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Usefulness of palynomorphological characteristics for the identification of species *Cyanus* Mill. (Asteraceae) in Turkey: a taxonomic approach

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Abstract: Palynological characters of 20 taxa belonging to the Turkish subgenus *Cyanus* were studied in details using light and scanning electron microscopy. Pollen grains of the subgenus *Cyanus* were tricolporate or syncolporate, subprolate or prolate-spheroidal, 26.15 to 53 µm in polar size, 22.84 to 46.86 µm in equatorial size, isopolar, and radially symmetrical. The only type of pollen grain ornamentation observed was scabrate-perforate. Principal component analysis and cluster analysis were performed to investigate the relationships of the studied *Cyanus* taxa. As a result of the phenetic analyses, the polar axis, equatorial axis, colpus length, and apocolpium were found to be significant palynomorphological characteristics for taxonomic use.

Key words: Compositae, *Cyanus*, pollen morphology, numerical analysis, systematics

1. Introduction

The family Asteraceae is the largest and the most cosmopolitan of the flowering plants and is presumably the most widespread in the Mediterranean (Attard and Cuschieri, 2009). *Centaurea* L., is a large genus belongs to the tribe Cardueae, comprises approximately 250 species (Susanna and Garcia-Jacas, 2009) and of its 181 species are growing in Turkey (Uysal, 2012), and since then, the number of the taxa has reached 205, with the latest additions (Şirin et al. 2020). Worldwide, the subgenus *Cyanus* is represented by about 25 species (Hellwig, 2004) and 21 of them are distributed in Turkey (Uysal, 2012).

Recent definitions of *Centaurea* (Susanna and Garcia-Jacas, 2009) include the subgenera *Acrocentron*, *Centaurea*, and *Cyanus*, and although the sister relationship of the latter two has been clearly established, the relationships between them and *Acrocentron* remain unclear (Susanna and Garcia-Jacas, 2009). Miller (1754) first taxonomically described *Cyanus* as a genus. Following that, then reassigned the group as a section of *Centaurea* (De Candolle, 1838), which has been accepted extensively by taxonomists (Bentham, 1873; Boissier, 1875; Wagenitz, 1975). *Cyanus* is now generally accepted as a subgenus (Hilpold et al., 2014) and very rarely, as a group (Wagenitz and Hellwig, 1996; Garcia-Jacas et al., 2001) within *Centaurea*, although some researchers have still insisted on it being a genus (Greuter, 2003; Bancheva and Greilhuber, 2006).

Distribution of the *Cyanus* group lies across central and southern Europe, North Africa, Anatolia, and the Caucasus. Moreover, some species occur in areas of Iran and Afghanistan (Boršić et al., 2011). Within the scope of the current research, *Cyanus* was considered as a subgenus, based on current molecular studies (Şirin et al., 2019).

Florets in this group are blue or purplish blue in color, with a few taxa having been reported as cream or pale pink, which is highly exceptional for the subtribe Centaureinae. Additionally, the most unusual characteristic is the phyllary appendages, which are non-spiny and decurrent almost down to the base (Wagenitz and Hellwig, 1996). This group also has some significant characteristics in common with groups *Jacea* and *Acrocentron*. It has marginal florets that are sterile and do not comprise staminodes, and the seed has a lateral hilum (Garcia-Jacas et al., 2001; Şirin et al., 2017).

Aside from its morphological characteristics, the *Cyanus* group is also distinguished by having two pollen types. In a study by Wagenitz (1955), it was reported that of the eight types of pollen in *Centaurea* s.l., two types were identified in *Cyanus* subgroups. One of the subgroups comprises annuals that have the *Cyanus* pollen type, while the other subgroup comprises perennials that have the *Montana* pollen type (Figure 1).

Species of the subgenus *Cyanus* have been examined by various researchers in recent years (Pınar and İnceoğlu, 1996; Özler et al., 2009; Bakels, 2012; Atar

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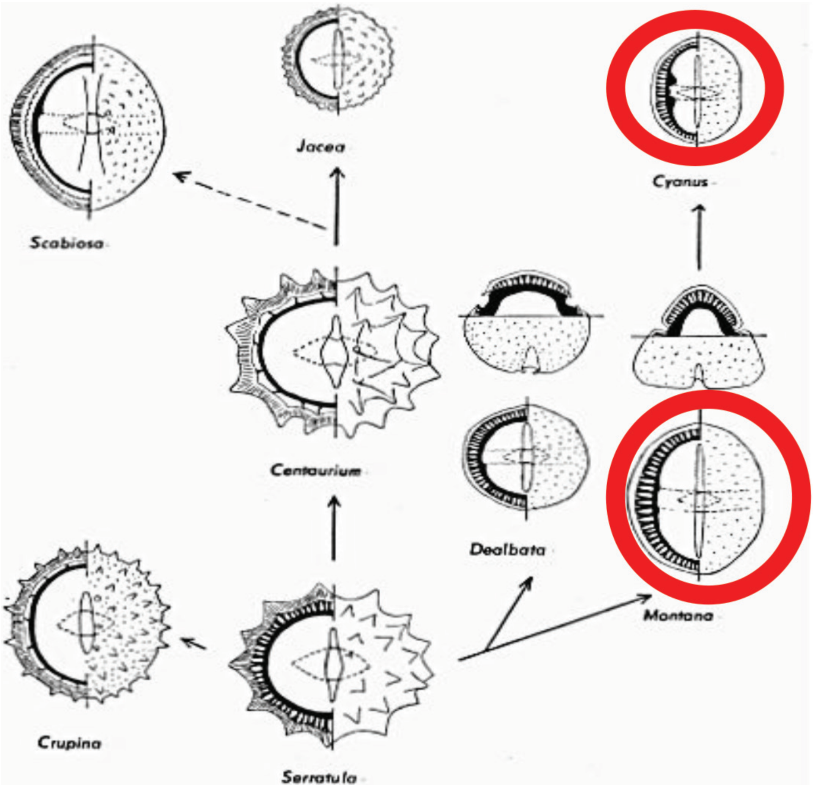

Figure 1. Wagenitz's (1955) pollen types.

et al., 2018; Taşar et al., 2018). Although these studies have reported palynological analyses of these species of *Cyanus*, no correlation results have been reported with other *Cyanus* subgenus species. To date, there remain some Turkish *Cyanus* subgenus species that have not been palynomorphologically investigated in detail. Therefore, the main aim herein was to: i) examine Turkish *Cyanus* subgenus species to identify their pollen characteristics, ii) clarify the systematic value of their palynological characteristics via numerical analysis, and iii) contribute to the systematic position of the examined taxa.

2. Materials and methods

2.1. Plant materials

The plant samples investigated in this study were obtained during doctoral studies of the second author from various phytogeographical regions in Turkey. Information about the voucher specimens and other details are given in Table 1. The *Cyanus* subgenus plant samples were kept in the Department of Biology, Faculty of Science, Selçuk University Faculty Herbarium (KNYA).

2.2. Microscopic studies

For LM studies, pollen grains were prepared according to Wodehouse (1935) method. This method allows very easy observation of the pollen grains within glycerin jelly and safranin. They were investigated under a light microscope (LM) and photographed at 100× magnification using immersion oil. Measurements were made using Kameram 21 software based on at least 30 or more pollen grains from each specimen. The apocolpium (t) measurements was done according to Ruiz-Domínguez et al. (2019) and amb dimensions was done according to Erdtman (1969).

For the SEM analysis, the dried nonacetolysis pollen grains were placed directly onto aluminum stubs and coated with gold using a sputter coater (Çitak et al., 2019). They were photographed at several magnifications under Zeiss Evo LS 10 SEM (Carl Zeiss Microscopy GmbH, Oberkochen, Germany) at the Advanced Technology Research and Application Center of Selçuk University in Konya, Turkey.

2.3. Terminology

The pollen terminology used was from Erdtman (1954, 1969), Wagenitz (1955), Faegri and Iversen (1975), Punt et al. (2007), Punt and Hoen (2009), Yurtseva et al. (2014), Halbritter et al. (2018) , and Ruiz-Domínguez et al. (2019).

