ANATOMY AND MORPHOLOGY OF THE SEED COAT IN THE TEXAS SPECIES OF *ARGEMONE* (PAPAVERACEAE)

by

Shelby LeeAnn Conway, B.S.

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Committee Members:

David E. Lemke, Chair

Paula S. Williamson

Jason P. Martina

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ABSTRACT

Argemone is one of the largest genera within Papaveraceae, comprising 32 species of annual, biennial, or perennial herbs. The most recent complete taxonomic revision of the Texas species of Argemone is included in the work of Ownbey, dating back to 1958, who recognized eight species occurring in the state: *A. aenea*, *A. albiflora*, *A. aurantiaca*, *A. chisosensis*, *A. mexicana*, *A. polyanthemos*, *A. sanguinea*, and *A. squarrosa*. Ownbey used a variety of morphological characters to delimit species within the genus, including petal, latex, stamen and stigma color; presence or absence of clasping leaves; shape of the bud and capsule; presence or absence of prickles on foliage bracts or on stems; and seed dimensions, and most treatments of the genus in published Texas floras have been based on Ownbey's work. Several authors, however, have noted that Ownbey's taxonomic distinctions are not necessarily always clear due, at least in part, to the largely continuous morphological variation seen in many of these characters. Because studies of seed coat morphology have often proven useful in the delimitation of taxa (including other genera in Papaveraceae) and in providing data for the generation of phylogenetic hypotheses, I chose to study whether or not morphological and anatomical features of the seed coat would prove useful for distinguishing among the Texas species of this genus.

Seed morphology was examined using light and scanning electron microscopy, while standard botanical histological procedures were used to study development of the seed coat. Although the seeds of all Texas species of Argemone are similar in gross morphology, consistent differences in seed size and shape, surface microsculpturing

patterns, relative prominence of the hilum, raphe, and chalazal umbo, and certain anatomical features appear to provide characters useful for species delimitation.

I. INTRODUCTION

Papaveraceae is a large, primarily north temperate angiosperm family considered by recent authors to be a core member of the basal eudicots (Hoot et al., 1997; Wang et al., 2009; Hoot et al., 2015). Members of the family are herbs or shrubs characterized by the presence of laticifers and white, yellow, orange, or red sap or specialized elongate secretory cells and clear, mucilaginous sap. The leaves are usually alternate and spirally-arranged, simple, entire to lobed or dissected, occasionally spinose; stipules are lacking. The inflorescence type is varied. The flowers are bisexual, radially, or bilaterally symmetrical. The sepals number 2 or 3, and are usually distinct and early deciduous, large, and enclosing the bud or small and bract-like. The petals are 4 or 6 or occasionally numerous, and are often crumpled in bud and appearing wrinkled when expanded; occasionally the inner 2 or 3 are differentiated from the outer 2 or 3 and sometimes have a prominent basal nectar spur or pouch. The stamens are numerous, or may be 6 in number in two groups of 3 with the filaments distinct to connate. The carpels are 2 to numerous with parietal placentation, and the ovary is superior with the stigma 1 or equal in number to the carpels. The ovules are numerous or occasionally reduced to 1 or 2. The fruit is a capsule that opens typically by valves or apical pores. The seeds are small, numerous, and sometimes arillate (Judd et al., 2016). Monophyly of the group is supported by molecular data (Hoot et al., 1997; Wang et al., 2009), as well as by the morphological characters of a syncarpous gynoecium of two or more carpels with parietal placentation (Wang et al., 2009).

The family includes approximately 40 genera and 770–825 species and has a worldwide distribution with an emphasis on the Northern Hemisphere, specifically the

north temperate zone. The family is economically important as a source of cultivated ornamentals (e.g., *Eschscholzia, Papaver, Corydalis, Dicentra*), while the opium poppy (*Papaver somniferum*) is the source of opium and its derivative alkaloids such as morphine, heroin, and codeine (Judd et al., 2016).

Generic delimitation among members of Papaveraceae is not controversial, the genera being easily distinguished such as growth habit, phyllotaxy, pubescence, color of latex and petals, presence or absence of prickles on vegetative parts, inflorescence type, and carpel number. Within genera, however, species delimitation can be more challenging, as exemplified by certain members of the large genus *Argemone*.

Argemone (prickly poppy) is one of the largest genera within Papaveraceae; only *Corydalis* (ca. 400 spp.), *Papaver* (ca. 100 spp.) and *Fumaria* (ca. 50 spp.) are larger. *Argemone* comprises 32 species of annual, biennial, or perennial herbs. The stems are leafy and branching with sessile leaves in a basal rosette and alternate cauline leaves. The leaves are either unlobed or shallowly to deeply pinnately lobed with dentate margins, each tooth terminated by a prickle. The leaf surfaces are glaucous, often mottled on the veins, and can be glabrous and unarmed, prickly, or hispid. The sap ranges from white to yellow, orange, or reddish orange. The inflorescences are terminal, cymose and bracteate. The flowers have 2 or 3 sepals, either prickly or unarmed but with an erect, subapical prickle. There are 6 petals in 2 whorls of 3. Stamens range in number from 20–250, the filaments usually pale yellow but occasionally red to lavender. The gynoecium is syncarpous, of 3–7 carpels, the ovary unilocular and the stigma 3–7 lobed. The fruit is an erect capsule that dehisces from the apex to about one-third of the length. The seeds are numerous, blackish brown, subspherical to subglobulose, ca. 1–3 mm in the longest

dimension, and conspicuously reticulate (Ownbey, 1958; Harris, 1979).

Argemone has a distribution that ranges from North America to South America and the West Indies, with one species (*A. glauca)* endemic to Hawaii and another (*A. mexicana*) that has been introduced in many tropical and subtropical regions of the world.

The most recent complete taxonomic revision of the Texas species of *Argemone* is included in the work of Ownbey (1958), who recognized eight species occurring in the state: *A. aenea, A. albiflora, A. aurantiaca, A. chisosensis, A. mexicana, A. polyanthemos, A. sanguinea,* and *A. squarrosa*. Ownbey used a variety of morphological characters to delimit species within the genus, including petal, latex, stamen and stigma color; presence of clasping leaves; shape of the capsule and bud; presence of prickles on foliage bracts or on stems; and seed dimensions; and most treatments of the genus in published Texas floras have been based on Ownbey's work (e.g., Correll and Johnston, 1970; Diggs et al., 1999; Powell and Worthington, 2018). Several authors, however (e.g., Schwarzbach and Kadereit, 1999; Powell and Worthington, 2018), have noted that Ownbey's taxonomic distinctions are not necessarily always clear due, at least in part, to the largely continuous morphological variation seen in many characters. The characteristics provided by Ownbey make it difficult to identify the white flowered *Argemone* species from one another, so a revision is needed to clearly delimitate the species.

Studies of seed coat morphology among the vascular plants have proven useful within a taxonomic context, for example, in the delimitation of tribes (Whiffin and Tomb, 1972), genera (Mulligan and Bailey, 1976), and species (Chuang and Heckard, 1972; Ehler, 1976; Hill, 1976; Seaver et al., 1977), and in providing data for the generation of

phylogenetic hypotheses (Prain, 1895; Fedde, 1936; Kadereit et al., 1994). Within the widely-distributed, largely temperate family Papaveraceae, seed coat characters have been used to separate species and to provide data toward the resolution of evolutionary questions in the genus *Eschscholzia* (Clark and Jernstedt, 1978), while Araii et al. (2010) discovered that seed ornamentations and color can be used to distinguish among species in the genus *Fumaria.* Seed coat development has been used to separate species in *Corydalis* and *Dicentra* and to link primitive traits with families (Fukuhara, 1992). Scanning electron microscopy has been used to identify *Meconopsis* species based on seed coat pattern (Sulaiman, 1995).

Gross seed morphology in the North American species of *Argemone* was examined by Gunn and Seldin (1976), who noted that seeds are dull or shiny, brown to black and spherical with an emergent to basal nipple with secondary reticulation and moderate to bold reticulum walls. Cresson (1986) used scanning electron microscopy to examine surface sculpturing in the Texas species of the genus, however, this work has not been published. Meunier (1891) and Sachar (1955) examined the structure of the seed coat in *A. mexicana*, and Cresson and Schneider (1988) described the seed coat anatomy of *A. aurantiaca*, however, no studies of seed coat structure have been published for the other Texas species of *Argemone*.

Argemone aurantiaca and *A. mexicana* are the only two species in which seed morphology and anatomy have been examined in detail. Cresson and Schneider (1988) reported the seeds of *A. aurantiaca* to be dull to shiny brown in color, ±1–3 mm in length, and non-arillate. Characteristics of potential value in distinguishing among species included the presence or absence of a ridged raphe, micropylar beak, and the

distribution of prominent reticulations over the external surface of the seed (Cresson and Schneider, 1988). Sachar (1955) provided a detailed description of the seed anatomy of *A. mexicana*, noting that the ovules are bitegmic, with an inner integument consisting of three layers of cells and an outer integument consisting of two layers. Both layers of the outer integument take part in the formation of the seed coat. According to Sachar (1955), as the seed matures, the cells of the outer epidermis enlarge, their nuclei increase in size and are surrounded by numerous starch grains, and a thick cuticle develops on the outer tangential wall. Cells of the inner layer of the outer integument enlarge greatly in the radial direction, with their nuclei migrating towards the outer tangential wall and eventually disappearing. Numerous crystalline bodies appear in these cells and the cells are eventually impregnated with a tannin-like substance (Sachar, 1955).

This study will seek to determine: (1) if the observations of seed anatomy and morphology made by Cresson and Schneider (1988) and Sachar (1955) for *A. aurantiaca* and *A. mexicana*, respectively, remain consistent for other members of the genus; (2) if variation exists among the Texas species of *Argemone* with respect to morphological and anatomical characteristics of the seed; and (3) if any variation noted proves to be useful for species delimitation and identification purposes.

II. MATERIALS AND METHODS

Seeds for this study have been obtained from recent field collections, as well as from specimens deposited in the herbaria of Texas State University (SWT) and the Billie L. Turner Plant Resources Center, University of Texas at Austin (TEX-LL). Voucher specimen data for the seeds used in this study are presented in Table 1.

Morphological examination of whole seeds was carried out with a Nikon SMZ1000 stereomicroscope at magnifications of 10-80X. Whole seed images were recorded on a Canon EOS R5 camera fitted with a Novoflex macro bellows and Mitutoyo 5X plan apochromat objective lens, mounted on a Novoflex Castel-Micro focusing rack and illuminated by a Canon MR-14EX II macro ring light. Each image is a composite of 25–30 separate photographs taken at 50 µm intervals and combined using the Helicon Focus software package.

