© 2012 Triveni Enterprises Vikas Nagar, Lucknow, INDIA editor@jeb.co.in Full paper available on: www.jeb.co.in



J. Environ. Biol. 33, 343-353 (2012) ISSN: 0254-8704 CODEN: JEBIDP

Morphological, anatomical and ecological studies on some *Orchis* (Orchidaceae) taxa of Mediterranean region, Turkey

Author Details

Ece Sevgi (Corresponding author)	Department of Pharmaceutical Botany, Faculty of Pharmacy, Bezmialem Vakif University, Istanbul, 34093, Turkey e-mail: ecesevgi1@yahoo.com
Ernaz Altundag	Department of Biology, Faculty of Arts and Sciences, Düzce University, Düzce, 81620, Turkey
Omer Kara	Soil Science and Ecology Department, Faculty of Foresty, Bartin University, Bartin, 74100, Turkey
Orhan Sevgi	Soil Science and Ecology Department, Faculty of Foresty, Istanbul University, Istanbul, 34473, Turkey
Huseyin Baris Tecimen	Soil Science and Ecology Department, Faculty of Foresty, Istanbul University, Istanbul, 34473, Turkey
Ilyas Bolat	Soil Science and Ecology Department, Faculty of Foresty, Bartin University, Bartin, 74100, Turkey

Abstract

Publication Data

Paper received: 25 October 2010

Revised received: 02 July 2011

Accepted: 30 July 2011

In this study, nine species mainly distributed in Mediterranean (+ Aegean region) region of Turkey were investigated in terms of 15 morphological, 16 anatomical and 5 ecological characteristics. Those species are *Orchis anatolica* Boiss., *Orchis italica* Poiret, *Orchis laxiflora* Lam., *Orchis morio* L. subsp. *morio*, *Orchis provincialis* Balbis ex DC., *Orchis purpurea* Hudson, *Orchis sancta* L., *Orchis simia* Lam., *Orchis tridentata* Scop. In conclusion, we found that *O. laxiflora* was characterized by the longest plant height and *O. purpurea* had the biggest tuber; whereas *O. italica* was identified by the highest number of leafs and *O. purpurea* had the longest leaf length and widest leaf width. Additionally, the other species which were characterized by different morphological and anatomical parameters are as follow: *O. sancta* with the longest bract length, *O. italica* and *O. simia* with the longest sepal lengths, *O. laxiflora* with the shortest and widest labellum, *O. simia* with the longest petal length, *O. sancta* with longest caudiculum length, *O. anatolica* with the longest spur length and *O. provincialis* with the longest ovary length. Particularly, *O. laxiflora* and *O. purpurea* species present essential divergence from the aspect of anatomical features of leaf surface in comparison with the other species. Morphological and anatomical traits of the species were attributed to the habitat selections of the species since that character differs along with each species.

Key words

Leaf anatomy, Morphology of Orchis sp., Orchidaceae, Turkey

Introduction

The family Orchidaceae comprises approximately 19,500 species distributed all around the world. Turkey is a rich country of terrestrial orchids and represented by 150 taxa. 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).

Turkish orchids were introduced in the volume 8 and 11 of Flora of Turkey. *Orchis* is a major genus of Orchidaceae family in Flora of Turkey, represented by 22 species (Renz and Taubenheim, 1984; Kreutz, 2000). In the last description study on *Orchis* (Kreutz, 2009), some species were classified under the genus *Anacamptis* and *Neotinea* (Kreutz, 2009). In this study, the nomenclature of *Orchis* is according to the Flora of Turkey (Renz and Taubenheim, 1984).

Leaf surface, shape and dimension of epidermis cells, type of stomata and stomata index are important characteristics for identifying some taxa (Zarinkamar, 2006; Jakubska, 2007;

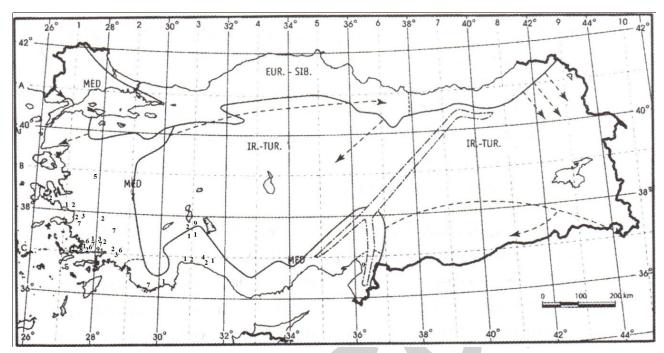


Fig. 1: Map of collected taxa: EUR.-SIB.= Euro-Siberian phytogeographical region, IR.-TUR.= Irano-Turanian phytogeographical region, MED.= Mediterranean phytogeographical region, X= Middle European part of Euro-Sibirian phytogeographical region. ——> Mediterranean elements.

1. O. anatolica, 2. O. italica, 3. O. morio subsp. morio, 4. O. laxiflora, 5. O. purpurea, 6. O. provincialis, 7. O. sancta, 8. O. simia, 9. O. tridentata

Foroughbakhch *et al.*, 2008; Hidayat and Kusdianti, 2009). Few studies are present about vegetative anatomy of *Orchis* species (Sgargi and Del Prete, 2005, Altundag and Sevgi, 2010, Aybeke *et al.*, 2010). In a recent study, Aybeke *et al.* (2010) studied the vegetative anatomy of *O. tridentata*, *O. purpurea*, *O. simia*, *O. morio* subsp. *morio* and *O. laxiflora* distributed in Thracian region of Turkey. But a detailed and comprehensive examination of the ecological, morphological and anatomical properties of the taxa was not attempted previously. Therefore, in this study, we investigated the morphological, anatomical and ecological characteristics of 9 *Orchis* taxa, distributed in Mediterranean region of Turkey, which will be basic knowledge for future studies in terrestrial orchid systematics.

Materials and Methods

In this study 9 *Orchis* species, collected from 40 natural populations in Mediterranean part of Turkey in years 2007-2009, were investigated. The locations of the samples were shown on the map (Fig. 1) and also listed in Table 1. 304 location data had been extracted from the previous publications (Sezik 1984; Dusen, 2001; Duran, 2002; Unal and Gokceoglu, 2003; Deniz and Sumbul, 2004; Varol *et al.*, 2004; Aytepe, 2007; Kreutz, 2009) and EGE (Faculty of Science Herbarium of Ege University), ISTE (Faculty of Pharmacy Herbarium of Istanbul University) Herbarium records samples are stored in the Faculty of Forestry Herbarium of Istanbul University (ISTO No: 35082-35093).

