Pollen Morphology of the Oklahoma Endemic Plants Leavenworthia aurea (Brassicaceae/Cruciferae) and Phlox pilosa Subsp. Longipilosa (Polemoniaceae), With Special Reference To Their Natural History

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POLLEN MORPHOLOGY OF THE OKLAHOMA ENDEMIC PLANTS *LEAVENWORTHIA AUREA* (BRASSICACEAE/CRUCIFERAE) AND *PHLOX PILOSA* SUBSP. *LONGIPILOSA* (POLEMONIACEAE), WITH SPECIAL REFERENCE TO THEIR NATURAL HISTORY

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ABSTRACT. The morphology, taxonomy, ecology, distribution, and conservation status of the Oklahoma endemics, *Leavenworthia aurea* (Brassicaceae/Crucifereae) and *Phlox pilosa* subsp. *longipilosa* (Polemoniaceae) Pennell, are reviewed and supplemented with original pollen morphological descriptions that include comparisons to related taxa. These descriptions are considered extensions of the original taxonomic descriptions. *Leavenworthia aurea* is a glabrous winter annual found on limestone glades in Choctaw and McCurtain counties. The taxon is considered imperiled, with Natural Heritage rankings of G2S2. The pollen has three colpi with a reticulate surface enclosing polygonal lumina (openings within reticulations on the pollen surface) with lengths varying from 0.5–2.5 μm, that are either empty or contain inclined free standing columellae approximately 0.5–0.8 μm in height. Granules less than 0.5 μm in diameter also occur in some lumina, but are most common in the colpus. The reticulate surface of *Leavenworthia* pollen is similar to other Cardamineae, as well as to the pollen of other members of the Brassicaceae. *Phlox pilosa* subsp. *longipilosa*, found on granitic soils in the Quartz and Wichita mountains of Greer and Kiowa counties, is a branching spring perennial ranging from 20.0–45.0 cm in height. Like *L. aurea*, it is considered to be imperiled, with a Natural Heritage ranking of G2S2. *Phlox* pollen morphology has been broadly characterized but has not been described in *P. pilosa* subsp. *longipilosa*. The pollen of *P. pilosa* subsp. *longipilosa* is pantoporate and reticulate, with enclosed polygonal lumina often exceeding 5.0 μm. The exine (pollen surface) is multireticulate because all lumina, except those containing pores, enclose smaller reticulate networks composed of thin rods of sporopollenin. In porate lumina, sporopollenin rods are curved, with one end attached to the surrounding ridges, as they are in non-porate lumina; the other end either hangs freely above the pores or is attached to the pore margins. Pollen morphology is similar to well-established *Phlox* pollen types.

Key Words: exine, pollen, Oklahoma endemic, *Phlox*, Polemoniaceae, *Leavenworthia*, Brassicaceae, Cruciferae, scanning electron microscopy, SEM

The state of Oklahoma has over 2800 taxa of vascular plants but few endemics (B. Hoagland, Oklahoma Natural Heritage Inventory, pers. comm.; J. Singhurst, Texas Parks and Wildlife Department, pers. comm.; Taylor and Taylor 1994; USDA, NRCS 2013). Two of these endemic plants are *Leavenworthia aurea* Torr. (golden gladecress; Al-Shehbaz 2012) and *Phlox pilosa* L. subsp. *longipilosa* (Waterf.) Locklear, *comb. et stat. nov.* (longhair phlox; C. Ferguson, Kansas State University, pers. comm.). Our studies of *L. aurea* and *P. pilosa* subsp. *longipilosa* are specifically intended to enhance the original morphological descriptions in which pollen data are absent. Further, the data supplement the pollen morphological databases for their respective families. *Leavenworthia* has not been previously described and *Phlox* pollen, although broadly portrayed in the literature, is herein complimented by scanning electron microscopy (SEM) of whole and fractured grains after acetolysis treatment (Erdtman 1960).

Leavenworthia aurea. The genus *Leavenworthia* Torr. is a member of the Cardamineae Dumort. tribe in the Brassicaceae (Al-Shehbaz 2012). It includes 12 species that are confined to the southern and southeastern US (Al-Shehbaz 2010). *Leavenworthia aurea* was originally described by Torrey (1837) as occurring in Fort Towson, Arkansas (now Oklahoma), Texas, and in Jefferson County, Alabama. The Alabama specimens were subsequently reexamined and identified as *L. exigua* Rollins (as *L. exigua* var. *lutea* Rollins; Baskin and Baskin 1984; Rollins 1963), whereas the plants from the Texas populations were referred to *L. texana* by Mahler (1987). Rollins (1993) reduced this taxon to a variety of *L. aurea*—*L. aurea* Torr. var. *texana* (Mahler) Rollins—but Al-Shehbaz (2010) recognized that the two taxa are distinct species based on differences in chromosome number, petal shape and color, and apical leaf segments.

