

An SEM Survey of the Leaf Epidermis in Danthonioid Grasses (Poaceae: Danthonioidae)

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ABSTRACT. This study comprised a scanning electron microscope survey of abaxial epidermal features in subfamily Danthonioidae, with emphasis on North American *Danthonia*. The survey included 21 taxa, encompassing seven genera (*Austroanthonia*, *Cortaderia*, *Danthonia*, *Merxmuellera*, *Notodanthonia*, *Rytidosperma*, *Tribolium*) and the eight North American *Danthonia* species. We investigated micromorphological characters to determine taxonomic utility and whether North American representatives of *Danthonia* are distinct. We examined micromorphological epidermal features: macrohairs, bicellular microhairs, prickle hairs, silica bodies, and stomata. We found no distinguishing characters at the subfamilial level. *Danthonia* is characterized by the absence of abaxial stomata and presence of bicellular microhairs with basal and terminal cells of equal length and microhairs with long basal cells relative to terminal cells. Prickle hairs in the abaxial epidermal costal regions of four species of North American *Danthonia* are reported for the first time.

KEYWORDS: Danthonioidae, epidermal characters, macrohairs, microhairs, prickle hairs, silica bodies.

The Danthonioidae (Poaceae) comprise a single tribe, Danthoniaceae, with approximately 250 species and 19 genera [Grass Phylogeny Working Group (GPWG) 2001]. The subfamily occurs predominantly in the Southern Hemisphere and is considered south-temperate, but the greatest diversity is found in Africa (nine genera and 125 species). In the Australian grasslands of New South Wales, Victoria, and Tasmania, danthonioid grasses frequently form the dominant vegetation cover and are economically important as native forage species.

Danthonioidae were formerly included in Aveneae (Hubbard 1934) or within Arundinoideae, a mix of unrelated taxa that do not fit into other subfamilies (Watson and Dallwitz 1992). Molecular evidence supports the polyphyly of Arundinoideae s.l. (Barker et al. 1995, 1999) and the recognition of Danthonioidae at the subfamilial level as a distinct lineage from Arundinoideae (GPWG 2001). The monophyly of Danthonioidae is supported by morphological and molecular data. Haustorial synergids, bilobed prophylls, and ovaries with distant styles are subfamilial synapomorphies (Linder and Verboom 1996). In addition, several characters from the chloroplast genome provide strong evidence for monophyly (Barker et al. 2003). Currently, the phylogenetic position of this subfamily is within the Panicoideae-Arundinoideae-Centothecoideae-Chloridoideae-Aristidoideae-Danthonioidae (PACCAD) clade and sister to Aristidoideae (GPWG 2001). Though Danthonioidae are monophyletic (GPWG 2001), several genera in the subfamily, including *Cortaderia*

Stapf. (Barker et al. 2003) and *Merxmuellera* Conert (Barker et al. 2000), are para- or polyphyletic.

Historically, the generic circumscription of danthonioid grasses, in particular *Danthonia* DC., has been controversial due to the large number of species worldwide and the wide overlap of morphological attributes. The latest studies recognize nine species in South America, eight in North America [*D. californica*, *D. compressa*, *D. decumbens*, *D. intermedia*, *D. parryi*, *D. sericea*, *D. spicata*, and *D. unispicata* (Darbyshire 2003)], three in Europe, two in Africa, and two in Asia (Linder and Verboom 1996). Of these, one species (*D. decumbens*) is native to Europe but is naturalized in North and South America and Australia (Darbyshire 2003) for a total of 23 species (Linder and Verboom 1996).

Early morphological studies of *Danthonia* revealed an extensive range of interspecific variability and highlighted the need for a new taxonomic scheme (deWet 1954, 1956). The taxonomic rearrangement of *Danthonia* s.l. began with the formal establishment of *Chionochloa* Zotov, *Notodanthonia* Zotov, *Erythranthera* Zotov, and *Pyrranthera* Zotov to describe the New Zealand taxa (Zotov 1963). In the following decade the South African taxa were circumscribed within the genera *Dregeochloa* Conert (Conert 1966), *Karoochloa* Conert & Türpe (Conert and Türpe 1969), *Merxmuellera* Conert (Conert 1970), and *Pseudopentameris* Conert (Conert 1971). Nicora (1973) placed several South American taxa in the genus *Rytidosperma* Steud. Furthermore, Conert (1987) argued that the basis for the separation of the Australian taxa from the genus *Danthonia* was unfounded because the lemma hair characters used to separate them from *Danthonia* s.l. were inconsis-

tent. More recently, Linder and Verboom (1996) advocated the recognition of *Austrodanthonia* H. P. Linder, *Joycea* H. P. Linder, *Notochloe* Domin., and *Plinthanthesis* Steud. to describe Australasian danthonioid grasses and recognized the African genus *Schismus* P. Beauv. The same study supported separation of *Danthonia* and *Rytidosperma* based on morphological and anatomical data, such as the presence of tufted lemma hairs in *Rytidosperma* and cleistogamous florets in the upper leaf sheaths of *Danthonia*. A more recent re-examination of the relationships within danthonioid grasses identified seven informal groups: the basal *Merxmuellera* assemblage and the *Pentastichis*, *Pseudopentameris*, *Chionochloa*, *Cortaderia*, *Rytidosperma*, and *Danthonia* clades (Barker et al. 2000).

Despite the extensive work in para- or polyphyletic taxa, such as *Merxmuellera* and *Cortaderia*, the intrageneric taxonomic boundaries in the subfamily remain unclear (Barker et al. 2000, 2003). Likewise, studies of the subfamily have not included sufficient taxonomic sampling within *Danthonia* to adequately test its monophyly. Wright (1984) suggested that *Danthonia* is monophyletic, but the morphological and anatomical traits supporting the clade, such as scattered lemma hairs, costal short cells in rows, undivided phloem, and the presence of bulliform cells, are not unambiguous synapomorphies. More recently, two synapomorphies have been reported for *Danthonia*, the presence of cleistogenes in the lower leaf sheaths and a base chromosome number of $x = 18$ (Linder and Verboom 1996), but there are some exceptions. For example, cleistogenes are rarely present in *D. intermedia* (Darbyshire 2003), and an unusual chromosome count of $2n = 31$ was reported in *D. spicata* (Darbyshire and Cayouette 1989).

