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ARTICLE

Pollen morphology of the genus Pyrus (*Rosaceae*) in Iran

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ABSTRACT In this study, pollen morphological characters of nine species of the genus *Pyrus* L. belonging to four sections, *Argyromalon*, *Pashia*, *Pyrus* and *Xeropyrenia* were examined by light (LM) and scanning electron (SEM) microscope. Regarding pollen shape, two forms can be recognized in the same specimen: the first form ranges from prolate- spheroidal, subprolate to prolate, while the second form includes triangular, trilobate and circular shapes, the apertures structure usually consists of three ectocolpi and three endopores. colpi occupy 85- 91% of length of pollen, often arranged meridionally but also parallel pattern can be recognized, endopore is located in the middle of colpi. Regarding sculpturing of the exine in proximal face, striate sculpturing is observed that according to some characters such as degree of slope of ridges, percentage of ridges and perforations, diameter of perforations is subdivided to four main types. Results of pollen grain fertility studies in the genus showed high percentage of fertility among studied species except in one species. **Acta Biol Szeged 54(1):51-56 (2010)**

KEY WORDS

Iran
Pollen grain
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The genus *Pyrus* L. (Pear) that initially was defined by Linnaeus (1753) covering not only pear- trees but also apple-trees in that time, is a tree member of subtribe Pyrinae (formerly subfamily Maloideae), family Rosaceae (Campbell et al. 2007). *Pyrus* probably originated in the mountainous regions of western and southwestern China in Tertiary or, possibly, even more ancient time and evolved and spread eastward and westward (Rubtsov 1944), and nowadays consists of 41 species in the temperate zones of Northern Hemisphere (except for North America) and exceptionally enters the most northwestern tip of Africa (Browicz 1993; Zamani et al. 2009; Zamani and Attar 2010). The highest number of taxa is found in Caucasus (specifically in Armenia) where more or less half of the species occur (Browicz 1993). Iran comprises of 7 (Schönbeck-Temesy 1969), 12 (Khatamsaz 1992) to 18 species (Zamani 2009; Zamani et al. 2009a, b; Zamani and Attar 2010) species that eight of them are endemic. The genus consists of two main centers of distribution in Iran, namely Alborz (N Iran) and Zagros (NW to S Iran) Mts.; however a few locations are reported from east and south (Browicz 1982) of Iran. The most important studies on the genus were those of Schönbeck-Temesy (1969), Browicz (1972a, 1972b, 1973, 1993) and Maleev (1971) that are mainly based on morphology and distribution of the genus. Pollen morphology has been proved to be useful in systematic of the Rosaceae (Hebda and Chinnappa 1990) as well as some of its particular genera and species (Reitsma 1966; Ueda and Tomita 1989; Arzani et al. 2005; Bednorz et al. 2005; Wronska- Pilarek and Boratynska 2005; Wronska- Pilarek and Lira 2006; Polyakova and Gataulina 2008). However it has been emphasized (Moore et al. 1991) that pollen morphological characters of the family are variable, even among several populations of the same species. Regarding *Pyrus*, there are only a few works dealing with pollen morphology of *Pyrus* (Westwood and Challice 1978; Fang and Yi-Xuan 1990). For this reason and also lacking any study in Iran, this survey aims to: (1) present detailed quantative and qualitative data on pollen morphology of the genus and (2) evaluate taxonomical value of these data.

Materials and Methods

Pollen of 16 populations belonging to nine species of *Pyrus* was studied by light microscope (LM) and scanning electron microscope (SEM). These species include 1- *P. boissieriana* Buhse, 2- *P. longipedicellata* Zamani and Attar, 3- *P. pashia* Hamilton ex D. Don. from sect. *Pashia*, 4- *P. hyrcana* Fed., 5- *P. grossheimii* Fed., 6- *P. farsistanica* Browicz from sect. *Pyrus* , 7- *P. mazanderanica* Schönbeck-Temesy, 8- *P. syriaca* Boiss. from sect. *Xeropyrenia* and 9- *P. salicifolia* Pall. from sect. *Argyromalon*. Voucher specimens were deposited in TUH (acronym according to Holmgren et al. 1990) and Herbarium of research institute of forests and rangelands of Orumieh. Pollen obtained from flower buds at anthesis was prepared for light microscope (LM) using methods described in Harley (1992) with some modifications and mounted in glycerol jelly on glass slides and sealed. Because the surface of the pollen grains was not adequately cleaned which made an exact study of sculpturing patterns difficult, the time of acetolysis was set at 7–8 min. For LM measurements, at least

