Seed morphology of the *Centaurea* species (*Asteraceae*) in Iran

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Abstract. The macro- and micromorphological characters of seed and seed surface ornamentations were investigated in nine *Centaurea* species by using stereomicroscope and scanning electron microscopy. The seed shape was ovoid in *C. gigantea*, *C. nemecii* and *C. behen*, narrowly oblong in *C. aucheri* subsp. *szowitsii*, *C. albonitens* and *C. amadanensis* var. *gymnoclada,* and widely oblong in *C. aucheri* subsp. *aucheri*, *C.aucheri* subsp. *elbursensis* and *C. regia* subsp. *regia*. SEM showed that the achene surface had a striate sculpture and was strongly depressed on both dorsal and margin surfaces, without any hairs. However, in *C. behen* the achene had rectangular cells with long hairs on the surface. The seed length and width ranges differed significantly among the studied species. The obtained data were used in UPGMA clustering and PCoA plot to divide the studied species into distinct groups. Therefore, they were taxonomically important to the genus *Centaurea*.

Key wods: scanning electron microscopy, seed micromorphology, *Centaurea*

Introduction

The genus *Centaurea* L., which belongs to tribe *Cardueae* (*Asteraceae*), is represented by 500–600 taxa across the world (Rendle 1976; Heywood 1979; Hickey & King 1981; Susanna & Garcia-Jacas 2007; Kubitzki 2011), and particularly in Asia, Europe, North Africa and America (Wagenitz & Davis 1975; Davis & al. 1988). The endemism rate of the genus in Iran is high (42.35 %) (Wagenitz 1980). *Centauera* has 86 species in Iran, 36 of which are endemic (Ghahreman & Attar 1999). The fact that it has a high proportion of endemism corroborates the opinion that Iran is an important centre of diversity for the genus *Centaurea* (Wagenitz 1986). Many authors have regarded the fruit and seed ornamentations as a useful tool for taxonomic considerations (Sheikh Akbari & Azizian 2006; Kaya & Dirmenci 2008; Pinar & al. 2009; Bayrakdar & al. 2010). The morphological characters of the seed have been viewed as an essential aid in the taxonomic treatment of the genus *Centaurea* L. The achene shape and size, pappus size, the shape of indumentum and cells are some of these informative characters. For example, in *C. consanguinea* DC the achene is 3–3.5 mm long and the pappus is (0.5-) 1–2 (-2.5) mm. The surface of seeds is serrated, the SEM study showed that the achene surface is hairy and cells form a striate sculpture, with strongly depressed dorsal and margin surfaces. In the micromorphological study of *C. polyclada* DC., the achene is slightly obovate-rectanglar and very slightly compressed on both sides. The apex of the achene is semicircular. It is generally very small, 1.6–5 mm scales. The SEM study showed that all cells form a striate sculpture, strongly depressed on both dorsal and margin surfaces. The indumentum is sparsely covered with long hairs and the achene seems glabrescent. While studying *C. ptosimopappa* Hayek and *C. ptosimopappoides* Wagenitz, the achenes were recorded as 5.6 ± 1.9 mm long, 3.4 ± 0.6 mm wide, rectangular, while the pappus was semi-deciduous and 6–11 mm long. The inner row was indistinct. (Celik & al. 2005; Uysal & al. 2005; Celik & al. 2008). As such studies have not been carried out on

Table 1. List of examined taxa, collectors and herbarium number.

