Morphological Variation and Palynomorphology of *Rosa laxa* in Xinjiang, China

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ABSTRACT. *Rosa laxa* is widely distributed in the Xinjiang Uygur Autonomous Region of China and is highly adaptable and rich in variation. In this study, we investigated the morphology, habitats, and palynomorphology of *R. laxa* botanical varieties from Xinjiang, China. In addition to *R. laxa* var. *laxa*, there were three other botanical varieties of *R. laxa* growing in southern Xinjiang, including var. *mollis*, var. *kaschgarica*, and var. *tomurensis*. Together, these four botanical varieties exhibited morphological variation, mainly in the morphology of prickles and the glandular trichome and in flower color. The pollen grains of the *R. laxa* botanical varieties, all medium in size (21.77–48.39 μm), came in three shapes: perprolate, prolate, and subspheroidal. Their pollen exine sculptures were characterized by either a striate-perforation pattern or striate pattern, but perforation varied in terms of diameter and density and striae varied in depth and density. Palynomorphological assessment showed that three types of evolution, i.e., primitive, transitive, and evolved, were present among *R. laxa* botanical varieties, and pollen dimorphism was observed in the same botanical variety. Perprolate pollen with a dense striate pattern was the most evolved type. Based on morphological and palynomorphological investigations, var. *tomurensis* was considered to be the most evolved one among the studied botanical varieties.

As one of the biodiversity centers of wild *Rosa*, China is home to 95 species (65 endemic) of *Rosa*, accounting for nearly half of the world total (Ku and Kenneth, 2003). The Xinjiang Uygur Autonomous Region is a large and sparsely populated area located in the northwest of China covering 1.6 million km^2 (about one-sixth of the total land area of China). Separated by the Tianshan Mountains, north Xinjiang is characterized by temperate climate, whereas south Xinjiang possesses a warm temperate climate. These complex environments make Xinjiang host to a rich variety of plant species. Although officially 14 species (including three botanical varieties) of wild *Rosa* are found in this region (Ku and Kenneth, 2003), there may be as many as 22 wild *Rosa* species in the region (Bao, 1993; Liu, 1993; Liu and Cong, 2000; Ma and Chen, 1990).

There are about 50 species of *Rosa* section *Cinnamomeae*, 36 of which are in China (26 endemic) (Ku and Kenneth, 2003; Yu et al., 1985). *Rosa laxa* var. *laxa* is one of these 36 species, and it grows extensively in the Xinjiang region only. In addition to the var. *laxa*, three other botanical varieties of *laxa* are also known to grow in Xinjiang (Liu and Cong, 2000), although only *R. laxa* var. *mollis* is recorded in the *Flora Republicae Popularis Sinicae* (Yu et al., 1985) and *Flora of China* (Ku and Kenneth, 2003). In the *Flora of China*, *R. laxa* is described as follows:

Shrubs 1–2 m tall. Branchlets terete, straight or slightly curved, glabrous; prickles in pairs below leaves or scattered. Leaves including petiole 4.5–10 cm; leaflets 7–9, elliptic, oblong, or ovate, rarely obovate, 1.5–4 cm long, 1–3 cm wide, glabrous or pubescent. Flowers often 3–6 in corymb, sometimes solitary, 1.5–3 cm in diam.; pedicel 1–1.8 (–3) cm, glandular; bracts ovate, pubescent, glandular hispid, apex acuminate. Sepals 5, ovate-lanceolate, leaflike, abaxially sparsely pubescent and glandular hispid, adaxially densely pubescent, margin entire. Petals 5, white or pink, obovate, apex erose. Hip red, oblong or ovoid, 1–1.8 cm in diameter, glabrous, often shiny, apex with a short neck and persistent, erect sepals.

Other recorded botanical varieties of *R. laxa* include 1) var. *mollis*, 2) var. *kaschgarica*, and 3) var. *tomurensis*. *Rosa laxa* varies considerably in terms of morphological characteristics. There is abundant inter- and intrapopulation genetic diversity among six natural populations of *R. laxa* var. *laxa* in the Tianshan Mountains of north Xinjiang (Guo, 2010). However, there is little information in the literature focused on the detailed descriptions (and specimens) of these botanical varieties in the Xinjiang region. Furthermore, only a relatively small area has been previously surveyed for *R. laxa*, although it is well known that Xinjiang is a biodiversity center of *Rosa*.

In this study, we reported the results of a recent survey (2007–18) of the distribution of *R. laxa* botanical varieties and their morphological characteristics in the Xinjiang region. In addition to the usual morphological characters, we also measured pollen traits. Because the morphological characteristics of pollen grains are generally unaffected by environmental factors but exhibit strong genetic stability, considerable evolutionary information regarding species and botanical varieties may be inferred from

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pollen characteristics (Wang and Wang, 1983; Wrońska-Pilarek and Jagodziński, 2011).

