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Type specimen of *V. kermanense* Hub.-Mor. (collected from Kerman by Soják in 1973, no. 3883).

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Focusing on three *Verbascum* L. taxa (Scrophulariaceae) of the *Flora of Iran*

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KEY WORDS
Scrophulariaceae,
Iran,
Bornmüller,
Celsia,
Kerman,
Baluchistan,
Taftan,
new synonym,
new subspecies.

ABSTRACT
We analyzed *Verbascum kermanense* Hub.-Mor., *V. carmanicum* (Bornm.) Hub.-Mor. and *V. gabrielae* (Bornm.) Hub.-Mor. (Scrophulariaceae) to resolve their classification using morphological traits and molecular evidence. *Verbascum kermanense* previously synonymized with *V. gabrielae* is presented as a subspecies of *V. carmanicum*, and *V. gabrielae* as a valid species based on morphological and molecular characteristics. The present article discusses morphological and hierarchical clustering analyses of key characters of these taxa, and provides their conservation status assessment and their distribution map for Iran.

MOTS CLÉS
Scrophulariaceae,
Iran,
Bornmüller,
Celsia,
Kerman,
Baluchistan,
Taftan,
synonymes nouveaux,
sous-espèces nouvelles.

RÉSUMÉ
Focus sur trois taxa de du genre Verbascum *L. (Scrophulariaceae) de la* Flore d'Iran.
Nous avons examiné trois espèces de *Verbascum* : *Verbascum kermanense* Hub.-Mor., *V. carmanicum* (Bornm.) Hub.-Mor. et *V. gabrielae* (Bornm.) Hub.-Mor. (Scrophulariaceae) pour établir leurs relations de parenté en utilisant à la fois des traits morphologiques et moléculaires. *Verbascum kermanense* qui avait été mis en synonymie avec *V. gabrielae*, est une sous-espèce de *V. carmanicum*, alors que *V. gabrielae* est bien une espèce valide d'après ses caractéristiques morphologiques et moléculaires. Le présent article traite des analyses de classifications morphologique et hiérarchique des caractères-clés de ces taxons, et fournit une évaluation de leur état de conservation et leur répartition pour l'Iran.

TABLE 1. — Morphological comparison of *Verbascum kermanense* Hub.-Mor., *V. carmanicum* (Bornm.) Hub.-Mor. and *V. gabrieliae* (Bornm.) Hub.-Mor. based on *Flora Iranica* and new observations of types and herbarium specimens.

Characters	*V. kermanense*	*V. carmanicum*	*V. gabrielae*
Height	50-65 cm	100-200 cm	40-50 cm
Indumentum	dense stellate hairs	dense stellate hairs	dense stellate and branched with mixture of glandular hairs in upper inflorescence parts
Stem	robust, leafy	robust, leafy	multi-stem, leafy in lower part (dense rosette form)
Petiole of basal leaves	long, 4-10 cm	long, 4-10 cm	short and narrow, 1-3 cm
Basal leaves	up to 20 cm long, 4-7 cm wide, ovate or ovate-oblong, sinuated, obtuse	up to 20 cm long, 4-7 cm wide, ovate or ovate-oblong, sinuated, obtuse	up to 10 cm long, 3 cm wide, oblong, not sinuated, acute
Lower leaves	similar to basal, shortly petiolate	similar to basal, shortly petiolate	similar to basal, shortly petiolate, subsessile
Bracts	ovate or triangular-ovate, entire, 5-10 mm long. stellate and glandular hairs; longer than fruiting pedicel	ovate or triangular-ovate, entire, up to 5 mm long. stellate and glandular hairs; almost same length as fruiting pedicel	ovate, entire, 2-5 mm long, stellate and glandular hairs; equal or a little longer than fruiting pedicel
Pedicel (fruiting)	up to 2 mm	2.5-5 mm	2-4 mm
Calyx segments	4-7 mm long, oblong-linear, acute, with stellate and glandular hairs		up to 4 mm long, oblong, acute, dense glandular, sparsely stellate
Corolla	18-22 mm diam.	18-22 mm diam.	10 mm diam.

TABLE 2. — Primers and references for the molecular markers.

