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A localized morphometric study of Panicum virgatum and sister taxa (Poaceae: Panicoideae: Paniceae)

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ABSTRACT

Morphological distinctions were evaluated between taxa of *Panicum* section *Virgata* Nees as recognized by Hitchcock and Chase (1951) and Freckmann and Lelong (2002b): *P. virgatum* var. *virgatum, P. virgatum* var. *cubense*, *P. amarum* subsp. *amarum*, and *P. amarum* subsp. *amarulum*, using morphometrics and greenhouse experiments on material from the southeastern United States, a region with the most extreme overlap in distribution. 31 characters for 104 specimens were subject to univariate, bivariate, and multivariate analyses. Spikelet bivariate graphs showed two groups with large (C) and small (A+B) spikelets. When habitat, distribution, and phenology was superposed on these graphs, non-exclusive trends polarized the small spikelet group into two groups (A) and (B). Morphogroups A, B, and C did not overlap on PCA graphs, but were not separated by gaps. Results show two morphogroups present: A+B (*P. virgatum* var. *virgatum,* var. *cubense*, *P. amarum* subsp. *amarulum*) and C (*P. amarum* subsp. *amarum*).

Key words: *Panicum virgatum, Panicum amarum*, morphometric analysis, PCA

OBJECTIVE

Evaluate the morphological distinctions between southeastern United States taxa of *Panicum* section *Virgata* through multivariate analysis and greenhouse experiments to revise the taxonomy as necessary.

INTRODUCTION

Panicum section *Virgata* is a group of about a dozen species, the most prominent of which is *Panicum virgatum* L., switchgrass, which is receiving increasing attention because of its potential as a source of biofuel. Despite the considerable interest in *P. virgatum*, the distinctions between it and other members of the section are unclear. This is particularly true in the southeastern United States, where *P. virgatum* grows near what have been called *P. amarum* Elliott subsp. *amarum*, and *P. amarum* subsp. *amarulum* (Hitchcock and Chase) Freckmann and Lelong.

The genus *Panicum*

Panicum in the strict sense (i.e. former *Panicum* subg. *Panicum*, sensu Zuloaga and Morrone (1987)) encompasses plants with a tufted growth form, leaves with membranous-ciliate ligules, and open panicles with spikelets on long pedicels/stalks. The upper glume and lower lemma of the spikelets have seven to thirteen nerves. The upper lemma and palea surrounding the upper flower harden during development; compound papillae, which can be seen using a scanning electron microscope, ornament the apex of the upper palea. In some species, these papillae are also present at the apex of the upper lemma (Zuloaga and Morrone 1987, Aliscioni et al. 2003). Species of *Panicum* have a

base chromosome number of x=9 and have the C_4 NAD-me subtype photosynthetic pathway (Aliscioni et al. 2003).

Recent molecular work has shown that the large genus *Panicum* is polyphyletic, and the name *Panicum* is now restricted to a monophyletic group corresponding to the former subgenus *Panicum*. Based on molecular sequence data from the chloroplast gene *ndh*F, Aliscioni et al. (2003) found that the five sections of subgenus *Panicum* (*Dichotomiflora, Virgata, Panicum, Rudgeana,* and *Urvilleana*) form a highly supported monophyletic group. *Panicum* s. str., as newly circumscribed, includes approximately 100 species, a great reduction from the previously heterogeneous group of 450 species (Aliscioni et al. 2003). Species outside subgenus *Panicum* have been assigned to new genera (Sede et al. 2008, Zuloaga et al. 2007, Zuloaga et al. 2006, Bess et al. 2005, Bess et al. 2006).

The most parsimonious tree of Aliscioni et al. (2003) showed that the sections *Panicum* and *Rudgeana* are each monophyletic, but sections *Dichotomiflora, Virgata*, and *Urvilleana* are not so clearly resolved. Sections *Virgata* (represented by *Panicum tricholaenoides* and *P. virgatum*), *Urvilleana* (represented by *P. chloroleucum* and *P. racemosum*), and two ungrouped species (represented by *P. mystasipus* and *P. olyroides*) form a clade. Within this clade, sections *Virgata* and *Urvilleana* form a well-supported clade with 91% BS support (Aliscioni et al. 2003; see also Zuloaga et al. 2007). Aliscioni et al. (2003) summarized "further studies are needed to understand relationships among *Dichotomiflora*, *Virgata*, and *Urvilleana*", and that molecular and morphological work was still needed within the newly delimited genus *Panicum*.

The distribution of section *Virgata* is worldwide, while section *Urvilleana* is found throughout the Americas (Zuloaga and Morrone 1987, Stevens, 2001, Freckmann and Lelong 2002b, Aliscioni et al. 2003) (Table 1). Of the species discussed in this study, *Panicum urvilleanum* grows in the southwestern region of the United States, and does not overlap with *P. virgatum*, *P. amarum* subsp. *amarum,* or *P. amarum* subsp. *amarulum* (Figure 2). However, *P. virgatum*, *P. amarum* subsp. *amarum,* or *P. amarum* subsp. *amarulum* all overlap in the southeast United States (Figure 2)

Panicum* sect. *Virgata

Hitchcock and Chase (1951) grouped *P. repens* L., *P. gouini* E. Fourn., *P. virgatum* L., *P. havardii* Vasey, *P. amarum* Elliott, and *P. amarulum* Hitchc. and Chase in the informal group *Virgata*. Freckmann and Lelong (2002b) referred to the equivalent group of taxa as section *Repentina* Stapf., which includes *P. repens, P. virgatum* var. *virgatum, P. virgatum* var. *cubense* Griseb., P. *virgatum* var. *spissum* Linder, *P. havardii*, *P. amarum* ssp. *amarum*, and *P. amarum* ssp. *amarulum* in North America.

Extrapolating from their molecular results, Aliscioni et al. (2003) included ten taxa in *Panicum* sect. *Virgata*: *P. altum* Hitch. and Chase, *P. amarum* var. *amarum*, *P. amarum* var. *amarulum* (Hitch. and Chase) Palmer, *P. deciduum* Swallen, *P. glabripes* Döll, *P. havardii*, *P. longissimum* (Mez) Henrard, *P. tricholaenoides* Steud. var. *tricholaenoides*, *P. tricholaenoides* var. *flavomarginatum* (Mez) Zuloaga, and *P. virgatum* (Aliscioni et al. 2003). *Panicum virgatum* and *P. amarum* have been consistently grouped together due to their similar morphology.

Previous classifications have disagreed regarding the existence, rank and number of infraspecific taxa of *P. virgatum*, *P. amarum*, and *P. amarulum*. All told, 15 varieties have been described under *P. virgatum*: var. *breviramosum* Nash, var. *confertum* Vasey, var. *cubense* Griseb., var. *diffusum* Vasey, var. *elongatum* Vasey, var. *glabrum* Döll, var. *glaucephyllum* Cassidy, var. *longiglume* Thell. and Zimm., var. *macranthum* Vasey, var. *obtusum* Alph. Wood, var. *pilosum* Döll, var. *scorteum* Linder, var. *spissum* Linder, and var. *thyrsiforme* Linder. Borhidi raised *P. virgatum* var. *cubense* to the subspecific rank as *P*. ssp. *cubense* (Griseb.) Borhidi. Most of the others have been synonymized with *P. virgatum* and were not recognized as distinct by Zuloaga and Morrone in the Catalogue of New World Grasses (Zuloaga et al. 2003). Hitchcock and Chase (1951) were the last to recognize the variety *cubense*. Freckmann and Lelong (2002b) discussed variation of *P. virgatum*, but recognized no infraspecific taxa. *Panicum virgatum* var. *cubense*, var. *spissum*, and *P. havardii* were all synonymized by them with *P. virgatum*, and they noted that plants formerly identified as *P. virgatum* var. *cubense* are found only in the southeastern U.S.

Panicum virgatum is morphologically similar to *P. amarum*. As currently circumscribed (Freckmann and LeLong 2002b), *P. amarum* includes two subspecies, *P. amarum* subsp. *amarum* and *P. amarum* subsp. *amarulum*. This latter taxon was originally described at the species rank by Hitchcock and Chase (1910), and then was reduced to a variety of *amarum* by Palmer (1975). More recently, Freckmann and LeLong (2002a,b), recognized these two taxa at the rank of subspecies. In addition, Vasey and Scribner described *P. amarum* var. *minus*, which is currently placed in

synonymy with *P. amarum* var. (=subsp.) *amarum* by Zuloaga and Morrone in Zuloaga et al. (2003).

Morphological variation within section *Virgata* has been well documented by Hitchcock and Chase (1910), Silveus (1942), Palmer (1972, 1975) and Zuloaga and Morrone (1987). *Panicum virgatum* and *P. amarum* are morphologically different from the rest of the group in the acuminate apex of the spikelet. The spikelets of these two taxa appear quite similar although *P. amarum* has more nerves on the first glume and slightly longer spikelets, but the values overlap (Figure 1). Other characters, such as contracted or spreading inflorescences, erect or decumbent culms, and spikelet density, have been used to separate the taxa, but often these observations are ambiguous and some characters have not been analytically documented (Hitchcock and Chase 1910, Silveus 1942, Palmer 1972). Palmer (1975) found morphological overlap in several characters such as spikelet length and branching of the inflorescence, which were two key features in Hitchcock's (1951) description of *P. amarum* and *P. amarulum*; as a result, she reduced the rank of these two taxa to variety. Freckmann and Lelong (2002b) acknowledge problems with the current classification, and note that the species intergrade morphologically in parts of their ranges. Clearly, *P. amarum* and *P. virgatum* are not readily distinguished, and Freckmann and Lelong (2002b) raised the possibility that the two species might not be truly distinct.

Previous classifications were based on general observations on specimens collected across a large area of distribution. This broad sampling may complicate the classification. The *P. virgatum* and *P. amarum* complexes have been shown to exhibit morphological clines and were thought to hybridize; in habitats of overlap, they can then

be indistinguishable (Freckmann and Lelong 2002b, Palmer 1975). Palmer (1975) reported variation in morphology of the *P. amarum* complex along the Atlantic and Gulf coasts. Members of the two subspecies were more similar along the Gulf, but in localities farther north along the Atlantic coast, beginning in North Carolina, they became more distinguishable (Palmer 1972).

Due to the confusion regarding taxon boundaries in *P. virgatum* and its close relatives in North America, a focused morphological study is clearly needed. This study concentrates on the plants of *Panicum* section *Virgata* found in the southeastern United States, the region in which the taxa share the widest range of reported overlap. To date, no one has carried out multivariate analysis on this complex. Here I evaluate the morphological distinctions between the following North American taxa of *Panicum* section *Virgata*: *P. virgatum* var. *virgatum, P. virgatum* var. *cubense*, *P. amarum* subsp. *amarum*, and *P. amarum* subsp. *amarulum*, through multivariate analysis, field observations, and greenhouse experiments. In addition, morphological variation was compared to distribution and phenology.

METHODS

Plant material

Four hundred and ninety-one specimens of *P. virgatum*, *P. virgatum* var. *cubense*, *P. amarum* ssp. *amarum*, and *P. amarum* ssp. *amarulum* were borrowed from the following herbaria: Missouri Botanical Garden (MO), New York Botanical Garden (NY), the Smithsonian Institution (US), North Carolina State (NCSC), North Carolina University (NCU) and Duke University (DUKE). Herbarium abbreviations follow Index Herbariorum (Holmgren and Holmgren 1998). Specifically, specimens collected from

the southeastern United States were requested because this region represents the area where the taxa have the most extreme reported overlap in distribution. From NY and US, I limited my request to specimens from Virginia, North Carolina, and South Carolina, and from the remaining herbaria I requested all material. Type specimens were not requested on loan, but isotypes collected by Elliott and Vasey from the Missouri Botanical Garden were used for visual comparison along with type images from the New York Botanical Garden and Smithsonian Institution.

Additional specimens were collected in the field, where I was able to observe habitat, abundance, surrounding plant community, and phenology of plants of this complex. Leaf fragments were stored in silica for DNA preservation, spikelets were stored in FAA, and soil samples were taken. *Panicum urvilleanum* was collected in Barstow, Imperial, and Riverside counties, in southern California in May, 2007. *Panicum virgatum*, *P. amarum* ssp. *amarum* and ssp. *amarulum* were collected in Columbus and New Hanover counties, North Carolina, in August 2007, and in Onslow County, North Carolina in October 2007.

Rhizomes from some specimens were brought back to the University of Missouri-St. Louis greenhouse for growth in a common environment. Leaves and culms were cut back and the rhizomes wrapped in moistened paper towels and then several layers of dry paper towels, and placed in plastic bags on ice. The plants were planted in 10" plastic pots and were top watered several times a week. For additional observations, seeds were obtained from the United States Department of Agriculture of *P. virgatum* (PI 476290 01 SD), *P. virgatum* var. *cubense* (PI 315728 01 SD), *P. amarum* (PI 561721 01 SD), and *P.*

amarum var. *amarulum* (PI 476815 01 SD), and grown in the University of Missouri-St. Louis greenhouse.

Morphometric measurements

Morphological data were collected from one hundred and four specimens (Appendix I, Figure 3). Thirty-one characters (twenty-six quantitative and five qualitative) characters were initially chosen, based on previous literature and personal observations (Table 3). Characters are also indicated schematically in Figure 4.

Measurements of the leaves and stem were to the nearest mm unless otherwise noted and were made using a fifteen-centimeter Westcott clear plastic ruler. Stem height (CL) was measured from the emergence of the shoot from the rhizome to the base of the lowest inflorescence branch. Inflorescence length (IL) was measured from the lowest branch to the tip of the inflorescence. When measurements for CL and IL were added together, this gave the entire plant height (PL). Inflorescence width (IW) was measured at the widest part of the inflorescence. Inflorescence basal branch length (IBB) was determined by the average of three lateral branches at the base of the inflorescence. Inflorescence middle branch length (IBM) was the average of three branches at the center of the inflorescence axis. Inflorescence upper branch length (IBT) was determined by the average of three branches at the apex of the inflorescence. Width of stem at first internode (CWINT) was the maximum width at the center of the internode between the flag leaf node and the node of the lowest inflorescence branch. Width of stem at uppermost node or first node (CWNOD) was the width of the flag leaf node. Length of first internode (INTL_1) was the length of the internode between the flag leaf node and the node of the lowest inflorescence branch. Similarly, the length of the second node (INTL_2) was the distance between the flag leaf node and the next lower node down the culm. Flag leaf length (FLL) was measured from the junction of the blade and the sheath to the tip of the blade. Flag leaf width (FLW) was measured at the widest point. Rhizome length between shoots (RL) is measured as the distance from one vertical shoot to the adjacent one.

