

## A new species of *Onobrychis* sect. *Onobrychis* (Fabaceae) from Iran

Atefe AMIRAHMADI<sup>1</sup>, Shahrokh KAZEMPOUR OSALOO<sup>1\*</sup>, Maryam KHOSHSOKHAN MOZAFFAR<sup>2</sup>,  
Mohammad Mehdi CHARKHCHIAN<sup>3</sup>

<sup>1</sup>Department of Plant Biology, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

<sup>2</sup>Department of Biology, Qom Branch, Islamic Azad University, Qom, Iran

<sup>3</sup>Qazvin Natural Resource Research Center, Qazvin, Iran

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**Abstract:** *Onobrychis alamutensis* Amirah., Kaz. Osaloo & Charkhch. is described as a new species. It is restricted to the northwestern Alborz mountain range in Rudbar-Alamut, Qazvin, Iran. The diagnostic characters and relevant description of the species are given. The new species is distinguished from its closely related taxa, *Onobrychis verae* Širj., *O. ptychophylla* Širj. & Rech.f., and *O. sosnowskyi* Grossh. in both morphological and molecular evidence (nrDNA ITS sequences). The distribution map and an illustration of the new species are also provided.

**Key words:** Leguminosae, new species, nrDNA ITS, *Onobrychis*, taxonomy

### 1. Introduction

The genus *Onobrychis* Mill. includes more than 130 annual and perennial species distributed in Eurasia and NE Africa (Lock, 2005; Mabberley, 2008). The section *Onobrychis*, with approximately 74 species, displays a wide range of distribution in Eurasia (Širjaev, 1925; Ball, 1968; Hedge, 1970; Grossheim, 1972; Rechinger, 1984). In the *Flora Iranica*, this section was represented by 14 species (Rechinger, 1984). Recently, Ranjbar et al. (2007, 2009, 2011, 2012), Amirabadizadeh (2011), and Toluei et al. (2012, 2013a, 2013b) increased the number of species of sect. *Onobrychis* to 21 in Iran by adding *O. assadii* Ranjbar, Tolui & Amirab.; *O. chaldoranensis* Toluei, Ranjbar & Wink; *O. neychalanensis* Ranjbar, Hadadi & Karamian; *O. patula* Ranjbar, Joharchi & Karamian; and *O. mucronifolia* Ranjbar & Hadadi as new species and *O. sosnowskyi* Grossh., *O. cyri* Grossh., *O. viciifolia* Scop., and *O. araxina* Schischk. as new records. The nrDNA ITS region has been widely used to elucidate phylogenetic relationships at the generic and species levels (e.g., Coşkunçelebi, 2012; Lewke Bandara et al., 2013; Vafadar et al., 2014; Naderi Safar et al., 2014). The present study aimed to distinguish a new species of *Onobrychis* using both morphological and molecular evidence. Considering the new findings together with the new species described herein, the species number of this section in Iran is increased to 22.

### 2. Materials and methods

#### 2.1. Plant material

The materials of the new species were collected during a recent botanical collection by us. The specimens were cross-checked with the various *Onobrychis* accounts given in the relevant references (Ball, 1968; Hedge, 1970; Grossheim, 1972; Rechinger, 1984; Ranjbar et al., 2007, 2011, 2012; Toluei et al., 2012). The new species belonging to sect. *Onobrychis* is described as *O. alamutensis* Amirah., Kaz. Osaloo & Charkhch. The specimens in vegetative and fruiting stages were deposited at the herbaria of TARI, TUH, Tarbiat Modares University, and the Qazvin Natural Resource Research Center.

#### 2.2. Molecular studies

Total genomic DNA was isolated from fresh or dried materials using the modified CTAB method of Doyle and Doyle (1987). The nrDNA ITS region was amplified using the primers ITS5m (Sang et al., 1995) and ITS4 (White et al., 1990). Polymerase chain reaction (PCR) was carried out in 20 µL of final volume of mixture containing 1.0 µL of template DNA (5 ng/µL), 0.5 µL of each primer (10 pmol/µL), 10 µL of the 2X Taq DNA polymerase Master Mix Red (Amplicon, Cat. No. 180301, Germany), and 8.0 µL of sterile water. PCR cycles consisted of 30 cycles of 50 s at 94 °C for template denaturation, 40 s at 53 °C for primer annealing, and 55 s at 72 °C for primer extension, followed by 7 min at 72 °C for completion of primer extension. PCR products

\* Correspondence: skosaloo@modares.ac.ir

were separated by electrophoresis in 1% agarose gel stained with ethidium bromide and were photographed with a UVI gel documentation system (UVItec, UK). The nrDNA ITS region was then sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) with the same ITS5m and ITS4 primers in an ABI Prism 3730xl DNA Analyzer (Applied Biosystems, USA).

