Geographic patterns of genomic diversity and structure in the C₄ grass Panicum hallii across its natural distribution

Juan Diego Palacio-Mejía¹², Paul P. Grabowski³, Edgardo M. Ortiz⁴, Gustavo Adolfo Silva-Arias⁵, Taslima Haque¹, Dave L. Des Marais⁶, Jason Bonnette¹, David B. Lowry⁶, Thomas E. Juenger¹

¹Department of Integrative Biology, The University of Texas at Austin, Austin, Texas, USA.
²Currently, Corporación Colombiana de Investigación Agropecuaria – AGROSAVIA, Centro de Investigación Tibaitatá, Mosquera, Cundinamarca, 250047, Colombia.
³Genome Sequencing Center, HudsonAlpha Institute for Biotechnology, Huntsville, Alabama, USA.
⁴Ecology & Ecosystem Management, Plant Biodiversity Research, Technical University of Munich, Freising, Germany.
⁵Professorship for Population Genetics, Department of Life Science Systems, School of Life Sciences, Technical University of Munich, Freising, Germany
⁶Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA.
⁶Department of Plant Biology, Michigan State University, East Lansing, Michigan, USA.

Corresponding author:
Juan Diego Palacio-Mejía, Corporación Colombiana de Investigación Agropecuaria – AGROSAVIA, Centro de Investigación Tibaitatá, Mosquera, Cundinamarca, 250047. E-mail jdpalacio@utexas.edu
Orcid ID: 0000-0002-7958-1586

© The Author(s) 2021. Published by Oxford University Press on behalf of the Annals of Botany Company.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.
Abstract

Geographic patterns of within-species genomic diversity are shaped by evolutionary processes, life history, and historical and contemporary factors. New genomic approaches can be used to infer the influence of such factors on the current distribution of infra-specific lineages. In this study, we evaluated the genomic and morphological diversity as well as the genetic structure of the C₄ grass *Panicum hallii* across its complex natural distribution in North America. We sampled extensively across the natural range of *P. hallii* in Mexico and the United States to generate ddRAD sequence data for 423 individuals from 118 localities. We used these individuals to study the divergence between the two varieties of *P. hallii*, *P. hallii* var. *filipes* and *P. hallii* var. *hallii* as well as the genetic diversity and structure within these groups. We also examined the possibility of admixture in the geographically sympatric zone shared by both varieties, and assessed distribution shifts related with past climatic fluctuations. There is strong genetic and morphological divergence between the varieties and consistent genetic structure defining seven genetic clusters that follow major ecoregions across the range. South Texas constitutes a hotspot of genetic diversity with the co-occurrence of all genetic clusters and admixture between the two varieties. It is likely a recolonization and convergence point of populations that previously diverged in isolation during fragmentation events following glaciation periods.

**Keywords:** *Panicum*, phylogeographic structure, ecological genomics, ddRAD-seq, evolution, genetic admixture, habitat suitability modeling.
Introduction

Genetic variation is the raw material necessary to understand evolution and adaptation to diverse environmental conditions (Conner & Hartl, 2004; Jump et al., 2009; Randall et al., 2008). Evolutionary processes generate new diversity through random mutations which are under the influence of microevolutionary forces such as genetic drift, migration and selection (Vellend & Geber, 2005). A plant species’ life history, including breeding system and dispersal mechanisms, has an influence on the genetic diversity and distribution of species (Ellegren & Galtier, 2016; Hamrick & Godt, 1996). Current plant species distributions are the result of geological events that have had influence on soil patterns and changes in climate. For example, the soil composition of the Atlantic coastal plains in North America has been influenced by sediment deposits from the Late Cretaceous to recent deposits from the Pleistocene (Noss et al., 2015). Also, climatic fluctuations during the Quaternary have caused the contraction and expansion of species distribution, with remarkable genetic consequences (Hewitt, 2000). Shifts in range distributions can result in the loss of alleles because of bottlenecks during range expansion or the introgression of novel allele by gene flow from secondary contacts of genetic lineages differentiated in allopatry during the contractions (Hewitt, 2001, 2004). Finally, the current ecological conditions covering a species’ distribution can affect genetic structure as a result of local adaptation to these environments. Linking these processes across spatial and temporal scales can help identify the drivers of the current genetic structure of plant populations.

Here, we explored the genetic effect of different environmental factors on the genetic variation of the Hall’s panicgrass, *Panicum hallii* Vasey at the population level. This species is a perennial C$_4$ grass native to North America with a distribution that spans from the southeastern part of Mexico through the South-Central and Southwestern regions of the United States (Figure 1A). In its natural range, *P. hallii* is found across several environmental gradients such as mesic to xeric (east to west), semitropical to temperate hardiness zones (south to north), and altitudinal (from sea level along the coastal shore of the Gulf of Mexico to over 2,200 meters above sea level in the Guadalupe Mountains, Texas). This species is also found in a variety of soil and ecological conditions, including nine
ecoregions as defined by Olson, et al., (2001) (Figure 1B). The species has two varieties that are morphologically well differentiated: the widespread *P. hallii* var. *hallii* (hereafter var. *hallii*) and the more restricted *P. hallii* Vasey var. *filipes* (hereafter var. *filipes*). Both varieties occur sympatrically in a small part of their ranges. Variety *hallii* has a native distribution that extends from southern Colorado south into Mexico and from western Arizona to eastern Texas, and typically occurs on a variety of soil substrates, including sandy to shallow, dry, rocky, and calcareous soils. In contrast, variety *filipes* is typically found in areas of transition between clay soils and mesic depressions along the Western Gulf Coastal Grassland ecoregion and the Pine Oak Forest ecoregion at the Rio Grande Valley (Lowry et al., 2013; Waller, 1976). In these two ecoregions of south-central Texas, both varieties can be found in sympatry. Although *P. hallii* is a highly inbred species (Lowry et al., 2012), hybrids of both varieties can be obtained under controlled conditions in the greenhouse (Lowry et al., 2015). Nevertheless, the existence of hybridization between varieties in natural populations is unknown.

The two varieties of *P. hallii* were described at the end of the 19th century as two different species, *P. hallii* and *P. filipes*. A century later, in a taxonomic treatment of the species of *Panicum* section *Diffusa*, the taxonomic status of these two species changed to varieties of *P. hallii* based on panicle morphology (Waller, 1974) with clear morphological separation between the varieties. The *filipes* variety is generally larger than var. *hallii* with the exception of seed size (Waller, 1976). More recently, a set of 18 specific microsatellite markers was developed and validated for *P. hallii* (Lowry et al., 2012), revealing a low heterozygosity in the species, which suggests a self-fertilization pollination system (Lowry et al., 2013). Morphological and genetic analyses of 39 populations collected along a longitudinal transect from the deserts of Arizona and New Mexico through the savannas of central Texas showed strong geographic population structure for var. *hallii* (mean $F_{ST}=0.6$) with a split that occurs in western Texas (Lowry et al., 2013). Beyond these two studies, little is known about the drivers of genomic diversity of *P. hallii* across its native geographical range. Here we combine genomics, environmental niche models and common garden experiments using more than 400 samples to characterize the patterns and distribution of diversity in *P. hallii* across its
natural range. This includes 351 newly collected samples, incorporating novel material from Mexico, West and Central Texas, and natural collections from four close relative species. We used both ddRAD, a reduced representative genotyping method, and seven discriminant morphological markers to address three major questions: 1) What is the degree of divergence between the two varieties of *P. hallii*? 2) How is the genomic and morphological diversity distributed within each variety? 3) Is there evidence of admixture between individuals from these two varieties, especially when they occur in sympatry? Answering these evolutionary questions will help us to understand how evolutionary and ecological processes have shaped species diversity.

**MATERIALS AND METHODS**

**Plant material collection**

The plant material used in this study comes from two sources. First, we used *P. hallii* seed collections derived from previous studies (Lowry et al., 2012, 2013), which provides a representative sampling of the species distribution from the Southwestern U.S. Second, we conducted new field collections between 2012 and 2015 in Mexico and several Texas ecoregions (Western Gulf coastal grasslands, pine oak forest, East Central Texas forest, Texas Blackland prairies, Chihuahuan desert, and Edwards Plateau savanna), adding 351 new collections to the 298 previously collected plants. These new collections complement the previous collection effort and provide an exhaustive sampling across the natural distribution of this species.

Newly collected seeds and whole plants were transported to The University of Texas at Austin greenhouse facilities for propagation. Herbarium vouchers were collected and deposited in the Billie L. Turner Plant Resources Center at The University of Texas at Austin, and leaf tissue was collected from these plants and stored in a -80°C freezer for DNA extraction. Seeds from the collected plants were harvested and stored in a seed collection. Overall, we include 649 samples from these collections in this study (Table 1, Figure 1B, and Supporting Information Figure S1).
Habitat suitability modeling (HSM)

We implemented an ensemble modeling approach using the biomod2 package in R (Araújo & New, 2007; Thuiller et al., 2009) to explore habitat suitability based on occurrence and climate data. Our approach included the model obtained with five cross-validation replicates of seven presence-absence algorithms and five pseudo-absence sampling (a total of 175 models). We implemented independent habitat suitability modeling runs for var. hallii and var. filipes using presence records, in situ confirmed (real) absence locations, as well as pseudo-absences points sampled in the whole species distribution area (Supporting Information Figure S2). Considering the wide geographic distribution of var. hallii covering extensive environmental heterogeneity we also performed additional HSMs for the two largest infra-specific genetic clusters (West and Tex-Mex; see results). As explanatory variables, we included eight (largely independent) bioclimatic variables from the WorldClim v.1.4 (Hijmans et al., 2005). To evaluate the possible influence of historical and climate change processes on the observed patterns of genetic diversity and differentiation in P. hallii, we projected the HSMs onto three past climate scenarios including the mid-Holocene (~6 Kya), Last Glacial Maximum (LGM, ~22 Kya) and Last Inter-Glacial (LIG, 120-140 Kya) available in the WorldClim v.1.4 climatic database.

For our distribution hindcast distribution analyses, we assessed the intrinsic uncertainty of the simulation of past climatic scenarios by projecting the HSMs using climatic data derived from three different global circulation models (GCMs). A complete description of the data and modeling methods can be found in the Supporting Information 2.

Genotyping

High-quality DNA was extracted from the leaf tissue of each individual plant using a modified CTAB protocol (Allen et al., 2006). DNA concentration was quantified using a Qubit® 2.0 fluorometer and dsDNA BR Assay Kit (Life Technologies, Carlsbad, CA). The quality and purity of the genomic DNA was evaluated by running the samples on a 1% agarose gel for comparison to a low molecular weight ladder (New England BioLabs). All samples were normalized at 1 µg of DNA and stored at -20°C until used.
In order to obtain a genome-wide representation of genetic diversity at low cost, we used double-digestion restriction-associated DNA sequencing (ddRAD) (Peterson et al., 2012). Based on in silico digestions (Lepais & Weir, 2014; Mora-Márquez et al., 2017) of the Panicum hallii var. filipes reference genome v. 2.0 (DOE-JGI, https://phytozome.jgi.doe.gov), we chose the combination of NspI and Mlucl restriction enzymes for the ddRAD method.

DNA samples were submitted in three sets to the Genomics and Bioinformatics Service at Texas A&M University (College Station, Texas). Library preparation followed an in-house protocol. Briefly, for each sample, around 1 μg of genomic DNA was digested with the restriction enzymes NspI and Mlucl and then adaptor ligation fragments were selected in a range of 375-650 bp using Pippin Prep technology (Sage, Beverly, MA). Finally, the library samples were sequenced using the Illumina HiSeq 2500 and 4000 (San Diego, CA), producing around 2.6 billion of 2 x 100 or 2 x 125 pair-end raw reads.

Filtering sequencing data for nucleotide polymorphism calling and quality control

We obtained a total of ~2.6 billion raw reads for the 649 samples submitted, with a mean of ~4 million pair-end raw reads per sample (Mean= 3.961.472 ±3.265.748). Raw fastq read quality was evaluated with FastQC v. 0.11.3 (Andrews, 2018). Sequencing reads were pre-processed using BBTools v. 37.50 in five steps: 1. Trimming of adaptor sequence. 2. Removal of reads corresponding to the phiX viral genome and other common contaminants. 3. Trimming of the cutsite "AATT". 4. Merging of overlapping paired reads, and 5. Trimming of low-quality bases, retaining reads of at least 35 bp after processing.

