

## Labellum micromorphology of some orchid genera (Orchidaceae) distributed in the Black Sea region in Turkey

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**Abstract:** A detailed micromorphological analysis of the labellum was carried out using both light and scanning electron microscopy on 14 genera (*Anacamptis*, *Cephalanthera*, *Coeloglossum*, *Dactylorhiza*, *Gymnadenia*, *Himantoglossum*, *Limodorum*, *Neottia*, *Orchis*, *Ophrys*, *Platanthera*, *Serapias*, *Spiranthes*, and *Steenia*) of Orchidaceae in Turkey. In the samples, various epidermal features were identified in the spur that developmentally are invaginations of the labellum and the adaxial surface of the labellum. Typical secretory cells and inclusions such as starch or calcium oxalate crystals of different sizes and shapes were found within the structure of individuals. In many genera, characteristically shaped secretory trichomes and papillae were concentrated at the base part close to the gynostemium or the distal part of the labellum and on all surfaces of the spur. In the genus *Ophrys*, the labellum surface was covered with many hairs. Furthermore, the labellum surface striation varied among the orchid genera. UPGMA of cluster analysis was used to evaluate the morphological and micromorphological features of the labellum and spur among the analyzed taxa.

**Key words:** Labellum, micromorphology, orchid, spur, SEM, Turkey

### 1. Introduction

The family Orchidaceae includes a high variety of epiphytes or parasites that have adapted to a wide range of ecological conditions all over the world (Leake, 2005). It contains many types of species in a wide geographic area from the tropical regions to the temperate zone. The wild orchids show rich biodiversity in Turkey. The family is represented by 24 genera. Together with hybrids, the number of taxa in Turkey is recorded as 229 (Güler and Deniz, 2012). Furthermore, some of the species selected for the present study are important in terms of salep production.

The pollination strategy is one factor giving rise to orchid biodiversity (Rudall and Bateman, 2002; Box et al., 2008). These unique plants are noteworthy for their flower variations and pollination methods (Cozzolino and Widmer, 2005). Due to traits such as energy-rich food rewards or deceptive strategies, orchid flowers attract pollinators and increase pollination efficiency (Burns-Balogh et al., 1987; Neiland and Wilcock, 1998; Cozzolino and Widmer, 2005; Schiestl, 2005; Bell et al., 2009; Vereecken, 2009). With respect to their colors and the odors that they produce, many orchid flowers perform pollination by mimicking insect foods (Cozzolino and Widmer, 2005). Sexual imitation is the most advanced form of deceptive pollination observed in *Ophrys* (Schiestl, 2005).

Orchid flowers have some common features (Rudall and Bateman, 2002; Kreutz and Çolak 2009). The perianth, consisting of three sepals and petals, covers the fertile gynostemium composed of the gynoecium and androecium cohesion called pollinia with pollen mass. The central petal differs from the lateral petal in terms of some characteristics such as shape, size, and color. This part, called the labellum, extends and forms the structure called the spur, which is thought to be involved in deceptive pollination for nectar nonproducing species or can usually be considered to be associated with nectar production (Fulton and Hodges, 1999). In orchid flowers, the labellum also serves as a resting place, a runway, or deceptive sexual organs during the pollinator visitation; it also plays a critical role in attracting pollinators and therefore many researchers pay attention to its traits (Burns-Balogh et al., 1987; Ayasse et al., 2000; Rudall and Bateman, 2002; Box et al., 2008; Chase et al., 2009).

Because of the complex floral characteristics and economic value of orchids, they have been the subject of many research fields such as experimental (Darwin, 1862; Kullenberg, 1961; Paulus and Gack, 1990), phylogenetic, and reproductive biology (Devey et al., 2008, 2009; Chase et al., 2009), especially of the genus *Ophrys*. However, there have been a limited number of studies on the

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micromorphology of flowers of temperate orchids, except for *Ophrys* and a few other deceptive genera. Some micromorphological studies explained that successful flower–pollinator interactions occur through the floral micromorphological features such as nectar secreting trichomes, odor-producing osmophores, or secretory epidermal cells under the cuticle in the labellum and spur (Stpiczyńska, 1993, 2003; Anton et al., 2012). Stpiczyńska (2001) analyzed osmophores on the labellum of *Gymnadenia conopsea*, a European species with a nectary spur and pollinated by Lepidoptera, and suggested their secretion mechanism using SEM and TEM. Furthermore, a study was conducted on four clades in Orchidinae and the relationship between nectar production and epidermal cell properties was investigated (Bell et al., 2009). Its results indicated that nectar production was associated with epidermal papillae size and cuticle striation. Moreover, in micromorphological research on four sympatric *Serapias* species using deceptive pollination, the characteristic secretory cells and the hairs were identified on the labellum of each species and it is emphasized that both visual and tactile properties could be effective in pollinator selection due to these structures (Lumaga et al., 2012). Recent research on the genus *Bulbophyllum* revealed that epidermal features were unusual unicellular trichomes having an osmophore function and also emphasized the importance of data with respect to species distinction (Nunes et al., 2015).

The importance of micromorphological features for the taxonomic consideration of orchids has been investigated by several researchers (Lumaga et al., 2006; Verma et al., 2014). Aybeke (2012) examined anther wall development in *Ophrys mammosa* and emphasized that this finding was important in regard to taxonomical factors. However, the labellum micromorphology of many species in the present study has not been studied previously. The labellum properties have been determined for the first time.

The main objectives of the current study were to (1) determine the micromorphological features of the labellum that are more diagnostic for species differentiation on orchids distributed in Turkey and to (2) characterize the diversity of labellum micromorphology in orchids from Turkey and its association with different pollination strategies or their taxonomical status. For this purpose, we investigated the floral micromorphological characteristics of 14 nectariferous and nectarless species belonging to photosynthetic and saprophytic taxa that have several floral traits and different ecological requirements (*Anacamptis pyramidalis* (L.) Rich., *Cephalanthera kotschyana* Renz & Taubenheim, *Coeloglossum viride* (L.) Hartman, *Dactylorhiza saccifera* (Brongn) Soo' subsp. *saccifera*, *Gymnadenia conopsea* (L.) R.Br., *Himantoglossum caprinum* (M.Bieb.) Spreng., *Limodorum abortivum* (L.)

