# STUDIES ON THE MORPHOLOGY, ANATOMY AND ECOLOGY OF *OPHRYS LUTEA* CAV. SUBSP. *MINOR* (GUSS.) O. DANESCH & E. DANESCH EX GÖLZ & H.R. REINHARD (ORCHIDACEAE) IN TURKEY

# CENK DURMUSKAHYA<sup>1</sup>, CANAN OZDEMIR<sup>2</sup>, BAHATTIN BOZDAG<sup>2</sup> AND MÜNIR ÖZTÜRK<sup>3</sup>

<sup>1</sup>Celal Bayar University Education Faculty, Demirci Manisa, Turkey <sup>2</sup>Celal Bayar University, Art and Science Faculty, Biology Department, Manisa, Turkey <sup>3</sup>Botany Department, Ege University, Izmir, Turkey & ICCBS, University of Karachi, Karachi, Pakistan.

### Abstract

In this study, morphological, anatomical and ecological characteristics of *Ophrys lutea* subsp. *minor* in Turkey were investigated. The plant material was collected from 30 different populations during 2010-2013. In all 19 morphological and 20 anatomical features were investigated, and habitat characteristics recorded. The soil samples were also collected from the plant sampling sites and subjected to an analysis for 18 characteristics. The minimum plant length was 69 mm and maximum 323 mm, minimum length of underground part was 29 mm, and maximum 49 mm, and the number of leaves varied between 1-10. The ecological characteristics revealed that *O. lutea* subsp. *minor* flourishes well from sea level to 800 m, and the most common habitats of *O. lutea* subsp. *minor* are rocky limestones, phrygana, macchie, olive gorves, woodland margins.

# Introduction

Orchids are among the rarest and fascinating plants in the world, stimulating great enthusiastic interest from botanists (Kreutz, 2009). They are the most researched family of the plant kingdom. The genus *Ophrys* is one of the very attractive groups among orchids due to their vivid colours, shapes and interesting pollinating system, therefore commonly known as bee orchids due to the resemblance of flowers of some species to the furry bodies of bees and other hymenopteras. The scientific name *Ophrys* means "eyebrow" in Greek language referring to the furry edges of the lips of several species (Pridgeon, 1992).

The name was first mentioned in the book "Natural History" by Pliny the Elder (23-79 AD) (Pliny et al., 1956), then categorized as a special group in 1753 by Linneus (Linneus, 1753). *Ophrys* species generally are terrestrial orchids distributed from Scandinavia in the north to northern Africa in the south and from Ireland, Portugal and the Canary Islands in the west, to Russia and Iran in the east, with its main concentration of species in the Mediterranean countries.

The genus includes wintergreen species with a rosette of leaves formed in the autumn followed by flowers in March/April. Its summer dormancy begins during the flowering period and sometimes before the leaves die back.

A combination of different colours together with the hairs and odours of the labellum, they attract male bees, wasps and bumblebees - promising them a virgin female to mate with (Arditti, 1992). The unusual flower structure is attracting not only male bees but also many botanists, horticulturists and naturalists.

*Ophrys* genus has two sections: Euophrys and Pseudophrys. Euophrys is paraphyletic, because it is not clear whether all descendants are of a recent common ancestor (which ties the group together). Therefore molecular analysis and a possible review of the genus is overdue. One of the results is that species, subspecies and hybrids are constantly revised, making identification a difficult task. The two sections were proposed by Godfery in 1928 and adopted by others (Delforge, 2006; Devillers & Devillers-Terschuren, 2004). The members of the sections were assigned on the

basis of how the insect mounts the flower. Pseudophrys species attach pollinia to the pollinator's abdomen – this has been confirmed by comparison of flower features (Pedersen, & Faurholdt, 2007).

