Acta Biologica Szegediensis

Volume 62(1):37-43, 2018
Acta Biologica Szegediensis
http://abs.bibl.u-szeged.hu/index.php/abs
DOI:10.14232/abs.2018.1.37-43

ARTICLE

Anatomy study of the genus *Cirsium* Mill. in Iran

Masoud Sheidai, Saeedeh Shojaei, Fahimeh Koohdar*

Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran

ABSTRACT The genus *Cirsium* Mill. (Asteraceae) also known as plume thistles with about 250 perennial, biennial or rarely annual spiny species is a phylogenetically unresolved and paraphyletic genus. *Cirsium* species grow in different ecological conditions and tend to form interspecific hybrids. Some species are morphologically very similar and need to be delineated by additional anatomical and molecular characters. About 28 *Cirsium* species have been reported in Flora Iranica; they were classified in five sections. Taxonomic investigation of these species was confined to morphology and molecular study of RAPD and ISSR markers. The present study carried out anatomical investigation (leaf and stem characters) of *Cirsium* species in the country. PCoA analysis of anatomical characters could delimit the studied species and the grouping obtained was almost in agreement with morphological and sectional delineation of the genus. The results obtained are in agreement with several other investigations and all together suggestive of the continued gene flow and introgression between *Cirsium* species that make taxonomy and phylogenetic relationship of the species difficult.
Acta Biol Szeged 62(1):37-43 (2018)

KEY WORDS
anatomy
Cirsium
taxonomy

ARTICLE INFORMATION
Submitted
30 October 2017.
Accepted
30 March 2018.
***Corresponding author**
E-mail: f_koohdar@yahoo.com

Introduction

The genus *Cirsium* Mill. (Asteraceae) contains about 250 perennial, biennial or rarely annual spiny species that grow in different ecological conditions in the Northern hemisphere, Europe, North Africa, Siberia, Central Asia, West and East Africa, as well as Central America (Zwölfer 1994; Bures et al. 2004). The genus *Cirsium* is one of several genera known commonly as thistles. They are more precisely known as plume thistles and differ from other thistle genera (*Carduus*, *Silybum* and *Onopordum*) in having feathered hairs to their achenes. The other genera have a pappus of simple unbranched hairs (Francis 1981). There are about 28 species, 4 subspecies and 10 varieties of *Cirsium* in Iran that have been placed in 5 sections and 7 subsections (Ghoreyshi-Alhosseini et al. 2004).

Cirsium species can adapt to various ecological conditions with variable elevation, temperature and edaphic factors due to their genetic adaptability and plasticity. Their high genetic variability and ecological adaptability partly comes from inter-specific hybridization that frequently occurs in the genus (Bureš et al. 2004; Sheidai et al. 2013). These events cause difficulties in taxonomic delimitation of the *Cirsium* species. For example, *C. arvense* (Bull thistle) exhibits variation in several morphological characteristics that have been described as subspecies by some authors; however, the Flora Europaea does not recognize these taxa, because they lack sufficient morphological or geographical delimitation (Forcella et al. 1994). Moreover, several hybrids of bull thistle have been described in Europe (Klinkhamer and De Jong 1993) and some new varieties have been identified based on morphological and molecular studies in other places (Seif et al. 2012). The occurrence of inter-specific genetic introgression was recognized between *C. aduncum* and *C. haussknechtii* (Sheidai et al. 2016). Taxonomic complication of the *Cirsium* species is also to some degree due to intraspecific genetic and morphological variability. For example, Sheidai et al. (2013) extensively studied different geographical populations in four *Cirsium* species by morphological, cytogenetic and molecular data. They reported extensive morphological variability, genetic admixture, molecular diversity, and variation in chiasma frequency, and also the occurrence of heterozygote translocations. These features all together bring about a high degree of genetic and phenotypic variability in each species.

