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Morphoanatomical study of *Matricaria* L. (Asteraceae) in Turkey

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ABSTRACT: In the Turkish flora, the genus *Matricaria* is present with four taxa, namely *M. aurea*, *M. chamomilla* var. *chamomilla*, *M. chamomilla* var. *recutita* and *M. matricarioides*. This study presents an evaluation of selected diagnostic characters and anatomical traits of the achene (cypsela) of *Matricaria* in Turkey using univariate analysis (one-way analysis of variance) and multivariate analysis (cluster analysis, principal component analysis) to obtain new information. Three groups are found within the genus *Matricaria* based on morphoanatomical characteristics. The colour of disc florets, that of ribs on the achenes, the presence or absence of a slime envelope and pericarp thickness are useful for delimitation of *Matricaria* taxa, and a key to taxa based on these characters together with other diagnostic traits is provided.

Keywords: *Matricaria*, morphometry, achene anatomy, Turkey, taxonomy

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INTRODUCTION

Matricaria L. is a small genus of the tribe Anthemideae, with six species mostly distributed in Europe, North Africa, Macaronesia, Asia (western, southwest and central) and western North America (Oberprieler *et al.* 2007). It was revised for the *Flora of Turkey and the East Aegean Islands* (Grierson 1975), with five taxa recognised as *M. aurea* (Loefl.) Sch. Bip., *M. chamomilla* L. var. *chamomilla*, *M. chamomilla* var. *recutita* (L.) Fiori, *M. chamomilla* var. *pappulosa* Margot & Reuter and *M. macrotis* Rech. f. Later, *M. matricarioides* (Less.) Porter ex Britton was added to the *Flora of Turkey* (Davis *et al.* 1988). However, Oberprieler & Vogt (2006) made a new combination for *M. macrotis*, transferring it to the genus *Anthemis* L. as *A. macrotis* (Rech.f.) Oberprieler & Vogt. This classification was followed by Inceer (2012). On the other hand, *M. chamomilla* var. *pappulosa* was reduced to the status of a synonym of *M. chamomilla* var. *coronata* J. Gay ex Boiss. (Applequist 2002), and this taxon was noted as a doubtful record for the Turkish flora (Inceer 2012). Additionally, a recent molecular study based on ITS and ETS sequence data confirmed the systematic position of these taxa (Inceer *et al.* 2018).

The taxonomy of *Matricaria* is controversial and confused. Depending on the authors, several species are classified in *Matricaria* or *Tripleurospermum* Sch. Bip. (Inceer 2011). As a result, they have been confused with each other, both taxonomically and nomenclaturally (Applequist 2002). Additionally, the systematic position of *Matricaria* taxa has been determined differently by a number of authors. In particular, the opinions of various authors on *M. chamomilla* and *M. recutita* L. are very different. Some authors recognised a single species as *M. recutita* (= *M. chamomilla*) (Meikle 1985; Podlech 1986; Bremer & Humphries 1993; Pobedimova 2000) or *M. chamomilla* (= *M. recutita*) (Greuter & von Raab-Straube 2008), while others preferred *M. chamomilla* with two varieties as var. *recutita* (Grierson 1974, 1975; Applequist 2002, and synonyms therein) and var. *pappulosa* (Grierson 1974, 1975, and synonyms therein) or var. *coronata* (Applequist 2002, and synonyms therein). Taxonomic delimitation of these taxa is difficult because of overlap in diagnostic characters such as shape and size of the achenes. Documented delimitation of individual taxa supported by a specific combination of diagnostic traits is therefore necessary for correct identification, but no comprehensive mor-

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phometric analysis based on diagnostic characters has yet been carried out on these taxa.