*Ophrys lutea* is one of the common *Ophry* species included in the Pseudophrys section and Ophrys lutea group and is found in many Mediterranean countries (Kreutz, 2009; Delforge, 2006) (Fig. 1). A total of 7 species and one subspecies have been placed in this group namely; *O. melena, O. sicula, O. phryganae, O. lutea, O. lutea subsp. quarteirae, O. corsica, O. praemelena* and *O. galilaea* (Devillers & Devillers-Terschuren, 2013).

*Ophrys lutea* group is represented by *O. lutea* subsp. *minor* and *O. lutea* subsp. *phryganae* in Turkey. In this study; we have tried to enlighten the morphological, anatomical and ecological characteristics of *O. lutea* subsp.*minor*, known locally as sari salep or kucuk salep in Turkey (Tuzlaci, 2006). It is one of the well known species of orchids followed by the locals.

#### Materials and Methods

*Ophrys lutea* Cav. subsp. *minor* (Guss.) O.Danesch & E.Danesch ex Gölz & H.R.Reinhard samples were collected from 30 populations in Turkey during the years 2010-2013. The herbarium specimens are deposited in the Education Faculty Herbarium of Celal Bayar University, Manisa, Turkey. The morphological features were recorded on randomly selected 96 individuals. 20 morphological parameters were observed including 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.

During the field studies, besides plant sample collection for recording morphological features, soil samples were also collected and habitat characteristics were also recorded. Plant samples were fixed in 70% alcohol for anatomical studies. Anatomical Sections of the root, stem and leaf were taken. Sartur reactive was applied to the sections for better tissue differentiation.



Fig. 1. Distrubution of Ophrys lutea in the World.

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 was measured with micrometer ocular as minimum, maximum, and medium and standard deviation calculated.

Soil samples were collected from the localities given in the Table 1. The surface layer of the soil was removed and the soil samples taken from 0-5 and 5-15 cm depths for analysis. These were kept in the polyethylene bags and immediately brought to the laboratory. The samples were air dried, ground, passed through a 2 mm sieve and subjected to 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 Bremmer (Bremmer, 1965), organic carbon according to Nelson & Sommers (Nelson & Sommers, 1982) and C:N ratios were calculated.

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

# **Result and Discussion**

**Description:** Plants slender 7-30 (-40) cm. leaves  $\pm$  basal, 3-5, broadly lanceolate to ovate. Spike 1-7 flowered. Sepals green, ovate; laterals spreading; dorsal erect, somewhat hooded. Petals are linear, obtuse.  $\frac{1}{2}$  -1/3 x sepals, yellowish – green. Labellum spreading , 3-lobed above middle, to 8(-11) x 12 mm, with an intensely yellow, glabrous, flattened marginal zone to mm broad (sometimes indistinct or much reduced); lateral lobes not overlapping; middle lobe notched or bilobed at apex, with

two dark purplish-browns divergent patches; speculum pale greyish-blue, shining, surrounded by a brown or purplish brown velvety zone. Column obtuse. Flowering time; 3-4. It prefers rocky limestone slopes, phrygana, macchie, olive groves, cemeteries, woodland margins, open *Pinus brutia* forests, grows between 1-600 m (Renz & Taubenheim, 1984).

**Morphological features:** In all 20 morphological features of *O. lutea* subsp. *minor* were examined. For each feature, range, minimum, maximum, mean and standard deviation are given in the Table 2. Numerical description of *O. lutea* subsp. *minor* is not given completely in the Flora of Turkey by Renz & Taubenheim (Davis, 1988).

The results of anatomical features were compared with the information published in the Flora of Turkey (Davis, 1988) and European *O. lutea* using "The Bee Orchids of Europe" (Pedersen & Faurholdt, 2007) and Bee Orchids of Greece (Antonopaulos, 2009).

