

MORPHOLOGICAL, ANATOMICAL AND CYTOLOGICAL STUDIES ON ENDEMIC *LAMIUM PISIDICUM*

PELİN BARAN^{1*} AND CANAN ÖZDEMİR²

¹Uşak University, Faculty of Science and Art, Department of Biology, 1 Eylül Campus, D2 Academic Block, Uşak 64200-Turkey;
²Celal Bayar University, Faculty of Art and Science, Department of Biology, Muradiye Campus, Muradiye-Manisa 45140-Turkey
*Corresponding author's e-mail: pelin.baran@usak.edu.tr

Abstract

Morphological and anatomical features of endemic *Lamium pisidicum* R. Mill (Lamiaceae) were studied. *L. pisidicum* is a perennial with a taproot system. The stem is ascending to erect in position and clearly quadrangular in shape. The leaves are ovate to broadly ovate or rarely reniform in shape. Verticillasters are 2-14 flowered. The corolla is pinkish-purple to mauve and the upper lip is deeply bifid and the tube is without annulus. The stigma is bifid. The stamens are didynamus. Anatomically, spring and autumn woods are distinguishable in the root structure of the perennial taxon. There is a well-developed collenchymatous layer at the corners of the stem and a pith hollow in the centre. In the petiole cross-section, 1-2 layered collenchyma layer is located at the corners while 3 central vascular bundles are located in the middle and 1 vascular bundle at the petiolar wings. The leaf is bifacial. Each anther is of two thecae and each theca is of 2 pollen sacs. Pollen type is trizonocolpate and pollen shape is spheroidal. The cross-section of seed is triangular. Glandular hairs distributed on the plant species are classified into 2 main types, peltate and capitate. Furthermore, capitate glandular hairs are separated into 2 types, type I and type II. Somatic chromosomes are counted as $2n=18$ for the first time in this study.

Introduction

The type genus of Lamiaceae is *Lamium* L. (Harley 2003). The genus *Lamium* comprises about 40 species of herbaceous annuals and perennials occurring from North Africa to Eurasia (Mennema, 1989; Mabberley, 1997). The distribution area of the genus reaches from Western Europe to Eastern Asia, including Northern Africa, approximately between 65 and 30° Northern latitude. Some of the taxa are introduced in Greenland Iceland, America, Australia and Tropical and South Africa. The centre of diversity of the genus is obviously found in the Irano-Turanian and the Mediterranean regions (Mennema 1989). In Turkey, about 30 *Lamium* species naturally exist and approximately 23 taxa including varieties and subspecies are endemic. *Lamium pisidicum* R. Mill is one of the endemic species to Turkey (Mill, 1982; Davis *et al.*, 1988; Güner *et al.*, 2000). It is categorized as LR(cd) endemic (Ekim *et al.*, 2000).

Some *Lamium* species have been used in the official and folk medicines as astringent, anti-proliferative, antispasmodic in some parts of Europe, China and also in Anatolia as well as other medicinal plants (Khattak 2012) and have useful remedies in menorrhagia, prostrate, scrofula, leukorrhea, paralysis, regulation of sebaceous secretions and traditional usage as food (Bremness, 1995; Baytop, 1999; Cui *et al.*, 2003). On the other hand, *Lamium* species which are frequently visited by honeybees and bumblebee queens are ecologically important hosts for a number of insect species and they are self-pollinating and self-compatible plants (Savchenko *et al.*, 2001; Macior, 1978, Sönmez & Altan, 1992; Sıralı & Deveci, 2002; Sabuncu *et al.*, 2002; Eltz 2006). In addition, some *Lamium* species are ornamental and well suited to a variety of growing conditions (Rudy, 2004).

A taxonomical revision of the genus *Lamium* mainly based on the study of herbarium collections has been made by Mennema (1989). A few studies on floral forms (Lord, 1980; 1982), chromosome numbers (Gill, 1983), systematic implications of pollen morphology (Abu-Asab

& Cantino, 1994) of some *Lamium* species and a revision (Park & Kim, 1995) are also available in the literature. There has not been any morphological and anatomical study resembling this work belonging to *Lamium* species, except the studies recently done on endemic perennial *L. lycium* (Baran & Özdemir, 2009) and endemic annual *L. moschatum* var. *rhodium* (Baran & Özdemir, 2011).

Materials and Methods

Plant samples were collected from natural populations and some of them were used for morphological and anatomical studies while others were dried as herbarium samples. Voucher samples are sited in the herbarium of Science and Art Faculty of Celal Bayar University. The samples were collected from the following location:

Isparta: Şarkikaraağaç, Kızıldağ National Park, dry places on limestones, 1391 m, 22.iv.2009, Baran 252.

The taxonomical description of the species followed Mill (1982). Morphological measurements were based on 15-20 plant specimens. Anatomical studies were carried out on the samples kept in alcohol 70%. The paraffin method (Algan, 1981) using microtome was applied for preparing the anatomical cross-sections of root, stem, petiole, leaf, corolla, anther and seed. Safranin O and Fast green was used for dyeing the cross-sections. Handle-blade sections were taken for calyx, pistil, filament and stem collenchyma tissue, dyeing with Sartur reactive (Baytop, 1981). Anatomical measurements were based on 30 cells. Classification of glandular hairs followed Werker (1993). Pollen specimens were prepared using the technique of Wodehouse (1965). Measurements were based on 30 pollen grains. Classification of pollen type and shape followed Moore *et al.*, (1991) and Punt *et al.*, (2007) respectively.

Cytological study was carried by following the technique of Elçi (1994). Microscopic examinations were made on an OLYMPUS BX50 microscope with photographic camera.

Results

Morphological characters: Perennial plant species has a taproot. Stem is clearly quadrangular, ascending to erect and branched at the base (Figs. 1C, 2A), glandular, sparsely puberulent to pubescent or to sparsely villous. Leaves are simple, ovate to broadly ovate or rarely to reniform (Fig. 1C), deeply incised-crenate (Figs. 1A-D), obtuse at apex, cordate at base, reticulate-pinnate in venation (Fig. 1D), glandular, pilose to villous, puberulent to pubescent. Petiole is glandular, pubescent to villous or sparsely villous. Bracts are ovate to broadly ovate, glandular, pilose to pubescent (Figs. 1D, 2B-C). Bracteoles are needle shaped, glandular, pilose (Fig. 2D). Verticillasters are 1-4 and the number of flowers in

each verticillaster is 2-14 (Figs. 1A-D & 2B-D). Calyx has 5 teeth which are narrowly triangular, glandular, patent-ciliate (Fig. 2D). Corolla is bilabiate, pinkish-purple to mauve, light pink to purple. Corolla tube is straight, striate without annulus (Figs. 1D, 2E), glandular, pubescent or villous. The veins on the inner surface of corolla tube are bright while the ones on the outer surface are pale (Fig. 1D). Upper lip is deeply bifid, forming two lobes, glandular, pilose to villous or pubescent (Figs. 1D, 2E). Lower lip is deeply emarginate, glandular, villous (Fig. 2E). Stigma is bifid. Stamens are didynamous. Anther thecae are divaricate, hairy. Nutlets are acutely triquetrous, dark brownish-olive (Figs. 1E, 2F). Table 1 shows morphological measurements of the plant species.



Fig. 1. View (A-B), habit (C), flower (D) and seeds (E) of *Lamium pisidicum* (Scale Bar 5 cm for C, 1 cm for D, 2 mm for E) r. root, s. stem, p. petiole, l. leaf, v. verticillaster, b. bract, ca. calyx, cot. corolla tube, u. upper lip of corolla, lu. lobe of the upper lip, ll. lower lip of corolla, ll. lobe of lower lip.

