Seed Morphological and Anatomical Structures as Taxonomy Tool for Turkish Hyacinthella Schur (Asparagaceae) Taxa

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Abstract

This paper offers the first taxonomic assessment of the morphological and anatomical characters of the seeds of 11 *Hyacinthella* taxa from Turkey, 10 of them are endemic, with the cluster analysis and principal component analysis. Macro-morphologically, the outcomes display that the species are different from each other as per seed shape and size. The studied seeds are separated into 5 shapes; triangularis, circularis, ellipticus, ellipticus-late and ovatus. Seed sizes range from 1.51 mm to 3.06 mm in length, from 1.06 mm to 2.04 mm in width. Micro-morphologically, seed surface ornamentation is grouped into 7 types: rugose, reticulate-pusticulate, verrucate, tuberculate, ruminate, reticulate-foveate and alveolate. The most common type is verrucate, as rugose (in *H. acutiloba*), reticulate-pusticulate (in *H. campanulata*), ruminate (in *H. hispida*) and reticulate-foveate (in *H. lineata*) ornamentation types are taxon specific. Anatomically, testa structures of the studied seeds are usually consisted of 1 layer, the epidermis, formed in the sclerenchymatous tissue. The epidermis layer demonstrates significant differences in cell form, comprising of crushed, flat or polygonal cells, in 1–7 layers, and has surged or straight wall form. Also, the subepidermis layer is found in some of the studied taxa (*H. glabrescens*, *H. heldreichii* and *H. siirtensis*). The thickness of testa layers varies between 15.77 µm (in *H. micrantha*) and 49.76 µm (in *H. campanulata*). In the systematics of the genus *Hyacinthella*, the seed morphological and anatomical characters are very important characteristics that reveal inter-specific relationships among the studied species. Also, a dichotomous key is provided for the identification of the examined species based on seed characters.

Introduction

The family Asparagaceae is of 7 subfamilies, 114 genera and circa 2900 taxa in worldwide (Chase et al 2009; Christenhusz and Byng 2016). It represents with 19 genera and 182 species in the Flora of Turkey (Güner et al 2012). *Hyacinthella* Schur consists of 17 species, 1 subspecies and 1 variety belonging to 2 sections, and it disperses from Balkan Peninsula to Turkey and along northern Iran (Persson and Wendelbo 1981; Speta 1998; Govaerts et al 2021). According to Mathew (1987), Turkey is one of the diversity centers of the genus. The genus was placed within the family Liliaceae in its first classification based on limited macromorphological characters. As a result of molecular studies, *Hyacinthella* was first transferred to the family Hyacinthaceae and then to the family Asparagaceae (APG III 2009).

The genus *Hyacinthella* was reported as 10 taxa with 9 species and 1 hybrid species (*H. micrantha* (Boiss.) Chouard × *H. heldreichii* (Boiss.) Chouard.) to Flora of Turkey (Persson and Wendelbo 1984). Subsequently, it was defined that the samples belonging to the declared hybrid species were not hybrid and they were new species (*H. lazulina* K. Perss. and Jim. Perss.) (Persson and Persson 1992). Afterwards, another new species was published from Turkey (Persson 2000). *Hyacinthella millingenii* (Post) Feinbrun endemic to Cyprus was involved in the latest update flora checklist performed in Turkey; however, no specific specimens or location of the species was included, and it was noted that "the status of the species needs to be clarified" (Ekim 2012). Therefore, the number of taxa belonging to the genus *Hyacinthella* in Turkey has updated as 11 species, including 10 endemic species.

The main characters utilized in taxonomy of the genus *Hyacinthella* are pedicel presence-absence and pedicel length, flower color, lobe-flower ratio, anther-filament ratio, flower shape, leaf shape, leaf edge, secondary leaf to primary leaf ratio (Persson and Wendelbo 1984; Persson 2000). According to Persson and Wendelbo (1984), the characters used in the taxonomy of the genus may not be consistent in terms of features such as color, length and number. Accordingly, new distinctive and consistent characters are needed to contribute to the systematics of the genus.

The seeds are of the characteristic morphological features such as shape, colour, size and surface ornamentation types, and they can offer valuable data in of the elucidating taxonomic problems and in of the establishing systematic relationships (Barthlott 1981; Karaismailoğlu and Erol 2018). Besides, the anatomical characters are typically as helpful as morphological characters for plant taxonomy, and they often are valuable in the separation of closely correlated taxa (Karamian et al 2012; Karaismailoğlu 2015). The importance of the seed in genera within the family Asparagaceae has often been overlooked. The only study showing the importance of comprehensive seed morphology and anatomy within the family was carried out by Eroğlu et al. (2021) on Turkish *Muscari*, which is a genus closely related to *Hyacinthella*. In the mentioned study, it was seen that seed morphological and anatomical characters easily distinguished 36 *Muscari* taxa from each other.

Some morphological, anatomical, cytological and palynological studies on some taxa of the genus *Hyacinthella* have been made, so far (Feinbrun 1961; Persson and Wendelbo 1981, 1982; Puizina et al 2003; Selvi et al 2008; Yetişen et al 2012; Tekin and Meriç 2013; Tzonev and Panovska 2017; Doğu 2019). However, the morphological and anatomical characteristics of seeds have been ignored in the classification of taxa belonging to the genus. The aim of this work is to examine the morphological and anatomical characters of seeds of Turkish *Hyacinthella* taxa for the first time, and discuss the systematic practice of these characteristics.

Material And Methods

Plant materials

Species were gathered from different phytogeographical areas of Turkey and were stored at VANF (Van Yüzüncü Yıl University Herbarium). The studied species and their locations were offered in Table 1.

Table 1
The collections data of the investigated *Hyacinthella* taxa and their locations.

Species	Locality	Voucher no.
Hyacinthella acutiloba	B7 Malatya: Yazıhan, Girmana Canyon, rocky places, 1063 m, 31 March 2021.	H.Eroglu 1593
H. campanulata	C4 Konya: Meram, Konya-Beyşehir road, 5 km to Altınapa Dam, marly steppe, 1310 m. 26 May 2021.	H.Eroglu 1719
H. glabrescens	C5 Mersin: Tarsus, south of Cevizliçeşme Village, rocky *Pinus* yards, 1030 m, 17 March 2020.	H.Eroglu 1530
H. heldreichii	C3 Antalya: Korkuteli, from the old road from Antalya to Korkuteli, northwest of Güllük Mountain, *Pinus* yards, 702 m. 19 March 2020.	H.Eroglu 1532
H. hispida	C5 Mersin: Tarsus, Çamalan Village, southeast of martyrdom, *Pinus* and *Juniperus* yards, 776 m, 17 March 2020.	H.Eroglu 1529
H. lazulina	C4 Mersin: Silifke, between Demircili and İmamlı villages, 1 km to İmamlı Village, maquis yards, 718 m, 18 March 2020.	H.Eroglu 1531
H. lineata	C2 Denizli: Honaz, south of Gürleyik Village, around of marble quarry, marly steppe, 424 m, 20 March 2020.	H.Eroglu 1533
H. micrantha	A4 Kastamonu: Kastamonu University Kuzeykent campus, around of football stadium, *Pinus* yards, 820 m, 18 March 2019.	H.Eroglu 1450
H. nervosa	C6 Gaziantep: Şahinbey, Gaziantep-Kilis road, 1 km from Küçükkızılhisar, stony-rocky steppe, 859 m, 16 March 2020.	H.Eroglu 1527
H. siirtensis	C8 Mardin: Artuklu, between Mardin and Diyarbakır, Akresta pass, Hut Farm road, streamside, steppe, 1138 m, 16 March 2020.	H.Eroglu 1524
H. venusta	C4 Konya; between Taşkent and Başyayla, Bolay highland, roadside, steppe, 1677 m, 26 May 2021.	H.Eroglu 1717