Sw., *Neottia nidus-avis* (L.) Rich., *Ophrys oestrifera* M.Bieb. subsp. *oestrifera*, *Orchis papilionacea* L. subsp. *papilionacea*, *Platanthera chlorantha* (Cruster) Rchb., *Serapias bergonii* E.G.Camus, *Spiranthes spiralis* (L.) Chevall, and *Steveniella satyrioides* (Spreng.) Schltr.) in the Black Sea region.

## 2. Materials and methods

Specimens were collected from different localities in the Central and Eastern Black Sea regions between March and August coinciding with the flowering period of plants in 2012 and 2015. The list of localities of the specimens is given in Table 1. Ten flowers of each specimen after anthesis collected from different localities per taxon were examined. All species were identified according to *Flora of Turkey and the East Aegean Islands* and *Türkiye Bitkileri Listesi (Damarlı Bitkiler)* (Davis, 1965; Güler and Deniz, 2012). Some of the samples were dried as herbarium material. The labellum and spurs of flowers in individuals of each genus were dissected and were maintained in 70% ethanol. Samples were passed through ethanol series for light microscopy. With the aid of a Zeiss polarized light microscope, inclusions such as starch or calcium oxalate crystals, trichome, papillary structures, and epidermal cell shapes were examined and photographed with an Axiocam camera. SEM analysis was performed on herbarium specimens. The entire labellum and spur structures were used, avoiding any deformation as far as possible. All dissected lobes and basal parts of the labellum and spurs were placed on stubs and were coated with 15-nm-thick gold–palladium using a Quorum Technologies SC7620 Mini Sputter Coater and visualized with a JEOL JSM-7001F field emission scanning electron microscope in a current of 10 kV. Epidermal cell shape, papillae, trichomes of the internal spur surface, and labellum adaxial surface were examined. A character matrix was created and grouping of taxa was carried out utilizing the clustering analysis method (UPGMA) based on morphological and micromorphological characters of the labellum and spur (Table 2).

## 3. Results

Labellum surfaces were examined for features such as epidermal cell shapes, striation of cuticle, the presence of secretory structures like papillae and hairs originating from the epidermis, and inclusions such as starch or calcium oxalate crystals. The micromorphological features of the labellum were comparatively described and it was determined that these characteristics could be helpful for differentiation of selected species. Investigations were carried out only on the labellum surface in species such as *Cephalanthera kotschyana*, *Neottia nidus-avis*, *Ophrys oestrifera* subsp. *oestrifera*, *Serapias bergonii*, and *Spiranthes spiralis* having no spur. Both spur and labellum

**Table 1.** List of investigated taxa and their localities.

Taxa	Locality	Date
<i>Anacamptis pyramidalis</i> (L.) Rich.	Samsun, Kurupelit	June 2015
<i>Anacamptis pyramidalis</i>	Samsun, Avdan, Canik	June 2013
<i>Anacamptis pyramidalis</i>	Sinop, Ayancık	June 2013
<i>Anacamptis pyramidalis</i>	Trabzon, Çaykara	May 2012
<i>Cephalanthera kotschyana</i> Renz & Taubenheim	Samsun, Çakallı	May 2015
<i>Cephalanthera kotschyana</i>	Samsun, Kavak	April 2013
<i>Cephalanthera kotschyana</i>	Ordu, Gököy	May 2012
<i>Coeloglossum viride</i> (L.) Hartman	Giresun, Kulakkaya	June 2014
<i>Coeloglossum viride</i>	Trabzon, Köprübaşı	July 2012
<i>Coeloglossum viride</i>	Ordu, Çambaşı	June 2012
<i>Dactylorhiza saccifera</i> (Brongn.) Soó	Rize, Ayder	June 2015
<i>Dactylorhiza saccifera</i>	Trabzon, Köprübaşı	June 2014
<i>Dactylorhiza saccifera</i>	Bolu, Abant	July 2012
<i>Gymnadenia conopsea</i> (L.) R.Br.	Trabzon, Köprübaşı	July 2015
<i>Gymnadenia conopsea</i>	Ordu, Çambaşı	July 2013
<i>Himantoglossum caprinum</i> (M.Bieb.) Spreng.	Sinop, Bayabat	June 2015
<i>Himantoglossum caprinum</i>	Samsun, Kurupelit	June 2015
<i>Limodorum abortivum</i> (L.) Sw.	Samsun, Kurupelit	June 2015
<i>Limodorum abortivum</i>	Samsun, Kavak	June 2013
<i>Limodorum abortivum</i>	Sinop, Ayancık	June 2012
<i>Neottia nidus-avis</i> (L.) Rich.	Bolu, Abant	June 2013
<i>Neottia nidus-avis</i>	Ordu, Mesudiye	May 2012
<i>Neottia nidus-avis</i>	Samsun, Kavak	May 2012
<i>Ophrys oestrifera</i> M. Bieb. subsp. <i>oestrifera</i>	Samsun, Kurupelit	April 2015
<i>Ophrys oestrifera</i> subsp. <i>oestrifera</i>	Samsun, Kavak	May 2015
<i>Ophrys oestrifera</i> subsp. <i>oestrifera</i>	Ordu, Ulubey	May 2015
<i>Orchis papilionaceae</i> L.	Samsun, Avdan, Canik	May 2015
<i>Orchis papilionaceae</i>	Samsun, Kurupelit	May 2015
<i>Platanthera chlorantha</i> (Cruster) Rchb.	Samsun, Kurupelit	June 2015
<i>Platanthera chlorantha</i>	Samsun, Kavak	June 2014
<i>Platanthera chlorantha</i>	Bolu, Mengen	July 2013
<i>Platanthera chlorantha</i>	Giresun, Kulakkaya	June 2012
<i>Serapias bergonii</i> E.G.Camus	Samsun, Kurupelit	May 2015
<i>Serapias bergonii</i>	Sinop, Ayancık	May 2013
<i>Spiranthes spiralis</i> (L.) Chevall	Samsun, Kurupelit	September 2015
<i>Spiranthes spiralis</i>	Trabzon, Köprübaşı	September 2015
<i>Stevniella satyrioides</i> (Spreng.) Schltr.	Samsun, Kurupelit	May 2015
<i>Stevniella satyrioides</i>	Ordu, Ünye	May 2015
<i>Stevniella satyrioides</i>	Samsun, Bafra	May 2014
<i>Stevniella satyrioides</i>	Bolu, Abant	June 2012

