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Contribution to the biosystematics of the wild *Vaccinium* species distributed in Turkey

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Summary. The genus *Vaccinium* L. (Ericaceae) consists of shrubs or dwarf shrubs species widely distributed in Southeast Asia, America, and Europe. In the present paper, members of the genus (*V. arctostaphylos*, *V. myrtillus*, *V. uliginosum*, *V. vitis-idaea*) naturally grown in Northeast Anatolia were compared in terms of leaf venation, seed micromorphology, and palynological properties based on 16 populations in detail. Present findings revealed that number of areoles (the small area bounded by secondary veinlet) is 8–9 for *V. arctostaphylos*, 5–7 for *V. myrtillus*, 3–4 for *V. uliginosum* and *V. vitis-idaea*. Scanning electron microscope observation shows that the leaf adaxial surface is fiberfluted in *V. arctostaphylos*, ruminant in *V. myrtillus*, *V. uliginosum*, and *V. vitis-idaea* due to epicuticular waxes. In addition, the seed shape of *V. arctostaphylos* in outline varies from triangular, tetragonal to oval, whereas they are lunate to reniform or falcate in the rest of the examined taxa. Epidermal cells of the seed are quadrilateral with straight anticlinal walls in *V. arctostaphylos*, however, they are most elongate rectangle with weakly undulate anticlinal walls in the rest species. The mean tetrad diameter (D) of pollen belonging to all examined species is 29.89 (21.47–43.22) µm while the lowest mean D was found in *V. uliginosum* (24.59 µm) and the highest mean D was found in *V. arctostaphylos* (36.74 µm). Principal component analysis based on 47 traits (flower: 9, leaf: 18, seed and fruit: 9 and pollen: 11) showed that the leaf length and width, teeth numbers, tetrad diameter, equatorial axis, and polar axis were the most valuable characters to distinguish the examined taxa in the present paper.

К биосистематике диких видов *Vaccinium*, распространенных в Турции

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Ключевые слова: вересковые, лист, плод, пыльцевое зерно, семя.

Аннотация. Род *Vaccinium* L. (Ericaceae) состоит из кустарниковых и кустарничковых видов, широко распространенных в Азии (гл. обр. Юго-Восточной), Америке и Европе. В настоящем исследовании было прове-

дено сравнительное изучение жилкования листьев, микроморфологии семян и палинологических особенностей 16 популяций четырёх представителей *Vaccinium* (*V. arctostaphylos*, *V. myrtillus*, *V. uliginosum*, *V. vitis-idaea*), естественно произрастающих в Северо-Восточной Анатолии. Полученные результаты показали, что для *V. arctostaphylos* характерно наличие 8–9 ареол, для *V. myrtillus* – 5–7, для *V. uliginosum* и *V. vitis-idaea* – 3–4 ареолы, образованные вторичной жилкой. Данные СЭМ показали, что адаксиальная поверхность листа у *V. arctostaphylos* покрыта волокнистыми бороздками, у *V. myrtillus*, *V. uliginosum* и *V. vitis-idaea* она рифленая из-за эпикутикулярных восков. Кроме того, форма семян *V. arctostaphylos* варьирует от треугольной и четырехугольной до овальной, в то время как у остальных исследованных таксонов она лунообразная, почковидная или серповидная. Эпидермальные клетки семени четырехугольные, с прямыми антиклинальными стенками у *V. arctostaphylos*, однако у остальных видов представляют собой вытянутый прямоугольник со слабоволнистыми антиклинальными стенками. Средний диаметр тетрады (D) пыльцы всех исследованных видов составляет 29,89 (21,47–43,22) мкм, наименьший у *V. uliginosum* (24,59 мкм), а наибольший – у *V. arctostaphylos* (36,74 мкм). Анализ основных компонент, основанный на 47 признаках (9 признаков цветка, 18 – листа, 9 – семени и плода и 11 – пыльцы), показал, что длина, ширина и число зубцов листа, диаметр тетрады, экваториальная ось и полярная длина пыльцевых зерен являются наиболее ценными признаками для разграничения исследованных видов.

Introduction

The genus *Vaccinium* L., the third largest genus in Ericaceae, contains approximately 450 species worldwide (Cronquist, 1981). The genus is mostly distributed in Southeast Asia, America and Europe including subtropical or tropical montane regions on most continents (Vander Kloet, 1988). Members of *Vaccinium* have been subjected to several taxonomical studies on both regional and global scale up to now. Sleumer (1941) reported that the inflorescence type, calyx tube shape, corolla shape, and type of stamen appendages are valuable taxonomical traits for the members of *Vaccinium*. Likewise, Palser (1961) confirmed the sectional classification for *Vaccinium* taxa distributed in the United States mainly based on the floral structure. However, according to Stevens (1971), many floral characters like calyx shape, corolla shape, and stamen supply limited information to distinguish taxa within the genus *Vaccinium*. On the other hand, Nekrasova (1952) emphasized that leaf indumentum is valuable to distinguish *Vaccinium* members in the “Flora of the USSR”. However, it is reported that the vegetative characters are much less important than floral characters in circumscribing sections in the genus (Odell, Vander Kloet, 1991). Schultz (1944) reported that the only difference between *V. scoparium* Leiberg ex Coville and *V. myrtillus* L. is berry color at a lower elevation. Both puberulent and glabrous twigs may occur in both taxa (often on the same plant) and puberulent twigs are not restricted to *V. myrtillus*. Fang and Stevens (2005) used leaf venation and fruit size to distinguish *V. myrtillus*, *V. uliginosum* L., and *V. vitis-idaea* L. as key characters in the “Flora of China”. It is well known that the carpological investigation provides large

number of diagnostic features for the classification of taxa at several level (Szkudlarz, 1999b) however there are limited carpological studies related to members of *Vaccinium* at a specific level (Vander Kloet, 1988; Szkudlarz, 1999a, 2001).

The genus *Vaccinium* is known to have a basic chromosome number of $x = 12$ (Darrow et al., 1944). Although diploid chromosome numbers are common in the genus, several polyploidy cytotypes were also recorded in *V. uliginosum*, *V. arctostaphylos* L., and *V. myrtillus* (Hagerup, 1928; Darrow et al., 1944). However, there seems to be no correlation between morphological characters and ploidy level (Young, 1970; Vander Kloet, 1988).

Sarwar et al. (2006) evaluated palynological features of 37 *Vaccinium* species in detail and reported that palynological characters are not in accordance with the current infrageneric classification, but they provide useful information to figure out the taxonomic problem within the genus. Goldy et al. (1984) also reported that the exine sculpture contributes to the delimitation of *Vaccinium* taxa. According to Cockerham and Galletta (1976), tetrad diameter was calculated 11 % larger in the tetraploid pollen than diploid pollen in the genus *Vaccinium*. Similarly, slight differences in tetrad size and exine sculpture were recorded between the New World blueberry species and Old World blueberry species by Sarwar et al. (2006). The smallest pollen grains were also found in *V. uliginosum* by Sarwar and Takahashi (2007).

The most recent and widely used molecular phylogenetic data also improved our knowledge of the taxonomy of *Vaccinium*. Firstly, Sleumer (1941) revealed the polyphyletic origin of the genus. However, the most comprehensive phylogenetic studies on the genus at sectional level (sect.

Macropelma Klotzsch and sect. *Myrtillus* Dumortier and sect. *Hemimyrtillus* Sleumer) and on the tribe *Vaccinieae* Rchb. were performed by Powell and Kron (2002) and Kron et al. (2002), respectively. The latter two molecular studies also revealed that *Vaccinium* is a polyphyletic genus as previously reported by Sleumer (1941). *Vaccinium arctostaphylos* is placed within a clade sister to *V. uliginosum* (Powell, Kron, 2002). Correspondingly, Sultana et al. (2020) reported that *V. myrtillus* and *V. macrocarpon* Ait. are closely related species and formed a sister clade. Kron et al. (2002) suggested that more morphological and molecular data are necessary to recognize members of *Vaccinium* within the tribe *Vaccinieae*. Despite many advantages of phylogenetic works, the representation of the *Vaccinium* in phylogenetic studies is still too limited to draw taxonomic conclusions, so Pedraza-Penalosa and Luteyn (2011) described seven new species based on traditional circumscription from Andean South America.

The first comprehensive taxonomic study on the Turkish native species of *Vaccinium* (*V. arctostaphylos*, *V. myrtillus*, *V. uliginosum*, and *V. vitis-idaea*) was presented by Stevens (1978). Afterward, the systematics of the Turkish *Vaccinium* was updated by Terzioğlu (2012) in the checklist of the “Flora of Turkey” without any further taxonomic contributions. Subsequently, many researchers mostly focused on chemical properties of the Turkish

Vaccinium. Ayaz et al. (2001) firstly examined the levels of fructose and glucose in *V. arctostaphylos* and *V. myrtillus* at three different maturity stages in NE Turkey. Similarly, anthocyanin and antioxidant contents in the berries of two species were reported from Turkey (Çolak et al., 2016). Furthermore, altitude variation in the volatile composition of blueberry species (*V. arctostaphylos*, *V. uliginosum*, *V. vitis-idaea*, and *V. myrtillus*) distributed in the NE Anatolia was examined by Erik et al. (2020). Some phenological characteristics and distribution patterns of the Turkish *Vaccinium* were compiled from accessible sources by Çelik and Serçe (2015).

In the present paper, we aimed to explore surface and venation patterns of leaf, seed macro-morphology, and pollen features of the *Vaccinium* species distributed in NE Anatolia, contribute filling the gaps in the biosystematics features of the examined taxa and determine valuable characters to distinguish the examined *Vaccinium* species.

