



Taxonomic studies on endemic species from Turkey: *Helianthemum nummularium* (L.) Miller subsp. *lycaonicum* Coode&Cullen (Cistaceae)

Emine Burcu YEŞİLYURT ^{*1}, Sadık ERİK ¹, Edibe ÖZMEN BAYSAL ¹, Galip AKAYDIN ²

¹ Hacettepe University, Department of Biology, Beytepe, Turkey

² Hacettepe University, Department of Biology Education, Beytepe, Turkey

Abstract

The genus *Helianthemum* Miller, includes 12 annual or perennial species in the Flora of Turkey, 4 of which are endemic. In this study, macro and micromorphological, anatomical and palynological characteristics of the endemic *Helianthemum nummularium* (L.) Miller subsp. *lycaonicum* Coode&Cullen are studied. This species is grown on steppe, chalky and limestone slopes in Turkey. In morphological studies, detailed descriptions of the *H. nummularium* subsp. *lycaonicum* and characteristic features are given. Also, identification key of four subspecies added. In anatomical studies, cross sections of root, stem and leaf were examined. Besides, seed morphology is studied by SEM. According that, seeds are ovate. The seed coat ornamentation is verrucate. The pollen grains are tricolporate, medium sized and prolate. Apocolpial area is rather narrow. Exine is very thin. Exine ornamentation is identified as striate-perforate on SEM studies.

Key words: *Helianthemum*, Cistaceae, morphology, anatomy, palynology

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Türkiye'deki endemik *Helianthemum nummularium* (L.) Miller subsp. *lycaonicum* Coode&Cullen (Cistaceae) üzerinde taksonomik çalışmalar

Özet

Helianthemum Miller cinsi Türkiye Florası'nda tek veya çok yıllık 12 tür içermekte olup, bunlardan 4'ü Türkiye için endemiktir. Bu çalışmada, endemik olan *Helianthemum nummularium* (L.) Miller subsp. *lycaonicum* Coode&Cullen türünün morfolojik, mikromorfolojik, anatomik ve palinolojik özellikleri çalışılmıştır. Bu tür Türkiye'de step, kireçli ve kireç taşlı yamaçlarda yetişmektedir. Morfolojik çalışmalarda *H. nummularium* subsp. *lycaonicum*'un ayrıntılı morfolojik betimleri ve karakteristik özellikleri verilmiştir. Ayrıca türün 4 alttürüne ait teşhis anahtarları eklenmiştir. Anatomik çalışmalarda kök, gövde ve yaprağın enine kesitleri incelenmiştir. Ayrıca, tohum morfolojisi taramalı elektron mikroskobu (SEM) ile çalışılmıştır. Buna göre, tohumlar ovat, tohum kabuğu ornamentasyonu verrukat (siğilli)'tir. Polen taneleri trikolporat, orta boyutlu ve prolattır. Kolpuslararası bölge oldukça dardır. Ekzin çok incedir. SEM ile yapılan incelemelerde ekzin ornamentasyonu striat-perforat olarak tanımlanmıştır.

Anahtar kelimeler: *Helianthemum*, Cistaceae, morfoloji, anatomi, palinoloji

1. Introduction

The largest genus *Helianthemum* Miller includes about 110 heliophytic species of shrubs, subshrubs and annual herbs in the family Cistaceae and shows a wide spread in Europe, America and from North Africa to Central Asia on marl, gypsum, limestone, sand dunes, coastal scrub, steppe, macchie, rocky slopes. The Western Mediterranean area is the highest diversity of taxa. The Iberian Peninsula and Baleric Islands harbours nearly half of the total number of species of *Helianthemum* (Parejo-Farnés et al., 2013). Numerous studies which are in the field of pollen morphology, seed morphology, anatomy, phylogeny, phylogeography and ecology on the genus *Helianthemum* has been done until

* Corresponding author / Haberleşmeden sorumlu yazar: Tel.: +903122976774; Fax.: +903122976774; E-mail: eburcu.yesilyurt@gmail.com

now (Proctor, 1958; Rivas, 1979; Georghiou et al., 1992; Ukrantseva, 1993; Nandi, 1998a; Nandi, 1998b; Gonzales-Benito and Perz-Garcia, 2006; Guzman and Vargas, 2009; Soubani, 2010; Sanderson et al., 2011; Parejo-Farnés et al., 2013). The palynological, anatomical and micromorphological features of *H. nummularium* subsp. *lycaonicum* have not been studied previously. Thus, it is the aim of this study to elaborate morphological description, investigate anatomical characteristics and search out micromorphologically of the pollen and seed of the taxon on LM and SEM.