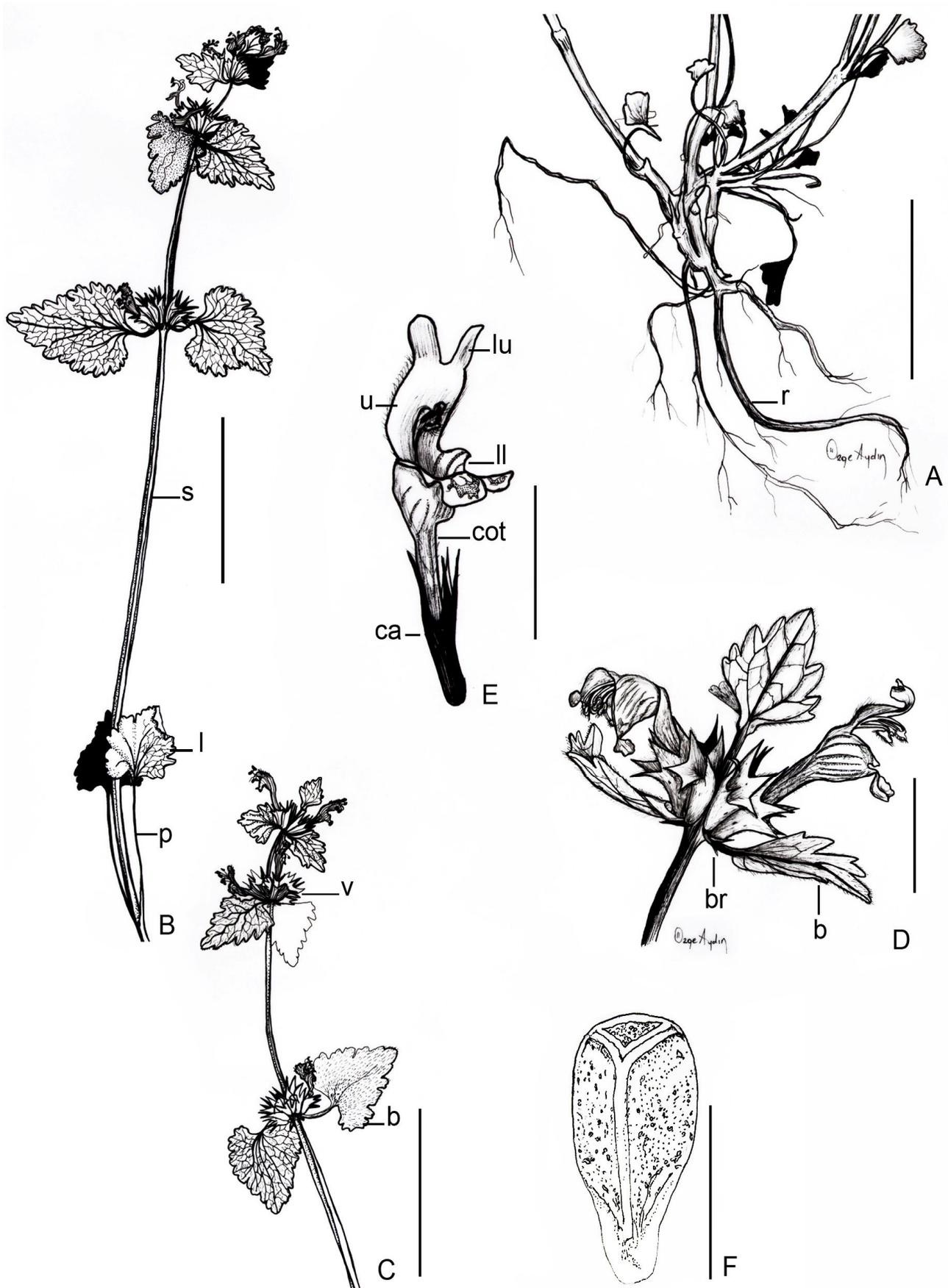


Fig. 2. Morphological drawings of parts of *Lamium pisidicum*.

A. Root (Scale Bar: 3 cm), B-C. Stem flowered (Scale Bar: 5 cm), D. Inflorescence (Scale Bar: 1 cm), E. Flower (Scale Bar: 1 cm), F. Seed (Scale Bar: 2 mm). s. stem, p. petiole, l. leaf, v. verticillaster, b. bract, br. bracteol, ca. calyx, cot. corolla tube, u. upper lip of corolla, lu. lobe of the upper lip, ll. lower lip of corolla, r. root.

Table 1. Morphological measurements of *Lamium pisidicum*.

N [■] = 20	Min. [▲] -Max. [●] (cm)	Mean±S.D*(cm)		Min. [▲] -Max. [●] (cm)	Mean±S.D*(cm)
Root			Corolla		
Root length	15.00-45.00	29.25±12.34	Tube width	0.06-0.15	0.10±0.03
Stem			Upper lip length	0.50-1.10	0.75±0.14
Stem length	18.00-50.00	34.05±9.91	Lobe of upper lip	0.20-0.50	0.35±0.11
Leaf			Lower lip length	0.55-0.85	0.70±0.08
Leaf length	1.50-3.80	2.89±0.74	Upper Filament	0.70-0.80	0.76 ± 0.05
Leaf width	1.20-3.00	2.42±0.58	Lower Filament	0.85-1.20	1.02±0.13
Petiole			Anther length	0.10-0.13	0.11±0.01
Petiole length	1.50-6.00	3.67±1.38	Pistil length	1.25-2.80	2.03±0.53
Calyx			Bract		
Calyx length	0.80-1.10	0.93±0.12	Bract length	1.20-4.50	2.34±1.07
Teeth length	0.25-0.40	0.34±0.06	Bract width	0.80-3.50	2.03±0.91
Calyx tube	0.30-0.65	0.50±0.11	Bracteol	0.25-0.50	0.40±0.09
Corolla			Seed		
Corolla length	1.80-2.80	2.35±0.34	Seed length	0.20-0.35	0.29±0.05
Tube length	0.90-2.00	1.53±0.28	Seed width	0.12-0.15	0.14±0.01

■: Sample Number, ▲: Minimum, ●: Maximum, *: Standard Deviation

Anatomical characters

Root: Peridermis is 6-19 layered at the outermost of the cross-section (Fig. 3A). Parenchymatous cortex under peridermis is 18-25 layered with large and oval or rectangular shaped cells in the root. Endodermis is indistinguishable. Phloem is large and parenchyma cells in phloem are orderly placed. Sclerenchyma is absent in the cortex. Cambium is distinguishable. Vessel elements are enlarged and vessels are homogenously scattered from the inner to the outer. Xylem rays are 1-2 layered and heterogenous. Primary xylem elements are placed in the center of root xylem. Spring and autumn woods are clearly distinguishable (Fig. 3A, Table 2).

Stem: Stem is quadrangular in cross-section (Fig. 3B). Epidermis is single layered and formed by variously shaped cells. Angular collenchyma is 8-12 layered and located only at the corners of stem (Fig. 3C). Cortex is formed by 5-9 layered parenchyma. These cells are oval or sometimes nearly roundish in shape. The cells near epidermis is smaller than those near vascular bundles. Phloem is located in a small part upon the xylem. Vascular cambium is 1-3 layered. Vessels are orderly arranged in xylem. Basically, 4 large and many small vascular bundles are located. The large pith region consists of roundish parenchymatous cells with intercellular spaces. Centre of stem cross-section is hollow (Fig. 3B, Table 2).

Petiole: Epidermis, the outermost layer, is formed by cells of various shapes and sizes (Fig. 4A). Epidermis bears glandular hairs. Parenchyma with circular cells and intercellular spaces is 5-9 layered under epidermis. Chlorenchyma is 1-5 layered under epidermis. Discontinuous plate collenchyma is 1-3 layered at the

corners of the cross-section. Three central collateral vascular bundles are present in the middle and one small bundle in the petiolar wings (Fig. 4A-B, Table 2).

Leaf: Epidermis formed by various shaped cells is single layered on the adaxial and abaxial surface. Stoma cells are visible in the abaxial epidermis (Fig. 4C). The adaxial epidermis is thicker than the abaxial. Leaf is bifacial consisting of a palisade and spongy parenchyma. Palisade parenchyma is 2 or 3 layered with vertically elongated and parallelly ordered cells. Spongy parenchyma is 3-4 layered and consists of various shaped cells with large intercellular spaces. Mesophyllous cells adjacent to the median vein are round shaped (Fig. 4C, Table 2).

Calyx: Adaxial and abaxial epidermis cells are various shaped. The outer surface of epidermis cells is smooth walled and without papilla. Abaxial epidermis is thicker than the adaxial (Fig. 4D). Parenchyma is 3-5 layered and with large intercellular spaces. Parenchymatous cells are usually roundish or oval shaped. Vascular bundles are arranged regularly along the cross-section. Parenchyma cells bear chloroplasts (Fig. 4D, Table 2).

Corolla: The outer surfaces of epidermis cells are smooth walled. A thin cuticle surrounds epidermis. Adaxial and abaxial epidermis cells are various shaped and are of chloroplasts. Parenchyma with large intercellular spaces is 2-8 layered (Fig. 4E). Intercellular spaces within parenchyma adjacent to the vascular bundles are much smaller than those in other parts. Diameter of parenchymatous cells is getting smaller in parts towards the vascular bundles. Parenchymatous cells are various shaped (Fig. 4E, Table 2).