About 28 *Cirsium* species have been reported in Flora Iranica (Rechinger 1979) and classified in five sections. *C. pyramidale* is endemic species with confined geographical distribution and just growing in Kerman province. The previous studies performed in *Cirsium* species of Iran were mainly concerned with morphometric, cytogenetic and population genetic analysis (Nouroozi et al. 2013; Seif et al. 2012; Sheidai et al. 2012, 2013, 2016). In only one attempt species relationship were investigated by RAPD

Table 1. The species studied their section and locality.

No	Species	Section	Locality
1	*C. pyramidale* Bornm.	*Pseudoepitrachys*	Kerman
2	*C. spectabile* DC.	*Pseudoepitrachys*	Kerman
3	*C. congestum Fisch. & C.A. Mey.* ex *DC.*	*Pseudoepitrachys*	West Azarbaijan
4	*C. vulgare* (Savi) Ten.	*Epitrachys*	West Azarbaijan
5	*C. bornmülleri* Sint. ex Bornm.	*Epitrachys*	Khorassan
6	*C. ciliatum* (Murray) Moench.	*Epitrachys*	West Azarbaijan
7	*C. lappaceum* Lam.	*Epitrachys*	West Azarbaijan
8	*C. bracteosum* DC.	*Epitrachys*	Boyerahmad
9	*C. osseticum* (Adams) Petr.	*Epitrachys*	Mazandaran
10	*C. strigosum* (M.Bieb.) Fisch.	*Epitrachys*	West Azarbaijan
11	*C. arvense* (L.) Scop.	*Cephalonoplos*	West Azarbaijan
12	*C. obvallatum* (M.Bieb.) M.Bieb.	*Cirsium*	West Azarbaijan
13	*C. alatum* (S.G.Gmel.) Bobrov.	*Cirsium*	West Azarbaijan
14	*C. elodes* M.Bieb.	*Cirsium*	Tehran
15	*C. hygrophilum* Boiss.	*Cirsium*	Ardabil
16	*C. libanticum* DC.	*Cirsium*	West Azarbaijan
17	*C. echinus* (M.Bieb.) Hand-Mazz.	*Echenais*	West Azarbaijan

(Random Amplified Polymorphic DNA) and ISSR (Inter Simple Sequence Repeats) molecular markers (Nouroozi et al. 2013). Having in mind above said difficulties and limitations in the published articles on taxonomy of the genus, we carried out detailed population based anatomical investigation of the *Cirsium* species in Iran with the aim produce data for species delimitation and reveal the species relationship.

Materials and Methods

Plant materials

In total, 17 *Cirsium* species (3 individual of each species) could be obtained for the present investigation (Table 1). These species are from all sections of the Iranian *Cirsium*. According to Petrak (1979), the genus *Cirsium* has five sections in Iran, including *Pseudoepitrachys* Petrak, *Epitrachys* DC., *Echenais* (Cass.) Petrak, *Cirsium*, and *Cephalonoplos* (Necker) DC.

Anatomy study

Embedded materials were prepared as follows: Three adult plants samples were excised and immediately fixed in formalin-acetic acid-alcohol (FAA) (formalin: acetic acid:ethanol (90%) = 5:5:50%) (Jensen 1962) for 48 to 72 hours, and stored at 4 °C until sectioning, after dehydrated in a graded ethanol series and embedded in 70% ethanol.

Table 2. Anatomical characteristics of stem in *Cirsium* species.

No	Characters
1	Number of epidermis layers
2	The width of the epidermis layer
3	Cuticle width
4	The length of the pith cells
5	Cortex thickness
6	The number of cortex layers
7	Swinging collenchymatic cells groups
8	Width of lower phloem
9	Width of upper phloem
10	Width of xylem
11	Angular collenchymas thickness
12	Inner sclerenchyma thickness
13	External sclerenchyma thickness
14	Number of stem corners
15	The number of bundles of vascular

Table 3. Anatomical characteristics of leaf in *Cirsium* species.

