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Fruit and seed micromorphology of the genus *Iberis* L. (Brassicaceae) in Turkey and its utility in taxonomic delimitation

Micromorfología de frutos y semillas del género *Iberis* L. (Brassicaceae) en Turquía y su utilidad en la delimitación taxonómica

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Abstract

Background: This paper investigates whether the fruit and seed micromorphology of eight Turkish *Iberis* (Brassicaceae) species can be used as a further aid in their taxonomic delimitation.

Questions: Are the micro-morphological characters of the fruits and seeds of *Iberis* useful to support the taxonomic delimitation of its species?

Studied species / data description /Mathematical model: The eight species of *Iberis* present in Turkey were examined. The fruit and seed characteristics were obtained and statistical analysis was performed using UPGMA.

Study site and dates: Fifteen localities in Turkey, from 2015 to 2019.

Methods: Samples of fruit and seed of eight species of *Iberis* were collected, stored, and later measured and described with aid of light and scanning electron microscopies.

Results: All eight species had different fruit and seed characters; particularly differing from each other with respect to fruit and seed size, seed shape and seed ornamentation. Two fruit ornamentation types were evident, smooth and striated. The seed coat surface were separated into three types: reticulate, reticulate-rugose and reticulate-papillate, being reticulate the most common. Further differences were noted for the seed surface epidermal structures, notably species had rectangular, tetragonal, or pentagonal patterning. An identification key was constructed based on a similarity analysis.

Conclusions: Fruit morphology, seed dimensions, colour, and epidermal cell patterning are useful microcharacters that enabled species-level determinations in the *Iberis* species sampled.

Keywords: candytufts, Iberideae, mustard family, numerical analysis, reticulate surface, taxonomy.

Resumen

Antecedentes: Este trabajo investiga si la micromorfología de frutos y semillas de ocho especies de *Iberis* turcas (Brassicaceae) puede usarse como apoyo adicional en la delimitación taxonómica de especies dentro de ese género.

Preguntas y / o Hipótesis: ¿Son de utilidad los caracteres micro-morfológicos de los frutos y semillas de género *Iberis* para apoyar la delimitación taxonómica de sus especies?

Especies de estudio / Descripción de datos / Modelo matemático: Se examinaron ocho especies de *Iberis* de Turquía. Se obtuvieron las características de frutos y semillas y UPGMA se empleó como método de agrupamiento.

Sitio y años de estudio: 15 localidades en Turquía, 2015 a 2019.

Métodos: Se recolectaron frutos y semillas de ocho especies de *Iberis*, se almacenaron y posteriormente se midieron y describieron con la ayuda de la microscopía óptica y electrónica de barrido.

Resultados: Las ocho especies tuvieron diferentes caracteres en los frutos y semillas; particularmente en el tamaño del fruto y la semilla, así como en la forma y ornamentación de la semilla. Hay dos tipos de ornamentación en los frutos, lisos y estriados. La superficie de la cubierta de la semilla tuvo tres tipos: reticulada, reticulada-rugosa y reticulada-papilada, de las cuales la más común fue la reticulada. Una clave de identificación se construyó con base en el análisis de similitud.

Conclusiones: La morfología del fruto, las dimensiones de la semilla, el color y el patrón de las células epidérmicas, son útiles para apoyar la delimitación taxonómica de las especies de *Iberis* estudiadas.

Palabras clave: análisis numérico, brasicáceas, Iberideae, superficie reticulada, taxonomía.

Brassicaceae comprises nearly 38 genera and 3,700 species (Warwick *et al*. 2006) that includes *Arabidopsis thaliana* (L.) Heynh. used as the flowering plant model system (Mühlhausen *et al*. 2013). In Turkey, the Brassicaceae is represented by 91 genera and 686 species (Güner *et al*. 2012). The genus *Iberis* is represented by nine species in Turkey (Mutlu 2012, Oskay 2017, Citak 2019, Citak & Crespo 2019), that are widely distributed, especially across middle Anatolia.

The fruit and seeds of plants provide a raft of morphological features that have the taxonomic potential to delimit taxa (Kaya *et al*. 2011). Following the advent of scanning electron microscopy (SEM) with the ability to produce high-resolution systems imagery, the use of ultrastructural characters to analyse fruit and seed characters has increased significantly in taxonomic studies (Heywood 1971, Kaya *et al*. 2011). For decades, micromorphological characters have been of crucial importance in detecting taxonomic and phylogenetic relationships of particular plant groups and have been successfully used in the Brassicaceae (Khalik & van der Maesen 2002, Pinar *et al*. 2007, Atçeken *et al*. 2016, Karaismailoğlu & Erol 2018, Kaya *et al*. 2019). The distinctive morphological characters of fruit and seed in *Iberis* are valuable for species delimitation (Hedge 1965). However, there has been no taxonomic research conducted on the fruit and seed structures of the genus *Iberis* in Turkey. The fruit and seed coat morphological features of *Iberis* species growing in Turkey have not yet been correctly stood out to indicate the importance of these characters in the taxonomy and identification of Turkish *Iberis* species. Thus, the main aims of this study were to 1) identify and examine the fruit and seed characteristics of Turkish *Iberis* species and 2) elucidate the systematic value of the micromorphological traits via numerical analysis.

