

Taxonomic and Ecologic Properties of the Endangered *Iris pamphylica* (Iridaceae) Endemic to S.W. Anatolia

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Abstract

In this research, the morphological, anatomical, palynological, karyological and ecological properties of the locally endemic *Iris pamphylica* Hedge, which were collected or observed between 2008 and 2014 in different localities of the Manavgat, Alanya and Akseki districts of Antalya province were examined. After field studies, a detailed morphological description was prepared with morphological observations and measurements carried out on bulbs, leaves, falls and standards of this rare endemic species. The species was recorded in new areas and at different altitudes. The seed morphology and pollen grains of *I. pamphylica* were examined using SEM techniques. Karyotypic ideogram and microphotographs of somatic metaphases were also obtained. To establish ecological preferences of *I. pamphylica*, certain parameters including distributions, habitat types, soil analyses and flowering times were studied and evaluated.

Keywords: Antalya, Conservation, Ecology, Iris, Taxonomy.

INTRODUCTION

In terms of rich plant biodiversity, Antalya is one of Turkey's richest provinces. Even considering the ecological richness of our country as a whole, Antalya has an important role with its rare species and ecological habitat diversity. The presence of sudden altitude changes within short distances, climatic variations from coastal to inland areas, as well as variable topography ensure rich diversity of species. Antalya province is home to 773 of the country's endemic plant taxa. 244 of them are best described as locally endemic as they are found only in the Antalya region. One of the important geophyte family of Antalya flora is Iridaceae with its 19 endemic taxa, 4 of them belonging to *Iris*.

Iris L. is one of the largest genus of the Iridaceae family and in the world, comprises over 300 species. Distributed in the Northern Hemisphere [1], *Iris* species have been used as ornamental plants in parks and gardens since ancient times, due to the very beautiful and colorful flowers [2]. Totally 55 taxa of the genus *Iris* (46 species, 5 subspecies, 3 variety, 1 hybrid) distribute in Turkey, of which 23 are endemic [3]. *I. pamphylica*, a rare endemic species found in South-west Anatolia (Turkey), was first collected by Davis and O. Polunin in 1956. The species is located in subg. *Hermodactyloides* and no closely related to any other species in the subgenus [4].

Sadly, despite rich biodiversity, and as a result of various factors in Antalya, about 60 of the locally endemic taxa are endangered. When distribution data gathered from field studies of the endangered species were collated, it was clear that some species required urgent active conservation. The volume of studies intended to identify endangered plants, carried out by the authors, have increased in recent years. Through these studies, threat categorisation,

distribution areas and descriptions of the species have been revised. Endangered species, like *Iris pamphylica* located in or nearby settlements were prioritized. In this investigation, during conservation studies, the taxonomic and ecologic characteristics of *I. pamphylica* were examined and recorded in order to provide opportunity for further studies in the future.

MATERIALS AND METHODS

In this research, morphological, anatomical, palynological, karyological and ecological properties of the rare endemic *Iris pamphylica* Hedge which were collected or observed between the years of 2008 and 2014 from different localities of Manavgat, Alanya, Gündoğmuş and Akseki districts of Antalya province were investigated. The detailed morphological description was prepared with morphological observations and measurements carried out on bulb, leaves, falls and standards during the field studies. Collected specimens were dried in accordance with standard techniques and deposited in Akdeniz University's Herbarium (AKDU). Deposited root, leaves, scape and flower parts preserved in 70% ethyl alcohol or fresh materials were used for the anatomical studies. Cross and surface sections taken from the materials were examined under optical microscope and photographed.

Seed morphology and pollen grains of *I. pamphylica* were examined using SEM (Scanning electron microscopy) techniques. For SEM study, the seeds and pollens were covered with gold and mounted on stubs. The microphotographs were taken with a Zeiss LEO-1430 Scanning Electron Microscope. LM studies measured the long axis (LA), short axis (SA), exine and intine thickness. The measured pollen diameters were based on 50 samples. In order to examine the exine sculptures and seed coat in

detail, scanning electron microscopy (SEM) was also used. For SEM study, pollens were first treated with 70% alcohol, and then dried before mounting on stubs and covered with gold. Pollen shapes and ornamentation were identified according to relevant literature [5, 6, 7].

