A PHYLOGENETIC STUDY OF *DANTHONIA* DC. (POACEAE) IN NORTH AMERICA

A thesis submitted to the

College of Graduate Studies and Research

in partial fulfillment of the requirements

for the degree of Master of Science

in the Department of Biology,

University of Saskatchewan

Saskatoon, Saskatchewan, Canada

By

Elizabeth Reimer

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department of the Dean of the College in which my thesis work was done. It is understood that any copying, publication, or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make use of other material in this thesis in whole or in part should be addressed to:

Head of the Department of Biology
University of Saskatchewan
112 Science Place
Saskatoon, Saskatchewan
S7N 5E2

ABSTRACT

The Danthonioideae (Poaceae) has been the focus of several systematic studies. Previous studies have shown the subfamily is monophyletic, but relationships within several groups, including *Danthonia*, have not been examined in depth. To address the question of the monophyly in North American representatives of *Danthonia*, this study utilized a holistic approach, examining both molecular and morphological features. A molecular phylogeny was constructed based on the *trn*L-F region of the chloroplast genome, and micromorphological characters of the leaf epidermis, caryopsis, and lodicules were examined by scanning electron microscopy.

This study involved a phylogenetic examination of the subfamily Danthonioideae, with emphasis on North American *Danthonia*. The objectives were twofold: 1) to determine whether representative species of *Danthonia* in North America form a monophyletic assemblage based on molecular and morphological characters, and 2) to examine relationships of the North American *Danthonia* species to putative related species in the Southern Hemisphere and Europe. The survey included the genera *Austrodanthonia*, *Cortaderia*, *Danthonia*, *Merxmuellera*, *Notodanthonia*, *Rytidosperma*, *Tribolium*, and eight North American *Danthonia* species. South American representatives of *Danthonia* were included for comparative purposes.

Several micromorphological epidermal features for danthonioid grasses were examined, including macrohairs, bicellular microhairs, prickle hairs, silica bodies, and stomatal complexes. Macrohairs are large, unicellular basifixed structures. Bicellular microhairs are of three types: 1) microhairs with a long basal cell relative to the terminal cell, 2) microhairs with basal and terminal cells approximately equal in length, and 3) microhairs with a short basal cell relative to the terminal cell. Prickle hairs in the costal regions of the leaf epidermis of four species of North American *Danthonia* are reported for the first time. Epidermal silica bodies in danthonioid grasses are dumbbell-shaped, tall and narrow, or cross-shaped. The stomatal complexes are paracytic with two dome-shaped subsidiary cells. No distinguishing characters were found at the subfamily level. *Danthonia* is characterized by the absence of abaxial stomata, presence of bicellular microhairs with basal and terminal cells of equal length, as well as microhairs with long

basal cells relative to terminal cells. These findings provide a new framework useful for interpretating and re-evaluating taxonomic and phylogenetic relationships in the Danthonioideae.

Caryopsis features were useful in reconstructing the phylogeny of the Danthonioideae. Three features associated with the caryopsis, hilum, and surface pattern of *Danthonia* included: 1) ovoid to obovoid caryopsis shape, 2) linear hilum, and 3) undulating or straight reticulate surface pattern. No other taxon examined in this study possesses this combination of characters. Secondly, *Rytidosperma* is characterized by 1) ovoid caryopses that are generally smaller than the caryopses in *Danthonia*, 2) punctate hila, and 3) undulating reticulate or substriate caryopsis surface patterns. Finally, *Tribolium* has 1) small obovoid caryopses ≤1.2 mm in length, 2) punctate hila, and 3) a substriate caryopsis surface pattern. Though *Cortaderia* shares the linear hilum and undulating reticulate surface pattern with *Danthonia*, the lanceolate caryopsis differs from the ovoid to obovoid caryopsis of *Danthonia*.

The subfamily Danthonioideae is monophyletic. Within this subfamily two monophyletic clades, i.e. *Danthonia* and *Rytidosperma*, were identified. *Cortaderia selloana* is basal to the aforementioned clades. Although the *Danthonia* clade is monophyletic, this study does not support the separation of North and South American and European species, and there is poor resolution of terminal branches within the clade. Interspecific relationships within *Danthonia* are not clear, but evidence suggests the taxonomic separation of *Danthonia* from *Rytidosperma*, two genera that were previously considered to be closely related. The *Rytidosperma* clade is composed of *Austrodanthonia*, *Notodanthonia*, *Rytidosperma*, *Tribolium*, and *Merxmuellera*. Though taxonomic sampling of South American *Rytidosperma* only included one species, the *trn*L-F strict consensus tree shows strong support for its inclusion in the *Rytidosperma* clade, demonstrating that South American representatives of *Rytidosperma* are distinct from *Danthonia*. Within the *Rytidosperma* clade, the genus *Rytidosperma* may be paraphyletic.

ACKNOWLEDGEMENTS

This project would not have been possible without the assistance of many organizations and individuals. Primarily, I extend my heartfelt appreciation to my supervisor, Dr. Hugo Cota-Sánchez, who has provided unlimited guidance and inspiration. I also thank my committee members, Dr. Arthur Davis and Dr. Vernon Harms for their many contributions.

I also appreciate the individuals and institutions who provided material for this study, including: Dr. Liliana Giussani, Dr. Carlos Marcelo Baeza, U.S. Department of Agriculture Plant Introduction Center, Ada Hayden Herbarium, and Herbaria at Missouri Botanical Garden, New York Botanical Garden, Institito de Botánica Darwinion, Universidad de Concepción, and Universidad Nacional Autónoma de México. Thanks also to the staff of the University of Saskatchewan Herbarium for housing and processing loans, and providing general aid. Dewey Litwiller and Dr. Peter Linder provided helpful comments on a manuscript based on my thesis work. I am grateful for assistance from members of the Biology Department, especially Jeaniene Smith, Dennis Dyck, and Xiaochu Wu. I extend a special thanks to Kumary Ubayasena and other members of the Plant Systematics lab (past and present) for advice, encouragement, friendship, and the occasional shared meal.

Financial assistance was provided by a Graduate Teaching Fellowship from the Department of Biology and a Graduate Student Fellowship from the College of Biotechnology at the University of Saskatchewan. The Plant Systematics Laboratory is supported financially by operating funds from the University of Saskatchewan and the National Geographic Society, which were instrumental to this project and ongoing research in our laboratory.

Finally, I am indebted to friends and family too numerous to mention for their continued kindness and understanding. I am especially thankful for the love and encouragement of my parents, William and Susan Reimer.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ACCTRAN: Accelerated transformation.

AT: Adenine and thymine.

atp: Chloroplast gene coding for adenosine triphosphate synthase.

BEP: Bambusoideae, Ehrhartoideae, Pooideae

BMA: Basal *Merxmuellera* Assemblage.

BOP: Bambusoideae, Oryzoideae, Pooideae

bp: Base pair.

CI: Consistency index.

CONC: Herbarium of the Universidad de Concepción.

cpDNA: Chloroplast DNA.

CTAB: 2X hexacetyl trimethylammonium bromide

DELTRAN: Delayed transformation.

dNTPs: Deoxynucleotide triphosphates.

GC: Guanine and cytosine.

GPWG: Grass Phylogeny Working Group

IR: Inverted repeat.

ISC: Ada Hayden Herbarium.

ITS: Internal transcribed spacer.

kb: Kilobase pair.

LSC: Large single copy region of the chloroplast genome.

mat: Chloroplast gene coding for a maturase.

MEXU: Herbarium of the Universidad Nacional Autónoma de México.

MO: Missouri Botanical Garden Herbarium.

Mya: Million years ago.

ndh: Chloroplast gene coding for NADH dehydrogenase.

NY: New York Botanical Garden Herbarium.

OG: Outgroup.

PACC: Panicoideae, Arundinoideae, Centothecoideae, Chloridoideae

PACCAD: Panicoideae, Arundinoideae, Centothecoideae, Chloridoideae, Aristidoideae, Danthonoideae.

PAUP: Phylogenetic Analysis Using Parsimony software program.

PCR: Polymerase chain reaction.

PI: U.S. Department of Agriculture Plant Introduction Center.

psb: Chloroplast gene coding for Photosystem II proteins.

rbc: Chloroplast gene coding for of ribulose 1,5-bisphosphate carboxylase-oxygenase (Rubisco).

RI: Retention index.

rpo: Chloroplast gene coding for RNA polymerase.

SASK: University of Saskatchewan Herbarium.

SEM: Scanning electron microscope or scanning electron microscopy.

SI: Herbarium of the Institito de Botánica Darwinion.

s.l.: Sensu lato.

s.s.: Sensu stricto.

SSC: Small single copy region of the chloroplast genome.

TBR: Tree-bisection reconnection.

TE: tris ethylene diaminetetra-acetic acid EDTA.

trn: Chloroplast gene coding for transfer RNA.

USDA: United States Department of Agriculture.

1.0 INTRODUCTION

The classification and phylogeny of the Poaceae have been the focus of numerous studies, because the family is economically important and includes many crops, such as wheat, corn and rice. The grass family includes over 10,000 species in 600 to 700 genera (Watson and Dallwitz 1992; Kellogg 2001). From an ecological perspective, grasses are the dominant component of grassland biomes, which cover roughly 20% of the Earth's surface (Kellogg 2001). Owing in part to its ecological and economic importance, the evolution and phylogeny of the Poaceae has been widely studied.

The grass family has been considered a natural group, and is characterized by glumes, paleas, lemmas, and caryopses, and several molecular synapomorphies, including characteristic structural rearrangements of the chloroplast genome [Grass Phylogeny Working Group (GPWG) 2001]. The Poaceae is currently divided into 12 subfamilies (GPWG 2001). Three form the most basal lineages, namely the Anomochloiodeae, Pharoideae, and Pueloideae. Three other subfamilies form the BEP clade (Bambusoideae, Ehrhartoideae, and Pooideae) (GPWG 2001). The six remaining subfamilies are circumscribed within the PACCAD clade (Panicoideae, Aristidoideae, Chloroideae sensu lato (s.l.), Centothecoideae, Arundinoideae sensu stricto (s.s.), and Danthonioideae). The phylogenetic position of *Danthonia* DC., the subject of this study, is within the Danthonieae, the only tribe of the subfamily Danthonioideae (GPWG 2001).

Danthonia sensu deWet (1954) is a morphologically variable with over 100 species. The genus has subsequently undergone extensive taxonomic rearrangement. After revisions by various authors, *Danthonia* as currently described is a genus consisting of 23 species distributed in North America (8), South America (9), Europe (3), northeastern Africa (2), and Asia (2) (Linder and Verboom 1996).

The genus *Danthonia* has not been adequately studied to test its monophyly conclusively. Nonetheless, it has been suggested that *Danthonia* forms a monophyletic assemblage based on the presence of scattered lemma hairs and bulliform cells, two putatively primitive features shared with *Cortaderia* Stapf. (Wright 1984). Cleistogenes (autogamous florets) in the lower leaf sheaths and a base chromosome number of x=18 are also synapomorphies for *Danthonia* (Linder and Verboom 1996). Some exceptions to these findings have been documented. For example, cleistogenes are rarely seen in *D. intermedia* (Darbyshire 2003), and an unusual chromosome count of 2n=31 has been reported in *D. spicata* (Darbyshire and Cayouette 1989). Even though the systematics of *Danthonia* have been addressed in previous studies, no clear picture has emerged regarding its phylogenetic and evolutionary history. To address the issues identified, this study combines morphological and molecular techniques to answer two fundamental questions, namely the monophyly of *Danthonia*, with emphasis on the North American species, and the phylogenetic relationships of the genus with other members of the subfamily.

1.1 Research Objectives

Several issues have been identified in the Danthonioideae, including the lack of morphological synapomorpies, and the unresolved position of North American representatives of *Danthonia*. To that end, this study incorporated molecular and morphological data to elucidate relationships within the Danthonioideae with two primary objectives:

1. To determine whether the representative species of *Danthonia* in North America form a monophyletic assemblage based on molecular and morphological characters.
2. To examine the relationships of North American *Danthonia* species to putative related species in the Southern Hemisphere and Europe.

1.2 Importance of this Study

This study provides new information on the morphology of the lodicules, caryopsis, and leaf epidermis of members of the Danthonioideae. Furthermore, through a holistic approach using both molecular and morphological data, this study examines the phylogenetic relationships within the subfamily with emphasis on North American

representatives of the genus *Danthonia*. The monophyly of the genus has never been tested, thus this study provides relevant information on this group for which taxonomy has been problematic.

2.0 LITERATURE REVIEW

2.1 Phylogeny and Classification of the Poaceae

The Poaceae is classified within the Order Poales, which includes the Poaceae, Flagellariaceae, Joinvilleaceae, Restionaceae, Centrolepidaceae, Anarthriaceae, and Ecdeiocoleaceae (Michelangeli et al. 2003). Within the Poales, the Ecdeiocoleaceae is sister to the Poaceae. These two families share several features, namely a 6 kilobase (kb) inversion in the chloroplast genome, and operculate, annulate pollen without scrobiculi (Michelangeli et al. 2003). The Joinvilleaceae is closely related to the two aforementioned families and has been considered a sister of the Poaceae (GPWG 2001). The Joinvilleaceae, Ecdeiocoleaceae, and Poaceae share a 28 kb inversion in the chloroplast genome, the differentiation of long and short cells on the leaf epidermis, and the presence of a ligule (Michelangeli et al. 2003). The 28 kb chloroplast inversion is polymorphic in the Restionaceae, which is sister to the Centrolepidaceae and Anarthriaceae. The Centrolepidaceae and Anarthriaceae lack the inversion, suggesting the character was reversed in these families, or it may have evolved independently in some members of the Restionaceae. Alternatively, the Restionaceae may not be monophyletic, and the phylogenetic position of the taxa with the 28 kb inversion may warrant reexamination (Michelangeli et al. 2003).

The Poaceae is a strongly-supported monophyletic group, characterized by several unambiguous morphological and molecular synapomorphies (GPWG 2001). The coleoptile, coleorhiza, and epiblast are unique characters of the grass embryo (Campbell and Kellogg 1987; Rudall et al. 2005). Within the Poales, the laterally positioned embryo is a synapomorphy for the family (GPWG 2001). Several structural rearrangements of the chloroplast genome, namely the RNA polymerase beta″ subunit (*rpo*C2) insert (GPWG 2001) and the transfer RNA Threonine (*trn*T) inversion (GPWG 2001; Michelangeli et al. 2003; Hilu 2004), are characteristic of the Poaceae.

Early attempts at intrafamilial classification systems divided the Poaceae (then the Gramineae) into two subfamilies: the Paniceae, and a large and heterogeneous Poaceae (Kellogg 1998). This division was based largely on gross morphological features of the inflorescence, and is now known to be an artificial arrangement that does not reflect the evolutionary history of the grass family. Examination of cytological, micromorphological, physiological, and anatomical characters led to a more natural system of classification (Hilu and Wright 1982). Molecular data have also helped to clarify relationships within the grass family (Davis and Soreng 1993; Soreng and Davis 1998; Mathews et al. 2000).

The most current description of subfamilies within the Poaceae was proposed in 2001 (GPWG 2001), based on morphological, anatomical, cytological, and biochemical data, plus nuclear and chloroplast DNA sequence data, and restriction site analysis. The basal lineages comprise three subfamilies: the Pueloideae, Pharoideae, and Anomochloideae. The Bambusoideae, Ehrhartoideae, and Pooideae comprise the BEP clade. This group is equivalent to the Bambusoideae, Oryzoideae, plus Pooideae (BOP clade) of the GPWG (2000) treatment, but the subfamilial name of the Ehrhartoideae is given priority over the Oryzoideae (GPWG 2001).

The remaining subfamilies (Panicoideae, Arundinoideae, Chloridoideae, Centothecoideae, Aristidoideae, and Danthonioideae) form the PACCAD clade (GPWG 2001). The PACCAD clade is equivalent to the Panicoideae, Arundinoideae, Centothecoideae, and Chloridoideae (PACC clade) of Davis and Soreng (1993), in which the Arundinoideae included the Danthonioideae and Aristidoideae. This arrangement is polyphyletic, thus the Danthonioideae and Aristidoideae were given subfamilial recognition (GPWG 2001). Support for the monophyly of the subfamilies within the PACCAD clade is strong, with the exception of the Centothecoideae; however, the relationship between the Panicoideae and Centothecoideae is strongly supported (GPWG 2001).

2.2 Origin and Evolution of the Poaceae

The date of the origin of grasses is controversial. Fossil evidence suggests that the Poaceae likely originated in tropical areas during the Late Cretaceous period, 70-55 million years ago (mya) (Kellogg 2001). Recent studies contradict this date of origin of

the Poaceae. A molecular clock estimates that grasses diverged approximately 83 mya (Janssen and Bremer 2004), while recently-discovered silica bodies of grasses in fossilized dinosaur dung suggest that the BEP and PACCAD clades diverged before 80 mya, much earlier than previously thought (Prasad et al. 2005).

It is believed that grasses originated at the tropical forest margin, from which the bamboos radiated into forest habitats, while other grasses adapted to open habitats (Renvoize and Clayton 1992). A biogeographical study of the Poales suggests the ancestral area of the grass family included South America, and possibly regions of Africa or Australia (Bremer 2002). Based on the present geographic distribution of the basal lineages of the Poaceae, grasses may have arisen in Gondwanaland (Clark et al. 1995), and they may have established their current range through long-distance dispersal across the Indian and Atlantic Oceans, or by vicariance through the breakup of the Gondwanan continent (GPWG 2001). Grasses were not likely abundant in the Northern Hemisphere until the Oligocene or Miocene era (Stebbins 1987), and grass-dominated ecosystems appeared in the mid-Miocene (GPWG 2001). Radiation into dry habitats, facilitated by the evolution of drought tolerance, occurred millions of years after the origin of the Poaceae, and is thought to be one of the contributing factors to the abundance and diversity of grasses worldwide (Kellogg 2001).

The earliest grasses are thought to have been herbaceous, rhizomatous, broad-leaved, wind-pollinated, and possessed six anthers and three stigmas (Clark et al. 1995). The caryopsis was present in the earliest grass lineages, but the spikelet evolved in a series of steps after the origin of grasses (Kellogg 2001). Early grasses had bracteate inflorescences, but lacked a true palea and lemma, which evolved some time before the divergence of the Pharoideae (GPWG 2001).

Evolutionary patterns in the Poaceae are not always obvious. The difficulty arises partly from lack of availability of appropriate outgroups. Comparing the morphology of the Poaceae to sister groups is often difficult because grass structures are so highly derived that homology is difficult to assess. For example, it is unclear whether the caryopsis of the grasses is homologous to the dry, indehiscent fruit of *Ecdeiocolea* F. Muell. (Rudall et al. 2005). Grasses show very complex patterns of evolution;

hybridization, polyploidy, and reticulation have all influenced the evolution of the Poaceae.

2.3 Morphological Studies of the Poaceae

Grasses have several distinctive morphological features. They are the only plant group with paleas, lemmas, glumes, and a caryopsis as a fruit type (GPWG 2001). Even though nearly all grasses share these features, remarkable diversity of forms can be observed in virtually every character. For example, grasses range in size from woody bamboos, such as *Dendrocalamus sinicus* Chia et J.L. Sun that can attain a height of 30 m (Clayton et al. 2002 onwards), to Arctic grasses such as *Phippsia algida* (Sol.) R. Br., which is only 2 to 15 cm high at maturity (Cody 2000). In addition to a vast range of variability in stature, grasses have adapted to a wide range of environmental conditions from obligate aquatics to desert species, which has also led to morphological variability. To add even more complexity, many morphological characters have evolved from multiple independent origins within the grass family, and this homoplasy causes difficulty in reconstructing phylogenetic relationships in the Poaceae (Stebbins and Crampton 1959). Nonetheless, phenetic studies of the Poaceae, based on large morphological data sets, have provided the basis for grass taxonomy (e.g. Hilu and Wright 1982; Watson and Dallwitz 1992).

Although more modern molecular techniques have been developed to infer phylogeny, morphological characters continue to play an important role in studies of the Poaceae. Examples of recent studies that incorporate morphological data abound. For instance, a recent analysis of American species of *Tripogon* Roem. & Schult. was based on leaf anatomy and micromorphological features of spikelets (Rúgolo de Agrasar and Vega 2004). Similarly, morphological studies of the Chloridoideae have categorized the genera within this subfamily based on whether the inflorescence is panicate, digitate, racemose, or spicate (Liu et al. 2005a), as well as caryopsis features such as the ventral face, hilum, surface sculpture, and stylopodium type (Liu et al. 2005b). A phylogenetic study of *Psathyrostachys* Nevski combined molecular data with information on culm pubescence, spikelet length, length of the lemma awn, and leaf epidermal cell wall width to elucidate relationships within this genus (Petersen et al. 2004).

Inflorescences in the Poaceae are morphologically diverse, ranging from spikes to many-branched panicles (Doust and Kellogg 2002). Inflorescences vary in the number of branches, the number of orders of branching, and the degree of elongation of axes (Doust et al. 2005). Differences in inflorescence architecture relate to axis ramification, differentiation of inflorescence primordia, and axis elongation (Doust and Kellogg 2002). Based on branching patterns, inflorescences may be termed spikes, racemes, or panicles, but some inflorescence types have arisen independently in multiple lineages (Doust and Kellogg 2002). Despite homoplasy being evident at the familial level, patterns of inflorescence branching are used in traditional grass classifications because these characters are easily observed (Doust et al. 2005).

The majority of grasses have reproductive structures arranged into florets, which are organized within a spikelet structure. The arrangement of florets into spikelets that are subtended by sterile, bract-like glumes is characteristic of the Spikelet Clade, which includes all of the grasses except for the Anomochlooideae (GPWG 2001). Each floret typically possesses a lemma, palea, lodicules, androecium, and gynoecium (GPWG 2001). Lemmas are the sheath-like bracts lowest on the floret axis (Soreng and Davis 1998). Paleas are considered prophylls, and are inserted above lemmas (GPWG 2001). Micromorphological traits of the palea and lemma have been useful in recent phylogenetic studies of *Bromus* L. (Acedo and Llamas 2001), and *Melica* L. (Mejia-Saules and Bisby 2003).

Lodicules are considered homologous to petals in eudicots, and function to open florets for fertilization (Bommert et al. 2005). Grass florets typically possess two lodicules, but a third lodicule, when present, is inserted higher on the floral axis than the two anterior lodicules (Clifford 1987). The number, shape, and vascularization of lodicules have traditionally been useful for grass classification (Stebbins and Crampton 1959). Vascularization of lodicules is reduced or lost in the Pooideae and PACC clade of Soreng and Davis (1998). Lodicule morphology may be informative in phylogenetic studies, as Hsu (1965) indicated for *Panicum* L. Similarly, *Melica* and *Glyceria* R.Br. share characteristic fusion of the anterior pair of lodicules (GPWG 2001).

Early lineages of grasses have six stamens, therefore this is considered the plesiomorphic state in grasses. Thus the inner whorl of stamens was lost before the

divergence of the PACC clade (equivalent to the PACCAD clade) and the Pooideae (Soreng and Davis 1998), resulting in a reduction to three stamens in most grasses. Reversal to six stamens has occurred in at least three independent events in the Bambusoideae and Ehrhartoideae (GPWG 2001).

The caryopsis is a unique fruit type, and it is a distinguishing feature of the Poaceae (GPWG 2001). The caryopsis is similar to an achene in that both are dry indehiscent fruits; however, the pericarp is adnate to the seed coat in the caryopsis. Even though the pericarp is free in some grasses, these fruits are not considered achenes, but rather the free pericarp represents a modification of the caryopsis (Brandenburg 2003). Other examples of variability in the pericarp (caryopsis surface) in grasses include differences in textural patterns, including reticulate, verrucate, striate, substriate, tuberculate, regulate, echinate, psilate, lophate, and foveolate (Jordan et al. 1983). Variability of surface texture of the caryopsis is also taxonomically informative at specific and generic levels when examined with scanning electron microscopy (SEM) (Sendulsky et al. 1987). Some grass genera are clearly distinguished because all the species share a common caryopsis surface pattern type, such as *Digitaria* Haller, which is characterized by a verrucate caryopsis surface (Jordan et al. 1983). Additionally, caryopsis characters such as length and width of epicarp cells, the degree of concavity of the periclinal walls, and the shape of cell wall undulations, are useful to differentiate among European species of *Echinochloa* Beauv. (Costea and Tardif 2002), and species of *Eragrostis* N.M. Wolf in Australia (Lazarides 1997). Within the Chloridoideae, caryopsis traits, such as the ventral face and hilum morphology are useful at the tribal and generic levels (Liu et al. 2005b). Similarly, caryopsis size, shape, beak, and degree of pericarp fusion are useful in differentiating North and South American species of *Diarrhena* P. Beauv. (Brandenburg et al. 1991).

The structure of transverse cells of the pericarp is useful for identifying fossilized caryopses of *Triticum* L. and *Secale* L. (Körber-Grohne 1981). Furthermore, SEM examination of surface patterns on fossilized caryopsis remnants has been important in determining the evolution and origin of cultivation in crops such as *Eleusine coracana* (L.) Gaertn. subsp. *coracana* (finger millet) (Hilu et al. 1979). Thus, ample evidence indicates that the surface pattern of the caryopsis and its associated microstructure are

informative. In addition, caryopsis characters may represent an important link to the fossil record for a better understanding of the conditions under which cereals were domesticated.

Other characters of the gynoecium are also taxonomically informative at the subfamily level in the Poaceae, including number and shape of stigma branches, embryo length relative to the length of the caryopsis, and the shape of the hilum (Stebbins and Crampton 1959). Plumose stigmas arose after the divergence of the Phareae and are presumed to have evolved in concert with wind pollination in the Poaceae (Soreng and Davis 1998). The loss of stigmatic branching is seen in many diverse lineages, and it is not necessarily indicative of phylogenetic relationships; however, secondary stigmatic branching is a synapomorphy for the Meliceae (Soreng and Davis 1998). Embryos tend to be longer relative to the caryopsis length in pooids, versus smaller embryos in panicoid grasses (Sendulsky et al. 1987). The linear hilum is plesiomorphic, and is characteristic of all basal lineages (GPWG 2001). Short hila are characteristic of the PACC clade, with reversals to the long, linear state in some taxa in the Arundinoideae s.l. (Soreng and Davis 1998).

Reeder (1959) identified six groups of grasses based on embryonic characters: 1) true festucoids, 2) true panicoids, 3) chloridoid-eragrostidoids, 4) bambusoids, 5) oryzoid-olyroids, and 6) arundinoid-danthonioids. Informative embryonic characters include the divergence of the scutellar vascular traces, the presence or absence of an epiblast, the fusion of the scutellum to the coleorhiza, and whether the margins of the embryonic leaf meet or overlap. The PACCAD clade is characterized by the presence of an elongated internode between the scutellum and the embryonic leaf (GPWG 2001), which corresponds to the panicoid-type divergence of the scutellar vascular system described by Reeder (1959). The epiblast is commonly present in representatives of the Pharoideae, Bambusoideae, the Ehrhartoideae, Pooideae, Centothecoideae, and Chloridoideae. Loss of the epiblast has been suggested as a synapomorphy for the PACC clade [equivalent to the PACCAD clade of the GPWG (2001)], which implies the presence of an epiblast in some members of the Chloridoideae and Centothecoideae is a reversal (Soreng and Davis 1998).

Micromorphological characters of the leaf epidermis have been taxonomically informative throughout the plant kingdom, and are especially important for comparing extant taxa to relatives in the fossil record (Stace 1984). Morphological and anatomical descriptions of grass leaf blades were compiled by Metcalfe (1960), including detailed descriptions of the leaf epidermis across a broad, though incomplete, taxonomic sampling. Several features of the leaf epidermis, including:intercostal long and short cells, stomatal cell type and shape, type of papillae, prickle hairs, macro- and microhairs, and silica bodies are taxonomically informative in grasses (Metcalfe 1960; Ellis 1979).

The leaf blade epidermis in the Poaceae is divided into the costal and intercostal zones (Metcalfe 1960). The zones are more obvious in species that have well-developed sclerenchyma strands associated with the vascular bundles just below the epidermis (Ellis 1979). The leaf epidermis is composed of long cells and short cells, named for their degree of elongation (Metcalfe 1960; Ellis 1979).

Generally, long cells comprise more surface area of the leaf blade (Ellis 1979). Cell walls of long cells vary in their degree of undulation and pitting (Metcalfe 1960). Anticlinal walls of long cells may be parallel, forming rectangular cells, angled outward to form hexagonal cells, or bowed outward to form inflated cells (Ellis 1979). Long cells show a high degree of phenotypic and developmental variation, thus taxonomic significance of these characters must be inferred with caution (Ellis 1979).

Short cells occur in rows in the costal region, and in pairs or individually between long cells in the intercostal region. Prat (1948, p. 342) described short cells as "differentiated elements," and described four categories: 1) silica cells, 2) exodermic elements (i.e. hairs and prickles), 3) cork cells, and 4) stomata. Silica cells in the Poaceae are specialized cells in the leaf epidermis that accumulate silica in a crystalline form, such that a silica body, a type of phytolith, forms and occupies most of the lumen (Piperno and Pearsall 1998). Other plants may also accumulate silica. In the Commelinidae, silica deposits in the leaf are hypothesized to serve as a protection against fungal infection (Prychid et al. 2003).

The significance and implications of silica bodies in taxonomy of grasses have been addressed. Certain shapes of silica bodies are characteristic of grass subfamilies, e.g. dumbbell-shaped in panicoid grasses, saddle-shaped in most pooid grasses, and

vertically oriented silica bodies in the Bambusoideae (Piperno 1988). Distribution and shape of silica bodies is taxonomically informative at the tribal level in the Stipeae (Barkworth 1981). Silica bodies are not restricted to leaves, and an SEM survey of lemmas in *Melica* indicated that the presence or absence of these structures is valuable for differentiating species (Mejia-Saules and Bisby 2003). Silica bodies are preserved in the soil for up to 600,000 years (Piperno and Pearsall 1998), and they were used to reconstruct changes in vegetation patterns in Argentinean grasslands (Gallego and Distel 2004).

Hairs and prickles have also been examined for taxonomic utility. Microhairs are informative at the subfamilial level. Broad-tipped microhairs of the epidermis are restricted to the Chloridoideae (Amarasinghe and Watson 1990; Takeota et al. 1959), and microhairs are absent in the Pooideae, with the exception of *Lygeum* Loefl. ex L. and *Nardus* L. (GPWG 2001). At the generic level, morphology of epidermal papillae is useful for delineating groups within *Sorghastrum* Nash (Dávila and Clark 1990).

Although cork cells have not received much attention, they may also provide taxonomic information. Cork cells are short cells containing deposits of organic material and suberized cell walls (Acedo and Llamas 2001). The shape of cork cells in paleas were useful in differentiating among subgenera of *Bromus* (Acedo and Llamas 2001).

The stomatal complexes in the Poaceae are unique. The lumina of guard cells are enlarged at either end and constricted in the middle (Metcalfe 1960). Subsidiary cells lie in the same plane as the guard cells, but the two cell types do not arise from the same mother cell (Metcalfe 1960). Several types of subsidiary cells have been identified, using their shape. They may be triangular, parallel-sided, low dome-shape, tall dome-shape, or variable (Ellis 1979). These differences are useful for taxonomic purposes at the subfamily level. For example, triangular subsidiary cells are common in the Panicoideae, and uncommon in other subfamilies (Ellis 1979).

Leaf anatomy is also taxonomically useful at the subfamily level in grasses. Prat (1936) identified the panicoid leaf anatomy type, with large parenchyma sheath cells, and radial arrangement of chlorenchyma cells. The festucoid type has a poorly developed parenchyma sheath and irregularly arranged chlorenchyma cells (Prat 1936). Anatomy is related to biochemistry, and C_4-type grasses usually have Kranz anatomy.

Radiate mesophyll is characteristic of C_4 grasses, but C_4 photosynthesis has multiple independent origins in the Poaceae (Kellogg 2000; Giussani et al. 2001). Thus similarities in anatomy may be due to parallel evolution.

2.4 Cytological Studies in the Poaceae

Chromosomes in the Poaceae vary in number and size (Hunziker and Stebbins 1987). Base chromosome numbers in the Poaceae range from x=2 to x=18 (Hilu 2004), while diploid chromosome numbers are as high as 2n=265 in *Poa* L. (deWet 1987). Autopolyploidy, allopolyploidy, and aneuploid losses and gains have all occurred frequently within the grass family and contributed to the high variability within the family (Hunziker and Stebbins 1987). Variation in chromosome size likely relates to the quantity of repetitive DNA (Hunziker and Stebbins 1987).

Elucidating the evolutionary trends in chromosome number in the Poaceae has proven difficult. Chromosome number has undergone frequent reductions and increases, and past disagreements on phylogenetic relationships in grasses have caused confusion (Hilu 2004). Phylogenetic disagreements at the familial level are now mostly resolved GPWG study in 2001 (Hilu 2004). Using the phylogeny produced by the GPWG (2001), it can be hypothesized that the shared ancestor of the Poaceae and Ecdeiocoleaceae had a chromosome number of x=11 (Hilu 2004). Subsequent aneuploid reduction led to x=9, followed by doubling to x=18 in the Anomochlooideae; aneuploid increase from the ancestral chromosome number led to x=12 in the Puelioideae and Pharoideae. Further modification through polyploidy and aneuploidy led to the diversity of chromosomal numbers in grasses. Smaller chromosome numbers are found in terminal taxa such as *Colpodium versicolor* (Stev.) Woronow, where 2n=4, while basal species maintain higher basic chromosome numbers. These findings refute the "secondary polyploidy hypothesis" which states that higher chromosome numbers are derived from lower base chromosome numbers. This evidence supports the "reduction hypothesis" that the ancestral chromosome number in grasses is x=12, and that lower chromosome numbers were derived from ancestral grasses through aneuploidy events (Hilu 2004).

2.5 The Utility of the Chloroplast Genome in Grass Systematics

The examination of DNA sequences is successful for determining phylogenetic relationships when morphological characters are homoplasious, as is the case in the

Danthonioideae (Wright 1984; Barker et al. 2000), and the Poaceae as a whole (Stebbins 1987; GPWG 2001). Compared to the nuclear genome, the chloroplast genome is small [between 120 and 217 kilobase pairs (kbp)], relatively homogeneous, has a semi-conservative rate of evolution, and is independent of changes in ploidy level (Hilu 1987). These factors account for its utility in reconstructing plant phylogenies. In addition, the uniparental inheritance is particularly useful in grasses, many of which have undergone multiple changes in ploidy levels through hybridization (Stebbins 1987; Olmstead and Palmer 1994).

The chloroplast genome is a circular molecule separated into a small single copy region (SSC), and a large single copy region (LSC) by two 25 kbp inverted repeats (Olmstead and Palmer 1994). The plastid genome encodes about 100 functional genes, several of which are critical for photosynthesis (Clegg et al. 1994). The size of the chloroplast genome varies from 120 kbp in the Pinaceae, to 217 kbp in *Pelargonium* L'Her. ex Ait in green plants (Downie and Palmer 1992). In the Poaceae, the chloroplast genome varies from 135.5 kbp in *Avena* L. (Murai and Tsunewaki 1987) to138 kbp in *Sorghum bicolour* (L.) Moench (Dang and Pring 1986). Several regions of the chloroplast genome are useful for elucidating relationships within the Poaceae, including coding and noncoding regions. Useful coding regions in phylogenetic studies include *rpo*C2 (e.g. GPWG 2001), the NADH dehydrogenase ND5 subunit (*ndh*F) (e.g. Clark et al. 1995), *rbc*L (e.g. Duvall and Morton 1996), and the maturase within the transfer RNA-Lysine (*trn*K) intron (*mat*K) (e.g. Hilu and Alice 1999). Useful noncoding regions include the transfer RNA-Leucine (*trn*L) intron and the intergenic spacer between *trn*L and the transfer RNA-Phenylalanine (*trn*F) (*trn*L-F) (Brysting et al. 2000), the Photosystem II protein D1 (*psb*A) to transfer RNA-Histidine (*trn*H) spacer, the ATP synthase beta subunit (*atp*B) to *rbc*L spacer, and the *trn*H intron (Vaio et al. 2005).

Two major structural rearrangements characterize the chloroplast genome of the Poaceae, namely an insertion in the *rpo*C2 region, and an inversion in the transfer RNA-Threonine (*trn*T) region (GPWG 2001). These molecular synapomorphies set the grasses apart from the Ecdeiocoleaceae, the closest relative of the Poaceae (Michelangeli et al. 2003). Within the Poaceae, chloroplast DNA (cpDNA) sequences and restriction sites have been used to examine phylogenetic relationships at all levels of organization, from

family (e.g. Davis and Soreng 1993; Duvall and Morton 1996; GPWG 2001), subfamily (e.g. Barker et al. 1995; Catalán et al. 1997), tribe (e.g. Ge et al. 2002), subtribe (e.g. Catalán et al. 2004), genus (e.g. Baumel et al. 2002), and species (e.g. Hodkinson et al. 2002).

Although coding and noncoding regions of the chloroplast genome have been used in phylogenetic studies of the Poaceae, noncoding regions have been more useful at lower taxonomic levels because they are less subject to functional constraint, and have a higher substitution rate (Clegg et al. 1994). Structural rearrangements of the chloroplast genome, such as insertions and deletions (indels), and inversions are also a potential source of phylogenetic information. They are especially useful in noncoding regions of the chloroplast where structural rearrangements may be more common than point mutations (Hilu 1987).

The *trn*L intron and *trn*L-F intergenic spacer are noncoding regions located in the large single copy region of the chloroplast genome (Hiratsuka et al. 1989). In grasses, this marker ranges from about 300 to 800 bp in length (Brysting et al. 2000; Baumel et al. 2002). Following the development of universal primers for the *trn*L-F region (Taberlet et al. 1991), this marker has been widely used in phylogenetic studies. The phylogenetic utility of this region has been shown at the intrageneric level in the Poaceae, specifically in *Poa* (Brysting et al. 2000; Stoneberg Holt et al. 2004), *Spartina* Schreb. (Baumel et al. 2002), *Saccharum* L. and *Miscanthus* Anderss. (Hodkinson et al. 2002), and *Ixophorus* (J. Presl) Schltdl. (Kellogg et al. 2004). This marker has provided sufficient signal to address phylogenetic issues at the proposed taxonomic level in other plant groups such as the Cyperaceae (Yen and Olmstead 2000; Muasya et al. 2001), Liliaceae (Zomlefer et al. 2001), Restionaceae (Eldenäs and Linder 2000), and in various dicotyledon groups (e.g. Böhle et al. 1994).

2.6 Phylogeny of the Danthonioideae

The phylogenetic position of the Danthonioideae is better understood now than ever before, due in part to relatively recent studies of the Poaceae. In early studies, the Danthonieae was included in the Aveneae (Hubbard 1934), or the Arundinoideae, a group described by Watson and Dallwitz (1992) as a "rag-bag," a jumble of unrelated taxa that do not fit well into other subfamilies. Molecular evidence has demonstrated the

polyphyly of the Arundinoideae s.l. (Barker et al. 1995), and recent studies support the recognition of the Danthonioideae at the subfamilial level, distinct from the Arundinoideae (GPWG 2001). The Danthonioideae is sister to the Aristidioideae within the PACCAD clade, which also includes the Panicoideae, Arundinoideae, Centhothecoideae, and Chloridoideae (GPWG 2001). In addition to molecular characters, morphological synapomorphies, such as the presence of haustorial synergids (Verboom et al. 1994), bilobed prophylls, and ovaries with widely separated styles support the monophyly of the Danthonioideae (Linder and Verboom 1996).

The Danthonioideae, as conventionally delimited, comprises a single tribe, the Danthonieae, with approximately 250 species in 19 genera (GPWG 2001). Seven informal groups (the Basal *Merxmuellera* Assemblage (BMA), plus the *Pentaschistis*, *Chionochloa*, *Pseudopentameris*, *Cortaderia* "A", *Rytidosperma*, and *Danthonia* clades) have been identified within the Danthonieae using chloroplast DNA sequence data from *rbc*L, *rpo*C2, ITS, and morphological evidence (Barker et al. 2000). These seven groups are probably monophyletic, but different data sets vary in terms of relationships among them, presumably due to limited overlap in taxonomic sampling (Barker et al. 2000). Although the affinities among groups remain uncertain, this preliminary survey provides a framework for examining intergeneric relationships within the danthonioid grasses. The aforementioned study contends that several genera within the Danthonioideae are para- or polyphyletic. For instance, New Zealand representatives of *Cortaderia* Stapf. are included in the *Danthonia* clade along with *Danthonia*, *Notochloe* Domin., and *Plinthanthesis* Steud., while South American species of *Cortaderia* fall within the *Cortaderia* "A" clade (Barker et al. 2000). The paraphyly of *Cortaderia* was later confirmed by Barker et al. (2003). In addition, several species of *Merxmuellera* Conert form the BMA, while the remaining *Merxmuellera* species are placed within the speciose *Rytidosperma* clade, which occupies the terminal position in the strict consensus tree (Barker et al. 2000).

Part of the difficulty in determining phylogenetic relationships within the Danthonioideae arises from the unstable taxonomy. Several authors have contested the taxonomic rearrangements within the Danthonioideae. For example, Conert (1987) argued that separating the Australian taxa from the genus *Danthonia* is unwarranted

because the distinguishing features (i.e. lemma hair and hilum characters) are inconsistent. Furthermore, Zotov (1963) described the genus *Notodanthonia*, but Connor and Edgar (1979) gave priority to *Rytidosperma* Steud., an earlier name. Veldkamp (1980) favored conserving the name *Notodanthonia* Zotov, a proposal rejected by Jacobs (1982).

Recent examination of the generic limits of the *Rytidosperma* complex recognized 11 genera, including *Danthonia*, *Erythranthera* Zotov, *Joycea* H. P. Linder, *Monostachya* Merr., *Notochloe*, *Notodanthonia*, *Plinthanthesis*, *Pyrrhanthera* Zotov, *Rytidosperma*, *Schismus* P. Beauv., and *Thonandia* H. P. Linder (Linder and Verboom 1996). The remaining danthonioid genera have their own distinctive features. Long, deep red anthers are characteristic of *Joycea*. *Plinthanthesis* has villous palea margins, and lacks bulliform cells in the adaxial leaf epidermis. *Pyrrhanthera* has a thick, hard pericarp, while *Rytidosperma* is characterized by tufted lemma indumentum, at least in the upper row of hairs. *Schismus* is distinguished by a reduced apical awn of the lemma, and like *Plinthanthesis*, it lacks tufted lemma indumentum (Linder and Verboom 1996). Lemma indumentum of short uneven hairs, terminating in long tufted hairs in the upper characterize the genus *Thonandia*. Linder (1997) later corrected the nomenclature of *Notodanthonia* when it became evident that the type specimen of *Notodanthonia* had been erroneously included in *Thonandia*, an invalid name. Consequently, a new genus, *Austrodanthonia* H. P. Linder, was erected to describe the taxa with a long, pointed callus, thus *Thonandia* became a synonym for *Notodanthonia* (Linder 1997).

