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THE COMPARATIVE MORPHOLOGICAL, ANATOMICAL AND PALYNOLOGICAL STUDIES ON THE GENUS *HELLEBORUS* (RANUNCULACEAE) GROWING IN TURKEY

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Abstract

In the present study, the morphological, anatomical, and palynological *characteristics* of *H. orientalis* Lam. and *H. vesicarius* Aucher (endemic) were studied and compared. The root structures, branching shape, the distance between internodes, basal and stem leave dimensions, detailed descriptions of leaves, detailed new morphological characters of flowers and sepals were added to plant descriptions. Their anatomical structures were visualized under a light microscope and compared for the first time with detailed micro-anatomical measurements. Pollen images were shown for the first time by using the Scanning Electron Microscopy. It was found that the pollen types were tricolpate and tricolporate (rarely tricolpate), their shapes were subprolate and prolate-spheroidal, and their ornamentations were reticulate and microreticulate-foveolate.

Key words: Ranunculaceae; *Helleborus*; Morphology; Anatomy; Palynology.

Introduction

Helleborus genus, which is included in the Ranunculaceae family, consists of 22 taxa. Taxa of this genus grown in some parts of Europe and Western Asia (Tutin, 1964; Nowicke & Skvarla, 1983; Gabryszewska, 2016). The genus has two species growing in Turkey, namely *H.orientalis* Lam. is widely spread in the Northern part of Turkey while *H. vesicarius* Aucher is endemic plant in South Anatolia (Kusmenoglu *et al*., 1995; Davis, 1965).

Helleborus is easily recognised and not readily mistakable for other genera. It is characterised by a fruit consisting of clustered follicles, multiovular carpels and the persistence of the perianth segments throughout the life of the flower (McLewin & Mathew, 1995; Sun *et al*., 2001). The taxa belonging to the genus are all rhizomatous, and sympodial rhizomes with long-stalked basal leaves and individual flowers in a terminal producing area with advanced sympodial branching with leaves and leaves and stiff branches. The flowers have five large sepals arranged asymmetrically until the fruit is formed. The inner whorl consists of ≤ 32 tubular nectaries (modified petals), numerous (variable in number) stamens, and 2-10 free or slightly basally fused carpels, which develop into a group of several-seeded follicles (Mathew, 1989). *Helleborus* pollen is tricolpate and has a finely or coarsely reticulate tectum and is easily distinguished from that of other genera of Ranunculaceae, the vast majority of which have a spinulose and punctate/perforate tectum (Nowicke and Skvarla, 1983).

In literature, although some species of *Helleborus* have been widely researched and reported, such as chromosomes (Omduff, 1967;Strid, 1981; Yuan & Peng, 1987, etc.); palynology (Nowicke & Skvarla, 1983; Savitsky, 1989), embryology (Ba, 1974), phytochemistry (Colombo *et al*., 1991) further evidence is needed, especially molecular information, to resolve aspects of taxonomy, phylogeny, and biogeography (McLewin & Mathew, 1995). In addition to these, Sezer, Öztürk, Tanker, and Bingöl reported morphological, anatomical, and palynological investigation on the *Helleborus* from Turkey (Sezer, 1985; Öztürk, 2020; Tanker & Bingöl, 1984).

In this paper, the comparative morphological, anatomical, and palynological characteristics on the genus *Helleborus* growing in Turkey were investigated. It is expected that it will provide important information to the taxonomic studies as a monograph and will contribute to further investigation in the future.

Materials and Methods

Plant material: The plant specimens were collected from natural habitats of Sinop and Hatay, Turkey in 2020 (Table 1). For morphological studies, 20 samples were collected from each taxon.

Morphological studies: The taxonomic identification of the plant was made using the flora of Turkey (Davis, 1965). The morphological and morphometrical characters are presented in (Table 2). Forty-one morphological characters (9 quantitative and 33 qualitative) were selected and measured for morphometric analysis.

Table 1. Taxa used for studies and localities of specimen collection.

Taxa	Collection areas and collector's number
H. orientalis	Turkey, A5, Sinop: Bostancılı village, roadside, 20m, 15.03.2021, ÖE-1165
H. vesicarius	Turkey, C5, Hatay: Samandağ, roadside, 600m, 25.03.2021, ÖE-1170

Table 2. Comparative morphology of *H. orientalis* and *H. vesicarius*.