Macro And Micro-morphological Analysis

Macro-morphological structures of the seeds containing colour, shape and size were studied with 100 seeds of 10 specimens per species using a Leica EZ4 binocular microscope with a HD camera (Fig. 1, Table 2). Micro-morphological structures of the seeds including surface ornamentation, anticlinal and periclinal cell walls, and epidermal cells were analyzed with a Scanning Electron Microscope (SEM) (Fig. 2, Table 2). Firstly, seeds were positioned on the stubb with a carbon tape and covered with gold, then observed with a Zeiss LEO 440 SEM (Karaismailoğlu 2015).

Table 2
The seed morphological characters of the studied Hyacinthella taxa.

Taxa	Color	Shape	Seed sizes		L/W	Seed surface	Anticlinal	Periclinal	Epidermal
			L (mm)	W (mm)		ornamentation	cell wall	cell wall	cell structure
H. acutiloba	Black	Triangularis	2.58 ± 0.10	1.94 ± 0.12	1.32	Rugose	Unclear	Unclear	Unclear
H. campanulata	Black	Circularis	1.51 ± 0.08	1.27 ± 0.06	1.18	Reticulate-Pusticulate	Raised	Concave	Polygonal cells
H. glabrescens	Black	Ellipticus	2.43 ± 0.12	1.85 ± 0.10	1.31	Verrucate	Flat	Convex	Unclear
H. heldreichii	Black	Ovatus	1.95 ± 0.06	1.55 ± 0.12	1.25	Tuberculate	Sunken	Concave	Alveolar cells
H. hispida	Black	Triangularis	1.98 ± 0.04	1.59 ± 0.06	1.24	Ruminate	Unclear	Unclear	Unclear
H. lazulina	Black	Ellipticus-late	2.09 ± 0.04	1.73 ± 0.08	1.20	Verrucate	Flat	Convex	Unclear
H. lineata	Black	Ovatus	1.59 ± 0.06	1.06 ± 0.04	1.50	Reticulate-Foveate	Raised	Concave	Polygonal and alveolar cells
H. micrantha	Brown	Ovatus	2.01 ± 0.04	1.48 ± 0.06	1.35	Alveolate	Raised	Concave	Alveolar cells
H. nervosa	Black	Ovatus	1.82 ± 0.08	1.35 ± 0.04	1.34	Verrucate	Flat	Convex	Unclear
H. siirtensis	Black	Triangularis	3.06 ± 0.12	2.04 ± 0.08	1.50	Alveolate	Raised	Concave	Alveolar cells
H. venusta	Black	Ellipticus-late	1.80 ± 0.06	1.61 ± 0.08	1.11	Tuberculate	Sunken	Concave	Alveolar cells

L = length, W = width

Anatomical Analysis

The examination of the seed anatomical structures was performed with dry herbarium materials. Cross-sections were obtained from the middle of the seed with a fully automatic microtome (Thermo Shonda Met Finesse, Thermo). They were treated with a series of alcohol and xylene, stained with hematoxylin and eosin-Y in a staining device (ASC 720 Medite) and covered with using Entellan (Fig. 3, Table 3) (Karaismailoğlu 2015; Karaismailoğlu and Erol 2018). Anatomical structures were surveyed with an Olympus CX31 light microscope and Kameram Imaging Software (KAMERAM12 CCD, Argenit Micro System Ltd., Turkey).

The terminology utilized for seed morpho-anatomical features is suitable with Stearn (1985).

Statistical analysis

Clustering of taxa was made with utilizing the grouping analysis method (UPGMA) in MultiVariate Statistical Package (MVSP) as per the 42 characteristics in Tables 2–3 (Fig. 4). The characters in statistical analysis were utilized seed colour: black (1), brown (2); seed shape: triangularis (3), circularis (4), ellipticus (5), ovatus (6), ellipticus-late (7); seed size: length (8), width (9), length/width (10); seed ornamentation type: rugose (11), reticulate-pusticulate (12), verrucate (13), tuberculate (14), ruminate (15), reticulate-foveate (16), alveolate (17); anticlinal cell wall: unclear (18), flat (19), raised (20), sunken (21); periclinal cell wall: unclear (22), concave (23), convex (24); epidermal cell structure: unclear (25), polygonal (26), alveolar (27); anatomical structure of epidermis: crushed (28), flat (29), polygonal (30); presence of subepidermis layer (31); anatomical structure of subepidermis: flat (32), polygonal (33); testa thickness (34); cotyledon size: length (35), width (36); embryo shapes: elongated (37), ellipticus (38), ovatus (39), circularis (40); embryo size: length (41), width (42). The dissimilarity matrix of the examined species was formed with MVSP (Kovach 2007) (Table 4). A dendrogram was formed. Also, the cophenetic correlation coefficient was calculated to explain the relation between the dendrogram and dissimilarity matrix (Table 4, Fig. 4).

Table 3 The seed anatomical characters of the examined *Hyacinthella* taxa

Taxa	Epidermis layers			Cotyledon sizes		Embryo	Embryo sizes	
	Epidermis structures	Subepidermis structures	Thickness (μm)	L (μm)	W (μm)	shape	L (μm)	W (μm)
H. acutiloba	3-5 layers, crushed cells	-	32.59±1.44	1851.49±4.97	1279.35±4.88	Elongated	859.31±1.35	297.14±1.23
H. campanulata	5-7 layers, crushed cells	-	49.76±2.31	1913.63±11.86	1605.52±10.79	Ellipticus	487.92±3.08	339.26±4.88
H. glabrescens	3-4 layers, crushed cells	1 layer, flat cells	39.25±4.99	1652.47±5.70	1327.18±8.64	Elongated	940.25±4.95	290.69±3.29
H. heldreichii	4-5 layers, flat cells	1 layer, flat cells	44.08±1.16	1928.54±2.65	1428.39±3.27	Ovatus	446.08±2.21	152.10±1.08
H. hispida	2-3 layers, flat cells	-	25.61±0.88	1975.48±7.29	1425.76±3.15	Ellipticus	245.17±1.26	314.22±3.14
H. lazulina	1-2 layers, flat cells	-	20.13±0.53	1869.36±3.89	1196.25±2.76	Elongated	532.15±1.43	180.16±0.89
H. lineata	2-3 layers, crushed cells	-	29.89±1.35	1854.82±6.55	1441.38±4.84	Elongated	901.66±2.42	193.51±1.35
H. micrantha	1 layer, flat cells	-	15.77±0.92	1679.51±3.64	856.44±5.89	Ovatus	484.19±1.29	179.22±2.11
H. nervosa	2 layers, polygonal cells	-	26.14±0.99	1964.69±2.88	1409.22±2.54	Ellipticus	625.11±0.95	368.49±3.07
H. siirtensis	2-3 layers, flat cells	1-2 layers, polygonal cells	47.91±1.27	1848.44±3.71	1382.63±3.66	Elongated	753.04±1.83	195.66±2.48
H. venusta	3-4 layers, flat cells	-	36.29±1.56	1792.35±3.24	1231.37±1.09	Circularis	471.66±2.05	332.32±2.59

