SYSTEMATIC AND EVOLUTIONARY STUDIES IN THE *DICHANTHELIUM ACUMINATUM* (POACEAE:PANICEAE) COMPLEX

A Dissertation

by

RICKY LEE HAMMER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2010

Major Subject: Botany

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Approved by:

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	Stephan Hatch
Committee Members,	Alan Pepper
	Hugh Wilson
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ABSTRACT

Systematic and Evolutionary Studies in the *Dichanthelium acuminatum* (Poaceae:Paniceae) Complex. (May 2010)

Ricky Lee Hammer, B.B.A., The University of Texas-Permian Basin; M.S., Texas A&M University

Co-Chairs of Advisory Committee: Dr. James Manhart
Dr. Stephan Hatch

Taxonomic boundaries and systematic relationships in the grass subspecific complex *Dichanthelium acuminatum* were investigated with both morphological and molecular methods. Circumscription of subspecific taxa comprising the complex has been difficult due to a continuum of morphological character variation among taxa and possibly due to infraspecific and interspecific hybridization. Qualitative and quantitative morphological character data was collected from herbarium specimens and field-collected specimens and analyzed using multivariate statistical techniques. Representative specimens were selected for molecular phylogenetic analysis of DNA sequences from the GBSSI (*waxy*) nuclear gene. Subspecific boundaries as circumscribed in the most recent taxonomic treatment (10 subspecies) were tested from: 1) a morphological perspective with results of the multivariate statistical analysis to determine if the study specimens formed natural groupings that corresponded to the recent treatment; and, 2) with molecular phylogenetic analysis to estimate the evolutionarily significant lineages present and to determine if such lineages supported the natural groupings revealed from the

multivariate morphological analysis. A separate investigation was conducted using a molecular technique to screen for putative hybrid specimens from DNA obtained from field-collected specimens.

Multivariate statistical analysis of the morphological data provided support for four of the 10 taxa tested and additional support for two taxa considered as a single unit. Further research is needed to determine the appropriate status of the remaining six taxa of the ten taxa tested. Molecular phylogenetic analysis provided support for recognizing four evolutionarily significant units and provided parallel support for four of the five taxa recognized from the morphological analysis. The hybridization investigation identified two putative hybrid specimens, which were confirmed as hybrids with GBSSI sequence data and also with multivariate statistical analysis of morphological data to provide provisional evidence for the role of hybridization in producing specimens with intermediate morphological phenotypes. A taxonomic treatment and dichotomous key was produced for the 10 subspecific taxa of the *Dichanthelium acuminatum* complex.

DEDICATION

I would like to dedicate this work to the memory of my late parents, Jack Leroy Hammer and Leola Francis Williamson Hammer. Countless fishing, camping, and backpacking trips with my father as I was growing up in west Texas provided the genesis and spark for my deep interest in the natural history of Texas and farther afield. That early immersion in natural history has matured and merged into a focus on evolutionary history, an interest which continues to pay rich intellectual rewards. Such gifts often start from the smallest of seeds. Thanks to my mother for encouraging and nurturing the development of a child who would eventually aspire to a career in science and teaching. We are all, in some ways big and small, the products of our parents.

I also dedicate this disstertation, which represents many hours and years of work, to my beloved wife, Tiffany Kristen Hammer. Without her constant support and tolerance of many late nights in the laboratory and many Saturdays and Sundays spent in research and writing I would not have been able to complete my doctorate. I will always value her constant love and support. I also dedicate this work to my beloved son, Nathan, who came into the world at the midpoint of my doctoral journey. Time away from both my wife and son has been the only detrimental aspect of my research.

I would also like to thank my sister, Charleen Ikeler Jones, for her love and encouragement over the years.

ACKNOWLEDGEMENTS

I would like to thank my committee co-chairs, Dr. James Manhart (Biology) and Dr. Stephan Hatch (Ecosystem Science and Management), for their guidance and support throughout the course of this research. I have benefited greatly from their knowledge and experience. My other committee members, Dr. Alan Pepper and Dr. Hugh Wilson, were also instrumental in the success of my doctoral research at Texas A&M. I thank all of my committee members for the friendship they have extended beyond professional advisement.

Significant financial support was provided by several grants from the Fank W. Gould Research Award in Plant Systematics. Dr. Gould left a substantial legacy concerning the study of the genus *Dichanthelium* at Texas A&M including many fine herbarium specimens from which a novice could begin the difficult task of "learning" how to identify the various members of *Dichanthelium*. Dale (72') and Phoebe Watts kindly provided a grant to assist in the early stages of the molecular work and their contribution is greatly appreciated.

Recognition is also due the Department of Biology for providing assistance in the form of teaching assistantships over the years. I would also like to thank Dr. Terry Thomas of the Laboratory for Functional Genomics in the Department of Biology for use of the lab's Linux cluster for computational support for the molecular phylogenetic portion of my research. Thanks also go to Dr. Robert Freckmann, retired from the

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Thanks go to several entitities that allowed me to conduct field research in support of dissertation: Texas Parks & Wildlife Department (various parks and WMAs), Marysee Prairie Preserve (Hardin County, TX), and Texas Nature Conservancy (Roy Larsen Sandylands Preserve, Hardin County, TX).

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CHAPTER I

INTRODUCTION

Dichanthelium (Poaceae:Panicoideae) is a genus of approximately 72 species with 34 of these species occurring in North America (Freckmann & Lelong 2003). The center of diversity for *Dichanthelium* is the southeastern United States (Crins 1991). All members of the genus are C_3 perennials (Brown and Smith 1972; Smith and Brown 1973), with two distinct periods of flowering and culm growth, one in the spring (vernal) and one in the summer/fall (autumnal). Plants are typically cespitose or rhizomatous in growth habit. Leaves are basal and cauline with the basal leaves often differentiated into a rosette in most species. This basal rosette of leaves differentiates the species of *Dichanthelium* from all other North American grasses (Clark and Gould 1975).

Culm leaves have blades usually longer and narrower than the leaves of the basal rosette. Culm leaf blade cross-sections show non-Kranz anatomy. Leaf blades and sheaths are glabrous to variously pubescent. Ligules are membranous or a line of hairs or rarely absent. Inflorescences are panicles, located at the culm apex in vernal-phase plants and at the branch apex in the autumnal-phase of the plant. Spikelets have sterile or staminate lower florets and bisexual upper florets. Spikelets produced on autumnal plants may not produce a caryopsis due herbivory by thrips. All *Dichanthelium* species are diploid (2n = 18; Gould and Clark 1978) with the exception of three taxa that are tetraploid (2n = 36; Gould and Clark 1978).

This dissertation follows the style of *Systematic Botany*.

The grass genus *Dichanthelium* has been the subject of much study and debate as to its proper taxonomic status, similar to the situation to be described shortly for the *D. acuminatum* subspecific complex. Linnaeus (1753), in *Species Plantarum*, described the first

three species of grasses that would eventually be included in *Dichanthelium*— *Panicum dichotomum, P. clandestinum*, and *P. latifolium*. In their monograph of the genus *Panicum* Hitchcock and Chase (1910) treated the species of *Dichanthelium* as a subgenus of *Panicum*, with subgenus *Dichanthelium* being further subdivided into related groups. Gould (1974) elevated *Dichanthelium* to generic rank. Clark and Gould (1975) found consistent epidermal differences in the palea of the upper floret of *Dichanthelium* and *Panicum*. Recent molecular phylogenetic analysis provides further support for the recognition of *Dichanthelium* as a distinct genus (Guissani et al. 2001).

Dichanthelium acuminatum (Sw.) Gould and C. Clark-commonly called "rosettegrass"- is a subspecies or varietal complex of grasses common to much of North America with the range of the taxa extending into northern South America. All subspecific taxa in the *D. acuminatum* complex are diploid (2n=18) (Gould and Clark 1978). Determination of the number of taxa to include in the "*acuminatum*" complex has been problematic. Hitchcock and Chase (1950; Table 1) recognized 170 species—based on the earlier monograph of the genus *Panicum* by Hitchcock and Chase (1910)—in the genus *Panicum*, grouping the species into several subgenera within the subgenus *Dichanthelium* and further subdividing subgenus *Dichanthelium* into 17 "sections", two of which (*Spreta* and *Lanuginosa*) together comprise 24 species (Table

1) and which would essentially be equivalent to the *D. acuminatum* subsp. *acuminatum* complex. Gould (1975) produced a treatment of the genus *Dichanthelium* in Texas. Gould and Clark (1978) produced the first major monograph of the genus *Dichanthelium* since the monograph of *Panicum* by Hitchcock and Chase (1910). In this treatment Gould and Clark recognized eight varieties in the *D. acuminatum* complex: *D. acuminatum* vars. *villosum, acuminatum, thurowii, implicatum, wrightianum, densiflorum, lindheimeri*, and *longiligulatum*. Gould and Clark (1978) moved some of the 24 species of the *acuminatum* complex, as circumscribed by Hitchcock and Chase (1950), to species outside of the "*acuminatum*" complex while others species are synonomized. The most recent treatment of the complex is that of Freckmann and Lelong (2003) in their treatment of the genus *Dichanthelium* for the *Flora of North America*. Here they recognize 10 subspecies in the *D. acuminatum* complex (Table 1).

The synonomy of *D. acuminatum* is burdensome as many authors have proposed numerous realignments of the subspecific taxa, including shifts of some varieties to the species level and vice versa (Gould and Clark 1978; Freckmann 1981; Lelong 1984; Freckmann and Lelong 2003). For example, Gould and Clark (1978) placed 46 names in synonomy in their treatment of *D. acuminatum*.

Regional floras as well contain considerable variation in how this group is circumscribed for a given region. For example, in Texas, Correll and Johnston (1979) describe the species of *Dichanthelium acuminatum* (Correll and Johnston classify all species of *Dichanthelium* as species of *Panicum*) found in Texas with three taxa, all treated as distinct species: *Panicum leucothrix, P. lanuginosum*, and *P. lindheimeri*.

Gould, in his *Grasses of Texas* (1975), treated the Texas plants as comprising only two taxa: *Dichanthelium lindheimeri*, *D. lanuginosum* var. *lanuginosum*. Gould also included another taxon, *D. lanuginosum* var. *villosissimum*, as a part of *D. lanuginosum* but this variety is classified as another species, *D. ovale* subsp. *villosissimum*, in the *Flora of North America* treatment (Freckmann & Lelong 2003). Compare this with the current Flora of North America treatment, which would give Texas five to six taxa, all as subspecies of *D. acuminatum*. In the *Illustrated Flora of East Texas* (Diggs et al. 2006) the authors relegate all taxa to the infraspecific level, recognizing four varieties in one species for the complex.

According to Lelong (1986) *D. acuminatum* "is probably the most polymorphic and troublesome species in the genus." The difficult and confusing synonomy and circumscription is the result of extensive morphological variation found among members of the complex (Shinners 1944, Freckmann 1981, Lelong 1986). Lelong (1965) studied a number of groups in *Dichanthelium* including the *acuminatum* complex and concluded that hybridization most likely played a major role in obscuring taxon boundaries in the group. Spellenberg (1975) studied western U.S. populations of some of the subspecies, finding one suspected hybrid, and proposed that autogamy and hybridization are a common means that account for much of the morphological variation and thus the taxonomic difficulty encountered in the complex.

Another problem, making study of this group of taxa difficult, is that the specimens comprising the *D. acuminatum* complex have been poorly, if at all, annotated in most herbaria. Many specimens received on loan for this study had never been annotated.

Thus, most herbarium specimens of *D. acuminatum* are labeled with synonymns, with many having a specific epithet other than "*acuminatum*." Therefore, it is difficult for a taxonomist to obtain herbarium specimens for this group for taxonomic and revisionary studies.

To the author's knowledge there has been no taxonomic analysis specifically of the *D. acuminatum* complex using a morphometric approach based on herbarium and field-collected specimens. Similarly, the author is not aware of molecular systematic analysis of *D. acuminatum* employing DNA sequence data. Both of these techniques have the potential to provide valuable taxonomic insight to the ongoing difficulties within the *D. acuminatum* complex.

In light of the foregoing taxonomic difficulties that the *D. acuminatum* complex poses, the goal of the present study is to assess the specific and subspecific boundaries of this complex using both morphometric and molecular techniques, with data gathered from both herbarium specimens and specimens from field-collected populations. The *Flora of North America* treatment of the genus *Dichanthelium* (Freckmann & Lelong 2003) and its treatment of the *D. acuminatum* complex and accompanying keys are used as a starting hypothesis for circumscription of the taxa of the complex. Plant specimens examined and measured for morphological and morphometric data and specimens from which tissues were sampled for use in DNA analysis were identified to the subspecific level using the *Flora of North America* key. The taxonomic hypothesis is subjected to multivariate statistical analysis of morphological/morphometric data and by phylogenetic molecular analysis of DNA sequence data.

CHAPTER II

MORPHOLOGICAL ANALYSIS

INTRODUCTION

Ten taxa were studied overall. Taxa included in the current study are as follows from the *Dichanthelium acuminatum* subspecies complex (Freckmann and Lelong 2003): *D. acuminatum* (Sw.) Gould & C. Clark subsp. *acuminatum*, *D. acuminatum* subsp. *columbianum* (Scribn.) Freckmann & Lelong, *D. acuminatum* subsp. *fasciculatum* (Torr.) Freckmann & Lelong, *D. acuminatum* subsp. *implicatum* (Scribn.) Freckmann & Lelong, *D. acuminatum* subsp. *leucothrix* (Nash) Freckmann & Lelong, *D. acuminatum* subsp. *lindheimeri* (Nash) Freckmann & Lelong, *D. acuminatum* subsp. *longiligulatum* (Nash) Freckmann & Lelong, *D. acuminatum* subsp. *sericeum* (Schmoll) Freckmann & Lelong, *D. acuminatum* subsp. *spretum* (Schult.) Freckmann & Lelong, *D. acuminatum* subsp. *thermale* (Bol.) Freckmann & Lelong. All specimens were identified using the Flora of North America treatment of the *Dichanthelium acuminatum* subspecies complex (Freckmann and Lelong 2003). For puposes of discussion the subspecies of the *D. acuminatum* complex will be referred to using only the "subsp." designation.

MATERIALS AND METHODS

Morphological Characters Studied–A total of 389 specimens (see Excel data file) were examined from the ten subspecies in the *Dichanthelium acuminatum* complex. Specimens were obtained from several herbaria (ISC, NY, TAES, WIS, UWSP, and US) and from field collection. Field-collected specimens include population samples of the following subspecies: *acuminatum*, *fasciculatum*, *lindheimeri*, and *longiligulatum*.

Specimens from populations were combined with non-population field specimens and with herbarium specimens for computing univariate statistics and initial multivariate analysis. Analysis at the population level was performed where it was helpful in explaining patterns derived in the multivariate analysis. As a starting point, fifteen macromorphological characters, five qualitative and 10 continuous quantitative, were measured for each specimen and are listed in Table 2. For leaf quantitative and qualitative data the third leaf down from thw apex was used. Morphological characters chosen for study included those which have been used in floristic keys to separate the taxa plus additional general descriptive characters.

Statistical Analyses–Individual plant specimens were treated as independent operational taxonomic units (OTUs) for all statistical analyses. However, OTUs were grouped into subspecies and similarity/dissimilarity was analyzed on these groupings for the major part of the study. Also, some subspecific OTU groupings were broken into natural component parts (such as field-collected populations and herbarium collections) and analyzed as such.

Descriptive statistics were generated at two levels for plant specimens: 1) grouped by subspecies designation and 2) plant specimens within a subspecies grouped by populations. Univariate statistical analyses were generated using SPSS (2007) software to summarize general quantitative and qualitative variation among the specimens and OTU groups. General univariate exploratory analyses included descriptive statistics of variation by subspecies group and boxplots of some quantitative variables were generated to highlight specific morphological character variation both at the subspecies

and population level. For qualitative variables tables were generated to summarize frequency and distribution of character types within and among the subspecific OTU groupings. Box plots were generated with R statistical package (R Development Core Team).

In preparation for multivariate analysis the five qualitative variables were designated to be ordinal variables with each representing an ordered ranking of the character being evaluated. For each of these variables Table 2 lists the ordinal categories in increasing order of degree, with state "1" being of less degree than state "2" and so forth, with up to *N* states to describe the qualitative variation for a given character. For example, leaf sheath pubescence has 6 character states: 1 = glabrous; 2 = sparsely pubescent; 3 = puberulent; 4 = pubescent; 5 = pilose; 6 = villous. State '1' is glabrous or without any sheath hairs while state '6' is villous representing a densely hairy sheath. States '2' through '5' represent increasing states of leaf sheath hair density between the two extremes of glabrous and villous. It should be noted that no assumption is made that there are equal densities of increasing hairiness between succeeding states as one moves from state '1' to state '6'. In other words, state '4' is not necessarily twice as hairy as state '2'.

Multivariate statistical analysis was conducted on the entire data matrix (both quantitative and qualitative data together) by using the ordination method of Principal Coordinates Analysis (PCoA). PCoA was chosen as the ordination method as it is suitable for the analysis of datasets composed of both quantitative and qualitative variables. The OTU data matrix was standardized by ranging. A dissimilarity distance

matrix was generated from the OTU data matrix using the *daisy* module of the R statistical package (R Development Core Team) with the Gower Similarity Coefficient metric (Gower, 1971) for mixed data. The resulting dissimilarity matrix was used as input for the PCoA computations. PCoA eigenvector computations and scatter plot graphics were generated using the NTSYSpc software package (Rohlf 2005). Eigenvalues for each PCoA axis were compared to expected eigenvalues predicted by the broken stick null model (Legendre and Legendre 1998; Frontier 1976) in order to determine the statistical significance of each axis. Spearman's rank correlation coefficients (*r*) were computed between the individual morphological characters and the first three PCoA axes using SPSS for Windows to estimate correlation. Appendix A lists detailed steps for data preparation and computation of the PCoA. Results from PCoA were used to assess multivariate relationships among the OTU subspecies groupings in order to evaluate taxonomic morphological boundaries among the subspecies.

RESULTS

Univariate Statistical Analysis–The five qualitative characters that were scored for the OTUs primarily deal with the degree of vestiture or hairs on culm, leaf blade and leaf sheath surfaces. Tables 3-6 summarize the frequency and distribution of the qualitative morphological data among the 10 subspecies in the *D. acuminatum* complex. Two major groups emerge from this data. First, a "glabrous" group composed of subsp. *lindheimeri*, subsp. *longiligulatum* and subsp. *spretum* clearly separate from the other subspecies based on leaf sheath pubescence (Table 3), culm internode pubescence (Table 4), leaf blade adaxial pubescence (Table 5), and leaf blade abaxial pubescence (Table 6),

while the remaining seven taxa form a pubescent group composed of various combinations of the above characters. Table 7 summarizes morphometric variation in 10 continuous quantitative characters across subspecific taxa of the *D. acuminatum* complex. Within subspecies groupings, box plots were generated at the population level for the 10 quantitative characters (Figs. 1-5). Figures 6-8 show box plots for subsp. *acuminatum* populations. These descriptive results show that there is some variation among populations and grouped herbarium specimens for some of the quantitative characters. For spikelet length in populations of subsp. *acuminatum* (Fig. 6A) there was no clear separation of populations, however, populations 1, 8 and 15 do tend to have longer spikelet lengths than the other populations. Peduncle length in populations of subsp. *acuminatum* (Fig. 6C) shows a separation of populations 4, 8, 15, and 17 from population 1 and the group of herbarium specimens, although there is some overlap among outlier specimens from each of the populations. Similarly, peduncle hair length in populations 8, 15, and 17 of subsp. *acuminatum* (Fig. 6D) shows a trend toward longer lengths for this character as compared to populations 1, 4, and the group of herbarium specimens. Leaf sheath hair length among populations of subsp. *acuminatum* (Fig. 7C) shows a trend towards separation of populations 1 and 4 from populations 8, 15, and 17; however, the group of herbarium specimens has a wide level of variation that overlaps with all of the other populations. The remaining quantitative characters, culm length (Fig. 6B), leaf blade length (Fig. 7A), leaf blade width (Fig. 7B), ligule length (Fig. 7D), panicle length (Fig. 8A), and panicle length (Fig. 8B) each showed considerable overlap among populations.

Figures 9-11 show box plots for subsp. *lindheimeri* populations. Other than in spikelet length (Fig. 10A) the quantitative character measurements show no clear separation among populations or the group of herbarium specimens. Although there is some overlap with a few outlier specimens, spikelet lengths for subsp. *lindheimeri* population 3 specimens (Fig. 10A) are longer when compared to the other populations.

Figure 12 shows box plots for subsp. *fasciculatum* populations. Only one population of subsp. *fasciculatum* was encountered and sampled during the three seasons of field work conducted in southeast and east Texas and western Louisiana for the author's dissertation research. When quantitative character measurements (Fig. 12) from this population (*Hammer* population specimens 230-1 to 230-15) are compared to the group of subsp. *fasciculatum* herbarium specimens there are observable differences when comparing the medians but the overall range of variation and overlap is considerable.

Figures 13-14 show box plots for subsp. *longiligulatum* populations. Populations 5 (*Hammer* population specimens 5-1 to 5-20), and 7 (*Hammer* population specimens 7-1 to 7-20) show some separation from the group of subsp. *longiligulatum* herbarium specimen based on spikelet length (Fig. 13A), culm length (Fig. 13B), and panicle length (Fig. 14C); however there is considerable overlap among outlier specimens between the two populations and the herbarium specimens for all of these characters.

A cross-tabulation summarizing the qualitative character data for the subsp. *acuminatum* populations was generated and this is presented in Tables 8-12. Leaf blade abaxial pubescence type (Table 8) does show significant differences in distribution of the character types across the populations and with a tendency of most populations to be

composed predominantly of only one pubescence type. The exceptions are populations 1 and 4 which are almost equally split between two pubescence types. To a lesser extent there is some variation in the distribution of character types across populations for leaf blade adaxial pubescence type (Table 9) but several populations (1, 4 and 17) do not have a dominant character type for the specimens sampled. Table 10, showing the distribution of culm pubescence types across the subsp. *acuminatum* populations, shows only minor variation in the distribution of the two character types. Both leaf blade margin type (Table 11) and leaf sheath pubescence type (Table 12) show little variation in character type across the subsp. *acuminatum* populations. The distribution and variation of both the quantitative and qualitative characters across the subsp. *acuminatum* populations will be more fully explored in the context of the multivariate results in the *Discussion* section.

Bivariate Statistical Analysis–The scatterplot matrix shown in Fig. 15 shows bivariate plots for all possible pairings of the 10 quantitative characters. All combinations of characters show continuous distributions of data points and no perceptible groups or clusters of points. Several plots show character combinations that indicate a relatively strong positive linear correlation, such as the plot for culm length versus panicle length, and to a lesser degree, the plot for panicle length versus leaf length. Note that the three glabrous subspecies, subsp. *lindheimeri*, subsp. *longiligulatum*, and subsp. *spretum* have zero values for two of the continuous quantitative characters, peduncle hair length (pedH) and leaf sheath hair length (lvfShH). These zero values will cause the glabrous OTUs to cluster in either a vertical or

horizontal line on the subplots in which one of the axes is peduncle hair length or leaf sheath hair length.

Multivariate Statistical Analysis–Eigenvalues for the principal coordinates analysis (PCoA) of the quantitative and qualitative data for the *Dichanthelium acuminatum* complex as a whole (10 subspecies) are reported in Table 13. The first three axes of the PCoA explained 85.8% (56.9%, 21.2% and 7.7%, respectively) of the total variation among the OTUs. Each of the first three PCoA axes are also statistically significant under the (random) broken stick distribution (Legendre and Legendre 1998; Frontier 1976). Figure 16 shows a plot of the first two PCoA axes which explain a total of 78.1% (56.9% + 21.2%) of the variation. Axis 1 separates the three "glabrous" taxa-subsp. *lindheimeri*, *longiligulatum* and *spretum* (represented on the far left-hand side of the plot)-from the most pubescent taxon-subsp. *acuminatum* (represented on the far right-hand side of the plot). The remaining six subspecies are located in between these two extremes and are separated from them along axis 2. These six subspecies represent intermediates in overall degree of pubescence as compared to the glabrous group and the pubescent subsp. *acuminatum*. Thus, the abscissa (axis 1) represents a gradation of increasing pubescence or "hairiness" from left to right. The axis1/axis2 PCoA plot provides satisfactory separation of subsp. *acuminatum* from the other taxa. Among the glabrous taxa, subsp. *lindheimeri* is separated into two groups and is mostly distinct from subsp. *longiligulatum* and subsp. *spretum*. Subspecies *longiligulatum*, *spretum* and one of the two groups of subsp. *lindheimeri* cluster closely together and are not well separated from one another. The remaining six intermediate subspecies cluster in

between the glabrous group and subsp. *acuminatum* and are not clearly differentiated into subspecies groupings in the axis1/axis2 PCoA plot.

Figure 17 shows a plot of PCoA axes 1 and 3 which account for 56.9% and 7.7% of the total variation, respectively. This plot does show separation of subsp. *columbianum* from most of the other taxa although a few outliers of subsp. *leucothrix* and one individual of subsp. *sericeum* do cluster closely with the subsp. *columbianum* group. Axis 1 and axis 3 separate two of the three specimens of subsp. *thermale* from the other taxa while the third specimen of subsp. *thermale* clusters with the group of subsp. *fasciculatum* specimens. The separation of subspecies *columbianum* from the other taxa as well as two of the subsp. *thermale* specimens is still supported in the plot of PCoA axes 2 and 3 (Fig. 18).

In the PCoA analysis of the 10 subspecies Spearman's correlations ($P < 0.01$; Table 14) show that axis 1 was most strongly correlated to leaf sheath pubescence type ($r = 0.93$), followed by culm pubescence type ($r = 0.91$), peduncle hair length ($r = 0.90$) and leaf sheath hair length ($r = 0.89$). Axis 2 is most strongly correlated with culm length ($r = 0.71$) and panicle length ($r = 0.69$).

In order to better elucidate the relationships among the pubescent group of taxa a second PCoA analysis was done with only the seven pubescent subspecies included in the data matrix. The resulting data matrix consisted of 230 specimens from the following "pubescent" subspecies: subsp. *acuminatum*, subsp. *columbianum*, subsp. *fasciculatum*, subsp. *implicatum*, subsp. *leucothrix*, subsp. *sericeum*, and subsp. *thermale*. Eigenvalues are listed in Table 15. The first three axes of the PCoA were

statistically significant under the broken stick distribution (Legendre and Legendre 1998; Frontier 1976).

The first three axes accounted for a total of 80.7% of the variation (Table 15). A plot of axis 1 and axis 2 of the PCoA is presented in Fig. 19. Axis 1 accounted for 54.2% of the total variation. Several characters were strongly correlated with the first axis of the PCoA analysis ($P < 0.01$; Table 16). Leaf margin type ($r = 0.86$) was the most highly correlated character followed by leaf sheath pubescence type ($r = 0.83$), panicle length ($r = 0.79$) and culm pubescence type ($r = 0.78$). Whereas in the first PCoA, which included all 10 subspecies (axis 1/axis2, Fig. 16), showed the subsp. *acuminatum* specimens as one group, the PCoA of the 7 pubescent subspecies (Figs. 19-21) shows the subsp. *acuminatum* specimens as two somewhat distinct groups. Four specimens of the subsp. *sericeum* are clustered with one of the subsp. *acuminatum* groups on the axis1/axis 2 (Fig. 19) plot. The axis 1/axis 3 plot (Fig. 20) shows a delineation of the subsp. *acuminatum* specimens from the remaining subspecies except for a few outlier specimens. The axis1/axis 3 plot also shows subsp. *sericeum* clustering with these outlier subsp. *acuminatum* specimens. Subspecies *columbianum* clusters with subsp. *leucothrix* on the axis1/axis 2 plot (Fig. 19) with subsp. *leucothrix* having some outlier specimens outside of this cluster. A majority of the subsp. *fasciculatum* specimens cluster together on the axis 2/axis 3 (Fig. 21) plot but start to intergrade into a mixed group of subsp. *implicatum*, subsp. *leucothrix* and subsp. *acuminatum* on the right-hand side of the cluster. There were no highly-correlated characters on either axis 2 or axis 3 (all $r < 0.50$, Table 16).

The PCoA plots produced for the pubescent subspecies of the *D. acuminatum* complex (Figs. 19-21) were redrawn with unique plot symbols chosen for each of the subsp. *acuminatum* populations in the data matrix of 230 specimens. These redrawn plots are presented in Figs. 22-24. The unique plot symbols for each of the populations allow for the substructure of the subsp. *acuminatum* specimens to be visualized. Table 17 lists each subsp. *acuminatum* herbarium specimen (providing herbarium accession number and location of collection) that is part of the pubescent data matrix and which are analyzed alongside the populations of subsp. *acuminatum* in PCoA Figs. 22-24.

PCoA analysis was also conducted on a subset of the data matrix that included only the 117 subsp. *acuminatum* specimens, composed of five geographically distinct populations and 15 non-geographically associated herbarium specimens (Table 17). The PCoA plots are presented in Figs. 25-27. In support of this population-based analysis of the subsp. *acuminatum* data Table 18 was generated to summarize the variation for each of the seven quantitative characters across the populations. Table 18 gives population size (*N*), mean, median, minimum value, maximum value and range for each of the characters.

A separate PCoA was conducted on the 161 specimens comprising the glabrous taxa (Figs. 28-30). For purposes of this study the glabrous taxa are the field-collected populations and herbarium specimens of subsp. *lindheimeri*, subsp. *longiligulatum* and subsp. *spretum*. Eigenvalues for the PCoA of the glabrous taxa are presented in Table 19 and Spearman correlation values are presented in Table 20.

The axis 1/axis 2 PCoA plot of the glabrous subspecies (Fig. 28) shows two main clusters of specimens and a number of outlier specimens. The leftmost cluster on Fig. 28 is composed subsp. *longiligulatum* population 5 (plot symbol 6; specimens: *Hammer* 5-1 to 5-20), subsp. *longiligulatum* population 7 (plot symbol 7; specimens: *Hammer* 7-1 to 7-20), subsp. *longiligulatum* herbarium specimens (plot symbol 8) and subsp. *spretum* herbarium specimens (plot symbol 9). On the right side of this cluster is a small cluster of 12 subsp. *lindheimeri* specimens.

The large cluster of specimens to the right of the leftmost cluster of Fig. 28 is composed almost entirely of subsp. *lindheimeri* specimens with the exception of two outlier herbarium specimens of subsp. *longiligulatum*. The large subsp. *lindheimeri* cluster is composed of specimens from all four of the subsp. *lindheimeri* populations included in the study: population 3 (specimens: *Hammer* 3-1 to 3-20); population 19 (specimens: *Hammer* 19-1 to 19-20); population 20 (specimens: *Hammer* 20-1 to 20-20); and population 210 (specimens: 210-1 to 210-20). Also included in the large subsp. *lindheimeri* cluster are several herbarium specimens of subsp. *lindheimeri*.

Finally, regarding Fig. 28, there are two specimens (*Hammer 303* and *Hammer 328*) of subsp. *lindheimeri* located on the extreme right-hand side of the plot.

DISCUSSION

Recent monographic treatments of the *Dichanthelium acuminatum* complex have produced morphological-based keys that take a similar approach to dividing the taxa into groups and eventually to individual subspecies or varieties. Both Gould & Clark (1978) and Freckmann & Lelong (2003) first divide the taxa of the complex into two groups,

one with culms and leaf sheaths mostly glabrous and the other with pubescent culms and sheaths. Both univariate and multivariate analyses of the present study tend to support this initial division of the taxa.

Qualitative characters such as leaf sheath pubescence (Table 2), culm internode pubescence (Table 3), and leaf adaxial and abaxial pubescence (Tables 4-5) separate out the mostly glabrous subspecies group consisting of subsp. *lindheimeri*, subsp. *longiligulatum* and subsp. *spretum* from the remaining seven pubescent subspecies group. This group consists of subsp. *acuminatum*, subsp. *columbianum*, subsp. *fasciculatum*, subsp. *implicatum*, subsp. *leucothrix*, subsp. *sericeum*, and subsp. *thermale*.

Bivariate analysis of the 10 continuous quantitative characters (Fig. 15) shows that there is no one character or group of characters that will separate one subspecies from another as there is much overlap among the taxa for most characters. Further, the quantitative characters, when taken individually or in total, provide no separation of taxa when considering the three glabrous taxa as a starting group or the seven pubescent taxa as another group.

Multivariate analysis of both the qualitative and quantitative character data begins to provide some insight into the relationships of the individual subspecies and of the populations themselves. The Principal Coordinates Analysis (PCoA) of the 10 subspecies of the *D. acuminatum* complex (Fig. 16) shows a general grouping of the three glabrous subspecies– subsp. *lindheimeri*, subsp. *longiligulatum* and subsp.

spretum–which is in accordance with the groupings revealed by simple tabular analysis of the OTU qualitative character data (Tables 1-5).

The three glabrous taxa are separated from the remaining seven pubescent taxa mainly on axis 1 (Fig. 16). The "bow" shape of Fig. 16 has been described as a "Gauch" curve (Gauch 1982) and this indicates that axis 2 is a quadratic distortion of the valid first axis of the PCoA. In other words, in this instance, axis 2 is invalid and is not providing informative information to the analysis.

The PCoA plot of axis1/axis3 (Fig. 17) does not show the Gauch distortion of the axis1/axis2 plot (Fig. 16) and thus provides better separation of several of the subspecies. There is a separation of subsp. *acuminatum* OTUs from those of subsp. *fasciculatum*. Specimens of subsp. *columbianum* are separated from most of the other OTUs but the cluster is fairly loose. Also, the subsp. *columbianum* grouping contains several specimens of subsp. *leucothrix* and one specimen of subsp. *fasciculatum*. Two of the three specimens representing subsp. *thermale* cluster together and are separated from the other OTUs. The OTUs of the three glabrous taxa, subsp. *lindheimeri*, subsp. *longiligulatum* and subsp. *spretum* form a tighter cluster and are more distinct as a group from the seven pubescent taxa along the axis 1/axis 3 PCoA plot (Fig. 17).

Subsp. *fasciculatum* + subsp. *implicatum* + subsp. *leucothrix* group–Specimens of subsp. *fasciculatum* generally exhibit good clustering on several of the PCoA plots given the wide distribution of this taxon in North America. This diverse geographical distribution is somewhat represented in the 68 specimens included in the morphological data set. Specimens in the data matrix represent 32 U.S. states including: AL, AR, CA,

CO, CT, DC, FL, GA, IA, IL, IN, KS, KY, LA, MA, ME, MO, MS, NC, NE, NH, NY, OH, OK, OR, PA, SC, TX, VA, VT, WI and WV. One specimen from Quebec, Canada is also included in the data set.

Subspecies *fasciculatum* forms a homogenous cluster along the axis 1/axis 3 PCoA plot (Fig. 17) of the10-subspecies analysis (N = 389) but does have a few intergrading specimens of subsp. *implicatum* and subsp. *leucothrix* in the right-hand side of the subsp. *fasciculatum* cluster. The axis 2/axis 3 PCoA plot (Fig. 18) of all 10 subspecies is similar to Fig. 17 in that subsp. *fasciculatum* specimens cluster together but start to intergrade with subsp. *implicatum* and subsp. *leucothrix* on one side of the cluster. Spearman correlations for the 10-subspecies PCoA analysis (Figs. 16-18) indicate that leaf sheath pubescence type was the character most associated with axis 1 of the plots (r = 0.93, Table 14). Table 3 shows that 91.2 % (N = 68) of the subsp. *fasciculatum* specimens had a leaf sheath vestiture character type of pilose while 100% (N = 9) of the subsp. *implicatum* specimens were also classified as pilose for this character. As a result some intergradation and overlap would be expected between the two taxa. In contrast, in the same PCoA analysis, only 27.3% (N = 11) of subsp. *leucothrix* specimens had a pilose leaf sheath vestiture type.

An almost identical situation is true for the pattern seen in culm internode pubescence type among the three subspecies. Both subsp. *fasciculatum* and subsp. *implicatum* have high percentages (89.7% and 100%, respectively) of their specimens classified with a pilose culm internode. And again subsp. *leucothrix* specimens show less overlap with only 54.5% of specimens classified as pilose for this character. This similarity would

contribute to the pattern of intergradation and overlap observed between subsp. *fasciculatum* and subsp. *implicatum* in the PCoA plots considering that culm internode pubescence type had the second highest Spearman correlation (r = 0.91, Table 14) for axis 1 of the 10 subspecies PCoA plots (Figs. 16-18).

Similarly, the axis 1/axis 2 PCoA plot (Fig. 19) of the seven pubescent subspecies analysis (N = 230) shows good separation of subsp. *fasciculatum* from the pubescent subspecies except for a number of subsp. *implicatum* and subsp. *leucothrix* specimens which are mixed in the subsp. *fasciculatum* cluster. Finally, the axis 2/axis 3 PCoA plot (Fig. 21) of the seven pubescent subspecies clusters the majority of subsp. *fasciculatum* specimens to the extreme left-hand side of the plot with the same intergradation of subsp. *implicatum* and subsp. *leucothrix* specimens on the right-hand-side of the plot. In both of these PCoA plots axis 1 was most correlated with leaf blade margin type (r = 0.86, Table 16) and leaf sheath vestiture type (r = 0.83, Table 16) and both subsp. *fasciculatum* and subsp. *implicatum* had a high percentage of specimens sharing the same character states for these two characters (Tables 2 and 6).

From the foregoing description of the various PCoA plots of subsp. *fasciculatum* it is suggested that many of the subsp. *fasciculatum* specimens are distinct morphologically in the multivariate analysis, but that there are consistently a number of subsp. *implicatum* and to a lesser extent subsp. *leucothrix* specimens that intergrade into the subsp. *fasciculatum* cluster, based on shared qualitative character states in leaf sheath pubescence and culm internode pubescence types and leaf blade margin type.

There are several conclusions that can be drawn from the foregoing results of the multivariate analysis for the subsp. *fasciculatum/implicatum/leucothrix* subspecies "complex."

First, possibly influencing the results, is the fact that sample sizes are low for both subsp. *implicatum* (N = 9) and subsp. *leucothrix* (N = 11). More specimens need to be examined for both subspecies and included in the multivariate analysis. Even though the sample size for subsp. *fasciculatum* is large relative to the other two subspecies the resolution of the multivariate analysis would most likely be improved with the addition of more specimens into the data matrix. It should be noted that the subsp. *fasciculatum* specimens in the data matrix include 53 herbarium specimens drawn from 32 states in North America and a single east Texas population of 15 specimens collected in Rusk County, TX. The box plots provided in Fig. 12 compare the 10 quantitative variables for both of these groups. The box plots for the Rusk County, TX population sample show considerable divergence from the box plots of the herbarium specimens, especially in spikelet length and peduncle hair length. These differences may be due solely to environmental or phenotypic factors especially when comparing a population sample from one location to a group of geographically divergent herbarium specimens. Nevertheless, collection of more population samples from Texas and surrounding states and inclusion of these specimens in the univariate and multivariate analyses might begin to shed some light on regional versus broader-scale geographic patterns in subsp. *fasciculatum* morphology.

Second, as a result of the small sample sizes for both subsp. *implicatum* and subsp. *leucothrix* it is difficult to draw conclusions as to their taxonomic status in the D. *acuminatum* complex. More specimens need to be examined for both taxa. However, differences in the qualitative characters discussed above seem to point to subsp. *leucothrix* as being morphologically distinct from both subsp. *fasciculatum* and subsp. *implicatum*. The PCoA results seem to favor this conclusion as well. As for subsp. *implicatum* there are similarities in several qualitative characters as discussed above that seem to show a trend towards morphological similarity between subsp. *implicatum* and subsp. *fasciculatum*. Again, subsp. *implicatum* requires examination of more morphological specimens.

As for the appropriate taxonomic status of subsp. *fasciculatum* the results of the multivariate analysis indicate that this taxon is appropriately classified as a subspecies in the *D. acuminatum* complex. Other than the forgoing discussion of intergradation of some subsp. *fasciculatum* specimens with specimens of subsp. *implicatum,* the member of the *D. acuminatum* complex that most resembles subsp. *fasciculatum* morphologically is subsp. *acuminatum*. However, it has been shown above that subsp. *fasciculatum* is reasonably separable from subsp. *acuminatum* in the results of the multivariate analysis.

Subspecies *columbianum*–The axis 1/axis 3 PCoA plot of Fig. 17 (389 specimens, all 10 subspecies) shows a distinct separation of subsp. *columbianum* specimens from the other subspecies. The subsp. *columbinaum* cluster does intergrade with three of the subsp. *leucothrix* specimens and one subsp. *fasciculatum* specimen on the left side of the cluster. However, the subsp. *columbianum* specimens do cluster as a group and exhibit

the pattern of separation expected in a matrix of closely-related taxa. Further, the three intergrading specimens of subsp. *leucothrix* are not overly problematic in that subsp. *leucothrix* is typically found in the southeast coastal plain while subsp. *columbianum* is restricted to the more northeastern part of the *D. acuminatum* species range. Only 15 specimens of subsp. *columbianum* were obtained for examination. The sample size needs to be enlarged to quantify the extent of character variation in this taxon.

Subspecies *sericeum*–Subspecies *sericeum* shows a separation from the other six pubescent subspecies along the axis 1/axis 3 PCoA plot (Fig. 20). There are four specimens of subsp. *acuminatum* that are in the center of the subsp. *sericeum* group of specimens. These subsp. *acuminatum* specimens are somewhat atypical for subsp. *acuminatum* as three of these outlier specimens have a leaf blade margin type of "entire" and a leaf sheath vestiture type of "pilose." The typical or predominant states for these characters in the data matrix are "ciliate at base up to ¼ of blade length" or "ciliate from base to at least ½ of blade length" for leaf blade margin type, and for leaf sheath vestiture type the predominant type for subsp. *acuminatum* is "villous." So, the four subsp. *acuminatum* specimens clustering in the subsp. *sericeum* group on the axis 1/axis 3 PCoA plot (Fig.20) are not typical for subsp. *acuminatum*.

Further supporting the separation of subsp. *sericeum* from subsp. *acuminatum* and the remaining pubescent subspecies is the fact that subsp. *sericeum* has a very restricted geographical distribution and physical habitat. This subspecies grows in warm or hot ground around geysers and hot springs in the Rocky Mountains from Banff, Alberta southward to Yellowstone National Park in Wyoming and eastward to Bighorn County,

Wyoming (Freckmann & Lelong 2003). Gould & Clark (1978) classify subsp. *sericeum* as a segregate under their *D. acuminatum* var. *acuminatum* taxon. Prior to Gould and Clark this taxon was treated under the name *Panicum thermale* (Table 21) by Hitchcock and Chase (1950).

Subspecies *thermale*–Subspecies *thermale* consists of a cluster of three specimens (*N* = 3) in the axis 1/axis 2 PCoA plot (Fig. 19). Sample size is small due to several reasons. First, as stated in the introduction, obtaining specimens for study is difficult for the *D. acuminatum* group. Second, subsp. *thermale*, like subsp. *sericeum*, grows in a restricted geographic region and specific physical habitat. Freckmann & Lelong (2003) describe subsp. *acuminatum* as only occurring in warm, moist soil at the Geysers, Sonoma County, CA. One of the three studied specimens is from Sonoma County, CA and the other two specimens were collected in Lassen National Volcanic Park, Shasta County, CA which is known for its large-scale geothermal areas.

The three specimens of subsp. *thermale* cluster relatively close together along the axis 1/axis 2 PCoA plot (Fig.19) and are located just above a tight cluster of subsp. *fasciculatum* and subsp. *implicatum* specimens but do intergrade with a few specimens of subsp. *fasciculatum* and subsp. *columbianum*. With such a small sample size it is difficult to clearly delineate this taxon as distinct on the basis of the multivariate results alone. However, due to its known restricted geographic distribution and apparent ecological and physical habitat it is not unreasonable to designate subsp. *thermale* as a distinct taxon in the *D. acuminatum* subspecies complex.

However, for future research, the multivariate morphological analysis for this subspecies needs to be strengthened by obtaining and including more specimens in the analysis. The two specimens from Shasta County, CA (Lassen National Volcanic Park) provide documentation for occurrence of subsp. *thermale* beyond the range stated in Freckmann & Lelong (2003) and acquisition of further specimens would likely help to further delineate the range of this subspecies.

Subspecies *acuminatum*–Figure 19 (axis1/axis 2) shows a separation of subsp. *acuminatum* from the other pubescent taxa. Subspecies *acuminatum* clusters into two somewhat distinct groups on the axis 1/axis 2 plot. Several of the subsp. *sericeum* specimens cluster with the lower subsp. *acuminatum* group in this plot. However, on the axis 1/axis 3 plot (Fig. 20) the subsp. *sericeum* specimens are shifted to the left of almost all subsp. *acuminatum* specimens. Thus, subsp. *acuminatum* is distinct from the other subspecies on the basis of the axis 1/axis 2 and axis 1/axis 3 PCoA plots (Figs. 19 and 20).

To further investigate the two distinct clusters of subsp. *acuminatum* specimens that are shown on the axis 1/axis 2 plot (Fig. 19) the plot was redrawn using unique plotting symbols for each geographical population represented in the data set for subsp. *acuminatum*. This version of the axis 1/axis 2 plot (Fig. 22) reveals a distinct population substructure and thus a degree of morphological variation to the two clusters of subsp. *acuminatum* OTUs. The lower cluster is composed primarily of specimens from the subsp. *acuminatum* population collected from Montgomery County, TX (plot symbol 11). The upper cluster is made up of specimens representing four geographically

separated populations: Leon County, TX (plot symbol 1), Hardin County, TX (plot symbol 8), Vernon Parish, LA (plot symbol 2), and Vernon Parish, LA (plot symbol 10). From these plots it appears that there is a geographic structuring to the morphological variation seen in the plot. Also shown on the plot are 15 non-population specimens from herbarium sheets (plot symbol 12). The majority of the plot symbols representing herbarium specimens are positioned to the left of the two main clusters of subsp. *acuminatum* on Fig. 22. Table 17 lists the collection location for each herbarium specimen and shows that five specimens were collected outside of the United States and represent the following countries: Columbia, Jamaica, Mexico, Panama, and Puerto Rico. However, four of the subsp. *acuminatum* herbarium specimens (Table 17)–*Hammer 212*, *Hammer 221*, *Hammer 308*, *Hammer 389*–were all collected in east Texas and cluster in the upper subsp. *acuminatum* group of populations.