15 morphological parameters were measured on mature tuber, leaf and flower at each samples collected from the field.

Morphological characters of investigated *Orchis* species were plant length, tuber length and width, longest leaf length and width, leaf number, bract length, the elements of flower are as; dorsal and lateral sepal length, labellum length and width, petal length, spur length, ovary length and caudiculum length.

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 (Vardar et al., 2006; Ruzin, 1999). The well-staining sections were photographed on Leica DFC 295 color camera type, Leica DM 2500 light microscope. For each taxa, the anatomical features such as stomata size (width-length) and stomata index, epidermal cell shape and size on the abaxial and adaxial surface (width-length) at surface sections; cuticular type and thickness, epidermal cell shape, anticlinal and periclinal walls of epidermal cells, chlorenchyma cells shape and layers, lacunae and raphide in mesophyll cells, characters of vascular bundle in cross sections were recorded.

The variants used for ecological studies on the habitats of *Orchis* species were noted as altitude, habitat type, slope position and surface stoniness.

Statistical evaluation: The normality of measurements regarding the morphological features were tested by Kolmogorov-Smimov Z test. The comparison of abnormally distributed data groups were analyzed by Kruskal-Wallis multi-test, significantly differing groups were re-arranged by Whitney-U binary test. In normally distributed groups homogeneity of variances was tested. Dunnett T3 test was

applied to the rest of homogenous groups (Kalipsiz, 1981; Ozdamar, 2002; Senol, 2004). Statistical analyses were executed in SPSS 13v software. Besides, morphological features were evaluated in CAP 4 (Community Analysis Package) program as agglomerative, complete linkage and distant based Bray-Curtis. Anatomical data was analyzed and evaluated within descriptive characteristics and their presence - absence status.

Results and Discussion

Morphological characters: The plant length was divided into 3 groups according to Dunnett T3 test. *O. laxiflora* was noted as the longest plant species among others with average length 494.6 mm. *O. morio* subsp. *morio* has the shortest leaf length with average length 299.5 mm. Tuber length results can be grouped into 3 according to Dunnett's T3 test. *O. purpurea* diversed from the rest of the species with the size 36.7 x 44.8 mm. *O. anatolica* has the smallest tuber size measuring 11.3 x 17.3 mm (Table 2).

Leaf numbers of the species showed 4 sub-groups according to Kruskal-Wallis multi-test. The longest leaf length and width showed abnormal distribution and 4 sub-groups were

determined according to Dunnett's T3 test. *O. anatolica* has the lowest leaf number with 4.5 and *O. italica* has the highest leaf number with 10.4. The longest and widest leaf belongs to *O. purpurea*. Although *O. laxiflora* has the longest leaves its width ranked among the average levels. The shortest leaves were found in *O. morio* subsp. *morio* and the narrowest leaf was in *O. sancta* (Table 2).

The statistical analysis on characteristics of flowers elucidated that bract length results could be assembled into 4 subgroups according to Kruskal-Wallis multi-test, and additionally only limited transitions achieved among groups. *O. purpurea* has the lowest and *O. sancta* has the highest bract length. The characteristics of flowers revealed that dorsal and lateral sepal length demonstrated 4 sub-groups according to Kruskal-Wallis multi-test. The lowest sepal lengths were found in *O. anatolica* and *O. italica*. *O. simia* has the highest sepal length. Labellum length and width measurements showed 2 and 4 assemblages according to Dunnett's T3 test. The lowest labellum length was found in *O. laxiflora* and the highest in *O. italica*. The highest labellum width was recorded in *O. laxiflora* and the narrowest in *O. simia*.

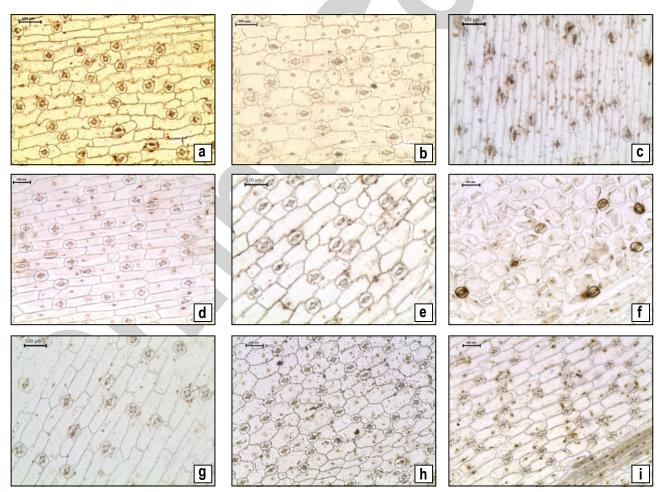


Fig. 2: Abaxial surface of leaves: (a) O. anatolica, (b) O. italica, (c) O. laxiflora, (d) O. morio subsp. morio, (e) O. provincialis, (f) O. purpurea, (g) O. sancta, (h) O. simia, (i) O. tridentata

