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Pollen morphology of two *Lysimachia* L. (Primulaceae) species from Işıklar (Ganos) mountain of Tekirdağ

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Abstract
The pollen morphology of two species of *Lysimachia*, *L. atropurpurea* and *L. punctata* from Işıklar (Ganos) mountain were investigated using light and scanning electron microscopy. The pollen grains of *L. atropurpurea* are trizonocolporate, while those of *L. punctata* are trizonocolporate and tetrazonocolporate; prolate spheroidal or oblate spheroidal in shape and small in size. Ectoapertures are elongated colpi, endoapertures are lalongate pori. Exine is semitectate; the ornamentation reticulate with polygonal lumina which are larger equatorially and smaller towards the colpi ends. Intine is very thin. Palynological analysis revealed that the pollen grains of the two species are quite similar, but there are differences in the sizes of different characters. The obtained results would contribute to the future taxonomic revision of the genus in Turkey.
Keywords: *Lysimachia*, Primulaceae, pollen, LM, SEM, European Turkey.

Introduction

Lysimachia L. comprises about 190 species which are distributed in temperate and subtropical regions of the Northern Hemisphere; with some species in Africa, Australia and South America. The diversification center of the genus is China where 138 species occur (Huang et al. 2019). Although *Lysimachia* was included in Myrsinaceae based on molecular and morphological phylogenetic analyses (Källersjö et al. 2000), it was nested again in Primulaceae as a genus of Myrsinoideae subfamily (Stevens 2001 onwards).

In traditional Chinese medicine the species are used as diuretic, antibiotic, bile juice secretion stimulant, swelling disperser; also in the treatment of jaundice, eczema, burn, infected wound and snakebite (Hempen and Fischer 2009), as anticholecystitic and cholagogic (Yang et al. 2011). In traditional folk medicine in Turkey, the infusion of *Lysimachia* is used as antipyretic and expectorant (Baytop 1999). In recent years, the species of this genus have gained great importance in terms of phytochemicals as they contain flavonoids and triterpene saponins (Yusukawa et al. 1990, Tóth et al. 2012, Podolac et al. 2013, Toth et al. 2014). Most recent studies have revealed the anticarcinogenic effect of the plant extracts of different species (Liu et al. 2010, Aydemir et al. 2019).

In Turkey, the genus is represented with nine species: *L. atropurpurea* L., *L. dubia* Sol., *L. japonica* Thunb., *L. linum-stellatum* L., *L. nummularia* L., *L. punctata* L., *L. verticillaris* Spreng., *L. vulgaris* L. and *L. savranii* Başköse & Keskin (Leblebici 1978, Terzioğlu and Karaer 2009, Coşkunçelebi 2012, Başköse et al. 2016). *Lysimachia* species of Turkey are perennial or annual herbs. The leaves are

opposite, alternate or rarely whorled, sessile or petiolate, occasionally are dotted with reddish glands. Inflorescence is a panicle, raceme or spike, or flowers are in axillary whorls. Calyx is deeply 5-partite; teeth are linear to ovate, shorter or rarely longer than corolla. Corolla is rotate, yellow, pink or dark purple (Leblebici 1978).

In the classification of the genus by Bennel and Hu (1983) [following the classification of Handel-Mazzetti (1928) and as modified by Chen and Hu (1979)], *L. atropurpurea* is in the subgen. *Palladia* (Moench) Hand.-Mazz., *L. punctata* in the subgen. *Lysimachia* Hand.-Mazz. The most prominent characters which differ between the two species are flower color, inflorescence type and the leaf arrangement. The flowers of *L. atropurpurea* are purple in a terminal spike and the leaves are alternate and sessile, whereas the flowers of *L. punctata* are yellow in axillary whorls and the leaves are opposite or whorled and short-petiolate.

