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A Comparative Anatomical Study on Two Endemic *Sempervivum taxa* (Crassulaceae) in Türkiye

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Abstract − This paper comparatively examines the anatomical features of *Sempervivum brevipilum* and *Sempervivum gillianiae* using cross-sections from roots, stems, rosette leaves, sepals, and petals, alongside surface sections from rosette leaves, sepals, and petals. It identifies key distinguishing characters including: root periderm and cortex layer numbers; periderm width and length; root tracheid diameter; cortex parenchyma cell diameter; root tannin density (dense/not dense); stem cuticle layer structure; micropapillae presence/absence; stem cortex layer count; xylem configuration (straight/wavy); stem epidermis cell diameter; pith parenchyma and xylem tracheid diameter; stem cuticle thickness; mesophyll parenchyma diameter; stomatal width and length; rosette leaf upper/lower epidermis cell length, shape, and anticlinal wall structure; mesophyll layer number and shape in rosette leaves and sepals; margin structure of irregular cells adjacent to stomata in sepals and rosette leaves; sepal upper epidermis cell length; sepal lower epidermis cell width and length; petal cuticle thickness (thick/thin); petal epidermis cell diameter; petal epidermis anticlinal wall structure; petal mesophyll layer number; and tannin quantity in vegetative organs. This study confirms statistically significant differences ($p<0.05$) in select anatomical features. It also establishes that these findings align with earlier anatomical research within Crassulaceae and the genus *Sempervivum*, validating the diagnostic utility of these characters.

Keywords − *Sempervivum taxa, endemic, anatomical features, Crassulaceae*

1. Introduction

The Crassulaceae family is nearly cosmopolitan, and its species are distributed in South Africa, Mexico, Macaronesia, the Mediterranean, and the Himalayas [1, 2]. Mort et al. [3] suggested that this family spread from Southern Africa to the Mediterranean, Eastern Europe, and Asia, while the North African species spread to Macaronesia and where they later diversified. Family members generally consist of herbs, shrubs, and succulent plants. They are remarkable plants because they have water-storage tissue in their leaves and stems [4]. The majority of members of the family are leaf succulents. Due to their biological characteristics, these plants adapt well to life in ecologically harsh conditions.

The closeness of the Crassulaceae family to other families has caused some problems distinguishing its subfamilies and genera. On the other hand, the species of the family have high similarities in vegetative and generative organs (especially flower and embryonic features) [5]. Ham and $'\tau$ Hart [6] conducted chloroplast DNA restriction-site variation analyses to eliminate the problems related to subfamilies. They identified four

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subfamilies within the family: Cotyledonoideae, Sempervivoideae, Sedoideae, and Echeverioideae. Furthermore, Mort et al. [3] comprehensively studied the molecular phylogeny and evolution of the Crassulaceae family. Their research revealed that the family is divided into two subfamilies, Crassuloideae and Sempervivoideae, while Thiede and Eggli [2] divided this family into three subfamiles (Sempervivoideae, Crassuloideae and Kalanchoideae). Nevertheless, *Sempervivum* L., both according to Ham and $'\tau$ Hart [6] and Mort et al. [3] belong to Sempervivoideae subfamily.

The genus *Sempervivum* consists of approximately fifty species and seventeen hybrids [7-10]. The natural habitat of the species of this genus is the rocky and dry terrains of the high mountains of Central and Southern Europe, Southwest Asia, the Caucasus, and the Mediterranean [11, 12]. The endemism rate of the genus is 70% in the world and 87% in Türkiye. The first revision of the *Sempervivum* genus in Türkiye was made by Muirhead [13, 14], and eleven species and one variety were described. Since the publication of the Türkiye Flora, new taxa have been described from Türkiye, and the number of taxa has reached nineteen [7, 8, 15]. Thirteen of these are endemic to Türkiye. Besides, *Serpervivum minus* Turrill var. *glabrum* Wale was elevated to species level as *Sempervivum ekimii* by Karaer and Celep [16]. Detailed descriptions of taxa from Türkiye and information on ecology, distribution, and naturally occurring hybrids have been published by Neeff [15]. According to the Türkiye Flora [14], most *Sempervivum* species in Türkiye are local endemics, but differences between taxa are minimal.

Members of the Crassulaceae family (Orostachys L., *Sedum* L., *Hylotelephium* L., *Sempervivum*, etc.) have been preferred in the food industry, medicine, and ornamental plants for a long time. *Sempervivum* species (*Sempervivum marmoreum* Griseb.) are frequently used in landscape design and applications in home gardens, rock, and dry wall gardens as succulent ground covers [17, 18]. Additionally, some species (*Sempervivum davisii* Muirhead, *Sempervivum armenum* Boiss. Et Huet., *Sempervivum marmoreum,* and *Sempervivum tectorum* L.) are also preferred as folk medicine [19, 20]. Fresh juice obtained from the leaves of *Sempervivum* species treats skin complaints such as burns, wounds, and painful areas. Drinking tea prepared from *S. tectorum* leaves is recommended for ulcer treatment. The basal leaves of *Sempervivum sosnowskyi* Ter-Chatsch. are consumed as a salad by locals in NE Anatolia [21-23]. The biological activities and chemical composition of essential oils in fresh flowers, leaves, and stems of *Sempervivum brevipilum* Muirhead have been investigated. It has been reported that it has high antituberculostatic activity against *Mycobacterium smegmatis* [24].

Tannins are essential polyphenols found naturally in plants. They can be found in various plant structures such as wood, bark, root, leaf, fruit, and seed, and they regulate the development of these structures. They also protect vegetative organs against freezing, herbivores, pathogens, UV rays, and allelopathic and bactericidal effects [25]. Metcalfe and Chalk [26] suggested that tannins are widespread in non-woody stems, especially in the cortex, pith, and phloem of representatives of the Crassulaceae family. On the other hand, the presence of tannins has been detected in numerous members of representatives of this family, such as *Crassula multicava* Lemaira, *Echeveria venezuelensis* Rose, *Pachyphytum* sp., *Compactum* sp., *Kalanchoe* Adans. sp., *Bryophyllum daigremontianum* (Raym.-Hamet et perrier) A. Berger, *Sedum* sp., *Aeonium* sp. and *Sempervivum* sp. [27]. Although some morphological and palynological studies have been carried out on the *Sempervivum* genus, ecological, anatomical, and micromorphological studies on its species are insufficient. Therefore, this study aims to compare the anatomical features of the vegetative and generative organs and tannin content of the two aforementioned endemic *Sempervivum* taxa and evaluate whether any of the analyzed traits possess taxonomical significance.

The rest of the paper is organized as follows: Section 2 identifies plant specimens used in this study and the methods. Section 3 describes the anatomical features of the vegetative and generative organs of the examined taxa. Furthermore, the anatomical results obtained from this study were discussed in the literature, and the anatomical results of studies conducted with other members of the Crassulaceae family. In section 4, the study's conclusions are presented, and the future studies that need to be undertaken on taxa are mentioned.

2. Materials and Methods

Plant samples were taken from areas where taxa naturally spread, during flowering periods. Collection data related to plant materials are presented in Table 1. The identification of the collected taxa was conducted using the descriptions provided by Muirhead [14] and Eggli [28]. In *S. brevipilum*, sections were taken from 10 number of samples with a length of 20-22 cm in the summer season, while sections of *S. gillianiae* were taken from 10 number of samples with a length of 16-18 cm in the summer season. In anatomical examinations, cross-sections of the root, stems, rosette leaves, sepals, petals, and surface sections from the upper and lower surfaces of rosette leaves, sepals, and petals were taken and photographed. To enhance the visibility of tissues and cells in the vegetative and generative organs of the examined taxa, sartur reagent was applied to the cross and surface sections [29]. Width and length measurements of cells in each species' layers of stems, rosette leaves, sepals, and petals were made using an ocular micrometer. The means and standard deviations were determined according to the IBM SPSS Statistics 27.0 program. Similarities and differences in anatomical features of the examined taxa are given in Tables 2 and 3. Since the obtained data did not show normal distribution, the Mann-Whitney U test was used to reveal differences between similar groups more clearly. The test results are shown in Table 4. Tannin contents of taxa were determined by the Folin-Denis method [30, 31]. The measured absorbance value was converted to tannin amount in $GA_{mg}/extract_{mL}$ using the previously established standard curve (Figure 3).

Table 1. Locality information of the areas where *S. brevipilum* and *S. gillianiae* were collected

Taxa	Locality	Coordinates	Altitude	Collectors
S. brevipilum	Akdağ (Derebaşalan- Amasya) 07.08.2023	40°52'34.4"N 35°51'34.3"E	1500 m.	Tuğba Şahin
S. gillianiae	Sakarat Mountain (Yuva Village-Amasya) 02.07.2023	40°38'47.2"N 36°10'49.1"E	1100 m.	Tuğba Şahin

Table 2. Comparison of qualitative anatomical features of *S. brevipilum* and *S. gillianiae*

	Features of plant organs	*S. brevipilum*	*S. gillianiae*
ROOT	Periderm*	3-4 layered	4-5 layered
	Endodermis	Single-layered	Single-layered
	Cortex layer*	10-13 layered	19-22 layered
	Tannins in parenchyma cells*	Very dense	Sparse
	Xylem (trachea)	Chain shaped	Chain shaped
STEM	Epidermis cell*	Single-layered, quadrangular-shaped and large celled	Single-layered, quadrangular-shaped, and medium celled
	Cuticle layer*	Cuticle thick and densely micropapillated	Cuticle thick and without micropapillae
	Cortex layer*	20-23 layered, oval or hexagonal, large and small parenchymatic celled	20-22 layered, rounded shaped and large parenchymatic celled
	Pith region*	Wide area and large, oval, or hexagonal parenchyma celled	Wide area and large rounded parenchyma celled
	Vascular bundle elements*	Phloem narrow, xylem wide and straight	Phloem narrow, xylem wide and undulate
	Vascular bundle type	Open collateral	Open collateral
	Tannins in parenchyma cells	+	+
ROSETTE LEAF	Mesophyll structure	Unifacial	Unifacial
	Tannins in mesophyll	+	+
	Upper epidermis cell*	Rectangular-shaped and large-celled	Quadrangular-shaped and medium-celled
	Lower epidermis cell*	Rectangular-shaped and large-celled, anticlinal walls undulate	Quadrangular-shaped and large-celled anticlinal walls slightly undulate
	Margin structure of ordinary irregularly shaped cells next to the stomata *	Straight	Straight and slightly undulate
	Stomata type	Anisocytic	Anisocytic
	Tannins in ordinary irregularly shaped cells next to the stomata	+	+

