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Notes on the genus *Viscum* (Viscaceae) in Iran: A new combination based on morphological evidence

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The genus *Viscum* L. (Viscaceae) with 150 species in the world was known in Iran only by *V. album* L. subsp. *album*. The plants of *Viscum* are hemiparasites growing on various host trees such as *Crataegus* sp., *Parrotia persica* and *Pyrus* sp. To investigate the range of morphological variation in different populations of these subspecies authors conducted detailed morphological and micromorphology studies. Furthermore, anatomical features of stem, leaf, petiole and fruit were investigated in different populations of this taxon. The presence of calcium oxalate crystals and platelet ornamentation of wax crystalloid structures on leaf surfaces were interesting features that could aid separating one population distributed in Golestan forest (NE, Iran) from others. The ornamentations of seed and fruit surface provide further evidence in recognizing this population as a separate taxon. The seed surface in *V. album* subsp. *album* is smooth while wrinkled in the population from Golestan forest. Furthermore, the fruit surfaces are smooth in the former, but furnished with platelet crystalloid ornamentations in the latter. These micromorphological differences were associated with important evidence from gross-morphology: the prophylls are two at the axils of each leaf in *V. album* subsp. *album*, but four in the population from Golestan forest. In conclusion, the results of the present study suggest recognizing the population distributed in Golestan forest as a separate taxon rather at subspecific level. A new subspecies is described for this new taxon: *V. album* subsp. *golestanicum* Saeidi, Shahi and Golmohammadi.

Key words: *Viscum album* subsp. *album*, Viscaceae, calcium oxalate crystals, Iran.

INTRODUCTION

The genus *Viscum* L. (Viscaceae) contains about 150 species (Heide-Jørgensen, 2008) in both the northern and southern hemispheres, distributed in Europe, Africa, Asia and Australia (Nickrent et al., 2010). This genus, known locally in Iran as "Darvash" which is represented by one species: *V. album* L.

Viscum is a hemi-parasitic dioecious shrub growing on the branches of deciduous trees as well as conifers. It is an evergreen plant with leathery and opposite leaves. The inflorescences are axillary or terminal. Male flowers consist of four perigones. Anthers are implanted on perigones and dehisce by numerous pores. The female flowers are smaller than the male ones. The ovary of

mistletoes (*Viscum* sp.) generally demonstrates striking reduction phenomena. No ovules exist; instead, a central ovarian papilla, or mamelon, protrudes into the ovarian cavity and produces two or more embryo sacs (Zaki and Kuijt, 1995). Fruit is white, ovoid or elliptic, one seeded berry, with bitter and unpleasant odor. In addition to the embryo and the endosperm, each mistletoe berry contains a mucilaginous tissue called viscin that consists of cellulose in a mixture of acidic and neutral polysaccharides (Gedalovich et al., 1988; Sallé, 1983). Mistletoe berry is not part of the seed morphologically, even though it is closely associated with it (Gedalovich and Kuijt, 1987). This composite substance with its sticking nature plays a crucial role for the dissemination of mistletoe by birds (Azuma et al., 2000). Numerous attempts have been made to divide *Viscum album* L., the most widespread species of the genus, into different

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subspecies, varieties, etc. These divisions were usually made on the basis of morphological characters; for example, leaf size (Ball, 1993; Singer, 1958), fruit morphology and seed number and differences in coloration of shoots. However, the morphological characters cannot provide a clear-cut toll in recognizing subspecific taxa in this species (Becker and Exner, 1980). In most treatments of this species three subspecies are recognized differing in the structure of ripe berries (Grazi and Urech, 1981). If the ripe berries are squeezed, the seeds of *V. album* subsp. *abietes* (Wiesb.) Abromeit. and *V. album* subsp. *austriacum* (Wiesb.) Vollmann. are easily freed from the skin and from the viscous layer, but in *V. album* subsp. *album* the viscous layer remains attached to the skin and the seed by squeezing. The pattern of flavonoid aglycones may be different for certain samples. But like morphological features, there is no clear-cut distinction between three different subspecies (Becker and Exner, 1980).

Extensive surveys on host specificities accompanied by infection experiments, carried out by Tubeuf (1923) and Hass et al. (2003), resulted in recognizing three subspecies: (i) *V. album* subsp. *album*, growing on various dicotyledonous trees, (ii) *V. album* subsp. *abietis*, growing on fir (*Abies* spp.) and (iii) *V. album* subsp. *austriacum*, growing on pine (mainly *Pinus sylvestris* and *P. nigra*).

