MICRO - MACRO MORPHOLOGY OF THE GENUS *GEUM* L. (ROSACEAE) IN IRAN AND THEIR TAXONOMIC SIGNIFICANCE

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In the current survey, pollen, seed and fruit micro-morphological characters of the genus Geum L., comprising 5 species from two subgenera, *Orthostylus* Fisch & Mey. and *Geum* were examined using scanning electron microscope (SEM) and light microscope (LM). The pollen grains are monad, radially symmetrical, isopolar, tricolporate, medium in size, elliptical (from equatorial view) and triangular to circular (from polar view) in outline, subprolate to prolate in shape. The exine ornamentation is striate with micro perforation. Seeds coat micro - morphology revealed two types of sculpturing (including sulcat-ribbed and sulcat-foveolate) and fruits morphological analysis showed the importance of joint position and hairs on the style, beak length and hook in this genus. Principal component analysis (PCA) was carried out using a total of 20 characters. The result supports current classifications and emphasizes the importance of micro- macro-morphologocal traits for separating the two subgenera and species of the genus. Based on the important distinguishing characters a key is presented

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Key words: Morphology; micro morphology; pollen, seed coat, fruit, *Geum*, Rosaceae

مطا لعه ریز ریخت شناسی و ریخت شناسی سرده Geum در ایران و اهمیت کاربرد آنها در تاکزونومی

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مطالعه حاضر به بررسی صفات ریزریخت‌شناسی گرده ، پوسته بذر و ریخت‌شناسی میوه ۵ گونه از سرده Geum متعلق به دو زیر جنس Orthostylus Fisch & Mey. و Geum با استفاده از میکروسکوپ الکترونی نگاره (SEM)و نوری (LM) اختصاص دارد .گرده این گیاهان به صورت موناد، متقارن‌شعاعی، جور قطب، سه شیاری– منفذی و دارای اندازه متوسط، طرح کلی بیضی (از نمای استوایی) و سه‌گوش تا تقریباً مدور (از نمای قطبی) و به شکل تقریباً استوانه ای – استوانه ای هستند .تزئینات اگزین راه‌راه با منافذ میکروسکوپی است .مطالعه ریز ریخت شناسی پوسته بذر دو نوع تزیینات شامل پشته ای– نواری و پشته ای – موجدارآشکار ساخت و بررسی ریخت شناسی میوه اهمیت موقعیت مفصل روی خامه، طول منقار و قلاب در این جنس را نشان داد. در مجموع ۲۰ صفت به روش تجزیه مولفه اصلی (PCA)مورد ارزیابی قرار گرفتند .نتایج این آنالیز ضمن حمایت از رده بندی های موجود، بر اهمیت صفات ریز ریخت شناسی و ریخت شناسی در تفکیک زیر سرده‌ها و گونه‌ها ی این سرده تاکید دارد. بر اساس صفات مهم تشخیصی یک کلید شناسایی ارایه شده است.

INTRODUCTION

The genus *Geum* mainly includes a group of herbaceous perennials and a few species of small shrubs with a thick caudex and rosette of imparipinnate leaves. They are distributed in temperate and arctic regions of the world, especially in the northern hemisphere. However it has a few representatives in South America, New Zealand, Australia, and Tasmania. The genus was introduced by Linnaeus (1753) with five species. The taxonomical delimitation of *Geum* has changed greatly over several years. Scheutz (1870) monographed *Geum* and included 43 species and eight

sections. Focke (1894) divided *Geum* into two subgenera *Geum* (partly deciduous styles) and *Sieversia* Willd. (non-deciduous styles). The second monograph of the genus was published by Bolle (1933). He divided *Geum* into several genera, comprising species with plumose styles, harpoon type and fish-hook fruits (Iltis 1913). Yuzepchuk (1941) divided *Geum* in to two sections (*Caryophyllata* Seringe and *Cary ophyllastrum* Seringe), four series and two additional genera including *Woronowia* Juz. (with straight, glabrous, articulate styles) and *Orthurus* Juz (with the harpoon type fruit species e.g. *O. heterocarpus* (Boiss.) Juz and *O. kokanicus* ((Rgl. et Schmalh.) Juz. Gajewski (1957, 1968) classified *Geum* based on a major cytogenetic study. His classification (1957, 1958, and 1968) was very influential for later authors (Huber 1961; Schulze-Menz 1964; Hutchinson1967; Robertson 1974; Kalkman 1988). Schönbech-Temesy (1969) arranged the genus into three subgenera (*Orthosylus* (Fisch & C. A. Mey.) Bolle (with two species), *Geum* (with four species) and *Acomastylis* (E.L. Greene) Gayewski ex Schönbeck-Temesy (with the single species) in Flora Iranica. khatamsaz (1993) divided the Iranian species of *Geum* into two subgenera: *Orthosylus* (Fisch & C. A. Mey.) Bolle and *Geum*. She placed *G. heterocarpum* Boiss., *G. kokanicum* Regel & Schmalh. ex Regel and *G. iranicum* Khatamsaz in the first and *G. rivale* L. and *G. urbanum* L. in the second subgenus. Iranian species of the genus are mainly distributed in N, NW, W, NE and C of the country at altitudes minimum 200 (e.g. *G. urbanum* L.) to maximum 3400m (e.g. *G. kokanicum* Regel and Schmalh. ex Regel).

Geum belongs to a critical and taxonomically difficult group, which forms an interesting object for several taxonomical studies. However these studies were focused on the morphology (e.g. Yuzepchuk 1941; Huber 1961; Schulze-Menz 1964; Hutchinson1967; Robertson 1974; Kalkman 1988; Schönbech-Temesy (1969); cytology (e.g. Gajewski 1957, 1958, 1968); fruit evolution and allopolyploidy (Smedmark and Eriksson 2006) and phylogeny (Smedmark and Eriksson 2002; Smedmark & al. 2003) of the genus. The main aim of this research were to describe palynological, seed and fruits micro - morphological details of Iranian species of the genus and verify their taxonomic implications.

MATERIALS AND METHODS

Pollen, seed and fruits were obtained from freshly collected plants (during 2013-2015) and herbarium specimens of Research Institute of Forests and Rangelands, Tehran (TARI), Faculty of Pharmacy, Tehran University of Medical Sciences (THE) and Guilan University Herbarium (GUH). The voucher specimens were deposited in Guilan University Herbarium (GUH). Species sampled are listed in table 1.

In the current survey the pollen grains of five species of *Geum* in Iran were studied using light and scanning electron microscope. For LM, pollen grains were acetolysed using Harley's method (1992). The observation were carried out using light Olympus BH-2 microscope and photographed by Nikon camera model Coolpix S10. For each sample at least 20 grains were measured. The palynological data are presented in table 2. For SEM observation, grains were mounted on the stubs with double-sided cellophane tape and then coated in a sputter coater with 25nm of gold-palladium at an accelerating voltage of 10–15 kv. The micrographs were prepared by scanning electron microscope Tescan SEM Vega. The pollen terminology in general follows Erdtman (1952), Punt & al. (2007), and Ueda and Tomita (1989).

The dried seeds (10-15 for each sample) were examined by LM and SEM analysis as described above. The terminology used here follows Barthlott (1981, 1984); Abdel Khalik (2006) and Svetlana & al. (2009) The seed morphological characters were presented in table 3.

Mature and immature fruits samples were taken for investigation (10-20 for each taxon), tables 4 and 5. Measurements and optical observations of fruits were carried out under Olympus BH-2 microscope and the photographs were taken by digital microscope, Dino-Lite, AN-413T model and to have a clear images from achene characteristics, they were illustrated carefully. Fruits were observed under Olympus BH-2 microscope and the photographs were taken by digital microscope, Dino-Lite, AN-413T model .To obtain a clear image from achene characteristics, they were illustrated carefully.

Data analysis was carried out, using 19 quantitative and single qualitative characters, comprising the mean of quantitative and coded qualitative characters, as binary/ multistate characters. The standardized variables were employed for multivariate statistical analysis. The pollen, seed and fruits character states used in the numerical analysis of 5 species and their different populations of *Geum* in Iran are presented in table 6. PCA analysis was conducted using the general linear model (GLM) in Minitab statistical software (Ryan and Joiner 2001).

