

Araştırma Makalesi / Research Article

Türkiye’de Bulunan *Cirsium aggregatum* Ledeb. Üzerinde Sistemik Araştırmalar**Melahat OZCAN**Artvin Coruh University, Faculty of Forestry, Department of Forest Engineering, Artvin
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Geliş Tarihi: 25.03.2016; Kabul Tarihi: 31.08.2016

Anahtar kelimeler**Özet**“*Cirsium aggregatum*”;
“Morfoloji”;
“Anatomi”;
“Kromozom sayısı”;
“SEM”Bu çalışmada Türkiye’den yalnızca iki noktadan kaydı bilinen *Cirsium aggregatum* Ledeb. Artvin ili Ardanuç, Kordevan Dağı’ndan ilk defa toplanmıştır. Türün morfolojik özellikleri detaylandırılmış, gövde ve kipsela anatomi ve mikromorfolojisi ile sitolojik özellikleri incelenmiştir. Çalışmadan elde edilen sonuçlar literatür verileriyle ilişkilendirilmiştir.**Systematical Investigations on *Cirsium aggregatum* Ledeb. (Asteraceae, Cardueae) from Turkey****Keywords****Abstract**“*Cirsium aggregatum*”;
“Morphology”;
“Anatomy”;
“Chromosome
number”; “SEM”In this study, *Cirsium aggregatum* Ledeb. which is known from only two localities in Turkey, was collected for the first time from Artvin, Ardanuç town, Kordevan Dagı. Morphological characteristics were detailed, stem and cypsela anatomy, cypsela micromorphology, and cytological characteristics were investigated. Results obtained from this study were compared the data present in literature.

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1. Introduction

Cirsium Miller, one of the largest genera of the Asteraceae, contains more than 250 taxa or variable number depending on the authors (Smith, 1977; Zomlefer, 1994). It has a holarktic distribution with several centers of diversity. The largest extents from the northern Mediterranean area over Turkey to Caucasus. Minor centers are in the Tjan san, China, Japan, the Rocky Mountains and Mexico (Häffner, 2000).

Turkey is one of the gene centers and new taxa have been determined day by day for the Turkish flora. According to recent studies, this genus is represented by 78 taxa at the species, subspecies, and variety level in Turkey (Davis and Parris 1975; Guner *et al.* 2000; Yıldız, 2012; Yıldız *et al.* 2013).

Recently 7 species (*C. ekimianum* Yıldız & Dirmenci, *C. handaniae* Yıldız, Dirmenci & Arabacı, *C. peshmenianum* Yıldız, Dirmenci & Arabacı, *C. sivasicum* Yıldız, Arabacı & Dirmenci, and *C. yildizianum*, *Cirsium balikesirense* Yıldız, Arabacı & Dirmenci and *C. nerimaniae* Yıldız, Dirmenci & Arabacı Arabacı & Dirmenci) have added to the Flora of Turkey (Daşkın *et al.* 2006; Yıldız, 2012; Yıldız *et al.* 2013) and endemism ratio reached 35.89%.

Cirsium aggregatum Ledeb. is a late and short term flowering perennial species in the genus *Cirsium*. It is distributed NE Anatolia and Georgia. This species is known two localities in Turkey. In the present study, it was collected from Artvin province for the first time as a third locality. It is aimed to amend

morphological description, to investigate stem and cypsela anatomical characteristics and cypsela micromorphology and to count chromosome number of this species. Data obtained from this study were evaluated to their potential value and in relation to the previous work in the genus *Cirsium*.

2. Material and Methods

2.1. Sampling

Plant samples were collected from two different localities in August 2011, 2014;

Soğanlı Pass, Çaykara road, Bayburt - Trabzon, humid areas, 2100-2210 m, 40°17'25.1"E, 40°32'59.1"N, M. Ozcan 459.

Kordevan Dağı, Ardanoç, Artvin, among alpine grasses, 2085 m, 42°11'35.51"E, 41°07'18.36"N, M. Ozcan 693.

2.2. Morphology

Species identification was made according to Davis & Parris (1975). Specimens for morphological examinations were dried according to standard herbarium techniques and deposited in Artvin Coruh University Herbarium (ARTH). Morphological examinations and calculations were carried out under stereomicroscope (Leica M60 with digital camera attachment DFC 295). Leaf corolla, outer and inner phyllaries were drawn from herbarium specimens and illustrated using stereomicroscopy.