Torrey (1837, p. 89) described *Leavenworthia aurea* as occurring on ''wet places on the priaries [*sic*].'' The genus as a whole is adapted to open areas with shallow soils over thin beds of limestone. These ''glade'' areas are able to temporarily maintain a

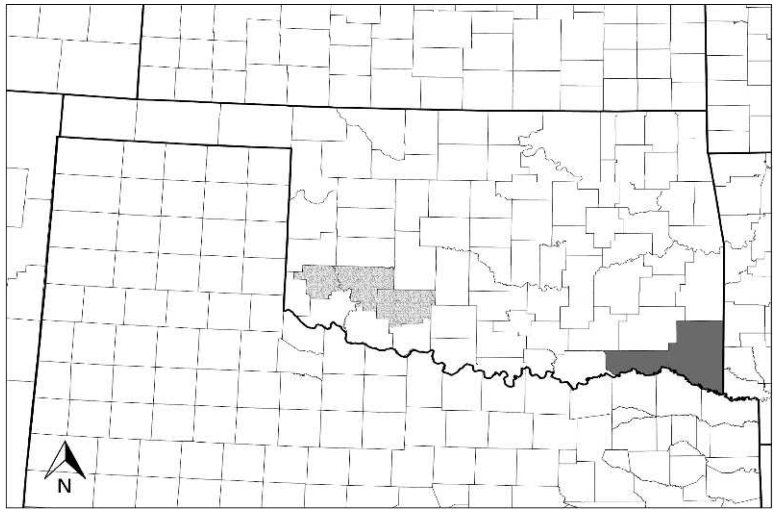

Figure 1. County level distribution of *Leavenworthia aurea* (in gray stipple) and *Phlox pilosa* subsp. *longipilosa* (solid gray) in Oklahoma (shaded counties have occurrences of the taxon).

higher moisture content at their surface, resulting in wetter soils in the late winter and early spring—a necessity for *L. aurea* and the rest of the genus (Rollins 1963). Today, *L. aurea* is found at 25 sites in Choctaw and McCurtain counties (Figure 1; Hoagland et al. 2004; NatureServe 2013). These sites are relatively flat, and include disturbed roadsides, pastures, and creek margins (Rollins 1963, 1993). A single population may include hundreds of individuals.

Leavenworthia aurea is a small winter annual, 5.0–15.0 cm tall (Figure 2). The plant is glabrous, with a rosette of pinnatifid leaves. Early season flowers are scapose, but later in the season, flowers are found in racemes along lateral branches. Sepals are purple-tinged and petals are yellow. The fruit is a silique about 2.5 cm long (Rollins 1993; Torrey 1837). The plant begins flowering in mid-March and continues through April. Germination occurs in September, with the plant growing as a rosette throughout the winter (Rollins 1963). The plant is a polyploid ($n = 24$; Rollins 1963). *Leavenworthia aurea* is tracked by the Oklahoma Natural Heritage Inventory. It has a global rank of G2 and a subnational rank of S2, implying that it is ‘‘imperiled in the jurisdiction because of rarity due to very restricted range, very few populations, steep

Figure 2. Mature stems of *Leavenworthia aurea* in flower.

declines, or other factors making it very vulnerable to extirpation'' (NatureServe 2013; Oklahoma Natural Heritage Inventory 2013).

The Brassicaceae has long been regarded as a stenopalynous (with morphologically similar pollen; Erdtman 1952) family, primarily due to a virtually universal reticulate exine and 3-colpate apertures nearly reaching the poles (El-Ghazali 1993; Erdtman 1952; Reille 1992). However, the Brassicaceae has also been considered as eurypalynous (morphologically dissimilar; Erdtman 1952) with aperture systems varying from 3-colpate (the most common) to dicolpate, syncolpate, and nonaperturate, with shapes ranging from peroblate to prolate spheroidal (Al-Shehbaz et al. 2006; Erdtman 1952; Inceoglu and Karamustafa 1977; Jonsell 1979; Lahham and Al-Eisawi 1987) and with a surface that is finely punctuate and spinulose (in *Heliophila* L.; Rollins and Banerjee 1979) rather than characteristically reticulate. Based on the diameters of the lumina, Inceoglu and Karamustafa (1977) recognized three patterns: 1) faintly reticulate with lumina $<$ 0.3 μm, 2) reticulate with lumina 0.3–2.0 μm, and 3) coarsely reticulate with lumina $>$ 2.0 μm. These patterns differed slightly from the three patterns recognized by Abdel Khalik et al. (2002) as: 1) microreticulate with lumina $<$ 1.0 μm, 2) reticulate with lumina 1.0–2.0 μm, and 3) coarsely reticulate with lumina $>$ 2.0 μm.

Studies of Brassicaceae pollen are numerous at both familial and generic levels. Familial studies include those of Abdel Khalik et al. (2002), Perveen et al. (2004), Rollins and Banerjee (1979), and Tarnavschi et al. (1987). Studies of individual genera include *Exhalimolobos* Al-Shehbaz & C.D. Bailey (Bailey et al. 2007); *Arabidopsis* (DC.) Heynh. (Khan 2004), *Brassica* L., *Lepidium* L., *Physaria* (Nutt. *ex* Torr. & A. Gray) A. Gray, *Stanleya* Nutt., *Streptanthus* Nutt., and *Thysanocarpus* Hook. (Martin and Drew 1969, 1970); *Draba* L. (Mulligan 1976); *Arabis* L. (Mutlu and Erik 2012); *Hesperis* L. (Pinar et al. 2009); and *Dimorphocarpa* Rollins and *Dithyrea* Harv. (Rollins 1979). Basic pollen information on genera ascribed to the Cardamineae (*Armoracia* G. Gaertn., B. Mey. & Scherb., *Barbarea* W.T. Aiton, *Cardamine* L., *Nasturtium* W.T. Aiton, *Ornithocarpa* Rose, *Rorippa* Scop., and *Selenia* Nutt.), is included in Table 1.