Materials and methods

Species sampled. The fruit and seed samples used in this study were collected between 2015 and 2019 from 15 localities in Turkey. The collection data is given in Table 1. The plant specimens of the studied species were housed in the herbarium of Department of Biology, University of Selcuk (KNYA).

Microscopic investigations. The macro- and micro characters of at least 20 or more fruits and seeds per taxon were analysed using a stereo microscope. For the SEM mature and dry fruits and seeds were placed on the double sided tape and then sputter coated with gold for five minutes, and observed under a Zeiss Evo LS10 SEM (Carl Zeiss Microscopy GmbH, Jena, Germany).

Terminology. The fruit and seed terminology used here is adapted from Koch *et al*. (2009), Pinar *et al*. (2009), Mühlhausen *et al*. (2013) and Ghaempanah *et al*. (2013).

Statistical analysis. The qualitative and quantitative characters were coded for the numerical analysis to evaluate the similarity relationships of the Turkish *Iberis* species.

Table 1. Location data of the investigated taxa of *Iberis*.

Taxa	Location	Collector number
I. carica Bornm.*	C2 Muğla: Marmaris, 130 m, 25.05.2019	B. Çıtak-345
I. carnosa Willd.	C2 Muğla: Köyceğiz, 100 m., 25.05.2019	B.Çıtak-345-a
	B5 Nevşehir: Ortahisar, 1,300 m, 17.05.2015	B.Çıtak-167-a
	C5 Adana:Pozantı, Horozköy, 1,030 m, 22.05.2018	B.Çıtak-339
I. halophila Vural & H. Duman*	C4 Konya: Cihanbeyli, The Salt Lake, 920-950 m, 19.05.2018	B.Çıtak-335-a
	C4 Aksaray: Eskil, The Salt Lake, 920-950 m, 19.05.2017	B.Çıtak-335-b
I. odorata L.	C6 Kahramanmaraş: Pazarcık, 800 m. 23.04.2018	B. Çıtak-334
I. saxatilis subsp. *magnesiana* Oskay*	B1 Manisa: Soma, 1,000 m, 26.05.2018	B.Çıtak-337
I. saxatilis L. subsp. *saxatilis*	B1 Balıkesir: Edremit, Kaz Dağı, 1,600 m, 25.05.2018	B.Çıtak-336
I. sempervirens L.	C4 Konya: Beyşehir, Dumanlı Mountain, 1,800 m, 07.06.2018	B.Çıtak-340
	C3 Antalya: Akseki, Atlarkırı Mountain, 2,100 m, 04.06.2019	B. Çıtak-348
	C3 Antalya: Akseki, Ürküden Mountain, 2,200 m, 04.06.2019	B. Çıtak-349
I. simplex DC.	B5 Nevşehir, Ürgüp-Göreme road, Akdağ, 1,300 m, 29.05.2016	B.Çıtak-180
	C4 Konya, Altınapa-Başarakavak road, 1,380 m, 16.06.2017	B.Çıtak-315
	C5 Adana: Pozantı, 920 m., 22.05.2018	B. Çıtak-332

*endemic taxa

Characters are listed in Table 2. For the multivariate analysis, a primary matrix was created for the eight taxa using twelve characters. The clustering analysis was based on Gower's (1971) general coefficient similarity, which can be used directly with a mixture of character types (binary, qualitative, and quantitative characters). The UPGMA was selected because it produces an accurate reflection similarity matrix, as measured by the cophenetic correlation coefficient of Sokal & Rohlf (1962) and symmetrical hierarchical structure (Sokal & Rohlf 1962, McNeill 1979), and has congruence with the classification derived by traditional methods (Ward 1993). Untransformed, not centered and standardised data were used to create a covariance matrix (Baldemir *et al*. 2018). MVSP 3.22 software was used for all of the computations.

Results

Fruit micromorphology. The fruit and seed exomorphological characters (colour, shape, size, and surface) were evaluated. The fruit colours were observed as green, purple and yellow (Figure 1, Table 2). The fruit shapes were ovate and ovate-orbicular without wing in examined species. The end of the wing varied from acute to obtuse. The greatest wing width was observed in *I. odorata* (Figure 1 G), while the least was in *I. simplex* (Figure 1 F). The smallest fruit was observed in *I. saxatilis* subsp. *saxatilis* (mean value: 4.69 mm; Figure 1 C) and the greatest was in *I. sempervirens* (mean value: 8.03 mm; Figure 1A, Table 2). Fruit surface are determined as striated and smooth among the examined species (Table 2, Figures 2, 3). The fruit of *I. halophila*, *I. carnosa* and *I. carica* have a distinct striated ornamentation (Figures 2 B, E-H). The remaining species have smooth ornamentation (Figures 2 A, C, D, F, G).

