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Pollen and floral micromorphological Studies of the genus *Cotoneaster* Medik. (Rosaceae) and its systematic importance

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Abstract. The micromorphology of petal and pollen grain of 16 species of the genus *Cotoneaster* Medik., belonging to two subgenera (*Cotoneaster* and Chaenopetalum) has been studied by light and scanning electron microscopy. We used different multivariate statistical methods to reveal the species relationships. Results showed that in comparison to most genera of the family Rosaceae, both tri- and tetracolporate pollen grains are observed in one specimen. Palynological observation revealed that shape of tricolporate pollen in most species is prolate-spheroidal, but also subprolate and prolate pollen grains can be recognized. In the other hand, tetracolporate pollen is quadrangular. The main ornamentation type was mainly striate which in turn can be subdivided to several categories; however, psilate one is also recognized also (*C. persicus*). The results revealed that pollen traits are probably effective in separating the sections and using these traits for placing a species in a particular section is probably helpful. Apomixis is one of the reasons for not changing the ornamentation of the both surface of the petals and their similarity to each other in different species. Totally, the studied micromorphological characters of petal cannot be used as diagnostic tools for *Cotoneaster* in Iran.

Keywords: Cotoneaster, Iran; pollen, petal, Rosaceae, SEM.

INTRODUCTION

The genus Cotoneaster Medik. which is mostly a shrubby member of the subtribe Pyrinae, tribe Pyreae, subfamily Spiraeoideae, family Rosaceae (Campbell *et al.* 2007). *Cotoneaster* consists of about 90 species widespread in temperate Asia (except Japan), Europe and North Africa (Yü and Lu 1974; Lu and Brach 2003), although other authors consider the number of species 260 (Mabberley 2008) to 400 (Fryer and Hylmö 2009). The center of diversity for the genus is in Tibet and the Himalayas, though species are native across Asia, North Africa, and Europe (Bartish *et al.* 2001; Dickore and Kasperek 2010; Fryer and Hylmö 2009). In Iran, 19 species of the genus are mainly distributed in Alborz Mts., elevations in NW (Azerbaijan province) and NE (Khorasan province) (Raei Niaki *et al.* 2009). Among these species, the *C. assadii*, *C. esfandiarii* and *C. persicus* are endemic to Iran (Riedl 1969; Khatamsaz 1985; Khatamsaz 1992).

The petal traits or the number of flowers in the cyme is the main characters used to determine interspecific relationships in *Cotoneaster* (Koehne 1893, Yu 1963). In some families, petal morphology is one of the most important diagnostic characters (Sharma *et al.* 2005, Campbell *et al.* 2007, Akcin 2009, Arianmanesh *et al.* 2016). The patterns of petal epidermis in angiosperms particularly Rosaceae family have important characters for identification of close species (Christensen and Hansen 1998). Several researchers have focused on petal micromorphology of different genera of Rosaceae (Tahir *et al.* 2010, Sharifnia and Behzadi Shakib 2012, Omer *et al.* 2017).

Regarding pollen morphology, it has been proved to be beneficial in systematic of the family Rosaceae (Hebda and Chinnappa 1990); however some others deny such an application (Moore *et al.* 1991) which is caused by easy hybridization among several species and even genera of the family. Regarding *Cotoneaster*, some a few studies (Kumar and Panigrahi 1995; Hsieh and Huang 1997; Perveen and Qaiser 2014) have reported some common features of pollen such as size, aperture number, exine thickness and ornamentation of surface. According to these studies, ornamentation of pollen surface including striate, sub-psilate and regulate ones is the most important feature in separating species. However, generally they emphasized these characters only play a little role in separating a few species and pollen morphology is not a useful tool in classification of the genus.

A comprehensive study on morphological and micro-morphological characters in *Cotoneaster* is almost lacking, moreover, the potential application of these characters in taxonomy of the genus has not been illustrated yet. Therefore, the objectives of the present study were 1) to provide detailed morphological and micro-morphological information on petal and pollen morphology of *Cotoneaster*, and 2) to evaluate application of these characters to find out the inter species relationships and delimit the species taxonomically.

MATERIAL AND METHODS

Pollen sampling

Totally 42 populations were collected and studied from 16 taxa of *Cotoneaster* from different habitats in Iran for study the pollen features (Table 1). 5-8 individuals of each location were studied and examined for 2 qualitative and 13 quantitative features (Table 2 and 3). Voucher specimens were deposited in TUH and FUMH (Table 1). Pollen obtained from flower buds at anthesis were prepared for light microscope (LM) using methods described by Harley (1992) with some modifications, mounted in glycerol jelly on glass slides and sealed. For LM measurements, at least 20–25 pollen grains were measured by Nikon light microscope model 200 M with aid of a × 100 eyepiece. For scanning electron microscopy (SEM) examinations, pollen grains were not acetolysed according to the method of Erdtman (1960). The pollen were suspended in a drop of water for a while, and then directly transferred to a metallic stub by a fine pipette, and double sided cello tape were used and then the pollen were sputtered in chamber coated with gold (Sputter Coater BALTEC, SCDOOS). Coating with gold by the physical vapor deposition method (PVD) was restricted to 100 Å. The SEM examination was carried out on a TESCAN microscope. For detailed examination of sculpturing, the classification presented in Ueda and Tomita (1989) was used. For estimation of pollen fertility, the pollen from fresh collected herbarium materials were stained by acetocarmine glycerin jelly, as described by Radford *et al.* (1974).

Petal sampling

In the current study, the micromorphological characteristics of petals of 16 species belonging to two subgenera of the *Cotoneaster* (*Cotoneaster* and *Chaenopetalum*) were studied for the first time. The collected specimens were deposited in the herbarium of Tehran University (TUH), Ferdowsi University of Mashhad Herbarium (FUMH) (Table 1). The voucher specimens are listed in Table 1. 122 specimens from 42 different populations of *Cotoneaster* taxa were collected from their natural habitats in Iran. Five micromorphological characters were examined; among the five characters, four were qualitative and one character was quantitative (Table 4).

The materials for SEM observation were directly mounted on stubs without any treatment, and sputter coated with gold-palladium. Petals and sepals of fully opened flowers were investigated using a HITACHI model SU 3500 electron microscope at 15 kV accelerating voltage; After a number of specimens had been compared under SEM. Both petal surfaces were examined. The epidermis of the petals was classified based on cell ornamentation, shape of cell (the primary sculpture), visibility of the boundary between cells using the terminology of Barthlott (1981) and Kay *et al.* (1981).

Table 1. List of the investigated taxa including origin of voucher specimens.

Taxon	Voucher information
C.* subgen. *Cotoneaster	
C. melanocarpus (Ledeb.) Lodd., G. Lodd. & W. Lodd. ex M. Roem.	Mazandaran: Firoozkooh road, 30 km after Veresk village to Tehran, after Dogol station. Raei Niaki & Mahdigholi. 46888-TUH
C.* subgen. *Chaenopetalum	
C. multiflorus Bunge.	Kurdistan: Nushoor olia village; Attar, Raei Niaki & Maroofi, 46870-TUH
C. suavis Pojark.	Khorasan: Gifan, Misino mountain, 20790-FUMH
C. hissaricus Pojark.	Azerbayjan: after Peygham village to Kaleybar; Attar, Zamani & Raei Niaki, 37261-TUH.
C. morulus Pojark.	Azerbayjan: Orumiyeh, Marmishu lake, Attar & Zamani, 40614-TUH
C. tytthocarpus Pojark.	Mazandaran: Siah-bishe, 7 km after Pole-Zanguleh to Chalus. Raei Niaki & Mahdigholi, 46887-TUH
C. luristanicus G. Klotz	Luristan: Aleshtar. Ghahraman, Attar & Ghaffari. 21658-TUH
C. turcomanicus Pojark.	Mazandaran: Firoozkooh Road, 30 Km after Veresk village to Tehran; Raei Niaki & Mahdigholi. 46890-TUH
C. nummularioides Pojark.	Mazandaran: Chalus road, between Reyzamin and Asara village; Attar, Zamani & Raei Niaki; 37203-TUH
C. kotschyi (C.K.Schneid.) G.Klotz	Mazandaran: Haraz Road, Yush village. Raei Niaki & Mahdigholi, 46897-TUH
C. assadii khat.	Mazandaran: Siah-bishe, Gachsar village; Raei Niaki & Mahdigholi, 46898-TUH
C. nummularius Fisch. & C.A.Mey.	Mazandaran: Firoozkooh Road, Seleben Village; Raei Niaki & Mahdigholi, 46901-TUH
C. ovatus Pojark.	Mazandaran: Firoozkooh Road, Seleben Village; Raei Niaki & Mahdigholi, 46892-TUH
C. esfandiarii khat.	Mazandaran: Firoozkooh, Arjmand village, Attar & Raei Niaki, 46886-TUH
C. discolor Pojark.	Mazandaran: Firoozkooh Road, 30 km after Veresk village to Tehran, Raei Niaki & Mahdigholi, 46889-TUH
C. persicus Pojark.	Khorasan: Pivehjan village, 23394-FUMH

Data analysis

The characters of pollen grains of the studied species are summarized in Tables 2 and 3. Multi-state qualitative characters converted into presence-absence descriptions. 13 pollen grain quantitative data were noted and treated statistically to determine average values for each species. PCA analysis were performed to check the similarity and dissimilarity between different taxa of the tribe, after linear standardization by range of each variable of the original data set. Principal Components Analysis (PCA) was performed to check the dissimilarity between the studied species based on palynological features useful for the delimitation of the species. To calculated Euclidean and taxonomic distance between different species of the genus, PCA ordination plot was performed (Podani 2000). PAST version 2.17 (Hammer *et al.* 2012) was used for multivariate statistical analyses of morphological data.