Seed morphology was examined using a JEOL JSM-6010 Scanning Electron Microscope (SEM), housed in the College of Science and Engineering Analysis Research Service Center at Texas State University, to capture images at magnifications of approximately 270X. I examined raw seeds under low vacuum without gold coating which allows for clear resolution without seed impairment. Approximately 30 seeds per species were examined. Using the JEOL SEM micrographs, I measured the papillae density by creating 2500 µm² squares and measured 10 squares for each seed type (figure 2) and I measured the size of the reticulations among the different species. Seed characteristics examined include length of seed in longest dimension, presence or absence of micropylar beak, hilum (funicular scar), and chalazal umbo; along with the length and presence or absence of raphe (figure 1).

Anatomy of the seed coat was studied in cross-sections prepared following standard techniques for botanical material (Johansen, 1940). Immature seeds were fixed in FAA (formalin: acetic acid:50% ethanol) or FPA (formalin: propionic acid:50% ethanol), dehydrated in a graded series of alcohols, and embedded in paraffin. Mature seeds were soaked in 5% ethylenediamine for a period of several weeks to soften the seed coat following the procedure of Carlquist (1982), then dehydrated and embedded as above. Sections were cut at 12 µm on an AO Spencer rotary microtome, mounted onto 75 x 25 mm glass slides, and stained following Johansen's safranin and fast green procedure as outlined in Ruzin (1999).

Anatomical material was examined under a Nikon Eclipse 50i compound microscope with brightfield illumination at magnifications of 40–100X and imaged with a Nikon DS-Fi1 digital camera.

Data collected from the scanning electron micrographs, light micrographs and morphological characteristics will be used to compare the *Argemone* species and develop a key that integrates the data with the previous characteristics described by Ownbey (1958).

Table 1. Voucher specimens from the Billie L. Turner Plant Resources Center (TEX-LL) and the Texas State University Herbarium (SWT) used in the present study.

Species Name	**Collector Number**	**Location (County)**
A. aenea	Lemke s.n. (SWT)	Reeves
A. albiflora	Bergman 420 (TEX)	Lee
A. albiflora	Carr 23034B (TEX)	Goliad
A. albiflora	Correll & Correll 38826	Gonzalez
A. aurantiaca	Rodriguez et al. 109 (TEX)	Comal
A. aurantiaca	Lemke s.n. (SWT)	Hays
A. chisosensis	Warnock & McBryde 14903A (LL)	Pecos
A. chisosensis	Henrickson 24253 (TEX)	Presidio
A. mexicana	Runyon 5430 (TEX)	Cameron
A. polyanthemos	Correll & Johnston 19041 (LL)	Crane
A. polyanthemos	Tharp 4368 (TEX)	Wheeler
A. sanguinea	Vargas & Atha 2 (TEX)	Maverick
A. sanguinea	Lemke s.n. (SWT)	Wilson
A. sanguinea	Ramirez & Cardenas 86 (TEX)	Webb
A. squarrosa	Hansen 6655 (TEX)	Crockett

Table 2. Fixation, dehydration, embedding and staining schedule followed in the present study, modified after Johansen (1940).

Fixation and Dehydration	**Staining and Mounting**
1. Soak material in FAA/FPA - 24 hours	1. Safeclear - 10 minutes
2. 50% Ethanol - 2 hours (minimum)	2. Safeclear-100% EtOH - 10 minutes
3. 50% Alcohol - 2 hrs	3. 100% EtOH - 10 minutes
4. 70% Alcohol - 2 hrs	4. 95% EtOH -10 minutes
5. 85% Alcohol - 2 hrs	5. 70% EtOH - 10 minutes
6. 95% Alcohol - 2 hrs	6. 1% Safranin in 50% EtOH - 2hr to 24 hrs
7. 100% Alcohol - 2 hrs	7. H2O Rinse (2 times)- 10 dips
8. TBA (tert-Butanol)- Two 2-hour and one overnight	8. 1% Chromic acid in H2O- 10 dips
9. Embed in Paraffin - One overnight session and three changes for two hours	9. 95% EtOH + few drops of ammonia - 10 dips
	10. 100% EtOH - 10 dips
	11. 0.05% Fast green - 10 seconds
	12. Clearing solution (2 times) - 10 dips
	13. Safeclear + a few drops of 100% EtOH - 10 dips
	14. Safeclear - 15 minutes
	15. Safeclear - 2 hrs
	16. Mount with Permount

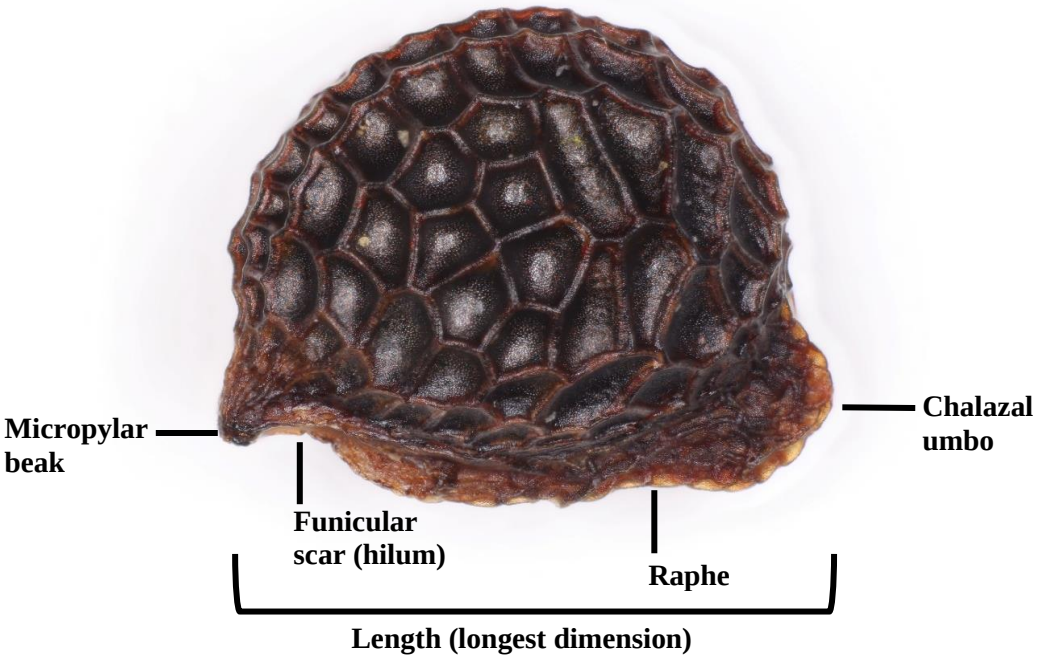

Figure 1. Morphological features of the seed in *Argemone* examined in the present study.

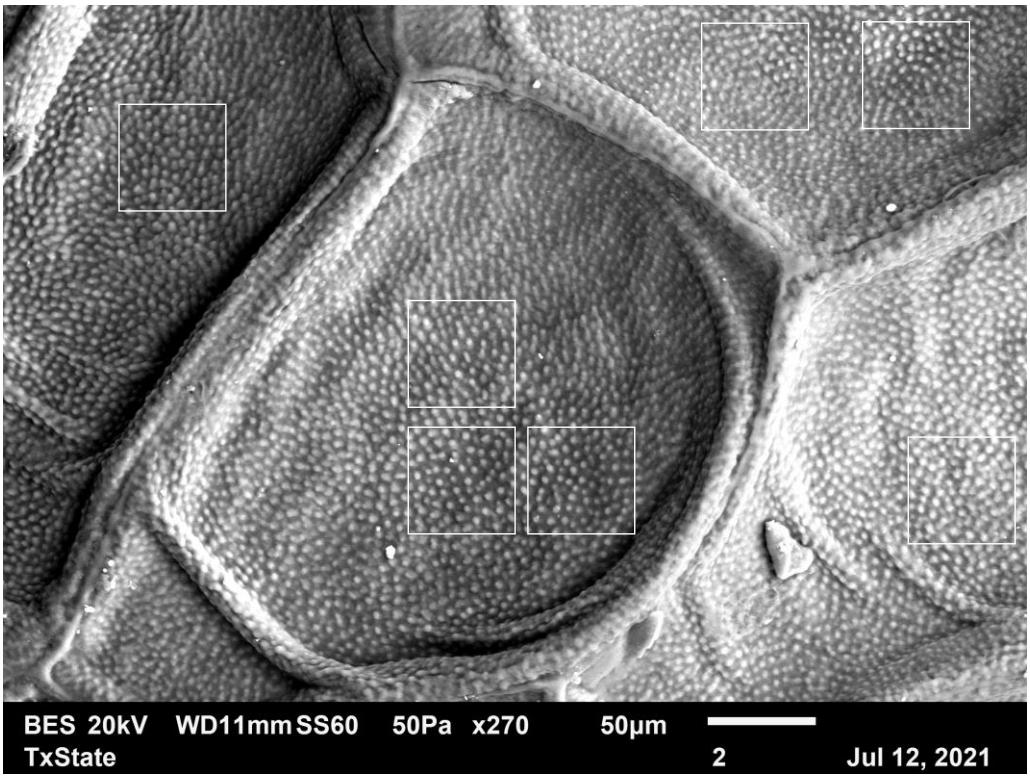

Figure 2. Scanning electron micrograph illustrating the methodology for determining the density of cuticular papillae. Scale bar = 50 µm.

III. RESULTS AND DISCUSSION

Seed Coat Anatomy

The anatomy of the developing seeds of five species (*A. albiflora, A. aurantiaca, A. chisosensis, A. sanguinea,* and *A. squarrosa*) was examined under light microscopy to determine if any differences that might be useful for species delimitation occur among the species. Seed structure was fairly uniform among the Texas species. As noted by Sachar (1955) for *A. mexicana* and by Cresson and Schneider (1988) for *A. aurantiaca*, the mature ovules for all the Texas species are anatropous, bitegmic, and crassinucellate (figure 3). Early in development, the inner integument of the ovule consists of three layers of cells that are more or less cuboidal and approximately equal in size (figure 4), while the outer integument consists of two or three layers of cells. The innermost cell layer of the outer integument comprises cells that are also more or less cuboidal, but that have a dense cytoplasm and a nucleus that occupies a large proportion of the cell volume (figures 4, 5). The outer layer of the outer integument consists of large, thin-walled cells containing a large nucleus and abundant starch grains and characterized by a papillate cuticle (figure 5). In some seeds, a middle layer of somewhat radially-flattened cells can be seen (figure 6).