L=length, W=width

Table 4 The dissimilarity matrix of the examined taxa

Taxa	1	2	3	4	5	6	7	8	9	10	11
H. acutiloba (1)	0	-	-	-	-	-	-	-	-	-	-
H. campanulata (2)	500.259	0	-	-	-	-	-	-	-	-	-
H. glabrescens (3)	220.327	593.938	0	-	-	-	-	-	-	-	-
H. heldreichii (4)	469.139	261.575	591.528	0	-	-	-	-	-	-	-
H. hispida (5)	643.679	310.305	773.267	263.080	0	-	-	-	-	-	-
H. lazulina (6)	357.922	444.546	493.273	257.240	405.344	0	-	-	-	-	-
H. lineata (7)	197.022	472.466	254.995	463.769	678.517	443.988	0	-	-	-	-
H. micrantha (8)	602.783	801.702	665.816	626.211	697.988	392.232	739.987	0	-	-	-
H. nervosa (9)	299.449	247.763	457.996	284.402	384.312	314.003	346.740	665.353	0	-	-
H. siirtensis (10)	180.315	380.598	292.653	323.504	538.976	291.533	160.999	615.634	246.873	0	-
H. venusta (11)	396.656	393.956	500.079	300.937	350.818	185.054	502.22	421.107	293.772	352.165	0

Results

Macro-morphologically, this study evaluates the seed structures of the studied species containing colour, shape and size. Seed color is black in all examined taxa except *H. micrantha*, which is of the brown seeds. The shape and size of seeds differ noticeably. The studied seeds are separated into 5 shapes; triangularis, circularis, ellipticus, ellipticus-late and ovatus. Ovatus is the most common type (noted in 4 species). However, circularis and ellipticus are typical forms for *Hyacinthella campanulata* and *H. glabrescens*, respectively. Seed sizes vary between 1.51 mm and 3.06 mm in length, and between 1.06 mm and 2.04 mm in width. As *H. siirtensis* is of the largest seeds, *H. campanulata* and *H. lineata* have the smallest seeds (Table 2, Fig. 1).

Micro-morphologically, the surface ornamentation, anticlinal and periclinal cell walls, and epidermal cell forms of the studied seeds have been assessed in this work. Seed surface ornamentation is clustered into 7 forms: rugose, reticulate-pusticulate, verrucate, tuberculate, ruminate, reticulate-foveate and alveolate. The most common type is verrucate, as rugose, reticulate-pusticulate, ruminate and reticulate-foveate ornamentation types are found as taxon-specific (Table 2, Fig. 2). The rugose, reticulate-pusticulate, ruminate and reticulate-foveate ornamentation types are shown by only one taxon. The structures of the anticlinal cell walls of the examined species are unclear, raised, flat and sunken. As raised cell walls are generally observed in the reticulate-pusticulate, reticulate-foveate and alveolate ornamentation types, the tuberculate ornamentation type is found where epidermal cells are surrounded by sunken walls. Rugose and ruminate types are connected with unclear type (Table 2). There is no connection between concave or convex periclinal cell walls and surface ornamentation type. In the ruminate and rugose forms are seen only unclear periclinal cells. Also, the shape of epidermal cells on the seed surface has exhibited variety. It is polygonal, alveolar and unclear structures. The most common cell type are unclear and alveolar, as polygonal is fairly rare (Table 2).

The anatomical characters of the seeds are shown in Fig. 3 and Table 3. Testa structures of the studied seeds are usually consisted of 1 layer, the epidermis, formed in the sclerenchymatous tissue. The epidermis layer demonstrates significant differences in cell form, comprising of crushed, flat or polygonal cells, in 1–7 layers, and has surged or straight wall form. The most common kind are flat and crushed while the fewest one is the polygonal type (Table 3, Fig. 3). Also, the subepidermis layer is found in some of the studied taxa (*H. glabrescens*, *H. heldreichii* and *H. siirtensis*). The subepidermis layer comprises of flat or polygonal cells in 1–2 layers. The most frequently found type is flat, however; the rarest one is polygonal type. The thickness of testa layers ranges from 15.77 µm (in *H. micrantha*) to 49.76 µm (in *H. campanulata*). The cotyledon sizes vary between 1652.47 µm (in *H. glabrescens*) and 1913.63 µm (in *H. campanulata*) in length, between 856.44 µm (in *H. micrantha*) and 1605.22 µm (in *H. campanulata*) in width. In addition to these findings, embryo sizes of the seeds range from 245.17 µm (in *H. hispida*) to 940.25 µm (in *H. glabrescens*) in length, from 152.10 µm (in *H. heldreichii*) to 368.49 µm (in *H. nervosa*) in width, and embryos in seeds are formed elongated (*H. acutiloba*, *H. glabrescens*, *H. lazulina*, *H. lineata* and *H. siirtensis*), elliptical (*H. campanulata*, *H. hispida* and *H. nervosa*), oval (*H. heldreichii* and *H. micrantha*) and circular (*H. venusta*) in shapes (Fig. 3).

The numerical assessment of the seed morpho-anatomical characters permits the formation of a dendrogram, which reveals the differences-similarities among the studied species. A dendrogram is built as a consequence of the cluster analysis of the studied taxa of *Hyacinthella* based on the variation of 42 characteristics in 11 species. The cophenetic correlation coefficient is determined to learn the relative between the dendrogram and dissimilarity matrix (Fig. 4, Table 4). The cophenetic correlation between the dissimilarity matrix and dendrogram has been computed as 0.64, representing a good match.

Our cluster analysis has separated the taxa into 2 main groups of A and B: Cluster A comprises *H. glabrescens*, *H. siirtensis*, *H. lineata* and *H. acutiloba*. Cluster B1 includes *H. hispida*, *H. heldreichii*, *H. nervosa* and *H. campanulata*. Cluster B2 includes *H. venusta* and *H. lazulina. H. micrantha* has produced a clade outside these clusters in the dendrogram (Fig. 4). *H. siirtensis* and *H. lineata* are the most closely related species (dissimilarity coefficient: 160.999), as *H. micrantha* and *H. campanulata* are the most distantly related species (dissimilarity coefficient: 801.702) (Table 4). Cluster B has the highest number of species compared to another cluster (Fig. 5).