RESULTS

In the present investigation different micro morphological characters of the petal and pollen grain of *Cotoneaster* have been studied in detail. The investigated specimens are given in Table 1. The petal and pollen morphological characters are summarized in Table 2, 3 and 4.

Pollen morphology

The most important characters are given in table 2 and 3. Selected micrographs are presented in (Figures 1, 2, 3). Pollen grains are shed as monad, medium-sized (P= 29.26–35.13). One important and interesting feature observed frequently in most of the studied species, is the presence of tri- and tetracolporate pollen grains in the same specimen (e.g. Figures 1. e, o; Figures 2. e, k, q), while most species of family Rosaceae consist of tricolporate pollen. Percentage of this character is variable in different species, so that tetracolporate pollen in some species such as *C. melanocarpus* Fisch. ex A.Blytt , *C. kotschyi* (C.K.Schneid.) G.Klotz and *C. multiflorus* Bonge. is frequent, while the percentage of tricolporate pollen in some others such as *C. tytthocarpus* Pojrk. and *C. morulus* Pojark. constitute the major percentage of pollen grains. Regarding symmetry, both tricolporate and tetracolporate pollen grains are isopolar (e.g. Figures 1. i, k, m; Figures 2. c, e, f, g, m) and heteropolar (e.g.Figures 1. c, e, q). In the case of the shape of tri-

Table 2. Evaluated characters of pollen grains in *Cotoneaster* species studied (values M ± SD μm). M- Mean value; SD- Standard deviation. Abbreviations: P, polar axis length; E, equatorial axis length; P/E, proportion of polar axis to equatorial axis length; M, Mesocolpium length; A, Apocolpium length; C, Colpus length; E, Exine thickness

Taxa	P (μm)	E (μm)	P/E(μm)	M (μm)	A (μm)	C (μm)	E (μm)
C. Subgen.*Cotoneaster*							
C. Sect.*Cotoneaster*							
C. Ser. *Cotoneaster*							
C. integerrimus	31.0(33.73±2.12)39.0	23.0(28.20±3.76)35.0	1.3	12.00(18.53±4.61)25.00	5.00(6.60±1.40)9.00	27.00(29.47±2.26)35.00	0.70(0.97±0.12)1.10
C. Ser. *Melanocarpi*							
C. melanocarpaus	32.0(35.00±1.93)39.0	23.0(27.47±3.46)34.0	1.3	13.00(16.93±2.43)23.00	5.00(6.75±1.04)8.00	28.00(30.07±1.62)33.00	0.70(1.69±0.42)2.00
C. Subgen.*Chaenopetalum*							
C. Sect.*Chaenopetalum*							
C. Ser.*Racemiflori*							
C. persicus	29.00(33.40±2.85)39.00	18.00(25.20±4.77)35.00	1.37	11.00(17.93±5.19)30.00	4.00(5.87±1.12)8.00	25.00(29.47±2.39)34.00	1.00(1.71±0.32)2.00
C. discolor	30.00(31.10±1.28)34.00	25.00(28.20±2.73)33.00	1.11	17.00(21.73±4.32)30.00	5.00(6.33±1.18)8.00	22.00(22.47±1.99)30.00	0.70(0.97±0.12)1.10
C. assadii	30.00(32.43±2.35)35.00	23.00(26.00±3.67)33.00	1.25	16.00(20.53±5.73)25.00	4.00(6.12±1.32)9.00	23.00(26.05±1.25)28.00	0.85(1.34±0.56)1.30
C. nummularius	31.00(33.73±2.12)39.00	23.00(31.00±3.82)40.00	1.1	15.00(22.67±5.33)33.00	4.00(7.47±1.46)10.00	28.00(30.13±1.96)35.00	0.70(1.30±0.43)2.00
C. esfandiarii	28.00(30.93±2.31)35.00	23.00(28.20±3.76)35.00	1.11	16.00(21.93±4.88)32.00	4.00(9.27±1.44)8.00	23.00(26.00±2.03)30.00	1.00(1.79±0.45)2.60
C. ovatus	29.00(33.47±1.76)36.00	25.00(30.87±3.87)36.00	1.1	11.00(20.60±4.92)28.00	4.00(6.07±1.39)9.00	26.00(29.87±2.53)36.00	0.80(1.39±0.48)2.00
C. Ser.*Hissarici*							
C. hissaricus	30.00(33.80±2.01)38.00	21.00(27.27±3.55)34.00	1.26	12.00(18.53±4.61)25.00	5.00(6.93±1.03)9.00	27.00(29.47±2.26)35.00	0.90(1.09±0.20)1.70
C. turcomanicus	31.00(35.13±3.11)43.00	21.00(33.20±5.72)40.00	1.1	10.00(25.80±8.21)35.00	5.00(7.47±1.19)9.00	23.00(30.20±4.34)40.00	0.80(1.38±0.46)2.50
C. morulus	25.00(30.20±2.98)37.00	13.00(21.20±3.51)26.00	1.47	7.00(13.47±3.58)20.00	5.00(6.60±1.40)9.00	21.00(26.13±3.02)33.00	1.00(1.26±0.20)1.70
C. tytthocarpus	25.00(29.26±3.01)35.00	18.00(22.93±3.79)32.00	1.29	9.00(13.33±2.77)20.00	5.00(6.25±2.50)10.00	20.00(25.33±3.11)30.00	0.90(1.44±0.46)2.10
C. luristanicus	31.00(33.53±2.20)37.00	27.00(33.40±3.16)38.00	1.01	17.00(26.20±5.68)37.00	5.00(6.97±1.26)10.00	25.00(28.80±2.73)33.00	1.20(1.75±0.33)2.50
C. kotschyi	30.00(33.20±2.40)38.00	21.00(24.27±3.06)32.00	1.39	11.00(16.13±4.75)30.00	4.00(5.10±0.96)7.00	26.00(29.53±2.10)34.00	0.80(1.23±0.33)1.90
C. nummularioides	25.00(31.27±2.68)37.00	21.00(23.53±2.03)28.00	1.34	12.00(15.80±2.24)20.00	4.00(6.53±1.36)9.00	21.00(27.53±2.88)33.00	1.00(1.40±0.38)2.00
C. Sect.*Multiflori*							
C. Ser. *Multiflori*							
C. multiflorus	30.00(34.78±2.82)37.00	20.00(26.89±5.04)36.00	1.32	8.00(14.56±4.19)21.00	5.00(7.21±1.25)9.00	25.00(29.67±3.08)34.00	1.50(1.76±0.22)2.00

Table 3. Continue evaluated characters of pollen grains in *Cotoneaster* species studied (values M ± SD μm). M- Mean value; SD- Standard deviation. Abbreviations: C/P, proportion of colpus to polar axis length; S, Shape of pollen; Sc, Sculpturing of pollen; FP, Fertility percentage

