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Myosotis persica (Boraginaceae), a new species from alpine wetlands of NW Iran

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ABSTRACT: A new species, *Myosotis persica*, is described and illustrated from the alpine wetlands of the Talesh Mountains in northwestern Iran, a transitional zone between the Irano-Turanian and Euro-Siberian floristic regions. Morphological distinctiveness and phylogenetic analysis based on nuclear ribosomal ITS sequences support its recognition as a species new to science. Morphological comparisons indicate that *M. persica* is closely allied to *M. koelzii, M. ramosissima,* and *M. diminuta* but can be distinguished by its dwarf habit, branched filiform stems, patent to erect leaf hairs, deeply lobed calyx, and nutlet morphology. Phylogenetic reconstruction confirms its placement within *Myosotis* subgen. *Myosotis,* forming a supported clade with *M. koelzii* and *M. ramosissima.* With an estimated extent of occurrence less than 10 km² and a total population size under 1500 individuals, the species is assessed as Critically Endangered (CR) under IUCN criteria. The findings underscore the importance of conserving alpine wetland habitats and provide new insights into species diversification within the genus *Myosotis* in south-western Asia.

KEY WORDS: alpine wetland, Ardabil, forget-me-nots, Irano-Turanian region, taxonomy, Talesh Mountains.

INTRODUCTION

The genus *Myosotis* L. commonly referred to as "forget-me-nots," is a member of the Family Boraginaceae Juss., comprises approximately 100 species primarily distributed across temperate regions of the Northern Hemisphere (Weigend *et al*., 2016; Hao *et al*., 2017). *Myosotis* is generally regarded as a monophyletic group (Sherafati *et al*., 2014), and is characterized by features such as ovoid, smooth, glossy brown to black nutlets. Traditionally, the genus has been divided into two subgenera: *Strophiostoma* and *Myosotis*, based on characters such as inflorescence architecture, nutlet morphology, and the presence or absence of a caruncle (Popov, 1953; Riedl, 1967; Khatamsaz, 2002). However, the delimitation of many taxa within the genus remains challenging due to morphological convergence and ecological plasticity.

Riedl (1967) had recorded 13 species from Iran in the 48th volume of Flora Iranica (Riedl, 1967). The number of *Myosotis* species in Iran was raised to 15 by the treatment of "Flora of Iran" (Khatamsaz, 2002), and subsequently to 16, after the introduction of *M. diminuta*, a rare species, from NW Iran, by Mahmoodi *et al*. (2015). Many of the Iranian species of *Myosotis* belong to subgenus *Myosotis*, namely *M. asiatica* Schizchk & Serg, *M. alpestris* Schmidt, *M. caespitosa* Schultz, *M. lithospermifolia* (Willd) Hornem, *M. olympica* Boiss, *M. palustris* (L.), *M. sylvatica* Ehrh. ex Hoffm, *M. diminuta* Grau, *M. koelzii* Riedl, *M. ramosissima* Rochel ex Schultes, *M. stricta* Link ex Roem. & Schult., *M. refracta* Boiss. and the new species, *M. persica* sp. nov. Other species of the genus belong to the subgenus *Strophiostoma*, including *M. propinqua* (Turcz.) Fisch. & C.A.Mey, *M. pseudopropinqua* Popov, *M. anomala* Riedl and *M. sparsiflora* J.C.Mikan ex Pohl.

During a long-term floristic survey of the alpine wetlands in the Backro-Dagh region of the Talesh Mountains (northwestern Iran), we encountered a morphologically unusual population of *Myosotis* that could not be attributed to any known species based on existing literature and herbarium specimens. This population exhibited consistent and distinct morphological traits, prompting further investigation.

The present study aims to (1) assess the taxonomic identity of this putatively new taxon through comparative morphological analyses with closely related species, and (2) determine its phylogenetic placement using nuclear ribosomal internal transcribed spacer (nrITS) sequences. Our results support the recognition of a new species, *Myosotis persica*, which we describe herein.

