

## **Verbascum albidiflorum (Scrophulariaceae), a new species from W Iran**

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## *Verbascum albidiflorum* (Scrophulariaceae), a new species from W Iran

### Abstract

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*Verbascum albidiflorum* Ranjbar & Nouri (Scrophulariaceae) is described and illustrated as a new species of *V* [sect. *Bothrosperma* Murb.] subsect. *Singuliflora* Murb. from Kermanshah Province, W Iran. It is morphologically most similar to *V. alceoides* Boiss. & Hausskn. and the two species are compared with respect to gross morphology, leaf anatomy and pollen morphology. The chromosome number  $2n = 38$  is reported for the first time in *V. albidiflorum* and  $2n = 48$  is reported in *V. alceoides*, both from Kermanshah Province, Iran.

Additional key words: chromosome number, endemic, Kermanshah Province, leaf anatomy, pollen morphology, taxonomy, *Verbascum alceoides*

### Introduction

The genus *Verbascum* comprises some 360 species worldwide (Heywood 1993), 42 species in Iran (Huber-Morath 1981; Sharifnia & Assadi 2011) and 49 species in the *Flora iranica* (Huber-Morath 1981), which includes parts of Afghanistan, Iraq, Pakistan, Talish and Turkmenistan. The main centre of *Verbascum* diversity and evolution is Turkey and Iran (Zohary 1973; Huber-Morath 1978). Among species distributed in Iran, 15 are endemic (Huber-Morath 1981; Sharifnia 2007). In recent years the relationships between *Verbascum* species based on morphological characteristics (Karavelioğulları & al. 2004, 2008; Vural & Aydoğdu 1993; Al-Hadeethy & al. 2014; Cabi & al. 2011; Parolly & Eren 2008; Parolly & Tan 2007; Kaynak & al. 2006; Sharifnia & Assadi 2007), anatomical characteristics (Petković & al. 1997; Kheiri & al. 2009), cytological characteristics (Ghaffari 2008; Malik & al. 2011;

Marhold & Breitwieser 2009) and pollen-morphological characteristics (Pehlivan & al. 2008; Kheiri & Khayami 2006) have been somewhat explored in various studies. The present study focuses on the morphological, leaf-anatomical, pollen-morphological and karyological analysis of a species of *Verbascum* apparently endemic to W Iran, in the Zagros Mountains in Kermanshah Province. These plants have been cross-checked with the keys provided by Murbeck (1933), the floras of the neighbouring countries and other taxonomic literature (Boissier 1879; Fedtschenko 1955; Ferguson 1972; Huber-Morath 1978, 1981) and finally compared with herbarium material. These studies have allowed us to recognize the plants as a new and distinctive species, which is described here as *V. albidiflorum* Ranjbar & Nouri, and to consider *V. alceoides* Boiss. & Hausskn., which is distributed from NE Iraq to W Iran (Huber-Morath 1981; Fig. 5), as the most similar species and possible nearest relative.

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All Iranian species of *Verbascum* belong to *V.* sect. *Bothrosperma* Murb., in which has been recognized two main groups: “A” and “B” (Huber-Morath 1978, 1981; Sharifnia & Assadi 2011). In Flora iranica (Huber-Morath 1981), *V. alceoides* Boiss. & Hausskn. is in group A, characterized by having a solitary flower in the axil of each bract, and subgroup b, characterized by being covered, at least partially, with stellate and dendroid hairs. In Flora of Turkey (Huber-Morath 1978), *V. alceoides* is in *V.* [sect. *Bothrosperma*] subsect. *Singuliflora* Murb.

## Material and methods

### Material

The material used for this study represents four populations (Table 4). *Verbascum albidiflorum* and the collection of *V. alceoides* referred to here as “*V. alceoides* 35707” were collected during field exploration in Kermanshah Province, Iran, during May 2014 and were deposited in the BASU herbarium (herbarium codes according to Thiers 2015+). The holotype of *V. alceoides* (at G), from NE Iraq, was also studied. In addition, micromorphological data from a further collection of *V. alceoides*, also from NE Iraq and here referred to as “*V. alceoides* 235”, were taken from Al-Hadeethy & al. (2014); this collection was selected in that work because it is the nearest to the type locality of *V. alceoides*.

