Pollen Morphology of the genus *Clypeola* (*Brassicaceae*) in Iran

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Abstract. The pollen morphology and exine structure of four *Clypeola* species were investigated using light microscopy and scanning electron microscopy. The pollen grains of three of the species were 3-colpate, while in *C. aspera* both 3- and 4-colpate pollen grains were observed in one individual. The pollen grains varied in shape from prolate-spheroidal, subprolate to prolate. The results indicated that pollen characters, such as the polar axis, average diameter of lumen, average diameter of muri, length of mesocolpium, width of colpus, form of lumen, shape of colpi, and P/E of the *Clypeola* species are valuable diagnostic features in species delimitation.

Key words: *Clypeola*, pollen morphology, scanning electron microscopy, systematic

Introduction

Brassicaceae is a family comprising about 338 genera and 3709 species (Franzke & al. 2010) occurring mainly in the temperate regions of northern hemisphere (Hedge 1976). In Iran the family is represented by 120 genera and 358 species (Khosravi & al. 2009). *Clypeola* L. includes nine species of annual herbs. These species are easily distinguished from one another by their morphology and geographical distribution (Chaytor & Turill 1935). This genus is distributed mainly in the Mediterranean region and SW Asia (Zohary 1966; Jafri 1977; Townsend 1980), but *Clypeola jonthlaspi* L. has a vast distribution range in relation to the other species of the genus and is spread further in the Central and West Europe and North Africa (Jefry 1977). *Flora Iranica* reports five species (Rechinger 1968), although in this research authors believe that there are only four species: *C. jonthlaspi*, *C. aspera* (Grauer) Turill, *C. lappacea* Boiss., and *C. dichotoma* Boiss. distributed across different habitats of Iran.

Brassicaceae is a stenopalynous family, the pollen grains are usually tricolpate, with a reticulate exine (Abdel-Khalik 2002). Pollen morphological studies of *Brassicaceae* have been carried out by several authors (Abdel-Khalik 2002; Pinar & al. 2009; Perveen & al. 2004; Bolurian 2009). In this family, pollen morphology has provided an approach to the systematic relationships among the genera and species. Orcan & Binzet (2004) had considered morphology and palynology of *Alyssum floribundum*, studying the normal and abnormal pollen of this genus. Bolurian (2009) studied eight species of *Alyssum* L. and has observed three types of pollen: subprolate, prolate-spheroidal and prolate, with reticulate exine ornamentation. So far no one has studied the pollen grains of *Clypeola* species in Iran. The objectives of this paper were to provide a detailed account of the pollen morphology of *Clypeola* in general by light microscopy (LM) and scanning electron microscopy (SEM), and to determine to what extent these palynological data can be used as a taxonomic character in the genus. The present investigations are based on the pollen morphology of four species of *Clypeola* in Iran.

Material and methods

Plant material

The material used for this study was collected from wild populations and herbarium speciments (Table 1). The observations were made with an Olympus DP12 light microscope, without any prior treatment. The measurements were based on 20 or more pollen grains per population.

SEM studies were done on pollen grains suspended in a drop of water and then directly transferred by a fine pipette to a metallic stub, using a double sided cello tape and coated with gold in a sputtering chamber (Sputter Coater BAL-TEC, SCDOOS). Coating was restricted to 100 A. SEM examination was carried out with a XL30 Philips microscope. For numerical taxonomy analysis, 14 qualitative and quantitative morphological characters were studied (Table 2). To compare the differences or similarities in pollen size between the studied taxa, simple variance analysis was performed on the polar axis (P) and equatorial diameter (E). The ratio between the mean polar axis and the mean equatorial diameter can be used to assign the pollen grains to shape classes, according to Erdtman (1943); pollen terminology of Punt & al. (1994) was used.

Table 1. Collection data for populations used in this study. ALUH stands for Herbarium of Alzahra University, **FAR** for Herbarium of Tarbiyat Moalem University; **FUMA** stands for Herbarium of Ferdousi University; **HSBU** stands for Herbarium of Shahid Beheshti University.