Seed micromophology. With regards to the shapes of the seeds of the *Iberis* species, four types were determined: orbicular, broadly ovate, ovate, and elliptic (Table 2). Orbicular is characteristic only in *I. sempervirens* (Figure 3 A) and ovate only in *I. saxatilis* subsp. *saxatilis* (Figure 3 C). *I. halophila*, *I. saxatilis* subsp. *magnesiana*, *I. carnosa* and *I. simplex* have broadly-ovate seeds. Otherwise, *I. carica* and *I. odorata* have elliptic shaped seeds. The seed size is ranged from 2.08 mm to 3.69 mm in length and 1.37 mm to 2.52 mm in width (Table 2). The biggest seeds were observed in *I. sempervirens*, while the smallest were observed in *I. carica* (Figure 3 H). The seed colour was yellow in *I. saxatilis* subsp. *magnesiana* (Figure 3 D) and *I. carica*. Otherwise, *I. saxatilis* subsp. *saxatilis*, *I. simplex* and *I. odorata* have light brown-yellow coloured seeds. Additionally, *I. sempervirens* has light brown and *I. carnosa* has brownish-black coloured seeds and *I. halophila* brown seeds. The surfaces of the *Iberis* species were determined as reticulate-papillate in *I. simplex* (Figure 4 K, L), reticulate-rugose in *I. odorata* (Figure 4 M, N) and reticulate in remaining species (Table 2). Epidermal cell varied among the species, as tetragonal-elongated in

Table 2. The fruit and seed characteristics of examined Turkish *Iberis* species.1-L×W (mm) for fruit; 2- Fruit shape (ovate = o, ovate-orbicular = o-or); 3- Fruit colour (P-g = Purple-green; G-y = Green yellow); 4- The width of wing (mm); 5- Fruit surface (smooth = sm, striated = st); 6- L×W (mm) for seed; 7- Seed shape (orbicular = or, broadly ovate = bo, ovate = o, elliptic = e); 8- Seed colour (light brown = lb, brown = b, light brown-yellow lby, brownish-black = b-b, yellow = y); 9- Seed surface (reticulate = r, reticulate-papillose = r-p, reticulate-rugose = r-r); 10- Cell types of seed surface TE = tetragonal, TE-E tetragonal-elongated, TE-E & P, tetragonal-elongated & polygonal); 11- Anticlinal walls (not distinct = nd, distinct, straight, exposed = dse, distinct, straight, sunken = dss); 12- Outer periclinal wall (convex = cx, concave = co).

Taxa	1	2	3	4	5	6	7	8	9	10	11	12
I. carica	5.30 × 4.61	o	P-g	1.36	st	2.08 × 1.49	e	y	r	te-e	dse	co
I. carnosa	5.97 × 4.66	o-or	P-g	1.5	st	2.86 × 1.83	bo	b-b	r	te & p	dse	co
I. halophila	6.38 × 5.05	o	P-g	1.35	st	3.09 × 1.97	bo	b	r	te-e	dse	cx
I. odorata	6.44 × 5.74	o	G-y	0.74	sm	2.57 × 1.79	e	lb-y	r-r	te & p	dss	cx
I. saxatilis subsp. *saxatilis*	4.69 × 3.77	o	P-g	0.59	sm	2.49 × 1.37	o	lb-y	r	te-e	dse	co
I. saxatilis subsp. *magnesiana*	6.11 × 5.76	o-or	P-g	0.73	sm	3.19 × 2.36	bo	y	r	te-e & p	dss	cx
I. sempervirens	8.03 × 6.67	o	G-y	1.42	sm	3.69 × 2.52	or	lb	r	te	nd	co
I. simplex	4.85 × 5.04	o	G-y	0.85	sm	2.84 × 1.89	bo	lb-y	r-p	te-p	dss	cx

I. halophila (Figure 4 D), *I. saxatilis* subsp. *saxatilis* (Figure 4 F), *I. carica* (Figure 4 P), tetragonal-polygonal in *I. simplex* (Figure 4 L), tetragonal and polygonal in *I. carnosa* (Figure 4 J) and *I. odorata* (Figure 4 N), tetragonal-elongated and polygonal in *I. saxatilis* subsp. *magnesiana* (Figure 4 H), tetragonal in *I. sempervirens* (Figure 4 B).The anticlinal cell walls were determined as distinct, straight and exposed in *I. halophila*, *I. saxatilis* subsp. *saxatilis*, *I. carnosa*, and *I. carica* (Figure 4 D, F, I, P), distinct, straight and sunken in *I. saxatilis* subsp. magnesiana, *I. simplex*, and *I. odorata* (Figure 4 H, L, M) and not distinct in *I. sempervirens* (Figure 4 A). *I. saxatilis* subsp. *magnesiana* had a wax plate that differ from other examined *Iberis* species (Figure 4 H).The outer periclinal cell walls were observed as convex in *I. halophila*, *I. saxatilis* subsp. *magnesiana*, *I. simplex*, and *I. odorata*, concave in *I. saxatilis* subsp. *saxatilis*, *I. carnosa I. carica* and *I. sempervirens*.