Other measurements were taken to the nearest 0.01 cm using a dissecting microscope with an ocular micrometer. Ligule length (LIGL) was measured from the base of the ligule membrane to the end of the membrane hairs. Spikelets were measured at anthesis when possible; all spikelets were briefly boiled in water before measuring, and three spikelets from each specimen measured, the results being averaged. Spikelet length (SL) was measured from the base to the apex of the spikelet. Spikelet width (SW) was the maximum width of the spikelet. First glume (G1L), second glume (G2L), first lemma (L1L), and second lemma (L2L) were each measured from the base to the apex. Anther length (AL) was the maximum length of the anther sac.

Also, several characters were measured by counting, such as the number of nerves on the first glume (G1NERV). The structure and degree of branching of the inflorescence was recorded by counting the number of primary branches from the apex (IS_PB), number of secondary (IS_20), tertiary (IS_30), quaternary (IS_40), and quinary (IS_50) branches from that first branch. The total number of spikelets (TOTSPK_PB) and the highest degree of branching order (IS_ORD) were counted on the first branch. Consequently, the length of the first branch at the base of the inflorescence (IS_LB) with a 15 cm ruler was measured to compare density of spikelets.

The following five qualitative measurements were recorded from the specimens: a description of the membrane and hairs of the ligule, the presence or absence of hairs on the spikelets, the presence or absence of purple pigmentation on the spikelets, the shape of the spikelets, and the presence or absence of hairs at the inflorescence nodes.

Histograms and bivariate graphs were created using all characters and combinations of characters. Qualitative measurements were overlaid onto bivariate graphs that showed signs of a correlated relationship or taxonomic pattern.

Ecology and Phenology

Characters regarding habitat and flowering times were recorded from specimen labels. If not recorded on the label, the coordinate information for each specimen was determined using Google Earth. Elevation (ELV) was recorded either directly from the label or estimated using Google Earth and locality information. In addition, Google Earth was used to measure the distance from the collecting site to the ocean shore (DIST_COAST). All locality information recorded from herbarium specimens is available upon request. The reproductive developmental stage (FLR_FRT) of each specimen was recorded using numbers one through five (1-5): 1=Pre-flowering, 2=Flowering (anthers and stigmas exposed in at least some lower flowers of spikelets), 3=Late flowering (both flowers sexually developed with anthers and stigmas exposed), 4=Fruiting (anthers often not present, stigmas withering, upper palea and lemma hardening), 5=Post-fruiting (no evidence of anthers or stigmas, upper palea and lemma hardened, often with many spikelets fallen off) (Table 5).

Ploidy

My sample of specimens included 17 voucher specimens of *P. amarum* ssp. *amarum* and ssp. *amarulum* that had been used for chromosome squashes by Palmer (1972). In addition, fresh leaf samples of 13 greenhouse-grown plants were sent to the

lab of Dr. Charles Brummer at the University of Georgia for estimation of DNA content, and hence ploidy, using flow cytometry (Table 4).

Statistics

All analyses used SPSS version 12.0 for Windows. Histograms were created for all 26 quantitative characters to examine the frequency of values or gaps in values for a given character.

Principal components analysis (PCA) was conducted initially on all specimens and all quantitative characters. Any missing values were replaced with the means and values of three factors were extracted and saved. Following these preliminary analyses, one character, rhizome length (RL) was omitted because this measurement was not available for a majority of specimens. The character IS_LB, the length of the first primary inflorescence branch, was not included because it was the same measurement as IBB, which was a measured of the average length of basal inflorescence branches. Inclusion of IS_LB would result in overweighting that one character. In addition, five characters (IS_PB1NOD, IS_PB, IBT, and AL)) were omitted because they did not vary. Also, meristic characters IS_ORD, IS_2O, IS_3O, IS_4O, IS_5O, TOTSPK_PB, and G1NER were not included in PCA because they violate the assumption of normality required for multivariate statistical analysis. This left 19 characters that were included in the analyses presented here: PL, CL, IL, IW, IBB, IBM, CWINT, CWNOD, INTL_1 INTL_2, LIGL, FLL, FLW, SL, SW, G1L, G2L, L1L, and L2L (Table 3).

RESULTS

Morphometrics

The initial analysis included *P. urvilleanum*, but specimens were not included in the following analyses to enable the slight morphological differences within section *Virgata* to be studied in more detail. *Panicum urvilleanum* is quite distinct from other taxa in section *Virgata* because it is found only in the southwest region of North America. Also, a qualitative character measured for this study, presence or absence of pubescence on spikelets, strictly separated the pubescent spikeleted plants of *P. urvilleanum* from all other taxa in section *Virgata*, which have glabrous spikelets. However, *P. urvilleanum* is similar in that all taxa were found to grow in sandy soils. Subsequent analyses focused on the *P. virgatum* complex from the Southeast, and included 95 specimens. My observations initially suggested that three groups of specimens, hereafter called morphogroups, could be recognized; these correspond approximately to A) *P. virgatum*, B) *P. amarum* subsp. *amarulum*, and C) *P. amarum* subsp. *amarum*.

Data for all quantitative characters are summarized in Table 3, showing the range, mean, and standard deviation of characters. After measuring sixty-nine specimens, it became clear that the length of the top branches of the inflorescence (IBT) and the number of primary branches (IS_PB) were variable, but not correlated with other characters, such as spikelet characters, that seemed to best distinguish the provisional morphogroups, so these were not considered further, leaving a total of 24 quantitative characters for analysis.

A histogram was produced for each character to determine if the values were bimodal (Figure 5A-D). Most characters showed a unimodal distribution, and none

clearly delimited more than one group. Although IBM and IS_PB appeared somewhat bimodal, the measurements varied only slightly and did not show consistent patterns among morphogroups.

All characters were then plotted pair-wise. Inflorescence structure characters such as the length of the primary branch (IS_LB) plotted against the total number of spikelets (TOTSPK) (Figure 6), and the number of secondary branches (IS_O2) plotted against the number of tertiary branches (IS_O3) on the first primary branch (Figure 7) show no taxonomic groupings. The spikelet characters SL, G1L, G2L, L1L, and L2L were all positively correlated, with the strongest correlation between SL and L1L (R^2=0.9385). The plot of spikelet length (SL) against first lemma length (L1L) suggested the possible presence of two morphogroups, A+B with small + medium spikelets (2.8-6.2 mm long) and C with larger spikelets (6.6-8.1 mm long) or perhaps three groups of specimens, with A small (SL 2.8-4.5 mm long), B medium (SL 4.5-6.2 mm long), and C large (6.6-8.1 mm long) spikelets, which correspond to observations in the field and approximately to taxa *P. virgatum*, *P. amarum* subsp. *amarulum*, and *P. amarum* subsp. *amarum* respectively (Figure 8). Ploidy (Figure 9), flowering times (Figure 10) and distance from the coast (Figure 11) are labeled on all SL by L1L. To further examine habitat in relation to morphology, spikelet length was plotted against distance from the Atlantic coast (Figure 12).

A principal components analysis (PCA) using 19 characters produced the result shown in Figures 13-18. The first three components explained 67.6% of the total variance (Component 1=36.3%, 2=22.6%, and 3=8.7%, R^2=4E-13). Lines on Figure 14 represent morphogroups A, B, and C, which correspond to morphogroups found in the

spikelet bivariate graph (Figure 8). Thus, in the multivariate analysis, similar trends exist, but there is even less evidence for distinct groups.

Two plants were measured both in the wild (W) and subsequently in the greenhouse (GH) to determine the extent of plasticity of morphological characters. Each pair of plants is represented by two dots on the PCA and are connected by lines in Figure 13. Greenhouse plants are clearly larger and more robust than the original field collections. This was most likely due to the greenhouse soil, which was rich compared to the nutrient poor sand in which plants grew in the wild.

The number of nerves on the first glume has been used to distinguish taxa. However, when the number of nerves on the first glume is labeled on the PCA, this does not delimit groups A, B, and C (Figure 15). The number of nerves on the first glume is positively correlated with the length of the spikelet. Generally speaking, the small spikelets have three to five nerves and larger ones have seven to nine nerves, which is expected, although both small and large spikelets can have seven nerves. When other qualitative characters were labeled on the PCA graphs such as ligule morphology (membrane, hairs), the presence or absence of purple pigmentation on the spikelets, the shape of the spikelets, and the presence or absence of hairs at the inflorescence nodes, no trends were observed.

In Figure 9, ploidy levels were overlaid on the bivariate graph of spikelet length (SL) against length of the first lemma (L1L), and also on the plot of the first two principal components (Figure 16). Plants in morphogroup A with small spikelets are predominantly tetraploid (4x), whereas those with large spikelets, morphogroup C, are

octoploid (8x). Morphogroup B, between these two extremes, exhibits a mix of 4x, 6x, and 8x ploidy levels.

Flowering time also shows a weak correlation with spikelet size. Plants with smaller spikelets (A) were at anthesis in July and August, whereas those with medium (B) and large (C) spikelets are generally in anthesis around September and October (Figure 17). Flowering time does not distinctly divide groups.

When overlaying distance from the ocean on the multivariate plots in Figure 18, the plants of morphogroup C are indeed concentrated along the coast, as suggested by field observations. However, spikelet size shows a gradual decrease as one moves away from the coast. Although, there is virtually no overlap between the habitats of the large spikelet group C and small spikelet group A, and the medium size spikelet plants in group B overlap in habitats with both groups A and C. The PCA also shows that plants of morphogroup C grow on the beach or fore dunes, while plants of morphogroup A were never found in these habitats and were often found on the edge of forests or roadsides. Again, plants of morphogroup B were intermediate, growing in a range of habitats from the beach to the edges of forests, and disturbed areas and roadsides.

Ploidy levels

Results for the ploidy level analysis show that plants collected from the field and requested from GRIN varied in ploidy levels from tetraploid to octoploid. Plants of morphogroup A+B tended to have lower ploidy levels then those of morphogroup C (Table 4). In general, plants with higher ploidy levels had larger spikelets.

DISCUSSION

My results indicate that vegetative morphology varies along a continuum, whereas spikelet morphology may permit delimitation of at most two taxa, or species, based primarily on differences in spikelet size. The four taxa examined here are extremely similar in morphology and may not deserve separation. Only spikelet characters seem to delimit morphological groups while other previously used taxonomic characters do not appear useful. Consequently, the multivariate analysis was strongly influenced by spikelet characters, as reflected the bivariate graphs of spikelet length (SL) by first lemma length (L1L) (Figure 8). Spikelet size was loosely correlated with ploidy level, flowering times, and distance from the coast in the PCA analysis. Based on only a slight discontinuity in measurements shown in spikelet character bivariate graphs and trends in PCA graphs of all 19 characters (Figure 13), the specimens can be divided into two (A+B and C) weakly delimited morphogroups, which also differ slightly in habitat. The classifications by Hitchcock and Chase (1951) and Freckmann and Lelong (2002b) combined included four taxa for the southeastern United States, *Panicum virgatum*, *P. virgatum* var. *cubense* + *P. amarum* subsp. *Amarulum*, all members of morphogroup A+B, and *P. amarum* subsp. *amarum* belonging to morphogroup C; this treatment seems overly divided. Two species groups, *P. virgatum* and *P. amarum,* is more appropriate based on morphology. *Panicum virgatum* subsp. *amarulum* seems to fit the morphological patterns shown here better then *P. amarum* subsp. *amarulum* because groups A and B were not separated by a significant spatial gap in spikelet graphs, although each showed individual trends in localities and morphologies, whereas *P. amarum* subsp. *amarum*, C, was a distinct cluster in spikelet graphs.

Habitat

The one representative of *Panicum* sect. *Urvilleanum* studied here, *P. urvilleanum*, grows in sand dunes that have been deposited on the sides of mountains and in sand and rock mixtures along empty riverbanks and riverbeds. This species is scattered in colonies in the southwestern deserts of the U.S, in California, Nevada, and Arizona. Growing in sandy soil is a feature in common with the taxa studied in section *Virgata*.

The North American members of sect. *Virgata* grow in a wide range of habitats. *Panicum virgatum* is reported to extend east of the Rocky Mountains to the Atlantic coast and slightly north into Canada and south into Mexico and grows in many soil types from grassland prairies to sandy moist coastal swales (Freckmann and Lelong 2002b). *Panicum amarum* subsp. *amarum* and subsp. *amarulum* are coastal dune grasses confined to the Atlantic and Gulf coasts (Palmer 1972). *Panicum virgatum* overlaps these distributions in southeastern U.S.

In the southeastern U.S., some plants of putative *P. virgatum* grow in fields, pine forests and in ditches along roadsides. Such plants appeared to have relatively small spikelets and bloomed in midsummer. Other putative plants of *P. amarum* are found on the beach, foredunes, and on the backside and top of the first dunes. Some dune plants are distinctive because of their relatively large spikelets, condensed and sparsely flowered panicles and shrunken stature, but others on the coast have smaller spikelets more similar to *P. virgatum*. These plants are more waxy and robust than their neighbors with condensed and densely flowered panicles and grow in decumbent deeply rhizomatous clumps.

My collecting trips originally suggested that there were distinct morphotypes since, in a localized region in the southeast United States, plants were distinguishable even in similar habitats. *Panicum virgatum* and *P. amarum* seeds are probably dispersed by wind and animals, as has been suggested for other *Panicum* taxa (Davidse 1986) so dispersal between different habitats is likely to be quite easy. For example, on the coastal foredunes plants with both medium (B) and large spikelets (C) were found, and away from the coast by pine forests I found plants with the smallest spikelets (A). However, as more numerous specimens were examined, it became apparent that there was great overlap in habitat between plants with different spikelet morphologies.