Several measurements recorded during this study differ from the records on *O. lutea* subsp. *minor*. According to the Flora of Turkey (Davis, 1988) and Bee Orchids of Europe (Pedersen & Faurholdt, 2007), the flower number is mentioned as 1-6. In our study, flower numbers were recorded as 1-10. Labellum length has been given as 8-12 mm in the Flora of Turkey, 9-12 mm in *Ophrys* of Andalusia (Renz & Taubenheim, 1984), 14-18 mm in Bee Orchids of Greece (Antonopaulos, 2009) and 13-19 mm in Bee Orchids of Europe (Pedersen & Faurholdt, 2007). In our study it was recorded as 10-18 mm. In the former studies the lengths of the plant have been reported to lie between 5-40 cm. In this study it was recorded as 6. 9-32.3 cm.

Among the 96 *O. lutea* subsp. *minor* samples observed by us and evaluated for 20 morphological characters, a correlation analysis was done which revealed that both generative (1-7) as well as vegetative characters (8-20) (Table 2) are of diagnostic nature for introducing the species numerically.

Sampling point	Plant	Soil	Altitude (m)	Slope (%)	Aspect	Locality
1	2	+				
2	2		100	5	Northeast	Izmir Çeşme Dalyan
3	1	+	1	5	West	Izmir Çeşme Çiftlik
4	5	+	90	25	Northeast	Izmir Karaburun Sarpıncık
5	1		61	10	East	Izmir Karaburun Bozköy
6	2	+	12	40	East	Izmir Balıklıova
7	3	+	25	10	Southwest	Izmir Seferihisar
8	1		90	35	West	Izmir Selçuk
9	2	+	178	15	Southwest	Izmir Selçuk Şirince
10	3		560	60	South	Izmir Sabuncubeli
11	2	+	116	55	West	Izmir Kemalpaşa Karabel
12	1		23	35	South	Izmir Gümülüdür
13	3	+	200	25	West	Izmir Foça
14	2	+	110	15	South	Izmir Çicekli Village
15	2	+	330	50	South	Izmir Kemalpaşa Kavaklıdere
16	5		090	65	South	Aydin Söke Priene
17	9	+	210	20	North	Aydin Kuşadası Dilek Yarımadasi
18	6	+	2	10	Northwest	Aydın Didim Akköy
19	7	+	125	18	North	Muğla Milas Bafa
20	5	+	270	33	West	Muğla Milas Güllük
21	2	+	54	16	Northwest	Muğla Milas Ovakışlacık
22	3		120	40	West	Muğla Milas Euromos
23	10	+	800	25	South	Muğla Yerkesik
24	3	+	197	20	South	Muğla Bodrum Faralya
25	4		510	40	Southwest	Muğla Fethiye Çukurincir Village
26	2		600	60	Southeast	Antalya Emiraşıklar
27	5	+	20	45	South	Antalya Kas Ağullu
28	1		70	20	Northeast	Çanakkale Bozcaada Tuzburnu
29	1	+	40	10	North	Çanakkale Bozcaada Merkez
30	1	+	10	5	Northeast	Çanakkale Gokceada Kuzulimanı

Table 1. List of the sampling sites.

# Table 2. Morphological characters of Ophrys lutea subsp. minor.

Characters	Ν	Min	Max	Mean	Std. Dev.
Length (mm)	30	69	323	206.97 + 1.20	66.20
Underground part (mm)	30	29	49	38.10 + 1.02	5.62
Tuber length (mm)	30	16	39	30.13 + 1.00	5.51
Tuber width (mm)	30	12	33	21.73 + 1.08	5.91
Leaf number	37	2	6	4.24 + 018	1.14
Length of longest leaf (mm)	30	37	129	$89.21 \pm 0.48$	2.63
Width of longest leaf (mm)	30	10	19	10.33 + 055	3.04
Length of shortest leaf (mm)	31	21	55	39.64 + 1.63	9.11
Width of shortest leaf (mm)	30	9	31	17.10 + 1.06	5.83
Bract length (mm)	30	10	24	18.36 + 060	3.32
Dorsal sepal length (mm)	30	4	11	7.20 + 0.42	2.35
Lateral sepal length (mm)	30	6	19	$9.80 \pm 0.51$	2.84
Labellum length (mm)	30	9	20	14.86 + 0.62	3.40
Labellum width (mm)	30	5	11	8.20 + 0.33	1.82
Petal length (mm)	30	3	7	$4.83 \pm 0.22$	1.23
Ovary length (mm)	30	8	19	13.20 + 0.57	3.15
Caudiculum	30	1	3	$1.83 \pm 0.15$	0.83
Pollinia	30	2	3	2.46 + 0.92	0.50
Number of flowers	30	1	10	4.20 + 0.41	2.28

