

Systematics of *Alhagi*: molecular phylogeny and morphology revisited

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Alhagi is a small genus of desert or semi-desert shrubs with about six species widely distributed throughout northern Africa, eastern Mediterranean, the Middle East to the Central Asia, and small parts of India and China. Despite previous molecular and morphological studies on the genus, the taxonomy of *Alhagi* has been remained controversial. In order to delimit natural groups as well as to clarify interspecific relationships of *Alhagi* species distributed in Iran, 45 ingroup taxa representing 30 accessions of the genus *Alhagi* and 15 species from the sister genera, as well as two species, namely, *Astragalus arthuri* and *Colutea persica* as outgroups were selected. The nuclear ribosomal DNA internal transcribed spacer was employed for phylogenetic reconstructions. Phylogenetic analyses were performed using maximum parsimony, maximum likelihood and Bayesian inference. The obtained results recovered *Alhagi* accessions as a well-supported monophyletic group. All three traditionally recognized species in Iran split into two distinct morphologically well-supported lineages similar to splits graph. The results showed that, *Alhagi maurorum* and *A. graecorum* are recognized as two distinct species distributed in two phyto geographical regions of Irano-Turanian and Saharo-Sindian. As a complementary taxonomic treatment, the diagnostic key and description of the species are also provided herewith.

Keywords: *Alhagi graecorum*, *A. maurorum*, *Fabaceae*, phylogenetic relationships, taxonomy**سیستماتیک جنس خارشتر: بررسی مجدد فیلوژنی مولکولی و ریخت‌شناسی***

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خلاصه

خارشتر (*Alhagi*) جنسی کوچک از تیره باقلائیان شامل شش گیاه بوته‌ای بیابانی و نیمه‌بیابانی می‌باشد که به طور گسترده‌ای در سراسر شمال آفریقا، شرق مدیترانه و از خاورمیانه به سمت آسیای مرکزی و بخش‌های کوچکی از هند و چین پراکنش یافته است. به‌رغم مطالعات ریخت‌شناختی و مولکولی گذشته روی این جنس، مباحث مربوط به تاکسونومی آن به صورت بحث‌برانگیز باقی مانده است. در راستای تعیین گروه‌های طبیعی و روشن شدن روابط بین گونه‌های پراکنش یافته در ایران، در این مطالعه، ۴۵ آرایه به عنوان درون‌گروه شامل ۳۰ نمونه از جنس خارشتر و ۱۵ گونه از جنس‌های خویشاوند به عنوان درون‌گروه و دو گونه به اسامی *Astragalus arthuri* و *Colutea persica* به عنوان بیرون‌گروه انتخاب شدند. از توالی‌های ریبوزومی هسته‌ای ناحیه فاصله‌گذار رونویسی‌شونده داخلی برای مطالعات فیلوژنی استفاده شد. آنالیزهای فیلوژنی با استفاده از روش‌های بیشینه صرفه‌جویی، بیشینه درست‌نمایی و Bayesian انجام شد. نتایج حاصل نشان داد که اعضای جنس مذکور، در یک گروه تک‌تبار با حمایت خوب قرار گرفته‌اند، به طوری که سه گونه شناخته شده در مفهوم Reching برای ایران، به دو دودمان مجزا با حمایت و ویژگی‌های متمایز ریخت‌شناختی تقسیم شده‌اند که مشابه با نتایج حاصل از splits graph است. طبق نتایج به دست آمده، *Alhagi maurorum* و *A. graecorum* به عنوان دو گونه متمایز شناخته شدند که در دو ناحیه جغرافیایی گیاهی، ایران-تورانی و صحرا-سندی، پراکنش یافته‌اند. همچنین، پس از بررسی کامل تاکسونومیک، کلید شناسایی و شرح این گونه‌ها آرایه گردید.

واژه‌های کلیدی: باقلائیان، تاکسونومی، روابط فیلوژنتیکی، *A. maurorum*، *Alhagi graecorum*

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Introduction

The genus *Alhagi* Gagnebin (*Fabaceae*, *Hedysareae*) consists of six species growing as shrubs or subshrubs (Hassler 2019) distributed throughout northern Africa, the eastern Mediterranean and the Middle East, eastwards to western China (Chamberlain 1970, Townsend 1974, Meikle 1977, Yakovlev 1979, Rechinger 1984, Lock & Simpson 1991, Awmack & Lock 2002, Langran & Larsen 2010). Potential vegetation for this genus is largely desert and semi-desert areas with salty or gravelly soils (Rechinger 1984, Zahran & Willis 1992). The species of *Alhagi* have economic relevance both as weeds and in medicine as herbals (Townsend 1974). The genus is characterized by spiny branches, glabrous and simple leaves, articulate-moniliform and indehiscent legumes with 1–9 seeds (Rechinger 1984, Awmack & Lock 2002).