For karyological study, the squash preparation method was used for the chromosome study of this species. Root tips were pretreated in α -monobromonaphthalene at 4°C overnight and then washed with distilled water and finally fixed in Carnoy's solution (3:1 absolute ethanol: glacial acetic acid) for a minimum of 5 hours. The root tips were hydrolysed for 12 minutes in 1N HCl at 60°C, stained using the standard Feulgen technique and squashes were prepared. Slides were made permanent with Entellan. Slides were examined under a Nikon E200 microscope with photographic camera. The ideograms were drawn from mitotic metaphase. The nomenclature used for the description of chromosome morphology is that proposed by Levan [8].

Without harming the populations in nature, a certain number of individuals were transferred to the Botanic Garden of Akdeniz University in order to protect the generation of the species. A few plant samples were collected and dried according to the herbarium techniques and archived in the Akdeniz University Herbarium (AKDU). To demonstrate the ecological preferences of *I. pamphylica*, certain parameters including their distributions, habitat types and flowering times were studied and evaluated. Five soil samples were taken from the exact locations where plants were established, and analysed in the soil analysis laboratories of the Western Mediterranean Forestry Research Institute. All soil samples were taken from 0–20 cm depth as demonstrated by Jackson [9], and then air dried and passed through a 2 mm sieve. The physical and chemical properties of soil samples were analyzed, and data concerning the habitat, elevation, aspect, slope and vegetation were recorded during the field studies. Geographic information system GlobalMapper and Google Earth Pro software, digital elevation maps and 1/25000 raster maps were made use of. These maps are O27a3, O27b4, O27d2 and O27c1. The 3D Analyst module of the program was used for the creation of incline and elevation maps. The coordinates of the individuals were transferred onto the maps by using the relevant modules of the program.

RESULTS AND DISCUSSION

Morphological features

Iris pamphylica Hedge, Notes Roy. Bot. Gard. Edinburgh 23:557 (1961). (Fig. 1).

Description

Plant 15–25 cm tall at flowering time. Bulba 25–33 x 18–22 mm, ovoid, reticulate-fibrose, producing few small bulblets (4–13 x 7–10 mm). Leaves 1–2, 15–45 x 0.2–0.25 cm at anthesis, elongating later to 80 cm, 4-angled, linear, glaucous, scabridulous at margins. Bracts and bracteoles almost equal, 5–6 cm long, closely sheathing perianth tube, ventricose, green, herbaceous, membranous at apex and margins, acute. Flowers solitary, bicolored, dark brown to blue; perianth tube 2–2.5 cm long. Outer tepals (falls) lanceolate, dark purple veins evident on the claw and limb, adaxial surface minutely papillate; claw 25–26 x 3–3.5 mm, yellow with black-spotted, longer than limb; limb 11–15 x 8–11 mm, mucronate or acuminate at apex, dark purple to dark brown. Inner tepals (standart) lanceolate, 40–43 cm

long, 2 mm wide at the base and up to 11 mm wide towards the apex, apex obtuse or slightly emerginate, dark purple to dark brown towards the apex and dark purple veins evident, yellow with dark-spotted towards the base, adaxial surface minutely papillate. Style branches 42–45 x 3 mm (including style crests), yellow with dark brown-spotted; style crests 9–12 x 3 mm, veins evident; stigmatic lip 0.5 x 1.5–2 mm, clearly narrower than style branch. Stamens 23–24 mm long; filament 12–13 x 1 mm, pale yellow with dark brown spotted; anther 11–13 x 2 mm, creamy white. Capsule oblong-elliptic, c. 25–37 x 10–14 mm, beak c. 1 cm long, many seeded. Seeds turbate to subglobose, 3.1–5.2–2.1–3.2 mm, with caruncle at apex and funicular protuberance at the base. The rafe is often inconspicuous, sometimes appears as a flat or raised area of the testa where the epidermal cells are elongated along the longitudinal axis of the seed. Caruncle coat surface microstructure with reticulate colliculate. Seed surface irregularly rugose, walls undulate (Fig. 2).



