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COMPARATIVE ANATOMICAL CHARACTERISTICS OF THE SUBGENUS *CYANUS* (MILL.) HAYEK (ASTERACEAE) IN TURKEY

BURCU YILMAZ ÇITAK*, EMRAH ŞIRIN, HÜSEYIN DURAL AND KUDDISI ERTUĞRUL

Department of Biology, Faculty of Science, University of Selçuk, Konya, Turkey

Keywords: Morphoanatomy; *Cyanus*; Numerical analysis; Plant systematics.

Abstract

The identification of species based on anatomical characters is valuable to investigate their taxonomic status, phylogeny and understand their autoecology. The current study analysed the stem and leaf properties of 20 species of the subgenus *Cyanus* (Mill.) Hayek in Turkey. The *Cyanus* species exhibited xeromorphic anatomical features, such as tight palisade parenchyma and induced spongy parenchyma, which indicated adaptations to the arid soil and a forest ecosystem in which they occurred, as well as to high light intensity and precipitation. Anatomical features such as the midrib shape and number of vascularization patterns differed among the species. Similar anatomical characters were analysed for the investigated species. These properties can provide an important database for future studies including the phylogeny within the subgenus *Cyanus*.

Introduction

The group *Cyanus* (Mill.) Hayek of the family Asteraceae was first described as a genus (Miller, 1754), and then later reduced to a section of the genus *Centaurea* L., a position that was accepted by some taxonomists (Candolle, 1838; Bentham, 1873; Stefanov, and Gheorghiev, 1931; Wagenitz, 1975). However, by some other taxonomists, it was recognized as a subgenus of *Centaurea* based on molecular evidence (Susanna and Garcia-Jacas, 2009; Hilpold *et al*., 2014a, 2014b). However, some botanists also recognized *Cyanus* as a separate genus based on morphological evidence (Soják, 1972; Greuter, 2003, 2008; Hellwig, 2004; Wagenitz, 2006; Olšavská *et al*., 2009, 2011; Stoyanov, 2016; Negaresh, 2018).

In Turkey, the subgenus *Cyanus* was rearranged as a subgenus (Ertuğrul *et al*., 2018) as a result of molecular studies (Susanna and Garcia-Jacas, 2009; Hilpold *et al*., 2014a, b).
The florets of the subgenus *Cyanus* are blue or purplish (rarely creamy or pale pinkish), which are extremely unusual colours in the subtribe Centaureinae (Boršıć *et al*., 2011). The appendages that are decurrent and not ending with mucro are characteristic for *Cyanus*, and their involucrum and leaf features are effective in species distinction (Wagenitz, 1975).

Anatomical studies can provide useful characters that could aid in the identification of problematic species, and also establish their taxonomical relationships (Metcalfe and Chalk, 1957; Janaćković *et al*., 2019a,b; Janaćković *et al*., 2019b). Although there have been many studies on the anatomy of *Centaurea* species (Metcalfe and Chalk, 1950; Esau, 1977; Metcalfe and Chalk, 1979; Uysal, 1991; Bhattacharya and Johri, 1998; Kaya *et al*., 2000; Celik *et al*., 2005; Uysal *et al*., 2005; Celik *et al*., 2008; Altundağ and Gürdal, 2009; Aydin *et al*., 2013; Özcan, 2013; Taşar *et al*., 2018; Aydın *et al*., 2019), there are very few studies regarding *Cyanus* species (Çakırlar *et al*., 2005; Ozcan *et al*., 2014; Ozcan, 2018).

Examining subgenus *Cyanus* species to identify their anatomical characteristics, clarifying the systematic value of their anatomical characteristics by way of numerical analysis, and contributing to the systematic position of the examined taxa were the main objectives herein.

*Corresponding author, E-mail: burcuyilmaz@selcuk.edu.tr

Materials and Methods

In this study 20 species of subgenus *Cyanus* in Turkey have been investigated (Table 1). The voucher specimens of each species, collected from at least five individuals, were stored in the Herbarium of Konya (KNYA). At least five fully-grown leaves and the middle parts of the stems were used in the analysis. Only sections of *C. lanigera* were taken from the herbarium specimen.

All procedures were performed at the Plant Anatomy Laboratory, Department of Biology, Selçuk University, Konya, Turkey, with either herbarium material or material fixed in 70%. Cross-sections of the stem, leaf blade and midrib were obtained using a rotary microtome. The paraffin method was applied and the parts of stem and leaf were treated through alcohol and xylene series. The anatomical tissues were stained with safranin-fast green, which was used as an established method (Johansen, 1940). The vascularization patterns of the stems and leaves, parenchyma pattern, and arrangement of the midrib vascular system were analysed. The microphotographs of the anatomical slides were obtained using a Canon EOS450D digital camera (Ota City, Tokyo, Japan) attached to Leica 1000 DM light microscope (Wetzlar, Germany).

A total of nine anatomical characters (six quantitative and three qualitative) were measured (µm) or scored using Kameram v.21 analysis system software (Argenit, Istanbul, Turkey) (Tables 2 and 3). A data matrix was set using the recorded qualitative and quantitative characters. Based on the anatomical characters, the coefficients of correlation among the 20 species were determined and these species were grouped using the clustering analysis method (unweighted pair group method with arithmetic mean (UPGMA), dissimilarity, standardized variables). The clustering analysis was based on Gower's (1971) general coefficient similarity (Sneath and Sokal, 1973), which was used directly with a mixture of character types (binary, qualitative, and quantitative. Untransformed, centered, and unstandardized data were used to create a covariance matrix. MVSP 3.22 software (Kovach Computing Services, Anglesey, Wales) was used for all of the computations.

Table 1. Locality information of the investigated taxa of *Cyanus* subgenus.

