



Ecology, anatomy and morphology of *Orchis spitzelii* in Turkey

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Abstract

In the present study reveals the morphological, anatomical and ecological characteristic of *Orchis spitzelii* in Turkey. Plant materials of *Orchis spitzelii* were collected from 17 different population, between 2010-2013 in Turkey. *Orchis spitzelii* samples were analyzed for 19 morphological, 20 anatomical and 18 soil characters and habitat properties. As a result, the minimum length of plant was 200 mm and maximum 500 mm, minimum length of underground part was 25 mm and maximum 94 mm. Number of leaves varied between 3-9. According to habitat definition, *Orchis spitzelii* grew up to 500 m to 2100 m. Also, *Pinus nigra* forests and glades were most common habitat of *Orchis spitzelii*, found generally over calcareous soils.

Key words

Orchis spitzelii, Ecology, Anatomy, Morphology

Introduction

Orchidaceae is the most famous and attractive plant family among all plant families of the world (Arditti, 1992; Prigdeon, 1992). The name of family comes from genus *Orchis* Tourn. ex L. According to Greek language *orchis* is derived from *orkhis* means testicle. Because of double tubers that scientific name were given them.

Orchids are nearly found in all ecosystems around the world except for glaciers, true desert and open water, although tropical areas especially in Asia, Africa and America are the hot spots of diversity. Most of them grow as epiphytes on other plants, rocks or static objects for support and derive their nutrients and water from the atmosphere and debris, however many species grow in forest or grassland areas. Some are parasites of fungi. Some, such as species in the subfamily *Vanilloideae* grow as lianas that can reach up to 20 m size. or more in length; the tiny *Bulbophyllum minutissimum* is only 3-4 mm (0.16-0.2 inches) tall.

The charisma of orchids and their biology have attracted botanists and general public as many varieties and hybrids are widely cultivated; this passion has inspired intrepid collecting expeditions and spawned hundreds of orchid societies and clubs around the world, spawning a global cultivation industry worth

nine-billion dollars annually (Ziegler, 2007). Most cultivars are tropical or sub-tropical. Many orchid species are threatened in wild due to over collection and habitat degradation. Therefore, orchid studies have got considerable importance.

As well as providing significant botanical interest, some orchids have edible use. Vanilla, for example, is a commercially important and widely used flavouring agent extracted from dried pods of several orchid species. Some orchids produce edible tubers example Australian desert and forest orchids are eaten by Aborigines. Orchids also have ancient origins in traditional medicine in many cultures, including Chinese medicine (Bulpitt *et al.*, 2007). In Turkey several orchid species are used in making traditional beverage and ice cream.

Genus *Orchis* has 36 taxa (Webb *et al.*, 2010). This genus is limited in its distribution exclusively to the northern hemisphere. Indeed, the center of its range lies unambiguously in the Mediterranean area, where maximum density of species are found (Kretzschumar *et al.*, 2007).

Orchis genus comprises of 7 section and *Orchis spitzelii* is found in Robustocalcare section. According to Hautzinger, the species of this section are characterized with big-sac like, conical spur and big stigma (Hautzinger, 1978). This section has got only

two species viz. *Orchis spitzelii* and *Orchis patens* Desf.

Orchis spitzelii was discovered by a German forest officer, Anton von Spitzel, in Austria in 1835. Therefore, the name of *spitzelii* comes from first collector of this species (Kretzschumar et al., 2007). *Orchis spitzelii* is one of the rarest *Orchis* species among other terrestrial orchids because of some ecological characteristics. It generally grows during winters, rich in snow, with high moisture content of soil during spring and alkaline recting subsoil. All these features are limited to distribution of the species. As a result, it is generally found in sub-alpine or alpine biotopes (Kretzschumar et al., 2007). In Turkey it can be seen generally in forest vegetation as it mostly prefers black pine (*Pinus nigra*) woods. Another important features of this species, it is occasionally found solitary and it doesn't generate big groups. Therefore, *Orchis spitzelii* cannot be found easily.

This species is known as salep in Turkey likes many other members of Orchidaceae (Baytop, 1997; Tuzlaci, 2006). Dried and powdered roots of this species are used to produce hot beverage "salep" and in preparing ice cream. For this reason many individuals of this species are collected and sold by local people (Toroglu et al., 2010). As a result, *Orchis spitzelii* and other species of orchid are alarmingly decreasing day by day, especially in West Anatolia.