Species	Voucher No	Origine	Collector
	18917. ALUH	Iran,Tehran, Boumehn	Abbasian
	18921. ALUH	Iran,Ghazvin, 20 km Ange road, Shekarnab village	Abbasian
	18925. ALUH	Iran,Tehran, Modiriyat bridge	Keshavarzi, Abbasian, Habibi
	18923. ALUH	Iran, Kermanshah, Tagh-e-Bostan	Gholami
C. jonthlaspi	18915. ALUH	Iran, Fars, 35 Km to Shiraz,1482 m	Abbasian
	18913. ALUH	Iran,Yazd,Tabas, Neyzar village, 1010 m	Abbasian
	18911 .ALUH	Iran,Yazd,Tabas, Eshqabad road, Ozbak Kuh	Abbasian
	1895. ALUH	Iran,Yasd,Tabas, Kherv village	Abbasian
	1899. ALUH	Iran,South Khorasan, Boshrouyeh, Neyganan village	Abbasian
	18916. ALUH	Iran,Fars,35 km to Shiraz,1482 m	Abbasian
	18914. ALUH	Iran,Yazd, Tabas, Neyzar village, 1010 m	Abbasian
	18912. ALUH	Iran,Yazd, road Eshqabad, Ozbak Kuh	Abbasian
	18910. ALUH	Iran, South Khorasan, Boshrouyeh, Neyganan village	Abbasian
	1898. ALUH	Iran, Kerman, 320 km to Shiraz, 1770 m	Abbasian
C. aspera	1894. ALUH	Iran,Yazd, 40 km to Deyhuk, 1361 m	Abbasian
	1891. ALUH	Iran,Yazd, Tabas, Kalshane village,1092 m	Abbasian
	1893. ALUH	Iran, Yazd, Tabas, Abid village	Abbasian
	18917. ALUH	Iran, Gazvin, Abyek	Abbasian
	1896.ALUH	Iran, Fars, 35 km to Neyriz, 1480 m	Abbasian
	5044. ALUH	Iran, Fars, 35 km to Marvdash	Rastipishe
	16609 FUMA.	Iran, South Khorasan, S.W. Bojnurd, 1050 m	Joharchi & Zanguee
	17287. FUMA	Iran, Khorasan, E. of Birjand, Gzyk mountains,1400-1500 m	Jouharchi & Zanguee
	13743. FUMA	Iran, Khorasan, Ferdus-Boshrouyeh	Ayatollahi & Jouharchi
C. dichotoma	21809. FUMA	Iran, Khorasan, Birjand, Hamand-Give road, 1400 m	Faghihi nia & Zanguee
	15109, FUMA	Iran, Khorasan, Birjand , 8 Km after Hamand, 1300-1350 m	Rashed & Zanguee
	24743, FUMA	Iran , road Khorasan-Boshrouyeh , 1400 m	Rafei & Zanguee
	26738, FUMA	Iran, Khorasan, SE of Birjand, 1650 m	Rafei & Zanguee
	Anonym, FUMA	Iran, Khorasan, Birjand,Tabas, 1150 m	Anonymus

Table 1. Continuation

Species	Voucher No	Origine	Collector
C. lappacea	8624402. HSBU	Iran, Chahar mahal and Bakhtiari, between Gandoman and Ardal, 2350-2450 m	Zehzad
	36. HSBU	Iran, Kermanshah, Songhor-Asad abad,1650 m	Zehzad
	87410. HSBU	Iran, Isfahan, between Shahreza and Vanak, 2300 m	Khosravi
	87532. HSBU	Iran,Chaharmahal and Bakhtiari, between Kharadgee and Gandoman, 2150-2300 m	Zehzad
	1890. FARH	Iran,Takab	Anonymus
	Anonym. HSBU	Anonym	Anonymus

Table 2. Studied pollen features in *Clypeola* species of Iran.

