# Taxonomic Utility of Lemma Micromorphological Characters in the Sporobolus compositus and Sporobolus Vaginiflorus Complexes (Poaceae)

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# TAXONOMIC UTILITY OF LEMMA MICROMORPHOLOGICAL CHARACTERS IN THE *SPOROBOLUS COMPOSITUS* AND *SPOROBOLUS VAGINIFLORUS* COMPLEXES (POACEAE)

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**Abstract:** Five lemma micromorphological characters including bicellular microhairs, hooks, long cells, prickles, and silica cells of six *Sporobolus* taxa representing the *S. compositus* and *S. vaginiflorus* complexes in Texas were investigated using scanning electron microscopy (SEM). Our study supplements earlier SEM treatments of *Sporobolus* in Valdés-Reyna & Hatch and Liu et al. and colleagues. The micromorphological differences found by our study support the taxonomic treatment of these two complexes by Peterson et al.

**Keywords:** Poaceae, *Sporobolus*, *Sporobolus* sect. *Clandestini*, Lemma micromorphology, flora of Texas.

The importance and utility of micromorphological features of the lemmas of grass florets in species identification and circumscription as well as in determining phylogenetic relationships is widely known and documented (see, for instance, Liu et al. 2010, Ortúñez & de la Fuente 2010, and the references cited in each). The taxonomic utility of these microcharacters in the grass genus *Sporobolus* R.Br. has not been fully explored. Liu et al. (2010, p. 201) noted that "full characterization of lemma micromorphology of a few large genera such as *Eragrostis* Wolf and *Sporobolus* awaits further study."

The present study looks at lemma micromorphological characters and their taxonomic utility in two species complexes present in Texas (Table 1): the *Sporobolus compositus* (Poir.) Merr. complex, which includes the perennial species S. *clandestinus* (Biehler) Hitchc. and *S. compositus* [with three varieties, the typical variety plus *S. c.* var. *drummondii* (Trin.) Kartesz & Gandhi and *S. c.* var. *macer* (Trin.) Kartesz & Gandhi]; and the *S. vaginiflorus* (Torr. ex A. Gray) A.W. Wood complex, with the annuals *S. vaginiflorus*, *S. ozarkanus* Fernald, and *S. neglectus* Nash. All of these taxa occur in Texas. Recent molecular data (Peterson et al. 2014) indicate that these two complexes together form the monophyletic *Sporobolus* section *Clandestini* P.M. Peterson. These species are characterized by generally narrow, relatively few-flowered spike-like panicles which are often partly included in the upper sheaths and which produce cleistogamous flowers. Peterson et al. (2014) also note that laterally flattened caryopses are an important character of this group.

This study arose from observations by the first author that numerous Texas specimens of these two groups in the TEX-LL herbarium appeared to be misidentified, with annotation disagreement among grass specialists a common theme. This problem seemed especially acute in plants that could either be annuals or first-year perennials (although it was not restricted to these plants), such that species of the perennial group might be identified as annual species and vice versa. In many of the most used keys for *Sporobolus* in Texas (e.g., Gould, 1975; Diggs et al. 1999, 2006; Peterson et al. 2003; Shaw 2012), the annual versus perennial distinction forms the first or second couplet of the key and has probably been a contributing factor to the misidentifications. In addition, although Peterson et al. (2003) distinguished *S. clandestinus* and

TABLE 1. Taxa and localities for the six *Sporobolus* specimens selected for SEM analysis on the basis of surface features determined to be typical for that taxon.

| |
|---|
| ***Sprorbolus compostus*** complex |
| *S. clandestinus* (Biehler) Hitchc. Texas, Hays Co., *Harms 18* (TEX) |
| *S. compositus* (Poir.) Merr. var. *compositus*. Texas, Lampasas Co., *Hatch 5826* (TEX) |
| *S. compositus* var. *drummondii* (Trin.) Kartesz & Gandhi. Texas, Hays Co., *Harms 19* (TEX) |
| *S. compositus* var. *macer* (Trin.) Kartesz & Gandhi. Texas, Bastrop Co., *Harms 30* (TEX) |
| ***Sprorbolus vaginiflorus*** complex |
| *S. neglectus* Nash. Texas, Grayson Co., *Riggins 396* (TEX) |
| *S. vaginiflorus* (Torr. ex A. Gray) A.W. Wood. Texas, Hays Co., *Harms 44a* (TEX). |