MATERIALS AND METHODS

Morphological study. Field investigations were carried out in the alpine wetland habitats of the Backro-Dagh altitudes (Talesh Mountains, NW Iran) from 2013 to 2025. Plant specimens were collected during peak flowering and fruiting seasons (June–July). The collected individuals were pressed, dried, and deposited as voucher specimens in the herbaria T (Herbarium of the Faculty of Biological Sciences, Kharazmi University) and GILAN

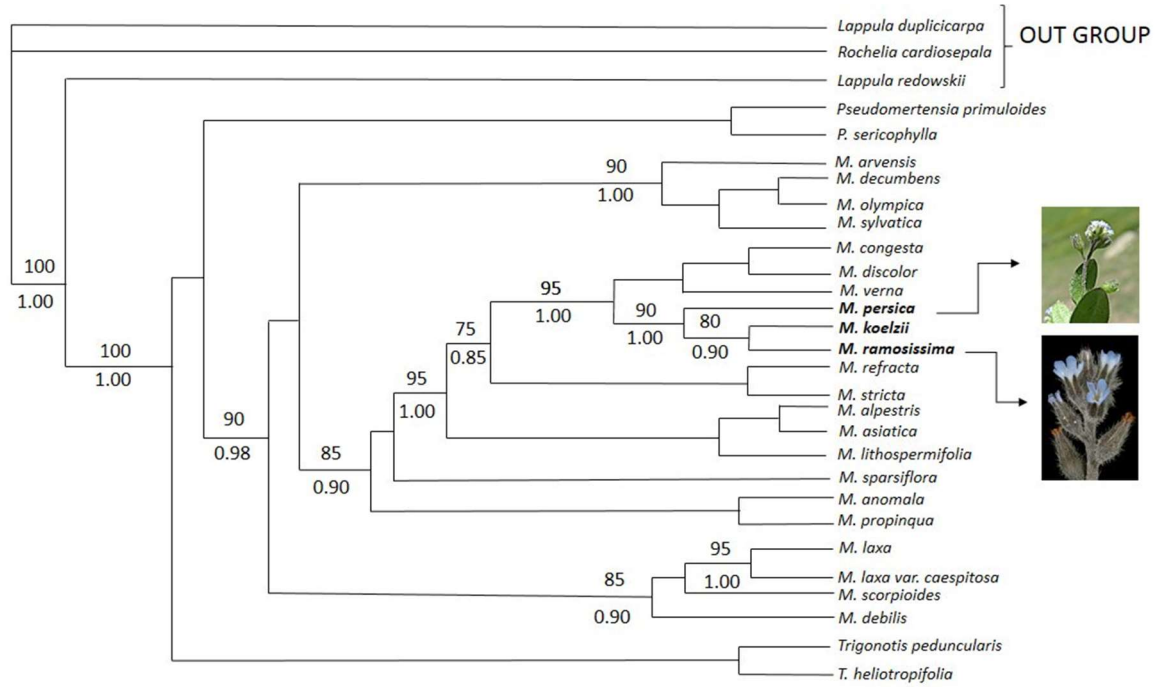

Fig. 1. Tree derived from analysis of the nrITS sequences, shows the relationship of ***Myosotis persica*** and other related taxa, numbers along branches provide nonparametric bootstrap values and Bayesian posterior probabilities are indicated above and below the branches, respectively, Photo of ***Myosotis ramosissima*** from POWO (2025).

Table 1. Taxa used in ***Myosotis*** molecular analyses and their GenBank accession numbers.

Species	Accession NO.	References
Rochelia cardiosepala	KU927781.1	Chacón *et al*., 2016
Lappula redowskii	KP027121.1	Serrano *et al*., 2016
Lappula duplicicarpa	JX976781.1	Huang *et al*., 2013
Trigonotis peduncularis	MT922933.1	Song *et al*., 2023
Trigonotis heliotropifolia	MN821523.1	Xu *et al*., 2020
Pseudomertensia sericophylla	KF849123.1	Otero *et al*., 2014
Pseudomertensia primuloides	KU927706.1	Chacón *et al*., 2016
Myosotis anomala	AB989073.1	Sherafati *et al*., 2014
Myosotis laxa var. caespitosa	AY092914.1	Winkworth *et al*., 2002
Myosotis olympica	AB989057.1	Sherafati *et al*., 2014
Myosotis ramosissima	KX165541.1	De Vere & Ford, 2016
Myosotis scorpioides	EU594655.1	Pihu *et al*., 2009
Myosotis stricta	AB989059.1	Sherafati *et al*., 2014
Myosotis verna	AY092936.1	Winkworth *et al*., 2002
Myosotis arvensis	AY092908.1	Winkworth *et al*., 2002
Myosotis congesta	AY092916.1	Winkworth *et al*., 2002
Myosotis debilis	AY092917.1	Winkworth *et al*., 2002
Myosotis discolor	AY092919.1	Winkworth *et al*., 2002
Myosotis stenophylla	KU927751.1	Chacón *et al*., 2016
Myosotis persica	PX046107.1	This study
Myosotis alpestris	AY092907.1	Winkworth *et al*., 2002
Myosotis koelzii	AB989067.1	Sherafati *et al*., 2014
Myosotis lithospermifolia	KX894531.1	Karimov *et al*., 2018
Myosotis propinqua	AY092928.1	Winkworth *et al*., 2002
Myosotis refracta	AY092930.1	Winkworth *et al*., 2002
Myosotis sparsiflora	KU927750.1	Chacón *et al*., 2016
Myosotis sylvatica	MT636726.1	Stojilkovič, 2021
Myosotis asiatica	AB989068.1	Sherafati *et al*., 2014
Myosotis laxa	JX128892.1	Meudt *et al*., 2015
Myosotis decumbens	MT636715.1	Stojilkovič 2021