These more clearly defined generic circumscriptions within the Danthonioideae make it possible to establish a current concept of *Danthonia*. The genus is composed of eight species in North America (Darbyshire 2003), nine in South America, three in Europe, two in Africa, and two in Asia (Linder and Verboom 1996). Of these, one species (*D. decumbens* (L.) DC.) is native to Europe, but it is naturalized in North and South America and Australia, for a total of 23 *Danthonia* species globally (Linder and Verboom 1996).

2.7 Origin and Geographic Distribution of the Danthonioideae

A biogeographical study of the Danthonieae suggests that this group evolved shortly after the origin of grasses in the late Cretaceous period (Linder and Barker 2000).

Two explanations are possible for the current distribution of the tribe. The distribution may be a product of long-range dispersal or vicariance via breakup of Gondwana (Linder and Barker 2000). If the geographic distribution is assumed to be due to vicariance, and the origin of the group is in Australia and New Zealand, then the origin must antecede the isolation of New Zealand approximately 80 mya, close to the date estimated for the origin of grasses (Linder and Barker 2000). An alternative explanation is that the danthonioid grasses dispersed to New Zealand, but that Australia and South America maintained contact until 30 mya when Australia separated from Antarctica (Linder and Barker 2000). The ancestors to the North American *Danthonia* likely radiated northward from South America about 3 mya when the two continents collided (Conert 1987), and the Central American landbridge emerged as an important corridor for many plant and animal migrations. Currently, *Danthonia* covers most of North America north of Mexico (Fig. 2.1) (Darbyshire 2003).

The modern-day distribution of the Danthonioideae is primarily based in the Southern Hemisphere, covering Australia, New Zealand, Africa, and South America. *Danthonia* is the only representative of the Danthonioideae in the Northern Hemisphere; it occurs in North and South America, and Eurasia (Linder and Barker 2000). In the New World, *Danthonia* occurs in Argentina, Bolivia, Brazil, Canada, the Caribbean, Chile, Columbia, Costa Rica, Ecuador, Guatemala, Mexico, Peru, USA, Uruguay, and Venezuela (Zuloaga et al. 2003). If the North American species have evolved from a single ancestor that dispersed from South America, then the most parsimonious explanation would be that they represent a monophyletic group closely related to, but distinct from South American representatives of the genus. Consequently, the North American species would be more distantly related to Australian and African danthonioid grasses than they are to South American species.

The origin of *Danthonia* remains unresolved, but it has been suggested that the putative origin of the Danthonioideae is New Zealand and Australia (Linder and Barker 2000). North American taxa are presumed to have dispersed from South America (Conert 1987). However, before the questions of origin of the tribe can be addressed, it is necessary to elucidate the phylogeny of several key groups, especially the questions regarding the paraphyly of *Merxmuellera* Conert (Barker et al. 2000) and *Cortaderia*

Stapf. (Barker et al. 1999, 2003), which are poly- or paraphyletic. Recent studies have not included adequate sampling of *Danthonia* to elucidate intrageneric relationships.

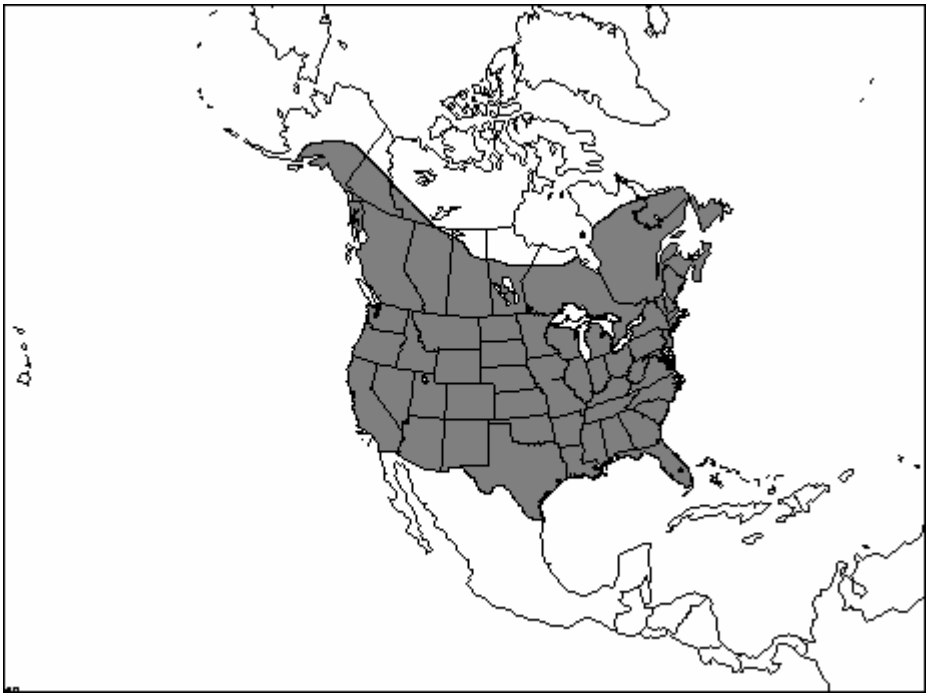

Fig. 2.1. Geographic distribution of *Danthonia* in North America north of Mexico. Adapted from Darbyshire (2003).

2.8 Morphological and Anatomical Survey of *Danthonia*

Various vegetative and reproductive characters have been used to characterize *Danthonia* s.s. The genus is distinguished from *Rytidosperma* by a long linear hilum, scattered lemma hairs that are not tufted or in transverse rows, and glabrous lodicules (Watson and Dallwitz 1992). *Danthonia* s.l. is characterized by a two-lobed lemma with a single awn arising between the lobes; the basal portion of the awn is tightly spiralled while the apical region of the awn is straight (Wright 1984). Vickery (1956) reported that cleistogenes were present in North and South American taxa in the Danthonioideae, but these structures were absent in Australian species. Dobrenz and Beetle (1966) observed cleistogenes in North American *Danthonia* species, but also reported these structures in unrelated taxa, for example, *Sporobolus* R. Br. Leaf anatomy and lodicule morphology of *Danthonia* and 14 segregate genera suggested that North American and European species were anatomically and morphologically uniform, while some South American species had paired costal short cells not found in other species of *Danthonia*

(Tomlinson 1987). This difference in South American species was not correlated with shape of vascular bundles, shape of bulliform cells, and presence or absence of abaxial sclerenchyma associated with the vascular bundles that Tomlinson (1987) examined. Therefore, no clear-cut divisions within *Danthonia* s.s. were evident.

Previous work supported a division between *Danthonia* and *Rytidosperma* based on the presence of microhairs on the lodicules of *Rytidosperma* that were lacking in *Danthonia* (Tomlinson 1987). Hairs were absent on the lodicules of *Danthonia* in Canada (Findlay and Baum 1974). Conert (1987) argued that *Rytidosperma* showed insufficient differences to warrant its separation from *Danthonia*, contrary to what was reported by Blake (1972), and Zotov (1963). Within this group, anatomical differences were not necessarily correlated with morphological features, leading to difficulty in establishing a consistent taxonomic treatment (Tomlinson 1987).

2.9 Circumscription of *Danthonia*

The systematics of the genus *Danthonia* has undergone major changes over the years, due to its taxonomic complexity. *Danthonia* was first described by De Candolle in 1805 based on European and North American species (Conert 1987). Following its original description, over 100 species were added to the genus worldwide, resulting in a complex and artificial treatment (Conert 1987). The problematic taxonomy of *Danthonia* s.l. was highlighted in deWet's (1954) description of the genus based on cytology and leaf anatomy. The taxonomic rearrangement of *Danthonia* s.l. began with formal establishment of *Chionochloa* Zotov, *Notodanthonia*, *Erythranthera*, and *Pyrranthera* Zotov to describe the New Zealand taxa (Zotov 1963). This generic separation is based on the presence of deep grooves on the leaves, tufted lemma indumentum, and punctate hilum of the caryopsis. In turn, some Patagonian species with tufted lemma indumentum were reclassified as *Rytidosperma* Steud., while those with marginal and scattered lemma hairs remained within *Danthonia* (Nicora 1973; Baeza 1996). Similarly, Blake (1972) concluded tufted lemma indumentum distinguished the Australasian species from *Danthonia* s.l. Alternatively, Conert (1987) proposed that African species should be excluded from *Danthonia* s.l., and described three new genera (*Karroochloa* Conert and Túrpe, *Merxmuellera*, and *Dregeochloa* Conert). *Karroochloa* is segregated based on the lack of bulliform cells, caryopsis size, and the form of the prophyllum, while

Merxmuellera differs from *Danthonia* in the arrangement of hairs on the lemma, the presence of single-veined glumes, and indurate lower leaf sheaths (Wright 1984). In *Dregeochloa* the pericarp is free from the seed and the embryo is larger than in *Danthonia* (Wright 1984). Indeed *Dregeochloa* is not a member of the Danthonioideae, but belongs in the Chloridoideae (Hsaio et al. 1998).

Baeza (1996) recognized seven species of *Danthonia* in North America. Darbyshire (2003) recognized eight species in North America north of Mexico, one naturalized from Europe. Hitchcock (1951) recognized the same seven species as the most recent taxonomic treatment (Darbyshire 2003), but treats *D. decumbens* as a segregate genus (*Sieglingia* Bernh.). *Sieglingia* differs from *Danthonia* in that it a flattened, twisted, apical lemma awn (Blake 1972; Watson and Dallwitz 1992).

Austin (1872) described *Danthonia alleni* Austin, a new species for North America. He considered this species distinct from *D. spicata* because *D. alleni* is more robust. Zuloaga et al. (2003) considered *D. alleni* a synonym of *D. compressa*; however, Darbyshire (2003) contends that *D. alleni* is misapplied to robust specimens of *D. spicata*. Darbyshire (2003) included *D. epilis* Scribn. in *D. sericea*, while Quinn (2003) argued that differences in leaf pubescence, ribbing, distribution of stomata on the leaf, lemma length, and number of spikelets per panicle are sufficient to warrant specific status for *D. epilis*.

Baum and Findlay (1973) constructed a taxonomic treatment for Canadian *Danthonia*. They argued that previous treatments were inadequate because used continuous characters, and thus species circumscription was difficult. They examined 52 vegetative and reproductive characters. A phenetic analysis found that lodicules were taxonomically informative (Baum and Findlay 1973), and erected a new species, *D. canadensis*, which was described as having club-shaped lodicules with a truncate apex. *D. californica* was described as having an irregularly lobed apex, and *D. parryi* possessed fan-shaped lodicules. Lodicules were lacking in *D. sericea* and *D. spicata*. These two species were differentiated by the lack of setae on the tip of the lemma in *D. spicata* (Findlay and Baum 1974). The Great Plains Flora Association (1986) rejected Findlay and Baum's (1973) treatment, on the basis that lodicule characters do not correlate with any other characters. Boivin (1981) refuted Findlay and Baum's (1973)

because the treatment results in a sympatric distribution of all Canadian *Danthonia* species. Philipson (1986) reported that cleistogamous and chasmogamous aerial florets were present in the same inflorescence of *D. spicata*. If lodicules are present in chasmogamous florets, and absent in cleistogamous florets, as Dore and McNeill (1980) suggest, then lodicule characters become unreliable. Zuloaga et al. (2003) considered *D. canadensis* a synonym of *D. intermedia*. This taxonomic disagreement illustrates the debate surrounding the circumscription of *Danthonia*.

2.10 Cytology of *Danthonia*

Darbyshire (2003) and deWet (1954) described the diploid chromosome number of North American *Danthonia* as $2n=36$. For Australian and South African species of *Danthonia* s.l., deWet (1954) reported chromosome numbers of $2n=12$, 24, 36, and 48, but new genera have since been erected to describe these species (Linder and Verboom 1996). *D. decumbens* has a diploid chromosome number of $2n=24$, 36, or 124 (Darbyshire 2003). Hunziker and Stebbins (1987) reported a base chromosome number of $x=6$ for all grasses, doubling to a base number of $x=12$ in arundinoid grasses (including the Danthonieae).

3.0 LEAF EPIDERMAL CHARACTERS IN THE DANTHONIOIDEAE

3.1 Introduction

The grass subfamily Danthonioideae, as conventionally delimited, comprises a single tribe, the Danthonieae, with approximately 250 species and 19 genera [Grass Phylogeny Working Group (GPWG) 2001]. The subfamily grows predominantly in the Southern Hemisphere and is considered a south-temperate group, but the greatest diversity is found in Africa with nine genera and 125 species. In the Australian grasslands of New South Wales, Victoria, and Tasmania, native danthonioid grasses frequently form the dominant vegetation cover and they are economically important as forages.

The Danthonioideae was formerly included in the Aveneae (Hubbard 1934) or within the Arundinoideae, a group described as a mix of unrelated taxa that do not fit into other subfamilies (Watson and Dallwitz 1992). Recent molecular evidence has demonstrated the polyphyly of the Arundinoideae sensu latu (s.l.) (Barker et al. 1995) and supports recognition of the Danthonioideae at the subfamilial level as a distinct lineage from the Arundinoideae (GPWG 2001). The monophyly of the Danthonioideae 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 the monophyletic origin of this group (Barker et al. 2003). At present, the phylogenetic position of this subfamily within the Poaceae is within the Panicoideae-Arundinoideae-Centothecoideae-Chloridoideae-Aristidoideae-Danthonioideae (PACCAD) clade and sister to the Aristidoideae (GPWG 2001). Even though DNA sequences, restriction sites, and morphological data support the monophyly of the Danthonioideae (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, generic circumscription of danthonioid grasses, in

particular *Danthonia* DC., has been controversial due to the large number of species worldwide and the overlap of morphological attributes among genera and species. Early morphological studies of *Danthonia* revealed an extensive range of interspecific variability and highlighted the need for a new taxonomic scheme for the genus (deWet 1954, 1956). The taxonomic rearrangement of *Danthonia* s.l. began with formal establishment of *Chionochloa* Zotov, *Notodanthonia* Zotov, *Erythranthera* Zotov, and *Pyrranthera* Zotov to describe New Zealand taxa (Zotov 1963). Later, South African taxa were circumscribed within the genera *Dregeochloa* Conert, *Karroochloa* Conert & Turpe, *Merxmuellera*, and *Pseudopentameris* Conert (Conert 1987). In turn, Nicora (1973) placed several South American taxa in the genus *Rytidosperma* Steud. Furthermore, as part of the disagreements regarding the taxonomy of danthonioid taxa, 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 inconsistent. More recently, Linder and Verboom (1996) advocated the recognition of *Austrodanthonia* H. P. Linder, *Joycea* H. P. Linder, *Notochloe* Domin., *Plinthanthesis* Steud., and *Schismus* P. Beauv. to describe Australasian danthonioid grasses. The same study supported separating *Danthonia* and *Rytidosperma* using morphological and anatomical characters, such as tufted lemma hairs in *Rytidosperma* and cleistogamous florets in the upper leaf sheaths of *Danthonia*. Finally, a recent re-examination of relationships within danthonioid grasses identified seven informal groups, including the basal *Merxmuellera* assemblage, and the *Pentaschistis*, *Pseudopentameris*, *Chionochloa*, *Cortaderia*, *Rytidosperma*, and *Danthonia* clades (Barker et al. 2000).

Despite extensive work in several conflicting taxa, such as *Merxmuellera* and *Cortaderia*, intrageneric taxonomic boundaries in the subfamily remain unclear (Barker et al. 2000, 2003). Likewise, studies of the subfamily have not included a sufficient taxonomic sampling within *Danthonia* to test its monophyly adequately. *Danthonia* is a cosmopolitan genus and grows in a diversity of tropical, semi-tropical and temperate habitats. The latest studies recognized nine species in South America, eight in North America, 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 *Danthonia* species globally (Linder and Verboom 1996). In North America, seven of eight species (*D. californica*, *D. compressa*, *D. intermedia*, *D. parryi*, *D. sericea*, *D. spicata*, and *D. unispicata*) are native while *D. decumbens* is introduced from Europe (Darbyshire 2003). Wright (1984) suggested that the genus *Danthonia* forms a monophyletic assemblage and that 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 primitive. However, these characters are homoplasious as they are shared with other danthonioid taxa, such as *Cortaderia*. More recently, two synapomorphies have been reported for *Danthonia*, namely the presence of cleistogenes in the lower leaf sheaths and a base chromosome number of x=18 (Linder and Verboom 1996). There are, nonetheless, some exceptions. For example, cleistogenes are rarely seen in *D. intermedia* (Darbyshire 2003), and an unusual chromosome count of 2n=31 distinguishes *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) reported detailed descriptions of the leaf epidermis in numerous taxa, pointing out the significance of these characters in the systematics of the Poaceae. At present, 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 (Metcalfe 1960; Ellis 1979). Also, 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. SEM also revealed distinguishing characters at the subfamily level in the Poaceae, such as the fact that broad-tipped epidermal microhairs are restricted to the Chloridoideae (Takeota et al. 1959; Amarasinghe and Watson 1990), while microhairs are absent in the Pooideae, except in *Lygeum* Loefl. ex L. and *Nardus* L. (GPWG 2001). Similarly, at the generic level, morphology of epidermal papillae

delineates species groups within *Sorghastrum* Nash (Dávila and Clark 1990). At the specific level, Hilu (1984) revealed distinguishing epidermal patterns in most *Andropogon* L. sect. *Leptopogon,* and phylogenetically informative characters have been reported in the epidermis of paleas and lemmas in *Bromus* L. (Acedo and Llamas 2001), *Melica* L. (Thomasson 1986), and *Zea mays* L. (Prat 1948), highlighting the utility of electron microscopy in grass systematics.

SEM technology is important in investigating the morphology of silica bodies in plants. The significance and implications of silica bodies in the taxonomy of grasses have been widely addressed. Silica bodies are a common type of phytolith in plants. They are mineral deposits that form inside specialized epidermal cells in the Poaceae (Piperno and Pearsall 1998). Certain shapes of silica body are characteristic of grass subfamilies. For instance, silica bodies are dumbbell-shaped in panicoid grasses, saddle-shaped in most pooid grasses, and vertically oriented in the Bambusoideae (Piperno 1988). At the tribal level, the distribution and shape of silica bodies is taxonomically informative in the Stipeae (Barkworth 1981). Similarly, an SEM survey of lemmas indicates that the presence or absence of silica bodies is valuable for distinguishing species of *Melica* L. (Mejia-Saules and Bisby 2003). These structures are preserved in the soil for up to 600,000 years (Piperno and Pearsall 1998), allowing reconstruction of changes in vegetation patterns in Argentinean grasslands (Gallego and Distel 2004).

Despite numerous references detailing the macro- and micromorphological leaf epidermal structures in grasses, these studies are scanty in the Danthonioideae, and several relevant taxonomic and phylogenetic questions remain unanswered. The apparent taxonomic complexity, the paucity of morphological synapomorphies, and relatively unexplored micromorphological structures required an SEM survey of epidermal features in danthonioid representatives with emphasis on North American representatives of the genus *Danthonia* to identify potentially informative characters in this poorly known group of the Poaceae. The primary objectives were of this study were to: 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 from the rest of the world danthonioid

taxa using microstructures of the leaf epidermis. This study represents the first formal SEM examination of leaf epidermal characters of danthonioid grasses.

3.2 Materials and Methods

3.2.1 Taxonomic Sampling and Plant Material

Twenty one taxa, including *Austrodanthonia*, *Cortaderia*, *Danthonia*, *Merxmuellera*, *Notodanthonia*, *Rytidosperma*, *Tribolium* Desv. as recognized by Barker et al. (2000), were investigated in this study (Table 3.1). Sampling encompassed the eight North American *Danthonia* species. ***D. decumbens*** [syn. ***Sieglingia decumbens*** (L.) Bernh.] is introduced from Europe, and seven native species (*D. californica*, *D. compressa*, *D. intermedia*, *D. parryi*, *D. sericea*, *D. spicata*, *D. unispicata*), which are recognized in the Flora of North America (FNA) North of Mexico (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 in this survey; however, they are excluded from the FNA treatment. Although limited in sampling, three South American species of *Cortaderia* and *Rytidosperma* were added to the survey to assess potential differences between North American taxa and those from Central and South America. Several Danthonioideae genera from Australia, New Zealand, and South Africa (Table 3.1) were included for comparative purposes. Taxonomy for North American *Danthonia* follows Darbyshire (2003). Taxonomic authorities for Latin names of the remaining danthonioid taxa investigated in this study are based on the Missouri Botanical Garden W^{3}TROPICOS (2005) nomenclatural database.

Seeds from danthonioid species from Australia, New Zealand, Africa, and South America were obtained from the Western Regional Plant Introduction Station (PI) in Pullman, Washington (Table 3.1). The seeds were grown in pots in the University of Saskatchewan greenhouses to obtain leaf tissue for SEM analysis and later transferred outdoors. Voucher specimens were prepared from seed-grown specimens for their inclusion in the herbarium collection of the University of Saskatchewan (SASK). Where fresh material was unavailable, portions of leaves were removed from herbarium specimens for investigation as indicated in Table 3.1. Fresh plant material was air dried at room temperature before analysis.

Table 3.1. New World and Old World danthonioid taxa investigated, including geographic distribution and source of material. ISC: Ada Hayden Herbarium; MO: Missouri Botanical Garden; NY: New York Botanical Garden; PI: U.S. Department of Agriculture Plant Introduction Center; SASK: University of Saskatchewan; SI: Institito de Botánica Darwinion.

Taxon	Herbarium Number	Locality, Collector, Collection Number, Date
New World Danthonioideae		
Cortaderia bifida Pilg.		
	MO 3400731	PERU: Pasco, Oxapampa. Clump of 5-6 tillers; inflorescence silvery purple. Elev. 2100 - 2650 m. *D. N. Smith 4115*, 19/05/1983.
	MO 3632045	BOLIVIA: La Paz, Inquisivi. 1-1.5 m high grass on open ground above falls ravine. 17°00'S 67°09'W. Elev. 3550 - 3600 m. *M. Lewis 88645*, 19/05/1988.
C. hapalotricha (Pilg.) Conert		
	NY 721441	VENEZUELA: Apure: A lo largo del río Talco (Oirá) y sus afluentes, en páramo entre Alto de Cruces y Tierra Negra, Páramo de Pata de Judío, en la frontera Colombia – Venezolana, 30 kms. al sur de San Vincente de la Revancha, 32 kms. Al sur de Alquitrana, sureste del Páramo de Tamá, suroeste de Santa Ana. Elev. 3000-3200 m. *J.A. Steyermark & E. Dunsterville 101094*, 19/01/1968.
C. selloana (Schult. & Schult. F.) Asch. & Graebn.		
	NY 472542	BOLIVIA: Santa Cruz: Florida. Gorge of Río Bermejo, along highway from Samaipata to Santa Cruz, 1.5 km E of Río Las Cruces (Río Vicoquín) bridge, 5.5 km (by road) WSW of Bermejo. Elev. 980 m. 18º08'S 063º41'W. *M. Nee 48310*, 15/02/1998.
Danthonia californica Bol.		
	MO 3140100	USA. CA: Marin Co. *J.G. Moore 51* 10/5/1969
	MO 3179929	USA. WY: Grand Teton National Park. *J.A. Steyermark 4328*, 14/07/1961.
	MO 793389	USA. CA: Humboldt Co. *J.P. Tracy 3650*, 9/6/1912.

Table 3.1. Continued.

Taxon	Herbarium Number	Locality, Collector, Collection Number, Date
D. chilensis E. Desv.		
	SI	CHILE: Lago Rauco Río Calcurrupe. *O. Boelke 329*, 19/12/1944.
D. compressa Austin		
	MO 1710981	USA. TN: Bevier Co. *J.A. Steyermark 65742*, 23/06/1948.
	MO 3685617	USA. MA: Franklin Co. *H.E. Ahles 67393*, 4/6/1967.
	MO 870462	USA. NY: Tompkins Co. *A.J. Eames & K.W. Weigand 11330*, 13/07/1919.
D. decumbens (L.) DC.		
	ISC 278020	COSTA RICA. Alajuela: Volcán Poas. Cordillera Central, 1 km below main crater. Elev. 2310 m. Chromosome number n=18. *R.W. Pohl & G. Davidse 10813*, 3/8/1968.
	SI	SPAIN. *E. Leroy 5637*, 18/06/1925.
D. filifolia F.T. Hubb.		
	ISC 356119	GUATEMALA: El Quiche. *M.J. Metzler 34*, 15/12/1978.
D. intermedia Vasey		
	MO 1010084	USA. WY: Grand Teton National Park. *J.G. Moore & J.A. Steyermark 3736* No date
	MO 1734377	USA. CA: Fresno Co. *P.H. Raven s/n*, 11/7/1954.
	MO 1577546	CANADA. SK: Cypress Hills. *A.J. Breitung 4864*, 15/07/1947.
D. parryi Scribn.		
	ISC 23982	USA. CO: Ruxton Dell. *F.E. Clements & S.E. Clements s/n*, 18/07/1901.
	ISC 282513	CANADA. AB: Crowsnest Forest Reserve. *R.G.H. Cormack 102*, 12/7/1955.

Table 3.1. Continued.

Taxon	Herbarium Number	Locality, Collector, Collection Number, Date
		D. sericea Nutt.
	ISC 231221	USA. NC: Stokes Co., 1.5 mi. N. of Belews Creek. *A.E. Radford 33480*, 4/6/1958.
	ISC 250186	USA. VA: Prince George Co. *G.L. Pyrah 260*, 30/05/1967.
	ISC 262071	USA. MS: Kemper Co. *D. Isely, S.L. Welsh & D. Isely 10510*, 17/05/1967.
D. spicata (L.) P. Beauv. *ex* Roem. & Schult.		
	ISC 254805	USA. NC: Durham Co. *A.E. Radford 44755*, 13/06/1966.
	ISC 324402	USA. NC: Transylvania Co. *W.W. Overholt 14*, 24/06/1972.
D. unispicata (Thurb.) Munro *ex* Vasey		
	MO 1259856	USA. WA: Lincoln Co. *H.T. Rogers 496*, 21/05/1940.
	MO 1751098	USA. CA: Park Co. *C.L. Porter 5446*, 12/7/1950.
	MO 1971209	USA. CA: Trinity Co. *J.T. Howell 30382*, 13/06/1955.
Rytidosperma glabra (Phil.) Nicora		
	NY 721442	CHILE: Malleco: Near southern boundary, Fundo Solano, Los Alpes, Cordillera de Nahuelbuta. Cattle camp outpost. *W.J. Eyerdam 10224*, 16/01/1958.
R. violacea (E. Desv.) Nicora		
	SI	ARGENTINA. Neuquen: Departamento de Ñorquín, Copahue. *Troiani & Steibel 15830*, 14/1/2004.
R. virescens (E. Desv.) Nicora var. *virescens*		
	MO 04979480	ARGENTINA: Río Negro: West facing summit. Elev. 1700 - 1850 m. *Ward s/n*, 22/01/1964.
	MO 3937897	ARGENTINA: Río Negro: Slight depression catching meltwater, seep. Plant cover to 40%. East facing slope. Elev. 5600 ft. *Ward s/n*, 23/01/1964.

Table 3.1. Continued.

Taxon	Herbarium Number	Locality, Collector, Collection Number, Date
Old World Danthonioideae		
Austrodanthonia pilosa (R. Br.) H.P. Linder		
	ISC 210942	USA. CA: Alameda Co. Introduced from Australia. *R.W. Pohl 7201*, 11/9/1952.
	ISC 311043	USA. CA: Santa Clara Co. Introduced from Australia. *J.T. Howell 35482*, 29/06/1960.
Merxmuellera disticha (Nees.) Conert		
	PI 364332	SOUTH AFRICA: Original material collected from mountain road, 40 km SE. of Maseru. Elev. 2250 m. *A. Oakes 1377*, 6/15/1971.
	SASK 168165	Cultivated in University of Saskatchewan garden plot, 29/09/2004.
Notodanthonia semiannularis (Labill.) Zotov		
	PI 210172	AUSTRALIA: Original material collected from Capital Terr. *W. Hartley s/n*, 9/16/1953.
	SASK 168160	Cultivated in University of Saskatchewan greenhouse, 22/04/2004.
R. unarede (Raoul) Connor & Edgar		
	PI 237160	NEW ZEALAND: Original material collected from Christchurch. *Dept. of Scientific and Industrial Res*. 2/4/1957.
	SASK 168157	Cultivated in University of Saskatchewan garden plot, 29/09/2004
Tribolium hispidum (Thunb.) Desv.		
	PI 368889	SOUTH AFRICA: Original material collected from Langgewens Experimental Farm, north of Malmsbury. 5/20/1909.
	SASK 168158	Cultivated in University of Saskatchewan greenhouse, 02/09/2004.

Selection of leaf material followed that of Ellis (1979). For each specimen, three leaves were chosen for comparison, each from a different plant specimen when possible. For consistency, the sample was always selected from mature, non-flag leaves from an area midway between the apex and ligule following Ellis (1979). A portion of the lamina, approximately 5 mm long, was selected and mounted on a stub, then sputter-coated with gold in an Edwards Sputter Coater S150B. Abaxial epidermal surfaces of leaves were examined with a Philips 505 SEM and photographed at 65X and 300X using Polaroid 665 Positive/Negative film.

3.2.2 Selection and Examination of Characters

Selection of micromorphological characters was based primarily on those included in previous studies of the Poaceae (Metcalfe 1960; Ellis 1979). Three specimens per species were examined whenever plant material was available. Structural features of the abaxial leaf epidermis were investigated, including 1) macrohairs, 2) microhairs, 3) cells of microhairs, 4) prickle hairs, 5) shape of silica bodies, in the costal and intercostal regions, and 6) stomata. A list of characters and character states is provided in Table 3.2. Previous studies (Metcalfe 1960; Ellis 1979; Watson and Dallwitz 1992), focussed on the taxonomic importance of the abaxial surface. In this survey the features of the leaf abaxial epidermis surface were also examined to standardize methodology and because adaxial structures may be obscured by dense hairs, waxes, and furrows (pers. obs.). The terminology used to describe leaf epidermal characters follows that of Ellis (1979).

3.3 Results

3.3.1 Macrohairs

Macrohairs can be observed with the naked eye; however, details cannot be distinguished without microscopic aid. At the SEM level, macrohairs of danthonioid grasses are typically large, unicellular basifixed structures, as seen in *Danthonia sericea* (Fig. 3.1A), *D. intermedia* (Fig. 3.1B), *D. unispicata* (Fig. 3.1C), *Tribolium hispidum* (Fig. 3.2C), and *Austrodanthonia pilosa* (Figs. 3.2A, 3.2D). Macrohairs are also present in *D. californica, Rytidosperma glabra*, and *R. virescens* (Table 3.3). Macrohairs are absent in *Cortaderia bifida, C. hapalotricha* (Fig. 3.2B), *C. selloana, D. chilensis*, *D. compressa*, *D. decumbens*, *D. filifolia*, *D. parryi*, *D. spicata, Merxmuellera disticha,*

Table 3.2. Characters and character states of the abaxial leaf epidermis of danthonioid grasses examined in this study.

Character	Character states
Macrohairs	1) Present; 2) Absent
Bicellular microhairs	1) Present; 2) Absent
Cells of microhairs	1) Not applicable (absent); 2) Long basal cell relative to terminal cell; 3) Cells of microhair approximately equal in length; 4) Short basal cell relative to terminal cell
Prickle hairs	1) Present; 2) Absent
Costal silica bodies	1) Dumbbell-shaped; 2) Absent
Intercostal silica bodies	1) Absent; 2) Dumbbell-shaped; 3) Cross-shaped; 4) Tall and narrow
Abaxial stomata	1) Present; 2) Absent

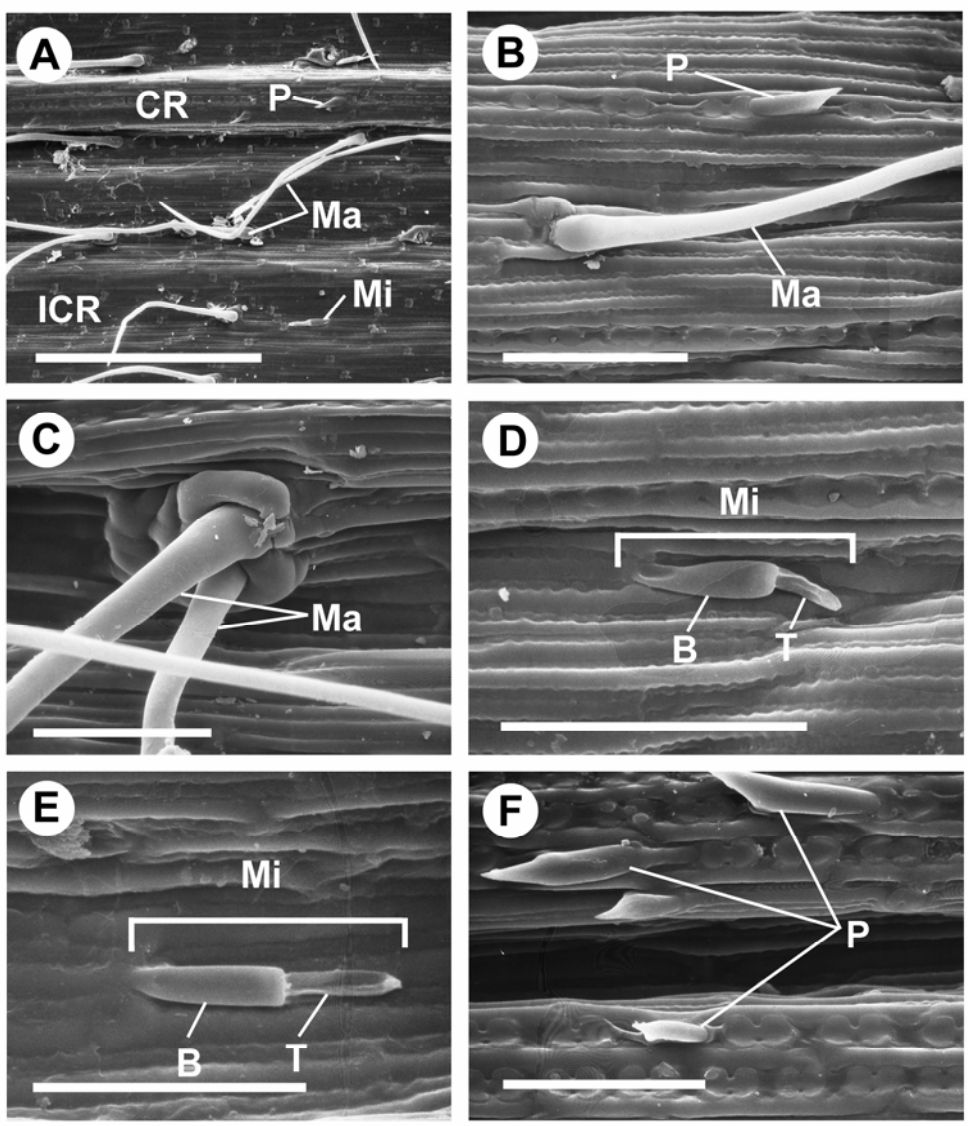

Fig. 3.1. Micrographs showing hair types on the abaxial leaf epidermis of *Danthonia* species. A) *D. sericea* showing costal (CR) and intercostal regions (ICR), unicellular macrohairs (Ma), bicellular microhairs (Mi), and a prickle hair (P). B) *D. intermedia* showing a unicellular macrohair (Ma) and a prickle hair (P). C) *D. unispicata* showing unicellular macrohairs (Ma). D) *D. intermedia* showing bicellular microhairs (Mi) with a long basal (B) cell relative to the terminal (T) cell. E) *D. decumbens* showing bicellular microhairs (Mi) with terminal (T) cell and basal (B) cell approximately equal in length. F) *D. californica* showing prickle hairs (P). Scale bar in Fig. 3.1A = 0.5 mm. Scale bar in Figs. 3.2B to 3.2F = 0.1 mm.

Table 3.3. 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	Costal Silica Bodies	Intercostal Silica Bodies	Abaxial Stomata
Austrodanthonia pilosa	Present	Present	Short basal cell	Absent	Dumbbell-shaped	Absent	Present
Cortaderia bifida	Absent	Absent	N/A	Absent	Dumbbell-shaped	Absent	Absent
C. hapalotricha	Absent	Absent	N/A	Absent	Dumbbell-shaped	Absent	Absent
C. selloana	Absent	Absent	N/A	Absent	Dumbbell-shaped	Absent	Absent
Danthonia californica	Present	Present	Long basal cell	Present	Dumbbell-shaped	Absent	Absent
D. chilensis	Absent	Present	~ Equal in length	Absent	Dumbbell-shaped	Absent	Absent
D. compressa	Absent	Present	Long basal cell	Absent	Dumbbell-shaped	Dumbbell-shaped	Absent
D. decumbens	Absent	Present	~ Equal in length	Absent	Dumbbell-shaped	Absent	Absent
D. filifolia	Absent	Absent	N/A	Absent	Dumbbell-shaped	Dumbbell-shaped	Absent
D. intermedia	Present	Present	Long basal cell	Present	Dumbbell-shaped	Cross-shaped	Absent
D. parryi	Absent	Absent	N/A	Absent	Dumbbell-shaped	Tall and narrow	Absent
D. sericea	Present	Present	~ Equal in length	Present	Dumbbell-shaped	Cross-shaped	Absent
D. spicata	Absent	Present	~ Equal in length	Absent	Dumbbell-shaped	Absent	Absent
D. unispicata	Present	Present	~ Equal in length	Present	Dumbbell-shaped	Absent	Absent
Merxmuellera disticha	Absent	Present	Short basal cell	Absent	Dumbbell-shaped	Tall and narrow	Present
Notodanthonia semiannularis	Absent	Present	Short basal cell	Absent	Dumbbell-shaped	Cross-shaped	Present
Rytidosperma glabra	Present	Present	Short basal cell	Absent	Dumbbell-shaped	Tall and narrow	Absent
R. unarede	Absent	Present	Short basal cell	Absent	Dumbbell-shaped	Absent	Present

Table 3.3. Continued.

Taxon	Macrohairs	Bicellular Microhairs	Cells of Microhairs	Prickle Hairs	Costal Silica Bodies	Intercostal Silica Bodies	Abaxial Stomata
R. violacea	Absent	Absent	N/A	Absent	Dumbbell-shaped	Absent	Present
R. virescens	Present	Present	Long basal cell	Absent	Dumbbell-shaped	Absent	Present
Tribolium hispidum	Present	Present	Short basal cell	Absent	Dumbbell-shaped	Absent	Present

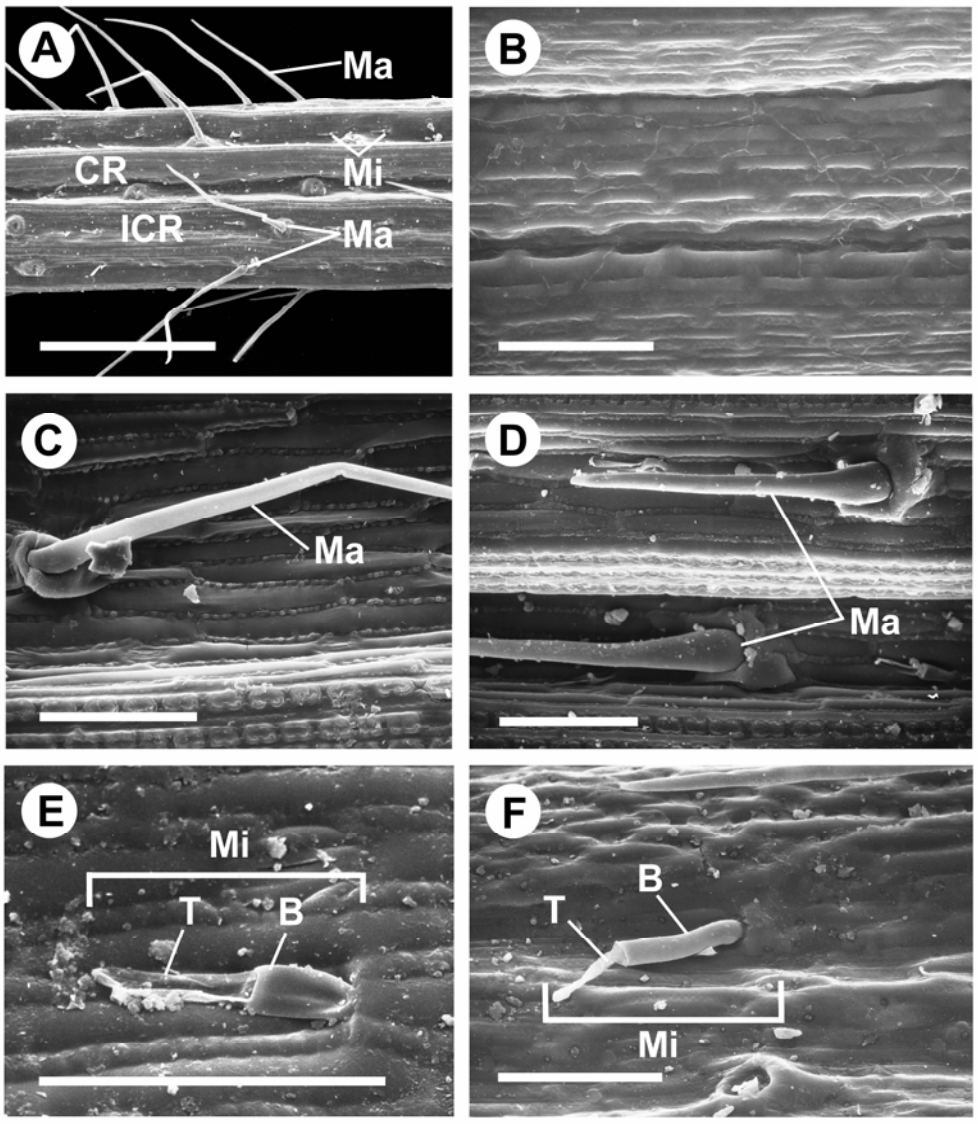

Fig. 3.2. Micrographs showing hair types on the abaxial leaf epidermis of selected danthonioid taxa outside the genus *Danthonia*. A) *Austrodanthonia pilosa* showing the costal region (CR), intercostal region (ICR), unicellular macrohairs (Ma), and bicellular microhairs (Mi). B) *Cortaderia hapalotricha* lacking hairs. C) *Tribolium hispidum* showing unicellular macrohair (Ma). D) *A. pilosa* showing unicellular macrohairs (Ma). E) *Rytidosperma unarede* showing a bicellular microhair (Mi) with a short basal (B) cell relative to the terminal (T) cell. F) *R. virescens* showing a bicellular microhair (Mi) with a long basal (B) cell relative to the terminal (T) cell. Scale bar in Fig. 3.2A = 0.5 mm. Scale bar in Figs. 3.2B to 3.2F = 0.1 mm.

