MICROMORPHOLOGY CHARACTERIZATION POLLEN GRAINS OF SUBGENERA *PRUNUS* AND *CERASUS* (ROSACEAE) IN KURDISTAN REGION-IRAQ

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ABSTRACT
This investigation was aimed to study pollen grains of subgenera *Prunus* and *Cerasus*. The polleniferous materials were collected from different places of Kurdistan region of Iraq living specimens. Pollen grains of all investigated taxa of subgenera *Prunus* and *Cerasus* were isopolar, monad, symmetric, tricolporate, dicolporate and tetracolporate pollens also observed, but very rarely. The pollen grains shape of taxa of subgenera *Prunus* and *Cecasus* divided in two groups subprolate and prolate depending on P/E value. While, the size were medium with the exception of *P. domestica* subsp. *italica* grains were found to be small in size. The percentage of repeat morphological shape of pollen grains in polar view showed that the triangular outlines shapes of pollen grains were the most repeated, while, in the equatorial view the elliptic outline shapes was dominant in all taxa. Pollen grains surface of studied taxa was showed striate pattern with perforations in the grooves except *P. domestica* subsp. *insititia* which appeared striate-reticulate type of exine sculpture. Principal component analysis can recognize 67.7 % of the total variability thus can help prediction of taxa.

Keywords: taxa, tricolporate, isopolar, monad, symmetric.
***Part of Ph.D. dissertation of 1st author.**

وصف الشكل الدقيق لحبوب اللقاح لجنيس subgenera *Prunus* و*Cerasus* (Rosaceae) في كردستان العراق

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المستخلص
هذا البحث يهدف الى دراسة حبوب اللقاح لنباتات تحت الجنس *Prunus* و *Cerasus*. جمعت حبوب اللقاح من العينات الحية من أماكن مختلفة من إقليم كردستان العراق. وقد اتضح ان حبوب اللقاح لجميع الأنواع المدروسة تحت الجنس *Prunus* و *Cerasus* كانت متساوية القطب، أحادية، متماثلة، من الطراز ثلاثي الثقوب الأخاديد، وكذلك ثنائي و رباعي الثقوب و الأخاديد أيضًا وجدت ولكن بشكل نادر. شكل حبوب اللقاح لانواع تحت الجنس *Prunus* و *Cerasus* قسمت إلى مجموعتين المتطاول و شبه المتطاول اعتمادًا على قيمة P/E. بينما كان حجمها متوسط الحجم باستثناء *P. domestica* subsp. *italica* كانت حبوب لقاحها صغيرة الحجم. أظهرت النسبة المئوية لتكرار الشكل المورفولوجي لحبوب اللقاح في المنظر القطبي أن شكل المثلث كان الأكثر تكرارًا، بينما في المنظر الاستوائي كان شكل اهليجي هو السائد في جميع الأنواع. سطح الخارجي لحبوب اللقاح الأنواع المدروسة ذات نمط مخطط مع ثقوب في الأخاديد باستثناء *P. domestica* subsp. *insitia* التي ظهرت على شكل مخطط شبكي. تحليل المكون الرئيسي يمكن أن يميز 67.7 ٪ من التباين الكلي وبالتالي يمكن أن يساعد في التنبؤ.

الكلمات المفتاحية: الاصناف, الطراز ثلاثي الثقوب الأخاديد, متساوية القطب، أحادية، متماثلة.
* جزء من أطروحة الدكتوراه للباحث الأول.

Received:13 /2/2023, Accepted:16/7/2023, Published:December 2025

INTRODUCTION

The genus *Prunus* L. belongs to the subfamily Prunoideae in the Rosaceae family, it is a genus of deciduous or evergreen trees and shrubs. According various authors *Prunus* comprise from 200 to 430 species: higher than 200 (26), 250 (32), 400 (18) and 430 species (22). A pollen grain is a noticeable natural material that has a crucial function in plant reproduction. The importance of morphology characters of pollens in plant systematics has been exhibited by a many of researchers, mostly by several researchers (2, 7, 10, 20, 21, 28). They have unique biological features, such as contain a high amount of genetic information and show strong genetic conservation therefore it can be used for species identification (13, 27, 30). Pollen grain features mainly comprise pollen shape, size, surface morphology, and ultrastructure which consider high importance for description of the pollen grains (19). At the same time pollen plays serious roles in phylogenetic investigations of fruit trees (9). According to Elysiane *et al*. (5) the complication and difference of exine ornamentation of pollen grains among individuals, might be used for plant identification and classification. The exine of pollen grains was genetically stable for different species of Rosaceae family and might be used for identification of species (31). Hebda *et al.,* (14) point out that the pollen surface sculpturing, aperture and aperture structure, pollen shape, size were benefit features to differentiate the various genera and even species of the Rosaceae. According to Kuiling *et al*. (16) study of the pollen morphology of plants of the Rosaceae forms the fundamental for systematic classification of some species. Genus *Prunus* considered one of the most complicated genera in the Rosaceae because of the polymorphism, the wide ecological tolerance of the species, and the presence of a high numbers of cultivars (4). Also (13) indicated that because of high variety of fruit tree cultivars of the genus *Prunus*, the microscopic features of pollens, among others characters are a tool for their identification. The research of morphological features of pollens could be a suitable method to identify genotypes in different species of the genus *Prunus* (11, 15). Therefore the aims of the present study were conducted to characterize the pollen morphology of subgenera *Prunus* and *Cerasus* species and infra species in Kurdistan region of Iraq and to evaluate the utility of pollen features in order to develop an additional plant identification tool to differentiate between the highly related taxa.

MATERIALS AND METHODS

The present study was done in the Scientific Researches Center Laboratory, College of Agricultural Engineering Sciences, University of Dohuk, Kurdistan Region, Iraq. The polleniferous materials used in this investigation were collected from different places of Kurdistan region of Iraq from living specimens. Some anthers were removed from the flowers and putted into the 70% alcohol, kept in the refrigerator at 4Cº for later use, and other were dried. For preparation of sides for light microscope (LM), pollen grains were mounted in glycerin jelly and drop of methyl green stain (1g methyl green dissolved in 100 ml alcohol 95%) added to it (25), after then samples have been putted on the slides and covered by a cover slide. Pollen grains were observed by light microscope (OLYMPUS CX21) and photographed using digital camera (Sony, 16 mega pixels). The following parameters were analyzed: polar axis length in the equatorial view (P), equatorial axis diameter in the equatorial view (E), mesocolpium (Meso) in the equatorial view, Colpus length (Colpus) in equatorial view, exine thickness (Exine) in polar view, apocolpium (Apo) in the polar view, P/E ratio, pollens size, percentage of repeat morphological shape of pollen grains/ $^{1}/_{2}$ mm^2 in polar and equatorial view. The mean of each character was based on 25 observations. The samples of pollen grains were also examined and photographed by using scanning electron microscope (SEM) model MIRA 3 TESCAN. Size classes of Erdtman (6) were assessed, while shape classes of Erdtman (7) were followed. The terminology follows that of Erdtman and Sorsa (8) and Punt *et al.* (24). Minitab Version 21.2 was used to analyze the data for principal component analysis method.

