

# POLLEN MORPHOLOGY OF THE GENUS FUMARIA L. (PAPAVERACEAE) IN IRAN

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The pollen morphology and exine structure of seven *Fumaria* L. species were investigated using light microscopy and scanning electron microscopy. The pollen grains of all seven species were porate with protruding on annulus. Pollen grains have six to twelve pores varying in shape from oblate spheroid and prolate spheroid to euprolate. The results indicate that the pollen characters of the *Fumaria* species may provide diagnostic features for taxonomic applications.

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**Key words.** *Fumaria*, scanning electron microscopy, systematic, pollen morphology, Iran.

ریخت شناسی دانه گرده در جنس *Fumaria* L. (*Papaveraceae*) در ایران

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ریخت شناسی دانه گرده و ساختار آگزین ۷ گونه از شاتره با استفاده از میکروسکوپ نوری و الکترونی (نگاره) مورد بررسی قرار گرفت. دانه های گرده ۷ گونه مورد بررسی از نوع porate و دارای بیرون زدگی هایی در محل annul بود. این دانه ها واجد ۶ تا ۱۲ منفذ و با اشکالی متفاوت از oblate spheroid و prolate spheroid تا euprolate بودند. نتایج این بررسی موید آنست که صفات گرده شناسی گونه های *Fumaria* صفات افتراقی مناسبی را برای کاربردهای رده بندی این گونه ها فراهم می آورد.

## Introduction

*Fumariaceae* family previously treated as a small family of about 19 genera and 400 species (Lidén 1986) occurring mainly in North America, Europe, Asia and Africa. In Iran, it is represented by two genera (Wendelbo 1974). Nowadays most of the authors are treating *Fumariaceae* as a subfamily of *Papaveraceae*. Lidén (1986) described general features of the pollen morphology of *Fumarioideae* in his monographic studies. The genus *Fumaria* L., comprising almost 50 species of herbaceous annual plants, is a member of the subfamily *Fumarioideae*. In Flora Iranica 7 *Fumaria* species have been reported from Iran (Wendelbo 1974). However, Lidén has recently added *F. officinalis* as a new species for the flora of Iran (Lidén 2000). *Fumaria* species are distributed chiefly in the temperate and cooler regions of the Old World and often as weeds (Jafri 1974).

Several authors have carried out pollen morphological studies on *Papaveraceae*. Lidén in his monograph mentioned some misinterpretations and taxonomical errors due to pollen grains study in *Fumaria*. Perveen & Qaiser (2004) investigated the pollen morphology of the nine species of *Fumaria* from Pakistan, but so far, no one has studied pollen grains of *Fumaria* species in Iran. The only study in this field related to the Sajedi & Assadi (2008). They found that pollen characteristics are of taxonomic value in *Corydalis* subgenera and sections in Iran. They also provided an identification key based on pollen features of studied *Corydalis* species. The objectives of this paper were to provide a detailed account of the pollen morphology of *Fumaria* as a whole by light microscopy (LM) and scanning electron microscopy

Table 1. Voucher specimens of *Fumaria* species used in this study.

Species	Voucher specimens
<i>F. vaillantii</i> Loisel.	Mazandaran: Abeask, Ebrahimzadeh 8610 AUH; Veresk, Ebrahimzadeh 8611 AUH; Gadok, Keshavarzi 8612 AUH. -Tehran: Jajrod, Ebrahimzadeh 8614 AUH; Vanak, Ebrahimzadeh 8615 AUH; Tehran, Modiriat bridge, Ebrahimzadeh 8626 AUH. -Hamedan: Heydareh, Keshavarzi 8628 AUH.
<i>F. parviflora</i> Lam.	Tehran: Vanak, Ebrahimzadeh 8636 AUH; Abali, Mobarak abad, Nataj 8639 AUH. -Lorestan: Dasht Chegene, Direkvandi 8642 AUH. -Fars: Fasa, Rastipisheh 8646 AUH. -Yazd: Yazd, Keshavarzi 8647 AUH.
<i>F. indica</i> (Hauskn.) Pugsley	Tehran: Chitgar park, Ebrahimzadeh 8635 AUH.
<i>F. asepalata</i> Boiss.	Tehran: Darabad, Rastipisheh 8656 AUH; Taleghan, Falatory 8657 AUH.
<i>F. densiflora</i> DC.	Mazandaran: Kelardasht, Ebrahimzadeh, 8659 AUH.
<i>F. officinalis</i> L.	Mazandaran: Galogah highlands, Habibi Tirtash 8910 AUH.
<i>F. schleicheri</i> Soyer-Willment	Kerman: Sirjan, Habibi Tiratsh, 8911, AUH.