Greenhouse experiments and taxonomic characters

Vegetative characters and order of branching were not useful taxonomic characters because they did not form any consistent clusters in morphometric analysis. Greenhouse experiments helped show what characters are potentially the most taxonomically useful. Plants taken from the field and grown in the greenhouse under presumably more nutrient rich conditions grew taller, thicker, and were more branched. Both greenhouse specimens had more tertiary branches, and developed quaternary branches, which were not seen at all in the wild sandy habitats in which they were collected. The degree of branching and inflorescence density, originally thought to be a useful taxonomic character, may be subject to environmental variation. In contrast, spikelet size remained relatively constant and so it is potentially taxonomically more useful. Finally, the greenhouse-grown progenitors differ greatly in morphometric space from their wild clones (Figure 13). This environmentally dependent morphology may

account for the lack of distinguishable or consistent morphogroups when all specimens are examined. Clearly, more growth comparisons are needed.

Ploidy variation

Chromosome numbers and North American distributions of the named taxa are summarized in Table 2. In regions where *Panicum amarum* var. (=subsp.) *amarum* and P. *amarum* var. (=subsp.) *amarulum* are indistinguishable morphologically, there are both hexaploids and tetraploids (Palmer 1975). *Panicum amarum* subsp. *amarum* is hexaploid, while the subspecies *amarulum* is tetraploid (Palmer 1975). *Panicum virgatum* is most commonly tetraploid in the northern range of its distribution, further south, the ploidy levels are often higher (Freckmann and Lelong 2002b, Palmer 1975). However, ploidy levels may even vary from diploid to duodecaploid (n=108) within a "small" region (Freckmann and Lelong 2002b, Palmer 1975).

Palmer (1972) suggests that *P. virgatum* is the ancestral form of *P. amarum* subsp. *amarum*, *P. amarum* subsp. *amarulum*, and *P. havardii*. Also, subsp. *amarum* seems to have originated in the Gulf region, where the subspecies *amarulum* and *amarum* are the most similar (Palmer 1972). Furthermore, her study suggests *P. amarum* subsp. *amarum,* which was found to grow on fore dunes where subsp. *amarulum* was absent, may be a specialized autoploid derivative of subsp. *amarulum*.

Conclusions

I suggest morphogroup C, which corresponds to *P. amarum* subsp. *amarum,* is a separate taxon because of character and habitat consistency. Specimens with larger spikelets (C) tended to have higher chromosome numbers, later flowering times, and grew only on the beach and fore dunes. In addition, morphogroup C is clearly separated from other specimens in bivariate graphs by spikelet length. Morphogroup A+B corresponds to *P. virgatum*, *P. virgatum* var. *cubense* and *P. amarum* subsp. *amarulum* and is a separate taxon from C. Morphogroup A+B was more heterogeneous than morphogroup C. There were no consistent ploidy levels or other morphological characters that unified the group, only trends. In the bivariate graphs of spikelet length, specimens of morphogroups A and B did not overlap, but were not separated by a large gap. Specimens of morphogroup A were never found closer than 300 m from the coast and did not grow on the coastal foredunes or beach. However, specimens of morphogroup B with their medium sized spikelets showed extreme overlap in morphology with A and in habitat with both A and C.

Previous classifications may have recognized different taxa based on several morphological characters that seem to be influenced by environmental factors. This may account for some of the differences observed in the field. However, an explanation for the more or less continuous patterns of morphological variation is gene transfer via hybridization, a process well known in grasses. As speculation, perhaps the plants on the coast and plants growing inland are best adapted to those environments or are simultaneously occupying a middle area in which hybridization is occurring. The slight difference in flowering times within the complex with the general trend of morphogroup A flowering the earliest, transitioning to B, then C seems to be related to distance from

the coast. Although the opposite has been reported in colder climates such as Canada inc which plants on the beach and fore dunes are exposed to a milder maritime climate, which promotes earlier flowering (Delisle-Oldham et al. 2008).

Several limitations of this study make a formal taxonomic revision premature. I chose to look at specimens from a localized area, focusing on morphological diversity in a region of putative species and subspecies overlap. This was to allow the degree of morphological diversity and discontinuity at a biologically significant scale to be evaluated. If there are taxa, discontinuities will normally be apparent at that scale, and that is the scale at which any interactions between them will appear. Broader patterns of variation were not studied. Furthermore, my observations were made during only a single growing season, and the few plants, which flowered in the greenhouse, only allowed limited inferences regarding plasticity. Finally, I did not look at features such as ploidy level in context of pollen fertility or breeding systems. Nevertheless, I suggest that despite the claims that four taxa are exist in the area on which I focused my study, that there are at most two morphological groups (A+B and C) present. These data will be a basis for highlighting important taxonomic characters and relating morphology to ploidy, habitat, and timing of anthesis.

Solving the taxonomic problem of the limits of the sister taxa *P. virgatum* and *P. amarum* will have implications for conservation and biotechnology. From a conservation standpoint, and according to the USDA PLANTS Database (2009), *P. virgatum* (A+B) is considered "weedy or invasive", while *P. amarum* subsp. *amarum* (C) and subsp. *amarulum* (A+B) are listed as threatened and endangered. If the groups suggested here are confirmed, these listings will need to be reconsidered. Since plants with larger (C)

and medium spikelets (included in A+B) generally grow closer to the coast, which is especially at risk for over-development, the preservation of habitat is important for the survival of this complex.

In addition, *P. virgatum* (included in A+B) is a popular candidate for biofuel production via vegetative tissues due to its high tolerance for nutrient poor soils, rhizomatous habit, and robustness, and because it is not a food crop (Bouton 2007). Also, this taxon has the potential to play a role in the production of bioplastics (Lindberg 2008). Although the more robust varieties of *P. virgatum* such as var. *havardii*, primarily found in Texas, are the most likely candidates for bio-products, this study provides useful information on other taxa in the complex, which are also capable of growing in harsh conditions and have a more widespread distribution. Obviously, morphology and habitat are important considerations when choosing a crop plant, and this study provides insight on both topics.

In future studies, the combination of morphological data with molecular work, perhaps utilizing microsatellite markers, will confirm whether groups are taxonomically independent or not. Developing these microsatellites markers will also be a useful tool in breeding switchgrass as a productive biofuel and or bioplastics crop as advances in biotechnology continue (Tobias 2006). Molecular work will provide clear evidence for the absence, presence, and perhaps degree of hybridization in section *Virgata*. At the population level, molecular work may also prove or disprove if *P. virgatum* is really one species or many subspecies, a question raised half a century ago by McMillan and Weilder (1959).

LIST OF TABLES AND SPECIMEN APPENDIX

TABLE 1: Species habitat and distribution (Freckmann & Lelong 2002)

Species	Type locality	Habitat	New World Distribution	North American Distribution
P. amarum subsp. *amarum* Elliott	South Carolina, USA	Coastal dunes, wet sandy soils, margins of swamps; foredunes, occasionally in swales	Belize, Caribbean, Honduras, Mexico, USA	East coast (CT to TX)
P. amarum subsp. *amarulum* (Hitchc. & Chase) Freckmann & Lelong	Virginia Beach, Virginia, USA	Coastal dunes, wet sandy soils, margins of swamps; swales behind first dune and sandy borders of wet areas	Belize, Caribbean, Honduras, Mexico, USA	East coast (NJ to TX)
P. virgatum L.	Virginia, USA	Tallgrass prairies, sand, open oak or pine woodlands, shores, riverbanks brackish marshes	Canada, Caribbean Mexico, USA	Plains, east of Rocky Mountains, south of Canada

P. urvilleanum	Chile	Desert sand dunes	Argentina, Chile, Mexico, Peru, USA	Southwest (CA, NV, AZ)

TABLE 2: Reported chromosome nubers of North American species of sections *Virgata* and *Urvilleanum* (Palmer 1972; Freckmann & Lelong 2002).

Species	Specified ploidy ranges for North American distributions	Ploidy and sterility
P. virgatum	North of TX	Lower; varies but most Tetraploid 2n=36; fertile
P. virgatum	South, including TX	Higher; varies from Tetraploid (2n=36 to duodecaploid (2n=108); fertile
P. amarum subsp. *amarum* (suspected origin hybridization with *P. virgatum*)	N/A	Hexaploid (2n=54), Tetraploid (2n=36); partially sterile
P. amarum subsp. *amarulum*	N/A	Tetraploid (2n=36); fertile
P. urvilleanum	N/A	Tetraploid (2n=36); fertile

TABLE 3: Unit, Number (N), range (minimum and maximum), Mean, and standard deviation (Std dev) of 32 characters used for morphometric analysis

Character	Unit	N	Min	Max	Mean	Std dev
Plant length (PL)	cm	69	35.4	216.1	109.58	40.76
Culm length (CL)	cm	69	16.6	177.5	76.46	35.40
Inflorescence length (IL)	cm	95	9.6	76.2	32.85	13.59
Inflorescence width (IW)	cm	95	0.8	76.2	6.10	6.37
Number of primary inflorescence branches at first node (IS_PB1NOD)		92	1	6	1.79	0.94
Ligule length (LIGL)	mm	93	1.4	7.1	2.99	1.06
Number of primary		28	11	31	19.89	6.51

branches on inflorescence (IS_PB)						
Highest order of inflorescence branching (IS_ORD)		93	3	5	3.81	0.50
Length of first inflorescence branch (IS_LB)	cm	94	2.4	33.2	12.10	6.51
Number of secondary branches on first inflorescence branch (IS_2O)		94	2	26	9.36	4.08
Number of tertiary branches on first inflorescence branch (IS_3O)		94	2	128	22.77	21.24
Number of quaterary branches on first inflorescence branch (IS_4O)		94	0	67	12.05	14.35

Number of quinary branches on first inflorescence branch (IS_5O)		4	0	12	8.25	2.99
Total number of spikelets on first inflorescence branch (TOTSPK_PB)		94	4	169	44.53	38.73
Average length of inflorescence basal branches (IBB)	cm	93	1.6	29.35	10.03	5.56
Average length of inflorescence middle branches (IBM)	cm	93	1.85	27.75	10.07	5.51
Average length of inflorescence top branches (IBT)	cm	69	0.8	11.75	3.56	2.10
Culm width at first node (CWNOD)	mm	94	0.4	7.9	3.46	1.60

Culm width at first internode (CWINT)	mm	95	0.35	4.9	2.13	0.91
Length of first internode (INTL_1)	cm	92	2.8	50.2	15.86	10.79
Length of second internode (INTL_2)	cm	88	2.5	61.1	12.63	8.14
Spikelet length (SL)	mm	93	2.80	8.17	4.89	1.08
Spikelet width (SW)	mm	93	1.07	2.50	1.74	0.34
Number of nerves on the first glume (G1NER)		93	3	9	5.92	1.49
First glume length (G1L)	mm	93	1.40	4.77	3.38	0.95
Second glume length (G2L)	mm	92	2.37	7.77	4.57	1.05
First lemma length (L1L)	mm	92	2.20	6.63	4.14	0.97
Second lemma length	mm	92	1.90	3.83	2.73	0.48

(L2L)						
Anther sac length (AL)	mm	57	0.6	3.4	2.25	0.57
Flag leaf length (FLL)	cm	91	7.2	80.3	33.60	12.80
Flag leaf width (FLW)	cm	95	0.27	1.26	0.66	0.20
Rhizome length (RL)	cm	47	0.23	30.1	4.63	6.57

TABLE 4: Ploidy estimations obtained from flow cytometry using living leaf tissue from personal collections and USDA GRIN material. Morphogroup information refers to the morphogroup the collected specimen from either the wild or it's greenhouse clone. All clones measured were consistant with their wild progenitors.

Sample	Morphogroup in PCA	Putative Ploidy*	Note
Youngstrom-1 P. urvilleanum	N/A	4x or 6x	
Youngstrom-2 P. urvilleanum	N/A	4x or 6x	
Youngstrom -6 (W and GH)	A+B	4x	
Youngstrom -7 (W and GH)	A+B	8x	Or higher?
Youngstrom -8	A+B	8x	
Youngstrom -9	A+B	8x	
Youngstrom -10	A+B	8x	
Youngstrom -11	A+B	6x or 8x	
Youngstrom -12	A+B	8x	

PI 315728 *P. virgatum* var. *cubense*	A+B	4x	
PI 476290 *P. virgatum*	A+B	4x	
PI 476815 *P. amarum* var. *amarum*	A+B	4x or 6x	**
PI 561721 *P. amarum*	A+B	6x	**
Lab callibration (*P. virgatum*)	N/A		
PI 421520 Blackwell	N/A	8x	
PI 421521 Danlow	N/A	4x	
PI 469228 Cave-In-Rock	N/A	8x	

*Assuming the same genome size as *P. virgatum*

**These samples had a second peak that could be mitosis/endoreduplication or a mixture of ploidies if more than one plant was included

TABLE 5: Descriptions used to record reproductive stage of spikelets on specimens.

Label	Description of reproductive stage
1	"Pre-flowering" Spikelets not sexually mature, anthers may or may not be present, if present anthers are small and pollen not mature
2	"Flowering" Spikelets of at least lower floret sexually mature, beginning of anthesis
3	"Late flowering" Spikelets well into anthesis, stigmas usually exposed
4	"Fruiting" Upper lemma and palea beginning to harden, anthers usually not present, stigmas can be persisting
5	"Post-fruiting" Upper floret very hard (caryopsis), spikelets may have fallen off inflorescence

Appendix I: Specimens used in morphometric analysis. Specimen No. column are collector numbers unless ° indicates herbarium accession or other identifying number and ⊗ indicates if the specimen was cultivated in the greenhouse, and ♦ indicates the specimen is a Type specimen, ♦♦ indicates the specimen is an isotype. Conf. (confidence) for N and W coordinates * denotes estimate, ** estimation is very approximate.

