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Foliage leaf and floral bract micromorphology vary by habitat and habit in *Guadua* (Poaceae: Bambusoideae: Bambuseae)

Elizabeth K. McMurchie [a,*], Josephine A. Crock [a], Devin Molnau [a], Haldre S. Rogers [a,b], Dean C. Adams [a], Lynn G. Clark [a]

[a] Department of Ecology, Evolution, and Organismal Biology, Iowa State University, 251 Bessey Hall, Ames, IA 50011-4009, USA
[b] Department of Fish and Wildlife Conservation, Virginia Tech, 152 Cheatham Hall, Blacksburg, VA 24060, USA

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ABSTRACT

Micromorphology of bamboo foliage leaves is a valuable tool for determining taxonomic placement of species. Members of the Neotropical woody bamboo subtribe Guaduinae, including *Guadua*, are unusual in that most have stomata on both leaf surfaces. Foliage leaves of savanna-dwelling *Guadua* also often feature papillae on both surfaces, while the plants share a suite of macromorphological characteristics. We sought to evaluate statistically whether there were patterns in micromorphological variation in *Guadua* foliage leaves that aligned with known habitat- or habit-based patterns in macromorphological variation. Additionally, we investigated the micromorphology of *Guadua* floral bracts to determine whether they displayed variation similar to that of foliage leaves. By analyzing scanning electron microscopy images of these structures, we constructed binary datasets recording presence or absence of micromorphological features on foliage leaves and floral bracts of *Guadua* specimens belonging to 28 and 24 described species, respectively, as well as four and two putative species, respectively, for foliage leaves and floral bracts. We produced distance matrices using simple matching coefficient for the micromorphology datasets, visualized our data using principal coordinates analysis, and determined whether there were significant differences in foliage leaf and floral bract micromorphology based on habitat and habit using factorial multivariate analyses of variance. Our results indicate that *Guadua* foliage leaf and floral bract micromorphology differs significantly between *Guadua* found in savanna, forest, or river habitats and between *Guadua* of small arching, climbing, or larger erect habits.

1. Introduction

The presence of certain micromorphological features in foliage leaves appears to confer advantages to plants in different habitats (Haworth and McElwain, 2008). Papillae around stomatal complexes might help to protect stomata from water damage, while trichomes could prevent water loss (Haworth and McElwain, 2008), and silica bodies possibly reduce drought stress and damage from ultraviolet radiation (Klančnik et al., 2014; Shakoor, 2014). Numerous studies involving comparisons of foliar micromorphology across ecological gradients or between different habitats have historically focused on assessments of characters within individual species (e.g., McNeilly et al., 1987; Sevik et al., 2019; Yigit et al., 2019). Studies of foliar micromorphology focused on taxonomic differences between species have sometimes hinted at the role that ecology may nor may not play in the prevalence of certain characters (e.g., Adebowale et al., 2014; Ashfaq et al., 2019; Xiang et al., 2010), although measurements of some features vary based on the habitat in which the plant grew, including trichome size (Jurišić Grubešić et al., 2007) and density (Perez-Estrada et al., 2000) and stomatal density (Zhang et al., 2012). Studies of the micromorphology of floral parts have similarly demonstrated the taxonomic usefulness of characters such as presence of certain trichome and idioblast types in a wide variety of flowering plants (e.g., Angulo and Dematteis, 2014; Giuliani et al., 2018; Nunes et al., 2015; Piwowarczyk and Kasińska, 2017). Interspecific differences in floral micromorphology, however, have been studied largely in the context of differences in pollination strategy (e.g., Besi et al., 2021; Coiro and Barone Lumaga, 2018; Nunes et al., 2015) rather than genetically-controlled variation in micromorphology associated with the habitat in which various species grow. The incongruence between floral micromorphology and pollinator syndrome in diverse taxa indicates that other factors, such as hydrophobicity, may affect floral micromorphology

* Corresponding author.
E-mail address: elkamcmurchie@gmail.com (E.K. McMurchie).

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(Kraaij and van der Kooi, 2020), highlighting the role that habitat may play in these features.

Variations in patterns of foliage leaf micromorphology have proven taxonomically useful in bamboos of the Neotropics, delineating species and groups within genera (Leandro et al., 2016; 2017; Lima et al., 2020). Presence and morphology of papillae and micromorphology of the stomatal apparatus, including subsidiary cell shape and presence of stomata on the adaxial leaf surface, are often taxonomically significant (Leandro et al., 2016; 2017; 2019). The shapes of silica bodies in foliage leaves also help to define groups within bamboo genera (Leandro et al., 2017; Lima et al., 2021); these features may be especially taxonomically significant because silica body shapes do not appear to be affected by environment within a particular species (Prychid et al., 2003). The presence or absence of micromorphological features observed in the foliage leaves of different bamboo species could be related to the habitats in which species evolved and is unlikely to be explained by environmental plasticity; however, this has largely gone untested in the bamboos (Adebowale et al., 2014; Leandro et al., 2016; 2017).