In addition, the amount of embryos per berry varies. While *V. album* subsp. *album* tends to be diembryonal, the percentage of monoembryonal berries is higher in both other subspecies.

Nagl and Stein (1989) characterized DNA from mistletoes grown on various hosts. Small but not significant differences were found between the cpDNA contents of the three subspecies and the base composition. Significant differences were detected in the patterns of sequence organization. Other genetic evidence for host specificity was reported by Zuber and Widmer (2000) and Piotrowski et al. (2003).

Rechinger (1979) has reported *V. album* widely in Northern Iran without any taxonomic treatment at subspecific level. Parsa (1950) reported also *V. laxum* as an independent species in NE Iran. The latter is known today as a synonym of *V. album* subsp. *austriacum* growing on several genera of Pinaceae (Ball, 1993). So, the occurrence of this taxon in Iran is very unlikely.

Metcalfe and Chalk (1957) provide some information regarding the anatomical characteristics of the leaf, petiole and stem of *V. album* and *V. articulatum*. Anatomical study of *V. album* has been also investigated by Andronache et al. (2006), Khan et al. (2009) and Mbagwu et al. (2009).

The micromorphology of epicuticular waxes of leaves in subspecies of *V. album* has also been investigated (Hass et al., 2003). Barthlott et al. (1998) discussed the classification, terminology and the taxonomic value of plant epicuticular waxes in 13,000 plant species. The epicuticular

waxes often form complex three-dimensional crystalline microstructures such as platelets, rods, and tubules (Koch and Barthlott, 2006).

The present study reports a new subspecies and describes the morphological and anatomical features of the stem, leaf and fruit anatomy of *Viscum* subspecies growing in Iran as well as their seed morphology.

MATERIALS AND METHODS

Taxonomic studies

Ten samples of two subspecies were investigated from field gatherings and herbarium specimens. Voucher specimens are kept in the Herbarium of University of Guilan (Table 1). The descriptions of the morphological features of both subspecies were made using experimental observations. The chorological study of subspecies is based on Zohary (1973).

Anatomical studies

Plant samples were first embedded in FAA and then fixed in 70% ethanol for 3 days. Anatomical investigations were performed on cross-sections of stems, leaves, petioles and fruits produced using hand cutting. For each taxon, replicates were collected from several populations, from which three was selected for further analysis. The cross-sections were stained with methylene blue (to study xylem and fiber tissue) and congo red (to study phloem, epidermis and parenchyma tissue) and mounted with glycerin jelly to make permanent slides (Vardar, 1987). Well stained sections were photographed with an olympus BX-51 light microscope (LM).

For measuring stomatal index, dried leaves were used. The leaves were placed in a tube filled with 70% lactic acid for 4 to 5 days. Lactic acid softens the leaf and it was possible to scrap the leaf surface with sharp scalpel or by hand. The length and width of the stomata were measured with a LM. The stomatal index was calculated according to the method described by Meidner and Mansfield (1968).

Micromorphological studies

The seeds were detached from herbarium sheets and directly placed on aluminium stubs and covered with gold for SEM studies. For removing any pollution, leaves and fruits were put into water for 12 and 1 h and subsequently dried with freeze dryer (ALPHA 1-2 LD) for 3 h at -34°C and with oven (WTF- binder) for 1 h at 30°C , respectively. Photographs were taken with SEM (LEO 1430 VP). Seed and fruit terminology followed by Bojnansky and Fargasova (2007), while the terminology of leaf epicuticular waxes was according to Barthlott et al. (1998).

RESULTS

Identification key for the subspecies

- 1) Leave with two acute, ciliate and membranous prophylls
 *V. album* subsp. *Album*
 - leave with four small acute, ciliate and membranous prophylls

Table 1. Voucher specimens for taxonomic studies (Bold); Anatomical studies (underline) and micromorphological studies (stars).