### Morphology

The present study is mainly based on herbarium material examined at B, BASU, E, G, JE, LD, P, W and WU. At first glance, *Verbascum albidiflorum* seemed to be similar to *V. alceoides*. Measurements, colours and other details given in the description are based on living material, alcohol-preserved specimens, and data derived from field notes. Morphological characters were surveyed, including stem shape and size, leaf shape and size, type of leaf pubescence, calyx colour and shape, corolla colour and size, and capsule width.

### Leaf anatomy

Micromorphological characters, especially those related to hairs and stomata, can be considered as useful taxonomic features in species of *Verbascum* (Al-Hadeethy & al. 2014). The importance of anatomical studies was emphasized by Lersten & Curtis (2001), and it seems that epidermis peels have also proved to be diagnostically helpful. In this study, mature leaves from dried specimens were chosen and softened in a mixture of distilled water/glycerine/ethanol 70% (1:1:1) for 2 weeks. Indumentum and epidermis were removed from the middle part of the leaves and petiole using commercial razor blades, stained by methyl blue and carmine and mounted on slides using Canada balsam. Hairs and stomata on the leaves were

examined using an Olympus BX-51 photomicroscope at 400× magnification and photographed with an Olympus digital camera.

### Pollen morphology

Pollen samples were obtained from herbarium specimens and prepared using the standard method of acetolysis (Erdtman 1960). They were mounted in unstained glycerin jelly and observations were made with a Nikon Type-2 microscope. The following measurements were made on 25 pollen grains from each specimen: polar axis (P), equatorial diameter (E), distance between colpi, colpus length, pore length, pore width and shape index (= P/E).

### Karyology

The chromosome number of mitotic stages was studied in *Verbascum alceoides* and *V. albidiflorum*, both collected from Kermanshah Province, Iran, during May 2014, and deposited in the BASU herbarium. Flower buds at an appropriate stage of development were fixed in 96% ethanol, chloroform and propionic acid (6:3:2) for 24 hours at room temperature and then stored in 70% alcohol at 4 °C until used. Anthers were squashed and stained with 2% acetocarmine. All slides were made permanent by Venetian turpentine. Photographs were taken on an Olympus BX-51 photomicroscope initially at 1000× magnification. Chromosome counts were made from well-spread metaphases in intact cells, by direct observation and from photomicrographs.

## Results and Discussion

*Verbascum albidiflorum* Ranjbar & Nouri, **sp. nov.** – Fig. 1.

Holotype: Iran, Kermanshah Province, Gahvareh to Khosro Abad, along road from Naylak to Beryakhani, eastern road from Khosro Abad to Kerend-e Gharb, 34°18'10.6"N, 46°23'26.7"E, 1450 m, 12 May 2014, S. Nouri 35706 (BASU!; isotypes: BASU!, W!).

*Diagnosis* — *Verbascum albidiflorum* Ranjbar & Nouri (Fig. 1) is most similar to *V. alceoides* Boiss. & Hausskn. (Fig. 2) but differs from *V. alceoides* in having stems 70–80 (vs 16–22) cm tall; basal leaf petiole 10–14 (vs 2–3) cm long; bracts 4–12 (vs 4–5) mm long; corolla milk-coloured or yellowish white (vs yellow) with dense purplish red spots in throat (vs unspotted); and filament hairs white to grey (vs purple-violet) (see Table 1).

*Description* — *Herbs* perennial, slightly woody at base, 70–80 cm tall. *Stems* several, robust, erect, unbranched or pyramidally branched from 15–20 cm above base toward apex, light green to somewhat purplish brown at base, cylindric, densely leafy, densely covered with



Fig. 1. *Verbascum albidiflorum* – A & B: habitat and habit, plants with inflorescences; C: basal leaves showing surfaces and margin; D: cauline leaves, parts of inflorescence, partly opened flower and flower buds; E: as D but with fully opened flower; F: fully opened flower, apical view; G: inside of flower showing stamens. – Scale bars: A = 5 cm; B–F = 3 cm; G = 5 mm. – Type locality, 12 May 2014, photographed by S. Nouri.