Notodanthonia semiannularis, R. unarede, and *R. violacea* (Table 3.3).

Length and abundance of macrohairs in the leaf epidermis varies among individuals in *A. pilosa*, *D. californica, D. sericea,* and *D. unispicata*, among other taxa. Unlike the majority of specimens investigated, the leaf epidermis of *Cortaderia* species was covered with waxes. Initially, it was thought that the waxes could have obstructed the observation of epidermal structures. However, macrohairs are absent in *Cortaderia*. This waxy surface pattern with no macrohairs was consistent among the three species investigated [as seen in *C. hapalotricha* (Fig. 3.2B)], and there was no evidence indicating that the waxy layer could disguise the relatively large macrohairs observed in other danthonioid taxa.

3.3.2 Microhairs

Microhairs are sparsely distributed throughout the leaf epidermis, as seen in *Danthonia sericea* (Fig. 3.1A) and *Austrodanthonia pilosa* (Fig. 3.2A). They are present on the abaxial surface of most danthonioid grasses examined, except in *Cortaderia* species (Fig. 3.2B), *D. filifolia*, *D. parryi*, and *Rytidosperma violacea* (Table 3.3). Microhairs are typically small, bicellular, rod-shaped structures with a cylindrical basal cell and a slender terminal cell as seen in *D. intermedia* (Fig. 3.1D). Three types of microhairs were observed in danthonioid grasses including, 1) microhairs with a long basal cell relative to the terminal cell, characteristic of *D. californica*, *D. compressa*, *D. intermedia* (Fig. 3.1D), and *Rytidosperma virescens* (Fig. 3.2F) (Table 3.3), 2) microhairs with basal and terminal cells approximately equal in length, observed in *D. chilensis, D. decumbens* (Fig. 3.1E), *D. sericea, D. spicata,* and *D. unispicata* (Table 3.3), and 3) microhairs with a short basal cell relative to the terminal cell, occurring in *Austrodanthonia pilosa*, *Merxmuellera disticha*, *Notodanthonia semiannularis*, *Rytidosperma glabra, R. unarede* (Fig. 3.2E), and *Tribolium hispidum* (Table 3.3). The relative length of microhair cells appears to be consistent among individuals of the same species, but the intraspecific variation was not quantified.

3.3.3 Prickle Hairs

Prickle hairs are distributed sparsely throughout the costal regions of the epidermis. They are short, relatively stiff structures with sharply pointed ends, as seen in *Danthonia californica* (Fig. 3.1F). These structures are exclusive to only four North

American *Danthonia* species, namely *D. californica* (Fig. 3.1F), *D. intermedia* (Fig. 3.1B), *D. sericea* (Fig. 3.1A), and *D. unispicata*. Prickle hairs in *Danthonia* have not been previously reported. Prickle hairs vary in size within a single leaf, as observed in *D. californica* (Fig. 3.1F), and are consistently present in all specimens investigated of these four taxa. The remaining *Danthonia* species investigated lack these structures (Table 3.3). Prickle hairs are also absent in all South American and Old World danthonioid species examined (Table 3.3). Therefore, their presence is unique and restricted to the four North American *Danthonia* species indicated above.

3.3.4 Silica Bodies

Epidermal silica bodies in the leaves of danthonioid taxa are dumbbell-shaped, tall and narrow, or cross-shaped. They are distributed either in the costal or intercostal leaf regions, or in both leaf regions. In general, costal silica bodies in the leaf epidermis of danthonioid grasses are arranged in rows of short cells and are consistently dumbbell-shaped, as observed in *Danthonia californica* (Fig. 3.3C), *D. sericea* (Fig. 3.3D), *D. compressa* (Fig. 3.3E), *D. parryi* (Fig. 3.3F), *Tribolium hispidum* (Fig. 3.4A), *Merxmuellera disticha* (Fig. 3.4B), and *Rytidosperma glabra* (Fig. 3.4D). Conversely, the intercostal silica bodies were more variable, and included three shapes, cross-shaped, dumbbell-shaped to tall and narrow. Normally, silica bodies are lacking in the leaf intercostal regions of *Austrodanthonia pilosa* (Fig. 3.2D), *Cortaderia bifida, C. hapalotricha* (Fig. 3.2B), *C. selloana* (Fig. 3.4C), *D. californica* (Fig. 3.3C), *D. chilensis*, *D. decumbens* (Fig. 3.1E), *D. spicata*, *D. unispicata* (Fig. 3.1C), *Rytidosperma unarede* (Fig. 3.2E), *R. violacea*, *R. virescens* (Fig. 3.2F), and *Tribolium hispidum* (Fig. 3.4A; Table 3.3). The cross-shaped intercostal silica bodies are restricted to *D. intermedia, D. sericea* (Fig. 3.3D), and *Notodanthonia semiannularis* (Table 3.3). Similarly, dumbbell- shaped intercostal silica bodies have limited taxonomic distribution. They were observed only in *D. compressa* (Fig. 3.3E) and *D.*

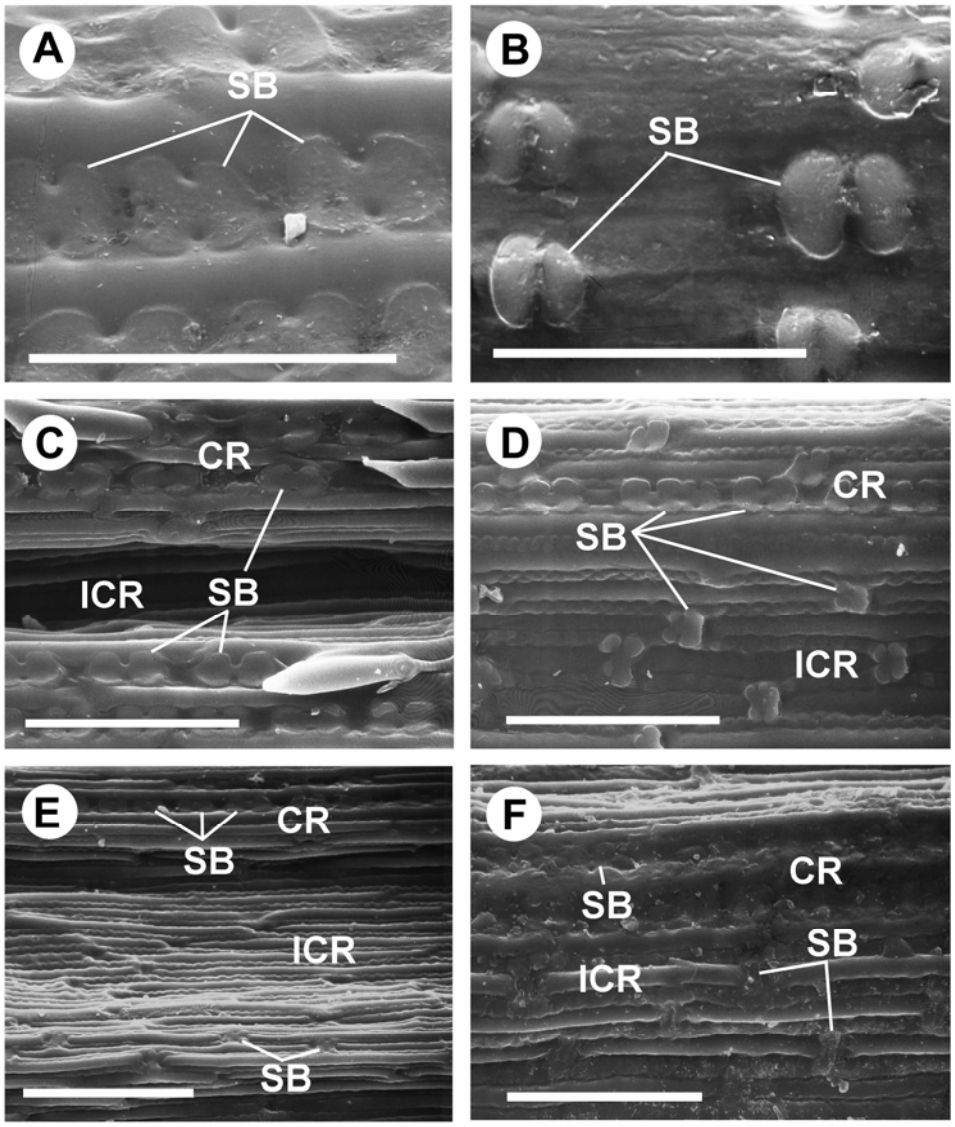

Fig. 3.3. Micrographs showing silica bodies on the abaxial leaf epidermis of *Danthonia* species. A) *D. unispicata* showing dumbbell-shaped silica bodies (SB) in the costal region. B) *D. filifolia* showing dumbbell-shaped silica bodies (SB) in the costal region. C) *D. californica* showing dumbbell-shaped silica bodies (SB) in the costal region (CR) and no silica bodies in the intercostal region (ICR). D) *D. sericea* showing dumbbell-shaped silica bodies (SB) in the costal region (CR) and cross-shaped silica bodies (SB) in the intercostal region (ICR). E) *D. compressa* showing dumbbell-shaped silica bodies (SB) in the costal region (CR) and the intercostal region (ICR). F) *D. parryi* showing dumbbell-shaped silica bodies (SB) in the costal region (CR) and tall and narrow silica bodies (SB) in the intercostal region (ICR). Scale bar in Fig. 3.3A and 3.3B = 0.05 mm. Scale bar in Fig. 3.3C to 3.3F = 0.1 mm.

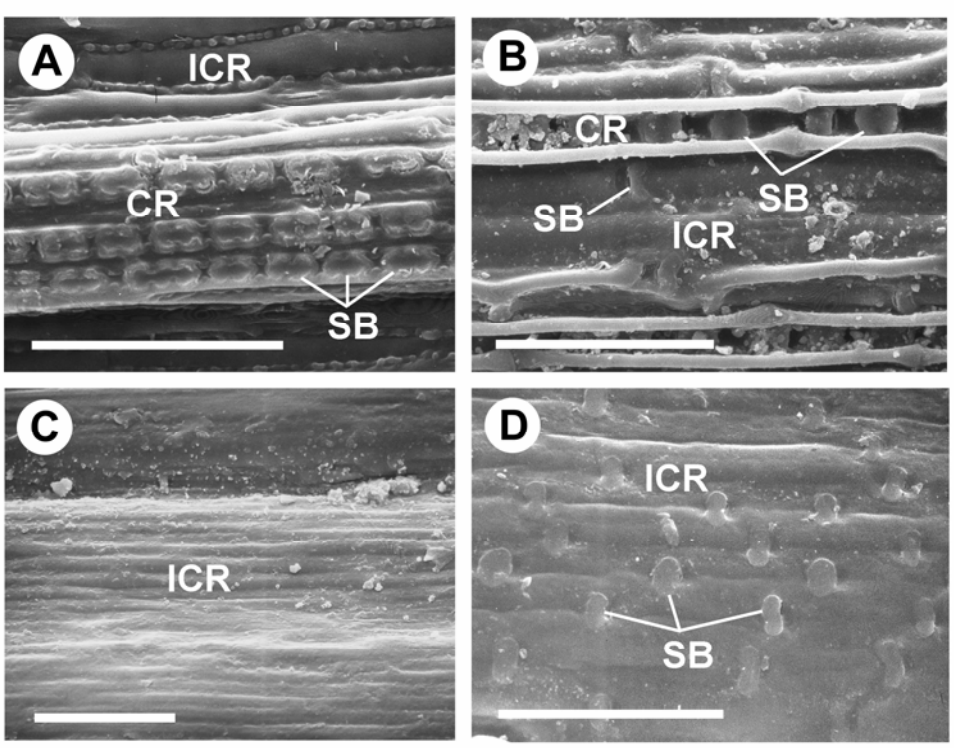

Fig. 3.4. Micrographs showing silica bodies on the abaxial leaf epidermis of selected danthonioid taxa outside the genus *Danthonia*. A) *Tribolium hispidum* showing dumbbell-shaped silica bodies (SB) in the costal region (CR) and no silica bodies in the intercostal region (ICR). B) *Merxmuellera disticha* showing dumbbell-shaped silica bodies (SB) in the costal region (CR) and tall and narrow silica bodies in the intercostal region (ICR). C) *Cortaderia selloana* lacking silica bodies in the intercostal region (ICR). D) *Rytidosperma glabra* showing tall and narrow silica bodies in the intercostal region (ICR). Scale bar = 0.1 mm.

filifolia (Table 3.3). Finally, tall and narrow silica bodies are characteristic in the intercostal regions of *D. parryi* (Fig. 3.3F), *Merxmuellera disticha* (Fig. 3.4B), and *Rytidosperma glabra* (Table 3.3). Slight variations in size and shape of silica bodies were evident in the same leaf (*D. sericea*, Fig. 3.3D), in which the outlines of the silica bodies exhibit subtle variation.

3.3.5 Stomatal Complexes

Stomatal complexes are of the paracytic type in the Poaceae, and have two differentiated subsidiary cells (Watson and Dallwitz 1992). Paracytic types were observed in *Rytidosperma virescens* (Fig. 3.5A) and *R. violacea* (Fig. 3.5B). Stomatal complexes have dome-shaped subsidiary cells (Figs. 3.5A, 3.5B) and occur sparsely on the abaxial leaf surface, specifically, in the intercostal region between long cells.

Stomatal complexes are not present on the abaxial surface of any of the *Danthonia* or *Cortaderia* specimens examined (Table 3.3). Instead, abaxial stomatal complexes seem to be restricted to other danthonioid genera, such as *Austrodanthonia, Merxmuellera, Notodanthonia, Rytidosperma* (except *R. glabra*), and *Tribolium* (Table 3.3). Even when stomatal complexes are present on the abaxial surface, there are few, making intraspecific variation difficult to estimate. However, no obvious differences in size, arrangement, and shape of subsidiary cells were evident from the material examined.

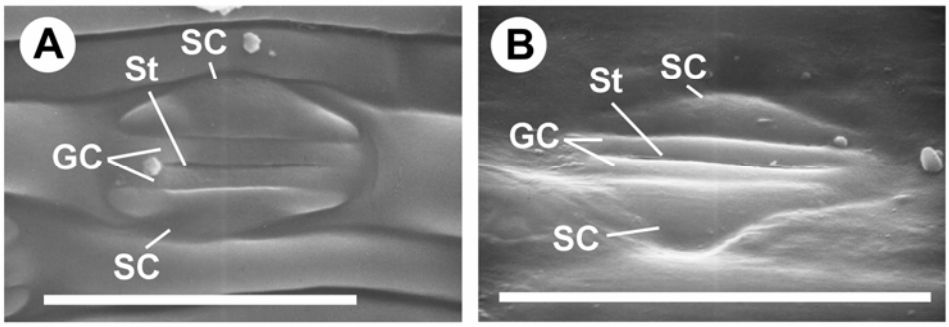

Fig. 3.5. Abaxial paracytic stomatal complexes in selected danthonioid taxa. A) *Rytidosperma virescens* showing subsidiary cells (SC), guard cells (GC), and stomatal aperture (St). B) *R. violacea* showing subsidiary cells (SC), guard cells (GC), and stomatal aperture (St). Scale bar = 0.05 mm.

3.4 Discussion

Certain micromorphological features of the abaxial leaf epidermis are of taxonomic value at the generic level in the Danthonioideae, but inferences at the subfamily level are limited due to the lack of comparative data from members of other subfamilies of the Poaceae. Also, many epidermal traits overlap, limiting their systematic value.

3.4.1 Epidermal Hairs

Macrohairs have been useful in distinguishing species of *Hordeum* L. in the Poaceae (Cai et al. 2003). Similar cases have been reported in *Aristida stricta* Michaux (Kesler et al. 2003) and *Elymus glaucus* Buckley (Wilson et al. 2001), in which variation in leaf pubescence is used to separate subspecies. Nevertheless, neither the presence nor the absence of macrohairs appears to reflect taxonomic relationships within danthonioid taxa. In fact, these structures are widespread in the Poaceae, but their presence and abundance on the leaf surface has been attributed to environmental factors

as they confer adaptive advantages in adverse conditions (Metcalfe 1960) and reduce transpiration rates and water loss in arid habitats (Meinzer and Goldstein 1985). In spite of the apparently well-known ecological role of leaf pubescence, macrohairs are of limited value in the systematics of danthonioid grasses. The most parsimonious inference is that the Danthonioideae is characterized by macrohairs on the leaf epidermis, a feature that has apparently been lost in *Cortaderia* and several species within the other genera examined.

Microhairs also provide limited taxonomic information and only a few assumptions can be made. Except for *Danthonia filifolia* and *D. parryi*, *Danthonia* is characterized by bicellular microhairs with basal and terminal cells of equal length or with long basal cells relative to the terminal cells. These structures are absent in *D. filifolia* and *D. parryi*, and have presumably been lost. *Cortaderia* species lack bicellular microhairs, but the remaining danthonioid genera, except for *Rytidosperma virescens*, possess bicellular microhairs with short basal cells (Table 3.3). Microhairs are also purportedly absent in *Merxmuellera* (Watson and Dallwitz 1992 onwards) but they were present in the specimen of *M. disticha* examined here. It is likely microhairs are a plastic character and display interspecific variability.

The long basal cell of microhairs in *Rytidosperma virescens* is shared with *Danthonia*, but the occurrence of this structure in these two genera is probably due to homoplasy rather than a close intergeneric relationship. DNA sequences indicate that these genera do not share a recent common ancestor (Barker et al. 2000; Reimer and Cota-Sánchez unpubl. data). It is also possible that this character is more variable than observations suggest, because terminal cells of the microhair are apparently equal in length to the basal cell in *Rytidosperma* and *Tribolium* (Watson and Dallwitz 1992 onwards). 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*, except for *D. secundiflora*, has a linear hilum (Linder and Verboom 1996). The data for South American *Rytidosperma* species is inconsistent, making the interpretation complex due in part to the variability of the microhairs and the limited number of species investigated.

Wright's (1984) study using light microscopy indicated that the bicellular microhairs in *Danthonia* have long basal cells relative to the short basal cells found in *Rytidosperma*. The current study partially agrees with Wright's observations because several *Danthonia* species have bicellular microhairs with long basal cells. At the same time, the thin-walled terminal cell is present in the microhairs, an observation not reported by Wright (1984). This terminal cell was invariably observed intact in all samples investigated. Results presented here are more congruent with Palmer and Tucker's (1981) SEM examination of grass epidermis, in which intact terminal cells were present in microhairs. Wright (1984) may have been unable to describe this character because the terminal cell of the bicellular microhair was absent or damaged. This discrepancy is likely due to differences in preparation techniques between epidermal peels for light microscopy and SEM. Absence of terminal cells in the microhairs of epidermal peel studies may be the result of physical damage inflicted during the removal of the epidermis from the mesophyll. SEM is a more effective technique for specimens with thin-walled terminal cells of the bicellular microhairs or, in general, for delicate epidermal structures requiring exhaustive inspection (Palmer and Tucker (1981).

Prickle hairs represent a potential distinguishing feature within *Danthonia*. They are restricted to *D. californica*, *D. intermedia*, *D. sericea*, and *D. unispicata* (Table 3.3). With the exception of *D. sericea*, in which the lemmas have hairs scattered across the back, North American species of *Danthonia* with prickle hairs have lemmas with glabrous backs (Darbyshire 2003). The combination of these two morphological characters supports the close relationship among *D. californica*, *D. intermedia*, and *D. unispicata,* as suggested by Darbyshire (2003). This premise is also supported by the sympatric distribution of these three species in the western region of North America. The presence of prickle hairs in *Danthonia* is only of taxonomic value for differentiating the four species mentioned above. Their occurrence may indicate that this character evolved relatively recently in these North American species.

Prickle hairs between veins on the abaxial leaf surface of the danthonioid *Cortaderia selloana* were previously reported (Metcalfe 1960). Nevertheless, the current study including three *Cortaderia* species shows that prickle hairs are absent in this

genus. Moreover, in an anatomical and morphological survey of the Danthonioideae, Wright (1984) made no mention of prickle hairs in *Cortaderia*. The presence of prickle hairs in *Cortaderia* should not be ruled out until extensive SEM analysis is conducted in a wider taxonomic sampling.

3.4.2 Silica Bodies

Silica bodies in some groups of monocots has been informative (Mejia-Saules and Bisby 2003; Prychid et al. 2003). Specific types of silica bodies distinguish the Bambusoideae, Panicoideae, and Pooideae (Piperno 1988). Nonetheless, in spite of the degree of variability observed in the intercostal silica bodies of the leaf epidermis of danthonioid grasses (Table 3.3), silica body shape is not of taxonomic significance in the subfamily. Conversely, even though costal silica bodies are reportedly variable in the Danthonioideae and may be crenate, tall and narrow, cross-shaped, rounded, or dumbbell-shaped (Watson and Dallwitz 1992 onwards), only dumbbell-shaped silica bodies were observed in the costal region. The consistent pattern of dumbbell-shaped costal silica bodies in the epidermal costal regions is not unique to the subfamily Danthonioideae. Dumbbell-shaped silica bodies are also present in a few representatives of the Bambusoideae and are common in the subfamilies of the PACCAD clade (Watson and Dallwitz 1992 onwards). The shape of silica bodies in the epidermis of the grasses examined does not appear to be taxonomically informative, but it is noteworthy that the occurrence of tall and narrow silica bodies between 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 reported tall and narrow silica bodies in the intercostal regions of *Austrodanthonia, Cortaderia, Merxmuellera,* and *Tribolium*, as well as *Danthonia decumbens*. Taxonomic implications of these structures are unclear. Furthermore, 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 Danthonioideae remains ambiguous and deserves further investigation involving more species.

Overall, no major differences in silica body types and shapes were observed between North and South American *Danthonia*, as well as Old World and South

American danthonioids. Althogh deWet's (1956) argued that danthonioid taxa could be grouped on the basis of microhairs and silica body shapes, this study does not support the such a division in the Danthonioideae. The apparent lack of taxonomic information on silica bodies necessitates further research in the subfamily. Finally, it is likely that the shape and frequency of silica bodies in the abaxial leaf epidermis may also be related to water conducting systems that influence the availability of silica in the surrounding environment. A similar case was described in species of *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).

3.4.3 Abaxial Stomatal Complexes

Except for *Austrodanthonia, Merxmuellera, Notodanthonia, Rytidosperma unarede, R. virescens, R. violacea,* and *Tribolium*, the abaxial leaf surfaces of danthonioid taxa tend to lack stomata (Table 3.3). Nonetheless, Watson and Dallwitz (1992 onwards) reported 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 results presented here, except that stomata were observed in *Merxmuellera*. In addition, this study does not support the presence of stomata on the abaxial leaf surface of *Danthonia decumbens* and *D. sericea* as indicated by Metcalfe (1960) and Gray et al. (1969), respectively. Stomata may be present in the species, but they were absent in the particular areas of the leaf samples examined. The frequency of stomata on the leaf is strongly correlated with environmental factors (Ellis 1979). Abaxial stomata are more common in species in wetter and cooler environments, while stomatal density decreases under water stress in *Leymus chinensis* (Trin.) Tzvel. (Xu and Zhou 2005). Based on this result, abaxial stomata may be absent or present in small numbers in taxa occurring in drier conditions, which is the case of the Danthonideae. More studies are necessary to evaluate the distribution of abaxial and adaxial stomata in leaf surfaces of danthonioid taxa. Clearly, the taxonomic relevance of the shape of subsidiary cells cannot be assessed solely on the examination of the abaxial leaf surface.

3.4.4 Final Remarks

Overall, this study provides new and relevant information regarding the morphology of leaf epidermal structures in danthonioid grasses. It also shows that the systematic utility of epidermal attributes is limited in the subfamily when they are treated independently. The wide range of overlapping of characters at the intergeneric and interspecific levels, and the apparent multiple origins of various epidermal features increases the levels of homoplasy, making taxonomic inferences difficult. None of the characters investigated provide distinctive information at the subfamilial and generic levels, nor do they assist in separating Old and New World danthonioid taxa. Nevertheless, these findings provide a framework that can be used in a future interpretation and re-evaluation of taxonomic and phylogenetic relationships of the Danthonioideae, in particular for North American *Danthonia*. For instance, *Danthonia* is characterized by the absence of abaxial stomata, bicellular microhairs with long basal cells or cells of equal length. Furthermore, prickle hairs occur in four North America species. When these micromorphological characters are evaluated together with other evidence, the significance of leaf epidermis traits in danthonioid grasses becomes more apparent. The optimization of morphological characters onto the strict consensus obtained from preliminary analyses of DNA sequences from the *trn*L-F chloroplast marker shows that the monophyly of *Danthonia* is robust, even though the morphological characters are homoplasious (Reimer and Cota-Sánchez, unpubl. data). Similarly, the monophyly of *Danthonia* is supported by additional characters, including ovoid to obovoid caryopsis shape, linear hilum, and undulating or straight-walled reticulate surface pattern (discussed in Chapter 4). Nevertheless, a broader taxonomic sampling and investigation of other micromorphological structures are needed to generate a more explicit hypothesis about the phylogenetic intergeneric relationships within the subfamily.

4.0 LODICULE AND CARYOPSIS CHARACTERS IN THE DANTHONIOIDEAE

4.1 Introduction

The subfamily Danthonioideae is found predominantly in the Southern Hemisphere and is considered a south-temperate group. The only genus with representatives in the Northern Hemisphere within the subfamily is *Danthonia* DC. (Linder and Barker 2000). The greatest diversity of danthonioid grasses is in Africa with nine genera and 125 species. Danthonioid representatives frequently form the dominant cover and are economically important as native forage grasses in the Australian grasslands of New South Wales, Victoria, and Tasmania (Linder and Verboom 1996).

Within the Poaceae, the Danthonioideae is sister to the Aristidoideae, and these two subfamilies form a basal clade to the Arundinoideae and Chloridoideae. The Centhothecoideae and the sister Panicoideae are the basal-most lineages of these six subfamilies that collectively form the PACCAD clade [Grass Phylogeny Working Group (GPWG) 2001]. The Danthonioideae, as conventionally delimited, comprises a single tribe, the Danthonieae, with approximately 250 species and 19 genera (GPWG 2001).

The danthonioid taxa were formerly included in the subfamily Aveneae based on the characteristic long glumes and lemmas with geniculate awns (Hubbard 1934). Alternately, danthonioid grasses have been recognized at the tribal level and included within the Arundinoideae (Watson and Dallwitz 1992). Molecular evidence has demonstrated the polyphyly of the Arundinoideae sensu lato (s.l.) (Barker et al. 1995), supporting recognition of the Danthonioideae as a subfamily, distinct from the Arundinoideae (GPWG 2001).

The monophyly of the Danthoniodeae is supported by both morphological and molecular data. A phylogeny based on chloroplast and nuclear DNA sequence data provides strong evidence for the monophyletic origin of this subfamily (Barker et al. 2003). Additionally, haustorial synergids, bilobed prophylls, and ovaries with widely

separated styles are considered morphological synapomorphies for the Danthonioideae (Linder and Verboom 1996).

Morphological characters in the Danthonioideae are homoplasious and few unambiguous synapomorphies have been identified at the generic and specific levels (Wright 1984; Barker et al. 2000). Generic delineation and selection of diagnostic characters has been difficult due to morphological and anatomical variation in danthonioid grasses. However, a recent study based on morphological, nuclear, and chloroplast sequence data recognized seven informal groups within the Danthonioideae: the Basal *Merxmuellera* Assemblage (BMA), the *Chionochloa* clade, the *Pentaschistis* clade, the *Cortaderia* "A" clade, the *Danthonia* clade, the *Pseudopentameris* clade, and the *Rytidosperma* clade (Barker et al. 2000). These seven groups are probably monophyletic, but results vary between data sets, presumably due to limited overlap in taxonomic sampling (Barker et al. 2000). Although relationships among the groups remain uncertain (Barker et al. 2000), the preliminary survey provides a framework for examining intergeneric relationships while identifying several genera within the Danthonioideae that are para- or polyphyletic. For example, the New Zealand representatives of *Cortaderia* Stapf. are included in the *Danthonia* clade along with *Danthonia*, *Notochloe* Domin., and *Plinthanthesis* Steud., while the South American species of *Cortaderia* fall within the *Cortaderia* "A" clade (Barker et al. 2000). The paraphyly of *Cortaderia* was confirmed later (Barker et al. 2003). In addition, several species of *Merxmuellera* Conert form a paraphyletic assemblage at the base of the clade that comprises the entire Danthonioideae. The remaining species of *Merxmuellera* are placed within the speciose *Rytidosperma* clade, which occupies the terminal position of the strict consensus tree (Barker et al. 2000).

Unlike *Cortaderia*, *Danthonia* has not been adequately studied to test its monophyly conclusively. Nonetheless, it has been suggested that *Danthonia* forms a monophyletic assemblage based on the presence of scattered lemma hairs and bulliform cells, two putatively primitive features shared with *Cortaderia* (Wright 1984). The presence of cleistogenes (autogamous florets) in the lower leaf sheaths and a base chromosome number of x=18 are also synapomorphies for *Danthonia* (Linder and Verboom 1996). Some exceptions to these findings have been documented, for example,

cleistogenes are rarely seen in *D. intermedia* (Darbyshire 2003), and an unusual chromosome count of 2*n*=31 has been reported in *D. spicata* (Darbyshire and Cayouette 1989). Though *Danthonia* systematics has been addressed in previous studies (summarized chronologically below), to date no clear picture has emerged regarding its phylogenetic and evolutionary history. Consequently, more research is required to identify additional synapomorphies and generate a more robust and coherent phylogenetic hypothesis about *Danthonia*.

Over the years, the systematics of *Danthonia* has undergone major changes. *Danthonia* was first described by De Candolle in 1805 based on European and North American species (Conert 1987). Following original description of *Danthonia*, over 100 species were added to the genus worldwide, creating a complex and artificial treatment (Conert 1987). DeWet's (1954) description of cytology and leaf anatomy in *Danthonia* s.l. highlighted the problematic taxonomy. Since then, the taxonomy of *Danthonia* has undergone extensive rearrangements. First, the extreme morphological variation in *Danthonia* s.l. led to the exclusion of all the New Zealand species, with the subsequent description of new genera (*Notodanthonia*, *Chionochloa* Zotov, *Erythranthera*, and *Pyrrhanthera*) to describe the segregated taxa (Zotov 1963). This generic separation is based on the presence of deeply grooved leaves, the tufted lemma indumentum, and the punctate hilum of the caryopsis. Nicora (1973) reclassified some South American species with tufted lemma indumentum as *Rytidosperma* Steud, while other taxa with scattered lemma hairs remained within *Danthonia*. This arrangement was supported by Baeza (1996). Similarly, Blake (1972) concluded the presence of tufted lemma indumentum in Australasian species distinguished these taxa from *Danthonia* s.l.. Nevertheless, Conert (1987) argued that the lemma indumentum characters were inconsistent and that *Danthonia* and *Rytidosperma* should not be separated. However, he concluded that African species should be excluded from *Danthonia* s.l. and he described three new genera (*Karroochloa* Conert and Túrpe, *Merxmuellera*, and *Dregeochloa* Conert). *Karroochloa* was segregated based on the lack of bulliform cells, caryopsis size, and the form of the prophyllum, while *Merxmuellera* differs from *Danthonia* in the arrangement of hairs on the lemma, the presence of single-veined glumes, and indurate lower leaf sheaths (Wright 1984). A large embryo and free pericarp distinguishes

Dregeochloa from *Danthonia* (Wright 1984), and molecular work has shown that *Dregeochloa* is more accurately placed in the Chloridoideae (Hsaio et al. 1998).

The taxonomy of several groups within the Danthonoideae has also been controversial. Zotov (1963) described the genus *Notodanthonia*, but Connor and Edgar (1979) gave priority to *Rytidosperma*, an earlier valid name. Veldkamp (1980) favored conserving the name *Notodanthonia*, a proposal disputed by Jacobs (1982) and subsequently rejected. More recently, generic limits of the *Rytidosperma* complex have been re-examined and 11 genera were recognized: *Danthonia*, *Erythranthera*, *Joycea* H. P. Linder, *Monostachya* Merr., *Notochloe*, *Notodanthonia*, *Plinthanthesis*, *Pyrrhanthera*, *Rytidosperma*, *Schismus* P. Beauv., and *Thonandia* H. P. Linder (Linder and Verboom 1996). Linder (1997) later corrected the nomenclature of *Notodanthonia* when it became evident that the type specimen of *Notodanthonia* had been erroneously included in *Thonandia*. T*honandia* was then not a validly published name. A new genus, *Austrodanthonia* H. P. Linder, was erected to describe taxa with a long pointed callus (Linder 1997). *Notodanthonia* was also re-examined and included taxa with short, uneven lemma indumentum, terminating in long tufted hairs in the upper row of lemma hairs (Linder and Verboom 1996). The remaining danthonioid genera have their own distinctive features. For instance, long, deep red anthers are characteristic of *Joycea*; *Plinthanthesis* has villous palea margins, and lacks bulliform cells in the adaxial leaf epidermis. *Pyrrhanthera* is separated because of the thick, hard pericarp, while *Rytidosperma* is characterized by tufted lemma indumentum, at least in the upper row of hairs. *Schismus* is distinguished by a reduced apical lemma awn, the absence of tufted lemma hairs, and it lacks tufted lemma indumentum (Linder and Verboom 1996).

To a large extent, Conert's (1987) concerns regarding the lack of clear-cut morphological distinctions between genera are justified. One example of the debate surrounding the selection of features that best characterize danthonioid genera can be illustrated by the taxonomic value of the hilum character. Zotov (1963) used differences in hilum length to separate *Notodanthonia* from *Danthonia*, but Blake (1972) argues that the distinction between short punctate hila and a long linear hila is not clear-cut, thus emphasizing the value of lemma vestiture. Other morphological characters in the Danthonioideae are also highly homoplasious, highlighting the need to identify

additional morphological characters to clarify taxonomic boundaries and phylogenetic relationships within the subfamily.

The utility of floret characters such as caryopsis and lodicule traits in elucidating phylogenetic relationships within the grass family has been demonstrated. Lodicules are considered homologous to petals in eudicots, and they function to open grass florets for fertilization (Bommert et al. 2005). Lodicule morphology has also been taxonomically informative and these structures have the potential to infer phylogenetic relationships in the grass family. For example, lodicules are thick and heavily vascularized in panicoid grasses, as compared to thin, membranous lodicules in the Pooideae (Hsu 1965). *Melica* L. and *Glyceria* R. Br. share characteristic fusion of the anterior pair of lodicules (GPWG 2001). Lodicule morphology in North and South American danthonioid species is reported by Baeza (1996), while Old World danthonioid lodicule information is available from Watson and Dallwitz (1992 onwards).

Lodicule characters in the Danthonioideae have been investigated. Within the Danthonioideae, ciliate lodicules are characteristic of *Rytidosperma*, versus glabrous lodicules in *Danthonia* (Veldkamp 1980). Findlay and Baum (1974) proposed a taxonomic treatment of Canadian *Danthonia* based on lodicule attributes, arguing that previous taxonomic schemes were based on characters with continuous variation, making species circumscription difficult. Since a phenetic analysis indicated that lodicule attributes were taxonomically informative (Baum and Findlay 1973), a subsequent study provided a new taxonomic treatment for Canadian *Danthonia* species (Findlay and Baum 1974). A new species, *D. canadensis*, was described based on club-shaped lodicules with truncate apices (Findlay and Baum 1974). The same study reported a wide range of lodicule variation. *D. californica* was characterized by "*californica*" type lodicule apices, *D. parryi* had fan-shaped lodicules, and lodicules were wanting in *D. sericea* and *D. spicata* (Findlay and Baum 1974). The Great Plains Flora Association (1986) contested this treatment and asserted that lodicule characters did not correlate with any other morphological characters examined, and thus rejected Findlay and Baum's (1974) taxonomic treatment.

As with lodicule attributes, fruit characters may also be taxonomically informative. The caryopsis is a unique fruit type and it is one of the distinguishing

features of the Poaceae (GPWG 2001). The caryopsis is similar to an achene because both are dry, indehiscent fruits; however, the pericarp is adnate to the seed coat in the caryopsis. Even though the pericarp is free in some grasses, these fruits are not considered achenes, but rather the free pericarp represents a modification of the caryopsis (Brandenburg 2003). Other examples of the variability of the pericarp (caryopsis surface) in grasses include differences in textural patterns (reticulate, verrucate, striate, substriate, tuberculate, regulate, echinate, psilate, lophate, and foveolate) (Jordan et al. 1983). Variability of surface texture of the caryopsis is taxonomically informative at specific and generic levels when examined with scanning electron microscopy (SEM) (Sendulsky et al. 1987). Some grass genera are clearly distinguished because all species share a common caryopsis surface pattern type (Jordan et al. 1983). Additionally, a suite of caryopsis characters including length and width of epicarp cells, the degree of concavity of the periclinal walls, and the shape of cell wall undulations, allow differentiation among European species of *Echinochloa* Beauv. (Costea and Tardif 2002) and Australian *Eragrostis* N.M. Wolf species (Lazarides 1997). Within the Chloridoideae, caryopsis traits, such as the ventral face and hilum morphology are useful at the tribal and generic levels (Liu et al. 2005). Similarly, caryopsis size, shape, beak, and degree of pericarp fusion are useful in differentiating species of *Diarrhena* P. Beauv. (Brandenburg et al. 1991). The structure of the transverse cells of the pericarp was useful in identifying fossilized grains of *Triticum* L. and *Secale* L. (Körber-Grohne 1981), and caryopsis morphology may represent an important link to the fossil record and assist in reconstructing phylogenetic relationships.

Though caryopsis micromorphology is poorly documented in danthonioid grasses, these characters may also be of taxonomic significance in this group. Seed and fruit characters have been useful in diverse groups of angiosperms, notably in the Hydrocharitaceae (Shaffer-Fehre 1991), Iridaceae (Manning and Goldblatt 1991), and Orchidaceae (Molvray and Kores 1995. In general, caryopsis morphology and its associated features are relatively poorly understood in danthonioid grasses, but previous studies have demonstrated its taxonomic utility. For instance, the obovoid caryopsis shape is a character shared by *Rytidosperma* and *Danthonia*, providing evidence of a close phylogenetic relationship between these two genera (Wright 1984). Moreover,

Barker (1994) described types of surface patterns in seven danthonioid genera, reporting the rugose type in *Karroochloa* and *Tribolium* Desv., scalariform-reticulate patterns in *Chaetobromus* Nees, *Pseudopentameris* Conert, *Merxmuellera*, and some species of *Pentaschistis* (Nees) Spach., and a deeply reticulate pattern in other species of *Pentaschistis*. The colliculate type was documented in *Pentameris* P. Beauv. A separate study reported the straight reticulate type in *Danthonia californica* (Jordan et al. 1983). Additional caryopsis characters of taxonomic utility in the Danthonioideae include the hilum, which is punctate in *Notodanthonia* and linear in *Danthonia* (Zotov 1963).

Because morphological traits are quite variable in the Danthonioideae, and it is often difficult to delimit species based solely on gross morphological and reproductive characters, an SEM survey of lodicules and caryopses in selected danthonioid grasses was undertaken. These two structures and their associated traits have not been examined thoroughly in the Danthonioideae, and previous reports regarding their taxonomic utility are inconsistent and add uncertainty to the systematics of this group. The general morphology and systematic utility of lodicule and caryopsis structures is examined for the subfamily. The goals of this study were 1) to present an overview of the micromorphological characters of lodicules and caryopses in danthonioid grasses, 2) to identify distinguishing features at the generic and specific levels, with emphasis on North American *Danthonia*, and 3) to assess the systematic significance and applicability of lodicule and caryopsis characters in this subfamily.

4.2 Materials and Methods

4.2.1 Taxonomic Sampling and Plant Material

A total of 23 taxa were investigated in this study. Of these, 21 belong to the Danthonioideae and include *Austrodanthonia*, *Cortaderia*, *Danthonia*, *Merxmuellera*, *Notodanthonia*, *Rytidosperma*, *Tribolium*, encompassing both Old and New World representatives (Table 4.1). Sampling included the eight North American *Danthonia* species (Table 4.1) recognized by Darbyshire (2003), *Danthonia decumbens* [syn. *Sieglingia decumbens* (L.) Bernh.] introduced from Europe, and seven species (*D. californica*, *D. compressa*, *D. intermedia*, *D. parryi*, *D. sericea*, *D. spicata*, *D. unispicata*) native to North America. In addition, *D. filifolia* [syn. *Danthonia secundiflora* subsp. *secundiflora* J. Presl.], a Mexican species, *D. cirrata* and

Table 4.1. Taxa investigated in this study, including their geographic distribution, and source of material. Institution acronyms: MO: Missouri Botanical Garden; ISC: Ada Hayden Herbarium; PI: U.S. Department of Agriculture Plant Introduction Center; SASK: University of Saskatchewan; SI: Instituto Botánica Darwinion; UAS: Universidad Autónoma de Sinaloa.

Taxon	Institution Acronym	Location, collector, date
Aristida purpurea Nutt.		
	PI 598971	USA. WA: Okanogan Co. 9.7 miles west of highway 155 on road to Omak Lake. Latitude: 48.220 North, Longitude: 119.251 West. *R. Johnson & T. Jones W6 16294.* 30/09/1994.
Arundo donax L.		
	UAS	MEXICO. Sinaloa. Km. 8 Carretera Los Mochis - Ahome. Plant about 3 m tall, growing along the roadside by a ditch. *J. H. Cota-Sánchez s/n.* 28/10/2005.
Austrodanthonia pilosa (R. Br.) H.P. Linder		
	ISC 210942	USA. CA: Alameda Co. (Introduced from Australia) *R. W. Pohl 7201* 11/09/1952.
	ISC 311043	USA. CA: Santa Clara Co. (Introduced from Australia) *J. T. Howell 35482.* 29/06/1960.
Cortaderia selloana (Schult. & Schult. F.) Asch. & Graebn.		
		Purchased from Whatcom Seed Company, native to South America.
Danthonia californica Bol.		
	PI 232247	USA. Original material collected in Arizona, California, Colorado, Idaho, Montana, Nevada, Oregon, Utah, Washington and Wyoming. *F. Hermann & B. Leese s/n.* July-September 1955.
D. cirrata Hack. & Arechav.		
	SI	ARGENTINA. Cordoba. Punilla entre copina y Pampa de Achida. *Krapovickas 7414.* 13/01/1951.
D. compressa Austin		
	MO 3179930	USA. NY: Old Farge, NY. Holly Cottages, larch. *Anonymous 3830*. 25/08/1960.
	MO 4269636	USA. SC: Greenville Co. Table Rock Reservoir watershed. Buzzard Mountain summit to Slicking Creek. Gneissic-granitic steep slope. Elev. 2000-2800 ft. *S. R. Hill 23571.* 26/07/1992.