Specimen No.	**Herbarium**	**Collector**	**Date collected**	**State**	**County**	**N Coordinates**	**W Coordinates**	**Conf.**
71	MO	Agnes Chase	10-May-10	CA	Barstow	34°54'4.04"	117° 5'7.00"	**
29023°	MO	Jones	11-Apr-32	CA	Imperial	32°43'1.25"	114°48'40.14"	**
3541	MO	F. J. Hermann	25-Jul-32	NJ	Burlington	39°51'27.49"	74°38'50.90"	**
489	MO	Jean Wooten	25-Jul-66	FL	Wakulla	30°13'57.40"	84°13'52.98"	**
14111°	MO	A. A. Heller	17-Jul-26	NC	Columbus	34°17'59.09"	78°17'6.12"	**
482	MO	Olga Lakela	Jun-66	FL	Dade	25°27'26.48"	80°33'54.70"	**

11255°	MO	M. L. Fernald & Bayard Long	19-Sep-39	VA	Charles City	37°13'34.40"	76°56'44.88"	**
10	MO	Churchill	6-Sep-08	DE	Rehoboth	38°42'57.74"	75° 4'59.20"	**
7551	MO	Fogg	3-Oct-34	NJ	Salem	_ 39°39'26.03"	_ 75°31'29.64"	*
19	MO	Donald W. Woodard	29-Oct-70	TX	Cameron	_ 26° 9'8.27"	_ 97°10'13.14"	*
5527	MO	Curtiss	2-Sept-1895	FL	Lake Worth	_ 26°36'54.52"	_ 80° 2'12.09"	*
812965°	MO	?	Oct-1896	FL	Carrabelle	_ 29°51'0.13"	_ 84°39'58.41"	*
183	MO	Mckenzie	14-Aug-85	FL	Broward	_ 26° 7'12.10"	_ 80° 6'53.06"	*
3840	MO	Michael O'C. Moore & Barbara S. Reed	18-Nov-83	GA	Liberty	31°41'26.24"	81° 7'52.48"	*
27	MO	Churchill	3-Aug-27	VA	Norfolk	36°55'48.17"	76° 1'18.01"	**

51082°	MO	R. K. Godfrey & William B. Fox	5-Oct-50	NC	Currituck	36° 4'48.32"	75°47'44.44"	**
21°	MO	Churchill	24-Jul-27	VA	Warwick/ York	37° 8'52.87"	76°23'25.54"	**
44°	MO	A. S. Hitchcock	2-Sep-05	VA	Lynnhaven	36°52'35.42"	75°59'1.08"	*
10788°	MO	Demaree	21-Jul-34	OH	Scioto	38°47'35.74"	82°57'37.27"	**
109	MO	Niles E. Wallen	5-Nov-72	TX	Matagorda	28°35'49.69"	95°58'42.68"	**
64078	MO	Clyde F. Reed	5-Sep-63	MD	AA	38°41'21.80"	76°33'30.66"	**
1	N/A	Sarah Youngstrom	23-May-07	CA	Riverside	33°47'28.70"	116°56'23.39"	
2	N/A	Sarah Youngstrom	23-May-07	CA	Riverside	33°46'51.98"	116°55'49.65"	
3	N/A	Sarah Youngstrom	25-May-07	CA	Barstow	34°54'23.19"	117° 1'11.73"	

4	N/A	Sarah Youngstrom	26-May-07	CA	Imperial	32°43'25.44"	114°54'50.47"	
5	N/A	Sarah Youngstrom	4-Aug-07	NC	Brunswick	34° 3'55.71"	78° 1'57.75"	
6	N/A	Sarah Youngstrom	8-Aug-07	NC	Columbus	34°11'34.30"	78°21'32.60"	
5635	N/A	Sara DeGroot	24-May-07	CA	San Bernardo	115°38'57.08"	34°58'57.83"	
5635	N/A	Sara DeGroot	24-May-07	CA	San Bernardo	115°44'10.15"	34°52'49.77"	
5515	US	L.B. Smith	15-Sep-51	VA	Lancaster	37°36'55.30"	76°16'49.82"	*
128	US	Collins	24-Aug-41	NC	Dare	36° 4'31.50"	75°42'7.09"	**
2488420°	MO	Hitchcock						**
2488419°	MO	Hitchcock						**
1762387°	US	C. Munoz	3-Aug-40	NC	Brunswick	33°55'39.59"	78° 1'36.07"	*

953459°	US	Herb A. Cuthbert	Aug-05	SC	St. Helena Island	32°22'34.80"	80°32'50.77"	**
57	US	R.K. Godfrey/ R.M. Tryon	23-Jun-39	SC	Georgetown	33°31'26.39"	79°18'1.65"	*
9470	DUKE	R. L. Wilbur	18-Aug-67	NC	Brunswick	34°14'30.25"	77°57'43.97"	*
13696	DUKE	H. L. Blomquist	30-Jun-45	NC	Johnston	35 28' 18.9"	78 11' 34.69"	*
6651	DUKE	R. L. Wilbur & E. O. Beal	21-Oct-61	NC	Onslow	34°38'27.15"	77° 7'39.29"	*
6569	DUKE	R. L. Wilbur & E. O. Beal	6-Sep-61	NC	Onslow	_ 34°37'46.29"	_ 77° 9'9.46"	*
6872	DUKE	R. L. Wilbur	28-Jun-63	NC	Brunswick	_ 33°54'29.95"	_ 78° 4'25.82"	*
1612	DUKE	Don Correll	25-Jun-35	NC	Beaufort	_ 34°43'26.69"	_ 76°38'51.60"	*
3957	DUKE	R. L. Wilbur	26-Jul-55	NC	Tyrrell	_ 35°55'15.78"	_ 76° 1'1.17"	*
6493	DUKE	R. L. Wilbur & E. O. Beal	5-Sep-61	NC	Onslow	34°38'26.55"	77° 7'39.81"	*

1725	NY	Kenneth K. Mackenzie	19-Sep-1905	VA	Virginia Beach	36°51'25.62"	75°58'37.64"	**
16710	DUKE	H. L. Blomquist & James Ebert	15-Jun-55	NC	Nash	35°57'50.30"	77°47'55.63"	*
2063	US	Thos. H. Kearney, Jr.	1-Oct-1898	VA	Princess Anne (near Virginia Beach)	36°51'23.48"	75°58'36.37"	**
168	US	A. S. Hitchcock	Aug-1902	VA	Virginia Beach	36°51'35.15"	75°58'39.82"	**
3090	US	T. A. Williams	24-Sep-1900	VA	Virginia Beach	36 51' 41.16"	75 58' 36.13"	**
936	US	Ellsworth P. Killip & Emery C. Leonard	30-Aug-19	VA	Cape Henery	_ 36°55'44.33"	_ 76°10'16.56"	**
149302°	DUKE	Katharine Rondthaler	Aug-51	NC	Hyde	_ 35° 7'51.05"	_ 75°55'15.65"	*

304	DUKE	W. R. Anderson	1-Aug-62	NC	Carteret	_ 34°43'7.09"	_ 76°43'22.69"	*
723	DUKE	R. K. Godfrey & R. M. Tryon, Jr.	19-Jul-39	SC	Charlesto n	_ 33° 9'56.79"	_ 79°30'36.34"	*
107	DUKE	H. L. Blomquist	18-Aug-30	NC	Brunswic k	_ 33°55'16.63"	_ 78° 1'11.08"	**
71_133	NCSC	Patricia G. Palmer	6-Oct-71	AL	Mobile	_ 30°15'1.77"	_ 88° 7'52.56"	*
70_1	NCSC	Patricia G. Palmer	19-Aug-70	NC	Brunswic k	33°54'29.39"	78° 6'21.03"	*
22	NCSC	Jimmy Dickerson	22-Oct-77	NC	Onslow	34°37'54.19"	77° 8'37.86"	*
71_2⊗	NCSC	Patricia G. Palmer	4-Apr-71	NC	Onslow	34°28'16.48"	77°27'50.16"	*
70_9	NCSC	Patricia G. Palmer	22-Aug-70	NC	Onslow	34°31'31.82"	77°20'43.31"	*
70_8	NCSC	Patricia G. Palmer	22-Aug-70	NC	Pender	34°27'22.13"	77°29'36.76"	*
1901	NCSC	Wells & Shrunk	3-Sep-37	NC	New Hanover	34° 2'25.13"	77°53'20.36"	**

70_19	NCSC	Patricia G. Palmer	12-Sep-70	NC	Carteret	34°41'51.32"	76°44'21.13"	*
195	NCSC	Susan L. Corda	3-Oct-81	NC	Beaufort	35°23'49.79"	76°35'32.59"	**
70_16	NCSC	Patricia G. Palmer	29-Aug-70	NC	Dare	36° 9'6.02"	75°44'31.86"	*
1708	NCSC	George P. Johnson	10-Oct-83	NC	Carteret	34°41'39.12"	76°41'42.44"	**
70_18	NCSC	Patricia G. Palmer	12-Sep-70	NC	Carteret	34°41'51.30"	76°44'27.17"	*
71_1⊗	NCSC	Patricia G. Palmer	4-Apr-71	NC	Pender	34°24'53.36"	77°33'45.63"	*
71_179	NCSC	Patricia G. Palmer	24-Oct-71	SC	Georgetown	33°25'26.09"	79° 7'33.28"	*
71_86	NCSC	Patricia G. Palmer	2-Oct-71	SC	Beaufort	32°11'32.56"	80°42'13.31"	*
°	NCSC	A. Krings s.n.	10-Sep-01	NC	Wake	35°48'28.49"	78°43'14.30"	*
7011	NCSC	Patricia G. Palmer	22-Aug-70	NC	Pender	34°25'27.90"	77°33'2.65"	*
7010	NCSC	Patricia G. Palmer	22-Aug-70	NC	Pender	34°25'27.95"	77°33'3.14"	*

71_177	NCSC	Patricia G. Palmer	23-Oct-71	NC	New Hanover	34°13'56.00"	77°57'18.34"	*
1523	NCSC	David M. DuMond	23-Aug-68	NC	Jackson	35° 3'23.33"	83° 6'15.96"	*
71_84	NCSC	Patricia G. Palmer	1-Oct-71	SC	Colleton	32°30'17.74"	80°17'38.29"	*
71_85	NCSC	Patricia G. Palmer	2-Oct-71	SC	Beaufort	32°11'21.94"	80°41'57.39"	*
71_75	NCSC	Patricia G. Palmer	10-Sep-71	VA	Northhampton	37°15'46.28"	76° 1'29.99"	*
71_99	NCSC	Patricia G. Palmer	3-Oct-71	FL	St. John's	29°58'15.78"	81°18'34.97"	*
71_124	NCSC	Patricia G. Palmer	5-Oct-71	FL	Bay	30° 7'34.30"	85°44'10.59"	*
71_102	NCSC	Patricia G. Palmer	4-Oct-71	FL	Pinellas	28° 0'13.75"	82°49'41.65"	*
71_132	NCSC	Patricia G. Palmer	6-Oct-71	AL	Mobile	30°14'40.71"	88° 5'48.65"	*
71_136	NCSC	Patricia G. Palmer	7-Oct-71	AL	Mobile	30°14'34.00"	88° 6'38.11"	*
71_98	NCSC	Patricia G. Palmer	3-Oct-71	FL	Nassau	30°38'43.21"	81°26'5.41"	*

71_103	NCSC	Patricia G. Palmer	4-Oct-71	FL	Pinellas	27°55'31.24"	82°50'36.93"	*
70_12	NCSC	Patricia G. Palmer	29-Aug-70	NC	Dare	35°53'41.44"	75°38'39.67"	*
71_111	NCSC	Patricia G. Palmer	5-Oct-71	FL	Franklin	29°54'12.36"	84°25'2.33"	*
71_101	NCSC	Patricia G. Palmer	3-Oct-71	FL	Volusia	29°18'5.80"	81° 2'37.25"	*
71_88	NCSC	Patricia G. Palmer	2-Oct-71	GA	Glynn	31° 8'37.09"	81°22'17.38"	*
71_94	NCSC	Patricia G. Palmer	3-Oct-71	GA	Glynn	31° 2'51.79"	81°25'17.36"	*
71_151	NCSC	Patricia G. Palmer	9-Oct-71	LA	Lafourche	29°13'53.04"	89°59'45.71"	*
381578♦	NY	G. Vasey	1879	VA	Fortress Monroe	37° 0'59.00"	76°17'58.73"	**
3853♦	NY	Stephan Elliott	no info	no info	no info	no info	no info	***
7	N/A	Sarah Youngstrom	22-Oct-07	NC	Onslow	34°41'38.55"	76°40'52.70"	

8	N/A	Sarah Youngstrom	22-Oct-07	NC	Onslow	34°41'41.51"	76°40'37.92"	
9	N/A	Sarah Youngstrom	22-Oct-07	NC	Onslow	34°40'45.44"	76°55'55.32"	
10	N/A	Sarah Youngstrom	22-Oct-07	NC	Onslow	34°37'23.77"	77° 9'57.74"	
11	N/A	Sarah Youngstrom	22-Oct-07	NC	Onslow	34°37'32.06"	77° 9'39.16"	
12	N/A	Sarah Youngstrom	22-Oct-07	NC	Onslow	34°38'8.01"	77° 8'17.04"	
7_1⊗	N/A	Sarah Youngstrom	18-Aug-08	NC	Onslow_GH	GH		
6_1⊗	N/A	Sarah Youngstrom	18-Aug-08	NC	Columbus	GH		
476815°⊗	N/A	GRIN	18-Aug-08			GH		
315728°⊗	N/A	GRIN	18-Aug-08			GH		

476290° ⊗	N/A	GRIN	18-Aug-08			GH		
796292° ♦♦	MO	Elliot	1879	VA				**
780868° ♦♦	MO	Vasey	1879	VA	Hoffman?			**
887♦	US-Internet	S. B. & W. F. Parish	June 1886	CA	San Bernardo	35° 0'50.10"	115°55'42.07"	**

FIGURE 1: Distribution maps of a) *Panicum amarum* (including *P. amarum* subspecies *amarulum*), b) *P. virgatum*, and c) *P. urvilleanum*; from Freckmann and LeLong (2002), copyright Utah State University.

FIGURE 2: Similarities between spikelets (a) *Panicum amarum* subspecies *amarulum* (b) *P. amarum* subspecies *amarum* and (c) *P. virgatum.* Illustrations by Linda A. Vorobik and Cindy Roché for Freckmann and LeLong (2002); copyright privileges owned by Utah State University.