(Gilan Agricultural and Natural Resources Research and Education Center, Rasht) (Thiers, 2025). The holotype is preserved at T (T 10026), and the isotype at GILAN (GILAN 10026).

Specimens were examined and identified using regional floras, including Flora Iranica (Riedl, 1967), Flora of the USSR (Popov, 1953), Flora Orientalis (Boissier, 1879), Flora of Turkey (Grau, 1978), and Flora of Iran (Khatamsaz, 2002). Morphological traits were assessed on both live and herbarium material using a Zeiss Stemi SV6 stereomicroscope. Nutlet surface and areola morphology were further studied using scanning electron microscopy (SEM) with a LEO 1430VP at the Central Laboratory of Ardabil University. Samples were sputter-coated with gold before imaging. Terminology and descriptive style follow standard taxonomic conventions, particularly those used in recent treatments of Boraginaceae (e.g., Meudt *et al*., 2015; Sherafati *et al*., 2014).

Molecular data. Total genomic DNA was extracted from silica-gel dried leaf tissue using the DNeasy Plant Mini Kit (Qiagen), following the manufacturer's protocol. The internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrITS) was amplified using universal primers ITS4 and ITS5 (White *et al*., 1990). PCR amplification was conducted in 25 µl volumes with a Techne Thermal Cycler, using the following program: initial denaturation at 94°C for 3 min; 35 cycles of 94°C for 30 sec, 52°C for 45 sec, and 72°C for 1 min; final extension at 72°C for 7 min (Khoshsokhan Mozaffar *et al*., 2013; Kamrani and Riahi, 2018). Amplicons were

purified and sequenced bidirectionally. Chromatograms were assembled and edited using Geneious v.10.0.9. Sequences were aligned using ClustalW v.1.4 (Thompson *et al*., 1994). Phylogenetic relationships were inferred using Maximum Likelihood (ML) and Bayesian Inference (BI) methods implemented in RAxML and MrBayes v.3.2, respectively. Model selection was based on the Akaike Information Criterion (AIC) using jModelTest.

The aligned matrix consisted of 23 taxa of *Myosotis*, including *M. koelzii*, *M. ramosissima*, and *M. diminuta*, and six outgroup species from *Rochelia, Lappula*, *Trigonotis*, and *Pseudomertensia* (Table 1). The final alignment was 664 base pairs long, with 158 parsimony-informative sites. Bootstrap support values >70% and posterior probabilities >0.90 were considered strong support. The aligned matrix consisted of 23 taxa of *Myosotis*, including *M. koelzii*, *M. ramosissima*, and *M. diminuta*, and six outgroup species from *Rochelia, Lappula*, *Trigonotis*, and *Pseudomertensia* (Table 1). The final alignment was 664 base pairs long, with 158 parsimony-informative sites. Bootstrap support values >70% and posterior probabilities >0.90 were considered strong support. Newly generated sequences were deposited in GenBank under accession numbers: PX046107.

RESULTS

Morphological results, the specimens collected from the Backro-Dagh alpine wetlands differ significantly from all previously known Iranian species of *Myosotis*. Diagnostic traits include a dwarf, branched, ascending to prostrate habit; filiform stems; oblong to obovate leaves with patent to erect hairs; campanulate calyx divided nearly two-thirds of its length; and ovoid nutlets with marginally grooved areolae. These features are consistent and distinct across the observed population. A comparative overview with the morphologically closest species (*M. koelzii*, *M. ramosissima*, *M. diminuta*) is summarized in Table 2.