The morphological features of O. lutea subsp. minor are as follows; the plant and underground part lengths vary between 69-323 mm, number of leaves lies between 2-6. Length of basal leaves varies between 37-129 mm, width of longest leaf is 10-19 mm, that of shortest leaf between 21-55 mm., and width of longest leaf was 9-31mm. Flowers were 1-10, generally bright yellow in colour (Ascensao et al., 2005). Labellum had brown markings on the central part and was covered with yellow stripe on the edge. The width of this stripe was 1-4 mm. In a few samples another small, rounded, brown markings on the lateral sides of the labellum were observed. This feature has not been recorded in the previous studies. Dorsal sepal length was 4-11 mm. and lateral sepal length 6-13 mm. Labellum was 9-20 mm. x 5-11mm. (width x length). Petal length was 3-7 mm. Ovarium length was 8-19 mm. Caudiculum was 1-3 mm. pollinium was 2-3 mm.

Besides the aerial parts, underground parts of *O*. *lutea* subsp. *minor* were also examined and tuber length was recorded to lie between 16-39 mm and width varied between 12-29 mm.

Leaf anatomy: Transversal section of the leaf of *O. lutea* subsp. *minor* shows that there is a cuticle layer covering the adaxial and abaxial epidermis. Adaxial epidermal cells are bigger than abaxial ones, located outwardly making a chamber and are periclinally elongated, whereas abaxial epidermal cells are anticlinally elongated (Table 3). No differentiation visible between the spongy and palisade parenchyma in the mesophyll layer. Mesophyll layer is generally thin and sometimes made up of single cell layer. Thick mesophyll layer present only adjacent to the vascular bundles. Air lacunas are found regularly in the leaves (Fig. 2a).

**Stem anatomy:** The anatomical sections of the stem depicts that it is covered with a thin cuticle layer,

followed by single cell layer of epidermis. These are wider than long, some epidermal cells have got papilla. The epidermis is followed by cortex made up of parenchymatous cells followed by thick cell walled collenchyma cells which give durability to the stem. The central part of the stem is filled with pith cells. There are 2-5 layered parenchyma cells lying between the vascular bundles and collenchyma. Vascular bundles are collateral, resembling the dicotyledonous plants and arranged with regular spaces. Xylem elements are clearly visible but not the phloem elements because of the thin cell walls. The pith has many lacunas in the centre of stem due to the breakup of pith into pieces (Fig. 2b).

Existence of collenchyma cells in the *Ophrys* has been reported in other studies as well (Aybeke *et al.*, 2010), but it is reported to consist of 8-11 layers, but in our study it was seen that they consist of 5-8 layers, some starch grains are observed in the sections.

**Root anatomy:** An evaluation of the root anatomy revealed that outermost layer has 1-2 cell rows. These cells are sometimes periclinally, sometimes anticlinally elongated or equal sized. Epidermis layer did not show a stable shape. Generally formed of 5-9 layers. Cortex cells generally rounded in shape but some cells were uniform and their length reached up to 165 µm. smaller cortex cells were usually found closer to the pith and epidermis. Bigger cortex cells were usually found in the middle part. Fungal peletons and hypae were detected only in the outer and middle cortical cells (Aybeke *et al.*, 2010; Carlsward & Stern, 2009). Similar results have been reported by several authors with different material infected by fungi or by bacteria (Willamson & Handley, 1969; Barnasso & Pais, 1990) (Fig. 2c).