2. Materials and methods

Plant materials were collected from a natural population in Turkey by corresponding author and preserved as herbarium samples in the Hacettepe University Herbarium (HUB). Morphological studies were carried out on the herbarium samples. The detailed description of *H. nummularium* subsp. *lycaonicum* was created by measuring about 80 morphological characters.

Palynological studies were carried out on pollen material obtained from the herbarium samples. The pollen slides were prepared according to Erdtman (1969). Under LM, at least 50 pollen grains were measured in terms of P, E, Clg, Clt, Plg, Plt, Amb, Apocolpium, Apoporium and Exine until obtaining a Gaussian curve. The pollen grains were photographed by the imaging system Olympus CX41-E330. For SEM studies, the pollen grains were directly placed on stubs and sputter coated with gold (Özmen et al., 2012). Then, SEM images were taken by Zeiss EVO-50 EP. For the pollen terminology we followed Punt et al. (2007).

For anatomical studies, living material was kept in 70% ethanol. The paraffin method was used for the cross sections of root, stem and leaf. The specimens were embedded in paraffin wax and then sectioned at 10,0-20,0 µm thickness with a Leica RM2125RT rotary microtome. All sections were stained with safranin-astra blue and then mounted with Canada balsam (Johansen, 1944). Also cross-section of roots, stems and leaves were performed by hand from material preserved in 70% ethanol. Sartur reagent was applied to the sections. The sartur reagent was comprised of KI-I, aniline, sudan-III, lactic acid, ethanol and water (Çelebioğlu and Baytop, 1949). Measurements and photos were taken using a Leica DM4000 B binocular light microscope with Leica DFC320 camera.

Seeds were observed by using the Tronic-Kameram 122CU stereomicroscope to provide that they were of normal size and maturity. Twenty mature seeds were measured in order to determine the average seed size. For SEM, mature seeds were placed on stubs directly, covering with gold and then analyzed as to their surface sculpturing by Zeiss EVO-50 EP SEM. For the terminology of seed surface ornamentation Stearn (1992) is followed.

3. Results

3.1. Identification key for subspecies

1. Leaves green on both surfaces, without stellate hairs.....subsp. *ovatum*
1. Leaves stellate-canescens, at least on lower surface
 2. Stem blackish, white-tomentose.....subsp. *lycaonicum*
 2. Stem green, pilose or puberulous
 3. Stem pilose; petals 11,0-18,0 mm.....subsp. *tomentosum*
 3. Stem puberulous; petals 8,0-11,0 mm.....subsp. *nummularium*

3.2. Morphological characteristics

H. nummularium (L.) Miller subsp. *lycaonicum* Coode&Cullen in Notes R. B. G. Edinb. 26: 200 (1965).

Type: Steppe bei Korasch, 1600 m, Ende vi 1912, Lycaonia, Vilayet Konia, Siehe 456 (as *H. kotschyannum* Boiss.; holo. E!).