Numerical analysis of the fruit and seed morphology. The dendrogram is presented in Figure 5. This dendrogram revealed two main groups plus *I. sempervirens*. Group A comprised five taxa with 71 % similarity and Group B included *I. carica* and *I. simplex* with 78 % similarity. Notably, *I. saxatilis* subsp. *saxatilis* and *I. saxatilis* subsp. *magnesiana* belong to different subgroups. *I. saxatilis* subsp. *magnesiana* and *I. carnosa* had the highest value of similarity (87 % similarity).

Key to species on the basis of fruit and seed morphology

1a:The fruit colour green-yellow	2a
1b: The fruit colour purple-green	4a
2a: The fruit length less than 5 mm	*I. simplex*
2b: The fruit length more than 5 mm	3a
3a: The fruit surface smooth	*I. sempervirens*
3b: The fruit surface striated	*I. odorata*
4a: The fruit shape ovate-orbicular	5a
4b: The fruit shape ovate	6a
5a: The fruit surface smooth, the seed colour yellow, the anticlines distinct straight and sunken	*I. saxatilis* subsp. *magnesiana*
5b: The fruit surface striated, the seed colour brown-black, the anticlines distinct straight and exposed	*I. carnosa*
6a: The fruit surface striated, the seed broadly ovate	*I. halophila*
6b: The fruit surface smooth, the seed shape ovate or elliptic	7a
7a: The seed shape ovate and light brown-yellow colour	*I. saxatilis* subsp. *saxatilis*
7b: The seed shape elliptic and yellow colour	*I. carica*

Discussion

This study examined the utility of using the fruit and seed micromorphology of *Iberis* species in Turkey. The sculpturing of surfaces has been used for problems in taxonomy to observe relationships of species in Brassicaceae (Khalık & van der Maesen 2002, Khalik *et al.* 2002, Pinar *et al*. 2007, Atçeken *et al*. 2016, Karaismailoğlu & Erol 2018, Karaismailoğlu 2019, Gönen *et al*. 2019). The outcomes showed that the seed micromorphology was more useful than fruit micromorphology to separate *Iberis* taxa in Turkey.

The fruit morphology of the *Iberis* species investigated included characters believed essential for species level distinction. Gabr (2018) had a detailed study of the fruit micromorphology in the Brassicaceae family. He stated that the fruit characters such as fruit shape, surface, and beak traits separated the family into tribes and groups. In *Iberis*, the fruit size and colour were good characters (Table 2). The largest fruits were determined in *I. sempervirens*. The fruit colour of *I. halophila*, *I. saxatilis* subsp. *saxatilis*, *I. saxatilis* subsp. *magnesiana*, *I. carnosa* and *I. odorata* was observed as purple-green. The fruit colour of *I. sempervirens*, *I. simplex* and *I. carica* was observed as green-yellow. When considering the surface ornamentation of the fruit, two surface sculptures were determined, smooth and striated. Three species, *I. halophila*, *I. carnosa* and *I. odorata*, had striated ornamentation on their fruit surface, while the remaining species had smooth surface sculpturing.