***Phlox pilosa* subsp. *longipilosa*.** The genus *Phlox* L. is a member of the tribe Phlocideae Dumort. in the Polemoniaceae and includes approximately 69 species of small shrubs and annual and perennial herbs found in North America and Siberia (Grant 1959; Porter and Johnson 2000). *Phlox longipilosa* was originally described by Waterfall (1971) from material referred to by Wherry (1955) as *P. pilosa* L. subsp. *riparia* Wherry and *P. pilosa* L. subsp. *pilosa*, based on the long, jointed hairs on the calyx, stem, and leaves, the multiple stems per plant, the alternate upper leaves, and the short, congested internodes (Waterfall 1971). Taylor and Taylor (1981, p. 183) reduced the taxon to a variety of *P. pilosa* (*P. pilosa* L. var. *longipilosa* [Waterf.] R. John Taylor & C.E.S. Taylor), noting that it "falls well within the spectrum of variation found in the *Phlox pilosa* complex…" Locklear (2009) referred to the taxon as a subspecies of *P. pilosa*: *P. pilosa* subsp. *longipilosa* (Waterf.) J. Locklear, and it will be maintained as such in the upcoming *Flora of North America* treatment (C. Ferguson, Kansas State University, pers. comm.).

Phlox pilosa subsp. *longipilosa* occurs exclusively on soils derived from granitic rock and is associated with mixed grass prairie or live oak or post oak woodlands (NatureServe 2013). Historically, it is known from 10 to 20 populations in the Quartz and Wichita Mountains of southwestern Oklahoma in the counties of Greer and Kiowa (Figure 1); however, many of these populations cannot be re-located (A. Buthod, unpubl. data). A collection from adjacent

Table 1. Comparative pollen morphology of *Leavenworthia aurea* and other members of the Brassicaceae. Tribal affiliation is given after taxon name (Al-Shehbaz 2012). In order to calculate the PA/EA when a range of values was given in the literature, the arithmetic mean, calculated as: (the lowest value in the range + the highest value) ÷ 2, was used. Pollen Shape: OS = oblate spheroidal, P = prolate, PSPH = prolate spheroidal, SP = subprolate. Axes: PA = polar axis, EA = equatorial axis, PA/EA = polar axis/equatorial axis ratio. Lumen type: CR = coarsely reticulate, M = microreticulate, R = reticulate. Columellae: + = present, 0 = absent, NA = not available. References (Ref): 1 = Al-Shehbaz 1989; 2 = Buthod and Skvarla (this study); 3 = Hesse et al. 2009; 4 = Inceoglu and Karamustafa 1977; 5 = Abdel Khalik et al. 2002; 6 = Rollins and Banerjee 1979; 7 = Tarnavschi et al. 1987. [1]Dash (–) indicates a range of lengths (Axes) or thicknesses (Exine).

			Axes						
			Length[1]		Ratio	Colpi	Exine Thickness[1]	Lumen	Stranded
Taxon	Ref	Shape	PA (μm)	EA (μm)	PA/EA	(N)	(μm)	Type	Columellae
CARDAMINEAE									
Leavenworthia aurea Torr.	2	PSPH	26–29	22–26	110	3	1.4–1.8	R-CR	+
Armoracia rusticana G. Gaertn., B. Mey. & Scherb.	7	SP	29–36	26–29	120	3	3.6–4.2	R-CR	NA
A. macrocarpa (Waldst. & Kit.) Kit. *ex* Baumg.	7	P	31–36	22–25	140	3	3.6–4.2	R-CR	NA
Barbarea minor K. Koch	4	SP	20	17	120	3	1.7	R	NA
B. stricta Andrz.	7	P	29–34	19–24	150	3	2.4–2.9	R-CR	NA
Cardamine hirsuta L.	4	SP	25	21	120	3	2.7	R	NA
C. bulbifera (L.) Crantz	7	SP	36–41	28–38	120	3	2.4–3.6	R-CR	NA
Nasturtium officinale W.T. Aiton	7	SP	26–31	22–24	120	3	2.4–3.6	R	NA
Ornithocarpa torulosa Rollins	1	P	15–17	8–10	180	3	NA	M	0
O. fimbriata Rose	1	P	13–15	8–9	160	3	NA	M	+
Rorippa prolifera Neilr.	7	P	29–41	20–25	160	3	2.8–3.6	R	NA
R. palustris (L.) Besser	5	P	26–32	15–19	170	3	NA	R	NA

Table 1. Continued.

Taxon	Ref	Shape	Axes			Colpi (N)	Exine Thickness[1] (μm)	Lumen Type	Stranded Columellae
			Length[1]		Ratio				
			PA (μm)	EA (μm)	PA/EA				
Selenia dissecta Torr. & A. Gray	6	P	42	25	170	3	NA	R	NA
S. grandis R. F. Martin	6	P	41	22	190	3	NA	R	+
OTHER TRIBES									
Aethionema armenum Boiss.; Aethionemeae	4	P	21	15	140	3	1.7	M	?
Arabis hirsuta (L.) Scop.; Arabideae	4	SP	23	19	120	3	1.9	R	?
Capsella bursa-pastoris (L.) Medik.; Camelineae	4	PSPH	21	19	110	3	1.7	M-R	?
Draba verna L.; Arabideae	3	PSPH	24–26	21–23	110	3	1.5–2.0	CR	+
Eremobium aegyptiacum Asch. *ex* Boiss; Anastaticeae	5	P	20–24	11–15	170	3	NA	M-R	NA
Erysimum repandum L.; Erysimeae	5	SP	20–25	18–20	120	3	NA	M-R	NA
Matthiola longipetala (Vent.) DC.; Anchonieae	4	OS-PSPH	27	27	100	0	3.4	CR	NA
Nasturtiopsis coronopifolia (Desf.) Boiss; Brassiceae	5	PSPH	21–25	19–22	110	3	NA	R-CR	NA

Figure 3. *Phlox pilosa* subsp. *longipilosa*. Inset shows distinctive jointed hairs on calyx, stems, and leaves.

