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TAXONOMIC IMPORTANCE OF POLLEN MICROMORPHOLOGY IN PRUNUS L. SUBGENUS CERASUS PERS. (ROSACEAE) FROM IRAN

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Prunus subgenus *Cerasus* belongs to the Rosaceae family with about 100 species in the world, of which 19 taxa are distributed in Iran. Pollen micromorphological traits of the Iranian taxa belonging to three sections, (*Microcerasus*, *Cerasus*, and *Mahaleb*) were examined using light and scanning electron microscopes. The hierarchical clustering method and principal component analysis of the pollen traits were performed using InfoStat. The results showed that in the subgenus *Cerasus* pollen grains are monad, radially symmetrical, isopolar, tricolpate to tricolporate, and medium-sized. Pollen shape is rectangular to cylindrical in the equatorial view and triangular to circular in the polar view. The exine ornamentation is striate with or without perforations. The studied pollen can be divided into two types and six subtypes. The rugulate exine ornamentation was only found in *P. pseudoprostrata*. Ward's hierarchical clustering method and principal component analysis results did not support the existing sectional delimitation of taxa. However, a high variation in pollen grain characteristics could be observed in taxa within the subgenus *Cerasus*, especially in dwarf cherries (sect. *Microcerasus*). These differences are remarkable among closely related species, such as *P. microcarpa* and its allies. Finally, an identification key to the species is provided.

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Keywords: Rosaceae; *Cerasus*; pollen; statistical analyses; exine ornamentation; Iran

اهمیت آرایه‌شناسی ریزریخت‌شناسی دانه گرده در زیرسرده Prunus L. subgen. Cerasus Pers. (تیره گل سرخیان) **از ایران**

میثم حبیبی: فارغ التحصیل دکتری سیستماتیک گیاهی، دانشگاه تهران، تهران و مربی گروه زیست شناسی دانشگاه گنبد کاووس، گنبد

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زیرسرده‌ی Cerasus متعلق به سرده‌ی Prunus و تیره گل‌سرخیان (Rosaceae) است و شامل ۱۰۰ آرایه در جهان می‌باشد که از این میان ۱۹ آرایه آن در ایران پراکنش دارد. صفات ریزریخت‌شناسی دانه گرده آرایه‌های ایرانی زیرسرده Cerasus متعلق به سه بخش Microcerasus، Cerasus و Mahaleb با استفاده از میکروسکوپ نوری و میکروسکوپ الکترونی نگاره برای اولین بار مطالعه شد. تحلیل داده‌ها با استفاده از نرم‌افزار InfoStat انجام شد. نتایج نشان دادند که دانه‌های گرده Cerasus، موناد، متقارن شعاعی، جورقطب، سه‌شیاری یا سه‌شیار منفذی و دارای اندازه متوسط هستند. شکل دانه گرده در نمای استوایی چهارگوش تا استوانه‌ای و در نمای قطبی سه‌گوش تا مدور است. تزئینات اگزین مخطط،

دارای منفذ یا بدون منفذ بودند که از این جهت می‌توان آنها را به دو گروه اصلی و شش زیرگروه تقسیم کرد. تزئینات rugulate فقط در گونه P. pseudoprostrata مشاهده شد. روش خوشه‌بندی سلسله‌مراتبی Ward و تجزیه به مولفه‌های اصلی (PCA) در اغلب صفات از تعیین حدود بخش‌ها حمایت نکرد. با این‌حال، تنوع زیادی در صفات دانه گرده درون زیرسرده آلبالو، به‌ویژه در آلبالوهای کوتاه قد (بخش Microcerasus) مشاهده شد. این تفاوت‌ها به‌ویژه در برخی آرایه‌ها با روابط خیلی نزدیک همچون P. microcarpa و خویشاوندانش قابل توجه بود. در نهایت، یک کلید تشخیصی برای گونه‌ها تهیه شد.

INTRODUCTION

Prunus L. (Rosaceae, subfamily Spiraeoideae formerly Amygdaloideae) consists of ca. 250- 400 species of deciduous and evergreen trees and shrubs widely distributed in the temperate, subtropics and tropics of both the New and Old Worlds (Shi & al. 2013). The genus is divided into five subgenera in modern plum taxonomy, *Prunus*, *Amygdalus* (L.) Focke., *Cerasus* Pers., *Padus* (Moench) Koehne, and *Laurocerasus* Koehne (Rehder 1940; Morway and Werner 1990). However, several other classifications segregated the complex group into multiple genera (e.g., Yu & al. 1986; Lu & al. 2003). The subgenus Cerasus was known based on several taxonomic traits, including the presence of 1 or 3 axillary winter buds, simple, alternate, or fascicled leaves on short branchlets, axillary inflorescences, fasciculate or corymbose inflorescence with 1- or 2- flowers, superior ovary, solitary carpel with two pendulous anatropous ovules, drupe fruit with fleshy mesocarp without a longitudinal groove or splitting when ripe (Pojarkova 1941; Browicz 1969). The subgenus Cerasus includes about 100 species of trees or shrubs which are mainly distributed in the temperate regions of the Northern Hemisphere, including Asia, Europe, and North America (Looney & Jackson 1999; Chaoluan & al. 2003). Several *Cerasus* species are cultivated for their edible fruits and ornamental purposes (Browicz & Zielinski 1982; Dehshiri & al. 2012). Subgenus *Cerasus* is divided into four sections: sect. *Microcerasus* Spach, sect. *Cerasus*, sect. *Mahaleb* Roemer. and sect. *Pseudocerasus* (Ersicli 2004). In Flora Iranica (Browicz 1969) and Flora of Iran (Khatamsaz 1992), 10-11 species, three subspecies, and two varieties were reported in the subgenus in Iran; which was classified into three sections (*Microcerasus*, *Cerasus*, and *Mahaleb*). In recent years, some new species have been described and a few records have been added to the existing numbers of species as follows: two new records *C. araxina* Pojark. and *P. jacquemontii* Hook.f., and three new species, *P. mazandaranica* Habibi, Maleki & Attar., *Prunus yazdiana* (Mozaff.) Falat, and *P. paradoxa* (Dehshiri & Mozaff.) Falat. have been described from Iran (Mozaffarian 2002; Dehshiri & al. 2012; Habibi & al. 2022). The total number of the species and the subspecific taxa now stands at 14 and 19, respectively. The wild cherries inhabit the open habitats of forest margins and shrublands on rocky and stony slopes and are distributed across the southern slopes of the Alburz and Zagros Mountains in Iran (Browicz 1969; 1972; Pojarkova 1941; Nuri Nas & al. 2012).

Several studies have investigated the importance of pollen morphology in Rosaceae. For instance, Hebda & Chinnappa (1990, 1994) showed that pollen shape and size, exine ornamentations, aperture, and aperture zone structure are diagnostic features for several genera and species in the family. Furthermore, there are other studies on the pollen of Rosaceae (Reitsma 1967; Ueda and Tomita 1989; Arzani & al. 2005; Bednorz & al. 2005; Wronska-Pilarek and Boratynska 2005; Wronska-Pilarek and Lira 2006; Vafadar & al. 2010). Similarly, various authors, such as Moore & Webb (1978), Richard (1970), Ramos-Zamora & al. (1987), and Moore & al. (1991) studied *Prunus* pollen grains using light microscopy (LM). Also, Reitsma (1966) reported pollen of some West European species and provided images and illustrations based on LM observations. Hebda & al. (1991), Kang & al. (1997), Zhou & al. (1999), and Li & al. 2021 observed the pollen morphology of *Prunus* from Canada and China using both LM and scanning electron microscopy (SEM), respectively. Kang & al. (1997) and Zhou & al. (1999) observed the pollen morphology of *Prunus* from China using SEM. Eide (1981) examined the pollen of some *Prunus* species from northwest Europe with LM and SEM. These studies suggested that pollen grains of *Prunus* are isopolar, monads, radially symmetrical usually tricolporate, and mostly subspheroidal to prolate; their exine is tectate and perforate; the surface sculpturing is striate to rugulate with muri that are predominantly parallel to apertures, and the polar axis ranges 20–44.12 µm, and equatorial axis ranges 17.85–36.95 µm. These studies showed that several pollen-related traits in *Prunus* including pollen shape, size, exine sculpturing, and aperture features might have taxonomic value. Hitherto, there are only a few studies dealing with the pollen morphology of *P*. subgenus *Cerasus.* The objectives of the present study are: (1) to present detailed quantitative and qualitative data on the pollen of the subgenus *Cerasus*, (2) to evaluate the taxonomical value of these data among the species, and (3) to provide a diagnostic key to the species based on these data.