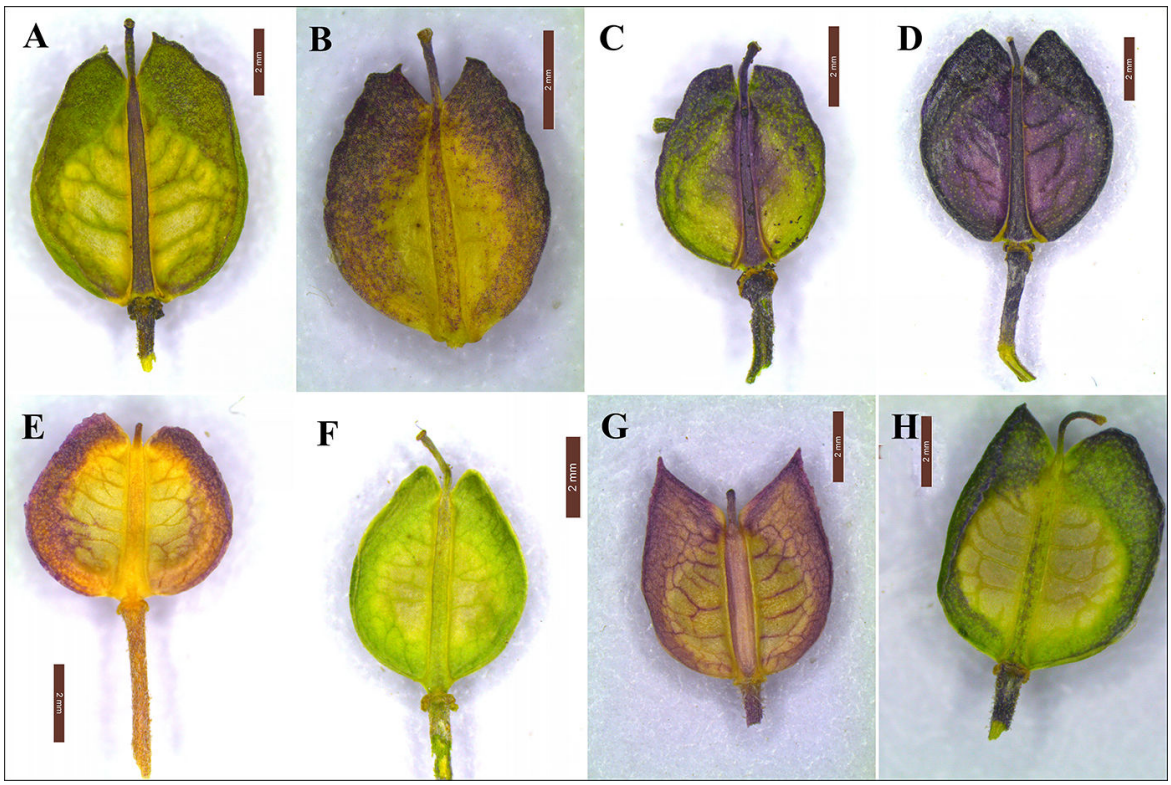

Figure 1. The fruits of Turkish *Iberis* species (LM). A) *I. sempervirens* B) *I. halophila* C) *I. saxatilis* subsp. *saxatilis* D) *I. saxatilis* subsp. *magnesiana* E) *I. carnosa* F) *I. simplex* G) *I. odorata* H)*I. carica* (Scale bars = 2 mm).

The fruit morphological characteristics just mentioned were found to be valuable to distinguish among species. These characters are presented here for the first time for the taxa of *Iberis* of Turkey and were important to construct the identification key.

The macro- and micromorphology of the seeds were determined as the most distinctive characters for distinguishing the eight *Iberis* taxa. The seed colour and size, and ornamentation provided great potential for distinguishing seed morphological variations at species level as in previous studies (Barthlott 1984, Khalik & van der Maesen 2002, Pinar *et al*. 2007, 2009, Ghaempanah *et al*. 2013, Atçeken *et al*. 2016, Karaismailoğlu & Erol 2018, Karaismailoğlu 2019, Gönen *et al*. 2019). In the current study, the light brown-yellow seed colour in *I. saxatilis* subsp. *saxatilis*, *I. simplex* and *I. odorata*, light brown in *I. sempervirens*, brown in *I. halophila*, brown-black in *I. carnosa*, yellow in *I. saxatilis* subsp. *magnesiana*, and *I. carica* were observed (Table 2). These seed colour variation was described as a new character for *Iberis* taxa in the present study.

The features of the seed coat, such as surface sculpturing, anticlinal and periclinal wall cells, and epidermal cell shape have been determined as useful in the delimitation of taxa within some genera in the family Brassicaceae (Moazzeni *et al*. 2007, Atçeken *et al*. 2016, Gabr 2018, Karaismailoğlu & Erol 2018, Karaismailoğlu 2019, Gönen *et al*. 2019). In this research, the seed coat ornamentation, which was reticulate, reticulate-rugose and reticulate-papillate can be considered essential diagnostic features at species level. For example, reticulate-rugose seed coat is distinctive in *I. odorata*, while reticulate-papillate is unique in *I. simplex*. In Brassicaceae, reticulate surface ornamentation is the most common character at the generic level (Zeng *et al*. 2004, Moazzeni *et al*. 2007, Atçeken *et al*. 2016, Karaismailoğlu & Erol 2018, Karaismailoğlu 2019, Gönen *et al*. 2019) as it was confirmed since six of the eight taxa studied show reticulate seed coat. In taxonomy, epidermal cell shape on the seed surface is important at generic and also subgeneric level in the family Brassicaceae (Karaismailoğlu 2019). These seed characteristics showed great variation in the *Iberis* species examined. In the examined species, the epidermal cells were tetragonal-elongated with anticlines distinct, straight and exposed in *I. halophila*, *I. saxatilis* subsp. *saxatilis* and *I. carica*; tetragonal-polygonal in *I. carnosa* and *I. odorata*; tetragonal and polygonal in *I. simplex*; tetragonal with anticlines nondistinct in *I. sempervirens*; and tetragonal-elongated and polygonal with anticlines distinct, straight and sunken in *I. saxatilis* subsp. *magnesiana*. With this study, we confirmed that the epidermal traits of the seed could be a good taxonomic character to separate *Iberis* species.