Analysis of both the quantitative and qualitative characters on a population level for each of the subsp. *acuminatum* populations does suggest differentiation in several morphological characters that helps explain the pattern of clustering for these populations in the multivariate analysis. First, the quantitative characters will be discussed followed by the qualitative characters.

Figures 6-8 show box plots of each of the 10 quantitative variables, with the plots for each of the five geographic populations of subsp. *acuminatum* and the grouping of herbarium specimens displayed side-by-side. Based on these plots there is differentiation in the populations for some of the variables. Peduncle hair length (Fig. 6 plot “D”) clearly separates populations 8, 15, and 17 from populations 1, 4 and the group

of herbarium specimens. Similarly, peduncle length (Fig. 6 plot "C") separates both the herbarium specimen group and population 1 from the remaining populations. Leaf sheath hair length (Fig. 7 plot "C") separates populations 8 and 15, and to a lesser extent population 17 from populations 1 and 4.

Tables 8-12 show comparisons of the subsp. *acuminatum* populations for the five qualitative variables. Leaf blade abaxial pubescence-type (Table 8) groups populations 8, 15 and 17 together based on the almost constant vestiture type of velutinous, with hairs > 0.5 mm in length" with only population 17 showing some slight variability in this character. Both populations 1 and 4 and the herbarium specimen group show almost equal variability in this character with each population split roughly 50:50 between "velutinous, hairs < 0.5 mm" or with "velutinous, hairs > 0.5 mm."

For leaf blade adaxial vestiture type (Table 9) there is a similar grouping of populations 1 and 4, with both populations showing individual specimens split among three character types: sparsely puberulent/pubescent; pilose; and sparsely pilose only near base. Conversely, populations 8, 15 and 17 are mostly constant for this character with both populations 8 and 15 having 100% of specimens classified as "pilose" and population 17 showing 85% of individuals as "pilose" and 15% as "sparsely puberulent/pubescent."

Finally, the remaining qualitative variables, culm vestiture type (Table 10), leaf blade margin type (Table 11), and leaf sheath vestiture (Table 12) type do not show any major differences among the individual populations for these characters.

From the foregoing discussion of the distribution of both quantitative and qualitative characters among the individual populations of subsp. *acuminatum* a general pattern emerges that groups populations 1 and 4 together and populations 8, 15 and 17 together, based on degree and similarity of vestiture in peduncle hair length, leaf sheath hair length, leaf blade adaxial vestiture and leaf blade abaxial vestiture. Comparing these results to the PCoA (Figs. 22) of the pubescent subspecies there is not total agreement as this PCoA (population substructure version) only separates population 4 from the cluster of populations 1, 8, 15 and 17. This difference may be partially explained by the data for culm vestiture character types in Table 10 which shows population 4 with all pilose culms and the other populations with high percentages of villous culms. Spearman's correlation values (Table 16) for the PCoA indicate that leaf blade margin type was most highly correlated with axis 1. Interestingly, the summary of leaf blade margin type (Table 11) shows little variation among any of the populations or the group of herbarium specimens. However, this PCoA plot (Fig. 22) includes all seven of the pubescent subspecies, among which leaf blade margin type would be informative in separating subsp. *acuminatum* specimens from the remaining pubescent subspecies.

To further quantify the extent of morphological variation among the individual populations of subsp. *acuminatum* a PCoA was generated (Figs. 25-27) from the 117 specimens of subsp. *acuminatum* in the data matrix, which includes five individual geographical populations and a group of 16 non-geographically-related specimens.

From the PCoA (Fig. 25) of subsp. *acuminatum* populations it is appears that only population 4 (plotting symbol "2") forms a cluster that is distinct from the other

populations and the group of herbarium specimens. The PCoA plot of axis 2/axis 3 (Fig. 27) best illustrates the separation of population 4 and is also apparent to a lesser extent in the plot of axis 1/axis 2 (Fig. 25). Populations 8, 15 and 17 form a concentrated cluster in the far right-hand portion of both the axis 1/axis 2 PCoA plot (Fig. 25) and the axis 1/axis 3 plot (Fig. 26). This same group of populations is clustered in the center of part of the axis 2/axis 3 plot (Fig. 27).

The specimens of subsp. *acuminatum* in population 1 are more distinct from the cluster of specimens made up of populations 8, 15 and 17 in the PCoA of only the subsp. *acuminatum* populations and herbarium specimens (Figs. 25-27). The PCoA of the seven pubescent subspecies (Figs. 22-24), which includes subsp. *acuminatum*, does not clearly differentiate the specimens of population 1 from those of populations 8, 15, and 17.

The PCoA (Figs. 25-27) grouping of the populations along with the distribution of both the qualitative and quantitative characters types for these populations demonstrates a marked degree of morphological variability in the subsp. *acuminatum* taxon in southeast Texas and western Louisiana, where these populations were collected. Populations 8, 15, 17 are more hairy than populations 1 and 4 on a quantitative basis, with peduncle hair length, peduncle length, and leaf sheath hair length supporting this separation of populations. Leaf blade abaxial vestiture type, a qualitative character, suggests a separation of populations 8, 15 and 17 from populations 1 and 4. Spearman's correlations (Table 18) derived from the final PCoA analysis (Figs. 25-27), which analyzed only the populations of subsp. *acuminatum*, support the foregoing quantitative

and qualitative univariate statistics. Axis 1 (Fig. 25) was most highly associated with leaf sheath hair length (r = 0 .77, Table 18), followed by peduncle hair length (r = 0.75) and leaf sheath abaxial pubescence (r = 0.69). Leaf sheath adaxial pubescence type was weakly correlated with axis 1 (r = - 0.16, p > 0.05) and this is borne out in the high degree of variability in the distribution of pubescence types among the subsp. *acuminatum* populations shown in Table 9.

The data and multivariate analysis presented for subsp. *acuminatum* indicate a wide range in character variation among populations and this variability should be addressed at least regionally in taxonomic treatments and subsequent keys. The Flora of North America treatment of *Dichanthelium acuminatum* (Freckmann & Lelong 2003) states that leaf sheath hairs do not exceed 3 mm for any of the subspecies of the complex. The data for the subsp. *acuminatum* populations collected for this study include specimens that exceed 3 mm in length. Population 8 includes seven specimens with leaf sheath hairs equal to or greater than 3 mm and population 15 includes seven such specimens including one individual with leaf sheath hairs to 4 mm in length. In contrast, of the 21 specimens measured for population 1, the maximum leaf sheath hair length was 2.1 mm. Twenty specimens were measured for population 4 with the maximum leaf sheath hair length of 2.1 mm as well.

Gould & Clark (1978) in their treatment of the genus *Dichanthelium*, state that *var. acuminatum* have leaf sheaths with hairs usually less than 2 mm in length. Gould and Clark (1978) treated specimens with leaf sheath hairs of 2-5 mm lengths and spikelet lengths of 1.8-2.7 mm as *D. acuminatum* var. *villosum* (=*D. ovale* subsp. *villosissimum*

(Nash) Freckmann & Lelong, in Freckmann & Lelong 2003). As a result many of the specimens in the subsp. *acuminatum* populations of this study would be classified as *D. ovale* var. *villosum* based on leaf sheath hair length.

Populations 1 and 4, in terms of leaf sheath hair length, do fit the criteria for both the Gould & Clark (1978) and Freckmann & Lelong (2003) treatments of subsp. *acuminatum* and these two populations might be considered typical for this character. Populations 8 and 15, both with a number of specimens exceeding 3 mm in length and both with average leaf sheath hair lengths of 2.5 mm, do not seem to fit into either treatment based on the leaf sheath vestiture character. Population 17 has leaf sheath hair lengths within the range of variation described by Freckmann & Lelong (2003) with seven specimens of 2 mm or greater in length plus one specimen with a 3.0 mm long leaf sheath hair. Population 17 would be atypical for leaf sheaths in the Gould & Clark (1978) treatment of subsp. *acuminatum*.

Neither the Freckmann & Lelong nor the Gould & Clark treatments of subsp. *acuminatum* describe a typical hair length for the peduncle and this character is not included in their respective keys for identifying the subspecific taxa of the complex. As mentioned previously, peduncle hair length ($r = 0.75$, Table 18) was the most highly correlated character after leaf sheath hair length ($r = 0.77$, Table 18) in the PCoA (Figs 25-27) of the subsp. *acuminatum* population data and thus is a significant character in distinguishing and clustering the populations in the multivariate analysis.

Although the number of populations examined is small, peduncle hair length does seem to correlate to leaf sheath hair length in separating the more "typical" subsp.

acuminatum populations 1 and 4 from the atypical populations 8, 15 and 17. The box plots (Fig. 6D) for peduncle hair length for the subsp. *acuminatum* populations show populations 1 and 4 to have much shorter hairs than those of populations 8, 15 and 17. More specifically, Table 19 shows population 1 with an average peduncle hair length of 1.4 mm and maximum of 2.1 mm; population 4 with average length of 1.3 mm and maximum of 2.2 mm. In contrast is population 8 with an average peduncle hair length of 2.2 mm and maximum of 3.1 mm; population 15 with average of 2.7 mm, maximum of 4.0 mm; population 17 with average of 2.5 mm and maximum of 4.2 mm. It is recommended that peduncle hair length be included as a character in regional floras and keys to this subspecies as this character, along with leaf sheath hair length, are useful in identifying the more atypical populations of this taxon.

As mentioned earlier with the discussion of the subsp. *acuminatum* PCoA results, leaf sheath abaxial pubescence type was the character most associated with axis 1 of the axis 1/axis 2 PCoA plot (Fig. 25). Table 8 shows the distribution of leaf sheath abaxial vestiture types for the subsp. *acuminatum* populations. Almost all specimens of populations 8, 15 and 17 have a leaf blade abaxial vestiture type of "velutinous, with hairs > 0.5 mm in length," while populations 1 and 4 are split almost equally between this same pubescence type and "velutinous, with hairs <= 0.5 mm in length." The more atypical populations of subsp. *acuminatum*, namely populations 8, 15 and 17, are almost constant in their type of leaf blade abaxial vestiture and this character, in conjunction with leaf sheath hair length and peduncle hair length, would adequately identify atypical subsp. *acuminatum* populations and specimens.

One last character to consider in regards to the atypical status of subsp. *acuminatum* populations 8, 15 and 17 is spikelet length. The box plots of Fig. 6A compare spikelet length for each of the populations and the group of herbarium specimens. There is no apparent differentiation in this character when comparing the populations although population 15 does trend toward slightly longer spikelet lengths. So, for spikelet length, populations 8, 15 and 17 are typical or comparable to populations 1 and 4.

It was noted earlier that Gould & Clark (1978) would classify as *D. ovale var*. *villosum* the specimens from populations 8, 15 and 17 that had leaf sheath hairs exceeding 2.0 mm in length or greater. For population 8 this would classify 17 of the 20 specimens as *D. ovale* var. *villosum*. For population 15 this would again classify 17 of the 20 specimens as *D. ovale* var. *villosum*. Seven of the 20 population specimens would fall under *D. o*. var. *villosum*. In contrast, the treatment of Freckmann & Lelong (2003) would classify all specimens of populations 8, 15 and 17 as *D. acuminatum* subsp. *acuminatum* with spikelet length as the critical character in the determination. None of the five populations of subsp. *acuminatum* had any specimen with spikelet length greater than 2.0 mm (Fig. 6A; Table 19) with the mean for each population about 1.8 mm in length.

In essence both Gould & Clark (1978) and Freckmann & Lelong (2003) have recognized the atypical or intermediate nature of subsp. *acuminatum* specimens that constitute a large percentage of populations 8, 15 and 17. But they have handled the taxonomic determination of these specimens in different ways. Gould & Clark classify them as part of another species complex (*D. ovale* var. *villosum*) while Freckmann &

Lelong retain them in subsp. *acuminatum* by defining wider latitude in characters such as leaf sheath hair length and culm vestiture type. Morphologically, most of the specimens of populations 8, 15 and 17 resemble specimens of *D. ovale* subsp. *villosissimum* in terms of leaf sheath hair length, culm internode vestiture type and peduncle hair length.

However, spikelet lengths in *D. ovale* subsp. *villosissimum* specimens is 2.1 mm or greater. The specimens of populations 8, 15 and 17 have retained the spikelet character of *D. acuminatum* subsp. *acuminatum* and thus their "intermediate" characterization. Based on spikelet length it is recommended to classify the specimens of these populations as in the past as subsp. *acuminatum* and make appropriate adjustments in regional floras and keys to accommodate and recognize the intermediacy of these populations.

Subspecies *lindheimeri*–The specimens of subsp. *lindheimeri* are very clearly separated from specimens of the other two glabrous subspecies. Specimens from the four field-collected populations and the herbarium specimens cluster together on the PCoA plot for axis 1/axis 2 (Fig. 28), with several outlier specimens to the right of the main subsp. *lindheimeri* cluster. To the left side of this cluster is a group of 12 subsp. *lindheimeri* specimens that are clustered together and alongside a group of subsp. *longiligulatum* specimens. Examination of the qualitative character data shows that this outlier group of subsp. *lindheimeri* specimens differs from the main cluster in terms of leaf blade margin type. The specimens composing this outlier group, which is positioned alongside the subsp. *longiligulatum* specimens, all have a leaf blade margin type of

"scabridulous" (at 30x) while the main cluster of subsp. *lindheimeri* specimens have a leaf blade margin type of "ciliate at base up to one-fourth of blade length". This is most likely the character that is separating these two groups and this is supported by the Spearman correlation values for the PCoA (Fig. 28), which indicate that leaf blade margin type was the most highly correlated character on axis 1 ($r = 0.85$, $p < 0.01$; Table 22). The leaf blades of subsp. *lindheimeri* typically have conspicuous, long, papillose-based hairs at the base and this character is present on the main subsp. *lindheimeri* cluster of Figure 28. All leaf characters were scored from the third leaf down from the apex. It was noted by the author that on some subsp. *lindherimeri* specimens the ciliate hairs were not present on the lower leaves but were often present on the younger first or second leaf down from the apex. This observation offers a reasonable explanation for the positioning of the outlier group of specimens (in the PCoA of Fig. 28) apart from the main subsp. *lindheimeri* cluster. Therefore, the results of the multivariate analysis show that subsp. *lindheimeri* is morphologically distinct from the other two glabrous taxa, subsp. *longiligulatum* and subsp. *spretum.*

Subspecies *longiligulatum* and subsp. *spretum*–Since these two putative subspecies are very similar morphologically they are discussed together in this section. The left-most cluster of specimens in the axis 1/axis2 PCoA plot of Fig. 28 contains specimens of subsp. *longilgulatum* on the lower portion and specimens of subsp. *spretum* on the uppermost portion of the cluster. There are also two outlier specimens of subsp. *spretum* towards the top margin of the plot. On the right-side of the cluster of subsp. *longiligulatum* and subsp. *spretum* specimens is the small group of outlier subsp.

lindheimeri specimens which were discussed in the foregoing section on subsp. *lindheimeri*. The cluster of specimens constituting subsp. *longiligilatum* is actually composed of specimens from two separate field-collected populations and a small group of herbarium specimens, while the bordering cluster of subsp. *spretum* specimens is composed strictly of herbarium specimens. Despite the close proximity of the overall cluster representing subsp. *longiligulatum* specimens and the cluster representing subsp. *spretum* there is minimal intergradation or mixing of specimens from the two subspecies groups. This could be an artifact of the multivariate analysis and thus have no real taxonomic significance. However, given the considerable morphological similarity between subsp. *longiligulatum* and subsp. *spretum*, it is reasonable to expect the multivariate clustering pattern represented in the PCoA plot in Fig. 28.

The main morphological difference used to separate subsp. *longiligulatum* from subsp. *spretum* in the FNA treatment (Freckmann & Lelong 2003) is the degree to which the panicles are open or congested (closed) and the length of the panicles compared to their width. The individual plants from both populations of subsp. *longiligulatum* that were collected from the field during the spring 2005 field season were not handled optimally to preserve the characteristics of the panicles for morphological study. The plants of subsp. *longiligulatum* were not pressed when collected from the field but rather were stored in ice chests for transportation to the lab and upon arrival at the lab were then stored in refrigerated coolers (at approximately 4°C) for a period of 7-14 days, until fresh leaf material was removed for use in extraction of DNA. At that time the plants were then pressed. At the time the plants were pressed there was a noticeable decrease

in the overall "quality" of the plant specimen in terms of tendency of the leaf blades to fold inward along the midvein and also in a more wilted panicle. Therefore, the panicle width character, as measured for individual plant specimens from the two populations of subsp. *longiligulatum*, is likely not representative of the panicle width at the time the plants were collected in the field.

Summary of Conclusions from Multivariate Statistical Analysis of Morphological Data–Table 26 summarizes these conclusions for the 10 subspecies comprising the *D. acuminatum* complex (*sensu* Freckmann & Lelong 2003). Interpretation of the PCoA results in six morphologically diagnosable taxa (MDT): subsp. *acuminatum*, subsp. *columbianum*, subsp. *fasciculatum*, subsp. *lindheimeri*, subsp. *longiligulatum* + subsp. *spretum*, and subsp. *sericeum*.

CHAPTER III

MOLECULAR PHYLOGENETIC ANALYSIS

INTRODUCTION

To the author's knowledge there has been no molecular phylogenetic analysis completed for the *D. acuminatum* complex or for the genus *Dichanthelium*. A small number of *Dichanthelium* species have been included in broader molecular phylogenetic studies. Giussani et al. (2001) included molecular data from the chloroplast gene *ndhF* for *D. koolauense* (H. St. John & Hosaka) Gould & C. Clark and *D. sabulorum* (Lam.) Gould & C. Clark in their molecular phylogeny of the grass subfamily Panicoideae. Aliscioni et al. (2003), focusing more narrowly in the Paniceae tribe, included *D. acuminatum* (subspecific taxon unknown), *D. clandestium* (L.) Gould, *D. cumcubana* (Renvoize) Zuloaga, *D. koolauense,* and *D. sabulorum* in their molecular phylogenetic study (based on chloroplast gene *ndhF*) of the grass genus *Panicum (*sensu lato*).* As a result of these two studies, some chloroplast DNA sequence data has been generated for *Dichanthelium* species, but the author is not aware of previous DNA sequence data from the *Dichanthelium* nuclear genome. Thus, the present study is the first attempt to sequence nuclear DNA from any species of *Dichanthelium*.

The goals of the present study were to assess the genetic affinities and species/subspecies boundaries among the taxa of the *D. acuminatum* complex and to provide independent evidence to test the results of the morphometric study based on morphological data in Chapter II.

The choice of a molecular marker must be made in relation to the level of taxonomic resolution that is required for a particular study. For the *D. acuminatum* subspecies complex, with the primary goal of testing a particular hypothesis regarding circumscription of taxon boundaries, a marker appropriate for lower taxonomic boundaries was needed. A molecular marker or gene appropriate for evaluating species and subspecies boundaries must be one in which at least some regions of the gene are free to accumulate nucleotide mutations at a rate sufficient to allow gene sequence discrimination between closely related members of an evolving subspecies complex.

Markers for single or low-copy nuclear genes have recently been developed and successfully employed in a number of studies at lower taxonomic levels in plants. One of the major advantages low-copy nuclear genes in phylogenetic applications at lower taxonomic levels is their potential increased rate of sequence evolution (or rate of nucleotide mutation) relative to that of genetic markers from the chloroplast genome (cpDNA) or nuclear ribosomal DNA (rRNA) (Small et al. 2004). This higher rate of sequence evolution should give single and low-copy nuclear genes a greater number of phylogenetically informative characters relative to that of cpDNA or rRNA genes (Small et al. 1998; Sang 2002; Small et al. 2004) and is therefore an appropriate type of molecular marker for this study.

One such low-copy nuclear gene, the granule-bound starch synthase gene (GBSSI or *waxy*), has been applied at the species-level and higher levels in phylogenetic studies in the Poaceae (e.g., Mason-Gamer et al. 1998; Mason-Gamer 2001; Baumel et al. 2002; Mathews et al. 2002; Mason-Gamer 2004) and other plant families (e.g., Miller et al.

1999; Evans et al. 2000; Peralta and Spooner 2001). GBSSI is single-copy in the Poaceae (Mason-Gamer et al. 1998) and other plant families except in the Rosaceae, which has two copies per diploid genome (Evans et al. 2000). GBSSI structure consists of 13 translated exons and one untranslated exon (van der Leij et al. 1991). The parts of the GBSSI gene (or any nuclear gene in general) most appropriate for lower level phylogenetic studies (species/subspecies within genera) are the intron sequences (Mason-Gamer et al. 1998), occurring between the 13 exons of the gene. The intron portions of the GBSSI gene were thus the focus for obtaining sequence data for the molecular portion of this study.

MATERIALS AND METHODS

Plant Material–Plant material for DNA sequencing was obtained from both herbarium specimens and live tissue sampled from field-collected plants (Table 21). For field-collected tissue a small portion of green leaf tissue from a single individual was removed and placed in a 1.5-mL microcentrifuge tube containing desiccant material (t.h.e. dessicant, EM Science) for preservation. The entire plant was collected and preserved as a voucher specimen. Voucher specimens were deposited at TAES. For herbarium specimens a small portion of leaf tissue was removed and placed in a 1.5-mL microcentrifuge tube for storage until ready for DNA extraction. All 10 taxa of the *Dichanthelium acuminatum* complex were included in the analysis. Additionally, plant tissue was sampled from the closely related taxa *Dichanthelium ovale* subsp. *praecocius* (Hitchc. & Chase) Freckmann & Lelong, *D. ovale* subsp. *villosissimum* (Nash) Freckmann & Lelong, and *D. wrightianum* (Scribn.) Freckmann.

Genomic DNA Isolation–DNA was extracted from leaf tissue by a simple, micropreparation method. Fresh leaf tissue (appx. 1 cm2) was placed in a 1.5-mL microcentrifuge tube and macerated using a Teflon pestle (VWR, West Chester, Pennsylvania, USA) attached to a power-drill. After the addition of 0.5 mL of extraction buffer (200 mmol/L Tris pH 7.5, 250 mmol/L NaCl, 25 mmol/L EDTA pH 8, 0.5% SDS), tissues were further hand-macerated for 10 sec. After brief centrifugation to remove intact solids, nucleic acids were precipitated with the addition of 0.5 mL of isopropyl alcohol. After centrifugation for 5 min at 16 000 3 g, the pellet was resuspended in 0.5 mL 50 mmol/L Tris pH 7.5, 10 mmol/L EDTA, then briefly centrifuged to remove undissolved solids. Following addition of NaOAc pH 5.2 to a final concentration of 0.3 mol/L, nucleic acids were again precipitated with 0.5 mL of isopropyl alcohol. After further centrifugation, the nucleic acid pellet was resuspended in 0.1 mL of 10 mmol/L Tris pH 7.5, 1 mmol/L EDTA. Samples of extracted DNA were electrophoresed on 1.5% agarose TBE gels, stained with ethidium bromide and visualized under UV light to verify extraction success and to visually estimate DNA concentration.

DNA Sequence Analysis–Sequence data was obtained from the nuclear-encoded granule-bound starch synthase I gene (GBSSI). Polymerase chain reaction (PCR) and sequencing primers are given in Table 22. PCR was performed in 25 µL total reaction volumes containing1 µl (~ 5 ng) DNA template, 12.5 µL Go-Taq Green PCR Master Mix (Promega) and 0.25 mM of each primer. PCR cycling conditions were: 1 cycle of 2 min @ 94°C of initial denaturation, followed by 35 cycles of denaturation at 94°C,

primer annealing at 65°C for 30 sec, primer extension at 72°C for 2 min. A final extension step consisted of one cycle of 10 min @ 72°C. PCR products were electrophoresed on 1.5% agarose TBE gels, stained with ethidium bromide and visualized under UV light to verify amplification products. PCR products were purified prior to sequencing with the Wizard Gel and PCR Clean-Up Kit (Promega).

Prior to sequencing, 2.0 μl of purified PCR product along with known concentrations of lambda DNA were electrophoresed on 1.5% agarose TBE gels to estimate relative post-PCR DNA concentrations. Direct sequencing from the purified double-stranded PCR product was performed using 20-50 ng of template for 60 cycles of sequencing using BigDye terminator chemistry (Applied Biosystems, Foster City, California, USA). Products from the BigDye sequencing reactions were purified with Sephadex columns and then electrophoresed and detected on an ABI (model?) automated sequencer (Institute for Plant Genomics and Biotechnology, Texas A & M University). All fragments were sequenced in both directions and contigs constructed from the forward and reverse fragments using Sequencher 8.0 (GeneCodes Ann Arbor, Michigan, USA). When direct sequencing of larger fragments failed to yield high quality sequence the fragment in question was re-amplified into two shorter fragments with alternative primer pairs (i.e., this was usually necessary when amplifying template DNA obtained from herbarium tissue). All sequences have been (will be) deposited in GenBank.

Phylogenetic Analysis–Alignment of DNA sequences was initially performed with ClustalX (Thompson et al. 1997), with subsequent refinement by eye using the software program BioEdit v.7.0.9 (Hall 2007). Alignments across insertion/deletion differences

(indels) were vetted manually. Indels were not utilized in the phylogenetic analysis. FastGap (Borchsenius 2009) was used to format the aligned data matrix into nexus format files for import into PAUP* v.4.0b10 x86 Linux (Swofford 2002). Phylogenetic analyses were performed under the optimality criterion of maximum parsimony using PAUP*. Searching of tree space was performed with the heuristic tree search algorithm of PAUP*, with ACCTRAN character state optimization, and gaps treated as missing data. Relative support for clades was estimated using bootstrap analysis (1000 replicates with full heuristic searches). Phylogenetic trees produced by PAUP were viewed and manipulated with FigTree v. 1.2.2 (Rambaut 2009).

The data sets and phylogenetic trees generated in this study have been (will be) deposited in TreeBASE (?? accession numbers).

RESULTS

Sequence Variation in the *Dichanthelium acuminatum* Complex–The GBSSI data set generated in this study for the *D. acuminatum* complex consisted of a total of 1,203 aligned nucleotides–from translated exon 6 through exon 13 and intron 7 through intron 13–with the majority being intron sequence. A total of 67 characters were variable among the sequences of the *D. acuminatum* group, and 46 of these were phylogenetically informative. Fourteen indels (ranging from 1 to 15 bp) were inferred with minimal difficulty in aligning ingroup sequences. One primer pair (E2-for/F2f-bac) did not produce an amplification product for subsp. *sericeum*, resulting in 138 bp of missing data for this taxon.

An extended GBSSI data set was created to analyze the phylogenetic relationships of specimens of the *D. acuminatum* complex to two taxa of the closely related *D. ovale* subspecies complex: *D. ovale* subsp. *praecocius* and *D. ovale*. subsp. *villosissimum*. The extended data set had a total of 75 variable characters with 47 of these being phylogenetically informative.

Phylogenetic Analyses–Phylogenetic analysis of the GBSSI sequences of the *D. acuminatum* complex resulted in six equally parsimonious trees with the majority-rule consensus shown in Fig. 31 (tree length = 79, CI = 0.85, RI = 0.92). This analysis includes all of the taxa of the *D. acuminatum* subspecies complex, except for subsp. *leucothrix* (not able to obtain DNA sequence data). The majority-rule consensus tree provides little basal resolution but does provide relatively high bootstrap support (> 50%) for the terminal clades.

Phylogenetic analysis of the extended GBSSI data set (*D. acuminatum* complex plus *D. ovale* subsp. *villosissimum* and *D. ovale* subsp. *praecocius*) resulted in six equally parsimonious trees with the majority-rule consensus shown in Fig. 32 (tree length = 89, CI = 0.84, RI = 0.91). The majority-rule consensus tree again provides little basal resolution but does provide relatively high bootstrap support (> 50%) for the terminal clades.

DISCUSSION

The majority-rule consensus tree (Fig. 31) provides a partially-resolved phylogenetic hypothesis for the nine taxa (no data for subsp. *leucothrix*) of the *D. acuminatum* complex. Three of these taxa, subsp. *acuminatum*, subsp. *lindheimeri*, subsp.

fasciculatum are placed into well-resolved monophyletic taxon groups, which correspond to the hypothesis being tested in the present study. Two morphologically similar taxa, subsp. *longiligulatum* and subsp. *spretum*, are grouped together into a monophyletic clade and are well-resolved from each other by a high bootstrap value. The remaining four taxa, subsp. *columbianum*, subsp. *implicatum*, subsp. *sericeum*, and subsp. *thermale* are not well-resolved by analysis of the present GBSSI data set.

As mentioned earlier the goal of the molecular phylogenetic analysis was to provide an estimation of genetic divergence among the taxon groups as circumscribed by the most recent morphology-based taxonomic treatment (Freckmann & Lelong 2003), which is being tested in this study. Multiple accessions were sampled for each taxon except for subsp. *implicatum*, for which molecular data for only one accession was generated, and for subsp. *leucothrix*, for which molecular data were not obtained. For purposes of this study genetic divergence was considered significant when multiple accessions of a putative taxon formed monophyletic clades (= evolutionarily significant units) in the majority-rule consensus tree with at least 50% bootstrap support (Figs. 31 and 32). Similar methodology has been employed in other plant and algal taxonomic groups to initially delineate lineages or evolutionarily significant units (for example, Baldwin 2000; Verbruggen et al., 2007).

Discussion and interpretation of the results for each subspecific taxon follows. Individual plant specimens are referenced by the collector and collection number (e.g., *Hammer 212*) and individual specimens collected as part of a population sample are

referenced with collector, population number, and specimen number (e.g., *Hammer 1-12* = specimen number *12* from population number *1*).

D. acuminatum* subsp. *acuminatum–Specimens of subsp. *acuminatum* appear in four separate monophyletic clades (A, B, C, and D) in Fig. 31. Clade "A" consists of three accessions of subsp. *acuminatum* (*Hammer 4-7*, *1-12*, and *308*) in Fig. 31. All three of the accessions are from different Texas counties. Bootstrap support for the clade is high at 99%. Additionally, all three of the accessions in clade "A" share four unambiguous GBSSI mutations at phylogenetically informative sites that are not shared with any other specimens in the data matrix. Morphologically, specimens *1-12* and *4-7* would be considered "typical" (in terms of pubescence) for subsp. *acuminatum* while specimen *308* would be morphologically "atypical" (= more pubescent). Specimen *308* has no GBSSI mutations at phylogenetically informative sites in common with the two "atypical" specimens (*Hammer 212* and *389*; clade "B" Fig. 31) in the data matrix and this is possibly due to allele segregation.

However, subsp. *acuminatum*—as represented in this study by populations sampled from the field and from herbarium specimens—as a whole is more complicated both at the molecular and morphological level. Recall from the discussion of the morphological analysis of subsp. *acuminatum* from Chapter II that there were three field-collected populations and several additional field-collected specimens that exhibited variability in several morphological characters that is atypical for subsp. *acuminatum*. Specimens representative of these three populations—*Hammer* populations *8, 15*, and *17*—were more pubescent than specimens from typical subsp. *acuminatum* populations—*Hammer*

populations *1* and *4*—and this divergence was quantified and supported statistically (see PCoA Figs. 25-27) using the following characters: peduncle hair length, peduncle length, and leaf sheath hair length. The two specimens from clade "B" (*389* and *212*, Fig. 31) are representative of this atypical morphology and will be discussed further shortly.

Attempts to obtain sequence data from specimens from populations *8*, *15*, and *17* were not successful due to either multiple PCR bands or multiple alleles (sequence polymorphism) that were detected in the DNA sequence for some primer pairs. Fig. 33 shows the gel electrophoresis results for primer pair E2f+G2b1 for several specimens from these populations. The heterogeneity observed in the PCR products of these populations (Fig. 33: multiple PCR bands for specimens *17-6* and *24-10*; and multiple alleles for specimens *8-13* and *15-10*) may possibly be the result of a hybrid swarm or hybrid. Follow-up studies are planned to revisit the population locations to confirm the persistence of each population. If these populations have persisted, plans are to make extensive field collections for morphological and molecular study and to survey for the putative parental taxa (subsp. *acuminatum* and possibly a member of the *D. ovale* complex).

Specimens of similar morphology (*Hammer 212*, *308*, and *389*) to those of populations *8*, *15*, and *17* (but not members of these populations) were collected in the field. PCR analysis of these three specimens revealed no sequence polymorphism in the GBSSI fragments analyzed and amplification products, which showed single bands on the gels. GBSSI sequence data from specimens *212*, *308*, and *389* was added to the data

matrix used in the phylogenetic analysis just presented for the *D. acuminatum* complex (Fig. 31). In addition, GBSSI sequence data was included for two specimens from the *D. ovale* subspecies complex: one from *D. ovale* subsp. *villosissimum* and *D. ovale* subsp. *praecocius*. The *D. ovale* specimens were included in the analysis on the basis of the author's opinion that the atypical subsp. *acuminatum* specimens showed similarity in several morphological characters when compared to the two *D. ovale* subspecies. The species distributions of subsp. *acuminatum* and *D. ovale* subsp. *villosissimum* overlap in eastern Texas so the potential for gene flow between the taxa exists. Results of this extended phylogenetic analysis are presented in Fig. 32. Two of the atypical specimens of *D. acuminatum* subsp. *acuminatum* (*212* and *389*) along with the two specimens of subsp. *sericeum*, which comprised clade "B" in Fig. 31 are now grouped into a clade with the *D. ovale* specimens in Fig. 32 (however, bootstrap support is < 50%). Morphological analysis of the "*D. acuminatum* subsp. *acuminatum*/*D. ovale*" complex combined with the phylogenetic results of Fig. 32 is insightful and is presented next.

Specimens of subsp. *acuminatum* which were part of the GBSSI data matrix (*1-12, 4-7, 212, 308,* and *389*) were combined with the specimens from subsp. *acuminatum* field-collected populations (populations *1, 4, 8, 15,* and *17*), subsp. *acuminatum* herbarium specimens, a field-collected population of *D. ovale* subsp. *villosissimum,* and one herbarium specimen of *D. ovale* subsp. *villosissimum* (*SP11,* representing the GBSSI sequence in Fig. 32) to create a morphological data matrix of 138 total specimens which was analyzed using principal coordinates analysis (PCoA) identical to that of Chapter II. Figs. 34-36 present the graphical results of the PCoA and Tables 23 and 24 present the

eigenvalues from the PCoA and the Spearman's correlation values between the morphological characters, respectively.

When considered together, the results of the phylogenetic analysis (Fig. 32) and the results from the multivariate analysis of morphological data provide preliminary data for explaining the atypical morphology of subsp. *acuminatum* populations *8*, *15*, and *17*. Fig. 37 shows an annotated version of the PCoA plot for axis 1/axis 2 (Fig. 34) which includes taxonomic boundaries between *D. ovale* subsp. *villosissimum* and *D. acuminatum* subsp. *acuminatum* specimens, Spearman's correlation values for the two most informative characters for axis 1 and axis 2, and labels for the symbols that represent specimens which are included in molecular phylogenetic analysis of Fig. 32.

In Fig. 37 specimens of the "atypical" populations cluster on the upper right-hand corner of the plot while specimens from the typical subsp. *acuminatum* populations cluster in the lower right-hand section of the plot. Note that labeled specimens *212* and *389*, which are the morphologically atypical specimens, clearly cluster with the specimens from the atypical populations and specimen *308*, with typical or characteristic subsp. *acuminatum* morphology, also clusters with this same atypical group. Specimens *1-12* and *4-7*, with typical or characteristic subsp. *acuminatum* morphology, cluster in the lower right-hand corner of the plot with the other typical specimens. Specimens of *D. ovale* subsp. *villosissimum* cluster in the mid to upper left-hand side of the plot with specimen *SP-11* representing the GBSSI sequence for *D. ovale* subsp. *villosissimum* in Fig. 32.

The PCoA analysis of morphological data summarized in Fig. 37 and phylogenetic analysis presented in Fig. 32 provide preliminary evidence that the atypical populations of subsp. *acuminatum* are possibly of hybrid origin, with introgressed genes from *D. ovale* subsp. *villosissimum* or *D. ovale* subsp. *praecocius*. Further analysis of the intermediate populations, in the form of cloning and sequencing the multi-band and multiple-allele PCR products from *Hammer* populations *8, 15*, and *17*, should be pursued to further elucidate the genetic relationships of these populations to subsp. *acuminatum*. The major question to be answered is whether these populations are distinct evolutionarily significant units apart from the typical subsp. *acuminatum* specimens. If so, these populations should be recognized as distinctive from a taxonomic standpoint, and segregated at the subspecific level in the *D. acuminatum* complex or as a new species of *Dichanthelium*. Sufficient diagnostic macromorphological variability exists to allow a practical floristic separation of these lineages.

D. acuminatum* subsp. *lindheimeri–The five accessions of subsp. *lindheimeri* form a relatively well-supported clade with a bootstrap value of 68% in Fig. 31 (68% in Fig. 32). However, the six specimens share only one GBSSI mutation at a phylogenetically informative site not shared by other specimens in the data matrix. Five of the specimens (*SP16, Hammer 343, Hammer 292, Hammer 3-14*, and *Hammer 19-8*) all share an identical deletion sequence (TGCGGCGAGCAATGT) beginning at position 656 in the GBSSI alignment. The other subsp. *lindheimeri* specimen (294) has a deletion identical to the first 10 base pairs of the 15 base pair deletion, then contains the next four base pairs, then has the 15th base pair missing. This 15 base pair deletion sequence is present

with some minor polymorphism in all of the non-subsp. *lindheimeri* specimens in the data matrix except for subsp. *spretum* specimen S1 which shares the identical 15 base pair deletion.

The accessions represent specimens collected from three counties in Texas, one parish in Louisiana, and one county in Wisconsin, which demonstrates the phylogenetic cohesiveness of this subspecies on a broad geographic scale, albeit from limited sampling and molecular analysis. Further field collection and molecular analysis from a broader geographic sampling are needed to further substantiate the phylogeography of this subspecies.

D. acuminatum* subsp. *sericeum–As noted in the results section above, there are 138 base pairs of data absent from the GBSSI data matrix for the two specimens of subsp. *sericeum*. This equates to five missing phylogenetically informative sites out of a total of 47 for the aligned data matrix. The missing sequence data is from primer pair E2f (forward) and F2b (reverse). Approximate amplicon size for E2f+F2b is 200 bp. PCR analysis with this primer pair for both of the two subsp. *sericeum* specimens actually produced faint bands on the gel. The amount of PCR product was judged to be insufficient for sequencing. There was a considerable size difference in the subsp. *sericeum* bands, which were approximately 600-700 bp in length, compared to the uniform length of about 200 bp in the other *D. acuminatum* subspecies.

In the phylogenetic analysis of Fig. 31 (*D. acuminatum* complex, no *D. ovale* specimens) the two accessions of subsp. *sericeum* form a monophyletic pair that forms a sister clade with the two "atypical" accessions of subsp. *acuminatum* (*Hammer 212* and

Hammer 389). In the phylogenetic analysis shown in Fig.32, which includes *D. ovale* subsp. *villosissimum* and *D. ovale* subsp. *praecocius* specimens, the two subsp. sericeum specimens are grouped into a clade that includes the *D. ovale* specimens and the atypical subsp. *acuminatum* specimens (*212* and *389*). However, the bootstrap value is less than 50% for the basal portion of this clade. The two specimens of subsp. *sericeum* share some morphological similarities with both the atypical subsp. *acuminatum* populations and the subsp. *acuminatum Hammer 212* and *389* specimens. These shared morphological similarities may signal some underlying genetic affinities, which are being revealed in the phylogenetic analysis.

From a molecular genetic standpoint the relationship of subsp. *sericeum* to that of the other subsp. in the *D. acuminatum* complex remains unresolved at present. Further work should focus on obtaining sequence data for the 138 base pair region (primers E2 f+F2b) that is missing from the GBSSI data for this taxon. As discussed at the beginning of this section, this region of the GBSSI gene appears to be approximately 500 bp longer in subsp. *sericeum* when compared to the same region for the other members of the *D. acuminatum* complex. Substantiation of this size difference from molecular analysis of additional subsp. *sericeum* specimens alone would provide evidence for genetic differentiation when considered from a non-phylogenetic perspective.

D. acuminatum* subsp. *fasciculatum–Three specimens of subsp. *fasciculatum*, *Hammer 230-1, SP10,* and *A1* plus a specimen of subsp. *columbianum, C1,* form a monophyletic clade in Fig. 31. However, there is weak support (bootstrap value of 63%) for grouping the subsp. *columbianum* specimen with the three subsp. *fasciculatum*

specimens. With respect to the subsp. *fasciculatum* specimens there is strong support for grouping them together (bootstrap value of 87%). All three subsp. *fasciculatum* specimens share three unambiguous GBSSI mutations at phylogenetically informative sites that are not shared with any other specimens in the data matrix. The subsp. *columbianum* specimen (*C1*) shares one GBSSI mutation at one phylogenetically informative site with the three subsp. *fasciculatum* specimens that is not shared with any other specimens in the data matrix. Overall, there is weak support for including the subsp. *columbianum* specimen in the monophyletic clade with the three subsp. *fasciculatum* specimens.

It should be noted that subsp. *fasciculatum* is the most widely distributed taxon in the *D. acuminatum* complex with specimens representing 33 states included in the morphological part of this study (Ch. 2). Freckmann & Lelong (2003) define the geographic distribution of subsp. *fasciculatum* as essentially the eastern half of the United States, overlapping the much smaller distribution of subsp. *columbianum*. Given these distributions there would certainly be opportunity for gene flow between these two subspecies and the shared phylogenetically informative site between the three subsp. *fasciculatum* specimens and the single subsp. *columbianum* specimen could certainly have resulted from such interaction. Given this, it is difficult to *not* regard the three subsp. *fasciculatum* specimens in this clade as an evolutionarily significant unit and representative of the genetic distinctiveness of this taxon from the other taxa in the *D. acuminatum* complex.

D. acuminatum* subsp. *longiligulatum* + subsp. *spretum–The three specimens of subsp. *longiligulatum*, *Hammer 206, Hammer 214,* and *Hammer 5-12* and the two specimens of subsp. *spretum, S1* and *SP5,* form a monophyletic clade in Fig. 31. Bootstrap support for this clade is 62%. The three subsp. *longiligulatum* specimens form a subordinate clade to the subsp. *spretum* specimens with a high bootstrap value of 98%. The three subsp. *longiligulatum* specimens and two subsp. *spretum* specimens all together share two unambiguous GBSSI mutations at phylogenetically informative sites not shared by any other specimens in the GBSSI data matrix. One individual specimen of subsp. *spretum* (*SP5*) and the three subsp. *longiligulatum* specimens are closely related genetically as they share four GBSSI mutations at phylogenetically informative sites that are not shared by any other taxa in the data matrix. This is a disjunct group spatially and temporally as the subsp. *spretum* specimen (*SP5*) was collected in 1908 in Massachusetts and the three subsp. *longiligulatum* specimens were all collected in 2005 in Texas (*214*, *5-12*) and Louisiana (*206*). Finally, the three subsp. *longiligulatum* specimens share three GBSSI mutations at phylogenetically informative sites not shared by other taxa.

As a group, the three subsp. *longiligulatum* specimens and the two subsp. *spretum* specimens constitute an evolutionarily significant unit (ESU) in relation to the other specimens analyzed in the GBSSI dataset. However, the subsp. *longiligulatum* specimens cannot be resolved from the subsp. *spretum* specimens with the current data. This is not surprising from a morphological standpoint since variation between the two taxa is almost cryptic, being separated morphologically by only panicle width in

published keys. With one subsp. *spretum* specimen (*SP5*) more closely related to the three subsp. *longiligulatum* specimens than to the other specimen of subsp. *spretum*, this possibly calls into question how representative these subsp. *spretum* specimens are, at least genetically. For example, subsp. *spretum* specimen *S1* shares two phylogenetically informative sites with some of the subsp. *lindheimeri* specimens, suggesting possible gene flow between specimen *S1* and sympatric populations of subsp. *lindheimeri*. Specimen *S1* was collected in Trinity County, TX and subsp. *lindheimeri* would certainly be expected in this area.

Results of phylogenetic analysis of the current GBSSI data only allow recognition of an ESU containing both subsp. *longiligulatum* and subsp. *spretum* together. Additional specimens representing both subsp. *longiligulatum* and subsp. *spretum* need to be examined to further resolve the taxonomic relationship between these two taxa and their appropriate taxonomic relationship to the other taxa of the *D. acuminatum* complex.

Fig. 31 Clade "C" (subsp. *acuminatum*, subsp. *fasciculatum*, subsp. *columbianum*)–This monophyletic clade from Fig. 31 is somewhat of an enigma, at least initially. Three specimens make up the clade—*Hammer 302*, *SP8*, and *SP14*—and these have not been treated so far in the discussion. These three specimens share seven GBSSI mutations at phylogenetically informative sites that are not shared by any other specimens in the data matrix and this provides strong support (bootstrap support of 98%) for grouping them together as an evolutionarily significant unit. Yet, identifications based on traditional morphological characters place each specimen as a different subspecies in the *D. acuminatum* complex.

Both specimen *SP8* (subsp. *fasciculatum*) and specimen *SP14* (subsp. *columbianum*) were collected in Wisconsin in separate counties while specimen *302* was collected in Liberty County, TX. Thus, gene flow could explain the close genetic similarity between the two Wisconsin specimens but not the Texas specimen. The two Wisconsin specimens (*SP8* and *SP14*) have identical sequences in the GBSSI alignment and may likely represent the same taxon or lineage despite some morphological differences. The Texas specimen (*302*), which is more pubescent than the Wisconsin specimens, undoubtedly exhibits morphological traits of subsp. *acuminatum,* and from a morphological standpoint, is properly classified as such. However, the subsp. *acuminatum* traits in this specimen (*302*) appear to be attributable to gene flow from members of the *D. ovale* subspecies complex.