**Table 2.** Character list based on morphological and micromorphological characters of labellum and spur for cluster analysis (UPGMA).

Characters	States			
Labellum shape	Entire (0)	Lobed (1)	Epichile-hypochile (2)	Characteristic (3)
Labellum epidermal cell shape	Isodiametric oval, rectangular or polygonal (0)	Elongated, oval, rectangular, or polygonal (1)	Amorphous cell with wavy edge (2)	
Labellum epidermal cell shape difference	Absent (0)	Present (1)		
Surface structure	Absent (0)	Papillose (1)	Papillae (2)	Hairs (3)
Labellum hair/papillae distribution	Absent (0)	Tips and lobes (1)	Base (2)	All surface (3)
Labellum surface striation	Absent (0)	Striate (1)	Vermiform (2)	Reticulate (3)
Labellum crystal morphology	Absent (0)	Raphide cluster (1)	Crystal sand (2)	Prismatic crystal (3)
Labellum crystal distribution	Absent (0)	Median axis or middle lobe (1)	Base or lobes (2)	All surface (3)
Spur	Absent (0)	Present (1)		
Spur morphology	Absent (0)	Long (1)	Tapered (2)	Saccat (3)
Spur epidermal cell	Absent (0)	Isodiametric oval, rectangular or polygonal (1)	Elongated oval, rectangular or polygonal (2)	
Spur epidermal cell difference	Absent (0)	Present (1)		
Spur surface structure	Absent (0)	Papillose (1)	Papillae (2)	
Spur hair/papillae distribution	Absent (0)	Around midrib (1)	All surface (2)	
Spur surface striation	Absent (0)	Striate (1)	Vermiform (2)	Reticulate (3)
Spur crystal morphology	Absent (0)	Raphide cluster (1)	Crystal sand (2)	Prismatic crystal (3)
Spur crystal distribution	Absent (0)	Base (1)	All surface (2)	
Spur starch	Absent (0)	Present (1)		
				Bisacat (4)
				Spherical crystal (4)

surface were divided into three main areas including the base, central area, and margin or lobes in other species with a spur. Detailed micromorphological descriptions of the labellum for the 14 orchid genera are presented for each species in Tables 3 and 4.

Some species have a different shaped labellum such as deeply parted and having two or three lobes in *Anacamptis pyramidalis*, *Coeloglossum viride*, *Dactylorhiza saccifera* subsp. *saccifera*, *Gymnadenia conopsea*, *Neottia nidus-avis*, and *Steniella satyrioides*. However, the labellum is divided into two parts called the epichile and hypochile in *Cephalanthera kotschyana*, *Limodorum abortivum*, and *Serapias bergonii*. The labellum has a morphologically distinct appearance similar to the female members of the pollinator. There are two lateral appendices adjacent to the margin resembling insect limbs. Moreover, a small addition is obvious on the distal part in *Ophrys oestrifera* subsp. *oestrifera*. The labellum is broadly unshattered with an undulate margin in *Orchis* and *Spiranthes*. *Platanthera chlorantha* has a characteristic structure with a short, ligulate labellum. The labellum morphologically is very characteristic. The labellum of *Himantoglossum caprinum* has wavy margins and distally two long, thin, often twisted appendices. In the labellum epidermal cells are elongated and oval, rectangular, or polygonal, but they are usually isodiametric polygonal in others. Epidermal cells on the labellum surface of *Anacamptis pyramidalis*, *Cephalanthera kotschyana*, *Himantoglossum caprinum*, and *Limodorum abortivum* are different shapes. For example, the margins have differentiated epidermal cells with irregular shapes and undulate walls (Figure 1a), while in the central part of the hypochile they are elongated, rectangular, or polygonal (Figure 1b); on the other hand, they are shortened, oval, or polygonal on the central epichile (Figure 1c). In almost all species the labellum is covered with different shaped epidermal structures such as conical papillae with expanded base, large, short, conical papillose structure, or papillose with spherical head (Figures 1d–1g). In addition, long, unicellular hairs are rare on *Himantoglossum caprinum* and are dense on *Ophrys oestrifera* subsp. *oestrifera* labellum (Figure 2a). In *Serapias bergonii* the labellum's surface is covered with long hairs concentrated in the center. Hairs also show typical dome-like protuberances on the surface (Figure 2b). Long glandular hairs are noteworthy on two lateral ridges at the base of the labellum of *Spiranthes spiralis*. All labellum surfaces are covered with cuticles showing different shapes from smooth to undulate (Figure 2c). Striation on the epidermal cell surface is deeply vermiform in *Neottia nidus-avis* (Figure 2d). Striation varies from densely reticulate to vermiform in *Steniella satyrioides* (Figure 2e). A great majority of the species have raphide clusters on different parts of the labellum (Figure 3a–3e).