Ecology, morphology and anatomy of several Turkish Orchid species have been studied earlier (Durmuskahya, 2013; Durmuskahya et al., 2014; Sevgi et al., 2012a; Sevgi et al., 2012b; Altundag et al., 2012a; Altundag et al., 2012b; Aybeke et al., 2010). But information about various orchid species is merge. 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.

Materials and Methods

Plant materials of *Orchis spitzelii* were collected from 17 native population of Nif Mountain, Yamanlar Mountain, Spil Mountain, Savaştepe, Honaz and Aydoğdu Mountain, Çameli Upland, Bayır, Kavaklıdere, Cevizli, Kuyucak, Korkuteli Pass, Murat Mountain, Dirmil Upland, Yalvaç, Abant Lake Side and Ilgaz Pass, between 2010-2013 (Table 1). For each plant sample, morphological and ecological features were recorded. In this study, total 57 plant samples were collected for anatomical studies. Herbarium specimens were kept in the Herbarium of Celal Bayar University.

Some morphological features were noted in field and some were studied in laboratory. About 19 morphological features viz; length of aerial part, length of underground part, tuber width and length, leaf number, width and length of longest leaf, width and length of shortest leaf, bract length, dorsal and lateral bract length, width and length of labellum, petal length, spur length, ovary length, caudiculum and the length of pollinium.

Plant samples were fixed in 70% alcohol for anatomical studies. Sections of root, stem and leaf were taken for anatomical study sartur reactive was applied to the sections for better tissue differentiation (Celebioglu and Baytop, 1949). Paraffin infiltrated tissues were prepared and sectioned with microtome. These anatomical sections were photographed using Leica DC3000 motorized microscope. The size of root, stem and leaf cells were measured with micrometer ocular as minimum, maximum and medium.

Table 1 : List of the sampling points

Sampling point	Sample		Altitude (m)	Slope (%)	Aspect	Locality
	Plant	Soil				
1	3	+	1400	5	South	Izmir Nif Dağı
2	4	+	1075	5	Northeast	Izmir Yamanlar Dağı
3	8	+	1350	10	West	Manisa Spil Dağı
4	2	+	710	30	West	Balıkesir Savaştepe
5	3	+	1230	10	East	Denizli Honaz Dağı
6	1	+	1190	35	West	Denizli Aydoğdu Dağı
7	4	+	1250	25	North	Denizli Çameli
8	1		960	20	West	Muğla Bayır
9	4	+	1245	15	South	Muğla Kavaklıdere
10	3	+	980	30	Southwest	Antalya Cevizli
11	2	+	1550	45	North	Antalya Kuyucak
12	5		1210	15	East	Antalya Korkuteli
13	2	+	1100	35	West	Kütahya Muratdağı
14	6	+	1330	25	North	Burdur Dirmil Yaylası
15	2	+	1030	20	West	Isparta Yalvaç
16	5	+	1610	45	East	Kastamonu Ilgaz Geçidi
17	2	+	1390	20	North	Bolu Abant

Soil samples were collected from the localities given in Table 1. The surface layer of soil was removed and soil samples taken from 0-5 and 5-15 cm depth were analysed. The soil samples were kept in polyethylene bags and immediately brought to the laboratory. 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 Ozturk *et al.* (1997). The total nitrogen was determined according to Bremner (1965), organic carbon according to Nelson and Sommers (Nelson and Sommers, 1982) and C: N ratio was calculated.

The ecological features recorded from each habitat during the study were; altitude, direction, habitat type, inclination and position of slope and surface stoniness. For statistical evaluations, SPSS 20 software was used (Demir, 2012).