short-stalked stellate and simple eglandular hairs, densely pubescent at base. *Leaves* alternate, mostly congested at base in a dense persistent rosette; *basal leaves*: *petiole* 10–14 cm long; *leaf blade* dark green when fresh, purplish brown when dried, oblong to elliptic, 20–26 × 3.4–4.4 cm, loosely yellowish white tomentose on both surfaces, with stellate hairs and glandular hairs on both surfaces, sometimes with dendroid hairs, veins indistinctly pinnately reticulate, more densely covered with hairs, base gradually attenuate into petiole, margin clearly obtuse-crenulate, apex acute or acuminate; *cauline leaves* decreasing in size toward flowering part of stem; *lower cauline leaves*: *petiole* 3.5–4 cm long; *leaf blade* 13–18 × 3.3–4.3 cm, margin crenulate to serrulate; *upper cauline leaves* subsessile; *leaf blade* elliptic, 4.5–7 × 2–2.4 cm, margin slightly crenulate to entire. *Inflorescence* racemose or loosely paniculate, 30–35 cm long; *lateral branches* erect, loose, green, elongating to 25–30 cm long, many-flowered, with pale white or grey eglandular stellate hairs; *internodes* 1.5–2 cm at inflorescence base; *inflorescence leaves* ovate to elliptic, 8–30 × 3–6 mm, similar to floral bracts, apex acute or acuminate; *bracts* green when fresh, purplish brown when dried, linear-lanceolate, 4–12 × 1–1.5 mm, densely covered with stellate hairs, sparsely covered with glandular and dendroid hairs, margin entire, apex acute to acuminate. *Flowers* solitary at each node (i.e. each bract embracing 1 flower), subsessile, ebracteolate, horizontal to ascending. *Calyx* green to greenish white, broadly ovoid or infundibuliform, 7–8 × 5.5–6 mm, lobed for ½ or more of length, densely tomentose with stellate hairs



Fig. 2. *Verbascum alceoides* 35707 – A: habitat and habit, plant with inflorescence; B: part of inflorescence with fully opened flowers and flower bud; C: cauline leaves upper surface. – Scale bars: A–C = 3 cm. – See Table 4 for locality, 2 May 2014, photographed by S. Nouri.

outside; *lobes* lanceolate-ovate, 3–5 mm long, subequal, distal ½ with deciduous hairs, apex acute and mucronulate. *Corolla* milk-coloured or yellowish white when fresh, pale yellow when dried, with dense purplish red spots in throat, rotate, 31–35 mm in diam.; *tube* 1–1.5 mm

Table 1. Diagnostic morphological characters of *Verbascum albidiflorum* and the holotype of *V. alceoides*.

Characters	<i>Verbascum albidiflorum</i>	<i>Verbascum alceoides</i>
Stem colour	light green to somewhat purplish brown at base	greyish purple
Stem height [cm]	70–80	16–22
Basal leaf petiole length [cm]	10–14	2–3
Basal leaf blade shape	oblong to elliptic	ovate to elliptic
Basal leaf blade size [cm]	20–26 × 3.4–4.4	2–4 × 1–2.5
Basal leaf blade margin	clearly obtuse-crenulate	slightly obtuse-crenulate
Cauline leaf arrangement	dense	loose
Lower cauline leaf blade size [cm]	13–18 × 3.3–4.3	3–4.5 × 1.5–2
Upper cauline leaf blade size [cm]	4.5–7 × 2–2.4	2–3 × 0.8–1.5
Bract size [mm]	4–12 × 1–1.5	4–5 × 1–4
Calyx size [mm]	7–8 × 5.5–6	4–5 × 3.5–4
Pedicel length [mm]	flowers subsessile	0.5–2.5(–4)
Corolla colour	milk-coloured or yellowish white, with dense purplish red spots in throat	yellow, without spots in throat
Filament hairs colour	white to grey	purple-violet

Table 2. Diagnostic anatomical characters (hairs and stomata) on leaves of *Verbascum albidiflorum* and *V. alceoides* 235. Dimensions are in  $\mu\text{m}$ . Mean is shown in brackets.

