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BIOLOGY OF REDISCOVERED RARE ENDEMIC *COTA FULVIDA* (GRIERSON) HOULB. IN ESKIŞEHIR-TURKEY

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Key words: Cota fulvida, Anatomy, Karyology, Taxonomy, SEM, Endemic

Abstract

The present study determines the morphological, anatomical, palynological, karyological, chorological features and conservation status of the rediscovered rare endemic Turkish taxon *Cota fulvida* (Grierson) Holub. (Asteraceae) after ca.110 years. *C. fulvida* distributes naturally in Gölcük Plateau (Türkmen Mountain), Eskişehir-Turkey. In the cross-sections of the root of *C. fulvida*, the pith was found completely covered by xylem cells. As to stem, it was observed that large parenchymatic cells were present in the pith of the stems. Leaves are equifacial and amphistomatious having amaryllis type of stomata. They are mesomorphic. Stomata are anisocytic. The results of the light and scanning electron microscope investigation revealed that the pollen grains of *C. fulvida* are tricolporatae, amb shape triangular, pollen shape spheroidal. Exine tectatae-echinate. The somatic chromosome number of *C. fulvida* was found to be 2n =18 without any satellite. It is considered "Critically Endangered" (CR) based on IUCN category.

Introduction

The generic and infrageneric concepts of *Anthemis* have been changed several times. Recently, *Anthemis* sect. *Cota* was accepted as a generic name, *Cota* J. Gay by Oberprieler (2001), Greuter *et al.* (2003), Oberprieler *et al.* (2007, 2009), Lo Presti *et al.* (2010) and Özbek and Vural (2011).

Cota J. Gay is represented by about 53 species throughout the world. These species are distributed in Europe, Southwest Asia and North Africa. The last new species is *Cota hamzaoglui* Özbek & Vural in Anthemideae tribe from Turkey. The total number of the species in the genus *Cota* has now reached 16 together with this record in Turkey. This genus includes 22 taxa in Turkey, of which 11 taxa are endemic. The endemism value is assessed 50% in Turkey (Grierson and Yavin 1975, Özbek and Vural 2011, Özbek *et al.* 2011).

Cota fulvida (Grierson) Houlb. (Syn. *Anthemis fulvida* Grierson). Fam. Asteraceae was collected from the type locality by Bornmüller in 1899 from Turkey. However, in the later period was not found in the this area anymore. Therefore, the species had been the category of DD (IUCN 2001). But during the excursion for the project Flora of Eskisehir, the species was found after ca. 110 years for the first time in Türkmendağı (Eskişehir-Turkey). In this study, anatomical, palynological, karyological and some morphological characteristics of hitherto rediscovered *C. fulvida* have been determined for the first time. The species distribution have been discussed phytogeographically. Evaluating population, new risk category and conservation measures is proposed.

Materials and Methods

The specimens were collected from Gölcük Plateu in the Türkmen Mountain, N: 39°43'03.6"-E, 030°38'51.6", 1800 m, during the summer of 2009. The plant was identified based on fresh material and the dried samples were preserved in Eskişehir Osmangazi University Herbaria

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(OUFE). The characters of the specimens were compared to Flora of Turkey, OUFE 16464 (Grierson and Yavin 1975) and presented in this paper (Table 1).

Samples for anatomical studies were kept in 70% alcohol until the sections were prepared. For the determination of anatomical characteristics of the plants, cross-sections of roots, stems and leaves were carried out manually. The sections were photographed by the Kameram digital camera fitted to a Nikon 80i type microscope.

For palynological studies pollen material was obtained from dried flower specimens. The pollen morphology of the taxon was investigated through light and scanning electron microscope (SEM). Faegri and Iversen's (1975) terminology for the names of the exine layers were used. Light microscopy of the pollen samples was carried out after Wodehouse (1935) and Erdtman (1969). For scanning electron microscopy (SEM), unacetolyzed pollen grains were directly placed onto stubs, sputter-coated with gold and examined with a Jeol 5600 LV scanning electron microscope (Walker 1974a,b). Terminologies used for pollen morphology were proposed by Wodehouse (1935), Erdtman (1954), Pokrovskaia (1958), Erdtman (1969), Skvarla (1966) and Faegri and Iversen (1975) and Pehlivan (1995).