RESULTS AND DISCUSSION

In the present research, pollen grains of investigated species and intraspecific taxa of both subgenera *Prunus* and *Cerasus* were isopolar, monad, symmetric, colporate, the characters of investigated pollen grains show in Table (1) and Figures (1-12). All pollen grains were tricolporate, dicolporate and tetracolporate pollen also appeared, but very rarely, this agreed with the results found by many researchers who referred that the pollen grains of many species of Rosaceae categorized as tricolporate (seldom tricolpate) (33,34). Chwil (3) incubated that the pollen grains in the flowers of fruit trees of the genus *Prunus* was tricolporate. Also (17) founded that the pollen grains of 175 accessions of *P. armeniaca* were isopolar monads, radially symmetric, medium to large in size, prolate or subprolate and tricolporate. The pollen grains of genus *Prunus* have dicolporate and tetracolporate pollens (15). The mean length of the polar axis of taxa of both subgenera *Prunus* and *Cerasus* was range from 22.73 µm in *P. domestica* subsp. *italica* to 33.32 µm in *P. armeniaca* var. *armeniaca*, The smallest mean of aquatorial diameter was found in *P. cerasifera* 16.52 µm, while the largest mean of aquatorial diameter (25.97 µm) was found in *P. domestica* subsp. *insititia*. Apocolpium the shorter polarity area as mean (25.01µm) was registered in *P. microcarpa* var. *microcarpa*, while, the longest one (39.95µm) was registered in *P. domestica* subsp. *insititia*. Mesoculpium varies between taxa, the smallest mean area between two adjacent colpi was 4.00 µm in *P. cerasus*, while the largest mean area recorded in *P. armeniaca* cv. Saeede Mariame (10.24 µm). The mean colpus length in pollen grain of both subgenera was different among investigated taxa, it was (16.43 µm) as a shorter mean colpus length was observed in *P. domestica* subsp. *italica*, but longer mean colpus length (28.49 µm) was showed in *P. armeniaca* var. *armeniaca*. The exine thickness of *P. domestica* subsp. *insititia* (2.26 µm) was considered to be thick, while the exine thickness (1.18 µm) of *P. microcarpa* var. *pubescens* was considered to be thin. And the rest taxa of both subgenera were between these two values for all pollen grains characters dimensions as appeared in Table (1). Pollen grains have varied surface sculpture and a multi cell wall layer, which consists of an inner intine and an outer exine, the exine thickness was one of the characters which used in distinguish between taxa, however pollen grains exine sculpture by SEM become important feature for distinguish between plants. In the general pollen grains surface of all the studied taxa of subgenera *Prunus* and *Cerasus* was showed striate pattern except *P. domestica* subsp. *insititia* which appeared striate-reticulate type of exine sculpture with perforations in the grooves. The arrangement of ornamentation (muri or ridges), separated by the gaps between them (grooves) and the puncta (perforations) present in the grooves of all taxa of subgenera *Prunus* and *Cerasus*. Similar results have been reported by Hebda and Chinnappa (12) distinguished six pollen kinds within Rosaceae: I, striate and macroperforate, II, striate and microperforate, III, tuberculate and perforate, IV, microverrucate, V, verrucate and VI, perforate, without supratectal characters. As well as (11) stated numerous taxa of the genus *Prunus* were distinguished by striate exine sculpture of their pollen grains. *P. armeniaca* pollen grains contain a perforated tectum (1, 11), also present in the plum pollen grains (11, 15), and sour cherry (23). According to Erdtman (7) shape class, the shape of pollen grains of taxa of subgenera *Prunus* and *Cecasus* divided in two groups subprolate and prolate depending on P/E value. Similar results were reported by some other research workers Geraci *et al*. (11) have observed that *P. domestica* taxa have prolate pollen shape. Similar results have been reported by Arzani *et al*. (1) who examined pollen grains of eleven apricot cultivars of *P. armeniaca* in Iran by SEM and found that the pollen grains were isopolar monads, radially symmetric, of medium size, prolate, and tricolporate. As well as (3) pointed out that the shape subprolate of pollen grains was founded in two cultivars of sour cherry. While the size the investigated pollen grains of all taxa of both subgenera depending on the size classes of Erdtman (7) were medium size with the exception of *P. domestica* subsp. *italica* grains were found to be small in size. This in accordance with the current results that indicated the size of apricot pollen grains was medium (31). Also (3) observed by using SEM the pollen grains of the two cultivars of apricot were medium in size. The percentage of repeat morphological shape of pollen grains amb (polar view) showed that the triangular outlines shapes of pollen grains were the most repeated in all taxa of both subgenera, the ovate outlines comes next, whilst few shapes was circular and not present in all accessions, and no elliptic shapes was observed. In addition tetrangular shapes appear only in the polar view of *P. armenaica* var. *armeniaca, P. armeniaca* cv. Saeede Mariame, and *P. cerasus*. While, in the equatorial view the elliptic outline shapes was dominant in all taxa of both subgenera, whereas circular and ovate outlines shapes comes next. In the same time triangular and tetrangular not observed in this view for all taxa, as shows in Table (2). These results were similar to those of other researchers (1, 11) who explained that the most pollen grains of the genus *Prunus* in polar view were triangular outline, whereas in equatorial view an elliptical outline was abundant. Also found to be in agreement with the results of other authors (29) reported that subgenus *Cerasus* polar view triangular to circular; equatorial view subspheroidal to prolate shape.

Table 1. Quantitative characters of pollen grains (µm).