Taxa	C/P	S	Sc	FP	pore number	ridge width	inter ridge width	pore width
C. Subgen.*Cotoneaster*								
C. Sect.*Cotoneaster*								
C. Ser. *Cotoneaster*								
C. integerrimus	0.83	subprolate	TypeII-A	96%	2.00(8.87±3.00)13.00	0.19(0.22±0.03)0.28	0.04(0.08±0.02)0.14	0.05(0.14±0.05)0.22
C. Ser. *Melanocarpi*								
C. melanocarpaus	0.85	subprolate	TypeII-A	95%	7.00(10.33±2.47)16.00	0.18(0.31±0.08)0.47	0.05(0.14±0.07)0.37	0.05(0.21±0.09)0.38
C. Subgen.*Chaenopetalum*								
C. Sect.*Chaenopetalum*								
C. Ser.*Racemiflori*								
C. persicus	0.88	subprolate	Type VI	85%	4.00(6.11±1.80)9.00	0.08(0.18±0.05)0.24	0.07(0.13±0.04)0.22	0.07(0.16±0.03)0.26
C. discolor	0.72	prolate-spheroidal	Type V	97%	1.00(4.47±2.36)9.00	0.12(0.21±0.05)0.29	0.10(0.22±0.07)0.33	0.08(0.16±0.07)0.29
C. assadii	0.82	subprolate	Type III	92%	7.00(9.47±2.10)15.00	0.20(0.26±0.04)0.35	0.06(0.08±0.02)0.11	0.07(0.16±0.04)0.26
C. nummularius	0.89	prolate-spheroidal	Type I	98%	2.00(6.93±2.41)10.00	0.09(0.18±0.04)0.25	0.06(0.13±0.04)0.23	0.06(0.14±0.05)0.24
C. esfandiarii	0.84	prolate-spheroidal	Type I	98%	4.00(6.13±1.82)9.00	0.08(0.15±0.04)0.24	0.07(0.13±0.04)0.22	0.13(0.18±0.03)0.25
C. ovatus	0.89	prolate-spheroidal	Type III	98%	7.00(12.53±3.52)17.00	0.12(0.17±0.03)0.24	0.05(0.08±0.02)0.12	0.07(0.10±0.03)0.17
C. Ser.*Hissarici*								
C. hissaricus	0.87	subprolate	Type I	96%	6.00(8.27±2.41)14.00	0.10(0.16±0.04)0.22	0.07(0.16±0.07)0.30	0.09(0.19±0.06)0.34
C. turcomanicus	0.85	prolate-spheroidal	Type II-A	99%	2.00(5.93±2.29)9.00	0.17(0.27±0.07)0.45	0.13(0.20±0.04)0.29	0.18(0.23±0.03)0.30
C. morulus	0.86	prolate	Type II-B	96%	9.00(12.40±3.16)21.00	0.15(0.22±0.05)0.33	0.07(0.14±0.04)0.20	0.08(0.15±0.07)0.33
C. tytthocarpus	0.86	subprolate	Type I	90%	0.00(5.73±4.12)14.00	0.18(0.29±0.06)0.38	0.06(0.10±0.02)0.13	0.08(0.16±0.08)0.39
C. luristanicus	0.85	prolate-spheroidal	Type III & IV	99%	5.00(7.73±1.65)10.00	0.13(0.21±0.05)0.29	0.07(0.14±0.05)0.23	0.10(0.31±0.14)0.59
C. kotschyi	0.88	prolate	Type V	95%	3.00(5.07±1.12)7.00	0.13(0.18±0.03)0.26	0.09(0.14±0.03)0.21	0.08(0.19±0.06)0.30
C. nummularioides	0.88	prolate	Type V	88%	0.00(12.20±5.13)20.00	0.14(0.24±0.05)0.34	0.08(0.11±0.02)0.15	0.08(0.12±0.04)0.24
C. Sect.*Multiflori*								
C. Ser. *Multiflori*								
C. multiflorus	0.85	subprolate	Type I & IV	96%	0.00(4.13±3.01)9.00	0.14(0.25±0.05)0.35	0.08(0.12±0.02)0.16	0.07(0.17±0.07)0.34

Table 4. Distribution and coding of main petal characteristics in studied species.

Taxon	Number of conical projections in 50 μm²	Distinct or not distinct Boundaries between cells	Closely or not closely conical projections	Folding or not folding of top of conical projections	Oriented or not oriented of conical projections
C. subgen. *Cotoneaster*					
C. melanocarpus	12	_	+	+	_
C. subgen.*Chaenopetalum*					
C. multiflorus	9	+	_	_	+
C. suavis	9	_	+	+	_
C. hissaricus	13	+	_	+	+
C. morulus	12	+	_	+	+
C. tytthocarpus	19	_	+	_	_
C. luristanicus	16	_	+	+	_
C. turcomanicus	11	_	+	+	+
C. nummularioides	12	+	_	_	+
C. kotschyi	17	_	+	_	_
C. assadii	17	+	_	+	_
C. nummularius	25	_	+	_	_
C. ovatus	8	+	_	+	_
C. esfandiarii	18	_	+	+	+
C. discolor	17	+	+	_	+
C. persicus	13	+	_	_	_

colporate pollen in equatorial view, prolate- spheroidal (e.g. Figures 2. m), subprolate (e.g. Figure 2. i) and prolate (Figure 1. i) shapes (column S in Table 3) are recognized, while in polar view triangular (e.g. Figures 1.a, q) and trilobate (e.g Figures 1. h, Figures 2. a, k) shapes can be recognized. In the other hand, tetracolporate pollen are quadrangular (e.g. Figures 1. c, e, Figures 2. e, q). Shape of apex varies from obtuse (e.g. Figures 1. c, g, m, Figure 2. e) to truncate (e.g. Figure 1. E; Figures 2. g, i). Colpi which occupy 72% in *C. discolor* Pojark. to 89% in *C. nummularius* Fisch. and *C. ovatus* Pojark. of length of the polar axis, are arranged meridionally (e.g. Figure 1. G; Figure 2. e) or parallel (e.g. Figures 1 i, k; Figures 2. g, i). Endopores which are located in the middle of ectocolpi, consist of distinct (e.g. Figures 1. c, e, g) or indistinct (e.g. Figures 2. c, g, i) projections. The mean of polar axis length (column P in Table 2) varies from 29.26 μm in *C. tytthocarpus* to 35.13 μm in *C. turcomanicus* Pojark. while the mean of equatorial axis length (column E in Table 2) varies from 21.20 μm in *C. morulus* to 33.40 μm in *C. luristanicus* G. Klotz. The mean of mesocolpium axis length (column M in Table 2) varies from 13.33 μm in *C. tytthocarpus* to 26.20 μm in *C. luristanicus*. Regarding apocolpium axis length (column A in Table 2), range is from 5.10 μm in *C. kotschyi* to 9.27 μm in *C. esfandiarii*. The mean of colpus length (column C in Table 2) varies from 22.47 μm in *C. discolor* to 30.20 μm in *C. turcomanicus*. The thickness of exine (column E in Table 2) which is clearly composed of two layers (ectexine and endexine) varies from 0.97 μm in *C. discolor* to 1.79 μm in *C. esfandiarii* Khat. Results of fertility test showed that most species have high percentage of fertility so that this character (column FP in Table 3) ranges from 85% in *C. persicus* to 99% in *C. turcomanicus*. With regard to sculpturing, the prominent ornamentation is striate (e.g. Figures 1. d, f, l, p; Figure 2. h); however some others such as psilate (Figures 2. o, p) can be recognized. Also the sterile pollen grains have deformed shape (Figure 3. q).

As illustrated above, main feature of several species (i.e. sculpturing) is very homogenous in different species. But type of sculpturing, number of perforation and the perforation size is different in same species. For example, series *Hissarici* members represent a rather uniform group but different types of sculpturing are observed in these species. On the basis of this character, *C. persicus* is separated from other species with its psilate sculpturing (Figures 2. o, p). This species is closely related to *C. discolor*, but differs from it by subglabrous upper leaf surface (very sparsely pilose – strigose in *C. persicus*), red vein and petiole (green in *C. persicus*).

According to exine sculpturing pattern, two main types (striate) and non-striate (psilate) were recognized in the *Cotonoster*. Most of the specimens belong to types striate.

