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RESEARCH ARTICLE

MORPHOLOGICAL, ANATOMICAL, AND PALYNOLOGICAL INVESTIGATION OF THREE *LEGOUSIA* TAXA (CAMPANULACEAE) FROM TURKEY

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ABSTRACT

In this study, morphological, anatomical and palynological features for three taxa of *Legousia* Durande were investigated in Turkey. The morphological measurements were compared with the "Flora of Turkey". This study measured all morphological characteristics that have taxonomical value to clarify the similarities and differences between these taxa for the first time. Root, stem, and leaf cross-sections as well as leaf surface sections of these taxa were examined under a light microscope. The usual features of Campanulaceae anatomy were observed in all the studied taxa. The outer surfaces of the roots in all taxa were covered by peridermis. Cambium was distinguishable but discontinuous. Laticifers were located in the pith region of stems in all taxa. The endodermis in the stem of all examined taxa consisted of large,circular, continous, parenchymatous cells, surrounding vascular bundles. The leaves were bifacial and amphistomatical. Stoma type was amaryllis and anomocytic for all the taxa. Pollen grains of all studied taxa were radially symmetrical and spheroidal in shape. The type of aperture in all examined taxa was porate, but the number of pores were variable. The pollen grains of *L. pentagonia* were60% pentaporate, 30% tetraporate, and 10% triporate, whereas in *L. falcata* they were 70% pentaporate, 20% tetraporate, and 10% triporate. On the other hand, in *L. speculum-veneris,* the pollen grains were 30% pentaporate, 50% tetraporate, and20% triporate.

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INTRODUCTION

The Campanulaceae family consists of approximately 90 genera and 2500 species worldwide (Heywood *et al.,* 2007). Family members are herbaceous plants growing in the tropics and subtropics, as well as trees and shrubs (Evans, 2000). Campanulaceae is rich in species containing flavonoids (Cuendet *et al.,* 2001; Yaylı *et al.,* 2003; Dumlu *et al.,* 2008), triterpenoids (Yaylı *et al.,* 2005; Kim *et al.,* 2006; Son *et al.,* 2007), phenylpropanoid (Lee *et al.,* 2004), phenolic glycosides (Kuang *et al.,* 1991), polyacetylenes (Dumlu *et al.,* 2008), alkaloids (Shibano *et al.,* 2001; Ishida *et al.,* 2008), cyanogenic glycosides (Harborne *et al.,* 1996) and essential oil (Morteza-Semnani *et al.,* 2008). The first study on general anatomical characteristics of the Campanulaceae family. However, no anatomical studies on the *Legousia* genus of the family were found in the literature. Pollen morphology has been studied in several species of Campanulaceae, but the application of pollen morphological characters in a comprehensive study to find relationships between species or natural groups of species in *Campanula* are few. Most palynological studies have focused on generic delimitation in Campanulaceae. Chapman (1967) conducted one of the oldest relevant palynological assessments of Campanulaceae and analyzed31 species representing 21 genera using light microscopy (LM). Avetisjan (1967) also described the palynology of the order Campanulales. Dunbar (1975a) studied the pollen morphology of Campanulaceae and related families with special reference to ultra structure. Other investigations included the species of selected geographical areas, for example, India (Sahay, 1969), Pakistan (Perveen and Qaise, 1999), Armenia (Avetisjan, 1967), Turkey (Erkara *et al.,* 2008; Inceoglu, 1976), and Romania (Tarnavschi, 1919). Detailed pollen morphological studies on Campanulaceae using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were performed by Dunbar (Dunbar, 1973; 1975a; 1975b;1984; Dunbar and Wallentinus, 1976). These studies revealed three major aperture types in Campanulaceae: colpate, colporate, and porate. Campanulaceae is represented by seven genera (*Campanula* L., *Symphyandra* A. DC., *Asyneuma* Griseb. & Schenk, *Michauxia* L'Herit., *Legousia* Durande., *Jasione* L., *Trachelium* L.) and 138 species in the "Flora of Turkey". The *Legousia* genus in the Campanulaceae family is closely related to the genera *Michauxia* and *Jasione*. Seven species and 25 taxa of *Legousia* are synonyms in the world. This genushas four species in Turkey (Damboldt, 1 978). *Legousia* species are locally known as "Kadınaynası" in Turkey. Morphological, palynological, and anatomical studies for *Legousia* were carried out for the first time in this study. In this study, we provide detailed information about *Legousia* species by

evaluating the results obtained from morphological, anatomical, and palynological investigations.