No	Characters
1	Upper epidermis cell length
2	Upper epidermis cell width
3	Lower epidermis cell length
4	Lower epidermis cell width
5	Mesophile thickness
6	Upper collenchymas thickness
7	Lower collenchymas thickness
8	Xylem thickness
9	Upper phloem thickness
10	Lower phloem thickness
11	Number of vascular of vein
12	Trachea thickness
13	Spongy parenchyma thickness
14	Palisade parenchyma thickness
15	Upper sclerenchyma thickness
16	Lower sclerenchyma thickness
17	Upper cortex thickness
18	Lower cortex thickness

Table 4. Details of stem anatomical characters in *Cirsium* species (species and character numbers are according to Tables 1 and 2).

Character (µm)	Species																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	1	2	2	1	1	2	2	1	2	2	1	2	1	2	2	2	2
2	25.6	15.9	15.1	7.5	3.5	12.3	9.5	5.3	13.3	24.6	5.6	12.0	6.3	27.0	13.3	32.7	23
3	11.0	5.4	2.9	3.5	2.4	3.4	24.6	2.3	2.4	5.8	3.5	2.8	6.2	4.7	2.1	7	6
4	21.4	19.6	18.0	24.8	17.3	25.5	18.2	13.8	11.0	17.3	24.9	27.8	18.8	29.2	25.5	14	24.3
5	219.8	179.5	180.9	94.9	98.5	142.7	232.1	125.5	128.1	100.9	74.1	114.6	124.3	222.2	91.1	141.3	112
6	25	11	22	12	7	12	17	17	14	10	10	15	12	12	11	19	10
7	8	11	8	14	9	11	9	6	7	6	8	13	10	9	8	8	12
8	31.8	30.7	28.5	21.4	30.1	17.0	16.2	13.6	11.9	11.1	15.6	39.2	20.2	22.7	21.3	29.0	15.8
9	85.9	81.1	105.1	47.0	87.1	45.6	49.5	33.9	23.5	42.6	28.9	54.2	48.2	32.5	33.2	33.7	38.2
10	51.0	69.4	65.4	44.9	91.7	43.8	34.8	37.0	23.7	33.7	22.0	52.9	27.3	34.4	34.3	59.7	30.9
11	69.9	33.2	62.2	36.0	16.5	34.9	30.3	35.2	33.8	36.2	29.9	38.5	44.1	46.2	39.1	15.0	22.1
12	23.6	19.7	16.9	9.0	13.9	16.8	12.2	13.2	11.02	12.7	15.02	37.05	6.1	4.5	21.2	13.3	15.4
13	32.4	22.0	15.4	14.1	13.8	19.1	15.9	5.0	12.6	12.2	14.9	14.3	14.0	14.9	17.1	10.0	14.7
14	8	10	8	14	9	10	8	6	7	6	8	12	10	9	8	8	12
15	41	42	72	44	28	42	28	34	27	31	22	38	31	32	35	56	40

Table 5. Details of leaf anatomical characters in *Cirsium* species (species and character numbers are according to Tables 1 and 2).

