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Pollen morphology and anatomical features of *Lilium* (Liliaceae) taxa from Turkey

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Abstract: In this study, pollen grains and anatomical features of Turkish lilies were investigated under the electron (SEM) and light (LM) microscope. LM and SEM observations showed that the pollen grains are monosulcate, heteropolar, elliptical in polar view and oblate. Numerical results based on combined palynological and anatomical characters were discussed and compared with traditional taxonomic treatments. It was found that the midrib shape, mesophyll type, P/E (polar/equatorial), sulcus length, and lumina width are the most valuable traits in separating the examined taxa. The numerical analysis showed that *Lilium candidum* L. differs from the rest Turkish *Lilium* and also confirmed a close relationship between *L. szovitsianum* Fisch. & Avé-Lall. and *L. armenum* Miscz. ex Grossh. Also this study is the first report dealing with anatomical and palynological features of all Turkish lilies.

Key words: *Lilium*; anatomy; pollen; systematics; Turkey

Introduction

The genus *Lilium* L. (Liliaceae) includes about 100 species distributed throughout the cold and temperate regions of Northen Hemisphere (McRae 1998). Most of the *Lilium* species consisting of fragrant, bulbous and perennial herbs, form an important group of flowering garden plants. The long-standing popularity of *Lilium* as ornamental plants is due to their large, showy flowers that often have a strong fragrance (Woodcock & Stearn 1950).

Recently, many taxonomic viewpoints regarding the members and infrageneric classification of genus *Lilium* have been put forward. Traditionally, it has been subdivided into 5–11 sections (Endlicher 1840; Baker 1871; Wilson 1925; Baranova 1988) based on the morphological characters such as flower shape and position. But a more detailed and acceptable classification was proposed by Comber (1949) with seven sections based on combination of 13 morphological characteristics and two germination types. The infrageneric treatment of Comber (1949) has been supported by some molecular studies of Nishikawa et al. (2001), Ikinci (2005, 2011), Ikinci et al. (2006), Rønsted et al. (2005), Resetnik et al. (2007), Muratovic et al. (2010b), Lee et al. (2011) and Gao et al. (2012).

It is well known that pollen features have a great taxonomic value, and have been used in the classification of different genera (Troia et al. 2012; Ceter et al. 2013) and also closely related Liliaceae taxa (Tekşen et al. 2010; Kameshwari 2011; Masoumi 2012). Additionally, palynological studies showed that several features (Kosenko 1999; Muratović et al. 2010a; Pupuleku et al. 2010) and carbohydrate content of pollens in the genus *Lilium* (Clement & Audran 1995) provide important information, but to our knowledge there are no records on pollen morphology and contents of lilies distribution in Turkey. Similarly, as stated by Kim & Lee (1990), Kaviani et al. (2008), Dhyani et al. (2009) and Muratović et al. (2010a), the features related to stem and leaf anatomy have considerable taxonomic value in the systematic of *Lilium*. The first taxonomic treatment of Turkish *Lilium* was made by Davis & Henderson (1984), who recognized four species and four varieties. Since then, *L. akkusianum* R. Gämperle has been recorded as a new species from Turkey (Gämperle 1998). Recently the most comprehensive phylogenetic study on Turkish lilies based on phenetic and molecular data published (İkinci 2005). The recent study reveals, genus *Lilium* is represented by 7 species and 8 taxa in Turkey (İkinci 2012). Inceer et al. (2002) and Coskuncelebi et al. (2005) reported the caryological properties of some lily taxa (*L. candidum*, *L. martagon* L., *L. ponticum* K. Koch (= *L. carniolicum* subsp. *ponticum* (C.Koch) Davis & Henderson), *L. ciliatum* P.H. Davis) distributed in Turkey. Additionally, some anatomical

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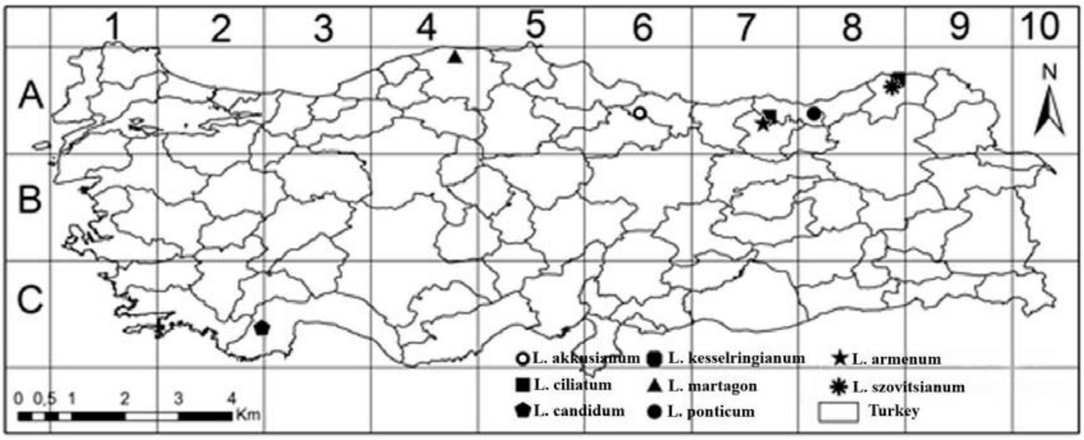

Fig. 1. Distribution map of the examined taxa.

Table 1. Locality information of Turkish lilies.

No	Taxon	Locality	Altitude (m)	Collection numbers
1	*Lilium candidum* L.	Muğla, Fethiye-Dalaman eski yolu, Göçek Geçidi	298	Makbul 203
2	*Lilium martagon* L.	Kastamonu-Küre-Akdivan köyü	1990	Makbul 164
3	*Lilium ponticum* K. Koch	Trabzon-Çaykara-Kabataş köyü	1250	Makbul 161
4	*Lilium ciliatum* P. H. Davis	Trabzon-Maçka-Hamsiköy üstü	1400	Makbul 152
5	*Lilium szovitsianum* Fisch. & Avé-Lall.	Artvin, Karabel-Ercan kayalığı üstleri, Aksaz Gölü	1912	Makbul 163
6	*Lilium armenum* Miscz. ex Grossh.	Trabzon-Zigana Dağı-Gümüşhane bölümü	1643	Makbul 154
7	*Lilium kesselringianum* Miscz.	Artvin-Borçka-Karagöl üstleri	1848	Makbul 162
8	*Lilium akkusianum* R. Gämperle	Ordu, Akkuş-Gökçebayır arası	1200	Makbul 208

features of *L. ciliatum* (Coskuncelebi & Beyazoglu 1999; Özdemir 2003), *L. ponticum* (= *L. carniolicum* Bernh. ex W.Koch var. *artvinense* (Miscz.) Davis & Henderson) (Aktaş et al. 2009) and *L. candidum* (Özen et al. 2012) have been recorded from Turkey. These all preliminary studies do not provide detailed anatomical information for all Turkish *Lilium* species. Thus, the present study aims; (1) to explore the anatomical and palynological properties and (2) contribute systematic position of eight *Lilium* taxa from Turkey.