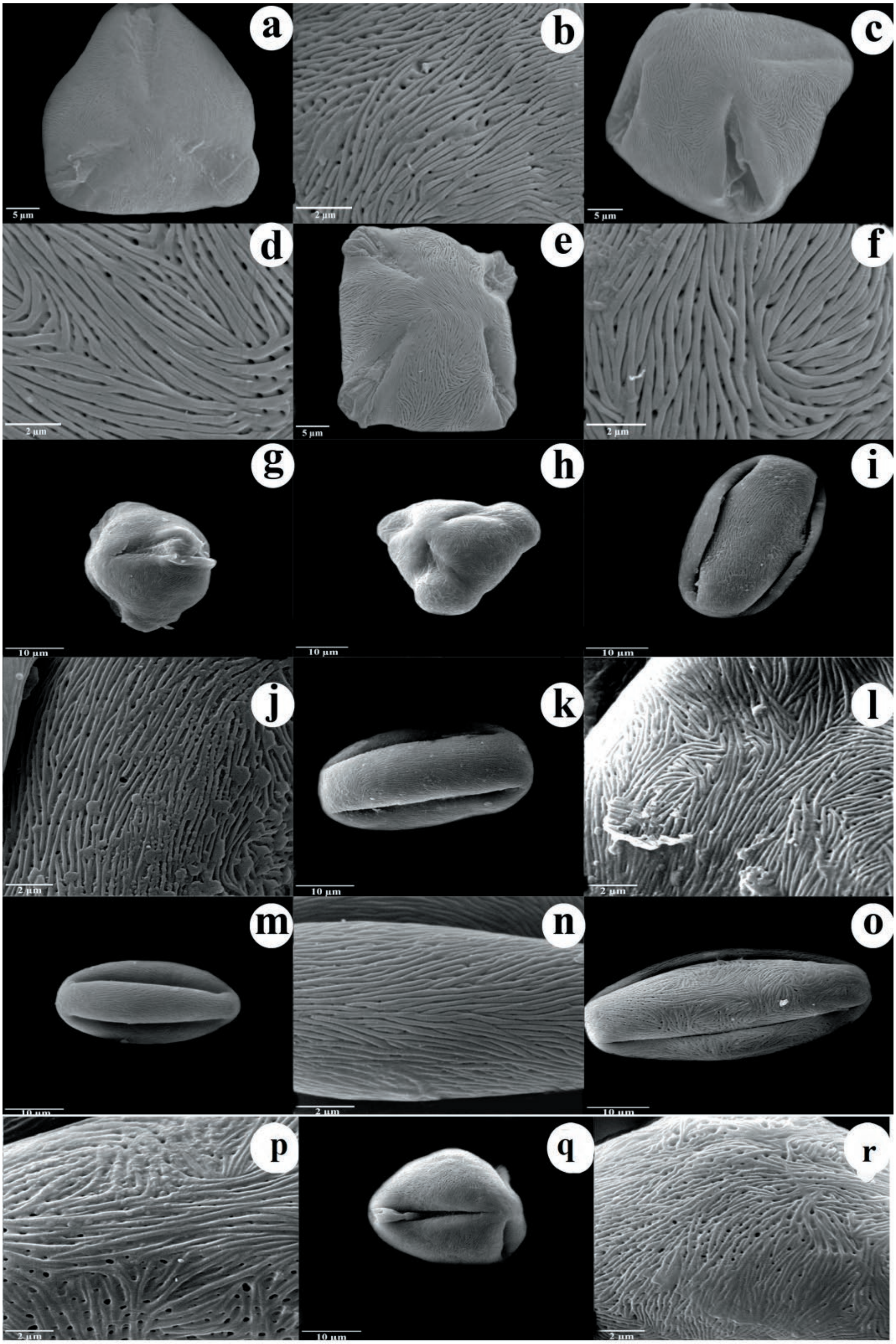

Figure 1. SEM micrographs of pollen grains of *C. integerimus* (a-b), *C. melanocarpus* (c-d), *C. turcomanicus* (e-f), *C. morulus* (g-j), *C. ovatus* (k-l), *C. assadii* (m-n), *C. luristanicus* (o-p), *C. nummularioides* (q-r).

Type (I): striate

This type is recognized by distribution of lira throughout pollen surface. This type is subdivided according to the pattern of perforation between lira.

Subtype I

Striate pollen, which has clear fingerprint-like ridges with few small perforations and with long intervals of ridges. This subtype is observed in these species: *C. nummularius*, *C. esfandiarii*, *C. hissaricus* Pojark., *C. tytthocarpus* and *C. multiflorus* (Figures 2 f, h, j, l, n).

The first two species surely belong to the subgenus *Chaenopetalum*, section *Chaenopetalum*, series *Racemiflori* (classification according Fryer and Hylmo, 2009). They have semi-dense inflorecensc and red fruit (except *C. esfandiarii*) and the two latter species are members of the *Hissarici* series that they have lax inflorecens and black fruit. This type also is observed in the last species; *C. multiflorus* from subgenus *Chaenopetalum*, section *Multiflori*, series *Multiflori* with open inflorescence and lower surface of leaves scarcely hairs but pollen of this species has very large perforation similar to that is seen in subtype IV.

Subtype type II (A-B)

This subtype differs from subtype I by having prominent perforations between ridges. This type is subdivided according to interval of ridges; subtype II-A and type subtype II- B with short and long intervals, respectively. Subtype II-A is observed in *C. integerrimus* Medik., *C. melanocarpus* and *C. turcomanicus* (Figures 1. b, d, f). Subtype II-B is seen in *C. morulus* (Figure 1. j)

Subtype III

This subtype differs from subtype II, subtype III has short ridges (0.15 to 0.30 µm) and can be seen in *C. assadii* Khat., *C. ovatus* and *C. luristanicus* (Figures 1. l, n, p). The first two species are members of the series *Racemiflori*, the latter species is the member of the *Hissarici* series.

Subtype IV

This subtype is diagnosed by having very large perforations.

Subtype V

This subtype is recognized by having obscure ridges due to very moderate slope of ridge. This subtype is seen in *C. discolor*, *C. kotschyi* and *C. nummularioides* Pojark. (Figures 2. b, d; Figure 1. r). First species belongs to the series *Racemiflori* and the latter two species are in *Hissarici* series.

Type (II): Psilate

This type is diagnosed by having no ridge on the pollen surface. This type is seen in *C. persicus* (Figures 2. o-r). This species is a member of the series *Racemiflori* and lacks any perforation on the surface.

Petal morphology

The micromorphological characters of petals of 16 species belonging to two subgenera of the *Cotoneaster* were studied. Also, according to previous studies on petals of other genera of Rosaceae, the ornamentations of the adaxial surface and the lower surface of the petals are described.

Adaxial surface of petals:

On the adaxial surface of all petals conical (finger-like or tubercle) shape projection are observed.

C. melanocarpus: The epidermal cells of the petal surface are loosely packed with distinct outline. This species exhibits irregular folds and rugose tuberculate pattern. The surface of each cell exhibits striate to rugose pattern. The ruga and striae are condensed and forming ruminate pattern on the tubercle of folds (Figure 4. a).

C. multiflorus: The petal surface cells are distinct and loosely packed with distinct cell walls. The margin of cells is smooth. The central part of the cells is raised into small regular finger-like projections. A tubercle is formed in the middle of the finger-like projection with ruminate patterns. The surface of each cell exhibiting striate to rugose pattern (Figure 4. b).

C. suavis: Adaxial surface has loosely packed cells with prominent cell boundaries and more or less thick folds, forming tubercle in the middle of the folds. The surface as a whole is striate to rugose but at the tubercle becomes ruminate (Figure 4. c).

C. hissaricus: Petal surface of this species exhibits closely packed epidermal cells. The cell surfaces are raised into broad finger-like projections or tubercles. The surface as a whole shows striate pattern which is paral-

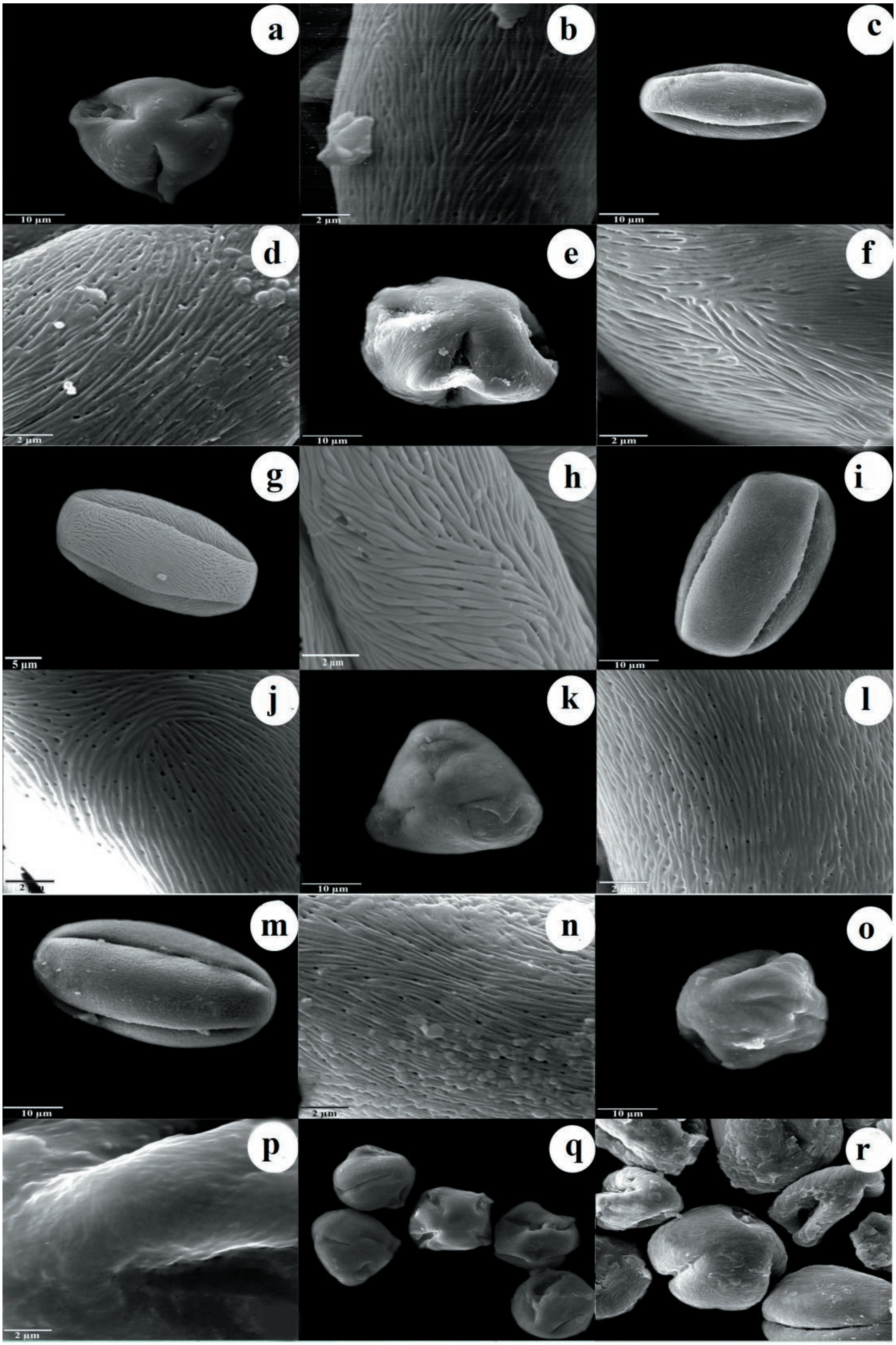

Figure 2. SEM micrographs of pollen grains of *C. kotschyi* (a-b), *C. discolor* (c-d), *C. multiflorus* (e-f), *C. tytthocarpus* (g-h), *C. hissaricus* (i-j), *C. nummularius* (k-l), *C. esfandiarii* (m-n), *C. persicus* (o-p), *multiflorus* (q), *C. discolor* (r).

