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Araştırma makalesi

Multivariate analysis of some species of the genus *Lathyrus* L. (Papilionoideae, Fabaceae) based on anatomical, micromorphological and macromorphological data

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Abstract

Lathyrus L. (Papilionoideae, Fabaceae), a cosmopolitan genus, has great significance in terms of food and agricultural areas. There are too few studies on the vegetative anatomy and the floral and foliar micromorphology of the genus. The present study is therefore carried out by means of multivariate cluster analysis (CA) and principal coordinate analysis (PCoA) on the basis of the anatomical, micromorphological as well as macromorphological characteristics of *L. aureus* (Steven) Bornm., *L. czeczottianus* Bässler (endemic to Turkey), *L. inconspicuus* L., *L. pratensis* L., and *L. sphaericus* Retz. assigned to this genus. This study is the first comprehensive report dealing with the vegetative anatomy and the petal, sepal and leaf epidermal micromorphology of these species. In general, the results of the current analysis clearly reveal the usefulness of these characters in inference of species delimitation and phylogenetic relationships.

Keywords: *Anatomy, Lathyrus, macromorphology, micromorphology, multivariate analysis.*

1. Introduction

Fabaceae (=Leguminosae), commonly known as the legume, pea or bean family, is the third largest family of flowering plants with over 700 genera and 19.000 species (Lewis et al., 2005). In the Turkish flora, it is the second most economically important family after Poaceae and the second largest family in terms of the number of species (Güner et al., 2012). It is an important component of almost all terrestrial biomes on all continents, except Antarctica.

Fabaceae comprises three subfamilies, two of which are monophyletic Papilionoideae (=Faboideae) and Mimosoideae and each is derived from a paraphyletic Caesalpinioideae. The subfamily Papilionoideae is the largest of the three subfamilies with about two-thirds of all the genera and species of the family. It is composed of 28 tribes, 478 genera and over 13.800 species, many of which are significant as food crops (Miller et al., 2011; The Legume Phylogeny Working Group, 2013).

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Lathyrus L. is the largest genus in the economically significant tribe Fabeae (syn. Vicieae) of the subfamily Papilionoideae, with nearly 160 annual or mostly perennial herbaceous species and 47 subspecies, most of which are self-pollinating (Ghorbel et al., 2014). The genus is widespread in the temperate regions of both the northern and southern hemispheres and extends into tropical East Africa, Australia, and South America (Bennett and Cocks, 1999). The eastern Mediterranean and Irano-Turanian regions are its main centers of diversity, with smaller centres in North and South America (Kupicha, 1983). For genetic and ecological research, *Lathyrus* species are economically important as food and fodder crops, ornamentals, soil nitrifiers, dune stabilizers, significant agricultural weeds, and model organisms (Chittenden, 1951; Kenicer et al., 2005).

Lathyrus is separated into 12 or 13 sections by many researchers (Czefranova, 1971; Kupicha, 1983; Asmussen and Liston, 1998; Croft et al., 1999; ILDIS, 2005; Leht, 2009). Kupicha's (1983) infrageneric classification is the only worldwide treatment of the genus. Based on the diversity of morphological attributes, she divided it into 13 sections: *Aphaca* (J.Mill.) Dumort., *Clymenum* (J.Mill.) DC. ex Ser., *Lathyrostylis* (Griseb.) Bässler, *Lathyrus* L., *Linearicarpus* Kupicha, *Neurolobus* Bässler, *Nissolia* (J.Mill.) Dumort., *Notolathyrus* Kupicha, *Orobastrum* Boiss., *Orobon* Tamamsch., *Orobus* (L.) Godr., *Pratensis* Bässler, and *Viciopsis* Kupicha. Her classification (1983) is generally supported by the most current molecular phylogenetic studies (Croft et al., 1999; Kenicer et al., 2005).

On the basis of Kupicha's (1983) infrageneric system, *Lathyrus* growing in Turkey is represented by 66 species and 76 taxa belonging to 11 sections, except for the sections *Neurolobus* and *Notolathyrus* (Davis, 1970; Davis et al., 1988; Güneş and Özhatay, 2000; Genç and Şahin, 2011; Güneş and Çırpıcı, 2012; Güneş 2014). 24 taxa of *Lathyrus* are endemic to Turkey that is a major center of diversity for the genus in Eurasia with a high number of species.

There are limited studies on the anatomy and micromorphology of *Lathyrus*, except for its seed and pollen micromorphology (Metcalfe and Chalk, 1950, Kupicha, 1975; Stirton, 1981; Hammett et al., 1994; Christensen and Hansen, 1998; Gaboreanu et al., 1998; Krstić et al., 2002; Mantar et al., 2002, 2003; Leht, 2009; Ojeda et al., 2009; Celep et al., 2011; Çildir et al., 2012, 2017; Kahraman et al., 2014). Therefore, the main objectives of the present study are to carry out multivariate correspondence analysis (e.g. cluster analysis and principal coordinate analysis) of *L. aureus* (Steven) Bornm., *L. czeczottianus* Bässler (endemic to Turkey), *L. inconspicuus* L., *L. pratensis* L., and *L. sphaericus* Retz. based on the anatomical and micromorphological characteristics as well as the macromorphological properties of the vegetative and generative structures, to address their taxonomic significance in species delimitation, to elucidate the phylogenetic relationships among the species, and to contribute the taxonomic knowledge of the genus. The results of multivariate analysis based on combined anatomical, micromorphological and macromorphological characters are discussed and compared with traditional taxonomic treatments. This is to date the first detailed study on the vegetative anatomy and the petal, sepal and leaf epidermal micromorphology of these species.

2. Material and Methods

Specimens of *L. aureus* in sect. *Orobus*, *L. czeczottianus* and *L. pratensis* in sect. *Pratensis*, and *L. inconspicuus* and *L. sphaericus* Retz. in sect. *Linearicarpus* were collected from their natural habitats during extensive field studies in Turkey. The collected specimens were stored in the herbarium of the Department of Biological Sciences, at the Middle East Technical University (METU) in Ankara. The voucher specimens are presented in Appendix 1.

Anatomical investigations were performed using fresh specimens kept in 70 % ethyl alcohol. The paraffin wax method was applied for preparing cross-sections of fully mature roots, stems and leaves. The sections were stained with safranin and fast green (Johansen, 1944) with some modifications relating to staining time and amount of additions to the stains, and then mounted on slides using Entellan mounting medium. Next, they were examined and photographed with a Leica DM1000 Light Microscope (LM) (Figures 1-3).

The epidermal micromorphology of petals and sepals of fully opened flowers, and leaves was investigated with the help of a Leica S8AP0 Stereo Microscope (SM) and a QUANTA 400F Scanning Electron Microscope (SEM). After a number of specimens had been compared under SM, three to six petals, sepals and leaves were examined for each species under SEM at 15-20 kV (Figures 4-6). One kind of petals, two lateral petals (=wings), was examined. The epidermal types of the petals were classified on cell size, shape (the primary sculpture), and on the fine relief of the cell wall, using the terminology of Kay et al. (1981). The trichome types and density are described. The general trichome terminology follows Metcalfe and Chalk (1950) and Kahraman et al. (2014). Terminology for surface sculpturing of the leaves follows Stearn (2004).

The macromorphological characters were chosen from investigations of the material collected and from the species descriptions of several floras (Mouterde, 1966; Ball, 1968; Davis, 1970; Zohary, 1972; Townsend, 1974; Rechinger, 1979; Greuter, 1997), as well as from taxonomic studies (Czefranova, 1971; Kupicha, 1983).

