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ANATOMICAL FEATURES OF SIX ONOSMA L. (BORAGINACEAE) SPECIES FROM TURKEY

S. Güven, O. Beyazoğlu, S. Makbul, Z. Türkmen & A. Kandemir

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In the present study, *Onosma discedens* Hausskn. & Bornm., *Onosma tenuiflora* Willd., *Onosma aucheriana* DC., *Onosma roussaei* DC., *Onosma rigida* Ledeb. and *Onosma trapezuntea* Boiss. & A.Huet ex Hand.-Mazz. were examined by light microscopy (LM) in terms of stem and leaf anatomy. The anatomical characters were numerically analyzed by cluster analysis (CA) and principal component analysis (PCA). Although general stem and leaf anatomical traits are very similar, some anatomical characters such as the ratio of cortex/diameter of stem and phloem/xylem, the average row number of collenchyma, palisade and spongy cells, and stomata index are more important for delimiting the investigated taxa. These results indicated that anatomical features would be useful in separating the examined species.

Seher Güven (correspondence <cakmakseher@hotmail.com>) & Serdar Makbul, Recep Tayyip Erdoğan University, Faculty of Arts and Sciences, Department of Biology, Rize, Turkey. -Osman Beyazoğlu, Yeni Yüzyıl University, Faculty of Engineering and Architecture, Department of Biomedical Engineering, Istanbul, Turkey. -Zafer Türkmen, Giresun University, Faculty of Arts and Sciences, Department of Biology, Giresun, Turkey. –Ali Kandemir, Erzincan University, Faculty of Arts and Sciences, Department of Biology, Erzincan, Turkey.

Key words. Anatomy, *Onosma*, systematics, Turkey.

مطالعه ویژگیهاي آناتومي شش گونه جنس **Onosma L. (Boraginaceae)** از ترکیه

سحر گوون، عضو هیئت علمي گروه زیست‌شناسي دانشگاه رجب طیب اردوغان، رایز، ترکیه.

سردار مقبول، عضو هیئت علمي گروه زیست‌شناسي دانشگاه رجب طیب اردوغان، رایز، ترکیه.

عثمان بیاض اوغلو، عضو هیئت علمي گروه مهندسي زیست‌شناسي پزشکي، دانشگاه یوزییل، استانبول، ترکیه.

ظفرترکمن، عضو هیئت علمي گروه زیست‌شناسي دانشگاه گیرسون، گیرسون، ترکیه.

علي کاندمیر، عضو هیئت علمي گروه زیست‌شناسي ارزینجان، ارزینجان، ترکیه.

در مطالعه حاضر گونه‌های زیر از نظر آناتومی ساقه و برگ با استفاده از میکروسکوپ نوری مورد بررسی قرار گرفته است.

Onosma discedens, Onosma tenuiflora, Onosma aucheriana, Onosma roussaei, Onosma rigida, Onosma trapezuntea.

ویژگیهای آناتومی از طریق تجزیه خوشه‌ای و تجزیه به عامل‌ها مورد تجزیه و تحلیل قرار گرفته است. اگرچه ویژگیهای آناتومی عمومی ساقه و برگ گونه‌ها شبیه است، لیکن برخی ویژگیهای آناتومی مثل نسبت پوست به قطر ساقه و آوند آبکش به آوند چوبی، متوسط تعداد ردیف‌های سلولهای کلانشیمی، نردبانی و اسفنجی و همچنین شاخص روزنه‌ها اهمیت بیشتری برای تعیین حدود گونه‌های مورد بررسی دارند. این نتایج نشان داد که ویژگی‌های آناتومی در جدا کردن گونه‌های مورد بررسی می تواند مفید باشد.

Introduction

Onosma L. (Boraginaceae) is a widespread genus consisting of about 150 species, distributed from Spain and Morocco to China, with areas of high diversity in Turkey, Iran and Central Asia (Teppner 1996). The genus comprises 88 species belonging to three sections in the Flora of Turkey (Riedl 1978). Except for *O. rostellatum* Lehm. (sect. *Protonosma* M. Popov) and *O. orientale* L. (sect. *Podonosma* (Boiss.) Gürke), all species in Turkey belong to sect. *Onosma* which fall

Into 2 subsections based on the indumentum type: subsect. *Asterotricha* (Boiss.) Gürke and subsect. *Haplotricha* (Boiss.) Gürke (Riedl 1978). Since the genus was revised by Riedl (1978), many new taxa have been recorded from Turkey (Davis & al. 1988, Güner 2000, Riedl & al. 2005, Binzet & Orcan 2007, Kandemir & Türkmen 2010, Aytaç &Türkmen 2011). The latest studies indicated that *Onosma* is represented by about 104 taxa (99 species) in Turkey and the endemism rate is 50% (Binzet & Akçin 2012).