Comanche County was made in 1937, but neither this population, nor others in the county, have been found (A. Buthod, unpubl. data; Hoagland et al. 2004). Plants in extant populations are locally abundant, with hundreds of individuals occurring at a given site (NatureServe 2013).

Phlox pilosa subsp. *longipilosa* is a highly branched, erect perennial herb, 20.0–45.0 cm tall (Figure 3). Leaves are linear at the base of the plant, but become lanceolate toward the top of the plant. The inflorescence is a multi-flowered panicle with pale purple flowers. Flowering begins in late May and continues through early June. The fruit is a capsule, 10.0–12.0 mm long. Worcester et al. (2012) noted that the taxon is diploid ($n = 7$; citing unpublished data). *Phlox pilosa* subsp. *longipilosa* is tracked by the Oklahoma Natural Heritage Inventory. It has a global rank of G2 and a subnational rank of S2 (NatureServe 2013; Oklahoma Natural Heritage Inventory 2013).

Pollen morphology is well known in the Polemoniaceae, a eurypalynous family of approximately 26 genera and 379 species (Porter and Johnson 2000; Stuchlik 1967a). The highly diverse pollen morphology is notably characterized by striate, striato-reticulate,

reticulate, and eutectate pollen sculpturing (Erdtman 1952; Ludlow-Wiechers 1982; Marticorena 1961; Quiroz-García et al. 2002; Taylor and Levin 1975). Generic pollen studies include work on *Eriastrum* Wooton & Standl., *Gilia* Ruiz & Pav., and *Linanthus* Benth. (as *Leptodactylon* Hook. & Arn.; Martin and Drew 1970); *Gilia* (Grant and Grant 1960); *Collomia* Nutt. (Chuang et al. 1978; Loeblich 1964); *Cantua* Juss. *ex* Lam. (Monofils 2004; Stuchlik 1967a); *Cobaea* Cav. (Stuchlik 1967a); and *Loeselia* L. (Porter and Steinmann 2009). With its strikingly complex, reticulate exine, *Phlox* pollen is often used to exemplify the family (e.g., Hesse et al. 2009; Kapp 1969). The most comprehensive analysis of Polemoniaceae pollen was by Stuchlik (1967a, b), wherein he described the morphology of nearly 150 species and established 26 pollen types, placing *Phlox* in his *Phlox* pollen type. This type was characterized by spherical pollen with diameters ranging from 22.0–56.0 μm, with pantoporate apertures and a reticulate surface with polygonal lumen having diameters of 1.4–15.0 μm (Table 2).

MATERIALS AND METHODS

Pollen was removed from herbarium sheets (specimens are enumerated in Table 3), acetolyzed to clean the pollen surface (Erdtman 1960), stained in osmium and thiocarbohydrazide to enable secondary electron conductivity (Chissoe et al. 1995), mounted on rectangular specimen holders (Chissoe and Skvarla 1996a), pulse sputter-coated with gold/palladium to increase electron conductivity (Chissoe and Skvarla 1996b), and examined in a JEOL 880 scanning electron microscope (JEOL Ltd., Tokyo, Japan) equipped with a lanthanum hexaboride (LaB_6) electron source. Measurements of at least 10 grains for each specimen were made directly from SEM images. All images were digitally obtained, stored, and eventually reconstructed in Adobe Photoshop 6 (Adobe Systems Inc., San Jose, CA).

RESULTS

***Leavenworthia aurea* pollen morphology.** Pollen shape was prolate-spheroidal to subprolate. Pollen grain sizes ranged from 25.8–29.0 μm for the polar axis and 22.2–26.0 μm for the equatorial axis (Figure 4A–C). The apertures were tricolpate, with a number-position-character (NPC) analysis classification of 343. Colpus

Table 2. Comparative morphology of pollen from *Phlox pilosa* subsp. *longipilosa* and other *Phlox* taxa. Sph = spheroidal. Lumen columns: NA = information not available; F = micromesh in all lumen; F* = micromesh in lumen but extent unknown; O = no micromesh in any lumen; S = micromesh in some lumen. References (Ref): 1 = Buthod and Skvarla; 2 = Taylor and Levin 1974; * = Stuchlik 1967b.

Taxon	Ref	Shape	Diameter (μm)	Number of Pores	Exine Thickness (μm)	Lumen	
						Diameter (μm)	Content
Phlox pilosa L. subsp. *longipilosa* (Waterf.) Locklear, *comb. et stat. nov.*	1	Sph	21–41	15–30	4.0–6.0	4.0–6.0	F
P. aculeata A. Nelson	*	Sph	39–59	19–25	5.2	1.5–15.0	S
P. adsurgens Torr. *ex* A. Gray	*	Sph	42–53	22–25	6.1	2.8–13.0	S
P. alyssifolia Greene	*	Sph	42–50	20–27	5.8	NA	S
P. amplifolia Britton	*	Sph	42–51	25–30	4.5	3.6–8.0	F
P. austromontana Coville	*	Sph	31–53	12–16	5.1	3.9–12.7	S
P. bifida L.C. Beck	*	Sph	29–40	23–27	5.1	3.1–9.6	S
P. bryoides Nutt.	*	Sph	28–48	20–23	4.7	3.3–11.7	S
P. caespitosa Nutt.	*	Sph	31–55	25–28	5.4	3.4–10.4	F
P. carolina L.	*	Sph	43–56	23–29	6.0	3.9–14.8	S
P. colubrina Wherry & Constance	*	Sph	28–45	20–26	5.1	3.1–9.3	F
P. diffusa Benth.	*	Sph	29–48	17–19	5.3	3.2–8.3	F
P. divaricata L.	*	Sph	25–33	21–26	3.4	3.4–8.8	O
P. douglasii Hook.	*	Sph	27–38	22–27	4.0–5.1	1.4–8.5	S
P. drummondii Hook.	*	Sph	25–36	20–21	4.0	4.6–13.5	O
P. glaberrima L.	*	Sph	37–48	24–32	5.6	2.5–15.0	S
P. hoodii Richardson	*	Sph	30–48	23–27	5.4	1.8–12.7	F
P. longifolia Nutt.	*	Sph	31–45	16–18	5.5	4.1–12.4	F
P. maculata L.	*	Sph	40–53	23–29	5.3	5.2–15.0	O

Table 2. Continued.