Marker	Primer	Reference
psbA-trnH	psbA F	Sang *et al.* 1997
	trnH (GUG)	Tate & Simpson 2003
trnL-L-F	trnL (UAA)	Taberlet *et al.* 1991
	trn F (GAA)	Taberlet *et al.* 1991
trnS-G	trnS GCU	Hamilton 1999
	trnG UCC	Hamilton 1999
matK	390F	Cuénoud *et al.* 2002
	1326R	Cuénoud *et al.* 2002

INTRODUCTION

The genus *Verbascum* L. (Scrophulariaceae) is composed of ca. 400 species in the Old World (Sotoodeh 2015). In Iran, 44 species and a few hybrids have been described (Huber-Morath 1981; Sharifnia 2011; Sotoodeh 2015; Sotoodeh *et al.* 2014, 2015, 2016, 2017), which 21 out of the 44 species are endemic. Classification of the genus *Verbascum* in *Flora Iranica* (Huber-Morath 1981) and other references is based on indumentum type (simple, stellate, dendroid or glandular hairs), flower numbers per axil, stamen numbers, hair color of stamen filament, and length of bract, pedicel and capsule. The geographical distribution of *Verbascum* in Iran is very wide; most species occur in alpine or steppes of the Irano-Turanian region. However some taxa are also found in the Hyrcanian region such as the widely distributed *V. thapsus* L., and a few are found in the Sudanian region, in the south of Iran (e.g. *V. scoparium* Mozaffarian). The present study compares two taxa from the Kerman province (south of Kerman city) and one from the Taftan Mountain (Fig. 1), whose nomenclature is problematic. Taxonomically, these three species belong to the "single-flowered and four stamens" groups (Murbeck 1933; Huber-Morath 1978), and are endemic to Iran. *Verbascum kermanense* Hub.-Mor. was put in synonymy with *V. gabrielae* (Bornm.) Hub.-Mor. by Sharifnia (2007), only based on morphological characters. In the course of the first author's Ph.D thesis, during examination of *Verbascum* specimens from Iran, we found that we disagreed with this classification. Therefore we compared *V. gabrielae* to *V. carmanicum* (Bornm.) Hub.-Mor. and *V. kermanense* to establish a better classification, and to provide their conservation status assessment.

MATERIALS AND METHODS

During studying of *Verbascum* type specimens and other samples in different herbaria (BM, HKS, K, P, TARI, THE, and TUH), we concluded that ambiguities in the descriptions of *Verbascum kermanense*, *V. carmanicum* and *V. gabrielae* specimens require further investigations. We conducted these investigations using type specimens and other specimens from herbaria, different floras (Murbeck 1933; Parsa 1952; Huber-Morath 1978; Huber-Morath 1981; Fedtschenko 1997; Sharifnia 2011) and newly defined species from neighbouring countries (Firat 2015, 2017a, b; Karavelioğulları *et al.* 2014), and we defined, measured and compared their morphological characters (Table 1).

EXTRACTION PCR AND SEQUENCING

For the molecular genetic analysis, leaf samples were collected on field trips and dried in silica gel, or from herbarium specimens from the Tehran University Herbarium (TUH) and from Research Institute of Forest and Rangelands' Herbarium (TARI). Total DNA was extracted using the DNeasy plant mini kit (QIAGEN), following the manufacturer's protocol. PCRs were performed using the Taq PCR Master Mix Kit (QIAGEN). For the *ITS* nuclear marker we used the primers *ITSlcF* and *ITSlcR* (Khamar *et al.* 2017) and the PCR program given in Khamar *et al.* (2017). For chloroplastic regions, *psbA-trnH, trnL-L-F, trnS-G and matK*, primers and references are given in the Table 2. PCR products were confirmed on 1.2%

Fig. 1. — Distribution map of *Verbascum carmanicum* (Bornm.) Hub.-Mor., *V. kermanense* Hub.-Mor., and *V. gabrielae* (Bornm.) Hub.-Mor.

Table 3. — Voucher specimens used for DNA extraction and sequencing analysis. Abbreviations: **TUH**, Tehran University Herbarium; **TARI**, Research Institute of Forest and Rangelands.

