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MORPHOLOGICAL AND ANATOMICAL FEATURES OF CYPSELA OF SOME *CREPIS* TAXA (ASTERACEAE) FROM TURKEY AND THEİR TAXONOMIC IMPORTANCE

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Abstract

Fruit morphology and anatomy have taxonomic importance in Asteraceae. The fruits structures of *Crepis* from Turkey, which include five species (C. *alpina*, *C. smyrnaea*, *C. pulchra*, *C. zacintha, C. sancta*) and two subspecies (*C. foetida* subsp. *foetida* and *C. foetida* subsp. *rhoeadifolia*) were studied for fruit morphological and anatomical characters with one-way analysis of variance, cluster analysis and principal component analysis. Fruit size, shape, color, and the presence of beak were observed with stereomicroscopy. Whereas the surface patterns of fruit and pappus were examined using scanning electron microscopy (SEM). Furthermore, pericarp structure, thicknesses of testa and endosperm, and number of rib, cotyledon width in cypsela were studied anatomically. Results indicated that cypsela sizes, the presence or absence of beak on the cypsela, fruit and pappus surfaces, pericarp. Thickness of testa and endosperm, and number of ribs are of major importance to illustrate interspecific relations among the examined taxa. Also, this investigation is a preliminary study, which was performed to use fruit morphological and anatomical characters for their practicality on the classification of taxa within the genus.

Key words: Cichorieae, *Crepis*, Cypsela.

Introduction

The Genus *Crepis* L. (Asteraceae, Cichorieae) has more than 200 species which are distributed over a wide area in all continents, excluding Australia (Lack, 2007). The centre of origin of the Genus is presumably recognized as Pamir/Altai area in central Asia (Babcock, 1947; Yildirim *et al.,* 2011). The *Crepis* has about 47 species in Turkey and 8 of them are endemic to Turkey (Guner *et al.,* 2012). However, there is no updating of the monograph of the Genus; it is difficult to comment on the number of taxa.

Molecular studies show that there are some genera included within *Crepis* (Enke & Gemeinholzer, 2008). Morphological reevaluation of the genus and definition of distinctive characters are needed to obtain a revised classification (Enke, 2008). This statement has been verified by different researches such as Sennikov & Illarionova (2007), Torres & Galetto (2007), and suggested that new useful characters are needed for determination of the natural limits of the genus.

The morphological and anatomical observations on the fruit provide suitable characters in the classification and phylogeny of the taxa in Asteraceae (Kallersjo, 1985; Funk *et al.,* 2009; Abid & Qaiser, 2009; Abid & Ali, 2010; Lo Presti *et al.,* 2010; Inceer *et al.,* 2010). Morphological characters of the cypsela such as size, beak presence or absence, number of the rib, surface structure (smooth or striate) and branching of the pappus were taken into account for diagnosis of *Crepis* species (Babcock, 1947; Lamond, 1984). Nevertheless, there are solely few taxonomic investigations on the cypsela structure of *Crepis* and they are restricted to few characters. The purpose of this study was to perform a preliminary study to examine possible morphological (macro and micro) and anatomical characteristics of some *Crepis* taxa such as *C. foetida* L. subsp. *foetida*, *C. foetida* L. subsp. *rhoeadifolia* (Bieb.) Čelak., *C. alpina* L., *C. smyrnaea* DC. ex Froehlich, *C. pulchra* L., *C. zacintha* (L.) Babcock and *C. sancta* (L.) Babcock for their utility in the classification within genus by utilizing multivariate analyses such as one-way analysis of variance, cluster analysis and principal component analysis.

Material and Methods

The dry cypselae of 7 taxa of *Crepis* were used for the morphological and anatomical studies. The samples were collected from natural populations and herbarium specimens, the ripe cypsela of each taxon were taken from the center of the 10-15 capitulas and their origins were presented in Table 1. Voucher specimens were taken from ISTF (Herbarium Facultatis Scientiarum Universitatis Istanbulensis).

Cypsela surface: Macromorphological characteristics of the cypselae (including size, shape, color, and the presence of beak were studied by Olympus SZX7 stereomicroscope and Kameram Imaging Software (Fig. 1). The micromorphological characters of the cypselae (including rib surface, cell type and frequency on the outer surface, diameter of pappus and number of protrusions in 100 µm on the pappus bristle of the fruits) were observed. The samples were prepared for electron microscopy by mounting to stub with silver adhesive, plated with gold and examined with JEOL Neoscope-5000 scanning electron microscope.