Table 1. The species used in the current study.

Species	IRAN: Province, Collector, Date	Accession No.
G. heterocarpum Boiss.	Golestan: Park Jahan nama Almeh,1850m, Wandelbo and Froghi, 1974/6/9	012647(TARI)
	Tehran: Darband, 1730m, Baba khanloo and Amin, 1973/5/8	20506(TARI)
	Tehran: 2500m, 1972/ 6/19	20708(TARI)
	Khorasan: Sarcheshmeh, Rooin, 2100m, Monsef, 2009/6/3	6738(TEH)
G. kokanicum Regel & Schmalh ex Descer.	Khorasan: Esfarayen, Koohe Shah Jahan nama, 1700-2500m, Mozafarian, 1984/ 6/27	048435(TARI)
	Tehran: Darband sar, 2700-3400m. Mozafarian and Mohammadi, 1984/8/14	49183(TARI)
	Tehran:Gajreh, 2500-300m, Baba khanloo and Amin, 1972/8/8	20423(TARI)
	Khorasan: Bojnord to Esfarayen road, gardaneh Assadi, 1738m, Shahi, 2014/ 3/9	5751(GUH)
	Khorasan: Bojnord, Near Petroshimi, 659m, Shahi, 2014/ 3/7	5752(GUH)
G. iranicum Khtamsaz.	Khorasan: Graiil region, Emamzadeh Zakaria, Shirvan, Monsef, 2009/ 6/6	6714(THE)
	Khorasan: Graiil region, Emamzadeh Zakaria, Shirvan, Monsef, 2009/ 6/6	6715(THE)
G. rivale L.	Mazandaran:Chaloos road, Kandavan, 2600m, Froghi	531(TARI)
	Tehran: Karai valley, Asm vark, 2440m, Froghi 1970/6/20	526(TARI)
	Mazandaran: 20 k south of Ramsar, 200-3000m, Assadi and Masoomi, 1984/ 7/4	51385(TARI)
	Azarbaijan: Arasbaran, 2500-2800, Assadi and Sardabi, 1977/ 7/13	24037(TARI)
G. urbanium L.	Khorasan: Mashhad, Zoshk, 1560m, Froghi, 1971, 5,3	1447(TARI)
	Kermanshah, Bakhtaran,45 k to West Marand, Rijab, 900m, Lashkar bloke and Khatamsaz, 1982/ 4/24	0189(TARI)
	Golestan: 11k to south Shahpasand, 280m , Papo, 1966/5/10	7521(TARI)
	Guilan: Astara to Ardabil, Moshn, 800m, Khatamsaz and Salehnia, 1987/ 6/ 19	56779(TARI)

Table 2. Pollen morphology data: Numbers refer to (minimum-) mean ± standard deviation (-maximum), Polar axis (P), Equatorial axis (E), Polar axis/Equatorial axis (P/E) ratio, Pollen shape (Ps) and Size (Si), Distance between the apices of two ectocolpi/equatorial diameter (d/D), Mesocolpium (Meso), Colpus length/polar axis (Cl/P), Colpus length (Cl), Number of Colpi (No. Cl), Exine thickness (Et), Sculpturing type (ScT), Striate –microperforation (St mp), Prolate-spheroidal (PS), Prolate (Pr), Subprolate (Subp), Small (S), Medium (M). sexinal flaps (Sf);* large perforation

Species	**P µm**	**E µm**	**P/E µm**	**PS**	**d/D µm**	**S**	**CL µm**	**ET µm**
G. heterocarpum	29.2-31.4 (30.37 ±1.10)	21.84-25.78 (23.94 ±1.69)	1.18-1.33 (1.23±0.079)	Subp	0.34-0.43 (0.38±0.49)	M	26.84-28.9 (27.82±0.75)	1.76-1.87 (1.8±0.07)
G. kokanicum	28.63 -29 (28.81±0.26)	20.45-20.90 (20.67±0.31)	1.36-1.38 (1.38±0.03)	Pr	0.25-0.29 (0.27±0.020)	M	25-29.9 (27.01±1.72)	1.25-1.38 (1.3±0.09)
G. iranicum	28-30 (28 ±1)	23.5-24.36 (24±0.7)	1.07-1.27 (1.17±0.14)	Subp	0.24-0.28 (0.27.5±0.01)	M	20-21.8 (20.9±1.27)	0.93-1.25 (1±0.2)
G. rivale	25.5-26 (25.5±0.35)	16.5-17.2 (16.8±0.261)	1.4-1.6 (1.5±0.264)	Pr	0.20-0.33 (0.26±0.063)	M	23.7-24.5 (24.14±0.35)	0.83-0.93 (0.88±0.0)
G.urbanum	23.02-24.1 (23.5±0.46)	15.3-16 (15.6±0.39)	1.47-1.52 (1.5±0.026)	Pr	0.34-0.38 (0.36±0.018)	M	20.2-21.3 (20.79±0.46)	0.9-1 (1.19±0.24)

Table 2 Continues of characters.

Species	CL//P µm	Meso µm	No. CL	ScT	SF	Rigid+ valley
G. heterocarpum	0.91-0.97 (0.94±0.02)	14.7-16.3 (15.5±0.8)	3	*St.m.p	3.1-3.9 (3.5±0.40)	0.43 -0.92 (0.62±0.16)
G. kokanicum	0.87-1.03 (0.93±1)	14-15.2 (14.7±0.64)	3	*St.m.p*	3.1-3.8 (3.51±0.33)	0.65 -0.85 (0.71±0.04)
G. iranicum	0.72-0.76 (0.74±0.0)	10-13.75 (11.87)	3	*St.m.p	3-3.1 (3±0.07)	0.24-0.94 (0.62±0.203)
G. rivale	0.94-0.945 (0.942±0.003)	11.6-13.92 (12.58±1)	3	*St.m.p	3.3-5.71 (3.53±1.60)	0.62-1.21 (0.92±0.21)
G. urbanum	0.881-0.885 (0.87±0.0026)	9.46-11.42 (10.66±0.84)	3	*St.m.p	1.91-3.2 (2.65±0.55)	0.49-1.43 (0.80±0.37)

Table 3. Seed SEM morphological characteristics in 5 species of *Geum* from Iran: Seed shape (SS); Seed length (SL); Seed width (SW);); Seed apical width (SAW); Seed basal width (SBW); Seed sculpturing (SSC), Rigid interval (RI); Rigid height (RH).

Species	SS	SL mm	SW mm	SAW mm	SBW mm	SSC	RI mm	RH mm
G. heterocarpum	Oblong	3.9-4.2 (4.08±0.16)	1.3-1.5 (1.41±0.12)	0.86-1.50 (1.10±0.34)	0.56-0.63 (0.59±0.03)	SR	0.013-0.028 (0.023±0.00)	0.006-0.018 (0.010±0.00)
G. kokanicum	Fusiform	5.02-6.8 (6.03±0.91)	2.07-4.3 (2.94±1.19)	1.2-1.33 (1.28±0.07)	0.40-1.15 (0.84±0.39)	SR	0.017-0.027 (0.020±0.00)	0.002-0.009 (0.005±0.00)
G. iranicum	Oval	4.18-4.93 (4.57±0.37)	2.31-2.67 (2.52±0.19)	1.1-1.76 (1.45±0.42)	0.6-1.03 (0.844±0.2)	SR	0.01-0.026 (0.016±0.00)	0.001-0.005 (0.0030.001)
G. rivale	Oval	2.2-2.5 (2.42±0.23)	0.87-0.93 (0.90±0.04)	0.66-0.707 (0.68±0.03)	0.43-0.46 (0.44±0.02)	SF	0.008-0.017 (0.014±0.00)	0.002-0.0029 (0.0027±0.00)
G. urbanum	Fusiform	2.7-2.94 (2.86±0.11)	1.30-1.31 (1.307±0.00)	1.02-1.08 (1.05±0.046)	0.3-0.5 (0.40±0.13)	SF	0.010-0.013 (0.017±0.00)	0.0029-0.0054 (0.0041±0.00)

RESULTS

General pollen grain features

The most important pollen morphological features of the studied species are summarized in table 2 and the microphotographs taken LM by and SEM presented in figures 1-2.