Taxon	Collector (s)	Coll. date	Voucher	Locality (Iran), altitude	Accession numbers (ITS, trnL-F, trnS-G, matK, trnH-psbA)
V. kermanense Hub.-Mor.	Ghahreman, Attar, Mehdigholi	2002	28554TUH	Kerman, Kuhpayeh, 2550 m	MH844089, MH885338, MH885342, MH885330, MH885334
V. kermanense Hub.-Mor.	Ghahreman, Attar, Mehdigholi	2002	28583TUH	Kerman, South of Gugher 2552 m	MH844090, MH885339, MH885343, MH885331, MH885335
V. carmanicum (Bornm.) Hub.-Mor.	–	–	26011TUH	Kerman, Mount. Jupar, –	MH844091, MH885340, MH885344, MH885332, MH885336
V. gabrielae (Bornm.) Hub.-Mor.	Mozaffarian	1985	53182TARI	Baluchistan, Taftan Mountain, 2300-2500 m	MH844092, MH885341, MH885345, MH885333, MH885337

agarose gels and sent for purification and sequencing to GATC Biotech (Germany).

Alignment and correction

Forward and reverse sequences were edited and corrected using Sequencher 5.2.4 software (GeneCode Corporation, Ann Arbor, Michigan, USA) and were aligned in PAUP 4.0b10 for Macintosh (Swofford 2002) including gaps. The sequences are deposited in GenBank (Table 3).

Then we also performed hierarchical clustering (R function hclust) based on five gene sequences (one nuclear and four chloroplastic regions) and morphological characters, and visualized the results in a dendrogram. This method was used in another publication by Sotoodeh *et al.* in 2016 and proved useful to distinguish species. The dendrogram was constructed on the genomic regions and also on morphological characteristics. The morphological characters taken into account are given in Table 1, and the matrix made by morphological and molecular data in Fig. 6. The distance tree was created using Euclidian distance and Ward's hierarchical clustering method implemented in the hclust R function (Dunn & Everitt 2004; Parikh *et al.* 2010, Paradis 2011; Baselga & Orme 2012; Murtagh & Legendre 2014; Sotoodeh *et al.* 2016).

Examined specimens

Verbascum kermanense

Iran. Kerman, Mt. Jebal Barez, near Deh Bakri, in rocky slopes, 1700-2700 m, 2.V.1973, *Soják 3883* (holotype PR!); Rabor, Hanza, Takhte-Sartashtak, 3300 m, 8.VII.2014, *Mirtadzadini. s.n.* (TUH!); Kuhpaye, Simk Village, 2550 m, 26.V.2002, *Ghahreman, Attar, Mehdigholi 28554* (TUH!); 110 km SW of Kerman, Gughar Village, 28.V.1995, *Mirtadzadini 23477* (TUH!).

Fig. 2. — Type specimen of *Verbascum gabrielae* (Bornm.) Hub.-Mor. (collected from Taftan by Gabriel in 1937, no. 117).

