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Research Article

MORPHO-ANATOMICAL AND PALYNOLOGICAL CHARACTERIZATION OF *Jasione heldreichii* Boiss. & Orph.

Okan Sezer [1,a,*], İsmühan Potoğlu Erkara [1,b]

[1] *Eskisehir Osmangazi University, Faculty of Science and Letters, Department of Biology, 26040 Eskisehir, Turkey*

**Corresponding Author:*
E-mail: oksezer@ogu.edu.tr

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a: ORCID 0000-0001-7304-1346, b: ORCID 0000-0001-5780-4999

ABSTRACT. In this study, morpho-anatomical and palynological features of *Jasione heldreichii* Boiss. & Orph. which is distributed in Marmara, Egean, Mediterranean regions and West parts of Central Anatolia has been investigated. Anatomical features, a detailed morphological description and a distribution map of *Jasione heldreichii*. For identification of anatomical features, tranverse sections of the roots, stems and leaves were investigated. Obtained morpho-anatomical characteristics of *J. heldreichii* were compared with its description given in Flora of Turkey. *J. heldreichii* showed oblate-spheroidal characteristics and had triporate pollen type.

Keywords: *Campanulaceae, Jasione heldreichii, anatomy, morphology, palynology*

INTRODUCTION

The Campanulaceae L. family is a cosmopolitan family and is represented by 84 genera and approximately 2400 species in 5 subfamilies. The family name is also derived from the largest genus of the Family, *Campanula* L. [1]. In the flora of Turkey, the family of Campanulaceae, Asyneuma Griseb. & Schenk, *Campanula* L., *Jasione* L., *Legousia* Durande, *Michauxia* L'Hér., *Sachokiella* Kolak., *Solenopsis* C. Presl, and *Theodorovia* Kolak. ex Ogan. represented by their genus. Among these genera, *Campanula* is the richest in terms of number of taxa, and it contains 139 taxa, 73 of which are endemic. When we look at the Campanulaceae genera in our country in terms of endemism rate, the Jasione genus ranks first with 71.4 % (5 of them endemic 7 taxa) [2].

The genus *Jasione* is represented by 16 species in the world. Considering the distribution areas of Jasione members, it is known that they spread up to Alpine areas at an altitude of 3500 meters above sea level, especially in Europe, Northwest Africa and the northern and western parts of Turkey [3]. In the flora of Turkey, the existence of 7 *Jasione* taxa (4 species and 3 subspecies) is known. 5 of these 7 taxa are endemic to Turkey, and 4 of these endemic taxa are subspecies of *Jasione supina* Sieber ex Spreng. Except for the flora of Turkey, the number of reference sources that can be referenced in the classification of endemic *Jasione* taxa is very few [4, 5, 6, 7, 8, 9, 10]. With this study, it is aimed to reveal the morphological, anatomical and palynological characters of *Jasione heldreichii*, which is in the flora of our country. It is hoped that the data obtained within the scope of this study will be a reference for future taxonomic studies on Turkish *Jasione* taxa.

MATERIALS AND METHODS

Plant Material

Jasione heldreichii specimens evaluated within the scope of the study were collected between 2019-2020 from their locations stated in the literature as a result of field studies carried out during vegetation periods [4, 5, 6, 7, 8, 9, 10]. Within the scope of the study, *Jasione* specimens found in domestic and foreign herbariums were examined through herbarium visits (ANK, OUFE, HUB, BULU, EDTU, EGE), borrowed specimens and digital herbarium catalogs (GOET, Herb. Muséum Paris) and were used as references in species identification. After the samples collected within the scope of the field studies were identified, they were turned into herbarium material in accordance with the herbarium rules and kept in Eskişehir Osmangazi University Herbarium (OUFE).

Localities were collected plant samples:

Turkey, B3 Kütahya: Sökmen village, Calcareous rock and soils, 1050 m, 20.07.2020, *O. Sezer,* O.S. 2152 (OUFE); Turkey B3 Eskişehir: Türkmendağı, Aşağı Kalabak, ca. 900, step, 12 July 2020, *O. Sezer*, O.S. 2104 (OUFE); Turkey B3 Eskişehir: Türkmendağı, Aşağı Kalabak, ca. 900, step, *T. Ekim*, (ANK-2005); Turkey B3 Eskişehir: Türkmendağı, kavak dere, ca. 1300, 09 July 1977, *T. Ekim*, (ANK-????).

Morphological Studies

Morphological features of *Jasione heldreichii* specimens were determined by Leica EZ-40 stereomicroscope using fresh specimens and herbarium materials (ANK, HUB, BULU, EDTU, EGE). The variation ranges of descriptive morphological characters were determined by measuring 25 individuals taken from different points of the determined natural populations.

Anatomical Studies

Anatomical examinations were carried out on fresh plant materials collected from type localities and determined in 70% alcohol. Root and stem cross-sections were taken from these materials with the help of hand and razor blade. Various literatures have been used both as a reference in tissue typing and for comparison purposes in anatomical examinations [11, 12, 13, 14, 15, 16, 17].