MATERIALS AND METHODS

Plant materials

Pollen grains of 12 species, three subspecies, and one variety from three different sections of the *P.* subgen. *Cerasus* from Iran were studied using LM and SEM. Pollen grains were collected from herbarium specimens deposited in the Central Herbarium of the University of Tehran (TUH) and TARI (Table 1).

Microscopic observation (LM & SEM)

To conduct light microscopy measurements, pollen samples were acetolysed following the method described in Erdtman (1952). The pollen grains were mounted in glycerin jelly on glass slides after acetolysis and were studied with an Olympus microscope (model BX-50) and photographed with a Nikon camera model 200 M. All measurements were performed on about 20 pollen grains for each taxon. The polar axis (P), equatorial diameter (E), apocolpium index (AP), and mesocolpium length (ML) were observed under the light microscope (Table 2). For scanning electron microscopy, specimens were mounted on 12.5 mm diameter stubs after acetolysis and then coated with 25 nm of gold-palladium in a sputtering chamber (BAL-TEC, SCDOOS) at an accelerating voltage of 10-15kv. The specimens were examined and photographed with SEM (model-JSM-6380A, JEOL). All measurements of the other quantitative pollen features, including the width of the ridge (WR), the thickness of ridges (TR), the width of valleys (WV), the diameter of perforations (DP), and the number of perforations on an area of 25 μm^2 were made using AxioVs40 V 4.8.2.0. software. Various terminologies including exine sculpturing patterns (exine morphology) and the shape of pollen were adopted from published taxonomic literature for the pollen grains Erdtman (1952), Walker and Doyle (1975) Punt & al. (1999, 2007), Reitsma (1966), Ueda and Tomita (1989), Hebda & Chinnapa (1990), Zhou & al. (1999), Halbritter & al. (2007) and Hesse & al. (2009).

Statistical analyses

The correlation between quantitative characters was tested using Pearson's coefficient (Sokal and Rohlf 1995). Principal component analysis (PCA) was performed using InfoStat ver. 2020 to determine whether the quantitative pollen-related traits are efficient to split the taxa into different groups. For these analyses, the arithmetic mean of 13 variables was used (Table 2). The first three principal components (PCs) with an eigenvalue larger than one were represented. In addition, a two-dimensional plot of the first and second principal components was presented. Similarly, Ward's hierarchical clustering method was used to compute the relationship among taxa using InfoStat.

RESULTS

Pollen size and shape

The grains of *Prunus* subgen. *Cerasus* species were 3-zonocolpate (Figs. 1E, F, S; 2H, K; 3H, K; 5D, G, H, Table 2) or 3-zonocolporate (Figs. 1A, C, G, K; 2A, D; 3A, D; 4A, D, G, J; 5A, J, K; Table 2). All pollen grains were medium (26.9-46 μm) according to Erdmann's (1952) pollen size classification. The average length of the polar axis (P) was 36.68, ranging from 26.91-46.04 μm for *P. cerasus* and *P. mazandaranica*, respectively. The majority of small pollen was observed in *P. cerasus* 26.91 (range 25-28 μm) and in *P. mahaleb* 28.10 (range 26-31 μm). On the other hand, the longest pollen grains (with polar axis ≥ 40 μm) were found in *P. mazandaranica* 46.04 (45-46) and *P. microcarpa* subsp. *diffusa* 2 44.85 (41-48.5) *P. turcomanica* 42.18 (40-45) and in *P. paradoxa* 41.02 (41-45), (Table 2).

Pollen apertures

Two major types of apertures were observed in pollen grains of the examined species including 3-zonocolpate (with three colpi) or 3-zonocolporate (with three ectocolpi and three endopori). The simple colpi or ectocolpi were distributed symmetrically, elongated, and narrowed toward the poles, with granular colpus membranes. The mean colpus length (CL) was 32.53, ranging from 23.43-41.12 in *P. cerasus* and *P. mazandaranica*, respectively. The CL strongly correlates with the length of the polar axis across all species (r=0.98, $P<0.01$). The colpus ends are mostly acute. On average, the length of the colpi included 87.6% of the polar axis (RCLP). The colpus membrane is different from the exine surface and is covered with irregularly shaped granules. Each endopore is placed in the middle part of the ectocolpus that consists of either distinct (Figs. 2A, D; 3A, D; 4 A, J; 5A, J) or indistinct (Figs. 4D, G) projections.

Table 1. Voucher specimens of Iranian species of *Prunus* subgenus *Cerasus* used in pollen grain study.

Species	**Collection Data**	**Collector(s)**
P. paradoxa (Dehshiri & Mozaff.) Falat.	Lorestan: ca. 60 km SE Aligudarz, Durak fountain, growing on the ticket place, 9570-TARI	Dehshiri & Ahmadi
P. yazdiana (Mozaff.) Falat.	Yazd: Mehriz, Dumgahan valley, 97467-TARI	Mirvakili &
P. incana Pall.	Azerbaijan: ca 18 km after Ahar to Tabriz, 42354-TUH	Attar, Hamzee & Zamani
P. turcomanica Pojark.	Northern Khorasan: Bojnourd, Raz road, 2 km after Tazeh-Ghaleh to Gifan, 48294-TUH	Attar, Mehdigholi, Habibi & Bayat
P. chorassanica Pojark.	Golestan: Darkesh, ca. 5 km to Haver, 48305-TUH	Attar, Mehdigholi,
P. pseudoprostrata Pojark. 1	Mazandaran: ca, 70 km to Tehran from Gazanak, 48309-TUH	Attar, Mehdigholi, Habibi & Bayat
P. brachypetala var. brachypetala Boiss.	Lorestan: Khoram-Abad, Sefid-Kuh, 24166-TUH	Veiskarami
P. brachypetala (Boiss.) Walp var. *Bornmulleri* (C. K Schneider) Hand. -Mzt.	Kurdistan: North of Mirabad, 37772-TUH	Attar & Zamani
P. microcarpa C. A. Mey. ssp. *tortuosa*	Kurdistan: Secondary road of Sanandaj to Marivan, Ariz pass, 37767-TUH	Zamani & Maleki
P. microcarpa C. A. Mey. ssp. *diffusa* 1	Fars: ca 35 km to Marvdasht from Saadat-Shahr, 48071-TUH	Attar & Habibi
P. microcarpa C. A. Mey. ssp. *diffusa* 2	Fars: Sarvestan, Tangeh-Ghanimeh, 48074-TUH	Attar & Habibi
P. microcarpa C. A. Mey ssp. *microcarpa* 1	Kurdistan: Saqez to Baneh, road of Mirdeh village to Pir-Omran village, 37749-TUH	Attar & Zamani
P. microcarpa C. A. Mey ssp. *microcarpa* 2	Northern Khorassan: Bojnourd to Shoqan, after Hessar-Hosseini, 38059-TUH	Attar, Zamani & Maleki
P. microcarpa C. A. Mey ssp. *microcarpa* 3	Fars: Margoon Waterfall, 48104- TUH	Attar & Habibi
P. mazanderanica Habibi, Maleki & Attar	Alborz: Karaj-Chalous road, ca 50 km to Chalous, 37189-TUH	Attar, Maleki & Zamani
P. jacquemontii Hook. f.	Northern Khorasan, Darkesh, ca 5 km to Haver, 48307-TUH	Attar, Mehdigholi, Habibi & Bayat
P. cerasus L.	Gilan: Talish, ca 2 km to Birin village from Dizagh village, 43481-TUH	Attar, Zamani & Sotoudeh
P. mahaleb L.	Kurdistan: ca. 65 km after Baneh to Marivan vicinity of Gug-Jeh village, 37743-TUH	Attar, Maroofi & Zamani

Sculpturing pattern

The pollen grains mainly exhibited striate exine ornamentation in all species (Figs. 2C, F, I, L; 3F, I, L; 4C, F, I, L; 5C, F, I, L; Table 2), except in *P. pseudoprostrata*; in which, rugulate exine ornamentation is expressed (Fig. 3C; Table 2). The species studied were classified by Ueda and Tomita (1989) and Hebda and Chinnappa (1990) into three types and six subtypes of pattern sculpturing isolated for the subgenus *Cerasus*.