The Neotropical woody bamboo genus *Guadua* Kunth is the most speciose and wide-ranging of the six genera belonging to the Neotropical woody bamboo subtribe Guaduinae, currently at 37 described species (McMurchie et al., 2025; Ruiz-Sanchez et al., 2021). As species of *Guadua* are associated with several different habitat and habit types, this genus represents a valuable opportunity to examine the effect of these factors on the evolution of foliar micromorphology (Judziecwicz et al., 1999). Species of *Guadua* include some of the most economically (Judziewicz et al., 1999; Killeen, 1990; Young and Judd, 1992) and ecologically (Chaves et al., 2021; Griscom and Ashton, 2006; Veldman, 2008) important plants found within their range, from central Mexico to southern Brazil and northern Argentina and Uruguay (Judziewicz et al., 1999). Most *Guadua* species live primarily in forested or river habitats (Judziewicz et al., 1999; Young and Judd, 1992), where they are expected to experience direct competition from trees but relatively low water stress, enabling them to remain evergreen. However, a few species belonging to the *G. paniculata* Munro complex predominately live in savanna habitats where they experience a prolonged dry season that results in yearly leaf loss (Veldman, 2008). Based on the environmental stresses present in the habitats in which *Guadua* is found, certain micromorphological features might have evolved to become more common in certain species. Unlike the density of trichomes, trichome type appears to be species-specific (Adebowale et al., 2014), much like papillar morphology and silica body type (Leandro et al., 2016; 2017); however, the habitat differences potentially driving the variation in trichome type, silica body type, and papillar morphology in *Guadua* and other bamboos remains unstudied.

Species of *Guadua* belonging to the *G. paniculata* complex, which include *G. paniculata, G. refracta* Munro, and *G. venezuelae* Munro, appear to be adapted to open savanna or cerrado habitats that experience frequent fire (Veldman, 2008). These species generally have narrower foliage leaves than their forest congeners, with blade widths typically less than 2.5 cm. In addition, they are of relatively small stature, generally less than 10 m tall and 4 cm in diameter, and have culm leaves with subequal sheaths and deciduous blades rather than the much longer sheaths and persistent blades typical of their forest congeners (Shirasuna et al., 2020). *Guadua venezuelae* is easily distinguished from *G. paniculata* and *G. refracta* when in flower as it is the only member of the species complex to have pubescent pseudospikelets; *G. paniculata* and *G. refracta* are more difficult to differentiate, with *G. paniculata* originally described as having darker-colored and smaller pseudospikelets than *G. refracta*, as well as narrower, sharper foliage leaves (Munro, 1868; Shirasuna et al., 2020). *Guadua* species of forests and river habitats, which comprise the rest of the genus excluding the *G. paniculata* species complex, typically have foliage leaves with blades over 2.5 cm wide and culms that are taller and larger in diameter than species of savannas, excluding some species with climbing habits. The unusual branching flowering structures of *Guadua,* known as pseudospikelets (Londoño and Clark, 2002), also show general macromorphological differences based on habitat. Excluding a few species with few fertile florets (Olivier and Poncy, 2009), the pseudospikelets of forest species of *Guadua* tend to be large, often over 4 cm long, while pseudospikelets of savanna species of *Guadua* are less than 4 cm long (Shirasuna et al., 2020).

Although the presence or absence of various micromorphological features has been demonstrated to have evolutionary implications, few studies analyze these features in isolation from quantitative measurements of other features, such as trichome and stomatal index, that are relatively plastic and frequently affected by habitat (Ashfaq et al., 2019). Studies of floral micromorphology are largely descriptive, with the mathematical analyses (e.g., Kraaij and van der Kooi, 2020) focused on measurements such as cell height rather than presence or absence of different features. In this study, we instead sought to use quantitative methods to compare the micromorphological patterns of presence or absence to determine whether differences seen in the macromorphology of *Guadua* species from forest, river, and savanna habitats correspond to differences in micromorphology of leaves. Due to the differing environmental pressures such as light and water availability in forest, river, and savanna habitats, we predicted that the foliage leaf micromorphology of these groups of *Guadua* species would differ significantly. We also examined whether there were significant differences in foliar micromorphology based on the habits of *Guadua* by categorizing species by primary habit type: tall and erect, leaning and/or climbing, or small and arching. As *Guadua* flowering structures also differ macromorphologically between forest and savanna-dwelling species and may have evolved different traits to protect floral parts under different environmental conditions, we additionally hypothesized that we would see significant differences between patterns of floral bract micromorphology in *Guadua* lemmas and paleas based on habitat or habit.

2. Materials and methods

2.1. Scanning electron microscopy (SEM)

Images used for analysis of foliage leaf micromorphology were obtained using scanning electron microscopy (SEM) on dried herbarium specimens from A, ISC, MO, and US (Thiers, 2025), as recorded in the specimen data in Supplemental File 1. Specimens were sampled from as many of the 37 described species as possible, aiming for at least ten specimens per species for foliage leaves and five specimens per species for floral bracts, each from a variety of populations across species range. Due to a historical lack of collection and the rarity of flowering in some *Guadua*, some species were represented by fewer specimens. A total of 184 *Guadua* specimens belonging to 28 described species and four putative species yet to be described were used for foliage leaf analysis, including 13 species that primarily live in forests, 12 primarily riparian or riverine species (generalized as “river” species), and three savanna species. A total of 87 specimens belonging to 24 described species and two putative species yet to be described were used for floral bract analysis, including 12 primarily forest species, 9 primarily river species, and three savanna species. Specimens belonging to species yet to be described were included in the analysis with habit and habitat assigned based on label information from the associated herbarium vouchers. Of the four foliage leaf specimens representing undescribed species, two were from forest habitats and two from river habitats, while two were small and arching, one leaning and/or climbing, and one tall and erect. Both floret specimens belonging to undescribed species had small arching habits, while one was from a forest habitat and the other from a river habitat.