Material and methods

Specimens

Both polleniferous and anatomical materials (stem and leaf) were collected from the natural habitats in Turkey during 2009 and 2010 (Fig. 1). The collection data for the examined taxa are given in Table 1. The voucher specimens were stored in the Herbarium of Recep Tayyip Erdoğan University, Department of Biology (RUB).

Anatomical studies

The anatomical materials were fixed in FAA (formaldehyde : acetic acid : alcohol) for 24 h and then preserved in ethanol (70%). Cross section of stem and leaf were taken with the microtome of Shandon Cryotome SME at 25–30 µm thickness. Surface sections of the leaves were cut by free hand. All sections except for surface ones were stained with hematoxylen for 30 minutes and mounted with aqua witrexia in order to obtain permanent slides (Vardar 1987). The sections were photographed with an Olympus BX51 from permanent slides. All measurements and observations were performed on 5 species for each population.

Palynological studies

Polleniferous materials were removed from living specimens in the field. The pollen grains were prepared for light microscope (LM) by standard methods described by Erdtman (1952). All measurements were based on at least 30 pollen grains per taxa. After pollen grains were coated with a thin layer of gold for 3 min with a Poloron SC502 Sputter Coater, they were examined and photographed with a JEOL-JSM 5600 scanning electron microscopy (SEM) at The Scientific and Technical Research Laboratories of Kırıkkale University (Turkey). Pollen terminology mainly follows Punt et al. (2007).

Numerical analysis

Two types of multivariate analyses were performed by Syn-Tax PC 5.0 (Podani 1993): Cluster analysis (CA) and principal components analysis (PCA). For CA (UPGMA), a pairwise matrix of resemblance values was calculated from raw standardized data matrix, using Gower's coefficient of resemblance designed for mixed data sets (Sneath & Sokal 1973). A dendrogram was generated by the unweighted pair-group method by using arithmetic averages (UPGMA). Also, cophenetic correlation coefficient (rcs) was calculated (Sneath & Sokal 1973). For PCA, firstly the raw data were used to create a correlation matrix and then two eigenvectors (a set of coordinates) were extracted by Eigen analysis from this correlation matrix. Two coordinates (axes) were projected to give a two-dimensional plot of the taxa and the characters. Thirteen anatomical and eleven palynological characters were assessed by numerical analysis: Three

Table 2. Anatomical and palynological characters used in numerical analysis.

Symbol	Characters
X_1	Width/length of epidermal cells of stem (μm/μm)
X_2	Width/length of collenchyma cells of stem (μm/μm)
X_3	Width of cortex tissue / Width of scleranchymatic tissue (μm/μm)
X_4	Width of phloem / Width of xylem (μm/μm)
X_5	Plant; glabrous or simple hairy:0, strigose hairy:1
X_6	Midrib; triangular-shaped or semicircular:0, circular:1
X_7	Mesophyll; unifasial:0, bifasial:1
X_8	Width of mesophyll (μm)
X_9	Width/length of lower epidermal cells of leaf (μm/μm)
X_{10}	Width/length of lower stomata (μm/μm)
X_{11}	Stomata index of lower epidermis
X_{12}	Width/length of upper epidermal cells of leaf (μm/μm)
X_{13}	Average number of upper epidermal cells of leaf (mm^2)
X_{14}	Polar axis (P) (μm)
X_{15}	Equatorial axis (E) (μm)
X_{16}	P/E rate
X_{17}	Muri (μm)
X_{18}	Lumina (μm)
X_{19}	Sulcus length (Slg) (μm)
X_{20}	Sulcus width (Slt) (μm)
X_{21}	Length/width of sulcus (Slg/Slt) (μm/μm)
X_{22}	Exine (μm)
X_{23}	Sexine (μm)
X_{24}	Nexine (μm)

characters were nominally scored as 0 or 1 and the remaining twenty one characters were quantitative including linear measurements and numbers (Table 2). All anatomical and palynological measurements used in the numerical analysis are given in the Appendix as a raw data matrix.

Results

Anatomical results

The general anatomical properties of stem and leaves were very similar in all examined taxa (Figs 2, 3). Therefore the detailed anatomical description of each species was not given separately here.

The cross sections taken from the stems revealed the following elements. There is one-layered epidermis consisting of rectangular or orbicular cells in all the examined taxa. Epidermal cells are smooth or covered with strigose and simple hairs. A single layer of collenchyma is located beneath the epidermis. The cortex consists of 2–5 rows and thin-walled parenchymatous cells having small intercellular spaces. A continuous sclerenchymatic ring surrounds the pith including the scattered vascular bundles in the central ground tissue. Sclerenchyma cells delimiting the cortex and pith have lignified thick walls. There is also large pith composed of cylindrical parenchymatic cells with several large intercellular spaces in the stem centre.

Transverse sections of the midrib and lamina, and surface preparations of the leaves of eight lily taxa were also analyzed (Fig. 3). As seen in Fig. 3, the upper and lower surfaces consist of orbicular and/or rectangular epidermal cells; the midrib is circular, semicircular or triangular-shaped. A small vascular bundle is surrounded by thin-walled orbicular parenchymatous cells. The mesophyll is bifacial or unifacial in all the examined taxa. Although bifacial leaves consist of a single layer of arm-shaped palisade cells and 6–7 layers of isodiametric spongy parenchymatic cells, there is homogenous mesophyll in the unifacial leaves. All leaves are hypostomatic leaves with anomocytic stomata.

Palynological results

The color of the pollen grains of the examined taxa were determined from the polleniferous materials collected in the field, however it was not used in numerical analysis in the present study. According to our observations, the color of pollen grains is yellow in *L. candidum, L. ciliatum, L. szovitsianum, L. armenum,* but it is orange in the rest of the examined taxa.