Thirty-two diagnostic anatomical, micromorphological and macromorphological characters scored were assessed by multivariate analysis (Table 1). The anatomical, micromorphological and macromorphological data matrix are given in Appendix 2. For the multivariate analysis, a similarity matrix was created first using Gower's (1971) general coefficient of similarity (Sneath and Sokal, 1973), which can be used directly with a mixture of character types (e.g. binary, qualitative, and quantitative characters) as well as taking into account missing values (St-Laurent et al., 2000). These similarity matrices were then clustered using UPGMA (Unweighted Pair-Group Method Using Arithmetic Averages) and the results were demonstrated in the dendrogram (Figure 7). UPGMA is the most frequently used method (Romesburg, 1984) and also appears to produce the best results (Radford, 1986) in terms of following criteria: accurate reflection of the similarity matrix, symmetrical hierarchical structure, and congruence with classification derived by traditional methods (Ward, 1993). The characters used in the analysis were assumed to be as important as each other and were unweighted. Principal coordinates analysis (PCoA), using Gower's general similarity coefficient, was performed to summarize relationship among specimens and to see their distribution as 3-dimensional plots (Figure 8). For these analyses, the MVSP software (A MultiVariate Statistical Package) was applied (Kovach, 1999).

3. Results

For the *Lathyrus* taxa studied, selected LM micrographs of anatomical transverse sections of the root, stem and leaf and chosen SEM micrographs of the petal, sepal and leaf epidermal micromorphology are presented in Figures 1-6. Moreover, the macromorphological characteristics of the vegetative and generative organs are provided.

3.1 Root anatomy

The root cross-sections show that *L. inconspicuus* and *L. sphaericus* have the pattern of primary root growth. The primary structure is composed of epidermis, cortex, and central

cylinder formed by xylem and phloem elements. The root is covered outside by the epidermis. The small area is occupied by the parenchyma of the cortex, comprising thin-walled live cells variable in size and shape. There are sclerenchymatous cells in the cortex but there are more sclerenchymatous cells in the cortex of *L. inconspicuous*. The number of the rows of the xylem rays is 1-2(-3). The root cross-sections of *L. aureus*, *L. czeczottianus* and *L. pratensis* reveal that the root exhibits the pattern of secondary growth. In the secondary structure, the vascular cambium develops and gives rise to the secondary xylem and phloem, and the cork cambium, called the phellogen, is formed and produces the periderm. The outermost layer of the root is covered outside by a thin or thick periderm protecting other internal tissues from disease and damage. The periderm is mostly composed of crushed or broken cells during the expansion growth of the root. A multiple cortex found below the periderm is formed by parenchymatous cells that are different in shape and size. In *L. pratensis*, the cortex comprises more numerous layers of cells filled with starch grains. Sclerenchymatous cells are situated in the cortex. The cambium is located between the primary xylem and phloem, adopting a circular shape by the addition of secondary vascular elements. The vascular tissue of *L. pratensis* is thicker, showing greater development of the phloem and xylem. In *L. aureus*, the xylem and phloem are much less developed. Vessel elements in the xylem are oval, rounded or polygonal in shape and have thick cell walls. The vessels and tracheids are between 15 and 60 μm and between 5 and 20 μm in size, respectively. Xylem rays are uniseriate or multiseriate with 1-6 radial rows of rectangular or almost square parenchymatous cells. In *L. czeczottianus* and *L. pratensis*, the maximum number of the ray rows is 6. On the other hand, the maximum number of the ray rows is 3 in *L. aureus*. There are no parenchymatous pith cells since the xylem forms a solid mass in all the central part of the root; however, the root centre is occupied by the parenchymatous cells in *L. pratensis* (Figure 1).

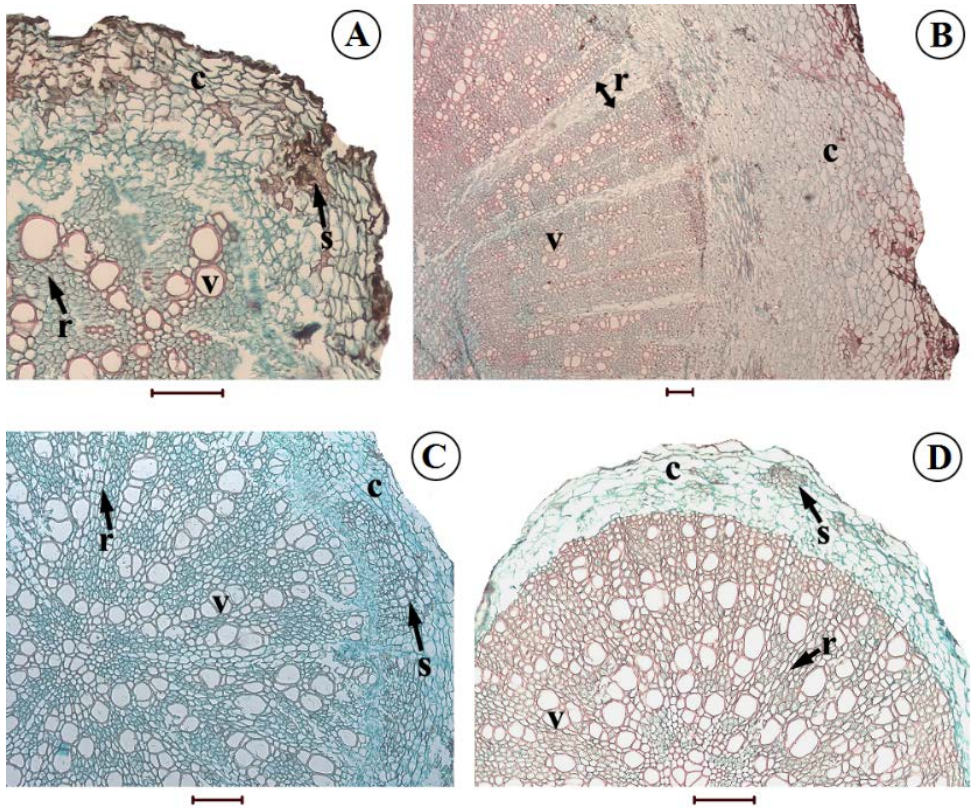

Figure 1. Cross-section sections of the roots in the *Lathyrus* species studied. (A) *L. aureus*, (B) *L. pratensis*, (C) *L. inconspicuus*, (D) *L. sphaericus*. c: cortex, r: rays, s: sclerenchymatous cells, v: vessel element. *Scale bars* = 100 µm.

3.2 Stem anatomy

The stem cross-sections reveal that the stems are mostly angled, or rarely more or less circular. The epidermis is composed of 1(-2)-layered rectangular, square or oval cells (8-45 µm wide and 5-30 µm long), with a uniform cuticle covering its outer surface. It is covered with simple, uniseriate, unicellular or multicellular, patent or adpressed, sparsely or densely eglandular hairs, and without or with few glandular hairs. In *L. inconspicuus* and *L. sphaericus*, chlorenchyma tissue lies adjacent to the epidermis. Under the epidermis the cortex comprises 1-9 layers of thin-walled parenchymatous cells that are of various shapes (oval, elongated, rectangular, polygonal or rounded) and sizes (12-75 µm wide and 7-50 µm long) with intercellular spaces. The cortex is significantly wider in *L. aureus*, *L. czeczottianus*, and *L. pratensis* than the other species. Similarly, it has more than three layers in *L. aureus*, *L. czeczottianus*, and *L. pratensis* whereas it is 1-3-layered in *L. inconspicuus* and *L. sphaericus*. There occur cortical vascular bundles in the cortex of *L. aureus* and *L. czeczottianus*. 3-9-layered sclerenchymatous cells are recognized above the phloem, with thicker cell walls. Fascicular cambium between the xylem and phloem within vascular bundles is observed. Interfascicular cambium between the bundles is clearly recognized in the stem of *L. aureus*. The center part of the stem is of great pith comprising polygonal and rounded parenchymatous cells (15-100 µm in diameter). However, in *L. aureus* the broken cells of the pith form a pith cavity in the center of the stem (Figure 2).