Characters	<i>Verbascum albidiflorum</i>	<i>Verbascum alceoides</i> 235
Length of neck of glandular hairs	4–[7.33]–12	5–[5.62]–6.25
Length of stomata on lower surface	10–[11.8]–13	22.7–[25.6]–29.2
Width of stomata on lower surface	6–[7.66]–8	22.5–[23.5]–25
Stomatal index on lower surface	c. 20	c. 12.9
Length of stomata on upper surface	9–[10.86]–12	25–[28.5]–30
Width of stomata on upper surface	7–[8]–9	20–[23.3]–25
Stomatal index on upper surface	c. 22	c. 12.5

Table 3. Quantitative analysis of the pollen morphology of *Verbascum albidiflorum* and *V. alceoides* 35707. Dimensions are in  $\mu\text{m}$ . Mean is shown in brackets.

Characters	<i>Verbascum albidiflorum</i>	<i>Verbascum alceoides</i> 35707
Polar axis (P)	22–[23.2]–24	24–[27.2]–29
Equatorial diameter (E)	19–[20.08]–23	22–[24.5]–26
Distance between colpi	7–[10.26]–13	12–[13.26]–20
Colpus length	10–[16.93]–21	16–[19.93]–25
Pore length	1–[1.66]–3	1–[2.66]–4
Pore width	2–[4.8]–8	3–[6.8]–9
Shape index (= P/E)	c. 1.15	c. 1.11

long; lobes orbicular, 17–19  $\times$  8–10 mm, subequal, often 2 smaller than others, stellate tomentose outside. *Stamens* 5, fertile, subequal; *filaments* free, bluish purple, 9–10 mm long, glabrous at base, remainder densely covered with clavate villous hairs, with white to grey glandular hairs near anthers, 2 anterior filaments more sparsely haired than others; *anthers* yellowish white, 1.5–2 mm long, connate with connective, base decurrent on anterior anthers and reniform on others. *Ovary* 2-locular, ovoid to globose, densely stellate hairy; *style* terminal,

In addition, some other plant species occurring in the habitat included *Aegilops triuncialis* L., *Cousinia dalahensis* Attar & Ghahr., *Gundelia tournefortii* L., *Hedysarum criniferum* Boiss., *Onobrychis melanotricha* Boiss., *Poa trivialis* L. and *Quercus brantii* Lindl.

*Conservation status* — *Verbascum albidiflorum* is known only from the type locality in Kermanshah Province. The estimated area of occupancy is less than 2 km<sup>2</sup> and the known number of mature individuals is fewer than five.

filiform, 7–8 mm long, pubescent with stellate hairs at base; *stigma* clavate, 2–3  $\times$  c. 0.5 mm, apex semiglobose. *Capsule* olive-brown, shining, ovoid or ellipsoid to globose, 7–8  $\times$  6–7 mm, scarcely longer than calyx, pubescent-velutinous, densely or rarely sparsely covered with stellate hairs to glabrescent, dehiscing by a longitudinal groove, apex shortly beaked. *Seeds* numerous, greenish to light brown when immature, rugose, uniformly transversely sulcate.

#### *Distribution and ecology*

— The new species is a rare endemic of W Iran, in the Zagros Mountains between Gahvareh and Khosro Abad in Kermanshah Province, about 100 km E of the Iraq border (Fig. 5). It is an Irano-Turanian geoelement. It grows on hills of clay and stone in open *Quercus* forest at altitudes of 1400–1500 m.

Table 4. Origin of material used in this study.