As in the anatomical part of this study, polar views including surface ornamentation taken by SEM are given in Fig. 4. All pollen grains are monosulcate; heteropolar, elliptical in polar view and oblate (Fig. 4). Polar axis is 40.34 (± 4.23)–48.80 (± 8.78) μm, equatorial axis is 65.93 (± 6.11)–79.98 (± 3.41) μm. P/E ratio is 0.58–0.68. The sulcus is located in the distal pole, with a length (Slg) of 56.39 (± 4.62)–67.98 (± 4.95) μm; sulcus width (Slt) is 6.79 (± 2.14)–8.60 (± 3.43) μm. The sulcus is narrow, deep by rounded ends, almost as long as the equatorial axis, the Slg/Slt ratio is 6.81–9.00. The thickness of the muri is 1.68 (± 0.34)–2.34 (± 0.44) μm and width of the lumina is 3.86 (± 0.97)–5.81 (± 1.45) μm. The exine thickness is 2.37 (± 0.42)–2.74 (± 0.35) μm, and the sexine thickness ranges from 1.37 (± 0.33) to 1.74 (± 0.40) μm, whereas the nexine thickness is between 0.84 (± 0.09) and 1.04 (± 0.20) μm. Surface ornamentation is reticulate.

Numerical results

The dendrogram resulting from UPGMA based on 24 combined variables is represented in Fig. 5. The first

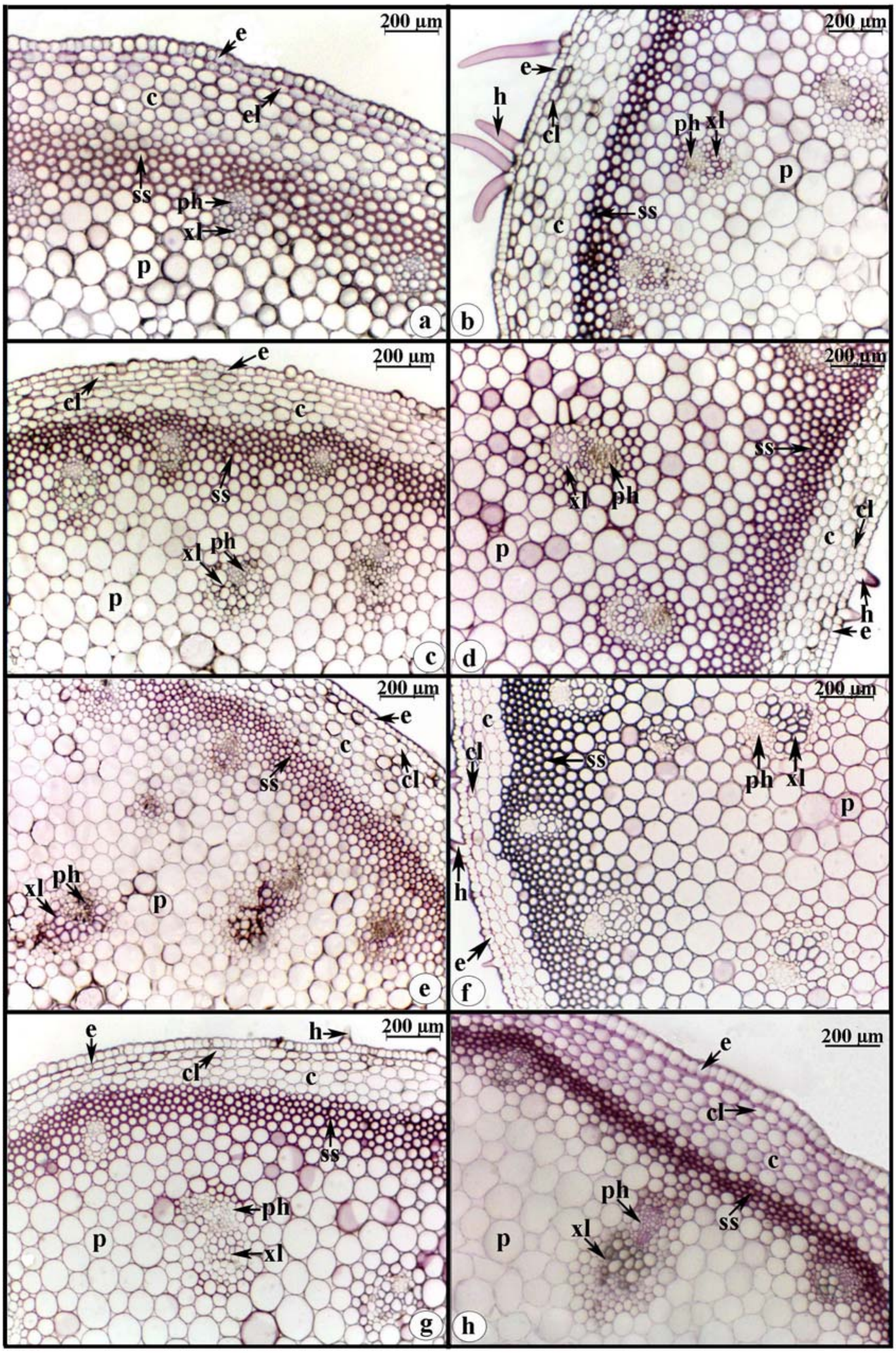

Fig. 2. Cross sections of stem: (a) – *L. candidum*; (b) – *L. martagon*; (c) – *L. ponticum*; (d) – *L. ciliatum*; (e) – *L. szovitsianum*; (f) – *L. armenum*; (g) – *L. kesselringianum*; (h) – *L. akkusianum.*
Abbreviations: e, epidermis; cl, collenchyma; c, cortex; h, hair; p, pith; ph, phloem; ss, sclerenchymatic sheath; xl, xylem.

cluster, labeled as "a" is linked to the other examined taxa with a 98.8% dissimilarity level, consists of *L. candidum.* The second largest group labeled as "b" is divided into smaller clusters, including all the remaining seven taxa. The cophenetic correlation coefficient is 0.91.