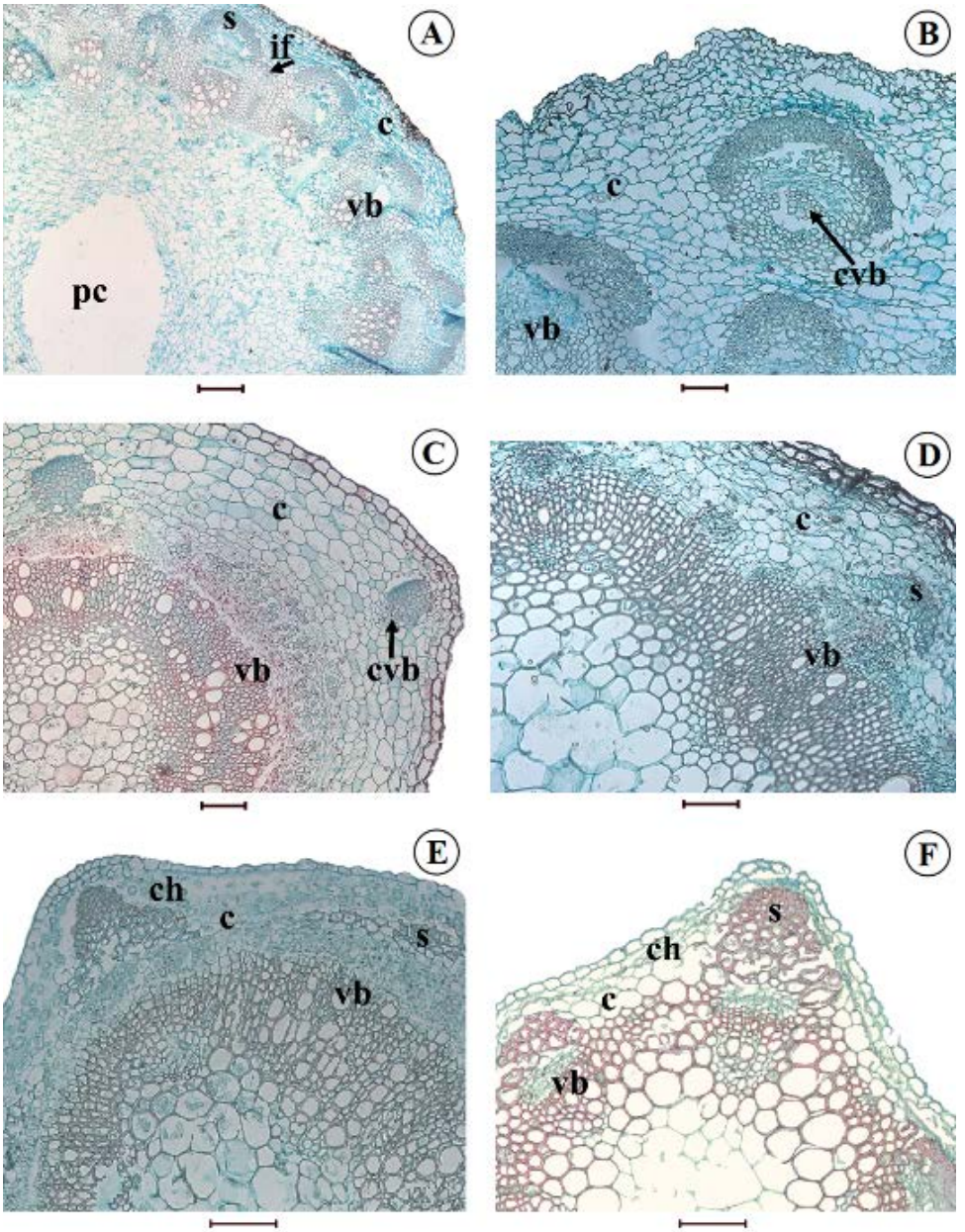

Figure 2. Cross-section sections of the stems in the *Lathyrus* species studied. (A-B) *L. aureus*, (C) *L. czeczottianus*, (D) *L. pratensis*, (E) *L. inconspicuus*, (F) *L. sphaericus*. c: cortex, ch: chlorenchyma, cvb: cortical vascular bundle, if: interfascicular cambium, pc: pith cavity, s: sclerenchymatous cells, vb: vascular bundle. *Scale bars* = 100 µm.

3.3 Leaf anatomy

The leaf cross-sections indicate that the epidermis is one layer of isodiametric, somewhat squarish or elongated cells on both adaxial and abaxial surfaces. Thickness of the leaf ranges from 140 to 330 µm. The upper and lower epidermises are covered with a cuticle, approximately 1-5 µm thick. The upper and lower epidermal cells are nearly equal in size (15-45 µm wide and 10-35 ± 2.46 µm). The leaf is dorsiventral and stomata are placed at almost the same level as the adjoining epidermal cells. The mesophyll (100-270 µm thick) consists of one or two layer of vertically elongated rectangular palisade cells having numerous chloroplasts and of three to five layers of irregular and loosely arranged spongy cells containing a smaller number of chloroplasts and large intercellular spaces. The palisade parenchyma is exclusively one-layered in *L. czeczottianus* and *L. pratensis* but it is 1-2-layered in the other species. The spongy parenchyma is 4-5-layered in *L. aureus* while it is 3-5-layered in the remaining species. The mesophyll contains vascular tissue organized in small and large collateral vascular bundles, each surrounded by sclerenchymatous fibre caps on both phloem and xylem. The vascular bundles are surrounded by one layer of parenchymatous bundle sheat cells. Bundle sheath extensions project as ribs on the abaxial side. In *L. aureus*, prismatic crystals of calcium oxalate are seldom observed in the mesophyll (Figure 3).

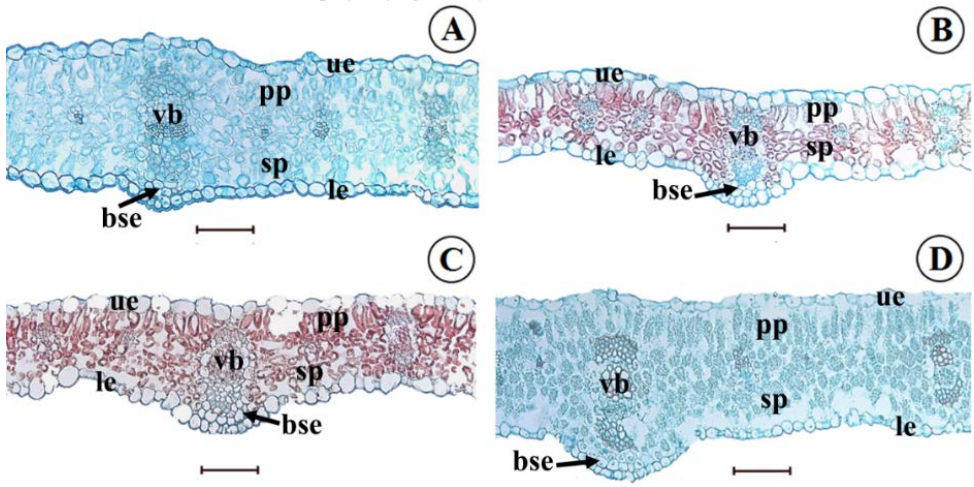

Figure 3. Cross-section sections of the leaves in the *Lathyrus* species studied. (A) *L. aureus*, (B) *L. czeczottianus*, (C) *L. pratensis*, (D) *L. inconspicuus*. bse: bundle sheath extensions, le: lower epidermis, pp: palisade parenhyma, sp: spongy parenchyma, ue: upper epidermis, vb: vascular bundle. *Scale bars* = 100 µm.