MATERIALS AND METHODS

Plant materials: Plant materials were collected from natural habitats from 2014 to 2015. The voucher specimens were deposited at the Firat University Herbarium (FUH). The taxonomic descriptions of the plants were prepared according to "Flora of Turkey" (Damboldt, 1978). Table 1 lists the taxa investigated in this study with their localities and vouchers and Figure 1 summarizes their distribution. The morphological and morphometrical characters are evaluated in Table 2 and herbarium specimens of these taxa are shown in Figure 2.

Anatomical investigation: Anatomical studies were carried out using specimens fixed in 70% alcohol. The cross sections of the roots, stems, and leaves surface sections of the leafwere done by hand and stained with Floruoglycine-HCl (Yakar-Tan, 1982). The sections were observed under light microscopy "Olympus BX51"and photographed with an "Olympus Camedia C-4000 digital camera".

Palynological investigation: Pollen materials were obtained from the herbarium samples for the palynological investigations. Pollen slides were prepared using the technique of Wodehouse (1935). For LM studies, measurements were taken from 30 mature pollen grains for each morphological characteristic using an ocular micrometer on a light microscope at 1000×magnification.

Table 1. List of the investigated taxa with their localities and voucher specimens.

Taxa	Locality	Voucher specimens
L. falcata	C5/Icel; Fındıkpınarı Plateau, 1200m, 08.05.2015	Dogan, 1820
L. speculum-veneris	C5/ Icel; Gülek Gateway, 1650 m, 17.06.2014	Dogan, 1783
L. pentagonia	B7/Elazig; Harput, Anguzababa Mountain, 1400 m, 11.06.2014	Dogan, 1780

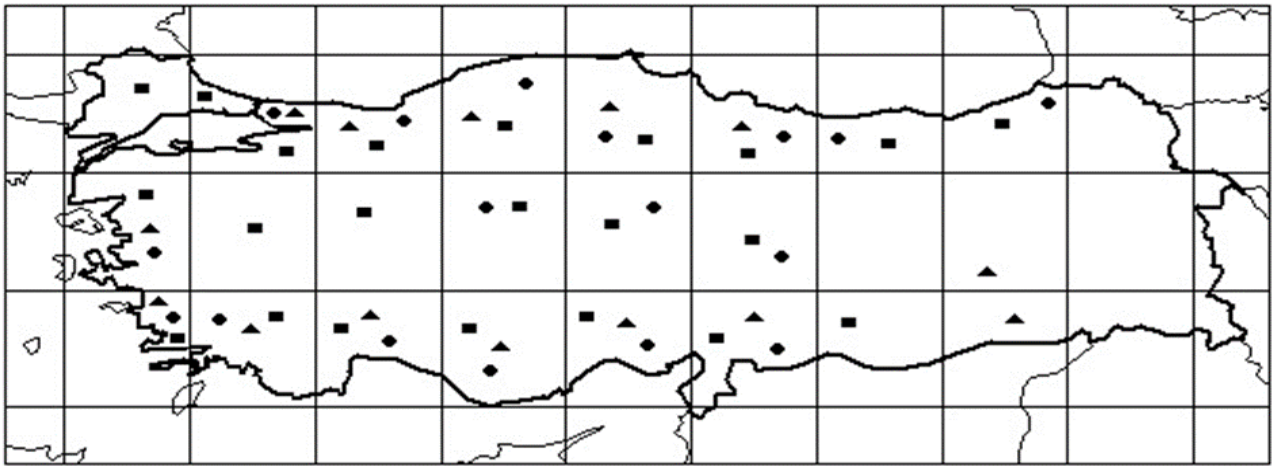

Figure 1. Distribution of studied *Legousia* taxa in Turkey (*L. falcata* ▲, *L. speculum-veneris* ●, *L. pentagonia* ■)