In Type I, striate exine ornamentation is expressed, without or with only a few perforations (≤10). Type I can be subdivided into three subtypes (A, B, C). In type I-A the striae run parallel to the colpus which continues over the pole, and in type, I-B ridges do not extend the whole length of the grain and loop or turn to the perpendicular intersection with the colpus and frequently they formed fingerprint-like twists usually in the subpolar zone. In Type I-C ridges are short, weaving, and crossing. Ridges usually extend less than 5µm before ending or changing direction. Their orientation is neither preferentially perpendicular nor parallel to the colpus. In Iranian *Cerasus* species type I-A is observed in *P. microcarpa* subsp. *tortuosa* (Figs. 5B-5C). Type I-B in *P. yazdiana*, *P. microcarpa* subsp. *microcarpa* 1-3, *P. mahaleb* (Figs. 2F; 4I, L, F), and Type I-C in *P. jacquemontii*, *P. cerasus* (Figs. 2C, 5 L).

Table 2. An overview of pollen grain morphological features of the studied species of *Cerasus*. Numbers refer to mean value, ± SE, and minimum and maximum. P: length of polar axis; E: length of equatorial diameter; P/E: ratio of the polar axis to equatorial diameter; Shape: the shape of pollen grain; EXS: exine sculpture; ApT: aperture type; Ap- length of apocolpium; CL: colpus length; RCLP: ratio of colpus length to the polar axis; ML: mesocolpium length; DP: diameter of perforations; NP: number of perforations; NR: number of ridges; WR: width of ridges; TR: the thickness of ridges; WV-width of valleys.

Species	P	E	P/E	Shape	EXS	ApT	Ap	CL	RCLP	ML
Sect. *Microcerasus*										
P. paradoxa	41.015 ± 1.64 (41- 45)	23.4 ± 1.75 (23- 28)	1.76	prolate	striate-Type II-B	trizonocolpate	8.35±0.8 (7 - 9)	36.32 ± 2.6(29 - 41)	0.88	14.36 ± 2.75 (13-19)
P. yazdiana	34.25 ± 1.7 (24 - 40)	16.77 ± 1.15 (11 - 22)	2.04	perprolate	striate-Type I-B	trizonocolpate	8.47±1.1 (5.50 - 11.50)	31.51 ± 2.85(35-26)	0.92	11.67 ± 1.9 (9.50-14)
P. incana	31.93 ± 2.05 (29 - 35)	22.82 ± 2.65 (18 - 29)	1.41	prolate	striate- Type II-B	trizonocolporate	8.74±2.6(5 - 14)	27.73 ± 3.15(22-33.50)	0.86	14.80 ± 2.8(6 - 20)
P. turcomanica	42.18 ± 1.32 (40-45)	24.57 ± 1.10 (23 - 26)	1.71	prolate	striate-Type II-C	trizonocolpate	7.13±1.72 (5 - 11)	38.73 ± 1.62 (34-41.50)	0.91	15.71 ± 0.45 (15-16)
P. chorassanica	42.68 ± 2.62 (31 - 47)	21.19 ± 1.15 (18 - 23.50)	2.01	perprolate	striate-Type II-B	trizonocolpate	9.28±1.65 (6.50 - 12)	36.62 ± 2.05 (33-42)	0.85	15.11 ± 2.32 (11-18)
P. pseudoprostrata	30.87 ± 1.42 (28 - 34)	20.73 ± 2.26 (17 - 31)	1.50	prolate	regulate-Type III	trizonocolporate	6.80± 1.36 (5 - 10)	26.10 ± 2.05 (23-28)	0.84	11.89 ± 0.80 (11-14)
P. brachypetala var. *brachypetala*	36.45 ± 2.60 (28 - 44)	17.8 ± 1.82 (14.50-19)	2.06	perprolate	striate-Type II-A	trizonocolpate	15.21± 1.12 (13 - 16)	31.55 ± 2.35 (23-35)	0.86	18.9 ± 1.05 (17-20)
P. brachypetala var. *Bornmullerii*	41.01 ±1.76 (38 - 46)	21.56 ± 1.40 (20-23.5)	1.9	prolate	striate-Type II-B	trizonocolpate	9.18± 1.22 (7 - 12)	35.29 ± 2.35 (31-39)	0.86	14.46 ± 1.36 (13-17)
P. microcarpa subsp. *tortuosa*	39.51 ±2.58(35 - 43.50)	22 ± 1.75 (20 - 25)	1.78	prolate	striate-Type I-A	trizonocolporate	8.74± 1.63 (5.50-10.50)	36.27 ± 2.43 (30-39)	0.91	16.42 ± 1.20 (15-17)
P. microcarpa subsp. *diffusa* 1	32.95 ± 1.72 (31 - 35)	21 ± 1.80 (19 - 25)	1.56	prolate	striate-Type II-B	trizonocolporate	6.27± 1.17 (5.50 - 8)	29.58 ± 2.40 (27-32)	0.89	15.05 ± 1.10 (14-16)
P. microcarpa subsp. *diffusa* 2	44.85 ±2.15(41 - 48.50)	23.65 ± 1.42 (22 - 25)	1.89	prolate	striate-Type II-C	trizonocolporate	9.34± 0.85 (7 - 10.50)	39.76 ± 2.65 (35-44)	0.88	14.33 ± 1.42 (12-17)
P. microcarpa subsp. *microcarpa* 1	40.91 ±1.52 (36 - 43.50)	23.03 ± 1.25 (20 - 25)	1.77	prolate	striate-Type I-B	trizonocolporate	7.40± 0.60 (6 - 9)	37.65 ± 1.25 (36-38)	0.92	15.64 ± 0.45 (15-16)
P. microcarpa subsp. *microcarpa* 2	33.75 ±1.86 (31 - 36)	24.65 ± 1.35 (22 - 27)	1.36	prolate	striate-Type I-B	trizonocolporate	9.36± 0.30 (9 - 10.50)	28.36 ± 2.65 (26-31)	0.84	16.94 ± 1.60 (15-19)
P. microcarpa subsp. *microcarpa* 3	38.76 ±1.73 (36 - 41)	22.92 ± 1.20 (21 - 25)	1.68	prolate	striate-Type I-B	trizonocolporate	7.80± 1.10 (9 - 10.50)	33.76 ± 1.60 (31-36)	0.87	14.78 ± 1.55 (13-17)
P. jacquemontii	32.50 ±1.70 (29 - 35.50)	27.53 ± 1.55 (25 - 30)	1.11	prolate spheroidal	striate-Type I-C	trizonocolporate	9.20± 0.45 (8 - 10.50)	27.03 ± 1.25 (24-31.50)	0.83	19.33 ± 0.45 (17-21)
P. mazandaranica	46.04 ±1 (45 - 46)	22.86 ± 1.65 (21 - 24)	2.04	perprolate	striate-Type II-C	trizonocolpate	8.84± 1.15 (7 - 12)	41.12 ± 1.85 (40-44)	0.89	15.4 ± 0.40 (15-16)
Sect. *Cerasus*										
P. cerasus	26.91 ±1.25 (25- 28)	17.28 ± 1.75 (16 - 18)	1.55	prolate	striate-Type I-C	trizonocolporate	4.6± 1.40 (3 - 7)	23.43 ± 1.85 (21-26)	0.87	9.30 ± 1.46 (7.50-11)
Sect. *Mahaleb*										
P. mahaleb	28.10 ±1.78 (26 - 31)	25.4 ± 1.20 (24 - 27)	1.04	prolate spheroidal	Type I-B	trizonocolporate	7.05± 1.48 (5 - 8)	24.61 ± 1.6 (23-27)	0.87	19.84 ± 1.35 (18-21)

Table 2. continued.