As the contents of the nucellus develop and enlarge, the cells of the innermost layer of the inner integument begin to enlarge and apparently become devoid of contents, while the two outer cell layers are compressed against the outer integument (figure 5) and ultimately degenerate. Within the cuboidal cells that form the inner layer of the outer integument, the nuclei migrate to the distal ends of the cells and rod- and polyhedral-shaped crystals, presumably of calcium oxalate (Brückner, 1985) appear (figure 6). These

cells then begin to elongate radially to form a distinctive palisade layer in which the crystalline bodies become aggregated towards the distal ends of the cells and the nuclei ultimately disappear (figure 7). During this elongation, the cells of the inner layer of the outer integument also become impregnated with a dense tannin-like substance (figure 8). The much larger cells of the outer layer of the outer integument develop a thickened outer tangential wall with numerous simple papillae and a thick cuticle (figures 5–8).

In a mature seed, the testa (outer integument) is composed of the two or three cell layers covered by a thick, papillate cuticle. Mechanical strength is provided by the palisade layer that, impregnated with tannin-like substances and capped distally with a layer of crystals, becomes indurated (figures 8, 9). As the seed matures and dries, the outer tangential walls of the testa become concave so that the surface of the mature seed appears to be covered with reticulated depressions having a papillate surface (figure 10).

The most comprehensive published study of seed anatomy in Papaveraceae is that of Meunier (1891), who provided descriptions of seed structure for thirteen genera, including *Argemone*. Meunier's illustration of seed structure in several genera of the family is presented in figure 11. Meunier reported that ovule and seed structure was fairly uniform among the species that he examined. Ovules were anatropous and bitegmic. Structural integrity of the mature seed was provided by a palisade layer of radially elongate cells containing crystals of calcium oxalate, while the surface sculpturing pattern of the seed developed from the collapse of the enlarged cells of the outer cell layer of the outer integument. The occurrence of calcium oxalate crystals in the inner layer of the outer integument of the seed was noted to be characteristic of almost all members of Papaveraceae by Brückner (1985) and was used as a character in the data set for a recent

phylogenetic analysis of the family (Hoot et al., 2015).

Sachar (1955) and Cresson and Schneider (1988) provided detailed descriptions of seed anatomy for *Argemone mexicana* and *A. aurantiaca*, respectively. In these species, the mature ovules were reported to be anatropous, bitegmic, and crassinucellate and the mature seed was found to be characterized by a tegmen (inner integument) composed of one layer of enlarged cells and a testa (outer integument) composed of two or three cell layers. The innermost layer of the testa is formed by a palisade layer of radially elongate cells impregnated with tannin-like materials and containing abundant rod-shaped and polyhedral crystals; this layer provides structural integrity to the mature seed. The outermost layer of the testa forms from greatly enlarged cells with a thick, papillate cuticle; collapse of the outer tangential wall of these cells as the seed dries produces a characteristic reticulate surface sculpturing pattern. The present study has documented similar patterns of seed development and structure in four additional species of *Argemone*: *A. albiflora*, *A. chisosensis*, *A. sanguinea*, and *A. squarrosa*.

Morphology and Microsculpturing

Argemone aenea G. Ownbey

Annual, biennial and sometimes perennial herbs with bright yellow latex. Stems to 0.8 m tall sparingly to distinctly prickly with slender perpendicular or reflexed prickles. Leaves glaucous with conspicuous blue veins; lower leaves pinnatifid, the middle and upper ones less lobed but with acute prickle-tipped marginal teeth; surface of the leaf somewhat prickly underneath and sparingly prickly on the veins above. Flower buds elliptic to oblong, to 24 mm long by 16 mm broad and moderately prickly. Flowers

to 9 cm in diameter and closely subtended by foliar bracts; sepal horns approximately round to 14 mm long, smooth or with few prickles on the base, spine tipped; petals yellow to golden or bronze; stamens about 150; filaments red or purplish; anthers purplish-yellow; stigma purple, by 2–3 mm high, 3–6 mm wide, with sinuate lobes. Capsules 4- or 5- carpellate, narrow elliptic to elliptic-oblong, length 35 mm (including the stigma), width to 16 mm, coarsely prickly, the larger prickles interspersed with smaller ones with longest about 8 mm long, capsular surface visible.

Seeds (figures 12A, 16A) 1.9 ± 0.1 mm in overall length, the body spherical; micropylar beak prominent; chalazal umbo prominent; raphe prominent, forming a conspicuous wing extending 3/4 length of seed; hilum on side of micropylar beak or between micropylar beak and raphe, inconspicuous. Seed coat shiny brown/black, reticulate; reticulation bold, composed of regular rectangular cells 184–304 x 243–293 µm. Radial walls thin, wavy; outer tangential walls depressed, moderately covered (54 ± 8 per 2500 µm²) with minute cuticular papillae 5.4 µm in diameter.

Argemone aenea is distributed in the central region of the Trans-Pecos, and in scattered counties of the western Edwards Plateau, northern Coastal Sand Plain and South Texas Brush Country (Turner et al., 2003).

Argemone albiflora Hornem. subsp. *texana* G. Ownbey

Annual or biennial with a deep taproot and yellow latex. Stems to 1.5 m tall sparsely to moderately prickly. Basal and lower cauline leaves lobed to the midrib, middle and upper leaves less lobed and entirely smooth or with a few weak prickles on midrib above. Flower bud broadly elliptic to subspherical, 28–33 mm long, 15–17 mm broad and sparingly covered with slender prickles. Flowers closely subtended by foliar

bracts 9–12 cm in diameter; sepal horns to 1 cm long, smooth or sparingly prickly at the base; petals white; stamens more than 150, equal to or shorter than the pistil at anthesis, filaments white–yellow; anthers white; stigma 1.5–2 mm high, 2–3.5 mm broad; style sometimes evident. Capsules 3- to 5- carpellate, typically elliptic, 4 cm long (including the stigma), 14 mm wide (exclusive of armature), moderately armed with slender herbaceous spines, surface partially obscured.

Seeds (figures 12B, 16B) 1.6 ± 0.1 mm in overall length, the body spherical; micropylar beak elongate; chalazal umbo prominent; raphe prominent, forming a conspicuous ridge extending 2/3 length of seed; hilum between micropylar beak and raphe, inconspicuous. Seed coat shiny brown/black, reticulate; reticulation moderate/bold, composed of irregular rectangular cells 207–250 x 174–326 µm. Radial walls thin, wavy; outer tangential walls depressed, moderately covered (54 ± 8 per 2500 µm²) with minute cuticular papillae 6.5 µm in diameter.

Argemone albiflora is found in the eastern and southern parts of Texas, specifically in the Gulf Coast Prairies and Marshes, Oak Woods and Prairies and Blackland Prairie regions, with scattered localities in the Coastal Sand Plains and Rolling Plains (Turner et al., 2003).

Argemone aurantiaca G. Ownbey

Annual or biennial herbs with reddish-orange latex. Stem to 0.8 m tall, moderately or copiously armed with slender perpendicular prickles. Leaves bluish, deeply lobed toward the base and less lobed on the uppermost leaves, with spines on the margin and veins. Flower buds oblong, to 18 mm broad by 25 mm wide, sparingly to densely prickly with large compound prickles. Flowers subtended by 1 or 2 reduced leaves, 8–12

cm in diameter, sepal horns angular, dorsiventrally flattened 8–12 mm long, prickly with an apical spine; petals white; stamens 150 or more, filaments pale-yellow, the anthers yellow; stigma purple, 2.5–3.5 mm high, 3.5–5 mm wide, 5-or-6 lobed. Capsules 5- or 6-carpellate, ovate, 4–5 cm long (including the stigma), 15–25 mm wide (exclusive of armature), densely covered by herbaceous erect or reflexed often branched spines to 35 mm long, capsule also provided with small simple prickles arising between the large spines, the surface partially obscured.

Seeds (figures 13A, 17A) 2.3 ± 0.1 mm in overall length, the body subspherical; micropylar beak elongate; chalazal umbo prominent; raphe prominent, forming a conspicuous ridge extending 2/3 length of seed; hilum between micropylar beak and raphe, conspicuous. Seed coat shiny brown/black, reticulate; reticulation bold, composed of regular rectangular cells 283–435 x 222–407 µm. Radial walls thin, wavy; outer tangential walls depressed, densely covered (74 ± 6 µm²) with minute cuticular papillae 6.5 µm in diameter.

Argemone aurantiaca is found in the central part of Texas in the eastern Edwards Plateau and southern Blackland Prairie regions with scattered collections from the Rolling Plains (Turner et al., 2003).

Argemone chisosensis G. Ownbey

Biennial or perennial herbs with yellow latex. Stems to 0.8 m tall, sparsely to copiously prickly with slender perpendicular prickles. Lower leaves lobed to near the midrib, middle and upper leaves less deeply lobed; lower leaf surface stoutly armed on the midvein and less so on the secondaries; upper surface weakly armed on the main veins or unarmed. Flower buds broadly elliptic, to 2 cm long and 1.5 cm wide,

moderately prickly with strong perpendicular prickles, subtended by 1 or 2 foliar bracts. Flowers 7–10 cm in diameter; sepal horns terete or angular in cross section, to 12 mm long; petals white to pale lavender; stamens ± 150 or more, filaments pale yellow or red, anthers yellow or purplish; stigma purple, 2.5–3.5 mm wide, 1.5–2.5 mm high. Capsule 3- or 4- carpellate, narrowly elliptic-lanceolate to elliptic-ovate, to 45 mm long (including the stigma), 8–13 cm wide and armed with scattered stout spreading or slightly recurved prickles (the largest to about 10 mm long) interspersed with smaller prickles, the capsular surface visible.

Seeds (figures 13B, 17B) 2.1 ± 0.1 mm in overall length, the body spherical; micropylar beak prominent; chalazal umbo not prominent; raphe prominent, forming a conspicuous wing extending 2/3 the length of seed; hilum between micropylar beak and raphe, inconspicuous. Seed coat shiny, brown with red undertones, reticulate; reticulation moderate, composed of regular rectangular cells 141–283 x 157–283 µm. Radial walls thin, wavy; outer tangential walls depressed, densely covered (52 ± 16 per 2500 µm²) with minute cuticular papillae 6.5 µm in diameter.