Molecular phylogenetic results, the nrITS phylogenetic analysis places *Myosotis persica* within a strongly supported clade that includes *M. koelzii* and *M. ramosissima*, confirming their close relationship. Both ML and BI analyses yielded congruent topologies. Bootstrap values and posterior probabilities for the *M. koelzii*, *M. persica*, *M. ramosissima* clade were 92% and 0.98, respectively (Fig. 1).

DISCUSSION

The family Boraginaceae has been the subject of extensive morphological and molecular studies, shedding light on its evolutionary history and diversity (Noroozi *et al*., 2022; 2024). The morphological and molecular results obtained in this study provide compelling evidence that the population discovered in the Backro-Dagh alpine wetlands represents a previously undescribed species of *Myosotis*. Key morphological traits—such as dwarf habit with branched, filiform, prostrate to ascending stems; obovate to oblong leaves with patent hairs; a deeply lobed pilose calyx; and nutlets with marginally grooved areolae—set *M. persica* apart from its closest relatives, including *M. koelzii*, *M. diminuta*, and *M. ramosissima*. These species differ significantly in their stature, calyx shape and indumentum, and nutlet morphology (Table 2).

Phylogenetic analysis based on the nrITS region confirms that *M. persica* forms a well-supported clade with *M. koelzii* and *M. ramosissima*, both of which are morphologically allied to the new species. The analysis corroborates the placement of *M. persica* within subgenus *Myosotis* (sensu Riedl, 1967; Sherafati *et al*., 2014), which is characterized by features such as scorpioid cymes, unappendaged nutlet attachment scars, and the absence of thickening below the calyx on the peduncle.
The alpine wetlands of the Talesh Mountains, where *M. persica* was found, represent a highly specialized and fragile ecosystem. The area supports a rich flora, including species from the Irano-Turanian, Hyrcanian (Zohary, 1973), Caucasian, and Alborz floristic elements. Despite long-term floristic research in this region, *M. persica* has only been recorded from a single site, indicating its restricted distribution and habitat specialization.

Climate change and anthropogenic pressures, especially overgrazing and road construction, threaten the delicate balance of alpine wetland habitats. Based on IUCN Red List criteria (IUCN, 2019), *M. persica* meets the conditions for Critically Endangered (CR) status, as it is known from a single location with an extent of occurrence (EOO) and area of occupancy (AOO) both under 10 km², and a total number of mature individuals estimated to be fewer than 1,500.

The discovery of *M. persica* not only adds to the taxonomic diversity of the genus in Iran but also highlights the importance of conserving high-altitude wetlands as refugia for endemic and narrowly distributed plant species. Further investigations into neighboring alpine habitats may reveal additional isolated populations or closely related taxa, improving our understanding of diversification patterns within *Myosotis* in Southwest Asia.

TAXONOMIC TREATMENT

Myosotis persica Bidarlord, Akbarnejad & F. Ghahrem., ***sp. nov.*** **Fig. 2**

Type: Iran, Ardabil–Gilan border, Lissar Protected Area, Backro-Dagh, Neor to Subatan, 37°56′19″N, 48°36′47″E, 3050 m a.s.l., 26 June 2016, *Bidarlord s.n.* (holotype: T 10026!; isotype: GILAN 10026!)

Diagnosis. Differs from *M. koelzii*, *M. diminuta*, and *M. ramosissima* by its dwarf habit, branched filiform ascending to prostrate stem, obovate to oblong leaves