*S. compositus* in part, based on "lemmas minutely pubescent or scabridulous" vs. "lemmas usually glabrous and smooth," these contrasting character-states seem to reflect real differences which are not, however, always easy to ascertain. Furthermore, not all variation in lemma pubescence has been taken into account in keys and descriptions; for instance, Peterson et al. (2003) distinguished *S. vaginiflorus* from *S. neglectus* partly by the strigose lemmas of the former versus the glabrous ones of the latter, even though Riggins (1969) documented the occurrence of forms of *S. ozarkanus* (treated by Peterson et al. 2003 as a variety of *S. vaginiflorus*) with glabrous lemmas. We thus decided to look in more depth at the lemma characteristics of these species as a way to clarify the keys and, hopefully, to solve the problem of distinguishing annual from perennial plants in doubtful cases.

An earlier SEM lemma survey by Valdés-Reyna & Hatch (1991) of 57 species (30 genera) then placed in the tribe Eragrostideae included nine *Sporobolus* taxa, including four in the above complexes – i.e., *S. asper* (Michx.) Kunth var. *asper* [= *S. compositus* var. *compositus*], *S. clandestinus* var. *canovirens* (Nash) Shinners [= *S. clandestinus*], *S. neglectus*, and *S. ozarkanus*. Images and discussion were provided only for *S. compositus* and *S. ozarkanus* and focused on the presence or absence of characters. Liu et al. (2010) included SEM studies of three species of *Sporobolus* but none of the species here under study.

Earlier light microscope study of the *Sporobolus* lemma surface conducted by V. L. Colbry (1957) and Rhonda Riggins (1969) produced results indicative of differences at the species level but which are often difficult to interpret. These are discussed below.

## MATERIALS AND METHODS

All (ca. 200) specimens representing the *Sporobolus compositus* and *S. vaginiflorus* complexes from Texas in TEX/LL were identified following the treatment in Peterson et al. (2003) with the exception of *S. vaginiflorus*, which followed Riggins' 1969 annotations on TEX/LL collections (cf., Riggins 1969, Yatskievych 1999). Riggins' seminal works on the annual (1969) and perennial (1977) species complexes were consulted repeatedly. These specimens were examined under a dissecting microscope at 40–45X for abaxial lemma surface characters, especially those corresponding to macrohairs, prickles and hooks. For each of the six taxa represented in TEX/LL one specimen was chosen as having lemmas deemed typical for that taxon based on micromophological distinctions discernable at 45X; i.e., a surface scabridulous (with prickles) or minutely papillate (with hooks) (Fig. 4, Table 1). Several lemmas from each selected specimen were removed for SEM study. For *S. clandestinus* only scabridulous lemmas which lacked apparent pubescence were selected. *Sporobolus ozarkanus*, cited by Riggins (1969) for Bowie Co. in the NE corner of Texas (*G.W. Letterman 77* [MO]), but not represented from Texas in TEX/LL, was regrettably not included.