The filtered reads were mapped to the P. hallii var. hallii v. 2.0 reference genome (DOE-JGI, https://phytozome.jgi.doe.gov), using bbmap v. 37.50 (Bushnell, 2014). We used the maximum likelihood statistical model in Stacks v. 1.47 (J. Catchen et al., 2013; J. M. Catchen et al., 2011) to call SNPs, following the pipeline designed for ecology and population genomics (Rochette & Catchen, 2017). The populations program in Stacks was used to divide the samples into three biological groups: one set for all P. hallii samples, one set for var. hallii, and one set for var. filipes. We used a minimum minor allele frequency of 0.05 to process a nucleotide at a locus. We were concerned about
poor mapping of short RAD reads given the repetitive aspects of many plant genomes. As such, we filtered for paralog loci by removing markers with an excess of heterozygosity and excluding heterozygous loci with strong allele ratio deviations using the *HDplot* method (McKinney et al., 2017). We removed markers with heterozygosity >6%, which was the maximum heterozygosity obtained using microsatellite markers in this species (Lowry et al., 2013) and the read ratio deviation (1:4, D>4) to minimize the presence of loci that consist of repetitive regions that have been co-assembled (da Fonseca et al., 2016). We used paralog-finder v. 1.0 (Ortiz, 2018) to identify loci that likely contain co-assembled paralogs (Supporting Information Figure S3). In addition, we removed markers with more than 50% missing data and, subsequently, individuals with more than 80% missing data (Supporting Information Table S1). Final VCF files were modified for different downstream population genomics analysis using vcfR v. 1.0.5 (Knaus & Grünwald, 2017).

To test for reference genome bias against var. *filipes* samples, we mapped reads from var. *filipes* to the var. *filipes* reference genome (*P. hallii* var. *filipes* v3.1 v. at DOE-JGI, http://phytozome.jgi.doe.gov/) and found that the number of SNPs is very similar (Supporting Information Table S2) and phylogenetic trees are concordant when mapping to either genome (Supporting Information Figure S4).

**Species assignment via Sanger sequencing**

As the taxonomic identification of species from *Panicum* section *Diffusa* is notoriously difficult (Waller, 1976), we sequenced two chloroplast and one nuclear region to aid in taxonomic confirmation. Samples of *Panicum* section *Diffusa* species determined by taxonomic specialists at the Billie L. Turner Plant Resources Center (TEX) at The University of Texas at Austin were used as a control for species identification. A total of 31 samples were sequenced for the nuclear Internal Transcribed Spacer (ITS1) along with two new developed suitable chloroplast regions. The first marker, cp45676, spans the 3’end of the intergenic region between *ycf3* and *trnS* and includes the 5’ portion of *trnS*. The second marker, cp1204, spans the 3’ end of the intergenic region between *psbA* and *trnK* and includes the 5’ portion of *trnK*. These markers were specially designed to differentiate species from the section *Diffusa* of the genus *Panicum*. The ingroup contained 17 control samples
belonging to 11 species of the genus *Panicum*, section *Diffusa*. Also, six samples acted as known controls for both varieties of *P. hallii*. A sample of *Setaria viridis* was used as an outgroup for phylogenetic inference.

Approximately 570 bp of ITS1, 650 bp of cp1274, and 550 bp of cp45676 were amplified by polymerase chain reaction (PCR) using the same DNA samples that were sent for ddRAD. For ITS1, we used newly designed primers ddITS1F (5’- CCG TGA ACG TGT CAT CCA TG -3’) and ddITS1R (5’- GGT CCG AGC ACC AAG GCG -3’). For cp1204: 1204F (5’- GGC TTG TAC TTT CGC GTC TC -3’) and 1204R (5’- CGG TAC GAA CTT TTA TGC AAC G -3’). For cp45676: 45676F (5’- TAG GCA TAA TTC CCA ACC CA -3’) and 45676R (5’- CTA ACC CTC GGT AAA CAA AA -3’). PCR mixtures were in 15 µL and were run on the MJR PTC-200 thermocycler. For ITS, we used the following conditions: 35 cycles of 95 °C denaturation for 60s, 56 °C annealing for 45s and 72 °C extension for 60s. For the cp1204 and cp45676, we used a two-step PCR with 20 cycles of 95 °C for 60s, 54 °C for 30s, reduced by 0.1 °C every cycle, and 72 °C for 60s, followed by 20 cycles of the same conditions but with a constant annealing temperature of 52 °C. PCR amplicons were visualized with SYBRSafe (Invitrogen, Eugene, OR) on 1% agarose gels and prepared for cycle sequencing by treatment with a 10:1 mixture of Shrimp Alkaline Phosphatase and Exonuclease I (both from USB; Cleveland, OH). Cleaned PCR products were submitted to The University of Texas at Austin ICMB Core Facility for cycle sequencing and visualization. The chromatograms were inspected by eye, manually trimmed for quality and aligned, then, the sequences were manually assembled, using Geneious v. 7.1.9 (Kearse et al., 2012). A resulting alignment of 1,982 nucleotides was analyzed, using Bayesian phylogenetic inference as implemented in MrBayes v. 3.2.2. The resultant trees were compared, exported and edited, using FigTree v. 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). In addition, because the species in the *Diffusa* section also differ in ploidy, the ploidy level was estimated in these samples using flow cytometry analysis (BD LSRFostesca SORP).
Population genomic structure

We calculated expected heterozygosity for all samples using VCFtools (--het) (Danecek et al., 2011). As a result of preliminary analyses, we selected San Antonio Texas as a landmark geographic location as it exhibited the maximum diversity of *P. hallii* in our sample. We calculated the distance of all samples to this landmark using the R package ‘geosphere’ v1.5-10 (Hijmans et al., 2019). We used the R function cor.test (R Core Team, 2018) to test for correlation between expected heterozygosity and distance to San Antonio, Texas for the var. *hallii* gene pools; the var. *filipes* gene pools lacked adequate sample sizes for the test.

Analyses of phylogeographic structure

Phylogenetic estimation: Relationships between *Panicum* species and the *P. hallii* varieties were assessed by performing maximum likelihood analyses implemented in IQ-TREE v. 1.5.5 (Nguyen et al., 2015). Because only variable sites were analyzed, we used the model GTR+R+ASC to compensate for ascertainment bias; to provide nodal support we used the ultrafast bootstrap (Minh et al., 2013) implemented in IQ-TREE (Option –bb) with 10,000 pseudo-replicates.

PCA: The high-quality SNP dataset was used to identify *de novo* the optimal number of clusters and their relationship using Discriminant Analysis of Principal Component (DAPC) implemented in the Adegenet v. 2.1.1 package (Jombart, 2008) for the R software (R Core Team, 2018). DAPC centers on a discriminant function to maximize the differences between groups and minimizing variation within clusters (Jombart, 2008). We ran the k-means clustering (find.clusters) to assess groups using the Bayesian information criterion (BIC); we used the cross-validation test (xvalDapc) to retain the number of PCs with the lowest mean squared error using 100 replicates. We used three different SNP data matrices for several independent analyses including the full set of 16,397 SNPs for all 423 samples of *P. hallii* species, one set of 16,595 SNPs for var. *hallii*, and one set of 13,167 for var. *filipes*.
Phenotypic divergence between *Panicum hallii* varieties

To explore the phenotypic divergence between var. *hallii* and var. *filipes*, a common garden was established on May 15 2014. 480 individuals from 76 different localities (ranging from 1 to 22 individuals per locality) were planted at Pickle Research Campus, The University of Texas at Austin, Texas, US (Latitude 30.3838 N, longitude -97.9296 W), following the seedling method described at Lowry *et al.* (2012). The experimental planting design followed a honeycomb layout with an interplant distance of 0.85 m. In total, seven phenotypic traits were chosen to evaluate divergence between the *P. hallii* varieties. During the months of July and August of 2014, one plant for each sampled locality was chosen to measure seed mass and panicle structure. For seed mass, 20 seeds per individual were weighed. Two representative mature panicles were collected and photographed to measure primary and secondary branch number and panicle length, using the Panicle Phenotyping tool P-trap (AL-Tam *et al.*, 2013). On August 29th 2014, all samples were tested for cold tolerance using the electrolyte leakage method (Campitelli *et al.*, 2013; Pérez-Harguindeguy *et al.*, 2013) in leaves after a -3Cº frozen treatment. Early in Fall 2014, we harvested all plants and obtained above-ground biomass after drying samples for one week in a 65ºC oven. Finally, for 368 days after planting (between May 15 2014 and May 18 2015), we measured leaf senescence every two weeks for each plant, using a qualitative scale from 1 to 4 (1 > 50% of leaf canopy green, 2 > 50% of leaf canopy senesced, 3 > 90% of leaf canopy senesced, and 4 complete canopy senescence). The average quality state was taken as the output for each plant across the year.

**Correlation between genetic and phenotypic distances**

A principal component analysis was performed on the seven phenotypic traits using R v3.6.3 (R Core Team, 2018) to identify the major axes of variation. We used the first two components from this analysis to build a matrix of pairwise phenotypic distances between populations. Similarly, we built a matrix of pairwise genetic differentiation between populations using the two first linear discriminants components (LD1 and LD2) from the DAPC analyses. Finally, a matrix of pairwise geographic distances between populations was constructed using the R package ‘geosphere’ v1.5-10 (Hijmans *et al.*, 2019).
To test correlations between any two of these matrices, we performed a Mantel Tests using the R package ‘vegan’ v2.5-6 (Oksanen et al., 2019) as well as a Partial Mantel Test to evaluate associations between genomic and phenotypic distances while controlling by geographic distance. Mantel tests were performed using the Spearman correlation method and 9999 permutations.

**Admixture evaluation between Panicum hallii varieties**

Genotypic and phenotypic analyses were conducted to explore the relationship between var. *filipes* and var. *hallii* in their region of sympatry. At the genotypic level, a new matrix of SNP was made including all var. *filipes* samples (27 individuals from 14 localities), samples from var. *hallii* in sympatry with var. *filipes* (24 individuals from 14 localities), and a sample of var. *hallii* samples allopatric to var. *filipes* (26 individuals from 12 localities). We assigned the allopatric var. *hallii* samples to one population, HAL, and a random subset of var. *filipes* samples to another population, FIL. We then ran STRUCTURE (Pritchard et al., 2000) using the USEPOPINFO model parameter and the “HAL” and “FIL” population assignments for 12 runs. The USEPOPINFO model parameter included in the program STRUCTURE (Pritchard et al., 2000) was used to detect putative admixture individuals. In this approach, samples from known varieties are defined as "learning samples" (PopFlag=1), and STRUCTURE attempts to group the unknown samples (PopFlag=0) to assign the membership probability associated with each variety (Porras-Hurtado et al., 2013). For the phenotypic analysis, a principal component analysis (PCA) was conducted with the 'stats' package in R v3.6.3 (R Core Team, 2018), using the phenotypic dataset obtained for the common gardens, but samples of var. *hallii* were divided into allopatric and sympatric samples.

**RESULTS**

**Sampling**

This study included a total of 649 individuals primarily representing *P. hallii*. The sampling covered the entire geographic range of *P. hallii*, including collections from previously unsampled Mexican states and Texas counties (Figure 1 and Table 1). From these field collections, twenty-eight localities were represented by a single individual, but most localities were represented by two to 22 individuals
(4.5 individuals in average). Species identification for undetermined samples was completed using nuclear and plastid markers. Bayesian phylogenetic trees constructed with nuclear and plastid sequences clearly grouped with strong support our new collections against the control samples, confirming that the vast majority of our collected samples included three species belonging to the Diffusa section (Figure 2). While the presence of P. hallii in Louisiana was recently reported (Reid & Urbatsch, 2012) our sample from this state corresponds to P. diffusum not P. hallii. One collection in central Texas was also assigned to P. diffusum, despite resembling P. hallii in the field. All samples collected in the central part of Mexico in the states of Mexico and Michoacan formed a clade with the P. lepidulum control sample, which had been collected in the same area. All samples from northern Mexico, from the states of Durango and Coahuila, clustered with P. hallii. Lastly, one sample in the eastern edge of the P. hallii was P. capillare. In addition, to the phylogenetic analysis, the ploidy level measured with flow cytometry showed the polyploid status of the undetermined samples (P. lepidulum and P. diffusum are putatively 4x tetraploids), in contrast with the diploid condition of P. hallii (Supporting Information Table S3). We limited our subsequent analyses to 600 samples which had a strong phylogenetic assignment to P. hallii.

**ddRAD sequencing**

We used the ddRAD approach to genotype the P. hallii samples. Almost all (99%) of the raw reads passed our quality filtering criteria. These high quality reads were mapped against the P. hallii v. 2.1 reference genome assembly (DOE-JGI, http://phytozome.jgi.doe.gov/; Lovell et al., 2018). After genotyping, SNPs were filtered to cope with presumed paralogs and missing data at the markers and individual levels (Supporting Information Figure S3). Finally, after quality control steps, a matrix with 16,397 SNPs from 423 individuals of P. hallii belonging to 127 localities was obtained. In addition, individual SNP matrices were made for each variety with 16,595 SNP for var. hallii (396 samples from 104 localities) and 13,167 for var. filipes (27 individuals from 14 localities). The high-confidence SNPs were fairly evenly distributed across the nine nuclear chromosomes of P. hallii (Figure S3C).
Natural distribution of *Panicum hallii* and habitat suitability modeling

Our sampling included regions where *P. hallii* has been described but for which contemporary samples are lacking. Several collection records show the presence of *P. hallii* samples in eastern Texas and Louisiana (Figure 1A). However, after extensive field collection trips (16 localities), the only samples we collected were either *P. diffusum* or *P. capillare*. The absence of *P. hallii* from the East Central Texas Forest, Western Gulf coastal grasslands, and Pine Wood forest could indicate ecological constraints for establishment and persistence at the eastern edge of its distribution (Figure 1B). A similar situation was found at the southern part of the natural distribution, where extensive sampling was conducted in the central states of Mexico, including the states of Mexico and Michoacan. However, both morphology and molecular markers indicate that collections made in 11 localities are *P. lepidulum* (Figure 2). With these findings, the southern distribution of *P. hallii* seems to be restricted to the north of Mexico in the Meseta Central matorral ecoregion.

