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A Re-Examination of the Morphology of Two Endemic Species of *Centaurea* L. (Asteraceae) from Turkey, with Implications for the Taxonomy of the Genus

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Abstract

In this study, two cornflowers endemic to Turkey, Centaurea sericea Wagenitz and Centaurea cankiriensis A.Duran & H.Duman were studied from both a morphological and a palinological view. The results suggest that a close systematic relationship exists between the two local endemic and, more importantly, imply a need to alter subgeneric divisions within the genus and sink one of these species into the other.

Key words: Endemic, morphology, palinology, taxonomy, Turkey

INTRODUCTION

Turkey is one of the main centers of diversity in the genus *Centaurea* L. (Asteraceae), which ranks at number three in the list of major genera of Turkish Flora [1]. It is listed among the genera having the highest endemic taxon number in this country, with a total species count of 181, a total taxon count of around 240, 32 subspecies and 28 varieties. The proportions of endemic taxa are as follows: 112/181 species; 18/32 subspecies; and 16/28 varieties. The genus *Centaurea* has traditionally been a problematic taxon, and none of the early attempts to subdivide it were widely accepted [2-6]. However, more recent molecular analyses of this genus, together with studies of morphology, pollen type and karyology, have enabled this taxon's natural limits and intraspecies differences to be established with greater confidence [7-10].

Within the Turkish flora, some sections of *Centaurea* are very close taxonomically. However, the lack of absolutely certain limiting of characteristics has resulted in examples such as *Pseudoseridia* Wagenitz and *Cheirolepis* (Boiss.) O.Hoffm. Being separated by very poor vegetative specifications, with some molecular systematists even suggesting in recent years that the sections delimitations are absolutely artificial [11, 12]. Certainly, modern analyses methods provide an important contribution in the resolution of this kind of problem. Moreover, a more favorable established relationship between molecular data and morphologic characters needs to be encouraged.

Centaurea sericea Wagenitz is a very local and rare endemic species of Central Anatolia (Turkey). Until the time of this present research, only one insufficient specimen has been available for study. This sample was collected a long time ago by Sevim & Mehpare from antique woodland belonging to the town of Dursunbey (Balıkesir) in Turkey. Post-collection, *C. sericea* was submitted by Wagenitz [11] as a new species resembling *C. def exa* Wagenitz. In the intervening period, while *Centaurea sericea* has been scanned by a lot of researchers, it has not been reharvested / collected again from the same locality since 1950. Fortunately, we have succeeded in collecting samples from a different locality, Eskişehir, quite far from the original harvesting site. As a result, this rare endemic species will be submitted again into the world of science as detailed in this article.

Centaurea cankiriensis A.Duran & H.Duman is another local species endemic to Turkey, and is very close to *C. sericea* in terms of its involucre of bracts, as well as its appendage. Recent analysis of DNA sequences confirmed the close relationship of *C. cankiriensis* to the complex of sections of *Cheirolepis-Pseudoseridia* Wagenitz [11], a group defined by Garcia-Jacas *et al.* on a molecular basis [9].

The sectional classification of *Centaurea* relies heavily on the morphology of the appendage of bracts and of the achenes [10]. Moreover, the palinologic datas have been used effectively for taxonomy of the genus *Centaurea* in the recent years.

The purpose of the study was to examine morphological and palinological features relevant to two closely relative *Centaurea* species. The study also presented extensively a description and the rediscovery of the *Centaurea sericea* which had not been collected since 1947 years.

MATERIAL AND METHODS

Materials; We have based our study on samples taken from our own collections in Turkey and ten samples of each taxon were collected. Our collection's localities are given in the descriptions of morphological parts.

Morphological methods; Achenes were examined under an Olympus SZX9 binocular microscope, while the bristles

of the pappus were examined under an Olympus U- TV1X microscope. In both cases microphotographs were taken with an Olympus Camedia Master C3030 electronic camera. Digital images of plants, involucrums, and of the bracts were obtained with a Sony digital camera.

Palinological methods; Pollen material was obtained from dried flower specimens. For scanning electron microscopy (SEM) investigations, unacetolyzed pollen grains were directly placed onto stubs, sputter-coated with gold, and examined with a Jeol 5600 LV scanning electron microscope [13, 14]. Terminologies for pollen morphology proposed by Skvarla [15], Erdtman [16], Walker [13, 14], Charpin *et al.* [17] and Faegri and Iversen [18] were employed.