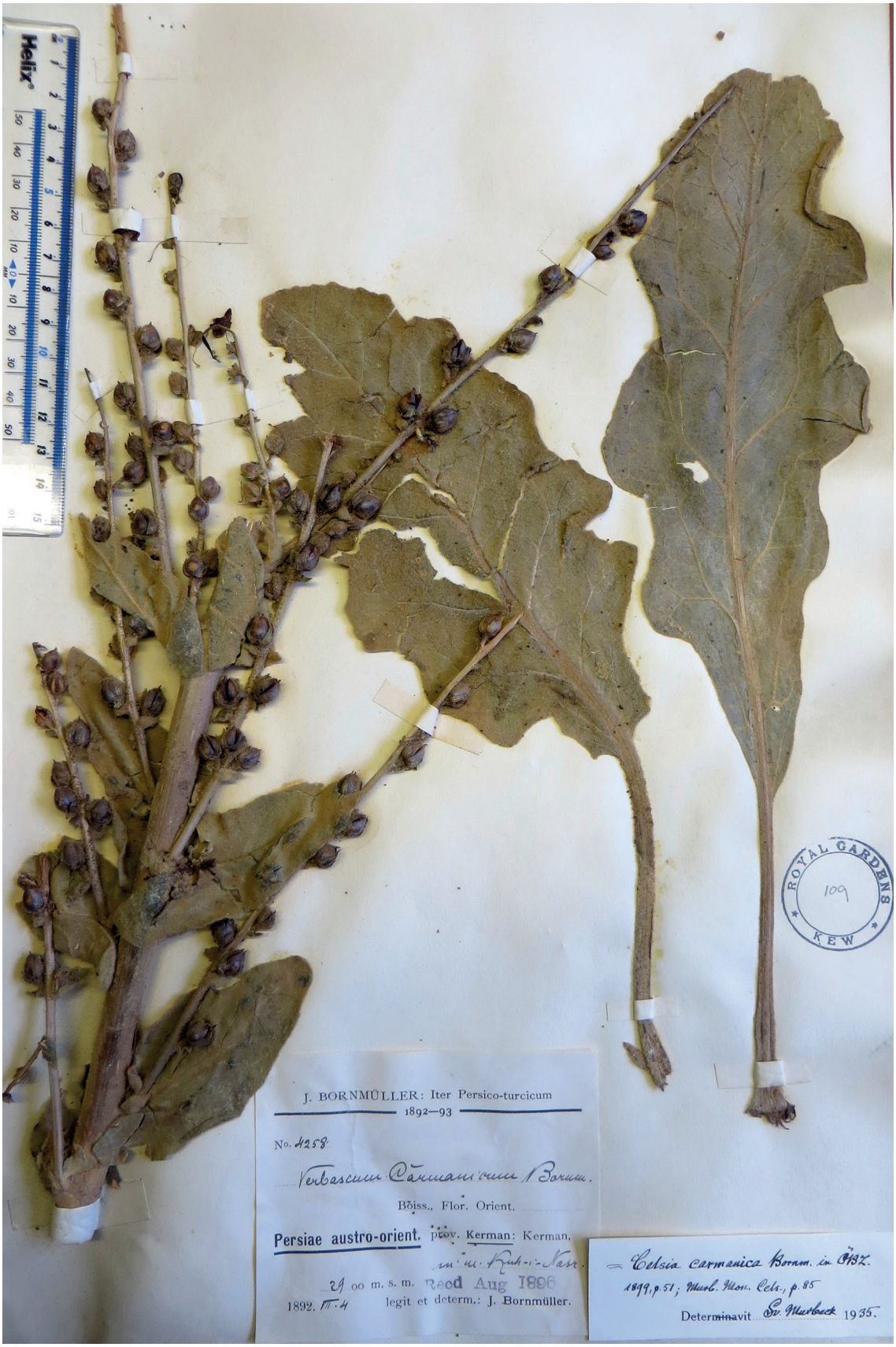

Fig. 3. — Type specimen *Verbascum carmanicum* (Bornm.) Hub.-Mor. (collected from Kerman by Bornmüller in 1892, no. 4259).

