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POLLEN MORPHOLOGY OF *COTONEASTER* (ROSACEAE) SPECIES IN TÜRKİYE AND ITS SYSTEMATIC SIGNIFICANCE

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Pollen morphology of 14 specimens from all nine Turkish native species along with one undescribed species of *Cotoneaster* was examined by light microscopy (LM) and scanning electron microscopy (SEM). The most frequent pollen types are trizonocolporate and tetracolporate, respectively and they occur together. Moreover, trisyncolporate, 3-parasyncolporate, 4-parasyncolporate, and pentacolporate pollen grains were observed in *Cotoneaster* for the first time in this study. Trizonocolporate pollen grains are radially symmetrical, isopolar, 22.5–35 × 26.25–42.5 µm in size and suboblate in shape. The amb is triangular, except *C. integerrimus*, which has a circular outline. Two endoaperture types can be distinguished. One is rectangular/square, the other is circular (endopore) and present only in *C. integerrimus*, *C. melanocarpus* and *C. tomentosus*. The exine pattern can be divided into two types, namely striate-perforate and rugulate-perforate. The quantitative pollen characteristics were analyzed using principal component analysis (PCA) and all pollen features were considered for hierarchical cluster analysis (HCA). Principal component analysis revealed that colpus width, endoaperture length, and endoaperture width are the most powerful metrical pollen traits in *Cotoneaster* identification. According to hierarchical cluster analysis, pollen traits enable division of *Cotoneaster* into two subgenera. The present study provides insights into pollen morphology of *Cotoneaster*, as well as its use for taxonomic purposes.

Keywords: *Cotoneaster*, hierarchical cluster analysis, pollen morphology, principal component analysis, taxonomy

INTRODUCTION

Rosaceae, comprising ca. 90 genera and 3000 species, is a relatively large family, notable for its taxonomic difficulty due to polyploidization, hybridization, apomixis, and radial evolution (The Angiosperm Phylogeny Group, 2016). As a consequence of complex breeding system, the genus *Cotoneaster* Medik. is one of the complicated taxa in Rosaceae and it includes 50 to 370 species according to different taxonomic approaches (Flinck and Hylmö, 1966; Phipps et al., 1990; Fryer and Hylmö, 2009; Dickoré and Kasperek, 2010). The genus is a woody member of Malinae subtribe (referred to Maloideae in Morgan et al., 1994; Evans and Campbell, 2002 and Pyrinae in Campbell et al., 2007; Potter et al., 2007; Li et al., 2012; Lo and Donoghue, 2012) and chiefly distributed in the northern hemisphere (Fryer and Hylmö, 2009). Some species of *Cotoneaster* are cultivated for ornamental usage because of the flower, colored fruit and cold tolerance (Li et al., 2012).

The complex breeding system allows to increase morphologically intermediate offspring. For instance, apomictic breeding is a well

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known phenomenon in *Cotoneaster* as in other genera of Rosaceae (Li et al., 2012; Rothleutner et al., 2016). Likewise, hybridization is a frequent occurrence in cultivation and the genus is composed of more than 50% tetraploids species (Fryer and Hylmö, 2009; Dickinson, 2018), although more comprehensive studies are needed to prove natural hybridization in *Cotoneaster* (Dickoré and Kasperek, 2010). Relationships within *Cotoneaster* are not deeply resolved, owing to complex species groups and lack of diagnostic morphological characters (Li et al., 2012; Meng et al., 2021). Initially, some flower characters such as petal color (white vs. pink or red), flower number and spreading or erect petals were used to divide *Cotoneaster* into two subgenera/sections, *Chaenopetalum* and *Orthopetalum* (syn. *Cotoneaster*) (Koehne, 1893; Flinck and Hylmö, 1966; Phipps et al., 1990; Fryer and Hylmö, 2009). Recently, molecular phylogenetic studies (Li et al., 2012; Meng et al., 2021) have focused on infrageneric taxonomy, hybridization and character evolution in the genus and showed the incongruence between the nrITS and cpDNA data indicating hybridization.

Rosaceae pollen morphology regarding subfamilies, tribes and genera has been described by many researchers (Zhou et al., 1999; 2000; Song et al., 2016) and pollen morphology of *Cotoneaster* has been studied by several authors (Eide, 1981; Hebda and Chinnappa, 1990; Sáez and Rosselló, 2012; Perveen and Qaiser, 2014; Ghosh and Saha, 2017). Some researchers (Hsieh and Huang, 1997; Chang et al., 2011a; Chang et al., 2011b) also examined pollen morphology of four Taiwanese *Cotoneaster* species, while four species from China were studied by Jarvis et al. (1992) and Zhou et al. (2000). Pollen morphology of several *Cotoneaster* species that occur in Iran (16 species) was examined by Raei Niaki et al. (2020), five of which (*C. integerrimus* Medik., *C. melanocarpus* (Bunge) Fischer, *C. nummularius* Fisch. & C.A.Mey., *C. morulus* Pojark. and *C. multiflorus* Bunge.) also grow in Türkiye and were investigated in our study. It is known that pollen morphology contains unique characters with a large amount of genetic information and they can play important roles for species identification and phylogenetic studies of fruit trees (Song et al., 2017). Some researches focused on taxonomic importance of pollen characters and showed valuable palynological knowledge for taxonomy of the target taxa (Işık and Dönmez, 2004; Oybak Dönmez, 2008; Ullah et al. 2022). However, pollen morphology of Turkish *Cotoneaster* taxa has been poorly understood from a taxonomic perspective.

The main object of the study was to investigate pollen morphology of all Turkish native *Cotoneaster* species and to evaluate pollen features in respect of taxonomy of the genus. Pollen features were examined by light and scanning electron microscopy, and multivariate analyses were performed using nine quantitative and four qualitative pollen characters. More specifically, palynological knowledge was employed to understand the taxonomic implications of pollen morphology in *Cotoneaster*.

MATERIALS AND METHODS

PLANT MATERIAL

The pollen material used in the present study was collected in Türkiye by the second and third authors (Table 1), and the voucher specimens are deposited at the Herbarium of Hacettepe University (HUB). For pollen morphological studies, a total of 13 specimens from eight known species, and one newly described species in preparation for publication were investigated. Whenever possible, more than one specimen was investigated for each species. The following specimens were examined: one from *C. integerrimus*, *C. melanocarpus*, *C. meyeri*, *C. transcaucasicus*, *C. morulus* and *Cotoneaster sp.*, two from *C. multiflorus* and *C. tomentosus*, and four from *C. nummularius*.