Anatomy: The cross sections from the middle of the cypsela were done with a full automatic microtome (Thermo Shonda Met Finesse). The materials were placed in FAA for a minimum of 24 h, dehydrated through an ethanol and xylene series, and dyed with haematoxylin (harris-RRSP67-E) in a dyeing apparatus (ASC 720 Medite), and were coated by Entellan to observe anatomical structures (Algan, 1981; Inceer *et al.,* 2010; Karaismailoglu, 2015). The anatomical characters (including rib number and size, pericarp structure, testa and endosperm thicknesses, and cotyledon width) were observed and photographed using Olympus CX21FS1 microscope and Kameram Imaging Software.

Table 1. Voucher details of *Crepis* taxa.

Number	Voucher	Taxa	Origin
C1	ISTF41008	*Crepis foetida* L. subsp. *foetida*	Trabzon
C2	ISTF32819	*C. foetida* L. subsp. *rhoeadifolia* (Bieb.) Čelak.	Usak
C3	ISTF1314	*C. alpina* L.	Ankara
C4	ISTF4185	*C. smyrnaea* DC. ex Froehlich	Bursa
C5	ISTF3218	*C. pulchra* L.	Istanbul
C6	ISTF33548	*C. zacintha* (L.) Babcock	Balikesir
C7	ISTF34101	*C. sancta* (L.) Babcock	Kütahya

Clustering analysis: Data analysis was carried out with SPSS computer program and Duncan's multiple-range test was used to identify the statistical importance of distinctions among the data (Table 2). Grouping of taxa was performed using the clustering analysis method (UPGMA) (Fig. 3). Besides, coordination and similarity matrix based upon principal component analysis (PCA) were performed (Fig. 4 and Table 3).

Results and Discussion

A detailed morphological (macro and micro) study performed on the cypselae of the examined taxa showed a great variety of characters (Table 2). Shape, colour and size characters of taxa were evaluated macromorphologically. Cypselae are fusiform (C1, C2 and C4) or straight (C3, C5, C6 and C7) in shape, some of them are slightly curved (C4 and C5), and coloured in brown and tones (C1, C2, C3 and C7), reddish-brown (C4), straw or greenish (C5) and yellowish (C6). Also, cypsela are strongly beaked (C1, C2, C3 and C7), or unbeaked (C4, C5 and C6), and their surfaces are straight (C5 and C6) or striped. Besides, as can be seen in Table 2, there are also differences in terms of cypsela dimensions which vary from 17.3 to 2.85 mm in length, and from 0.71 to 0.41 mm in width. Especially, C3 and C6 have greater variability among the examined species.

Cypsela morphology in the examined *Crepis* taxa vary mostly in colour, size, and presence or absence of beak. Similar results have also been reported in earlier studies (Lamond, 1984; Pak, 1993; Enke, 2008). However, shape of cypselae is determined to be less important in delimiting the examined taxa of *Crepis*. At the same time, the determined variations in the cypselae conform to the diagnostic characters in Flora of Turkey (Davis, 1984) for the *Crepis* species.

Cypselae surface of the studied taxa was evaluated micromorphologically, and considerable diversity was observed considerably in terms of surface structures such as rib surface and cell shape on the outer surface of the cypsela (Fig. 1 and Table 2). Accordingly, rib surfaces among the examined taxa are generally prominent; however, C5 and C6 have a smooth surface. In addition, cell shapes on the cypsela surfaces are highly variable and densely composed of dentate tipped cells (C1, C2 and C3), blunt tipped cells (C4) and spiky tipped cells (C5 and C6). At the same time, the dentate cells on the beak in C1, C2 and C3 are rarely arranged in a line. The surface of C7 also consists of a rough, grooved structure. Besides, there are sparse flat hairs on the surface of C4 in contrast to other taxa.

Fruit or seed surface structure have been variously used for solving systematic problems, interpretation of evolutionary interactions, and illumination of the adaptive features of the fruit surface (Heywood, 1971; Sulaiman, 1995; Pinar *et al.,* 2007; Abid & Qaiser, 2009). Furthermore, Torres & Galletto (2007) opined that micromorphological characters could be used in systematics at various levels in the Cichorieae. In the literature survey, no studies were conducted about micromorphological structures of the cypsela of *Crepis*. Cypselae surface in almost all of the examined taxa are found to be different. Their surfaces are composed of dentate, blunt and spiky cell tips. Taxa having dentate cell type are separated from each other with the cell arrangement on the beak. Also, C7 has a rough, grooved surface and there is no clear cell shape on surface. These variations in the cypsela surfaces of the studied taxa show that micromorphology of the achene surface of *Crepis* genus has a fairly high taxonomic value and a detailed study covering all of the taxa within the genus should be carried out to determine its phylogenetic role in the genus. Additionally, the unique morphology of the studied microcharacters mirrored a correlation between a molecular study (Enke & Gemeinholzer 2008) and the morphological characters used in classification at the present time.

