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## Studies on the leaf epidermal structure of genus *Cyanus*, sect. *Napuliferae* (*Compositae*)

### Abstract

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In order to assess the taxonomic significance of the epidermal structure, seven taxa from genus *Cyanus* Mill. (sect. *Napuliferae*) have been studied: *Cyanus pseudaxillaris*, *C. orbelicus*, *C. velenovskyi*, *C. napulifer*, *C. nyssanus*, *C. tuberosus* and *C. thirkei*. The investigated taxa possess thick covering of trichomes. The hairs consist of two or more basal cells (stalk) and a long whip cell. Five types of trichomes, very well distinguished by the number, form, size and the wall thickening of the stalk, were established. The cuticle is thin and easily could be distorted under gentle maceration. Some taxa possess cuticle corrugations, somewhere parallel to long cell axis. There are striations on the whole cuticle or only around the stomata and the trichome bases. The main stomatal type is anomocytic, but there are some stomata from anisocytic type, frequently untypical: the three cells do not encircle the stoma. There are stomata with only one subsidiary cell, which is rounded, smaller and well distinguishable by the other epidermal cells. The comparative study shows the epidermal features possess taxonomic significance and could be used as additional characters to differentiate the species into sect. *Napuliferae* of genus *Cyanus*.

### Introduction

The genus *Cyanus* Mill. belongs to the subtribe *Centaureinae* and is among the most taxonomically complicated genera of *Compositae*. Many taxonomists consider *Cyanus* as a subgenus or section in the genus *Centaurea* (Gajić 1975; Wagenitz 1975; 1975a; Dostál 1976; Strid & Tan 1991; Kožuharov 1992). In the last 10 years they have accumulated a lot of molecular data confirming the split of the genus *Centaurea* s. l. into several smaller genera (Susanna & al. 1995; Häfner 2000; Garcia Jacas & al. 2000, 2001). As a basis of the new taxonomic scheme of the *Centaurea* s. l. and *Centaureinae* (Greuter & al. 2001; Greuter 2003) serve the Wagenitz's pollen type classification, the base chromosome numbers (Wagenitz & Hellwig 1996), and the newly accumulated molecular data. In this scheme *Cyanus* has a generic status.

The representatives of the sect. *Napuliferae* are well distinguished from the other *Cyanus* species by its fusiform or napiform roots. All *Napuliferae* taxa, except *C. thirkei*

are Balkan endemics. The origin center of this group is the Balkan Peninsula. The main evolutionary trend is the adaptation of the more xerophytic habitats. Regard to this, the species possesses more or less developed white-lanate hair cover.

During the biosystematic studies on sect. *Napuliferae* (Bancheva & Raimondo 2003) have been detected some differences between the leaf epidermal structures of the investigated taxa. The taxonomic significance of epidermal structure, especially the type of the trichomes, has been emphasized by many authors (Metcalf & Chalk 1965; Stace 1965, 1968; Uzunova & al. 1997).

The aims of the present comparative study are: (1) to examine the leaf epidermal structure of seven *Cyanus* species: *Cyanus pseudaxillaris* (Stef. & Georgiev) Holub, *C. orbelicus* (Velen.) Soják, *C. velenovskyi* (Adamović) Wagenitz & Greuter, *C. napulifer* (Rochel) Soják, *C. tuberosus* (Vis.) Soják, *C. thirkei* (Sch. Bip.) Holub and *C. nyssanus* (Petrović) Soják, and (2) to estimate the taxonomic value of the leaf epidermal features.

There are not much informations about the epidermal characteristics of this family so rich of taxa. Metcalf & Chalk (1965) point out anomocytic and anisocytic stomatal types and nine types of non-glandular trichomes. They describe for *Centaurea* multicellular trichomes with uniseriate "pedestal" and whip like terminal cells. Hellwig (1992) determines different types of hairs of tribe *Astereae*. Sahu (1984) determines 13 types of non-glandular and 7 types of glandular trichomes of genus *Vernonia* Schreb., and uses the trichome complement to establish intra-generic relationships. Ogundipe & Adegbite (1991) examine the leaf epidermis of genus *Aspilia* Thouars, and elaborate a key for identification of the species on the basis of epidermal features.

