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Genetics / Genética

A population genetics study of three native Mexican woody bamboo species of *Guadua* (Poaceae: Bambusoideae: Bambuseae: Guaduinae) using nuclear microsatellite markers

Estudio de genética de poblaciones de tres especies de bambúes leñosos nativos de Mexico del género *Guadua* (Poaceae: Bambusoideae: Bambuseae: Guaduinae) utilizando microsatélites nucleares

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Abstract
Background: Sporadic flowering contributes significantly to genetic diversity and connectivity among populations. Woody bamboos present sporadic or gregarious flowering patterns with long flowering cycles. In this study, we analyze the genetic diversity of three *Guadua* species distributed along the Gulf of Mexico slope that have different patterns of flowering.
Questions: (1) Are the three *Guadua* species genetically differentiated? (2) Does the vulnerable species *G. inermis* have low levels of genetic diversity? (3) What is the relative contribution of geographic and environmental factors to the genetic structure of *G. inermis*?
Species studied: *Guadua amplexifolia*, *G. inermis* and *G. tuxtlensis.*
Study site and dates: During 2014 and 2015, we collected samples of *G. inermis* in Puebla and southeastern Mexico, *G. amplexifolia* in Veracruz and Oaxaca, and *G. tuxtlensis* in southern Veracruz.
Methods: We successfully amplified five of nine SSR markers, and genotyped a total of 155 samples**.**
Results: The three *Guadua* species were genetically differentiated. For *G. inermis*, we found high levels of population genetic diversity, which are relatively higher than those of other monocot species. Genetic differentiation was high and three groups were detected: north, central and south. We found a significant association between genetic distances and the maximum temperature of the warmest month, but not with geographic distance.
Conclusions: Our study is the first to analyze levels of genetic diversity in Mexican bamboos and confirms their taxonomic identity. *G. inermis* has a strong genetic structure, even when populations are geographically close.
Keywords: Bamboos, genetic diversity, genetic structure, sporadic and massive flowering, polyploid.

Resumen
Antecedentes: La floración esporádica puede influenciar la estructura genética de las poblaciones. Los bambúes presentan patrones de floración esporádica o gregaria. En este estudio analizamos la diversidad genética de tres especies de *Guadua* con diferentes patrones de floración distribuidos en la Vertiente del Golfo de México.
Preguntas: 1) ¿Están las tres especies de *Guadua* (*G. inermis*, *G. amplexifolia* y *G. tuxtlensis*) genéticamente diferenciadas? 2) ¿Presenta *G. inermis* bajos niveles de diversidad genética? 3) ¿Cuál es la contribución relativa de los factores geográficos y ambientales en la estructura genética de *G. inermis*?
Especies de estudio: *Guadua amplexifolia*, *G. inermis* y *G. tuxtlensis.*
Sitio y años de estudio: En 2014 y 2015, colectamos muestras de *G. inermis* en Puebla y sureste de México; *G. amplexifolia* en Veracruz y Oaxaca, y *G. tuxtlensis* en el sureste de Veracruz.
Métodos: Amplificamos exitosamente cinco marcadores SSR de un total de nueve y genotipificamos 155 muestras.
Resultados: Las tres especies analizadas están genéticamente diferenciadas. *G. inermis* presentó altos niveles de diversidad genética, y superiores a otras especies de monocotiledóneas. Los niveles de diferenciación genética fueron altos y se detectaron tres grupos: norte, sur y centro. Encontramos una asociación significativa entre las distancias genéticas con la temperatura máxima del mes más caliente, pero no con la distancia geográfica.
Conclusiones: Nuestro trabajo es el primer estudio que evalúa los niveles de diversidad genética en bambúes mexicanos y confirma su identidad taxonómica. *G. inermis* tiene una estructura genética alta, incluso cuando las poblaciones están cerca geográficamente.
Palabras clave: Bambúes, diversidad genética, estructura genética, floración esporádica y masiva, poliploidía.

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Understanding the processes leading to speciation and biodiversity in plants is one of the main research topics in evolutionary biology. Flowering time has a strong effect on gene flow among populations (Fitter & Fitter 2002). For example, floral synchrony results in the simultaneous and massive flowering of individuals, offering abundant floral resources that can attract numerous pollinators and favoring outcrossing rates (Domínguez & Dirzo 1995, Rodríguez-Pérez & Traveset 2016). In the same way, there is evidence that flowering synchrony increases reproductive success in wind-pollinated species (Bogdziewicz *et al.* 2020). In contrast, plant asynchrony or sporadic flowering might reduce the availability of mates and thus reproductive success in both insect and wind-pollinated species (Domínguez & Dirzo 1995, Bogdziewicz *et al.* 2020). Asynchrony in flowering time across populations should result in lower levels of gene flow and greater genetic structure, even for populations that are geographically close together (Kirkpatrick 2000, Reisch & Posclod 2009).

Geographic distance and the environment represent key components influencing species' genetic structure (Rousset 1997, Wang & Bradburd 2014). Several species exhibit a significant pattern of isolation by distance (IBD), which suggests that genetic differentiation increases with geographic distance. On the other hand, isolation by environment (IBE) has been proposed to describe the relationship between environmental heterogeneity and the spatial distribution of genetic variation (Wang & Bradburd 2014). Thus, genetic differentiation among populations increases with their environmental differentiation (Wang & Bradburd 2014). In contrast, limited dispersal constrains gene flow among populations and reduces the possibility of counteracting genetic drift (Slatkin 1993, Rousset 1997).

Woody bamboos (Poaceae: Bambusoideae: Bambuseae) are a large taxonomic group with notable economic and ecological importance. Flowering in woody bamboos is peculiar because many of them remain in a vegetative phase for a long period of time, as long as decades or a century, followed by mass synchronous flowering and subsequent death (Janzen 1976). Other woody bamboos are characterized by sporadic flowering with only a few individuals reproductively active in their populations. Flower phenology in woody bamboos falls into four spatio-temporal patterns: 1) Flowering distribution, with a small percentage of individuals flowering the first or second year before and after the main flowering; 2) Flowering wave, with gregarious flowering occurring in patches in successive years; 3) Variation in periodicity between populations, leading to diffuse flower temporality within a species; 4) Sporadic flowering, random or other non-gregarious patterns of flowering (Troup 1921, Janzen 1976, Gadgil & Prasad 1984, Banik 1998, Franklin 2004, Bhattacharya *et al.* 2009, Zheng *et al.* 2020). Understanding how asynchronous flowering phenology can influence patterns of gene flow in woody bamboos is crucial to elucidating its role in population genetic divergence and speciation.