The considerable morphological similarities and broad geographical distribution among *Alhagi* species have complicated the identification and delimitation of species, as this status is appeared in several synonyms and several nomenclatural problems (Yakovlev 1979, Rechinger 1984, Awmack & Lock 2002, Sheidai *et al.* 2001). Fedtschenko (1972) and Rechinger (1984) recognized five species (*A. canescens* (Regel.) Shap. ex Keller & Shap., *A. kirghisorum* Schrenk, *A. persarum* Boiss. and Buhse, *A. pseudalhagi* (M.Bieb.) Desv., and *A. mannifera* Desv.) in the area covered by the corresponding flora, whereas the majority of other floras (e.g. Townsend 1974, Meikle 1977) recognized two widespread species of *Alhagi* in the western part of the range of the genus, *A. graecorum* Boiss. and *A. maurorum* Medik. Moreover, only one species with three subspecies have been recognized by Yakovlev (1979): *A. maurorum* subsp. *kirghisorum* (Schrenk) Yakovlev, *A. maurorum* subsp. *canescens* (Regel) Yakovlev, and *A. maurorum* subsp. *maurorum*, the last subspecies being divided in two varieties, viz. var. *maurorum* and var. *turcomanica* Yakovlev.

Hair characteristics in genus *Alhagi*, like majority of the other members of *Fabaceae* (Zarre 2003,

Ghahremaninejad 2004), have taxonomic importance. Also, based on the hairiness of the ovary and pod, Awmack & Lock (2002), reduced two species recognized by Meikle (1977) to the subspecies level. Accordingly, *A. mannifera* Desv. has been synonymized with *A. maurorum* Medik. subsp. *graecorum* (Boiss.) Awmack & Lock, and *A. persarum* Boiss. & Buhse with *A. pseudalhagi* (M.Bieb.) Desv. were treated as new combinations under *A. maurorum* Medik. subsp. *maurorum*.

Despite recent phylogenetic attempts to address the relationships across tribe *Hedysareae* (Ahangarian *et al.* 2007, Amirahmadi *et al.* 2014, Duan *et al.* 2015), some ambiguities in the phylogeny and taxonomy of *Alhagi* remained. According to the most recent molecular phylogenetic studies (Amirahmadi *et al.* 2014, Duan *et al.* 2015), the monophly of this genus is confirmed and the analyzed species of *Alhagi* formed a well-supported clade as the first diverging lineage of the tribe *Hedysareae*. The main problem in taxonomy of *Alhagi* is the delimitation of the species, which is addressed in the present study. We employed a comprehensive dataset of nuclear ribosomal DNA internal transcribed spacer (nrDNA ITS) from several accessions representing different taxa and different geographical areas in Iran to assess the phylogenetic relationships among members of *Alhagi* and to delimit *Alhagi* species. In addition, we used morphological characters to discriminate the taxa of this genus present in Iran.

Materials and Methods

- Field survey and herbarium studies

In order to prepare a diagnostic key for members of *Alhagi*, a total of 110 individuals from 22 populations were collected and examined in Iran. Voucher specimens are deposited at Islamic Azad University Herbarium (IAUH). For phylogenetic study, the 45 ingroups represent 30 accessions of the genus *Alhagi* (three species), and 15 species of the sister genera *Caragana*, *Chesneya*, *Ebenus*, *Gueldenstaedtia*, *Halimodendron*, *Hedysarum*, *Onobrychis*, and *Taverniera*. Two other

species, viz. *Astragalus arthuri* and *Colutea persica* were selected as outgroups according to previous molecular studies (Amirahmadi *et al.* 2014, Duan *et al.* 2015, Liu *et al.* 2017). Twenty two nrDNA ITS sequences of

Alhagi are newly generated in this study and the remaining sequences were downloaded from GenBank. Voucher specimens and GenBank accession numbers of the analyzed sequences are listed in table 1.

Table 1. Localities and vouchers of *Alhagi* species and the related genera used in the current study