This large genus consists of several closely related species causing taxonomic confusions. The complex taxonomy of the genus is not due only to the large number of the phenetically related taxa, but also to the lack of morphological characters available to define species (Riedl 1978). The classification of *Onosma* is based mainly on sort of indumentum, leading in the past to various systematic errors (Ball 1972). Also, this trait was found to be inadequate in distinguishing the *Onosma* species by Riedl (1978) who pointed out the need of additional characters for proper identification.

In recent years, several karyological (Teppner 1981, 1991); micromorphological (Akçin 2007a, Akçin & Binzet 2011); palynological (Qureshi & Qaiser 1987, Türkmen 2006, Maggi & al. 2008, Binzet & al. 2010, Binzet 2011), chemical (El-Shazly & al. 2003, Özgen & al. 2004, Cadirci & al. 2007) and molecular (Cecchi & al. 2011, Mehrabian & al. 2011) studies have been carried out on the genus. Anatomical characteristics are also very important within the *Onosma* taxa (Watson & Dallwitz 1991). Metcalfe & Chalk (1979) defined the general anatomical characteristic of the family Boraginaceae, including some *Onosma* species. In addition, there have been some morphological and anatomical studies about Turkish *Onosma* species (Beyazoğlu & al. 2008, Coşkunçelebi & al. 2008, Makbul & al. 2008a, Akçin & Binzet 2010, Binzet & Akçin 2012). But, the six examined *Onosma* taxa were investigated in terms of anatomical traits for the first time. Consequently, the aim of the study was to survey internal traits of the six Turkish *Onosma* taxa in order to contribute to the future taxonomic studies.

Materials and Methods

The plants were collected from the natural habitats in Turkey in the year of 2003–2005. The collection data for the examined taxa are given in Table 1. Specimens were dried according to standard herbarium techniques and stored in the Herbarium of Karadeniz Technical University, Department of Biology (KTUB).

The anatomical materials were fixed in FAA (Formaldehyde: Acetic Acid: Alcohol) for 24 hours and then preserved in ethanol (70%). Cross sections taken from stem and leaves and surface sections from leaves were cut by free hand. All sections were stained with hematoxylen for 30 minutes and mounted with glycerine-gelatine in order to obtain permanent slides (Vardar 1987). The well-stained sections were photographed with an Olympus BX51 from permanent slides. All measurements and observations were performed five times from different slides.

Sixteen anatomical characters were assessed by numerical analysis (Table 2). Cluster analysis (CA) and principal components analysis (PCA) were performed by SYN-TAX PC 5.0 (Podani 1993). For CA, a pair-wise matrix resemblance values was calculated from raw standardized data matrix, using Gower's coefficient of resemblance designed for mixed data sets (Sneath & Sokal 1973). A dendrogram was generated by the unweighted pair-group method by using arithmetic averages (UPGMA). Also, cophenetic correlation coefficient (rcs) was calculated (Sneath & Sokal 1973). For PCA, the raw data were used to create a correlation matrix, and two eigenvectors were extracted, providing two axes onto which the raw data were projected to give a two-dimensional plot of the taxa and characters.

Results

Anatomical Results

Anatomical features of the six examined *Onosma* species based on transverse sections of the stem and the leaf, and also surface preparation of the lamina were studied. All detailed measurements related to stem and leaf anatomy are given in Table 3.

The cross sections taken from the stem of the examined taxa revealed the following elements (Figs. 1 a–f). One-layered epidermis consisting of orbicular or oval cells is covered with glandular, simple or stellate hairs in all taxa. The collenchyma is located close to the epidermis with 1–6 rows. The cortex generally consists of 4–13 layers of oval or transversely elongated parenchymatous cells. Also, some species have 2–3 layered compressed parenchymatic cells located between the collenchyma and parenchyma tissue. The width of cortex varies among the examined taxa. The vascular bundles are continuous along the stem. Phloem consists of thin-layered parenchymatous cells. Xylem has dense scleranchymatous cells. Phloem/xylem ratio varies among the examined taxa. Cambium occurs between the phloem and xylem. Pith consists of large and cylindrical parenchymatous cells.

Table 1. Locality information of the examined *Onosma* taxa.