The genus *Guadua* Kunth is one of the six genera of the subtribe Guaduinae (Judziewicz *et al.* 1999, Clark *et al.* 2015, Tyrrell *et al.* 2018). It includes 33 species and is one of the most diverse genera in the subtribe (Clark *et al.* 2015, Ruiz-Sanchez *et al.* 2021). Species of *Guadua* are distributed from Mexico to Argentina, most of the species occur in South America, and this region is considered its center of diversification (Medina & Medina 1965, Judziewicz *et al.* 1999, Londoño 2001, 2011, Clark *et al.* 2015). Mexico has seven species of *Guadua*: *G. aculeata* Rupr. ex Fourn., *G. amplexifolia* J. Presl., *G. longifolia* (E. Fourn.) R.W. Pohl, *G. paniculata* Munro, *G. inermis* Rupr. ex Fourn, *G. velutina* Londoño & L.G. Clark, and *G. tuxtlensis* Londoño & Ruiz-Sanchez. The last three species are endemic to Mexico, and except for *G. paniculata*, which is distributed along the Pacific coast of Mexico, the other species are distributed along the Gulf of Mexico in the states of Campeche, Chiapas, Hidalgo, Oaxaca, Puebla, San Luis Potosí, Tabasco, Tamaulipas, and Veracruz (Londoño & Ruiz-Sanchez 2014, Ruiz-Sanchez *et al.* 2020). Most of the species are used to build houses, fences, kiosks, and to make handicrafts (Londoño & Ruiz-Sanchez 2014).

Few studies have reported the flowering cycles of *Guadua* species. Guerreiro (2014), Guerreiro *et al.* (2020) and Vega & Hernández (2008) recorded massive flowering events and estimated flowering cycles for three South American *Guadua* species: *Guadua chacoensis* (Rojas) Londoño & P. M. Peterson (28-31 years), *G. paraguayana* Döll (38 years), and *G. trinii* (Nees) Nees ex Rupr. (30-33 years). Liebsch & Reginato (2009) estimated cycles of 27-28 years between flowering events for *G. sarcocarpa* Londoño & P. M Peterson and *G. weberbaueri* Pilg, while sporadic flowering and massive events have been recorded in *G. angustifolia* Kunth (Londoño 2002) and only sporadic flowering in *G. inermis* Rupr. ex Fourn (Aguilera López 2020).

Herbarium records and field collections show that *G. amplexifolia* has massive flowering cycles of unknown periodicity. We collected flowering plants of *G. aculeata* in

2012, and some from Puebla state that were flowering in 2020 (Morocho pers. comm.). *G. inermis* with flowers have been collected in different localities of Veracruz in different years (2009, 2012, 2014, and 2015), and from isolated blooming individuals (Ruiz-Sanchez pers. obs.). Finally, flowering has not been recorded in *G. tuxtlensis* (Londoño & Ruiz-Sanchez 2014).

Even though some bamboo species are of economic and ecological importance, there is little information on their patterns of genetic diversity and structure in Mexico. Knowledge of the genetic diversity resources of woody bamboos would provide valuable information for conservation strategies as the habitat of bamboos is highly vulnerable due to human practices such as livestock farming. In particular, the size of *G. inermis*' populations are small in comparison with those of other *Guadua* species (i.e., *G. velutina*) and, according to Ruiz-Sanchez *et al.* (2018), the projected distribution of *G. inermis* in 2050 could be reduced by as much as 42 % as a consequence of global warming. Increasing habitat fragmentation, habitat vulnerability due to climate change, and the sporadic flowering of *G. inermis* will threaten the prevalence of this species. Therefore, analyzing its genetic diversity is crucial to develop effective conservation efforts.

In this study, we used nuclear microsatellites for three *Guadua* species (*G. amplexifolia*, *G. inermis*, and *G. tuxtlensis)* to answer the following questions: (1) Are the three *Guadua* species genetically differentiated? (2) Does the vulnerable species *G. inermis* have low levels of genetic diversity? (3) What are the relative contributions of geographic and environmental factors to the genetic structure of *G. inermis*?

Materials and methods

Study species. *Guadua amplexifolia*, *G. inermis,* and *G. tuxtlensis* are morphologically similar, and some of their populations are sympatric within the state of Veracruz (Londoño & Ruiz-Sanchez 2014, Ruiz-Sanchez *et al.* 2015, 2020). The first two species are the most morphologically similar and are difficult to distinguish. It has been suggested that *G. tuxtlensis* is of hybrid origin and one of the potential parent species could be *G. inermis* (Londoño & Ruiz-Sanchez 2014) (Figure. 1). *Guadua inermis* is endemic to Mexico, occurring in the states of Campeche, Chiapas, Oaxaca, Tabasco, and Veracruz; in tropical subdeciduous forests (Cortés-Rodríguez 2000, Londoño & Ruiz-Sanchez 2014). This species has culms 4 to 12 m tall, 3 to 10 cm in diameter, and solid or thick-walled culms. *G. amplexifolia* occurs from Mexico to Colombia. Culms 10-15 m tall, 6-10 cm in diameter, solid at the base, and hollow in its distal portion. Flowering phenology is massive. The distribution of *G. tuxtlensis* is restricted to Los Tuxtlas, Veracruz in Mexico. The culms are 10 to 20 m tall and 8 to 14 cm in diameter. This species has hollow culms, walls are thick, thorny branches from the first node, culm leaves are persistent basally (Figure 1). Flowering has not been recorded for this species. According to Guo *et al.* (2019), Neotropical woody bamboos are tetraploid and wind-pollinated species.

Species sampling. We collected fresh leaves from these three *Guadua* species during 2014 and 2015. For *G. inermis,* we collected from thirteen populations in Veracruz, Chiapas, and Puebla. The Puebla population is probably a cultivated population, because the native distribution of *G. inermis* does not include this state. *G. amplexifolia* was collected from three populations in Veracruz and Oaxaca, and *G. tuxtlensis* from two populations in southern Veracruz (Appendix 1, Figure 2). In total 155 individuals were analyzed, and the number of individuals collected per site varied from 2 to 16 (Appendix 2). Foliar tissue of adult and juvenile plants was collected from plants separated by at least 10 m to reduce the possibility of sampling the same genotype. The material was preserved in silica gel prior to DNA extraction. GPS coordinates were recorded for each population.