The GBSSI sequence alignment provides several lines of preliminary evidence supporting possible gene flow from the *D. ovale* subspecies complex into the subsp. *acuminatum* specimen (*302*). First, specimen *302*, specimen *SP11* (*D. ovale*. subsp. *villosissimum*), *SP12* (*D. ovale* subsp. *praecocius*), specimen *212* (subsp. *acuminatum*), and specimen *389* (subsp. *acuminatum*) all have matching sequences for a four base pair deletion (ATGC) that is missing from all other specimens in the GBSSI alignment. Indels were ignored in the phylogenetic analysis but the presence of this indel suggests the possibility of gene flow between specimens *302*, *212*, and *389* and members of the *D. ovale* subspecies complex previously mentioned. Note that subsp. *acuminatum* specimens *212* and *389* were discussed earlier as part of the discussion for the subsp. *acuminatum* clade or ESU, and that preliminary support for gene flow between *212* and

389 was established from morphological and gene sequence data. Second, specimens *302*, *SP11* (*D. ovale*. subsp. *villosissimum*), and *SP12* (*D. ovale* subsp. *praecocius*) share unambiguous GBSSI mutations at two phylogenetically informative sites that are not shared by any other specimens in the data matrix. Together, evidence from the GBSSI indel and the two shared informative sites, may provide preliminary evidence to view specimen *302* as a product of hybridization. The pubescent morphological or phenotypic traits for this specimen appear to be derived from gene flow from the *D. ovale* subspecies complex, while genotypically this specimen is closely related to specimens *SP8* and *SP14*, both of which appear to represent the morphologically puberulent subsp. *columbianum*. It is emphasized that whether or not specimen *302* is a product of hybridization is at best speculative until further genetic data (preferably from another single or low copy nuclear gene) can be obtained and analyzed.

As mentioned earlier the three specimens that form this monophyletic clade are strongly supported by seven shared phylogenetically informative sites. The geographic separation of these specimens is noteworthy as well: two specimens from Wisconsin and one specimen from Texas. From a phylogeographic perspective it would be interesting to further explore the extent of this ESU in North America. It would also be interesting to attempt to further resolve the possible hybrid status of specimen *302* with data from another low-copy nuclear gene. Further sequencing of the GBSSI locus from specimens obtained from the location where specimen *302* was collected (Marysee Prairie Preserve, Liberty County, TX) would be a logical follow-up to extend the present research as well.

Fig. 31 Clade "D" (subsp. *implicatum*, subsp. *acuminatum*, subsp. *thermale*)–This monophyletic clade is composed of specimen *SP23* (subsp. *implicatum*), specimen *TR9* (subsp. *acuminatum*), specimen *SP3* (subsp. *thermale*) and specimen *SP2-1* (subsp. *thermale*). Fig 32, which extends the phylogenetic analysis of Fig. 31 with additional specimens, adds specimen *221* (subsp. *acuminatum*) to the clade of Fig. 31. Together, all four specimens share three phylogenetically informative sites not shared by other specimens in the GBSSI data matrix. This yields a monophyletic clade with a bootstrap value of 93%. The spatial distribution of the four specimens is somewhat helpful in understanding what appears to be an unnatural grouping of three subspecies in this clade.

Three of the California specimens, *TR9* (subsp. *acuminatum*, Monterey County), *SP2-1* (subsp. *thermale*, Lassen Volcanic National Park, Lassen County), and *SP3* (subsp. *thermale*, Lassen Volcanic National Park, Lassen County) were collected in California. Both subsp. *acuminatum* and subsp. *thermale* are morphologically similar with pubescent culms and leaves. However, subsp. *thermale* is restricted to the hot clay soils of Lassen County, CA. The GBSSI sequences of the two specimens of subsp. *thermale* are identical while the sequence of the subsp. *acuminatum* specimen differs by only one base (a transition of G to A) at position 1173 (a non-phylogenetically informative site) in the GBSSI alignment. Other specimens of subsp. *acuminatum* from this geographic region need to be sequenced to determine the extent of sequence divergence between these two taxa. The status of subsp. *acuminatum* in California and subsp. *thermale* as distinct evolutionarily significant units or lineages cannot be determined with only three

specimens. The main morphological difference between the two is the degree of panicle exsertion.

The remaining two specimens of this clade, *Hammer 221* (subsp. *acuminatum*, Hardin County, TX) and *SP23* (subsp. *implicatum*, Saline County, AR) have identical GBSSI sequences. Additional specimens of subsp. *implicatum* need to be sequenced and included in the genetic analysis to be able to resolve the phylogenetic status of this taxon relative to the other taxa of the *D. acuminatum* complex.

D. wrightianum–This taxon was included in the phylogenetic analysis for comparative purposes since *D. wrightianum* has traditionally been included as a subspecies of *D. acuminatum*. The most current treatment of the group (Freckmann & Lelong 2003) treats this taxon as a distinct species apart from the *D. acuminatum* complex, based primarily on *D. wrightianum*'s smaller spikelet length.

The two specimens of *D. wrightianum* included in the analysis, *TR7* (Polk County, TX) and *Hammer 216* (Montgomery County, TX) share four GBSSI mutations at phylogenetically informative sites that are not shared by any other specimens in the data matrix, with associated high bootstrap support of 99%. Thus, based on the two specimens in the current data matrix, *D. wrightianum* represents a distinct evolutionarily significant unit (ESU) when compared to the other taxa in the data matrix.

The question of whether or not *D. wrightianum* should be treated as a separate species from the taxa of the *D. acuminatum* complex is not satisfactorily resolved by the results of the phylogenetic analysis of the GBSSI locus. *Dichanthelium wrightianum*'s status as an ESU is unquestioned (but could be strengthened by the inclusion of more specimens

beyond Texas). However, when comparing the ESUs in terms of GBSSI mutations at phylogenetically informative sites unique to a specific ESU, clade "C" of Fig. 31 (most likely representing subsp. *columbianum*) had seven such sites compared to four unique sites for both *D. wrightianum* and *D. acuminatum* subsp. *acuminatum* (clade "A" in Fig. 31). From this observation a legitimate case could be made for elevation of subsp. *columbianum* to species level based solely on genetic data. But, from a morphological standpoint, there may not be sufficient discernable differentiation from subsp. *fasciculatum* (and reportedly from some specimens of subsp. *lindherimeri*) to make such a segregation practical for floristic use. Baldwin (2000) makes a valid point that, "adherence to the belief that plant systematics is a science that seeks to discern real entities of nature, i.e., evolutionary groups, dictates that plant taxonomy should reflect rigorous hypotheses of relationship rather than convenient but artificial oversimplistic assemblages." In terms of "macromorphological diagnosability" (Baldwin 2000), the reduced spikelet length of *D. wrightianum*, when compared to the taxa of the *D. acuminatum* complex, likely offers sufficient justification for segregation.

Summary of Conclusions From Phylogenetic Analysis of GBSSI Sequence Data– Monophyletic clades or lineages resulting from the phylogenetic analysis of GBSSI data which are appropriately recognized as evolutionarily significant units (ESUs) are: *D. acuminatum* subsp. *acuminatum*, *D. a*. subsp. *fasciculatum*, *D. a*. subsp. *lindheimeri*, *D. a*. subsp. *longiligulatum* + *D. a*. subsp. *spretum*, and *Dichanthelium wrightianum*. Table 25 summarizes this data for the 10 subspecies comprising the *D. acuminatum* complex (*sensu* Freckmann & Lelong 2003). Note that neither subsp. *longiligulatum* (as per the

discussion above) nor subsp. *spretum* could be resolved individually, but taken together they do constitute an evolutionary significant unit.

CHAPTER IV

HYBRIDIZATION IN *DICHANTHELIUM*

INTRODUCTION

According to Lelong (1986) *Dichanthelium acuminatum* "is probably the most polymorphic and troublesome species in the genus." The difficult and confusing synonomy and circumscription is the result of extensive morphological variation found among members of the complex (Shinners 1944, Freckmann 1981, Lelong 1986). Lelong (1965) studied a number of taxa in *Dichanthelium* including the *acuminatum* complex and concluded that hybridization most likely played a major role in obscuring taxon boundaries in these groups. Spellenberg (1975) studied western U.S. populations of some of the subspecies and proposed that autogamy and hybridization are a common means that account for much of the morphological variation and thus the taxonomic difficulty encountered in the complex.

This study was undertaken as an attempt to document putative hybridization among members of the *D. acumiantum* complex and of the genus, given its likely evolutionary role. To the author's knowledge there has been no study to document hybridization at the molecular level for this group of plants. A major impetus for this work was the discovery of a "ready-made" molecular marker in subsp. *lindheimeri* from DNA sequence data (see molecular phylogenetic analysis in Chapter III). Lindheimer's rosettegrass is one of the more common taxa in the group and is easily found in the field,

often growing sympatrically with other species of *Dichanthelium* taxa, adding to the utility of this taxon for studies of hybridization.

DNA sequencing of the nuclear granule-bound starch synthase gene (GBSSI or *waxy*) in the *D. acuminatum* subspecies complex and other *Dichanthelium* species for the molecular genetic portion of this dissertation provided preliminary sequence data for this study. The GBSSI gene has shown its utility for phylogenetic studies in plants (e.g., Mason-Gamer et al. 1998; Aliscioni et al. 2003) and the introns in GBSSI have been shown to be variable at lower taxonomic levels (Mason-Gamer et al. 1998; Small 2004). The GBSSI gene was first characterized by van der Leij et al. (1991) with gene structure consisting of one untranslated and 13 translated exons, with gene structure appearing to be conserved. Sequence data from the intron region between exon 10 and exon 11 (using "F-for" and "K-bac" primers from Mason-Gamer et al. 1998) revealed a 15 bp deletion found only in subsp. *lindheimeri* thus far. The nucleotide sequence of the 15 bp stretch can be determined from the GBSSI alignment (see the molecular phylogenetic study in Chapter III) and is 5'-TGCGGCGAGCAATGT-3'.

DNA sequencing of numerous individuals of subsp. *lindheimeri* revealed that the 15 bp deletion is homozygous. This provides a serendipitous molecular marker useful to indicate the haploid presence of subsp. *lindheimeri* in a particular *Dichanthelium* diploid genome. Given the possibility that hybridization with other species in the genus has contributed to the intraspecific and intra-population morphological variation observed in the complex (and in grasses in general) it seemed worthwhile to develop a molecular marker based on the subsp. *lindheimeri* deletion and to use the marker to analyze DNA

samples collected from *Dichanthelium* populations collected in the wild. A putative hybrid individual would contain one copy of the GBSSI gene with the 15 bp deletion and one copy of the gene that does not contain the deletion at this locus. For field surveys of numerous individual plants it would be too costly and laborious to sequence the GBSSI region from each plant specimen to determine presence or absence of the deletion. Thus, a less-laborious and less-expensive non-sequence-based method was needed in order to take advantage of this marker. Analysis of the DNA sequence data in the fragment amplified with the F-f and K-b primers (Table 22) revealed a DNA restriction site for the restriction endonuclease *Fnu*4HI (*Fusobacterium nucleatum)* within the GBSSI region containing the 15 bp deletion. This finding presented the opportunity to use a relatively fast and inexpensive technique of genetic analysis called Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to assay for presence or absence of the marker.

MATERIALS AND METHODS

Marker Development–An alignment of GBSSI sequence data representing the majority of taxa in the *D. acuminatum* complex was analyzed for single nucleotide polymorphisms (SNPs) using the software SNP2CAPS (Thiel et al. 2004). The results of this analysis identified a restriction site for the restriction enzyme *Fnu*4HI within the 15 bp deletion identified in subsp. *lindheimeri* specimens. The SNP2CAPS analysis indicated no other *Fnu*4HI restriction site within approximately 100 bp upstream or downstream from the position of the15 bp deletion. Based on this information PCR primers (L1f and L4r in Table 22) were designed using the software program OligoCalc

(Kibbe 2007) which flanked the deletion site to amplify an approximately 200 bp fragment from the GBSSI gene.

The SNP2CAPS software was also used to digest the representative sequences of the *D. acuminatum* complex *in silico* with *Fnu*4HI to identify expected genotypes. From this analysis three *Fnu*4HI genotypes were identified along with the predicted number of restriction fragments and their (*in silico*) lengths. Genotypes are:

1. homozygous subsp. *lindheimeri*: 1 fragment of 187 bp
2. homozygous non-subsp. *lindheimeri*: 2 fragments, 106 bp and 96 bp
3. heterozygous subsp. *lindheimeri* + non subsp. *lindheimeri* : 4 fragments, 202 bp heteroduplex fragment, 187 bp fragment from subsp. *lindheimeri* parent; 106 bp and 96 bp fragments resulting from restriction of the 15 bp indel sequence present in the non subsp. *lindheimeri* parent.

The third *Fnu*4HI genotype above (heterozygote) is actually deduced from knowledge of the restriction patterns of genotypes 1 and 2. The heteroduplex fragment present in the heterozygote post-restriction reaction pool arises from annealing of some of the 187 bp fragments from the subsp. *lindheimeri* parent to complementary strands (post-restriction) of both the 106 bp and 96 bp fragments from the non subsp. *lindheimeri* parent. This heteroduplex fragment does not cut with *Fnu*4HI because of its hybrid nature. A single-stranded loop is formed on the 187 bp subsp. *lindheimeri* fragment when it encounters the DNA sequence for the 15 bp deletion which is present on parts of the 106 bp and 96 bp fragments. This partial single-stranded character of the heteroduplex molecule affects its electrophoretic mobility during gel electrophoresis.

Plant Material–A small portion of leaf tissue from a single individual of subsp. *lindheimeri* or other *Dichanthelium* species found in the same field population as subsp. *lindheimeri* was removed and placed in a 1.5-mL microcentrifuge tube containing desiccant material (t.h.e. dessicant, EM Science) The entire plant was collected and preserved as a voucher specimen. Voucher specimens were deposited at TAES. A total of 91 plants were collected and sampled from 14 populations in southeast and east Texas during the spring of 2005 and 2006.

Genomic DNA Isolation–DNA was extracted from leaves of individual plant specimens by a simple, micropreparation method. Fresh leaf tissue (1 cm2) was placed in a 1.5-mL microcentrifuge tube and macerated using a Teflon pestle (VWR, West Chester, Pennsylvania, USA) attached to a power-drill. After the addition of 0.5 mL of extraction buffer (200 mmol/L Tris pH 7.5, 250 mmol/L NaCl, 25 mmol/L EDTA pH 8, 0.5% SDS), tissues were further hand-macerated for 10 sec. After brief centrifugation to remove intact solids, nucleic acids were precipitated with the addition of 0.5 mL of isopropyl alcohol. After centrifugation for 5 min at 16 000 3 *g*, the pellet was resuspended in 0.5 mL 50 mmol/L Tris pH 7.5, 10 mmol/L EDTA, then briefly centrifuged to remove undissolved solids. Following addition of NaOAc pH 5.2 to a final concentration of 0.3 mol/L, nucleic acids were again precipitated with 0.5 mL of isopropyl alcohol. After further centrifugation, the nucleic acid pellet was resuspended in 0.1 mL of 10 mmol/L Tris pH 7.5, 1 mmol/L EDTA.

PCR Amplification–Nuclear *Waxy* fragments were amplified using primers "L1f" and "L4r". Polymerase chain reaction (PCR) was performed in 25 mL total reaction

volumes of 5 ng DNA template, 12.5 mL Go-*Taq* Green PCR Master Mix (Promega) and 0.25 mM of each primer. PCR cycling conditions were: 1 cycle of 2 min @ 94 °C of initial denaturation, followed by 35 cycles of denaturation at 94°C, primer annealing at 65°C for 30 sec, primer extension at 72°C for 2 min. A final extension step consisted of one cycle of 10 min @ 72 °C. PCR products were electrophoresed on 1.5% agarose TBE gels, stained with ethidium bromide and visualized under UV light to verify amplification products.

Restriction Analysis–Successful PCR amplifications were digested for 1 hr @ 37 °C with the restriction enzyme *Fnu*4HI (New England BioLabs) in 10 µL total reaction volumes of 0. 5 µL Fnu4HI, 1.0 µL 10X buffer, 5.0 µL of *waxy* PCR product, and 3.5 µL reaction grade water. Digestion products were electrophoresed on 4% agarose TBE gels, stained with ethidium bromide and visualized under UV light to reveal banding patterns.

RESULTS

Fig. 38 shows the results of the restriction analysis of the 91 plants examined, representing subsp *lindheimeri* and other *Dichanthelium* specimens. For illustration the three predicted *Fnu*4HI genotypes are shown together in gel "D" (far right side of gel) of Fig. 38: specimen 352 is *D. a*. subsp. *lindheimeri* which represents the homozygous subsp. *lindheimeri* genotype; specimens 303 and 328 represent the heterozygous subsp. *lindheimeri* + non subsp. *lindheimeri* genotype so are putative hybrids of subsp. *lindheimeri* with another subspecies of the *D. acuminatum* complex or species of

Dichanthelium; and specimen 353 is *D. a*. subsp. *acuminatum* which represents the homozygous non-subsp. *lindheimeri* genotype.

The results of the restriction analysis shown in Fig. 38 revealed the following genotypes from the 91 plant specimens that were screened: 55 specimens had a homozygous subsp. *lindheimeri* genotype, 34 specimens had a homozygous non-subsp. *lindheimeri* genotype, and 2 specimens had a putative heterozygous subsp. *lindheimeri* + non–subsp. *lindheimeri* genotype.

DISCUSSION

Molecular Evidence for Hybridization–The two putative hybrid specimens will be the focus of the discussion. The PCR-RFLP results for specimens *Hammer 303* and *Hammer 328* in Fig. 38 show the predicted GBSSI restriction fragment pattern for a diploid genotype with one copy of the locus contributed by a subsp. *lindheimeri* parent and the other copy of the locus contributed by a *Dichanthelium* parent other than subsp. *lindheimeri*. To confirm the hybrid status of these specimens DNA samples from both specimens were amplified using the F-for and K-bac primer pairs to produce a larger amplicon more suitable for direct sequencing, but which still contained the smaller amplicon amplified using the L1f+L4r primer pair. The resulting PCR amplifications were sequenced both forward and reverse using the F-for and K-bac primers (for protocol see Materials and Methods in Chapter III) and the sequence chromatograms were inspected for evidence of sequence heterogeneity in the form of multiple peaks along stretches of nucleotides at the location of the deletion. Fig. 39 shows the DNA sequence chromatogram for specimen 328, which displays the pattern of multiple peaks

that would be expected from sequence data produced from a PCR pool that contains a heterogeneous mixture of fragments, that is, some containing the 15 bp sequence and other fragments not containing this sequence. In Fig. 39 the secondary base peaks are labeled on both the forward and reverse strands and the sequences of both are in agreement with nucleotide sequence for the 15 bp deletion (TGCGGCGAGCAATGT). This same pattern of double peaks is present in the chromatograms for the other specimen identified as a putative hybrid, specimen 303, with the PCR-RFLP analysis. The GBSSI DNA sequence data provide confirmation that both specimen 303 and 328 are the products of hybridization between a *D. acuminatum* subsp. *lindheimeri* parent and another unknown species of *Dichanthelium*.

Morphological Evidence for Hybridization–It should be noted that the phenotypes for both specimens *303* and *328* exhibit a mostly typical subsp. *lindheimeri* morphotype but detailed morphological analysis revealed that they did not group with other subsp. *lindheimeri* specimens (Figs. 40 and 41). When collected by the author in the field these specimens were identified both as subsp. *lindheimeri*. However, as indicated by notes in the author's field collection log (entry for Apr. 28, 2006 for Ft. Boggy State Park), there was some doubt as to the exact identity for specimen 328: "subsp. *lindheimeri* or subsp. *fasciculatum*, lower culms fuzzy." Contributing to this noted pubescence, specimen 328 has one morphological character, a peduncle hair length of 2.3 mm, which is atypical in that subsp. *lindheimeri* specimens typically have little if any pubescence on the peduncle. In contrast, the other subsp. *lindheimeri* hybrid specimen (303) has no measurable pubescence on the peduncle. Specimens of subsp. *fasciculatum* typically

have a peduncle hair length of 1-2 mm (see Fig. 12B). Both specimens 303 and 328 share an additional character state that is atypical for subsp. *lindheimeri* in that both have an internode hair length of approximately 2.0 mm (1.9 mm for specimen 303 and 2.1 mm for specimen 328). Specimens of subsp. *lindheimeri* are typically glabrous along the culm internodes. Other than these exceptions to the typical morphotype of subsp. *lindheimeri*, both specimens *303* and *328* exhibit a predominant subsp. *lindheimeri* phenotype and their herbarium specimens are annotated accordingly (*Hammer 303* = TAES 246125; *Hammer 328* = TAES 246126).

The morphometric analysis carried out in Chapter II included a principal coordinates analysis (PCoA) of 389 specimens representing the *D. acuminatum* subspecies complex (Figs. 16-18). The two confirmed hybrid specimens of subsp. *lindheimeri*, specimens 303 and 328, were not included in that analysis. A new PCoA was generated by adding morphometric data for specimens 303 and 328 to the original dataset of 389 specimens. The results of this PCoA are presented in Figs. 40-41.

The PCoA results shown in Fig. 40 (axis 1/axis 2) show a definite divergence of the main cluster of subsp. *lindheimeri* specimens away from both specimens 303 and 328. Overall degree of pubescence increases from left to right across Fig. 40. In the axis 1/axis 3 plot of Fig. 41 specimens 303 and 328 both cluster between the glabrous subsp. *lindheimeri* group to the left and the pubescent subsp. *fasciculatum* specimens to the right-hand side. The PCoA results seem to support the hybrid status of the genotypes as confirmed by the PCR-RFLP results. In addition, the morphometric analysis provides preliminary evidence that subsp. *fasciculatum* might possibly be the second parent

contributing to the hybrid genotypes of specimens 303 and 328. However, the working hypothesis that specimens 303 and 328 have a "subsp. *lindheimeri* x subsp. *fasciculatum*" genotype would need to be corroborated by additional research. Specifically, PCR products should be cloned to deconstruct the GBSSI sequences into individual alleles. Complete sequences of one clone representing each allele could then by added to the GBSSI data matrix for phylogenetic analysis as was carried out in Chapter III.

CONCLUSION

First, this study has provided documentation of hybridization at the molecular level between subsp. *lindheimeri* and another taxon of the genus *Dichanthelium*. Multivariate morphometric statistical analysis of the specimen data has seemed to detect a statistically significant "hybrid signal" in the morphometric data set which has provided complementary evidence to support the molecular evidence of hybridization for specimens *303* and *328*. Second, and more generally, evidence for at least occasional outcrossing among subsp. *lindheimeri* and other members of the *D. acuminatum* complex has been demonstrated and reported.

CHAPTER V

TAXONOMIC TREATMENT AND SUMMARY

INTRODUCTION

This chapter presents a current taxonomic treatment of the *Dichanthelium acuminatum* complex based on examination of the results of the morphometric and phylogenetic analyses conducted in this study. As discussed in the introductory chapter, the aim of the present study was to evaluate and account for the meaningful morphological variation present in the *Dichanthelium acuminatum* complex, along with an estimation of the genetic divergence among the taxonomic entities of the complex as well. Table 25 summarizes the results of the molecular phylogenetic analysis and Table 26 summarizes the results of the multivariate statistical analysis of the morphological data. For the present study decisions on how to circumscribe the morphological variation present in the *Dichanthelium acuminatum* complex was based on two major considerations: 1) morphological diagnosability and where relevant, uniqueness of ecological habitat and 2) genetic distinctiveness as supporting evidence. An important item taken into consideration was the lack of adequate morphological and genetic data for several of the taxa studied.

As a starting point for the present study the most recent treatment of the *Dichanthelium acuminatum* complex (Freckmann & Lelong 2003) was used as the taxonomic hypothesis to be tested. Taxonomic identities for all herbarium specimens and field-collected specimens secured for the study were determined using the key presented

in Freckmann & Lelong (2003). In their treatment and resulting key, the morphological variation present in the complex was categorized using the *subspecies* infraspecific concept. Earlier treatments (Gould and Clark 1978; Freckmann 1981; Lelong 1986) circumscribed the morphological variation of the *Dichanthelium acuminatum* complex as different varieties or (selected taxa) as species. The number of taxa recognized varied among these treatments. Editorial policy for Flora of North America (FNA) taxonomic treatments required that infraspecific taxa be treated as subspecies as opposed to varieties, and as a result, Freckmann and Lelong (2002) created new nomenclatural combinations (as subspecies) for the infraspecific taxa of the *Dichanthelium acuminatum* complex in preparation of their treatment of the genus *Dichanthelium* for the Flora of North America. This is noted to make the point that the shift from *varietal* recognition to *subspecific* recognition of the infraspecific taxa of the *Dichanthelium acuminatum* complex was not done on the basis of taxonomic considerations. The appropriateness of this infraspecific taxonomic rank will not be debated at the present but may be pursued in the future as a more complete picture of the morphological and genetic relationships among the infraspecific taxa of the *Dichanthelium acuminatum* complex and their broader relationship to the species of the genus *Dichanthelium* becomes available.

A taxonomic treatment follows along with summary comments following each subspecies description.

TAXONOMIC TREATMENT AND SUMMARY

A Key to Subspecies of *Dichanthelium acuminatum* and Related Taxa [Modified from Freckmann & Lelong 2003]–Key is based on vernal plant material.

1. Spikelets 0.8–1.1 mm long, puberulent to subglabrous; culms delicate, 0.3–0.8 mm thick……...……***D. wrightianum***

1. Spikelets 1.1–3.0 mm long, variously pubescent; culms not delicate, usually more than 1 mm thick.

2. Spikelets 1.1–2.1 mm long; sheaths glabrous or pubescent with hairs to 3.0 mm long……....……***D. acuminatum***

3. Lower portion of culms and lower sheaths usually glabrous or sparsely pubescent.

4. Primary panicles contracted, more than twice as long as wide; spikelets ascending to appressed…...…...…….....….....subsp. ***spretum***

4. Primary panicles open, less than twice as long as wide; spikelets diverging to ascending.

5. Blades green or purplish, margins not conspicuously ciliate at the base; spikelets 1.1–1.5 mm long, usually ellipsoid ……..…….……....subsp. ***longiligulatum***

5. Blades often yellowish-green, margins usually with long papillose-based cilia at the base; spikelets 1.3-1.6 mm long, usually obovoid……..…….……subsp. ***lindheimeri***

3. Lower portion of culms and lower sheaths densely and variously pubescent or puberulent.

6. Culms 15-30 cm tall; midculm sheaths nearly as long as internodes; blades usually 2-6.5 cm long, less than 8 times longer than wide

…...…..…….……...……..……subsp. ***sericeum***

6. Culms usually 31-100 cm tall; midculm sheaths about ½ as long as internodes; blades usually 6-12 cm long, more than 8 times longer than wide.
    7. Culms and lower sheaths densely covered with spreading, villous hairs or soft, thin, papillose-based hairs, often with shorter hairs underneath; blades softly pubescent to velvety on abaxial surfaces.
        8. Primary panicles usually poorly exserted, on peduncles less than 6 cm long; blades suberect, the margins lacking cilia on distal ½ …..…….……...…..subsp. ***thermale***
        8. Primary panicles usually well-exserted, on peduncles more than 8 cm long; blades ascending to spreading, margins ciliate most of their length……..……..subsp. ***acuminatum***
    7. Culms and sheaths pilose with papillose-based hairs to hispid, with mostly ascending hairs, or densely puberulent with a few longer hairs also present; blades appressed-pubescent or puberulent abaxially, not velvety to touch.
        9. Sheaths and culms densely puberulent, scattered long hairs often present ……...……...……..subsp. ***columbianum***
        9. Sheaths and culms pilose with papillose-based hairs, hairs mostly ascending, occasionally with incomspicuous, shorter hairs underneath
            10. Blades usually 6-12 mm wide, spreading to ascending, abaxial

surfaces nearly glabrous or with hairs shorter than 3 mm long; spikelets 1.5-2 mm long……...……subsp. ***fasciculatum***

10. Blades usually 2-6 mm wide, erect to ascending, spreading or reflexed, adaxial surfaces glabrous or with hairs 3-6 mm long; spikelets 1.1-1.6 mm long.

11. Blades erect to ascending, adaxial surfaces long-pilose; spikelets 1.3-1.6 mm long, usually broadly obovoid …...……..…..subsp. ***implicatum***

11. Blades ascending, spreading, or reflexed, adaxial surfaces glabrous or sparsely pubescent; spikelets 1.1-1.5 mm long, usually ellipsoid…….…….……..subsp. ***leucothrix***

2. Spikelets 1.8–3 mm long; leaf sheaths with haris to 4.5 mm long……...…… ……***D. ovale***

12. Lower sheaths and lower culm internodes with soft, spreading or retrose papillose-based hairs, the longer hairs oftern longer than 4 mm long; spikelets 1.8–2.5 mm long.

13. Spikelets 2.1–2.5 mm long; culms usually more than 1 mm thick, stiff; largest blades usually 6–10 mm wide……..……....subsp. ***villosissimum***

13. Spikelets 1.8–2.1 mm long; culms usually less than 1 mm thick, wiry; largest blades usually 2–6 mm wide…….subsp. ***praecocius***

12. Lower sheaths and lower culm internodes with ascending or appressed, non-papillose-based hairs shorter than 4 mm or nearly glabrous; spikelets

2.1–3 mm long.

14. Spikelets 2.5–3 mm long; basal blades with long hairs on or near the margins and bases……..…subsp. ***ovale***

14. Spikelets 2.1–2.6 mm long; basal blades usually without long hairs on or near the margins and bases…….....subsp. ***pseudopubescens***

Dichanthelium acuminatum (Sw.) Gould & C. Clark–Plants usually densely cespitose. Basal rosettes usually well differentiated; blades ovate to lanceolate. Culms 15-100 cm (rarely taller), usually thicker diameter than 1 mm, weak and wiry or relatively stout and rigid, erect, ascending or decumbent; nodes occasionally swollen, glabrous or densely pubescent, often with a glabrous or viscid ring below; internodes purplish or olive green or grayish-green or yellowish-green, variously pubescent, with hairs of 2 lengths or glabrous; autumnal phase erect, spreading, or decumbent, branching usually extensively at all but uppermost nodes, ultimately forming dense fascicles of branchlets with reduced, flat or involute blades and reduced secondary panicles with few spikelets. Cauline leaves 4-7; sheaths usually shorter than the internodes, glabrous or densely and variously pubescent with hairs shorter than 3 mm, margins ciliate or glabrous; ligules and pseudoligules 1-5 mm, of hairs; blades 2-12 cm long (rarely longer), 2-12 mm wide (rarely wider), firm or lax, spreading to reflexed or stiffly ascending, yellowish-green or grayish-green to olivaceous, densely to sparsely and variously pubescent, margins similar or occasionally whitish-scabridulous, margins often with papillose-based cilia, at least basally, bases rounded or subcordate. Primary panicles 3-12 cm long, ¼ - ¾ as wide as long, usually open, well-exserted, rather dense;

rachises glabrous, puberulent, or more or less densely pilose, at least basally. Spikelets 1.1-2.1 mm long, obovoid to ellipsoid, yellowish-green to olivaceous or purplish, variously pubescent, obtuse or subacute. Lower glumes usually 1/4-1/2 as long as spikelets, obtuse to acute; upper glumes and lower lemmas subequal, equaling the upper florets at maturity, or occasionally the upper glumes slightly shorter, not strongly veined; lower florets sterile; upper florets 1.1-1.7 mm long, 0.6-1 mm wide, ellipsoid, obtuse to acute or minutely umbonate or apiculate. 2N = 18.

Dichanthelium acuminatum (Sw.) Gould & C. Clark subsp. ***acuminatum*** (fragment and photo of holotype: US!)–Plants grayish olive green, densely velvety-villous throughout. Cauline nodes densely villous, with a glabrous ring below; autumnal phase branching extensively from midculm nodes, forming conspicuous flabellate fascicles of branches. Cauline sheaths densely soft spreading-villous, often with inconspicuous smaller hairs underneath, with hair lengths 0.8-4.0 mm long (> 2.0 mm in atypical populations); midculm sheaths about 1/2 as long as internodes; blades 6-12 cm long, to 10 mm wide, ascending to often spreading and slightly incurved, softly pubescent on both surfaces, with papillose-based cilia for most of their length. Primary panicles usually well-exserted, on peduncles longer than 8 cm; peduncle hair lengths 0.1-4 mm long (> 2.0 mm in atypical populations). Spikelets 1.6-2.0 mm long, broadly ellipsoid or obovoid.

Dichanthelium. acuminatum. subsp. *acuminatum* is distinct from the other taxa of the complex both from a morphometric and phylogenetic standpoint. However, as discussed in both the morphometric (Chapter II) and phylogenetic analyses (Chapter III), several of

the populations collected for the study (*Hammer* populations 8, 15, and 17) are atypical morphologically and preliminary genetic data indicates a possible hybrid origin for these populations. As indicated in the above description, these populations can be delineated on the basis of peduncle hair length and leaf sheath hair length as follows:

Key to populations of *D. acuminatum* subsp. ***acuminatum***

1. Peduncle hair lengths less than or equal to 2.0 mm long; leaf sheath hair lengths less than or equal to 2.0 mm long……..……..…….……typical subsp. ***acuminatum***

1. Peduncle hair lengths greater than 2.0 mm long; leaf sheath hair lengths greater than 2.0 mm long……...…….……..……..…atypical subsp. ***acuminatum***

Dichanthelium acuminatum subsp. ***columbianum*** (Scribn.) Freckmann & Lelong (isotype: NY!)—Plants cespitose, pale bluish- or grayish-green. Culms erect to ascending, densely puberulent, longer hairs often present also, at least on lower portion of culms; nodes puberulent; autumnal phase with spreading or decumbent culms, branching early from most nodes, secondary blades not as greatly reduced or as densely crowded as in subsp. *acuminatum*, subsp. *fasciculatum*, subsp. *implicatum*, and subsp. *leucothrix*. Cauline sheaths pubescent, pubescence similar to that of the culms but somewhat less dense; midculm sheaths about 1/2 as long as the internodes; ligules 1-1.5 mm long; blades 3-7 cm long, 3-7 mm wide, relatively firm, often ascending, abaxial surfaces densely puberulent to nearly glabrous, adaxial surfaces glabrous or sparsely

pilose near the base, margins whitish-scabridulous. Spikelets 1.5-2.0 mm long, broadly ellipsoid or obovoid, puberulent.

The results of the current study provide support for recognizing *Dichanthelium acuminatum* subsp. *columbianum* as a distinct subspecies from a morphometric standpoint. Results of the molecular phylogenetic analysis were inconclusive for this taxon and follow-up study is needed. The two specimens of subsp. *columbianum, C1* (*Freckmann* = WSU) and *SP14* (*Freckmann* = WSU 20234) included in the phylogenetic analysis end up as members of different monophyletic clades in Fig. 31. Specimen *C1* is a member of the subsp. *fasciculatum* clade but the statistical support for grouping this specimen with the statistically well-supported group (bootstrap = 87%) of three subsp. *fasciculatum* specimens is weak (bootstrap = 63%). Specimen *C1* displays typical morphological traits for a subsp. *columbianum* specimen, but, as was discussed in Chapter III, introgression from subsp. *fasciculatum* may be responsible for the inclusion of specimen *C1* with the subsp. *fasciculatum* specimens.

The placement of the *SP14* specimen of subsp. *columbianum* in a strongly supported clade (Fig. 31) of three total specimens consisting of one specimen of subsp. *fasciculatum, SP8* (*Freckmann 6301*) and one specimen of subsp. *acuminatum* (*Hammer 302*) is almost the reverse situation for specimen *C1* just discussed. As detailed in the Chapter III discussion, there is preliminary evidence that gene flow is responsible for these apparent contradictory results from a morphological standpoint.

In conclusion, the results from the present study support recognition of subsp. *columbinaum* based on morphometric data. Molecular phylogenetic evidence for this

taxon is inconclusive based on the current data set. Follow up research is planned in terms of collection of additional field-collections of subsp. *columbianum* specimens in the future. Gene sequence data from these specimens will be added to the GBSSI data set for further analysis.

Dichanthelium acuminatum subsp. ***fasciculatum*** (Torr.) Freckmann & Lelong– Plants yellowish-green to olivaceous or purplish. Culms 15-75 cm, ascending or spreading; nodes often with spreading hairs, occasionally with a glabrous ring below. Cauline sheaths with ascending to spreading, papillose-based hairs, occasionally with shorter hairs underneath; midculm sheaths about 1/2 as long as internodes; blades 5-12 cm long, 6-12 mm wide, spreading to ascending, bases with papillose-based cilia, abaxial surfaces usually pubescent, adaxial surfaces pilose or glabrous, hairs shorter than 3 mm. Spikelets 1.5-2 mm long (tending to be longer in western part of its range), obovoid to ellipsoid.

Dichanthelium. acuminatum. subsp. *fasciculatum* is distinct from the other taxa of the complex both from a morphometric and phylogenetic standpoint.

Dichanthelium acuminatum subsp. ***implicatum*** (Scribn.) Freckmann & Lelong (holotype: US)–Plants densely cespitose. Culms seldom over 50 cm, slender, ascending or spreading; nodes more or less densely pubescent; autumnal phase branching extensively from lower and midculm nodes, with conspicuous, flabellate fascicles of branches and reduced blades. Cauline sheaths shorter than internodes, lower sheaths usually pilose with papillose-based hairs, upper sheaths often short-pubescent; midculm sheaths about 1/2 as long as internodes; blades usually 2-6 mm wide, more than 8 times

longer than wide, relatively firm, erect to ascending, often yellowish-green, abaxial surfaces densely pubescent with short papillose-based appressed hairs or short-pubescent with subappressed hairs, adaxial surfaces usually densely pilose, hairs to 6 mm, conspicuous, erect or ascending, occasionally with shorter hairs underneath. Spikelets 1.3-1.6 mm long, usually broadly obovoid.

Both morphometric and molecular phylogenetic data are incomplete at present for *Dichanthelium acuminatum* subsp. *implicatum* based on the results of the present study. The taxonomic status for this taxon awaits the results of further morphometric and molecular studies.

Dichanthelium acuminatum subsp. ***leucothrix*** (Nash) Freckmann & Lelong (holotype: NY!) –Plants cespitose, pale olive green, often purplish-tinged. Culms usually 30-100 cm, erect to ascending, sparsely pubescent to almost glabrous, hairs appressed, thin, silvery, papillose-based; nodes sparsely pubescent; autumnal phase branching extensively from lower and midculm nodes, with conspicuous, flabellate fascicles of branches and reduced blades. Cauline sheaths shorter than internodes, sparsely pilose to nearly glabrous, hairs papillose-based, occasionally with shorter soft hairs underneath, margins ciliate; midculm sheaths about 1/2 as long as internodes; blades usually 2-7 cm long, 2-7 mm wide, relatively firm, ascending, spreading, or reflexed, abaxial surfaces densely puberulent, adaxial surfaces glabrous or sparsely appressed-villous, sometimes with a few longer hairs intermixed. Primary panicles 30-65 cm long, open, long-exserted, dense. Spikelets 1.1-1.5 mm long, usually ellipsoid, densely short-pubescent.

The multivariate analysis (PCoA) shows separation for some of the subsp. *leucothrix* specimens (Fig. 19) alongside a cluster of subsp. *columbianum* specimens. However, only eight specimens of subsp. *leucothrix* were obtained for morphological analysis. More specimens must be obtained and included in the multivariate analysis in order to satisfactorily represent the potential morphological variation in this taxon. Also, attempts to obtain DNA for phylogenetic analysis from the eight subsp. *leucothrix* specimens were unsuccessful. Therefore, resolution of the taxonomic status of this taxon awaits further morphological and phylogenetic evidence.

Dichanthelium acuminatum subsp. ***lindheimeri*** (Nash) Freckmann & Lelong (holotype: NY!)–Culms often yellowish-green, usually glabrous; nodes glabrous; autumnal phase usually with stiffly spreading culms with dense fascicles of branches with reduced, often involute blades. Cauline sheaths often yellowish-green, usually glabrous or the lowest sheaths sparsely ascending-pubescent; blades 4-9 cm long, 4-8 mm wide, stiffly ascending or spreading, often yellowish-green, glabrous on both surfaces or puberulent abaxially, bases rounded, margins faintly whitish-scabridulous, with conspicuous, long, papillose-based cilia at base. Primary panicles 3.5-7 cm long, open, less than twice as long as wide. Spikelets 1.3-1.6 mm long, diverging to ascending, usually obovoid, obtuse.

Recognition of *Dichanthelium acuminatum* subsp. *lindheimeri* as a taxon distinct from the other taxa of the *Dichanthelium acuminatum* complex is well supported by both morphological and phylogenetic analysis.

Dichanthelium acuminatum subsp. ***longiligulatum*** (Nash) Freckmann & Lelong (holotype: NY!)–Very similar to subsp. *spretum* vegetatively. Autumnal phase branching profusely from the lower and midculm nodes, producing dense fascicles of reduced branches, blades, and secondary panicles. Cauline blades green or purplish. Primary panicles 3-8 cm long, to ¾ as wide as long, normally expanded; branches numerous, slender, ascending, spikelets densely packed. Spikelets 1.1-1.5 mm long, usually ellipsoid, puberulent.

See the discussion under *Dichanthelium acuminatum* subsp. *spretum*.

Dichanthelium acuminatum subsp. ***spretum*** (Schult.) Freckmann & Lelong–Culms usually glabrous; nodes often swollen, glabrous; autumnal phase often with reclining culms, ultimately with fascicles of branches with greatly reduced blades and secondary panicles. Cauline sheaths usually glabrous; blades 3-9 mm wide, usually firm, ascending to reflexed, puberulent or glabrous abaxially, glabrous adaxially, with sparse papillose-based cilia at bases. Primary panicles 4-12 cm long, 1/4-1/2 as wide as long, usually narrow, congested. Spikelets 1.3-1.9 mm long, ascending to appressed, usually ellipsoid, usually puberulent (rarely glabrous).

From a molecular phylogenetic standpoint both *Dichanthelium acuminatum* subsp. *spretum* and subsp. *longiligulatum* together comprise an evolutionarily significant unit (ESU) and are thus distinct from the other taxa in the *Dichanthelium acuminatum* complex. The current GBSSI dataset does not allow either taxon to be separated from the other. However, as noted in the Chapter III discussion of the molecular phylogenetic

results, there is some question as to how representative our research specimens were for both subsp. *longiligulatum* and subsp. *spretum*.

The morphological analysis (PCoA, Fig. 28) showed an expected multivariate clustering pattern for two taxa with very similar vegetative features; that is, there was some discernable separation between the two taxa when looking at the clustering pattern. As noted in the Chapter II (morphological analysis) discussion, the field-collected specimens of subsp. *longiligulatum* were not handled properly to maintain qualitative and quantitative dimensions of several of the morphological characters of prime importance for distinguishing subsp. *longiligulatum* from subsp. *spretum*. Therefore, pending further investigation, both subsp. *longiligulatum* and subsp. *spretum* will be maintained as distinct taxa in the *Dichanthelium acuminatum* complex.

Dichanthelium acuminatum subsp. ***sericeum*** (Schmoll) Freckmann & Lelong– Plants more or less densely cespitose. Culms usually less than 30 cm, stiffly ascending to spreading, densely pubescent. Midculm sheaths nearly as long as the internodes; midculm blades usually 2-6.5 cm, usually less than 8 times as long as wide. Primary (autumnal) panicles 40-70 cm long, usually well-exserted. Spikelets mostly 1.6-1.8 mm long.

The multivariate statistical analysis of subsp. *sericeum* showed good separation of this taxon from the other taxa of the *D. acuminatum* complex. The molecular phylogenetic position of subsp. *sericeum* relative to the other taxa in the complex remains unresolved. Based on it's morphological separation and restricted geographic

distribution and unique ecological habitat subsp. *sericeum* is properly retained as a distinct taxon in the *Dichanthelium acuminatum* complex.

Dichanthelium acuminatum subsp. ***thermale*** (Bol.) Freckmann & Lelong–Plants often densely cespitose, densely and softly pubescent throughout, with soft, thin, spreading, papillose-based hairs on culms and lower sheaths. Culms usually over 30 cm tall. Midculm sheaths about 1/2 as long as the internodes; blades at midculm generally 6.5-12 cm long, usually more than 7 times as long as wide, spreading or ascending, softly pubescent on the abaxial surface, without papillose-based cilia on the distal 1/2. Primary panicles usually poorly exserted, peduncles shorter than 6 cm. Spikelets mostly 1.8-2 mm long.

Only three specimens of subsp. *thermale* were obtained for morphological analysis. Multivariate statistical results (PCoA Fig. 19) showed the three subsp. *thermale* specimens intergrading with specimens of subsp. *fasciculatum* and subsp. *columbianum*. More specimens need to be analyzed to satisfactorily evaluate the position of this taxon from a morphological standpoint relative to the other taxa in the *D. acuminatum* complex. The molecular phylogenetic position of this taxon is unresolved and awaits further analysis with the addition of additional specimens. However, due to its known restricted geographic distribution and unique ecological habitat it is not unreasonable to designate subsp. *thermale* as a distinct taxon in the *Dichanthelium acuminatum* complex.

Dichanthelium ovale (Elliott) Gould & C. Clark–Plants cespitose. Basal rosettes well-differentiated; blades 1-8 cm, lanceolate, often conspicuously ciliate. Culms 15-60 cm, usually more than 1 mm thick, not delicate, mostly ascending or spreading, often

decumbent; nodes densely to sparsely bearded with spreading, retrorse, or appressed hairs; internodes, particularly the lower internodes, usually long-hairy with appressed or ascending hairs, occasionally with spreading hairs, occasionally with shorter hairs, rarely nearly glabrous; fall phase with decumbent to prostrate culms, branching developing early and forming dense fascicles with erect, slightly reduced blades and greatly reduced secondary panicles. Cauline leaves 4-7; sheaths shorter than the internodes, pilose, hairs to 4 mm, occasionally with shorter, spreading hairs underneath; ligules and pseudoligules 1-5 mm, of hairs; blades 4-10 cm long, 3-10 mm wide, relatively firm, mostly ascending or spreading, 1 or both surfaces sparsely to densely pubescent with appressed or erect hairs, hairs to 5 mm, bases rounded or slightly narrowed, margins often whitish, ciliate basally, scabridulous elsewhere. Primary panicles 3-10 cm long, nearly as wide when fully expanded; rachises and branches often stiffly ascending or spreading, usually pilose basally. Spikelets 1.8-3 mm, ellipsoid or obovoid, densely to sparsely pilose or papillose-pilose, obtuse or slightly acute. Lower glumes 1/3-1/2 as long as the spikelets, often triangular, not strongly veined, usually acute or subacute; upper glumes usually slightly shorter than the lower lemmas and upper florets at maturity, not strongly veined; lower florets sterile; upper florets 1.6-2.5 mm, ellipsoid (slightly less than 1/2 as wide as long, or wider in subsp. *praecocius*), subacute. 2n = 18.