Taxon	Locality
Subsect. ***Haplotricha*** *O. discedens* Hausskn. & Bornm.	**B7** Erzincan: Kemaliye Salihli Köyü'nden Erzincan'a 7. km, 12.06.2004, Kandemir 6085, KTUB.
O. tenuiflora Willd.	**A8** Artvin: Yusufeli-İspir, Yusufeli çıkışı, Kocaküre mahallesi, 632 m, 07.06.2005, Türkmen 071, KTUB.
Subsect. ***Asterotricha*** *O. aucheriana* DC.	A7 Trabzon: Araklı Dağbaşı, Soğuksu, 1853 m, 07.07.2004,Türkmen 041, KTUB. A7 Giresun: Alucra'ya 10 km, 1443 m, 13.06.2005, Türkmen 075, KTUB. **A8** Rize: İkizdere-İspir yolu, 1858 m, 06.06.2005, Türkmen 063, KTUB. **A9** Artvin:Ardanuç, 609 m, 07.06.2005, Türkmen 072, KTUB.
O. roussaei DC.	**A7** Bayburt: Kop Dağı, 2200 m, 15.07.2003, Türkmen 015, KTUB. **A7** Bayburt: Bayburt-Aşkale yolu 1640 m, 30.06.2004, Türkmen 035, KTUB.
O. rigida Ledeb.	**A7** Gümüşhane: Köse Dağı, 1912 m, 04.06.2003, Türkmen 06–079, KTUB. **A8** Artvin: Şavşat yolu, Yavuzköy, 1550 m,16.07.2003, Türkmen 021, KTUB.
O. trapezuntea Boiss. & A.Huet ex Hand.-Mazz.	**A7** Trabzon: Şinik, 150 m, 07.06.2004, Türkmen 030, KTUB.

Table 2. Anatomical characters used in the numerical analysis.

Symbol	Characters
X_1	Width/length of epidermal cells of stem (µm/µm)
X_2	Average row number of collenchyma cells of stem
X_3	Width of cortex/Width of stem (µm/µm)
X_4	Width of vascular bundles/Width of stem (µm/µm)
X_5	Width of pith/Width of stem (µm/µm)
X_6	Width of phloem/Width of xylem on stem (µm/µm)
X_7	Average row number of collenchyma on midrib
X_8	Width of phloem/width of xylem on midrib (µm/µm)
X_9	Average row number of palisade cells beneath the upper epidermis
X_{10}	Average row number of palisade cells beneath the lower epidermis
X_{11}	Average row number of spongy cells
X_{12}	Width of spongy tissue/Width of mesophyll tissue (µm/µm)
X_{13}	Width/length of lower epidermal stomata of leaf (µm/µm)
X_{14}	Stomata index of lower epidermis
X_{15}	Width/length of upper epidermal stomata of leaf (µm/µm)
X_{16}	Stomata index of upper epidermis

Transverse and surface preparations of the leaves were also investigated (Figs. 2, 3). Both upper and lower surfaces consisting of one-layered oval or rectangular epidermal cells are covered with tuberculate setae, setules and glandular hairs. Idioblastic cells are seen clearly in the basis of setae. Also basal parts of the setae are globrous or have stellately setuled tubercles. Midrib consists of collenchyma cells located close to the lower epidermis. Average row number of the collenchyma varies among the examined taxa. Vascular bundle surrounded by thin-walled, orbicular parenchymatous cells occurs in the centre of midrib.

Phloem and xylem are seen clearly. *Onosma* leaves are ecvifacial type. Width of palisade and spongy parenchyma vary among the examined taxa. Equifacial leaves have anisocytic or anomocytic stomata on both surfaces. Stomata index vary for the upper and lower surface among the examined taxa.

Numerical Results

The dendrogram resulting from UPGMA based on 16 variables is represented in Fig. 4. The figure shows that all investigated taxa fall into 2 clusters at 98.5% dissimilarity levels. The first cluster, labeled as "a", consists of *O. discedens* and *O. roussaei* linked at 36.5% dissimilarity level. The second cluster labeled as "b", divided into two subclusters including all the remaining four taxa, linked at 65% dissimilarity level. The cophenetic correlation coefficient is 0.88 in our analysis. To determine which traits are important in