Over the last four decades, micromorphological characters of the leaf epidermis have been scrutinized in several plant groups. These attributes have been informative at various taxonomic levels and valuable to differentiate among groups of extant taxa with putative relatives available in the fossil record (Stace 1984). Watson and Dallwitz (1992 onwards) report detailed descriptions of the leaf epidermis in numerous taxa, emphasizing the significance of these characters in the systematics of Poaceae. Previous studies have found the abaxial surface of the leaf blade taxonomically informative (e.g., Dávila and Clark 1990; Watson and Dallwitz 1992). Several features of the leaf epidermis are useful in grass taxonomy, including the intercostal long and short cells, stomatal cell type and shape, type of papillae, prickle hairs, macro- and microhairs, and silica bodies (Metcalf 1960; Ellis 1979). Comparative studies of epidermal

characters using scanning electron microscopy (SEM) to investigate East African grasses (Palmer and Tucker 1981, 1983; Palmer et al. 1985; Palmer and Gerbeth-Jones 1986, 1988) have proven the taxonomic and phylogenetic utility of this technique, which provides detailed three-dimensional views. SEM has also been important in investigating the morphology of silica bodies, a common type of phytolith in plants. These structures are mineral deposits that form inside specialized epidermal cells in the Poaceae (Piperno and Pearsall 1998). The significance and implications of silica bodies in the taxonomy of grasses have been widely addressed. Certain silica body shapes are characteristic of grass subfamilies and tribes.

Previous studies have examined leaf blades in other genera and species within Danthonioidae (Ellis 1980a, 1980b, 1985; Barker and Ellis 1991; Barker 1993, 1995); however, several relevant taxonomic and phylogenetic questions remain unanswered. Due to taxonomic complexity, the paucity of morphological synapomorphies, and the relatively unexplored micromorphological structures, we undertook an SEM survey of epidermal features in danthonioid representatives with emphasis on North American representatives of the genus *Danthonia* to identify potentially informative characters. Our primary objectives were 1) to investigate micromorphological characters to assess their taxonomic value at the generic and specific levels and 2) to determine whether the North American representatives of *Danthonia* can be distinguished based on microstructures of the leaf epidermis. Our study represents the first formal SEM examination of leaf epidermal characters of danthonioid grasses.

MATERIALS AND METHODS

Taxonomic Sampling. Twenty-one taxa, including seven danthonioid genera (*Austrodanthonia*, *Cortaderia*, *Danthonia*, *Merxmuellera*, *Notodanthonia*, *Rytidosperma*, *Tribolium* Desv.) as recognized by Barker et al. (2000) were investigated in this study (Appendix 1). Our sampling encompasses the eight North American *Danthonia* species: *D. decumbens* [syn. *Sieglingia decumbens* (L.) Bernh.] introduced from Europe, and the seven native species (*D. californica*, *D. compressa*, *D. intermedia*, *D. parryi*, *D. sericea*, *D. spicata*, *D. unispicata*; Darbyshire 2003). In addition, *D. filifolia* [syn. *Danthonia secundiflora* subsp. *secundiflora* J. Presl.], a Mexican species, and *D. chilensis*, native to Chile, were included in the American group. Although limited in sampling, three South American species each of *Cortaderia* and *Rytidosperma* were included to assess potential differences between North American taxa and those from Central and South America, as well as several Danthonioidae genera from the Old World (Australia, New Zealand, and South Africa). The taxonomy for North American *Danthonia* follows Darbyshire (2003).

Plant Material. Seeds from danthonioid species from Australia, New Zealand, Africa, and South America were obtained from the Western Regional Plant Introduction

TABLE 1. Micromorphological characters of the abaxial leaf epidermis of danthonioid grasses examined with scanning electron microscopy. N/A = Not applicable.

Taxon	Macrohairs	Bicellular Microhairs	Cells of Microhairs	Prickle Hairs	Intercostal Silica Bodies	Abaxial Stomata
<i>Austrodanthonia pilosa</i>	Present	Present	Short basal cell	Absent	Absent	Present
<i>Cortaderia bifida</i>	Absent	Absent	N/A	Absent	Absent	Absent
<i>C. hapalotricha</i>	Absent	Absent	N/A	Absent	Absent	Absent
<i>C. selloana</i>	Absent	Absent	N/A	Absent	Absent	Absent
<i>Danthonia californica</i>	Present	Present	Long basal cell	Present	Absent	Absent
<i>D. chilensis</i>	Absent	Present	~ Equal in length	Absent	Absent	Absent
<i>D. compressa</i>	Absent	Present	Long basal cell	Absent	Dumbbell-shaped	Absent
<i>D. decumbens</i>	Absent	Present	~ Equal in length	Absent	Absent	Absent
<i>D. filifolia</i>	Absent	Absent	N/A	Absent	Dumbbell-shaped	Absent
<i>D. intermedia</i>	Present	Present	Long basal cell	Present	Cross-shaped	Absent
<i>D. parryi</i>	Absent	Absent	N/A	Absent	Tall and narrow	Absent
<i>D. sericea</i>	Present	Present	~ Equal in length	Present	Cross-shaped	Absent
<i>D. spicata</i>	Absent	Present	~ Equal in length	Absent	Absent	Absent
<i>D. unispicata</i>	Present	Present	~ Equal in length	Present	Absent	Absent
<i>Merxmüllera disticha</i>	Absent	Present	Short basal cell	Absent	Tall and narrow	Present
<i>Notodanthonia semiannularis</i>	Absent	Present	Short basal cell	Absent	Cross-shaped	Present
<i>Rytidosperma glabra</i>	Present	Present	Short basal cell	Absent	Tall and narrow	Absent
<i>R. unarede</i>	Absent	Present	Short basal cell	Absent	Absent	Present
<i>R. violacea</i>	Absent	Absent	N/A	Absent	Absent	Present
<i>R. virescens</i>	Present	Present	Long basal cell	Absent	Absent	Present
<i>Tribolium hispidum</i>	Present	Present	Short basal cell	Absent	Absent	Present

Station (USDA) in Pullman, Washington (Appendix 1). The seeds from some taxa were grown in pots in the University of Saskatchewan greenhouses to obtain leaf tissue for SEM analysis and later transferred to an outdoor experimental garden plot. Voucher specimens were prepared from seed-grown specimens for their inclusion in the herbarium collection of the University of Saskatchewan (SASK). Fresh plant material was air dried at room temperature prior to analysis. When fresh material was unavailable, portions of leaf blades were removed from herbarium specimens for investigation as indicated in Appendix 1. Selection of leaf material followed Ellis (1979). For each species, three leaf blades were chosen for comparison, each from a different plant specimen whenever possible. For consistency, the sample was selected from mature, non-flag leaf blades from an area midway between the apex and ligule. A portion of the lamina approximately 5 mm long was mounted on a stub, then sputter-coated with gold in an Edwards Sputter Coater S150B. Leaf abaxial epidermal surfaces were examined with a Philips 505 SEM and photographed at 65X and 300X using Polaroid 665 Positive/Negative film.