2.3. Anatomy

Anatomical observations were performed in the stem and cypsela anatomies of the species. Fresh parts of stem were fixed in the field with formaldehyde, acetic acid–alcohol (FAA). Handmade cross-sections were prepared from the median part of the stem by using commercial razor blades. Mature cypselas were obtained from at least three individual plants, softened by boiling water for 3-4 days and stored in glycerin. Cryostat was used for cross-sections of cypselas. Sections were cut to a thickness of 15- 20 µm. All sections were stained in Haematoxylin for about 15 min (Algan, 1981). Semi-permanent slides were

mounted in glycerin. Well stained sections were examined under a light microscopy (LM) and photographed using an Olympus research microscope with digital camera attachment DP73.

Five cross-sections from at least three different individual plants were measured to assess the consistency of anatomical characters and to calculate the means and standard error among different cross-sections using LM.

2.4. Micromorphology

Micro and macromorphological data of cypselas were studied using a stereomicroscope (Leica M60 with digital camera attachment DFC 295) and a scanning electron microscope (SEM; Zeiss Evo LS 10, ACU- Biltekmer). The cypselas were examined at first by using a stereomicroscope to ensure size, shape, color and maturity and photographed. For SEM, mature cypselas were placed on stubs using double-sided adhesive tape, coated with gold in cressington sputter coater 108 auto coating apparatus for 2-3 minutes.

The terminology of the cypselar characters proposed by Barthlott (1981) and Stearn (1985) have been adopted to describe the fruit coat, size and shape, cell arrangements and primary sculpturing.

2.5. Chromosome count

For mitotic chromosome observation root tips germinated in petri dishes were cut off and pretreated with saturated solution of 1-Bromonaphthalene at 4°C temperature for 16 h (Ozcan *et al.* 2011), then fixed in fresh Carnoy absolute alcohol-glacial acetic acid (3:1) for 24 h at 4°C. For chromosome counts, root tips were hydrolyzed in 5N HCl for 4-5 min at room temperature and then rinsed with distilled water for 2-3 min (Ozcan *et al.* 2011). Staining was carried out in lacto-propionic orcein at least for 2 h at room temperature. Permanent slides were prepared from at least ten well-spread cells. The best metaphase plates were photographed with

Olympus BX-53 microscope with digital camera attachment DP-73.

3. Results

3.1. Morphological characteristics

C. aggregatum Ledeb., Fl. Ross. 2:737 (1846). le: Dimitrieva, Opred. Rast. Adjarii: t. 31 (1959).

Perennial, 70-120 mm. Stem unbranched, unwinged, sparsely arachnoid to floccose. Median cauline leaves semi-amplexicaul, ovate-oblong, pinnatisect, lateral lobes bifid, c. 4-6-paired, triangular to narrowly triangular, lateral and terminal lobes with weak c. 1 mm apical spine, spinose-strigose above, with setae 0-17-0.7 mm, otherwise glabrous: arachnoid below, Uppermost leaves 1-3, equal to or longer than involucre. Median cauline leaves size 9-13 x 12.2-21 cm. $\frac{3}{4}$ pinnatisect (13-18 mm undissected part). Involucres c. 5-13, sessile in dense corymb. stem apex, obovoid, 14-17 x 12-16 mm. Capitula length 20-26 mm. Phyllaries purplish, sparsely arachnoid, outer \pm erect, 8,5-9,5 x 1,75-2,25 mm, incl. weak 0.5-0.58 mm apical spine ; median \pm erect, 11.75-14.33 x 1.75-2.08 mm, incl. weak 0,5-1mm apical spine. Corollas purple, 14-18 mm, corolla tube length 10.25-11 mm. Cypselas 2-2.5 x 5.10-6.67 mm. Pappus 13-16 mm, deciduous (Figure 1).

Type: [Georgia] in provinciis caucasicis (Guria, Nordmann) (LE).

Flowering time: August

Growing area: Marshy meadows and steep moist hayfields, 2000-21000 m.

Distribution: NE Anatolia, euxine elements.