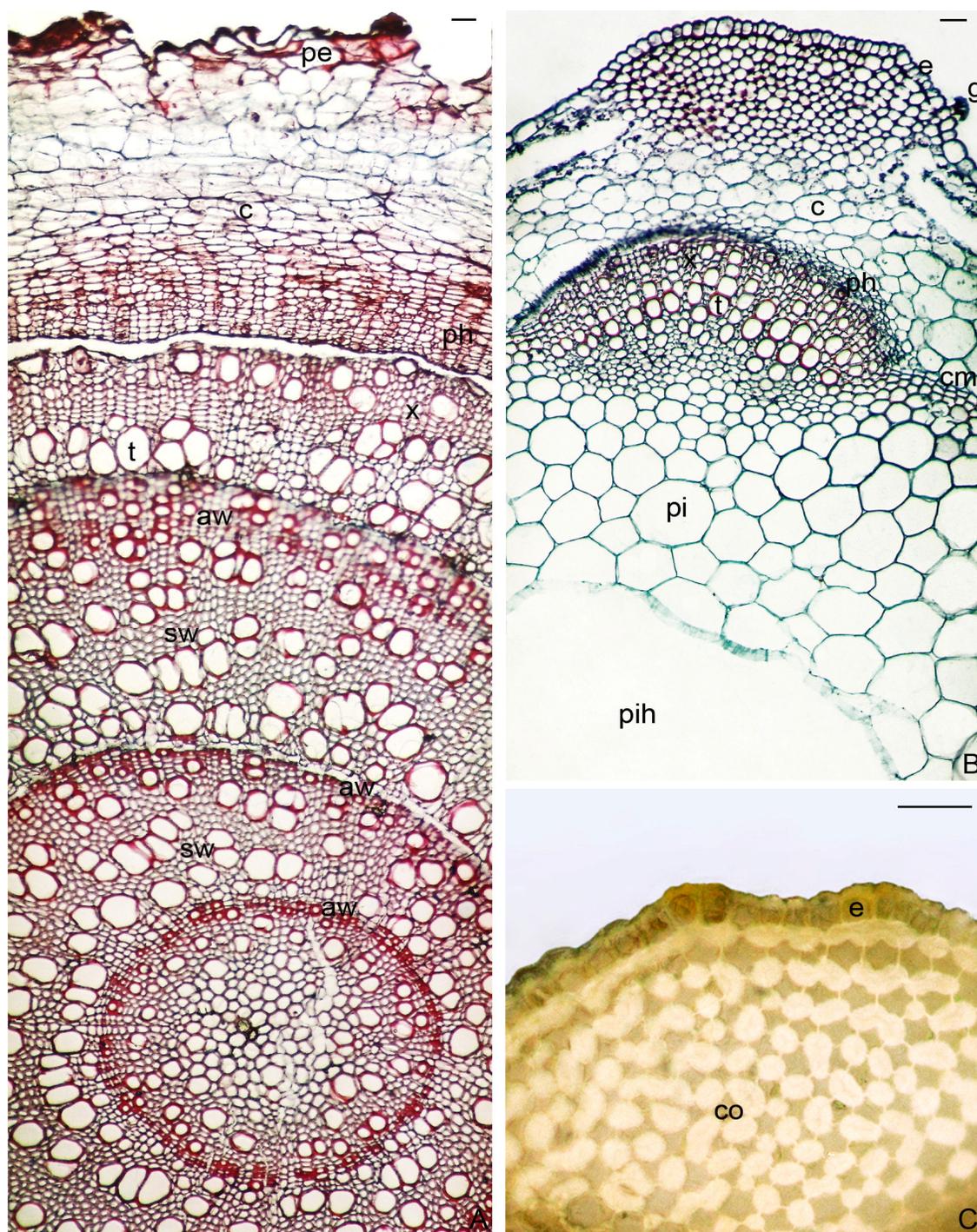


Fig. 3. The root (A) and stem (B-C) cross-sections of *Lamium pisidicum*

e. epidermis, g. glandular hair, pe. periderm, c. cortex parenchyma, co. collenchyma, ph. phloem, cm. cambium, x. xylem, t. trachea, sw. spring wood, aw. autumn wood, pi. pith, pih. pith hollow (Scale Bars: 35 μ m)

Generative organs: Pistil cross-section is nearly circular in shape at the median level and covered by an epidermis (Fig. 5). Pistil bears a central sclerenchymatous axis and two small lateral vascular bundles and is filled with roundish parenchymatous cells (Fig. 5A). Filament cross-section at the median level is roundish. An epidermis outermost and parenchyma and one vascular bundle in the middle, respectively are located (Fig. 5B). Each anther bears two thecae with two pollen sacs (Fig. 5C). Pollen sacs are filled with a number of pollen grains (Fig. 5D). The tapetum covers pollen grains. Pollen type classified according to Moore *et al.*, (1991) is trizonocolpate. Pollen

shape classified according to Punt *et al.*, (2007) is spheroidal (Fig. 5E, Table 3).

Seed cross-section consists of an embryo in the center, endosperm, seed coat, sclerenchyma layer and a cork layer in the outermost layer, respectively. The endosperm consists of large nearly polygonal adjacent cells. The seed coat surrounds the endosperm. The sclerenchyma layer gives general shape of the seed. The cork layer consists of longitudinally elongated cells. Both sclerenchyma and cork layer, the outermost 2 layers, function as a protective tissue for the seed (Fig. 5F).

Table 2. Anatomical measurements of various organs of *Lamium pisidicum*.

N [■] = 30	Width (µm)		Length (µm)	
	Min. [▲] - Max. [●]	Mean ± S.D.*	Min. [▲] - Max. [●]	Mean ± S.D.*
Root				
Peridermis cell	29.10-90.00	54.25 ± 19.21	15.90-42.40	27.81 ± 8.68
Parenchyma cell	18.50-95.30	48.35 ± 20.47	15.90-63.50	27.28 ± 14.54
Vessel	15.90-79.40	43.42 ± 21.90		
Endodermis cell	10.60-58.20	24.85 ± 13.68	10.60-26.50	20.58 ± 4.97
Stem				
Epidermis cell	5.30-26.50	15.61 ± 7.13	10.60-29.10	17.75 ± 5.87
Parenchyma cell	10.60 -84.70	51.87 ± 27.30	10.60-66.20	35.73 ± 19.38
Collenchyma cell	4.10-32.70	14.91 ± 8.67	5.50-35.50	16.75 ± 8.37
Vessel	7.90 -31.80	20.69 ± 8.62		
Pith cell	29.10-127.10	75.8 ± 36.50		
Petiole				
Adaxial Epidermis cell	10.60-37.10	23.57 ± 9.48	10.60-31.80	22.78 ± 7.20
Abaxial Epidermis cell	13.20-42.40	24.62 ± 10.76	10.60-37.10	25.17 ± 9.62
Parenchyma cell	13.20-95.30	47.86 ± 29.04		
Vessel	7.90-26.50	16.85 ± 7.43		
Leaf				
Adaxial cuticle	2.70-10.10	5.92 ± 2.72		
Abaxial cuticle	1.60-10.60	4.46 ± 3.72		
Adaxial epidermis cell	18.50-68.80	42.87 ± 16.67	11.70-42.40	28.98 ± 9.89
Abaxial epidermis cell	7.90-63.50	33.24 ± 21.65	10.60-37.10	22.25 ± 9.21
Spongy cell	15.90-47.60	25.66 ± 8.99	13.20-26.50	17.73 ± 4.34
Palisade cell	10.60-21.20	17.20 ± 3.36	18.50-47.60	30.44 ± 9.60
Vessel	5.30-21.20	14.19 ± 4.78		
Calyx				
Adaxial epidermis cell	10.60-37.10	21.98 ± 8.93	10.60-37.10	18.80 ± 7.44
Abaxial epidermis cell	21.20-58.20	36.81 ± 11.71	18.50-39.70	26.21 ± 6.89
Parenchyma cell	10.60-42.40	21.72 ± 10.52	7.90-18.50	12.69 ± 3.91
Vessel	4.20-10.60	7.80 ± 2.14		
Corolla				
Adaxial epidermis cell	10.60-42.40	24.37 ± 11.03	13.20-37.10	26.75 ± 7.45
Abaxial epidermis cell	10.60-31.80	21.45 ± 8.05	7.90-37.10	20.12 ± 9.78
Parenchyma cell	10.60-105.90	36.54 ± 27.76	7.90-68.80	30.44 ± 19.50
Vessel	5.30-13.20	8.89 ± 2.96		

■: Sample Number, ▲: Minimum, ●: Maximum, *: Standard Deviation

Trichome: Glandular and non-glandular hairs are distributed on the stem, leaf, petiole, bracts, calyx and corolla of *L. pisidicum* (Fig. 6). Non-glandular hairs are uniseriate and 1-2 celled. Glandular hairs point out 2 main types as capitate and peltate. Capitate glandular hairs are divided into two types, type I and II, which correspond to Werker (1993)'s classification. The capitate glandular hairs are of a head 1,2,4 celled and a stalk 1 or 2 celled (Fig. 6A-F). Type I capitate hairs are of a round shaped head 1,2,4 celled (Fig. 6A-D) while type II capitate hairs are of a slightly elongated head only 1 celled (Fig. 6E-F). Peltate glandular hairs are of a large head 4,6 or 8 celled and a central cell and an additional stalk 1 or 2 celled (Fig. 6G-P). Capitate and peltate glandular hairs are distributed on the aerial parts like stem, petiole, leaf, bracts, calyx and corolla.