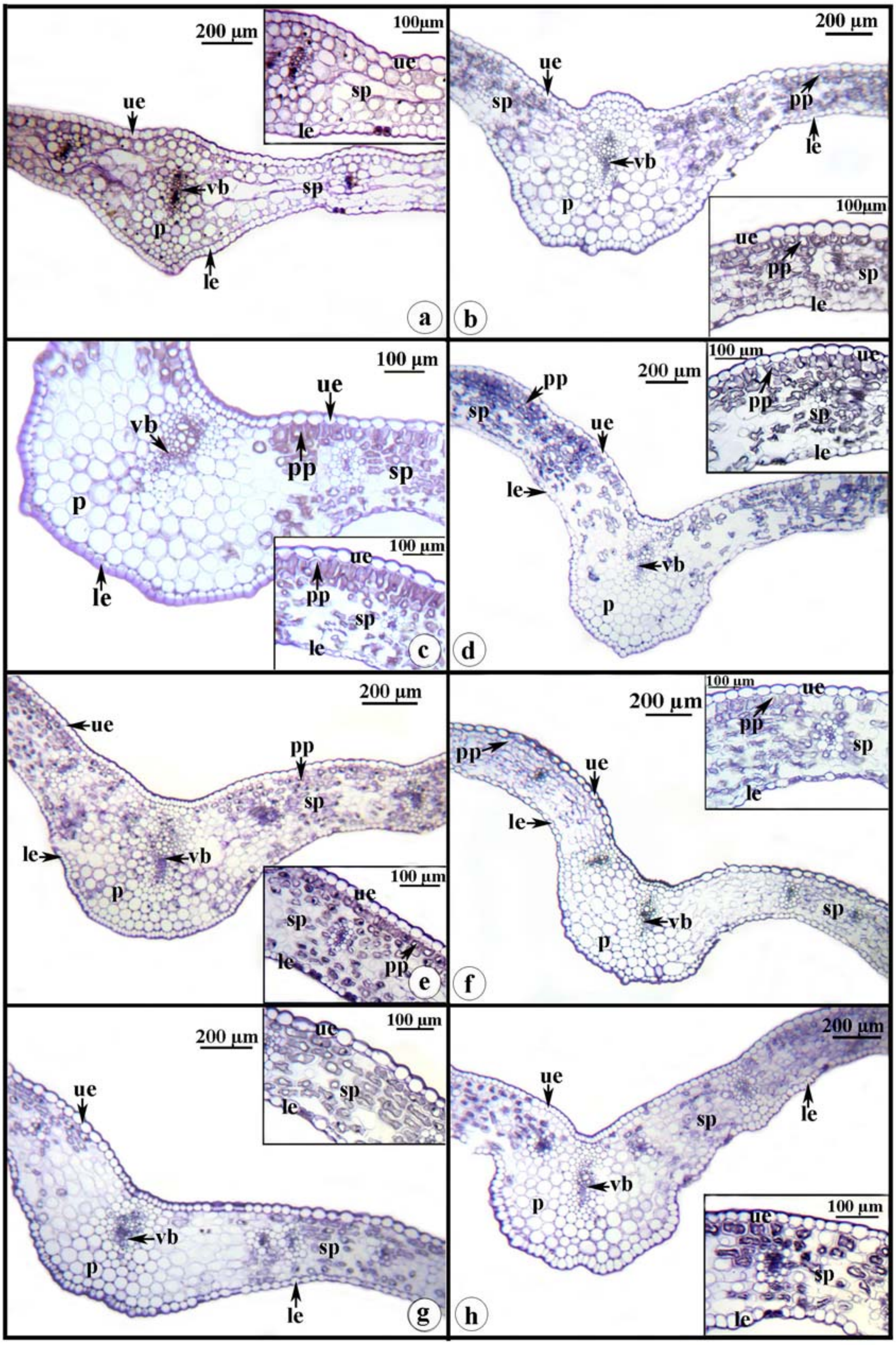

Fig. 3. Cross sections of leaves: (a) – *L. candidum;* (b) – *L. martagon;* (c) – *L. ponticum*; (d) – *L. ciliatum*; (e) – *L. szovitsianum*; (f) – *L. armenum*; (g) – *L. kesselringianum*; (h) – *L. akkusianum.*
Abbreviations: le, lower epidermis; p, parenchyma; pp, palisade parenchyma; sp, spongy parenchyma; ue, upper epidermis; vb, vascular bundle.

Biplot graph resulting from PCA analysis based on 24 variables are given in Fig. 6. This figure shows the distribution of taxa together with the variables onto the first two components (axes). Only the first three components were taken into account because of their Eigenvalue which expressed the most of the variance

