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LINDELOFIA STYLOSA (BORAGINACEAE), A NEW RECORD FOR THE FLORA OF IRAN, BASED ON MORPHOLOGICAL AND MOLECULAR EVIDENCE

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Lindelofia stylosa (Boraginaceae) is reported for the first time from Iran. The specimen of this species has been collected from Kuh-e Karkasi (Dibaj, N Damghan, Semnan province) during ongoing floristic studies and research journeys to the southern slopes of the Alborz Mountains. Molecular evidence obtained from DNA extraction and Internal Transcribed Spacer (ITS) sequence analysis confirm this new record. Description, photographs of living plant and some associated species are provided.

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Key words: New record; *Lindelofia stylosa*; *Cynoglossum kandavanensis*; Damghan; flora of Semnan province

گزارش جدید گونه Lindelofia stylosa (خانواده گاوزبان) برای فلور ایران بر پایه شواهد ریخت‌شناسی و مولکولی

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گونه Lindelofia stylosa (خانواده گاوزبان) برای اولین بار از ایران گزارش می‌شود. نمونه این گونه از کوه کرکسی (دیباج، شمال دامغان، استان سمنان) در حین مطالعات فلوریستیک مداوم و سفرهای تحقیقاتی به شیب‌های جنوبی رشته کوه البرز جمع‌آوری شده است. شواهد مولکولی بدست آمده از استخراج DNA و آنالیز توالی ITS این گزارش را تایید می‌کند. توصیف، تصاویری از گیاه زنده و فهرستی از گونه‌های همراه ارائه می‌شود.

INTRODUCTION

The genus *Lindelofia* Lehm. with about ten perennial species is distributed in C and W Asia, including Afghanistan, N India, Kashmir, Kazakhstan, Kyrgyzstan, Mongolia, Pakistan, Tajikistan, Turkmenistan, Uzbekistan and China (Zhu & al. 1995). Riedl (1967) introduced 8 species for the flora Iranica region. According to Riedl's account, *Lindelofia kandavanensis* Bornm. & Gauba was the only species in Iran, distributed in Mazandaran province as an Euro-Siberian element. Akhani (1998) transferred *L. kandavanensis* into the new combination *Cynoglossum kandavanensis* (Bornm. & Gauba) Akhani, based on the type material of *L. kandavanensis*, the material studied by Riedl for the Flora Iranica and specimens collected from Golestan National Park. Akhani (1998) emphasized that the styles of all studied plants are included into the corolla, at most they are as long as the tube and thus these plants belong to *Cynoglossum* L. However, Khatamsaz (2002) reported *L. kandavanensis* from the Hyrcanian forest of Iran without considering the new combination. In this study *L. stylosa* (Kar. & Kir.) Brand is reported for the first time from the alpine area of Eastern Alborz (Iran).

MATERIAL AND METHODS

Morphology

During expedtions to the southern slopes of Eastern Alborz, the specimen of *Lindelofia stylosa* has been collected from Kuh-e Karsi (Dibaj, N Damghan), an alpine area ranging from 3000-3700 m a.s.l. The specimen is kept in DU. The recorded locality of *L. stylosa* was investigated again to collect more materials. The specimen was cross-checked with different taxonomic keys and floras (Popov 1953;

Riedl 1967; Zhu & al. 1995; Nasir 1989; Khatamsaz 2002). Likewise, digital photos of *Lindelofia* and *Cynoglossum* species in the virtual herbaria such as W, B and LE and data available on Global Biodiversity Information Facility [GBIF] were seen.