The pollen morphology of *L. punctata* was analyzed by light microscopy (LM) by Aytuğ et al. (1971). Punt et al. (1974) studied the pollen morphology of 7 *Lysimachia* species of the northwest European Flora; and, based on the P/E ratio, endoaperture features and sexine types classified them in three pollen types: *L. ephemerum* type, *L. nemorum* type and *L. vulgaris* type. In the investigation of the pollen morphology of 98 species and varieties of *Lysimachia* by Bennell and Hu (1983) ten major pollen-types and four subtypes were recognized on the basis of exine sculpture and stratification and overall shape and size of the grains. The pollen grains of *L. punctata* were classified in the Congestiflora-type, those of *L. atropurpurea* were included in the Taliensis-type. Wronska-Pilarek and Morozowska (2009) studied the morphology and variability of pollen of *L. vulgaris* on the base of the plant material coming from 21 native localities in Poland.

Detailed pollen morphology of *L. atropurpurea* and *L. punctata* which have natural distribution in Işıklar (Ganos) mountain of Tekirdağ has not been studied so far. The aim of the present study is to examine comparatively in detail the pollen morphology as well as the micromorphology of the exine. The obtained data would contribute to the future taxonomic revision of the genus in Turkey.

Material and Methods

Plant material

Pollen samples were taken from dried herbarium specimens. Specimens are deposited at the Tekirdağ Namık Kemal University Herbarium. Collection data of examined *Lysimachia* species: *L. atropurpurea*: A1(E) Tekirdağ: Işıklar (Ganos) Mountain, Yeniköy-Mermer, 3 km from Yeniköy, 246 m, 13.06.2020, N. Şafak Odabaşı, NŞO 896; *L. punctata:* A1(E) Tekirdağ: Işıklar (Ganos) Mountain, Yeniköy-Mermer, 1 km from Yeniköy, 257 m, 13.06.2020, N. Şafak Odabaşı, NŞO 897.

Methods

The pollen was obtained from dried herbarium specimens collected by the author (NŞO). For light microscopy investigations pollen slides were prepared following Wodehouse's (1935) glycerin-jelly method. The slides were observed under ×100 magnification using an Olympus CX41 light microscope with a camera and Cell* imaging software. Eleven morphometric characters including polar axis (P), equatorial diameter (E), colpus length (clg) and width (clt), porus length (plg) and width (plt), distance between colpi apices (t), sexine (se), nexine (ne), exine (ex), and intine (in) were chosen for morphological characterization of the pollen grains. As observed in equatorial view, P/E ratio was employed to determine the pollen shape. At least fifty measurements were made until Gaussian curve was obtained for each character; the arithmetic average (M) and standard deviation (σ) were calculated (Table 1). In order to define the morphology, the terminology of Reitsma (1970) and Punt et al. (2007) was followed.

For scanning electron microscope (SEM) imaging, the pollen grains were mounted onto metal stubs with double-sided adhesive tape and observed without coating. The micrographs of general morphology

and detailed exine ornamentation were obtained with FEI QUANTA FEG 250 SEM at a magnification of ×10000-40000.

Table1. Morphometric data of the pollen grains of *L. atropurpurea* and *L. punctata* (M mean, σ standard deviation, V variance; dimensions are given in µm).

Species / Character	*L. atropurpurea*			*L. punctata*		
	M	σ	V	M	σ	V
P	19.88	±0.79	17.0-21.0	20.80	±0.57	19.0-22.0
E	18.47	±0.62	17.0-20.0	21.63	±0.64	20.0-23.0
clg	16.39	±0.61	14.5-17.5	16.46	±0.72	15.0-18.0
clt	5.91	±0.59	4.5-7.0	6.13	±0.56	5.0-7.5
plg	5.00	±0.57	4.0-6.5	6.52	±0.69	4.9-8.1
plt	6.15	±0.48	5.4-7.5	6.54	±0.42	5.6-7.4
t	5.78	±0.47	4.5-7.0	7.39	±0.56	6.0-8.5
se	0.45	±0.07	0.4-0.6	0.74	±0.07	0.6-0.9
ne	0.66	±0.09	0.5-0.8	0.78	±0.09	0.6-1.0
ex	1.11	±0.08	0.9-1.4	1.52	±0.08	1.2-1.9
in	0.29	±0.04	0.2-0.3	0.43	±0.11	0.3-0.6

Results

General pollen morphology

The grains of the two species are isopolar, trizonocolporate, tetrazonocolporate and small sized. The shape is prolate spheroidal or oblate spheroidal; polar view amb. triangular obtuse convex. The ectoapertures, colpi, are long with acute ends; the endoapertures, pori, are lalongate with acute ends. Costae present. Exine semitectate, reticulate; margo present.

