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A new *Aceria* species (Acari:Trombidiformes: Eriophyoidea) from West Asia, a potential biological control agent for the invasive weed camelthorn, *Alhagi maurorum* Medik. (Leguminosae)

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**ABSTRACT**

A new species of eriophyoid mite *Aceria alhagi* n. sp. inhabiting the weed *Alhagi maurorum* Medik., is described from the type locality in Iran, but it was also collected from Uzbekistan, Turkey and Armenia. This mite causes changes of the leaves and inflorescence. Infested plants develop cauliflower-like galls on the inflorescence and leaves deforming the reproductive structures and inhibiting seed production. The potential reduction in seed set suggests that this mite could constitute a potential biological control agent against this noxious weed. To investigate intraspecific variability between *A. alhagi* n. sp. populations from Iran, Turkey and Armenia, we analysed molecular sequences of the mitochondrial cytochrome oxidase subunit I (mtCOI). These results indicated that there are no significant intraspecific divergences among populations of *A. alhagi* n. sp. from the five different localities in three West Asia countries. This finding can be used in the future research of certain mite populations as biological control agent.

**Keywords** Eriophyidae, weed biological control, invasive weed, mtCOI, intraspecific variability, phytophagous mites

**Zoobank** [http://zoobank.org/0675CBF0-2A56-4F77-B1EC-7615D45983F0](http://zoobank.org/0675CBF0-2A56-4F77-B1EC-7615D45983F0)

**Introduction**

The plant genus *Alhagi* belongs to the tribe Hedysareae of the Leguminosae together with another eight genera: *Corethrodendron*, *Ebens*, *Eversmannia*, *Greuteria*, *Hedysarum*, *Onobrychis*, *Sala* and *Taverniera* (Duan et al. 2015). According to the Plant List database (2016), *Alhagi* has 9 plant species. Our target species, *Alhagi maurorum* Medik., is a shrub commonly known as camelthorn, camelthorn-bush, Caspian manna, or Persian manna (CABI 2015). The
native range of this plant extends from Cyprus and Egypt in the West, to Mongolia and China in the East and South to India and Saudi Arabia (ILDIS 2002, Li et al. 2010). In its region of origin, camelthorn is used as a medicinal herb (Suthar et al. 2016). However no uses have been reported from the areas where it was introduced. Conversely in these areas it has become an invasive weed: in the USA it is listed as a noxious weed in seven states (USDA–NRCS 2002); Australia has declared it a state prohibited weed in Victoria (Munakamwe 2016) and in South Africa it is a declared Category 1 invasive species (AGIS–WIP 2006). No records of biological control agents are reported for this weed, although there is a potential in this field (Rassoul et al. 1988).

All Eriophyoidae mites are phytophagous, many of them can reach pests status on crops, whereas many others are associated with weeds. Eriophyoid mites have high potential as classical biological control agents of weeds due to their strict host plant specificity (Lindquist et al. 1996, Smith et al. 2010).

According to the world catalogue (Amrine & Stasny 1994), Fauna Europaea (De Lillo 2004) and published records (Denizhan et al. 2007, Xue et al. 2012, Lotfollahi et al. 2014), six eriophyoid mites have been reported on Hedysarum hosts. These include, on Hedysarum type hosts, Aculus hedysari (Liro 1941) from Hedysarum sibiricum Ledeb. (junior synonym of Hedysarum alpinum L.); Aculus longifilis (Canestrini 1891) from Onobrychis vicifolia Scop.; Aceria novellae from Hedysarum sp. (Denizhan et al. 2007) and Aculodes alhagis Xue, Sadeghi & Hong from A. maurorum (Xue et al. 2012). The final two species, Aceria medicaginis (Keifer 1941) and Aculops allotrichus (Nalepa 1894) were recorded on Hedysarum coronarium L. and A. maurorum respectively as alternate hosts. Besides these six species one more was recorded as nomen nudum, i.e. Aceria alhagi from A. maurorum (Kamali 2011, Doryanizadeh et al. 2013).