Palynological Studies

Pollen samples obtained from the herbarium materials of *J. heldreichii* were stored in OUFE. For palynological investigations, pollen grains of 10-15 from each plant specimen collected from different localities were used. Light microscopy and SEM were used for investigation of their morphology. The terminologies used by Faegri and Iversen (1975) were followed to name the exine layers. For the purpose of investigations by light microscope, pollen grains of *J. heldreichii* were prepared following Wodehouse (1935) and Erdtman (1969) techniques. For identifications and counting of pollen grains simply 10 and 40x objectives were used. Detailed investigations on their identifications were made by 100x plan oil-immersion objective. Thickness of exine and intine of seeds of the taxa was measured following Wodehouse (1935) and Erdtman (1969). Terminologies for pollen morphology were used as par Wodehouse (1935), Kuprianova (1967), Erdtman (1969) and Faegri and Iversen (1975) [18, 19, 20, 21].

RESULTS

Morphological findings (Figs. 1-6, Table 1)

***Jasione heldreichii* Boiss. & Orph. in Boiss., Diagn. ser. 2(6): 120 (1859).**
Sinonym: *Jasione dentata* (A.DC.) Halácsy, Consp. Fl. Graec. 2: 280 (1902); *Jasione dentata* f. *canescens* Gajic, Glasn. Prir. Mus. Beogradu, Ser. B, Biol. Nauke 28: 64 (1973); *Jasione dentata* f. *dubravkiana* Gajic, Glasn. Prir. Mus. Beogradu, Ser. B, Biol. Nauke 28: 64 (1973); *Jasione dentata* f. *integrifolia* Gajic, Glasn. Prir. Mus. Beogradu, Ser. B, Biol. Nauke 28: 63 (1973); *Jasione glabra* Velen., Oesterr. Bot. Z. 34: 424 (1884); *Jasione heldreichii* var. *glabra* Nyman, Consp. Fl. Eur., Suppl. 2: 212 (1889); *Jasione heldreichii* var. *papillosa* J.Parn., Watsonia 16: 266 (1987); *Jasione jankae* Neilr., Aufz. Ungarn Slavon. Gefässpfl., Nachtr.: 43 (1870); *Jasione montana* var. *dentata* A.DC. in A.P.de Candolle, Prodr. 7: 415 (1839); *Jasione montana* var. *heldreichii* (Boiss. & Orph.) Nyman, Consp. Fl. Eur.: 486 (1879); *Jasione montana* f. *heldreichii* (Boiss. & Orph.) Schmeja, Beih. Bot. Centralbl. 48(2): 33 (1931); *Jasione montana* f. *jankae* (Neilr.) Schmeja, Beih. Bot. Centralbl. 48(2): 33 (1931).

Description: Biannual herbaceous plants. Stem one or several, erect or ascending, to 10-50 cm, ± pilose, sparsely branched from basal or upper parts. Leaves 8-30 × 1.5-5(7) mm, lanceolate to lineer-lanceolate, apex acute-oblong, margins undulate, dentate, apical or submarginal parts hairy or not. Capitula 10-20(30) mm diam. Peduncle 1-30 cm, apical parts hairy or not. Involucral bracts oblong-lanceolate, margins deeply dentate or incised, mostly incrassate, green-purplish. Pedicel 0-5 mm. Calyx 2-3.2 mm, linear-lanceolate, glabrous. Corolla 3-5 mm, blue. Stylus 4.7-5.9 mm. Flowering 6-8. Sandy places, dunes, stony areas, rocks, 1-1100 m.