*: different anatomical features between taxa

Table 2. (Continued) Comparison of qualitative anatomical features of *S. brevipilum* and *S. gillianiae*

SEPAL	Epidermis cell	Rectangular-shaped and large-celled, slightly undulate	Rectangular-shaped and large-celled, slightly undulate
	Mesophyll type*	7-8 layered, large oval parenchyma celled	5-6 layered, small parenchyma celled
	Tannins in the mesophyll	+	+
	Stomata type	Anisocytic	Anisocytic
PETAL	Epidermis cell*	Single layered, large, quadrangular-shaped, and dense undulate	Single layered, large, quadrangular-shaped, and slightly undulate
	Cuticle layer*	Thick and dense micropapillated	Medium thickness and micropapillated
	Mesophyll structure*	8-10 layered, oval-shaped, large and small parenchymatic celled	8-9 layered, oval-shaped, large and small parenchymatic celled
	Tannins in the mesophyll	+	+

*: different anatomical features between taxa

Table 3. Mean and standard deviation of the analyzed anatomical traits (µm) in the organs of *S. brevipilum* and *S. gillianiae*

	Features of plant organs	Number of samples	*S.brevipilum*	*S. gillaniae*
			Mean±Standard Deviation	
ROOT	Length of peridermal cell	10+10	28.40±5.48	47.50±5.89
	Width of peridermal cell	10+10	37.60±5.71	83.00±13.37
	Cortex parenchyma cell	10+10	35.20±6.47	63.00±11.59
	Phloem cell	10+10	18.20±2.20	-
	Xylem (Trachea cell)	10+10	18.08±1.99	14.30±2.11
	Cambium	10+10	9.90±1.52	-
	Pith parenchyma cell	10+10	106.00±20.11	76.00±12.64
STEM	Epidermis cell	10+10	39.00±6.58	30.20±2.04
	Cortex parenchyma cell	10+10	79.00±16.63	90.00±16.32
	Pith parenchyma cell	10+10	106.00±20.11	76.00±12.64
	Cambium	10+10	16.10±2.96	-
	Cuticle	10+10	12.60±1.89	12.00±1,16
	Phloem cell	10+10	16.60±3.06	-
	Xylem (Trachea cell)	10+10	28.80±3.15	11.20±1.75
ROSETTE LEAF	Upper epidermis cell width	10+10	64.00±8.43	70.50±8.23
	Upper epidermis cell length	10+10	43.50±3.37	31.20±4.77
	Lower epidermis cell width	10+10	58.50±10.55	54.00±5.67
	Lower epidermis cell length	10+10	44.00±4.59	26.70±2.31
	Mesophyll (Parenchyma cell)	10+10	202.00±52.02	154.00±20.65
	Stomata length	10+10	46.00±6.32	40.80±4.54
	Stomata width	10+10	37.60±3.86	21.36±2.10
FLOWER	Sepal cuticle	10+10	4.68±0.77	-
	Sepal upper epidermis cell length	10+10	34.00±3.94	24.80±4.13
	Sepal upper epidermis cell width	10+10	58.50±7.83	52.00±4.21
	Sepal lower epidermis cell width	10+10	58.50±7.83	38.40±4.29
	Sepal lower epidermis cell length	10+10	35.00±4.08	24.00±3.26
	Sepal mesophyll (parenchyma cell)	10+10	106.00±12.64	-
	Petal cuticle	10+10	7.20±1.03	-
	Petal upper epidermis cell diameter	10+10	50.40±13.62	40.00±5.96
	Petal lower epidermis cell diameter	10+10	46.40±7.82	28.40±3.97
	Petal mesophyll (parenchyma cell)	10+10	56.80±8.59	64.00±13.59

Table 4. Mann-Whitney U results of anatomical measurements of *S. brevipilum* and *S. gillianiae*

	Features of plant organs	Number of samples	Mann-Whitney U test	
			U	p (*p<0.05)
ROOT	Cortex parenchyma cell	20	0.000	0.000*
	Width of peridermal cell	20	0.000	0.000*
	Length of peridermal cell	20	0.000	0.000*
	Xylem-Trachea cell	20	8.000	0.001*
STEM	Cortex parenchyma cell	20	34.000	0.219
	Pith parenchyma cell	20	9.000	0.002*
	Epidermis cell	20	10.000	0.002*
	Xylem-Trachea cell	20	0.000	0.000*
ROSETTE LEAF	Cuticle	20	40.000	0.441
	Lower epidermis cell width	20	33.000	0.191
	Lower epidermis cell length	20	0.000	0.000*
	Upper epidermis cell width	20	28.500	0.096
	Upper epidermis cell length	20	2.000	0.000*
	Cuticle	20	17.500	0.012*
	Mesophyll (parenchyma cell)	20	20.500	0.025*
	Stomata width	20	0.000	0.000*
	Stomata length	20	24.500	0.047*
SEPAL	Upper epidermis cell width	20	25.000	0.056
	Upper epidermis cell length	20	4,000	0.000*
	Lower epidermis cell width	20	0.000	0.000*
	Lower epidermis cell length	20	0.000	0.000*
PETAL	Upper epidermis cell diameter	20	26.000	0.067
	Lower epidermis cell diameter	20	1.000	0.000*
	Mesophyll	20	33.500	0.208

3. Results and Discussion

In this study, the anatomical features of root, stem, rosette leaf, sepal, and petal, potantially important in distinguishing *S. brevipilum* and *S. gillianiae* distributed in Türkiye, are given in detail and comparatively. Since there are not many anatomical studies in the literature (only three anatomical studies were found) on the species of this genus, our findings were additionally compared with the findings of studies conducted with other members of the Crassulaceae family.

In the root cross sections of the two taxa, the periderm is 3-4 layered in *S. brevipilum* and 4-5 layered in *S. gillianiae* Muirhead. The cortical parenchyma cells are oval-shaped, and while parenchyma is 10-13 layered in *S. brevipilum*, it is 19-22 layered in *S. gillianiae* (Figures 1 A-C and Figures 2 A, B). More tannin was observed in the parenchyma cells in *S. brevipilum* than in the other taxa analyzed (Figure 3). In both analyzed

taxa, the xylem is large and clearly visible in the vascular bundles of the pith region, while the phloem occupies a much narrower area. The trachea is in the radial form in both taxa (Figure 1 D and Figures 2 C-E). Similar anatomical features were reported in the root of *Sedum telephium* L. ssp. *maximum* (L.) Krock., which is characterized by a thin periderm and numerous vascular tissue bundles [32]. Ulcay [33] found the periderm of *Sedum acre* L. to be 7-8 layered, *Sedum album* L. to be 3-4 layered, and *Sedum pallidum* M. Bieb. to be 2-3 layered. Kirilenko [34] saw radial conduction tissue bundles in the roots of *Crassula perforata* Thunbg and *Crassula socialis* Schonland. The root anatomical features observed in this study are consistent with the above-mentioned results regarding family members.

Shahrestani et al. [35] reported that the features that provide essential taxonomic evidence for the separation of genera are the presence or absence of trichomes on the stem epidermis, the presence or absence of tannin secretory cells in the stem cortex and pith region, the number of collenchyma layer, the presence or absence of endodermis, the presence or absence of xylem and stomata in the stem. Some researchers have suggested that anatomical characteristics in stems and leaves are important in distinguishing plant taxa [36, 37].

When the cross sections of the stems were examined, the cuticle of *S. brevipilum* was thick and micropapillated, and the epidermis was single layered, consisting of large, quadrangular cells. The cuticle of *S. gillianiae* was medium-thick, and the epidermis was single-layered, quadrangular cells, and consisting of medium large. The cortex was 20-23 layered in *S. brevipilum* and 20-22 layered in *S. gillianiae* (Figures 1 E, F and Figures 2 F, G, K). Tannins and large parenchyma cells were densely seen in the cortex of both taxa. In these taxa, contrary to phloem, the xylem occupied a large area (Figure 1G and Figure 2 H). In *S.gillianiae*, the xylem is wavy (Figure 2 F), while the xylem is straight in *S. brevipilium.* The pith region is wider in *S. brevipilium* (Figure 1 H). Anatomical features such as the structure of the cuticle layer in the stem, whether the epidermis cells are large or small, whether micropapillae are present or not, and whether the xylem is wavy or not can be used as useful characters in distinguishing these two taxa.

Kirilenko [38] determined that the outermost part of the stem of *S. tectorum* has a thick cuticle and a single-layered epidermis. The cortex is composed of large 17-22-layered parenchyma cells. 1-2-layered collenchyma was found immediately under the epiderma. Numerous vascular bundles were observed in the stem. The presence of large intercellular spaces in the cortex was detected. Similar features were seen in the stem of the examined taxa (except for large intercellular spaces).

Vorobojev et al. [39] examined, and compared, anatomical features of stem of *Sempervivum globiferum* L. (=*Jovibarba globifera* (L.) J.Parn.), *S. tectorum* and *Sempervivum ruthenicum* Schnittsp. & C. B. Lehm. distributed in Ukraine [39]. The anatomical differences between *S. globiferum* from *S. tectorum* and *S. ruthenicum* are the grooved shape of the two-layered epidermis on the stem. The circular shape of the vascular bundles on the stem, the poor development of the xylem and phloem tissues were reported to be similar features in these three *Sempervivum* taxa. The results of Vorobej et al. [39] are consistent with the findings of this study.