Character
Equatorial axis length (μm)
Polar axis length (μm)
Colpus length (μm)
Average diameter of lumen (μm)
Average diameter of muri (μm)
Colpus width (μm)
Mesocolpium length (μm)
P/E ratio
Pollen shape (prolate-spheroidal, subprolate, prolate)
Lumen shape (polygonal 1, rounded angles 0)
Muri surface (protruding prominent 1, not so 0)
Columella (prominent 1, not so 0)
Colpi shape (linear and symmetrical 1, wide and no symmetry 0)

Statistical analysis

In order to reveal species relationships, cluster analysis and principal component analysis (PCA) were applied. For multivariate analysis, the mean of quantitative characters were used, while qualitative characters were coded as binary/multi-state characters. Standardized variables were employed for multivariate statistical analysis. The average taxonomic distances and squared Euclidean distances were considered as dissimilarity coefficient in the cluster analysis of morphological data. SPSS Ver.16 and NTSYS software were applied for statistical analysis.

Results

Pollen morphology

The characters of pollen grains of the studied *Clypeola* species are summarized in Tables 3 and 4 by using LM and SEM (respectively) data, and shown in Figs 1 and 2.

Size, symmetry and shape

Light Microscopy study

The minimum and maximum polar axis of *C. lappacea* and *C. jonthlaspi* are 19.6 μm and 39.8 μm respectively. The minimum and maximum equatorial axes of *C. dichotoma* and *C.aspera* are 15 μm and 30.5 μm respectively (Table 3). The pollen grains of *Clypeola* are isopolar and radial symmetric. They are spheroid, prolate-spheroid, subprolate and prolate. Outline is elliptic in the equatorial view and circular in the polar view (Fig. 1).

Scanning Electron Microscopy study

The range of polar and equatorial axes is congruent with the light microscopic data. The mean diameter of lumen in all studied species of this genus is below 1μm (Table 4), and the largest lumen belongs to *C. dichotoma.*

Aperture

In three species of *Clypeola* pollen grains were tricolpate, but in *C. aspera* the pollen grains in the majority of studied populations were either tri- or tetracolpate (Figs 1–2). The colpi were linear and occasionally ended into a widened area, instead of a sharp point (Fig. 2). The Bakhteeyari population of *C. lappacea* was filled by columella-shaped particles inside the lumina, while in the other samples of this species the lumina were open and small parts of columella were observed.

Ornamentation

The basic ornamentation of the *Clypeola* pollen was reticulate. The lumen size was equal in the equatorial and polar zones, but in *C. dichotoma*, the largest lumina were located in the equatorial zone and decreased towards the poles (Fig. 2). Lumina were polygonal or nearly rounded. As some samples have protrudings on muri, their lumina were inside, as in *C. aspera*

and *C. jonthlaspi* too. The average thickness of the exine was 1.64 µm. Exine thickness is shown in Table 3. *C. lappacea* and *C. dichotoma* have a thicker exine than *C. jonthlaspi* and *C. aspera*.

According to the classification of Abdel-Khalik (2002) based on variation in the diameter of the lumina, the exine ornamentation type in all studied species is micro-reticulate (lumina below 1 µm).

Tabel 3. Pollen data by light microscopic study.

