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## Comparative Floral Structure of Four New World *Allium* (Amaryllidaceae) Species

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**Abstract**—Scanning electron microscopy has allowed the characterization of cell pattern and ornamentation of the bulb coat, leaf, and seed coat, thereby improving and providing consistency to the taxonomy of *Allium*. However, the pollination biology and taxonomic understanding of *Allium* is far from complete, in part because floral structures have been investigated in detail for only a few species. Accordingly, this study provides a description and micro- and macromorphological comparative analysis of floral characteristics (including the adjacent bulbils of *A. geyeri* var. *tenerum*) of four New World *Allium* species (*A. cernuum*, *A. geyeri* var. *tenerum*, *A. stellatum*, and *A. textile*). Observations of fresh material demonstrated that *A. geyeri* var. *tenerum* is distinguished from the closely related *A. textile* by a larger perianth with elliptical tepals (as opposed to ovate to oblong), filaments slightly shorter than tepals (as opposed to 2/3 as long as tepals), and globose young inflorescences (as opposed to ellipsoid). Ridged cuticles from a variety of floral parts have often been reported in *Allium* as the main type of epidermal cell covering; results from this study indicate that shape and distribution of cuticles are of systematic significance in conjunction with location of the septal nectary opening, development of ovarian processes, and shape of anther apices. The presence of ovarian processes is considered a recently derived character; we hypothesize that these structures function as non-secretory visual attractants to pollinators, and in concert with broader inner filaments and clearly concave inner tepals may also represent a special adaptation to facilitate retention of abundant nectar secreted in nodding flowers of *A. cernuum* and in widely spreading flowers of *A. stellatum*.

**Keywords**—Cell pattern, cuticle ornamentation, ovarian process, SEM, septal nectary opening.

The genus *Allium* L. is a member of the family Amaryllidaceae, subfamily Allioideae, tribe Allieae (Fay and Chase 1996; APGIII 2009; Chase et al. 2009), although some recent authors treated this genus as its own family, Alliaceae (Takhtajan 1997; Kubitzki 1998). After Fay and Chase (1996), Friesen et al. (2000), and Chase et al. (2009), *Allium* (including *Caloscordum* Herb., *Milula* Prain, and *Nectaroscordum* Lindl.) is the only genus in tribe Allieae. The genus is characterized by bulbs enclosed in membranous (sometimes finely fibrous) tunics, free or almost free tepals, and often a subgynobasic style (Friesen et al. 2006). With over 800 species, *Allium* is naturally distributed in the northern hemisphere and South Africa, mainly in seasonally dry regions (de Sarker et al. 1997; Friesen et al. 2006; Nguyen et al. 2008; Neshati and Fritsch 2009). The primary centre of diversity for *Allium* lies in the Mediterranean basin and southwestern and central Asia, but a smaller secondary area of diversification is found in North America (Friesen et al. 2006; Nguyen et al. 2008).

It has been suggested that *Allium* has existed in the New World since at least the Tertiary Period (Raven and Axelrod 1978) and that approximately 1/6 of *Allium* diversity is found in North America north of Mexico; that is, ca. 96 species, of which 12 are known from Canada (McNeal 1992; McNeal and Jacobsen 2002). Among these 12 taxa, only one species, *A. schoenoprasum* L., is widespread in the native floras of both the Old and New World (McNeal 1992; McNeal and Jacobsen 2002). All North American species have a base chromosome number of  $x = 7$ , except *A. schoenoprasum*, *A. tricoccum* Solander, and *A. victorialis* L., which share a base chromosome number ( $x = 8$ ) with the majority of Old World species (McNeal 1992; McNeal and Jacobsen 2002).

Even with recent progress and the advent of molecular systematics, taxonomic understanding of *Allium* has been limited, in part, because many New World species are poorly represented in herbaria (McNeal 1992; H. J. Choi, pers. obs.). The lack of well-preserved specimens documenting the geographic range of the genus, and the polymorphic nature of some vegetative and reproductive structures has led to misinterpretation of patterns of morphological variation in

numerous taxa, with subsequent confusion about species boundaries and distribution. While extensive collecting has added valuable material to American systematic collections, thereby allowing for reappraisal of morphological characters used in *Allium* classification (McNeal 1992), most Canadian herbaria are less diverse but contain valuable historical specimens collected by various botanists from the 1950s to the 1980s (Choi and Cota-Sánchez 2010a). The relatively narrow representation of Canadian *Allium* specimens in North American herbaria is, in part, correlated with few systematic studies that include Canadian species. Systematic studies of Canadian *Allium*, excluding the Flora of North America (McNeal and Jacobsen 2002) and Choi and Cota-Sánchez (2010a, 2010b), have mainly focused on the western or eastern region of the country, areas with relatively more taxonomic history (Choi and Cota-Sánchez 2010a).

In view of the scanty information involving morphology of vegetative and reproductive structures, Choi and Cota-Sánchez (2010a) have recently reviewed the taxonomy, rarity, and conservation status of *Allium* for the Canadian prairie provinces (CPP: Alberta, Saskatchewan, and Manitoba) based on analyses of herbarium specimens and fieldwork. Their study recognizes five species: *A. schoenoprasum* (sect. *Schoenoprasum* Dumort.), *A. geyeri* S. Watson var. *tenerum* M. E. Jones (sect. *Amerallium* Traub; Fig. 1A), *A. textile* A. Nelson & J. F. Macbride (sect. *Amerallium*; Fig. 1B), *A. cernuum* Roth (sect. *Lophioprason* Traub; Fig. 1C), and *A. stellatum* Ker Gawler (sect. *Lophioprason*; Fig. 1D). Among these, *A. schoenoprasum* is an S2 rare species (typically six to 20 occurrences, or total remaining individuals of 1,000–3,000) in Saskatchewan; *A. geyeri* var. *tenerum* is the rarest *Allium* taxon in the CPP and currently listed as S2 in Alberta, while *A. cernuum* is currently recommended as an S1S2 (more critical than S2) in Saskatchewan (Kershaw et al. 2001; Harms 2003; Choi and Cota-Sánchez 2010a).

*Allium geyeri* var. *tenerum* is a rare species in Canada with a distribution limited to the Waterton Lakes area of Alberta and southern Vancouver Island, British Columbia (Straley et al. 1985; McNeal and Jacobsen 2002; Choi and Cota-Sánchez