Materials and methods

Specimens

Samples for biosystematic studies were collected from sixteen populations of *V. arctostaphylos*, *V. uliginosum*, *V. vitis-idaea*, and *V. myrtillus* in Turkey within the scope of the MSc thesis of the first author

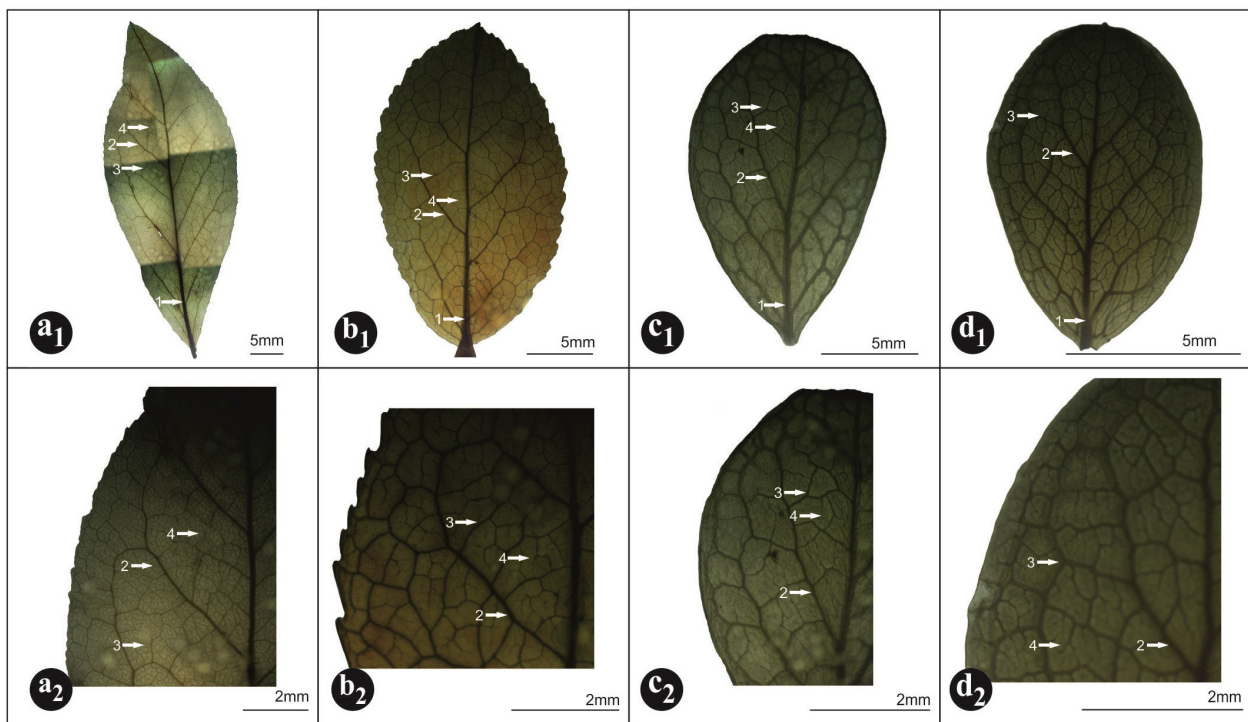


Fig. 1. Leaf areolar (vein islets) pattern: a₁₋₂ – *Vaccinium arctostaphylos*; b₁₋₂ – *V. myrtillus*; c₁₋₂ – *V. uliginosum*; d₁₋₂ – *V. vitis-idaea*. Arrow shows the primary (1), secondary (2), tertiary (3), and quaternary veins (4).

(Table 1). The specimens were labeled, numbered, annotated with the date of collections, the locality and the name of the collector during the field studies. After identification of the samples according to Stevens (1978), all vouchers were stored in the herbarium of the Department of Biology at Karadeniz Technical University (KTUB). Additionally, healthy leaves and fruits including seeds for morphological studies and flowers for palynological studies were also collected from the same population during the field trips. Taxonomic names followed Terzioğlu (2012), and distribution details were given according to the Turkey Grid System (Davis, 1965).

Protocols for leaf venation studies

At least 3–4 entire mature leaves from each investigated population of the Turkish *Vaccinium* were immersed in 2.5 % Sodium Hydroxide for 15–20 days (Panda, Kirtania, 2016). After that, all leaves were rinsed with distilled water and then stained with safranin for 15 minutes (Vardar, 1962). After staining, all leaves were again rinsed with distilled water before taking photos with a Leica DFC 490 digital camera attached to a stereomicroscope. Areoles number (the area bounded by veins) and divergence angle of secondary, tertiary, and quaternary veins were calculated from at least 3–4 different part

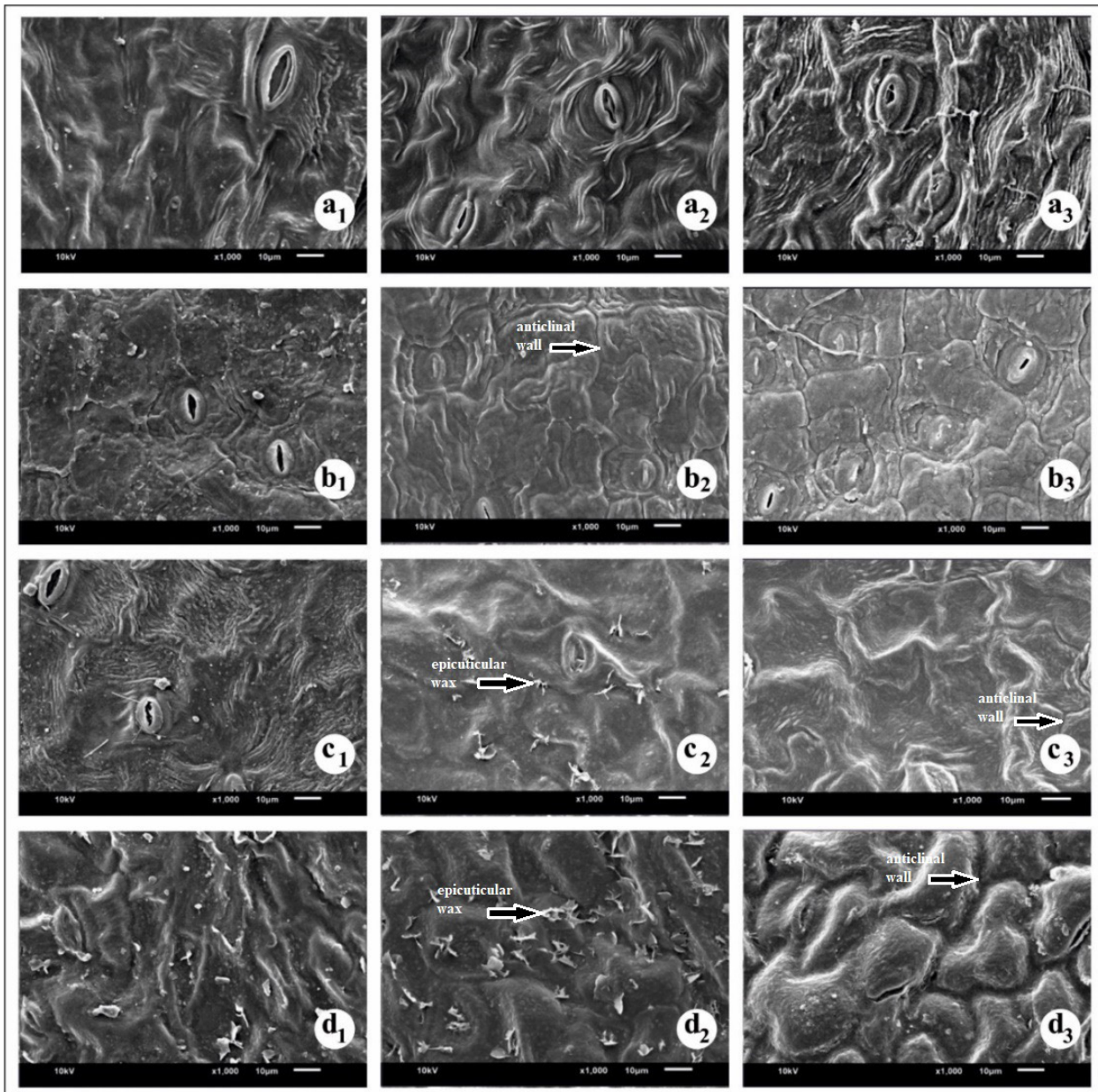


Fig. 2. Adaxial leaf surface, scanning electron microscope: a₁₋₃ – *Vaccinium arctostaphylos*; b₁₋₃ – *V. myrtilillus*; c₁₋₃ – *V. uliginosum*; d₁₋₃ – *V. vitis-idaea*.

of each stained leaves belonging to each investigated population according to Saibaba and Rao (1990) and Panda and Kirtania (2016).

Protocols for leaf micromorphology studies

Firstly at least three pieces of dried leaves about 1 cm² belong to each investigated population were cut out from the middle part by a razor blade and then all pieces were transferred onto aluminum stubs, coated with gold in a sputter-coater and examined under the JEOL-JSM 6610 scanning electron microscope (SEM) at the Central Research Laboratory of Recep Tayyip Erdogan University at a voltage 10 kV. All micrographs were taken 200× and 1000× magnification. At least ten leaf micro-

graphs taken 200× and 1000× magnification per investigated population was observed for micromorphological description. The terminology of Simpson (2012) and Stearn (1985) was adopted for the leaf micromorphological descriptions.