(SEM), and to determine the extent to which these palynological data can be used as a taxonomic character in the genus. Present investigations are based on the pollen morphology of seven species of *Fumaria* in Iran.

## Materials and Methods

### PLANT MATERIALS

Pollen samples were obtained from field. The voucher details of 18 sampled populations are given in table 1. Voucher specimens are deposited at herbarium of Alzahra University (AUH). For light microscopy, observations were made with an Olympus DP12 microscope without any treatment. Pollen morphological analysis was based on samples of 20 anthers from different specimens of each population. The terminology of pollen grains and sexine sculpturing mainly follows Paldat.

SEM studies were done by pollen grains, which suspended in a drop of water and were directly transferred by fine pipette to a metallic stub using double sided cello tape and coated with gold in a sputtering chamber (Sputter Coater BAL-TEC, SCDOOS). Coating was restricted to 100 Å. The SEM examination was carried out on a Philips microscope XL30. The measurements were based on 10-20 readings from each specimen. Pollen diameter, polar axis (P) and equatorial diameter (E), aperture size and margo thickness were measured. For numerical taxonomy analysis, 11 qualitative and quantitative morphological characters were studied (Table 2). In order to compare the differences or similarities in pollen size between studied taxa a simple analysis of variance was performed on P and E parameters.

### STATISTICAL ANALYSIS

Table 2. Studied pollen grain characters and states.

To reveal species relationships based on pollen grains data, cluster analysis and principal component ordination (PCO) were used. For multivariate analysis the mean of quantitative characters were used while qualitative characters were coded as binary/multi-state characters. Standardized variables were used for multivariate statistical analysis. The average taxonomic distances and squared Euclidean distances were considered as dissimilarity coefficient in cluster analysis of morphological data. SPSS ver. 9 (1998) and NTSYS softwares were used for statistical analysis.

## Results

Our observations showed that pollen grains of *Fumaria* species are usually radially symmetric, iso-polar or rarely apolar, porate, prolate to sub-prolate or oblate-spheroidal, rarely sub-oblate with a regulate-fossulate or fossulate-foveolate tectum. Characteristic pollen grains in studied *Fumaria* species are panto-porate with distinctly colliculate surface and thick collars around the pores. Pollen grains have annuli with bubbles protruding from the pores. The characters of pollen grains in studied *Fumaria* specie are summarized in table 3.

Light microscopy results indicated that maximum polar and equatorial axis is observed in *F. vaillantii* and the minimum ones belonged to *F. asepalata* (Table 3). Minimum margo thickness and the maximum annulus protruding were observed in *F. densiflora* and the minimum annulus protruding was observed in *F. vaillantii*. Erdtman (1943) defined some groups of pollen grains shape due to the ratio between polar to equatorial axis. *F. asepalata* and *F. densiflora* population showed oblate-prolate, populations of *F. parviflora* showed oblate-spheroidal and prolate spheroidal pollen grains. Chitgar population despite morphological

Characters	States
Equatorial axis length	Micron
Polar axis length	Micron
Number of pollen aperture	Number
Thickness of Margo	Micron
Length of protruding	Micron
Ratio of 1st diameter to 2nd diameter of annulus	Ratio
Ratio of 1st diameter to 2nd diameter of annulus protruding	Ratio
Pollen outline from polar view	Semi-symmetric pentagonal 1, cubic 2, semi orbicular 3, quadrangular 4, hexangular 5
Pollen outline from equatorial view	Semi-ellipsoid 1, cubic 2, orbicular 3, semi orbicular 4, ellipsoid 5
Sexine sculpture	Verrucate-perforate 1, nearly wrinkled and undulating 2, reticulate 3, granular with buds on protruding 4, smooth to granulate 5, granular 6
Annulus surface ornamentation pattern	T-shape 1, nearly smooth 2, smooth 3, wrinkled with small folding 4, plicate and star- shaped 5

similarities to *F. parviflora* showed some differences in its pollen grains (euprolate shape). Populations of *F. vaillantii* showed oblate-spheroidal and prolate-spheroidal pollen grains (Table 4).