Some of the species have different shaped spurs. They are long and tapered in *Anacamptis pyramidalis*, *Gymnadenia conopsea*, *Limodorum abortivum*, and *Platanthera chlorantha*. The spur is short and gently bisaccate or saccate in *Coeloglossum viride*, *Dactylorhiza saccifera* subsp. *saccifera*, *Himantoglossum caprinum*, *Orchis papilionacea* subsp. *papilionacea*, and *Steniella satyrioides*. Spur epidermal cells are different, being elongated, rectangular, polygonal, or isodiametric oval, polygonal along the species. Long, tapered, cylindrical papillae are intensive on the spur in many species (Figure 4a), but short, cylindrical secretory structures with bulbous tips are homogeneously dispersed on the spur in *Gymnadenia conopsea* (Figure 4b). Secretory cells and papillae are not observed on long spurs but spherical protubers are evident on epidermal cells in *Limodorum abortivum* (Figure 4c). Epidermal cell surfaces are smooth to slightly striate, striate, undulate, or reticulate (Figure 4d). Interestingly, slight striation is present on epidermal cells but the papillae surface is very smooth in *Platanthera chlorantha* (Figure 4e). Moreover, the crystal morphology differs among the species. In addition to the raphide clusters, styloid crystals, crystal sands, or prismatic crystals (Figure 4f) are evident. Characteristically shaped spherical crystals are present in *Himantoglossum caprinum* and *Steniella satyrioides*.

An unweighted pair group method with arithmetic mean (UPGMA) dissimilarity clustering dendrogram for orchid taxa according to the examined qualitative characters in Table 2 is given in Figure 5. Accordingly, branches in the dendrogram are divided into three main branches and subbranches. *Platanthera* composes the first main branch and differs clearly from other taxa; other taxa make up the second (including *A. pyramidalis*, *H. caprinum*, *L. abortivum*, *D. saccifera* subsp. *saccifera*, *G. conopsea*, and *O. papilionacea*) and third branches (including *C. viride*, *S. satyrioides*, *C. kotschyana*, *N. nidus-avis*, *O. oestrifera* subsp. *oestrifera*, *S. bergonii*, and *S. spiralis*).

#### 4. Discussion

In the present study, 14 orchid genera having important features with respect to pollination strategies were investigated and compared in terms of labellum structures and micromorphologies. Nectar production generally occurs within the spur, as in *Platanthera chlorantha* and *Gymnadenia conopsea*, but other flower parts such as petals and bracts can also produce nectar, as in *Platanthera stricta* and *Disa elegans* (Patt et al., 1989; Hobbhahn et al., 2013). Secretory structures such as surface papillae and smooth surface are the typical structures of nectar-producing plants. Among the species in this study, *Coeloglossum viride*, *Gymnadenia conopsea*, *Limodorum abortivum*, *Neottia nidus-avis*, *Platanthera chlorantha*, and *Spiranthes*

Table 3. Epidermal features on the labellum of species investigated in this study.

Taxa	Life form	Pollination strategies	Epidermal cells	Hairs or papillae	Hairs or papillae distribution	Striation	Mineral inclusion	Mineral inclusion distribution
<i>Anacamptis pyramidalis</i> (L.) Rich.	Photosynthetic	Food deceptive	Isodiametric polygonal on lobes Elongated rectangular-polygonal on base	Conical papillae with expanded base	Intensive on labellum tip	Slightly striated	Raphide cluster	Middle lobe
<i>Cephalanthera kotschyana</i> Renz & Taubenheim	Photosynthetic	Floral mimesis	Amorphous cell with wavy edge on labellum periphery Elongated, rectangular or polygonal on hypochile center Oval-polygonal on epichile tip	Papillose	Epichile tip	Absent	Raphide cluster	Hypochile base Epichile tip
<i>Coeloglossum viride</i> (L.) Hartman	Photosynthetic	Nectar rewarding	Elongated oval, polygonal or rectangular	Absent or papillose	All surface	Smooth to undulate	Raphide cluster	Dense along the median axis of the labellum
<i>Dactylorhiza saccifera</i> (Brongn) Soo' subsp. <i>saccifera</i>	Photosynthetic	Food deceptive	Short rectangular to polygonal	Conical papillae with expanded base	All surface	Striate	Raphide cluster Sand crystals	All surface and dense on the middle lobe
<i>Gymnadenia conopsea</i> (L.) R.Br.	Photosynthetic	Nectar rewarding	Short polygonal	Large, short, conical papillose structure	All surface	Striate	Raphide cluster	Dense on all surface
<i>Himantoglossum caprinum</i> (M.Bieb.) Spreng.	Photosynthetic	Food deceptive	Elongated rectangular or polygonal on appendix Isodiametric on base	Elongated, unicellular hairs Papillae	Middle part and base Margin	Striate	Raphide cluster Sand crystals Spherical crystal	All surface
<i>Limodorum abortivum</i> (L.) Sw.	Saprophytic	Nectar rewarding	Elongated rectangular on hypochile Short, polygonal on epichile	Conical papillae with expanded base	Hypochile base and epichile	Striate	Raphide cluster	Rare on all surface
<i>Neottia nidus-avis</i> (L.) Rich.	Parasitic	Nectar rewarding	Short oval polygonal	Conical papillose with expanded base Papillose with spherical head and secretory hairs	Margin and lobes of labellum Base and middle part	Deeply vermiform	Raphide cluster	Dense on the base Rare on the lobes
<i>Ophrys oestrifera</i> M.Bieb. subsp. <i>oestrifera</i>	Photosynthetic	Sexual deceptive	Short polygonal	Long cylindrical hairs Narrow, conical papillae	Appendix Decrease from margin to the middle	Striate Undulate-reticulate	Raphide cluster Sand crystals Prismatic crystals	All surface Appendix Densely all surface