<i>V. album</i> subsp. <i>Album</i>	<i>V. album</i> subsp. <i>golestanicum</i>
Golestan: Gorgan to chahar bagh, 1900 m, Mozaffarian 77844 (TARI); Golestan National Park, Zelkova region, 86 km East North of Gonbad, 770 m, Pbout 78145 (TARI).	Golestan: <u>Bandar Gaz</u> (*), 850 m, Golmohammadi and Mazandarani 3991 (GUH). Golestan National Park, 950 m, Sabeti 77452 (TARI); <u>Kordkuy</u> (*), 900 m, Golmohammadi and Mazandarani 3991/1 (GUH); <u>Golestan National Park</u> , 840 m, Assadi and Azadi 76086 (TARI).
Mazandaran: Kelardasht, Rudbarak 1700-1800 m, Runemark and Mozaffarian 25232 (TARI); <u>Chaloos</u> (*), Sinua village, 1500 m, Golmohammadi and Daneshfar 3990/3 (GUH). Amol, Kre sang jungle, without collector name and number (TARI); Amol, Khoshdash jungle, Alireza kord, without number (TARI); Noor, Geland road, Sabeti 30550 (TARI); Kojur, Rechinger and Manoochehri 30506 (TARI); Noshahr, Keirood کنار jungle, 400 m, Assadi 33390 (TARI).	
Guilan: <u>Emamzade hashem</u> (*), 800 m, Golmohammadi and Rankouhi 3990/1 (GUH); <u>Chaboksar</u> , Sarve lat, Gang sara village, 1000 m, 3990/2 (GUH); Asalem, Nave jungle, 1000 m, Asefi 24307 (TARI); Talesh, Khotbe sara jungle, without collector name 53847 (TARI).	
Azarbaijan: Khalkhal to Ardebil, 15 km to Ardebil, Havashan mountain, 1500-2000 m, Termeh and Mousavi 30498 (TARI).	
Kordestan: Marivan to Saghez, 50 m to Saghez, 2000 m, Termeh 30499 (TARI).	
Khorasan: Esfrayan, Rein, Shahrokhi 30494 (TARI).	
Semnan: around Shahrood, without collector name and number (TARI).	
Tehran: <u>Lavasanat</u> , 2000 m, Shadman rad 3990/4 (GUH); Ghazvin; Alamot, Sedighi 30501 (TARI).	

.....*V. album* subsp. *golestanicum*

Fl. and Fr. Time: March to October.

Viscum album L. subsp. *album*

Shrub 20 to 25 cm tall; dark green. Internodes 1.5 to 4 cm long. Leaves dark green; 3 to 5 × 1 to 1.5 cm; with two acute, ciliate and membranous prophylls, 0.1 to 0.5 cm length. Seeds reniform, green, 0.3 to 0.4 × 0.3 to 0.35 cm.

Ecology: Wet and temperate regions with lime soils.

Distribution: North, West and Central of Iran (Figure 1).

Hosts: *Carpinus betulus*, *P. persica*, *Zelkova carpinifolia*, *Ulmus* sp., *Crataegus* sp., *Malus vulgaris*, *Amygdalus* sp. and *Prunus* sp., *Quercus* sp., *Pyrus* sp., *Armeniaca vulgaris*.

Viscum album L. subsp. *golestanicum* Saeidi, Shahi and Golmohammadi, subsp. nov.

Type: Golmohammadi and Mazandarani 3991. Iran, Golestan, Gaz Bandar, 36°46'27" N, 53°56'53" E, 850 m, 10 August 2010 (Holo. Herbarium of University of Guilan). Frutex 23 to 27 cm altus lux viridis. Internodes 2 to 12 cm longa. Folia viridi lumine, 3.5 to 7 × 0.5 to 2 cm, cum quatuor parvis acutis et membranous prophylls ciliatis,, 0.1 to 0.2 cm longa. Semina elliptico, viridis, 0.4 to 0.5 × 0.1 to 0.3 cm. Shrub 23 to 27 cm tall; light green. Internodes 2 to 12 cm long. Leaves light green; 3.5 to 7 × 0.5 to 2 cm; with four small acute, ciliate and membranous prophylls, 0.1 to 0.2 cm length. Seeds elliptic, green, 0.4 to 0.5 × 0.1 to 0.3 cm.

Ecology: Wet and temperate regions with lime soils.