Perennial; stem blackish, woody at the base, suffrutescent, ascending, 8,0-36,0 cm, adpressed stellate tomentose hairy (Figure 1); leaves opposite, narrowly elliptic-oblong, 5,0-25,0×1,5-4,5 mm, pinnat venation, scarcely revolute, lower surface shortly spreading stellate canescens, longly stellate hairy on the vein, upper surface shortly stellate tomentose and longly bifurcate-stellate hairy (Figure 2a); petiole 1,5-3,5 mm; stipule elliptic-lanceolate, sessile, 2,5-10,0×0,75-1,5 mm, obtus to acute; inflorescence raceme, 4-16-flowered; bract elliptic-lanceolate, exstipulate, 2,0-6,0×0,75-2,0 mm; pedicel erect in flower, 6,0-10,0 mm, pendulous in fruit, 10,0-11,0 mm, not articulation; petals longer than inner sepals, yellow, 11,0-13,0×9,0-12,0 mm, obovate; inner sepals ovate, acute, inner surface glabrous, outer surface shortly spreading stellate hairy, longly spreading simple-bifurcate hirsute on the vein, 4-veined, hyaline margine 1,5-1,75 mm, longer than capsule, 7,0-8,0×4,0-5,0 mm in flower, 8,0-9,0×4,5-5,0 mm in fruit; outer sepals elliptic-lanceolate, obtuse, inner surface glabrous, rarely simple hairy, outer surface glabrous, rarely longly simple-bifurcate hirsute, simple-bifurcate hirsute on margine, 2,5-3,5 × 1,0-1,75 mm in flower, 3,5-4,0×1,0-2,0 mm in fruit; style curved below, 3,5-4,5 mm in flower, 4,5-5,0 mm in fruit (Figure 2b); stigma 3, capitate, papillate capsule ovate, 5,0-7,0×3,5-4,0 mm, shortly spreading stellate tomentose; stamens multi seriate, numerous, filament 3,25-5,0 mm, anther 0,5×0,5

mm (Figure 2c); seeds surface densely verrucate on LM, 1,25-1,5×1,0-1,25 mm, brown, ovate, 12-17 seed in per capsule.



Figure 1. Habitus of *H. nummularium* subsp. *lycaonicum*

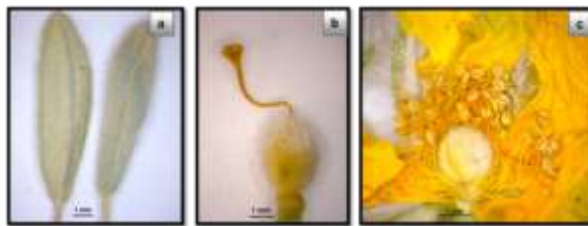


Figure 2. *H. nummularium* subsp. *lycaonicum*: a. leaf indumentum, b. style, c. multiseriate stamens.

Phenology: Flowering between May and July; fruiting between July and August.

Distribution and Ecology: Cosmopolitan in Central Anatolia (Figure 3). Chalky and limestone slopes, steppe, *Pinus nigra* and *Quercus spp.* opening, 400-2000 m.

Conservation status: LC

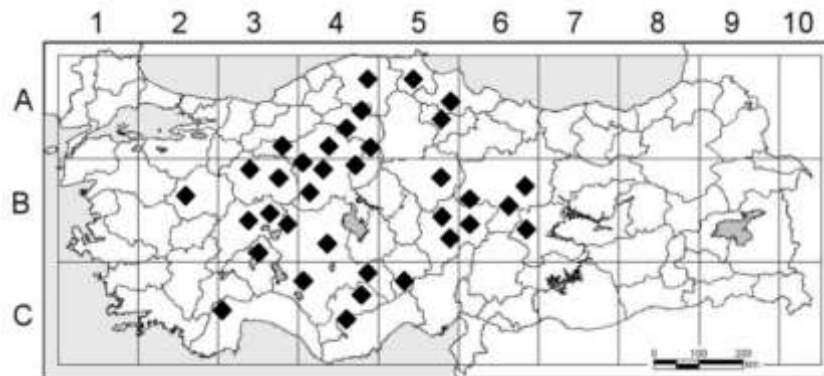


Figure 3. Distribution of *H. nummularium* subsp. *lycaonicum*

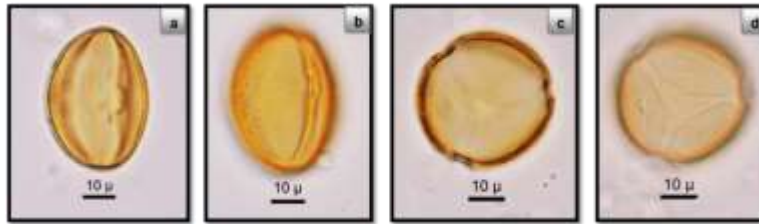
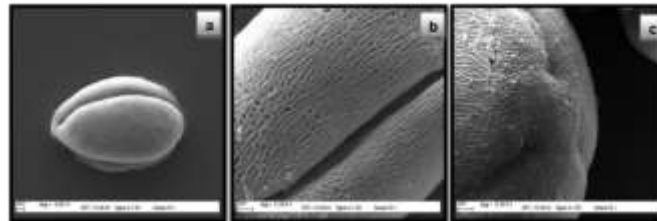
3.3. Pollen Micromorphology