2.4. Statistical analysis

The determined qualitative and quantitative characters were scored for numerical analyses. A total of 16 pollen characteristics (see Table 2) were used to evaluate the taxonomical relationships of the *Cyanus* taxa. Twenty taxa

Table 1. Location data of the examined 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
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
C5	EŞ-640-MŞ	*C. woronowii*	A8 Artvin: Hatila Valley National Park, 500 m, 11.06.2016
C6*	EŞ-654-MŞ	*C. eflanensis* (Kaya & Bancheva) Şirin & Ertuğrul	A4 Karabük: Bartın-Safranbolu road, 1078 m, 15.06.2016
C7	EŞ-601-MŞ	*C. thirkei* Sch. Bip.	C2 Denizli: Tavas, 1260 m, 28.04.2016
C8	EŞ-591-MŞ	*C. cheiranthifolia* Willd. var. *cheiranthifolia*	A9 Ardahan: Çıldır-Aktaş road, 2100 m, 14.08.2015
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
C11	EŞ-577-MŞ	*C. pichleri* Boiss. subsp. *pichleri*	A5 Amasya: Merzifon, 1502 m, 07.07.2015
C12*	EŞ-635-MŞ	*C. pichleri* Boiss. subsp. *extrarosularis* (Hayek & Siehe) Wagenitz	C5 Niğde: Demirkazık mountain, 1849 m, 02.06.2016
C13	EŞ-621-MŞ	*C. triumfettii* subsp. *axillaris* (Čelak.) Stef. & T. Georgiev	B2 Kütahya: Akdağ, 1610 m, 14.05.2016
	KE-5067-HD-TU	*C. triumfettii* subsp. *axillaris* (Čelak.) Stef. & T. Georgiev	A9 Ardahan: Göle, 2150 m, 24.07.2015
C14	EŞ-648-MŞ	*C. huetii* Boiss.	A8 Erzurum: Erzurum-İspir road, 2351 m, 13.06.2016
C15*	EŞ-618-MŞ	*C. mathiolifolia* Boiss.	C2 Denizli: Honaz mountain, 1829 m, 12.05.2016
C16*	EŞ-586-MŞ	*C. germanicopolitana* Bornm.	A4 Karabük: Eflani, Kavak village, 919 m, 03.08.2015
C17	EŞ-543-MŞ	*C. depressa* Bieb.	C2 Denizli: Muğla-Tavas road, 998 m, 14.05.2015
C18	EŞ-598-MŞ	*C. pinardii* Boiss.	C3 Burdur: İlyas village, 870 m, 28.04.2016
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.

were clustered using Gower's (1971) general coefficient similarity and a dendrogram was created using the clustering analysis method, i.e. the unweighted pair group method with arithmetic mean (UPGMA), in MVSP 3.22 software. Principle coordinate analysis (PCA) was performed in the same program according to the method of Baldemir et al. (2018).

3. Results

All of the examined samples were quantitatively and qualitatively summarized in Table 2, and illustrated in Figures 3–9.

3.1. Size, symmetry, and shape

The pollen grains of *Cyanus* subgenus taxa were determined as monad, radially symmetrical and isopolar. They were elliptic and not compressed at the poles in equatorial view, while they were subcircular or slightly triangular with obtuse angles in polar view. Fifteen of the studied taxa, had subprolate-shaped pollen grains with polar axis ranging from 39.2 to 49.34 µm and equatorial axis ranging from 32.41 to 43.76 µm. The remaining taxa had prolate-spheroidal-shaped pollen grains with polar axis ranging from 39.65 to 53 µm and equatorial axis ranging from 34.95 to 46.86 µm. The dimensions were smaller in

Table 2. Pollen characteristics of *Cyanus* subgenus in Turkey.

Taxa	Chromosome number (2n)/ ploidy level (x)*	Aperture type	Polar axes (P) (μ)		Equatorial axes (E) (μ)		P/E	Pollen shape	Exine at poles (μ)	Exine at equator (μ)	Intine (μ)
			Min-max	Mean±SD	Min-max	Mean±SD					
C. reuteriana var. *reuteriana*	2n = 20	100% Tricolporate	39.39–44.66	41.13 ± 1.56	33.82–39.18	35.57 ± 1.54	1.15	Subprolate	4.11	5.04	0.75
C. reuteriana var. *phrygia*	2n = 22	100% Tricolporate	41.24–46.43	44 ± 1.46	35.15–41.22	38.11 ± 1.52	1.15	Subprolate	3.26	3.45	0.85
C. lanigera	2n = 20	100% Tricolporate	39.65–49.8	42.75 ± 2.57	34.95–39.4	37.6 ± 1.29	1.13	Prolate-spheroidal	2.66	3.94	0.99
C. nigrofimbria	2n = 20	100% Tricolporate	42.46–46.92	44.75 ± 1.36	36.7–40.38	38.93 ± 0.94	1.14	Subprolate	3.49	4.16	0.82
C. woronowii	2n = 20	100% Tricolporate	39.2–46.07	42.95 ± 2.3	32.41–37.9	35.57 ± 1.23	1.20	Subprolate	3.08	4.27	0.87
C. eflanensis	2n = 20	100% Tricolporate	40.05–45.28	42.26 ± 1.16	34.69–39.52	36.58 ± 1.07	1.15	Subprolate	3.73	3.98	0.82
C. thirkei	2n = 22 ** B chromosome	97% Tricolporat, 3% Syncolporate	45.99–52.49	48.17 ± 1.7	38.88–43.76	40.91 ± 1.69	1.17	Subprolate	4.37	4.82	0.8
C. cheiranthifolia var. *cheiranthifolia*	2n = 20	100% Tricolporate	41.94–45.36	43.78 ± 1.10	36.53–40.56	38.67 ± 1.09	1.13	Prolate-spheroidal	3.51	3.81	0.84
C. cheiranthifolia var. *purpurascens*	2n = 4x = 40	100% Tricolporate	49.93–53	51.5 ± 1.03	43.32–46.86	45.33 ± 1.16	1.13	Prolate-spheroidal	2.75	4.40	0.89
C. bourgaei	2n = 4x = 40	100% Tricolporate	46.87–49.34	47.99 ± 0.86	38.36–42.45	40.18 ± 1.21	1.19	Subprolate	4.43	4.78	0.90
C. pichleri subsp. *pichleri*	2n = 4x = 40	100% Tricolporate	39.86–48.03	43.86 ± 2.08	36.32–40.98	39.62 ± 1.41	1.10	Prolate-spheroidal	4.20	4.83	0.89
C. pichleri subsp. *extrarosularis*	2n = 20	100% Tricolporate	40.07–46.79	43.68 ± 1.54	34.78–39.55	37.67 ± 1.02	1.15	Subprolate	3.12	3.85	0.82
C. triumfetti subsp. *axillaris*	2n = 4x = 40	100% Tricolporate	41.3–46.17	43.40 ± 1.29	35.37–38.87	36.64 ± 0.96	1.18	Subprolate	4.00	4.56	0.86
C. huetii	2n = 4x = 40	100% Tricolporate	40.51–46.13	44.27 ± 1.26	35.37–39.49	38.14 ± 1.07	1.16	Subprolate	3.17	3.93	0.73
C. matthiolifolia	2n = 4x = 40	86% Tricolporate, 14% Syncolporate	41.65–47.07	44.62 ± 1.42	36–40.79	38.83 ± 1.42	1.14	Subprolate	3.17	4.08	0.78
C. germanicopolitana	2n = 4x = 40	100% Tricolporate	40.25–46.82	44.21 ± 1.44	36.36–41.2	39.02 ± 1.33	1.13	Prolate-spheroidal	3.61	4.39	0.84
C. depressa	2n = 16	100% Tricolporate	33–36.67	34.64 ± 0.97	26.89–29.97	27.8 ± 0.87	1.24	Subprolate	1.83	2.46	0.72
C. pinardii	2n =16	100% Tricolporate	26.15–30.58	29.38 ± 1.05	22.84–26.3	24.93 ± 0.72	1.17	Subprolate	1.89	2.81	0.64
C. tchihatcheffii	2n = 20	100% Tricolporate	32.01–37.07	34.54 ± 1.62	23.76–29.08	26.51 ± 1.8	1.30	Subprolate	2.76	4.28	0.75
C. cyanus	2n = 24	100% Tricolporate	31.68–34.07	32.95 ± 0.66	24.19–26.8	25.54 ± 0.72	1.28	Subprolate	1.81	2.56	0.67

According to *Şirin et al. (2019), ** Bancheva and Greilhuber (2006).