Projections of the HSMs to the current climatic conditions encompass most of the known localities with high suitability scores. As expected, the areas with highest suitability match with the region of highest occurrence density located in south Texas. The consensus HSM for var. *filipes* includes the predictions of 134 (out of 175) independent models that showed true skill statistic (TSS) values above 0.75. The mean TSS over the full set of models was 0.83 and the area under the curve (AUC) score of 0.92 (Supporting Information Figure S5). Among the selected climate predictors, the mean temperature of warmest quarter was estimated as the most important variable to predict the presence of var. *filipes* (Supporting Information Figures S6 and S7). The ensemble model for var. *filipes* shows high suitable areas from south Texas in the US to the Nuevo Leon and Tamaulipas states in Mexico with decreasing suitability towards the south (Figure 1, Supporting Information Figure S8). Also, distribution models show a clear limit to the west in the foothills of the Sierra Madre Oriental.

The consensus HSM for var. *hallii* includes the predictions of 134 (out of 175) independent models that showed TSS values above 0.75. The mean TSS over the full set of models was 0.78 and the AUC 0.94 (Supporting Information Figure S9). Among the selected climate predictors, temperature seasonality was estimated as the most important variable to predict the presence of *P. hallii*
(Supporting Information Figures S10 and S11). The projected suitable distribution for var. *hallii* shows a bigger continuous area with high suitability values encompassing the region of Texas and the states of Coahuila and Nuevo Leon in Mexico (Figure 1, Supporting Information Figure S12). The projection for var. *hallii* also shows a nearly isolated suitable area in the far southwest between Arizona and New Mexico states in the US.

The projections to past climatic scenarios of the HSMs for both varieties show similar distribution patterns for the mid-Holocene period compared to current climate and high agreement between the results from the three GCMs (Supporting Information Figures S8 and S12). Conversely, the projection to LGM and LIG periods show a consistent southward shift and fragmentation in the distribution of suitable areas. Specifically, the predicted past distribution of var. *filipes* shows a strong reduction to scattered areas in the coast of the east of the Gulf of Mexico (Supporting Information Figure S8).

Interestingly, there was almost no agreement among the projections obtained from the three GCMs for the LGM suggesting that this result should be interpreted with caution. For var. *hallii*, we found a well-supported suitable region that remain constant along all projected climatic scenarios in the northeast of Mexico (Supporting Information Figure S12).

**Divergence between Panicum hallii var. hallii and Panicum hallii var. filipes**

Together, DAPC and phylogenetic analyses revealed substantial divergence between the *P. hallii* varieties as well as population structure within the varieties (Figure 3). Phylogenetic analyses confirmed that each variety of *P. hallii* forms a monophyletic group (Figure 3A). Summary statistics show that var. *filipes* has greater diversity (*H*<sub>e</sub>) than var. *hallii*, despite its narrow natural distribution (Table 1). Additionally, a Mantel test between DAPC genetic differentiation among populations and their corresponding geographic distances was highly significant (*p* value<0.0001) and had a positive Mantel statistic (*r*=0.36) (Table 5, Supporting Information Figure S13A), supporting an isolation by distance pattern for both varieties.
Population structure in *Panicum hallii* var. *hallii*

To examine the population structure of var. *hallii*, we investigated a set of 16,595 SNPs in 396 individuals from 104 localities. Using clustering methods, four groups were revealed by both the maximum likelihood tree and DAPC. The four genetic clusters present a clear geographic pattern. There is a large cluster in the southwestern United States (Arizona, New Mexico, and West Texas), hereafter called the "West" cluster. In addition, there is a cluster including the Mexican samples and some Texas samples collected close to the Mexican border, identified as the "Tex-Mex" cluster. In central Texas, samples collected in the Edwards plateau and the Central Forest Grassland transition ecoregions form a group that we call the "Central Texas" cluster. Finally, we identify a cluster occurring in South Texas where both varieties coexist under sympatric conditions that we name the "sympatric" cluster. One hypothesis is that this population structure is the result of a northward and westward expansion of the species range following the last glacial maximum. To examine this hypothesis in var. *hallii*, we examined the correlation between expected heterozygosity and geographic distance to San Antonio. We expect a decreased genetic diversity as the distance from modern San Antonio (i.e. genetic diversity hotspot) increases. The West cluster shows a significant negative correlation between expected heterozygosity and distance from the hotspot. The other three clusters also show a negative, albeit non-significant, correlation between expected heterozygosity and distance to San Antonio (Table 2).

In addition to the broad geographic structure of the genetic diversity in var. *hallii*, individuals collected in each locality usually group together as a monophyletic group in the phylogenetic tree (Figure 4A).

Population structure in *Panicum hallii* var. *filipes*

Despite the narrow distribution of var. *filipes* in South Texas, this variety shows strong population structure divided into three clusters (Figure 5): one genetic cluster has a coastal distribution, another group has an inland distribution, and a third widespread group with individuals collected in both inland (Pine oak forest ecoregion) and coastal areas (Western Gulf coastal grasslands ecoregion) (Figure 5).
Phenotypic divergence between *Panicum hallii* var. *filipes* and *Panicum hallii* var. *hallii*

Principal component analysis (PCA) using seven phenotypic characters showed a high divergence between var. *filipes* and var. *hallii* (Figure 6). In general, var. *filipes* had larger morphological features (excepting seeds) than var. *hallii* (Table 3). Also, var. *filipes* was more tolerant to cold stress (measured as a percentage of electrolyte leakage). Lastly, the lifespan (measured as senescence score across time) was longer for var. *filipes* than var. *hallii*.

The seven phenotypic characters also illustrate phenotypic divergence between genetically distinct geographic clusters (Figure 6). With PCA, we see clustering differences between geographic regions, especially in var. *filipes*, where the samples collected in the coastal and inland form two different clusters. Interestingly, the var. *hallii* samples belonging to the sympatric group form a morphology-based cluster that is intermediate between the var. *filipes* and the rest of the var. *hallii* samples. Our Mantel test revealed a positive correlation between phenotypic distance of populations and their geographic distance ($r_s = 0.23$, $P < 0.001$; Table 5 and Supporting Information Figure S13B). Thus, confirming our field observations of increased morphological differentiation as a function of geographic distance. Similarly, we also found support for the correlation between the DAPC genetic distances and the PCA phenotypic distances supported by a Mantel test ($r=0.24$, $p$ value=0.0005; Table 5, and Supporting Information Figure S13C). This correlation held even after controlling by geographic distance in a Partial Mantel test ($r=0.17$, $p$ value=0.0094) (Table 5).

**Genotypic and phenotypic analyses in the sympatric area**

In Southwest Texas, where var. *filipes* and var. *hallii* coexist in sympatry, some samples appear intermediate between the two varieties when genotypes and phenotypes are clustered (Figure 6). Though *P. hallii* is a highly self-fertilizing species (Lowry et al., 2012, 2013), the two varieties can be crossed in the greenhouse (Lowry et al., 2015), and outcrossing in greenhouse conditions has been observed (X. Weng, 2018 pers. comm.). To determine if intermediate samples resulted from gene flow between the two varieties, we generated a new matrix of 12,463 SNP from all samples collected in the sympatric region (27 var. *filipes* from 14 localities and 24 var. *hallii* from 14 localities) plus
some var. hallii samples (26 individuals from 12 localities) collected in allopatric regions to serve as non-admixed controls.

After running the program STRUCTURE (Pritchard et al., 2000) to evaluate admixture between varieties, six individuals labeled as var. hallii collected from four localities in the sympatric zone were assigned membership to both var. filipes and var. hallii groups under four different arrangements of var. filipes learning samples (Table 4), suggesting that these samples are admixed and there is at least periodic gene flow between var. hallii and var. filipes in sympatry.

Morphological analysis of the samples of var. hallii collected in the sympatry area showed intermediate phenotypic characteristics between samples of var. filipes and samples of var. hallii growing in allopatry. Phenotypic traits such as secondary branch number, biomass, tiller number, seed mass and senescence presented intermediate values between these two varieties. In addition, the PCA analysis of the phenotypic data showed that the sympatric localities of var. hallii formed a group between the samples of var. filipes and var. hallii in allopatry. This intermediate behavior is especially true for two (BEE.1A and UVL.1A) of the six samples with admixed compositions in the genotypic analysis (Figure S14).

DISCUSSION

Genetic and morphological divergence between Panicum hallii varieties

We used ddRAD to genotype more than 400 P. hallii individuals across the natural range of the species. We detected strong genomic differentiation between the two varieties of P. hallii, as indicated by the phylogenetic tree (Figure 3A) and the discriminant analysis (Figure 3B). More generally we found a strong signal of genetic divergence as a function of geographic distance as shown by a Mantel test (Table 5 and Supporting Information Figure S13A). This was consistent with the genetic architecture of differentiation detected with QTL (Lowry et al., 2015) and morphology (Lowry et al., 2013; Waller, 1976). The two varieties are typically found in contrasting habitats, with var. hallii growing in xeric habitats and var. filipes found in more mesic habitats. This habitat preference was
observed in the field and confirmed with our collections. Indeed, Gould et al., (2018) found that climatic variables related with extreme conditions such as minimum temperature and temperature daily range are key variables correlated with individual genetic distances in *P. hallii* as a whole. The most important climatic predictors estimated for the HSM corroborates that variables representing temperature variation through the year (e.g. temp. annual range for var. *filipes* and temp. seasonality for var. *hallii*), extreme temperature conditions (e.g. mean temp. of warmest quarter for var. *filipes* and mean temp. of wettest quarter for var. *hallii*) are the better predictors of the *P. hallii* varieties distribution. We also corroborate annual precipitation as important predictor for var. *hallii* which almost occupies the geographic range of the entire species, but not for var. *filipes* that is more restricted to coastal and foothill environments and should be less tolerant to low precipitation conditions (Supporting Information Figures S7 and S11).

We also detected morphological differentiation between the varieties, particularly in traits that may confer ecological adaptation. For instance, var. *hallii* has heavier seeds than var. *filipes* (Supporting Information Figure S14), which is a common pattern for plants that are typically exposed to drought conditions after germination in their natural habitat (Baker, 1972). We also detected differences in cold tolerance between the varieties (Supporting Information Figure S14), with the var. *hallii* showing greater cold sensitivity than the var. *filipes*. This was surprising, given that var. *hallii* inhabits colder regions and higher altitudes than var. *filipes*. In addition, although *P. hallii* has been described as a perennial grass, life span evaluations show that var. *filipes* has longer life span than var. *hallii* under controlled common garden conditions (Supporting Information Figure S14, senescence). Short life span in var. *hallii* might be associated with other life history characters important for plant survival in dry environments, such as early flowering time, summer dormancy, and bigger seed mass (Lowry et al., 2012, 2013). In combination, these traits may allow var. *hallii* to rapidly complete its life cycle during the short-wet seasons and avoid the stresses of the dry seasons and winters.

Interestingly, the morphological differentiation pattern was found between and within varieties as indicated by the Partial Mantel Tests (Table 5, Supporting Information Figure S13B-C) which show a significant positive correlation between phenotypic distance and geographic distance as well as...
between phenotypic and genetic distance (which remains highly significant and positive even after controlling by geographic distance in a Partial Mantel Test, Table 5). An isolation by distance pattern can be deduced from these correlations, where we can observe that as the geographic distance increases between populations, so does genetic divergence and the concomitant phenotypic separation.