Characteristics		*Helleborus orientalis*		*Helleborus vesicarius*	
		Flora of Turkey	**In our study**	**Flora of Turkey**	**In our study**
Way of life		Perennial, herbaceous	Perennial, herbaceous	Perennial, herbaceous or suffruticose	Perennial, herbaceous or suffruticose
Throughout the plant		*	45-65cm	*	26-55cm
The root type		*	Taproot	*	Taproot
Basal leaves	Width-length	*	5-6 x 9-19mm	*	1-2 x 10-45mm
	Petiolate length	Long-petiolate *	Long-petiolate 12-23cm	*	Long-petiolate 16-28cm
	Leaf shapes	Narrowly elliptic	Narrowly elliptic	Lanceolate to narrowly elliptic	Lanceolate to narrowly elliptic
	Leaf apices	*	Obtuse	*	Obtuse
	Leaf margins	Coarsely toothed segments	Coarsely toothed segments	*	Entire
	Leaf base	*	Obtuse	*	Obtuse
	Shapes of the upper facial hairs	*	Pilose	*	Not seen facial hairs
	Shapes of the lower facial hairs	Glabrescent to shortly pilose	Shortly pilose	*	Not seen facial hairs
	Leaf attachment	*	Semi-amplexicaule	*	Semi-amplexicaule
	Leaf venation	*	Branched	*	Branched
	Leaf colours	Evergreen	Evergreen	Evergreen	Evergreen
Stem	Forms	*	Erect	*	Erect
	Lenght	Up to 60cm	Up to 45-65cm	Up to 60cm or more	Up to 57 cm or more
	Branching	*	Not branched	*	Branched
	Hair condition	*	Glabrous	Glabrous	Glabrous
	Length of internodes	*	*	*	2-5cm
Cauline leaves	Lenght	-	-	*	8-10 x16-22cm
	Leaf shapes	-	-	*	Linear
	Leaf margins	-	-	*	Entire
	Leaf apices	-	-	*	Acute
	Shapes of the upper facial hairs	-	-	*	Lanate
	Shapes of the lower facial hairs	-	-	*	Lanate
	Leaf attachment	-	-	*	Decurrent
	Color	-	-	*	Grayish-green
Flowers	Flower shapes	*	Capitulum	*	Capitulum
	State flowers of capitulum	*	Loose corymbose	*	Lanate corymbs
	Piece of flowers	*	3-5	*	8-10
	Length of flowers	*	18-30mm	*	17-25mm
	Color	*	Greenish yellow	*	Greenish to purple
Sepals	Lenght	20-30mm	5-11x15-26mm	16-18mm	10-14 x 15-20 mm
	Shapes	Ovate	Ovate to lanceolate	*	Ovate
	Base	*	Rounded	*	Rotundate
	Margins	*	Entire	*	Entire
	Apices	*	Acute to Apiculate	*	Rotundate to obtuse
	Shapes of the upper facial hairs	*	Tomentose	*	Glabrescent to shortly pilose of leaf apices
	Shapes of the lower facial hairs	*	Tomentose	*	Glabrescent to shortly pilose of leaf apices
	Colour	Greenish, sometimes fading purplish	Greenish, sometimes fading purplish	Green to greenish yellow, tipped purple or brownish purple	Green to greenish yellow, tipped purple or brownish purple
	Arrangement	*	Sepals-free	*	Sepals-free

*: Not in the Flora of Turkey,-: Not available

Anatomical studies: Anatomical studies were carried out on specimens kept in 70% alcohol. The paraffin method was used for the sections of the leaves and stems. The specimens were embedded in the paraffin wax and then sectioned with a SAKURA, SRM 200 rotary microtome. All sections were stained with Safranin and Fast Green and then mounted with Entellan (Johansen, 1944; Kahraman *et al*., 2009). A Leica DM 2500 binocular light microscope with a Leica DFC450 camera was used for anatomical measurements and photographs. Fifty measurements for each anatomical character were made.

Palynological studies: For palynological investigations, pollen material was obtained from dried flower specimens. The Wodehouse technique was used while preparing the pollen preparations (Wodehouse, 1935). Measurements and observations were made using the Leica DM 2500 binocular light microscope with a Leica DFC450 camera. The values of P (polar axis length), E (equatorial diameter), Clg (Colpus longitude [length]), Clt (Colpus latitude [width]), Plg (pore width, Plt (pore length), Ex (Exine thickness) and in (Intine thickness) were measured and the P/E ratio was calculated. Plant pollen grains were observed and photographed using the Zeiss EVO40 scanning electron microscope. Pollen terminology of Faegri and Iverson (Faegri *et al*., 1975) has been used (Kahraman *et al*., 2009).

Results

Morphological characteristics: *H. orientalis* plants are perennial herbs with a height of 45-65cm and have taproot with stems up to 45-65 cm, without branches and glabrous. Basal leaves are 5-6x9-19mm with long petiolate (12-23cm), apices obtuse, margins with coarsely toothed segments, leaf base obtuse, shapes of the upper and lower facial hairs shortly pilose, semi-amplexicaule, branched, and evergreen. Flower status is capitulum, loose corymbose, piece of flowers is 3-5, length of flowers is 18-30mm with greenish yellow color. Sepals are 5-11x15-26mm, ovate-to-lanceolate, base rounded, margins entire, apices acute-to-apiculate, shapes of the upper and lower facial hairs tomentose, sepals are free 5-11x15-26mm and ovate-to-lanceolate (Table 2).

H.vesicarius plants are perennial, herbaceous or suffruticose, throughout the plant 45-65cm, taproot. Stems are up to 57cm or more, below-branched, glabrous, and the length of internodes is 2-5cm. Basal leaves are 1-2x10-45mm with long petiolate (16-28cm), apices obtuse, margins entire, base obtuse, semi-amplexicaule, branched, and evergreen. Cauline leaves are 8-10x16-22cm, shapes are linear, margins entire, apices acute, shapes of the upper and lower facial hairs lanate and shortly pilose, decurrent, and grayish-green. Flower shape is capitulum, lanate, corymb, piece of flowers is 8-10, length of flowers is 17-25mm, and are greenish to purple in color. Sepals are 10-14x15-20mm, ovate, leaf base is rotundate, margins entire, apices rotundate-to-obtuse, shapes of the upper and lower facial hairs are glabrescent to shortly pilose in leaf apices, and sepals are free (Table 2).

Anatomical characteristics: ***H. orientalis***: Some fragmentations are detected in the epidermis and cuticle layer in the cross-section taken from the root of the plant. The arrangement of the cells is circular. Under the epidermis, there are endodermis cells arranged in a single row and are larger than the epidermis cells. The cortex cells between the endodermis layers consist of an average of 13-14 rows of cells. Cortex cells are close to each other in size and partly angular. There are very few spaces among the cells and starch grains are seen in the cells. There are pericyclic cells arranged in a single row smaller than the cortex cells around the xylem and phloem. The xylem consists of 3 branches, trachea, and tracheid cells (Fig. 1).

There is a very thin outermost cuticle layer, under which there is a single row of epidermis cells arranged regularly and smaller than the cortex cells in the cross-section taken from the stem of the plant. There are large gaps in some parts of the cortex layer consisting of an average of 20 rows of cells. Xylem and phloem bundles are arranged regularly on the cambium around the core region. Sclerenchyma cells that consist of smaller cells are seen in the heads of the conduction bundles. Sclerenchyma cells are denser in the parts of the vascular bundles towards the cortex. There is an open collateral conduction bundle. The cells are larger in the core region, and the intercellular spaces are more (Fig. 2).