Taxon	Statistics	Polar length	Equatorial diameter	P/E	Apocolpium	Mesocolpium	Colpus length	Exine thickness	Pollen shape
P. cerasifera	Mean	25.58	16.52	1.57	31.09	4.83	19.20	1.35	Prolate
	Min	22.16	12.05	1.25	25.29	2.00	16.07	0.82	
	Max	31.73	19.26	2.25	36.10	9.97	28.75	1.83	
P. domestica* subsp. *domestica	Mean	25.23	19.75	1.29	25.12	5.53	18.69	1.48	Subprolate
	Min	22.42	15.72	1.08	17.15	2.38	13.76	1.19	
	Max	30.41	22.58	1.66	36.34	8.92	25.47	2.14	
P. domestica* subsp. *syriaca	Mean	27.90	24.00	1.23	28.04	7.61	17.90	1.36	Subprolate
	Min	20.74	18.89	0.64	17.52	2.33	10.71	0.88	
	Max	45.19	45.19	2.39	39.29	17.21	26.64	1.83	
P. domestica* subsp. *insititia	Mean	33.11	25.97	1.32	39.95	6.61	19.57	2.26	Subprolate
	Min	19.49	18.38	0.79	28.71	3.38	12.69	1.31	
	Max	48.25	35.92	2.36	55.00	11.32	24.62	2.79	
P. domestica* subsp. *italica	Mean	22.73	20.17	1.15	32.49	6.16	16.43	1.46	Subprolate
	Min	17.92	15.03	0.78	27.68	1.97	11.47	1.00	
	Max	36.52	26.11	1.76	37.98	11.08	22.86	2.19	
P. armeniaca* var. *armeniaca	Mean	33.32	21.76	1.55	37.53	6.46	28.49	1.22	Prolate
	Min	26.01	17.96	1.00	30.42	1.92	17.99	0.82	
	Max	59.03	27.62	2.43	45.25	13.14	47.25	1.61	
***P. armeniaca* cv. Saeede Mariame**	Mean	26.27	21.53	1.24	36.81	10.24	18.68	1.52	Subprolate
	Min	17.86	16.44	0.78	26.81	2.90	12.41	1.04	
	Max	31.28	25.71	1.64	46.23	20.14	24.13	2.19	
P. microcarpa* var. *pubescens	Mean	27.43	17.66	1.58	29.63	6.48	20.28	1.18	Prolate
	Min	22.81	13.33	1.21	24.24	2.23	15.83	0.82	
	Max	34.86	21.89	2.22	39.27	11.79	32.49	1.62	
P. microcarpa* var. *microcarpa	Mean	30.42	22.81	1.35	25.01	5.47	22.81	1.26	Prolate
	Min	22.80	16.84	0.88	21.98	1.55	12.16	0.82	
	Max	37.60	27.41	1.78	30.09	12.95	31.43	1.62	
P. brachypetala	Mean	32.03	22.73	1.45	35.41	4.93	22.67	1.33	Prolate
	Min	25.77	17.80	0.97	24.28	1.55	9.94	1.04	
	Max	44.94	28.88	2.52	44.90	11.36	38.60	1.67	
P. mahaleb	Mean	28.36	17.89	1.62	29.86	4.39	19.19	1.55	Prolate
	Min	21.53	14.04	1.06	24.60	1.77	9.43	0.82	
	Max	42.61	22.06	2.70	33.58	8.03	34.54	2.67	
P. cerasus	Mean	27.38	21.19	1.30	30.28	4.00	19.31	1.44	Subprolate
	Min	22.04	17.43	0.99	22.60	1.87	14.38	0.93	
	Max	36.37	25.99	1.68	42.15	7.11	27.68	2.08	

***Mean (the sum of all observations value divided by the total *number of* observations).**

Table 2. Percentage of repeat morphological shape of pollen grains.

Taxon	Polar view					Equatorial view				
	Triangular	Ovate	Circular	Elliptic	Tetrangular	Triangular	Ovate	Circular	Elliptic	Tetrangular
P. cerasifera	****	**	*	-	-	-	**	*	****	-
P. domestica subsp. *domestica*	****	***	*	-	-	-	**	***	***	-
P. domestica subsp. *Syriaca*	****	***	*	-	-	-	*	**	***	-
P. domestica subsp. *Insititia*	***	*	***	-	-	-	*	**	***	-
P. domestica subsp. *Italic*	****	**	-	-	-	-	*	**	***	-
P. armeniaca var. *armeniaca*	****	**	-	-	*	-	*	***	***	-
P. armeniaca cv. Saeede Merieme	****	**	*	-	*	-	*	***	****	-
P. microcarpa var. *pubescens*	****	**	*	-	-	-	*	**	***	-
P. microcarpa var. *microcarpa*	****	*	*	-	-	-	**	*	****	-
P. brachypetala	****	**	*	-	-	-	**	*	***	-
P. mahaleb	****	***	-	-	-	-	**	*	****	-
P. cerasus	****	**	***	-	*	-	**	***	***	-

*1-20 %, ** 20-30%, *** 30- 40 % and **** more 40%, - not present

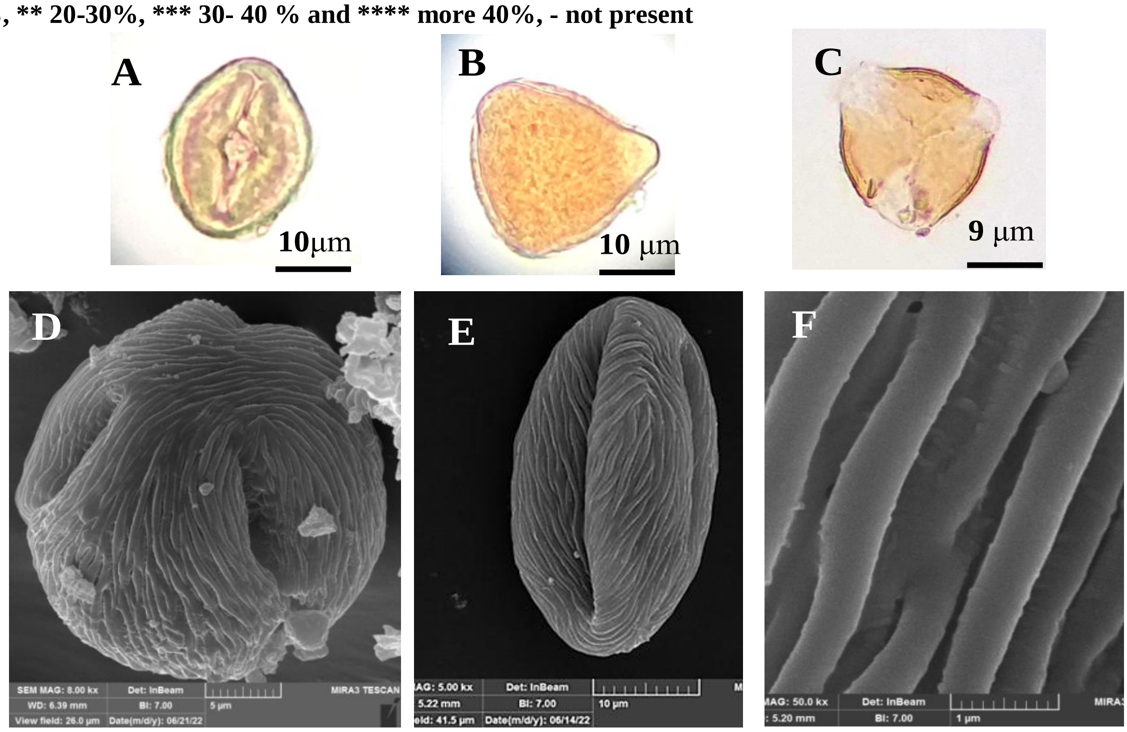

Figure 1. LM and SEM micrographs of pollen grains of *P. ceracifera*: A-C. LM micrographs: A. Elliptic equtorial view, B. Triangular polar view, C. Triangular polar view; D-F SEM micrographs: D. Semicircular polar view, E. Elliptic equtorial view, F. Exine sculpture