Across much of the range of *P. hallii*, there are strong geographic and ecological barriers to gene flow which likely promote and/or reinforce differentiation between the varieties. However, in southeast Texas, var. *filipes* and var. *hallii* grow in sympatry and are subject to similar climate conditions. Climate-related selection pressures promoting phenotypic differentiation may be relaxed, because var. *hallii* samples from this sympatric region are less morphologically differentiated from var. *filipes* than they are from var. *filipes* from allopatric regions (Figure S14). In this sympatric area, where geographic and ecological barriers to gene flow are weakened, we detect evidence of admixture between the varieties in at least six samples from four localities. Our results suggest the existence of inter-mating between *P. hallii* varieties despite an estimated divergence of ~1 M years (Lovell et al., 2018). For instance, in the eastern locality close to Gonzalez, Texas, genetic analysis of nine samples showed that individuals from both varieties are present at the same location (one for var. *filipes* and six var. *hallii*). Two samples from that site appear to be admixed, suggesting natural crosses of these two varieties. The Gonzalez locality is particularly notable because the morphological and ecological characteristics are not typical for *P. hallii*. Morphologically, we were not able to definitively differentiate the two varieties in the field at this locality. Ecologically, this is the only locality in the Texas Blackland Prairie ecoregion where we collected both varieties of *P. hallii*, despite extensive collecting effort at other localities in the ecoregion. However, while there is detectable hybridization in the Gonzalez locality, samples of both varieties with no evidence of admixture are also found in this locality, indicating that hybridization is not ubiquitous. Potential reproductive barriers between the two varieties have yet to be quantified, as they have been for other plant species (Lowry et al., 2008; Sobel & Chen, 2014). However, Lowry et al. (2015) did identify a two-locus Dobzhansky-
Muller hybrid incompatibility causing sterility in a cross between var. hallii and var. filipes, which suggests that there is intrinsic postzygotic isolation between the two varieties.

Population structure within varieties

The two varieties of P. hallii are adapted to different environments, but we observe evidence of considerable differentiation within each variety as well. In a broad sense, var. hallii has a geographical break at the genetic structure level in West Texas, coinciding with the Upper Cretaceous sediment to the West and most recently Paleogene, Neogene, and Quaternary deposits to the East around the 100° W meridian. This break isolates the West and TexMex groups from the other genetic clusters. This geological boundary suggests that the type of soil can also explain the genetic pattern found in var. hallii. To the east of this geological boundary is the Central Texas group, which is almost exclusively restricted to the Edwards Plateau and Central forest-grassland transition ecoregions, suggesting that there are either physical or ecological barriers constraining this group to these ecoregions. Within var. filipes, the Coastal and Inland genetic groups are found only in the Western Gulf Coastal Grasslands and Pine Oak Forest ecoregions, respectively, suggesting that their differentiation is related to ecological differences between those regions. Interestingly, the third var. filipes group, which is found in both Western Gulf Coastal Grasslands and Pine Oak Forest ecoregions, has the most divergent morphological traits of any group and has a clear perennial life span, suggesting that the differentiation of this group is related to life history strategy. This relationship between population structure and ecoregions suggests that adaptive differences play a major role in shaping genetic diversity not only between varieties but also within varieties of P. hallii.

New insights of the species historical demography

There is strong population genetic structure within P. hallii, with two divergent intra-specific lineages already classified as varieties, and a total of seven genetic clusters. A diversity hotspot was found in southern Texas, where at least one representative of each genetic cluster occurs within a radius of 200 km from San Antonio, Texas (Figure 3). There are at least three non-exclusive reasons why this region is the center of genetic diversity for P. hallii: diverse ecological conditions, complex demographic processes in response to the Quaternary climatic fluctuations, and potential admixture.
between the varieties. Given that the projections of the HSMs to past climate scenarios do not support San Antonio area as a climatic stable region, we propose a scenario of strong distribution shifts and fragmentation but with mild bottlenecks and that the high diversity in the San Antonio region is a joint product of post-glacial recolonization, high recent ecological diversification and hybridization. Below, we discuss the evidence supporting this plausible scenario.

From the ecological perspective, the ecological heterogeneity of Central Texas can be a key promoter of diversification. This region belongs to the recently proposed North American Coastal Plain (NACP) global biodiversity hotspot (Noss et al., 2015), characterized by its high endemism of vascular plants, especially in its grassland coastal biomes (MacRoberts et al., 2002; Noss, 2014). Moreover, this area contains seven ecoregions, defined as a land area containing a particular assembly of natural communities and species (Olson et al., 2001) (Figure 1). In addition, at the plant diversity level, a mix of three floristic provinces coincide in Central and South Texas, where around 20% of the endemic plants are grasses (Sorrie & Weakley, 2001). Specifically, the area around San Antonio, Texas, contains five grassland community types (Fayette, Upper Coastal, Coastal, Blackland, and San Antonio Prairie) characterized by seven major soil associations (Diamond & Smeins, 1985). Taken together, these ecological factors likely contribute to the high diversity of *P. hallii* by promoting multiple local adaptation processes over short time scales in this narrow area in Central Texas and the Rio Grande Valley.

It is well known that the Quaternary climatic fluctuations in the form of multiple contraction-expansion cycles of species distribution shaped the contemporary genetic composition of the biodiversity (Hewitt, 2000, 2004; Razgour et al., 2013). Strong distribution shifts during the LGM as well as disagreement between the predicted distribution areas resulted from the projections using different global circulation models were already reported in plants from South Texas (Rebernig et al., 2010). During the glacial cycles of the Pleistocene, South Texas was a glacial refugium belt for boreal displaced vegetation from the northern boreal latitudes (Holmgren et al., 2007) and for lowland taxa during the flooding due to sea level fluctuations (Noss et al., 2015). It served as a refugium for a variety of plants and animals promoting species richness and genetic diversity (Bryson et al., 2014;
Elias, 1992; Majure et al., 2012; Seal et al., 2015; Swenson & Howard, 2005). A large-scale aridification of the southwestern parts of North America started during the Holocene at the end of the last glacial maximum (~21,000 ybp), allowing the range expansion of drought-adapted genotypes from the refuge areas to the new arid regions (Rebernig et al., 2010) following the Northwest outward-direction, post-glacial expansion route proposed by Swenson and Howard (2005) for Central Texas refugia. An expansion of the var. hallii during Holocene might explain the lack of diversity in the recently colonized regions (i.e., West and TexMex genetic clusters). In fact, there is a significant negative correlation between diversity (heterozygosity) and distance from southern Texas (San Antonio) for the var. hallii West cluster, consistent with a post-glacial expansion (Table 2). A similar pattern has been found in blackfoot daisy (Melampodium leucanthum, Asteraceae)(Rebernig et al., 2010; Stuessy et al., 2004) and members of the Humifusa clade of Opuntia genus (Majure et al., 2012).

Finally, diversity may be high in Central Texas due to hybridization between the varieties. During the Pleistocene, there were recurrent glacial cycles and the species range likely contracted and expanded multiple times. These successive contraction-expansion events led to the formation of contact zones, hybrid zones, and phylogeographic break areas in North America (Suture zones)(Hewitt, 2001; Remington, 1968), one of them in Central Texas (Swenson & Howard, 2005, 2004), which correspond to the genetic diversity hotspot found for P. hallii in this study. Thus, the observed high diversity may be due to a history of secondary contact at this location between groups that diverged in allopatry during previous glacial cycles. More extensive sampling in the sympatric zone for both varieties and the use of plastid genomic information can be used to address this question.

**Panicum hallii distribution**

The results from our field sampling suggested that the natural distribution of *P. hallii* is narrower than previously considered at the southern and eastern edges of the species distribution. Previous literature and collection records indicate that the natural distribution of *P. hallii* spans from the Southwest United States (Oklahoma, Colorado, Texas, New Mexico, and Arizona) through Mexico to the southern Mexican state of Oaxaca (Estrada & Villareal, 2010; F. W. Gould, 1975; Herrera-Arrieta &
Cortés-Ortiz, 2010; Herrera & Pámanes, 2006; Sánchez-Ken, 2011; Valdés-Reyna et al., 2015; Waller, 1976). However, our collection efforts in the southern portion of this reported range, in the Mexican states of Mexico and Michoacán, failed to find *P. hallii*. The plants we found that most resemble *P. hallii* have morphological characteristics not found in *P. hallii* samples collected in the United States, and molecular markers indicate that these plants correspond to *P. lepidulum* (Figure 2).

We focused our Southern Mexican collection efforts in areas where herbarium samples were previously collected, and the absence of *P. hallii* in these Mexican states suggests that the southern boundary of the distribution of *P. hallii* is currently the Meseta Central Matorral and Chihuahua Desert ecoregions in the Northern Mexican states (Figure 1B).

At the eastern part of the distribution, several collection records indicate that *P. hallii* grows in eastern Texas close to the Louisiana border and even in northwest Louisiana (Reid & Urbatsch, 2012). However, our results showed that these individuals genetically grouped with two different *Panicum* species (*P. diffusum* and *P. capillare*). We also made at least 14 collection trips east of the Edwards Plateau ecoregion in central Texas without finding *P. hallii*, suggesting that the eastern distribution of *P. hallii* is restricted to the Texas Blackland Prairies and East Central Texas forest ecoregions (Figure 1B).

The projections of the HSMs for current conditions also support these proposed eastern and southern distribution limits (Figure 1). Despite the fact that we trained our species distribution models including putative occurrences located beyond these limits (based on secondary reports), the distribution limits suggested by the HSM agrees with the limits of the mentioned ecoregions and exclude doubtful records beyond these limits.

Overall, var. *hallii* is associated primarily with six ecoregions: the Meseta Central Matorral in the South, the Chihuahuan Desert and the Arizona Mountain Forest ecoregion to the West, the Western Short Grasslands and Central Forest Grasslands Transition to the North, and finally, the Central and Southern Mixed Grasslands and Edwards Plateau Savanna to the East (Figure 1B). The distribution of var. *filipes* is restricted to the Western Gulf Coastal Grasslands and Pine Oak Forest ecoregions. The var. *hallii* was also collected in these ecoregions, but it was by far less abundant. According to the
projections of the HSMs, the distribution of var. filipes encompasses a subset of the var. hallii distribution in its eastern range. Moreover, the habitat suitability model supports a conspicuous western limit near to the foothills of the Sierra Madre Oriental for the var. filipes (Figure 1).

Lessons for diversity studies in morphologically and ecologically diverse systems

One challenge we encountered while trying to sample diversity across the range of P. hallii was to correctly distinguish P. hallii from closely related species. P. hallii grows in a variety of habitats, and its morphology in natural and controlled settings can differ substantially. We collected 45 samples from the originally reported range of P. hallii that were members of other species in Panicum section Diffusum. There are many research systems that share this characteristic: ecologically and morphologically diverse taxa that are difficult to distinguish from closely related taxa in natural setting (Rebernig et al., 2010; Tyler et al., 2016). Sampling the breadth of genetic, morphological, and ecological diversity is important, but can lead to the collection of non-target taxa. While including all collected samples in sequencing-based genotyping methods (e.g. ddRAD, GBS) can resolve the relationships among target and non-target taxa, that may not be the most efficient use of sequencing, computing, or analysis resources. For instance, sequencing effort of the target taxa is decreased when non-target taxa are included. In addition, in our experience, methods for identifying SNPs and calling genotypes in non-model species can be sensitive to phylogenetic diversity (Mastretta-Yanes et al., 2015; Shafer et al., 2017), and starting strictly with the target taxa reduces the filtering steps necessary to produce a useful set of SNPs for subsequent analyses.

Therefore, we recommend that, if possible, samples be checked in a controlled environment in order to identify samples that do not match the standard description of the taxon, though we recognize this is not possible for many systems. Then a quick method should be used to verify that suspicious samples are within the intended taxonomic level for the study. For this study, Sanger sequencing of nuclear and chloroplast loci were fast and provided the resolution required to differentiate species. Finally, the next-generation sequencing (NGS) efforts should focus on the verified samples. While the initial steps take time and may delay NGS efforts, they are a valuable investment as they improve the sequencing results and can save substantial time during bioinformatic analysis.
In conclusion, the genetic and morphological evaluation in this study represents the most extensive sampling of the *P. hallii* varieties. This evaluation made it possible to distinguish genetic structure at different biological levels. At the species level, divergence was found between both varieties using molecular and morphological data. Within varieties, it was possible to characterize population structure across the diverse geological and ecological native range of *P. hallii*. Within the sympatric zone, this set of data allowed us to identify putative admixed individuals between var. *filipes* and var. *hallii*. Taken together, these data illustrate the geographical distribution of genetic diversity in *P. hallii*. A practical application of these results could be the creation of a core collection of these samples to streamline screening for particular traits of interest. Future work could include increased sampling efforts for var. *filipes*, especially in the Rio Grande Valley and Mexico to explore the genetic diversity in the Western and South boundary of the distribution. In addition, to confirm admixture between the varieties, the plastome from samples in the sympatric area can be examined to confirm admixture between the varieties. Furthermore, studies of local adaptation could be conducted by crossing members of different populations (Lowry et al., 2015; Milano et al., 2016), or by reciprocal transplants across ecoregions to identify the traits that underlie ecotype formation (Hereford, 2009). Together, our data and analyses provide a foundation for understanding the natural history and evolution of *P. hallii* and highlight some of the important factors leading the observed structure and genetic diversity in the group.
Data Availability

The raw reads were submitted to the National Center for Biotechnology Information (NCBI) Short Read Archive (SRA) under project number SRP110713.

Sources of Funding

Funding support for the research was provided by The National Science Foundation, Plant Genome Research Program Award (IOS-0922457 and IOS-1444533) and US Department of Energy (SC-0008451) to T.E.J. In addition, founds from Linda Escobar Award and Texas EcoLab research grant to J.D.P-M. made field collections possible.