DNA extraction and microsatellite genotyping. Genomic DNA was isolated from 100 mg of leaf tissue following Doyle & Doyle's (1987) CTAB procedure. The DNA was dissolved in 100 µL of Milli-Q water. We tested seven microsatellite primers developed for *Guadua angustifolia* (Pérez-Galindo *et al.* 2009) and two developed for *Aulonemia aristulata* (Abreu *et al.* 2011). PCR reactions contained 2.5 µl of a multiplex solution, 5-100 ng of DNA template, and 0.3 µM of each primer. The final volume was 5.5 µl. PCR reactions were performed using an AERIS™ thermal cycler (Esco Healthcare, Singapore) under the following conditions: 96 ºC - 15 min; 35 cycles of 94 ºC - 30s, a gradient of annealing temperature from 50 to 60 ºC - 1:30 min, 72 ºC - 1 min. The final extension lasted 30 min at 60 ºC. We successfully amplified five primers. The forward primers FJ444929 and FJ444936 were labeled with HEX™, and the forward primers Aar12, FJ476075, and FJ444930 were labeled with 6-FAM™ dyes. The PCR amplification for labeled primers consisted of 2.5 µl of a multiplex solution, 5 - 100 ng of template DNA, and

Figure 1. Morphological differentiation in three *Guadua* species from eastern Mexico. A-B. *Guadua tuxtlensis*, C-D. *G. amplexifolia,* and E-F. *G. inermis*. Photos by Eduardo Ruiz-Sanchez.

0.5 µl of the primer mix. The primer mix included 2 µM for each primer and two pairs of primers per reaction were combined except for one of them (FJ444929 + Aar12; FJ444936 + FJ444930; and FJ476075). PCR temperature cycling conditions were as follows: 96 ºC for 15 min; 35 cycles of 94 ºC - 30 s, 57 ºC - 1:30 min, 72 ºC - 1 min. The final extension included 60 ºC for 30 min. We diluted each PCR reaction (1:30) and sent them to Illinois University, UIUC Core Sequencing Facility to conduct the fragment analysis using GeneScan™-500 LIZ Size Standard. GeneMapper v4.1 (Applied Biosystems) was used to carry out genotype scoring. Because *G. inermis* is a tetraploid species, the genotype configuration for each allele was determined using the MAC-PR method, microsatellite DNA allele counting - peak ratios (Esselink *et al.* 2004). This method infers the number of alleles as a function of the peak area for each individual and each locus.

Genetic diversity analyses. We estimated the average null allele for each locus based on De Silva's method (De Silva *et al.* 2005) implemented in Polysat (Clark & Jasieniuk 2011). We calculated genetic diversity statistics, including observed (H_O), Nei's gene diversity corrected for sample size (H_E), number of alleles per locus (N_A), and effective number of alleles per locus (N_{Ae}) using the software Spatial Pattern Analysis of Genetic Diversity (SPAGeDi) v1.3a (Hardy & Vekemans 2002). We also estimated allelic richness using the rarefaction method implemented in the Allelic Diversity Analyzer (ADZE) (Szpiech *et al.* 2008). This method trims unequal sample sizes to the same standardized sample size (*g*), taking a value that is less than or equal to the smallest sample size for the whole dataset. We set g = 4 to have a more comparable measurement of genetic diversity among populations.

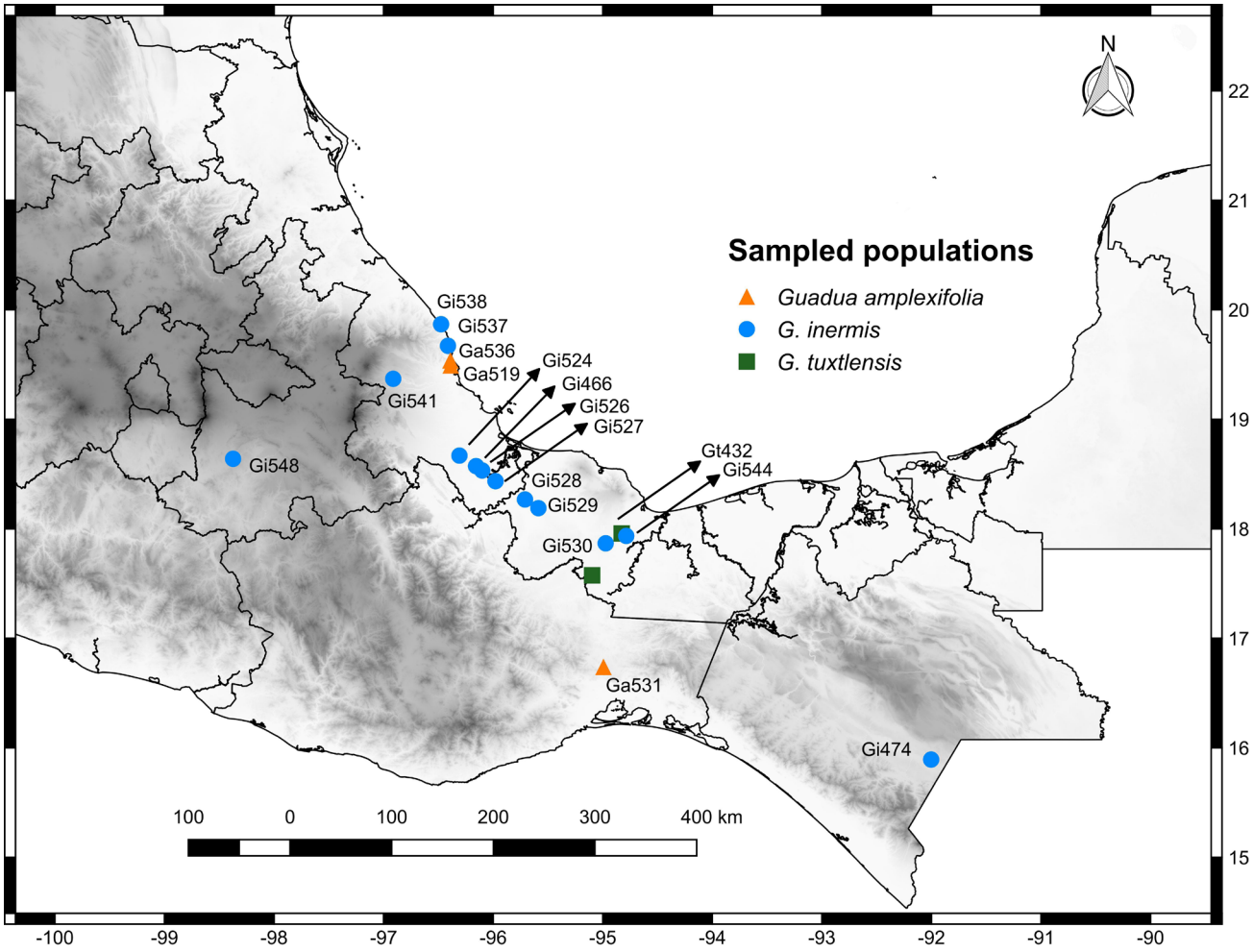

Figure 2. Collecting sites of three *Guadua* species in eastern Mexico.