Character (µm)	Species																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	9.9	6.3	9.3	11.2	9.5	9.3	10.4	9.2	12.3	14.0	11.1	14.5	10.8	11.6	18.33	8.0	8.8
2	12.1	7.2	12.3	13.3	9.0	12.2	13.6	12.0	20.4	12.5	13.3	15.1	10.9	14.1	17.67	8.5	10.2
3	10.5	6.3	7.8	8.7	7.8	11.0	10.7	10.0	9.7	6.5	8.5	12.4	14.0	11.5	19.33	8.0	7.0
4	11.1	7.0	10.0	11.7	9.7	13.5	13.0	16.0	13.9	11.5	9.1	12.6	18.4	12.4	17.01	7.2	8.6
5	239.8	119.5	178.2	90.7	89.1	135.0	108.8	189.5	229.0	271.0	201.9	154.3	195.2	211.0	140	225.0	130.6
6	76.8	57.7	63.2	58.2	28.7	49.0	70.9	38.6	69.8	102.0	24.5	21.1	38.0	29.6	39.57	16.5	26.1
7	56.5	53.7	63.1	66.5	51.0	73.9	50.5	35.4	72.7	60.5	26.6	26.8	47.7	29.1	51.56	13.5	16.1
8	31.6	24.7	38.8	51.5	30.0	55.8	29.0	37.0	84.2	69.5	40.1	38.2	57.8	29.7	55	37.0	43.7
9	36.3	25.0	41.0	33.7	21.0	43.7	27.0	58.0	21.2	77.1	29.9	65.1	29.0	41.6	65	41.0	29.5
10	64.7	28.0	54.2	68.4	34.5	69.8	34.2	69.0	60.8	68.0	58.2	50.6	78.2	39.8	75.67	31.5	38.4
11	1	3	3	6	7	3	1	1	1	2	3	1	3	1	1	3	1
12	52.9	42.1	71.8	79.0	44.0	100.4	53.2	78.0	113.2	86.0	68.5	41.0	86.7	52.4	84	75.0	49.9
13	141.7	57.9	106.3	43.7	65.1	58.0	42.4	92.9	106.0	146.0	100.4	56.6	107.3	112.3	57.42	53.5	48.7
14	98.7	63.3	70.0	49.7	29.7	73.7	50.8	75.5	119.0	117.0	88.7	49.5	110.3	104.9	74.95	43.0	63.3
15	19.8	17.0	18.7	13.8	0.0	18.4	6.3	6.4	16.0	23.0	9.0	6.8	3.7	6.3	35	14.0	9.3
16	20.7	18.0	19.4	22.0	25.0	24.4	5.7	5.5	24.5	52.5	10.0	5.9	16.8	5.8	40	9.0	10.5
17	5.1	2.7	4.3	5.3	4.0	4.2	5.4	1.8	5.6	5.2	4.8	3.5	6.5	3.3	5.41	3.7	4.2
18	9.9	6.3	9.3	11.2	9.5	9.3	10.4	9.2	12.3	14.0	11.1	14.5	10.8	11.6	18.33	8.0	8.8

After preparation of free transverse hand sections of the lamina and stem samples were washed with distilled water and placed in 5% sodium hypochlorite solution for 20 min for clearing and rinsed with distilled water. The sections were stained with methyl blue and carmine and mounted on the slides using Canada balsam. Thin cut sections were observed under a microscope fitted with digital camera. For anatomical studies totally 33 characters were used from which 18 leaf characters were identical and 15 stem ones showed variation (Table 2, 3). Anatomical data were standardized (Mean = 0, Variance = 1) and used for multivariate analyses. Principal coordinate analysis (PCoA) was used for grouping of the species. PCA (Principal Components Analysis) was performed to identify the most variable anatomical characters (Podani 2000). For these analyses, we used PAST (Paleontological Statistical software) version 3 (Hammer et al. 2016).

Results

Anatomy

Details of stem and leaf anatomical characters are given in Figure 1 and Table 4. Cross-section of the stem in all investigated species had multidimensional. Swingeing collenchymatic cells groups, which are 6 (*C. bracteosum*