The seeds were germinated at 25ºC on moist filter paper in Petri plates for karyological studies. Actively growing root tips of about 1 cm in length were excised from the germinating seeds and pretreated in α-bromonaphthalene solution for 4 hrs at 4ºC followed by fixation in ethanol-acetic acid (3 : 1) for 24 hrs. The root tips were then hydrolyzed in 1 N HCl at 60ºC for 14 min and washed in distilled water. Staining was carried out in aceto orsein for 4 times heating and squashed with 45% acetic acid. Five good quality photomicrographs of metaphase plates were considered for the study. The long arm (l), short arm (s) and total chromosome lengths (c) of each chromosome were measured on enlarged microphotographs. The relative lengths, arm ratios (r = l/s) and centromeric index (I = 100 s/c) were calculated and used to classify and determine homologous chromosomes. The chromosome nomenclature proposed by Levan *et al.* (1964) is used. A karyogram was constructed by arranging the homologous pairs from largest to smallest. The variation in chromosome length and chromosome arm ratio within the karyotype was estimated by calculating the standard deviation (Sd) of these parameters (Elçi 1982).

Results and Discussion

Morphological properties: Rootstocks somewhat woody, densely branched at base. Stems up to 45 cm, unbranched, 1-headed, leafless and pedunculate above; stems and leaves covered with whitish sericeous hair. Lower leaves petiolate, petiole 2 - 3 mm long, lamina 2-pinnatisect, oblanceolate or broadly elliptic in outline, 1.5 - 5 × 0.5 - 1.5 cm, lower surface densely lanate; primary segments 4 - 6 paired, 0.5 - 1 cm long, with additional smaller, simple, apex acuminate-setaceous; secondary segments 1 - 3-paired, up to 3 mm, lanceolate, acuminate-setaceous. Upper cauline leaves similar to lower ones but smaller and entire, lanceolate. Peduncle 0.5 - 1.9 cm long, becoming slightly thickened at maturity below capitulum. Capitula homogamous and discoid. Involucre 1.5 - 2.2 cm broad; phyllaries imbricate, outer ones lanceolate to linear lanceolate, 3.5 - 5 × 1 - 1.5 mm, ± densely pubescent, inner ones oblong, oblong to lanceolate 6.5-7 × 1.5-2 mm, obtuse at apex, margins brownish ciliate, glabrous surface. Corolla bright yellow, flowers 5-lobed, 3.5 - 4.5 × 0.5 - 1 mm; lobes obtuse at apex. Style bifurcate, 2.5 - 3.5 mm long. Paleae golden yellow, stiff, acuminate, c. 7 - 9 × 1 - 1.5 mm, 2 - 3 mm longer than central flowers. Achenes creamy, dorsiventrally compressed, transversely rhombic in cross section, 2 - 2.7 mm long, obscurely 10-ribbed, 2 lateral wings up to 0.4 mm broad; corona crenulate, 0.3 - 0.5 mm long. Fl. 6 - 8, Fr. 8 - 9, Alt. c.1800 - 1850 m (Table 1, Fig. 1).

Table 1. Comparison of some morphological characters of *Cota fulvida* based on the present study and Grierson and Yavin (1975).

Characters	Grierson and Yavin (1975)	Present study
Stems	Up to 30 cm tall, unbranched leafless	Up to 45 cm tall, unbranched leafless
Indumentum	Yellowish sericeous	Whitish sericeous
Indumentum of lower surface of leaf lamina	-	Densely lanate
Leaf lamina	1.5 - 5 × 0.75 - 1.5 cm	1.5 - 5 × 0.5 - 1.5 cm
Primary segments of leaf lamina	4 - 6 pairs	4 - 6 pairs
Secondary segments of leaf lamina	4 - 5 pairs	1 - 3 pairs
Involucre broad	1.5 - 2 cm	1.5 - 2.2 cm
Peduncle (leafless part below capitula)	-	(0.5 - 1.9 cm long
Outer phyllaries	Lanceolate, 4.5 mm long	Lanceolate to linear lanceolate, 3.5 - 5 × 1 - 1.5 mm
Inner phyllaries	Oblong, 10 mm	Oblong, oblong to lanceolate 6.5-7 × 1.5-2 mm
Style	-	Bifurcate, 2.5 - 3.5 mm long.
Paleae	c. 7 × 1.25 mm	c. 7 - 9 × 1 - 1.5 mm
Achenes	-	2 - 2.7 mm long, creamy, obscurely 10-ribbed, 2 lateral wings up to 0.4 mm broad
Corona	-	Crenulate, 0.3 - 0.5 mm long
Flowering	6 - 7	6 - 8
Fruiting	-	8 - 9
Altitude	c.1850 m	1800 - 1850 m



Fig. 1. *Cota fulvida* with fruits.