Dichanthelium ovale subsp. ***villosissimum*** (Nash) Freckmann & Lelong–Basal blades 3-7 cm, evenly long pilose. Culms more than 1 mm thick, stiff, often decumbent or prostrate in the fall; internodes with soft, spreading or retrorse, papillose-based hairs, hairs longer than 4 mm. Cauline sheaths with soft, spreading or retrorse hairs, hairs

longer than 4 mm, papillose-based; ligules 2-5 mm; blades 6-10 mm wide, both surfaces densely pilose, hairs longer than 4 mm, margins short-ciliate basally, scabridulous and faintly whitish elsewhere. Spikelets 2.1-2.5 mm, usually ellipsoid, with dense, spreading, papillose-based hairs. Lower glumes 1/3-1/2 as long as the spikelets, usually acute. 2n = 18.

Dichanthelium ovale subsp. ***praecocius*** (Hitchc. & Chase) Freckmann & Lelong–Basal blades 1-3 cm, sparsely to densely evenly pilose. Culms less than 1 mm thick, wiry; internodes with soft, spreading or retrorse papillose-based hairs longer than 4 mm. Cauline sheaths with soft, spreading or retrorse hairs, hairs usually longer than 4 mm, papillose-based; ligules 3-4 mm; blades 2-6 mm wide, both surfaces densely pilose. Spikelets 1.8-2.1 mm, obovoid or ellipsoid, pilose with papillose-based hairs.

Dichanthelium ovale (Elliott) Gould & C. Clark subsp. ***ovale***–Basal blades 3-8 cm, rigid, with long hairs on or near the bases and margins. Culms more than 1 mm thick, stiff; lower internodes pilose; upper internodes short-pilose to nearly glabrous. Cauline sheaths with ascending hairs, hairs to 4 mm, not papillose-based: ligules 1-4 mm; blades 5-12 mm wide, firm, ascending, abaxial surfaces appressed-pubescent, adaxial surfaces nearly glabrous except for the long hairs on or near the scabridulous margins and bases. Spikelets 2.5-3 mm, ellipsoid, sparsely to densely pilose. 2n = 18.

Dichanthelium ovale subsp. ***pseudopubescens*** (Nash) Freckmann & Lelong–Basal blades 2-6 cm, evenly pilose. Culms more than 1 mm thick, stiff; lower internodes sparsely pubescent, with ascending or appressed hairs, hairs shorter than 4 mm, not papillose-based. Cauline sheaths with sparse, ascending or appressed hairs, hairs shorter

than 4 mm, often with shorter hairs underneath, not papillose-based; ligules 1-4 mm; blades 3-8 mm wide, both surfaces sparsely appressed-pubescent, margins ciliate basally, scabridulous elsewhere. Spikelets 2.1-2.6 mm, ellipsoid or obovoid-ellipsoid, with papillose-based hairs.

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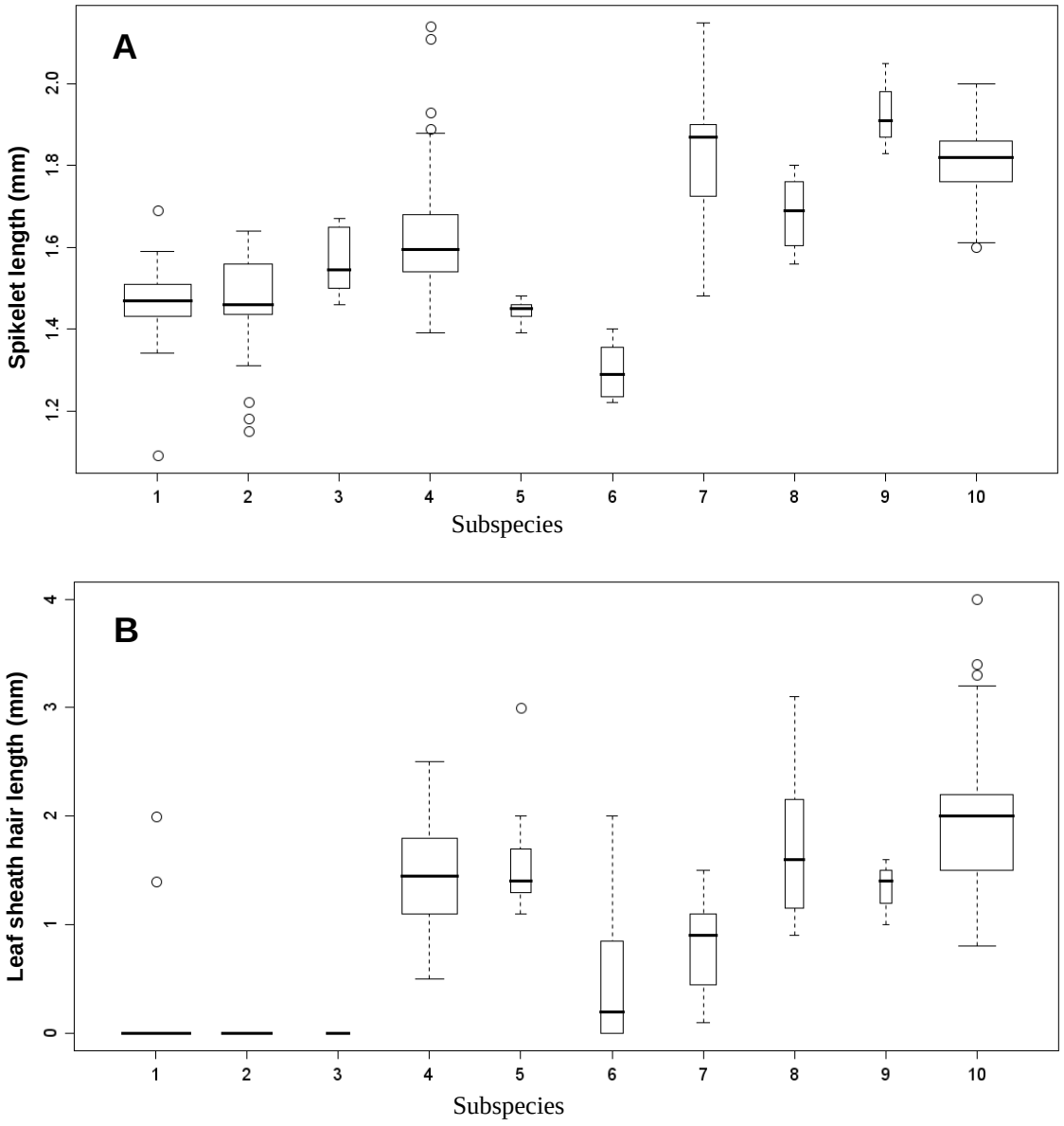
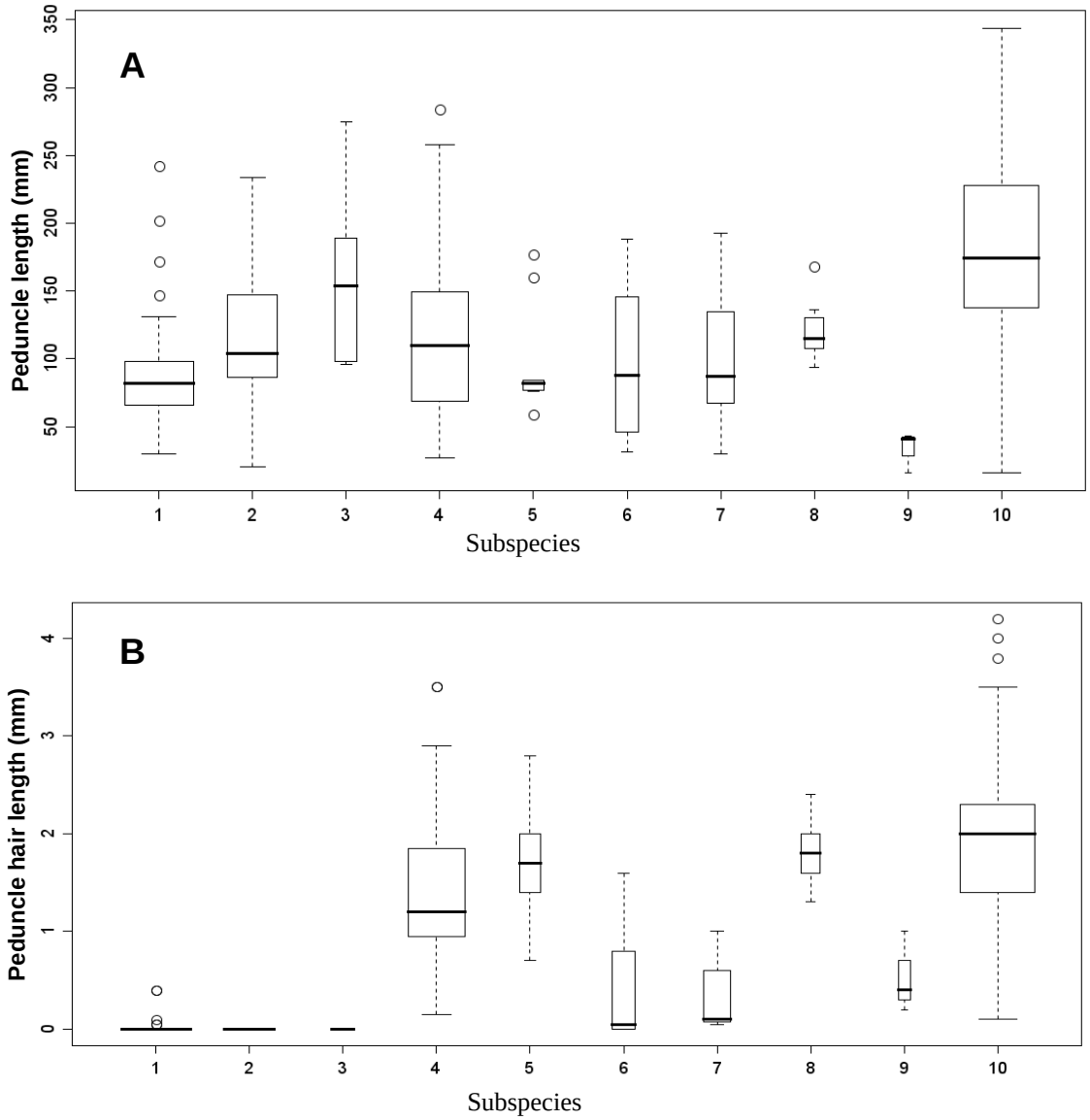
APPENDIX A

FIGURES



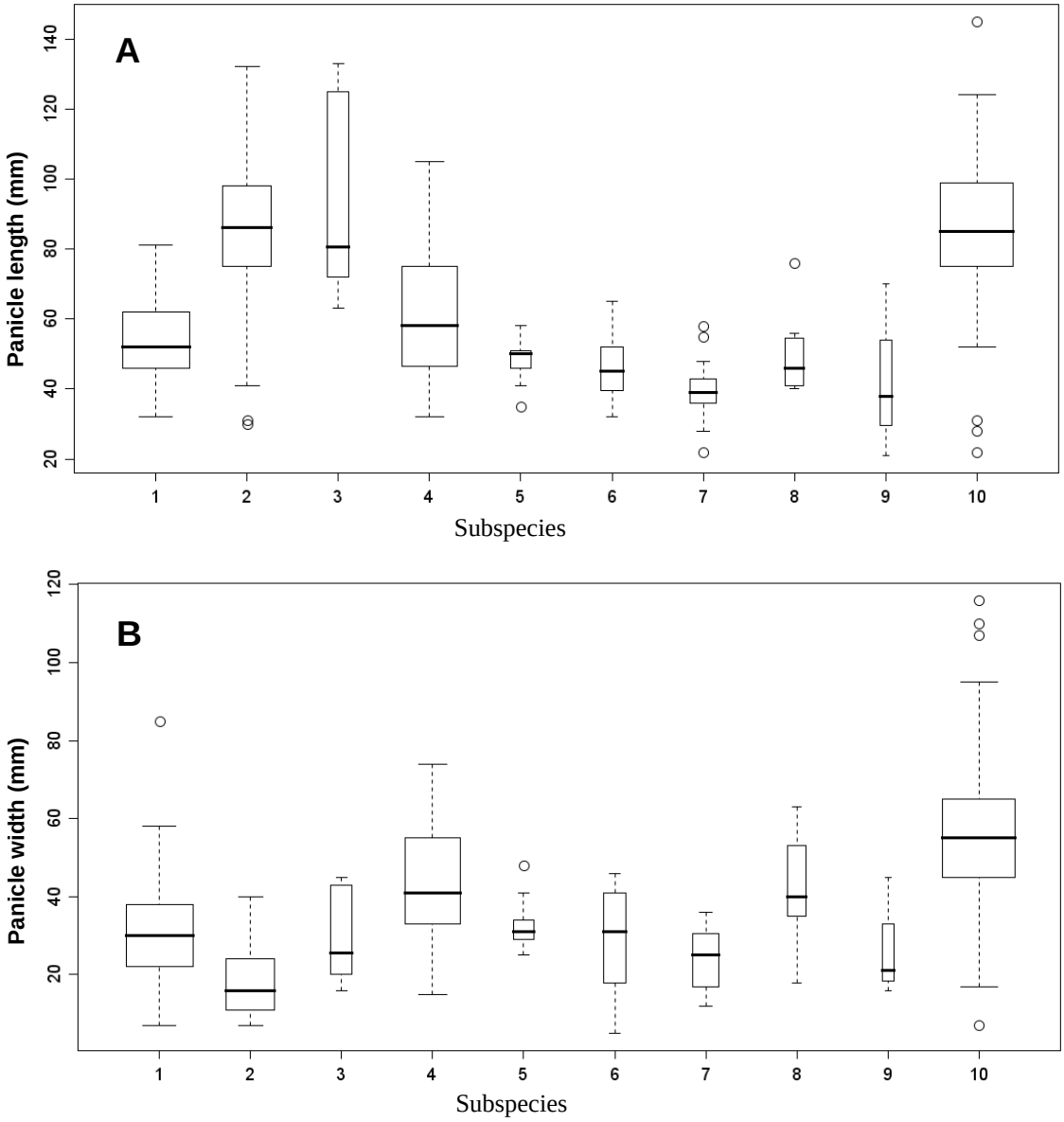
Subspecies legend: **1** – *D. acuminatum* subsp. *lindheimeri* (N=98)*;* **2** – *D. a*. subsp. *longiligulatum* (N=52); **3** – *D. a*. subsp. *spretum* (N=10); **4** – *D. a*. subsp. *fasciculatum*; (N=67) **5** – *D. a*. subsp.. *implicatum* (N=9); **6** – *D. a*. subsp. *leucothrix* (N=11); **7** – *D. a*. subsp. *columbianum* (N=15); **8** – *D. a*. subsp. *sericeum* (N=7); **9** – *D. a*. subsp. *thermale* (N=3); **10** – *D. a*. subsp. *acuminatum* (N=117*)*.

Fig. 1. Standard box and whisker plots of quantitative continuous variables by subspecies groupings in the *Dichanthelium acuminatum* complex. A. Spikelet length. B. Leaf sheath hair length. The box encompasses the 25^{th}-75^{th} percentile points of data and the midline represents the median. Box plot widths are drawn proportional to the square root of the samples sizes.



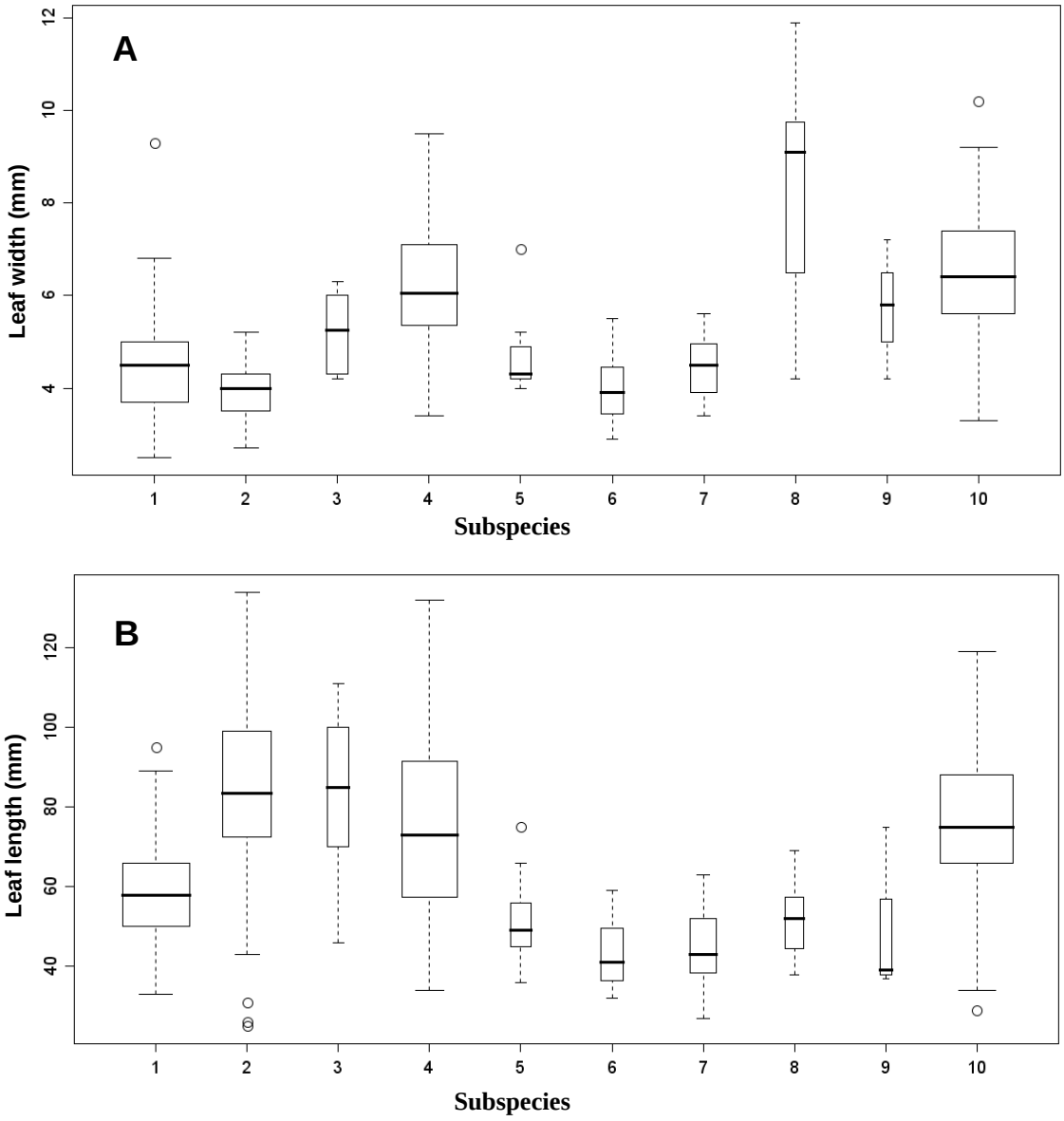
Subspecies legend: **1** – *D. acuminatum* subsp. *lindheimeri* (N=98)*;* **2** – *D. a*. subsp. *longiligulatum* (N=52); **3** – *D. a*. subsp. *spretum* (N=10); **4** – *D. a*. subsp. *fasciculatum*; (N=67) **5** – *D. a*. subsp.. *implicatum* (N=9); **6** – *D. a*. subsp. *leucothrix* (N=11); **7** – *D. a*. subsp. *columbianum* (N=15); **8** – *D. a*. subsp. *sericeum* (N=7); **9** – *D. a*. subsp. *thermale* (N=3); **10** – *D. a*. subsp. *acuminatum* (N=117*).*

Fig. 2. Standard box and whisker plots of quantitative continuous variables by subspecies groupings in the *Dichanthelium acuminatum* complex. A. Peduncle length. B. Peduncle hair length. The box encompasses the 25^{th}-75^{th} percentile points of data and the midline represents the median. Box plot widths are drawn proportional to the square root of the samples sizes.



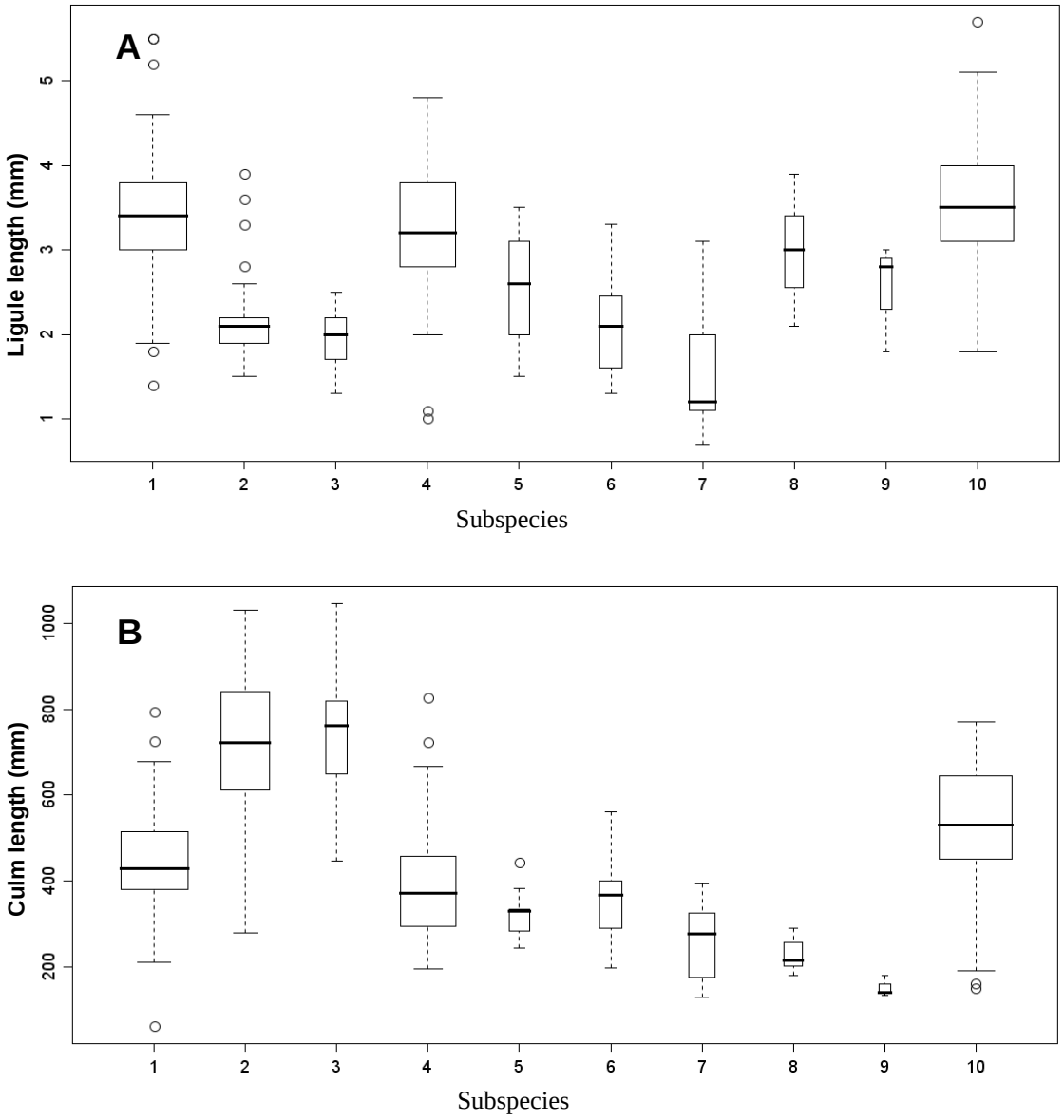
Subspecies legend: **1** – *D. acuminatum* subsp. *lindheimeri* (N=98)*;* **2** – *D. a*. subsp. *longiligulatum* (N=52); **3** – *D. a*. subsp. *spretum* (N=10); **4** – *D. a*. subsp. *fasciculatum*; (N=67) **5** – *D. a*. subsp.. *implicatum* (N=9); **6** – *D. a*. subsp. *leucothrix* (N=11); **7** – *D. a*. subsp. *columbianum* (N=15); **8** – *D. a*. subsp. *sericeum* (N=7); **9** – *D. a*. subsp. *thermale* (N=3); **10** – *D. a*. subsp. *acuminatum* (N=117*)*.

Fig. 3. Standard box and whisker plots of quantitative continuous variables by subspecies groupings in the *Dichanthelium acuminatum* complex. A. Panicle length. B. Panicle width. The box encompasses the 25^{th}-75^{th} percentile points of data and the midline represents the median. Box plot widths are drawn proportional to the square root of the samples sizes.



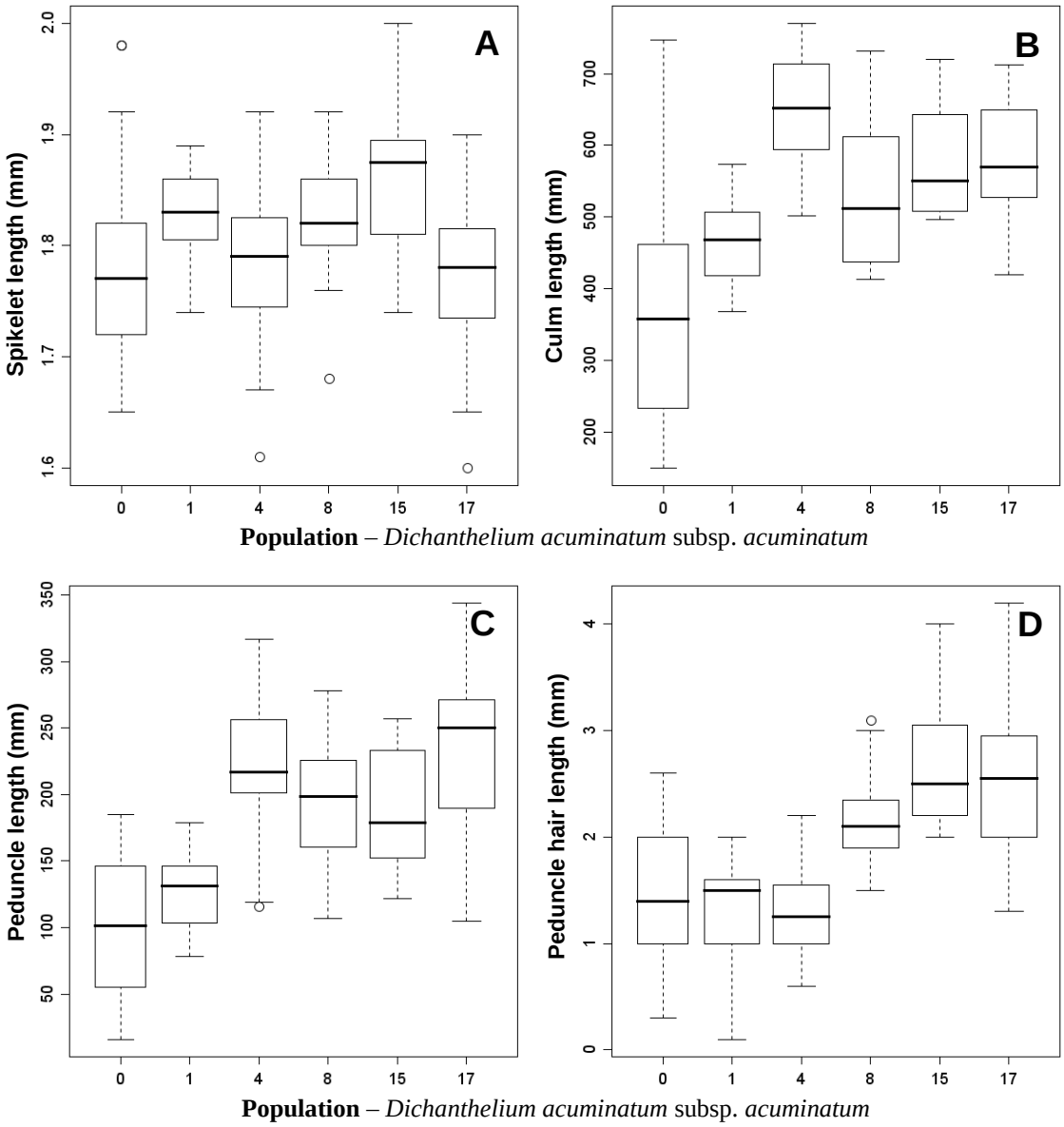
Subspecies legend: **1** – *D. acuminatum* subsp. *lindheimeri* (N=98)*;* **2** – *D. a*. subsp. *longiligulatum* (N=52); **3** – *D. a*. subsp. *spretum* (N=10); **4** – *D. a*. subsp. *fasciculatum*; (N=67) **5** – *D. a*. subsp.. *implicatum* (N=9); **6** – *D. a*. subsp. *leucothrix* (N=11); **7** – *D. a*. subsp. *columbianum* (N=15); **8** – *D. a*. subsp. *sericeum* (N=7); **9** – *D. a*. subsp. *thermale* (N=3); **10** – *D. a*. subsp. *acuminatum* (N=117*)*.

Fig. 4. Standard box and whisker plots of quantitative continuous variables by subspecies groupings in the *Dichanthelium acuminatum* complex. A. Leaf width. B. Leaf length. The box encompasses the 25^{th}-75^{th} percentile points of data and the midline represents the median. Box plot widths are drawn proportional to the square root of the samples size.



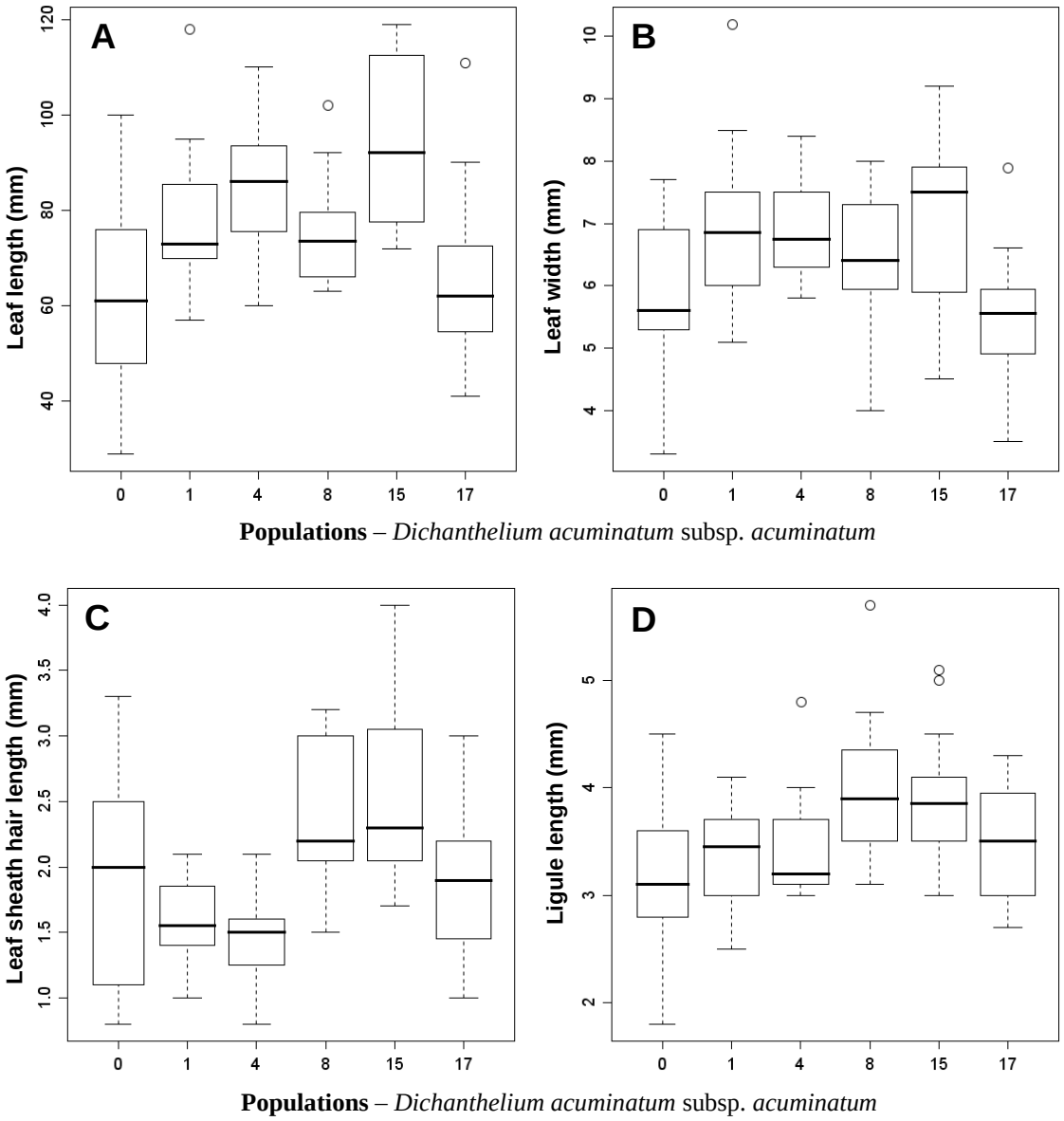
Subspecies legend: **1** – *D. acuminatum* subsp. *lindheimeri* (N=98)*;* **2** – *D. a*. subsp. *longiligulatum* (N=52); **3** – *D. a*. subsp. *spretum* (N=10); **4** – *D. a*. subsp. *fasciculatum*; (N=67) **5** – *D. a*. subsp.. *implicatum* (N=9); **6** – *D. a*. subsp. *leucothrix* (N=11); **7** – *D. a*. subsp. *columbianum* (N=15); **8** – *D. a*. subsp. *sericeum* (N=7); **9** – *D. a*. subsp. *thermale* (N=3); **10** – *D. a*. subsp. *acuminatum* (N=117*)*.

Fig. 5. Standard box and whisker plots of quantitative continuous variables by subspecies groupings in the *Dichanthelium acuminatum* complex. A. Ligule length. B. Culm length. The box encompasses the 25^{th}-75^{th} percentile points of data and the midline represents the median. Box plot widths are drawn proportional to the square root of the samples sizes.



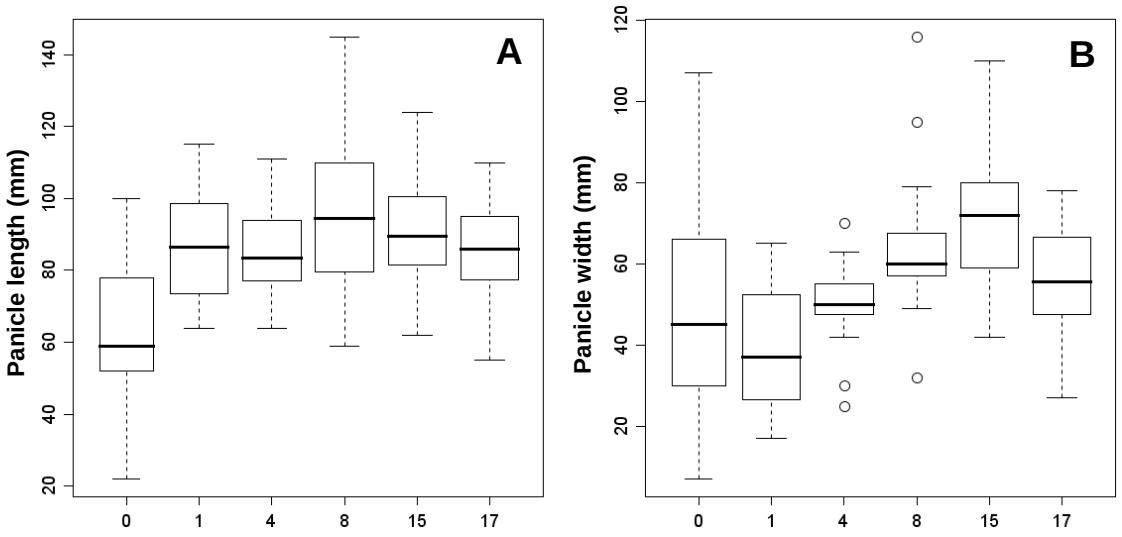
Legend for *Dichanthelium acuminatum* subsp. *acuminatum* populations: **0** – Herbarium specimens (N=16)*;* **1** –Population 1, TX-Leon, (N=20); **4** – Population 4, TX-Montgomery, (N=20); **8** – Population 8, TX-Hardin, (N=20); **15** – Population 15, LA-Vernon, (N=20); **17** – Population 17, LA-Vernon (N=20)

Fig. 6. Standard box and whisker plots of quantitative continuous variables by population groupings for *Dichanthelium acuminatum* subsp. *acuminatum* specimens. A. Spikelet length. B. Culm length. C. Peduncle length. D. Peduncle hair length. The box encompasses the 25th-75th percentile points of data and the midline represents the median. Box plot widths are drawn proportional to the square root of the samples sizes. The legend for each population reports: population collection number; state and county/parish of collection; number of specimens.



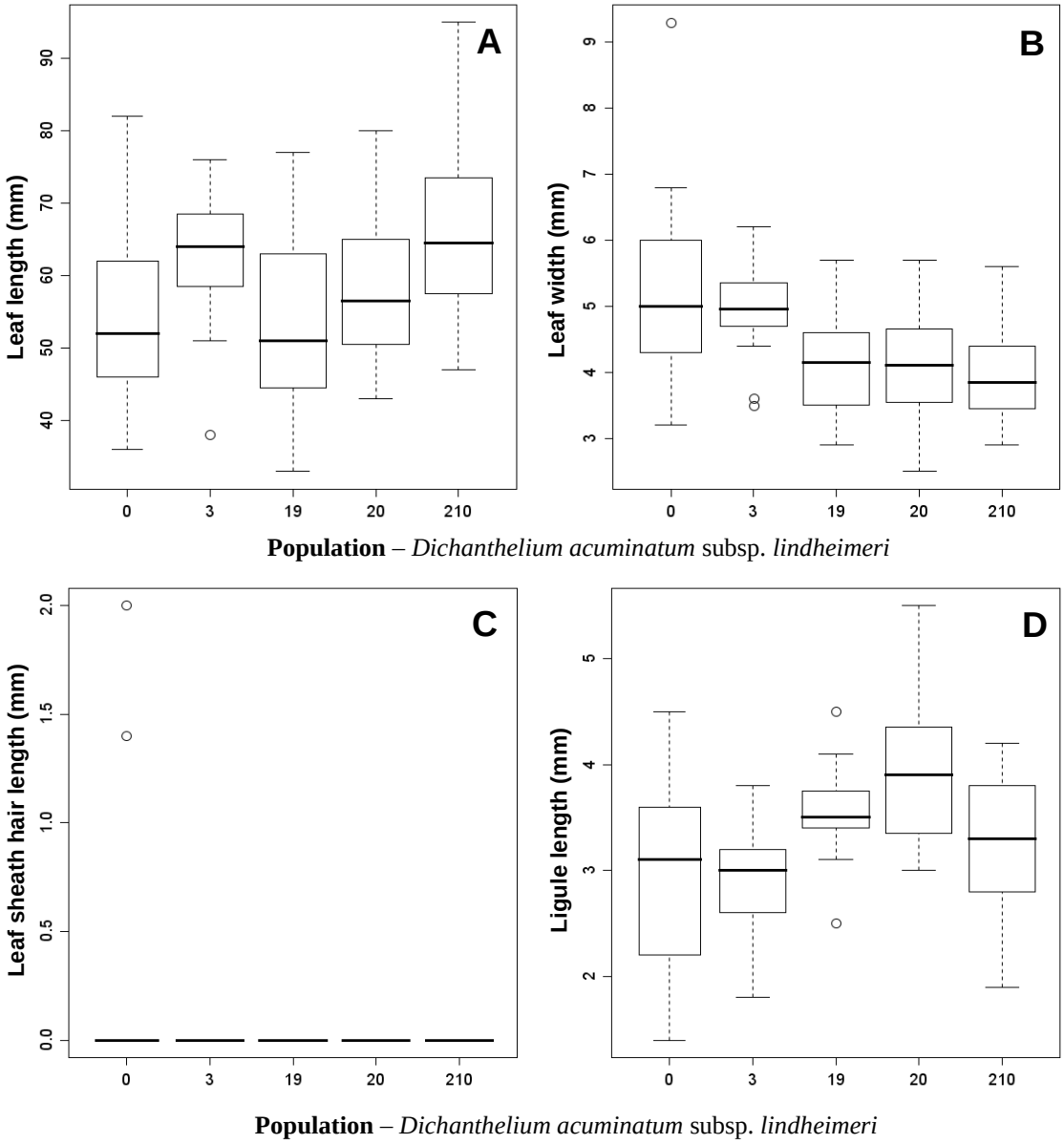
Legend for *Dichanthelium acuminatum* subsp. *acuminatum* populations: **0** – Herbarium specimens (N=16)*;* **1** –Population 1, TX-Leon, (N=20); **4** – Population 4, TX-Montgomery, (N=20); **8** – Population 8, TX-Hardin, (N=20); **15** – Population 15, LA-Vernon, (N=20); **17** – Population 17, LA-Vernon (N=20)

Fig. 7. Standard box and whisker plots of quantitative continuous variables by population groupings for *Dichanthelium acuminatum* subsp. *acuminatum* specimens. A. Leaf length. B. Leaf width. C. Leaf sheath hair length. D. Ligule length. The box encompasses the 25^{th}-75^{th} percentile points of data and the midline represents the median. Box plot widths are drawn proportional to the square root of the samples sizes. The legend for each population reports: population collection number; state and county/parish of collection; number of specimens.



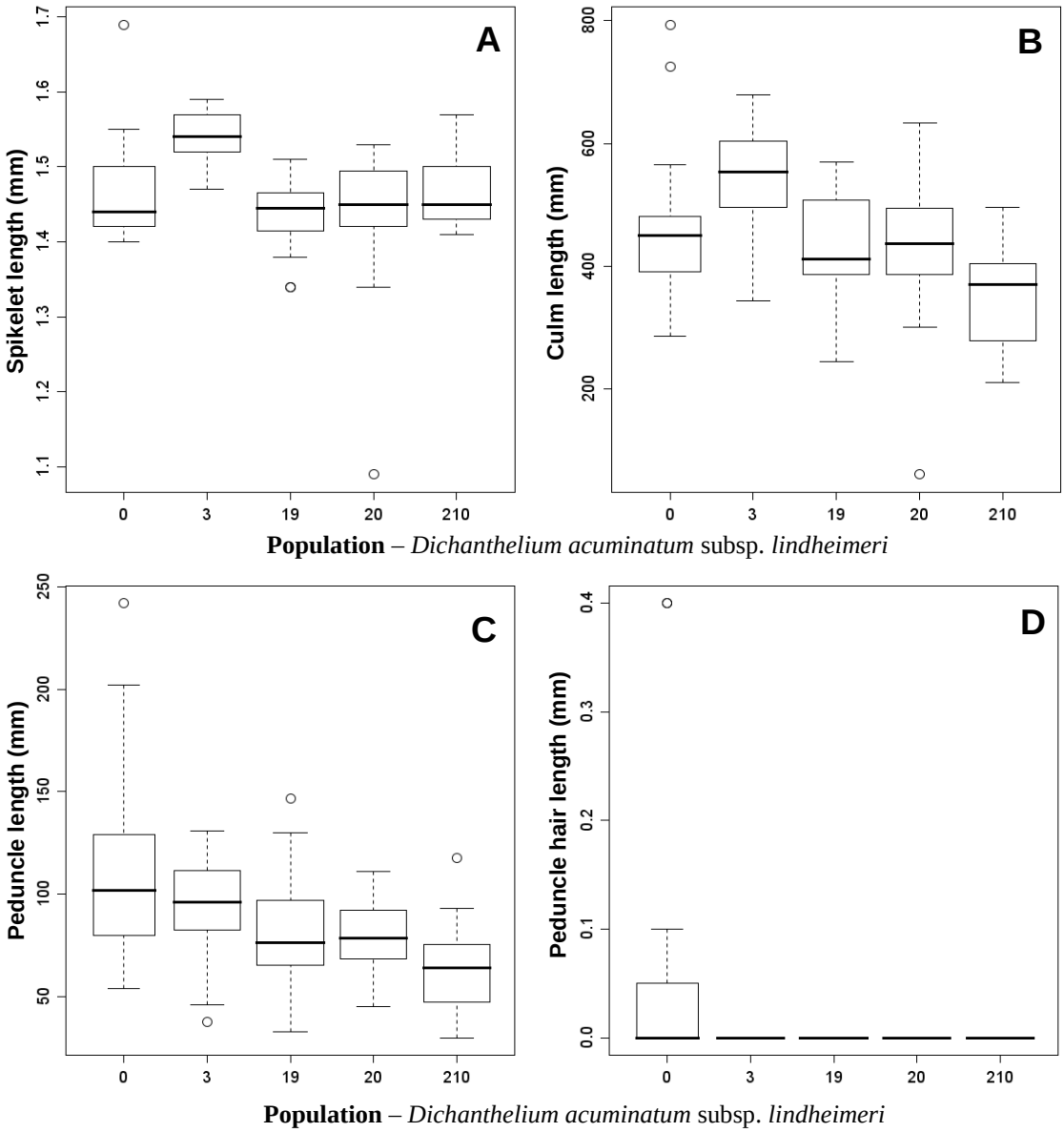
Legend for *Dichanthelium acuminatum* subsp. *acuminatum* populations: **0** – Herbarium specimens (N=16)*;* **1** –Population 1, TX-Leon, (N=20); **4** – Population 4, TX-Montgomery, (N=20); **8** – Population 8, TX-Hardin, (N=20); **15** – Population 15, LA-Vernon, (N=20); **17** – Population 17, LA-Vernon (N=20)

Fig. 8. Standard box and whisker plots of quantitative continuous variables by population groupings for *Dichanthelium acuminatum* subsp. *acuminatum* specimens. A. Panicle length. B. Panicle width. The box encompasses the 25^{th}-75^{th} percentile points of data and the midline represents the median. Box plot widths are drawn proportional to the square root of the samples sizes. The legend for each population reports: population collection number; state and county/parish of collection; number of specimens.