Acronym	Collection number	Species	Locality
C1	**EŞ-574-MŞ	*Centaurea reuteriana* Boiss. var. *reuteriana*	C2 Muğla: Köyceğiz, Sandras mountain, 1763 m, 29.06.2015
C2*	**EŞ-554-MŞ	*C. reuteriana* Boiss. var. *phrygia* Bornm.	B3 Afyon: Sultandağları, 1850 m, 21.05.2015
	EŞ-582-MY	*C. reuteriana* Boiss. var. *phrygia* Bornm.	A4 Karabük: Keltepe, step, 1800 m, 09.07.2015
	EŞ-562-MŞ	*C. reuteriana* Boiss. var. *phrygia* Bornm.	C3 Isparta: Davraz Dağı, stony places, 1800 m, 16.06.2015
C3*	**EŞ-659-MŞ	*C. lanigera* DC.	B5 Aksaray: Hasan mountain, 1979 m, 29.06.2016
C4	**EŞ-668-MŞ	*C. nigrofimbria* (K. Koch) Sosn.	A8 Trabzon: Çaykara, Soğanlı mountain, 2300 m, 12.07.2016
	EŞ-595-AG,HG	*C. nigrofimbria* (K. Koch) Sosn.	A8 Trabzon: Çaykara: North of Soğanlı Mountain, steppe, 2300 m, 12.07.2016
C5	**EŞ-642-MŞ	*C. woronowii*	A9 Artvin: Ardanuç, 2 km to Rabat Church, 1273 m, 11.06.2016
C6*	**EŞ-656-MŞ	*C. eflanensis* (Kaya & Bancheva) Şirin & Ertuğrul	A4 Karabük: Bartın-Safranbolu road, 1078 m, 15.06.2016

Table 1 Contd.

Acronym	Collection number	Species	Locality
C7	**EŞ-605-MŞ	*C. thirkei* Sch. Bip.	B1 Manisa: Spil Mountain, Merdivencik, *P. Nigra* openings, 951 m, 30.04.2016
C8	EŞ-591-MŞ	*C. cheiranthifolia* Willd. var. *cheiranthifolia*	A9 Ardahan: Çıldır-Aktaş road, 2100 m, 14.08.2015
	**EŞ-672-MŞ	*C. cheiranthifolia* Willd. var. *cheiranthifolia*	A9 Ardahan: Hanak, Aydere Village, step, 2326 m, 14.07.2016.
C9	**EŞ-643-MŞ	*C. cheiranthifolia* Willd. var. *purpurascens* (DC.) Wagenitz	A9 Ardahan: Değirmenli village, 2287 m, 11.06.2016
C10	**EŞ-622-MŞ	*C. bourgaei* Boiss.	C4 İçel: Mut, 1561 m, 15.05.2016
	EŞ-572-MŞ	*C. bourgaei* Boiss.	C3 Antalya: Elmalı, Kızlar Sivrisi dağcılar şenlik alanı, road sides, 1900 m, 29.06.2015
C11	EŞ-577-MŞ	*C. pichleri* Boiss. subsp. *pichleri*	A5 Amasya: Merzifon, 1502 m, 07.07.2015
	EŞ-567-MŞ	*C. pichleri* Boiss. subsp. *pichleri*	C2 Antalya: Korkuteli, Kırkpınar Plateau, 1600 m, 17.06.2015
	**EŞ-535-MŞ	*C. pichleri* Boiss. subsp. *pichleri*	C3 Isparta: Davraz Mountain, steppe, 1600 m, 12.05.2015
C12*	**EŞ-635-MŞ	*C. pichleri* Boiss. subsp. *extrarosularis* (Hayek & Siehe) Wagenitz	C5 Niğde: Demirkazık Mountain, 1849 m, 02.06.2016
	EŞ-576-MŞ	*C. pichleri* Boiss. subsp. *extrarosularis* (Hayek & Siehe) Wagenitz	B2 Kütahya: Murat Mountain, near summit, *Pinus nigra* openings, 1850 m, 01.07.2015
	EŞ-613-MŞ	*C. pichleri* Boiss. subsp. *extrarosularis* (Hayek & Siehe) Wagenitz	B3 Eskişehir: Kütahya - Eskişehir road, steppe, 820 m, 03.05.2016
	EŞ-616-MŞ	*C. pichleri* Boiss. subsp. *extrarosularis* (Hayek & Siehe) Wagenitz	B5 Kayseri: Yahyalı - Aladağ road, red-brown soils, 1727 m, 05.05.2016
	EŞ-568-MŞ	*C. pichleri* Boiss. subsp. *extrarosularis* (Hayek & Siehe) Wagenitz	C4 Konya: Konya-Beyşehir road, turnout of Ayışığı At Çiftliği, steppe, 1270 m, 27.06.2015
	EŞ-623-MŞ	*C. pichleri* Boiss. subsp. *extrarosularis* (Hayek & Siehe) Wagenitz	C5Niğde: Niğde/Adana; Aladağ, Narpuz Boğazı, steppe, 2224 m, 16.05.2016
C13	**EŞ-583-MŞ	*C. triumfettii* subsp. *axillaris* (Čelak.) Stef. & T. Georgiev	B2 Kütahya: Akdağ, 1610 m, 14.05.2016
	EŞ-579-MŞ	*C. triumfettii* subsp. *axillaris* (Čelak.) Stef. & T. Georgiev	A4 Bolu: Mengen, 1 km to Arak Village, *P. nigra* forest, 812 m, 08.07.2015
	EŞ-548-MŞ	*C. triumfettii* subsp. *axillaris* (Čelak.) Stef. & T. Georgiev	A4 Çankırı: Step, 750 m, 20.05.2015
C14	**EŞ-645-MŞ	*C. huetii* Boiss.	A9 Ardahan: Çıldır, 1 km to Gökbelen Village, sides of fields, 1991 m, 12.06.2016
	EŞ-666-MŞ	*C. huetii* Boiss.	B7 Sivas: Divriği, Göl Mountain, summit, steppe, 1926 m, 01.07.2016
C15*	**EŞ-618-MŞ	*C. mathiolifolia* Boiss.	C2 Denizli: Honaz Mountain, 1829 m, 12.05.2016
	EŞ-549-MŞ	*C. mathiolifolia* Boiss.	B3 Afyon: Sultandağı, Dereçine-Büyükyayla road, road sides, 1350 m, 21.05.2015
	EŞ-599-MŞ	*C. mathiolifolia* Boiss.	C2 Burdur: from Tefenni to Korkuteli, stony places, 1351 m, 28.04.2016

Table 1 Contd.