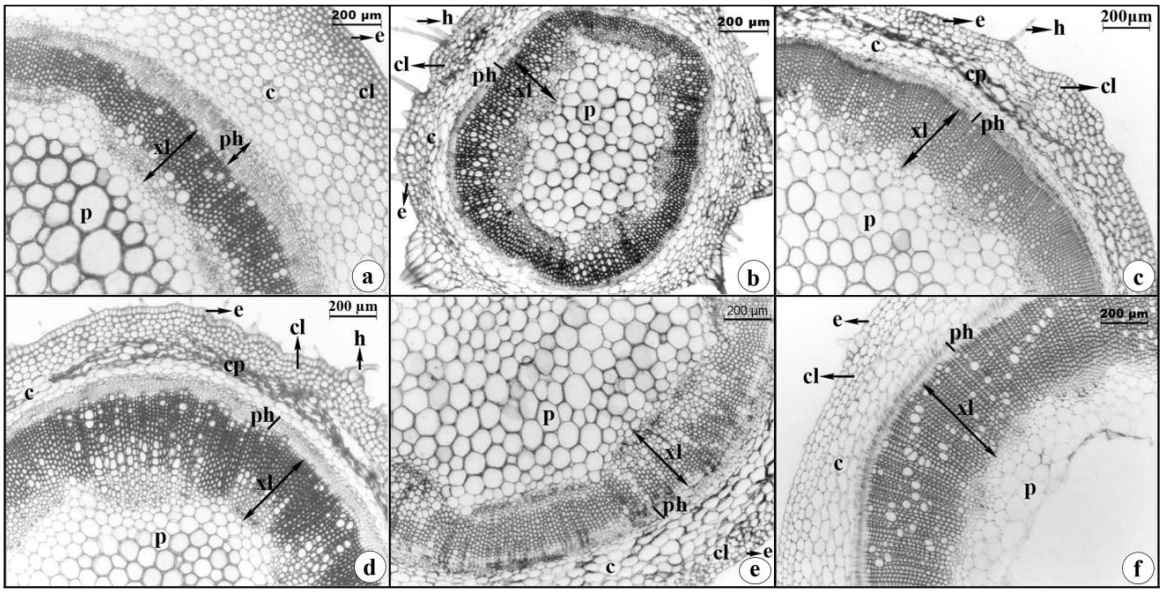

Fig. 1. Cross sections of stem. a: *O. discedens*; b: *O. tenuiflora*; c: *O. aucheriana*; d: *O. roussaei*; e: *O. rigida*; f: *O. trapezuntea*. e: epidermis, c: cortex, cl: collenchyma, cp: compressed parenchyma, h: hair, p: pith, ph: phloem, xl: xylem.

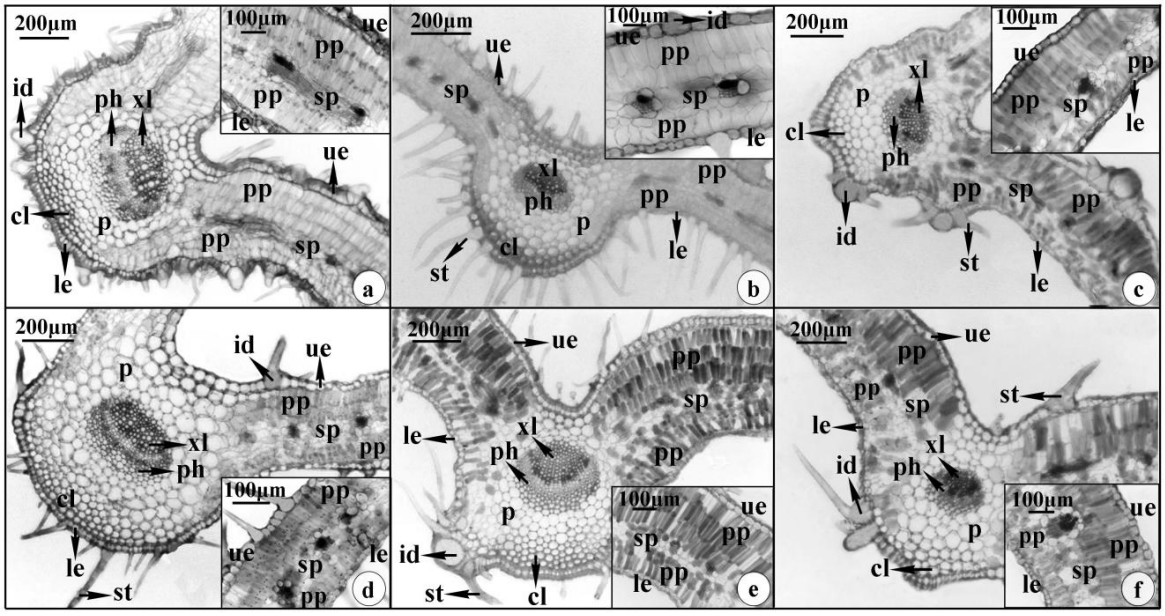

Fig. 2. Cross sections of leaves. a: *O. discedens*; b: *O. tenuiflora*; c: *O. aucheriana*; d: *O. roussaei*; e: *O. rigida*; f: *O. trapezuntea*. id: idioblastic cell, le: lower epidermis, p: parenchyma, pp: palisade parenchyma, sp: spongy parenchyma, st: seta, ue: upper epidermis.