LIGHT AND SCANNING ELECTRON MICROSCOPIC STUDIES

For light microscopic (LM) studies, pollen placed on a microscope slide was first treated with 2–3 drops of 70% ethyl alcohol to remove oily substances (like pollenkitt). After the ethyl alcohol on the slide had evoparated on a hot plate, the pollen material was embedded in glycerine-jelly, stained with basic fuchsine, following the method of Wodehouse (1935). The aperture form was determined for a minimum of 100 grains per specimen, and the frequency occurrence of each aperture type was then calculated. Size measurements were made on the most frequent trizonocolporate pollen grains. The following param-

TABLE 1. Voucher information of the studied *Cotoneaster* specimens in this study.

Specimens	Voucher information
C. integerrimus Medik.	Sivas: 1662 m, 40°10'11.0"N, 037°52'715"E, 11.6.2014, AAD 19111
C. melanocarpus (Bunge) Fisch.	Kars: Kağızman, 1331 m, 40°03'54.3"N, 042°51'163"D, 05.05.2013, AAD 18486
C. multiflorus Bunge.	Erzurum: 2172 m, 40°40'00.7"N, 040°32'824"E, 29.06.2012, AAD 18020-M. Beilstein & J. Brock
	Sivas: Divriği, 1691 m, 39°36'991"N, 037°43'113"E, 11.6.2014, AAD 19101
C. meyeri Pojark.	Erzurum: Oltu, 1660 m, 40°23'99.9"N, 041°32'906"E, 30.05.2012, AAD 17960 - Z. Uğurlu
C. nummularius Fisch. & C.A. Mey.	Sivas: from Refahiye to Sivas, 25.05.2012, AAD 17912 - Z. Uğurlu
	Bingöl: 1145 m, 38°56'431"N, 040°38'105"E, 27.05.2012, AAD 17934 - Z. Uğurlu
	Kars: 1195 m, 40°10'815"N, 043°08'557"E, 30.05.2012 AAD 17947- Z. Uğurlu
	Çankırı: Kurşunlu, 1431 m, 40°55'78.7"N, 033°14'743"E, 30.05.2013, AAD 18530
C. transcaucasicus Pojark.	Kars: 1195 m, 40°10'815"N, 043°08'557"E, 30.05.2012, AAD 17944 - Z. Uğurlu
C. morulus Pojark.	Artvin: Yaylabaşı, 1468 m, 40°52'95.0"N, 041°20'795"E, 03.06.2013, AAD 18565
C. tomentosus (Aiton) Lindl.	Çankırı: Kurşunlu, 1431 m, 40°55'78.7"N, 033°14'743"E, 30.5.2013, AAD 18528.
	Çankırı: 30.05.2014, AAD 19045
Cotoneaster sp.	A4 Çankırı: Kurşunlu, 13.05.2014, AAD 19036

eters were measured: pollen size, given by the polar axis (P) and equatorial axis (E); equatorial diameter in polar view; distance between two ectocolpi at poles; colpus length (Clg) and colpus width (Clt); endoaperture length (Enaplg) and endoaperture width (Enaplt); exine and intine thickness. The pollen shape was determined by the ratio of mean length of polar axis to the mean length of equatorial axis (P/E). The polar area index (PAI) was calculated as the ratio of the distance between the apices of two ectocolpi to its equatorial diameter in polar view. P and E were measured for 50 pollen grains per specimen, and the other measurements were made on ten grains. IBM SPSS Statistics version 23 programme (George and Mallery, 2016) was utilized to calculate the means (M), standard deviations (SD) and ranges (V) for pollen size (P and E). Photomicrographs were taken with a Leica DFC 320 digital camera connected to a Leica DM 4000 B microscope.

For scanning electron microscopic (SEM) study, the pollen was first treated with 70% ethyl alcohol, then air-dried before being mounted on stubs, subsequently coated with gold. The photomicrographs were taken using a JSM 6490 LV electron microscope.

The palynological terminology mainly follows Punt et al. (2007) and Hesse et al. (2009). In addition, Van Bergen et al. (1995) and Grímsson et al. (2017) were used as sources for describing 3-parasyncolporate and 4-parasyncolporate pollen types.

MULTIVARIATE DATA ANALYSES

Thirteen pollen features, nine quantitative (polar axis, equatorial axis, polar area index, colpus length, colpus width, endoaperture length, endoaperture width, exine thickness, intine thickness) and four qualitative (pollen shape, amb shape, endoaperture shape, exine pattern) were analyzed for fourteen specimens of *Cotoneaster*.

For principal component analysis (PCA), arithmetic means of quantitative variables were used and the results were shown in a two-dimensional plot of the first and second PCs with eigenvalues of the characters. Vector values are provided in the results. All computations were run with the statistical software R version 4.0.5 using the FactoMineR and Factoextra packages (R Core Team, 2020). Hierarchical cluster analysis (HCA) based the Gower coefficient (Gower, 1971) was performed to group the examined taxa based on all the obtained quantitative and qualitative pollen features presented in Tables 2–3. The multivariate data analysis was conducted using Past version 2.17c (Hammer et al., 2001).

TABLE 2. Quantitative pollen characteristics of the studied Turkish *Cotoneaster* taxa.

	Pollen Type (%)						P (µm)			E (µm)		
Taxa-Voucher	**trizc**	**tetrac**	**trisync**	**3-psc**	**4-psc**	**pentac**	**M**	**SD**	**V**	**M**	**SD**	**V**
C. integerrimus-AAD 19111	59	22	15	3	–	1	28.85	±0.59	26.25–32.5	34.1	±0.83	28.75–37.5
C. melanocarpus-AAD 18486	65	20	12	2	1	–	27	±0.81	22.5–32.5	31.2	±0.94	26.25–37.5
C. multiflorus-AAD 18020 – M. Beilstein & J. Brock	99	1	–	–		–	26.33	±0.67	22.5–30	31.88	±0.95	26.25–35
C. multiflorus-AAD 19101	26	40	13	17	4	–	26.25	±0.57	22.5–26.25	32.05	±0.81	26.25–37.5
C. meyeri-AAD 17960 – Z. Uğurlu	56	32	2	10	–	–	26.58	±0.61	24.3–29.16	31.03	±0.98	26.73–34.02
C. nummularius-AAD 17912 – Z. Uğurlu	94	6	–	–	–	–	30.08	±0.7	26.73–34.02	36.94	±1.11	29.16–41.31
C. nummularius-AAD 17934 – Z. Uğurlu	49	47	2	2	–	–	26	±0.6	24.3–29.16	30.57	±1	26.73–36.45
C. nummularius-AAD 17947 – Z. Uğurlu	99	1	–	–	–	–	27.41	±0.68	24.3–30.38	32.08	±0.88	26.73–36.45
C. nummularius-AAD 18530	85	15	–	–	–	–	27.25	±0.54	25–30	32.33	±0.85	27.5–37.5
C. transcaucasicus-AAD 17944 – Z. Uğurlu	40	59	–	1	–	–	27.27	±0.6	24.3–29.16	31.54	±0.82	26.73–36.45
C. morulus-AAD 18565	88	11	1	–	–	–	26.48	±0.65	22.5–31.25	32.4	±0.94	26.25–38.75
C. tomentosus-AAD 18528	65	25	10	–	–	–	27.55	±0.47	25–30	31.68	±0.85	27.5–37.5
C. tomentosus-AAD 19045	46	53	1	–	–	–	31.33	±0.75	27.5–35	36	±0.84	32.5–41.45
C. sp.-AAD 19036	61	22	3	14	–	–	27.25	±0.66	22.5–31.25	33.65	±1.24	27.5–42.5