Material and Methods

SURVEY AREA. Cities, counties, or districts were selected from the following 13 areas across the Xinjiang region (Fig. 1; Table 1), including all regions where climatic conditions are suitable for *Rosa* species: 1) Altay city, Habahe County, Burjin County, and Fuhai County (Altay Prefecture, Ili Kazakh Autonomous Prefecture); 2) Tacheng city, Emin County, and Yumin County (Tacheng Prefecture, Ili Kazakh Autonomous Prefecture); 3) Bole city and Jinghe County (Bortala Mongol Autonomous Prefecture); 4) Kuitun city, Yining city, Gongliu County, Tekes County, and Nilka County directly under the control of Ili Kazakh Autonomous Prefecture; 5) Dushanzi

district of Karamay city; 6) Manas County (Shihezi city); 7) Tianshan district and Urumqi County (Urumqi city); 8) Turpan city and Toksun County (Turpan Prefecture); 9) Korla city and Luntai County (Bayingolin Mongol Autonomous Prefecture); 10) Aksu city, Wensu County, and Kuqa County (Aksu Prefecture); 11) Artux city (Kizilsu Kirghiz Autonomous Prefecture); 12) Kashgar city, Shule County, Shufu County, and Yingjisha County (Kashgar Prefecture); and 13) Hotan city (Hotan Prefecture).

PLANT MATERIALS. *Rosa laxa* pollen samples were collected and preserved in the specimen bank of the China National Engineering Research Center for Floriculture, Beijing, China (Table 1).

METHODS AND ANALYSIS. The morphology of each *R. laxa* botanical variety was identified and assessed to assign each collection to a specific *R. laxa* using the *Flora of China* (Ku and Kenneth, 2003), *Flora Republicae Popularis Sinicae* (Yu et al.,

Fig. 1. Survey areas in 13 prefectures/cities of Xinjiang, China (2007–18): (A) Altay Prefecture (subordinate to Ili Kazakh Autonomous Prefecture); (B) Tacheng Prefecture (subordinate to Ili Kazakh Autonomous Prefecture); (C) Bortala Mongol Autonomous Prefecture; (D) directly controlling area of Ili Kazakh Autonomous Prefecture; (E) Karamay city; (F) Shihezi city; (G) Urumqi city; (H) Turpan Prefecture; (I) Bayingolin Mongol Autonomous Prefecture; (J) Aksu Prefecture; (K) Kizilsu Kirghiz Autonomous Prefecture; (L) Kashgar Prefecture; (M) Hotan Prefecture.

Table 1. Pollen samples of *Rosa laxa* for palynomorphologic study.

No.	Latin name	Voucher specimen	Location
R1	<i>R. laxa</i> var. <i>laxa</i>	Luo le, bjfu-xj-09008	Sandy land and slope shrub, Kuitun city, 685 m
R2	<i>R. laxa</i> var. <i>laxa</i>	Luo le, bjfu-xj-10035	Brook bank and forest edge, Wensu County, Aksu city, 1,108 m
R3	<i>R. laxa</i> var. <i>laxa</i>	Luo le, bjfu-xj-10085	Saline-alkaline land, Xiaocaohu, Turpan city, 330 m
R4	<i>R. laxa</i> var. <i>mollis</i>	Luo le, bjfu-xj-09024	Forest edge, Xiaodonggou, Altay city, 982 m
R5	<i>R. laxa</i> var. <i>kaschgarica</i>	Luo le, bjfu-xj-10101	Gravel land, Kashgar city, 1,001 m
R6	<i>R. laxa</i> var. <i>kaschgarica</i>	Luo le, bjfu-xj-10083	Saline-alkaline land, Tiemenguan, Korla city, 1,012 m
R7	<i>R. laxa</i> var. <i>kaschgarica</i>	Luo le, bjfu-xj-10095	Brook bank, Nature Tuomuer, Aksu city, 1,399 m
R8	<i>R. laxa</i> var. <i>kaschgarica</i>	Luo le, bjfu-xj-10072	Seasonal river valleys, Wensu County, Aksu city, 1,312 m
R9	<i>R. laxa</i> var. <i>tomurensis</i>	Luo le, bjfu-xj-10113	Seasonal river valleys, Nature Tuomuer, Aksu city, 2,103 m
R10	<i>Rosa</i> sp.	Luo le, bjfu-xj-10115	Seasonal river valleys, Nature Tuomuer, Aksu city, 2,103 m