Taxa	Date	Collector & herbarium number
C. aucheri subsp. *aucheri*	10.7.2011	Bagheri, HSBU-1030
C. aucheri subsp. *szowitsii*	9.7.1991	Mozaffarian, TUH-69907
C. aucheri subsp. *elbursensis*	7.6.1988	Ghaffari, TUH-11594-3
C. albonitens	4.4.1990	Ghahreman&Mozaffarian, TUH-9672
C. regia subsp. *regia*	3.7.1996	Ghahreman & Attar, TUH-20565
C. gigantea	18.6.1998	Ghahreman& Attar, TUH-21840
C. amadanensis var. *gymnoclada*	8.7.1995	Ghahreman&Mozaffarian, TUH-18307
C. nemecii	7.7.1995	Ghahreman&Mozaffarian, TUH-18952
C. behen	24.7.2006	Attar, TUH-36373

Legend. HSBU: Herbarium of Shahid Beheshti University; **TUH**: Tehran University Herbarium.

the *Centauera* species in Iran, the objective of this paper was to provide a detailed account of the macro and micro seed morphology in some selected taxa of *Centaurea* in Iran and see if such data can be used in the taxonomy of the genus. The paper presents for the first time a detailed account of the seed morphology of nine species/subspecies of *Centaura* growing in Iran.

Material and methods

Plant material

Seed studies by stereomicroscope and scanning electron microscope (SEM) were performed on nine species/subspecies of the genus *Centauera,* belonging to four sections (Wagenitz 1980), namely: (12) Sect. *Phaeopappus* including *C. aucheri* subsp. *aucheri*, *C. aucheri* subsp. *szowitsii* (Boiss.) Wagen., *C. aucheri* subsp. *elbursensis* Wagen., *C. albonitens* Turril; (13) Sect. *Cynaroides* including *C. regia* subsp. *regia*, *C. gigantea* Chutz-Bip. ex Boiss.; (14) Sect. *Paraphysis* including *C. amadanensis* var. *gymnoclada* (Jaub. & Spach.) Wagenitz, *C. nemecii* Nab.; and (15) Sect. *Microlophus,* including *C. behen* L. Details of the voucher

Table 2. Seed morphological parameters of the examined taxa.

Taxa	SL	SW	SL/SW	PL	PW	PL/PW	PL/SL	HDE	HD	S	SC
Sect. *Phaeopappus*											
C. aucheri subsp. *aucheri*	7.23±0.63	2.55±1.08	2.83	13.76±1.82	13.93±1.84	0.98	1.89	0.61±0.08	1.66±0.21	Widely oblong	White
C. aucheri subsp. *szowitsii*	7.33± 0.65	2.16±0.95	3.43	13.43±1.65	14.70±1.94	0.90	1.82	0.11±0.01	1.13±0.14	Narrowly oblong	Brown
C. aucheri subsp. *elbursensis*	5.66±0.48	2.06±0.93	2.74	15.30±2.02	14.30±1.89	1.06	2.69	0.13±0.02	0.65±0.08	Widely oblong	Brown
C. albonitens	7.00± 0.53	1.84±0.85	3.78	10.56±1.39	10.23±1.25	1.03	1.50	0.51±0.06	0.83±0.10	Narrowly oblong	Brown to yellow
Sect. *Cynaroides*											
C. regia subsp. *regia*	8.03± 0.78	2.91±1.14	2.75	12.20±1.61	15.53±2.05	0.78	1.51	0.04±0.005	0.74±0.09	Widely oblong	White
C. gigantea	6.53± 0.5	4.62±4.53	1.40	10.20±1.27	8.56±1.13	1.18	1.55	0.12±0.01	1.33±0.17	Ovoid	White
Sect. *Paraphysis*											
C. amadanensis var. *gymnoclada*	9.00± 0.84	2.79±1.11	3.23	15.53±2.15	17.60±2.32	0.87	1.72	0.05±0.006	0.9±0.11	Narrowly oblong	Brown
C. nemecii	6.16± 0.49	4.69±4.55	1.31	10.36±1.35	15.26±1.86	0.67	1.67	0.11±0.01	1.5±0.19	Ovoid	Brown
Sect. *Microlophus*											
C. behen	4.06± 0.32	3.55±2.09	1.14	6.56±0.86	7.2±0.95	0.90	1.61	0.61±0.08	1.15±0.15	Ovoid	Brown to yellow