Results and Discussion

Morphological features : In the present study, 19 morphological features of *Orchis spitzelii* were examined. For each feature, minimum, maximum, mean and standard deviation are given in the Table 2. Numerical description of *Orchis spitzelii* was not given clearly in the Flora of Turkey by Renz and Taubenheim (Davis, 1984). On the other hand, general morphological features of *Orchis spitzelii* were described in the Flora of Turkey and East Aegean Island (FOT) (Davis, 1984), Our Orchids, (OOT) (Sezik, 1984), The Orchid Genera, *Anacamptis* Rich., *Orchis*, *Neotinea* Reichb. fil. (TOG) (Kretzschumar *et al.*, 2007) and Orchids of Turkey (OT) (Kreutz, 2009) before. But according to the result of the present study, were found some differences between the

former knowledge. In FOT, plant length was mentioned to be 20-40 cm. but in the present study, it was found to be 20-50 cm. However, no information about number of leaves, number of flowers in FOT and OT, where in OOT and TOG it is mentioned. According to these literatures the inflorescences comprised 10-25 flowers, but in the present study it was found to be 8-30. Another important difference identified was the marks seen on the labellum. In FOT and OOT, there is not enough description about labellum pattern. Whereas according to our study some species have got labellum pattern and some not, and also the color of the labellum was red, pink and sometimes white.

Orchis spitzelii, 20-50 cm in height is one of a medium sized orchid species (Sezik, 1984) At the base of stalk between 2-9 oval to lanceolate, pale to dark green, shiny and unspotted leaves are arranged in the rosette. The width of the leaves are 1.5-3 cm and length 6-15 cm. Besides, the plant has got 1 -2 vaginating leaves, which loosely wrap the lower part of the stalk. However, during flowering period, they mainly stand away in varying degrees from the stalk (Kretzschumar *et al.*, 2007). Inflorescence is cylindrical and narrow shape like a pipe. It consists of 10-25 single flowers. Flowers are dark red to mauve in colour and occasionally white. (Davis, 1984) Sepals are oval shaped reddish in colour. On inner side they are olive-green with dark red spots. Labellum is broadly ovate and tri-lobed. It is generally 11-18 x 9-12 mm. Labellum exhibits a highly variable dark spotting which is normally found only in the middle of the central lobe but in some individuals no spotting is present. The spur is conical, directed downwards and 6-10 mm in length. Stigma is large and redish-brown colour. Mace-shaped pollinaria have one small common sac. The seeds are relatively long and thin. This species is pollinated through wild bees.

Table 2 : Morphological characters of *Orchis spitzelii*

Characters	N	Minimum	Maximum	Mean	S.D.
Length (mm)	17	200	500	33.30±1.98	81.68
Underground part (mm)	17	25	94	56.17±5.47	22.55
Tuber length (mm)	17	20	45	31.41±1.70	7.04
Tuber width (mm)	17	20	25	22.29±0.44	1.82
Leaf number	17	3	9	5.67±0.38	1.60
Length of longest leaf (mm)	17	60	150	100±6.07	25.06
Width of longest leaf (mm)	17	15	35	25.52±1.44	5.93
Length of shortest leaf (mm)	17	60	92	73.41±2.55	10.54
Width of shortest leaf (mm)	17	15	23	18.76±0.65	2.70
Bract length (mm)	17	13	19	14.76±0.37	1.56
Dorsal sepal length (mm)	17	6	9	7.47±0.28	1.17
Lateral sepal length (mm)	17	6	9	7.35±0.19	0.78
Labellum length (mm)	17	9	13	10.94±0.31	1.29
Labellum width (mm)	17	11	18	14.70±0.58	2.39
Petal length (mm)	17	8	10	8.67±0.18	0.75
Ovary length (mm)	17	15	20	17.01±0.38	1.58
Caudiculum	17	1	4	2.58±0.22	0.93
Pollinia	17	2	4	3.05±0.18	0.74
Number of flower	17	8	30	14.88±1.55	6.39

57 samples of *Orchis spitzelii* were studied for 19 morphological characters. All these characters are given in Table 2. According to Table 2; length of aerial part varied between 200-500 mm, number of leaves ranged between 3-9. Length of basal leaves varied between 60-150 mm, width of longest leaf was 15-35 mm while, that of shortest leaf was 60-92 mm. Flowers were 8-30 mm. *Orchis spitzelii* labellum was generally pinkish or dark bluish colour with dark spotting and sometimes creamish and dull yellow colour. But occasionally they were found without spotting. In the present study species with light colours were hardly found. Labellum was tri-lobed lip. It was longer than wider. (11-18 x 9-13 mm.). It had highly variable dark spotting, which was normally found only in the middle of the central lobe. However, it also covered the whole lip, or totally absent. Length of dorsal and lateral sepals were same (6-9 mm). Ovarium length was 15-20 mm. Beside aerial parts, underground parts of *Orchis spitzelii* were also examined and tuber length ranged between 20-45 mm and width varied between 20-25 mm.

