Taxonomic significance of morphological and elemental characteristics of achenes of Artemisia genus from Turkey

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August 17, 2023

Abstract

In the study, the achene macro and micro morphological characters of the genus *Artemisia* distributed in Turkey have been researched with the target to know systematically important carpological structures for the examined species. Color, shape and dimension of achene have macro-morphologically shown variations. The examined achenes are separated into 4 shapes; fusiform-oblong, oblong, oblong-ovate and ovate. Oblong-ovate is the most common type. However, oblong is typical form for *A. abrotanum*. Achene dimensions range from 0.62 mm to 2.48 mm in length, from 0.30 mm to 1.21 mm in width. As *A. santonicum* subsp. *patens* is of the largest achenes , *A. annua* and *A. verloitorum* have the smallest achenes. Also, carpopodium diameter vary between 0.10 mm and 0.19 mm. The largest carpopodium diameter is found in taxa *A. fragrans* and *A. taurica* var. *vanensis* , while the narrowest one is *A. alpina* species. Achene surfaces of the examined taxa are micro-morphologically assessed, and the substantial differences are noticeably detected on behalf of the surface structures for instance, surface ornamentation, anticlinal and periclinal cell walls, epidermal cells and presence of secondary structures of the achenes. Surface ornamentation is separated into 10 types: irregularly sulcate, regularly sulcate, ruminate, sulcate-scalariform, rugose, favulariate, slightly sulcate, alveolate, tuberculate and reticulate. The most common types are irregularly sulcate (in 7 taxa) and regularly sulcate (in 7 taxa), while ruminate (in *A. abrotanum*), rugose (in *A. chamaemelifolia*), favulariate (in *A. arborescens*), alveolate (in *A. santonicum* subsp. *patens*), tuberculate (in *A. taurica* var. *vanensis*) and reticulate (in *A. bashkalensis*) ornamentation types are found as taxon-specific. A percentage comparison of the elements in the achene pericarp of the studied taxa have performed with SEM-EDS. Accordingly, pericarps in taxa include C, Ca, K, Mg, Cl, Si, Na and S elements. The most common seen element is C, which ranges from 77.4 (in *A. austriaca*) to 96.2 (in *A. absinthium*). Na element is observed only in *A. santonicum* subspecies and *A. arborescens* species. On the other hand, S element is found in low amounts only in *A. alpina* species. In the taxonomy of the genus *Artemisia*, the achene morphological characters are very significant characteristics that disclose inter-specific relations among the examined taxa. Moreover, a dichotomous key is offered for the identification of the studied taxa based on achene characters.

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Running title: Morphological and elemental characteristics of achenes of *Artemisia*

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ACKNOWLEDGEMENT

We are indebted to TÜBİTAK (Project no. TBAG106T559) and Bitlis Eren University Scientific Research Projects Coordinatorship (Proje No BEBAP 2023.09) for its financial support. In addition, this study includes achenes results from the master's thesis of Nagihan POLAT ALKAN.

ABSTRACT

In the study, the achene macro and micro morphological characters of the genus *Artemisia* distributed in Turkey have been researched with the target to know systematically important carpological structures for the examined species. Color, shape and dimension of achene have macro-morphologically shown variations. The examined achenes are separated into 4 shapes; fusiform-oblong, oblong, oblong-ovate and ovate. Oblong-ovate is the most common type. However, oblong is typical form for *A. abrotanum* . Achene dimensions range from 0.62 mm to 2.48 mm in length, from 0.30 mm to 1.21 mm in width. As *A. santonicum* subsp. *patens* is of the largest achenes, *A. annua* and *A. verloitorum* have the smallest achenes. Also, carpopodium diameter vary between 0.10 mm and 0.19 mm. The largest carpopodium diameter is found in taxa *A. fragrans* and *A. taurica* var. *vanensis* , while the narrowest one is *A. alpina* species. Achene surfaces of the examined taxa are micro-morphologically assessed, and the substantial differences are noticeably detected on behalf of the surface structures for instance, surface ornamentation, anticlinal and periclinal cell walls, epidermal cells and presence of secondary structures of the achenes. Surface ornamentation is separated into 10 types: irregularly sulcate, regularly sulcate, ruminate, sulcate-scalariform, rugose, favulariate, slightly sulcate, alveolate, tuberculate and reticulate. The most common types are irregularly sulcate (in 7 taxa) and regularly sulcate (in 7 taxa), while ruminate (in *A. abrotanum*), rugose (in *A. chamaemelifolia*), favulariate (in *A. arborescens*), alveolate (in*A. santonicum* subsp. *patens*), tuberculate (in *A. taurica* var. *vanensis*) and reticulate (in *A. bashkalensis*) ornamentation types are found as taxon-specific. A percentage comparison of the elements in the achene pericarp of the studied taxa have performed with SEM-EDS. Accordingly, pericarps in taxa include C, Ca, K, Mg, Cl, Si, Na and S elements. The most common seen element is C, which ranges from 77.4 (in *A. austriaca*) to 96.2 (in *A. absinthium*). Na element is observed only in *A. santonicum* subspecies and *A. arborescens* species. On the other hand, S element is found in low amounts only in *A. alpina*species. In the taxonomy of the genus *Artemisia* , the achene morphological characters are very significant characteristics that disclose inter-specific relations among the examined taxa. Moreover, a dichotomous key is offered for the identification of the studied taxa based on achene characters.

KEYWORDS: *Artemisia* , Cypsela, EDS, SEM, Turkey

RESEARCH HIGHLIGHTS:

- The achenes of Turkish *Artemisia* taxa have been examined in depth.
- The morphological characteristics of achenes of Turkish*Artemisia* taxa have been studied using SEM and LM for the first time, and debated the systematic practice of these characters.
- The elemental content of the achene pericarp has been systematically evaluated for the first time.

1. INTRODUCTION

The *Artemisia* L. is of the largest genera in the tribe Anthemideae Cass. of the family Asteraceae Dumort., comprises 550 species distributed in the Northern Hemisphere (Mungalov, 2004; Beer, 2005; Boyko, 2011; Vakulenko et al., 2020) with aromatic herbaceous plant, subshrubs, or shrubs (Al-Ajmi et al., 2021). Taxa of the genus*Artemisia* are usually utilized in folk and modern medicine for curing illnesses like malaria, hepatitis, cancer, and infections induced by fungi, bacteria, and viruses (Willcox, 2009; Abad et al., 2012; Al-Ajmi et al., 2021). The genus *Artemisia* has been separated into 5 sections (*Absinthium, Artemisia, Dracunculus, Seriphidium* , and *Tridentatae*) (Torrell et al., 1999). There are 26*Artemisia* taxa distributed in different phytogeographic regions in Turkey (Güner et al., 2012), belonging to *Artemisia* ,*Dracunculus* and

Seriphidium sections. This infrageneric classification of the genus does not represent as a normal grouping (Valles & McArther, 2000). The genus includes the taxonomical difficulties due to the infra-spesific diversity of the used diagnostic morphological characters in the classification. This triggers the search for new diagnostic characteristics.

The fruits or seeds have the typical morphological characters such as shape, color, dimension and surface ornamentation structures, and they can reflect valuable in the clarifying of taxonomic difficulties and in the establishment of systematic relationships (Barthlott, 1981; Karaismailoğlu & Erol, 2018). Scanning electron microscopy (SEM) is very significant for the carpological micromorphology. It permits to examine the ultrastructure of integuments in more data and to clarify extra fruit characters, such as the features of the carpopodium and the apical bowl, primary and secondary surface structures ultrastructure of the surface (Mukherjee & Nordenstam, 2010; Boyko, 2011; Vakulenko et al., 2020).