Specimens for the study had all been air dried and were on herbarium sheets or

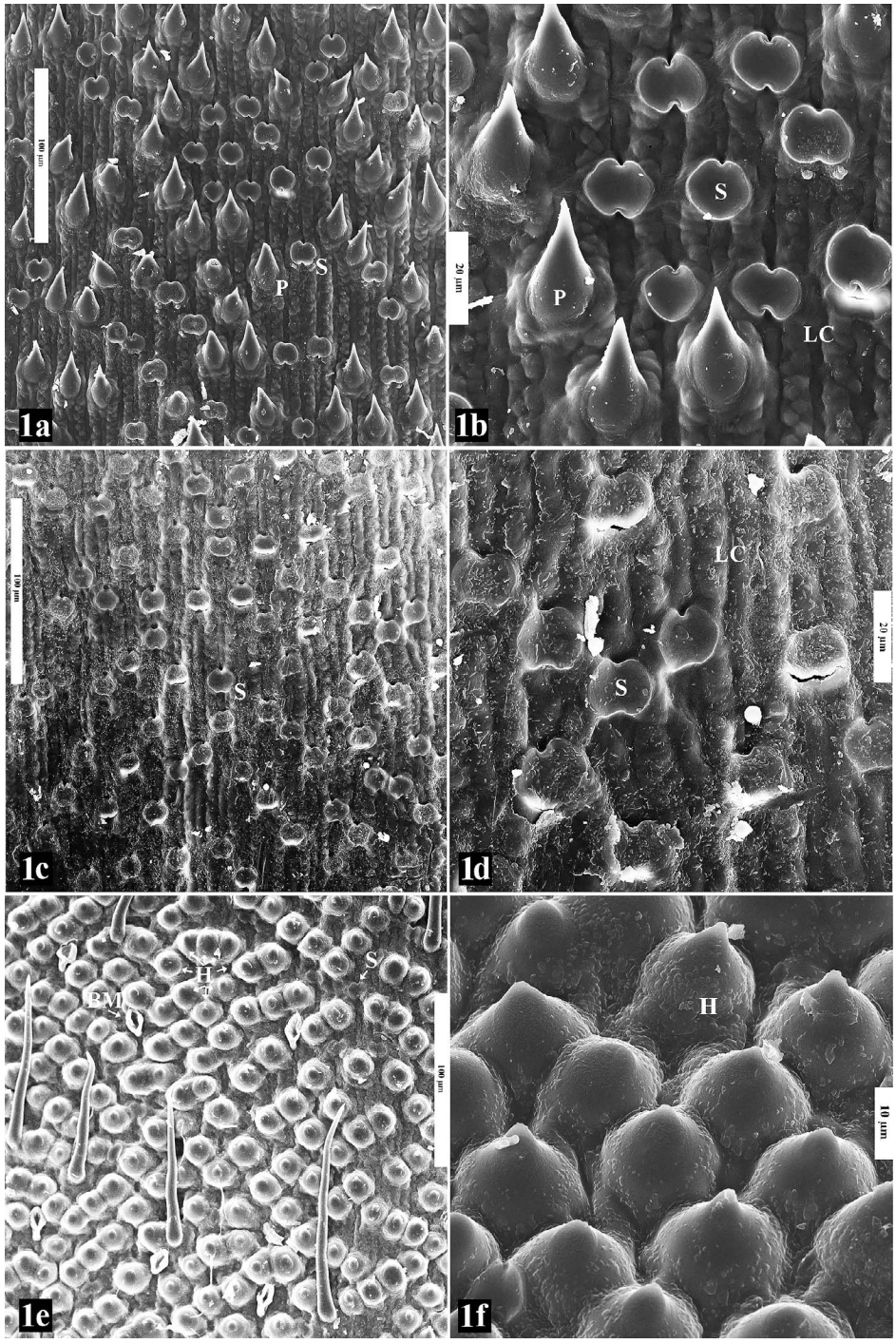



FIG. 1. Intercostal area. a–b: *Sporobolus clandestinus*; c–d: *S. compositus* var. *compositus*; e–f: *S. vaginiflorus*. Scale bars: left column, 100 µm; right column b & d, 20 µm; f, 10 µm. BM = bicellular micro-hair; H = hook; LC = long cell; P = prickle; S = silica cell.