Table 4.1. Continued.

Taxon	Institution Acronym	Location, collector, date
D. decumbens (L.) DC.		
	ISC 278438	COSTA RICA. Cartago. Upper slopes of Volcán Turrialba. Elev. 3000 m. *R. W. Pohl & G. Davidse 11053.* 27/08/1968.
D. filifolia F.T. Hubb.		
	ISC 356119	GUATEMALA. El Quiché. *M. J. Metzler 34*. 15/12/1978.
D. intermedia Vasey		
	MO 1639729	USA. CO: White River National Forest. West slope, Independence Pass. *G. B. Van Schaack 2629.* 06/08/1949.
	MO 2960585	USA. CA: San Mateo. Crystal Springs Lake. *A. D. E. Elmer 4707.* 12/04/1903.
D. montevidensis Hack. & Arechav.		
	SI	ARGENTINA. Buenos Aires. General Belgrano Rt. 3. *Burkart 28943.* 05/12/1971.
D. parryi Scribn.		
	ISC 242287	USA. WY: Albany Co. Hwy 130. 1/2 mi. W. of Centennial. Elev. 7500 ft. *J. P. Smith, Jr. 929.* 19/08/1964.
D. rhizomata Swallen		
	SI	URUGUAY. *Rosengurtt B-5267*. 21/11/1948.
D. sericea Nutt.		
	MO 1283726	USA. VA: Fortress Monroe. *J. W. Chickering, Jr. s/n* 30/05/1878.
	MO 2320822	USA. NC: Macon Co. Fodderstock Mountain, SE of highlands E. of Satalah Mountain. *D. Boufford 14673* 23/06/1974.
	MO 3735055	USA. AL: Houston Co. Sandy clay of longleaf pineland by US 84, 2 mi. W. of Columbia. *R. Kral 46175* 02/05/1972.

Table 4.1. Continued.

Taxon	Institution Acronym	Location, collector, date
D. spicata (L.) P. Beauv. *ex* Roem. & Schult.		
	MO 2120609	USA. MA: Bristol Co. Berkley. *F. C. Seymour 19334* 07/07/1961.
	ISC 347979	USA. MA: Hampshire Co. Northampton. *H. E. Ahles 86958* 25/06/1979.
	PI W6 19122	USA. NY: Blydenburgh County Park, Smithtown. Habitat: In very sandy soil along a horse trail. Latitude: 40°50'30" N, Longitude: 073°13'30" W. *D. Taub. 95-30* 13/07/1995.
D. unispicata (Thurb.) Munro *ex* Vasey		
	ISC 348077	USA. CA: Lassen Co. Open rocky flat W. of Susanville near confluence of Willard Creek & Susan River. Elev. 4700 ft. *J. T. Howell 52406* 22/06/1977.
	ISC 411417	USA. CA: Nevada Co. On old Hwy 40, 1/2 mi. S. of Norden near county line. Small bunch grass ~15 cm. Elev. 6900 ft. *G. Turner 86* No date.
	MO 1124121	USA. CA: Park Co. Clarks Fork of the Yellowstone River near Crazy Woman Creek, Shoshone National Park. Elev. 7000 ft. *L .O. & R. P. Williams 3692* 26/07/1937.
	MO 2960792	USA. WY: Laramie Park. *A. Nelson 1630* 07/08/1895.
Merxmuellera disticha (Nees.) Conert		
	PI 364332	SOUTH AFRICA. Original material collected from mountain road, 40 km SE of Maseru . Elev. 2250 m. *A. Oakes 1377* 6/15/1971.
	SASK 168165	Cultivated in U of S garden plot 29/09/2004.
Notodanthonia semiannularis (Labill.) Zotov		
	PI 210172	AUSTRALIA. Original material collected from Capital Terr. *W. Hartley s/n* 9/16/1953.
	SASK 168160	Cultivated in U of S Greenhouse 22/04/2004.

Table 4.1. Continued.

Taxon	Institution Acronym	Location, collector, date
Rytidosperma unarede (Raoul) Connor & Edgar		
	PI 237160	NEW ZEALAND. Original material collected from Christchurch. *Dept. of Scientific and Industrial Res*. 2/4/1957.
	SASK 168157	Cultivated in U of S garden plot 29/09/2004.
R. violacea (E. Desv.) Nicora		
	SI	ARGENTINA. Neuquen. Departamento de Ñorquín, Copahue. *Troiani & Steibel 15830*. 14/1/2004.
R. virescens (E. Desv.) Nicora		
	SI	ARGENTINA. Chubut. Tehuelches, Lago Vinter. *Nicora 10054*. 26/1/1995.
Tribolium echinatum (Thunb.) Renvoize		
	PI 238332	SOUTH AFRICA. Original material collected 26/03/1957.
	SASK 168162	Cultivated in U of S Greenhouse 02/09/2004.
T. hispidum (Thunb.) Desv.		
	PI 368889	SOUTH AFRICA. Original material collected from Langgewens Experimental Farm, north of Malmsbury. 1966.
	SASK 168158	Cultivated in U of S Greenhouse 02/09/2004.

D. montevidensis from Argentina, and *D. rhizomata* from Uruguay were included in the American group in this survey, but they are excluded from the FNA treatment. Although limited in sampling, South American taxa (one species of each of *Cortaderia* and *Rytidosperma*) were added to the survey for assessing potential trends in evolution between North American taxa and those from Central and South America. Also, a number of Danthonioideae genera from outside North America (Australia, New Zealand, South Africa, and South America) (Table 4.1) were included for comparative purposes. Caryopses of *Aristida purpurea* (Aristidoideae), a member of the sister subfamily to the Danthonioideae were examined in order to elucidate evolutionary trends of the morphological characters. Lodicules of *Arundo donax* in the Arundinoideae (within the PACCAD clade) were also examined (Table 4.1). Scientific names and taxonomic authorities follow the W^{3}TROPICOS nomenclatural database (Missouri Botanical Garden 2005).

Caryopses of *Danthonia spicata*, *D. intermedia*, and *D. californica*, as well as genera in the Danthonioideae (*Austrodanthonia*, *Cortaderia*, *Notodanthonia*, *Rytidosperma*, and *Tribolium*) and Aristidoideae (*Aristida*), were obtained from the Western Regional Plant Introduction Station (PI) in Pullman, Washington (Table 4.1). Caryopses of *Cortaderia selloana*, a cultivated ornamental grass, were ordered online from Whatcom Seed Company. The remaining samples of caryopses and lodicules were obtained from herbarium specimens preserved at the Missouri Botanical Gardens Herbarium (MO), Ada Hayden Herbarium (ISC), and the Instituto Botánica Darwinion (SI) (Table 4.1). Florets of *Arundo donax* were collected in Mexico (Table 4.1).

4.2.2 Specimen Selection and Preparation

Three specimens per species were selected at the anthesis stage of development for lodicule examination. Florets were chosen from the middle to lower third of each spikelet. The lemma of each floret was removed to expose the lodicules, and the floret was placed on an SEM stub with the palea facing down. Caryopsis length was measured with a stage micrometer using a stereomicroscope. Ten mature caryopses were measured whenever possible. Three mature and well-developed caryopses of each species were randomly selected for SEM examination. The caryopses were taken from different plant specimens whenever sufficient material was available. When fewer than three plant

samples were available, caryopses were chosen from different spikelets for examination. The caryopses were mounted on a stub with double sided tape. Some fruits were mounted with the hilum facing up and others with the embryo side facing up to allow examination of the ventral and dorsal surfaces of the caryopsis. The selected lodicule and caryopsis specimens were sputter-coated with gold in an Edwards Sputter Coater S150B and examined with a Philips 505 SEM. Photographs were taken with Polaroid 665 positive and negative film. Images of the entire caryopsis were recorded at magnifications between 20X and 85X depending on the size of the fruit. Surface detail was documented at 300X from the abaxial region, distal to the embryo and proximal to the style. Caryopsis length was measured from the tip of the point of attachment to the base of the stylar region using a stage micrometer and a stereomicroscope. Lodicule terminology follows Baeza (1996), but the term "lobed" is introduced here to describe lodicules with a lobed apex. Baum and Findlay (1973) described lobed lodicules as the "*californica* type", but this term was not adopted in this study because it is not restricted to *Danthonia californica*. Terminology for lodicule vestiture follows Watson and Dallwitz (1992 onwards). Although the term "bristly" has also been used in the Danthonioideae to describe lodicules with hair (Linder and Verboom 1996), Watson and Dallwitz (1992 onwards) were followed because broader taxonomic sampling in the latter facilitates comparison using standard terminology across the Poaceae. Similarly, although Barker (1994) described caryopsis surface textures for some danthonioid grasses, the terminology used here to describe caryopsis surface patterns follows Jordan et al. (1983). The latter included a wider taxonomic sampling, but Barker's (1994) study was based on limited sampling within the Poaceae, and did not encompass the range of variability that was observed. Descriptions of caryopsis shape are based on Clayton et al. (2002 onwards).

4.3 Results

4.3.1 Lodicules

The grass floret includes two outer bracts, a lemma and palea, plus the lodicules, androecium and gynoecium. The lodicules are fleshy flaps of tissue inside grass florets that are inserted on the floral axis distal to the palea and proximal to the androecium and

Table 4.2. Micromorphological characters investigated in the caryopsis of danthonioid grasses using scanning electron microscopy. The dash "-" indicates that material was not available. N/A: Not applicable. * immature caryopsis, single specimen examined

Taxon	Lodicules	Lodicule Apex	Lodicule Vestiture	Lodicule Hair Type	Mean Caryopsis Length (mm)	Caryopsis Shape	Hilum	Caryopsis Color	Caryopsis Surface Pattern
Aristida purpurea	Absent	N/A	N/A	N/A	10.5±1.2	Lanceolate	Linear	Brown	Substriate
Arundo donax	Present	Truncate	Glabrous	N/A	-	-	-	-	-
Austrodanthonia pilosa	Present	Truncate	Ciliate	Multicellular	1.6±0.10	Obovoid	Punctate	Brown	Substriate
Cortaderia selloana	-	-	-	-	2.5±0.18	Lanceolate	Linear	Brown	Undulating reticulate
Danthonia californica	Present	Lobed	Ciliate	Unicellular	4.6±0.18	Ovoid	Linear	Brown	Straight reticulate
D. cirrata	Present	Cuneate	Ciliate	Unicellular	2.4±0.42	Ovoid	Linear	Brown	Undulating reticulate
D. compressa	Present	Truncate	Glabrous	N/A	2.3±0.17	Obovoid	Linear	Brown	Undulating reticulate
D. decumbens	Absent	N/A	N/A	N/A	1.8±0.21	Ovoid	Linear	Brown	Straight reticulate
D. filifolia	Present	Truncate	Ciliate/ glabrous	Unicellular	1.9±0.19	Obovoid	Linear	Brown	Undulating reticulate
D. intermedia	Present	Truncate	Glabrous	N/A	3.4±0.25	Ovoid	Linear	Brown	Undulating reticulate
D. montevidensis	Absent	N/A	N/A	N/A	1.9±0.36	Ovoid	Linear	Brown	Undulating reticulate

Table 4.2. Continued.

Taxon	Lodicules	Lodicule Apex	Lodicule Vestiture	Lodicule Hair Type	Mean Caryopsis Length (mm)	Caryopsis Shape	Hilum	Caryopsis Color	Caryopsis Surface Pattern
D. parryi	Present	Truncate	Glabrous	N/A	4.3±0.39	Ovoid	Linear	Brown	Straight reticulate
D. rhizomata	Present	Cuneate	Glabrous	N/A	2.2±0.42	Ovoid	Linear	Brown	Undulating reticulate
D. sericea	Present	Lobed	Glabrous	N/A	1.8±0.21	Obovoid	Linear	Brown	Undulating reticulate
D. spicata	Present	Cuneate	Glabrous	N/A	2.4±0.20	Obovoid	Linear	Brown	Undulating reticulate
D. unispicata	Present	Cuneate	Ciliate/ glabrous	Unicellular	3.4±0.26	Ovoid	Linear	Brown	Straight reticulate
Merxmuellera disticha	-	-	-	-	3.5±0.22	Lanceolate	Linear	Brown	Substriate
Notodanthonia semiannularis	-	-	-	-	1.7±0.13	Obovoid	Punctate	Brown	Substriate
Rytidosperma unarede	-	-	-	-	2.0±0.15	Ovoid	Punctate	Brown	Substriate
R. violacea	Present	Truncate	Ciliate	Multicellular	1.1*	Ovoid	Punctate	Brown	Undulating reticulate
R. virescens	-	-	-	-	1.9±0.24	Ovoid	Punctate	Brown	Undulating reticulate
Tribolium echinatum	Present	Truncate	Ciliate	Multicellular	1.2±0.61	Obovoid	Punctate	Brown	Substriate
T. hispidum	-	-	-	-	1.1±0.53	Obovoid	Punctate	Brown	Substriate

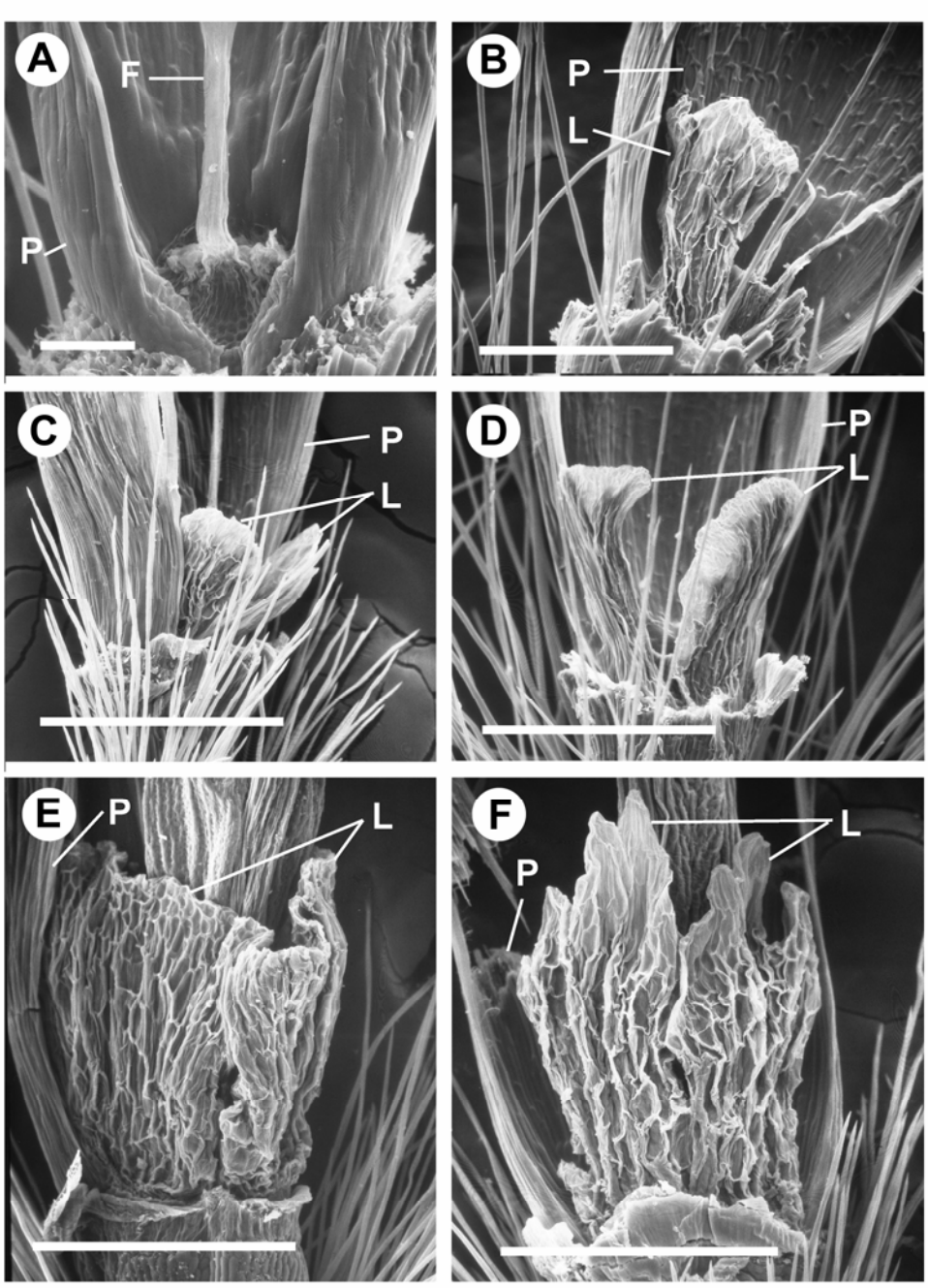

Fig. 4.1. Micrographs showing lodicule apex types in danthonioid grasses. A) Floret of *Danthonia montevidensis* showing the palea (P) and filament (F) of an anther. Lodicules are absent. B) Cuneate lodicule (L) apex in front of the palea (P) in *D. rhizomata*. C) Cuneate lodicule (L) apices in front of the palea (P) in *D. unispicata*. D) Truncate lodicule (L) apices in front of the palea (P) of *D. filifolia.* E) Truncate lodicule (L) apices in front of the palea (P) of *D. parryi*. F) Lobed lodicule (L) apices in front of the palea (P) of *D. sericea*. Scale bar in Fig. 4.1A = 0.1 mm. Scale bar in Figs. 4.1B to 4.1F = 0.5 mm.

gynoecium (Figs. 4.1A to 4.1F and 4.2A to 4.2D). They may be present or absent in the grass florets. Lodicules are absent in *Aristida purpurea*, *Danthonia decumbens* and *D. montevidensis* (Fig. 4.1A), and present in *Arundo donax*, *Austrodanthonia pilosa*, *D. californica*, *D. cirrata*, *D. compressa*, *D. filifolia* (Fig. 4.1D), *D. intermedia*, *D. parryi* (Fig. 4.1E), *D. rhizomata* (Fig. 4.1B), *D. sericea* (Fig. 4.1F), *D. spicata*, *D. unispicata* (Fig. 4.1C), *Rytidosperma violacea*, and *Tribolium echinatum* (Table 4.2). Due to the lack of representative material, these structures could not be studied in *Cortaderia selloana*, *Merxmuellera disticha*, *Notodanthonia semiannularis*, *Rytidosperma unarede*, *R. virescens*, and *Tribolium hispidum* (Table 4.2).

4.3.2 Lodicule Apex

The lodicule apices in the florets of danthonioid grasses are variable. Three lodicule apex types were identified in the subfamily: 1) cuneate, 2) truncate, and 3) lobed.

The cuneate lodicule apex is characterized by a lodicular apex that tapers to a point as in *Danthonia cirrata*, *D. rhizomata* (Fig. 4.1B), *D. spicata*, and *D. unispicata* (Fig. 4.1C; Table 4.2). In the truncate lodicule apex, the lodicular apex is straight across the top. The truncate apex seems to be the most common type and is found in *Arundo donax*, *Austrodanthonia pilosa*, *Danthonia compressa*, *D. filifolia* (Fig. 4.1D), *D. intermedia*, *D. parryi* (Fig. 4.1E), *Rytidosperma violacea*, and *Tribolium echinatum* (Table 4.2). The lobed lodicule apex has several irregular lobes. It was only observed in *Danthonia californica* and *D. sericea* (Fig. 4.1F; Table 4.2). The lobed lodicule apex as described here corresponds to the "*californica*" type described by Baum and Findlay (1973). The term "lobed" is introduced here for the first time. This term is more appropriate to describe this type of lodicule apex because it is not limited to *D. californica*, and it may also occur in other species.

4.3.3 Lodicule Vestiture

Lodicules may be glabrous (Figs. 4.2A and 4.2B) or have vestiture of unicellular or multicellular hairs (Figs. 4.2C to 4.2F). Glabrous lodicules are quite common in the taxa examined and are characteristic of *Arundo donax* (Fig. 4.2B), *Danthonia compressa*, *D. filifolia*, *D. intermedia*, *D. parryi*, *D. rhizomata*, *D. sericea*, *D. spicata* (Fig. 4.2A), and *D. unispicata* (Table 4.2). The vestiture of ciliate lodicules may be

composed of unicellular (Figs. 4.2C and 4.2D) or multicellular hairs (Figs. 4.2E and 4.2F). Ciliate lodicules with unicellular hairs were observed in *D. californica*, *D. cirrata* (Fig. 4.2D), *D. filifolia* (Fig. 4.2C), and *D. unispicata* (Table 4.2). Vestiture with multicellular hairs is characteristic of *Austrodanthonia pilosa* (Fig. 4.2F), *Rytidosperma violacea*, and *Tribolium echinatum* (Fig. 4.2E; Table 4.2). In addition, lodicule vestiture may be polymorphic in some taxa. For example, *D. filifolia* (Fig. 4.2C), and *D. unispicata* have both glabrous lodicules and lodicules with unicellular hairs in different individuals within the same species (Table 4.2). The glabrous lodicule condition seems prevalent in *Danthonia*, but when lodicule vestiture is present, hairs are unicellular. Lodicule vestiture is not applicable in *D. decumbens* and *D. montevidensis* (Table 4.2) because lodicules are absent in these species. Material was unavailable for *Cortaderia selloana*, *Merxmuellera disticha*, *Notodanthonia semiannularis*, *Rytidosperma unarede*, *R. virescens*, *Tribolium echinatum*, and *T. hispidum* (Table 4.2), hence this character remains unknown for these danthonioid taxa.

4.3.4 Caryopsis Length

In general, caryopsis length is quite variable in the study group, ranging from 1.1 mm in *Tribolium hispidum* to 10.5 mm in *Aristida purpurea*. Among danthonioid taxa, caryopses were longest in *Danthonia californica* (4.6 mm), less than one-half the length of *A. purpurea* caryopses. Within the Danthonioideae, caryopsis length ranges from 1.1 mm in *Tribolium hispidum* to 4.6 mm in *D. californica*. Caryopsis length was continuous in the danthonioid genera. Most danthonioid taxa fall within the range observed in *Danthonia* (1.8 to 4.6 mm), with the exception of *Notodanthonia seminannularis* (1.7 mm) and *Tribolium*, which is characterized by a small fruit (*T. hispidum* at 1.1 mm, and *T. echinatum* at 1.2 mm) (Table 4.2). Caryopsis length in *Rytidosperma violacea* is only 1.1 mm, but this caryopsis was immature and cannot be compared to the remainder of the caryopses examined.

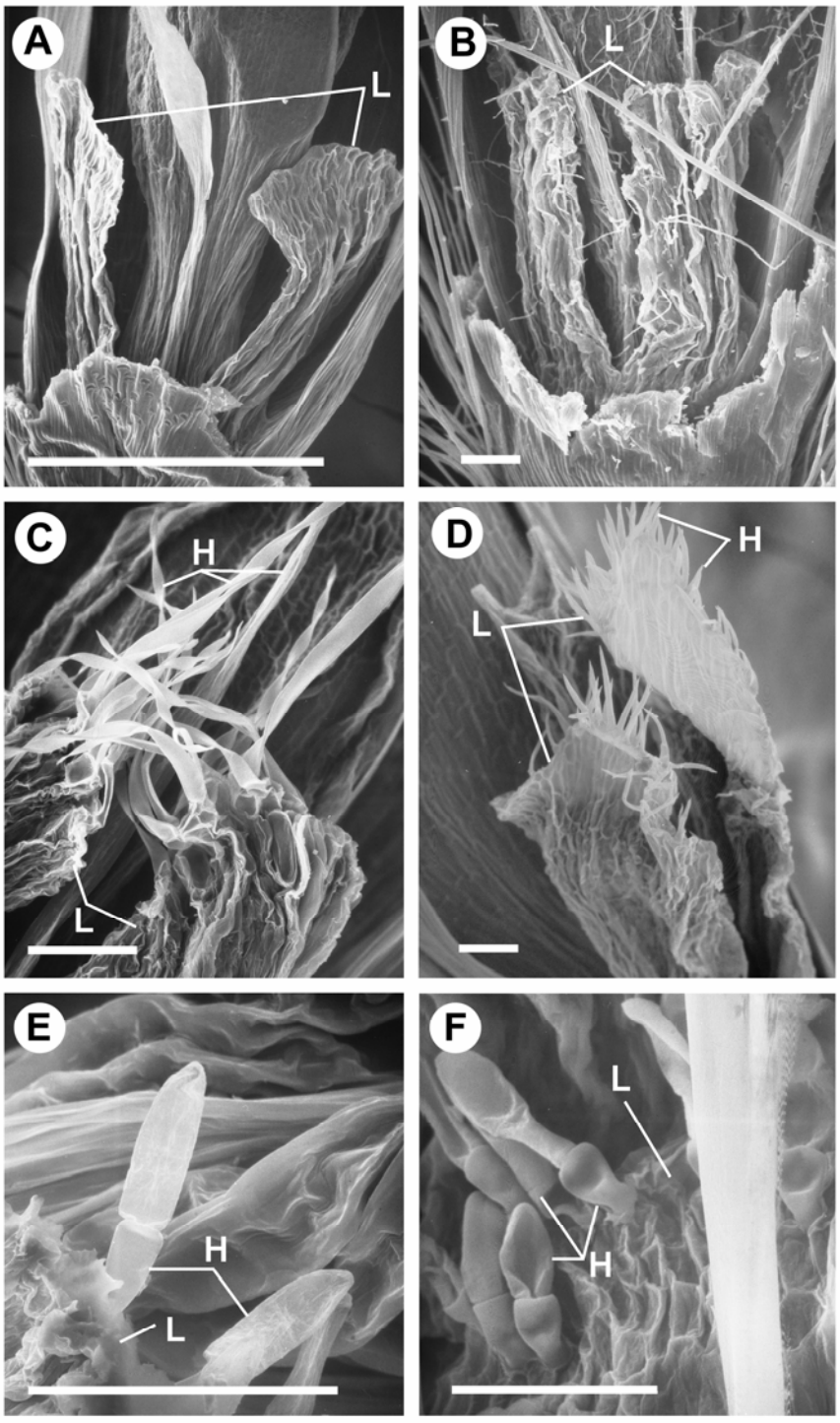

Fig. 4.2. Micrographs showing lodicule vestiture types in grasses. A) Glabrous lodicules (L) in *Danthonia spicata*. B) Glabrous lodicules (L) in *Arundo donax*. C) Ciliate lodicule (L) vestiture with unicellular hairs (H) in *D. filifolia*. D) Ciliate lodicule (L) vestiture with unicellular hairs (H) in *D. cirrata*. E) Ciliate lodicule (L) vestiture with multicellular hairs (H) in *Tribolium echinatum*. F) Ciliate lodicule (L) vestiture with multicellular hairs (H) in *Austrodanthonia pilosa*. Scale bar in Fig. 4.2A = 0.5 mm. Scale bar in Figs. 4.2B to 4.2D = 0.1 mm. Scale bar in Figs. 4.2E and 4.2F = 0.05 mm.

4.3.5 Caryopsis Shape

Caryopsis shape is described by the outline of the caryopsis in surface view (Clayton et al. 2002 onwards). In this survey, three major types of caryopsis shapes were identified: obovoid, ovoid, and lanceolate (Figs. 4.3 and 4.4).

Obovoid caryopses are typically narrowed towards the pedicel, but they are widest past the middle region and towards the stylar end, as seen in *Danthonia spicata* (Fig. 4.3A), *D. sericea* (Fig. 4.3B), *Austrodanthonia pilosa* (Fig. 4.4C), *R. virescens* (Fig. 4.4F), and *Tribolium echinatum* (Fig. 4.4D). Obovoid caryopses appear to be relatively common in danthonioid taxa and were also observed in *D. compressa*, *D. filifolia*, *Notodanthonia semiannularis*, and *Tribolium hispidum* (Table 4.2). At the generic level, the obovoid caryopsis is characteristic of *Tribolium*.

Ovoid caryopses are widest at the mid-point, tapering at both ends. This caryopsis shape is the most common type observed in the danthonioid taxa examined, and is present in *Danthonia californica* (Fig. 4.3D), *D. cirrata*, *D. decumbens*, *D. intermedia* (Fig. 4.3C), *D. montevidensis*, *D. parryi*, *D. rhizomata*, *D. unispicata*, *Rytidosperma unarede* (Fig. 4.4E), and *R. violacea* (Table 4.2). *Danthonia* and *Rytidosperma* exhibit a combination of ovoid and obovoid caryopses.

Lanceolate caryopses are at least three times as long as broad. Lanceolate caryopses are uncommon in the Danthonioideae, and were observed in *Cortaderia selloana* (Fig. 4.4B) and *Merxmuellera disticha*. This shape was also observed in the Aristidoideae represented by *Aristida purpurea* (Fig. 4.4A; Table 4.2).

4.3.6 Hilum Shape

The hilum in grasses is the point of attachment of the funiculus to the inner ovary wall, and is visible on the ventral surface of the caryopsis (Figs. 4.3 and 4.4). In this survey, linear and punctate hilum shapes were identified in the Danthonioideae.

The linear hilum is narrow and elongated along the main axis of the caryopsis. This was the most common type observed in this survey and is seen in the Aristidoideae represented by *Aristida purpurea* (Fig. 4.4A), and in the danthonioid representatives, namely *Cortaderia selloana* (Fig. 4.4B), *D. californica* (Fig. 4.3D), *D. cirrata*, *D. compressa*, *D. decumbens*, *D. filifolia*, *D. intermedia* (Fig. 4.3C), *D. montevidensis*, *D. parryi*, *D. rhizomata*, *D. sericea* (Fig. 4.3B), *D. spicata* (Fig. 4.3A), *D. unispicata*, and *Merxmuellera disticha* (Table 4.2). As a genus *Danthonia* is characteristically

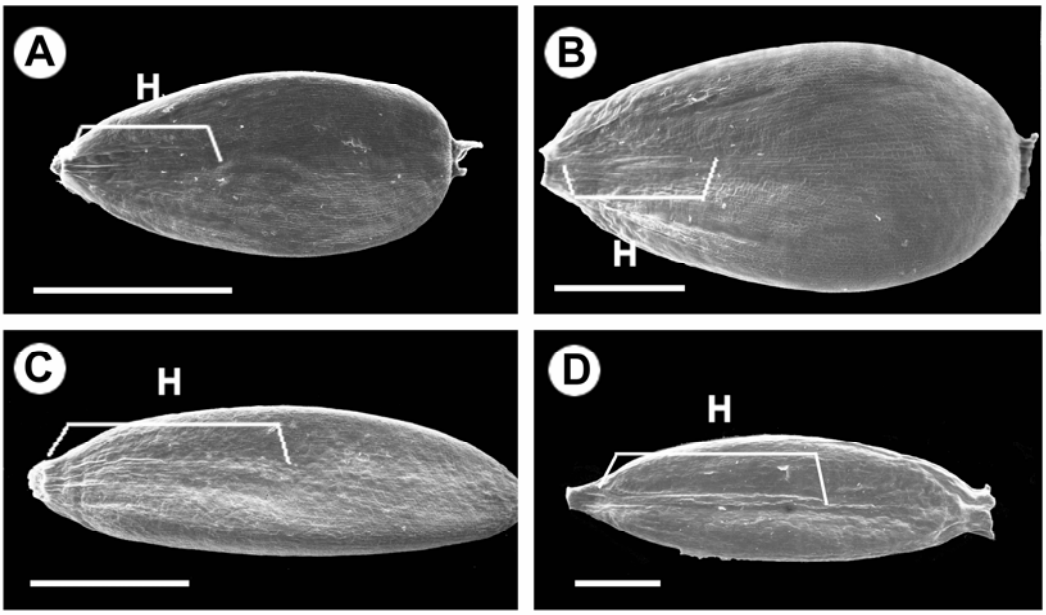

Fig. 4.3. Micrographs showing caryopsis shapes with linear hilum in *Danthonia*. A) Obovoid caryopsis with linear hilum (H) in *D. spicata.* B) Obovoid caryopsis with a linear hilum (H) in *D. sericea*. C) Ovoid caryopsis with linear hilum (H) in *D. intermedia*. D) Ovoid caryopsis with linear hilum (H) in *D. californica*. Scale bar = 1 mm.

distinguished by the linear hilum type, but this type also occurs in *Cortaderia* (Fig. 4.4B), *Merxmuellera*, and *Aristida purpurea* (Fig. 4.4A; Table 4.2).

A short punctate hilum is broad, and partially sunken into the caryopsis as seen in *Austrodanthonia pilosa* (Fig. 4.4C), *Notodanthonia semiannularis*, *Rytidosperma unarede* (Fig. 4.4E), *R. violacea*, *R. virescens* (Fig. 4.4F), *Tribolium echinatum* (Fig. 4.4D), and *T. hispidum* (Table 4.2).

4.3.7 Caryopsis Color

Overall, caryopsis color is homogeneous among the taxa sampled. All specimens examined have brown caryopses (Table 4.2), indicating that color is not a taxonomically informative character in the study group.

4.3.8 Caryopsis Surface Pattern

The caryopsis surface pattern is created by the shape and distribution of cells in the epicarp layer of the caryopsis (Figs. 4.5 to 4.6). Surface detail was observed on the abaxial surface, between the embryo and style. Descriptions of the caryopsis surface patterns in the Danthonioideae are scarce. This survey indicates that the caryopsis

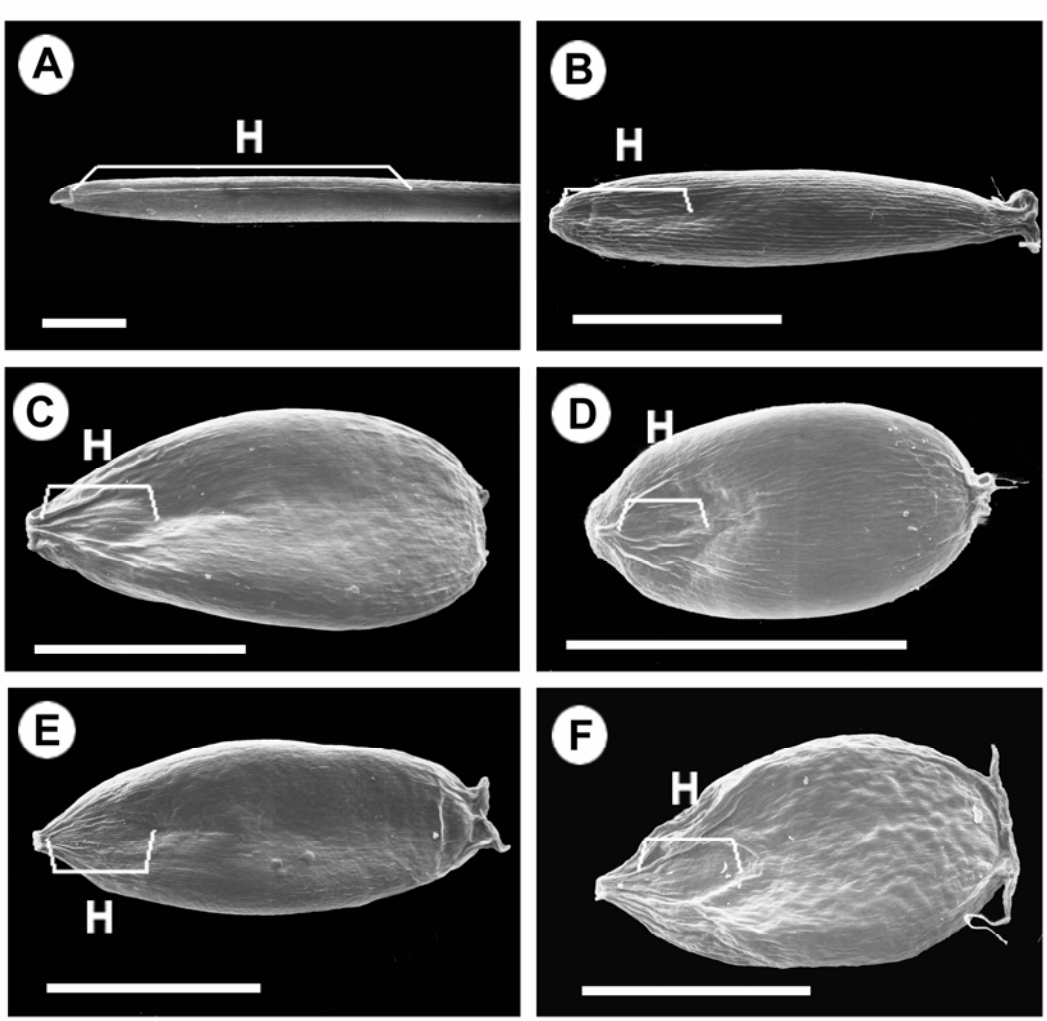

Fig. 4.4. Micrographs showing caryopsis shapes and hilum types in selected representatives of the Poaceae. A) Lanceolate caryopsis with linear hilum (H) in *Aristida purpurea*. B) Lanceolate caryopsis with linear hilum (H) in *Cortaderia selloana*. C) Obovoid caryopsis with short punctate hilum (H) in *Austrodanthonia pilosa*. D) Obovoid caryopsis with short punctate hilum (H) in *Tribolium echinatum*. E) Ovoid caryopsis with short punctate hilum (H) in *Rytidosperma unarede*. F) Obovoid caryopsis with short punctate hilum (H) in *Rytidosperma virescens*. Scale bar = 1 mm.

surface pattern is variable among danthonioid genera. Three caryopsis surface patterns were identified in the species investigated, including straight reticulate, undulating reticulate, and substriate.

The straight reticulate caryopsis surface pattern is characterized by the presence of straight walls with cells arranged in an imbricate pattern as seen in *Danthonia parryi* (Fig. 4.5A) and *D. unispicata* (Fig. 4.5B). This type of pattern was also observed in *D. californica* and *D. decumbens* (Table 4.2).

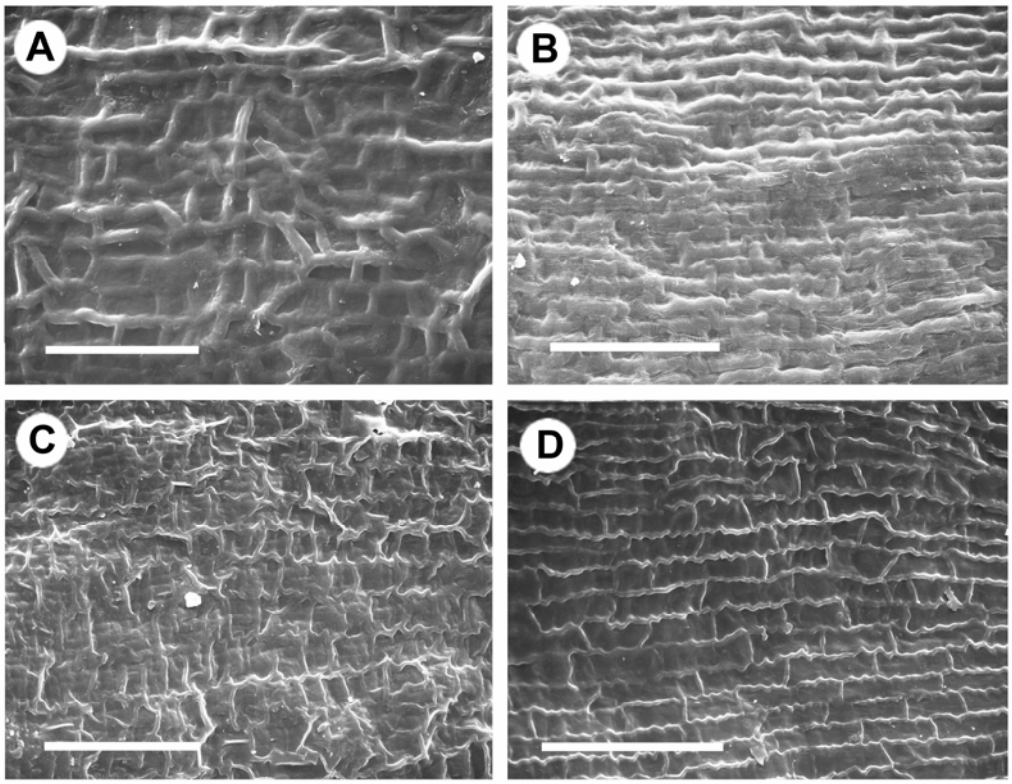

Fig. 4.5. Micrographs showing caryopsis surface patterns in *Danthonia*. A) Straight reticulate pattern in *D. parryi*. B) Straight reticulate pattern in *D. unispicata*. C) Undulating reticulate pattern in *D. intermedia*. D) Undulating reticulate pattern in *D. spicata*. Scale bar = 0.1 mm.

The undulating reticulate pattern is similar to the straight reticulate surface, but the anticlinal and periclinal walls of the cells on the caryopsis surface are characteristically undulating as seen in *Cortaderia selloana* (Fig. 4.6D), *Danthonia intermedia* (Fig. 4.5C), and *D. spicata* (Fig. 4.5D). This is the most common caryopsis surface pattern among the danthonioid taxa investigated. It was also observed in *D. cirrata*, *D. compressa*, *D. filifolia*, *D. montevidensis*, *D. rhizomata*, *D. sericea*, *Rytidosperma violacea*, and *R. virescens* (Table 4.2). The periclinal and anticlinal cell walls on the caryopsis surface in the undulating reticulate pattern vary in the amplitude of undulation. It is possible that the caryopsis surface patterns exhibit a continuous range of variation from very obvious undulations to completely straight walls. An intermediate pattern wherein the cells form a reticulate pattern (i.e. brick wall-like pattern) and the walls are only slightly undulating was observed in *D. unispicata* (Fig. 4.5B), which were included in the straight reticulate group.