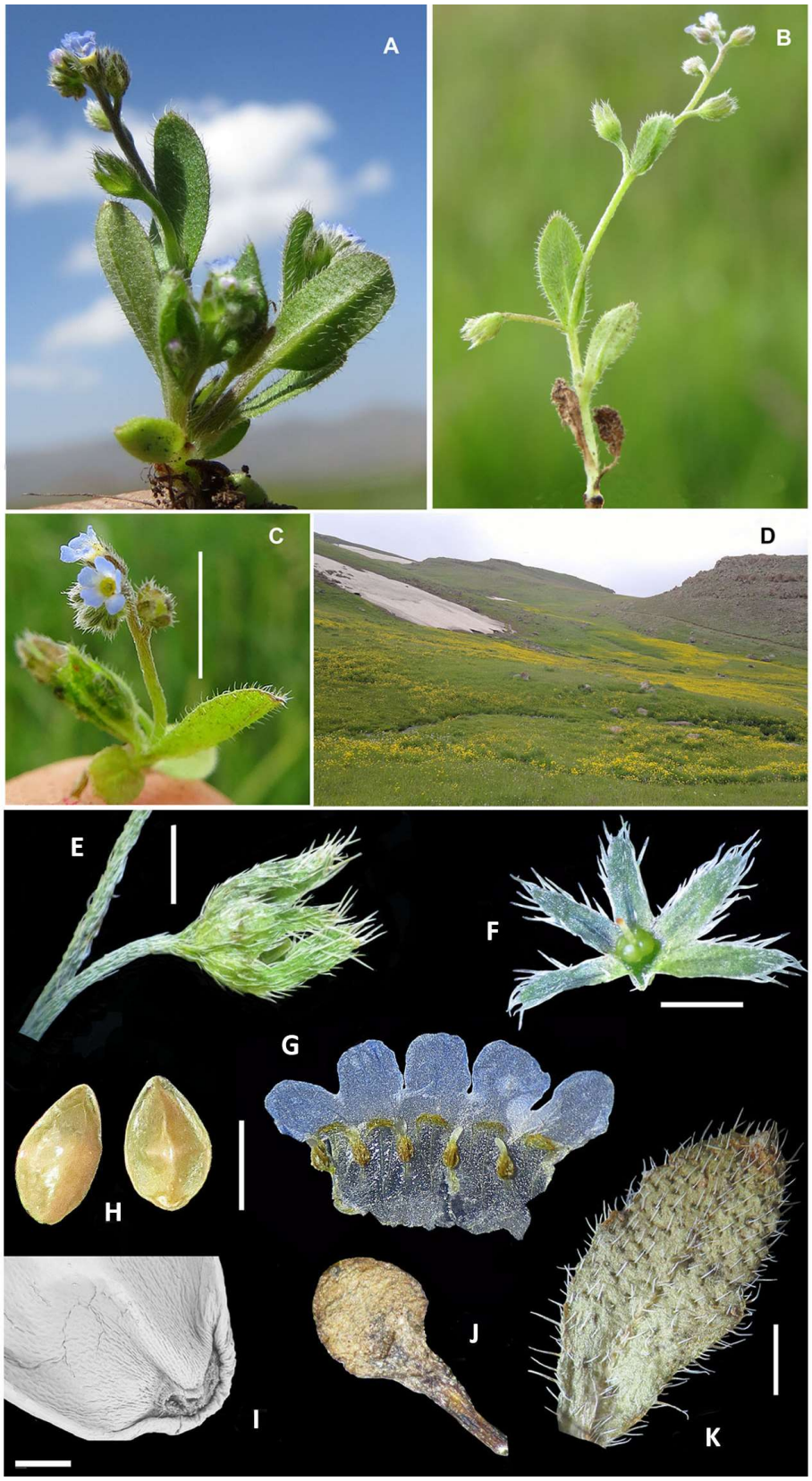

Fig. 2. ***Myosotis persica***: **A.-B.** in flower (bar 1 cm), **C**. in fruit, **D.** habitat, **E.** pedicel and calyx in fruiting, .**F.** calyx in flowering and gynoecium (bar 1 mm), **G.** corolla with anthers and faucal scales (bar 1 mm), **H.** erem (nutlet) in both side (bar 1 mm), **I.** SEM photos of nutlet attachment scar (bar 0.1 mm), **J.** basal leaf, **K.** stem leaf (bar 1 mm), from the holotype.

Table 2. Morphological differences of ***Myosotis persica*** with ***M. diminuta***, ***M. koelzii***, and ***M. ramosissima***

Characters	***M. persica***	***M. diminuta***	***M. koelzii***	***M. ramosissima***
Plant high	1–6(–8) cm	up to 4 cm	10–15 cm	8–40 cmm
Stem	branched, erect to prostrate	unbranched, erect	unbranched, erect	unbranched, erect
Leaves shape	late elliptic-obovate or oblong	narrowly oblong	oblong-lanceolata	lanceolate
Leaves indumentum	on both surfaces patent to erect hairs to glabrous	on both surfaces sparsely adpressed pilose	on both surfaces sparsely adpressed pilose (dense in upper one)	on both surfaces patent straight hairs
Leaves apex	round or obtuse	obtuse	obtuse or acuminate	obtuse
Flowers	many	only 1–2 (3)	many	many
Pedicels	erect, up to 7 mm	erect, up to 10 mm	patent to reflex, up to 15 mm	patent or recurved, 3-5 mm
Flowering calyx	1–2 mm	2–3	2–2.5 mm	5 mm
Calyx splitting	2/3	1/3	1/3	1/2
Calyx indumentum	dense adpressed pilose	sparsely adpressed pilose, especially at the base	sparsely adpressed pilose	hooked hairs at base
Corolla	1.5–2.5 mm	up to 4 mm	2–2.5 mm	4 mm
Areolas (Nutlet attachment scar)	with marginal grooves	roundish to elliptic	roundish to elliptic	roundish to elliptic