DNA extraction, amplification and sequencing

Total genomic DNA was isolated using the modified CTAB method of Doyle & Doyle (1987). The nrDNA ITS region was amplified using the primers ITS5m (Sang & al. 1995) and ITS4 (White & al. 1990). The PCR amplification was carried out in the volume of 20 µl, containing 8 µl deionized water, 10 µl of the 2× Taq DNA polymerase master mix Red (Amplicon) 0.5 µl of each primer (10 pmol/µl), and 1 µl of template DNA. PCR cycles consisted of pre-denaturation at 94 °C for 3 min followed by 31 cycles, each consisting of denaturation at 94 °C for 1 min, annealing at a temperature 61 °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 1X TBE as the gel buffer) stained with ethidium bromide. PCR products along with the primers used for amplification were sent for Sanger sequencing at Macrogen (Seoul, South Korea). The sequences were aligned using the clustal W 1.8 (Thompson & al. 1994) followed by manual adjustment. The best models of sequence evolution were selected using the program MrModeltest version 2.3 (Nylander 2004) based on the Akaike information criterion (AIC) (Posada & Buckley 2004). Phylogenetic analyses of the sequence data were performed by the Bayesian inference (BI) and Maximum parsimony (MP) method as implemented in MrBayes v.3.2.4 (Ronquist & al. 2012) and PAUP* version 4.0b10 (Swofford 2002). Branch support was assessed via the non-parametric bootstrap (Felsenstein 1985) using 1,000 bootstrap replicates each with simple sequence addition. In the Bayesian phylogenetic analyses, the final tree is accompanied with posterior probability (PP) values. List of species included in the phylogenetic analysis is given in table 1. Furthermore, the sequences of new record were analayzed by using the Software BLASTn (Basic Local Alignment Search Tool; Altschul & al. 1990) for similarity and compared with the nrDNA sequences of accession number JX976809 availabe on GenBank which was collected from China (Huang & al. 2013).

Table 1. Taxa included in the nrDNA ITS analyses.

Taxa	Source, Voucher	Accession numbers nrDNA ITS
Lindelofia stylosa (Kar. & Kir.) Brand.	Iran: Naderi 2662 (DU001183)	LC628083
Lindelofia stylosa (Kar. & Kir.) Brand.	China: Huang 20090299 (XJBI)	JX976809
Lindelofia longiflora (Benth.) Baill.	Serrano & al. (2016)	KP027115
Cynoglossum kandavanensis (Bornm. & Gauba) Akhani	Iran: Mozaffarian 65137 (TARI)	LC410065
Cynoglossum officinale L.	Iran: Assadi 73526 (TARI)	AB758292
Asperugo procumbens L.	Iran: Kazempour Osaloo 581244 (Tarbiat Modares University Herbarium)	AB758290
Nonea caspica (Willd.) G.Don	China: Huang 20090288 (XJBI)	JX976811

RESULT AND DISCUSSION

Lindelofia stylosa (Kar. & Kir.) Brand, Pflanzenr. (Engler) 4, 252: 85 (1921). Figs. 1-4.

Type: Kazakhstan: In pratensibus subalpinis Alatau ad fl. Sarchan, 1841, Karelin & Kiriov 1745 (LE, W photo!).

Specimen seen: Iran, Semnan province, Damghan, Dibaj, Sarband prohibited area, Kuh-e Karkasi, 3000-3700 m, 15 Jun 2015, Naderi 2662 (DU001183).

Distribution: Iran, Afghanistan, Pakistan, Tajikistan, Kyrgyzstan, Uzbekistan, Kazakhstan, Mongolia, Tibet, China, West Himalaya.

Lindelofia conspicuously differs from *Cynoglossum* by its long style exerted from corolla and the stamens exerted from the throat in contrast to the included style and stamens in *Cynoglossum* (Popov 1953; Zhu & al. 1995; Nasir 1989; Khatamsaz 2002). *Lindelofia stylosa* is distinguished from the closely related taxa such as *L. micrantha* Rech. f. & Riedl, *L. platycalyx* Riedl, *L. campanulata* Riedl and *L. ancusoides* (Lindl.) Lehm. by corolla tube ca. as long as calyx, equal to or longer than the limb and dark purple or purplish red color (Riedl 1967; Zhu & al. 1995; Nasir 1989).

Some associated species with *L. stylosa* in Kuh-e Karkasi are: *Astragalus* (sect. *Caprini* DC.) *perdurans* Podlech, *Polygonum serpyllaceum* Jaub. & Spach, *Onobrychis cornuta* (L.) Desv. (both purple and white flowers), *Alopecurus textilis* Boiss., *Melica jacquemontii* Decne., *Dielsiocharis kotschyi* (Boiss.) O.E.Schulz, *Drymocallis damghanensis* Naderi & Faghir, *Allium cristophii* Trautv., *Taraxacum brevirostre* Hand.-Mazz., *Nepeta fissa* C.A.Mey., *Galium decumbens* (Ehrend.) Ehrend. & Schönb.-Tem., *Artemisia melanolepis* Boiss. and *Jurinella microcephala* (Boiss.) Wagenitz. The specimens of all associated species are kept in DU (see Bardsiri & al. 2017).