## Material and methods

The study is based on material collected from 11 natural populations, 10 from Bulgaria and 1 from Serbia (Tab. 1). The preparation of the leaves for observation in SEM has been accomplished according to Huttunen & Laine's protocol (1983). Observations were carried out with a SEM Leica S420 at 15 kV in the Department of Botany, Palermo University (Italy). For investigations of the stomatal type semi-permanent glycerin-jelly mounts have been prepared after clearing in perhydrol and washing in water. For obtaining better results the trichomes were removed. The terminology used is after Dilcher (1974) and Payne (1978). The vouchers are kept in the Herbarium of Institute of Botany, Sofia (SOM).

## Results and discussion

The investigated species from genus *Cyanus* have some common epidermal features: amphystomatal leaves, sunken stomata from anomocytic, anisocytic and hemiparacytic type, thick covering of trichomes. All observed trichomes could be described as multicellular, uniseriate flageliforme. They consist of two or more basal cells (stalk) ending with one very long slightly twisted upper cell (whip) (Fig. 2, 3, 8, 10). The thick lanate covering on the leaves is due to these cells. We consider that the various structures of the foot cells could be used as a base for differentiation of 5 trichome types in sect. *Napulifera* of genus *Cyanus*:

Table 1. Origin of the populations examined: BU - Bulgaria, SR - Serbia, Banch. - Bancheva, M. N. - M. Niketić

<i>Cyanus</i>	Origin	Coll. date	Coll.
<i>pseudaxillaris</i>	BU: Thracian plain: Besaparski Ridove near Sinitevo, 490 m asl	26.05.1998	Banch. Sh9801
<i>orbelicus</i>	BU: Pirin Mt. above Hiža Banderica, 1850 m asl	22.07.1995	Banch. Sh9562
	BU: Rila Mt. above Hiža Grančer, 2350 m asl	11.07.1996	Banch. Sh9627
<i>napulifer</i>	BU: C Stara Planina Mt.: near Hiža Planinski Izvori, 1700 m asl	04.08.1995	Banch. Sh9605
	BU: C Stara Planina Mt.: near Hiža Vasil Levski, 1600 m asl	03.08.1997	Banch. Sh9724
<i>velenovskiyi</i>	BU: C Stara Planina Mt.: Djuzata	24.08.1997	Banch. Sh9733
<i>tuberosus</i>	BU: Slavjanka Mt.: Konjarite, open grassy places, 1700 m asl	28.08.1996	Banch. Sh9660
<i>thirkei</i>	BU: E Rhodope Mts: Mandrica - Likan Češma, c. 700 m asl	28.04.2003	Banch. Sh03011
	BU: E Stara Planina Mt.: Kačula, c. 900 m asl	18.06.1996	Banch. Sh9616
<i>nyssanus</i>	SR: Suva Planina Mt.: Golaš: Divna Gorica, 1120-1300 m asl		M. N.

Type I: 2-3 basal cells, 10.0-25.0  $\mu\text{m}$  in diameter, sometimes with fine striations (*C. orbelicus*, *C. velenovskiyi*, *C. napulifer*, *C. tuberosus*) (Fig. 1, 8, 9, 11).

Type II: 12-15 basal cells, 30.0-35.0  $\mu\text{m}$  in diameter, frequently by one sunken and one swollen cells (*C. pseudaxillaris*) (Fig. 10).

Type III: 7 cells 20.0- 22.0  $\mu\text{m}$  in diameter, thin-walled, filiform (*C. thirkei*).

Type IV: 5-8 large basal cells, 55.0-68.0  $\mu\text{m}$  in diameter, thin-walled. The first one likes goblet (*C. thirkei*) (Fig. 3).

Type V: 5-7 large, thick-walled, rounded cells, 40.0- 65.0  $\mu\text{m}$  in diameter (*C. pseudaxillaris*) (Fig. 2, 4).