Legend. SL: seed length; **SW**: seed Width; **SL/SW**: ratio of seed length to seed width; **PL**: pappus length; **PW**: pappus width; **PL/PW**: ratio of pappus length to pappus width; **PL/SL**: ratio of pappus length to seed length; **HDE**: hillum depth; **HD**: hillum diameter; **S**: shape of seed; **SC**: seed colour.

numbers are provided in Table 1. Voucher specimens are deposited in the Tehran University Herbarium (TUH) and Herbarium of Shahid Beheshti University (HSBU). Altogether, 10 quantitative and qualitative seed morphological characters were studied (Table 2). Characters were selected on the basis of those reported earlier in the articles (Chehregani & Mahanfar 2007; Celik & al. 2008), and of our own observations. Samples were studied at first by a Dino-Lite pro hand stereomicroscope (Figs 1, 4, 7, 9, 12, 15, 18, 21, 24). For SEM studies, the samples were suspended in a drop of water and directly transferred to a metallic stub using double-sided adhesive tape and coated with gold by a sputter coater. The specimens were examined and photographed with a XL30 Philips scanning electron microscope (Figs 3, 6, 11, 14, 17, 20, 23, 26), and the measurements were based on 10 to 20 readings from at least three achenes from each specimen with Image Tools software (ver. 3, 2002).

Statistical analysis

ANOVA (Analysis of Variance) test was used to show the significant differences in seed morphological characters among the studied species and sections. SPSS, version 9 (1998) was used for statistical analysis.

Results and discussion

The general shape of the seeds in all studied species was almost alike. The seed shape in *Centaurea* was determined by the seed's length-to-width ratio. Therefore, the seed shape was ovoid in *C. gigantea*, *C. nemecii* and *C. behen* (Figs 12, 21, 24), narrowly oblong in *C. aucheri* subsp. *szowitsii*, *C. albonitens* and *C. amadanensis* var. *gymnoclada* (Figs 7, 1, 18), and widely oblong in *C. aucheri* subsp. *aucheri*, *C.aucheri* subsp. *Elbursensis,* and *C. regia* subsp. *regia* (Figs 4, 9, 15).

Seed colour was brown to yellow in *C. albonitens* and *C. behen*, white in *C. aucheri* subsp. *aucheri*, *C. gigantea* and *C. regia* subsp. *Regia,* and brown in *C. aucheri* subsp. *elbursensis*, *C. aucheri* subsp. *szowitsii*, *C. amadensis* var. *gymnoclada,* and *C. nemecii* (Figs 1,4,7,9,12,15,18,21,24).

SEM study showed that the achene surface had a striate sculpture and was strongly depressed on both dorsal and marginal sides, without any hairs (Figs 3, 6, 11, 14, 17, 20, 23). However, in *C. behen* the achene had rectangular cells with long hairs on the surface (Fig. 26).

Seed length (SL) ranged from 4.06 mm in *C. behen* to 9 mm in *C. amadensis* var. *gymnoclada*; seed width (SW) ranged from 1.84 mm in *C. albonitens* to 4.69 mm in *C. nemecii*. Similarly, pappus length ranged from 6.56 mm in *C. behen* to 15.53 mm in *C. amadanensis* var. *gymnoclada*, while, pappus width ranged from 7.2 mm in *C. behen* to 15.53 mm in *C. regia* subsp. *regia*. Hillum depth (HD) ranged from 0.04 mm in *C. regia* subsp. *regia* to 0.61 mm in *C. aucheri* subsp. *aucheri* and *C. behen*.

The ANOVA test performed on nine quantitative seed morphological characters (Table 2) showed a significant difference among the studied species (Sig. <0.05). Thus these data could be used in the genus taxonomy and could differentiate the species from each other.