Detailed pollen morphology for each species

L. atropurpurea (Table 1; Figs. 1 a - f, 3 a - c)

Pollen type: Trizonocolporate.

Pollen shape: Prolate spheroidal, P/E=1.08

Pollen dimensions: P × E = 19.88 × 18.47 µm

Apertures: Ectoaperture is long colpus with acute ends and distinct margins, margo distinct along colpus, clg: 16.39 µm, clt: 5.91 µm and clg/clt=2.77. Endoaperture is lalongate porus, plg: 5.00 µm, plt: 6.15 µm and plg/plt=0.81. Costae present.

Exine: Semi-tectate; 1.11 µm thick, nexine is thicker than sexine: se/ne=1/1.47.

Ornamentation: Reticulate. Lumina are polygonal in shape, decreasing towards the colpi (forming a margo) and the poles. Inside the lumina there are small scattered granules. In the mesocolpium area, muri are narrower than the lumina and broader towards the colpi and the poles. At the mesocolpium area the lumina diameter is 0.3-1.6 µm and the muri width is 0.2-0.5 µm; at the polar area the lumina diameter is 0.1-0.6 µm and the muri width is 0.4-0.9 µm.

Intine: Very thin, ex/in = 3.83/1.

Outlines: Equatorial view – elliptic; polar view – triangular obtuse convex.

Figure 1. Light microscope micrographs of *L. atropurpurea*. **a.** Ecto- and endoaperture: colpus and porus in equatorial view. **b.** Outline in equatorial view, costae distinct. **c.** Pointed ends of endoaperture and distinct margo. **d.** Reticulum in mesocolpium. **e.** Apocolpium **f.** Outline in polar view, costae distinct. Scale bar 10µm

L. punctata (Table 1; Figs. 2 a - f, 3 d - f)
Pollen type: Trizonocolporate, few tetrazonocolporate.
Pollen shape: Oblate spheroidal, P/E=0.96
Pollen dimensions: P × E = 20.80 × 21.63 µm
Apertures: Ectoaperture is long colpus with acute ends and distinct margins, clg: 16.46 µm, clt: 6.13 µm and clg/clt=2.69. Endoaperture is lalongate porus, plg: 6.52 µm, plt: 6.54 µm and plg/plt=1. Costae present.
Exine: Semi-tectate; 1.52 µm thick, nexine is almost as thick as sexine: se/ne=1/1.05.
Ornamentation: Reticulate. Lumina are polygonal or almost circular in shape, decreasing towards the colpi and the poles. Inside the lumina there are numerous small scattered granules. In the mesocolpium area, muri are narrower than the lumina and broader towards the colpi and the poles. At the mesocolpium area the lumina diameter is 0.3-1.9 µm and the muri width is 0.3-0.8 µm; at the polar area the lumina diameter is 0.2-1.0 µm and the muri width is 0.3-1.1 µm.
Intine: Very thin, ex/in = 3.53/1.
Outlines: Equatorial view – elliptic to almost circular; polar view – triangular obtuse convex (trizonocolporate grain). Equatorial view – elliptic to almost circular; polar view – quadrangular obtuse convex (tetrazonocolporate grain).