Cross section of root showed single layered epidermal cells. The cell length was longer than width (20-45 × 35-50 µm). Cortex consisted of 8-10 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. Paracymatic cells located far from center were smaller than the ones close to center (Fig. 1A). These cells contained starch grains and cluster raphide crystals (Fig. 1F). Fungal pelotons and hyphae were observed in big cortical cells. Similar results have been earlier reported by several authors in members of Orchidaceae family (Aykebe et al., 2010; Altundag et al., 2012; Durmuskahya et al., 2014). Endodermal cells (25.5-40 × 20-40 µm) with thickened wall were seen but were not visible clearly. Pericycle was single-layered and located under endodermis. Xylem consisted of radially arranged 6 to 7 vessels, while pith was made up of parenchymatous cells. (Fig. 1A, Table 3).

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, 20-60 × 10-30 µm. 4-7 layers of cortex cells were found under epidermis with 45-75 µm diameter. Few collenchyma cells with thick and lignified cell walls were found in cortex layer. 5-6 layered collenchyma cells gave durability to the stem. Cortical parenchyma, a thinner layer than collenchyma was found with large amount of starch grains (Fig. 1 D). Presence of collenchyma cells in *Ophrys* L. and *Dactylorhiza* Necker ex Nevski have been reported previously in other studies (Altundag et al., 2012; 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. 1B,E).

Leaves : 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 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. According to former studies, adaxial epidermal cells of *O. spitzelii* were transformed to water-storage tissues. Mesophyll layer was homogenous, without any differentiation in to palisade and sponge parenchyma tissues.

These cells were rounded and 25-35 µm in diameter. Vascular bundles could be seen among the mesophyll cells (Fig. 1C).

Ecology : Colonies of *Orchis spitzelii* have been found in Pyrenees, over the Alps including foothills, Italy, the Balkan

Table 3: Anatomical characters of *Orchis spitzelii*

	Width (µm)				Length (µm)			
	Min.	-	Max.	Mean ±S.D.	Min.	-	Max.	Mean ±S.D.
Root								
Epidermis	20.00	-	45.00	35.87±7.95	35.00	-	50.00	45.25±5.11
Cortex parenchyma	55.00	-	110.00	74.00±16.75				
Endodermis	25.50	-	40.00	35.00±6.12	20.00	-	40.00	34.60±8.41
Stem								
Cuticle length					1.33	-	4.67	3.05±1.07
Epidermis	20.00	-	60.00	40.71±11.70	10.00	-	30.00	22.50±6.54
Cortex parenchyma	45.00	-	75.00	61.66±12.11				
Trachea (diameter)	12.00	-	20.00	17.42±3.35				
Leaves								
Adaxial epidermis	50.00	-	110.00	73.00±23.61	80.00	-	85.00	82.00±2.73
Mesophyll cells	25.00	-	35.00	33.00±9.30				
Abaxial epidermis	55.00	-	80.00	60.83±17.72	35.00	-	50.00	45.50±9.02
Trachea (diameter)	5.00	-	10.00	11.00±9.50				