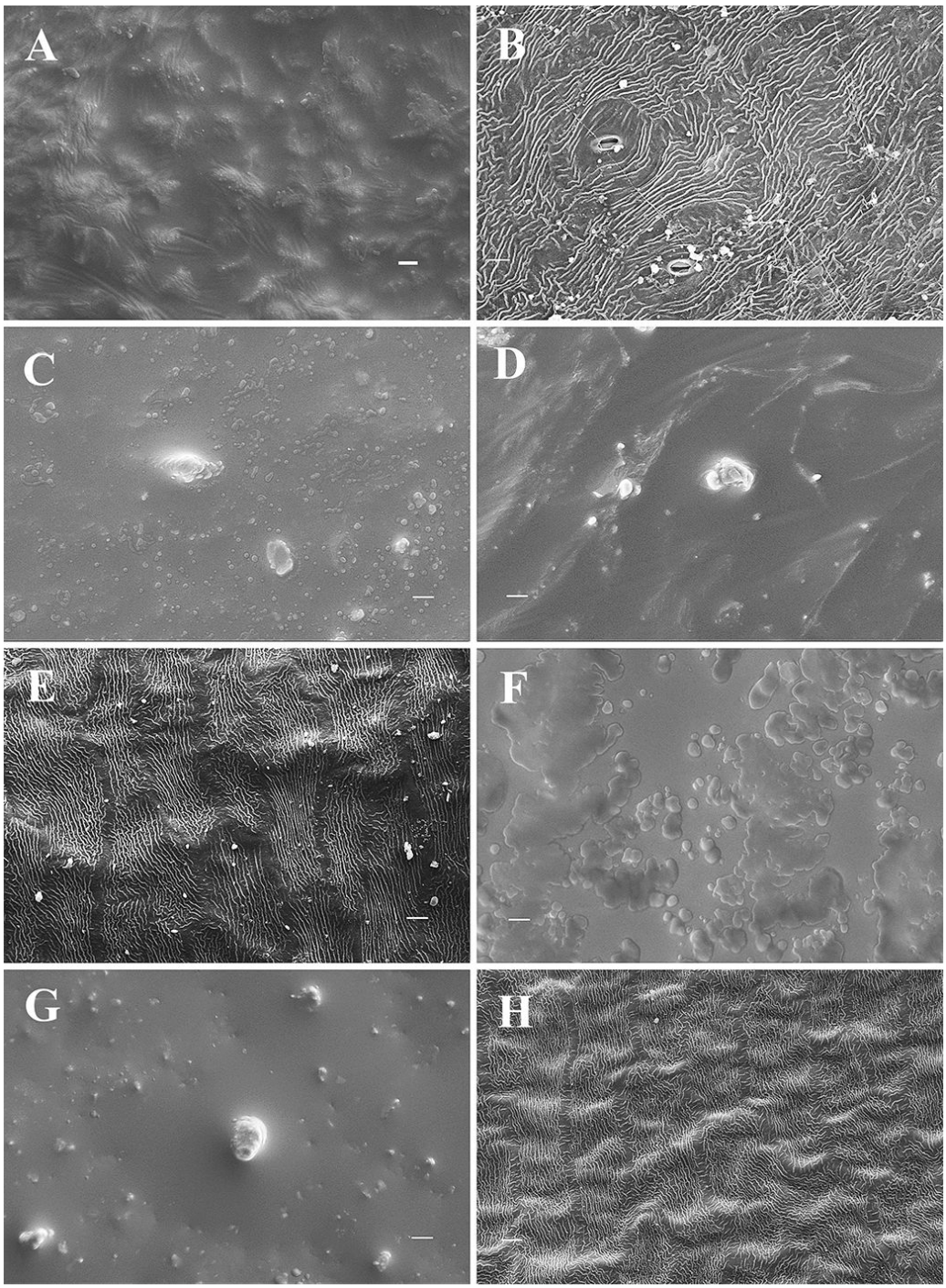

Figure 2. The fruit surface of Turkish *Iberis* species (SEM). A) *I. sempervirens* B) *I. halophila* C) *I. saxatilis* subsp. *saxatilis* D) *I. saxatilis* subsp. *magnesiana* E) *I. carnosa* F) *I. simplex* G) *I. odorata* H) *I. carica* (Scale bars = 10 µm).