Figure 1. General appearances of flower (a), habit (b), capsule (c) and habitat (d) characters of *Iris pamphylica*

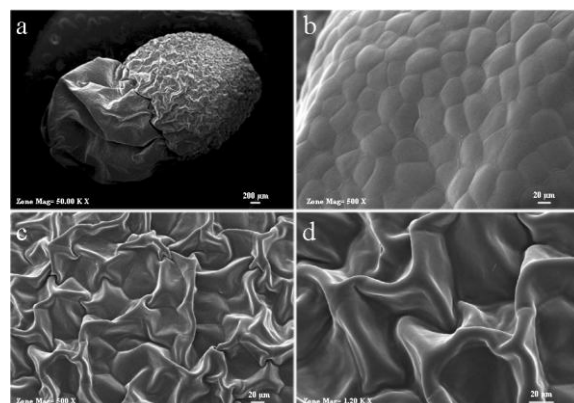


Figure 2. SEM microphotographs of the seed (a), caruncle (b) and seed surfaces (c, d) of *I. pamphylica*.

I. pamphylica shows distinctive morphological characters and according to Mathew, is not closely related to any other species in the *Hermodactyloides* subgenus [4]. It is easily recognisable by its flower colours, short perianth tube and fruiting characters. For this reason, it has not been compared with the relative species in the present study. The literature of examination with SEM on the seed surface of Turkish *Iris* species are quite inadequate, except *Crocus*

[10] and *Gladiolus* [11, 12] which have been reported previously. Although the seed surface of *I. pamphylica* is conformable with the results obtained from these studies, it is distinguish with large caruncle and inconspicuous rafe.

Anatomical features

Root anatomy: The cross section of the root (Fig. 3AB) shows that the cortex parenchyma is 7-8 layered and the cells are elliptic-rounded in shape. The thin walled endodermis and pericycle are located inside the cortex parenchyma. There are 8-11 metaxylems at the centre of the root. Xylem strands are present on the periphery of the vascular cylinder, they are four in number.

Scape anatomy: The scape is rounded in shape (Fig. 3CD) and the epidermis cells are surrounded by thin layered cuticle. The stomata are dipped into the epidermis cells. The 7-10 layered cortex parenchyma consists of parenchymatic cells. These cells are oval and circular in shape. Pith area is present at the centre of the scape. There are numerous and scattered vascular bundles are around the pith.

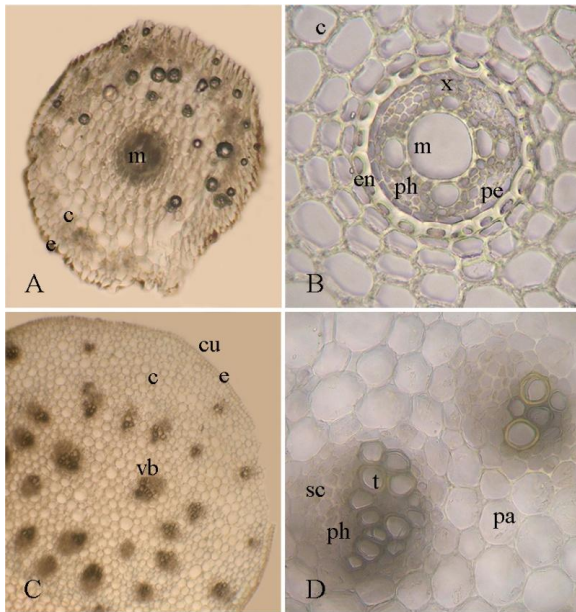


Figure 3. Cross-sections of the root (A-B) and scape (C-D) of *Iris pamphylica*. (c: cortex, cu: cuticle, e: epidermis, en: endodermis, m: metaxylem, pa: palisade parenchym, pe: pericycle, ph: phloem, t: trachea, vb: vascular bundle, x: xylem strands).