Morphological and anatomical features of the achene (cypsela) play an important role in systematics and phylogeny of the tribe Anthemideae of the family Asteraceae (Kynčlova 1970; Källersjö 1985; Bruhl & Quinn 1990; Oberprieler *et al.* 2007; Funk *et al.* 2009; Lo Presti *et al.* 2010; Inceer *et al.* 2012). Morphological features of the achene such as size, shape, number of ribs and slime envelope formation were previously considered useful for delimitation of *Matricaria* taxa (Kynčlova 1970; Grierson 1974; Lo Presti *et al.* 2010). On the other hand, peripheral and central achenes are morphologically different from each other in some species. Failure of some authors to distinguish achenes formed from ray and disc florets appears to have been the cause of confilicting data for *Matricaria* (Kynčlova 1970). However, little is known about anatomy of the achenes of *Matricaria*, and it has not been described in detail to date. The aim of the present study was to investigate variation of selected diagnostic morphological characters and anatomy of the achenes of *Matricaria* in Turkey in order to obtain new information. Detailed anatomical features of the achenes in the studied taxa as well as the data of univariate and multivariate analyses are presented here for the first time.

MATERIALS AND METHODS

Plant material. The plant materials examined were collected from natural populations in Turkey (Table 1). Vouchers were deposited in the herbarium of Karadeniz Technical University, Department of Biology (KTUB) or H. Inceer collections. Names of the investigated taxa, localities according to the grid system adopted for the *Flora of Turkey* by Davis (1975), accession numbers and collection information are given in Table 1. The nomenclature adopted by Grierson (1974, 1975) and Applequist (2002) was followed.

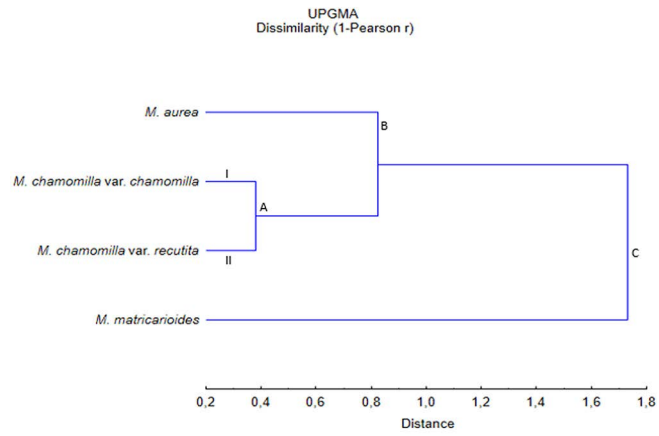


Fig. 1. UPGMA clustering of *Matricaria* taxa on the basis of morphoanatomical characters.

Morphometry. The diagnostic morphological characters obtained from five specimens for each taxon were used for morphometric analyses. The selection of characters was made so as to include particularly those commonly used in the relevant literature (Grierson 1974, 1975; Pobedimova 2000). The studied characters are listed in Table 2.

Achene anatomy. Five achenes obtained from five specimens for each taxon were embedded in paraffin (melting points 62-66°C) for microtoming. Sections were cut to a thickness of 10-15 µm with a microtome. Cross sections were stained in hematoxylin and then mounted in Entellan (Inceer *et al.* 2012). Drawings were made with a camera lucida.

Statistical analyses. To evaluate diagnostic characters (Table 2), univariate and multivariate analyses were performed using Statistica (version 12). For all characters

Table 1. Origins of materials studied.

Taxon	Locality	Voucher
M. aurea (Loefl.) Sch. Bip.	C6 Gaziantep/Şanlıurfa: Between Nizip and Birecik, Dutlu, roadsides, near cultivated area, 440 m a.s.l., 08.V. 2007.	Inceer 322
M. chamomilla L. var. *chamomilla*	C1 Muğla: Marmaris, between Marmaris and Köyceyiz, roadsides, 20 m a.s.l., 18. IV. 2007.	Inceer 305
M. chamomilla L. var. *recutita* (L.) Fiori	C1 Muğla: Marmaris, between Kizilkaya and Fethiye, roadsides, 24 m a.s.l., 18. IV. 2007.	Inceer 307
M. matricarioides (Less.) Porter ex Britton	A9 Kars (Ardahan): Between Ardahan and Göle, roadsides, 1800 m a.s.l., 18.VII.2007.	Inceer 420