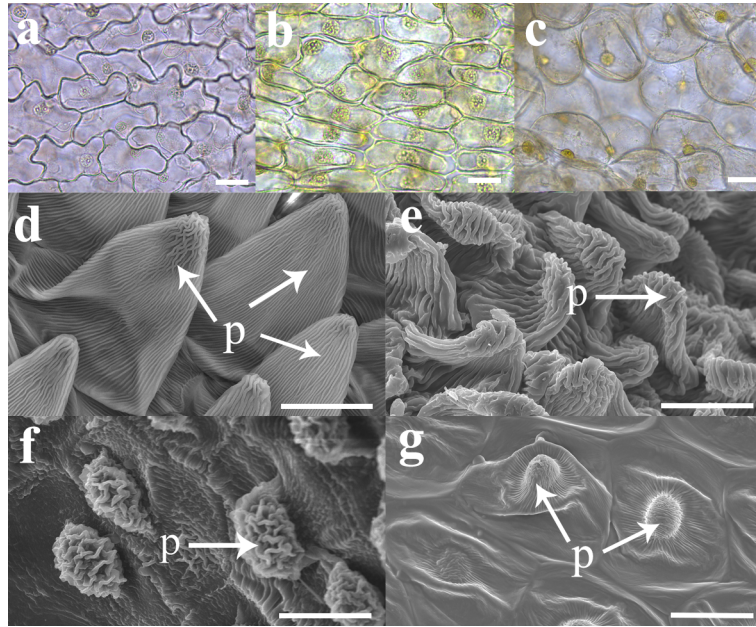
Table 3. (Continued).

	Photosynthetic	Food deceptive	Short oval polygonal	Middle-sized, conical papillae with expanded base	All surface	Deeply striate	Raphide cluster	Densely on the middle lobes and axis
<i>Orchis papilionacea</i> L. subsp. <i>papilionacea</i>			Short oval polygonal		All surface	Deeply undulate on cell borders and reticulate on cell surface		
<i>Platanthera chlorantha</i> (Cruster) Rchb.	Photosynthetic	Nectar rewarding	Long polygonal	Absent	-		Absent	-
<i>Serapias bergonii</i> E.G.Camus	Photosynthetic	Resting place	Isodiametric polygonal	Long, cylindrical hairs Long, conical papillae Papillose with spherical head	Base part Rare on all surface Dense on all surface	Striate	Raphide cluster	Densely all surface
<i>Spiranthes spiralis</i> (L.) Chevall	Photosynthetic	Nectar rewarding	Isodiametric polygonal	Glandular hairs Long, cylindrical hairs	Ridge on the base part Ridge on the base part and labellum tip	Slightly striate on hairs and smooth on other surface	Raphide cluster	Densely on all surface
<i>Stevintella satyrioides</i> (Spreng.) Schltr.	Photosynthetic	False-prey syndrome	Isodiametric polygonal	Papillose with expanded base	All surface except the base	Densely reticulate-vermiform	Raphide cluster	Middle labellum and intensify to the base

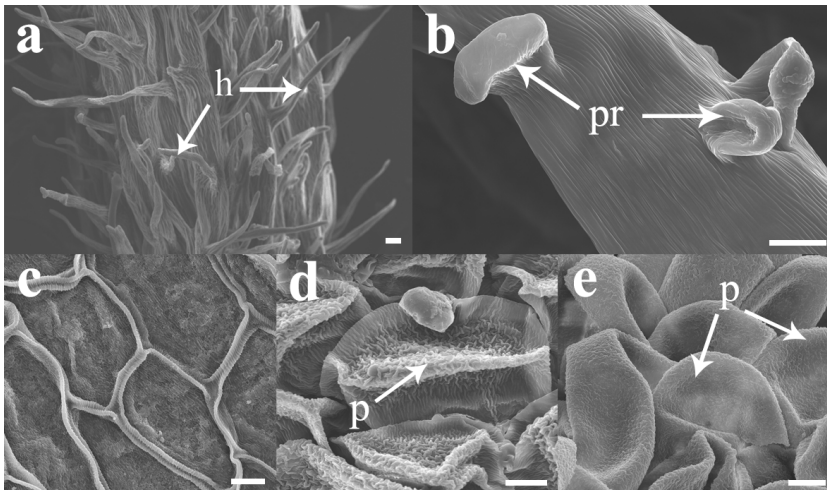
**Table 4.** Epidermal features of spurs belonging to species of genera with spur in this study.

Taxa	Epidermal cells	Hairs or papillae	Hairs or papillae distribution	Striation	Mineral inclusion	Mineral inclusion distribution
<i>Anacamptis pyramidalis</i> (L.) Rich.	Isodiametric polygonal	Absent	Absent	Striate	Styloid	Rare
<i>Coeloglossum viride</i> (L.) Hartman	Long rectangular polygonal	Absent	Absent	Reticulate	Absent	Absent
<i>Dactylorhiza saccifera</i> (Brongn) Soo' subsp. <i>saccifera</i>	Long rectangular polygonal	Long cylindrical papillae	All surface	Striate to undulate	Styloid, crystal sand	Rarely all surface
<i>Gymnadenia conopsea</i> (L.) R.Br.	Long rectangular polygonal on tip Isodiametric polygonal on base	Short, cylindrical secretory structures with bulbous tips	All surface	Smooth to slightly striate	Raphide cluster	Spur base
<i>Himantoglossum caprinum</i> (M.Bieb.) Spreng.	Shortly polygonal	Long, tapered, cylindrical papillae	Densely all surface	Striate	Styloid Spherical crystals	Moderately all surface
<i>Limodorum abortivum</i> (L.) Sw.	Square to polygonal	Slightly papillose with spherical head	All surface	Reticulate	Prismatic crystals Crystal sand	Densely all surface
<i>Orcis papilionacea</i> L. subsp. <i>papilionacea</i>	Long polygonal on tips isodiametric oval on base	Absent	Absent	Undulate	Raphide cluster	Rarely middle axis
<i>Platanthera chlorantha</i> (Cruiser) Rchb.	Long rectangular	Long and narrow, cylindrical papillae	Dense around the midrib	Slightly striate on epidermal cells Absent on papillae	Single raphides	Spur base
<i>Steveniella satyrioides</i> (Spreng.) Schltr.	Distinctly isodiametric polygonal	Absent	Absent	Undulate	Raphide cluster Spherical crystals	Densely all surface