Pollen grains are radially symmetrical, monads, isopolar, tricolpate. The outline of the pollen grains is elliptical (from equatorial view, fig. 1 A-C and G-H) and triangular to circular (from polar view, fig. 1 D-F and I-J). The shape of pollen grains varies from subprolate (1.4-1.6) to prolate (1.18-1.33). The minimum and maximum polar axis (P) changes from 23.02. µm in *G. urbanum* to 30.37µm in *G. heterocarpum*. The maximum (24.00 µm) equatorial axis is found in *G. iranicum* and minimum (15.6 µm) equatorial (E) axis is reported in *G. urbanum*. According to Erdtman (1952), the pollen grains of all the studied species are medium in size (25-50 µm), except *G. urbanum* which has small pollen (23.5- 15.6 µm). Among the studied species, *G. heterocarpum* has the largest and *G. urbanum* possess the smallest pollen grains (table 2). The mean apocolpium index (Punt & al. 2007), or d/D ratio (the distance between the apices of two ectocolpi (d)/ equatorial diameter D) is measured. The apocolpium index ranges over an interval of 0. 26 µm in *G. rivale* to 0.38 µm in *G. heterocarpum.* Maximum (15.5 µm) and minimum (10.66 µm) mesocolpium thickness were measured in *G. heterocarpum* and *G. urbanum* respectively.

The ratio of colpus length / to polar axis varies from minimum (0.74) in *G. iranicum* to maximum (0.94) in *G. heterocarpum* and *G. rivale*. The apertures were uncovered in majority of the studied species (fig. 2 D and I-K). But in some species e.g. G. *heterocarpum* and *G. iranicum* protruded apertures were identified (fig. 1 A-C and fig. 2 C, F -G).

In All the examined taxa, granulated colpus membranes (fig. 2 G), sexinal flaps or pore flaps (fig. 2 G-K), equatorial bridges (fig. 1 D-F and I –J) were recorded. The exine thickness was measured along the polar and equatorial axes and maximum (1.8 µm) and minimum (0. 88µm) exine thickness were identified in G. *heterocarpum* and *G. rivale* respectively.

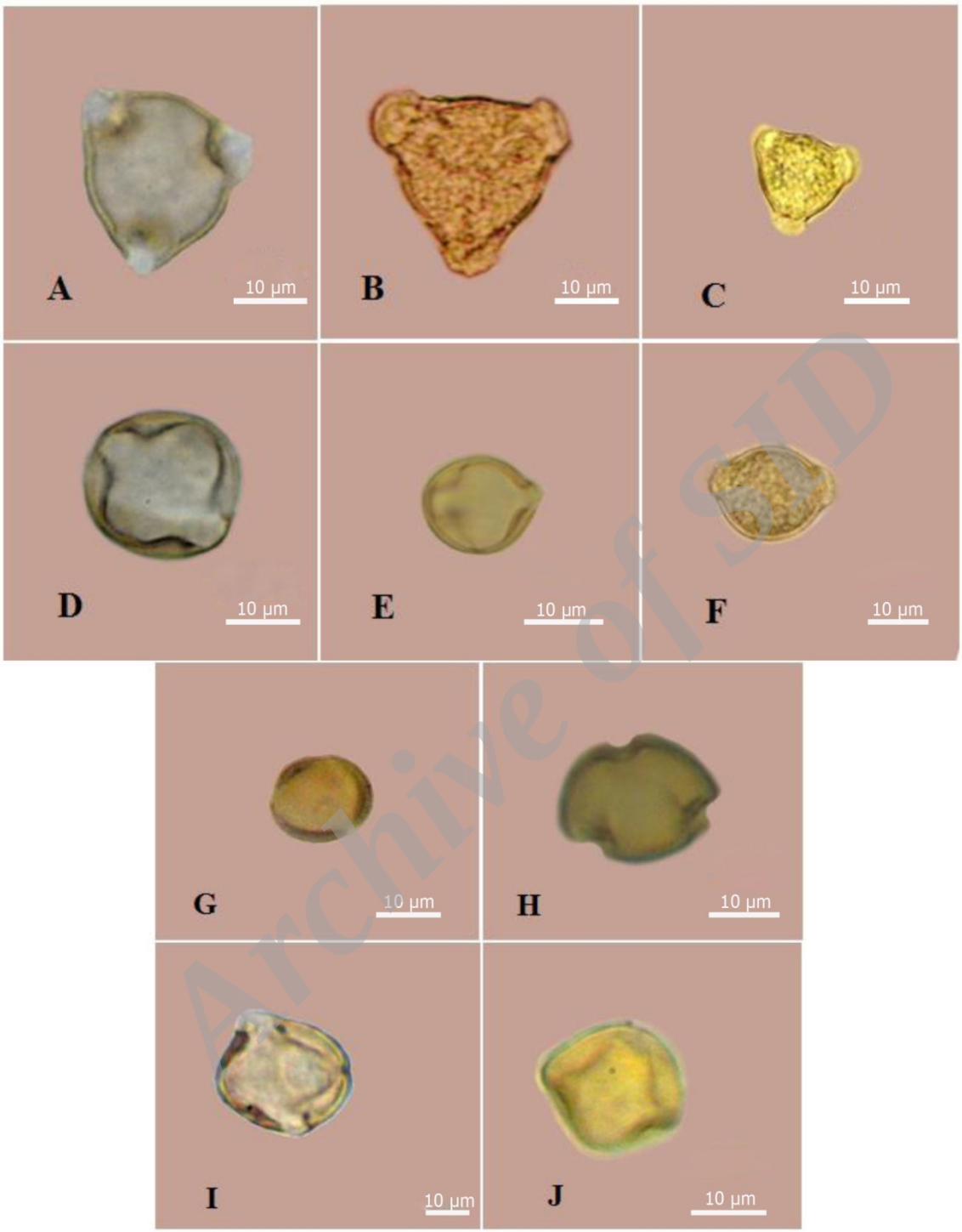

Fig. 1. The LM micrographs of pollen grains in *Geum.* A and D, *G. heterocarpum*; B and E, *G. kokanicum*; C and F, *G. iranicum*; *G* and I, *G. rivale*; H and L, *G. urbanum.*