The presence of various trichome types and peculiarities of the cuticle, different forms of epidermal cells and stomata types allow the investigated species to be distinguished by their epidermal structure.

*Cyanus pseudaxillaris* (Stef. & Georgiev) Holub is characterized by cuticle with almost parallel corrugations, isodiametric to elongate epidermal cells of the upper epidermis (Fig. 2, 4). The epidermal cells of the lower epidermis have wavy anticlinal walls (Fig. 10). Stomatal type is mixed - anomocytic, anisocytic and hemiparacytic. The trichomes belong to the types II and V.

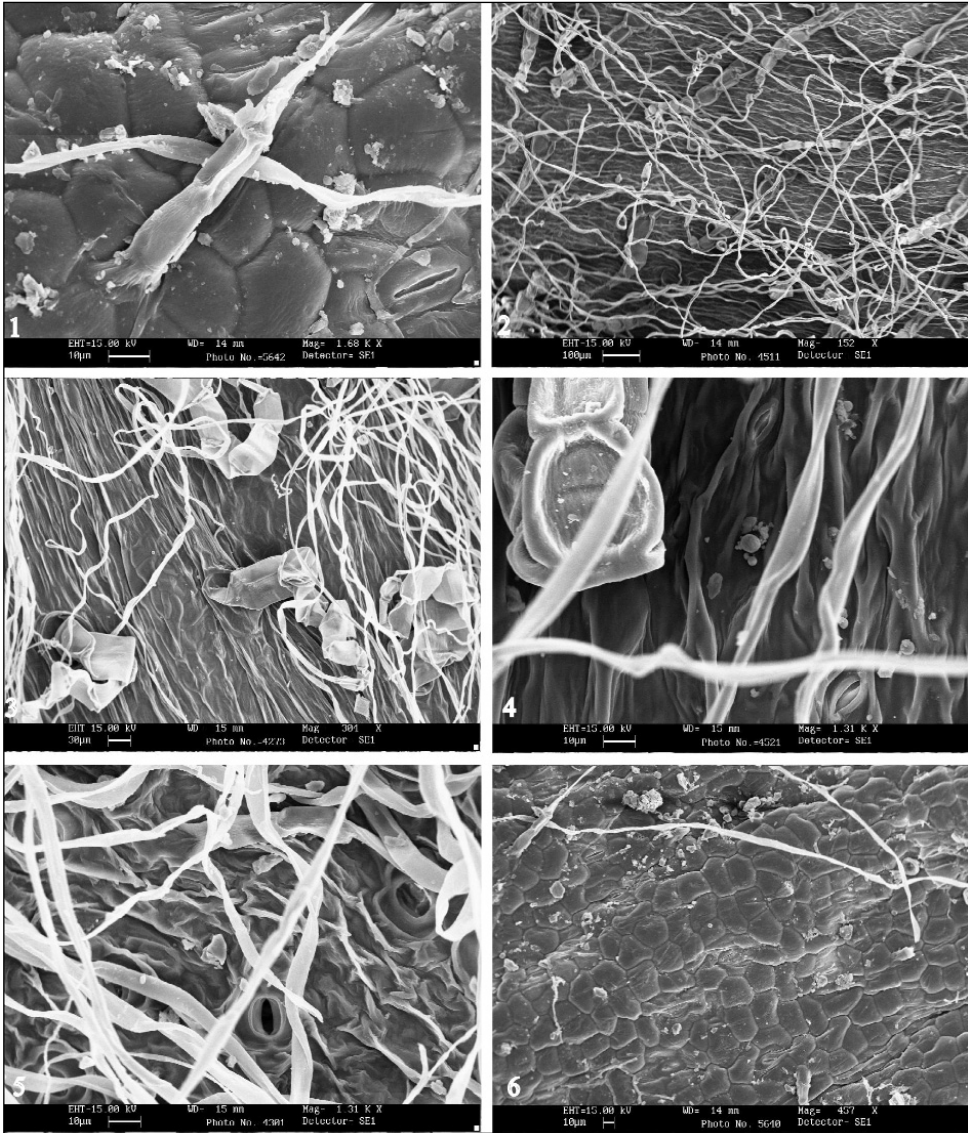


Fig. 1-6. Epidermal structure: 1 & 6 *Cyanus napulifera*, 2 & 4 *C. pseudaxillaris*, 3 *C. thirkei*, 5 *C. tuberosus*.