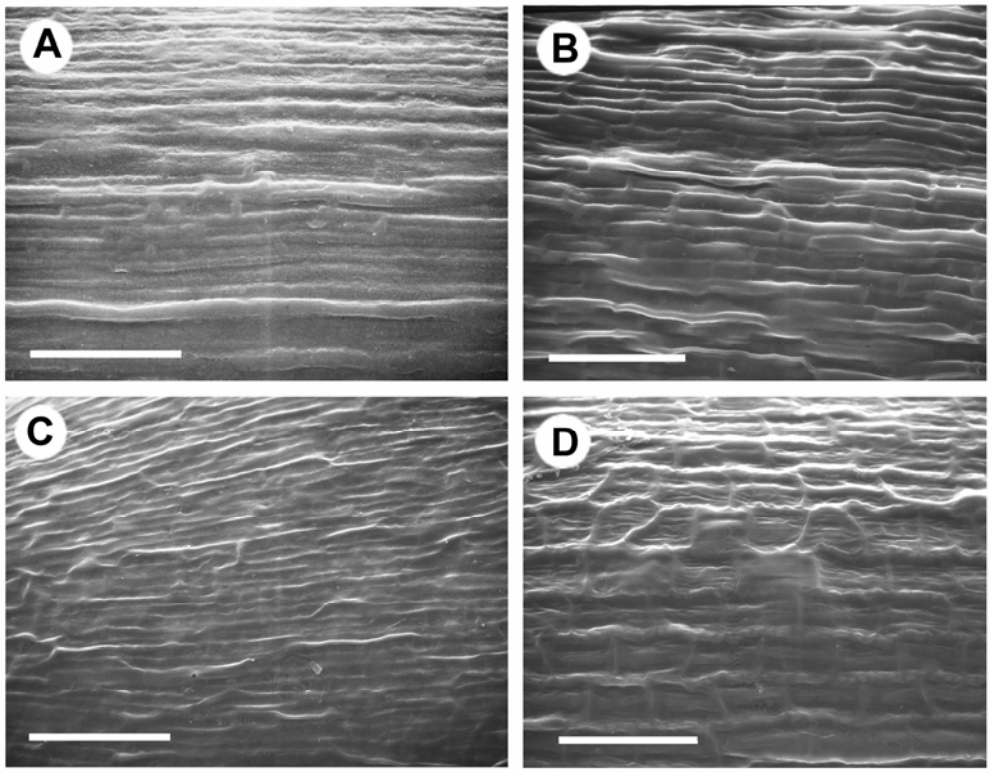

Fig. 4.6. Micrographs showing caryopsis surface patterns in selected representatives of the Poaceae. A) Substriate pattern in *Aristida purpurea*. B) Substriate pattern in *Merxmuellera disticha*. C) Substriate pattern in *Tribolium hispidum*. D) Undulating reticulate pattern in *Cortaderia selloana*. Scale bar = 0.1 mm.

Cells on the caryopsis surface in the substriate pattern type are long and narrow, and they are arranged in longitudinal rows to create a striped pattern. This pattern was observed in *Aristida purpurea* (Fig. 4.6A), *Austrodanthonia pilosa*, *Merxmuellera disticha* (Fig. 4.6B), *Notodanthonia semiannularis*, *Rytidosperma unarede*, *Tribolium echinatum*, and *T. hispidum* (Fig. 4.6C; Table 4.2).

4.4 Discussion

This survey provides new information regarding the caryopsis and lodicule characters, expanding our knowledge of the systematics of the Danthonioideae. Several characters, such as the length and shape of the hilum, caryopsis shape and surface pattern provide insight into the taxonomy of some taxa examined.

4.4.1 Lodicule Characters

The presence or absence of lodicules and characters associated with these structures may provide useful taxonomic information (Hsu 1965; Baum and Findlay

1973; Findlay and Baum 1974). However, the present study does not support these observations. The lodicule data in this study suggests lodicule characters are of little use to delimit genera or species complexes in the Danthonioideae.

Lodicules may be present or absent. Their absence may be due to a variety of developmental factors, especially floral age. Typically, lodicules are not retained in mature florets. Additionally, lodicules fail to develop in many cleistogamous grasses such as *Microlaena* R.Br., *Thyridolepis* S.T. Blake, and *Calyptochloa* C.E. Hubb. (Connor 1979). Philipson (1986) reported that cleistogamous and chasmogamous aerial florets are present in the same inflorescence of *D. spicata*. Furthermore, lodicules are present in chasmogamous florets of *Danthonia* species, and absent in cleistogamous florets of the same species (Dore and McNeill 1980). The apparent absence of lodicules does not necessarily indicate degree of phylogenetic relationships because lodicules may be present elsewhere in the inflorescence of the same plant. Findlay and Baum's (1974) taxonomic treatment has not been widely accepted for these reasons. Zuloaga et al. (2003) did not recognize *D. canadensis*, a species described by Findlay and Baum (1974) using lodicule characters, but instead they consider it a synonym of *D. intermedia*. Similarly, the Great Plains Flora Association (1986) has rejected such treatment because lodicule characters do not correlate with any other character examined. Furthermore, Findlay and Baum's (1974) treatment results in a sympatric distribution of the Canadian *Danthonia* species (Boivin 1981), contradicting the current accepted geographical ranges of North American *Danthonia* as proposed by Darbyshire (2003). Lodicule characters seem to be unreliable diagnostic features at the specific level because of developmental variability.

In general, lodicule attributes provide no phylogenetic resolution in the Danthonioideae at the generic and specific levels. The lodicule apex is not a useful character for distinguishing any danthonioid genera. The truncate apex type is a common character in *Danthonia*, but it is also present in *Arundo donax*, *Austrodanthonia pilosa*, and *Rytidosperma violacea* (Table 4.2). Contrary to Baum and Findlay (1973), lodicules are present in *D. sericea* and *D. spicata*. The presence, shape, and size of lodicules are apparently not consistent within a species. In addition, Baum and Findlay (1973) reported fan-shaped lodicules in *D. parryi*, whereas lodicules with truncate apices were

observed in this species in the present study (Fig. 4.1E). The disagreement between the present study and Baum and Findlay's (1973) suggests high variability in the lodicule apex within a species or between florets at various developmental stages. Overall, variability in the presence and shape of lodicules within species limits the taxonomic utility of lodicule attributes.

As with the lodicule apex, the lack of data for several taxa makes interpretation of lodicule vestiture problematic. In spite of missing representative taxa, the present study indicates that ciliate lodicules are present in *Danthonia* (Table 4.2), and they may not be as rare as previously reported by Baeza (1996). *D. californica*, *D. cirrata*, *D. filifolia*, and *D. unispicata* have ciliate lodicules (Table 4.2). Linder and Verboom (1996) prefered the term "bristly" in *D. unispicata*. Lodicules of *D. filifolia* and *D. unispicata* are ciliate in some specimens and glabrous in others. The occurrence of both ciliate and glabrous lodicules was documented in other danthonioid taxa, such as *Merxmuellera*, *Schismus*, and *Chionochloa* (Wright 1984). Lodicule vestiture shows intraspecific variation and it may not provide reliable taxonomic information. Quite possibly, the presence or absence of hairs on the lodicule may be environmentally influenced or may be affected by specimen preparation.

The distinctive multicellular hairs on the lodicules of *Austrodanthonia pilosa*, *Rytidosperma violacea*, and *Tribolium echinatum* characteristically have two (Fig. 4.2E) or three cells (Fig. 4.2F). These structures were not observed in any other danthonioid specimen examined in this survey (Table 4.2). Wright (1984) previously reported multicellular hairs on the lodicules in the Danthonioideae, specifically in *Chionochloa*, *Karroochloa*, *Pyrrhanthera*, *Rytidosperma* and some species of *Merxmuellera*. The presence of multicellular hairs in *Rytidosperma* was confirmed by this study. Furthermore this feature was reported here in *Austrodanthonia* (*A. pilosa*) and *Tribolium* (*T. echinatum*) for the first time. On the other hand, multicellular hairs were absent in *Danthonia*, *Plinthanthesis*, and *Cortaderia* (Wright 1984). *Danthonia* and *Plinthanthesis* are included in the *Danthonia* clade proposed by Barker et al. (2000). It is interesting that both of these taxa lack multicellular microhairs on the lodicules. This clade also includes *Notochloe*, which was not included in Wright's (1984) study. Conversely, *Austrodanthonia*, *Cortaderia*, *Notodanthonia*, and *Tribolium* have ciliate lodicules

(Watson and Dallwitz 1992 onwards), but it is unclear whether this report refers to unicellular or multicellular hairs. Multicellular hairs on the lodicule may have taxonomic utility in the Danthonioideae, but a much broader taxonomic sampling and detailed observations are required before conclusions can be drawn about the diagnostic value of this character.

It is unfortunate that no taxonomic conclusions can be drawn from lodicule shape, vestiture, and other attributes in the present study. Lodicules are apparently lacking in *Aristida purpurea*, but these structures are present in other representatives of the genus, e.g. *A. pubescens* Caro & E. A. Sánchez (Clayton et al. 2002 onwards). Lodicules are also absent in unrelated species in other subfamilies, therefore, the loss of lodicules has likely occurred several times in the grass family, and may have undergone multiple losses in the Danthonioideae as well. In all, although lodicule characters do not provide substantial taxonomic information, this study provides an overview of these structures that can be used in future studies.

4.4.2 Caryopsis Characters

Caryopsis size has been one of several taxonomically useful characters in *Diarrhena* (Brandenburg et al. 1991), and in the Triticeae (Terrell and Peterson 1993). Similarly, caryopsis length is taxonomically informative at the generic level in the Danthonioideae. A caryopsis less than 4 mm in length is small, compared to a range of 4 to 10 mm for medium caryopses, and over 10 mm for large caryopses (Watson and Dallwitz 1992 onwards). Thus, compared to other grasses such as *Aristida purpurea* (with a caryopsis length of 10.5 mm), danthonioid grasses generally have small caryopses (from 1.1 mm to 4.6 mm), though *Danthonia californica* (4.6 mm) and *D. parryi* (4.3 mm) have medium caryopses. Likewise, caryopses tend to be larger in *Danthonia* relative to other danthonioid genera, i.e. *Austrodanthonia*, *Notodanthonia*, *Rytidosperma*, and *Tribolium* (Table 4.2). Veldkamp (1980) concluded that *Danthonia* caryopses are larger than those of *Notodanthonia* (although Veldkamp's circumscription of *Notodanthonia* included *Rytidosperma* and *Austrodanthonia*). This survey indicates that the caryopses in *Tribolium* species and *Notodanthonia semiannularis* are the smallest within the danthonioid taxa, a difference which may be of taxonomic importance (Table 4.2). The two species of *Tribolium* investigated in this survey have

the smallest caryopses in the Danthonioideae (Table 4.2). Further inferences based on caryopsis size cannot be made because of the limited number of specimens and small sample sizes were not ideal for conducting statistical analyses. Caryopsis lengths are provided as general information that may be useful in subsequent studies of the subfamily.

Caryopsis color is not of taxonomic relevance as this character does not discriminate among danthonioid grasses. The caryopses of danthonioid grasses are consistently brown in color, though this character is not restricted to the Danthonioideae, as seen by its appearance in *Aristida purpurea* (Table 4.2). Brown caryopses have also been reported in the Chloridoideae (Liu et al. 2005), and Pooideae (Watson and Dallwitz 1992 onwards), in which color has no further taxonomic application.

Conversely, caryopsis shape provides some insight into the systematics of danthonioid grasses. *Danthonia*, *Austrodanthonia*, *Notodanthonia*, and *Rytidosperma* all share the characteristic ovoid or obovoid caryopsis, which has been cited as evidence of a close taxonomic relationship among these taxa (Wright 1984). The taxonomic implications of caryopsis shape are as follows: *Danthonia* and *Rytidosperma* have ovoid and obovoid caryopses, while the species of *Tribolium* investigated have obovoid caryopses (Table 4.2). The obovoid caryopsis shape was also observed in *Austrodanthonia pilosa*, *Notodanthonia semiannularis* (Table 4.2), which concurs with previous reports in these genera (Watson and Dallwitz 1992 onwards); however, these authors report obovoid caryopses in *Rytidosperma*, which is in disagreement with the ovoid type found in this survey. Caryopsis shape is likely variable because *Rytidosperma* has many species.

Cortaderia, *Merxmuellera*, and *Aristida* have the uncommon lanceolate caryopsis shape (Table 4.2). This shared character is likely due to convergence. The presence of this character state in the representative of the Aristidoideae suggests that this may be the plesiomorphic condition; however, a single representative of each genus was examined, limiting the ability to assess the taxonomic distribution of this trait.

This survey provides further evidence that the hilum can be used as a distinguishing feature at the generic level in the Danthonioideae, as corroborated by earlier studies (Zotov 1963; Baeza 1996). Previous literature also stated that *Danthonia*

has long, linear hila, versus short hila in *Austrodanthonia*, *Notodanthonia*, *Rytidosperma*, and *Tribolium* (Watson and Dallwitz 1992 onwards). However, *D. secundiflora* reportedly possesses a short oblong hilum (Linder and Verboom 1996), but this species was not included in the present study. This species may represent an exception to the general trend of linear hila in *Danthonia*.

Short punctate hila are reported in *Austrodanthonia*, *Notodanthonia*, *Rytidosperma*, and *Tribolium* (Watson and Dallwitz 1992 onwards), four genera that belong to the *Rytidosperma* clade (Barker et al. 2000). Although the *Rytidosperma* clade is large and diverse, the short punctate hilum is prevalent in this group. A single exception, *Merxmuellera disticha* (Table 4.2), has a linear hilum. Thus, the hilum type provides support for the monophyly of the *Rytidosperma* clade, which is further supported by the presence of tufted lemma indumentum seen in *Austrodanthonia*, *Notodanthonia*, and *Rytidosperma*, as opposed to the scattered or marginal lemma hairs in *Danthonia* (Linder and Verboom 1996; E. Reimer, pers. obs.).

The evolution of hilum type in the Danthonioideae cannot be discerned in this study due to lack of taxonomic sampling in the basal *Merxmuellera* assemblage as described by Barker et al. (2000). However, it is feasible that the punctate hilum observed in the *Rytidosperma* clade may have evolved from an ancestor with a linear hilum. The *Cortaderia* "A" clade includes *Cortaderia selloana*, and this clade is basal to all the taxa included in this survey. Thus the linear hilum present in *Cortaderia selloana* (Fig. 4.4B) may represent the plesiomorphic character state in the subfamily. In addition, linear hila are also observed in the Aristidoideae, sister to the danthonioid grasses, as seen in *Aristida purpurea* (Table 4.2). Further evidence of the plesiomorphic condition of the linear hilum is given by the presence of this character state in all the basal lineages (Anomochlooideae, Pharoideae, and Puelioideae) of the Poaceae (GPWG 2001). However, recent data indicates that hilum shape appears to have undergone several reversals in the family. Within the PACCAD clade, the Panicoideae, Arundinoideae, Centothecoideae, and Chloridoideae are distinguished by a short hilum, but this character reverts back to the linear state in the Danthonioideae and Aristidoideae (GPWG 2001).

Only a few examples of caryopsis surface patterns in the Danthonioideae are available in the literature. *Danthonia californica* has a straight reticulate pattern (Jordan et al. 1983), but the surface patterns for other species in this genus have not been reported. The possible taxonomic utility of the caryopsis surface pattern in danthonioid grasses was first reported by Barker (1994), who concluded that *Chaetobromus* and *Pseudopentameris* are related based on a shared rugose caryopsis surface pattern. Although Barker (1994) described caryopsis surface patterns for *Merxmuellera* and *Tribolium* as scalariform-reticulate and rugose respectively, the substriate surface pattern was observed in these taxa in the present study. This discrepancy may reflect inconsistencies in the terminology used to describe surface patterns of caryopses. Terminology in the present study follows Jordan et al. (1983), who included neither the rugose nor scalariform-reticulate types of caryopsis surface patterns. It is therefore difficult to compare the surface patterns to Barker's (1994). Perhaps this suggests the need to establish a standard and consistent general terminology for types of caryopsis surface patterns in grasses.

Several taxonomic inferences can be made using the caryopsis surface pattern. *Danthonia* species have undulating reticulate or straight reticulate patterns. The undulating reticulate pattern is also present in *Cortaderia selloana*, *Rytidosperma violacea*, and *R. virescens* (Table 4.2). From a biogeographic perspective, the substriate pattern seems to be restricted to Old World danthonioids (i.e. *Austrodanthonia pilosa*, *Merxmuellera disticha*, *Notodanthonia semiannularis*, *Rytidosperma unarede*, and the two species of *Tribolium*), but it is also observed in at least one member of the Aristidoideae (*Aristida purpurea*). The danthonioid taxa with the substriate caryopsis surface patterns form part of the *Rytidosperma* clade described by Barker et al. (2000), but the South American representatives of *Rytidosperma* have undulating reticulate patterns. Thus no single surface pattern on the caryopsis characterizes this clade, but it is useful to separate Old World and New World members of the *Rytidosperma* clade. Even though the substriate caryopsis surface pattern apparently distinguishes both species of *Tribolium*, the rugose surface pattern has also been reported for the genus (Barker 1994). Therefore, a broader sampling is required to assess the utility of this feature for separating species at the generic and subfamilial level.

Caryopsis surface pattern provides further insight into the taxonomy of *Danthonia*. The genus can be split into two species complexes based on this character: the first group has straight reticulate surface patterns, and the second group has undulating reticulate surface patterns (Table 4.2). The straight reticulate pattern is restricted to *D. californica*, *D. decumbens*, *D. parryi*, and *D. unispicata.* Interestingly, *D. unispicata* is sometimes included as a subspecies of *D. californica*, and *D. parryi* has been suggested as a putative hybrid of *D. californica* and *D. intermedia* (Darbyshire 2003). Species possessing the straight reticulate pattern also share long pointed calluses and marginal lemma hair (Darbyshire 2003), with the exception of *D. intermedia* (Fig. 4.5C), which has an undulating reticulate pattern. Caryopsis surface patterns observed in this survey of danthonioid grasses suggests that *D. parryi*, *D. unispicata*, *D. californica* and *D. intermedia* are closely related species, a view supported by Darbyshire (2003). Furthermore, *D. decumbens* (a European species) shares the straight reticulate pattern present in North American *Danthonia*, which supports Conert's (1987) hypothesis that European species may have originated in North America.

To date, the evolution of caryopsis surface patterns in the Poaceae remains obscure because the plesiomorphic condition cannot be inferred from available data. The same premise applies to this study. The frequency of morphological traits in grasses has some utility in character state polarization. Common character states are thought to be plesiomorphic, while rare character states are apomorphic (Crisci and Steussy 1980). The straight reticulate caryopsis surface pattern was the most common in grasses (48.3% of species out of a total of 118 species) (Jordan et al. 1983), suggesting that the straight reticulate surface pattern might be the plesiomorphic condition. The undulating reticulate pattern was reported in a further 14.4% of species, making this the second most common caryopsis surface type. Although the predominance of the straight reticulate pattern might identify this type as the plesiomorphic condition, Stebbins (1982) cautioned that selection pressure tends to increase the relative abundance of highly advantageous apomorphic characters. Therefore, other methods of character state polarization are recommended, especially comparing extant taxa to fossil evidence, or comparing characters to an appropriate outgroup. In this study, the plesiomorphic condition in the Aristidoideae and Danthonioideae may be the substriate pattern, given

that this character state is present in both groups. Alternatively, this pattern may have arisen independently in the Aristidoideae and the Danthonioideae.

The Ecdeiocoleaceae is sister to the Poaceae (Michelangeli et al. 2003) and would be the ideal outgroup for further examination of the evolution of caryopsis surface patterns. However, taxonomic inferences must be made with caution because homology is difficult to assess due to the highly specialized nature of fruit structures in grasses. Within the Ecdeiocoleaceae, *Ecdeiocolea monostachya* F. Muell. shares a characteristic dry, indehiscent fruit with the Poaceae, but unlike grasses, the pericarp in *Ecdeiocolea* F. Muell. is free from the seed and forms an achene rather than a caryopsis (Rudall et al. 2005). A well-differentiated seed coat in *Ecdeiocolea* suggests that the transformation from capsular fruit to dry indehiscent fruit may have occurred recently in evolutionary history, while the caryopsis in grasses is thought to have evolved in very early lineages (Rudall et al. 2005). Nevertheless, the transition from capsular fruit to indehiscent fruit may have multiple, independent origins in the Poales, and thus the achene of *Ecdeiocolea* and the caryopsis of grasses are not necessarily directly comparable (Rudall et al. 2005). Finally, the caryopsis surface patterns in the basal elements of the Poaceae [Anomochooideae, Puelioideae, Pharoideae (GPWG 2001)] remain unknown. As a consequence, no appropriate inferences can be made regarding the evolution of this character in the grass family in general, and the Danthonioideae in particular, until further investigations are conducted in the basal lineages of the Poaceae. Future studies examining caryopsis characters of grasses in basal subfamilies of the Poaceae would be instrumental in determining trends in evolution within the family, and could provide valuable information for phylogenetic inference within the Danthonioideae.

4.4.3 Final Remarks

No single characters examined in isolation distinguished any of the genera in this study. When the data are examined as a whole, several genera can be distinguished using a suite of characters. Foremost, *Danthonia* has the following three features associated with the caryopsis, hilum, and surface pattern: 1) ovoid to obovoid caryopsis shape, 2) linear hilum, and 3) undulating or straight reticulate surface pattern. No other taxon examined in this study possesses this combination of characters. Secondly, *Rytidosperma* is characterized by 1) ovoid caryopses that are generally smaller than the

caryopses in *Danthonia*, 2) punctate hila, and 3) undulating reticulate or substriate caryopsis surface patterns. Finally, the genus *Tribolium* has 1) small obovoid caryopses ≤1.2 mm in length, 2) punctate hila, and 3) a substriate caryopsis surface pattern. Though *Cortaderia* shares the linear hilum and undulating reticulate surface pattern with *Danthonia*, the lanceolate caryopsis shape is quite different from the ovoid to obovoid caryopsis of *Danthonia*.

Overall, the differences in hilum length are useful in differentiating *Rytidosperma* from *Danthonia*. Moreover, molecular studies also support the separation of these genera (Barker et al. 2000). In addition, preliminary analysis of *trn*L-F sequences indicates that *Danthonia* forms a distinct lineage from *Rytidosperma*. The same ongoing studies support the monophyly of the Danthonioideae and the genus *Danthonia*. Other distinguishing morphological features of the genus *Danthonia* include presence of epidermal multicellular microhairs with basal and terminal cells that are equal in length or with the basal cell longer than the terminal cell (Reimer and Cota-Sánchez, in review). These morphological characters provide support for the molecular phylogeny, and when taken as a whole, this suite of characters provides strong evidence for the monophyletic origin of the genus *Danthonia*.

5.0 MOLECULAR PHYLOGENY OF THE DANTHONIOIDEAE

5.1 Introduction

The subfamily Danthonioideae is found predominantly in the Southern Hemisphere and is considered a south-temperate group. The genus *Danthonia* DC. is the exception, and is the only member of the subfamily represented in the Northern Hemisphere (Linder and Barker 2000). Africa has the greatest diversity of danthonioid grasses with 9 genera and 125 species, but in the Australian grasslands of New South Wales, Victoria, and Tasmania these plants are economically important forages and frequently form the dominant cover (Linder and Verboom 1996).

The phylogenetic position of the Danthonioideae is better understood now than ever before, due in part to relatively recent studies of the Poaceae. In early studies, the Danthonieae was included in the Aveneae (Hubbard 1934), or the Arundinoideae, a group described by Watson and Dallwitz (1992) as an assortment of unrelated taxa that do not fit well into other subfamilies. Molecular evidence has demonstrated the polyphyly of the Arundinoideae sensu lato (s.l.) (Barker et al. 1995), and recent studies support recognizing the Danthonioideae at the subfamilial level, distinct from the Arundinoideae [Grass Phylogeny Working Group (GPWG) 2001]. The Danthonioideae is sister to the Aristidioideae within the PACCAD clade, which also includes the Panicoideae, Arundinoideae, Centhothecoideae, and Chloridoideae (GPWG 2001. Morphological synapomorphies, such as the presence of haustorial synergids (Verboom et al. 1994), bilobed prophylls, and ovaries with widely separated styles also support the monophyly of the Danthonioideae (Linder and Verboom 1996).

Though the monophyly of the Danthonioideae is evident, intergeneric relationships within the subfamily are less clear. The Danthonioideae includes a single tribe, the Danthonieae, with approximately 250 species in 19 genera (GPWG 2001). Seven informal groups including the Basal *Merxmuellera* Assemblage (BMA), *Pentaschistis*, *Chionochloa*, *Pseudopentameris*, *Cortaderia* "A", *Rytidosperma*, and

Danthonia clades have been identified within the Danthonieae using molecular and morphological characters (Barker et al. 2000). These seven groups are probably monophyletic, but within each of the groups, relationships are less clear. This lack of consistency among data sets is presumably due to limited overlap in taxonomic sampling (Barker et al. 2000). Although the affinities among groups remain uncertain, this preliminary survey provides a framework for examining intergeneric relationships within the danthonioid grasses. The aforementioned study contends that several genera within the Danthonioideae are para- or polyphyletic. For instance, New Zealand representatives of *Cortaderia* Stapf. are included in the *Danthonia* clade along with *Danthonia*, *Notochloe* Domin., and *Plinthanthesis* Steud., while South American species of *Cortaderia* fall within the *Cortaderia* "A" clade (Barker et al. 2000). The paraphyly of *Cortaderia* was later confirmed by Barker et al. (2003). In addition, several species of *Merxmuellera* Conert form the BMA, while the remaining *Merxmuellera* species are placed within the speciose *Rytidosperma* clade, which occupies the terminal position in the strict consensus tree (Barker et al. 2000).

Even though *Cortaderia* and *Merxmuellera* are clearly paraphyletic (Barker et al. 2000; Barker et al. 2003), the monophyly of *Danthonia* remains unresolved. A previous study suggested that the genus is monophyletic, but its monophyly is difficult to ascertain conclusively because of ambiguous morphological synapomorphies (Wright 1984). Several characteristic features of *Danthonia*, such as scattered lemma hairs, costal short cells in rows, undivided phloem bundles in the vascular tissue, and the presence of bulliform cells, are primitive and shared in parallel with *Cortaderia* (Wright 1984). More recently, two other synapomorphies have been suggested for *Danthonia* including cleistogenes (autogamous florets in the leaf sheaths), and a base chromosome number of 18 (Linder and Verboom 1996). Some exceptions to these findings have been documented. For example, cleistogenes are rarely seen in *D. intermedia* (Darbyshire 2003), and an unusual chromosome count of $2n=31$ has been reported in *D. spicata* (Darbyshire and Cayouette 1989). Furthermore, other distantly related grasses (e.g. representatives of the Stipeae) also have cleistogenes, thus these structures are not restricted to *Danthonia* (Dobrenz and Beetle 1966).

The genus *Danthonia* as described by de Wet (1954, 1956) is not monophyletic, but many rearrangements have occurred since that time. The taxonomic rearrangement of *Danthonia* s.l. began with formal establishment of *Chionochloa* Zotov, *Notodanthonia* Zotov, *Erythranthera* Zotov, and *Pyrranthera* Zotov to describe the New Zealand taxa (Zotov 1963). This generic separation is based on the presence of deep grooves on the leaves, tufted lemma indumentum, and punctate hilum of the caryopsis. In turn, some Patagonian species with tufted lemma indumentum were reclassified as *Rytidosperma* Steud., while those with marginal and scattered lemma hairs remained within *Danthonia* (Nicora 1973; Baeza 1996). Similarly, Blake (1972) concluded tufted lemma indumentum distinguished the Australasian species from *Danthonia* s.l. Alternatively, Conert (1987) proposed the exclusion of African species from *Danthonia* s.l., and described three new genera (*Karroochloa* Conert and Túrpe, *Merxmuellera*, and *Dregeochloa* Conert). *Karroochloa* is segregated based on the lack of bulliform cells, caryopsis size, and the form of the prophyllum, while *Merxmuellera* differs from *Danthonia* in the arrangement of hairs on the lemma, the presence of single-veined glumes, and indurate lower leaf sheaths (Wright 1984). In *Dregeochloa*, the pericarp is free from the seed and the embryo is larger than in *Danthonia* (Wright 1984). A recent molecular study showed that *Dregeochloa* is not a member of the Danthonioideae, and it belongs in the Chloridoideae (Hsaio et al. 1998).

Part of the difficulty in determining phylogenetic relationships within the Danthonioideae arises from unstable taxonomy. Several authors have contested the taxonomic rearrangements within the Danthonioideae. For example, Conert (1987) argues that separating the Australian taxa from *Danthonia* is unfounded because the distinguishing features used to identify them (i.e. lemma hair and hilum characters) are inconsistent. Furthermore, Zotov (1963) described the genus *Notodanthonia*, but Connor and Edgar (1979) gave priority to *Rytidosperma*, an earlier valid name. Veldkamp (1980) favored conserving the name *Notodanthonia*, a proposal rejected by Jacobs (1982).

A more recent examination of the generic limits of the *Rytidosperma* complex recognized 11 genera: *Danthonia*, *Erythranthera*, *Joycea* H. P. Linder, *Monostachya* Merr., *Notochloe*, *Notodanthonia*, *Plinthanthesis*, *Pyrrhanthera*, *Rytidosperma*,

Schismus P. Beauv., and *Thonandia* H. P. Linder (Linder and Verboom 1996). The remaining danthonioid genera have their own distinctive features. Long, deep red anthers are characteristic of *Joycea*; *Plinthanthesis* has villous palea margins, and lacks bulliform cells in the adaxial leaf epidermis. *Pyrrhanthera* is separated on the basis of the thick, hard pericarp, while *Rytidosperma* is characterized by tufted lemma indumentum, at least in the upper row of hairs. *Schismus* is distinguished by a reduced apical lemma awn, and like *Plinthanthesis*, it lacks tufted lemma indumentum (Linder and Verboom 1996). Lemma indumentum of short uneven hairs, terminating in long tufted hairs in the upper row characterize the genus *Thonandia*. Linder (1997) later corrected the nomenclature of *Notodanthonia* when it became evident that the type specimen of *Notodanthonia* had been erroneously included in *Thonandia*. Consequently, a new genus, *Austrodanthonia* H. P. Linder, was erected to describe the taxa with a long pointed callus. *Thonandia* became a synonym for *Notodanthonia* (Linder 1997).

These more clearly defined generic circumscriptions within the Danthonioideae make it possible to describe a current concept of *Danthonia*. The genus *Danthonia* as currently circumscribed comprises eight species in North America (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. This non-native species is naturalized in North and South America and Australia, for a total of 23 *Danthonia* species globally (Linder and Verboom 1996).

Despite the progress towards understanding the relationships within the Danthonioideae, several key issues remain unresolved. Phylogenetic relationships within several genera, including *Danthonia*, have not been examined in the context of current knowledge of relationships within the Danthonioideae. Furthermore, Conert's (1987) concerns relating to the lack of clear-cut morphological distinctions between genera are somewhat justified because morphological attributes overlap. The value of the hilum character illustrates the debate surrounding selection of features that best characterize danthonioid genera. Zotov (1963) separated of *Notodanthonia* from *Danthonia* because of differences in hilum length, but Blake (1972) argued that the distinction between a short hilum and a long linear hilum is not clear-cut, and instead emphasized the value of lemma indumentum. Other morphological characters in the Danthonioideae also show

high degrees of homoplasy (Barker et al. 2000), highlighting the need to identify additional morphological traits to elucidate taxonomic boundaries and phylogenetic relationships within the subfamily.

Examination of DNA sequence data has been successful in determining phylogenetic relationships when morphological characters are homoplasious, as is the case in the Danthonioideae (Wright 1984; Barker et al. 2000), and the Poaceae as a whole (Stebbins 1987, GPWG 2001). Compared to the nuclear genome, the chloroplast genome is small [between 120 and 217 kilobase pairs (kb)], relatively homogeneous, has a semi-conservative rate of evolution, and is independent of changes in ploidy level (Hilu 1987). These factors account for its utility in reconstructing plant phylogenies. In addition, uniparental inheritance is particularly useful in grasses, many of which have undergone multiple changes in ploidy levels through hybridization events (Stebbins 1987; Olmstead and Palmer 1994).

The chloroplast genome is a circular molecule (Fig. 5.1), separated into a small single copy region (SSC), and a large single copy region (LSC) by two 25 kb inverted repeats (Olmstead and Palmer 1994). The plastid genome encodes about 100 functional genes, several of which are critical for photosynthesis (Clegg et al. 1994). The size of the chloroplast genome varies in photosynthetic land plants from 120 kb in the Pinaceae, to 217 kb in *Pelargonium* L'Her. ex Ait (Downie and Palmer 1992). In the Poaceae, the chloroplast genome varies from 135.5 kb in *Avena* L. (Murai and Tsunewaki 1987) to 138 kb in *Sorghum bicolour* (L.) Moench (Dang and Pring 1986). Several regions of the chloroplast genome have proven useful for elucidating relationships within the Poaceae including coding and noncoding regions. Useful coding regions in phylogenetic studies include *rpo*C2 (e.g. GPWG 2001), the NADH dehydrogenase ND5 subunit (*ndh*F) (e.g. Clark et al. 1995), *rbc*L (e.g. Duvall and Morton 1996), and the maturase within the transfer RNA-Lysine (*trn*K) intron (*mat*K) (e.g. Hilu and Alice 1999). Useful noncoding regions include the transfer RNA-Leucine (*trn*L) intron and the intergenic spacer between *trn*L and the transfer RNA-Phenylalanine (*trn*F) (*trn*L-F) (Brysting et al. 2000), the Photosystem II protein D1 (*psb*A) to transfer RNA-Histidine (*trn*H) spacer, the ATP synthase beta subunit (*atp*B) to *rbc*L spacer, and the *trn*H intron (Vaio et al. 2005).

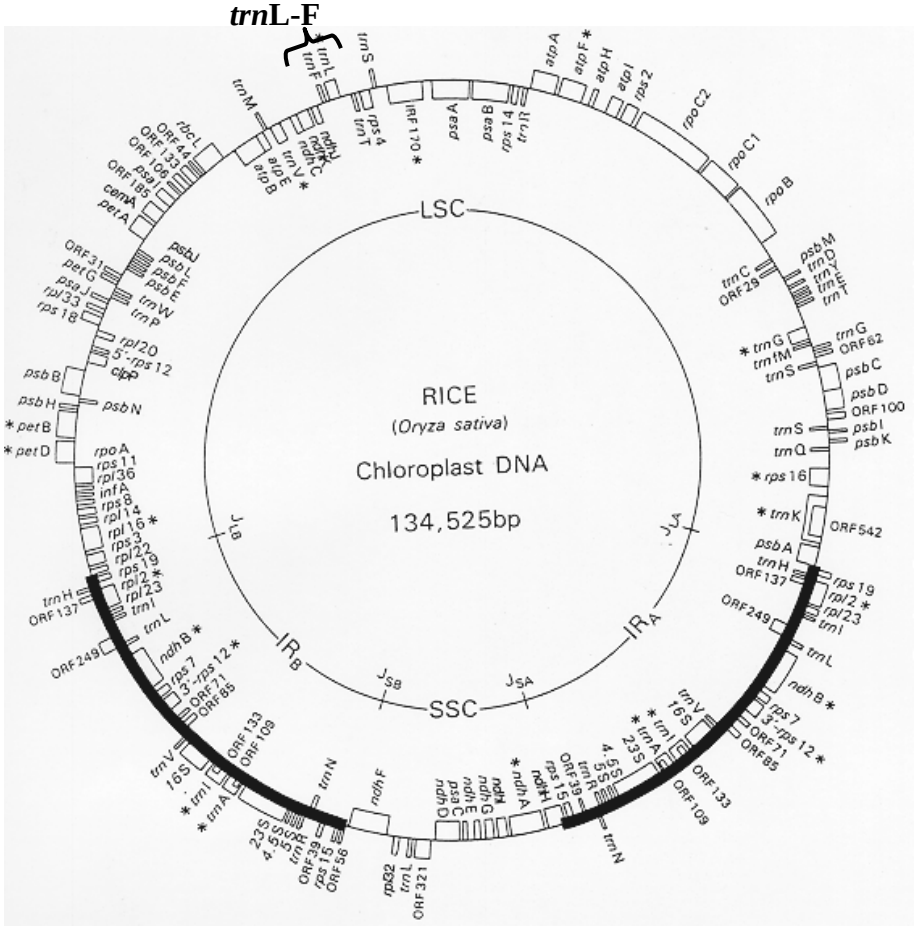

Fig. 5.1. Genetic map of the rice (*Oryza sativa* L.) chloroplast genome. Genes shown outside the circle are encoded on the A strand and transcribed counter-clockwise. Genes shown inside are encoded on the B strand and transcribed clockwise. Asterisks denote split genes. LSC=large single copy region; IR=inverted repeat (thick lines); SSC=small single copy region. The *trn*L-F region is indicated by the bracket. Modified from: Hiratsuka et al. (1989).

Two major structural rearrangements characterize the chloroplast genome of the Poaceae, namely an insertion in the *rpo*C2 region, and an inversion in the transfer RNA-Threonine (*trn*T) region (GPWG 2001). These molecular synapomorphies set the grasses apart from the Ecdeiocoleaceae, the closest relative of the Poaceae (Michelangeli et al. 2003).Within the Poaceae, chloroplast DNA (cpDNA) sequences and restriction sites have been used to examine phylogenetic relationships at all levels of organization, from family (e.g. Davis and Soreng 1993; Duvall and Morton 1996; GPWG 2001), subfamily (e.g. Barker et al. 1995; Catalán et al. 1997), tribe (e.g. Ge et al. 2002), subtribe (e.g.

Catalán et al. 2004), genus (e.g. Baumel et al. 2002), and species (e.g. Hodkinson et al. 2002).

Although coding and noncoding regions of the chloroplast have been used extensively in phylogenetic studies of the Poaceae, noncoding regions have been more useful at lower taxonomic levels because they are less subject to functional constraint, leading to a higher substitution rate (Clegg et al. 1994). Structural rearrangements of the chloroplast genome, such as insertions and deletions (indels), and inversions are a also potential source of phylogenetic information, and are especially useful in noncoding regions of the chloroplast where structural rearrangements may be more common than point mutations (Hilu 1987).

The *trn*L intron and *trn*L-F intergenic spacer are noncoding regions located in the large single copy region of the chloroplast genome (Hiratsuka et al. 1989; Fig. 5.1). In grasses, this marker ranges from approximately 300 to over 800 bp in length (Brysting et al. 2000; Baumel et al. 2002). This region is phylogenetically useful at the intrageneric level in the Poaceae, particularly in *Poa* (Brysting et al. 2000; Stoneberg Holt et al. 2004), *Spartina* Schreb. (Baumel et al. 2002), *Saccharum* L. and *Miscanthus* Anderss. (Hodkinson et al. 2002), and *Ixophorus* (J. Presl) Schltdl. (Kellogg et al. 2004). This marker has provided sufficient signal to address phylogenetic issues at the proposed taxonomic level in other plant groups as well, such as the Cyperaceae (Yen and Olmstead 2000; Muasya et al. 2001), Liliaceae (Zomlefer et al. 2001), Restionaceae (Eldenäs and Linder 2000), and in various dicotyledon groups.

In this study the utility of the *trn*L-F region was explored. Objectives were 1) to test the monophyly of the genus *Danthonia*, and 2) to assess relationships of danthonioid species in the Americas and the Old World. Morphological and molecular data were combined to address the issues surrounding the placement of *Danthonia* within the Danthonioideae, and to identify characters providing additional support.

5.2 Materials and Methods

5.2.1 Taxonomic Sampling and Outgroup Selection

A total of 21 taxa, including three ougroup taxa, *Sorghum halepense* and *Themeda triandra* (subfamily Panicoideae), and *Phragmites australis* (subfamily Arundinoideae). The outgroup taxa belong to the PACCAD clade, but are placed outside

the Danthonioideae as defined by the GPWG (2001). In addition, 18 danthonioid taxa, including seven genera (*Austrodanthonia*, *Cortaderia*, *Danthonia*, *Merxmuellera*, *Notodanthonia*, *Rytidosperma*, *Tribolium* Desv.) (Watson and Dallwitz 1992 onwards) were investigated in this study (Table 5.1). Sampling encompasses the eight North American *Danthonia* species (Table 5.1) recognized by Darbyshire (2003) in the Flora of North America (FNA) North of Mexico: *Danthonia decumbens* [syn. *Sieglingia decumbens* (L.) Bernh.] introduced from Europe, and seven species (*D. californica*, *D. compressa*, *D. intermedia*, *D. parryi*, *D. sericea*, *D. spicata*, *D. unispicata*) native to North America. Two additional taxa, *D. filifolia* [syn. *Danthonia secundiflora* subsp. *secundiflora* J. Presl.], a Mexican species, and *D. chilensis* native to Chile, were included in the American group for comparative purposes; however, they are excluded from the FNA treatment. Although limited in sampling, one South American species of *Cortaderia* and one *Rytidosperma* species (Table 5.1) were added to the survey for assessing potential differences between North American taxa and danthonioid taxa of Central and South America.

Seeds from danthonioid species from North America, Australia, New Zealand, and Africa were obtained from the United States Department of Agriculture Western Regional Plant Introduction Station (PI) (Table 5.1). Seeds for *Cortaderia selloana*, a cultivated ornamental species, were purchased from Whatcom Seed Company (Eugene, OR). Seeds from some taxa were grown in pots in the University of Saskatchewan greenhouses to obtain leaf tissue for DNA extraction, and later transferred outdoors. Voucher specimens were prepared from seed-grown specimens and were included in the University of Saskatchewan (SASK) herbarium. Where fresh material was unavailable, portions of leaves were removed from herbarium specimens for investigation as indicated in Table 5.1.

5.2.2 DNA Extraction

Total DNA was extracted from fresh leaves where available, otherwise from herbarium material, using a modified CTAB method, based primarily on Saghai-Maroof et al. (1984). Tissue was powdered with liquid nitrogen, and added to an Eppendorf tube containing hot 2X hexacetyl trimethylammonium bromide (CTAB) and 2-mercaptoethanol, followed by organic extraction with chloroform-isoamyl (24:1).

Table 5.1. New World and Old World danthonioid taxa investigated, including their geographic distribution, and source of material. Herbarium Acronyms: CONC: Universidad de Concepción; ISC: Ada Hayden Herbarium; MEXU: Universidad Nacional Autónoma de México; MO: Missouri Botanical Garden; PI: U.S. Department of Agriculture Plant Introduction Center; SI: Institito de Botánica Darwinion.

