



## Morphology, anatomy and cytology of critically endangered endemic *Asperula daphneola* from, West Anatolia, Turkey

Salih Gucel\*

Near East University, Environmental Sciences Inst, Nicosia-99138, Turkish Republic of Northern Cyprus

\*Corresponding Author E-mail: [sgucel@hotmail.com](mailto:sgucel@hotmail.com)

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### Abstract

*Asperula daphneola*, which belongs to Rubiaceae family, is only distributed on Nif Mountain. The present study investigates the species anatomically, morphologically and cytologically, laying the basis for future biosystematic studies as well as introducing this endemic taxa. *A. daphneola* seeds were 1.3-1.8 X 2.3-2.9 mm; ovate; seed surface was prominent and channelled; dorsal type was convex, hispid hairs all over; hylar zone type recessed; yellowish green colour. Type of pollen were stephanocolpate and had 6 colpus with tectate structure. The chromosome number in *A. daphneola* was counted as  $2n=20$

### Key words

*Asperula daphneola*, Nif Mountain, Kemalpaşa, İzmir, Turkey

### Introduction

Family Rubiaceae is predominantly tropical, consisting of ~ 650 genera and 13,000 species. Genus *Asperula* has 195 species widely distributed in Europe, northern Africa, temperate and subtropical Asia to Australasia (Bremer B. J.-F. Manen 2000). *Asperula daphneola* is a rare taxon and known from type gathering only. According to the records published in the Flora of Turkey and the East Aegean Islands (Davis, 1982), the first known population of *A. daphneola* grew at an open site 1500 m asl; however, Gucel and Secmen (2009) recorded 4 new locations of this taxon. It is related closely to *Asperula pulvinaris* (Boiss.) Heldr. ex Boiss. and *Asperula icarica* Ehrend. & Schonb, which belongs to the Cynanchicae section located in the mountains of southern Greece (Davis, 1982). The distribution area of these species possibly fragmented before the last glaciation period (Verdier, 1963). In order to enlighten the historical distribution and interrelationships of all these three species, there is a need for further detailed studies. There are no studies about Cynanchicae section and few studies about anatomical and morphological studies of *Asperula* genus. The aim of the present study was to investigate *A. daphneola* anatomically, morphologically and cytologically, laying the basis for future biosystematic studies and investigations, among Cynanchicae section.

### Materials and Methods

**Plant material :** The material consisted of *Asperula daphneola* O.Schwarz Feddes Rep., 36: 139 (1934). (Davis, 1988). It's an endemic perennial herb restricted to the peak of Nif Mountain (1500 m asl) in Western Anatolian part of Turkey, approximately 40 km East of Izmir. *A. daphneola* is a perennial cushion-forming, low-growing herb, with maximum height of 60 cm. It has imbricate leaves, producing up to 8 flowers in the axils of leafy bracts. The flowers lack calyx, but corollas are bright pink, 10-12 mm long, and exhibit an autogamous syndrome. Usually there are 4, and rarely 5 or 6 stamens epipetalous. Flowering begins in May and continues till June.

**Morphological studies :** Seed morphology of the species was assessed according to Stearn (1996), with the help of microphotographs obtained from the microscope images. About 100 seeds from 25 different plants were evaluated for morphological study. Microphotographs of the seeds were taken.

**Anatomical studies :** Anatomical studies were carried out in the samples kept in 70% alcohol, using paraffin method (Algan, 1981). The cross-sections were also taken by hand using razor blade.

**Palynological studies :** Flowers of *A. daphneola* were collected from the peak of Nif mountain, next to fire observation building. The mature pollen grains were acetolysed according to Ertman (1960) and then left to dry in centrifuge tubes. One month later, different pollen parts such as polar diameter (P), equatorial diameter (E), exine thickness, colpus length (Clg) or pore width and colpus width (Clt) were measured by light microscopy. About 50 pollens from 10 different plants were used while obtaining these measurement values. Arithmetic average and standard deviation was calculated. Terminology of data obtained was done according to Punt *et al.* (1994) and Moore *et al.* (1997).

**Cytological studies :** Mature seeds were collected from the plants in the field and placed in envelopes. Herbarium specimens of all taxa were deposited at the Ege University Herbarium. The seeds were germinated on filter paper, root-tips were cut and prepared following the method of Elçi (1994). The preparations obtained were examined and photomicrographs were taken. Somatic chromosome images obtained were redrawn by hand using tracing paper. Large number of seeds were taken from at least 5 plant specimens, and not less than 20 cell divisions were observed in each preparation. Chromosome numbers were evaluated following the method of Darlington and Wylie (1955); Löve and Löve (1961); Federov 1974); Löve (1978a); Löve (1978b), Moore (1982) and Davis (1988), respectively.