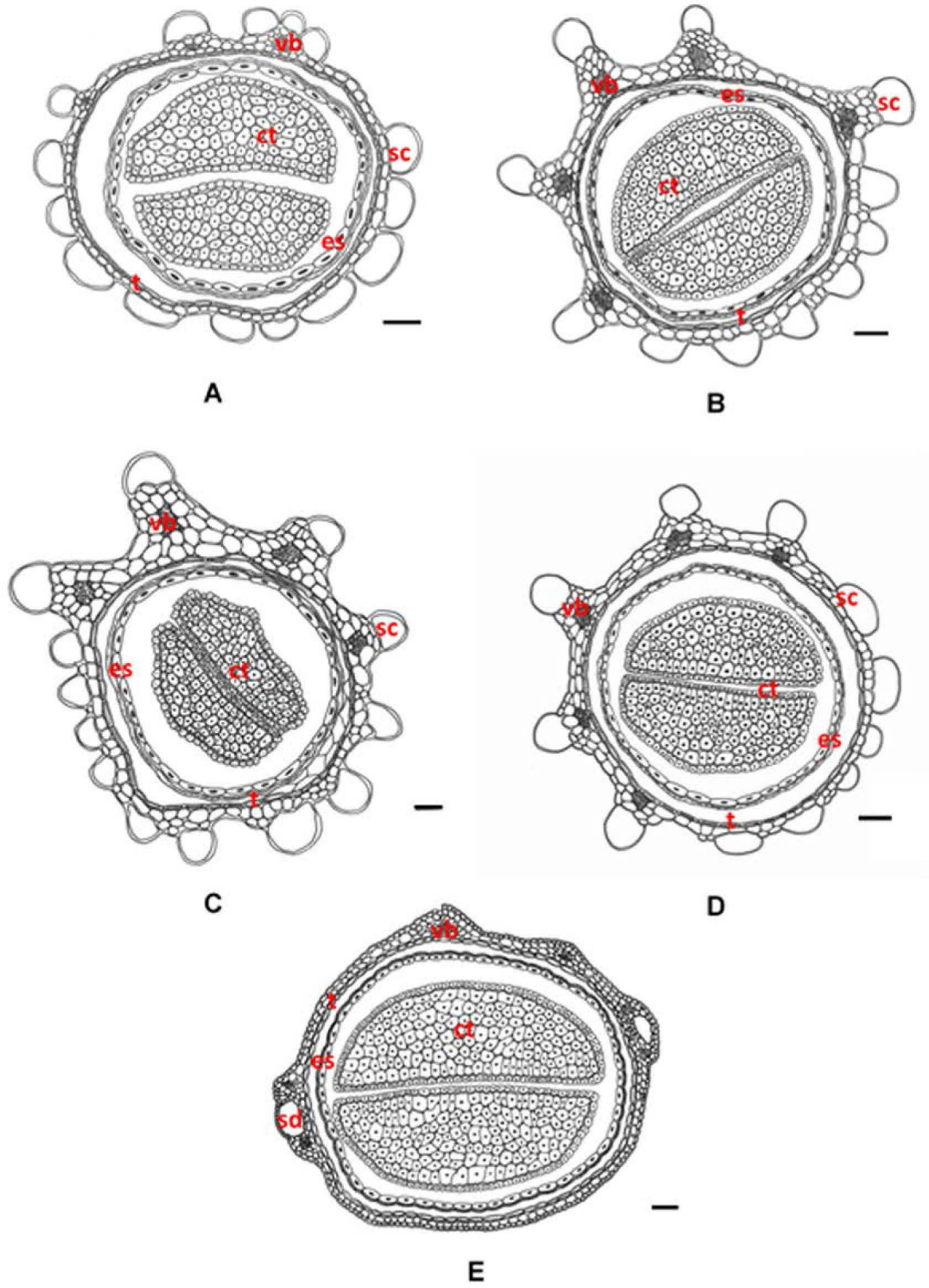


Fig. 2. Cross sections in the middle part of achenes in *Matricaria* (**A**-**E**): **A**. *M. aurea*, **B**. *M. chamomilla* var. *chamomilla* (peripheral achene), **C**. *M. chamomilla* var. *chamomilla* (central achene), **D**. *M. chamomilla* var. *recutita*, **E**. *M. matricarioides*, **ct.** cotyledons, **es**. endosperm, **sc**. slime cell (mucilaginous cell), **sd**. secretory duct, **vb**. vascular bundle, **t**. testa. Scale bars: 40 μm.

Table 2. Characters and character states used in multivariate analyses of the *Matricaria* taxa.

1	Capitulum discoid (0), radiate (1)
2	Disc florets 4-lobed (0), 5-lobed (1)
3	Disc florets greenish (0), yellow (1),
4	Achene ribs reddish in only laterally (0), white in all surface (1)
5	Slime in achene absent (0), present (1)
6	Corona absent (0), present (1)
7	Mean pericarp thickness (μm)
8	Mean pericarp width (μm)
9	Mean vascular bundle length (μm)
10	Mean testa thickness (μm)
11	Mean endosperm thickness (μm)

of the taxa, grouping was performed by cluster analysis (UPGMA, dissimilarity, standardised variable) and by ordination based on principal component analysis (PCA). Duncan's multiple-range test from one-way analysis of variance (ANOVA) was employed to determine the statistical significance of differences between the means of quantative characters.

RESULTS

The clustering dendrograms of UPGMA obtained from the studied characters of the *Matricaria* taxa are presented in Fig. 1. As can be seen on the dendrograms, the studied taxa are connected with each other at several levels on the basis of their diagnostic characteristics and structure of their achenes. Three groups can be identified within *Matricaria* based on morphoanatomical characters:

Group A. This group is represented by *M. chamomilla*. The delimiting character of the group is the presence of ligulate florets, i.e., a radiate capitulum. Female ray florets have a white limb and there are 11-20 per capitulum. Disc florets are numerous, hermaphroditic and yellow, with five lobes. Achenes are coronate or ecoronate, brown and mucilaginous. Their anterior surface is smooth, while the posterior surface has 4-5 whitish ribs. This group is further divided into two sub-groups.

Sub-group I. It is composed of *M. chamomilla* var. *chamomilla*, which is characterised by the fact that only its peripheral achenes are with coronas, whereas its central achenes are ecoronate. Peripheral and central achenes are also mucilaginous. In this taxon, pericarp structure differs in peripheral and central achenes: in peripheral achenes, the pericarp has five ribs and is mainly composed of several layers of thin-walled parenchymatous cells with one vascular bundle in each rib. Its surface has slime cells. The pericarp is 4–6 cells thick at the ribs but thin, 1–2-celled between the ribs (Fig. 2B). In central achenes, the pericarp has four ribs, is moderately thickened and is mainly composed of several layers of thin-walled parenchymatous cells with one vascular bundle in each rib. It is 4–6 cells thick at the ribs, 1–2-celled between the ribs (Fig. 2C). In both peripheral and central achenes, the outer testa layer is lignified, whereas the inner testa layer is crushed. No vascular bundle is found in the testa. The seed lobes (cotyledons) are formed by more or less oval or round cells, which are larger than the square cells of the surface layer.