3.2. Stem anatomy

Epidermis is a single layered, size $17.27\pm 0.39 \times 17.2\pm 0.41 \mu\text{m}$. There are eglandular multicellular hairs on its. Parenchymatic cortex has been observing 18-24 series ($432.0\pm 24.85 \mu\text{m}$) beneath the epidermis. Swinging collenchymatic cells groups which are 10-12 layered ($226.4\pm 12.91 \mu\text{m}$) are seen at the protruding sides of the stem. It is possible to observe both of small and large vascular

bundles at the interfascicular area arranged in one circle. Vascular bundles of corners are larger than others. Phloem thickens is $246.40\pm 21.61 \mu\text{m}$ and xylem thickens is $272.27\pm 15.51 \mu\text{m}$. Sclerenchymatous caps ($174.27\pm 15.48 \mu\text{m}$) were present above vascular bundles. Parenchymatic cells layer are present above the bundles. Large collenchyma tissues are found in both phloem and xylem parts. Some large parenchymatous cells or gaps contain secretion material near the phloem parts. Cambium is distinguishable. Large pith formed of cylindrical and thin walled big parenchymatic cells ($118.57\pm 3.00 \mu\text{m}$) are present in the stem. Mean diameter of the tracheas is $26.35\pm 1.15 \mu\text{m}$ (Figure 2).

3.3. Cypselas anatomy

It composed of pericarp, testa, endosperm and cotyledons. The mature pericarp is differentiated into two zones; exocarp and mesocarp, and shows a variation in the structure.

Pericarp (26.48 ± 0.79) includes thick wall rectangular epidermal cell ($12.15\pm 1.14 \times 7.13\pm 0.71$, exocarp) and parenchymatous cells (mesocarp). Four carpel bundle traces ($76.81\pm 5.70 \times 34.17\pm 2.16$) underlaid by a layer of parenchymatous cells (in pericarp) and one-two raphe bundles (in testa) placed in opposite sides are present. Tetragonal crystals were found in some sections. Testa body consists of two differentiated cell types with a single row longitudinally elongated, lignified cells (outer subzone, 70.42 ± 2.42) and crushed parenchymatous cell group (inner zone, 35.17 ± 0.96). A subjacent layer of the testa is endosperm (9.59 ± 0.27) or only remnants composed of one layer of elongated and thin-walled parenchymatous cells. The cotyledons occupy large area of seed interior (Figure 3). Tetragonal crystals were observed in mesocarp of some sections.