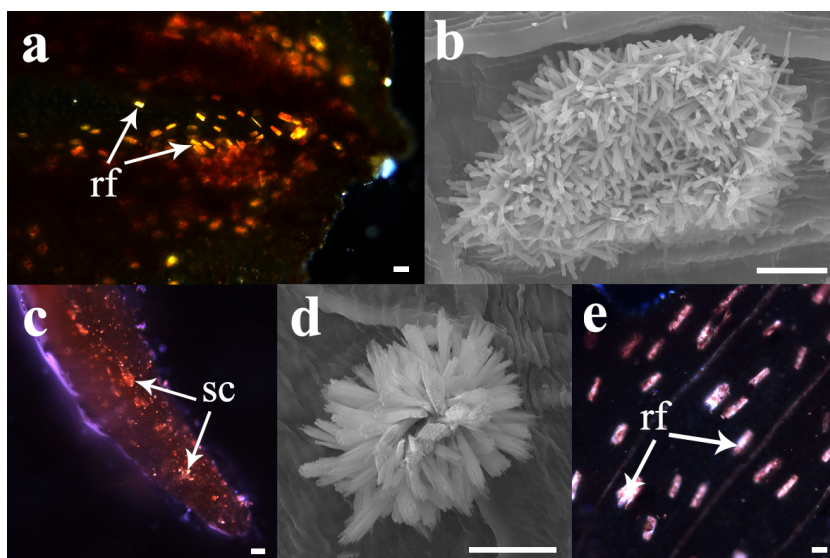




**Figure 1.** a–c, Light microscope image of epidermal cells with different shapes on margin, middle part of hypochile, and middle part of epichile in *Cephalanthera kotschyana*, respectively. d, SEM image of conical papillae (arrows, p) with expanded base and linear striation on the labellum of *Dactylorhiza saccifera* subsp. *saccifera*. e, SEM details of papillae (arrow, p) with linear striation were concentrated on the hypochile of *Limodorum abortivum*. f, SEM image of labellum surface showing short papillose (arrow, p) with expanded base on lobes of *Neottia nidus-avis*. g, SEM image of papillose structures (arrows, p) with spherical head on epichile of *Serapias bergonii*. Scale bars, 20  $\mu\text{m}$ .



**Figure 2.** a, SEM image of lateral appendix having quite long, cylindrical hairs of *Ophrys oestrifera* subsp. *oestrifera* (arrow, h). b, Details of middle portion of labellum having long hairs with characteristic dome-like protuberances (arrows, pr) on the surface of *Serapias bergonii*. c, Photomicrograph of *Coeloglossum viride* labellum surface showing vermiform striation. d, SEM image of *Neottia nidus-avis* labellum surface showing short papillae (arrow, p) with expanded base and vermiform striation on lobes. e, SEM image of *Steveniella satyrioides* labellum having papillose structure (arrows, p) with reticulate striation on distal part. Scale bars, 10  $\mu\text{m}$ .



**Figure 3.** a, Polarized light microscope image of labellum tip having many raphide clusters (arrows, rf) of *Spiranthes spiralis*. b, SEM details of spherical crystals with unique shape on the labellum of *Himantoglossum caprinum*. c, Polarized light microscope image of lateral addition including the crystal sands (arrows, sc) of *Ophrys oestrifera* subsp. *oestrifera*. d, Photomicrograph of uniquely shaped spherical crystals on the labellum and spur entry of *Steveniella satyrioides*. e, Polarized light microscope image of labellum including raphide clusters (arrows, rf) homogenously distributed around vessels on *Steveniella satyrioides*. Scale bars, 10  $\mu$ m.

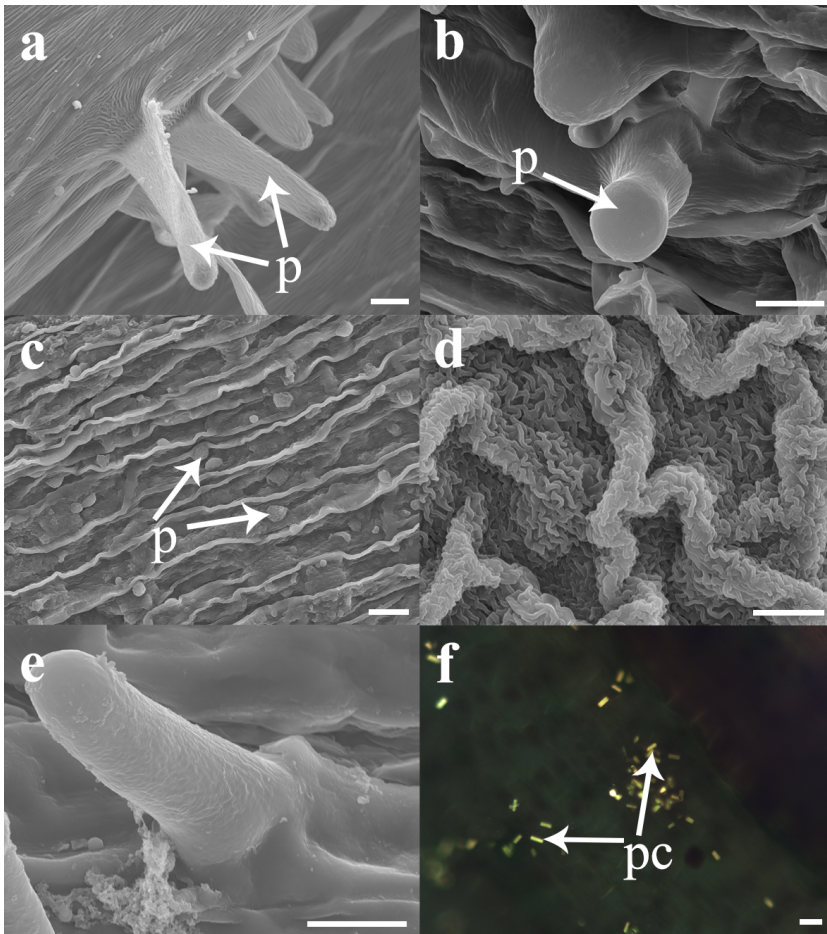
*spiralis* attract the pollinators through nectar reward (Godfrey, 1922; Nilsson, 1978; Willems and Lahtinen, 1997; Neiland and Wilcock, 1998; Willems and Melsner, 1998; Stpiczyńska, 2003; Stpiczyńska et al., 2005). In the present study, on the long spur of *Platanthera chlorantha* papillae are noteworthy around vessels. Intense secretory structures were present on the spur of *Gymnadenia conopsea* and in both species there were no or very few striations. However, we observed that the labellum and spur of *Limodorum* and *Coeloglossum* did not have the characteristics of nectar-secreting structures.