Leaf anatomy: The leaf is square-shaped in cross section and distinctly 4-angled (Fig. 4A). The epidermis is 1-layered and oblong in shape; covered by cuticle (Fig. 4B). The surface of cuticle is papillose (Fig. 4C). Stomata are large, oval and ordered. The type of stoma apparatus is tetracytic. (Fig. 4D). The sclerenchyma creates boundaries between epidermis and palisade parenchyma at the apex of the leaf arms. The palisade parenchym cells are oblong in shape and generally 2-3 layered. There are vascular bundles in palisade parenchyma. The aeranchyma with large air spaces is at the centre of the leaf. There are so few styloid crystals square in shaped was observed around the sclerenchyma.

Anatomical structure of the roots of *I. pamphylica* is typically monocotyledonous. The metaxylem number in root and arrangement of vascular bundles can serve as distinguishing characteristics for the *Iris* species [13]. There are 8-11 metaxylems are in the root and four xylem

strands are present in the species. It is reported that, pericyclic sclerenchyma forms a whole ring, which separates primary cortex from central cylinder in the cross section of the scape of *I. pseudacorus* and *I. sibirica* [14]. This ring is not observed in *I. pamphylica* also not in *I. iberica* and *I. sintenisii* [13].

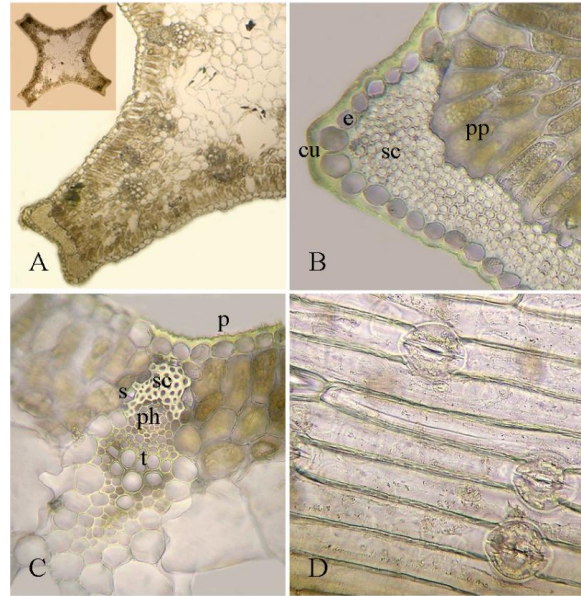


Figure 4. Leaf anatomy of *Iris pamphylica*. A- Quadrangular leaf, B- Leaf apex (e: epidermis, cu: cuticle, pp: palisade parenchym, sc: sclerenchyma), C- Papillary growths, vascular bundle and styloid crystals (p: papillae, ph: phloem, s: styloid, sc: sclerenchyma, t: trachea), D- tetracytic type of stomata apparatus.

The 4-angled and square leaf shape is characteristic for the species. *I. pamphylica* can be clearly distinguished from other species thank to this feature. In some genera in Iridaceae the larger vascular bundles have sclerenchyma girders connecting them to the epidermis [15], in *I. pamphylica* such the girders always absent. The sclerenchyma creates boundaries between epidermis and palisade parenchyma at the apex of the leaf arms in the species. The similar sclerenchymatic boundaries were observed also in the leaf of *I. schachtii* [16]. The shape and the size of the styloid crystals are diagnostic characters for anatomy of the genus *Iris* according to Wu and Cutler [17]. There are so few styloid crystal were observed in the leaf of the species.

Palynological studies

As results of the palynological studies, the pollen grains are monad, large (51-100 µm), suboblate to oblate-spheroidal, monosulcate, elliptic in equatorial view, reticulate exine sculpturing. The detailed measurements of the pollen grains of *I. pamphylica* collected from two different localities is shown in the Table 1. While the pollen shape of the species was reported as prolate-spheroidal [18], the shape of pollen grains was evaluated as suboblate to oblate spheroidal by the present study. The aperture of *I. pamphylica* is different from the other taxa included in the subg. *Hermodactyloides*. While in subg. *Hermodactyloides* the pollen is semitectate zonaaperturate generally, with the exception of *I. pamphylica* which has semitectate monosulcate pollen [18, 19]. Therefore, the pollen grains of the taxa classified in the subg. *Hermodactyloides* were evaluated as insulate and semitectate-columellate [19].