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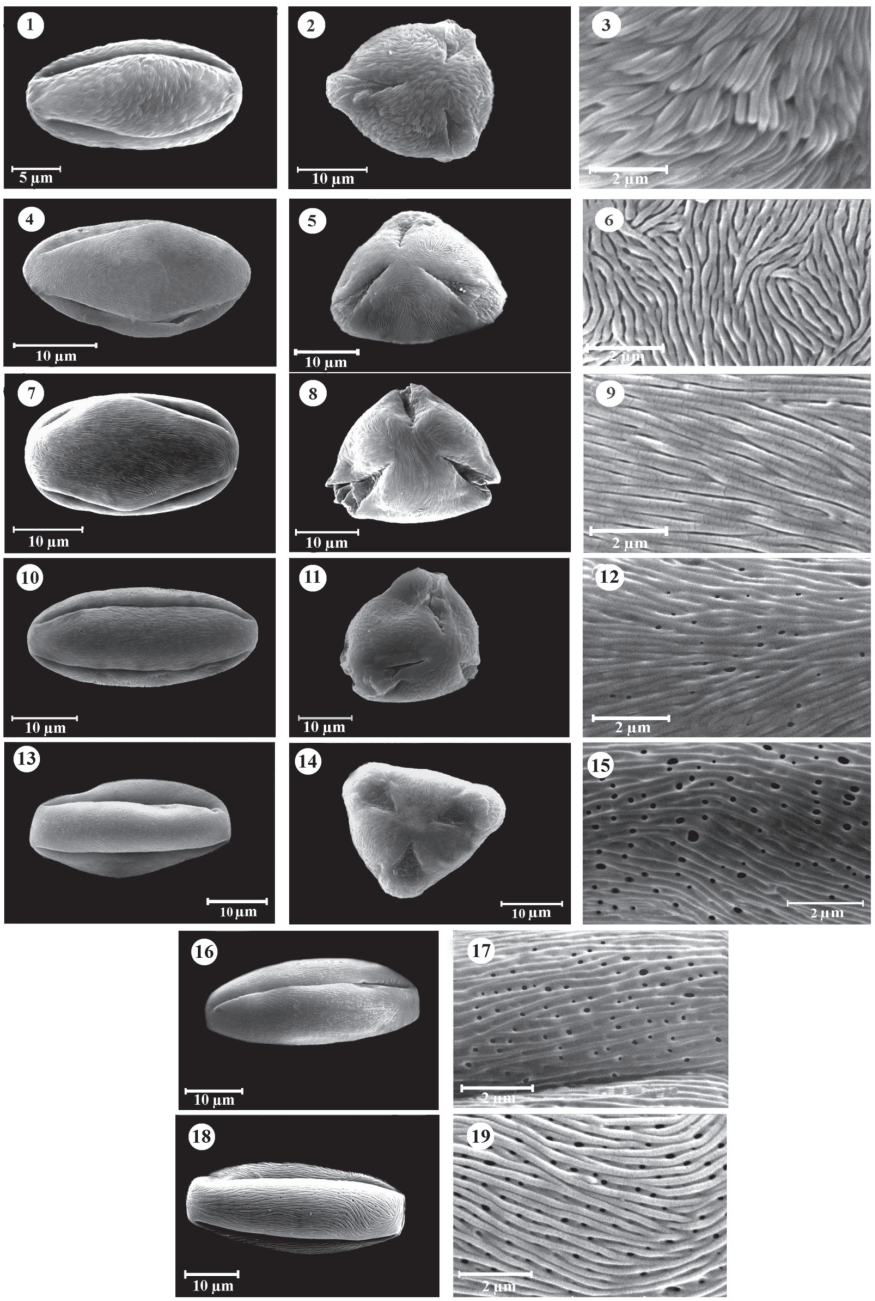
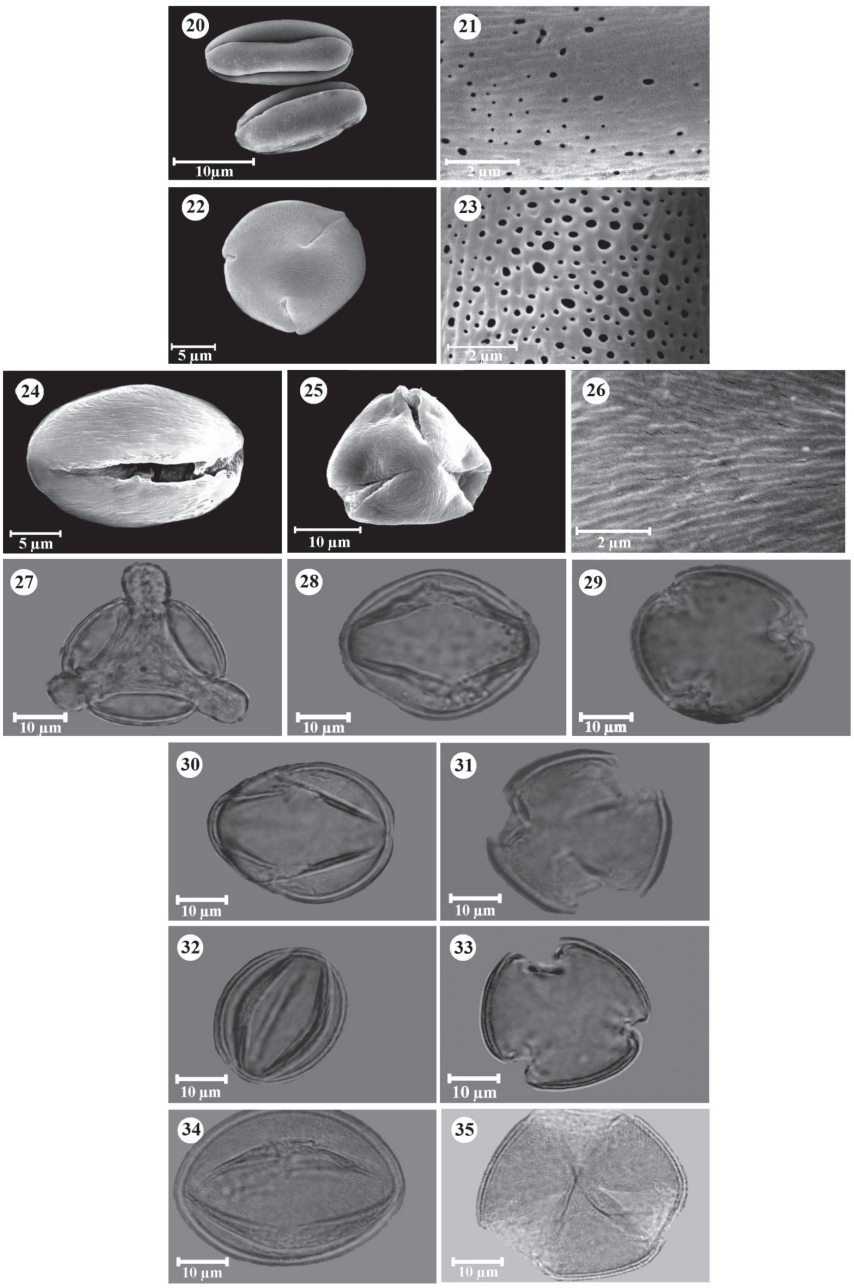