Dorsiventral leaf type is seen in the leaf cross-section. There is a cuticle layer on the lower and upper epidermis. The cuticle layer is thicker in the upper epidermis. There are stomatal cells in the lower epidermis, but there are no in the upper epidermis. The facial hairs are rarely seen in the lower epidermis. Palisade parenchyma cells are more regular than sponge parenchyma cells with fewer intercellular spaces (Fig. 3).

Stomatal cells are detected on the lower surface, but not on the upper surface in the leaf surface sections. The shapes and arrangement of the lower and upper epidermis cells are similar (Fig. 4, Table 3).

***H. vesicarius*:** There is fragmentation in the epidermis cells and the cuticle layer in the transverse section taken from the root of the plant. The cell arrangement is not exactly circular. Endodermis cells are larger than epidermis cells and their arrangement is not regular. The cells constituting the cortex have different sizes and their arrangement is irregular. The endodermis cells around the conduction bundles are smaller, regular, and tightly arranged compared to the cortex cells. Pericycle cells are larger than endodermis cells. The xylem consists of 8 branches on average (Fig. 5).

There is a thin cuticle layer in the outermost part in the section taken from the stem of the plant. Some parts of the epidermis and cuticle are fragmented. In general, it is also seen that the circular structure has deteriorated. An average of 12 rows of cells are detected in the cortex region. The vascular bundles are arranged regularly around the cambium. There is an open collateral conduction bundle. The sizes of the cells that form the cortex are close to each other and the spaces between the cells are small (Fig. 6).

In the leaf cross-sections, cuticle layers are detected on the upper and lower epidermis. The cuticle layer in the upper epidermis is more prominent. Upper epidermis cells are arranged more regularly and consist of larger cells compared to the lower epidermis. Stomas are detected in the lower epidermis. No feather structures are detected. Cells in the lower epidermis are different in size, and there is no linear arrangement. Dorsiventral leaf type is also seen. Palisade parenchyma is arranged well and intercellular spaces are small. The intercellular space is large and the cell arrangement is irregular in the sponge parenchyma (Fig. 7).

In the superficial sections of the leaves, stomatal cells are detected on the lower surface, but not on the upper surface. The cells in the upper epidermis are smaller than those in the lower epidermis and have a more regular arrangement (Fig. 8, Table 3).

Palynological characteristics: The pollens of the taxa were photographed under light and SEM and measurements were made, in which the characteristics of the polar axis, equatorial axis, colpus length, colpus width, exine thickness, and intine thickness were measured.

It was found that the *H. orientalis* pollen types were tricolpate, their shapes were subprolate, and their ornamentation was reticulate. At the same time, *H. vesicarius* pollen types were tricolporate (rarely tricolpate), their shapes were prolate-spheroidal, and their ornamentation was microreticulate-foveolate (Figs. 9-10, Table 4).

Table 3. Comparative anatomy of the root, stem, and leaf of *H. orientalis* and *H. vesicarius*.

	H.orientalis				***H.vesicarius***			
	Width (..)		**Length (lm)**		**Width (..)**		**Length (lm)**	
	Min.-Max.	**Mean±SD**	**Min.-Max.**	**Mean±SD**	**Min.-Max.**	**Mean±SD**	**Min.-Max.**	**Mean±SD**
Root								
Epidermis cell	14.5-33.7	24.3±5.9	9.6-29.6	19.5±7.2	16.2-42.8	29.1±8.4	16.4-38.7	27.6±6.5
Endodermis cell	24.5-32.8	28.6±3.1	11.3-19.9	16±3.2	15.7-33.5	26.2±5.2	10.0-19.5	14.9±2.9
Cortex cell	48.6-87.4	65.7±11.6	41.3-80.1	59.1±11.7	35.1-62	47.4±8.8	30.8-58.2	40.8±8.9
Xylem cell	12.0-20.0	15.9±2.8	9.9-18.8	15.1±2.8	14.8-36.4	24.8±6.6	21.7-40.4	32±5.6
Floem cell	10.3-24.2	16.8±4.8	11.9-24.3	17.2±3.9	8.9-16.6	12.7±2.6	14.7-21.4	18.4±2.5
Stem								
Cuticle	1.4-5.6	4.3±1.1			3.4-8.5	5.9±1.4		
Epidermis cell	13.1-24.0	18.2±3.1	12.5-20.2	16.4±2.8	16.0-26.7	21.6±4.1	12.6-22.6	18.0±3.4
Endodermis cell	19.8-35.2	29.5±4.9	16.6-46.3	29.9±8.6	30.2-48.7	39.4±5.7	23.4-40.5	34.6±7.2
Xylem cell	11.8-16.5	13.6±1.6	11-16.5	14.3±1.9	7.7-18.7	12.1±3.0	10.5-16.8	13.4±2.0
Floem cell	7.7-16.0	11.6±2.7	9.7-14.1	11.8±1.7	8.3-14.7	12.2±2.1	7.7-14.6	11.0±2.4
Pith region(diameter)		56.5-81.5*69±17.6				80.4-101.3*87±7.6		
Cortex (diameter)		955.9-1024.1*987.4±25.6				628.0-944.5*805.1±92.0		
Leaf (Transverse)								
L. epidermis cell	33.9-62.1	48.0±7.6	50.5-62.5	56.5±4.8	26.1-62.1	44.9±12.1	25.3-51.2	32.5±7.2
Lower cuticle	1.9-7.0	5.5±1.6			2.5-6.3	4.6±1.0		
U. epidermis cell	13.3-47.1	26.1±12.1	13.2-22.4	17.3±3.3	17.1-47.5	28.3±7.8	15.1-24.2	19.0±3.6
Upper cuticle	6.3-14.3	10.2±2.5			2.0-3.9	2.89±0.6		
P. parenchyma cell	58.4-74.1	66.3±5.7			41.8-74.9	60.3±10.5		
S. parenchyma cell	97.7-115.4	106.5±5.8			111.7-134.7	122.2±7.2		
Leaf (lower)								
Stoma (diameter)	31.2-43.3	37±3.7			27.3-42.9	35.1±11.0		

-50 measurements for each anatomical character were taken

Table 4. Comparative pollen morphology of *H. orientalis* and *H. vesicarius* showing mean value ± standard deviation.