1985), *Rosa in Xinjiang* (Liu and Cong, 2000), and *Flora Xinjiangensis* (Han, 1995). In addition, several samples were assessed for pollen traits (Table 1). All pollen samples were prepared and analyzed following published protocols (Erdtman, 1952; Moore et al., 1991). Pollen grains were randomly collected from a flower bud that was close to opening. Then, the single pollen grain was prepared in an air-dried condition in the shade for 2–3 d and vacuum-coated with gold (Hesse et al., 2009). A scanning electron microscope (S-3400 N; Hitachi Science Systems, Tokyo, Japan) was used to scan the pollen, and representative visual fields at $\times 500$, $\times 2000$, and $\times 6000$ of the pollen under equatorial or polar views and the exine sculpture, respectively, were photographed. Twenty well-developed pollen grains were randomly selected from each sample, and their polar length (P), equatorial length (E), P/E ratio, aperture length, aperture width, perforation diameter, foveae density, and striae width, distance, and density were measured using Image-Pro Plus 6.0 (Media Cybernetics, Rockville, MD). Duncan's multiple range test was used to compare the means of pollen characters among the specimens, and SPSS (version 13.0, IBM Corp., Armonk, NY) was used to assess both intra- and interpopulation variation. The palynomorphological terminology used follows Erdtman (1952), Reitsma (1970), Wang and Wang (1983), Moore et al. (1991), and Punt et al. (2007).

Results

General descriptions of *R. laxa* botanical varieties

The survey results confirmed that in addition to *R. laxa* var. *laxa*, three other botanical varieties (var. *mollis*, var. *kaschgarica*, and var. *tomurensis*) were also present in Xinjiang.

ROSA LAXA VAR. LAXA. *Rosa laxa* var. *laxa* represents the original botanical variety of *R. laxa*. It is widely distributed in sandy or rocky areas, north-slope shrub, dry riverbeds, stream sides, forest edge, and near farmland at altitudes of 500–2500 m in Altay, Tacheng, Ili, and Urumqi in north Xinjiang. This botanical variety was also found in the Gobi desert, saline-alkaline land and flood land, and near farmland at altitudes of 700–2700 m in Turpan, Korla, Aksuand, and Kashi in south

Xinjiang (Fig. 2A–F). Its morphological characteristics varied greatly, as follows:

Leaflet: oval or oblong in areas with sufficient water, with smooth petioles or a few small and scattered prickles; subrotund or obovoid in arid areas, with smaller leaf size and glandular pubescence or puberulous on petioles (Fig. 2A).

Prickle: Recurved, broadly dilated at base, especially in burgeon, but to varying degrees; varying frequency of prickles with higher frequency in arid areas (Fig. 2B).

Fig. 2. *Rosa laxa* var. *laxa* [(A) leaflet glabrous; (B) prickle recurved with broad base; (C) bud with sepals, pedicel glandular pubescent or glabrous; (D) flower white; (E) bud and flower pink, often 3–6 in corymb; (F) hip red, oblong or ovoid, apex with a short neck and persistent, erect sepals]; *R. laxa* var. *mollis* [(G) leaflet densely pubescent on both surfaces; (H) hip red, 3–6 in corymb]; *R. laxa* var. *kaschgarica* [(I) leaflet thickness, almost leathery, mostly oval, base subrounded or cuneate; (J) old branch 10–15 cm in diameter; (K) prickle significantly and strongly hooked; (L) large shrub near the water with 3.8 m shrub crown; (M) flower white or pale pink; (N–O) bud often with pink mark; (P) hip red, ovoid or nearly spherical; (Q) a variant in Shenmu Park, leaflet almost leathery, very narrow oval, flower mostly solitary].

Glandular pubescence: Greatest changes in pedicel, receptacle, and sepals (Fig. 2C); pedicel glandular pubescent or glabrous; sometimes premature glandular pubescence shedding, leading to smooth surface of stalk and hip during the fruiting stage.

Flower: Often three to six in corymb, sometimes solitary, mostly in south Xinjiang; white petals, but buds often with pink tips just before flowering (Fig. 2D and E).

R. LAXA VAR. MOLLIS. *Rosa laxa* var. *mollis* is easily distinguished from *R. laxa* var. *laxa* by its dense pubescence

on both adaxial and abaxial leaflet surfaces. It contains white flowers in the corymb, with glandular pubescence on the pedicels and sepals. This botanical variety was only found in wet meadow or poplar forests at altitudes of 580–1100 m in Altay-Xibodu in north Xinjiang (Ku and Kenneth, 2003; Liu and Cong, 2000; Yu et al., 1985) (Fig. 3G and H). However, it was found to be more widespread throughout Xinjiang than previously reported. *R. laxa* var. *mollis* was frequently found at

Fig. 3. *Rosa laxa* var. *tomurensis* [(A) leaflet glabrous, elliptic or oblong; prickles with nonobvious camber in tip, branchlet often purplish red; (B) pedicel glandular pubescent or glabrous, long; (C–D) flower white and big with long pedicel, mostly solitary; (E) habitat near river bank; (F) hip large, often pendulous, stalk slightly swelling]; new variant of *R. laxa* in the Tuomuer Nature Reserve [(G–I) flower pale pink in corymb, sometimes solitary; (J) leaflet almost leathery, oblong; (K) hip oval or flat round, with erect sepals persistent or not; (L) prickles slightly curved]; *R. beggeriana* [(M) hip round or flat round; sepal deciduous]; *R. albertii* [(N) hip oblong or pyriform; sepal deciduous].