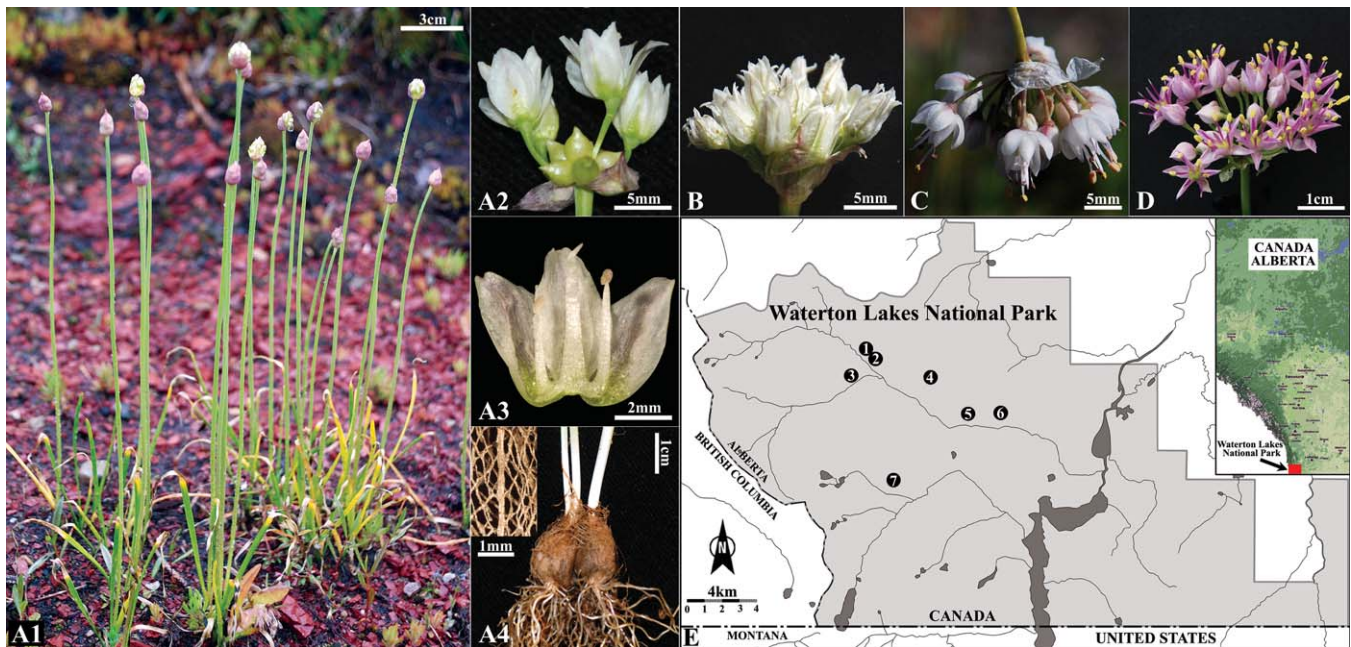


FIG. 1. *Allium geyeri* var. *tenerum* (A, E), *A. textile* (B), *A. cernuum* (C), and *A. stellatum* (D). A1. Habit. A2, B–D. Inflorescence. A3. Tepal and filament arrangement. A4. Underground structure and outer tunica (inset) of bulb. E. Geographic distribution map in Waterton Lakes National Park of Canada (1–7 indicating localities of field studies in 2010; voucher information provided in Table 1).

2010a). As in the province of Alberta, this taxon is designated as R2 in British Columbia (Straley et al. 1985). Although relatively common in the U. S. A. (McNeal and Jacobsen 2002), the rarity of *A. geyeri* var. *tenerum* in Canada may be correlated with the northernmost range limit of this species. Regardless of this distributional pattern, proactive research, such as population monitoring, should be implemented to protect this species in its northernmost range near the U. S. A. and Canada border in an effort to understand the geographic range limits, and ecological and climatic parameters governing the northern populations, a key issue in conservation biology. Indeed, biological data for this taxon in Canada are scarce, due in part to its rarity status and restricted distribution. Further, recent studies have shown that the taxonomic understanding of *Allium* and the accurate recognition of species has been limited, to some extent, by difficulty in determining their identity using only specimens preserved in herbaria (Choi and Cota-Sánchez 2010a). Therefore, the examination of plant material in-situ is required to address taxonomic questions and better understand floral structure in relation to pollination biology.

Several criteria have been used historically in classifying *Allium*. For instance, sexuality of plants, structure and shape of underground parts (including rhizome and bulb), anatomical features of root, leaf, scape, and ovary, as well as base chromosome number have been useful at the subgeneric and sectional levels (Fritsch 1992; Hanelt et al. 1992; Kruse 1992; McNeal 1992; Friesen et al. 2006; Gurushidze et al. 2008; Nguyen et al. 2008; Choi 2009). Shape and size of floral organs (e.g. perianth, filament, pistil, capsule, and seed), combined with somatic chromosome number, have provided diagnostic characters at the species level (McNeal 1992; Choi et al. 2007; Ko et al. 2009; Choi and Cota-Sánchez 2010a, 2010b), and scanning electron microscopy (SEM) has allowed the characterization of cell pattern and ornamentation of the bulb coat, leaf, and seed coat, improving the taxonomy of *Allium* (Kruse 1992; McNeal 1992; Choi et al. 2004; Fritsch et al. 2006; Choi and Cota-Sánchez 2010a). However, despite existing

variation in floral morphology among taxa, floral microcharacters have been investigated in just a few *Allium* species (Žuraw et al. 2009).

To enhance general understanding and patterns of morphological variability of floral features in *Allium*, we have conducted an investigation of four New World species (*A. cernuum*, *A. geyeri* var. *tenerum*, *A. stellatum*, and *A. textile*) from the CPP. Our study provides a general synopsis of flower structure, including new qualitative and quantitative data from fresh flowers of *A. geyeri* var. *tenerum*; new comparative micromorphological data of taxonomic significance of various floral parts and bulbils of these species; and a phylogenetic approach regarding the evolution of ovarian processes in the genus *Allium*. This information will be significant in future systematic treatments and phylogenetic inferences on character evolution of *Allium*, in particular, and the Amariyllidaceae, in general. Additionally, habitat photos and a distribution map of *A. geyeri* var. *tenerum* from Waterton Lakes National Park of Canada are provided for documentation and to assist in development of protection plans for this species.

## MATERIALS AND METHODS

**Plant Material**—Field surveys (from May to August) were carried out during the summers of 2009 and 2010, leading to a total of ten collections representing four *Allium* species from the CPP (Fig. 1; Table 1). All species were also grown in the experimental plot at the Department of Biology, University of Saskatchewan, Canada. A minimum of five buds, ten open flowers, and five post-anthesis flowers per species, as well as ten bulbils of *A. geyeri* var. *tenerum*, were fixed in FAA (Sass 1958; formaldehyde: acetic acid: 96% alcohol: water; 10:5:50:35) for microscopic examination.

**Morphological Analyses**—Floral samples were observed and photographed using a TESSOVAR photomacrographic zoom system with a Nikon D100 camera. Detailed analyses of the perianth, stamens, and pistils were undertaken in *Allium geyeri* var. *tenerum* with fresh open flowers because this species has been one of the least documented in terms of floral characters. Observations and measurements were based on a minimum of 20 specimens, from which the mean and standard deviations were calculated (Table 2). Line drawings were generated from photos of voucher specimens using Adobe Photoshop 7.01.

TABLE 1. Collection data and voucher information of *Allium* species examined in this study (AB = Alberta; SK = Saskatchewan; MB = Manitoba). Vouchers deposited in the W. P. Fraser Herbarium (SASK) collection.

Taxon	Collection site and date	Voucher at SASK
<i>A. geyeri</i> var. <i>tenerum</i>	AB: Waterton Lakes National Park, 16 June 2010	<i>H. J. Choi-ab-1</i> : Figs. 1A1–A4, E (1); 2A–X
	AB: Waterton Lakes National Park, 16 June 2010	<i>H. J. Choi-ab-2</i> : Figs. 1E (2); 6A
	AB: Waterton Lakes National Park, 16 June 2010	<i>H. J. Choi-ab-7</i> : Fig. 1E (3)
	AB: Waterton Lakes National Park, 17 June 2010	<i>H. J. Choi-ab-8</i> : Fig. 1E (4)
	AB: Waterton Lakes National Park, 17 June 2010	<i>H. J. Choi-ab-9</i> : Fig. 1E (5)
	AB: Waterton Lakes National Park, 17 June 2010	<i>H. J. Choi-ab-10</i> : Fig. 1E (6)
	AB: Waterton Lakes National Park, 18 June 2010	<i>H. J. Choi-ab-11</i> : Fig. 1E (7)
	AB: Waterton Lakes National Park, 16 June 2010	<i>H. J. Choi-ab-6</i> : Figs. 3E–U; 6B
	SK: East of University Bridge, Saskatoon, 3 June 2009	<i>H. J. Choi-sk-1</i> : Figs. 1B; 3C, D
	MB: Brandon, 24 May 2010	<i>H. J. Choi-mb-1</i> : Fig. 3A, B
	<i>A. textile</i>	AB: Waterton Lakes National Park, 16 June 2010
SK: Cypress Hills Provincial Park, 22 July 2009		<i>H. J. Choi-sk-10</i> : Figs. 1C; 6C
<i>A. cernuum</i>	SK: Martensville, 11 August 2009	<i>H. J. Choi-sk-16</i> : Figs. 1D; 6D
	SK: Oakshela, 16 July 2010	<i>H. J. Choi-sk-18</i> : Fig. 5A–G, I–W
<i>A. stellatum</i>	MB: Riding Mountain National Park, 4 July 2010	<i>H. J. Choi-mb-2</i> : Fig. 5H

**Vegetative and Reproductive Microstructures**—For observation of micromorphological structures of bulbils and floral parts (inner tepal, pistil, and stamen), tissues were rinsed twice with 0.1 M phosphate buffer (pH 6.8), refixed in 2.5% glutaraldehyde, dehydrated in an ethanol-acetone series, critical-point dried with a Polaron E3000 Series II, mounted on stubs, and coated with gold in an Edwards S150B ion sputter coater. In all cases, at least five samples in each flowering stage (bud, open flower, and post-anthesis flower) were analyzed for each taxon, characterized, and photographed with a Philips 505 SEM. All data presented in Table 3 were obtained from open flowers.