Species	**P (µm)**	**E (µm)**	**P/E ratio shape**	**Clt (µm)**	**Clg (µm)**	**Plg (µm)**	**Plt (µm)**	**Exine (µm)**	**Intine (µm)**
H. orientalis	19.33 ± 1.43	14.07 ± 0.95	subprolate	2.06 ± 0.58	14.96 ± 1.63	1.22 ± 0.22	1.15 ± 0.31	1.00 ± 0.16	0.77 ± 0.20
H. vesicarius	15.48 ± 1.76	13.67 ± 1.37	prolate-spheroidal	1.20 ± 0.36	10.64 ± 1.47	1.08 ± 0.35	1.49 ± 0.77	0.57 ± 0.13	0.53 ± 0.16

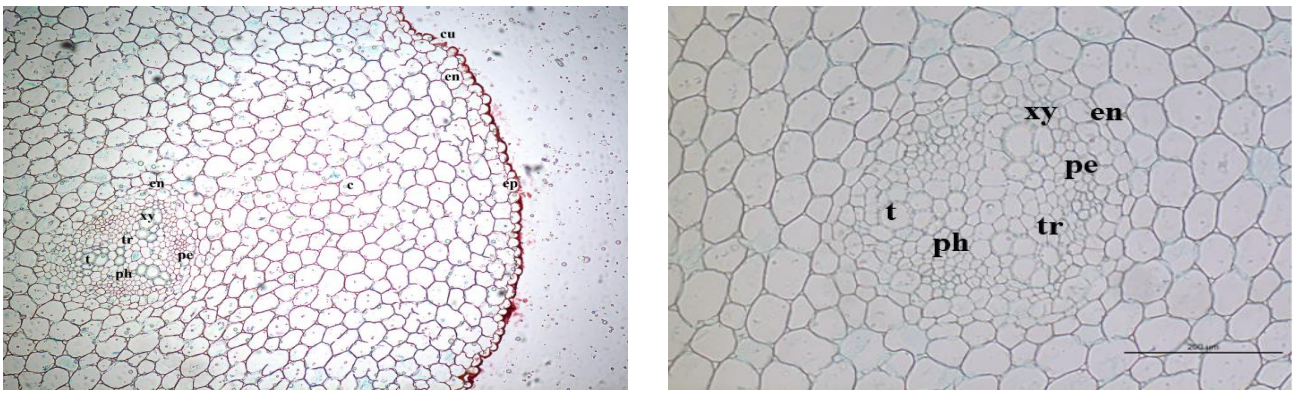

Fig. 1. The transverse section of the root of *H. orientalis;* cu: cuticle, ep:epidermis, en: endodermis, c: cortex, pe: pericycle, ph: phloem, xy: xylem, s: starch grains, t: trachea, tr:tracheids.

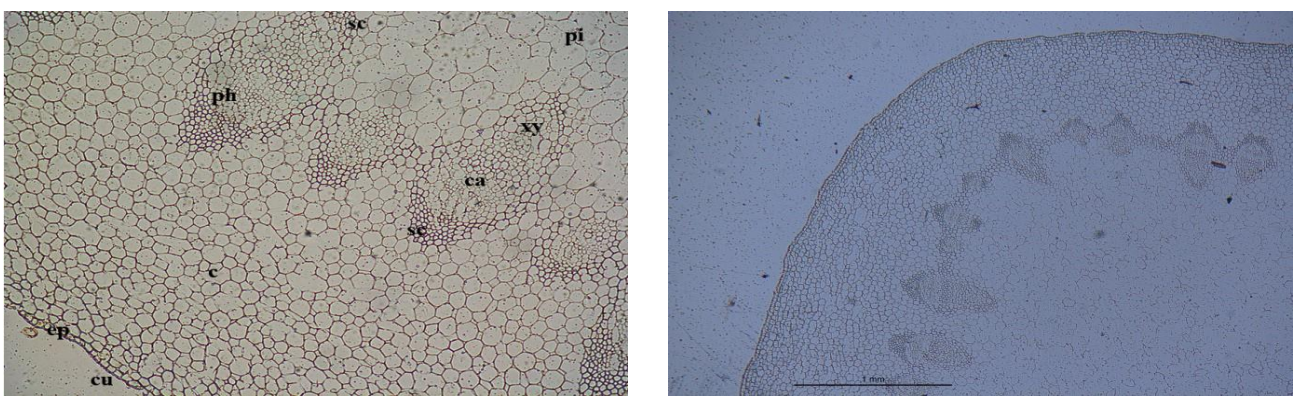

Fig. 2. The transverse section of the stem of *H. orientalis;* cu: cuticle, ep:epidermis, c: cortex, sc:sclerenchyma , ca:cambium, ph: phloem, xy: xylem, pi: pith region.

