Pollen morphology in *Lysipomia* (Campanulaceae: Lobelioideae) and interpretation of shape artifacts

Kevin J. Price and Tina J. Ayers*

Department of Biological Sciences, Northern Arizona University, P.O. Box 5640, Flagstaff, AZ 86011-5640, USA; *corresponding author; e-mail: tina.ayers@nau.edu

Abstract. The pollen of 32 species of *Lysipomia* was examined by light, scanning, and transmission electron microscopy. Two pollen types occur in the genus: 3-colporate and 6-colporate. The 3-colporate condition occurs in only two species, *L. laciniata* and *L. pumila*. The remaining 30 species are 6-colporate, a condition known from only one other genus in the Campanulaceae. Surface sculpturing among the species is uniformly striate. Pollen shape was highly variable within a single individual in comparisons of pollen gathered from herbarium specimens, FAA preserved material collected in the field, and fresh pollen from cultivated individuals grown from seed. Shape may change from oblate spheroidal to subprolate as a result of drying time and temperature, and should not be used as a morphological character in systematic studies if infraspecific variation is seen. When fresh or preserved pollen is not available, rehydrated pollen should be compared to reduce the possibility of inadvertent artifact production confounding the analysis of morphological data.

Key Words: Campanulaceae, Lobelioideae, *Lysipomia*, pollen, morphology, SEM, TEM.

Lysipomia Kunth (Campanulaceae: Lobelioideae) comprises about 40 species restricted to the páramo and wet puna of the Andes from 3000 to 5000 meters elevation. Two species, *L. laciniata* A. DC. and *L. sphagnophila* Griseb., are widespread and comprise many infraspecific taxa. The remaining 38 species show little or no infraspecific variation and have extremely narrow distributions, with many endemic to a single ridge or volcanic cone.

Within the subfamily Lobelioideae (sensu Lammers, 2007), *Lysipomia* is unique in possessing minute capsules that dehisce via an apical operculum. This derived feature has been the key generic character since Kunth (1819) established the genus and is good evidence that the genus is monophyletic. The generic limits of *Lysipomia* have expanded over the past 150 years to include Weddell's (1858) *Rhizocephalum* (delimited by a pubescent corolla tube and an ovary that is unilocular at the apex but bilocular at the base) and Wimmer's (1953) *Dominella* (delimited by a corolla tube split on the dorsal side as in the genus *Lobelia* L. and the lack of anther trichomes). The only detailed revision of the entire genus was prepared by McVaugh (1955). His treatment was based on macromorphological data gathered from herbarium specimens.

Micromorphological characters of the pollen exine have been reported for three species of *Lysipomia* as part of a fossil pollen flora from Quaternary sediments near Bogotá, Colombia (van der Hammen & Cleef, 1978). Pollen characteristics from the remaining species have not been investigated. The goal of this study is to describe the pollen morphology throughout the genus *Lysipomia* and evaluate its utility as a source of phylogenetic information. Pollen morphology was found to be an excellent indicator of relationship in Dunbar's (1975, 1984) thorough generic-level survey of pollen in the family Campanulaceae. Pollen exine morphology also supported a sister species relationship between *Lobelia gypsophila*

Ayers (formerly *Heterotoma pringlei* B. L. Robins.) and *Lobelia margarita* F. E. Wimmer (Ayers, 1988).

Materials and methods

Pollen from 32 of the 40 known species of *Lysipomia* was examined with the SEM and photographed for comparison. The remaining eight species were not included in this study because they are only known from the type material or because the available herbarium specimens were too scanty to justify removal of anthers. Pollen grains were removed from field collected specimens or from specimens on loan from the following herbaria: AAU, F, MO, NY (acronyms from Holmgren et al., 1990). Pollen was mounted directly onto aluminum stubs without acetolysis, and sputter coated with gold-palladium. Morphological characterization and observations were conducted with a Jeol 1200EX at 15 KV at magnifications of 1000×. Images were captured and saved to disc in order to analyze colpi number, apertures, size, shape, and sculpturing.