Table 2 (continued). Additional pollen characteristics of *Cyanus* subgenus in Turkey.

Taxa	Costae (μ)	Amb (μ)	t (μ)	The length of colpus (μ)	The width of colpus (μ)	The length of porus (μ)	The width of porus (μ)	Pollen type
C. reuteriana var. reuteriana	2.34	15.07	7.00	34.11	13.42	9.27	12.43	Montana type
C. reuteriana var. phrygia	2.33	11.83	8.56	30.71	12.57	9.15	11.43	Montana type
C. lanigera	2.61	17.88	10.89	31.30	13.68	9.96	12.70	Montana type
C. nigrofimbria	1.91	14.39	7.79	33.58	13.60	11.48	14.05	Montana type
C. woronowii	2.42	13.61	8.68	33.15	11.88	9.29	11.89	Montana type
C. eflanensis	2.18	13.65	8.04	32.08	11.87	9.15	11.93	Montana type
C. thirkei	2.16	19.13	10.83	33.89	12.78	8.85	12.11	Montana type
C. cheiranthifolia var. cheiranthifolia	2.21	15.10	10.60	34.48	13.56	11.31	13.44	Montana type
C. cheiranthifolia var. purpurascens	2.73	19.07	9.85	46.29	16.89	11.93	15.92	Montana type
C. bourgaei	2.49	13.6	9.88	39.61	13.15	10.06	13.1	Montana type
C. pichleri subsp. pichleri	2.28	17.18	7.81	34.07	11.27	11.33	13.33	Montana type
C. pichleri subsp. extrarosularis	2.39	14.54	8.78	33.62	12.71	9.84	13.13	Montana type
C. triumfetti subsp. axillaris	2.11	14.70	8.79	33.88	10.94	9.36	11.45	Montana type
C. huetii	2.37	14.42	8.13	33.90	12.36	10.56	12.94	Montana type
C. matthiolifolia	2.26	16.77	10.22	34.35	13.32	10.52	13.23	Montana type
C. germanicopolitana	1.93	17.24	10.63	33.34	11.91	10.70	12.08	Montana type
C. depressa	1.98	12.54	8.33	27.69	9.79	6.52	8.98	Cyanus type
C. pinardii	1.63	11.93	6.06	22.95	7.00	5.87	7.59	Cyanus type
C. tchihatcheffii	1.84	12.04	7.91	30.14	10.62	6.98	9.80	Cyanus type
C. cyanus	1.70	9.53	5.66	25.99	7.88	5.16	7.77	Cyanus type

t: apocolpium, amb: the measurements of polar view of pollen grains.

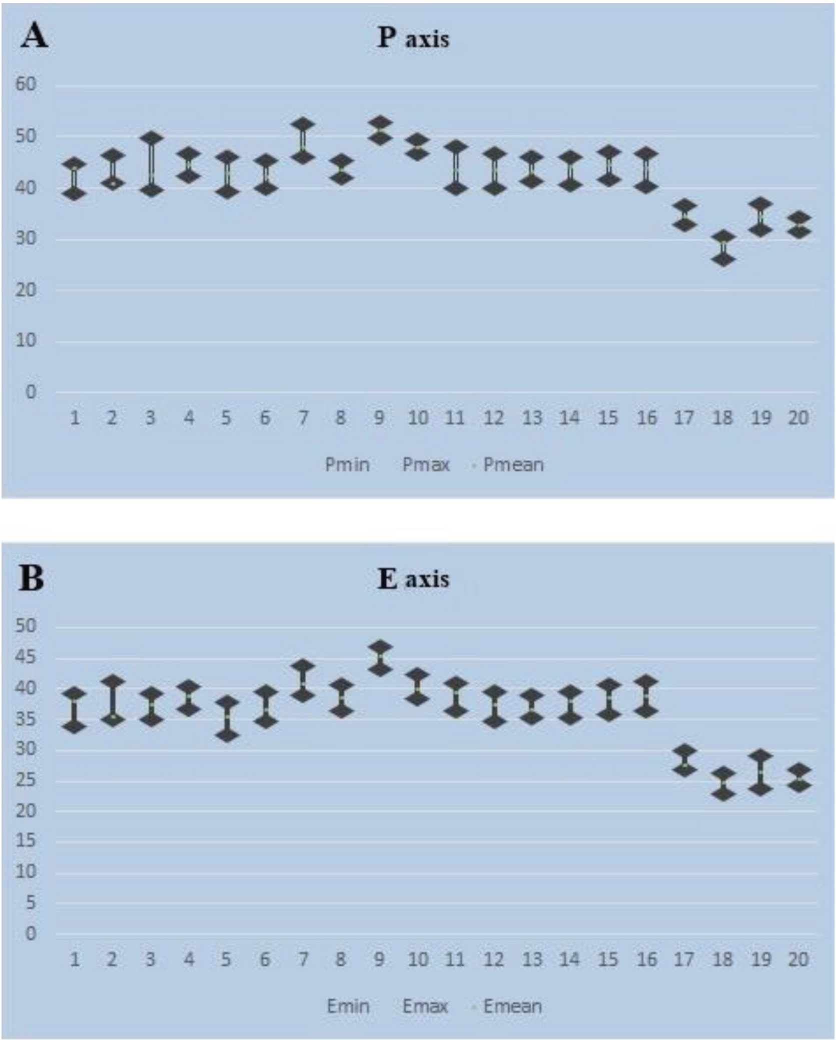

Figure 2. The Simpson and Roe test for the *Cyanus* subgenus species A. Polar axis (P); B. Equatorial axis (E).

Centaurea pinardii (and larger in *C. cheiranthifolia* var. *purpurascens* (Table 2, Figure 2). Apolcolpium is smallest in *C. cyanus* (9.53 µm) and is largest in *C. cheiranthifolia* var. *purpurascens* (19.07 µm).

3.2. Exine and intine

The intine was very thin and the intine thickness varied from 0.64 µm to 0.99 µm. The exine was semitectate. and the exine thickness at the equator was slightly thicker than that at the poles (Table 2). The scabrate-perforate sculpturing was determined in the studied *Cyanus* taxa and the spinule length was 0.18–0.23 µm (Figures 8 and 9). All of the taxa had a costae thickness ranging from 1.63 µm to 2.73 µm (Table 2, Figure 3).

3.3. Apertures

All of the specimens of the subgenus examined generally had 3-zonocolporate or rarely syncolporate pollen grains, which were taxonomically important. *C. thirkei* and *C. matthiolifolia* had heteromorphic pollen features. *C. thirkei* had 3% syncolporate pollen grains and 97% 3-zonocolporate pollen grains (Figures 4–9) and *C. matthiolifolia* had 14% syncolporate pollen grains and 86% 3-zonocolporate pollen grains (Figure 6).

The colpus in the observed species was sunken (Figures 3–9). The colpus was long (22.95–46.29 µm) and narrow (7.00–16.89 µm) and the ora were circular or lolongate in all of studied species. The highest values were seen in *C. cheiranthifolia* var. *purpurascens*, which had the largest measured colpus. Margins were distinct, straight, and the ends were acute to slightly obtuse in all of the investigated taxa. The colpus membrane was granulose in all of the taxa. The porus length changes from 8.85 µm to 11.93 µm and the porus width also changes from 7.59 µm to 15.92 µm.