Acronym	Collection number	Species	Locality
	EŞ-561a-MŞ	*C. mathiolifolia* Boiss.	C2 Isparta: Davraz Mountain, stony places, 1800 m, 16.06.2015
	EŞ-564-MŞ	*C. mathiolifolia* Boiss.	C2 Isparta: Davraz Mountain, around ski resort, steppe, 1600 m, 16.06.2015
C16*	**EŞ-614-MŞ	*C. germanicopolitana* Bornm.	A4 Çankırı: Eldivan, before 3 km to Oğlaklı Village, road sides, 854 m, 04.05.2016
C17	**EŞ-547-MŞ	*C. depressa* Bieb.	A4 Çankırı: Hacı Ali Dinlenme Tesisi, road sides, 700 m, 20.05.2015
	EŞ-637-MŞ	*C. depressa* Bieb.	A5 Amasya: Akdağ, near summit, road sides, 1003 m, 09.06.2016
	EŞ-545-MŞ	*C. depressa* Bieb.	B3 Afyon: Sinanpaşa, Düzağaç Town, road sides, 1150 m, 15.05.2015
	EŞ-557-MŞ	*C. depressa* Bieb.	B4 Ankara: Hacı Hasan Village, 'Sevgi çiçeği' protected area, fallow places, 950 m, 28.05.2015
	EŞ-541-MŞ	*C. depressa* Bieb.	C2 Denizli: 950 m, 13.05.2015
C18	**EŞ-598-MŞ	*C. pinardii* Boiss.	C3 Burdur: İlyas village, 870 m, 28.04.2016
	EŞ-603-MŞ	*C. pinardii* Boiss.	B2 Uşak: Southwest of Uşak, near Karabol stream, steppe, 580 m, 29.04.2016
	EŞ-602-MŞ	*C. pinardii* Boiss.	B3 Afyon: Dazkırı, Sarıkavak Village, edge of fields, 864 m, 29.04.2016
C19*	**EŞ-560-MŞ	*C. tchihatcheffii* Fisch. & C. A. Mey.	B3 Afyon: Dazkırı, 864 m, 15.06.2015
C20	**EŞ-604-MŞ	*C. cyanus* L.	B1 Manisa: Spil Mountain, 647 m, 30.04.2016

*Endemic taxa, ** Selected taxa.

Table 2. Anatomical traits used in statical analysis of *Cyanus* subgenus.

Vegetative organ	Acronyms	Definition of anatomical traits
Stem	A1	Number of vessels
	A2	Diameter of pith cells
	A3	Width of collenchyma
Leaf	A4	Sclerenchyma cells on phloem
	A5	Mesophyll type
	A6	Mesophyll thickness
	A7	Length of palisade cells
	A8	Width of palisade cells
	A9	Width of spongy parenchyma/mesophyll
	A10	Row of palisade tissue
	A11	Width of spongy parenchyma
	A12	The shape of midrib (triangular: 0, linear: 1, oval:2, v-shaped:3)

Table 3. The anatomical measurements and observations of *Cyanus* subgenus.

Species/Anatomical characters	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
C. reuteriana var. *reuteriana*	18	93.51	146.43	1	0	690.37	67.39	18.45	0.12	3	88.52	2
C. reuteriana var. *phrygia*	20	82.19	57.74	1	0	230.76	40.85	12.90	0.46	3	108.44	1
C. lanigera	18	38.25	128.43	1	0	152.79	18.56	7.04	0.27	2	42.41	2
C. nigrofimbria	19	80.71	79.80	1	1	203.39	31.80	20.64	0.31	2	63.35	0
C. woronowii	20	61.58	125.14	0	1	105.23	23.35	13.36	0.28	2	29.71	0
C. eflanensis	16	76.53	103.4	1	0	227.13	32.04	16.55	0.28	2	65.81	2
C. thirkei	20	40.58	97.09	0	0	209.63	25.34	11.07	0.21	3	44.63	0
C. cheiranthifolia var. *cheiranthifolia*	26	114.73	175.63	1	0	202.09	34.55	16.22	0.28	2	58.53	0
C. cheiranthifolia var. *purpurascens*	24	82.23	88.72	1	0	421.05	60.37	24.16	0.24	2	103.02	1
C. bourgaei	13	50.05	62.46	1	0	262.81	48.92	14.49	0.17	2	45.41	2
C. pichleri subsp. *pichleri*	19	57.64	116.33	1	0	165.38	26.22	10.82	0.27	3	44.76	0
C. pichleri subsp. *extrarosularis*	16	65.31	111.99	1	0	231.94	29.23	14.55	0.22	2	52.53	3
C. triumfettii subsp. *axillaris*	x	x	x	x	0	68.86	21.83	7.89	0.50	2	34.71	2
C. huetii	21	73.64	107.3	1	0	269.87	41.89	15.21	0.21	2	58.18	0
C. mathiolifolia	14	55.93	107.9	1	0	257.08	40.01	14.26	0.21	2	54.83	0
C. germanicopolitana	16	95.15	162.33	1	0	268.47	40.42	21.85	0.12	3	33.87	3
C. depressa	16	101.94	130.21	1	0	230.03	39.81	13.55	0.15	2	34.97	0
C. pinardii	17	68.20	82.46	1	0	354.67	70.98	25.33	0.20	2	72.62	0
C. tchihatcheffii	21	50.79	56.45	0	0	236.21	53.20	18.8	0.14	2	34.83	2
C. cyanus L.	16	81.97	145.26	1	0	217.05	47.30	17.80	0.17	2	38.25	3

Results and Discussion

Stem anatomy

The stem cross-section varied in size among the species (Table 3). In most, it was oval-shaped with leaf blade parts (Figs 1-5). In the examined species, the stems were densely covered by unicellular trichomes (Figs 1-5). Collenchyma was present at protrusion areas in stem cross-sections. Cortex parenchyma had two types of cells; the first was cylindrical-shaped with abundant chlorophyll, the second was oval-shaped with less chlorophyll. Sclerenchyma was placed above the phloem in the cortical parenchyma of most of the species.