Fig. 4. Scanning electron microscope images of pollen grains. (A–H) Pollen grains of *Rosa laxa* var. *laxa* [(A) perprolate pollen grain view for sample R1 ($\times 500$); (B) pollen grain view with dimorphic shape of sample R2, prolate and subspheroidal ($\times 500$); (C) polar view of sample R2, obtuse-triangular of subspheroidal pollen on the left, trilobate-circular of prolate pollen; exine sculpture with tricolpus ($\times 2000$); (D) equatorial view of prolate pollen of sample R2, long-elliptic; elongated, narrow colpus nearly as long as the polar axis ($\times 2000$); (E) equatorial view of subspheroidal pollen of sample R2, circular or subcircular; spindle-shaped colpus ($\times 2000$); (F) sculpture view of perprolate pollen of sample R1, thin striate, almost parallel with pollen axis, with light muri and mostly large perforation between wide gaps ($\times 6000$); (G) sculpture view of prolate pollen of sample R2, deep, high-density striate-perforate ($\times 6000$); (H) sculpture view of subspheroidal pollen of sample R2, striae running almost parallel with pollen axis, scarcely perforate, like multiple ornamentation with striae and subsidence puncture ($\times 6000$); (I–M) pollen grains of *R. laxa* var. *mollis* of sample R4 [(I) polar view, trilobate-circular; exine sculpture with tricolpus ($\times 2000$); (J–K) equatorial view long-elliptic, prolate shape, with flat axis ($\times 6000$); (L–M) exine sculpture densely striate; perforation irregular (fewer and smaller in L, denser and larger near colpus in M) ($\times 6000$); (N–O) pollen grains of *R. laxa* var. *kaschgarica* [(N) perprolate pollen grain view for sample R7 ($\times 500$); (O) prolate pollen grain view of sample R5 ($\times 500$)].

the Xiaodonggou Forest Farm and the Habahe River in north Xinjiang coexisting with *R. laxa* var. *laxa*, *Berberis*, *Cotoneaster*, *Caragana*, and *Populus*. The sporadic presence of *R. laxa* var. *mollis* was also observed along the river from Aksu to

Atushi in south Xinjiang, often in clusters of two to five plants or coexisting with *R. laxa* var. *kaschgarica* at altitudes of 1500–2000 m.

***R. LAXA* VAR. *KASCHGARICA*.** The traits that separate *R. laxa* var. *kaschgarica* from *R. laxa* var. *laxa* include branchlets often purplish or reddish-brown; prickles in pairs below leaves, strongly hooked on old branches, and dilated proximally at the base (Fig. 2K); leaflets smaller than those of *R. laxa* var. *laxa*, thick, almost leathery, mostly ovoid or ovate-oblong, margin entire below middle, base subrotund or cuneate, pubescent often on both adaxial and abaxial surfaces but fewer on young leaflets, adaxial surface of old leaves covered with white waxy powder (Fig. 2I–K); white or pale pink small flowers, three to five flowers per corymb (Fig. 2M–O); and ovoid or subrotund hips, smaller than those of *R. laxa* var. *laxa* (Fig. 2P). The flowering period lasts for 6–8 months and the fruiting stage for 8–10 months.

This botanical variety was found only in the Tarim Basin, as reported in *Rosa in Flora Xinjiangensis* (Han, 1995) and *Rosa in Xinjiang* (Liu and Cong, 2000). It was present only at altitudes of 750–1700 m, mainly in arid plains (e.g., the Gobi, arid slopes, seasonal river valleys, gravel land, and saline-alkaline land, especially in the Kashgar area) in south Xinjiang. It is also reported to grow in central Asia, Siberia, and Mongolia (Han, 1995; Liu and Cong, 2000).

A new variant type (narrow leaf shape) of this botanical variety was found in Shenmu Park in Wensu County during this survey (Fig. 2Q). The leaflets of this variant of *R. laxa* var. *kaschgarica* are almost leathery, but narrow oval in shape. Flowers are mostly solitary, white, buds with pink tips, and pedicels are short, about 0.5–1 cm. Furthermore, large shrubs (3–4 m in shrub canopy, 2–4 m in height, with old branches 10–15 cm in diameter) of this variant of *R. laxa* var. *kaschgarica* were found in habitats close to water (Fig. 2J–L). The almost leathery leaflets distinguish this variant from the original botanical variety.

***R. LAXA* VAR. *TOMURENSIS*.** This botanical variety is characterized by the following features: branchlets often purplish red; prickles with nonobvious camber in tip (Fig. 3A); flowers often solitary, rarely in clusters of 2–3 flowers; often large solitary

flowers (1.5–2.5 cm in diameter, Fig. 3B–D); long pedicels, up to 2.5 cm (Fig. 3B and F); pedicel glandular pubescent or glabrous; large and shiny hips, 1.5–2 cm long, 1–1.5 cm wide, and often pendulous; and fruit-stalks slightly swelling (Fig. 3E and F). The flowering period lasts for 6–7 months and the fruiting stage for 8–10 months.