Discussion

The morphological structures of the seeds present significant data in relation to evolutionary relations of the flowering plants (Corner 1976; Karaismailoğlu and Erol 2018). However, seed morpho-anatomical characters have so far not been comprehensively applied to clarify inter-species relations within genera of the family Asparagaceae, except the genus *Muscari* (Eroğlu et al 2021). This is the first research to exhibit the morpho-anatomical structures of the seeds of the genus *Hyacinthella*, and it will be a model for following surveys for various closely related genera.

The macro-morphological characteristics of the seeds demonstrate differences among the studied *Hyacinthella* species (Fig. 1 and Table 2). The species studied in our research are not very distinct with regard to seed color. Black dominates in the genus, whereas *H. micrantha* has brown seeds unlike other species. *H. glabrescens* (ellipticus)-*H. hispida* (triangularis), *H. campanulata* (circularis)-*H. heldreichii* (ovatus), *H. acutiloba* (triangularis)-*H. lineata* (ovatus) and *H. siirtensis* (triangularis)-*H. nervosa* (ellipticus-late) taxa are more or less similar in terms of leaf, scape and flower structures, but they can be simply distinguished with applying of seed shape.

The researches including surface micro-morphological structures of seeds in various plant families provide systematically useful information (Karaismailoğlu 2015; Candan et al 2016; Karaismailoğlu and Erol 2018; Karaismailoğlu et al. 2018; Karaismailoğlu and Güner 2019; Eroğlu et al 2021; Şirin and Karaismailoğlu 2021; Gavrilović and Janaćković 2022). Also, the importance and effectiveness of scanning electron microscopy in clarifying of taxonomic difficulties and in characterizing of species have been highlighted by many researchers (Heywood 1971; Barthlott 1981; Eroğlu et al 2021). However, there are few papers on the significance of seed micromorphology in the family Asparagaceae (Yıldırım 2015; Eroğlu et al 2021). This research on 11 *Hyacinthella* species reveals that seed micro-structures are helpful characters in discrimination of the taxa within the family, as in the genus *Muscari* (Eroğlu et al 2021). All of the examined species have been analyzed for the first time. We noted seven seed surface ornamentation types in this work. In the genus, the most common seed ornamentation type is verrucate. Unlike this work, reticulate or reticulate-areolate ornamentation forms have been frequently found among species from many angiosperm families (Barthlott 1981; Erol et al 2006; Bona 2013; Karaismailoğlu 2015; Özbek et al 2018; Karaismailoğlu et al 2018; Karaismailoğlu and Erol, 2018). The rugose (in *H. acutiloba*), reticulate-pusticulate (in *H. campanulata*), ruminate (in *H. hispida*) and reticulate-foveate (in *H. lineata*) ornamentation types are taxon specific. Also, ornamentation types are effective in distinguishing closely related taxa from each other, such as *H. glabrescens* (verrucate)-*H. hispida* (ruminate), *H. campanulata* (reticulate-pusticulate)-*H. heldreichii* (tuberculate), *H. acutiloba* (rugose)-*H. lineata* (reticulate-foveate) and *H. siirtensis* (alveolate)-*H. nervosa* (verrucate) taxa (Fig. 2 and Table 2). Besides, past seed surface surveys have shown that the views and forms of anticlinal and periclinal cell walls are diagnostic characteristics in the creation of inter-taxa interactions (Barthlott 1981; Eroğlu et al 2021). The types of anticlinal and periclinal cell walls, and epidermal cell structures of the studies species reveal variations among taxa. The macro and micro-morphological outcomes of this investigation are separated all taxa examined from each other, and they are suitable with the former studies done with exomorphic features of seeds of the family Asparagaceae (Yıldırım 2015; Eroğlu et al 2021; Tugay et al 2021).

Testa anatomical studies are effective in answering of the taxonomical problems in many plant families (Vaughan et al 1976; Manning and Staden 1987; Meyer 1991; Karaismailoğlu 2015; Karaismailoğlu and Erol 2018; Eroğlu et al 2021). Koul et al. (2000) and Eroğlu et al. (2021) have stated that testa forms can be used as a useful characters in the discrimination of the species and in the explanation of their phylogenetic relations. This study is the first such research for the genus and is the precursor to following works. In this study, it is observed that the testa layers usually contain one layer as the epidermis in the sclerotic form. The epidermis structures display variations among the species. This 1–7 layered epidermis can compose of flat, crushed, or polygonal cells. The most common form is crushed, as the rarest is polygonal type. Some of the studied species, which are *H. glabrescens*, *H. heldreichii* and *H. nervosa*, have the subepidermis layer under epidermis occurring polygonal or flat cells. The presence of the subepidermis layer is sufficient to distinguish some closely related taxa such as *H. glabrescens*-*H. hispida*, *H. heldreichii*- *H. campanulata* and *H. nervosa*- *H. siirtensis*. Also, the differences in testa thicknesses and cotyledon sizes of the seeds of Turkish *Hyacinthella* have been detected. The most distant species are *H. micrantha* and *H. campanulata*. The embryos of *Hyacinthella* taxa display a broad variation in terms of shapes and sizes. Embryos are elongated form in *H. acutiloba*, *H. glabrescens*, *H. lazulina*, *H. lineata* and *H. siirtensis*, elliptical form in *H. campanulata*, *H. hispida* and *H. nervosa*, oval form in *H. heldreichii* and *H. micrantha*, circular form in *H. venusta*. Closely related taxa such as *H. glabrescens*-*H. hispida*, *H. campanulata*-*H. heldreichii*, *H. siirtensis*- *H. nervosa* that are morphologically similar can easily be distinguished from each other according to their embryo shape (Table 3 and Fig. 3).

In this study, the seed anatomical characters such as the structures of the epidermis and subepidermis, thickness of the testa, cotyledon sizes and embryo shapes are rather efficient and useful in separating almost all of the studied *Hyacinthella* taxa. This can be explained as follows: the seed anatomical characters are useful additional characteristics in the *Hyacinthella*, and they are able to assist in the taxonomy of this genus.

The obtained outcomes are compatible with parallel earlier works conducted on seed structure of some taxa of the genera *Crocus* L. and *Romulea* Maratti in the closely related family Iridaceae, and genus *Muscari* in the family Asparagaceae (Grilli Caiola et al 2010; Karaismailoğlu 2015; Karaismailoğlu et al 2018; Eroğlu et al 2021).