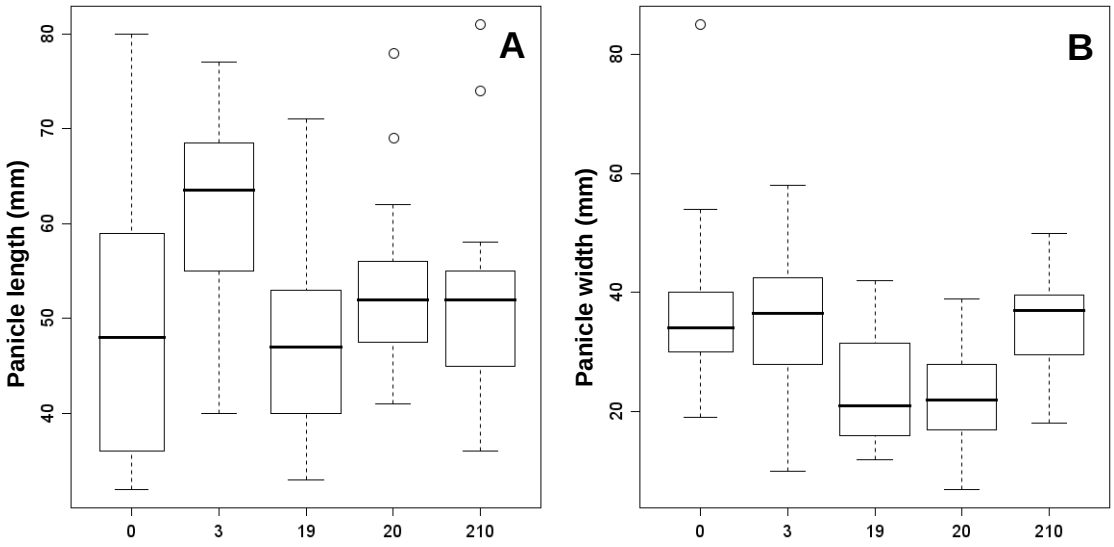
Legend for *Dichanthelium acuminatum* subsp. *lindheimeri* populations: **0** – Herbarium specimens (N=17)*;* **3** –Population 3, TX-Brazos, (N=20); **19** – Population 19, LA-Vernon, (N=20); **20** – Population 20, TX-Leon, (N=20); **210** – Population 210, TX-Lee, (N=20).

Fig. 9. Standard box and whisker plots of quantitative continuous variables by population groupings for *Dichanthelium acuminatum* subsp. *lindheimeri* specimens. A. Leaf length. B. Leaf width. C. Leaf sheath hair length. D. Ligule length. The box encompasses the 25^{th}-75^{th} percentile points of data and the midline represents the median. Box plot widths are drawn proportional to the square root of the samples sizes. The legend for each population reports: population collection number; state and county/parish of collection; number of specimens.



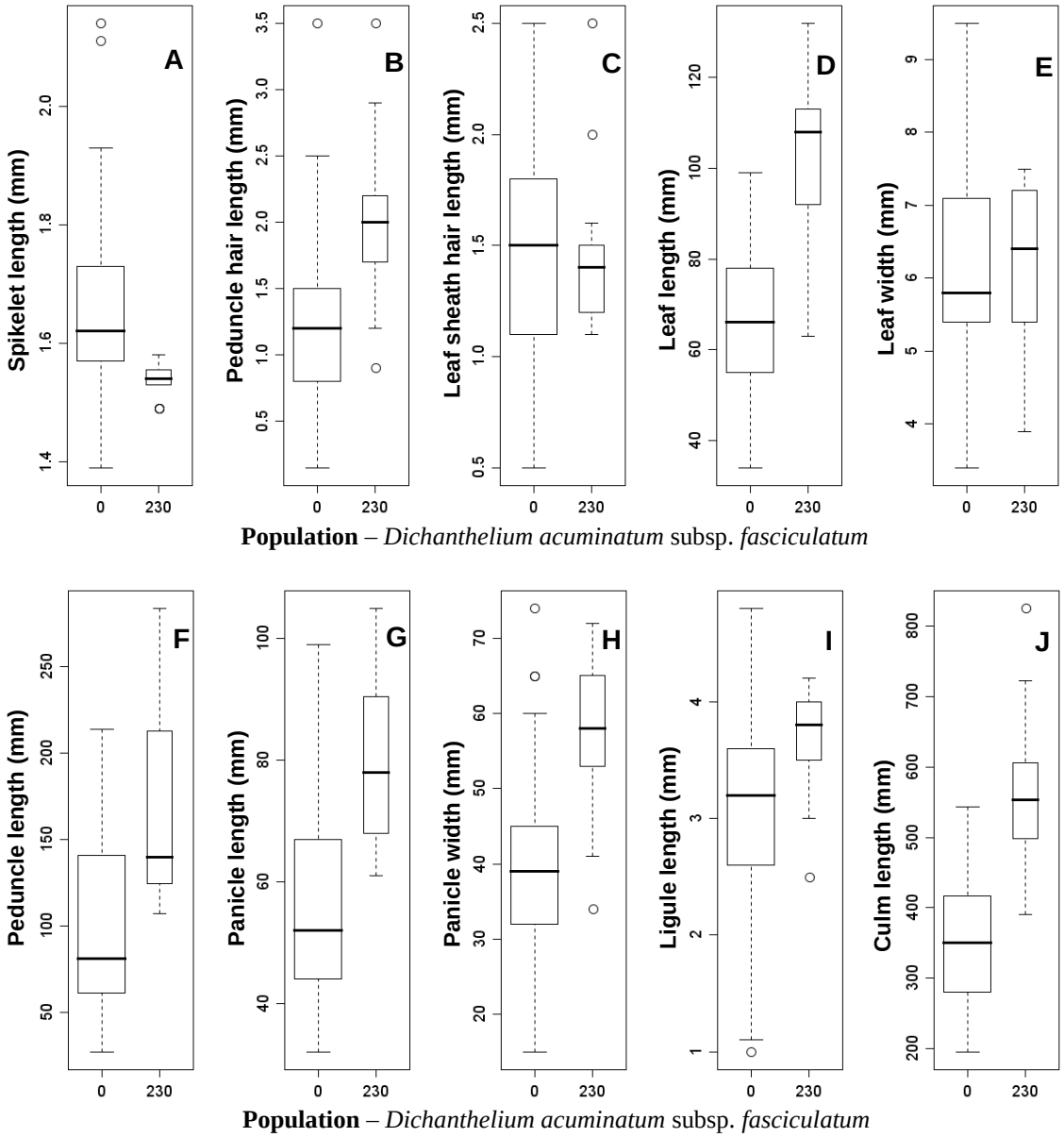
Legend for *Dichanthelium acuminatum* subsp. *lindheimeri* populations: **0** – Herbarium specimens (N=17)*;* **3** –Population 3, TX-Brazos, (N=20); **19** – Population 19, LA-Vernon, (N=20); **20** – Population 20, TX-Leon, (N=20); **210** – Population 210, TX-Lee, (N=20).

Fig. 10. Standard box and whisker plots of quantitative continuous variables by population groupings for *Dichanthelium acuminatum* subsp. *lindheimeri* specimens. A. Spikelet length. B. Culm length. C. Peduncle length. D. Peduncle hair length. The box encompasses the 25th-75th percentile points of data and the midline represents the median. Box plot widths are drawn proportional to the square root of the samples sizes. The legend for each population reports: population collection number; state and county/parish of collection; number of specimens.



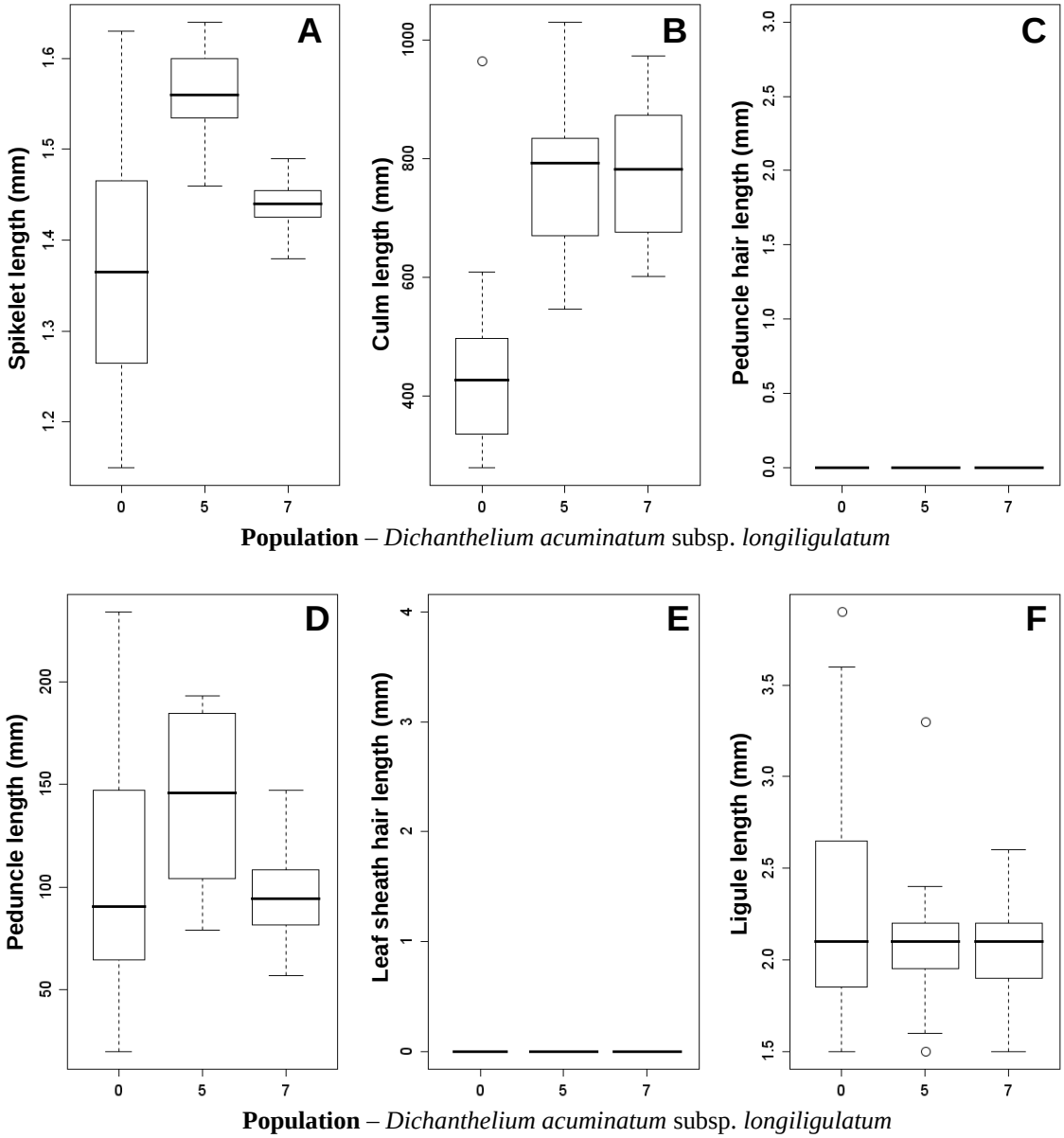
Legend for *Dichanthelium acuminatum* subsp. *lindheimeri* populations: **0** – Herbarium specimens (N=17)*;* **3** –Population 3, TX-Brazos, (N=20); **19** – Population 19, LA-Vernon, (N=20); **20** – Population 20, TX-Leon, (N=20); **210** – Population 210, TX-Lee, (N=20).

Fig. 11. Standard box and whisker plots of quantitative continuous variables by population groupings for *Dichanthelium acuminatum* subsp. *lindheimeri* specimens. A. Panicle length. B. Panicle width. The box encompasses the 25th-75th percentile points of data and the midline represents the median. Box plot widths are drawn proportional to the square root of the samples sizes. The legend for each population reports: population collection number; state and county/parish of collection; number of specimens.



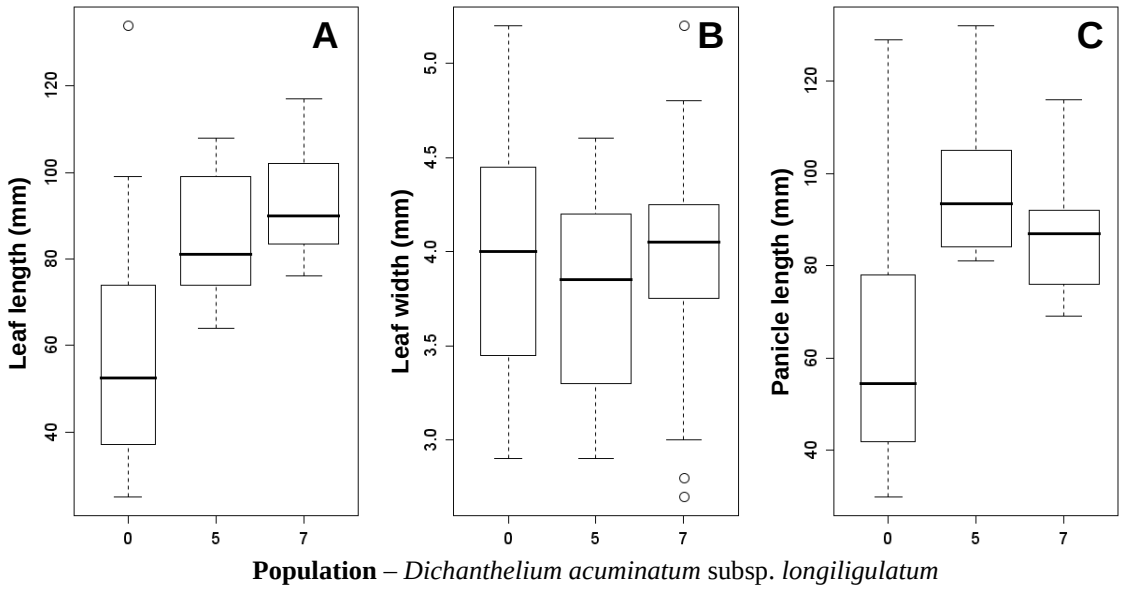
Legend for *Dichanthelium acuminatum* subsp. *fasciculatum* populations: **0** – Herbarium specimens (N=53)*;* **230** –Population 230, TX-Rusk, (N=15).

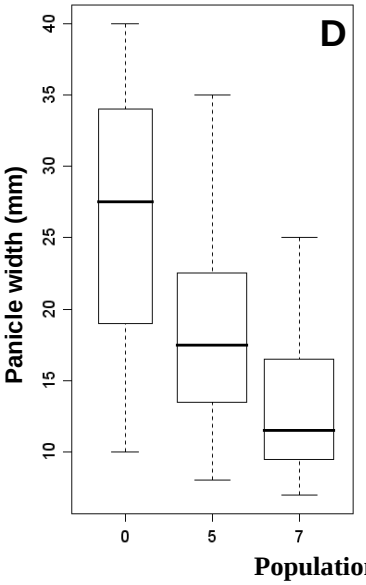
Fig. 12. Standard box and whisker plots of quantitative continuous variables by population groupings for *Dichanthelium acuminatum* subsp. *fasciculatum* specimens. A. Spikelet length. B. Peduncle hair length. C. Leaf sheath hair length. D. Leaf length. E. Leaf width. F. Peduncle length. G. Panicle length. H. panicle width. I. Ligule length. J. Culm length. The box encompasses the 25^{th}-75^{th} percentile points of data and the midline represents the median. Box plot widths are drawn proportional to the square root of the samples sizes. The legend for each population reports: population collection number; state and county/parish of collection; number of specimens.



Legend for *Dichanthelium acuminatum* subsp. *longiluglatum* populations: **0** – Herbarium specimens (N=12)*;* **5** –Population 5, TX-Montgomery, (N=20); **7** – Population 7, TX-Hardin, (N=20).

Fig. 13. Standard box and whisker plots of quantitative continuous variables by population groupings for *Dichanthelium acuminatum* subsp. *longiligulatum* specimens. A. Spikelet length. B. Culm length. C. Peduncle length. D. Peduncle hair length. E. Leaf sheath hair length. F. Ligule length. The box encompasses the 25^{th}-75^{th} percentile points of data and the midline represents the median. Box plot widths are drawn proportional to the square root of the samples sizes. The legend for each population reports: population collection number; state and county/parish of collection; number of specimens.

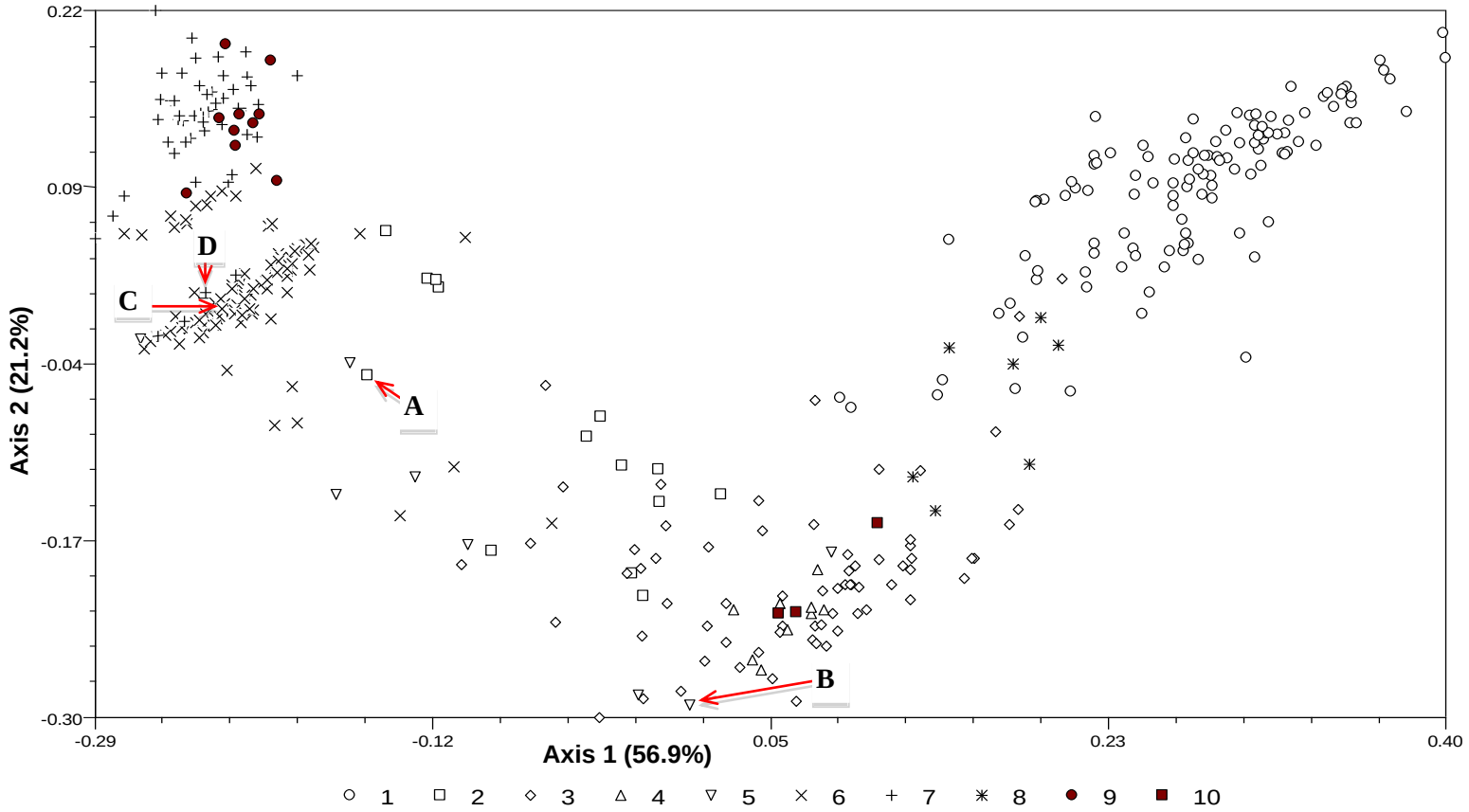




Legend for *Dichanthelium acuminatum* subsp. *acuminatum* populations: **0** – Herbarium specimens (N=12)*;* **5** –Population 5, TX-Montgomery, (N=20); **7** – Population 7, TX-Hardin, (N=20).

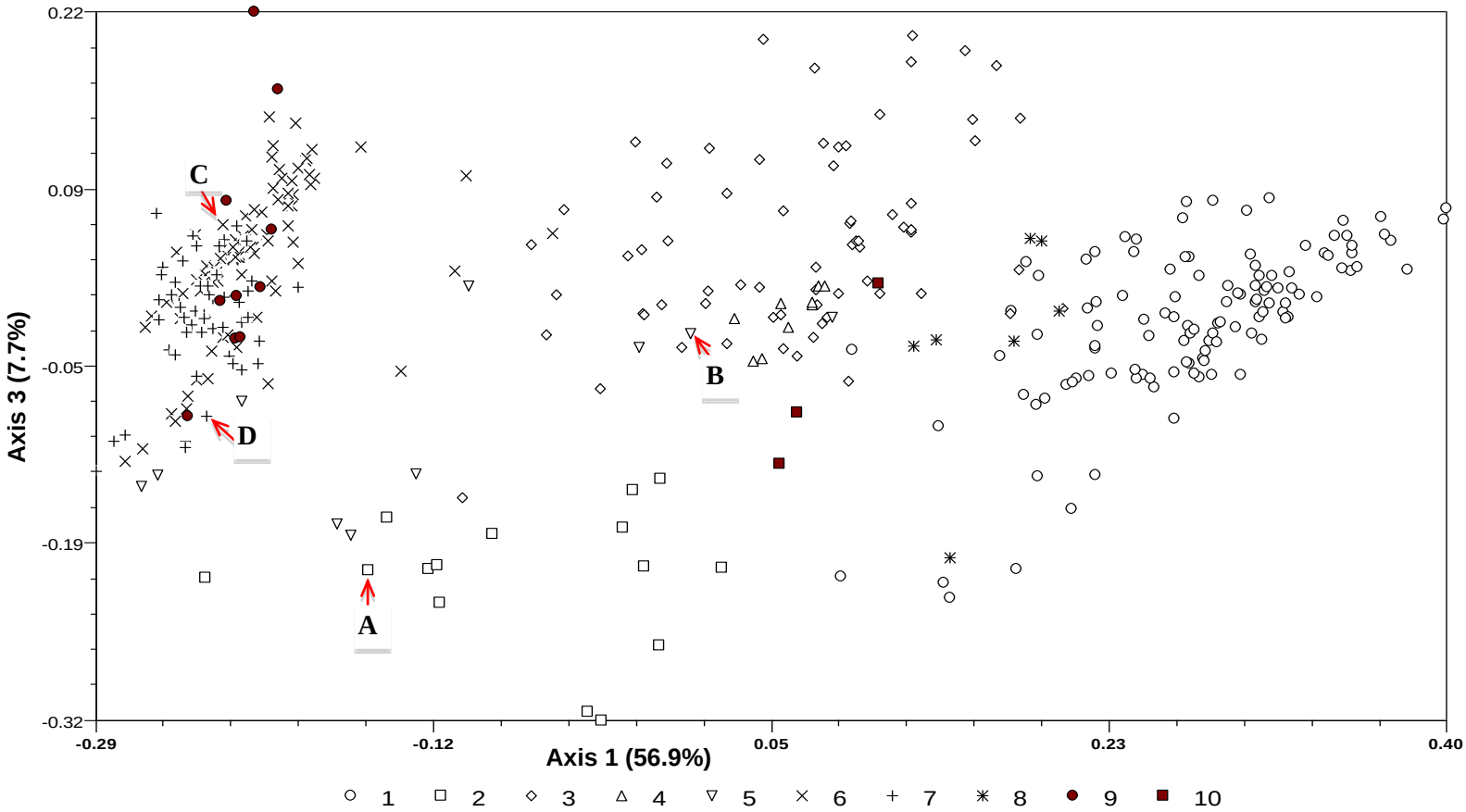
Fig. 14. Standard box and whisker plots of quantitative continuous variables by population groupings for *Dichanthelium acuminatum* subsp. *longiligulatum* specimens. A. Leaf length. B. Leaf width. C. Panicle length. D. Panicle width. The box encompasses the 25^{th}-75^{th} percentile points of data and the midline represents the median. Box plot widths are drawn proportional to the square root of the samples sizes. The legend for each population reports: population collection number; state and county/parish of collection; number of specimens.

Fig. 15. Scatterplot matrix of quantitative morphological character data for the *Dichanthelium acuminatum* subspecies complex. Character codes correspond to those of Fig 1. (*N* = 389 specimens).



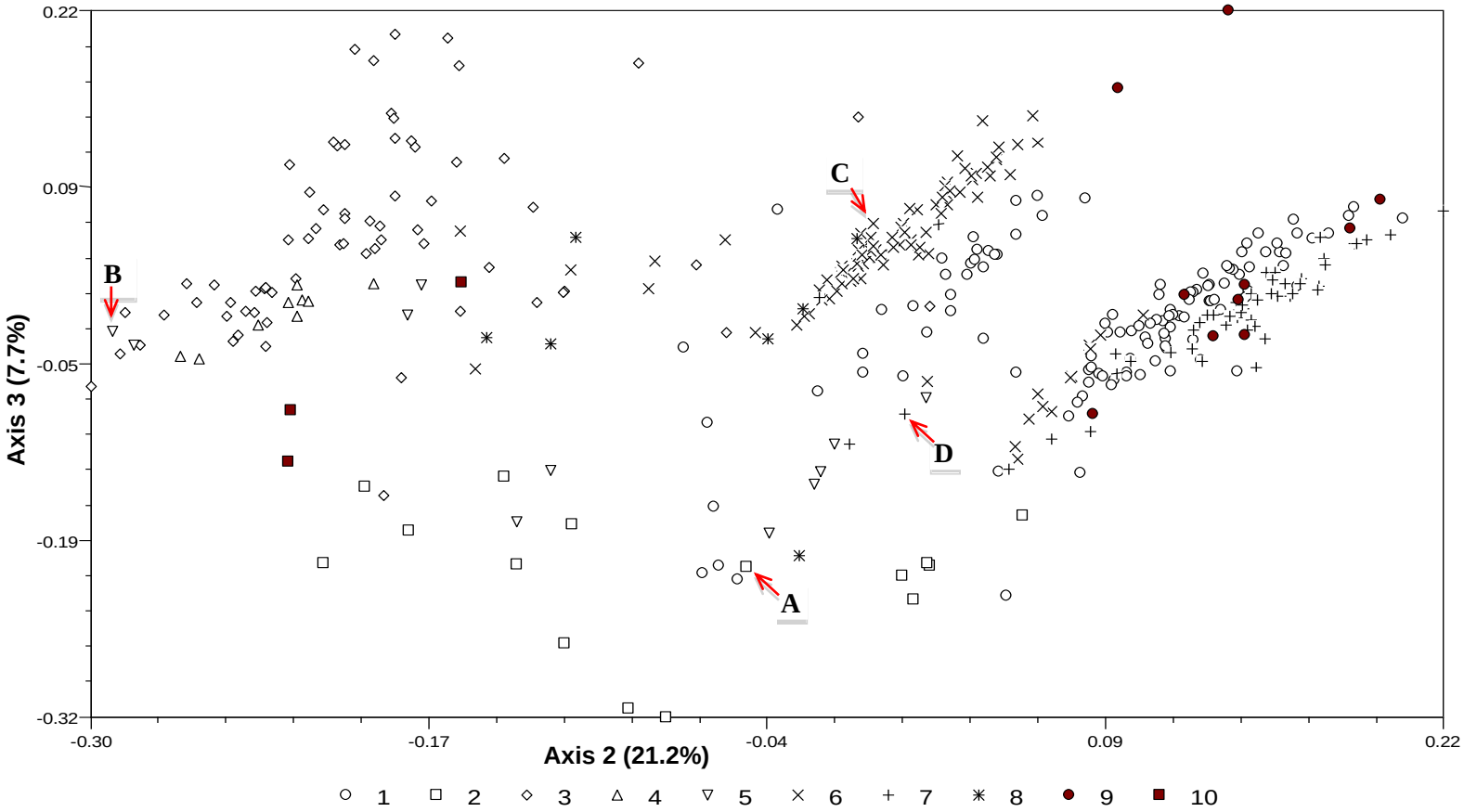
Legend: **1** – *D. acuminatum* ssp. *acuminatum;* **2** – *D. a*. ssp. *columbianum*; **3** – *D. a*. ssp. *fasciculatum*; **4** – *D. a*. ssp. *implicatum*; **5** – *D. a*. ssp.. *leucothrix*; **6** – *D. a*. ssp. *lindheimeri*; **7** – *D. a*. ssp. *longiligulatum*; **8** – *D. a*. ssp. *sericeum*; **9** – *D. a*. ssp. *spretum*; **10** – *D. a*. ssp. *thermal*e.

Fig. 16. Principal coordinates analysis (PCoA) of morphological character data of the *Dichanthelium acuminatum* complex, based on 15 characters (*N* = 389 specimens). Upper case letters identify type specimens: A - subsp. *columbianum*; B - subsp. *leucothrix;* C – subsp. *lindheimeri*; D – subsp. *longiligulatum*.



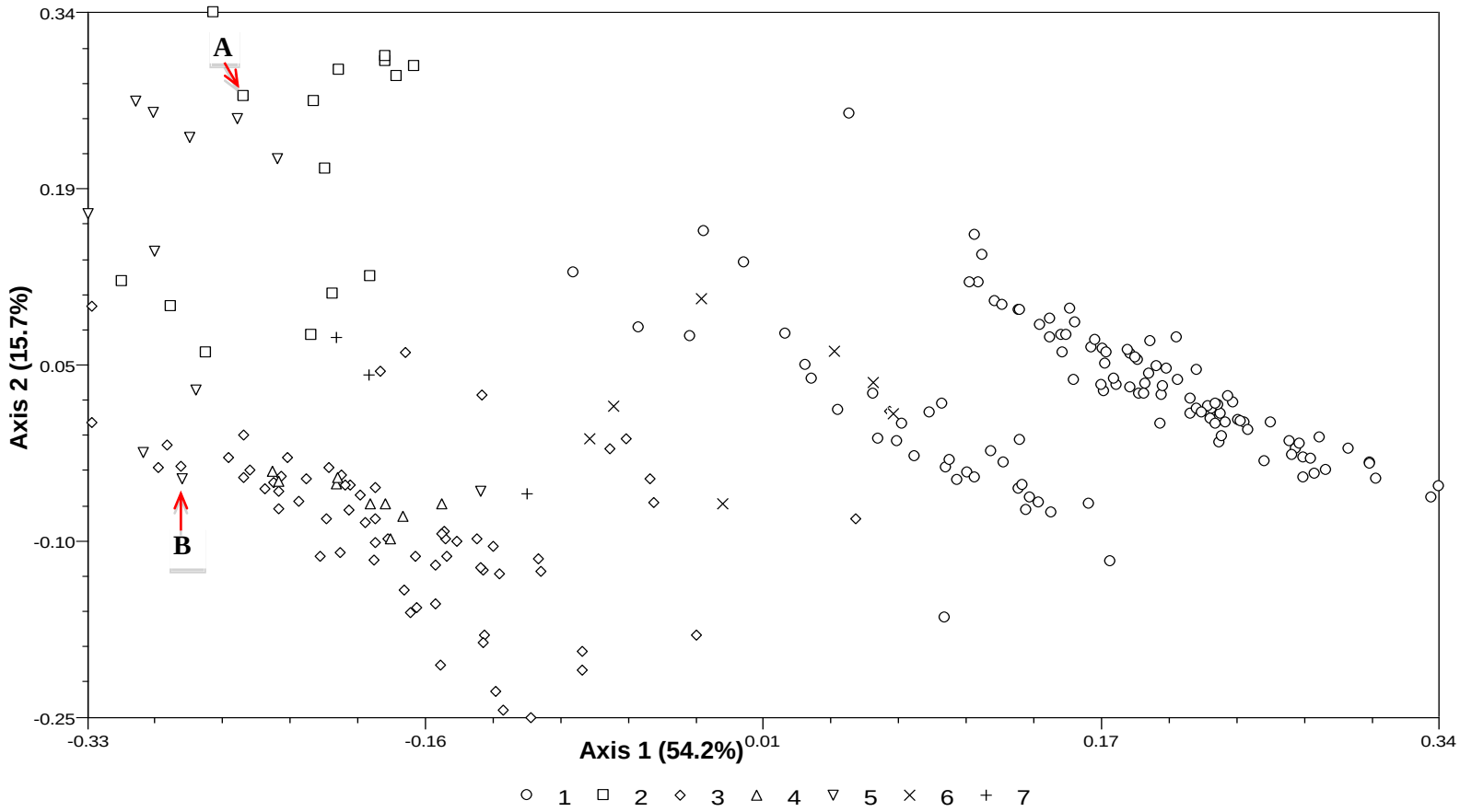
Legend: **1** – *D. acuminatum* ssp. *acuminatum;* **2** – *D. a*. ssp. *columbianum*; **3** – *D. a*. ssp. *fasciculatum*; **4** – *D. a*. ssp. *implicatum*; **5** – *D. a*. ssp.. *leucothrix*; **6** – *D. a*. ssp. *lindheimeri*; **7** – *D. a*. ssp. *longiligulatum*; **8** – *D. a*. ssp. *sericeum*; **9** – *D. a*. ssp. *spretum*; **10** – *D. a*. ssp. *thermal*e.

Fig. 17. Principal coordinates analysis (PCoA) of morphological character data of the *Dichanthelium acuminatum* complex, based on 15 characters (*N* = 389 specimens). Upper case letters identify type specimens: A - subsp. *columbianum*; B - subsp. *leucothrix;* C – subsp. *lindheimeri*; D – subsp. *longiligulatum*.



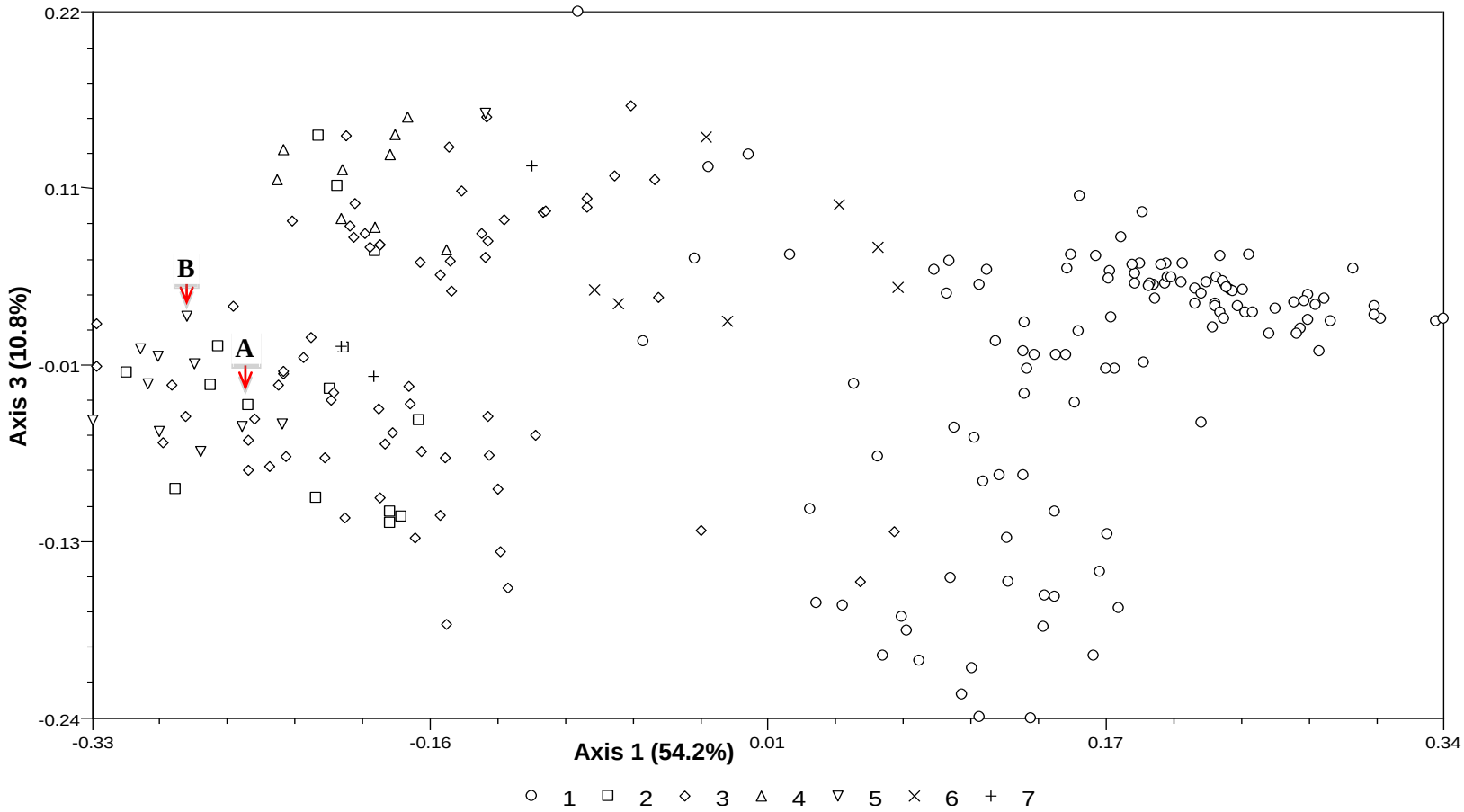
Legend: **1** – *D. acuminatum* ssp. *acuminatum;* **2** – *D. a*. ssp. *columbianum*; **3** – *D. a*. ssp. *fasciculatum*; **4** – *D. a*. ssp. *implicatum*; **5** – *D. a*. ssp.. *leucothrix*; **6** – *D. a*. ssp. *lindheimeri*; **7** – *D. a*. ssp. *longiligulatum*; **8** – *D. a*. ssp. *sericeum*; **9** – *D. a*. ssp. *spretum*; **10** – *D. a*. ssp. *thermal*e.

Fig. 18. Principal coordinates analysis (PCoA) of morphological character data of the *Dichanthelium acuminatum* complex, based on 15 characters (*N* = 389 specimens). Upper case letters identify type specimens: A - subsp. *columbianum*; B - subsp. *leucothrix;* C – subsp. *lindheimeri*; D – subsp. *longiligulatum*.



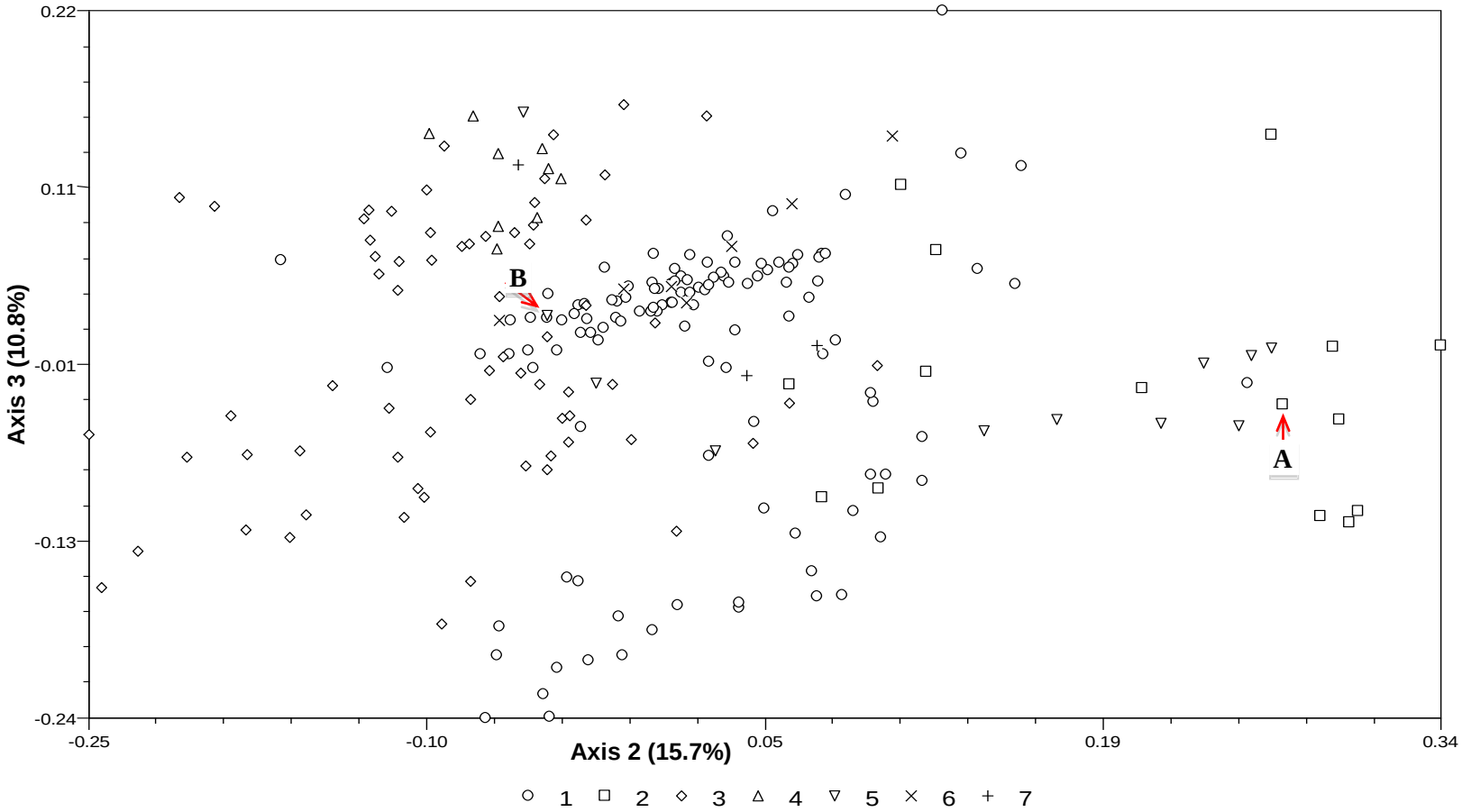
Legend: **1** – *D. acuminatum* ssp. *acuminatum;* **2** – *D. a*. ssp. *columbianum*; **3** – *D. a*. ssp. *fasciculatum*; **4** – *D. a*. ssp. *implicatum*; **5** – *D. a*. ssp.. *leucothrix*; **6** – *D. a*. ssp. *sericeum*; **7** – *D. a*. ssp. *thermal*e.

Fig. 19. Principal coordinates analysis (PCoA) of morphological character data of the pubescent taxa of the *Dichanthelium acuminatum* complex, based on 15 characters (*N* = 230 specimens). Upper case letters identify type specimens: A - subsp. *columbianum*; B - subsp. *leucothrix*.



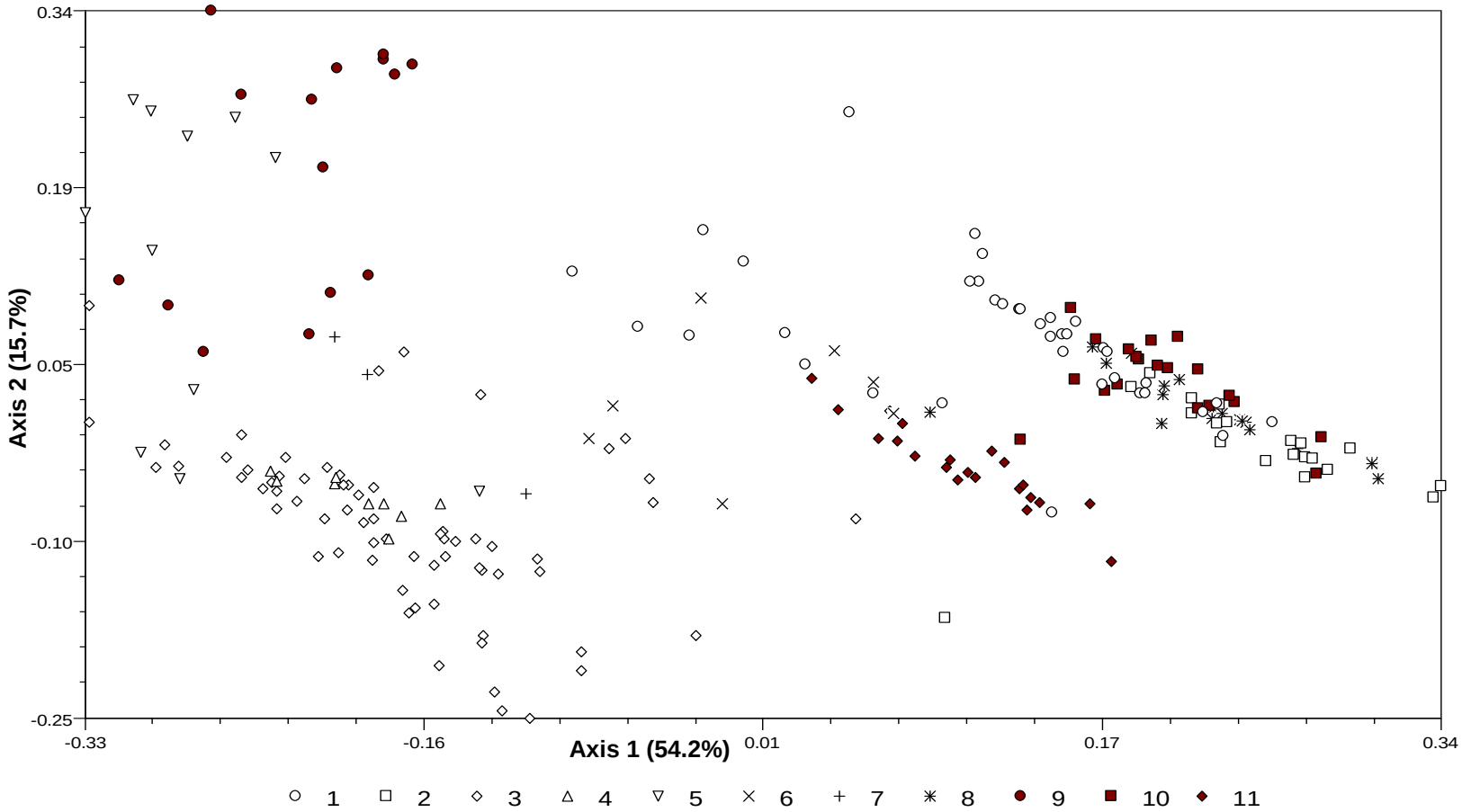
Legend: **1** – *D. acuminatum* ssp. *acuminatum;* **2** – *D. a*. ssp. *columbianum*; **3** – *D. a*. ssp. *fasciculatum*; **4** – *D. a*. ssp. *implicatum*; **5** – *D. a*. ssp.. *leucothrix*; **6** – *D. a*. ssp. *sericeum*; **7** – *D. a*. ssp. *thermal*e.