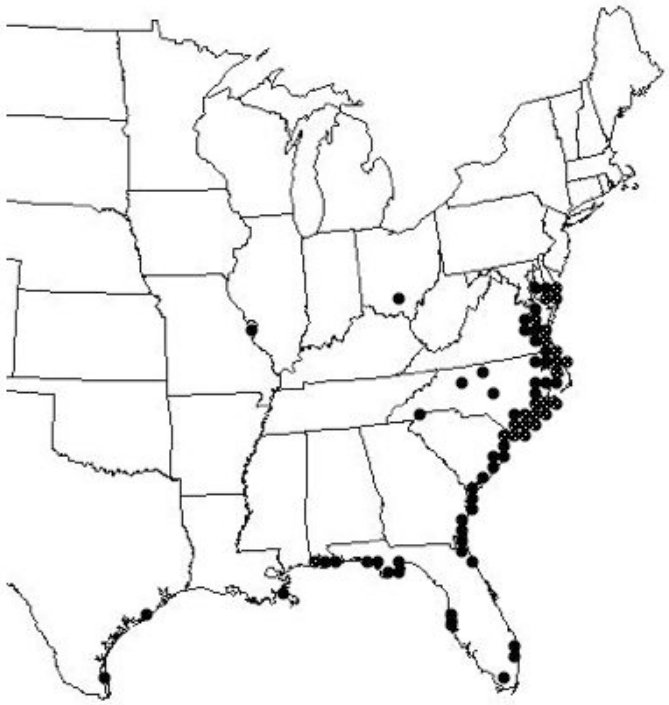


FIGURE 3: Locations of 104 measured specimens used for morphometric analysis. Each black dot represents one specimen and black and white dots represent two or more specimens on the map.

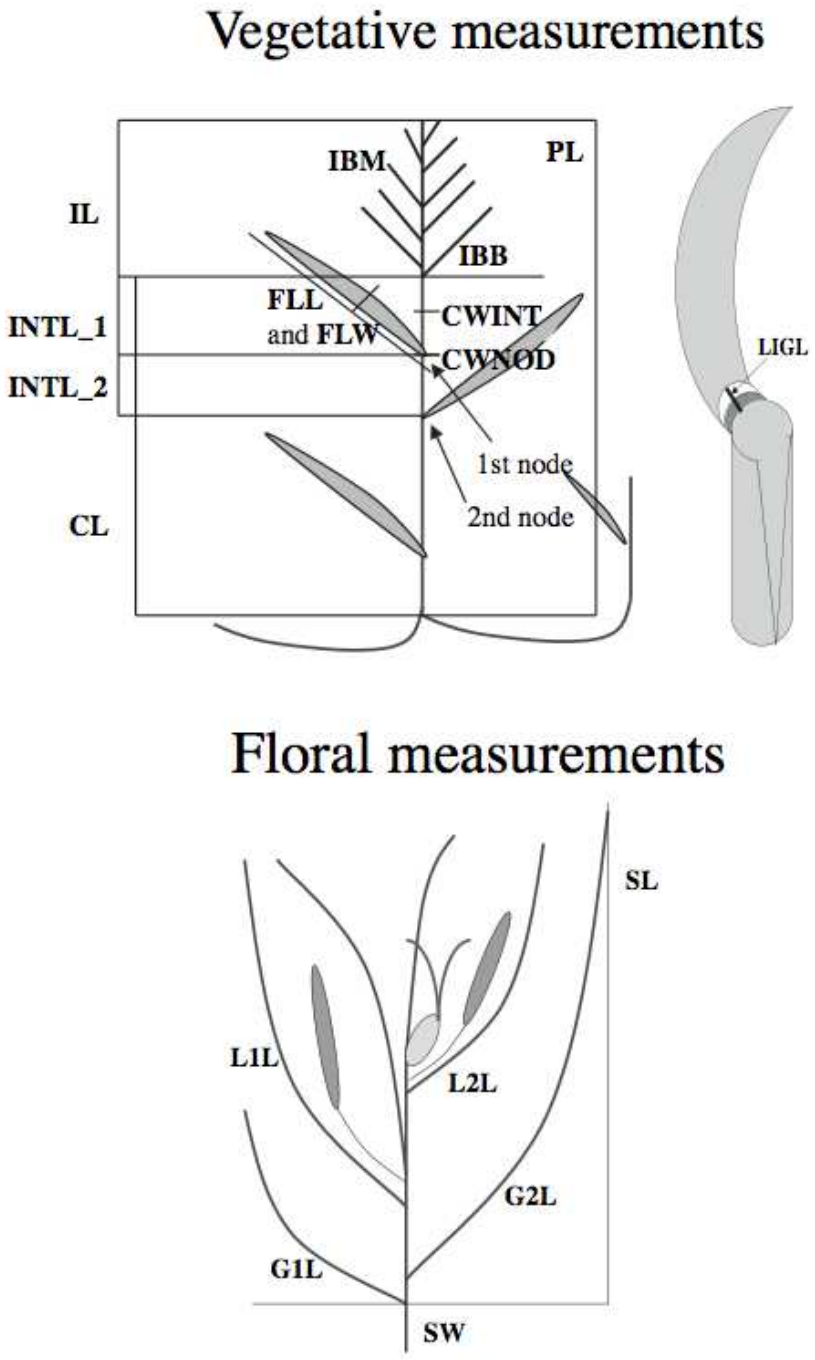
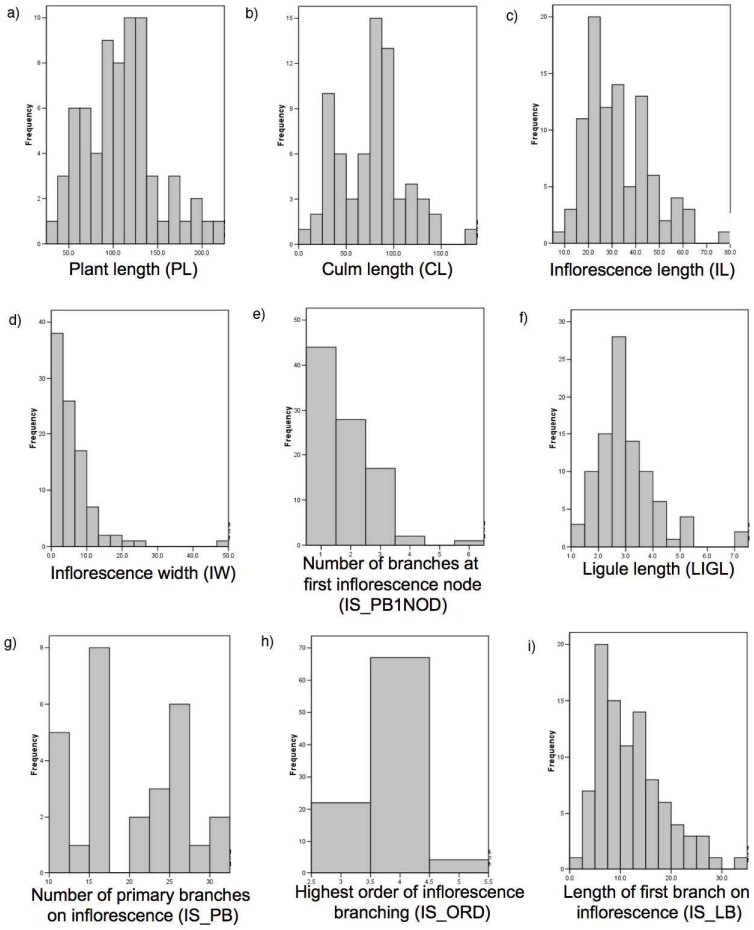
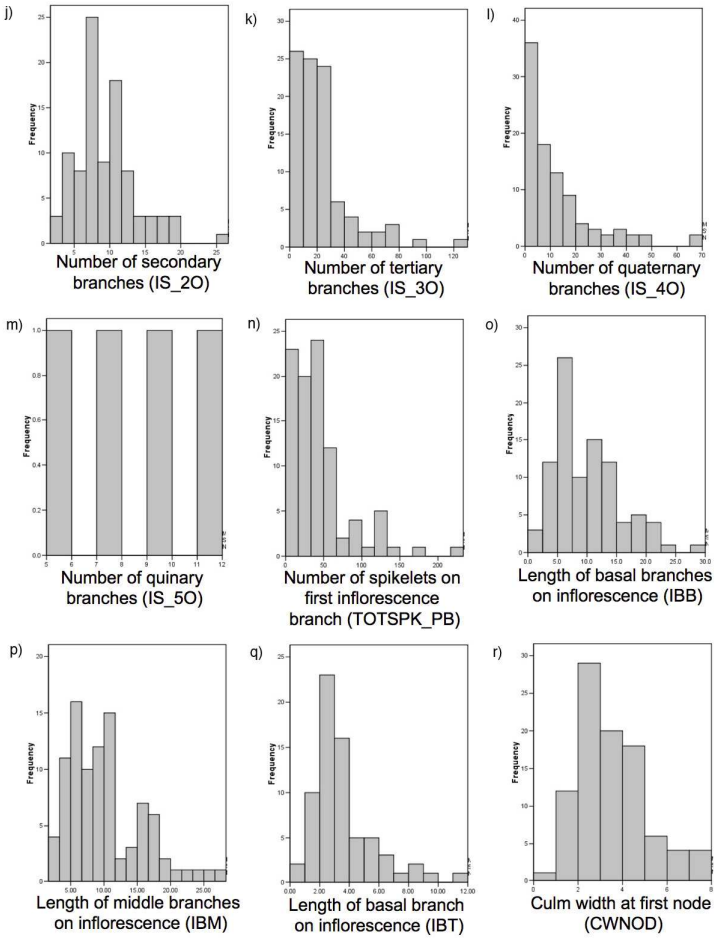
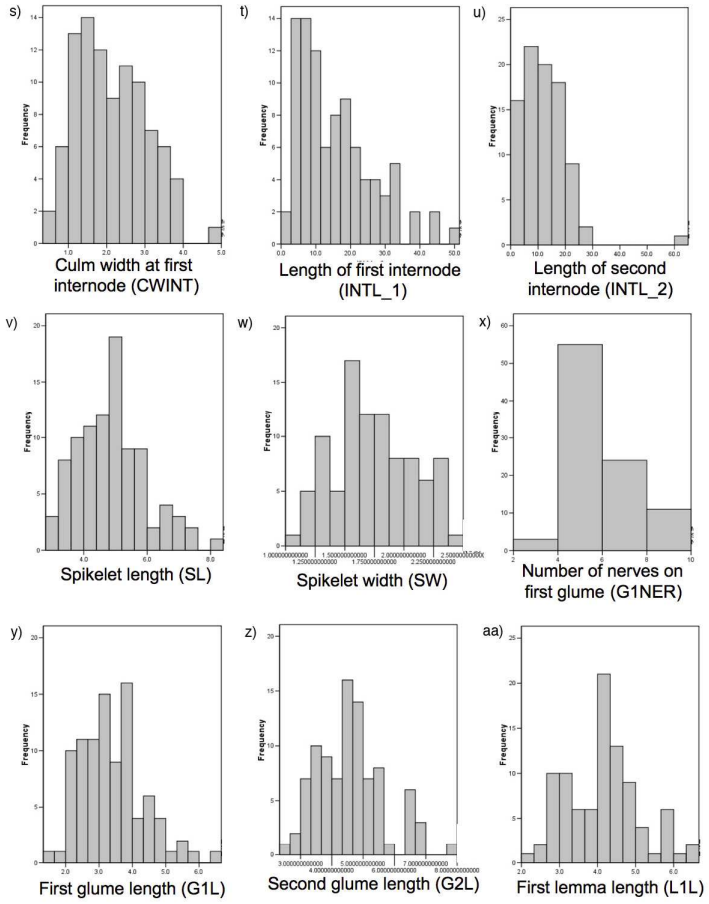


FIGURE 4: Illustrations of 19 characters measured for morphometric analysis and incorporated in Principle components analysis (PCA). Plant length (PL), culm length

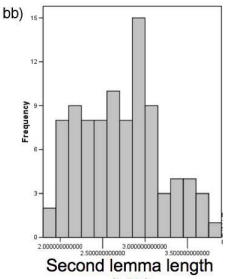
(CL), inflorescence length (IL), inflorescence width (IW), inflorescence basal branch (IBB), inflorescence middle branch (IBM), culm width at first internode (CWINT), culm width at first node (CWNOD), length of first internode (INTL_1), length of second internode (INTL_2), length of ligule (LIGL), flag leaf length (FLL,) flag leaf width (FLW) spikelet length (SL), spikelet width (SW), first glume length (G1L), second glume length (G2L), first lemma length (L1L), second lemma length (L2L).


a)
Frequency
Plant length (PL)
b)
Frequency
Culm length (CL)
c)
Frequency
Inflorescence length (IL)
d)
Frequency
Inflorescence width (IW)
e)
Frequency
Number of branches at first inflorescence node (IS_PB1NOD)
f)
Frequency
Ligule length (LIGL)
g)
Frequency
Number of primary branches on inflorescence (IS_PB)
h)
Frequency
Highest order of inflorescence branching (IS_ORD)
i)
Frequency
Length of first branch on inflorescence (IS_LB)


j)
Frequency
Number of secondary branches (IS_2O)
k)
Frequency
Number of tertiary branches (IS_3O)
l)
Frequency
Number of quaternary branches (IS_4O)
m)
Frequency
Number of quinary branches (IS_5O)
n)
Frequency
Number of spikelets on first inflorescence branch (TOTSPK_PB)
o)
Frequency
Length of basal branches on inflorescence (IBB)
p)
Frequency
Length of middle branches on inflorescence (IBM)
q)
Frequency
Length of basal branch on inflorescence (IBT)
r)
Frequency
Culm width at first node (CWNOD)

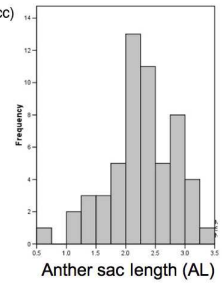

s)
Frequency
Culm width at first internode (CWINT)
t)
Frequency
Length of first internode (INTL_1)
u)
Frequency
Length of second internode (INTL_2)
v)
Frequency
Spikelet length (SL)
w)
Frequency
Spikelet width (SW)
x)
Frequency
Number of nerves on first glume (G1NER)
y)
Frequency
First glume length (G1L)
z)
Frequency
Second glume length (G2L)
aa)
Frequency
First lemma length (L1L)

bb)



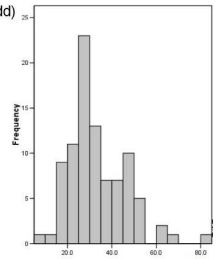
Second lemma length (L2L)

cc)



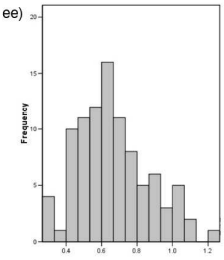
Anther sac length (AL)

dd)



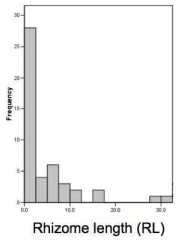
Flag leaf length (FLL)

ee)



Flag leaf width (FLW)

ff)



Rhizome length (RL)

FIGURE 5: Histograms for all measured characters a) Plant length (PL), b) culm length (CL), c) inflorescence length (IL), d) inflorescence width (IW), e) number of branches at the first inflorescence node (IS_PB1NOD), f) length of ligule (LIGL) g) number of primary branches on inflorescence (IS_PB), h) highest order of inflorescence branching (IS_ORD) i) length of first branch on the inflorescence (IS_LB), j) number of secondary branches on first inflorescence branch (IS_2O) k) number of tertiary branches on first inflorescence branch (IS_3O), l) number of quaternary branches on first inflorescence branch (IS_4O) m) number of quinary branches on first inflorescence branch (IS_5O) n) total number of spikelets on first inflorescence branch, o) inflorescence basal branch (IBB), p) inflorescence middle branch (IBM), q) inflorescence top branch (IBT) r) culm width at first node (CWNOD), s) culm width at first internode (CWINT) t) length of first internode (INTL_1), u) length of second internode (INTL_2), v) spikelet length (SL), w) spikelet width (SW), x) number of nerves on the first glume (G1NERV), y) first glume length (G1L), z) second glume length (G2L), aa) first lemma length (L1L), bb) second lemma length (L2L), cc) anther sac length (AL), dd) flag leaf length (FLL) ee) flag leaf width (FLW) ff) rhizome length (RL).