RESULTS

Morphological results

Centaurea sericea Wagenitz (Fig. 1, 2A, 3A, 3C)

20–50 cm, perennial, with woody rootstock and sterile shoots. STEM simple or branched; erect herbs. LEAVES entire, linear-lanceolate, lateral veins not prominent, tomentose, margins scabrous; Basal leaves lanceolate, 11–12 x 0.6–0.7 cm, not withered at flowering time; stem leaves similar to basal leaves but reduced upwards, upper not envoloping capitula. INVOLUCRE ovoid, 20–35 x 10–20 mm, bracts large, lanceolate and hairy. APPENDAGE orbiculate, 4–5 x 2–4 mm, including lateral fimbriae, concealing basal parts of the bracts, reddish coloured with brownish center, very weakly hooded in the middle, laterally fimbriate with short fimbriae 1-2, 5 mm, apical fimbria reduced in a mucro, 1-3 mm. FLOWERS 2, 5-3 cm, lobes c.6 mm. ACHENES lanceolate, 5–6 x 2.5-3 mm, blackish brown with lighter stripes, with creamish stria. PAPPUS 10-15 mm, simple, barbellate, brown, easily deciduous. **Fl.** July, **Mature fr.** August-September, *Pinus* and *Quercus* forest clearings, **Alt.** 1100–1150 m.

Chromosome number 2n= 36 [19].

Distribution and Conservational Status: This species is endemic to Central Anatolia (Eskişehir province), Irano-Turanian element. The specimens were collected in B3 Eskişehir-İnönü, a forested area where the species is very rare and localised, from an area c. 100 acres. The species has an abundant population in the area. *Centaurea sericea* is an endemic species and only known from the type gathering. The range of this species limited to a single localition and area of occupancy estimated to be less than 5 km (criteron B2a), the mature individual plants number being less than 300 (criteron C), [20]. Therefore it can be included in CR (Critically Endangered) category.

Habitat and Life Form: *Centaurea sericea* grows in meadows and clearings in a *Pinus nigra* J.F.Arnold plantation at 1100-1150 m altitude, alongside *Potentilla recta* L., *Polygala pruinosa* Boiss., *Dianthus cibrarius* Clem., *Silene otites* (L.) Wibel, *Rumex tuberosus* L., *Rumex nepalensis* Spreng., *Malva neglecta* Wallr., *Astragalus condensatus* Ledeb., *Astragalus lycius* Boiss., *Anthemis cretica* L., *Bellis perennis* L., *Centaurea thracica* (Janka) Hayek, *Legousia speculum-veneris* (L.) Chaix, *Cyclamen cilicicum* Boiss. & Heldr. var. *intaminatum* Meikle, *Convolvulus holosericeus* M.Bieb., *Veronica hederifolia* L., *Globularia trichosantha* Fisch. & C.A.Mey., *Salvia vir gata* Jacq., *Scutellaria orientalis* L. subsp. *santolinoides* (Hausskn. & Bornm.) J.R.Edm., *Stachys tmolea* Boiss. and *Lamium purpureum* L. Chamaephyte.

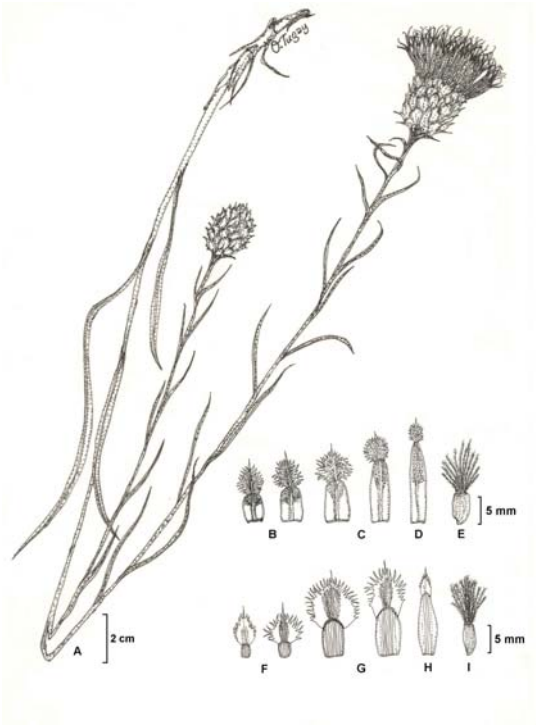

Figure 1. *Centaurea sericea* Wagenitz ─ **A:** Habit. ─ **B:** Outer phyllaries. ─ **C:** Median phyllaries. ─ **D:** Inner phyllaries. ─ **E:** Achene, *Centaurea cankiriensis* A.Duran & H.Duman. ─ **F:** Outer phyllaries. ─ **G:** Median phyllaries. ─ **H:** Inner phyllaries. ─ **I:** Achene