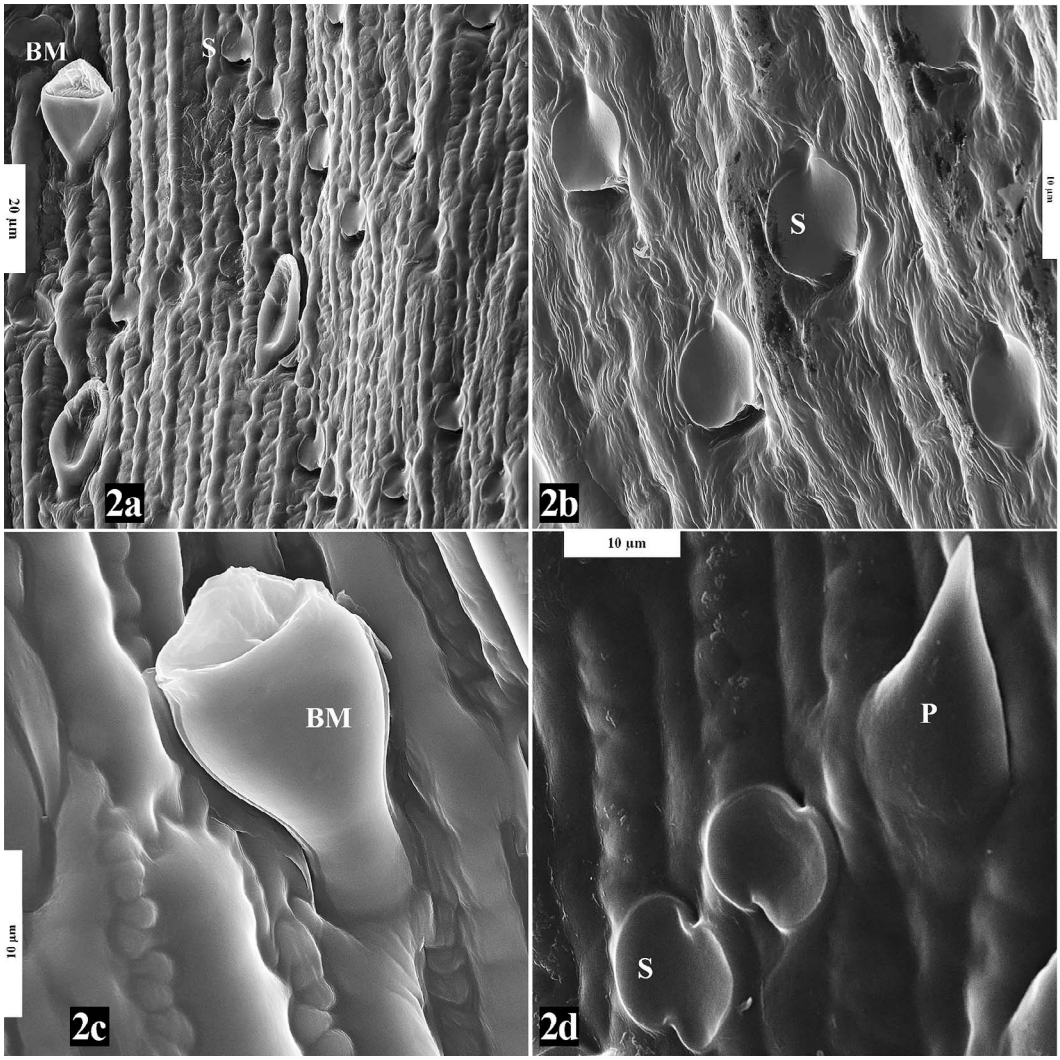



FIG. 2. Intercostal area. a–c: *Sporobolus neglectus*; d: *S. clandestinus*. Scale bars: a, 20 µm; b–d, 10 µm. BM = bicellular micro-hair; P = prickle; S = silica cell.

awaiting mounting on sheets. Lemmas were extracted from the florets and placed on conductive adhesive tabs, which were then mounted on aluminum stubs. These were then sputter coated in argon plasma with a 60/40 platinum/palladium target. The samples were examined with a Zeiss SUPRA 40 field emission SEM at 15 to 20 kV at magnifications of 1.7–49.4 kX. Tilt angles of the sample stub were set at 0.0°, 66.3°, 90.0° depending upon the best perspective of the given lemma surface, which was generally not flat. Only one of the lemmas was used in the counts for Table 2.

Micromorphological characters found in the taxa studied were (1) long cells, (2) silica cells, (3) prickles, (4) hooks, and (5) bicellular microhairs. Macrohairs, typical for *Sporobolus clandestinus*, *S. ozarkanus* and *S. vagniniflorus,* were not considered. Terminology for the characters noted is based primarily on the detailed treatment of Ellis 1979 and does not differ significantly from that in other treatments. Ellis (1979 p.

TABLE 2. Density of cell types in a 100 μm$^2$ intercostal area.

| Taxon | Silica cells | Prickles | Hooks | Microhairs |
|---|---|---|---|---|
| *S. clandestinus* | 6 | 8 | — | 0 |
| *S. compositus* var. *compositus* | 13 | 0 | — | 0 |
| *S. neglectus* | 20 | 0 | — | 1 |
| *S. neglectus* | 21 | 0 | — | 4 |
| *S. vaginiflorus* | 2 | 0 | 24 | 1 |

666) considered prickles and hooks to be two main types of prickle hairs, prickles having an oval or elliptical base and larger size, and hooks having a rounded base, a smaller size, and generally being in an intercostal zone. Valdés-Reyna & Hatch (1991) distinguish 'prickle hair' (or 'prickle') from 'hook;' Liu et al. (2010) use 'microprickle.' In a study of *Melica* L. lemma vestiture Meija-Saules & Bisby 2003 distinguished prickles, hooks, and hooked papillae. Although *Sporobolus* hooks at low magnification closely resemble papillae, hooks are much larger and terminate in short barbs directed toward the lemma apex. In costal areas, prickles and hooks sometimes appear to intergrade (Fig. 3f), perhaps owing to prickle immaturity.