Ulcay [33] investigated the anatomical features of some *Sedum* species distributed in Türkiye. When the stem cross-section was examined, it was found that the epidermis was single-layered and round-shaped in *Sedum acre*, single-layered and epidermis cells rectangular or round-shaped in *Sedum album,* and single-layered, with rectangular or circular shaped epidermis cells in *Sedum pallidum.* The cortex is reported to be 13-14 layered in *S. album*, 10-11 layered in *S. pallidum,* and quite wide in *S. acre*. The vascular bundles in the stems were in the form of regular rings. The anatomical features of *S. acre*, which is distributed under different ecological conditions in Southern and Central Kazakhstan, were investigated by Akhmetzhanova et al. [40]. It has been found that the vascular bundles in the stem are in a regular ring, single-layered of the epidermis and 7-8 layered of cortex.

Shahrestani et al. [35] reported anatomical features of 22 taxa of *Sedum s.l.* (Crassulaceae) distributed in Iran that could provide useful data in solving the current problem regarding the taxonomy and nomenclature of *Sedum s.l.* Their study found that species belonging to the genera *Phedimus* Raf., *Prometheum* (A. Berger) H.

Ohba, and *Hylotelephium* H. Ohba could be readily distinguished from other *Sedum s.l.* taxa based on their anatomical characteristics. In addition, it was found that *Sedum* and *Epeteium* sections were close to each other regarding anatomical features. Tannins and starches with different sizes and densities were detected in the parenchyma cells of some species' cortex and pith region of the stem. With the exception of *Sedum caespitosum* (Cav.) DC., 1-3 layered collenchyma was observed beneath the stem epidermis in analyzed taxa. The parenchymatic cortex is found between collenchyma and endoderma. The pith region was observed in the stem of all examined taxa (except *Sedum callichroum* Boiss.). Stomata were observed only in the stem epidermis of *Phedimus obtusifolious* (C. A. Meyer) *'τ* Hart*, Sedum tenellum* M. Bieb., *Sedum subulatum* (C. A. Meyer) Boiss. and *Sedum annum* L. It was also emphasized that the xylem in the stem of the taxa differed, and this feature was important in distinguishing the taxa. The stem anatomy of *Sedum s.l.* taxa and examined *Sempervivum* taxa showed similarities, such as xylem structure and presence of tannins, but also differences, including the presence of collenchyma layers and starch.

Abdel-Raouf [41] studied the anatomical features of 15 *Kalanchoe* species and their taxonomic importance. It was observed that the cuticle on the stem was relatively thick in eight taxa, as in *Kalanchoe tubiflora* Raym.-Hamet, and thin in other taxa. Hypodermis was recorded in *Kalanchoe daigremontiana* Raym.-Hamet et H.Perrier, and cork tissue examined were found only in *Kalanchoe blossfeldiana* Poelln. and *Kalanchoe beharensis* Drake. The cortex was in a wide area in *Kalanchoe pumila* Baker, and a narrow area in *K. tubiflora*, among others. The cortex of most taxa has well-developed collenchymatic cells, as in *K. tubiflora*. There are vascular bundles in three taxa, as in *Kalanchoe roseleaf* Adans., secretory ducts in *K. beharensis* and some species, and druse crystals in some species. Similar results (the stem cortex is generally composed of succulent and strongly developed and watery parenchyma or contains weakly developed collenchyma, the cork tissue being in the epidermis or in the subepidermal layer as in members of *Kalanchoe* and *Sedum* genera) were also determined by Metcalfe and Chalk [26] in other members of the Crassulaceae family. Abdel-Raouf [41] reported several stem features in *Kalanchoe* taxa, including the presence of cork tissues and hypodermis layers, pigmented epidermis cells, collenchymatic and storage cells in the cortex, secretory channels, and druse crystals, that differed from those observed in the examined taxa in this study.

Comprehensive studies on the leaf anatomical features and epidermal structures of species in the genus *Sempervivum* are quite limited [38, 39, 42]. However, studies have been conducted on the leaf epidermal structures of genera such as *Kalanchoe, Crassula*, *Rhodiola, Echeveria*, and *Sedum*, etc. [33-35, 41, 43-48]. It has been determined that leaves are amphistomatic and stomata are mostly anisocytic [2, 32-34, 38-40, 42, 44, 45]. In addition, it has been reported that stomata are generally more numerous in the lower epidermis in many species of the Crassulaceae family [49].

In cross-section, the rosette leaves of taxa analyzed in this study exhibit upper and lower epidermis cells of nearly equal size (Table 3). In S. *brevipilum*, the epidermis cells are rectangular, whereas in *S. gillianiae*, they are quadrangular. The anticlinal walls of the lower epidermis cells in *S. gillianiae* are slightly undulated. In the surface sections of the rosette leaves, anisocytic stomata were found in the upper and lower epidermis of the two taxa (Tables 2 and 3, Figure 1N and Figure 2 N). In both taxa, stomata are denser in the lower epidermis than in the upper epidermis. Abundant tannins were found in both taxa's epidermis cells around the stomata. While the anticlinal walls of adjacent stomatal cells are mostly straight in *S. brevipilum*, they are slightly undulate in *S. gillianiae* (Figure 1N and Figure 2N). In both taxa, the rosette leaves are unifacial, with a mesophyll composed of cells that are relatively uniform in shape, showing no clear differentiation into palisade and spongy parenchyma (Figures 1 K, L, M, and Figures 2 L, M) and abundant tannins are found in the parenchyma cells. The mesophyll layer of *S. brevipilium* comprises of 15-17 layered, large, oval, or hexagonal-shaped parenchyma cells (Tables 2 and 3, Figures 1 K, L). The mesophyll layer of *S. gillianiae* is quite wide and consists of 17-19 layered, large, oval-shaped parenchyma cells (Figures 2 L and M). The vascular bundles in the middle of the mesophyll are smaller in *S. gillianiae*. The findings in the literature showed that both taxa preserved the characteristic leaf anatomical features of the *Sempervivum* genus.

Jovanović et al. [42] studied the epidermal structures of rosette leaves of yellow-flowered *Sempervivum* species distributed in the Balkan Peninsula and grouped under two complexes as *Sempervivum ciliosum* (*Sempervivum ciliosum* Craib*, Sempervivum jakucsii* Penzes*, Sempervivum klepa* Micevski*, Sempervivum octopodes* Turrill and *Sempervivum galicicum* (Sm.) Micevski) and *S. ruthenicum* (*Sempervivum ruthenicum, Sempervivum leucanthum* Pancic, *Sempervivum kindingeri* Adamovic and *Sempervivum zeleborii* Schott). Eighteen quantitative characters describing the lower and upper epidermal structures were analyzed. Differences between the species of the two complexes were observed in the anticlinal walls and the number and length of epidermis cells. Statistical analysis showed that the differences in quantitative characteristics of the epidermal structures were significant ($p<0.05$), supporting their relevance in the differentiation of the complexes. Furthermore, the epidermis cells of the leaves of species in the *S. ciliosum* complex (especially those on the abaxial surface) are mostly undulated, with anticlinal walls that are straight or rarely undulated (*S. ciliosum)*

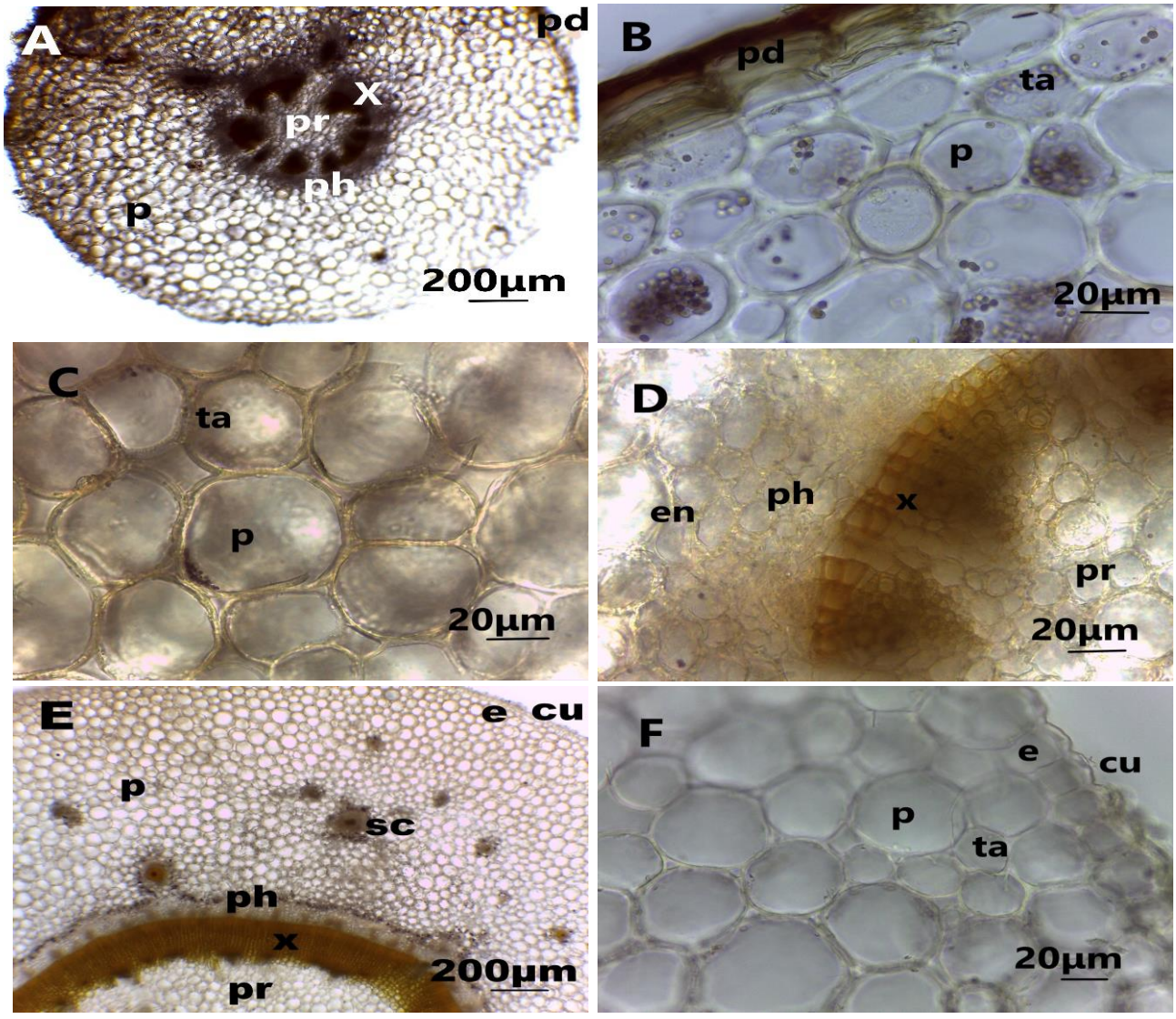

Figure 1. Anatomical structures of vegetative and generative organs of *Sempervivum brevipilum*. A. General structure of the root, B. Periderm and cortex regions of the root, C. Cortex region of the root, D. Phloem, xylem and pith regions of the root, E. General structure of the stem, F. Epidermis and cortex regions of the stem, pd: periderm; ph: phloem; x: xylem; p: parenchyma; ta: tannin; pr: pith region; en: endodermis; cu: cuticle; e: epidermis; sc: secretory channels.