For the phylogenetic reconstruction, 12 species belonging to sect. *Onobrychis* were analyzed using the nrDNA ITS sequence data. *Eversmannia subspinosa* (Fisch.) B.Fedtsch. and *Hedysarum formosum* Fisch. & C.A.Mey. ex Basin. were chosen as outgroups according to the study by Amirahmadi et al. (2014). The locality information of the taxa used in phylogenetic analysis and GenBank accession numbers are given in Table 1. The sequences for these species were edited using BioEdit ver. 7.0.9.0 (Hall, 1999) and aligned using MUSCLE (Edgar, 2004), followed by manual adjustment. Phylogenetic analyses of the sequence data were performed by the neighbor-joining method (NJ) using the K2P model (Kimura, 1980) and maximum parsimony (MP) methods as implemented in PAUP\* version 4.0b10 (Swofford, 2002). Branch support values were calculated with 1000 bootstrap replicates (Felsenstein, 1985).

### 3. Results

***Onobrychis alamutensis*** Amirah., Kaz.Osaloo & Charkhch. **sp. nov.** (Figures 1 and 2).

**Type:** Iran, Qazvin, Rudbar-Alamut, toward Juladak village, between Shahrak and Aftabdar villages, 36°23'11.7"N, 50°31'49.3"E, 1438 m, 10.6.2013, *K. Osaloo & Bahadori* 98194 (holotype: TARI, isotypes: Tarbiat Modares Univ. Herb., TUH).

**Diagnosis:** *Onobrychis alamutensis* is more closely related to long-winged species, including *O. verae*, *O. ptychophylla*, and *O. sosnowskyi*, than to short-winged species, e.g., *O. shahpurensis*. It differs from *O. verae*, *O. sosnowskyi*, and *O. ptychophylla* in having creamy white-yellowish corolla with pale-colored veins (not red-pink with deeper colored veins) and pod crest of 7–10 dentate (not 4–6 dentate), and also from the latter with unfolded leaves (not folded along midrib). *O. alamutensis* mainly differs from *O. shahpurensis* with a dorsoventrally flattened pod (not a dorsoventrally convex pod), spineless disc (not spiny), with crest of short dentate of 0.2–0.5 mm (not long dentate of 1–2 mm) and long wings of 11 mm long (not short wings 4.5 mm long).

**Description:** Ascending-erect perennial with woody rootstock, branched at the base, up to 70 cm long, covered

**Table 1.** Taxa included in the nrDNA ITS.

Species	Voucher, source	GenBank accession no.
<i>Eversmannia subspinosa</i> (Fisch.) B.Fedtsch.	Iran: Freitag & Mozaffarian 28397 (TARI)	AB329692*
<i>Hedysarum formosum</i> Fisch. & C.A.Mey. ex Basin.	Iran: Mozaffarian 9778 (TUH)	AB854494*
<i>Onobrychis alamutensis</i> Amirah., Kaz.Osaloo & Charkhch.	Iran: Kazempour Osaloo & Bahadori 98194 (TARI)	AB911415
<i>O. alba</i> (Waldst. & Kit.) Desv.	Yugoslavia: Podlech 28272 (MSB)	AB911416
<i>O. araxina</i> Schischk.	Iran: Toluei & Ranjbar 23157 (BASU)	JQ780470*
<i>O. bungei</i> Boiss.	Iran: Rechinger 43484 (MSB)	AB911417
<i>O. carduchorum</i> C.C.Towns.	Iran: Kazempour Osaloo et al. 2012-1 (Tarbiat Modares Univ. Herb.)	AB911418
<i>O. gontscharovii</i> Vassilcz.	Iran: Toluei & Ranjbar 23119 (BASU)	JQ780471*
<i>O. ptychophylla</i> Širj. & Rech.f.	Iran: Toluei & Ranjbar 23152 (BASU)	JQ780472*
<i>O. shahpurensis</i> Rech.f.	Iran: Kazempour Osaloo et al. 2012-2 (Tarbiat Modares Univ. Herb.)	AB911419
<i>O. sosnowskyi</i> Grossh.	Iran: Mozaffarian, 93762 (TARI)	AB911420
<i>O. transcaspica</i> V.V.Nikitin	Iran: Ghahraman & Mozaffarian 5859 (TUH)	AB911421
<i>O. verae</i> Širj.	Iran: Kazempour Osaloo et al. 2011-1 (Tarbiat Modares Univ. Herb.)	AB854511*
<i>O. vicifolia</i> Scop.	Spain: Podlech 24883 (MSB)	AB854512*

BASU: Herbarium of Bu-Ali-Sina University, Hamedan, Iran; MSB: Herbarium of Ludwig-Maximilians-Universität, Munich, Germany; TARI: Herbarium of the Research Institute of Forests and Rangelands, Tehran, Iran; TUH: Tehran University Herbarium, Tehran, Iran;

\*: sequences from GenBank.