	<i>Verbascum albidiflorum</i>	<i>V. alceoides</i> 35707	<i>V. alceoides</i> 235	<i>V. alceoides</i> holotype
Locality	Iran: Kermanshah Province: Gahvareh to Khosro Abad, along road from Naylak to Beryakhani, eastern road from Khosro Abad to Kerend-e Gharb	Iran: Kermanshah Province: Bisoton mountain	Iraq: Sulaymaniyah Province	Iraq: Sulaymaniyah Province: Pir Omar Gudrun (Pira Magrun)
Coordinates	34°18'10.6"N, 46°23'26.7"E	34°23'47.7"N, 47°25'47.6"E	35°50'20.4"N, 45°19'50.3"E	35°46'14.1"N, 45°13'41.1"E
Altitude [m]	1450	2000	–	1200
Date	12 May 2014	2 May 2014	–	Jun 1867
Specimen	<i>S. Nouri</i> 35706 (BASU)	<i>S. Nouri</i> 35707 (BASU)	<i>M. Al-Hadeethy</i> 235 (BAG)	<i>Hausknecht</i> 713 (G)

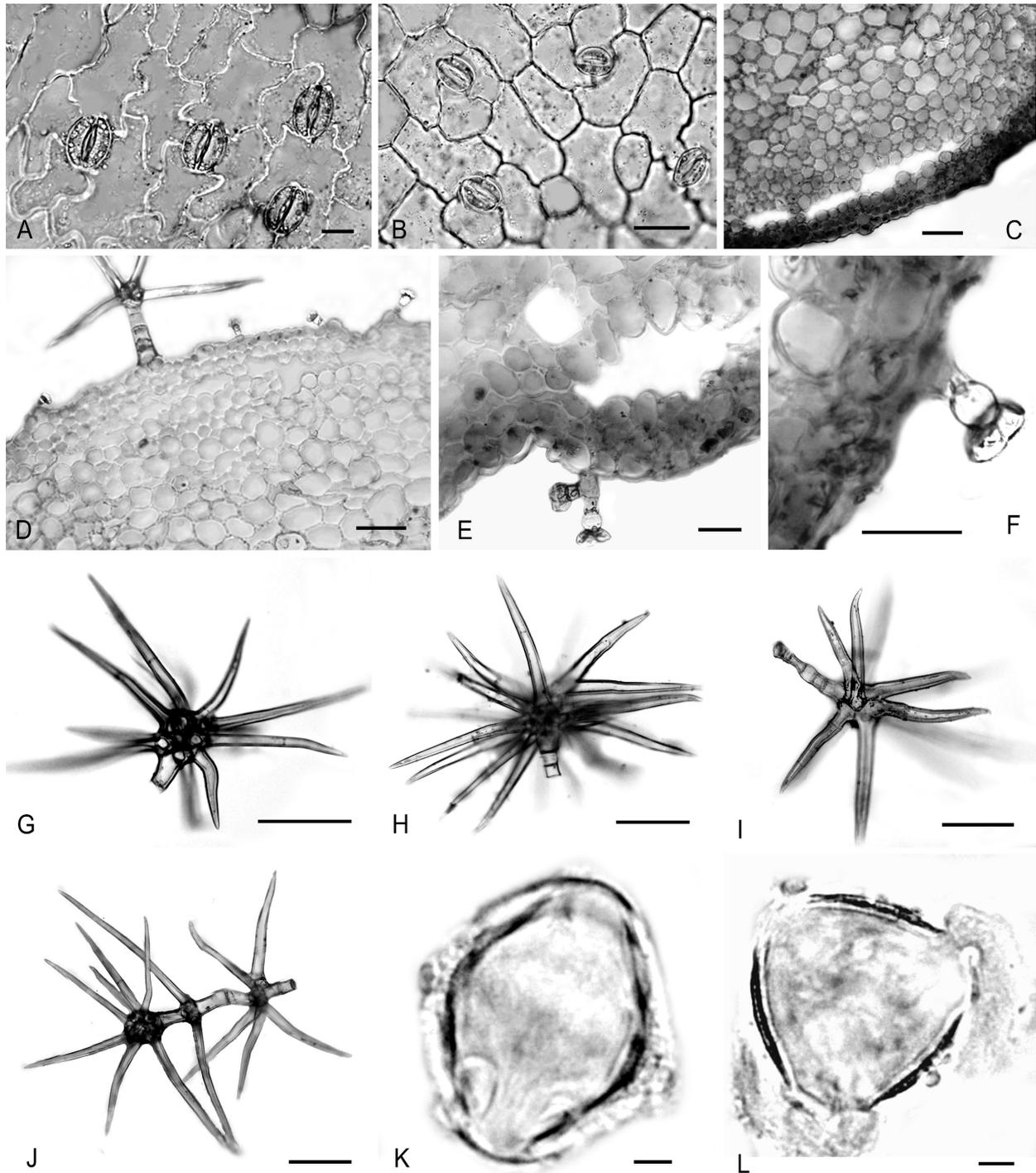


Fig. 3. Photomicrographs of leaf epidermis, leaf hairs, petiole epidermis and pollen grains in *Verbascum albidiflorum* – A: surface section of leaf with anomocytic stomata in lower epidermis; B: surface section of leaf with anomocytic stomata in upper epidermis; C: transverse section of petiole with cavities in lower epidermis; D: eglandular and glandular hairs on leaf with cavities in lower epidermis; E: dendroid glandular hairs with multicellular base on leaf with cavity in lower epidermis; F: glandular hair on leaf; G: stellate hair with unicellular base; H: stellate hair with tricellular base; I: stellate hair with multicellular base; J: dendroid hair; K: pollen grain, equatorial view; L: pollen grain, polar view. – Scale bars: A & E = 10  $\mu\text{m}$ ; B & F = 20  $\mu\text{m}$ ; C & D = 50  $\mu\text{m}$ ; G, H, I & J = 100  $\mu\text{m}$ ; K & L = 3  $\mu\text{m}$ .

