MORPHOLOGICAL AND PALYNOLOGICAL STUDIES ON CIRSIUM BAYTOPAE DAVIS&PARRIS AND CIRSIUM BULGARICUM DC. (ASTERACEAE)

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Abstract: Cirsium baytopae Davis&Parris has been recently (1975) described as a new species with the specimen collected from Turkish Thrace and introduced to the world. This new species is closely related to C. bulgaricum DC., from which it differs in several morphological characteristics. These two species have been observed morphologically and palynologically in details and the differences between them have been discussed.

Keywords: Asteraceae, Cirsium baytopae, Cirsium bulgaricum, morphology, palynology, Turkey

Introduction

Cirsium Mill. genus belongs to subtribe Carduinae of the Cardueae tribe, family Compositae (Asteraceae). Cirsium species have distribution in Mediterranean, also are widespread in Europe, Asia and North America. There are about 250 species throughout the world, while in Turkey the genus has a species number of 59 (Sell and Murrell, 2006; Yıldız et al. 2009).

Cirsium baytopae Davis & Parris has been recently described as a new species with the specimen collected from Tekirdağ-Saray, Güngörmez village. This new species is closely related to *C. bulgaricum* DC., from which it differs in its smaller involucre with all the phyllaries markedly recurved, the median ones without a narrow membranous margin towards the apex. Also, in the discussion part of the description in Flora of Turkey, it was mentioned that an immature specimen from Bulgaria resembles the type of *C. baytopae* (Davis, 1975).

In order to find out more features which distinguish these two closely related species and clarify this subject under discussion, new distribution areas have been investigated. The specimens collected from these areas have been observed morphologically and palynologically in details and the differences between them have been revealed.

Materials and methods

Taxonomical material and methods

Prior to collection of the samples from the field, preliminary information about the species was obtained from floristic literature (Davis, 1975; Stojanov, 1967; Werner 1976) and ISTE herbarium.

The specimens were collected from different localities from Turkish Thrace in 1999-2000. The dried material is kept in the MUFE Herbarium. Some parts of plants were preserved in 70% ethanol in order to use in morphological studies. Flora of Turkey and the

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East Aegean Islands (Davis, 1975) and Flora Europaea (Werner, 1976) were used for the identification of the species. The studied species were compared with the specimens of different herbaria (ISTE, GAZI and K).

The general plant habit of the species in the field is presented on photographs (Fig. 1). Main diagnostic characteristics of these two species such as plant height, stem, leaves, capitula, involucre, phyllaries, corolla, achene, pappus, flowering time, habitat, phytogeographical region are given in a table (Table 1). The distribution of the species in Bulgaria, Turkey and in the studied area is indicated on map (Fig. 2). Illustrations are hand drawn from fresh and alcohol preserved material by the author (Figs. 3, 4).

Palynological material and methods

Pollen samples were taken from the dried specimens. The preparations were done using two methods: fresh pollen preparation method of Wodehouse (1935) and the acetolysis method of Erdtman (1960). The slides were observed using Olympus BH-2 light microscope with x100 objective. For the measurement of the pollen grains as well as their morphological characterization, 12 characters (polar axis (P), equatorial diameter (E), length (clg) and width (clt) of colpi, length (plg) and width (plt) of pori, base (dt) and length (dh) of echinae, distance between colpi apices (t), distance between two echinae (V), exine (Ex) and intine (In)) were determined. The terminology used is according to Punt et al. (Punt et al. 1994). The pollen shape is determined using P/E ratio as observed in equatorial view where echinae are included. Approximately 50 measurements were done for each character. Arithmetic average (M) and standard deviation (σ) were estimated for the morphometric data (Table 2) using the following formulas:

$$M = m + a.1/n \sum xy$$
, $\sigma = a \sqrt{1/n \sum x^2y - u^2}$, $u = 1/n \sum xy$

Results and Discussions

1. Morphological Properties

C. bulgaricum and C. baytopae are very close morphologically. The main differences between these two species are summarised on Table 1.