Figure 1. Habit of *Onobrychis alamutensis* (from holotype: TARI).



Figure 2. *Onobrychis alamutensis* (from the holotype). A- calyx, B- standard, C- wings, D- keel, E- androecium, F- gynoecium, G- pod.

with white short, soft appressed hairs. Stipules connate at the base, triangular-subulate, membranous with brownish stripes, 3–7 mm long, covered by spreading hairs. Leaves imparipinnate, the lower leaves with 4–7 pairs of leaflets,

10–23 cm long, the upper leaves 4–12 cm long, leaflets 3–5 pairs, obovate-oblong to linear, obtuse-rounded-mucronate at the apex, densely with appressed hairs at lower surface, more or less glabrous or sparsely appressed

hairs at upper surface,  $9\text{--}22 \times 1.5\text{--}4$  (6.5) mm; terminal leaflets 7–28 mm long, leaflet sessile to petiolulate, at most 1 mm long. Peduncle longer than the leaves. Inflorescence approximately 30-flowered,  $\pm$ loose. Calyx 5–7 mm long; acute-subulate teeth, longer than tube, 3–4 (5) mm long. Corolla milky yellowish with pale-colored veins; standard elliptic-obovate, emarginated at apex,  $12\text{--}14 \times 7\text{--}7.5$  mm; wings with claw,  $10\text{--}11 \times$  approximately 3 mm; keel 14 mm long, almost equal to standard; with claw 2–2.5 mm long. Pod  $9\text{--}12 \times 5\text{--}7$  mm, semiovoid, dorsoventrally flattened, pale yellow-green, with very short appressed hairs; disc spineless, areoles of disc 7–9, at 2 rows; crest 0.5–3 mm long, areoles of crest rectangular, with 7–10 very short teeth up to 0.2–0.5 mm long.

**Paratypes:** Iran, Qazvin, Rudbar-Alamut, Shahrak toward Juladak, 1430 m, 3.6.2009, *Charkhchian s.n.* (Tarbiat Modares Univ. Herb., Herb. of Qazvin Natural Resource Research Center): toward Juladak village, between Shahrak and Aftabdar villages,  $36^{\circ}23'11.7''\text{N}$ ,  $50^{\circ}31'49.3''\text{E}$ , 1434 m, 10.6.2013, *K.Osaloo & Bahadori* 98195 (TARI, Tarbiat Modares Univ. Herb.).

**Etymology:** The specific epithet corresponds to the type locality, Rudbar-Alamut, and also is related to Alamut

Mountain, which is located in the northwestern Alborz mountain range.

**Distribution and habitat:** *Onobrychis alamutensis* is a local endemic species, growing in the grasslands of the northwestern Alborz mountain range, known from many individuals collected in the type locality and vicinities (Figure 3).

#### 4. Discussion

The nrDNA ITS data matrix for 14 analyzed species includes 641 nucleotide sites, of which 173 (27%) are variable and 83 (13%) parsimoniously informative. The MP analysis resulted in a single most parsimonious tree with the length of 133 steps, a consistency index of 0.789, and a retention index of 0.872, along with bootstrap values (Figure 4). The MP tree is almost the same, in terms of topology and bootstrap support, as the NJ tree (not shown). The analysis of nrDNA ITS data generated 2 well-supported clades of ingroup taxa. One clade, A, was composed of 6 species including *O. gontscharovii* Vassilcz., *O. verae* Širj., *O. ptychophylla* Širj. & Rech.f., *O. araxina*, *O. sosnowskyi*, and *O. alamutensis*. The second clade, B, consisted of 6 species: *O. shahpurensis* Rech.f., *O. carduchorum* C.C.Towns., *O. alba* (Waldst. &