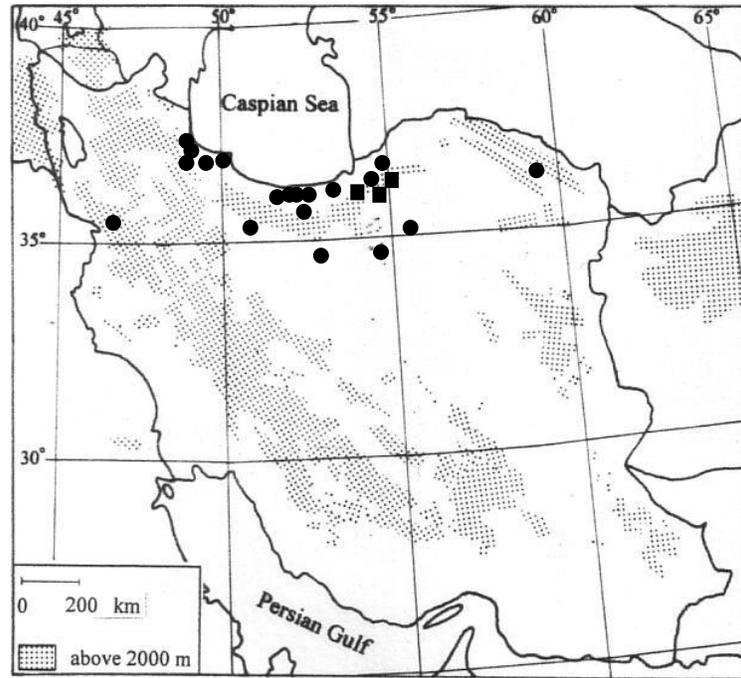


Figure 1. Map showing the distribution of the sampled population of *V. album* subspecies. *album* (●) and *V. album* subspecies. *golestanicum* (■) in Iran.



Figure 2. *V. album* subspecies. *golestanicum* (Golmohammadi and Mazandarani 3991).

Distribution: Only in North of Iran (Figure 2).

Hosts: *C. betulus*, *P. persica*, *Ulmus* sp., *Amygdalus* sp., *Pyrus* sp., *Prunus* sp., *M. vulgaris*, *Crataegus* sp.

Fl. and Fr. Time: March to October.

The anatomical studies

Stem: In cross sections, the stem is circular and undulate; it is sometimes winged in both subspecies. Outermost layer is composed of one layer of epidermal cells in different sizes and shapes (for example, triangular