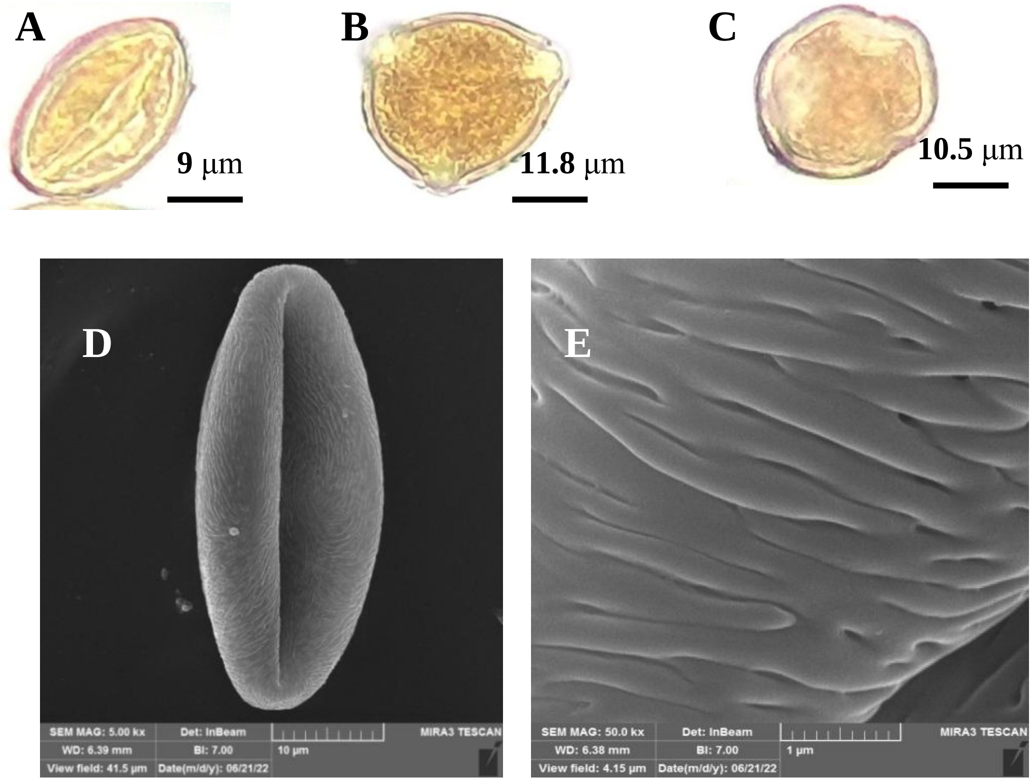

Figure 2. LM and SEM micrographs of pollen grains of *P. domestica* subsp. *domestica*: A-C. LM micrographs: A. Elliptic equtorial view, B. Triangular polar view, C. Smicircular polar view; D-E SEM micrographs: D. Elliptic equtorial view, E. Exine sculpture

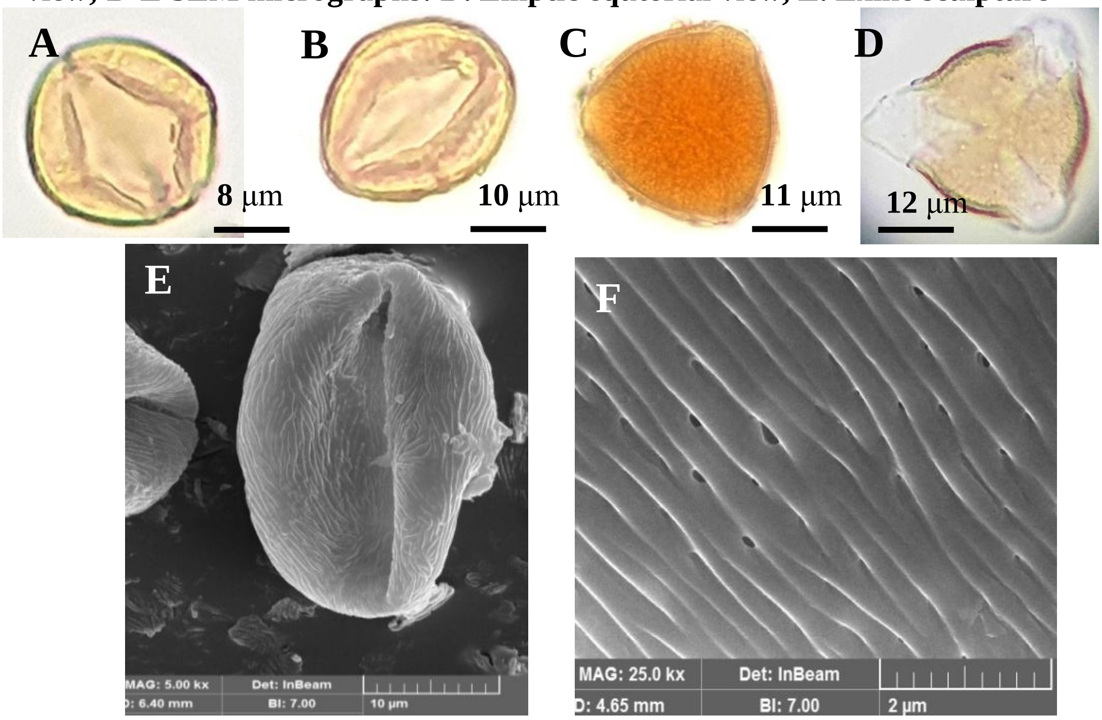

Figure 3. LM and SEM micrographs of pollen grains of *P. domestica* subsp. *syriaca*: A-D. LM micrographs: A. Semicircular equatorial view, B. Ovate equtorial view, C. Triangular polar view; D. Triangular polar view; E-F SEM micrographs: E. Ovate equatorial view, F. Exine sculpture