*Anacamptis pyramidalis*, *Cephalanthera kotschyana*, *Dactylorhiza saccifera* subsp. *saccifera*, *Himantoglossum caprinum*, *Orchis papilionacea* subsp. *papilionacea*, and *Steveniella satyrioides* are food deceptive species that attract the pollinator by releasing some cues like odor (Vogel, 1972; Dafni and Ivri, 1981; Dafni, 1987; Nazarov, 1995; Fritz and Nilsson, 1996; Proctor et al., 1996; Neiland and Wilcock, 1998; Cozzolino and Widmer, 2005; Kowalkowska et al., 2012). Scent in the flower is released from structures called osmophores. In many studies, osmophore structures were examined with light microscopy and in both SEM and TEM (Stern et al., 1987; Curry et al., 1991; Stpiczyńska 1993, 2001). Osmophores may be located on the adaxial surface of sepals, petals, or parts of the lip. Such surfaces are covered by papillae or papillose cells with smooth cuticles. Secretion residues in

the epidermal cell surface are evident (Anton et al., 2012). The papillae or secretory structures and striation were not observed on the spur but on the labellum surface the papillae and deep striations were intense in *Anacamptis pyramidalis*, *Orchis papilionacea* subsp. *papilionacea*, and *Steveniella satyrioides*. While the striate and conical papillae were densely present in the *Himantoglossum caprinum* spur, they were long and cylindrical with smooth surfaces in the *Dactylorhiza saccifera* subsp. *saccifera* spur. On the labellum surface, striate and conical papillae with expanded bases were present in both species. In the spurless species *Cephalanthera kotschyana*, striation is very little and secretory structure is indistinct papillose. Whether these structures in the labellum of this species have an osmophore function should be determined by detailed examination of the cell content, which could be identified by TEM.

Epidermal cells shapes were different both among the species and between different parts of the labellum and spur of the species. Interestingly, there were amorphous cells with wavy edges on the labellum periphery but elongated, rectangular, or polygonal cells on the hypochile center and oval-polygonal cells on the epichile center in *Cephalanthera kotschyana*.

Calcium oxalate (CaOx) crystal scattering on the middle lobe or basal part of the labellum surface has drawn attention in many species and it was intense on the



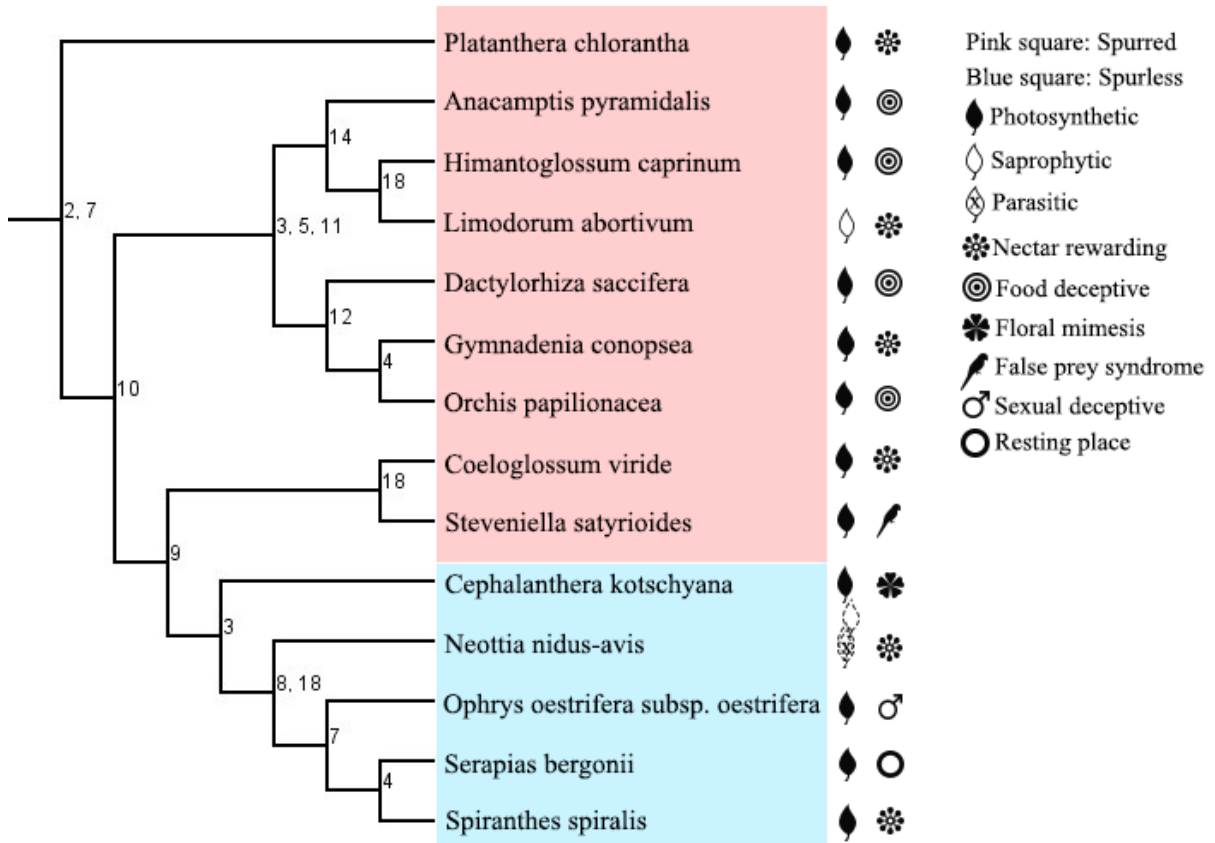
**Figure 4.** a, SEM image of narrow cylindrical papillae (arrows, p) with linear striation on the spur of *Dactylorhiza saccifera* subsp. *saccifera*. b, SEM image of *Gymnadenia conopsea* spur showing cylindrical, secretory structures with bulbous tips (arrow, p). c, Photomicrograph of *Limodorum abortivum* epidermal cell with spherical protubers (arrows, p) and reticulate striation. d, Photomicrograph of spur surfaces showing reticulate striation in *Coeloglossum viride*. e, Photomicrograph of *Platanthera clorantha* spur surface having narrow, cylindrical papillae around the midrib with no striation. f, Polarized light microscope image of *Limodorum abortivum* spur including prismatic crystal (arrows, pc). Scale bars, 10  $\mu$ m.