Species	Voucher No	Polar axis (µm)		Equatorial axis (µm)		Length of exine (µm)		P/E		Shape of pollen
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	
1	2	3	4	5	6	7	8	9	10	11
	18913	20–32.2	24.26	16.6–30.4	22.19	1–2.7	1.63	0.86–1.64	1.12	Prolate-spheroidal
	1895	21.6–2	23.2	16.7–23.4	21	0.6–2.3	1.2	0.94–1.4	1.11	Prolate-spheroidal
	18911	19.8–28.3	23.8	16–26.3	20.6	0.6–1.8	1.3	0.96–1.6	1.2	Prolate-spheroidal
C. jonthlaspi	18915	19.6–25.2	22.6	18–28	21.3	0.6–2.1	1.5	0.9–1.3	1.1	Prolate-spheroidal
	18925	21.2–24.4	23.1	20.1–23.3	22	1.4–2	1.8	1–1.1	1	spheroidal
	1899	19.7–23.8	22.4	18–23.3	20.5	1.1–1.9	1.5	1–1.2	1	Spheroidal
	18923	24.3–28.4	26	18.8–23	21	1.1–1.9	1.6	1.1–1.4	1.2	Prolate-spheroidal
	18921	20.9–24.2	22.8	18.7–22.7	21.4	1.1–1.8	1.4	0.98–1.1	1	spheroidal
	18914	25.7–31.3	29	16.1–30.5	23.5	1.1–1.9	1.5	1–1.7	1.3	subprolate
	1983	23.6–32.9	28.6	20.6–26.7	23.3	1.2–2.9	1.5	1–1.5	1.2	Subprolate
	18912	27.7–30.6	28	21–24.1	22.6	1.2–2.4	2	1.1–1.3	1.2	Subprolate
	1891	25.8–30.3	27.8	19.1–26.8	22.3	1.4–1.9	1.7	1.1–1.5	1.2	Subprolate
	1894	22.9–29.8	27.7	15.6–26.5	20.9	1.1–1.8	1.3	1.2–1.6	1.4	Prolate
C. aspera	18916	21.2–33	27.9	19.7–28.8	24.3	0.87–2.2	1.5	1–1.4	1.1	Prolate-spheroidal
	18917	24.7–30.5	28.2	18.8–26	21.8	1.5–2	1.7	1–1.5	1.3	Subprolate
	1898	24.9–36.2	28.4	19.2–24	20.9	1.2–2.2	1.7	1–1.9	1.3	Subprolate
	18910	24–32	27.4	20–27.6	23.5	0.99–2.7	1.6	1–1.3	1.13	Prolate-spheroidal
	5044	21.9–28.3	25.4	20.3–25.9	23.1	1.3–2.7	1.8	0.99–1.3	1.1	Prolate-spheroidal
	1896	22.3–29.4	27.3	18.8–25.1	21.8	0.99–2.6	1.8	1–1.4	1.2	subprolate
	87532	28.2–37.7	31.8	19.6–27.5	22.7	1–1.8	1.4	1.1–1.8	1.4	Prolate
	36	29.8–36.7	33.4	20.1–25.1	23.2	1–2.2	1.5	1.2–1.8	1.4	Prolate
C. lappacea	anonym	28.6–36.3	32.2	22.4–26.3	23.9	1.1–2	1.6	1.2–1.4	1.3	Subprolate
	87410	24.6–34.6	30	20.8–23.8	22.6	1.1–1.8	1.5	1–1.6	1.3	Subprolate
	18906	28.5–35.4	32	20.2–25.5	23	1–2.6	1.8	1.2–1.5	1.4	Prolate
	21809	24–29.4	26	19.6–25.2	21.4	1.4–2.3	1.8	1.1–1.3	1.2	Subprolate
	13743	24.6–29	27	18.9–21.8	20.2	1.2–2.2	1.6	1.2–1.4	1.3	Subprolate
	17287	24.2–30.2	26.1	19.4–22.1	20.9	1.3–2.1	1.7	1.1–1.5	1.2	Subprolate
C. dichotoma	16609	25.5–29.8	27.5	21.1–24.28	22.2	1.2–2.2	1.5	1–1.3	1.2	Subprolate
	15109	22.3–27.5	25.3	18.7–22.1	20.6	1.48–2.26	1.8	1.1–1.2	1.2	Subprolate
	annonym	23.65–31.8	27.4	18.2–24	21.1	1.2–1.8	1.5	1–1.4	1.2	Subprolate
	26738	24.1–27.6	26	18.5–22.1	20	1.3–1.9	1.7	1–1.4	1.2	Subprolate
	24743	22.3–29.5	25.8	15.1–21.3	19.2	0.98–1.9	1.5	1.1–1.8	1.3	Subprolate

Table 4. Pollen quantitative data in studied *Clypeola* species based on SEM study.