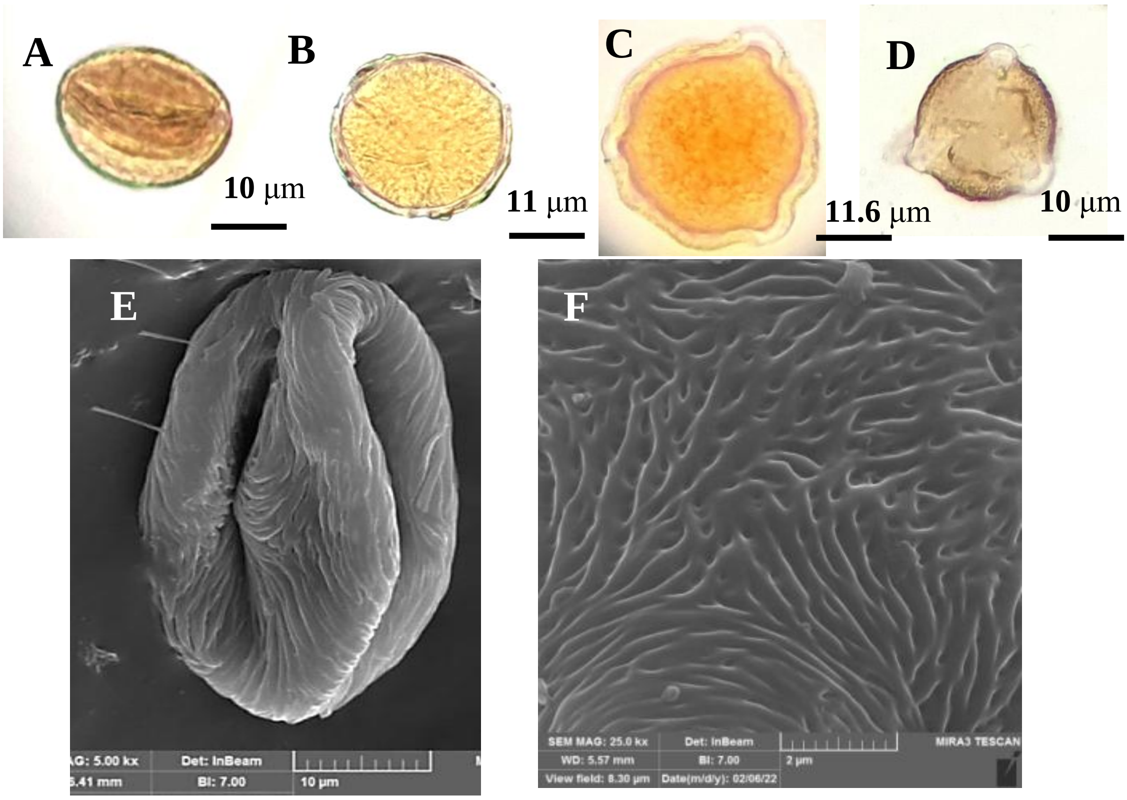

Figure 4. LM and SEM micrographs of pollen grains of *P. domestica* subsp. *insititia*: A-D. LM micrographs: A. Elliptic equtorial view, B. Circular polar view, C. Smitriangular polar view, D. Triangular polar view; E-F SEM micrographs: E. Elliptic equatorial view, F. Exine sculpture

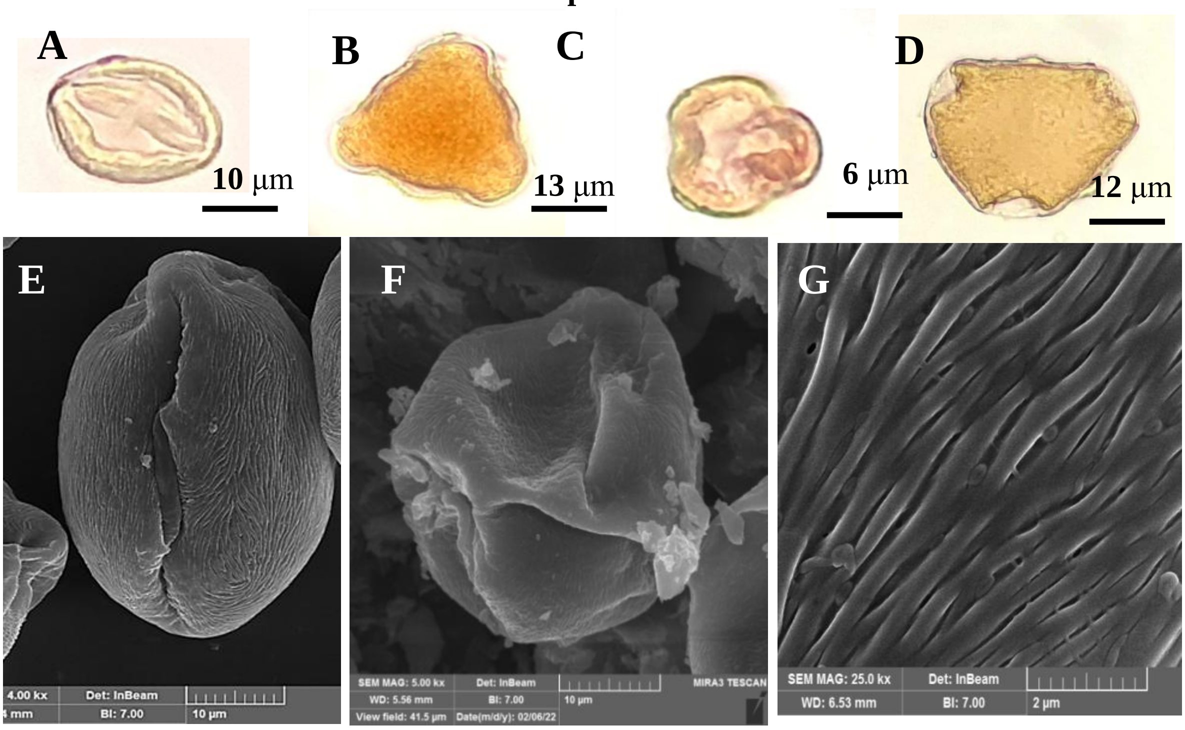

Figure 5. LM and SEM micrographs of pollen grains of *P. domestica* subsp. *italica*: A-D. LM micrographs: A. Elliptic equtorial view, B. Triangular polar view, C. Semicircular polar view, D. Triangular polar view; E-G SEM micrographs: E. Elliptic equatorial view, F. Semicircular polar view, G. Exine sculpture