| Taxon | Locality, source and voucher | GenBank accession No. |
|---|--|-----------------------|
| <i>Alhagi graecorum</i> Boiss. | Iran: Hormozgan province, Amirkhosravi 15103 (IAUH) | * |
| <i>A. graecorum</i> Boiss. | Iran: Ilam province, Amirkhosravi 15117 (IAUH) | * |
| <i>A. graecorum</i> Boiss. | Iran: Kohgiluyeh & Boyer-Ahmad province, Amirkhosravi 15107 (IAUH) | * |
| <i>A. graecorum</i> Boiss. | Iran: Bushehr province, Amirkhosravi 15121 (IAUH) | * |
| <i>A. graecorum</i> Boiss. | Saudi Arabia | KF805117 ^a |
| <i>A. maurorum</i> Medik. | Iran: Tehran province, Amirkhosravi 15100 (IAUH) | * |
| <i>A. maurorum</i> Medik. | Iran: Kerman province, Amirkhosravi 15101 (IAUH) | * |
| <i>A. maurorum</i> Medik. | Iran: Fars province, Amirkhosravi 15104 (IAUH) | * |
| <i>A. maurorum</i> Medik. | Iran: Khorasan (N) province, Amirkhosravi 15105 (IAUH) | * |
| <i>A. maurorum</i> Medik. | Iran: Kermanshah province, Amirkhosravi 15106 (IAUH) | * |
| <i>A. maurorum</i> Medik. | Iran: Hamadan province, Amirkhosravi 15108 (IAUH) | * |
| <i>A. maurorum</i> Medik. | Iran: Khorasan (S) province, Amirkhosravi 15109 (IAUH) | * |
| <i>A. maurorum</i> Medik. | Iran: Khorasan (RZ) province, Amirkhosravi 15110 (IAUH) | * |
| <i>A. maurorum</i> Medik. | Iran: Khorasan (RZ) province, Amirkhosravi 15111 (IAUH) | * |
| <i>A. maurorum</i> Medik. | Iran: Khorasan (RZ) province, Amirkhosravi 15112 (IAUH) | * |
| <i>A. maurorum</i> Medik. | Iran: Kurdistan province, Amirkhosravi 15113 (IAUH) | * |
| <i>A. maurorum</i> Medik. | Iran: Yazd province, Amirkhosravi 15114 (IAUH) | * |
| <i>A. maurorum</i> Medik. | Iran: Isfahan province, Amirkhosravi 15115 (IAUH) | * |
| <i>A. maurorum</i> Medik. | Iran: Sistan & Baluchistan province, Amirkhosravi 15116 (IAUH) | * |
| <i>A. maurorum</i> Medik. | Iran: Golestan province, Amirkhosravi 15118 (IAUH) | * |
| <i>A. maurorum</i> Medik. | Iran: Azarbajejan (W) province, Amirkhosravi 15119 (IAUH) | * |
| <i>A. maurorum</i> Medik. | Iran: Khorasan (S) province, Amirkhosravi 15120 (IAUH) | * |
| <i>A. maurorum</i> Medik. | Iran: Khuzestan province, Amirkhosravi 15123 (IAUH) | * |
| <i>A. maurorum</i> Medik. | Iran: Kazempour Osaloo 2008-2 (TMUH) | AB854477 ^a |
| <i>A. maurorum</i> Medik. | Iran: Kazempour Osaloo 2007-1 (TMUH) | AB374181 ^a |
| <i>A. maurorum</i> Medik. | China: Shihezi, Xinjiang | KJ486540 ^a |
| <i>A. maurorum</i> Medik. | * | GQ246125 ^a |
| <i>A. maurorum</i> Medik. | Iran: Kazempour Osaloo 2008-1 (TMUH) | AB854476 ^a |
| <i>A. sparsifolia</i> Shap. | * | KX942204 ^a |
| <i>A. sparsifolia</i> Shap. | * China: S.M.Duan TLF001 (TURP) | KP338103 ^a |
| <i>Astragalus arthuri</i> Jones. | USA: Wojciechowski 509 | AF121739 ^a |
| <i>Caragana arborescens</i> Lam. | * | DQ311963 ^a |
| <i>Chesneya astragalina</i> Jaub. & Spach | Iran: Assadi & Maassoumi 55503 (TARI) | AB051906 ^a |
| <i>Colutea persica</i> Boiss. | Iran: Foroughi 17434 (TARI) | AB051907 ^a |
| <i>Ebenus stellata</i> Boiss. | Iran: Ahangarian & Kazempour Osaloo 2006-1 (TMUH) | AB329691 ^a |
| <i>Gueldenstaedtia verna</i> (Georgi) Boriss. | USSR: Ulanova & Bassargin 39025 (MSB) | AB854489 ^a |
| <i>Halimodendron halodendron</i> (Pall.) Voss | Iran: Maassoumi <i>et al.</i> 86046 (TARI) | AB329693 ^a |
| <i>Hedysarum boreale</i> Nutt. | * | KY366158 ^a |
| <i>H. damghanicum</i> Rech.f. | Iran: Kazempour Osaloo <i>et al.</i> 2009-2 (TMUH) | AB854493 ^a |
| <i>H. hedysaroides</i> (L.) Schinz & Thell. | Russia: Zaslavskaja 6184 (TARI) | AB854495 ^a |
| <i>Onobrychis afghanica</i> Sirj. & Rech.f. | Afghanistan: Podlech 15931 (MSB) | AB854501 ^a |
| <i>O. atropatana</i> Boiss. | Turkey: Nydegger 28212 (MSB) | AB854503 ^a |
| <i>O. montana</i> DC. | * | KF015324 ^a |
| <i>O. ptolemaica</i> DC. | Iran: Salehi & Zahrabi 395 (KNRCH) | AB854507 ^a |
| <i>O. transcaucasica</i> Grossh. | * | HM542539 ^a |
| <i>O. viciifolia</i> Scop. | Spain: Podlech 6892 (MSB) | AB854512 ^a |
| <i>Taverniera spartea</i> DC. | Iran: Mozaffarian 49325 (TARI) | AB329707 ^a |

(*) Data not available in GenBank; (°) Sequences from GenBank; Abbreviations used: IAUH, Islamic Azad University Herbarium, Tehran, Iran; TARI, Herbarium of the Research Institute of Forests and Rangelands, Tehran, Iran; TMUH, Tarbiat Modares University Herbarium, Tehran, Iran; MSB, Herbarium of Ludwig-Maximilians-Universitat, Munchen, Germany; KNRCH, Khuzestan Natural Resource Research Center Herbarium of Khuzestan, Ahwaz, Iran; TURP, Turpan Eremophytes Botanical Garden, China.

- DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from silica gel-dried leaf material with the Dneasy plant mini kit (Qiagen, Inc., Germany). The nrDNA ITS region was amplified using the primers AB101F and AB102R (Douzery *et al.* 1999).

The PCR amplification was carried out in the volume of 20 μ l, containing 8 μ l deionized water, 10 μ l of the 2 \times Taq DNA polymerase master mix Red (Amplicon) 0.5 μ l of each primer (10 pmol/ μ l), and 1 μ l of template DNA. PCR cycles consisted of pre-denaturation at 94 °C for 3 min followed by 28–35 cycles, each consisting of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and elongation at 72 °C for 1 min followed by a final elongation step of 7 min at 72 °C. The quality of the PCR products was checked by electrophoresis on a 1% (w/v) agarose gel (using 1 \times TBE as the gel buffer) stained with ethidium bromide. PCR products along with the primers used for amplification were sent for Sanger sequencing at StaSEQ (Mainz, Germany).

- Alignment and phylogenetic analyses

The single marker data set was aligned using the web-based version of MUSCLE (Edgar 2004) under default parameters followed by manual adjustment. Indels were treated as missing data in all analyses.

Phylogenetic analyses were performed using maximum parsimony (MP) and Bayesian inference (BI). Parsimony analyses were conducted using PAUP* 4.0b10 (Swofford 2002). The heuristic search option was employed for each data set, using tree bisection-reconnection (TBR) branch swapping, 100 random sequence addition sequence replicates and no maximum tree limit. Branch support was assessed via the non-parametric bootstrap, MPB, (Felsenstein 1985) using 1,000 bootstrap replicates each with simple sequence addition. For likelihood analysis, the best models of sequence evolution were selected using MrModeltest 2.3 (Nylander, 2004) based on the Akaike information criterion (AIC) (Posada & Buckley 2004). This program identified GTR+G for nrDNA ITS. Maximum likelihood

(ML) analyses were performed using RAxMLGUI Ver. 1.5 (Silvestro & Michalak 2012). Nonparametric bootstrap values (MLB) were calculated in RAxMLGUI based on 1,000 bootstrap replicates with one search replicate per bootstrap replicate. The program MrBayes 3.2.4 (Ronquist *et al.* 2012) was used for the Bayesian phylogenetic analyses. Posteriors on the model parameters were estimated from the data, using the default priors and running the MCMC chains for 10 million generations. MrBayes performed two simultaneous analyses starting from different random trees (Nruns = 2) each with four Markov chains (i.e., one cold and three heated) and parameters sampled every 1000 generations. The first 25% trees were discarded as the burn-in. The remaining trees were then used to build a 50% majority rule consensus tree accompanied with posterior probability (PP) values. Tree visualization was carried out using TreeView version 1.6.6 (Page 2001). Mean pairwise sequence divergence and calculating variable sites were done in MEGA Ver. 6 (Tamura *et al.*, 2013).

Phylogenetic networks: Neighbor-Net (NN), a distance-based network construction method, allows for graphical representation of conflicting phylogenetic signals and interpretation of evolutionary histories which are not tree-like (Bryant & Moulton 2004). NN splits graphs have been used with varying success to detect reticulate history (Carine *et al.* 2007, Frajman & Oxelman 2007, Grimm & Denk 2008, Weiss-Schneeweiss *et al.* 2008, Ramdhani *et al.* 2010, Schulte *et al.* 2010). Splits graphs were constructed using the NN algorithm with uncorrected p-distances in SplitsTree Ver. 4.14.4 (Huson 1998).

Results

Visual inspection of all clades and sister group relationships revealed no major differences among tree topologies obtained from MP and BI, respectively. Therefore, we will focus on the better resolved BI trees. The aligned nrDNA ITS data set contained 669 characters of which 300 were variable and 229

parsimony informative. Mean pairwise sequence divergence among nrDNA ITS haplotypes was 0.106. The number of variable sites within *Alhagi* sequences was 52. Heuristic search yielded 10000 most parsimonious trees (length=624 steps, CI=0.683, RI=0.893). In the obtained cladogram (Fig. 1), the accessions representing the genus *Alhagi* were inferred as monophyletic (PP=1.00, MPB=100, MLB=100) and found to be composed of two well-supported clades. One clade (PP=1.00, MPB=100, MLB=99) included most

representatives of *Alhagi* split into several subclades, and the other clade contained the remaining *Alhagi* accessions in one well-supported clade (PP=1.00, MPB=100, MLB=96). Similar to the Bayesian consensus tree (Fig. 1), the splits graph revealed two main groups (Fig. 2). Mean pairwise sequence divergence among these two main groups was 0.048. The number of variable sites in the larger and smaller clades contained twenty and six, respectively.