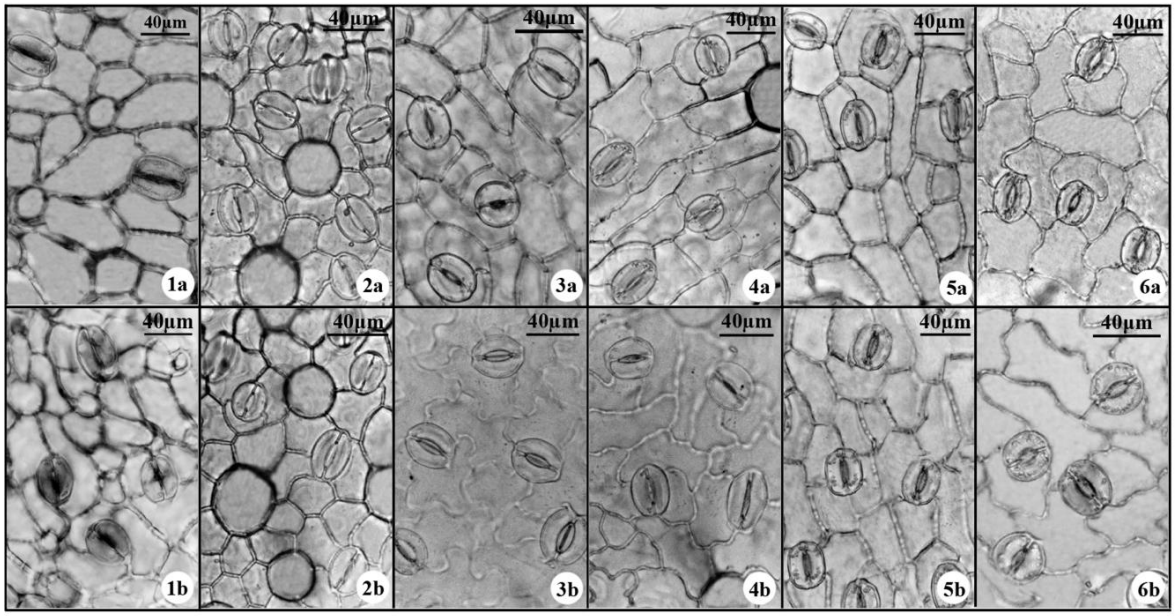

Fig. 3. a: Superficial section of upper epiderma; b: Superficial section of lower epiderma, 1: *O. discedens*, 2: *O. tenuiflora*, 3: *O. aucheriana*, 4: *O. roussaei*, 5: *O. rigida*, 6: *O. trapezuntea*.