Reliable identification is one of the key elements in the search for biological control agents of pests, including target weeds, in order to avoid unfavourable non-target effects. Phenotypic differences among eriophyoid mites belonging to the same genus associated with the closely related host plants are usually small. Intraspecific variability of morphological traits and the existence of cryptic species makes species determination difficult (Amrine et al. 1994, Skoracka et al. 2002, Navia et al. 2006). Recently, molecular analyses are widely accepted and applied as supplementary methods that help to avoid errors in systematics in such situations (Navajas & Navia 2010). The current study presents the morphological description of a new eriophyoid mite species, A. alhagi n. sp., found on A. maurorum as well as mt-COI nucleotide sequences of populations from different geographical areas.

Materials and methods

Plant samples of Alhagi maurorum Medik. (Leguminosae) were collected from Iran, Uzbekistan, Turkey and Armenia during 2012, 2013 and 2015 and examined at the laboratory under a dissection stereomicroscope. Since this mite species was recorded first in Iran (Kamali 2011), we decided to use this material as the type material for morphological description and measurements. Mite specimens from samples representing certain populations were used for morphological and molecular analyses and fixed in 75% and 96% ethanol respectively.

Morphological analysis

Mites were extracted from the plants using a fine pin with the aid of direct examination under a dissection stereomicroscope and/or using extraction methods described by de Lillo (2001) and Monfreda et al. (2007). The mites were mounted in Keifer’s F medium (Amrine & Manson 1996) and then examined using a Leica DMLS research microscope with phase-contrast. The morphology and nomenclature follows Lindquist (1996) and genus classification is based on Amrine et al. (2003). Measurements and illustrations were made according to Amrine & Manson (1996) and de Lillo et al. (2010). Morphometry was performed using the software Vidović B. et al. (2018), Acarologia 58(2): 302-312; DOI 10.24349/acarologia/20184243 303
package IM 1000 (Leica, Wetzlar, Germany) and for drawings a camera lucida was used. All measurements are given in micrometers (μm) and, unless stated otherwise, are the lengths of the structures. Morphometric data of *A. medicaginis* which were used for comparison with *A. alhagi n. sp.*, were obtained from the literature (Keifer 1941). Plant names are in accordance with The Plant List (2016) on-line database.

The holotype and the paratype slides are deposited in the collections of the Acarology Laboratory, Department of Entomology and Agricultural Zoology, Faculty of Agriculture, University of Belgrade, Serbia; one paratype slide is deposited in the Department of Plant Protection, Razavi Agricultural and Natural Resources Research Centre, Mashhad, Iran.

Scanning electron micrographs (SEM) were taken according to Alberti & Nuzzaci (1996). Live mites were collected individually using a fine pin from fresh plant material under a stereomicroscope. For sample preparation before being placed on the SEM holder, mites were sputter-coated with gold for 100 s under 30 mA ion current.

The samples were then studied in the vacuum chamber of a JEOL Scanning Electron Microscope (SEM, JEOL-JSM6390) at the Laboratory of Electron Microscopy, Faculty of Agriculture, University of Belgrade, Serbia.

**Molecular analysis**

Material collected for molecular analysis included samples of populations from Iran, Turkey and Armenia (Table 1). Prior to molecular analysis, mite specimens were preserved in 96% ethanol and stored at 4°C until DNA extraction. Total DNA was extracted from a pool of 15-20 whole specimens using QIAGEN DNeasy® Blood & Tissue Kit (QIAGEN, Hilden, Germany), according to the manufacturer’s instructions. The barcoding region of the mitochondrial cytochrome oxidase subunit I gene was amplified by polymerase chain reaction (PCR) using a forward primer LCO1490 (Folmer et al. 1994), and reverse primer HCOd (Chetverikov et al. 2015). Polymerase chain reactions (PCR) were conducted using High Yield Reaction Buffer A with Mg (1x), 2.5 mM MgCl₂, 0.6 mM of each dNTP, 0.5 μM of each primer and 1 U of KAPA TaqDNA polymerase (Kapa Biosystems, London, UK) in a 25 μL final volume. PCR was carried out in a Master cycler ep gradient S thermal cycler (Eppendorf, Hamburg, Germany) applying the following steps: 95°C for 5 min (initial denaturation), 35 cycles at 94°C for 1 min, 1 min at 54°C (annealing), 1 min 30 s at 72°C, with a final extension at 72 °C for 7 min. PCR amplicons were purified using the QIAquick PCR purification Kit (QIAGEN) according to the manufacturer’s instructions, and sequenced on automated equipment by Macrogen (Seoul, South Korea) with the same primer pairs as in the initial PCR procedure. The sequences were manually edited using FinchTV v.1.4.0 (www.geospiza.com), and aligned by CLUSTAL W integrated in MEGA5 software (Tamura et al. 2011). Uncorrected pairwise genetic distances were used to calculate the average genetic distance between *A. alhagi n. sp.* populations associated with *A. maurorum* collected from different localities (Table 1).