Pappus bristles, their diameter and protrusion number per 100 µm of the examined taxa are demonstrated in Fig. 1 and in Table 2. Pappus of the taxa is usually very fine, soft and persistent. The diameter of the pappus bristles are different, it varies from 26.43 µm to 10.04 µm. While C1 has the widest bristle diameter, C7 has the narrowest. The protrusion number per 100 µm on the bristle has shown variations among the examined taxa. It ranges between 2 and 12. While C1 has the most intense protrusion, C5 is the sparsest. In addition, the examined pappus surfaces are generally smooth, though C4 and C5 have a rough pappus surface.

Kilian *et al.* (2008) and Enke (2008) stated that the pappus usually provide a significant character to differentiate groups on all of the taxonomic levels in Cichorieae. This study has offered pappus structure as probable delimiting character at the infrageneric level. Accordingly, both pappus bristle diameter and protrusion number per 100 µm on it have shown large variations in the studied taxa. These characters in spite of some exceptions such as there is no difference between C2 and C4 in the number of protrusions on pappus, support the use of the present morphological characters in identification (Davis, 1984), and can be used as a supportive character in separating the taxa from each other within the genus.

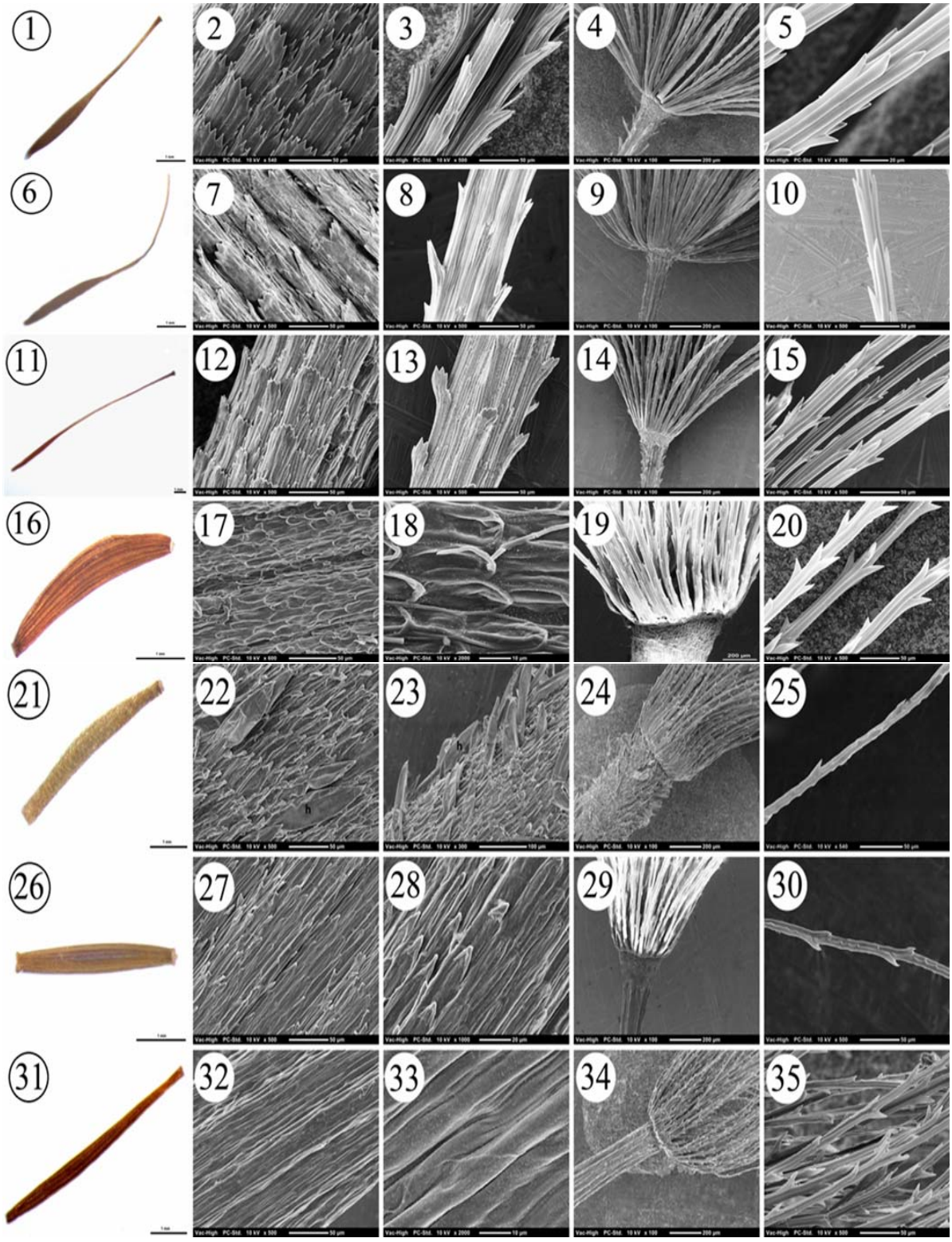

Fig. 1. Macro and micro morphological features of the cypsela *Crepis* taxa (for taxa abbreviations see Table 1); C1: **1-** overall appearance, **2-** cypsela surface, **3-** cell arrangement on the beak, **4 and 5-** pappus and bristle; C2: **6-** overall appearance, **7-** cypsela surface, **8-** cell arrangement on the beak, **9 and 10-** pappus and bristle; C3: **11-** overall appearance, **12-** cypsela surface, **13-** cell arrangement on the beak, **14 and 15-** pappus and bristles; C4: **16-** overall