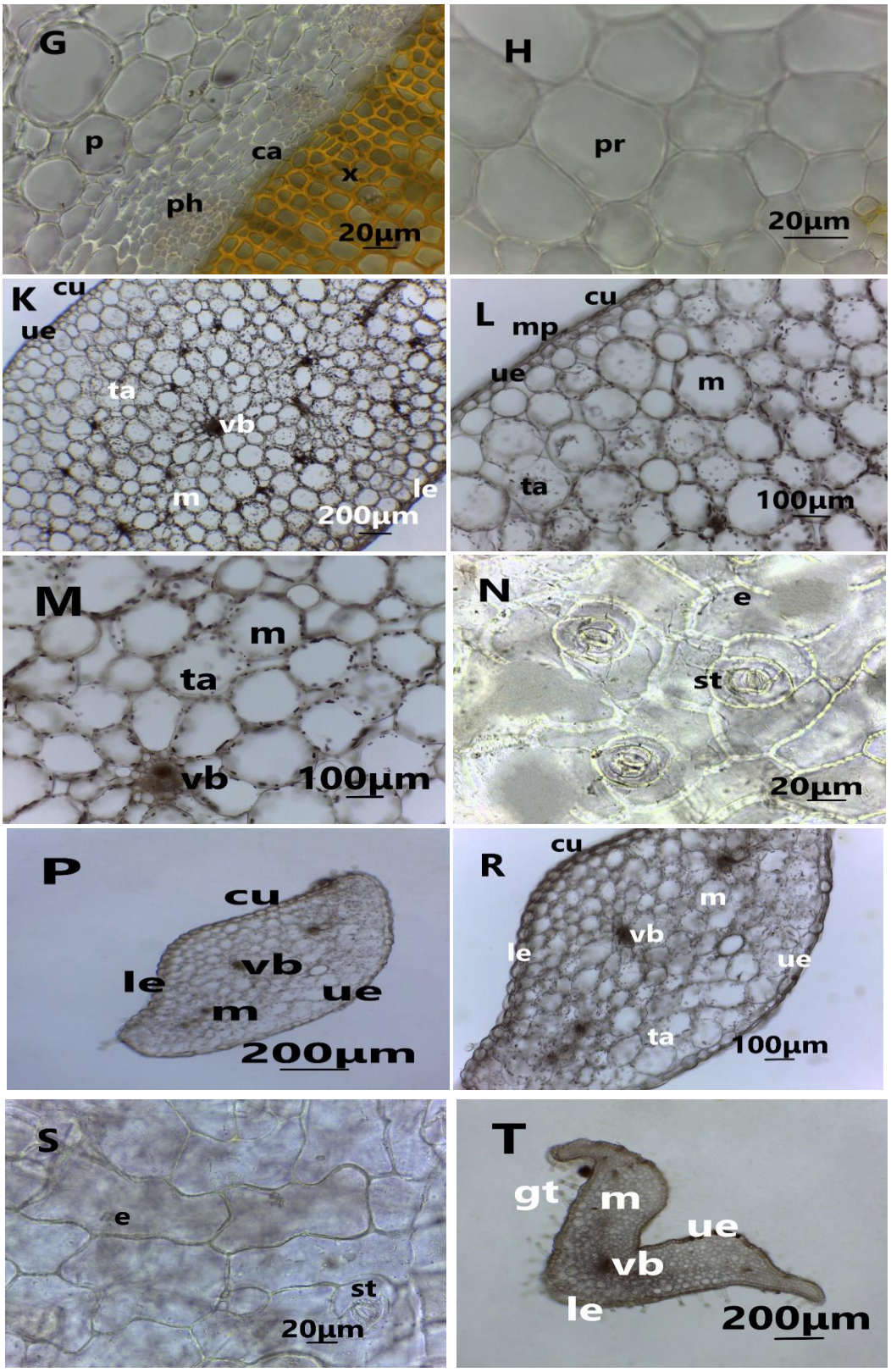

Figure 1. (Continued) Anatomical structures of vegetative and generative organs of *Sempervivum brevipilum*. G. Phloem and xylem regions of the stem, H. Pith region of the stem, K. General structure of rosette leaf, L. Mesophyll region of rosette leaf, M. Mesophyll region and vascular bundle, N. Stomata of rosette leaf, P and R. General structure of sepal, S. Epidermis and stomata cells of sepal, T. General structure of petal, ph: phloem; x: xylem; p: parenchyma; ta: tannin; pr: pith region; cu: cuticle; e: epidermis; ca: cambium; m: mesophyll; vb: vascular bundle; ue: upper epidermis; le: lower epidermis; st: stomata; mp: micropapillae; gt: glandular trichomes

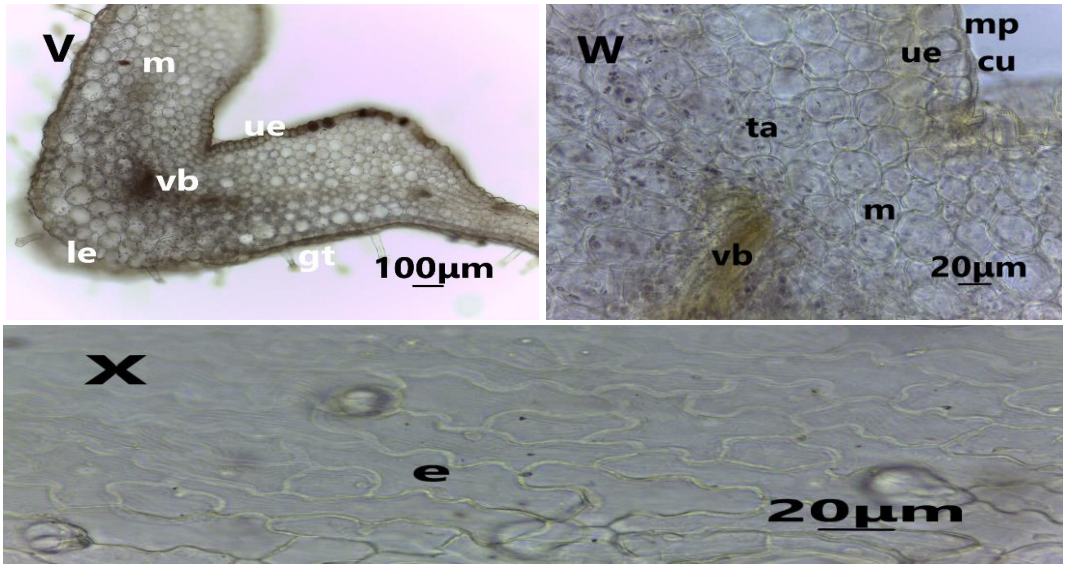

Figure 1. (Continued) Anatomical structures of vegetative and generative organs of *Sempervivum brevipilum*. V. General structure of petal, W. Epidermis and mesophyll region of petal, X. Epidermis cells of the petal, ta: tannin; cu: cuticle; e: epidermis; m: mesophyll; vb: vascular bundle; ue: upper epidermis; le: lower epidermis; mp: micropapillae; gt: glandular trichomes

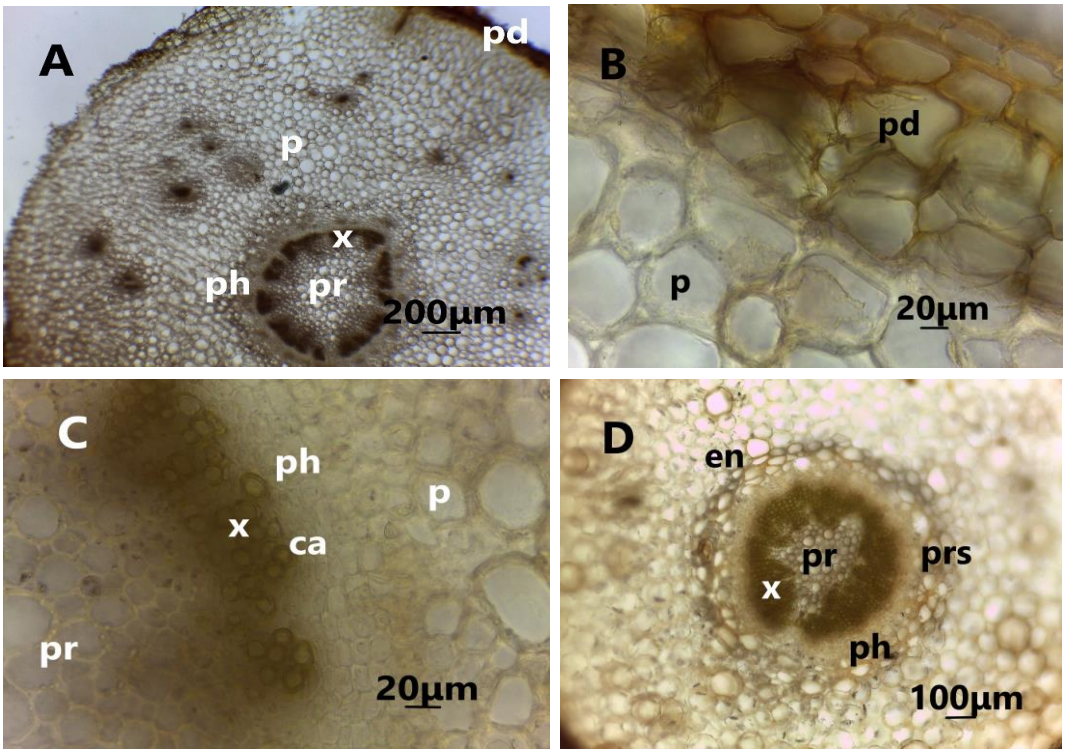

Figure 2. Anatomical structures of vegetative and generative organs of *Sempervivum gillianiae*. A. General structure of the root, B. Periderm and cortex regions of the root, C and D. Phloem, xylem, and pith regions of the root, pd: periderm; ph: phloem; x: xylem; p: parenchyma; pr: pith region; en: endodermis; prs: pericycle; ca: cambium.