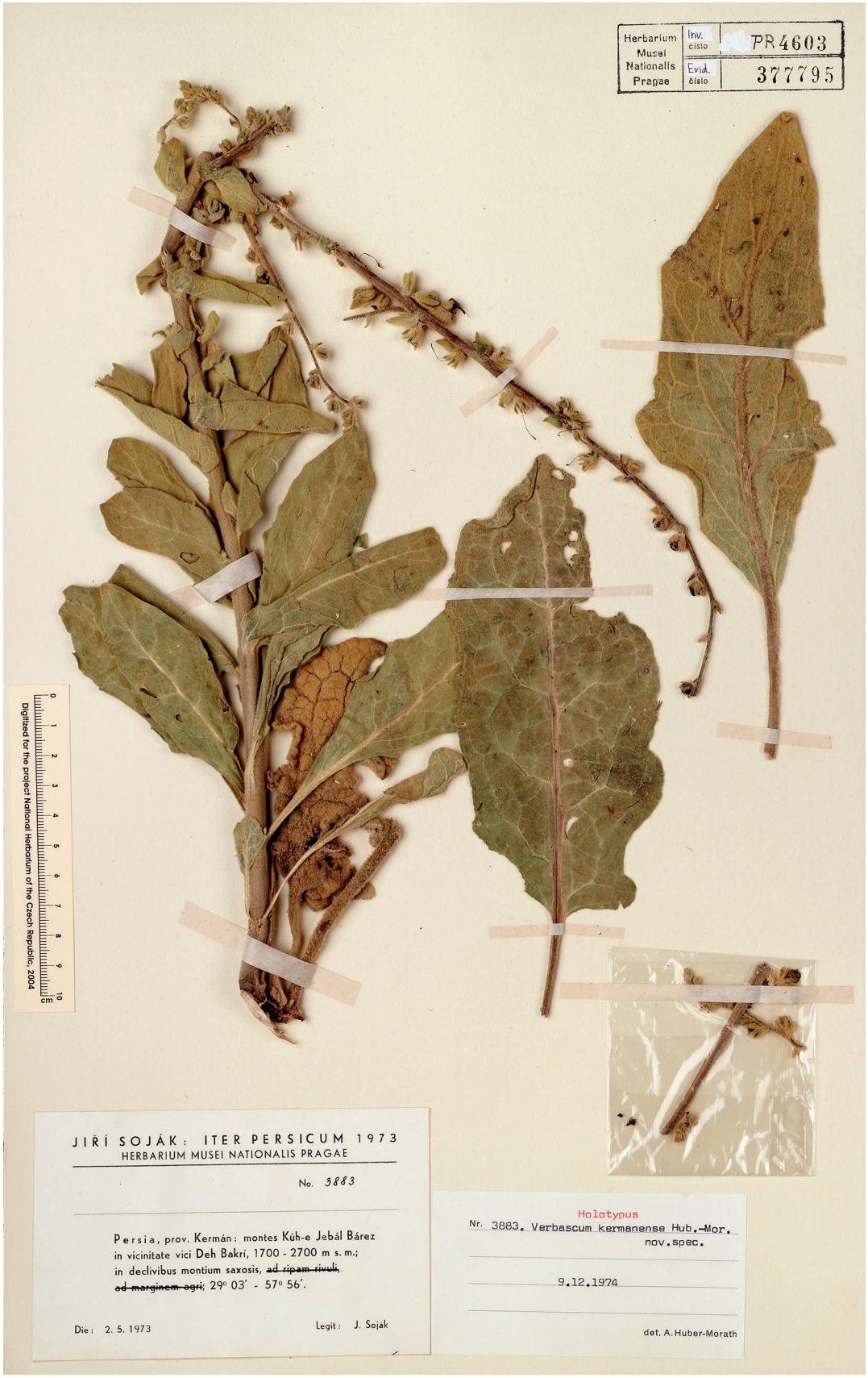

Fig. 4. — Type specimen of *V. kermanense* Hub.-Mor. (collected from Kerman by Soják in 1973, no. 3883).