Species	Voucher No	Average diameter of lumen (µm)		Average diameter of muri (µm)		Length of colpus (µm)		Width of colpus (µm)		Width of mesocolpium µm)	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
C. jonthaspi	18926,18923	0.35–0.7	0.49	03–0.4	0.35	6.5–18.7	12.74	1–2.51	1.6	4.6–6.2	5.5
C. aspera	18910	0.53–0.69	0.58	0.44–0.54	0.48	17–19	17.8	0.19–0.32	0.23	7–7.35	7.13
C. lappacea	36, 87532	0.53–0.78	0.64	0.4–0.53	0.46	15.4–24.1	20	0–0.95	0.28	4.2–9.9	7
C. dichotoma	15109	0.77–0.92	0.85	0.41–0.43	0.42	18.8–22.5	20.5	0.06–1.6	0.9	7.5–7.6	7.5

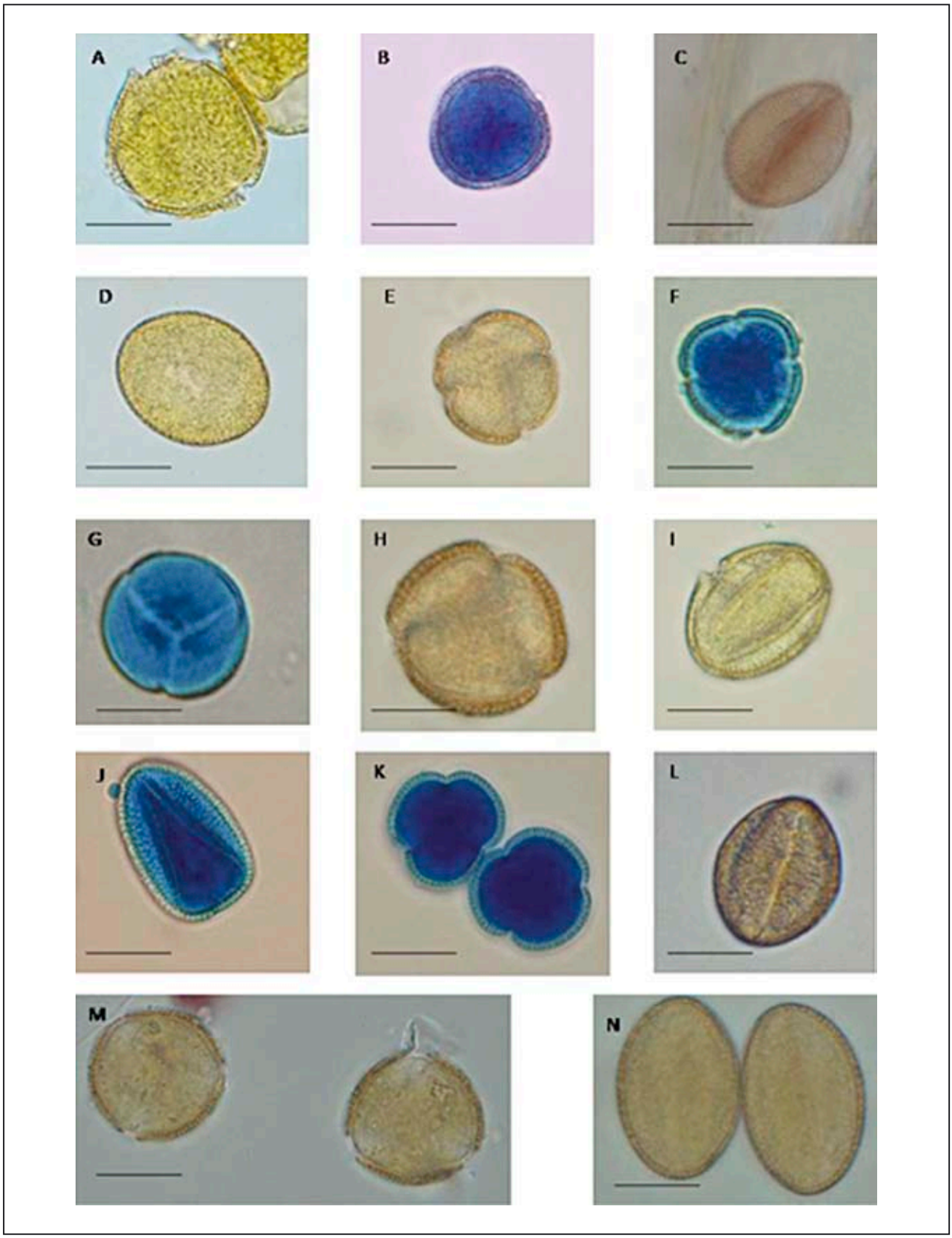

Fig. 1. Light micrographs of *Clypeola* pollen grains. **A–D**. *C. jonthlaspi*: A–B, polar view, C–D. equatorial view; **E–J**. *C. aspera*: E–H. polar view, I–J. equatorial view; **K–L**. *C. dichotoma*: K. polar view, L. equatorial view; **M–N**. *C. lappacea*: M. polar view, N. equatorial view.