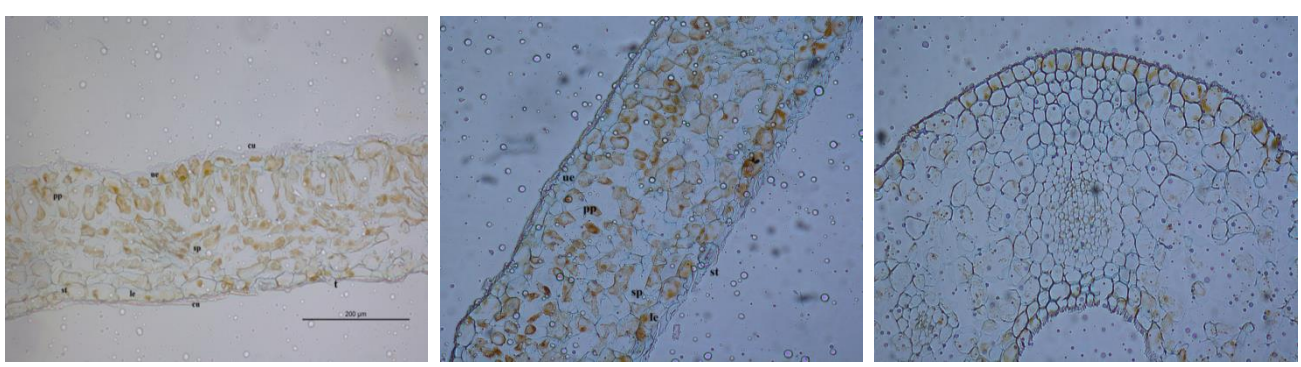

Fig. 3. The transverse section of the leaf of *H. orientalis;* cu: cuticle, t: trichom, ue: upper epidermis, pp: palisade parenchyma, sp: spongy parenchyma, st: stomata, le: lower epidermis.

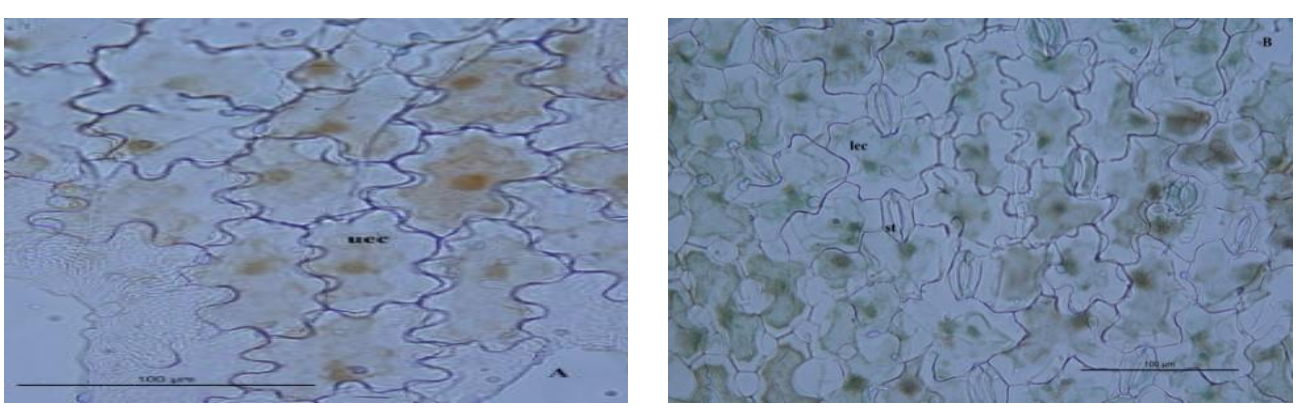

Fig. 4. Leaf surface section of *H. orientalis.* A: Upper surface, B:Lower surface (uec: upper epidermal, lec: lower epidermal, st: stomata).

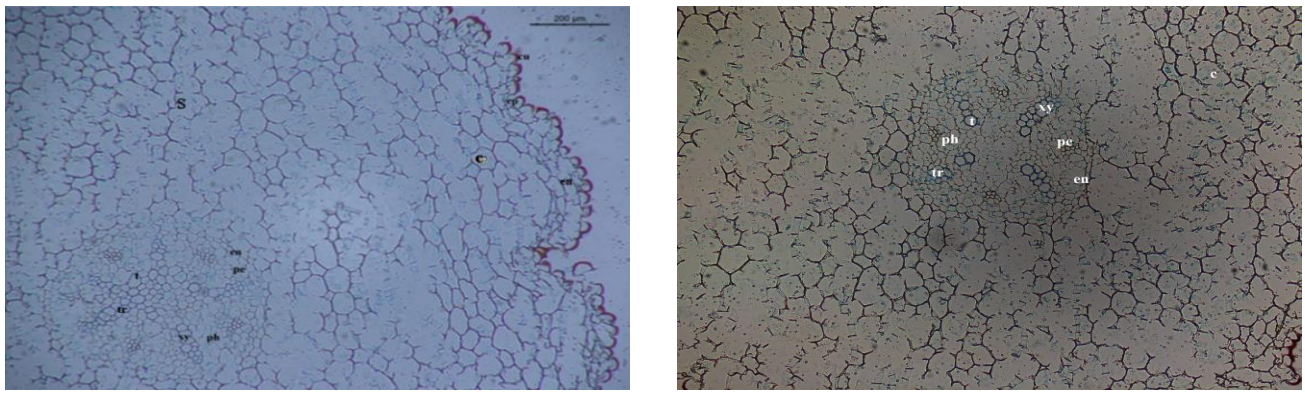

Fig. 5. The transverse section of the root of *H. vesicarius*; cu: cuticle, ep:epidermis, , en: endodermis, c, cortex, pe: pericycle, ph: phloem, xy: xylem.