**Results**

**Taxonomy**

**Family: Eriophyidae Nalepa, 1898**

*Aceria alhagi* n.sp. Vidović et Kamali, 2016 (Figures 1-2)

Zoobank: B78B39BC-C1E7-44F2-9C4E-9AE24D293F5C


Table 1 Collection data for *Aceria alhagi* n. sp. inhabiting *Alhagi maurorum* populations.

<table>
<thead>
<tr>
<th>Country</th>
<th>Locality, – date</th>
<th>GPS coordinates</th>
<th>Name of collector</th>
<th>Accession numbers COI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Iran</td>
<td>Shirvan, -- June 2015</td>
<td>37°24ʹ28ʺN; 57°59ʹ12ʺE</td>
<td>Cristofaro M.</td>
<td>MF150169</td>
</tr>
<tr>
<td>2 Iran</td>
<td>Honameh, -- July 2014</td>
<td>37°31ʹ12ʺN; 58°01ʹ42ʺE</td>
<td>Cristofaro M.</td>
<td>MF150170</td>
</tr>
<tr>
<td>3 Turkey</td>
<td>Sarayhan, -- November 2013</td>
<td>38°33ʹ25ʺN; 33°53ʹ32ʺE</td>
<td>Cristofaro M.</td>
<td>MF150171</td>
</tr>
<tr>
<td>4 Turkey</td>
<td>Cavusin, -- June 2015</td>
<td>38°41ʹ10ʺN; 34°52ʹ01ʺE</td>
<td>Cristofaro M.</td>
<td>MF150172</td>
</tr>
<tr>
<td>5 Armenia</td>
<td>Vedi, -- July 2015</td>
<td>39°58ʹ05ʺN; 44°52ʹ23ʺE</td>
<td>Cristofaro M.</td>
<td>MF150173</td>
</tr>
</tbody>
</table>

Prodorsal shield — 26 (24 – 27) including frontal lobe, 35 (30 – 38) wide, semi-elliptical, basally-flexible frontal lobe, over gnathosomal base. Shield pattern almost smooth, without median line, with two short admedian lines and shorter submedian lines I and II; scapular setae sc dorsal tubercles on rear shield margin, 19 (17 – 20) apart, scapular setae sc 34 (28 – 37), directed posteriorly.

Legs — with all usual segments and setae present. Leg I 29 (25 – 32), femur 7 (6 – 9), genu 6 (5 – 7), tibia 7 (5 – 7), tarsus 8 (6 – 8), tarsal solenidion (ω) 8 (8 – 9) distally rounded, tarsal empodium (em) simple, 5 (5 – 7), 6-rayed; basiventral femoral setae (bv) 16 (12 – 16), antaxial genual setae (l″) 8 (6 – 85 (5 – 7), paraxial fastigial tarsal setae (ft‘) 13 (11 – 14), antaxial fastigial tarsal setae (ft″) 24 (21 – 28). Leg II 26 (25 – 29), femur 6 (6 – 9), genu 5 (4 – 6), tibia 5 (4 – 6), tarsus 8 (6 – 8), tarsal solenidion (ω) 8 (6 – 9) distally rounded, tarsal empodium (em) simple, 6 (4 – 6), 6-rayed; basiventral femoral setae (bv) 13 (12 – 16), antaxial genual setae (l″) 11 (10 – 14), paraxial fastigial tarsal setae (ft′) 6 (5 – 7), antaxial fastigial tarsal setae (ft″) 24 (20 – 28).