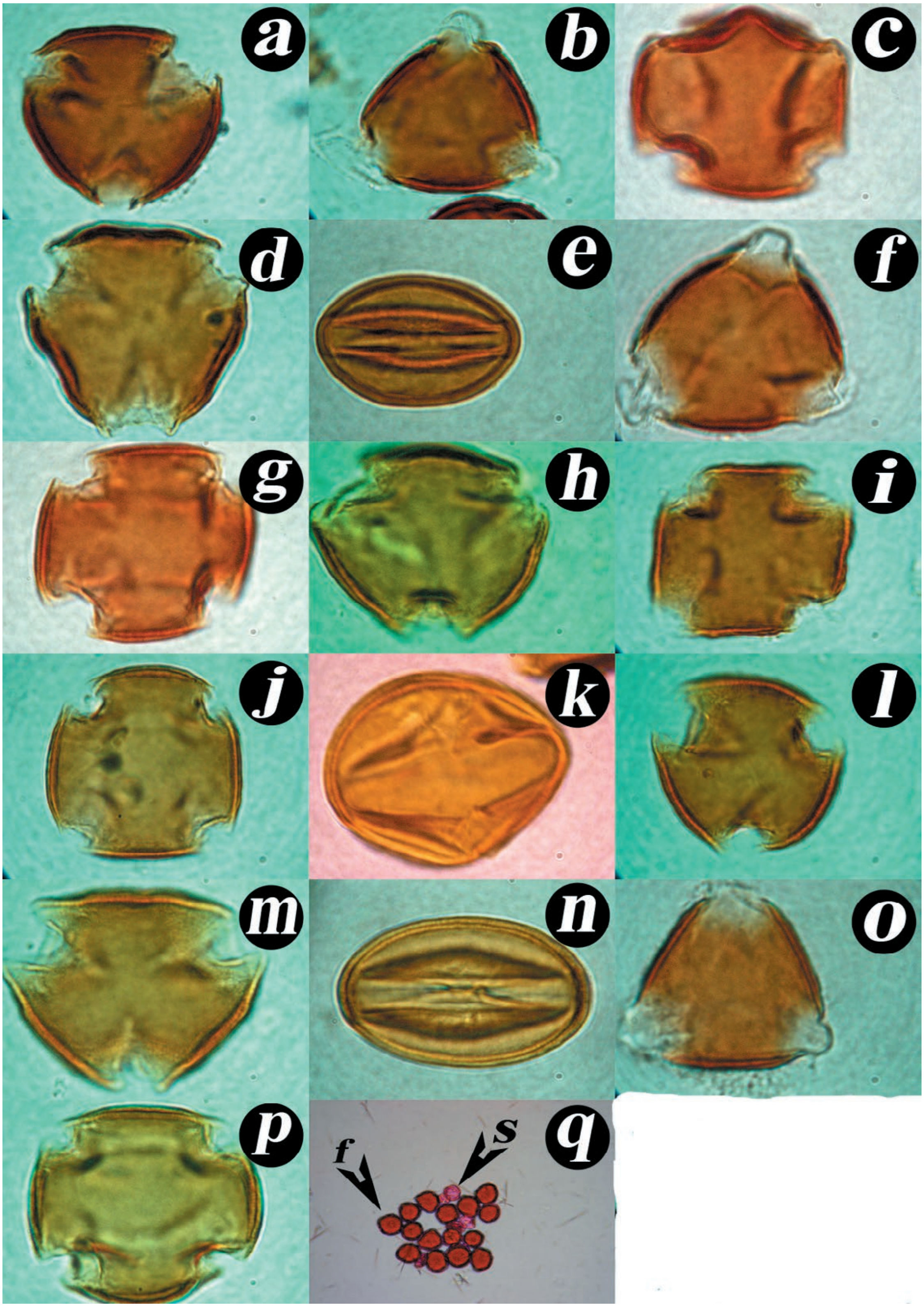

Figure 3. LM micrographs of pollen grains of *C. integerimus* (a), *C. melanocarpus* (b), *C. persicus* (c), *C. discolor* (d), *C. assadii* (e), *C. nummularius* (f), *C. esfandiarii* (g), *C. ovatus* (h), *C. hissaricus* (i), *C. turcomanicus* (j), *C. morulus* (k), *C. tytthocarpus* (l), *C. luristanicus* (m), *C. kotschyi* (n),*C. nummularioides* (o), *C. multiflorus* (p), fertil and steril pollen grain (q).

lel all over the surface except at the tubercles which are intermingled with together in these pearts (Figure 4. d).

C. morulus: The epidermal cells of the petal surface were loosely packed. Central part of each cell is raised into a fold surrounded by thick flat boundaries with distinct outline and rugose-tuberculate surface pattern which is condensed in the central fold or tubercle giving ruminate appearance. Cell margin is flat with smooth patterns (Figure 4. e).

C. tytthocarpus: The petal surface of this species exhibits closely packed epidermal cells. The cell surface is raised into broad finger-like projections and more or less thick folds, forming tubercle in the middle of the cell. The surface as a whole is rugose but at the tubercle becomes ruminate (Figure 4. f).

C. luristanicus: The epidermal cells of the petal surface are closely packed without distinct outline, showing rugose tuberculate pattern. The cell surface is raised into irregular projection giving appearance of simple folds or V-shaped folds. Ruga are observed all over the surface running parallel to each other or intermingling at the tubercle (Figure 4. g).

C. turcomanicus: The epidermal cells of petal surface are closely packed with rugose-tuberculate surface pattern and distinct outline. The cell surfaces are raised into big regular projections giving appearance of folds (Figure 4. h).

C. nummularioides: The petal surface exhibits rugose-ruminate pattern. The epidermal cells are distinct and loosely packed with thin walls. The elevated radial walls also show smooth pattern. The central part of the cells is raised into small finger-like projection (Figure 4. i).

C. kotschyi: Petal surface of this species exhibits closely packed without distinct outline. The cell surface is raised into broad finger-like projections and more or less thick folds, forming tubercles in the mid of the cell. The surface as a whole shows rugose to striate pattern which is parallel all over the surface except at the tubercle where these are intermingled with together (Figure 4. j).

C. assadii: The epidermal cells of the petal surface are loosely packed with distinct outline, showing rugose tuberculate pattern. The surface between radial walls of each cell exhibit striate pattern, the central part of the cells is raised into small irregular projections giving appearance of folds. The striae are condensed and forming ruminate pattern on the folds (Figure 4. k).

C. nummularius: Petal surface is composed of closely packed cells. Surface of the cell is raised into finger-like to folded projections or tubercles. The surface as a whole is rugose but at the tubercle becomes dense ruminate and cell boundaries are not clear (Figure 4. l).

C. ovatus: Petal surface of this species shows the cell boundaries prominently, the surface exhibits finger-like projections with rugose pattern (Figure 4. m).

C. esfandiarii: The petal surface of this species exhibits closely packed epidermal cells. The cell surface is raised into broad finger-like projections or tubercles, sometimes flattened into folds. The surface as a whole shows striate pattern except the top of the projections or tubercles where the striate show parallel and ruminate pattern (Figure 4. n).

C. discolor: The petal surface of this species exhibit closely packed epidermal cells. The cell surface is raised into broad finger-like projections or tubercles, sometimes flattened into folds. The surface as a whole shows striate pattern except the top of the projections or tubercles where the striate are ruminate pattern (Figure 4.o).

C. persicus: Petal surface of this species is loosely packed with traceable cell boundaries. The epidermal cell appears to be polygonal with raised folds. The central part of the cells is raised into small semi-regular projection giving appearance of folds. Sometimes the ruga are condensed forming ruminate pattern on the folds (Figure 4. p).

Abaxial surface of petal:

On the abaxial surface of petal two basic types of ornamentation are seen:

1) The striate surface as a whole, parallel and does not show the cell boundaries prominently (This form is observed in: *C. melanocarpus, C. multiflorus*, *C. luristanicus, C. kotschyi, C. ovatus, C. discolor*) (Figures 5. A, b, g, j, m, i).

2) The hive-shape with four to seven-sided houses in this form the boundaries between cells are clear. This form can be seen in the rest of the species studied (Figures 5.c, d, e, f, h, k, l , n, p).