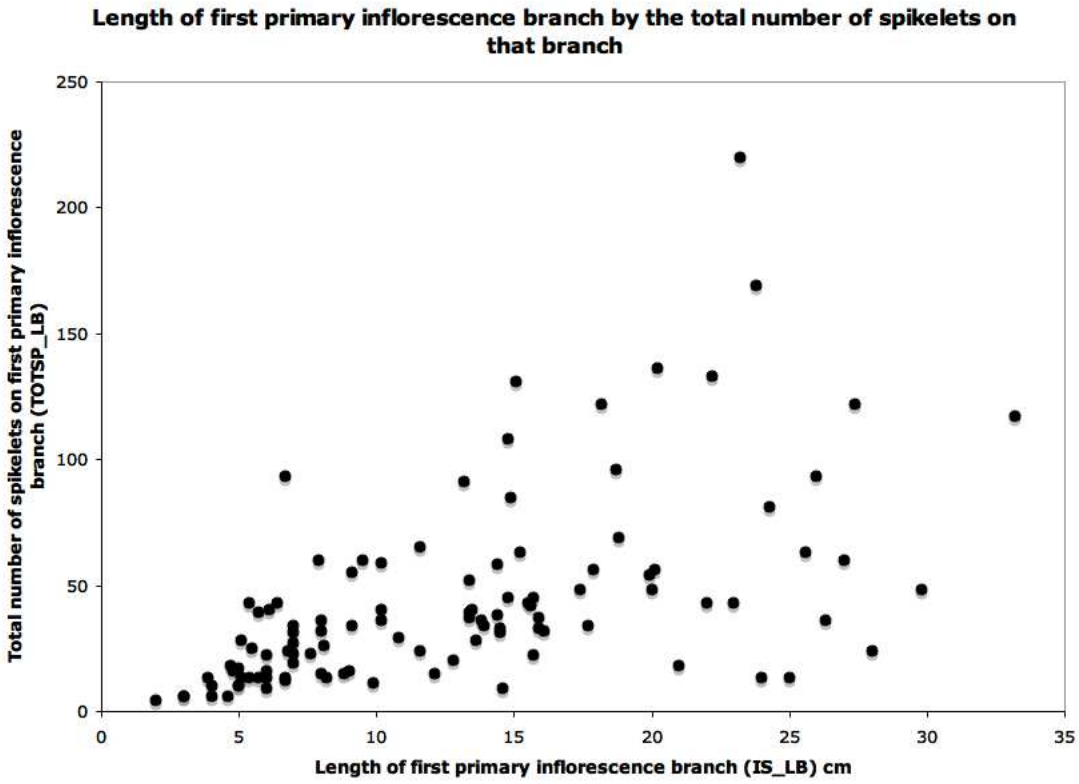


FIGURE 6: The length of first branch (IS_LB) x Total number of spikelets on first branch (TOT_SPK) do not show taxonomic groupings.

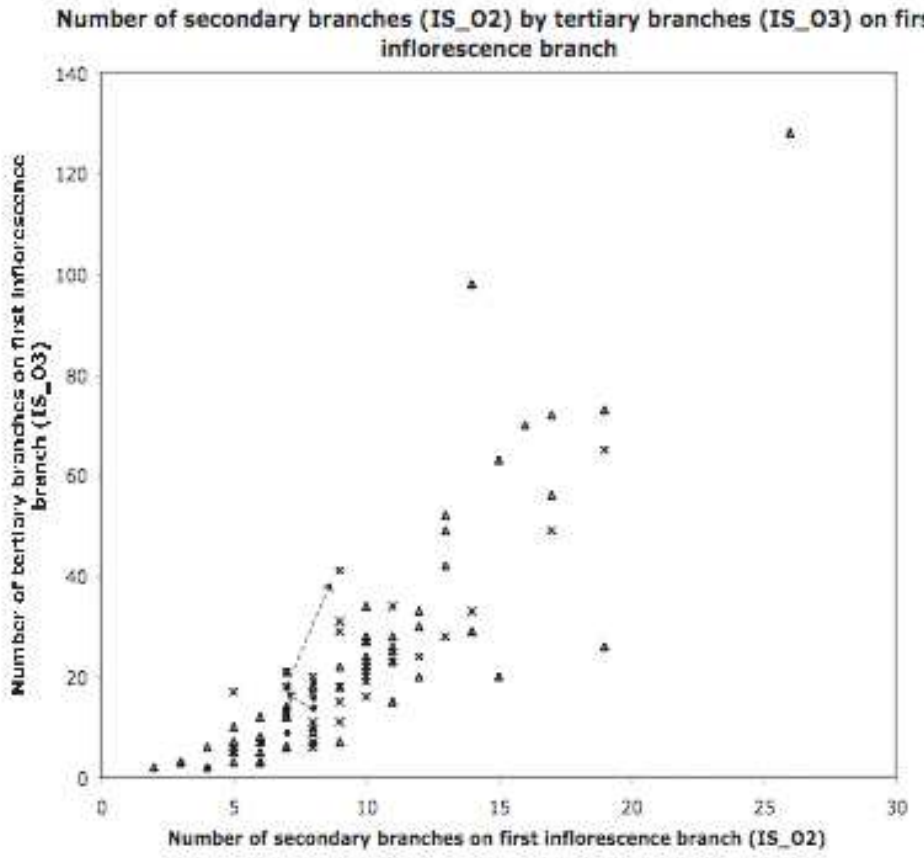


FIGURE 7: Number of secondary branches on first branch (IS_O2) x Number of tertiary branches on first branch (IS_O3). On the graph, small spikeleted specimens are represented by "x", medium by a triangle, and large by a filled in circle. Arrows point to clone grown in the greenhouse, both increased in degree of tertiary branching.

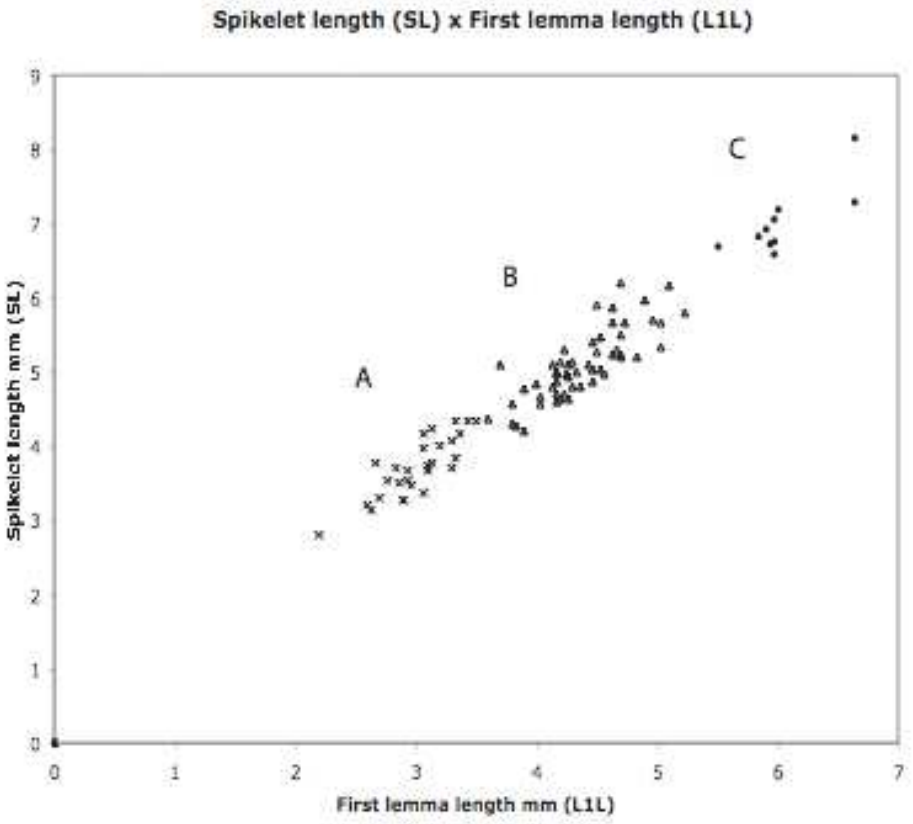


FIGURE 8: Spikelet length (SL) x length of first lemma (L1L). There are three correlated but not strongly defined morphogroups corresponding to specimens with small (A), intermediate (B), and large (C) spikelets which roughly correspond to putative taxa *P. virgatum*, *P. amarum* subsp. *amarulum,* and *P. amarum* subsp. *amarum*. On this graph, small spikeleted specimens are represented by "x", medium by a triangle, and large by a filled in circle.

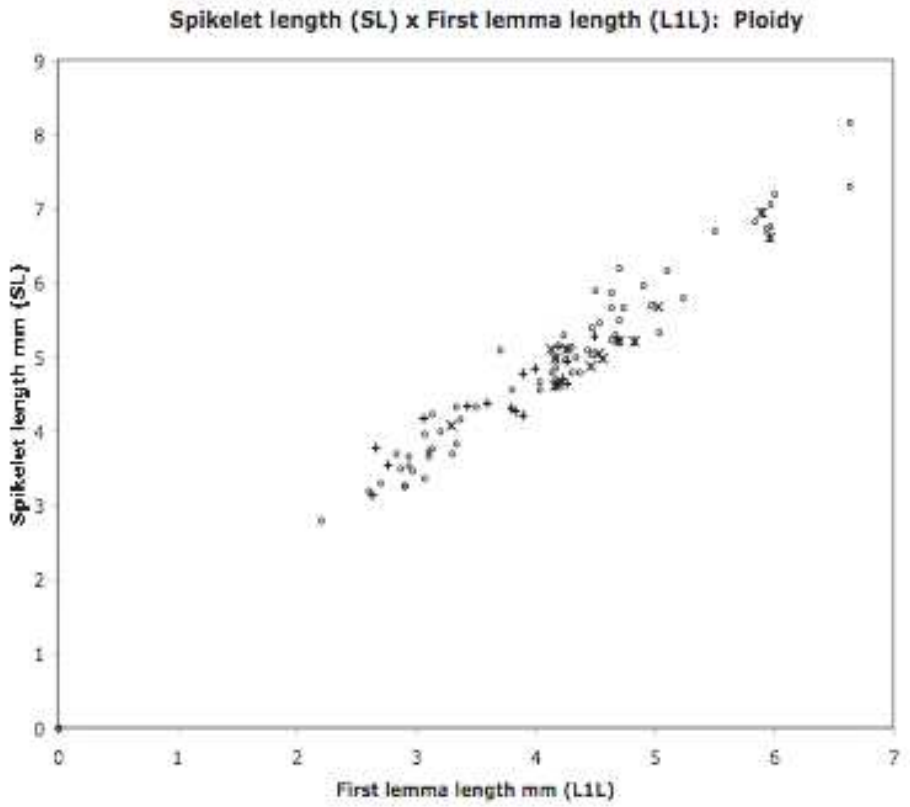


FIGURE 9: Spikelet length by first lemma length labeled with estimated ploidy levels. Larger spikelets tend to have higher ploidy levels. On this graph, specimens without ploidy estimations are represented by an empty circle, those specimens with a ploidy estimation of 4x are represented by a "+", 6x by an "x", and 8x by a three line star.