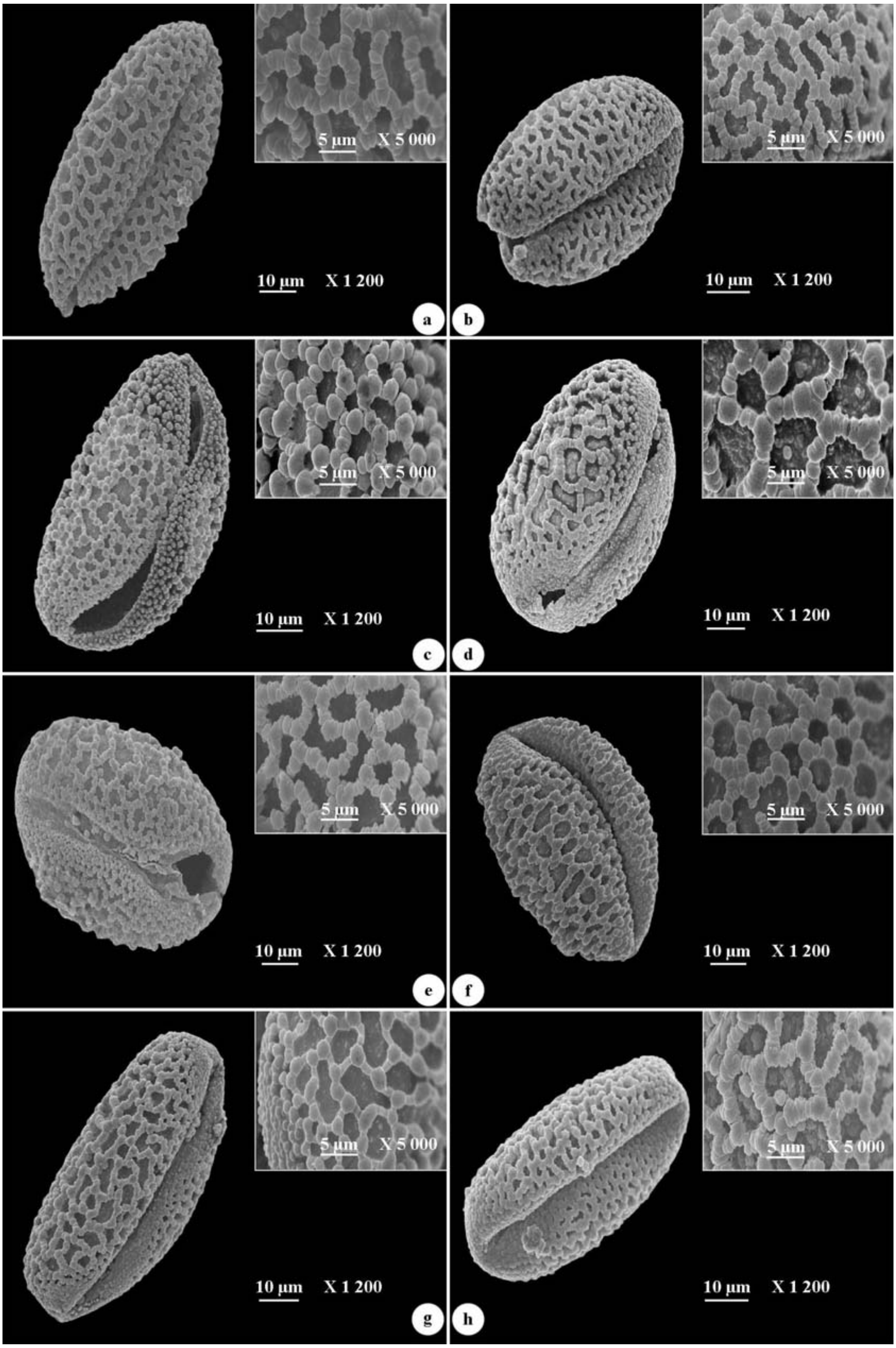

Fig. 4. Pollen grains of the examined taxa in polar view: (a) – *L. candidum*; (b) – *L. martagon*; (c) – *L. ponticum*; (d) – *L. ciliatum*; (e) – *L. szovitsianum*; (f) – *L. armenum*; (g) – *L. kesselringianum*; (h) *L. akkusianum.*

explained by the associated eigenvectors. The eigenvalue of the first, second and third component in percentages is 28.55%, 19.89%, 16.16%, respectively (Table 3).

Discussion

Anatomical studies provide taxonomically significant data in many different plant genera especially for the monocots (Lersten & Curtis 2001). Similarly, anatom-

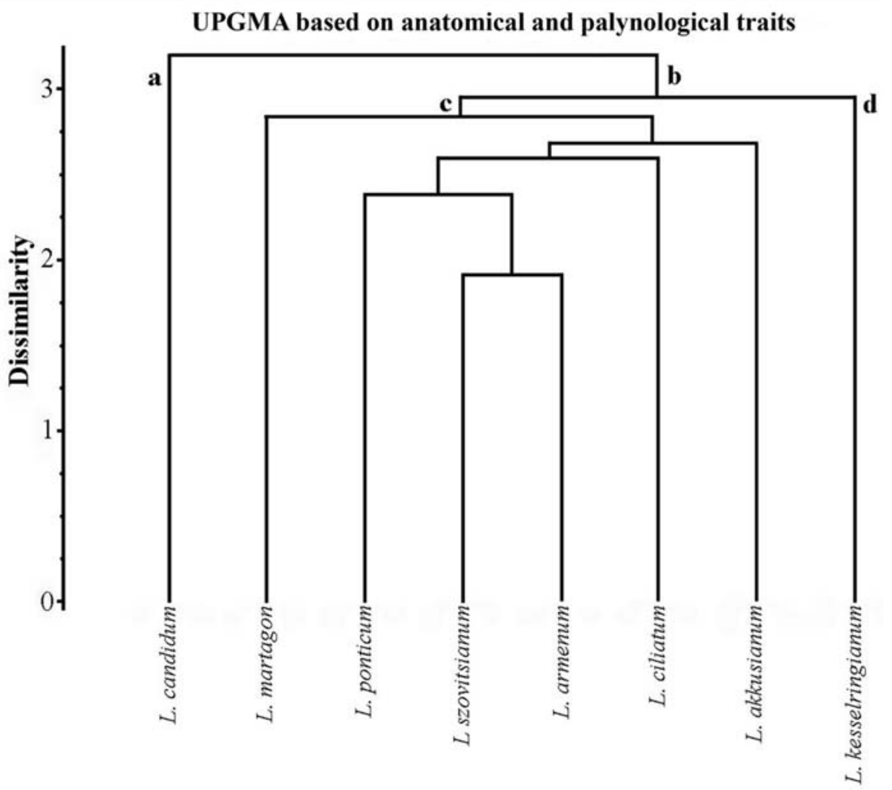

Fig. 5. Cluster analysis – UPGMA.

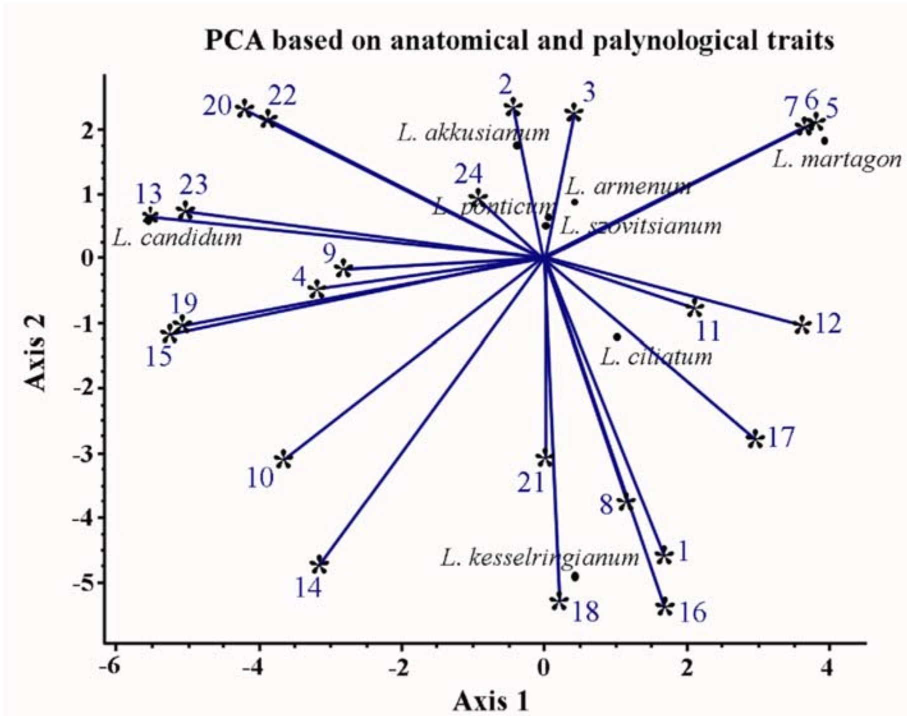

Fig. 6. Principal component analyses of 8 taxa and 24 variables projected onto the axis 1 and axis 2.