Patterns of exine sculpture show many variations between studied species. Populations of *F. asepala* evolved smooth to granulate texture while *F. officinalis*, *F. schleicheri* and *F. densiflora* populations provided a granular pattern. Studied population of *F. parviflora* show more variation in exine sculpture. Fasa population comprised wrinkled and wavy pattern, Yazd population a granular pattern with some tuber shaped protruding. *F. indica* showed a special pattern with some gaps and pores between protruding, it could be grouped as reticulate texture. In populations of *F. vaillantii* exine sculpture are of verrucate- perforate type (Figs. 1 & 2).

Annulus showed some protruding with different shape in studied taxa, which seemed to be of taxonomic importance. *F. asepala* populations showed a smooth annulus without any wrinkle. *F. densiflora* and *F. officinalis* populations showed star shaped protruding deeply wrinkled. Population of *F. parviflora* showed variations in this feature too (Fig. 1). Fasa population showed a smooth surface, while Yazd showed a very small protruding. Pollen grains of *F. indica* and *F. schleicheri* populations showed globular protruding with finely wrinkled surface (Fig. 1). *F. vaillantii* populations showed a T- shaped ornamentation in annulus protruding (Fig. 2).

Pollen grains showed differences in equatorial view outline as follows (Fig. 1): Orbicular in *F. asepala*, semi-orbicular in *F. densiflora*, elliptic (Fasa population) and cubic (Yazd population) in *F.*

*parviflora*, semi-elliptic in *F. vaillantii*, elliptic in *F. indica*, and cubic in *F. officinalis* and *F. schleicheri*.

Polar view of pollen grains were quadrangular in *F. vaillantii*, *F. densiflora*, *F. asepala* and *F. indica*, cubic (Yazd population) or pentangular (Fasa population) in *F. parviflora*, cubic in *F. schleicheri*, and hexagonal in *F. officinalis* (Fig. 1).

In order to define the diagnostic value of pollen grains in species delimitations in *Fumaria*, cluster analysis by UPGMA method were done by 11 qualitative and quantitative features (Fig. 3). As it could be seen, *F. vaillantii* is clearly separated from other species. *F. parviflora* from Fasa and Yazd populations comprise a separate cluster. *F. indica* showed more similarities to *F. asepala* from Taleghan region. These two are more similar and related to *F. densiflora*. PCO based on qualitative and quantitative features of pollen grains (Fig. 4) confirms the cluster analysis by UPGMA method.

## Discussion

Palynological evidences showed some variations in different populations of *F. parviflora* in pollen shape, exine sculpture and annulus protruding. *F. indica* is more related to *F. asepala* populations. Jafri (1974) mentioned the hybrid position of *F. indica* with the proposed progenitors as *F. asepala* and *F. parviflora*. Wendelbo (1974) in Flora Iranica mentioned the high morphological similarities between *F. indica* and *F. parviflora*. The pollen grains features of this project confirmed this opinion. Yazd and Fasa populations of *F. parviflora* are separated in Figs. 3 and 4, the former

