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Morphological, anatomical and ecological studies on *Orchis simia* (Orchidaceae) taxon of Eskişehir, Turkey

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Abstract: In this study, in the present study reveals the morphological, anatomical and ecological characteristic of *Orchis simia* Lam. in Turkey. Plant materials of Orchis L. species were collected from one population, between 2018 in Eskişehir/Turkey. *Orchis simia* Lam. samples were analyzed for 5 morphological, 6 anatomical and soil characters and habitat properties. In morphological investigations, the structure of flower, lateral sepal, petal, dorsal sepal, lip, anther cap and column was determined. The findings were compared with those in Flora of Turkey. to habitat definition, *Orchis simia* grew up to 800 m to 1100 m. Also, Quercus cerris forests and glades were most common habitat of *Orchis simia*, found generally over calcareous soils.

Keywords: Anatomy, Morphology, *Orchis simia*, Eskişehir, Turkey.

1. Introduction

The flora of Turkey is represented 1.220 genus and 11.707 species and sub-species which belong to 154 family (Ataşlar, 2018) . Turkey is one of the country that has the richest flora in the World with 11.707 species and sub-species taxa. The family Orchidaceae comprises approximately 19,500 species distributed all around the world. Orchidaceae is the most famous and attractive plant family among all plant families of the world (Arditti, 1992; Pridgeon, 1992; Zarinkamar, 2006). Turkish orchidaceae were introduced in the volume 8 and 11 of Flora of Turkey. In Turkey, Orchidaceae is represented by 26 genus and about 166 species, of which 60 are endemic (Davis et al. 1978; Güner et al. 2000; Akman et al. 2014; Ataşlar, 2018; Dreesler, 1993; Güler, 2016). Terrestrial orchids have creeping, much reduced, fibrous or fleshy rhizomes or tuber like roots. Orchis L. taxa are terrestrial orchids and diagnosed by a basal rosette and terminal, unbranched infloresence that is composed of small to moderately large resupinate flowers. This genus belonged to the Orchidinae tribe of which 30 species demonstrate their main distribution in the Northern regions of Europe and Asia (Renz and Taubenheim, 1984; Dressler, 1993; Kreutz, 2000; Kreutz, 2009; Bateman, 1987-1989; Fay, 2013). Most cultivars are tropical or sub-tropical. This species is known as salep in Turkey likes many other members of Orchidaceae (Baytop, 1997; Tuzlaci, 2006).

Orchid is erect perennials with globose to ellipsoid undivided tubers. Leaves unspotted or spotted, ±arranged near base. Emerging spike by spathe-like leaves, many flowered, ±cylindrical. Flowers in various shades of red, purple, and yellow, rarely white. Bracts membranous. Lateral sepals spreading to reflexed or all sepals connivent with petals, forming a hood. Labellum ±directed down-wards, entire or 3-lobed, with entire or ±divided middle lobe, glabrous or ±papillose above, with saccate to filiform spur. Anther firmly attached to short, erect column, folded median part of rostellum placed between the parallel anther cells (loculi). Pollinia 2, clavate, narrowed below to caudicles, attached to separate viscidia, which are anclosed in a single pouch (bursicula). Ovary cylindrical, sessile, twisted, glabrous (Davis et al. 1965-1988).

Ecology, morphology and anatomy of several Turkish Orchid species have been studied earlier (Durmuskahya, 2013; Durmuskahya et al., 2014; Aybeke et al., 2010). Turkey has a rich biodiversity and has got more than 170 taxa (Kreutz, 2009). In high of the above, the aim of the present study was to investigate new ecological, morphological and anatomical information about another orchis taxa and to provide base knowledge for further studies.