SEM with energy dispersive X-ray spectrometry (SEM-EDS) is an elemental microanalysis technique broadly practical across a wide range of the physical and biological sciences, technology, and forensic studies (Goldstein et al., 2003). In this technique, electron-excited characteristic X-ray peaks offer characterization and quantification for all elements of the periodic table (Dale & Ritchie 2013). SEM-EDS is able to quantitative analysis with correctness and precision equivalent to wavelength dispersive spectrometry (WDS) analysis (Ritchie et al., 2012). This application has not been used for systematic purposes, so far. A percentage comparison of the elements in the achene pericarp would be helpful in systematically determining the correlation.

Some morphological, anatomical, cytological and palynological studies on some taxa of the genus *Artemisia* have been made, so far(Pınar & Adıgüzel, 1998; Nourbakhsh et al., 2008; Kursat & Civelek, 2011; Kurşat et al., 2011a, 2011b; Tabur et al., 2012; Tabur et al., 2014; Kursat et al., 2014; Kurşat et al., 2015; Kurşat et al., 2018; Sancar et al., 2021). Also, morphological characters of the achenes have been taken into account for diagnosis of a small number of*Artemisia* taxa in different countries (Boyko, 2013; Abdel-Hamid, 2020; Vakulenko et al., 2020; Al-Ajmi et al., 2021). However, the significance of the achene in the genus has often been ignored in Turkey, where is one of the centers of diversity for the genus. The target of this study is to examine the macro and micro morphological characteristics of achenes of Turkish *Artemisia* taxa for the first time, and debate the systematic practice of these characters. Also, the taxa within the genus will be compared for the first time, with elemental analyzes in the achene pericarp.

2. MATERIAL AND METHODS

The studied Artemisia taxa were collected from various phytogeographical areas of Turkey by Dr. Murat KÜRŞAT and Dr. Şemsettin CİVELEK, and stored in Fırat University. The studied taxa and their locations were presented in Table 1.

Macro-morphological structures of the achenes including color, shape, dimension and carpopodium diameter were studied with 100 achenes of 10 specimens per taxa utilizing a Leica EZ4 binocular microscope with a HD camera (Figure 1 and Table 2).

Micro-morphological features of the achenes containing surface ornamentation, anticlinal and periclinal cell walls, epidermal cells and presence of secondary structures were examined with a Scanning Electron Microscope (Figure 2, Table 3). Initially, achenes were located on the stubb with a carbon tape or silver agar and covered with gold-palladium, then detected with a TESCAN MAIA3 XMU SEM (Karaismailoğlu, 2015).

EDS (Oxford Instruments, INCA ENERGY) analyzes were performed with a TESCAN MAIA3 XMU SEM. EDS analyzes were carried out by selecting the same spot on the sample surface at 80 sec under 30 μm aperture size, with 20 kV acceleration voltage, 8 mm operating distance, high current and processing time conditions. All analyzes were performed at three different points, and the average percentage of the quantitative values obtained were used (Table 4).

The grouping of the examined taxa was performed with using the clustering analysis method (UPGMA) in MultiVariate Statistical Package (MVSP) according to the 44 characters in Tables 2-3 (Figure 4). The

characteristics in statistical analysis were used achene color: brown-dark brown (1), straw yellowish-light brown (2), light brown-brown (3), yellowish-light brown-brown (4), dark brown (5), dark brown-blackish brown (6); achene figure: fusiform-oblong (7), oblong (8), oblong-ovate (9), ovate (10); achene dimension: length (11), width (12), length/width (13); achene surface structures: bare, deeply longitudinally striate (14), bare, finely longitudinally striate (15), rough, deeply longitudinally striate (16); carpopodium diameter (17); achene ornamentation type: irregularly sulcate (18), regularly sulcate (19), ruminate (20), sulcate-scalariform (21), rugose (22), favulariate (23), slightly sulcate (24), alveolate (25), tuberculate (26), reticulate (27); anticlinal cell wall: unclear (28), raised (29), sunken (30); periclinal cell wall: unclear (31), concave (32), convex (33), flat (34); epidermal cell structure: unclear (35), polygonal (36), alveolar (37), rectangular (38), arched-rectangular (39); presence of secondary structures: irregular lines (40), irregular epicuticular wrinkles (41), irregular epicuticular protrusions (42), epicuticular wrinkles (43), dome-shaped protrusion (44). The dissimilarity matrix of the studied taxa was formed with MVSP (Kovach 2007) (Table 5). A dendrogram was designed. Moreover, the cophenetic correlation coefficient was calculated to clarify the relative between the dendrogram and dissimilarity matrix (Table 5 and Figure 4).

3. RESULTS

This work evaluates macro-morphologically the achene structures of the examined taxa containing color, figure, dimension and carpopodium diameter. Achene color has shown variation; brown-dark brown (in 3 taxa), straw yellowish-light brown (in 2 taxa), light brown-brown (in 12 taxa), yellowish-light brown-brown (in 6 taxa), dark brown (1 taxon) and dark brown-blackish brown (in 2 taxa). The figure and dimension of achenes vary markedly. The examined achenes are separated into 4 shapes; fusiform-oblong, oblong, oblong-ovate and ovate. Oblong-ovate is the most common type (noted in 20 taxa). However, oblong is typical form for*A. abrotanum* . Achene dimensions range from 0.62 mm to 2.48 mm in length, from 0.30 mm to 1.21 mm in width. As *A. santonicum* subsp.*patens* is of the largest achenes, *A. annua* and *A. verloitorum* have the smallest achenes (Table 2, Figure 1). Achene macromorphological surface structures are of 3 different types; (1) bare, deeply longitudinally striate (in 3 taxa), (2) bare, finely longitudinally striate (in 18 taxa) and (3) rough, deeply longitudinally striate. Carpopodium diameter vary between 0.10 mm and 0.19 mm (Table 2). The largest carpopodium diameter is found in taxa *A. fragrans*and *A. taurica* var. *vanensis* , while the narrowest one is*A. alpina* species (Table 2).

Achene surfaces of the examined taxa are micro-morphologically assessed, and the substantial differences are noticeably detected on behalf of the surface structures for instance, surface ornamentation, anticlinal and periclinal cell walls, epidermal cells and presence of secondary structures of the achenes. Surface ornamentation is separated into 10 types: irregularly sulcate, regularly sulcate, ruminate, sulcate-scalariform, rugose, favulariate, slightly sulcate, alveolate, tuberculate and reticulate. The most common types are irregularly sulcate (in 7 taxa) and regularly sulcate (in 7 taxa), while ruminate (in *A. abrotanum*), rugose (in *A. chamaemelifolia*), favulariate (in*A. arborescens*), alveolate (in *A. santonicum* subsp.*patens*), tuberculate (in *A. taurica* var. *vanensis*) and reticulate (in *A. bashkalensis*) ornamentation types are found as taxon-specific (Table 3, Figure 2). The structures of the anticlinal cell walls of the studied taxa are unclear, raised and sunken. As raised cell walls are mostly detected in the irregularly sulcate, ruminate, sulcate-scalariform, alveolate and reticulate ornamentation types, the favulariate and tuberculate types are found where epidermal cells are enclosed by sunken walls. Rugose type is connected with unclear type (Table 3). There is no link between concave or convex periclinal cell walls and surface ornamentation type. In the rugose type is observed only unclear periclinal cells. Moreover, the structure of epidermal cells on the achene surface has displayed variation. It is rectangular, arched-rectangular, polygonal and unclear forms. The most common cell type is rectangular, while arched-rectangular and polygonal are fairly rare (Table 3). 13 of the examined taxa have secondary structures, which are irregular lines, irregular epicuticular wrinkles, irregular epicuticular protrusions, epicuticular wrinkles and dome-shaped protrusion. The most common type is epicuticular wrinkles; however, dome-shaped protrusions and epicuticular wrinkles are for characteristic for *A. marschalliana* .