The SEM images of each taxon were studied and the micromorphological features noted. Although we did not study *Sporobolus ozarkanus*, Valdés-Reyna & Hatch (1991) included an SEM image of a lemma of this species from a specimen collected and determined by Riggins (Jefferson Co., Missouri collection, *Riggins 444* [TAES]), and we used that image for our data for *S. ozarkanus*. Herbarium material at TEX-LL of all taxa (including non-Texas material of *S. ozarkanus*) was then studied with a dissecting microscope at 40–45X to ascertain the consistency of the characters observed in the SEM images.

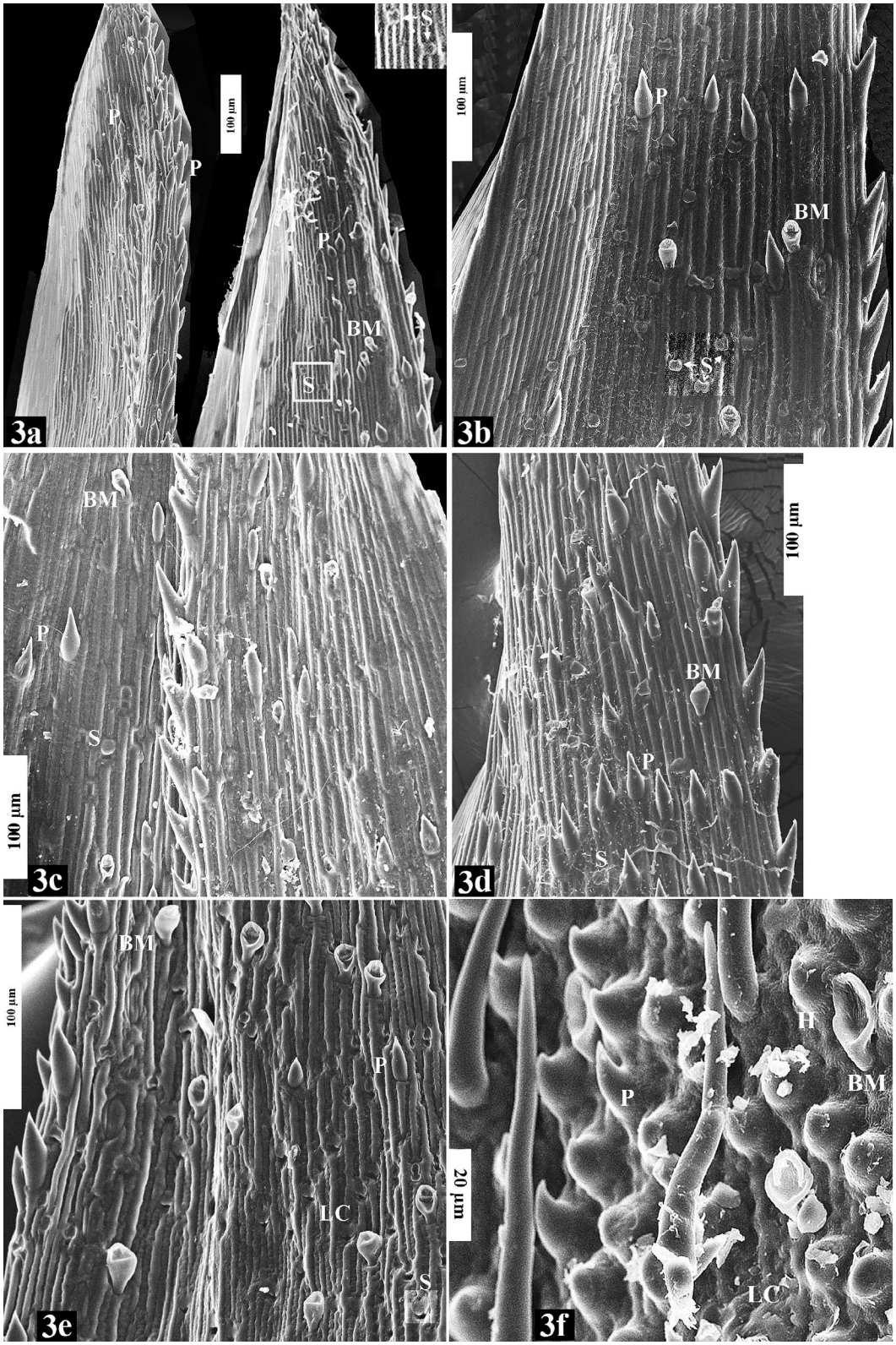
## RESULTS

The SEM images are shown in Figures 1–3 and the following microcharacters labeled.

**Long cells** (Fig. 3e) were apparent for all species except *Sporobolus vaginiflorus* because of its dense covering of hooks. Although at lower magnifications (e.g., 1750–3000X) the anticlinal walls appear slightly undulating (following Ellis 1979) with higher magnification (>12,000X) the edges appear lined with one or more series of tiny teeth-like projections (Figs. 1b, 2c), and even a somewhat twisted appearance (*S. neglectus*; Fig. 2a). Without a larger sample at high magnification, we are unable to determine distinctions among the taxa examined.

**Silica cells** were found on both lemma intercostal (Figs. 1, 2) and tip (Fig. 3) areas for all taxa. They are approximately equidimensional dumb-bell shaped with a wide central portion, often asymmetrically indented or not at all on one side, and with rounded ends. They are flat on the same plane as the long cells, but in *Sporobolus neglectus* seemingly tilted with the proximal end slightly raised (Figs. 2a-b). *Sporobolus neglectus* had the densest distribution and *S. vaginiflorus* the sparsest (Table 2). *Sporobolus clandestinus* and *S. compositus* var. *compositus* had the largest silica cells (ca. 14–15 μm wide); *S. neglectus*, the smallest (ca. 9 μm wide), followed by *S. compositus* var. *macer* and *S. compositus* var. *drummondii* (ca. 10 μm wide). In *S. vaginiflorus* they were nearly the same size as the base of the abundant hooks, ca. 11 μm wide (Fig. 1e).