Figure 2. Herbarium specimens of studied *Legousia* taxa A *L. falcata*B *L. speculum-veneris*C*L. pentagonia*

Photographs were obtained using the Olympus BX51 and photographed with the Olympus Camedia C-4000 digital camera. All of the morphological parameters investigated are given in Table 3. For SEM, dry pollen grains were mounted on stubs and pulse sputter coated for 3.5 min with gold. Pollen grains were examined with a Zeiss EVO MA10 SEM operated at 15kV.The pollen terminology of Faegri and Iversen (1964) and Hesse *et al.,* (2009) were used. The class of pollen shape, based partly on P/E ratio, was identified using Erdtman's (1969) system.

RESULTS

Morphological properties

***Legousia falcata* (Ten.) Fritsch ex Janch.**
= *Prismatocarpus falcatus* Ten., Prodr. Fl. Nap. 1:16, t. 20 (1812)
= *Speculariafalcata*(Ten.) A. DC., Monogr. Camp. 345 (1830)

Description: Plantannual, 18-60 cm tall, stems mostly simple, rarely branched, glabrous, rarely almost pubescent. Leaves ovate to ovate-lanceolate, lower shortly petiolate, entire or almost undulate-crenate, 5-12x 10-25mm, leaf apices acute-rotund. Flowers solitary in upper part or in pairs in leaf axils, forming a long lax spike. Calyx lobes linear-lanceolate, long-acuminate, 0.4-0.9 x 11-15 mm,patent, almost falcate-recurved. Corolla broadly campanulate, 4-10 mm, violet. Filaments ciliate. Ovary 10 mm, Capsule not constricted at apex, cylindrical, 15-20 mm. Seeds orbicular, 1mm (Figure 2A).

Flowering: April to July

Habitat: Macchie, steppe, fields, roadsides, sand dunes, at 1400 m above sea level.
Turkish Name: Eğrikadınaynası.

***Legousia speculum-veneris* (L.) Durande ex Vill.**
= *Campanula speculum-veneris* L.,Sp. Pl. 168 (1753)
= *Specula*riaspeculumA. DC., Monogr. Camp. 346 (1830)
= *Legousia speculum* Fischer ex A. DC., op. cit. 347 (1830)

Description:Plant annual, 8-35 cm tall, glabrous or rarely shortly hispid. Stems erect or erect-ascending, branched. Leaves ovate to ovate-lanceolate, lower shortly petiolate, entire or almost undulate-crenate, 2-20 x 6-40 mm, leaf apices acute-rotund. Flowers solitary or more often, in a panicle. Calyx lobes linear-lanceolate, 1-4 x 6-20 mm, about as long as ovary at flowering time. Coralla rotate, 10-15x10-25 mm, with obtuse lobes, violet, rarely white or lilac. Filaments short, broadened at base, glabrous. Ovary 10-12 mm, Capsule cylindrical, constricted at apex, 10-15 mm. Seeds broadly ellipsoid, 1.1-1.2mm (Figure2B).

Flowering: April to June
Habitat: Pinus sylvestris forest, fields, open places, at 1900 m above sea level.
Turkish Name: Hoşkadınaynası

***Legousia pentagonia* (L.) Thell.**
= *Campanula pentagonia* L.,Sp. Pl. 169 (1753)!
= *Specula*riapentagonia (L.) A. DC., Monogr. Camp. 344 (1830)!
= *Specula*riacoa A. DC., Monogr. Camp. 350 (1830)!

Description: Plant annual, 8-25 cm tall, stems simple or branched from base, erect or erect-ascending, usually hispid, rarely glabrous. Leaves ovate to ovate-lanceolate, lower shortly petiolate, entire or almost undulate-crenate, 2-10 x 8-28 mm, leaf apices acute. Flowers solitary or in a panicle. Calyx lobes 0.3-1 x 7-16 mm. Coralla broadly campanulate, 10-23 mm, white at base, blue in middle, apex violet. Filaments with broad ciliate base. Ovary over 15 mm, much elongated after flowering. Capsule 20-30 mm, cylindrical, not constricted at apex. Seeds orbicular, 0.9-1mm (Figure2C).