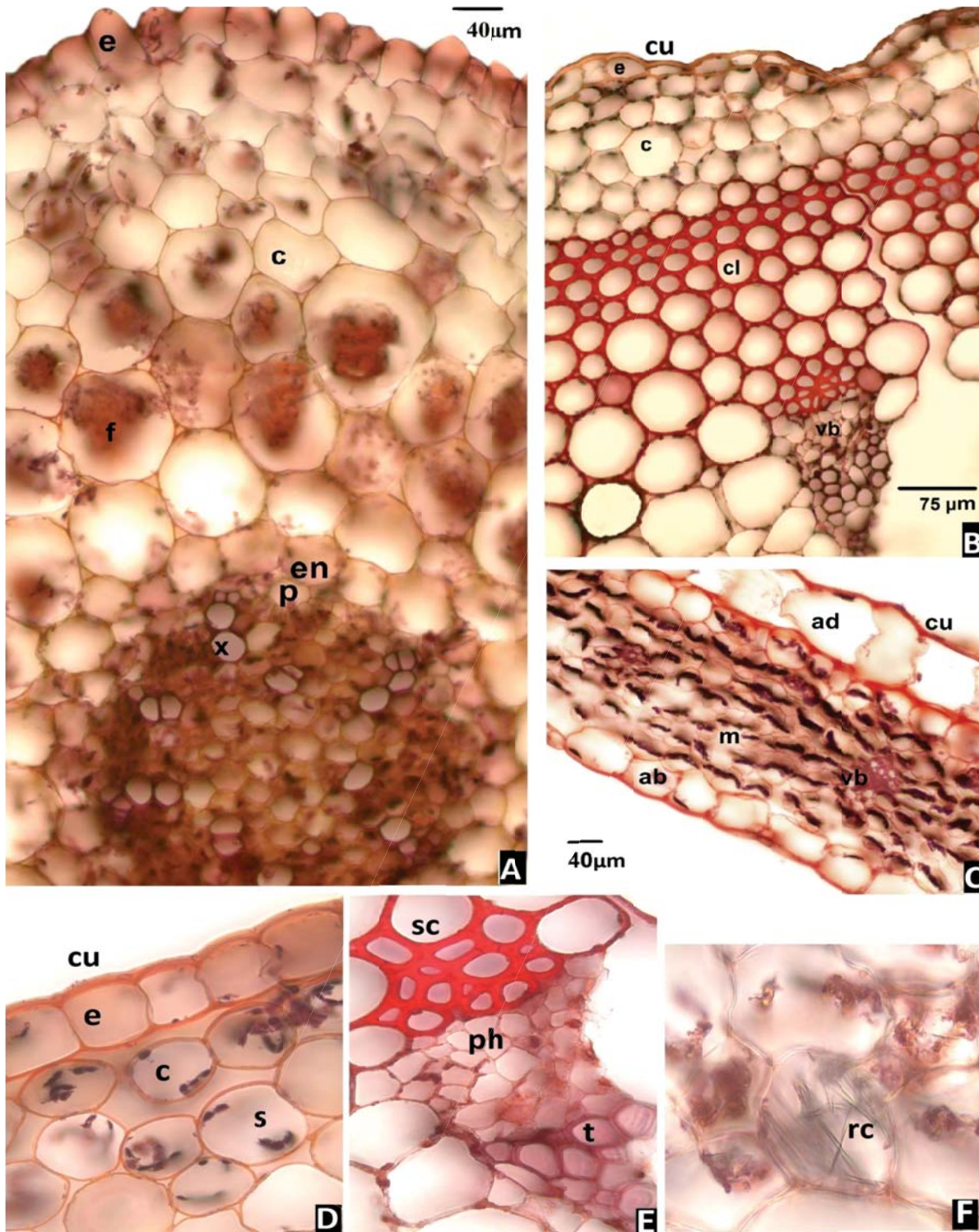


Fig. 1: Transverse sections of *O. spitzelii* root (A), stem (B,D), leaf (C), vascular bundle of stem (E) and raphide crystals of root (F); ab: abaxial epidermis, ad: adaxial epidermis, c: cortex, cl: collenchyma, cu: cuticle, e: epidermis, en: endodermis, f: fungal peloton, m: mesophyll p: pericycle, ph: phloem, rc: raphide crystals, s: starch grain, sc: sclerenchyma, t: tracheae, x: xylem, vb: vascular bundle