Pollen grains of *H. nummularium* subsp. *lycaonicum* are isopolar, medium sized and tricolporate. Shape of pollen grains are prolate, P 44,78 ($\pm 2,24$) μ , E 32,14 ($\pm 1,88$) μ , P/E 1,39. Pollen grains are circular in polar view, Amb 33,90 ($\pm 2,54$) μ . Apocolpial areas are rather narrow, Apocolpia 8,36 ($\pm 1,56$) μ , Apoporia 26,80 ($\pm 1,89$) μ . Colpi are long and narrow, Clg 36,00 ($\pm 2,44$) μ , Clt 1,0 ($\pm 0,00$) μ . Pori are lolongate and wider than colpi, Plg 6,10 ($\pm 0,58$) μ , Plt 3,96 ($\pm 0,57$) μ . Exine is very thin, 2,06 ($\pm 0,16$) μ m. Sexine and nexine is not distinguished from each other. Exine ornamentation is identified as perforate under LM. Despite that, the SEM analysis showed that the exine ornamentation was striate-perforate at equatorial area and rugulate-perforate at polar area.

Measurements of pollen grains of *H. nummularium* subsp. *lycaonicum* are given in Table 1. LM and SEM micrographs of pollen grains are placed respectively in Figure 4 and Figure 5.

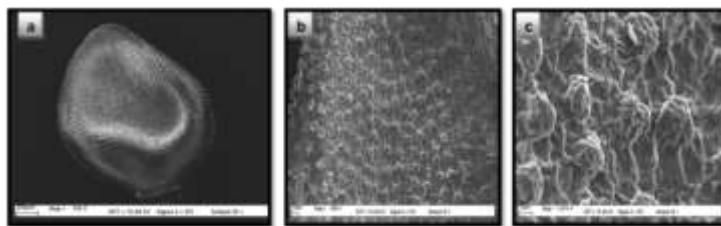
Table 1. Palynological measurements of *H. nummularium* subsp. *lycaonicum*

	<i>N</i>	<i>Minimum</i>	<i>Maximum</i>	<i>Mean</i>	<i>Standard deviation</i>
<i>E</i>	50	28,00 µm	37,00 µm	32,14 µm	±1,88
<i>P</i>	50	40,00 µm	50,00 µm	44,78 µm	±2,24
<i>Clg</i>	50	31,00 µm	42,00 µm	36,00 µm	±2,44
<i>Clt</i>	50	1,00 µm	1,00 µm	1,00 µm	±0,00
<i>Plg</i>	50	5,00 µm	7,00 µm	6,10 µm	±0,58
<i>Plt</i>	50	3,00 µm	5,00 µm	3,96 µm	±0,57
<i>Amb</i>	50	30,00 µm	42,00 µm	33,90 µm	±2,54
<i>Apocol</i>	50	5,00 µm	11,00 µm	8,36 µm	±1,56
<i>Apopor</i>	50	23,00 µm	31,00 µm	26,80 µm	±1,89
<i>Ekzin</i>	50	2,00 µm	2,50 µm	2,06 µm	±0,16

Figure 4. LM images of *H. nummularium* subsp. *lycaonicum* pollen grains: a. Equatorial view (high focus), b. Equatorial view (low focus), c. Polar view (high focus), d. Polar view (low focus)Figure 5. SEM images of *H. nummularium* subsp. *lycaonicum* pollen grains: a. General view, b. Equatorial view, c. Polar view

3.4. Seed Micromorphology

There are 12-17 seeds per capsule. Their shapes are ovate, size between 1,25-1,5×1,0-1,25 mm and the colour is brown. SEM studies showed that the seed coat ornamentation was densely verrucate. The anticlinal walls are occasionally distinct (Figure 6).