## RESULTS

**General Floral Morphology**—The inflorescence of *Allium geyeri* var. *tenerum* has a globose shape before anthesis (Fig. 2A). In contrast, young inflorescences in *A. textile*, *A. cernuum*, and *A. stellatum* are ellipsoid (Figs. 3A; 4A; 5A). Bulbils are developed only in the inflorescence of *A. geyeri* var. *tenerum* (Figs. 1A2; 2C).

Flowers of the four taxa investigated have a perianth with six tepals (Figs. 2D; 3C; 4D; 5D), a three-carpellate superior

TABLE 2. Comparison of macromorphological floral characters between *Allium geyeri* var. *tenerum* and *A. textile*. The asterisk (\*) indicates data from Choi and Cota-Sánchez (2010a).

Character	Measurement minimum (Mean ± SD) and maximum	
	<i>A. geyeri</i> var. <i>tenerum</i>	<i>A. textile</i> *
Young inflorescence	globose	ellipsoid
Inner tepal		
Shape	elliptical	ovate to oblong
Apex	obtuse	obtuse
Length (L, mm)	6.7 (7.41 ± 0.37) 8.7	4.5 (6.45 ± 1.40) 8.8
Width (W, mm)	2.9 (3.36 ± 0.29) 4.1	2.0 (2.43 ± 0.23) 2.8
L/W (ratio)	1.9 (2.05 ± 0.21) 2.2	1.8 (2.67 ± 0.54) 3.1
Outer tepal		
Shape	elliptical	ovate to lanceolate
Apex	obtuse	acute to obtuse
Length (L, mm)	6.6 (7.38 ± 0.40) 8.6	4.0 (5.94 ± 1.20) 7.8
Width (W, mm)	3.0 (3.38 ± 0.31) 4.2	2.5 (3.04 ± 0.47) 3.9
L/W (ratio)	1.9 (2.02 ± 0.23) 2.3	1.4 (1.97 ± 0.37) 2.6
Filament		
Inner length (mm)	6.5 (7.32 ± 0.31) 8.3	3.2 (4.21 ± 0.77) 5.3
Outer length (mm)	6.0 (6.98 ± 0.52) 8.3	2.7 (3.44 ± 0.68) 4.5
Anther length (mm)	1.4 (1.48 ± 0.17) 1.6	0.9 (1.19 ± 0.23) 1.5
Ovary		
Length (mm)	1.5 (1.83 ± 0.27) 2.2	1.3 (1.72 ± 0.29) 2.0
Width (mm)	1.6 (1.87 ± 0.30) 2.3	1.4 (1.63 ± 0.18) 1.9

ovary (Figs. 2E, G, J, O; 3H; 4E, H; 5E, H), and two whorls comprising six stamens (Figs. 2D; 3D; 4C, D; 5C, D), fused at the base with the perianth (Figs. 1A3; 2E; 3D; 4E; 5E). The pistil is borne on a small gynophore, forming a distinct stalk from the base of the androecium to the ovary (Figs. 2G; 3H; 4G, H; 5G, H). The ovary is trigonous with six distinct regions of suture and septum internally, and three septal nectaries (Figs. 2O, P; 4N; 5L, M). In addition, distinctive crest-like apical processes develop in *A. cernuum* (Fig. 4G, H) and *A. stellatum* (Fig. 5G, H). The nectar, which is secreted through an opening (canal end) at variable positions on the side (septum region) of the ovary per species, accumulates in the cavity between the ovary and the adnation area of the filament bases and tepals (Figs. 2E; 4E; 5E). The filaments of the stamens comprise two regions: a distinct elongated upper portion and an expanded basal part. The lower portions of the filaments of the inner and outer whorls are strongly expanded or swollen (Figs. 2S; 3O; 4R; 5P), and expansions of both whorls are fused together (Figs. 1A3; 3D; 4C; 5C). The elliptical anthers are dorsifixed and introrse (Figs. 2D, E; 3C; 4C, D; 5C, D).

Qualitative and quantitative macromorphological floral characters of *Allium geyeri* var. *tenerum* were gathered from live material (Table 2). In all flowers of this taxon the perianth is campanulate to urceolate and mostly white with reddish midveins (Figs. 1A2; 2D); the inner tepals (Figs. 1A3; 2E) are elliptical with obtuse apex and 6.7–8.7 × 2.9–4.1 mm; the outer tepals (Figs. 1A3; 2E) are nearly equal to the inner ones, 6.6–8.6 × 3–4.2 mm; the filaments (Figs. 1A3; 2E) are 6.0–8.3 mm long; the anthers (Fig. 2W) are elliptical and 1.4–1.6 mm long; the ovary (Fig. 2E, G, J) is subglobose without apical crest-like processes and 1.5–2.2 × 1.6–2.3 mm. Additional information on floral characters and their variability in the other three species, as well as other *Allium* species, is presented in Choi and Cota-Sánchez (2010a).

**Micromorphology of Bulbils and Flowers**—We evaluated the external structure of flowers and bulbils using SEM (Figs. 2–5), and the micromorphological data are summarized in Table 3. Epidermal cells of the bulbils in *A. geyeri* var. *tenerum* are rectangular with straight anticlinal walls and smooth cuticular covering (Fig. 2C2, C3). Stomata are mostly found in the upper part of the bulbils (Fig. 2C2).

Inner tepals of these species have epidermal cells that are usually elongated and linear in shape with straight anticlinal

TABLE 3. Micromorphological characters of bulbils and floral organs in four North American *Allium* species (EC = epidermal cell; + = developed; – = absent).

Character	sect. <i>Amerallium</i>		sect. <i>Lophioprason</i>	
	<i>A. geyeri</i> var. <i>tenerum</i>	<i>A. textile</i>	<i>A. cernuum</i>	<i>A. stellatum</i>
<b>Bulbil</b>				
Shape of EC	rectangular	–	–	–
Cuticle of EC	smooth	–	–	–
Stomata	+ (at upper part)	–	–	–
<b>Inner tepal</b>				
Shape of EC	linear	linear	linear	linear
Cuticle of EC	ridged	ridged	nearly smooth	nearly smooth
<b>Ovary</b>				
Cuticle of EC (apical process)	–	–	ridged	ridged
Cuticle of EC (upper part)	smooth to weakly ridged	ridged	ridged	ridged
Cuticle of EC (suture region)	smooth	ridged	smooth	smooth
Cuticle of EC (septum region)	smooth	smooth	smooth	smooth
Stomata	+ (basally)	–	–	–
<b>Septal nectary</b>				
Position of nectary opening	basal	basal	middle	middle
Shape of secretory cell	papillate	papillate	papillate	papillate
<b>Stigma</b>				
Shape of EC	irregular	irregular	irregular	irregular
Cuticle of EC	scarcely ridged	scarcely ridged	scarcely ridged	scarcely ridged
<b>Style</b>				
Shape of EC	linear	linear	linear	linear
Cuticle of EC	ridged	ridged	ridged	ridged
<b>Filament</b>				
Basal projection (expanded part)	–	–	+	+
Shape of EC (expanded part)	irregular	irregular	irregular	irregular
Shape of EC (elongated part)	rectangular to linear	rectangular to linear	rectangular to linear	rectangular to linear
Cuticle of EC (expanded part)	smooth	smooth	smooth	smooth
Cuticle of EC (base of elongated part)	minutely ridged	minutely ridged	minutely ridged	minutely ridged
Cuticle of EC (middle of elongated part)	ridged	ridged	ridged	ridged
<b>Anther</b>				
Apex	small, pointed	small, pointed	broad, rounded	broad, rounded
Shape of EC	nearly isodiametric	nearly isodiametric	nearly isodiametric	nearly isodiametric
Cuticle of EC	ridged	ridged	ridged	ridged

walls; stomata are absent (Figs. 2F; 3E; 4F; 5F). Within species, cell shape and sculpturing are similar on the adaxial and abaxial surface of these tepals. The cuticular cell sculpture pattern is ridged (with parallel arrangement of ridges) in *Allium geyeri* var. *tenerum* (Fig. 2F) and *A. textile* (Fig. 3E) or nearly smooth (slight microrelief may be evident) in *A. cernuum* (Fig. 4F) and *A. stellatum* (Fig. 5F). This character varies among species but is consistent within the same taxon. In *A. textile* the wall (Fig. 3E) has relatively thick cuticular ridges compared to *A. geyeri* var. *tenerum*, with thin ridges.