Taxon	Ref	Shape	Diameter (µm)	Number of Pores	Exine Thickness (µm)	Lumen	
						Diameter (µm)	Content
P. missoulensis Wherry	2	Sph	26–32	20–30	3.1	NA	F*
P. multiflora A. Nelson	*	Sph	31–44	20–26	4.7	3.5–13.5	S
P. nana Nutt.	*	Sph	26–35	12–17	4.2	2.0–8.6	F
P. oklahomensis Wherry	2	Sph	22–28	20–27	3.5	NA	F*
P. paniculata L.	*	Sph	42–56	29–34	4.4	3.4–13.0	O
P. paniculata L.	2	Sph	34–43	26–33	5.0	3.0–8.0	F*
P. pilosa L.	*	Sph	22–31	22	3.6	2.6–7.8	S
P. sibirica L.	*	Sph	31–48	21	5.2	2.3–7.8	F
P. subulata L.	*	Sph	23–36	14–17	4.2	2.8–8.0	S

Table 3. Herbarium specimens utilized in this study.

Species	Specimen
Leavenworthia aurea Torr.	U.S.A. Oklahoma: Choctaw County, 1.9 mi E of junction of highway 70 and 147 on highway 70, 15 May 1978, *Amos & Barber 741* (OKL)
Leavenworthia aurea	U.S.A. Oklahoma: Choctaw County, 2.5 mi W of Ft. Towson, 3 mi E of Sawyer along US 70, 16 Apr 1988, *Loconte 695* (OKL)
Leavenworthia aurea	U.S.A. Oklahoma: Choctaw County, 1.4 mi E of junction of highways 147 and 70, N side of road, 2 Mar 2000, *Folley s.n.* (OKL)
Leavenworthia aurea	U.S.A. Oklahoma: McCurtain County, 0.5 mi E of Goodwater, 16 Apr 1988, *Loconte 684* (OKL)
Phlox pilosa L. subsp. *Longipilosa* (Waterf.) Locklear, *comb. et stat. nov.*	U.S.A. Oklahoma: Comanche County, Wichita National Forest, 9 May 1937, *Eskew 1714* (OKL)
Phlox pilosa subsp. *longipilosa*	U.S.A. Oklahoma: Greer County, 0.4 mi N of intersection of Mountain Avenue and Quarry Road on Quarry Road, Granite, 10 May 1978, *Amos & Sherwood 747* (OKL)
Phlox pilosa subsp. *longipilosa*	U.S.A. Oklahoma: Kiowa County, 1.4 mi N of Quartz Mountain State Lodge, 10 May 1978, *Amos & Sherwood 780* (OKL)

length was nearly equal to that of the polar axis and the colpus was filled with granules less than 1.0 μm in diameter (Figure 4B). The pollen surface had 4–6 sided reticulate networks enclosing lumina 1.0–3.0 μm long. Lumina contents varied, with some being empty, others containing granules similar to but smaller in diameter ($<$ 0.50 μm) than those in the colpus, and others containing ill-defined, inclined spear-shaped protrusions approximately 0.3 μm in height (Figure 4E). Reticulum ridges were supported by massive columellae approximately 1.4 μm in height and 0.4 μm in width (Figure 4D, F; Figure 5). The remarkably thin exine, approximately 1.5 μm thick, is shown in Figure 5, wherein the lower or inner surface distinctly shows a clear, almost transparent outline of the reticulate outer surface.

***Phlox pilosa* subsp. *longipilosa* pollen morphology.** Pollen shape was spheroidal, with pollen grain sizes ranging from 21.0–41.0 μm. The apertures were pantoporate, with an NPC classification of

764 and approximately 15–30 evenly spaced pores separated by approximately 8.0–10.0 μm (Figure 6A). The pores were 2.5–3.0 μm in diameter, each with a prominent costa (Figure 6B). The reticulate surface had 4–6 sided reticulate networks, each with lumen 4.0–6.0 μm long and surrounded by ridges supported by thickened columellae, approximately 2.0 μm in height, that were expanded basally (Figure 7A–C). All lumen encompassed a complex mesh of delicate-appearing sporopollenin rods approximately 0.3 μm thick; the proximal end connected to the ridges about 0.5–1.0 μm beneath the surface, whereas the distal end formed numerous complex central meshworks suspended over the lumen floor and connected to it by short vertical extensions (Figure 7A). The individual meshworks were less than 1.0 μm in width (Figure 7A–C). The complex pollen surface was multireticulate. In those lumen containing pores, the sporopollenin rods originating from the ridges were either markedly bent at the attached pore margin, or hung freely over the pore (Figure 6A; Figure 7B, C). The exine was approximately 4.0–6.0 μm thick (Figure 6B; Figure 7C).