Figure 6. SEM images of *H. nummularium* subsp. *lycaonicum* seeds: a. General view, b. Close view, c. Surface ornamentation

3.5. Anatomy

3.5.1. Root anatomy: It is circular shaped. In cross section of the root, a thick periderm layer is on the outermost surface. The periderm cells are crushed or breaking up. Underneath the periderm, 4-11 layered parenchymatic cortex is located. There are sclerenchymatous cell groups above the phloem elements. Cambium is indistinguishable. The xylem is composed of tracheas and tracheids. Tracheas are circular or hexagonal. The xylem rays consist of 1-3 rowed cells. As the xylem occupies the pith, it is very narrow (Figure 7).

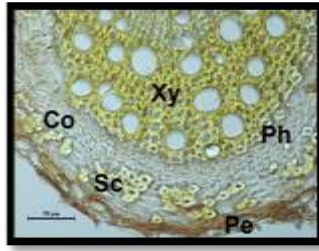


Figure 7. The cross section of the root of *H. nummularium* subsp. *lycanicum* (by Sartur reagent), Pe: Periderm, Co: Cortex, Sc: Sclerenchyma, Ph: Phloem, Xy: Xylem

3.5.2. Stem anatomy: The cross sections of the stem show that the 1-layered epidermis with stellate hairs covered by a thin cuticle. The epidermis consists of squarish or rectangular cells. The upper and lower walls of the epidermis cells are thicker than the lateral walls. Stomata are mesomorph type. There is a 3-4-layered chlorenchyma with intercellular spaces is under the epidermis. Under the chlorenchyma, 2-3-layered collenchyma cells are found. The innermost of cortex, which is called endodermis, is clear under the collenchyma tissue. Vascular bundles are next to each other. 1-4-layered sclerenchymatous cells are located above the phloem. The cambium is distinguishable. The xylem consists of thick-walled trachea and tracheids. The trachea cells are hexagonal. The pith is large and comprised of hexagonal parenchymatic cells (Figure 8).

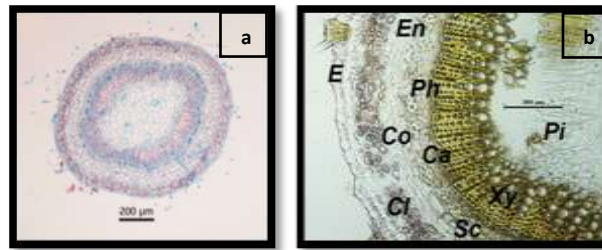


Figure 8. The cross section of the stem of *H. nummularium* subsp. *lycanicum* a. general view (by paraffin method), b. detailed view (by Sartur reagent), E: Epidermis, Cl: Chlorenchyma, Co: Collenchyma, En: Endodermis, Sc: Sclerenchyma, Ph: Phloem, Ca: Cambium, Xy: Xylem, Pi: Pith, St: Stellate hair, S: Stomata

3.5.3. Leaf anatomy: Upper and lower epidermis are covered by a thin cuticle layer. Both epidermises consist of uniseriate, squarish or rectangular cells. Occasionally, upper epidermis cells are larger than lower ones. Additionally, upper surface of the leaf is coated with bifurcate-stellate hairs, but lower surface has only stellate hairs. The leaf type is monofacial. Palisade parenchyma is 1-3-layered above and 1-layered below. The spongy parenchyma is 1-3-layered. Collenchyma is 4-5-layered above the xylem and 9-10-layered under the phloem. The type of the vascular bundles is open-collateral. Xylem is 5-6-layered and phloem is 3-4-layered. Druse crystals were occurred in the phloem of leaf (Figure 9).

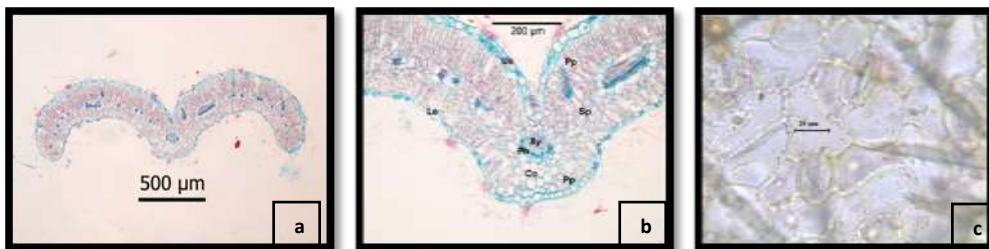


Figure 9. a-b. The cross section (by paraffin method), c. The surface section (by Sartur reagent) of the leaf of *H. nummularium* subsp. *lycanicum*, Ue: Upper epidermis, Le: Lower epidermis, Pp: Palisade parenchyma, Sp: Spongy parenchyma, Co: Collenchyma, Ph: Phloem, Xy: Xylem