Two rectangular pieces of foliage leaf with an area of about 1 cm^2 were cut for use for each set of images, with one piece used to image the abaxial surface of the leaf and the other used to image the adaxial surface. Sixty-seven leaf samples from ISC and US were imaged by Lynn G. Clark following the procedures of Clark (1990). Fourteen leaf samples

from A, ISC, and MO were imaged by Elizabeth K. McMurchie (EKM) using the procedures of McMurchie et al. (2022) with a Hitachi SU4800 field emission scanning electron microscope at the Iowa State University Roy J. Carver High Resolution Microscopy Facility, while 71 leaf samples were imaged using a slightly modified version of McMurchie et al. (2022) in which iridium was used to coat leaf material rather than platinum; both sets of leaves were also submerged in xylene three times to remove epicuticular wax, rather than the two periods of submergence used by McMurchie et al. (2022). To image floral bracts, we followed the procedure similar to that used by McMurchie et al. (2022) to image foliage leaves, but with a lemma and palea cut from the pseudospikelet and, once treated three times with xylene, affixed to the double-sided carbon tape on the cylindrical sample holders so that the lemma was flattened with the abaxial surface visible and the palea with one margin extended to the side so that the sulcus and abaxial margin were both visible. Twenty-one floral bract samples from ISC were coated with platinum while 40 from A, ISC, and MO were coated with iridium.

2.2. Data visualization and analysis

Using the SEM images of *Guadua* foliage leaves, we recorded the presence or absence of 45 micromophological features including simple, bifurcated, and branching papillae, different types of trichome, silica bodies of various shapes, and stomata seen on the abaxial and adaxial leaf surfaces, as listed in the data dictionary for foliage leaf micromorphology, Appendix A. We similarly recorded presence or absence of 35 micromorphological features on the abaxial surfaces of the lemmas and paleas, as listed in the data dictionary for floral bract micromorphology, Appendix B. Classification of micromorphological features mainly followed Ellis (1979), with the addition of shapes of silica bodies and papillae described in Cunha Santana (2017), as detailed in Appendices A and B. As in McMurchie et al. (2022), silica bodies that Ellis (1979) described as "dumbbell-shaped" were classified using the International Committee for Phytolith Taxonomy (ICPT) (2019) term "bilobate". Multicellular microhairs were counted as "bicellular microhairs" even if they appeared to potentially have three cells, such as the "mushroom" composites observed by Kalliola et al. (2019) in *G. sarcocarpa* Londoño & P.M.Peterson and *G. weberbaueri* Pilg. or tricellular microhairs observed in *G. lacerata* Ruiz-Sanchez & McMurchie by McMurchie et al. (2025), due to the frequency in which these hairs were observed with damage preventing enumeration of cells.

We conducted a broad survey of herbarium specimens to determine the most common habits and habitats for each studied species, as many individual specimens lack habitat and/or habit information. All specimens identified to species were categorized based on habit most common to the species, dividing species into three groups based on whether they were leaning and climbing, small arching, and tall and erect. We divided specimens identified to species into three habitat groups: savanna, forest, and river. For specimens of uncertain taxonomic placement, we assigned habit and habitat based on individual specimen label information.

Data were wrangled, visualized, and analyzed in R version 4.4.2 "Pile of Leaves" (R Core Team, 2024). Data wrangling and visualization involved the 'tidyverse' and 'readxl' R packages (Wickham et al., 2019; 2023; Wickham and Bryan, 2023).

To determine which micromorphological features were most strongly associated with each other, we analyzed the correlation between presence of each feature with that of all other features assessed and visualized the resulting correlation tables using 'ggcorrplot' (Kassambara, 2023; Kassambara and Patil, 2023). We noted some of the species-, habit- and habitat-specific micromorphological trends we observed and, for some features, made pie charts using the 'tidyverse' R package to visualize the percentage of specimens of different habitat types to show these features (Wickham et al., 2019; 2023). We then converted the floret and foliage leaf binary datasets into distances using simple matching coefficient (Sokal and Michener, 1958) and created individual-level distance matrices. From these distance matrices, we used the R packages 'ade4' (Bougeard and Dray, 2018; Chessel et al., 2004; Dray et al., 2007; Dray and Dufour, 2007; Thioulouse et al., 2018) and 'vegan' (Oksanen et al., 2025) to conduct principal coordinates analysis (PCoA) (Gower, 1966) to visualize how micromorphology of different *Guadua* voucher specimens appeared to group based on the two axes of the PCoA, with specimens categorized by habitat and habit the species is associated with, as well as by species. In addition to 'ade4' and 'vegan', 'tidyverse' was also necessary for visualization of the first two axes in the PCoA. Colors used in visualization of the PCoA were chosen using ColorBrewer 2.0 (Brewer et al., 2013). We additionally ran a scree plot (Cattell, 1966) to show the percent variation explained among each PCoA axis.

Although a recent phylogeny is available from which a phylogenetic comparative analysis could be made, this phylogeny excludes most of the taxa we examined in this study (Ruiz-Sanchez et al., 2025). Thus, we used a nonphylogenetic approach to evaluate within- and among-species trends in *Guadua* micromorphology. To analyze whether there were significant differences in the micromorphology of *Guadua* foliage leaves or floral bracts of different habits (small arching, tall and erect, or leaning and climbing) or habitats (forest, savanna, or river), we conducted factorial multivariate analysis of variance (MANOVA) using randomization of null model residuals in a procedure with 1000 permutations on the binary data with the R packages 'RRPP' and 'geomorph' (Adams et al., 2025; Baken et al., 2021; Collyer and Adams, 2024; 2018). We used 'RRPP' to generate pairwise comparisons of habit and habitat types using the distance between vectors statistic to explore whether there were significant differences in means and variances between different habits and habitats. To determine which model (habit or habitat alone, both habitat and habit ignoring potential interaction, or habitat and habit considering the interaction of these two factors) best fit the data, we used the log likelihood model comparison method from the 'RRPP' package with the 'pander' package (Daróczi and Tsegelskyi, 2022) to find the model with the best fit based on lowest Akaike Information Criterion (AIC) value (Akaike, 1973).