These decorations were probably immature decorations. Because in some species such as *C. esfandiarii*, there was an intermediate of these two forms, and in species such as *C. ovatus* and *C. discolor*, in different individuals, there was one of the two forms.

Infrageneric variation

Both clustering and PCA analyses of the *Cotoneaster* species studied produced similar groupings and therefore only PCA analyses tree characters are presented here (Figures. 6 and 7).

The result based on pollen morphological: In this plot (Figures, 6), it can be seen that the two *Hissarici*

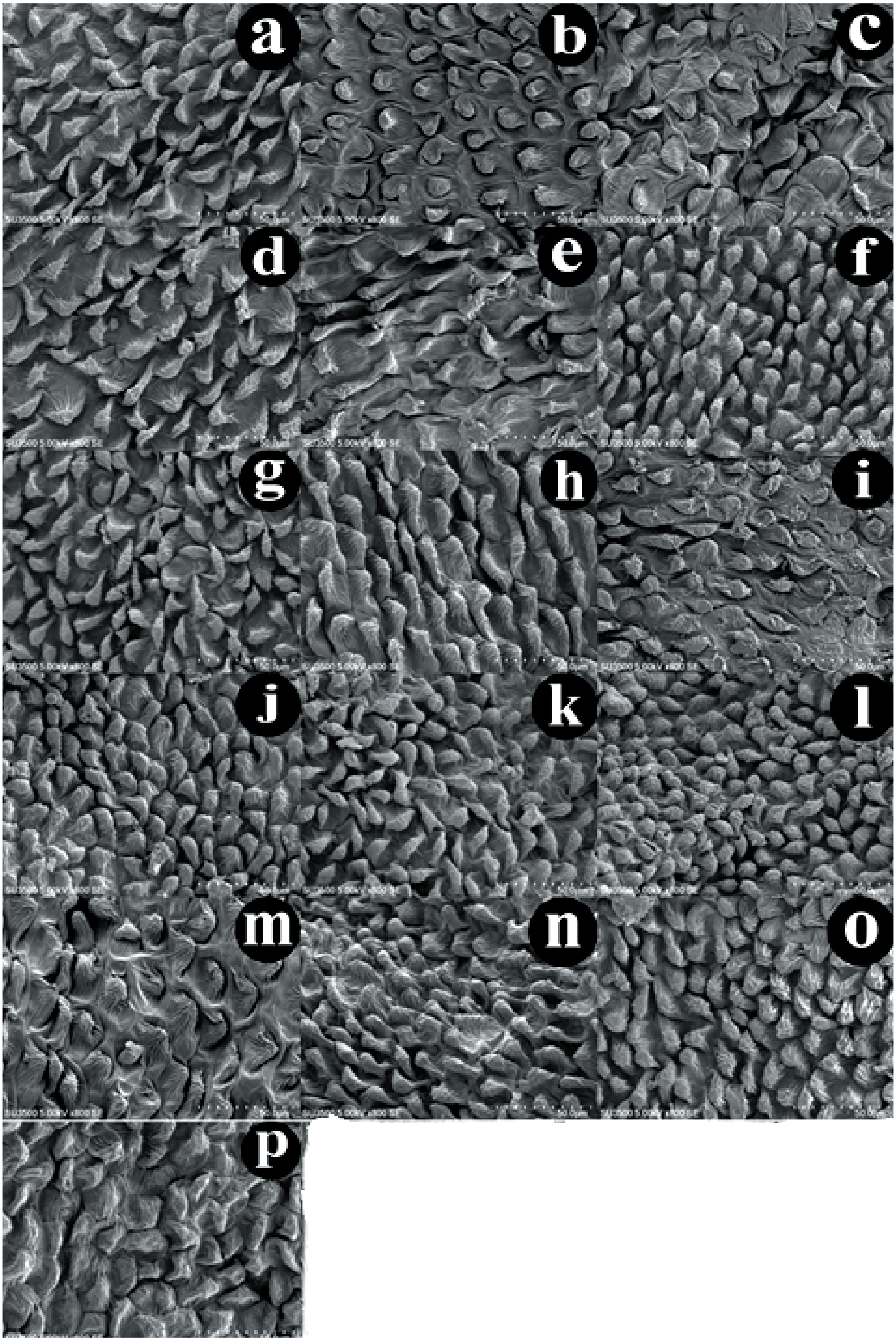

Figure 4. a-o. Micromorphological micrographs of ornamentation of adaxial surface in the studied species of *Cotoneaster*. *C. melanocarpus* (a), *C. multiflorus* (b), *C. suavis* (c), *C. hissaricus* (d), *C. morulus* (e), *C. tytthocarpus* (f), *C. luristanicus* (g), *C. turcomanicus* (h), *C. nummularioides* (i), *C. kotschyi* (j), *C. assadii* (k), *C. nummularius* (l), *C. ovatus* (m), *C. esfandiari* (n), *C. discolor* (o), *C. persicus* (p).

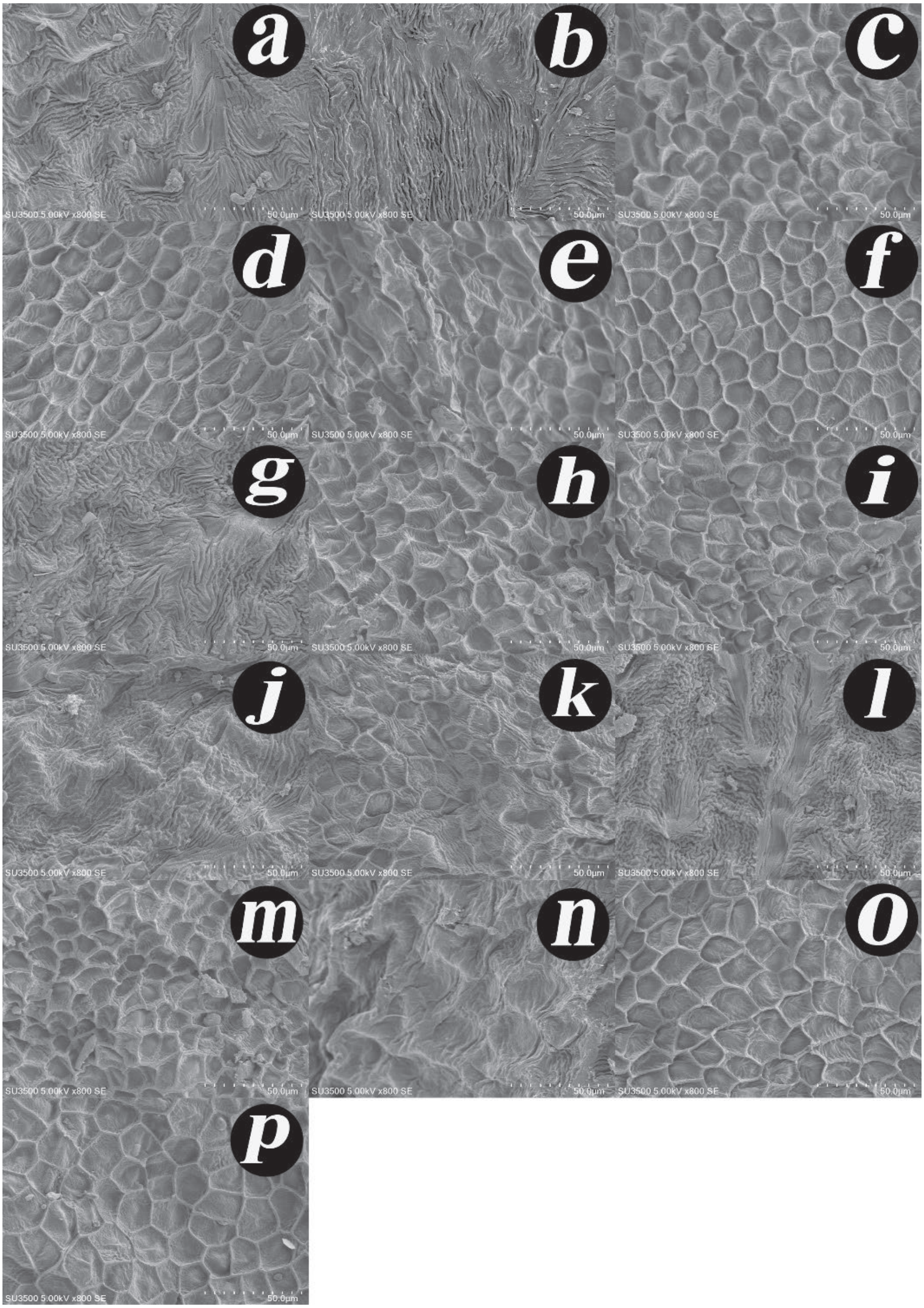

Figure 5. a-o. Micromorphological micrographs of ornamentation of abaxial surface in the studied species of *Cotoneaster*. *C. melanocarpus* (a), *C. multiflorus* (b), *C. suavis* (c), *C. hissaricus* (d), *C. morulus* (e), *C. tytthocarpus* (f), *C. luristanicus* (g), *C. turcomanicus* (h), *C. nummularioides* (i), *C. kotschyi* (j), *C. assadii* (k), *C. nummularius* (l), *C. ovatus* (m), *C. esfandiari* (n), *C. discolor* (i), *C. persicus* (p).