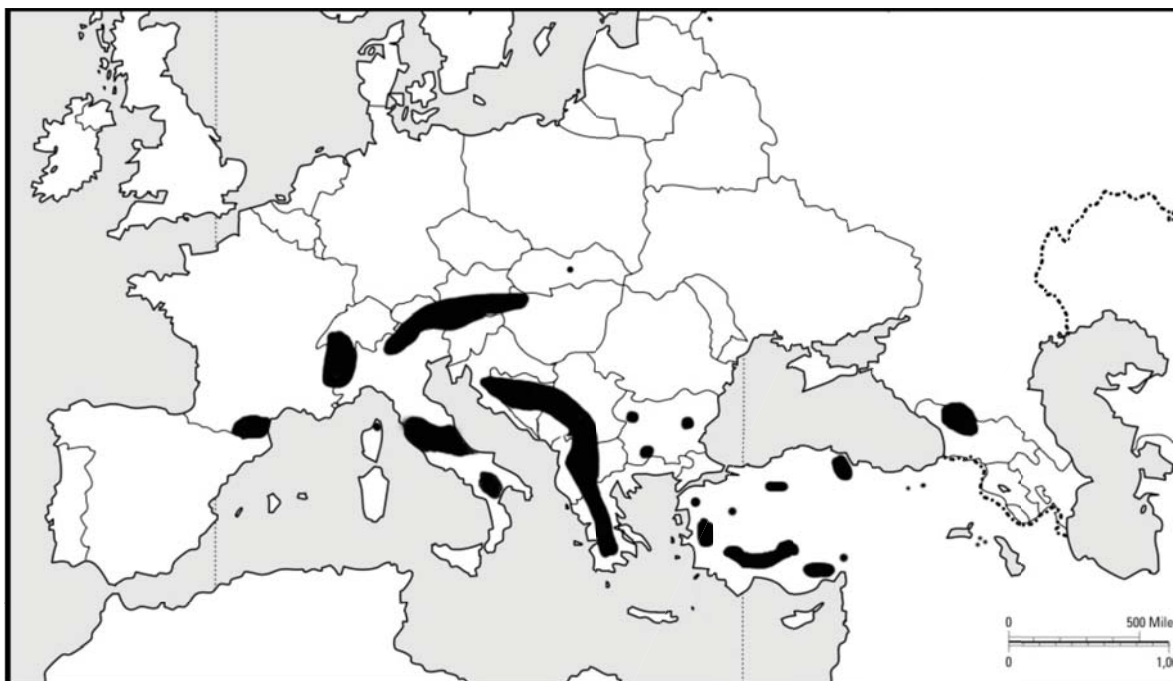


Fig. 2 : Distribution of *Orchis spitzelii*

Peninsula and Asia Minor as far as Caucasus. Outposts were found on the Island of Gotland, in Levant and North Africa (Fig. 2). *O. spitzelii* is also distributed in the alpine and subalpine zone of West and Middle part of Anatolia and is rarely found in the Central Black Sea Mountains and East Anatolia (Kreutz, 2009). The main population have been found in Manisa, Denizli, Muğla, Antalya and Bolu provinces. This species usually make small population, generally less than hundred individuals. Sometimes it was also found solitary.

Orchis spitzelii has been reported to grow naturally at an altitude of 500 m. to 2100 m (Davis, 1984, Delforge, 2006). But in the present study, it was observed that this species could grow up to 710-1610 m, but on the other hand it was reported 320 m in Balıkesir province (Ozen, 2000) and at 166 m in Muhat Villiage of Tarsus (Erzurumlu and Sogut, 2012). As indicated in Fig. 3, *Orchis spitzelii* was found mostly between 1200-1400 m (Fig. 3)

Orchis spitzelii are found in variety of biotopes, calcareous substrate with pH factor between 6,5 – 7 (Hautzinger, 1978), over limestone, less often over dolomite, winters rich in snow and relatively cold, as well as sufficient water supply in spring. These demands are mainly fulfilled at high mountains, especially alpine edges, at low attitude it occurs in coniferous and mixed forests that have small open glades, or in areas settled by bracken fern, where it grows in full sun just as well as in semi-shade (Kretzschumar et al., 2007).

In the present study *O. spitzelii* was usually found in *Pinus*

nigra forests and glades growing along with *Orchis purpurea* Huds., *Orchis laxiflora* Lam., *Orchis anatolica* Boiss., *Epipactis helleborine* (L.) Crantz, *Himantoglossum comperianum* (Steven) P. Delforge and *Neotinea maculate* (Desf.) Stearn. It was also found growing with *Dactylorhiza romana* (Seb.) Soo subsp. *romana*, *Ophrys isaura* Renz et Taub. and *Anacamptis morio* (Poir.) R. M. Bateman subsp. *caucasica* (K.Koch) H.Kretzschmar, Eccarius & H.Dietr. in Antalya region (Kreutz, 2009).