Cytological property: We counted the somatic chromosome number of *Lamium pisidicum* as $2n=18$ on ten cells (Fig. 7 A-P).

Discussion

We aimed to investigate *Lamium pisidicum*, an endemic species, morphologically, anatomically and cytologically in order to introduce in detail. The results obtained from the morphological studies are comparable with the description of the taxon in "Flora of Turkey" (Mill, 1982) and with the study of Mennema (1989). Our morphological findings of *L. pisidicum* were generally consistent with the morphological description of the taxon, given in the Flora of Turkey (Mill, 1982), with some exceptions of the numerical data. Mill (1982) reported the stem length as 12-22 cm, the petiole length as 1.8-4.0 cm, the leaf length as 1.8-2.5 cm, the leaf width as 1.5-1.7 cm, the width of corolla tube 0.08 (-0.15) cm and the lobe of upper lip as 0.1-0.3 cm while we have determined them respectively as 18-50 cm, 1.5-6.0 cm, 1.5-3.8 cm, 1.2-3.0 cm, 0.06-0.15 cm, 0.2-0.5 cm (Table 1). Those findings showed that the intervals between the upper and the lower limits of the characters mentioned above seem to be enlarged. The different number of plant samples observed in "Flora of Turkey" and also variation in the climatical conditions for years may be responsible for some differences in our numerical data.

Table 3. Pollen measurements of *Lamium pisidicum*.

N [#] = 30	Min. [▲] - Max. [●] (μm)	Mean ± S.D.* (μm)
Polar axis (P)	26.00-32.50	29.21 ± 01.46
Equatorial diameter (E)	28.60-33.80	31.11 ± 01.59
P/E ratio	00.85-01.09	00.94 ± 00.06
Pollen shape	Spheroidal	

■: Sample Number, ▲: Minimum, ●: Maximum,*: Standard Deviation

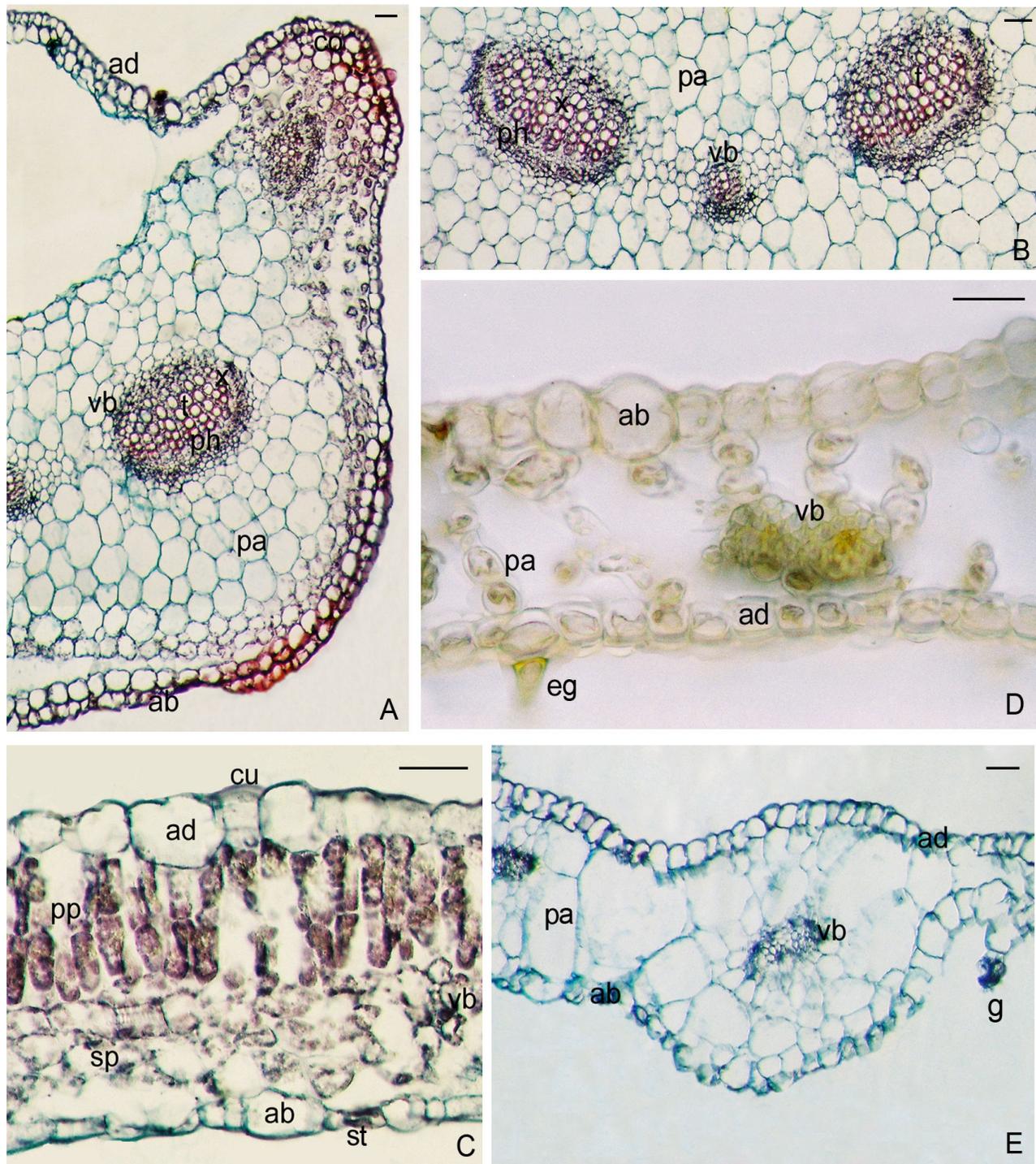


Fig. 4. The petiole (A-B), leaf (C), calyx (D), corolla (E) cross-sections of *Lamium pisidicum*

eg. eglandular hair, g. glandular hair, ab. abaxial epidermis, ad. adaxial epidermis, cu. cuticle, ph. phloem, x. xylem, t. trachea, pa. parenchyma, vb. vascular bundle, pp. palisade parenchyma, sp. spongy parenchyma, st. stoma (Scale Bars: 35μm).