4. Conclusions

H. nummularium subsp. *lycanicum* is very similar with *H. kotschyanum* and two taxa are usually misidentified each other. In fact, in the beginning the type specimen of *H. nummularium* subsp. *lycanicum* was misidentified as *H. kotschyanum* by Hayek. After that, Coode&Cullen has corrected the identification to *H. nummularium* subsp. *lycanicum*. At the first look, simple-bifurcate hirsute hairs of *H. kotschyanum* are remarkable on the upper surface of leaf. On the other hand, *H. nummularium* subsp. *lycanicum* has stellate tomentose hairs on upper

surface of leaf. The other diagnostic character is the hairy outer surface of inner sepals. *H. kotschyianum* has very few stellate hairs or glabrous between veins and *H. nummularium* subsp. *lycaonicum* has densely stellate hairs (Table 2).

Table 2. Comparison of morphological characteristics of *H. nummularium* subsp. *lycaonicum* and *H. kotschyianum*

Characters	<i>H. nummularium</i> subsp. <i>lycaonicum</i>	<i>H. kotschyianum</i>
Stem	long stellate tomentose hairs	sparsely long adpressed stellate hairs, spreading simple, bifurcate hirsute hairs
Upper surface of leaves	short stellate hairs and long stellate, bifurcate tomentose hairs	sparsely short adpressed stellate hairs, adpressed simple, bifurcate hirsute hairs
Pedicel length on flowering time	6,0-10,0 mm	5,5-6,5 mm
Petal length	11,0-13,0 mm	13,5-15,0 mm
Outer surface of inner sepals	densely short adpressed stellate hairs between veins	± sparsely short adpressed stellate hairs between veins
Capsule length	5,0-7,0 mm	4,0-5,5 mm

The subspecies of *H. nummularium* differ from each other based on the type of their indumentum and petal length. Leaves of *H. nummularium* subsp. *ovatum* are green on both surfaces without stellate hairs. This subspecies is distributed frequently in East and Central Black Sea Region on the moisted fields between 300-2500 m. Stem of *H. nummularium* subsp. *lycaonicum* is blackish and covered all over white tomentose hairs, distributed in Central Anatolia on steppe, limestone and rocky slopes and between 400-2000 m. Besides *H. nummularium* subsp. *nummularium* and subsp. *tomentosum* are green, pilose or puberulous hairs on the stem. *H. nummularium* subsp. *tomentosum* has longer petals (11,0-18,0 mm), longer capsules (5,0-8,0 mm), broader leaves (2,0-10,0 mm), more flowers (2-12-flowered) and all veins of inner sepals are hispid hairs. *H. nummularium* subsp. *nummularium* has shorter petals (8,0-11,0 mm), shorter capsules (4,75-6,0 mm), narrower leaves (0,75-4,5 mm) and two veins of inner sepals are hispid hairs.

There are several studies on the pollen morphology of family Cistaceae (Rivas, 1979; Ukraintseva, 1993; Perveen and Qaiser, 1998; Hassan, 2011, Yeşilyurt et al., 2012; Yeşilyurt et al., 2015). In terms of overall palynological features, the taxon is corresponding with the previous studies. But the details of exine ornamentation differ according to researchers' opinion.

Rivas (1979) studied 10 species from genus *Helianthemum* and determined the pollen grains as 3-colporate, prolate to subprolate and striate or rugulate. But also pollen SEM images in the paper of Rivas (1979) are clearly striate-perforate. In this case, the equatorial area of pollen grains should be analyzed for deciding the sculpture and also the perforations between stria was ignored by Rivas. In this way, our results are appropriate to Rivas's (1979).