The aligned nrDNA ITS data matrix for 7 examined species are 581 nucleotide sites long of which 48 sites are parsimoniously informative. Maximum parsimony (MP) analysis of the nrDNA dataset (characters equally weighted) generated the most parsimonious tree with a length of 67 steps, a consistency index (CI) 0.851, and a retention index (RI) 0.818. In the phylogenetic tree (fig. 5), *Nonea caspica* (Willd.) G.Don. and *Asperugo procumbens* L. were chosen as outgroup. The remaining taxa in this study as ingroup formed a clade with high support (PP=1, MP=100). Within this clade, there are 2 subclades. In the first subclade, 2 representatives of the genus *Cynoglossum* as *C. kandavanensis* and *C. officinale* L. formed a group (PP=0.76, MP=63). In the second subclade, 3 representatives of the genus *Lindelofia* formed a group. Within this group, *L. stylosa* (China) and *L. stylosa* (Iran) established a robust clade (PP=0.95, MP=93) that *L. longiflora* (Benth.) Baill. is sister group to it (PP=0.78, MP=66). Moreover, BLASTn result showed that the similarity of nrDNA ITS sequences of the new record from Iran to the accession number JX976809 from China was 99.19%. Both morphological and molecular evidence strongly confirm the record of *L. stylosa* for the flora of Iran.

Description

Perennial herbs. Root stout to 2 cm in diam. Stems sub-simple, villous-pubescent, 20-60 cm long. Basal leaves petiolate, 13-30 × 2-5 cm, oblong-lanceolate, acute-obtuse, covered on both surfaces with short sub-appressed hairs; lower stem leaves petiolate, sublinear-lanceolate; upper stem leaves sessile, narrowly lanceolate. Inflorescence often composed of 2 terminal cymes, at first dense, one-side, drooping, 3-7 cm long, elongating in fruit to 20 cm long and straightening; pedicle gray-tomentose. Calyx 5-6.5 mm long, gray-tomentose, elongating in fruit, with lanceolate-linear acute lobes; lobes slightly unequal, split to the base. Corolla dark purple or purplish red, tubular-funnelform, 8-12 mm long; tube straight, hardly longer than calyx, 5-7 mm long; limb as long as or shorter than tube, ca. 5-5.5 mm long, oblong-rhomboid, obtuse; throat appendages scaly, glabrous, ovate-triangular, 1 mm long. Filaments inserted below the appendages; stamens exerted from throat but not from corolla, sagittate at the base, 3 mm long, ca. as long as filaments. Style exerted from corolla, slightly thickened at the base, 12-16 mm long. Nutlets (not seen in the recorded specimen) 6-7 mm long, ovoid; basal disc triangular-ovate, with reduced and a prominent center line, margin and abaxially with dense glochids.

Note: The habitat of *L. stylosa*, was investigated again in July 20th 2020, at the geographical coordinates N 36° 27′ 31.3″, E 54° 26′ 44.9″, 3232 m a.s.l., to find a specimen in fruiting state, but the plants were in the vegetative growth stage, with no fruit.

Fig. 1. Herbarium specimen of *Lindelofia stylosa*, Naderi 2662 (DU001183).

Fig. 2. *Lindelofia stylosa* in natural habitat in Kuh-e Karkasi (Dibaj, N Damghan, Semnan province), 15 Jun 2015, Naderi 2662 (DU001183).