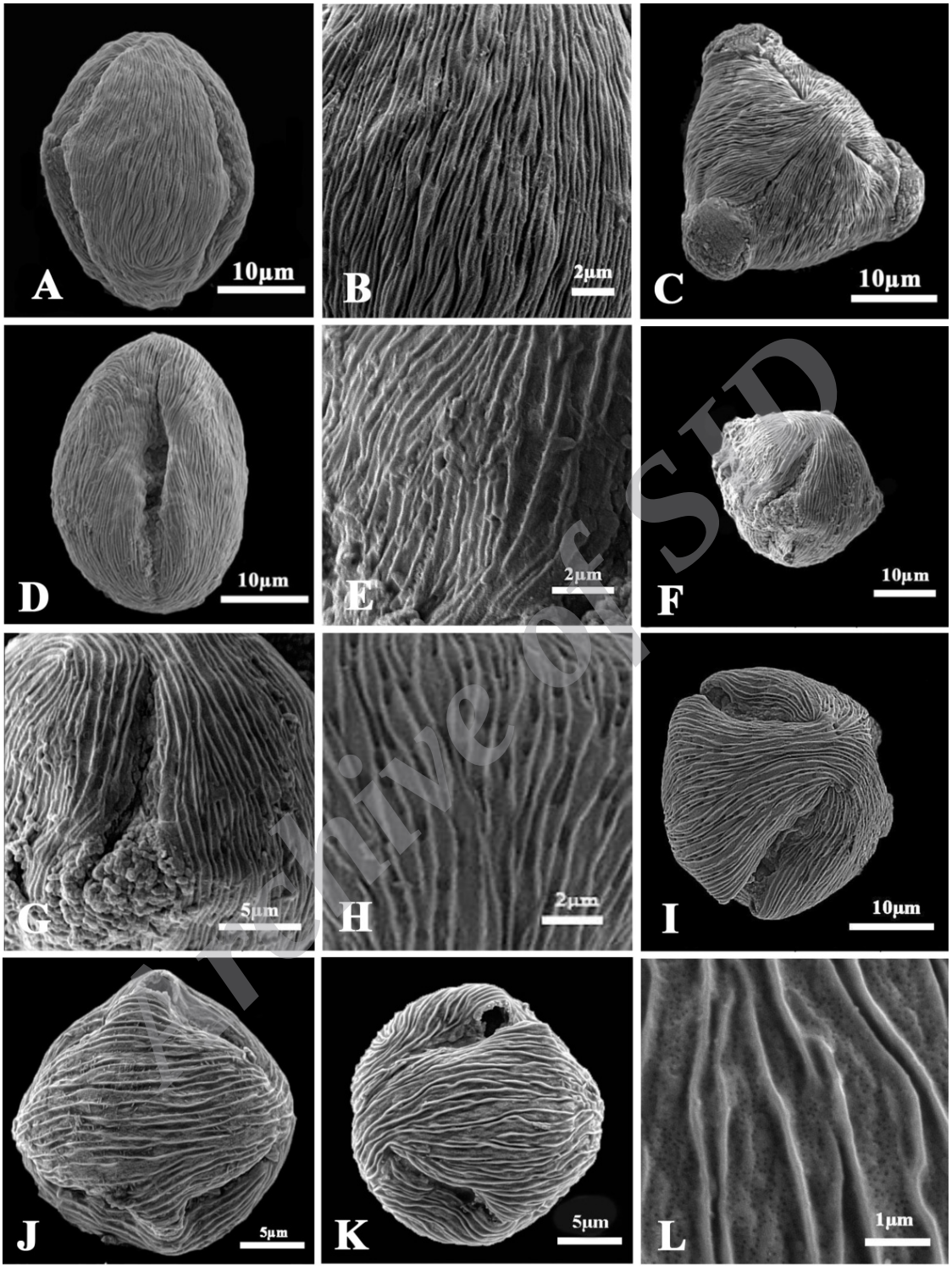

Fig. 2.The SEM micrographs of pollen grains in *Geum.* A-D, *G. heterocarpum*; E-G, *G. iranicum*; H-I, *G. kokanicum*; J, *G rivale*; K-L, *G. urbanum.*

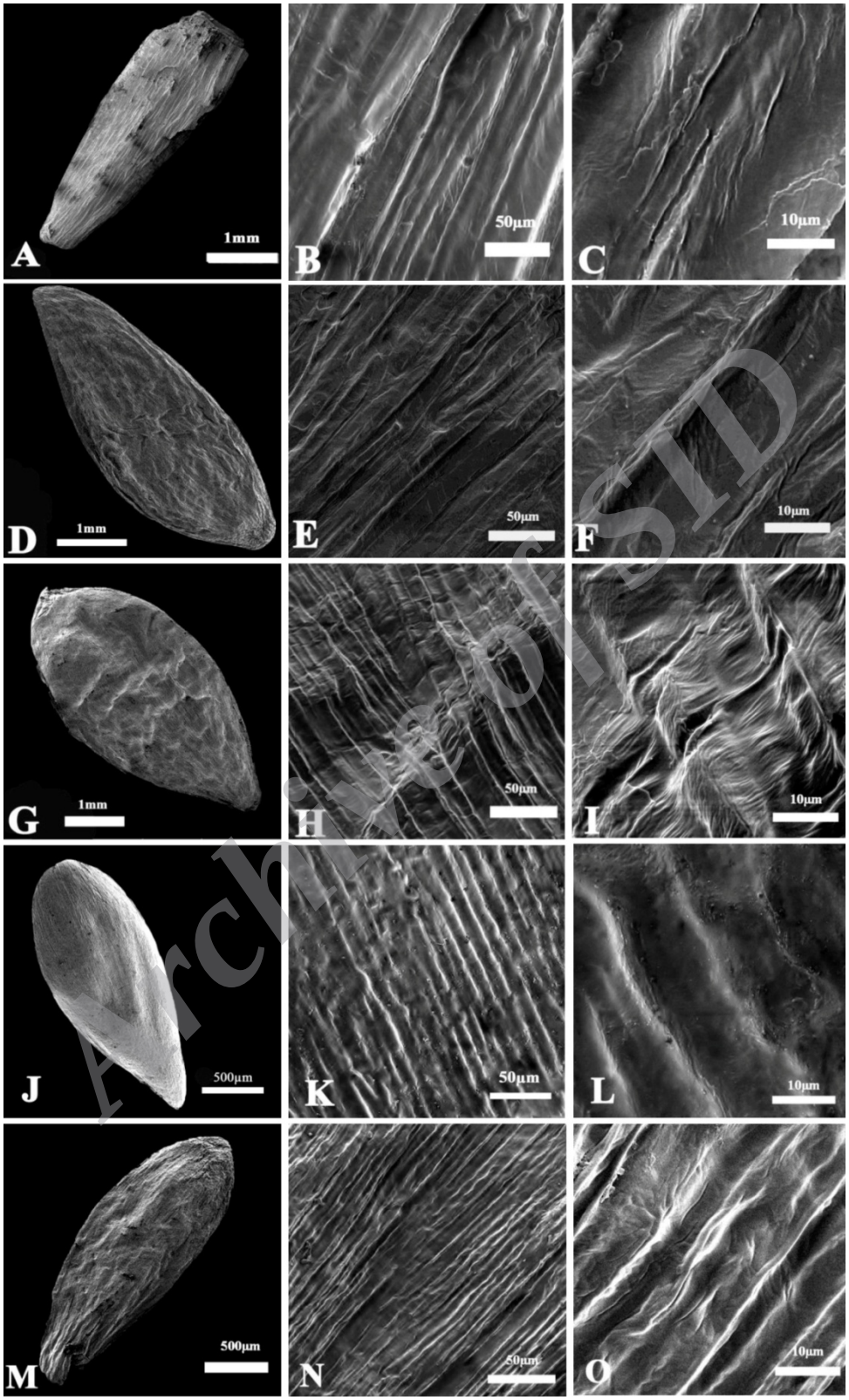

Fig. 3. The SEM micrographs of seed and seed coat in *Geum.* A – C, *G. heterocarpum*; D-F, *G. kokanicum*; G-I, *G. iranicum*; J-L, *G rivale*; M-O, *G. urbanum*..

Exine sculpture types

In all the studied species, the exine sculpturing type (fig. 2 B, E, H and L) is of striate (with ridges and valleys) with randomly scattered micro perforation between the valleys. However, some differences have been observed regarding the ridges intervals and perforation sizes. In *G. heterocarpum* ridges are too compact (fig. 2 B) with minimum ridge intervals of 0.58 µm and in some area the micro perforation are hardly visible. In contrast, in G. *rivale* , the valleys width reaches to its maximun (0.92 µm), (fig. 2 L). In some species *G. iranicum* (fig. 2 E) and *G. kokanicum* (fig. 2 H) the valleys are covered by both small and large perforations. The large perforations are quite abundant in *G. kokanicum.*

Seed micro and macro morphology

The seed morphological data of the studied species and their micrographs are presented in table 3 and fig. 3.

In general the outline of seeds is symmetrical in some species (*G. iranicum* and *G. rivale*) and asymmetrical in others (fig. 3 A, D, G, J and M). Their shape varies from oblong (*G. heterocarpum*, fig. 3 A), to fusiform (*G. kokanicum* and *G. urbanum*, fig. 3 D and M) and oval in *G. iranicum* and *G. rivale* (fig. 3 G and J). The seed dimensions changes from minimum 2.42 × 0.90 mm *G. rivale*) to maximum 4.8 ×1.4mm (*G. kokanicum*). In all the examined representatives, the color of seeds varies from light to dark brown (not given in table).

The epidermal cells are isodiametric, regular elongated, smooth in all the studied taxa.

Two types of cell wall boundaries were identified: 1) Raised, entirely fused smooth and straight. The first type was identified in 3 species of subgenus *Orthstylus* (fig. 3 B-C, E-F and H-I). 2) Raised, entirely fused, smooth, straight and wavy (fig. 3 K-L and N-O). This was recognized in two representatives (*G. rivale* and *G. urbanum*) of subgenus *Geum.*

Outer periclinal cell walls are mainly smooth, flat, in the examined species. However we observed some slightly concave area in *G. iranicum* (fig. 3 I).