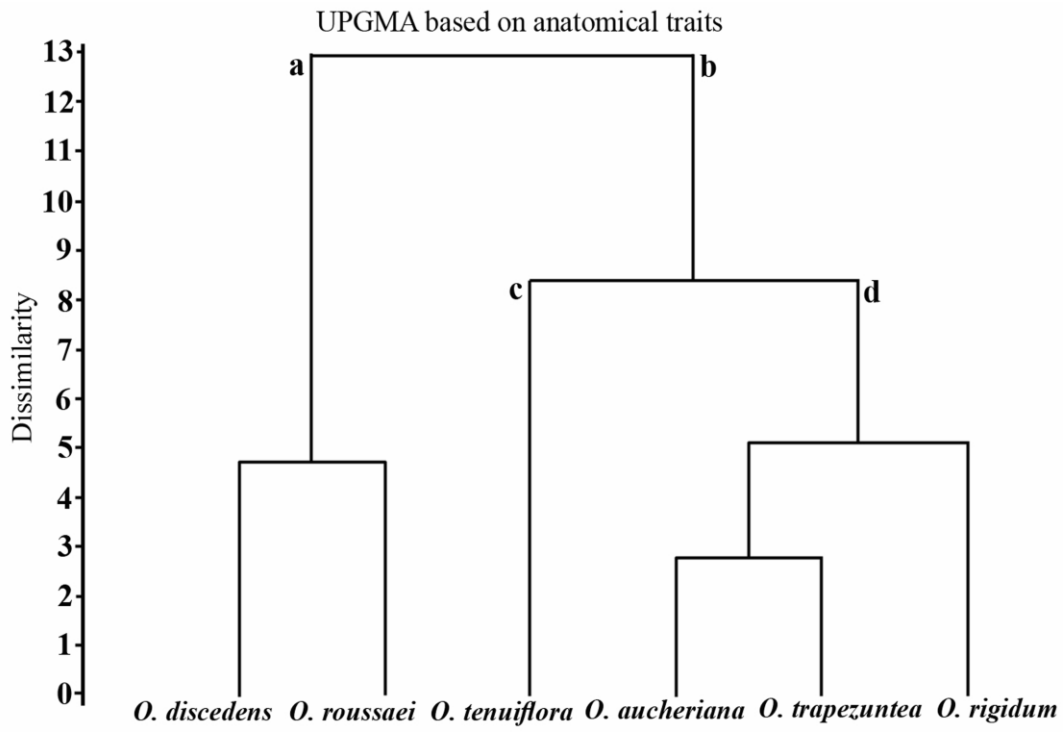

Fig. 4. Cluster analysis- UPGMA.

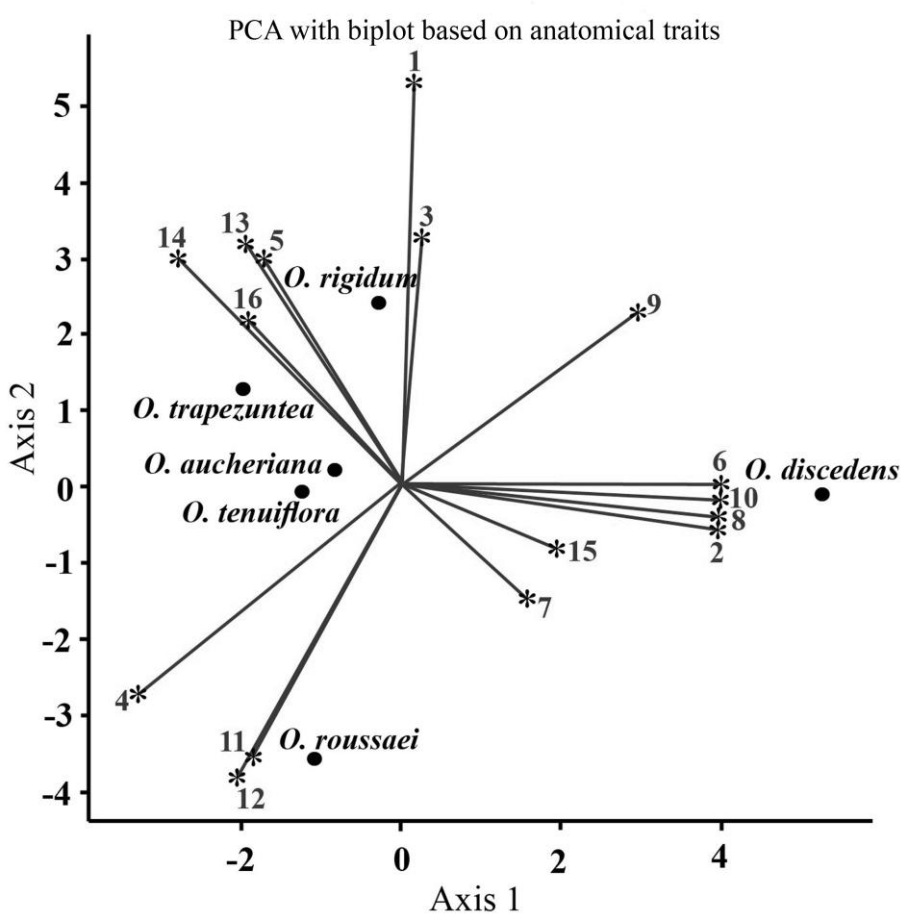

Fig. 5. Principal component analyses of 6 *Onosma* taxa and 16 variables projected onto the axis 1 and axis 2 (see Table 2 for the character numbers).

explaining total variation among the examined species, PCA analysis was performed. PCA results based on 16 traits are given in Fig. 5. This figure shows the distribution of taxa together with the variables on the first two components. Only the first three components were taken into account because of their eigenvalues. The first, second and third component were accounted for 43.88%, 25.47%, 17.92%, respectively (Table 4).

Discussion

In this study, internal structure of the six *Onosma* species was explored by numerical taxonomic methods in order to provide contributions to the systematic of the genus. Among the examined taxa, *O. discedens* and *O. trapezuntea* are endemic taxa for Turkey. This is the first comparative anatomical report on the six *Onosma* taxa.