3.4 Petal, sepal and leaf epidermal micromorphology

The epidermal micromorphology of lateral petals of the *Lathyrus* species studied shows three main epidermal cell types: tabular rugose striate cells (TRS), areolate cells with more or less striations (AS) and papillose conical striate cells (PCS). TRS is detected in the lateral petals of *L. aureus* and *L. czeczottianus*. However, the lateral petals of *L. aureus* have elongated cells with dense striations whereas those of *L. czeczottianus* have elongated cells with less-dense striations. AS is recognized in *L. inconspicuus* and *L. sphaericus*. However, the lateral petals of *L. inconspicuus* consist of isodiametric cells with straight or weakly curved anticlinal walls while those of *L. sphaericus* comprises elongated cells with straight

anticlinal walls. PCS is found on the lateral petals of *L. pratensis*. Stomata are not found on the petals of all species studied. (Figure 4).

The sepals of the species studied bear three different trichome types on the epidermal cells: peltate glandular (type 1), capitate glandular (type 2), and non-glandular trichome (type 3). The type 1 shows the peltate glandular trichomes with a basal epidermal cell, one neck cell and multicellular secretory head. The type 2 indicates the capitate glandular trichomes with a basal epidermal cell, a short stalk, a neck cell and a cutinized, unicellular (or rarely multicellular, as in *L. aureus*) secretory head. The types 1 and 2 are situated on the sepals of *L. aureus*, *L. czeczottianus*, and *L. pratensis* but absent on those of the remaining species. The type 3 shows uniseriate, unbranched, short to long and unicellular to multicellular non-gladular trichomes. Also, these trichomes are straight or curved, and sharp-pointed or rounded at the tip. The size, structure and density of the non-glandular trichomes on the sepals vary among the species. Trichome density decreases with sepal growth. The sepals of *L. aureus* densely bear long flagelliform non-glandular trichomes; however, short and more or less straight trichomes are less densely distributed. *L. czeczottianus* and *L. pratensis* possess long non-glandular trichomes together with short non-glandular trichomes on their sepals, especially on the margins and ribs. The sepals of *L. inconspicuus* only bear short non-glandular trichomes consisting of one to several cells and bent in different directions. On the sepals of *L. sphaericus*, short non-glandular trichomes are very sparcely distributed (Figure 5).

According to leaf epidermal characteristics of the *Lathyrus* species investigated, epidermal cells are isodiametric or mostly elongated in shape. These cells usually have undulate to sinuate anticlinal walls. However, some epidermal cells have almost straight anticlinal walls. All the studied species are amphistomatic leaves as stomata are present on the abaxial and adaxial surfaces. Most of the stomata are opened or partially closed. The leaf epidermis of all species is covered by various trichome types but in *L. inconspicuus* and *L. sphaericus* it is glabrous or subglabrous. Three types of trichomes are observed: peltate glandular (type 1), capitate glandular with a short stalk (type 2), and non-glandular trichome (type 3). Trichome density decreases with leaf expansion. The types 1 and 2 are only found in *L. aureus*. The uniseriate, adpressed, straight or curved, unicellular or multicellular, short or long non-glandular trichomes are recognized in *L. aureus*, *L. czeczottianus*, and *L. pratensis*. They are often distributed evenly on the leaf but sometimes occur more frequently on the veins or margins (Figure 6).

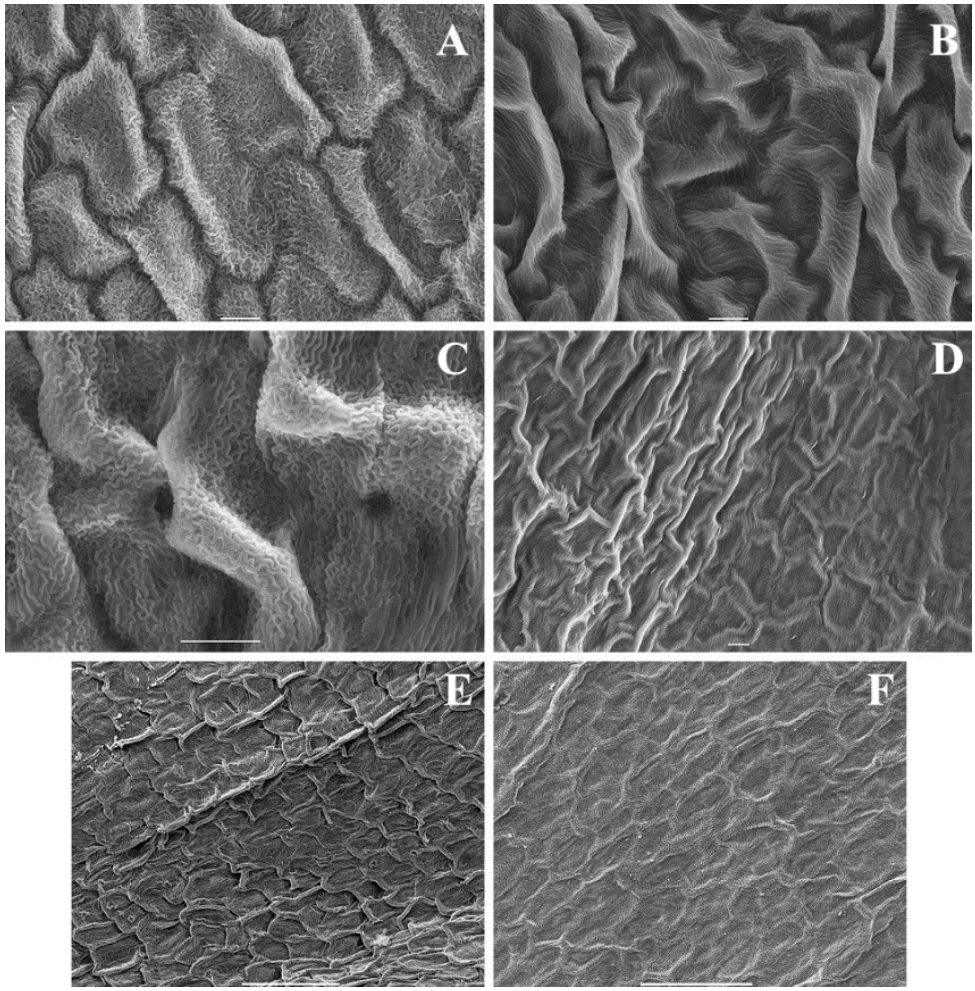

Figure 4. SEM micrographs showing the petal micromorphology of the *Lathyrus* species studied. (A) *L. aureus*, (B) *L. czeczottianus*, (C) *L. pratensis*, (D) *L. inconspicuus*, (E-F) *L. sphaericus*. *Scale bars:* A-D = 10 µm, E-F = 100 µm.