3. Results

3.1. Observations of characteristics

In all specimens of the three species belonging to the savanna-dwelling *G. paniculata* species complex, simple papillae were found on the abaxial foliage leaf surface in both the stomatal and interstomatal regions (Figs. 1a, 2). Bifurcated and branching papillae were additionally found in the stomatal and interstomatal zones of the abaxial foliage leaf surfaces of most savanna *Guadua* (Figs. 1a, 2), although these types of papillae were never found in the absence of simple papillae; branching and bifurcating papillae on the abaxial surface were lacking in one *G. paniculata* specimen, and several *G. venezuelae* specimens lacked abaxial branching papillae in the stomatal zones, interstomatal zones, or both. Simple papillae were relatively uncommon on the abaxial surfaces of *Guadua* from forest and river habitats (Figs. 1b, 2), although they were commonly found in specimens identified as the river species *G. ciliata* Londoño & Davidse and *G. superba* Huber, as well as on one of the two examined specimens of *G. virgata* (Trin.) Rupr. and the forest-dwelling *G. weberbaueri* and *G. sarcocarpa.* Bifurcated and branching papillae were rare on the abaxial surface of foliage leaves of non-savanna species, and commonly found only in *G. weberbaueri* and *G. sarcocarpa,* as well as one of the two specimens of *G. virgata* (*Clark & Morel 710*). Prickles, bicellular microhairs, and macrohairs were found on the abaxial surfaces of most specimens, but macrohairs appeared somewhat less common in species found in forested or river habitats (Figs. 1a, 2). In the intercostal zone of the abaxial foliage leaf surface, smooth saddle-shaped silica bodies were found in many savanna-dwelling species but were typically absent in species that primarily live in forests and along rivers, with the exception of the small arching

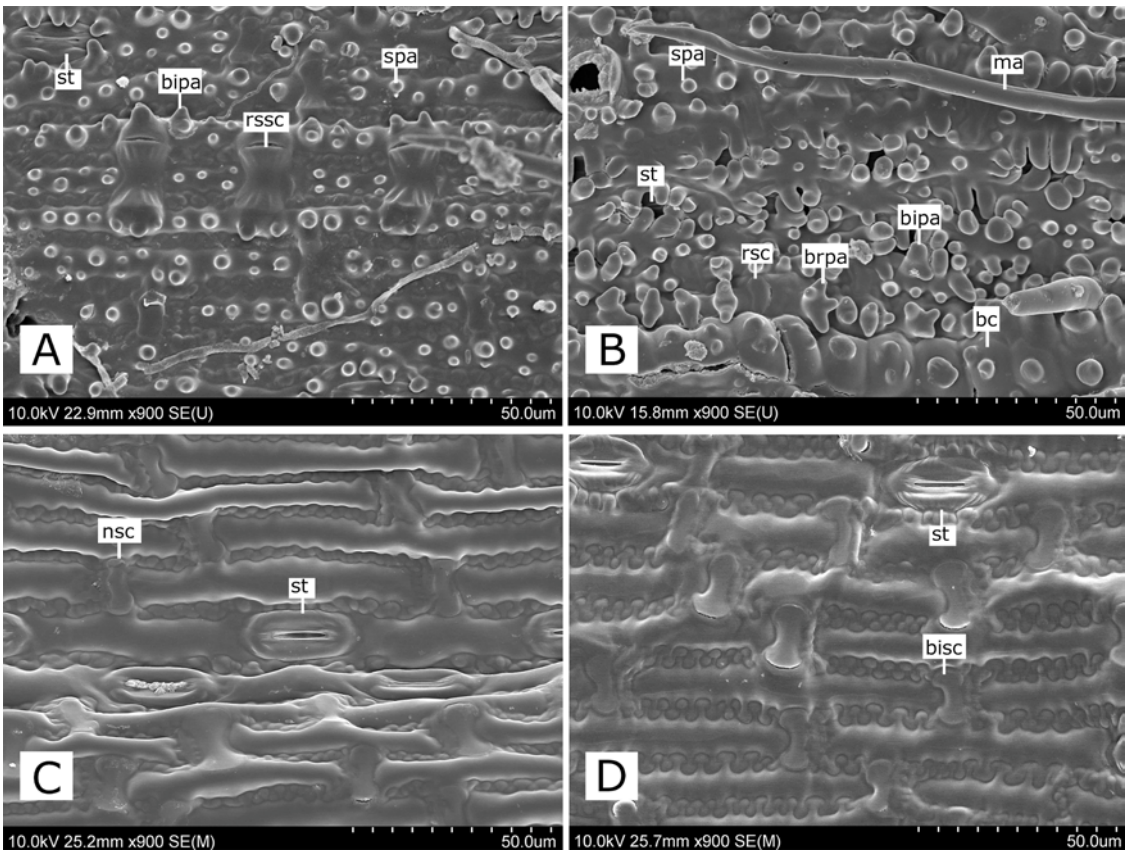


Fig. 1. Foliage leaf micromorphology of *Guadua* from SEM. Abaxial surface (A, C), adaxial surface (B, D). A *G. refracta* (*Irwin & Soderstrom 5696*). B *G. refracta* (*Irwin & Soderstrom 6331*). C-D *G. chacoensis* (*Davidse et al. 11233*). Abbreviations: bc = bulliform cell; bipa = bifurcated papilla; bisc = vertical bilobate silica body; brpa = branched papilla; ma = macrohair; nsc = nodular silica body; rssc = ridged saddle-shaped silica body; spa = simple papilla; st = stomatal complex.

species *G. longifolia* (E.Fourn.) R.W.Pohl, *G. virgata,* and *G. trinii* (Nees) Rupr. (Figs. 1a–3). Nodular silica bodies were not found in the abaxial leaf surfaces of any specimens of savanna-dwelling but were commonly found in specimens of species from forests and less commonly in specimens from river habitats (Figs. 1b, 2). Similarly, bilobate silica bodies were common in the costal zone of the abaxial leaf surface in forest and river species but typically absent from most specimens from savannas (Figs. 1a, b, 2). However, on the abaxial leaf surface, ridged saddle-shaped silica bodies were found primarily in costal zones of species that grow primarily in savannas, though they were also found in a small number of specimens from forest and river-dwelling species (Figs. 1a, 2).