As a useful tool for taxonomy, anatomy has been successfully used since the 19. Century in addition to morphological characters in order to indicate phylogenetic relationships among taxa (Metcalfe & Chalk 1979). Also, it is well known that anatomical traits provide taxonomically significant data in the numerous Angiosperms including Boraginaceae family (Watson & Dallwitz 1991, Beyazoğlu & al. 2008, Coşkunçelebi & al. 2008, Makbul & al. 2008a, Akçin & Binzet 2010, Binzet & Akçin 2012). As indicated above literature, anatomical traits are very important among *Onosma* taxa. We found that some anatomical characters supported by numerical analysis such as the ratio of cortex/diameter of stem and phloem/xylem, the average row number of collenchyma, palisade and spongy cells, and stomata index are the most important for delimitation of the examined taxa. The position and average row number of collenchyma tissue were considered meaningful diagnostic characters for the comparative anatomical studies in plants (Özörgücü & al. 1991, Makbul & al. 2008b, 2011a). In our study, it was determined that the average row number of collenchyma differs among the

Table 3. Anatomical measurements of the examined taxa.

	Onosma discedens	*Onosma tenuiflora*	*Onosma aucheriana*	*Onosma roussaei*	*Onosma rigida*	*Onosma trapezuntea*
X_1	0.9±0.17	0.86±0.23	0.92±0.11	0.63±0.12	1.09±0.2	0.99±0.15
X_2	5.5±1.58	2.3±0.82	1.8±0.78	2.3±0.48	2.2±0.78	1.7±0.67
X_3	0.33±0.03	0.22±0.04	0.31±0.3	0.18±0.02	0.19±0.03	0.19±0.02
X_4	0.21±0.03	0.32±0.07	0.28±0.03	0.33±0.04	0.25±0.03	0.29±0.02
X_5	0.44±0.04	0.45±0.09	0.48±0.05	0.47±0.05	0.54±0.06	0.49±0.04
X_6	0.4±0.07	0.17±0.09	0.14±0.02	0.16±0.03	0.19±0.02	0.14±0.01
X_7	2.6±0.51	2.3±0.8	2.4±0.5	2.7±0.67	2.8±0.63	1.2±0.42
X_8	0.8±0.11	0.6±0.15	0.61±0.12	0.96±0.17	0.57±0.19	0.5±0.04
X_9	3	2	2	2	3	2
X_{10}	2	1	1	1	1	1
X_{11}	2.4±0.51	2.7±0.48	3.5±0.5	5.3±0.48	3.4±0.69	3.2±0.42
X_{12}	0.19±0.02	0.3±0.03	0.25±0.03	0.58±0.04	0.3±0.04	0.33±0.04
X_{13}	0.79±0.09	0.8±0.05	0.84±0.06	0.79±0.1	0.85 ±0.1	0.93±0.09
X_{14}	11.8±0.45	22.57±0.88	23.13±0.67	12.78±1.25	19.25±0.75	24.57±1.71
X_{15}	0.83±0.11	0.79±0.08	0.83±0.05	0.8±0.1	0.78±0.06	0.81±0.07
X_{16}	11.9±1.06	24.51±0.31	15.37±0.94	11.48±0.46	16.9±0.9	17.39±2.09

Table 4. Percentage of variance accounted for by the first three components.

Components	Based on anatomical traits	
	Square roots of eigenvalues	Variance (%)
PC1	264.98	43.88
PC2	201.88	25.47
PC3	169.32	17.92
Total	-	87.28

investigated taxa. The cortex consists of usually parenchymatic oval cells with thin walls. However, *O. aucheriana* and *O. roussaei* have compressed parenchymatous cells in cortex. Akçin (2007b) and Binzet & Akçin (2012) reported that squashed parenchyma cells are common amongst the some *Onosma* taxa. Yentür (2003) expressed that arrangement of bundles provides valuable information in comparative anatomical studies. In our study, arrangement of the vascular bundles does not vary in examined taxa. However, phloem/xylem rate varies among the species.