Taxon	Institution Code	Locality, Collector, Collection Number, Date
Outgroup Taxa		
Sorghum halepense (L.) Pers.		
	GenBank AY116244	Living collection at Kew Botanical Garden, Surrey, UK.
Themeda triandra Forssk.		
	GenBank AY116261	Living collection at Kew Botanical Garden, Surrey, UK.
Phragmites australis (Cav.) Trin. ex Steud.		
	GenBank AY651843	Germany.
New World Danthonioideae		
Cortaderia selloana (Schult. & Schult. F.) Asch. & Graebn.		
		Seed from Whatcom Seed Company.
Danthonia californica Bol.		
	PI 232247	USA. AZ, CA, CO, ID, MT, NV, OR, UT, WA, and WY. *F. Hermann & B. Leese s/n*, July, August and September, 1955.
D. chilensis E. Desv.		
	CONC	CHILE. VIII Región. Provincia de Nuble. Camino Las Achiras hacia Quirihue. 355 m. *C.M. Baeza, P. Lopez & M.J. Parra 2099*, 28/01/2000.
D. compressa Austin		
	ISC 254702	USA. NC: Ashe Co. Rich woods, Bluff Mountain. *Radford 44899*, 07/07/1966.

Table 5.1. Continued.

Taxon	Institution Code	Locality, Collector, Collection Number, Date
D. decumbens (L.) DC.		
	MO 870809	CANADA. NS: Yarmouth. Upper border of sandy, peaty beach, Tefry's Lake, Arcadia. *M.L. Fernald & B. Long 19982*, 27/07/1920.
D. filifolia F.T. Hubb.		
	ISC 356119	GUATEMALA. El Quiche. *M.J. Metzler 34*, 15/12/1978.
D. intermedia Vasey		
	PI 232248	USA. AZ, CA, CO, ID, MT, NV, OR, UT, WA, and WY. *F. Hermann & B. Leese s/n*, July, August and September, 1955.
D. parryi Scribn.		
	MEXU	USA. CO: 26 mi. N. of Mill City, 7 km N. of Nederland. 9100 ft. *C.W. Morden 183*, 09/08/1984.
D. sericea Nutt.		
	ISC 285254	USA. FL: Walton Co. Elgin Air Force Reservation, at edge of Choctawhatchee Bay, just E. of mouth of Eagle Creek, 2 mi. E of Okaloosa Co. line. *D.B. Ward 7180*, 18/04/1969.
D. spicata (L.) P. Beauv. *ex* Roem. & Schult.		
	PI W6 19122	USA. NY: Blydenburgh County Park, Smithtown. Habitat: In very sandy soil along a horse trail. Latitude: 40 deg. 50 min. 30 sec. N, Longitude: 073 deg. 13 min. 30 sec. W. *D. Taub 95-30*, 13/07/1995.
D. unispicata (Thurb.) Munro *ex* Vasey		
	ISC 384077	USA. CA: Lassen Co. Open rocky flat W. of Susanville near confluence of Willard Creek & Susan River, 4700 ft. *J.T. Howell 52406*, 22/06/1977.
Rytidosperma violacea (E. Desv.) Nicora		
	SI	ARGENTINA. Neuquen. Departamento de Ñorquín, Copahue. *Troiani & Steibel 15830*, 14/1/2004.

Table 5.1. Continued.

Taxon	Institution Code	Locality, Collector, Collection Number, Date
Old World Danthonioideae		
Austrodanthonia pilosa (R. Br.) H.P. Linder		
	PI 212237	NEW ZEALAND. 12/01/1954.
	SASK 168161	Cultivated in University of Saskatchewan greenhouse. 03/12/2003.
Merxmuellera disticha (Nees.) Conert		
	PI 364332	SOUTH AFRICA. Original material collected from mountain road, 40 km SE. of Maseru. Elev. 2250 m. *A. Oakes 1377*, 6/15/1971.
	SASK 168165	Cultivated in University of Saskatchewan greenhouse. 11/08/2004.
Notodanthonia semiannularis (Labill.) Zotov		
	PI 210172	AUSTRALIA. Original material collected from Capital Territory. *W. Hartley s/n*, 9/16/1953.
	SASK 168160	Cultivated in University of Saskatchewan greenhouse 03/12/2003.
Rytidosperma unarede (Raoul) Connor & Edgar		
	PI 237160	NEW ZEALAND. Original material collected from Christchurch. *Department of Scientific and Industrial Research*, 2/4/1957.
	SASK 168157	Cultivated in University of Saskatchewan greenhouse. 11/08/2004.
Tribolium echinatum (Thunb.) Renvoize		
	PI 238332	SOUTH AFRICA. 26/03/1957.
	SASK 168162	Cultivated in University of Saskatchewan greenhouse. 03/12/2003.
T. hispidum (Thunb.) Desv.		
	PI 368889	SOUTH AFRICA. Original material collected from Langgewens Experimental Farm, north of Malmsbury. 5/20/1909.
	SASK 168158	Cultivated in University of Saskatchewan greenhouse. 03/12/2003.

DNA was precipitated in 95% ethanol, then washed in 70% ethanol. The resulting pellet was dried and resuspended in tris ethylene diaminetetra-acetic acid EDTA (TE) buffer. Total DNA was quantified with a BioPhotometer UV spectrophotometer (Eppendorf AG) and was run on 1% agarose gel and stained with ethidium bromide for visualization and further quantification before amplification.

5.2.3 Amplification and Sequencing of Target Molecular Marker

The *trn*L-F region was amplified via the polymerase chain reaction (PCR) using bidirectional universal primers described in Taberlet et al. (1991) and F downy, edf, and Ci designed in the Cota-Sánchez laboratory (Fig. 5.2; Table 5.2). An appropriate volume of total DNA at a concentration of approximately 20 ng/µL was selected (3-5 µL for most taxa), and added to a tube along with 5 µL 25 mM magnesium chloride ($MgCl_2$), 5 µL 10X Taq buffer, 0.5 µL dNTPs, 2 µL of 5 µM forward primer (C or Ci) and the same volume of reverse primer (F or F downy) (Table 5.2; Fig. 5.2), plus 0.5 µL Taq polymerase (Promega). For DNA obtained from some herbarium specimens, the amplification was conducted in two separate reactions using two primer sets, i.e. from C to D, and edf to F downy (Fig. 5.2). Double distilled water was added to yield a 50 µl reaction.

The tubes containing the PCR reaction were placed in a PTC-200 Peltier Thermal Cycler (MJ Research Inc.). The PCR protocol included an initial cycle for five minutes at 94ºC, followed by denaturing for 30s at 94ºC, annealing at 48ºC for 60s, and extension at 72ºC for 90s, repeated for a total of 40 cycles, followed by a final extension cycle at 72ºC for seven minutes. The reaction tubes were then held at 4ºC. The PCR products were resolved on a 1 % agarose gel alongside a standard DNA ladder, stained with ethidium bromide, and illuminated with ultraviolet light using a BioDoc-It System (UVP Inc.). When bands were evident on the gel indicating that the PCR had successfully amplified the region of interest, then the PCR products were purified with a QIAquick PCR Purification Kit (Qiagen Inc.) and quantified on an agarose gel next to a standard of known concentration (pGEM).

Purified PCR products were sequenced using ABI Prism Big Dye Terminator v.3.0 Cycle Sequencing Ready Reaction Kit (Applied Biosystems). Forward and reverse cycle sequencing was conducted in 10 µl reactions as follows: 1.6 µL primer (Ci, D, E,

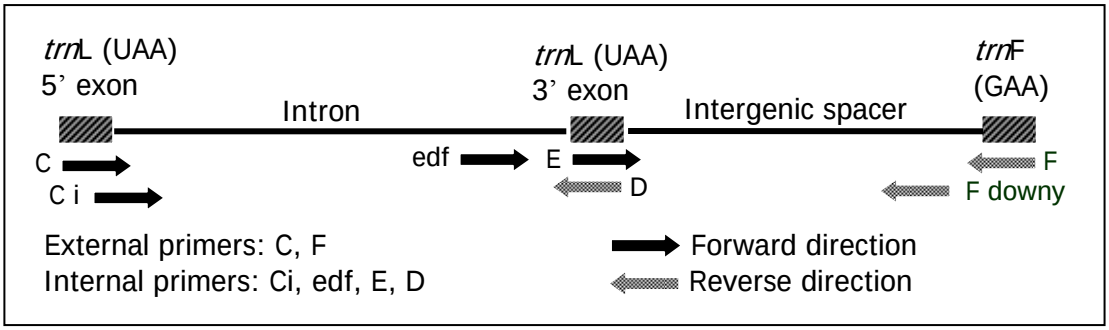

Fig. 5.2. General diagram with positions and directions of universal primers used to amplify noncoding *trn*L-F region of the chloroplast genome.

Table 5.2. List of primers and primer sequences used to amplify and sequence the *trn*L-F region of the chloroplast genome. Primer sequences for C, D, E, and F are based on Taberlet et al. (1991), the remainder were specifically designed in the Plant Systematics laboratory.

Primer	Sequence (5' to 3')	Used in amplification (A) or sequencing (S)	Internal/External
C	CGAAATCGGTAGACGCTACG	A	E
C i	TCGGTAGACGCTACGGACTT	A/S	I
edf	GGAGCAGAATGAAGATAGAG	A	I
D	GGGGATAGAGGGACTTGAAG	A/S	I
E	GGTTCAAGTCCCTCTATCCC	S	I
F	ATTTGAACTGGTGACACGAG	A	E
F downy	CAGTCCTCTGCTCTACCAGC	A/S	I

or F downy), 2 µL Big Dye, 2.0-2.5 µL buffer, 2-4.4 µL DNA (volumes of DNA and buffer were dependant on DNA concentration). If required, double distilled water was added to bring the reaction to a final volume of 10 µL. Cycle sequencing was conducted on a PTC-200 Peltier Thermal Cycler (MJ Research) as follows: 1) an initial cycle of 80ºC for four minutes, followed by 2) heating at 1ºC/s to 96ºC to reach denaturing temperature, 3) held for 10s at 96ºC, 4) cooling at a rate of 1ºC/s to 50ºC and 5) held at 50ºC for 5s (annealing), 6) heating at 1ºC/s to 60ºC, and 7) held at 60ºC for four minutes (extension). Steps 2 through 7 were repeated for a total of 25 cycles. Extension products were purified using an ethanol precipitation protocol to remove excess dye and primer

dimers, then stored at -20ºC. Sequence data were generated with electrophoresis using a polyacrylamide gel in an automated ABI Prism 377 DNA Sequencer (Applied Biosystems).

5.2.4 Sequence Alignment and Phylogenetic Analysis

DNA sequences of the *trn*L-F intron and intergenic spacer for the outgroup taxa *Sorghum halepense* (Accession number AY116244), *Themeda triandra* (Accession number AY116261), and *Phragmites australis* (Accession number AY651843) were obtained from GenBank of the National Center for Biotechnology Information (NCBI). For the ingroup, the raw sequences obtained from individual primers were assembled into contiguous sequences (contigs) and edited in Sequencher software v.3.0 (Gene Codes Corp.). Approximately 20 bases were removed from the 5' and 3' end of the sequences to avoid ambiguous areas potentially affected by excess dye or signal loss. The sequences were aligned manually one at a time relative to the outgroup using sequence alignment software, Se-Al (Rambaut 1995) to make a sequence data matrix (Appendix 1). Aligned *trn*L-F sequences were imported in NEXUS format into the Phylogenetic Analysis Using Parsimony (PAUP) v.4.0b10 software (Swofford 2004).

Trees were constructed invoking maximum parsimony methods, using default settings for the heuristic search in PAUP. Branches were collapsed if maximum length was zero, the branch swapping tree-bisection-reconnection (TBR) algorithm was used, with ACCTRAN (accelerated transformation) optimization, and starting trees were created by stepwise addition. The ingroup was made monophyletic, with the outgroup (*Sorghum halepense* and *Themeda triandra*) rooted at the base of the tree. Two separate analyses were run. In the first analysis, gaps were treated as missing information, and in the second analysis, gaps were included as a fifth character state. When gaps were treated as a fifth base, species for which only partial sequences were available (*Danthonia chilensis*, *D. decumbens*, *D. filifolia*, and *D. parryi*) were removed from the analysis. For each analysis, a strict consensus tree was constructed based on the most parsimonious trees that resulted from the heuristic search in PAUP. Support for monophyletic clades was tested using 1000 replicates of bootstrap analysis (Felsenstein 1985) with "fast" stepwise addition searching. Decay values [Bremer support (Bremer 1988)] were calculated for trees up to three steps longer than the most parsimonious tree

in PAUP. Phylogenetic trees were imported into MacClade (Madison and Madison 1999) to test the number of additional steps necessary to make the North American species of *Danthonia* monophyletic. Taxa were removed from the phylogenetic analysis one at a time in PAUP to test for changes in clade support.

The hilum length of the caryopsis was optimized on one of the most parsimonious trees using both ACCTRAN (ACCelerated TRANsformation) and DELTRAN (DELayed TRANsformation) (Fitch 1971) to provide support for the monophyly of groups within the Danthonioideae, and to infer character evolution. Other morphological characters (caryopsis shape, lodicule characters, silica body shape and distribution on the abaxial epidermis) were examined, but could not be optimized on the consensus tree due to high levels of homoplasy.

5.3 Results

5.3.1 Length of trn*L-F Sequences*

In the danthonioid taxa investigated, multiple alignment of *trn*L-F sequences is 947 bp in length (Appendix 1). Sequences for the outgroup taxa range from 811 bp (*Sorghum halepense*) to 905 bp (*Phragmites australis*) (Table 5.3). The sequences of the ingroup taxa vary between 870 bp (*Danthonia sericea*) and 890 bp (*Austrodanthonia pilosa* and *Rytidosperma unarede*) (Table 5.3). The *trn*L intron ranges from 454 bp (*Sorghum halepense*) to 548 bp (*Phragmites australis*) in the outgroup, and 534 bp (*Tribolium echinatum* and *T. hispidum*) to 544 bp (*A. pilosa*, *D. chilensis*, *D. filifolia*, *D. intermedia*, *Notodanthonia semiannularis*, *Rytidosperma unarede*, and *R. violacea*) in the Danthonioideae (Table 5.3). The *trn*L to *trn*F intergenic spacer region for the outgroup taxa ranges from 357 bp (*Sorghum halepense*) to 361 bp (*Themeda triandra*), and in the ingroup taxa from 336 bp (*Cortaderia selloana*) to 348 bp (*Tribolium echinatum* and *T. hispidum*) (Table 5.3).

5.3.2 Tree Statistics

Of the 947 characters analysed with PAUP when gaps were coded as "missing", 825 are constant, 53 are variable and uninformative, while 69 (7.3%) are parsimony informative. The phylogenetic analysis produced 74 most parsimonious trees, from which PAUP generated a strict consensus tree of 146 steps with a consistency index (CI) of 0.911, and a retention index (RI) of 0.940 (Fig. 5.3).

Table 5.3. Sequence length of the *trn*L-F region of the chloroplast. The asterisk (*) denotes taxa with partial sequences available.

Species	**Length of *trn*L intron (bp)**	**Length of the *trn*L to *trn*F intergenic spacer (bp)**	**Total length of the *trn*L-F region (bp)**
Outgroup			
Sorghum halepense	454	357	811
Themeda triandra	456	361	817
Phragmites australis	548	357	905
Ingroup			
Austrodanthonia pilosa	544	346	890
Cortaderia selloana	537	336	873
Danthonia californica	538	336	874
D. chilensis	543	333	876
D. compressa	544	335*	879*
D. decumbens	538	336	874
D. filifolia	535	176*	711*
D. intermedia	544	335*	879*
D. parryi	544	335	879
D. sericea	538	228*	766*
D. spicata	534	336	870
D. unispicata	538	336	874
Merxmuellera disticha	540	334	874
Notodanthonia semiannularis	544	340	884
Rytidosperma unarede	544	346	890
R. violacea	544	340	884
Tribolium echinatum	534	348	882
T. hispidum	534	348	882

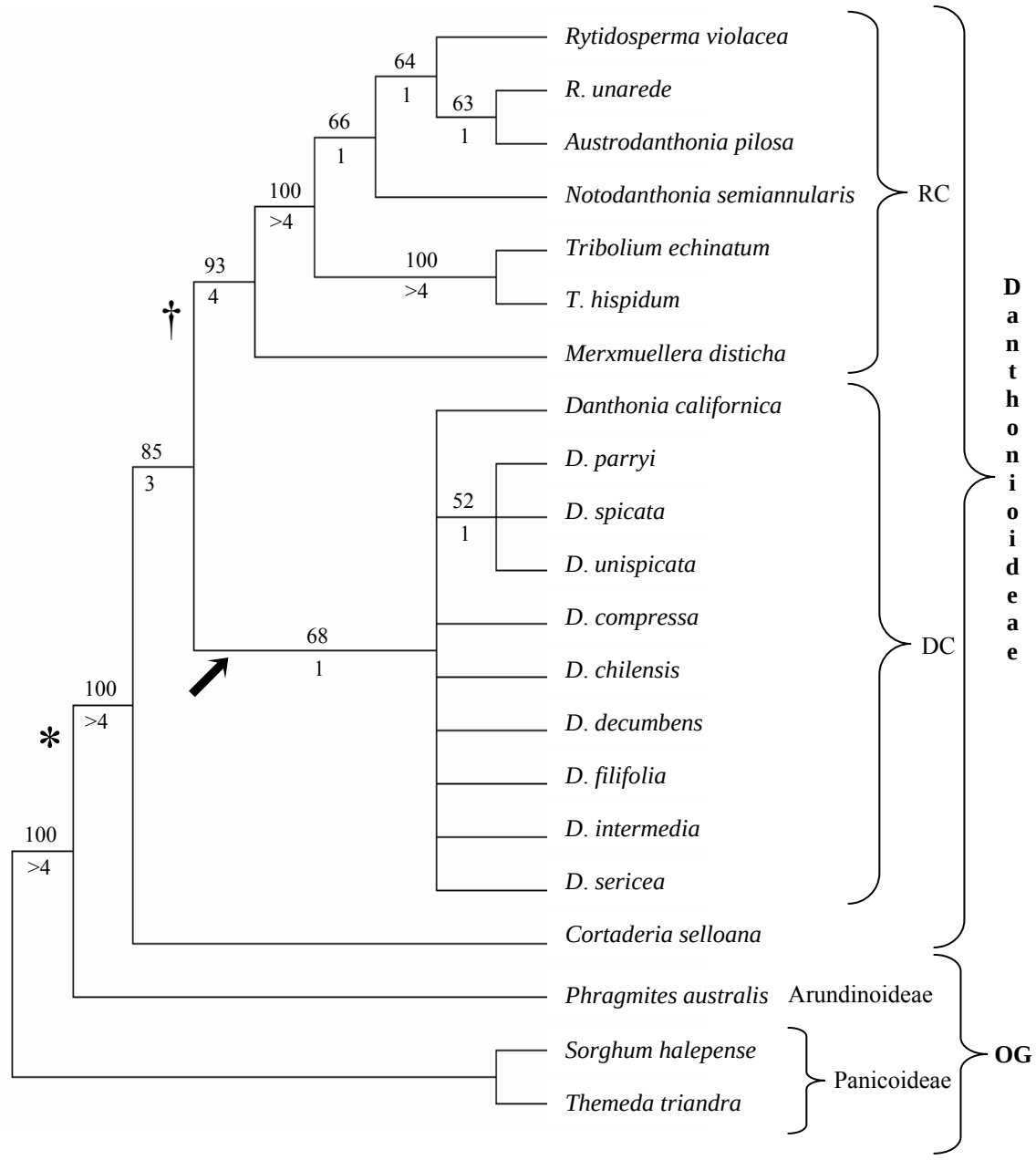

Fig. 5.3. Strict consensus of 74 most parsimonious trees (length = 146 steps, CI=0.911, RI=0.940) obtained from phylogenetic analysis of *trn*L-F sequence data, with gaps treated as "missing". The multiple sequence alignment consists of a total of 947 unordered characters, of which 825 characters are constant, 53 variable characters are uninformative, and 69 are parsimony informative. Bootstrap values (1000 replicates) are indicated above the branches, and decay values below the branches. The asterisk (*) indicates the monophyly of the Danthonioideae, the dagger (†) indicates the monophyly of the *Rytidosperma* clade (RC). The arrow indicates the monophyly of the *Danthonia* clade (DC). Outgroup (OG) taxa represent the Panicoideae and Arundinoideae.

When gaps are coded as a "fifth base", 652 out of 947 characters are constant, 81 variable characters are uninformative, and 214 (22.6%) are parsimony informative. The strict consensus of two most parsimonious trees is 359 steps (CI=0.925 and RI=0.936) (Fig. 5.4). Partial sequences (*Danthonia chilensis*, *D. decumbens*, *D. filifolia*, and *D. parryi*) were excluded from this analysis because PAUP treats missing portions of the sequence as gaps.

5.3.3 Phylogeny of the Danthonioideae

Based on the strict consensus obtained from the analysis of the most parsimonious trees generated from aligned sequences of the *trn*L-F region, the Danthonioideae forms a strongly supported monophyletic group. This result (100% bootstrap support and decay value >4) was obtained consistently in both analyses, i.e. with gaps coded as "missing" (Fig. 5.3) and coded as a "fifth base" (Fig. 5.4). In fact, the branching order and topology of the two strict consensus trees from both analyses (Figs. 5.3 and 5.4) is the same.

Within the Danthonioideae, *Cortaderia selloana* is a basal member of the subfamily. The remaining members of the subfamily form two sister clades. One clade includes all the representatives of the genus *Danthonia*, hereafter referred to as the *Danthonia* clade. The second clade is comprised of *Merxmuellera disticha*, *Tribolium echinatum*, *T. hispidum*, *Notodanthonia seminannularis*, *Austrodanthonia pilosa*, *Rytidosperma unarede*, and *R. violacea*, hereafter the *Rytidosperma* clade (Figs. 5.3 and 5.4). These two clades are discussed in more detail below.

The strict consensus tree generated in the phylogenetic analysis of the *trn*L-F data when gaps are treated as "missing" (Fig. 5.3) suggests that *Danthonia* is monophyletic with modest clade support of 68% bootstrap and decay value of 1. The *Danthonia* clade is more robust in the strict consensus obtained when gaps are coded as a "fifth base" (74% bootstrap and decay value of 4) (Fig. 5.4). Hence, both analyses support the monophyly of *Danthonia*. In addition, *D. decumbens* was excluded from a separate analysis with gaps coded as "missing", and clade support increased to 86% bootstrap and decay value of 4 (tree results not shown). Analyses with gaps treated as a "fifth base" and excluding *D. decumbens* provide strong support for monophyly of *Danthonia*, and overall topology of the trees remains unchanged in all analyses.

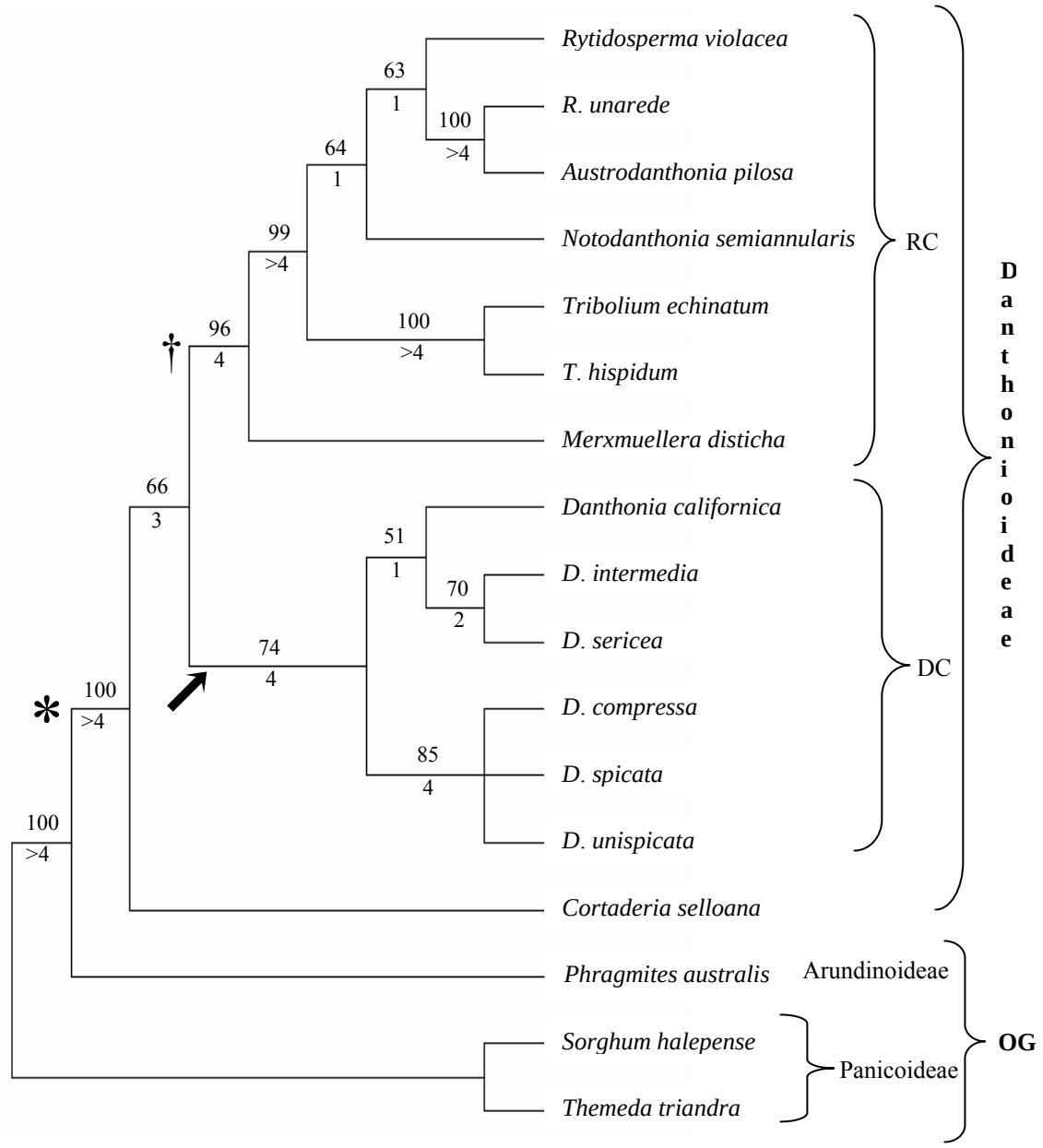

Fig. 5.4. Strict consensus of two most parsimonious trees (length = 359 steps, CI=0.925, RI=0.936), obtained from phylogenetic analysis of *trn*L-F sequence data, with gaps treated as "fifth base". The multiple sequence alignment consists of a total of 947 unordered characters, of which 652 characters are constant, 81 variable characters are uninformative, and 214 are parsimony informative. Bootstrap values (1000 replicates) are indicated above the branches, decay values below the branches. The asterisk (*) indicates the monophyly of the Danthonioideae, the dagger (†) indicates the *Rytidosperma* clade (RC). The arrow indicates the monophyly of the *Danthonia* clade (DC). Outgroup (OG) taxa represent the Panicoideae and Arundinoideae. Taxa with partial sequences (*Danthonia chilensis*, *D. decumbens*, *D. filifolia*, and *D. parryi*) were excluded from this analysis.

Interspecific relationships within *Danthonia* are unclear when the entire taxonomic sampling is included in analyses. *D. californica*, *D. chilensis*, *D. compressa*, *D. decumbens*, *D. filifolia*, *D. intermedia*, and *D. sericea* form a polychotomy (Fig. 5.3). The South American species of *Danthonia*, represented by *D. filifolia* and *D. chilensis*, are included within the polychotomy, as is *D. decumbens*. Within *Danthonia* a single clade (*D. parryi*, *D. spicata*, and *D. unispicata*) is evident supported by 52% bootstrap and decay value of 1 when gaps are treated as "missing".

The relationships within the *Danthonia* clade are better resolved in the analysis with gaps coded as "fifth base" (Fig. 5.4). As indicated previously, taxa with partial sequences (i.e. *Danthonia chilensis*, *D. decumbens*, *D. filifolia*, and *D. parryi*) were excluded from this analysis. Nonetheless, the remaining *Danthonia* species form two distinct subclades. *D. compressa*, *D. spicata*, and *D. unispicata* form one subclade (85% bootstrap and decay value of 4; Fig. 5.4). Within the other subclade, *D. californica* is basal to *D. intermedia* and *D. sericea* (51% bootstrap and decay value of 1; Fig. 5.4), which appear as sister species (70% bootstrap and decay value of 2; Fig. 5.4).

Danthonia filifolia from Mexico and *D. chilensis* from Chile form part of the polychotomy in the *Danthonia* clade (Fig. 5.3). When *D. chilensis* and *D. filifolia* are constrained to the outgroup, and the North American *Danthonia* are forced to be monophyletic, PAUP cannot generate a most parsimonious tree. The tree length increased from 117 to 122 steps when these two taxa were removed from the North American clade in MacClade to make the North American representatives of *Danthonia* monophyletic. An analysis excluding *D. decumbens* from the *Danthonia* clade produced a tree length of 119 steps.

Monophyly of the *Rytidosperma* clade is well resolved in both analyses (Figs. 5.3 and 5.4). When gaps are treated as "missing", the monophyly of the *Rytidosperma* clade is strongly supported (93% bootstrap and decay value of 4) (Fig. 5.3). Support for the *Rytidosperma* clade remains unchanged (94% bootstrap and decay value of 4) in the analysis with gaps treated as a "fifth base" (Fig. 5.4). The *Rytidosperma* clade forms two sister subclades, to which *Merxmuellera disticha* is basal (Figs. 5.3 and 5.4). Australian (*Notodanthonia semiannularis*) and New Zealand taxa (*Austrodanthonia pilosa* and *Rytidosperma unarede*), plus the South American species of *Rytidosperma* (*R. violacea*)

form a monophyletic subclade (66% bootstrap and decay value of 1). *Tribolium* forms another monophyletic subclade (100% bootstrap and decay value >4).

5.3.4 trn*L-F Sequence Evolution*

The base pair composition of the *trn*L-F region is somewhat variable in the taxa examined. In general, AT content is higher than GC content (roughly 2:1) in all sequences investigated (Table 5.4). The mean AT content for all taxa examined is 67.81%, and mean GC content is 32.19%. The mean AT content for outgroup taxa is 68.00% and the mean GC content is 32.00%, while the mean AT/GC content in the danthonioid taxa is 67.78%/32.22% respectively. Within the Danthonioideae, the mean AT content in *Danthonia* is slightly higher (67.82%) than the subfamily average (67.78%), while the average for the *Rytidosperma* clade is somewhat lower (67.71%). The mean AT content in *Cortaderia* is 67.89%. The highest AT content (and consequently lowest GC content) in any sequence is 68.32% in *D. chilensis*, followed by 68.30% in *Themeda triandra* (Table 5.4). The lowest AT content is *D. decumbens* at 66.95%, followed by *Rytidosperma violacea* at 67.42%. AT content differs by 1.37% across the entire taxonomic sampling.

Thirty one length mutations ranging from 1 bp to 87 bp in length were evident in the multiple alignment of *trn*L-F sequences of the Danthonioideae and outgroup taxa (Table 5.5). The starting positions of all the length mutations are given relative to the first base pair (bp) of the multiple alignment shown in the data matrix (Appendix 1). Thirteen length mutations (1, 3, 4, 7, 12, 14, 16, 18, 21, 22, 23, 24, and 26) are unique molecular autapomorphies to the taxa in which they occur, while 18 (2, 5, 6, 8, 9, 10, 11, 13, 15, 17, 19, 20, 25, 27, 28, 29, 30, and 31) are molecular synapomorpies shared by two or more taxa (Table 5.5). The Danthoniodeae and Arundinoideae (*Phragmites australis*) representatives share five length mutations (2, 25, 27, 29, and 30) relative to the panicoid outgroup taxa (Fig. 5.5; Table 5.5). *Phragmites australis* is characterized by three length mutations relative to the panicoid outgroup taxa, i.e. (3, 4, 7) (Fig. 5.5; Table 5.5). The Danthonioideae are characterized by four length mutations: 10, 19, 28, 31 (Fig. 5.5; Table 5.5). *Cortaderia selloana* is characterized by a six bp deletion at position 257 (length mutation 16) (Fig. 5.5; Table 5.5). No length mutations characterize the genus *Danthonia*, but *D. compressa*, *D. parryi*, *D. spicata*, and *D. unispicata* share a

Table 5.4. Relative base pair content of *trn*L-F sequences of taxa examined.

Taxon	**A (%)**	**T (%)**	**AT (%)**	**G (%)**	**C (%)**	**GC (%)**	**#sites**
Sorghum halepense	33.33	34.81	68.14	15.01	16.85	31.86	813
Themeda triandra	33.91	34.39	68.30	15.06	16.65	31.70	817
Phragmites australis	33.66	33.89	67.55	15.89	16.56	32.45	906
Outgroup Mean	**33.63**	**34.36**	**68.00**	**15.32**	**16.68**	**32.00**	**845.33**
Danthonia californica	35.01	32.95	67.96	15.51	16.53	32.04	877
D. chilensis	35.49	32.83	68.32	14.91	16.76	31.68	878
D. compressa	34.82	32.88	67.70	15.58	16.72	32.30	873
D. decumbens	36.43	30.52	66.95	16.88	16.17	33.05	911
D. filifolia	35.08	32.80	67.88	15.49	16.63	32.12	878
D. intermedia	35.08	32.92	68.00	15.38	16.63	32.01	878
D. parryi	34.77	32.94	67.71	15.69	16.60	32.29	876
D. sericea	35.21	33.03	68.24	15.08	16.69	31.76	869
D. spicata	34.82	32.88	67.70	15.58	16.72	32.30	873
D. unispicata	34.82	32.88	67.70	15.58	16.72	32.30	873
***Danthonia* Mean**	**35.15**	**32.66**	**67.82**	**15.57**	**16.62**	**32.18**	**878.6**
Austrodanthonia pilosa	34.61	33.03	67.64	15.73	16.63	32.36	890
Merxmuellera disticha	34.36	33.10	67.47	15.81	16.72	32.53	873
Notodanthonia semiannularis	34.73	33.03	67.76	15.61	16.63	32.24	884
Rytidosperma unarede	34.49	33.15	67.64	15.73	16.63	32.36	890
R. violacea	34.50	32.92	67.42	15.72	16.86	32.58	884
Tribolium echinatum	34.58	33.45	68.03	15.08	16.89	31.97	882
T. hispidum	34.58	33.45	68.03	15.08	16.89	31.97	882
***Rytidosperma* Clade Mean**	**34.55**	**33.16**	**67.71**	**15.54**	**16.75**	**32.29**	**881**
Cortaderia selloana	34.52	33.37	67.89	15.48	16.63	32.11	872
Danthonioideae Mean	**34.88**	**32.90**	**67.78**	**15.55**	**16.67**	**32.22**	**883.57**
Overall Mean	34.71	33.10	67.81	15.52	16.67	32.19	875.4

5 bp length mutation (length mutation 15) (Fig. 5.5; Table 5.5). *D. californica* is characterized by a one bp deletion at position 201 (length mutation 14), and *D. sericea* is the only taxon with a nine bp deletion beginning at position 522 (length mutation 21) (Fig. 5.5; Table 5.5). The *Rytidosperma* clade is characterized by a single bp deletion at position 419 (length mutation 20); within the *Rytidosperma* clade, *A. pilosa* and *R. unarede* share a six bp insertion at position 604 (length mutation 6), and the *Rytidosperma* clade except *Merxmuellera disticha* is distinguished by two length mutations: (length mutation 5 and 11) (Fig. 5.5; Table 5.5). The genus *Tribolium* is characterized by two length mutations: a 4 bp deletion at position 159 and a 6 bp deletion at position 313 (length mutations 13 and 17, respectively) (Fig. 5.5; Table 5.5). In addition, a six bp insertion beginning at position 745 (length mutation 8 in Table 5.5) is shared in parallel between *Sorghum halepense* and *Tribolium*, and a 2 bp insertion at position 751 (length mutation 9 in Table 5.5) is shared by *S. halepense*, *Themeda triandra*, and *Tribolium* (Fig. 5.5; Table 5.5).

5.3.5 Optimization of Morphological Characters

Attempts were made to optimize several morphological characters from the caryopsis, lodicule, and silica bodies onto the molecular phylogeny, but only the hilum type provided taxonomic information supporting phylogenetic inferences. The remaining characters overlapped across taxonomic groups, and did not help to elucidate relationships within *Danthonia*. The hilum type, related to length, was optimized on one of the 74 most parsimonious trees obtained from phylogenetic analysis of *trn*L-F sequence data (Fig. 5.6). The outgroup taxa, *Phragmites australis*, *Sorghum halepense* and *Themeda triandra*, have a short hilum. *Danthonia* species possess a long linear hilum, which is also observed in *Cortaderia selloana* and *Merxmuellera disticha*. Except for *M. disticha*, the species within the *Rytidosperma* clade (i.e. *Austrodanthonia pilosa*, *Notodanthonia semiannularis*, *Rytidosperma unarede*, *R. violacea*, *Tribolium echinatum*, and *T. hispidum*) are characterized by a short hilum (Fig. 5.6).

Table 5.5. List of length mutations found in the Danthonioideae. Relative positions of the length mutations are based on the multiple alignment of the *trn*L-F sequences examined (Appendix 1). The taxa indicated refer exclusively to those included in this study.

Mutation Number	Starting Position	Length (bp)	Taxa with mutations at this position
Insertions			
1	56	1	*Sorghum halepense*
2	156	87	Danthonioideae and *Phragmites australis*
3	202	1	*P. australis*
4	292	4	*P. australis*
5	344	1	*Rytidosperma* clade except *Merxmuellera disticha*
6	604	6	*Austrodanthonia pilosa* and *Rytidosperma unarede*
7	726	12	*P. australis*
8	745	6	*S. halepense* and *Tribolium*
9	751	2	*S. halepense*, *Themeda triandra*, and *Tribolium*
10	810	5	Danthonioideae
11	815	6	*Rytidosperma* clade except *M. disticha*
Deletions			
12	70	3	*S. halepense*
13	159	4	*Tribolium*
14	201	1	*Danthonia californica*
15	235	5	*D. compressa, D. parryi, D. spicata, D. unispicata*
16	257	6	*Cortaderia selloana*
17	313	6	*Tribolium*
18	343	1	*M. disticha*
19	408	1	Danthonioideae
20	419	1	*Rytidosperma* clade
21	522	9	*D. sericea*
22	596	1	*Themeda triandra*
23	598	4	*S. halepense*
24	616	3	*M. disticha*
25	697	4	Danthonioideae and *P. australis*
26	701	5	*S. halepense*
27	706	4	Danthonioideae and *P. australis*
28	715	11	Danthonioideae
29	756	5	Danthonioideae and *P. australis*
30	801	2	Danthonioideae and *P. australis*
31	853	3	Danthonioideae

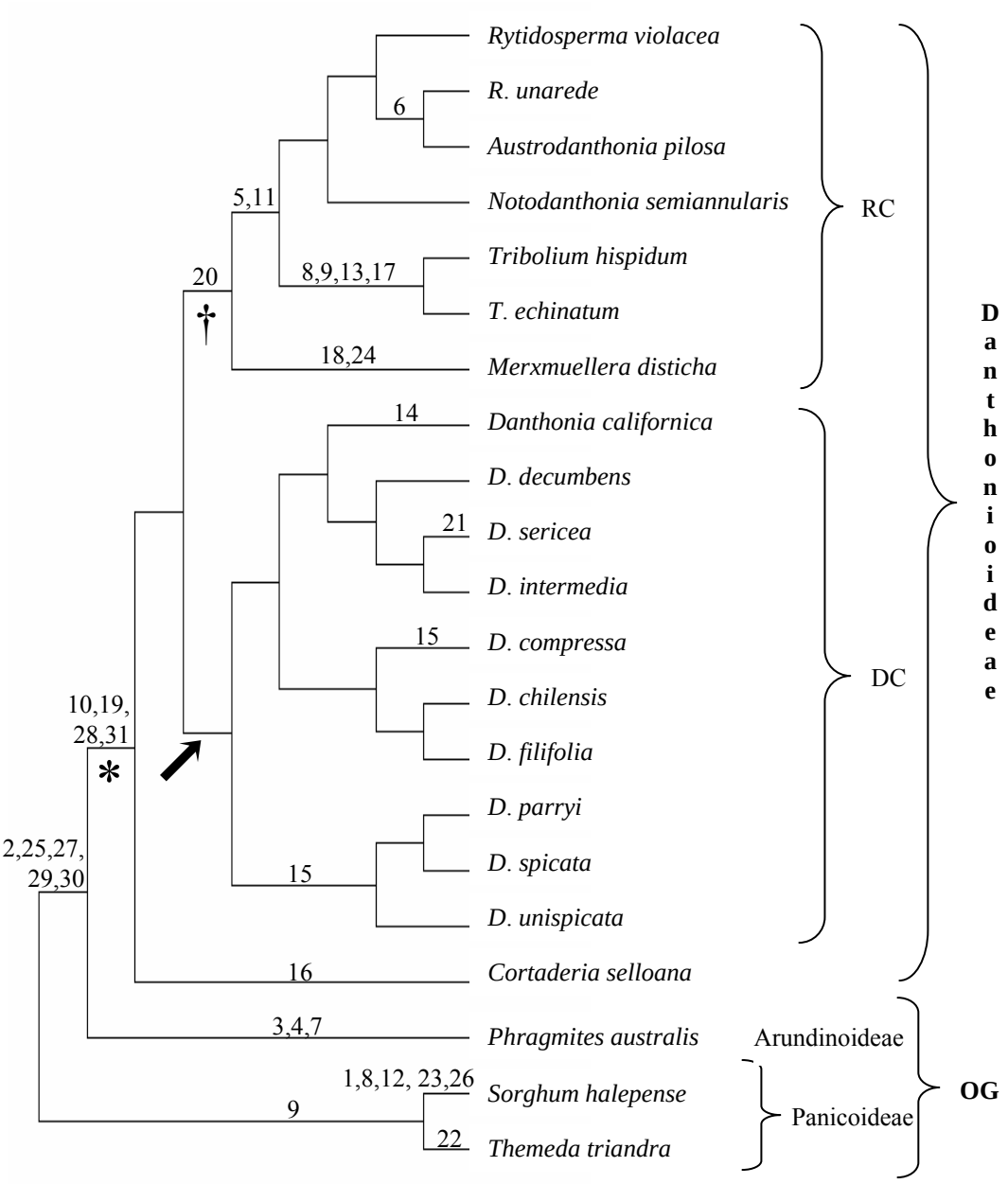

Fig. 5.5. Length mutations optimized on one of 74 most parsimonious trees (length = 146 steps, CI=0.911, RI=0.940) obtained from phylogenetic analysis of *trn*L-F sequence data. Length mutation number (as indicated in Table 5.5) is indicated above the branches. The asterisk (*) indicates the monophyly of the Danthonioideae, the dagger (†) indicates the *Rytidosperma* clade (RC), and the arrow indicates the monophyly of the *Danthonia* clade (DC). The outgroup (OG) taxa represent the Panicoideae and Arundinoideae.