Simple papillae were always present on the long cells of the adaxial stomatal region in savanna species (Figs. 1c, 3) and, while present over half of the time in river species, typically absent from forest species (Figs. 1d, 3). Bifurcated and branching papillae were sometimes also found when simple papillae were present (Fig. 1c). Bicellular microhairs were typically present on the adaxial surface of foliage leaves of savanna-dwelling species, but often absent from the adaxial surface of leaves of forest and river species (Figs. 2, 3). Macrohairs were less common than bicellular microhairs on the adaxial surface of foliage leaves of savanna species (Figs. 1c, 3) and typically absent from the adaxial surface of foliage leaves of species from forest or river habitats, except in small arching species, where they were seen in the river species *G. guzmanii* Ruiz-Sanchez, Anaya & Londoño and *G. longifolia*, as well as *G.* aff. *virgata*, and the forest species *G. trinii.* Similar patterns in silica body shape were seen on the adaxial surface of foliage leaves as on the adaxial surface (Fig. 1c, d), but smooth saddle-shaped silica bodies were less common on the adaxial surface of foliage leaves of species from all habitats than on the abaxial surface (Figs. 1d–3).

In floral bracts, fewer micromorphological features differentiated species that predominantly live in savannas from those that primarily live in forests or river habitats than in foliage leaves. Lemmas of savanna species always had simple papillae (Fig. 4a), but presence of simple papillae on lemmas of forest and river species was variable (Figs. 4b, 5). All paleas of savanna species had simple papillae present (Fig. 4c), while only one specimen of a forest-dwelling species, tentatively identified as *G. macclurei* R.W.Pohl & Davidse (*Pohl & Erickson 12700*), had papillae on its paleas (Fig. 5). Bilobate horizontally elongated silica bodies were absent from all paleas of specimens belonging to savanna species, but were present in slightly more than half of the specimens from forest species and slightly fewer than half of the specimens from river species (Figs. 4d, 5).

3.2. Correlation of micromorphological features

In both foliage leaves and floral bracts, the strongest correlations were found between the presence of simple and branching and bifurcated papillae, as well as presence of all types of papillae in stomatal and interstomatal zones (Figs. C.1, C.2), as branching and bifurcated papillae were only found in the presence of simple papillae. Simple papillae were observed to be more common in all species found to have papillae than their branching and bifurcated counterparts on foliage leaves and floral bracts.

For foliage leaves, there were typically fairly high levels of correlation between similar silica body shapes in the costal and intercostal zones and adaxial and abaxial surfaces of the leaf (Fig. C.1). The presence of ridged saddle-shaped silica bodies in the costal zone was highly correlated with the presence of papillae (e.g., on the adaxial leaf surface, $r = 0.69$ for the presence of papillae in the interstomatal zone and the presence of ridged saddle-shaped silica bodies in the costal zone)