This botanical variety, which was not reported in *Flora Reipublicae Popularis Sinicae* (Yu et al., 1985) or *Flora of China* (Ku and Kenneth, 2003), was proposed by Liu and Cong (2000). This survey confirmed that this botanical variety has a stable trait profile and is present only at altitudes of 1650–2200 m in shrubs at river banks (Fig. 3E) and in river valleys in arid, hilly areas in the Tuomuer Nature Reserve in Wensu County, south Xinjiang. This area has an arid climate, and the temperature and rainfall vary greatly at different altitudes. *Rosa laxa* var. *tomurensis* was often found coexisting with *R. laxa* var. *kaschgarica*, *Rosa beggeriana*, *Rosa albertii*, *Berberis*, and *Caragana* under these conditions. Liu and Cong (2000) described this botanical variety as having fewer prickles than the original botanical variety, which was, however, not supported by the present survey.

New variant

A new variant of *R. laxa* was found in the Tuomuer Nature Reserve (Fig. 3G–L) and is characterized by the following: small leaflets, almost leathery, base cuneate, entire margin (Fig. 3J); prickles slightly recurved (Fig. 3L); glandular on sepals and bracts, gradually shedding at later stages; and flowers pale pink, in groups of three to five on stems or sometimes solitary, 1.5–2 cm in diameter, with petal tip slightly curling and splitting (Fig. 3G–I). This was originally thought to be a variant of *R. laxa* var. *kaschgarica*. However, the new variant's hips were globular or elliptic. Nearly half of the sepals at the apex were persistent and erect, but the other half shed completely (Fig. 3K). *R. beggeriana* (Fig. 3M) and *R. albertii* (Fig. 3N) were also found nearby, with the apical part of the hypanthium and sepals deciduous after the hips ripened. Based on these observations, we suggest that this new variant was a natural hybrid of *R. beggeriana* or *R. albertii* with *R. laxa* var. *kaschgarica* and that *R. laxa* var. *kaschgarica* was likely to be the female parent.

Pollen grain morphology

R. LAXA VAR. LAXA. R1 was perprolate in shape (P/E = 2.04) and 32.80 to 39.86 × 16.58 to 19.48 μm in size (Fig. 4A–H; Table 2). Its shape in the equatorial view was long-elliptic and in the polar view was trilobate-circular with tricolporate, which extended toward the polar axis. Thin striae on the exine sculpture were almost parallel to the polar axis, with light muri and perforations in wide intervals between striae.

R2 and R3 were dimorphic and were prolate (P/E = 1.72) and subspheroidal (P/E = 1.01), respectively, in shape. The prolate pollen grains were 22.73 to 36.74 × 16.27 to 20.58 μm in size. Their equatorial and polar views were the same as those of R1. The prolate pollen grains were also similar to those of R1 in exine sculpturing but with much thicker and denser striae at 0.56–0.83 μm². The subspheroidal pollen grains were 19.15 to 4.44 × 16.80 to 22.67 μm in size and were circular or subcircular in the equatorial view and obtuse-triangular with tricolporate in the polar view. The colpus was spindle-shaped, with a width ranging from 0.71 to 4.53 μm. There was a significant difference in sculpturing between R2 and R3. R3 had the densest striae on the sculpture, ranging from 1.03 to

Table 2. The palynomorphology characters of *Rosa laxa* botanical varieties.