Flowering: April to June
Habitat: Dry open places, fields, at 2000 m above sea level.
Turkish Name: Kadınaynası

Table 2. Morphological and morphometric characters of studied *Legousia* taxa in "Flora of Turkey" and our study

Taxa	Characters	Flora of Turkey	Our study
L. falcata	Plant tall	20-50 cm	18-60 mm
	Leaf	---	5-12 x 12-25 mm
	Leaf apices	---	acute-rotund
	Calyx lobes	over 10 mm	0.4-0.9 x 11-15 mm
	Coralla	---	4-10 mm
L. speculum-veneris	Plant tall	10-30 cm	8-35 cm
	Leaf	---	2-20 x 6-40 mm
	Leaf apices	---	acute-rotund
	Calyx lobes	(6-)8-12(-15) mm	1-4 x 6-20 mm
	Coralla	10-15 x 20-25 mm	10-15 x 10-25 mm
L. pentagonia	Plant tall	15-30 cm	8-25 cm
	Leaf	---	2-10 x 8-28 mm
	Leaf apices	---	acute
	Calyx lobes	(7-)10-16(-20) mm	0.3-1 x 7-16 mm
	Coralla	15-20 mm	10-23 mm

Anatomical properties

Root anatomy: Peridermis was 1-3 layered in the root. Cortex parenchyma was 3-5 layered just below the periderm. Cambium was distinguishable but discontinuous. Xylem was interrupted by parenchymatous large pith rays. Parenchymatous pith rays disintegrated in some areas. Trachea cells in secondary xylem were huge and clear. Pith region was small consisting of round shaped cells. In addition, primary xylem elements sometimes entered into the pith region. We did not observe any laticifer in the root of two examined taxa, however laticifers were observed in the root of *L. speculum-veneris* (Figure 3).

Stem anatomy:The stem was usually circular with many projections. The epidermis was composed of a single layer. The outer wall of the epidermis was covered with a thin cuticle. The 3-4 layers of collenchyma tissue were located immediately under the epidermis. Under the collenchyma, endodermis of all the examined taxa was circular, continuous and consisted of large parenchymatous cells, surrounding vascular bundles. Cambium was distinguishable. Xylem elements were arranged in parallel rows. Laticifer was seen at the same places in the pith region. Pith with intercellular spaces was formed by spheroid parenchymatous cells (Figure 3).

Leaf anatomy: The leaves were covered with a thin cuticle for both upper and lower epidermis in the cross-sections. Epidermal cells were covered with simple eglandular trichomes. The leaves were bifacial, consisting of palisade and spongy parenchyma. The palisade cells were usually 1-2 layered, cylindrical cells. Spongy parenchyma consisted of 3-4 layers with rare intercellular spaces (Figure 4A).Stoma type was amaryllis and because neighboring cells were undifferentiated, they were anomocytic.

Table 3.Details of pollen morphological characters in studied taxa.

Characters	*L. falcata*	*L.speculum-veneris*	*L. pentagonia*
Polar axix (□m)	(10.40-12.30)11.90	(11.10-12.70)12.30	(9.70-11.90)10.80
Equatorial axis (□m)	(9.90-11.80)11.10	(10.30-12.30)11.20	(9.20-11.60)10.30
P/E	1.07	1.09	1.04
Pollen shape	spheroidal	spheroidal	spheroidal
Echinae width (□m)	0.61±0.18	0.72±0.22	0.47±0.10
Echinae length (□m)	0.54 ± 0.11	0.65±0.15	0.41±0.14
Echinae density (25□m^2)	22-32 (27.09±3.58)	18-26 (21.5±3.60)	28-38 (33.61±2.86)
Exine (□m)	0.52	0.76	0.48
Por diameter (□m)	2.12	2.42	2.63
Ornemantation	Rugulate-microechinatae	Rugulate-microechinatae	Rugulate-microechinatae
Pore number	3-5	3-5	3-5