The UPGMA dendrogram and PCoA plot based on the morphological characters of seed surface (Figs 27 & 28) differentiated the *Centaurea* species from each other. According to Lavialle (1912), the achene morphological characters are very useful in distinguishing the different genera of tribe *Cardueae* and are very important in the classification of this tribe. According to Lavialle (1912) and Dittrich (1985), the achene morphological characters are useful in the differentiation of subtribe *Echinopsinae* and *Carlinineae* from *Carduinae* and *Centaureinae*. Also, Bancheva & Gorgorov (2010) used achene features (length and width of the achene, length of the pappus) in the statistical variance analysis of the studied populations of *Centaurea davidovii* and *C. nervosa*. Our results have also shown that the achene features are very good for the differentiation of species in this genus and support the taxonomic treatment of the genus in the *Flora Iranica* (Wagenitz 1980). According to UPGMA dendrogram (Fig. 28), the three subspecies of *C. aucheri* fall into different but closest clades; that is, the morphological features of the achenes of *Centaurea* could be used in a taxonomic context only in combination with other features.

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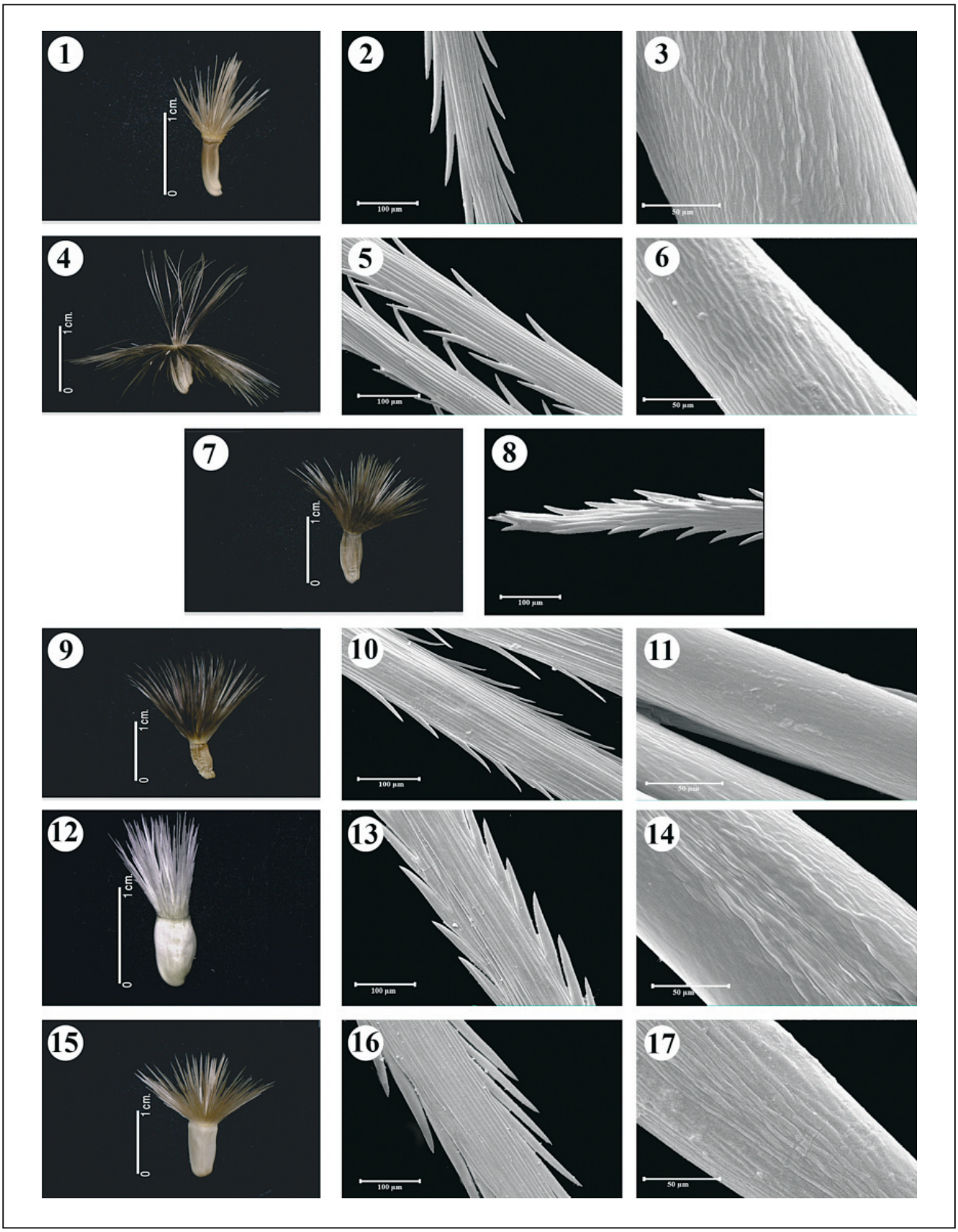
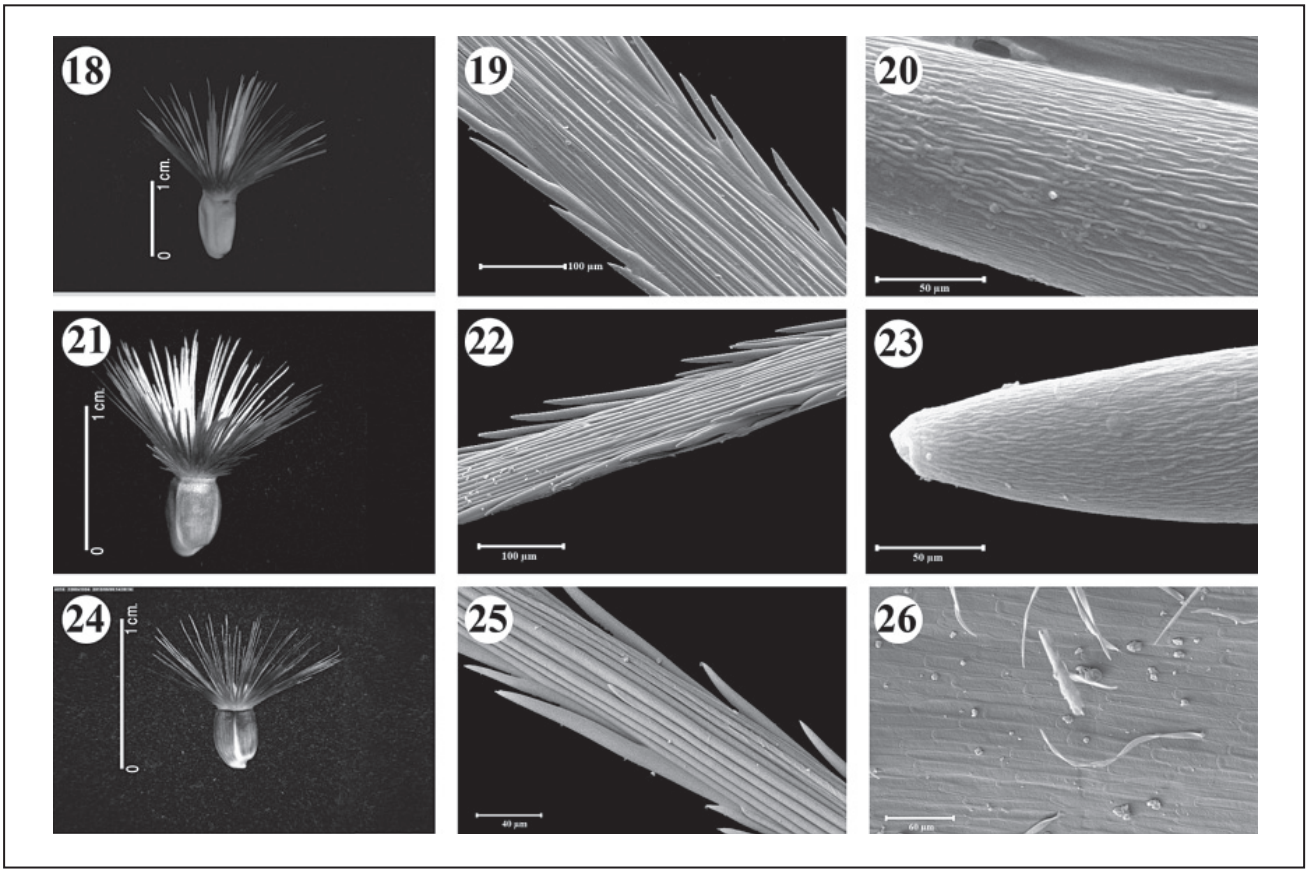