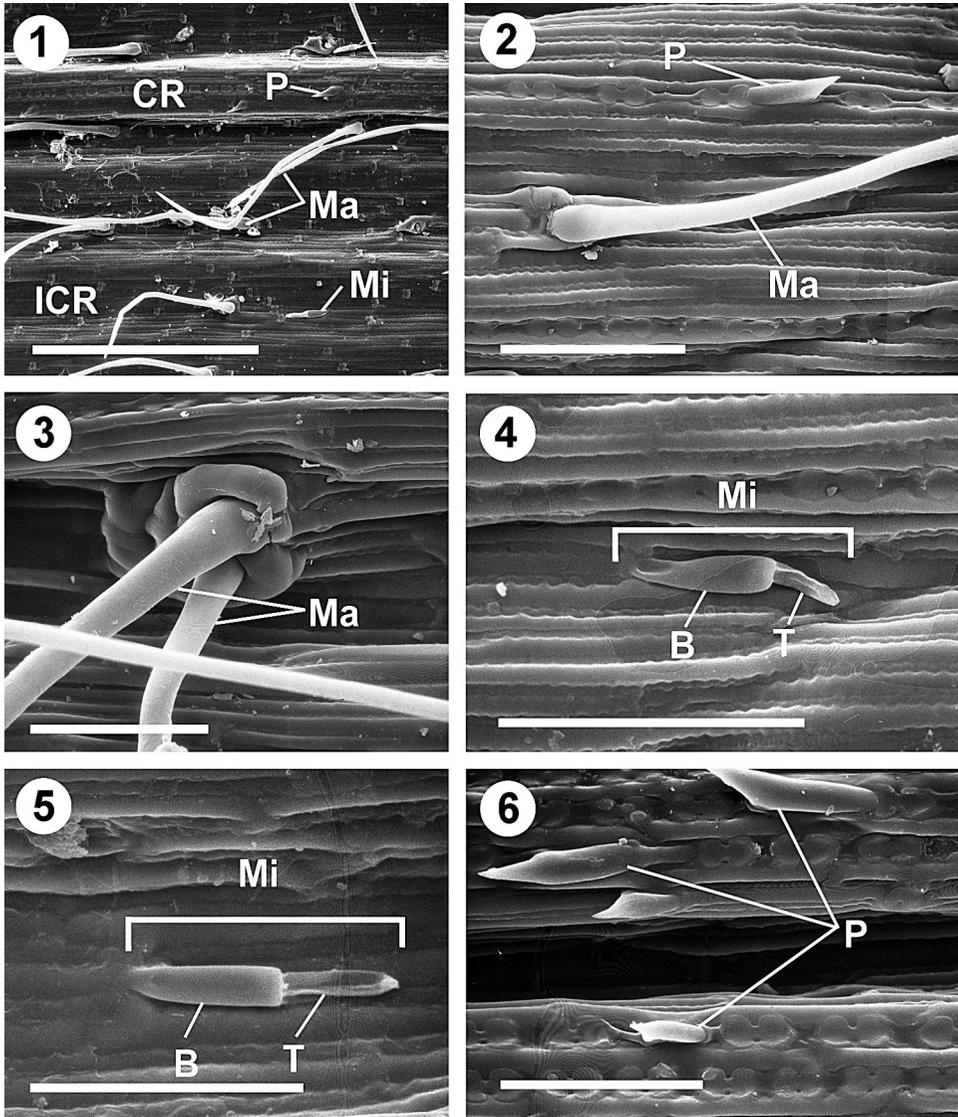
Selection and Examination of Characters. The selection of micromorphological characters was based primarily on Metcalfe (1960) and Ellis (1979). We selected characters observed on the abaxial surface as this facilitates comparison with other information available, especially the *World Grass Species – Descriptions* (Clayton et al. 2002 onwards) and *Grass Genera of the World* (Watson and Dallwitz 1992 onwards). These include 1) macrohairs, 2) microhairs, 3) cells of microhairs, 4) prickle hairs, 5) shape of silica bodies, both in the costal and intercostal regions, and 6) stomata. Terminology used to describe leaf epidermal characters follows Ellis (1979).

RESULTS

The abaxial leaf epidermis of danthonioid grasses exhibits several micromorphological structures (Table 1), described below.

Macrohairs. Macrohairs can be observed with the naked eye and are up to 5 mm long, but details require microscopic aid. At the SEM level, the macrohairs of danthonioid grasses are typically unicellular basifixed structures, as in *Danthonia sericea* (Fig. 1), *D. intermedia* (Fig. 2), *D. unispicata* (Fig. 3), *Tribolium hispidum* (Fig. 9), and *Austrodanthonia pilosa* (Figs. 7, 10). Macrohairs are also present in *D. californica*, *Rytidosperma glabra*, and *R. virescens* (Table 1). The length and abundance of macrohairs on the leaf epidermis varies among individuals of the same species (e.g., *A. pilosa*, *D. californica*, *D. sericea*, and *D. unispicata*). Unlike the majority of specimens investigated, the leaf epidermis of *Cortaderia* species was covered with wax and lacks macrohairs. This waxy surface pattern with no macrohairs was consistent among the three species investigated (e.g., *C. hapalotricha*; Fig. 8), and we found no evidence indicating that the waxy layer could disguise the macrohairs observed in other danthonioid taxa.