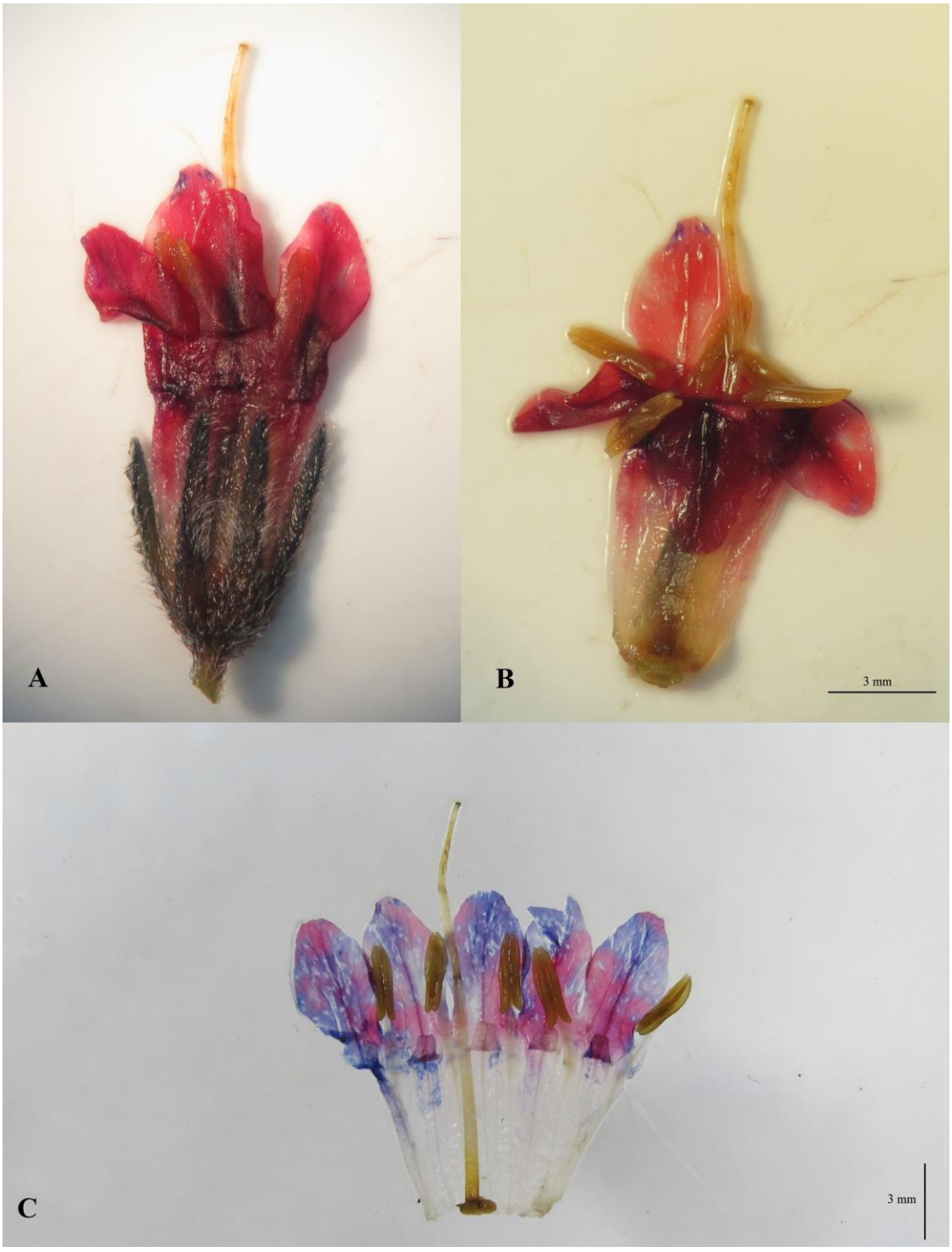

Fig. 3. Dissection of flower in *Lindelofia stylosa*. A & B, the style exerted from the corolla; C, the stamens exerted from the corolla throat.