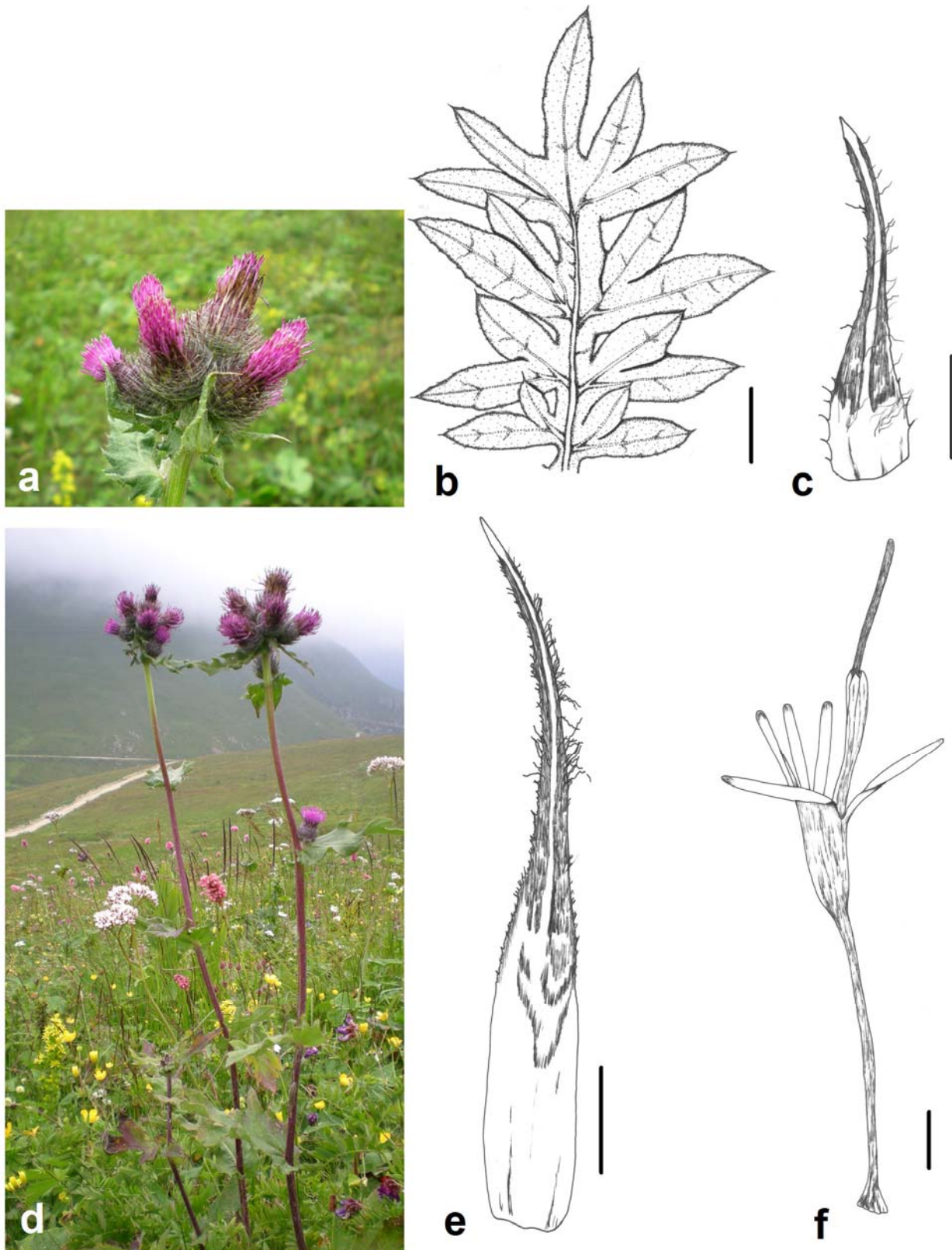


Figure 1. *Cirsium aggregatum*. a, d: photograph, b, c, e, f: drawing. a: capitula, b: cauline leaf, c: outer phyllary, d: habitus, e: inner phyllary, f: corolla. Scale bars (b): 2 cm, (c, e, f): 2 mm.

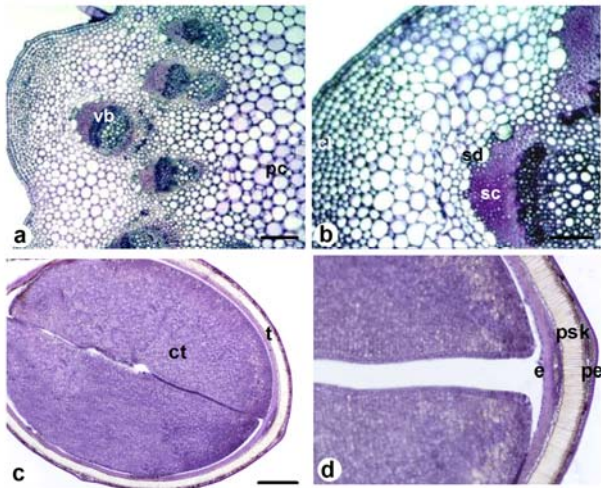


Figure 2. Cross section. a, b: stem, c, d: cypselas. cl: collenchyma, ct: cotyledon, e: endosperm, pc: pith cell, pe: pericarp, psk: palisade sclerenchyma, sc: Sclerenchymatic cap, sd: secretory duct, t: testa, vb: vascular bundle, Scale bars (a): 200 μ m, (b, d): 100 μ m, (c): 500 μ m.

3.4. Cypselas micromorphology

Cypselas color is yellowish brown to brown. It is compressed slightly on both sides, symmetric or slightly asymmetric in outline, and has non-ribbed structure. It has narrow base, sublateral hilum, thin and acute apex. Mean lengths of cypselas varied from 5.10 to 6.67 mm. Their mean width varied between 2.0 and 2.5 mm. The cypselas shape was widely oblong.

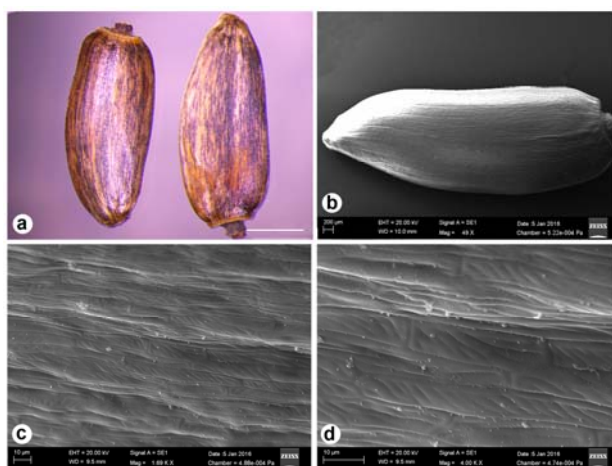


Figure 3. Cypselas micrograph. a: LM, b-d: SEM. Scale bar (a): 1 mm.

Epidermal surface not included trichomes, completely glabrous. Anticlinal cell walls observed

in the taxa are straight, while periclinal cell walls flat and smooth. According to the surface ornamentation in SEM investigations scalariform type was determined.