Ovaries are composed of irregular epidermal cells with surfaces covered by a smooth or weakly to strongly ridged cuticular layer (Figs. 2G, I–N; 3F–L; 4H–M; 5H–K). The ridged cells are frequently observed in the upper part of the ovary, including the crest-like processes. Occasionally, few stomata are found in the basal part of ovaries in *Allium geyeri* var. *tenerum* (Fig. 2M). The apical processes found in *A. cernuum* and *A. stellatum* frequently have conically shaped epidermal cells with a protruding outer wall; their surface is covered by a distinct layer of cuticle with strongly marked ridges (Figs. 4H, M; 5H, K). The position of nectary openings is either basal or middle, with the former found in *A. geyeri* var. *tenerum* and *A. textile* (Figs. 2G, J; 3H), and the latter in *A. cernuum* and *A. stellatum* (Figs. 4G, H; 5G, H). Flat epidermal cells with a well-developed, smooth cuticle cover the openings of all investigated species.

Our field observations indicate that nectar secretion begins after flower opening (Fig. 4I–K). Based on analyses of cross sections of the ovary, the single-layered palisade secretory epidermis of the nectary (secretory epithelium) surrounds a narrow cavity, in which nectar accumulates (Figs. 2P; 4N; 5L). Longitudinal section of the nectary indicates that the secretory cells are papillate (Figs. 4O; 5M, N); sometimes the cuticle layer of the cells is slightly lifted to form a papillate shape (Fig. 5N). The stigmas have irregular epidermal cells with surfaces covered by a scarcely ridged cuticular layer (Figs. 2Q; 3M; 4P). Conversely, the styles have elongated linear epidermal cells with surfaces covered by a prominent ridged cuticular layer (Figs. 2R; 3N; 4Q; 5O).

Expansions in the basal part of the filaments have moderate projections only in *Allium cernuum* and *A. stellatum* (compare Figs. 4R and 5P with 2S and 3O). The irregularly shaped adaxial epidermal cells at the base of the filaments are covered by a smooth cuticle in all taxa (Figs. 2T; 3P; 4S; 5Q). On the other hand, elongated parts of the filaments have rectangular to elongated linear epidermal cells covered by a ridged cuticle in all taxa (Figs. 2S, U, V; 3O, Q, R; 4R, T, U; 5P, R, S). The cuticular ridges are thicker and denser in the upper portion compared to the basal part of each filament.

The anthers exhibit two main patterns of apex shape: small and pointed in *Allium geyeri* var. *tenerum* and *A. textile* (Figs. 2W; 3S, T); broad and rounded in *A. cernuum* and *A. stellatum* (Figs. 4V; 5T, U). The epidermal cells covering the

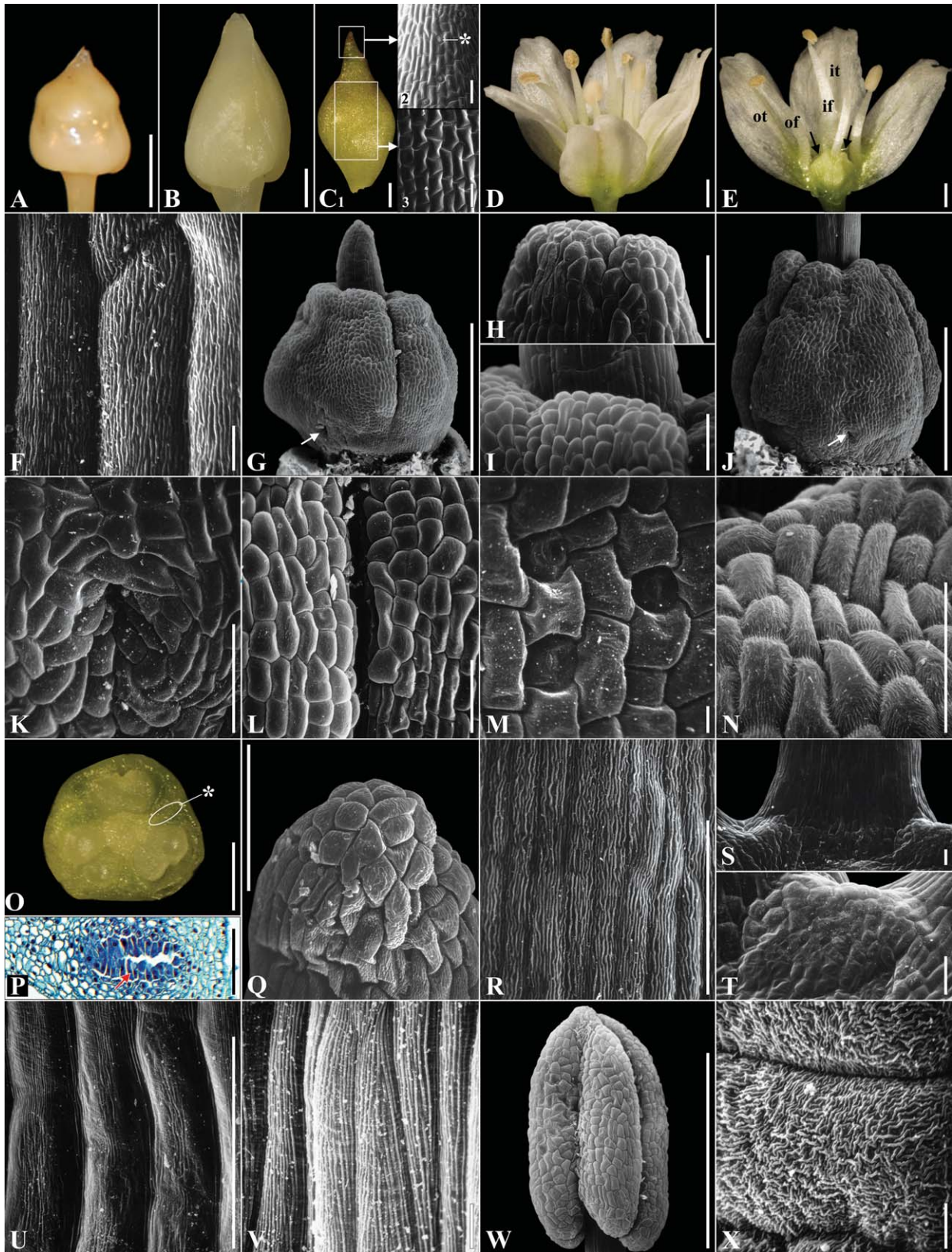


FIG. 2. Floral characters of *Allium geyeri* var. *tenerum*. A. Young inflorescence. B. Floral bud. C. Bulbil and epidermal cells (\* = stomata). D. Open flower. E. Internal structure in D (if = inner filament; it = inner tepal; of = outer filament; ot = outer tepal; arrow = nectar droplet). F. Detail of abaxial surface of inner tepal in D. G. Pistil in B (arrow = nectary opening). H. Detail of stigma in G. I. Detail of upper part of ovary in G. J. Ovary in D (arrow = nectary opening). K. Detail of nectary opening in J. L. Detail of suture in J. M. Stomata in J. N. Detail of upper part of ovary in J. O, P. Cross section of ovary in D (\* = septal nectary; arrow = secretory epithelium). Q. Detail of stigma in D. R. Detail of middle part of style in D. S. Basal part of an inner filament in D. T. Detail of fused area between inner and outer filaments in S. U, V. Detail of basal (U) and middle (V) adaxial surfaces of inner filament in D. W. Anther in D. X. Detail of epidermal cells in W (scale bars: 5 mm, Fig. A; 1 mm, Figs. B, C1, D, E, G, J, O, W; 0.1 mm, Figs. C2, C3, H, I, K, L, N, P-U; 10  $\mu$ m, Figs. F, M, V, X).