Fig. 20. Principal coordinates analysis (PCoA) of morphological character data of the pubescent taxa of the *Dichanthelium acuminatum* complex, based on 15 characters (*N* = 230 specimens). Upper case letters identify type specimens: A - subsp. *columbianum*; B - subsp. *leucothrix*.



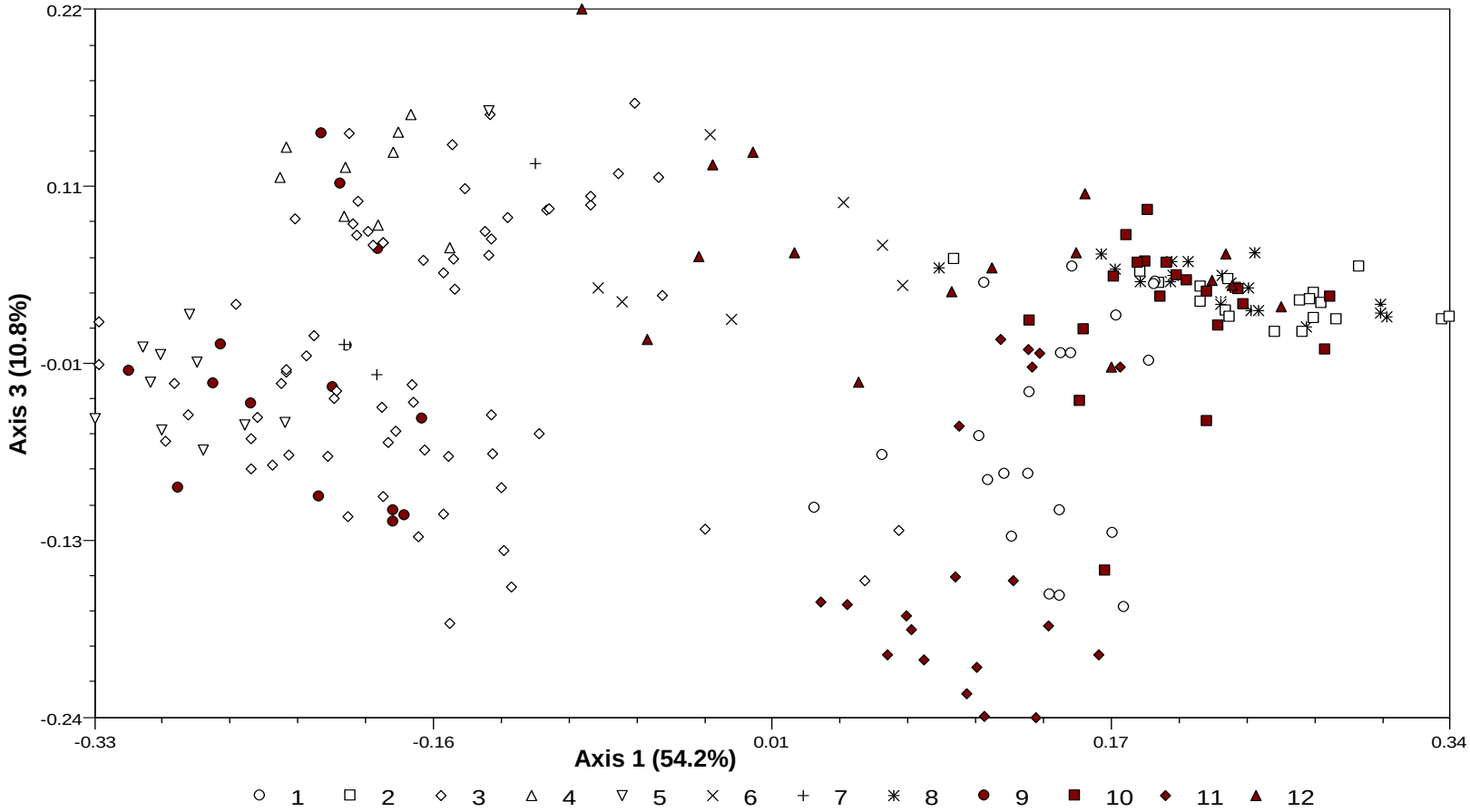
Legend: **1** – *D. acuminatum* ssp. *acuminatum;* **2** – *D. a*. ssp. *columbianum*; **3** – *D. a*. ssp. *fasciculatum*; **4** – *D. a*. ssp. *implicatum*; **5** – *D. a*. ssp.. *leucothrix*; **6** – *D. a*. ssp. *sericeum*; **7** – *D. a*. ssp. *thermal*e.

Fig. 21. Principal coordinates analysis (PCoA) of morphological character data of the pubescent taxa of the *Dichanthelium acuminatum* complex, based on 15 characters (*N* = 230 specimens). Upper case letters identify type specimens: A - subsp. *columbianum*; B - subsp. *leucothrix*.



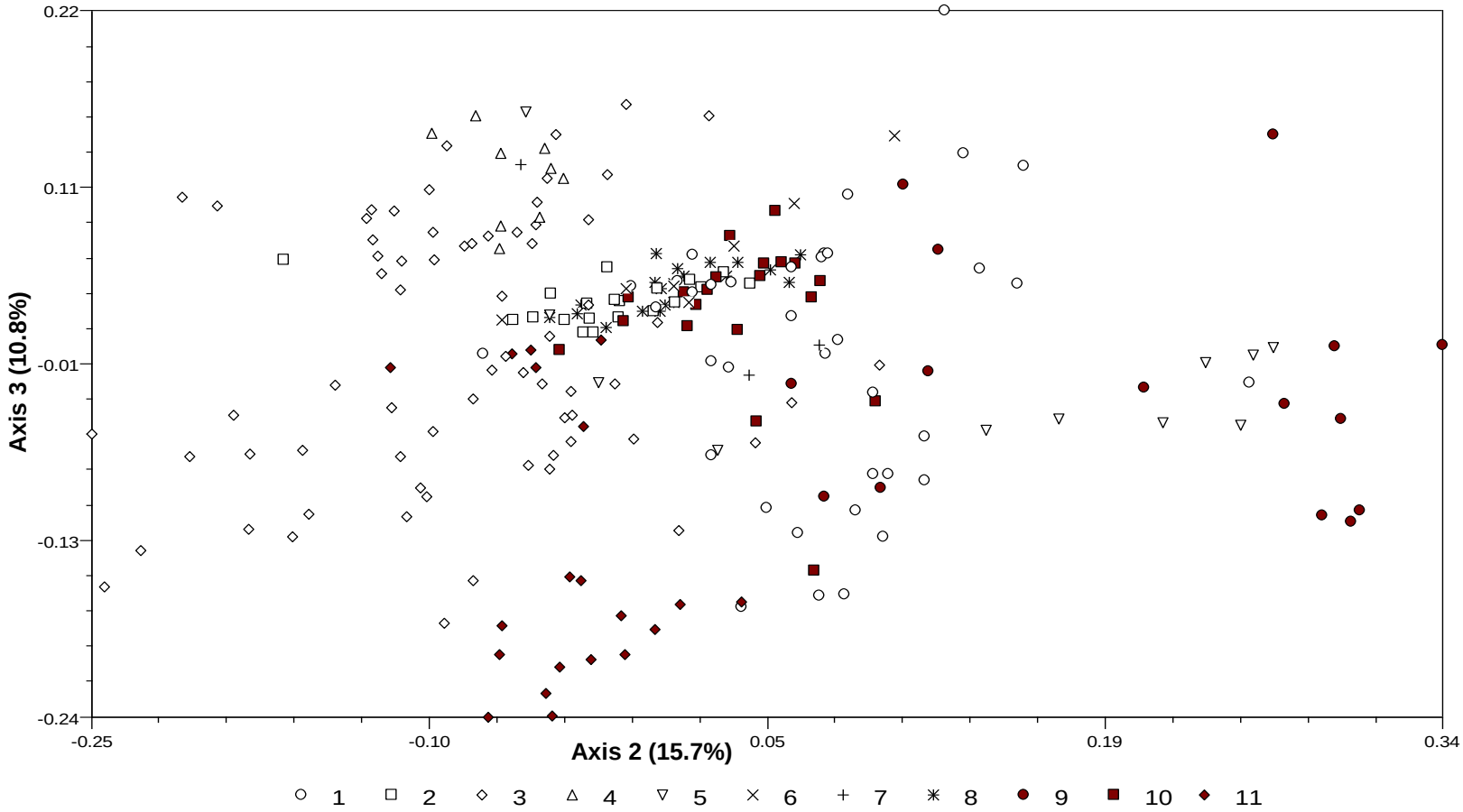
Legend: **1** – *D. acuminatum* ssp. *acuminatum* pop 1, TX-Leon*;* **2** – - *D. a.* ssp. *acuminatum* pop 15, LA-Vernon; **3** – *D. a*. ssp. *fasciculatum*; **4** – *D. a*. ssp. *implicatum*; **5** – *D. a*. ssp.. *leucothrix*; **6** – *D. a*. ssp. *sericeum*; **7** – *D. a*. ssp. *thermale*; **8**- *D. a.* ssp. *acuminatum* pop 8, TX-Hardin; **9** - *D. a*. ssp. *columbianum;* **10** - *D. a.* ssp. *acuminatum* pop 17, LA-Vernon; **11** - *D. a.* ssp. *acuminatum* pop 4, TX-Montgomery.

Fig. 22. Principal coordinates analysis (PCoA) of morphological character data of the pubescent taxa of the *Dichanthelium acuminatum* complex, based on 15 characters (*N* = 230 specimens). Populations of subspecies *acuminatum* are plotted with unique symbols.



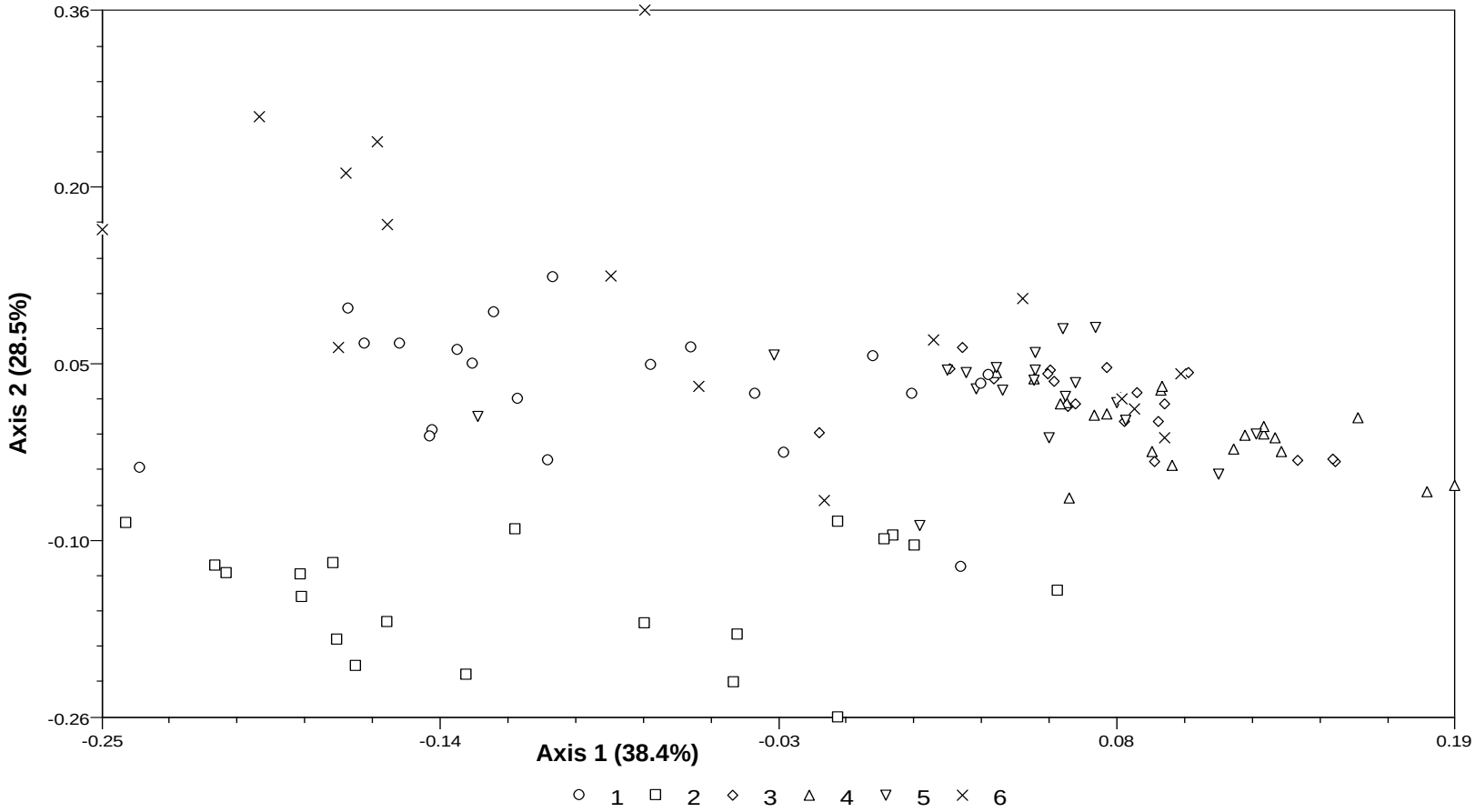
Legend: **1** – *D. acuminatum* ssp. *acuminatum* pop 1, TX-Leon; **2** – - *D. a.* ssp. *acuminatum* pop 15, LA-Vernon; **3** – *D. a*. ssp. *fasciculatum*; **4** – *D. a*. ssp. *implicatum*; **5** – *D. a*. ssp.. *leucothrix*; **6** – *D. a*. ssp. *sericeum*; **7** – *D. a*. ssp. *thermale*; **8**- *D. a.* ssp. *acuminatum* pop 8, TX-Hardin; **9** - *D. a*. ssp. *columbianum;* **10** - *D. a.* ssp. *acuminatum* pop 17, LA-Vernon; **11** - *D. a.* ssp. *acuminatum* pop 4, TX-Montgomery; **12** – *D. a*. ssp. *acuminatum* herbarium specimens.

Fig. 23. Principal coordinates analysis (PCoA) of morphological character data of the pubescent taxa of the *Dichanthelium acuminatum* complex, based on 15 characters (*N* = 230 specimens). Populations of subspecies *acuminatum* are plotted with unique symbols.



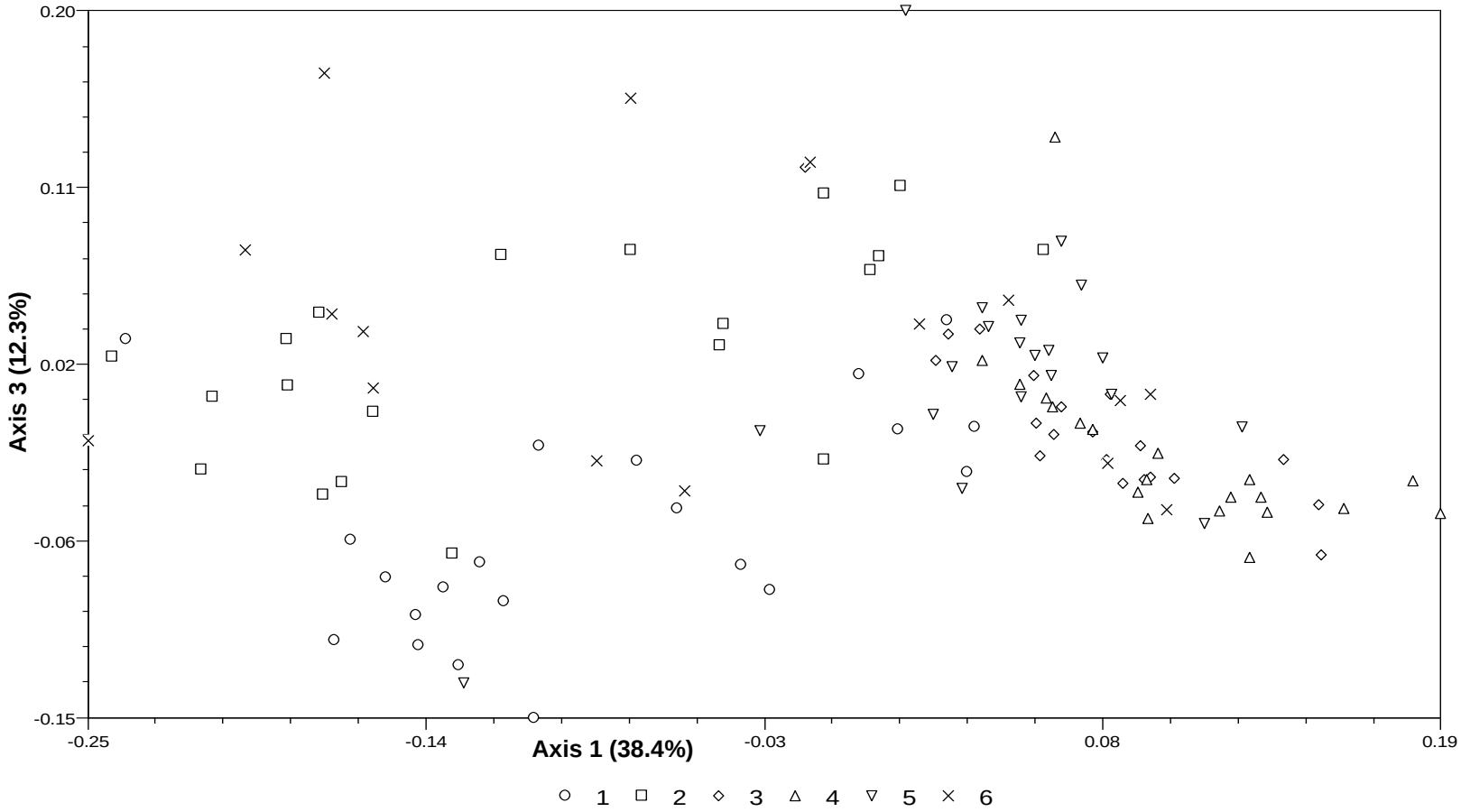
Legend: **1** – *D. acuminatum* ssp. *acuminatum* pop 1, TX-Leon; **2** – - *D. a.* ssp. *acuminatum* pop 15, LA-Vernon; **3** – *D. a*. ssp. *fasciculatum*; **4** – *D. a*. ssp. *implicatum*; **5** – *D. a*. ssp.. *leucothrix*; **6** – *D. a*. ssp. *sericeum*; **7** – *D. a*. ssp. *thermale*; **8**- *D. a.* ssp. *acuminatum* pop 8, TX-Hardin; **9** - *D. a*. ssp. *columbianum;* **10** - *D. a.* ssp. *acuminatum* pop 17, LA-Vernon; **11** - *D. a.* ssp. *acuminatum* pop 4, TX-Montgomery.

Fig. 24. Principal coordinates analysis (PCoA) of morphological character data of the pubescent taxa of the *Dichanthelium acuminatum* complex, based on 15 characters (*N* = 230 specimens). Populations of subspecies *acuminatum* are plotted with unique symbols.



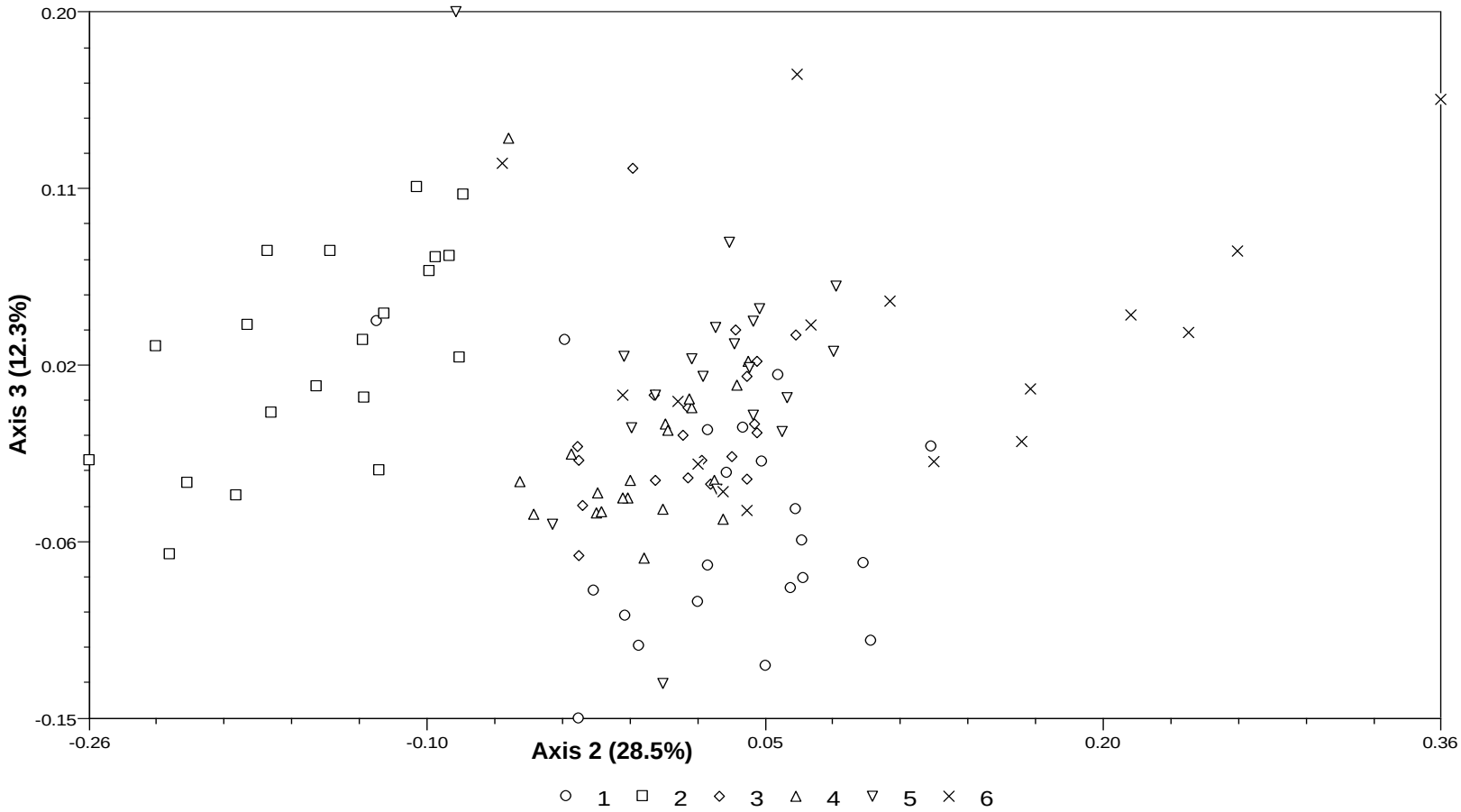
Key to *D. acuminatum* subsp. *acuminatum* populations: **1** – *Hammer* pop 1, TX-Leon; **2** – *Hammer* pop 4, TX-Montgomery; **3** – *Hammer* pop 8, TX-Leon;**4** – *Hammer* pop 15**,** LA-Vernon Parish; **5** – *Hammer* pop 17**,** LA-Vernon Parish; **6** – herbarium specimens. State and county of collection are included for each population.

Fig. 25. Principal coordinates analysis (PCoA) of morphological character data for *Dichanthelium acuminatum* subsp. *acuminatum* populations, based on 15 characters (*N* = 117 specimens).



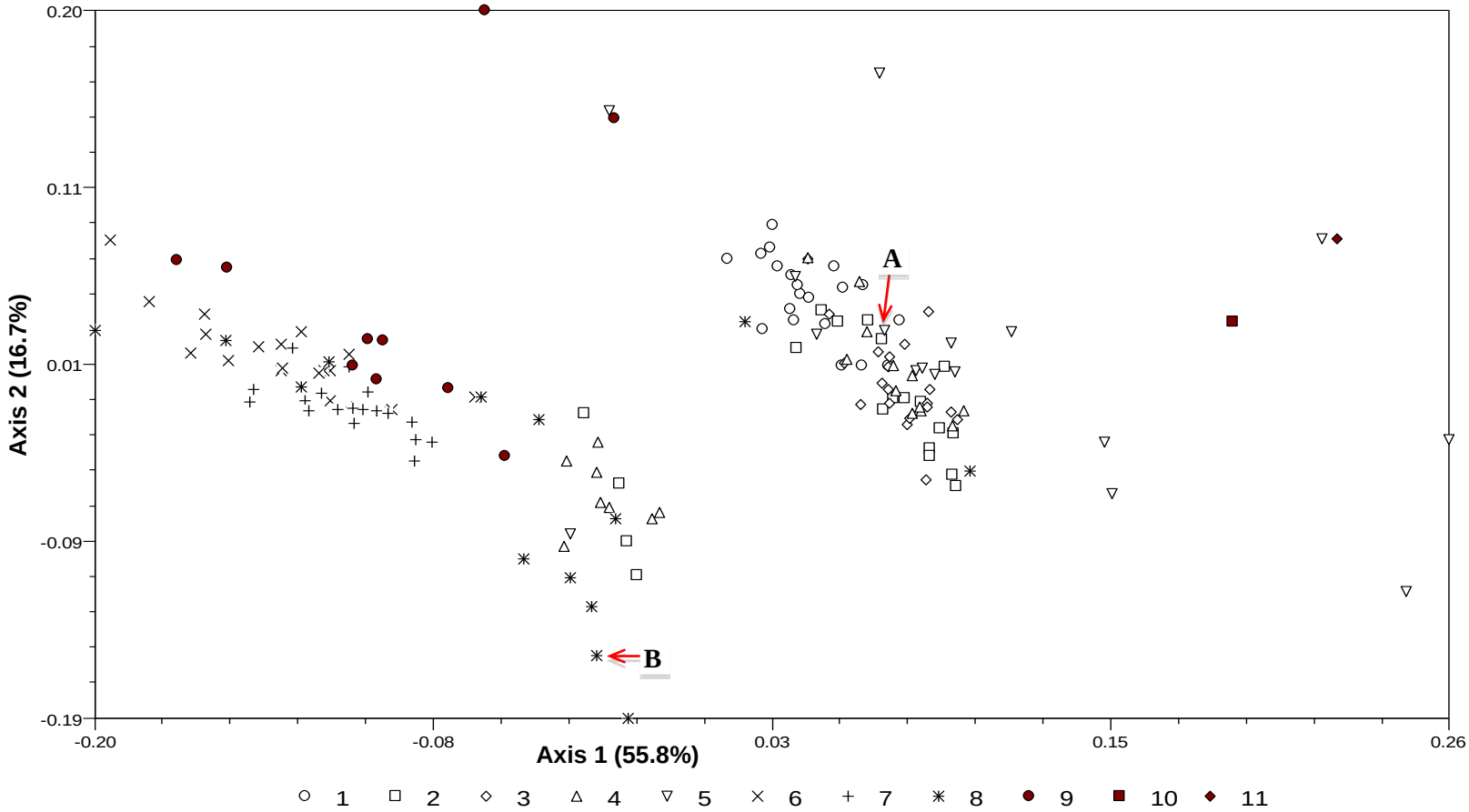
Key to *D. acuminatum* subsp. *acuminatum* populations: **1** – *Hammer* pop 1, TX-Leon; **2** – *Hammer* pop 4, TX-Montgomery; **3** – *Hammer* pop 8, TX-Leon;**4** – *Hammer* pop 15**,** LA-Vernon Parish; **5** – *Hammer* pop 17**,** LA-Vernon Parish; **6** – herbarium specimens. State and county/parish of collection are included for each population.

Fig. 26. Principal coordinates analysis (PCoA) of morphological character data for *Dichanthelium acuminatum* subsp. *acuminatum* populations, based on 15 characters (*N* = 117 specimens).



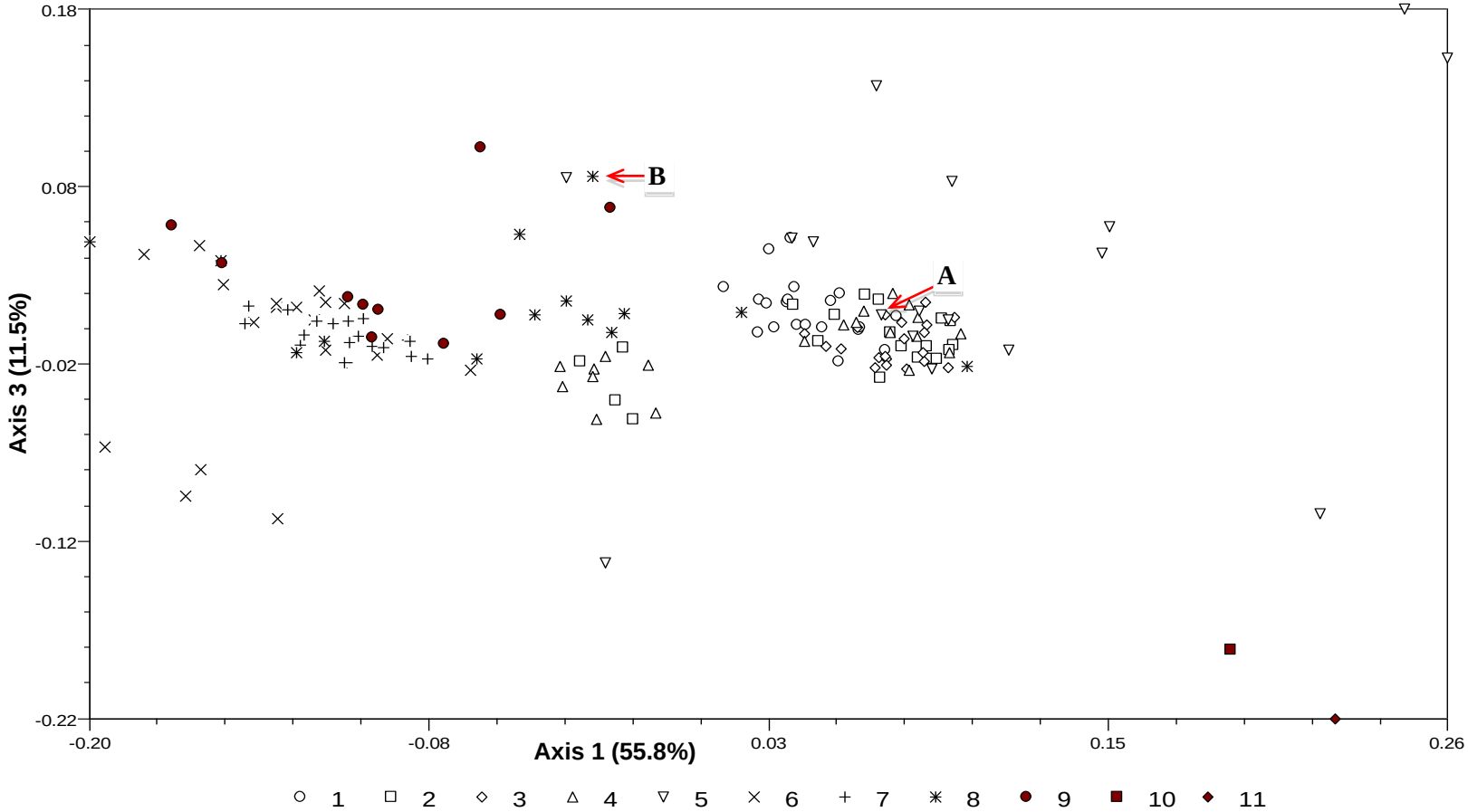
Key to *D. acuminatum* subsp. *acuminatum* populations: **1** – *Hammer* pop 1, TX-Leon; **2** – *Hammer* pop 4, TX-Montgomery; **3** – *Hammer* pop 8, TX-Leon;**4** – *Hammer* pop 15**,** LA-Vernon Parish; **5** – *Hammer* pop 17**,** LA-Vernon Parish; **6** – herbarium specimens. State and county/parish of collection are included for each population.

Fig. 27. Principal coordinates analysis (PCoA) of morphological character data for *Dichanthelium acuminatum* subsp. *acuminatum* populations, based on 15 characters (*N* = 117 specimens).



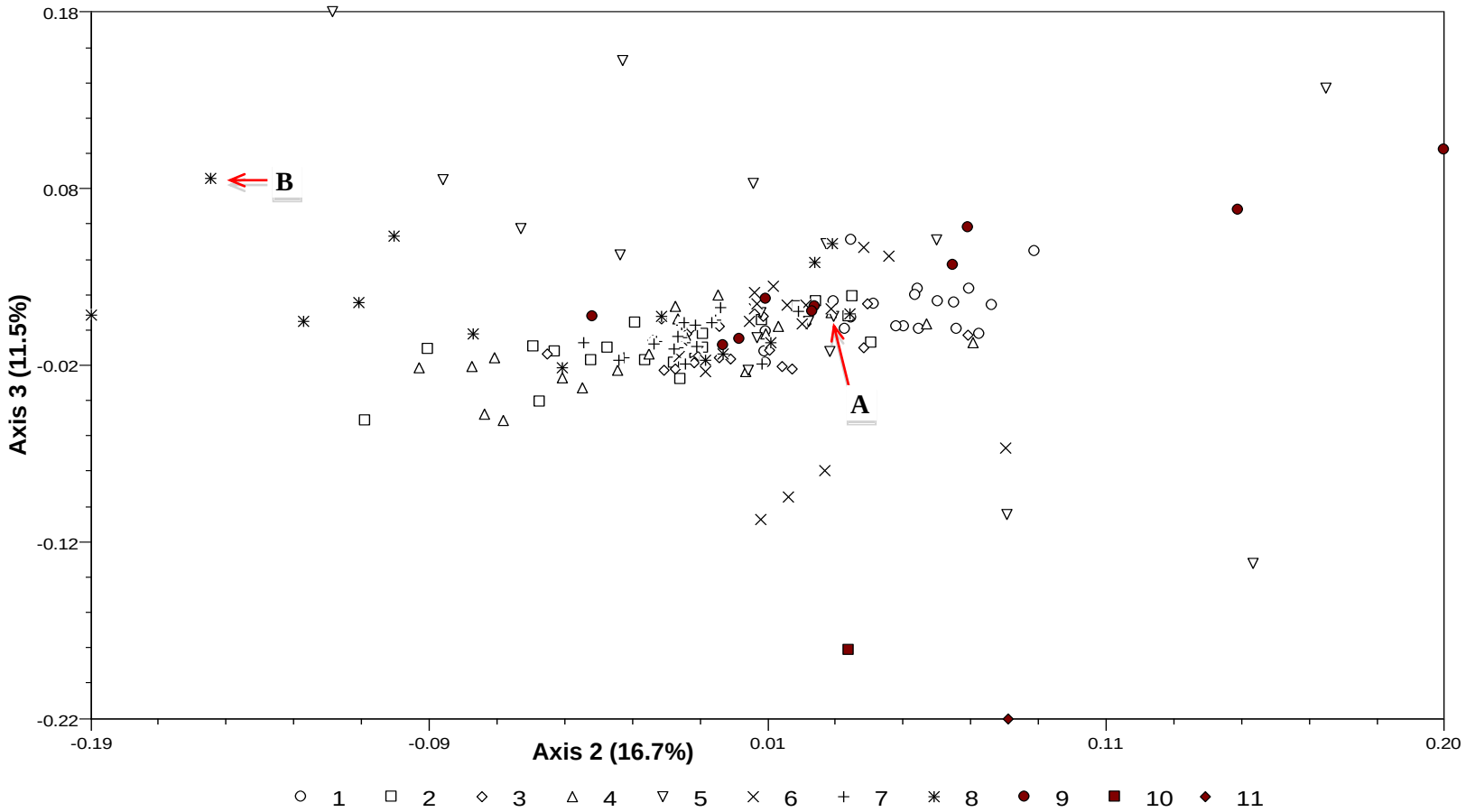
Key to subspecies: **1** – subsp. *lindheimeri*, *Hammer* pop 3, TX-Brazos; **2** – subsp. *lindheimeri*, *Hammer* pop 19, TX-Montgomery; **3** – subsp. *lindheimeri*, *Hammer* pop 20, TX-Leon;**4** – subsp. *lindheimeri*, *Hammer* pop 210**,** LA-Vernon Parish; **5** - subsp. *lindherimeri* herbarium specimens; **6** – subsp. *longiligulatum*, *Hammer* pop 5, TX-??; 7 – subsp. *longiligulatum*, *Hammer* pop 7, TX-??; 8 – subsp. *longiligulatum* herbarium specimens; 9 – subsp. *spretum* herbarium specimens; 10 – subsp. *lindherimeri*, *Hammer* 303; 11 – subsp. *lindheimeri*, *Hammer* 328.

Fig. 28. Principal coordinates analysis (PCoA) of morphological character data for *Dichanthelium acuminatum* subsp. *lindheimeri*, subsp. *longiligulatum* and subsp. *spretum* populations and herbarium specimens, based on 15 characters (*N* = 161 specimens). Upper case letters identify type specimens: A - subsp. *lindheimeri*; B - subsp. *longiligulatum.*



Key to subspecies: **1** – subsp. *lindheimeri*, *Hammer* pop 3, TX-Brazos; **2** – subsp. *lindheimeri*, *Hammer* pop 19, TX-Montgomery; **3** – subsp. *lindheimeri*, *Hammer* pop 20, TX-Leon;**4** – subsp. *lindheimeri*, *Hammer* pop 210**,** LA-Vernon Parish; **5** - subsp. *lindherimeri* herbarium specimens; **6** – subsp. *longiligulatum*, *Hammer* pop 5, TX-??; 7 – subsp. *longiligulatum*, *Hammer* pop 7, TX-??; 8 – subsp. *longiligulatum* herbarium specimens; 9 – subsp. *spretum* herbarium specimens; 10 – subsp. *lindherimeri*, *Hammer* 303; 11 – subsp. *lindheimeri*, *Hammer* 328.

Fig. 29. Principal coordinates analysis (PCoA) of morphological character data for *Dichanthelium acuminatum* subsp. *lindheimeri*, subsp. *longiligulatum* and subsp. *spretum* populations and herbarium specimens, based on 15 characters (*N* = 161 specimens). Upper case letters identify type specimens: A - subsp. *lindheimeri*; B - subsp. *longiligulatum.*



Key to subspecies: **1** – subsp. *lindheimeri*, *Hammer* pop 3, TX-Brazos; **2** – subsp. *lindheimeri*, *Hammer* pop 19, TX-Montgomery; **3** – subsp. *lindheimeri*, *Hammer* pop 20, TX-Leon;**4** – subsp. *lindheimeri*, *Hammer* pop 210**,** LA-Vernon Parish; **5** - subsp. *lindherimeri* herbarium specimens; **6** – subsp. *longiligulatum*, *Hammer* pop 5, TX-??; 7 – subsp. *longiligulatum*, *Hammer* pop 7, TX-??; 8 – subsp. *longiligulatum* herbarium specimens; 9 – subsp. *spretum* herbarium specimens; 10 – subsp. *lindherimeri*, *Hammer* 303; 11 – subsp. *lindheimeri*, *Hammer* 328.

Fig. 30. Principal coordinates analysis (PCoA) of morphological character data for *Dichanthelium acuminatum* subsp. *lindheimeri*, subsp. *longiligulatum* and subsp. *spretum* populations and herbarium specimens, based on 15 characters (*N* = 161 specimens). Upper case letters identify type specimens: A - subsp. *lindheimeri*; B - subsp. *longiligulatum.*

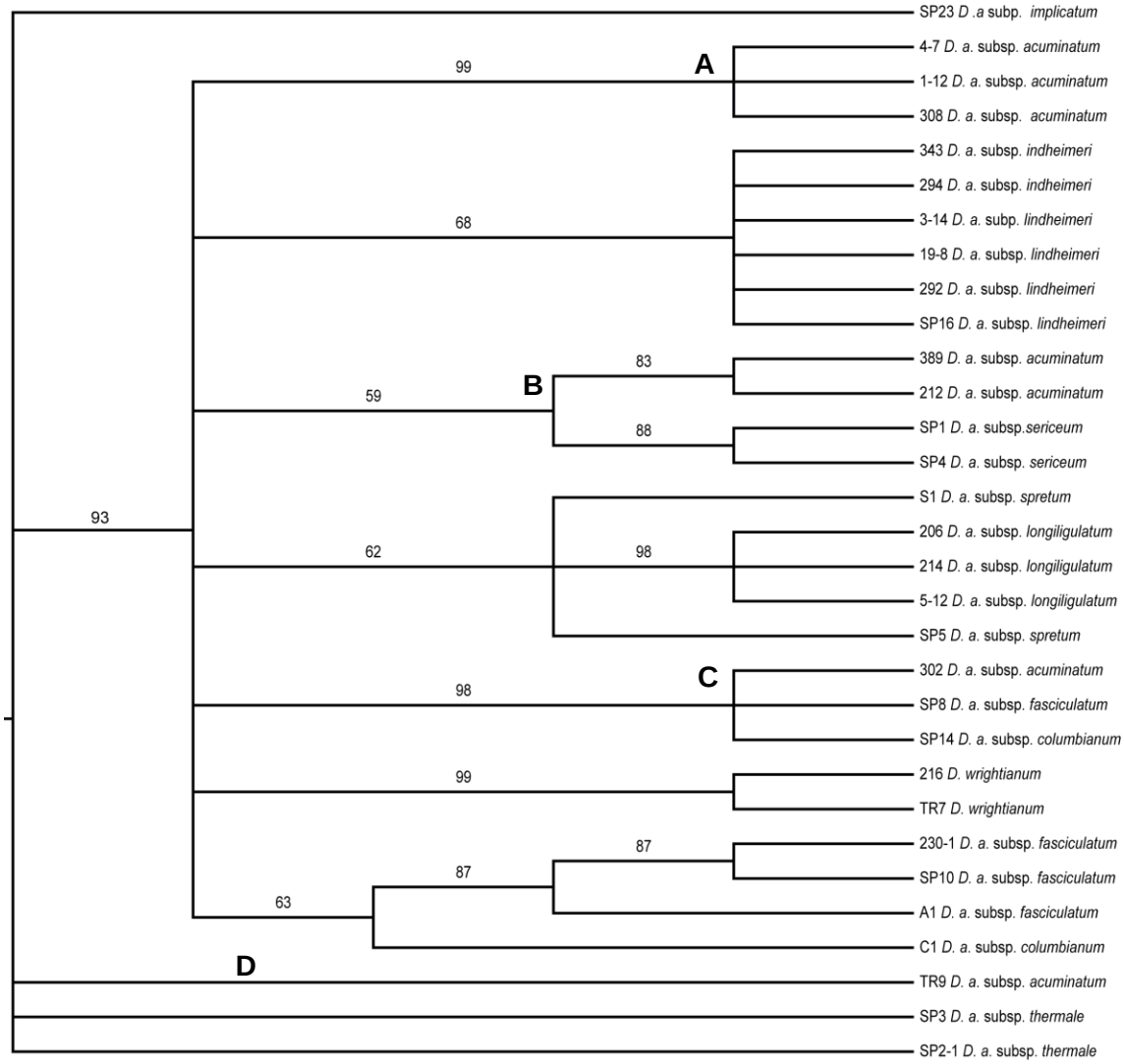


Fig. 31. Majority-rule consensus of six equally parsimonious trees showing phylogenetic relationships/genetic divergence among the taxa of the *Dichanthelium acuminatum* subsp. *acuminatum* complex from analysis of GBSSI sequences. Tree length = 79, CI = 0.85, RI = 0.92. Bootstrap values greater than 50% are shown above each branch.

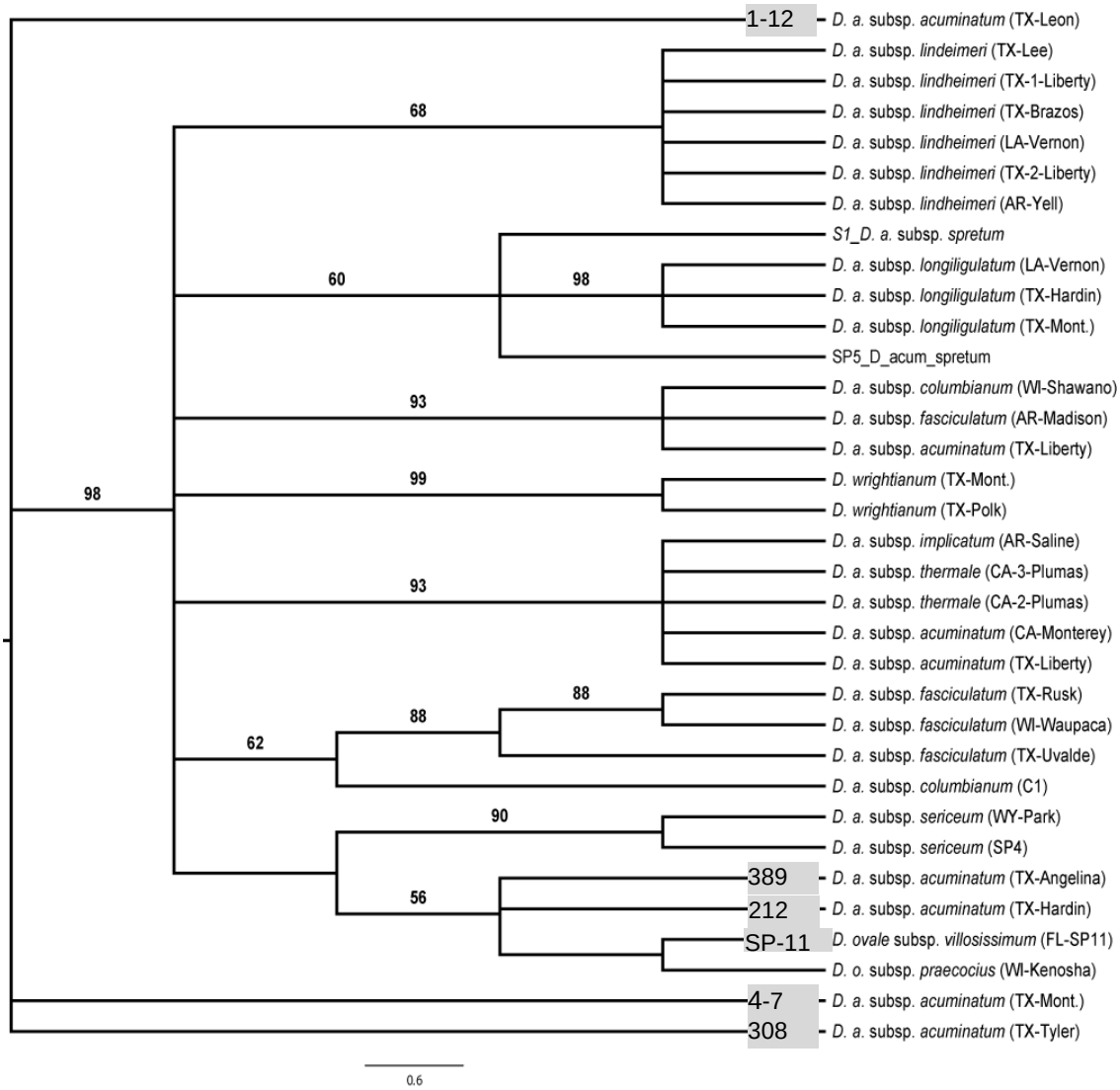


Fig. 32. Majority-rule consensus of six equally parsimonious trees showing phylogenetic relationships/genetic divergence among the taxa of the *Dichanthelium acuminatum* subsp. *acuminatum* complex and selected taxa of the *D. ovale* subspecies complex from analysis of GBSSI sequences. Tree length = 89, CI = 0.84, RI = 0.91. Bootstrap values greater than 50% are shown above each branch. Specimen labels are provided at the tips of some nodes for reference and comparison to Fig. 34.