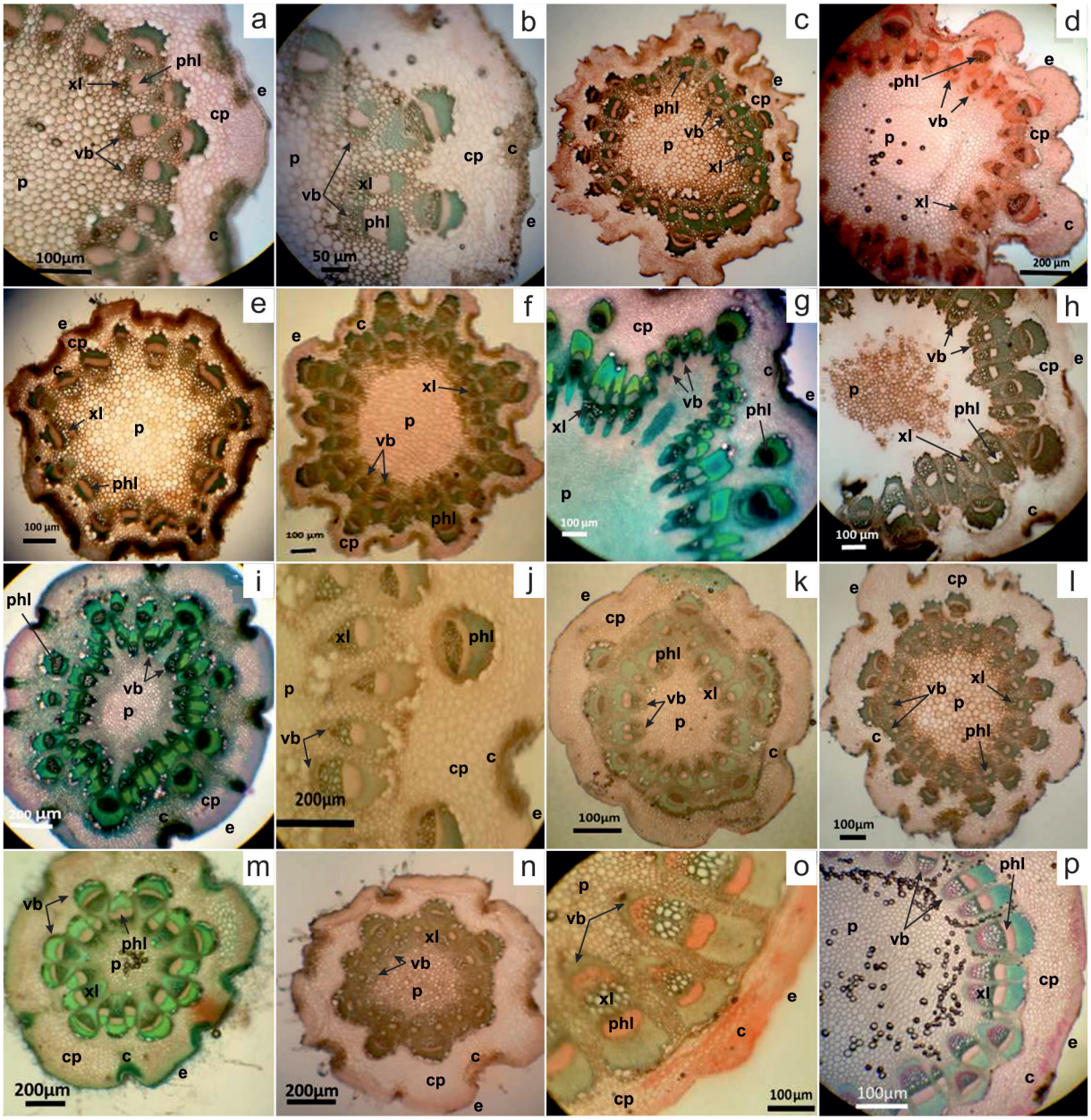

Figure 1. Appearance of stem in *Cirsium* species. a: *C. alatum*; b: *C. elodes*; c: *C. hygrophilum*; d: *C. obvallatum*; e: *C. arvense*; f: *C. echinus*; g: *C. congestum*; h: *C. spectabile*; i: *C. pyramidale*; j: *C. ciliatum*; k: *C. strigosum*; l: *C. vulgare*; m: *C. lappaceum*; n: *C. osseticum*; o: *C. bornmülleri*; p: *C. bracteosum*; e: epidermis; cp: cortex parenchyma; c: collenchymas; phl: phloem; xl: xylem; p: pith.

and *C. strigosum*) to 15 (*C. vulgare*) layered are seen at the protruding sides of the stem.

The highest stem epiderm width was observed in *C. libanticum* (32. 7 µm), while *C. bornmullerii* had the lowest value (3.5 µm). This indicate extensive variation genus. The cortex layer varied from 7 in *C. bornmullerii* to 25 series in *C. pyramidale*. Similarly, the highest value for cortex thickness occurred in *C. lappaceum* (232.1 µm), while *C. arvens* had lowest value of the same (74.1 µm). Details of leaf anatomical characters are given in Figure 2 and Table 5. All of, the leaves in the sections were bifacial (dorsiventral mesophyll) type and are composed of a one or two layered epidermis. The number of vascular of vein varies among different species occurring in either one layer or two to seven layers. The highest value for cortex thickness occurred in *C. bornmülleri* (271 µm), while C. *osseticum* had lowest value of the same (89.1 µm).