Protocols for seed macro and micromorphology studies

A total of 12 dried fruits from each investigated population were boiled in water and then all seeds were extracted from fruits. Then, the seeds were stored in anhydride-sulfuric acid (9 : 1) for 24 hour at room temperature. After that, all seeds were rinsed with distilled water, dried in oven at 50 °C for 24 hour and photographed by a Leica DFC 490



Fig. 3. Seed morphology, light microscope: a – *Vaccinium arctostaphylos*; b – *V. myrtillus*; c – *V. uliginosum*; d – *V. vitis-idaea*.

stereomicroscope attached to a digital camera. Dried seeds also were transferred on aluminum stubs with double-sided adhesive carbon tape and coated with gold for 4 min in a sputter-coater for SEM studies. Seed micrographs were observed by a JEOL-JSM 6610 microscope at the Central Research Laboratory of Recep Tayyip Erdogan University at 50 \times , 300 \times , 1000 \times , and 2000 \times magnification. The terminology of Barthlott (1981), Stearn (1985), Coşkunçelebi et al. (2000, 2016) and Khorasani et al. (2020) was mainly followed for the seed macro-micromorphological descriptions. Qualitative characters of seed were described according to terminology provided by Khorasani et al. (2020).

Protocols for palynological studies

Several randomly selected flowers were collected from each population belonging to investigated species. Pollen grains were extracted from the flower according to the acetolysed method proposed by Erdtman (1952) and then one half was immersed in an alcohol solution of glycerine (for LM) to obtain

permanent slides and the other in ethyl alcohol 96 % (for SEM). Permanent slides were examined using Leica S6D light microscope to measure or determine the following parameters: Tetrad axis (D), equatorial axis (E or d), polar axis (P), P/E ratio, D/d ratio, colpus length (2f), colpus width (W), septum (total exine) thickness (Se), exine (Ex), 2f/W ratio, 2f/D ratio. For each analyzed population, thirty mature and correctly formed pollen grains were measured by light microscopy. For SEM studies, air-dried acetolysed pollen grains were transferred onto aluminum stubs and coated with gold for 4 min in a sputter-coater and examined by a JEOL-JSM 6610 microscope at the Central Research Laboratory of Recep Tayyip Erdogan University at a voltage 10 kV. Pollen photographs and micrographs were taken using Leica S6D light microscope and JEOL-JSM 6610 electron microscope, respectively. For the pollen grains, the description terminology of Sarwar (2007), Punt et al. (2007) and Park and Song (2010) were mainly followed.

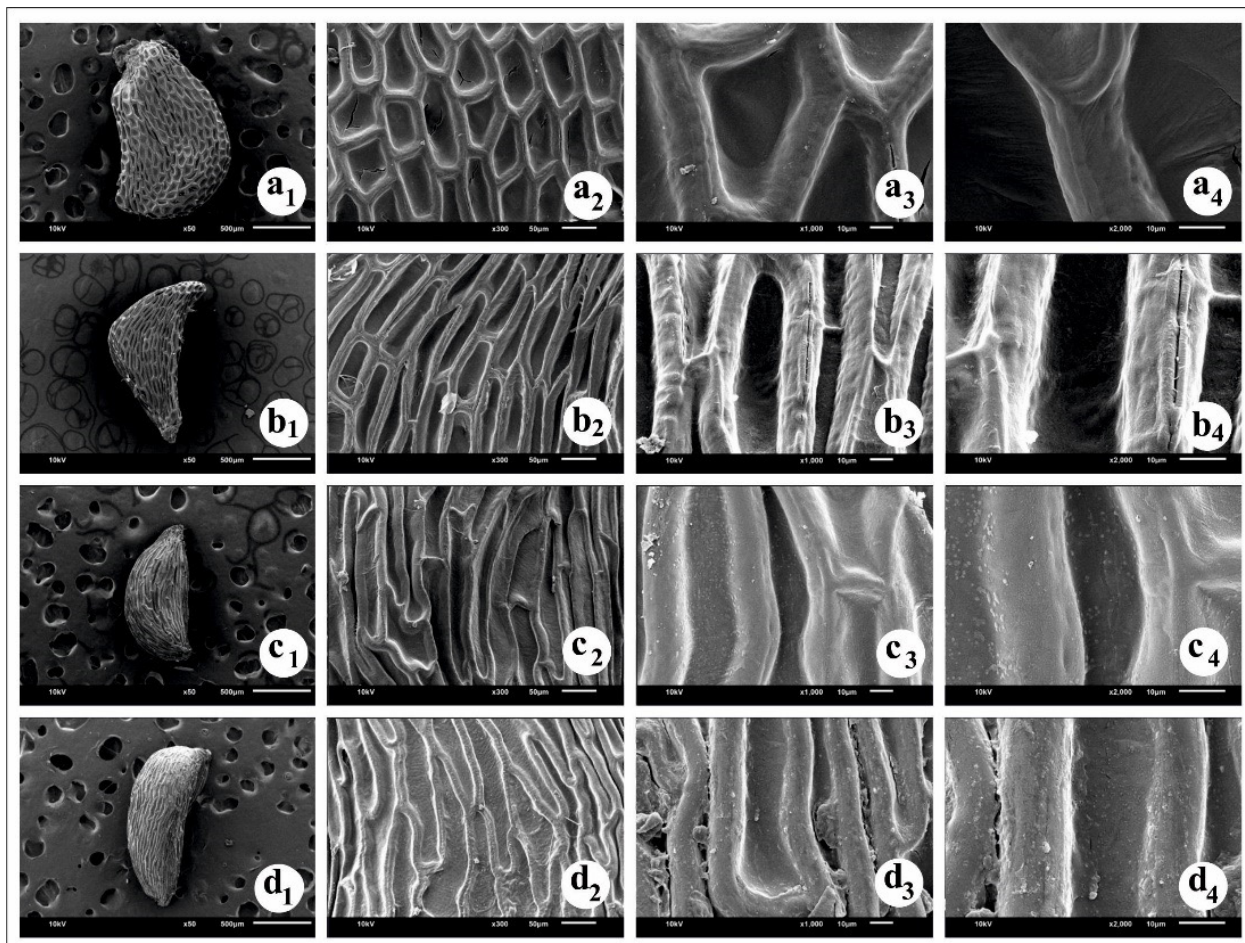


Fig. 4. Seed morphology, scanning electron microscope: a₁₋₄ – *Vaccinium arctostaphylos*; b₁₋₄ – *V. myrtillus*; c₁₋₄ – *V. uliginosum*; d₁₋₄ – *V. vitis-idaea*.

Numerical analysis

Forty-seven traits (Table 2) related to leaf, flower, fruit, seed and pollen grains were measured or scored using a light microscope. All scores or measurements were checked at least three or four times for each population. Average values and standard deviations (SD) of the all traits were given in Table 4 for each population. A data matrix including scores of 47 traits was subjected to cluster analysis (CA) and principal component analysis (PCA). For CA, a pair-wise matrix of resemblance values was calculated from the standardized data matrix, using Gower's coefficient of resemblance designed for mixed data sets (Sneath, Sokal, 1973). For PCA, the standardized data were used to create a covariance matrix. Five eigenvectors were extracted, providing axis onto which the standardized data were projected to give a two-dimensional plot of the examined taxa. All analyses were performed using MVSP computer programs for multivariate data analysis in ecology and systematics (Kovach, 2007).

Results

Traits of leaf, seed and pollen grains

Findings related to leaf (shape, margins, and venation), seed, and pollen grains of each investigated taxon were given alphabetically in the following.

Vaccinium arctostaphylos L. 1753, Sp. Pl. 351

Leaf: Elliptic-broadly lanceolate with serrate or serrulate margin in shape (Fig. 1a₁). The secondary veins are opposite at the base of a primary vein and then alternatively arranged with a divergence angle of 25°–45° towards the primary vein tip and free-ending. The numbers of areoles formed by the secondary, tertiary, and quaternary veins are 8–9, 5–6 and 3–6, respectively (Fig. 1a₂). The leaf adaxial surface under SEM is weakly striate; the epidermal cell borders are hardly indistinct (Fig. 2a_{1–3}).

Seed: Seeds are 1.3–1.5 mm length, brown, triangular, tetragonal or oval (Fig. 3a). Epidermal cells of seed coat are mostly polygonal in outline,