A dendrogram has been created to assess the morpho-anatomical characteristics of the seeds of Turkish *Hyacinthella* taxa with UPGMA cluster analysis (Fig. 4). The dendrogram, displaying two main clusters, is partly consistent with the results of Persson and Wendelbo (1984). According to the descriptions in the Flora of Turkey, the systematic affinity in the closely related *H. campanulata*-*H. heldreichii*, and *H. acutiloba*-*H. lineata* taxa is partially preserved. However, *H. siirtensis*-*H. nervosa* and *H. glabrescens*-*H. hispida* taxa are located in different clusters in the dendrogram (Figs. 4–5 and Table 4). In addition, *H. micrantha* has been described as an isolated species with a very different description because of its unique leaf and veining properties within the genus in the Flora of Turkey. This species has showed similar characteristics in the dendrogram created according to seed morphological and anatomical features, and formed a clade apart from 2 main clusters.

As a result, this work display that morphological and anatomical seed characteristics of Turkish *Hyacinthella* species presents significant and consistent insights into the taxonomy of species within the genus.

Key to studied *Hyacinthella* taxa, based on seed characters

1.Seed colour is brown………………………..……………………………… *H. micrantha*

1.Seed colour is black……………………………………………………………………2

2.Seed shape is circularis, ellipticus or ellipticus-late……………………………………...3

2.Seed shape is triangularis or ovatus……………………………………………………6

3.Seed shape is circularis……………..…………………………………..…. *H. campanulate*

3.Seed shape is ellipticus or ellipticus-late..……………………………………………...4

4.Ellipticus……………………………………………………………….….. *H. glabrescens*

4.Ellipticus-late……………………………………………………………………………..5

5.Seed surface ornamentation is verrucate………………………………………. *H. lazuline*

5.Seed surface ornamentation is tuberculate……………………………………….. *H. venusta*

6.Seed shape is triangularis…………………..…………………………………………….7

6.Seed shape is ovatus……………………………………………………….………………9

7.Seed surface ornamentation is alveolate……………………………………… *H. siirtensis*

7.Seed surface ornamentation is rugose or ruminate……………………………………….8

8.Rugose…………………………………………………………………….. *H. acutiloba*

8.Ruminate………………………………………………………….………. *H. hispida*

9.Seed surface ornamentation is tuberculate or verrucate…………………………………10

9.Seed surface ornamentation is alveolate or reticulate-foveate……………………………..11

10.Tuberculate……………………………………………………..………… *H. heldreichii*

10.Verrucate……………………………………………………...…………. *H. nervosa*

11.Alveolate………………………………………………………………… *H. micrantha*

11.Reticulate-foveate……………………………………………..……………… *H. lineata*

Declarations

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Contributions

M.C. Karaismailoğlu and H. Eroğlu contributed to the study conception and design. Material preparation, data collection and analysis were performed by M.C. Karaismailoğlu, S.M. Pınar, H. Eroğlu, and M. Fidan. The first draft of the manuscript was written by M.C. Karaismailoğlu, and H. Eroğlu and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

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

Competing Interest

The authors have no competing interests

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

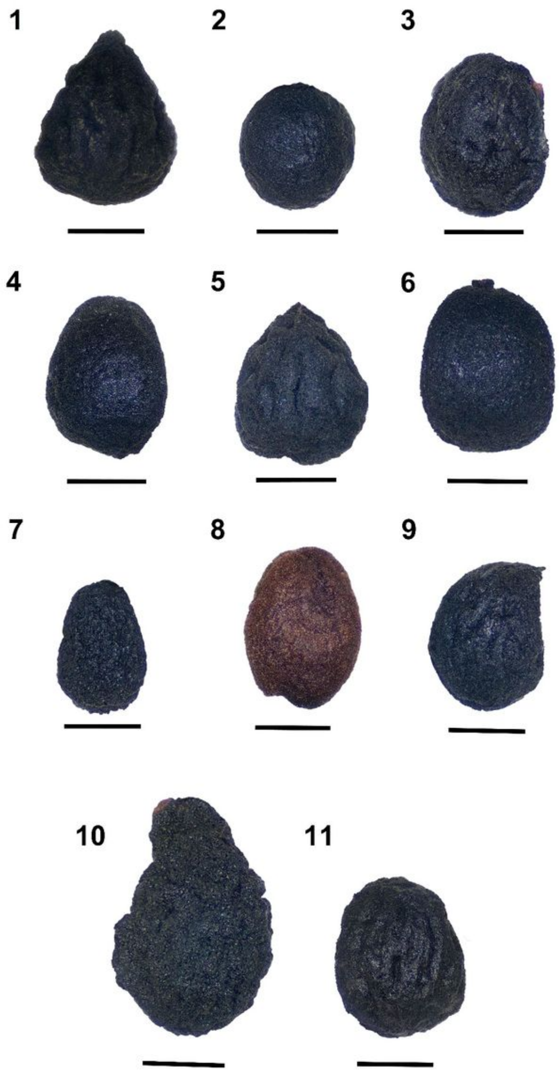

Figure 1

The seeds of the studied *Hyacinthella* taxa; **1:** *H. acutiloba*, **2:** *H. campanulata*, **3:** *H. glabrescens*, **4:** *H. heldreichii*, **5:** *H. hispida*, **6:** *H. lazulina*, **7:** *H. lineata*, **8:** *H. micrantha*, **9:** *H. nervosa*, **10:** *H. siirtensis* and **11:** *H. venusta* (Scale bars= 1 mm).