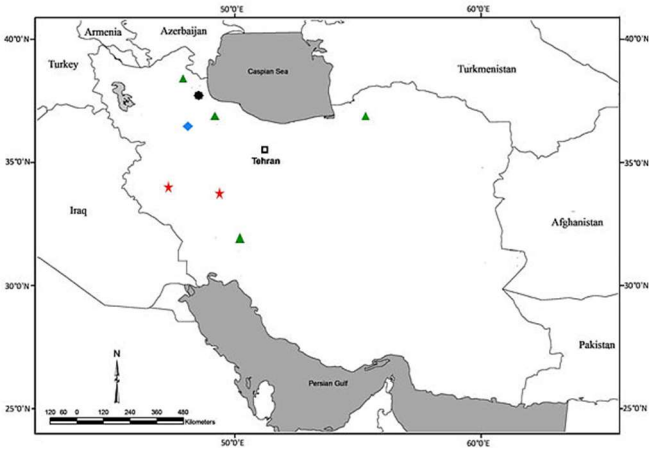

Fig. 3. The distribution of ***Myosotis diminuta*** (rhombus), ***M. koelzii*** (star), ***M. ramosissima*** (triangle), and ***M. persica*** (circle) in Iran.

with patent to erect hairs, deeply lobed calyx with dense appressed straight hairs, and nutlets with marginally grooved areolae (Table 2).

Description. Annual herb, 1–6(–8) cm tall. Stems initially partially erect then ascending to prostrate, filiform, branched from base, densely antrorsely adpressed hairy. Basal leaves 7–13 × 3–5 mm, oblong toelliptic or spathulate, petiolate, margin and surface with patent to erect hairs to glabrous, apex obtuse to rounded. Stem leaves 4–14 × 2–6 mm, elliptic to orbicular, sessile. Inflorescence a scorpioid cyme, numerous flowers. Pedicels up to 1 mm in flower, up to 7 mm in fruit. Calyx campanulate, 1–2 mm in flower, 2–3 mm in fruit, divided to 2/3, with dense straight antrorse hairs. Corolla 1.5–2.5 mm, blue, limb saucer-shaped, up to 2 mm in diameter, with yellow faucal scales; stamens included. Style 0.5 mm; stigma clavate. Nutlets ovoid to ellipsoid, 1.3 × 0.7 mm, smooth, glossy, pale brown, with marginally grooved areola.

Phenology. Flowering June; fruiting June–July.

Etymology. The epithet "persica" refers to the country of origin, Iran.

Distribution and habitat. This species seems to be a very local endemic, as flora and vegetation of the area is well studied by the first author and this species is only recorded from a single locality (Fig. 3). The new species is adapted to the wetland habitat of alpine zone in the Talesh Mountains, near to borderline of Irano-Turanian and Hyrcanian phytochoria and elevation ranges from 2900 to 3100 m a.s.l. It is an Irano-Turanian element (Fig. 3). The associated species are some other wetland species such as *Poa pratensis* L., *Erigeron acer* L., *Carex songarica* Schrank, *Dichodon kotschyi* (Boiss.) Ikonn., *Gagea confusa* A.Terracc, *Veronica pusilla* Kotschy & Boiss., *Trifolium repens* L. and, *Ranunculus amblyolobus* Boiss. & Hohen., *Ranunculus kotschyi* Boiss.

Its worthy to mention that the species diversity of the genus *Myosotis* in Talesh mountains could be evaluated as high, for the presence of species such as *Myosotis anomala* (restricted to Talesh altitudes), *M. alpestris, M. asiatica*, *M. palustris*, *M. lithospermifolia*, *M. minutiflora*, recorded for the first time from these mountains (NW Iran), *M. olympica* subsp. *demawendica*, *M. persica, M. sylvatica* Ehrh. & Hoffm. and *M. ramosissima* Rochel. Due to being located in the floristic transition area and also benefiting from the Caucasus, the Alborz, and Zagros floristic elements, Talesh highlands have a high plant diversity (Ghahremaninejad *et al*., 2012).

Conservation status. Critically Endangered (CR) based on IUCN (2019) criteria B1ab(iii)+B2ab(iii)+C2a(i).

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