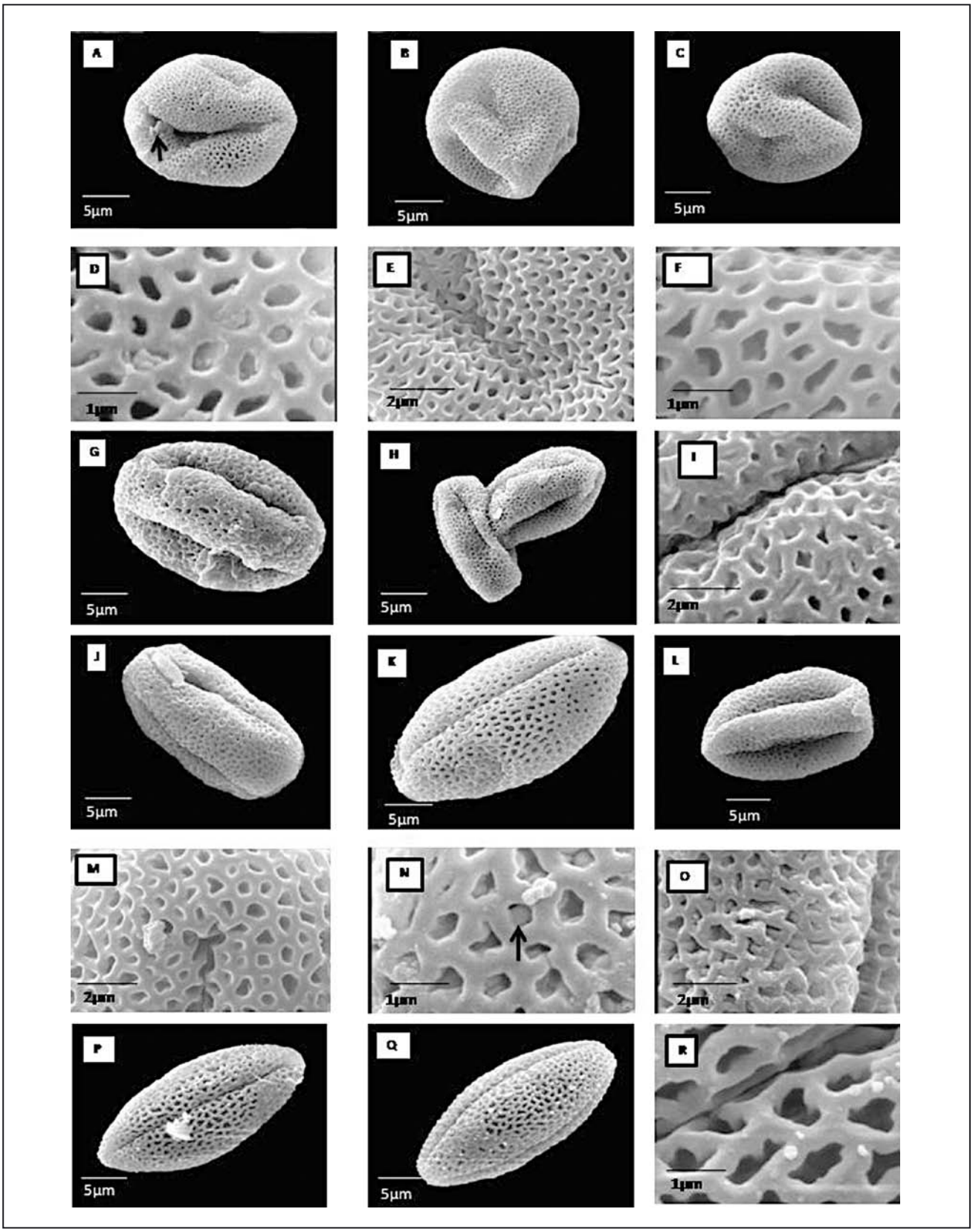

Fig. 2. SEM micrographs of *Clypeola* pollen. **A–F**. *C. jonthlaspi;* **G–I**. *C. aspera*; **J–O**. *C. lappacea*; **P–R**. *C. dichotoma.* A: the arrow shows the presence of column- shaped objects; C: colpus with flattened end; D: muri with protruding. H–K and P–Q: colpi are linear and in all parts have same measure. N (Chaharmahal and Bakhtiari populations): arrow shows inside filled lumen, also observed in O. Q–R sample: the lumens are bigger and the size of holes toward ends are gradually becoming smaller.

Discussion

Brassicaceae is a stenopalynous family with reticulate exine ornamentation. The results obtained in the present study are in concordance with the earlier data. Minor differences in size of the lumina, muri, polar and equatorial axes, and shape of the lumen have been observed. Our statistical analysis has shown that these characters are of taxonomic importance for the studied species. Our results are congruent with those of Abdel-Khalik (2002), although he did not study representatives of *Alysseae,* but mentioned the importance of lumina size in delimitation of different genera in Matthioleae and between the species in the Malcolmia alliance. The studied species also differed significantly in most of their pollen quantitative characters, as revealed by ANOVA and LSD tests. This was supported by clustering (Fig. 3) of the studied species based on quantitative and qualitative pollen features. Fig. 3 shows that *Clypeola jonthlaspi* makes a separate cluster and the populations of *C. aspera*, *C. lappaceae* and *C. dichotoma* make another one. The cluster analysis and PCA ordination of *Clypeola* species in Iran, based both on quantitative and qualitative pollen characters, have produced similar results (Fig. 4). In the overall analysis, two major clusters were formed. The first major cluster comprised C. *jonthlaspi*. The second major cluster comprised two sub-clusters: in the first were the populations belonging to *C. aspera* and *C. lappaceae*; in the second *C. lappacea* was closely related with the separate group of *C. dichotoma*. Apparently, the different populations of *C. johnthlaspi* are closely related. Presumably, *C. lappaceae* has a lot of variations and thus its populations are included in different clades.

In order to determine the most variable characters among the studied species, a factor analysis based on PCA was performed, revealing that the first three factors comprise about 79.76 % of total variation. In the first factor, with about 53.5 % of the total variation, such characters as the polar axis, average diameter of lumen, average diameter of muri, length of mesocolpium, width of colpus, form of lumen, shape of colpi, and P/E had the highest correlation (≥0.6) (Table 5).