The aim of the present study is to describe the morphological and anatomical structure and in addition to investigate the epidermal cell and stomata of leaves of *Orchis simia.*

2. Materials and Method

Orchis simia was collected from Eskişehir: Sarıcakaya-Hekimdağ, below Quercus trees, 1000 m., 19.05.2018 and stored in the Eskişehir Osmangazi University Herbarium as a herbarium specimen (OUFE 12509). Determination of the species was made according to the Davis et al. 1965-1988. Morphological descriptions are based on living plants and herbarium specimens.

Anatomical investigations were applied on the leaves of samples fixed in 70% alcohol. The transverse sections discarded from mid-vessels of leaves were stained with safranin and upper and lower surface layers were investigated in the media with 20% gliserin (Altundag et al., 2012; Sevgi et al., 2012). The well-staining sections were photographed on Leica DFC 295 color camera type, Leica DM 2500 light microscope. In this study, total 5 plant samples were collected for anatomical studies.

The surface layer of soil was removed and soil samples taken from 0-5 and 5-15 cm depth were analysed. The samples were air dried, ground, passed through a 2 mm sieve and subjected to physico-chemical analysis. Total soluble salts, pH, calcium carbonate content and texture were determined by the methods outlined in detail by Hartikainen H, Kerko E. (2009).

3. Results

Plant 15-45 cm. Basal leaves usually broadly lanceolate and unspotted. Spike ovoid to conical, withnflowers opening from top downwards. Flowers whitish pink, with an upswept hood; lip conspicuous and in shape of a man with body.

Fig. 1 *Orchis simia* habitat view

Orchis simia is labellum 15-20 mm, centre whitish-rose, deeply segmented near base into linear lateral lobes, middle lobe ligulate, divided into 2 linear lobules; all lobules darker purple towards apex. Spur cylindrical. Fl. 4-5. Grassy hillsides, Scrub, on calcerous soils, Quercus scrub, 900-1000 m. (P.H. Davis, 1965-1988) (Fig 1-2).

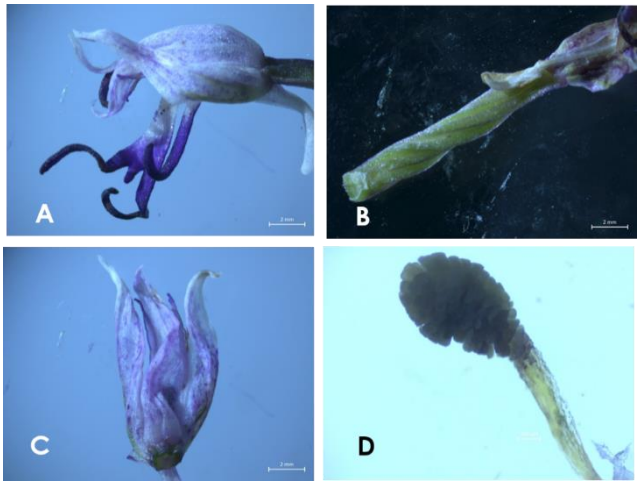

Fig. 2 *O. simia* morphology: A flower, B Spur, C lateral lobes, D lip, anther cap and column

Cross section of root showed single layered epidermal cells. The cell length was longer than width (30-60 × 45-100 µm). Cortex consisted of 7-12 layered parenchymatous cells. The shape of cortex cells ranged from oval to globular. These cells were 60-100 µm in diameter and their walls were thin. Parancymatic cells located far from center were smaller than the ones close to center (Fig. 1A). Similar results have been earlier reported by several authors in members of Orchidaceae family (Aykebe et al., 2010; Sevgi et al., 2012; Durmuskahya et al., 2014). Endodermal cells (35-50 × 45-100 µm) with thickened wall were seen. Pericycle was single-layered and located under endodermis. (Fig. 3, Tab. 1).