*C. orbelicus* (Velen.) Soják is very thick hairy. The cuticle has corrugations and fine striations somewhere. Stomata are rotund, anomocytic type. The trichomes are from type I (Fig. 9).

*C. velenovskyi* (Adamović) Wagenitz & Greuter – the upper and lower cuticles have corrugations and rough striations. The stomata are rotund, slightly sunken, anomocytic

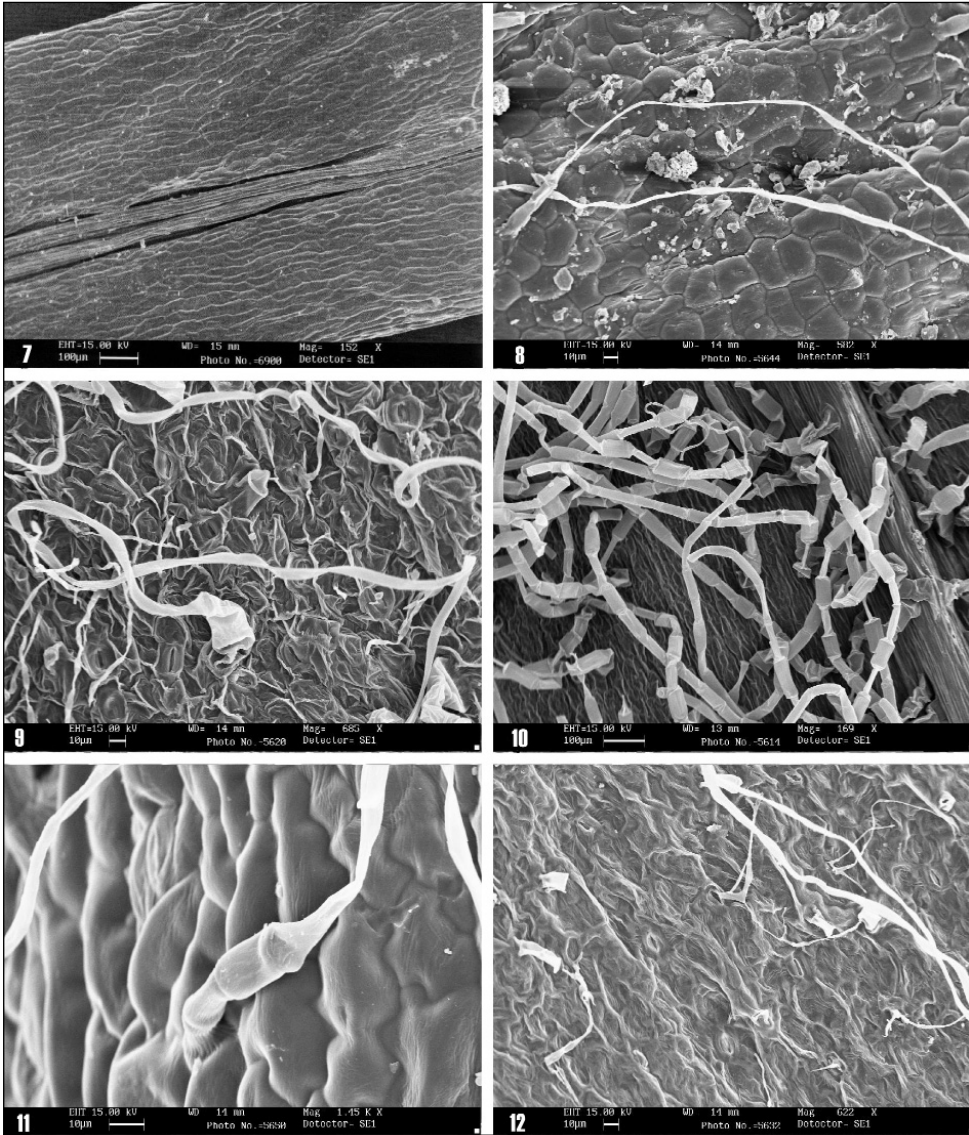


Fig. 7-12. Epidermal structure: 7 *Cyanus nyssanus*, 8 *C. napulifera*, 9 *C. orbelicus*, 10 *C. pseudaxillaris*, 11 *C. tuberosus*, 12 *C. velenovskyi*.

type. The trichomes, which basal cells have striations, belong to the type I (Fig. 12).

*C. napulifer* (Rochel) Soják – the upper epidermis consists of polygonal epidermal cells and sunken ellipsoidal stomata from anomocytic type (Fig. 1, 8). The epidermal cells of lower epidermis possess low wavy anticlinal walls, arranged like “puzzle” (Fig. 6). The trichomes are from type I. The basal cells have fine striations, including around the stomata.

*C. tuberosus* (Vis.) Soják – the upper epidermis consists of isodiametric, slightly swollen cells. The stomata are situated almost on the same level as the epidermal cells. The cuticle has rough striations (Fig. 5, 11). The trichomes are from type I.

*C. thirkei* (Sch. Bip.) Holub – the cuticle possesses fine corrugations and striations. Epidermal cells have low undulate anticlinal walls. The stomata belong to the anomocytic type and are situated almost on the same level as the epidermal cells. The trichomes are from types III and IV (Fig. 3).

*C. nyssanus* (Petrović) Soják – the cuticle on the upper and lower epidermis is completely smooth (Fig. 7). The epidermal cells and stomata are arranged in almost parallel rows. The trichomes, from the type I, are distributed only on the midvein on the lower surface. The upper surface is entirely without trichomes.