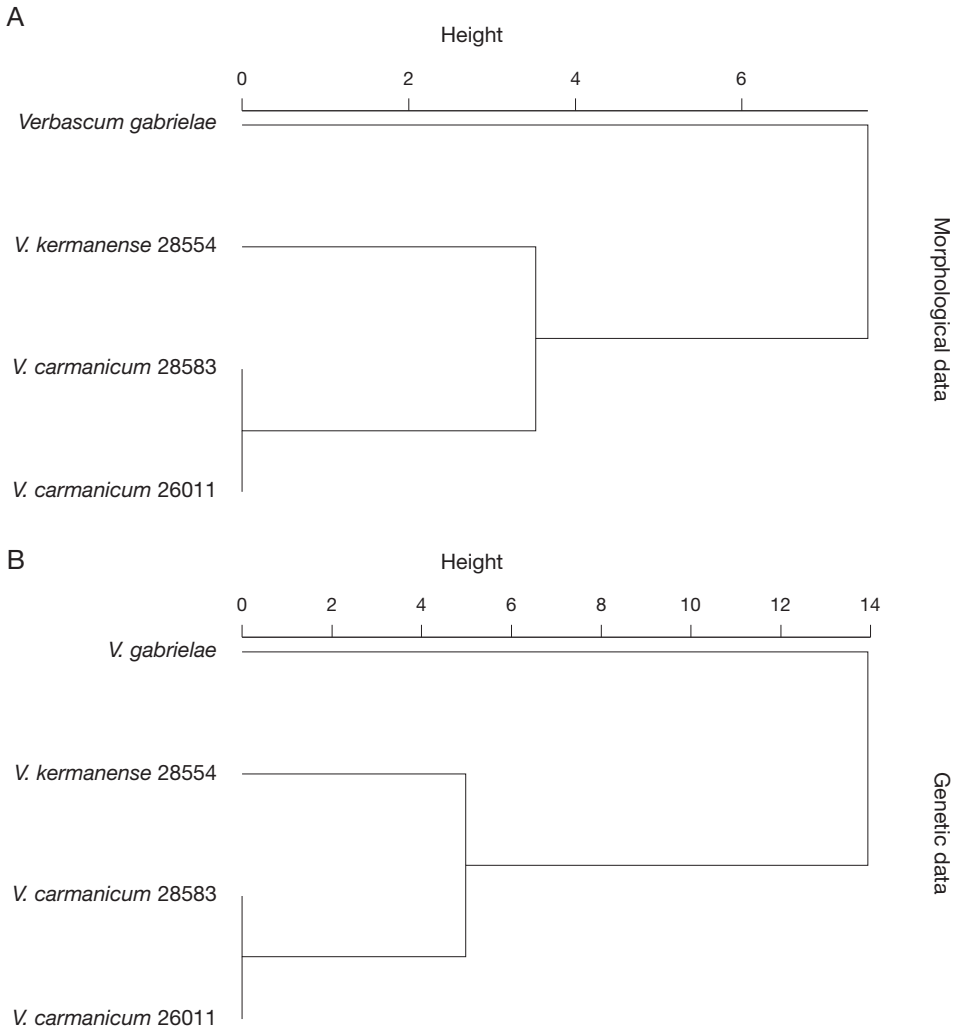

Fig. 5. — Distance trees based on morphological (**A**) and genetic (**B**) characters used to differentiate *Verbascum carmanicum* (Bornm.) Hub.-Mor., *V. kermanense* Hub.-Mor., and *V. gabrielae* (Bornm.) Hub.-Mor. species.

Verbascum carmanicum
Iran. Kerman, Mt. Kuh-Hazar, 3400 m, 1892, *Bornmüller 4259* (syn-, BM!, K!, B, P, image!); Kuh-Lalezar, 3600 m, 1892, *Bornmüller 4257* (syn-, B!, image!); Kuh-Nasr, 2900 m, 1892, *Bornmüller 4258* (syn-, K!, B!, image!); mt. Jupar, *26011*; Kuhpaye, Bidou, 23.VI.2004, *Mirtadzadini s.n.* (TUH!); 40 km from Baft, south of Gugher, 2552 m, 28.V.2002, *Ghahreman, Attar, Mehdigholi 28583* (TUH!).

Verbascum gabrielae
Iran. Baluchistan, Taftan mountain, Taftan Kuh-Garsh, 2000-3000 m, 1937, *Gabriel 117* (holo-, B image!); Taftan mountain, Temendan valley, 2300-2500m, 30.V.1985, *Mozaffarian 53182* (TARI!).

RESULTS

Description of *V. carmanicum, V. kermanense*, and *V. gabrielae* given in Latin language by *Flora Iranica* are translated and completed by new observations of types and herbarium specimens along with their distribution and conservation status assessment:

Verbascum carmanicum (Bornm.) Hub.-Mor.

In Bauhinia 5: 11 (1973).

Celsia carmanica Bornm., *Oesterreichische botanische Zeitschrift* 49: 51 (1899).

DESCRIPTION
Plant biennial, 50 to 200 cm height, covered in densely stellate, yellowish-gray hairs.

Stem
Robust, leafy, round at the bottom, but a bit angular, and branched at top.

Basal leaves
Have long petioles, lamina up to 20 cm long, 4-7 cm wide, ovate or ovate-oblong, sinuated, 2-4 lobes, obtuse.