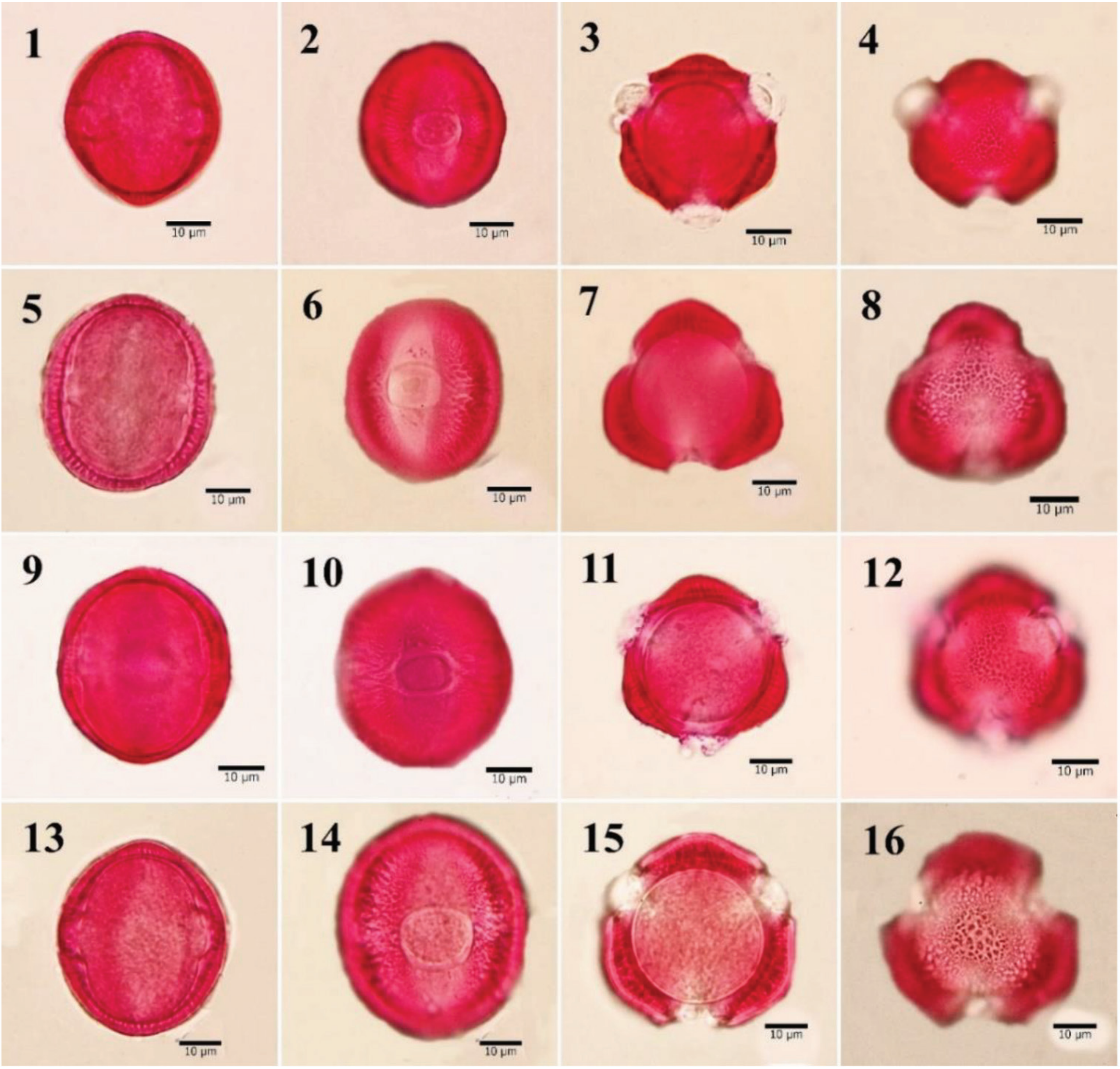

Figure 3. Pollen grains of Turkish *Cyanus subgenus* under LM. 1–4: *Centaurea reuteriana var. reuteriana* 5–8: *C. reuteriana* var. *phyrgia*, 9–12: *C. lanigera*, 13–16: *C. nigrofimbria*.

3.4. Numerical analysis of the palynological characteristic states

The dendrogram obtained from the cluster analysis using the UPGMA based on the 5 palynological variables (equatorial axis, outline in polar view (amb), colpus length, and porus length and width) of the 20 *Cyanus* subgenus taxa is presented in Figure 10. This dendrogram reflected the similarities among the investigated taxa and revealed 2 main groups: group A (with 78% similarity), which comprised 4 species of annual *Cyanus* taxa, and group B (with 64% similarity), which comprised the remaining 16 taxa. Group B comprised 2 main clusters, which were described further as clusters C and D. Cluster C contained only *C. cheiranthifolia* var. *purpurascens* (with 64% similarity). Cluster D contained 15 taxa with 2 subgroups, namely D1 and D2. Cluster D1 contained 2 subclusters, namely D1a and D1b. Subcluster D1a contained only *C. bourgaei* and subcluster D1b contained 2 subclusters, wherein only *C. thirkei* was in subcluster E (with 89% similarity). *C. germanicopolitana*, *C. mathiolifolia*, *C. cheiranthifolia* var. *cheiranthifolia*, and *C. lanigera* were all in subcluster F (with different similarity rates), which contained 2

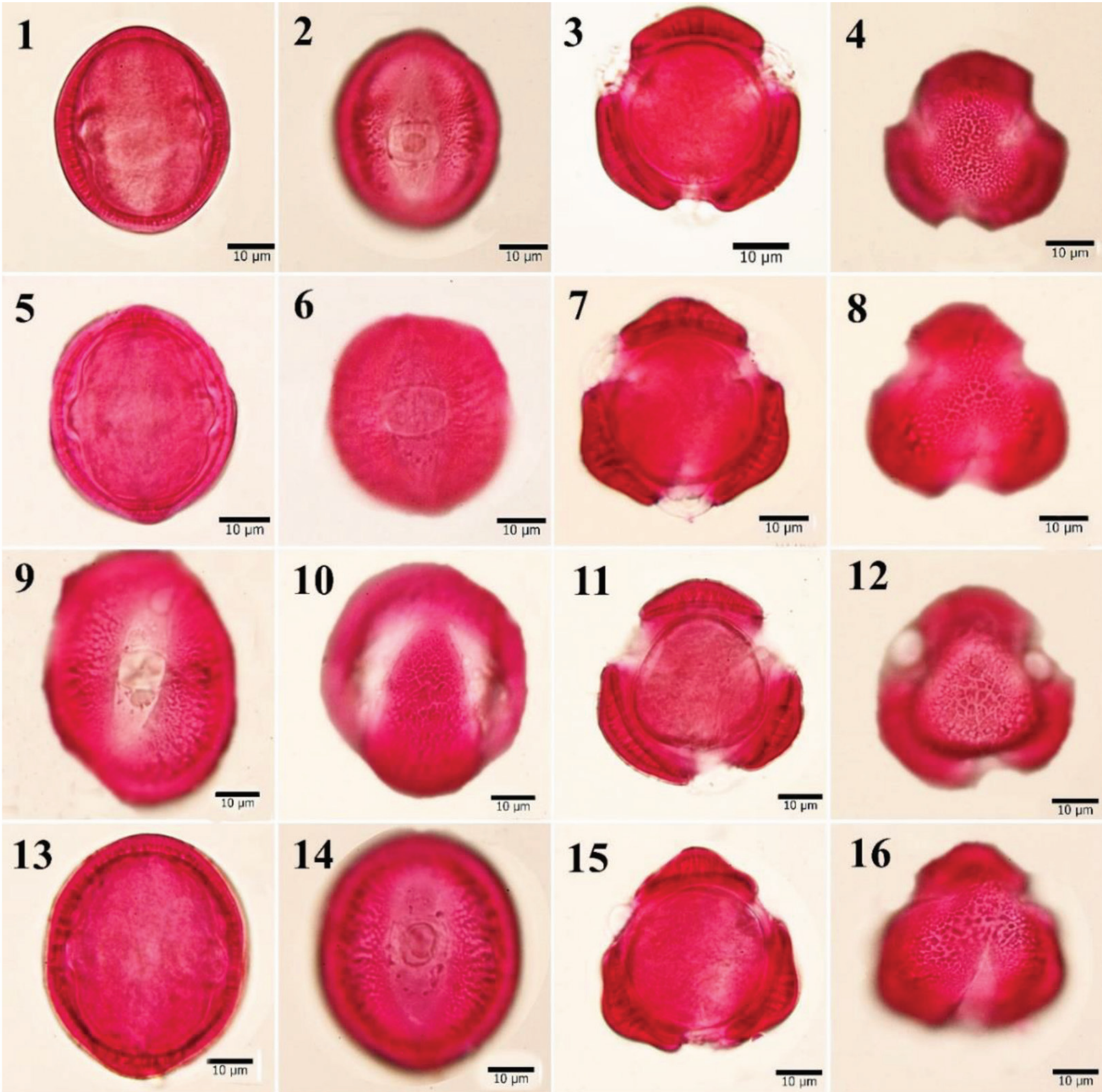

Figure 4. Pollen grains of Turkish *Cyanus subgenus* under LM. 1–4: *Centaurea woronowii,* 5–8: *C. eflanensis*, 9–12: *C. thirkei*, 13–16: *C. bourgaei.*