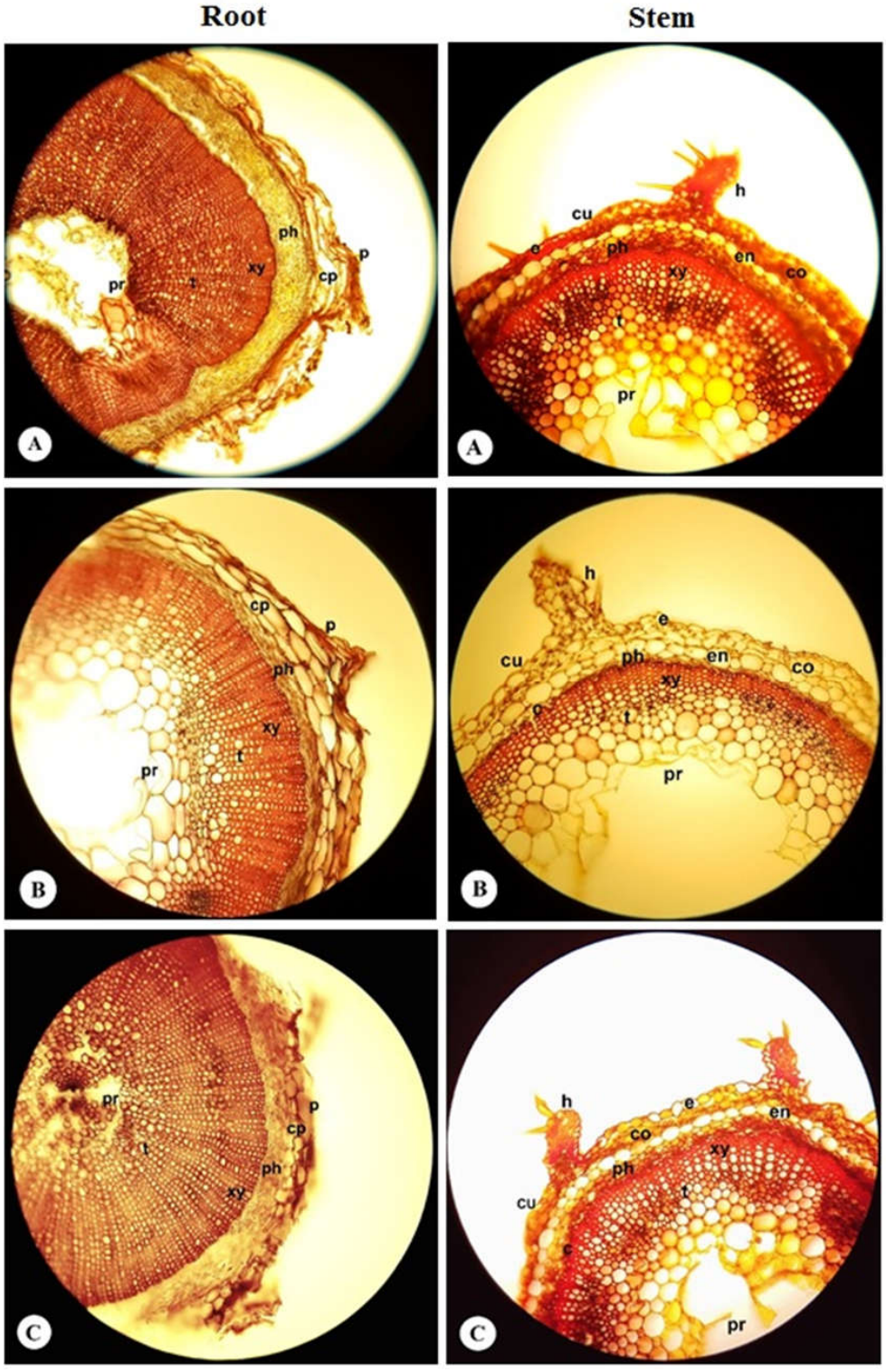

Figure3. Cross-sections of roots and stemsA*L. falcata*B*L. speculum-veneris* C *L. pentagonia* (p=periderm, cp=cortex parenchyma, ph=phloem, xy=xylem, t=trachae, pr=pith region, h=hair, cu=cuticula, e=epidermis, co=collenchyma, en=endodermis, c=cambium)