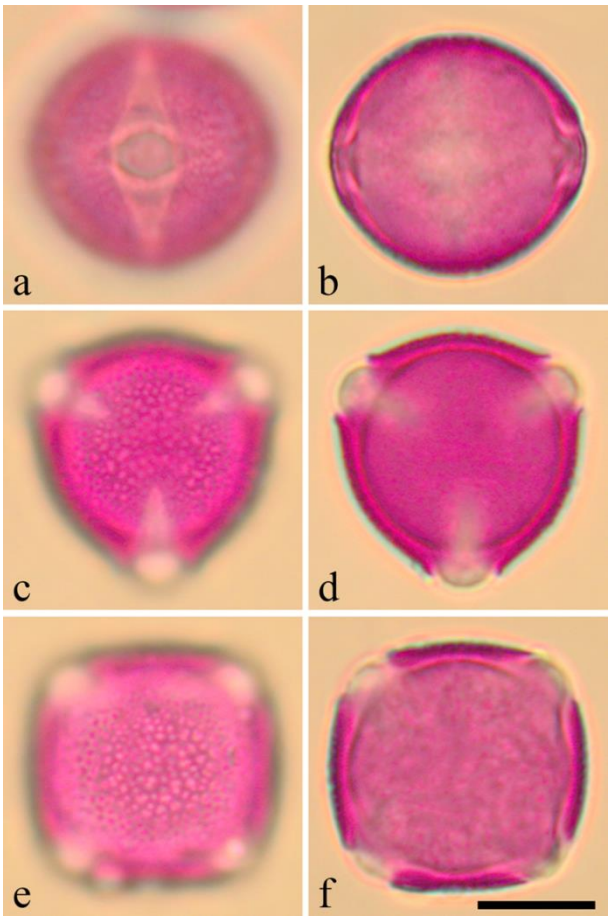

Figure 2. Light microscope micrographs of *L. punctata*. **a.** Ecto- and endoaperture: colpus and porus in equatorial view. **b.** Outline in equatorial view, costae distinct. **c.** Apocolpium **d.** Outline in polar view, costae distinct. **e.** Tetrazonocolporate pollen with reticulate ornamentation **f.** Outline of tetrazonocolporate pollen in polar view. Scale bar 10µm

Discussion

While *L. atropurpurea* and *L. punctata* are in different subgenera, the pollen analysis observations revealed that the pollen morphologies of the two species are similar. Although there are differences in sizes of different characters, it has been revealed that there are some overlaps among some of them. The most remarkable differences are the size of pollen (P and E), the shape of the pollen (P/E ratio), distance between the colpi apices (t), exine (ex) thickness and its reticulation. Both species have pollen of small size; in *L. atropurpurea* P × E= 19.88 × 18.47 and in *L. punctata* P × E= 20.80 × 21.63. Punt et al. (1974) placed the pollen of *L. punctata* in the *L. vulgaris*-type and the size for the group consisting of five species was indicated for all as: P: 13-33 µm, and E: 17-28 µm. Bennell and Hu (1983) classified the pollen of the 98 species in ten major pollen-types and four subtypes using a dichotomous key. Considering that the pollen grains are medium-sized and the tectum coarsely reticulate, they have included the pollen of *L. punctata* in the Congestiflora-type, together with twenty-four species; on the other hand, considering that the pollen grains are prolate, they included the