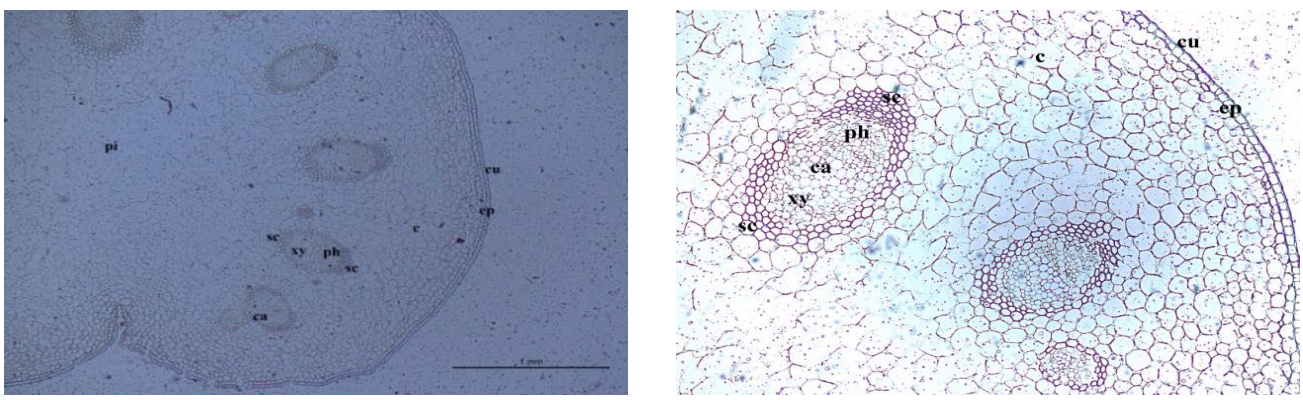

Fig. 6. The transverse section of the stem of *H. vesicarius;* cu: cuticle, ep: epidermis, c: cortex, sc: sclerenchyma, ca: cambium, ph: phloem, xy: xylem, pi: pith region.

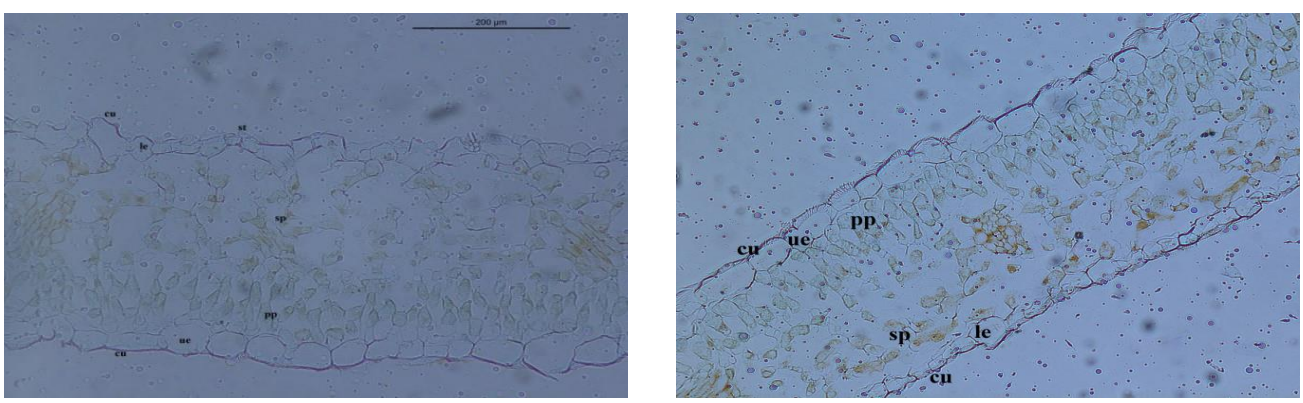

Fig. 7. The transverse section of the leaf of *H. vesicarius;* cu: cuticle, ue: upper epidermis, pp: palisade parenchyma, sp: spongy parenchyma, st: stomata, le: lower epidermis.

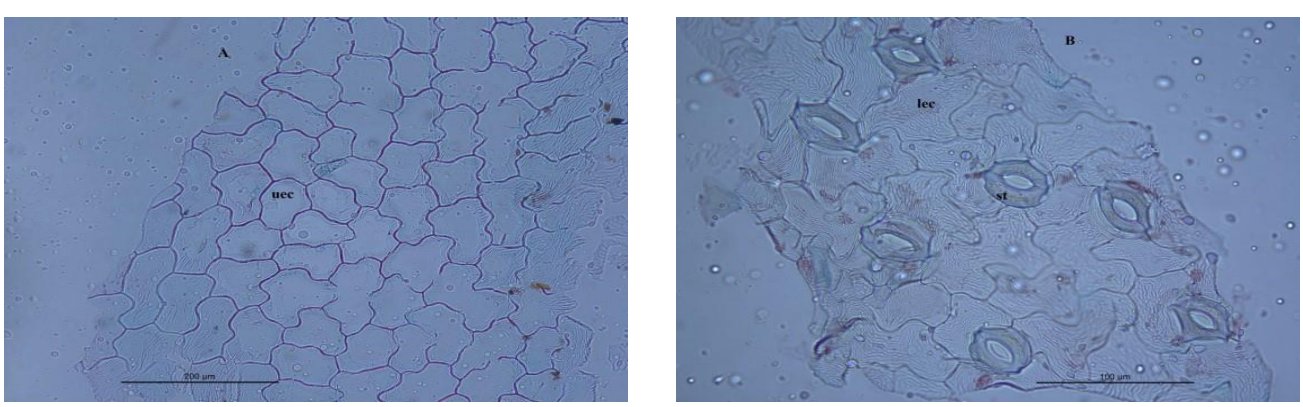

Fig. 8. Leaf surface section of *H. vesicarius*. A: Upper surface, B: Lower surface (uec: upper epidermal, lec: lower epidermal, st: stomata).