Table 1 Root anatomy measurement of *O. simia*

	Width (µm)		Length (µm)	
	Min.	**Max.**	**Min.**	**Max.**
Root				
Epidermis	35	50	55	80
Cortex parenchyma	60	100	100	160
Endodermis	30	45	50	70

The anatomical section of stem showed thick cuticle layer. Under this layer there was a single layered epidermis. Epidermal cells were square or rectangular shaped, 35-150 × 30- 50 µm. 8-16 layers of cortex cells were found under epidermis with 20-30 µm diameter. Few collenchyma cells with thick and lignified cell walls were found in cortex layer. 10-24 layered collenchyma cells gave durability to the stem. (Fig. 4). Vascular bundles were collateral and located in one ring. The bundle sheath consisted of sclerenchymatic cells, at the phloem pole of vascular bundles. Pith had many lacunas in the centre of stem due to the breakup of pith into pieces (Fig. 4, Tab. 2).

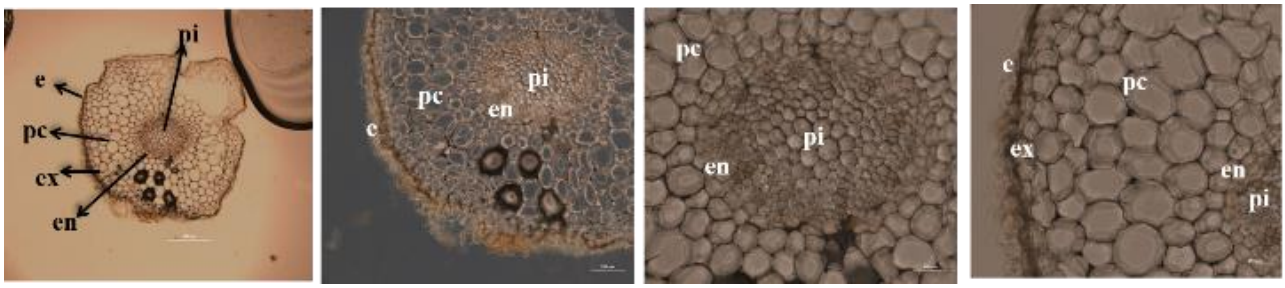

Fig. 3 Root transverse section of *O. simia* epidermis, ex exodermis, pc parenchymatic cells, en endodermis, pi pith (Bar 100 µm)

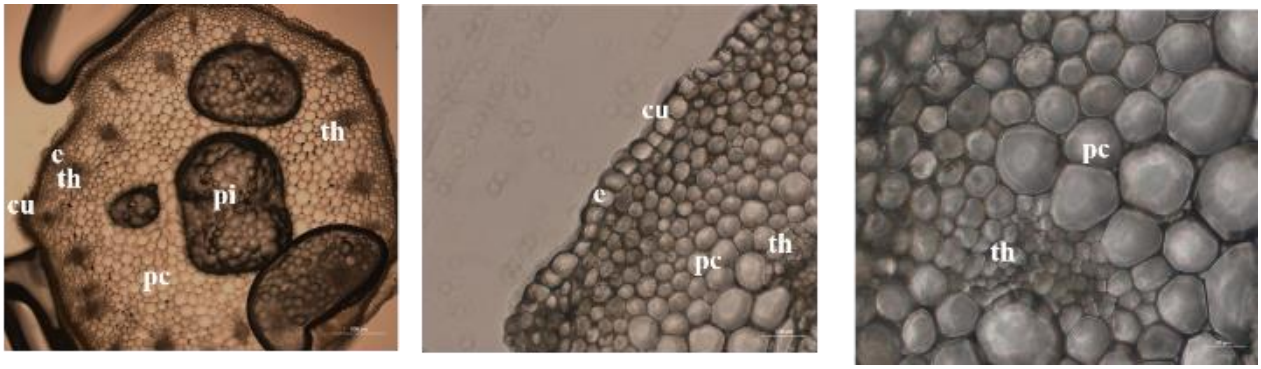

Fig. 4 Stem transverse section of *O. simia* cu cuticle, e epidermis, pc parenchymatic cells, th trachea, pi pith (Bar 100 µm)