Species	DP	NP	NR	WR	TR	WV
Sect. *Microcerasus*						
P. paradoxa	0.44 ± 0.09 (0.24-0.79)	22 (18-25)	8.80 (8 - 9)	0.22±0.1 (0.15 - 0.35)	0.29±0.06 (0.23 - 0.36)	0.25±0.07 (0.16 - 0.34)
P. yazdiana	0.15 ± 0.08 (0.14-.18)	2 (0 - 3)	13.57 (11 - 16)	0.29±0.1 (0.26 - 0.33)	0.12±0.02 (0.10 - 0.14)	0.08±0.04 (0.04 - 0.14)
P. incana	0.22 ± 0.08 (0.07-0.49)	25.2 (22 - 30)	10.75 (9 - 12)	0.33±0.3 (0.23 - 0.47)	0.27±0.05 (0.18 - 0.39)	0.15±0.02 (0.15 - 0.21)
P. turcomanica	0.20 ± 0.1 (0.07-0.55)	35.28 (32 - 38)	11.11 (8 - 13)	0.19±0.4 (0.15 - 0.26	0.15±0.35 (0.12 - 0.19)	0.09±0.04 (0.05 - 0.15)
P. chorassanica	0.22 ± 0.11 (0.06-0.42)	26 (8 - 45)	14 (13-15)	0.21±0.08 (0.13 - 0.30)	0.28±0.04 (0.21 - 0.33)	0.28±0.03 (0.25 - 0.32)
P. pseudoprostrata	0.14 ± 0.07 (0.07-0.27)	9.80 (6 - 15)	?	?	?	?
P. brachypetala var. *brachypetala*	0.19 ± 0.04 (0.14-0.29)	37 (30 - 52)	12.45 (11 - 14)	0.36±0.06 (0.30 - 0.43)	0.41±0.11 (0.29 - 0.58)	0.28±0.07 (0.19 - 0. 40)
P. brachypetala var. *Bornmulerii*	0.17 ± 0.06 (0.07-0.32)	52.80 (44 - 62)	7.58 (6 - 9)	0.27±0.06 (0.21 - 0.35)	0.36±0.05 (0.28 - 0.46)	0.25±0.05 (0.15 - 0.33)
P. microcarpa subsp. *tortuosa*	0.17 ± 0.05 (0.12-0.22)	5.41 (0 - 12)	10 (9 - 12)	0.36±0.09 0.26 - (0.51)	0.43±0.05 (0.41 - 0.69)	0.23±0.05 (0.16 - 0.29)
P. microcarpa subsp. *diffusa* 1	0.12 ± 0.04 (0.08-0.21)	14 (8 - 18)	9.66 (9 - 11)	0.30±0.04 (0.26 - 0.35)	0.30±0.03 (0.27 - 0.36)	0.10±0.02 (0.07 - 0.15)
P. microcarpa subsp. *diffusa* 2	0.31 ± 0.15 (0.14-0.66)	30 (20 - 35)	6.7 (5 - 9)	0.31±0.04 (0.27 - 0.36)	0.35±0.06 (0.29 - 0.42)	0.12±0.02 (0.10 - 0.14)
P. microcarpa subsp. *microcarpa* 1	0.19 ± 0.08 (0.12-0.32)	1.90 (0 - 5)	9.1 (8 - 10)	0.17±0.02 (0.15- 0.19)	0.23±0.01 (0.21 - 0.25)	0.21±0.04 (0.15 - 0.28)
P. microcarpa subsp. *microcarpa* 2	0.17 ± 0.06 (0.12-0.30)	1.75 (0 - 4)	9.27 (7 -11)	0.32±0.06 (0.24 - 0.39)	0.29±0.04 (0.22 - 0.36)	0.14±0.03 (0.10 - 0.18)
P. microcarpa subsp. *microcarpa* 3	0.17 ± 0.05 (0.11-0.33)	2.3 (0 - 6)	9.4 (8 - 12)	0.19±0.03 (0.16 - 0.24)	0.23±0.02 (0.17 - 0.25)	0.07±0.02 (0.06 - 0.10)
P. jacquemontii	0.23 ± 0.08 (0.15-0.33)	9.07 (4 - 15)	7.4 (6 - 9)	0.27±0.04 (0.23 - 0.33)	-	0.09±0.02 (0.07 - 0.12)
P. mazandaranica	0.23 ± 0.08 (0.11-0.55)	38 (32 - 45)	6.30 (5 - 7)	0.49±0.03 (0.45 - 0.55)	0.42±0.02 (0.40 - 0.50)	0.28±0.03 (0.24 - 0.36)
P. cerasus	-	-	17.44 (16 - 19)	0.13±0.02 (0.11 - 0.17)	0.24±0.03 (0.20 - 0.27)	021±0.02 (0.19 - 0.23)
P. mahaleb	-	-	13 (11 - 15)	0.29±0.04 (0.26 - 0.41)	0.27±0.04 0.22 - (0.34)	0.07±0.01 (0.06 - 0.09)

Fig. 1. A-U, LM micrographs of pollen grains in species of *Prunus* subgenus Cerasus. A-B, *P. cerasus*, A, polar view; B. equatorial view. C-D, P. mahaleb, C, polar view; D, equatorial view. E-F, *P. brachypetala* var. *brachypetala*, E, Polar view; F, equatorial view, G-H, *P. pseudoprostrata*. G, polar view; H. equatorial view. I-J, *P. microcarpa* subsp. *microcarpa2*, I, polar view; J, equatorial view. K-L, *P. microcarpa* subsp. *microcarpa3*, K, polar view; L. equatorial view. M-N, *P. microcarpa* subsp. *tortuosa*, M, polar view; N, equatorial view. O-P, *P. paradoxa*, O, Polar view; P, equatorial view. Q-R, *P. microcarpa* subsp. *diffusa*1, Q, polar view; R, equatorial view. S, *P. mazandaranica*, equatorial view. T, *P. jacquemontii*, polar view, U P. incana, polar view. (Scale bar= 5 µm for A, B, D, E, G, H, J, K; 1 µm for C, F, I, L). var. brachypetala, E, Polar view; F, equator.

Type II contains prominent perforations (14-58) between ridges; a character that is different from type I. Similar to Type I, three exine patterns are recognized within this type. In type II-A, ridges and valleys are usually parallel to the colpi to the polar axis. In type II-B, ridges formed fingerprint-like twists usually in the subpolar zone. In type II-C, ridges are short, weaving and crossing. Ridges usually extend less than 5µm before ending or changing direction. Their orientation is neither preferentially perpendicular nor parallel to the colpus. Type II-A is observed in *P. brachypetala* var. *brachypetala* (Figs. 2 H, I); type II-B in *P. brachypetala* var. *Bornmullerii*, *P. incana*, *P. chorassanica*, *P. paradoxa* (Figs. 2K, L; 3E, F, K, L; 5H, I), and type II-C in *P. turcomanica P. microcarpa* subsp. *diffusa* 1&2, and *P. mazandaranica* (Figs. 3H, 3I, 4B, 4C, 4D, 4F).

In Type III the largest width of ridges was observed in *P. mazandaranica* 0.49±0.03 (0.45-0.55 µm; in Table 2), whereas *P. cerasus* has the smallest width of ridges 0.13±0.02 (0.11-0.17; in Table 2). The ridge width significantly correlated with the thickness of ridges across all species (r= 0.67; *P*< 0.001). The mean thickness of ridges was 0.28 µm across all species and ranged from 0.12-0.43 µm in *P. yazdiana* and *P. microcarpa* subsp. *tortuosa*, respectively. The number of ridges was similar in all species (with an average of 6-13 per 25 µm^2), except in *P. cerasus* (with an average number of 17). Width of valleys varied from 0.07 µm in *P. microcarpa* subsp *microcarpa*-3 to 0.28 µm in *P. brachypetala* var. *brachypetala*, *P. chorassanica*, and *P. mazandaranica*.