Centaurea cankiriensis **A. Duran & H. Duman**

10–40 cm, perennial with woody rootstock and sterile shoots. STEM erect, generally simple herbs. LEAVES entire, lateral veins not prominent, tomentose, undulate, glandular; margines scabrous. Basal leaves linear-lanseolate, 6–11 x 0.5-0.7 cm, not withered at flowering time, petiolate; stem leaves linear, 1-4 x 0.1-0.3 mm, uppermost not enveloping capitula. INVOLUCRE 2-3 x 0.8-1.7 cm, narrow oblong-ovoid; bracts without line, linear- lanseolate. APPENDAGE membranous, decurrent, glabrouse, with orbicular lamina; 3–6 x 4–5 mm, creamish, with brownish center at the middle of the lamina, laterally lacerated with very short laciniae 0.5-1.5 mm, very weak, apical spine further reduced, 2-4 mm. FLOWERS yellow, marginal; not radiant, with nectar, lobes with stria brownish red longitudinally. ACHENES glabrous, 4–5 x 2–2.5 mm, creamish-brown, irregular with white stria. PAPPUS double, outer series plumose; 8-10 mm, inner series barbellate 2-3 mm, brown. Fl. July, Mature fr**.** August. Steppe with rocky areas, **Alt.** 1400–1500 m.

Chromosome number 2n=18 [19].

Figure 2. The capitulums of (A) *Centaurea sericea* and (B) *Centaurea cankiriensis*.

Distribution and Conservational Status: Endemic in transition territory of Central and North Anatolia. Irano-Turanian Element. The range of *Centaurea cankiriensis* is restricted to a single location and an area of less than 5 km^2 (Criterion B2a), [20]. As a result, we suggest that *C. cankiriensis* should be placed under the IUCN category Critically Endagered (CR).

Habitat and Life Form: Occurs in steppe on stony slopes at 1400-1600 m. Flowers and fruit June, July and August. This species grows with *Galium verum* subsp. *glabrescens* EHREND., *Achiella teretifolia* WILLD., *Inula britannica* L., *Scariola viminea* (L.) F.W.Schmidt, *Sedum obtusifolium* C. A. Meyer, *Salvia verticillata* L., *S. frigida* Boiss., *Silene supine* Bieb. and *Hypericum linarioides* Bosse. [21].

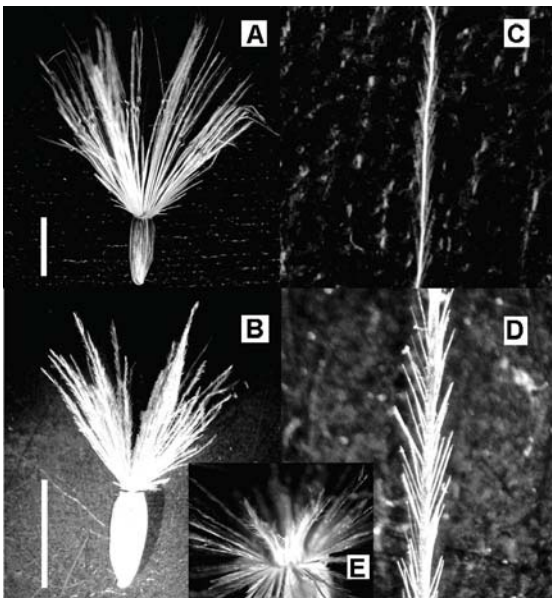

Figure 3. Microphotographs of the achenes of (A) *Centaurea sericea*, (B) *Centaurea cankiriensis* Scale bars = 5mm. Detail of pappus bristles (C) *Centaurea sericea*, (D) *Centaurea cankiriensis*. Pappus inner view (E) *Centaurea cankiriensis*.

Palinological Results

The pollen grains of *C. sericea* are tricolporate, microechinate-scabrate, spheroidal-subprolate, the amb triangular. The exine has one layer of columellae beneath spines, microspine length 2 µm, spinule width 3 µm. Exine tectate-scabrate, 36 spinule in 100 µ^2 and average distance between spinules 0, 5 µm (Fig. 4. A1, A2, A3).

The pollen grains of *C. cankiriensis* are tricolporate, microechinate-scabrate, spheroidal-subprolate and the amb triangular. The exine has one layer of columellae beneath spines, microspine length 1 µm, spinule width 3,04 µm. Exine tectate-scabrate, 28 spinule in 100 µ^2 and average distance between spinules 0,25 µm (Fig. 4. B1, B2, B3).

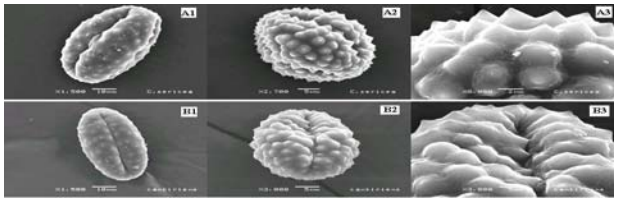

Figure 4. Scanning electron micrographs of pollen grains of C. sericea (A1, A2, A3) and C. cankiriensis (B1, B2, B3); A1, A2, B1, B2, general views of pollen grains; A3, B3, close up of pollen grains.