To analyze variation in pollen shape and size, pollen from 18 species was obtained fresh from plants in cultivation in a growth chamber at Northern Arizona University and from specimens preserved in formalin/acetic acid/ethanol 1:1:3 (FAA) collected in the field. This material was then compared to dried material removed from herbarium specimens collected during field work in 1995–98. FAA preserved pollen grains from the 18 species were mounted directly into Hoyer's solution. Dimensional characteristics were noted on FAA preserved pollen using light microscopy (LM). Measurements of polar and equatorial axes were performed with a Zeiss Axioskop microscope at 400× coupled to a Hitachi video camera and monitor. The polar and equatorial axes of 10 pollen grains were measured for each species. Means, standard deviations, ranges, and polar/equatorial (P/E) ratios were subsequently calculated.

To analyze the effects of temperature and chemical dehydration on pollen shape, fresh pollen was first observed immediately upon removal from the anthers of live plants and then dried at room temperature (25°C), at 37°C, and at 65°C in a Fisher Isotemp laboratory oven. SEM photos of these dehydrated samples were compared to fresh and FAA preserved materials.

To investigate whether exine structure is correlated with the ability of pollen to change shape as a function of the drying process, FAA preserved pollen was sectioned and observed using transmission electron microscopy (TEM). The pollen grains were fixed with 2% glutaraldehyde in a 0.1 M phosphate buffer at pH 7.2 followed by treatment with 0.2% OsO_4 in phosphate buffer without rinsing. The fixed pollen was then embedded in epoxy resin (Spurr, 1969), ultrathin sectioned, and stained with a uranyl acetate/lead citrate combination. The exine of the prepared samples was examined utilizing a LEO 435 transmission electron microscope. Micrographs of pollen sections were taken and images were captured and saved to disc in order to analyze exine structure.

Results

Analysis of the SEM photos of the 32 species showed that *Lysipomia* pollen is colporate with uniformly striate surface sculpturing (Table I). These characters are common in the Campanulaceae, subfamily Lobelioideae (Dunbar, 1975, 1984; Dunbar & Wallentinus, 1976). The germinal furrows have a granular texture. Only two species in the genus are 3-colporate (Fig. 1A): *L. laciniata* and *L. pumila* (Wedd.) E. Wimm. The other 30 species examined are 6-colporate (Fig. 1B). Each species has one of two types of aperture arrangements distinguished here. In type I, the colpi remain separate (Fig. 1C) at the poles as in the 3-colporate grains; in type II, the colpi have furrow pairs that fuse just below each pole (Fig. 1B).

A more extensive study of shape was necessary due to observed variations in shape within a single species. Unlike Hong's (1983) study, where changes in shape could be correlated with age of the herbarium specimen from which the pollen was removed, the pollen of *Lysipomia* was extremely variable from herbarium specimens of similar age. Moreover, fresh and FAA preserved pollen often varied significantly from the field collected herbarium specimens (Table II). Fresh and FAA preserved pollen were similar, retaining a uniform, generally subspheroidal

TABLE I
POLLEN MORPHOLOGY DATA FOR 32 SPECIES OF *LYSIPOMIA* FROM DRIED HERBARIUM SPECIMENS.