Perforations were observed in all taxa except *P. carasus* and *P. mahaleb.* The distribution patterns of the perforations are divided into scarce or abundant. According to the mean number of perforations per 25 μm^2, the species can be divided into three distinct groups including no perforation (*P. carasus* and *P. mahaleb*), 0-10 perforations (*P. yazdiana*, *P. pseudoprostrata*, *P. microcarpa* subsp. *tortuosa*, *P. microcarpa* subsp. *microcarpa* 1-3, and *P. jacquemontii*), and 14-52 perforations (*P. brachypetala* var. *Bornmullerii P. microcarpa* subsp. *diffusa*, *P. brachypetala* var. *brachypetala*, *P. mazandaranica*, *P. incana*, *P. turcomanica*, *P. chorassanica*) (Table 2). The perforation diameter ranged from 0.12-0.44 µm in *P. microcarpa* subsp. diffusa (pollen with striate, type II-C) and *P. paradoxa,* respectively (Table 2).

Interspecific variation in pollen traits

Variation among taxa in the quantitative pollen data was explored using principal component analysis (Fig 6). The first two principal components explained 56.7% of the total variance of the analyzed data (Table 3). The PC1 accounted for 35.9% of the variance based on the values of the P, P/E, WV, WR, CL, NP, DP, and EXS (Table 3, Fig 6). The PC2 explains 20.8% of the data variability, NR, Ap, and E are the significant variables for the ordination of taxa (Table 3, Fig 6). The two-dimensional plot revealed clusters of taxa according to the mean number of perforations. Almost, all of the taxa with 14-52 perforations, are grouped on the positive and negative sides of the PC1 axis. The taxa without perforation, the OTUs from section *Mahaleb* (*P. mahaleb*), section *Cerasus* (*P. cerasus*), and some taxa of section *Microcerasus* (*P. microcarpa* and *P. yazdiana*) with 0-10 perforation, are grouped on the positive and negative side of PC2. Also, *P. pseudoprostrata*, which has unique exine sculpturing (rugulate), is positioned on the negative side of the PC 2 axis and more separated from the other taxa in the plot (Fig. 6). The cophenetic correlation obtained from cluster analysis using the ward's method of agglomeration based on Euclidean distance was 0.607. The phenogram divided the cherry species into two main groups, each of which is further subdivided into two or three subgroups (Fig 7). The variable related to the number of perforations (NP), range of the number of perforations, aperture type, exine sculpturing, and pollen grain size provided more data for species distinction.

The first cluster includes *P. mahaleb, P. jacquemontii*, and *P. microcarpa* subsp *microcarpa* 2 (subgroup 1), *P. cerasus*, *P. yazdiana*, and *P. pseudoprostrata* (subgroup 2). The second one includes *P. mazandaranica*, *P. chorassanica*, *P. brachypetala* var. *brachypetala*, *P. brachypetala* var. *bornmullerii* (subgroup 3), *P. microcarpa* subsp. *diffusa* 1, *P. incana*, *P. turcomanica*, *P. microcarpa* subsp. *diffusa* 2, *P. paradoxa* (subgroup 3), and *P. microcarpa* subsp. *microcarpa* 1, 2 and *P. microcarpa* subsp. *tortuosa* (subgroup 4).

Table 3. The results of the principal component analysis of the 13 quantitative pollen morphological characters of the *Prunus* subgenus *Cerasus.*

Trait	PC 1 (35.9%)	PC 2 (20.8%)	PC 3 (16.6%)
P	0.90	-0.19	-0.21
E	0.05	-0.58	0.60
P/E	0.73	0.24	-0.58
Ap	-0.03	-0.80	-0.37
ML	0.44	-0.31	0.65
CL	0.88	-0.11	-0.27
RCLP	0.31	0.41	-0.42
WR	0.59	0.23	0.52
TR	0.68	0.30	0.54
WV	0.71	0.40	0.10
DP	0.65	0.47	-0.06
NR	-0.09	0.89	0.14
NP	0.70	-0.19	-0.07

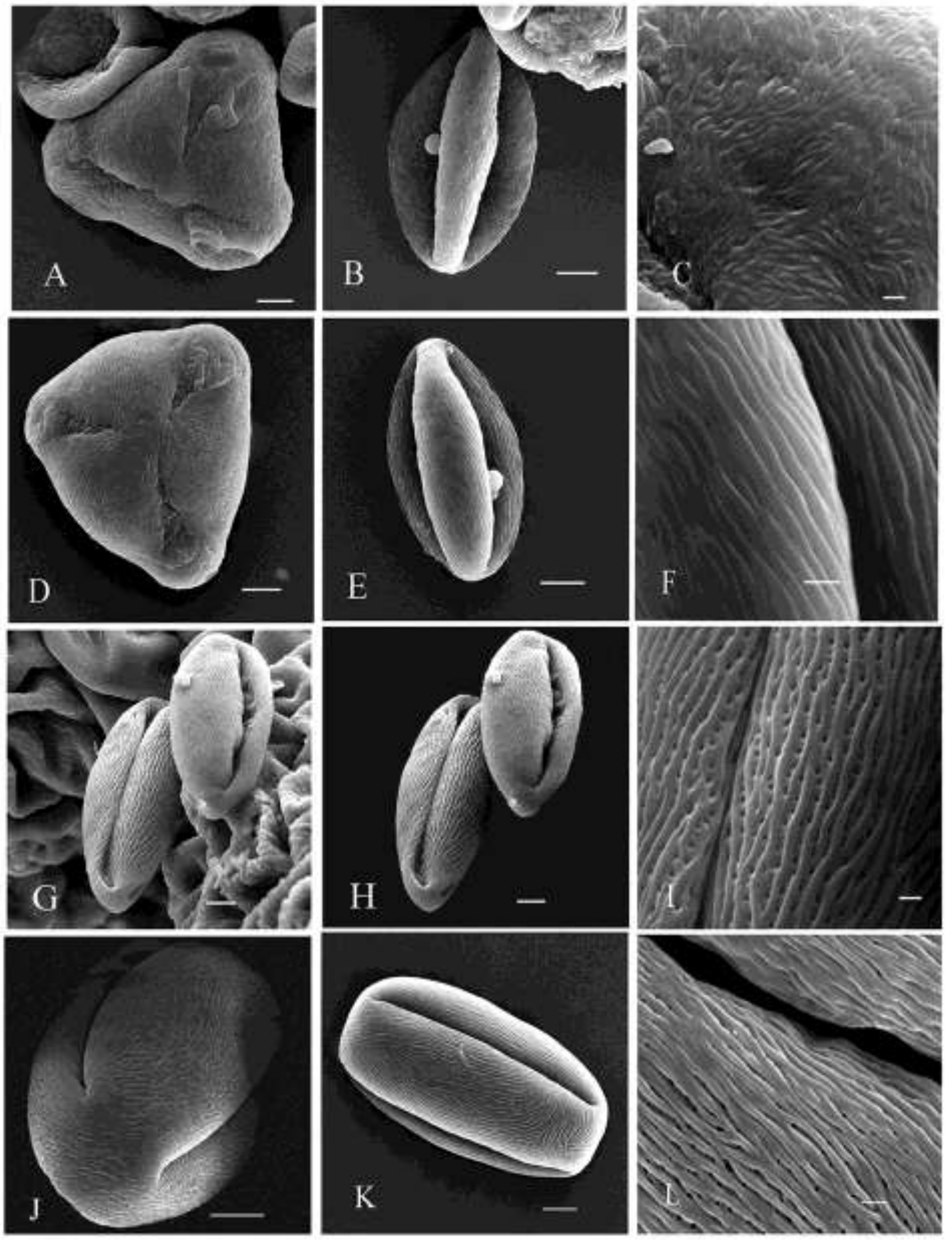

Fig. 2. A-L, SEM micrographs of pollen grains in species of Prunus subgenus Cerasus. A-C, *P. cerasus*, A, polar view; B, equatorial view; C, details of the exine sculpture. D-F, *P. mahaleb*, D, polar view; E, equatorial view; F, details of the exine sculpture. G-I, *P. brachypetala* var *brachypetala*, G, polar view; H, equatorial view; I, details of the exine sculpture. J-L, *P. brachypetala* var. *Bornmulleri*, J. polar view; K, equatorial view; L, details of the exine sculpture. (Scale bar= 5 μm for A, B, D, E, G, H, J, K; 1 μm for C, F, I, L).