A percentage comparison of the elements in the achene pericarp of the studied taxa have performed with

SEM-EDS. Accordingly, pericarps in taxa include C, Ca, K, Mg, Cl, Si, Na and S elements. The most common seen element is C, which ranges from 77.4 (in *A. austriaca*) to 96.2 (in *A. absinthium*). Along with C, Ca element is also found in the pericarps of all taxa examined. It is between 0.6 (in *A. arborescens*) and 12.2 (in *A. austriaca*). K element is observed in other taxa except *A. abrotanum* and *A. marschalliana* , albeit in low amounts. Mg element is found in the pericarps of 12 of the examined taxa; the amount varies between 0.6 (in *A. tournefortiana*) and 1.2 (in *A. austriaca*). Si element is only found in trace amounts in taxa *A. chamaemelifolia* (0.6), *A. tournefortiana* (0.8) and *A. fragrans* (0.5). Na element is observed only in *A. santonicum*subspecies and *A. arborescens* species. On the other hand, S element is found in low amounts only in *A. alpina* species (Table 4).

The numerical evaluations of the achene morphological characteristics allow the creation of a dendrogram, which discloses the differences-similarities among the examined taxa. A dendrogram is made as a result of the cluster analysis of the examined taxa of*Artemisia* based on the variation of 44 characters in 26 taxa. The cophenetic-correlation coefficient is defined to explain the relationship between the dendrogram and dissimilarity matrix (Figure 3, Table 5). The cophenetic-correlation between the dissimilarity matrix and dendrogram has been calculated as 0.61, signifying a good-match. Cluster analysis has detached the taxa into 2 major groups of A and B: Cluster A includes *A. araratica, A. scoparia* and *A. verloitorum* (A1), *A. abrotanum* , *A. annua* , *A. austriaca* , *A. campestris* , *A. incana* , *A. alpina* ,*A. spicigera* , *A. tournefortiana* , *A. absinthium* ,*A. fragrans* and *A. armeniaca* (A21), *A. splendens* ,*A. taurica* var. *taurica* , *A. haussknechtii* , *A. sieberi* subsp. *sieberi* , *A. marschalliana* and *A. taurica* var. *vanensis* (A22). Cluster B1 comprises A. *chamaemelifolia* and *A. arborescens* . Cluster B2 contains *A. santonicum* subsp. *santonicum* , *A. bashkalensis* and*A. santonicum* subp. *patens* . *Artemisia vulgaris* has formed a clade outside these clusters in the dendrogram (Figure 3).*A. splendens* and *A. taurica* var. *taurica* are the most closely correlated taxa (dissimilarity coefficient: 0.069), while*A. vulgaris* and *A. marschalliana* are the most distantly correlated taxa (dissimilarity coefficient: 0.716) (Table 5). Cluster A has the highest number of taxa compared to another cluster (Figure 3).

4. DISCUSSION

The morphological structures of the achenes offer important data in connection with evolutionary relationships of the flowering plants (Corner, 1976; Karaismailoğlu, 2015). Achene morphological characteristics have so far been comprehensively performed to elucidate inter-species relatives within various genera of the family Asteraceae (Karaismailoğlu, 2015; Şirin et al., 2017; Ghimire et al., 2018; Bona, 2020; Bona et al., 2023). This is the first investigation to display the morphological features and elemental contents of the achenes of Turkish*Artemisia* , and it could be a pioneering work for next studies in many related genera.

The macro-morphological characters of the achenes reveal variances among the examined *Artemisia* taxa (Figure 1 and Table 2). The taxa examined in our study are very different with regard to achene color. Light brown-brown dominates in the genus, whereas brown-dark brown, straw yellowish-light brown, dark brown and dark brown-blackish brown have a distinctive character among taxa by having a small number of taxa. *Artemisia annua* (ovate) and *A. tournefortiana*(fusiform-oblong) taxa are more or less similar in terms of the population appearance and flower structures, but they can be basically distinguished with relating of achene shape.

The investigates containing surface micro-morphological features of the fruits in various plant families such as Asteraceae, Umbelliferae, Chenopodiaceae, Boraginaceae, Scrophulariaceae, Brassicaceae, Lamiaceae, Solanaceae offer taxonomically valuable information (Juan et al., 2000; Özcan, 2004; Karcz et al., 2005; Binzet & Akçin, 2009; Kaya et al., 2011; Karaismailoğlu, 2015b; Khafagi et al., 2018; Karaismailoğlu & Güner, 2019). Also, the significance and usefulness of SEM in solving of taxonomic problems and in describing of taxa have been emphasized by many researchers (Heywood, 1971; Karaismailoğlu & Erol, 2018; Eroğlu et al., 2022). However, there are few studies on the importance of achene micromorphology in the genus *Artemisia* (Boyko, 2013; Abdel-Hamid, 2020; Vakulenko et al., 2020; Al-Ajmi et al., 2021). In this study, Turkish *Artemisia* taxa have been examined for the first time. We determined ten types of achene surface ornamentation in this study. In the genus, the most common achene ornamentation type is irreg-

ularly or regularly sulcate. Contrary to this study, reticulate or reticulate-areolate surface ornamentation types have been commonly encountered among species from many angiosperm families (Erol et al., 2006; Karaismailoğlu, 2015a; Özbek et al., 2018; Karaismailoğlu & Erol, 2018; Eroğlu et al., 2021; Eroğlu et al., 2022). The ruminate (in*A. abrotanum*), rugose (in *A. chamaemelifolia*), favulariate (in *A. arborescens*), alveolate (in *A. santonicum* subsp.*patens*), tuberculate (in *A. taurica* var. *vanensis*) and reticulate (in *A. bashkalensis*) ornamentation types are taxon specific. Moreover, ornamentation types facilitate the separation of closely correlated taxa like *A. annua*(sulcate-scalariform)-*A. tournefortiana* (irregularly sulcate), *A. alpina*(sulcate-scalariform)- *A. splendens* (irregularly sulcate),*A. santonicum* subsp. *santonicum*(sulcate-scalariform)-*A. santonicum* subsp. *patens*(alveolate) and *A. taurica* var. *taurica* (irregularly sulcate)- *A. taurica* var. *vanensis* (tuberculate) taxa (Figure 2 and Table 3). Also, previous seed or fruit surface examinations have displayed that the types and arrangements of the anticlinal and periclinal cell walls are diagnostic features in the formation of inter-taxa relations (Barthlott, 1981; Karaismailoğlu et al., 2018; Özbek et al., 2018; Eroğlu et al., 2022). The kinds of anticlinal and periclinal cell walls, and epidermal cell forms of the examined taxa disclose differences among taxa. The macro and micro-morphological results of this search are separated all taxa studied from each other, and they are consistent with the previous works including the exomorphic characters of achenes of the family Asteraceae (Karaismailoğlu, 2015; Coşkunçelebi et al., 2016; Şirin et al., 2017; Özbek et al., 2018; Bona, 2020; Ayaz et al., 2020; Vakulenko et al., 2020; Bona et al., 2023).

The elemental data of the examined taxa show that the achene samples are composed of over 77% C and a lesser amount of Mg, Cl, Si, Na and S. The distribution of elements in each achene is given in Table 4. The amounts of elements present have showed considerable variation among the taxa studied. *A. austriaca* contains more than 5 times more Ca element than the average of other studied taxa. Elements or percentage of elements contained by closely related taxa are different, for example*A. annua* (C, Ca and K)-*A. tournefortiana* (C, Ca, K, Mg, Cl and Si), *A. alpina* (C, Ca and K)-*A. splendens* (C, Ca, K, Mg and Cl), and *A. taurica* var. *taurica* (C, Ca and K)-*A. taurica* var. *vanensis* (C, Ca, K, Mg and Cl) taxa (Table 4). *A. alpina* species is also distinguished from the others by having the S element among the studied taxa. In general, achene elemental content or frequency varies in all studied taxa.