Differences between some of the character traits (length of stem, indumentum, leaf lamina, broad involucre and paleae) in the present investigation and identified in the type have been observed (Table 1). These may be due to soil type, climate and habitat changes. On the other hand, some morphological characteristics, such as indumentum of lower surface of leaf lamina, peduncle (leafless part below capitula), style, achenes and corona were determined for the first time (Table 1).

Distribution and ecology: C. fulvida is endemic to Central Anatolia (Turkey) and was collected from type locality (Turkey, Afyon: Sultandagi) in 1899 by Bornmüeller. Despite all seanches the species could not be found in the type locality since 1899 (Özbek and Vural 2011).

The main reason of the absence of the species in the type locality is the anthropogenic effects. The present rediscovered locality of the species is in close proximity to the type locality, which is obvious because of an identical climatological geographical featrures (Fig. 3).

Type locality: Turkey B3 Afyon.: Sultandagh in jugis alpinis supra Engeli (Geneli?), 1850 m, 28 vi 1899, Bornmüller 4656 (holo. E! iso, K! W!), Endemic, Ir.-Tur. Element (Fig. 2).

New locality: B3 Eskişehir: Gölcük Plateu, in the Türkmen Mountain (Turkey), N: 39° 43' 03.6"-E: 030° 38' 51.6", 1800 m, *Pinus brutia* Ten. open forests (Fig. 2).

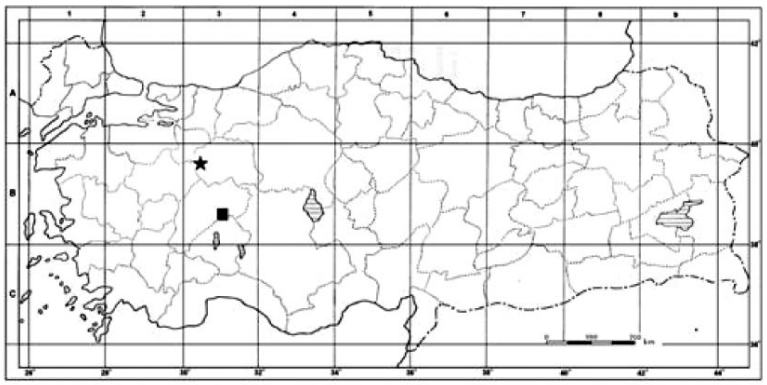


Fig. 2. Distribution map of *Cota fulvida*. (■) Type locality. (*) New locality in Turkey.

C. fulvida grows among the subalpine shrubs, alpine meadows and rocky slopes with *Pinus brutia* Ten., *Juniperus oxycedrus* L. subsp. *oxycedrus*, *Cistus laurifolius* L., *Chamaecytisus hirsutus* (L.) Link, *Quercus robur* L. subsp. *robur*, *Hypericum linarioides* Bosse, *Lathyrus digitatus* (M. Bieb.) Fiori, *Eryngium campestre* L. var. *virens* Link, *Dipsacus laciniatus* L., *Cirsium vulgare* (Savi) Ten., *Chondrilla juncea* L. var. *acantholepis* (Boiss.) Boiss., *Teucrium orientale* L. var. *orientale*, *Poa pratensis* L., and *Stipa bromoides* (L.) Dörfler at altitudes between 1800 and 1850 m in Gölcük Plateu in the Türkmen Mountain.