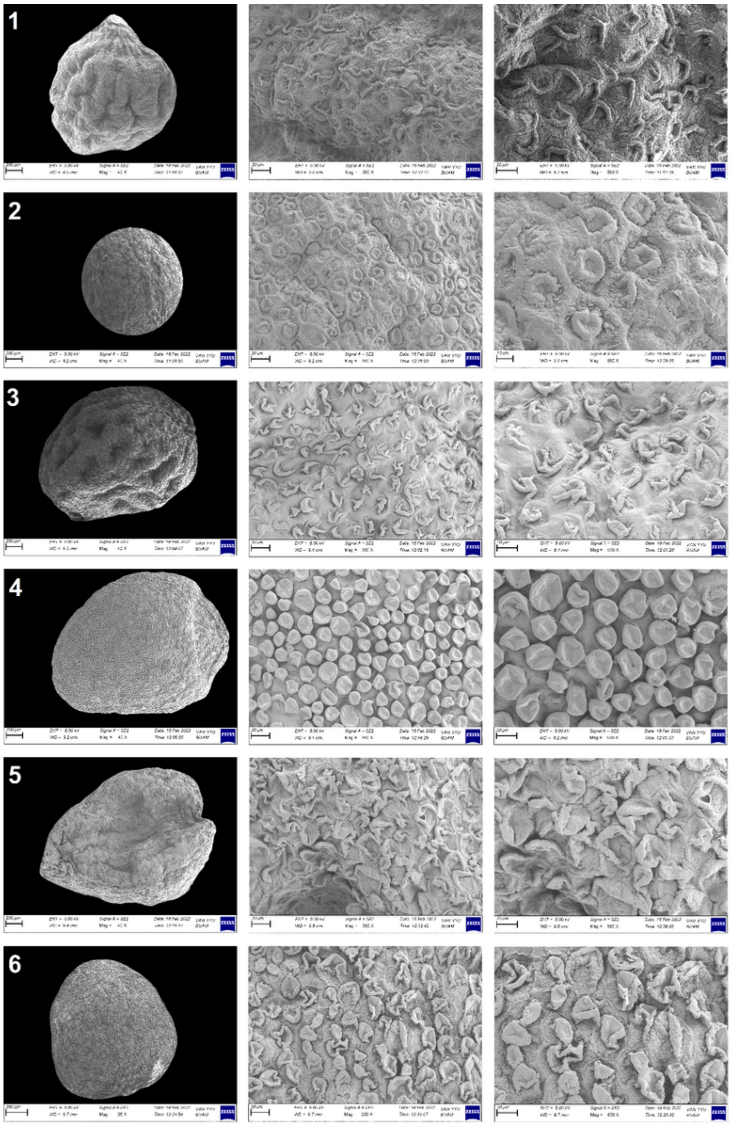

Figure 2

The SEM pictures of the examined *Hyacinthella* taxa; **1:** *H. acutiloba*, **2:** *H. campanulata*, **3:** *H. glabrescens*, **4:** *H. heldreichii*, **5:** *H. hispida*, **6:** *H. lazulina*.

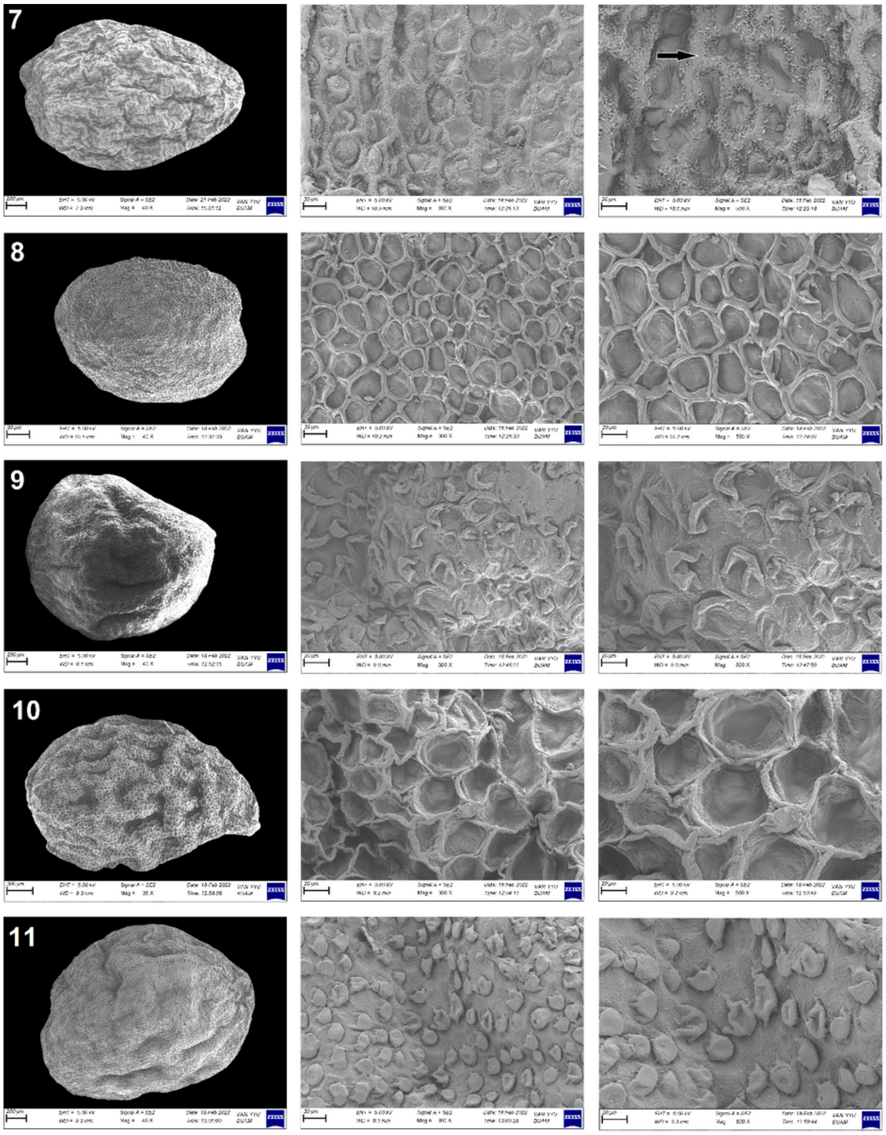

Figure 3

The SEM pictures of the examined *Hyacinthella* taxa; **7:** *H. lineata*, **8:** *H. micrantha*, **9:** *H. nervosa*, **10:** *H. siirtensis* and **11:** *H. venusta* (black arrow indicates secondary structures on the surface).

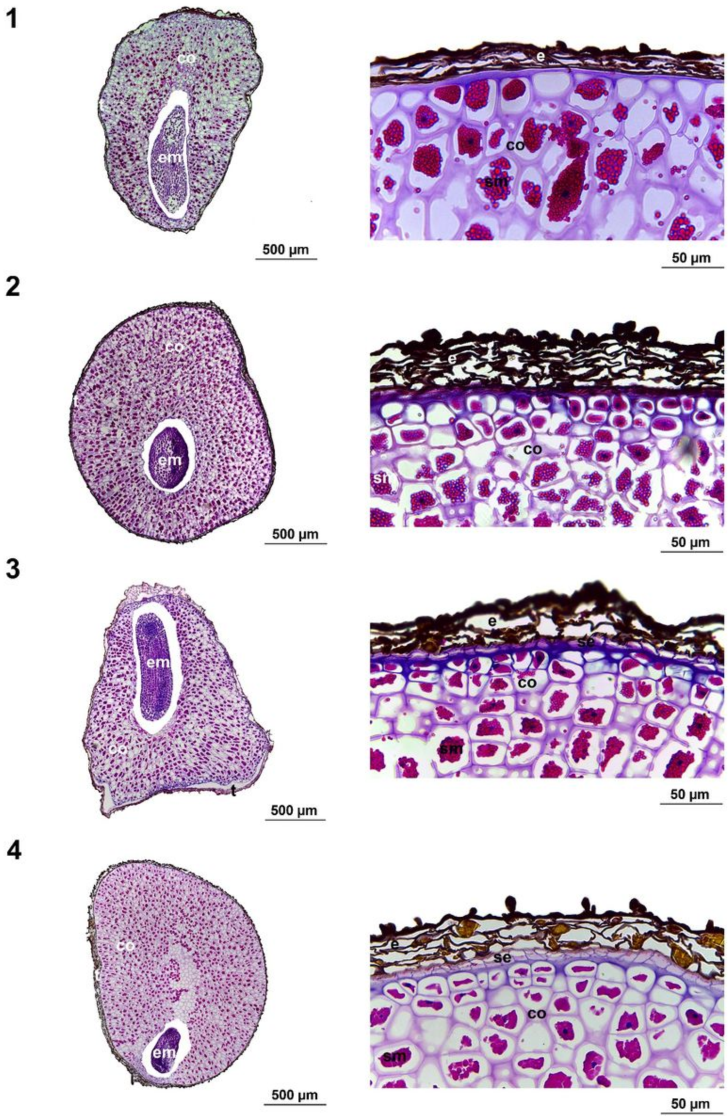

Figure 4

The seed anatomical structures of the examined *Hyacinthella* taxa; **1:** *H. acutiloba*, **2:** *H. campanulata*, **3:** *H. glabrescens*, **4:** *H. heldreichii* (t= testa, co= cotyledon, e= epidermis, se= subepidermis, sm= storage material, em= embryo).