subgroups, namely F1 and F2. Subgroup F1 contained 2 subgroups (with 95% similarity), namely F1a and F1b. Subgroup F1a contained only *C. germanicopoliatana*. Subgroup F1b contained *C. matthiolifolia* and *C. cheiranthifolia* var. *cheiranthifolia*. Subgroup F2 contained only *C. lanigera* (with 90% similarity). Cluster D2 also contained 2 subclusters, namely D2a and D2b. Subcluster D2a contained two subgroups, namely G and H. Subgroup G contained *C. huetii* and *C. pichleri subsp. extrarosularis* (with 95% similarity). Subgroup H contained *C. pichleri* subsp. *pichleri* and *C. nigrofimbria* (with 97% similarity). Subcluster D2b contained 2 subgroups, namely I and J. Subgroup I contained only *C. reuteriana* var. *reuteriana* (with 89% similarity). Subgroup J contained 2 subclusters, namely J1 and J2. Subcluster J1 contained only *C. eflanensis* (with 95% similarity). Subcluster J2 contained three species in 2 subgroups. Subgroup J2a included only *C. reuteriana* var. *phyrigia*, while subgroup J2b contained *C. woronowii* and *C. triumfetti* subsp. *axillaris* (with 96% similarity).

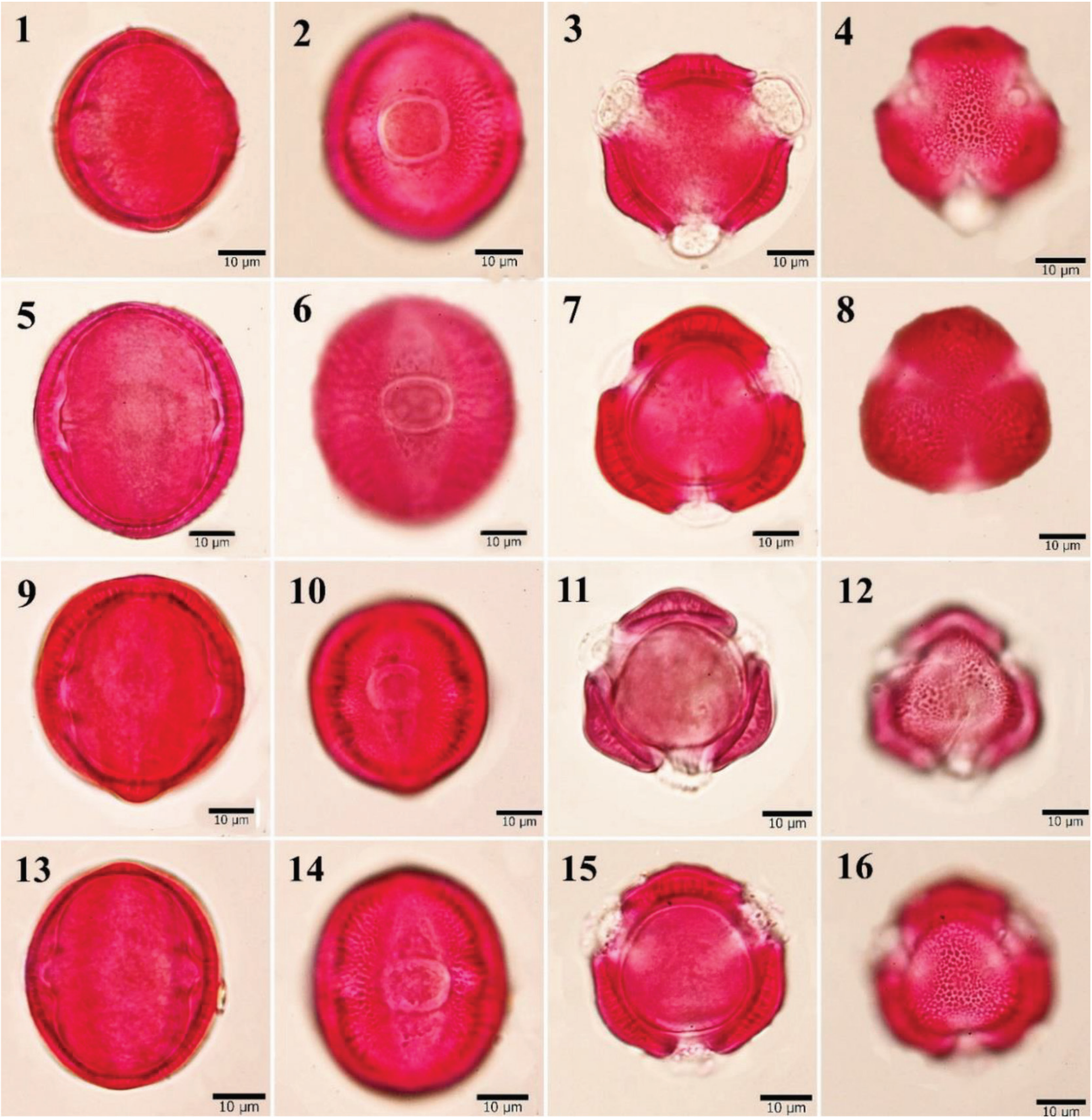

Figure 5. Pollen grains of Turkish *Cyanus subgenus* under LM. 1–4: *Centaurea cheiranthifolia* var. *cheiranthifolia* 5–8: *C. cheiranthifolia* var. *purpurascens,* 9–12: *C. pichleri* subsp. *pichleri*, 13–16: *C. pichleri* subsp. *extrarosularis*.

PCA analysis was performed to determine which variables were important for explaining the total variation among the 20 taxa examined. The PCA explained 99.897% of the variation with the first three axis (Figure 11). The first principal component explained 99.7% of the total variation in the examined species. The polar and equatorial axis, and colpus length were the most significant variables in the first principal component because they had the highest relative variation rate. The apocolpium and amb had the strongest influence on the species in the second principal component, which explained 99.859% of the total variation. The third principal component explained 99.897% of the total variation, mainly through variables such as the intine thickness. The first three components that underlined the three primary valuable variables for separating the species of *Cyanus* subgenus examined in this study were the polar and equatorial axis, and colpus length. The apocolpium, amb, and intine thickness were secondary important characteristics.