A dendrogram has been formed to assess the morphological characters of the achenes of Turkish *Artemisia* taxa with UPGMA cluster analysis (Figure 3). The dendrogram, showing 2 major groups, is partially suitable with the consequences of Cullen (1975), Davis (1975) and Davis et al. (1988). As stated by the descriptions in the Flora of Turkey, the taxonomic closeness in the *A. annua* -*A. tournefortiana* ,*A. alpina* -*A. splendens* , *A. santonicum* subsp.*santonicum* -*A. santonicum* subsp. *patens* and *A. taurica* var. *taurica* -*A. taurica* var. *vanensis* taxa is somewhat conserved. On the other hand, a logical relationship could not be established in determining the section boundaries (Figures. 3-4 and Table 5). Moreover, *A. vulgaris* has been defined as an isolated species with a very dissimilar description as a consequence of its unique leaf features within the genus in the Flora of Turkey. This species has exhibited parallel characters in the dendrogram formed according to achene morphological features, and consisted of a clade apart from two major groups.

5. CONCLUSION

This study demonstrate that macro and micro morphological achene characters of Turkish *Artemisia* taxa offer important and reliable insights into the systematic of taxa within the genus.So much so that a dichotomic key is given at the end of this section that allows to distinguish the taxa studied only according to these characters. In addition, elemental content of achene pericarps has played an active role in separating some taxa from each other.

Key to Turkish *Artemisia* taxa, based on achene characters

1. Achene color brown-dark brown, dark brown or dark brown-blackish brown. 2

2. Achene shape fusiform-oblong. 3

3. Surface ornamentation type irregularly sulcate. Anticlinal cell wall raised. Achene length > 2 mm. Secondary structures irregularly lines.

A. vulgaris

3. Surface ornamentation type regularly sulcate. Anticlinal cell wall sunken. Secondary structures absent.

A. verloitorum

2. Achene shape oblong-ovate. **4**

4. Achene color dark brown-blackish brown. **5**

5. Ornamentation type slightly sulcate. Epidermal cell unclear. Secondary structures dome-shaped protrusions and epicuticular wrinkles.

A. marschalliana

5. Ornamentation type regularly sulcate. Epidermal cell rectangular type. Secondary structures absent.

A. araratica

4. Achene color dark brown or brown-dark brown. 6

6. Achenes dark brown. Ornamentation type slightly sulcate. Secondary structures absent.

A. campestris

6. Achenes brown-dark brown. Ornamentation type regularly sulcate. Secondary structures irregular lines.

A. scoparia

1. Achene color light brown-brown, straw yellowish-light brown or yellowish-light brown-brown. 7

7. Straw yellowish-light brown, yellowish-light brown-brown. 8

8. Straw yellowish-light brown. 9

9. Achene shape oblong. Ornamentation type ruminate.

A. abrotanum

9. Achene shape ovate. Ornamentation type sulcate-scalariform.

A. annua

8. Yellowish-light brown-brown. 10

10. Achene shape ovate. Ornamentation type regularly sulcate.

A. armeniaca

10. Achene shape oblong-ovate. Ornamentation types rugose, favulariate, sulcate-scalariform, alveolate or reticulate. 11

11. Reticulate or alveolate. 12

12. Reticulate.

A. bashkalensis

12. Alveolate. . . . . .

A. santonicum **subsp. *patens***

11. Rugose, favulariate or sulcate-scalariform. 13

13. Rugose. . . . . .

A. chamaemelifolia

13. Favulariate or sulcate-scalariform. 14

14. Favulariate. . . . . .

A. arborescens

14. Sulcate-scalariform. . . . . .

A.santonicum **subsp. *santonicum***

7. Light brown-brown. 15

15. Achene shape fusiform-oblong. . . . . .

A. tournefortiana

15. Achene shape oblong-ovate. 16

16. Achene length > 2 mm. 17

17. Ornamentation types irregularly sulcate or regularly sulcate. 18

18. Regularly sulcate. Epidermal cells rectangular. . . . . .

A. fragrans

18. Irregularly sulcate. Epidermal cells unclear. . . . . .

A. splendens

17. Ornamentation types tuberculate or sulcate-scalariform. 19

19. Tuberculate. . . . . .

A. taurica **var. *vanensis***

19. Sulcate-scalariform. . . . . .

A. spicigera

16. Achene length < 2 mm. 20

20. Ornamentation type sulcate-scalariform. 21

21. Secondary structures absent. . . . . .

A. incana

21. Secondary structures irregular epicuticular protrusions. . . . . .

A. alpina

20. Ornamentation type irregularly sulcate or regularly sulcate. 22

22. Regularly sulcate. . . . . .

A. sieberi **subsp. *sieberi***

22. Irregularly sulcate. 23

23. Epidermal cells rectangular. 24

24. Secondary structures absent. . . . . .

A. austriaca

24. Secondary structures irregular epicuticular wrinkles. . . . . .

A. absinthium

23. Epidermal cells unclear 25

25. Apocolpidium diameter 0.15 mm. . . . . .

A. taurica **var. *taurica***

25. Apocolpidium diameter 0.10 mm. . . . . .

A. haussknechtii

Conflict Of Interest

On behalf of all authors, the corresponding author states no conflict of interest.

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TABLES

TABLE 1. The examined taxa and their locations.

No	Subgenus	Taxa	Location
1	*Artemisia*	*Artemisia vulgaris*	Mus: Airport crossroad, Toprakbaba Park, 1300 m, 23.11.2007
2		*A. verloitorum*	Giresun: Espiye, Gelevare Stream Sides, 2m, 10.12.2007
3		*A. abrotanum*	Mus: Center Entrance, Passing Vocational School, Roadsides, 12(
4		*A. austriaca*	Van: Gurpinar, Slopes Between Hamurkesen Village and Isikpina
5		*A. incana*	Mus: Manzikert, Aktuzla Village-Karncalı Village Between, 1550
6		*A. armeniaca*	Agri: Dogubeyazit, Igdır Road, South of Kori Mountains slopes,
7		*A. chamaemelifolia*	Agri: Dogubeyazit, Igdır Road, South of Kori Mountains slopes,
8		*A. annua*	Trabzon: Maçka Road, Around Esiroglu Kenan Oltan High Schoo
9		*A. tournefortiana*	Van: Gürpınar, Hamurkesen Village, Water The Edges of the Tre
10		*A. absinthium*	Mus: Manzikert, Aktuzla Village-Karncalı Village Between, 1550
11		*A. arborescens*	Hatay: Samandagi, Musa Mountain, Çevlik Locality, Titus Tunn(
12		*A. splendens*	Van: Gürpınar, Sapakonak Village, Slopes, 2692m, 25.07.2008
13		*A. alpina*	Nigde: Çamardı, Around Demirkazık Village, Steppe Fields, 1560
14		*A. haussknechtii*	Hakkari: Hakkari, Kırıkdag Village, Kırbas Castle, Kırbas Distric
15	*Dracunculus*	*A. campestris*	Ankara: Polatlı Highway, 3 km from Temelliye, Roadside, 843m,
16		*A. marschalliana*	Bitlis: Tatvan- Ahlat Arası, Tatvan Çıkısı (Sorgun), Askeri Alan
17		*A. araratica*	Malatya:Dogansehir, Dedeyazı Köyü, Çanakcı Mevkii, Step, 1495
18		*A. scoparia*	Mus: Malazgirt, Malazgirt- Aktuzla Arası, Nuretin Köyü Çevresi
19	*Seriphidium*	*A. santonicum* subsp. *santonicum*	Ankara: Sereflikoçhisar- Ankara Highway, 6 km from Sereflikoçhis
20		*A. santonicum* subsp. *patens*	Çanakkale: Çanakkale- Kesan Road, Old İstanbul Road, Kavak D
21		*A. taurica* var. *taurica*	Ankara: Sereflikoçhisar, Hamzalı village, Kayacık (Mutlucan) sur
22		*A. taurica* var. *vanensis*	Van: Van -Hakkari Highway, North Slopes of Zernek Dam, 24.11.
23		*A. spicigera*	Mus: Malazgirt, Between Aktuzla Village and Karncalı Village, S
24		*A. sieberi* subsp. *sieberi*	Şanliurfa: Between Islet and Mezrah, Exit of Adacik Village, Slo
25		*A. fragrans*	Van: Between Tatvan and Van, Kuzgun Kıran Pass,2142 m,31.10
26		*A. bashkalensis*	Hakkari: Van - Hakkari Highway, 58 km from Hakkariye, Slopes,1

TABLE 2. Macromorphological features of the studied *Artemisia* taxa.