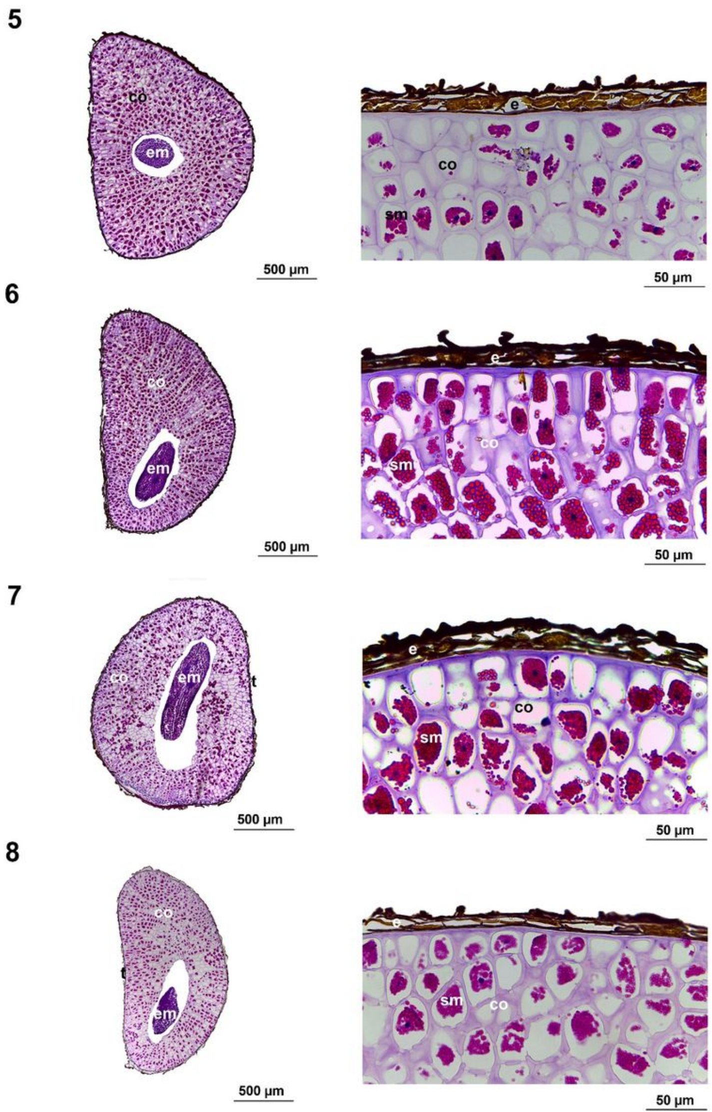

Figure 5

The seed anatomical structures of the examined *Hyacinthella* taxa; **5:** *H. hispida*, **6:** *H. lazuline*, **7:** *H. lineata*, **8:** *H. micrantha* (t= testa, co= cotyledon, e= epidermis, se= subepidermis, sm= storage material, em= embryo).

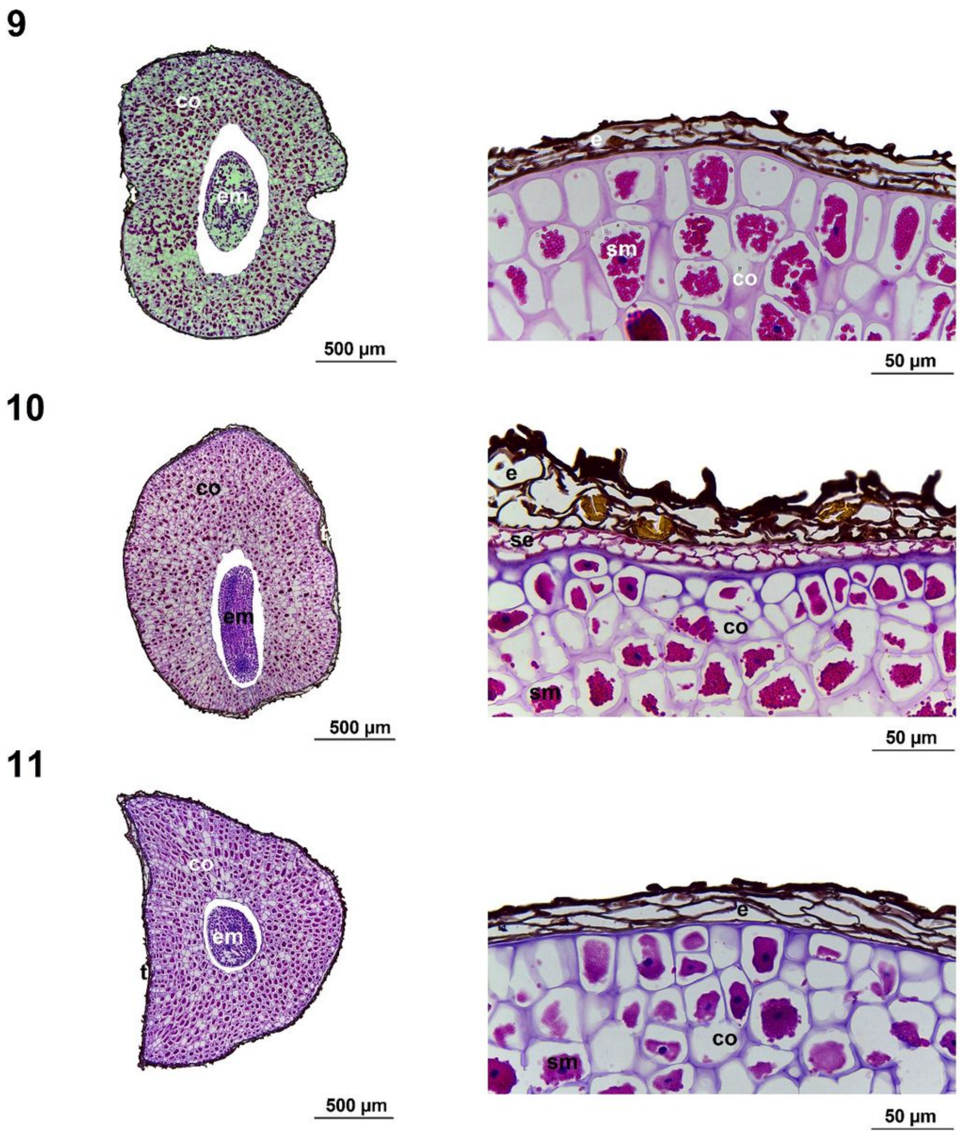

Figure 6

The seed anatomical structures of the examined *Hyacinthella* taxa; **9:** *H. nervosa*, **10:** *H. siirtensis* and **11:** *H. venusta* (t= testa, co= cotyledon, e= epidermis, se= subepidermis, sm= storage material, em= embryo).