Coxigenital area — coxisternal plates granulated; anterolateral setae on coxisternum I (1b) 10 (9 – 13), 7 (7 – 8) apart; proximal setae on coxisternum I (1a) 23 (21 – 26), 5 (4 – 6) apart; proximal setae on coxisternum II (2a) 35 (33 – 46), 18 (17 – 18) apart. Prosternal apodeme 5 (5 – 7).

Opisthosoma — with subequal annuli, 67 (66 – 76) dorsal and 74 (67 – 74) narrow ventral annuli (counted from first annulus after coxae II), 5 (4 – 6) coxigenital annuli. Microutubercles elliptical on dorsal annuli and circular on ventral annuli. Setae c2 28 (24 – 28) on ventral annulus 12 (9 – 12), 46 (38 – 47) apart; setae d7 65 (60 – 80) on ventral annulus 26 (22 – 26), 35 (31 – 37) apart; setae e17 (14 – 18) on ventral annulus 41 (36 – 41), 18 (15 – 19) apart; setae f27 (24 – 27) on ventral annulus 68 (61 – 68), 19 (15 – 20) apart. Last 5 ventral annuli with numerous elongated linear microtubercles. Setae h2 122 (110 – 144), setae h1 6 (4-6).

Genital cover flap 12 (9 – 12), 20 (17 – 20) wide, with 16 (15 – 17) longitudinal striae; proximal setae on coxisternum III (3a) 34 (30 – 36), 13 (12 – 14) apart. Internal genitalia — Anterior, transversal apodeme trapezoidal, longitudinal bridge relatively long, the post spermathecal part of the longitudinal bridge is reduced; spermathecae tubes directed latero-posterad, spermathecae egg-shaped, globose.

Figure 1 Aceria alhagi n.sp.: AD – Antero-dorsal mite; AL – Antero-lateral view of mite; CG – Coxigenital region of female; em – Empodium; GM – Genital region of male; IG – Internal female genitalia; L1 – Leg I of female; LO – Lateral opisthosoma; PM – Postero-lateral mite. Scale bar: 20μm for AD, AL, CG, GM, IG, LO, PM; 10μm for L1; 5μm for em.
Figure 2 SEM images of *Aceria alhagi* n. sp.; A – prodorsal shield; B – tarsal empodia on legs I and II; C – ventral view of coxigenital area of female; D – ventral view of coxigenital area of male.

Nymph and Larva — Not found

Host plant — *Alhagi maurorum* Medik (Fabaceae) commonly known as camelthorn, camelthorn-bush, Caspian manna, and Persian manna plant.

Relation to the host plant — This mite caused changes of the leaves and inflorescence. The edge of the leaves become twisted and the inflorescence do not develop fully, with deformation of the reproductive structures. The flowers are replaced by cauliflower-like galls (Figure 3).

Type Material — Holotype: female, Shirvan, Iran (37°24′28″N; 57°59′12″E), 27 June 2015. coll. Cristofaro M. Paratypes: 20 females, 4 males, same data.

Additional studied material — Mashad, Iran (37°30′81″N; 58°01′18″E), 12 September 2012, 30 slides; Honameh, Iran (37°31′12″N; 58°01′42″E), 12 July 2014, 10 slides; Ohalik, Uzbekistan (39°32′01″N; 66°53′43″E), 18 August 2013, 25 slides; Bukhara, Uzbekistan (39°46′37″N; 64°24′02″E) 21 August 2013, 30 slides; Sarayhan, Turkey (38°33′25″N; 33°53′32″E), 10 November 2013, 30 slides; Cavusin, Turkey (38°41′10″N; 34°52′01″E) 20 June 2015, 8 slides; Vedi, Armenia (39°58′05″N; 44°52′23″E) 07 July 2015, 7 slides; coll. Cristofaro M. All slides are deposited in the Department of Entomology and Agricultural Zoology, Faculty of Agriculture, University of Belgrade, Serbia.

Etymology — The species designations *alhagi* is from the genus name of the host plant.

GenBank accession numbers — The domain mtCOI was successfully amplified and sequenced. The sequence of this region in *A. alhagi* n. sp. is available from GenBank under accession number MF150169 – MF150173.