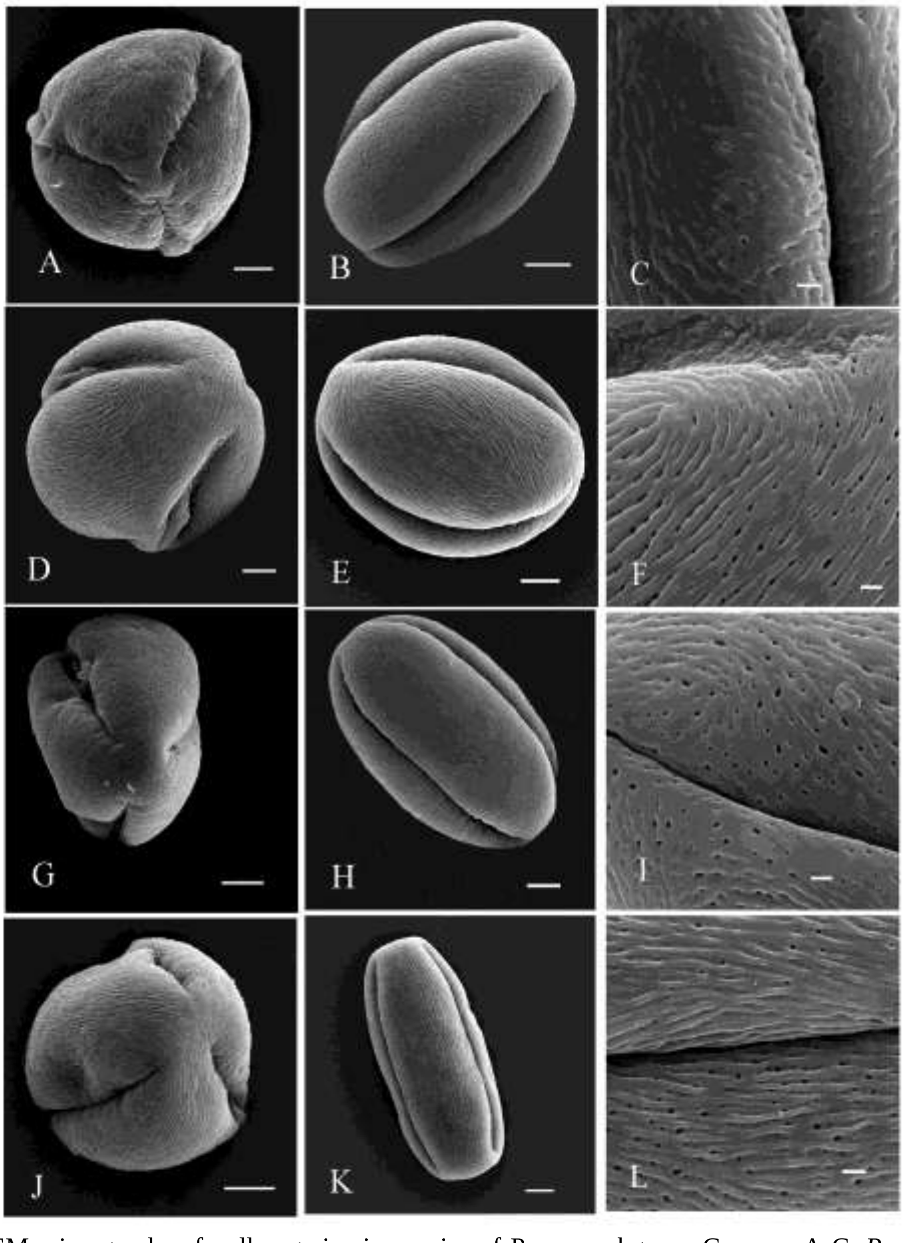

Fig. 3. A-L, SEM micrographs of pollen grains in species of Prunus subgenus Cerasus. A-C, *P. pseudoprostrata*, A, polar view; B, equatorial view; C, details of the exine sculpture. D-F, *P. incana*, D, polar view; E, equatorial view; F, details of the exine sculpture. G-I, *P. turcomanica*, G, polar view; H, equatorial view; I, details of the exine sculpture. J-L, *P. chorassanica*, J, polar view; K, equatorial view; L, details of the exine sculpture. (Scale bar= 5 µm for A, B, D, E, G, H, J, K; 1 µm for C, F, I, L).

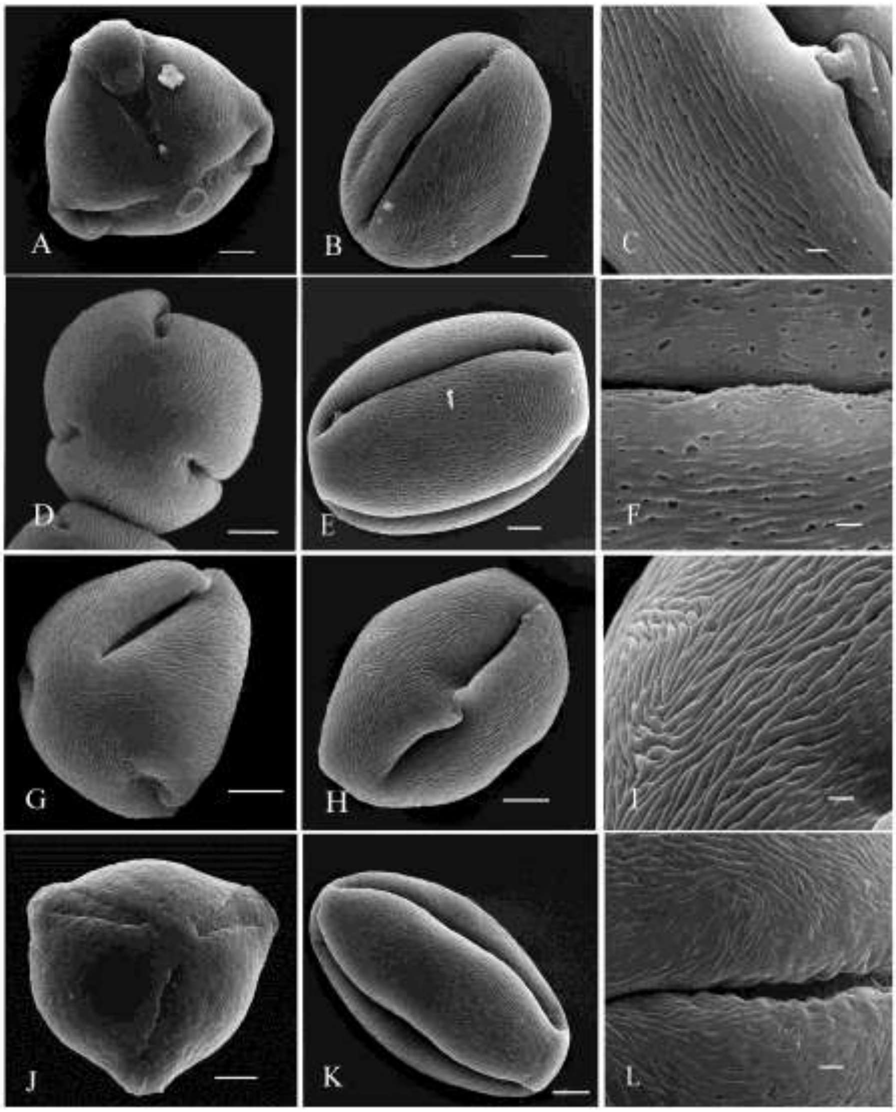

Fig. 4. A-L, SEM micrographs of pollen grains in species of *Prunus* subgenus Cerasus. A-C, *P. microcarpa* subsp. *diffusa* 1, A, polar view; B, equatorial view; C, details of the exine sculpture. D-F, *P. microcarpa* subsp. diffusa 2, D, polar view; E, equatorial view; F, details of the exine sculpture. G-I, *P. microcarpa* subsp. *microcarpa* 2, G, polar view; H, equatorial view; I, details of the exine sculpture. J-L, *P. microcarpa* subsp. *microcarpa*3, J, polar view. K. equatorial view; L. details of the exine sculpture. (Scale bar= 5 µm for A, B, D, E, G, H, J, K; 1 µm for C, F, I, L).