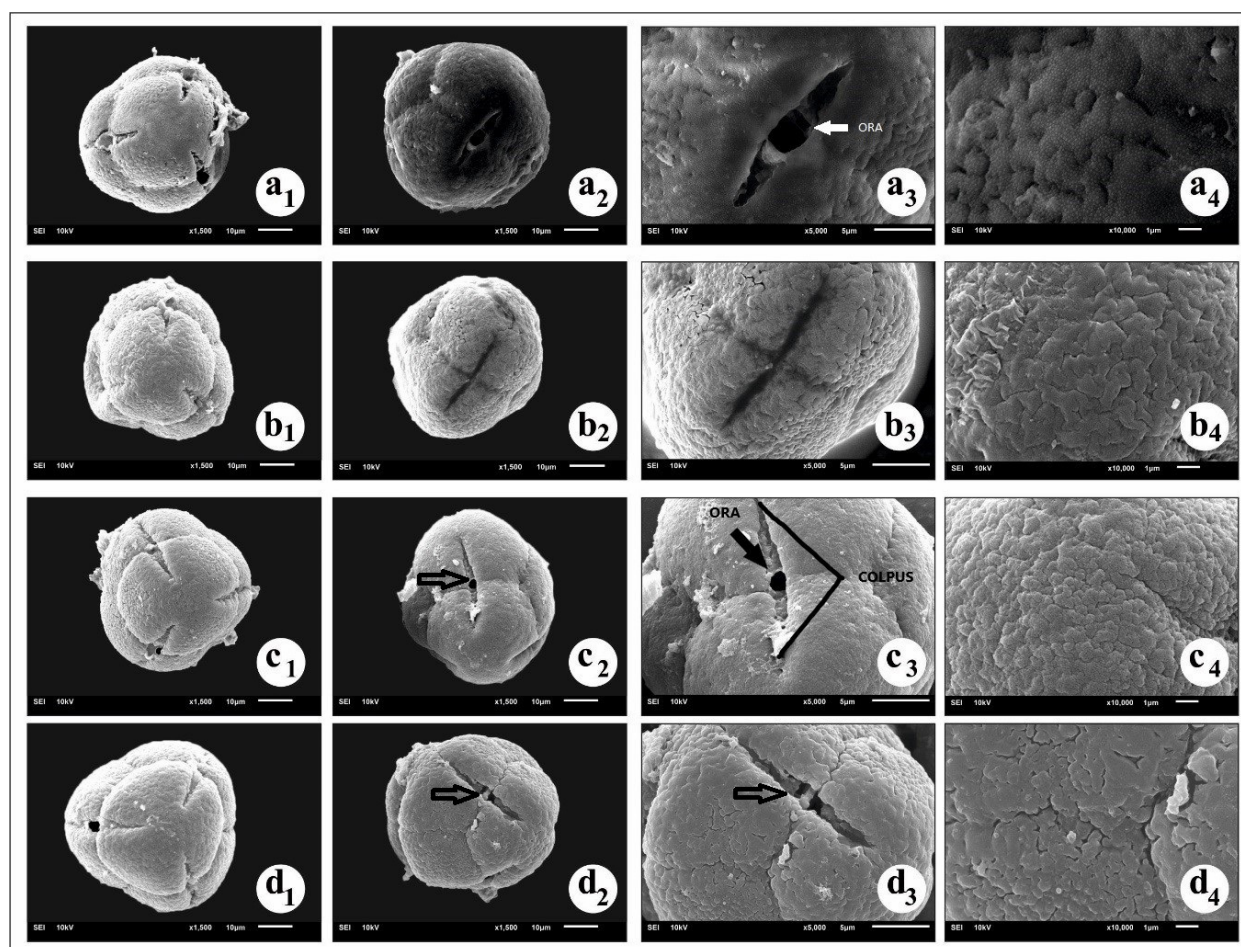


Fig. 5. Pollen morphology, scanning electron microscope: a_{1–4} – *Vaccinium arctostaphylos*; b_{1–4} – *V. myrtillus*; c_{1–4} – *V. uliginosum*; d_{1–4} – *V. vitis-idaea*.

anticlinal walls straight, raised, smooth, strongly thickened, periclinal cell walls smooth and concave (Fig. 4a₁₋₄).

Pollen grains: Pollen grains are in tetrahedral tetrads, tetrad axis (D) 28.55–43.22 (36.7) μm , oblate-spheroidal, equatorial diameter (E or d) 22.34–31.02 (29.86) μm , polar axis (P) 15.15–23.99 (20.58) μm , P/E ratio 0.63–0.94 (0.81) μm , D/d ratio 1.27–1.38 (1.34) μm . Colpus is narrowing, colpus margin distinct. Ora is in the middle of the colpus, length (2f) 17.45–24.23 (21.95) μm , width (W) 1.98–3.65 (2.69) μm . Septum thickness (Se) 1.22–1.89 (1.46) μm thick, exine (Ex) 1.52–1.9 (1.72) μm thick (Fig. 5a₄). 2f/W ratio 9.04–9.45 (9.16) μm , 2f/D ratio 0.33–0.62 (0.47). Pollen grains are verrucate, microgranulate in

the population of VA1, regulate in the rest examined populations. Representative micrographs of pollen under the SEM are given in Fig. 5a₁₋₄.

***Vaccinium myrtillus* L. 1753, Sp. Pl. 349**

Leaf: Broadly elliptic or ovate, sharply serrate margin in shape (Fig. 1b₁). The secondary veins are alternatively arranged with a divergence angle of 30–55° toward a primary vein tip and free-ending. The numbers of areoles formed by the secondary, tertiary, and quaternary veins are 5–7, 5–9, and 3–6, respectively (Fig. 1b₂). The leaf adaxial surface under SEM is without striate; epidermal cell borders are slightly visible (Fig. 2b₁₋₃).

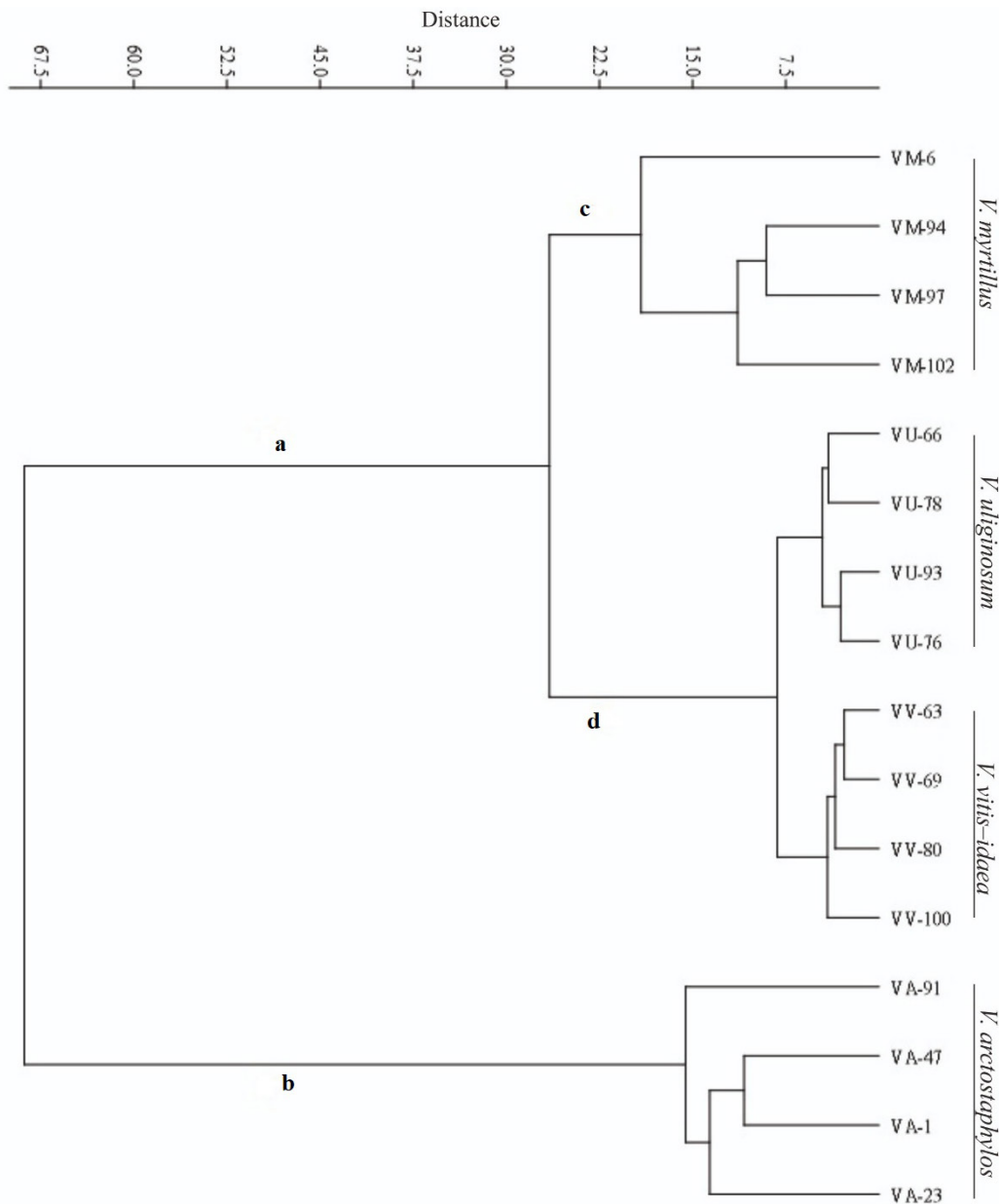


Fig. 6. UPGMA dendrogram of *Vaccinium* taxa based on morphological data (Details of the population numbers are given in Table 1).

Seed: Seeds are 1.2–1.3 mm length, light or dark brown, lunate to reniform in outline (Fig. 3b). Epidermal cells of seed coat are elongated, mostly rectangle in shape, anticlinal walls straight, raised, slightly striated, thickened, the surface of outer periclinal cell wall is smooth and concave (Fig. 4b₁₋₄).

Pollen grains: Pollen grains are in tetrahedral tetrads, tetrad axis (D) 25.68–35.4 (31.08) μm , oblate, equatorial axis (E or d) 20.58–29.14 (24.13) μm , polar axis (P) 13.7–26.17 (18.32) μm , P/E ratio 0.65–0.97 (0.82), D/d ratio 1.13–1.36 (1.27). Colpus is narrowing, colpus margin distinct. Ora is in the middle of the colpus, length (2f) 9.45–19.78 (17.04) μm , width (W) 1.43–3.08 (2.35) μm . Septum thickness (Se) 1.32–2.09 (1.69) μm thick, exine (Ex) 1.44–1.68 (1.59) μm thick, regulate. 2f/W ratio 6.08–8.35 (7.09) μm , 2f/D ratio 0.37–0.62 (0.51) μm . Representative micrographs of pollen under the SEM are given in Fig. 5b₁₋₄.