Differential diagnosis and remarks — Until now, only one eriophyoid mite was described
Table 2 Comparison of available key morphological characters between females of *Aceria alhagi* n. sp. and *Aceria medicaginis* (Keifer, 1941). The characters that are different between the two species are highlighted in bold.

<table>
<thead>
<tr>
<th>Morphometric characters</th>
<th><em>Aceria alhagi</em> n. sp.</th>
<th><em>Aceria medicaginis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of body</td>
<td>178 (174 – 225)</td>
<td>150 – 180</td>
</tr>
<tr>
<td>Width of body</td>
<td>55 (45 – 55)</td>
<td>40 – 50</td>
</tr>
<tr>
<td>Length of gnathosoma</td>
<td>24 (19 – 24)</td>
<td>23</td>
</tr>
<tr>
<td>Length of prodorsal shield</td>
<td>26 (24 – 27)</td>
<td>30</td>
</tr>
<tr>
<td>Width of prodorsal shield</td>
<td>35 (30 – 38)</td>
<td>35 – 40</td>
</tr>
<tr>
<td>Length of setae <em>sc</em></td>
<td>34 (28 – 37)</td>
<td>24</td>
</tr>
<tr>
<td>Tubercles of <em>sc</em> apart</td>
<td>19 (17 – 20)</td>
<td>27</td>
</tr>
<tr>
<td>Length of leg I</td>
<td>29 (25 – 32)</td>
<td>32</td>
</tr>
<tr>
<td>Length of tibia I</td>
<td>7 (5 – 7)</td>
<td>7,5</td>
</tr>
<tr>
<td>Length of tarsus I</td>
<td>8 (6 – 8)</td>
<td>8</td>
</tr>
<tr>
<td>Length of empodium I <em>em</em></td>
<td>5 (5 – 7)</td>
<td>8</td>
</tr>
<tr>
<td>Number of rays on tarsal empodium</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Length of leg II</td>
<td>26 (25 – 29)</td>
<td>28,5</td>
</tr>
<tr>
<td>Length of tibia II</td>
<td>5 (4 – 6)</td>
<td>5,5</td>
</tr>
<tr>
<td>Length of tarsus II</td>
<td>8(6 – 8)</td>
<td>6,5</td>
</tr>
<tr>
<td>Length of empodium II <em>em</em></td>
<td>6 (4 – 6)</td>
<td>8,5</td>
</tr>
<tr>
<td>Length of female genitalia</td>
<td>12 (9 – 12)</td>
<td>15</td>
</tr>
<tr>
<td>Width of female genitalia</td>
<td>20 (17 – 20)</td>
<td>21</td>
</tr>
<tr>
<td>Number of ridges</td>
<td>16 (15 – 17)</td>
<td>10 – 12</td>
</tr>
<tr>
<td>Length of setae <em>3a</em></td>
<td>34 (30 – 36)</td>
<td>16</td>
</tr>
<tr>
<td>Length of setae <em>c2</em></td>
<td>28 (24 – 28)</td>
<td>46</td>
</tr>
<tr>
<td>On annulus no:</td>
<td>12 (9 – 12)</td>
<td>7</td>
</tr>
<tr>
<td>Length of setae <em>d</em></td>
<td>77 (65 – 80)</td>
<td>63</td>
</tr>
<tr>
<td>On annulus no:</td>
<td>26 (22 – 26)</td>
<td>19</td>
</tr>
<tr>
<td>Length of setae <em>e</em></td>
<td>17 (14 – 18)</td>
<td>23,5</td>
</tr>
<tr>
<td>On annulus no:</td>
<td>41 (34 – 41)</td>
<td>36</td>
</tr>
<tr>
<td>Length of setae <em>f</em></td>
<td>27 (24 – 27)</td>
<td>27</td>
</tr>
<tr>
<td>Number of complete annuli</td>
<td>67 – 76</td>
<td>60</td>
</tr>
</tbody>
</table>

A. *alhagi* n. sp. is close to *A. medicaginis*, but it can be distinguished by the following characters: number of rays on the tarsal empodium (*A. alhagi* n. sp. = 6; *A. medicaginis* = 5); number of striae on the female genital cover flap (*A. alhagi* n. sp. with 15 – 17; *A. medicaginis* with 10 – 12); prodorsal shield design (*A. alhagi* n. sp. with almost smooth prodorsal shield, with short admedian and submedian lines on the rear margin of the shield, while *A. medicaginis* with completely smooth prodorsal shield without any lines). The differing morphometric characters between *A. alhagi* n. sp. and *A. medicaginis* are presented in Table 2.