TABLE 2. Continued

Taxa-Voucher	**PAI**	**Clg (µm)**	**Clt (µm)**	**Enaplg (µm)**	**Enaplt (µm)**	**Exine thickness (µm)**	**Intine thickness (µm)**
C. integerrimus-AAD 19111	0.18	19 (21) 23	8 (10.7) 16	7 (10.8) 13	8 (10.6) 14	1.75 (1.92) 2	0.75 (0.77) 1
C. melanocarpus-AAD 18486	0.23	18 (19.60) 22	8 (8.8) 10	6 (8.6) 11	7 (8.4) 10	1.5 (1.78) 2	0.75 (0.78) 1
C. multiflorus-AAD 18020 – M. Beilstein & J. Brock	0.21	18 (20.7) 23	9 (11.7) 14	12 (15.5) 18	11 (12) 14	1.5 (1.7) 1.75	0.75 (0.8) 1
C. multiflorus-AAD 19101	0.21	18 (19.80) 21	9 (12.7) 14	12 (15.2) 17	10 (12.6) 14	1.5 (1.67) 1.75	0.75 (0.8) 1
C. meyeri-AAD 17960 – Z. Uğurlu	0.20	19 (21.80) 24	12.5 (13.75) 17.5	11 (14.8) 19	11 (13.4) 17	1.5 (1.8) 2	0.75 (0.8) 1
C. nummularius-AAD 17912 – Z. Uğurlu	0.21	23 (24.20) 25	15 (18) 20	19 (20.4) 23	15 (16.3) 20	1.5 (1.83) 2	0.75 (0.78) 1
C. nummularius-AAD 17934 – Z. Uğurlu	0.22	19 (21.20) 23	12.5 (15.5) 20	12 (15.8) 18	12 (13.3) 16	1.5 (1.78) 2	0.75 (0.8) 1
C. nummularius-AAD 17947 – Z. Uğurlu	0.23	21 (23.4) 25	12.5 (15.25) 17.5	14 (15.5) 18	12 (14.2) 16	1.5 (1.8) 2	0.75 (0.8) 1
C. nummularius-AAD 18530	0.25	19 (21.20) 24	9 (11.6) 13	10 (12.1) 15	9 (11.5) 13	1.75 (1.8) 2	0.75 (0.8) 1
C. transcaucasicus-AAD 17944 – Z. Uğurlu	0.22	20 (21.38) 25	9 (11.6) 13	10 (12.1) 15	9 (11.5) 13	1.75 (1.8) 2	0.75 (0.8) 1
C. morulus-AAD 18565	0.26	20 (22.20) 25	10 (11.8) 15	11 (13.7) 18	10 (12.2) 16	1.5 (1.8) 2	0.75 (0.78) 1
C. tomentosus-AAD 18528	0.29	21 (23.3) 26	6 (7.6) 10	6 (7.8) 10	6 (7.3) 9	1.5 (1.83) 2	0.75 (0.78) 1
C. tomentosus-AAD 19045	0.34	22 (23.3) 26	6 (8.30) 10	5 (8.2) 10	5 (8.05) 10	1.5 (2) 2.5	0.75 (0.8) 1
C. sp.-AAD 19036	0.29	21 (21.90) 24	9 (12.7) 17	9 (12.6) 15	8 (11.4) 15	1 (1.55) 2	0.50 (0.63) 0.75

Abbreviations: P – polar axis, E – equatorial axis, M – mean value, SD – standard deviation, V – variation, trizc – trizonocolporate, tetrac – tetracolporate, trisync – trisyncolporate, psc – parasyncolporate, pentac – pentacolporate, PAI – polar area index, Clg – colpus length, Clt – colpus width, Enaplg – endoaperture length, Enaplt – endoaperture width.

TABLE 3. Qualitative pollen characteristics of the studied Turkish *Cotoneaster* taxa.

Taxa-Voucher	Pollen shape	Amb	Endoaperture shape	Exine pattern
C. integerrimus-AAD 19111	suboblate	circular	circular (endopore)	Striate-perforate
C. melanocarpus-AAD 18486	suboblate	triangular	circular (endopore)	Rugulate-perforate
C. multiflorus-AAD 18020 – M. Beilstein & J. Brock	suboblate	triangular	rectangular/square	Striate-perforate
C. multiflorus-AAD 19101	suboblate	triangular	rectangular/square	Striate-perforate
C. meyeri-AAD 17960 – Z. Uğurlu	suboblate	triangular	rectangular/square	Striate-perforate
C. nummularius-AAD 17912 – Z. Uğurlu	suboblate	triangular	rectangular/square	Striate-perforate
C. nummularius-AAD 17934 – Z. Uğurlu	suboblate	triangular	rectangular/square	Striate-perforate
C. nummularius-AAD 17947 – Z. Uğurlu	suboblate	triangular	rectangular/square	Striate-perforate
C. nummularius-AAD 18530	suboblate	triangular	rectangular/square	Striate-perforate
C. transcaucasicus-AAD 17944 – Z. Uğurlu	suboblate	triangular	rectangular/square	Striate-perforate
C. morulus-AAD 18565	suboblate	triangular	rectangular/square	Rugulate-perforate
C. tomentosus-AAD 18528	suboblate	triangular	circular (endopore)	Rugulate-perforate
C. tomentosus-AAD 19045	suboblate	triangular	circular (endopore)	Rugulate-perforate
C. sp.-AAD 19036	suboblate	triangular	rectangular/square	Rugulate-perforate