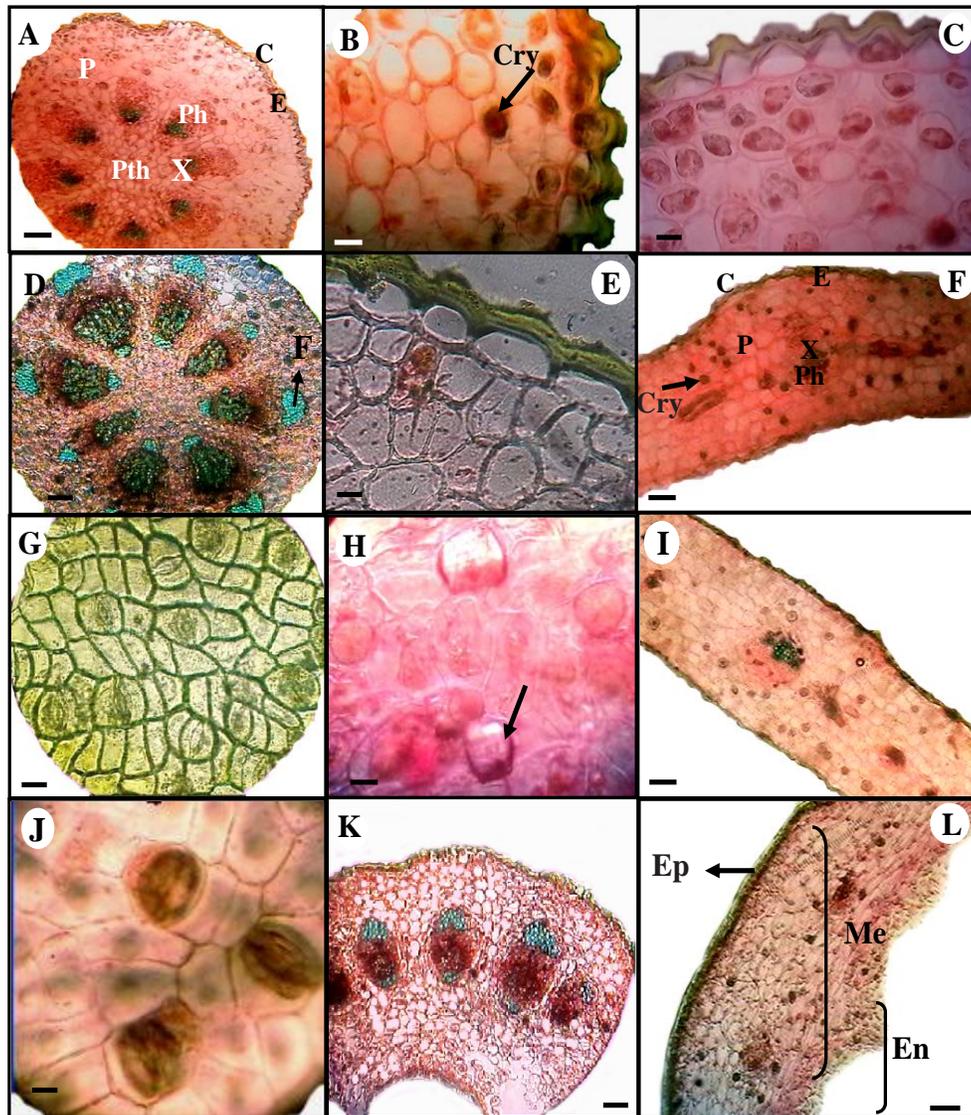


Figure 3. Transverse sections of stem: *V. album* subsp. *album*: A) general aspect of stem, B) a part of stem with cubical crystal (arrow), C) epidermis of stem with triangular cells; *V. album* subsp. *golestanicum*: D) central part of stem, E) epidermis of stem with quadrangular cells. Transverse sections of leaf: *V. album* subsp. *album*: F) a part of lamina, G) stomatal architecture, H) cubical calcium oxalate crystals in the mesophyll; *V. album* subsp. *golestanicum*: I) general aspect, J) stomatal architecture. Transverse section of petiole: *V. album* subsp. *golestanicum*: K) general aspect. Transverse sections of fruit: *V. album* subsp. *album*: L) a part of pericarp. – Abbreviations: E: Epidermis; Ph: Phloem; X: Xylem; P: Parenchyma; E: Epidermis; C: Cuticle; Cry: Crystal; F: Fiber; Ep: Epicarp; Me: Mesocarp; En: Endocarp. - Scale bars: A, D, F, G, I, K and L = 0.04 mm; B, C, E, H and J = 0.01 mm.

in *V. album* subsp. *album* and quadrangular in *V. album* subsp. *golestanicum*). Epidermal cells are covered with a thick layer of cuticle. Cortex is made up of circular cells and intercellular spaces. Cubical, prismatic and stellate calcium oxalate crystals were observed in mesophyll of *V. album* subsp. *album*, but prismatic crystals were not observed in *V. album* subsp. *golestanicum*. In the cortex, there are bundles of fibers above the primary phloem and xylem of the vascular bundle. Stem shows primary

growth; eight large vascular bundles are arranged in a ring. In the central part there is a parenchymatous pith (Figure 3A to E).

Leaf blade: In cross section, the leaf is quadrangular and dorsiventral. Epidermal cells are irregular quadrangular in both subspecies. Cuticle is present on epidermis. Mesophyll is undifferentiated and encompasses large intercellular spaces. There are prismatic, stellate and

cubical calcium oxalate crystals in mesophyll of *V. album* subsp. *album*, while there are only stellate crystals in *V. album* subsp. *golestanicum*. Vascular bundles are collateral. There are also fibers associated with vascular bundles. Leaves are amphistomatic and stomata are paracytic type (Figure 3F to J).

Petiole: In cross section, the petiole is elliptic and winged in both taxa. Epidermal cells of *V. album* subsp. *album* are elliptic in shape, but they are quadrangular in *V. album* subsp. *golestanicum*. There is a cuticle on both sides of the epidermis. Cubical and stellate calcium oxalate crystals were observed in the cortex of *V. album* subsp. *album*, but *V. album* subsp. *golestanicum* has only stellate crystals. There are five unequal size vascular bundles arranged in an arc. As in the leaf, bundles are collateral and there are bundles of fibers above the phloem and xylem of the vascular bundle (Figure 3 K).

Fruit: Epicarp consists of one layer of epidermal cells. Mesocarp is parenchymatous with elliptic cells, intercellular spaces and fiber bundles. There are cubical and stellate calcium oxalate crystals in mesocarp of *V. album* subsp. *album*, but cubical calcium oxalate crystals were not observed in *V. album* subsp. *golestanicum*. Endocarp in both taxa consists of viscin layer (Figure 3 L).

Micromorphological studies

Leaf: On upper and lower surface of leaf in both subspecies platelet crystalloids were observed with parallel orientation to each other and perpendicular to the surface, however, the crystalloids were more condensed on adaxial surface (Figure 4 A to F).

Fruit: fruit is a unilocular, globose white berry, with leathery surface and one seed. The size of berries ranges from 0.6 to 0.8 × 0.4 to 0.6 cm (for example, *V. album* subsp. *album*) and 0.5 to 0.8 × 0.3 to 0.4 cm (for example, *V. album* subsp. *golestanicum*) (Table 2). The berry surface of *V. album* subsp. *album* is smooth, while it is platelet crystalloid in *V. album* subsp. *Golestanicum* (Figure 4 G to I).