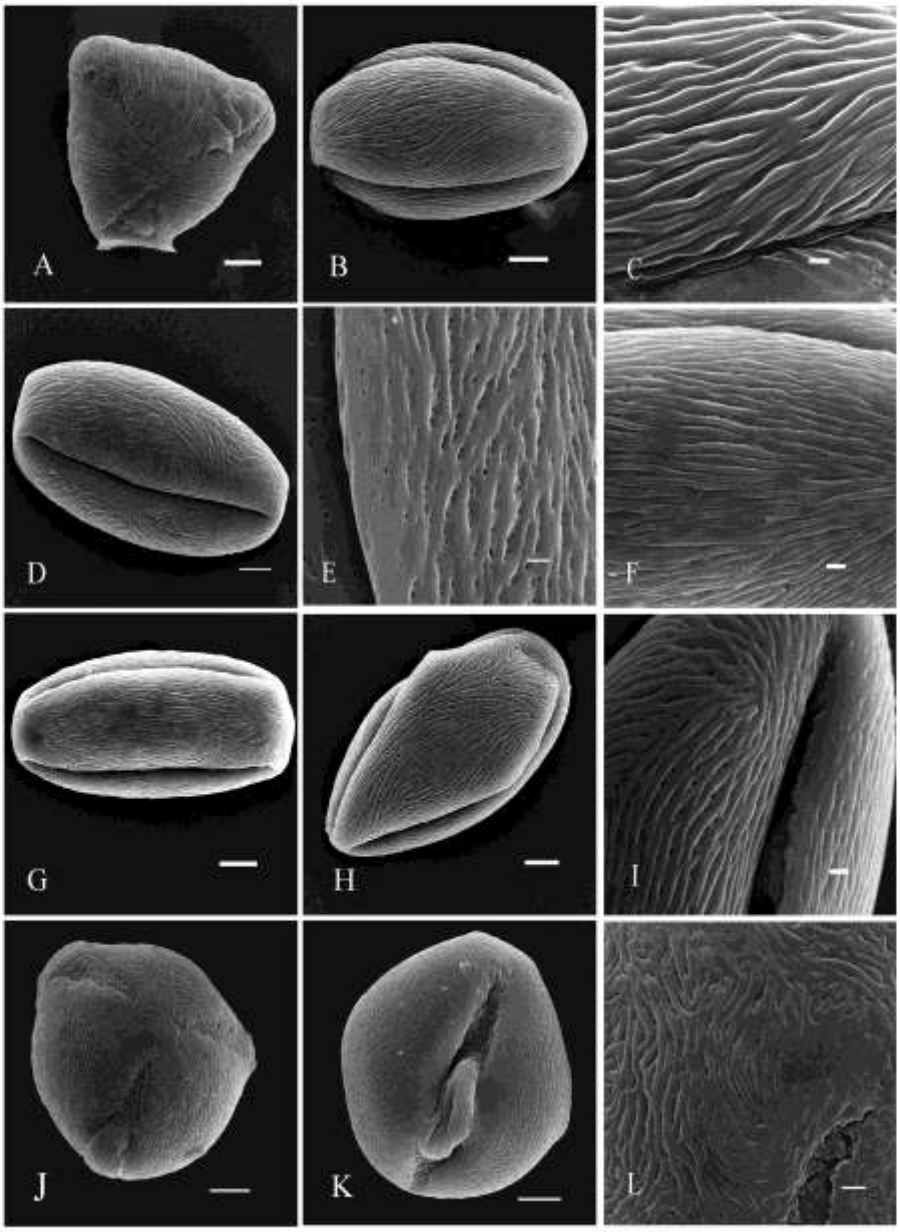

Fig. 5. A-L, SEM micrographs of pollen grains in species of *Prunus* subgenus Cerasus. A-C, *P. microcarpa* subsp. *tortuosa*, A, polar view; B, equatorial view; C, details of the exine sculpture. D-E, *P. mazandaranica*, D, equatorial view; E, details of the exine sculpture. F-G, *P. yazdiana*, F, details of the exine sculpture; G, equatorial view. H-I, P. paradoxa, H, equatorial view; I, details of the exine sculpture. J-L, *P. jacquemontii*, J, equatorial view; L, details of the exine sculpture. (Scale bar= 5 µm for A, B, D, G, H, J, K; 1 µm for C, E F, I, L).

Identification Key to the species based on pollen traits

1. Exine sculpture regulate............. *P. pseudoprostrata*
- Exine sculpture striate 2
2. Exine with no perforations 3
- Perforations present 4
3. Pollen shape prolate, mean of P/E ratio 1.54 .... *P. cerasus*
- Pollen shape prolate spheroidal, mean of P/E ratio 1.04 .... *P. mahaleb*
4. Average number of perforations 0-10 5
- Average number of perforations 14-58 7
5. Ridges narrow .... *P. yazdiana*
- Ridges wide 6
6. Pollen shape prolate, mean of P/E ratio .... 1.3-1.8 *P. microcarpa* subsp. *microcarpa* and 1-3, *P. microcarpa* subsp. *Tortuosa*
- Pollen shape prolate spheroidal, mean of P/E ratio 1.11 .... *P. jacquemontii*
7. Mean of P value > 40 µm 8
- Mean of P value < 40 µm 9
8. Ridges formed fingerprint-like, twists usually in the subpolar zone (Type IIB)... .... *P. paradoxa*, *P. chorassanica*
- Ridges short, weaving and crossing (Type IIC) .... *P. turcomanica*, *P. microcarpa* subsp. *diffusa* 2, *P. mazandaranica*,
9. Pollen shape perprolate to prolate, mean of P/E > 1.9, ridges and valleys usually parallel to colpi to polar axis (Type IIA) .... *P. brachypetala* var. *brachypetala*, *P. brachypetala* var. *Bornmullerii*
- Pollen shape prolate, mean o P/E < 1.5, ridges formed fingerprint-like twists usually in the subpolar zone (Type IIB) .... *P. incana*, *P. microcarpa* subsp. *diffusa* 1

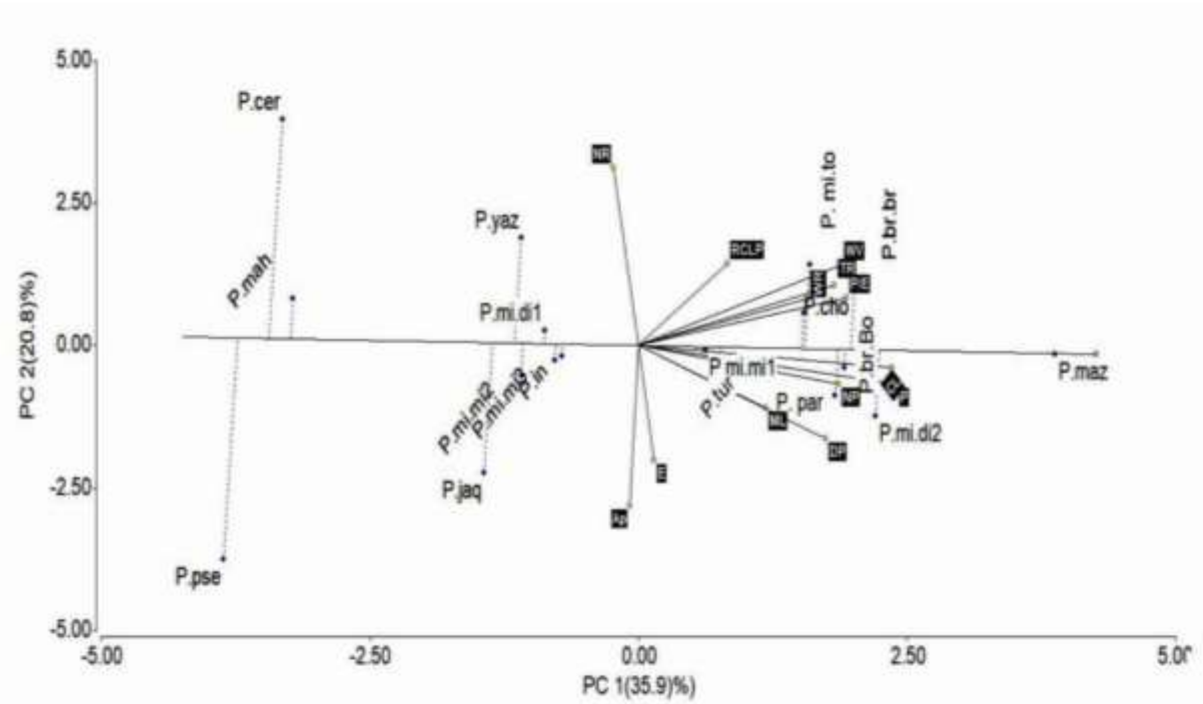

Fig. 6. Principal component analysis (PCA) performed with 13 pollen quantitative traits from *Prunus* subgenus Cerasus (see Table I for the abbreviation of quantitative pollen traits).