DISCUSSION

The morphology of two species in the genus is narrowly connected, according to their vegetative characters and pollen features. *Centaurea cankiriensis* has an interesting taxonomical character which hasn't been seen in any section of *Cheirolepis* before: its pappus is both plumose and double series. Thus, the species exhibits an intermediary form between sections of *Cheirolepis* and *Pseudoseridia*; the taxonomical character could supply morphological evidence for the introduction of molecular diagnosis on the *Cheirolepis-Pseudoseridia* complex.

In previous studies, the sectional classification of *Centaurea* relied heavily on the morphology of the appendage of bracts and of the achenes [10, 22]. In terms of Turkish flora, the genus *Centaurea* was revised by Wagenitz and the sections *Pseudoseridia* and *Cheirolepis* were separated according to the characteristics of their achenes and pappus. In a later revision of Wagenitz, the pappus of sect. *Pseudoseridia* was found to be double series and scabrous. On the other hand, the pappus of sect. *Cheirolepis* was simple and plumose. Aside from these, no other morphological differences were described between the two sections [23]. Due to the closeness of the two sections taxonomically, the interesting characteristic of the pappus in *Centaurea cankiriensis* justifies re-examination of the two sections in order to find the answer to two vital questions. Firstly, precisely where *Centaurea cankiriensis* should be placed in the sectional level? Secondly, whether or not the pappus features really justify the separation of the two sections. According to our findings, the answer to the first question is completely related to the second question. Since just one taxonomical character is inadequate in morphologically terms for the separation of these two sections, it is clear that the delimitation of the two sections is artificial, and that *C. cankiriensis* should be placed in the section *Cheirolepis* according to the priority principles of plant taxonomy. Similarly, a monotypic species *C. paphlagonica* (Bornm.) Wagenitz (Sect. *Plumosipappus*) displays similar taxonomic features to *C. cankiriense* , with

basically no differencies morphologically among these sections' species. In this case, we again advocate the merging of these sections on the basis that the singular feature of the pappus being used as a pretext for sectional separation is insufficient. In previous studies, the hypothesis of evolution we have submitted in molecular ground explains the close connections found in the DNA sequence analyses of the sections *Cheirolepis, Pseudoseridia* and *Plumosipapus* (Czerep.) Wagenitz [11].

The species investigated are very similar morphologically, but there are some important differences. The basal leaves of *C. sericea and C. cankiriensis* are linear-lanceolate but they show different length/width ratio. Clearly, the capitulum of *Centaurea cankiriensis* is smaller than that of *Centaurea sericea.* Although the appendages of *Centaurea cankiriensis* are decurrent with irregularly lacerated margins, the appendages of *Centaurea sericea* are simple with regularly ciliated margins. Both of the appendages are of a membranish texture, but the terminal spinule of *Centaurea cankiriensis* is slightly longer than that of *Centaurea sericea*. The sizes and shapes of pappus are clearly different for *Centaurea sericea* and *Centaurea cankiriensis* . The pappus of *Centaurea sericea* is simple and barbellate, but the pappus of *Centaurea cankiriensis* is double and plumosus. Furthermore, *Centaurea cankiriensis* grows in steps, while *Centaurea sericea* grows in woodland areas.

Ertuğrul *et al.* indicated that *Cheirolepis* section's species had 3 type appendages at the start of their evolution [23]. It was also suggested in the same article that the appendages of *C. def exa* and *C. paphlagonica* had a second model, in which the apical spine was of a vulnerant type and that the margin was lacerate-fimbriate. The appendage of the first type can be described as having a wide orbicular lamina with an irregularly lacerate margin, such as that seen in *C. ensiformis* P.H.Davis. In ultimate appendage types, the appendix is reduced to a set of palmate spines, with the terminal spine being only slightly longer. As a result, the two species studied have second model appendage types, with this appendage model demonstrating the transition between the other two appendage models.

The pollen morphology of the two species exhibits a close relationship to the other *Centaurea* species inhabiting Turkey [24, 25]. In particular, the morphological pollen characteristics of *C. cankiriensis* showed only some minor differences, such as those seen on the pollen surface, spinule dimensions and spinule density in comparison with *C. sericea*.

As a result of this study, we can declare that these species are very close taxonomically. Morphological results have demonstrated that these close species could be easily separated according to the characteristics of the pappus. There are minor differences palinologically, but palinology of the species isn't sufficient in itself to justify the separation of these close species.

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