Seed: Morphological characters and SEM patterns of seed coat in examined taxa are presented in Table 2. The seeds were reniform in *V. album* subsp. *album* and elliptic in *V. album* subsp. *golestanicum*. Seed size was also almost similar in both subspecies. The patterns of sculpturing in *V. album* subsp. *golestanicum* were wrinkled, but in *V. album* subsp. *album* were almost smooth with very superficial longitudinal grooves (Figure 4 J to L).

DISCUSSION

This study provides useful anatomical and micromor-

phological information and additional perspective to the systematics of the examined *Viscum* subspecies in Iran. Morphological characters proved that *Viscum* has two subspecies in the country. *Viscum album* subsp. *golestanicum* described here as a new subspecies matches well with the distribution area and the description of *V. laxum* sensu Parsa (1950).

The variation of leaf anatomy is significant and revealed that this character possesses many attributes of potential taxonomic importance that are diagnostic at the genus and species levels (Carlquist, 1961; Edeoga and Iklem, 2001; Mbagwu and Edeoga, 2006; Nwachukwu and Mbagwu, 2007). Examined subspecies are similar in general aspects of the leaf anatomy. Leaf mesophyll was undifferentiated in both of subspecies; while Andronache et al. (2006) have reported palisade parenchyma under upper epidermis and spongy parenchyma under lower epidermis in *V. album* subsp. *album*. According to Metcalfe and Chalk (1957) the mesophyll of the biennial leaves of *V. album* consist of isodiametric cells during the first year, but a single layer of palisade tissue develop towards both surfaces in the second year; so it allows us to speculate that the leaves examined here were within the first year of growth.

The result of this study shows that the distribution and shape of the calcium oxalate crystals could provide valuable characters in delimitation of subspecific taxa in *V. album*. The use of calcium oxalate crystals in solving taxonomic problems has been suggested in previous studies of other plant groups such as Papilionoideae (Mbagwu and Edeoga, 2006) and Dioscoraceae (Edeoga and Okoli, 1992, 1995). Prismatic calcium oxalate crystals in stem and prismatic and cubical calcium oxalate crystals in leaf of *V. album* subsp. *album* and their absence in *V. album* subsp. *golestanicum* is an important diagnostic character. No crystals were reported in previous studies in *V. album* subsp. *album* (Khan et al., 2009).

The shape of epidermal cells of the stem provides another diagnostic character in recognizing both subspecies. They are triangular in *V. album* subsp. *album*, while quadrangular-elliptic in *V. album* subsp. *golestanicum*. The stomatal index and the type of stomata (paracytic) observed in our study confirm the previous results in *V. album* (Metcalfe and Chalk, 1957; Andronache et al., 2006).

The stomatal index in *V. album* subsp. *album* was two times greater than in *V. album* subsp. *golestanicum* (Table 2). It may be however related to the environmental factors. *Viscum album* subsp. *golestanicum* grows in dryer regions than the typical subspecies.

The results of petiole study are generally similar, however, the type of calcium oxalate crystals in cortex varies; the cubical and stellate crystals were observed in *V. album* subsp. *album*, while there were stellate crystals in *V. album* subsp. *golestanicum*; Khan et al. (2009) have not reported any crystal in petiole of *V. album*.