Figures 1-19. SEM micrographs of pollen grains in *Pyrus* spp. Type I: (1-3) *P. syriaca* 37098, (4-6) *P. grossheimii*, (7-9) *P. hyrcana* 38144, Type II: (10-12) *P. syriaca* 37106, (13-15) *P. pashia*, (16-17) *P. salicifolia*, (18-19) *P. farsistanica*.

20–25 pollen grains were measured by Nikon light microscope model 200 M with aid of a ×100 eyepiece. For SEM, after acetolysis, the specimens were mounted on 12.5mm diameter aluminum stubs and then coated in a sputter coater with approximately 25 nm of gold-palladium. The specimens were examined and photographed with Philips scanning electron microscope model XL30 at an accelerating voltage of 20 kV. The terminology follows mainly that of Ertdman (1952) and Halbritter et al. (2007). For detailed examination of sculpturing, we used the classification presented in Ueda and Tomita (1989). For estimation of pollen fertility, the pollen from fresh collected herbarium material was stained by acetocarmin in glycerine jelly, as described by Radford et al. (1974). In total, 11 populations belonging to nine species were analyzed.



Figures 20-35. SEM and LM micrographs of pollen grains in *Pyrus* spp. Type III: (20-21) *P. mazanderanica*, (22- 23) *P. boissieriana*, Type IV (24-26) *P. longipedicellata*, (27-29) *P. hyrcana*, (30- 31) *P.longipedicellata*, (32-33) *P.salicifolia*, (34-35) *P.farsistanica.*

Results and Discussion

The main features of the investigated pollen grains are summarized in Table 1. Selected SEM and LM micrographs of examined pollen grains are presented in Figures 1-35.

General pollen morphological features

Main pollen characters vary even in different populations of the same species (see Figs. 1-3, 10-12, Table 1). Pollen grains are shed as monad, medium-sized (P= 30.62-34.05 µm), trizonocolporate, isopolar. Regarding shape of pollen, equatorial view includes prolate- spheroidal (Figs. 7, 24), subprolate (Figs. 1, 4) to prolate (Figs. 10, 13, 16, 18) shapes, while polar view includes triangular (Figs. 5, 8, 25) and trilobate shapes (Figs. 2, 11). Ectocolpi converge close to the polar ends, arranged meridionally (Figs. 1, 4, 7, 10) or parallel (Fig. 13, 18). In the middle of ectocolpi, there is endopore that consists of distinct (Figs. 2, 11, 27, 28) or indistinct (Fig. 5) projec-

Table 1. Details of examined characters of *Pyrus* species in this study.

Species	P	E	P/E	C	C/P	M	A	ET	ECTE	ENDE
P. Sect. *Argyromalon*										
P. salicifolia	28.00 (31.45± 1.93) 36.00	26.00 (27.35± 1.37) 31.00	1.15	24.00 (28.75± 3.04) 36.00	0.91	19.00 (22.05± 1.50) 25.00	6.00 (8.05± 1.76) 12.00	1.00 (1.46± 0.36) 2.00	0.77 (1.06± 0.11) 1.25	0.73 (0.96± 0.13) 1.29
P. Sect. *Pashia*										
P. boissieriana	29.00 (33.10± 2.24) 38.00	24.00 (27.55± 1.4) 30.00	1.20	24.00 (28.25± 2.71) 34.00	0.85	14.00 (19.95± 2.60) 25.00	5.00 (6.30± 1.13) 8.00	1.00(1.21± 0.27) 2.00	0.58 (0.82± 0.13) 1.02	0.66 (0.90± 0.14) 1.18
P. longipedicellata	25.00 (30.62± 2.27) 34.00	18.00 (27.34± 3.76) 32.00	1.12	16.00 (26.85± 3.62) 31.00	0.88	11.00 (17.90± 3.71) 23.00	4.00 (5.67± 1.85) 11.00	1.00 (1.63± 0.51) 3.00	0.74 (0.86± 0.067) 1.02	0.78 (1.04± 0.14) 1.30
P. pashia	26.00 (31.23± 2.05) 34.00	20.00 (28.35±2.67) 32.00	1.10	22.00 (28.26± 2.56) 33.00	0.90	17.00 (21.13± 2.45) 25.00	4.00 (6.73± 1.24) 10.00	1.00 (1.34± 0.45) 2.00	0.76 (0.85±0.10) 1.20	0.80 (1.10±0.15) 1.35
*P.*Sect. *Pyrus*										
P. farsistanica	30.00 (34.05± 2.84) 40.00	26.00 (30.50± 2.76) 36.00	1.14	24.00 (29.65± 3.01) 35.00	0.87	16.00 (21.50± 3.15) 27.00	4.00 (5.90 ± 1.52) 9.00	1.00 (1.48± 0.32) 2.00	0.75 (0.93± 0.12) 1.23	1.22 (1.38± 0.11) 1.58
P. grossheimii	29.00 (31.50± 1.82) 36.00	26.00 (27.88± 1.54) 32.00	1.13	24.00 (27.00± 1.72) 31.00	0.86	18.00 (21.20± 2.14) 27.0	5.00 (11.00± 3.80) 19.00	1.00 (1.53± 0.36) 2.00	0.76 (0.89± 0.10) 1.13	0.92 (1.01± 0.10) 1.30
P. hyrcana	30.50 (34.78± 2.93) 41.50	25.00 (30.62± 2.76) 35.50	1.15	26.50 (29.79± 2.40) 35.00	0.86	19.00 (22.86± 2.57) 27.50	4.50 (6.42± 1.28) 9.50	0.95 (1.39± 0.50) 2.50	0.84 (0.98± 0.11) 1.22	0.83 (1.05± 0.09) 1.22
P. sect. *Xeropyrenia*										
P. mazanderanica	26.50 (31.27± 2.26) 34.50	24.00 (28.00± 2.10) 31.50	1.13	22.00 (26.95± 2.33) 30.50	0.86	16.50 (20.85± 2.45) 26.50	4.50 (6.05± 1.28) 9.00	1.00 (1.50± 0.37) 2.10	0.80 (0.97± 0.11) 1.17	0.84 (1.09± 0.15) 1.35
P. syriaca	29.57 (33.51± 2.00) 37.43	23.57 (29.21± 2.66) 33.57	1.17	25.43 (29.02± 2.00) 33.00	0.87	14.86 (21.23± 3.15) 26.71	5.71 (7.18± 1.10) 9.57	0.97 (1.40± 0.32) 2.03	0.84 (1.03± 0.27) 1.26	0.72 (1.04± 0.12) 1.29