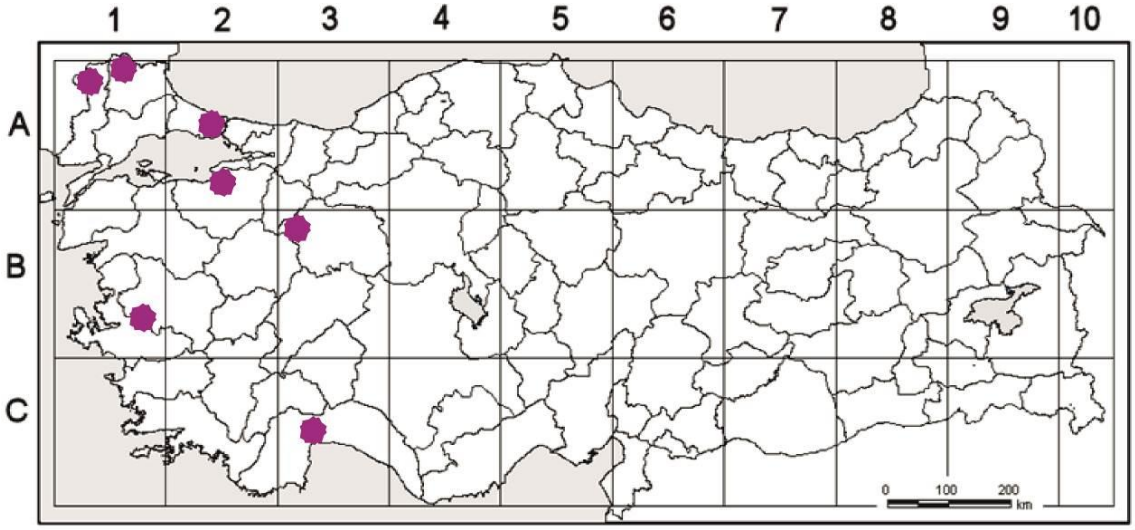

Fig. 1. *Distribution of Jasione heldreichii in Turkey*

Fig. 2. *General appearance of Jasione heldreichii in nature*

Fig. 3. *Capitula of Jasione heldreichii*

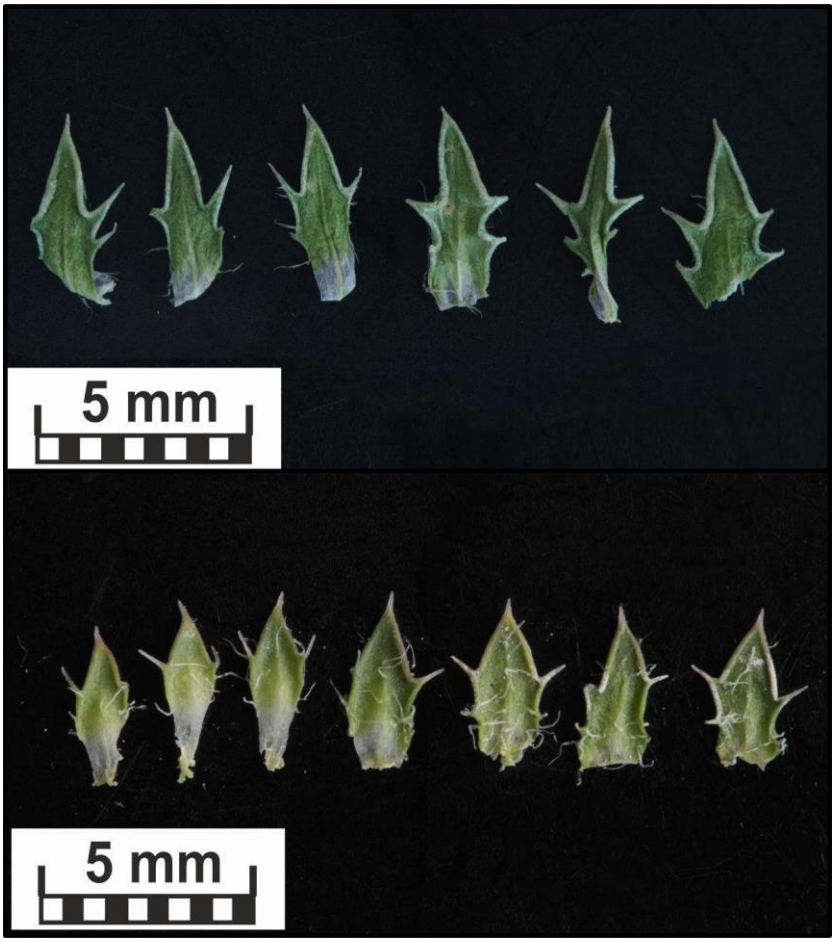

Fig. 4. *Involucral bracts of Jasione heldreichii* (A: Lower surface; B: Upper surface)