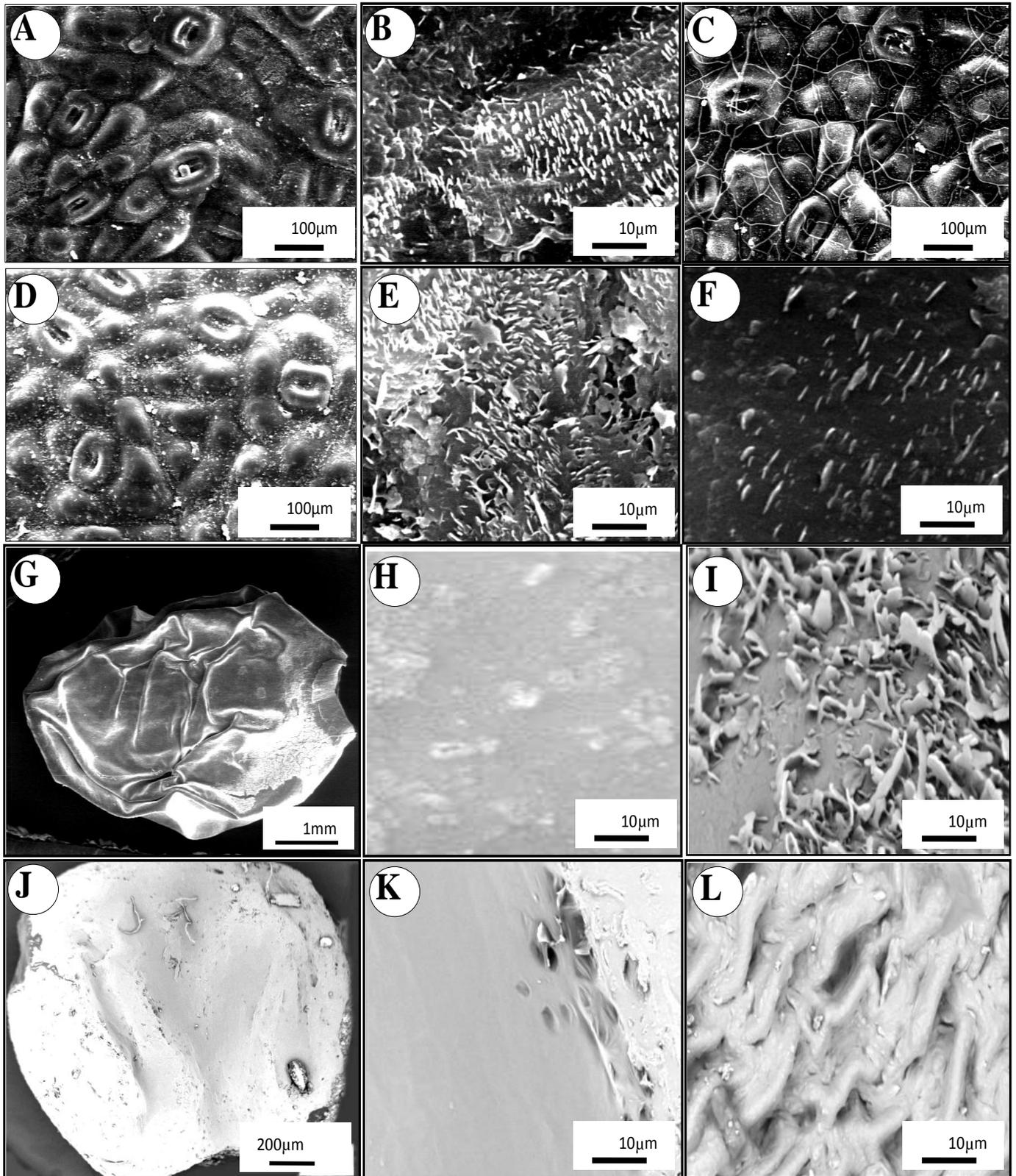


Figure 4. SEM photographs of *Viscum* subspecies. -Surface views of leaf cuticle: *V. album* subsp. *album*: A, B) adaxial cuticle, C) abaxial cuticle, *V. album* subsp. *goletanicum*: D, E) adaxial cuticle; F) abaxial cuticle. Fruit surface ornamentation: *V. album* subsp. *album*: G) pericarp surface, H) ultrastructure of pericarp surface; *V. album* subsp. *goletanicum*: I) ultrastructure of pericarp surface. Views of seed: *V. album* subsp. *album*: J) seed shape, K) smooth ornamentation; *V. album* subsp. *goletanicum*: L) wrinkle ornamentation.

Table 2. A comparison of the anatomical and micromorphological characters of *V. album* subsp. *album* and *V. album* subsp. *golestanicum*.