RESULTS

GENERAL POLLEN MORPHOLOGY

A summary of pollen morphological observations of the Turkish *Cotoneaster* species examined in this study under LM and SEM is given Tables 2–3 and Figs. 1–2. Pollen grains occur as monads. The most frequent pollen types are trizonocolporate (26–99%) (Fig. 1a, b and Fig. 2a, b) and tetracolporate (1–59%) (Fig. 1c and Fig. 2c). However, in *C. multiflorus* (AAD 19101), *C. transcaucasicus* Pojark. and *C. tomentosus* (Aiton) Lindl. (AAD 19045) samples, the ratio of tetracolporate pollen type is higher than the ratio of trizonocolporate pollen. In all specimens both trizonocolporate and tetracolporate pollen grains occur together. In addition, some *Cotoneaster* specimens also have trisyncolporate (1–15%; *C. integerrimus*, *C. melanocarpus*, *C. multiflorus*-AAD 19101, *C. meyeri* Pojark., *C. nummularius*-AAD 17934, *C. morulus*, *C. tomentosus*, *C. sp.*) (Fig. 1d), 3-parasyncolporate (1–17%; *C. integerrimus*, *C. melanocarpus*, *C. multiflorus*-AAD 19101, *C. meyeri*, *C. nummularius*-AAD 17934, *C. transcaucasicus*, *C. sp.*) (Fig. 1e), 4-parasyncolporate (1–4%; *C. melanocarpus*, *C. multiflorus*-AAD 19101) (Fig. 1f) and pentacolporate (1%; *C. integerrimus*) (Fig. 1g) pollen grains. Trizonocolporate pollen grains are radially symmetrical, isopolar and small to medium in size: the polar axis (P) measures 22.5–35 µm, and the equatorial axis (E) measures 26.25–42.5 µm. The pollen grains are suboblate in shape. The equatorial outline is transversely elliptic. The amb (the outline of a pollen grain or spore seen in polar view) is triangular (Fig. 1a), with the exception of *C. integerrimus,* which has a circular amb (Fig. 1b). The polar area index (PAI) ranges from 0.18 to 0.34. The apertures situated at the equator and the aperture structure consist of a colpus and endoaperture. Margins of colpi are straight. Colpus length (Clg) ranges from 18 to 26 µm and colpus width (Clt) ranges from 6 to 20 µm. Colpus ends are acute (Fig. 1a) and colpus membrane is without any sculpturing element. Endoapertures are distinct and two endoaperture types can be distinguished. One is rectangular/square in outline (Fig. 1h), the other is circular (endopore) (Fig. 1i), and occurs only in *C. integerrimus*, C. *melanocarpus* and *C. tomentosus*. Rectangular endoapertures are slightly equatorially elongate (lalongate) in some pollen grains of *C. multiflorus* and *C. morulus*. Endoaperture length (Enaplg) ranges from 5 to 23 µm and endoaperture width (Enaplt) ranges from 5 to 20 µm. The exine wall is tectate-columellate and 1–2.5 µm in thickness. Exine pattern types are striate-perforate (Fig. 2d-h) and rugulate-perforate (Fig. 2i-l). The striate-perforate type consists of lirae (which vary in size, orientation and anastomosing) separated by grooves (varied in size), having perforations (which vary in size and frequency) and the rugulate-perforate type consists of elongated exine elements longer than 1 µm, irregularly arranged (with perforations). Intine thickness is ≤ 1 µm.

EVALUTION OF MULTIVARIATE ANALYSES

The metric data of pollen grains were presented using PCA to reveal taxonomic relationship among the taxa (Fig. 3, Table 4). The first and second axes from the PCA explained 80.9% of the accumulated variance of the analyzed data. The first axis (PC1) accounted for 36.8% of the variance, mainly based on pollen size (P and E). Cumulative variance of the second axis (PC2) totaled 44.1% and it was associated with the endoaperture size (Enaplg and Enaplt) and colpus width. Most of the target taxa were nested on the negative side of axis 1 and had the highest size of colpus width and endoaperture. The remaining taxa, i.e., *C. melanocarpus*, *C. tomentosus*, *C. integerrimus* and *Cotoneaster sp.*,