Specimens	Pollen grain (mean ± SD) ²				Exine sculpture (mean ± SD)						Aperture (colpus) (mean ± SD)			
	Axis		Axis		Striae		Striae		Perforation		Perforation		Density	
	Polar length (P)	Equator length (E)	P/E	P/E	Width (μm)	Distance (μm)	Density (μm ⁻²)	Diam (μm)	Density (μm ⁻²)	Diam (μm)	Density (μm ⁻²)	Length (μm)	Width (μm)	Range of width (μm)
R1	35.63 ± 2.27 d ³	17.52 ± 1.15 a	2.04 ± 0.20 de	2.04 ± 0.20 de	0.25 ± 0.03 de	0.46 ± 0.14 g	0.44 ± 0.11 a	0.18 ± 0.04 de	3.17 ± 0.42 d	0.18 ± 0.04 de	3.17 ± 0.42 d	32.85 ± 2.09 de	0.77 ± 0.16 ab	0.56–1.16
R2–1	30.64 ± 3.99 c	17.89 ± 1.61 a	1.72 ± 0.27 c	1.72 ± 0.27 c	0.13 ± 0.01 a	0.29 ± 0.05 cd	0.56 ± 0.01 abc	0.14 ± 0.03 bc	2.64 ± 0.22 bcd	0.14 ± 0.03 bc	2.64 ± 0.22 bcd	27.36 ± 3.36 c	1.14 ± 0.20 b	0.79–1.16
R2–2	21.77 ± 1.96 a	21.58 ± 1.25 d	1.01 ± 0.09 a	1.01 ± 0.09 a	0.23 ± 0.02 c	0.27 ± 0.05 cd	0.54 ± 0.02 abc	0	0	0	0	19.98 ± 1.62 a	2.00 ± 0.76 c	0.99–4.53
R3–1	27.38 ± 2.43 b	18.08 ± 1.23 ab	1.52 ± 0.16 b	1.52 ± 0.16 b	0.15 ± 0.01 a	0.25 ± 0.04 bc	1.03 ± 0.15 d	0.13 ± 0.02 bc	2.02 ± 1.07 ab	0.13 ± 0.02 bc	2.02 ± 1.07 ab	24.03 ± 1.96 b	1.16 ± 0.30 b	0.62–1.94
R3–2	21.93 ± 0.88 a	19.75 ± 1.31 c	1.11 ± 0.08 a	1.11 ± 0.08 a	0.14 ± 0.01 a	0.12 ± 0.02 a	1.76 ± 0.07 e	0	0	0	0	18.03 ± 1.53 a	1.88 ± 0.90 c	0.71–3.62
R4	38.63 ± 1.85 e	23.89 ± 1.51 e	1.62 ± 0.11 bc	1.62 ± 0.11 bc	0.23 ± 0.06 c	0.21 ± 0.04 b	0.71 ± 0.19 c	0.08 ± 0.02 a	2.21 ± 0.56 bc	0.08 ± 0.02 a	2.21 ± 0.56 bc	33.94 ± 2.06 e	0.44 ± 0.07 a	0.27–0.62
R5	37.82 ± 2.48 e	19.30 ± 0.98 bc	1.97 ± 0.19 d	1.97 ± 0.19 d	0.22 ± 0.06 c	0.28 ± 0.05 cd	0.67 ± 0.12 bc	0.11 ± 0.03 ab	2.95 ± 0.22 cd	0.11 ± 0.03 ab	2.95 ± 0.22 cd	33.77 ± 2.83 e	0.90 ± 0.16 ab	0.59–1.25
R6	43.95 ± 3.34 f	21.11 ± 2.34 d	2.11 ± 0.29 de	2.11 ± 0.29 de	0.17 ± 0.03 b	0.38 ± 0.12 f	0.52 ± 0.12 abc	0.20 ± 0.06 e	2.22 ± 0.46 bc	0.20 ± 0.06 e	2.22 ± 0.46 bc	37.54 ± 2.59 f	0.62 ± 0.13 a	0.47–0.88
R7	34.87 ± 2.30 d	17.02 ± 1.49 a	2.06 ± 0.15 de	2.06 ± 0.15 de	0.13 ± 0.02 a	0.34 ± 0.05 ef	0.57 ± 0.08 abc	0.13 ± 0.02 bc	2.17 ± 0.21 bc	0.13 ± 0.02 bc	2.17 ± 0.21 bc	31.30 ± 2.37 d	0.70 ± 0.18 ab	0.56–1.27
R8	38.20 ± 1.88 e	17.65 ± 1.47 a	2.17 ± 0.15 e	2.17 ± 0.15 e	0.14 ± 0.02 a	0.34 ± 0.05 ef	0.66 ± 0.05 bc	0.15 ± 0.03 cd	2.47 ± 0.25 bcd	0.15 ± 0.03 cd	2.47 ± 0.25 bcd	33.84 ± 1.96 e	0.86 ± 0.19 ab	0.71–1.43
R9–1	48.39 ± 2.73 g	22.39 ± 1.00 d	2.17 ± 0.17 e	2.17 ± 0.17 e	0.24 ± 0.05 cd	0.36 ± 0.10 f	0.49 ± 0.05 ab	0.22 ± 0.08 e	1.24 ± 0.42 a	0.22 ± 0.08 e	1.24 ± 0.42 a	43.49 ± 3.63 g	0.92 ± 0.20 ab	0.41–1.39
R9–2	27.14 ± 1.74 b	26.91 ± 1.57 f	1.01 ± 0.06 a	1.01 ± 0.06 a	0.26 ± 0.03 e	0.15 ± 0.04 a	0.52 ± 0.05 abc	0	0	0	0	24.51 ± 2.45 b	1.92 ± 1.52 c	0.31–5.12
R10	35.26 ± 1.60 d	17.55 ± 1.27 a	2.02 ± 0.15 de	2.02 ± 0.15 de	0.15 ± 0.02 ab	0.31 ± 0.08 de	0.62 ± 0.14 abc	0.13 ± 0.03 bc	1.83 ± 0.37 ab	0.13 ± 0.03 bc	1.83 ± 0.37 ab	31.72 ± 1.36 de	0.93 ± 0.29 ab	0.56–1.40

²Shape of pollen grain (Erdman, 1952). Subspheroidal: P/E ratio of 0.75–1.33. Prolate: P/E ratio of 1.33–2. Perprolate: P/E ratio >2.