Argemone chisosensis is widely distributed in the Trans-Pecos and also occurs in scattered counties in the Sand Hills and western Edwards Plateau (Turner et al., 2003). According to Powell and Worthington (2018), *A. chisosensis* is the most common and widespread prickly poppy in the Trans-Pecos. These authors note that throughout most of the Trans-Pecos, the flowers have white petals, while those in the southern part of the region may often have lavender petals.

Argemone mexicana L.

Annual herbs with bright-yellow latex. Stem to 0.8 m tall, sparingly prickly with

perpendicular prickles. Leaves glaucous with conspicuous blue veins, lobed to half the distance or more to the midrib in lower leaves with shallower lobing in upper leaves, marginal teeth tipped with a spine, middle and upper leaves clasping. Leaves distinctly prickly on upper surface, sparingly prickly on lower surface. Flowers up to 7 cm in diameter, subtended by 1 to 2 foliar bracts; sepal horns terete 5–10 mm long, smooth; petals bright or pale yellow; stamens 30 to 50, filaments pale-lemon-yellow; anther bright yellow; stigma purple 1.5–4 mm wide to 1–2 mm high lobes appressed to the style at anthesis; style usually 1, up to 3 mm long in fruit. Capsule 4- to 6- carpellate, oblong to broadly elliptic 25–45 mm long (including stigma and style), 12–20 mm wide and surface with mostly large spines 6–10 mm long with few lesser spines, the capsular surface visible.

Seeds (figures 14A, 18A) 1.4 ± 0.1 mm in overall length, the body spherical; micropylar beak prominent; chalazal umbo prominent; raphe prominent, forming a conspicuous wing extending 2/3 length of seed; hilum between micropylar beak and raphe, inconspicuous. Seed coat shiny brown with red undertones, reticulate; reticulation moderate, composed of regular rectangular cells 120–178 x 109–163 µm. Radial walls thin, straight; outer tangential walls depressed, sparsely covered (38 ± 8 per 2500 µm²) with minute cuticular papillae 6.7 µm in diameter.

Argemone mexicana occurs in the central and southern parts of Texas with scattered localities in the Edwards Plateau, Gulf Coast Prairies and Marshes and South Texas Brush Country (Turner et al., 2003).

Argemone polyanthemos (Fedde) G. Ownbey

Annual or biennial herbs with a deep taproot and bright-yellow latex. Stems up to

1.2 m, sparingly prickly with stout perpendicular or recurved prickles. Leaves glaucous, succulent, the lower ones lobed two-thirds to the distance to the midrib, the middle and upper leaves more shallowly lobed and the upper leaves clasping the stems, smooth on upperside with scattered prickles near veins on underside. Flower buds elliptic–oblong, to 22 mm long and 15 mm broad, sparingly to moderately prickly with spreading prickles. Flowers mostly 7-10 cm in diameter, closely subtended by 1 or 2 foliar bracts but sometimes bracts distant; sepal horns essentially terete to 15 mm long, devoid of prickles; petals white, very rarely lavender, the inner ones broadly obovate-obcuneate, the outer ones suborbicular, the distal margins minutely erose; stamens 150 or more; filaments lemon-yellow, anthers bright-yellow; stigma purple, 2–3 mm high, 3–4.5 mm broad, 3 or 4 lobed. Capsules 3- or 4- carpellate, narrowly to broadly elliptic, 3 cm long (including the stigma) to 17 mm wide (exclusive of armature), copiously spinescent with widely spreading or recurved simple prickles that are interspersed with smaller prickles, capsular surface visible.

Seeds (figures 14B, 18B) 1.9 ± 0.1 mm in overall length, the body spherical; micropylar beak emergent; chalazal umbo prominent; raphe prominent, forming an inconspicuous ridge extending 2/3 length of seed; hilum on side of micropylar beak, inconspicuous. Seed coat dull brown with red undertones, reticulate; reticulation bold, composed of regular rectangular cells 180–326 x 165–326 µm. Radial walls thin, straight; outer tangential walls depressed, sparsely covered (30 ± 5 per 2500 µm²) with minute cuticular papillae 6.5 µm in diameter.

Argemone polyanthemos can be found primarily in the northern regions of the state in the High Plains, Rolling Plains, Blackland Prairie, Oak Woods and Prairies, and

Edwards Plateau as well as Trans-Pecos (Turner et al., 2003).

Argemone sanguinea Greene

Annual, biennial or short-lived perennial herbs with pale to bright yellow latex. Stems to 1.2 meters tall, sparingly prickly with perpendicular to recurved prickles. Leaves glaucous, the veins conspicuously light blue-lined, the basal and lower cauline leaves deeply lobed four-fifths distance to the midrib, the middle and upper cauline leaves less deeply lobed with marginal teeth on the lobes and prickles on underside and sparingly prickled or smooth on upper surface. Flower buds broadly elliptic–oblong to 2 cm long by 15 mm broad, moderately prickly. Flowers 6–9 cm in diameter, closely subtended by 1 or 2 foliar bracts; sepal horns basally slightly prickly, 5–10 mm; petals white to lavender, broadly obovate-obcuneate to suborbicular, distally erose; stamens 150 or more, equaling the ovary in length, filaments lemon yellow to red, anthers yellow to lavender; stigma purple, not sinuate 1.5–3 mm high, 2.5–5 mm wide. Capsules 3- to 5-carpellate, narrowly to broadly elliptic, 5 cm long (including the stigma) and 18 mm wide, armed with stout often recurved prickles, the longest 5–10 mm in length, capsular surface clearly visible.

Seeds (figures 15A, 19A) 1.8 ± 0.1 mm in overall length, the body ovoid; micropylar beak prominent; chalazal umbo prominent; raphe prominent, forming a conspicuous wing extending whole length of seed; hilum on side of micropylar beak inconspicuous. Seed coat dull brown with red undertones, reticulate; reticulation moderate, composed of irregular rectangular cells 141–211 x 163–322 μm. Radial walls thin, wavy; outer tangential walls depressed, sparsely covered (44 ± 10 per 2500 μm^2) with minute cuticular papillae 6.5 μm in diameter.

Argemone sanguinea can be found primarily in the South Texas Brush Country and Coastal Sand Plain regions but is scattered among Edwards Plateau and the Trans-Pecos regions as well (Turner et al., 2003).

Argemone squarrosa Greene subsp. *glabrata* G. Ownbey

Perennial herbs with yellow latex. Stems to 0.6 m tall, moderately to copiously prickly with slender prickles. Leaves distinctly bluish, deeply lobed below and less lobed above with the uppermost leaves clasping the stem and leaf margins with acute points or secondary lobes with a terminal rigid prickle, sparingly prickly on veins and smooth or very sparingly prickly on the upper side. Flower buds subspherical, 1.6–2 cm long and broad, sparingly prickly. Flowers closely subtended by 1 or 2 reduced leaves, 8–11 cm in diameter and sepal horns more or less angular, 8–11 mm long, very sparsely prickly with a terminal spine; petals white, stamens 150 or more, the filaments pale yellow, anthers yellow, stigma purple, 2–3 mm high, 3–4 mm broad, 4- or 5 lobed. Capsules 4-or 5-carpellate, oblong–elliptic to lance–ovate, 2.5–5 cm long (including the stigma) and 14–18 mm wide, armed with stout scattered prickles.

Seeds (figures 15B, 19B) 2.4 ± 0.1 mm in overall length, the body spherical; micropylar beak not prominent, emergent; chalazal umbo prominent; raphe not prominent, forming a conspicuous ridge extending 2/3 length of seed; hilum between micropylar beak and raphe, inconspicuous. Seed coat dull brown, reticulate; reticulation moderate/bold, composed of irregular rectangular cells 265–337 x 337–441 µm. Radial walls thin wavy; outer tangential walls depressed, densely covered (73 ± 8 per 2500 µm²) with minute cuticular papillae 6.7 µm in diameter.

Argemone squarrosa occurs widely in the Trans-Pecos region and scattered

throughout the High Plains, Rolling Plains, and Edwards Plateau (Turner et al., 2003).

Systematic Considerations

As noted previously, the most recent complete taxonomic revision of the Texas species of *Argemone* is included in the work of Ownbey (1958), who recognized eight species occurring in the state: *A, aenea, A. albiflora, A. aurantiaca, A. chisosensis, A. mexicana, A. polyanthemos, A. sanguinea* and *A. squarrosa*. Ownbey used a variety of morphological characters to delimit species within the genus, including latex, petal, stamen, and stigma color; the presence or absence of clasping leaves; the shape of the unopened flower bud and the mature capsule; the presence of prickles on foliage bracts or on stems; and seed dimensions. Most treatments of the genus in published Texas floras have been based on Ownbey's work (e.g., Correll and Johnston, 1970; Diggs et al. 1999; Powell and Worthington, 2018). Several authors, however (e.g., Schwarzbach and Kadereit, 1999; Powell and Worthington, 2018), have noted that Ownbey's taxonomic distinctions are not necessarily always clear due, at least in part, to the largely continuous morphological variation seen in many characters. The present study seeks to determine if anatomical and morphological characteristics of the seed are useful in the identification of the Texas species of the genus.

Flower color is a useful characteristic for the identification of several Texas species of *Argemone*. Petal color of *Argemone* species in Texas ranges from pale-lemon yellow to white to purplish-red. Two Texas species have flowers that are yellow or yellowish-bronze in color, *Argemone aenea* and *A. mexicana*. These species can easily be distinguished based on flower size and stamen number. *Argemone aenea* has flowers that

are between in 7 and 12 cm in diameter and bear approximately 150 stamens, while *A. mexicana* has flowers between 3 and 7 cm in diameter and around 75 stamens. In the absence of flowering material, seed size can be used to separate the two species; it averages 1.9 ± 0.1 mm in the longest dimension in *A. aenea* and 1.4 ± 0.1 mm in *A. mexicana*.

Pink, red, or purplish-red flowers can be found in two Texas species, *A. chisosensis* and *A. sanguinea* and, although these species are largely allopatric, their distributions do overlap in the western Edwards Plateau and Trans-Pecos regions (Turner et al., 2003). Characters used to separate these species (Correll and Johnston, 1970) include the abundance of prickles on the stems ("often closely prickly" in *A. chisosensis* vs. "not closely prickly" in *A. sanguinea*) and shape of the mature capsule (narrowly ovate-elliptic to elliptic-lanceolate in *A. chisosensis* vs. narrowly to broadly elliptic in *A. sanguinea*). Seed morphology seems to provide a less subjective character for distinguishing between the species. The seeds of *A. chisosensis* average 2.1 mm in longest dimension, have a seed coat that is densely papillate (82 ± 16 per 2500 μm²), and lack a prominent chalazal umbo, whereas the seeds of *A. sanguinea* average 1.6 mm in the longest dimension, have a sparsely papillate seed coat (44 ± 10 per 2500 μm²), and have a prominent chalazal umbo.