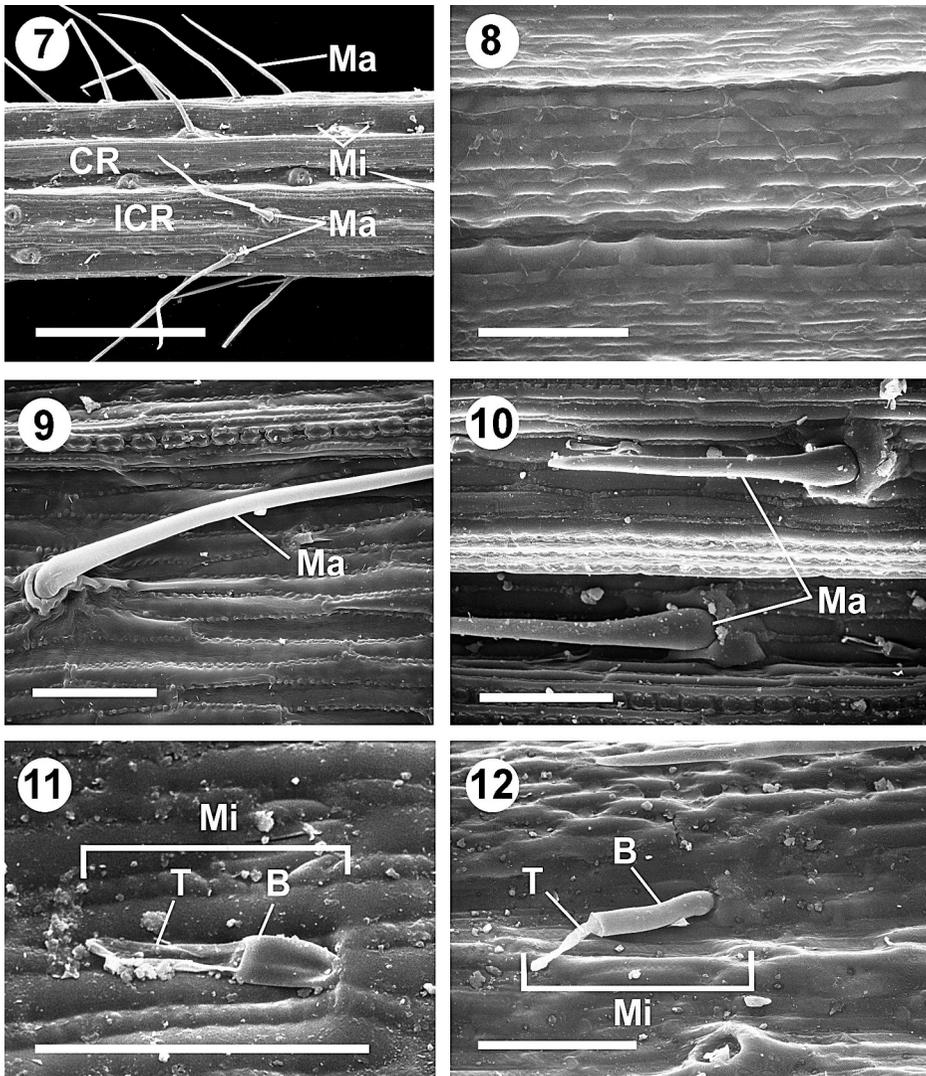
Microhairs. These structures, ranging from 0.06 to 0.1 mm, are sparsely distributed throughout the leaf epidermis, as in *Danthonia sericea* (Fig. 1) and *Austrodanthonia pilosa* (Fig. 7), and are present on the abaxial surface of most danthonioid grasses examined, except in *Cortaderia* (Fig. 8), *D. filifolia*, *D. parryi*, and *Rytidosperma violacea* (Table 1). Microhairs are typically bicellular, rod-shaped structures with a cylindrical basal cell and a thin-walled terminal cell as in *D. intermedia* (Fig. 4). Three types of microhairs occur in danthonioid grasses: 1) long basal cell relative to the terminal



FIGS. 1–6. Micrographs of hairs on the abaxial leaf epidermis of *Danthonia* species. 1. *D. sericea*: costal (CR) and intercostal regions (ICR), unicellular macrohairs (Ma), bicellular microhairs (Mi), and a prickly hair (P). 2. *D. intermedia*: unicellular macrohair (Ma) and prickly hair (P). 3. *D. unispicata*: unicellular macrohairs (Ma). 4. *D. intermedia*: bicellular microhairs (Mi) with a long basal (B) cell relative to the terminal (T) cell. 5. *D. decumbens*: bicellular microhairs (Mi) with terminal (T) cell and basal (B) cell approximately equal in length. 6. *D. californica*: prickly hairs (P). Scale bar: Fig. 1 = 0.5 mm; Figs. 2–6 = 0.1 mm.

cell in *D. californica*, *D. compressa*, *D. intermedia* (Fig. 4), and *Rytidosperma virescens* (Fig. 12); 2) basal and terminal cells approximately equal in length in *D. chilensis*, *D. decumbens* (Fig. 5), *D. sericea*, *D. spicata*, and *D. unispicata*; and 3) short basal cell relative to the terminal cell in *Austrodanthonia pilosa*, *Merxmuellera disticha*, *Notodanthonia semiannularis*, *Rytidosperma glabra*, *R. unarede* (Fig. 11), and *Tribolium hispidum*. The relative length of microhair cells is consistent within individuals of the same species, but intraspecific variation was not evaluated.

Prickle Hairs. Prickle hairs are sparsely distributed throughout the costal regions of the epidermis. They are very short, relatively stiff hairs with sharply pointed apices, as in *Danthonia californica* (Fig. 6). Prickle hairs are recorded in only four North American *Danthonia* species: *D. californica* (Fig. 6), *D. intermedia* (Fig. 2), *D. sericea* (Fig. 1), and *D. unispicata*. The occurrence of prickly hairs in *Danthonia* is a new finding. Prickle hairs vary in size on a single leaf blade, as in *D. californica* (Fig. 6) and are present in all specimens investigated of these four taxa. The remaining *Danthonia*