In all of the species examined, vascular bundles were collateral type, forming either continuous or discontinuous rings, arcs, or rings with arcs inside (Figs 1-5). Sclerenchymatic caps were found around the vascular bundles in the examined species, except in *C. tchihatcheffii*, *C. woronowii*, and *C. thirkei* (Figs 1,3,4). Vascular bundles were observed near the abaxial surface of the stems and varied in number among the species.

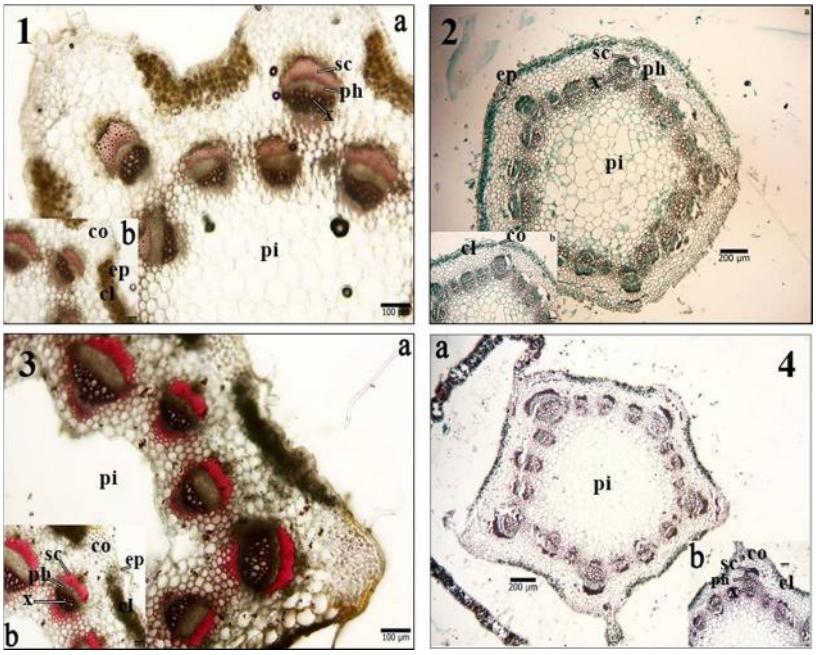

Fig. 1. The stem anatomical photographs of *Cyanus* subgenus. (a) general view, (b) close view 1. *C. reuteriana* var. *reuteriana* 2. *C. reuteriana* var. *phyrgia,* 3. *C. lanigera,* 4. *C. nigrofimbria.* ep:epidermis, cl:chlorenchyma, co:collenchyma, sc:sclerenchyma, ph:phloem, x:xylem, pi:pith

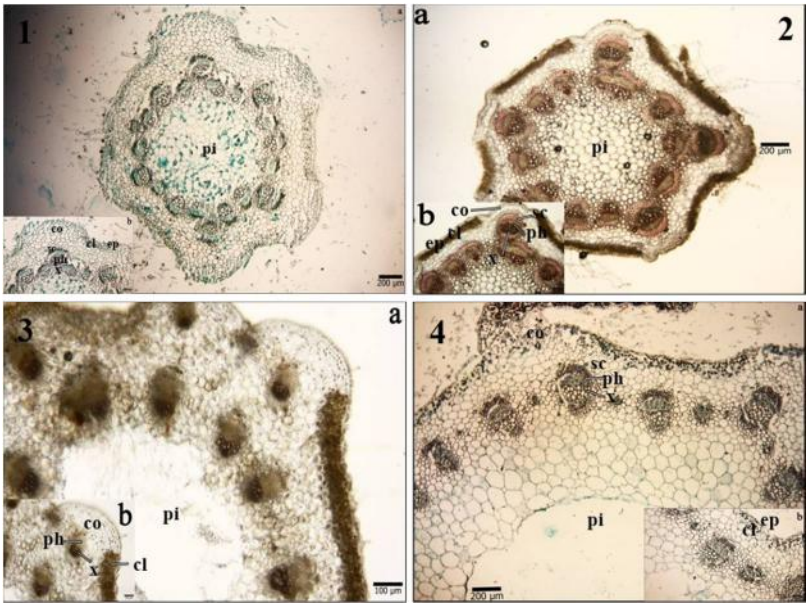

Fig. 2. The stem anatomical photographs of *Cyanus* subgenus. (a) general view, (b) close view 1. *C. woronowii*, 2. *C. eflanensis,* 3. *C. thirkei,* 4. *C. bourgaei.* ep:epidermis, cl:chlorenchyma, co:collenchyma, sc:sclerenchyma, ph:phloem, x:xylem, pi:pith

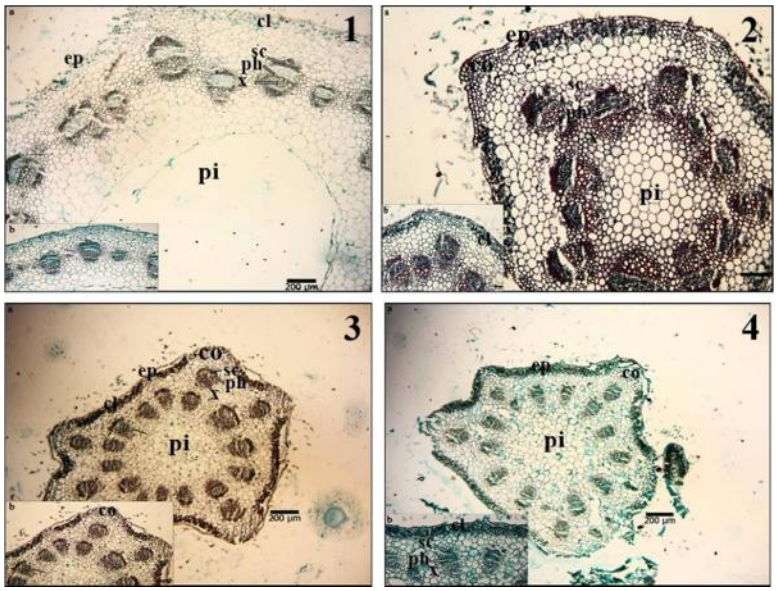

Fig. 3. The stem anatomical photographs of *Cyanus* subgenus. (a) general view, (b) close view. 1. *C. cheiranthifolia* var. *cheiranthifolia*, 2. *C. cheiranthifolia* var. *purpurascens* 3. *C. pichleri* subsp. *pichleri* 4. *C. pichleri* subsp. *extrarosularis.* ep:epidermis, cl:chlorenchyma, co:collenchyma, sc:sclerenchyma, ph:phloem, x:xylem, pi: pith