Contributions by the Authors

T.E.J. and J.D.P-M. conceived and designed the study. D.B.L. collected most of the plant material. E.M.O., T.H., P.P.G., D.L.D.M. G.A.S-A and J.D.P-M performed the bioinformatic, genetic data, and niche modeling analysis. J. B. coordinated the field work. J.D.P-M., T.E.J. and P.P.G. wrote the manuscript with contribution from all authors.

Conflict of Interest

None declared.

Acknowledgements

The authors would like to thank J. Heiling, S. Merrell, A. Diaz, O. Vargas, N. LeClear, I Vargas, Q. Hiers, L. Adhikari, L. Mayer, and D. Miller, S. for help in the common garden field and at the glasshouse and S. Razzaque for his help with analyses and field observations. We also thank the following for providing seed and logistic help during the collections: J. L. Reilly from the USDA
Kika de la Garza Plant Materials Center; S. Segura from Universidad Autónoma Chapingo, Morelia, México; M. Socorro González-Elizondo from Instituto Politécnico Nacional, CIIDIR, Durango, México. The Genomics and Bioinformatics Service at Texas A&M University (College Station, TX), specially to R. Metz for its collaboration generating the ddRAD data. The Texas Advanced Computing Center (TACC) provided computational resources. Phylogenetic species assignment method was made possible by collaboration with E. Meyer, T. Quedensley, A. Díaz, O. Thornton, and M. Schill. The flow cytometry data was collected by B. Watson. The students of The University of Texas Freshman Research Initiative for help with phenotyping, especially M. Villalpando, T. Schroyer, E. Alger. Invaluable paleontological and ecological advice was brought by L. Boucher and N. Fowler. This manuscript was improved following comments from A. Diaz, A. MacQueen, and B. Campitelli.
Literature Cited

AL-Tam, F., Adam, H., Anjos, A. D., Lorieux, M., Larmande, P., Ghesquière, A., Jouaunic, S., & Shahbazkia, H. R. (2013). P-TRAP: A Panicle Trait Phenotyping tool. *BMC Plant Biology, 13*(1). https://doi.org/10.1186/1471-2229-13-122

Allen, G. C., Flores-Vergara, M. A., Krasyanski, S., Kumar, S., & Thompson, W. F. (2006). A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. *Nature Protocols, 1*(5), 2320–2325. https://doi.org/10.1038/nprot.2006.384

Andrews, S. (2018). *FastQC: a quality control tool for high throughput sequence data.* https://qubeshub.org/resources/fastqc

Araújo, M. B., & New, M. (2007). Ensemble forecasting of species distributions. *Trends in Ecology & Evolution, 22*(1), 42–47. https://doi.org/10.1016/j.tree.2006.09.010

Baker, H. G. (1972). Seed Weight in Relation to Environmental Conditions in California. *Ecology, 53*(6), 997–1010. https://doi.org/10.2307/1935413

Bryson, R. R. W., Prendini, L., Savary, W. E. W., Pearman, P. B., Hewitt, G. M., Avise, J., Hewitt, G., Webb, T., Bartlein, P., Hewitt, G., Hoffmann, R., Avise, J., Walker, D., Waltari, E., Hijmans, R., Peterson, A., Nyari, A., Perkins, S., Guralnick, R., … Hedin, M. (2014). Caves as microrefugia: Pleistocene phyllogeography of the troglophilic North American scorpion Pseudouroctonus reddelli. *BMC Evolutionary Biology, 14*(1), 9. https://doi.org/10.1186/1471-2148-14-9

Bushnell, B. (2014). *BBMap: a fast, accurate, splice-aware aligner.* Ernest Orlando Lawrence Berkeley National Laboratory, Berkeley, CA (US).

Campitelli, B. E., Gorton, A. J., Ostevik, K. L., & Stinchcombe, J. R. (2013). The effect of leaf shape on the thermoregulation and frost tolerance of an annual vine, Ipomoea hederacea (Convolvulaceae). *American Journal of Botany, 100*(11), 2175–2182. https://doi.org/10.3732/ajb.1300136

Catchen, J., Hohenlohe, P. a, Bassham, S., Amores, A., & Cresko, W. a. (2013). Stacks: an analysis tool set for population genomics. *Molecular Ecology, 22*(11), 3124–3140. https://doi.org/10.1111/mec.12354

Catchen, J. M., Amores, A., Hohenlohe, P., Cresko, W., & Postlethwait, J. H. (2011). Stacks: building and genotyping goci de novo from short-read sequences. *G3: Genes, Genomes, Genetics, 1*(August), 171–182. https://doi.org/10.1534/g3.111.000240

Conner, J. K., & Hartl, D. L. (2004). *A primer of ecological genetics.* Sinauer Associates Incorporated.

da Fonseca, R. R., Albrechtsen, A., Themudo, G. E., Ramos-Madrigal, J., Sibbesen, J. A., Maretty, L., Zepeida-Mendoza, M. L., Campos, P. F., Heller, R., & Pereira, R. J. (2016). Next-generation biology: Sequencing and data analysis approaches for non-model organisms. *Marine Genomics, 30,* 3–13. https://doi.org/10.1016/j.margen.2016.04.012

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., & Durbin, R. (2011). The variant call format and VCFtools. *Bioinformatics, 27*(15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330
Diamond, D. D., & Smeins, F. E. (1985). Composition, classification and species response patterns of remnant tallgrass prairies in Texas. *American Midland Naturalist*, 294–308.

Elias, S. A. (1992). Late Quaternary Zoogeography of the Chihuahuan Desert Insect Fauna, Based on Fossil Records from Packrat Middens. *Journal of Biogeography*, 19(3), 285. https://doi.org/10.2307/2845452

Ellegren, H., & Galtier, N. (2016). Determinants of genetic diversity. *Nature Reviews Genetics*, 17, 422. http://dx.doi.org/10.1038/nrg.2016.58

Estrada, E., & Villareal, J. Á. (2010). Flora del centro del estado de Chihuahua, México. *Acta Botanica Mexicana*, 92, 51–118.

Gould, B. A., Palacio-Mejia, J. D., Jenkins, J., Mamidi, S., Barry, K., Schmutz, J., Juenger, T. E., & Lowry, D. B. (2018). Population genomics and climate adaptation of a C4 perennial grass, Panicum hallii (Poaceae). *BMC Genomics*, 19(1), 792. https://doi.org/10.1186/s12864-018-5179-7

Gould, F. W. (1975). *The grasses of Texas*. Texas A&M University, Texas Agricultural Experiment Station.

Hamrick, J. L., & Godt, M. J. W. (1996). Effects of life history traits on genetic diversity in plant species. *Phil. Trans. R. Soc. Lond. B*, 351(1345), 1291–1298.

Hereford, J. (2009). A Quantitative Survey of Local Adaptation and Fitness Trade-Offs. *The American Naturalist*, 173(5), 579–588. https://doi.org/10.1086/597611

Herrera-Arrieta, Y., & Cortés-Ortiz, A. (2010). Listado florístico y aspectos ecológicos de la familia Poaceae para Chihuahua, Durango y Zacatecas, México. *Journal of the Botanical Research Institute of Texas*, 4(2), 711–738.

Herrera, A. Y., & Pámanes, D. S. (2006). Guía de pastos para el ganadero del Estado de Durango. *IPN-CONACYT-Fundación PRODUCE-Filo de Agua, Durango, México.*

Hewitt, G. M. (2000). The genetic legacy of the Quaternary ice ages. *Nature*, 405(6789), 907–913. https://doi.org/10.1038/35016000

Hewitt, G. M. (2001). Speciation, hybrid zones and phylogeography—or seeing genes in space and time. *Molecular Ecology*, 10, 537–549. https://doi.org/10.1046/j.1365-294x.2001.01202.x

Hewitt, G. M. (2004). Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 359(1442), 183–195. https://doi.org/10.1098/rstb.2003.1388

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, 25(15), 1965–1978. https://doi.org/10.1002/joc.1276

Hijmans, R. J., Williams, E., & Vennes, C. (2019). Package 'geosphere' (1.5-10; p. 45). CRAN.

Holmgren, C. A., Norris, J., & Betancourt, J. L. (2007). Inferences about winter temperatures and summer rains from the late Quaternary record of C4 perennial grasses and C3 desert shrubs in the northern Chihuahuan Desert. *Journal of Quaternary Science*, 22(2), 141–161. https://doi.org/10.1002/jqs.1023

Jombart, T. (2008). Adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24(11), 1403–1405. https://doi.org/10.1093/bioinformatics/btn129
Jump, A. S., Marchant, R., & Peñuelas, J. (2009). Environmental change and the option value of genetic diversity. *Trends in Plant Science*, 14(1), 51–58. https://doi.org/10.1016/j.tplants.2008.10.002

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thirier, T., Ashton, B., Meintjes, P., Drummond, A. A., & Heled, J. (2012). Geneious. In *Bioinformatics* (Vol. 28, Issue 12, pp. 1647–1649). https://doi.org/10.1093/bioinformatics/bts199

Knaus, B. J., & Grünwald, N. J. (2017). vcfr: A package to manipulate and visualize variant call format data in R. *Molecular Ecology Resources*, 17, 44–53. https://doi.org/10.1111/1755-0998.12549

Lepais, O., & Weir, J. T. (2014). SimRAD: a R package for simulation-based prediction of the number of loci expected in RADseq and similar genotyping by sequencing approaches. *Molecular Ecology Resources*, 13(14), 1314–1321. https://doi.org/10.1111/1755-0998.12273

Lovell, J. T., Jenkins, J., Lowry, D. B., Mamidi, S., Sreedasyam, A., Weng, X., Barry, K., Bonette, J., Campitelli, B., Daum, C., Gordon, S., Gould, B. A., Lipzen, A., MacQueen, A., Palacio-Mejia, J. D., Plott, C., Shakirov, E. V., Shu, S., Yoshinaga, Y., … Juenger, T. E. (2018). *The Panicum hallii genome reveals molecular adaptation to drought*.

Lowry, D. B., Hernandez, K., Taylor, S. H., Meyer, E., Logan, T. L., Barry, K. W., Chapman, J. A., Rokhsar, D. S., Schmutz, J., & Juenger, T. E. (2015). The genetics of divergence and reproductive isolation between ecotypes of Panicum hallii. *New Phytologist*, 205(1), 402–414. https://doi.org/10.1111/nph.13027

Lowry, D. B., Modliszewski, J. L., Wright, K. M., Wu, C. A., & Willis, J. H. (2008). The strength and genetic basis of reproductive isolating barriers in flowering plants. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1506), 3009 LP – 3021. http://rstb.royalsocietypublishing.org/content/363/1506/3009.abstract

Lowry, D. B., Purmal, C. T., & Juenger, T. E. (2013). A population genetic transect of Panicum hallii (Poaceae). *American Journal of Botany*, 100(3), 592–601. https://doi.org/10.3732/ajb.1200379

Lowry, D. B., Purmal, C. T., Meyer, E., & Juenger, T. E. (2012). Microsatellite markers for the native Texas perennial grass, Panicum hallii (Poaceae). *American Journal of Botany*, 99(3), 114–116. https://doi.org/10.3732/ajb.1100430

MacRoberts, M. H., Sorrie, B. A., MacRoberts, B. R., & Evans, R. E. (2002). Endemism in the West Gulf Coastal Plain: importance of xeric habitats. *SIDA, Contributions to Botany*, 20(2), 767–780.

Majure, L. C., Judd, W. S., Soltis, P. S., & Soltis, D. E. (2012). Cytogeography of the Humifusa clade of Opuntia s.s. Mill. 1754 (Cactaceae, Opuntioideae, Opuntieae): Correlations with pleistocene refugia and morphological traits in a polyploid complex. *Comparative Cytogenetics*, 6(1), 53–77. https://doi.org/10.3897/CompCytogen.v6i1.2523

Mastretta-Yanes, A., Arrigo, N., Alvarez, N., Jorgensen, T. H., Piñero, D., & Emerson, B. C. (2015). Restriction site-associated DNA sequencing, genotyping error estimation and de novo assembly optimization for population genetic inference. *Molecular Ecology Resources*, 15(1), 28–41. https://doi.org/10.1111/1755-0998.12291

McKinney, G. J., Waples, R. K., Seeb, L. W., & Seeb, J. E. (2017). Paralogs are revealed by proportion of heterozygotes and deviations in read ratios in genotyping-by-sequencing data from...
Milano, E. R., Lowry, D. B., & Juenger, T. E. (2016). The Genetic Basis of Upland/Lowland Ecotype Divergence in Switchgrass (Panicum virgatum). *Molecular Ecology Resources, 17*(4), 656–669. https://doi.org/10.1111/1755-0998.12613

Minh, B. Q., Nguyen, M. A. T., & von Haeseler, A. (2013). Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution, 30*(5), 1188–1195.