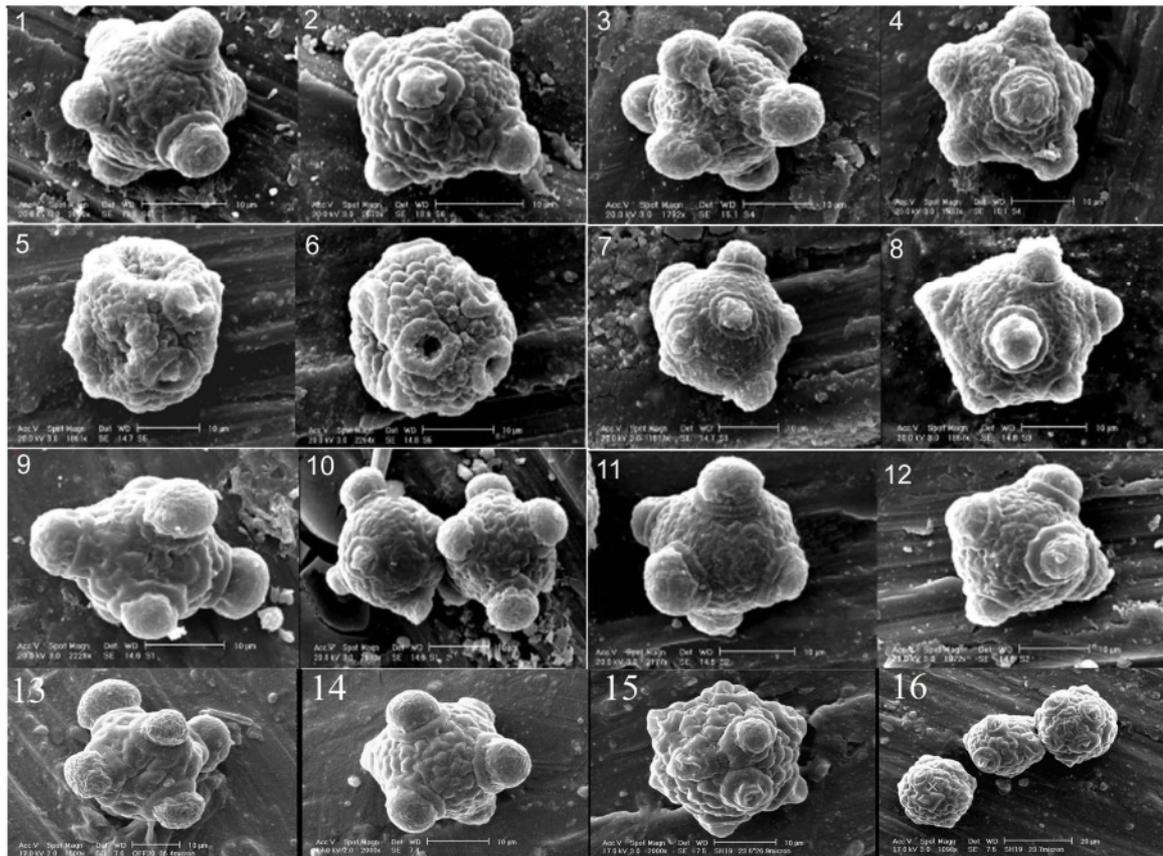


Fig. 1. Scanning electron micrographs of *Fumaria* pollen grains (equatorial & polar view). 1, 2 *F. asejala*, 3, 4 *F. densiflora*, 5, 6 Yazd population and 7, 8 Fasa population of *F. parviflora*, 9, 10 *F. indica*, 11, 12 *F. vaillantii*, 13, 14 *F. officinalis*, 15, 16 *F. schleicheri* (1, 3, 5, 7, 9, 11, 13, 15 equatorial, 2, 4, 6, 8, 10, 12, 14, 16 polar view).