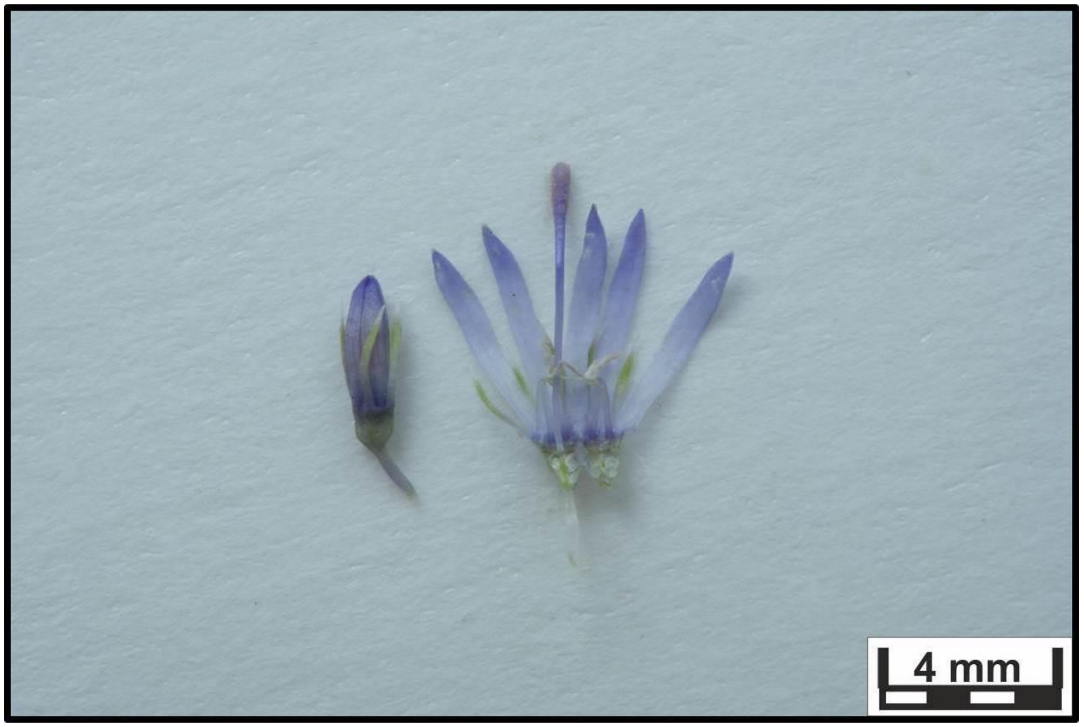

Fig. 5. *Flower of Jasione heldreichii*

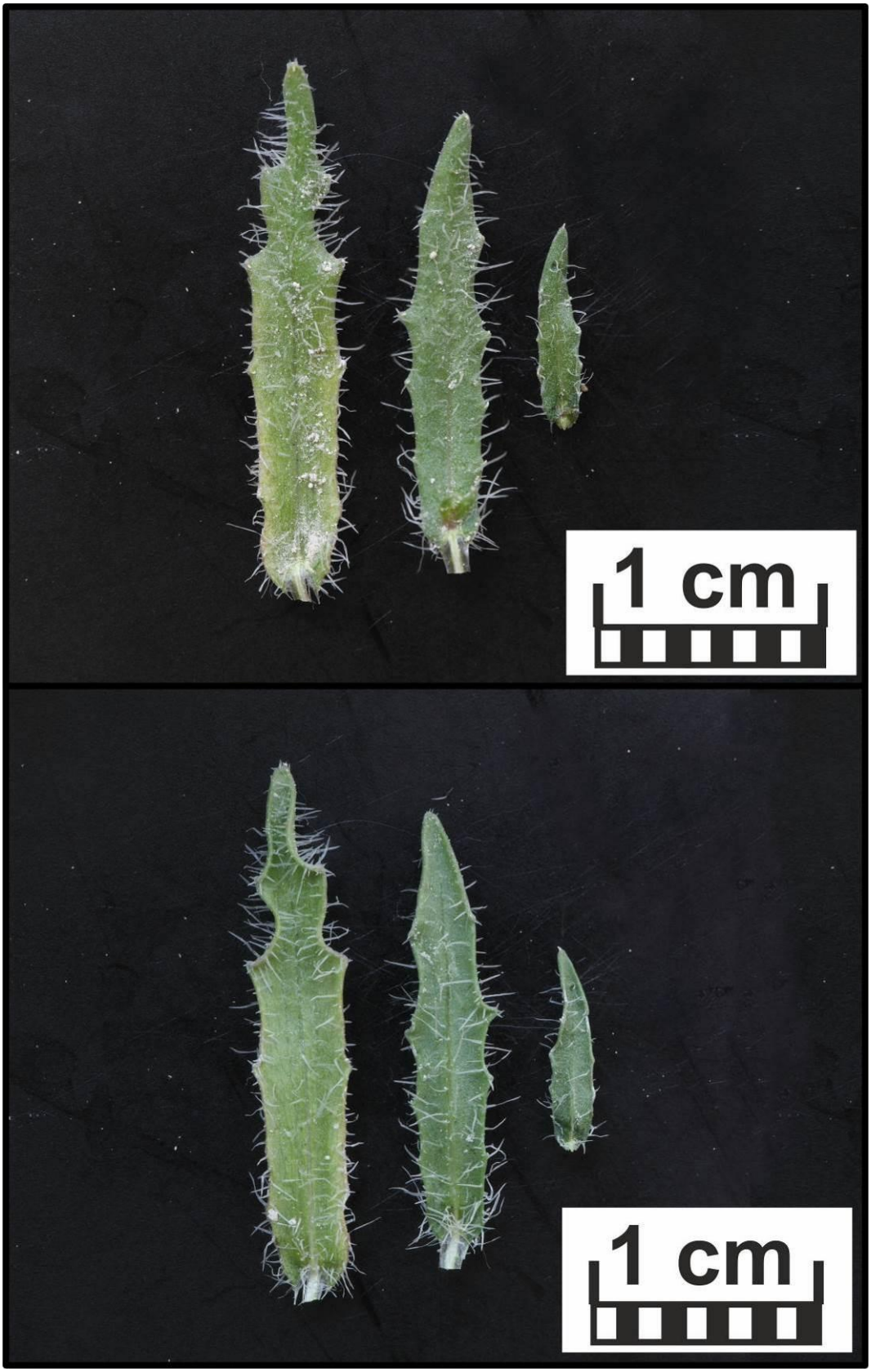

Fig. 6. *Upper and lower cauline leaves of Jasione heldreichii*

Fig. 6. *Stem indumentum of Jasione heldreichii* (**A:** Upper parts of stem; **B:** Lower parts of stem)