Table - 1: List of the sampling points*

No.	Taxa (Morphological measurement numbers)*	Locality
1	Orchis italica (1)	Mugla-38 km from Ören to Milas
2	Orchis italica (1)	Mugla-Milas - Alacam - Kargacik district
3	Orchis anatolica, Orchis italica (1)*	Mugla-Milas - Alacam - Mortas district where Kapiz river rotated to Kocaçay
4	Orchis italica (1), Orchis sancta	Mugla-Milas - Kiyikislacik – Grand Kara Kuyu
5	Orchis italica (5)	Mugla-Bodrum – Güvercinlikli
6	Orchis italica	Mugla-Kafaca – On the way departed to the right hand after Yesilyurt crossroad
7	Orchis italica	Mugla-Kafaca - On the way departed to the right hand after Yesilyurt crossroad
8	Orchis italica	Mugla-Marmaris - Datca way Kovalica towards Forest Fire Observation Towe
9	Orchis laxiflora	Mugla- Between Datca – Marmaris Hisarönü district
10	Orchis italica (2)	Mugla-300 m forward from left hand cross road before entrance to Ula Karabörtler
11	Orchis italica (2)	Mugla-300 m forward from left hand cross road before entrance to Ula Karabörtler
12	Orchis morio subsp morio (3)	Antalya-Kas - Irgenler village
13	Orchis anatolica, Orchis sancta	Mugla-Datca - Hizirsah village meadow
14	Orchis laxiflora	Mugla-Marmaris - 11km forward from Karacaköy crossroad departed from
		Gökova way Ovacik
15	Orchis anatolica (5)	Antalya-Kumluca - Gödene Way Altinyaka village outgoing
16	Orchis laxiflora*	Antalya – Left side of the way connecting Serik to Zeytintasi Cave
17	Orchis tridentata	Hatay - Dortyol - Kuzuculu district
18	Orchis anatolica (6)	Isparta - Sutculer – Candir way
19	Orchis anatolica (5)	Isparta - Sutculer entrance Ince Meryem hill
20	Orchis sancta*	Aydin - Karacasu entrance district
21	Orchis sancta	Aydin – 2 km to Ortaklar 25 km to Seluk
22	Orchis purpurea*	Manisa - Sipil Mount - At Yard
23	Orchis anatolica (10)	Mugla – The slopes on the left side of Mugla outgoing
24	Orchis sancta	Mugla- On the way from Akbuk to Gokova
25	Orchis anatolica (10)	Mugla -Yerkesik - Oren
26	Orchis provincialis,	Mugla -Oren - Yerkesik way 1 km to Oren Kizilcam dense forest on the right side
	·	of the way
27	Orchis anatolica (3), Orchis simia	Mugla -Yerkesik - Oren way 2.8 km to Kiran village
28	Orchis anatolica, Orchis italica (1), Orchis tridentata	Mugla -Yerkesik - Oren way 1.8 km to Kiran village
29	Orchis morio subsp morio (10)*, Orchis provincialis*	Mugla - Koycegiz Yayla village cemetery
30	Orchis italica	Aydin-Nazilli - Karacasu 5 km to Karacasu
31	Orchis anatolica (9)*, Orchis simia*, Orchis tridentata*	Isparta - Kovada lake View terrace surrounding
32	Orchis italica (5)	Izmir Cesme - Alacati village
33	Orchis anatolica (10)	Mugla - Koycegiz way The way on the left side after Ula 4-5 km
34	Orchis italica (9), Orchis morio subsp morio (2)	Izmir-Selcuk Kusadasi way the corner of earth road on the right
35	Orchis anatolica (10), Orchis italica	Antalya- Kizilcam reforestation land
36	Orchis anatolica, Orchis italica	Antalya-Serik - Zeytintasi cave left of the road in the splay
37	Orchis anatolica	Antalya 1 km to 5 km to Tepekoy sign
38	Orchis provincialis	Mugla-Milas – Mugla way 2 km further from Tuzabat village under Kizilcan
		forest
39	Orchis italica (20)	Mugla- Right side of Gokova-Marmaris way
40	Orchis anatolica, Orchis italica	Izmir- Menderes – Selcuk way

¥ = 52 punctual sampling was made from 40 sites belonged to 9 species, * = Anatomical measurements applied

Investigations on the petal length measurements showed that petal lengths have 3 sub-groups according to Kruskal-Wallis multi-test. *O. anatolica* has the lowest petal length and *O. simia* has the longest petals. The caudiculum measurements revealed 5 sub-groups according to Kruskal-Wallis multi-test. *O. tridentata* has the shortest and *O. sancta* longest caudiculum length. Spur measurements showed 6 sub-groups according to Kruskal-Wallis multi-test. *O. purpurea* has the shortest spur length while *O. anatolica* has the longest spur (4.5 and 20.2 mm, respectively). Ovary

measurement results showed 3 sub-groups according to Kruskal-Wallis multi-test. *O. sancta* has the shortest and *O. provincialis* has the longest ovary (10.4 and 17.3 mm, respectively) (Table 3).

Leaf anatomy: The leaf surfaces of all investigated species are glabrous and stomata cells are abaxially located being anomocytic or tetracytic type with 4 or 5 neighbouring cells (Fig. 2). The epidermal cell rows were parellel to the midrib. These characters were observed by Aybeke *et al.* (2010) as well. Upper epidermal

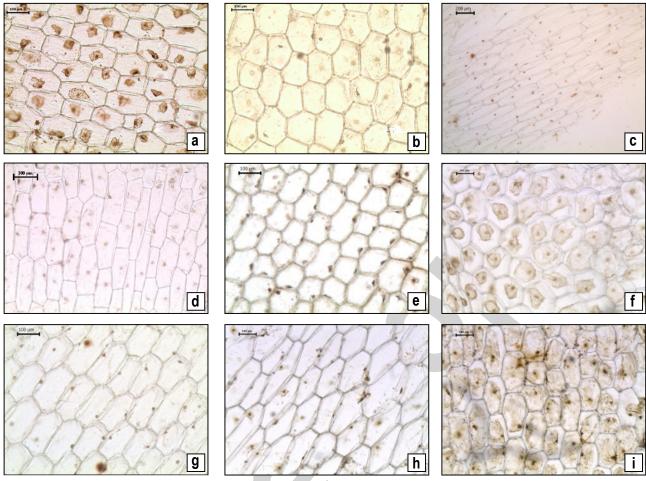


Fig. 3: Adaxial surface of leaves: (a) O. anatolica, (b) O. italica, (c) O. laxiflora, (d) O. morio subsp. morio, (e) O. provincialis, (f) O. purpurea, (g) O. sancta, (h) O. simia, (i) O. tridentata

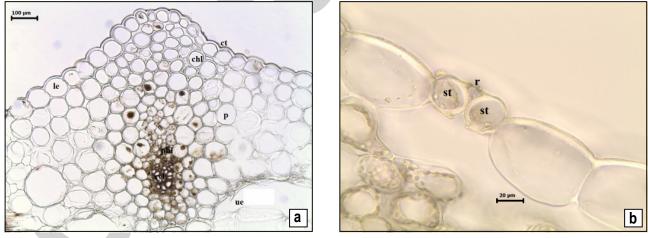


Fig. 4: Transverse section of (a) O. tridentata leaf; (b) stomata in transverse section of the leaf O. italica; cu= Cuticle, chl= Chlorenchyma, ue= Upper epidermis, le = Lower epidermis, p = Parenchyma, xyl = Xylem, phl = Phloem, r = Ridge, st = Stomata

cells on the midrib are elongated, rectangular (Table 4). Shapes of epidermal cells among the veins are rectangular, polygonal on abaxial and polygonal, isodiametric, rectangular on adaxial leaf surface (Fig. 2 and 3). O. laxiflora differs from other Orchis species

on the basis of its epidermal cell size: $37.50 \times 281.00 \,\mu\text{m}$ (abaxial) and $49.33 \times 320.65 \,\mu\text{m}$ (adaxial), with narrowest and tallest cells. O. purpurea has pentagonal and hexagonal isodiametric cells on abaxial side(Table 4). While O. purpurea and O. provincialis had