Genetic structure analyses. Genetic differentiation among the populations of all three species was estimated using the F_{ST} statistic implemented in the software SPAGeDi v1.3a (Hardy & Vekemans 2002). In addition, the G_{ST} statistic was calculated for each species using Genodive v 2.0b27 (Meirmans 2020). We performed assignment genetic analysis using a Bayesian method implemented in the software STRUCTURE v2.3.4 (Pritchard *et al.* 2000). We included an admixture model and uncorrelated allele frequencies because we analyzed three bamboos species. Additionally, we carried out a second analysis including only the populations of *G. inermis* using the admixture model and correlated allele frequencies. We ran the analysis with 1×10^6 Markov chain Monte Carlo (MCMC) steps and a burn-in of 1×10^5 steps, with 10 replicates for each *K* (number of clusters), where *K* = 1 to 10. To determine the most appropriate value of *K*, the average and standard deviation (SD) of the likelihood of each model were used to calculated Δ*K* (Evanno *et al.* 2005) using Structure Harvester (Earl & vonHoldt 2012). The visual output of the STRUCTURE results was generated using DISTRUCT (Rosenberg 2003) and CLUMPAK (Kopelman *et al.* 2015).

We also ran a Principal Components Analysis (PCA) in the R package *adegenet* (Jombart *et al.* 2010). PCA is a multivariate method free of Hardy-Weinberg and linkage disequilibrium assumptions. We carried out an Analysis of Molecular Variance (AMOVA) by grouping populations by species and only for *G. inermis* populations without a specific hierarchical structure. These analyses were run in the *popr* R package with significant values determined after 20,000 replicates (Kamvar *et al.* 2014).

Isolation by Distance (IBD) and Isolation by Environment (IBE) in G. inermis. We explored the influence of geographic and environmental variables on genetic differentiation to test IBD and IBE in *G. inermis*. Linearized F_{ST} values (F_{ST}/1 - F_{ST}) were used to obtain the pairwise genetic matrix. The geographic distance matrix was obtained using the great-circle distance in the *geosphere* R package v1.5.10 (Hijmans 2019). We obtained the 19 environmental variables from WorldClim (www.worldclim.org/) with a resolution of 30 arcsec (1 km^2). We performed a PCA on all bioclimate variables. The variables most strongly correlated with the first three PCA axes containing the highest variance were selected. We checked for multicollinearity among the selected variables with the variance inflation factor (VIF) and excluded variables with VIF > 10 using the *vifstep* function in the *usdm* R package v1.1.18 (Naimi 2015). The bioclimate variables selected were isothermality (Bio 3), max temperature of warmest month (Bio 5), mean temperature of warmest quarter (Bio 10), precipitation of wettest month (Bio 13), and precipitation of coldest quarter (Bio19). First, we tested for IBD using the Mantel test with 1000 permutations in the *vegan* package (Oksanen *et al*. 2013). Then the statistical relationships of IBD and IBE with the genetic distance matrix were evaluated with maximum-likelihood population effects model (MLPE; github.com/nspope/ corMLPE; Clarke *et al.* 2002). This approach explicitly accounts for the non-independence of values in regressions run on distance matrices (Clarke *et al.* 2002). Parameter estimation was performed using restricted maximum likelihood (REML). To select among competing univariate models, we utilized information theoretical criteria such as the Akaike information criterion corrected for sample size (AICc) and the Akaike model weights (w*i*) calculated using the *dredge* function from the MuMIn v1.4 package (Bartoń 2018).

Results

Diversity and genetic structure of three Guadua species. The average frequency of null alleles for each locus an-

Table 1. Genetic diversity statistics, genetic differentiation and inbreeding coefficient for three *Guadua* species for specimens from the states of Chiapas, Oaxaca, Puebla, and Veracruz, Mexico.

Species	Code	N	N pop	Ho	H_E	G_{ST}	G_{IS}	*P*
G. amplexifolia	Ga	41	3	0.21	0.38	0.10	0.39	0.001
G. tuxtlensis	Gt	16	2	0.33	0.38	0.04	0.06	0.09
G. inermis	Gi	98	13	0.20	0.30	0.29	0.12	0.001

N sample size; N pop number of populations, Ho observed heterozygosity; H_E expected heterozygosity; G_{ST} genetic differentiation; G_{IS} inbreeding; *P* probability of G_{IS} and Hardy-Weinberg equilibrium test.

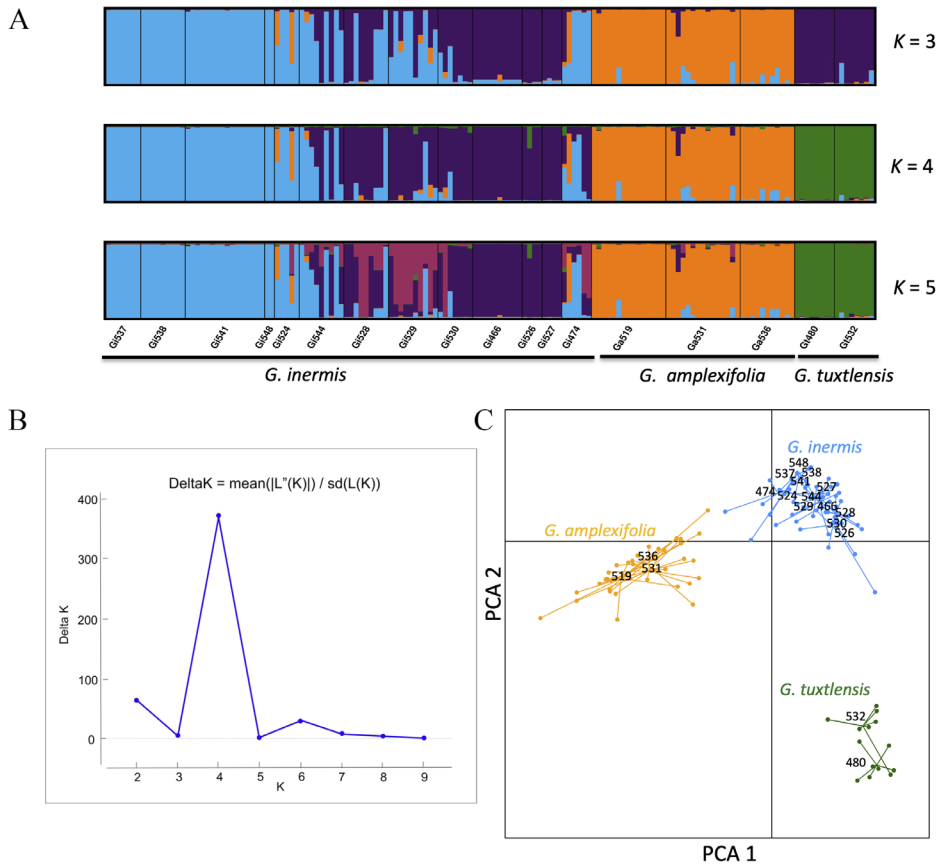

Figure 3. A. STRUCTURE plot (K = 3 to 5) for the assignment of individuals. B. K groups identified by Evanno's method. C. PCA for three species of *Guadua* based on five nuclear microsatellites.