tion. Regarding exine, exine thickness (column ET in Table 1) ranges from thin (ET= 1.21-1.40 µm) to medium (ET= 1.46-1.63 µm), clearly consists of two layers (Figs. 27–35); ectexine and endexine. In most species, thickness of endexine is more than ectexine (column ECTE/ENDE<1), except in *P. salicifolia* (ECTE/ENDE= 1.11). On the basis of arrangement of ridges to the colpus, parallel (Figs. 4, 7, 10, 16, 18) and perpendicular (Fig. 1) patterns are recognized. Moreover according to arrangement of ridges to each other, intersecting (Figs. 3, 6) and semi-parallel (Figs. 9, 12, 15, 17, 19) patterns were recognized. The most variable character in this study belongs to sculpturing of the exine so that on the basis of the exine sculpturing in proximal face, striate ornamentation can be recognized; however on the basis of differences in surface structure such as diameter and slope of ridges, interval between ridges, presence, number and size of perforations four main types can be recognized as following:

Type I

This type is recognized by having merely deep and fingerprint like ridges and lacking any perforations on the surface of the exine (Figs. 3, 6, 9). Thickness and slope of ridge in this type is clearly more than other types (Figs. 3, 6, 9; see also column TR in Table 1). This type can also be subdivided according to interval between ridges; type I-A and type I-B with short (*P. syriaca*, Fig. 1) and long (*P. grossheimii* and *P. hyrcana*, Figs. 6, 9) intervals, respectively.

Type II

This type is recognized by having perforations between ridges (Figs.12, 15, 17, 19). This type is subdivided according to density and diameter of perforations; type II-A with sparse and small (*P. syriaca*; Fig. 12) and II-B with dense and large perforations (*P. salicifolia*, *P. farsistanica*, *P. pashia*; Figs. 15, 17, 19).

Table 1. continued.