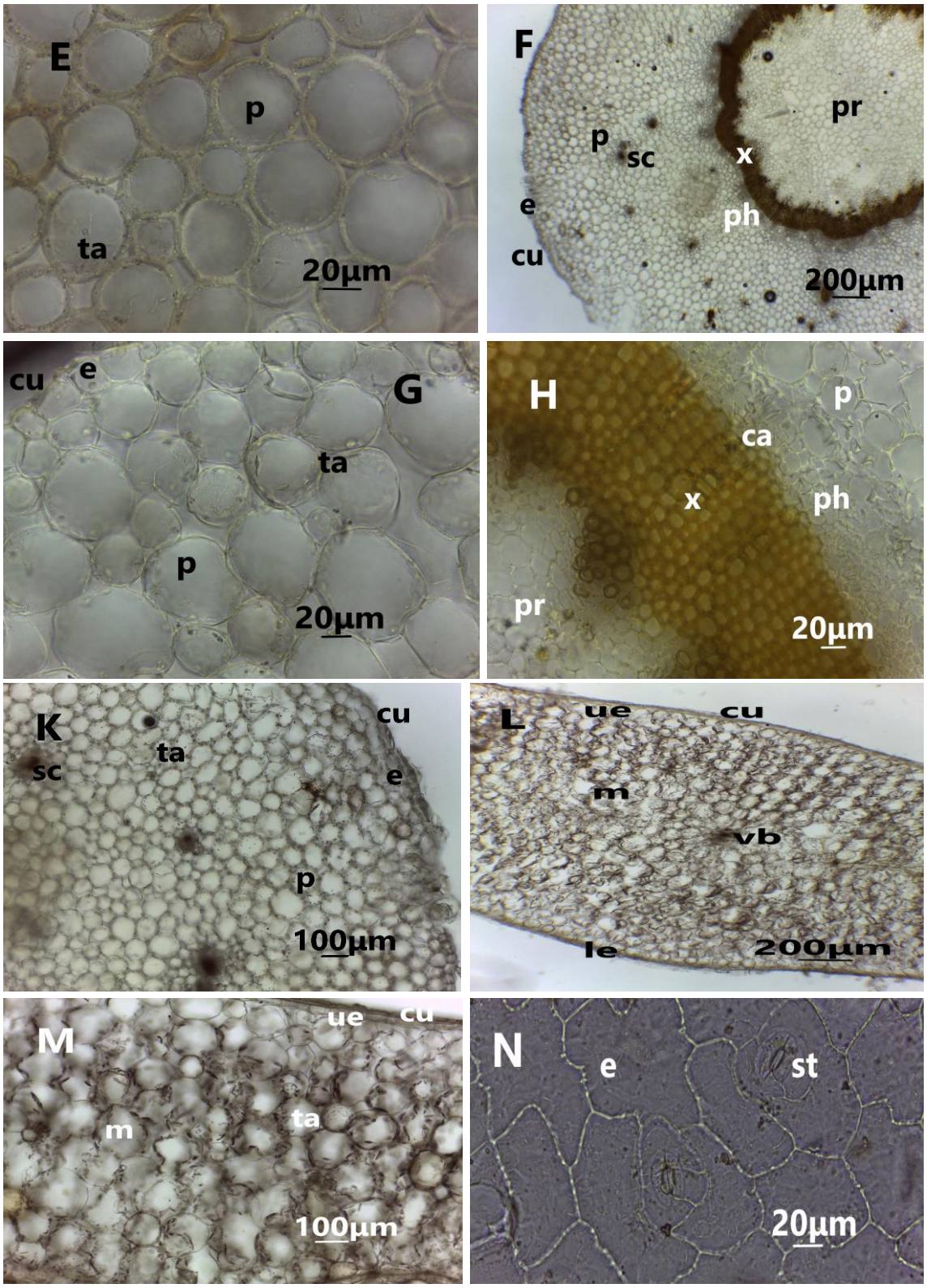

Figure 2. (Continued) Anatomical structures of vegetative and generative organs of *Sempervivum gillianiae*. E. Cortex region of the root, F. General structure of the stem, G and K. Epidermis and cortex regions of the stem, H. Phloem and xylem regions of the stem, L. General structure of rosette leaf, M. Mesophyll region of rosette leaf, N. Stomata of rosette leaf, ph: phloem; x: xylem; p: parenchyma; ta: tannin; pr: pith region; cu: cuticle; e: epidermis; ca: cambium; sc: secretory channels; m: mesophyll; vb: vascular bundle; ue: upper epidermis; le: lower epidermis; st: stomata.

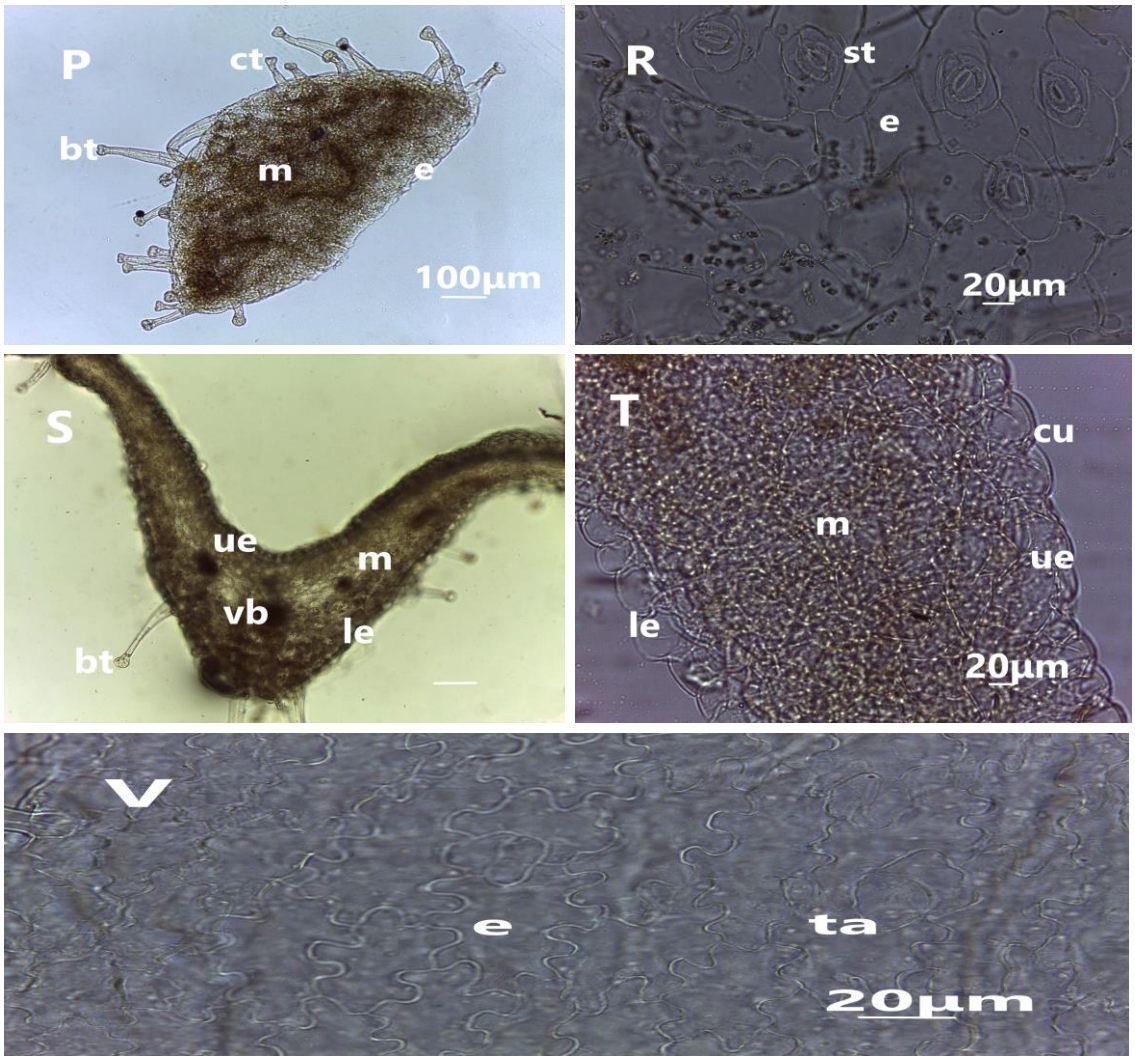

Figure 2. (Continued) Anatomical structures of vegetative and generative organs of *Sempervivum gillianiae*. P. General structure of sepal, R. Epidermis and stomata cells of sepal, S. General structure of petal, T. Epidermis and mesophyll region of petal, V. Epidermis cells of petals, ta: tannin; cu: cuticle; e: epidermis; m: mesophyll; vb: vascular bundle; ue: upper epidermis; le: lower epidermis; st: stomata; bt: multicellular biseriate trichome; ct: capitate trichome

In contrast, the anticlinal walls of the epidermis cells of species of the *S. ruthenicum* complex are straight and slightly undulate. Species of the *S. ciliosum* complex generally have longer and wider epidermis cells on both adaxial and abaxial surfaces. In the *S. ciliosum* complex, the highest number of epidermis cells was observed on the abaxial (lower) surface, while in the *S. ruthenicum* complex, the highest number of epidermis cells was observed on the adaxial (upper) surface. It was determined that the rosette leaves are amphistomatic, with anisocytic and scattered stomata in all species of analyzed complexes. However, there were differences in the length, width, and number of guard cells in stomata among species. It was reported that there is no significant difference in the length and width of the guard cells on the upper and lower surfaces of rosette leaves in individuals of the same species. The rosette leaves of species in the *S. ruthenicum* complex contain longer guard cells than those in the *S. ciliosum* complex. More numerous stomata were observed on the adaxial surface of the *S. ruthenicum* complex, while more stomata were observed on the abaxial surface of the *S. ciliosum* complex. In the examined taxa, the leaves are amphistomatic, and the stomata are anisocytic. It was determined that more stomata existed in the lower epidermis of the examined two taxa. Important distinguishing anatomical characters were detected between these two taxa in the length, shape, and wall structure of the upper and lower epidermis cells of the rosette leaf, the width and length of the stomata, and the wall structure of the neighboring cells of the stomata in the rosette leaves. These differences are also statistically significant

($p<0.05$). In this study, although the anticlinal walls of the cells adjacent to the stomata and the lower epidermis cells are slightly undulated in *S. gillianiae*, the edges of the cells adjacent to the stomata and the lower epidermis cells are straight in *S. brevipilum*. Thus, our data agrees with the data obtained by Jovanović et al. [42].