Mora-Márquez, F., García-Olivares, V., Emerson, B. C., & López de Heredia, U. (2017). ddradseqtools: a software package for in silico simulation and testing of double-digest RADseq experiments. *Molecular Ecology Resources, 17*(2), 230–246. https://doi.org/10.1111/1755-0998.12550

Nguyen, L. T., Schmidt, H. A., Von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution, 32*(1), 268–274. https://doi.org/10.1093/molbev/msu300

Noss, R. F. (2014). Forgotten grasslands of the south: Natural history and conservation. In Forgotten Grasslands of the South: Natural History and Conservation. Island Press. https://doi.org/10.5822/978-1-61091-225-9

Noss, R. F., Platt, W. J., Sorrie, B. A., Weakley, A. S., Means, D. B., Costanza, J., & Peet, R. K. (2015). How global biodiversity hotspots may go unrecognized: Lessons from the North American Coastal Plain. *Diversity and Distributions, 21*(2), 236–244. https://doi.org/10.1111/ddi.12278

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P., O’Hara, R. B., Simpson, G., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2019). Package ‘vegan’ (p. 296). CRAN.

Olson, D. M., Dinerstein, E., Wikramanayake, E. D., Burgess, N. D., Powell, G. V. N., Underwood, E. C., D’amico, J. A., Itoua, I., Strand, H. E., Morrison, J. C., Loucks, C. J., Allnutt, T. F., Ricketts, T. H., Kura, Y., Lamoreux, J. F., Wettengel, W. W., Hedao, P., & Kassem, K. R. (2001). Terrestrial Ecoregions of the World: A New Map of Life on Earth. *BioScience, 51*(11), 933. https://doi.org/10.1641/0006-3568(2001)051[0933:TEOTWA]2.0.CO;2

Ortiz, E. M. (2018). paralog-finder: a script to detect and blacklist paralog RAD loci (1.0). https://doi.org/10.5281/zenodo.1209328

Pérez-Harguindeguy, N., Díaz, S., Garnier, É., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M. S., Cornwell, W. K., Craine, J. M., Gurvich, D. E., Urcelay, C., Veneklaas, E. J., Reich, P. B., Poorter, L., Wright, I. J., Ray, P., Enrico, L., Pausas, J. G., de Vos, A. C., … Cornelissen, J. H. C. (2013). New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany, 61*, 167–234. https://doi.org/http://dx.doi.org/10.1071/BT12225

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species. *PLoS ONE, 7*(5), e37135. https://doi.org/10.1371/journal.pone.0037135

Porras-Hurtado, L., Ruiz, Y., Santos, C., Phillips, C., Carracedo, Á., & Lareu, M. V. (2013). An overview of STRUCTURE: Applications, parameter settings, and supporting software. *Frontiers in Genetics, 4*(MAY), 98. https://doi.org/10.3389/fgene.2013.00098
Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics, 155*(2), 945–959.

R Core Team. (2018). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Randall, H. A., D., I. B., J., J. M. T., Nora, U., & Mark, V. (2008). Ecological consequences of genetic diversity. *Ecology Letters, 11*(6), 609–623. https://doi.org/10.1111/j.1461-0248.2008.01179.x

Razgour, O., Juste, J., Ibáñez, C., Kiefer, A., Rebelo, H., Puechmaillé, S. J., Arlette, R., Burke, T., Dawson, D. A., Beaumont, M., & Jones, G. (2013). The shaping of genetic variation in edge-of-range populations under past and future climate change. *Ecology Letters, 16*(10), 1258–1266. https://doi.org/10.1111/ele.12158

Rebernig, C. A., Schneeweiss, G. M., Bardy, K. E., Schönswetter, P., Villaseñor, J. L., Obermayer, R., Stuessy, T. F., & Weiss-Schneeweiss, H. (2010). Multiple pleistocene refugia and holocene range expansion of an abundant southwestern American desert plant species (Melampodium leucanthum, Asteraceae). *Molecular Ecology, 19*(16), 3421–3443. https://doi.org/10.1111/j.1365-294X.2010.04754.x

Remington, C. L. (1968). Suture-zones of hybrid interaction between recently joined biotas. In *Evolutionary biology* (pp. 321–428). Springer.

Rochette, N. C., & Catchen, J. M. (2017). Deriving genotypes from RAD-seq short-read data using Stacks. *Nature Protocols, 12*(12), 2640–2659. https://doi.org/10.1038/nprot.2017.123

Sánchez-Ken, J. G. (2011). *Flora del valle de Tehuacán-Cuicatlán223* (R. Medina Lermos (ed.); Fascículo). UNAM.

Seal, J. N., Brown, L., Ontiveros, C., Thiebaud, J., & Mueller, U. G. (2015). Gone to Texas: comparative phylogeography of two Trachymyrmex species along the southeastern coastal plain of North America. *Biological Journal of the Linnean Society, 114*(May), 689–698.

Shafer, A. B. A., Peart, C. R., Tusso, S., Maayan, I., Brelsford, A., Wheat, C. W., & Wolf, J. B. W. (2017). Bioinformatic processing of RAD-seq data dramatically impacts downstream population genetic inference. *Methods in Ecology and Evolution, 8*(8), 907–917. https://doi.org/10.1111/2041-210X.12700

Shorthouse, D. (2018). *SimpleMappr, an online tool to produce publication-quality point maps*.

Sobel, J. M., & Chen, G. F. (2014). Unification of methods for estimating the strength of reproductive isolation. *Evolution, 68*(5), 1511–1522.

Sorrie, B. A., & Weakley, A. S. (2001). Coastal plain vascular plant endemics: phytogeographic patterns. *Castanea, 50*–82.

Stuessy, T. F., Weiss-Schneeweiss, H., & Keil, D. J. (2004). Diploid and polyploid cytotype distribution in Melampodium cinereum and M. leucanthum (Asteraceae, Heliantheae). *American Journal of Botany, 91*(6), 889–898. https://doi.org/10.3732/ajb.91.6.889

Swenson, & Howard. (2005). Clustering of Contact Zones, Hybrid Zones, and Phylogeographic Breaks in North America. *The American Naturalist, 166*(5), 581.
Swenson, N. G., & Howard, D. J. (2004). Do suture zone exist? Evolution, 58(11), 2391–2397.

Thuiller, W., Lafourcade, B., Engler, R., & Araújo, M. B. (2009). BIOMOD - A platform for ensemble forecasting of species distributions. Ecography, 32(3), 369–373. https://doi.org/10.1111/j.1600-0587.2008.05742.x

Tyler, L., Lee, S. J., Young, N. D., DeIulio, G. A., Benavente, E., Reagan, M., Sysopha, J., Baldini, R. M., Troia, A., Hazen, S. P., & Caicedo, A. L. (2016). Population Structure in the Model Grass Is Highly Correlated with Flowering Differences across Broad Geographic Areas. The Plant Genome, 9(2), 1–20. https://doi.org/10.3835/plantgenome2015.08.0074

Valdés-Reyna, J., Villaseñor, J. L., Encina-Domínguez, J. A., & Ortiz, E. (2015). The grass family (poaceae) in Coahuila, Mexico: Diversity and distribution. Botanical Sciences, 93(1), 119–129. https://doi.org/10.17129/botsci.79

Vellend, M., & Geber, M. A. (2005). Connections between species diversity and genetic diversity. Ecology Letters, 8(7), 767–781. https://doi.org/10.1111/j.1461-0248.2005.00775.x

Waller, F. R. (1974). A New Combination in Panicum (Gramineae). The Southwestern Naturalist, 19(1), 105. http://www.jstor.org/stable/3669794

Waller, F. R. (1976). A biosystematic study of Panicum section Diffusa (Poaceae) in North America (Vol. 37, Issue 3). Texas A&M University.
Figures

**Figure 1.** Natural distribution of *Panicum hallii*. A. Map of *Panicum hallii* distribution inferred from ensemble Habitat Suitability Modeling and occurrence points from personal field collections and observations and secondary records from the Global Biodiversity Information Facility (GBIF (05/12/2014) and herbarium visits. The map also shows confirmed absence locations of *P. hallii*. Detailed maps of the projections of the ensemble Habitat Suitability Models can be found in the Supporting Information Figures S8 and S12. B. Map of collections of *Panicum hallii* used for genetic and morphological analyses. The map includes the ecoregions showing the ecological preferences for *Panicum hallii* and the shaded area of the habitat suitability models. The East boundary of the distribution of *Panicum hallii* matches with the East Central Texas forest, where several failed collection trips were made. The South distribution was limited to the Meseta Central Matorral in Mexico (Olson et al., 2001). Red dots represent var. *hallii*, blues dots var. *filipes*, green dots localities were both varieties were collected, and black dots correspond to true absences. C. *Panicum hallii* varieties grew under greenhouse conditions, to the left variety *filipes* and to the right, variety *hallii*.

**Figure 2.** Species validation using nuclear and chloroplasts markers. A. Bayesian phylogenetic analyses of the ITS nuclear marker and two chloroplast regions distinguish the different species from within the *Panicum* section *diffusa* (Branch support values are a consensus from 1,000 bootstrap replicates). Green lines indicate *P. diffusum* samples. Red lines indicate *P. lepidulum* samples. Blue lines indicate *P. hallii* collections. Orange clade indicates *P. capillare* collections. B. Geographical distribution of the species collected.

**Figure 3.** Geographic and genetic structure of 423 samples of *Panicum hallii*. A. Maximum likelihood phylogenetic tree, with the blue cluster representing *Panicum hallii* var. *filipes* and the red, magenta, orange and yellow clusters representing *Panicum hallii* var. *hallii*. *Panicum virgatum* was used as an outgroup (Size of black dots on the nodes is proportional to the node support derived from 10,000 ultrafast bootstrap). B. Discriminant analysis of principal components (DAPC). C. Geographical representation of the phylogenetic tree and DAPC analysis using an ecoregion map. The green circle represents the *P. hallii* diversity hotspot 200 km around San Antonio, Texas. Maps produced using SimpleMappr (Shorthouse, 2018).

**Figure 4.** Geographic and genetic structure of 396 samples of *Panicum hallii* var. *hallii* from 104 localities. A. Maximum likelihood phylogenetic tree rooted according to Figure 3, including inset clades depicting the close relationship between individuals from single collection locations (Size of black dots on the nodes is proportional to the node support derived from 10,000 ultrafast bootstrap). B. Geographical and ecological representation of the clustering analysis. C. Discriminant analysis of principal components (DAPC).

**Figure 5.** Geographic and genetic structure of 27 samples of *Panicum hallii* var. *filipes* from 14 localities. A. Maximum likelihood phylogenetic tree rooted according to Figure 3 (Size of black dots on the nodes is proportional to the node support derived from 10,000 ultrafast bootstrap). B. Geographical and ecological representation of the clustering analysis. C. Discriminant analysis of principal components.

**Figure 6.** Principal component analysis of seven phenotypic characters in *Panicum hallii* var. *filipes* (blue) and var. *hallii* (Red, labels represent the genetic cluster).
Table 1. List of localities. Number of samples (N), expected heterozygosity (He)