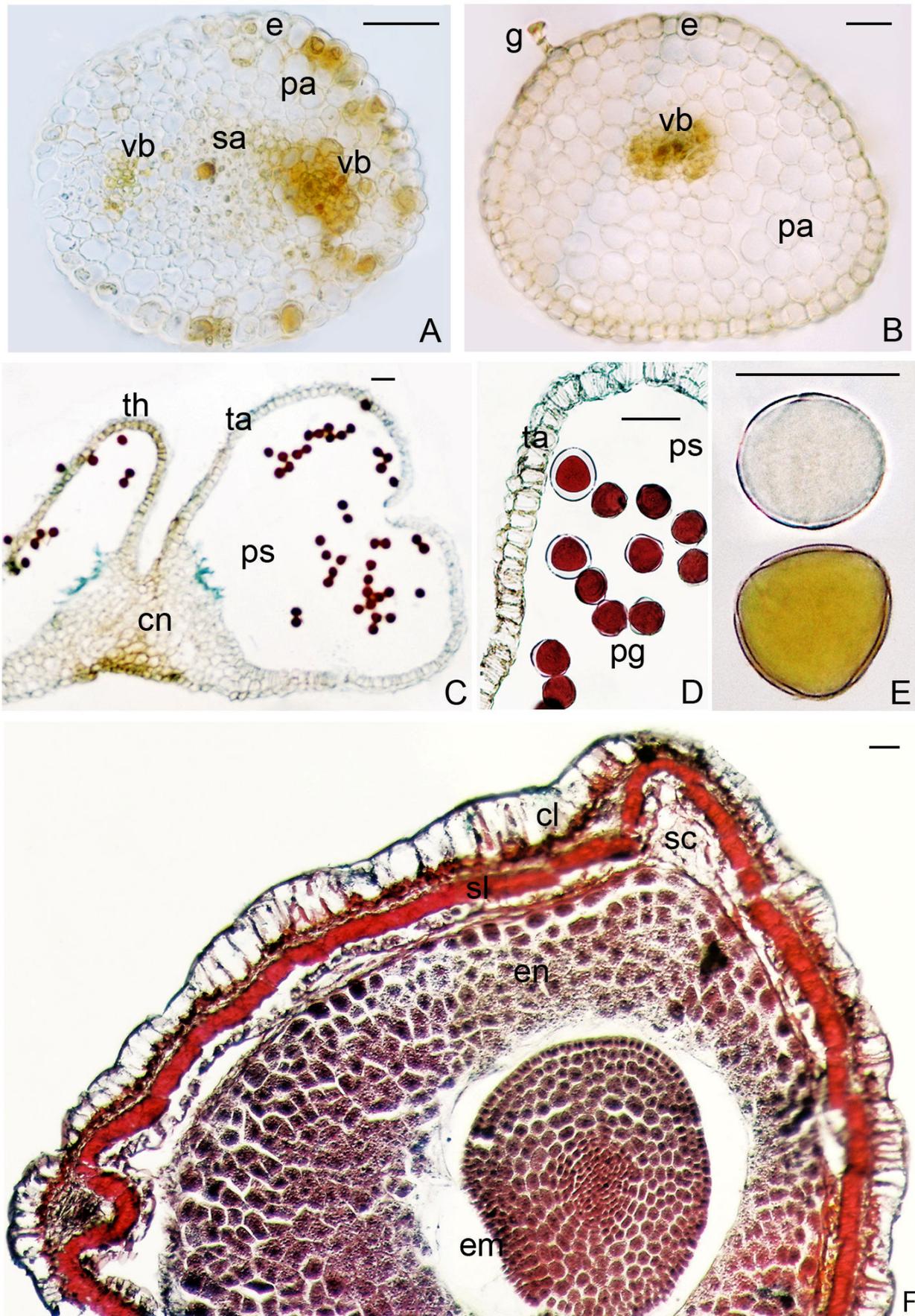


Fig. 5. The pistil (A), filament (B), anthers (C-D), equatorial and polar view of pollen (E) and seed (F) cross-sections of *Lamium pisidicum* g. glandular hair, e. epidermis, pa. parenchyma, vb. vascular bundle, sa. sclerenchymatic axis, th. theca, ta. tapetum, cn. connective tissue, pg. pollen grain, ps. pollen sac, cl. cork layer, sl. sclerenchyma layer, sc. seed coat, en. endosperm, em. embryo (Scale Bars: 35 μ m)

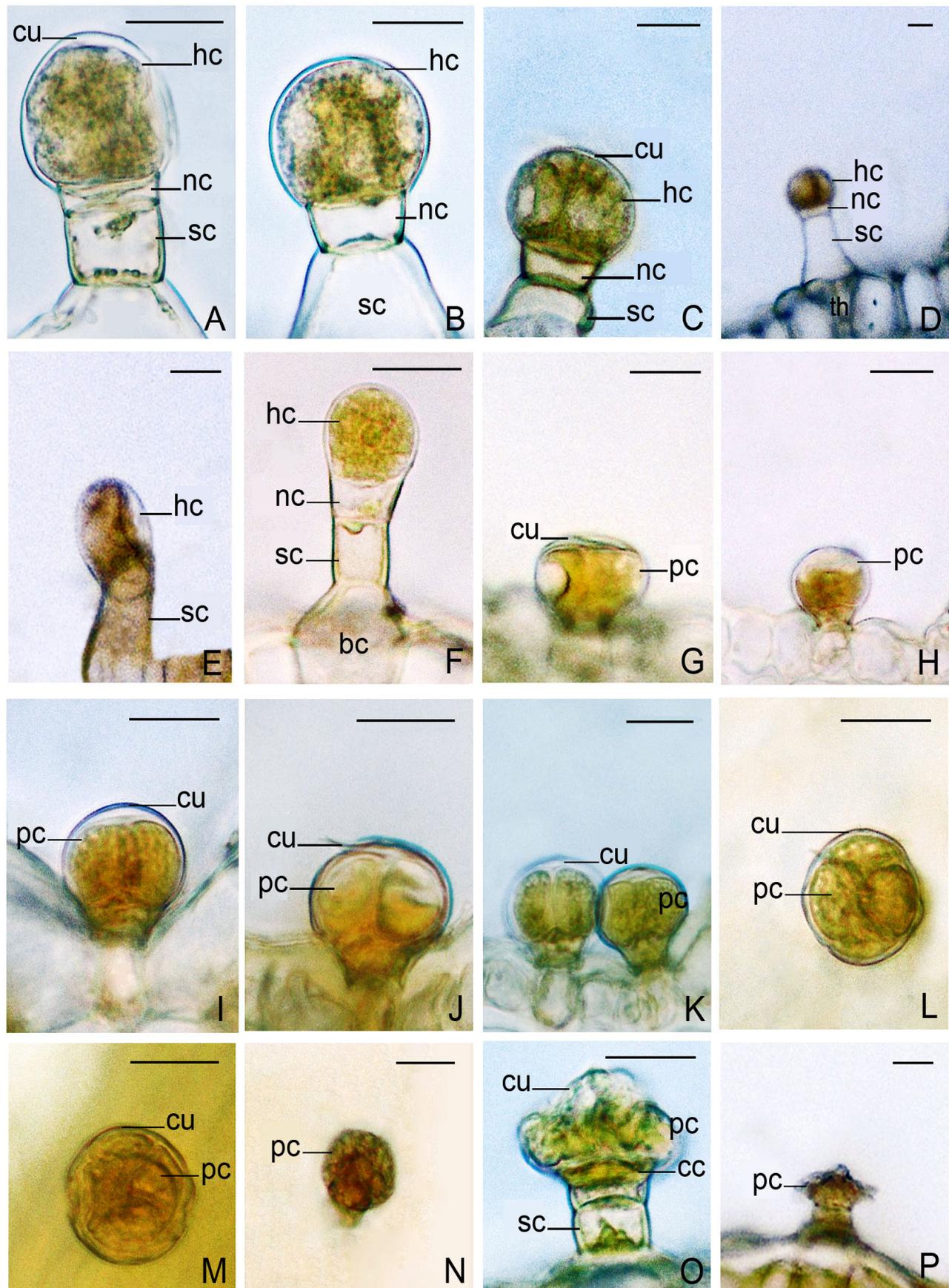


Fig. 6. Glandular trichomes distributed on the aerial organs of *Lamium pisidicum*

Type I capitulate glandular hairs (A-D), Type II capitulate glandular hairs (E,F), Peltate glandular hairs (G-P). A-E. corolla, F. filament, G. stem, H-I. leaf, J-K. petiole, L-P. calyx. hc. head cell cu. cuticle nc. neck cell sc. stalk cell bc. base cell pc. periphery cell cc. central cell (Scale bars: 20 μ m)