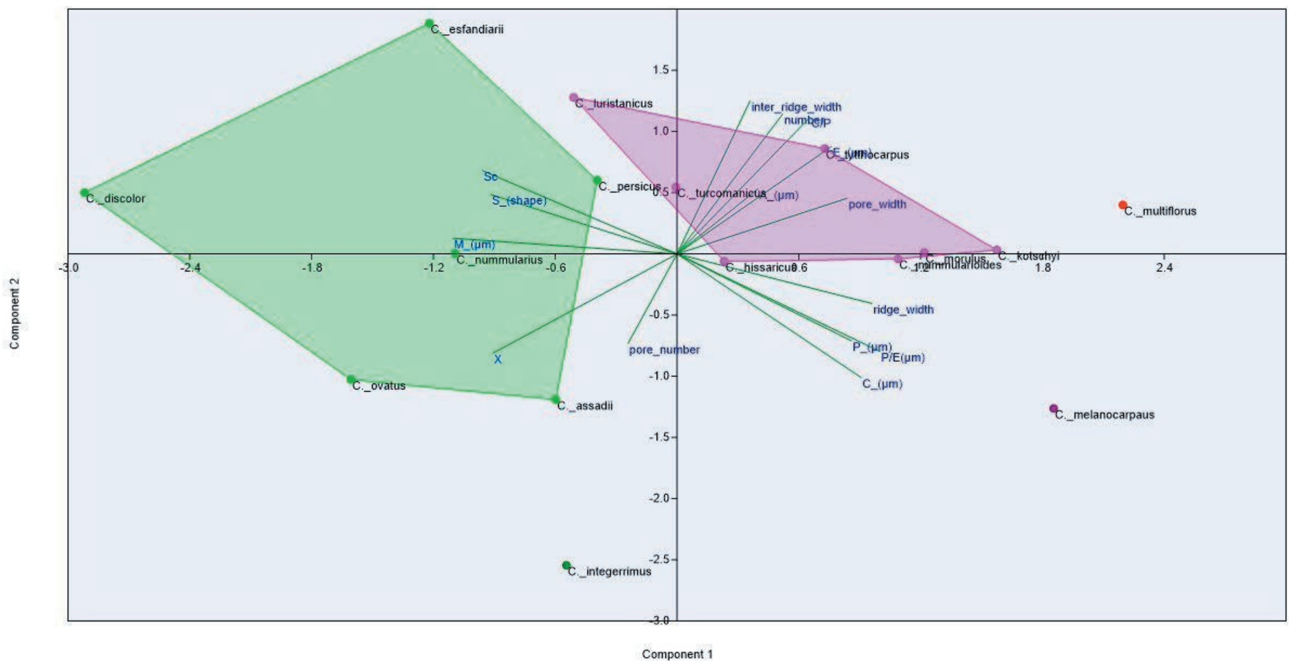

Figure 6. PCA plot of *Cotoneaster* species based on pollen morphological characters.

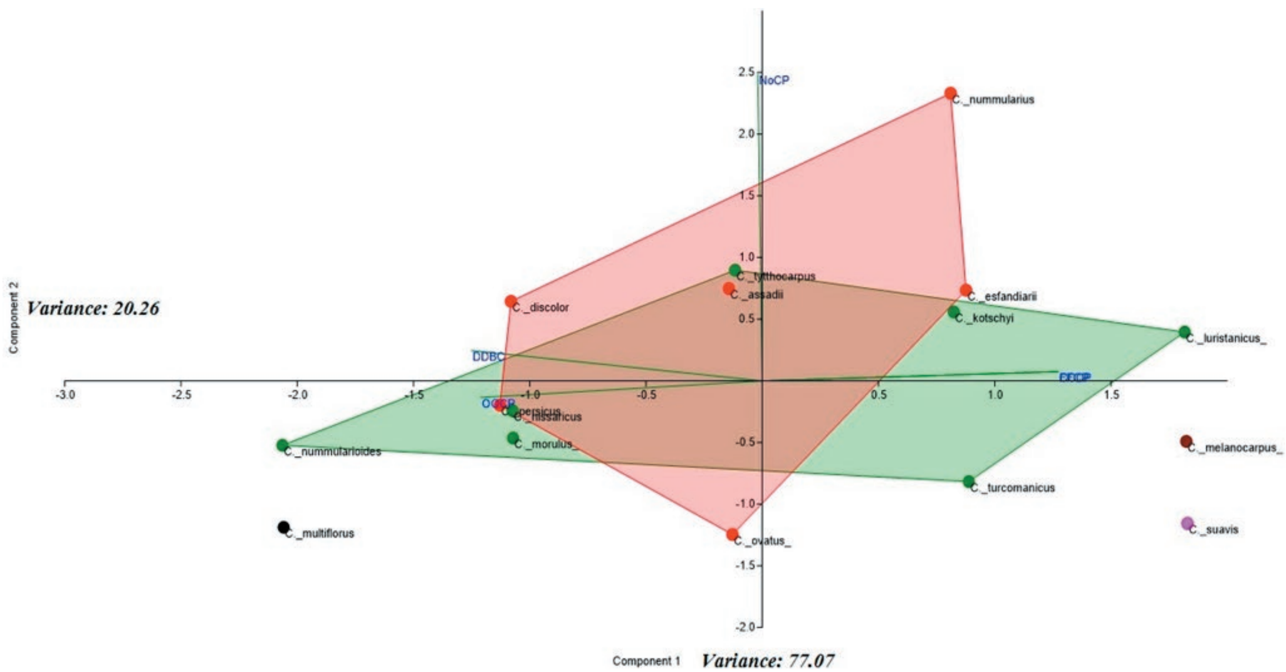

Figure 7. PCA plot of Cotoneaster species based on floral morphological characters.

and *Racemiflori* sections, which have the most species of this genus, are completely separated from each other and it can be said that using of pollen traits is probably effective in separating the sections and using these traits for placing a species in a particular section is probably helpful. The *Cotoneaster* subgenus members have considerable distance each other. *C. suavis* from the *Aitchisonioides* section and *C. multiflorus* from *Multiflori* subsection are also placed far from each other, which, due to the their few representatives in Iran, this separation cannot be interpreted as a meaningfull seperation. But, as we can see, the dispersal of the species in this chart indicates that pollen traits alone are not suitable for the separation, and that some species that are macromorphologically similar to each other, such as *C. discolor* and *C. persicus* are placed far from each other.

As shown in Figure 7, the first component variance is 77.07 and the second component variance is 20.26. The CCCP and FFCP traits have a significant positive correlation with the first component and the other three traits show a negative correlation with this component. Additionally, there is a significant positive correlation between the quantitative NoCP trait with the first component, and the remaining four qualitative traits show small positive and negative correlation with the second component.

Finally, the PCA analysis showed that petal traits in cotoneaster, as expected, are not separating traits, and the *Hissarici* and *Racemiflori* series species, which are the most common species in Iran, were overlapping in the terms of the separating petal traits. Subgenus *Cotoneaster* and some other series (*Multiflori, Aitchisonioides*), although they are separated, but because they have few representatives in Iran, it can be said that this separation is probably not meaningful and it can be relied only when more individuals of these subgenus, sections and series are studied.

DISCUSSION

Species delimitation and taxonomic consideration by pollen character

Many researchers have proven that taxonomic characters are of great interest for the correct identification of different plant groups (Ullah *et al*., 2018a; Ullah *et al*., 2018b).

The genus *Cotoneaster* like other tree and shrubby genera of *Rosaceae* such as *Amygdalus* L., *Pyrus* L., *Crataegus* L., *Rosa* L. is a morphologically difficult genus. Occurrence of hybridization which is a result of specific structure of flower, leads to appearance of individuals with intermediate characters. According to some studies on the family (Hebda and Chinnappa 1990) and also some genera such as *Amygdalus* (Vafadar *et al.* 2010), *Pyrus* (Xu and Yao 1990, Zamani *et al.* 2010), *Rubus* (Wronska- Pilarek *et al.* 2006), striate sculpturing is the predominant ornamentation in the family. An important feature in *Cotoneaster* different from other genera such as *Pyrus*, *Rubus*, *Amygdalus* and *Rosa* (Xu and Yao 1990; Wronska-Pilarek *et al.* 2006; Vafadar *et al.* 2010; Fatemi *et al.* 2012) is the presence of both tri- and tetracolporate pollen in the same specimen which is generally related to different levels of ploidy (Borsch and Wilde 2000).