The comparison between epidermal structures of the investigated *Cyanus* taxa shows the tendency of developing of xeromorphic features. As a rule, the trichomes are distributed on the lower epidermis. All *Cyanus* taxa (excluding *C. nyssanus*) possess thick trichome covering, on both surfaces. Moreover the specific trichome morphology helps the reduction of the evaporation. The stalks, which are formed of several cells, erect the flagelliforme long cells thus between the surface and trichome cover it remains a space, which protects the epidermis from extreme evaporation. Furthermore the cuticle corrugations and striations, and the sunken stomata restrict the cuticle transpiration.

The established epidermal features: the trichome types, the cuticle peculiarities and the different stomatal types, allow the identification of the species by their epidermal structure. According Cutler & al. (1978) the leaf surface is under strong genetic control and consequently the trichome type and the indumentum features are of taxonomical value (Stace 1965; Uzunova & al. 1997).

The current investigation shows that *C. nyssanus* can be easily differentiated from the other taxa by its glabrous upper surface (Fig. 7). The epidermal structures of *C. orbelicus* and *C. velenovskyi* are very similar (Fig. 9, 12). Both species, being close relatives, possess trichomes from type I, as well striations and corrugations, which bring them together. *C. napulifer* and *C. tuberosus* have trichomes from type I also, but the epidermal cells of the lower surface are wavy and arranged like “puzzle” (Fig. 1, 6, 8, 11). The last taxon could be distinguished by “comb like” upper epidermis (Fig. 11). *C. pseudaxillaris* has combination of trichomes from types II and V (Fig. 2, 10). So it could be very well differentiated by the other species. The specific combination of trichomes from types III and IV, and the cuticle structure (Fig. 3), allow us to distinguish *C. thirkei* from all other species.

The study establishes that the epidermal structure has an important role for the adaptation of the *Cyanus* species from sect. *Napuliferae* to the environmental xerophytic conditions and could be very useful to reveal their inter-generic relationships.

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