The greatest difficulties in identification of Texas *Argemone* species lies with the white-flowered species: *A. albiflora, A. aurantiaca, A. polyanthemos, A. squarrosa,* and occasionally, *A. chisosensis* and *A. sanguinea*. Among the species, *A. aurantiaca* and *A. squarrosa* can be recognized by the large size of their seeds, averaging more than 2.3 mm in the longest dimension. These two species can be distinguished from one another by the

color of the fresh latex (reddish-orange in *A. aurantiaca* vs. pale lemon-yellow to yellow in *A. squarrosa*), the length of the largest capsular spines (14-35 vs. 8-15 mm), and the micropylar beak on the seeds (prominent in *A. aurantiaca*, lacking in *A. squarrosa*). The remaining white-flowered species can be reliably distinguished using a combination of vegetative, floral, and seed morphological characters, including the abundance of cauline prickles, the extent of lobing of the lower cauline leaves, the orientation of the prickles on the surface of the sepals, the length of the sepal horns, the average seed length in the longest dimension, and the density of seed coat papillae.

The following key, based on a combination of vegetative, floral, fruit, and seed characters, can be used to distinguish among the eight species of *Argemone* currently known from Texas.

Key to the Texas Species of *Argemone*

1a. Petals pale lemon-yellow to bright yellow or bronze 2

1b. Petals lavender, red, pink or white 3

2a. Flowers 3–7 cm in diameter; stamens ≤ 75; seeds averaging 1.9 mm in longest dimension ..... *A. mexicana*

2b. Flowers 7–12 cm in diameter; stamens 150 or more; seeds averaging 1.4 mm in longest dimension ..... *A. aenea*

3a. Capsular prickles compound with few to many smaller prickles arising from the basal portion; stems usually closely prickly; seeds > 2.2 mm in longest dimension. 4

3b. Capsular prickles simple; stems usually with widely-spaced prickles or almost smooth; seeds < 2.2 mm in longest dimension 5

4a. Latex reddish-orange when fresh; largest capsular spines 14–35 mm long; seeds with prominent micropylar beak ..... *A. aurantiaca*

4b. Latex pale lemon-yellow to yellow when fresh; largest capsular spines 8–15 mm long; seeds lacking a prominent micropylar beak..... *A. squarrosa*

5a. Prickles of sepals ascending or occasionally absent; lower cauline leaves usually lobed less than one-half the distance to the midrib 6

5b. Prickles of sepals perpendicular to surface; lower cauline leaves usually lobed to

at least four-fifths the distance to the midrib 7

6a. Sepal horns 7–15 mm long; seeds averaging 1.9 mm in longest dimension; seed coat papillae averaging 30 ± 5 per 2500 μm² ..... *A. polyanthemos*

6b. Sepal horns 3–6 mm long; seeds averaging 1.6 mm in longest dimension; seed coat papillae averaging 54 ± 8 per 2500 μm² .....*A. albiflora*

7a. Stems closely prickly; seeds averaging 2.1 mm in longest dimension; seed coat densely papillate (82 ± 16 per 2500 μm^2); chalazal umbo not prominent; mostly Trans-Pecos.....*A. chisosensis*

7b. Stems not closely prickly; seeds averaging 1.6 mm in longest dimension; seed coat sparsely papillate (44 ± 10 per 2500 μm^2); chalazal umbo prominent; mostly southern Texas .....*A. sanguinea*

Conclusions

The present study aimed to determine: (1) if the seed anatomy and morphology observations made by Cresson and Schneider (1988) and Sachar (1955) for *A. aurantiaca* and *A. mexicana* remain consistent for other members of the genus; (2) if variation exists among the Texas species of *Argemone* with respect to morphological and anatomical characteristics of the seed; and (3) if any variation noted proves to be useful for species delimitation and identification.

The *Argemone* species examined showed little variation in seed anatomical characteristics, supporting the observations made previously by Cresson and Schneider

(1988) for *A. aurantiaca* and by Sachar (1955) for *A. mexicana*. However, potentially useful morphological variation in seed length, the presence or absence of a chalazal umbo, the presence or absence and prominence of the micropylar beak, and density of cuticular papillae was noted. The seed features have been combined with other morphological characteristics and distribution data to produce a revised key that should prove useful for making accurate identifications of the Texas of *Argemone*.

Today, global biodiversity is being lost at an unprecedented rate, primarily as a consequence of anthropogenic activities and climate change (Song et al., 2018). One method of combating this trend is the establishment of protected areas that support a diverse flora and fauna, yet how can decisions makers decide where to establish such areas if they do not know what is being protected? The field of systematics provides basic information on the components of biodiversity and, as such, is critical to making effective decisions regarding conservations issues and the sustainable use of resources. Vane-Wright (1996) has noted that there is a need to make taxonomic products, such as identification systems, more accessible and user-friendly, and it is hopes that the small contribution presented here will assist in that regard. The research described here contributes to this need of a more user-friendly identification system for the genus *Argemone*.

Table 3. Morphological features of the seed in the Texas Species of *Argemone.*

Species	**Size(mm) ± SD**	**Shape**	**Sculpturing**	**Size of cells**
A. aenea	1.9 ± 0.1	Spherical	Bold	Moderate
A. albiflora	1.6 ± 0.1	Spherical	Moderate-bold	Small-moderate
A. aurantiaca	2.3 ± 0.1	Subspherical	Bold	Large
A. chisosensis	2.1 ± 0.1	Spherical	Moderate	Moderate-large
A. mexicana	1.4 ± 0.1	Spherical	Bold	Small
A. polyanthemos	1.9 ± 0.1	Spherical	Bold	Moderate-large
A. sanguinea	1.6 ± 0.1	Subspherical	Moderate	Small
A. squarrosa	2.4 ± 0.1	Spherical	Moderate-bold	Moderate-large

Table 4. Size and density of seed coat papillae in the Texas species of *Argemone.*

Species	**Size µm**	**Density per 2500 µm^2 ± SD**
A. aenea	5.4	54 ± 8
A. albiflora	6.5	54 ± 8
A. aurantiaca	6.5	74 ± 6
A. chisosensis	6.5	82 ±16
A. mexicana	6.7	38 ± 8
A. polyanthemos	6.5	30 ± 5
A. sanguinea	6.5	44 ± 10
A. squarrosa	6.7	73 ±8

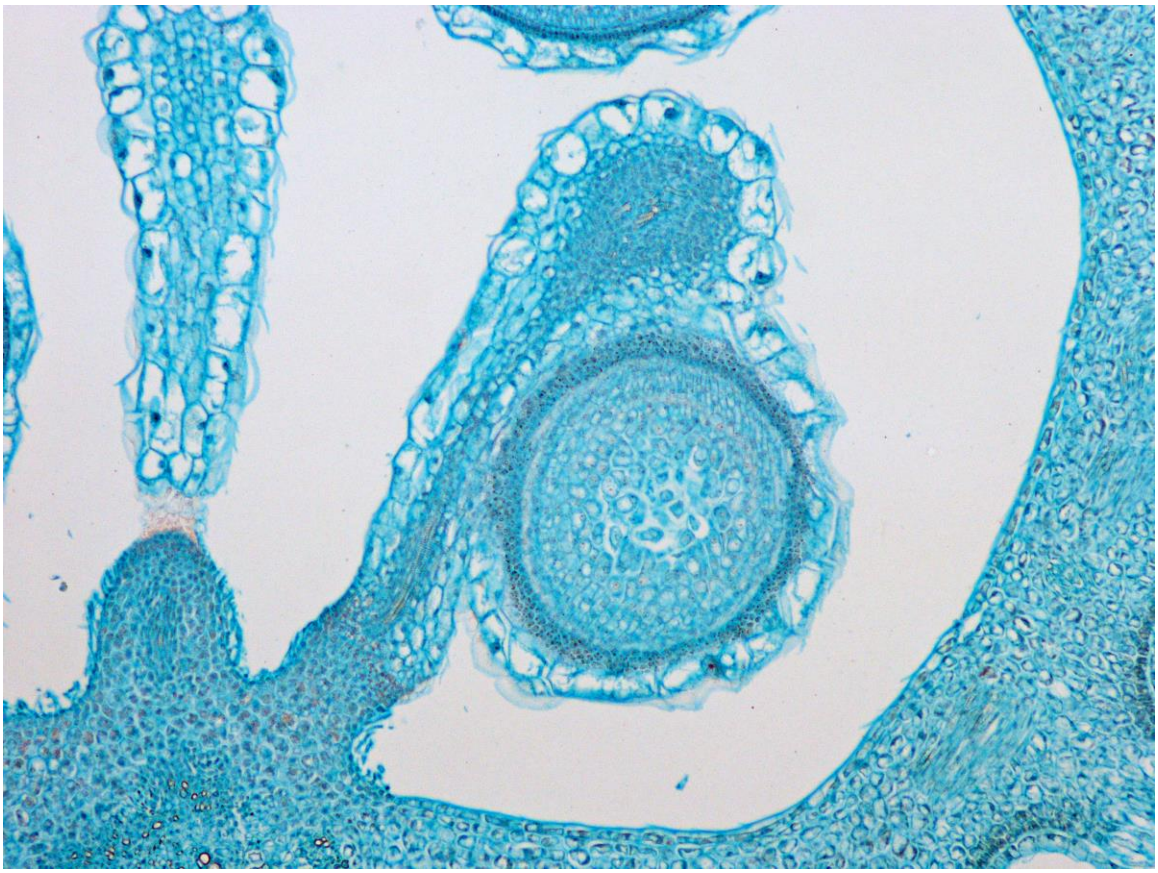

Figure 3. Young anatropous ovule of *Argemone albiflora*.