appearance, **17 and 18-** cypsela surfaces, **19 and 20-** pappus and bristles; C5: **21-** overall appearance, **22 and 23-** cypsela surfaces (h: flat hairs), **24 and 25-** pappus and bristle; C6: **26-** overall appearance, **27 and 28-** cypsela surfaces, **29 and 30-** pappus and bristle; C6: **31-** overall appearance, **32 and 33-** cypsela surfaces, **34 and 35-** pappus and bristles.

Table 2. Morphological and anatomical features of cypselae of the *Crepis* taxa.

Taxa	Colour	Shape	Cypsela size*		Cell types on the fruit surface	Diameter of pappus* (µm)	Number of protrusion* (in 100 µm)	Number of rib	Rib size*		Pericarp thickness* (µm)	Testa thickness* (µm)	Endosperm thickness* (µm)	Cotyledon width* (µm)	Examined cypselae number
			L (mm)	W (mm)					L (µm)	W (µm)					
C1	Brown	Fusiform	6.32 ± 0.2c	0.45 ± 0.2ab	Dentate	26.43 ± 2.1a	12 ± 2a	18	34.43 ± 4.1cd	52.17 ± 4.3b	38.31 ± 2.4g	4.46 ± 0.8bd	24.24 ± 2.3b	106.64 ± 8.4c	50
C2	Pale brown	Fusiform	7.46 ± 0.4b	0.41 ± 0.2bd	Dentate	15.21 ± 2.4c	8 ± 2ab	18	34.71 ± 8.3cd	52.41 ± 6.1b	46.42 ± 4.2ef	6.51 ± 0.4b	21.36 ± 2.8bc	146.19 ± 4.5a	50
C3	Pale brown	Straight	17.13 ± 0.3a	0.44 ± 0.2bc	Dentate	25.24 ± 2.1ab	6 ± 2bc	15-18	33.65 ± 6.2cd	38.34 ± 4.1cd	48.21 ± 3.6ee	5.03 ± 0.6bc	32.31 ± 3.4a	128.11 ± 9.7b	50
C4	Reddish-brown	Fusiform	4.15 ± 0.1f	0.71 ± 0.1a	Blunt	14.11 ± 1.8cd	8 ± 2ab	12-14	42.63 ± 4.2c	46.46 ± 9.8bc	53.48 ± 4.7bc	8.11 ± 0.4a	18.54 ± 2.1bd	112.63 ± 6.5bc	50
C5	Straw or greenish	Straight	5.37 ± 0.2d	0.52 ± 0.1ab	Spiky	10.23 ± 1.4ef	2 ± 1de	14-18	52.32 ± 3.4a	61.16 ± 2.5a	71.83 ± 8.4a	6.37 ± 0.8b	8.91 ± 2.4e	85.93 ± 9.4d	50
C6	Yellowish	Straight	2.85 ± 0.3g	0.43 ± 0.1b	Spiky	12.32 ± 1.3de	4 ± 1cd	10-18	48.48 ± 4.2ac	42.21 ± 4.6bc	51.36 ± 4.1bd	6.11±1.1b	23.36 ± 3.4b	84.46 ± 8.3de	50
C7	Brown	Straight	5.01± 0.2de	0.42 ± 0.1b	-	10.04 ± 2.2ef	7 ± 2bc	10-12	51.93 ± 3.2ab	54.11 ± 4.1b	58.34 ± 3.6b	5.08 ± 0.4bc	16.47 ± 2.7cd	148.06 ± 8.7a	50