Vaccinium uliginosum L. 1753, Sp. Pl. 350

Leaf: Ovate-obovate with entire margin in shape (Fig. 1c₁). The secondary veins are opposite at the base of a primary vein and then alternatively arranged with a divergence angle of 25–45° towards the primary vein tip and anastomose. The numbers of areoles formed by the secondary, tertiary, and quaternary veins are 3–4, 1–3, and 1–2, respectively (Fig. 1c₂). However the quaternary veins are generally freely ending without forming any areoles. The leaf adaxial surface under SEM is covered by a distinctly rare, more or less dispersed epicuticular wax; the epidermal cell borders are slightly visible (Fig. 2c₁₋₃).

Seed: Seeds are 1–1.1 mm length, light or dark brown, lunate to elliptic in outline (Fig. 3c). Epidermal cells of seed coat are elongated, irregular in shape, anticlinal walls obvious, smooth, thickened, slightly undulate, periclinal surfaces smooth and flat (Fig. 4c₁₋₄).

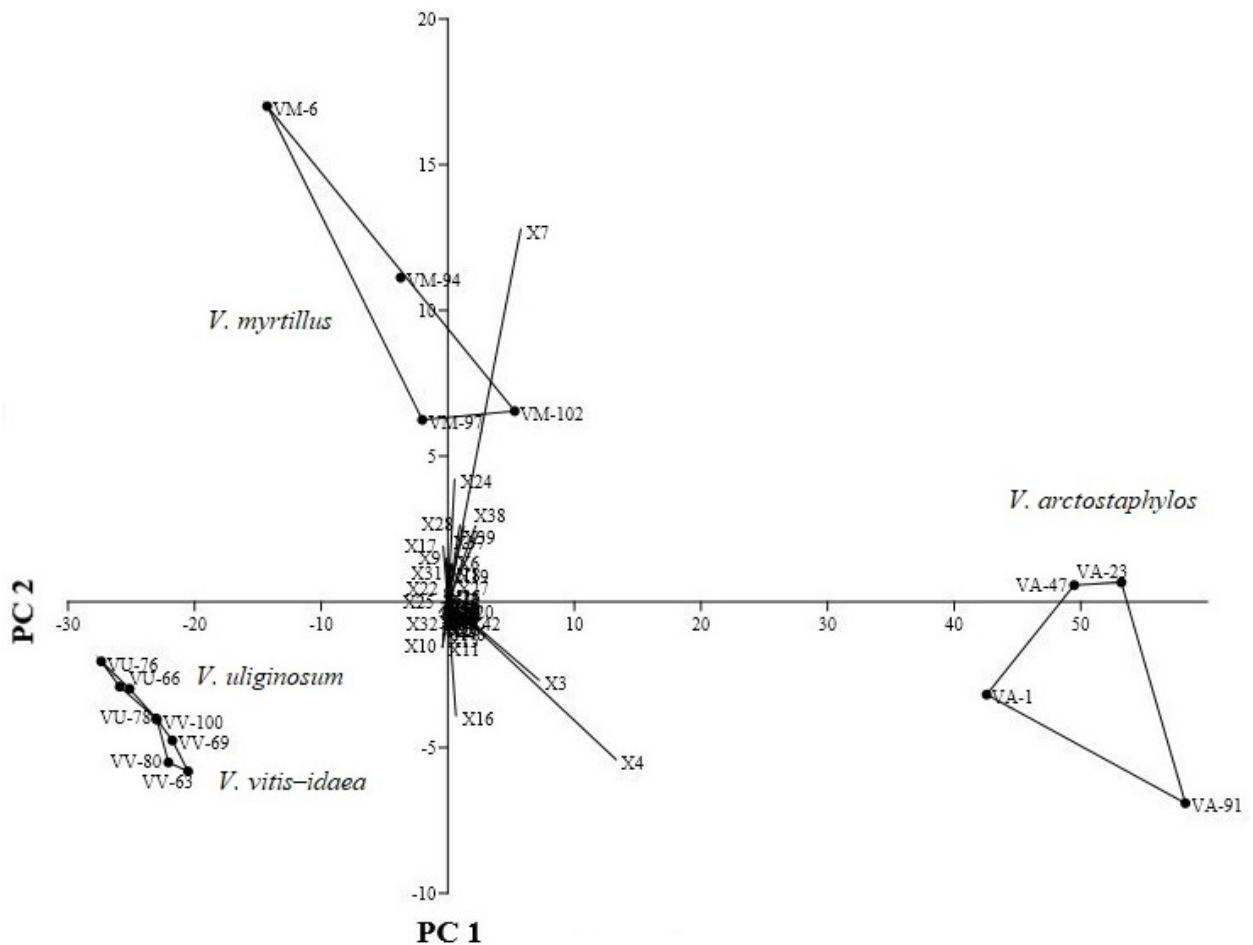


Fig. 7. Ordination resulting from principal components analysis (PCA) of *Vaccinium* based on 47 characters (Details of the population numbers and traits are given in Table 1, 2 respectively).

Table 1

Voucher data of the studied taxa *Vaccinium*

Taxa	Pop. Code	Grid of Davis (1970)	Locality	Altitude (m)	Voucher number
<i>V. arctostaphylos</i>	VA1	A7	Gümüşhane: Kürtün above Alan Plateau	1845	<i>Coşkunçelebi & Yılmaz 1</i>
	VA23	A7	Trabzon: Tonya above Kalınçam Village	1483	<i>Coşkunçelebi & Yılmaz 23</i>
	VA47	A8	Rize: Ardeşen, Siprona Plateau	1032	<i>Coşkunçelebi & Yılmaz 47</i>
	VA91	A8	Artvin: Murgul, Tiryal Mountain	2234	<i>Coşkunçelebi & Yılmaz 91</i>
<i>V. myrtillos</i>	VM6	A7	Gümüşhane: Kürtün, Süme Village	2081	<i>Coşkunçelebi & Yılmaz 6</i>
	VM94	A7	Trabzon: Multat Plateau	2811	<i>Coşkunçelebi & Yılmaz 94</i>
	VM97	A8	Artvin: Şavşat, Kirazlı Village	2533	<i>Coşkunçelebi & Yılmaz 97</i>
	VM102	A9	Ardahan: Hanak, Alabalık Plateau	2613	<i>Coşkunçelebi & Yılmaz 102</i>
<i>V. uliginosum</i>	VU66	A8	Artvin: Şavşat, Karagöl National Park	2537	<i>Coşkunçelebi & Yılmaz 66</i>
	VU76	A9	Ardahan: Posof, Üçlıçkın Hill	2405	<i>Coşkunçelebi & Yılmaz 76</i>
	VU78	A9	Ardahan: Hanak, Alabalık Plateau	2588	<i>Coşkunçelebi & Yılmaz 78</i>
	VU93	A7	Trabzon: Multat Plateau	2811	<i>Coşkunçelebi & Yılmaz 93</i>
<i>V. vitis-idaea</i>	VV63	A8	Artvin: Şavşat, Karagöl National Park	2533	<i>Coşkunçelebi & Yılmaz 63</i>
	VV69	A9	Ardahan: Posof, Sesödile Hill	2376	<i>Coşkunçelebi & Yılmaz 69</i>
	VV80	A9	Ardahan: Alabalık Plateau	2579	<i>Coşkunçelebi & Yılmaz 80</i>
	VV100	A8	Artvin: Şavşat, Kocabey Plateau	2491	<i>Coşkunçelebi & Yılmaz 100</i>

Table 2

Characters

Acronym	Examined characters and their states
X1	Leaf (evergreen: 0; deciduous: 1)
X2	Leaf (papery: 0; coriaceous: 1)
X3	Leaf width (cm)
X4	Leaf length (cm)
X5	Leaf shapes (elliptic-lanceolate: 1; elliptic-ovoid: 2; ovate-oblong: 3; ovate-oblong: 4)
X6	Leaf margin (entire: 1; serrate 2; serrulate: 3)
X7	Leaf teeth numbers in 1 cm long (digit)
X8	Leaf apex (acuminate: 1; acute: 2; obtuse: 3)
X9	Leaf base (oblique: 1; attenuate: 2; rounded: 3)
X10	Leaf abaxial surface (pubescent: 0; glabrous: 1)
X11	Leaf adaxial surface (pubescent: 0; glabrous: 1)
X12	Petiole length (mm)
X13	Petiole (glabrous: 0; hairy: 1)
X14	Leaf venation (reticulate: 0; anastomose: 1)
X15	Inflorescence (terminal: 0; axillary rasemes: 1)
X16	Flower number (digit)
X17	Corolla shape (campanulate: 1; urceolate: 2; spherical: 3)
X18	Corolla color (white-mock: 1; white - dark red: 2; dark red-yellowish green: 3)
X19	Corolla width (mm)
X20	Corolla length (mm)
X21	Calyx length (mm)
X22	Calyx margin (narrow-wide triangular: 1; wavy: 2; rounded-undulate: 3)
X23	Calyx (pubescent: 0; glabrous: 1)
X24	Pedicel length (mm)
X25	Fruit color (coal black: 1; black: 2; indigo: 3; sour cherry: 4)
X26	Average weight of 10 air dried fruit/mg
X27	Pedicel length in fruiting (cm)
X28	Dried fruit diameter (mm)

Table 2 (continuation)