Based on the cell arrangement patterns and their outline, two types of seed coats sculpturing were recognized:1) sulcat-ribbed, this type was recorded in *G. heterocarpum*, *G. kokanicum*, *G. iranicum* (fig. 3 B-C, E-F and H-I) . 2) sulcat-foveolate, this type was noticed in *G. rivale* and *G. urbanum* (fig. 3 K-L and N-O).

Fruit morphology

Morphological characters of immature and matured fruits of the studied species are presented in tables 4 -5 and fig. 4. The shape, color, length, ovary dimensions, beak length, position of joint and hairs on style, hook and fish hook characters were among the most outstanding examined traits.

The shape of immature fruit changes from fusiform (*G. heterocarpum* and *G. urbanum*) to fusiform –oval (*G. kokanicum* and *G. iranicum*) and fusiform - elliptical (*G. rivale*). While the mature achene are either fusiform (*G. heterocarpum*, *G. kokanicum* and *G. rivale*) or fusiform-oval (*G. urbanum* and *G. iranicum*) .The outline of mature achenes are symmetrical in *G. rivale*, *G. urbanum*, *G. heterocharpum* and *G. iranicum* and asymmetrical in *G. kokanicum.*

The color of immature fruits, in all the studied species are brown -purple (*G. heterocarpum* and *G. rivale*), dark brown (*G. kokanicum*) and light brown (i*n G. iranicum* and *G. urnanum*). These colors darken with fruit maturity.

The height of immature achene (ovary + style) varies from minimum 3.34 mm (*G. iranicum*) to maximum 13.5 mm (*G. kokanicum*) and the height of mature achene (ovary + beak) changes from minimum 8.53 (*G. rivale*) to maximum 14.25 (*G. kokanicum*). Minimum (1.5±mm) and maximum (3.5 ±mm) length of ovary were recorded in *G. iranicum* and in *G. urbanum* respectively. Minimum ovary width (0.8± mm), in both immature and mature fruits belonged to *G. rivale* and maximum (3± mm) ovary width were measured in *G. kokanicum*.

In all the studied taxa, the style is articulated. As the fruit matures joint moves up words, its upper part falls off and its lower remaining part elongates to form the "beak". The joint position varies among the two sub genus and also between the species. The ratio of style entire length/ joint position is measured in all the studied taxa. Three species of the subgenus *Orthostylus* have the harpoon type fruit in common (fig. 4 A-F), but they show variations regarding the position of the joint on the style. Among them, the style is jointed above the middle (5.7/9.53) in *G. heterocarpum* (fig. 4 A), almost middle (7.33/ 17.11; 9.7/17.15) in G. *kokanicum* (fig .4 B) and almost basal (0.7/5.8 mm) in *G. iranicum*(fig .4 C). The two representatives of the subgenus *Geum*, share fish-hook fruit (fig .4 G-J) and terminal jointed style (the ratio of style entire length/ joint position in 10/15.5 in *G. rivale* (fig 4. G) and 9.5 /11.2 in *G. urbanum* (fig. 4 H). The beak length differs among the studied taxa (table 4).

The position of trichome on immature (fig. 4 G-H) and mature fruits (fig. 4 I-J) differs between the two subgenera and their species.

Table 4 Immature fruits morphological characteristics in 5 species of *Geum* from Iran: Shape (S); Color(C), height (H); Ovary length(OL), Ovary width(OW); Beak length(BL); Joint; Hook (Ha), Fish-hook(FH); Harpoon(Ha)

Species	S	C mm	H mm	OL mm	OW mm	BL mm	J	Ha -FH
G. heterocarpum	Long fusiform	Brown -Purple	6.6	1-2.5 (2.04±0.90)	0.75-1 (0.87±0.13)	2	+	Ha
G. kokanicum	Fusiform-Oval	Dark Brown	13.5	3-3.5 (3.25±0.35)	1.9-2 (1.95±0.07)	5	+	Ha
G. iranicum	Fusiform-Oval	Light brown	3.34	1.2-1.8 (1.5±0.0)	1-1.33 (1.16±0.23)	1	+	Ha
G. rivale	Fusiform-elliptical	Brown -Purple	9.33	2-2.1 (2.03±0.05)	0.5-1 (0.8±0.26)	4.25	+	FH
G. urbanum	Fusiform	Light brown	7.1	2.5-4 (3.05±0.71)	0.75-1.25 (0.9±0.22)	5	+	FH

In the subgenus *Orthostylus* and in *G. heterocarpum* (fig .4 A), hairs appear on the style of immature fruits, especially on the either side of the joint, in two opposite directions and the ovary is also covered by dense long hairs. In the mature fruits, beak apex is covered by retrorse bristles and the mature ovary is more or less hairy (fig. 4 D).

In *G. kokanicumm*, hairs are present on the either side of the joint style (in two opposite direction) but it's distil part is hairless. The ovary of immature achenes are entirely covered by hairs (fig .4 B). In this species hairs position does not change in the mature fruit (fig. 4 E). In *G. iranicum*, the distal part of the style is hair less; the proximity of the join is covered with long erect trichome and the ovary of immature fruit is also covered by dense hairs (fig .4 C). In this species, the mature achen and beak are fully covered by dense hairs (fig. 4 D).

In two species of the subgenus *Geum*, the ovary of immature and mature is covered by long hairs. But portion of the style below the joint and distil part of the beak are hairless (fig. 4 G-J). However the distil part of fish-hook in *G. rivale* (fig. 4 G) is hairless, and in mature fruit long hairs covering ovary extended up to the middle of the beak (fig .4 I). In these two species the beak elongates, the region below the joint curves downward and a fish-hook like structure is formed. The distal part of the fish-hook is vertical in *G. urbanum* and horizontal in *G. rivale*

Data analysis

Principal components analysis (PCA) result shows the distribution of taxa and the variables of the first two components (fig. 4). The first principal component (PC1) scoring system, with 75.3% of the total variation, is characterized by the, fruits hook (h) and beak length (b), position of joint (JP) in immature fruit and rigid intervals (RI) of pollen that split the two subgenera and among them fruit hook (h) and rigid interval (RY) excluded the two species of subgenus *Geum* from others.

In the second component (PC2) scoring system, with 24,7% of the total variation rigid distance (RD) and height (DH) of seed, Joint position (JP) and ovary length (OL) of immature fruit, beak type (b) of mature achene, colpi length (Cl), mesocolpium thickness(Meso), exine thickness (ET), sexinal flap (SF) of pollen grains separated three species of the subgen. *Orthosyylus* (*G. heterocarpum*, *G. kokanicum* and *G. iranicum*). Among them, seed apical (Saw) and basal widths (Sbw), seed length(SL), pollen equatorial (E) and polar (P) axis, immature fruit ovary width (Ow) and fruit length (FL) isolated the *G. iranicum* from the two former species. While *G. heterocarpum* and *G. kokanicum* were separated by pollen exine thickness (ET), mesocolpium (Mes), colpi length (CL) and sexinal flaps *(*SF), seed rigid height (RH) and ovary length(OL) of immature fruit.