* Mean value ± Standard deviation: differences determined by Duncan's multiple-range test; means with dissimilar letters are important at P=0.05 level; L: Length, W: Width.

The results of anatomical studies are presented in Table 2 and Fig. 2. Accordingly, the anatomical characters of the pericarp are different in the examined taxa. The ribs on the pericarp among taxa have significantly different appearances; their number, thickness and width are of taxonomic value for the distinction among taxa. They are usually of rounded exterior surface with 10–20 rib formed of sclerenchymatous bunches (C5 is 20 ribbed; while C6 and C7 have 10 rib). Ribs are substantially formed of a few layers of thin or thick-walled parenchymatic cells and 3-6 layers of sclerenchyma, and their sizes vary from 52.32–33.65 µm to 61.16–38.34 µm. In addition, pericarp thickness in all of the studied taxa varies among the taxa even in sub-species level; in fact C1 and C2 can even be easily separated from each other by this character. At the same time, C5 has the widest pericarp; whereas, C1 has the narrowest pericarp. The exocarp layer of the pericarp has one layer with thin (C1) or thick (others) cell wall. Also, endocarp has two layers and it is usually collapsed in examined taxa. Furthermore, they contain more thrived palisade sclerenchyma between the ribs (developmental connection) in C5 and C7, on the other hand others don't have sclerenchymatous cells, and their interribs are collapsed. Also, some of them have very obvious and clearly tipped ribs (C1, C2 and C4) (Fig. 2). According to these findings, there is a potential developmental connection among the tested cypsela to phylogeny and it may supply other opinions. These results are useful to examine infrageneric or intergeneric correlations and compatible with previous studies, including cypsela anatomy, in the Asteraceae (Pak, 1993; Zhu *et al.,* 2006; Sennikov & Illarionova, 2008; Enke, 2008; Inceer *et al.,* 2010). According to the results obtained from this investigation, pericarp features such as rib number on the pericarp, its size and pericarp thickness can be utilized as supplemental or additional characters to support the current identification.

The testa epidermis in the examined taxa is sclerenchymatic type. Epidermis cells consist of U-shaped waved or straight and thick or thin-walled cells in the cross-sections (Fig. 2). In addition, the mean values of the testa thickness vary from 8.11 µm to 4.46 µm. The widest testa is noted in C4, whereas narrowest is in C1. Accordingly, testa covers the least location in cypsela based on other examined anatomical features. Tegel (2002) stated that testa epidermis cells, were reliable characters owing to lower pressure exposure when taken the cross section of cypsela, have a high taxonomic significance at the generic and subgeneric levels. However, variation among the examined taxa is very low in terms of testa epidermis structure. Also, endosperm cells in the examined taxa are usually two-layered and are composed of elongated rectangular cells. Endosperm thickness in examined taxa vary from 32.31 µm to 8.91 µm; the widest endosperm is noted in C3, whereas it is the narrowest in C5 (Table 2). According to these findings, testa and endosperm thicknesses can slightly be a helpful taxonomic character in separation of the *Crepis* taxa. Obtained results are in agreement with previous studies such as Bruhl & Quinn (1990) and Enke (2008).