The pollen morphology of 65 species from family Cistaceae were investigated under LM and 6 types of pollen are characterized by Ukraintseva (1993). According to the study, *Helianthemum* pollen type is characteristic only for the genus *Helianthemum* and the pollen grains are 3-colporate, prolate-spheroidal to prolate and finely reticulate (Ukraintseva, 1993). In our study we decided that the exine ornamentation seems perforate under LM. Difference between two studies is based on researcher opinions. According to Punt et al. (2007) reticulum is a network-like pattern but a pattern with irregular holes less than 1 µm is referred to perforate. Therefore, perforate is more suitable for the tectum of *Helianthemum* pollen grains.

Perveen and Qaiser (1998) studied the pollen morphology of *Helianthemum lippii* and reported that the pollens of *H. lippii* are 3-colporate, prolate-spheroidal, triangular in polar view and striate-rugulate. Also we found that the pollen grains of *H. nummularium* subsp. *lycaonicum* are prolate, circular in polar view and striate-perforate in equatorial area, rugulate-perforate in polar area. We saw that Perveen and Qaiser (1998) have ignored the perforation between striae. Hassan (2011) determined three pollen types for family Cistaceae and included the pollens of genus *Helianthemum* into the pollen type III. Besides, the researcher divided this pollen type to three subtypes such as striate, striate-reticulate, and striate-rugulate. Indeed, striate-reticulate subtype of Hassan (2011) should be striate-perforate because of the irregular holes less than 1 µm between striae. Under the circumstances, *H. nummularium* subsp. *lycaonicum* pollens can be inserted in striate-reticulate subtype.

Yeşilyurt et al. (2015) studied the pollen morphology of *H. germanicopolitanum* and *H. antitauricum*. According to these studies, pollen grains are tricolporate, prolate-spheroidal, circular in polar view. Yeşilyurt et al. (2012) identified the exine ornamentation of *H. germanicopolitanum* as striate at equatorial area and rugulate-perforate at polar area. After that, the researchers reported that the exine ornamentation is striate-perforate at equatorial area and rugulate-perforate at polar area for each species (Yeşilyurt et al., 2015). Also the ornamentation of pollen grains belong to *H. nummularium* subsp. *lycaonicum* is striate-perforate at equatorial area and rugulate-perforate at polar area under SEM. The results of these three studies are in agreement with each other.

The seed micromorphology of *H. germanicopolitanum* is rugulate-verrucate, warts are in irregular groups and anticlinal lines are unclear. On the other hand, anticlinal lines are distinct, anticlinal fields are striate and central fields have convex hunches with striate ornamentation on seed coat of *H. antitauricum* (Yeşilyurt et al., 2015). The seed coat of *H. nummularium* subsp. *lycaonicum* is also rugulate-verrucate, and warts are in an order and densely.

The consequence of our anatomical research is accordant with Metcalfe and Chalk (1957). According to Metcalfe and Chalk (1957), in Cistaceae, stomata present on both leaf surface, mesophyll is always including well-

developed palisade tissue. As regards our study, the surface anatomy of the leaf shows that the anomocytic stomata are framed by the epidermis cells. Furthermore, there are mesophytic stomata (amhistomatic) on the both surface. The mesophyll is composed of elongated rectangular palisade parenchyma cells and irregularly arranged spongy parenchyma cells. Mostly, a rounded structure is observed in the cross-sections of the stem. In the outer layer of annual species (*H. ledifolium*, *H. salicifolium* and *H. aegyptiacum*), there is a single epidermis layer. In the outer layer of perennial species, there are 1-2 epidermis layers in primary phase or fragmented in secondary phase. The epidermis is consisted of squarish or rectangular cells. The collenchyma and chlorenchyma layer are located under the epidermis. Endodermis is clearly defined in young stems of certain species of *Helianthemum*. Under this layer, there are phloem and xylem, respectively. Annual species of *Helianthemum* are observed secondary growth of stem. The cambium was located between the phloem and xylem. The type of vascular bundle is collateral open. Dispersion of the vascular bundles is circular and next to each other. Most species contain crystal druses around the vascular bundles.

As a conclusion, there was no detailed study on Turkish endemic *H. nummularium* subsp. *lycaonicum* before this research. Our study is accordingly the first extensive research of morphology, anatomy, pollen micromorphology, seed micromorphology, and distribution about the species. The results showed that the studied taxa are generally appropriate to each other in terms of palynology and anatomy. However, morphological characters and nutlet micromorphology of samples indicated some differences.

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