| Loc ID | Species                       | Region                  | Country | State | County   | Altitude m.a.s.l. | Latitude    | Longitude    | N  | He  |
|--------|-------------------------------|-------------------------|---------|-------|----------|-------------------|-------------|--------------|----|-----|
| HAR    | Panicum capillare             | USA                     | Texas   | Harris|          | 6                  | 29.7197     | -94.9502    | 5  |     |
| DSO    | Panicum diffusum             | USA                     | Louisiana| De Soto|          | 51                 | 32.3062     | -93.8076    | 1  |     |
| ENR    | Panicum diffusum             | USA                     | Texas   | Llano |          | 461.3             | 30.5085     | -98.8246    | 4  |     |
| ARA    | Panicum hallii var. filipes  | USA                     | Texas   | Aransas|          | 2                  | 28.2237     | -96.987     | 1  | 0.044|
| AWR    | Panicum hallii var. filipes  | USA                     | Texas   | Aransas|          | 2.7                | 28.2851     | -96.9457    | 2  | 0.076|
| CAM    | Panicum hallii var. filipes  | USA                     | Texas   | Cameron|          | 5                  | 26.0007     | -97.2691    | 3  | 0.039|
| DIM    | Panicum hallii var. filipes  | USA                     | Texas   | Dimmit |          | 162                | 28.5701     | -99.5245    | 1  | 0.025|
| EPS    | Panicum hallii var. filipes  | USA                     | Texas   | Maverick|          | 248                | 28.4826     | -100.21     | 1  | 0.041|
| FIL    | Panicum hallii var. filipes  | USA                     | Texas   | Nueces |          | 3                  | 27.6496     | -97.4039    | 7  | 0.059|
| LAR    | Panicum hallii var. filipes  | USA                     | Texas   | Webb  |          | 160                | 27.4773     | -99.2092    | 3  | 0.063|
| NDP    | Panicum hallii var. filipes  | USA                     | Texas   | San Patricio|          | 1                  | 27.9117     | -97.6076    | 3  | 0.052|
| PAB    | Panicum hallii var. filipes  | USA                     | Texas   | Cameron|          | 9                  | 26.0199     | -97.4745    | 1  | 0.054|
| ZAF    | Panicum hallii var. filipes  | USA                     | Texas   | Zapata|          | 97                 | 26.5759     | -99.1091    | 1  | 0.04  |
| ZAP    | Panicum hallii var. filipes  | USA                     | Texas   | Zapata|          | 116                | 27.021      | -99.1251    | 3  | 0.043|
| GNF    | Panicum hallii var. filipes  | USA                     | Texas   | Dewitt|          | 76.5               | 29.3119     | -97.3001    | 2  | 0.038|
| MCM    | Panicum hallii var. filipes  | USA                     | Texas   | McMullen|          | 100                | 28.1519     | -98.6385    | 1  |     |
| NUE    | Panicum hallii var. filipes  | USA                     | Texas   | Nueces|          | 15                 | 27.5684     | -97.7956    | 1  |     |
| ATC    | Panicum hallii var. filipes  | USA                     | Texas   | Atascosa|         | 786                | 28.824      | -98.6932    | 5  | 0.02 |
| ATC    | Panicum hallii var. hallii   | USA                     | Texas   | Atascosa|        | 786                | 28.824      | -98.6932    | 5  | 0.016|
| Loc ID | Species            | Region         | Country | State | County         | Altitude m.a.s.l | Latitude  | Longitude | N  | He  |
|--------|--------------------|----------------|---------|-------|----------------|------------------|-----------|-----------|-----|-----|
| RAR    | Panicum hallii var. hallii | Sympatric     | USA     | Texas | Live Oak       | 83               | 28.607    | -98.222   | 5   | 0.034 |
| RAR    | Panicum hallii var. filipes | Inland        | USA     | Texas | Live Oak       | 83               | 28.607    | -98.222   | 5   | 0.024 |
| ABI    | Panicum hallii var. hallii | Central Texas | USA     | Texas | Coleman        | 540              | 31.8597   | -99.4422  | 3   | 0.006 |
| ALI    | Panicum hallii var. hallii | West          | USA     | Texas | Potter         | 961              | 35.5739   | -101.7011 | 7   | 0.004 |
| ARB    | Panicum hallii var. hallii | Central Texas | USA     | Oklahoma | Murray | 305              | 34.4298   | -97.1467  | 4   | 0.007 |
| ART    | Panicum hallii var. hallii | West          | USA     | New Mexico | Chaves | 1441             | 32.8567   | -104.9316 | 10  | 0.004 |
| ATA    | Panicum hallii var. hallii | Tex-Mex       | USA     | Texas | Atascosa       | 113              | 28.7897   | -98.5413  | 1   | 0.015 |
| ATO    | Panicum hallii var. hallii | Tex-Mex       | Mexico  | Durango | Mezquital      | 1869             | 23.693    | -104.3961 | 5   | 0.027 |
| BAN    | Panicum hallii var. hallii | Central Texas | USA     | Texas | Bandera        | 679              | 29.8473   | -99.5734  | 5   | 0.008 |
| BBD    | Panicum hallii var. hallii | Tex-Mex       | USA     | Texas | Brewster       | 780              | 29.6235   | -103.1162 | 4   | 0.014 |
| BBF    | Panicum hallii var. hallii | Tex-Mex       | USA     | Texas | Brewster       | 1244.4           | 29.2736   | -103.3375 | 3   | 0.011 |
| BEE    | Panicum hallii var. hallii | Sympatric     | USA     | Texas | Bee            | 83               | 28.3828   | -97.7792  | 4   | 0.034 |
| BEL    | Panicum hallii var. hallii | Central Texas | USA     | Texas | Bell           | 240              | 30.9183   | -97.6581  | 5   | 0.006 |
| BEX    | Panicum hallii var. hallii | Central Texas | USA     | Texas | Bexar          | 341              | 29.7214   | -98.3542  | 5   | 0.005 |
| BME    | Panicum hallii var. hallii | West          | USA     | Oklahoma | Cimarron | 1319             | 36.8753   | -102.8874 | 6   | 0.005 |
| BOS    | Panicum hallii var. hallii | West          | USA     | Texas | Bosque         | 204              | 32.128    | -97.5596  | 5   | 0.006 |
| BSP    | Panicum hallii var. hallii | Sympatric     | USA     | Texas | Brown          | 440              | 31.8616   | -99.0258  | 1   | 0.015 |
| BUR    | Panicum hallii var. hallii | Sympatric     | USA     | Texas | Burnet         | 248              | 30.5468   | -98.1562  | 5   | 0.013 |
| BUS    | Panicum hallii var. hallii | West          | USA     | Texas | Potter         | 1119             | 35.2649   | -102.0605 | 8   | 0.004 |
| CBS    | Panicum hallii var. hallii | Central Texas | USA     | Texas | San Saba       | 346.4            | 31.0574   | -98.4839  | 5   | 0.008 |
| CCG    | Panicum hallii var. hallii | West          | USA     | Texas | Briscoe        | 754              | 34.4468   | -101.0823 | 6   | 0.006 |
| Loc ID | Species | Region         | Country | State  | County | Altitude m.a.s.l | Latitude  | Longitude   | N  | He  |
|--------|---------|----------------|---------|--------|--------|-----------------|-----------|-------------|----|-----|
| CCL    | Panicum hallii var. hallii | West | USA    | Texas  | Briscoe | 742            | 34.4441   | -101.0751  | 5  | 0.006 |
| CCO    | Panicum hallii var. hallii | West | USA    | Texas  | Briscoe | 749            | 34.4379   | -101.0561  | 2  | 0.005 |
| CDM    | Panicum hallii var. hallii | Tex-Mex | Mexico | Coahuila | Parras | 1262            | 25.5083   | -102.5699  | 5  | 0.016 |
| CHA    | Panicum hallii var. hallii | West | USA    | Texas  | Oldham  | 1069           | 35.6052   | -102.2966  | 8  | 0.004 |
| CKR    | Panicum hallii var. hallii | West | USA    | Arizona | Maricopa | 1817          | 34.2342   | -112.3154  | 10 | 0.004 |
| CLA    | Panicum hallii var. hallii | West | USA    | New Mexico | Union | 1463          | 36.6364   | -103.0375  | 1  | 0.004 |
| CLD    | Panicum hallii var. hallii | West | USA    | Texas  | Armstrong | 956          | 34.7984   | -101.4363  | 7  | 0.004 |
| CNF    | Panicum hallii var. hallii | West | USA    | Arizona | Santa Cruz | 1550        | 31.8021   | -110.9152  | 4  | 0.004 |
| COL    | Panicum hallii var. hallii | Sympatric | USA    | Texas  | Coleman  | 474            | 31.8063   | -99.2348   | 5  | 0.013 |
| COM    | Panicum hallii var. hallii | Tex-Mex | USA    | Texas  | Val Verde | 485          | 29.7018   | -101.2077  | 2  | 0.013 |
| COR    | Panicum hallii var. hallii | Tex-Mex | USA    | Arizona | Maricopa  | 1110          | 34.2862   | -112.179   | 17 | 0.01  |
| CRC    | Panicum hallii var. hallii | West | USA    | Texas  | Oldham  | 985            | 35.5298   | -102.2651  | 8  | 0.005 |
| CRS    | Panicum hallii var. hallii | West | USA    | Texas  | Briscoe | 740.6          | 34.4374   | -101.0568  | 4  | 0.005 |
| DOF    | Panicum hallii var. hallii | Tex-Mex | USA    | Texas  | Val Verde | 417          | 29.8946   | -100.9946  | 7  | 0.017 |
| DRB    | Panicum hallii var. hallii | Tex-Mex | USA    | Texas  | Val Verde | 477          | 29.9412   | -100.9706  | 5  | 0.015 |
| DRS    | Panicum hallii var. hallii | Tex-Mex | USA    | Texas  | Val Verde | 457.4        | 29.9051   | -101.0032  | 5  | 0.023 |
| DUV    | Panicum hallii var. hallii | Sympatric | USA    | Texas  | Duval    | 139          | 27.9141   | -98.7002   | 3  | 0.012 |
| EDW    | Panicum hallii var. hallii | Central Texas | USA    | Texas  | Edwards  | 628          | 29.8263   | -100.4664  | 5  | 0.006 |
| ELG    | Panicum hallii var. hallii | West | USA    | Arizona | Santa Cruz | 1531        | 31.6009   | -110.5585  | 10 | 0.005 |
| ESE    | Panicum hallii var. hallii | Central Texas | USA    | Texas  | Kimble   | 642          | 30.3482   | -99.5894   | 3  | 0.01  |
| GEO    | Panicum hallii var. hallii | West | USA    | New Mexico | Harding | 1780        | 36.0429   | -104.3092  | 1  | 0.006 |
| Loc ID | Species              | Region          | Country | State     | Altitude m.a.s.l. | Latitude  | Longitude  | N  | He  |
|--------|----------------------|-----------------|---------|-----------|-------------------|------------|------------|----|-----|
| GIL    | Panicum hallii var. hallii | West            | USA     | New Mexico | Grant             | 2101       | -108.057   | 25 | 0.003 |
| GMO    | Panicum hallii var. hallii | West            | USA     | Texas     | Culberson         | 2257.5     | -104.8391  | 6  | 0.006 |
| GRA    | Panicum hallii var. hallii | West            | USA     | Texas     | Kerr              | 642        | -99.2431   | 1  | 0.016 |
| HAL    | Panicum hallii var. hallii | Central Texas   | USA     | Texas     | Travis            | 244        | -97.874    | 2  | 0.007 |
| HAY    | Panicum hallii var. hallii | Central Texas   | USA     | Texas     | Hays              | 387        | -98.1804   | 6  | 0.004 |
| HWY    | Panicum hallii var. hallii | West            | USA     | Texas     | Briscoe           | 931        | -101.1103  | 5  | 0.004 |
| JHC    | Panicum hallii var. hallii | Tex-Mex         | USA     | Texas     | Blanco            | 361        | -98.307    | 4  | 0.02  |
| JWE    | Panicum hallii var. hallii | Sympatric       | USA     | Texas     | Jim Wells         | 169        | -98.0055   | 1  | 0.014 |
| KEN    | Panicum hallii var. hallii | Tex-Mex         | USA     | Texas     | Kendall           | 478        | -98.694    | 5  | 0.009 |
| KER    | Panicum hallii var. hallii | Central Texas   | USA     | Texas     | Kerr              | 806        | -99.3973   | 5  | 0.009 |
| KNT    | Panicum hallii var. hallii | West            | USA     | Texas     | Culberson         | 1326       | -104.212   | 3  | 0.006 |
| LBY    | Panicum hallii var. hallii | West            | USA     | Texas     | Hutchinson        | 918        | -101.5465  | 7  | 0.006 |
| LMA    | Panicum hallii var. hallii | West            | USA     | New Mexico | Guadalupe         | 1450       | -104.6406  | 3  | 0.005 |
| LME    | Panicum hallii var. hallii | West            | USA     | Texas     | Real              | 678        | -99.7314   | 4  | 0.007 |
| LOS    | Panicum hallii var. hallii | West            | Mexico  | Coahuila  |                  | 1550       | -101.0239  | 3  | 0.01  |
| MAP    | Panicum hallii var. hallii | Tex-Mex         | Mexico  | Durango   | Mapimi            | 1563       | -104.1051  | 5  | 0.014 |
| MCR    | Panicum hallii var. hallii | West            | USA     | Texas     | Jeff Davis        | 1865       | -104.1348  | 8  | 0.007 |
| MEZ    | Panicum hallii var. hallii | Tex-Mex         | Mexico  | Durango   | Mezquital         | 1600       | -104.4324  | 5  | 0.01  |
| MKF    | Panicum hallii var. hallii | Central Texas   | USA     | Texas     | Travis            | 174        | -97.7251   | 2  | 0.044 |
| NDD    | Panicum hallii var. hallii | Tex-Mex         | Mexico  | Durango   | Nombre de Dios    | 1866       | -104.335   | 5  | 0.013 |
| NMX    | Panicum hallii var. hallii | West            | USA     | New Mexico | Dona Ana          | 1656       | -106.5815  | 2  | 0.005 |
| Loc ID | Species | Region   | Country | State | County | Altitude m.a.s.l. | Latitude  | Longitude  | N  | He   |
|--------|---------|----------|---------|-------|--------|------------------|-----------|------------|----|------|
| NOH    | Panicum hallii var. hallii | USA     | Texas   | Willacy | 4.57   | 26.4949         | -97.5453  | 1          | 0.021 |      |
| PAJ    | Panicum hallii var. hallii | USA     | Texas   | Kimble | 532    | 30.4854         | -99.7376  | 1          | 0.019 |      |
| PCR    | Panicum hallii var. hallii | West    | USA     | Arizona | 1652   | 31.9802         | -109.3634 | 1          | 0.004 |      |
| PDB    | Panicum hallii var. hallii | West    | USA     | Texas   | 870    | 34.9287         | -101.6366 | 9          | 0.004 |      |
| PFD    | Panicum hallii var. hallii | West    | USA     | Texas   | 864    | 34.9437         | -101.6613 | 8          | 0.003 |      |
| PDK    | Panicum hallii var. hallii | West    | USA     | Texas   | 905    | 34.9476         | -101.689  | 1          | 0.004 |      |
| PDL    | Panicum hallii var. hallii | West    | USA     | Texas   | 892    | 34.9476         | -101.689  | 2          | 0.003 |      |
| PDS    | Panicum hallii var. hallii | West    | USA     | Texas   | 860    | 34.9395         | -101.6528 | 2          | 0.004 |      |
| PDT    | Panicum hallii var. hallii | West    | USA     | Texas   | 1040   | 34.9391         | -101.6305 | 8          | 0.004 |      |
| PFL    | Panicum hallii var. hallii | Central Texas | USA | Texas | Travis | 200 | 30.4506 | -97.6386 | 2 | 0.006 |
| PIN    | Panicum hallii var. hallii | West    | USA     | Arizona | 1642   | 34.3161         | -111.4006 | 3          | 0.003 |      |
| PIS    | Panicum hallii var. hallii | Sympatric | USA | Texas | Cameron | 5 | 26.0173 | -97.2735 | 1 | 0.009 |
| POI    | Panicum hallii var. hallii | Sympatric | USA | Texas | Cameron | 5 | 26.0643 | -97.2375 | 2 | 0.012 |
| PRT    | Panicum hallii var. hallii | West    | USA     | Arizona | 1478   | 31.9159         | -109.1547 | 8          | 0.003 |      |
| REA    | Panicum hallii var. hallii | Central Texas | USA | Texas | Real | 599 | 29.7683 | -99.8171 | 5 | 0.008 |
| SAN    | Panicum hallii var. hallii | Tex-Mex | USA     | Texas   | 981    | 30.1346         | -102.5633 | 3          | 0.007 |      |
| SAP    | Panicum hallii var. hallii | Tex-Mex | Mexico  | Durango | 1476   | 25.4331         | -103.7161 | 5          | 0.016 |      |
| SCG    | Panicum hallii var. hallii | Tex-Mex | USA     | Texas   | 421.4  | 29.6942         | -101.3221 | 5          | 0.014 |      |
| SEM    | Panicum hallii var. hallii | Tex-Mex | USA     | Texas   | 425    | 29.681  | -101.3099 | 4 | 0.016 |
| SEP    | Panicum hallii var. hallii | Central Texas | USA | Texas | Travis | 291.9 | 30.4013 | -97.797 | 7 | 0.018 |
| SEV    | Panicum hallii var. hallii | West    | USA     | New Mexico | 1544   | 34.3317         | -106.9735 | 24         | 0.003 |      |
| Loc ID | Species | Region          | Country | State | County     | Altitude m.a.s.l. | Latitude  | Longitude  | N  | He    |
|--------|---------|-----------------|---------|-------|------------|------------------|-----------|------------|----|-------|
| SLR    | Panicum hallii var. hallii | Sympatric     | USA     | Texas | Kimble     | 538              | 30.4453   | -99.7974   | 1  | 0.01  |
| SLS    | Panicum hallii var. hallii | Tex-Mex       | USA     | Texas | Burnet     | 391              | 30.9913   | -98.1008   | 4  | 0.012 |
| SMF    | Panicum hallii var. hallii | Central Texas | USA     | Texas | Lampasas   | 476              | 31.3052   | -98.4387   | 1  | 0.007 |
| SMT    | Panicum hallii var. hallii | Tex-Mex       | USA     | Texas | Mills      | 488              | 31.5616   | -98.5992   | 3  | 0.016 |
| SNO    | Panicum hallii var. hallii | West          | USA     | Arizona | Pima      | 1567             | 31.8005   | -110.7035  | 8  | 0.004 |
| SOM    | Panicum hallii var. hallii | Central Texas | USA     | Texas | Somervell  | 191              | 32.2756   | -97.6016   | 5  | 0.007 |
| SPE    | Panicum hallii var. hallii | West          | USA     | Texas | Crockett   | 744              | 30.6664   | -100.9707  | 23 | 0.005 |
| SPJ    | Panicum hallii var. hallii | USA           | Texas   |        | Kimble     | 602              | 30.47     | -99.7308   | 5  | 0.006 |
| SPR    | Panicum hallii var. hallii | West          | USA     | Texas | Jeff Davis | 1900             | 30.6723   | -104.1277  | 1  | 0.006 |
| SRG    | Panicum hallii var. hallii | Tex-Mex       | USA     | Texas | Val Verde  | 380.5             | 29.6608   | -101.3152  | 8  | 0.018 |
| STW    | Panicum hallii var. hallii | Central Texas | USA     | Texas | Palo Pinto | 332              | 32.6093   | -98.4896   | 8  | 0.006 |
| SYN    | Panicum hallii var. hallii | West          | USA     | New Mexico | Guadalupe | 1551             | 34.8311   | -104.8274  | 9  | 0.004 |
| THC    | Panicum hallii var. hallii | Central Texas | USA     | Texas | Travis     | 289              | 30.2419   | -98.0091   | 5  | 0.014 |
| TLA    | Panicum hallii var. hallii | Tex-Mex       | Mexico  | Durango | Tlahualilo | 1114             | 26.1281   | -103.4992  | 5  | 0.012 |
| TNK    | Panicum hallii var. hallii | West          | USA     | New Mexico | Guadalupe | 1398             | 34.9369   | -104.694  | 4  | 0.004 |
| TWC    | Panicum hallii var. hallii | Central Texas | USA     | Texas | Travis/Williamson | 360 | 30.6222 | -97.9719  | 5  | 0.004 |
| UVA    | Panicum hallii var. hallii | Sympatric     | USA     | Texas | Uvalde     | 304              | 29.2415   | -100.0972  | 1  | 0.014 |
| UVL    | Panicum hallii var. hallii | Sympatric     | USA     | Texas | Uvalde     | 550              | 29.5983   | -100.0253  | 2  | 0.037 |
| WBW    | Panicum hallii var. hallii | Sympatric     | USA     | Texas | Kimble     | 597              | 30.4329   | -99.7958   | 3  | 0.045 |
| ARR    | Panicum hallii var. hallii | West          | USA     | Arizona | Santa Cruz | 1460             | 31.6134   | -110.5063  | 2  |       |
| CMR    | Panicum hallii var. hallii | Sympatric     | USA     | Texas | Maverick   | 219              | 28.5835   | -100.1117  | 2  |       |
| Loc ID | Species | Region | Country | State | County | Altitude m.a.s.l. | Latitude | Longitude | N | $He$ |
|--------|---------|--------|---------|-------|--------|------------------|----------|-----------|---|------|
| GNH    | Panicum hallii var. hallii | Sympatric | USA     | Texas | Dewitt | 76.5             | 29.3119  | -97.3001 | 2 | 0.029 |
| HAM    | Panicum hallii var. hallii | Texas-Mex | USA     | Texas | Stonewall | 505     | 33.0098  | -100.1813 | 2 |
| HUE    | Panicum hallii var. hallii | Sympatric | USA     | Texas | Gillespie | 550      | 30.2255  | -99.0156 | 1 |
| KIC    | Panicum hallii var. hallii | Texas-Mex | USA     | Texas | Kinney | 561     | 29.6185  | -100.443 | 1 |
| MAV    | Panicum hallii var. hallii | Sympatric | USA     | Texas | Maverick | 215      | 28.2386  | -100.1592 | 3 |
| RAN    | Panicum hallii var. hallii | West     | USA     | Texas | Lubbock | 990      | 33.4602  | -101.9143 | 2 |
| CHR    | Panicum lepidulum | Mexico | Michoacan | Charo | 1975 | 19.6908 | -101.0312 | 5 |
| CUT    | Panicum lepidulum | Mexico | Michoacan | Morelia | 2113 | 19.7213 | -101.3424 | 2 |
| ERO    | Panicum lepidulum | Mexico | Michoacan | Erongaricuaro | 2101 | 19.6072 | -101.6871 | 5 |
| HTE    | Panicum lepidulum | Mexico | Michoacan | Huaniqueo | 2160 | 19.8959 | -101.4332 | 3 |
| HUA    | Panicum lepidulum | Mexico | Michoacan | Huaniqueo | 2141 | 19.876 | -101.4334 | 5 |
| MOR    | Panicum lepidulum | Mexico | Michoacan | Morelia | 1975 | 19.6761 | -101.3136 | 5 |
| PTZ    | Panicum lepidulum | Mexico | Michoacan | Patzcuaro | 2128 | 19.5581 | -101.5781 | 1 |
| TEM    | Panicum lepidulum | Mexico | Estado de Mexico | Temamatla | 2344 | 19.1887 | -98.8921 | 5 |
| TEN    | Panicum lepidulum | Mexico | Michoacan | Huaniqueo | 2207 | 19.8947 | -101.4284 | 3 |
| TZI    | Panicum lepidulum | Mexico | Michoacan | Morelia | 2127 | 19.7908 | -101.4445 | 1 |
| ALD    | Panicum sp | Mexico | Tamaulipas | Aldana | 269  | 22.863 | -98.2296 | 1 |
Table 2 Correlation between geographical distance and genetic diversity (He, heterozygosity) in *Panicum hallii* variety *hallii* genetic clusters from the diversity hotspot.