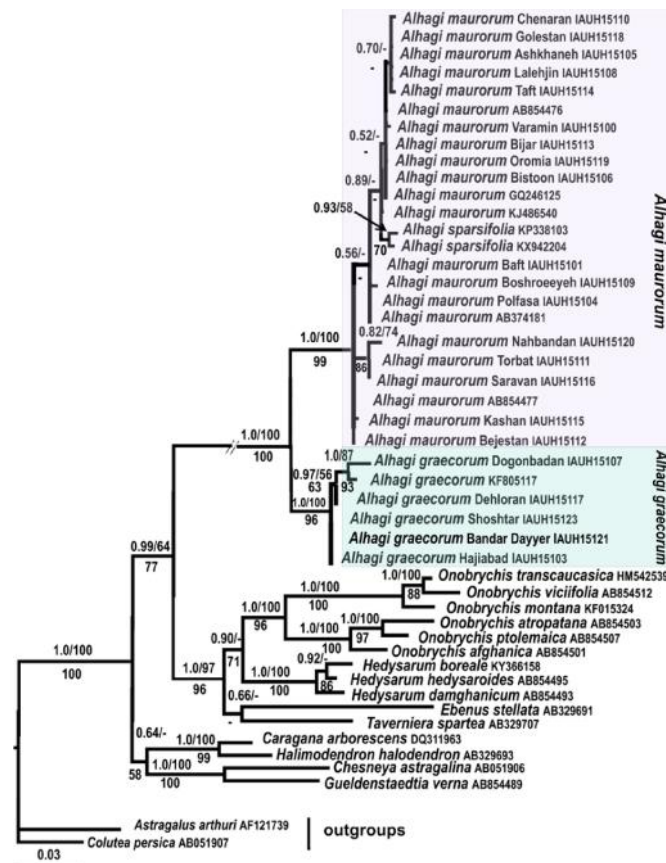


Fig. 1. Fifty percent majority rule consensus tree resulting from Bayesian inference of nrDNA ITS dataset of *Alhagi* and its related genera. Numbers above branches are posterior probability and parsimony bootstrap values, those below branches likelihood bootstrap values. Values <50% are not shown.

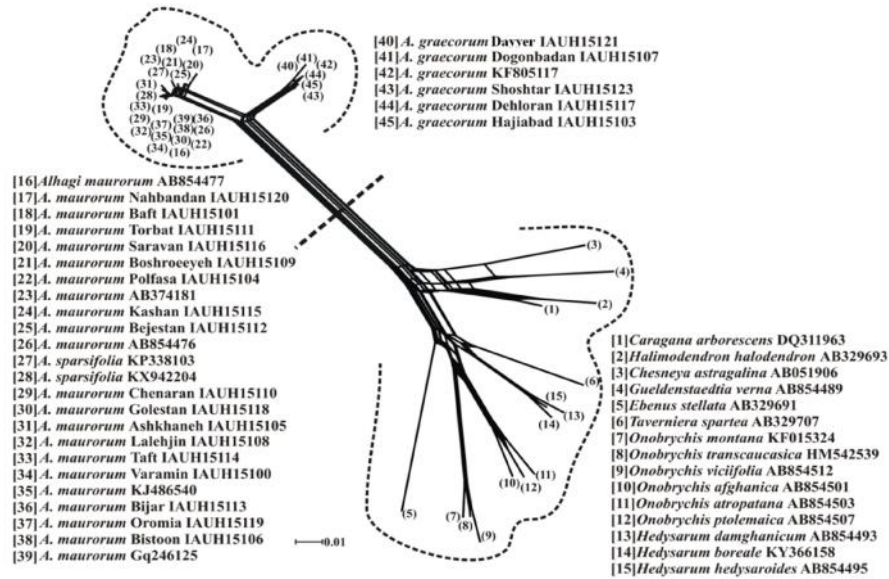


Fig. 2. Splits graph for nr DNA ITS data set of *Alhagi* and its related genera.