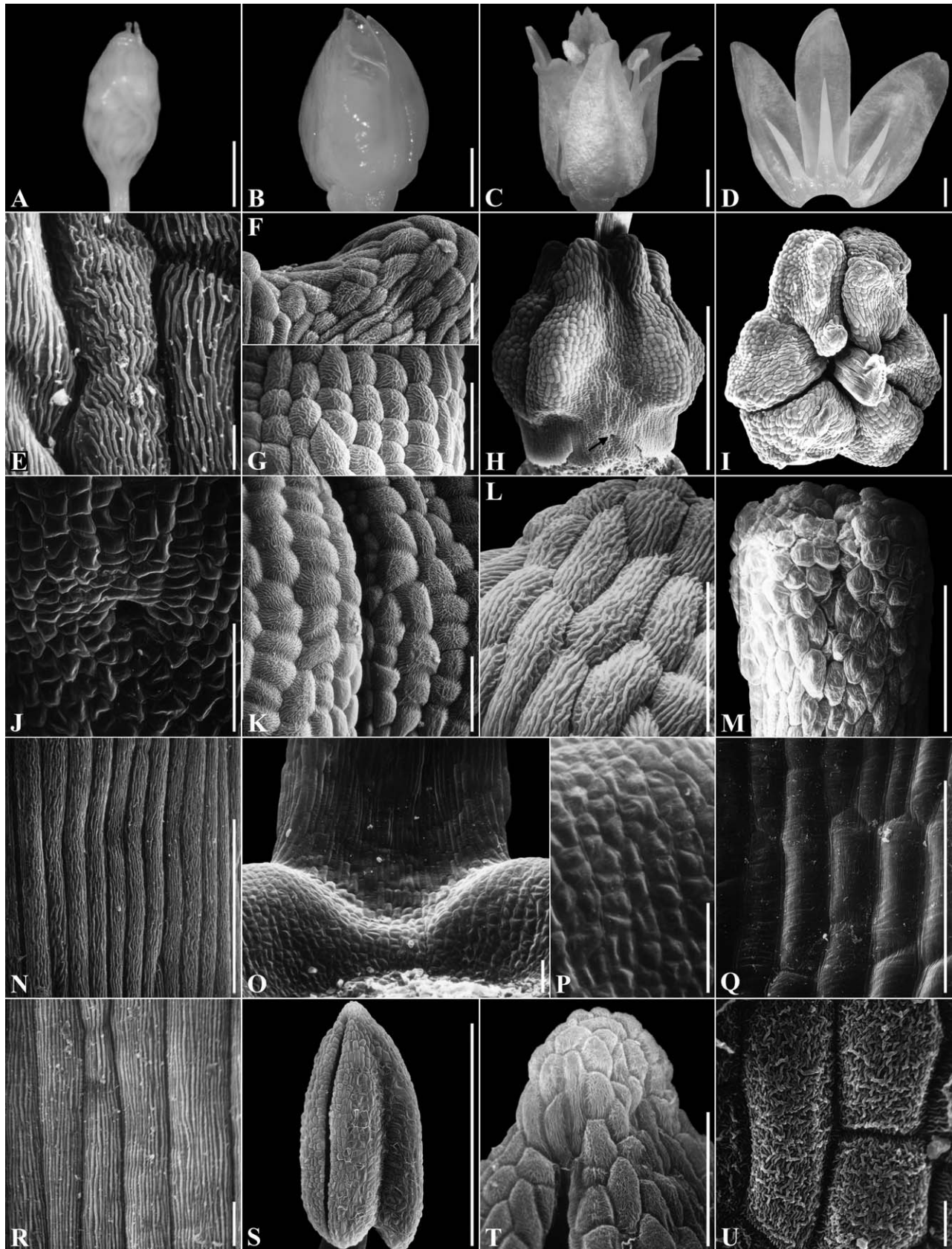


FIG. 3. Floral characters of *Allium textile*. A. Young inflorescence. B. Floral bud. C. Open flower. D. Tepal and filament arrangement in C. E. Detail of abaxial surface of inner tepal in C. F, G. Detail of upper (F) and middle (G) parts of ovary in B. H, I. Lateral (H) and top (I) views of ovary in C (arrow = nectary opening). J. Detail of nectary opening in H. K. Detail of suture in H. L. Detail of upper part of ovary in H. M. Detail of stigma in C. N. Detail of middle part of style in C. O. Basal part of an inner filament in C. P. Detail of fused area between inner and outer filaments in O. Q, R. Detail of basal (Q) and middle (R) adaxial surfaces of inner filament in C. S. Anther in C. T. Detail of apex in S. U. Detail of epidermal cells in S (scale bars: 5 mm, Fig. A; 1 mm, Figs. B–D, H, I, S; 0.1 mm, Figs. F, G, J–Q, T; 10  $\mu$ m, Figs. E, R, U).