Species	ECTE/ ENDE	FP	PN	DP	DR	TR	S	Sc	RP
P. Sect. *Argyromalon*									
P. salicifolia	1.11	%96.8	0.00(2.33± 1.80) 5.00	0.089(0.13± 0.037) 0.26	0.12(0.20± 0.042) 0.28	0.13(0.17± 0.021) 0.21	Subprolate, Triangular-Circular	Type II-B	Parallel
P. Sect. *Pashia*									
P. boissieriana	0.91	%97.1	2.00 (4.10± 1.02) 5.00	0.090(0.22± 0.093) 0.45	-	-	Subprolate, Trilobate- Circular	Type III	-
P. longipedicellata	0.83	%96.34	-	-	0.085(0.12± 0.017) 0.15	0.090(0.13± 0.030) 0.20	Prolate-Spheroidal, Triangular	Type IV	Parallel
P. pashia	0.77	%94.3	1.20 (1.98± 1.73) 4.50	0.10 (0.17± 0.045) 0.36	0.092 (0.15± 0.018) 0.16	0.097 (0.14± 0.026) 0.28	Prolate-Spheroidal, Triangular	Type III	Parallel
*P.*Sect. *Pyrus*									
P. farsistanica	0.68	%88.5	1.00 (2.95± 1.07) 5.00	0.13(0.20± 0.053) 0.35	0.092(0.13± 0.025) 0.20	0.14(0.20± 0.028) 0.24	Prolate-Spheroidal	Type II -B	Parallel
P. grossheimii	0.88	%52	-	-	0.076(0.097± 0.017) 0.14	0.18(0.24± 0.045) 0.34	Prolate-Spheroidal, Triangular	Type I-B	Intersecting
P. hyrcana	0.93	%97.57	-	-	0.060(0.087± 0.014) 0.12	0.19(0.23± 0.032) 0.33	Subprolate, Triangular-Circular	Type I -B	Parallel
P. sect. *Xeropyrenia*									
P. mazanderanica	0.89	%90.39	0.00(1.60± 1.34) 4.50	0.10(0.18± 0.071) 0.35	0.090(0.17± 0.070) 0.36	0.16(0.21± 0.047) 0.35	Prolate-Spheroidal, Circular	Type III	Parallel
P. syriaca	0.99	%92.79	0.00(0.75± 0.96) 3.00	0.07(0.11± 0.043) 0.25	0.082(0.11± 0.019) 0.15	0.14(0.21± 0.033) 0.27	Subprolate, Trilobate	Type I-A, II	Parallel, intersecting

Abbreviations: P, polar axis length; E, equatorial axis length; P/E, ratio of polar axis to equatorial axis; C, colpus length; C/P, ratio of colpus length to polar axis length; M, mesocolpium length; A, apocolpium length; ET, exine thickness; ECTE, ectexine thickness; ENDE, endexine thickness; ECTE/ENDE, ratio of ectexine thickness to endexine thickness; PN, perforations number in one square of micrometer; FP, fertility percentage; S, shape of pollen; Sc, pattern of sculpturing of pollen; RP, ridge arrangement's pattern; DP, diameter of perforation; DR, distance between ridges; TR, thickness of ridge; -, there is not the mentioned character; ?, is not observed the mentioned character.

Type III

This type is recognized by having dense (PN ranges from 1.92 to 4.10, Table 1) and large perforations (mean of perforations equals 0.20 µm) and also with obscure ridges (*P. boissieriana*, *P. mazanderanica*; Figs. 21, 23).

Type IV

This type is recognized by having obscure ridges due to moderate slope of ridges and lacking of any perforations (*P. longipedicellata*, Fig. 26).

The delimitation of the genus *Pyrus* L. in subgeneric and species level is difficult (Browicz 1993), so that the number of species has been increased to more than 80 (Browicz 1993). In the other hand, several authors have divided the genus to different subgeneric levels (race, section, subsection) (Browicz 1993). The main cause of this problem is the ease of hybridization among different species and different individuals of the same species (Rubtsov 1944; Browicz 1993), however some other reasons such as distributional differences among several individuals of the same species play role in this problem. Moore et al. (1991) have emphasized that Pollen morphology in taxa of Rosaceae is very variable, even among the populations within the same species that is related it 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; specifically in *P. syriaca* (Figs. 1-3, 10-12). On the basis of Fang and Yi-Xuan (1990), exine sculpturing provides useful index for identifying different species of the genus. According to Flora Iranica (Schönbeck-Temesy 1969), the genus includes four sections, *Xeropyrenia*, *Pyrus*, *Pashia*

and *Argyromalon.* In spite of morphologically similarities of the members of these sections (Schönbeck-Temesy 1969), in most cases any remarkable resemblance is not observed among species of these sections (Table 1). As it is clear in Table 1, percentage of fertility in all species is more than 90%, except *P. grossheimii*. In conclusion, pollen morphological features of the genus *Pyrus* can be a descriptive and not diagnostic evidence of the species, specifically in the widespread species.

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