Table - 2: Morphological features related to the plant size, tuber and leaves

Parts	ana⁺	ita⁺	lax⁺	mor*	pro⁺	pur*	san⁺	sim⁺	tri*	
Length**	Aver. N Min-Max. Range	296,7 ^a 78 160-490 330	344,6 ^{ab} 48 102-520 418	494,6° 13 300-660 360	299,5° 15 180-405 225	315,7° 21 205-380 175	449,2 ^{bc} 6 365-520 155	306,5 ^a 17 160-430 270	436,0 ^{bc} 5 325-610 285	392,3 ^{abc} 11 250-480 230
Tuber Length**	Aver. N Min-Max. Range	17,3° 74 9-26 17	30,9 ^b 48 17-55 38	22,9 ^{ab} 13 6-42 36	19,9 ^{ab} 15 10-42 32	21,1 ^{ab} 21 16-35 19	44,8° 6 32-55 23	21,7 ^{ab} 9 12-30 18	26,5 ^{ab} 4 15-36 21	26,2 ^{ab} 9 17-40 23
Tuber width**	Aver. N Min-Max. Range	11,7° 74 5-22 17	20,5 ^b 48 11-32 21	17,6 ^{ab} 13 10-25 15	17,5 ^{ab} 15 8-30 22	14,9 ^{ab} 21 10-18 8	36,7° 6 27-45 18	15,8 ^{ab} 9 10-20 10	21,8 ^b 4 15-28 13	17,1 ^{ab} 9 8-27 19
Leafnumber	Aver. N Min-Max. Range	4,5° 78* 3-8 5	10,4 ^d 47 6-15 9	7,2 ^{abc} 13 6-9 3	8,4 ^{bcd} 15 5-12 7	7,0 ^{abc} 21 5-9	8,3 ^{bcd} 6 7-9 2	8,0 ^{bcd} 17 5-15 10	6,0 ^{ab} 5 5-7 2	8,5 ^{bcd} 11 4-11 7
Length of longest leaf**	Aver. N Min-Max. Range	92,6 ^{ab} 77 45-148 103	108,3 ^{abc} 46 12-180 168	175,4 ^d 13 90-240 150	68,6ª 15 40-100 60	115,4 ^{bc} 21 86-146 60	176,2 ^d 6 150-200 50	80,0 ^{ab} 6 45-115 70	139,0 ^{∞l} 5 80-180 100	101,4 ^{abc} 11 62-120 58
Width of longest leaf **	Aver. N Min-Max. Range	15,0 ^{ab} 77 8-20 12	18,8 ^{ab} 46 14-27 13	14,8 ^{ab} 13 10-22 12	14,8 ^{ab} 15 9-22 13	16,6 ^{ab} 21 13-20 7	58,2 ^d 6 40-92 52	12,0° 6 5-18 13	33,4° 5 17-42 25	19,7 ^b 11 10-24 15

^{*)} ana= O. anatolica, ita = O. italica, lax = O. laxiflora, mor. O. morio subsp. morio, pur = O. purpurea, pro = O. provincialis, san = O. sancta, sim = O. simia, tri = O. tridentata

wide and large isodiametric cells, *O. simia* had some sinuous constrictions on tips (Fig. 3 e,f,h). Generally, stomata cell shapes are more or less circular (Fig. 2). *O. anatolica* stomata cells are circular with the size of $56.15 \times 57.70~\mu m$. In other species size was *O. provincialis* $66.50 \times 71.00~\mu m$ and *O. italica* $69.50 \times 74.50~\mu m$. *O. laxiflora* with the size of $40.00 \times 67.00~\mu m$ had the most different width and length (Table 4). The stomatal index varied from $29.12~\mu m$. (*O. sancta*) to $40.87~\mu m$. (*O. laxiflora*) (Table 4).

The cuticle surrounded both surfaces of the epidermal cells on both surfaces. Cuticle layers were striate and lyrate on the midrib and smooth on the margins on adaxial surface, even more smooth on abaxial surface. These results are almost similar to Aybeke et al. (2010). Although abaxial cuticle is thicker than adaxial, in O. anatolica and O. provincialis generally adaxial cuticle was thicker than abaxial cuticle. Aybeke et al. (2010) observed that O. laxiflora and O. purpurea had thicker cuticle on adaxial, but we oberved that these species had thicker cuticle on abaxial side. O. laxiflora had similar thickness on both adaxial and abaxial surface. The thickest cuticular layers were observed in O. provincialis (18.25 μ m/14.85 μ m abaxial/ adaxial) and O. sancta (18.30 μ m/14.90 μ m abaxial/ adaxial). O. purpurea, O. provincialis, O. sancta and O. tridentata were considerably different as regards the cuticular thickness on both surfaces (Table 5).

In the cross sections of lamina, the adaxial cells were larger than abaxial cells (Fig. 4a). On account of foliar xeromorphic features, adaxial epidermal cells are acting as water-storage tissues as reported by Aybeke et al. (2010) too. Adaxial epidermal cells are rectangular or rounded in shape, whereas abaxial epidermal cells are squared and rounded (Table 5). Anticlinal walls of epidermal cells are sinuous, curvilinear or smooth, periclinal walls are curvilinear. Stomata have a pore and ridge (Fig. 4b). The chlorenchyma cells are ovate, elliptic or round in shape with thin wall, and are homogenous (Fig. 4a). According to Aybeke et al. (2010) the mesophyll is heterogenous in O. laxiflora and O. morio subsp. morio, but in our investigations it was homogenous. They reported that the leaf mesophyll tissue of O. laxiflora and O. morio subsp. morio was heterogenous, but heterogenous leaves consisted of 2 types of cells such as spongy and palisade parenchyma cells (Stem, 1997b; Arditti, 1992; Yukawa and Stern, 2002; Stern et al., 2004; Stern and Carsward, 2006; Carlsward et al., 2006; Stern and Carsward, 2009). In this study, we found that the 2 species with homogenous leaves like other Orchis species also have spongy parenchyma cells.