PCoA plot of combined anatomical data (Fig. 3) almost

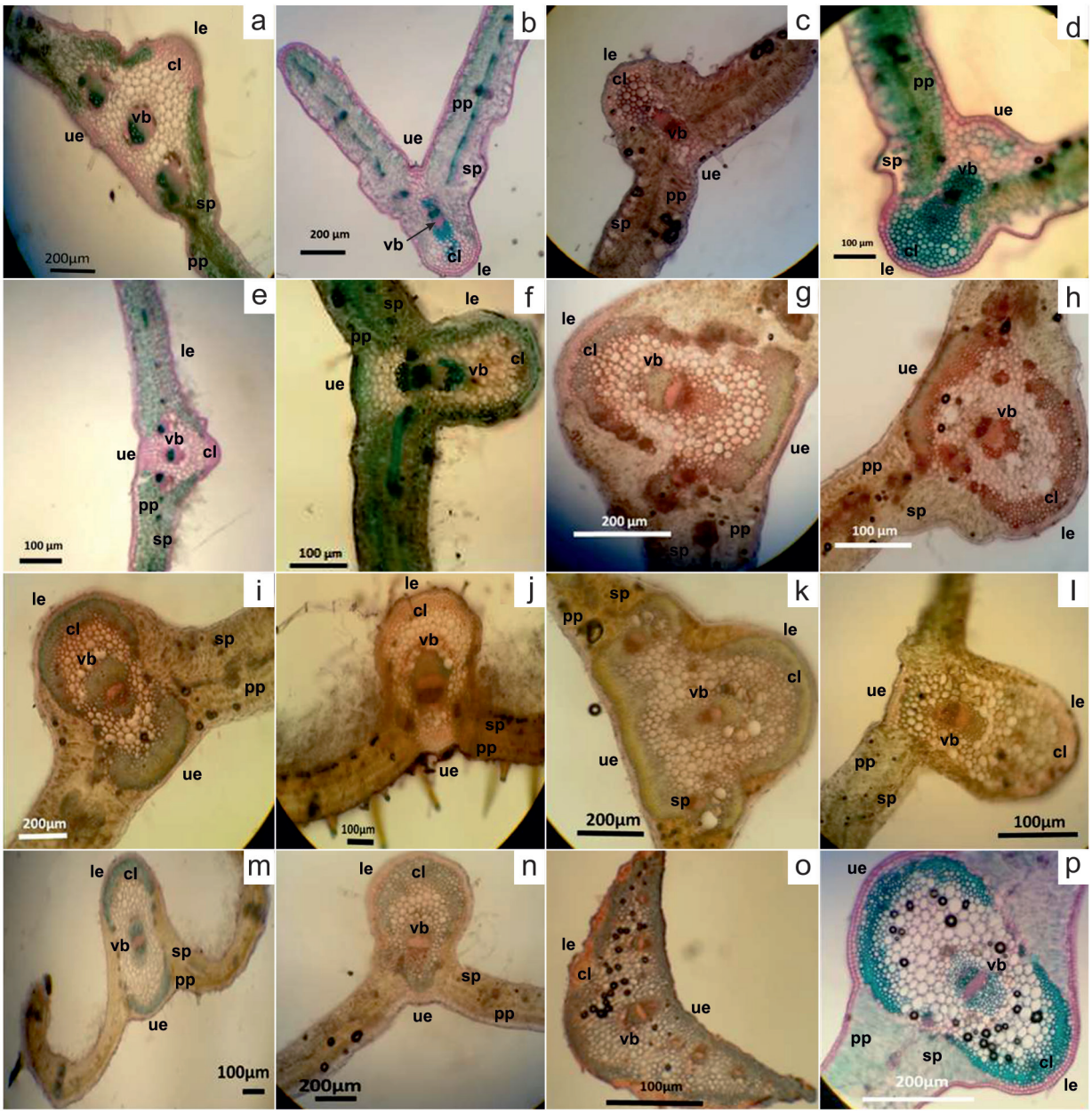

Figure 2. Appearance of stem in *Cirsium* species. a: *C. alatum*; b: *C. elodes*; c: *C. hygrophilum*; d: *C. obvallatum*; e: *C. arvense*; f: *C. echinus*; g: *C. congestum*; h: *C. spectabile*; i: *C. pyramidale*, j: *C. ciliatum*; k: *C. strigosum*; l: *C. vulgare*; m: *C. lappaceum*; n: *C.osseticum*; o: *C. bornmülleri*; p: *C. bracteosum*; le: lower epidermis; cl: collenchyma; vb: vascular bundle; pp: palisade parenchyma; sp: spongy parenchyma; ue upper epidermis.

separated the studied species and indicated the use of anatomical characters in species delimitation.

Considering sectional delimitation of the genus, we find that anatomical data separated the species almost according to their respective sections too. For example, *C. pyramidale*, *C. spectabile*, and *C. congestum* of the sect. *Pseudoepitrachys* Petrak was placed close to each other. Similarly, species of the sect. Epitrachys DC. *viz. C. bracteosum*, *C. bornmülleri*, *C. ciliatum*, *C. osseticum*, and *C. lappaceum* were placed close to each other. Three species studied each from different section *viz. C. echinus* (sect. *Echenais* (Cass.) Petrak), *C. obvallatum* and *C. alatum* (sect. *Cirsium*) and *arvense* (sect. *Cephalonoplos* (Necker) DC. C.) were placed far from the others. PCA analysis revealed that the first two PCA axes, comprised about 85% of total variation and characters like thickness of collenchyma in the lower leaf, thickness of xylem in stem, thickness of inner phloem in stem are the most variable characters

among the studied species. Therefore, these anatomical characters may be used along with morphological characters to delimit *Cirsium* species.

Discussion