Acronym	Examined characters and their states
X29	Seed length (mm)
X30	Leaf surface under SEM (fibros-striat: 1; ruminat: 2; concave-postulate :3)
X31	Areoles shape (ovoid: 0; diagonal: 1)
X32	Number of secondary veins (10–16: 0; 8–10: 1)
X33	Areoles number of secondary vein (16–18: 1; 10–14: 2; 6–8: 3)
X34	Seed surface under SEM (wrinkled: 0; wavy: 1)
X35	Seed epidermal cells shape (isolateral or quadrilateral: 0; elengote: 1)
X36	Anticlinal wall of the seed epidermal cells (smooth: 0; ruminat: 1)
X37	Tetrad axis (D) (μm)
X38	Equatorial axis (E or d) (μm)
X39	Polar length (P) (μm)
X40	P/E ($\mu\text{m}/\mu\text{m}$)
X41	D/E ($\mu\text{m}/\mu\text{m}$)
X42	Colpus length (2f) (μm)
X43	Colpus width (W) (μm)
X44	Septum thickness (Se) (μm)
X45	Exine (Ex) (μm)
X46	2f/W ($\mu\text{m}/\mu\text{m}$)
X47	2f/D ($\mu\text{m}/\mu\text{m}$)

Pollen grains: Pollen grains are in tetrahedral tetrads, tetrad axis (D) 21.47–28.95 (24.59) μm , oblate, equatorial axis (E or d) 17.83–25.43 (20.77) μm , polar axis (P) 11.96–17.94 (14.51) μm , P/E ratio 0.63–0.77 (0.64) μm , D/d ratio 1.14–1.39 (1.27) μm . Colpus is narrowing, colpus margin distinct. Ora is in the middle of the colpus, length (2f) 10.3–17.45 (15.47) μm , width (W) 1.83–2.88 (2.48) μm . Septum thickness (Se) 0.97–1.44 (1.25) μm thick, exine (Ex) 1.32–1.53 (1.42) μm thick, rugulose. 2f/W ratio 5.63–6.99 (6.26) μm , 2f/D ratio 0.49–0.88 (0.65) μm . Representative micrographs of pollen under the SEM are given in Fig. 5c₁₋₄.

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Leaf: Ovate-obovate with entire margin in shape (Fig. 1d₁). The secondary veins are opposite at the base of a primary vein and then alternatively arranged with a divergence angle of 25°–45° towards the primary vein tip and free-ending. The numbers of areoles formed by the secondary, tertiary, and quaternary veins are 3–4, 1–3, and 1–3, respectively (Fig. 1d₂). The leaf adaxial surface is covered by a distinctly dense dispersed epicuticular waxes; the epidermal cell borders are slightly visible (Fig. 2d₁₋₃).

Seed: Seeds are 1.3–1.5 (1.4) mm length, light or dark brown, lunate to falcate (Fig. 3d). Epidermal cells of seed coat are elongated, irregular in shape, anticlinal walls obvious, smooth, thickened, slightly undulate, periclinal surfaces smooth and flat (Fig. 4d).

Pollen grains: Pollen grains are in tetrahedral tetrads, tetrad axis (D) 24.38–34.74 (27.14) μm , oblate-spheroidal, equatorial axis (E or d) 12.96–22.48 (18.7) μm , polar axis (P) 12.03–18.32 (14.86) μm , P/E ratio 0.71–0.92 (0.84) μm , D/d ratio 1.32–1.39 (1.36) μm . Colpus is narrowing, colpus margin distinct. Ora is in the middle of the colpus, length (2f) 14.37–23.85 (17.68) μm , width (W) 1.52–3.15 (2.67) μm . Septum thickness (Se) 0.89–1.52 (1.18) μm thick, exine (Ex) 1.18–1.26 (1.2) μm thick, regulate. 2f/W ratio 5.45–9.12 (7.94) μm , 2f/D ratio 0.61–0.83 (0.81) μm . Representative micrographs of pollen under the SEM are given in Fig. 5d₁₋₄.

Numerical results

Scores related to leaf, flower, fruit and seed and pollen traits of 16 populations belonging to four wild *Vaccinium* were given in Table 4. The dendrogram resulting from the cluster analysis (UPGMA) based on the combined dataset (36 morphological: flower: 9, leaf: 18, seed and fruit: 9) and 11 palynological characters) is given in Fig. 6. The dendrogram revealed that the examined *V. arctostaphylos* specimens (label b) are distinctly separated from the rest of the examined *Vaccinium* specimens (label a) at a distance value of 67.5. Moreover, specimens of *V. uliginosum* and *V. vitis-idaea* are aggregated at a distance value of 7.5, which shows that they are the most closely related taxa based on both morphological and palynological characters (Fig. 6).

Table 3
Scores of forty seven traits of examined population belong to *Vaccinium* (Explanation of the population number and traits are given in Table 1,2 respectively

Traits	VA1	VA23	VA47	VA91	VM6	VM94	VM97	VM102	VU66	VU78	VU93	VU76	VV63	VV69	VV80	VV100
X1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
X2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
X3	Mean	33.00	41.50	46.60	9.00	12.75	14.25	20.33	6.43	5.60	8.58	6.72	8.62	7.75	7.92	6.50
	SD	±0.12	±0.14	±0.20	±0.26	±0.18	±0.16	±0.25	±0.55	±0.39	±0.47	±0.35	±0.15	±0.22	±0.18	±0.21
X4	Mean	75.99	67.3	77.87	13.25	24.00	27.75	32.30	12.01	9.49	13.65	10.09	16.36	14.89	14.61	13.68
	SD	±1.28	±1.17	±1.21	±0.95	±1.04	±0.93	±0.97	±0.24	±0.32	±0.28	±0.17	±0.22	±0.28	±0.19	±0.21
X5	1	1	1	1	2	2	2	2	3	3	3	3	3	3	3	3
X6	2	3	2	3	3	3	2	2	1	1	1	1	1	1	1	1
X7	Mean	30.53	29.11	25.16	19.5	19.25	13.5	17.00	0	0	0	0	0	0	0	0
	SD	±0.85	±0.96	±1.02	±0.65	±0.74	±1.17	±1.11	0	0	0	0	0	0	0	0
X8	1	1	1	1	2	2	2	2	1	3	3	3	3	3	3	3
X9	1	1	1	1	3	3	3	3	1	1	1	1	2	2	2	2
X10	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
X11	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
X12	Mean	0.1	0.1	0.1	0.98	1.06	0.8	0.95	0.94	1.11	0.95	1.06	1.7	1.72	1.61	2.01
	SD	±0.24	±0.19	±0.25	±0.17	±0.22	±0.14	±0.12	±0.01	±0.02	±0.04	±0.03	±0.02	±0.01	±0.03	±0.04
X13	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
X14	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
X15	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1
X16	Mean	5.30	4.70	5.90	0	0	0	0	1.50	2.00	1.50	1.50	4.00	4.00	5.50	4.00
	SD	±0.46	±0.75	±0.63	±0.92	±0.00	±0.00	±0.00	±0.21	±0.49	±0.73	±0.54	±0.25	±0.15	±0.42	±0.50
X17	1	1	1	1	3	3	3	3	2	2	2	2	1	1	1	1
X18	2	2	2	2	3	3	3	3	1	1	1	1	2	2	2	2
X19	Mean	5.7	4.02	5.89	4.5	3.87	4.25	5.48	3.02	2.72	2.87	2.95	3.35	3.4	4.07	3.43
	SD	±0.85	±0.92	±0.79	±0.24	±0.32	±0.28	±0.31	±0.45	±0.38	±0.42	±0.37	±0.23	±0.19	±0.25	±0.28

Table 3 (continuation)

Traits	Statistics	VA1	VA23	VA47	VA91	VM6	VM94	VM97	VM102	VU66	VU78	VU93	VU76	VV63	VV69	VV80	VV100
X20	Mean	7.1	7.19	7.8	3.86	3.86	4.3	3.96	4.67	3.06	3.15	3.7	3.23	4.66	4.26	4.25	4.4
	SD	±1.15	±1.18	±1.11	±0.94	±0.95	±0.93	±1.04	±1.08	±0.18	±0.23	±0.14	±0.16	±0.94	±0.79	±0.83	±0.97
X21	Mean	3.87	3.58	3.3	3.36	2.27	1.78	2.55	2.49	1.84	1.89	1.55	1.86	2.08	1.88	1.94	1.83
	SD	±0.12	±0.13	±0.14	±0.12	±0.24	±0.15	±0.32	±0.17	±0.11	±0.22	±0.14	±0.17	±0.25	±0.23	±0.19	±0.34
X22		1	1	1	1	2	2	2	2	3	3	3	3	1	1	1	1
X23		0	0	0	0	1	1	1	1	1	1	1	1	0	0	0	0
X24	Mean	4.53	7.19	4.82	6.68	12.39	2.05	6.83	7.12	2.89	3.16	2.6	2.87	2.6	3.68	3.22	2.62
	SD	±0.15	±0.18	±0.14	±0.11	±0.37	±0.32	±0.27	±0.31	±0.18	±0.13	±0.24	±0.16	±0.23	±0.19	±0.17	±0.25
X25		1	1	2	1	3	3	3	3	3	3	3	3	4	4	4	4
X26	Mean	0.87	0.82	0.86	0.63	1.12	0.93	0.78	0.65	0.36	0.46	0.38	0.43	0.22	0.28	0.32	0.25
	SD	±0.14	±0.19	±0.11	±0.18	±0.78	±0.74	±0.68	±0.15	±0.17	±0.09	±0.18	±0.21	±0.18	±0.22	±0.19	±0.19
X27	Mean	6.28	4.8	6.15	4.83	4.17	5.18	4.25	5.43	3.93	3.08	3.52	3.87	4.04	3.96	4.37	4.37
	SD	±1.27	±1.12	±1.25	±1.19	±1.09	±1.15	±1.11	±1.13	±0.72	±0.69	±0.75	±0.71	±0.85	±0.86	±0.89	±0.89
X28	Mean	6.77	5.67	7.13	7.14	4.25	7.11	5.94	9.12	2.25	2.67	2.13	2.26	2.81	2.03	1.98	2.78
	SD	±0.18	±0.08	±0.14	±0.10	±0.28	±0.21	±0.19	±0.27	±0.49	±0.39	±0.43	±0.48	±0.39	±0.24	±0.28	±0.35
X29	Mean	1.34	1.42	1.53	1.38	1.28	1.35	1.27	1.25	1.18	1.05	1.12	1.07	1.45	1.37	1.38	1.53
	SD	±0.08	±0.05	±0.10	±0.07	±0.05	±0.09	±0.07	±0.04	±0.19	±0.12	±0.07	±0.12	±0.01	±0.03	±0.01	±0.02
X30		1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	3
X31		0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0
X32		0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
X33		1	1	1	1	2	2	2	2	3	3	3	3	3	3	3	3
X34		0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1
X35		0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1
X36		0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
X37	Mean	36.11	38.01	35.22	37.65	30.07	32.13	31.15	30.97	25.29	24.52	23.93	24.64	26.83	27.39	25.94	28.42
	SD	±1.27	±2.20	±3.05	±1.87	±2.68	±3.84	±2.77	±5.03	±3.21	±4.80	±4.78	±5.02	±3.91	±4.03	±2.14	±4.12