	1_0: height 50-65 cm; 1: 100-200 cm.	2_0: dense stellate hairs; 1: stellate and branched hairs.	3_0: leaves not rosette form; 1: rosette form.	4_0: basal leaves, long petiolate; 1: short petiolate.	5_0: basal leaves, 20 cm L, 4-7 cm W, obtuse; 1: 10 cm L, 3 cm W, acute.	6_0: basal leaves, sinuated; 1: not sinuated.	7_0: pedicel up to 2 cm; 1: 2-4 cm.	8_0: calyx segments, 4-7 mm; 1: up to 4 mm.	9_0: corolla diam 18-22 mm; 1: 10 mm.	388 – trnL-F	441 – trnS-G	46 – ITS	113 – ITS	171 – ITS	268 – matK	348 – trnh-psbA
Verbascum kermanense Hub.-Mor. 28554	0	0	0	0	0	0	0	0	0	T	C	A	G	G	C	G
V. carmanicum (Bornm.) Hub.-Mor. 28583	1	0	0	0	0	0	0	1	0	0	C	A	G	G	C	G
V. carmanicum 26011	1	0	0	0	0	0	0	1	0	0	C	A	G	G	C	G
V. gabrielae (Bornm.) Hub.-Mor. 53182	0	1	1	1	1	1	1	1	1	1	A	G	A	G	T	G

Fig. 6. — The matrix made by morphological and molecular data.

Lower stem leaves
Similar to the basal ones, but with shorter petioles, upper leaves sub-sessile, oval, lobed-toothed; at top: sessile, sub-entire.

Inflorescence
Loose, many simple branches, up to 90 cm long.

Bracts
Small, ovate or triangular-ovate, entire, up to 10 mm, shorter, same as or longer than fruiting pedicel.

Pedicel (fruiting)
Sessile or up to 5 mm long, thick, ± erect.

Calyx
Lobes 4-6 mm long, oblong-linear, acute, covered with stellate and glandular hairs.

Corolla color
Yellow, but Huber-Morath (1981) described its color as violet. Nevertheless, we noticed repeatedly that corolla color changes after drying to turn very dark.

Corolla
18-22 mm diam., abaxial surface covered with sparse stellate hairs.

Stamens
Four, one third of top part of two anterior filaments are naked, the rest covered densely with long purple hairs.

Anthers
Reniform and medifix.

Capsule
Pyramid-ovoid, 7-10 mm long, 4.5-7 mm wide, hairy.

Verbascum carmanicum (Bornm.) Hub.-Mor. subsp. *carmanicum* (Hub.-Mor.) Sotoodeh, Attar, Andalo & Civeyrel, subsp. nov.

Verbascum carmanicum Hub.-Mor., *Bauhinia* 5: 11 (1973), **syn. nov.** — Basionym: *Celsia carmanica* Bornm., *Oesterreichische botanische Zeitschrift* 49: 51 (1899).

Type material. — *Bornmüller 4257*, *4258*!, *4259*! (syn-, P[P03287427, P03287428, P03287429], B) (Fig. 3).

Distribution. — Endemic of the irano-turanian region.

Conservation status assessment. — Field observations indicated that *V. carmanicum* plants were never observed in large populations. Their geographical range of occurrence is estimated to be less than 3000 km^2 and severely fragmented. They were collected in less than five locations. According to the rules for classifying the rare, threatened, and endemic plants, IUCN criteria (http://www.iucnredlist.org [accessed: 16 March 2018]), this species should be considered as endangered [EN A1ac; B1ab (iv)].

Description

Plant 100-200 cm height. Bracts up to 5 mm, same as fruiting pedicel. Pedicels (fruiting) 2.5-5 mm.

Verbascum carmanicum (Bornm.) Hub.-Mor. subsp. *kermanense* (Hub.-Mor.) Sotoodeh, Attar, Andalo and Civeyrel, subsp. nov.

Verbascum kermanense Hub.-Mor., *Bauhinia* 5: 149 (1975), **syn. nov.** — *Celsia carmanica* Bornm., *Oesterreichische botanische Zeitschrift* 49: 51 (1899).

Type material . — **Iran**. Kerman, Kuh-e Jebal Barez, near Deh Bakri, in rocky slopes, 1700-2700 m, *Soják 3883*! (holo-, PR image!) (Fig. 4).

Diagnosis. — *Verbascum carmanicum* subsp. *kermanense*, subsp. nov. can be distinguished from *V. carmanicum* subsp. *carmanicum*, subsp. nov. by a combination of the following characters: Plant 50-65 cm height (vs 100-200). Bracts ovate or triangular-oval, 5-10 mm long (vs up to 5), longer than fruiting pedicel, entire (vs same as fruiting pedicel). Pedicels (fruiting): sessile or up to 2 mm (vs 2.5-5).