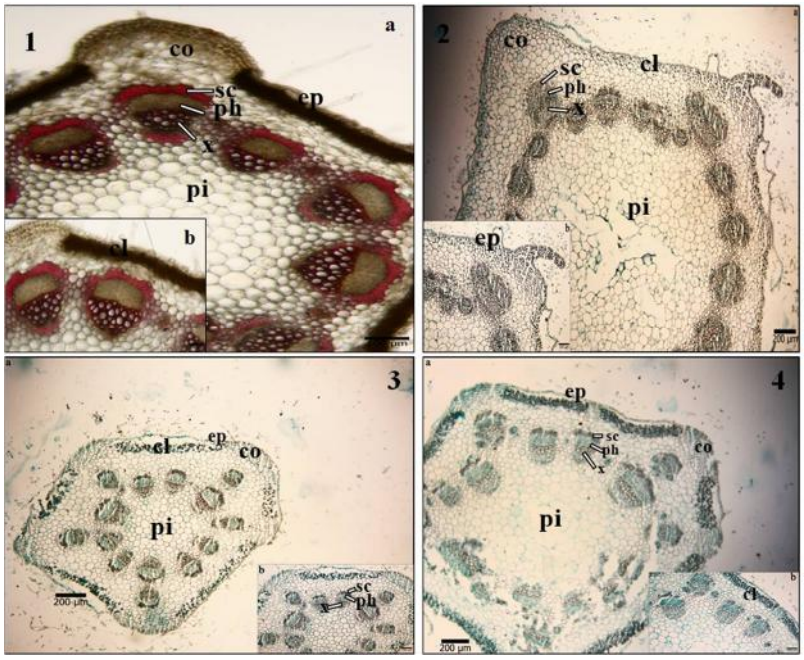

Fig. 4. The stem anatomical photographs of *Cyanus* subgenus. (a) general view, (b) close view. 1. *C. triumfetti* subsp. *axillaris*, 2. *C. huetii*, 3. *C. mathiolifolia*, 4. *C. germanicopolitana*. ep:epidermis, cl:chlorenchyma, co:collenchyma, sc:sclerenchyma, ph:phloem, x:xylem, pi:pith

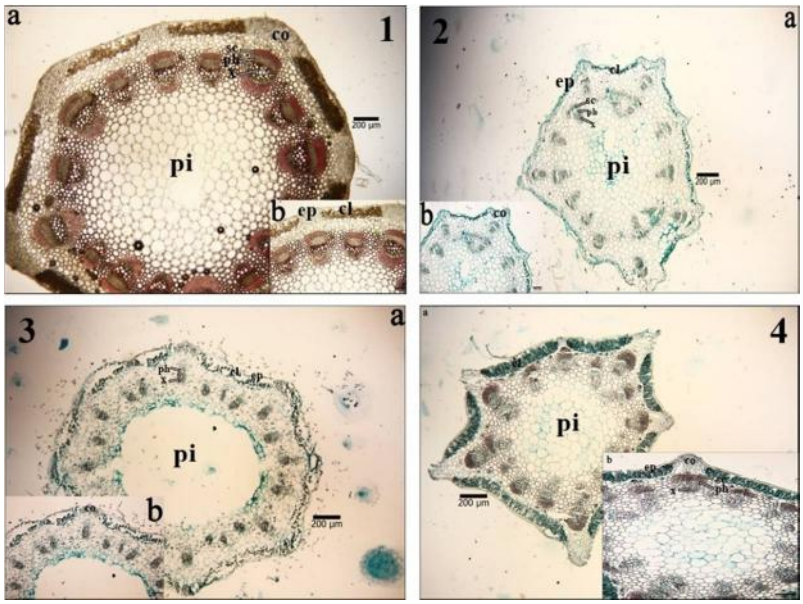

Fig. 5. The stem anatomical photographs of *Cyanus* subgenus. (a) general view, (b) close view. 1. *C. depressa*, 2. *C. pinardii*, 3. *C. tchihatcheffii*, 4. *C. cyanus.* ep:epidermis, cl:chlorenchyma, co:collenchyma, sc:sclerenchyma, ph:phloem, x:xylem, pi:pith

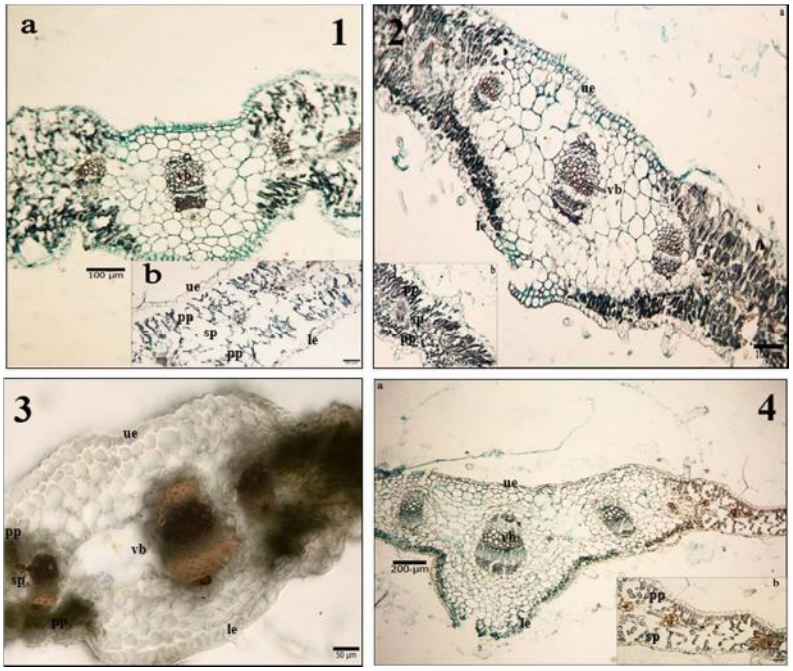

Fig. 6. The leaf anatomical photographs of *Cyanus* subgenus. (a) general view of midrib, (b) lamina view. 1. *C. reuteriana* var. *reuteriana*, 2. *C. reuteriana* var. *phyrgia*, 3. *C. lanigera*, 4. *C. nigrofimbria.* ue: upper epidermis, le:lower epidermis, pp:palisade parenchyma, sp: spongy parenchyma, vb:vascular bundle