alyzed was 0.26 for FJ444929, 0.08 for Aar12, 0.26 for FJ476075, 0.30 for FJ444936, and 0.22 for FJ444930. The number of alleles for *Guadua inermis*, *G. amplexifolia* and *G. tuxtlensis* was 28, 25, and 13 alleles, respectively. The multilocus statistic of genetic diversity based on H_O was 0.20 for *G. inermis*, 0.21 for *G. amplexifolia* and 0.33 for *G. tuxtlensis* (Table 1). Average levels of genetic diversity were lower for *G. inermis*, and most of the estimated statistics were higher for *G. tuxtlensis* (Appendix 2). The rarefaction statistic for AR was 1.45 for *G. inermis*, 1.71 for *G. amplexifolia*, and 1.71 for *G. tuxtlensis*. All populations of *G. amplexifolia* (Ga519, Ga531, and Ga536) exhibited significant values of inbreeding, but this only occurred in three populations of *G. inermis* (Gi524, Gi528, and Gi529) and in one *G. tuxtlensis* population (Gt532) (Appendix 2).

According to Delta K, the STRUCTURE analysis showed that the most likely number of clusters was K = 4 (Figure 3). Group 1 (color: green) corresponded to individuals of *Guadua tuxtlensis*; the second group (color: orange) included individuals of *G. amplexifolia*, while the third and fourth groups (color: blue and purple respectively) were composed of *G. inermis* plants. For these last two groups, group 3 included mainly individuals from populations Gi524, Gi537, Gi538, and Gi541, which are located in the northern part of the sampling region and Gi548 located in the state of Puebla. Group 4 was composed of plants from populations Gi466, Gi526, and Gi527, which are in the central part of the study region. The southern populations Gi544, Gi528, Gi529, Gi530 had plants assigned to groups 3 or 4 and some individuals had a mixed ancestry. Population Gi474, located in Chiapas, had a mixed ancestry from groups 2, 3, and 4; population Gi524 also had mixed ancestry with group 2 that corresponds to the *G. amplexifolia* cluster. The PCA for the three species had a pattern similar to that observed in STRUCTURE, where there is a distinction among the populations of the three species (Figure 3).

The AMOVA indicated that 59.89 % of the genetic variance was explained among the three species, in contrast to

Table 2. Analysis of molecular variance for a) three species of *Guadua* and b) *G. inermis*.

Source of variation	df	SS	MS	% CV
a) Among groups	2	107.24	53.62	59.89
Among populations within species	15	51.36	3.42	19.38
Within populations	104	51.72	0.49	20.71
Total	121	210.33	1.73	100
b) Among populations	12	111.93	9.32	71.74
Within populations	57	37.63	0.66	28.25
Total variation	69	149.57	2.16	100

19.39 % of the genetic variance among populations within species and 20.71 % of the variance within populations (Table 2). The G_{ST} for *G. amplexifolia*, *G. tuxtlensis* and *G. inermis* was 0.10, 0.04 and 0.29 respectively (Table 1). The highest levels of genetic differentiation were detected between one population of *Guadua tuxtlensis* (Gt480) and the populations of *G. inermis* located in the northern region of the sampling area in Veracruz (Gi537, Gi538, and Gi541; Appendix 3). The lowest levels of genetic differentiation were detected between Ga531 located in Oaxaca and Gi474 located in Chiapas; also Ga531 exhibited low levels of genetic differentiation with populations of *G. inermis* located mainly in the southern part of the sampling region in Veracruz (Appendix 3). These results agree with the PCA and STRUCTURE analyses, where *G. amplexifolia* and *G. inermis* had lower levels of genetic differentiation than *G. tuxtlensis* did.

Diversity, genetic structure, and connectivity of Guadua inermis. Average levels of genetic diversity of *G. inermis* were H_E = 0.23 and Ho = 0.20 (Appendix 2). The lowest levels of genetic diversity based on H_O, H_E, and N_{AE} were found in population Gi537 located in the northern part of the sampling region, and population Gi548 in Puebla, while the highest values of H_O, H_E, and N_{AE} were detected for Gi528 and Gi530 located in the central and southern part of the sampling region (Appendix 2).

According to Delta *K* for STRUCTURE, the most likely number of clusters was *K* = 2. The first group (orange, Figure 4) was composed of five populations (Gi537, Gi538, Gi541, Gi548) from the northern part of Veracruz and one population (Gi474) from Chiapas. The second group included three populations (Gi466, Gi526, and Gi527) from the central part of the sampling region in Veracruz, while five populations (Gi524, Gi544, Gi528, Gi529, and Gi530) included individuals with mixed ancestry from the two groups, located mainly in southern ranges (Figure 4). The PCA exhibited a pattern similar to the result of *K* = 3 from STRUCTURE, where some individuals are intermixed among populations. Specifically, one group included the most northern populations Gi541, Gi474, Gi524, Gi548, Gi544, Gi537, and Gi538. The second group included populations located mainly in southern ranges, Gi528, Gi528, Gi530 (purple group of

Table 3. Results from a multimodel inference on MLPE regression models in *G. inermis*. Log likelihood (LogLik), Akaike's information criterion corrected for sample size (AICc), relative difference between the best model and each of the other models in the set (ΔAIC), model weight representing the probability that a model is the best in the set (w_i), and the correlation coefficient rho (***ρ***).

Predictor	logLik	AICc	ΔAIC	wi	*P*
Bio 5	29.90	-51.3	0	1	0.333*
IBD	18.62	-28.7	22.57	0	0.109
Bio19	18.03	-27.5	23.73	0	0.096
Bio10	16.33	-25.1	26.12	0	0.041
Bio 3	16.28	-24.0	27.25	0	0.054
Bio 13	16.14	-23.7	27.54	0	0.050

*P = 0.0001.

$K = 3$) and the third group, those located in the central part of the sampling area, Gi527, and Gi526 (the same as the pure blue group in $K = 2$ and 3) (Figure 4). The Gi474 population (located in Oaxaca) included wide variation, with some individuals similar to other populations while others are divergent. The AMOVA results showed that 71.74 % of the genetic variance was explained by differences among populations, while 28.25 % could be attributed to the genetic variance within populations (Table 2).