ical investigations performed on *Lilium* supplied many useful taxonomically important traits (Kim & Lee 1990; Kaviani et al. 2008; Dhyani et al. 2009). In this study, the general stem structures are almost similar in all examined taxa but, there are few differences in the rate of cortex/scleranchymatic tissue and phloem/xylem. *L. akkusianum* has the highest value for both characters and is typically separated from the other examined taxa in terms of these anatomical features. However, these characteristics were not among the statistically impor-

Table 3. Percentage of variance accounted for by the first three components.

Components	Based on anatomical traits		Based on palynological raits		Based on anatomical and palynological raits	
	Square roots of eigenvalues	Variance (%)	Square roots of eigenvalues	Variance (%)	Square roots of eigenvalues	Variance (%)
PC1	195.91	29.52	199.94	34.36	261.78	28.55
PC2	184.82	26.28	181.96	30.10	218.50	19.89
PC3	148.26	16.91	131.10	15.63	196.94	16.16
Total	–	72.71	–	80.09	–	64.61

tant once. Our findings for stem structure were in accordance with the previous studies carried out on *L. ciliatum* (Coskuncelebi & Beyazoglu 1999; Özdemir 2003), *L. ponticum* (Aktaş et al. 2009), *L. candidum* (Özen et al. 2012), *L. ledebourii* (Baker) Bios (Kaviani et al. 2008) and *L. polyphyllum* D. Don ex Royle (Dhyani et al. 2009).

The leaf structure of the examined lilies supplies valuable traits to delimit the examined taxa. The midrib shape is circular in *L. martagon* (Fig. 3b), triangular in *L. candidum* (Fig. 3a) and semicircular in rest of the investigated taxa. In our study, it was determined that the arrangement of mesophyll tissue differs among the taxa. *L. candidum*, *L. kesselringianum* Miscz. and *L. akkusianum* have unifacial mesophyll, but the rest taxa have bifacial mesophyll. Yentür (2003) indicated that the arrangement of mesophyll is a valuable trait in comparative leaf anatomy. In previous studies, it was reported that mesophyll is more or less unifacial and the distinction between palisade and spongy parenchyma is not clear in *L. ciliatum* (Coskuncelebi & Beyazoglu 1999; Özdemir 2003) and *L. ponticum* (Aktaş et al. 2009). However, the present study clearly reveals the presence of single layered palisade parenchyma in *L. ciliatum* and *L. ponticum* (Fig. 3c, d). Özen et al. (2012) reported lobed palisade parenchyma cells in *L. candidum* but, in our study it is clearly seen that the mesophyll tissue consists of only uniform cells (Fig. 3a). However, we observed arm-shaped palisade parenchymatic cells in *L. martagon, L. ponticum, L. ciliatum, L. szovitsianum* and *L. armenum.* Similarly, Yentür (2003) reported that palisade parenchyma cells are branched and lobed in the genus *Lilium.*

All the examined taxa have hypostomatic leaves with anomocytic stomata. Similar stomata type was also observed in *L. ledebourii* (Kaviani et al. 2008), *L. polyphyllum* (Dhyani et al. 2009), *L. carniolicum* (Muratović et al. 2010a), *L. ciliatum* (Coskuncelebi & Beyazoglu 1999; Özdemir 2003), *L. ponticum* (Aktaş et al. 2009) and *L. candidum* (Özen et al. 2012). Stomata index varies from 26.5 to 44.8 in all examined taxa, but it is well known that this trait is among the environmentally influenced anatomical characters. According to Hodgson et al. (2010), stomata size is positively correlated to genome size across a wide range of major angiosperm taxa, so that the stomata characters are not so useful in the delimitation of the plant taxa; but *L. candidium* growing in the warmer western regions of Turkey, in a Mediterranean climate, differs from the other Turkish taxa grown in the Black Sea region in an oceanic climate (Akman & Ketenoğlu 1986). Kaviani et al. (2008) reported a very similar stomata index for *L. ledebourii* grown in an arid region of Iran.

In the second part of this study, detailed pollen features of the Turkish lilies was examined for the first time. As a result of palynolgical examination, it was found that pollen grains of all the examined taxa are monosulcate which is a primitive trait in seed plants, and occur widely among the monocotyledons (Furness & Rudall 2001). Similar results were reported in the genus *Lilium* by Kosenko (1999), Dhyani et al. (2009), Pupuleku et al. (2010) and Muratović et al. (2010a). P/E is a prominent character as reported in several palynological studies (Makbul et al. 2008; Tekşen et al. 2010). P/E varies from 0.58 to 0.68 in all examined Turkish *Lilium.* Muratović et al. (2010a) reported that there were no significant differences among the populations of *L. bosniacum* and *L. carniolicum* in terms of P/E ratio. It varies from 0.81 (± 0.01) to 0.86 (± 0.02) in different populations of those taxa. Similarly, Pupuleku et al. (2010) indicated that pollen size, influenced by biological factors, varies from one habitat to another. As seen in the above literature, P/E which was found to be statistically important trait could vary depending on the environmental conditions.

Palynological studies performed on the Liliaceae members indicated that some pollen characters such as sulcus, muri and lumina are taxonomically useful (Kuprianova 1983; Kosenko 1999). Our findings also supported this view and supplied additional information to separate the examined taxa. Previous studies on the lilies grains showed that the muri thickness varies from 1 to 3.4 μm (Kosenko 1999; Muratović et al. 2010a), but it was found that it varies from 1.68 (± 0.34) to 2.34 (± 0.44) in the present study.

Exine thickness should be another important palynological trait among the examined Turkish lilies. Kosenko (1999) reported that exine thickness varies from 2.2 to 3.7 μm in some members of *Lilium.* In the present stuy, the highest exine thickness (2.74 ± 0.35 μm) is determined in *L. candidum* and the lowest one (2.37 ± 0.42 μm) observed in *L. kesselringianum.* Pupuleku et al. (2010) determined that exine thickness ranges from 1.8 μm to 2.3 μm in *L. martagon,* but it was measured 2.44 (± 0.36) μm in the present study.