Leaf blade and Midrib

The shape of the epidermal cells from the adaxial face was either rectangular or oval (Figs 6-10). The epidermis was uniseriate in all of the species. Collenchyma occurred under the midrib epidermis in all of the species. The mesophyll was equifacial in 17 species with well-defined palisade and reduced spongy parenchyma, bifacial in three species: *C. woronowii*, *C. nigrofimbria*, and *C. reuteriana* var. *phrygia* (Figs 6 and 7). Palisade parenchyma was composed of two to three layers of elongated cylindrical cells (Table 3). Cells of spongy parenchyma were predominantly cuboid, variably compressed according to the species. Only three species were characterized by loose spongy parenchyma, with larger intercellular spaces. Stomata were observed at the level of the epidermal line (Figs 6-10) in the species.

Midrib shape in cross-sectional view also varied among the species (Figs 6-10). On the abaxial side, it was convex in all of the species. On the adaxial side, convex (Figs 6,7,9), concave (Figs 6, 8,10), and planar midribs were found (Figs 6,8,9,10). The midrib vascular system was organized into arches that were formed by collateral bundles arranged in groups of 1 or 3.

Statistical analysis

The dendrogram derived from the cluster analysis using the UPGMA based on the nine anatomical variables of the 20 *Cyanus* species is presented in Fig. 11, in which the similarities among the examined species were presented. The dendrogram revealed two main groups: Groups A and B. Group A (with 58% similarity) comprised one perennial plant, *C. reuteriana* var. *phrygia*. Group B (with 62% similarity) comprised the remaining 19 taxa of the annuals and perennials of Turkish *Cyanus*. Group B consisted of two main clusters, which were described further as Clusters B1 and B2. Cluster B1 included *C. germanicopolitana* and *C. reuteriana* var. *reuteriana* (with 72% similarity). Group C consisted of only *C. triumfetti* subsp. *axillaris*. Cluster D included two main clusters: Clusters D1 and D2. Cluster D1 included two species: *C. cheirantifolia* var. *purpurascens* and *C. pinardii*. D2 contained two main clusters: Groups E and F. Group E consisted of two species *C. nigrofimbria* and *C. woronowii* (with 84% similarity). Cluster F included 10 species in two different subclusters: F1 and F2. Sub-cluster F1 comprised two further subclusters: Groups G and H. Group G contained only *C. lanigera*. Group H included two subclusters: Group H1 and H2. Group H1 contained *C. eflanensis*, *C. pichleri* subsp. *extrarosularis*, and *C. cyanus* (with 88% similarity) under two different subclusters. Group F2 included two subclusters, namely Subclusters F3 and F4. Subcluster F3 contained only *C. cheiranthifolia* var. *cheiranthifolia*. Subcluster F4 comprised *C. matthiolifolia* and *C. huetii* and *C. depressa* under two different smaller subclusters (with 93% similarity).

The anatomical findings on the subgenus *Cyanus* herein supported the results of Sirin *et al*. (2017), Sirin *et al*. (2019), and Citak *et al*. (2021), from the point of view of the karyological, palynomorphological, and achene morphological data.

The stem anatomical characters were determined to vary among the species; however, patterns allowing distinctions among species were not detected. Çakırlar *et al*. (2005) presented differences in the vascularization patterns of the stems of *C. tchihatcheffii* and *C. depressa*, including variations in the vascular bundle type, and number and position of accessory bundles (Fig. 5). The shape of the stem cross-sections was rounded, semi-rounded, rectangle, circular, or irregular in the family Asteraceae (Celik *et al*., 2005, 2008; Aydin *et al*., 2013; Aydin *et al*., 2019) and also in the subgenus *Cyanus* (Çakırlar *et al*., 2005, Özcan *et al*., 2014, Özcan, 2018). The position and the number of layers of the collenchyma tissue are important in plant communities (Metcalfe and Chalk, 1950; Özörgücü *et al*., 1991; Lersten and Curtis, 1997; Makbul *et al*., 2008, Aydin *et al*., 2013, Özcan *et al*., 2014). In current study, it was observed that different rows of collenchymatic tissue (3–10 rows) were located under epidermis. The arrangement of the vascular

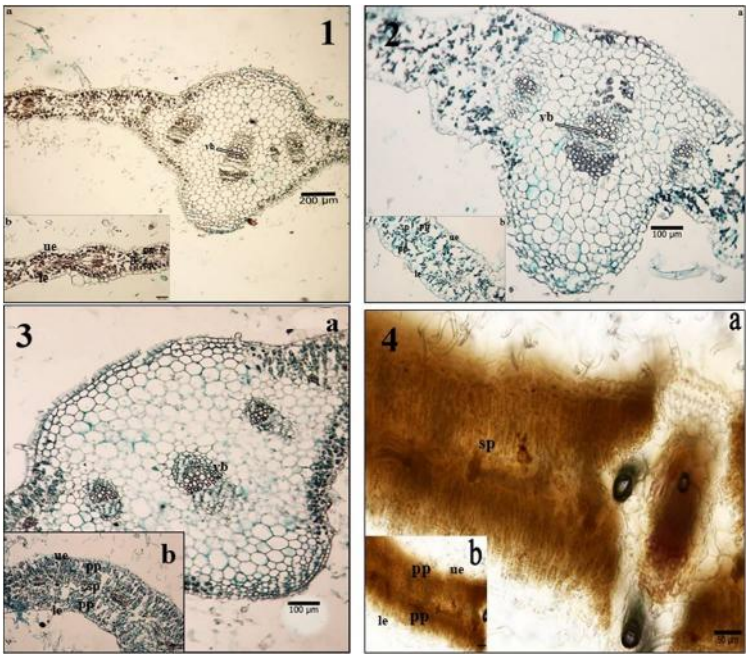

Fig. 7. The leaf anatomical photographs of *Cyanus* subgenus. (a) general view of midrib, (b) lamina view. 1. *C. woronowii*, 2. *C. eflanensis*, 3. *C.thirkei*, 4. *C. bourgaei*. ue: upper epidermis, le:lower epidermis, pp:palisade parenchyma, sp: spongy parenchyma, vb:vascular bundle