The present study revealed taxonomic implication of leaf and stem anatomy in both *Cirsium* species delimitation and sections differentiation. Anatomical characters, like thickness of collenchyma in the lower leaf, thickness of xylem, presence/absence of sclerenchyma, and thickness of inner phloem in stem, may be used along with morphological characters to delimit *Cirsium* species. Ozcan et al. (2015) described the leaf anatomical characters of 26 *Cirsium* taxa of Turkey. These species could be separated based on the midrib and lamina thickness, the height and width of vascular bundle, and number of stomata, and epidermal cell wall patterns in the abaxial and adaxial surfaces. They reported that the species grouping based on leaf anatomy was partly in accordance with their sectional delimitation in the flora of Turkey. In present study also, the species grouping was not in accordance to sectional division.

Ginko et al. (2016) investigated the root and rhizome anatomy implication in taxonomy of 59 species from 34 genera and 12 sub-tribes in Cardueae and Cichorieae They found that anatomical characters used can delineate subtribes Cardueae and Cichorieae. Moreover, combination of morphological, anatomical and molecular data provides efficient data to delimit *Cirsium* species.

References

Bodo Slotta TA, Horvath DP, Foley M (2012) Phylogeny of *Cirsium* spp. in North America: Host specificity does not follow phylogeny. Plants (Basel) 1:61-73.

Bureš P, Wang YF, Horová L, Suda J (2004) Genome size variation in central European species of *Cirsium* (Compositae) and their natural hybrids. Ann Bot 94:353-363.

Francis R (1981) The Wild Flower Key. Frederick Wame & Co, pp. 377-380.

Garcia-Jacas N, Garnatje T, Susanna A, Vilatersana R (2002) Tribal and subtribal delimitation and phylogeny of the Cardueae (Asteraceae): a combined nuclear and chloroplast DNA analysis. Mol Phyl Evol 22:51-64.

Ginko E, Dobeš C, Saukel J (2016) Suitability of root and rhizome anatomy for taxonomic classification and reconstruction of phylogenetic relationships in the tribes Cardueae and Cichorieae (Asteraceae). Sci Pharma 84:585-602.