Table 1. Comparative morphological diagnostic characters of C. baytopae and C. bulgaricum

DI 41 114	C. baytopae (Fig. 3)	C. bulgaricum (Fig. 4)			
Plant height	70-215 cm	40-200 cm			
Stem	Branched, sparsely arachnoid, unwinged.	Branched, sparsely arachnoid to			
Leaves Capitula	Oblong, pinnatisect, lateral lobes bifid, lateral and terminal lobes bearing 3-13 mm stout apical spine. Upper surface and the margins spinose-strigose with setae 0.2-1.8 mm; lower surface arachnoid to tomentose. In corymbs	Oblong, pinnatisect, lateral lobes bifid, lateral and terminal lobes bearing 3-18 mm stout apical spine. Upper surface and the margins spinose-strigose with setae 0.3-2.0 mm, lower surface arachnoid to tomentose. Lax or dense racemes or corymbs			
Involucre	Ovoid-globose, 15-20 × 18-23 mm.	Ovoid-globose, 18-25 × 18-30mm			
Phyllaries	Arachnoid, 10-11 seriate, markedly recurved, margin spinules 0.05 - 0.1 mm; median phyllaries 9 - 14 mm including 1.2 - 2.0×0.2 - 0.25 mm apical spine.	Arachnoid, 9-10 seriate, slightly reflexed, median phyllaries 15-20 mm, narrowed above base, widened to an appendage with spinulose margin, spinules 0.1-0.3 mm, ending by 1.3-3.2 × 0.2-0.6 mm apical spine.			

Corolla Purple, lilac; 15-24 mm Purple, lilac; 22-28 mm.

Achene 45-52 mm

50-65 mm Plumose, 12-16 mm Plumose, 20-26 mm **Pappus** Flowering time July-October July-October

Habitat Under Quercus, road banks, valley, damp Road banks, Pinus sylvestris forest, P. brutia

forest, 120-1000 m. forest, 400-1800 m.

Phytogeographical Endemic for Turkey Balkan-Anatolian element Distribution areas Turkey Turkey, Is. Samos, Bulgaria

(Fig. 2)



Figure 1. General habit of a. C. baytopae and b. C. bulgaricum

Distribution areas of *C. baytopae*

A1(E) Tekirdağ: Güngörmez village, A. Baytop, E. Tuzlacı, ISTE 26339! Tekirdağ: Saray: Güngörmez cemetery, A. Baytop, ISTE 38108!; Tekirdağ: Saray: Güngörmez cemetery, A. Baytop, E. Tuzlacı, K. Akpınar, ISTE 38096!; Kırklareli: Vize-Kıyıköy road, 380m, A. Baytop, E. Tuzlacı, K. Akpınar, ISTE 38134!; Tekirdağ: Saray-Midye, Güngörmez cemetery, 200 m, A. Baytop, A. Meriçli, ISTE 45957!; Kırklareli: Vize-Pınarhisar, 19 km to Pınarhisar, 200m, A. Baytop, A.Meriçli, ISTE 45959!; Tekirdağ: Saray: entrance to Güngörmez village, around the cemetery, 200m, E. Özhatay, F. Günes, N. Şafak, MUFE 5803!, Tekirdağ: Saray-Vize road, Saray exit, around the Saray valley bridge, 120m, E. Özhatay, F. Günes, N. Safak, MUFE 5805!, Kırklareli: Vize-Pınarhisar road 4. km, between Vize-Pazarlı, E. Özhatay, F. Güneş, N. Şafak, MUFE 5806! A2(E) Istanbul: between Saray-Sinekli, 38 km to Sinekli, A Baytop, E. Tuzlacı, K. Akpınar, ISTE 38139!; **C2** Denizli: Babadağ, Değirmendere village, valley, 900-1000m, 02.09.1999, M. Vural, N. Adıgüzel, H. Şağban 3429!

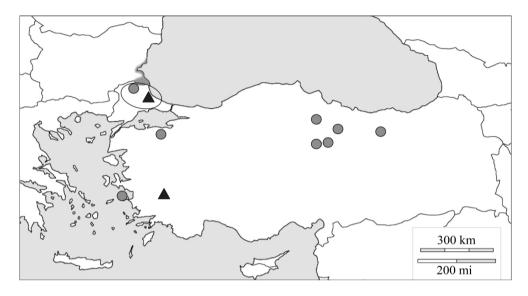


Fig. 2. Distribution of *C. baytopae* (▲) and *C. bulgaricum* (●) in Turkey. The darker area in Bulgaria indicates the distribution of *C. bulgaricum*. Study area is encircled.