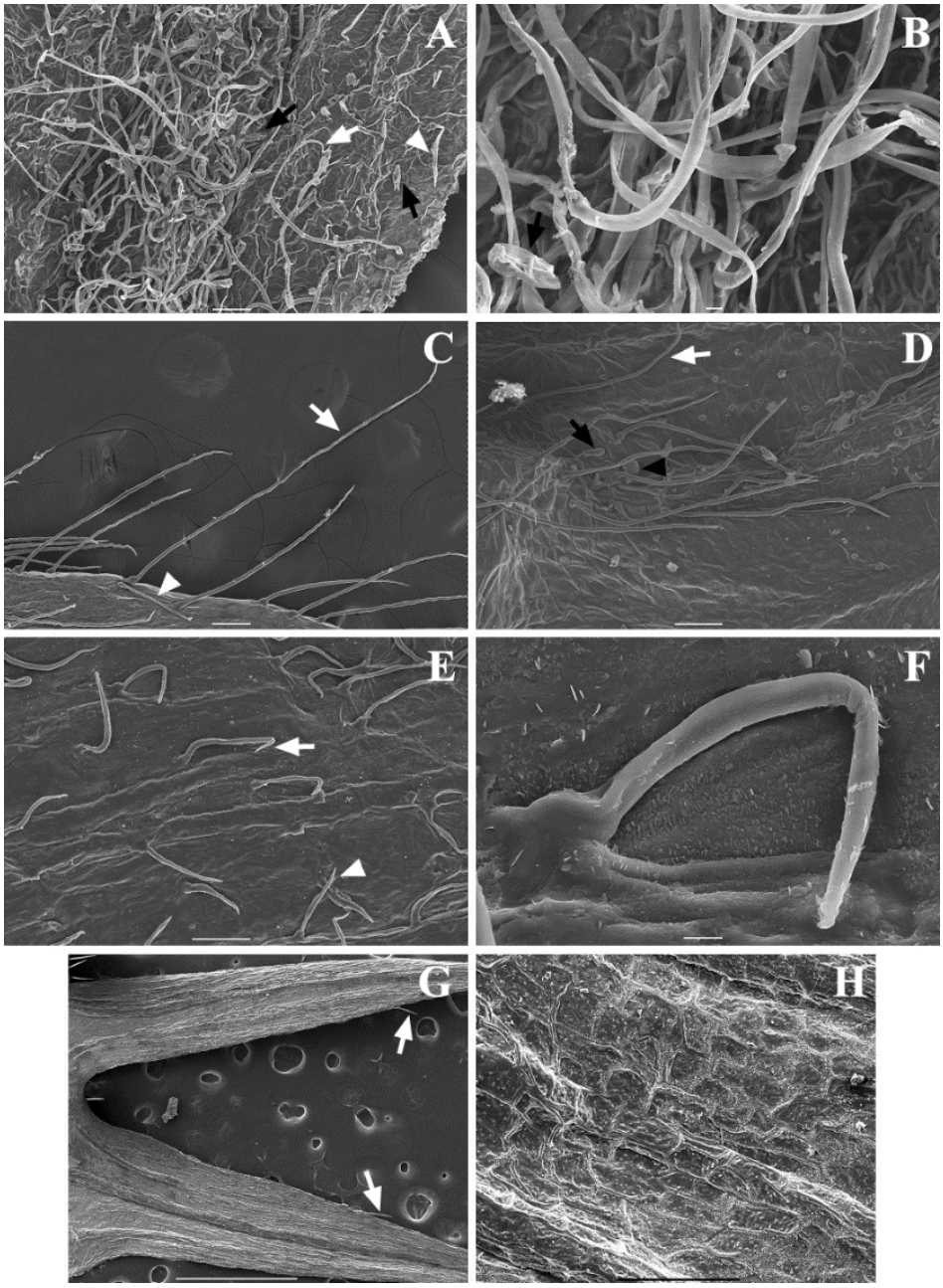

Figure 5. SEM micrographs showing the sepal micromorphology of the *Lathyrus* species studied. (A-B) *L. aureus*: A. short-stalked glandular trichomes (black arrow), multicellular, long flagelliform non-glandular trichomes (white arrow) and short non-glandular trichomes (white arrowhead), B. stalked glandular trichomes consisting of four head cells (black arrow); (C) *L. czeczottianus*: long, erect or bent non-glandular trichomes narrowed at the apical part (white arrow) and short non-glandular trichomes (white arrowhead); (D) *L. pratensis*: short-stalked glandular trichomes (black arrow), peltate glandular trichomes (black arrowhead) and long non-glandular trichomes (white arrow); (E-F) *L.*

inconspicuus: short, curved non-glandular trichomes (white arrow) and short, straight non-glandular trichomes (white arrowhead); (G-H) *L. sphaericus*: short non-glandular trichomes very sparsely situated on the sepal (white arrow). *Scale bars:* B, F = 10 µm, A, C-E = 100 µm, H = 200 µm, G = 1 mm.

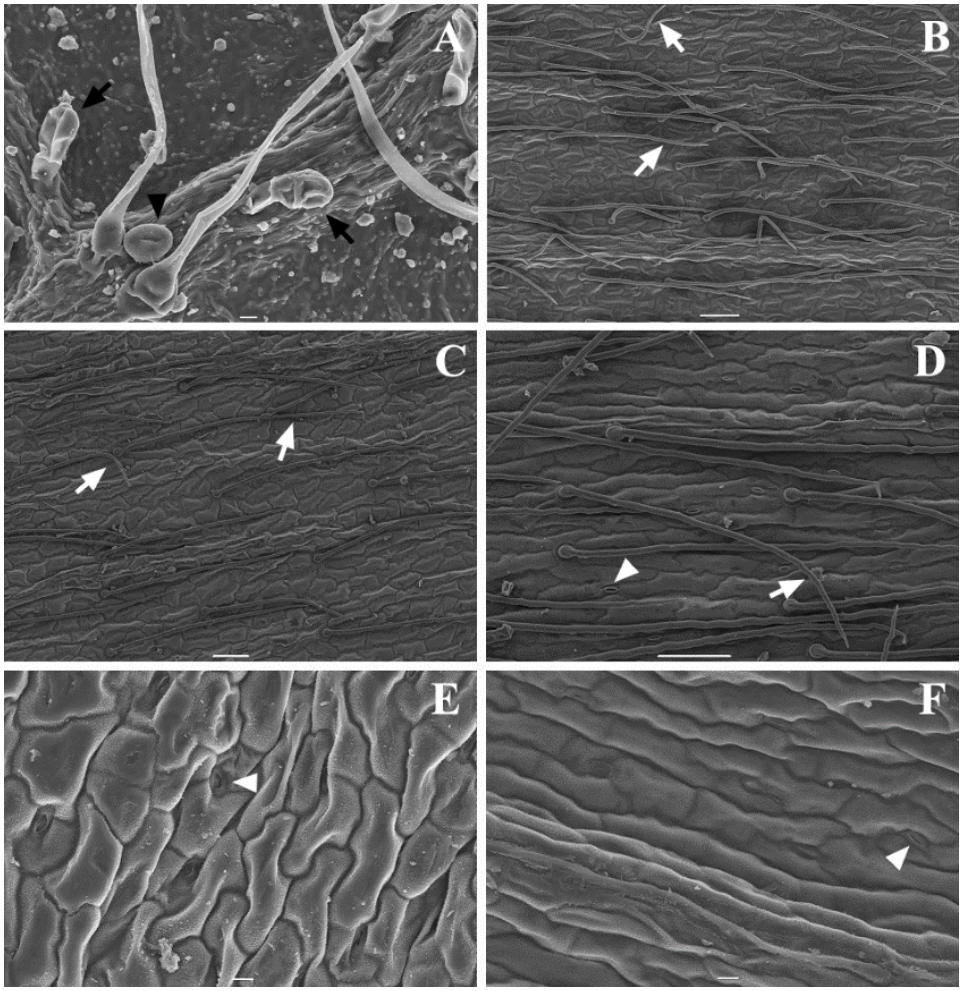

Figure 6. SEM micrographs showing the leaf micromorphology of the *Lathyrus* species studied. (A) *L. aureus*: short-stalked glandular trichomes (black arrow) and peltate glandular trichomes (black arrowhead); (B) *L. czeczottianus*: adpressed, straight or curved, unicellular or multicellular non-glandular trichomes (white arrow); (C-D) *L. pratensis*: adpressed, straight or curved, unicellular or multicellular non-glandular trichomes (white arrow) and stomata (white arrowhead); (E) *L. inconspicuus*: opened stoma (white arrowhead); (F) *L. sphaericus*: partially opened stomata (white arrowhead). *Scale bars:* A, E, F = 10 µm, B-D = 100 µm.