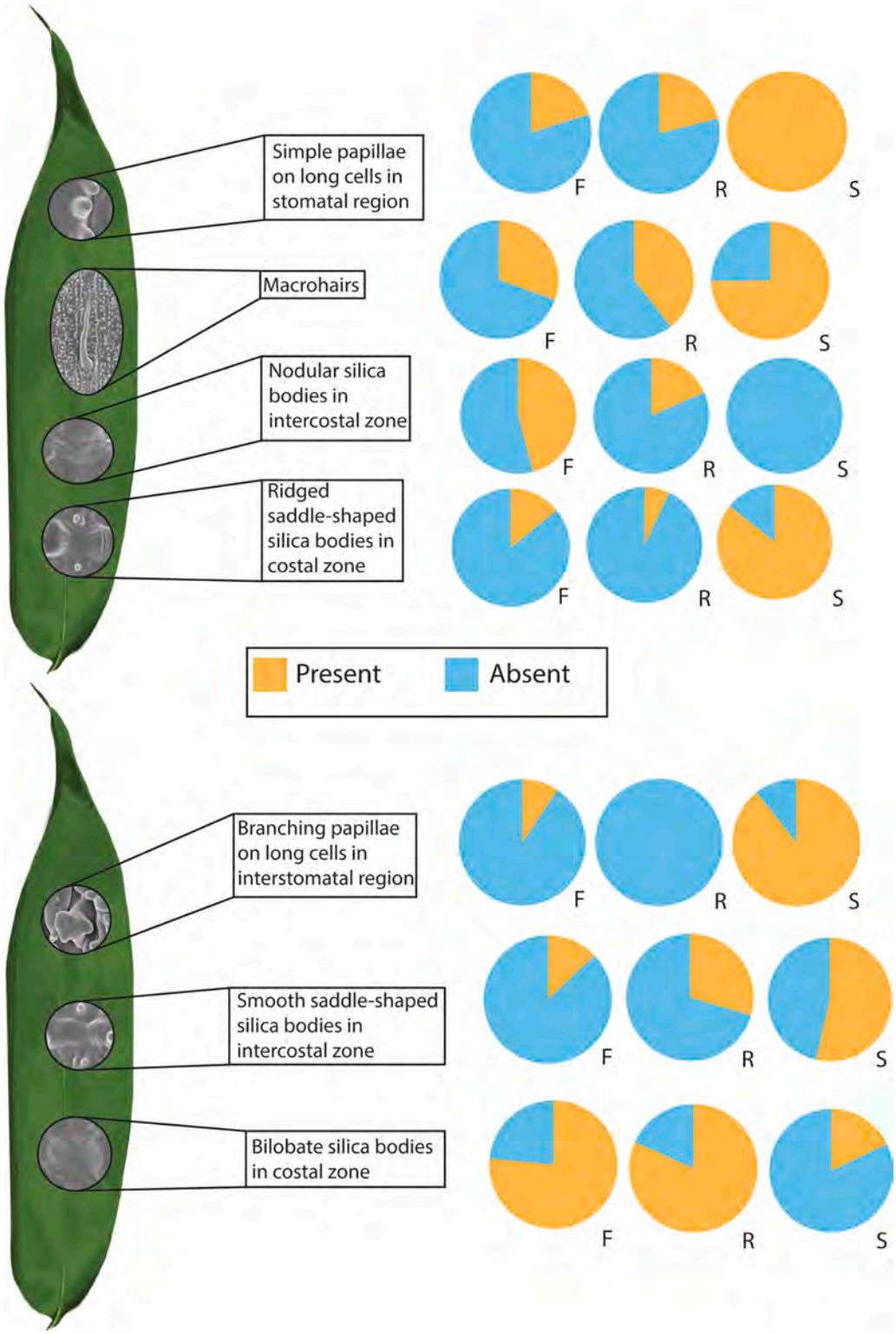


Fig. 2. Percentage of individual *Guadua* specimens belonging to forest, river, and savanna species in which selected micromorphological features are present or absent on abaxial foliage leaf surfaces. Pie charts for forest species are designated with the letter "F", river species with the letter "R" and savanna species with the letter "S".

(Fig. C.1). A low to moderate negative correlation was typical for presence of papillae and bilobate silica bodies (e.g., on the adaxial leaf surface $r = -0.60$ for the presence of branched papillae in the interstomatal zone and the presence of bilobate silica bodies in the costal zone) (Fig. C.1). Additionally, the presence of bilobate silica bodies in costal and intercostal zones and ridged saddle-shaped silica bodies in the costal zones were moderately negatively correlated (e.g., on the adaxial surface $r = -0.54$ for bilobate silica bodies in the intercostal zone or $r = -0.66$ for bilobate silica bodies in the costal zone when compared with ridged saddle-shaped silica bodies in the costal zone) (Fig. C.1). Low positive correlations were also seen between the presence of bicellular microhairs on the adaxial surface of the leaf and presence of papillae and

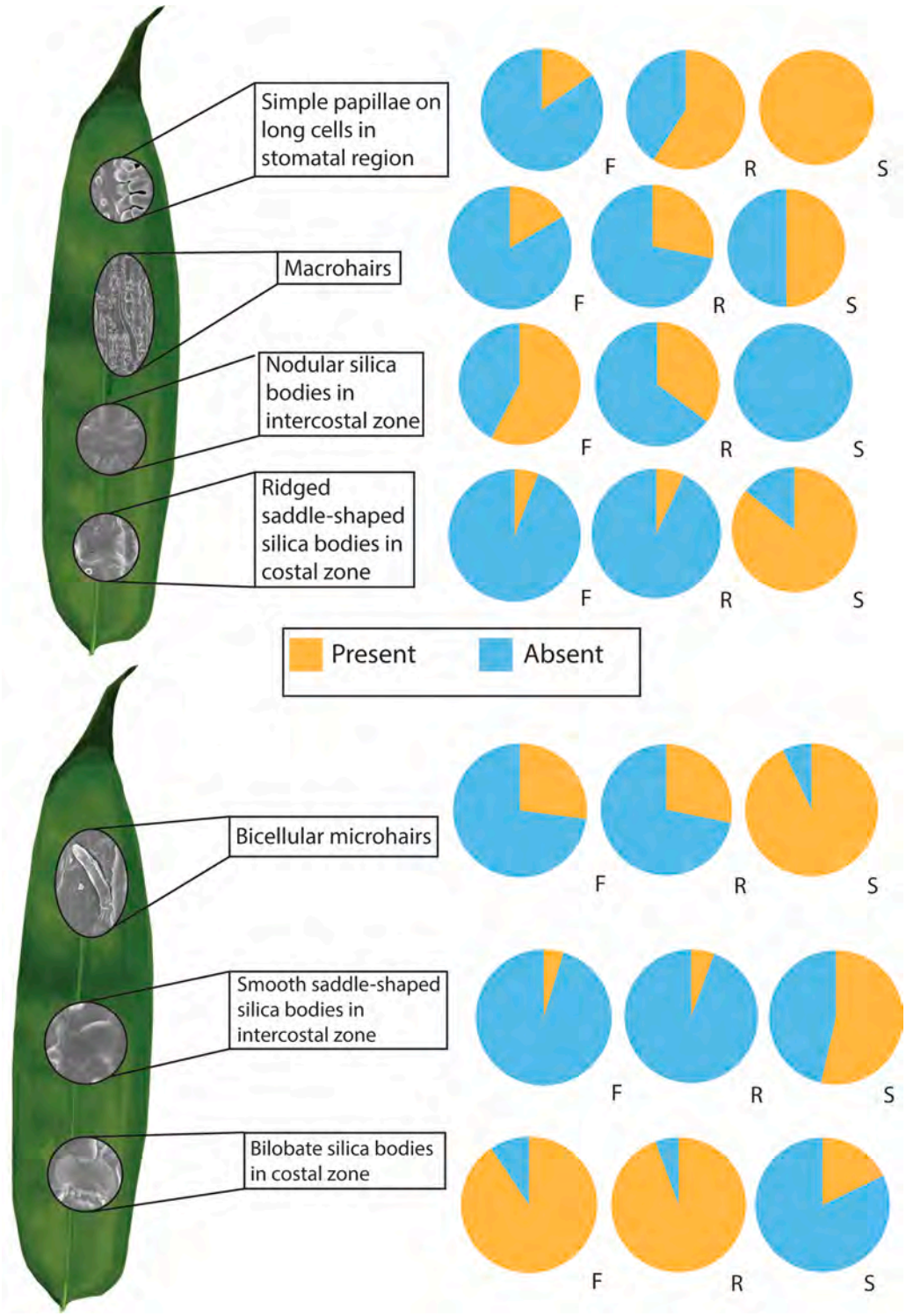


Fig. 3. Percentage of individual *Guadua* specimens belonging to forest, river, and savanna species in which selected micromorphological features are present or absent on adaxial foliage leaf surfaces. Pie charts for forest species are designated with the letter "F", river species with the letter "R" and savanna species with the letter "S".