3.5. Chromosome number

Chromosome number of this species is found as $2n = 34$ and also three B chromosomes are detected (Figure 4). This is the second count for this species. Previous count in the species was reported by Ozcan et al. (2011) as $2n = 34$. This diploid count agrees with only one former report from Turkish material. However B chromosomes were firstly reported for this species. B chromosomes were also previously determined in two different taxa in the genus *Cirsium* by Ozcan et al. (2011).

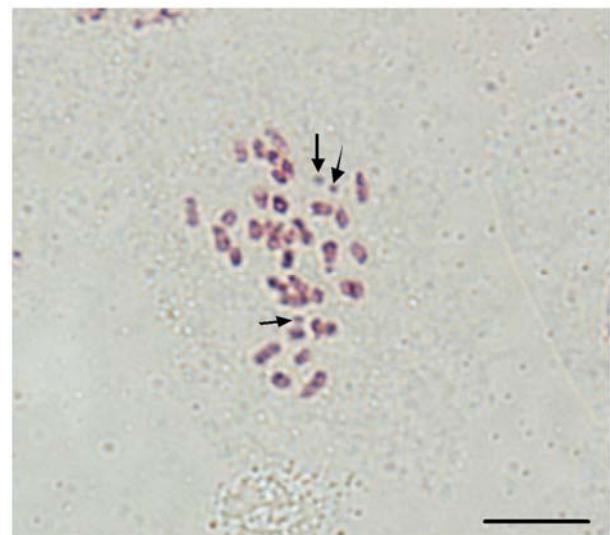


Figure 4. Somatic metaphase, $2n = 34+0-3B$. Scale bar: 10 μ m.

4. Discussion

Different systematical aspects of *Cirsium aggregatum* have been evaluated in this study. Morphological description was expanded, and median cauline leaf size, outer phyllary length and cypselas width were included. Some diagrammatic illustrations were supplied. The present results are found generally in agreement in The Flora of Turkey (Davis and Parris 1975). However some differences were noted, such as smaller setae

length in upper leaf surface, more involucre (5-13) in stem apex and smaller pappus.

Many anatomical investigations have been used by authors as an additional data for species identifications (Stace, 1984; Lu *et al.* 2008; Inceer and Ozcan 2011; Ozcan *et al.* 2014; Ozcan *et al.* 2015). Metcalfe and Chalk (1979) and mentioned general anatomical characteristics of Asteraceae, and Kadereit and Jeffrey (2007) have previously reported similar features. Ozcan and Demiralay (2013) studied stem anatomies of four taxa and important characters were reported. 9-14 ribbed stem with supporting tissue elements in the corners and large sclerenchymatic caps above vascular bundles and large parenchymatic cells containing secretory material in cortex were observed in this species. The present findings are in accordance with these previous reports.

Cypselas anatomy of taxa show five distinct parts; exocarp (sclerified epidermal cells), mesocarp, testa (elongated, lignified cells and crushed cells), one layer endosperma and large cotyledons. Some carpological studies on some taxa of the tribe Cardueae present in literature (Häffner, 2000; Zarembo and Boyko 2006). The present results are in accordance with these reports. Four main vascular traces were found in *C. aggregatum*. Four to five, sometimes six to seven vascular traces have been reported in the taxa of the tribe Cardueae by Häffner (2000). It is observed tetragonal crystals in mesocarp of cypselas. Zarembo and Boyko (2006) previously reported calcium oxalate crystals in some taxa. In addition, Häffner (2000) and Dormer (1961) indicated that these type crystals are common in the tribe Cardueae. One layered endosperm has been previously reported by Häffner (2000). Grau and Hopf (1985) supposed that this structure probably provides as an additional protection to the embryo.

Two different reports were present in literature about cypselas of some *Cirsium* taxa; one is the cypselas anatomy of *Cirsium eriophorum* (Häffner, 2000) and second is surface micromorphologies of

some *Cirsium* taxa of the Sect. *Cirsium* (Kostekci and Arabaci 2011). According to surface ornamentation, scalariform type was observed. This type was previously reported in *C. echinus* and *C. pubigerum* var. *spinatum* by Köstekci and Arabaci (2011).

Acknowledgements

This research was financed by the research foundation of Artvin Coruh University (Project number: 2011.F15.02.16). The author is thankful to the Research Foundation director for financial support and Prof. Dr. Özgür Eminağaoğlu for his help with the collection of plant materials from Kordevan Mountain.

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