Distribution areas of C. bulgaricum

A1(E) Kırklareli: Dereköy-border road, 3 kms after Dereköy, 530 m, N.&E. Özhatay, ISTE 70975!; Kırklareli-Dereköy, 1.5 kms to Dereköy-Vali fountain area, 480 m, N.&E. Özhatay, ISTE 70959!; Kırklareli-Dereköy road, Kapaklı limestone operation area, E. Özhatay, N. Şafak, F. Güneş, MUFE 5811! Kırklareli-Dereköy road, 5 kms to Dereköy, E. Özhatay, N. Şafak, F. Güneş, MUFE 5816! Dereköy: Kapaklı village entrance, E. Özhatay, N. Şafak, F. Güneş, MUFE 5822! Dereköy: Istraga stream arounds, E. Özhatay, N. Şafak, F. Güneş, MUFE 5823! A2(A) Bursa: Uludağ, Aucher 3381; Bursa: Uludağ road, by roadside, 1800 m, A.&T. Baytop, ISTE 20992! A5 Amasya: Akdağ, 1400 m, Bornm. 1890:1614; A6 Amasya: Kuzgeçe k., Issiztepe loc., c. 700 m, S. Peker, GAZI 1603!, Amasya: Aşbaraklı k. Hızırcık loc., by roadside, c. 800 m, S. Peker, GAZI! Tokat: Topçam loc., 1640 m, N. Adıgüzel et al., GAZI 1828! A7 Gümüşhane: Tempede, Sint. 1894:7440; B5 Yozgat: 28 km east of Akdağmadeni, 1400 m, 04.08.1971, J. Lemond 5055! Sivas: Yıldızeli- Akdağmadeni, 1400 m, Rech. 44435; Greece (2A) Is. Samos: Spadarion to Pyrgos, on Banks, 400-500 m, 07.10.1981, Davis 67763!

Distribution areas of the species in Bulgaria are given according to Flora Bulgarica and Conspectus of the Bulgarian Vascular Flora (BSBCP, 2002; Assyov et al. 2012).

2. Palynological Properties

In both species the pollen type is 3-zonocolporate as is specified by Beug for *Cirsium* pollen (Beug, 2004); the shape (P/E) is sphaeroidal. The structure of the exine is

tectate. The sculpture of the exine is echinate. Echinae large, conical, broad at the base, uniformly arranged over pollen surface. In fresh pollens colpi long with smooth margins, wide or narrower in the middle, the points prominent; pori circular or slightly elliptic, prominent. In acetolysed pollens colpi contracted; endocolpi prominent, oblong, the points acuminate; pori margins indistinct (Plates 1, 2)

The pollen of *C. baytopae* in polar view is three lobed, in equatorial view elliptical. On the other hand, *C. bulgaricum* pollen in polar view is three lobed, in equatorial view elliptical (fresh pollen) or almost circular (acetolysed pollen).

According to the measurements there are some distinctive differences between the pollen sizes of these two species (Table 2).

The distance between colpi apices (t) of the fresh pollens of *C. baytopae* 21.51 μ , while in *C. bulgaricum* is 22.54 μ ; in acetolysed pollens is 21.65 μ and 20.88 μ respectively.

The shrinkage of acetolysed pollens lead to changes in aperture shapes. The clg/clt ratio of *C. baytopae* fresh pollen is 2.28, in acetolysed pollen is 7.03. In *C. bulgaricum* this value is 2.71 and 8.87, respectively. The plg/plt ratio of *C. baytopae* fresh pollen is 1.14, in acetolysed pollen is 0.59. In *C. bulgaricum* this ratio is 1.26 ve 0.89, respectively.

Fresh pollen base width of the echinae (dt) is $10.78~\mu$ in *C. baytopae* and $10.22~\mu$ in *C. bulgaricum*. In acetolysed pollen of *C. baytopae* this value sligtly changes ($10.97~\mu$), while in *C. bulgaricum* it becomes $12.40~\mu$. Fresh pollen length of the echinae (dh) is $4.00~\mu$ in *C. baytopae* and $4.41~\mu$ in *C. bulgaricum*. In acetolysed pollen of *C. baytopae* this value is $5.92~\mu$, in *C. bulgaricum* it becomes $5.87~\mu$

Ex/Int ratio changes between these two species. In *C. baytopae* is 4.76, in *C. bulgaricum* 3.03.