DISCUSSION

Leavenworthia aurea. *Leavenworthia* pollen morphology is consistent with that of most members of the Brassicaceae, a family morphologically typified by pollen having a reticulate surface and 3-colpate apertures extending nearly the length of the polar axis. Lumen morphology is a character widely used in describing Brassicaceae pollen. In *L. aurea*, lumen sizes and shapes span all three classes of the systems recognized by Inceoglu and Karamustafa (1977) and Abdel Khalik et al. (2002), thus reflecting the heterobrochate (different sizes; Erdtman 1952) nature of the reticulations. Similar observations have been made for other members of the Brassicaceae (Table 1). Statistical parameters such as lumen area, spatial distances between lumen ridges, and consistency or inconsistency of lumen sizes in various areas of the pollen, such as the poles or colpus regions, have not been analyzed. Such analyses are beyond the scope of this study, but they offer a new dimension to understanding lumen morphology in the Brassicaceae, as well as in other families with reticulate pollen.

Other distinguishing structural features in *Leavenworthia aurea* are the inclined, spear-shaped protrusions in some lumina. These

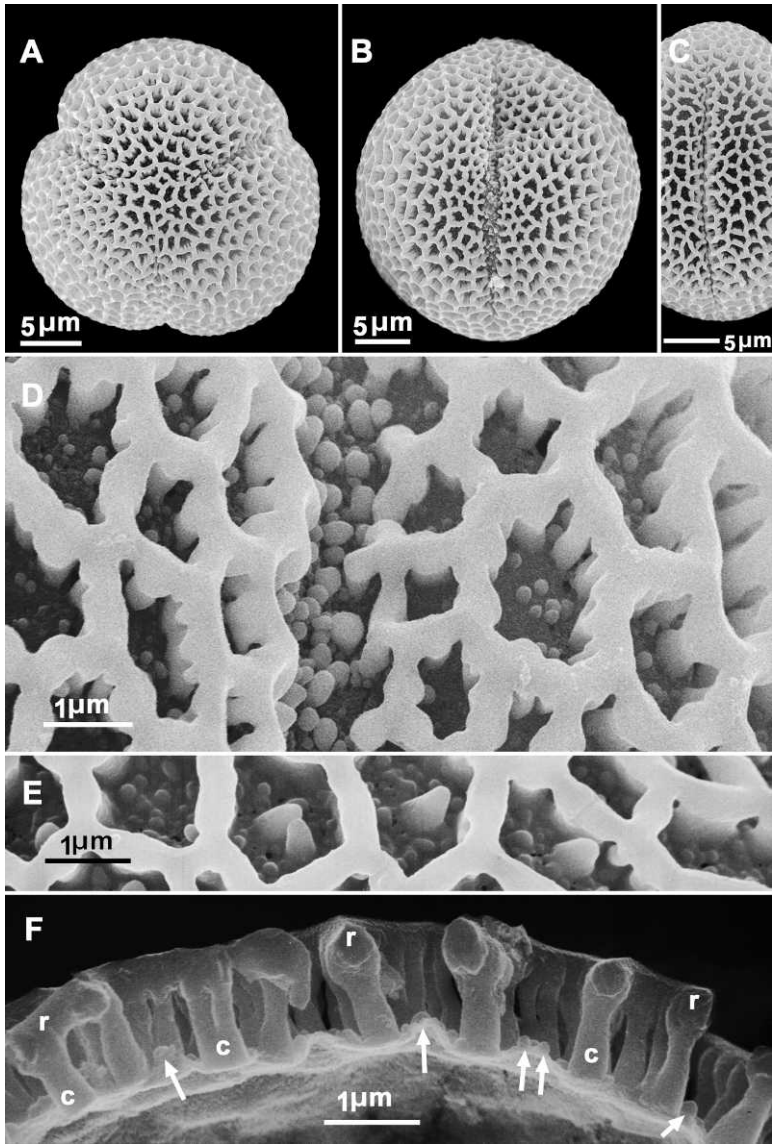

Figure 4. Scanning electron micrographs of *Leavenworthia aurea* pollen. A. Polar view (from voucher *Folley s.n.*). B. Equatorial view. The colpus nearly reaches the poles and is filled with granules (see Figure 4D for enlargement; from voucher *Folley s.n.*). C. Equatorial view. In contrast to open colpus in Figure 4B, the colpus is closed and appears as a narrow slit (from voucher

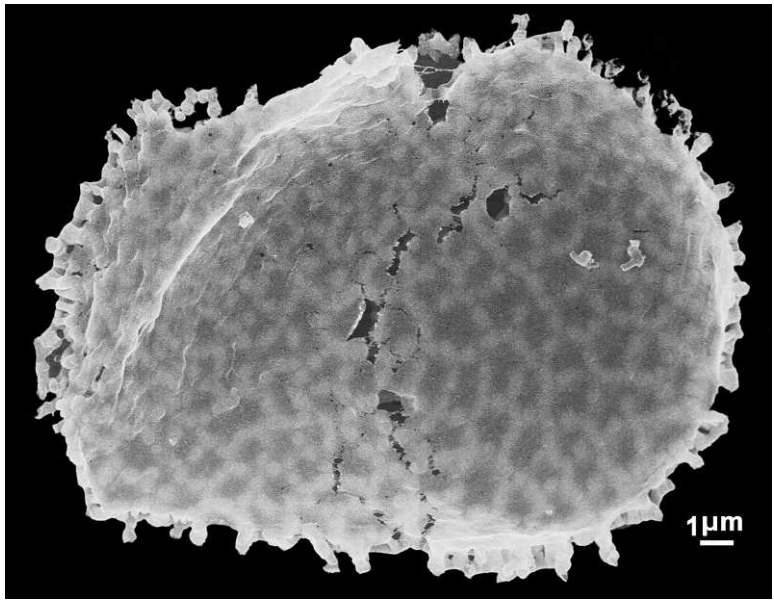

Figure 5. Scanning electron micrograph of *Leavenworthia aurea* pollen. The thin exine is emphasized by the underside of a fractured grain whereby the outline of the reticulate surface is clearly displayed. Note thick columellae along perimeter of grain (from voucher *Loconte 695*).