Character	<i>V. album</i> ssp. <i>album</i>	<i>V. album</i> ssp. <i>golestanicum</i>
Cross section shape	Circular, undulate and winged	Circular, undulate and winged
Cuticle diameter (μm) *	(36) 36.7 \pm 0.7 (37.5)	(25) 25.25 \pm 0.24 (25.5)
Epidermal cell shape	Triangular	Quadrangular
Epidermis diameter (μm) *	(30) 30 \pm 0 (30)	(29) 29.5 \pm 0.5 (30)
Parenchyma layer number	7	8
Stem		
Crystal type	Stellate, cubical and prismatic	Stellate, cubical
Cortex diameter (μm) *	(250) 250 \pm 0 (250)	(300) 327.5 \pm 2 (360)
Vascular fiber	+	+
Xylem fiber diameter (μm) *	(50) 57.5 \pm 2 (65)	(50) 55 \pm 2 (60)
Phleom fiber diameter (μm) *	(100) 105 \pm 2 (110)	(50) 75 \pm 2 (100)
Vascular bundle diameter (μm)*	(300) 317.5 \pm 2 (325)	(500) 562 \pm 2 (600)
Pith	+	+
Pith diameter (μm) *	(400) 400 \pm 0 (400)	(500) 505 \pm 2 (510)
Cuticle diameter (μm)*	(120) 122.5 \pm 2 (125)	(120) 122.5 \pm 2 (125)
Epidermal cell shape	Quadrangular	Quadrangular
Epidermis diameter (μm) *	(25) 25 \pm 0 (25)	(25) 25 \pm 0 (25)
Mesophyll type	Isobilateral	Isobilateral
Leaf		
Crystal type	Stellate, prismatic and cubical	Stellate
Stomata type	Paracytic	Paracytic
Vascular bundle diameter (μm) *	(250) 250 \pm 0 (250)	(220) 235 \pm 2 (250)
Lower surface stomatal index (mm^2)	89.3	42.8
Upper surface stomatal index (mm^2)	88.1	37.7
Stomata length to width ratio	2	2.7
Cross section shape	Elliptic and winged	Elliptic and winged
Cuticle diameter (μm) *	(20) 22.5 \pm 1.5 (25)	(12) 13.5 \pm 1.5 (15)
Epidermal cell shape	Elliptic	Elliptic
Epidermis diameter (μm) *	(37) 37.25 \pm 0.24 (37.5)	(25) 27.5 \pm 0.7 (30)
Petiole		
Crystal type	Stellate and cubical	Stellate
Vascular fiber	Phloem-Xylem fiber	Phloem-Xylem fiber
Vascular bundle number	5	5
Vascular bundle diameter (μm)*	(370) 405 \pm 2 (440)	(300) 312.5 \pm 2 (325)
Color	White	White
Shape	Globose	Globose
Surface sculpture	Smooth	Platelet
length \times Width (mm)*	(0.5) 0.67 \pm 0.1 (0.8) \times (0.3) 0.37 \pm 0.04 (0.4)	(0.6) 0.7 \pm 0.07 (0.8) \times (0.4) \pm 0.47 \pm 0.09 (0.6)
Fruit		
Epicarp thickness (μm)*	(25) 32 \pm 2 (40)	(25) 26 \pm 1.8 (30)
Mesocarp thickness (μm)*	(450) 462 \pm 1.5 (475)	(350) 425 \pm 1.5 (500)
Endocarp thickness (μm)*	(1000) 1125 \pm 1.8 (1250)	(1500) 1750 \pm 1.7 (2000)
Fiber	+	+
Crystal	Stellate and cubical	Stellate
Shape	Reniform	Elliptic
Seed		
Length \times Width (μm)*	(30) 32 \pm 0.04 (40) \times (25) 25 \pm 0.02 (27)	(40) 45 \pm 0.08 (50) \times (30) 42 \pm 0.04 (32)
Surface sculpture	Almost smooth	Wrinkle

*Numbers are (Min) mean value \pm standard deviation (Max).

The epicuticular waxes of leaves which could have potential taxonomic significance and are helpful as an additional taxonomic characters (Stace, 1965; Baronova,

1992) was another objectives of the present study. The platelet crystalloid microstructures were oriented parallel to each other and perpendicular to the surface in both

subspecies. This observation was similar to that reported in *V. album* subsp. *album* by Hass et al. (2003).

Fruit shape and its color were similar in both studied subspecies. However, the epicarp surface under SEM was considerably variable. The epicarp surface of *V. album* subsp. *album* is smooth, but in *V. album* subsp. *golestanicum* the pattern of epicuticular waxes is platelet crystalloid.

The importance of ultrastructure of seed surface, as a reliable approach for solving taxonomic problems has been well recognized (Heywood, 1971). The shape of the seeds in *V. album* subsp. *album* is elliptic, while it is reniform in *V. album* subsp. *golestanicum*. Furthermore, the seed surface in *V. album* subsp. *album* is smooth in *V. album* subsp. *album*, but wrinkled without distinct orientation in *V. album* subsp. *golestanicum*. The wrinkled seed surface with longitudinal orientation of walls was previously observed in other subspecies of *V. album* (Bojnansky and Fargasova, 2007).

In conclusion and based on data from several taxonomic resources it seems that two subspecies should be recognized in *Viscum album* in Iran: subsp. *album* and subsp. *golestanicum*.

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