Baranova (1985) and Du et. al (2014) classified *Lil-*

Table 4. Sectional classification according to different authors of the Turkish *Lilium*.

Taxa	Baker (1871)	Wilson (1925)	Comber (1949)	Baranova (1988)	İkinci (2011)
L. candidum	*Eulirion*	*Leucolirion*	*Liriotypus*	*Lilium*	*Liriotypus*
L. martagon	*Martagon*	*Martagon*	*Martagon*	*Martagon*	*Martagon*
L. ponticum	*Martagon*	*Martagon*	*Liriotypus*	*Eurolirium*	*Liriotypus*
L. ciliatum	*Martagon*	*Martagon*	*Liriotypus*	*Eurolirium*	*Liriotypus*
L. szovitsianum	*Martagon*	*Martagon*	*Liriotypus*	*Eurolirium*	*Liriotypus*
L. armenum	*Martagon*	*Martagon*	*Liriotypus*	*Eurolirium*	*Liriotypus*
L. kesselringianum	*Martagon*	*Martagon*	*Liriotypus*	*Eurolirium*	*Liriotypus*
L. akkusianum	*Martagon*	*Martagon*	*Liriotypus*	*Eurolirium*	*Liriotypus*

ium pollens into four morphological types called Martgon, Callose, Concolor and Formosanum. Martagon type pollen is characterized by the muri consisting of rectangular columellae. All examined *Lilium* pollens have been found to be in conformity with the Martagon type.

In the dendrogram resulting from UPGMA (Fig. 5), all investigated taxa fall into two major clusters at 98 % dissimilarity levels. One, labeled "a" consists of only *L. candidum* easily separated from the rest Turkish lilies by means of over-wintering basal leaves and open funnel-shaped flowers (İkinci 2005; Muratović et al. 2010b). Additionally while most Turkish lilies are mainly distributed in the region of Northern Turkey–Caucasus (Davis & Henderson 1984), *L. candidum* occurring naturally in SW Europe, Syria, Lebanon and Israel (Muratović et al. 2010c) is the only species distributed in the SW Turkey. Comber (1949) put *L. candidum* together with other Turkish species under the sect. *Liriotypus* Ascj. & Graebn. characterized by alternate leaf arrangements. But our anatomical and palynological findings do not support this treatment. However *L. candidum* was classified under sections of *Eulirion* Rchb., *Leucolirion* Wilson and *Lilium* Rchb. by Baker (1871), Wilson (1925) and Baranova (1988) respectively (Table 4). Also our findings supports to place *L. candidum* in a separate section. But recent molecular studies performed on Turkish (İkinci 2005, 2011; İkinci et al. 2006) and non Turkish *Lilium* representatives (Nishikawa et al. 2001; Rønsted et al. 2005; Resetnik et al. 2007; Muratovic et al. 2010b; Lee et al. 2011; Gao et al. 2012) supported Comber's (1949) view. In contrast to the above mentioned molecular studies, Muratović et al. (2010c) indicated that *L. candidum* presents a particular pattern quite different from other European representatives of the sect. *Liriotypus.* This means that molecular investigations do not provide similar results every time. Even in some case, morphologically similar two taxa may be placed in different clade according to molecular properties. So, for the resolving of taxonomic misunderstandings, morphological characters have the most important role in the plant systematics, and molecular, anatomical and palynological characters can be used to support the morphological characters.

Cluster "b" is divided into two subgroups (Fig. 5). While the first group labeled "d" consists of only *L. kesselringianum,* the second group labeled "c" includes the remainder taxa. *L. kesselringianum* is placed in the Caucasian *Monadelphum* group with *L. armenum, L. szovitsianum* and *L. monadelphum* M.Bieb. which have similar flower morphology (İkinci et al. 2006). But, Kesselring (1932) reported that *L. kesselringianum* with lighter petal, slightly reflexed petal segments and shorter chestnut-purple anthers differs from *L. szovitsianum* and *L. monadelphum.* Also, *L. kesselringianum* is separated from the other Turkish lilies including *Monadelphum* group by means of anatomical and palynological traits such as width/length of epidermal cells of stem, P/E ratio, width of lumina, and length/width of sulcus. While *L. armenum* and *L. szovitsianum* have bifacial mesophyll, *L. kesselringianum* has unifacial ones. The length/width of the sulcus varies among *L. armenum* (7.83), *L. szovitsianum* (7.80) and *L. kesselringianum* (8.21). Additionally, the highest P/E ratio (0.68) and lumina width (5.81 ± 1.45 µm) were recorded in *L. kesselringianum.* According to our palynological and anatomical results, *L. kesselringianum* clearly differs from the other related taxa and this separation is supported by our numerical analysis based on UPGMA and PCA (Figs 5, 6).

L. kesselringianum and *L. akkusianum* are similar by means of anatomical traits used in this study. İkinci (2011) reported that these two species are closely related because of the similarities in floral morphology such as color of flowers, shape of perianth segments and color of pollen. In contrast, the UPGMA analysis based on combined palynological and anatomical data showed that these two taxa are grouped in a different cluster (Fig. 5). This means that palynological traits supply useful information for delimiting these two closely related taxa. The most valuable palynological characters are thickness of muri, width of lumina and length/width of sulcus. While the highest muri thickness (2.34 ± 0.44 µm) and lumina width (5.81 ± 1.45 µm) were recorded in *L. kesselringianum,* the lowest muri thickness (1.68 ± 0.34 µm) and lumina width (3.86 ± 0.97 µm) were observed in *L. akkusianum.* Also, the length/width of the sulcus varies between *L. kesselringianum* (8.21) and *L. akkusianum* (7.60). Additionally, these two taxa were clustered in a different group based on cpDNA and nrDNA ITS (İkinci et al. 2006; İkinci 2011) and RAPD data (İkinci & Oberprieler 2010).

L. martagon differs from the other five Turkish lilies grouped in cluster "c" based on the anatomical and palynological properties. It is determined that some anatomical characters such as stem pubescence and the shape of midrib are useful for delimiting the investigated taxa. In the examined taxa, the stem is either simple hairy or glabrous and the midrib is semicircular or triangular, except for *L. martagon* having strigose hairs with circular midrib. In contrast to the anatomical properties, palynological traits are not supported to treat *L. martagon* as a distinct species. The present analysis showed that *L. martagon* is placed in sect. *Martagon* together with other Turkish lilies except for *L. kesselringianum* and *L. candidum*. Our palynological and anatomical results are not in accordance with Comber's (1949) view, but support the earlier classifications proposed by Baker (1871) and Wilson (1925).