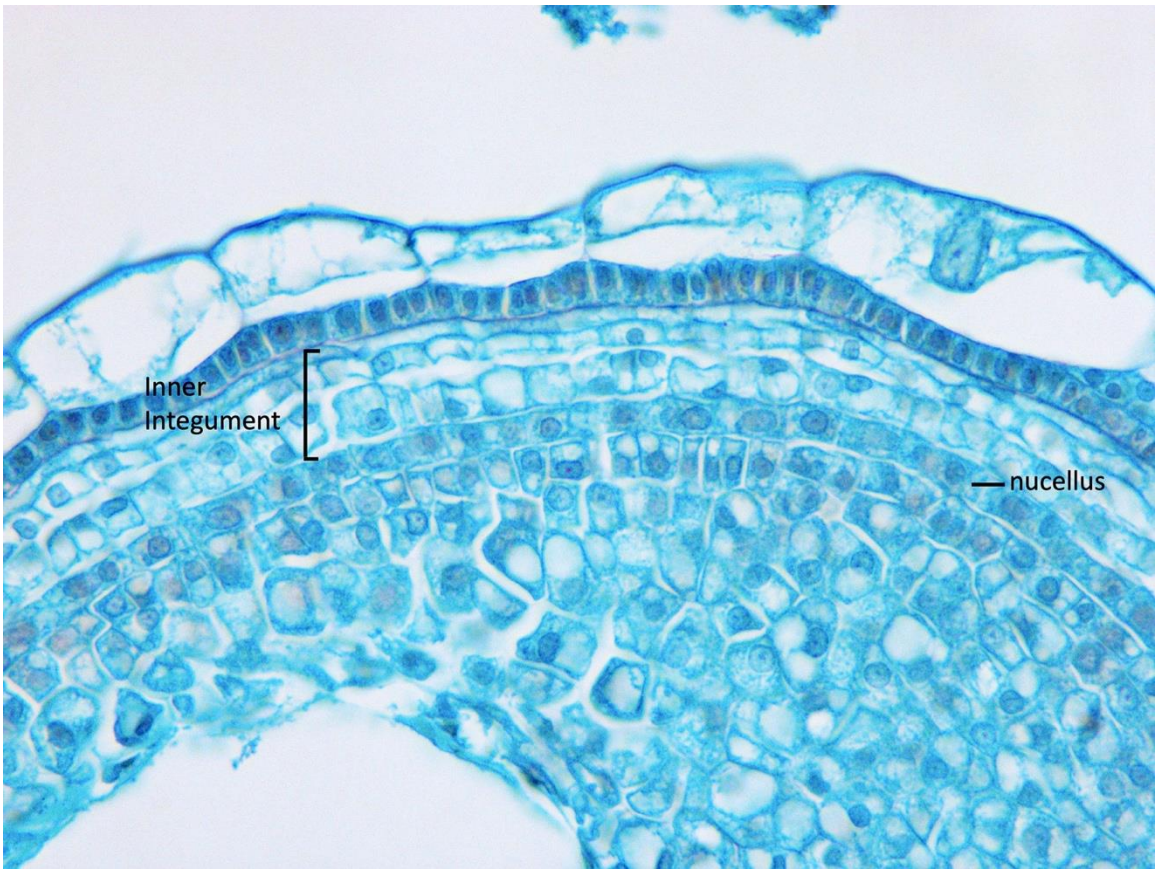

Figure 4. Section of a young developing ovule with the three layers of the inner integument visible just outside the nucellus or wall of the megasporangium. The cells of the inner integument are all more or less cuboidal in shape and of approximately the same size.

Figure 5. Section of a young developing ovule showing one of the large, thin-walled cells that form the outer layer of the outer integument. Starch grains, the large nucleus, and the papillate cuticle are clearly visible, as are the much smaller, crystal-containing cells of the inner layer of the outer integument. Note the enlargement of the cells of the innermost layer of the inner integument and the compression of the two outer layers.

Figure 6. Structure of the outer integument of the seed of *Argemone aurantiaca*. A third layer of radially-flattened cells is clearly visible between the palisade layer and the outer layer of tangentially enlarged cells.

Figure 7. Section of a developing seed showing the radial elongation of the inner layer of the outer integument to form a palisade layer. Nuclei have migrated to the distal end of the cell and polyhedral and rod-shaped crystals are beginning to accumulate.

Figure 8. Section of a developing seed showing the accumulation of tannin-like material in the cells of the inner layer of the outer integument.

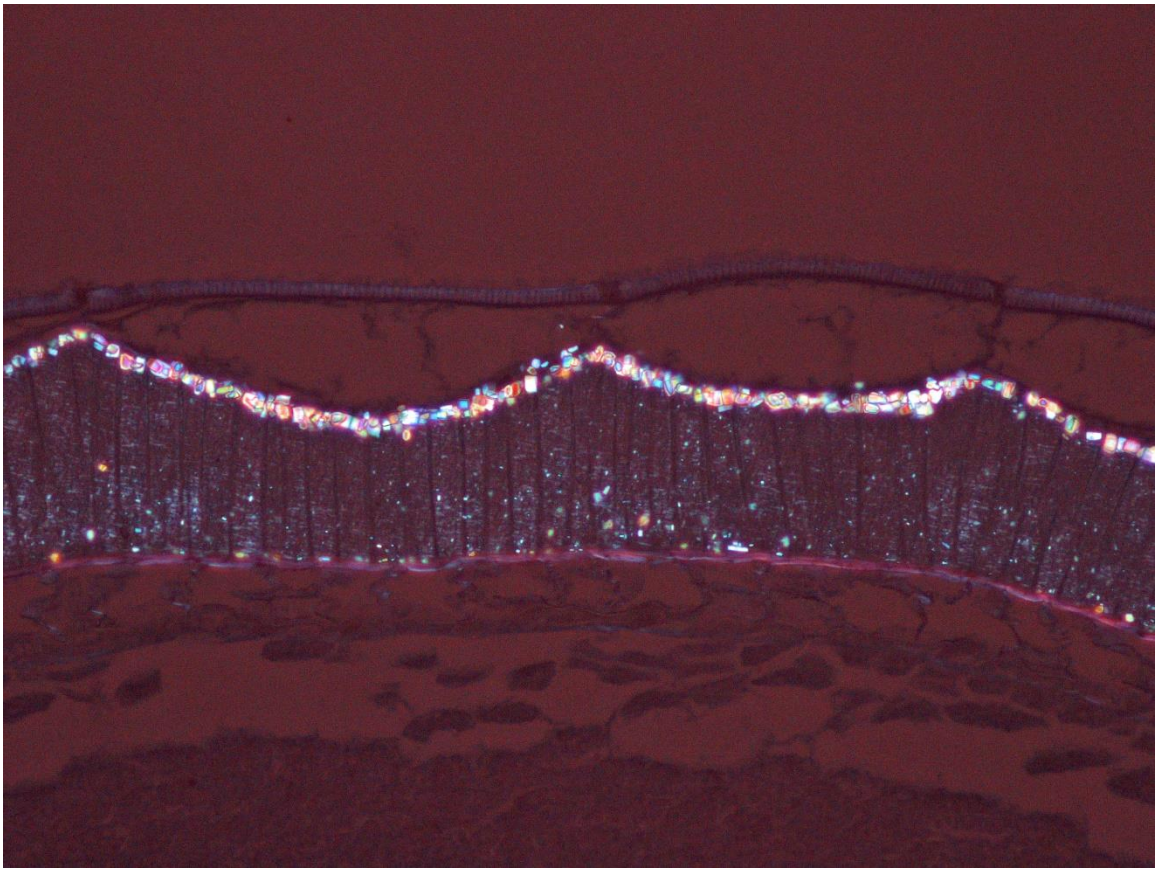

Figure 9. Section of a developing seed photographed under polarized light showing the accumulation of crystalline material at the distal end of the palisade layer formed by the cells of the inner layer of the outer integument.

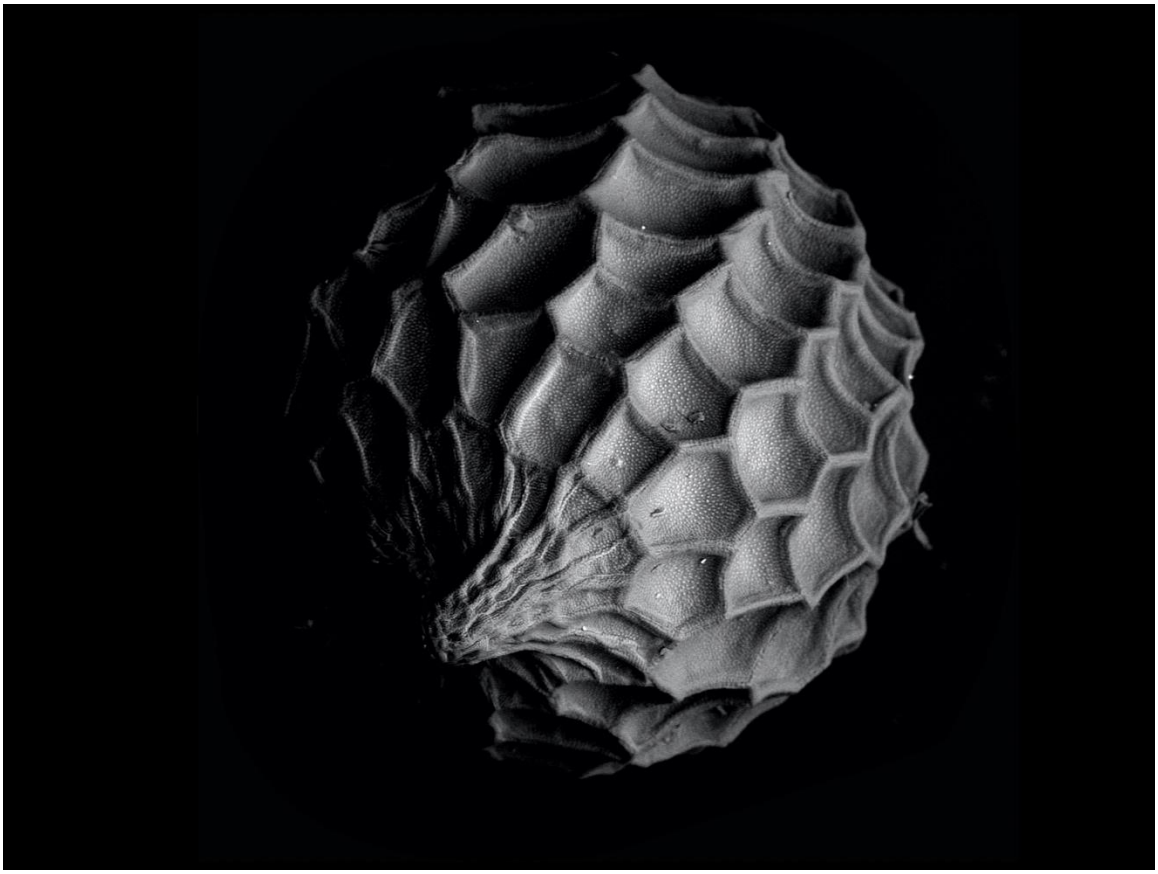

Figure 10. Scanning electron micrograph of a mature seed of *Argemone sanguinea* illustrating the reticulate surface sculpturing pattern and papillate microsculpturing characteristic of all Texas species of the genus.