Sub-group II. It includes *M. chamomilla* var. *recutita*, which is characterised by having ecoronate achenes. All of its achenes are also mucilaginous. Thus, it differs from *M. chamomilla* var. *chamomilla* in having only ecoronate achenes. The pericarp has five ribs and is mainly composed of several layers of thin-walled parenchymatous cells with one vascular bundle in each rib. Its surface has slime cells. The pericarp is 3–4 cells thick at the ribs but thin, 1–2-celled between the ribs. The outer testa layer is lignified, whereas the inner testa layer is crushed. No vascular bundle is found in the testa. The seed lobes are formed by more or less oval or round cells, which are larger than the square cells of the surface layer (Fig. 2D).

Group B. It is represented by *M. aurea,* which is characterised by possessing a discoid capitulum. This species is similar to *M. matricarioides*, but differs in having mucilaginous and ecoronate achenes. Ray florets are absent, and disc florets are numerous, yellow, with four lobes. Achenes are ecoronate, brown and copiously mucilaginous. Their anterior surface is smooth, while the posterior surface has three whitish ribs. The pericarp has three ribs and is mainly composed of several layers of thin-walled parenchymatous cells with one vascular bundle in each rib. Its surface has slime cells (mucilaginous cells). The pericarp is 3–4 cells thick at the ribs but very thin, single-celled between the ribs. The outer testa layer is lignified, whereas the inner testa layer is crushed. No vascular bundle is found in the testa. The seed lobes are formed by more or less oval or round cells, which are larger than the square cells of the surface layer (Fig. 2A).

Group C. This group is composed of *M. matricarioides*, which is characterised by possessing a discoid capitulum, non-mucilaginous achenes and greenish disc florets