It can therefore be categorized as Critically Endangered (CR) according to IUCN criterion D (IUCN 2012).

**Etymology** — The specific epithet *albidiflorum* means “whitish flowered”.

#### Leaf anatomy

Results from leaf-hair morphology in *Verbascum albidiflorum* showed that the leaves are covered with eglandular and glandular hairs (Fig. 3D–J). There are three

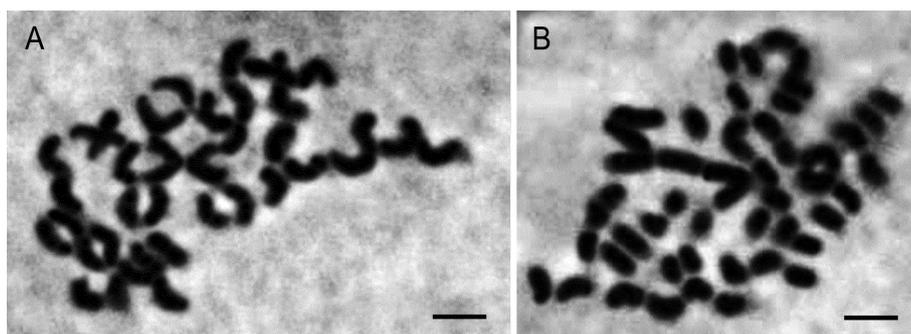


Fig. 4. Representative prophase in a mitotic cell. – A: *Verbascum albidiflorum*,  $2n = 38$ ; B: *V. alceoides* 35707,  $2n = 48$ . – Scale bars: A & B = 1  $\mu\text{m}$ .

types of hairs in this species: (1) eglandular stellate hairs with a unicellular or multicellular single stalk by which they are attached to the surface of the leaf (Fig. 3G–I); (2) eglandular many-branched dendroid hairs (Fig. 3J); and (3) glandular hairs that are simple or rarely branched (Fig. 3E & F). The glandular hairs have a short neck and a broad 4-celled head. For comparison with *V. albidiflorum* using hair and stomata characters, the collection *V. alceoides* 235 was used (Table 2). Both collections have glandular and eglandular hairs on the lower and upper surfaces of the leaves and there is no difference in the diameter of the glandular hairs, but they are different in the length of the neck. *Verbascum alceoides* 235 has a multicellular short neck ranging from 5–6.25  $\mu\text{m}$  long, which is often shorter than that of *V. albidiflorum* which ranges from 4–12  $\mu\text{m}$  long. Also the terminal cell of the glandular hair neck has a round shape in *V. albidiflorum* but is oblong in *V. alceoides* 235.