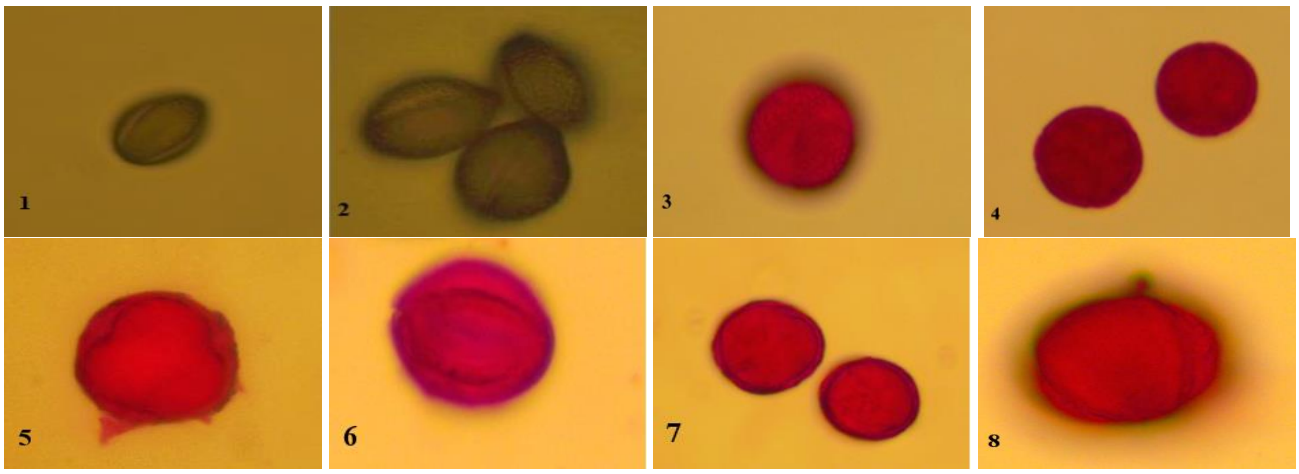

Fig. 9. Light microscope micrographs of pollen grains in the *Helleborus* taxa examined 1-4 *H. orientalis*, 5-8 *H. vesicarius.*

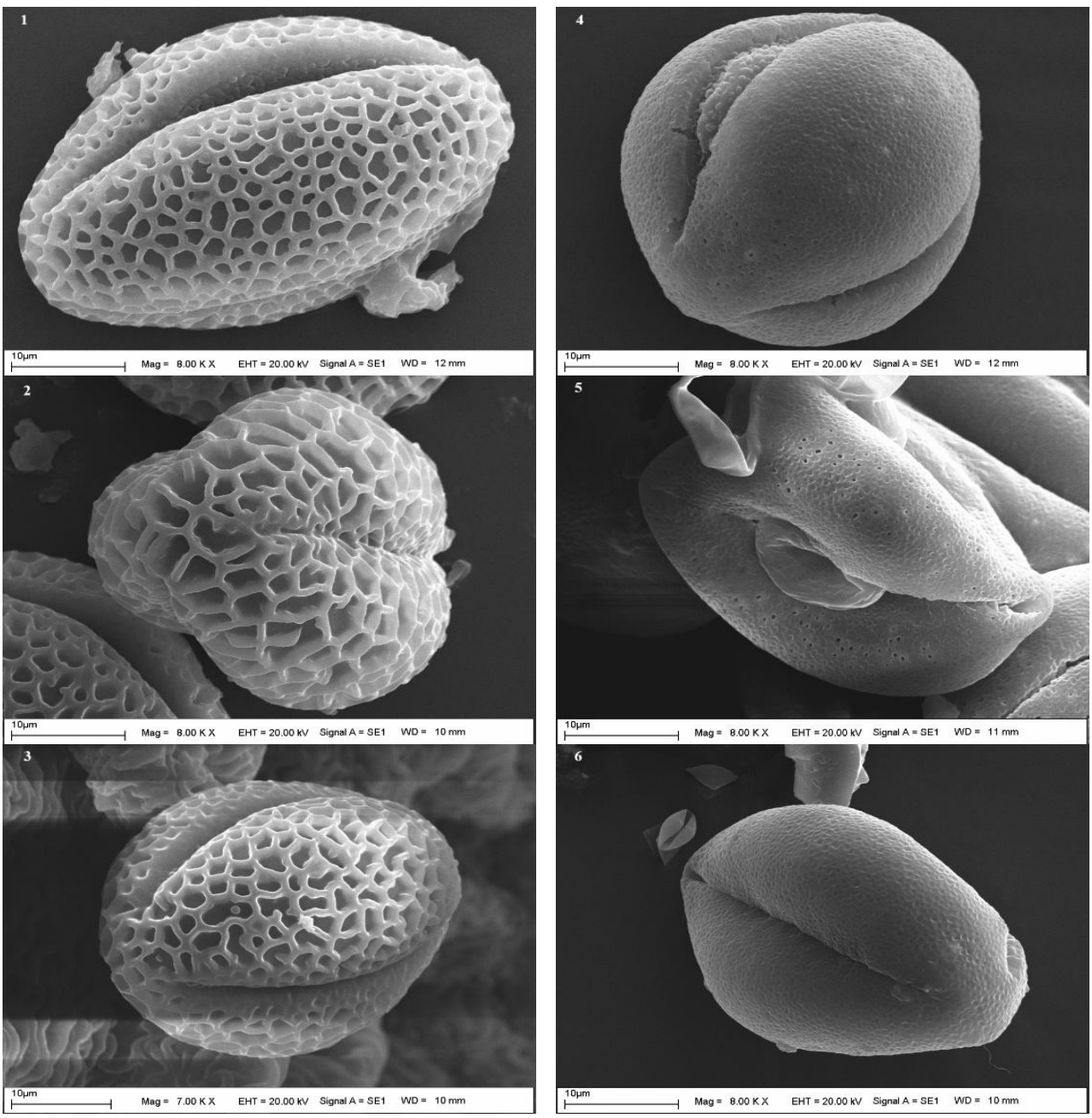

Fig. 10. SEM micrographs of pollen grains in the *Helleborus* taxa examined 1-3 *H. orientalis*, 4-6 *H. vesicarius.*

Discussion

In the present study, the morphological, anatomical, and palynological characteristics of *H.orientalis* and *H.vesicarius* that grows naturally in Turkey were compared. In addition to the characters used in the description of plants in morphological terms, new characters were also added. The leaves in the plant body, the junction points of the follicles, their sizes, and the number of seeds were the distinguishing characteristics. Also, the size of the basal leaves, type of leaf margins, hair condition, number of flowers, and detailed structures of the sepals were the morphological characteristics in our study. Basal leaves of *H. orientalis* were wider and shorter than those of *H. vesicarius*. Although there were rarely short and facial hairs on the upper and lower surfaces of the basal leaves of *H. orientalis*, these were not detected in *H. vesicarius*. The flowers of *H. orientalis* were generally longer than those of *H. vesicarius*. Although the bases and ends of the sepals were rounded, acute-to-apiculate in *H. orientalis*, they were rotundate, rotundate-to-obtuse in *H. vesicarius*, and although the hair structures on the lower and upper surfaces of the sepals were tomentose in *H. orientalis*, they were glabrescent-to-shortly pilose at the sepal tips in *H. vesicarius*.