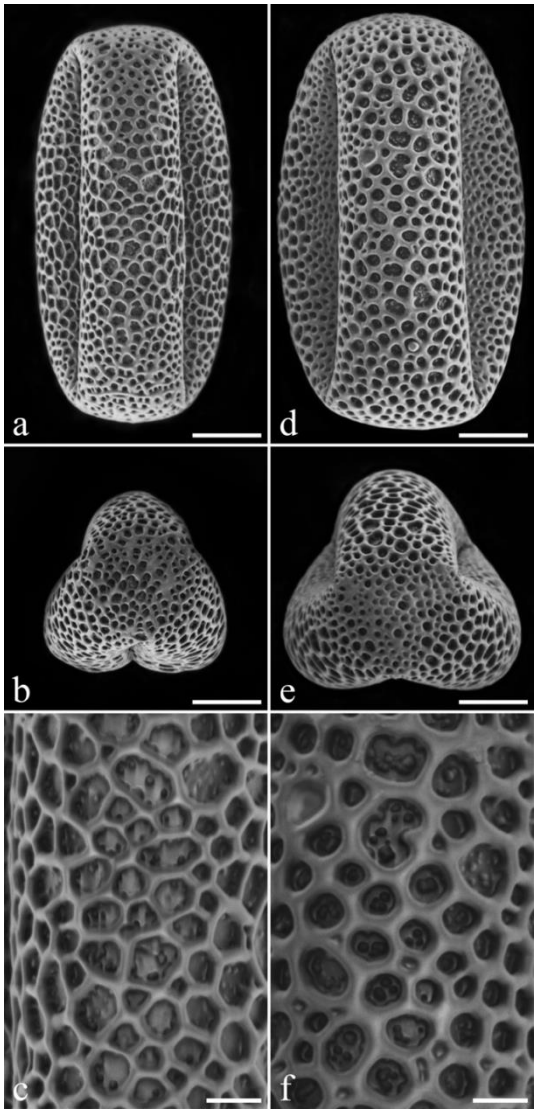

Figure 3. Scanning electron microscope micrographs of *L. atropurpurea* (**a-c**) and *L. punctata* (**d-f**). **a** and **d** equatorial view. **b** and **e** polar view. **c** and **f** exine close-up. Scale bar for **a**, **b**, **d** and **e** 5 µm; for **c** and **f** 1 µm

pollen of *L. atropurpurea* in the Taliensis-type, together with twenty species. The pollen of the two species of the present study cannot be identified using this dichotomous key; because, *L. punctata* pollen grains are not medium-sized, and, those of *L. atropurpurea* are not prolate (P/E>1.33) in shape. In the present study the pollen shape of *L. atropurpurea* and *L. punctata* is prolate spheroidal (P/E=1.08) and oblate-spheroidal (P/E=0.96), respectively.

Distance between the colpi apices (t) differs between the two species. It is 5.78 µm in *L. atropurpurea* and 7.39 µm in *L. punctata*. The results are in concordance with Aytuğ et al. (1971) for *L. punctata*.

The thickness of the exine, as well as its reticulation is another distinctive character between the pollen grains of the two species. It is 1.11 µm in *L. atropurpurea* and 1.52 µm in *L. punctata*. In both species it is semi-tectate with reticulate sculpturing. Tectum reticulate in both, lumina are large (especially in the mesocolpium), polygonal in shape and granulated. Muri are narrower in mesocolpium and wider in polar areas. Ornamentation features are congruent with those of Punt et al. (1974) and Bennell and Hu (1983). Punt et al. (1974), while commenting about *L. vulgaris*-type, stated that the pollen of the five species in the group is quite similar, and that the pollen of the *L. punctata* was distinguished from the others by lumina of the reticulum provided with small granules. Both species in this study possess

granules in the lumina of the reticulum and the granules of *L. punctata* are more numerous and larger than that of *L. atropurpurea*.

Bennell and Hu (1983) reported the ectoapertures as long and frequently syncolporate, the endoapertures as lalongate, simple, with diffuse ends, often extended to give a complete equatorial band in Congestiflora-type; and, the ectoapertures as long, tapering, sometimes syncolporate, the endoapertures as lalongate, simple to complex H-shaped in Taliensis-type; in the present study, the ectoapertures of both species are long, with distinct margins not syncolporate, and, endoapertures simple with distinct margins and the ends without forming an equatorial band or H-shape. Punt et al. (1974) reported diffuse endoaperture ends rarely with indistinct horns for *L. vulgaris*-type; in the studied two species such structure was not detected.

In *L. punctata*, along with trizonocolporate grains, tetrazonocolporate grains were observed in very few amounts. Tetrazonocolporate grains were observed in *L. nummularia* (Congestiflora-type) as "unusual grains which constitute 20% in one collection" (Bennell and Hu, 1983). Tetrazonocolporate grains were also recorded for *L. vulgaris*-type group, without specifying the species (Punt et al. 1974).

Presence of margo was indicated by Bennell and Hu (1983) in the group of *L. punctata*; in the present study, margo is distinct in *L. atropurpurea* (Fig. 1c) and not noticeable in *L. punctata* (Fig. 2a) in the light microscope.

The present article presents a detailed pollen analysis of two *Lysimachia* species from Işıklar (Ganos) mountain of Tekirdağ. The study would contribute to the further taxonomic revision of the genus in Turkey.

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