Ghoreyshi-Alhosseini J, Hosseini J, Amiri-Moghadam D (2004) The systematic study of *Cirsium* species (Asteraceae) in Khorasan of Iran on the base of morphological characters and numerical taxonomy. Iran J Biol 16:60-71.

Hammer Ø, Harper D, Ryan PD (2012) PAST: Paleontological Statistics software package for education and data analysis. *Paleoclimat Res* 4:1-9.

Jump A, Dawson DA, James CM, Woodward FI, Burke T (2002) Isolation of polymorphic microsatellites in the stemless thistle (*Cirsium acaule*) and their utility in other *Cirsium* species. Mol Ecol Notes 2:589-592.

Klinkhamer PGL, De Jong TJ (1993) *Cirsium vulgare* (Savi) Ten.: (*Carduus lanceolatus* L., *Cirsium lanceolatum* L.) Scop., Non Hill). J Ecol 81:177-191.

Kelch DG, Baldwin BG (2003) Phylogeny and ecological radiation of New World thistles (*Cirsium*, Cardueae-Compositae) based on ITS and ETS rDNA sequence data. Mol Ecol 12:141-151.

Lembicz M, Piszczałka P, Grzybowski T, Woźniak M, Jarmołowski A, Borkowska L, Falińska K (2011) Microsatellite identification of ramet genotypes in a clonal plant with phalanx growth: The case of *Cirsium rivulare* (Asteraceae). Flora 206:792-798.

Minaeifar AA, Sheidai M, Attar F, Noormohammadi Z, Ghasemzadeh-Baraki S (2016) Biosystematic study in the genus *Cousinia* Cass. (Asteraceae), section *Cousinia*. Biochem Biochem Syst Ecol 69:252-260.

Nouroozi M, Sheidai M, Seif E, Attar F, Noormohammadi Z (2013) ISSR and RAPD analyses of species and their relationships in the genus *Cirsium* (Asteraceae) in Iran. Phytol Balcan 19:225-232.

Ozcan M, Demiralay M, Kahriman A (2015) Leaf anatomical notes on *Cirsium* Miller (Asteraceae, Carduoideae) from Turkey. Plant Syst Evol 301:1995-2012.

Petrak F (1979) *Cirsium* Miller. In Rechinger KH, Ed., Flora Iranica. Vol. 139a, Compositae III-Cynareae. Akademische Druck-u. Verlagsanstalt, Graz, pp. 231-280.

Podani J (2000) *Introduction to the Exploration of Multivariate Data*. Backhuyes, Leiden, pp. 407.

Rechinger KH (1979) Cousinia. In Rechinger KH, Ed., Flora Iranica, No. 139A. Akademische Druk-u. Verlagsanstalt, Graz, Austria. pp. 108-153.

Seif E, Sheidai M, Norouzi M, Noormohammadi Z (2012) Biosystematic studies of *Cirsium arvense* populations in Iran. Phytol Balcan 18:305-314.

Sheidai M, Seif E, Nouroozi M, Noormohammadi Z (2012) Cytogenetic and molecular diversity of *Cirsium arvense* (Asteraceae) populations in Iran. *J Japan Bot* 87:193-205.

Sheidai M, Zanganeh S, Haji-Ramezanali R, Nouroozi M, Noormohammadi Z, Ghsemzadeh-Baraki S (2013) Genetic diversity and population structure in four *Cirsium* (Asteraceae) species. *Biologia* 68:384-397.

Sheidai M, Naji M, Noormohammadi Z, Nouroozi M, Ghasemzadeh-Baraki S (2016) Contemporary inter-

specific hybridization between *Cirsium aduncum* and *C. haussknechtii* (Asteraceae): Evidence from molecular and morphological data. Genetika 48:497-514.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2013) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731-2739.

Wiens JJ (2014) Character analysis in morphological phylogenetics: Problems and solutions. Sys Biol 50:689-699.

Zwölfer H (1988) Evolutionary and ecological relationships of the insect fauna of thistles. Ann Rev Entomol 33:103-122.