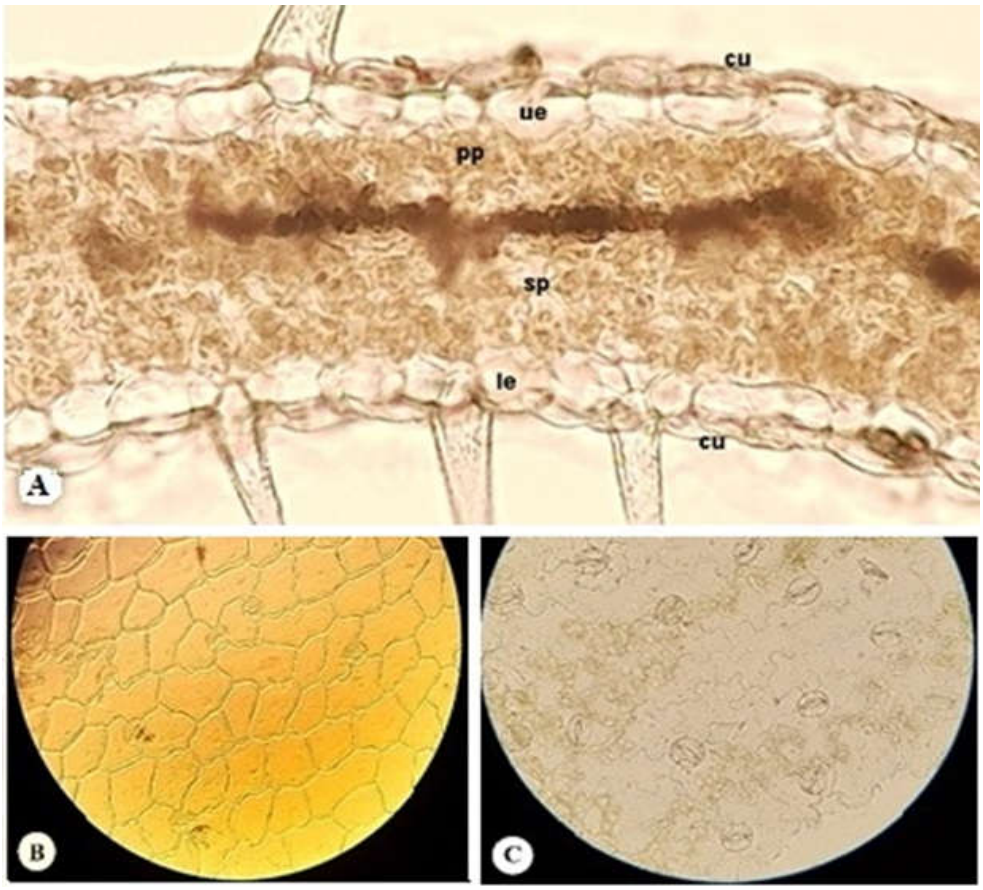

Figure 4. A Cross-section of leaves (cu=cuticula, ue=upper epidermis, pp=palisade paranchyma, sp=spongy paranchyma, le=lower epidermis) B Upper surface of leaves C Lower surface of leaves