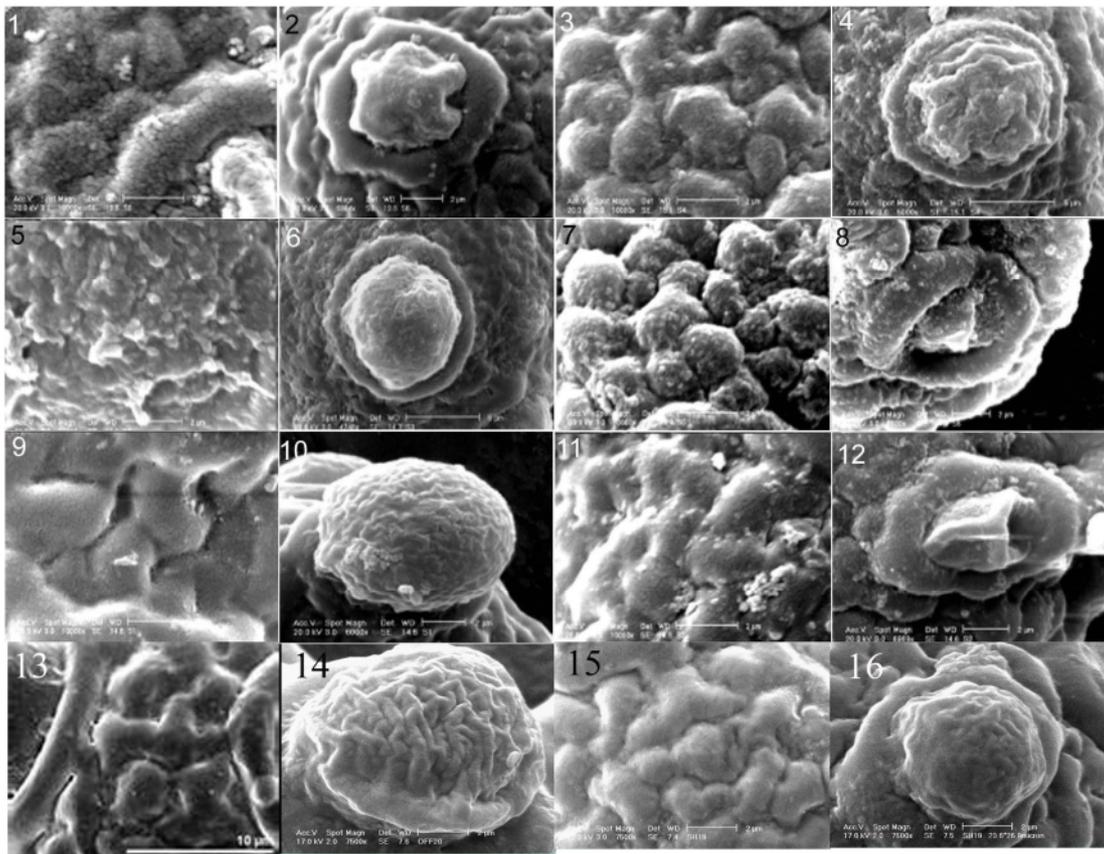


Fig. 2. Scanning electron micrographs of *Fumaria* pollen grains (1, 3, 5, 7, 9, 11, 13, 15 exine surface & 2, 4, 6, 8, 10, 12, 14, 16 annulus surface). 1, 2 *F. asepala*, 3, 4 *F. densiflora*, 5, 6 Yazd & 7, 8 Fasa populations of *F. parviflora*, 9, 10 *F. indica*, 11, 12 *F. vaillantii*, 13, 14 *F. officinalis* and 15, 16 *F. schleicheri*.

Table 3. Pollen quantitative morphological data of *Fumaria* species (scales in micron).

Species	P		E		No of Aperture	Annulus vertical to horizontal diameter	1 <sup>st</sup> to 2 <sup>nd</sup> annulus protruding diameter	Thickness of margo	Length of protruding
	Range	Mean	Range	Mean					
<i>F. vaillantii</i>	34.39-48.39	35.93	35.5- 56.2	38.55	6	0.68	0.62	2.41	1.95
<i>F. parviflora</i>	37.28 -45.95	40.49	32- 54.67	38.17	7	0.84	0.86	1.19	3.58
	31.66 -44	42.24	34.2 -49	47	6	0.8	0.76	2.15	999
<i>F. indica</i>	27.74 -36.05	30.26	35.66 -41	40.86	6	0.96	0.94	1.95	4.04
<i>F. asepala</i>	29.51- 44.67	33.61	34.19- 39	37.43	6	0.78	0.88	2.21	3.64
<i>F. densiflora</i>	33.94-44.18	39.03	34.51- 42	40.83	6	0.89	0.9	1.18	4.3
<i>F. officinalis</i>	15.7 – 23	20	20.55 -29	23.7	12	0.88	0.92	1.89	9.69
<i>F. schleicheri</i>	18.9-21	20.5	15-22.9	21	6	0.95	0.86	1.2	6.5

Table 4. Pollen qualitative morphological data of *Fumaria* species (Codes are indicated in table 2).

Species	Polar shape	Equatorial shape	Sexin sculpture	Annulus surface ornamentations pattern	Pollen shape
<i>F. vaillantii</i>	4	1	1	1	prolate spheroidal oblate spheroidal
<i>F. parviflora</i>	1	5	2	2	oblate spheroidal
	2	2	4	2	prolate spheroidal
<i>F. indica</i>	4	5	3	4	euoblate
<i>F. asepala</i>	4	3	5	3	oblate spheroidal
<i>F. densiflora</i>	4	4	6	5	oblate spheroidal
<i>F. officinalis</i>	5	2	6	5	sub oblate
<i>F. schleicheri</i>	2	2	6	4	oblate spheroidal

accession belongs to *F. parviflora* var. *parviflora* and the latter to *F. parviflora* var. *symmei* Loisel.

In our palynological investigation, we found unusual dimorphic pollen grains for some individuals of *F. parviflora* and *F. schleicheri*. This could be due to the sterility of pollen grains. There are also pollen grains with 6 and 12 annuli in both *F. vaillantii* and *F. schleicheri* species. In the latter 12 annuli is common but in *F. vaillantii* hexa-annulate pollen grains is usual form. It seems that there could be different ploidy levels and cytotypes of these species in Iran. To prove such a statement complementary meiotic study and pollen fertility tests should be done.