Table 3 (continuation)

Traits	Statistics	VA1	VA23	VA47	VA91	VM6	VM94	VM97	VM102	VU66	VU78	VU93	VU76	VV63	VV69	VV80	VV100
X38	Mean	28.15	30.27	31.85	29.17	23.44	24.7	25.46	22.93	21.14	20.12	20.18	21.65	17.99	19.09	18.63	19.12
	SD	±2.46	±4.13	±3.60	±3.45	±3.74	±1.25	±4.10	±2.88	±4.16	±3.43	±2.63	±2.15	±2.42	±1.80	±3.12	±1.15
X39	Mean	18.82	21.24	23.02	19.25	17.66	18.48	19.12	18.04	13.27	14.63	15.88	14.27	14.22	14.7	14.56	15.96
	SD	±3.11	±1.39	±1.55	±4.71	±4.12	±1.69	±1.18	±1.64	±3.11	±2.29	±2.17	±3.20	±1.12	±3.17	±2.39	±3.30
X40	Mean	0.64	0.93	0.86	0.83	0.87	0.83	0.77	0.82	0.56	0.62	0.75	0.66	0.86	0.87	0.88	0.75
	SD	±0.13	±0.24	±0.27	±0.5	±0.22	±0.17	±0.21	±0.13	±0.40	±0.28	±0.13	±0.23	±0.31	±0.44	±0.21	±0.23
X41	Mean	1.35	1.34	1.25	1.43	1.29	1.32	1.22	1.25	1.31	1.29	1.18	1.33	1.34	1.37	1.51	1.45
	SD	±0.41	±0.95	±0.93	±0.92	±0.91	±0.45	±0.94	±0.97	±1.02	±0.38	±0.99	±0.24	±0.44	±0.33	±0.98	±0.92
X42	Mean	20.85	23.37	22.45	21.15	16.13	17.57	16.91	17.55	14.6	15.04	16.32	15.91	17.07	18.09	18.01	17.58
	SD	±2.78	±4.11	±1.3	±4.12	±2.10	±2.52	±2.73	±3.14	±3.72	±0.17	±1.36	±3.18	±2.18	±2.5	±3.12	±2.84
X43	Mean	2.68	2.8	2.75	2.56	2.45	2.15	2.42	2.38	2.45	2.63	2.48	2.39	2.55	2.74	2.69	2.72
	SD	±0.30	±1.04	±0.84	±0.93	±0.75	±1.74	±1.13	±1.10	±1.25	±0.25	±0.44	±1.05	±1.43	±1.13	±1.15	±1.18
X44	Mean	1.55	1.33	1.46	1.52	1.63	1.81	1.79	1.53	1.25	1.17	1.22	1.38	1.06	1.3	1.22	1.29
	SD	±0.39	±0.15	±0.77	±0.27	±0.94	±0.38	±0.70	±0.19	±0.82	±0.71	±0.16	±0.94	±0.19	±0.10	±0.97	±0.82
X45	Mean	1.52	1.9	1.87	1.59	1.65	1.59	1.44	1.68	1.32	1.48	1.53	1.35	1.18	1.22	1.26	1.13
	SD	±0.92	±0.32	±1.09	±0.19	±0.44	±0.66	±0.81	±0.33	±0.74	±0.56	±0.90	±0.29	±0.74	±0.39	±0.54	±0.46
X46	Mean	9.22	9.15	9.15	9.14	6.57	6.52	8.29	7.01	5.8	5.71	6.98	6.55	6.91	6.8	8.99	9.06
	SD	±2.24	±1.24	±2.65	±1.84	±1.63	±2.74	±1.15	±2.25	±2.19	±1.84	±1.14	±1.15	±1.73	±1.14	±3.10	±1.75
X47	Mean	0.48	0.35	0.55	0.51	0.51	0.5	0.48	0.52	0.74	0.77	0.62	0.5	0.9	0.91	0.69	0.71
	SD	±1.83	±0.07	±0.24	±0.29	±0.25	±0.14	±0.13	±0.20	±0.31	±0.20	±0.13	±0.24	±0.18	±0.19	±0.29	±0.20

Table 4

Eigen values of the characters on the first five principal components
(Explanation of traits are given in Table 2)

Acronym	PC 1	PC 2	PC 3	PC 4	PC 5
X1	0.005	0.029	-0.062	-0.132	-0.027
X2	-0.005	-0.029	0.062	0.132	0.027
X3	0.430	-0.160	-0.781	0.312	0.029
X4	0.795	-0.323	0.332	-0.229	-0.123
X5	-0.025	-0.033	0.003	0.000	0.001
X6	0.018	0.078	0.025	0.001	-0.036
X7	0.344	0.769	0.089	0.028	0.104
X8	-0.022	-0.025	0.025	0.090	0.033
X9	-0.007	0.089	0.027	0.170	0.057
X10	-0.012	-0.046	0.010	-0.008	-0.006
X11	-0.012	-0.046	0.010	-0.008	-0.006
X12	-0.016	-0.012	0.043	0.107	0.039
X13	-0.005	-0.029	0.062	0.132	0.027
X14	-0.006	-0.017	-0.051	-0.141	-0.034
X15	0.000	-0.059	0.017	-0.018	-0.015
X16	0.038	-0.234	0.135	0.358	0.021
X17	-0.008	0.101	-0.087	-0.104	-0.004
X18	0.005	0.076	0.034	0.160	0.049
X19	0.021	0.051	-0.053	0.166	0.079
X20	0.043	-0.023	0.077	0.124	0.108
X21	0.021	-0.001	0.020	0.009	-0.050
X22	-0.014	0.024	-0.121	-0.265	-0.054
X23	-0.007	0.042	-0.069	-0.123	-0.019
X24	0.031	0.252	-0.014	0.279	-0.848
X25	-0.029	-0.002	0.018	0.167	0.086
X26	0.005	0.032	-0.003	-0.011	-0.004
X27	0.021	0.026	-0.011	0.085	0.174
X28	0.056	0.157	-0.195	0.067	0.273
X29	0.002	-0.001	0.019	0.042	0.022
X30	-0.019	-0.016	0.055	0.142	0.035
X31	-0.000	0.059	-0.017	0.018	0.015
X32	-0.012	-0.046	0.010	-0.008	-0.006
X33	-0.025	-0.033	0.003	0.000	0.001
X34	-0.013	0.012	-0.007	0.009	0.008
X35	-0.013	0.012	-0.007	0.009	0.008
X36	-0.005	-0.029	0.062	0.132	0.027
X37	0.143	0.143	0.247	0.219	0.098
X38	0.130	0.157	-0.093	-0.364	0.088
X39	0.075	0.155	0.018	0.046	0.228
X40	0.001	0.004	0.013	0.028	0.001
X41	7,70E-01	-0.005	0.010	0.018	-0.000
X42	0.073	-0.047	0.246	0.202	0.154
X43	0.001	-0.014	0.022	0.023	-0.019
X44	0.002	0.023	-0.010	-0.001	0.020
X45	0.005	0.016	-0.009	-0.026	-0.005
X46	0.028	-0.046	0.130	0.216	0.065
X47	-0.003	-0.011	-0.003	0.021	0.004
Eigenvalues	1013,18	45.638	7.787	5.054	4.212
Percentage	93.613	4.264	0.719	0.466	0.389
Cumulative Percentage	93.566	97.877	98.596	99.062	99.451

The positions of all investigated populations belonging to the Turkish *Vaccinium* and variable that explained most of the total variation using the first two components of the PCA are given in Fig. 7. Although the first extracted components explain 93.61 % of the total variation among the examined taxa, the first three components were considered due to height Eigenvalues and those account for 93.61 %, 4.21 %, and 0.71 % of the total variation, respectively (Table 3). As seen in Table 3, the first component emphasizes that the characters contributing most to the separation of the examined taxa are leaf length (0.79), leaf width (0.43), leaf teeth numbers in 1 cm long (0.34), tetrad axis (0.14), equatorial axis (0.13) and polar axis (0.07). The second component emphasizes that both leaf teeth numbers in 1 cm long (0.76) and leaf length (0.32) and also pedicel length (0.25) contributing most to the separation of the examined taxa. Besides the third component emphasized that leaf width (0.78) and leaf length (0.33) and both tetrad axis and colpus length (0.24) contribute most to the separation of the examined taxa (Table 3).