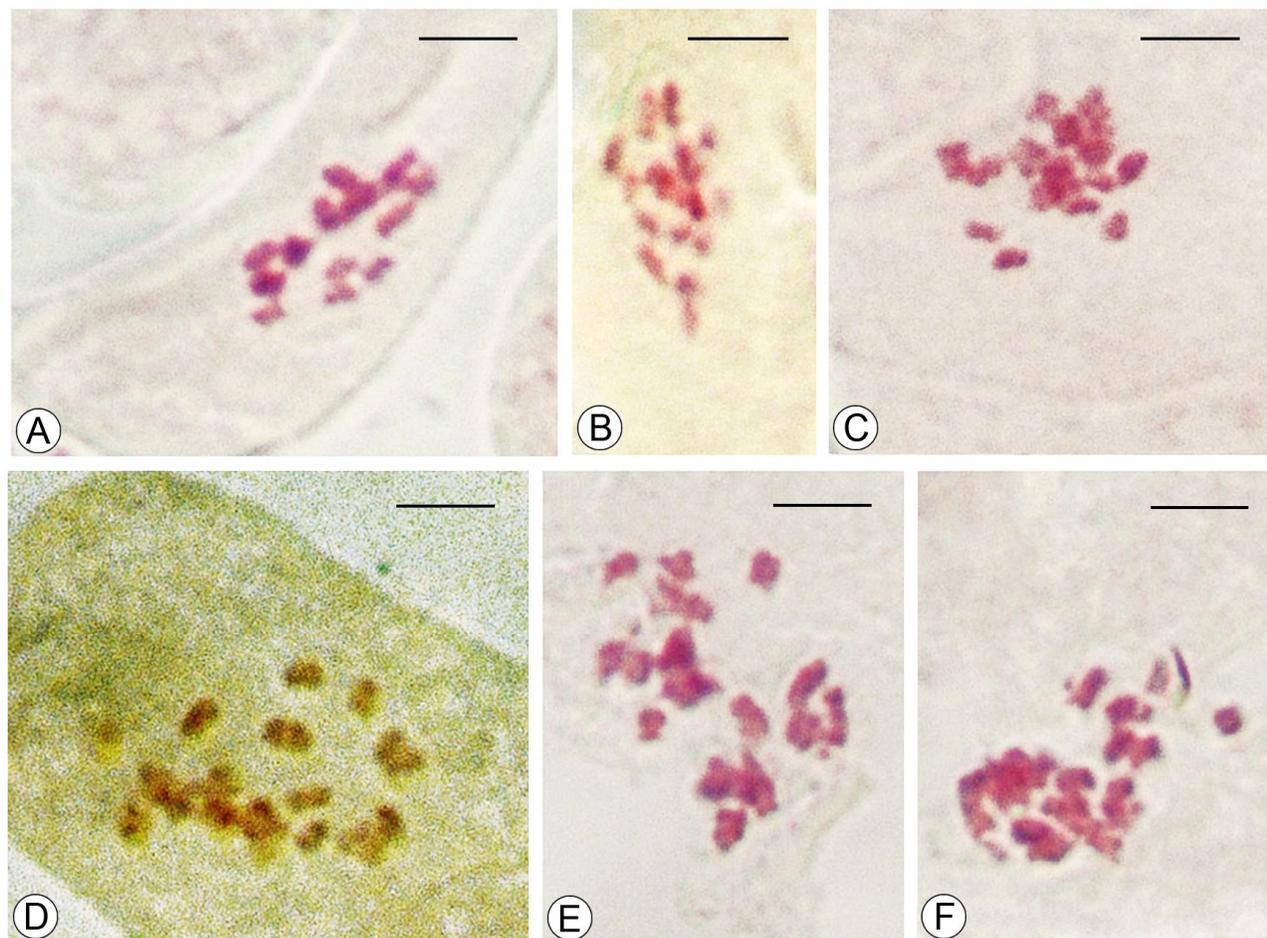


Fig. 7. Somatic chromosomes of *Lamium pisidicum* (A-F, Scale bars 4 μ m)

The perennial taxa of the genus *Lamium* possess more or less woody rhizomes, which are lacking in the annual taxa (Mennema, 1989). *L. pisidicum* is a perennial taxon and bears woody rhizomes (Figs. 1C, 2A) and is distributed in dry places on limestones (Fig. 1A-B). Our last study points out that *L. lycium*, which is another perennial endemic species of *Lamium* to Turkey, is of woody rhizomes, similar habitate features and altitude range when compared to *L. pisidicum* (Baran & Özdemir, 2009).

The anatomical analysis given in this work provides the first detailed description of *L. pisidicum* and is comparable with those of Metcalfe and Chalk (1972) and those of some other the investigated *Lamiaceae* members (Özdemir & Şenel, 1999, 2001; Kaya & Başer, 2002; Uysal, 2002; Baran & Özdemir, 2006; Dinç & Öztürk, 2008; Özdemir *et al.*, 2008, 2009). Our findings are especially comparable with perennial *L. lycium* (Baran & Özdemir, 2009), which is closed to *L. pisidicum*, and with annual *L. moschatum* var. *rhodium* (Baran & Özdemir, 2011) and a few *Lamium* species in the literature (Park & Kim, 1995). According to Metcalfe and Chalk (1972), pith rays of *Lamiaceae* family are 2-12 or more rowed and quite heterogeneous in structure. Analysis of the root cross-section of *L. pisidicum* showed that the pith rays were 1-2 rowed and heterogeneous. The pith rays of the root are 1-4 in *L. lycium* (Baran & Özdemir, 2009), and 1-2 in *L. moschatum* var. *rhodium* (Baran & Özdemir, 2011). The root centre of *L. pisidicum* is filled with xylem

elements (Fig. 3A) while the old root of *L. lycium* has parenchymatous cells in the centre in contrast to the young root that is filled with primary xylem in it (Baran & Özdemir, 2009). On the other hand, it can be clearly seen that the vessels in the root of *L. pisidicum* are more frequent throughout the cross-section (Fig. 3A) and more larger in diameter than those of *L. lycium* root (Baran & Özdemir, 2009). The root center of annual *L. moschatum* var. *rhodium* is filled with primary xylem which can be qualified as metaxylem in structure and the diameter of the vessels is normally larger than both the two perennials (Baran & Özdemir, 2011). It is reported in the literature that the root center is filled with primary xylem in some *Lamiaceae* members (Özdemir & Şenel, 1999; Uysal, 2002; Baran & Özdemir, 2006; Özdemir *et al.*, 2008, 2009) in contrast to some others (Özdemir & Şenel, 2001; Baran *et al.*, 2008). Endodermis of the root is indistinguishable as in the root of *L. moschatum* var. *rhodium* (Baran & Özdemir, 2011) while in contrast to that of *L. lycium* (Baran & Özdemir, 2009).

The characteristic feature of *Lamiaceae* family is a quadrangular stem and a well-developed collenchyma, supporting tissue at the corners of stem and a developed sclerenchymatic tissue surrounds the vascular tissue (Metcalfe & Chalk, 1972). A well-developed angular collenchyma at the corners of quadrangular stem and plate collenchyma of petiole were clearly distinguishable in *L. pisidicum* (Fig. 3B-C) as earlier reported in *L. lycium*

(Baran & Özdemir, 2009) and in *L. moschatum* var. *rhodium* (Baran & Özdemir, 2011). However, any extraxilar sclerenchymatic tissue is not seen in stem and root cross-sections of *L. pisidicum* (Fig. 3B), as not seen in *L. lycium* (Baran & Özdemir, 2009) and in *L. moschatum* var. *rhodium*, either (Baran & Özdemir, 2011). Many genera of Lamiaceae, even annual taxa, usually bear extraxilar sclerenchymatic tissues in their roots or stems (Uysal, 2002; Baran & Özdemir, 2006; Baran *et al.*, 2008; Dinç *et al.*, 2008; Dinç & Öztürk, 2008; Özdemir *et al.*, 2009; Güvenç & Duman, 2010; Kahraman *et al.*, 2010). Further investigations on the genus *Lamium* may illuminate that the lack of extraxilar sclerenchyma in *Lamium* taxa, whether they are perennial or annual, can be a descriptive feature throughout the genus. Endodermis in the stem is indistinguishable in contrast to the stem of *L. lycium* (Baran & Özdemir, 2009) and some *Sideritis* taxa (Kaya & Başer, 2002; Güvenç & Duman, 2010). The vascular cambium is distinguishable in stem of *L. pisidicum* (Fig. 3B) as well as in *L. lycium* (Baran & Özdemir, 2009) and *L. moschatum* var. *rhodium* (Baran & Özdemir, 2011), *Stachys yildirimli* M. Dinç (Dinç & Öztürk, 2008), *Salvia forskahlei* L. (Özdemir & Şenel, 2001), *Salvia argentea* L. (Baran *et al.*, 2008) in contrast to stems of *Sideritis galatica* (Kaya & Başer, 2002) and some other *Salvia* species (Özdemir & Şenel, 1999; Baran & Özdemir, 2006; Özdemir *et al.*, 2009). Another comparable feature between *L. pisidicum* and *L. lycium* in stem anatomy is pith. *L. lycium* has a large pith of parenchymatous cells (Baran & Özdemir, 2009), while *L. pisidicum* has a large hollow in the center of cross-section, which is surrounded by parenchymatous cells of pith (Fig. 3B).