In the second factor, with about 16.63 % of the total variation, such characters as the surface of muri and equator axis had the highest correlations. Therefore, these are the most variable morphological characters among the pollen grains of *Clypeola* species of Iran (Table 5).

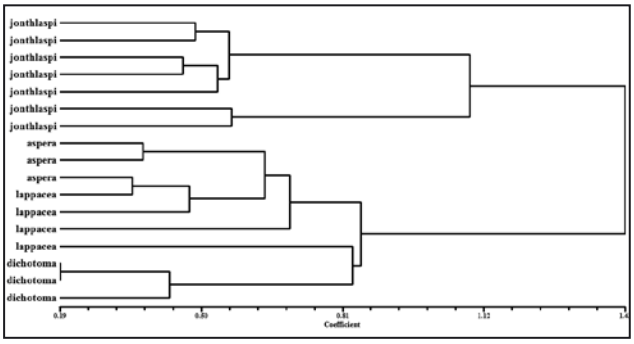

Fig. 3. Phenogram of the studied taxa, clustering with the UPGMA method.

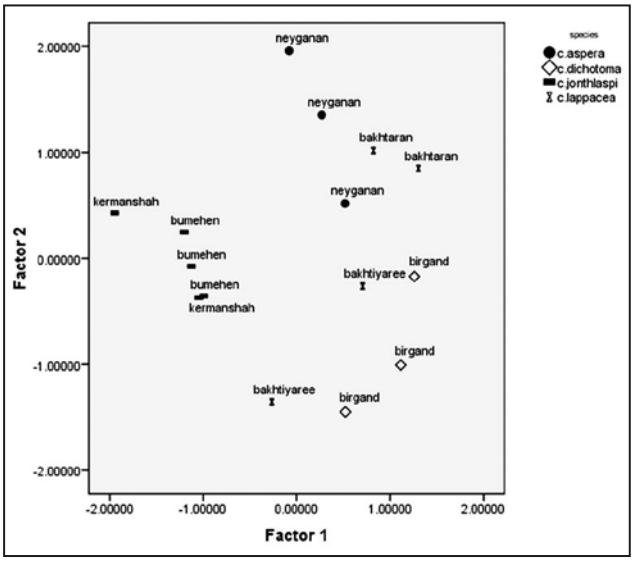

Fig. 4. PCA ordination of the studied *Clypeola* species based on pollen morphology.

Table 5. Factor Analysis Results based on pollen morphological characters of *Clypeola* populations of Iran.

Character	Factor		
	1	2	3
Length of colpus	926		
polar axis	942		
Polar axis/equator axis	896		
Average diameter of lumen	823		
Shape of colpus	822		
Length of mesocolpium	748		
Average diameter of muri	743		
Width of clolpus	–742		
Surface of muri		634	
Equator axis		628	
Columella			696

The multivariate analysis methods have proved very helpful in assessing the inter-specific affinities and intra-specific variability of the species. Such methods were used by Ng & al (1981) in the studies of

rice species to clarify the inter-specific relationships and to distinguish the species or geographical forms.

Clypeola jonthlaspi is the most polymorphic species in Iran. The polar axis, thickness of exine and P/E in the light microscopic data have a limited taxonomic value and can resonably separate the species into separate group. Thus in this study, the results obtained from SEM data are more reliable. The PCA based on qualitative and quantitative features of the pollen grains (Fig. 4) confirm the cluster analysis by the UPGMA method.

Palynological evidence shows some variations in the different populations of *Clypeola lappaceae*. *C. johnthlaspi* forms a compact group (Fig. 4), related rather to each other than to other species of this genus. Although some authors (Chaytor & Turrill 1935; Bristroffer 1936; Runemark 2002) believe that this species has some infra-specific levels, these differences are not as evident, at least in their pollen morphology. Four studied species of *Clypeola* have been efficiently delimited on the basis of the length of colpus, polar and equatorial axis, and P/E ratio.

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