G. inermis populations exhibited wide variation for F_{ST} pairwise comparisons, ranging from 0 to 0.67. Genetic differentiation at the species level was $F_{ST} = 0.47$ and $G_{ST} = 0.44$. The highest levels of genetic differentiation were detected between populations located in Chiapas, those in the center of the sampling area in Veracruz (Gi466, Gi526, and Gi527), and the northernmost populations of Veracruz (Gi537, Gi538, and Gi541). Overall, the lowest levels of genetic variation ($F_{ST} = 0$) were found in geographically close populations (Appendix 3).

We did not find a significant IBD pattern ($r = 0.13$, $P = 0.2$). Multimodal inference showed that the best performing MPLE model included the maximum temperature of the warmest month (Bio 5) as this variable had the maximum model probability ($w_i = 1$) and the lowest AICc score (-51.3) relative to the other models. This environmental variable explained 33 % of the genetic variation in population genetic distances (Table 3). None of the other bioclimate variables explained gene flow in *G. inermis*.

Discussion

Our main results confirm the taxonomic designation of the three *Guadua* species as they exhibited evident genetic differentiation (Londoño & Ruiz-Sanchez 2014). This was clear from the results of STRUCTURE and PCA that coherently separated the three species. Pairwise genetic differentiation among species was also high. The average levels of genetic diversity were lower for *G. inermis*, and the values for most of the statistics were larger for *G. tuxtlensis*. We found a strong genetic structure in *G. inermis*. We found a significant association of genetic distances with the maximum temperature of the warmest month, but not with geographic distance. Moreover, we did not find any evidence supporting the hybrid origin of *G. tuxtlensis* (which was thought to have *G. inermis* as one of its parental species), as suggested by Londoño & Ruiz-Sánchez (2014).

Genetic diversity and structure among Guadua species. Higher levels of genetic diversity were detected in *Guadua tuxtlensis* than in *G. inermis* or *G. amplexifolia* for most of the statistics, while the lowest levels of genetic diversity were detected in *G. inermis*. However, it was a population of *G. inermis* that exhibited the highest levels of genetic diversity, according to the Ho and H_E statistics for the Gi530 population, which is in the southern region of Veracruz. The PCA, STRUCTURE, and AMOVA indicated that each species is genetically different, supporting their taxonomic designation. The PCA, however, did reveal that *G. amplexifolia* and *G. inermis* are genetically closer to each other than to *G. tuxtlensis*. This result was supported by slightly lower F_{ST} pairwise comparisons between both species. According to Londoño (pers. comm.), *G. inermis* and *G. amplexifolia* would be grouped in the same genetic complex, while *G. tuxtlensis* would be grouped with other *Guadua* species. The inbreeding coefficient for most of the *G. inermis* populations we studied indicated an excess of heterozygotes, and the three populations of *G. amplexifolia* exhibited inbreeding. This result is partial, because *G. amplexifolia* has a wide distribution (Mexico to Colombia) and we only analyzed three populations of this species in Mexico.

Genetic diversity and connectivity in G. inermis. Multiple factors determine the genetic diversity of species, including flowering synchrony, geographic range, and mating system (Hamrick & Godt 1996, Nybom 2004). Genetic diversity in bamboos exhibits a wide range of variation. *Guadua inermis* harbors, on average, lower levels of genetic diversity (Ho = 0.20 and He = 0.23) compared to six other species of bamboos using SSR (Ho = 0.55 and $H_E = 0.46$) (Posso 2011, Attigala *et al.* 2017, Jiang *et al.* 2017, Yang *et al.* 2018, Meena *et al.* 2019, Huang *et al.* 2020). Among the highest levels of genetic diversity detected were those of *G. angustifolia* ($H_E = 0.56$ including 30 locations and 9 SSR markers in the Colombian Eje Cafetalero; Muñoz *et al.* 2010, Posso 2011).

G. angustifolia has a wide distribution range with massive and sporadic flowering; factors that could explain the high levels of genetic diversity in this species. In contrast, even though the bamboo *Dendrocalamus sinicus* Chia & J.L. Sun, flowers sporadically and has a narrow distribution, it has higher levels of genetic diversity than *G. inermis* does ($H_E = 0.54$, $H_O = 0.48$, including 8 SSR markers and 18 populations; Yang *et al.* 2018). Chen *et al.* (2017) found high levels of outcrossing rates in this species and another study estimated low levels of biparental inbreeding in *D. sinicus* (Xie *et al.* 2019). This explains the high values of diversity detected in this species. Other bamboos