In our study, the chlorenchyma has 7-13 layered spongy parenchyma, but is 9-19 layered around the midrib. According to Aybeke *et al.* (2010) thicker leaf chlorenchyma in *Orchis* taxa can

^{* =} Showed abnormal distribution (Kruskal-Wallis multi-test, Whitney-U binary test); ** = Variances are not homogenous (Dunnett T3) N= Number of plant individuals

Table - 3: Morphological features of flowers

Elements	ana⁺	ita⁺	lax⁺	mor⁺	pro⁺	pur⁺	san⁺	sim⁺	tri ⁻	
Bract length (mm)	Aver.	10,1 ^b	4,4ª	15,6 ^d	10,8 ^{bc}	11,3 ^{bc}	2,5ª	16,1 ^d	3,7ª	9,7 ^b
- , ,	N	68*	45	13	15	21	6	15	5	9
	Min-Max.	5-17	1-8	9-25	7-15	7-15	2-3	11-23	1,5-5	7-14
	Range	12	7	16	8	8	1	12	3,5	7
Dorsal sepal length (mm)	Aver.	8,0ª	12,5 ^d	8,8 ^{ab}	8,2a	9,5 ^{abc}	8,7ª	11,4 [∞]	12,6 ^d	10,9 ^{bcd}
,	N	68*	44	13	15	21	6	15	5	9
	Min-Max.	6-11	9-18	6-11	6-10	8-11	6-10	10-14	11-14	8-13
	Range	5	9	5	4	3	4	4	3	5
Lateral sepal length (mm)	Aver.	8,6ª	13,6 ^d	9,8 ^{ab}	9,1 ^{ab}	10,2 ^{abc}	9,7 ^{ab}	11,3 ^{bcd}	13,2 ^d	12,4 [∞]
. • ,	N	68*	44*	13	15	21	6	15	5	9
	Min-Max.	6-11	8-19	8-12	6-11	8-12	7-11	10-14	9-16	9-15
	Range	5	11	4	5	4	4	4	7	6
Labellum length **(mm)	Aver.	10,3ª	16,2⁵	7,9ª	9,4ª	9,8ª	10,2a	10,1ª	9,4a	10,2ª
0 ()	N	68	44	13	14	21	6	15	5	9
	Min-Max.	7-16	8-21	6-10	7-13	8-12	9-12	7-13	7-12	7-12
	Range	9	13	4	6	4	3	6	5	5
Labellum width**(mm)	Aver.	12,6 [∞]	7,9 ^b	14,6 ^d	9,9 ^{bc}	11,7 [∞]	10,8 ^{bc}	7,7 ^b	3,2ª	9,4 ^{bc}
()	N	68	44	13	14	21	6	15	5	9
	Min-Max.	9-16	3-14	11-19	7-13	8-15	9—12	7-9	2-4	5-15
	Range	7	11	8	6	7	3	2	2	10
Petal length (mm)	Aver.	6,2ª	6,7ª	7,3 ^{ab}	6,8ª	8,5 ^b	6,3ª	7,0a	10,0°	8,7 ^{bc}
	N	68*	44*	13	15	20*	6	15	5	9
	Min-Max.	4-9	4-9	6-10	5-8	7-9	5-7	6-8	8-11	7-10
	Range	5	5	4	3	2	2	2	3	3
Caudiculum (mm)	Aver.	1,7 ^d	1,8 ^d	1,6 ^{abc}	1,1 ^{ab}	1,5 ^{abc}	2,0 ^d	2,8e	1,2 ^{ab}	1,0ª
()	N	63*	36*	13	13*	21*	6	15*	5	8
	Min-Max.	1-2	1-2	1-2	1-1,5	1-2	2-2	2-3	1-2	1-1
	Range	1	1	1	0,5	1	0	1	1	0
Spur length (mm)	Aver.	19,9 ^f	6,5 ^{abc}	10,7 ^{de}	8,9 ^{cd}	13,5°	4,5ª	8,1 ^{bcd}	4,8 ^{ab}	8,2 ^{bcd}
opai iongai (iiiii)	N	68	44*	13	15	21	6	15	5	9
	Min-Max.	13-30	5-9	7-13	7-11	11-16	4-5	7-9	4-6	6-10
	Range	17	4	6	4	5	1	2	2	4
Ovary length (mm)	Aver.	16,6°	12,4 ^{ab}	15,3 ^{bc}	13,6 ^{abc}	5 17,3°	11,0ª	2 10,4ª	12,2 ^{ab}	11,8 ^{ab}
Ovary length (min)	N	68	45*	13,5	15,0	21	6	15,4	5	9
	Min-Max.	12-22	7-17	11-21	9-18	14-20	7-15	7-14	11-13	9-14
	Range	10	10	10	9	6	8	7-14	2	5

^{* =} Showed abnormal distribution (Kruskal-Wallis multi-test, Whitney-U binary test); ** = Variances are not homogenous (Dunnett T3); N= Number of plant individuals **Table - 4:** Leaf anatomical characters (surface)

Taxa	Epidermal cells	shape	Epidermal cells si	ze (width-length)	Stomata (width-length)		
Tuxu	Abaxial	Adaxial	Abaxial (μm)	Adaxial (µm)	Size (µm)	Index (%)	
ana⁺	thin rectangular	short-wide polygonal	60.25 × 153.58	94.42 × 170.25	56.15 × 57.70	34.88	
ita⁺	polygonal	polygonal isodiametric	80.00×180.00	129.50×211.80	69.50 × 74.50	33.82	
lax⁺	very thin very elongated rectangular	narrowed elongated rectangular	37.50 × 281.00	49.33 × 320.65	40.00 × 67.00	40.87	
mor*	thin, very elongated rectangular	elongated polygonal rectangular	39.50 × 212.50	77.00 × 218.00	50.00 × 67.50	30.94	
pro⁺	wide polygonal rectangular	wide short polygonal	73.50 × 154.50	106.50 × 146.00	66.50 × 71.00	29.41	
pur*	wide polygonal pentagonal hexagonal	polygonal circular pentagonal hexagonal	129.33 × 151.34	184.66 × 192.00	58.66 × 66.00	32.65	
san⁺	thin very elongated rectangular	wide polygonal	59.50 × 255.00	103.00 × 226.50	58.00 × 55.00	29.12	
sim ⁺	short polygonal rectangular	narrowed elongated polygonal	95.34 × 186.67	115.30 × 285.34	63.34 × 73.34	33.53	
tri*	rectangular,	wide elongated polygonal	69.34 × 210.00	137.67 × 234.65	48.00 × 61.39	36.99	

^{*)} ana= O. anatolica, ita = O. italica, lax = O. laxiflora, mor. O. morio subsp. morio, pur = O. purpurea, pro = O. provincialis, san = O. sancta, sim = O. simia, tri = O. tridentata;

Table - 5: Leaf anatomical characters (Cross-section)