	C. baytopae				C. bulgaricum			
Character	Fresh pollen (µm)	Standard deviation (σ)	Acetolysed pollen (µm)	Standard deviation (σ)	Fresh pollen (µm)	Standard deviation (σ)	Acetolysed pollen (µm)	Standard deviation (σ)
P	44.74	±1.30	58.26	±1.33	50.61	±1.77	61.04	±1.78
E	49.39	±1.20	61.42	±1.95	55.03	±1.46	61.99	±2.01
P/E	0.91	_	0.95	_	0.92	_	0.98	_
t	21.51	± 0.87	21.65	± 0.90	22.54	± 0.99	20.88	± 0.63
clg	28.13	±1.53	28.42	±1.53	33.01	±1.66	30.90	±1.04
clt	12.33	±0.54	4.04	± 0.92	12.17	± 1.26	3.48	± 0.78
clg/clt	2.28	_	7.03	_	2.71	_	8.88	_
plg	14.05	±0.71	8.79	± 0.91	15.14	±1.02	10.59	±0.65
plt	12.33	±0.54	14.73	±1.31	11.97	±1.30	11.77	±1.46
plg/plt	1.14	_	0.60	_	1.26	_	0.90	_
dt	10.78	±0.70	10.97	± 0.71	10.22	± 0.88	12.40	±1.13
dh	4.00	±0.36	5.92	±0.52	4.41	±0.36	5.87	± 0.60
\mathbf{V}	10.22	± 0.68	13.42	± 0.92	10.03	± 0.75	13.13	±1.11
Ex	3.19	±0.40	4.78	±0.43	2.70	±0.45	5.08	±0.53
In	0.67	±0.15	_	_	0.89	± 0.17	_	_
Ex/In	4.76	_	_	_	3.03	_	_	

Table 2. Comparative pollen grain characteristics of C. baytopae and C. bulgaricum

Conclusions

In conclusion, the morphological data shows that these two species have several diagnostic characters such as involucre, flower, achene and pappus size. The most important character of taxonomic value are the median phyllaries which are shorter and markedly recurved in one (*C. baytopae*), larger and only slightly reflected in the other (*C. bulgaricum*). Palynological observations revealed that the pollen morphology is similar, only the differences in pollen sizes are distinctive characters of these two species.

Morphological and palynological studies presented that these two species are closely related. Further observations, such as anatomical, cytological or molecular can reveal precise identification.

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REFERENCES

- Assyov, B., Petrova, A., Dimitrov, D., Vassilev, R. 2012. *Cirsium bulgaricum* DC., in: Assyov, B., Petrova, A. (Eds.). Conspectus of the Bulgarian Vascular Flora. Ed. 4: 143
- Beug, H.J., 2004. *Leitfaden der Pollenbestimmung für Mitteleuropa und angrenzende Gebiete*. Verlag Dr. Friedrich Pfeil, Munchen: p. 228
- BSBCP, 2002. Flora Bulgarica. An interactive CD-Rom. Bulgarian–Swiss Biodiversity Conservation Programme; Pro Natura Switzerland.
- Davis, P.H., Parris, B.S., 1975. *Cirsium Mill.*, in: Davis, P.H. (Ed.). *Flora of Turkey and the East Aegean Islands*, **5**. Edinburgh University Press: 370-412.
- Erdtman, G. 1960. The Acetolysis Method. Sven. Bot. Tidskr. 54: 561-564.
- Punt, W., Blackmore, Nilsson, S., Le Thomas, A. 1994. Glossary of Pollen and Spore Terminology. LLP Foundation, Utrecht.
- Sell, P., Murrell, G. 2006. Flora of Great Britain and Ireland, 4. Cambridge University Press, Cambridge.
- Stojanov, N., Stefanov, B., Kitanov, B. 1967. Flora of Bulgaria. Ed. 4, 2. Naouka&Izkoustvo, Sofia (in Bulgarian).
- Werner, K. 1976. *Cirsium Miller.*, in: Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.). *Flora Europaea*, **4**. Cambridge University Press.
- Wodehouse, R.P. 1935. Pollen Grains. Mc. Grew Hill, New York.
- Yıldız, B., Dirmenci, T., Arabaci, T. 2009. Cirsium handaniae (Asteraceae), a new species from Turkey. Annales Botanici Fennici, 46: 239-243.