Our observation indicated that there is a considerable variation in exine sculpture in *Fumaria* species. Seven studied species of *Fumaria* are efficiently separated based on shape, size, aperture number and exine sculpture patterns. Sometimes modifications were observed in annulus shape and protruding by careful laboratory observations. It does not seem to be a stable character of the pollen grains.

Our observations indicated that features as vertical and horizontal diameter of annulus and its protruding, margo thickness and sculpture patterns of exine and annulus surface are of taxonomic importance in studied species of Iran.

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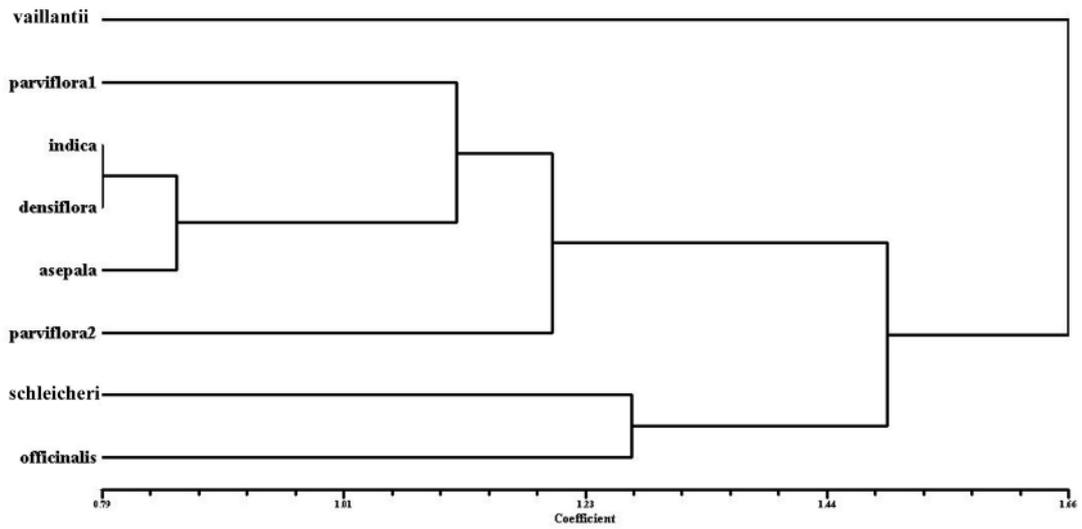


Fig. 3. Cluster analysis by UPGMA method based on pollen morphology results of studied *Fumaria* species.

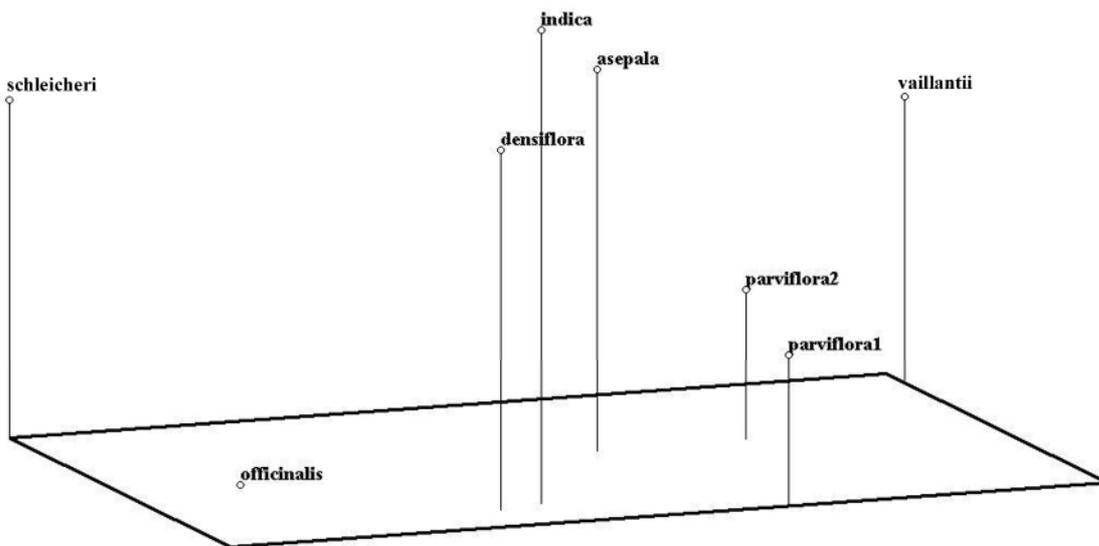


Fig. 4. PCO ordination of the studied *Fumaria* species based on pollen grain morphology.