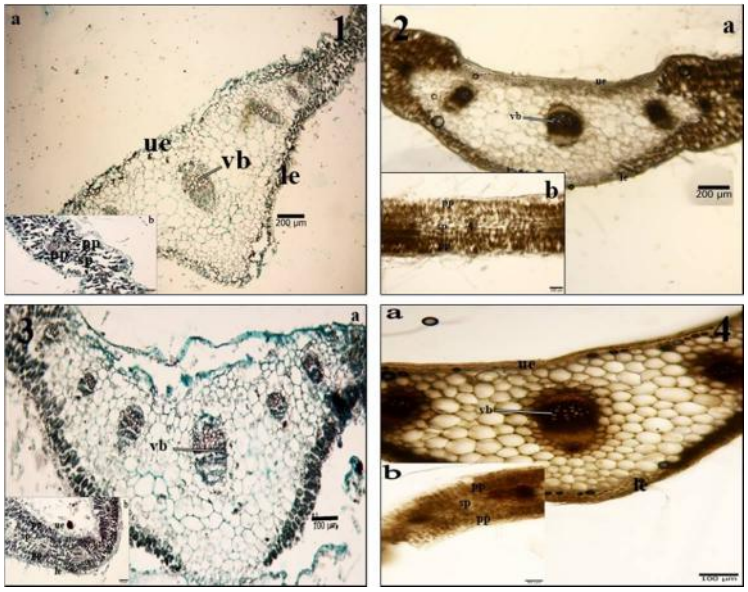

Fig. 8. The leaf anatomical photographs of *Cyanus* subgenus. (a) general view of midrib, (b) lamina view. 1. *C. cheiranthifolia var. cheiranthifolia*, 2. *C. cheiranthifolia var. purpurascens*, 3. *C. pichleri subsp. pichleri*, 4. *C. pichleri subsp. extrarosularis*. ue: upper epidermis, le:lower epidermis, pp:palisade parenchyma, sp: spongy parenchyma, vb:vascular bundle

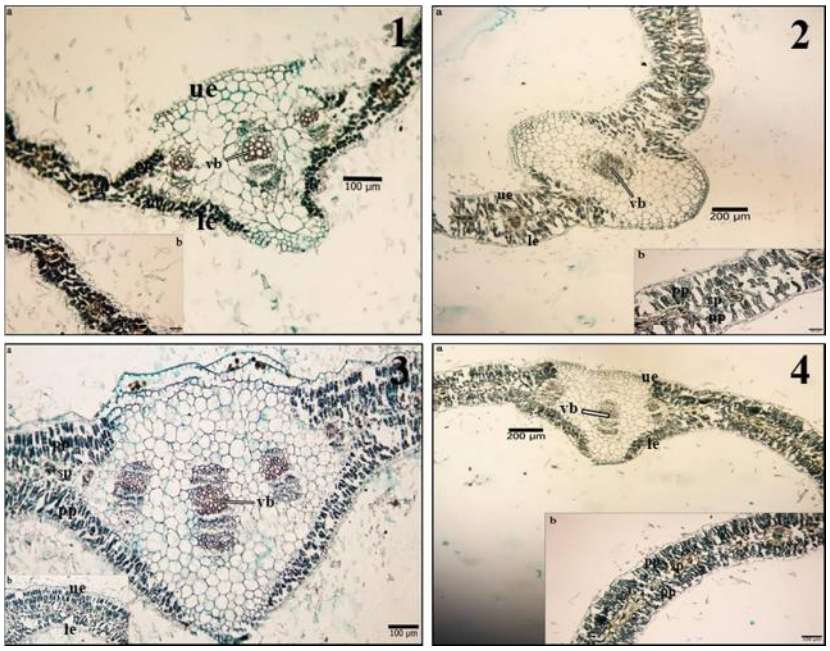

Fig. 9. The leaf anatomical photographs of *Cyanus* subgenus. (a) general view of midrib, (b) lamina view. 1. *C. triumfetti subsp. axillaris*, 2. *C. huetii*, 3. *C. mathiolifolia*, 4. *C. germanicopolitana*. ue: upper epidermis, le:lower epidermis, pp:palisade parenchyma, sp: spongy parenchyma, vb:vascular bundle.