**Prickles** were found on the lemma tip in all taxa (Fig. 3), but were numerous there in *Sporobolus clandestinus*. In the intercostal region these were found only in *S. clandestinus*, where they were more numerous than silica



cells (Table 2, Fig. 1a,b). In *S. vaginiflorus* there was little difference in size between the hooks and the few prickles noted (Fig. 3f), in contrast with the larger lemma-tip prickles of *S. neglectus* (Fig. 3e). The density of prickles plus silica cells for *S. clandestinus* was roughly the same as the density of silica cells alone for *S. compositus* var. *compositus*.

**Hooks** densely covered the surface of *Sporobolus vaginiflorus* (Table 2, Fig. 1e-f). These are distinctive for both *S. vaginiflorus* and *S. ozarkanus* (as shown in Valdés-Reyna & Hatch 1991). They are visible as minute papillae even at 40X with a dissecting microscope (Fig. 4).

**Bicellular microhairs** were scattered on the lemma tip in all taxa (Fig. 3), generally with the distal cell absent (as noted in Ellis 1979); in the intercostal area only in *Sporobolus neglectus* and *S. vaginiflorus*. The basal cells are more or less chalice-shaped (Fig. 2c), ca. 15–20 μm tall, and the cell walls much thicker than the short (ca. 3–4 μm) hood-shaped distal cell. These were the most salient feature on the otherwise glabrous lemma of *S. neglectus*.

Table 2 shows the density of different microcharacters based on the SEM images (not including *S. ozarkanus*). The general trends seen here were supported by study of herbarium material.

The lemma tip area differed from lower intercostal regions in the presence of prickles and microhair cells for all four species, but prickles were abundant there only for *S. clandestinus*. Hooks were present there only in *S. vaginiflorus* and *S. ozarkanus*.