Taxon (Collector number, acronym)	Aperture	Type I/II	Shape	Sculpturing
L. acaulis Kunth (*Jeppesen 8875*, AAU)	6-colporate	I	S	Striate
L. aretioides Kunth (*Ayers 1163*, ASC)	6-colporate	I	S	Striate
L. biliniata McVaugh (*Ayers 1427*, ASC)	6-colporate	I	P	Striate
L. bourgoinii Ernst (*Aristiguieta 2460*, NY)	6-colporate	I	S	Striate
L. brachysiphonia (A. Zahlbr.) E. Wimm. (*Balslev 3219*, NY)	6-colporate	I	P	Striate
L. caespitosa T. J. Ayers (*Ayers 1418*, ASC)	6-colporate	II	S	Striate
L. crassomarginata (E. Wimm.) S. Jeppesen (*Ayers 1419*, ASC)	6-colporate	II	P	Striate
L. cuspidata McVaugh (*Ayers 895*, ASC)	6-colporate	I	P	Striate
L. cylindrocarpa T. J. Ayers (*Ayers 1122*, ASC)	6-colporate	II	S	Striate
L. glandulifera (Wedd.) Schlect. (*SanchezVega 8870*, ASC)	6-colporate	I	S	Striate
L. gracilis (E. Wimm.) E. Wimm. (*Barbour 3422*, MO)	6-colporate	II	S	Striate
L. hutchinsonii McVaugh (*SanchezVega 8895*, ASC)	6-colporate	I	S	Striate
L. laciniata A. DC. subsp. *laciniata* (*Dotti 115*, ASC)	3-colporate	I	S	Striate
L. laricina E. Wimm. (*Ayers 887*, ASC)	6-colporate	I	S	Striate
L. lehmannii Hieron. ex A. Zahlbr. (*Ayers 1123*, ASC)	6-colporate	I	P	Striate
L. montioides Kunth (*Ayers 1165*, ASC)	6-colporate	I	S	Striate
L. multiflora McVaugh (*SanchezVega 8742*, ASC)	6-colporate	I	S	Striate
L. muscoides Hook. f. subsp. *muscoides* (*Ayers 1393*, ASC)	6-colporate	I	S	Striate
L. oellgaardii S. Jeppesen (*Ayers 1126*, ASC)	6-colporate	I	S	Striate
L. pumila (Wedd.) E. Wimm. (*Ayers 1184*, ASC)	3-colporate	I	S	Striate
L. sp. nov. 1 (*SanchezVega 8867*, ASC)	6-colporate	II	S	Striate
L. sp. nov. 2 (*Ayers 1121*, ASC)	6-colporate	I	P	Striate
L. sp. nov. 3 (*SanchezVega 8731*, ASC)	6-colporate	I	S	Striate
L. sp. nov. 4 (*Ayers 1161*, ASC)	6-colporate	I	S	Striate
L. sp. nov. 5 (*Dotti 128*, ASC)	6-colporate	I	P	Striate
L. sp. nov. 6 (*Sagastegui 12240*, F)	6-colporate	II	S	Striate
L. sparrei S. Jeppesen (*Ayers 886*, ASC)	6-colporate	I	S	Striate
L. speciosa T. J. Ayers (*Ayers 1124*, ASC)	6-colporate	I	P	Striate
L. sphagnophila subsp. *variabilis* McVaugh (*Ayers 1127*, ASC)	6-colporate	I	P	Striate
L. subpeltata McVaugh (*SanchezVega 8885*, ASC)	6-colporate	I	S	Striate
L. tubulosa McVaugh (*Øllgaard 38473*, AAU)	6-colporate	II	S	Striate
L. vitriola McVaugh (*Ayers 1433*, ASC)	6-colporate	II	S	Striate

Acronyms follow Holmgren et al. (1990). Type I pollen grain germinal furrows remain separate; type II grain furrows fuse at the poles. Shape designations: S for spheroidal; P for prolate.

shape, while the herbarium specimens were found to exhibit a wide variety of shapes within a single species (from oblate-spheroidal to prolate). Pollen shape in the herbarium specimens was not correlated with the age of the specimen. Because pollen removed from herbarium specimens was not consistent with respect to shape, dimensional characteristics presented in Table II are based upon FAA preserved material. All of the FAA preserved material viewed under the light microscope showed no shape variation, with all grains subspheroidal. The P/E ratios used to calculate shape ranged from 0.89 (*L. laricina* E. Wimm. and *L. pumila*) to 1.15 (*L. glandulifera* (Wedd.) Schlect.). Sizes ranged from 24.7 μm (*L. oellgaardii* S. Jeppesen) to 51.0 μm (*L. laciniata*) as measured along the polar axis. The largest oblate spheroidal pollen grains are the two 3-colporate species, *L. laciniata* and *L. pumila*.