Table 2 Stem anatomy measurement of *O. simia*

	Width (µm)		Length (µm)	
	Min.	**Max.**	**Min.**	**Max.**
Stem				
Epidermis	40	60	30	50
Cortex parenchyma	35	150	70	200
Trachea (diameter)	25	40	30	60

In leaves, adaxial epidermis consisted of a single layer of rectangular cells 25-80 × 30-60 µm with smooth cuticle. Abaxial epidermis was 55-80 × 35-50 µm. Adaxial epidermis cells were bigger than abaxial epidermis cells and adaxial cuticle thicker than adaxial. These features were observed in *Orchis laxiflora* Lam and *O. purpurea* Hudson by Aybeke et al. (2010). But Sevgi et al. (2012a) observed that these species had thicker cuticle on abaxial side and they had similar thickness on both adaxial and abaxial surface. Mesophyll layer was homogenous, without any differentiation in to palisade and sponge parenchyma tissues (Fig. 5, Tab. 3).

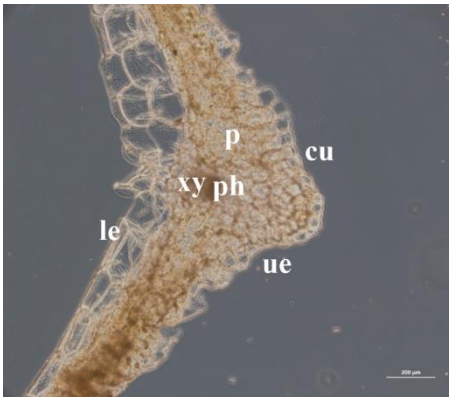

Fig. 5 Leaf transverse section of *O. simia* c cuticle, ue upper epidermis, p parenchyma, ph phloem, xy xylem, le lower epidermis (Bar 100 µm)

Leaves surface of investigated species was glabrous like many other orchid species. In leaves, adaxial epidermis consisted of a single layer of rectangular cells 50-110 × 80-85 µm with smooth cuticle. Abaxial epidermis was 45-100 × 55-155 µm. Adaxial epidermis cells were bigger than abaxial epidermis cells and adaxial cuticle thicker than adaxial. (Fig. 6, Tab. 4).

Table 3 Leaf anatomy measurement of *O. simia* (Cross-section)

	Width(µm)		Length (µm)	
	Min.	Max.	Min.	Max.
Leaves				
Adaxial epidermis	30	80	30	55
Mesophyll cells	25	55	30	40
Abaxial epidermis	25	50	35	60

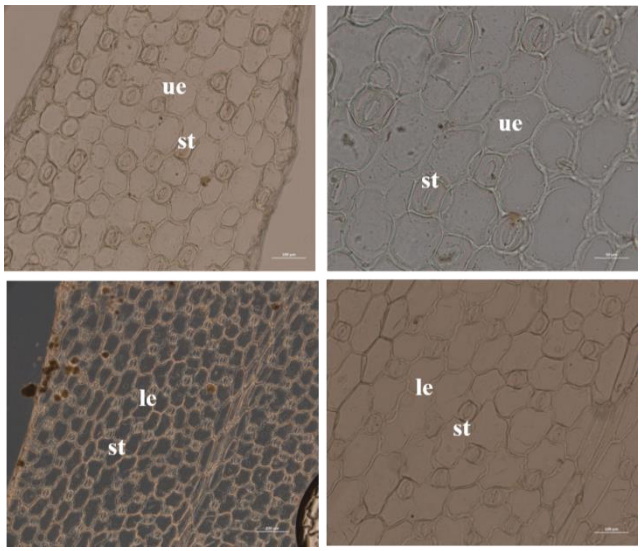

Fig. 6 Leaf epidermal peeling of *O. simia* ue upper epidermis, le lower epidermis, st stomata (Bar 100 µm)

Table 4 Leaf anatomy measurement of *O. simia* (surface)