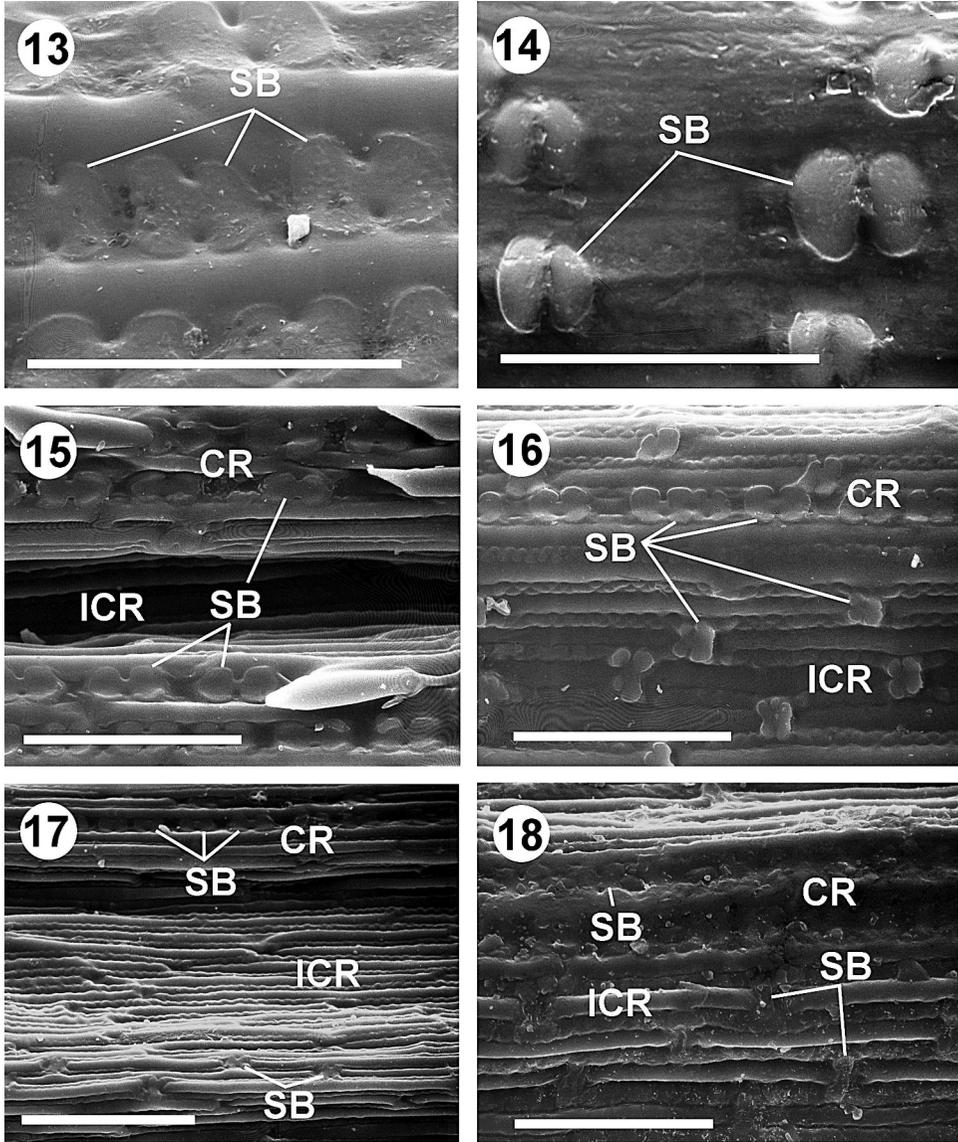


FIGS. 7–12. Micrographs of hairs on the abaxial leaf epidermis of selected danthonioid taxa. 7. *Austrodanthonia pilosa*: costal region (CR), intercostal region (ICR), unicellular macrohairs (Ma), and bicellular microhairs (Mi). 8. *Cortaderia hapalotricha*: lacking hairs. 9. *Tribolium hispidum*: unicellular macrohair (Ma). 10. *A. pilosa*: unicellular macrohairs (Ma). 11. *Rytidosperma unarede*: bicellular microhair (Mi) with short basal (B) cell relative to the terminal (T) cell. 12. *R. virescens*: bicellular microhair (Mi) with long basal (B) cell relative to the terminal (T) cell. Scale bar: Fig. 7 = 0.5 mm; Figs. 8–12 = 0.1 mm.

species and the South American and Old World danthonioid species examined lack these structures (Table 1).

Silica Bodies. Epidermal silica bodies in the leaf blades of danthonioid taxa are dumbbell-shaped, tall and narrow, or cross-shaped, and are distributed either on the costal and/or intercostal leaf regions. The costal silica bodies in the leaf epidermis of danthonioid grasses are dumbbell-shaped and arranged in rows of short cells (Figs. 13–20), as in *Danthonia californica* (Fig. 15), *D. sericea* (Fig. 16), *D. compressa* (Fig. 17), *D. parryi* (Fig. 18), *Tribolium hispidum* (Fig. 19), *Merxmüllera*

disticha (Fig. 20), and *Rytidosperma glabra* (Fig. 22). The intercostal silica bodies have more variability, ranging from cross-shaped to dumbbell-shaped to tall and narrow. Usually, silica bodies are lacking in the leaf intercostal regions of most species surveyed (e.g., Figs. 3, 5, 8, 10–12, 15, 19, 21; Table 1). Cross-shaped intercostal silica bodies are restricted to *D. intermedia*, *D. sericea* (Fig. 16), and *Notodanthonia semiannularis* (Table 1). Similarly, dumbbell-shaped intercostal silica bodies have a limited taxonomic distribution, observed only in *D. compressa* (Fig. 17) and *D. filifolia* (Table 1). Tall and narrow silica bodies are characteristic of