Numerical analysis showed that *L. armenum* and *L. szovitsianum* are the closest taxa based on our palynological and anatomical features. These findings are also consistent with the data in Flora of Turkey (Davis & Henderson 1984). Davis & Henderson (1984) determined that the taxa have similar morphological features and should be classified at variety level. In contrast to these results, some morphological studies (Mandenova 1940) and recent cytogenetic investigations (İkinci & Oberprieler 2010; İkinci 2011) indicated that these two taxa should be classified as separate species. Also, these two taxa have some different morphological characters such as size of flower, anther and style (Mandenova 1940). But according to findings of İkinci (2012), *L. szovitsianum* and *L. armenum*, morphologically closely related taxa, were treated as varieties of *L. monadelphum*. Our palynological and anatomical results support this view.

As it can be seen in Table 3, the first three components account together for 64.61 % total variation based on palynological (11 traits) and anatomical (13 traits) variables. According to the numerical analysis, some palynological characters such as P/E ratio, the sulcus length and the lumina width are more important than anatomical ones in explaining total variation. In conclusion, anatomical and palynological features supplied additional valuable information in separating the Turkish *Lilium*. Consequently, our results indicated that *L. candidum* differs from the other Turkish lilies, and also, *L. szovitsianum* and *L. armenum* are closely related in terms of anatomical and palynological features.

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Appendix. Anatomical and palynological measurements of examined taxa.

Characters	*L. candidum*	*L. martagon*	*L. ponticum*	*L. ciliatum*	*L. szovitsianum*	*L. armenum*	*L. kesselringianum*	*L. akkusianum*
X_1	0.7 ± 0.12	0.82 ± 0.11	1.01 ± 0.12	0.78 ± 0.09	0.9 ± 0.09	0.82 ± 0.02	1.2 ± 0.24	0.67 ± 0.11
X_2	1.5 ± 0.13	1.53 ± 0.16	1.49 ± 0.2	1.36 ± 0.2	1.21 ± 0.18	1.33 ± 0.18	1.3 ± 0.17	1.35 ± 0.13
X_3	1.12 ± 0.23	1.3 ± 0.3	0.72 ± 0.07	1.06 ± 0.2	1.26 ± 0.17	1.04 ± 0.05	1.03 ± 0.27	1.66 ± 0.31
X_4	0.76 ± 0.16	0.6 ± 0.22	0.56 ± 0.09	0.7 ± 0.09	0.59 ± 0.14	0.6 ± 0.05	0.68 ± 0.1	0.8 ± 0.08
X_5	0	1	0	0	0	0	0	0
X_6	0	1	0	0	0	0	0	0
X_7	0	1	1	1	1	1	0	0
X_8	186 ± 31	184 ± 23	228 ± 17	260 ± 21	210 ± 13	200 ± 10	235 ± 28	211 ± 18
X_9	0.3 ± 0.09	0.21 ± 0.03	0.26 ± 0.1	0.3 ± 0.09	0.17 ± 0.04	0.2 ± 0.04	0.2 ± 0.05	0.2 ± 0.06
X_{10}	0.83 ± 0.07	0.7 ± 0.05	0.8 ± 0.04	0.69 ± 0.04	0.74 ± 0.02	0.7 ± 0.03	0.84 ± 0.07	0.72 ± 0.03
X_{11}	26.5 ± 3.6	30.8 ± 2.4	42.1 ± 1.4	44.8 ± 5.1	39.2 ± 3.3	36.2 ± 3.1	31.5 ± 2.7	27.3 ± 2.9
X_{12}	0.2 ± 0 .06	0.27 ± 0.05	0.21 ± 0.04	0.26 ± 0.03	0.2 ± 0.05	0.19 ± 0.02	0.22 ± 0.06	0.18 ± 0.05
X_{13}	46.6 ± 3	32.4 ± 2	36.1 ± 3	35.4 ± 2	40.1 ± 3	36.7 ± 2	34.8 ± 2	34.5 ± 2
X_{14}	46.78 ± 4.23	40.34 ± 4.23	41.16 ± 4.47	42.97 ± 4.53	40.97 ± 5.92	40.56 ± 2.98	48.80 ± 8.78	41.62 ± 6.07
X_{15}	79.98 ± 3.41	66.30 ± 5.80	65.93 ± 6.11	70.30 ± 5.17	68.07 ± 9.02	69.21 ± 4.36	71.28 ± 9.88	68.96 ± 5.97
X_{16}	0.58	0.60	0.62	0.61	0.60	0.59	0.68	0.60
X_{17}	1.89 ± 0.46	2.27 ± 0.42	1.97 ± 0.52	1.93 ± 0.35	2.16 ± 0.48	2.15 ± 0.38	2.34 ± 0.44	1.68 ± 0.34
X_{18}	4.81 ± 1.30	4.77 ± 1.45	4.37 ± 1.20	4.80 ± 1.19	4.39 ± 1.38	4.40 ± 0.76	5.81 ± 1.45	3.86 ± 0.97
X_{19}	67.98 ± 4.95	56.49 ± 6.83	56.39 ± 4.62	61.23 ± 5.88	61.22 ± 5.81	61.33 ± 5.65	60.78 ± 8.25	58.48 ± 5.09
X_{20}	8.60 ± 3.43	7.43 ± 2.23	8.27 ± 4.10	6.79 ± 2.14	7.87 ± 1.61	7.83 ± 0.23	7.40 ± 2.47	7.68 ± 2.83
X_{21}	7.90	7.59	6.81	9.00	7.80	7.83	8.21	7.60
X_{22}	2.74 ± 0.35	2.44 ± 0.36	2.66 ± 0.36	2.50 ± 0.36	2.69 ± 0.53	2.46 ± 0.51	2.37 ± 0.42	2.44 ± 0.42
X_{23}	1.74 ± 0.40	1.37 ± 0.33	1.65 ± 0.23	1.52 ± 0.28	1.61 ± 0.21	1.59 ± 0.44	1.47 ± 0.35	1.47 ± 0.26
X_{24}	0.97 ± 0.20	0.95 ± 1.19	1.04 ± 0.20	0.92 ± 0.19	0.84 ± 0.09	0.85 ± 0.17	0.89 ± 0.20	0.89 ± 0.23