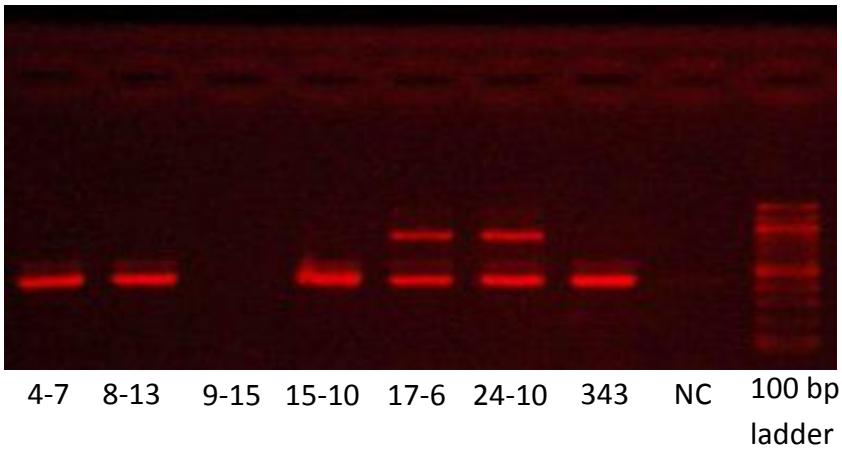
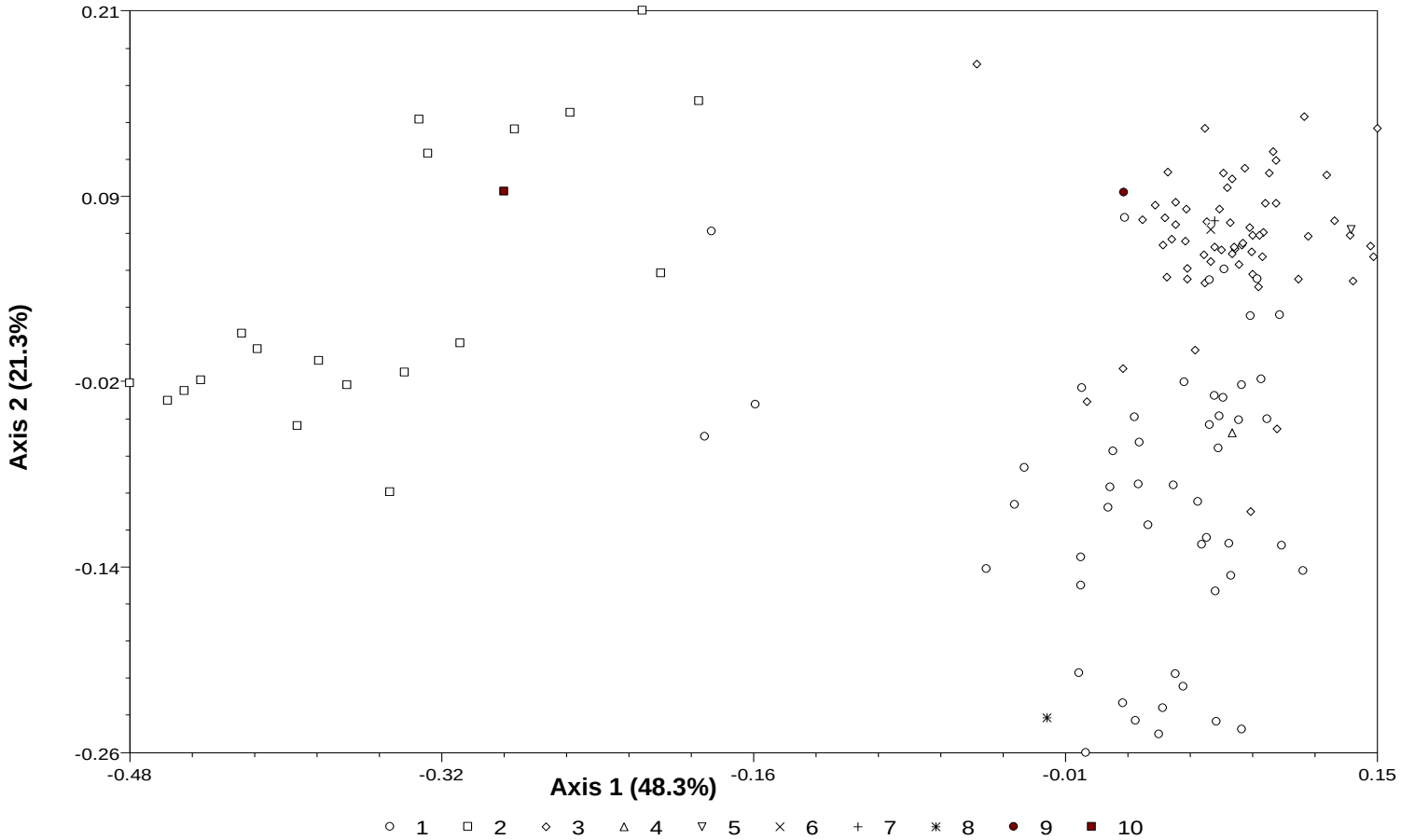
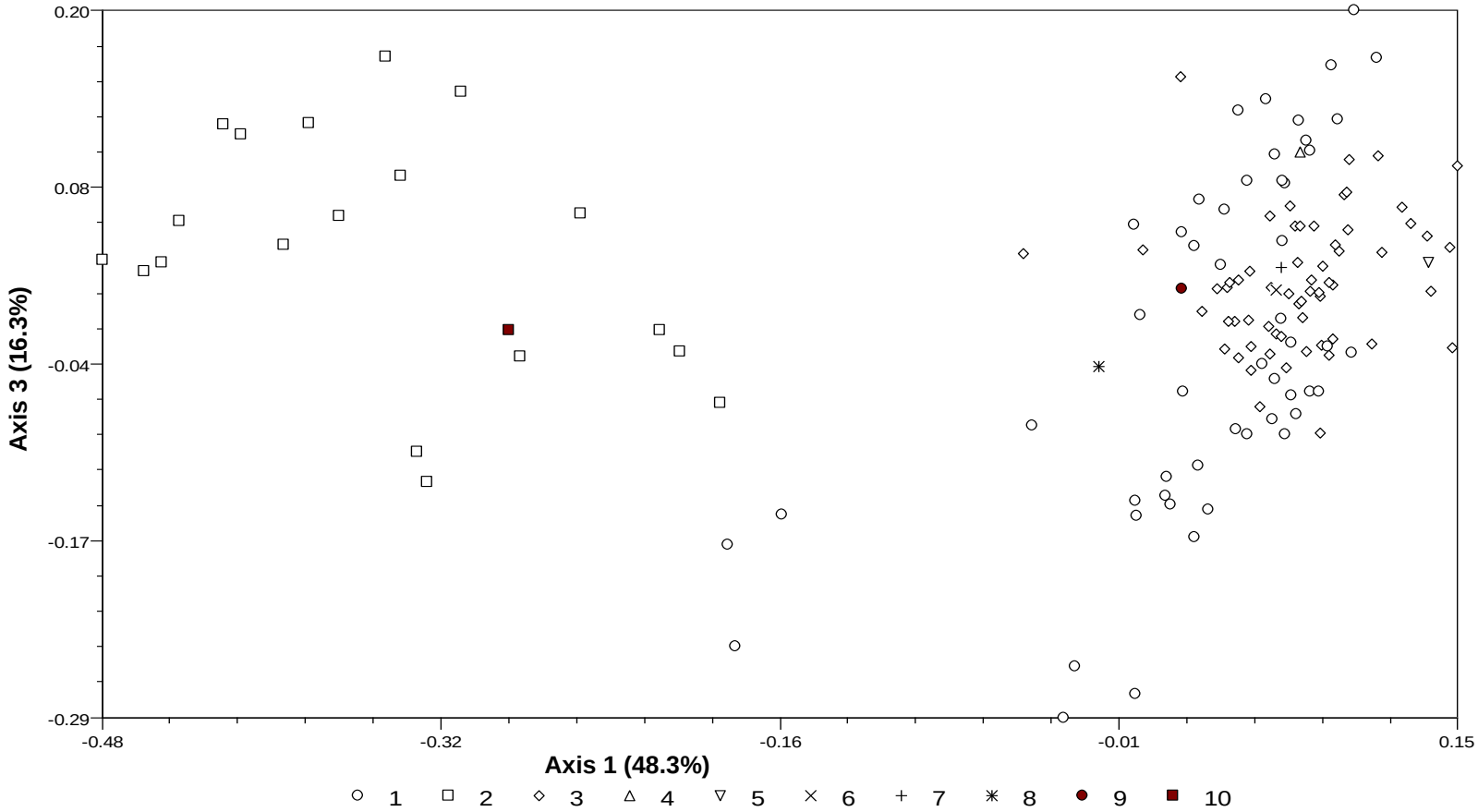


Fig. 33. Gel electrophoresis visualization of GBSSI PCR analysis with primer pair E2f + G2b1 for atypical specimens from *D. acuminatum* subsp. *acuminatum* populations. Gels are 1.5% agarose stained with ethidium bromide. Numbers below the gel bands indicate specimen ID. Loading wells are on the top side of the image.



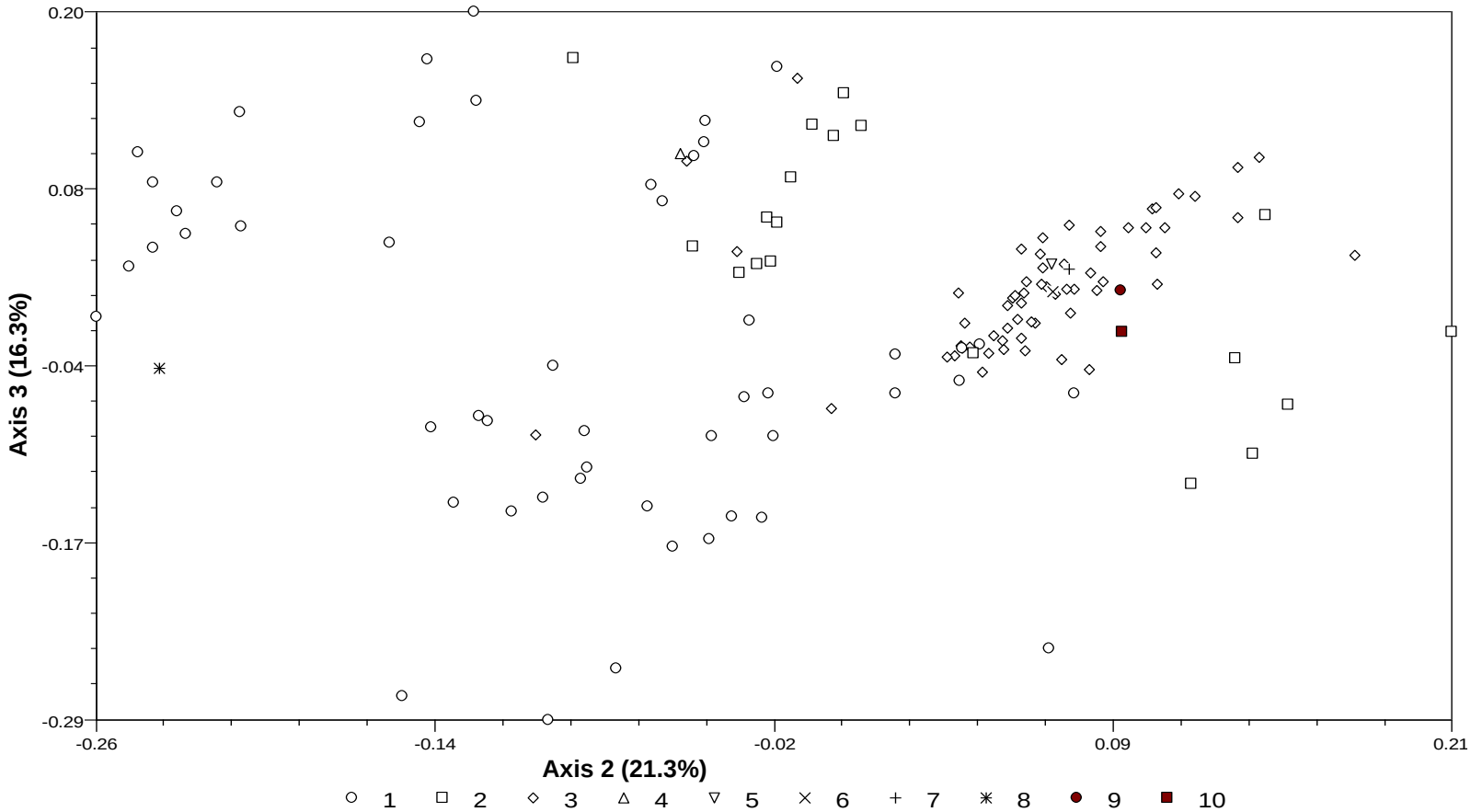
Key: **1** - subsp. *acuminatum (Hammer* pop 1 and pop 4, herb. specimens*)*; **2** – *D. ovale* subsp. *villosissimum* (*Hammer* pop 466, herb. specimens); **3** – subsp. *acuminatum* "intermediates" (*Hammer* pops 8, 15 and 17);**4** – subsp. *acum*. (*Hammer* 4-7); **5** – subsp. *acum. (Hammer* 389); **6** – subsp. *acum*. (*Hammer* 221); **7** – subsp. *acum*. (*Hammer* 308); **8** – subsp. *acum*. (*Hammer* 1-12); **9** – subsp. *acum*. (*Hammer* 212); **10** – *D. ovale*. subsp *villosissimum* (*Freckmann*)

Fig. 34. Principal coordinates analysis (PCoA) of morphological character data for *Dichanthelium acuminatum* subsp. *acuminatum* populations, *D. ovale* subsp. *villosissimum* populations and selected herbarium specimens, based on 15 characters (N = 138 specimens).



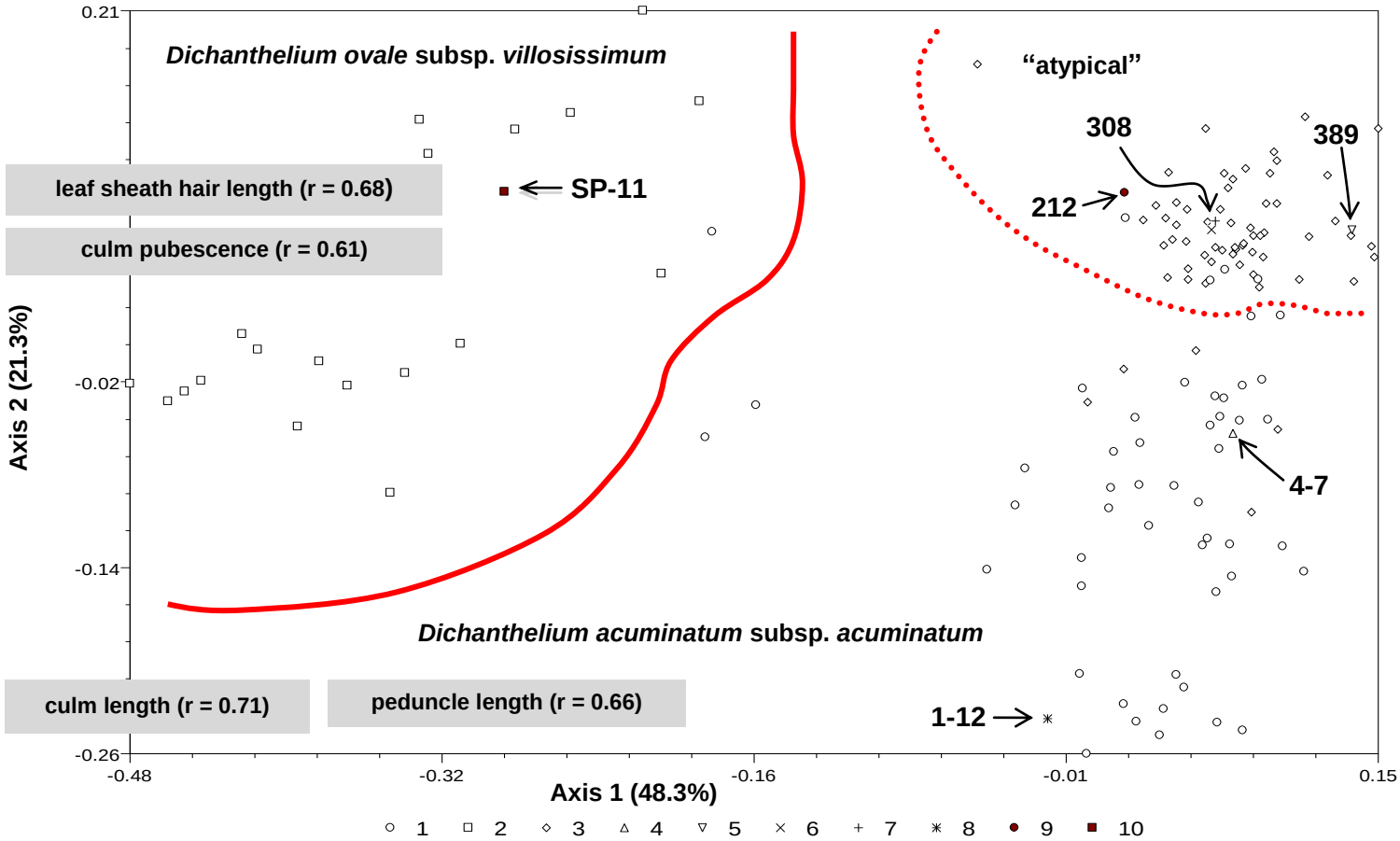
Key: **1** - subsp. *acuminatum (Hammer* pop 1 and pop 4, herb. specimens*)*; **2** – *D. ovale* subsp. *villosissimum* (*Hammer* pop 466, herb. specimens); **3** – subsp. *acuminatum* "intermediates" (*Hammer* pops 8, 15 and 17);**4** – subsp. *acum*. (*Hammer* 4-7); **5** – subsp. *acum. (Hammer* 389); **6** – subsp. *acum*. (*Hammer* 221); **7** – subsp. *acum*. (*Hammer* 308); **8** – subsp. *acum*. (*Hammer* 1-12); **9** – subsp. *acum*. (*Hammer* 212); **10** – *D. ovale*. subsp *villosissimum* (*Freckmann*)

Fig. 35. Principal coordinates analysis (PCoA) of morphological character data for *Dichanthelium acuminatum* subsp. *acuminatum* populations, *D. ovale* subsp. *villosissimum* populations and selected herbarium specimens, based on 15 characters (N = 138 specimens).



Key: **1** - subsp. *acuminatum (Hammer* pop 1 and pop 4, herb. specimens*)*; **2** – *D. ovale* subsp. *villosissimum* (*Hammer* pop 466, herb. specimens); **3** – subsp. *acuminatum* "intermediates" (*Hammer* pops 8, 15 and 17);**4** – subsp. *acum*. (*Hammer* 4-7); **5** – subsp. *acum. (Hammer* 389); **6** – subsp. *acum*. (*Hammer* 221); **7** – subsp. *acum*. (*Hammer* 308); **8** – subsp. *acum*. (*Hammer* 1-12); **9** – subsp. *acum*. (*Hammer* 212); **10** – *D. ovale*. subsp *villosissimum* (*Freckmann*)

Fig. 36. Principal coordinates analysis (PCoA) of morphological character data for *Dichanthelium acuminatum* subsp. *acuminatum* populations, *D. ovale* subsp. *villosissimum* populations and selected herbarium specimens, based on 15 characters (N = 138 specimens).



Key: **1** - subsp. *acuminatum (Hammer* pop 1 and pop 4, herb. specimens*)*; **2** – *D. ovale* subsp. *villosissimum* (*Hammer* pop 466, herb. specimens); **3** – subsp. *acuminatum* "intermediates" (*Hammer* pops 8, 15 and 17);**4** – subsp. *acum*. (*Hammer* 4-7); **5** – subsp. *acum. (Hammer* 389); **6** – subsp. *acum*. (*Hammer* 221); **7** – subsp. *acum*. (*Hammer* 308); **8** – subsp. *acum*. (*Hammer* 1-12); **9** – subsp. *acum*. (*Hammer* 212); **10** – *D. ovale*. subsp *villosissimum* (*Freckmann*)

Fig. 37. Principal coordinates analysis (PCoA) of morphological character data for *Dichanthelium acuminatum* subsp. *acuminatum* populations, *D. ovale* subsp. *villosissimum* populations and selected herbarium specimens, based on 15 characters (N = 138 specimens). Figure is annotated with Spearman's correlation values (gray boxes) and lines delineating taxonomic groupings

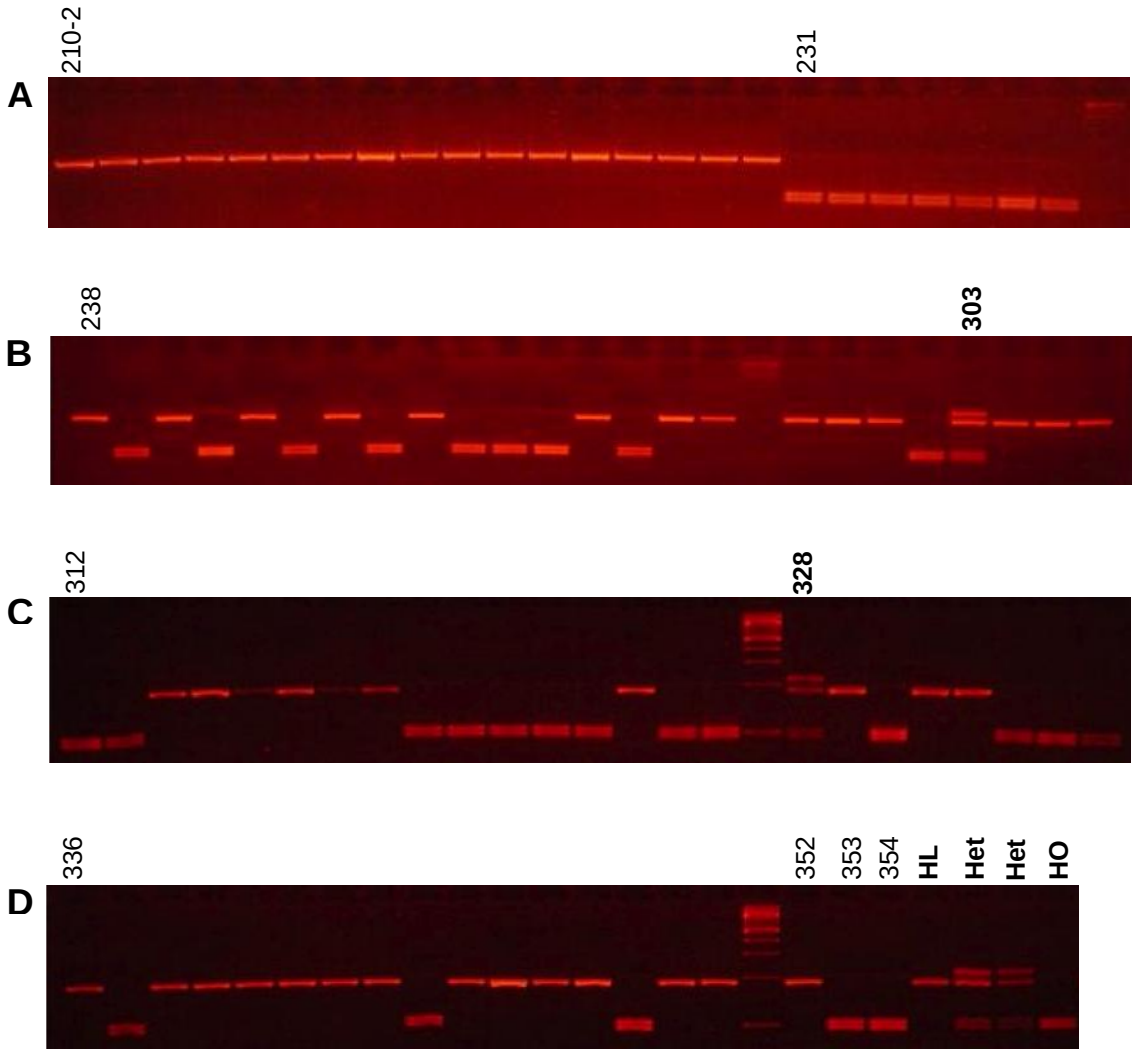


Fig. 38. PCR-RFLP electrophoresis results for *Fnu*4HI restriction digest of GBSSI fragments from 91 total *Dichanthelium* specimens: *Dichanthelium a.* subsp. *lindheimeri* : 57 specimens; *D. a*. subsp. *acuminatum*: 1 specimen; *D. a*. subsp. *fasciculatum*: 4 specimens; and other species of *Dichanthelium*: 29 specimens. DNA from *Dichanthelium* specimens was amplified with primers L1f+L4r (Table 22) and the resulting GBSSI amplicons were cut with the *Fnu*4HI enzyme. Numbers above the bands are specimen identifications. For reference gel "D" (far-right side) shows the three possible *Fnu*4HI GBSSI genotypes grouped together: homozygous subsp. *lindheimeri* (1 fragment of 187 bp fragment, specimen "HL"); homozygous non-subsp. *lindheimeri* (2 fragments, 106 bp and 96 bp, specimen "HO"); heterozygous subsp. *lindheimeri* + non-subsp. *lindheimeri* (4 fragments, 202 bp heteroduplex, 187 bp fragment from subsp. *lindheimeri* parent, and 106 bp plus 96 bp fragments from the non-subsp. *lindheimeri* parent, specimens "Het"). Putative hybrids are indicated for specimens possessing the heterozygous subsp. *lindheimeri* + non-subsp. *lindheimeri* genotype: specimens 303 and 328.

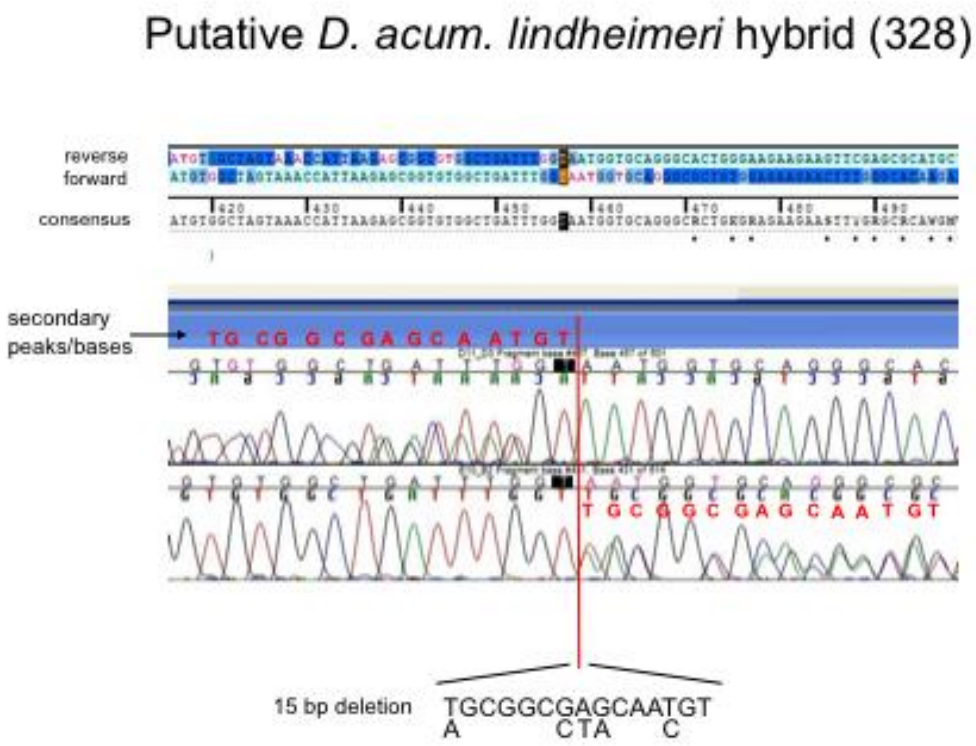
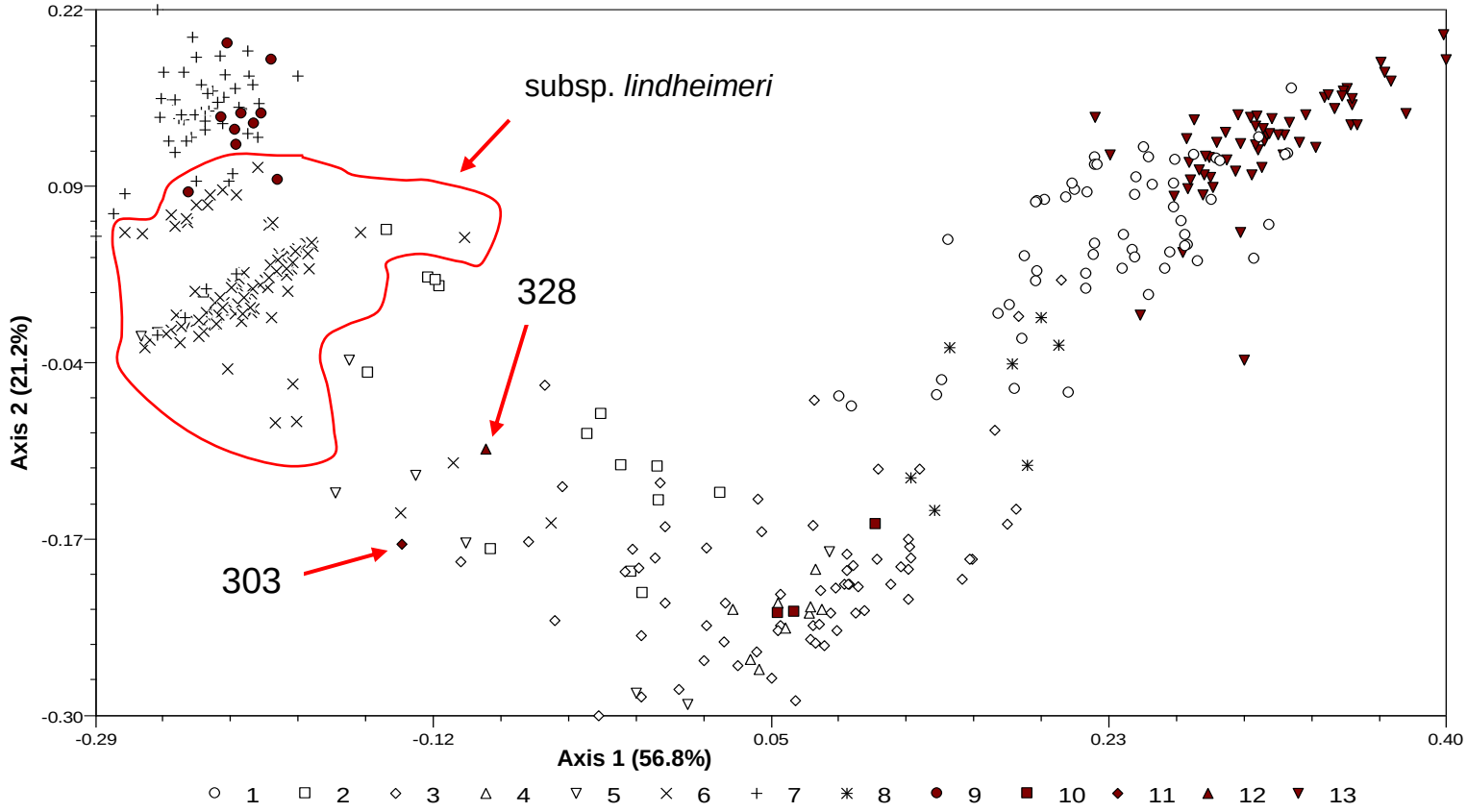
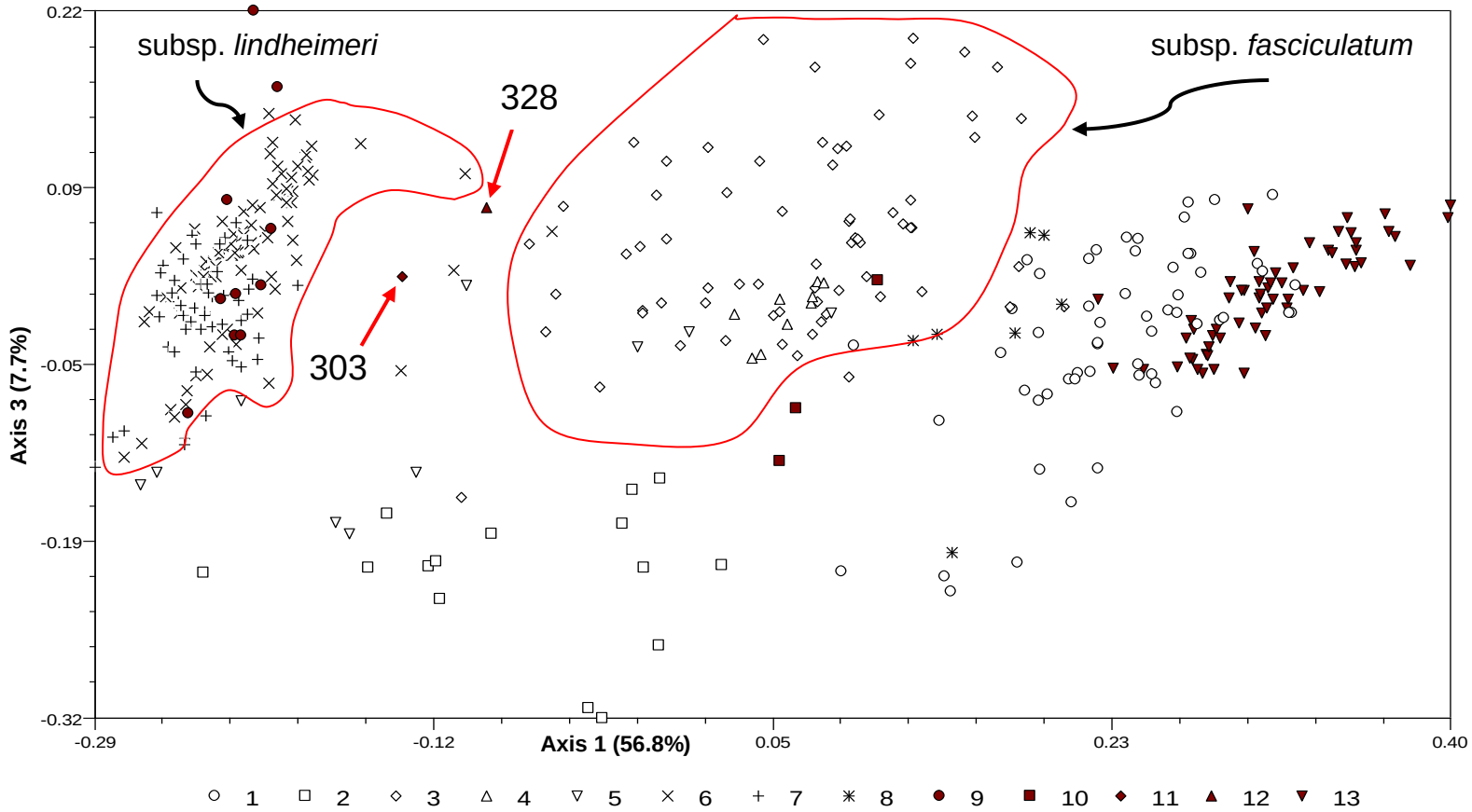


Fig. 39. DNA sequence chromatograms showing reverse, forward and consensus nucleotides for *D. acuminatum* subsp. *lindheimeri* specimen 328. The 15 bp deletion sequence is shown at bottom. The chromatograms for the reverse and forward sequences show "double" peaks. The secondary base peaks are labeled in red and the sequence on both the forward and reverse strands matches the known sequence of the 15 bp deletion.



Legend: **1** – *D. acuminatum* subsp. *acuminatum*; **2** – *D. a*. subsp. *columbianum*; **3** – *D. a*. subsp. *fasciculatum*; **4** – *D. a*. subsp. *implicatum*; **5** – *D. a*. subsp.. *leucothrix*; **6** – D. a. subsp. *lindheimeri*; **7** – D. a. subsp. *longiligulatum*; **8** – *D. a*. subsp. *sericeum*; **9** – *D. a*. subsp. *spretum*; **10** – *D. a*. subsp. *thermal*e; **11** – subsp. *lindheimeri* specimen 303; **12** – subsp. *lindheimeri* specimen 328; **13** – *D. a*. subsp. *acuminatum* populations 8, 15, and 17.

Fig. 40. Principal coordinates analysis (PCoA) of morphological character data of the *Dichanthelium acuminatum* complex, based on 15 characters (*N* = 391 specimens), including hybrid specimens of subsp. *lindheimeri*, 303 and 328.



Legend: **1** – *D. acuminatum* subsp. *acuminatum*; **2** – *D. a.* subsp. *columbianum*; **3** – *D. a.* subsp. *fasciculatum*; **4** – *D. a.* subsp. *implicatum*; **5** – *D. a.* subsp.. *leucothrix*; **6** – D. a. subsp. *lindheimeri*; **7** – D. a. subsp. *longiligulatum*; **8** – *D. a.* subsp. *sericeum*; **9** – *D. a.* subsp. *spretum*; **10** – *D. a.* subsp. *thermal*e; **11** – subsp. *lindheimeri* specimen 303; **12** – subsp. *lindheimeri* specimen 328; **13** – *D. a.* subsp. *acuminatum* populations 8, 15, and 17.

Fig. 41. Principal coordinates analysis (PCoA) of morphological character data of the *Dichanthelium acuminatum* complex, based on 15 characters (*N* = 391 specimens), including hybrid specimens of subsp. *lindheimeri*, 303 and 328.

APPENDIX B

TABLES

Table 1. Comparison of subspecies and species boundaries in the *Dichanthelium acuminatum* complex in selected treatments.

Hitchcock & Chase (1950)	**Gould & Clark (1978)**	**Freckmann & Lelong (2003)**
Panicum lanuginosum	*Dichanthelium. acuminatum* var*. acuminatum*	*D. acuminatum* subsp*. acuminatum*
P. auburne	*D. acuminatum* var*. implicatum*	"
P. thurowii	*D. acuminatum* var*. thurowii*	"
P. columbianum	*D. sabulorum* var*. thinium*	*D. acuminatum* subsp*. columbianum*
P. tsugetorum	"	"
P. huachucae	*D. acuminatum* var*. acuminatum*	*D. acuminatum* subsp*. fasciculatum*
P. huachucae var*. fasciculatum*	"	"
P. occidentale	"	"
P. pacificum	"	"
P. subvillosum	"	"
P. tennesseense	"	"
P. albemarlense	*D. acuminatum* var*. implicatum*	*D. acuminatum* subsp*. implicatum*
P. implicatum	"	"
P. meridionale	"	"
P. columbianum var*. thinium*	*D. sabulorum* var*. thinium*	"
P. oricola		"
P. leucothrix	*D. acuminatum* var*. implicatum*	*D. acuminatum* subsp*. leucothrix*
P. lindheimeri	*D. acuminatum* var*. lindheimeri*	*D. acuminatum* subsp*. lindheimeri*
P. longiligulatum	*D. acuminatum* var*. longiligulatum*	*D. acuminatum* subsp*. longiligulatum*
P. spretum	*D. acuminatum* var*. densiflorum*	*D. acuminatum* subsp*. spretum*
P. thermale	*D. acuminatum* var*. acuminatum*	*D. acuminatum* subsp*. sericeum*
"	"	*D. acuminatum* subsp*. thermale*
P. wrightianum	*D. acuminatum* var*. wrightianum*	*D. wrightianum*
P. villosissimum	*D. acuminatum* var*. villosum*	*D. ovale* subsp*. villosissimum*

Table 2. Morphological characters studied. All leaf characters are measured from third leaf down from apex.

1. Culm length (mm). **2. Culm internode pubescence**: 1 = glabrous; 2 = puberulent; 3 = pilose; 4 = villous. **3. Panicle length** (mm). **4. Panicle width** (mm) (measured at widest point). **5. Peduncle hair length** (cm) (average length over 5-10 mm below apex). **6. Peduncle length** (mm). **7. Spikelet length** (average of 5 spikelets) (mm). **8. Leaf blade margin**: 1 = smooth; 2 = scabridulous; 3 = ciliate (at least at leaf base). **9. Leaf sheath pubescence**: 1 = glabrous; 2 = sparsely pubescent; 3 = puberulent; 4 = pubescent; 5 = pilose; 6 = villous. **10. Leaf sheath hair length** (average length away from margins on upper half of sheath) (cm). **11. Leaf blade adaxial pubescence**: 1 = glabrous; 2 = sparsely pubescent/puberulent; 3 = appressed pubescent/puberulent; 4 = densely puberulent plus long pilose near base; 5 = velutinous, with hairs < = 0.5 mm long; 6 = velutinous, with hairs > 0.5 mm long; 7 = pilose; 8 = pilose only near margins; 9 = sparsely pilose only near base; 10 = villous. **12. Leaf blade abaxial pubescence**: 1 = glabrous; 2 = sparsely pubescent/puberulent; 3 = appressed pubescent/puberulent; 4= velutinous, with hairs < = 0.5 mm long; 5 = velutinous, with hairs > 0.5 mm long; 6 = pilose; 7 = villous. **13. Leaf blade length** (mm). **14. Leaf blade width** (mm). **15. Ligule length** (third leaf from apex) (mm).

Table 3. Frequency of leaf sheath vestiture character types for each subsp. in the *Dichathelium acuminatum* complex. $N = 389$. Key to leaf sheath pubescence types: 1 – glabrous; 2 – sparsely pubescent; 3 – puberulent; 4 – pubescent; 5 – pilose; 6 –villous.

Subsp.		Leaf sheath pubescence character type						Total
		1	2	3	4	5	6	
acuminatum	Count	0	0	0	0	6	111	117
	% within subsp.	.0%	.0%	.0%	.0%	5.1%	94.9%	100.0%
columbianum	Count	0	3	3	2	6	1	15
	% within subsp.	.0%	20.0%	20.0%	13.3%	40.0%	6.7%	100.0%
fasciculatum	Count	0	0	1	2	62	3	68
	% within subsp.	.0%	.0%	1.5%	2.9%	91.2%	4.4%	100.0%
implicatum	Count	0	0	0	0	9	0	9
	% within subsp.	.0%	.0%	.0%	.0%	100.0%	.0%	100.0%
leucothrix	Count	4	0	1	3	3	0	11
	% within subsp.	36.4%	.0%	9.1%	27.3%	27.3%	.0%	100.0%
lindheimeri	Count	95	1	0	0	1	0	97
	% within subsp.	97.9%	1.0%	.0%	.0%	1.0%	.0%	100.0%
longiligulatum	Count	48	4	0	0	0	0	52
	% within subsp.	92.3%	7.7%	.0%	.0%	.0%	.0%	100.0%
sericeum	Count	0	0	0	0	0	7	7
	% within subsp.	.0%	.0%	.0%	.0%	.0%	100.0%	100.0%
spretum	Count	10	0	0	0	0	0	10
	% within subsp.	100.0%	.0%	.0%	.0%	.0%	.0%	100.0%
thermale	Count	0	0	0	0	1	2	3
	% within subsp.	.0%	.0%	.0%	.0%	33.3%	66.7%	100.0%
Total	Count	157	8	5	7	88	124	389
	% of Total	40.4%	2.1%	1.3%	1.8%	22.6%	31.9%	100.0%

Table 4. Frequency of culm internode pubescence character types for each subsp. in the *Dichathelium acuminatum* complex. *N* = 389.

Subsp.		Culm internode pubescence character types				Total
		1	2	3	4	1
acuminatum	Count	0	0	27	90	117
	% within subsp.	.0%	.0%	23.1%	76.9%	100.0%
columbianum	Count	0	8	6	1	15
	% within subsp.	.0%	53.3%	40.0%	6.7%	100.0%
fasciculatum	Count	1	1	61	5	68
	% within subsp.	1.5%	1.5%	89.7%	7.4%	100.0%
implicatum	Count	0	0	9	0	9
	% within subsp.	.0%	.0%	100.0%	.0%	100.0%
leucothrix	Count	4	1	6	0	11
	% within subsp.	36.4%	9.1%	54.5%	.0%	100.0%
lindheimeri	Count	91	4	2	0	97
	% within subsp.	93.8%	4.1%	2.1%	.0%	100.0%
longiligulatum	Count	52	0	0	0	52
	% within subsp.	100.0%	.0%	.0%	.0%	100.0%
sericeum	Count	0	0	4	3	7
	% within subsp.	.0%	.0%	57.1%	42.9%	100.0%
spretum	Count	10	0	0	0	10
	% within subsp.	100.0%	.0%	.0%	.0%	100.0%
thermale	Count	0	0	3	0	3
	% within subsp.	.0%	.0%	100.0%	.0%	100.0%
Total	Count	158	14	118	99	389
	% Total	40.6%	3.6%	30.3%	25.4%	100.0%

Key to culm vestiture character codes: 1 – glabrous; 2 – puberulent/pubescent; 3 – pilose; 4 = villous.