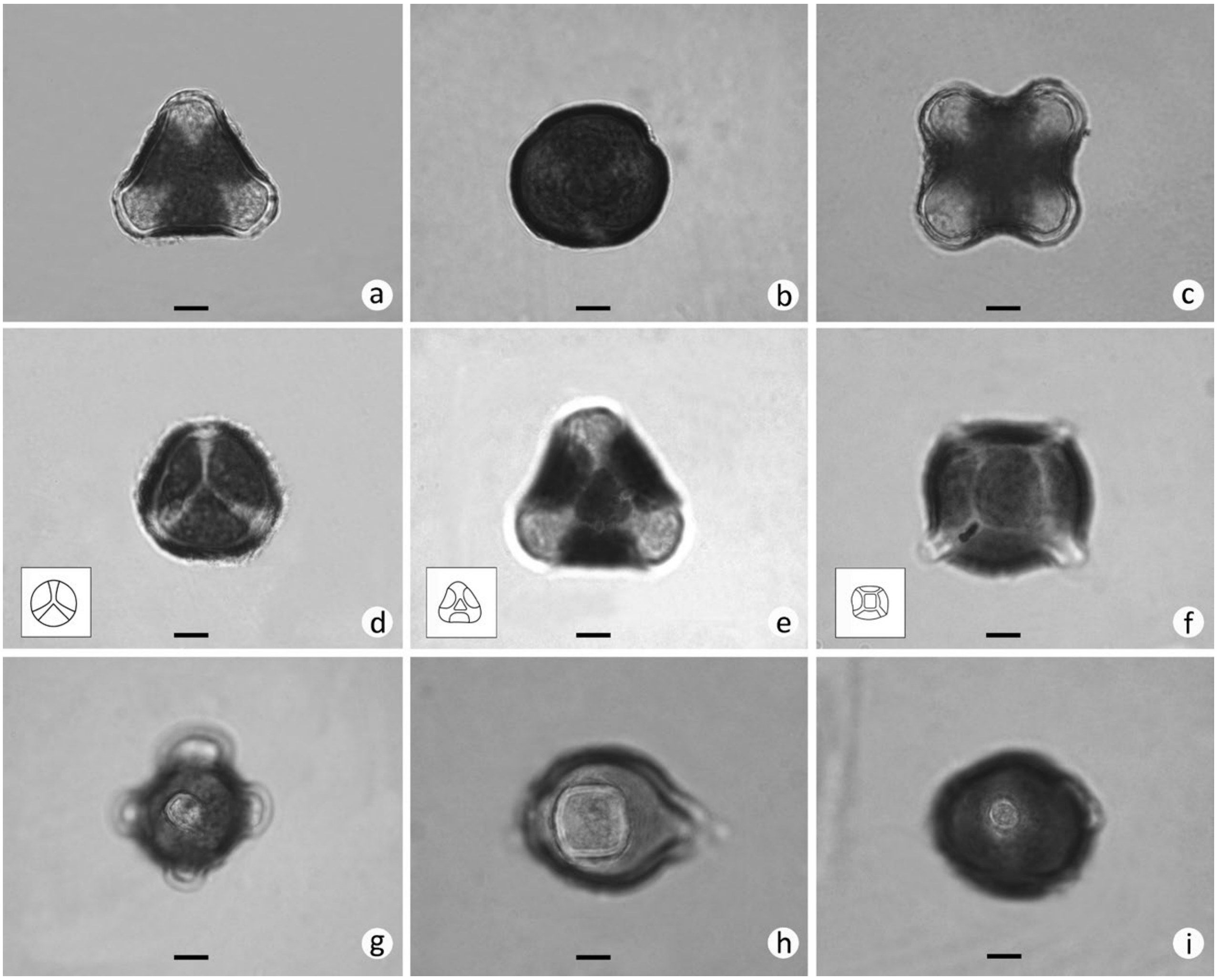

Fig. 1. Light microscopy (LM) photomicrographs of pollen grains of selected *Cotoneaster* species. **(a-b)** Trizonocolporate pollen grain in polar view **(a)** triangular in *C. morulus* (AAD 18565), **(b)** circular in *C. integerrimus* (AAD 19111). **(c)** Tetracolporate pollen grain in polar view in *C. multiflorus* (AAD 19101). **(d)** Trisyncolporate pollen grain in polar view in *C. melanocarpus* (AAD 18486). **(e)** 3-parasyncolporate pollen grain in polar view in *C. multiflorus* (AAD 19101). **(f)** 4-parasyncolporate pollen grain in polar view in *C. multiflorus* (AAD 19101). **(g)** Pentacolporate pollen grain in polar view in *C. integerrimus* (AAD 19111). **(h)** Rectangular/square endoaperture in equatorial view in *C. meyeri* (AAD 17960 – Z. Uğurlu). **(i)** Circular endoaperture (endopore) in equatorial view in *C. melanocarpus* (AAD 18486). Scale: 10 µm.

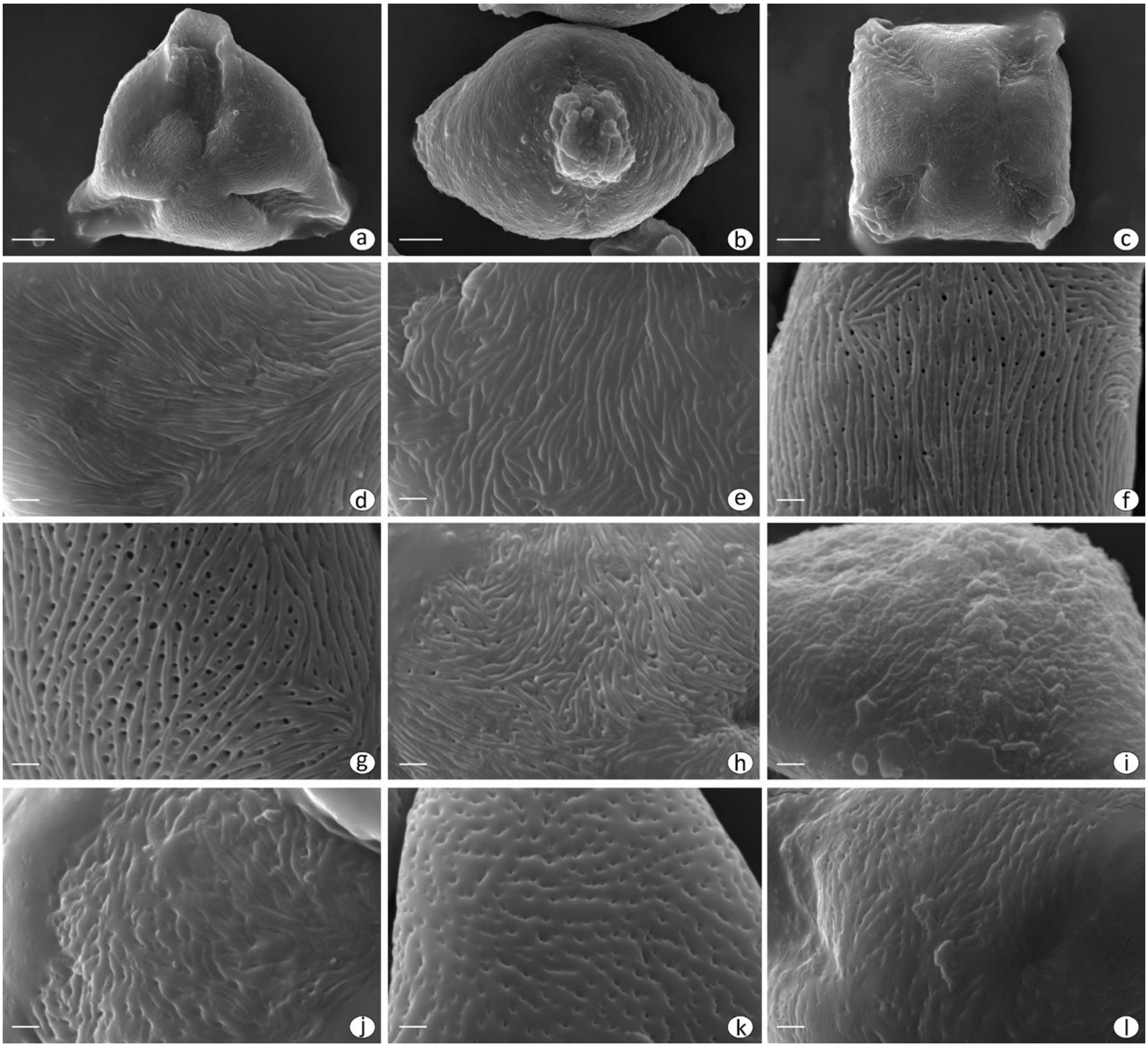

Fig. 2. Scanning electron microscopy (SEM) photomicrographs of pollen grains of *Cotoneaster* species. **(a–c)** *C. transcaucasicus* (AAD 17944 – Z. Uğurlu) **(a–b)** Tricolporate pollen grain **(a)** in polar view, **(b)** in equatorial view. **(c)** Tetracolporate pollen grain in polar view. **(d–h)** Striate-perforate exine patterns **(d)** *C. integerrimus* (AAD 19111), **(e)** *C. multiflorus* (AAD 19101), **(f)** *C. meyeri* (AAD 17960 – Z. Uğurlu), **(g)** *C. transcaucasicus* (AAD 17944 – Z. Uğurlu), **(h)** *C. nummularius* (AAD 17934 – Z. Uğurlu). **(i-l)** Rugulate-perforate exine patterns **(i)** *C. melanocarpus* (AAD 18486), **(j)** *C. morulus* (AAD 18565), **(k)** *C. tomentosus* (AAD 19045), **(l)** *C. sp.* (AAD 19036). Scale: a–c = 10 µm; d-l = 2 µm.