On the basis of a comprehensive study on pollen morphology of the family *Rosaceae* in Canada (Hebda and Chinnappa 1990) it has been stated that variation in sculpturing is a diagnostic tool by which taxa can be identified, usually at the generic and often at the specific level. According to sculpturing, two main types striate (ridges and valleys) and non-striate (mainly psilate and verrucate) were recognized in the family (Hebda and Chinnappa, 1990). Moore (1991) has emphasized that pollen morphology in taxa of Rosaceae is very variable, even among the populations of the same species. Also, the grain size is the least reliable feature that is related to the comparatively frequent occurrence of hybridization in this family. This problem is remarkable in this study in the case of shape and sculpturing, even in different specimens of the same species. The importance of pollen morphological characters and their fitness for the most actual subgeneric taxonomic grouping are discussed in the following.

Subgen. *Cotoneaster.* ser. *Cotoneaster*

In this research, two species of subgenus *Cotoneaster* were studied. As shown in Table 2 and 3, the pollen characters in these two species are very similar to each other. This confirms the previous results that said that subgenus *Cotoneaster* is monophyletic (Li *et al.* 2014). In addition, because the pollination of this subgenus is highly dependent on a particular group of bees, the similarity of pollen grains in this subgenus can be evolutionary.

Subgen. *Chaenopetalum.* ser. *Hissarici*

Similar ornamentation pattern in *C. nummularioides* and *C. kotschyi* and dissimilarity from others are in line with other similarities between these species (including subcoriaceous, small (15 × 13 mm) and ovate or broadly elliptic leaves, compact inflorescence and number of flower (2 -5) per inflorescence, black and small fruits, navel open and also the same geographical distribution in Iran). *C. hissaricus* and *C. tytthocarpus* are very similar to each other in having similar morphological (size and shape of leaves, color and size of fruit, villose and depressed calyx, open navel) and pollen characters (P/E, type of sculpturing, shape of pollen) which distinguish these taxa from other species of the series. *C. hissaricus* comes from Tajikistan and Afghanistan and *C. tytthocarpus* occurring in Tajikistan. Also, both of species are tetraploid (Fryer & Hylmo 2009). Morphological, micromorphological characters of pollen, origin center and chromosome number suggest they could be regarded as related species, although their area of distribution in Iran is not the same (first species distribution is in NW Iran and latter is in NE Iran). The pollen morphology in *C. morulus* is very heterogenus, the exine having variable sculpturing type, and does not provide much useful information for the interspecific delimitation within the series *Hissarici*.

Subgen. *Chaenopetalum*. ser. *Racemiflori*

Widely distributed *C. ovatus* (with ovate leaves and red fruit) and *C. assadii*. (with obovate leaves and red – orange fruit) are considered closely related by the similar ornamentation, polar axis length, pore number in area unit, inter ridge width and apocolpium length. With respect to their similar macromorphological characters (size of leaves and fruits, number of flowers in per infloresens, habit of plant) and distribution area, the overlapping pollen morphologies of *C. ovatus* and *C. assadii* provide support for the same origin of these species. According to Khatamsaz (1993), *C. esfandiarii* is placed in the *Cotoneaster* subgenus (by erect petals and 2-3 style) but Fryer and Hylmo placed it in the *Chaenopetalum* subgenus, *Racemiflori* section (by spread petals and 2- 3 style). In this survey, based on the exine sculpturing, *C. esfandiarii* resembles members of subgenus *Chaenopetalum* more to species of subgenus *Cotoneaster*, but the judgment in this case requires further studies. *C. persicus* and *C. discolor* are much alike in their pollen ornamentation. *C. discolor* pollen (with obscure ridge) differs from that of *C. persicus* (psilate sculpture) usually by having of a few number perforations in area unit.

Many of these species are relatively specific in their habitat requirements on the dry slopes (e.g. *C. persicus*, *C. prunoisus*, *C. kotschyi*) or wet regions (*C. assadii*) and may prove to be important habitat indicators. Also, the presence of pollen grains of these species in the depths of a region can be partly informed of the climate of that area in a particular geological period.

In conclusion, our findings revealed the palynological characteristics (e.g., perforation number, size and exine sculpturing) of the genus *Cotoneaster*. The similarity of exine structure and ornamentation, as well as the similarity of the various parameters analyzed at interspecific level makes it hard to establish taxonomical boundaries and clearly shows the affinity of species as far as morphological characteristics are concerned.

Species delimitation and taxonomic consideration by micromorphological petal

Taxonomic perspective

Shaheen *et al.* (2016) analyzed the shape of petal epidermal cells and their wall patterns within *Rosaceae* and concluded that family had a high degree of petal micromorphological variation, but we found only little differences among *Cotoneaster* species. Our result showed that there was not a significant variation at interspecific level in the 16 studied species. Unlike other genera of the Rosaceae, e.g. *Rubus*, *Crataegus* (Christensen 1992; Christensen and Hansen 1998; Sharifnia & Behzadi Shakib 2012; Hamzeh'ee *et al.* 2014), *Sibbaldia* (Tahir and Rajupt 2010) and *Rosa* (Sharma *et al.* 2005), *Cotoneaster* species petals decorating the microscopic level, did not show significant variation (exception number of conical projections). The petal epidermal features among species were fairly similar to each other. The shape of petal cells in the all of species was conical to finger-shape projection on the adaxial side. Conical cells may increase petal brightness and therefore increase pollinator visitation rates (Glover and Martin 1998; Comba *et al.* 2000; Dyer *et al.* 2006; Ojeda *et al.* 2009). The micromorphological properties of petal surfaces showed some variations. Number of conical projections is an important diagnostic character. The abaxial epidermis surface of these petals had a uniform pattern and cells with different sizes joined together in a fixed pattern.

Asexual seed production or apomixis, which is often associated with hybridization and polyploidy (Marshall & Brown 1981; Nogler 1984), has been reported in five Maloid genera e.g. *Amelanchier* Medik. (Campbell *et al.* 1985) and *Cotoneaster* (Hjelmqvist 1962). Such plants will therefore produce some completely maternal progeny through apomixes (Stebbins 1950). Consequently, apomixis genes can be much older than the clones they are currently contained in (Van Dijk 2003). Apomixis also has been reported frequently in *Cotoneaster* (Rothleutner *et al.* 2016). Since some of the maternal traits can be preserved for a long time through apomicies (Stebbins 1950), one of the reasons for not changing the ornamentation of the adaxial surface of the petals and their similarity to each other in different species is apomixis. The interesting thing is that micromorphological traits of petal in two species *C. hissaricus* and *C. morulus*, very similar to each other. These two species have similar macromorphological characters (shape and size of leaf, size and color of flower, shape and color of fruit) and regional distribution in Iran (Azerbaijan province).

Evolutionary perspective

Pollination is done by bees in *Cotoneaster* mainly by the short-tongued bumble bees (*Bombus terrestris* and *bombus lucorum*) and honey bees (*Apis mellifera*) which visited species in both subgenera of the genus, concentrating on the subgenus *Cotoneaster* during early summer and on *Chaenopetalum* after mid-June. The section *Cotoneaster* is recommended as particularly valuable for bee forage. Plants of the subgenus *Cotoneaster* were visited more by these bees in May and early June, a critical period when other forage may be scarce. The common carder bee *(Bombus pascuorum)* and the early bumble bee (*Bombus pratorum*) almost exclusively visited plants in the subgenus *Cotoneaster* throughout the season (Corbet *et al.* 1992). These findings and similar studies suggest that pollination of *Cotoneaster* and bee nutrition strongly linked together (Toth *et al.* 2011). Also, the *Cotoneaster* petals are white (especially in the section *Chaenopetalum*), for this reason the petal cell ornamentation on the adaxial surface is very important in attracting bees. The periclinal wall pattern of petal cells in all species studied is conical. Different species of *Cotoneaster* have the same pollinators and therefore there is not much difference between the adaxial surface ornamentation of the petals. Previous research has shown that flowers and their pollinators in many plants evolve together and has suggested that the rise of bees coincided with the largest flowering plant clade, the eudicots (Cappellari *et al.* 2013). Probably, pollination by certain species of bees is the only way to reproduce sexually in *Cotoneaster* and for this reason, the various species of this genus have evolved with each other in terms of petals, along with the particular species of this bee.

Ecological perspective

It seems that petal traits are stable in different species of this genus and do not change under the influence of the ecological conditions. Because all species that have been collected from different climates of Iran have almost the same ornamentation in their petals. As can be seen, in species with long and open inflorescences that have large flowers, the number of conical projections per unit area is lower and the boundary between the cells is quite distinct (*C. melanocarpus, C. multiflorus, C. suavis, C. ovatus*). This form of inflorescence and flower is found in species that have large, thin, and crusty leaves. On the other hand, these leaf traits are seen in mesophytic species. Thus, the high density of papillae on the adaxial surface of the petals can be a reason to deal with the dryness of the air. So mesophytic species do not have a high density of papillae. It is important to note that species with same traits, may also be found in semi-arid regions (*C. suavis, C. ovatus*). It can be concluded that petal traits maybe have been evolved once at the time of splitting species of this genus and in subsequent periods they have not been changed under the climate differences (similar results are obtained for leaves in the species of this genus).

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