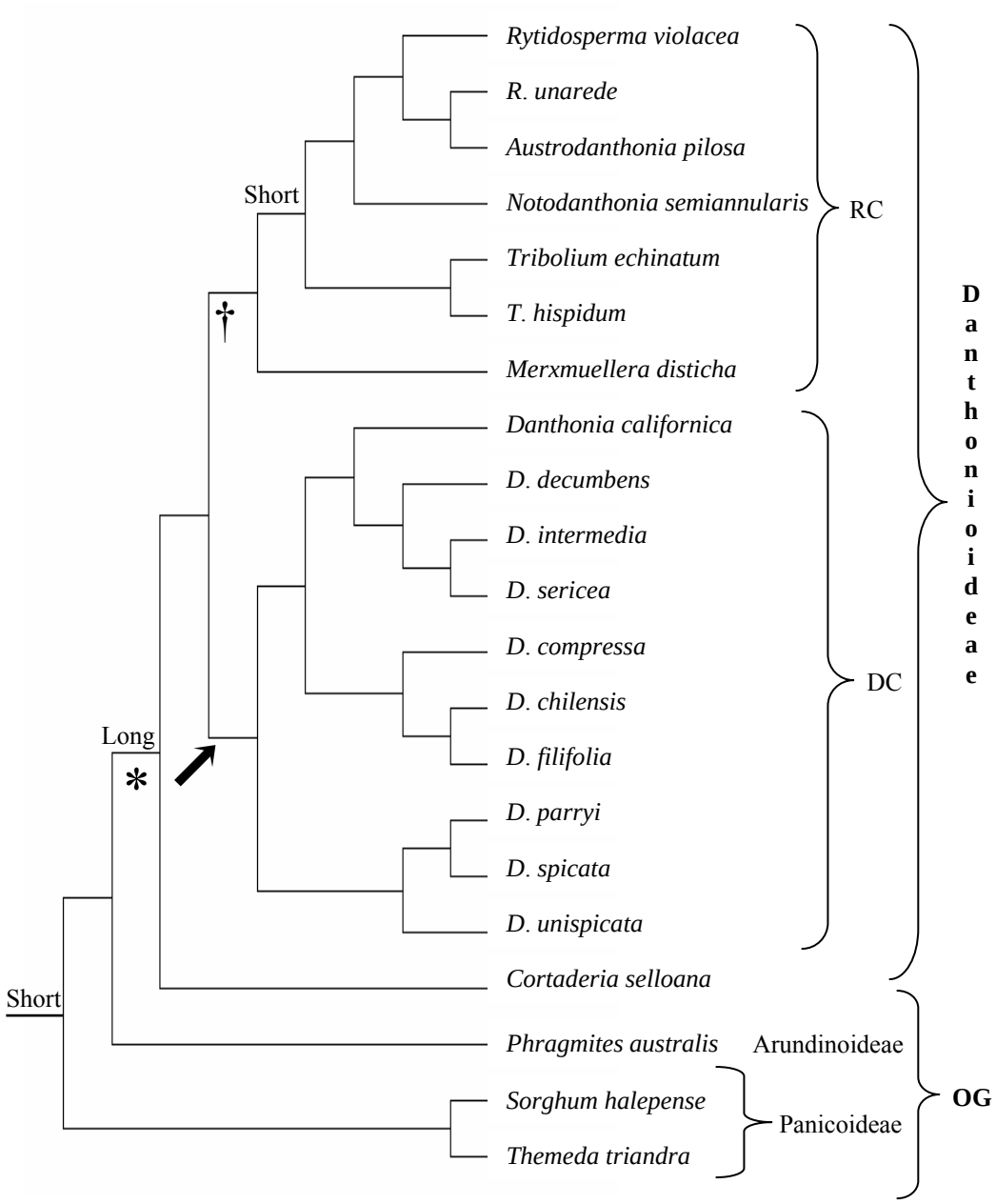

Fig. 5.6. Hilum length (indicated above the branches) optimized on one of 74 most parsimonious trees (length = 146 steps, CI=0.911, RI=0.940) obtained from phylogenetic analysis of *trn*L-F sequence data. The asterisk (*) indicates the monophyly of the Danthonioideae, the dagger (†) indicates the *Rytidosperma* clade (RC), and the arrow indicates the monophyly of the *Danthonia* clade (DC). The outgroup (OG) taxa represent the Panicoideae and Arundinoideae.

5.4 Discussion

5.4.1 Phylogeny of the Danthonioideae

The monophyletic origin of the subfamily is strongly supported by *trn*L-F sequence data [100% bootstrap and decay value of >4, whether gaps are treated as "missing" (Fig. 5.3) or as a "fifth base" (Fig. 5.4)]. The present study concurs with previous reports supporting the monophyly of the Danthonioideae (Verboom et al. 1994; Linder and Verboom 1996; Barker et al. 2000; GPWG 2001). From the morphological viewpoint, previous studies have shown that this group is characterized by haustorial synergids, bilobed prophylls, and ovaries with widely separated styles (Linder and Verboom 1996). Hence, morphological and molecular data strongly support the monophyly of the subfamily.

Within the Danthonioideae, two distinct monophyletic clades are evident in the consensus tree in both analyses: the *Rytidosperma* clade and *Danthonia* clade. *Cortaderia selloana* is basal to the *Rytidosperma* and *Danthonia* clades (Figs. 5.3 and 5.4). Australasian and South American *Rytidosperma* are closely related, and the distinction between the *Danthonia* and *Rytidosperma* clades is clear. The same relationships were observed in a study based on a combined analysis of molecular and morphological data (Barker et al. 2000). Other studies support the distinction between *Rytidosperma* and *Danthonia* (e.g. Zotov 1963; Blake 1972), however Conert (1987) objected to separating Australian and American taxa.

The present study indicates that the *Rytidosperma* clade is monophyletic, as previously reported (Barker et al. 2000). The *Rytidosperma* clade is composed of *Austrodanthonia*, *Joycea*, *Karroochloa*, *Notodanthonia*, *Rytidosperma*, *Schismus*, *Tribolium*, and some species of *Merxmuellera* (Barker et al. 2000). Although *Joycea*, *Karroochloa*, and *Schismus* were not included here, including these genera would not likely have changed the inferences evident from the *trn*L-F phylogeny. The same relationships observed in the present study were also evident in the strict consensus tree of five data sets including these taxa (Barker et al. 2000).

Within the *Rytidosperma* clade, several phylogenetic inferences can be made based on the molecular data. First, *Tribolium* is monophyletic (Figs. 5.3 and 5.4). Previous studies have also found that *Tribolium* is monophyletic when the generic

circumscription includes the now-synonymous genera *Lasiochloa* Kunth, *Plagiochloa* Adamson & Sprague, and *Urochlaena* Nees (Linder and Davidse 1997). Other inferences within the *Rytidosperma* clade imply that not all genera are monophyletic. *Austrodanthonia* is characterized by a long, pointed callus that is absent in *Rytidosperma* (Linder and Verboom 1996), but the results presented here suggest that *Austrodanthonia* is embedded within the genus *Rytidosperma*, sister to *R. unarede* (Fig. 5.3 and 5.4) making *Rytidosperma* paraphyletic. The relationship between the New Zealand taxa, *Austrodanthonia pilosa* and *R. unarede*, is moderately supported [63% bootstrap and decay value of 1 (Fig. 5.3) and share mutation number 6 (Fig. 5.5)]. This relationship may imply that geographical distribution is a better indicator of relationships than shared morphological characters for these genera. Whether *Rytidosperma* is paraphyletic needs to be tested, but it is quite possible that *Austrodanthonia*, *Notodanthonia*, and *Rytidosperma* are closely related. Zotov (1963) and Veldkamp (1980) did not make a taxonomic distinction between these genera. Perhaps these broader generic circumscriptions more accurately reflect the phylogenetic relationships within the Danthonioideae, but further studies are required to address these taxonomic issues.

The *Danthonia* clade described in the current study differs from the *Danthonia* clade defined by Barker et al. (2000). The *Danthonia* clade sensu Barker et al. (2000) included all *Danthonia* species sampled, plus two Australian genera (*Notochloe* and *Plinthanthesis*), and the New Zealand representatives of the genus *Cortaderia*. Neither *Notochloe*, *Plinthanthesis*, nor New Zealand representatives of *Cortaderia* were included in this analysis.

The *trn*L-F sequence indicates the genus *Danthonia* is monophyletic, supporting Wright's (1984) findings that were based on morphological data. Support for the monophyly of *Danthonia* is moderate (68% with gaps treated as "missing"; Fig. 5.3), but the support increases (86% bootstrap and decay value of 4) when *D. decumbens* is excluded from the analysis, likely because only partial sequences were available for this species, hence missing data decreases clade support. However, the possibility that weaker clade support could be due to sequence divergence in *D. decumbens* cannot be ruled out, an idea based on its geographic isolation from North and South American

species. *D. decumbens* has a European distribution and has become naturalized in the Americas relatively recently.

Interspecific relationships within *Danthonia* remain unresolved (Fig. 5.3). Treating gaps as a "fifth base" provides some resolution. *D. compressa*, *D. spicata*, and *D. unispicata* form a monophyletic subclade (85% bootstrap and decay value of 4; Fig. 5.4). Contrary to the findings presented here, Darbyshire (2003) suggested a close relationship between *D. californica* and *D. unispicata* based on shared morphological characters. The molecular phylogeny suggests that *D. californica* is more closely related to *D. sericea* and *D. intermedia* than to *D. unispicata* (Fig. 5.4). Despite the additional resolution provided by this analysis, the issue of character weighting is problematic when gaps are treated as a fifth base. A large deletion is likely the outcome of a single mutational event, but the analysis treats each base as a separate, independent character. Therefore, the relationships suggested by the analysis with gaps treated as a "fifth base" should be tested further.

North American and South American *Danthonia* species do not form separate geographic clades, as by Baeza (1996) using morphological phenetic analysis. This lack of geographic affinities suggests that North, Central and South American species may all have a common putative origin, as suggested previously (Dobrenz and Beetle 1966; Conert 1987).

The relationships between South American and North American species remain unclear, but the molecular phylogeny presented here suggests that *Danthonia* is monophyletic. Poor phylogenetic resolution may be caused by relatively low substitution rates or recent divergence time of taxonomic groups. Divergence of North and South American *Danthonia* species may be a relatively recent event. Lineages are thought to have radiated to the North American continent as recently as 3 million years ago, when the gap between the North and South American continents was connected by the Central American landbridge (Conert 1987).

5.4.2 trn*L-F Sequence Analysis*

Analysis of the *trn*L-F sequences indicate low levels of homoplasy as demonstrated by the tree statistics, i.e. a CI of 0.911 with gaps coded as "missing" and 0.925 with gaps coded as "fifth base" and RI of 0.940 with gaps coded as "missing" and

0.936 with gaps coded as “fifth base”. Low levels of homoplasy indicate that molecular characters provide synapomorphies for determining phylogenetic relationships. Thus, phylogenetic inferences that are based on molecular data are less likely to be confounded by parallelisms or reversals, although some mutations do occur in parallel. The low levels of homoplasy in molecular analyses contrast with the higher levels of homoplasy in the morphological data (Barker et al. 2000), suggesting that morphology is more difficult to interpret than molecular data in the Danthonioideae.

Sequence base pair composition is somewhat variable. AT content was 67.81% overall, indicating that sequences were AT rich (Table 5.4). Average GC content is higher in the subfamily relative to the outgroup (Table 5.4). The GC content of the outgroup taxa falls within the range of values seen in the ingroup taxa (Table 5.4). Therefore, base pair composition in the Danthonioideae is not the best character to infer phylogenetic relationships accurately. A comparative analysis of base pair content in nuclear and chloroplast markers would be ideal to use this data for phylogenetic inferences.

Chloroplast DNA tends to be AT enriched relative to the nuclear and mitochondrial genomes (Albert et al. 1992) because recombination leads to increased GC content through biased base conversion (Marais et al. 2004). AT content may influence the rate of transitions and transversions in the *trn*L-F region of the chloroplast, but base pair composition is not indicative of phylogenetic relationships in the Danthonioideae. High AT content can have several consequences in a genomic region. Relative nucleotide substitution rates are influenced by base pair composition in neighboring DNA regions, as was shown in noncoding regions of the Poaceae, in which higher proportions of A and T in the 5’ and 3’ flanking regions led to an increase in transversions (i.e. substitutions between purines and pyrimidines) relative to transitions (Morton 1995, 1997). Overall, base pair substitution favors transitions over transversions, but in the *trn*L-F region, the ratio of transitions to transversions ranges from 0.8 to 1 in representatives of five orders of flowering plants (Bakker et al. 2000).

5.4.3 trn*L-F Sequence Evolution*

Length mutations (insertions and deletions) can be useful characters in reconstructing phylogenetic relationships. In general, noncoding regions have a greater

proportion of indels than coding regions, and for taxa with low rates of sequence divergence, indel analysis may be phylogenetically informative. For example, in the Danthonoideae, indel events are useful in assessing phylogenetic relationships. In fact, the *trn*L-F sequences indicate that four indels (10, 19, 28, and 31) characterize the subfamily relative to the outgroup (Fig. 5.5; Table 5.5). Taxa in the *Rytidosperma* clade share one length mutation in the *trn*L-F region, and within this clade, the branch above *Merxmuellera disticha* is supported by two length mutations (Fig. 5.5). In addition, these taxa share a short, punctate hilum (Fig. 5.6). In previous studies, indels were useful in reconstructing the phylogeny of *Secale* L. (Petersen et al. 2004). Even at higher taxonomic levels, indels have been helpful, for example, a 6 bp deletion *mat*K region characterizes the PACC clade (Hilu and Alice 1999), and several indels in the *ndh*F region were also phylogenetically informative in panicoid grasses (Giussani et al. 2001). A problem in phylogenetic reconstruction may arise when non-homologous indels overlap, which can lead to misinterpretation of relationships (Morton 1995).

While some length mutations are informative in the Danthonioideae, others may be misleading. For instance, the length mutation shared by *Sorghum*, *Themeda*, and *Tribolium* (length mutation 9 from Table 5.5) likely has two independent origins, one in the common ancestor of *Sorghum* and *Themeda*, and a second in *Tribolium*. *Danthonia compressa*, *D. parryi*, *D. spicata*, and *D. unispicata* share a five bp deletion (length mutation 15) at position 233 of the multiple alignment (Table 5.5); however, there are no known morphological characters that are shared by these species. Thus the utility of this indel deletion as an informative character may be ambiguous if no true homology exists.

5.4.4 Morphology and Molecular Phylogeny

Several morphological characters obtained from studies presented in Chapters 2 and 3 were optimized on the *trn*L-F chloroplast phylogeny, but showed high levels of homoplasy. Among these, only hilum length appears to be an informative morphological attribute. The *Danthonia* clade and *Cortaderia selloana* share the same hilum type (linear) with the Aristidoideae (Fig. 5.6). The remaining members of the PACCAD clade have short hila (Clayton et al. 2002 onwards). The Danthonioideae and Aristidoideae are sister subfamilies (GPWG 2001), suggesting that the common ancestor of these two subfamilies had a long linear hilum. If this is the case, then the presence of a short hilum

in some taxa in the *Rytidosperma* clade (Fig. 5.6) represents a reversal to the putative ancestral condition.

Contrary to Blake (1972), who argued that hilum length was not an appropriate character, this character provided a great deal of taxonomic utility within the Danthonioideae. Although this trait has seemingly undergone several reversals in the Poaceae, it provided support for the phylogenetic relationships within the subfamily.

5.4.5 Final Remarks

The subfamily Danthonioideae is monophyletic. Within the subfamily two monophyletic clades, *Danthonia* and *Rytidosperma*, were identified. *Cortaderia selloana* is basal to the aforementioned clades. Caryopsis shape, lodicule characters, silica body shape and distribution on the abaxial epidermis were homoplasious and do not provide unambiguous synapomorphies for the clades identified within the Danthonioideae (Reimer and Cota-Sánchez in review). Examining new characters may identify morphological features that are helpful in elucidating relationships within this group. Even though cleistogenes have been suggested as a synapomorphy for the genus *Danthonia* (Linder and Verboom 1996), this character is difficult to observe on herbarium specimens because a thorough examination of all leaf sheaths damages specimens.

Although the *Danthonia* clade is monophyletic, this study does not support the separation of North and South American and European species. Interspecific relationships within *Danthonia* are not clear, and the use of additional molecular markers with a higher mutation rate is required for phylogenetic resolution of terminal taxa.

Finally, this study shows strong evidence for the taxonomic separation of *Danthonia* from *Rytidosperma*, two genera previously reported to be closely related (Wright 1984). This conclusion holds true for the South American *Rytidosperma* species, which do not form part of the *Danthonia* clade. Further, this study reinforces the relationships among the genera in the *Rytidosperma* clade defined by Barker et al. (2000). Within the *Rytidosperma* clade, *Rytidosperma* may be paraphyletic. Furthermore, *Tribolium* appears to form a monophyletic assemblage, but further studies with broader taxonomic sampling are needed. Though taxonomic sampling of South

American *Rytidosperma* only included one species, the *trn*L-F strict consensus tree shows strong support for its inclusion in the *Rytidosperma* clade. Conert's (1987) assertion that *Rytidosperma* should be included within *Danthonia* is not supported by the molecular phylogeny presented here. Instead, it appears that the geographic ranges of *Rytidosperma* and *Danthonia* overlap in South America, as Nicora (1963) suggested.

6.0 General Conclusions

Phylogenetic relationships within the Poaceae are currently an active area of investigation. The circumscription of subfamilies put forth by the GPWG (2001) is widely accepted, but many gaps remain in our understanding of relationships within these groups. In the context of broader, ongoing research into the evolution of the Poaceae, this study provides morphological and molecular information that is important to fill these gaps where knowledge is required.

Furthermore, this study provides new information on the morphology of the Danthonioideae. Character evolution is difficult to trace in the Poaceae due to the complex relationships often exhibited among members of this family, and because hybridization and polyploidy occur frequently. These processes may obscure patterns in the evolution of morphological traits. Therefore, studies of grass morphology, such as this survey, provide insight to the development and evolution of structures. In addition, the function of some grass features such as microhairs is not fully understood. Further research will lead to a better understanding of the evolution and ontogeny of these characters, and may provide insight into their function.

This research provides new information regarding the morphology of caryopsis and leaf epidermal structures in danthonioid grasses. The systematic utility of epidermal and caryopsis attributes is limited in the subfamily when these traits are treated independently. Overlap of morphological characters at the intergeneric and interspecific levels and the apparent multiple origins of various features increases the levels of homoplasy, making taxonomic inferences difficult.

None of the characters investigated provide distinctive information at the subfamilial and generic levels, nor do they separate Old and New World danthonioid taxa. Nevertheless, this study provides a framework that can be used in a future interpretations and re-evaluations of taxonomic and phylogenetic relationships of the Danthonioideae, in particular for North American *Danthonia*. For instance, several

diagnostic characters are distinctive of *Danthonia*, including the absence of abaxial stomata, presence of bicellular microhairs with basal and terminal cells of equal length and microhairs with long basal cells relative to terminal cells, and prickle hairs in four North American species. Furthermore, when these micromorphological characters are evaluated in concert with other data, the significance of leaf epidermal traits in danthonioid grasses becomes more relevant.

Similarly, the hilum and caryopsis surface pattern of danthonioid taxa, also provide support for phylogenetic relationships in the subfamily. When data are examined together, several genera can be distinguished. Foremost, *Danthonia* has the following three features associated with the caryopsis, hilum, and surface pattern: 1) ovoid to obovoid caryopsis shape, 2) linear hilum, and 3) undulating or straight reticulate surface pattern. No other taxon examined in this study possesses this combination of characters. Secondly, *Rytidosperma* is characterized by 1) ovoid or obovoid caryopses that are generally smaller than the caryopses in *Danthonia*, 2) short, punctate hila, and 3) undulating reticulate or substriate caryopsis surface patterns. Finally, the genus *Tribolium* has 1) small, obovoid caryopses ≤1.2 mm in length, 2) short, punctate hila, and 3) a substriate caryopsis surface pattern. Even though *Cortaderia* shares the linear hilum and undulating reticulate surface pattern with *Danthonia*, the lanceolate caryopsis shape is quite different from the ovoid to obovoid caryopsis of *Danthonia*. Morphological characters considered as a whole provide support for the molecular phylogeny, and this suite of characters provides strong evidence for the monophyletic origin of the genus *Danthonia*.

Contrary to previous findings (Baum and Findlay 1973; Findlay and Baum 1974; Veldkamp 1980), lodicule morphology was of little utility in characterizing genera and species within the Danthonioideae. Although lodicule vestiture could not be determined for several taxa, the specimens examined seem to indicate that multicellular hairs are lacking in *Danthonia*, but they are present in *Austrodanthonia*, *Rytidosperma*, and *Tribolium*. Multicellular lodicule hairs may provide support for a monophyletic *Rytidosperma* when this character is examined across a broader taxonomic sampling.

The subfamily Danthonioideae is monophyletic. Within this subfamily two monophyletic clades were identified, the *Danthonia* and *Rytidosperma* clades.

Cortaderia selloana is basal to the aforementioned clades. Caryopsis shape, lodicule characters, silica body shape and distribution on the abaxial epidermis are highly homoplasious, and do not provide unambiguous synapomorphies for the clades identified within the Danthonioideae. Examining new characters may be beneficial in identifying morphological features that elucidate relationships within this group. Even though cleistogenes have been suggested as a synapomorphy for the genus *Danthonia* (Linder and Verboom 1996), this character is difficult to observe on herbarium specimens without causing damage to specimens.

This study does not support the separation of North and South American and European species despite the monophyly of the *Danthonia* clade. Interspecific relationships within *Danthonia* are not clear, and the use of additional molecular markers with a higher mutation rate is required for phylogenetic resolution of terminal taxa.

Finally, these studies show that there is strong evidence for the taxonomic separation of *Danthonia* from *Rytidosperma*, two genera previously reported to be closely related (Wright 1984). This conclusion holds true for the South American *Rytidosperma* species, which are distinct from the *Danthonia* clade. Further, the present study reinforces the inclusion of *Austrodanthonia*, *Notodanthonia*, *Rytidosperma*, *Tribolium*, and some members of the genus *Merxmuellera* in a single monophyletic clade, the *Rytidosperma* clade (Barker et al. 2000), Within the *Rytidosperma* clade, *Rytidosperma* may be paraphyletic. Furthermore, *Tribolium* appears to form a monophyletic assemblage, as reported here, and in a previous study (Linder and Davidse 1997). Even though taxonomic sampling of South American *Rytidosperma* included only one species, the *trn*L-F strict consensus tree shows strong support for its inclusion in the *Rytidosperma* clade. The morphological and molecular analyses presented in this study do not support Conert's (1987) assertion that *Rytidosperma* should be included within *Danthonia*, but rather it appears that the geographic ranges of *Rytidosperma* and *Danthonia* overlap in South America, as Nicora (1963) suggested.

6.1 Future Studies

The molecular phylogeny presented here indicates that *Danthonia* is a monophyletic genus; however, intrageneric relationships within this group remain

obscure. The lack of phylogenetic resolution in the terminal branches of the genus could be resolved with additional molecular markers with higher mutation rates. At the intrageneric level, internal transcribed spacer (ITS) has been used in combination with chloroplast markers in phylogenetic studies of the Poaceae (e.g. Baumel et al. 2002; Hodkinson et al. 2002; Catalán et al. 2004; Hunter et al. 2004), and in other taxa, e.g. *Zigadenus* (Zomlefer et al. 2001). Including a broader taxonomic sampling from across all of the seven informal groups identified by Barker et al. (2000) would elucidate the phylogenetic structure of the subfamily. It would be especially helpful to include the closest putative relatives of *Danthonia,* including *Notochloe*, *Plinthanthesis*, and the New Zealand species of *Cortaderia*, to examine relationships within the *Danthonia* clade sensu Barker et al. (2000).

Further studies are required to identify morphological synapomorphies for clades within the Danthonioideae. The length of the terminal cell of the microhair, caryopsis surface pattern, and the presence of multicellular microhairs on the lodicules, should be examined across a broader sampling of the subfamily and optimized on the molecular phylogeny. In the case of the lodicule hairs, multicellular hairs were found only in the *Rytidosperma* clade (as defined in chapter 4 of this study), however, sampling was restrained by limited availability of material. Future research should focus on examining these characters in depth to corroborate or refute their taxonomic utility. Broader taxonomic sampling would be helpful in assessing phylogenetic relationships.

No morphological synapomorphies for *Danthonia* were identified in this study. Further examination of the distribution of cleistogamous florets in the leaf axils of danthonioid grasses may provide additional information to support the molecular phylogeny. Cleistogenes in the upper leaf sheaths is reported as a synapomorphy for *Danthonia* (Linder and Verboom 1996). Cleistogamy has been reported extensively in North and South American species of *Danthonia* (Weatherwax 1928; Vickery 1956; Dobrenz and Beetle 1966; Clay 1983; Linder and Verboom 1996). The present study was based largely on the examination of plant material from herbaria, and looking for cleistogenes in the leaf sheaths causes extensive damage to plant specimens. This same difficulty prevented Wright (1984) from conducting a complete examination of this character. High-resolution X-ray computed tomography (HRCT) was used to create an

accurate three-dimensional image of a tulip flower (*Tulipa* hybrid), and the inflorescence of *Leucospermum tottum* (Proteaceae) (Stuppy 2003). Unlike nuclear magnetic resonance imaging, HRTC does not require high water content of the tissue being examined, and HRTC is more applicable than confocal laser scanning microscopy because the tissue need not be semi-transparent to produce a high-quality image (Stuppy 2003). This technique holds potential for the non-destructive investigation of other delicate materials, such as the cleistogenes in danthonioid grasses.

7.0 LITERATURE CITED

ACEDO, C. and F. LLAMAS. 2001. Variation of micromorphological characters of lemma and palea of the genus *Bromus* (Poaceae). *Annals of Botany Fennici* 38: 1-14.

ALBERT, V. A., B. D. MISHLER, and M. W. CHASE. 1992. Character-state weighting for restriction site data in phylogenetic reconstruction, with an example from chloroplast DNA. Pp. 369-401 in *Molecular Systematics of Plants*, eds. P. S. Soltis, D. E. Soltis, and J. J. Doyle. New York: Chapman and Hall.

AMARASINGHE, V. and L. WATSON. 1990. Taxonomic significance of microhair morphology in the genus *Eragrostis* Beauv. (Poaceae). *Taxon* 39: 59-65.

AUSTIN, C. F. 1872. *Danthonia* DC. *D. Alleni*. *Bulletin of the Torrey Botanical Club* 3: 21-22.

BAEZA, C. M. 1996. Los géneros *Danthonia* DC. y *Rytidosperma* Steud. (Poaceae) en América – una revisión. *Sendtnera* 3: 11-93.

BAKKER, F. T., A. CULHAM, R. GOMEZ-MARTINEZ, J. CARVALHO, J. COMPTON, R. DAWTREY, and M. GIBBY. 2000. Patterns of nucleotide substitution in angiosperm cpDNA *trn*L (UAA)–*trn*F (GAA) regions. *Molecular Biology and Evolution* 17: 1146-1155.

BARKER, N. P. 1994. External fruit morphology of southern African Arundineae (Arundinoideae: Poaceae). *Bothalia* 24: 55-67.

BARKER, N. P., P. H. LINDER, and E. H. HARLEY. 1995. Polyphyly of Arundinoideae (Poaceae): Evidence from *rbc*L sequence data. *Systematic Botany* 20: 423-435.

BARKER, N. P., P. H. LINDER, and E. H. HARLEY. 1999. Sequences of the grass-specific insert in the chloroplast *rpo*C2 gene elucidate generic relationships of the Arundinoideae (Poaceae) *Systematic Botany* 23: 327-350.

BARKER, N. P., P. H. LINDER, C. M. MORTON, and M. LYLE. 2003. Paraphyly of the genus *Cortaderia* (Danthonioideae: Poaceae): Evidence from morphology and chloroplast and nuclear DNA sequence data. *Annals of the Missouri Botanical Garden* 90: 1-24.

BARKER, N. P., C. M. MORTON, and H. P. LINDER. 2000. The Danthonieae: Generic composition and relationships. Pp.221-230 in *Grasses: Systematics and Evolution*, eds. S. W. L. Jacobs, and J. Everett. Melbourne: CSIRO.

BARKWORTH, M. E. 1981. Foliar epidermes and taxonomy of North American Stipeae (Gramineae). *Systematic Botany* 6: 136-152.

BAUM, B. R. and J. N. FINDLAY. 1973. Preliminary studies in the taxonomy of *Danthonia* in Canada. *Canadian Journal of Botany* 51: 437-450.

BAUMEL, A., M. L. AINOUCHE, and R. J. BAYER. 2002. Molecular phylogeny of hybridizing species from the genus *Spartina* Schreb. (Poaceae). *Molecular Phylogenetics and Evolution* 22: 303-314.

BLAKE, S. T. 1972. *Plinthanthesis* and *Danthonia* and a review of the Australian species of *Leptochloa* (Gramineae). *Contributions of the Queensland Herbarium* 14: 1-19.

BÖHLE, U. R., H. HILGER, R.,CERFF, and W. F. MARTIN. 1994. Non-coding chloroplast DNA for plant molecular systematics at the infrageneric level. Pp 391-403 in *Molecular ecology and evolution: Approaches and applications*, eds. B. Schierwater, B. Streit, G. P. Wagner, and R. DeSalle. Basel: Birkhauser Verlag.

BOIVIN, B. 1981. Flora of the Prairie Provinces. A handbook to the flora of the provinces of Manitoba, Saskatchewan and Alberta. Part V. Gramineae. *Provancheria* 12: 1-108.

BOMMERT, P., N. SATOH-NAGASAWA, D. JACKSON and H. Y. HIRANO. 2005. Genetics and evolution of inflorescence and flower development in grasses. *Plant and Cell Physiology* 46: 69-78.

BRANDENBURG, D. M. 2003. Notes on free pericarps in grasses (Poaceae). *Journal of the Kentucky Academy of Science* 64: 114-120.

BRANDENBURG, D. M., J. R. ESTES, and S. L. COLLINS. 1991. A revision of *Diarrhena* (Poaceae) in the United States. *Bulletin of the Torrey Botanical Club* 118: 128-136.

BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42: 795-803.

BREMER, K. 2002. Gondwanan evolution of the grass alliance of families (Poales). *Evolution* 56: 1374-1387.

BRYSTING, A. K., A. HOLST-JENSEN, and I. LEITCH. 2000. Genomic origin and organization of the hybrid *Poa jemlandica* (Poaceae) verified by genomic in situ hybridization and chloroplast DNA sequences. *Annals of Botany* 85: 439-445.

CAI, L.-B., T.-L. ZHANG, and A. I. HSAIO. 2003. The circumscription and concept of *Hordeum* (Poaceae) based on some leaf epidermal characteristics. *Israel Journal of Plant Sciences* 51: 315-320.

CAMPBELL, C. S. and E. A. KELLOGG. 1987. Sister group relationships of the Poaceae. Pp. 217-227 in *Grass Systematics and Evolution*, eds. T. R. Soderstrom, K. W. Hilu, C. S. Campbell, and M. E. Barkworth. Washington: Smithsonian Institute Press.

CATALÁN, P., E. A. KELLOGG, and R. G. OLMSTEAD. 1997. Phylogeny of Poaceae subfamily Pooideae based on chloroplast *ndh*F gene sequences. *Molecular Phylogenetics and Evolution* 8: 150-166.

CATALÁN, P., P. TORRECILLA, J. A. L. RODRIGUEZ, and R. OLMSTEAD. 2004. Phylogeny of the festucoid grasses subtribe Loliineae and allies (Poeae: Pooideae) inferred from ITS and *trn*L-F sequences. *Molecular Phylogenetics and Evolution* 31: 517-541.

CLARK, L. G., W. ZHANG, and J. F. WENDEL. 1995. A phylogeny of the grass family (Poaceae) based on *ndh*F sequence data. *Systematic Botany* 20: 436-460.

CLAY, K. 1983. Variation and degree of cleistogamy within and among species of the grass *Danthonia*. *American Journal of Botany* 70: 835-843.

CLAYTON, W. D., K. T. HARMAN, and H. WILLIAMSON. 2002 onwards. World Grass Species: Descriptions, Identification and Information Retrieval. http://www.kew.org/data/grasses-db.html [Accessed 29 September 2005].

CLEGG, M. T., B. S. GAUT, G. H. LEARN, and B. H. MORTON. 1994. Rates and patterns of chloroplast DNA evolution. *Proceedings of the National Academy of Science U.S.A.* 91: 795-6801.

CLIFFORD, H. T. 1987. Spikelet and floral morphology. Pp. 21-30 in *Grass Systematics and Evolution*, eds. T. R. Soderstrom, K. W. Hilu, C. S. Campbell, and M. E. Barkworth. Washington: Smithsonian Institute Press.

CODY, W. J. 2000. *Flora of the Yukon Territory*, Second Edition. Ottawa: NRC Research Press.

CONERT, H. J. 1987. Current concept in the systematics of the Arundinoideae. Pp. 239-250 in *Grass Systematics and Evolution*, eds. T. R. Soderstrom, K. W. Hilu, C. S. Campell, and M. E. Barkworth. Washington: Smithsonian Institute Press.

CONNOR, H. E. 1979. Breeding systems in the grasses: A survey. *New Zealand Journal of Botany* 17: 547-574.

CONNOR, H. E. and E. EDGAR. 1979. *Rytidosperma* Steudel (*Notodanthonia* Zotov) in New Zealand. *New Zealand Journal of Botany* 17: 311-337.

COSTEA, M. and F. J. TARDIF. 2002. Taxonomy of the most common weedy European *Echinochloa* species (Poaceae: Panicoideae) with special emphasis on characters of the lemma and caryopsis. *SIDA* 20: 525-528.

CRISCI, J. V. and T. F. STEUSSY. 1980. Determining primitive character states for phylogenetic reconstruction. *Systematic Botany* 5: 112-135.

DANG, L. A. and D. R. PRING. 1986. A physical map of the *Sorghum* chloroplast genome. *Plant Molecular Biology* 6: 119-123.

DARBYSHIRE, S. J. 2003. *Danthonia* DC. Pp. 301-306 in *Flora of North America North of Mexico*, Vol. 25, eds. Flora of North America Editorial Committee. New York: Oxford University Press.

DARBYSHIRE, S. J. and J. CAYOUETTE. 1989. The biology of Canadian weeds. 92. *Danthonia spicata* (L.) Beauv. in Roem. & Schult. *Canadian Journal of Plant Science* 69: 1217-1233.

DÁVILA, P. and L. G. CLARK. 1990. Scanning electron microscopy survey of leaf epidermis of *Sorghastrum* (Poaceae: Andropogoneae). *American Journal of Botany* 77: 499-511.

DAVIS, J. I. and R. J. SORENG. 1993. Phylogenetic structure in the grass family (Poaceae) as inferred from chloroplast DNA restriction site variation. *American Journal of Botany* 80: 1444-1454.

DEWET, J. M. J. 1954. The genus *Danthonia* in grass phylogeny. *American Journal of Botany* 41: 204-211.

DEWET, J. M. J. 1956. Leaf anatomy and phylogeny in the tribe Danthonieae. *American Journal of Botany* 43: 175-182.

DEWET, J. M. J. 1987. Hybridization and polyploidy in the Poaceae. Pp. 188–194 in *Grass Systematics and Evolution*, eds. T. R. Soderstrom, K. W. Hilu, C. S. Campbell, and M. E. Barkworth. Washington: Smithsonian Institute Press.

DOBRENZ, A. K. and A. A. BEETLE. 1966. Cleistogenes in *Danthonia*. *Journal of Range Management* 19: 292-296.

DORE, W. G. and J. MCNEILL. 1980. *Grasses of Ontario*. Monograph No. 26. Ottawa: Research Branch, Agriculture Canada.

DOUST, A. N. and E. A. KELLOGG. 2002. Inflorescence diversification in the panicoid "bristle clade" (Paniceae, Poaceae): evidence from molecular phylogenies and developmental morphology. *American Journal of Botany* 89: 1202-1222.

DOUST, A. N., K. M. DEVOS, M. D. GADBERRY, M. D. GALE and E. A. KELLOGG. 2005. The genetic basis for inflorescence variation between foxtail and green millet (Poaceae). *Genetics* 169: 1659-1672.

DOWNIE, S. R. and J. D. PALMER. 1992. Use of chloroplast DNA rearrangements in reconstructing plant phylogeny. Pp 14-35 in *Molecular Systematics of Plants*, eds. P. S. Soltis, D. E. Soltis, and J. J. Doyle. New York: Chapman and Hall.

DUVALL, M. and B. MORTON. 1996. Molecular phylogenetics of Poaceae: an expanded analysis of *rbcL* sequence data. *Molecular Phylogenetics and Evolution* 5: 352-358.

EL NAGGAR, S. M. I. 2001. Systematic implications of seed coat morphology in Malvaceae. *Pakistan Journal of Biological Science* 4: 822-828.

ELDENÄS, P. K. and H. P LINDER, 2000. Congruence and complementarity of morphological and *trn*L-*trn*F sequence data and the phylogeny of the African Restionaceae. *Systematic Botany* 25: 692–707.

ELLIS, R. P. 1979. A procedure for standardizing comparative leaf anatomy in the Poaceae II: The epidermis as seen in surface view. *Bothalia* 12: 641-671.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783-791.

FINDLAY, J. N. and B. R. BAUM. 1974. The nomenclatural implications of the taxonomy of *Danthonia* in Canada. *Canadian Journal of Botany* 52: 1573-1582.

FITCH, W. 1971. Toward defining the course of evolution: Minimal change for a specific tree topology. *Systematic Zoology* 20: 406-416.

GALLEGO, L. and R. A. DISTEL. 2004. Phytolith assemblages in grasses native to central Argentina. *Annals of Botany* 94: 865-874.

GE, S., A. LI, B. R. LU, and S. Z. ZHANG. 2002. A phylogeny of the rice tribe Oryzeae (Poaceae) based on *mat*K sequence data. *American Journal of Botany* 89: 1967-1972.

GIUSSANI, L. M., J. H. COTA-SÁNCHEZ, F. O. ZULOAGA, and E. A. KELLOGG. 2001. A molecular phylogeny of the Panicoideae (Poaceae) shows multiple origins of C4 photosynthesis. *American Journal of Botany* 88: 1193-2012.

GRASS PHYLOGENY WORKING GROUP. 2000. A phylogeny of the grass family (Poaceae) as inferred from eight character sets. Pp. 3-7 in *Grasses: Systematics and Evolution*, eds. S. W. L. Jacobs, and J. Everett. Melbourne: CSIRO.

GRASS PHYLOGENY WORKING GROUP. 2001. Phylogeny and subfamilial classifications of the grasses (Poaceae). *Annals of the Missouri Botanical Garden* 88: 373-457.

GRAY, J. R., J. A. QUINN, and D. E. FAIRBROTHERS. 1969. Leaf epidermis morphology in populations of the *Danthonia sericea* complex. *Bulletin of the Torrey Botanical Club* 96: 525-530.

GREAT PLAINS FLORA ASSOCIATION. 1986. *Flora of the Great Plains*. Lawrence: University Press of Kansas.

HILU, K. W. 1984. Leaf epidermes of *Andropogon* sect. *Leptopogon* (Poaceae) in North America. *Systematic Botany* 9: 247-257.

HILU, K. W. 1987. Chloroplast DNA in the systematics and evolution of the Poaceae. Pp. 65-72 in *Grass Systematics and Evolution*, eds. T. R. Soderstrom, K. W. Hilu, C. S. Campbell, and M. E. Barkworth. Washington: Smithsonian Institute Press.

HILU, K. W. 2004. Phylogenetics and chromosomal evolution in the Poaceae (grasses). *Australian Journal of Botany* 52: 13-22.

HILU, K. W. and L. A. ALICE. 1999. Evolutionary implications of *mat*K indels in Poaceae. *American Journal of Botany* 86: 1735-1741.

HILU, K. W. and K. WRIGHT. 1982. Systematics of Gramineae: A cluster analysis study. *Taxon* 31: 9-36.

HILU, K. W., J. M. J. DEWET, and J. R. HARLAN. 1979. Archaeobotanical studies of *Eleusine coracana* ssp. *coracana* (finger millet). *American Journal of Botany* 66: 330-333.

HIRATSUKA, J., H. SHIMADA, R. F. WHITTIER, T. ISHIBASHI, M. SAKAMOTO, M. MORI, C. KONDO, Y. HONJI, C. R. SUN, B. Y. MENG, Y. Q. LI, A. KANNO, Y. NISHIZAWA, A. HIRAI, K. SHINOZAKI, and M. SUGIURA. 1989. The complete sequence of the rice (*Oryza sativa*) chloroplast genome: Intermolecular recombination between distinct tRNA genes accounts for a major plastid DNA inversion during the evolution of the cereals. *Molecular and General Genetics* 217: 185-194.

HITCHCOCK, A. S. 1951. *Manual of the Grasses of the United States*. U.S.D.A. Miscellaneous Publication 200. Washington, DC: U.S. Government Printing Office.

HODKINSON, T. R., M. W. CHASE, D. M. LLEDÓ, N. SALAMIN, and S. A. RENVOIZE. 2002. Phylogenetics of *Miscanthus*, *Saccharum* and related genera (Saccharinae, Andropogoneae, Poaceae) based on DNA sequenced from ITS nuclear ribosomal DNA and plastid *trn*L intron and *trn*L-F intergenic spacers. *Journal of Plant Research* 115: 381-392.

HSAIO, C., S. W. L. JACOBS, N. P. BARKER, and N. J. CHATTERTON. 1998. A molecular phylogeny of the subfamily Arundinoideae (Poaceae) based on sequences of rDNA. *Australian Systematic Botany* 11: 41-52.