	Cuticular	type	Cuticular thickness		Epidermal cel	l shape	Chlorenchyma	Mesophyll		
Taxa	Abaxial	Adaxial	Abaxial (µm)	Adaxial (μm)	Abaxial	Adaxial	General	On midrib	Lac.	Rap.
ana⁺	striate	striate	5.00	6.50	squared-circulary	elongated wide rectangular	8-10	11-13	-	+
ita⁺	lyriate	lyriate	12.30	10.60	squared-circulary	rectangular or circular ovate	8-9	11-13	+*	+
lax⁺	lyriate	lyriate	10.65	10.00	small squared	narrowed rectangular	8-10	15-17	+*	+
mor*	lyriate to smooth	lyriate to smooth	11.25	7.50	squared	ovate	7-10	9-11	-	+
pro⁺	lyriate	striate	15.00	18.34	squared-circulary	elongated rectangular	8-10	12-15	-	-
pur*	striate	striate	18.25	14.85	small squared rectangular	large rectangular	10-13	17-19	-	+
san⁺	striate	striate	18.30	14.90	squared-circulary	large rectangular	9-11	12-14	+*	+
sim⁺	obviously lyriate	obviously lyriate	8.40	6.65	squared	large rectangular	7-8	11-14	+*	+
tri⁺	obviously striate	obviously striate	15.00	11.65	squared-circulary	squared-rectangular	5-10	11-15		-

Lac. = Lacunae, Rap. = Raphide, * = rare

Table - 6: Distribution of Orchis species and their numbers according to their habitat*

Habitat	ana⁺	ita⁺	lax⁺	mor*	pro⁺	pur⁺	san⁺	sim⁺	tri ⁺
Olive farmland	1	2(2)					2(1)		1
Macchie (Shrubland)	25 (4)	13 (5)	2(2)	1			3	2(1)	5 (2)
Meadows(Pasture)	4(1)	3(1)	8 (3)	1 (1)	1		1 (1)		
Forest land	26 (8)	6 (4)	2	2 (1)**	3 (2)	2(1)	1(1)	3 (1)	4(1)
Abandoned Agr. Land	1(1)						2(2)		
Total	56	24	12	4	4	2	9	5	10

^{* =} Numbers in parenthesis refer to sites found in this study

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

Altitude belt	ana⁺	ita⁺	lax⁺	mor ⁺	pro⁺	pur⁺	san⁺	sim⁺	tri*
0 - 200	17 (6)	16 (10)	6 (2)	2(2)	3		14 (4)	3	1
200 - 400	9(2)	5 (2)	2(1)				2	1	1 (1)
400 - 600	16 (2)	2		2	2(1)		1 (1)	2	3
600 - 800	19(2)	2	4	6 (1)	4(2)		. ,	2	6
800 - 1000	16 (3)	1(1)	1	• •	` ,			6 (2)	6 (2)
1000 - 1200	15(2)		2						2
1200 - 1400	2		1			2(1)		2	
1400 - 1600	3			1		1(1)			
1600 - 1800					1 (1)				
Total	97	26	16	11	10	3	17	16	19

^{* =} Numbers in parenthesis refer to sites found in this study

be correlated with denser inflorescence, higher flower numbers, and good seed productivity irrespective of dry environmental conditions (Table 5).

O. provincialis, O.tridentata, O. laxiflora had raphide bundles in mesophyll cells. O. laxiflora had wide lacunae as observed by Aybeke et al. (2010) as well. In O. laxiflora growing in moist habitats, large intercellular spaces of O. laxiflora can be related

to its habitat (Aybeke et al., 2010). O. laxiflora samples were collected from moist meadows during this study (Table 6). The wide lacunaes in mesophyll are related with habitat of species. We also observed few lacunaes in mesophyll of O. italica, O. sancta, O. simia specimens. The lacunae are positioned besides the midrib (Table 5). Vascular bundles are collateral, comprised of xylem, phloem and sclerenchyma cells. In chlorenchyma these

^{** =} Forest gaps

^{*)} ana= O. anatolica, ita = O. italica, lax = O. laxiflora, mor. O. morio subsp. morio, pur = O. purpurea, pro = O. provincialis, san = O. sancta, sim = O. simia, tri = O. tridentata;

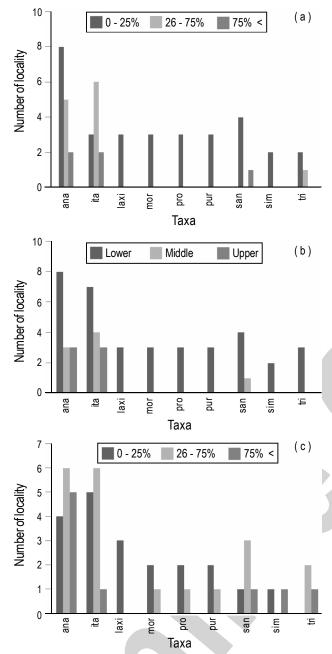


Fig. 5: Distribution of the *Orhcis* species according to (a) slope degree, (b) slope position and (c) surface stoniness; ana= O. anatolica, ita= O. italica, lax= O. laxiflora, mor= O. morio subsp. morio, pur:=O. purpurea, pro= O. provincialis, san= O. sancta, sim= O. simia, tri= O. tridentata.

are layered and vascular bundles alongside the midrib are larger than others.

Ecology: Habitat type selection of *Orchis* species according to different ecological conditions demonstrates variance. The more the ecological demands of the species come closer to each other the more they show inclination to grow on the same site, while individuals belonging to a different species could also appear as an exception. 162 sites are described on the basis of altitudinal characteristics. The altitudinal amplitude of the species is as follow: *O. anatolica* 0-

1600m, *O. italica* 0-1000m, *O. laxiflora* 0-1400m, *O. morio* subsp. *morio* 0-1600m, *O. provincialis* 0-1800m, *O. purpurea* 1200-1600m, *O. sancta* 0-600m, *O. simia* 0-1400m and *O. tridentata* 0-1200m (Table 7). While *O. sancta* generally is seen at lower altitudes, *O. purpurea* flourishes among the mountainous lands. Tsiftsaisa *et al.* (2008) stated that *Orchis* species demonstrate extensive vertical distribution. *O. purpurea* exists at lower altitudes such as 80 m in eastern Maccedonia which is located at upper meridians, it may be growing at lower altitudes in Tekirdag and Balikesir cities of Turkey (Tsiftsisa *et al.*, 2008). But nonetheless this situation is not true for the species *O. italica*.