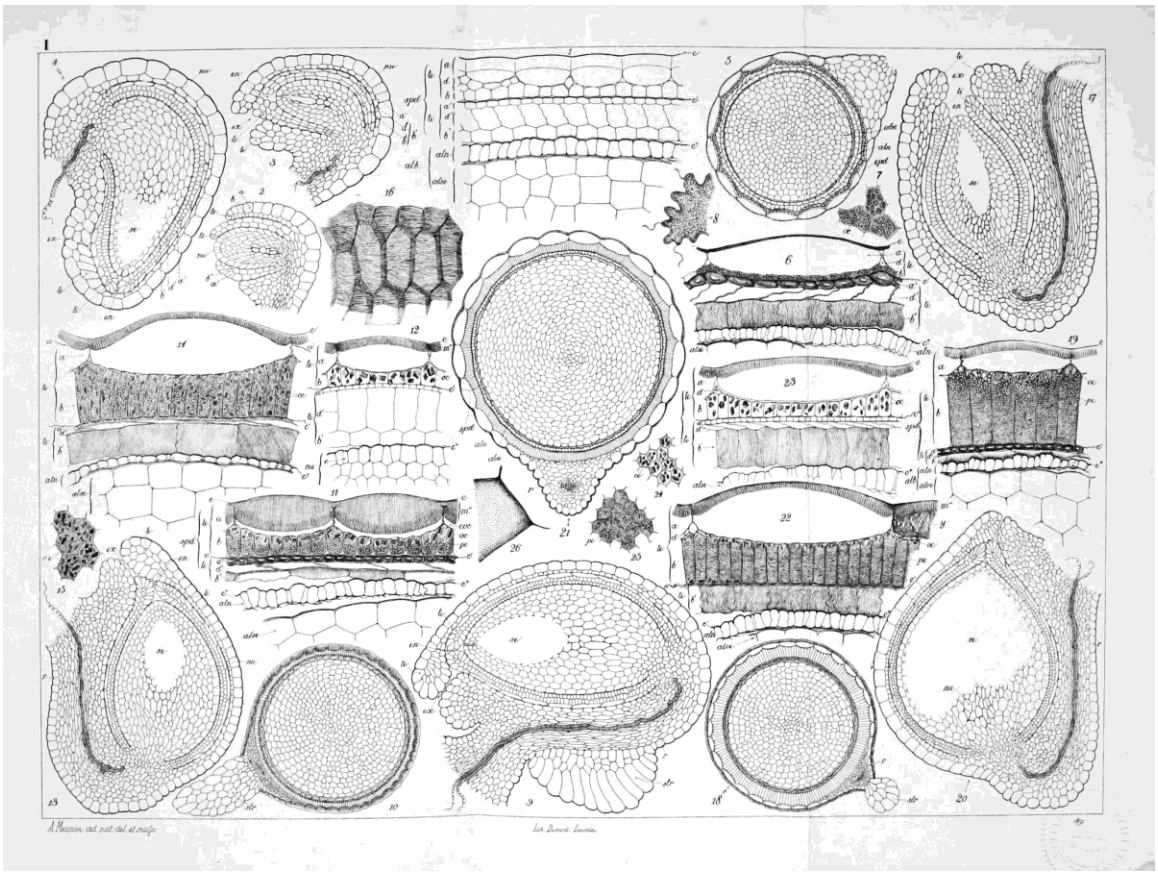

Figure 11. Illustrations of seed structure in several genera of Papaveraceae from Meunier (1891). Numbers 20–26 illustrate features of the seed of *Argemone mexicana*.

Figure 12. Seed morphology of the Texas species of *Argemone* (A) *Argemone aenea*; (B) *Argemone albiflora.*

Figure 13. Seed morphology of the Texas species of *Argemone* (A) *Argemone aurantiaca*; (B) *Argemone chisosensis.*

Figure 14. Seed morphology of the Texas species of *Argemone.* (A) *Argemone mexicana*; (B) *Argemone polyanthemos.*

Figure 15. Seed morphology of the Texas species of *Argemone.* (A) *Argemone sanguinea*; (B) *Argemone squarrosa*.

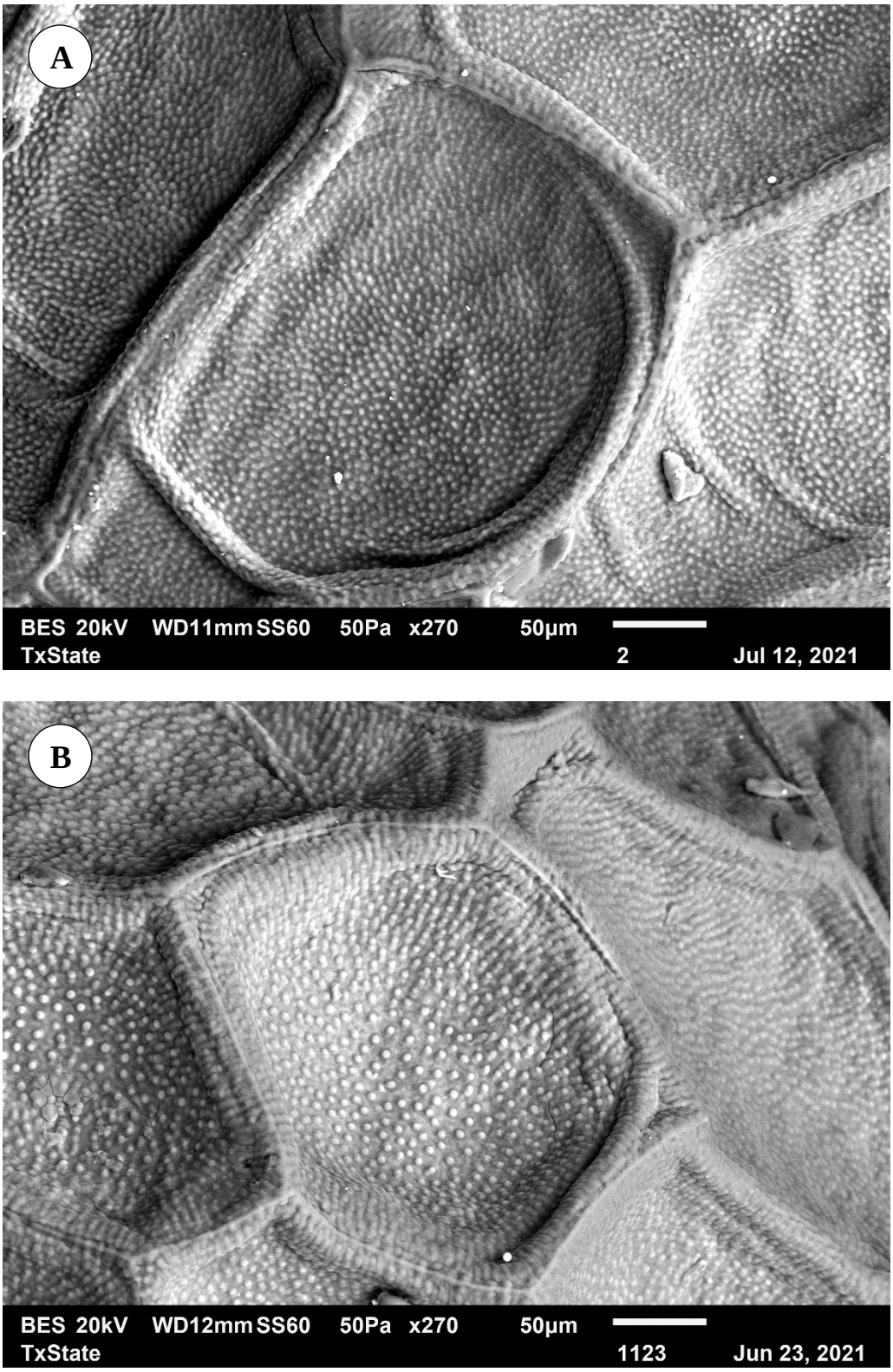

Figure 16. Scanning electron micrograph illustrating microsculpturing of the seed coat surface in *Argemone aenea* (A) and *Argemone albiflora* (B). Scale bar = 50 µm.