The dehydration experiment, in which fresh pollen was dried at various temperatures, produced shapes ranging from prolate to subspheroidal. Pollen that was dried at room temperature (25°C) and at 37°C became distinctly prolate (Fig. 1D). Pollen dried at the higher temperature (65°C) retained its original fresh shape (Fig. 1C). FAA preserved pollen that was subsequently dried at room temperature also looked identical to fresh pollen.

TEM examination of interaperatural areas confirms that the exine between furrow pairs in the 6-colporate taxa is comparable to that

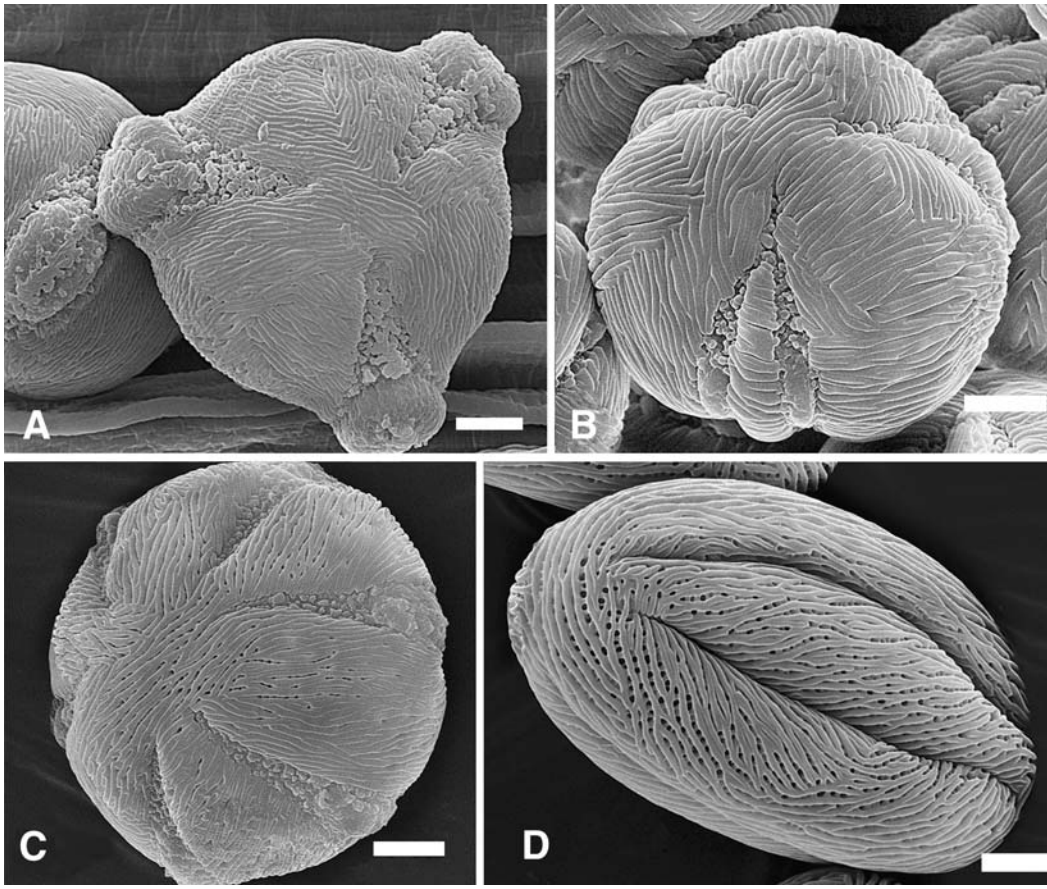

Fig. 1. Scanning electron micrographs of *Lysipomia* pollen. **A.** Three-colporate grain of *L. laciniata* (*Dotti 115*, ASC). **B.** Six-colporate, type II grain of *L. tubulosa* (*Øllgaard 38473*, AAU). **C.** Six-colporate, type I grain of *L. montioides* (*Ayers 1165*, ASC) dried at 65°C exhibiting spheroidal shape of fresh and FAA preserved pollen. **D.** *L. montioides* (*Ayers 1165*, ASC) dried at 25°C exhibiting prolate shape. All scale bars=5 μm.