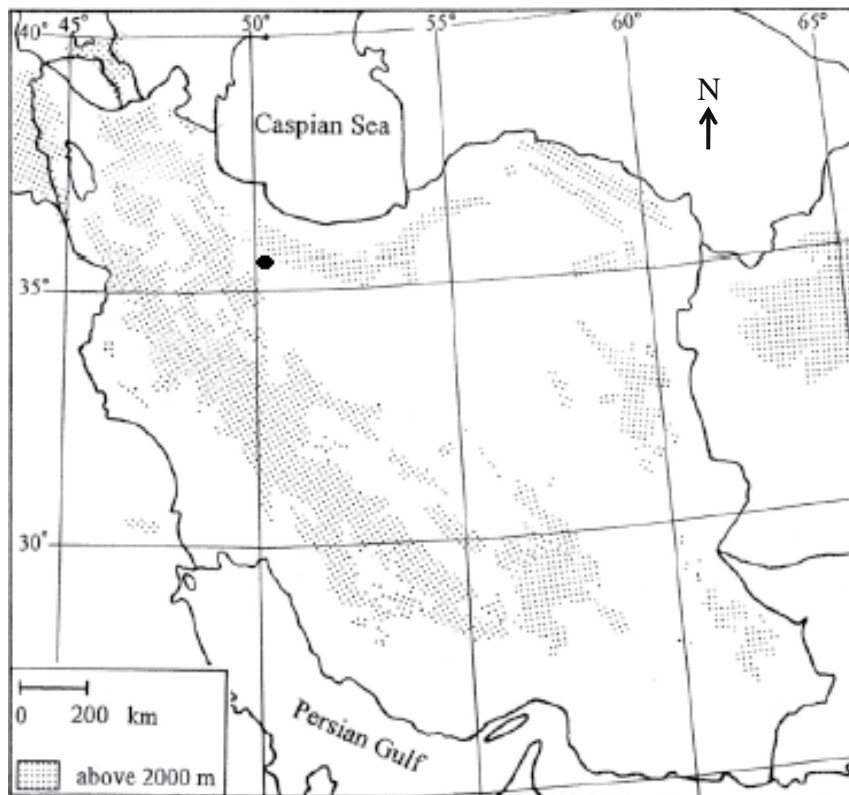
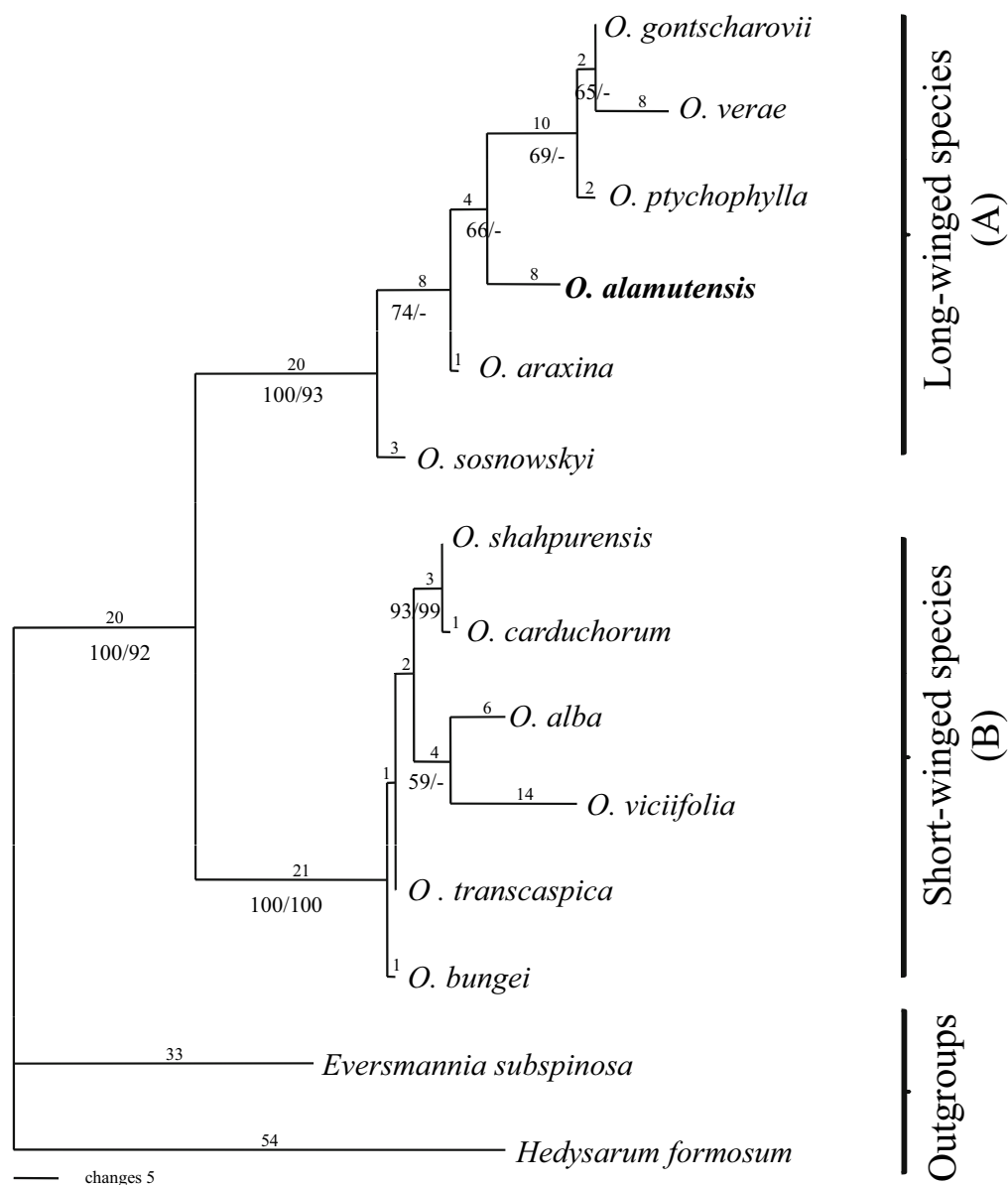