³Specimens with different lower case letters indicate significant differences at the 0.05 level.

1.76 μm^{-2} ; these were clear without puncture and ran almost parallel to the polar axis. The sculpturing of R2 looked like multiple ornamentation with striae and subsident foveae.

R. LAXA VAR. MOLLIS. R4 was prolate (P/E = 1.62), with a size of 36.31 to 42.35 $\times$ 21.19 to 26.79 μm , and was long-elliptic in the equatorial view and trilobate-circular with tricolporate extending toward the polar axis in the polar view (Fig. 4I–M). Sculpturing was characterized by a perforate-striate pattern, almost parallel to the polar axis, but the perforations were irregular, sometimes fewer and smaller and sometimes denser and larger near the colpus.

R. LAXA VAR. KASCHGARICA. R5 was prolate in shape (P/E = 1.97) and 33.78 to 40.32 $\times$ 17.98 to 21.15 μm in size (Figs. 4N, 4O, and 5A–E). R6–R8 were perprolate (P/E = 2.06–2.17), with a size of 29.20 to 50.46 $\times$ 14.96 to 24.33 μm . They were all long-elliptic with acute or obtuse poles in the equatorial view and trilobate-circular with tricolporate extending toward the polar axis in the polar view. Sculpturing was characterized by a striate pattern, with R5 having the densest striae at 2.95 μm^{-2} . R5–R8 differed in foveae or perforation: 1) R5 and R7 had more obvious striae, but fewer large perforations with a density of 0.85–1.44 μm^{-2} ; 2) thick striae were observed in R6 and R8, with dense perforations at 2.22–2.47 μm^{-2} ; and 3) no pits but perforations were seen in R6.

ROSA LAXA VAR. TOMURENSIS. R9 was dimorphic, with a perprolate shape (P/E = 2.17) measuring 42.86 to 51.87 $\times$ 20.67 to 23.63 μm in size, and a subspheroidal (P/E = 1.01) shape measuring 23.43 to 30.07 $\times$ 22.89 to 29.41 μm in size. Pollen grains were long-elliptic or subcircular in the equatorial view and trilobate-circular or subcircular, with deeper tricolpus, in the polar view. The exine sculpture exhibited prominent striae parallel to the equatorial axis and close to the colpus, but only perprolate pollen grains had perforations, with a density of 1.24 μm^{-2} . Subspheroidal pollen grains had thin striae that were wide in ridges and narrow in grooves, and the colpus was spindle-shaped with the width ranging from 0.31 to 5.12 μm .

Fig. 5. Scanning electron microscope images of pollen grains: (A–E) pollen grains of *Rosa laxa* var. *kaschgarica* [(A) polar view of sample R8, trilobate-circular with tricolpus ($\times 2000$); (B–C) equatorial view of samples R8 and R5, long-elliptic; linear, narrow colpus; dense striae parallel to pollen axis ($\times 2000$); (D) exine sculpture of sample R7, densely striate with more small, perforation-like pits ($\times 6000$); (E) exine sculpture of sample R8, striate-perforate; wide grooves with more larger, deeper perforations ($\times 6000$)]; (F–L) pollen grains of *R. laxa* var. *tomurensis* of sample R9 [(F) pollen grain view with dimorphic shape, perprolate and subspheroidal ($\times 500$); (G) polar view of perprolate pollen, trilobate-circular with tricolpus ($\times 2000$); (H) polar view of subspheroidal pollen, subcircular with tricolpus ($\times 2000$); (I) equatorial view of perprolate pollen, long-elliptic; striae nearly running along the equatorial axis near colpus; more deep perforations ($\times 2000$); (J) equatorial view of subspheroidal pollen, subcircular with spindle-shaped colpus ($\times 2000$); (K) exine sculpture of perprolate pollen, deeply striate-perforate ($\times 6000$); (L) exine sculpture of subspheroidal pollen, striae thin and wide, with more narrow grooves ($\times 6000$)]; (M–O) pollen grains of a variant of *R. laxa* [(M) pollen grain view with perprolate shape ($\times 500$); (N) polar view of sample R10, trilobate-circular with tricolpus ($\times 2000$); (O) exine sculpture, deeply striate-perforate ($\times 6000$)]].

New variant

R10, representing the special variant of *R. laxa*, was perprolate (P/E = 2.02) in shape and 33.32 to 37.68 $\times$ 16.21 to 20.15 μm in size (Fig. 5M–O). It was long-elliptic in the equatorial view and trilobate-circular with a deeper tricolporate

in the polar view. The sculpture was characterized by a perforate-striate pattern. There were no significant differences between R10 and *R. laxa* var. *kaschgarica* (R5–R8).