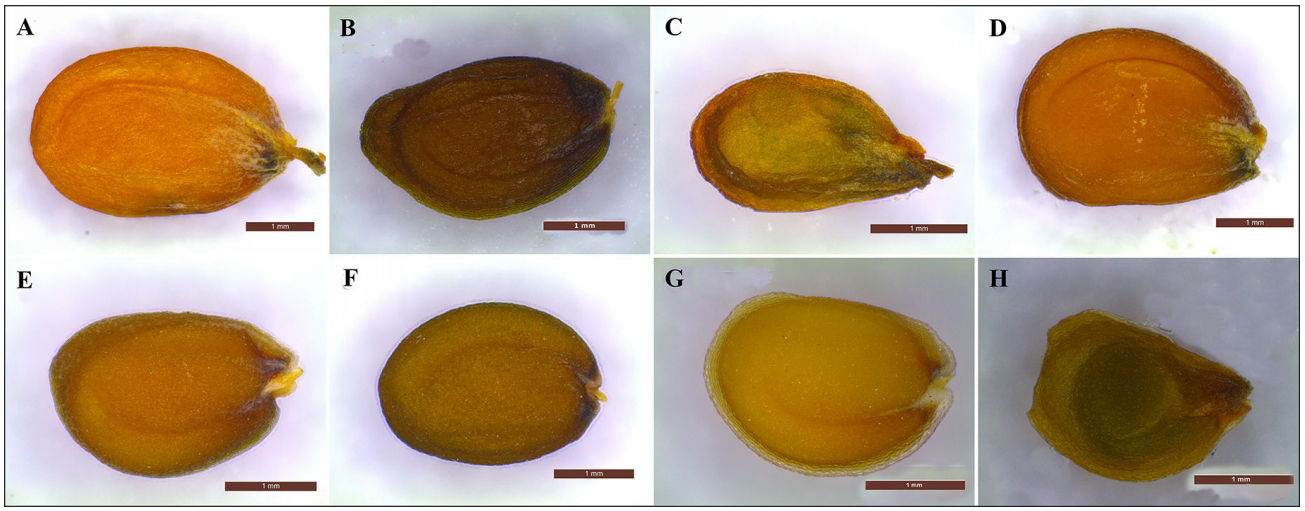

Figure 3. The light microscope seeds of *Iberis* species. A) *I. sempervirens* B) *I. halophila* C) *I. saxatilis* subsp. *saxatilis* D) *I. saxatilis* subsp. *magnesiana* E) *I. carnosa* F) *I. simplex* G) *I. odorata* H) *I. carica* (Scale bars = 1 mm).