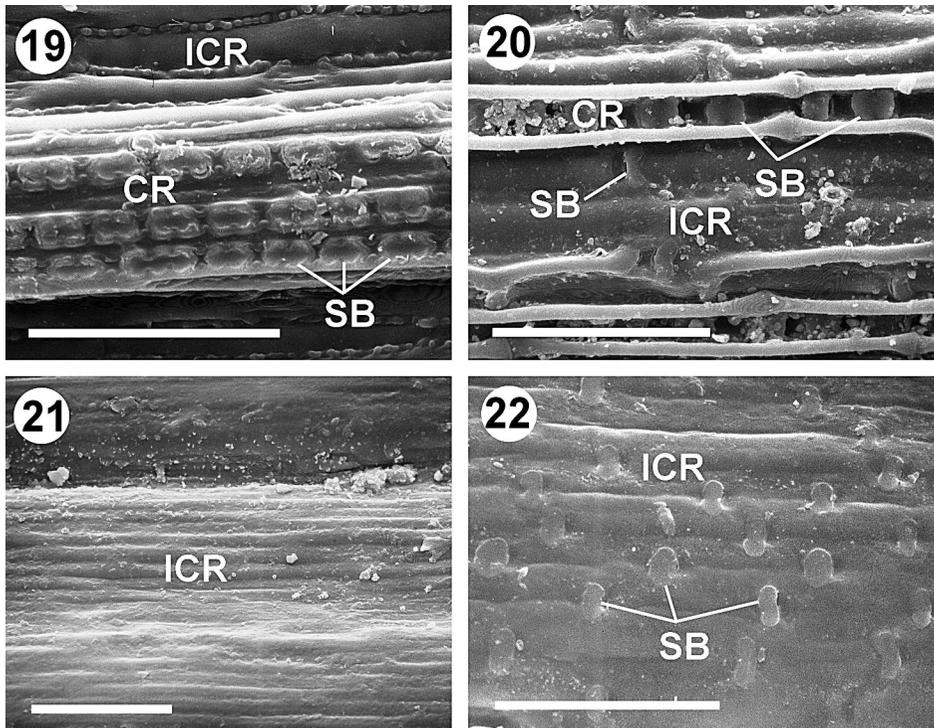


FIGS. 13–18. Micrographs of silica bodies on the abaxial leaf epidermis of *Danthonia* species. 13. *D. unispicata*: dumbbell-shaped silica bodies (SB) in costal region. 14. *D. filifolia*: dumbbell-shaped silica bodies (SB) in costal region. 15. *D. californica*: dumbbell-shaped silica bodies (SB) in costal region (CR) and no silica bodies in intercostal region (ICR). 16. *D. sericea*: dumbbell-shaped silica bodies (SB) in costal region (CR) and cross-shaped silica bodies (SB) in intercostal region (ICR). 17. *D. compressa*: dumbbell-shaped silica bodies (SB) in costal region (CR) and intercostal region (ICR). 18. *D. parryi*: dumbbell-shaped silica bodies (SB) in costal region (CR) and tall and narrow silica bodies (SB) in intercostal region (ICR). Scale bar: Figs. 13 and 14 = 0.05 mm; Figs. 15–18 = 0.1 mm.

the intercostal regions of *D. parryi* (Fig. 18), *Merxmüllera disticha* (Fig. 20), and *Rytidosperma glabra* (Table 1). Slight variations in size and shape of silica bodies were evident in the same leaf blade, as in *D. sericea* (Fig. 16). Due to the scope of this study, the degree of variability observed was not evaluated quantitatively.

Stomata. In the Poaceae, the stomata are paracytic with two differentiated subsidiary cells (Watson and Dallwitz 1992). The stomata have dome-

shaped subsidiary cells and occur sparsely on the abaxial leaf blade surface in the intercostal region between long cells. Stomata are not present on the abaxial surface of any of the *Danthonia* or *Cortaderia* specimens examined (Table 1). These structures are restricted to other danthonioid genera, such as *Austrodanthonia*, *Merxmüllera*, *Notodanthonia*, *Rytidosperma* (except *R. glabra*), and *Tribolium* (Table 1). Even when stomata are present on the abaxial surface, they are few, and no differences in size,



FIGS. 19–22. Micrographs of silica bodies on the abaxial leaf epidermis of selected danthonioid taxa outside the genus *Danthonia*. 19. *Tribolium hispidum*: dumbbell-shaped silica bodies (SB) in costal region (CR) and no silica bodies in intercostal region (ICR). 20. *Merxmuellera disticha*: dumbbell-shaped silica bodies (SB) in costal region (CR) and tall and narrow silica bodies in intercostal region (ICR). 21. *Cortaderia selloana*: lacking silica bodies in intercostal region (ICR). 22. *Rytidosperma glabra*: tall and narrow silica bodies in intercostal region (ICR). Scale bar = 0.1 mm.

arrangement, and shape of subsidiary cells were evident from the material examined.

DISCUSSION

Certain micromorphological features of the abaxial leaf blade epidermis in danthonioid grasses are of taxonomic value, especially at the generic level. This is particularly notable in *Danthonia*, but our inferences at the subfamily level may not be widely applicable due to limited taxonomic sampling. Also, a number of epidermal traits exhibit overlap among taxa limiting their systematic value, as discussed below.

Macrohairs. Macrohairs have been used to distinguish species in *Hordeum* L. (Cai et al. 2003) and subspecies of *Aristida stricta* Michaux (Kesler et al. 2003) and *Elymus glaucus* Buckley (Wilson et al. 2001). The presence/absence of macrohairs does not reflect taxonomic relationships within danthonioid taxa. Macrohairs are absent in *Cortaderia* and in a number of species within the other genera examined (Table 1).

Microhairs. Microhairs provide limited taxonomic information. *Danthonia* is characterized by bicellular microhairs with basal and terminal cells

of equal length or with a long basal cell relative to the terminal cell, except in *D. filifolia* and *D. parryi* (Table 1), in which these structures are absent. Other danthonioid taxa (e.g., *Cortaderia* species) lack bicellular microhairs, but the remaining danthonioid genera possess bicellular microhairs with a short basal cell, except *Rytidosperma virescens* (Table 1). Microhairs are also absent in *Merxmuellera* (Watson and Dallwitz 1992 onwards) but present in *M. disticha*. It is likely that this is a variable character within some species.

The long basal cell of the microhair in *Rytidosperma virescens* is shared with *Danthonia*, but this is probably due to homoplasy rather than an indication of a close relationship. Molecular data indicate that these genera do not share a recent common ancestor (Barker et al. 2000). This character may also be more variable than our observations suggest since terminal cells of the microhair are apparently equal in length to the basal cell in *Rytidosperma* and *Tribolium* (Watson and Dallwitz 1992 onwards). Our data for South American *Rytidosperma* species are inconsistent, making the interpretation complex due in part to the variability of the microhairs and the limited number of

species investigated. Likewise, various morphological characters used to distinguish *Rytidosperma* from *Danthonia* are homoplasious. For example, *Rytidosperma* has an oblong, punctate hilum of the caryopsis, while *Danthonia* has linear hilum, with the exception of the oblong hilum in *D. secundiflora* (Linder and Verboom 1996).