Distribution. — Endemic of the Irano-turanian region.

Conservation status assessment. — Field observations indicated that this species was never observed in large populations; its geographical range of occurrence is estimated to be less than 2000 km^2 and severely fragmented, or known to exist in no more than four locations. According to the rules for classifying the rare, threatened, and endemic plants, IUCN criteria (http://www.iucnredlist.org [accessed: 16 March 2018]), this species should be considered as endangered [EN A1ac; B1ab(iv)].

Verbascum gabrielae (Bornm.) Hub.-Mor.

In *Bauhinia* 5: 12 (1973).

Celsia gabrielae Bornm., *Beihefte zum Botanischen Centralblatt* 59 B: 307 (1939).

Type material. — **Iran**. Baluchistan, Kuh-e Taftan, Kuh-e Garok, 2000-3000 m, *Gabriel 117*! (holo-, B) (Fig. 2).

Locality. — **Iran**. South Baluchestan: In mountain Taftan, Garok, 2000-3000 m, *Gabriel 117*!.

Distribution. — Endemic of the Irano-turanian region.

Conservation status assessment. — Only two specimens were collected in close locations. Following IUCN criteria (http://www.iucnredlist.org/ [accessed: 16 March 2018]), *V. gabrielae* should be classified as Critically Endangered (CR A2; B2ab; C2ab; D) because its population size is suspected to have dropped to less than 50 mature individuals, and its area of occupancy is estimated to be less than 10 km^2.

Description

Plant (described from two samples) biennial or perennial, multi-stemmed, all covered by dense yellowish stellate and branched hairs.

Stems

Short, narrow and leafy at the bottom.

Basal leaves

Oblong, entire, forming a loose rosette, densely covered with yellowish hairs, up to 10 cm long, 30 mm wide, short and narrow petiole up to 2 cm.

Lower stem leaves

Look like the basal ones, but smaller, sub-sessile, oblong, obtuse or acute, upper leaves are sub-cordate and sessile.

Inflorescence

Loose, racemose, mixture of branched and glandular hairs in upper parts.

Bracts

Small, ovate, entire, equal in length or a little shorter than fruiting pedicels.

Pedicel (fruiting)

2-4 mm long.

Calyx

Lobes 4 mm long, oblong, acute, densely glandular and sparsely stellate hairs.

Corolla

Yellow, 10 mm diam., abaxial surface covered with non-dense stellate hairs.

Stamens

Four. One third of top part of front filaments is naked, the rest covered densely with long purple hairs.

Anthers

Reniform and medifix.

Capsule

Ovate, 6-7 mm long, 3.5-4 mm wide and covered with dense stellate hair.

Hierarchical clustering results

Two dendrograms of the hierarchical clustering analyses were produced, one with morphological characters and the other based on DNA sequences. They showed the distance or dissimilarity of *V. gabrielae* with *V. carmanicum* and *V. kermanense* (Fig. 5).

DISCUSSION

Verbascum carmanicum and *V. kermanense* are closely related and display a few differences in vegetative character states, like bract and fruiting pedicel size, and especially the ratio of bract to fruiting pedicel sizes (Table 1). This result is confirmed by the clustering analysis of both molecular and morphological data according to which the two taxa are clearly grouped together (Fig. 5). These differences are not enough to consider *V. kermanense* as a separate species, but due to geographical separation – the collected specimens were isolated in different mountain ranges at elevations of more than 2500 m – we propose to treat *V. kermanense* as a subspecies of *V. carmanicum*: *Verbascum carmanicum* (Bornm.) Hub.-Mor., subsp. *kermanense* (Hub.-Mor.), subsp. nov.

These taxa have more morphological differences with *V. gabrielae*, i.e. mixture of branched and glandular hairs in upper inflorescense parts in *V. gabrielae*, differences in petiole sizes of basal leaves, in basal leaf shape (sinuated or not), bract size and corolla size. The cluster analysis based on the two datasets clearly shows that *V. gabrielae* is isolated from *V. carmanicum* and *V. kermanense* (Fig. 5) and can be considered as a different species.

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