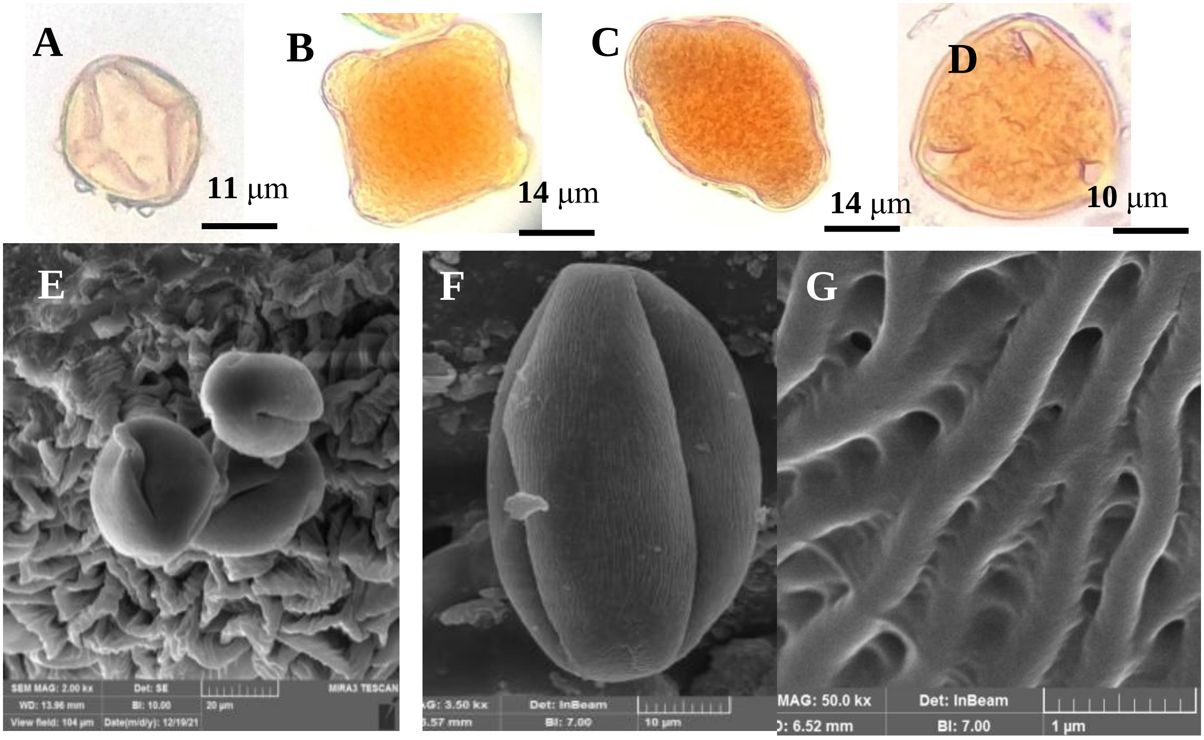

Figure 6. LM and SEM micrographs of pollen grains of *P. armeniaca* var. *armeniaca*: A-D. LM micrographs: A. Elliptic equtorial view, B. Tetracolporat polar view, C. Dicolporate polar view, D. Triangular polar view; E-G SEM micrographs: E. Triangular polar view, F. Elliptic equatorial view, G. Exine sculpture

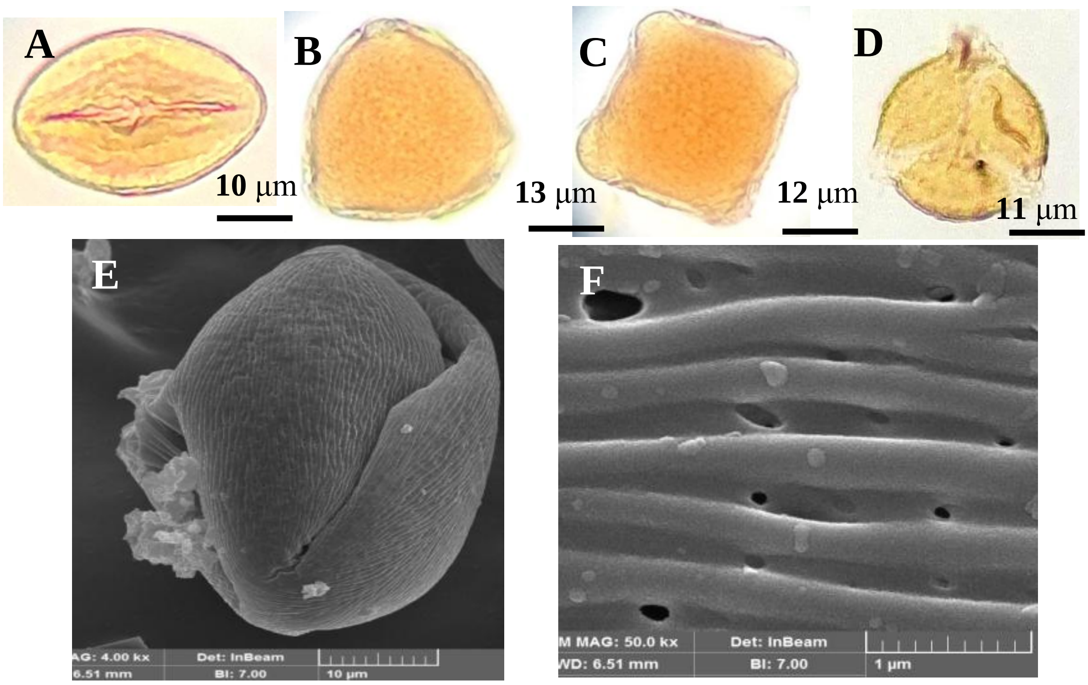

Figure 7. LM and SEM micrographs of pollen grains of *P. armeni aca* cv. Saeedy Mariame: A-D. LM micrographs: A. Elliptic equtorial view, B. Triangular polar view, C. Tetracolporat polar view, D. Triangular polar view; E-F SEM micrographs: E. Elliptic equtorial view, F. Exine sculpture

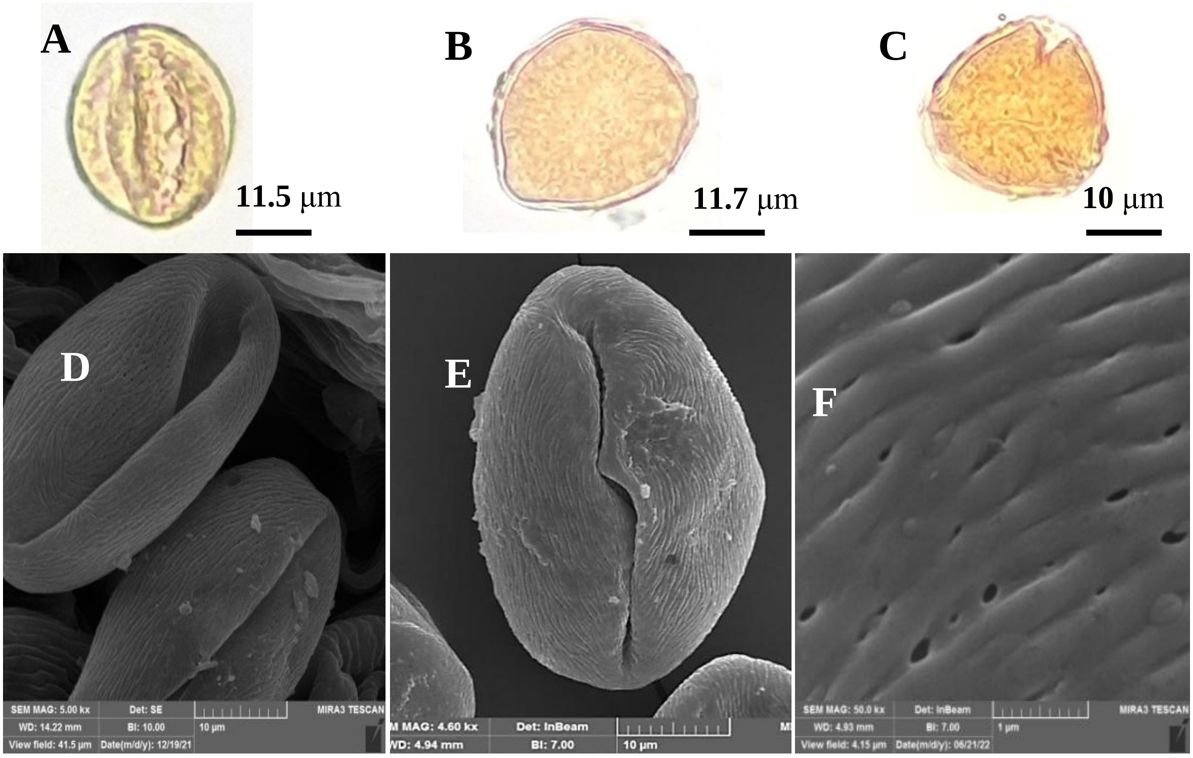

Figure 8. LM and SEM micrographs of pollen grains of *P. microcarpa* var. *pubescens*: A-C. LM micrographs: A. Elliptic equtorial view, B. Smitriangular polar view, C. Triangular polar view; D-F SEM micrographs: D. Elliptic equtorial view, E. Elliptic equtorial view, F. Exine sculpture