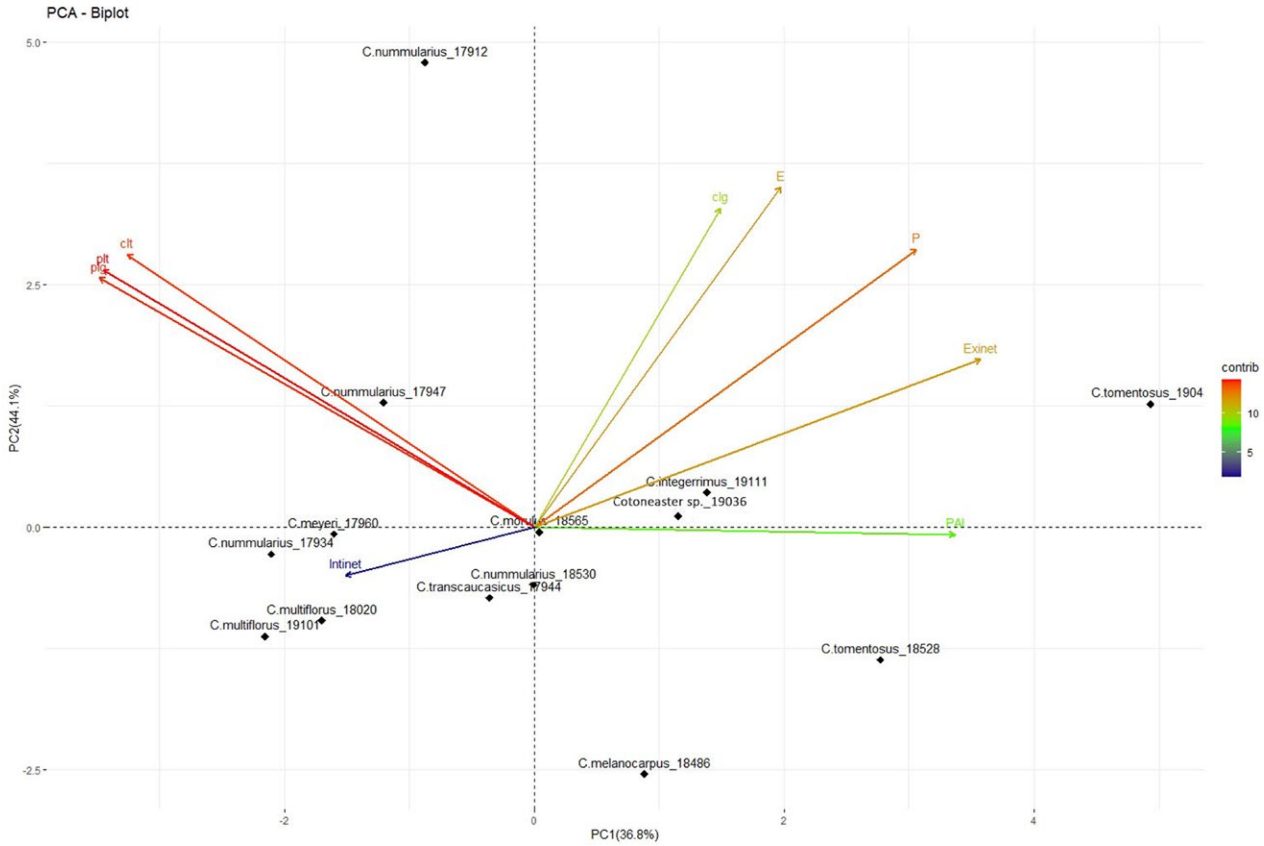

Fig. 3. Principal Component Analysis (PCA) performed with the pollen metric data of *Cotoneaster* taxa. Character codes follow Table 4. (contrib: contribution of variables to principal component).

TABLE 4. Vector values of the principal component analysis (PCA) of the nine quantitative pollen morphological characters of *Cotoneaster* taxa. The highest values are marked in bold.

Name of variables	PC1 (36.8%)	PC2 (44.1%)
Polar axis (P)	–0.042	**0.592**
Equatorial axis (E)	0.053	**0.702**
The polar area index (PAI)	–0.004	0.006
Colpus length (clg)	0.058	0.389
Colpus width (clt)	**0.553**	0.011
Endoaperture length (plg)	**0.677**	–0.039
Endoaperture width (plt)	**0.476**	–0.030
Exine thickness (Exinet)	–0.006	0.024
Intine thickness (Intinet)	0.040	–0.001

were positioned on the positive side of this axis. The common feature of these species was the highest value of polar and equatorial axes. Most of the *Cotoneaster* taxa were grouped around the centre of PC1 and PC2, while different specimens of *C. nummularius* and *C. tomentosus* were distributed at the extreme sides of PC1 and PC2.

Based on the qualitative and quantitative pollen characters, hierarchical cluster analysis (HCA) was performed to explore the relationship of *Cotoneaster* taxa. The phenogram produced three main sharp groups (Fig. 4). Group I includes *C. multiflorus*, *C. meyeri*, *C. nummularius* (AAD 17934, AAD 17947, AAD 17912) and *C. transcaucasicus*. Four species of *Cotoneaster*: *C. integerrimus, C. melanocarpus, C. nummularius* (AAD 18530) and *C. tomentosus* appear in Group II and the remaining two taxa (*C. morulus* and *C. sp.*) are placed in Group III.

Among the examined taxa, *C. morulus* and *C. sp.* show a rugulate-perforate exine pattern with a rectangular/square endoaperture shape. Likewise, *C. tomentosus* and *C. melanocarpus* have the same exine pattern (rugulate-perforate) but the endoaperture shape is circular (endopore). Only *C. integerrimus* is characterized by a striate-perforate exine pattern and a circular (endopore) endoaperture shape.