3.5 Macromorphology

Annuals or perennials, slender or sturdy, glabrous or pubescent. Stems 10-90 cm long, erect or ascending-erect, wingless, simple or branched, angled or not. Leaves pinnate,

aristate or tendrillous; leaflets 1-5-paired, ovate, lanceolate, linear, linear-lanceolate or elliptic-lanceolate, acute to acuminate, (-5)10-100 mm long, 0.5-50 mm broad, with parallel or pinnate veins; stipules ovate-lanceolate, lanceolate-subulate or lanceolate-acuminate, 3-30 mm long, sagittate or semi-sagittate. Peduncles shorter or longer than the leaves, raceme 1-25-flowered. Calyx 4-14 mm long; teeth equal to unequal, shorter or longer than the tube. Corolla orange, yellow, red or lavender-blue, 7-20 mm long. Legume linear or linear-oblong, 20-70 mm long, 4-8 mm broad. Seeds 4-14, smooth.

3.6 Multivariate Analysis

The dendrogram obtained from UPGMA clustering of similarity matrix is presented in Figure 7. The characters significantly differentiating *L. aureus* from the remaining species are stem with a pith cavity (4), sepal with flagelliform non-glandular trichomes (10), leaf with glandular trichomes (13), branched stem (16), leaf with 3-5-pairs of leaflets (20), ovate leaflet (21), leaflet more than 15 mm wide (22), pinnate leaf venation (23), flower more than 10 (25), very unequal calyx teeth (28) and gibbous calyx base (29). In turn, the greatest similarity is observed for *L. sphaericus* and *L. inconspicuus* assigned in sect. *Linearicarpus* (0.881) as well as for *L. pratensis* and *L. czeczottianus* belonging to sect. *Pratensis* (0.770).

The multivariate PCO analysis of *Lathyrus* species using Gower's general similarity coefficient shows the species studied in three groups corresponding to the sections *Linearicarpus*, *Pratensis* and *Orobus* (Figure 8). The first group includes one species, *L. aureus* in sect. *Orobus* that is very distant from the remaining groups. The second group consists of *L. czeczottianus* and *L. pratensis* in sect. *Pratensis* and the last one comprises *L. inconspicuus* and *L. sphaericus* in sect. *Linearicarpus*.

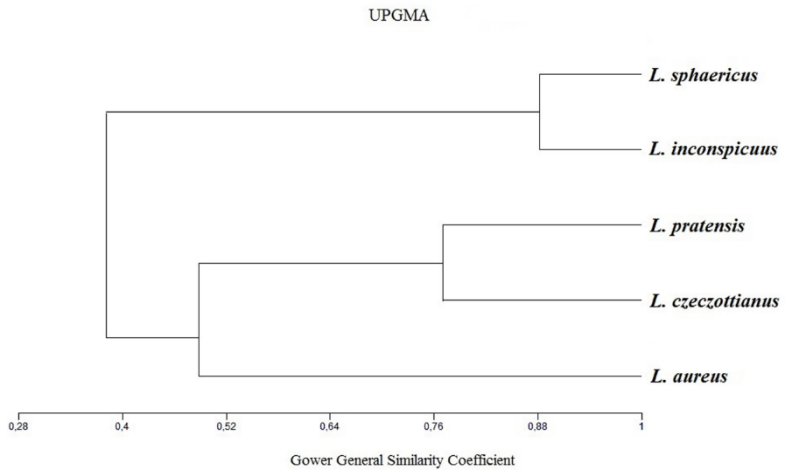

Figure 7. Dendrogram constructed by means of UPGMA algorithm and Gower's General Similarity Coefficient and showing relationships among the *Lathyrus* species studied.

Table 1
A list of anatomical, micromorphological and macromorphological characters scored for statistical analysis

No	Characters	Scores
1.	Presence of periderm at the root	Absent (0), present (1)
2.	Maximum number of rows of xylem rays at the root	3 (0), more than 3 (1)
3.	Number of rows of cortex cells at the stem	1-3 (0), 3-9 (1)
4.	Presence of pith cavity at the stem	Absent (0), present (1)
5.	Presence of cortical vascular bundles at the stem	Absent (0), present (1)
6.	Number of palisade parenchyma rows	Only 1 (0), 1-2 (1)
7.	Mesophyll thickness (µm)	Up to 150 (0), more than 150 (1)
8.	Type of petal epidermal micromorphology	Tabular rugose striate cells (0), areolate cells with more or less striations (1), papillose conical striate cells (2)
9.	Presence of glandular trichomes on the sepal	Absent (0), present (1)
10.	Presence of flagelliform non-glandular trichomes on the sepal	Absent (0), present (1)
11.	Presence of only short non-glandular trichomes on the sepal	Absent (0), present (1)
12.	Density of trichomes on the sepal	Absent/sparse (0), dense (1)
13.	Presence of glandular trichomes on the leaf	Absent (0), present (1)
14.	Presence of non-glandular trichomes on the leaf	Absent/almost absent (0), present (1)
15.	Life form	Annual (0), perennial (1)
16.	Stem branching	Branched (0), unbranched (1)
17.	Stem form	Rigid (0), not rigid (1)
18.	Presence of aristate leaves	Absent (0), present (1)
19.	Presence of tendrils on upper leaves	Absent (0), present (1)
20.	The number of leaflet pairs per leaf	1 (0), 3-5 (1)
21.	Leaflet shape	Ovate (0), lanceolate (1), elliptic-lanceolate (2), linear (3), linear-lanceolate (4)
22.	Leaflet width (mm)	Up to 15 (0), more than 15 (1)
23.	Leaflet venation	Pinnate (0), parallel (1)
24.	Stipule base	Sagittate (0), semi-sagittate (1)
25.	Number of flowers in a flower	Solitary (0), 3-10 (1), more than 10 (2)
26.	Calyx length (mm)	Up to 7 (0), more than 7 (1)
27.	Calyx base	Gibbous (0), not gibbous (1)
28.	Calyx teeth length	Equal/subequal (0), unequal (1)
29.	Corolla colour	Orange (0), lavander-blue (1), yellow (2), red (3)
30.	Corolla length (mm)	Up to 10 (mm), more than 10 (1)
31.	Legume shape	Linear (0), linear-oblong (1), linear-ensiform (2)
32.	Legume length (mm)	Up to 30 (0), more than 30

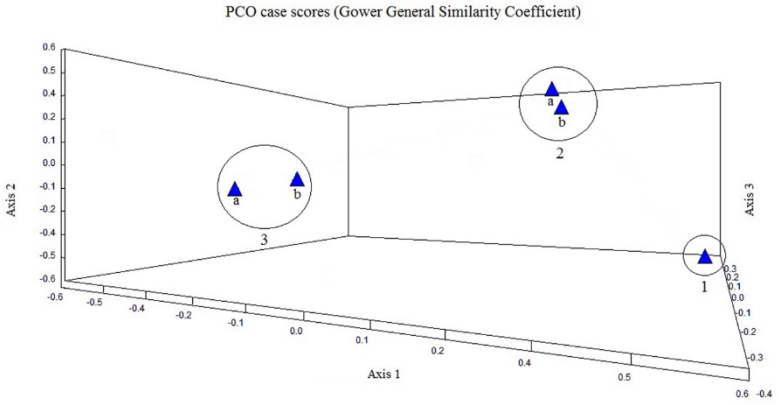

Figure 8. Principal coordinate analysis of *L. aureus* in sect. *Orobus* (1), *L. czeczottianus* in sect. *Pratensis* (2a), *L. pratensis* in sect. *Pratensis* (2b), *L. inconspicuus* in sect. *Linearicarpus* (3a) and *L. sphaericus* in sect. *Linearicarpus* (3b).