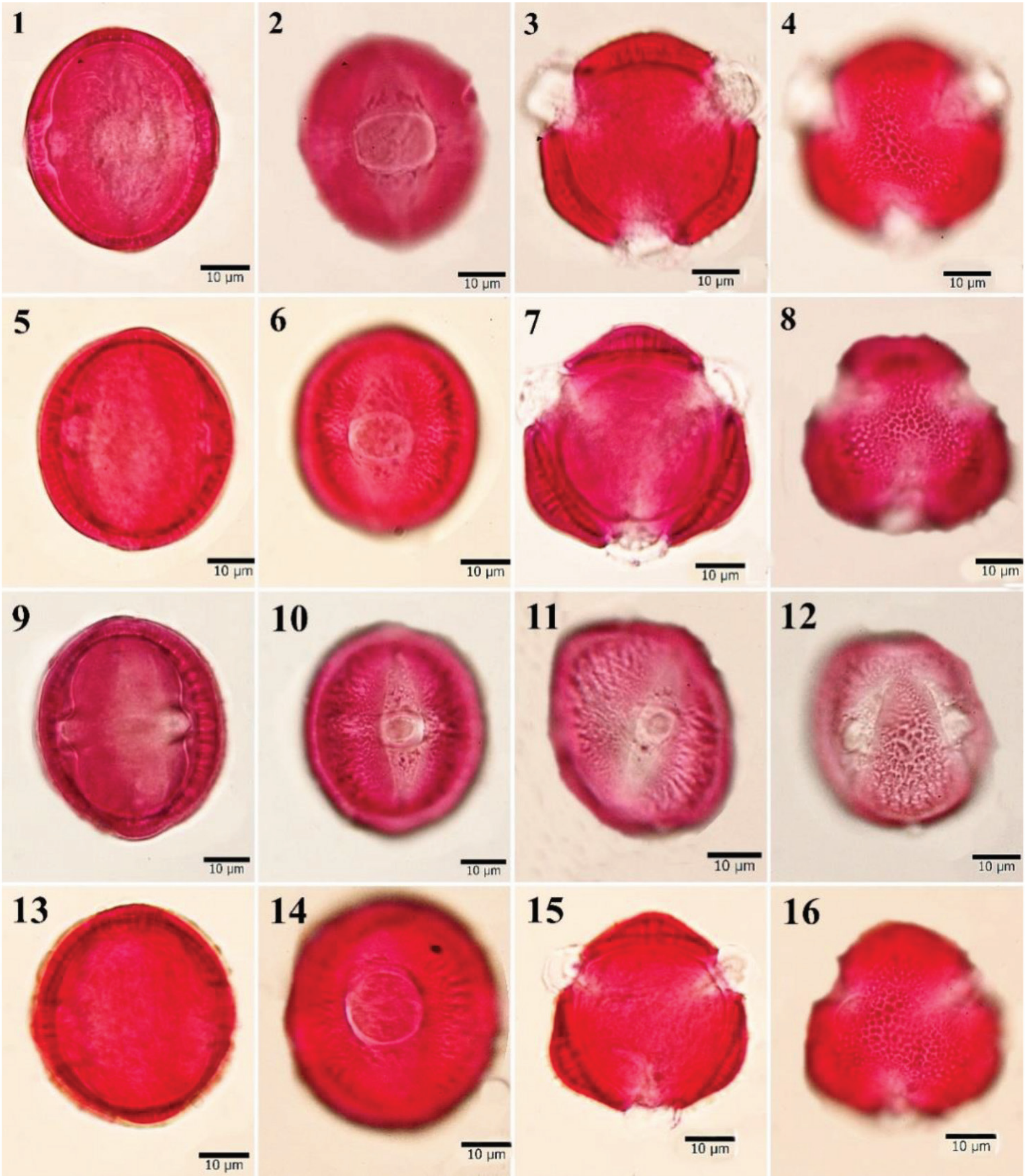

Figure 6. Pollen grains of Turkish *Cyanus subgenus* under LM. 1–4: *Centaurea triumfetti* subsp. *axillaris*, 5–8: *C. huetii*, 9–12: *C. matthiolifolia*, 13–16: *C. germanicopolitana*.

4. Discussion

Pollen grains of the subgenus *Cyanus* were tricolporate, rarely syncolporate, subprolate and prolate-spheroidal with scabrate-perforate ornamentation. Some members of the subgenus *Cyanus* were subjected to different examinations, which indicated that a subprolate pollen shape, tricolporate aperture, and scabrate ornamentation (Pınar and İnceoğlu, 1996; Bakels, 2012; Atar et al., 2018;

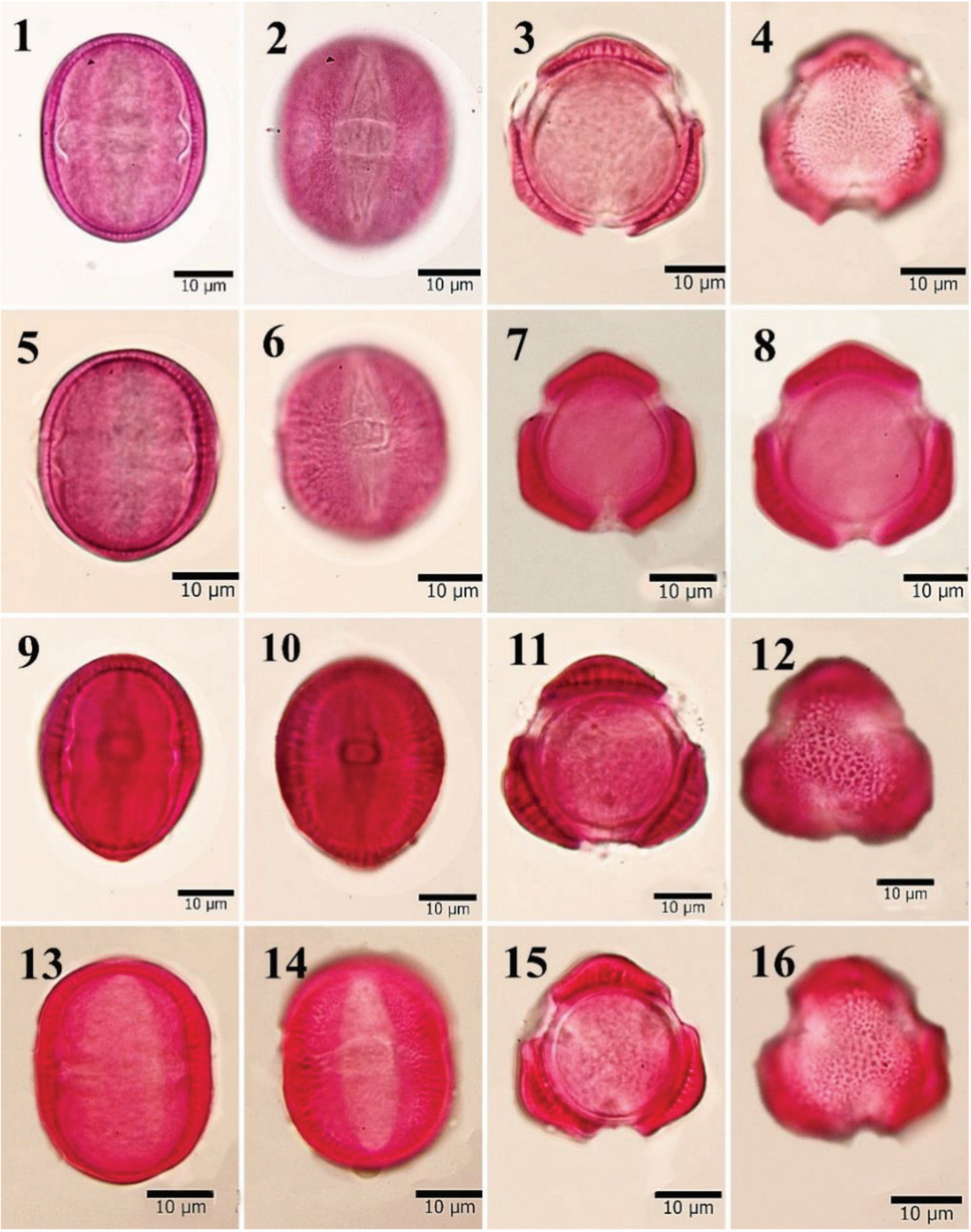

Figure 7. Pollen grains of Turkish *Cyanus* subgenus under LM. 1–4: *Centaurea depressa,* 5-8: *C. pinardii*, 9–12: *C. tchihatcheffii*, 13–16: *C. cyanus.*