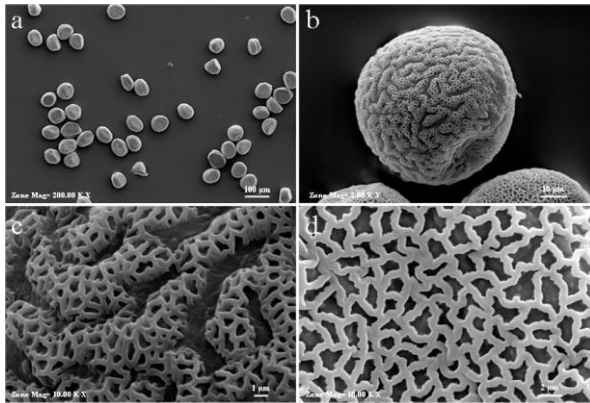


Figure 5. SEM microphotographs of the pollen and pollen surfaces of *I. pamphylica*.

Table 1. Some palynological measurements of *I. pamphylica*

Locality	Shape	P/E	P (µm)			E (µm)		
			M	SD	V	M	SD	V
Ahmetler	Suboblate	0.86	82.08	± 4.21	75-97	94.98	± 3.95	87-104
Çukurköy	Oblate spheroidal	0.93	91.28	± 4.15	85-100	97.3	± 6.06	85-110

Karyological studies

The somatic chromosome number of *Iris pamphylica* was determined to be $2n = 20$ (Fig. 6). This result is similar to previous counts [20, 21]. The karyotype formula of this species is $2mc+8smc+4stc+6tc$. The Stebbins symmetry types is 2A [22].

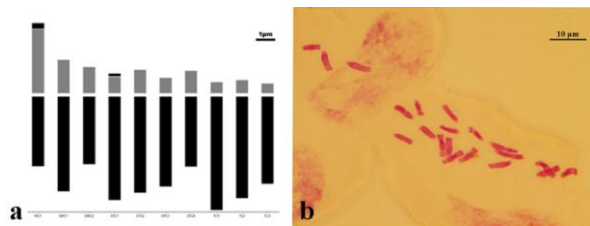


Figure 6. Ideogram of *Iris pamphylica* (a) and microphotograph of somatic metaphase (b).

I. pamphylica usually has sub-telocentric to telocentric chromosomes. These results are consistent with studies conducted on *Iris* taxa previously [20, 23, 24, 25]. In the present study, satellites were usually seen on the short arms

of metacentric and longest sub-telocentric (acrocentric) chromosomes of *I. pamphylica*. That satellites were seen on the short arms of the longest sub-telocentric chromosomes of *I. reticulata* and *I. histroides* is similar to *I. pamphylica*, whereas they were not seen on the metacentric chromosomes of these related taxa [23].

Ecological studies

I. pamphylica prefers forest clearings, maquis areas and olive groves. This state shows the structure of ecological tolerance depending on the species distribution in different habitats. Although previous studies had reported distribution at altitude range of 700-850 (1500) m previously [4], in the present study the species has been observed in Okurcalar and Alarahan which are near to sea level. External borders of distribution area rising gradually towards northward are presented in Fig. 7. The species distributes in old olive groves in Okurcalar, whereas in the Alarahan location, it prefers to be under *Pinus brutia* or in clearings of same tree. In this area, the species blooms during the last week of February. It was found to the north between Odaönü and Gündoğmuş road, only as groups comprising several individuals, whereas the most intense distribution and the holotype locality, of the species is in Güçlüköy (Fersinaları) and nearby surroundings. In this area, individuals flower in the second week of March at the two main identified locations. The Güçlüköy location shows wooded areas at the edge of farmland, Ahmetköy shows scrub clearance areas and in Çukurköy, limestone rock and grassy habitats under forested areas.