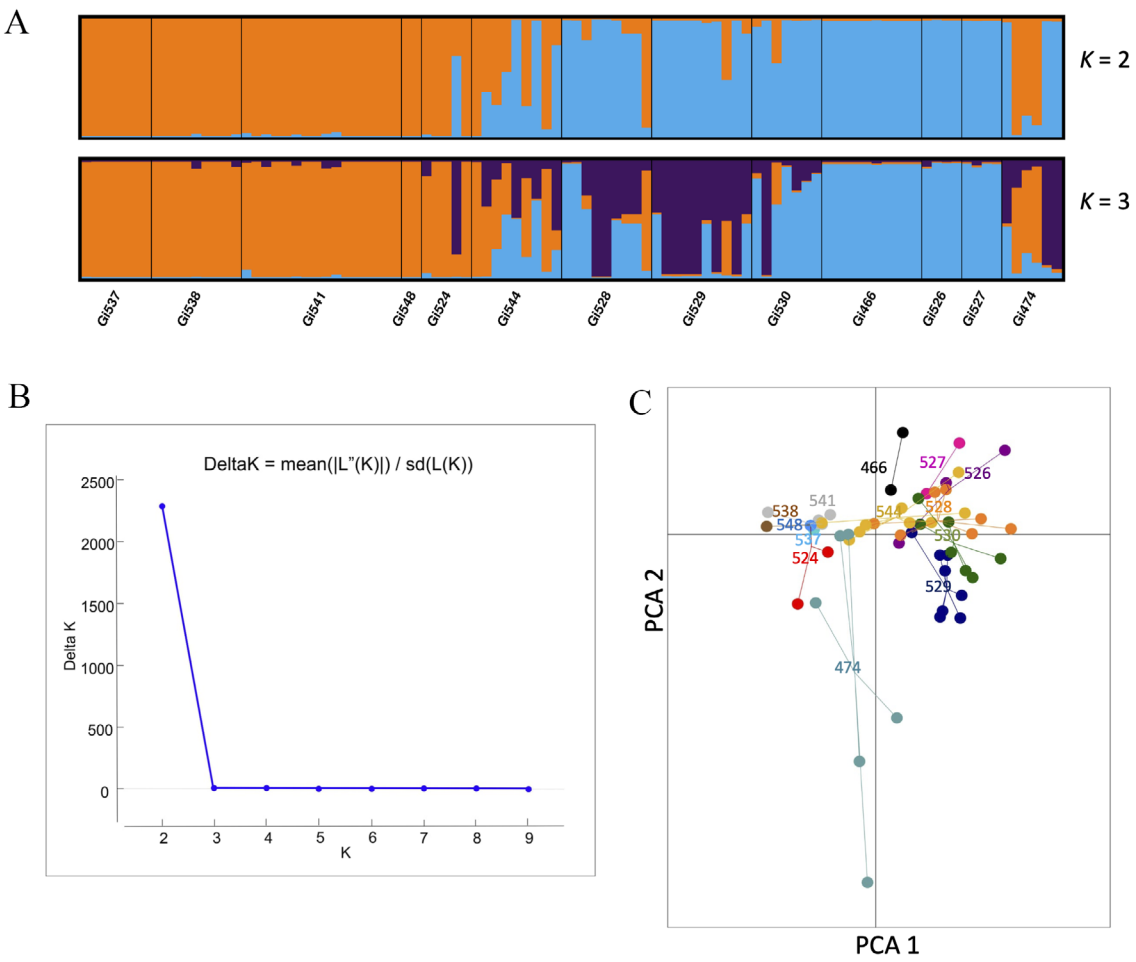

Figure 4. A. STRUCTURE bar plots (*K* = 2 and 3) for the assignment of individuals. B. K groups identified by Evanno's method. C. PCA for 13 populations of *Guadua inermis* based on five microsatellites.

also exhibit high levels of genetic diversity and excess heterozygosity even when flowering is sporadic (*Phyllostachys edulis* - Jiang *et al.* 2017, *Fargesia spathacea* complex - Huang *et al.* 2020). The authors suggested that even the sporadic flowering events can potentially contribute to the population diversity of these species.

Moreover, the flowering time interval within the sporadic phenology should vary among species, which potentially contributes to the variation in genetic diversity among bamboo species. In the case of *G. inermis*, during sample collection, we detected only six individuals flowering in six different populations. This highlights just how rare flowering is. However, it is necessary to further analyze flowering phenology to understand its role in the structure of population genetics.

G. inermis has lower levels of genetic diversity than other bamboo species. When it is compared to 79 other monocot outcrossing species, their levels of genetic diversity are higher (H_E = 0.15; Hamrick & Godt 1996). Polyploidy has been one of the factors that contribute to maintaining high levels of genetic diversity in bamboos. Theoretical and experimental data have shown a reduction of inbreeding in polyploid species as a consequence of a higher number of alleles per locus (Moody *et al.* 1993, Soltis & Soltis 2000, Baduel *et al.* 2018).

The higher genetic diversity and mixed ancestry of southern populations of *Guadua inermis* suggest that this region is the ancestral distribution. This result agrees with the southern origin of tropical bamboos (Clark 1997, Ruiz-Sanchez 2011). Northern range expansion could occur as a consequence of warming weather in the Late Pleistocene and Holocene. Other species inhabiting this region also migrated toward northern ranges (Ruiz-Sanchez & Ornelas 2014, Ornelas *et al.* 2016, 2019).

The AMOVA indicated high levels of genetic differentiation among populations. Further, we detected large genetic differences between northern and central populations, even when they are in proximity. Further research, including the use of more genetic markers, is needed to test this result. High genetic structure has been also detected in the tallest bamboo *Dendrocalamus giganteus* using ISSR (7 primers, 7 populations, G_{ST} = 0.84; Tian *et al.* 2012). The authors indicated that intensive selection of genotypes and their introduction into natural populations is one of the reasons for the high levels of genetic differentiation. Moreover, the long vegetative period and sporadic flowering are also important factors that influence the high degree of genetic structure and low levels of genetic diversity (H_E = 0.04 and H_O = 0.06; Tian *et al.* 2012).

Guadua inermis had higher levels of genetic differentiation than *G. angustifolia* (F_{ST} = 0.098; Posso 2011), *D. sinicus* (G_{ST} = 0.23; Yang *et al.* 2018), or *D. membranaceus* did (21.05 % of the variation was among populations; Yang *et al.* 2012). Even though *D. sinicus* has sporadic flowering, its genetic structure was not greater than that of *G. inermis*. It would be important to increase the number of genetic markers in future studies to increase accuracy when quantifying the levels of genetic diversity and structure of these species.

Anthropogenic habitat loss and fragmentation contribute to the reduction in population size and isolation of populations (Reed & Frankham 2003, Lowe *et al.* 2005, Chávez-Pesqueira *et al.* 2014, Schlaepfer *et al.* 2018), to which endemic species are more susceptible. Additionally, the loss of genetic diversity could have a negative impact on fitness, viability of individuals, and their ability to respond to environmental challenges (Reed & Frankham 2003). Habitat fragmentation, the sporadic flowering and the projected future reduction in the distribution of *G. inermis* (Ruiz-Sanchez *et al.* 2018) all threaten the prevalence of this species. Genetic diversity is an important aspect in the conservation and utilization of resources. Our study contributes to this knowledge and can be helpful when drawing up conservation management plans.

Precipitation and temperature are important factors influencing plant growth, development, survival, reproduction, and other ecological aspects (Manel *et al.* 2012). Our results show that the environment influenced the genetic structure of *G. inermis*. Specifically, the maximum temperature of the warmest month was significantly correlated with genetic distances, while geographic distance had no effect. This result was partially expected since geographically close populations are genetically differentiated, particularly in the center and northern ranges.

Our study is the first to evaluate levels of genetic diversity in Mexican endemic woody bamboos. We detected that the genetic diversity of *G. inermis* is on average lower than that reported in six other bamboo studies using SSR, and higher than that of other outcrossing monocots. Even with its small population size, patchy distribution and its sporadic flowering, *G. inermis* maintains its genetic diversity. Its sporadic flowering and habitat fragmentation could reduce genetic connectivity for this species. We detected a strong genetic structure between northern and central populations. Even when populations are geographically close, their genetic differences are as high as those in the comparisons of populations among species. Our study also confirms the taxonomic identity of three endemic bamboo species.

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Appendix 1. Population code (Code), location, longitude and latitude, elevation and voucher information for three *Guadua* species from eastern Mexico.