Taxa	Shape	Achene dimension Length	Achene dimension Width	L/W	Colour
Artemisia vulgaris	fusiform-oblong	2.22±0.21	0.53±0.08	4.18	brown-dark b
A. verloitorum	fusiform-oblong	0.80±0.07	0.30±0.08	2.66	brown-dark b
A. abrotanum	oblong	0.89±0.15	0.42±0.05	2.11	straw yellowi
A. austriaca	oblong-ovate	1.11±0.12	0.51±0.06	2.17	light brown-b
A. incana	oblong-ovate	1.36±0.12	0.54±0.08	2.51	light brown-b
A. armeniaca	ovate	1.68±0.17	0.79±0.14	2.12	yellowish- lig
A. chamaemelifolia	oblong-ovate	1.14±0.18	0.51±0.08	2.23	yellowish- lig
A. annua	ovate	0.62±0.10	0.38±0.05	1.63	straw yellowi
A. tournefortiana	fusiform-oblong	1.05±0.12	0.42±0.05	2.50	light brown-b
A. absinthium	oblong-ovate	0.98±0.10	0.41±0.08	2.39	light brown-b
A. arborescens	oblong-ovate	1.46±0.18	0.58±0.11	2.51	yellowish- lig
A. splendens	oblong-ovate	2.10±0.08	0.93±0.17	2.25	light brown-b
A. alpina	oblong-ovate	1.72±0.21	0.69±0.12	2.49	light brown-b

A. haussknechtii	oblong-ovate	1.26±0.12	0.53±0.05	2.37	light brown-b
A. campestris	oblong-ovate	1.15±0.11	0.56±0.08	2.05	dark brown
A. marschalliana	oblong-ovate	1.91±0.16	1.01±0.13	1.89	dark brown-b
A. araratica	oblong-ovate	1.21±0.14	0.55±0.09	2.20	dark brown-b
A. scoparia	oblong-ovate	1.01±0.08	0.50±0.06	2.02	brown-dark b
A. santonicum subsp. *santonicum*	oblong-ovate	2.33±0.40	1.15±0.23	2.02	yellowish- lig
A. santonicum subsp. *patens*	oblong-ovate	2.48±0.27	1.21±0.24	2.04	yellowish- lig
A. taurica var. *taurica*	oblong-ovate	1.68±0.24	0.76±0.18	2.21	light brown-b
A. taurica var. *vanensis*	oblong-ovate	2.24±0.21	0.92±0.18	2.43	light brown-b
A. spicigera	oblong-ovate	2.30±0.22	0.99±0.11	2.32	light brown-b
A. sieberi subsp. *sieberi*	oblong-ovate	1.46±0.16	0.60±0.09	2.43	light brown-b
A. fragrans	oblong-ovate	2.82±0.42	1.16±0.23	2.43	light brown-b
A. bashkalensis	oblong-ovate	1.54±0.10	0.54±0.06	2.85	yellowish- lig

TABLE 3. Micromorphological features of the studied *Artemisia* taxa.

Taxa	**Surface ornamentation type**	**Anticlinal cell wall**	**Periclinal cell wall**	**Epidermal cell structures**
Artemisia vulgaris	Irregularly sulcate	Raised	Concave	Rectangular
A. verloitorum	Regularly sulcate	Sunken	Convex	Arched-Rectangu
A. abrotanum	Ruminate	Raised	Concave	Unclear
A. austriaca	Irregularly sulcate	Raised	Flat	Rectangular
A. incana	Sulcate-scalariform	Raised	Convex or concave	Rectangular
A. armeniaca	Regularly sulcate	Raised	Flat or Concave	Rectangular
A. chamaemelifolia	Rugose	Unclear	Unclear	Unclear
A. annua	Sulcate-scalariform	Raised	Flat	Rectangular
A. tournefortiana	Irregularly sulcate	Raised	Concave	Rectangular
A. absinthium	Irregularly sulcate	Raised	Concave	Rectangular
A. arborescens	Favulariate	Sunken	Convex	Unclear
A. splendens	Irregularly sulcate	Unclear	Unclear	Unclear
A. alpina	Sulcate-scalariform	Raised	Concave	Rectangular
A. haussknechtii	Irregularly sulcate	Unclear	Unclear	Unclear
A. campestris	Slightly sulcate	Raised	Flat	Rectangular
A. marschalliana	Slightly sulcate	Sunken	Convex	Unclear
A. araratica	Regularly sulcate	Sunken	Convex	Rectangular
A. scoparia	Regularly sulcate	Sunken	Convex	Rectangular
A. santonicum subsp. *santonicum*	Sulcate-scalariform	Raised	Concave	Polygonal
A. santonicum subsp. *patens*	Alveolate	Raised	Concave	Alveolar
A. taurica var. *taurica*	Irregularly sulcate	Unclear	Unclear	Unclear
A. taurica var. *vanensis*	Tuberculate	Sunken	Convex	Unclear
A. spicigera	Sulcate-scalariform	Raised	Concave	Rectangular
A. sieberi subsp. *sieberi*	Regularly sulcate	Unclear	Unclear	Unclear
A. fragrans	Regularly sulcate	Raised	Concave	Rectangular
A. bashkalensis	Reticulate	Raised	Concave	Polygonal

TABLE 4. The EDS reports of the examined *Artemisia* achenes.

Taxa	**C (%)**	**Ca (%)**	**K (%)**	**Mg (%)**	**Cl (%)**	**Si (%)**	**Na (%)**	**S (%)**
Artemisia vulgaris	93.3	4.9	1.1	0.7	-	-	-	-

A. verloitorum	90.4	5.8	2.8	1.0	-	-	-	-
A. abrotanum	96.1	3.0	-	0.9	-	-	-	-
A. austriaca	77.4	12.2	9.2	1.2	-	-	-	-
A. incana	87.5	5.2	7.3	-	-	-	-	-
A. armeniaca	81.9	5.7	11.3	-	1.1	-	-	-
A. chamaemelifolia	93.1	4.8	0.7	0.8	-	0.6	-	-
A. annua	94.1	3.7	2.2	-	-	-	-	-
A. tournefortiana	89.5	4.4	3.8	0.6	0.9	0.8	-	-
A. absinthium	96.2	2.8	1.0	-	-	-	-	-
A. arborescens	91.2	0.6	3.9	-	2.9	-	1.4	-
A. splendens	87.0	3.6	8.0	0.9	0.5	-	-	-
A. alpina	91.6	5.7	1.8	-	-	-	-	0.9
A. haussknechtii	93.6	3.7	1.8	0.9	-	-	-	-
A. campestris	89.8	2.2	6.1	0.7	1.2	-	-	-
A. marschalliana	91.1	8.9	-	-	-	-	-	-
A. araratica	90.8	3.8	3.9	-	1.5	-	-	-
A. scoparia	94.2	4.5	0.7	0.6	-	-	-	-
A. santonicum subsp. *santonicum*	91.3	1.6	2.9	-	2.0	-	2.2	-
A. santonicum subsp. *patens*	92.6	0.9	4.2	-	0.6	-	1.7	-
A. taurica var. *taurica*	93.3	3.4	3.3	-	-	-	-	-
A. taurica var. *vanensis*	91.4	2.4	5.0	0.5	0.7	-	-	-
A. spicigera	91.4	2.5	6.1	-	-	-	-	-
A. sieberi subsp. *sieberi*	91.7	2.3	5.3	-	0.7	-	-	-
A. fragrans	90.5	1.8	5.5	0.7	1.0	0.5	-	-
A. bashkalensis	94.5	2.0	3.5	-	-	-	-	-

TABLE 5. The dissimilarity matrix of the examined *Artemisia* taxa (see Table 1 for taxon abbreviation).