	Width (µm)		Length (µm)	
	Min.	Max	Min.	Max.
Leaves				
Adaxial epidermis	50	150	160	355
Mesophyll cells	45	55	40	60
Abaxial epidermis	45	110	85	210

4. Discussion

Cross section of root showed single layered epidermal cells. The cell length was longer than width (35-50 × 55-80 µm). Cortex consisted of 8-12 layered parenchymatous cells. The shape of cortex cells ranged from oval to globular. These cells were 55-110 µm in diameter and their walls were thin. Parancymatic cells located far from center were smaller than the ones close to center (Fig. 2). Endodermal cells (30-45 × 50-70 µm) with thickened wall were seen but were not visible clearly. Pericycle was single-layered and located under endodermis. Xylem consisted of radially arranged 3 to 4 vessels, while pith was made up of parenchymatous cells. (Fig. 2).

The anatomical section of stem showed thick cuticle layer. Under this layer there was a single layered epidermis. Epidermal cells were square or rectangular shaped, 40-60 × 30- 50 µm. 7-14 layers of cortex cells were found under epidermis with 35-150 µm diameter. Few collenchyma cells with thick and lignified cell walls were found in cortex layer. Cortical parenchyma, a thinner layer than collenchyma was found with large amount of starch grains. Presence of collenchyma cells in *Ophrys* L. and *Dactylorhiza* Necker ex Nevski have been reported previously in other studies (Aybeke et al., 2010). Vascular bundles were collateral and located in one ring. The bundle sheath consisted of sclerenchymatic cells, at the phloem pole of vascular bundles. Pith had many lacunas in the centre of stem due to the breakup of pith into pieces (Fig. 3).Leaves surface of investigated species was glabrous like many other orchid species. In leaves, adaxial epidermis consisted of a single layer of rectangular cells (30-80 × 30-55 µm) with smooth cuticle. Abaxial epidermis was 25-50 × 35-60 µm. Adaxial epidermis cells were bigger than abaxial epidermis cells and adaxial cuticle thicker than adaxial. (Fig. 6).

Table 5 Leaf anatomical characters (surface)

Taxa	Epidermal cells size (width-length)	
	Abaxial (µm)	Adaxial (µm)
ita	80.00×180.00	126.50×210.50
pur	128.30 × 150.25	180.54 × 190.00
sim	90.04 × 182.30	110.40 × 282.65

ita = *O. italica*, pur = *O. purpurea*, sim = *O. simia*

It has been proven that the surface anatomy of the *O. simia* leaf has smaller cell sizes in the lower and upper epidermis surface compared to the *O. italica* and *O. purpurea* according to its systematic affinity (Tab. 5) (Sevgi at al. 2012b).

Table 6 Leaf anatomical characters (Cross-section)

Taxa	Epidermal cells size (width-length)	
	Abaxial (µm)	Adaxial (µm)
ita	60.00×130.00	109.50×180.20
pur	100.30× 120.40	184.66 × 192.00
sim	40.20 × 50.10	55.24 × 46.60

ita = *O. italica*, pur = *O. purpurea*, sim = O. simia

O. simia leaf anatomy is smaller in size than the lower epidermis cell and upper epidermis cell compared to *O. italica* and *O. purpurea* (Tab. 6) (Sevgi at al. 2012b).

Table 7 Distribution of *Orchis* species and their numbers on the basis of their altitude

Altitude	*ita*	*pur*	*sim*
0 - 200	+		+
200 - 400	+		+
400 - 600	+		+
600 - 800	+		+
800 - 1000	+		+
1000 - 1200			
1200 - 1400		+	+
1400 - 1600		+	

ita = O. italica, pur = O. purpurea, sim = O. simia

While *O. simia* spreads up to 1400 m. altitude, *O. italica* is determined to grow at altitudes between 0-1000 m and *O. purpurea* at heights between 1200-1600 m. (Tab. 7) (Sevgi at al. 2012b).