Discussion

The remarkable morphological similarities among the proposed species of *Alhagi* (*Fabaceae*, *Hedysareae*) have led to some complexities in the taxonomy and delimitation of the species of the genus (Chamberlain 1970, Townsend 1974, Meikle 1977, Yakovlev 1979, Rechinger 1984, Awmack & Lock 2002). The circumscription of *Alhagi* species has undergone several changes in the past (Rechinger 1984, Yakovlev 1979, Awmack & Lock 2002, Sheidai *et al.* 2001). Regarding potential vegetation of this genus on desert and semi-desert areas and salty or rocky soils, Iran is taken into account as one of intermediate distribution corridors of the genus consisting of three species sensu Rechinger (1984). In this survey, to test the hypotheses on circumscription and relationships among *Alhagi* spp., and to redefine and to establish a taxonomic treatment, a comprehensive taxon sampling from all habitats and geographical regions of Iran along with special focus on type localities were conducted. The current molecular phylogeny based on nrDNA ITS sequences provides a relatively well-supported scenario of relationships within *Alhagi*. In agreement with recent molecular studies, the genus was retrieved as a monophyletic group as the first diverging lineage of the tribe *Hedysareae* (Ahangarian

et al. 2007, Amirahmadi *et al.* 2014, Duan *et al.* 2015, Liu *et al.* 2017). Possessing spiny branches, glabrous, simple leaves, articulate and moniliform, indehiscent pods with 1–9 seeds are synapomorphic traits for diagnosing this genus from the other genera of *Hedysareae* (Rechinger 1984). However, the current study indicated that, morphological similarities cause some difficulties in diagnosing *Alhagi* species. This status is confirmed by previous morphological studies resulting in several synonymies (Yakovlev 1979, Rechinger 1984, Awmack & Lock 2002, Sheidai *et al.* 2001). Similar to the other *Alhagi* species, these taxonomic changes can be observed in the three recognized species of Iran sensu Rechinger (1984), viz. *A. mannifera*, *A. persarum*, and *A. pseudalhagi*. Following the botanical nomenclature priority rules (Article 11. 1–10, in Turland *et al.* 2018) the accepted name for *A. mannifera* is *A. graecorum*, since the characters used by both Boissier (1872) and Keller & Shaparenko (1933) to separate *A. graecorum* from *A. mannifera* had been proved to be unreliable (Chamberlain 1970). Based on a cytological and morphometric study, Sheidai *et al.* (2001) considered *A. persarum* as a synonymy of *A. pseudalhagi* and Iran housing only two species, i.e. *A. graecorum* and

A. pseudalhagi. However, in the western part of distribution of *Alhagi*, Awmack & Lock (2002) recognized a single variable species *A. maurorum*, *A. pseudalhagi*, and *A. persarum* were considered as synonyms of *A. maurorum* subsp. *Maurorum*, and *A. graecorum* as *A. maurorum* subsp. *graecorum*. In agreement with the previous morphological and cytological studies (Sheidai *et al.* 2001, Awmack & Lock 2002), the extensive morphological similarities between *A. persarum* and *A. pseudalhagi* was confirmed by the current molecular and morphological study, as the collected specimens from the localities reported by Rechinger (1984) for these two species did not indicate distinct molecular and morphological separation.

In agreement with the previous study (Amirahmadi *et al.* 2014), our analyses with the comprehensive taxon sampling from Iran indicated that, *Alhagi* accessions are divided into two well-supported clades which are geographically and morphologically distinct. The larger clade, distributed in the eastern part of the range of the genus, is confined to Irano-Turanian floristic region. As *Alhagi pseudoalhagi*, *A. persarum*, *A. sparsifolia*, and *A. maurorum* with remarkable morphological similarities joined together to form this larger clade. This clade is clearly distinct from the smaller clade with several morphological features such as the height and color of plant, the absence/presence hairs on stem, calyx, pod and ovary, the number of flowers on the axis of inflorescence. The smaller clade distributed in the western and south western of Iran related to Sahara-

Sindian region. Therefore, sufficient genetic and morphological differences confirmed these two clades as clearly distinct species. With regarding to the botanical nomenclature priority rules, the members of the larger and smaller clades are belonging to the species "*A. maurorum*" and "*A. graecorum*", respectively.

Based on the hairiness of the ovary and pod, Awmack & Lock (2002) with comprehensive taxon sampling treated these two last species as a single variable species with two subspecies. Likewise, Mabblerly (2017) introduced *Alhagi* as a monospecific genus, with *A. maurorum* as the only species. However, the present molecular and morphological evidence supported by adequate nucleotide difference in nrDNA ITS maker, the convincing number of diagnosed characters, and distinct biogeographic distribution patterns suggest recognizing two species of *Alhagi* in Iran, viz. *A. graecorum* and *A. maurorum*.

Taxonomic treatment

- *Alhagi* Gagnebin

Based on the molecular and morphological results of this study, it is quite clear that, there are only two distinct species in Iran. The two species differ morphologically and occur in different phytogeographical regions, i.e. Irano-Turanian and Saharo-Sindian. Comparing the results of this study with that of Awmack & Lock (2002), a key to these species is presented below.