The structure of vascular bundles in the cross-section of petiole in *Lamiaceae* species may be an important character of taxonomy (Metcalfe & Chalk, 1972). The analysis of the petiole cross-section, as illustrated in Fig. 4A-B, points out that three collateral vascular bundles are located in the middle and one bundle in the petiolar wings. A similar arrangement of vascular bundles in petiole is also seen in *L. lycium* (Baran & Özdemir, 2009), *L. moschatum* var. *rhodium* (Baran & Özdemir, 2011), *L. album* and *L. takesimense* taxa (Park & Kim, 1995). Apparently, vascular bundle arrangement in petiole do not seem to point out taxonomical significance among the reported *Lamium* taxa so far.

Anatomical structures of pistil, filament and anther of *L. pisidicum* (Fig. 5A-D) are consistent with those of *L. amplexicaule* L. (Lord, 1980, 1982). Any differences in the anatomical structure of the mentioned generative organs among *L. pisidicum*, *L. lycium* and *L. moschatum* var. *rhodium* does not exist (Baran & Özdemir, 2009, 2011).

Trichomes can be of great systematic significance and often even common types are used for diagnostic purposes in association with other characters (Khokhar *et al.*, 2012). Mennema (1989) have reported that glandular hairs are not observed on the leaves of the genus *Lamium*. According to the morphological description of *L. pisidicum* by Mill (1982), the calyx is glandular. However, our anatomical observations figured out that glandular hairs are present on the stem, petiole, leaf, bract, bracteole, calyx, corolla and even generative organs of the investigated taxon (Figs. 3B, 4E, 5B, 6A-P) as

earlier reported in *L. lycium* (Baran & Özdemir, 2009) and *L. moschatum* var. *rhodium* (Baran & Özdemir, 2011). In almost all species studied, two main types of glandular hair, peltate and capitate, occur which can be distinguished by head size and stalk length (Abu-Asab & Cantino, 1987). *L. pisidicum* bears capitate and peltate hairs as lately reported in some *Salvia* species in the literature (Baran *et al.*, 2010a,b; Kahraman *et al.*, 2010). We detected two different types of capitate hairs, type I and type II. Type I capitate glandular hairs have a large and clearly roundish head while type II capitate glandular hairs have slightly elongated head upon stalk. The type I capitate hairs in our work (Fig. 6A-D) corresponded to type I capitate glandular hairs described by Werker (1993), Ascensão *et al.* (1995), Ascensão and Pais (1998), Bisio *et al.* (1999) and to type II capitate glandular hairs described by Serrato-Valenti *et al.* (1997). The type II capitate hairs (Fig. 6E-F) corresponded to type II capitate glandular hairs described by Werker (1993). In addition to the peltate hairs with a four-celled head (Fig. 6G-M), as reported earlier for *Lamium galeobdolon* (L.) L. (Uphof & Hummel, 1962), we also detected the peltate hairs which are more than 4-celled and long stalked (Fig. 6N-P) in *L. pisidicum*. It is also reported by Corsi and Bottega (1999). *L. lycium* bears type I capitate hairs and both the peltate hairs (Baran & Özdemir, 2009) while *L. moschatum* var. *rhodium* bears type I, II and also type III capitate hairs which have cup-shaped head and both the peltate hairs (Baran & Özdemir, 2011).

Totally, 20 taxa of the genus *Lamium* are studied for the chromosome numbers and in each case the base number is reported as $n=9$ in the literature (Bernström, 1952; 1953; 1955; Bolkhovskikh *et al.*, 1969; Fedorov, 1969; Moore, 1982; Gill, 1983; Baran & Özdemir, 2011). We counted the chromosome number of *L. moschatum* var. *rhodium* as $2n=18$ in our previous study (Baran & Özdemir, 2011). Similarly, the chromosome number of *L. pisidicum* has been counted as $2n = 18$ for the first time in this study (Fig. 7A-F).

As a result of this study, we expanded the morphological description of endemic *L. pisidicum*, a perennial herbaceous species, contributing to the knowledge of the Flora of Turkey (Mill, 1982). Furthermore, we firstly reported numerical morphological data belonging to anther, filament, pistil, lower lip, upper lip lobes and calyx tube, calyx teeth and seed of *L. pisidicum* in this study (Table 1). On the other hand, this work points out that *L. pisidicum* is comparable with *L. lycium* studied in a previous work (Baran & Özdemir, 2009) and other Lamiaceae members in anatomical aspect and contributes to anatomical data of the family (Figs. 3-6, Tables 2,3). However, further investigations with the other *Lamium* species as in a recently done study (Banaras *et al.*, 2012) may illuminate whether any taxonomical implication is present or not within the genus *Lamium* or in the family, besides anatomical findings and the chromosome number of the taxon in this study supply the first data available for *L. pisidicum* in the literature.

Acknowledgements

We would like to thank Celal Bayar University, The Unit of Scientific Research Projects for financial support.