Orchis species have a wide range of habitats (Rasmussen, 1995). The companions in different habitats of Orchis species are: olive farmland 6, macchie (+ shrubland) 51, meadows (+pastureland) 18, forest land 48 and 3 species were recorded on abandoned agricultural lands (Table 6). Olive farmlands are common habitats for the Orchis species. O. anatolica and O. sancta are distributed on abandoned agricultural lands. O. laxiflora generally prefers meadows. Orchis species generally show their major distribution in macchie and forests when the gaps are closed the Orchis population disappears from the site. It can be concluded that habitat management carries importance from the aspect of sustainability of the diversity (Willems, 1981). We observed that O. italica and O. tridentata species generally select the vicinities of the brush group canopies where high organic matter is available. O. purpurea generally exists in the forest gaps (Jacquemyn et al., 2007). Tolerance to shade of the species reveals variance (Mckendrick, 1996). In general, tolerance ability to shade is efficient on habitat selection of the species.

Orchis species' distribution according to the slope inclinination of the field is as follow: on 31 sites slope ranges between 0-25%; in 12 sites slope ranges between 26-75% and on 5 sites slope ranges between >75%. Majority of species are distributed on flat type of field patches. Principally, the species O. anatolica and O. italica did not select specific slope degree (Fig. 5a).

According to the slope position, 36 sites were noticed at lower slopes, 8 at mid-slopes and 6 sites at upper slopes. Most of the species are distributed at down slope parts of the field. Principally the species *O. anatolica* and *O. italica* are found at all slope positions (Fig. 5b).

Distribution of the *Orchis* species according to surface stoniness reveals that 20 sites have 0-25% stoniness, 20 sites 26-75% and 9 sites 76%. *O. anatolica* and *O. italica* are distributed in all stony fields. *O. laxiflora* is distributed on the least number of stony sites (Fig. 5c).

Orchis species showed their major distribution between 0-25% slope groups. Since these sites coincide with the arable lands, sustainability of the Orchis species is threatened. Distribution areas of the Orchis species decrease as a consequence of shrinkage and degradation of habitats suitable for Orchis species (Cribb et al.

2003; Jacquemyn *et al.*, 2005; Kull and Hutchings, 2006; Tecimen *et al.*, 2010).

Ecologically convenient sites effect the morphological and anatomical features of the plants as is clear from the results presented in Table 3 and 4, Table 7 presenting ecological features; Table 6 and Figure 5 showing habitats. Ranges belonging to morphological characteristics widen along with selected habitat types. The morphological characteristic range of *O. provincialis* is less than the *O. italica* which is distributed on two and four respectively. A similar difference is observed in *O. italica* and *O. tridentata*.

The leaf anatomy of the family Orchidaceae is characteristic of the genus (Arditti, 1992; Stern, 1997b; Stern and Judd, 1999; Stern and Whitten, 1999; Yukawa and Stern, 2002; Stern *et al.*, 2004; Stern and Carsward, 2006; Carlsward *et al.*, 2006; Piwpuan and Thammathawom, 2008; Stern and Carsward, 2009). Anatomical features of the species demonstrate variance in relation with the altered habitat (Rada and Jaimez, 1992; Yukawa and Stern, 2002; Ciccarelli *et al.*, 2009). The results in this study revealed that there is a close correlation between anatomical features and ecological factors. Therefore, ecological conditions should be recorded while assessing the anatomical and morphological features.

Acknowledgement

Authors are grateful to Scientific and Technical Research Council of Turkey (TUBITAK) for financial support through Project number 1050530. We also thank Prof. Dr. Unal Akkemik for permitting us to work at the ISTO laboratory (Istanbul University, Faculty of Forestry, Department of Forestry Botany), EGE (Faculty of Science Herbarium of Ege University), ISTE (Faculty of Pharmacy Herbarium of Istanbul University), ISTF (Faculty of Sicence Herbarium of Istanbul University) Herbarium for their invaluable contribution and services; Master thesises (H.A. Aytepe, 2005; R.S. Gokturk, 1994; E. Kaya, 2004; S.T. Koruklu, 1997; B. Sahranc, 2001; D. Sari, 2003; L. Sik, 1992; H. Yolcu, 1998; A.H. Ucar, 2002; F. Palaz, 2006); Ph.D. thesises (E. Sezik, 1967; H. Demirelma, 2006); project reports; H. Pesmen and A. Guner, 1976; O. Secmen and E. Leblebici, 1982) for the locality records.

References

- Altundag, E. and E. Sevgi: Vegetative anatomy of *Orchis italica* Poiret. (Orchidaceae) in Turkey. XIII OPTIMA Meeting Proceedings, 22-26 (2010).
- Arditti, J.: Fundamentals of Orchid Biology. USA, pp.692. (1992).
- Aybeke, M., E. Sezik and G. Olgun: Vegetative anatomy of some Ophrys, Orchis and Dactylorhiza (Orchidaceae) taxa in Thrace region of Turkey. Flora, 205, 73-89 (2009).
- Aytepe, H.A.O. and Varol: Flora of Bencik mountain (Yatagan Mugla). *Ecology.* **63**, 41-61 (2007).
- Carlsward, B.S., W.L. Stern and B. Bytebier: Comparative vegetative anatomy and systematics of the angraecoids (Vandeae, Orchidaceae) with an emphasis on the leafless habit. *Bot. J. Linn. Soc.* **151**, 165-218 (2006).
- Ciccarelli, D., L.M.C. Forino, M. Balestri and A. Maria: Leaf anatomical adaptations of *Calystegis* soldanella, *Euphorbia paralis* and *Otanthus*