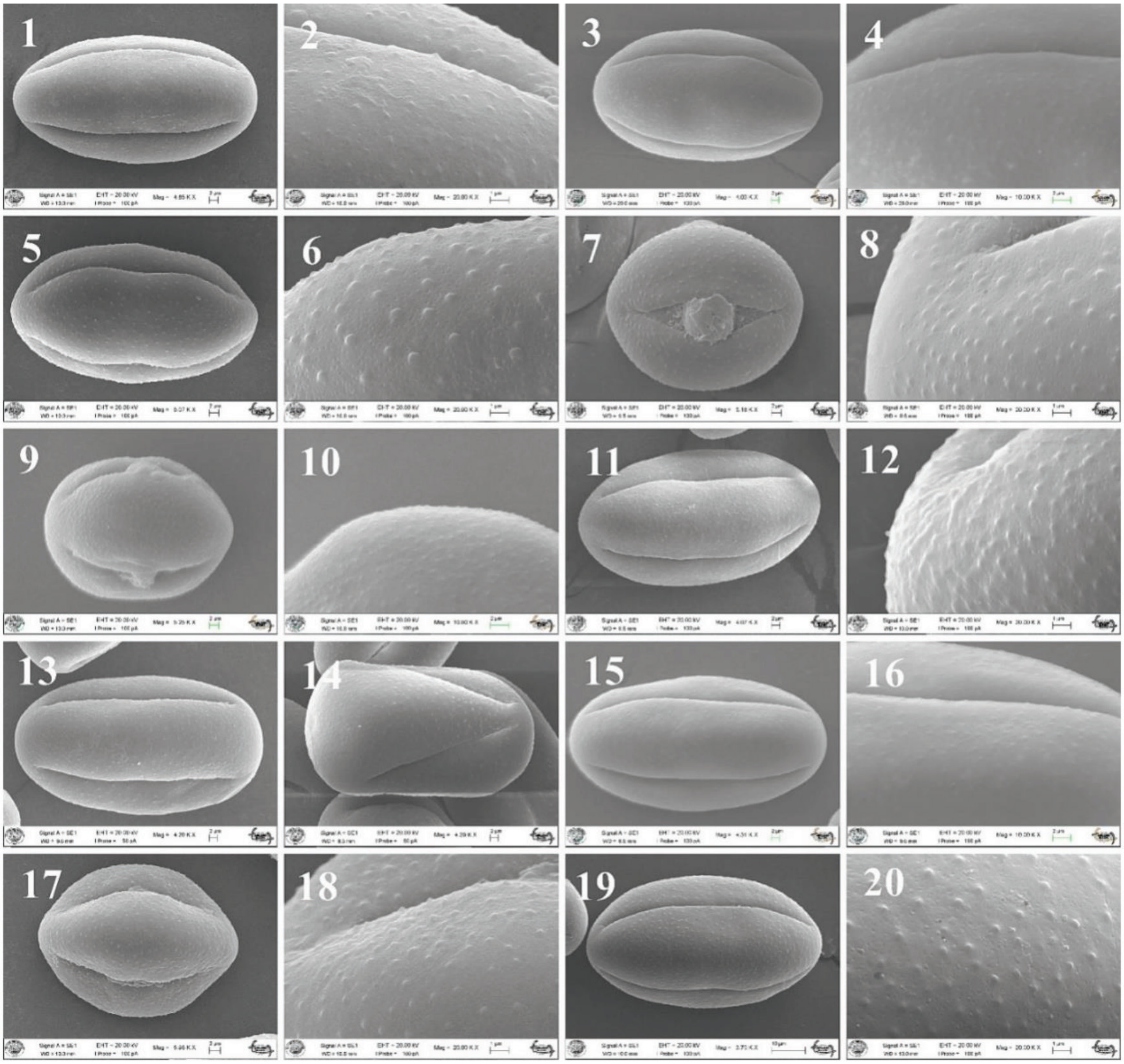

Figure 8. Pollen grains of Turkish *Cyanus* subgenus under SEM. 1–2: *Centaurea reuteriana var. reuteriana*, 3–4: *C. reuteriana var. phyrgia*, 5-6: *C. lanigera*, 7–8: *C. nigrofimbria,* 9–10: *C. woronowii.* 11–12: *C. eflanensis*, 13–14: *C. thirkei*, 15–16: *C. bourgaei,* 17–18: *C. cheiranthifolia* var. *cheiranthifolia,* 19–20: *C. cheiranthifolia* var. *purpurascens*.

Taşar et al., 2018) were also present in the current study. A scabrate-perforate ornamentation has not been previously described for the subgenus *Cyanus*. Furthermore, the subgenus *Cyanus* has rather small spines (0.18–0.23 µm), which is an evolved characteristic for the *Centaurea* group, with definition a of spine reduction (Wagenitz, 1976). Additionally, according to Wagenitz's pollen types (1955), 20 *Cyanus* taxa were grouped into two pollen types, as the *Montana* pollen type for perennial taxa and the *Cyanus* pollen type for annuals. *Montana* and *Cyanus* pollen types were larger in size and subprolate or prolate in shape. *Cyanus* pollen type had thicker costae than *Montana* pollen type. Similarly, this was also present in the results herein.

Different researchers performed on the palynomorphology of Asteraceae (Çeter et al., 2013; Çitak et al., 2019) reported that the aperture number was very useful for identification of members of the family. The heteromorphy of pollen grains occurs as the production of several pollen grain morphs in the same plant with a different aperture number (Till-Bottraud et al., 1995). It is thought that heteromorphy can be induced by

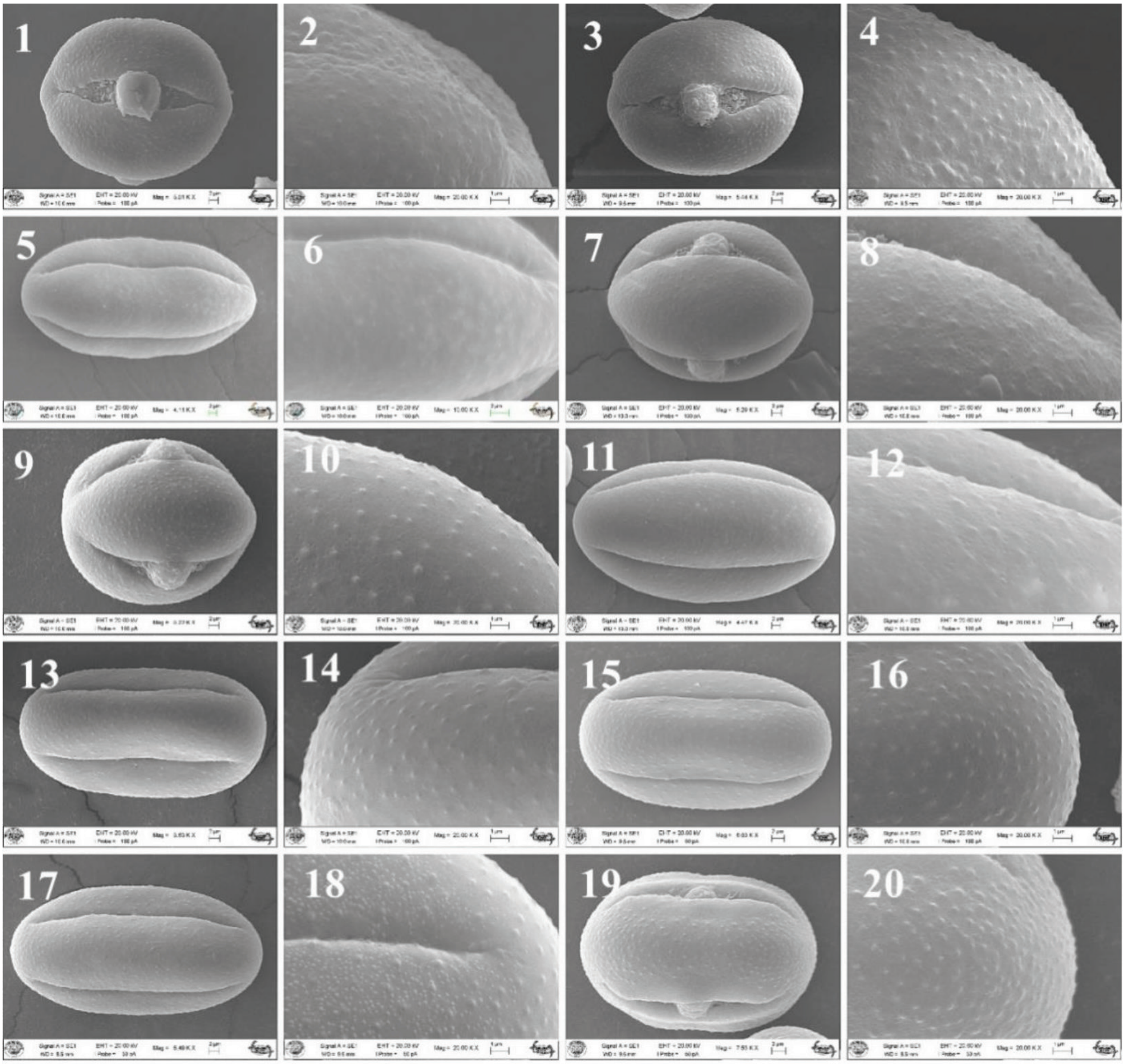

Figure 9. Pollen grains of Turkish *Cyanus* subgenus under SEM. 1–2: *Centaurea pichleri* subsp. *pichleri,* 3–4: *C. pichleri* subsp. *extrarosularis,* 5–6*: C. triumfetti* subsp. *axillaris,* 7–8: *C. huetii*, 9–10: *C. matthiolifolia*, 11–12: *C. germanicopolitana,* 13–14: *C. depressa,* 15–16: *C. pinardii*, 17–18: *C. tchihatcheffii,* 19–20: *C. cyanus*.

hybridization or meiosis defect (Matsuda, 1927; Aytuğ et al., 1971). Furthermore, Borsch and Wilde (2000) declared that variations in the pollen aperture type were generally related to different levels of ploidy. In the current study, *C. thirkei* had an aperture type that was 98% tricolporate and 2% syncolporate, and *C. matthiolifolia* had 86% tricolporate and 14% syncolporate. Şirin et al. (2019) reported that *C. matthiolifolia* was a 2n = 4x = 40 tetraploid species, which supports the results of the current study. The B chromosome that was found in *C. thirkei*, which was also reported by Bancheva and Greilhuber (2006), may have been the result of the formation of heteromorphy of the pollen grains in this study.