References

- Abu-Asab, M.S. and P.D. Cantino. 1987. Phylogenetic implications of leaf anatomy in subtribe Mellittidinae (Labiatae) and related taxa. *J. Arn. Arbor.*, 68: 1-34.
- Abu-Asab, M.S. and P.D. Cantino. 1994. Systematic implications of pollen morphology in subfamilies Lamioideae and Pogostemoideae (Labiatae). *Ann. Mo. Bot. Gard.*, 81: 653-686.
- Algan, G. 1981. Bitkisel Dokular İçin Mikroteknik. Fırat Üniv. Fen-Ed.Fak.Yayın. Bot. No:1, İstanbul.
- Ascensão, L., N. Marques and M.S. Pais. 1995. Glandular trichomes on vegetative and reproductive organs of *Leonotis leonurus* (Lamiaceae). *Ann. Bot.*, 75: 619-626.
- Ascensão, L. and M.S. Pais. 1998. The leaf capitate trichomes of *Leonotis leonurus*: histochemistry, ultrastructure and secretion. *Ann. Bot.*, 81: 263-271.
- Banaras, S., S. Aman, M. Zafar, M. Khan and S. Abbas. 2012. Molecular identification and comparative analysis of novel 18S ribosomal RNA genomic sequences of a wide range of medicinal plants. *Pak. J. Bot.*, 44(6): 2021-2026.
- Baran, P. and C. Özdemir. 2006. The morphological and anatomical characters of *Salvia napifolia* Jacq. (Lamiaceae) in Turkey. *Bangl. J. Bot.*, 35: 77-84.
- Baran, P., C. Özdemir and K. Aktaş. 2008. The morphological and anatomical properties of *Salvia argentea* L. (Lamiaceae) in Turkey. *Res. J. Agri. Biol. Sci.*, 4(6): 725-733.
- Baran, P. and C. Özdemir. 2009. The morphological and anatomical properties of *Lamium lycium* (Lamiaceae), endemic to Turkey. *Nord. J. Bot.*, 27: 1-9.
- Baran, P., K. Aktaş and C. Özdemir. 2010a. Structural investigation of the glandular trichomes of endemic *Salvia smyrnea* L. *S. Afr. J. Bot.*, 76: 572-578.
- Baran, P., C. Özdemir and K. Aktaş. 2010b. Structural investigation of the glandular trichomes of *Salvia argentea* L. *Biologia.*, 65: 33-38.
- Baran, P. and Özdemir, C. 2011. Morphological, anatomical and cytological investigation on endemic *Lamium moschatum* var. *rhodium*. *Biologia.*, 66(3): 439-447.
- Baytop, A. 1981. Bitkisel Droguların Anatomik Yapısı. İstanbul Üniv. Yay. 6, Baskı No: 32, İstanbul.
- Baytop, T. 1999. Türkiye’de Bitkilerle Tedavi (Geçmişte ve Bugün). Nobel Tıp Kitapevleri.
- Bernström, P. 1952. Cytogenetic Intraspecific Studies in *Lamium*. *Hereditas.*, 38(2): 163-219.
- Bernström, P. 1953. Increased crossability in *Lamium* after chromosome doubling. *Hereditas.*, 39(1-2): 241-256.
- Bernström, P. 1955. Cytogenetic studies on relationships between annual species of *Lamium*. *Hereditas.*, 41: 1-122.
- Bisio, A., A. Corallo, P. Gastaldo, G. Romussi, G. Ciarallo, N. Fontana, N. De Tommasi and P. Profumo. 1999. Glandular trichomes and secreted material in *Salvia blepharophylla* Brandegee ex Epling grown in Italy. *Ann. Bot.*, 83: 441-452.
- Bolkhovskikh, Z., V. Grif, T. Matvejeva and O. Zakharyeva. 1969. In: *Chromosome numbers of Flowering Plants*. (Ed.): A.A. Fedorov. Leningrad.
- Bremness, L. 1995. *The Complete Book of Herbs*. Dorling Kindersley Publishing, London.
- Corsi, G. and S. Bottega. 1999. Glandular trichomes of *Salvia officinalis*: new data on morphology localization and histochemistry in relation to function. *Ann. Bot.*, 84: 657-664.
- Cui, S.Y., X.G. Chen and Z. Hu. 2003. Identification and determination of ecdysone and phenylpropanoid glucoside and flavonoids in *Lamium maculatum* by capillary zone electrophoresis. *Biomed. Chromatogr.*, 17: 477-482.
- Davis, P.H., R.R. Mill and K. Tan. 1988. *Flora of Turkey and the East Aegean Islands* (suppl. 1). Edinburgh Univ. Press., Edinburgh.
- Diñç, M. and M. Öztürk. 2008. Comparative morphological, anatomical and palynological studies on the genus *Stachys* L. sect. *Ambleia* Benth (Lamiaceae) species in Turkey. *Turk. J. Bot.*, 32: 113-121.
- Diñç, M., A. Duran, M. Pınar and M. Öztürk. 2008. Anatomy, palynology and nutlet micromorphology of Turkish endemic *Teucrium sandrasicum* (Lamiaceae). *Biologia.*, 63(5): 637-641.
- Ekim, T., M. Koyuncu, M. Vural, H. Duman, Z. Aytaç and N. Adigüzel. 2000. *Red Data Book of Turkish Plants (Pteridophyta and Spermatophyta)*. Ankara: Turkish Association for the Conservation of Nature and Natural Resources.
- Elçi, Ş. 1994. *Sitogenetikte araştırma yöntemleri ve gözlemler*. Van: 100. Yıl Üniversitesi Yay. No:18, Fen Edebiyat Fakültesi Yay. No:16.
- Eltz, T. 2006. Tracing pollinator footprints on natural flowers. *J. Chem. Ecol.*, 32: 902-915.
- Fedorov, A.A. 1969. *Chromosome Numbers of Flowering Plants*. Leningrad.
- Gill, L.S. 1983. Cytotaxonomic studies of the tribe Stachydeae (Labiatae) in Canada. *Willdenowia.*, 13: 175-181.
- Güner, A., N. Özhatay, T. Ekim and K.H.C. Başer. 2000. *Flora of Turkey and The East Aegean Islands*. Vol.11, Edinburgh University Press, Edinburgh.
- Güvenç, A. and H. Duman. 2010. Morphological and anatomical studies of annual taxa of *Sideritis* L. (Lamiaceae), with notes on chorology in Turkey. *Turk. J. Bot.*, 34: 83-104.
- Harley, R.M. 2003. Validation of the *Lamioideae* (Labiatae). *Kew Bull.*, 58: 765-766.
- Kahraman, A., F. Celep and M. Doğan. 2010. Anatomy, trichome morphology and palynology of *Salvia chrysophylla* Stapf (Lamiaceae). *S. Afr. J. Bot.*, 76: 187-195.
- Khattak, K.F. 2012. 3 microbiological quality assessment of commercially available medicinal plants in Peshawar city, Pakistan. *Pak. J. Bot.*, 44(4): 1203-1208.
- Khokhar A.L., M.T. Rajput and S.S. Tahir. 2012. Taxonomic study of the trichomes in the some members of the genus *Convolvulus* (Convolvulaceae). *Pak. J. Bot.*, 44(4): 1219-1224.
- Kaya, A. and K.H.C. Başer. 2002. Endemik bir tür: *Sideritis galatica* Bormm. (Labiatae). 14. Bitkisel İlaç Hammaddeleri Toplantısı, Bildiriler, 29-31 Mayıs, Eskişehir.
- Lord, E. 1980. An anatomical basis for the divergent floral forms in the cleistogamous species, *Lamium amplexicaule* L. (Labiatae). *Am. J. Bot.*, 67: 1430-1441.
- Lord, E. 1982. Morphogenesis in *Lamium amplexicaule* L. (Labiatae) with a model for the evolution of the cleistogamous flower. *Bot. Gaz.*, 143: 63-72.
- Macior, L.W. 1978. Pollination interactions in sympatric *Dicentra* species. *Am. J. Bot.*, 65(1): 57-62.
- Mabberley, D.J. 1997. *The Plant Book. A Portable Dictionary For The Vascular Plants*. Cambridge University Press.
- Mennema, J. 1989. *A Taxonomic Revision of Lamium (Lamiaceae)*. Leiden Botanical Series.
- Metcalfe, J.R. and L. Chalk. 1972. *Anatomy of the Dicotyledons*. Vol. 2, Clarendon Press., Oxford.
- Mill, R.R. 1982. *Lamium* L. In: *Flora of Turkey and The East Aegean Islands*. (Ed.): P.H. Davis. Vol. 7. Edinburgh Univ. Press., Edinburgh, pp. 126-148.
- Moore, D.M. 1982. *Flora Europaea. Check-list and chromosome index*. Cambridge.
- Moore, P.D., J.A. Webb and M.E. Collinson. 1991. *Pollen Analysis*. Blackwell Science Ltd.
- Özdemir, C. and G. Şenel. 1999. The morphological, anatomical and karyological properties of *Salvia sclarea* L. *Turk. J. Bot.*, 23: 7-18.
- Özdemir, C. and G. Şenel. 2001. The morphological, anatomical and karyological properties of *Salvia forskahlei* L. (Lamiaceae) in Turkey. *J. Econ. Taxon. Bot.*, 19: 297-313.

- Özdemir, C., M. Özkan, K. Aktas and P. Baran. 2008. Morphological and anatomical properties of endemic *Salvia cryptantha* Montbret & Aucher ex Bentham (Lamiaceae) in Turkey. *Botanica Lithuanica.*, 14(4): 201-206.
- Özdemir, C., P. Baran and K. Aktaş. 2009. Anatomical studies in *Salvia viridis* L. (Lamiaceae). *Bangl. J. Pl. Taxon.*, 16: 65-71.
- Park, S.J. and Y.S. Kim. 1995. A taxonomic study on the genus *Lamium* (Lamiaceae) in Korea. *Kor. J. Pl. Taxon.*, 25: 103-122.
- Punt, W., P.P. Hoen, S. Blackmore, S. Nilsson and A. Le Thomas. 2007. Glossary of pollen and spore terminology. *Rev. Palaeobot. Palyno.*, 143: 1-81.
- Rudy, M.R. 2004. Plant evaluation notes. A comparative study of ground cover *Lamium*. *Chicago Bot. Gard.*, 23: 1-4.
- Sabuncu, I., A. Bıçakçı, S. Tatlıdil and H. Malyer. 2002. Microscopic analysis of pollens that are sold in markets and labelled as product of Uludag and Karacabey region in Turkey. *Uludag Bee Journal.*, 2: 3-9.
- Savchenko, T., M. Blackford, S.D. Sarker and L. Dinan. 2001. Phytoecdysteroids from *Lamium* spp: Identification and Distribution within Plants. *Biochem. Sys. Ecol.*, 29: 891-900.
- Serrato-Valenti, G., A. Bisio, L. Cornara and G. Ciarallo. 1997. Structural and histochemical investigation of the glandular trichomes of *Salvia aurea* L. leaves and chemical analysis of the essential oil. *Ann. Bot.*, 79: 329-336.
- Sıralı, R. and M. Deveci. 2002. Investigation of the important bee (*Apis mellifera* L.) plants in thrace region. *Uludag Bee Journal.*, 2(1): 17-26.
- Sönmez, R. and Ö. Altan. 1992. Teknik Arıcılık. Ege Üniversitesi Ziraat Fakültesi Yayınları. No:499, Bornova, İzmir. S: 246.
- Uphof, J.C.T. and K. Hummel. 1962. Plant hairs. In: *Encyclopedia of Plant Anatomy*, (Eds.): W. Zimmermann and P.G. Ozenda, Gebrüder Borntraeger, pp. 99-101.
- Uysal, İ. 2002. *Stachys cretica* L. subsp. *smyrnea* Rech Fill. endemik taksonunun morfolojisi, anatomisi ve ekolojisi üzerinde araştırmalar. *Ekoloji Çevre dergisi.*, 11(42): 16-20.
- Werker, E. 1993. Function of essential oil-secreting glandular hairs in aromatic plants of the Lamiaceae. *Flavour and Fragrance Journal.*, 8: 249-255.
- Wodehouse, R.P. 1965. *Pollen Grains*. Hammer Press.

(Received for publication 5 Januray 2012)