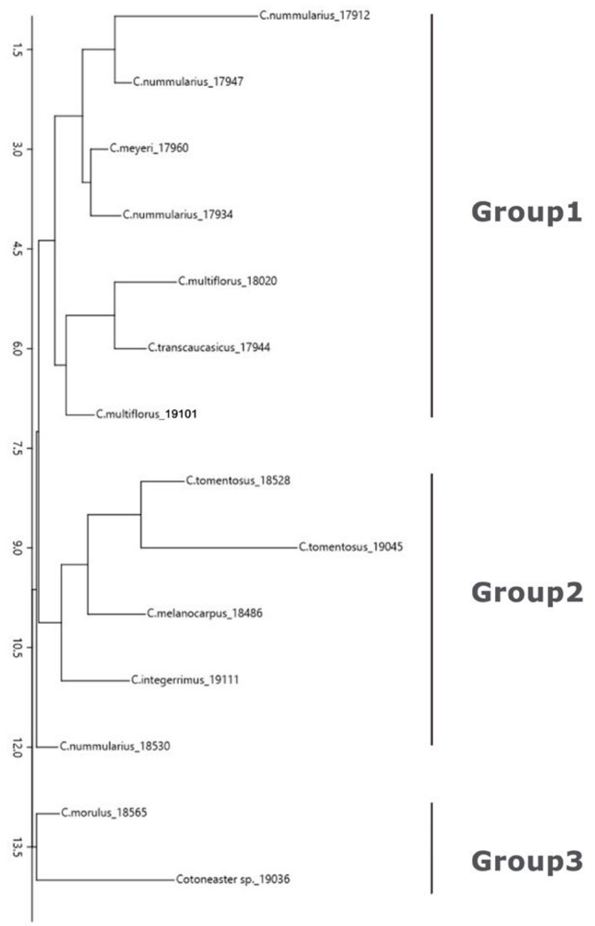

Fig. 4. Hierarchical Cluster Analysis performed with all pollen variables of *Cotoneaster* taxa.

The largest group of the phenogram including all populations of *C. nummularius, C. multiflorus*, *C. meyeri* and *C. transcaucasicus* is characterized by a striate-perforate exine pattern and rectangular/square endoaperture shape. It is clear that *Cotoneaster* taxa are classified based on the exine pattern features and endoaperture shape.

DISCUSSION

General outlines of the pollen grains in the Maleae tribe, which also comprises *Cotoneaster*, vary from oblate through spherical to prolate in equatorial view and the amb can be triangular through circular to three-lobed (Reitsma, 1966; Hebda et al., 1988; Perveen and Qaiser, 2014; Zhou et al., 2000; Ghosh and Saha, 2017). The main aperture type is tricolporate (Reitsma, 1966; Eide, 1981). However, some members (i.e., *Mespilus*, *Amelanchier*, *Crataegus*, *Sorbus*, *Malus*, ×*Malosorbus*, *Erilobus*) also have non-tricolporate pollen types such as tricolpate, tetracolporate, syncolpate, syncolporate, pericolp(or)ate and inaperturate (Reitsma, 1966; Hebda et al., 1988; Christensen, 1992; Işık and Dönmez, 2004; Oybak Dönmez, 2008). Pollen size in the tribe varies from 10.7 µm (*Cotoneaster*) to 56 µm (*Crataegus*, longest axis) (Christensen, 1992; Ghosh and Saha, 2017). Two basic types of sculpturing are observed: rugulate-striate and non-rugulate-striate. Rugulate to striate pollen grains are most frequent (Hebda and Chinnappa, 1990;1994; Zamani et al., 2010). Non-rugulate-striate pollen includes granulate-microscabrata (*Pyrus*) (Ghosh and Saha, 2017), perforate and microreticulate (*Crataegus*) (Oybak Dönmez, 2008), and reticulate (*Mespilus*, *Pyrus*, *Pyracantha*) (Byatt et al., 1977; Jarvis et al., 1992; Zhou et al., 2000).

Pollen grains of all the examined Turkish *Cotoneaster* species are suboblate in equatorial view (Table 3), unlike the spherical to prolate pollen grains recorded in previous *Cotoneaster* studies (Eide, 1981; Hsieh and Huang, 1997; Sáez and Rosselló, 2012; Perveen and Qaiser, 2014; Raei Niaki et al., 2020). The trizonocolporate pollen type is predominant in the Rosaceae family (Reitsma, 1966; Hebda and Chinnappa, 1990; Ghosh and Saha, 2017) as in *Cotoneaster* taxa. It was revealed that Turkish *Cotoneaster* species possess other pollen types along with trizonocolporate pollen within the same sack, including tetracolporate, trisyncolporate, 3-parasyncolporate, 4-parasyncolporate and pentacolporate ones. In all the specimens trizonocolporate and tetracolporate pollen grains co-occur but the others are present only in some specimens (Table 2). Tetracolporate pollen grains in three *Cotoneaster* species from Iran were also reported by Raei Niaki et al. (2020). However, in several previous pollen morphological studies on *Cotoneaster*, only trizonocolporate pollen was recorded (Eide, 1981; Hebda and Chinnappa, 1990; 1994; Jarvis et al., 1992; Hsieh and Huang, 1997; Zhou et al., 2000; Chang et al., 2011a; 2011b; Perveen and Qaiser, 2014; Ghosh and Saha, 2017). Trisyncolporate, 3-parasyncolporate, 4-parasyncolporate, and pentacolporate pollen grains were observed in *Cotoneaster* for the first time in this study (Fig. 1d-g). Polyploidy and apomixis are important phenomena among plants (Richards, 1990) and they are common in *Cotoneaster* species (Sax, 1954; Campbell and

Dickinson, 1990; Campbell et al., 1991; Bartish et al., 2001; Gregor, 2013; Rothleutner et al., 2016; Dickinson, 2018). Variation in pollen aperture numbers is generally related to different levels of ploidy (Borsch and Wilde, 2000) and the occurrence of pollen polymorphism associated with polyploid taxa has been recorded in some species (Chinnappa and Warner, 1982). In the case of the Turkish *Cotoneaster* samples, polyploidy and apomixis have not been examined yet. However, pollen type diversity observed in Turkish *Cotoneaster* species may be associated with polyploidy and apomixis.

The pollen grains of Turkish *Cotoneaster* are small to medium and 22.5–35µm (P), 26.25–42.5 µm (E) in size (Table 2). The range of (P) values is almost consistent with those of *Cotoneaster* species (23.2–34 µm) from Pakistan (Perveen and Qaiser, 2014) and 19.6–37.6 µm (Eide, 1981) from north-west Europe. However, Ghosh and Saha (2017) reported the smallest *Cotoneaster* pollen size (P: 10.4–11.2 µm, E: 7.2–8.8 µm) from India. There is no significant variation in pollen size between the Turkish species and within them, but only, *C. meyeri*, *C. transcaucasicus* and *C. tomentosus* show a narrower range of polar axis and equatorial axis.