| Genetic cluster          | Number of samples | Correlation | p-value     |
|--------------------------|-------------------|-------------|-------------|
| Central Texas            | 90                | -0.1377     | 0.1954      |
| West of the distribution | 187               | -0.3568     | 0.00000055  |
| Sympatry area            | 32                | -0.2335     | 0.1982      |
| Texas-Mexico             | 87                | -0.0722     | 0.5060      |
Table 3. Morphological traits mean (standard error) for *Panicum hallii* var. *filipes* and *Panicum hallii* var. *hallii* measured in two different seasons and three localities.

| Trait                  | var. *filipes*     | var. *hallii*  |
|------------------------|--------------------|----------------|
| Panicle length (cm)    | 24.75 (2.44)       | 26.38 (5.22)   |
| Primary branch number  | 13.83 (4.05)       | 9.72 (1.5)     |
| Secondary branch number| 45.25 (28.39)      | 17.31 (9.10)   |
| Biomass (gr)           | 247.76 (82.28)     | 151.92 (52.83) |
| Senescence score       | 1.81 (0.16)        | 2.43 (0.27)    |
| % electrolyte leakage  | 15.67 (9.67)       | 33.89 (12.39)  |
| Seed mass (mg)         | 0.77 (0.18)        | 1.41 (0.65)    |
Table 4. Membership probability of inferred ancestry component for individuals of *Panicum hallii* var. *hallii* collected in the sympatric zone using four sets of *Panicum hallii* var. *filipes* learning samples.

| Individual | var. *filipes* learning samples set 1 | var. *filipes* learning samples set 2 | Removing var. *filipes* misclassified samples | All var. *filipes* as a learning samples |
|------------|--------------------------------------|--------------------------------------|-----------------------------------------------|----------------------------------------|
|            | Fil  | Hal  | Fil  | Hal  | Fil  | Hal  | Fil  | Hal  | Fil  | Hal  |
| BEE_1AG    | 0.55 | 0.45 | 0.49 | 0.51 | 0.35 | 0.65 | 0.57 | 0.43 |       |      |
| BEE_3F     | 0.13 | 0.87 | 0.12 | 0.88 | 0.04 | 0.97 | 0.14 | 0.86 |       |      |
| GNZ_22F    | 0.42 | 0.58 | 0.38 | 0.63 | 0.48 | 0.52 | 0.43 | 0.57 |       |      |
| GNZ_24F    | 0.58 | 0.42 | 0.52 | 0.48 | 0.22 | 0.78 | 0.60 | 0.40 |       |      |
| RAR_8F     | 0.33 | 0.67 | 0.28 | 0.72 | 0.03 | 0.97 | 0.33 | 0.67 |       |      |
| UVL_1AG    | 0.27 | 0.73 | 0.24 | 0.76 | 0.57 | 0.43 | 0.28 | 0.72 |       |      |
Table 5. Mantel test statistics. * Partial Mantel test using geographic distance as a controlling variable.

| Test                                           | Mantel’s r | p value |
|------------------------------------------------|------------|---------|
| Genotypic DAPC ~ geographic                    | 0.3595504  | 0.0001  |
| Phenotypic PCA ~ geographic                     | 0.2339050  | 0.0002  |
| Phenotypic PCA ~ genotypic DAPC                 | 0.2363396  | 0.0005  |
| Phenotypic PCA ~ genotypic DAPC | geographic * | 0.1678044 | 0.0094 |
Figure 6