4. Discussion

This study includes significant findings on the macromorphology, anatomy, petal, sepal and leaf epidermal micromorphology of *L. aureus*, *L. czeczottianus*, *L. inconspicuus*, *L. pratensis*, and *L. sphaericus*. According to Kupicha's (1983) infrageneric classification of *Lathyrus* based upon macromorphological properties, such as life form, growth habit, stem shape, leaf arrangement, number of leaflets per leaf, leaf rachis ends, number of flowers per inflorescence and style contortion, are the most important characters used in delimiting the eleven sections of the genus in Turkey (Kahraman et al., 2014). The macromorphological results obtained in our study generally agree with earlier findings (Mouterde, 1966; Ball, 1968; Davis, 1970; Czefranova, 1971; Zohary, 1972; Townsend, 1974; Rechinger, 1979; Kupicha, 1983; Greuter, 1997; Leht, 2009). Leht (2009) pointed out that *L. inconspicuus* had stems with wings and leaves with no tendrils. However, our findings clearly reveal that this species has stems without wings and upper leaves with simple tendrils.

Metcalfe and Chalk (1950) offered the general anatomical characteristics of the family Fabaceae and their taxonomic value. Our findings on the root components are consistent with those of Metcalfe and Chalk (1950) and two members of *Lathyrus* investigated by Celep et al. (2011) and Kahraman et al. (2014). The root, stem and leaf cross-sections show some characters to be taxonomically significant in separating species. The surface tissue covering the root is the epidermis in *L. inconspicuus* and *L. sphaericus*. However, like *L. cilicicus* Hayek & Siehe (Celep et al., 2011) and *L. nissolia* L. (Kahraman et al., 2014), *L. aureus*, *L. czeczottianus*, and *L. pratensis* have the outer protective layer called the periderm replacing the epidermis. According to Metcalfe and Chalk (1950), xylem rays in the roots in the subfamily Papilionoideae of Fabaceae are composed of 1-12-rowed cells (mostly 2-3). *L. cilicicus* and *L. nissolia* have up to 5 rows of the rays (Celep et al., 2011; Kahraman et al., 2014). The xylem ray rows in the roots are partly significant for characterizing some species in our study. For example, there are 1-6 ray rows in the roots of *L. czeczottianus* and *L. pratensis* but the other investigated species have 1-3 ray rows in their roots. In most genera of the subfamily Papilionoideae, vessels are medium-sized (100-200 μm mean tangential diameter) (Metcalfe and Chalk, 1950). Our investigation reveals that the species investigated have small-sized vessels varying from 15 to 60 μm.

In agreement with earlier anatomical studies (Krstić et al, 2002; Mantar et., 2003; Celep et al., 2011; Kahraman et al., 2014), the stems of *L. inconspicuus* and *L. sphaericus* have a narrow cortex consisting of 1-3 cell layers. However, in *L. aureus*, *L. czeczottianus*, and *L. pratensis* the cortex covers a wider area composed of 4-9-layered cells. Cortical vascular bundles in the cortex occur in a few species of Papilionoideae, especially amongst those with winged or grooved stems (Metcalfe and Chalk, 1950). In our study, cortical vascular bundles are recognized in the cortex of two species, *L. aureus* and *L. czeczottianus*. The presence/absence and structure of the wing-like expansions in the stems of *Lathyrus* are of taxonomic value in separation of species (Metcalfe and Chalk, 1950). The stems of *L. maritimus* Bigel and *L. pratensis* L. are flattened on one side and provided with two wings each containing a vascular bundle, and their remaining vascular bundles are widely spaced and arranged in a flat circle around a pith cavity. *L. sylvestris* L. shows a similar stem structure but its wings are much longer than these species, with several vascular bundles in each wing (Metcalfe and Chalk, 1950). The stem cross-section of *L. latifolius* L. is elliptical with two expansions longer than the stem diameter (Krstić et al., 2002). There are two-winged stems in *L. hirsutus* L. and *L. sativus* L. (Mantar et al., 2003). The stems, on the contrary, have no wings in *L. cilicicus* (Celep et al., 2011) and *L. nissolia* (Kahraman et al., 2014) as well as the species studied in the present study. Whereas the pith occupying the center part of the stem is formed by large parenchymatous cells in *L. nissolia* (Kahraman

et al., 2014), it has a cavity in *L. cilicicus*, *L. maritimus* and *L. pratensis* (Metcalfe and Chalk, 1950; Celep et al., 2011). In our study there is a central pith cavity in the center of the stem of all species, except *L. aureus*.

Leaves are very variable in structure due to the wide range of leaf types in Fabaceae and usually dorsiventral and less frequently isobilateral (Metcalfe and Chalk, 1950). Unlike *L. latifolius* (Krstić et al, 2002), *L. hirsutus, L. sativus* (Mantar et., 2003) and *L. cilicicus* (Celep et al., 2011), the leaf of *L. nissolia* (Kahraman et al., 2014) is isobilateral. In our investigation, the species show dorsiventral leaf structure. *L. nissolia* (Kahraman et al., 2014) has leaves with the mesophyll ranging from 83.27-127.45 µm thick and composed of one-layered palisade cells. The mesophyll of *L. czeczottianus* and *L. pratensis* varies between 100 and 150 µm thick and comprises only one layer of palisade cells but that of the remaining species studied ranges from 160 to 150 µm thick and consists of 1-2 layers of palisade cells. In the subfamily Papilionoideae, crystals are predominantly solitary; however, are very variable in size and shape. Sometimes, they have a characteristic appearance and distribution, especially in the leaf epidermis. Crystalliferous cells frequently form a sheath along the outer boundary of the pericyclic sclerenchyma (Metcalfe and Chalk, 1950). In *L. aureus*, solitary crystals are rarely present in the mesophyll.

The epidermal surface of petals is significant in pollination since it affects the way where pollinators perceive and interact with the flower. Many legume species possess a specialized flower morphology that promotes pollinator specificity (Ojeda et al., 2009). Petal epidermal micromorphology exhibits a significant character in discrimination of species (Metcalfe and Chalk, 1950). The characteristics of the petal epidermal types have been used for taxonomic and phylogenetic analyses in Fabaceae (Stirton, 1981) and Compositae (Baagøe, 1977, 1980; Hansen, 1991). Stirton (1981) investigated the lateral petals of the papilionoids. Ojeda et al. (2009) studied a total of 175 species representing 26 tribes and 89 genera in all three subfamilies of Fabaceae. They also represented the epidermal types on the dorsal, lateral and ventral petals of seven species of *Lathyrus*. They concluded that papilionoids had high degree of petal micromorphological variations and great differences between the dorsal, lateral and ventral petals occurred but detected only smaller differences among *Lathyrus* species. As a result of Ojeda et al.'s (2009) research, tabular rugose cells with longitudinal striations (TRS) are found on the three petal types of some species of the genus, such as *L. latifolius*, *L. sativus* ve *L. sylvestris*. Similarly, all petals of *L. nissoli*a reveal TRS (Kahraman et al., 2014). The dorsal and lateral petals of *L. venetus* Reuy have papillose knobby cells with a rugose sculpture (PKR) whereas its ventral petals have TRS. In *L. vernus* (L.) Bernh., the dorsal and ventral petals have TRS while the lateral petals have PKR. The present study indicates that the lateral petals of the species studied have TRS, areolate cells with more or less striations (AS) and papillose conical striate cells (PCS). Between *L. aureus* and *L. czeczottianus* plus *L. inconspicuus* and *L. sphaericus*, significant differences, such as shape of epidermal cells, density of striations on the surface and pattern of anticlinal cell walls, are observed for TRS and AS. Stomata are rarely present on papilionoid flowers Ojeda et al. (2009). However, they are completely absent on the petals of the species studied.