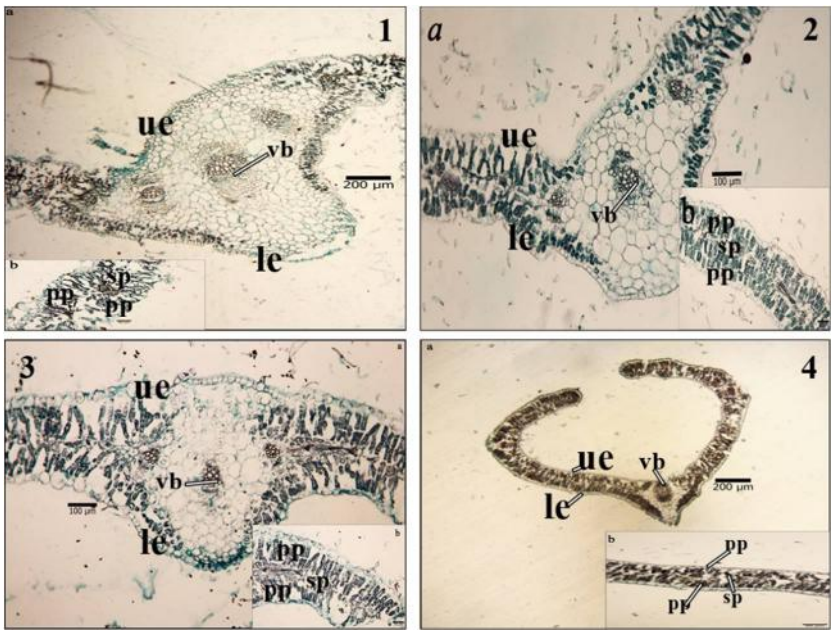

Fig. 10**.** The leaf anatomical photographs of *Cyanus* subgenus. (a) general view of midrib, (b) lamina view. 1. *C. depressa*, 2. *C. pinardii*, 3. *C. tchihatcheffii*, 4. *C. cyanus*. ue: upper epidermis, le:lower epidermis, pp:palisade parenchyma, sp: spongy parenchyma, vb:vascular bundle.

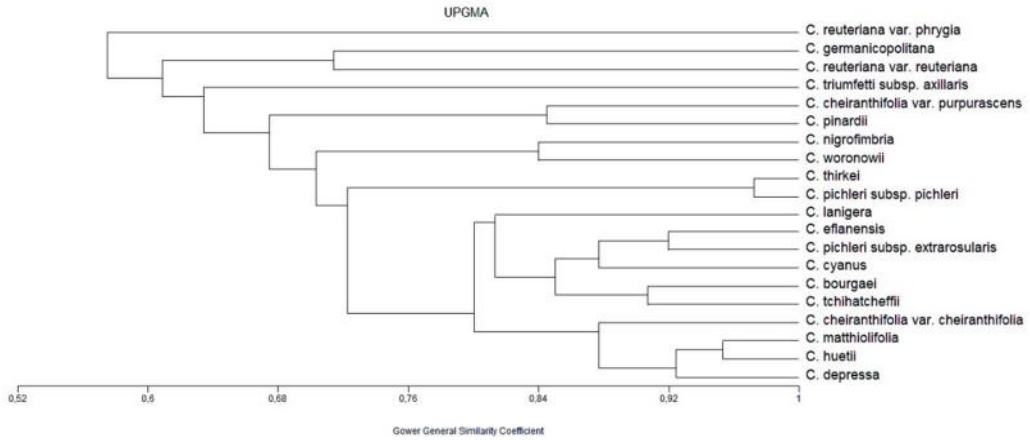

Fig. 11. The combined dendrogram according to anatomical characters of *Cyanus* subgenus

bundles was found to be in an arc shape in all of the studied species of the subgenus *Cyanus* by Çakırlar *et al*. (2005), Özcan *et al*. (2014), Özcan (2018) prior to this study. In the current study, this phenomenon was confirmed. The vascular bundles in the stem were generally arranged as two rings, and especially, those found under the collenchymatic area were bigger than the other vascular bundles. Celik *et al*. (2005, 2008) and Kaya *et al*. (2010) reported that the vascular bundles were scattered in a circular manner as one ring in the stem of some species of *Centaurea*. Clustered sclerenchymatic fibers were located on the upper sides of the vascular bundles in the examined taxa, except in *C. tchihatcheffii*, *C. woronowii*, and *C. thirkei* (Figs 2 and 5). Additionally, a chlorenchymatous tissue below the epidermis was observed in the stem cortex in the examined species. This kind of tissue was reported for the genus *Centaurea* in some previous studies performed by Uysal *et al*. (2005), Celik *et al*. (2005, 2008), and Kaya *et al*. (2010), and the subgenus *Cyanus* by Çakırlar *et al*. (2005), Özcan *et al*. (2014), and Özcan (2018). The stem cortex usually consisted of parenchymatic oval cells with thin walls in all of the examined taxa, but it varied from two to four rows.

It was found that the mesophyll of 17 species were equifacially oriented, with well-developed palisade parenchyma and reduced spongy parenchyma, and in contrast three species were dorsiventrally oriented. According to the observations of Özcan *et al*. (2014), the mesophyll of *C. cheiranthifolia* var. *purpurascens* and *C. woronowii* were equifacial. The present study confirmed that this was true of the mesophyll of *C. cheiranthifolia* var. *purpurascens* (Fig. 8); however, it was not true for *C. woronowii* (Fig. 7).

In the present study, dorsiventral mesophyll was only observed in *C. woronowii* (Fig. 7), *C. nigrofimbria* (Fig. 6), and *C. reuteriana* var. *phrygia* (Fig. 6) which grow in moist areas, while equifacial leaves were observed in the other investigated taxa, which mainly grow in the dry habitats of the Irano-Turanian phytogeographic region of Turkey. According to Yentür (2003), equifacial leaves were generally characteristic of xerophytic plants, which was in accordance with the observations made herein.

The varying midrib shape in *Cyanus* species can really contribute the systematics of this subgenus. Özcan *et al*. (2014) and Özcan (2018) reported that there were three vascular bundles in the midrib of *C. cheiranthifolia* var. *purpurascens* and *C. nigrofimbria*, and six in *C. woronowii*, which was in agreement with the results of the current study.

The UPGMA dendrogram derived from the anatomical traits of the stem and leaf discriminated the species of the subgenus *Cyanus* (Fig. 11). The positions of *Cyanus* species and their similarities reflected in the clusters were found to be partially agreeable with the previous classification of the subgenus based on the morphological data. *C. nigrofimbria* and *C. woronowii* were in the same clade according to their mesophyll type. The subspecies and varieties were close to each other in the dendrogram; however, their different positions in the different clusters can be explained by different ecological habitats, such as dry areas or forest ecosystems.

In conclusion, the number of vascularization patterns in stems, mesophyll type, and midrib shape were the most valuable variables for distinguishing the species of the subgenus *Cyanus*. The foliar and stem anatomical characters can help to improve the knowledge on *Cyanus*, separate its species more effectively, remerge the taxa, and provide an important database for future phylogenetic research within the group.

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