spur with typical morphologies in some species. Raphide clusters on the labellum or spur surface were observed in almost all species. The raphide and styloid crystals are considered to be particularly effective as a defense against herbivores in monocots (Molano-Flores, 2001). The nutritional, visual, or olfactory cues also cause attraction to unwanted guests. Therefore, the crystal inclusions on the spur and labellum that the nectar and odors release may prevent unwanted guests from eating the flower parts.

In *Serapias bergonii*, unusual trichomes with dome-like protuberances and striate surface were observed on the medial region of the labellum in addition to typical trichomes on its lateral region. In the literature, it has been reported that *Serapias* species do not use specific

strategies for attracting pollinators and pollinators prefer the labellum for their resting place (Neiland and Wilcock, 1998). However, recent research on floral micromorphology has shown that some *Serapias* species have sympatric distribution possessing different structures and secretory epidermal cells and typical trichomes present on the labellum (Lumaga et al., 2012). In addition to the nontypical secretory cells with intense striation, trichomes with bilobular or dome-like protuberances are mentioned in *Serapias vomeracea*. Our results differed from those of previous reports by Neiland and Wilcock (1998) while they confirm the findings published by Lumaga et al. (2012) with respect to pollination strategies, because these unusual trichomes also may secrete some compounds and





**Figure 5.** UPGMA clustering of some orchid taxa in Turkey based on morphological and micromorphological characters of the labellum and spur. The numbers on nodes show the characters separating the taxa in Table 2. The symbols on the right of the species names show the life form and the pollination strategies.

have an osmophore function. Thus *Serapias* species also have deceptive pollination strategies.

It has been observed that there are dense long trichomes with striations on the lateral appendix and short papillae with intense striation that may have a secretory function on all surfaces of the labellum in *O. oestriifera* subsp. *oestriifera*. Among the species in this study, the sexual deceptive *Ophrys oestriifera* subsp. *oestriifera* has the most specialized pollination strategies. *Ophrys* individuals attract the pollinator with sexual deception (Darwin, 1888). The labellum visually mimics the female pollinator. It is also known that the scent released from flowers mimics the female pheromones (Streinzner et al., 2009). Studies conducted on *Ophrys speculum* have reported that the cuticle striation on the speculum surface and the pigment in cells together contribute to the formation of iridescence (Vignolini et al., 2012). These results confirm the findings in the literature and *O. oestriifera* subsp. *oestriifera*'s labellum visually imitates the female's limb thanks to dense long trichomes and other micromorphological features.

In addition to the classical taxonomic approach, many molecular phylogenetic studies have been performed on the genera of the subtribe Orchidinae using different marker genes, but the results were not in all cases unequivocal. There are many studies where the floral structure is important in terms of speciation and phylogenetics (Curry et al., 1991; Cozzolino and Widmer 2005; Bateman and Rudall, 2006). According to the feature in Table 2, UPGMA of cluster analysis was used to evaluate the morphological and micromorphological features of the labellum and spur among the analyzed taxa. Accordingly, many micromorphological characteristics such as labellum surface striation, shape and distribution of epidermal structures such as papillae or hairs on the labellum and spur, labellum crystal type, spur morphology, epidermal cell shape on the spur, and absence and presence of starch could be systematically important among the genera. Generally, the branches containing nearly related taxa are compatible with the traditional taxonomic rank of orchid genus in the *Flora of Turkey and*

the East Aegean Islands (Davis, 1965). According to the UPGMA phenogram, the micromorphological features of epidermal cells in the species studied are not relevant to either the ecological requirements or the nectar production of these species, because nectariferous and nonnectariferous species are located in close branches of the phenogram. In addition, the saprophytic and parasitic species *Limodorum abortivum* and *Neottia nidus-avis* are distantly located on a different clade. Interestingly, however, the species having the spur are located as close to each other in adjacent branches. This result indicates that the micromorphological features in these species are relevant with presence of the spur whether it produces nectar or not.

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- Consequently, it was determined that there are significant differences among species in terms of labellum micromorphology in this research. The relationship between these differences and the pollination strategy of the species is thought to be important. These results are important in preparing further research to design experiments for functional characterization of anatomy and micromorphology, which affect pollination strategies and speciation in orchids.
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