of the exine structure of the 3-colporate taxa (Fig. 2). Cross sections of the exine of species that change from spheroidal when fresh to prolate upon drying were not significantly different in ultrastructure from sections of the taxa that are spheroidal when dry. TEM sections also show that there has been no increase in the number of nuclei in the grains with increasing colpi number. The pollen grains of the 6-colporate taxa remain two-nucleate.

Discussion

Investigations of the pollen of *Lysipomia* show that colpi number and not pollen shape or size is the more reliable characteristic to use in illuminating phylogenetic relationships. The evolution of 6-colporate pollen in *Lysipomia* appears to be unique in the subfamily Lobelioideae. The only other taxon in the family Campanulaceae so far reported to consistently possess six colpi is the monotypic Californian genus *Parishella* Greene (Dunbar, 1975), which also shares similar fruit dehiscence via an apical operculum. Colpi number is congruent with other morphological data (vertical, disc-like stem and contractile roots) supporting the two 3-colporate taxa, *L. laciniata* and *L. pumila*, as sister taxa. Both species are diploid and possibly less derived in the genus, while the 6-colporate species are either diploid or tetraploid (Ayers, unpublished data). If the colpi number is mapped on a molecular phylogeny

TABLE II
POLLEN MORPHOLOGY DATA FOR 18 SPECIES OF *LYSIPOMIA* FROM FAA PRESERVED MATERIAL.

Taxon	Polar axis (P) μm, mean ± SD (range)	Equatorial dia. (E) μm, mean ±S.D. (range)	P/E ratio	Shape
L. biliniata McVaugh	39.8±0.72 (37–41)	39.4±0.60 (38–40)	1.01	Prolate spheroidal*
L. cuspidata McVaugh	27.3±0.96 (25–29)	26.6±0.96 (24–28)	1.03	Prolate spheroidal
L. glandulifera (Wedd.) Schlect.	36.6±1.48 (33–40)	31.9±1.30 (30–37)	1.15	Subprolate
L. laciniata A. DC. subsp. *laciniata*	51.0±1.00 (50–55)	52.0±1.40 (50–55)	0.98	Oblate spheroidal
L. laricina E. Wimm.	36.8±2.84 (32–40)	41.3±1.16 (38–44)	0.89	Oblate spheroidal
L. lehmannii Hieron. ex A. Zahlbr.	36.0±0.80 (35–38)	33.2±1.56 (31–35)	1.08	Prolate spheroidal*
L. montioides Kunth	35.7±1.56 (32–38)	34.5±1.30 (32–37)	1.03	Prolate spheroidal*
L. multiflora McVaugh	48.2±1.40 (45–50)	44.2±0.96 (42–45)	1.09	Prolate spheroidal
L. muscoides Hook. subsp. *muscoides*	31.9±0.54 (31–33)	31.8±0.88 (30–34)	1.00	Oblate spheroidal
L. oellgaardii S. Jeppesen	24.7±1.64 (23–28)	26.7±1.78 (24 -30)	0.93	Oblate spheroidal*
L. pumila (Wedd.) E. Wimm.	41.5±1.40 (39–44)	46.4±1.68 (44–49)	0.89	Oblate spheroidal
L. sp. nov. 1 (*Sanchez Vega 8867*)	37.3±1.70 (35–40)	37.3±1.44 (34–40)	1.00	Oblate spheroidal
L. sp. nov. 2 (*Ayers 1121*)	28.7±0.76 (27–30)	28.6±1.12 (27–32)	1.00	Oblate spheroidal
L. sp. nov. 3 (*Sanchez Vega 8731*)	31.5±1.30 (30–35)	31.5±0.80 (30–33)	1.00	Oblate spheroidal*
L. sparrei S. Jeppesen	31.2±0.88 (30–34)	30.0±0.00 (30–30)	1.04	Prolate spheroidal
L. speciosa T. J. Ayers	40.2±0.32 (40–41)	38.1±0.72 (36–39)	1.06	Prolate spheroidal
L. sphagnophila Griseb. subsp. *variabilis* McVaugh	34.5±1.00 (32–36)	31.6±1.20 (30–33)	1.09	Prolate spheroidal*
L. subpeltata McVaugh	30.4±0.60 (29–31)	33.6±1.80 (31–37)	0.90	Oblate spheroidal