Taxa	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	0.551	0	-	-	-	-	-	-	-	-	-	-	-	-	-
3	0.623	0.571	0	-	-	-	-	-	-	-	-	-	-	-	-
4	0.570	0.572	0.478	0	-	-	-	-	-	-	-	-	-	-	-
5	0.555	0.552	0.462	0.343	0	-	-	-	-	-	-	-	-	-	-
6	0.535	0.529	0.517	0.463	0.484	0	-	-	-	-	-	-	-	-	-
7	0.674	0.571	0.524	0.522	0.546	0.552	0	-	-	-	-	-	-	-	-
8	0.676	0.586	0.434	0.386	0.436	0.441	0.577	0	-	-	-	-	-	-	-
9	0.482	0.546	0.457	0.341	0.373	0.493	0.586	0.475	0	-	-	-	-	-	-
10	0.538	0.586	0.455	0.265	0.308	0.493	0.545	0.470	0.214	0	-	-	-	-	-
11	0.663	0.512	0.554	0.549	0.522	0.569	0.404	0.613	0.607	0.570	0	-	-	-	-
12	0.638	0.626	0.538	0.431	0.495	0.607	0.429	0.601	0.510	0.466	0.535	0	-	-	-
13	0.546	0.604	0.474	0.354	0.221	0.48	0.553	0.453	0.385	0.325	0.524	0.483	0		-
14	0.643	0.591	0.505	0.401	0.477	0.609	0.400	0.564	0.478	0.429	0.524	0.142	0.483	0	-
15	0.616	0.575	0.479	0.302	0.406	0.461	0.523	0.384	0.458	0.404	0.550	0.524	0.414	0.503	0
16	0.716	0.571	0.553	0.543	0.520	0.624	0.543	0.606	0.612	0.574	0.471	0.457	0.554	0.474	0.49
17	0.639	0.438	0.525	0.427	0.402	0.507	0.522	0.493	0.503	0.455	0.456	0.521	0.461	0.501	0.42
18	0.606	0.413	0.544	0.453	0.436	0.534	0.545	0.507	0.527	0.480	0.487	0.552	0.495	0.526	0.45
19	0.604	0.630	0.535	0.521	0.441	0.466	0.474	0.557	0.552	0.512	0.484	0.547	0.421	0.577	0.51
20	0.642	0.604	0.587	0.572	0.542	0.518	0.484	0.645	0.601	0.564	0.444	0.549	0.523	0.585	0.56
21	0.644	0.607	0.517	0.410	0.482	0.603	0.409	0.576	0.491	0.443	0.526	0.069	0.479	0.076	0.50

22	0.661	0.554	0.547	0.489	0.450	0.611	0.531	0.614	0.558	0.519	0.446	0.371	0.485	0.402	0.53
23	0.537	0.619	0.485	0.359	0.220	0.464	0.556	0.469	0.397	0.340	0.562	0.454	0.182	0.484	0.41
24	0.668	0.556	0.510	0.457	0.477	0.568	0.403	0.572	0.527	0,483	0.522	0.241	0.479	0.216	0.50
25	0.580	0.619	0.572	0.411	0.414	0.489	0.590	0.566	0.493	0,451	0.588	0.416	0.383	0.473	0.46
26	0.548	0.577	0.500	0.492	0.456	0.467	0.441	0.571	0.509	0,466	0.455	0.561	0.457	0.550	0.49

FIGURES

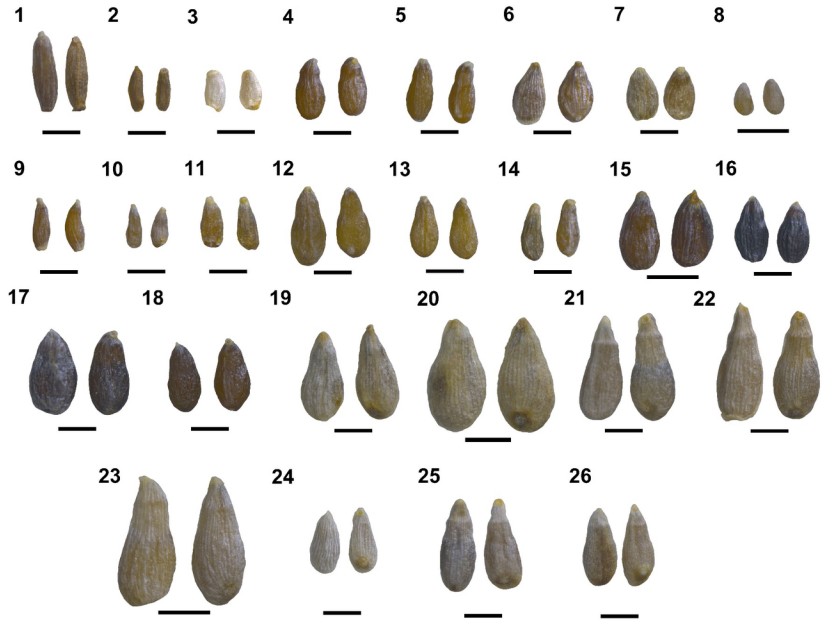

FIGURE 1. The achenes of the examined Artemisia taxa;**1:** *A. vulgaris* , **2:** *A. verloitorum* ,**3:** *A. abrotanum* , **4:** *A. austriaca* ,**5:** *A. incana* , **6:** *A. armeniaca* ,**7:** *A. chamaemelifolia* , **8:** *A. annua* ,**9:** *A. tournefortiana* , **10:** *A. absinthium* ,**11:** *A. arborescens* , **12:** *A. splendens* ,**13:** *A. alpina* , **14:** *A. haussknechtii* ,**15***: A. campestris,* **16***: A. marschalliana* , **17:** *A. araratica* , **18:** *A. scoparia* , **19:** *A. santonicum* subsp. *santonicum* ,**20:** *A. santonicum* subsp. *patens* , **21:***A. taurica* var. *taurica* , **22:** *A. taurica*var. *vanensis* , **23:** *A. spicigera* , **24:***A. sieberi* subsp. *sieberi* , **25:** *A. fragrans*and **26:** *A. bashkalensis* (Scale bars= 1 mm)

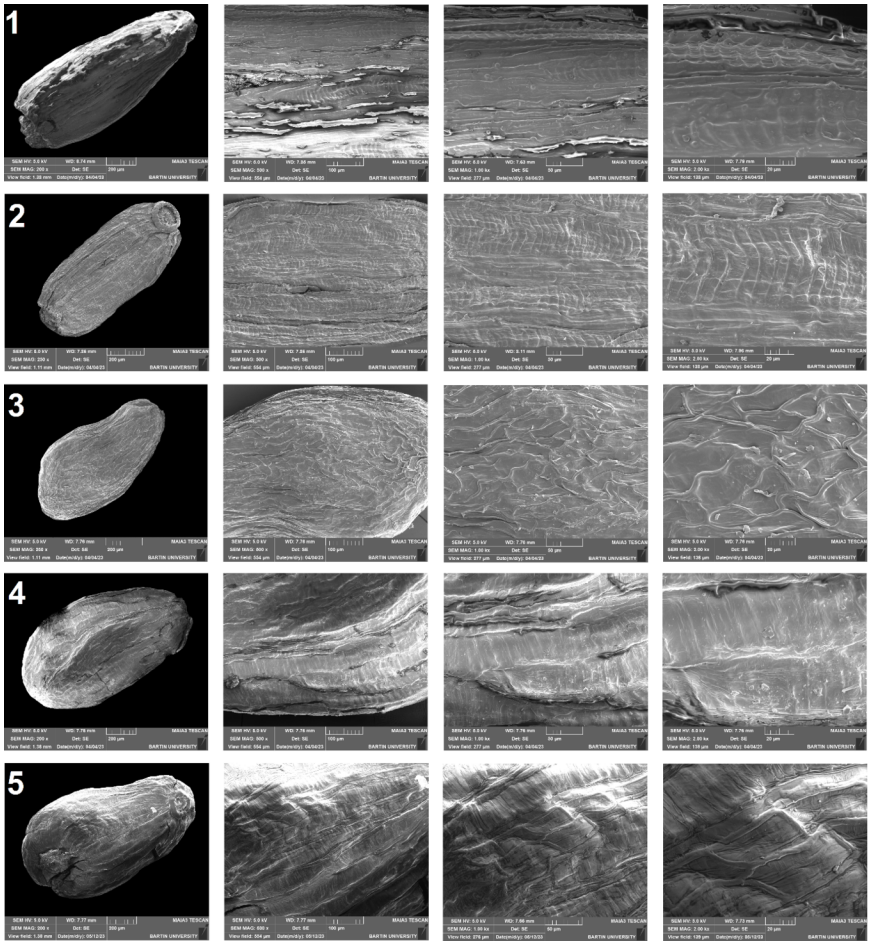

FIGURE 2. SEM pictures of the achene surfaces of the examined*Artemisia* taxa: **1:** *A. vulgaris* , **2:***A. verloitorum* , **3:** *A. abrotanum* , **4:***A. austriaca* and **5:** *A. incana.*