In this study, the contents of soil samples for *I. pamphylica* were analyzed and interpreted. To this end, five soil samples were taken from the exact locations where plants exist. The results of the soil analyses are given in Table 2 with sample's locations. Analysis results are conformable with soil characters of previously reported studies conducted on members of the genus *Iris* such as *I. danfordiae*, *I. histrio*, *I. reticulata* and *I. bakeriana* [2]. *I. pamphylica* prefers moderate alkaline to alkaline soils. Although it is reported that the species prefers only loamy soils [2], the results of the present study show it can distribute well in loam, silt loam, clay loam, sandy clay loam and clay soils. In addition, the amounts of $CaCO_3$ show significant variations in the investigated soils depending on the drainage in areas of species distribution. Levels in the Alarahan and Okurcalar locations are so high because of the altitude and land usage, whereas the amounts are very low in old soils of Güçlüköy, Ahmetköy and Çukurköy. In addition the investigated species grows well in soils moderate to rich in organic matter because of the general preference for forest habitats. The amount of organic matter is higher in the forested areas (Alarahan and Çukurköy) than habitats close to agricultural areas and surroundings. As reported previously *I. pamphylica* grows well in non saline soil. Likewise, it was determined that the species develops well with low nitrogen levels.

Table 2. Soil analysis of *I. pamphylica* locations.

No	Location	Altitude	Physical Parameters				Chemical Parameters				
			Sand %	Silt %	Clay %	Texture	pH 1:2.5	CaCO3 %	Organic matter	Total N, %	EC10 mS/cm
1	Alarahan	97 m	42.93	36.96	20.11	Loam	7.95	24.59	9.99	0.50	0.418
2	Okurcalar	52 m	47.98	35.88	16.14	Silt Loam	8.10	35.58	2.89	0.14	0.188
3	Güçlüköy	651 m	41.77	24.76	33.48	Clay Loam	7.79	2.59	4.27	0.21	0.186
4	Ahmetköy	708 m	52.43	23.79	23.79	Sandy Clay Loam	7.65	3.23	7.49	0.37	0.292
5	Çukurköy	802 m	35.70	23.78	40.52	Clay	7.5	1.94	10.78	0.54	0.235



Figure 7. Distribution map of *Iris pamphylica* (Numbers show outer boundary for EOO); Okurcalar (1), Alarahan (2), Odaönü (3), Saburlar (4), Halitağalar (5), Gündoğmuş Road (6), Ahmetköy (7), Çiçekoluk (8), Güçlüköy (9), Çukurköy (10).

Conservation status and category

I. pamphylica is one of the endangered endemic species growing in Antalya, Turkey. The first samples of the species were collected from Güçlüköy (Fersinalanı). The species which is attractive with its showy flowers, is generally located near settlements. Human pressures, on the populations of the *I. pamphylica*, have limited spread to separate narrow areas. During the study, it was noted that as well as the sheep, the shepherds also eat the plant's flowers, with the local name of "yoğurtotu" (Yoghurtweed). The expansion of agricultural lands and grazing pasture, and pressures from dam construction means that measures have to be taken to protect this species.

Conservation status of the *I. pamphylica* is assessed to IUCN Red List Categories and Criteria: Version 11 [26] and described below according to the rules of citation. Considering the IUCN criteria, *I. pamphylica* takes place in the CR (Critically Endangered) category; due to continuing habitat destruction in the locations mentioned above, is now confined to an area of approximately 10 km² in area, as observed in field studies since 2008. It was also noted that the species's limited habitat is close to towns. This data shows the extent that the already quite limited population of the species has decreased in the last 10 years (A2a+2c). To date, field observations, made by ourselves and different researchers, show that there are only 30-40 individuals in each location. The extent of occurrence (EOO) value of the species in Antalya is 95.61 km² according to boundaries of Fig. 7. And the area of occupancy value (AOO) by the sum of the occupied grid squares is limited 10 km² B1b(i,ii)+B2b(iii,iv).

CONCLUSION

In our country, especially in Antalya, the most important reason of human-induced environmental destruction is underdeveloped conservation awareness in the population. This is resolvable with species conservation and public education projects about species and environmental protection run by efficient and effective

institutions. Some conservation projects are carried out by the authors of the present study. The subject of one of the projects is increasing the number of individuals of *I. pamphylica* in their natural habitats using tissue culture methods. Conservation studies should be set up for the purpose of protecting the next generation of our endangered species, conducted on various taxonomic studies. Conservation projects mainly prepared by universities should be people-oriented, focused on target species.

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