**Molecular Analyses**

The final alignment included the whole mt-COI barcoding region. No insertions or deletions were found between the sequences. In total, 4/658 (0.6%) nucleotides were polymorphic, of which two were parsimony informative. Base pair frequencies show that the region is AT-rich (A: 0.228, C: 0.153-0.160, G: 0.157-0.158, T: 0.456-0.460). The translation of the nucleotide...
Table 3 Uncorrected p-distance among Aceria alhagi n. sp. populations of Alhagi maurorum from different localities.

<table>
<thead>
<tr>
<th>Population- Locality</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Iran–Shirvan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Iran–Honameh</td>
<td>0.005</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Turkey–Sarayhan</td>
<td>0.000</td>
<td>0.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Turkey–Cavusin</td>
<td>0.002</td>
<td>0.006</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Armenia–Vedi</td>
<td>0.003</td>
<td>0.002</td>
<td>0.003</td>
<td>0.005</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Population numbers refer to populations collected from locations as listed in Table 1.

A sequence resulted in a 219 amino acid positions, of which one was variable. The average mean divergence over all the sequence pairs was 0.3% and ranged from 0% to 0.6% (Table 3). Similar level of divergence was obtained within six populations of Aceria tulipae (Keifer), range 0.0 – 0.5% (Kiedrowicz et al., 2017), as well as within Phytoptus avellanae (Nal.) range 0.0 – 0.4% (Cvrković et al., 2016). In Tegolophus cells Guo, Li, Wong, Xue & Hong, 2015, divergence within the same gene, among protogynne and deutogynne females was 0% to 0.9% (Guo et al., 2015), while intraspecific nucleotide sequence divergence within each host strain of Abacarus hystrix ranged from 0.2% – 13.9% (Skoracka & Dabert 2010).

Results obtained in this study indicate that there are no significant intraspecific divergences among populations of A. alhagi n. sp. from the five different localities in western Asia.

Four different haplotypes were obtained for populations of A. alhagi n. sp. collected on A. maurorum from different geographic localities, and the sequences are available from GenBank under accession numbers MF150169 – MF150173.

Discussion

Alhagi maurorum is considered a weed of significance in many regions of the world where it has been introduced (AGIS–WIP 2006, Munakamwe 2016; USDA–NRCS 2002) and potential control options need to be considered. Biological control offers a safe and sustainable method, which can be used to control a weeds density and reduce spread (McFadyen 1998). The main mode of spread of camelthorn is through seed dispersal, where each self-fertile plant can produce up to 6000 seeds (Ambasht 1963). The eriophyoid, A. alhagi n. sp. has been observed causing serious damage on the reproductive output of A. maurorum in western Asia (five regions of the current study). The inflorescences of the infested plants are replaced by cauliflower-like galls and seed production is massively reduced. Although A. alhagi n. sp. is unlikely to reduce densities of the weed in the short term, a reduction in seed set in plants in the introduced range would limit the long distance dispersal of this weed through various abiotic (wind and water) (Ambasht 1963) and biotic (cattle, sheep, horses) (Kerr et al. 1965) factors.

The finding from this study allows us to conclude that only one species of Aceria is present on A. maurorum, with no doubt about its taxonomic status, A. alhagi n. sp. This is an important finding for the development of a biological control programme since eriophyoid mites are usually highly host specific and are gaining popularity in classical weed biological control (Lindquist et al. 1996, Smith et al. 2010). Future classical biological control studies targeting the reduction in reproductive output of A. maurorum should consider A. alhagi n. sp. a potential biological control agent with a high impact on inflorescence production, ultimately reducing seed set.
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References