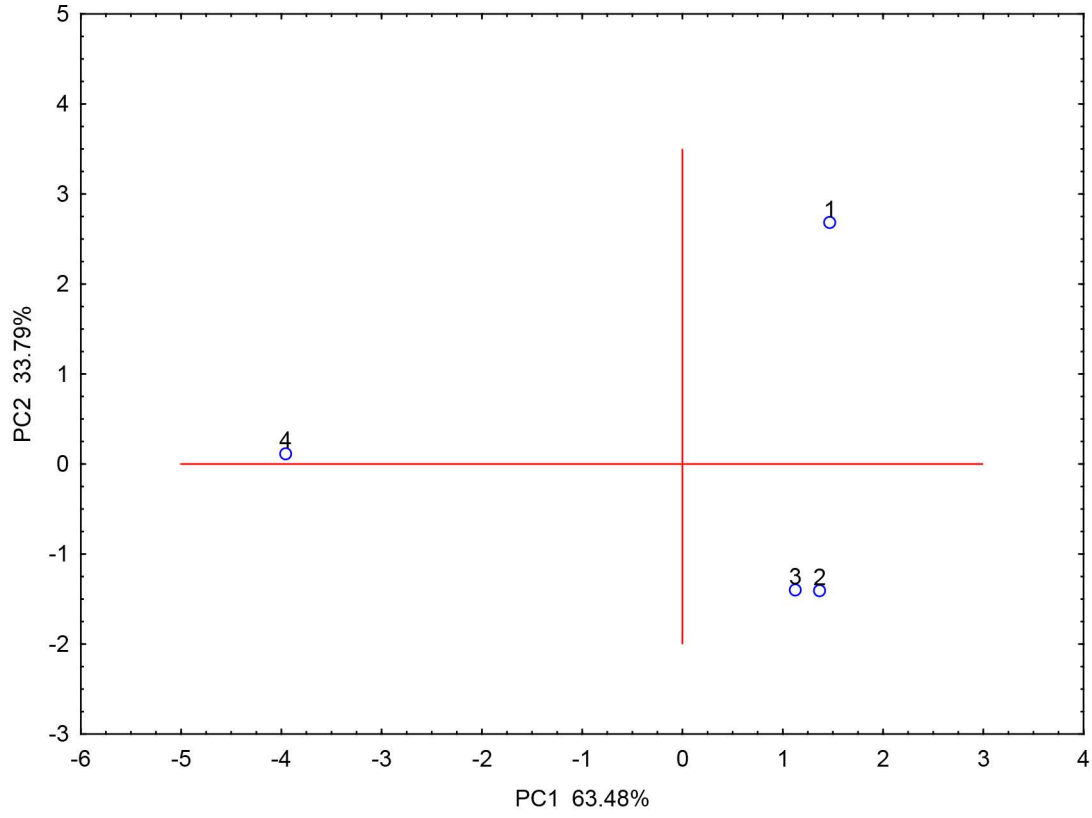


Fig. 3. Results of principal component analysis of *Matricaria* taxa based on morphoanatomical characters (**1-4**): **1.** *M. aurea*, **2.** *M. chamomilla* var. *chamomilla*, **3.** *M. chamomilla* var. *recutita*, **4.** *M. matricarioides*.

with 4-lobes. Achenes are brownish, their anterior surface is smooth, while posteriorly they are four-ribbed, the lateral ribs being reddish, especially above. The pappus is a short corona, with an entire margin. The pericarp has four ribs and is mainly composed of several layers of thin-walled parenchymatous cells with one vascular bundle in each rib. No slime cells are found in the surface of the pericarp. Lateral ribs have secretory ducts. The pericarp is 4–6 cells thick at the ribs but thin, 2–3-celled between the ribs. The outer testa layer is lignified, whereas the inner testa layer is crushed. No vascular bundle is found in the testa. The seed lobes are formed by more or less oval or round cells, which are larger than the square cells of the surface layer (Fig. 2E).

Principal component analysis of the studied characters reveals that two PC factors accounted for 97% of the total variance (Fig. 3). The first factor accounts for 64% of the total variance, with colour of disc florets, colour of ribs on the achenes and presence or absence of a slime envelope having the highest positive correlation ($r > 0.99$). The second factor accounts for approximately 34% of the total variance, with pericarp thickness having the highest negative correlation ($r > -0.91$).

DISCUSSION

Achene anatomy. The results of examining achene anatomy show that the achenes are clearly differentiated into pericarp, testa, endosperm and cotyledons (Fig. 2). The pericarp in all of the studied taxa has 3–5 ribs and is mainly composed of several layers of thin-walled parenchymatous cells with one vascular bundle in each rib. It is 4–8 cells thick at the ribs but very thin, one- or rarely two-celled between the ribs, thus being much less developed there (Fig. 2). These characteristics agree with data previously reported by Kynčlova (1970).

The pericarp has valuable taxonomical significance in the tribe Anthemideae (Kynčlova 1970; Kallerjö 1985; Bruhl & Quinn 1990; Inceer *et al.* 2012). Anatomical features of the pericarp differ significantly in the studied taxa (Table 3). The thickness and width of ribs in the pericarp vary from wide to narrow, and are of taxonomic value for differentiating among the taxa. The widest rib in the pericarp is found in *M. matricarioides*, while it is narrow in *M. aurea*. In addition, *M. chamomilla* var. *chamomilla* has the thickest pericarp, whereas *M. aurea* has the thinnest pericarp among the

Table 3. Differences in anatomical features (mean value ± standard deviation) of the achene in taxa of *Matricaria*, as determined by Duncan's multiple range test from one-way ANOVA. Among the taxa, mean values with different letters are significant at P = 0.05.

Taxon	Pericarp thickness (μm)	Pericarp width (μm)	Vascular bundle length (μm)	Testa thickness (μm)	Endosperm thickness (μm)
M. aurea	33.13±3.75 c	50.50±3.34 b	13.25±0.87 b	7.48±0.40 a	5.81±0.44 b
M. chamomilla var. *chamomilla* (peripheral achene)	64.00±3.00 b	71.50±4.65 a	23.25±2.06 a	7.30±0.87 a	6.72±0.69 a
M. chamomilla var. *chamomilla* (central achene)	66.13±2.84 b	69.75±3.10 a	17.25±3.77 c	9.65±0.51 b	6.75±0.20 a
M. chamomilla var. *recutita*	54.69±2.49 a	70.25±1.55 a	22.08±1.10 a	9.71±0.30 b	6.91±0.69 a
M. matricarioides	56.00±2.45 a	102.88±1.31 c	22.75±0.96 a	13,63±0.46 c	10.30±0.41 c

studied taxa. The results obtained in examining achene anatomy show that there are considerable differences in the pericarp among the studied taxa. It is concluded that pericarp thickness is useful for delimitation of the studied taxa.