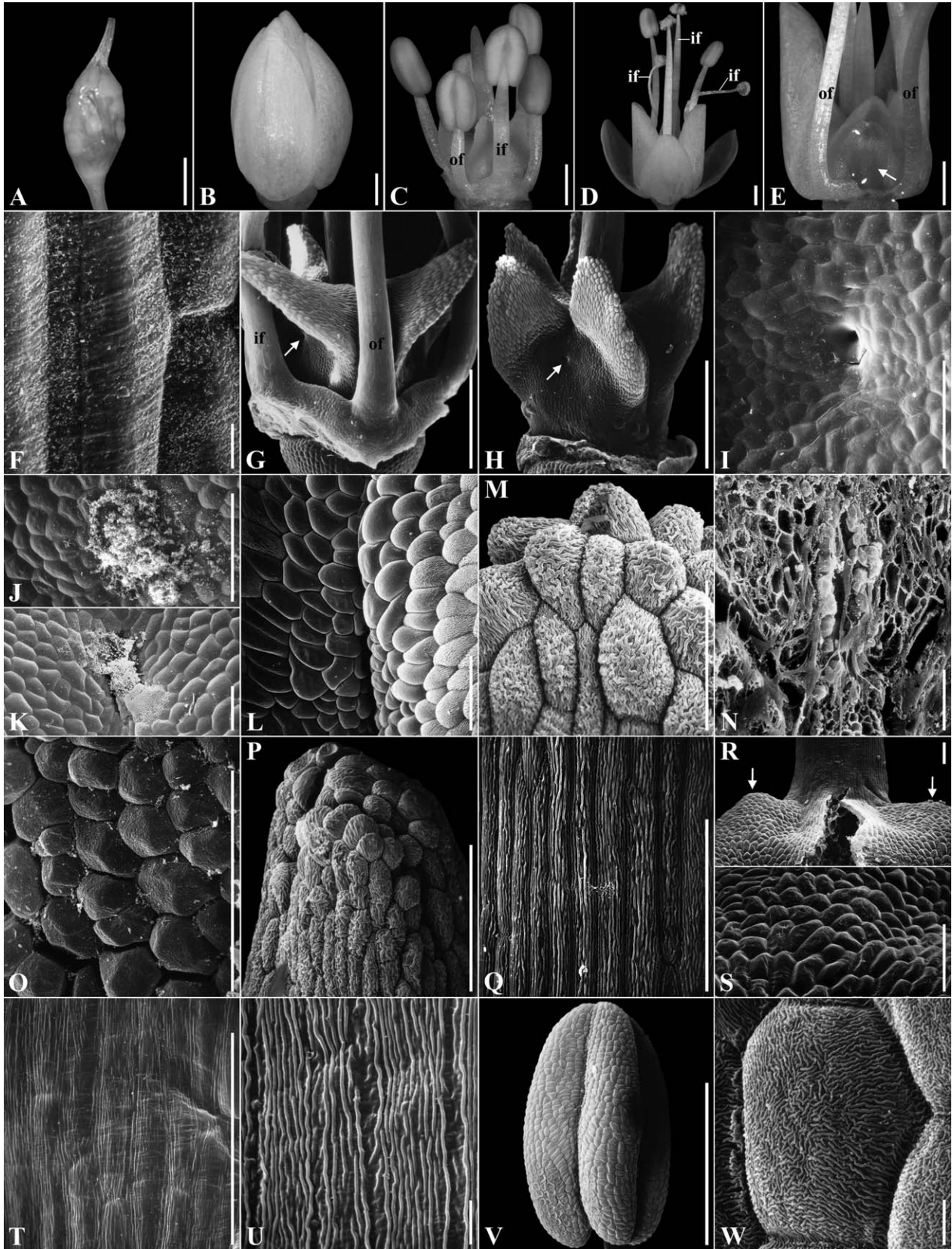


FIG. 4. Floral characters of *Allium cernuum*. A. Young inflorescence. B. Floral bud. C. Internal structure in B (if = inner filament; of = outer filament). D. Open flower (if = inner filament). E, G. Internal structure in D (if = inner filament; of = outer filament; arrow = nectar droplet). F. Detail of abaxial surface of inner tepal in D. H. Ovary in B (arrow = nectary opening). I–K. Detail of nectary opening in B (I), D (J), and flower in post-anthesis (K). L. Detail of suture in D. M. Detail of upper part of ovarian crest in D. N. Cross section of ovary showing septal nectary in D. O. Detail of secretory cells in longitudinal section of septal nectary in D. P. Detail of stigma in D. Q. Detail of middle part of style in D. R. Basal part of an inner filament in D (arrow = basal projection). S. Detail of fused area between inner and outer filaments in R. T, U. Detail of basal (T) and middle (U) adaxial surfaces of inner filament in D. V. Anther in D. W. Detail of epidermal cells in V (scale bars: 5 mm, Fig. A; 1 mm, Figs. B–E, G, H, V; 0.1 mm, Figs. I–T; 10  $\mu$ m, Figs. F, U, W).



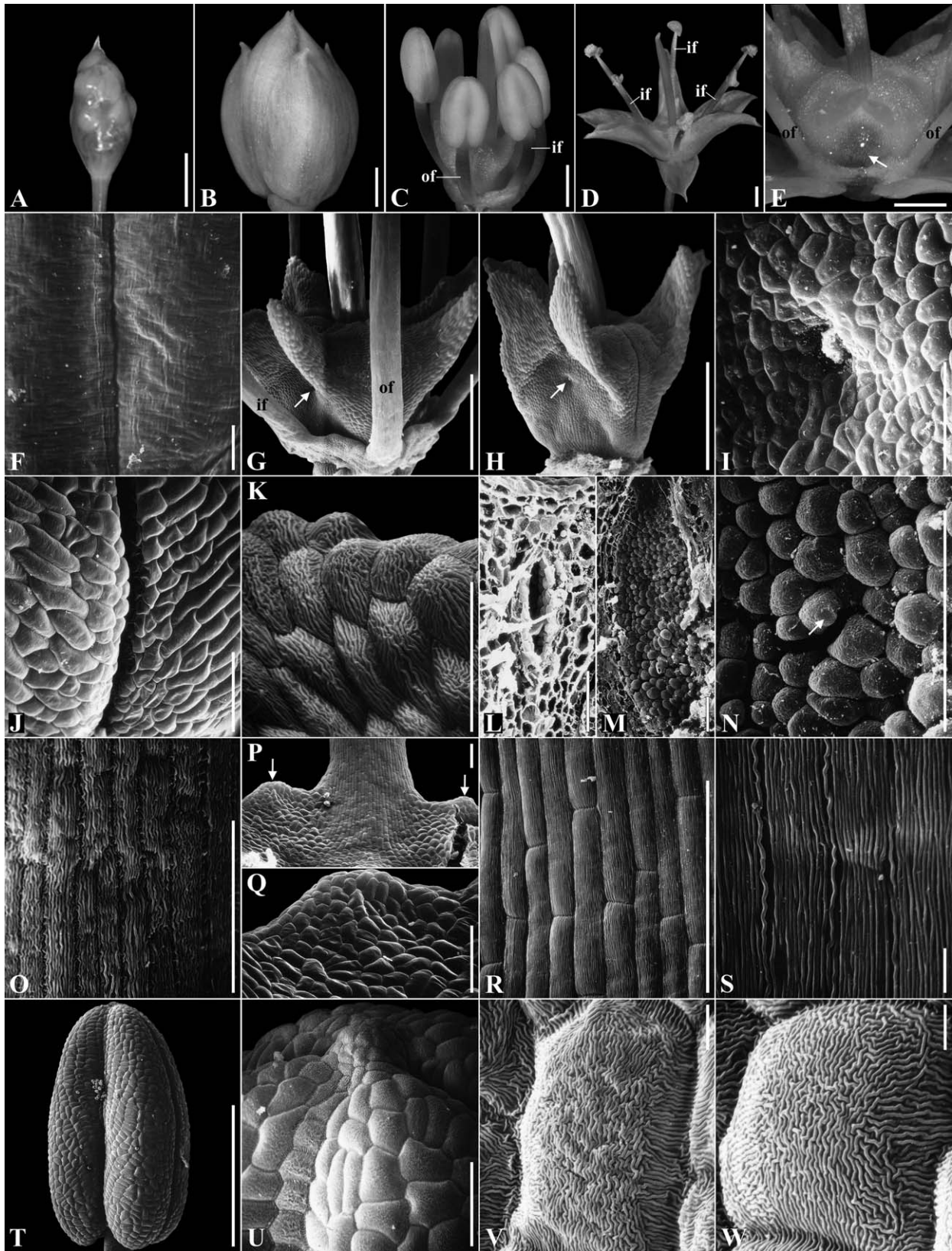


FIG. 5. Floral characters of *Allium stellatum*. A. Young inflorescence. B. Floral bud. C. Internal structure in B (if = inner filament; of = outer filament). D. Open flower (if = inner filament). E, G. Internal structure in D (if = inner filament; of = outer filament; arrow = nectar droplet in E, nectary opening in G). F. Detail of abaxial surface of inner tepal in D. H. Ovary in D (arrow = nectary opening). I. Detail of nectary opening in H. J. Detail of suture in H. K. Detail of upper part of ovarian crest in H. L. Cross section of ovary showing septal nectary in D. M. Longitudinal section of septal nectary in D. N. Detail of secretory cells in M (arrow = papillate projection of cuticle). O. Detail of middle part of style in D. P. Basal part of an inner filament in D (arrow = basal projection). Q. Detail of fused area between inner and outer filaments in P. R, S. Detail of basal (R) and middle (S) adaxial surfaces of inner filament in D. T. Anther in D. U. Detail of apex in T. V, W. Detail of epidermal cells in T (scale bars: 5 mm, Fig. A; 1 mm, Figs. B–E, G, H, T; 0.1 mm, Figs. I–R, U; 10  $\mu$ m, Figs. F, S, V, W).

anthers have an irregular to nearly isodiametric shape with straight anticlinal walls in all taxa (Figs. 2W; 3T; 4V; 5T). They have a clearly ridged cuticular layer with the ridges aligned predominantly in the longitudinal axis, but also developed continuously between adjacent cells (Figs. 2X; 3U; 4W; 5V, W).

#### DISCUSSION

***Allium geyeri* var. *tenerum* in Waterton Lakes National Park of Canada**—*Allium geyeri* var. *tenerum*, though rare, is widely distributed in meadows and damp sites along streams in mountainous regions of Waterton Lakes National Park (Fig. 1A1, E). Our 2009 and 2010 fieldwork yielded relevant information concerning the floral biology of this species. Foremost, our data indicate that this taxon has a relatively extended blooming period from the middle of June to the middle of July. It is locally abundant in some areas of the park, to the extent that it forms an herb mat (mean of  $52.8 \pm 4.8$  individuals per  $m^2$  in 10 randomly selected quadrat counts). This information is well matched with its current S2 status for Alberta. Further, the closely related *A. textile*, with which it can be taxonomically confused, occurs allopatrically in the prairie region of Waterton Lakes National Park, but has a relatively early blooming season (ca. 3 wk earlier in late May) (H. J. Choi, pers. obs. 2010).

Choi and Cota-Sánchez (2010a) investigated *Allium geyeri* var. *tenerum* only from herbarium specimens, but our recent examination of fresh material is inconsistent with the previous observation; that is, *A. geyeri* var. *tenerum* has elliptical (not oblong to lanceolate) tepals and ovaries without apical processes (Fig. 2E, G, J). Consequently, *A. geyeri* var. *tenerum* can be accurately distinguished from *A. textile* by its larger perianth with elliptical (versus ovate to oblong) tepals, filaments slightly shorter than tepals (versus 2/3 as long as tepals), and globose young inflorescences (versus ellipsoid) (Table 2). The presence of ovarian processes is not a key character difference between the two species.