### Results and Discussion

Our results coincide with seed measurements for *Asperula pseudochlorantha* (1.387 X 1.063 mm), *A. antalyensis* (1.405 X 1.081 mm), *A. brevifolia* (1.987 X 1.414 mm), *A. serotina* (1.009 X 0.672 mm) and *A. purpurea* ssp. *apiculata* (1.803 X 1.368 mm) which were examined by Ersin and Yildiz (2010), *Asperula serotina* (1.009 X 0.672 mm) and *A. purpurea* (1.803 X 1.368 mm) by Ersin *et al.* (2010) and *A. comosa* (2.3±0.26 X 0.84±0.11 mm) and *A. anatolica* (1.66±0.37 X 0.86±0.45 mm) by Ozturk (2013). Also seed coat patterns support the results of Chaw and Sivarajan (1989) in that seed coat patterns may not be of considerable taxonomic value for Rubiaceae family. Our results coincide with their seed measurements and support the results of Chaw and Sivarajan (1989) in that seed coat patterns may not be of considerable taxonomic value for Rubiaceae family.

*A. daphneola* seeds were 1.3-1.8 X 2.3-2.9 mm; ovate; seed surface was prominent and channelled; dorsal type was convex, hispid hairs all over; hylar zone type recessed; yellowish green colour (Fig. 1).

Polar diameter of pollen was  $18.47 \pm 0.78 \mu\text{m}$ , equatorial diameter was  $17.37 \pm 0.67 \mu\text{m}$ , colpus length was  $9.60 \pm 0.50 \mu\text{m}$ , colpus width was  $0.90 \pm 0.10 \mu\text{m}$  and exine was  $1.06 \pm 0.10 \mu\text{m}$ , respectively. Type of pollen were stephanocolpate. Pollen form was spheroidal and P/E ratio was 1.06. Pollen has 6 colpus with tectate structure. Ornamentation was granulate and granules were thin and regular. Similar figures for pollen measurements was observed in *Asperula pseudochlorantha*, *A. antalyensis*, *A. brevifolia*, *A. serotina*, *A. purpurea* ssp. *apiculata*, *A. serotina*, *A. purpurea*, *A. comosa* and *A. anatolica* was reported earlier by Ersin and Yildiz (2010); Ersin *et al.* (2010) and Ozturk (2013), however all other pollens had micro spines, *A. daphneola* had micro granules.

The root anatomical study of this specie showed secondary growth and outer surface of the root was covered by periderm. Thin layer of collenchymatic cortex with cells containing raphide crystals was found beneath periderm. Secondary phloem was followed by cambium above, secondary xylem had parenchymatic pith rays and the center had primary xylem, located in the sclerenchymatic pith region (Fig. 2).

The cross-section of stem showed thin layered periderm, followed by thin layered cortex, consisting of parenchymatic cells. There were cells that contained raphide crystals among parenchymatic cells. Thin plate chollenchyma layer with vascular bundles consisted of secondary phloem and xylem followed by endodermis. The center had sclerenchymatic pith cells. (Fig. 3).

Leaves were isolaral with single row of upper and lower epidermis and cuticle layer and glandular hairs above. Collenchymatic cells elongated towards lower epidermis along the midrib. Midrib and vascular bundles were surrounded by bundle sheath cells, mesophyll cells were found with raphide crystals. Mesophyll tissue had two layered elongated palisade parenchyma beneath upper and lower epidermis. Elliptic and compact spongy parenchyma cells were present between palisade parenchyma cells (Fig. 4). Our findings coincide with

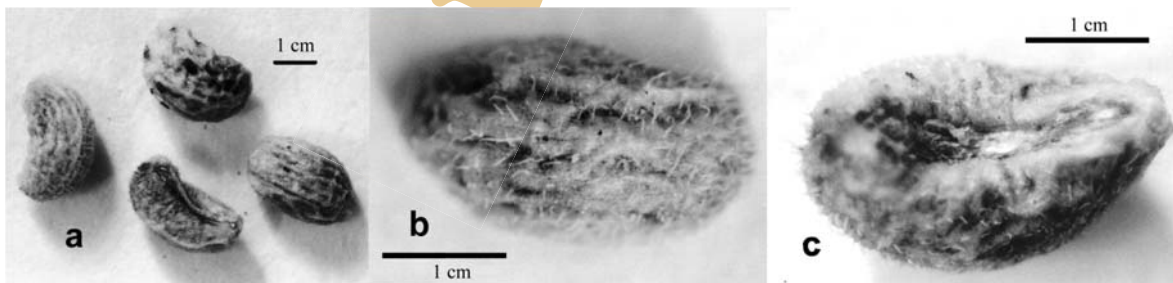
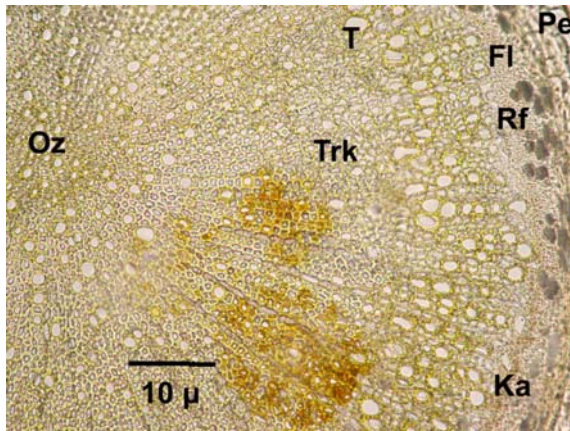
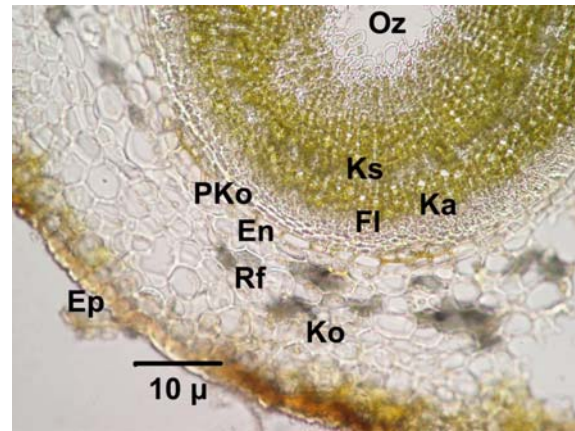