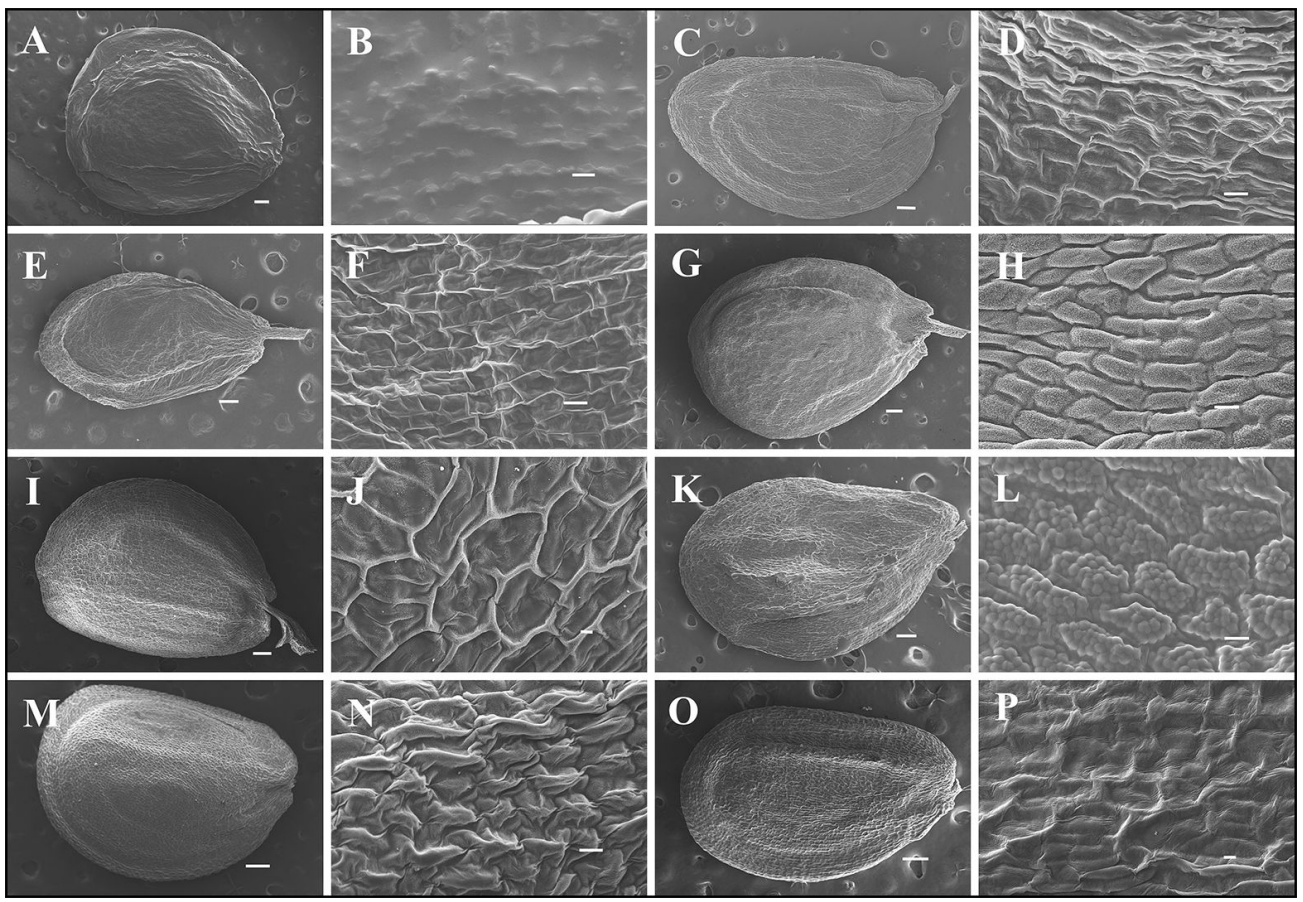

Figure 4. The seed surface of *Iberis* species (SEM). A-B) *I. sempervirens* C-D) *I. halophila* E-F) *I. saxatilis* subsp. *saxatilis* G-H) *I. saxatilis* subsp. *magnesiana* I-J) *I. carnosa* K-L) *I. simplex* M-N) *I. odorata* O-P) *I. carica* (Scale bars 200 µm in A,C,E,G,I, K,M,O; 20 µm in B,D,F,H,J,L,N,P)

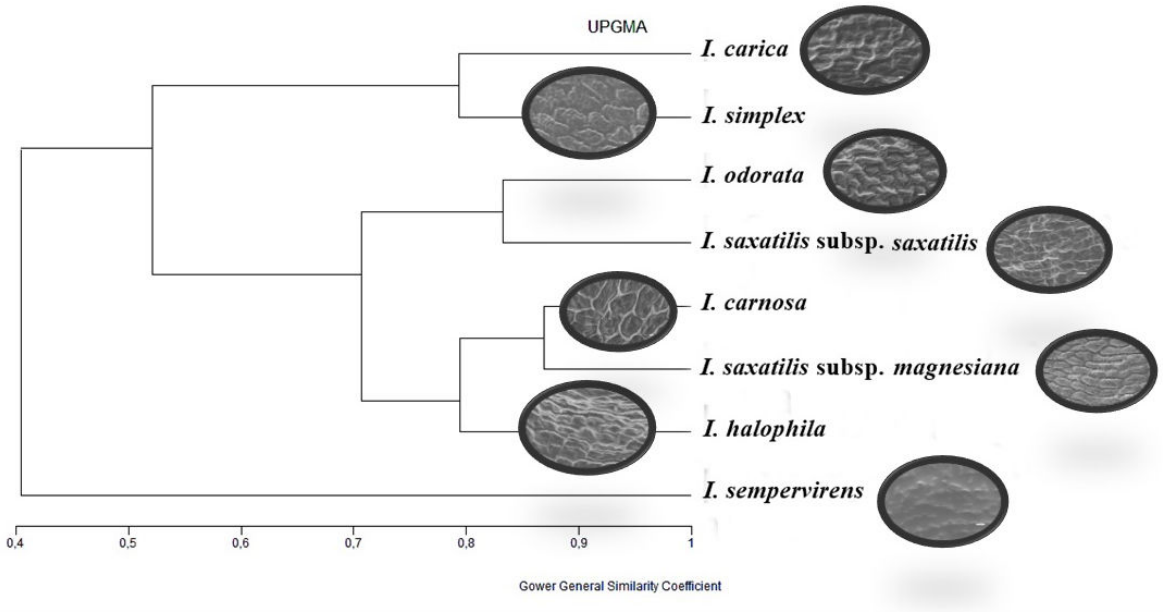

Figure 5. The dendogram indicating the similarity distance of Turkish *Iberis* species based on fruit and seed features.

The results from cluster analysis show that the examined species of *Iberis* that fall into two main groups coincide with fruit and seed morphology (Figure 5). According to UPGMA analysis based on fruit and seed morphological data, each species was distinctly separated from each other. *Iberis saxatilis* subsp. *saxatilis* and *I. saxatilis* subsp. *magnesiana* are in different groups. The two subspecies can be easily separated from each other by the retrorsely setulose stem indumentum in *I. saxatilis* subsp. *magnesiana*, while the stem of *I. saxatilis* subsp. s*axatilis* is glabrous and now several seed features of the anticlines and the outer periclinal walls also support their recognition. Additionally, *Iberis sempervirens* is a semi-shrub plant that has no close relatives in Turkish *Iberis* species and has the most distinct fruits and seeds of the species studied. These results were congruent with the palynomorphological analysis of Turkish *Iberis* species by Citak (2019). Seed length and width, colour of seed, fruit colour, and seed ornamentation are the most valuable variables for separating *Iberis* species. In further studies we predict that the systematic problems of the genus *Iberis* will be solved by providing anatomical, morphological and more molecular studies.

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