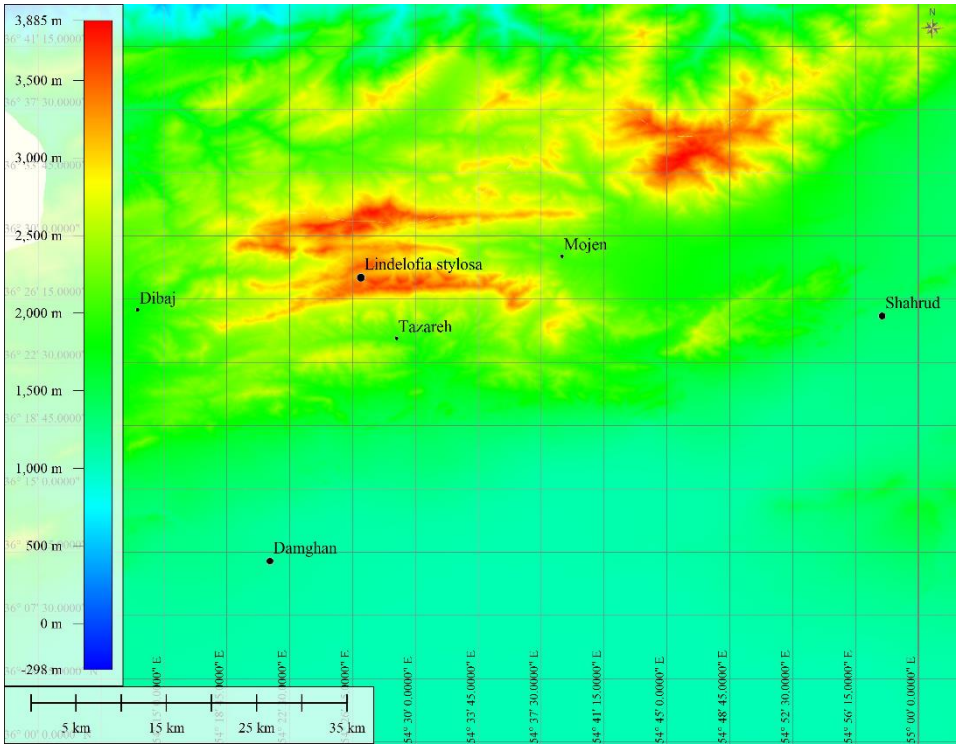

Fig. 4. Distribution map of *Lindelofia stylosa* (Kuh-e Karkasi, N Damghan, Semnan province). Retrieved from Global Mapper v.15.1.5.

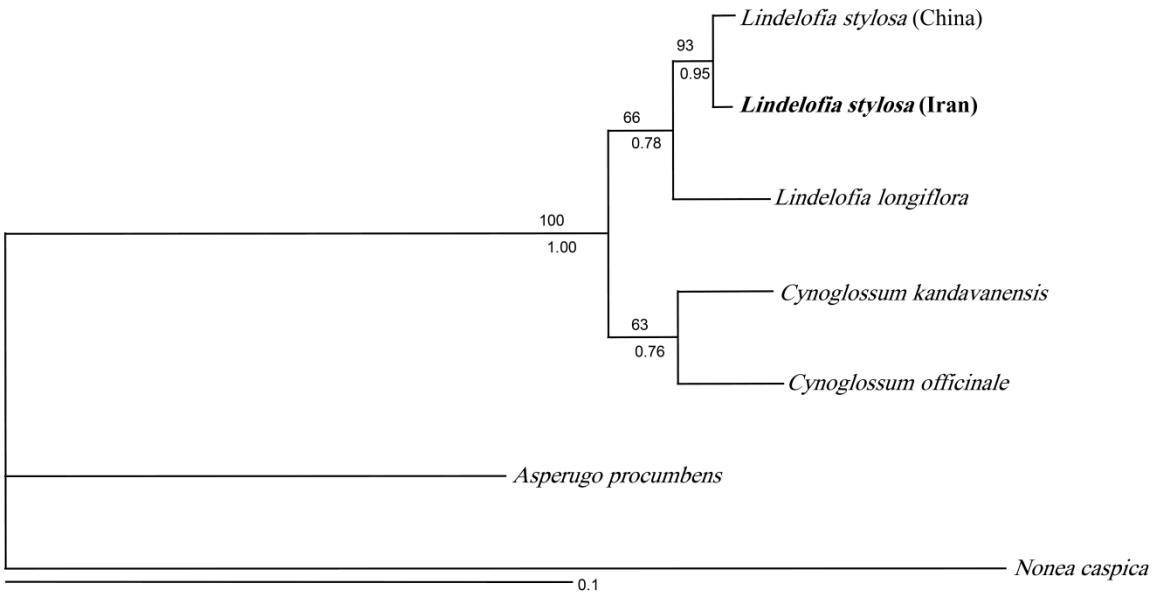

Fig. 5. Phylogenetic tree from Bayesian analysis of the nrDNA ITS sequences. Numbers above and below branches are parsimony bootstrap values and posterior probability, respectively.

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