Table 1. *Morphological features of Jasione heldreichii and related taxa*

	Flora of Turkey	***Obtained data from this study***	***Flora of Turkey***	***Obtained data from this study***
Taxon name	*Jasione montana*	*Jasione mantana*	*Jasione heldreichii*	*Jasione heldreichii*
Habit	Mostly biannual, rarely annual	Annual or biannual	Biannual	Biannual
Stem indumentum	± villous	Sparsely hairy, ± villous	Short staff hairy	± pilose
Inflorescence	Flowers in globose heads, 1-25 mm diam	Capitula 5-30 mm diam	Capitula c. 10 mm diam	Capitula 10-20(30) mm diam
Involucral bracts	Involucral bracts ovate to triangular, rarely lanceolate, entire, crenate, or dentate, usually with crisped hairs	Size and shape of the outer involucral bracts are very variable, green-lilac, (1)2-3 in rows, eliptical, oval to triangular, margins entire, serrate-cuspidate	Involucral bracts oblong-lanceolate, margins deeply acute-serrate	Involucral bracts oblong-lanceolate, margins deeply dentate or incised, mostly incrassate, green-purplish
Calyx	Calyx lobes lanceolate, glabrous or rarely ciliate	Calyx lobes 1.2-4.1 mm, linear-lanceolate, glabrous or rarely ciliate	-	Calyx lobes 2-3.2 mm, linear-lanceolate, glabrous
Corolla	Corolla blue, rarely reddish or white	Corolla 3-5 mm, lilac-dark blue	-	Corolla 3-5 mm, blue
Style	-	Style 4.7-6.3 mm		Style 4.7-5.9 mm
Stem	Erect or ascending, to 10-50 cm, simple or branched, leafless in upper part	Erect or ascending, to 5-60 cm, simple or sparsely branched from basal or upper parts	-	Stem one or several, erect or ascending, to 10-50 cm, ± pilose, sparsely branched from basal or upper parts
Cauline leaves	Leaves linear-oblong to linear-lanceolate, sessile, kenarları undulate at margin, entire or remotely crenate	Leaves 8-57 × 1.5-5 (10) mm, linear oblong to widely oblong, apex acute-oblong, sessile, margins entire or undulate	-	Leaves 8-30 × 1.5-5(7) mm, lanceolate to lineer-lanceolate, apex acute-oblong, margins undulate, dentate
Leaf indumentum	-	Apical or submarginal parts hairy or not	-	Apical or submarginal parts hairy or not
Flowering	6-8	5-8		6-8

Anatomical findings (Figs. 7-9)

Tissue typing was done by taking root, stem and leaf cross-sections of the examined taxon. Obtained data shows anatomical features proved to be inadequate in terms of classification. It is hoped that future detailed anatomical studies in supraspecies categories will help to shed light on taxonomic problems within the genus and family.

Anatomical examinations of *Jasione heldreichii* show that the body is permanently winged. The body is surrounded by a single layer of epidermis, and the outer face of the epidermis has a cutinized wall structure. The wall structures of epidermal cells are smooth. Stomata have been observed in the epidermis, and 3-10 rows of photosynthetic cortex tissue lies just below the epidermis. The cortex is composed of globular and semi-globular parenchyma cells. Lignification has been observed on the walls of old stems. A stratified endodermis was observed in all stems. The Kaspari strip is not prominent. Phloem is observed in the form of a thin ring and continuously. cells gradually shrink towards the vascular cambium. Except for the sections taken from very young stems, the xylem is in a continuous ring. The pith region is composed of parenchymatic cells, and especially in aged bodies, the cells in this region can be ruptured by tension. No sclereid was observed in the pith region (Fig. 7).