Table 5. Frequency of leaf blade adaxial pubescence character types for each subsp. in the *Dichathelium acuminatum* complex. *N* = 389.

Subsp.		Leaf blade adaxial pubescence character types						Total
		1	2	3	5	6	7	
acuminatum	Count	0	12	1	84	2	18	117
	% within subsp.	.0%	10.3%	.9%	71.8%	1.7%	15.4%	100.0%
columbianum	Count	4	0	0	3	2	6	15
	% within subsp.	26.7%	.0%	.0%	20.0%	13.3%	40.0%	100.0%
fasciculatum	Count	13	7	1	28	3	16	68
	% within subsp.	19.1%	10.3%	1.5%	41.2%	4.4%	23.5%	100.0%
implicatum	Count	0	0	0	9	0	0	9
	% within subsp.	.0%	.0%	.0%	100.0%	.0%	.0%	100.0%
leucothrix	Count	8	1	1	1	0	0	11
	% within subsp.	72.7%	9.1%	9.1%	9.1%	.0%	.0%	100.0%
lindheimeri	Count	95	0	0	0	0	2	97
	% within subsp.	97.9%	.0%	.0%	.0%	.0%	2.1%	100.0%
longiligulatum	Count	52	0	0	0	0	0	52
	% within subsp.	100.0%	.0%	.0%	.0%	.0%	.0%	100.0%
sericeum	Count	0	0	0	2	5	0	7
	% within subsp.	.0%	.0%	.0%	28.6%	71.4%	.0%	100.0%
spretum	Count	10	0	0	0	0	0	10
	% within subsp.	100.0%	.0%	.0%	.0%	.0%	.0%	100.0%
thermale	Count	0	0	0	1	2	0	3
	% within subsp.	.0%	.0%	.0%	33.3%	66.7%	.0%	100.0%
Total	Count	182	20	3	128	14	42	389
	% of Total	46.8%	5.1%	.8%	32.9%	3.6%	10.8%	100.0%

Key to leaf blade adaxial character codes: 1 – glabrous; 2 – sparsely puberulent/pubescent; 3 – appressed puberulent/pubescent; 4 – densely puberulent plus long pilose near base; 5 – pilose; 6 – pilose mainly near margins; 7 – sparsely pilose only near base; 8 – villous.

Table 6. Frequency of leaf blade abaxial pubescence character types for each subsp. in the *Dichathelium acuminatum* complex. *N* = 389.

Subsp.		Leaf blade abaxial pubescence character type						Total
		1	2	3	4	5	6	
acuminatum	Count	0	3	2	29	81	2	117
	% within subsp.	.0%	2.6%	1.7%	24.8%	69.2%	1.7%	100.0%
columbianum	Count	6	1	8	0	0	0	15
	% within subsp.	40.0%	6.7%	53.3%	.0%	.0%	.0%	100.0%
fasciculatum	Count	3	4	49	1	1	10	68
	% within subsp.	4.4%	5.9%	72.1%	1.5%	1.5%	14.7%	100.0%
implicatum	Count	0	0	4	1	0	4	9
	% within subsp.	.0%	.0%	44.4%	11.1%	.0%	44.4%	100.0%
leucothrix	Count	0	0	10	0	1	0	11
	% within subsp.	.0%	.0%	90.9%	.0%	9.1%	.0%	100.0%
lindheimeri	Count	91	0	6	0	0	0	97
	% within subsp.	93.8%	.0%	6.2%	.0%	.0%	.0%	100.0%
longiligulatum	Count	50	0	2	0	0	0	52
	% within subsp.	96.2%	.0%	3.8%	.0%	.0%	.0%	100.0%
sericeum	Count	0	0	0	1	6	0	7
	% within subsp.	.0%	.0%	.0%	14.3%	85.7%	.0%	100.0%
spretum	Count	10	0	0	0	0	0	10
	% within subsp.	100.0%	.0%	.0%	.0%	.0%	.0%	100.0%
thermale	Count	0	0	2	0	1	0	3
	% within subsp.	.0%	.0%	66.7%	.0%	33.3%	.0%	100.0%
Total	Count	160	8	83	32	90	16	389
	% of Total	41.1%	2.1%	21.3%	8.2%	23.1%	4.1%	100.0%

Key to leaf blade abaxial vestiture types: 1 – glabrous; 2 – sparsely puberulent/pubescent; 3 – appressed puberulent/pubescent; 4 – velutinous, with hairs <= 0.5 mm in length; 5 – velutinous, with hairs > 0.5 mm in length; 6 – pilose;

Table 7. Morphometric variation in 10 quantitative continuous morphological characters across subspecies in the *Dichanthelium acuminatum* complex.

		Morphological character code									
Subspecies		panL	panW	spkL	pedL	pedH	culL	lvfShH	lvfL	lvfW	ligL
acuminatum	N	117	117	117	117	117	117	117	117	117	117
	Mean	85.43	54.79	1.811	180.11	1.921	534.33	1.962	76.51	6.441	3.57
	Median	85.00	55.00	1.820	174.00	2.000	530.00	2.000	75.00	6.400	3.50
	SE	1.776	1.829	.0072	6.277	.0718	12.225	.0624	1.705	.1140	.056
	Minimum	22	7	1.6	16	.1	151	.8	29	3.3	2
	Maximum	145	116	2.0	344	4.2	770	4.0	119	10.2	6
columbianum	N	15	15	15	15	15	15	15	15	15	15
	Mean	39.67	23.87	1.819	99.80	.297	257.07	.773	44.60	4.400	1.59
	Median	39.00	25.00	1.870	87.00	.100	277.00	.900	43.00	4.500	1.20
	SE	2.427	2.118	.0503	12.101	.0805	21.877	.1140	2.644	.1875	.200
	Minimum	22	12	1.5	30	.1	130	.1	27	3.4	1
	Maximum	58	36	2.2	193	1.0	395	1.5	63	5.6	3
fasciculatum	N	68	68	68	68	68	68	68	68	68	68
	Mean	61.22	43.75	1.629	114.84	1.385	392.99	1.462	75.26	6.218	3.24
	Median	58.00	41.00	1.595	110.00	1.200	372.50	1.450	73.00	6.050	3.20
	SE	2.175	1.704	.0169	7.272	.0855	15.832	.0541	2.636	.1577	.089
	Minimum	32	15	1.4	27	.2	195	.5	34	3.4	1
	Maximum	105	74	2.1	284	3.5	826	2.5	132	9.5	5
implicatum	N	9	9	9	9	9	9	9	9	9	9
	Mean	47.78	32.89	1.444	97.56	1.711	324.22	1.644	51.89	4.733	2.59
	Median	50.00	31.00	1.450	82.00	1.700	329.00	1.400	49.00	4.300	2.60
	SE	2.247	2.469	.0087	13.712	.2044	21.168	.1908	4.283	.3100	.218
	Minimum	35	25	1.4	59	.7	244	1.1	36	4.0	2
	Maximum	58	48	1.5	177	2.8	444	3.0	75	7.0	4

Table 7. Continued

Subspecies		Morphological character code									
		panL	panW	spkL	pedL	pedH	culL	lvfShH	lvfL	lvfW	ligL
leucothrix	N	11	11	11	11	11	11	11	11	11	11
	Mean	45.55	28.82	1.297	99.45	.414	355.09	.536	43.36	3.955	2.15
	Median	45.00	31.00	1.290	88.00	.050	368.00	.200	41.00	3.900	2.10
	SE	3.102	4.076	.0207	18.110	.1714	30.376	.2120	2.764	.2246	.198
	Minimum	32	5	1.2	31	.0	197	.0	32	2.9	1
	Maximum	65	46	1.4	188	1.6	562	2.0	59	5.5	3
lindheimeri	N	97	97	97	97	97	97	97	97	97	97
	Mean	53.41	30.73	1.470	85.62	.010	440.75	.035	58.98	4.473	3.34
	Median	52.00	30.00	1.470	82.00	.000	430.00	.000	58.00	4.500	3.40
	SE	1.191	1.234	.0074	3.454	.0059	12.144	.0250	1.241	.1048	.077
	Minimum	32	7	1.1	30	.0	61	.0	33	2.5	1
	Maximum	81	85	1.7	242	.4	794	2.0	95	9.3	6
longiligulatum	N	52	52	52	52	52	52	52	52	52	52
	Mean	84.29	18.33	1.474	116.54	.000	701.19	.000	82.19	3.913	2.14
	Median	86.00	16.00	1.460	104.00	.000	721.50	.000	83.50	4.000	2.10
	SE	3.035	1.224	.0150	6.396	.0000	26.113	.0000	3.096	.0818	.064
	Minimum	30	7	1.2	20	.0	279	.0	25	2.7	2
	Maximum	132	40	1.6	234	.0	1030	.0	134	5.2	4
sericeum	N	7	7	7	7	7	7	7	7	7	7
	Mean	50.43	42.43	1.683	121.71	1.814	229.57	1.743	51.86	8.243	2.99
	Median	46.00	40.00	1.690	115.00	1.800	215.00	1.600	52.00	9.100	3.00
	SE	4.879	5.793	.0355	9.327	.1388	15.516	.3046	4.206	.9916	.237
	Minimum	40	18	1.6	94	1.3	181	.9	38	4.2	2
	Maximum	76	63	1.8	168	2.4	291	3.1	69	11.9	4

Table 7. Continued

Subspecies		Morphological character code									
		panL	panW	spkL	pedL	pedH	culL	lvfShH	lvfL	lvfW	ligL
spretum	N	10	10	10	10	10	10	10	10	10	10
	Mean	90.60	28.70	1.559	159.10	.000	746.30	.000	84.50	5.220	1.94
	Median	80.50	25.50	1.545	154.00	.000	762.50	.000	85.00	5.250	2.00
	SE	8.672	3.575	.0246	20.050	.0000	54.301	.0000	6.600	.2653	.121
	Minimum	63	16	1.5	96	.0	447	.0	46	4.2	1
	Maximum	133	45	1.7	275	.0	1047	.0	111	6.3	3
thermale	N	3	3	3	3	3	3	3	3	3	3
	Mean	43.00	27.33	1.930	33.33	.533	152.33	1.333	50.33	5.733	2.53
	Median	38.00	21.00	1.910	41.00	.400	141.00	1.400	39.00	5.800	2.80
	SE	14.364	8.950	.0643	8.686	.2404	14.438	.1764	12.347	.8667	.371
	Minimum	21	16	1.8	16	.2	135	1.0	37	4.2	2
	Maximum	70	45	2.1	43	1.0	181	1.6	75	7.2	3
Total	N	389	389	389	389	389	389	389	389	389	389
	Mean	68.47	38.45	1.619	126.63	.922	484.99	.979	69.50	5.380	3.06
	Median	68.00	35.00	1.580	112.00	.700	467.00	1.000	68.00	5.200	3.10
	SE	1.199	.993	.0096	3.348	.0513	9.285	.0497	1.069	.0794	.045
	Minimum	21	5	1.1	16	.0	61	.0	25	2.5	1
	Maximum	145	116	2.2	344	4.2	1047	4.0	134	11.9	6

Key to morphological character codes: panL – panicle length; panW – panicle width; spkL – spikelet length; pedL – peduncle length; culL – culm length; lvfShH – leaf sheath hair length; lvfL – leaf length; lvfW – leaf width; ligL – ligule length.

Table 8. Frequency of leaf blade abaxial pubescence types within populations of *Dichanthelium acuminatum* subsp. *acuminatum*. Key to populations: 1 – *D. acuminatum* subsp.. *acuminatum*, *Hamme*r 1-1 to 1-21, TX-Leon; 15 – *Hammer* pop 15-1 to 15-20, LA-Vernon Parish; 17 – *Hammer* pop 17-1 to 17-20, LA-Vernon Parish; 4-1 to 4-20 – *Hammer* pop 4, TX-Montgomery; 8-1 to 8-20 – *Hammer* pop 8, TX-Hardin. For each population the collector, collector population code, state and county/parish where collected is given.

Population	*N*	Leaf blade abaxial pubescence type (% within population)					
		sparsely puberulent/ pubescent	appressed puberulent/ pubescent	velutinous, with hairs <= 0.5 mm in length	velutinous, with hairs > 0.5 mm in length	pilose	Total
Herbarium	16	.0%	6.3%	37.5%	43.8%	12.5%	100.0%
1	21	9.5%	.0%	57.1%	33.3%	.0%	100.0%
15	20	.0%	.0%	.0%	100.0%	.0%	100.0%
17	20	.0%	5.0%	5.0%	90.0%	.0%	100.0%
4	20	5.0%	.0%	50.0%	45.0%	.0%	100.0%
8	20	.0%	.0%	.0%	100.0%	.0%	100.0%
Total	117	2.6%	1.7%	24.8%	69.2%	1.7%	100.0%

Table 9. Frequency of leaf blade adaxial pubescence types for populations of Dichanthelium acuminatum subsp. acuminatum. Key to populations: 1 – D. acuminatum subsp.. acuminatum, Hammer pop 1-1 to 1-21, TX-Leon; Hammer pop 15-1 to 15-20, LA-Vernon Parish; Hammer pop 17-1 to 17-20, LA-Vernon Parish; Hammer pop 4-1 to 4-20, TX-Montgomery; Hammer pop 8-1 to 8-20, TX-Hardin. For each population the collector, collector population code, state and county/parish where collected is given.

		Leaf blade adaxial pubescence type (% within population)					
Population	*N*	sparsely puberulent/ pubescent	appressed puberulent/ pubescent	pilose	pilose mainly near margins	sparsely pilose only near base	Total
Herbarium	16	.0%	6.3%	81.3%	12.5%	.0%	100.0%
1	21	38.1%	.0%	38.1%	.0%	23.8%	100.0%
15	20	.0%	.0%	100.0%	.0%	.0%	100.0%
17	20	15.0%	.0%	85.0%	.0%	.0%	100.0%
4	20	5.0%	.0%	30.0%	.0%	65.0%	100.0%
8	20	.0%	.0%	100.0%	.0%	.0%	100.0%
Total	117	10.3%	.9%	71.8%	1.7%	15.4%	100.0%

Table 10. Frequency of culm pubescence types for populations of Dichanthelium acuminatum subsp. acuminatum. Key to populations: D. acuminatum subsp.. acuminatum, Hammer pop 1-1 to 1-21, TX-Leon; Hammer pop 15-1 to 15-20, LA-Vernon Parish; Hammer pop 17-1 to 17-20, LA-Vernon Parish; Hammer pop 4-1 to 4-20, TX-Montgomery; Hammer pop 8-1 to 8-20, TX-Hardin. For each population the collector, collector population code, state and county/parish where collected is given.

			Culm pubescence type		Total
			pilose	villous	
population	herbarium	Count	2	14	16
		% within population	12.5%	87.5%	100.0%
	1-1 to 1-21	Count	2	19	21
		% within population	9.5%	90.5%	100.0%
	15-1 to 15-20	Count	1	19	20
		% within population	5.0%	95.0%	100.0%
	17-1 to 17-20	Count	1	19	20
		% within population	5.0%	95.0%	100.0%
	4-1 to 4-20	Count	20	0	20
		% within population	100.0%	.0%	100.0%
	8-1 to 8-20	Count	1	19	20
		% within population	5.0%	95.0%	100.0%
Total		Count	27	90	117
		% within population	23.1%	76.9%	100.0%

Table 11. Frequency of leaf blade margin types for populations of Dichanthelium acuminatum subsp. acuminatum. Key to populations: Hammer 1-1 to 1-21, TX-Leon; Hammer 15-1 to 15-20, LA-Vernon Parish; Hammer 17-1 to 17-20, LA-Vernon Parish; Hammer 4-1 to 4-20, TX-Montgomery; Hammer 8-1 to 8-20, TX-Hardin. For each population the collector, collector population code, state and county/parish where collected is given.

		Leaf blade margin type			Total
		scabridulous	ciliate at base up to one-fourth of blade length	ciliate from base to at least one-half of blade length	scabridulous
herbarium	Count	1	1	14	16
	% within population	6.3%	6.3%	87.5%	100.0%
1-1 to 1-21	Count	0	0	21	21
	% within population	.0%	.0%	100.0%	100.0%
15-1 to 15-20	Count	0	0	20	20
	% within population	.0%	.0%	100.0%	100.0%
17-1 to 17-20	Count	0	0	20	20
	% within population	.0%	.0%	100.0%	100.0%
4-1 tp 4-20	Count	0	0	20	20
	% within population	.0%	.0%	100.0%	100.0%
8-1 to 8-20	Count	0	0	20	20
	% within population	.0%	.0%	100.0%	100.0%
Total	Count	1	1	115	117
	% total	.9%	.9%	98.3%	100.0%

Table 12. Frequency of leaf sheath pubescence types for populations of Dichanthelium acuminatum subsp. acuminatum. Key to populations: Hammer 1-1 to 1-21, TX-Leon; Hammer 15-1 to 15-20, LA-Vernon Parish; Hammer 17-1 to 17-20, LA-Vernon Parish; Hammer 4-1 to 4-20, TX-Montgomery; Hammer 8-1 to 8-20, TX-Hardin. For each population the collector, collector population code, state and county/parish where collected is given.

			Leaf sheath pubescence type		Total
			pilose	villous	
Population	herbarium	Count	4	12	16
		% within population	25.0%	75.0%	100.0%
	1-1 to 1-20	Count	1	20	21
		% within population	4.8%	95.2%	100.0%
	15-1 to 15-20	Count	1	19	20
		% within population	5.0%	95.0%	100.0%
	17-1 to 17-20	Count	0	20	20
		% within population	.0%	100.0%	100.0%
	4-1 to 4-20	Count	0	20	20
		% within population	.0%	100.0%	100.0%
	8-1 to 8-20	Count	0	20	20
		% within population	.0%	100.0%	100.0%
Total		Count	6	111	117
		% within population	5.1%	94.9%	100.0%

Table 13. Eigenvalues from Principal Coordinates Analysis (PCoA) of the *Dichathelium acuminatum* complex. The first 20 of the total 389 eigenvalues are shown along with proportion of variance expected under a broken stick (random) distribution.

PCoA Axis	Eigenvalue	% Variation Explained	Cumulative Variation Explained	Expected Under Broken Stick Model
1	18.09846	56.9107	56.9107	1.6818
2	6.730969	21.1656	78.0763	1.4247
3	2.450641	7.7061	85.7824	1.2962
4	2.272205	7.145	92.9273	1.2105
5	1.420956	4.4682	97.3955	1.1462
6	1.143014	3.5942	100%	1.0948
7	0.94601	2.9747	100%	1.0519
8	0.769988	2.4212	100%	1.0152
9	0.61986	1.9492	100%	0.9831
10	0.554208	1.7427	100%	0.9545
11	0.510923	1.6066	100%	0.9288
12	0.423165	1.3306	100%	0.9055
13	0.352859	1.1096	100%	0.8840
14	0.319034	1.0032	100%	0.8643
15	0.285411	0.8975	100%	0.8459
16	0.279075	0.8776	100%	0.8288
17	0.263445	0.8284	100%	0.8127
18	0.243574	0.7659	100%	0.7976
19	0.224857	0.7071	100%	0.7833
20	0.2170101	0.6824	100%	0.7698

Table 14. Spearman's correlation coefficients (r) between individual morphological characters and axes computed from principal coordinates analysis (PCoA, Figs. 16-18) for the *Dichanthelium acuminatum* subspecies complex (N = 389).

Character	Axis 1 (56.9%)	Axis 2 (21.2%)	Axis 3 (7.7%)
Panicle length	.397 *	.694 *	.306 *
Panicle width	.737 *	0.054	.307 *
Spikelet length	.779 *	.195 *	-.138 *
Peduncle length	.555 *	.435 *	.226 *
Peduncle hair length	.897 *	-0.060	-0.031
Culm length	0.079	.708 *	.405 *
Culm pubescence type	.911 *	-0.005	-.189 *
Leaf margin type	.814 *	0.093	.131 *
Leaf sheath adaxial pubescence	.764 *	-.206 *	-.132 *
Leaf sheath abaxial pubescence	.871 *	-0.059	-.140 *
Leaf sheath pubescence type	.930 *	0.023	-.170 *
Leaf sheath hair length	.890 *	-0.060	-0.070
Leaf length	.231 *	.470 *	.470 *
Leaf width	.722 *	-0.028	.289 *
Ligule length	.423 *	-0.003	.376 *

* = Correlation significant at $p < 0.01$

Table 15. Eigenvalues from Principal Coordinates Analysis (PCoA; Figs. 19-21) of the pubesent taxa of the *Dichathelium acuminatum* complex. The first 20 of the total 230 eigenvalues are shown along with proportion of variance expected under a broken stick (random) distribution.

PCoA Axis	Eigenvalue	% Variation Explained	Cumulative Variation Explained	Expected Under Broken Stick Model
1	8.8505	54.1964	54.1964	2.6163
2	2.5719	15.7492	69.9456	2.1815
3	1.7672	10.8215	80.7670	1.9641
4	1.0806	6.6170	87.3840	1.8192
5	0.9409	5.7617	93.1457	1.7105
6	0.7651	4.6849	97.8306	1.6235
7	0.6713	4.1105	100%	1.5511
8	0.5809	3.5570	100%	1.4890
9	0.4873	2.9837	100%	1.4346
10	0.3812	2.3340	100%	1.3863
11	0.3235	1.9811	100%	1.3428
12	0.2788	1.7074	100%	1.3033
13	0.2582	1.5812	100%	1.2671
14	0.2342	1.4341	100%	1.2336
15	0.2244	1.3743	100%	1.2026
16	0.1802	1.1034	100%	1.1736
17	0.1718	1.0522	100%	1.1464
18	0.1629	0.9974	100%	1.1208
19	0.1510	0.9247	100%	1.0967
20	0.1406	0.8610	100%	1.0730

Table 16. Spearman's correlation coefficients (*r*) between individual morphological characters and axes computed from principal coordinates analysis (PCoA, Figs. 19-21) for the pubescent taxa of the *Dichanthelium acuminatum* subspecies complex (*N* = 230).

Character	**Axis 1 (54.2%)**	**Axis 2 (15.7%)**	**Axis 3 (10.8%)**
Panicle length	.787 *	-.152	-.213 *
Panicle width	.651 *	-.318 *	-0.039
Spikelet length	.591 *	.309 *	-0.041
Peduncle length	.629 *	-0.128	-.211 *
Peduncle hair length	.674 *	-.271 *	.230 *
Culm length	.676 *	-.222 *	-.338 *
Culm pubescence type	.779 *	.272 *	.167
Leaf margin type	.859 *	0.103	-0.036
Leaf sheath adaxial pubescence	.130	-0.102	-.151
Leaf sheath abaxial pubescence	.657 *	-0.027	.429 *
Leaf sheath pubescence type	.830 *	.179 *	-0.021
Leaf sheath hair length	.641 *	-.165	.310 *
Leaf length	.462 *	-.486 *	-.331 *
Leaf width	.424 *	-.398 *	-0.103
Ligule length	.568 *	-.287 *	-0.007

* = Correlation significant at $p < 0.01$

Table 17. Herbarium specimens of Dichanthelium acuminatum subsp. acuminatum examined for morphological analysis.

Accession	Country	State	County
TAES-143283	Columbia		
TAES-123828	Jamaica		
TAES-109849	Mexico (Chiapas)		
TAES-145985	Panama (Chiriqui)		
TAES-160560	Puerto Rico		
WSU-19628	USA	AR	Franklin
ISC-248997	USA	AR	Little River
ISC-285176	USA	LA	Boissier
ISC-243478	USA	NC	Beaufort
WIS-NA02	USA	SC	Berkeley
TAES-84662	USA	TX	Bastrop
Hammer 212	USA	TX	Hardin
Hammer 389	USA	TX	Jasper
Hammer 221	USA	TX	Tyler
Hammer 308	USA	TX	Tyler

Table 18. Morphometric variation in 7 quantitative continuous morphological characters across populations of *Dichanthelium acuminatum* subsp. *acuminatum*. Key to morphological character codes: panL – panicle length; panW – panicle width; spkL – spikelet length; pedL – peduncle length; culL – culm length; lvfShH – leaf sheath hair length; lvfL – leaf length; lvfW – leaf width; ligL – ligule length.

Population		Morphological character code									
		panL	panW	spkL	pedL	pedH	culL	lvfShH	lvfL	lvfW	ligL
1	N	21	21	21	21	21	21	21	21	21	21
	Mean	86.00	39.67	1.822	127.43	1.414	464.48	1.571	76.57	6.895	3.34
	Median	86.00	40.00	1.820	125.00	1.500	469.00	1.500	73.00	7.100	3.40
	Minimum	55	17	1.7	78	0.1	368	1.0	55	5.1	2
	Maximum	115	65	1.9	179	2.1	574	2.1	118	10.2	4
	Range	60	48	0.2	101	2.0	206	1.1	63	5.1	2
15	N	20	20	20	20	20	20	20	20	20	20
	Mean	92.00	70.40	1.862	188.70	2.680	581.35	2.525	93.55	7.080	3.87

Table 18. Continued.

Population		Morphological character code									
		panL	panW	spkL	pedL	pedH	culL	lvfShH	lvfL	lvfW	ligL
15	Median	89.50	72.00	1.875	179.00	2.500	551.00	2.300	92.00	7.500	3.85
	Minimum	62	42	1.7	122	2.0	497	1.7	72	4.5	3
	Maximum	124	110	2.0	257	4.0	720	4.0	119	9.2	5
	Range	62	68	0.3	135	2.0	223	2.3	47	4.7	2
17	N	20	20	20	20	20	20	20	20	20	20
	Mean	86.15	54.70	1.774	235.35	2.475	575.75	1.845	65.05	5.480	3.48
	Median	86.00	55.50	1.780	250.50	2.550	570.00	1.900	62.00	5.550	3.50
	Minimum	55	27	1.6	105	1.3	420	1.0	41	3.5	3
	Maximum	110	78	1.9	344	4.2	712	3.0	111	7.9	4
	Range	55	51	0.3	239	2.9	292	2.0	70	4.4	2
4	N	20	20	20	20	20	20	20	20	20	20
	Mean	85.25	50.40	1.787	220.05	1.300	656.25	1.440	84.85	6.880	3.44

Table 18. Continued.

Population		Morphological character code									
		panL	panW	spkL	pedL	pedH	culL	lvfShH	lvfL	lvfW	ligL
4	Median	83.50	50.00	1.790	217.00	1.250	652.00	1.500	86.00	6.750	3.20
	Minimum	64	25	1.6	116	0.6	502	0.8	60	5.8	3
	Maximum	111	70	1.9	317	2.2	770	2.1	110	8.4	5
	Range	47	45	0.3	201	1.6	268	1.3	50	2.6	2
8	N	20	20	20	20	20	20	20	20	20	20
	Mean	94.65	64.65	1.830	196.75	2.155	530.80	2.465	74.40	6.410	3.97
	Median	94.50	60.00	1.820	198.50	2.100	511.50	2.200	73.50	6.400	3.90
	Minimum	59	32	1.7	107	1.5	414	1.5	63	4.0	3
	Maximum	145	116	1.9	278	3.1	731	3.2	102	8.0	6
	Range	86	84	0.2	171	1.6	317	1.7	39	4.0	3
herbarium	N	16	16	16	16	16	16	16	16	16	16

Table 18. Continued

Population		Morphological character code									
		panL	panW	spkL	pedL	pedH	culL	lvfShH	lvfL	lvfW	ligL
herbarium	Mean	64.25	48.44	1.790	98.75	1.431	367.50	1.944	61.69	5.738	3.28
	Median	64.50	43.00	1.760	88.00	1.350	349.00	2.000	61.00	5.550	3.15
	Minimum	22	7	1.7	16	0.3	151	0.8	29	3.3	2
	Maximum	100	107	2.0	185	2.6	747	3.3	100	7.7	5
	Range	78	100	0.3	169	2.3	596	2.5	71	4.4	3
Total	N	117	117	117	117	117	117	117	117	117	117
	Mean	85.43	54.79	1.811	180.11	1.921	534.33	1.962	76.51	6.441	3.57
	Median	85.00	55.00	1.820	174.00	2.000	530.00	2.000	75.00	6.400	3.50
	Minimum	22	7	1.6	16	0.1	151	0.8	29	3.3	2
	Maximum	145	116	2.0	344	4.2	770	4.0	119	10.2	6
	Range	123	109	0.4	328	4.1	619	3.2	90	6.9	4

Table 19. Eigenvalues from Principal Coordinates Analysis (PCoA) of *Dichanthelium acuminatum* subsp. *lindheimeri*, subsp. *longiligulatum* and subsp. *spretum* populations and herbarium specimens. The first 15 of the total 161 eigenvalues are shown along with proportion of variance expected under a broken stick (random) distribution.

PCoA Axis	Eigenvalue	% Variation Explained	Cumulative Variation Explained	Expected Under Broken Stick Model
1	1.518978	55.8060	55.8060	3.5166
2	0.453883	16.6753	72.4813	2.8955
3	0.312947	11.4974	83.9787	2.5849
4	0.235923	8.6676	92.6463	2.3779
5	0.160222	5.8864	98.5327	2.2226
6	0.123017	4.5195	100%	2.0984
7	0.110090	4.0446	100%	1.9949
8	0.090438	3.3226	100%	1.9061
9	0.082723	3.0392	100%	1.8285
10	0.073478	2.6995	100%	1.7595
11	0.060160	2.2102	100%	1.6974
12	0.053980	1.9832	100%	1.6409
13	0.038697	1.4217	100%	1.5891
14	0.033053	1.2143	100%	1.5414
15	0.030477	1.1197	100%	1.4970

Table 20. Spearman's correlation coefficients (*r*) between individual morphological characters and axes computed from principal coordinates analysis (PCoA, Figs. 28-30) for *Dichanthelium acuminatum* subsp. *lindheimeri*, subsp. *longiligulatum* and subsp. *spretum* populations and herbarium specimens x (N = 161).

Character	**Axis 1 (55.8%)**	**Axis 2 (16.7%)**	**Axis 3 (11.5%)**
Panicle length	-.782 *	.411 *	.164
Panicle width	.314 *	.380 *	.470 *
Spikelet length	-.323 *	.563 *	0.104
Peduncle length	-.500 *	.438 *	.613 *
Peduncle hair length	.293 *	-0.063	.202
Culm length	-.745 *	.453 *	.308 *
Culm pubescence type	.348 *	0.086	0.010
Leaf margin type	.848 *	.314 *	0.108
Leaf sheath adaxial pubescence	.192(*)	-0.153	.192
Leaf sheath abaxial pubescence	.200	-.213 *	.251 *
Leaf sheath pubescence type	-0.045	.253 *	-.376 *
Leaf sheath hair length	.184	.234 *	-.269 *
Leaf length	-.690 *	.343 *	-0.069
Leaf width	0.098	.571 *	0.083
Ligule length	.687 *	-0.022	-.290 *

* = Correlation significant at $p < 0.01$

Table 21. Plant materials used for DNA sequencing, with voucher information.

D. acuminatum* subsp. *acuminatum—TX, Leon Co., *Hammer 1-12*; TX, Montgomery Co., *Hammer 4-7*; TX, Hardin Co., *Hammer 212* (TAES); TX, Tyler Co., *Hammer* 221 (TAES); TX, Liberty Co., *Hammer 302*; TX, Tyler Co., *Hammer 308*; TX, Angelina Co., *Hammer 389*; CA, Monterey Co., *Gould* (TAMU 123576); ***D. a.* subsp. *columbianum*—**SP14, WS, Shawano Co., *Freckmann* (WSU 20234); C1; ***D. a.* subsp. *fasciculatum***—TX, Uvalde Co., *Reed 2603* (TAMU 031817); SP10, WI, Waupaca Co., *Freckmann 5078* (WIS 10272); SP8, AR, Madison Co., *Freckmann 6301* (WIS 19646); TX, Rusk Co., *Hammer 230-1*; ***D. a.* subsp. *implicatum***—SP23, AR, Saline Co., *Freckmann 11418* (WSU 45271); ***D. a.* subsp. *lindheimeri***—TX, Brazos Co., *Hammer 3-14* (TAES); LA, Vernon Parish, *Hammer 19-8* (TAES); TX, Liberty Co., *Hammer 292* (TAES); TX, Liberty Co., *Hammer 294* (TAES); TX, Lee Co., *Hammer 343* (TAES); SP16, AR, Yell Co., *Freckmann 391* (WSU 10825); ***D. a.* subsp. *longiligulatum***—TX, Montgomery Co., *Hammer 5-12* (TAES); LA, Vernon Parish, *Hammer 206* (TAES); TX, Hardin Co., *Hammer 214* (TAES); ***D. a.* subsp. *sericeum***—SP1, WY, Park Co., *Freckmann 4597* (WSU 22781); SP4, (WSU 81044); ***D. a.* subsp. *spretum***—S1, (TAES); SP5, ??, (WSU 55029); ***D. a.* subsp. *thermale***—SP2-1, CA, Plumas Co., *Kearney* (WSU 81627); SP3, CA, Plumas Co., *Kearney* (WSU 81044); ***D. ovale*** subsp. ***villosissimum***—SP11, FL, (WSU 45318); ***D. ovale*** subsp. ***praecocius***—SP12, WI, Kenosha Co., *Freckmann 3003* (WSU 8963); ***D. wrightianum***—TX, Montgomery Co., *Hammer 216* (TAES); TR7, TX, Polk Co., *Brown* (TAES 246123)

Table 22. Amplification and sequencing primers for GBSSI used in this study.

Primer	Sequence (5' to 3')	Amp/Seq	Reference
E-for	GTG TTC GTC TGC AAC GAC TGG	Amp/Seq	this paper
G-bac	CGG CCT TCA TCC AGT TGA TCT T	Amp/Seq	this paper
F-for	TGC GAG CTC GAC AAC ATC ATG CG	Amp/Seq	Mason-Gamer et al. 1998
K-bac	GCA GGG CTC GAA GCG GCT GG	Amp/Seq	Mason-Gamer et al. 1998
K-for	CCA GCC GCT TCG AGC CCT G	Amp/Seq	Mason-Gamer et al. 1998
M-bac	GGC GAG CGG CGC GAT CCC TCG CC	Amp/Seq	Mason-Gamer et al. 1998
F2-for	CTC CGG GTA GTC CGA GAA G	Amp/Seq	this paper
G2b1-bac	CCT CGA TAA TCC CGG CCT TC	Amp/Seq	this paper
J-bac	ACG TCG GGG CCC TTC TGC TC	Amp/Seq	Mason-Gamer et al. 1998
I-for	GTT CGT CGG CAG GCT GGA G	Amp/Seq	this paper
L3-bac	TCC TCC GCG CTC ATC AGC ATG	Amp/Seq	this paper
L2-bac	CGC TGA GGC GGC CCA TGT GG	Amp/Seq	Mason-Gamer et al. 1998
L1U-for	GCC CTG CGT GTG TGC ATC C	Amp/Seq	this paper
L4U-bac	CGA CCT TGA TGG CGC GCT TC	Amp/Seq	this paper
L3U-for	GTG CAA GGT CGT GGA GCC G	Amp/Seq	this paper

Table 23. Eigenvalues from Principal Coordinates Analysis (PCoA) of *Dichanthelium acuminatum* subsp. *acuminatum* and *D. ovale* subsp. *villosissimum* populations and selected herbarium specimens. The first 15 of the total 138 eigenvalues are shown along with proportion of variance expected under a broken stick (random) distribution.

PCoA Axis	Eigenvalue	% Variation Explained	Cumulative Variation Explained	Expected Under Broken Stick Model
1	3.32849647	48.2681	48.2681	3.9914
2	1.47175707	21.3427	69.6108	3.2667
3	1.12579802	16.3257	85.9365	2.9044
4	0.63971776	9.2769	95.2134	2.6629
5	0.40588147	5.8859	100%	2.4817
6	0.27748504	4.0239	100%	2.3368
7	0.24053897	3.4882	100%	2.2160
8	0.20376812	2.9549	100%	2.1125
9	0.15456253	2.2414	100%	2.0219
10	0.14217028	2.0617	100%	1.9414
11	0.10756749	1.5599	100%	1.8689
12	0.10182391	1.4766	100%	1.8031
13	0.08902515	1.2910	100%	1.7427
14	0.08367281	1.2134	100%	1.6869
15	0.07655588	1.1102	100%	1.6352

Table 24. Spearman's correlation coefficients (*r*) between individual morphological characters and axes computed from principal coordinates analysis (PCoA, Figs. 34-36) for *Dichanthelium acuminatum* subsp. *acuminatum*, *D. ovale* subsp. *villosissimum* populations and herbarium specimens (N = 138).

Character	**Axis 1 (48.3%)**	**Axis 2 (21.3%)**	**Axis 3 (16.3%)**
Panicle length	0.641	0.204 *	0.319 *
Panicle width	0.364 *	0.399 *	0.514 *
Spikelet length	- 0.345 *	0.238 *	0.234 *
Peduncle length	0.661 *	0.120	0.430 *
Peduncle hair length	0.004	0.650 *	0.232 *
Culm length	0.706 *	0.038	0.476 *
Culm pubescence type	0.287 *	0.610 *	- 0.623 *
Leaf margin type	0.643 *	- 0.147	- 0.078
Leaf sheath adaxial pubescence	- 0.250 *	- 0.232 *	0.369 *
Leaf sheath abaxial pubescence	- 0.152	0.557 *	0.257 *
Leaf sheath pubescence type	0.487 *	0.190	- 0.049
Leaf sheath hair length	- 0.265 *	0.681 *	0.242 *
Leaf length	0.418 *	0.101	0.464 *
Leaf width	0.384 *	0.020	0.350 *
Ligule length	- 0.227 *	0.440 *	0.275 *

* = Correlation significant at $p < 0.01$

Table 25. Summary of phylogenetic analysis for taxa of the *Dichanthelium acuminatum* complex (*sensu* Freckmann & Lelong 2003).

Taxon	**Status as an evolutionary significant unit (ESU)**
D. a. subsp. *acuminatum*	ESU
D. a. subsp. *columbianum*	Not resolved
D. a. subsp. *fasciculatum*	ESU
D. a. subsp. *implicatum*	Not resolved
D. a. subsp. *leucothrix*	Not resolved
D. a. subsp. *lindheimeri*	ESU
D. a. subsp. *longiligulatum* + *subsp. spretum*	ESU
D. a. subsp. *sericeum*	Not resolved
D. a. subsp. *thermale*	Not resolved

Table 26. Summary of principal coordinates analysis of morphological characters among infraspecific taxa of the *Dichanthelium acuminatum* complex.

Taxon	**Status as an morphologically diagnosable taxon (MDT)**
D. a. subsp. *acuminatum*	MDT
D. a. subsp. *columbianum*	MDT
D. a. subsp. *fasciculatum*	MDT
D. a. subsp. *implicatum*	Not resolved
D. a. subsp. *leucothrix*	Not resolved
D. a. subsp. *lindheimeri*	MDT
D. a. subsp. *longiligulatum* + subsp. *spretum*	MDT
D. a. subsp. *sericeum*	MDT
D. a. subsp. *thermale*	Not resolved

APPENDIX C

COMPUTATIONAL DETAILS FOR MULTIVARIATE ANALYSIS OF MORPHOLOGICAL DATA

Data Preparation–Measurements for each of the morphological characters were scored on paper forms during the measurement process. Data from the forms was then entered into a Microsoft Excel spreadsheet (see CDROM supplement for data sets). Most statistical packages-such as SAS, SPSS (2007) and R (R Development Core Team 2008)-work most easily with a raw data matrix that is formatted with the OTUs or plant specimens listed as rows and the characters or variables that are measured listed as columns in the matrix. One exception is NTSYS-pc (Rohlf 2005) which by default will expect the character variables to be rows and the OTUs to be the columns for input of data. Most of the NTSYS-pc (2005) computational modules have a parameter for specifying how the data matrix is formatted, so either orientation can be handled.

No standardization or transformation of the data was done prior to the data being read into a particular statistical software package. Both SPSS (2007) and R statistical software version 2.7.0 (2008) were used for statistical analysis. For statistical analysis in R the data matrix contained in an Excel file was first saved as a tab-delimited file from the Excel 'Save' menu (R cannot read Excel files). For statistical analysis using SPSS (2007) the Excel data file was read directly by SPSS for data input. Dissimilarity matrices were calculated in R and output as square matrices to serve as NTSYS input.

Multivariate Analysis–Steps in performing principal coordinates analysis (PCoA) of the data matrix were as follows:

Read raw Data Into R Statistical Package-This step is done by first reading the tab-delimited data matrix into an R dataframe using the read.table() function. At this point R

now has read the data from the input file and has stored it in a R object called a dataframe with rows representing OTUs and columns representing the characters or variables. Next step is to define the attributes of the variables (columns). Columns containing the variable data were specified as character, numeric, or factor (categorical) in terms of their attribute type. The character and numeric variables are usually set correctly by default after the read.table() command has been executed. However, the categorical variables need to be specified as such by a series of special commands in R after initially reading the data. Attribute status of the variables can be checked with the dim() and names() commands. Most useful is the Hmisc package's (Hmisc is an optional package or module that can be added to any R base installation) contents() command for displaying current variable attributes.

The dissertation data set contains 10 continuous, quantitative variables or characters and five categorical variables which are treated as ordinal variables for multivariate analysis (Table 1). The quantitative variable are: culm length, panicle length, panicle width, peduncle hair length, peduncle length, spikelet length, leaf length, leaf width, leaf sheath hair length and ligule length. Each of the 10 quantitative variables is treated as a numeric or integer variable in R and will be set to this attribute type by default after initially reading in the data matrix.

The ordinal variables are: culm internode pubescence, leaf blade margin, leaf sheath pubescence, leaf blade adaxial pubescence and leaf blade abaxial pubescence. During character scoring of the OTUs each ordinal variable was scored as an integer value. As

a result, after reading the data matrix into R, these ordinal variable columns will be set to an integer attribute type and the attribute for each must be changed before commencing with multivariate analysis.

The factor() command in the R base installation is used to correctly set the attribute type for the ordinal variables. An example of the use of this command is given below for correctly setting the categorical variable "leaf sheath pubescence type" to the attribute type of ordinal after initially reading the data into R:

factor(lvfShP, levels = c("1", "2", "3", "4", "5", "6"), labels = c("glabrous", "sparsely pubes", "puberulent", "pubescent", "pilose", "villous"), ordered = TRUE)

In the above command note that "lvfShP" is the column name for leaf sheath pubescence type in the R data matrix and that there are six "levels", 1-6, that correspond to the six labels given. The parameter "ordered = TRUE" specifically states that the six levels are ordered, making this an ordinal variable. Similar factor() statements are issued in R to set the other four qualitative variables to type ordinal.

Calculate Dissimilarity Matrix-PCoA analysis-developed by Gower (Gower 1966)-is well suited to mixed data sets such as those containing both quantitative and qualitative variables. The goal of PCoA is allow the relationships among the OTUs to be represented in a two or three-dimensional Euclidian space (i.e. a representation in a Cartesian coordinate system) or more simply termed-a scatter plot (Legendre and Legendre). Before beginning a PCoA analysis a dissimilarity matrix-which is a matrix of the pairwise distances (dissimilarities) of all of the observations or OTUs in the dataset-

must be computed from the original data matrix. The most appropriate metric for computing distances in a mixed dataset is the Gower metric (Gower 1971), as modified and described in (Kaufman and Rousseeuw 2005; Podani 1999), as it allows for the inclusion of ordinal variables in computing the dissimilarities.

The Gower metric was used to compute pairwise dissimilarities (distances) between each of the 15 characters (Table 1) measured for each OTU. Dissimilarity matrices, with the Gower-computed distances, were generated with the *daisy*() command of the *cluster* module version 1.11.10 (Maechler et al. 2008) for R. The *daisy*() command is fully described in Kaufman and Rousseeuw (2005). All variables or columns of the data matrix are first range-standardized by *daisy*() with facilitates equal weighting of the variables. Next, the final dissimilarity between each pair of OTUs is computed as a Manhattan distance (city block) divided by the number of variables that are non-missing for all OTUs (there were no missing observations in the dissertation data matrix). Essentially, the "Gower metric" is combination of both the range-standardization and Manhattan computational steps on the data matrix.

The following *daisy*() command was used to generate dissimilarity matrices:

```
dissimilarity_matrix <- as.matrix(daisy(data_in_R,  metric = "gower"))
```

Note in the above command that "data_in_R" is the R dataframe containing the OTU data and that "dissimilarity_matrix" is the R object or representation of the *daisy*() output. Also note that the *as.matrix*() command is simply an intermediary R function that must be used to convert the result of *daisy*() from its R representation into a standard

matrix that can be written to a file for further processing. Dissimilarity matrix files were written to a file using the write.table() command:

write.table(dissimilarity_matrix, file = "C:\file_to_write" , row.names = TRUE, col.names = TRUE, quote = FALSE)

In the above command "file_to_write" is the name of the output file containing the dissimilarity matrix in square form. This file is now ready for import into NTSYS for PCoA computations.

PCoA Eigenvector Calculation-Several steps must be completed in NTSYS to generate the PCoA data: 1) the R-generated "dissimilarity_matrix", which is in the form of a square matrix, must be converted to a symmetrical form with the "transf" module (with the 'transfer by rows' option selected and 'transformation code' set to "symd"); 2) the symmetrical matrix produced in step 1 must be double-centered using the "dcenter" module (with the 'square distances' option selected); finally, eigenvectors and eigenvalues are calculated with the "eigen" module (with the 'vector scaling' option set to "SQRT(LAMBDA)", 'sample size' set to "0", 'degrees of freedom' set to "0", 'show details' option selected, and 'cutoff for roots' set to "0.00").

The computed eigenvectors were plotted using both the "matrix plot" and "Mod3D plot" modules in NTSYS.

VITA

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