In a study that was conducted by Tanker and Bingöl (Tanker & Bingöl, 1984), it was reported that *H. orientalis* had hairs only on the lower surface of the basal leaves; however, hairs were also detected on the upper surfaces of the leaves in our study. Other results show similarity. Also, the morphological results reported in the study by Sezer were similar to our study results (Sezer, 1985).

In anatomical terms, in the sections taken from the roots, stems, and leaves of the species and in the measurements, it was found that the cell arrangement was more regular in *H. orientalis* root anatomy than in *H. vesicarius*. It was also found that *H. orientalis* cortex cells were more angular and contained starch grains than the cells in *H. vesicarius*. The xylem branches forming the vascular bundles were more common in *H. vesicarius*. The numerical difference of the xylem arms is an important distinguishing character for the species in anatomical terms. It was also found in the measurements that *H. vesicarius* epidermis and xylem cells, *H. orientalis* endodermis, and cortex cells were larger (Table 3). Similar results were reported in the study that was conducted by Sezer (Sezer, 1985). However, the present study is the first study in which detailed measurements were made and photographed under a light microscope. In the study that was conducted by Balázs *et al.* with different taxa of the Helleborus genus, the number of xylem branches in root anatomy was stated as the distinguishing anatomical character (Balázs *et al*., 2020).

In the cortex, the high number of vascular bundles in *H. orientalis* were distinctive characteristics in the stem anatomy. Also, it was measured that epidermis and endodermis cells were larger in *H. vesicarius*, and xylem cells were larger in *H. orientalis*. When the general body anatomy shapes were evaluated, it was found that *H. orientalis* had a circular structure and *H. vesicarius* had an angular structure (Figs. 2, 6). Although similar results were reported in Sezer's study (Sezer, 1985), the distinctive characters used in our study were also noted in the studies of Balázs *et al*., (Balázs *et al*., 2020).

The lower epidermis cells of *H. orientalis* and *H. vesicarius* were larger than the upper epidermis cells in the cross-sections taken from the leaves of the species. It was measured that the cuticle on the upper epidermis was thicker than the cuticle on the lower epidermis in *H. orientalis*, and the lower cuticle was thicker than the upper one in *H. vesicarius*. Between these, the lower epidermis, lower and upper cuticle, and palisade parenchyma cells of *H. orientalis* were larger than those of *H. vesicarius*, and the lower epidermis and spongy parenchyma cells of *H. vesicarius* were larger. When the diameters of the stomata in the lower surface sections of the leaf were compared, it was found that they were larger in *H. orientalis*. In another study conducted by Özbucak *et al*., the leaf anatomy measurements of *H. orientalis* were found to be compatible with our study results (Özbucak *et al*., 2016). When the feather structures were examined, simple hairs were seen only on the lower epidermis of *H. orientalis*. For this reason, the amount of space between the sponge parenchyma and the presence of hair is the distinctive anatomical characteristic of these two species. The results of Sezer's study are compatible with our study results (Sezer, 1985).

Pollen studies on some genera in the Ranunculaceae family were researched and studied by Pollen morphology of some genera of the family Ranunculaceae has been examined by Erdtman & Sorsa (1952), Vishnu-Mittre Sharma (1962), Boet & Spoil (1968), Nowicke (1975), Kuprianova & Alyoshina (1978), Mkrtchyan & Agalabyan (1978), Skvarla & Nowicke (1979), Nowicke & Skvarla (1979, 1983) (Perveen & Qaiser, 2006). In previous studies conducted by Erdtman on 33 genera and 150 species, the pollen form in the Ranunculaceae family was found to be mostly dicolpate, tricolpate, 6 polyrugate, and polyforate. The grains are without aperture or irregular (panto) often with almost spiral-shaped aperture. The pollen shape varies from suboblate to prolate (Erdtman & Sorsa, 1952; Sezer, 1985). In our palynological study, the pollen type of *H. orientalis* was found to be tricolpate, the pollen shape subprolate, and the ornamentation reticulate. The pollen type of *H. vesicarius* was rarely tricolpate, generally tricolporate, pollen shape was prolate-spheroidal, and the ornamentation was microreticulate-foveolate. In the study by Sezer, it was determined that the pollen of *H. vesicarius* and *H. orientalis* was tricolpate, the pollen shape of *H. vesicarius* was oblate spheroidal, and suboblate and oblate spheroidal in *H. orientalis* with reticulate ornamentation (Sezer, 1985). Also, in another study that was conducted by Nowicke and Skvarla, it was reported that the ornamentations were reticulate, but they differed (Nowicke & Skvarla, 1983). The studies of Sezer and Erdtman had partially similar results with our study (Erdtman & Sorsa, 1952; Sezer, 1985).

Conclusions

In conclusion, new characters were added by expanding the morphological description of two taxa of *Helleborus* genus that grow naturally in Turkey. The images and detailed comparison of the anatomical findings were made for the first time in the light microscope. The number of xylem arms in root anatomies, and the presence of hairs can be considered as anatomical distinguishing characteristics for the species. In palynological terms, SEM images and detailed pollen measurements of the species were shown for the first time.

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