Figs 1-17. LM and SEM micrographs of *Centaurea albonitens* (1-3), *C. aucheri* subsp. *aucheri* (4-6), *C. aucheri* subsp. *szowitsii* (7-8), *C. aucheri* subsp. *elbursensis* (9-11), *C. gigantea* (12-14), *C. regia* subsp. *regia* (15-17); 2, 5, 8,10, 13, 16 and 3, 6, 11, 14,17 SEM micrographs of pappus and surface of each achene.



Figs 18-26. LM and SEM micrographs of *Centaurea amadanensis* var. *gymnoclada* (18–20), *C. nemecii* (21–23), *C. behen* (24–26); **Figs 19, 22, 25 and 20, 23, 26**. SEM micrographs of the pappus and surface of each achene.

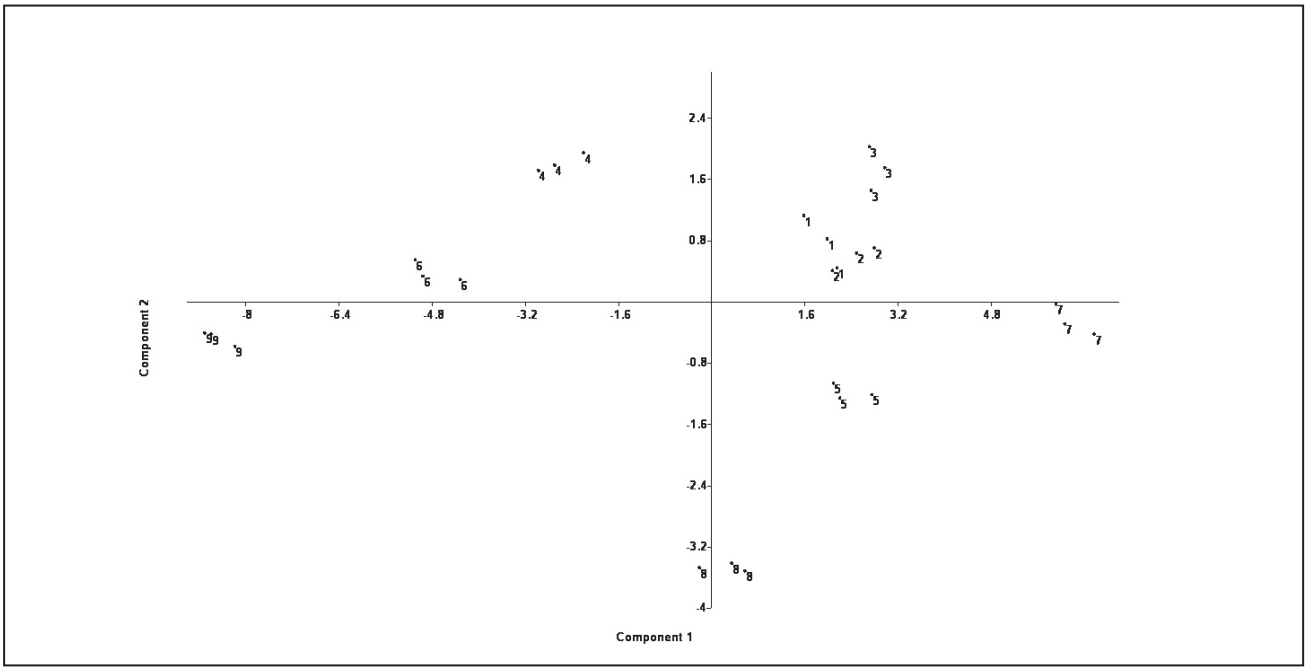


Fig. 27. PCA ordination of the *Centaurea* taxa based on seed morphology: **1.** *C. aucheri* subsp. *aucheri*, **2.