DISCUSSION

Pollen morphological data of the genus were primarily reported from general studies on the pollen of Rosaceae (Reitsma 1966; Eid 1981; Hebda & al. 1998; Hebda and Chinnappa 1990; Chung & al 2010). The current results revealed the most outstanding pollen morphological palynological characters within the genus *Geum.* Based on our findings, the polalynological data of Iranian species of *Geum* are in agreement with the previous studies (Hebda & al. 1988; Hebda and Chinnappa 1990). The pollen shape and outline, colpi length, equatorial bridge, marginal pore flaps are identical among the examined taxa and taxonomically uninformative. In contrast, pollen size, and Cl/P ratio, perforation size, sculpturing types are the most distinguishing characters that can provide

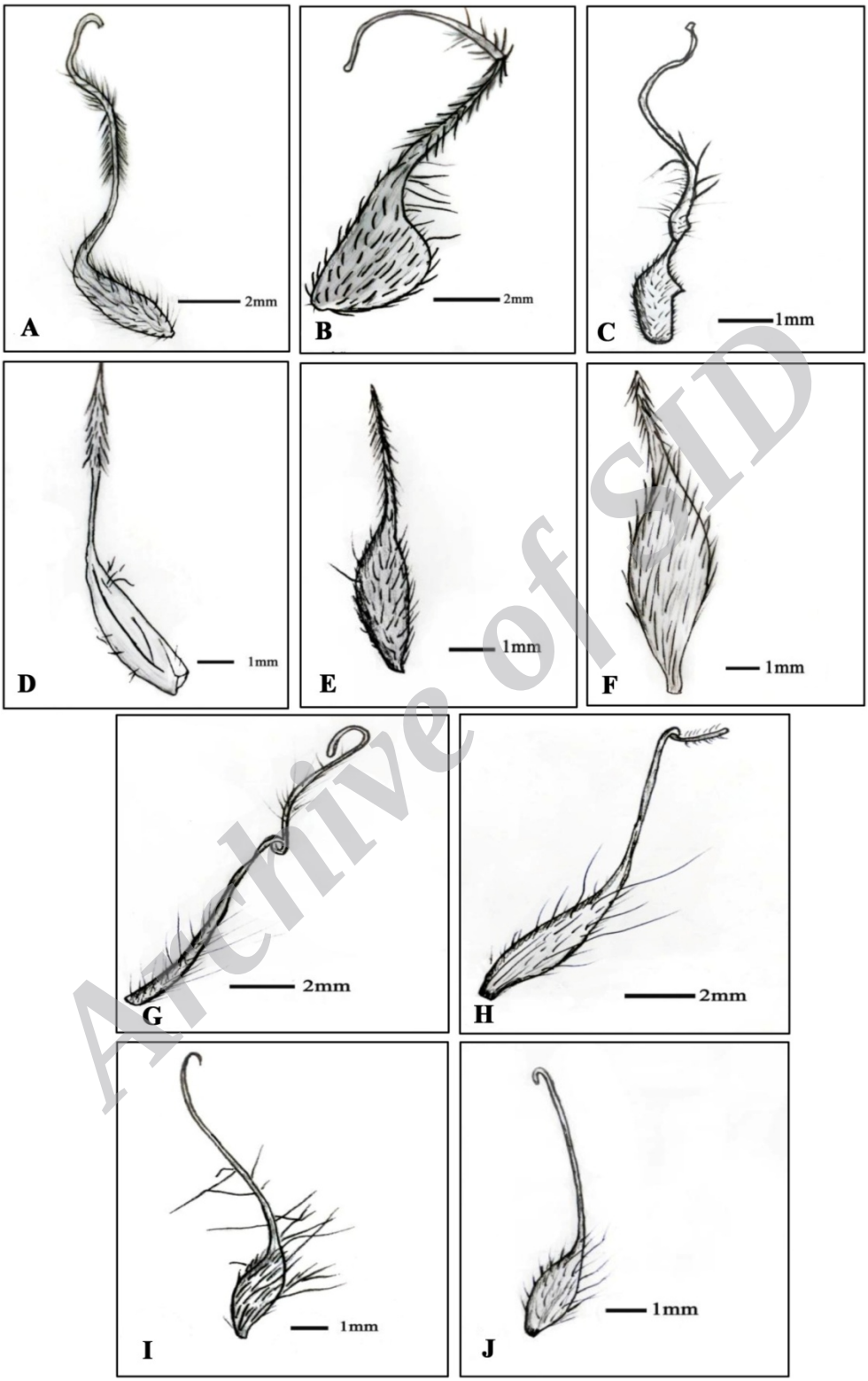

Fig. 4. The illustrated immature and mature fruits of *Geum*: A and D,. *G. heterocarpum*; B and E, *G. kokanicum*; C and E, *G. iranicum*; G and I, *G. rivale*; H and J, *G. urbanum.*

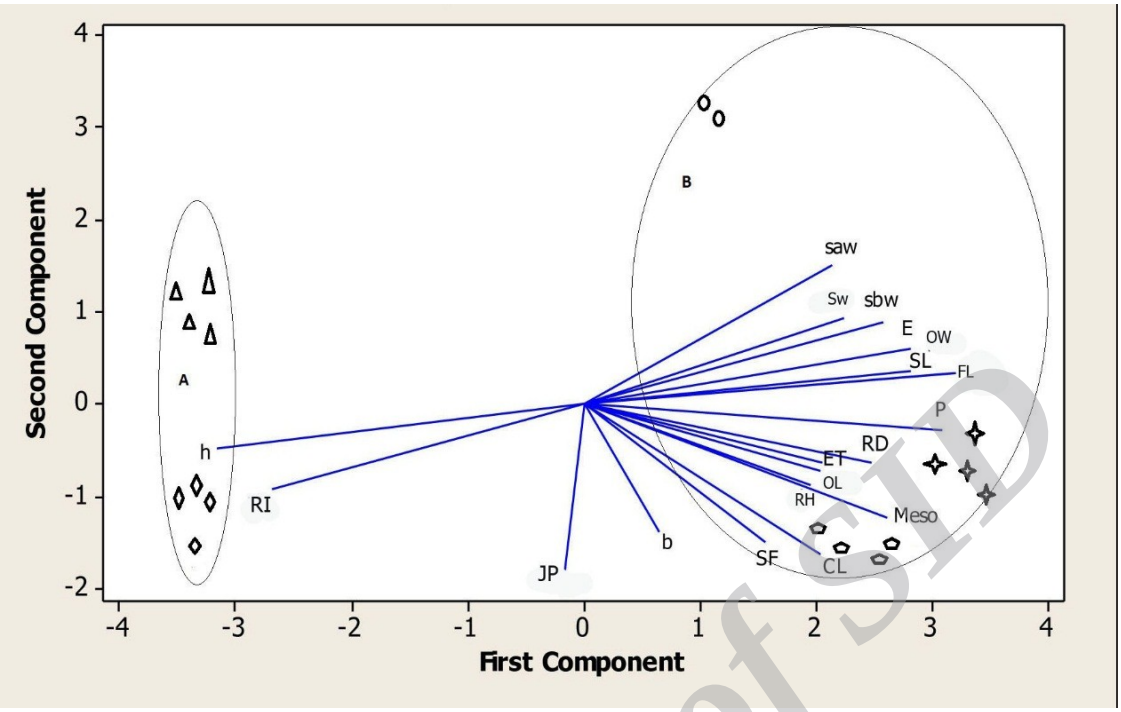

Fig. 5. Principal Component Analysis- Scatter plot expressing the morphological variation of 18 accession of 5 species of *Geum* from Iran, based on mean values obtained from the first two components of Principal Components Analysis (◇= *G. rivale*; △= *G. urbanum*; ✧= *G. kokanicum*; ○*G. iranicum*; ⬭= *G. heterocarpum*).

Table 6. Character and character states used in PCA analysis.* indicates the qualitative character

No	Seed characters	Abbreviations used
1.	Seed length(mm)	SL
2.	Seed width(mm)	Sw
3.	Seed apical width (mm)	Saw
4.	Seed basal width (mm)	Sbw
5.	Rigid distance(mm)	RD
6.	Rigid height(mm)	RH
	Fruits characters	
7.	Ovary length(mm)	OL
8.	Ovary width(mm)	OW
9.	Fruit length(mm)	FL
10.	Beak length(mm)	b
11.	Joint position on the style	JP
12.	presence or absence of fishhook*	h
	Pollen	
13.	Colpi length (mm)	Cl
14.	Exine thickness (mm)	ET
15.	Equatorial axis(mm)	E
16.	Polar axies(mm)	P
17.	Mesocolpium thickness (mm)	Meso
18.	Rigids interval (mm)	RI
19.	Size of exinal flaps, (mm)	SF

good help in species identification. These evidences were reported earlier in many Rosaceae taxa e.g. *Rubus* and *Rosa*, (Moore and Webb 1978; Hebda & al. 1988; Hebda and Chinnappa 1990). The pollen size was similar in 4 examined representatives but differed in *G. urbanum*. This character can be used for identification of this species. Based on the previous authors (Hebda and Chinnappa 1990) colpi length occupies 85 to 90 % of the pollen length and pore situated at the floor of the colpus in the equatorial zone. They also reported well-developed endoaperture (especially in *G. schofieldii* Calder and Taylor) and non operculate pollen in the *Geum* species. However, Chung & al (2010) reported protruded apertures, covered by opercula in *G. canadense.*

Based on our result, among the studied representatives, some pollen grains of *G. heterocarpun* and *G. iranicum* bear protruded apertures, covering colpus, not completely isolated from the remainder of the sexine (Hesse & al. 2009). The equatorial bridge was identified in all the representatives of the genus. The bridge is originated by marginal extension of exine on either side of the colpi in the equatorial zone, which led to formation of so called "equatorial flaps or sexinal flaps" (Moore and Webb 1978). This character has been reported by Hebda and Chinnappa (1990) in other genera of Rosaceae (*Rubus* and *Rosa*).