Wright's (1984) study using light microscopy indicated that bicellular microhairs in *Danthonia* have long basal cells relative to those in *Rytidosperma*. Our survey is partially in agreement: several *Danthonia* species have bicellular microhairs with long basal cells, but our data also indicate that the thin-walled terminal cell is intact in the microhairs, an observation not reported by Wright (1984). Our finding is more congruent with Palmer and Tucker's (1981) SEM examination of grass epidermis, which also found intact terminal cells in the microhairs. Wright likely had absent or damaged terminal cells in her samples. This discrepancy is reasonable due to the differences in preparation techniques between epidermal peels for light microscopy versus SEM examination of these delicate structures.

Prickle Hairs. Prickle hairs are restricted to a group of four North American *Danthonia* species (*D. californica*, *D. intermedia*, *D. sericea*, and *D. unispicata*) (Table 1). These North American species of *Danthonia* have lemmas with glabrous abaxial surfaces with the exception of *D. sericea*, in which the lemmas have hairs scattered across the abaxial surface (Darbyshire 2003). These two morphological characters support the relationship among *D. californica*, *D. intermedia*, and *D. unispicata*, as suggested by Darbyshire (2003), a premise also supported by their geographic distribution (sympatric in the western region of North America). Within *Danthonia*, the presence of prickles has taxonomic value for differentiating these four species.

Prickle hairs are also found in other genera in Danthonioidae, such as *Merxmuellera* (Barker and Ellis 1991), *Pentameris* (Barker 1993), and *Pseudopentameris* (Barker 1995), but the structures are located on the adaxial surface. The occurrence of intercostal prickle hairs on the abaxial leaf surface of *Cortaderia selloana* has been reported (Metcalfe 1960), but the three *Cortaderia* species that we examined lack prickle hairs. Wright (1984) also does not mention prickle hairs in *Cortaderia*. Due to our limited taxonomic sampling within *Cortaderia*, we suggest extensive SEM analysis over a wider taxonomic sampling.

Silica Bodies. The presence of silica bodies in some groups of monocots has been informative (Mejia-Saules and Bisby 2003; Prychid et al. 2003).

Also, specific types of silica bodies distinguish the bambusoid, panicoid, and pooid grasses (Piperno 1988). This survey (Table 1) suggests that silica body shape, although variable, is not of taxonomic significance in Danthonioidae. Although costal silica bodies in Danthonioidae are reportedly crenate, tall and narrow, cross-shaped, rounded, or dumbbell-shaped (Watson and Dallwitz 1992 onwards), we observed only dumbbell-shaped silica bodies in the costal region. This consistent pattern is not unique to this subfamily. It is also present in a few representatives of the Bambusoideae and is common in the subfamilies of the PACCAD clade (Watson and Dallwitz 1992 onwards). Though the shape of silica bodies in the epidermis of the grasses examined does not appear to be taxonomically informative, it is noteworthy that the occurrence of tall and narrow silica bodies between the long cells in the intercostal region is restricted to a few danthonioid representatives (*D. parryi*, *Merxmuellera disticha*, and *Rytidosperma glabra*). Watson and Dallwitz (1992 onwards) also report tall and narrow silica bodies in the intercostal regions of *Austrodanthonia*, *Cortaderia*, *Merxmuellera*, and *Tribolium*, as well as *Danthonia decumbens*. Crescentic silica bodies have been reported in *Rytidosperma* (Watson and Dallwitz 1992 onwards), a type not observed in this study. The systematic utility of the apparently enormous variability of silica bodies in the Danthonioidae remains ambiguous and deserves further investigation involving more species.

No major differences in silica body types and shapes were observed between North and South American *Danthonia* and Old World and South American danthonioids. Similarly, there is no significant correlation between the presence or absence of silica bodies and the geographic distribution and ecological requirements of danthonioid taxa. This lack of correlation contrasts with deWet (1956), who argued that danthonioid grasses could be grouped on the basis of microhairs and silica body shapes. Our study does not support this. It is also likely that the shape and frequency of silica bodies in the abaxial leaf epidermis is related to water conducting systems that absorb silica from the surrounding environment, as in *Oryza* L., in which the morphology of silica bodies is correlated with structural differences of veins transporting silicates in the leaf (Whang et al. 1998). Due to the apparent lack of taxonomic information of silica bodies, further research is required in the subfamily.

Abaxial Stomata. The abaxial leaf blade surfaces of danthonioid taxa tend to lack stomata,

except in *Austrodanthonia*, *Merxmuellera*, *Noto-danthonia*, *Rytidosperma unarede*, *R. virescens*, *R. violacea*, and *Tribolium* (Table 1). Watson and Dallwitz (1992 onwards) report that abaxial stomata are absent or very rare in *Cortaderia*, *Danthonia*, and *Merxmuellera*, while stomata are present in *Austrodanthonia*, *Rytidosperma*, and *Tribolium*. This pattern is somewhat consistent with our survey, except that we observed stomata in *Merxmuellera*. In addition, our study does not support the presence of stomata on the abaxial leaf blade surface of *Danthonia decumbens* and *D. sericea*, as indicated by Metcalfe (1960) and Gray et al. (1969), respectively. Stomata may have been present in the species but absent in the particular areas of the leaf blades, thus indicating that stomata position may vary. Ellis (1979) hypothesized that the frequency of stomata on the leaf blade is strongly correlated with environmental factors. Abaxial stomata are more common in species distributed in wetter, cooler environments, while stomata density decreases in severe water stress in the grass *Leymus chinensis* (Trin.) Tzvel. (Xu and Zhou 2005). Whether the absence of stomata in the Danthonioidae is related to environmental conditions is not known.

Our survey provides new information regarding the micromorphology of leaf epidermal structures in danthonioid grasses, but the systematic utility of epidermal attributes is limited within the subfamily. Overlap of character states makes taxonomic inferences difficult. None of the characters investigated provide clear synapomorphies at the subfamilial and generic levels, nor separate Old and New World danthonioid taxa.

Our findings provide a framework for re-evaluation of taxonomic and phylogenetic relationships in Danthonioidae, in particular North American *Danthonia*. Several diagnostic characters are distinctive in *Danthonia*, including 1) the absence of abaxial stomata, 2) the presence of bicellular microhairs with basal and terminal cells of equal length, 3) microhairs with long basal cells relative to terminal cells, and 4) prickle hairs in four North American species.