HSU, C. C. 1965. The classification of *Panicum* (Gramineae) and its allies, with special reference to the characters of lodicule, style-base and lemma. *Journal of the Faculty of Science, University Tokyo, section III (Botany)* 9: 43-150

HUBBARD, C. E. 1934. Gramineae. Pp. 199-229 in *The Families of Flowering Plants* Vol. II, ed. J. Hutchinson. London: McMillan and Co.

HUNTER, A. M., D. A. ORLOVICH, K. M. LLOYD, W. G. LEE, and D. J. MURPHY, 2004. The generic position of *Austrofestuca littoralis* and the reinstatement of *Hookerochloa* and *Festucella* (Poaceae) based on evidence from nuclear (ITS) and chloroplast (*trn*L-*trn*F) DNA sequences. *New Zealand Journal of Botany* 42: 253-262.

HUNZIKER, J. H. and G. L. STEBBINS. 1987. Chromosomal evolution in the Gramineae. Pp. 179-187 in *Grass Systematics and Evolution*, eds. T. R. Soderstrom, K. W. Hilu, C. S. Campbell, and M. E. Barkworth. Washington: Smithsonian Institute Press.

JACOBS, S. W. L. 1982. Comment on Proposal 520: Conservation of *Notodanthonia* Zotov (Gramineae). *Taxon* 31: 737–743.

JANSSEN, T. and K. BREMER. 2004. The age of major monocot groups inferred from 800+ *rbc*L sequences. *Botanical Journal of the Linnean Society* 146: 385-398.

JORDAN, J. L., L. S. JORDAN, and C. M. JORDAN. 1983. Prominent spermoderm patterns of Poaceae. *Botanical Magazine of Tokyo* 96: 269-272.

KELLOGG, E. A. 1998. Relationships of cereal crops and other grasses. *Proceedings of the National Academy of Science U.S.A.* 95: 2005-2010.

KELLOGG, E. A. 2000. The grasses: A case study in macroevolution. *Annual Review of Ecological Plant Systematics* 31: 217-238.

KELLOGG, E. A. 2001. Evolutionary history of the grasses. *Plant Physiology* 125: 1198-1205.

KELLOGG, E. A., K. M. HISER, and A. N. DOUST. 2004. Taxonomy, phylogeny, and inflorescence development of the genus *Ixophorus* (Panicoideae: Poaceae). *International Journal of Plant Science* 165: 1089-1105.

KESLER, T. R., L. C. ANDERSON, and S. M. HERMANN. 2003. A taxonomic reevaluation of *Aristida stricta* (Poaceae) using anatomy and morphology. *Southeastern Naturalist* 2: 1-10.

KÖRBER-GROHNE, U. 1981. Distinguishing prehistoric cereal grains of *Triticum* and *Secale* on the basis of their surface patterns using the scanning electron microscope. *Journal of Archeological Science* 8: 197-204.

LAZARIDES, M. 1997. A revision of *Eragrostis* (Eragrostideae, Eleusininae, Poaceae) in Australia. *Australian Systematic Botany* 10: 77-187.

LINDER, H. P. 1997. Nomenclatural corrections in the *Rytidosperma* complex (Danthonieae, Poaceae). *Telopea* 7: 269-274.

LINDER, H. P. and N. P. BARKER. 2000. Biogeography of the Danthonioideae. Pp. 231-238 in *Grasses: Systematics and Evolution*, eds. S. W. L. Jacobs, and J. Everett. Melbourne: CSIRO.

LINDER, H. P. and G. DAVIDSE. 1997. The systematics of *Tribolium* Desv. (Danthonieae: Poaceae). *Botanische Jahrbücher für Systematik Pflanzengeschichte und Pflanzengeographie* 119: 445-507.

LINDER, H. P. and G. A. VERBOOM. 1996. Generic limits in the *Rytidosperma* (Danthonieae, Poaceae) complex. *Telopea* 6: 597-627.

LIU, Q., N. X. ZHAO, and G. HAO. 2005a. Inflorescence structures and evolution in the subfamily Chloridoideae. *Plant Systematics and Evolution* 251: 183-198.

LIU, Q., N. X. ZHAO, G. HAO., X. Y. HU, and Y. X. LIU. 2005b. Caryopsis morphology of the Chloridoideae (Gramineae) and its systematic implications. *Botanical Journal of the Linnean Society* 148: 57-72.

MADISON, W. P. and D. R. MADISON. 1999. MacClade: Analysis of phylogeny and character evolution. Vers. 3.04. Sinauer, Sunderland, MA.

MANNING, J. C. and P. GOLDBLATT. 1991. Systematic and phylogenetic significance of the seed coat in the shrubby African Iridaceae, *Nivenia*, *Klattia*, and *Witsenia*. *Botanical Journal of the Linnean Society* 107: 387-404.

MARAIS, G., B. CHARLESWORTH, and S. I. WRIGHT. 2004. Recombination and base composition: the case of the highly self-fertilizing plant *Arabidopsis thaliana*. *Genome Biology* 5: R45.

MATHEWS, S., R. C. TSAI, and E. A. KELLOGG. 2000. Phylogenetic structure in the grass family (Poaceae): evidence from the nuclear gene phytochrome B. *American Journal of Botany* 87: 96-107.

MEINZER, F. and G. GOLDSTEIN. 1985. Some consequences of leaf pubescence in the Andean giant rosette plant *Espeletia timotensis*. *Ecology* 66: 512-520.

MEJIA-SAULES, T. and F. A. BISBY. 2003. Silica bodies and hooked papillae in lemmas of *Melica* species (Gramineae: Pooideae). *Botanical Journal of the Linnean Society* 141: 447-463.

METCALFE, C. R. 1960. *Anatomy of the Monocotyledons I Gramineae*. London, UK: Oxford University Press.

MICHELANGELI, F. A., J. I. DAVIS, and D. W. STEVENSON. 2003. Phylogenetic relationships among Poaceae and related families as inferred from morphology, inversions in the plastid genome, and sequence data from the mitochondrial and plastid genomes. *American Journal of Botany* 90: 93-106.

MISSOURI BOTANICAL GARDEN. 2005. W^{3}TROPICOS Nomenclatural Database. http://mobot.mobot.org/W3T/Search/vast.html. [Accessed 29 September 2005]

MOLVRAY, M. and P. J. KORES. 1995. Character analysis of the seed coat in Spiranthoideae and Orchidoideae, with special reference to the Diurideae (Orchidaceae). *American Journal of Botany* 82: 1443-1454.

MORTON, B. R. 1995. Neighboring base composition and transversion/transition bias in a comparison of rice and maize chloroplast noncoding regions. *Proceedings of the National Academy of Sciences of the U.S.A.* 92: 9717-9721.

MORTON, B. R. 1997. The influence of neighbouring base composition on substitutions in plant chloroplast coding sequences. *Molecular Biology and Evolution* 14: 189-194.

MUASYA, A. M., D. A. SIMPSON, M. W. CHASE, and A. CULHAM. 2001. A phylogeny of *Isolepis* (Cyperaceae) based on *rbc*L and *trn*L-F sequence data. *Systematic Botany* 26: 342-353.

MURAI, K. and K. TSUNEWAKI. 1987. Chloroplast genome evolution in the genus *Avena*. *Genetics* 116: 613-621.

NICORA, E. G. 1973. Novedades agrostológicas Patagónicas. *Darwiniana* 18: 80-106.

OLMSTEAD, R. G. and J. D. PALMER. 1994. Chloroplast DNA systematics: A review of methods and data analysis. *American Journal of Botany* 81: 1205-1224.

PALMER, P. G. and S. GERBETH-JONES. 1986. A scanning electron microscope survey of the epidermis of East African grasses, IV. *Smithsonian Contributions to Botany* 62: 1-120.

PALMER, P. G. and S. GERBETH-JONES. 1988. A scanning electron microscope survey of the epidermis of East African grasses, V, and West African supplement. *Smithsonian Contributions to Botany* 67: 1-157.

PALMER, P. G. and A. E. TUCKER. 1981. A scanning electron microscope survey of the epidermis of East African grasses, I. *Smithsonian Contributions to Botany* 49: 1-84.

PALMER, P. G. and A. E. TUCKER. 1983. A scanning electron microscope survey of the epidermis of East African grasses, II. *Smithsonian Contributions to Botany* 53: 1-72.

PALMER, P. G., S. GERBETH-JONES, and S. HUTCHINSON. 1985. A scanning electron microscope survey of the epidermis of East African grasses, III. *Smithsonian Contributions to Botany* 55: 1-136.

PETERSEN, G., O. SEBERG, and C. BADEN. 2004. A phylogenetic analysis of *Psathyrostachys* (Poaceae) based on one nuclear gene, three plastid genes, and morphology. *Plant Systematics and Evolution* 249: 99-110.

PHILIPSON, M. N. 1986. A re-assessment of the form of reproduction in *Danthonia spicata* (L.) Beauv. *New Phytologist* 103: 231-243.

PIPERNO, D. R. 1988. *Phytolith analysis: An archaeological and geological perspective*. San Diego: Academic Press, Inc.

PIPERNO, D. R. and D. M. PEARSALL. 1998. The silica bodies of tropical American grasses: Morphology, taxonomy, and implications for grass systematics and fossil phytolith identification. *Smithsonian Contributions to Botany* 85: 1-40.

PRASAD, V., C. A. E. STROMBERG, H. ALIMOHAMMADIAN, and A. SAHNI. 2005. Dinosaur coprolites and the early evolution of grasses and grazers. *Science* 310: 1177-1180.

PRAT, H. 1936. La systématique des Graminées. *Annales des Sciences Naturelles, Botanique, Séries X* 18: 165-258.

PRAT, H. 1948. General features of the epidermis in *Zea mays*. *Annals of the Missouri Botanical Garden* 35: 341-351.

PRYCHID, C. J., P. J. RUDALL, and M. GREGORY. 2003. Systematics and biology of silica bodies in monocotyledons. *Botanical Review* 69: 377-440.

QUINN, J. A. 2003. *Danthonia* Notes. In *Grass Manual on the Web*, ed. M. E. Barkworth. http://herbarium.usu.edu/webmanual/default.htm [Accessed 24 September 2005]

RAMBAUT, A. 1995. Sequence alignment program. Oxford: University of Oxford.

REEDER, J. R. 1959. The grass embryo in systematics. *Recent Advances in Botany* 1: 91-96.

REIMER, E. and J. H. COTA-SÁNCHEZ. In review. An SEM survey of the leaf epidermis in danthonioid grasses (Poaceae: Danthonioideae). *Systematic Botany*.

RENVOIZE, S. A. and W. D. CLAYTON. 1992. Classification and evolution of grasses. Pp. 37 in *Grass Evolution and Domestication,* ed. G. P. Chapman. Cambridge: Cambridge University Press.

RUDALL, P. J., W. STUPPY, J. CUNIFF, E. A. KELLOGG, and B. G. BRIGGS. 2005. Evolution of reproductive structure in grasses (Poaceae) inferred by sister-group comparison with their putative closest living relatives, the Ecdeiocoleaceae. *American Journal of Botany* 92:1432-1443.

RÚGOLO DE AGRASAR, Z. E. and A. S. VEGA. 2004. *Tripogon nicorae*, a new species and a synopsis of *Tripogon* (Poaceae: Chloridoideae) in America. *Systematic Botany* 29: 874-882.

SAGHAI-MAROOF, M. A., K. M. SOLIMAN, R. A. JORGENSEN, and R. W. ALLARD. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Science U.S.A.* 81: 8014-8018.

SENDULSKY, T., T. S. FILGUEIRAS, and A. G. BURMAN. 1987. Fruits, embryos, and seedlings. Pp. 31-36. in *Grass Systematics and Evolution,* eds. T. R. Soderstrom, K. W. Hilu, C. S. Campbell, and M. E. Barkworth. Washington: Smithsonian Institute Press.

SHAFFER-FEHRE, M. 1991. The position of *Najas* within the subclass Alismatidae (Monocotyledones) in the light of new evidence from seed coat structures in the Hydrocharitoideae (Hydrocharitales). *Botanical Journal of the Linnean Society* 107: 189-210.

SORENG, R. J. and J. I. DAVIS. 1998. Phylogenetics and character evolution in the grass family (Poaceae): Simultaneous analysis of morphological and chloroplast DNA restriction site character sets. *Botanical Review* 63: 1-85.

STACE, C. A. 1984. The taxonomic importance of the leaf surface. Pp. 67-94 in *Current Concepts in Plant Taxonomy*, eds. V. H. Heywood and D. M. Moore. London: Academic Press.

STEBBINS, G. L. 1956. Cytogenetics and evolution of the grass family. *American Journal of Botany* 43: 890-905.

STEBBINS, G. L. 1982. Major trends of evolution in the Poaceae and their possible significance. Pp. 3-36 in *Grasses and Grasslands: Systematics and Ecology*, eds. J. R Estes, R. J. Tyrl, and J. N. Brunken. Norman: University of Oklahoma Press.

STEBBINS, G. L. 1987. Grass systematics and evolution: Past, present and future. Pp. 359-367 in *Grass Systematics and Evolution*, eds. T. R. Soderstrom, K. W. Hilu, C. S. Campbell, and M. E. Barkworth. Washington: Smithsonian Institute Press.

STEBBINS, G. L. and B. CRAMPTON. 1959. A suggested revision of the grass genera of temperate North America. *Recent Advances in Botany* 1: 133-145.

STONEBERG HOLT, S. D., L. HOROVÁ, and P. BUREŠ. 2004. Indel patterns of the plastid DNA *trn*L-*trn*F region within the genus *Poa* (Poaceae). *Journal of Plant Research* 117: 393-407.

STUPPY, W. H., J. A. MAISANO, M. W. COLBERT, P. J. RUDALL, and T. B. ROWE. 2003. Three-dimensional analysis of plant structure using high-resolution X-ray computed tomography. *Trends in Plant Science* 8: 2-6.

SWOFFORD, D. L. 2004. PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods). Vers. 4.0b10. Sinauer, Sunderland, MA.

TABERLET, P., L. GIELLY, G. PAUTOU, and J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of cpDNA. *Plant Molecular Biology* 17:1105-1109.

TAKEOTA, T., S. INOUE, and S. KAWANO. 1959. Notes on some grasses. IX. Systematic significance of bicellular microhairs of leaf epidermis. *Botanical Gazette* 121: 80-91.

TERRELL, E. E. and P. M. PETERSON. 1993. Caryopsis morphology and classification in the Triticeae (Pooideae: Poaceae). *Smithsonian Contributions to Botany* 83: 1-25.

THOMASSON, J. R. 1986. Lemma epidermal features in the North American species of *Melica*, and selected species of *Briza, Catabrosa, Glyceria, Neostapfia, Pleuropogon* and *Schizachne* (Gramineae). *Systematic Botany* 11: 253-262.

TOMLINSON, K. L. 1987. Comparative anatomical studies in *Danthonia sensu lato* (Danthonieae: Poaceae). *Aliso* 11: 97-114.

VAIO, M, P. SPERENZA, J. F. VALLS, M. GUERRA, and C. MAZELLA. 2005. Localization of the 5S and 45S rDNA sites and cpDNA sequence analysis in species of the Quadrifaria group of *Paspalum* (Poaceae, Paniceae). *Annals of Botany* 96: 191-200.

VELDKAMP, J. F. 1980. Conservation of *Notodanthonia* Zotov (Gramineae). *Taxon* 29: 293-298.

VERBOOM, G. A., H. P. LINDER, and N. P. BARKER. 1994. Haustorial synergids: An important character in the systematics of Danthonioid grasses (Arundinoideae: Poaceae)? *American Journal of Botany* 81: 1601-1610.

VICKERY, J. W. 1956. A revision of the Australian species of *Danthonia* DC. *Contributions of the New South Wales National Herbarium* 2: 249-331.

WATSON, L. and M. J. DALLWITZ. 1992 onwards. The grass genera of the world: Descriptions, illustrations, identification, and information retrieval; including synonyms, morphology, anatomy, physiology, phytochemistry, cytology, classification, pathogens, world and local distribution, and references. Version: 27th June 2005. http://delta-intkey. com

WATSON, L. and M. J. DALLWITZ. 1992. *The Grass Genera of the World*. Wallingford: CAB International.

WEATHERWAX, P. 1928. Cleistogamy in two species of *Danthonia*. *Botanical Gazette* 85: 104-109.

WHANG, S. S., K. KIM, and W. M. HESS. 1998. Variation in silica bodies in leaf epidermal long cells within and among seventeen species of *Oryza* (Poaceae). *American Journal of Botany* 85: 461-466.

WILSON, B. L., J. KITZMILLER, W. ROLLE, and V. D. HIPKINS. 2001. Isozyme variation and its environmental correlates in *Elymus glauca* from the California Floristic Province. *Canadian Journal of Botany* 79: 139-153.

WRIGHT, K. L. T. 1984. Studies in the Danthonieae (Poaceae) [Ph. D. dissertation]. University of Oklahoma, Norman, OK. 142 pp. Available from University Microfilms, Ann Arbor, MI. AAT 8424002.

XU, Z.-Z. and G.-S. ZHOU. 2005. Effects of water stress and high nocturnal temperature on photosynthesis and nitrogen level of a perennial grass *Leymus chinensis*. *Plant and Soil* 269: 131-139.

YEN, A. C. and R. G. OLMSTEAD. 2000. Molecular systematics of Cyperaceae tribe Cariceae based on two chloroplast DNA regions: *ndh*F and *trn*L intron-intergenic spacer. *Systematic Botany* 25: 479-494.

ZOMLEFER, W. B., N. H. WILLIAMS, W. M. WHITTEN, and W. S. JUDD. 2001. Generic circumscription and relationships in the tribe Melanthieae (Liliales, Melanthiaceae), with emphasis on *Zigadenus*: Evidence from ITS and *trn*L-F sequence data. *American Journal of Botany* 88: 1657–1669

ZOTOV, V. D. 1963. Synopsis of the grass subfamily Arundinoideae in New Zealand. *New Zealand Journal of Botany* 1: 78-136.

ZULOAGA, F. O., O. MORRONE, G. DAVIDSE, T. S. FILGUEIRAS, P. M. PETERSON, R. J. SORENG, and E. JUDZIEWICZ. Chief Editors: R. J. Soreng and S. J. Pennington. 2003. Catalogue of New World Grasses (Poaceae): III. Subfamilies Panicoideae, Aristidoideae, Arundinoideae, and Danthonioideae. *Contributions of the United States National Herbarium* 46: 1-662.

APPENDIX 1. Multiple alignment of *trn*L-F sequence data for taxa investigated.

```
                                                                            50
Sorghum halepense           TTCCAAATTCAGAGAAACCCTGGAATGAAAAATGGGCAATCCTGAGCCAA
Themeda triandra            ··················································
Phragmites australis        ··························T·······················
Austrodanthonia pilosa      ··························T·······················
Cortaderia selloana         ··························T·······················
Danthonia californica       ··························T·······················
D. chilensis                ··························T·······················
D. compressa                ··························T·······················
D. decumbens                ··························T·······················
D. filifolia                ··························T·······················
D. intermedia               ··························T·······················
D. parryi                   ··························T·······················
D. sericea                  ··························T·······················
D. spicata                  ··························T·······················
D. unispicata               ··························T·······················
Merxmuellera disticha       ··························T·······················
Notodanthonia semiannularis ··························T·······················
Rytidosperma unarede        ··························T·······················
R. violacea                 ··························T·······················
Tribolium echinatum         ··························T·······················
T. hispidum                 ··························T·······················
                                                                           100
Sorghum halepense           ATCCACTTTTTTCAAAAAA---GTGGTTCTCAAACTAGAACCCAAAGGAA
Themeda triandra            ····-·······G······CAA·C··················A·······
Phragmites australis        ····-·······G······CAG··························G·
Austrodanthonia pilosa      ····-·······G······CAC·························A··
Cortaderia selloana         ····-·······G······CAA····························
Danthonia californica       ····-·······G······CAA····························
D. chilensis                ····-·······G······CAA····························
D. compressa                ····-·······G······CAA····························
D. decumbens                ····-·······G······CAA····························
D. filifolia                ····-·······G······CAA····························
D. intermedia               ····-·······G······CAA····························
D. parryi                   ····-·······G······CAA····························
D. sericea                  ····-·······G······CAA····························
D. spicata                  ····-·······G······CAA····························
D. unispicata               ····-·······G······CAA····························
Merxmuellera disticha       ····-·······G······CAG····························
Notodanthonia semiannularis ····-·······G······CAC·························A··
Rytidosperma unarede        ····-·······G······CAC·························A··
R. violacea                 ····-·······G······CAC·························A··
Tribolium echinatum         ····-·······G······CAC·························A··
T. hispidum                 ····-·······G······CAC·························A··
                                                                           150
Sorghum halepense           AAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACGAATCGAAGTAA
Themeda triandra            ··················································
Phragmites australis        ·············································G····
Austrodanthonia pilosa      ·············································G····
Cortaderia selloana         ·············································G····
Danthonia californica       ·············································G····
D. chilensis                ·············································G····
D. compressa                ·············································G····
D. decumbens                ·············································G····
D. filifolia                ·············································G····
D. intermedia               ·············································G····
D. parryi                   ·············································G····
D. sericea                  ·············································G····
D. spicata                  ·············································G····
D. unispicata               ·············································G····
Merxmuellera disticha       ·············································G····
Notodanthonia semiannularis ·············································G····
Rytidosperma unarede        ·············································G····
R. violacea                 ·············································G····
Tribolium echinatum         ·············································G····
T. hispidum                 ·············································G····
```

APPENDIX 1. Continued.

```
                                                                          200
Sorghum halepense           TAACG---------------------------------------------
Themeda triandra            ·····---------------------------------------------
Phragmites australis        ·T···TTGTGTTGGTAGTGAAACTCCCTCTAAATTAGAGAAAGAAGGGCT
Austrodanthonia pilosa      ·T···TTGTGTTGGTAGTGTAACGCCCTCTAAATTAGAGAAAGAAGGGCT
Cortaderia selloana         ·T···TTGTGTTGGTAGTGTAACTCCCTCTAAATTAGAGAAAGAAGGGCT
Danthonia californica       ·T···TTGTGTTGGTAGTGTAACTCCCTCTAAATTAGAGAAAGAAGGGCT
D. chilensis                ·T···TTGTGTTGGTAGTGTAACTCCCTCTAAATTAGAGAAAGAAGGGCT
D. compressa                ·T···TTGTGTTGGTAGTGTAACTCCCTCTAAATTAGAGAAAGAAGGGCT
D. decumbens                ·T···TTGTGTTGGTAGTGTAACTCCCTCTAAATTAGAGAAAGAAGGGCT
D. filifolia                ·T···TTGTGTTGGTAGTGTAACTCCCTCTAAATTAGAGAAAGAAGGGCT
D. intermedia               ·T···TTGTGTTGGTAGTGTAACTCCCTCTAAATTAGAGAAAGAAGGGCT
D. parryi                   ·T···TTGTGTTGGTAGTGTAACTCCCTCTAAATTAGAGAAAGAAGGGCT
D. sericea                  ·T···TTGTGTTGGTAGTGTAACTCCCTCTAAATTAGAGAAAGAAGGGCT
D. spicata                  ·T···TTGTGTTGGTAGTGTAACTCCCTCTAAATTAGAGAAAGAAGGGCT
D. unispicata               ·T···TTGTGTTGGTAGTGTAACTCCCTCTAAATTAGAGAAAGAAGGGCT
Merxmuellera disticha       ·T···TTGTGTTGGTAGTGTAACTCCCTCTAAATTAGAGAAAGAAGGGCT
Notodanthonia semiannularis ·T···TTGTGTTGGTAGTGTAACGCCCTCTAAATTAGAGAAAGAAGGGCT
Rytidosperma unarede        ·T···TTGTGTTGGTAGTGTAACGCCCTCTAAATTAGAGAAAGAAGGGCT
R. violacea                 ·T···TTGTGTTGGTAGTGTAACGCCCTCTAAATTAGAGAAAGAAGGGCT
Tribolium echinatum         ·T···TTG----GGTAGTGTAACGCCCTCTAAATTAGAGAAAGAAGGGCT
T. hispidum                 ·T···TTG----GGTAGTGTAACGCCCTCTAAATTAGAGAAAGAAGGGCT
                                                                          250
Sorghum halepense           -------------------------------------------ATTAATC
Themeda triandra            -------------------------------------------·······
Phragmites australis        TTATACATCTAATACACACCTATAGATACTGACATAGCAAATG·······
Austrodanthonia pilosa      T-ATACATCTAATACACACGTATAGATACTGACATAGGAAACG·······
Cortaderia selloana         T-ATACATCTAATACACACGTATAGATACTGACATAGGAAACG·······
Danthonia californica       --ATACATCTAATACACACGTATAGATACTGACATAGGAAACG·······
D. chilensis                T-ATACATCTAATACACACGTATAGATACTGACATAGGAAACG·······
D. compressa                T-ATACATCTAATACACACGTATAGATACTGACA-----AACG·······
D. decumbens                T-ATACATCTAATACACACGTATAGATACTGACATAGGAAACG·······
D. filifolia                T-ATACATCTAATACACACGTATAGATACTGACATAGGAAACG·······
D. intermedia               T-ATACATCTAATACACACGTATAGATACTGACATAGGAAACG·······
D. parryi                   T-ATACATCTAATACACACGTATAGATACTGACA-----AACG·······
D. sericea                  T-ATACATCTAATACACACGTATAGATACTGACATAGGAAACG·······
D. spicata                  T-ATACATCTAATACACACGTATAGATACTGACA-----AACG·······
D. unispicata               T-ATACATCTAATACACACGTATAGATACTGACA-----AACG·······
Merxmuellera disticha       T-ATACGTCTAATACACACGTATAGATACTGACATAGGAAACG·······
Notodanthonia semiannularis T-ATACATCTAATACACACGTATAGATACTGACATAGGAAACG·······
Rytidosperma unarede        T-ATACATCTAATACACACGTATAGATACTGACATAGGAAACG·······
R. violacea                 T-ATACATCTAATACACACGTATAGATACTGACATAGGAAACG·······
Tribolium echinatum         T-ATATATCTAATACACACGTATAGATACTGACATAGGAAACG·······
T. hispidum                 T-ATATATCTAATACACACGTATAGATACTGACATAGGAAACG·······
                                                                          300
Sorghum halepense           ACAGAACCCATATTATAATATAGGTTCTTTATTTTATTTTG----AGAAT
Themeda triandra            ········································T----·····
Phragmites australis        ·············C····················AT··A·TTTTT·····
Austrodanthonia pilosa      ·············C····················AT····T----·····
Cortaderia selloana         ······------·C····················AT····T----·····
Danthonia californica       ·············C····················AT····T----·····
D. chilensis                ·············C····················AT····T----·····
D. compressa                ·············C····················AT····T----·····
D. decumbens                ·············C····················AT····T----·····
D. filifolia                ·············C····················AT····T----·····
D. intermedia               ·············C····················AT····T----·····
D. parryi                   ·············C····················AT····T----·····
D. sericea                  ·············C····················AT····T----·····
D. spicata                  ·············C····················AT····T----·····
D. unispicata               ·············C····················AT····T----·····
Merxmuellera disticha       ·············C·····················T····T----·····
Notodanthonia semiannularis ·············C····················AT····T----·····
Rytidosperma unarede        ·············C····················AT····T----·····
R. violacea                 ·············C····················AT····T----·····
Tribolium echinatum         ·············C····················AT····T----·····
T. hispidum                 ·············C····················AT····T----·····
```

APPENDIX 1. Continued.

	350
Sorghum halepense	GAAATTAGGAATGATTATGAAATAGAAAATTCATAATTTTTTT-AGAATT
Themeda triandra	·································T········G-······
Phragmites australis	································TG·········-······
Austrodanthonia pilosa	········A·····A·················TG··A······T······
Cortaderia selloana	········A·····A·················TG·········-······
Danthonia californica	······C·A·····A·················TG·········-······
D. chilensis	······C·A·····A··················G·········-······
D. compressa	······C·A·····A··················G·········-······
D. decumbens	······C·A·····A··················G·········-······
D. filifolia	······C·A·····A··················G·········-······
D. intermedia	······C·A·····A··················G·········-······
D. parryi	······C·A·····A·················TG·········-······
D. sericea	······C·A·····A··················G·········-······
D. spicata	······C·A·····A·················TG·········-······
D. unispicata	······C·A·····A·················TG·········-······
Merxmuellera disticha	········A·····A·················TG·········-······
Notodanthonia semiannularis	········A·····A·················TG··A······T······
Rytidosperma unarede	········A·····A·················TG··A······T······
R. violacea	········A·····A·················TG··A······T······
Tribolium echinatum	······C·A···------··············TA··A······T······
T. hispidum	······C·A···------··············TA··A······T······
	400
Sorghum halepense	ATTGTGAATCTATTCCAATCGAATATTGAGTAATCAAATCCTTCAATTCA
Themeda triandra	A···
Phragmites australis	G·········C·······································
Austrodanthonia pilosa	A·········CG·······T···················T··········
Cortaderia selloana	A·········C········T······························
Danthonia californica	A··················T······························
D. chilensis	A·········C········T······························
D. compressa	A·········C········T······························
D. decumbens	A·········C········T······························
D. filifolia	A·········C········T······························
D. intermedia	A·········C········T······························
D. parryi	A·········C········T······························
D. sericea	A·········C········T······························
D. spicata	A·········C········T······························
D. unispicata	A·········C········T······························
Merxmuellera disticha	A·········C········T···················T··········
Notodanthonia semiannularis	A········TCG·······T···················T··········
Rytidosperma unarede	A·········CG·······T···················T··········
R. violacea	A·········CG·······T···················T··········
Tribolium echinatum	A·········C········T···················T··········
T. hispidum	A·········C········T···················T··········
	450
Sorghum halepense	TTGTTTTCGAGATCTTTTAAAAAGTGGATTAATCGGACGAGGATAAAGAG
Themeda triandra	··
Phragmites australis	·······T··
Austrodanthonia pilosa	··T····-··········-·····C·········AA·····A········
Cortaderia selloana	··T····-················C·························
Danthonia californica	··T····-················CA························
D. chilensis	··T····-···············ACA························
D. compressa	··T····-················CA························
D. decumbens	··T····-················CA························
D. filifolia	··T····-···············ACA························
D. intermedia	··T····-················CA························
D. parryi	··T····-················CA························
D. sericea	··T····-················CA························
D. spicata	··T····-················CA························
D. unispicata	··T····-················CA························
Merxmuellera disticha	··T····-··········-·····C················A········
Notodanthonia semiannularis	··T····-··········-·····C·········AA·····A········
Rytidosperma unarede	··T····-··········-·····C·········AA·····A········
R. violacea	··T····-··········-·····C·········AA·····A········
Tribolium echinatum	··T····-··········-·····C·········AA·····A········
T. hispidum	··T····-··········-·····C·········AA·····A········

APPENDIX 1. Continued.

```
                                                                          500
Sorghum halepense           AGAGTCCCATTCTACATGTCAATACTGACAACAATGAAATTTCTAGTAAA
Themeda triandra            ··················································
Phragmites australis        ··················································
Austrodanthonia pilosa      ··················································
Cortaderia selloana         ··················································
Danthonia californica       ··················································
D. chilensis                ··················································
D. compressa                ··················································
D. decumbens                ··················································
D. filifolia                ··················································
D. intermedia               ··················································
D. parryi                   ··················································
D. sericea                  ··················································
D. spicata                  ··················································
D. unispicata               ··················································
Merxmuellera disticha       ··················································
Notodanthonia semiannularis ··················································
Rytidosperma unarede        ··················································
R. violacea                 ··················································
Tribolium echinatum         ··················································
T. hispidum                 ··················································
                                                                          550
Sorghum halepense           AGGAAAATCCGTCGACTTTATAAGTCGTGAGGGTTCAAGTCCCTCTATCC
Themeda triandra            ··················································
Phragmites australis        ··················································
Austrodanthonia pilosa      ······················G···························
Cortaderia selloana         ······················G···························
Danthonia californica       ······················G···························
D. chilensis                ······················NNNNNNNNNNNNN···············
D. compressa                ······················G···························
D. decumbens                ······················G·····················NNNNNN
D. filifolia                ······················G···························
D. intermedia               ······················G···························
D. parryi                   ······················G···························
D. sericea                  ·····················---------····················
D. spicata                  ······················G···························
D. unispicata               ······················G···························
Merxmuellera disticha       ······················G···························
Notodanthonia semiannularis ······················G···························
Rytidosperma unarede        ······················G···························
R. violacea                 ······················G···························
Tribolium echinatum         ······················G···························
T. hispidum                 ······················G···························
                                                                          600
Sorghum halepense           CCAAACCCTCTTTTATTCGCTAACCATAGTTGTTATCCTTTTTTTTT---
Themeda triandra            C·················C····················T·····-CTTT
Phragmites australis        C················TA······C····AT·······T······CTTT
Austrodanthonia pilosa      C·················C···········AT·······A·····GATTT
Cortaderia selloana         C·················C···········AT·······T·······ATT
Danthonia californica       C·················C···········AT·······A·····GATTT
D. chilensis                C·················C···········AT·······A·····GATTT
D. compressa                C·················C···········AT·······A·····GATTT
D. decumbens                NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
D. filifolia                C·················C···········AT·······A······ATTT
D. intermedia               C·················C···········AT·······A······ATTT
D. parryi                   C·················C···········AT·······A·····GATTT
D. sericea                  C·················C···········AT·······A······ATTT
D. spicata                  C·················C···········AT·······A·····GATTT
D. unispicata               C·················C···········AT·······A·····GATTT
Merxmuellera disticha       C·················C···········AT·······A·····GATTT
Notodanthonia semiannularis C·················C···········AT·······A·····GATTT
Rytidosperma unarede        C·················C···········AT·······A·····GATTT
R. violacea                 C·················C···········AT·······A·····GATTT
Tribolium echinatum         C·················C···········AT·······A·····GATTT
T. hispidum                 C·················C···········AT·······A·····GATTT
```

APPENDIX 1. Continued.

```
                                                                      650
Sorghum halepense            -AT------CAATGGGTTTAAGATTCACTAGCTTTCTCATTCTACTCTTT
Themeda triandra             TAT------·········································
Phragmites australis         TAT------··················T······················
Austrodanthonia pilosa       TTGCAATTT··················T················T·····
Cortaderia selloana          TTT------··················T················T·····
Danthonia californica        TTT------··················T················TG····
D. chilensis                 TTT------··················T················TG····
D. compressa                 TTT------··················T················TG····
D. decumbens                 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
D. filifolia                 TTT------··················T················TG····
D. intermedia                TTT------··················T················TG····
D. parryi                    TTT------··················T················TG····
D. sericea                   TTT------··················T················TG····
D. spicata                   TTT------··················T················TG····
D. unispicata                TTT------··················T················TG····
Merxmuellera disticha        TTT------······---·········T················T·····
Notodanthonia semiannularis  TTT------··················T················T·····
Rytidosperma unarede         TTGCAATTT··················T················T·····
R. violacea                  TTT------··················T················T·····
Tribolium echinatum          TTT------··················T················T·····
T. hispidum                  TTT------··················T················T·····
                                                                      700
Sorghum halepense            CACAAAGGAGTGCGACAAGAACTCAATGAATCTTATGCTATTCATTAAAT
Themeda triandra             ·············G····································
Phragmites australis         ·············G·AG···········G·················----
Austrodanthonia pilosa       ·········C···A·AG···········G·················----
Cortaderia selloana          ·········A···A·AG········T··G·················----
Danthonia californica        ·········AA··A·AG·A···························----
D. chilensis                 ·········AA··A·AG·A·········G·················----
D. compressa                 ·········AA··A·AG·A·········G·················----
D. decumbens                 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
D. filifolia                 ·········AA··A·AG·A·········G·················----
D. intermedia                ·········AA··A·AG·A···························----
D. parryi                    ·········AA··A·AG·A·········G·················----
D. sericea                   ·········AA··A·AG·A···························----
D. spicata                   ·········AA··A·AG·A·········G·················----
D. unispicata                ·········AA··A·AG·A·········G·················----
Merxmuellera disticha        ·········C···A·AG····T······G·················----
Notodanthonia semiannularis  ·········C···A·AG···········G···········C·····----
Rytidosperma unarede         ···T·····C···A·AG···········G·················----
R. violacea                  ·········C···A·AG···········G·················----
Tribolium echinatum          ·········C···A·AG···········G·················----
T. hispidum                  ·········C···A·AG···········G·················----
                                                                      750
Sorghum halepense            -----AGATGATTTCTTTTTTATCT------------CTTTTTTTTTTAT
Themeda triandra             AAAAT·······TTCTTTTTTATTT------------·······------
Phragmites australis         AAATG----···CTTATGCTATTCATTAAATAGATTT·······------
Austrodanthonia pilosa       AAATG----···TT-----------------------·······------
Cortaderia selloana          AAATA----···TT-----------------------·······------
Danthonia californica        AAATG----···TT-----------------------·······------
D. chilensis                 AAATG----···TT-----------------------·······------
D. compressa                 AAATG----···TT-----------------------·······------
D. decumbens                 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
D. filifolia                 AAATG----···TT-----------------------·······------
D. intermedia                AAATG----···TT-----------------------·······------
D. parryi                    AAATG----···TT-----------------------·······------
D. sericea                   AAATG----···TT-----------------------·······------
D. spicata                   AAATG----···TT-----------------------·······------
D. unispicata                AAATG----···TT-----------------------·······------
Merxmuellera disticha        AAATG----···TT-----------------------·······------
Notodanthonia semiannularis  AAATG----···TT-----------------------·······------
Rytidosperma unarede         AAATG----···TT-----------------------·······------
R. violacea                  AAATG----···TT-----------------------·······------
Tribolium echinatum          AAACG----···TT-----------------------···········T·
T. hispidum                  AAACG----···TT-----------------------···········T·
```

APPENDIX 1. Continued.

```
                                                                          800
Sorghum halepense           TTATTAGAGTACAGTATCGGCAAGGAATCTCGATTATTAATTCGATTTTT
Themeda triandra            ·····AGAGT··········A······················T······
Phragmites australis        --···-----································A·······
Austrodanthonia pilosa      --···-----··············A····C·C····G·············
Cortaderia selloana         --···-----··············A······C·····C············
Danthonia californica       --···-----··············A······C··················
D. chilensis                --···-----··············A······C··················
D. compressa                --···-----··············A······C··················
D. decumbens                NNNNNNNNNNN·············A····C·C··················
D. filifolia                --···-----··············A······C··················
D. intermedia               --···-----··············A······C··················
D. parryi                   --···-----··············A······C·················G
D. sericea                  --···-----··············A······C··················
D. spicata                  --···-----··············A······C·················G
D. unispicata               --···-----··············A······C·················G
Merxmuellera disticha       --···-----··············A····C·C··················
Notodanthonia semiannularis --···-----··············A····C·C····G·············
Rytidosperma unarede        --···-----··············A····C·C····G·············
R. violacea                 --···-----··············A····C·C····G·············
Tribolium echinatum         ·····-----··············A····C·C····G·············
T. hispidum                 ·····-----··············A····C·C····G·············
                                                                          850
Sorghum halepense           TTAAGTATT-----------ATTAAGTAAGCCATCCACAATGCATAGGAC
Themeda triandra            ·········-----------······························
Phragmites australis        --·······-----------······························
Austrodanthonia pilosa      --·······TTTTTATTAAT······························
Cortaderia selloana         --·······ATTTT------······························
Danthonia californica       --·······ATTTT------······························
D. chilensis                --·······ATTTT------······························
D. compressa                --·······ATTTT------···················G··········
D. decumbens                --·······ATTTT------······························
D. filifolia                --·······ATTTT------······························
D. intermedia               --·······ATTTT------······························
D. parryi                   --·······ATTTT------················NNNNNNNNNNNNNN
D. sericea                  --·······ATTTT------······························
D. spicata                  --·······ATTTT------···················G··········
D. unispicata               --·······ATTTT------······························
Merxmuellera disticha       --·······ATTTT------······························
Notodanthonia semiannularis --·······TTTTTATTAAT······························
Rytidosperma unarede        --·······TTTTTATTAAT······························
R. violacea                 --·······TTTTTATTAAT······························
Tribolium echinatum         --·······TTTTTATTAAT······························
T. hispidum                 --·······TTTTTATTAAT······························
                                                                          900
Sorghum halepense           TACCCCTCCCCATTTCCTAATTTTGAATGGAATACTTTATTGATTTTTTA
Themeda triandra            T······················G··························
Phragmites australis        T···TCT·A········A·····G····A·····················
Austrodanthonia pilosa      T·---···········AA····AG········G·T····C··········
Cortaderia selloana         T·---············A····AG········A·T···············
Danthonia californica       T·---············A····AG········A·T···············
D. chilensis                T·---············A····AG········A·T···············
D. compressa                T·---············A····AG········A·T···············
D. decumbens                T·---············A····AG········A·T···············
D. filifolia                T·---············A····AG···A····A·T···············
D. intermedia               T·---············A····AG········A·T···············
D. parryi                   NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
D. sericea                  T·---············A····AG········A·T···············
D. spicata                  T·---············A····AG·····A··A·T···············
D. unispicata               T·---············A····AG········A·T···············
Merxmuellera disticha       T·---············A····AG········A·T········C······
Notodanthonia semiannularis T·---···········AA····AG········A·T····C··········
Rytidosperma unarede        T·---···········AA····AG········G·T····C··········
R. violacea                 T·---···········AA····CG········G·T····C··········
Tribolium echinatum         C·---···········AA····AG········A·T····C··········
T. hispidum                 C·---···········AA····AG········A·T····C··········
```

APPENDIX 1. Continued.

```
                                                                         947
Sorghum halepense           GTCCCTTTAATTGACATAGATGCAAATACTTTACTAAGATGATGCAC
Themeda triandra            ···············································
Phragmites australis        ······························C·····G··········
Austrodanthonia pilosa      A·····························C··G··G··········
Cortaderia selloana         A·····························C··C··G··········
Danthonia californica       A·····························C··G··G··········
D. chilensis                A·····························C··G··G··········
D. compressa                A·····························C·GA··G··········
D. decumbens                A·····························C··G··G··········
D. filifolia                A·····························C··G··G··········
D. intermedia               A·····························C··G··G··········
D. parryi                   NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
D. sericea                  A·····························C··G··G··········
D. spicata                  A·····························C··G··G··········
D. unispicata               A·····························C··G··G··········
Merxmuellera disticha       A·····························C··G··G··········
Notodanthonia semiannularis A·····························C··G··G··········
Rytidosperma unarede        A·····························C··G··G··········
R. violacea                 A·····························C··G··G··········
Tribolium echinatum         A·····························C··G··G··········
T. hispidum                 A·····························C··G··G··········
```