Table 8 Distribution of *Orchis* species according to their habitat

Habitat	***ita***	***pur***	***sim***
Olive farmland	+		
Macchie (Shrubland)	+		+
Meadows(Pasture)	+		
Forest land	+	+	+
Abandoned Agr.			

ita = *O. italica*, pur = *O. purpurea*, sim = O. simia

When we look at the distribution of Orchis species according to habitats, *O. simia* is found in maquis and forest areas, while *O. italica* grows in wet meadows, woodlands and maquis and olive fields, *O. purpurea* only grows in forest areas (Tab. 8) (Sevgi at al. 2012b).

5. Conclusion

In this study *Orchis simia* was investigated ecology, morphologically and anatomically. Morphological drawings of flower, lateral sepal, dorsal sepal, petal, lip, anther cap and column were made. The results obtained from morphological studies were generally consistent with the description given in the Flora of Turkey (Davis 1978 & 1988). The leaf is isolateral and compose of parenchymatic cells with intensively chloroplast. Vascular bundle is collateral and there hasn't bundle sheath. Stoma cells are located only on the abaxial side of the epidermis, as shown in Figure 4; just as Sevgi et al. (2012b) were reported. As a whole this study increase the knowledge of the morphology and anatomy in *Orchis* reporting data about the *O. simia.*

As far as ecological status is concerned, this species is rare as compared with other *Orchis* species like, *Orchis anatolica*, *Orchis italica* Poiret and *Orchis simia*. It is generally found in small groups. Due to its extreme habitat, its population are limited. Therefore, some scientist accept that it is an endangered species. Because of local use of this species like in preparing salep (Sevgi et al. (2012b), natural population is decreasing day by day. As compared to other orchid species, which are used for salep making, such as Serapias vomeracea, *Orchis anat*olica, *Anacamptis pyramidalis* (L.) Rich., it was found that in different habitat it blossoms in later time and these reason makes it easy target for plant collectors.

This species usually prefers calcareous soil. It is difficult to find them in clay or on alluvial soil. The soil analysis showed that at 0-5 cm soil depth, stoniness was around 16.8 % and these are mid stony soils (Cepel, 1988). The sand, silt and clay were 59. 25 and 22 % respectively at this depth. Soil was neutral with pH 6.86 and had high humus content. Total nitrogen content of the soil was 0.30 % and C:N ratio 35.15 (Table 2).

References

Akman Y, Ketenoğlu O, Kurt L, Vural M. 2014. The steppe vegetation of Inner Anatolia.

Altundag E, Sevgi E, Kara O, Sevgi O, Tecimen HB, Bolat I. (2012). Comparative morphological, anatomical and habitat studies on Dactylorhiza romana (Seb.) Soó subsp. romana and Dactylorhiza romana (Seb.) Soó subsp. georgica (Klinge) Soó ex Renz & Taub.(Orchidaceae) in Turkey. Pak J Bot, 44, 143-152.

Arditti J. 1992. Fundamentals of orchid biology. John Wiley & Sons.

Ataşlar E. 2018. Morpho-anatomical structure of *Orchis mascula* (L.) L. and its contribution to the taxonomy of Orchidaceae. EIJST. 7(6).

Aybeke M, Sezik E, Olgun G. 2010. Vegetative anatomy of some Ophrys, Orchis and Dactylorhiza (Orchidaceae) taxa in Trakya region of Turkey. Flora. 205(2): 73-89.

Bateman RM, Farrington OS. 1987. A morphometric study of× *Orchiaceras bergonii* (Nanteuil) Camus and its parents (*Aceras anthropophorum* (L.) Aiton f. and *Orchis simia* Lamarck) in Kent. Watsonia, 16, 397-407.

Bateman RM, Farrington OS. 1989. Morphometric comparison of populations of *Orchis simia* Lam.(Orchidaceae) from Oxfordshire and Kent. Botanical journal of the Linnean Society, 100(3), 205-218.