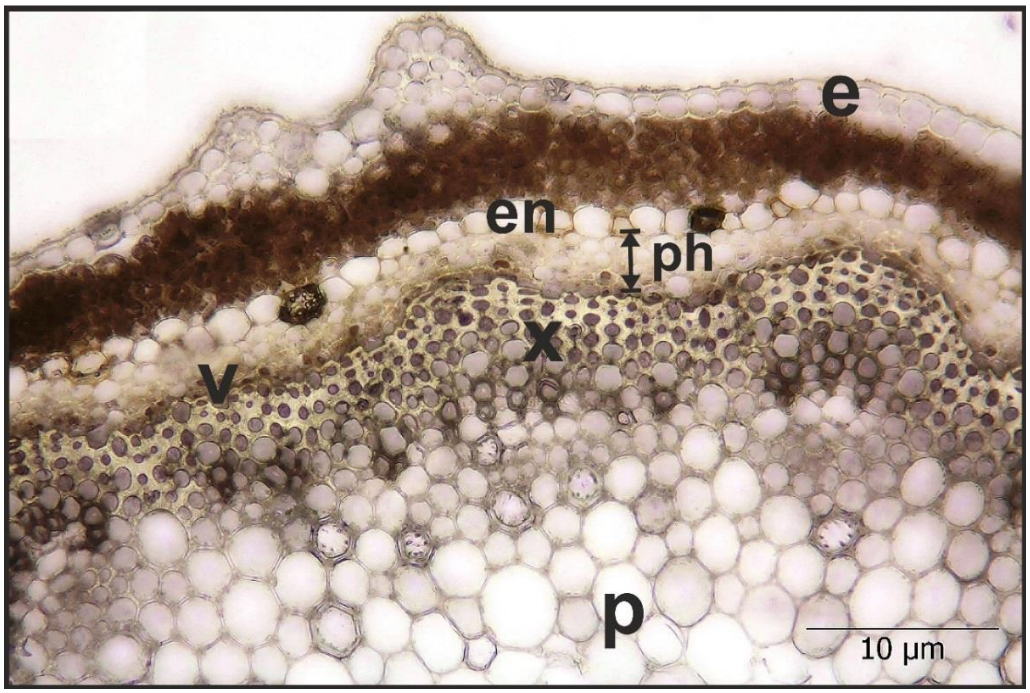

Fig. 7. *Stem cross section of Jasione heldreichii* (**e:** epidermis; **c:** cortex; **en:** endodermis; **ph:** phloem; **v:** vascular bundle; **x:** xylem; **p:** pith)

When the leaf cross-sections of the studied Jasione taxon are examined, the adaxial and abaxial surface cells are ± oval in shape and their sizes are approximately equal to each other. Their outer surfaces have a thin cutinized cell wall. There is no cutinization on the inward facing surfaces. Stomas are mesomorph type and are located on the lower and upper surfaces. There is no structural differentiation in the tissue between the upper and lower epidermis and is called mesophyll. It has been determined that the mesophyll consists of parenchymatic cells with very large intercellular spaces (Fig. 8).

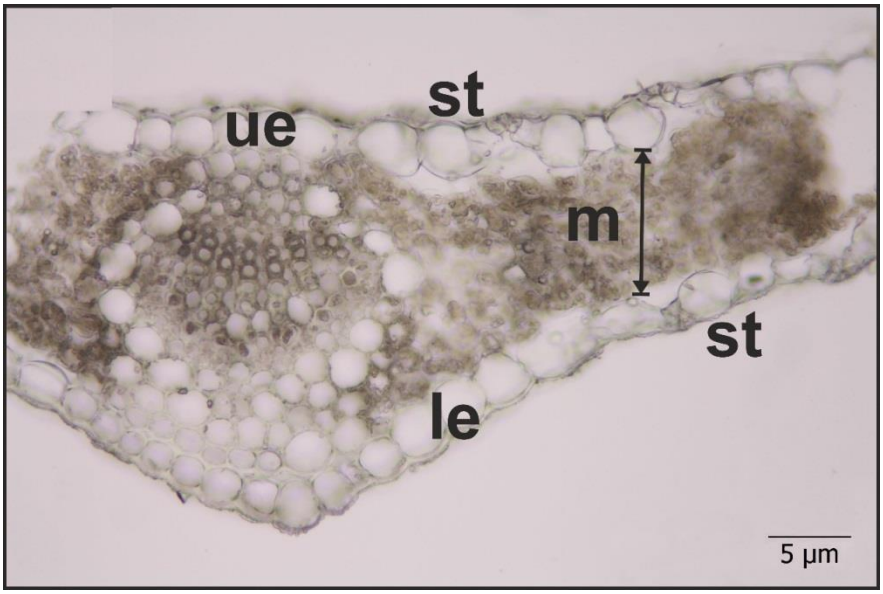

Fig. 8. *Leaf cross section of Jasione heldreichii* (**ue:** upper epidermis; **le:** lower epidermis; **m:** mesophyll; **st:** stoma)

In the studied taxon, it has been observed that a lignification has occurred in the roots, whether they are biennial or perennial, except for very young individuals. In woody roots, the outer part is covered with 3-5 layers of peridermis. The cortex is located under the peridermis and consists of parenchymatic cells. The distinction between cortex and phloem is not clear. Phloem sclerenchyma cells are dispersed within the phloem tissue. The vascular cambium formed by thin-walled and small-sized meristematic cells is clearly observed at the initial border of the xylem tissue between the phloem and the xylem. Depending on the age of the pith root, it may consist entirely of xylem tissue or sometimes contain parenchymatic cells in the center (Fig. 9).