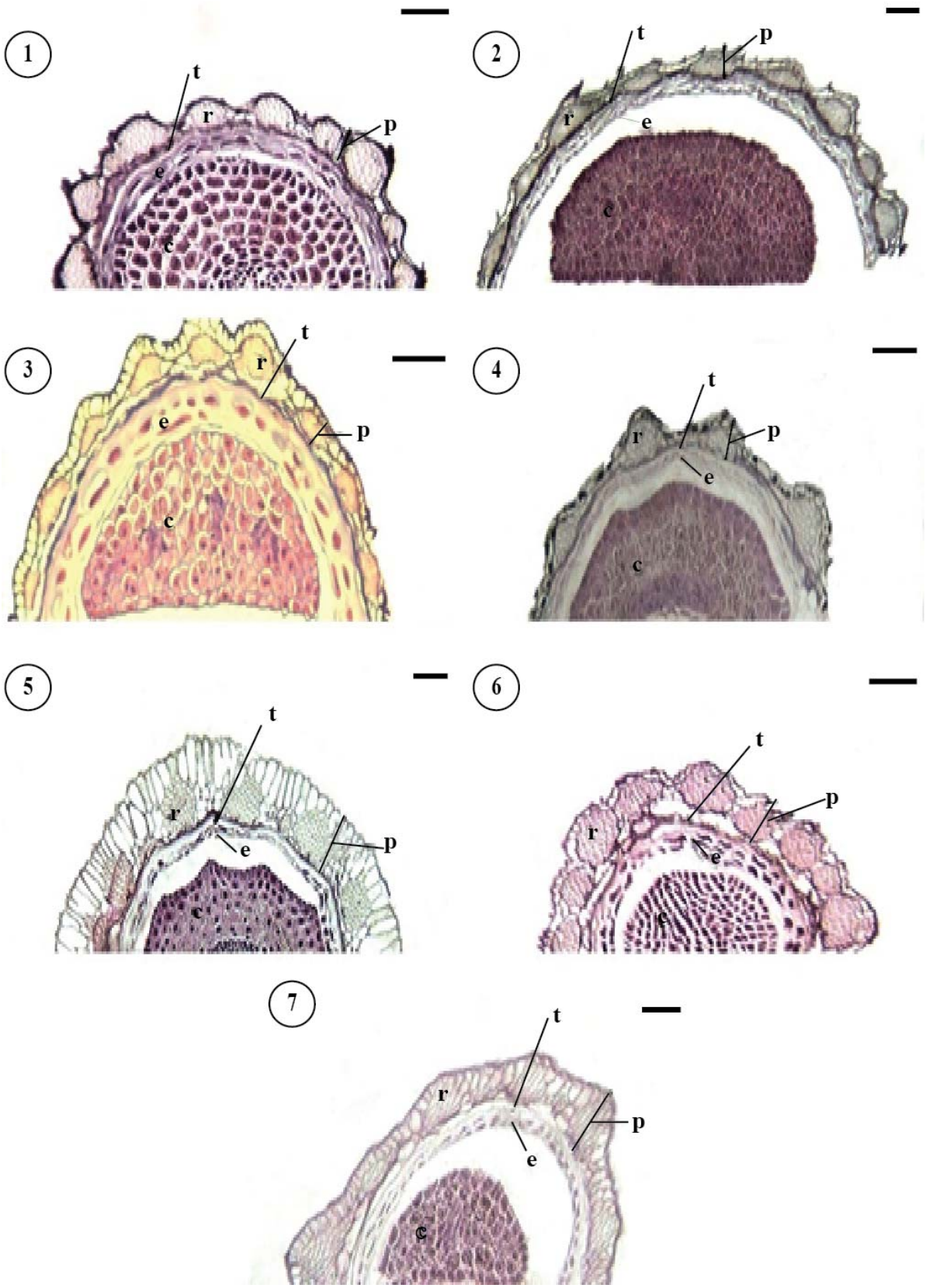

Fig. 2. Transverse sections of the cypselae in the *Crepis* taxa (for taxa abbreviations see Table 1): 1; C1, 2; C2, 3; C3, 4; C4, 5;C5, 6; C6, 7;C7. r: rib, p: pericarp, t: testa, e: endosperm, c: cotyledon, scale bars: 50 µm.