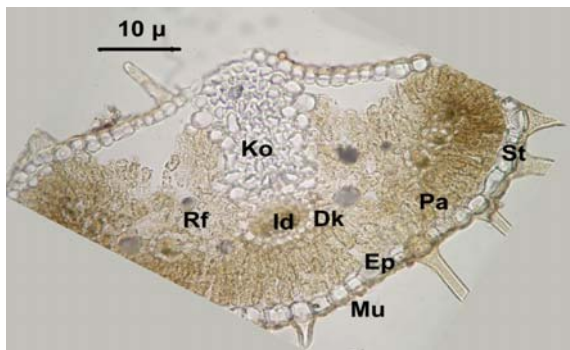
Fig. 1: *A. daphneola* seeds a. General view, b. Dorsal view, c. Ventral view



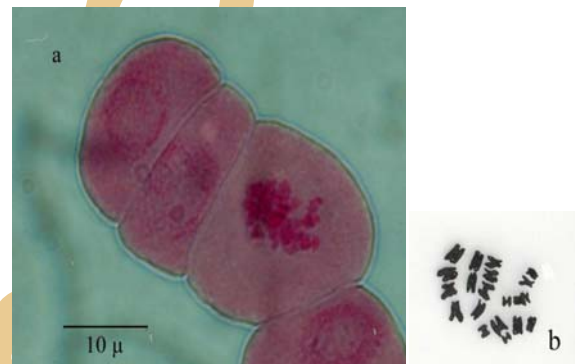
**Fig. 2 :** *A. daphneola* root cross-section Pe: Periderm, Rf: Raphide, Fl: Floem, Ka: Cambium, Ks: Xylem, T: Trache, Trk: Tracheid, Oz: Schlerenchymatic pith



**Fig. 3 :** *A. daphneola* stem cross-section Ep: Epidermis, Ko: Cortex, Rf: Raphide, En: Endodermis, PKo: Plate Collenchymatic Cortex, Fl: Floem, Ka: Cambium, Ks: Xylem, Oz: Parenchymatic pith



**Fig. 4 :** *A. daphneola* leaf cross-section; Ku: Cuticle; Ep: Epidermis; Pa: Palisade paranychma; St: Stoma; Dk: Bundle sheath; Id: Vascular bundles; Rf: Raphide; Ko: Collenchymatic cells



**Fig. 5 :** *A. daphneola* somatic chromosomes ( $2n=20$ ) a. Microscopic photo b. Drawing

those of Ozgen and Coskun (1999). The arrangement of different cell groups, showed the same order.

The chromosome counts of *A. daphneola* were determined for the first time. The diploid number of chromosomes in *A. daphneola* was 20 (Fig. 5). Cytological examination of 10 cells from 10 different specimens proved that there were no difference in chromosome numbers or chromosome abnormalities within the species.

The basic chromosome numbers of *Asperula* genus was counted as  $x=10, 11, 22$  by Darlington and Wylie (1955); Löve and Löve (1961); Federov 1974; Löve (1978a); Löve (1978b) and Moore (1982). Davis *et al.* (1988), in the Flora of Turkey, reported haploid chromosome number of only one specie, *i.e.*, *Asperula glomerata* (Bieb.) Griseb. 10.

The results obtained in the present study is in confirmation with the earlier reports of Darlington and Wylie (1955); Löve and Löve

(1961); Federov 1974; Löve (1978a); Löve (1978b); Moore (1982) and Davis *et al.*, (1988) on cytological studies on *Asperula* sp. where haploid chromosome number in *Asperula* species 10, 11, 22.

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