The aperture form is also an important feature of Rosaceae pollen. In the family, the aperture structure usually consists of a colpus and equatorial endoaperture, which varies from being well-defined to weakly defined (Hebda and Chinnappa, 1990). Although the endoaperture shape is rectangular/square in most Turkish *Cotoneaster* specimens, *C. integerrimus*, *C. tomentosus* and *C. melanocarpus* can be distinguished from other species by their circular endoaperture shape (endopore) (Table 3). In previous studies on *Cotoneaster*, there was no detailed information about the endoaperture shape feature. Only Jarvis et al. (1992) divided rosaceous pores into three main types and established that *C. horizontalis*, which grows in China, has a star-shaped pore region. Furthermore, Hebda and Chinnappa (1990) mentioned the presence of a pore flap in *Cotoneaster* pollen. However, a pore flap is known to be characteristic of *Amelanchier* and *Crataegus* in the Maleae tribe (Hebda et al., 1988).

All the Turkish *Cotoneaster* specimens have a triangular amb, as in the Iranian samples described by Raei Niaki et al. (2020), except *C. integerrimus,* which has a circular outline (Table 3). It was also noted that the pollen grains of some *Cotoneaster* species in China and Taiwan have a circular amb (Hsieh and Huang, 1997; Zhou et al., 2000; Chang et al., 2011b).

Polar area index (PAI) and aperture size (colpus length and width, endoaperture length and width) vary slightly between species (Table 2). Polar area index is often an informative differentiating character for distinguishing between types. It depends highly on the length of the ectocolpi. Long ectocolpi result in a small apocolpium index, short ectocolpi give large indices (Punt and Hoen, 2009). On the other hand, such a relationship was not observed in the studied Turkish *Cotoneaster* species. However, colpus width (Clt), endoaperture length (Enaplg) and width (Enaplt) values were observed to be shorter in *C. integerrimus*, *C. tomentosus* and *C. melanocarpus* species with a circular endoaperture (endopore), and the PCA shows that these features are important to group the analyzed species (Tables 2–4). The exine pattern is considered to be one of the most important distinguishing characters in Rosaceae (Eide, 1981; Hebda and Chinnappa, 1990; 1994; Ghosh and Saha, 2017). In the Turkish *Cotoneaster* species, the most frequent exine patterns are rugulate-perforate and striate-perforate (Table 3 and Fig. 2d–l). The examined specimens can be divided into two groups, based on the exine pattern. Also, in previous studies on *Cotoneaster*, striate perforate (Hebda and Chinnappa, 1994; Hsieh and Huang, 1997; Zhou et al., 2000; Raei Niaki et al., 2020) and rugulate perforate (Eide, 1981; Hsieh and Huang, 1997; Chang et al., 2011a) exine patterns were reported. However, psilate (Eide, 1981; Hebda and Chinnapa, 1990; Raei Niaki et al., 2020) and foveolate (Zhou et al., 2000) exine patterns are also seen in *Cotoneaster* species.

Two of multivariate analysis, namely PCA and HCA, were performed in this study. Most of the studied taxa were placed around the negative side of PC1, except *C. melanocarpus*, *C. tomentosus*, *C. integerrimus* and *Cotoneaster sp.* The PCA biplot in this study shows that pollen, colpus width and endoaperture size offer important information for *Cotoneaster* identification (Fig. 3). Similarly, Li et al. (2012) investigated palynological traits in *Prunus* taxa to assess taxonomic relationships, and found colpus width with the other pollen characteristics (equatorial size, colpus length and ridge width) to be the most powerful diagnostic characters for apricot identification.

The results of PCA based on metric variables are not strictly correlated with clustering analysis loadings for all pollen characters. All specimens are classified into two groups on the plot of PCA, which included only quantitative characters, while three sharp groups are nested on the phenogram, which is composed of both quantitative and qualitative characters (Fig. 4). Four species, *C. tomentosus*, *C. melanocarpus*, *C. integerrimus* and *C. morulus*, belonging to subgenus *Cotoneaster*, were grouped in the same group with *C. nummularius* (AAD 18530) and the unidentified *Cotoneaster* specimen. It is in accordance with previous studies (Li et al., 2012; Raei Niaki et al., 2020) and supports monophyly of subgenus *Cotoneaster*. Raei Niaki et al. (2020) consider the correlation between a particular pollinator group and pollen type to explain pollen similarity in subgenus *Cotoneaster*. Interestingly, one population of *C. nummularius* (AAD 18530) belonging to subgenus *Chaenopetalum* is placed in the same group with the taxa of subgenus *Cotoneaster*. This may be because it has smaller colpus width (Clt), endoaperture length (Enaplg) and endoaperture width (Enaplt) values than other *C. nummularius* populations. The remaining studied taxa belonging to subgenus *Chaenopetalum* are clustered into the second branch of the phenogram. From a taxonomic point of view, our results confirm that pollen features are important to divide *Cotoneaster* taxa into two subgenera.

CONCLUSION

The present study highlights pollen features of all nine Turkish native taxa such as pollen type, pollen size, pollen shape, polar area index, aperture size and characteristics, exine-intine thickness, exine pattern characteristics as well as taxonomic usefulness of pollen characters. Quantitative and qualitative pollen characteristics are approximately consistent between populations of the same taxa. Four new pollen types (trisyncolporate, 3-parasyncolporate, 4-parasyncolporate, and pentacolporate) for *Cotoneaster* were observed for the first time in this study, unlike in previous studies on the pollen morphology of the genus. PCA shows that pollen data are limited to resolve taxonomic relationships at the species level. The HCA results are more useful to split *Cotoneaster* taxa into two main subgenera based on the studied palynological features. Specifically, the exine pattern and endoaperture shape help to elucidate their taxonomic rank at the subgenus level. The unidentified *Cotoneaster* specimen was nested seperately in the phenogram and the pollen characterictics may provide additional clues to describe this taxon as a new taxonomic level. However, further knowledge is needed to clarify its systematic position.

AUTHORS' CONTRIBUTIONS

Serap Işık Seylan: pollen morphological studies and writing. Zübeyde Uğurlu Aydın: data analyses, fieldwork and writing. Ali A. Dönmez: Project administration, fieldwork, review and editing. The authors declare that there is no conflict of interest.

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