Exine sculpturing pattern is another taxonomically valuable criterion in the family Rosacea. The striate type of sculpturing has been recognized in several genera of this family as well as *Geum* (Reitsma 1966; Eide 1981; Hebda & al 1988; Ueda and Tomita 1989). Exine ornamentation showed variation among the examined species. In three representatives of the sub genus *Orthostylus*, the ridge intervals were relatively narrow. Among them, *G. heterocarpum* possess the most compactly arranged ridges and invisible micro perforation areas. The species of subgenus *Geum* have striate sculpturing with wide valleys covered by micro perforations and some scattered depression areas. However, our finding revealed larger pores in the valleys of *G. heterocrpum* and *G. iranicum*. The result shows, exine sculpturing is a diagnostic tool for separating the subgenus and species. Based on perforation diameter, Hebda and Chinnappa (1990), placed *Geum*, *Potentilla* and *Fragaria* in the same group.

The current survey showed the exine ornamentation of *Geum* is identical to the members of tribe *Potentillieae* especially that of *Potentilla*, *Argentina* (of subtribe *Potentillinae*), *Fragaria*, *Drymocallis*, *Schistophyllidium* and *Sibalddia* (of subtribe *Fragariinae*) (Faghir et –al. 2014). Several previous researches (Barthlott, 1984; Johnson & al., 2004) explained the importance of micro- morphological characters in species identification, phylogeny (Corner 1976, 1992; Rezk 1980, 1987; Smedmark & al 2002, 2003, 2006), taxonomy and classification of different families (Dowidar 2003; Tantawy and Naseri 2003).

Among the examined seed macro and micro morphological features, the seed color were almost identical in the studied species, therefore do not have taxonomic value. The seeds shape and symmetry varied among the studied taxa and carry diagnostic value for separation some species. The seed dimension is good criterion for separating the two subgenera. The two species of the subgenus *Geum* has smaller seed than the 4 representatives of the subgenus *Orthostylus*.

Tantawy, and Naseri (2003) reported the significance of seed coat morphological evidences in Rosoideae. Seed coat sculpturing of in Iranian species of the genus *Geum* is of two types: 1) sulcat-ribbed (found in 3 representatives of the subgenus *Orthostylus*), 2) sulcat-foveolate (seen in 2 species of the subgenus *Geum*). In addition, *G. heterocarpum* is identified based on it's the highest ridge and *G. iranicum* by its concave priclinal cell wall. Based on the features of the arrangement of epidermal cells, tenuicostate type of seed coats (Gontcharova et –al. 2009) was identified in all the examined species. The seed coat sculpturing and epidermal cells characters can be used for separating the two subgenera and some species. These traits have been used by previous researchers (Barthlott 1981) for separating different taxonomic ranks especially at subgenera and subfamilies.

Fruits morphological traits had significant role in classification of the family Rosacea. Traditionally this family is divided in to four sub families (Spiraeoideae, Rosoideae, Prunoideae and Maloideae) based on fruit characteristics (Fock 1894; Robertson 1974; Cronquist 1981).

According to the current research, among the examined traits, the fruits color and length (including mature ovary + beak) were almost identical and do not have systematic importance. The fruits outline was symmetrical (except in *G. kokanicum*) and their shape varied from species to species can be used for separating the species. However the position of the joint on the style (or the ratio of style entire length/ joint position in immature fruit), fruit types (harpoon or fish-hook), position of hairs and beak length are the most significant characteristic features of these species. These evidences also influenced the taxonomy of tribe Colurieae, as a unique tribe in Rosoideae, in having styles that do not wither at maturity, instead, they elongate into long villous plumes or stiff beaks (Smedmark 2006),.

Comparative ontogeny of Colurieae (Smedmark 2002, 2003, 2005, 2006), revealed how polyploidy can effect joint position on the style and fruit type in these plants and especially in *Geum* species which carry 94% of polyploidy within the family Rosaceae, (Vamosi and Dikinson 2006).

According to Smedmark and Eriksson (2006), The diploid ancestral species (G. *waldsteianiae*) of the genus has basal joint, completely deciduous style; the tetraploid taxa (*G. heterocarpum*) has joint on central position, partly deciduous style, beak with retrose bristles and harpoon shape fruit (Iltis 1913) and the hexaploid representatives (which constitutes about 40 species e.g. G. *urbanum* and *G. rivale*) have terminal joint, fully deciduous style and fruit with fish hook (Iltis 1913). Our observation were similar to that of Smedmark and Eriksson (2006) for three studied taxa including: G. *heterocarpum*, *G. urbanum* and *G. rivale*. But, regarding the position of joint on style, *G. kokanicum* (distributed in central Asia, C and E Iran) resembles to tetraploid species, and *G. iranicum* , an endemic to E Iran (Khatamsaz 1993) shows affinity to diploid ancestral species of the genus. The species of the genus are in urgent need of a cytological study (including the exact chromosome number or C-value analysis).

We used pollen, seed, and fruit morphological characters in PCA analysis. In this analysis, the examined species were divided in two groups using important pollen, seed, and fruit morphological characters. Among them fruit and pollen characters (especially fish-hook, beak length, Joint position, and rigid interval) are the most taxonomically informative traits for separating the two subgenera and species of the subgenus *Geum*. PCA analysis revealed that three species of subgenus share fruit, pollen and seed characters and among them, *G. heterocarpum* and *G. kokanicum* are distinguished from G. *iranicum* by seed length, apical and basal width, ovary width, pollen equatorial and polar axis and fruit beak length. While *G. heterocarpum* and *G. kokanicum* are identified by pollen and seed characters. The PCA result is most congruent with the current classification presented in Flora Iranica and Flora of Iran (Schonbeck-Temesy 1969; Khatamsaz 1993) and shows that seed, fruit and pollen morphological traits are reliable criteria for delimitation of subgenera and species of the genus *Geum* in Iran. An identification key is presented based on distinguishing studied characters as follows:

1- Fruit harpoon type, seed coat sculpturing sulcat-ribbed type 2
- Fruit with fish-hook, seed coat sculpturing sulcat-favularit type 4
2-Joint at the base of the style ..... *G. iranicum*
- Joint middle or above middle of the style 3
3-Entire beak length and ovary covered by hairs, exine sculpturing striate with micro perforation, with many large pore ..... *G. kokanicum*
- Distal portion of the beak covered by retrorse bristles, exine sculpturing compact striate with very few micro perforation or without it .... *G. heterocarpum*
4- Fishhook horizontal, seed outline asymmetrical and achene with dense hair extending up to the middle of the beak ..... *G. urbanum*
- Fishhook vertical, seed outline symmetrical; achene with dens long hairs, beak hairless ..... *G. rivale*

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REFERENCES

Abdel Khalik, K. N. 2006: Seed morphology of *Cuscuta* L. (Convolvulaceae) in Egypt and its systematic significance. - Feddes Repertorium. 117 (3–4): 217–224.

Barthlott, W. 1981: Epidermal and seed surface characters of plants: systematic applicability and some evolutionary aspects. - Nord. J. Bot. 1: 345–354.

Barthlott, W. 1984: Microstructural features of seed surfaces. In: Heywood, V.H., Moore, D.M. (Eds.), Current Concepts in Plant Taxonomy. 95– 105. Academic Press. - London.