DISCUSSION

We describe a detailed morphological character for pollen grains of *Prunus* subgenus *Cerasus* in its Iranian distribution range. According to Browicz (1969) and Khatamsaz (1993), Iranian cherries are classified into three sections (sect. *Microcerasus* Spach, sect. *Cerasus*, and sect. *Mahaleb* Roemer). In Rosaceae, pollen grain traits are viewed from two perspectives. Moor (1991) presented the first view. He emphasized that pollen grain morphology in the Rosaceae taxa varies greatly, even among populations of the same species. Furthermore, because pollen grain size is entirely dependent on frequent hybridization in this family, it is an unreliable trait. Hebda & Chinnapa (1990) presented the second point of view in a study on pollen grain in Rosaceae in Canada. According to their findings, diversity in exine sculpture can be used as a tool in identifying the genus or even species in this family. Our finding is congruent with the second point of view.

The main informative characteristic to identify pollen grains were the aperture and shape. Our results reveal that based on the definition described in Moore & al (1991), the pollen grains in species of subgenus *Cerasus* are 3-zonocolpate including three colpi or 3-zonocolporate including three ectocolpi and three endopori. Tricolporate aperture is dominant in studied species as in many members of Rosaceae (Reitsma 1967; Ueda and Tomita 1989; Hebda and Chinnappa 1990a, b, Xu and Yao 1991; Radice & al. 2003; Wornska-Pilarek & al. 2006; Donmez 2008; Polykova and Gataulina 2008;). The pollen grains of the studied species are medium in size with perprolate, prolate to prolate spheroidal shapes. These pollen characteristics were also reported in other Rosaceae genera (Ueda and Tomita 1989; Hebda and Chinnappa 1990a, b; Zhou & al 1999; Radice et al. 2003; Arzani & al. 2005; Wornska-Pilarek & al. 2006; Donmez 2008; Polykova and Gataulina 2008; Zamani & al. 2010; Nikolic & Milatovic 2015; Raei Niaki & al. 2020; Li & al. 2021). But this is not a diagnostic character in the other sections of the subgenus. Our results showed that the shape of pollen grains in three subspecies of *P. microcarpa* is prolate whereas its allies including *P. mazandaranica* and *P. yazdiana* exhibit perprolate pollen grains. All three pollen grain shapes mentioned above were observed in various taxa in the section *Microcerasus*. The interesting point was that the pollen grains in some relative taxa, particularly in three subspecies of *P. microcarpa*, were similarly apertured, and all of them were prolate and trizoncolporate. However, the type of aperture and the shape of pollen grains differed among *P. brachypetala* varieties and two morphologically similar species, *P. pseudoprostrata* and *P. chorassanica* (Table 2). The recently described, *Prunus mazandaranica*, (Habibi & al. 2022), differed in pollen shape and aperture type from its closest relatives, *P. microcarpa* subspecies. As previously stated, the aperture in most taxa was tricolporate, with three colpi and pores (Hebda & Chinnappa 1990a).

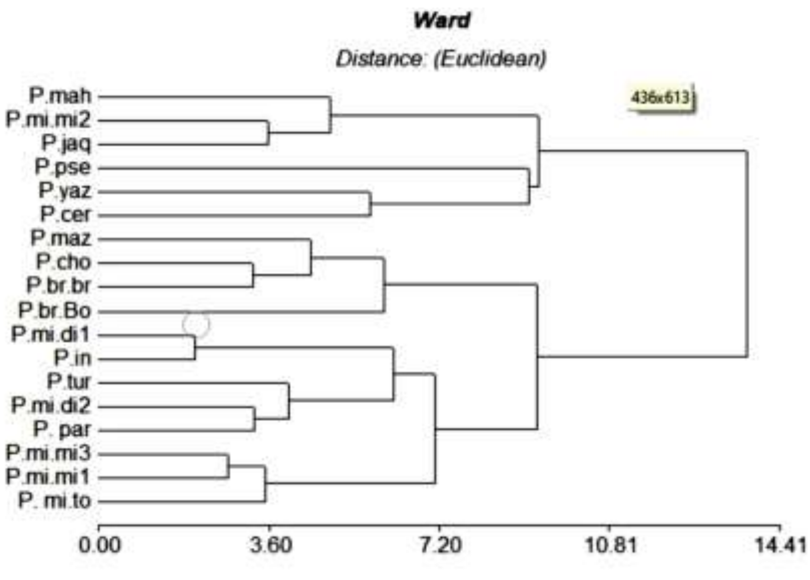

Fig. 7. Cluster analysis based on pollen grain morphological characters to demonstrate the grouping of species in subgenus Cerasus.

Exine sculpture types have been suggested as important diagnostic characteristics at various taxonomic levels in Rosaceae. The most frequent exine sculpturing in the family Rosaceae is striate or striate-perforate (Eide, 1981; Hebda & al. 1988a; Ueda and Tomita 1989; Hebda and Chinnapa 1990a, b, 1994; Ueda 1992; Lee & al. 1993; Chung & al. 2010; Lee & al. 2011; Wornska-Pilarek and Jagodzinski 2011; Geraci & al. 2012; Shi & al. 2013). Also, Hebda and Chinnappa (1990a, 1994) distinguished two types of perforations in Rosaceae (striate sculpturing, macroperforate, and nonstriated sculpturing, macroperforate, each with six subtypes). The included *cherries* in the first type with larger perforations often extending onto tectal striae. According to the above-cited study, pollen of *cherries* (with *Rosa*, *Rubus*, and *Spiraea*) belongs to the subcategory with striae separated by ridges and valleys, containing larger perforations (0.1-0.2 µm in diameter). Our studies demonstrated the inclusion of the *P*. subgen. *Cerasus* into one type is too general because firstly, there are cherries species with striate exine sculpture, among which, in some species, perforations are very scarce or do not occur at all (striate-perforate: type II), and rugulate (type III). However, most of the types we mentioned above were not explained by Hebda and Chinnappa (1990a, 1994). Also, our results suggest that the dwarf cherries (sect. *Microcerasus*) are the most heterogeneous taxa according to some pollen characters such as length of the polar axis (P), length of the equatorial diameter, aperture type (ApT), length of apocolpium (AP), colpus length (CL), mesocolpium length (ML), the diameter of perforations (DP), number of ridges (NR), and width of ridges (WR). However, there are distinct differences among closely related taxa, mainly three subspecies. of *P. microcarpa* or two varieties of *P. brachypetala*, that exhibit different pollen characteristics such as the number of perforations (NP), and exine sculpture type. Rugulate-non striate sculpture type was rarely found in Rosaceae and noted to be a unique characteristic of certain taxa. We could detect rugulate type (type III) in *P. pseudoprostrata* while other taxa had a striate type. Furthermore, different exine sculptures were observed in *P*. subgen. *Amygdalus* (L.) Benth. & Hook. (Vafadar & al. 2010) and other genera of family Rosaceae including Turkish *Crataegus* (Donmez 2008) and some rosaceous members in Canada (Hebda and Chinnappa 1990a). The results of our study showed variation in pollen morphology of subgenus *Cerasus* as a useful trait for species delimitation in the majority of species. But, because of some overlapping features among sections *Cerasus*, *Mahaleb*, and *Microcerasus*, did not provide diagnostic characters for the delimitation of the three sections. Furthermore, the results of this research do not support the existing sectional classification and taxonomic affinities of the Iranian species of the *P*. subgen. *Cerasus*.

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