**Floral Morphology of Four New World *Allium* Species**—Flower colors and shapes are considered to be signal attractants for pollinators (Mogford 1978; Weiss 1991; Arnold 1997). It is noteworthy that *Allium* species display a wide spectrum in the color of floral parts (e.g. perianth, filaments, style, and pedicel), which Żuraw et al. (2009) suggested could be important in their pollination biology. In addition, perianth shape, tepal color, and nectar guides in flowers have evolved in accord with physiological characteristics of the senses of their relevant pollinators (Weiss 1991; Dafni and Kevan 1996; Arnold 1997). Differences in color and shape of flowers and inflorescences, traits associated with floral isolation in closely related *Allium* species such as *A. geyeri* var. *tenerum* (few flowered with bulbils: Fig. 1A2) versus *A. textile* (congested without bulbils: Fig. 1B) of sect. *Amerallium*, and *A. cernuum* (nodding and pink to white, campanulate flowers; Fig. 1C) versus *A. stellatum* (erect and deep pink, spreading flowers; Fig. 1D) of sect. *Lophioprason*, may indicate different pollinating agents. Therefore, structural modifications may reflect a different pollination syndrome and may be correlated with a specific pollinator. Forthcoming studies on the comparative pollination biology in these four species will be instrumental in explaining floral reproductive isolation, breeding systems, and speciation mechanisms in *Allium*.

Some vegetative and reproductive outer surfaces of terrestrial plants are covered by a continuous water-resistant

cuticular membrane, which exhibits considerable variation in structural and chemical composition among lineages (Cheng et al. 1986; Christensen and Hansen 1998; Choi et al. 2004; Cheng and Walden 2005). Traditionally, this cuticular membrane has been interpreted variously as having an outer layer of epicuticular wax; a narrow, homogeneous cuticularized layer; or a thicker, reticular, cutinized layer (Cheng and Walden 2005). In addition to the six different structural types of cuticle (Holloway 1982), a ridged or rugose cuticle was reported from several genera, including *Sorghum* Moench., *Zea* L., *Avena* L., and *Oryza* L. (Cheng et al. 1979, 1986), and *Lycopersicon* Mill. (Sekhar and Sawhney 1984). In *Allium*, ridged cuticles have been reported as one of the main types of epidermal cell covering, especially from floral parts such as tepals, ovary, style, filaments, and anthers (Christensen and Hansen 1998; Choi et al. 2004; Choi 2009; Żuraw et al. 2009). The development of these ridges can be followed and observed during maturation of floral structures. For example, we observed that at an early developmental stage the outer epidermal surface is relatively smooth (Fig. 2H, I compared to Fig. 2N, Q; Cheng et al. 1986).

Micromorphology of tepals in flowering plants has been of interest to botanists (Kay et al. 1981; Christensen and Hansen 1998; Hong 2001). Barthlott (1990), in a general review of epidermal plant characters, confirmed the systematic significance of epidermal traits. It has been suggested that epidermal features are useful to determine mechanisms of floral protection and pollinator attraction (Gale and Owens 1983; Christensen and Hansen 1998), and that microstructure of petals might play a tactile role by providing an enticing microtextural nectar-guide for pollinators (Kevan and Lane 1985). It has also been proposed that the morphology of epidermal cells and cuticular surface of tepals is directly related to capture of incident light to produce maximum brightness of floral pigments (Hong 2001), and that the optical effect of light is dependent on the angle of incidence and shape of the barrier it strikes (Kay et al. 1981). Although information concerning tepal surface micromorphology in *Allium* species is scanty (Choi 2009), this feature may be relevant in reflecting light and guiding pollinators to the flower (Christensen and Hansen 1998). To date, only various shapes of ridged (longitudinally striated) epidermal cells have been reported in ca. 30 Old World *Allium* species (Christensen and Hansen 1998; Choi 2009; Żuraw et al. 2009), and this study documents the first observation of tepal microstructure in a New World species. It is possible that other, perhaps new, types of tepal epidermal cells are present in this genus, especially in *A. cernuum* and *A. stellatum* since these taxa share a nearly smooth cuticle covering on the epidermal cells (Figs. 4F; 5F). In contrast, *A. geyeri* var. *tenerum* and *A. textile* have ridged epidermal cells on the abaxial surface of the inner tepals (Figs. 2F; 3E).

Our data show that the ovaries of these four taxa are covered by both smooth and ridged epidermal cells (Figs. 2I, K–N; 3F, G, J–L; 4I–M; 5L–K; Table 3). Although the distribution pattern of ridged epidermal cells varies per taxon (Fig. 6), this is a dependable distinguishing character of *Allium geyeri* var. *tenerum*, which has weakly ridged cells only in the upper part of ovaries compared to its close relative *A. textile* with clearly ridged cells virtually throughout the ovaries (Fig. 6A, B; Table 3). Plants of *A. geyeri* var. *tenerum* possess bulbils that permit asexual reproduction, and rarely produce seeds in the wild. These weakly ridged structures may