Code	Location	Longitude N	Latitude W	Elevation (m asl)	Voucher
Ga519	Veracruz	-96.387222	19.492222	23	*E. Ruiz-Sanchez et al. 519*
Ga536	Veracruz	-96.390227	19.536512	24	*E. Ruiz-Sanchez & I. Guzmán 536*
Ga531	Oaxaca	-94.993222	16.738083	289	*E. Ruiz-Sanchez & A. Ortíz 531*
Gi466	Veracruz	-96.156139	18.572472	6	*E. Ruiz-Sanchez & W. Wysocki 466*
Gt480	Veracruz	-95.096293	17.575046	54	*E. Ruiz-Sanchez & A. Ortíz 480*
Gt532	Veracruz	-94.826361	17.957167	32	*E. Ruiz-Sanchez & A. Ortíz 532*
Gi474	Chiapas	-92.00225	15.892222	653	*E. Ruiz-Sanchez & E. Gándara 474*
Gi524	Veracruz	-96.308028	18.668417	13	*E. Ruiz-Sanchez & A. Ortíz 524*
Gi526	Veracruz	-96.100111	18.532306	6	*E. Ruiz-Sanchez & A. Ortíz 526*
Gi527	Veracruz	-95.978639	18.434972	5	*E. Ruiz-Sanchez & A. Ortíz 527*
Gi528	Veracruz	-95.709753	18.267389	3	*E. Ruiz-Sanchez & A. Ortíz 528*
Gi529	Veracruz	-95.587222	18.188528	8	*E. Ruiz-Sanchez & A. Ortíz 529*
Gi530	Veracruz	-94.973028	17.867306	7	*E. Ruiz-Sanchez & A. Ortíz 530*
Gi537	Veracruz	-96.412369	19.671034	45	*E. Ruiz-Sanchez & I. Valdivieso 537*
Gi538	Veracruz	-96.475312	19.867952	15	*E. Ruiz-Sanchez & I. Valdivieso 538*
Gi541	Veracruz	-96.912586	19.36973	934	*E. Ruiz-Sanchez & V. Piñeros 541*
Gi544	Veracruz	-94.785278	17.934722	21	*E. Ruiz-Sanchez & A. Ortíz 544*
Gi548	Puebla	-98.373861	18.639056	1348	*E. Ruiz-Sanchez & D. Angulo 548*

Appendix 2. Genetic diversity parameters and inbreeding coefficient per population for three *Guadua* (*G. amplexifolia, G. inermis,* and *G. tuxtlensis*) species from the states of Chiapas, Oaxaca, Puebla and Veracruz, Mexico.

Pop	Location	N	N_A	N_{Ae}	AR	$AR_{(ss4)}$	H_O	H_E	G_{IS}	*P*
G. inermis										
Gi466	VER	10	1.4	1.23	1.27	1.21	0.14	0.12	-0.20	0.02
Gi474	CHIS	6	2.4	1.82	1.97	1.67	0.21	0.31	0.33	0.23
Gi524	VER	5	2	1.38	1.7	1.47	0.16	0.25	0.36	0.00
Gi526	VER	4	1.8	1.51	1.67	1.50	0.26	0.27	0.02	0.52
Gi527	VER	4	1.6	1.29	1.48	1.30	0.22	0.18	-0.21	0.01
Gi528	VER	9	2.4	2.17	2.16	1.86	0.28	0.43	0.34	0
Gi529	VER	10	2.6	1.82	1.97	1.66	0.29	0.32	0.11	0.1
Gi530	VER	7	3	1.94	2.27	1.83	0.42	0.41	-0.03	0.28
Gi537	VER	7	1.2	1.13	1.19	1.14	0.10	0.07	-0.28	0.01
Gi538	VER	9	1.6	1.15	1.27	1.18	0.12	0.09	-0.23	0.00
Gi541	VER	16	2	1.16	1.31	1.20	0.13	0.10	-0.23	0
Gi544	VER	9	2.6	1.87	2.04	1.74	0.25	0.38	0.32	0.05
Gi548	PUE	2	1.2	1.14	1.2	1.15	0.1	0.08	-0.16	0.56
Average			**1.98**	**1.50**	**1.6**	1.45	**0.20**	**0.23**		
G. amplexifolia										
Ga519	VER	15	3.4	1.74	2.07	1.68	0.23	0.33	0.31	0
Ga531	OAX	15	3.6	2.06	2.45	1.90	0.26	0.41	0.38	0
Ga536	VER	11	3.2	1.70	1.88	1.56	0.12	0.26	0.55	0
Average			**3.4**	**1.83**	**2.1**	1.71	**0.20**	**0.33**		
G. tuxtlensis										
Gt480	VER	8	2.2	1.68	1.81	1.62	0.38	0.33	-0.15	0.03
Gt532	VER	8	2.4	2.25	2.18	1.80	0.29	0.38	0.25	0.00
Average			**2.3**	**1.96**	**1.99**	1.71	**0.33**	**0.35**		

N = sample size; $N_{A=}$ number of alleles; N_{Ae} = effective number of alleles; AR = allelic richness; Ho = observed heterozygosity; H_E = expected heterozygosity; G_{IS} = inbreeding; *P* = probability of G_{IS} and Hardy-Weinberg equilibrium test.
VER = Veracruz, OAX = Oaxaca, CHIS = Chiapas, PUE = Puebla.

Appendix 3. Pairwise genetic differentiation parameter of three species of *Guadua* (*G. amplexifolia*, *G. inermis*, and *G. tuxtlensis*) for specimens from the states of Chiapas, Oaxaca, Puebla and Veracruz, Mexico.

	466	**474**	**524**	**526**	**527**	**528**	**529**	**530**	**537**	**538**	**541**	**544**	**548**	**519**	**531**	**536**	**480**
474	0.48																
524	0.54	0.20															
526	0.30	0.38	0.41														
527	0.15	0.31	0.40	0.15													
528	0.28	0.16	0.21	0.12	0.16												
529	0.37	0.07	0.23	0.34	0.27	0.13											
530	0.17	0.14	0.22	0.08	0.09	0.02	0.10										
537	0.67	0.45	0.04	0.64	0.63	0.37	0.38	0.38									
538	0.66	0.47	0.05	0.63	0.62	0.38	0.39	0.39	0								
541	0.65	0.50	0.08	0.64	0.62	0.43	0.43	0.43	0	0							
544	0.39	0.20	0.11	0.15	0.24	0	0.21	0.10	0.26	0.24	0.28						
548	0.65	0.27	0	0.53	0.58	0.20	0.28	0.27	0	0	0	0.05					
519	0.61	0.43	0.43	0.54	0.52	0.44	0.42	0.43	0.59	0.60	0.64	0.48	0.52				
531	0.51	0.32	0.32	0.45	0.41	0.35	0.34	0.33	0.50	0.51	0.56	0.40	0.41	0.02			
536	0.68	0.46	0.44	0.59	0.58	0.50	0.51	0.50	0.63	0.64	0.67	0.51	0.54	0.22	0.12		
480	0.68	0.58	0.60	0.47	0.60	0.46	0.55	0.44	0.73	0.73	0.76	0.53	0.66	0.59	0.52	0.62	
532	0.65	0.45	0.49	0.44	0.55	0.39	0.47	0.38	0.64	0.64	0.68	0.44	0.53	0.53	0.47	0.57	0.06