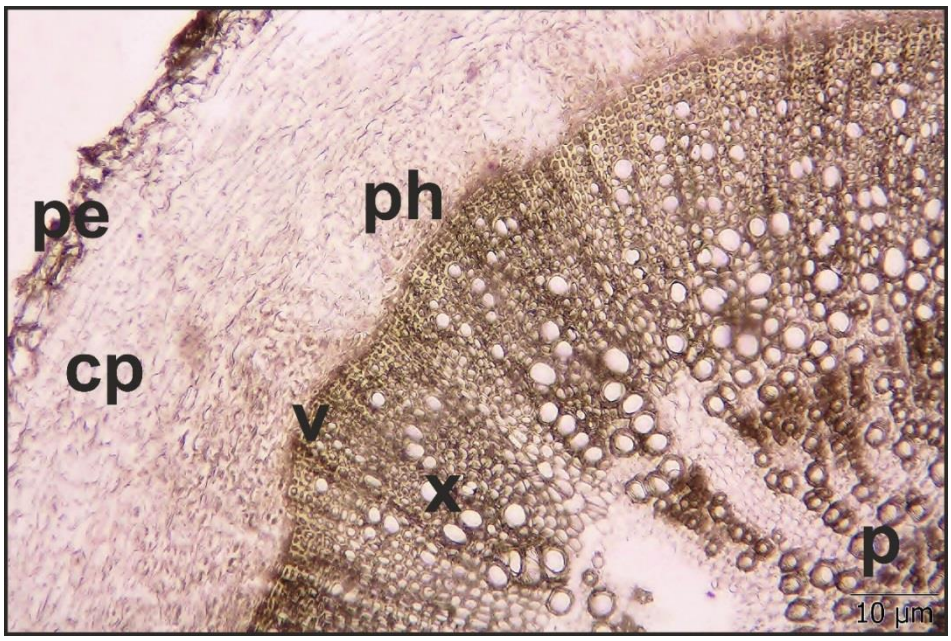

Fig. 9. *Root cross section of Jasione heldreichii* (**pe:** periderm; **cp:** cortex parenchyma; **ph:** phloem; **v:** vascular bundle; **x:** xylem; **p:** pith)

Palynological findings **(Figs. 10-11)**

In our study, it was determined that the taxon *J. heldreichii* distributed in our country showed oblate-spheroidal characteristics and had triporate pollen type. While the ratio (P/E) of the polar axis to the equatorial axis was 0.97 in the Woodehouse Method, it was observed to be 0.88 in the Erdtman Method. P value was determined as 21.54 µm in Woodehouse Method and 18.80 µm in Erdtman Method (Fig. 10-11, Table 2). Avetisjan (1973) states that the members of the Campanulaceae family are pollen in the tropical region as colpate, colporate or colpat porate, and the pollen in the temperate region is only porate. It also states that the increase in the number of pores evolves inversely with the length of the spinules. As it is known, our country is in the temperate climate zone. Therefore, our findings support Avetisjan's views on this subject [22, 23]. Çelemli (2020) examined the pollen grains of 7 *Jasione* (Campanulaceae) taxa distributed in Turkey. Pollen grains are in prolate-spheroidal and oblate-spheroidal shape; It has been determined that the apertures vary from triporate to tetraporate. The results obtained are similar to the results of our study [10].

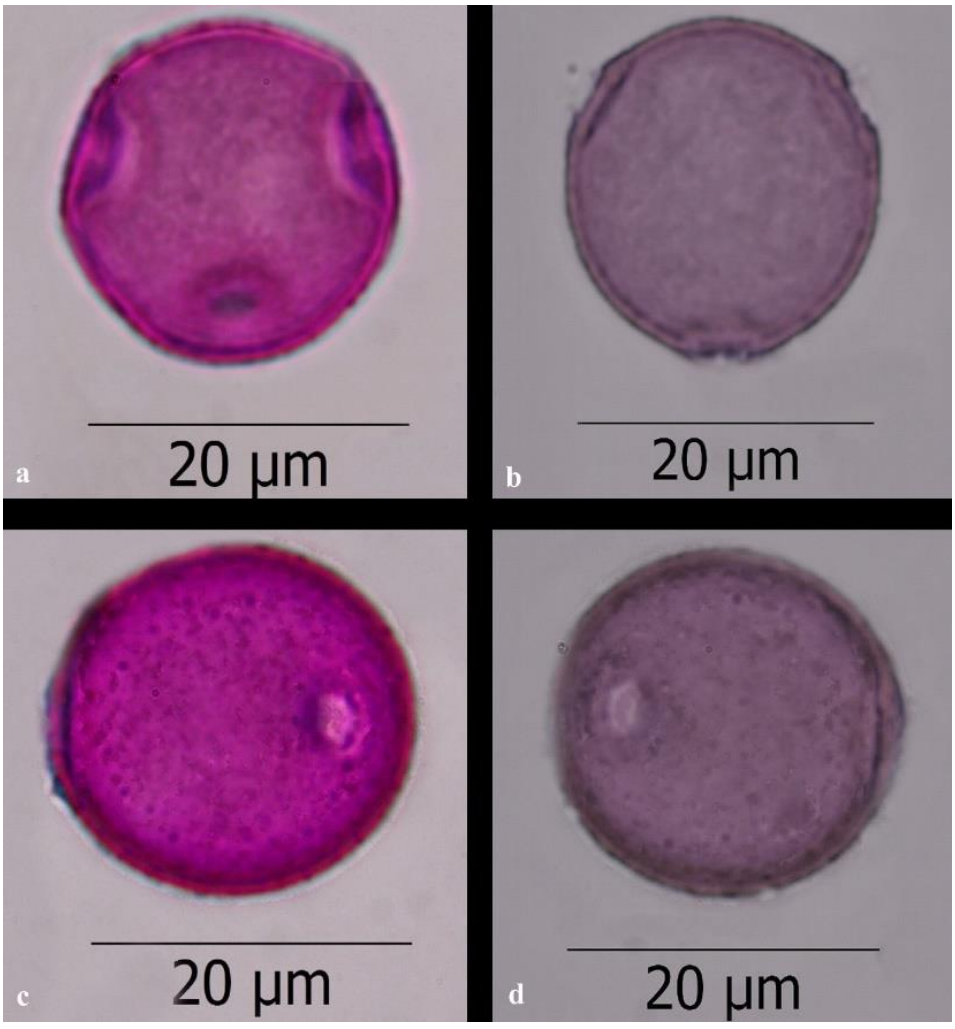

Fig. 10. *LM microphotographs of Jasione heldreichii* **a)** polar (W), **b)** polar (E), **c)** equatorial (W), **d)** equatorial (E) (**W:** non-acetolised; **E:** acetolised)