The results obtained via the statistical analyses used herein presented the importance of the similarity between different types of variables analyzed to group individuals with similar characteristics. This phenetic tree allowed us to define two groups of species based basically on the shape and exine of the pollen grains, in agreement with that of Wagenitz (1975), who was divided *Cyanus* into two main groups (annuals/perennials) in Flora of Turkey (Wagenitz, 1975), (Figure 10). However, the position of

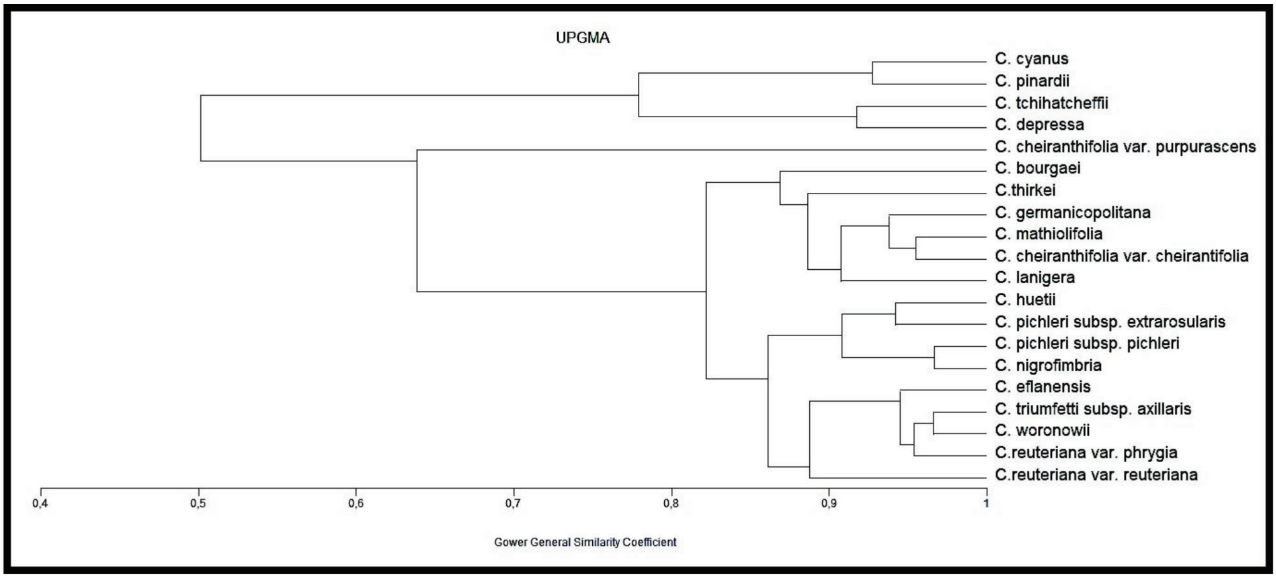

Figure 10. The obtained dendrogram showing similarity distance of *Cyanus* subgenus species.

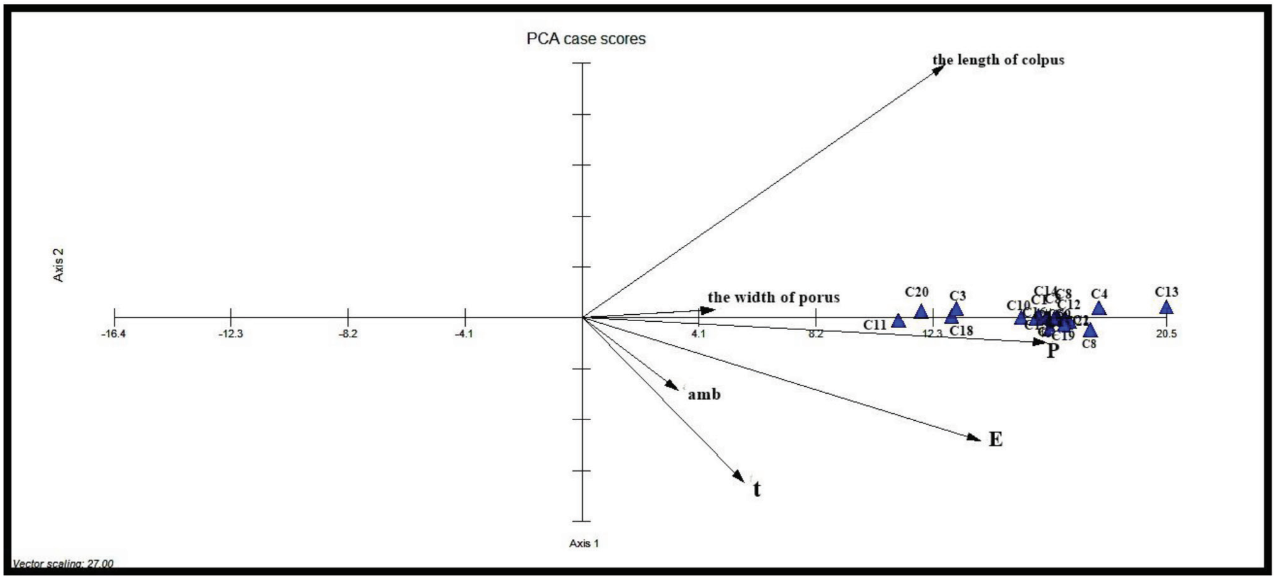

Figure 11. PCA of 20 taxa and 6 variables projected onto the first two axis. The signal (▲) is for taxa and variables are showed as vectors.

C. germanicopolitana, *C. bourgaei*, and *C. pichleri* subsp. *extrarosularis* in the same clade, but different clusters, brought to mind the question of whether the relationships of the taxa could be reviewed one more time with regards to their pollen characteristics.

In the PCA biplot analysis, *C. cheiranthifolia* var. *purpurascens* (perennial species) and *C. pinardii* (annual species) were found as the external taxa, first separating from the other taxa in the dendrogram and the plot (Figures 10–11).

The species were clustered according to their palynomorphological characteristics (Figure 10). A similarity rate of 51% was a low value among the species in the main clades and this was a sign of variation in the pollen types of the species. Some pollen characteristics, like the equatorial axis, length of colpus, and amb dimensions were very effective in distinguishing the species positioned in different clusters. When a general assessment was made of the dendrogram, all of the species seemed meaningfully similar and closely positioned. According to the dendrogram, the species were separated into two main clusters. While the first main cluster included only annual species, the second consisted of perennial species that were mainly located in two subclusters. The first main cluster was basically distinguished from other relatives by a specific pollen type. The taxa positioned within the second main clade could be divided into two main subclusters, with 63% similarity. The first subcluster consisted of only *C. cheiranthifolia* var. *purpurascens,* based on the length of the colpus. Outside of this second subcluster, 15 taxa occurred together. The positioning of close relative species (*C. reuteriana* and *C. pichleri*) showed the reliability of the results. *C. cheiranthifolia* var. *cheiranthifolia* and *C. cheiranthifolia* var. *purpurascens* were positioned in different subclusters, as in the study of achene micromorphology of *Cyanus* group (Şirin et al., 2017). Maybe the molecular studies about *Cyanus* subgenus will be solve this situation in progressing papers.

In conclusion, the morphological data together with numerical analysis procured essential information for separation of the 20 investigated taxa of the subgenus *Cyanus* of *Centaurea* in Turkey. Dimensions of the equatorial axis, length of the colpus, polar axis, and amb were determined as the most useful characteristics of systematic value. Data based on palynomorphology may have been inadequate in the distinction of the studied species of the subgenus *Cyanus*. However, for designation of the taxonomic position of the subgenus *Cyanus* with future evolutionary analyses, we opined that a solution could be found by presenting further anatomical, molecular, and morphological data.

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