The surface of the pericarp in the studied taxa has slime cells on and around each rib, except in the case of *M. matricarioides* (Fig. 2). Our results are in agreement with the data of Kynčlova (1970) and confirm the presence of slime cells on the achene pericarp in *Matricaria* (Bremer & Humphries 1993; Bremer 1994; Oberprieler *et al*. 2007; Inceer 2011).

The form of testa cells and the type of thickening of their walls provide taxonomically useful information for some tribes of Asteraceae (Inceer *et al*. 2012; Jana & Mukherjee 2014). In the sampled taxa, the outer testa layer is lignified, whereas the inner testa layer is crushed (Fig. 2). No vascular bundle is found in the testa. In addition, significant differences of testa thickness are found among the taxa, except between *M. chamomilla* var. *chamomilla* and *M. chamomilla* var. *recutita,* as well as between *M. aurea* and *M. chamomilla* var. *chamomilla* (Table 3). These findings show that testa thickness is useful for delimitation of some taxa.

The seed lobes of all taxa have a similar structure and are formed by more or less oval or round cells (Fig. 2). The seed lobes are in contact with the endosperm, which is formed by tangentially elongated cells. The walls of these cells are strongly light-refracting and appear white in cross section. The cell content is dark-brown to brown-green and of granular structure. These findings are in agreement with the data of Kynčlova (1970). Structure of the endosperm does not differ from one species to another, but the walls of its cells are relatively thickened and their thickness differs significantly between taxa, except between *M. chamomilla* var. *chamomilla* and *M. chamomilla* var. *recutita* (Table 3). The content of endosperm cells is dark brown to brownish green, presenting a granular structure. Inasmuch as the walls of its cells are thickened, the endosperm apparently serves as an additional protective layer for the embryo (Jeffrey 2007). According to Jeffrey (2007) and Karanović *et al*. (2016), the endosperm and seed consist of living cells that are rich in oil and protein, but devoid of starch, which is in line with our results.

Achene heteromorphism is very common in the family Asteraceae (Imbert 2002). Thus, the peripheral achenes in *M. chamomilla* var. *chamomilla* differ morphologically from the central ones, i.e., the achenes are dimorphic in this taxon. Its peripheral achenes have auriculate coronas, while its central achenes are naked (Grierson 1974). In addition, the peripheral achenes are smaller than the central ones. We found a significant difference of vascular bundle and testa thickness between peripheral and central achenes in the given taxon (Table 3). The presented results show that some differences of achene anatomy accompany achene dimorphism in *M. chamomilla* var. *chamomilla*.

Systematic implications of morphoanatomical characters. The results of cluster analysis show that three groups can be identified within *Matricaria* based on morphoanatomical characters (Fig. 1). As seen in the dendrograms, *M. chamomilla* is a major group, and two varieties, namely var. *chamomilla* and var. *recutita*, are found in different sub-groups within *M. chamomilla*. According to the dendrograms, there is low dissimilarity between var. *chamomilla* and var. *recutita*, whereas there is high dissimilarity between *M. aurea* and *M. matricarioides*. These findings are in accordance with the traditional classification of *Matricaria* (Grierson 1974, 1975; Applequist 2002; Inceer 2012; Inceer *et al*. 2018).