- maritimus to the ecological conditions od coastal sand dune systems. *Caryologia.*, **62**, 142-151 (2009).
- Cribb, P.J., S.P. Kell, K.W. Dixon and R.L. Barrett: Orchid conservation: A global perspective. (Eds.: K.W. Dixon, S.P. Kell, R.L. Barrett, P.J. Cribb). Orchid conservation, Natural History Publications, Kota, Kinabalu, Sabah, pp.1-24 (2003).
- Deniz, I.G. and H. Sumbul: Flora of the Elmali Cedar Research Forest (Antalya/Turkey), *Turk J. Bot.* **28**, 529-555 (2004).
- Dusen, O. D. and H. Sumbul: Flora of the region between Sarisu-Saklikent. Herb J. System. Bot., 8, 29-60 (2001).
- Dreesler, R.L.: Phylogeny and classification of the orchid family. Dioscorides Press, p. 314 (1993).
- Duran, A.: Flora of Tuzakli, Otluk, Gidefi mountains and surroundings (Akseki). Turk J. Bot. 26, 303-349 (2002).
- Foroughbakhch R., R.J. Ferry Sr, J.L. Hernández-Piñero, M.A. Alvarado-Vázquez and A. Rocha- Estrada: Quantitative measures of leaf epidermal cells as a taxonomic and phylogenetic tool for the identification of *Stanhopea* species (Orchidaceae), *Int. J.Exp. Bot.*, 77, 113-127 (2008).
- Hidayat, T. and K. Kusdianti: Stomata diversification and phylogenenetic analysis of 13 species of family Euphorbiaceae sensu lato, Biodiversitas, 10, 19-22 (2009).
- Jacquemyn, H., R. Brys, M. Hermy and J.H. Willems: Does nectar reward affect rarity and extinction probabilities of orchid species? An assessment using historical records from Belgium and the Netherlands *Biol. Conserv.*, 121, 257–263 (2005).
- Jacquemyn, H., R. Brys, K. Vandepitte, O. Honnay, I. Roldán-Ruiz and T. Wiegand: A spatially explicit analysis of seedling recruitment in the terrestrial orchid *Orchis purpurea*. New Phytologist, 176, 448–459 (2007).
- Jakubska, A.: The analysis of morphological differentiation of the epidermis of selected species of the genus *Epipactis Zinn*, 1757 (Orchidaceae: Neottieae). Wroclaw, 14, 41-45 (2007).
- Kalipsiz, A.: Statistical Methods. Istanbul University Forestry Faculty Publications Nu: 2837 / 294, (1981).
- Kreutz, C.A.J.: Orchidaceae. In: Flora of Turkey and the East Aegean islands (*Eds.*: A. Guner, N. Ozhatay, T. Ekim, K.H.C. Baser). Edinburg UniversityPress,Edinburgh, **11**, 274 303 (2000).
- Kreutz, C.A.J.: Orchids of Turkey, Botanical Properties, Ecological Requirements, Natural Spreading Sites, Vital Threats, Precautions for Protection (Trans. & Cont.: A. Colak), Rota Publications, pp. 55-848 (2009)
- Kull, T. and M.J. Hutchings: A comparative analysis of decline in the distribution ranges of orchid species in Estonia and the United Kingdom. *Biol. Conserv.*, 129, 31 –39 (2006).
- Mckendrick, S.L.: The effects of shade on seedlings of *Orchis morio* and *Dactylorhiza fuchsii* in chalk and clay soil. *New Phytol.* **134,** 343-352 (1996).
- Ozdamar, K.: Statistical data analysis with packed program 1 SPSS MINITAB. Kaan Kitabevi, (2002).
- Piwpuan, N. and A. Thammathaworn: Leaf and root anatomy of Acriopsis reinw. ex Blume (Orchidaceae) in Thailand. KKU Sci. J., 36, 58-63 (2008).
- Rada, F. and R. Jaimez: Comparative ecophysiology and anatomy of terrestrial and epiphytic Anthurium bredemeyeri Schott in a tropical Andean Cloud Forest. J. Exp. Bot., 43, 723-727 (1992).
- Rasmussen, H.N.: Terrestial orchids; from seed to mycotrophic plant. Cambridge University Press, (1995).
- Renz, J. and G. Taubenheim: Orchis L. (Orchidaceae), In: Flora of Turkey and the East Aegean islands (Eds.: P.H. Davis) . Edinburgh, University Press, Edinburgh. 8, 451-600 (1984).
- Ruzin, S.E.: Plant microtechnique and microscopy. Oxford University Press, Oxford, NewYork, (1999).
- Sezik, E.: Our orchids, The orchids of Turkey, Sandoz Cultural Publications, 6, P. 166 (1984).
- Stern, W.L.: Vegetative anatomy of subtribe Habenariinae (Orchidaceae). Bot. J. Linnean Soc., 125, 211-227 (1997 b).

- Stern, W.L. and W.S. Judd: Comparative vegetative anatomy and systematics of *Vanilla* (Orchidaceae), *Bot. J. Linnean Soc.*, 131, 353-382 (1999).
- Stern, W.L. and W.M. Whitten: Comparative anatomy of Stanhopeinae (Orchidaceae). *Bot. J. Linnean Soc.*, **129**, 87–103 (1999).
- Stern WL, W.S. Judd and B.S. Carlsward: Systematic and comparative anatomy of Maxillarieae (Orchidaceae), sans Oncidiinae. Bot. J. Linnean Soc., 144, 251–274 (2004).
- Stern, W.L. and B.S. Carlsward: Comparative vegetative anatomy and systematics of the Oncidiinae (Maxillarieae, Orchidaceae). *Bot. J. Linnean Soc.*, **152**, 91–107 (2006).
- Stem, W.L. and B.S. Carlsward: Comparative vegetative anatomy and systematics of Laeliinae (Orchidaceae). *Bot. J. Linnean Soc.*, **160**, 21-41 (2009).
- Senol, S.: Non-parametric statistical methods. Ege University Science Faculty Publications, 190, (2004).
- Tecimen, H.B., O. Sevgi, O. Kara E. Sevgi, E. Altundag and I. Bolat: The problems of *Salep* species of Turkey and solution suggestions. *J. West. Mediter. Fores. Institute*, **10**, 1-30 (2010)

- Tsiftsisa, S., I. Tsiripidisa, V. Karagiannakidoua and D. Alifragisb: Niche analysis and conservation of the orchids of East Macedonia (NE Greece). Acta Oecologica, 33, 27-35 (2008).
- Unal, O. and M. Gokceoglu: Flora of Akdeniz University Campus (Antalya Turkey). J. Akdeniz Uni. Agri. Faculty. 16, 143-154 (2003).
- Vardar, Y., Y. Gemici, N. Tort and M. Gemici: Plant Anatomy. Ege Univ. Faculty of Science Publications, 196, (1987).
- Varol, O., M. Isiloglu, R. Mammadov, A.R. Girgin and M. Yabanli: Research on Geohytes of Mugla. Mugla University Publications, 56, (2004).
- Willems, J. H.: Establishment and development of a population of *Orchis simia* Lamk. in the Netherlands, *New Phytol.*, **91**, 757-765 (1981).
- Yukawa, T. and W.L. Stern: Comparative vegetative anatomy and systematics of *Cymbidium* (Cymbidieae: Orchidaceae). *Bot. J. Linnean Soc.*, **138**, 383-419 (2002).
- Zarinkamar, F.: Density and size distribution of stomata in different monocotyledons. *Pak. J. Biol. Sci.*, **9**, 1650-1659 (2006).