Key to the species

1. Plant dwarf, shortly pubescent or hirsute, up to 40 cm (–60 cm) tall, pale green in colour; spines condensed and quadrangular in cross-section; inflorescence mostly with 1–3 flowers; calyx appressed hairy, the teeth distinct with acute sinuses; ovary sericeous; pods hairy, irregularly constricted between the seeds *A. graecorum* Boiss.
- Plant tall glabrous or subglabrous, mostly 75–150 cm tall, blue-green in color; spines sparsely branched, circled in cross-section; inflorescence mostly with 3–8 flowers; calyx glabrous, the teeth almost obsolete with obtuse sinuses; ovary glabrous; pods glabrous, more regularly and strongly constricted between the seeds *A. maurorum* Medik.

1. *Alhagi graecorum* Boiss., Diagn. Pl. Orient. Nov. ser.1 (9): 144 (1849); Fl. Orient. 2: 559 (1872); Townsend, Fl. of Iraq 3: 499, Pl. 96, Figs 1–10 (1974) Syn.: *Hedysarum alhagi* L., Spec. Plant. 745 (1753) p.p.; *A. mannifera* Desv., J. Bot. Agric. 1: 120, t. 4, Fig. 4 (1813) nom. nud.; *A. maurorum* sensu DC., Prodr. 2: 352 (1825), sensu Boiss., Fl. Orient. 2: 558 (1872) et sensu Rech. f., Ark. Bot. ser. 2, 1: 426 (1950), non Medik.; *Alhagi maurorum* Medik. subsp. *graecorum* (Boiss.) Awmack & Lock, Kew Bull. 57(2) 441 (2002).

Plant dwarf perennial subshrub, up to 40 cm (–60 cm), with green habit, erect to ascending or subspreading, shortly pubescent or hirsute; branches strong, condensed, 8–20 cm long, spines strong, with quadrant-section, up to c. 4.5 cm long and up to c. 2.2 mm in diameter, each tipped c. 2.5–6 mm in length, spines of the lateral stems slender than main stem; leaves simple, relatively hairy, rectangular to obovate, about 2–6 times shorter than spines, obtuse, petioles very short 0.5–1 mm, hairy; inflorescence racemose, axillary, axis spine-tipped, 1–3-flowered (–1–5), peduncles 1–2 mm long, hairy; bracts very minute 0.2–0.6 mm; calyx 2–4 mm long, appressed hairy, the teeth conspicuous, about one-third to one-quarter the length of the tube, with acute sinuses, 0.37–0.95 mm; corolla red to purplish; standard 5–9 mm, obovate, with a short claw; keel slightly shorter or subequal to the standard, slightly falcately oblong, with rounded apex; wings shorter or subequal to keel, oblong. Ovary linear, sericeous. Pods linear, moniliform, hairy, 1–5 on an axis ending to spine, irregularly constricted between the seeds, often variously twisted or falcate, 10–21 × 1–4 mm, stipe hairy with 1–3 mm long; seeds c 1–4 mm, subspherical to rhomboid, brown, smooth.

Distribution and habitat: Algeria, Bahrain, Chad, Cyprus, E. Aegean Is, Egypt, Greece, Iran, Iraq, Jordan, Kuwait, Lebanon, Libya, Niger, Palestine, Saudi Arabia, Sinai, Sudan, Syria, and Turkey (Hassler 2019). Most often on often edges of ditches and saline places at low elevation, mostly 100–500 m.

Selected specimens: Iran: Hormozgan province, Hajiabad, 5 km from Hajiabad to Baghat, 1109 m, 55°56.28 E, 28°22.56 N, Amirkhosravi IAUH-15103; Kohgiluyeh & Boyer-Ahmad province, Dogonbadan, 5 km from Dogonbadan to Talkhabshirin, 718.2 m, 50°47.37 E, 30°21.31 N, Amirkhosravi IAUH-15107; Ilam province, Dehloran, Bishehzar, 339.5 m, 46°56.36 E, 32°48.23 N, Amirkhosravi IAUH-15117; Khuzestan province, Shoshtar, 2 km from Shoshtar to Sardaran, 36.5 m, 48°50.58 E, 32°1.21 N, Amirkhosravi IAUH-15123; Bushehr province, Bandar-Dayyer, 2 km from Bandar-Dayyer to Bardestan, 10.7 m, 51°53.34 E, 27°50.3 N, Amirkhosravi IAUH-15121.

2. *A. maurorum* Medik., Vorles. Churpf. Phys.-Oecon. Ges. 2: 397 (1787)

Syn.: *Hedysarum alhagi* L., Spec. Plant. 745 (1753) p.p.; *H. pseudoalhagi* M. Bieb., Fl. Taur.-Cauc. 2: 174 (1808); *Alhagi pseudoalhagi* (M.Bieb.) Desv, Journ. Bot. 3: 120 (1813); *A. persarum* Boiss. & Buhse, Nouv. M6m. Soc. Imp. Naturalistes Moscou 12: 76 (1860); *A. camelorum* Fisch., Cat. Jard. Gorenki, ed. 2: 72 (1812); Boiss., Fl. Orient. 2: 559 (1872); *A. sparsifolia* Shap. ex Keller et Shap. in Sovjetskaja Bot. 3–4 (1933) 167, pro var.