Vorobej et al. [39] determined the leaf anatomical structure of *S. globiferum* and reported that the leaves were amphistomatic, the stomata were anisocytic, and the leaves were unifacial regarding the mesophyll position, which is congruent with findings of this study.

Kirilenko [38] reported that the rosette leaves of *S. tectorum* have a single-layered epidermis on both the upper and lower surfaces, covered by a thin cuticle. Anisocytic stomata were also present on both surfaces but were more numerous on the lower epidermis. The rosette leaves were isolateral regarding the mesophyll structure. In other words, there was a weak differentiation between palisade and spongy parenchyma. The intercellular spaces in the mesophyll were quite large. The vascular bundles were quite small, and parenchymatic bundle sheaths were observed around them. Mucilage-containing cells were observed in the epidermis cells and mesophyll cells. Although some results of Krilenko [38] are compatible with our results (e.g., anisocytic type stomata), other results (e.g. isolateral mesophyll structure, large intercelluar spaces, mucilage-containing cells in the mesophyll) differ from our observations. This can be attributed to the distribution of taxa in habitats with different climates and soil conditions.

Shahrestani et al. [35] determined that the cells of the lower epidermis were larger compared to the ones in upper epidermis in *Sedum lencoranicum* Grossh., and *Sedum gracile* C.A. Mey. Hypodermis was observed in *Phedimus* sp., *Prometheum* sp., *Hylotelephium* sp., *S. lenkoranicum, Sedum rubens* L. and *Sedum annum*. The mesophyll was unifacial in all analyzed taxa. Large mucilaginous cells associated with parenchymatous cells were detected in *Sedum hispanicum* L., *Sedum pentapetalum* Boriss., *Sedum album*. Storage cells were found in the mesophyll (especially around the vascular bundles) in members of the *Phedimus* genus. Our findings (unifacial mesophyll) are parallel to the findings of Shahrestani et al. [35]. Although the taxa are in different genera, their similar anatomical features can be attributed to their growth in similar habitats and climatic conditions.

Ulcay [33] reported that the anticlinal walls of the epidermis cells were undulated in the leaf of *Sedum acre, S. pallidum* and *S. album*. The mesophyll tissue was undifferentiated and consisted of round-shaped parenchyma cells. In the center of the leaf of *S. acre*, the vascular bundles were regular, and the number of vascular bundles was 5-6. *Sedum album* exhibited 10–13 vascular bundles, whereas *S. pallidum* had 9–10 vascular bundles that were scattered. Anisocytic stomata was observed in all three taxa. It was determined that there were significant differences in the width and length of the stomata located in the upper and lower epidermis of the species. Similar leaf anatomical features mentioned above were also found in the examined *Sempervivum* species.

The leaf anatomical features of 15 *Kalanchoe* taxa were investigated by Abdel-Raouf [41]. The cuticle layer is thick in *Kalanchoe thrysiflora* Raym et Hamet, but the cuticle layer is thin in *K. beharensis* and most other examined species. Large epidermis cells were seen only in *K. beauverdii*, and hypodermis was seen in *K. beharensis* and *K. tubiflora*. Multicellular branched trichomes were also recorded in *K. tomentosa* and *K. beharensis*. Same author confirmed that mesophyll was generally homogenous in most analysed *Kalanchoe* species, with exception of *K. tomentosa* Baker and *K. beauveradii* that had heterogenous mesophyll. Storage cells were found in the mesophyll of *K. tubiflora*, secretory ducts in *K. marmorata* Baker, and druse crystals in some taxa of *K. beharensis*. The xylem is arranged in crescent-shaped in *K. tomentosa* and ring-shaped in other taxa. Although well-developed bundle sheaths are not found in CAM plants, collenchymatic bundle sheaths were found in the *Kalanchoe* taxa. In addition, Balsamo and Uribe [50] reported that the thickened cuticle, bundle sheath, and mesophyll are not differentiated as palisade and spongy parenchyma in the leaf of *K. dagremontiana.* Findings of this study are almost compatible with the results of Abdel-Raouf [41] and Balsamo and Uribe [50].

The leaf characters of succulent species *Crassula perforata, Crassula socialis* (Crassulaceae), *Senecio rowleyanus* H. Jacobsen, and *Senecio herreianus* Moritz Kurt Din. (Asteraceae) collected from extreme habitats were comparatively studied [34]. Many common features (characteristic features of the external structure, weak differentiation of the mesophyll, significant development of water-carrying tissue) and different features (type of leaf mesophyll, type of stomatal apparatus, degree of development of conductive system) resulting from adaptation to growth conditions were reported in taxa.

The structure of the leaf of *Kalanchoe pumila* was studied by Chernetskyy and Weryszko-Chmielewska [44]. In *K. pumila*, the amphistomatic leaf had a striated thick cuticle layer and a single-layered epidermis. Stomata were anisocytic. Vascular bundles were collateral, and bundle sheaths of parenchyma cells were seen around them. There was no palisade and sponge differentiation in the mesophyll layer. However, the mesophyll layer comprises large-celled (water-carrying, CAM-type) and small-celled mesophyll cells. Some of our findings (anisocytic stomata, unifacial mesophyll, collateral vascular bundles, amphistomatic leaves) are compatible with those of Chernetskyy and Weryszko-Chmielewska [44].

Moreira et al. [45] investigated the leaf anatomy of *Kalanchoe pinnata* Pers. and *Kalanchoe crenata* (Andrews) Haw. members growing in shade and sun. In *K. pinnata*, the anticlinal walls of the epidermis cells are undulate in both sun and shade members. However, these folds are more pronounced on the adaxial surface than on the abaxial surface in sun leaves. In sun leaves of *K. crenata*, the anticlinal walls of the epidermis cells are straight or slightly undulated on the adaxial surface while the anticlinal walls of the epidermis cells are undulated on the abaxial surface. In both species, stomata are anisocytic, and leaves are amphistomatic. It was observed that the rosette leaves of the studied *Sempervivum* species were amphistomatic, and the stomata were anisocytic. The members growing in shade and sun of both *Kalanchoe* species have more stomata on the abaxial surface than on the adaxial. Epidermis cells on the leaf margins are larger than those in other parts of the leaf. The epidermis is covered with a thin cuticle. The epidermis of the forms of *K. pinnata* growing in sun conditions was found to be thicker than those of shade plants. However, no significant differences were observed in epidermis thickness characteristics in members of *K. crenata* growing in sun and shade. The mesophyll of both species is homogeneous. The mesophyll thickness was determined to be greater in sun plants due to the collenchyma layer. Hydathodes were observed at the edges of the leaf lamina of both species. As a result, some differences were determined despite the similarities in the anatomical features of these two species (*K. pinnata* and *K. creneta*).

When the cross-sectional of sepals were examined, large, rectangular-shaped lower and upper epidermis cells were obtained in both taxa. The anticlinal walls of the epidermis cells in *S. brevipilium* were slightly undulate, while the anticlinal walls of the epidermis cells were straight in *S. gillianiae*. The mesophyll tissue of sepals was composed of 10-12 layered, ovoid, large parenchyma cells in *S. brevipilium* and 5-6 layered, small parenchyma cells in *S. gillianiae* (Figures 1 P, R and Figures 2 P, R). Vascular bundles were seen in the mesophyll tissue of both taxa. The stomata of both taxa are anisocytic (Figure 1 S and Figure 2 R).

Regarding the anatomical features of the petals, in *S. brevipilum* anticlinal walls of the epidermis cells are very undulate (Figure 1 X), cells are quadrangulary shaped, and the cuticle is thick and densely micropapillated, while in *S.gillianiae*, the anticlinal walls of the epidermis cells are undulate (Figure 2V), cells are quadrangulary shaped, the cuticle is of medium thickness and micropapillated. The mesophyll tissue between the two epidermis layers comprises of 8-10 layered, oval-shaped, large, and small parenchyma cells in *S. brevipilum* (Figures 1 T, V and W). In *S. gillianiae*, the mesophyll tissue comprises of 8-9 layered, large, and small, oval-shaped parenchymatic cells (Figures 2 S, T). In both taxa, there are vascular bundles in the mesophyll. The differences in epidermal traits of flower part were found to be smaller compared to differences in vegetative parts. This could be explained by the less pronounced phenotypic plasticity of generative organs

The number of tannins in the roots, stems, rosette leaves, and flowers of analyzed taxa were determined as gallic acid equivalent (GAE, mg/mL) using the Folin-Denis method. However, it was found that there were differences in tannin amounts between vegetative and generative organs. The highest tannin content was found

in flowers in both taxa. The amount of tannin in both *S. brevipilum* and *S. gillianiae* flowers was calculated as 0.45 ± 0.02 mg/mL GAE (Figure 3).