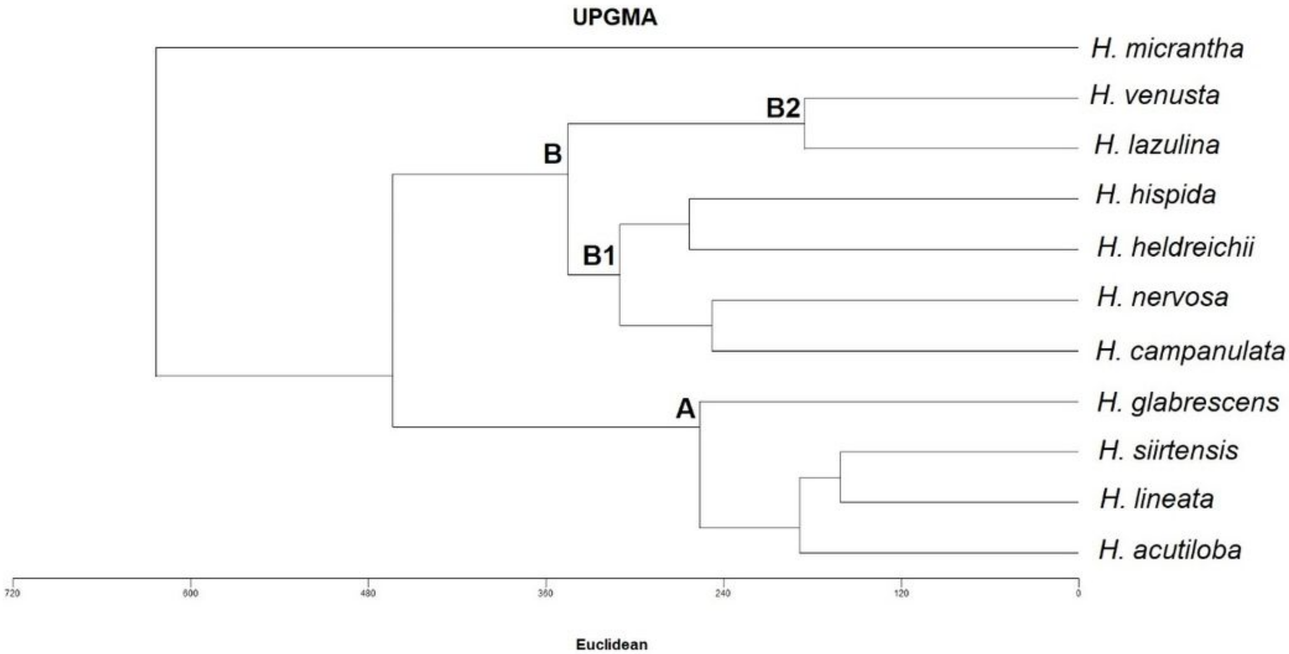

Figure 7

The dendrogram of the examined *Hyacinthella* taxa.

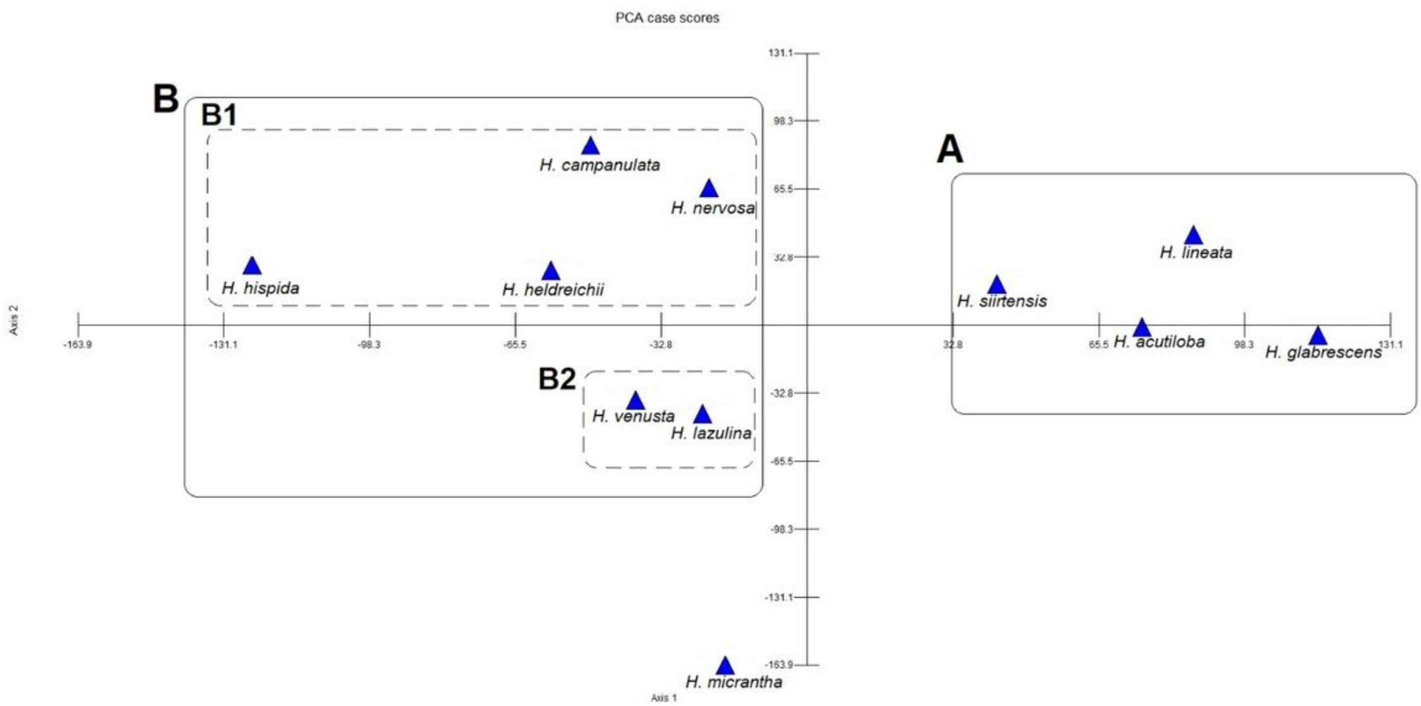

Figure 8

Principal component analysis of the examined *Hyacinthella* taxa.