The study of stomata characters carried out on *Verbascum albidiflorum* and *V. alceoides* 235 indicated

that they are uniformly amphistomatic (i.e. with stomata on both lower and upper leaf surfaces) and have the anomocytic type of stomata. The shape of the guard cells on the lower and upper surfaces is reniform in both *V. albidiflorum* and *V. alceoides* 235. There are three types of subsidiary cells in the leaves of *V. albidiflorum*: (1) stomata completely surrounded by 3 subsidiary cells, which are of more or less equal size or one of them is distinctly smaller (Fig. 3B); (2) stomata completely surrounded by 4 subsidiary cells, which are equal or unequal in size (Fig. 3A); and (3) stomata surrounded by 5 subsidiary cells, which are equal or unequal in size. Often the stomata are smaller on the upper surface with 3 subsidiary cells (Fig. 3B). Stomata with 4 subsidiary cells and 3 subsidiary cells with equal percentages (46.66) have the highest density and stomata with 5 subsidiary cells have the lowest density (6.66) on both leaf surfaces. All the subsidiary cells in leaves are monocyclic with ridged walls. The stomatal index on both surfaces in *V. albidiflorum* is larger than in *V. alceoides* 235. There are differences in length and width of stomata on the lower and upper leaf surfaces: the stomata length in *V. alceoides* 235 is larger than in *V. albidiflorum*. In addition, cavities were seen under the lower epidermis of the leaf blades and petioles of *V. albidiflorum* (Fig. 3C–E); these anatomical features might have taxonomic value.

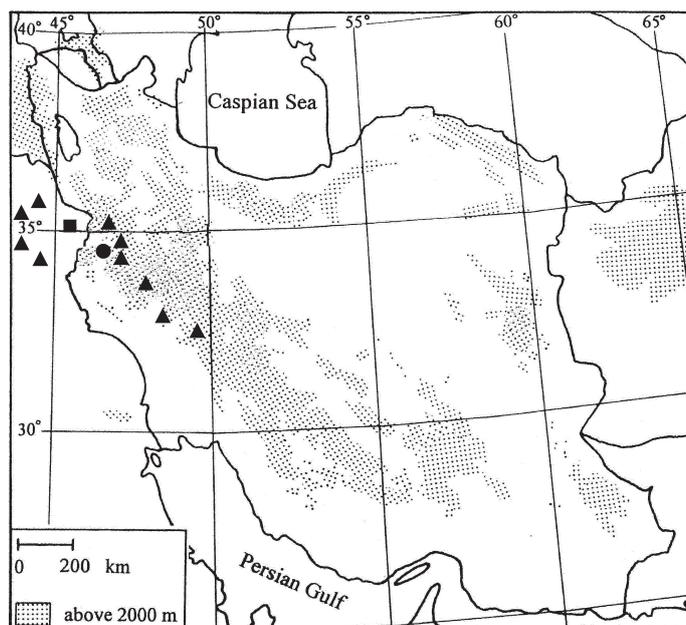


Fig. 5. Distribution of *Verbascum albidiflorum* (circle) and *V. alceoides* (triangles; square = type locality).

### Pollen morphology

The present study showed that most pollen grains of *Verbascum albidiflorum* (Fig. 3K & L) and *V. alceoides* 35707 are radially symmetrical, isopolar, oblate-spheroidal or sub-spheroidal or sub-prolate and tricolporate. Observations by light microscopy of seven pollen characters of *V. albidiflorum* and *V. alceoides* 35707 are reported here (Table 3). The pollen grains in *V. alceoides* 35707 are larger than in *V. albidiflorum*.

### Karyology

Few studies have been conducted on the chromosomes of *Verbascum* species (e.g. Mori 1957; Arts-Damler 1960; Nilsson & Lassen 1971; Koktay 1974; Dane & Yılmaz 2002) and, as has been pointed out (Yılmaz & Dane 2011), they have very small chromosomes. So far in Iran, only one chromosome number,  $2n = 2x = 30$ , has been reported for two *Verbascum* species: *V. sinuatum* (Ghaffari

2008) and *V. speciosum* (Cartier 1983). We are reporting the mitotic chromosome number  $2n = 38$  for *V. albidiflorum* (Fig. 4A) and, for the first time,  $2n = 48$  for *V. alceoides* 35707, from W Iran (Fig. 4B).

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