Plant erect, glabrous or subglabrous, spiny perennial shrub, mostly 75–150 cm, with blue-green habit; stems relatively strongly branching; spines sparsely branched with circle-section, erect-ascending to subcurved with broad angle, up to c. 5 cm long and up to c. 2.8 mm in diameter, each tipped c. 2–9 mm in length, spines of the lateral stems slender than main stem; leaves simple, rectangular to obovate, 8–22 mm long, 2–8 mm wide, obtuse or slightly acute, in both surfaces glabrous or more or less sparingly hairy, petioles short 1–2.5 mm; racemes 1–8-flowered, the axis spine like, peduncles 1.5–2 mm long; bracts minute 0.2–0.8 mm; calyx 1.5–5 mm, glabrous, the teeth almost obsolete, smaller than the tube, 0.18–2.47 mm, with obtuse sinuses; corolla red to purplish; standard 7–10.5 mm, obovate, abruptly narrowed to a short claw; keel slightly shorter or subequal to the standard, slightly falcate-oblong, with

rounded apex; wings shorter or subequal to keel, oblong. Ovary linear, glabrous. Pods linear, moniliform, glabrous, 1–9 on an axis ending to spine, regularly and strongly constricted between the seeds, 8–35 × 1–4 mm, stipe glabrous with 1–3 mm long; seeds c 1–4 mm, subspherical to rhomboid, brown, smooth.

Distribution and habitat: Afghanistan, Armenia, Azerbaijan, Bahrain, China, Cyprus, E. Aegean Is, India, Iran, Iraq, Jordan, Kazakhstan, Kirgizstan, Lebanon, Palestine, Pakistan, Russia, South Africa, Syria, Tadzhikistan, Turkey, Turkmenistan, and Uzbekistan (Hassler 2019). Most often on sand dunes, saline areas, field margins and as a weed in agricultural crops, mostly at 700–2300 m elevation.

Selected specimens: Iran: Tehran province, Varamin, 2 km from Varamin to Pishva, 923.1 m, 51°38.2 E, 35°19.59 N, Amirkhosravi IAUH-15100; Kerman province, Baft, Asiajofteh, 2304.7 m, 56°33.00 E, 29°14.58 N, Amirkhosravi IAUH-15101; Fars province, Polfasa, 1 km from Pol-e Fasa to Shiraz, 1475.2 m, 52°37.21 E, 29°31.16 N, Amirkhosravi IAUH-15104; Khorassan (N) province, Ashkhaneh, 1 km from Ashkhaneh to Mehmanak, 764.1 m, 56°54.39 E, 37°32.42 N, Amirkhosravi IAUH-15105; Kermanshah province, Biston, 15 km from Biston to Kermanshah, 1408.8 m, 47°14.11 E, 34°21.1 N, Amirkhosravi IAUH-15106; Hamedan province, Lalehjin, Dinarabad, 1713.2 m, 48°28.55 E, 34°58.18 N, Amirkhosravi IAUH-15108; Khorassan

(S) province, Boshroeyeh, 40 km from Ferdos to Deyhook, 1254.1 m, 57°11.27 E, 33°53.13 N, Amirkhosravi IAUH-15109; Khorassan (RZ) province, Chenaran, Radkan, 1180.3 m, 59°5.3 E, 36°38.57 N, Amirkhosravi IAUH-15110; Khorasan (RZ) province, Torbat Heydarieh, Robat Sang, 1710.4 m, 47°14.11 E, 34°21.1 N, Amirkhosravi IAUH-15111; Khorasan (S) province, Bajestan, 2 km from Bajestan to Gonabad, 1322.9 m, 58°10.23 E, 34°29.25 N, Amirkhosravi IAUH-15112; Kurdistan province, Bijar, 20 km from Bihar to Ghajoor, 1714 m, 47°46.42 E, 35°53.45 N, Amirkhosravi IAUH-15113; Yazd province, Taft, Cham, 1436.6 m, 54°15.29 E, 31°47.3 N, Amirkhosravi IAUH-15114; Esfahan province, Kashan, Taherabad, 984.5 m, 51°20.5 E, 34°1.54 N, Amirkhosravi IAUH-15115; Sistan & Baluchistan province, Saravan, 5 km from Saravan to Hooshak, 1275 m, 62°31.51 E, 27°17.21 N, Amirkhosravi IAUH-15116; Golestan province, Gonbad-e Kavous, Alghajar, 123.7 m, 55°20.25 E, 37°12.4 N, Amirkhosravi IAUH-15118; Azarbayejan (W) province, Urmia, 20 km from Urmia to Sero, 1349.7 m, 45°2.32 E, 37°35.27 N, Amirkhosravi IAUH-15119; Khorasan (S) province, Nehbandan, 10 km from Nehbandan to Sefidabeh, 1088.4 m, 59°49.36 E, 31°27.8 N, Amirkhosravi IAUH-15120.

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