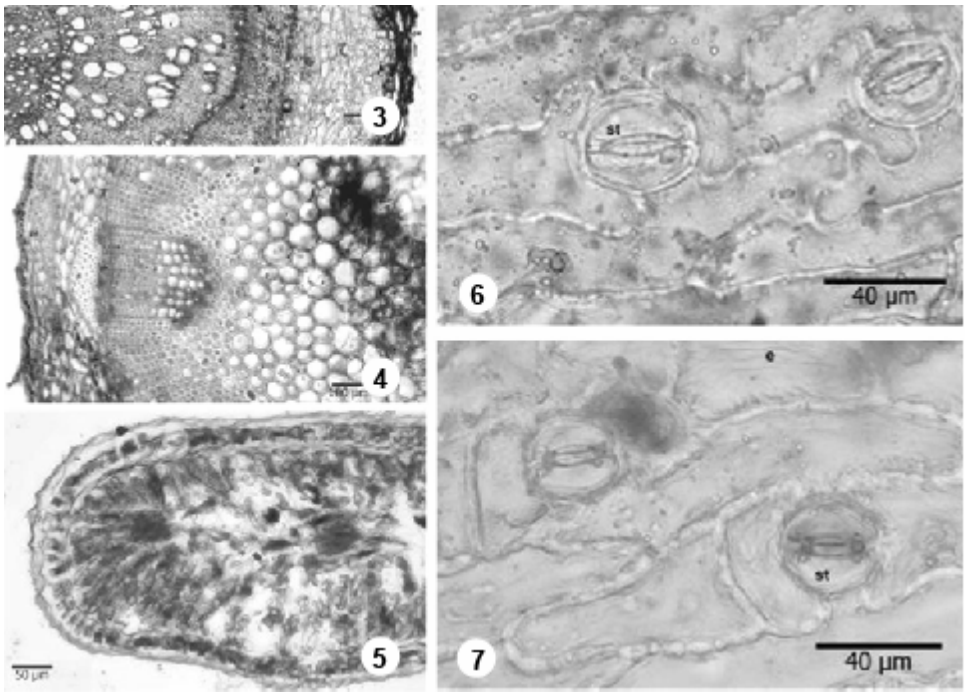
Conservation status: Today this species is known from only single locality with an extent of occurrence estimated to be less than 100 km^2, an area of occupancy estimated to be less than 10 km^2, and a continuing decline projected in the extent of occurrence, area of occupancy, extent and quality of habitat, and number of mature individuals. For these reasons, it should be classified as "Critically Endangered" (CR) based on criteria B1ab (i,ii,iii,v) + 2ab(i,ii,iii,v) + C2a (i,ii) (IUCN 2001).

Anatomical properties: There is a peridem layer on the outer surface of the root. Parenchymatous cortex is present under the periderm. Phloem occupies a narrow area with 2 - 3 layer cells. Cambium cells are distinguishable. The pith was determined to be completely covered by xylem cells (Fig. 3).

Stem has a thick cuticle with small-celled epidermis. Cortex is 5 - 6 layered and parenchymatous. There is a 5 - 6 layered collenchymatous tissue. Dense sclerenchymatous cells located on the phloem tissue which occupies a small region. Xylem consists of xylem vessels and a sclerencymatous parenchyma. A few primary xylem elements are present below the secondary xylem. Cambium is not distinguishable and the pith region is large and parencymatic (Fig. 5).

In leaves the upper epidermis consists of flat-ovoidal cells and the lower epidermis is made up of cells having same length and breadth. Stoma cells are present both in the upper and in the lower

epidermis. Palisade parenchyma cells are two-layered. The Leaves are equifacial and amphistomatious and they have amaryllis type stomata. They are mesomorphic. Stomata are anisocytic (Figs 5 - 7).



Figs 3-7. Transverse section of root (3), stem (4) and leaf (5). Upper surface (6) and lower surface (7) section of leaf.

Palynological properties: Pollen grains of *C. fulvida* are tricolporatae, amb shape triangular, pollen shape spheroidal. Exine tectatae-echinate. P/E=1.18 (N), 1.10 (A). The polar axis measured 35.48 µm (N), 32.2 µm (A), and the equatorial axis 30 µm (N), 29.28 µm (A), Amb was triangular and 32.28 µm (N), 28.46 µm (A) in diameter. The apocolpium was 11.44 µm (N), 10.64 µm (A) in diameter. Exine 1.62 µm (N), 1.86 µm (A). The exine has one layer of columellae beneath spines, microspine length 0.5 µm, and spinule width 1.1 µm. Exine tectate-echinate, 86 spinule in 100 µ2 and average distance between spinules 0.6 µm. Colpi ends are rounded Clg 26.28 µm (N), 23 µm (A), Clt 3.6 µm (N), 5.96 µm (A). The pores are transversely elongated; Plg 9.48 µm (N), 10.36 µm (A), Plt 7.28 µm (N), 7.2 µm (A). The pore latitude is wider than the colpi latitude. The surface ornamentation is tectatae-echinate under LM and SEM (Fig. 8).

Karyological properties: C. fulvida was found to be 2n = 18 without any satellite (Fig. 9). The haploid karyotype formula is n = 7m + 1sm + 1 st. Long and short arm with Sd, total length of the chromosomes, arm ratio (r = l/s) and relative length are given in Table 3. The range of metaphase chromosome lengths was 8,17 to 5,30 µm. The total length of the haploid set was 58,05 µm (Table 2). The karyogram and idiogram of haploid chromosome set are presented in Figs. 10,11.