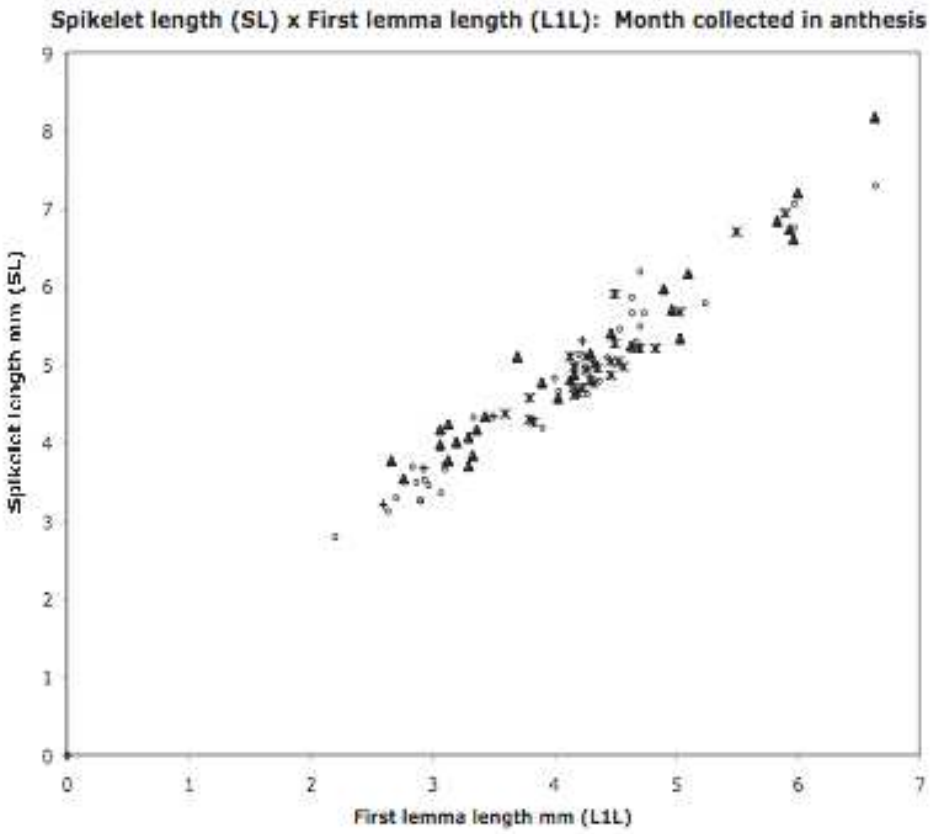


FIGURE 10: Spikelet length by first lemma length labeled with month the specimen was collected if it was in anthesis. Larger spikeleted plants flowered only slightly later then smaller spikeleted plants. On this graph, specimens that were not in anthesis are represented by an empty circle, June and July are represented by "+", August and September are represented by filled triangles, and October and November are represented by three lined stars.

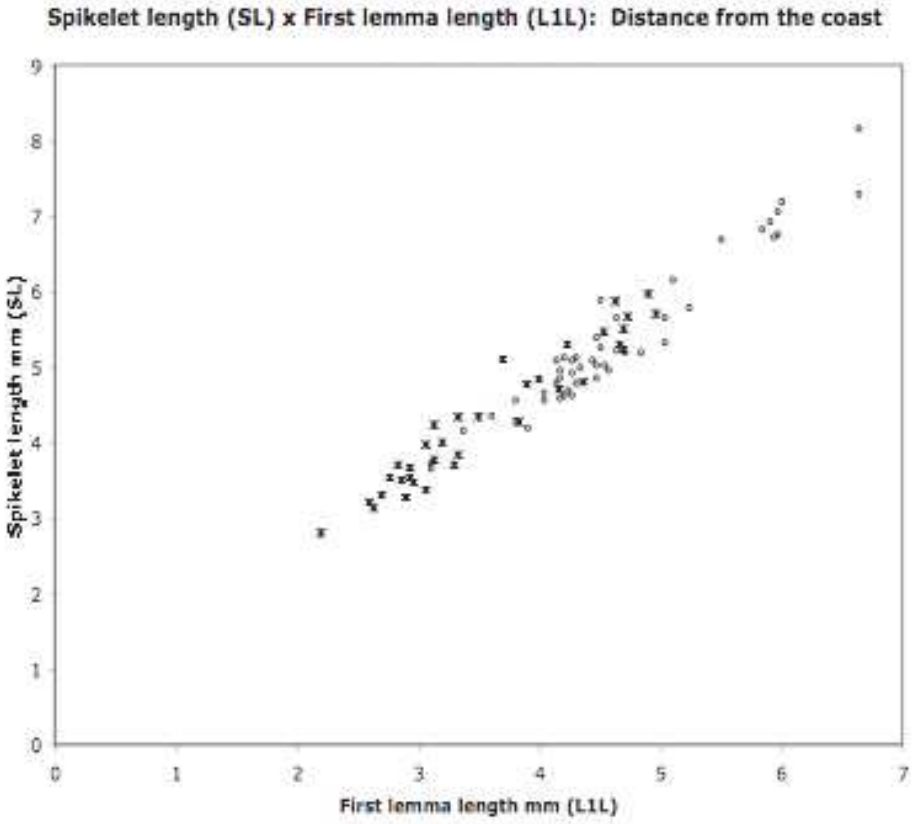


FIGURE 11: Spikelet length by first lemma length labeled with distance from the coast (Km). The plants with larger spikelets tend to grow closer to the coast. On this graph, specimens with localities closer then 300 m from the coast are represented by open circles and specimens found further than 300 m from the coast are represented by three lined stars.

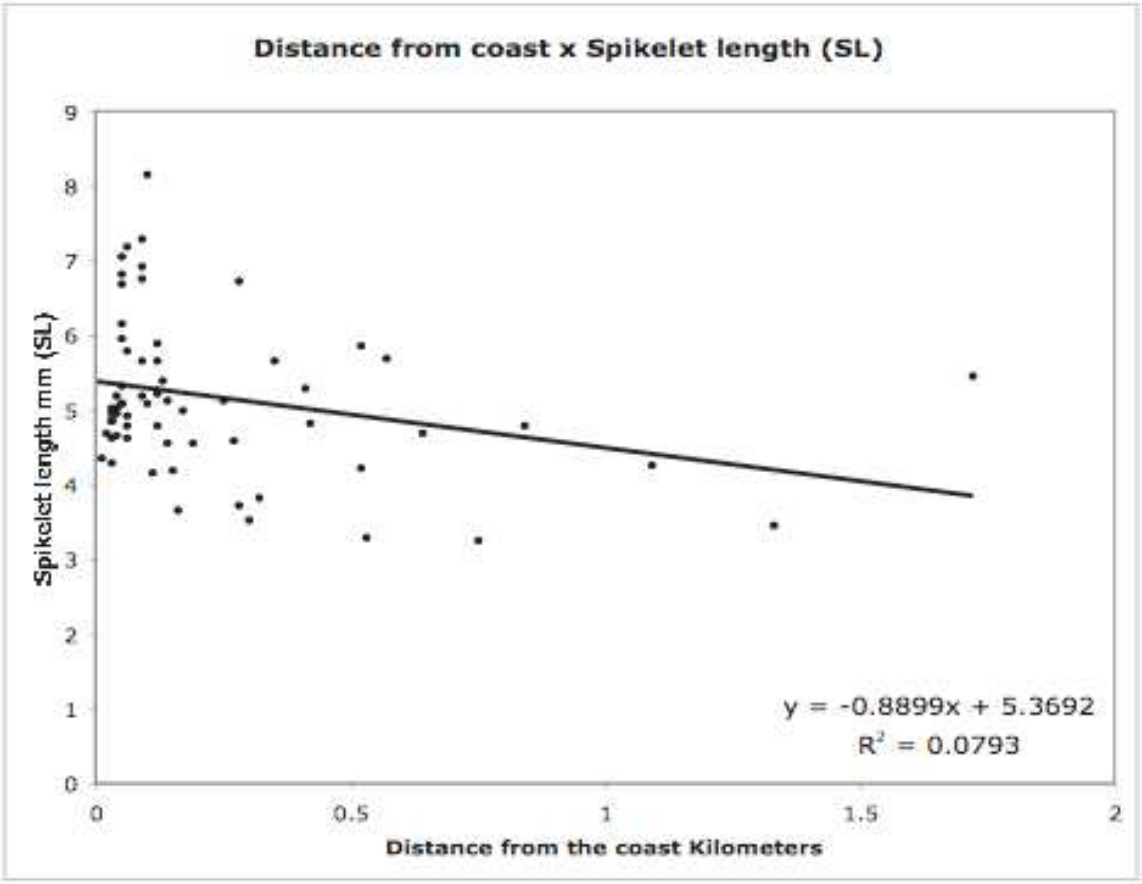


FIGURE 12: Distance from the coast (only localities less then two Km) by spikelet length. As localities became further from the coast, specimens decreased in spikelet length.

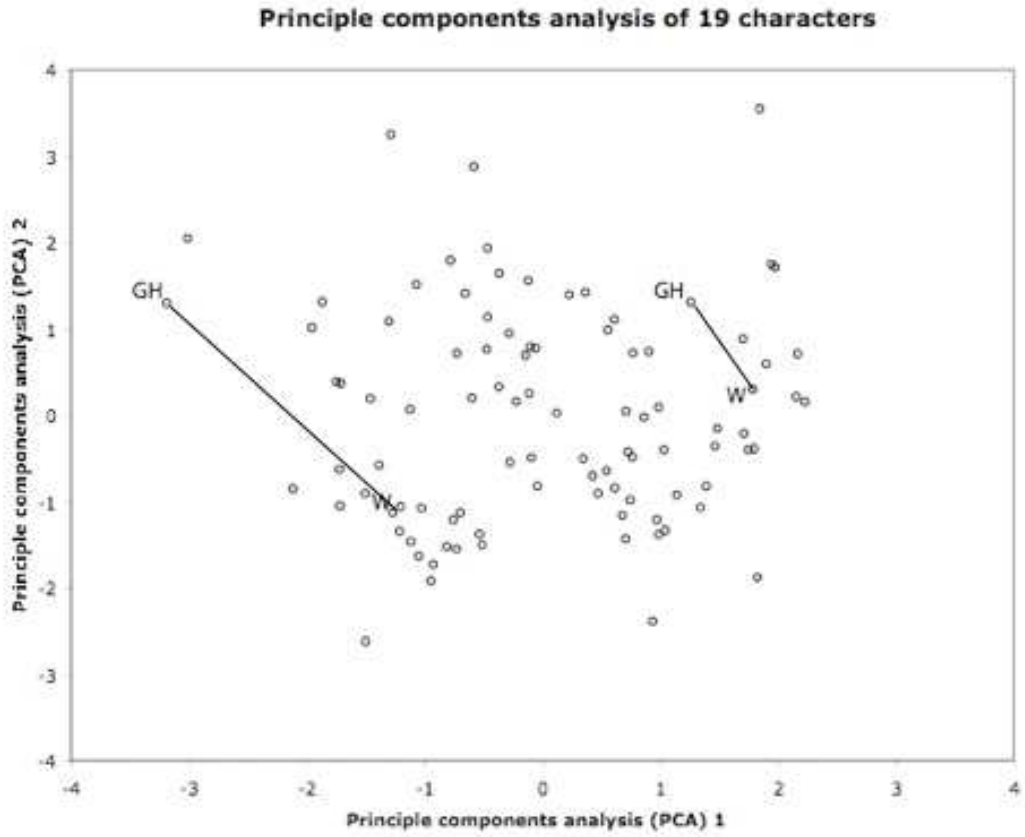


FIGURE 13: Principle components analysis (PCA) of all 19 characters displayed in bivariate graph of the first two components (Component 1: 36.34% variance explained by first lemma length (L1L), first glume length (G1L), and spikelet length (SL); Component 2: 22.57% variance explained; Inflorescence length (IL), flag leaf length (FLL), culm width at the first node (CWNOD)).

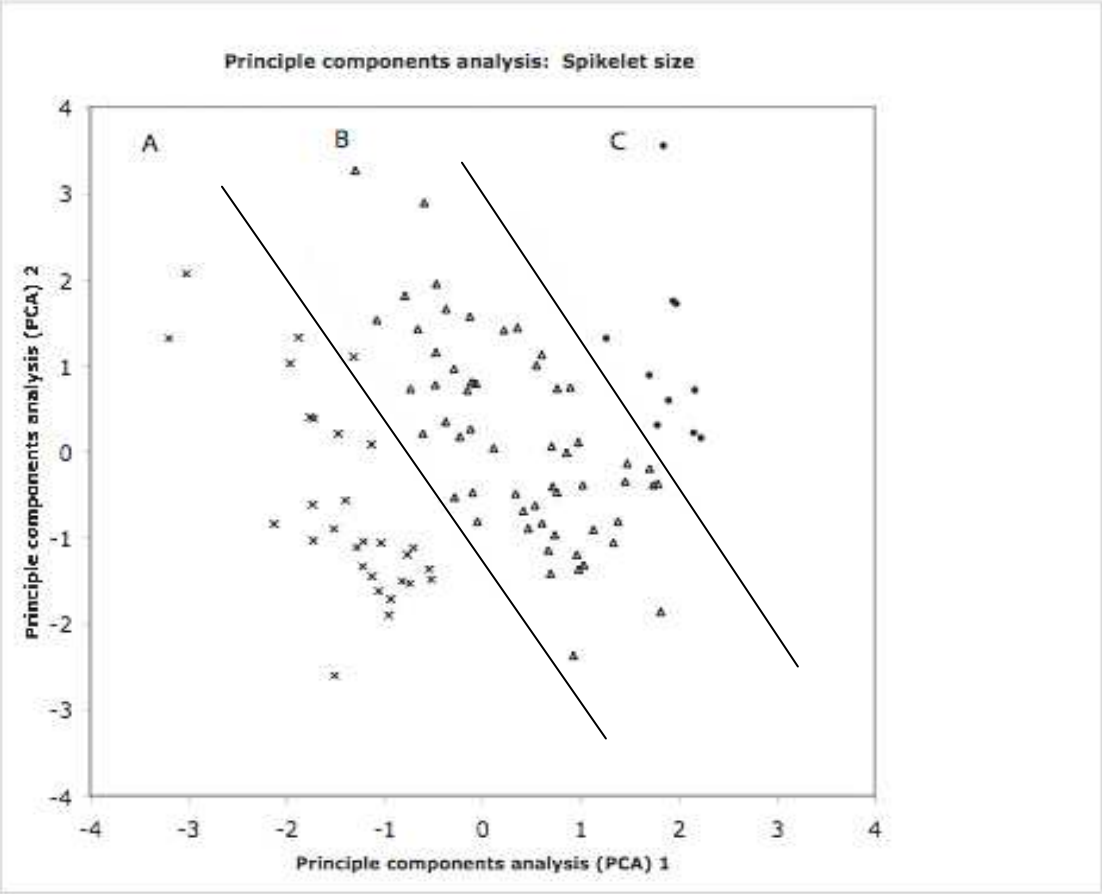


FIGURE 14: Principle components analysis (PCA) of 19 characters. On this graph, specimens with small spikelets are represented by "x", those with intermediate spikelets by a triangle, and those with large by a filled in circle (spikelets in Figure 6Bii bivariate spikelet character graphs (SL x L1L)). There is no overlap in graphical space of the specimens previously graphed in the bivariate graphs of spikelet length and first lemma length of small, medium and large spikeleted plants.