Legumes show a variety of unicellular and multicellular trichomes, both secretory and non-secretory, on the vegetative (Franceschi and Giaquinta, 1983; Retallack and Willson, 1988; Shaheed and Illoh, 2010) and floral organs (Guard, 1931; Bernard and Singh, 1969; Lersten and Brubaker, 1987; Gunasinghe et al., 1988; Tucker, 1997; Healy et al., 2005; de Freitas Mansano and de Pádua Teixeira, 2008). In a great number of genera belonging to the subfamily Papilionoideae, the leaf epidermis is characterized by the common occurrence of angular folds in the anticlinal walls; by the development of papillae, especially in the

lower surface (Metcalfe and Chalk, 1950). In agreement with those of the previous studies conducted on the legumes, the present results show peltate glandular, capitate glandular and non-glandular trichomes on the sepals and leaves of the species investigated. The presence/absence, type, size and density of sepal and leaf trichomes represent good taxonomic markers for their differentiation.

The UPGMA and PCO analyses indicate that the species studied can be easily separated. In general, morphological characters are more significant than anatomical and micromorphological characters. The UPGMA cluster analysis reveals a great anatomical and micromorphological affinity between *L. sphaericus* and *L. inconspicuus*. These species are differentiated by some macromorphological characteristics, such as density of trichomes on the sepal, presence/absence of aristate leaves, leaf shape, corolla colour and legume shape. Our results conducted on *L. sphaericus* and *L. inconspicuus* are similar to those of Leht (2009). The cluster analysis also shows the close relationship between *L. pratensis* and *L. czeczottianus*. According to the multivariate PCO analysis, there are three groups including the species studied. Both the UPGMA and PCO analyses support that *L. aureus* is more distinct than the other studied species due to its anatomical, micromorphological and macromorphological characteristics.

5. Conclusion

The present study is the first comprehensive report on the vegetative anatomy and the petal, sepal and leaf epidermal micromorphology of *Lathyrus* species studied. The results indicate some anatomical, micromorphological and macromorphological characters to be taxonomically informative in discriminating species. However, this study is somewhat limited at the infrageneric level as it is based on only the five species assigned in the sections *Linearicarpus*, *Pratensis* and *Orobus* of *Lathyrus*. Thus, investigations covering more species of the genus seem to be essential to construct a satisfactory infrageneric classification of the genus.

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Appendix 1

Collection data of *Lathyrus* specimens examined for anatomical, micromorphological and macromorphological studies

L. aureus
Ankara: Çubuk, Karagöl, 1512 m, 30.5.2010, H.Çildir 132. Sivas: Zara to Şerefiye, 1639 m, 22.6.2010, H. Çildir 163.

L. czeczottianus
Aksaray: Hasan Mountain, 1631 m, 23.5.2010, H. Çildir 126. Ankara: Kıcılcahamam Soğuksu National Park, 1111 m, 5.6.2010, H. Çildir 135; ibid. 1186, 5.6.2010, H. Çildir 136; ibid., 1392 m., 5.6.2010, H. Çildir 140. Ankara: Kızılcahamam Soğuksu National Park, Yanıksırtı road, 1222 m, 23.6.2009, H. Çildir 100. Ankara: Işık Montain, mountain peak road, 1651 m, 23.6.2009, H. Çildir 103. Ankara: Çubuk to Karagöl, 1208 m, 30.5.2010, H. Çildir 130; ibid., 1512 m, 30.5.2010, H. Çildir 132. Eskişehir: Mihalıççık, 1435 m, 16.6.2010, H. Çildir 148. Kayseri: Erciyes Mountain, 1780 m, 19.6.2010, H. Çildir 157. Konya: Akşehir, Sultan Mountain, 1290 m, 17.6.2010, H. Çildir 152. Sivas: Zara to Şerefiye, 1639 m, 22.6.2010, H. Çildir 162.

L. inconspicuus
Ankara: Çubuk to Karagöl, 1208 m, 30.5.2010, H. Çildir 131. Kayseri: Develer to Tomarza road, 1503 m, 19.6.2010, H. Çildir 158. Kayseri: Sorgun to Çekerek road, 1086 m, 24.6.2010, H. Çildir 176. Sivas: Zara to Şerefiye, 1639 m, 22.6.2010, H. Çildir 164. Sivas: Zara to İmranlı, 1587 m, 22.6.2010, H. Çildir 166. Yozgat: Sorgun to Alaca road, 1322 m, 24.6.2010, H. Çildir 177.

L. pratensis
Ankara: Beypazarı Eğriova detour, 1534 m, 28.6.2009, H. Çildir 113. Ankara: Kıcılcahamam Soğuksu National Park, 1111 m, 5.6.2010, H. Çildir 135; ibid. 1161 m, 5.6.2010, H. Çildir 134. Ankara: Kızılcahamam Soğuksu National Park, Yanıksırtı road, 1240 m, 23.6.2009, H. Çildir 101. Ankara: Işık Mountain, 1577 m, 23.6.2009, H. Çildir 102. Eskişehir: Yukarı Kalabak village, 1127 m, 27.6.2009, H. Çildir 108. Eskişehir: Mihalıççık, roadside, 1435 m, 16.6.2010, H. Çildir 147. Sivas: Divriği to Zara, Yeşildere Beydağ road, 1561 m, 21.6.2010, H. Çildir 161. Sivas: Zara to Şerefiye, 1639 m, 22.6.2010, H. Çildir 165. Sivas: Yıldızeli, near Kızılırmak river, 1368 m, 23.6.2010, H. Çildir171. Yozgat: Akdağmadeni to Şarkışla road, 1586 m, 23.6.2010, H. Çildir 173; ibid., 1484 m, 23.6.2010, H. Çildir 174. Yozgat: Yozgat National Park, 1492 m, 25.6.2010, H. Çildir 179.

L. sphaericus
C4 Konya: Akşehir, Çakıllı village, 1262 m, 12.6.2009, F. Güneş 2332.

Appendix 2

Data matrix of the coded anatomical, micromorphological and macromorphological characters

Characters	*L. aureus*	*L. czeczottianus*	*L. pratensis*	*L. inconspicuus*	*L. sphaericus*
1.	1	1	1	0	0
2.	0	1	1	0	0
3.	1	1	1	0	0
4.	1	0	0	0	0
5.	1	1	0	0	0
6.	1	0	0	1	1
7.	1	0	0	1	1
8.	0	0	1	2	2
9.	1	1	1	0	0
10.	1	0	0	0	0
11.	0	0	0	1	1
12.	1	1	1	1	0
13.	1	0	0	0	0
14.	1	1	1	0	0
15.	1	1	1	0	0
16.	1	0	0	0	0
17.	0	1	1	1	1
18.	1	1	0	1	0
19.	0	0	1	1	1
20.	1	0	0	0	0
21.	0	1	2	4	3
22.	1	0	0	0	0
23.	0	1	1	1	1
24.	1	0	0	1	1
25.	2	1	1	0	0
26.	1	1	0	0	0
27.	0	0	0	1	1
28.	1	0	0	0	0
29.	0	1	2	1	3
30.	1	1	1	0	0
31.	0	0	1	0	2
32.	0	0	1	0	0