Baytop T. 1997. Türkce bitki adları sözlügü. Türk Tarih Kurumu.

Çepel N. 1988. Toprak İlmi Ders Kitabı; Orman Topraklarının Karakteristikleri, Toprakların Oluşumu, Özellikleri ve Ekolojik Bakımdan Değerlendirilmesi. İÜ Orman Fakültesi Yayınları, Yayın, (3416-389).

Davis PH. 1965-1988. Flora of Turkey and the East Aegean Island. Vol: 1-10, Edinburgh: Ediburgh University Press.

Dreesler RL. 1993. Phylogeny and classification of the orchid family. Dioscorides Press, p. 314.

Durmuskahya C, & Öztürk M. 2013. Ethnobotanical survey of medicinal plants used for the treatment of diabetes in Manisa, Turkey. Sains Malaysiana, 42(10), 1431-1438.

Durmuskahya C, Ozdemir C, Bozdag B & Ozturk, M. 2014. Studies on the morphology, anatomy and ecology of Ophrys lutea cav. subsp. minor (guss.) o. danesch & e. danesch ex gölz & hr reinhard (orchidaceae) in Turkey. Pakistan J. Biol Sci, 46, 565-571.

Efimov PG, Kuropatkin VV. 2014. (2277) Proposal to conserve the name *Orchis italica* Poir.(Orchidaceae) with a conserved

type, and notes on the typification of O. simia Lam. Taxon, 63(2), 431-432.

Fay MF. 2013. 751. *Orchis militaris*: Orchidaceae. Curtis's Botanical Magazine, 30(1), 9-17.

Güler N. 2016. Seed micromorphology of Orchis Tourn. ex L.(Orchidaceae) and allied genera growing in Edirne province, Turkey. PhytoKeys, (68), 9.

Güner A, Özhatay N, Ekim T, Başer KHC. 2000. Flora of Turkey and the east Aegean Islands. Supplement 2: 28.

Hartikainen H, Kerko E. 2009. Lead in various chemical pools in soil depth profiles on two shooting ranges of different age.

Kreutz CAJ. 2009. Orchids of Turkey, Botanical Properties, Ecological Requirements, Natural Spreading Sites, Vital Threats, Precautions for Protection (Trans & Cont. A Colak), Rota Publications, pp 55-848.

Kreutz CAJ. 2000. Orchidaceae. In Flora of Turkey and the East Aegean islands (Eds.: A Guner, N Ozhatay, T Ekim, KHC Baser). Edinburg University Press, Edinburgh, 11: 274 – 303.

Pridgeon AM. 1992. The Illustrated encyclopedia of orchids.

Renz J, and G Taubenheim. 1984. Orchis L. (Orchidaceae), In: Flora of Turkey and the East Aegean islands (Eds.: P.H. Davis). Edinburgh, University Press, Edinburgh. 8: 451-600.

Sevgi E, Altundag E, Kara O, Sevgi O, Tecimen HB, Bolat I. 2012a. Studies on the morphology, anatomy and ecology of Anacamptis pyramidalis (L.) LCM Richard (Orcidaveae) in Turkey. Pak. J. Bot, 44, 135-141.

Sevgi E, Altundag E, Kara O, Sevgi O, Tecimen HB, Bolat I. 2012b. Morphological, anatomical and ecological studies on some Orchis (Orchidaceae) taxa of Mediterranean region, Turkey. JEB. 33(2): 343.

Tuzlacı E. 2006. Şifa niyetine: Türkiye'nin bitkisel halk ilaçları. Alfa yayınları.

Vardar Y. 1987. Botanikte preparasyon tekniği. Ege Üniversitesi, Izmir.

Zarinkamar F. 2006. Density, size and distribution of stornata in different monocotyledons. Pak J Biol Sci, 9: 1650-1659.