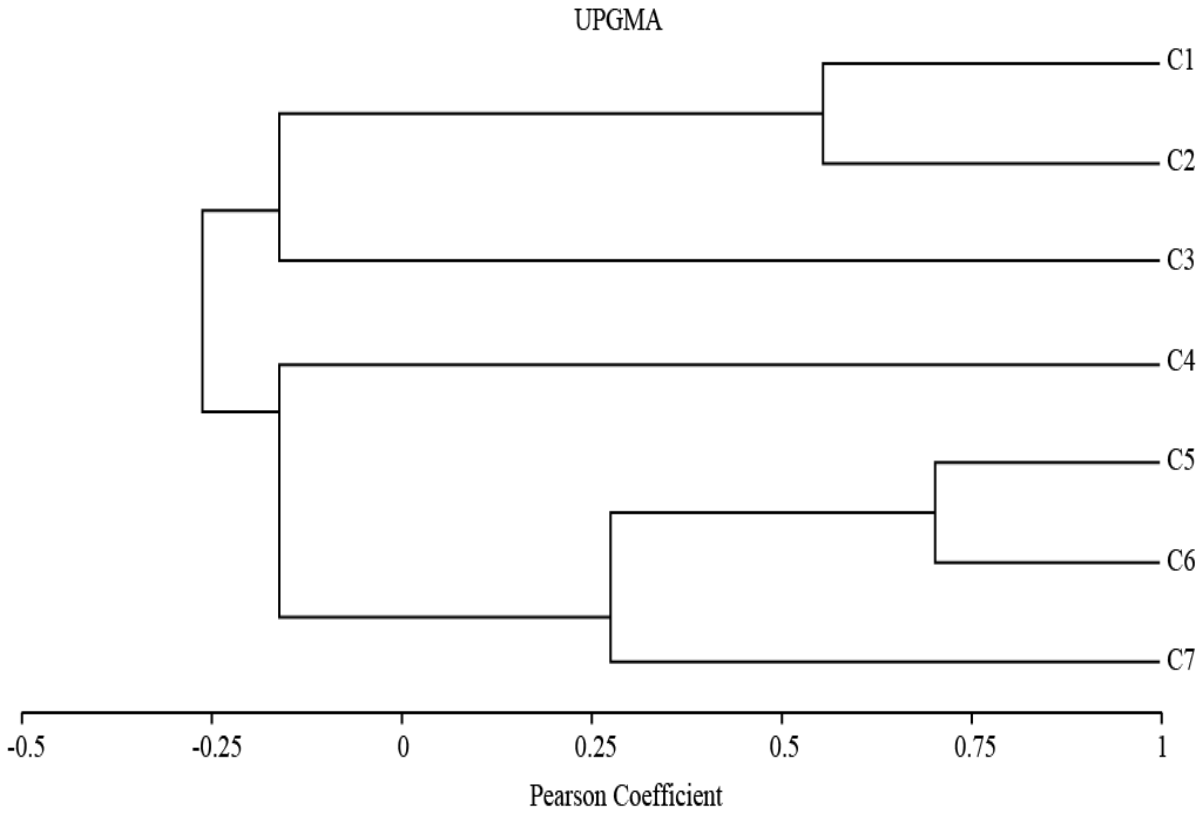

Fig. 3. UPGMA clustering of the *Crepis* taxa based on achene morphological and anatomical characters (for taxa abbreviations see Table 1).