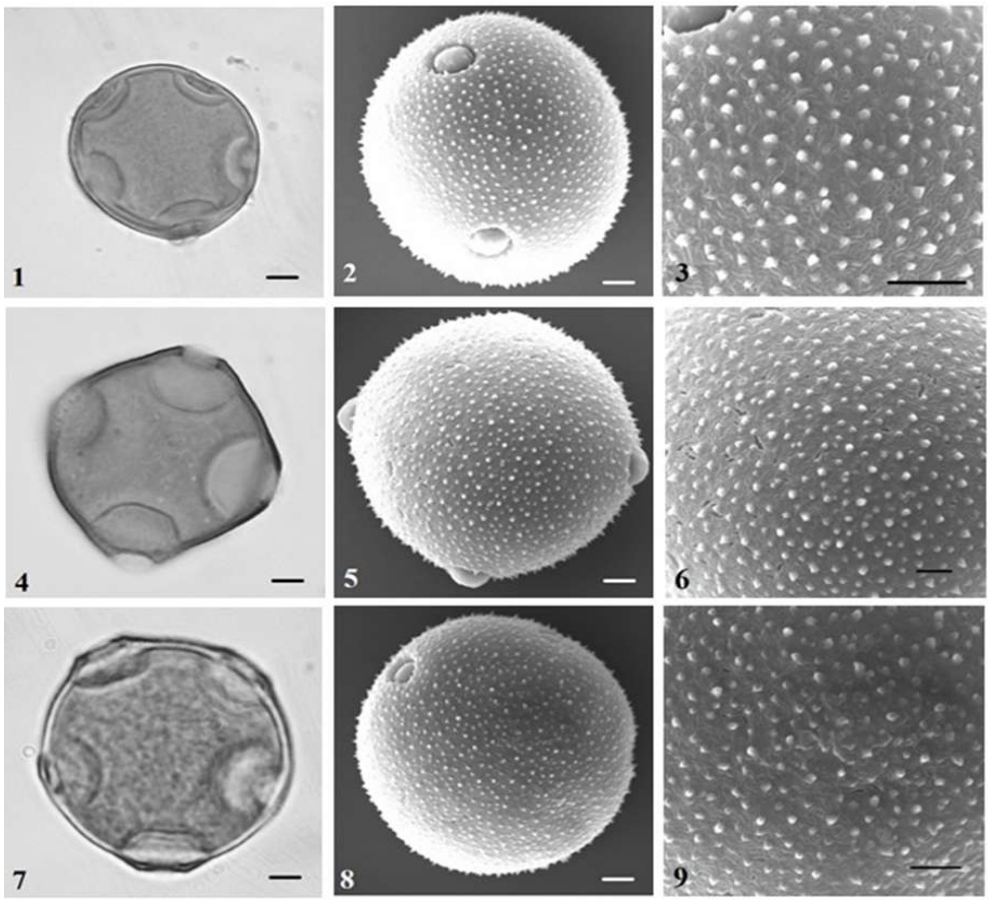

Figure 5. LM and SEM micrographs of pollen grains in studied taxa. 1-3:*L. falcate* 4-6: *L. speculum-veneris* 7-9: *L. pentagonia* (Scale bar: 2 µm)

Stomata were present on both surfaces of epidermis (Figures 4B-C), so the leaves were amphistomatic. We also observed more stomata on the lower surface of the leaves (Figure 4C).

Palynological properties

Selected LM and SEM micrographs of examined pollen grains are presented in Figure 5. The main pollen morphological features of studied taxa are summarized in Table 3. Pollen grains of all studied taxa were radially symmetrical and spheroidal in shape. The type of aperture in all examined taxa was porate, but the number of pores was variable. The pollen grains of *L. pentagonia* were 60% pentaporate, 30% tetraporate, and 10% triporate, whereas in *L. falcata* they were 70% pentaporate, 20% tetraporate, and 10% triporate. On the other hand, in *L. speculum-veneris* the pollen grains were 30% pentaporate, 50% tetraporate, and 20% triporate. Variation in number of pores was observed even among individual samples of one species. The smallest pore diameterobserved was 2.12 µm in *L. falcata,*whereasthe largest pores were 2.63 µm in *L. pentagonia.* With increased pore numbers, the size of the pore diameter decreased, as expected. Moreover, the size of the pore essentially correlated with the size of the pollen grains (Table 3). The pores were located equatorially and had an operculum in all species studied. The surface of the operculum was psilatae and sometimes disappeared, and only its remnants were observable under SEM (Figure5). The mean of the polar axis varied from 10.80µm in *L. pentagonia*to 12.30µm in *L. speculum-veneris*. The equatorial axis ranged from 10.30 µm in *L. pentagonia*to 11.20µm in *L. speculum-veneris*. The shape index (P/E) showed low variability, from 1.04 in *L. pentagonia*to 1.09 in *L. speculum-veneris* (Table 3). The thickness of exine rangedfrom 0.48 µm in *L. pentagonia* to 0.76µm in *L. speculum-veneris*. The sculpture of exine was rugulate-microechinatae and furnished with echini of different sizes and thickness. The lengthof echini onthe exine surface ranged from 0.41µm in *L. pentagonia* to 0.65µm in *L. speculum-veneris*. The width of echini ranged from 0.47µm in *L. pentagonia* to 0.72 µm in *L. speculum-veneris*. The mean density of echini in a sample area of 5 µm x 5 µm ranged from 18 in *L. speculum-veneris*, to 38 in *L. pentagonia* (Figure5).