Voucher designations are identical to those listed in Table I. Terminology and shape classes follow Erdtman (1952, 1969). Pollen of species marked with an asterisk (*) in the shape column was distinctly prolate in the field-collected voucher specimens. All measurements are given in micrometers.

based on nrITS sequences (Ayers, 2000; Ayers, in prep.), an increase in colpi number from three to six appears to have evolved independently at least two times. Increased ploidy level is often associated with increased physical grain size in pollen (Erdtman, 1969), but the opposite appears to be true in *Lysipomia,* where one of the diploid 3-colporate species (*L. laciniata*) has the largest pollen grains in the genus (Table II). The increase in colpi number from three to six does not appear to be correlated with an increase in the number of nuclei in the pollen as has been found in the Lamiaceae (Trudel & Morton, 1992). Type II colpi that fuse at the poles do not appear to reflect phylogenetic relationships when mapped on the existing phylogenies. Many species well supported as sister groups have different aperature arrangements as described here.

Researchers using herbarium specimens in phylogenic investigations should be aware that comparisons of dried materials, even those of identical ages, may inadvertently introduce error into their analyses. Pollen shape in *Lysipomia* appears to be correlated with the temperature (and possibly humidity) at which the specimens were dried. Most systematists who consult herbarium specimens do not know how the specimen was processed, although ethanol preservation of materials before drying is usually evident by the uniform color. Pollen data gathered solely

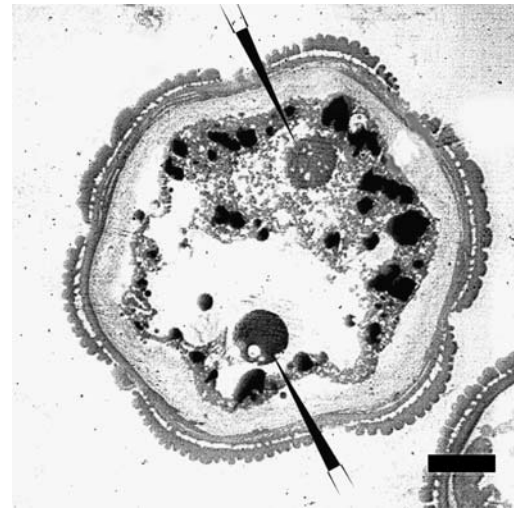

FIG. 2. Transmission electron micrograph of *Lysipomia* sp. nov. (*Ayers 1121*, ASC). Arrows point to the nucleus of each cell. Scale bar=2 μm.

from herbarium collections should thus be held suspect if significant intraspecific variation is detected among the specimens, especially if the variation is seen in herbarium specimens of similar ages. We recommend that multiple herbarium specimens of each species be consulted during the evaluation process. If intraspecific variation is detected, SEM photos of fresh pollen, preserved pollen, or pollen rehydrated in water and then fixed in absolute alcohol (Hong, 1983) should be compared to reduce the possibility of inadvertent artifact production confounding the analysis of morphological data.

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