Bolle, F. 1933: Eine U¨ bersicht u¨ber die Gattung *Geum* L. und die ihrnahestehenden Gattungen. Feddes Repertorium Beih 72:1–119.

Corner, E. J. H. 1976. The Seeds of the cotyledons. Cambridge University Press. - Cambridge

Cronquist, A. 1981: An integrated System of Classification of Flowering Plants. - Columbia University Press, - New York.

Chung, K. S., Elisens, W.J. & Skvarla, J. J. 2010: Pollen morphology and its phylogenetic significance in tribe Sanguisorbeae (Rosaceae). - Plant. Syst. Evol. 285:139–148

Dowidar, A. E., Loutfy, M. H. A., Kamel, E.A., Ahamed, A.M. & Hafez, H .L. 2003: Studies on the Rosaceae -Seed and/or achene macro and micromorphology. - Pakistan J. Biol. Sc. 6:1778-1791.

Eide, F. 1981: Key for Northwest European Rosaceae pollen. - Grana 20: 101-118.

Erdtman, G. 1952: Pollen morphology and plant taxonomy.Angiosperms. Almqvist and Wiksells. - Stockholm.

Faghir, M. B., Attar, F., Farazmand, A. & Kazempur Osaloo, S. 2014: Phylogeny of the genus *Potentilla* (Rosaceae) in Iran based on nrDNA ITS and cpDN *trnL*-F sequences with a focus on leaf and style characters' evolution. - Turk. J. Bot. 38: 417-429.

Fock, W. O. 1894: Rosaceae. Pp. 1–60 in Die Natu¨rlichen Pflanzenfamilien, ed. A. ngler. Vol. 3. - abteilung 3. Leipzig: Wilhelm Engelmann.

Gajewski, W. 1957: A cytogenetic study on the genus *Geum*. Monographiae Botanicae .4: 3–414.

Gajewski, W. 1958: Evolution in the genus *Geum*. - Evolution. 13: 378–388.

Gajewski, w. 1968: *Geum* L. Flora Europaea, eds. T. G. Tutin, V. H. Heywood, Burges, D. M. Moore, D. H. Valentine, S. M. Walters, and D. A. Webb., in Cambridge: Vol. 2: 34–36 .-Cambridge University.

Gontcharova, S.B. 2006: Sedoideae (Crassulaceae) of the Russian Far East flora. -Dalnauka, Vladivostok.

Gontcharova, S. B, Gontcharov, A.A, Yakubov, V.V. & Kondo, K. 2009: Seed surface morphology in some representatives of the Genus *Rhodiola* sect. Rhodiola (Crassulaceae) in the Russian Far East. - Flora. 204: 17—24

Harley, M. M. 1992. The potential value of pollen morphology as an additional taxonomic character in subtribe Ociminae (Ocimeae, Nepetoideae, Labiatae). In: Harley RM, Reynolds T, editors. Advances in Labiatae Science. 125–138. Richmond, UK: Royal Botanic Gardens, Kew. 125–138.

Hebda, R. J. & Chinnappa, C. C. 1990: Studies on the pollen morphology of Rosaceae in Canada. - Rev. Palaeobot. Palynol. 64(1-4):103-108.

Hebda, R. J., Chinnappa, C. C. & Smith B, M. 1988: Pollen morphology of the Rosaceae of Western Canada. - Grana. 27: 95-1 13

Hesse, M., Halbritter, H., Zetter, R., Weber, M., Buchner, R., Frosch-Radivo, A. & Ulrich, S., 2009: Pollen terminology, An Illustrated Handbook. Springer Wien, New York. 264 pp

Huber, H. 1961: Hegi—Illustrierte Flora von Mitteleuropa. Mu¨nchen: Carl Hanser. ed. 2. 4(2a):231–438.

Hutchinson, J. 1967: The genera of flowering plants. Oxford: Oxford University.

Iltis, H. 1913: U¨ ber das gynophor und die Fruchtausbildung beider Gattung *Geum*. Sitzungsberichte der Ko¨niglich Preussischen Akademie der Wissenschaften zu .Berlin .122: 1–36.

Johnson, L.A., Huish, K.H., & Porter, J. M. 2004: Seed surface sculpturing and its systematic significance in *Gilia* (Polem-oniaceae) and segregated genera. - Int. J. Plant Sci.165: 153–172.

Kalkman, C. 1988: The phylogeny of the Rosaceae. - Bot. J. Linn. Soc. 98: 37–59.

Khatamsaz, M. 1993: Flora of Iran. Rosaceae. Research Institute of Forests and Rangeland Press. 6:88-140. -Tehran.

Linnaeus. C. 1753: Species Plantarum. Stockholm: Laurentius Salvius.

Moore, P. D. & Webb, J. A. 1978: An illustrated guide to pollen analysis. - IIodder and Stoughton, London.

Moore, P. D., Webb, J. A. and Collinson, M. E., 1991: Pollen analysis, second edition. -Blackwell scientific publications. Oxford

Punt, W., Hoen, P. O., Blackmore, S., Nilsson, S. and Thomas, A. L. 2007: Glossary of pollen terminology. -Rev. Palaeobot. Palynol. 143: 1–81.

Ryan, B.F & Joiner, B.L. 2001: MINITAB Handbook, Fourth Edition. Duxbury.

Rezk, MR. 1980: Seed structure as phylogenetic criterion. A case of *Plantago* seed. -Egypt J. Bot. 23: 51-62.

Rezk, M. R. 1987: Variation in seed coat micro-sculpture in 5 species of *Plantago*. -Alex. Sci. Exch., 8(3).

Reitsma, T.J. 1966: Pollen morphology of some European Rosaceae. -Acta Bot. Neerl. 15:290 307.

Robertson, K. R. 1974: The genera of Rosaceae in the southeastern United States. -Journal of the Arnold Arboretum. 55: 611–662.

Smedmark, J. E. E. & Eriksson T. 2002: Phylogenetic relationships of *Geum* (Rosaceae) and relatives inferred from the nrITS and *trnL-trnF* regions. -Sys Bot. 27(2): pp. 303–317

Smedmark, J. E. E., Eriksson, T. & Bremer, B. 2005: Allopolyploid evolution in Geinae (Colurieae: Rosaceae): building reticulate species trees from bifurcating gene trees.- Org Divers. Evol. 5:275–283.

Smedmark, J. E. E., Eriksson, T., Evans, R. C. & Campbell, C.S. 2003: Ancient allopolyploid speciation in Geinae (Rosaceae): evidence from nuclear granule-bound starch synthase (GBSSI) gene sequences. -Syst Biol. 52:374–385

Smedmark, J. & Eriksson, T. 2006: early stage of development shed light on fruit evolution in allopolyploid species of *Geum* (Rosaceae). - Int. J. Plant Sci. 167(4):791–803.

Schönbech-Temesy , E. 1969: *Geum* (Rosaceae). Flora Iranica (ed. Rechinger, K. H.). 66 (30) 4: 116-121.

Scheutz, N. J. 1870: Monographiae Georum. Uppsala: Berling.

Schulze-Menz, G. K. 1964: Rosales. Pp. 193–242 in *A.* Engler's Syllabusder flanzenfamilien, ed. H. Melchior. Vol. 2. -Berlin: Gebru¨der Borntraeger.

Svetlana, BG, Andrey, AG, Valentin, VY . &

Yakubov, K K. 2009: Seed surface morphology in some representatives of the Genus Rhodiola sect. Rhodiola (Crassulaceae) in the Russian Far East. Flora, 204: 17-24.

Tantawy, M. E. & Naseri, M. E. 2003: Contribution to the Achene Knowledge of Rosoideae (Rosaceae) LM and SEM. – IJAB. 2:105–112

Ueda, Y. & Tomita, H. 1989: Morphometric analysis of pollen exine patterns in roses. – J. Japan. Soc. Hort. Sci. 58: 211–220

Vamosi, J.C. & Dickinson, T.A. 2006: Polyploidy and diversification: A Phylogenetic investigation in Rosaceae.-Plant Sci. 167(2):349–358.

Yuzepchuk, S. 1941. Subfamily Rosoideae. Flora of the USSR, ed. V. L. Komarov. Vol. 1 3–378. Moskva. Leningrad. Israel Program for Scientific Translations.