Several systematic investigations performed on different genera indicated that leaf anatomical properties have taxonomic value in distinguishing the taxa (Selvi & Bigazzi 2001, Diane & al. 2003). Among the observed foliar anatomical features, the average row number of palisade and spongy cells and stomata index was found to be important in delimiting the taxa. Makbul & al. (2008b, 2011b) reported that palisade and spongy parenchyma features differ in some genera. Also, Azizian & al. (2000) pointed out that the mesophyll is ecvifacial in sect. *Onosma* and dorsiventral in sect. *Protonosma* and sect. *Podonosma*. Our study is in agreement with the previous studies in terms of the mesophyll features. In our study, all examined leaves are ecvifacial. However, the row number of spongy and palisade parenchyma, which are statistically important characters, vary among the examined *Onosma* taxa. Yentür (2003) indicated that xeromorfic leaves have more dense palisade than the other type of leaves. *Onosma* taxa generally grow in xerophytic habitats (Cecchi & al. 2011). Therefore, presence of the dense palisade is the expected result for the examined *Onosma* leaves. All the investigated taxa have amphystomatic leaves with anomocytic and anisocytic stomata. Metcalfe & Chalk (1979) reported that anomocytic and anisocytic stomata are common in the family Boraginaceae. But, anomocytic type stomata are dominant in the family (Özörgücü 1991). It was found that all these mesophyll and stoma features are in accordance with the previous studies performed on the genus *Onosma* (Beyazoğlu & al. 2008, Coşkunçelebi & al. 2008, Makbul & al. 2008a, Akçin & Binzet 2010, Binzet & Akçin 2012). The stomata index varies from species to

species among the examined taxa. But, it is known that this trait is influenced from environmental factors (Özörgücü & al. 1991).

Among the diagnostic features, leaf hair characters are the most important in distinguishing the genus *Onosma*. The sect. *Onosma* was separated into two subsections by Riedl (1978) based on indumentum type: subsect. *Asterotricha* characterized by several setae on the leaves arising from stellately setuled tubercles (asterotrichous), and subsect. *Haplotricha*, leaves of which are covered by simple setae with glabrous or subglabrous tubercles (haplotrichous). Also, the porrect-stellate setae on leaves were used as taxonomic traits for determining the Italian *Onosma* species by Pignatti (1982). In our study, *O. discedens* and *O. tenuiflora* are in the subsect. *Haplotricha*, and the other examined *Onosma* taxa are in the subsect. *Asterotricha*. In addition, basal parts of the setae have idioblastic cells. The large idioblastic cells are named as cystolith-like structures by Metcalfe & Chalk (1979) and Watson & Dallwitz (1991). Additionally, Metcalfe & Chalk (1979) pointed out that the number and size of the cystolith-like bodies varies at different times of the year and according to the amount of calcareous material in the soil for the members of Boraginaceae. According to the UPGMA cluster analyses, the studied *Onosma* species are divided into two major groups at 98.5% dissimilarity levels (Fig. 4). The first, labeled "a", comprises of *O. discedens* and *O. roussaei,* the second, labeled "b", contains all remaining taxa. Riedl (1978) indicated that *O. discedens* and *O. tenuiflora* were closely related taxa in terms of indumentum type of leaves, but anatomical results presented in this study do not support this view. As seen in Fig. 4, these 2 taxa differ from each other mainly by the average row number of collenchyma, the rate between phloem and xylem on stem, mesophyll structure and stoma index. This means that anatomical traits can be useful to distinguish these two species.

Cluster "b" fall into 2 subgroups based on the anatomical data. While the first subgroup labeled "c" consists of only *O. tenuiflora,* the second subgroup labeled "d" includes the rest of the examined taxa. Türkmen (2006) reported that *O. tenuiflora* was separated from the other species in group "b" in terms of morphological and palynological properties. This view is correlated with our results obtained from UPGMA. As seen in Fig. 4, *O. aucheriana, O. roussaei* and *O. rigida*, which are phenetically related taxa (Riedl 1978), take place in the different group based on the anatomical traits. These three taxa are easily separated by means of the rate of cortex/stem and spongy tissue/mesophyll, stoma index and average row number of the palisade parenchyma beneath the upper epidermis. This shows that the anatomical traits supply useful information for delimiting these three species. Additionally, *O. aucheriana* and *O. roussaei* that are morphologically similar occur in different groups on the basis of nutlet ornamentation (Akçin 2007a) and some palynological characters (Türkmen 2006, Binzet & al. 2010).

The cophenetic correlation coefficient (rcs) was also calculated in this study. It has generally been found to vary from 0.6 to 0.95 (Sneath & Sokal 1973). Our dendrogram had a cophenetic correlation of 0.88. This means that the dendrogram provides a fairly accurate representation of the resemblances.

The PCA results of the six taxa based on 16 anatomical variables given in Table 2 are shown in Fig. 5. As seen in Table 4, the first, second and third components present 43.88%, 25.47%, 17.92%, respectively. So, the first three components explain 87.28% of the total variations among the examined *Onosma* species. This means that some characters such as the rate of cortex to diameter of stem, and phloem to xylem, the average row number of collenchyma, palisade and spongy cells, and stomata index are more important in separating the investigated *Onosma* taxa. In conclusion, anatomical features supply some valuable information in the systematics of examined *Onosma* species.

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