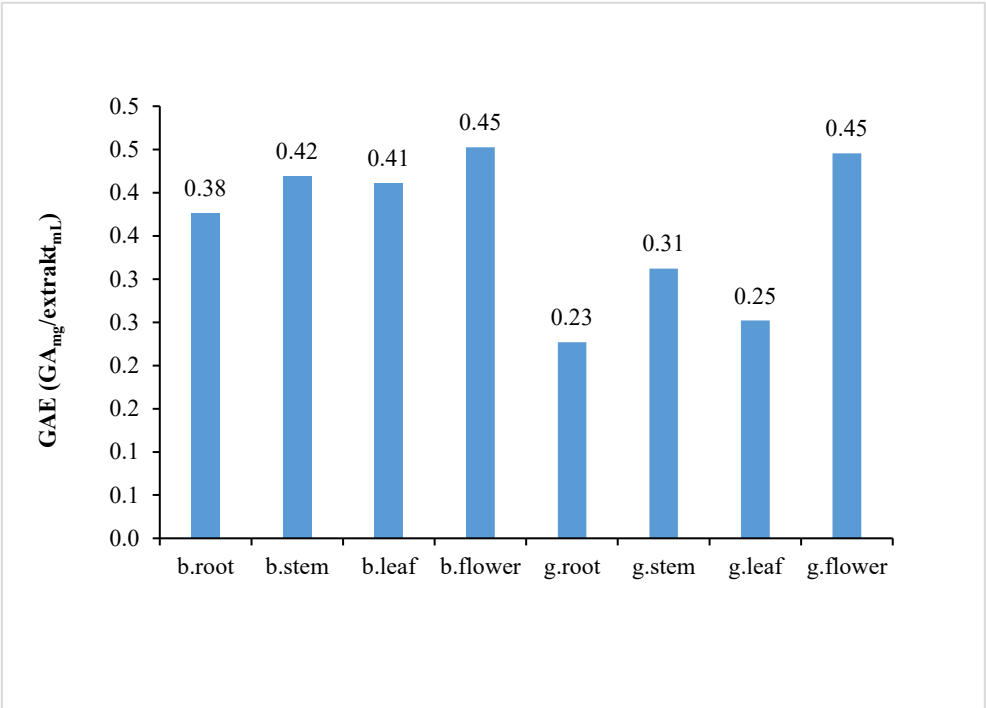

Figure 3. Tannin contents in vegetative and generative organs of *S. brevipilum* and *S. gillianiae*. b.root, b.stem, b.leaf, b.flower values of *S. brevipilum*. g.root, g.stem, g.leaf, g. flower values of *S. gillianiae*

Shahrestani et al. [35] reported that tannin storage cells in petioles and leaves provided important taxonomic evidence. In *K. pumila* leaves, anthocyanin pigments, tannin, epicuticular wax, and calcium deposits were seen. These structures serve to protect the leaves against harsh environmental conditions [44]. It was reported that there were differences in the distribution of phenolic compounds, especially in the subepidermal layer. It was stated that these different features could help identify species at the anatomical level and, therefore, the quality control of herbal medicines made by *K. pinnata* and *K. creneta* [45]. Tannins were found in all individuals of *Echeveria* aff. *gigantea* (a complex species) Rose and Purpus, especially in the epiderma, mesophyll, and vascular bundles of leaves. The distribution and amounts of tannins were essential in separating some forms of *E. gigantea* [48]. Metcalfe and Chalk [26] suggested that tannin cells were commonly found in non-woody stems, especially in the cortex, pith, and phloem of the Crassulaceae family. Proanthocyanidins (condensed tannins) are widely seen in the Crassulaceae family's herbaceous and woody taxa [2]. Stevens et al. [27] compared the alkaloid and tannin levels of 36 species of the Crassulaceae family. Tannins were found in *Crassula multicava*, *Echeveria venezuelensis, Pachyphytum* sp., *Compactum* sp., *Kalanchoe* sp., *Bryophyllum daigremontianum, Sedum* sp., *Aeonium* sp. and *Sempervivum* sp. Steven et al. [27] suggested that there is a dichotomy between the distribution of alkaloids and tannins, which is in good agreement with the major evolutionary trends within the family as inferred from chloroplast DNA restriction site variation. The distribution of tannins was reported as similar in the three *Sempervivum* taxa (S. *globiferum, S. tectorum,* and *S. ruthenicum*) [39]. Our findings regarding tannins support the tannin findings related to the family [26, 27, 35, 44, 45].

4. Conclusion

Consequently, the layer number of periderm, cortex layers in the root, the width and length of the periderm, the trachea diameter and the parenchyma cell diameter of the cortex, whether the tannins are dense or not, the cuticle layer structure in the stem and the presence or absence of micropapillae, number of cortex layers, straight or undulate xylem, epidermis cell diameter, pith parenchyma and xylem trachea diameter, cuticle

thickness, mesophyll parenchyma diameter, width and length of stomata, the length, shape and anticlinal walls structure of the upper and lower epidermis cells in the rosette leaves, the number of layers and shape of the mesophyll in the rosette leaf and sepal, the margin structure of ordinary irregularly shaped cells next to the stomata in the sepal and rosette leaves, length of upper epidermis cell in sepals, width and length of lower epidermis cell, the thickness or thinness of the cuticle layer in the petals, petal epidermis cell diameter, anticlinal walls structure of epidermis cell and the number of layers of the mesophyll in the petal, amount of tannin in vegetative organs were identified as important distinguishing anatomical characters between the two taxa. Moreover, differences in anatomical features were found to be statistically significant ($p<0.05$) (Table 4). At the same time, some important differences were reported in the morphological and trichomes characteristics of the vegetative and generative organs of these two taxa by Şahin and Kandemir [51, 52]. This study determined that the data on anatomical features obtained from the vegetative and generative organs of the *S. brevipilum* and *S. gillianiae* were congruent with the data obtained for other members of the Crassulaceae family and the *Sempervivum* genus. On the other hand, it has been emphasized in earlier studies that some anatomical features should be considered in solving some taxonomic problems between species and genera. Especially the anatomical features of stems and leaves are widely used as important distinguishing taxonomic characters in species classification [36, 37, 53-55]. It is recommended that detailed molecular (especially chloroplast DNA studies) and chromosome morphology studies be conducted on these two endemic taxa and other *Sempervivum* members in the future. Due to the tannin content in their vegetative and generative organs, the studied taxa are thought to have antioxidant and antimicrobial properties. Therefore, conducting antimicrobial and antioxidant studies on the taxa is deemed appropriate. The examined taxa have the potential to be used as ornamental plants (rock gardens and wall landscaping) due to their rosette structures and different colors of rosette leaves.

Author Contributions

All the authors equally contributed to this work. This paper is derived from the first author's master's thesis, supervised by the second author. They all read and approved the final version of the paper.

Conflict of Interest

All the authors declare no conflict of interest.

Ethical Review and Approval

No approval from the Board of Ethics is required.

References

[1] S. A. Sponberg, *The genera of Crassulaceae in the southeastern United States*, Journal of the Arnold Arboretum 59 (1978) 198–248.

[2] J. Thiede, U. Eggli, C*rassulaceae*, in: K. Kubitzki (Ed.), Families and Genera of Vascular Plants, Flowering Plants Eudicots, Springer, Hamburg, 2007, Ch. IX, pp. 83–118.

[3] M. E. Mort, D. E. Soltis, P. S. Soltis, J. Francisco-Ortega, A. Santos-Guerra, *Phylogenetic relationships and evolution of Crassulaceae inferred from matK sequence data,* American Journal of Botany 88 (1) (2001) 76–91.

[4] C. R. Metcalfe, L. Chalk, Anatomy of Dicotyledons, Claredon Press, Oxford 1972.

[5] L. C. Jensen, *Primary stem vascular patterns in three subfamilies of the Crassulaceae*, American Journal of Botany 55 (5) (1968) 553–563.

[6] R. C. Van Ham, H. ′τ Hart, *Phylogenetic relationships in the Crassulaceae inferred from chloroplast DNA restriction-site variation*, American Journal of Botany 85 (1998) 123–134.

[7] F. Karaer, F. Celep, *Taxonomic notes on Sempervivum armenum Boiss. and A. Huet (Crassulaceae) from Turkey with an amended description*, Bangladesh Journal of Botany 39 (2010) 103–105.

[8] F. Karaer, F. Celep, U. Eggli, *A taxonomic revision of the Sempervivum davisii complex (Crassulaceae)*, Nordic Journal of Botany 29 (2011) 49–53.

[9] J. T. Klein, J. W. Kadereit, *Phylogeny, Biogeography, and Evolution of Edaphic Association in the European Oreophytes, Sempervivum and Jovibarba (Crassulaceae)*, International Journal of Plant Sciences 176 (2015) 44–71.

[10] J. T. Klein, J W. Kadereit, *Allopatric hybrids as evidence for past range dynamics in Sempervivum (Crassulaceae), a western Eurasian high mountain oreophyte*, Alpine Botany 126 (2016) 119–133.

[11] H. ′τ Hart, B. Bleij, B. Zonneveld, *Sempervivum*. in: U. Eggli (Ed.), Illustrated handbook of succulent plants: Crassulaceae, Springer Verlag, Berlin, 2003, pp. 332–349.

[12] K. Topalov, M. E. Mort, P. Neeff, D. Lakušić, B. Zlatković, *Preliminary phylogenetic analyses of Sempervivum L. (Crassulaceae) inferred from DNA sequence data*, in: Botany 2006 Conference Abstract, Chico, 2006, pp. 300.

[13] C. W. Muirhead, Turkish species of *Sempervivum*, Notes from the Royal Botanic Garden Edinburgh 29 (1969) 15–28.

[14] C. W. Muirhead, *Sempervivum* L., in: P.H. Davis (Ed.), Flora of Turkey and East Aegean Islands, Edinburgh University, Edinburg, 1972, pp. 209–248.

[15] P. Neeff, *Neue Taxa der Gattung Sempervivum (Crassulaceae) in der Türkei*, Kakteen und andere Sukkulenten 56 (3) (2005) 71–72.