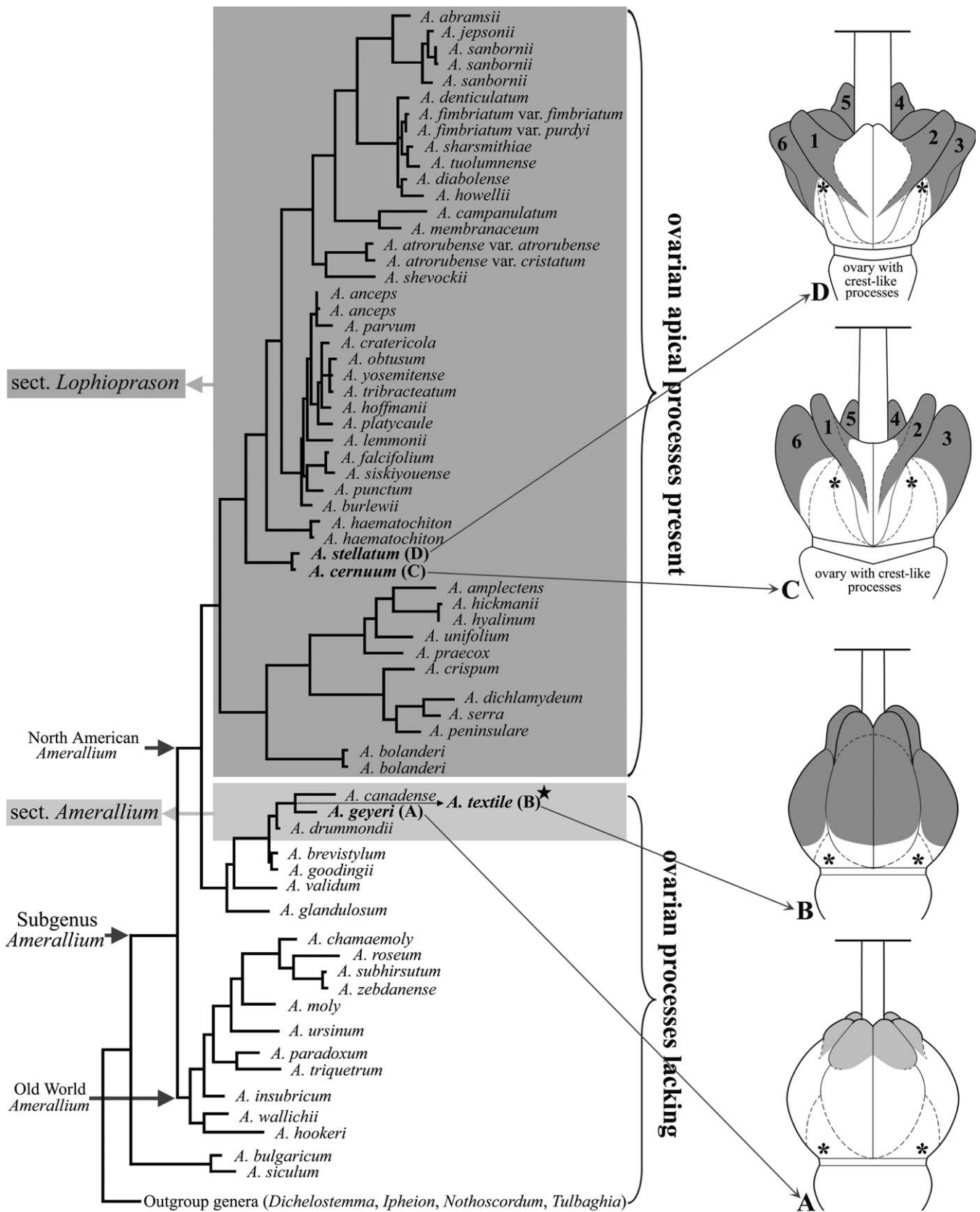


FIG. 6. Proposed evolution of ovarian apical processes in *Allium*. Nectary openings (\*), ovarian crest-like processes (1–6), and ridged ovarian cuticles (dark shaded area) of four *Allium* species (A–D in bold) have been optimized onto phylogeny of North American *Allium* (Nguyen et al. 2008). Note apparent derived position of ovaries with crest-like processes (C and D) in sect. *Lophioprason* from a putative ancestral ovary without processes (A and B) in basal lineages. Star (★) indicates putative phylogenetic position of *A. textile* based on *trnL-F* sequence data (H. J. Choi and J. H. Cota-Sánchez, unpubl. data). Shaded areas in A–D indicate distribution and type of cuticular ridges on the ovary [light shadow = weak cuticular ridges (Fig. A); dark shadow = strong cuticular ridges (Figs. B–D)].

be associated with their normally sterile condition and the partially developed ovary, although they do have active septal nectaries.

A remarkable characteristic found in some North American *Allium* species is the presence of ovarian apical processes composed of six flattened parts; that is, two processes on each of the three ovary lobes (Figs. 4G, H; 5G, H; 6C, D; McNeal 1992). These processes are sometimes quite prominent as in *A. cernuum* and *A. stellatum* (Figs. 4G, H; 5G, H), and their size, shape, and ornamentation is valuable in classification and phylogenetic inferences [e.g. McNeal (1992) described the ovarian crests as a plesiomorphism in North American *Allium*]. However, we consider the presence of two types (hood-like and basal in Old World species; crest-like and apical in New World species) of ovarian processes quite likely represents a recently derived character. Our hypothesis is based on optimization of ovary type on the molecular phylogenies from nuclear ribosomal (Nguyen et al. 2008; Fig. 6) and chloroplast (H. J. Choi and J. H. Cota-Sánchez, unpubl. data) DNA sequence data in relation to outgroup taxa. Preliminary analyses indicate that the putative recently derived *Allium* groups in the New and Old World have apical and basal processes, respectively, whereas the most basal groups lack ovarian processes, a condition also present in basal lineages such as *Dichelostemma* Kunth, *Ipheion* Raf., *Northoscordum* Kunth, and *Tulbaghia* L. (Traub and Moldenke 1955; Stearn 1980; Fay and Chase 1996; Mes et al. 1997; Jacobsen and McNeal 2002; McNeal and Jacobsen 2002; Pires 2002; Fig. 6; H. J. Choi and J. H. Cota-Sánchez, unpubl. data), further suggesting an ancestral ovary without processes. We further hypothesize that the processes play a role in pollination as signal attractants, as suggested by McNeal (1992) and Żuraw et al. (2009), but in concert with the characteristic broadened inner filaments and clearly concave inner tepals (see Figs. 6F and 7F of Choi and Cota-Sánchez 2010a) may also represent a special adaptation facilitating retention of the abundant nectar secreted in the nodding flowers of *A. cernuum* (Fig. 1C) and the widely spreading flowers of *A. stellatum* (Fig. 1D). In these two closely related species the inflorescence may often be nodding in bud stage (Figs. 4A; 5A), but in *A. stellatum* the inflorescence usually becomes erect during anthesis (Choi and Cota-Sánchez 2010a, 2010b).

In pollination biology, *Allium* flowers are classified in morphological terms as bowl-shaped (radially symmetrical) with hidden nectaries (Kugler 1970; Żuraw et al. 2009). Septal nectaries, included in the gynopleural type, are found in all major lineages of monocots except Liliales (sensu APG III) (Żuraw et al. 2009), and characterize the Alliaceae s. s., Asparagaceae, and Asphodelaceae (Weberling 1992; Vogel 1998; Smets et al. 2000; Weryszko-Chmielewska et al. 2006; Bernardello 2007). In fact, septal nectaries are the most frequently encountered type in monocotyledons (Smets et al. 2000; Żuraw et al. 2009). Additionally, Fritsch (1992) reported various shapes and positions of the nectaries and their openings in more than 160 *Allium* species. Their characteristic aspect is that nectar production and release occur in different sites of the ovary (Smets et al. 2000). In this study we found that there are three openings per ovary located in the basal (in *A. geyeri* var. *tenerum* and *A. textile*) or middle section (in *A. cernuum* and *A. stellatum*), which are regions associated with nectar release. Interestingly, this trait is congruent with the taxonomic pairings illustrated in Fig. 6; that is, *A. geyeri* var. *tenerum* and *A. textile*, placed in sect. *Amerallium*, have basal openings, and

*A. cernuum* and *A. stellatum* of sect. *Lophioprason* have middle openings (see also Table 3). Furthermore, Żuraw (2008) observed openings of septal nectaries halfway up the ovary in *A. flavum* L. and *A. sphaerocephalon* L., but in several other species the openings were in the basal part of the ovary (Żuraw et al. 2009). This is the first report documenting the location of nectary openings for any of the four North American species investigated here. The existing literature includes reports for taxa considered as members of the outgroup of *Allium* [i.e. *Nectaroscordum siculum* Lindl., *Nothoscordum gracile* (Aiton) Stern, and *Tulbaghia violacea* Harv.], in which the openings of septal nectaries are located in the upper part of the ovary (Figs. 22–24 of Fritsch 1992). Forthcoming studies of ovary anatomy in other monocotyledoneous taxa will be valuable in understanding character evolution of ovarian processes and septal nectaries, and their role in flower evolution and pollination biology of the Amaryllidaceae.

Stamens of the inner whorl of *Allium* flowers sometimes have filaments strongly expanded or toothed, double-sided at their bases (Choi et al. 2007; Żuraw et al. 2009; Choi and Cota-Sánchez 2010a). All species investigated here have filaments of the inner whorl broader than those of the outer whorl, a character indicated also by Choi and Cota-Sánchez (2010a). We consider this character to be a special adaptation of the inner filaments to facilitate retention of abundant nectar secreted in the flowers, in conjunction with their expanded basal projections and ridged epidermal surfaces (Figs. 2V; 3R; 4R, U; 5P, R, S; Żuraw et al. 2009). It is noteworthy that the inner filaments are located opposite the openings in the ovary from which nectar flows (Figs. 4G; 5G). Development of minute basal projections provides another useful taxonomic character as it distinguishes sect. *Lophioprason* from sect. *Amerallium* in this study (Table 3).

Although anther characters are conservative in many groups of angiosperms (Dnyansagar 1955; Hughes 1997), they are fairly variable and plastic at the subgeneric and sectional levels of the genus *Allium* (Choi 2009). The general types of anthers in *Allium* have been classified as oval, elliptical, and oblong (Choi 2009). In this study only elliptical anthers were observed, but their apices are clearly divided into two subtypes, which together with inner tepal topography and nectary opening position (Table 3) separate each section of the New World subgenus *Amerallium* Traub. However, microstructure of the anther epidermis shows consistency in possessing the ridged cuticular covering, which is the prevalent type, and one of the most conservative characters in *Allium*.

In conclusion, this comparative morphological study of four New World species of *Allium* represents a first attempt to investigate, in detail, floral structures within a taxonomic context. The taxonomic value, phylogenetic implications, and potential application of these macro- and micromorphological characters to recognize various groups at different taxonomic levels are clear. Future studies involving poorly investigated New and Old World species will provide a sound basis to understand the role of ovarian processes and other key floral innovations involved in the phylogeny, floral evolution, and pollination biology of *Allium*.

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