protrusions were considered equivalent to stranded columellae initially described in *Dithyrea* (tribe Physarieae B.L. Rob.) by Rollins (1979), differing only in that the latter displayed rounded ends. Rollins and Banerjee (1979) also found these protrusions dispersed among genera throughout the family: *Brassica* and *Rapistrum* Crantz (tribe Brassiceae DC.); *Capsella* Medik. (Camelineae DC.); *Lepidium* (Lepidieae); and *Dipoma* Franch. (tribe

←

Folley s.n.). D. Enlarged portion of exine from Figure 4B showing colpus filled with spherical granules less than 1.0 µm in diameter (from voucher *Folley s.n.*). The lumen contain scattered granules that are of smaller diameter than those in the colpus. E. Some lumen are filled with granules and other lumen also contain inclined, spear-shaped free standing columellae (from voucher *Loconte 684*). F. Cross section of fractured exine. White arrows show granules on extremely thin lumen floor; columellae (c); angular ridge (r; from voucher *Amos & Barber 741*).

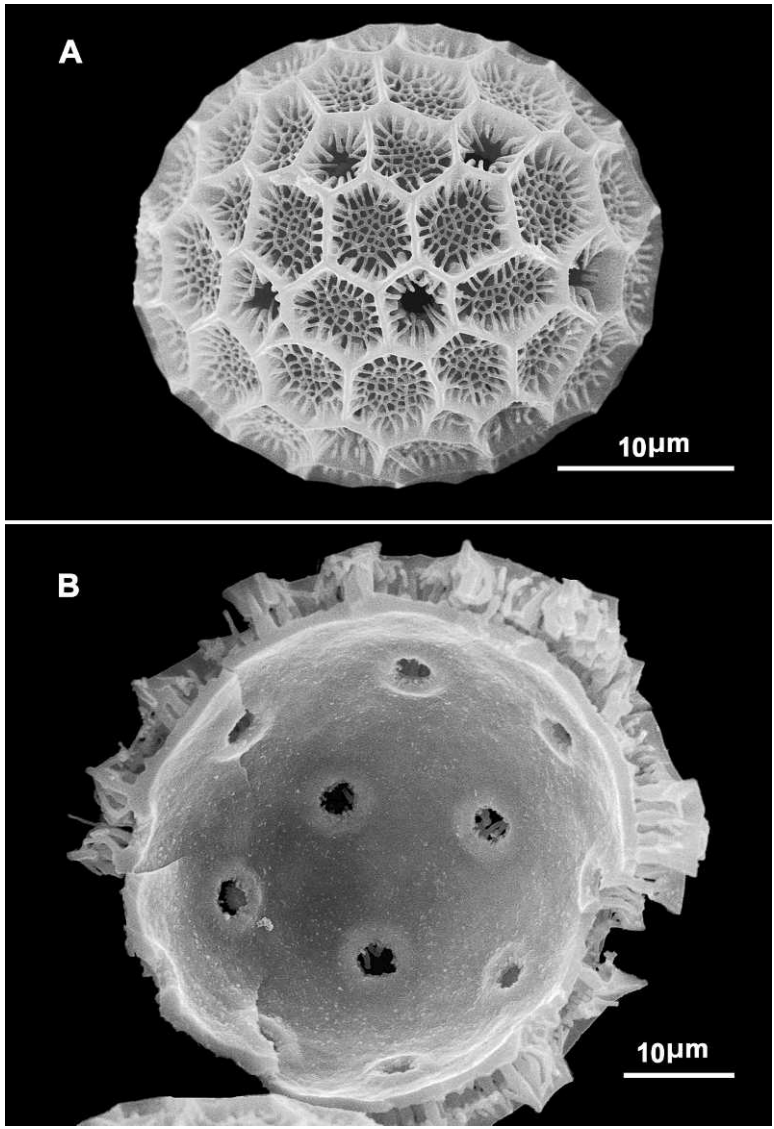

Figure 6. Scanning electron micrographs of *Phlox pilosa* subsp. *longipilosa* pollen. A. Pantoporate spheroidal grain showing at least five poral areas (from voucher *Amos & Sherwood 780*). B. Underside of fractured grain with at least 12 pores and distinctive costae. Note regularity of pore spacing (from voucher *Eskew 1714*).