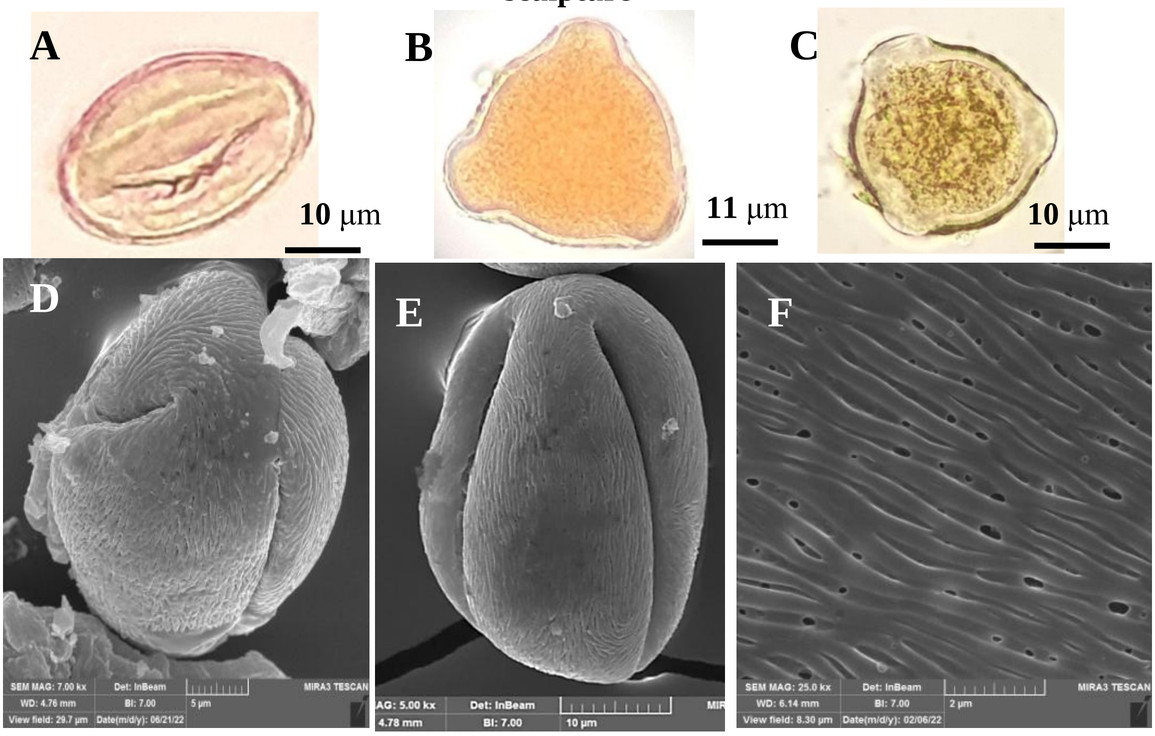

Figure 9. LM and SEM micrographs of pollen grains of *P. microcarpa* var. *microcarpa*: A-C. LM micrographs: A. Elliptic equtorial view, B. Triangular polar view, C. Triangular polar view; D-F SEM micrographs: D. Triangular polar view, E. Elliptic equatorial view, F. Exine sculpture

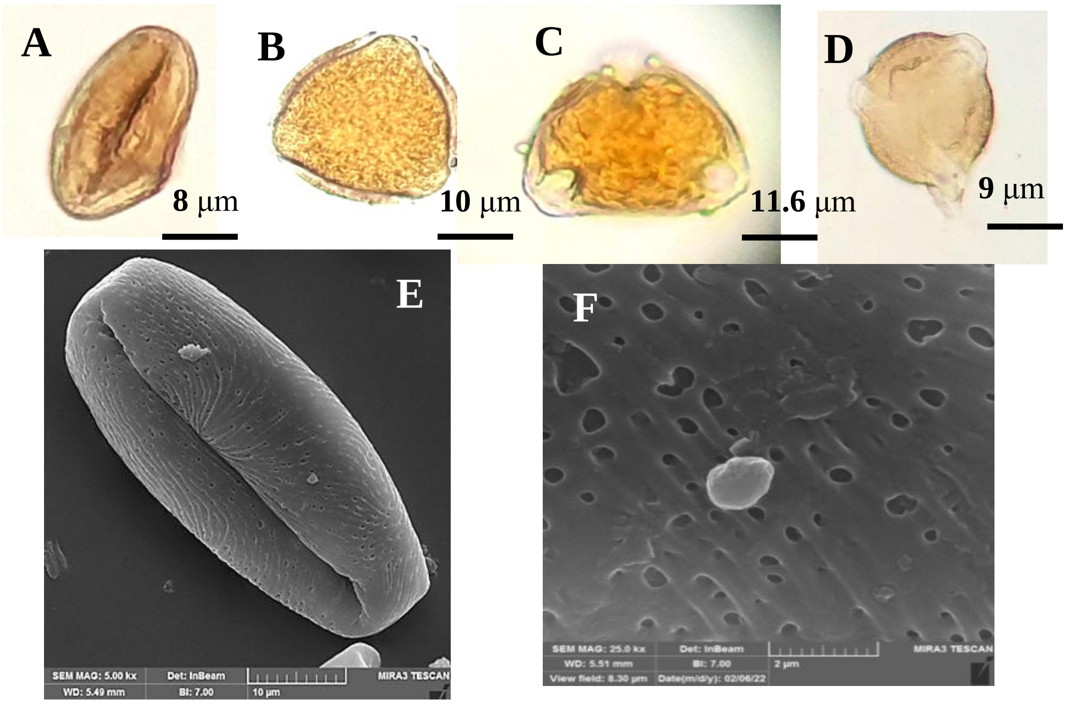

Figure 10. LM and SEM micrographs of pollen grains of *P. brachypetala*: A-D. LM micrographs: A. Elliptic equtorial view, B. Triangular polar view, C. Triangular polar view, D. Triangular polar view; E-F SEM micrographs: E. Elliptic equtorial view, F. Exine sculpture