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tion, and information retrieval; including synonyms,
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cytology, classification, pathogens, world and local
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APPENDIX 1. Danthonioid taxa investigated in this study,
including geographic distribution and source of material.
ISC: Ada Hayden Herbarium; MO: Missouri Botanical
Garden; NY: New York Botanical Garden; PI: U.S. De-
partment of Agriculture Plant Introduction Center;
SASK: University of Saskatchewan; SI: Instituto de Botánica
Darwinion.

New World Taxa. *Cortaderia bifida* Pilg., *D. N. Smith* 4115
(MO 3400731) 19/05/1983. PERU: Pasco, Oxapampa. *M.*
Lewis 88645 (MO 3632045), 19/05/1988. BOLIVIA: La Paz,
Inquisivi; *C. haplotricha* (Pilg.) Conert, *J. A. Steyermark & E.*
Dunsterville 101094 (NY 721441), 19/01/1968. VENEZUELA:
Apure; *C. selloana* (Schult. & Schult. F.) Asch. & Graebn., *M.*
Nee 48310 (NY 472542), 15/02/1998. BOLIVIA: Santa Cruz;
Danthonia californica Bol., *J. G. Moore* 51 (MO 3140100) 10/05/
1969. USA. CA: Marin Co. *J. A. Steyermark* 4328 (MO
3179929), 14/07/1961. USA. WY: Grand Teton National Park.
J. P. Tracy 3650 (MO 793389), 9/06/1912. USA. CA:
Humboldt Co.; *D. chilensis* E. Desv., *O. Boelke* 329 (SI), 19/
12/1944. CHILE: Lago Rauco Río Calcurrupe; *D. compressa*
Austin, *J. A. Steyermark* 65742 (MO 1710981), 23/06/1948.
USA. TN: Bevier Co. *H. E. Ahles* 67393 (MO 3685617), 4/06/
1967. USA. MA: Franklin Co. *A. J. Eames & K. W. Weigand*
11330 (MO 870462), 13/07/1919. USA. NY: Tompkins Co.; *D.*
decumbens (L.) DC., *R. W. Pohl & G. Davides* 10813 (ISC
278020), 3/08/1968. COSTA RICA. Alajuela: Volcán Poas. *E.*
Leroy 5637 (SI), 18/06/1925. SPAIN; *D. filifolia* F. T. Hubb., *M.*
J. Metzler 34 (ISC 356119), 15/12/1978. GUATEMALA: El
Quiche; *D. intermedia* Vasey, *J. G. Moore & J. A. Steyermark*
3736 (MO 1010084), no date. USA. WY: Grand Teton National
Park. *P. H. Raven s/n* (MO 1734377), 11/07/1954. USA. CA:
Fresno Co. *A. J. Breitung* 4864 (MO 1577546), 15/07/1947.
CANADA. SK: Cypress Hills; *D. parryi* Scribn., *F. E. Clements*
& *S. E. Clements s/n* (ISC 23982), 18/07/1901. USA. CO:
Ruxton Dell. *R. G. H. Cormack* 102 (ISC 282513), 12/07/1955.
CANADA. AB: Crownst Forest Reserve; *D. sericea* Nutt., *A.*
E. Radford 33480 (ISC 231221), 4/06/1958. USA. NC: Stokes
Co., 1.5 mi. N. of Belews Creek. *G. L. Pyrah* 260 (ISC 250186),
30/05/1967. USA. VA: Prince George Co. *D. Isely*, *S. L. Welsh*
& *D. Isely* 10510 (ISC 262071), 17/05/1967. USA. MS: Kemper
Co.; *D. spicata* (L.) P. Beauv. ex Roem. & Schult., *A. E. Radford*
44755 (ISC 254805), 13/06/1966. USA. NC: Durham Co. *W.*
W. Overholt 14 (ISC 324402), 24/06/1972. USA. NC: Trans-
ylvania Co.; *D. unispicata* (Thurb.) Munro ex Vasey, *H. T.*

Rogers 496 (MO 1259856), 21/05/1940. USA. WA: Lincoln Co. C. L. Porter 5446 (MO 1751098), 12/07/1950. USA. CA: Park Co. J. T. Howell 30382 (MO 1971209), 13/06/1955. USA. CA: Trinity Co.; *Rytidosperma glabra* (Phil.) Nicora, W. J. Eyerdam 10224 (NY 721442), 16/01/1958. CHILE: Malleco; *R. violacea* (E. Desv.) Nicora, Troiani & Steibel 15830 (SI), 14/01/2004. ARGENTINA. Neuquen: Departamento de Ñorquín, Copahue; *R. virescens* (E. Desv.) Nicora var. *virescens*, Ward s/n (MO 04979480), 22/01/1964. ARGENTINA: Río Negro. Ward s/n (MO 3937897), 23/01/1964. ARGENTINA: Río Negro.

Old World Taxa. *Austroanthonia pilosa* (R. Br.) H. P. Linder, R. W. Pohl 7201 (ISC 210942), 11/09/1952. USA. CA: Alameda Co. Introduced from Australia. J. T. Howell 35482 (ISC 311043), 29/06/1960. USA. CA: Santa Clara Co. Introduced from Australia; *Merxmüllera disticha* (Nees.) Con-

ert, A. Oakes 1377 (PI 364332), 15/06/1971. LESOTHO: Cultivated in University of Saskatchewan garden plot (SASK 168165), 29/09/2004; *Notodanthonia semiannularis* (Labill.) Zotov, W. Hartley s/n (PI 210172), 16/09/1953. AUSTRALIA: Original material collected from Capital Terr. Cultivated in University of Saskatchewan greenhouse (SASK 168160), 22/04/2004; *R. unarede* (Raoul) Connor & Edgar, *Dept. of Scientific and Industrial Res.* (PI 237160), 2/04/1957. NEW ZEALAND: Original material collected from Christchurch. Cultivated in University of Saskatchewan garden plot (SASK 168157), 29/09/2004; *Tribolium hispidum* (Thunb.) Desv., Anon. (PI 368889) 20/05/1909. SOUTH AFRICA: Original material collected from Langgewens Experimental Farm, north of Malmesbury. Cultivated in University of Saskatchewan greenhouse (SASK 168158), 2/09/2004.