ridged saddle-shaped silica bodies (e.g., $r = 0.49$ for bicellular microhairs and simple papillae in the interstomatal zone and $r = 0.48$ for bicellular microhairs and ridge saddle-shaped silica bodies in the intercostal zone on the adaxial leaf surface) (Fig. C.1).

For floral bracts, correlations were typically more subtle. Specimens with papillose paleas tended to lack bilobate silica bodies, although these silica bodies were elongated in the horizontal direction, rather than the vertical direction as seen in all silica bodies of the leaves ($r = -0.38$ for simple and bifurcated papillae and $r = -0.33$ for branched papillae when correlated with horizontally elongated bilobate silica bodies) (Fig. C.2). Saddle-shaped silica bodies on the lemma were moderately negatively correlated with rondel-shaped silica bodies on the lemma ($r = -0.49$); these two shapes essentially represented opposite extremes in silica body shape (Fig. C.2). Similar silica body

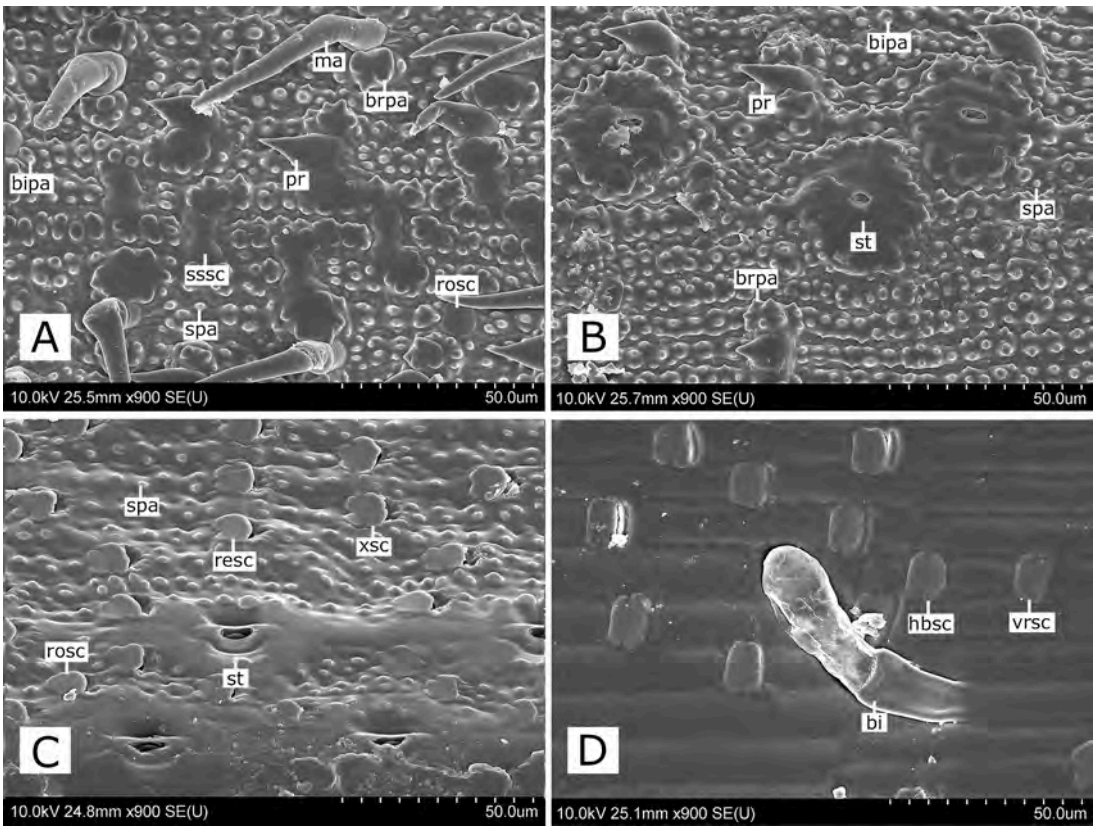


Fig. 4. Floral bract micromorphology of *Guadua* from SEM. Abaxial lemma surface (A, C), abaxial palea surface (B, D). A-B *G. venezuelae* (*Davidse & Huber 22496*). C-D *G. amplexifolia* (*Allen 4039*). Abbreviations: bi = bicellular microhair; bipa = bifurcated papilla; brpa = branched papilla; hbsc = horizontal bilobate silica body; ma = macrohair; pr = prickle; resc = reniform silica body; rosc = rondel-shaped silica body; spa = simple papilla; sssc = smooth saddle-shaped silica body; st = stomatal complex; vrsc = vertically elongated rounded silica body; xsc = cross-shaped silica body.