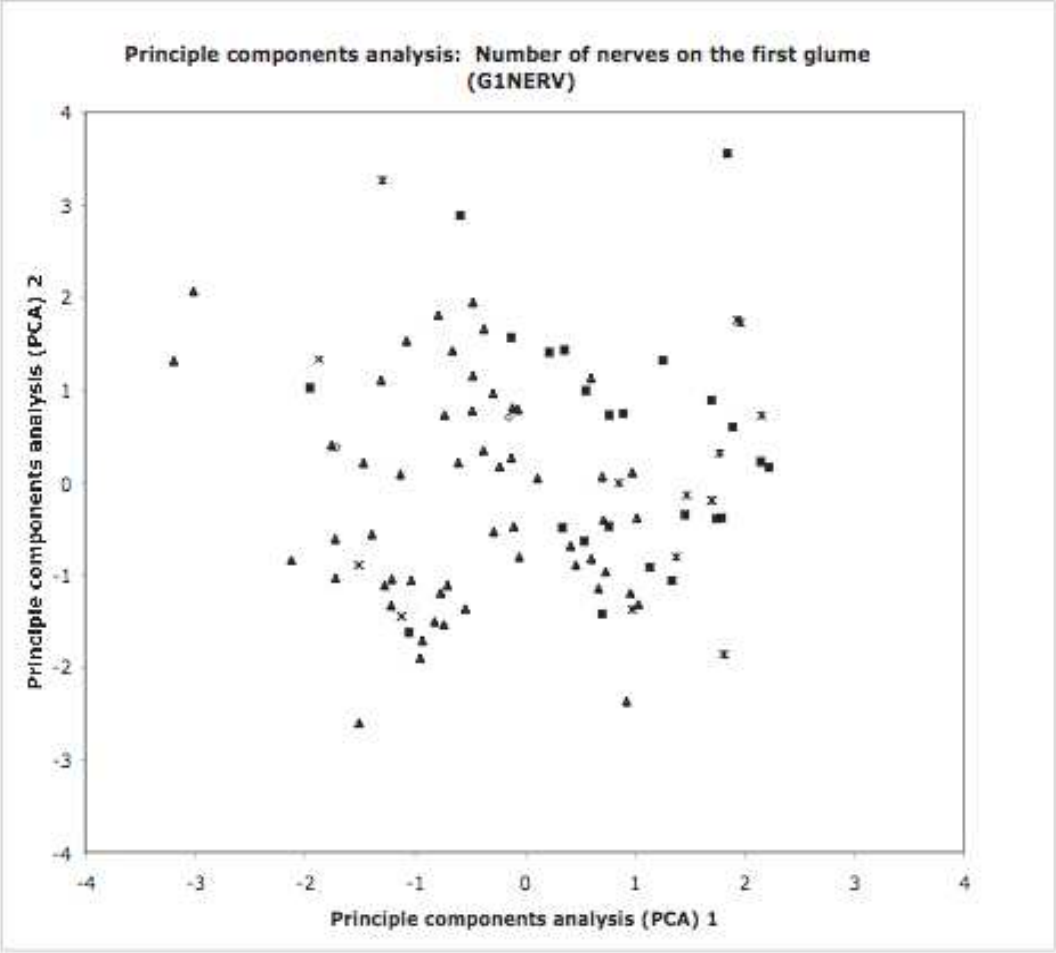


FIGURE 15: Principle components analysis (PCA) of 19 characters labeled with number of nerves on first glume, as spikelets increase in size so does the number of nerves on the first glume. On this graph, "x" represents three nerves, filled triangles represent five nerves, filled squares represent seven nerves, and three line stars represent nine nerves.

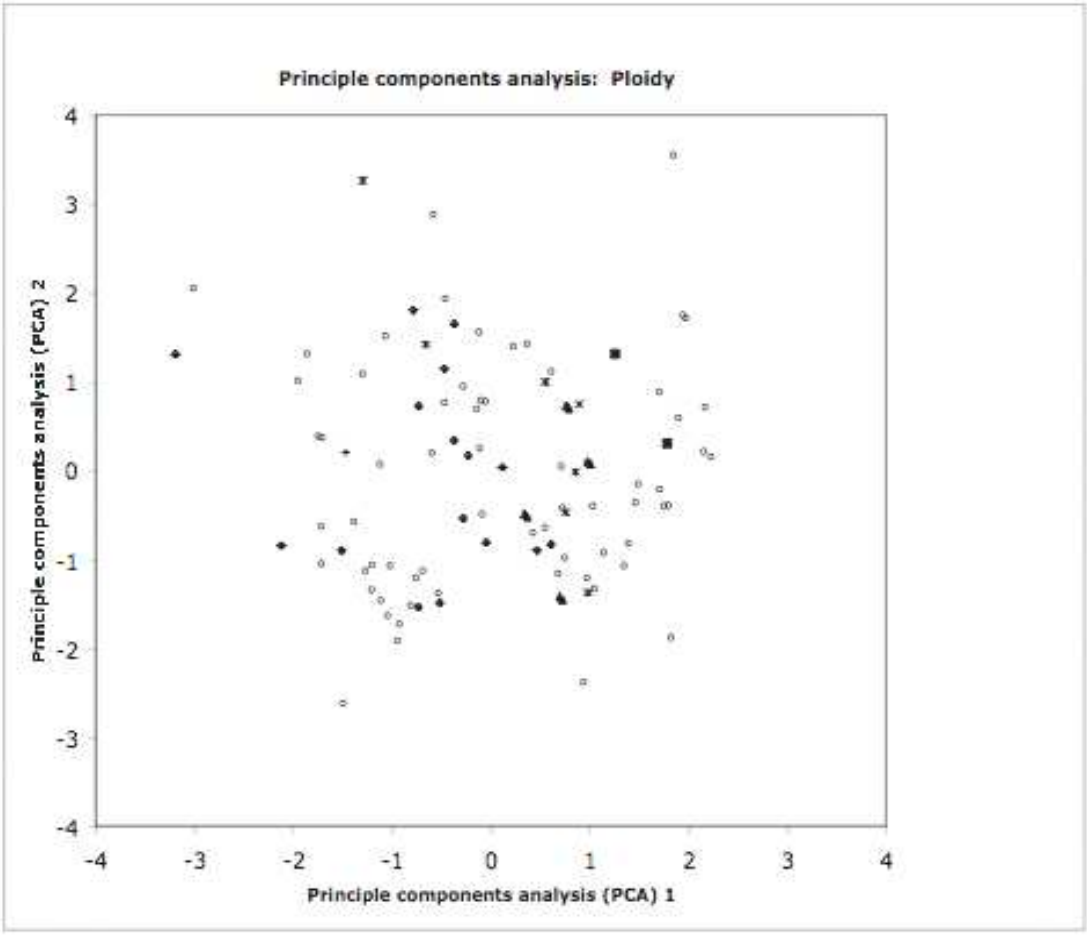


FIGURE 16: Principle components analysis (PCA) of 19 characters labeled with estimated ploidy. Ploidy levels increase from left to right, indicating larger spikeleted plants have higher ploidy levels. Black diamonds represent 4x, + represent 4x or 6x, black triangles represent 6x, x represent 6x or 8x, 6 point stars represent 8x, and black squares represent 8x or higher.

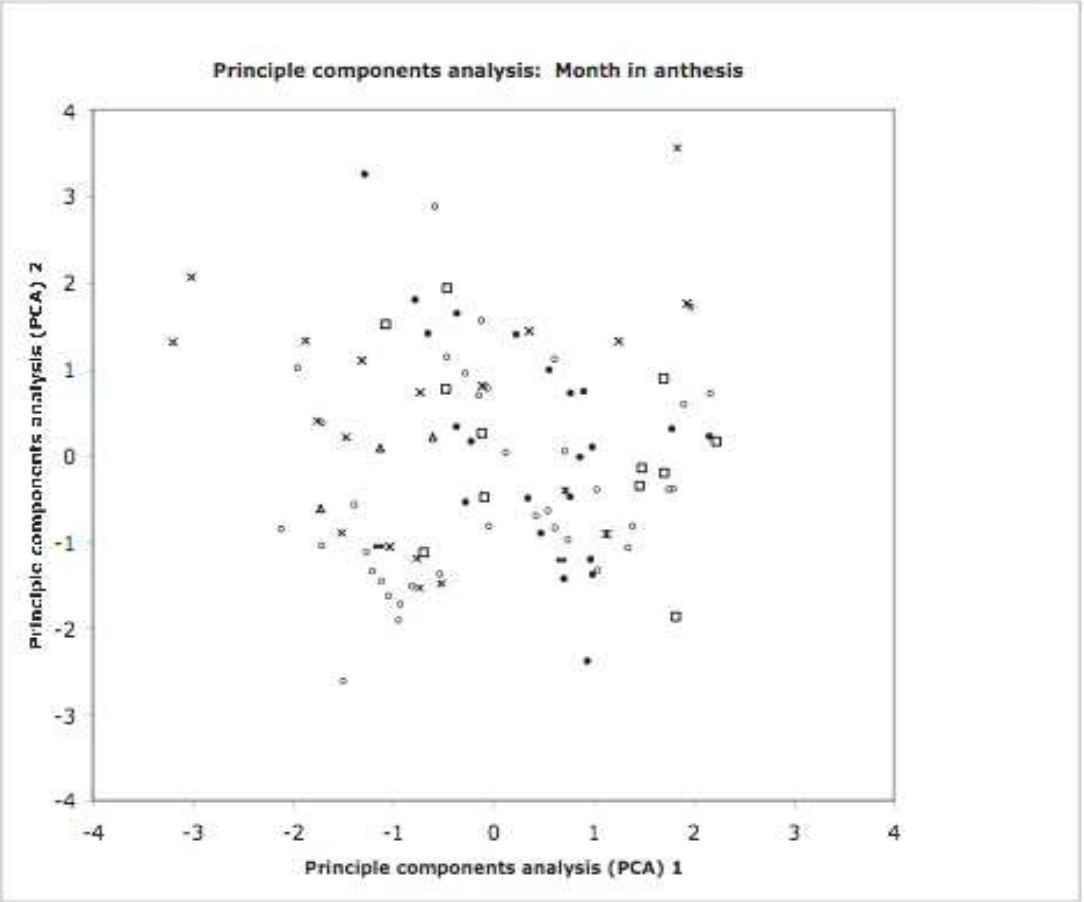


FIGURE 17: Principle components analysis (PCA) of 19 characters labeled with month in anthesis. The specimens are labeled if the specimen was in anthesis which month it was collected to make an estimation of reproduction timing. Black horizontal bar represent June, open triangle represent July, x represent August, open square represent September, black circle represent October, six point star represent November, and open circle represent specimens not in anthesis. Smaller spikelet plants appear to flower at a month or even earlier than those specimens on the right side of the graph. However, several specimens are exceptions, thus the statement is weakened.

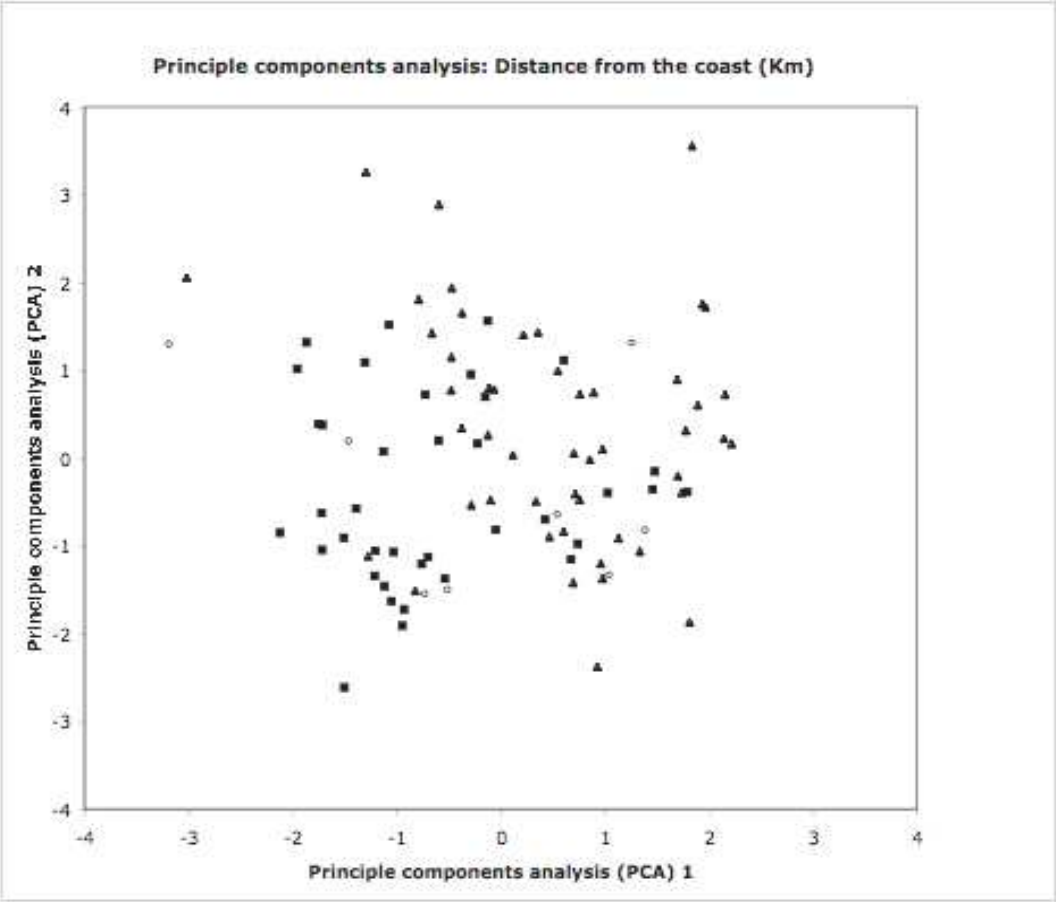


FIGURE 18: Principle components analysis (PCA) of 19 characters labeled with relative distance from the coast the specimen was located. Larger spikeleted plants are often found closer to the coast, while those plants with smaller spikelets populated regions further from the coast. Black squares represent specimens found further then 300 meters from the coast, black triangles represent specimens found closer then 300 meters from the coast, and open circles represent specimens without locality data.

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