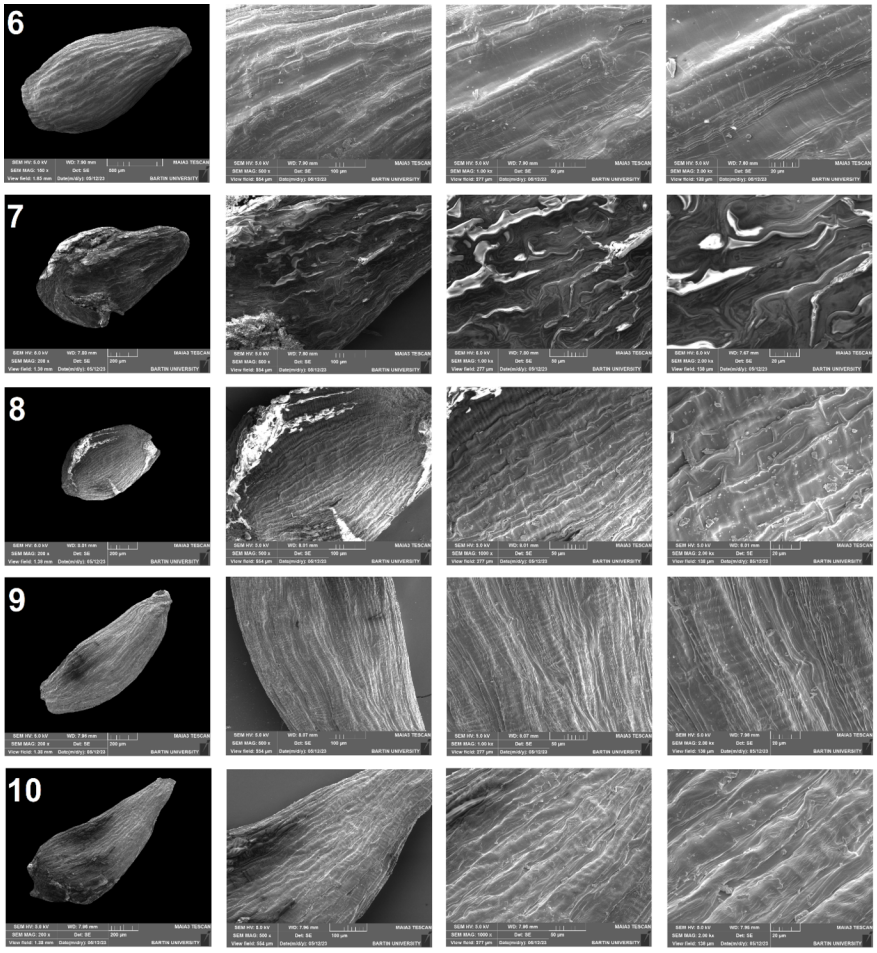

FIGURE 2. SEM pictures of the achene surfaces of the examined*Artemisia* taxa: **6:** *A. armeniaca* , **7:***A. chamaemelifolia* , **8:** *A. annua* , **9:***A. tournefortiana* and **10:** *A. absinthium.*

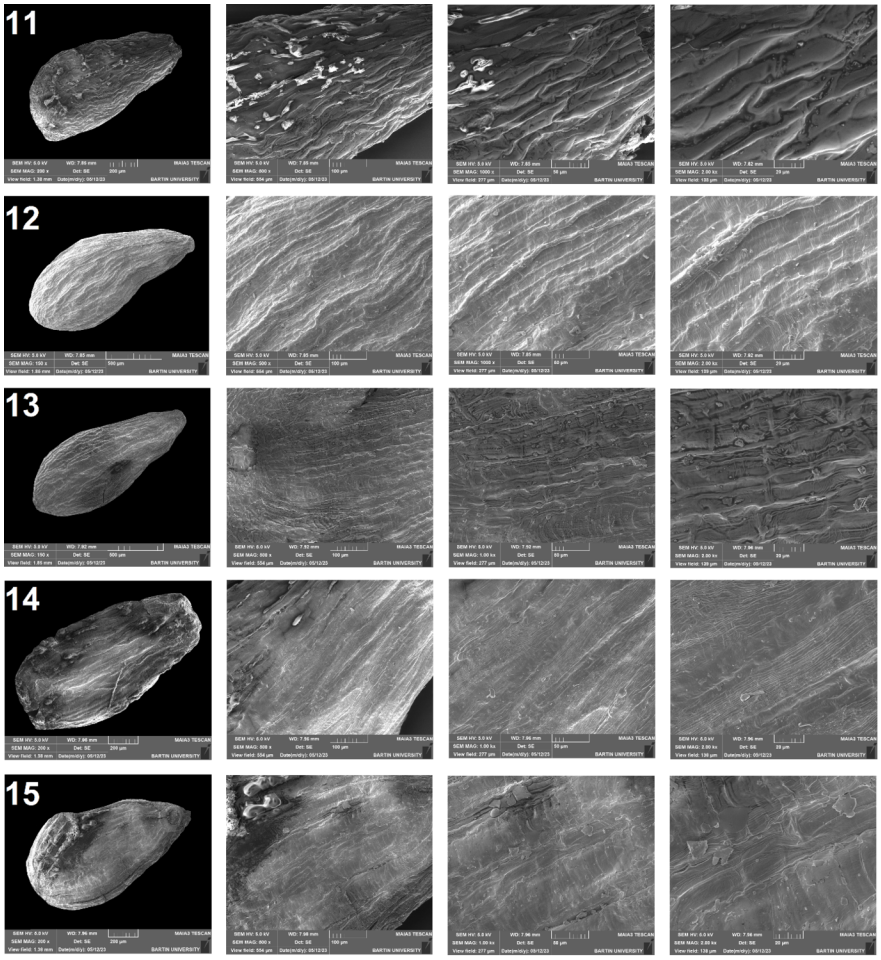

FIGURE 2. SEM pictures of the achene surfaces of the examined *Artemisia* taxa:**11:** *A. arborescens* , **12:** *A. splendens* ,**13:** *A. alpina* , **14:** *A. haussknechtii* and**15***: A. campestris.*