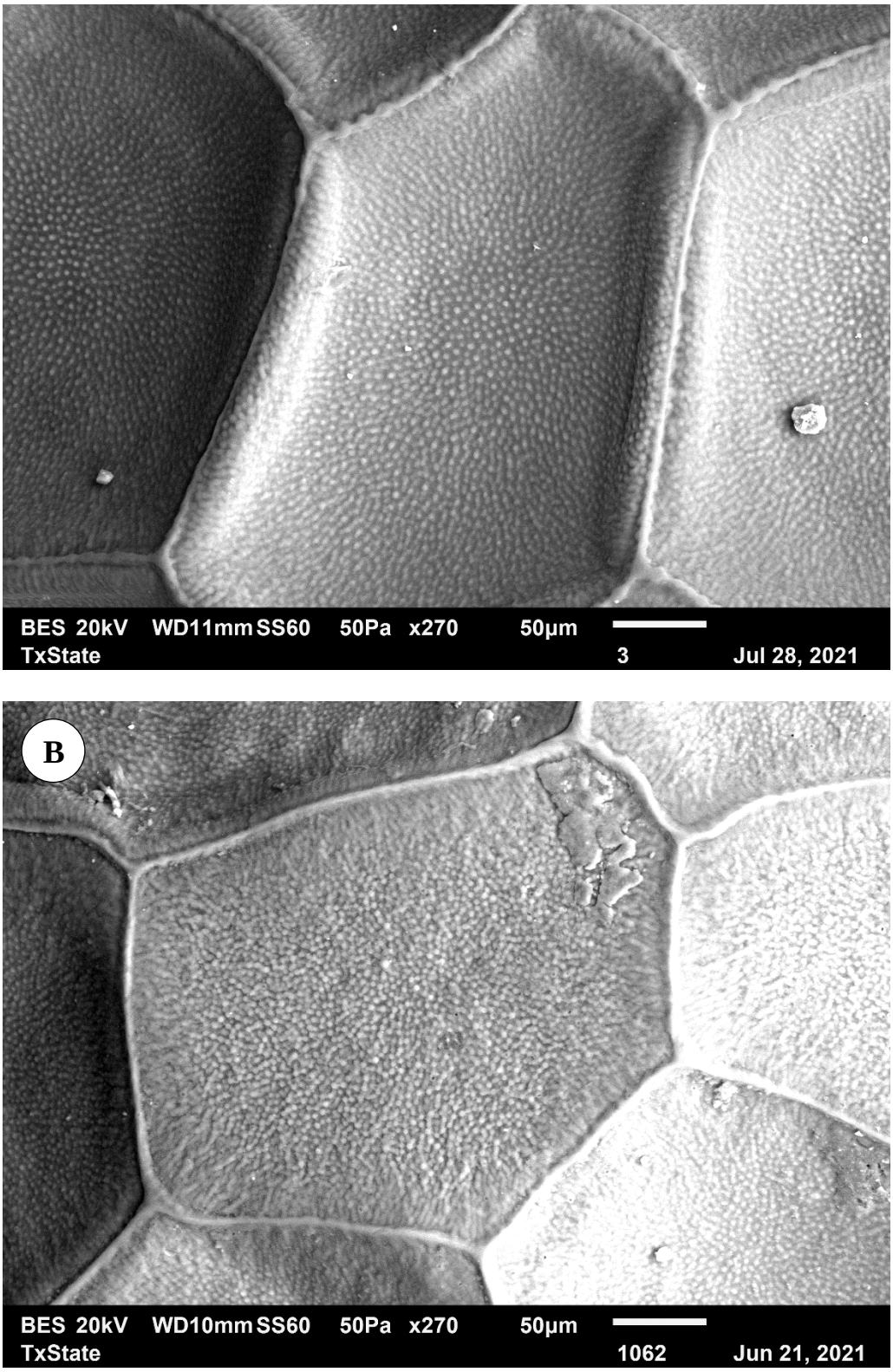

Figure 17. Scanning electron micrograph illustrating microsculpturing of the seed coat surface in *Argemone aurantiaca* (A) and *Argemone chisosensis* (B). Scale bar = 50 µm.

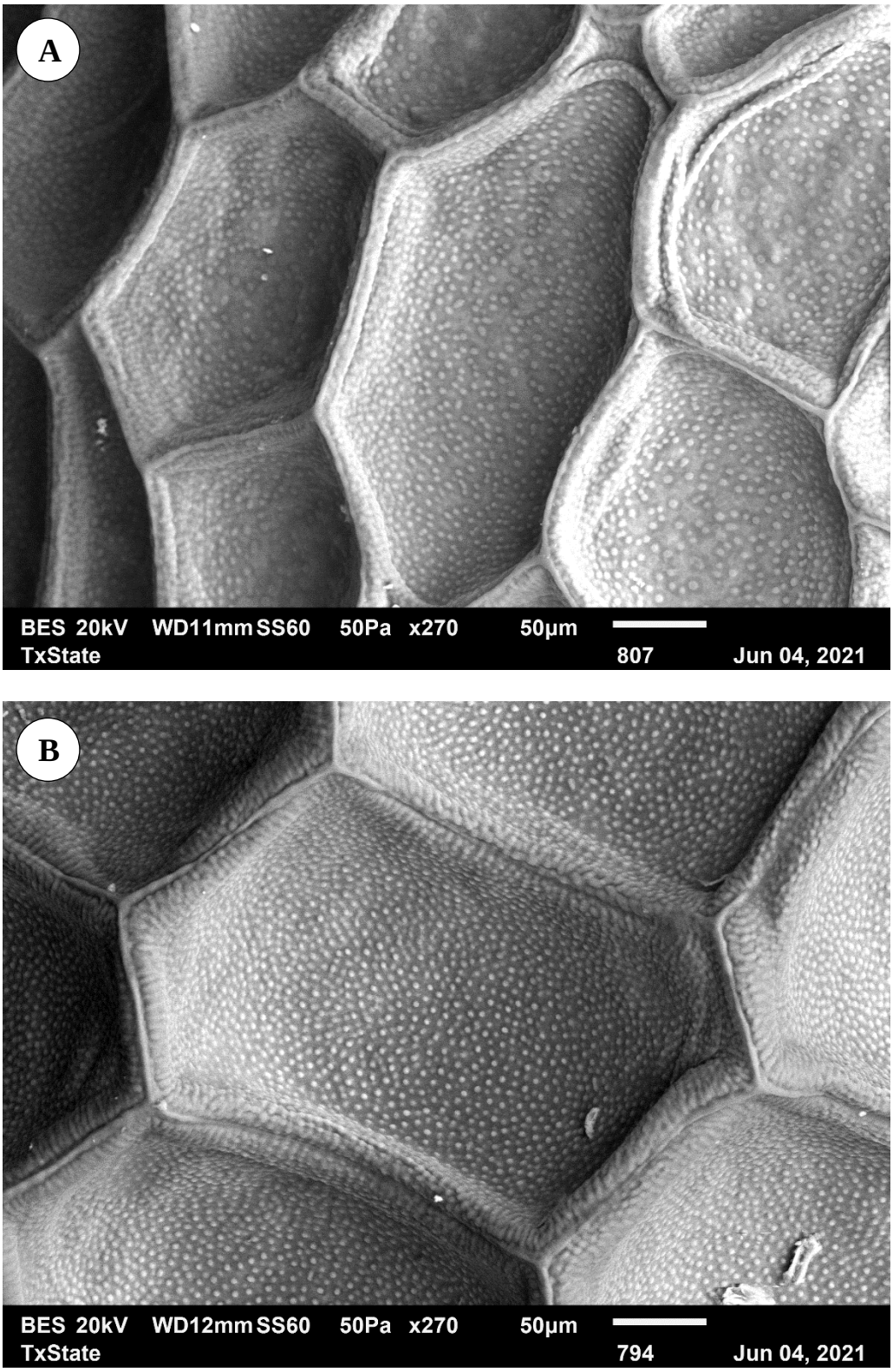

Figure 18. Scanning electron micrograph illustrating microsculpturing of the seed coat surface in *Argemone mexicana* (A) and *Argemone polyanthemos* (B). Scale bar = 50 µm.

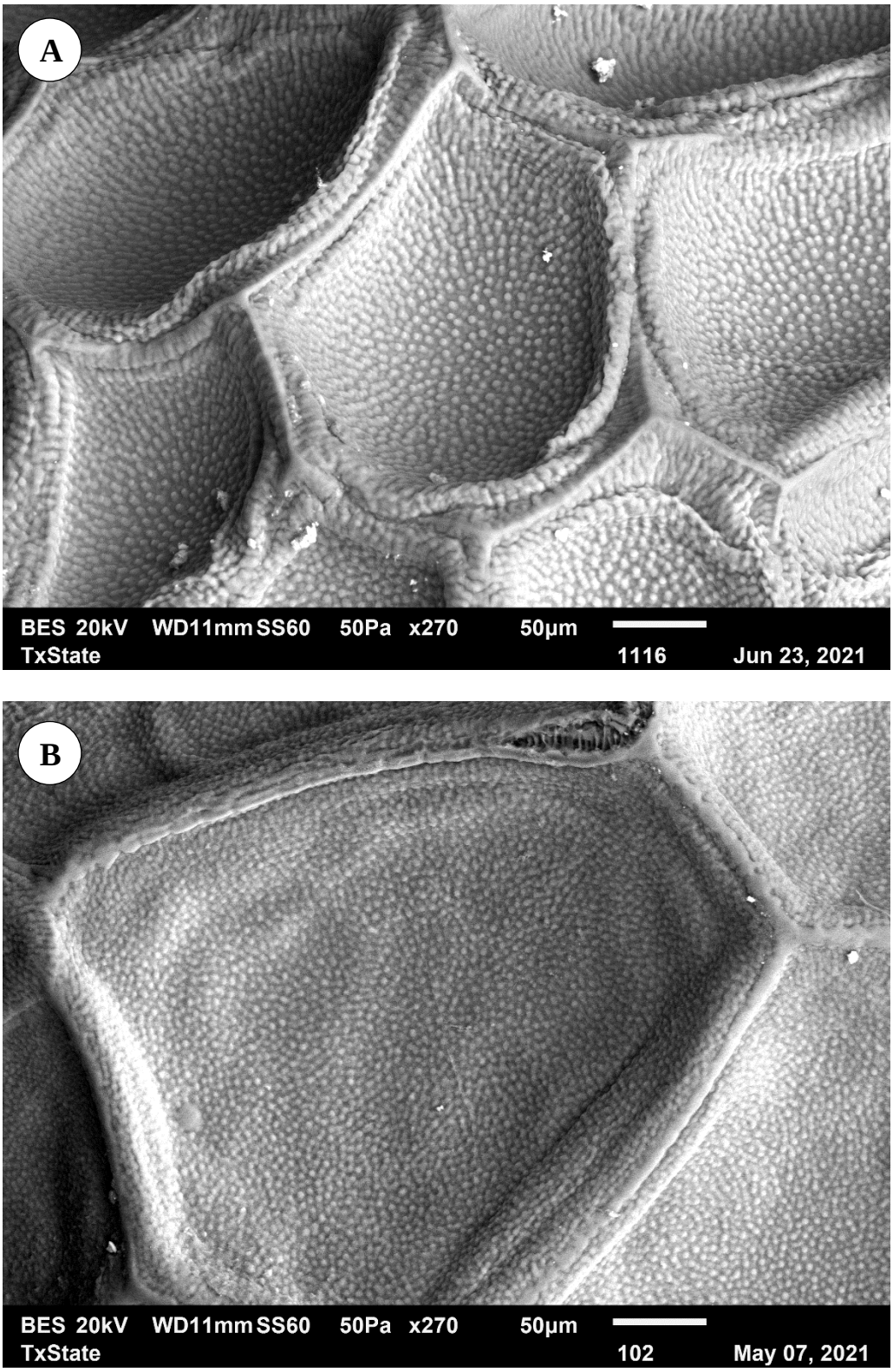

Figure 19. Scanning electron micrograph illustrating microsculpturing of the seed coat surface in *Argemone sanguinea* (A) and *Argemone squarrosa* (B). Scale bar = 50 µm.

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