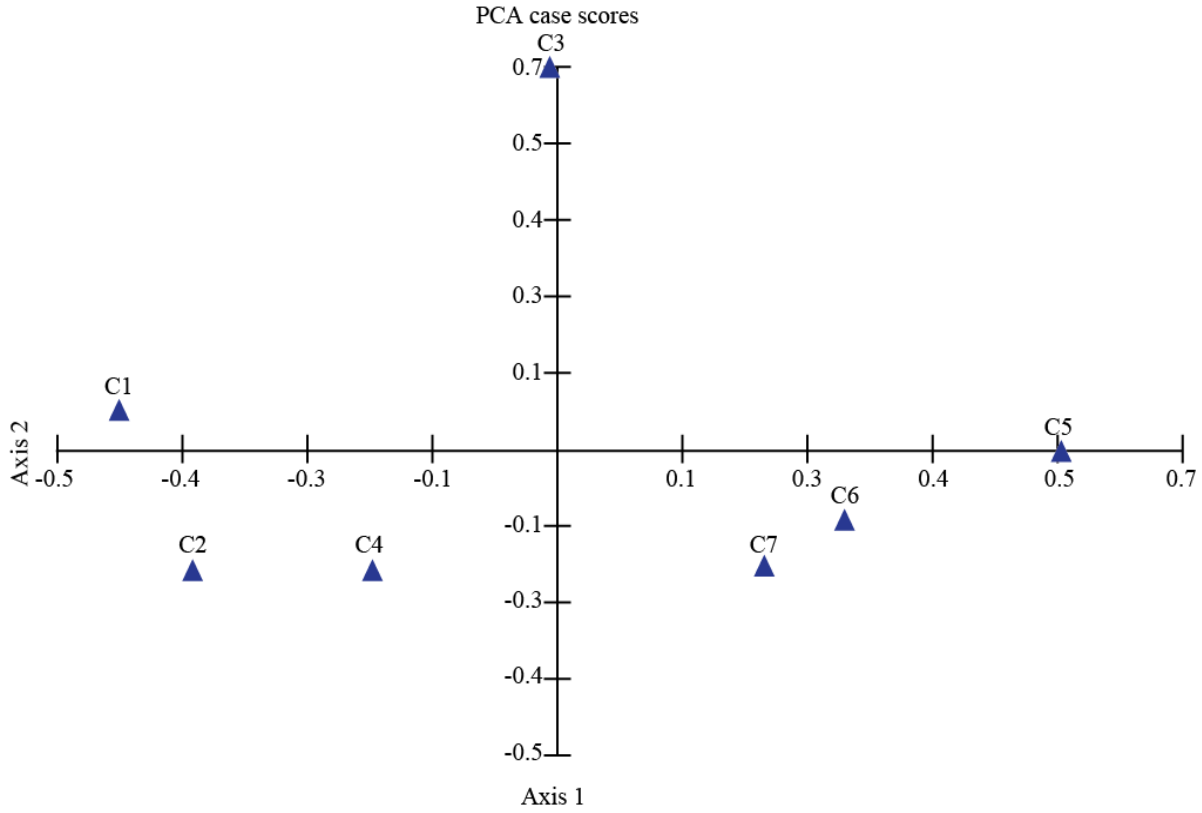

Fig. 4. Principal component analysis of the *Crepis* taxa (for taxa abbreviations see Table 1).

Table 3. Similarity matrix among the examined taxa.

Taxa	C1	C2	C3	C4	C5	C6	C7
C1	1	-	-	-	-	-	-
C2	0,557	1	-	-	-	-	-
C3	0,083	-0,161	1	-	-	-	-
C4	0,021	0,132	-0,161	1	-	-	-
C5	-0,263	-0,161	0,026	-0,161	1	-	-
C6	-0,046	0,089	-0,014	0,089	0,704	1	-
C7	-0,185	0,164	-0,121	-0,031	0,445	0,275	1

One of the other studied anatomical features are cotyledons (Table 2). The outline of the cotyledons has substantially a similar appearance and consists of round or oval cells, except for C4 and C5 which are of the angular. Besides, there are differences in terms of the width of the cotyledons among the studied taxa. C6 and C7 had greater variability. Their values range from 148.06 (C7) to 84.46 µm (C6). This character is usually not enough to distinguish between taxa, but it can be used as supporting other characters in some cases.

The unweighed pair group method with arithmetic mean (UPGMA) dissimilarity clustering dendrogram for the examined taxa is presented in Fig. 3. Accordingly, branches in dendrogram are divided into two main groups and subsets. C1, C2 and C3 form the first main group; other taxa compose the second main group. As could be seen from dendrogram, C3, C4 and C7 also differ markedly from other taxa based on the studied characters (Table 2). Generally, the branches included closely correlated taxa such as C1-C2 and C5-C6 conform to the traditional taxonomic rank of *Crepis* species in Turkey. Namely, the morphological and anatomical characteristics of the cypselae are compatible with the used characters in the infrageneric dispersion of the *Crepis* taxa in Flora of Turkey (Davis, 1984).

Principal component analysis (PCA) ordination and similarity matrix according to morphological and anatomical characteristics of cypselae are presented in Table 3 and in Fig. 4. Among the examined taxa, the closest and the most distant taxa are identified. This clearly demonstrates that C5 and C6 are the most nearly correlated taxa (similarity percentage: 0.704), while C1 and C5 are the most distant taxa (similarity percentage: -0.263) (Table 3 and Fig. 4). According to organized PCA axes in Fig. 4, obtained results indicated that the cumulative variance value of principal components achieved 61.22% (Axis 1: 34.79%, Axis 2: 26.43%). These ratios attribute that the examined characters in *Crepis* taxa can be useful in explanation of the phylogenetic correlations, and in clarifying taxonomic conflicts among the taxa on the account of variable values.

Conclusion

In this investigation, the practicality of the evaluated characters in the infrageneric delimitation in *Crepis* Genus has been questioned. The implementation of the some cypsela characteristics, which are mostly concerned with cypsela micromorphology and pappus structures, could useful and supportive in the infra generic classification. This is a preliminary study to determine the applicability of the tested characters, and further researches covering all taxa of the genus are needed for determining the entire variations and better understanding systematic of genus.

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