Discussion

The genus *Vaccinium* was investigated worldwide by several researchers (Sleumer, 1941; Camp, 1945; Stevens 1969, 1971) based on limited morphological characters (Vander Kloet, 1988). Although Odell and Vander Kloet (1991) reported that vegetative and generative characters are valuable to distinguish members of *Vaccinium*, Stevens (1971) emphasized that the many floral characters like calyx shape, corolla shape, and stamen supply limited information to distinguish taxa within the genus *Vaccinium*. Present study showed that leaf and pollen traits supply more informative data at the species level than flower and fruit traits to separate the examined *Vaccinium* taxa in Turkey (Table 2, 3).

The current study also supplies several new data for the surface sculpturing and venation type of leaf and seed length which are not specified before by Stevens (1978) in the “Flora of Turkey” and any other studies regarding *Vaccinium* in Turkey (Tables 2, 3). Although veining type was not investigated by Stevens (1978), its taxonomic importance was emphasized in the “Flora of China” (Fang, Stevens, 2005). Moreover, Pedraza-Penalosa et al. (2013) reported that leaf venation type is not affected by environmental conditions. In the present study, it was found that the vein islets of secondary veins are 8–9 in *V. arctostaphylos*, 5–7 in *V. myrtillus*, 3–4 in

V. uliginosum, and *V. vitis-idaea* (Fig. 1), which are compatible with the “Flora of China” (Fang, Stevens, 2005) and Pedraza-Penalosa et al. (2013). However, Powell and Vander Kloet (1997) reported that the foliar venation does not always supply valuable data at the species level as seen in *V. uliginosum* and *V. vitis-idaea* in the present study.

Odell and Vander Kloet (1991) used vegetative characters such as surface features and leaf blade size measurements to distinguish the *Vaccinium* taxa. Similarly, Pedraza-Penalosa and Luteyn (2011) described seven new species based on the leaves (shape, margins, indumentum) and floral traits (merosity, calyx, corolla, pedicel) from Andean South America. The numerical analyses indicated that leaf length (X4), leaf width (X3), and leaf teeth numbers in 1 cm long (X7) are the most valuable characters for separating the investigated *Vaccinium* species. Semerdjieva et al. (2003) indicated that the secondary sculpture (according to Barthlott, 1981) of leaf adaxial surface and adaxial cuticle thickness varied among *V. uliginosum*, *V. vitis-idaea*, and *V. myrtillus* however epicuticular structures were recorded only in *V. vitis-idaea* and *V. myrtillus*. Our observations also revealed that the leaf adaxial of *V. arctostaphylos* (Fig. 2a) is like fiber-fluted while it is ruminant in *V. myrtillus* (Fig. 2b) and *V. uliginosum* (Fig. 2c) and pustulate in *V. vitis-idaea* (Fig. 2d) due to epicuticular waxes. However epidermal cell borders are hardly indistinct in *V. arctostaphylos* (Fig. 2a), they are slightly visible in other species (Fig. 2b–d).

Szkudlarz (1999b) reported a relationship between the genus *Oxycoccus* Hill (*O. microcarpus* Turcz. ex Rupr. and *O. quadripetalus* Gilib.) and *V. vitis-idaea* based on number of locule in fruits as previously reported by Gray (1868 cited in Szkudlarz, 1999b) and frequently placing *V. vitis-idaea* in a separate genus or section. Additionally, chemotaxonomic studies also indicated differences of *V. vitis-idaea* from the remaining *Vaccinium* taxa (Gugnacka-Fiedor, 1994). However, the updated taxonomic position of *O. microcarpus* and *O. quadripetalus* Gilib. (The Plant List, 2010), the variation in the chemical composition of Turkish blueberries (Erik et al., 2020) and present findings did not support the view of Gray (1868 according to Szkudlarz, 1999b) and Gugnacka-Fiedor (1994).

The seed of *V. myrtillus*, *V. uliginosum*, and *V. vitis-idaea* varies from lunate to reniform or falcate in outline (Fig. 3b–d) and it is triangular, tetragonal or oval in *V. arctostaphylos* (Fig. 3a). Contrary Szkudlarz (2001) indicated that the seed shape varies

from oval to ovate in *V. myrtillus*, *V. uliginosum*, and *V. vitis-idaea*. However, the seeds length is very similar with finding of Szkudlarz (2001). The seed micromorphology of *V. arctostaphylos* was observed for the first time, however, the seed micromorphology of the rest species was investigated by Vander Kloet (1988) and Szkudlarz (2001). According to Vander Kloet (1988), Szkudlarz (2001) and Anisimova et al. (2005), the cell walls of exotesta cells are strongly lignified, thickened and obvious in *V. myrtillus*, *V. uliginosum*, and *V. vitis-idaea*. Present findings mainly support the researcher's observations.

It is well known that the dispersal unit of pollen (monads or tetrads) is an important feature in several genera of Ericaceae (e. g. *Erica* L.) (Wronska-Pilarek et al., 2018). On this basis, there are no differences in the examined *Vaccinium* taxa, however, there are significant differences among the tetrads axis. The mean measurement of tetrads is in general consistent with those reported by palynologists (Sarwar et al., 2006; Sarwar, Takahashi, 2007), although higher value ranges are generally obtained for the examined features.

Palynological features of *V. myrtillus*, *V. uliginosum*, and *V. vitis-idaea* were examined in many studies (Cockerham, Galletta, 1976; Kocon et al., 1981; Goldy et al., 1984, Sarwar et al., 2006; Sarwar, Takahashi, 2007). However, pollen features of *V. arctostaphylos* were documented for the first time and also detailed palynological observations were performed in detail based on multiple wild populations belonging to the rest of the examined *Vaccinium* taxa. The surface ornamentation of pollen grain varies from rugulate to psilate in *Vaccinium* (Sarwar et al., 2006). On the other hand, pollen surface is broadly fine verrucate in the Old World *Vaccinium* species and they have a wider variety of exine sculptures (Sarwar, Takahashi, 2007). Although pollen grains in *V. myrtillus* were recorded as verrucate by Kocon et al. (1981), the current study based on three populations showed that they are regulate (Fig. 5b). On the other hand, the pollen surface is verrucate in *V. arctostaphylos* (Fig. 5a), therefore it easily distinguishes itself from the other taxa based on the pollen surface ornamentation. Among the examined taxa, pollen grains of *V. arctostaphylos* have the longest tetrad axis (D: 28.55–43.22 μm) and that coincides with the palynological features of *V. sect. Hemimyrtillus* Sleumer (D: 30.1–45.0 μm) (Sarwar, Takahashi, 2007). Tetrad axis (X37) is among the most important palynological traits to delimitate the examined taxa (Table 2, 3). Correspondingly, all studied populations belonging

to *V. arctostaphylos* (label b) are exclusively grouped based on the combined dataset (Fig. 6). Sarwar and Takahashi (2007) noted that the lowest value of tetrad axis presents in *V. uliginosum* and also observed similarities in exine. In the present study, the smallest pollen grain was found in *V. uliginosum* (D: 21.47–28.95 μm), nevertheless, minimum values of exine were measured in *V. vitis-idaea* (Ex: 1.1–1.37 μm). Our results corroborate Sarwar et al. (2006) which consider slight differences in tetrad size and exine surface sculpture to be corresponding to the geographical distribution. Goldy et al. (1984) noted that exine sculpture may provide beneficial data on taxonomic relationships in *Vaccinium*. However, exine does not seem to be effective to separate *Vaccinium* taxa. Powell and Kron (2002), reported a relationship between *V. cylindraceum* Smith endemic to Azores and *V. arctostaphylos* based on both nuclear and chloroplast markers. However, present pollen findings of *V. arctostaphylos* (P: 20.58 μm) and *V. cylindraceum* (P: 44.92 μm) reported by Morgado et al. (2018) are not supporting a relationship between these species.

Stevens (1978) did not use any subgeneric classification in the treatment of Turkish *Vaccinium*. However, Nekrasova (1952) accepted sectional treating for the genus *Vaccinium* in the "Flora of the USSR". According to Nekrasova (1952), *V. myrtillus* and *V. uliginosum* belong to *V. sect. Euvaccinium* A. Gray, *V. vitis-idaea*, and *V. arctostaphylos* belong to *V. sect. Vitis-idaea* (Moench) Koch. However, present findings do not support treating *V. arctostaphylos* under the *V. sect. Vitis-idaea* contrary to Nekrasova (1952). The results clearly showed that *V. arctostaphylos* distinguishes from the rest of the examined species with several characters (e. g. leaf adaxial surface, the seed shape and epidermal cells of the seed) and it should be treated under the *V. sect. Hemimyrtillus* as stated by Powell and Kron (2002). Gailite et al. (2020) reported a close relationship between *V. myrtillus* and *V. uliginosum* based on genetic and geographic distribution. A similar relationship was reported for *V. uliginosum* and *V. vitis-idaea* by Vander Kloet and Avery (2010) based on phenological characters such as timing of flowering and fruiting, the number of plump seeds per berry. The present findings (Figs. 6–7) indicated that *V. uliginosum* and *V. vitis-idaea* are closely related taxa as stated by Vander Kloet and Avery (2010) and Bjedov et al. (2021). According to Powell and Kron (2002), molecular phylogeny, however, showed that *V. myrtillus* was neither closely related to *V. vitis-idaea* in Circumboreal Region.

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