Some bigger cortex parenchyma cells located in the middle were darker probably due to the deposition of parenchyma cells.

	Width			Lenght				
	Min	-	Max	Mean ± S.D.	Min	-	Max	Mean ± S.D.
Root								
Epidermis	26.67	-	100.00	$63.74\pm23.72$	6.67	-	86.67	$46.53\pm26.10$
Cortex parenchyma	40.00	-	140.00	$88.27 \pm 31.91$	20.00	-	113.33	$68.80\pm36.07$
Endodermis	16.67	-	33.33	$25.42\pm5.07$	6.67	-	20.00	$13.43\pm4.58$
Xylem (diameter)	6.67	-	26.67	$15.11\pm6.88$				
Stem								
Cuticle length					1.33	-	4.67	$3.05 \pm 1.07$
Epidermis	13.33	-	46.67	$31.19\pm9.22$	11.33	-	45.33	$26.00\pm10.53$
Cortex parenchyma	33.33	-	66.67	$53.33 \pm 8.84$	26.67	-	80.00	$55.69 \pm 18.49$
Collenchymas cells (diameter)	20.00	-	60.00	$37.33 \pm 11.00$				
Xylem cells (diameter)	3.33	-	20.00	$11.88 \pm 5.13$				
Pith	40.00	-	133.33	$92.35\pm27.72$	20.00	-	86.67	$59.10 \pm 19.76$
Leaves								
Adaxial cuticle					5.48	-	6.45	$5.95\pm0.29$
Adaxial epidermis	29.03	-	54.84	$40.61\pm7.06$	25,81	-	70,97	$47,\!17 \pm 14,\!40$
Mesophyll cells	25,81	-	48,39	$36,19 \pm 6,82$	12,90	-	41,94	$27,31 \pm 8,18$
Abaxial epidermis	22,58	-	48,39	$35,37 \pm 9,23$	16,13	-	35,48	$24,74 \pm 6,30$
Abaxial cuticle length					2,26	-	6,45	$4,19 \pm 1,11$

Table 3. Anatomical features of O. lutea subps. minor.

S.H. = Standard deviation



Fig. 2a. Transverse section of leaf.

ad. Adaxial epidermis cell, ab. Abaxial epidermis cell, k. cuticule, ko. cortex collenchyma m. Mesophyll layer, ks. Xylem cells, kp. cortex parenchyma, ö. pitchparenchyma, e. Epidermis cells, en. Endodermis cells



Fig. 2b. Transverse section of stem.



Fig. 2c. Transverse section of root.

**Ecology:** *O. lutea* subsp. *minor* is distributed in the Western part of Anatolia, starting from Istanbul in the northwest and reaching Hatay in the south. The main populations are seen in Izmir, Aydın and Mugla provinces. There are some populations distributed in Çanakkale and Antalya provinces too. This species to grows naturally from sea level to 600 m (Davis, 1988). But in this study it was observed that this species can grow up to 800 m. especially in the province of Mugla (Fig 3a). There are very crowded populations found in this province between 700-750 m (Durmuşkahya 2013). The species has been reported to grow at even 1800 m. in Europe (Webb *et al.*, 2010).

*O. lutea* subsp. *minor* is usually found in the phrygana together with some grassland species (Ozturk *et al.*, 2012), macchie, among the olive groves, around the cemeteries, in woodland margins and open *Pinus brutia* forests (Fig. 3b). The companion species in general are *O. tetrandhifera*, *O.mamosa*, *O. speculum* and *O. umblicata* and grow in the same habitat. The species is also seen together with the species from other orchid genera like *Orchis* and *Anacamptis* (Sevgi *et al.*, 2012; Sevgi *et al.*, 2012; Altundag *et al.*, 2012). It prefers phrygana formations too much than others. A similar finding has been reported from the Ioninan Island and Peloponesse (Antonopoulos, 2009), but studied species can be found in dry to moist grasslands or oak woodlands in Europe (Pedersen & Faurholdt, 2007).