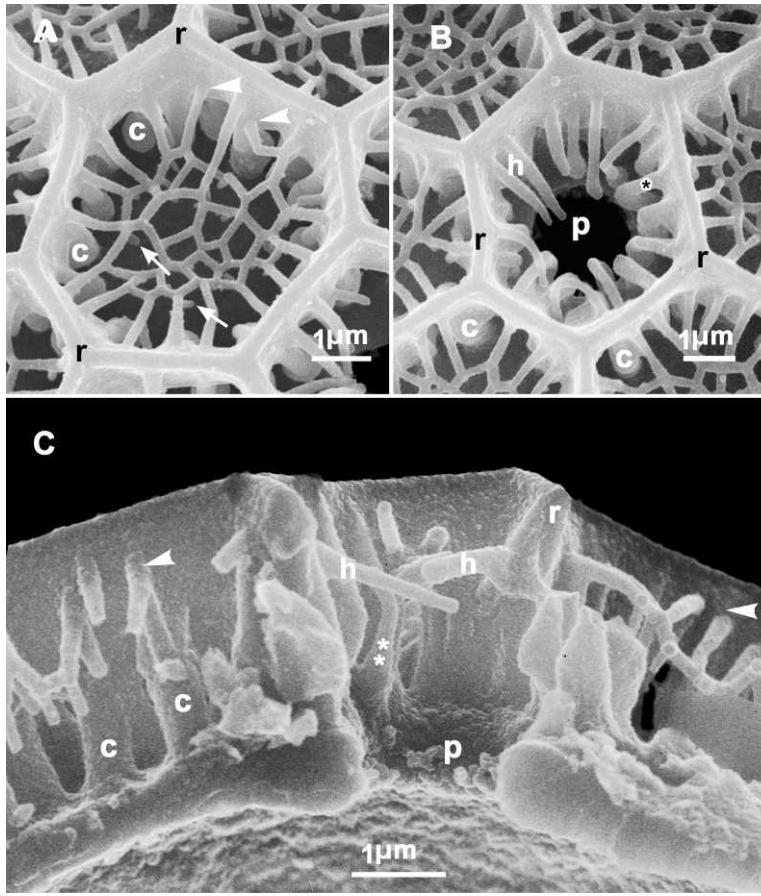

Figure 7. Scanning electron micrographs of *Phlox pilosa* subsp. *longipilosa* pollen. A. Multireticulate portion of exine. Note attachment of sporopollenin rods (white arrowheads) to ridges (r), the complex branching of rods in center of lumen, and their support above the lumen floor by short extensions (white arrows). Inflated structures (c) beneath ridges are basal portions of supporting columellae (from voucher *Amos & Sherwood 747*). B. Lumen containing pore (p). Rods are proximally attached to the ridges (r), distally some are attached to pore margin and others freely hanging (h). Support columellae (c; from voucher *Amos & Sherwood 780*). C. Section of fractured wall showing proximal attachment of rods (white arrowheads) to angular ridge (r), distal ends of rods (h) freely hanging above pore (p), and a curved rod (white asterisks) attached to pore margin. Support columellae (c) for ridges (from voucher *Amos & Sherwood 780*).

unassigned; Al-Shehbaz 2012). Their systematic value was initially demonstrated when they were used along with other criteria (viz., chromosome numbers, stigma shape, seed types, pollen shape) to separate the genus *Dithyrea* from *Dimorphocarpa* (Rollins 1979). In the Cardamineae, stranded columellae have also been proven of systematic value at the species level by distinguishing *Ornithocarpa fimbriata* Rose, with stranded columellae, from *O. torulosa* Rollins, without stranded columellae (Al-Shehbaz 1989). Stranded columellae have also been described in the colpus of *Selenia* by Rollins and Banerjee (1979). At this time, we do not have data from other members of the Cardamineae to extend observations on this character.

In summary, characters of *Leavenworthia aurea* pollen were comparable to *Amoracia*, *Barbarea*, *Cardamine*, *Nasturtium*, *Ornithocarpa*, *Rorippa*, and *Selenia* (Table 1)—all members of the Cardamineae. However, the data for *L. aurea* were also in accord with randomly selected taxa from other Brassicaceae tribes (Table 1). As clearly indicated in Table 1 and throughout other reports of Brassicaceae pollen, similar shapes, sizes, aperture types, exine thicknesses, and lumen types are found throughout the family. A character of potential value, stranded columellae, was difficult to evaluate.

***Phlox pilosa* subsp. *longipilosa*.** A variety of microscopic techniques has been applied to the study of *Phlox* pollen. Stuchlik (1967a) described acetolyzed pollen with light microscopy at different microscope levels using the LO analysis of Erdtman (1952), which focused on bright (Lux) and dark (Obscuritas) pollen features. He also described sections of unacetolyzed grains with phase contrast and transmission electron microscopy. Taylor and Levin (1975) included some of the first scanning electron micrographs of *Phlox*. Their unacetolyzed preparations of *P. oklahomensis*, *P. missoulensis*, and *P. paniculata* showed natural oils and waxes (pollenkitt) in the lumen on the exine surfaces; these substances are typically removed by acetolysis treatment. Their SEM images helped clarify the question of lumen content for *P. paniculata* by showing sporopollenin micromeshes saturated with pollenkitt (Table 2). In the present report, the acetolysis method clearly depicts individual sporopollenin rods composing the micromeshes, and allows their viewing in relation to their attachment to portions of the pollen grain walls and pores

(Figure 6A; Figure 7A, B). Further, broken grains (Figure 6B; Figure 7C), which result during some phase of pollen preparation, provide constructive sectional and internal views not achieved elsewhere, and supplement data from previous studies.

Phlox pilosa subsp. *longipilosa* pollen is similar to that described for *P. pilosa* by Stuchlik (1967a, b), with the exception that all lumen, rather than some, contain a complex micromesh of sporopollenin reticulations. Lumen content is the major distinguishing character among *Phlox* species (Table 2). In the 24 species included in the *Phlox* pollen type established by Stuchlik (1967a), three classes of lumen content were noted: 1) all lumen filled with micromesh complexes; 2) some lumen filled with micromesh complexes; and 3) lumen empty with no micromesh complexes (Table 2). This character was important in his pollen key (Stuchlik 1967a, p. 214), and it is noteworthy that 25% of the *Phlox* taxa in his study had pollen similar to that of *P. pilosa* subsp. *longipilosa*, in having all lumen filled (Table 2). The pollen size, shape, pore number, and exine thickness of *P. pilosa* subsp. *longipilosa* are comparable with those of other species of *Phlox* (Table 2) and fit within the parameters established for the genus by Stuchlik (1967a, b).

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