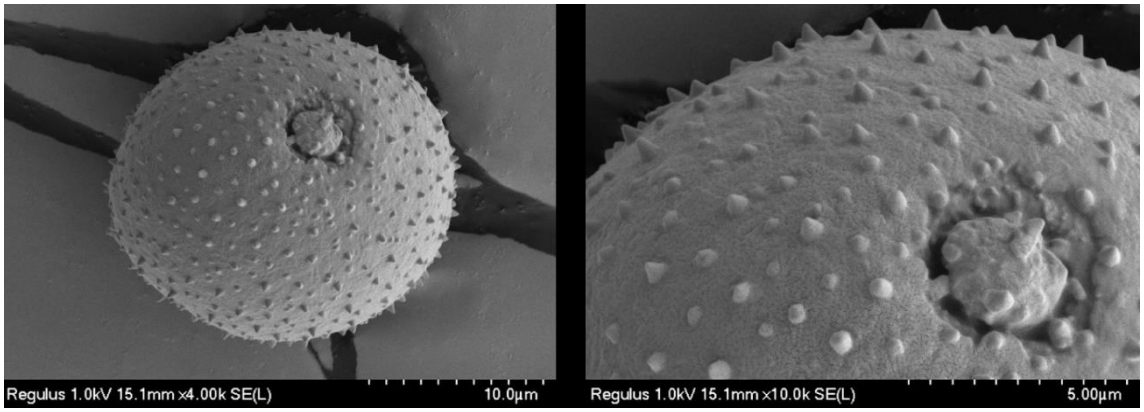

Fig. 11. *General view and exine ornamentation of Jasione heldreichii pollen in SEM*

Table 2. *Palynomorphological data of Jasione heldreichii*

	Non-acetolised		**Acetolised**	
	M	**S**	**M**	**S**
P	21,54 µm	± 1,22 µm	18,80 µm	± 2,10 µm
E	22,00 µm	± 1,08 µm	21,31 µm	± 0,80 µm
P/E	0,97 µm		0,88 µm	
plg	3,99 µm	± 1,39 µm	3,56 µm	± 0,86 µm
plt	3,01 µm	± 0,96 µm	3,10 µm	± 1,37 µm
Ex	1,23 µm	± 0,32 µm	0,86 µm	± 0,27 µm
i	0,32 µm	± 0,11 µm	-	-

DISCUSSION

Jasione taxa have a distribution area in Europe, Northwest Africa and Northern and Western parts of Turkey [3]. The fact that the genus has such a limited distribution area, and more than half of the taxa whose distribution in Turkey is endemic, reveals the necessity of researching Turkey in terms of *Jasione* taxa. However, contrary to this requirement, taxonomic studies on the genus *Jasione* within the borders of Turkey are extremely limited. The only reference source for the diagnosis of our country's endemic *Jasione* taxa is the flora of Turkey.

When the flora of Turkey is examined, it is seen that a classical classification based on morphological characters is used in the separation of *Jasione* taxa. The current key is sufficient for the separation of *Jasione* taxa whose natural distribution is determined in Turkey. However, the extremely limited descriptions in the flora and the absence of any pictures or illustrations of the related taxa and the mentioned morphological characters are insufficient to identify any *Jasione* taxon that is not included in the key and is located close to these taxa (Table 1).

J. heldreichii, one of the 2 *Jasione* taxa endemic to Turkey, could be observed in the natural environment in the habitats specified in the literature, especially in the locations given in the recent literature (Fig.1). Considering the size of the observed populations and the distribution of the taxon in our country, it is seen that the it has a relatively wide distribution area in Turkey [5, 10, 12, 22, 24, 25, 26].

The morphological and anatomical studies and literature reviews have shown that the structural similarity is quite high in the stem cross-sections of the members of the *Jasione heldreichii* complex [27]. Unfortunately, it reveals that the data obtained in this context will be insufficient alone in the differentiation of taxa. At this point; The contribution of palynological characters in the study of the taxon is indisputable.

Today, morphological characters are generally used to classify plants. In addition to these characters, more robust results can be obtained by using various auxiliary characters. Data obtained from palynological studies are also such auxiliary characters. The characteristics of pollen reveal that palynological studies are useful in phylogenetic studies of plants because they are reliable and stable reproductive characters. It has been reported in the literature that aperture features and exine structure are among the essential criteria for determining the phylogenetic relationships of the species in pollen morphology studies.

Acknowledgement. This study was supported and funded by the Commission of Scientific Research Projects of Eskişehir Osmangazi University (Project name: *Jasione* L. (Campanulaceae) Cinsinde Bazı Taksonomik Çalışmalar (In Turkish). Project Number: 2018-1900 (201819060)).

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