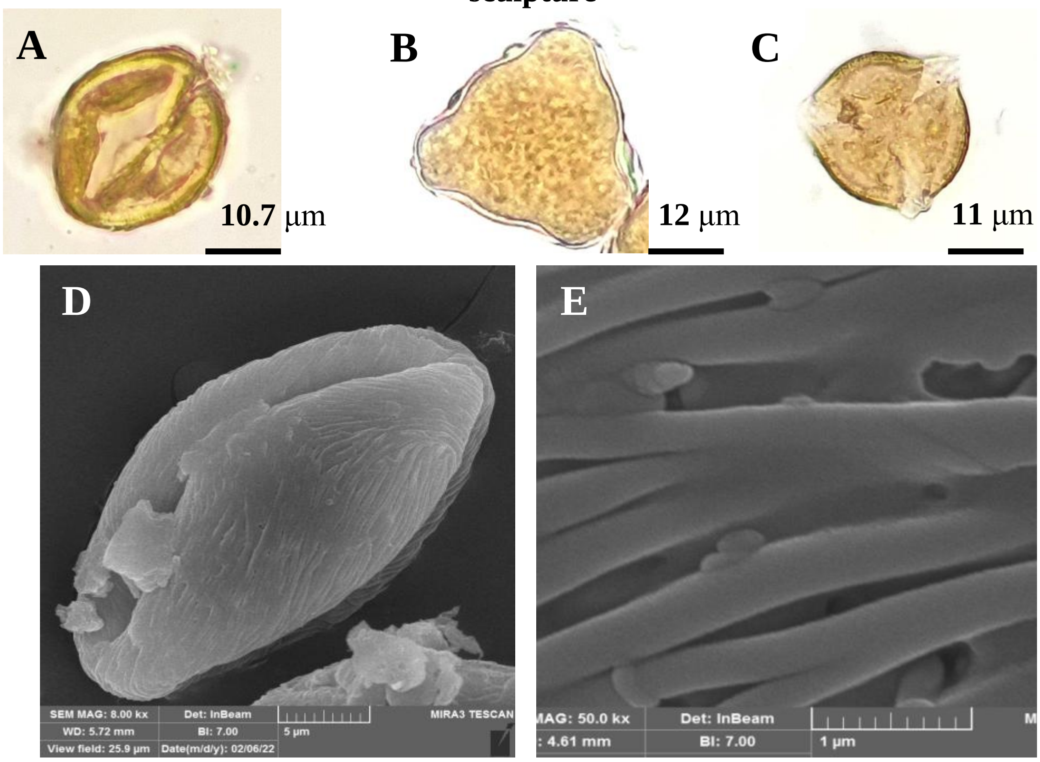

Figure 11. LM and SEM micrographs of pollen grains of *P. mahaleb*: A-C. LM micrographs: A. Ovate equtorial view, B. Triangular polar view, C. Triangular polar view; D-E SEM micrographs: D. Elliptic equatorial view, E. Exine sculpture

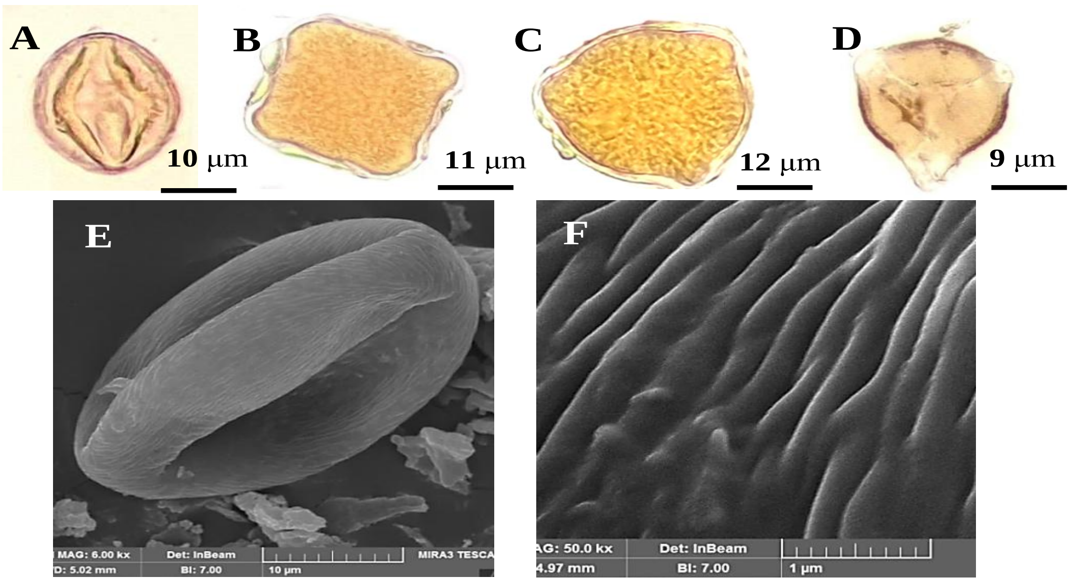

Figure 12. LM and SEM micrographs of pollen grains of *P. cerasus*: A-D. LM micrographs: A. Semicircular equtorial view, B. Tetracolporate polar view, C. Triangular polar view; D. Triangular polar view; E-F SEM micrographs: E. Elliptic equatorial view, F. Exine sculpture

Use of multivariate analysis for distinguishing subgenera *Prunus* and *Cerasus* (Rosaceae)

Principal component analysis: The purpose of this study (Principal component) is to obtain a small number of linear combinations of the 7 variables which account for most of the variability in this case, 3 components have been extracted, and together they account for 67.7% of the variability in the original data. The projection of the 7 studied pollen grain characters (Table 3) onto the plane defined by the 1-2 component planes show that the first principal component is influenced strongly by equatorial diameter and exine thickness, while the second principle component is highly influenced by apocolpium and polar length (Figure 13**).**

Table 3. Factors analysis

Component number	Eigenvalue	Percent of variance	Cumulative Percentage
1	1.8852	26.9	26.9
2	1.6713	23.9	50.8
3	1.1808	16.9	67.7
4	0.9126	13	80.7
5	0.7642	10.9	91.6
6	0.5599	8	99.6
7	0.026	0.4	100

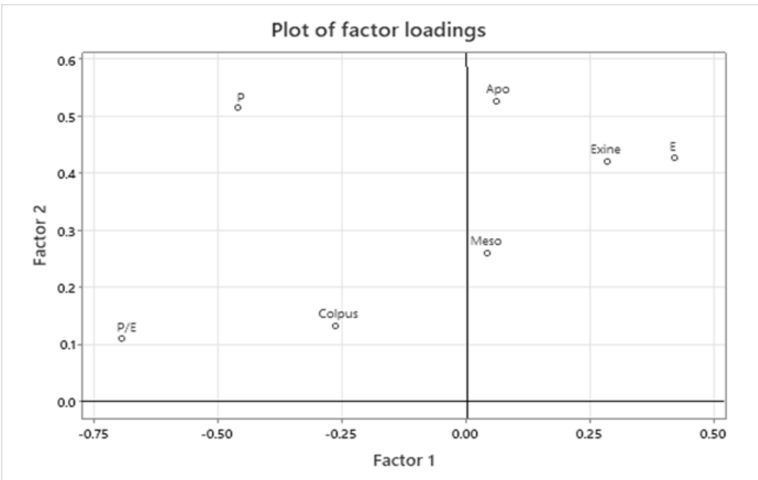

Figure 13. Principle component analysis for 7 pollen grain characters, polar axis length (P), equatorial axis diameter (E), mesocolpium (Meso), Colpus length (Colpus), exine thickness (Exine), apocolpium (Apo), P/E ratio

CONCLUSION

It is possible to depend on the micromorphological characteristics of pollen grain in identification and distinguishing of 12 taxa of subgenera *Prunus* and *Cerasus* of the genus *Prunus* L. (Rosaceae) by using light and scanning electron microscope.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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