As stated in Flora of Turkey by Renz and Taubenheim, *Orchis spitzelii* flowers blossom during April and May whereas,

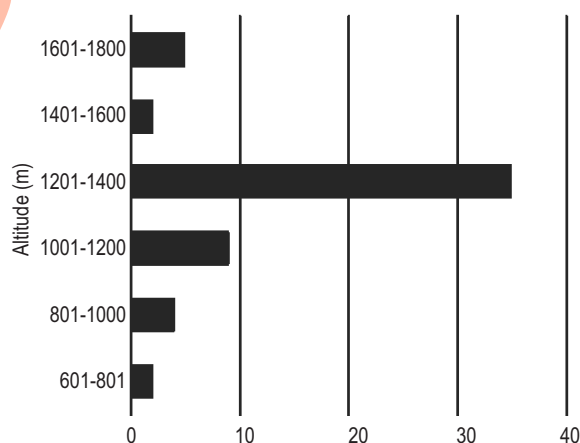


Fig. 3 : Record numbers of *Orchis spitzelii* according to altitude

Table 4: Descriptive statistic of characteristics of soil and sampling points

0-5 cm	Symbol	N	Min.	Max.	Mean	S.D.
	Volume weight (g/lt)	15	830.10	1296.30	1036.70±39.13	151.57
	Fine soil weight (g/lt)	15	665.63	1030.20	850.83±35.99	139.42
	Stone weight (gr/lt)	15	38.00	730.10	369.89±60.73	235.21
	Sand (%)	15	45.00	75.30	59.05±2.42	9.37
	Silt (%)	15	11.20	36.30	25.07±2.07	8.02
	Clay (%)	15	9.60	32.30	22.03±1.90	7.37
	pH	15	6.50	7.10	6.86±0.52	0.20
	Corg (%)	15	1.10	9.12	4.20±0.61	2.36
	Nt	15	0.08	0.63	0.30±0.04	0.18
	C/N	15	7.16	87.20	35.15±6.10	23.65
5-15 cm	Volume weight (g/lt)	15	910.00	1466.30	1150.30±46.95	181.85
	Fine soil weight (g/lt)	15	554.00	999.40	752.30±42.31	163.89
	Stone weight (gr/lt)	15	53.00	701.00	415.30±54.50	211.11
	Sand (%)	15	31.20	67.20	45.88±2.61	10.11
	Silt (%)	15	10.10	41.12	22.45±2.85	11.07
	Clay (%)	15	16.17	46.81	27.61±2.44	9.48
	pH	15	6.60	7.34	7.00±0.05	0.21
	Corg (%)	15	0.66	5.83	2.45±0.41	1.58
	Nt	15	0.11	0.51	0.25±0.03	0.13
	C/N	15	6.88	135.60	56.96±10.86	42.08
	Altitude	15	710.00	1610.0	1216.00±58.71	227.40
	Slope	15	5.00	45.00	21.66±3.07	11.90
	Surface stoniness	15	5.00	32.00	16.80±2.33	9.02

according to Sezik it flowers between April-July (Davis, 1984; Sezik, 1984). However, in the present study, it was observed that flowers blossomed from late April to early June.

As far as ecological status is concerned, this species is rare as compared with other *Orchis* species like, *Orchis anatolica*, *Orchis italica* Poirlet and *Orchis simia* Lam. 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.

In Turkey *Orchis spitzelii*, considered is as critical. Because of local use of this species like in preparing salep (Tecimen et al., 2010), natural population is decreasing day by day. As compared to other orchid species, which are used for salep making, such as *Serapias vomeracea*, *Orchis anatolica*, *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. Therefore, *Orchis spitzelii* need to protection in their habitat.

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 4).

At 5-15 cm soil depth stoniness was nearly same as in the upper layer, stressing the fact that this species preferred stony soil.

Many tubers were found at 5-11- cm depth, and textural classes of these soil consisted of 67 % sand, 41 % silt, and 46 % clay. Soil was neutral with average pH value of 7.00. Total nitrogen content in soil was 0.25 and C:N ratio was 56.96 (Table 4), respectively. In general *Orchis spitzelii* prefers neutral and partially rich soil are therefore at found high altitudes than *Orchis anatolica* and *Anacamptis pyramidalis* (Altundag et al., 2012a,b; Sevgi et al., 2012).

In this study, morphological, anatomical and ecological features of *Orchis spitzelii* were evaluated for the first time in Turkey. We believed that these ecological information and numerical data about species will be useful for further orchid studies.

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