Discussion

Rosa laxa botanical varieties represent stable variants in terms of certain morphological characteristics and are able to adapt to a range of geographical regions with varying climatic conditions in Xinjiang, China (Yu et al., 2014). Although *R. laxa* botanical varieties could be identified with these stable morphological variants, it remains difficult to distinguish them morphologically because of both intra- and interpopulation variation in these morphological characteristics, such as flower color and the morphology of prickles, indumentum, and glandular pubescence. A comparison of the morphological traits of these botanical varieties from different habitats suggests that flower color is not a suitable character for distinguishing *R. laxa* botanical varieties. In addition, prickles varied greatly in their shape and number. By contrast, branches, which showed stable growth, would be a preferred morphological character for classification. The indumentum and glandular pubescence of *R. laxa* are variable in number, even within the same plant; similarly, the constant shedding of the indumentum and glandular pubescence is also a problem worth noting to minimize errors in classification.

Palynomorphological data indicated that all pollen grain samples of *R. laxa* were monad with an NPC (aperture number, position, and character) = 345. There was a considerable amount of intraspecific variation in pollen grain morphological characters, and there was also shape dimorphism of pollen grains, particularly in *R. laxa* var. *laxa* and *R. laxa* var. *tomurensis*. In all botanical varieties, the colpus of the pollen grain was long, elliptic in outline, deeply set into the exine, and ended sharply. The colpus of the pollen grain was wide in the center and narrow at the ends, but this was not the case for prolate or perprolate pollen grains.

Previous research on plant geography in Xinjiang (Hui et al., 2003) found primitive, transitive, and evolved Rosaceae communities. Yu (1984) speculated that the Rosaceae had evolved mostly from a multiflowered compound inflorescence toward a few-flowered, simple inflorescence, and finally toward a solitary flower with large petals. In this sense, *R. laxa* var. *tomurensis* and *R. laxa* var. *kaschgarica* may be most recently diverged from the original botanical variety.

Ma and Chen (1992) reported that *R. laxa* has $2n = 2x = 14$ chromosomes in the shoot tips; by contrast, Krussmann (1981) and Yang et al. (2008) reported $2n = 4x = 28$ chromosomes in the root tips. Mixoploidy and intraspecific polyploidy are widespread in *Rosa* species (Luo et al., 2009; Ma and Chen, 1992; Simon et al., 2006) and polyploid pollen grains or spores are larger than diploid ones (Erdtman, 1952). Zlesak (2009) concluded that pollen diameter and guard cell length could be used to predict the ploidy level in *Rosa* species, with diploid pollen exhibiting a diameter $< 35.6 \mu\text{m}$, and tetraploid exhibiting a diameter of $35.6\text{--}43.7 \mu\text{m}$. In this study, pollen grains were all monads and medium in size ($21.77\text{--}48.39 \mu\text{m}$), with the maximum observed in *R. laxa* var. *tomurensis*. Based on the pollen diameters, we infer that *R. laxa* var. *kaschgarica* is tetraploid and that both diploids and tetraploids might exist for other botanical varieties. Plants with subspheroidal pollen grains might be the more primitive diploids.

In terms of palynology, Wodehouse (1935) inferred that longer pollen grains were stronger in regulatory function; hence, pollen grains with smaller volume/surface area ratios are likely to be more recently evolved. Pollen grains of primitive angiosperms are believed to be large in size, with pollen evolving toward a smaller size (Feng, 2007; Walker, 1976; Zhou et al., 2005). It has been hypothesized that the exine sculpture of the angiosperm pollen grain evolved from a nonstructural layer (smooth) toward a perforation foveola and then toward a striate pattern (Erdtman, 1952). Moreover, the perforation pattern might be the most primitive in the Rosaceae, with striate and striate-reticulate patterns being more recently evolved. The exine sculpture is also believed to be better able to provide evolutionary information than pollen size (Moore et al., 1991; Reitsma, 1966; Wronska-Pilarek, 2006; Wronska-Pilarek and Boratyńska, 2005; Li and Zhang, 2009; Zhou et al., 1999a, 1999b, 2000).

Based on the present study of *R. laxa* botanical varieties, exine sculpture and P/E ratio are the main characters that should be used for the identification of related botanical varieties. Small pollen grains with dense striae may be considered to be the most recently derived. For example, among the four samples of *R. laxa* var. *kaschgarica*, the R5 and R7 specimens with pink flowers, which exhibited denser striae, may be considered to be recently evolved. Although the pollen grains of *R. laxa* var. *tomurensis* are comparatively larger, it can still be regarded as recently evolved because of its dense striae and low-density foveae, in agreement with the aforementioned inference made on the basis of Yu's (1984) results.

The extensive adaptability of *R. laxa*, especially *R. laxa* var. *kaschgarica*, indicates that *R. laxa* botanical varieties may possess gene(s) conferring tolerance or resistance to environmental stresses. Same results on karyotype analysis were also found among these *R. laxa* species (Yu et al., 2014). Introduction of these genes into commercial breeding lines would greatly assist in the production of high-quality modern roses. Thus, effective conservation measures should be taken to preserve these genes.

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