Figure 3. Distribution map of *Onobrychis alamutensis* in Rudbar-Alamut, Qazvin, Iran.



**Figure 4.** Phylogenetic tree based on the nrDNA ITS sequences. Branch lengths are proportional to the number of nucleotide changes as indicated above branches. Bootstrap values resulting from maximum parsimony and neighbor-joining analyses are given under branches, respectively.

Kit.) Desv., *O. viciifolia*, *O. transcaspica* V.V.Nikitin, and *O. bungei* Boiss. Within clade A, *O. alamutensis* is allied with a subclade comprising *O. gontscharovii*, *O. verae*, and *O. ptychophylla*. The members of clade A are characterized by long-winged petals (7–12 mm), wings longer than calyx, a dorsoventrally flattened pod, and a mostly spineless pod disc. They belong to sect. *Onobrychis* subsect. *Macropterae* Hand.-Mazz. (Širjaev, 1925; Grossheim, 1972; Ranjbar et al., 2012; Toluei et al., 2012). This subsection has been considered to have 8 species in Iran, including *O. araxina*, *O. assadii*, *O. gontscharovii*, *O. mucronifolia*, *O. patula*, *O.*

*ptychophylla*, *O. sosnowskyi*, and *O. verae* (Ranjbar et al., 2012; Toluei et al., 2012). The position of *O. alamutensis* in the reconstructed molecular phylogeny is congruent with our interpretation of its morphological characters. The nrDNA ITS sequence of *O. alamutensis* is characterized by 8 singleton nucleotide changes, indicating that this is a distinct species. It is worth noting that *O. alamutensis* and *O. shahpurensis* are similar to each other in terms of corolla color of milky or creamy white-yellowish (Table 2), but they are positioned at separate clades. This indicates that this characteristic evolved in parallel in the 2 species.

**Table 2.** Comparison of the diagnostic characteristics of *Onobrychis alamutensis* with similar species.

Characters	<i>O. alamutensis</i>	<i>O. verae</i>	<i>O. ptychophylla</i>	<i>O. sosnowskyi</i>	<i>O. shahpurenensis</i>
Corolla color	creamy white-yellowish with pale-colored veins	red-pink with deeper colored veins	red to pink with deeper-colored veins	pink	milky with pale-colored veins
Pod disc	spineless	spiny or rarely spineless	spineless	spineless	spiny
Pod crest	7–10 dentate (0.2–0.5 mm)	4–6 dentate (0.4–0.7 mm)	6– dentate (0.4–0.7 mm)	4–6 dentate (0.5–1.5 mm)	3–6 dentate (1–2 mm)
Leaflet size (mm)	9–22 × 1.5–4	9–14 × 2.5–4.5	4–19 × 1.4–3	10–20 × 1.5–3	15–30 × 0.5–2
Standard length (mm)	12–14	7–16	8.7–10	9–11	8.5–9.5
Wings length (mm)	10–11	6–12.5	6.9–8.2	6–9	4
Calyx length (mm)	5–7	3.8–7	5.1–6.5	3–3.5	5–8

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