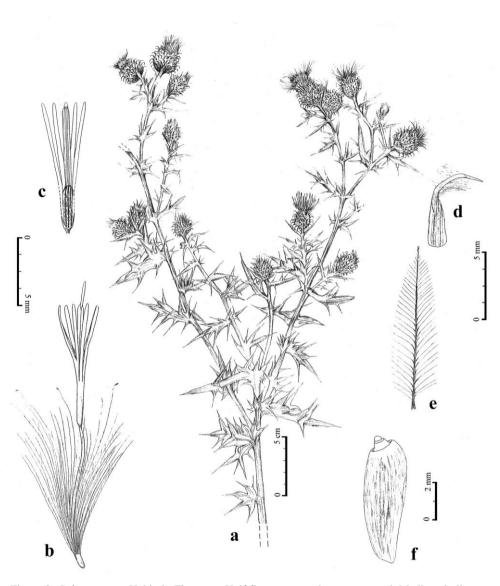


Figure 3. *C. baytopae* a. Habit, b. Flower, c. Half flower cut to show stamens, d. Median phyllary, e. Pappus, f. Achene

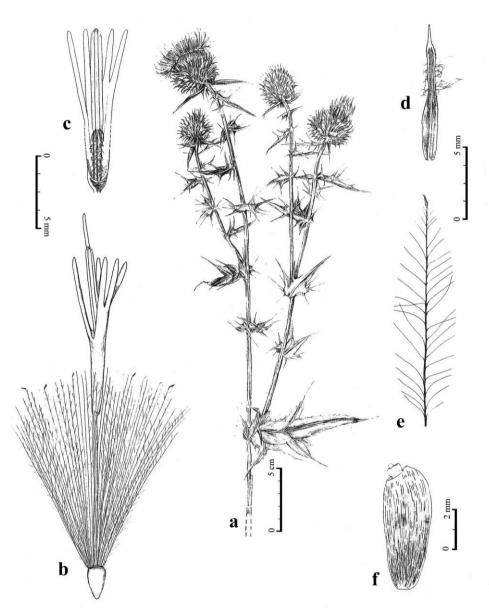


Figure 4. *C. bulgaricum* a. Habit, b. Flower, c. Half flower cut to show stamens, d. Median phyllary, e. Pappus, f. Achene

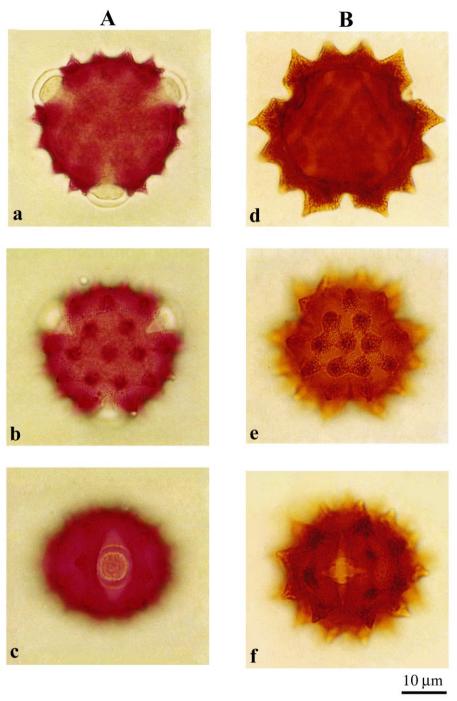


Plate 1. *C. baytopae* polen grain characteristics A. Fresh pollen a. margins, b. echinae, c. colpi and pori; B. Acetolysed pollen d. margins, e. echinae, f. colpi and pori

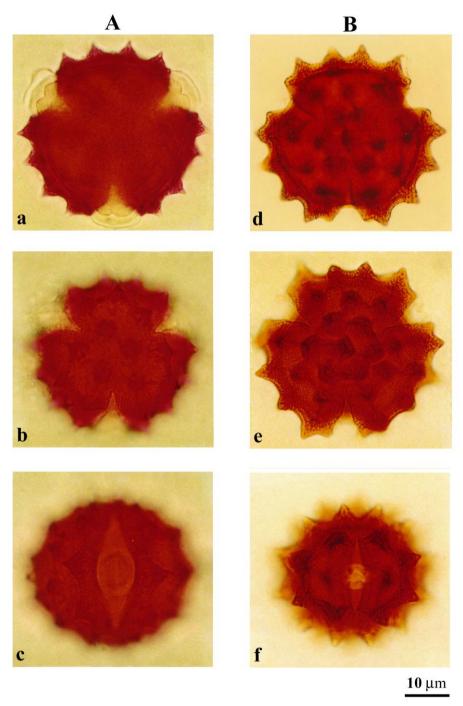


Plate 2. *C. bulgaricum* polen grain characteristics A. Fresh pollen a. margins, b. echinae, c. colpi and pori; B. Acetolysed pollen d. margins, e. echinae, f. colpi and pori