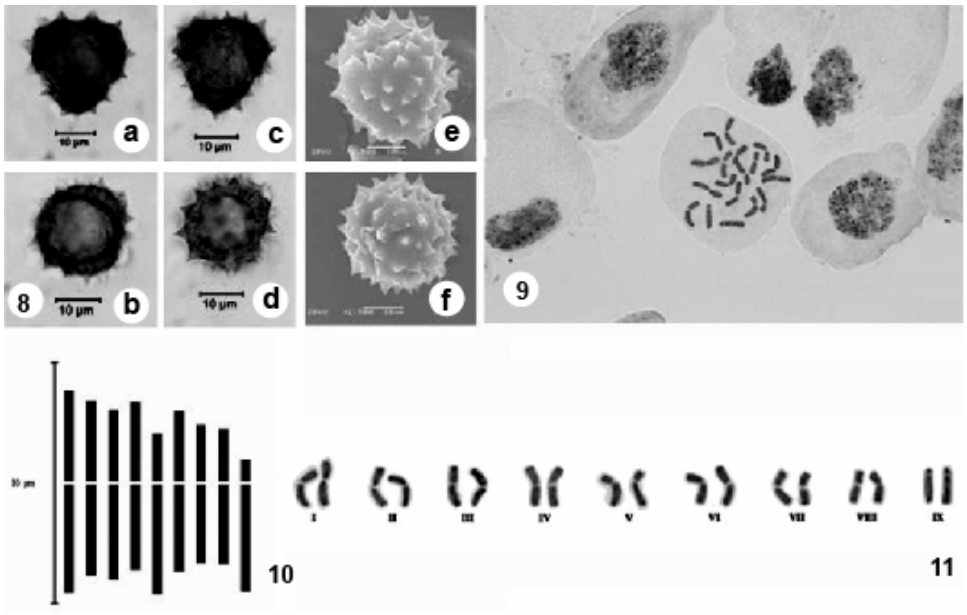
With few exceptions, the morphological characters of *C. fulvida* obtained in the present investigation is in agreement with other researches (Grierson and Yavin 1975). The species prefers

steppe habitats for their growth, which could be seen obviously by the widespread presence of xylem elements in the root anatomy.

Table 2. Morphometric data on chromosomes of *Cota fulvida.*

Chromosome pair	Long arm (µm)	Short arm (µm)	Total length (µm)	Arm ratio (r = l/s)	Centromeric index	Relative length	Centromeric position
I	4.46 ± 0.18	3.71 ± 0.14	8.17 ± 0.31	1.20	45.41	14.07	m
II	3.73 ± 0.30	3.33 ± 0.27	7.06 ± 0.03	1.12	47.09	12.16	m
III	3.90 ± 0.04	2.94 ± 0.13	6.84 ± 0.16	1.32	42.98	11.78	m
IV	3.52 ± 0.07	3.26 ± 0.02	6.78 ± 0.06	1.08	48.08	11.68	m
V	4.52 ± 0.03	1.98 ± 0.03	6.51 ± 0.11	2.28	30.51	11.21	sm
VI	3.58 ± 0.02	2.88 ± 0.08	6.46 ± 0.06	1.24	44.55	11.12	m
VII	3.23 ± 0.12	2.33 ± 0.25	5.56 ± 0.14	1.39	41.78	9.58	m
VIII	3.28 ± 0.19	2.16 ± 0.06	5.44 ± 0.13	1.52	39.69	9.36	m
IX	4.38 ± 0.02	0.92 ± 0.02	5.30 ± 0.05	4.78	17.30	9.12	st

m = Median, sm = Submedian, st = Subtelocentric and ± = Standard deviation.



Figs 8-11. 8. Pollen microphotography of *Cota fulvida*, (a) Polar view of a non acetolysed pollen in light microscope, (b) Equatorial view of a non acetolysed pollen in light microscope, (c) Polar view of an acetolysed pollen in light microscope, (d) Equatorial view of an acetolysed pollen in light microscope, (e) Polar view of a non acetolysed pollen in SEM, (f) Equatorial view of a non acetolysed pollen in SEM. 9. Somatic metaphase chromosomes of *Cota fulvida* (2n = 18). Bar = 10 µm. 10. Haploid idiogram of *Cota fulvida* N = 9 and 11. Diploid karyogram of *Cota fulvida.*

The essential criteria to determine the phylogenetic relationship between the characteristics of aperture and exine function of this species has been reported earlier (Kuprinova 1967, Cronquist 1968, Walker 1974a-b, Takhtajan 1980). In the present study, the karyological information for the species has been gathered for the first time which is consistent with other closer taxa (Cronquist

1968, Oberprieler 1998). The study enlights systematic-phylogenetic relationship of the species. Its anatomical, palynological and karyological structure contributes a better explanation to consider the usefulness of morphology in distinguishing the character traits possessed by a species.

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