** *C. aucheri* subsp. *szowitsii*, **3.** *C. aucheri* subsp. *elbursensis*, **4.** *C. albonitens*, **5.** *C. regia* subsp. *regia*, **6.** *C. gigantea*, **7.** *C. amadanensis* var. *gymnoclada*, **8.** *C. nemecii* and **9.** *C. behen*.

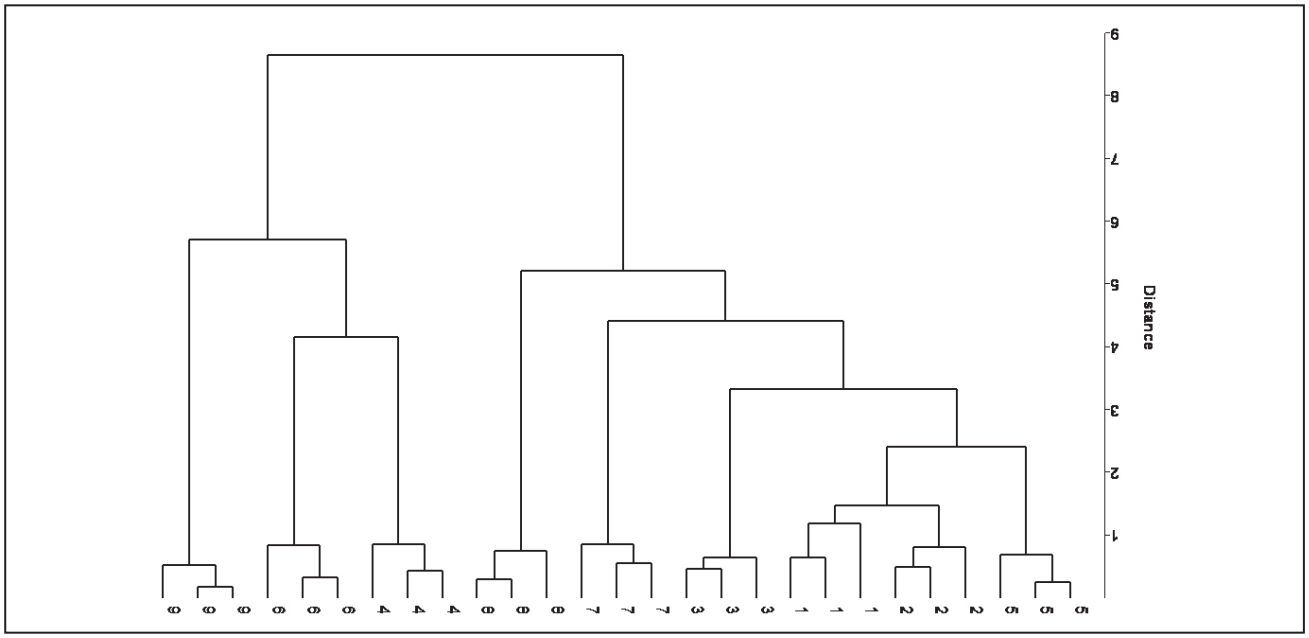


Fig. 28. UPGMA dendrogram based on the seed morphology of the *Centaurea* taxa: **1.** *C. aucheri* subsp. *aucheri*, **2.** *C. aucheri* subsp. *szowitsii*, **3.** *C. aucheri* subsp. *elbursensis*, **4.** *C. albonitens*, **5.** *C. regia* subsp. *regia*, **6.** *C. gigantea*, **7.** *C. amadanensis* var. *gymnoclada*, **8.** *C. nemecii* and **9.** *C. behen*.

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