The soil preference of this species is generally for rocky limestone habitats in Anatolia. It is very difficult to see them in the mould, clay or on alluvial soils. The soil analysis results show that at 0-5 cm soil depth, stoniness is around 39.2% and these are midstony soils. The sand, silt and clay were 52, 21 and 26% respectively at this depth. pH of the soils lies at 7.45 which means that they are alkaline in nature. The soils poossess higher than medium of humus as organic matter. Total nitrogen content of the soils is 0.235% and C:N ratio 14.21.

Between 5-15 cm soil depth stoniness is nearly same as in the upper layer, stressing the fact that this species prefers stony soils. Many tubers are found between in 6-18- cm depth, and textural classes of these soils consist of 46% sand, 24% silt, and 30% clay. pH of the soils is alkaline, average value being 7.89. Total nitrogen content of soils is 0.189 and C:N ratio is 27.8 (Table 4).

In general the *Ophrys* species seem to prefer alkaline and poor soils. This is the reason that the species of *Ophrys* genus are more limited than the species of *Orchis*, *Anacamptis* and *Dactylorhiza*. Some reports show that almost all *Ophrys* species in Turkey are distributed in the West and South of Turkey where Mediterranean climate predominates and soils are stony in nature (Kreutz, 2009; Durmuşkahya, 2013).

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Fig. 3. Record numbers of Ophrys lutea % values a: according to altitude; b: habitat selection.

		Symbol	Ν	Min.	Max.	Mean	Std. Dev.
0-5 cm	Volume weight (g/lt)		20	649.00	1287.00	$941.55 \pm 41.17$	184.12
	Fine soil weight (g/lt)		20	421.00	1072.00	$753.11\pm46.49$	207.91
	Stone weight (gr/lt)		20	36.00	674.00	$326.91\pm46.34$	207.25
	Sand (%)		20	26.50	76.70	$53.38\pm3.30$	14.79
	Silt (%)		20	7.42	39.60	$25.26 \pm 1.94$	8.68
	Clay (%)		20	10.33	39.90	$22.46 \pm 1.98$	8.87
	pН		20	6.46	7.89	$7.10\pm0.09$	0.40
	Corg (%)		20	0.86	7.12	$4.39\pm048$	2.14
	Nt		20	0.07	0.61	$0.32\pm0.04$	0.17
	C/N		20	4.96	67.80	$32.85\pm4.25$	19.00
5-15 cm	Volume weight (g/lt)		20	861.00	1466.30	$1162\pm44.04$	196.94
	Fine soil weight (g/lt)		20	496.00	1146.00	$785.46\pm50.86$	227.47
	Stone weight (gr/lt)		20	131.00	912.00	$520.16\pm57.39$	256.66
	Sand (%)		20	22.60	61.20	$40.70\pm2.84$	12.71
	Silt (%)		20	7.99	43.56	$23.86 \pm 2.48$	10.83
	Clay (%)		20	14.61	46.81	$28.31\pm2.27$	10.16
	pН		20	6.38	7.92	$7.03\pm0.08$	0.36
	Corg (%)		20	0.46	4.38	$2.56\pm0.29$	1.33
	Nt		20	0.04	0.52	$0.23\pm0.03$	0.14
	C/N		20	4.12	106.70	$48.72\pm7.63$	34.16
	Altitude		20	1.00	850.00	$227.90\pm 64.82$	289.91
	Slope		20	0.00	70.00	$25.25 \pm 4.59$	20.55
	Surface stoniness		20	5.00	60.00	$27.00\pm3.61$	16.17

<b>Fable 4. Descriptive</b>	e statistics of	characteristics	of soil a	nd sampling points.
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