The results of principal component analysis reveal that colour of the disc florets, colour of the ribs on the achenes, the presence or absence of a slime envelope and pericarp thickness account for most of the total variation among the taxa (Fig. 3). These results show that the given characters are the main diacritical traits among

the studied morphoanatomical characters. Together with other morphoanatomical traits, these characters can be used to separate the taxa, as shown in the key to taxa given below and in Fig. 3.

1a. Disc florets greenish; achenes without slime cells, the ribs reddish only laterally . . . . . ***M. matricarioides***
1b. Disc florets yellow; achenes with slime cells, the ribs white over their entire surface 2
2a. Capitulum discoid; pericarp 3-ribbed, rib width < 55 µm . . . . . ***M. aurea***
2b. Capitulum radiate; pericarp 4–5-ribbed, rib width > 65 µm 3
3a. Rib thickness < 58 µm . . . . . ***M. chamomilla* var. *recutita***
3b. Rib thickness > 60 µm . . . . . ***M. chamomilla* var. *chamomilla***

Matricaria chamomilla var. *chamomilla* and *M. chamomilla* var. *recutita* are distinguished from *M. aurea* and *M. matricarioides* by having ray florets in their synflorescence. The presence or absence of a corona in the peripheral achenes is the main diacritical character for these varieties of *M. chamomilla*. To date, the anatomy of achenes in var. *chamomilla* and var. *recutita* has not been described in detail. The presented results indicate that pericarp thickness differs significantly between these varieties of *M. chamomilla*. The variety *chamomilla* has thicker ribs than the variety *recutita*. These results are congruent with the classification of Grierson (1974, 1975) and Applequist (2002). Current studies on leaf anatomy (Inceer & Ozcan 2011), pollen morphology (Ceter *et al*. 2013) and molecular phylogeny (Inceer *et al*. 2018) of *Matricaria* have also supported the classification proposed by these authors. On the other hand, we suggest that pericarp thickness can also be used as a taxonomic criterion for delimitation of these varieties in *M. chamomilla*.

Matricaria aurea is similar to *M. matricarioides*, but differs in having mucilaginous and ecoronate achenes. Our results show that there is a significant difference between these species with respect to anatomical features of their achenes (Table 3). We suggest that results obtained in studying achene anatomy can be used as additional data to support the morphological separation of these species.

CONCLUSION

Numerical analyses of morphological characters and anatomical traits of the achenes provide some evidence for the delimitation of *Matricaria* taxa. The colour of disc florets, that of ribs on the achenes, the presence or absence of a slime envelope and pericarp thickness have significant taxonomic value at the inter/intraspecific level. In addition, differences in anatomy of the achenes accompany achene dimorphism, and results obtained in studying achene anatomy can be used as supplementary data to support the morphological separation of closely related species.

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Botanica SERBICA

REZIME

Morfoanatomska studija roda *Matricaria* L. (Asteraceae) u Turskoj

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Rod *Matricaria* je u turskoj flori prisutan sa četiri vrste: *M. aurea* (Loefl.) Sch. Bip., *M. chamomilla* L. var. *chamomilla*, *M. chamomilla* var. *recutita* (L.) Fiori, and *M. matricarioides* (Less.) Porter ex Britton. Ova studija se bavi procenom odabranih dijagnostičkih karaktera, kao i anatomskih karakteristika ahenija (cipsele) vrsta roda *Matricaria* u Turskoj pomoću univarijantnih i multivarijantnih analiza. Na osnovu morfo-anatomskih karakteristika, unutar roda *Matricaria* se mogu izdvojiti tri grupe. Boja cvetnog diska, boja rebara ahenija, prisustvo ili odsustvo sluzavog omotača kao i debljina perikarpa su korisni za razgraničenje vrsta roda *Matricaria*. Prikazan je ključ za razlikovanje vrsta, napravljen na osnovu ovih, kao i drugih dijagnostičkih karaktera.

Ključne reči: *Matricaria*, morfometrija, anatomija ahenija, Turska, taksonomija