[16] F. Karaer, F. Celep, *Sempervivum ekimii nom. et stat. nov. for S. minus var. glabrum (Crassulaceae), with an amplified description,* Annales Botanici Fennici 45 (3) (2008) 229–232.

[17] Y. Kobiv, R. Kish, R. Hleb, *Sempervivum marmoreum Griseb. (Crassulaceae) in the Ukrainian Carpathians: distribution, morphology, coenotic conditions, population parameters, and conservation*, Ukrainian Botanical Journal 64 (1) (2007) 22–29.

[18] D. Sarı, *Investigation of some species from the Crassulaceae family distributed in the flora of Türkiye in terms of landscape and ornamental plant qualities*, in: I. Cengizler, S. Duman (Eds.), Research and Evaluations in Agriculture, Forestry and Aquatic Products–I, Night library, 2021, Ch. 19, pp. 292–313.

[19] R. Novaretti, D. Lemordant, *Plants in the traditional medicine of the Ubaye Valley*, Journal of Ethnopharmacology 30 (1) (1990) 1–34.

[20] Z. Giczi, B. Sik, V. Kapcsandi, E. Lakatos, A. Mrazik, R. Szekelyhidi, Determination of the health-protective effect of different *Sempervivum* and *Jovibarba* species, Journal of King Saud University-Science 36 (1) (2024) 102998.

[21] A. Alberti, S. Beni, E. Lacko, P. Riba, M. Al-Khrasani, A. Kery, *Characterization of phenolic compounds and antinociceptive activity of Sempervivum tectorum L. leaf juice*, Journal of Pharmaceutical and Biomedical Analysis 70 (2012) 143–150.

[22] D. Stojković, L. Barros, J. Petrović, J. Glamoclija, C. Santos-Buelga, I. Ferreira, M. Soković, *Ethnopharmacological uses of Sempervivum tectorum L. in southern Serbia: Scientific confirmation for the use against otitis linked bacteria*, Journal Ethnopharmacol 176 (2015) 297–304.

[23] E. Yeşilada, E. Sezik, G. Honda, Y. Takaishi, Y. Takeda, T. Tanaka, *Traditional medicine in Turkey IX: folk medicine in north-west Anatolia*, Journal Ethnopharmacol 64 (1999) 195–210.

[24] N. Kahriman, Z. Şenyürek, V. Serdaroğlu, A. Kahriman, N. Yayli, *Chemical composition and biological activity of essential oils of Sempervivum brevipilum Muirhead*, Records of Natural Products 9 (4) (2015) 603–608.

[25] N. Silanikove, A. Perevoltsky, F. D. Provenza, Use of tannin-binding chemicals to assay tannins and their negative post ingestive effects in ruminants, Animal Feed Science and Technology 91 (2001) 69–81.

[26] C. R. Metcalfe, L. Chalk, Anatomy of the Dicotyledons I, Oxford University, 1950.

[27] J. F. Stevens, H. T. Hart, R. C. Van Ham, E. T. Elema, M. M. Van Den Ent, M. Wildeboer, Z. H. Zwaving, *Distribution of alkaloids and tannins in the Crassulaceae*, Biochemical Systematics and Ecology 23 (2) (1995) 157–165.

[28] U. Eggli, Sukkulentenlexikon, Crassulaceae (Dickblattgewächse) (in German), Herausgegeben in Zusammenarbeit mit der Internationalen Organisation für Sukkulenten-Forschung (IOS), Eugen Ulmer Verlag, Stuttgart, 2003.

[29] S. Çelebioğlu, T. Baytop, A new reagent for microscopical investigation of plant, Publication of the Institute of Pharmacognosy, 1949.

[30] V. L. Singleton, R. Orthofer, R. M. Lamuela-Raventos, *Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent*, Methods in Enzymology 299 (1999) 152–178.

[31] F. Bärlocher, M. A. S. Graça, *Total Phenolics*, in: M. A. S. Graça, F. Bärlocher, M. O. Gessner (Eds.), Methods for Studying Litter Decomposition, Springer International Publishing, 2020, Ch.14, pp. 155–160.

[32] M. Ardelean, I. Stanescu, D. Cachita-Cosma*, Sedum telephium L. spp. maximum (L.) Krock. Histo-anatomical aspects on the vegetative organs*, Scientific Annals of Alexandru Ioan Cuza University of Lasi, New Series 55 (2009) 75–80.

[33] S. Ulcay, *Comparative anatomy of some Sedum species (Crassulaceae) in Turkey and distinguishing characteristics of these species*, Journal of Animal and Plant Sciences 33 (1) (2023) 95–102.

[34] N. A. Kirilenko, *Anatomical and morphological features of the vegetative organs of succulent plants regards growth conditions*, Ukrainian Journal of Ecology 8 (1) (2018) 509–515.

[35] M. M. Shahrestani, M. B. Faghir, M. Assadi, *Comparative anatomical studies in relation to taxonomy of Sedum sl. (Crassulaceae) in Iran*, Turkish Journal of Botany 44 (3) (2020) 281–294.

[36] N. Kandemir, A. Çelik, F. Ullah, *Comparative micro-anatomical features of endemic Fritillaria taxa growing in the Mediterranean region (Turkey)*, Flora 290 (2022) 152049.

[37] N. Kandemir, A. Çelik, S. N. Shah, G. Albasher, M. Iqbal, *Taxonomic relevance of stem and leaf anatomy in 10 endemic Fritillaria species from the Mediterranean Region*, Flora 310 (2024) 152444.

[38] N. A. Kirilenko, *Structural peculiarities of vegetative organs of Sempervivum tectorum L. (Crassulaceae) and Salicornia europaea L. (Chenopodiaceae) – residents of extreme habitats*, Journal of Education, Health and Sport 6 (9) (2016) 883–892.

[39] P. Vorobej, O. Futorna, I. Olshanskyi, S. Zhygalova, O. Bezsmertna, *Micromorphological study (anatomical structure of lamina surface, ultrastructure of seeds) of Sempervivum globiferum L*, Ecological Sciences 1 (28) (2020) 316–323.

[40] A. I. Akhmetzhanova, A. K. Auyelbekova, D. K. Kyzdarova, *The anatomic characteristic of a Sedum acre L, caustic from various ecological conditions of South and the Central Kazakhstan,* Bulletin of the Karaganda University Biology Medicine Geography Series 3 (83) (2016) 43–48.

[41] H. S. Abdel-Raouf, *Anatomical traits of some species of Kalanchoe (Crassulaceae) and their taxonomic value*, Annals of Agricultural Sciences 57 (1) (2012) 73–79.

[42] M. Jovanović, D. Lakušić, B. Lakušić, B. Zlatković, *Diversification of yellow-flowered Sempervivum (Crassulaceae) species from the Balkan Peninsula: evidence from the morphometric study of the epidermal structures of rosette leaves*, Botanica Serbica, 45 (2) (2021) 163–176.

[43] E. Weryszko-Chmielewska, M. Chernetskyy, *Structure of trichomes from the surface of leaves of some species of Kalanchoe Adans*, Acta Biologica Cracoviensia Series Botanica 47 (2) (2005) 15–22.

[44] M. Chernetskyy, E. Weryszko-Chmielewska, *Structure of Kalanchoe pumila Bak. leaves (Crassulaceae DC.)*, Acta Agrobotanica 61 (2) (2008) 11–24.

[45] N. S. Moreira, L. B. S. Nascimento, M. V. Leal-Costa, E. S. Tavares, *Comparative anatomy of leaves of Kalanchoe pinnata and K. crenata in sun and shade conditions, as a support for their identification*, Revista Brasileira de Farmacognosia 22 (5) (2012) 929–936.

[46] M. Chernetskyy, *The role of morpho-anatomical traits of the leaves in the taxonomy of Kalanchoideae Berg. Subfamily (Crassulaceae DC.)*, Modern Phytomorphology 1 (2012) 15–18.

[47] B. Zlatković, Z. S. Mitić, S. Jovanović, D. Lakušić, B. Lakušić, J. Rajković, G. Stojanović, *Epidermal structures and composition of epicuticular waxes of Sedum album sensu lato (Crassulaceae) in Balkan Peninsula*, Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology 151 (6) (2017) 974–984.

[48] E. Sandoval-Zapotitla, D. M. Martinez-Quezada, J. Reyes-Santiago, M. D. L. A. Islas-Luna, U. Rosas, *Leaf morpho-anatomical diversity in Echeveria aff. gigantea (Crassulaceae)*, Botanical Sciences 97 (2) (2019) 218–235.

[49] J. Thiede, *Quantitative phytogeography, species richness, and evolution of American Crassulaceae*, in: H. $'\tau$ Hart, U. Eggli (Eds.), Evolution and systematics of the Crassulaceae, Backhuys, Leiden,1995, Ch. 6, pp. 89–123.

[50] R. A. Balsamo, E. G. Uribe*, Leaf anatomy and ultrastructure of the Crassulacean-acid-metabolism plant Kalanchoe daigremontiana*, Planta 173 (1988) 183–189.

[51] T. Şahin, N. Kandemir, *Glandular trichomes on the vegetative and generative organs of endemic two Sempervivum taxa*, Journal of New Result in Science 14 (1) (2025) 48–61.

[52] T. Şahin, N. Kandemir, *Comparison of morphological characters in vegetative and generative organs of endemic two Sempervivum L. taxa (Crassulaceae) in Türkiye*, International Journal of Agricultural and Natural Sciences 18 (1) (2025) 19–32.

[53] N. Kandemir, A. Çelik, S. N. Shah, A. Razzaq, *Comparative micro-anatomical investigation of genus Heliotropium (Boraginaceae) found in Turkey*, Flora 262 (2020) 151495.

[54] K. V. Koçak, N. Kandemir, *Taxonomic importance of anatomical, ecological and trichome features of Marrubium astracanicum subsp. astracanicum Jacq*. (Lamiaceae), Black Sea Journal of Engineering and Science 6 (3) (2023) 199–209.

[55] K. V. Koçak, N. Kandemir, *Anatomical, ecological and trichome micro-morphological features of two Marrubium L. taxa (Lamiaceae)*, Black Sea Journal of Engineering and Science 7(1) (2024) 43–54.