DISCUSSION

This study examined detailed morphological, anatomical, and palynological properties of three *Legousia* taxa. The following morphological properties were important in taxonomical identification: shape, length, falcate-recurved/or not of the calyx lobes, filaments glabrous/or ciliate, capsule constricted/or not constricted at apex, and shape of seeds. Although our results generally correspond with the description in "Flora of Turkey, a few differences were detected (Damboldt, 1978). These differences are described in Table 2 and may be the consequences of climate, ecological, and habitat conditions in which the examined taxa were studied. On the other hand, we determined morphological characteristics, including, width-length and apices of leaf,which were not included in the "Flora of Turkey" (Table 2). This study is the first to measure all taxonomically important morphological features, similarities, and differences in this genus. General anatomical characteristics of the Campanulaceae family were observed in the anatomical investigation of all taxa studied. Metcalfe and Chalk (1983) stated that endodermis and laticifers were distinguishable in the family Campanulaceae. The findings of this study support this assertion. Endodermis was indistinguishable in the root of all taxa in our study in agreement with the literature (Uysal *et al.,* 1984; Ocak and Tokur, 1996). Endodermis in the stem of all the examined taxa consisted of large, circular, continuous parenchymatous cells, surrounding the vascular bundles. Ocak and Tokur (1996) defined that some *Campanula* species have laticifers in the root and stem. We did not observe any laticifers in the root of two examined taxa, however we observed laticifers in the root of *L. speculum-veneris.* We observed laticifers located in the pith region of stems in all taxa, as stated by Metcalfe and Chalk (1983). The leaves were bifacial. Stoma type was amaryllis and because the neighboring cells were undifferentiated, they wereanomocytic for all examined taxa. Stomata were present on both surfaces of the epidermis, so the leaves were amphistomatic. We also observed more stomata on the lower surface of the leaves. Similar results have been reported by Ocak and Tokur (1996) and Alçıtepe (2013). Pollen grains of all studied taxa were radially symmetrical and spheroidal in shape. The type of aperture in all examined taxa was porate, but the number of pores was variable. The basic number of apertures in Campanulaceae usually is three (triporate) (Dunbar and Wallentinus, 1976). However, pollen grains are morphologically variable,from tetra-to pentaporate. In our study, we observed that tetra-and pentaporate pollen grains in *Legousia* were more frequent than triporate. It also varied between different individuals of the species. The exine sculpture in Campanulaceae was rugulate-echinate or, based on other terminology, rugulate-spinulose/verrucose (Dunbar, 1975a; Perveen and Qaiser, 1999). The taxa studied show only minor differences in this regard. As stated in the literature, we found that the exine sculpture in all three taxa was rugulate-microechinatae. The most valuable characters in pollen morphology of *Campanula* addressing its infrageneric classification were the size and density of echini on the exine surface. Characters such as pore number, size and shape of pollen grains, and several other features assessed here did not provide valuable characters for infrageneric classification of *Campanula*. The same situation was also observed for *Legousia* in this study. Moreover, detailed taxonomic studies of the genus in Turkey are absent. Thus, the present study sheds light on the taxonomy of the genus focusing on Turkish species, which may be useful in the systematics of *Legousia*.

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