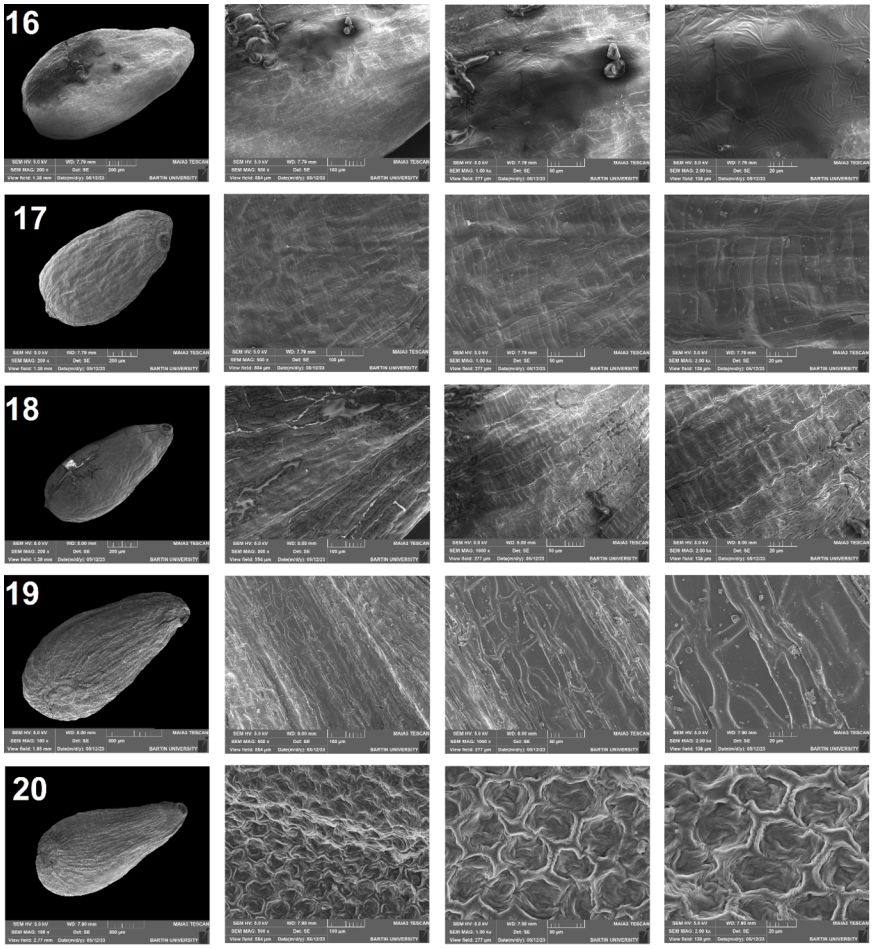

FIGURE 2. SEM pictures of the achene surfaces of the examined*Artemisia* taxa: **16***: A. marschalliana* ,**17:** *A. araratica* , **18:** *A. scoparia* ,**19:** *A. santonicum* subsp. *santonicum* and**20:** *A. santonicum* subsp. *patens*

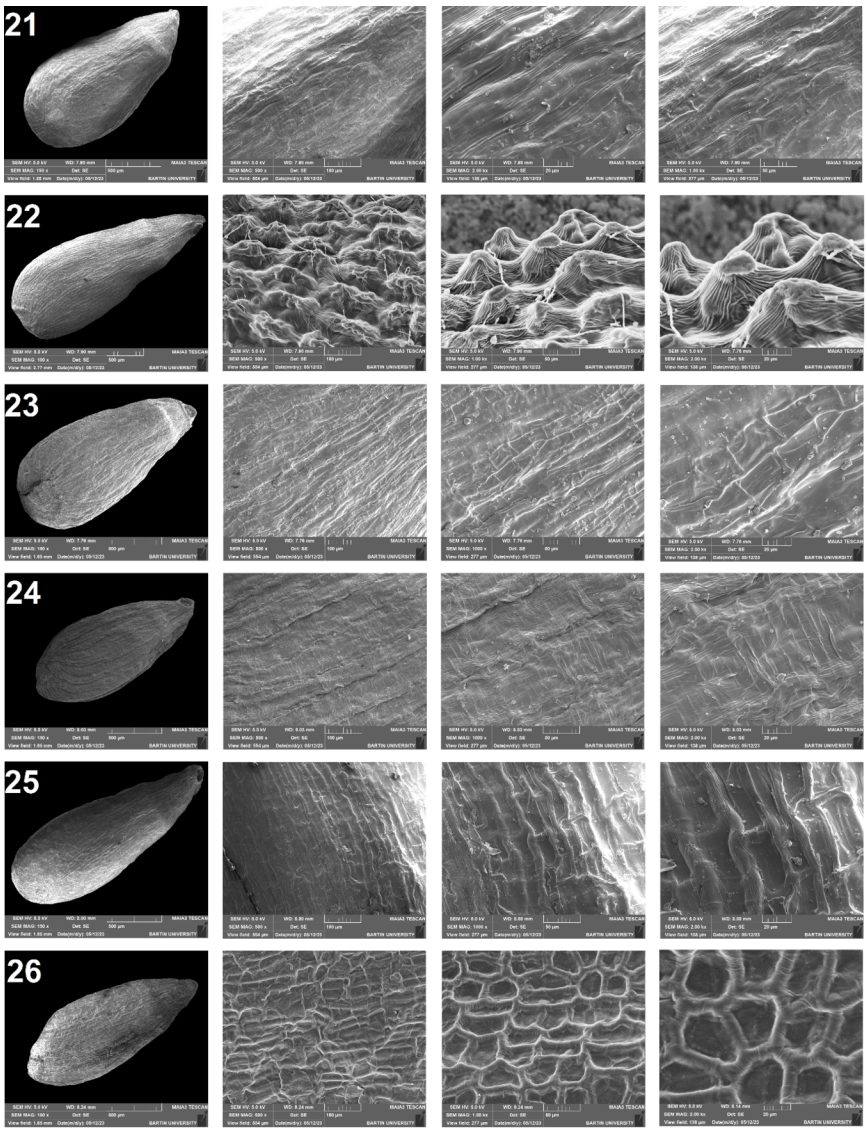

FIGURE 2. SEM pictures of the achene surfaces of the examined*Artemisia* taxa: **21:** *A. taurica* var.*taurica* , **22:** *A. taurica* var. *vanensis* ,**23:** *A. spicigera* , **24:** *A. sieberi* subsp.*sieberi* , **25:** *A. fragrans* and **26:** *A. bashkalensis.*

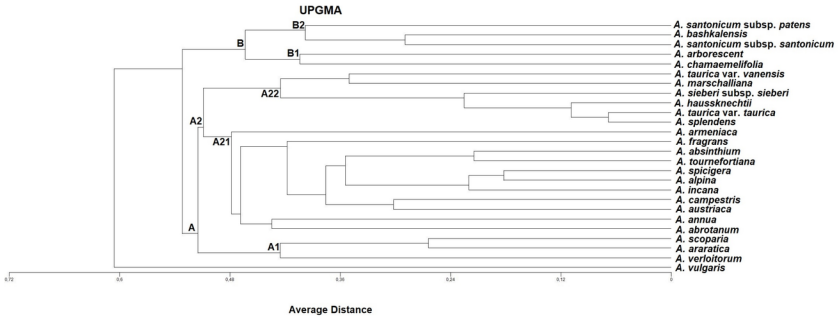

FIGURE 3. The cluster analysis of the examined *Artemisia*taxa.

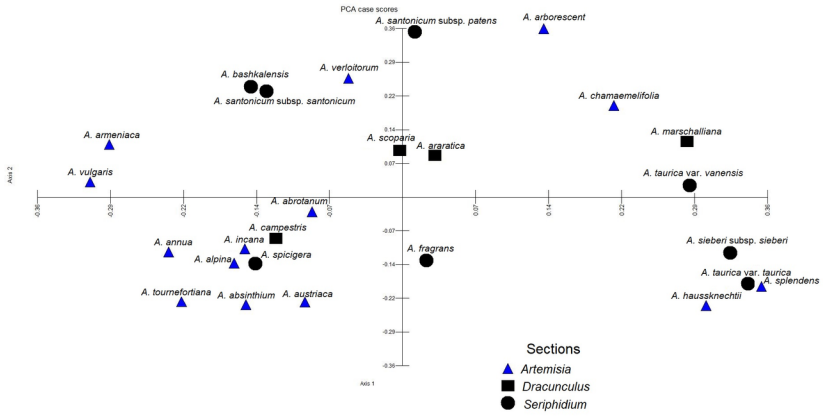

FIGURE 4. Principal component analysis of the examined*Artemisia* taxa.

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