## DISCUSSION

For those *Sporobolus* taxa surveyed by Valdés-Reyna & Hatch (1991), the presence of dumbbell-shaped silica cells, papillae, "long cells strongly sinuous with one papilla," hooks, and the absence of cork cells and prickle hairs were indicated (Valdés-Reyna & Hatch, 1991, p. 546: Table 2); no mention was made of bicellular microhairs in *Sporobolus*. Correspondingly for their *Sporobolus* taxa Liu et al. (2010) noted cross-shaped silica cells, long cells with U-shaped outline, oblong cork cells, bicellular microhairs with relatively narrow cap cells, and the absence of papillae, microprickles and macrohairs.

Our results for the taxa we examined were similar to both previous studies only with regard to the presence of silica cells and long cells, although the specific forms were not identical (see above); and our results differ with both in the presence of prickles in all our taxa. That we did not find either papillae or cork cells is assumed to be a result from our different sampled taxa.

The results of our SEM and subsequent herbarium studies suggest that lemma micromorphology may be a useful tool for distinguishing the species in *Sporobolus* sect. *Clandestini*, and we present a key below based on these results. The micromorphological differences found by our study (Table 2) support the taxonomic treatment of these two complexes in Peterson et al. 2003. The lemma surface of *S. clandestinus* with its abundant prickles, even in the absence of macro hairs, demonstrates the validity of the "scabridulous" differentia introduced by Peterson et al. 2003 in contrast with the glabrous lemma of *S. compositus*, which lacks these in intercostal areas. The separation of *S. neglectus* and *S. vaginiflorus* as distinct species, treated as varieties in Jones et al. (1997), is supported by distinctive *S. neglectus* characters; i.e.,

←

FIG. 3. Tip area. a: *Sporobolus compositus* var. *dummondii;* b: *S. compositus* var. *macer;* c: *S. compositus* var. *compositus;* d. *S. clandestinus;* e. *S. neglectus;* f. *S. vaginiflorus*. Scale bars: a–e, 100 μm; f, 20 μm. BM = bicellular micro-hair; H = hook; LC = long cell; P = prickle; S = silica cell.


FIG. 4. *Sporobolus vaginiflorus* lemma at 45X with dissecting microscope.

(1) the absence of macrohairs (noted in 'standard' treatments), (2) the absence of hooks (not noted in earlier treatments), (3) larger prickles and microhairs, (4) greater density of silica cells. The close relationship of *S. vaginiflorus* and *S. ozarkanus* (treated as a variety of the former by Peterson et al. [2003]) is supported by their shared density of hooks, a character not reported for other *Sporobolus* species.

A dissecting microscope examination (35X to 60X) of *Sporobolus* floret indumentum by Colbry (1957) identified a number of micromorphological characters, some seeming to match our findings and others difficult to interpret; as always, it is possible that taxonomic misidentification may play a role. Particularly interesting are the reports of lemmas "microscopically striate with shiny conical glands" in *S. clandestinus* and *S. vaginiflorus*. In her 1969 treatment of *S. ozarkanus*, Riggins noted that "glabrous florets are often covered with small conical projections." These, and Colbry's "shiny conical glands," might seem to describe the abundant hooks of the *S. vaginiflorus* lemma, noted also for *S. ozarkanus* by Valdés-Reyna & Hatch. These are visible as minute papillae at 40X (Fig. 4), although with the exception of Riggins (1969) they have never been noted in traditional taxonomic literature. We did not, however, find them in *S. clandestinus*, and thus Colby's "shiny conical glands" might refer to some other structures, such as bicellular microhairs, present in the apical portions of the lemmas of all the species studied.

### LEMMA MICROMORPHOLOGICAL KEY TO *SPOROBOLUS* SECT. *CLANDESTINI*

Micromorphological key based on lemma intercostal surface characters for the two complexes. *Sporobolus ozarkanus* and *S. vaginiflorus* are not distinguishable at this level, nor are the varieties of *S. compositus*. The key should be used in conjunction with the labeled cell types in Figs. 1–3.

1. Intercostal surface populated with numerous cells that terminate in points (prickles or hooks)
   2. Intercostal surface with hooks . . . . . *S. ozarkansus, S. vaginiflorus*
   2′. Intercostal surface with prickles . . . . . *S. clandestinus*
1. Intercostal surface not populated with cells that terminate in points
   3. Intercostal surface with bicellular microhairs common . . . . . *S. neglectus*
   3. Intercostal surface with bicellular microhairs absent or sparse . . . . . *S. compositus*

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