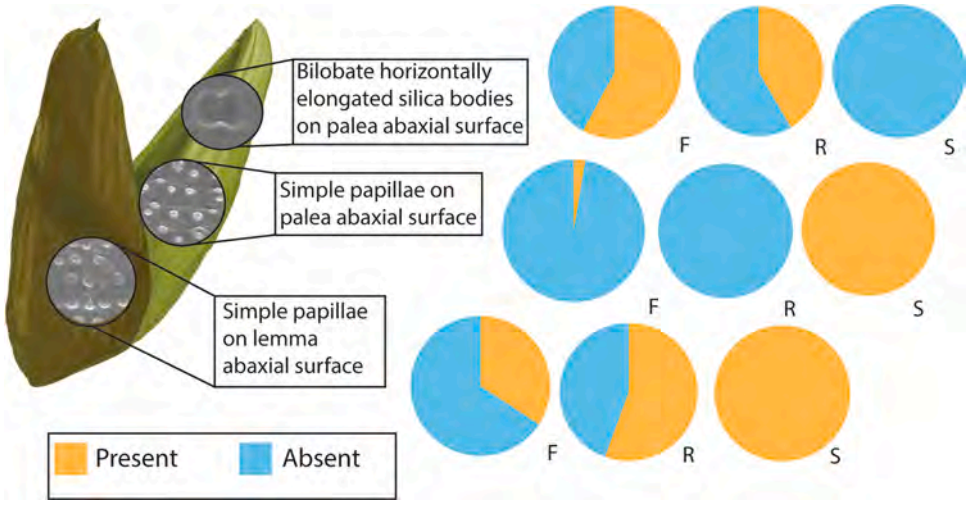


Fig. 5. Percentage of individual *Guadua* specimens belonging to species from forest, river, or savanna habitats in which selected micromorphological features are present or absent on lemmas or paleas. Pie charts for forest species are designated with the letter "F", river species with the letter "R" and savanna species with the letter "S".

shapes often had low but positive correlations; for example, bilobate horizontally elongated silica bodies on the lemma showed low positive correlations with horizontal reniform ($r = 0.37$) and cross-shaped silica bodies ($r = 0.40$) (Fig. C.2). Presence of branched papillae and macrohairs on the lemma also showed a low positive correlation ($r = 0.47$) and presence of prickles and bicellular microhairs on the lemma were positively correlated ($r = 0.61$) (Fig. C.2).

3.3. Principal coordinates analysis

Plots of principal coordinates analysis (PCoA) based on habitat for individual foliage leaf specimens showed that along the first two axes, micromorphology of specimens from savanna species was differentiated from that of specimens from forest and river species, despite some overlap (Fig. 6). Foliage leaf micromorphology of forest and river

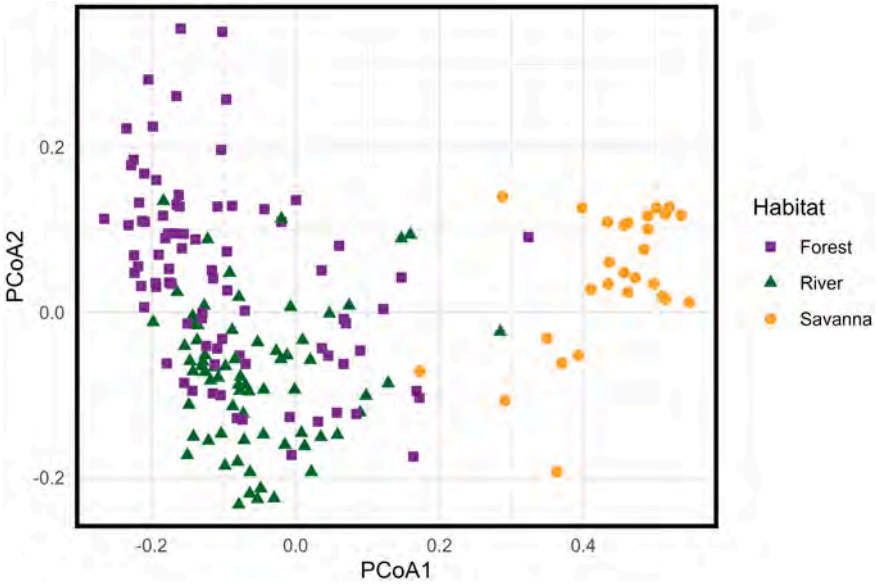


Fig. 6. Principal coordinates analysis scatterplot of 184 *Guadua* specimens belonging to 28 described species (13 primarily from forests, 12 primarily river, and 3 savanna species) and four putative species (two from forests and two river) based on a distance matrix obtained using the simple matching coefficient for the presence or absence of 45 foliage leaf micromorphological structures. Specimens are categorized by habitat: forest, river, or savanna.

species was less differentiated than micromorphology of savanna species from these two groups, although there appeared to be some differentiation between specimens of species from forest and river habitats (Fig. 6). Less differentiation was seen in foliage leaf micromorphology between specimens from species with small arching habits versus those with leaning/climbing or tall and erect habits (Fig. 7). Although specimens of many species appeared to group together, the tightness of these groupings varied between individual species (Fig. 8). The scree plot of the eigenvalues of principal coordinates involved in the analysis of foliage leaf micromorphology indicated that the first and second principal coordinate axes are responsible for most of the variation seen in the PCoA, with a steep drop in variation between these two coordinates (Fig. C.3). An "elbow" is seen at approximately the sixth coordinate axis, indicating that no more than the first five principal coordinates are likely significant (Fig. C.3).

Principal coordinates analysis based on habitat of individual floret specimens demonstrated strong differentiation between florets of specimens from species commonly found in savannas and those found in forest or river habitats (Fig. 9). Negligible differentiation was seen between specimens from forest and river habitats (Fig. 9). Between different habits, there was relatively little differentiation between floret specimens with small arching habits and those with tall and erect or leaning/climbing habits, despite a cluster of small arching specimens at low values along axes 1 and 2 (Fig. 10). As with foliage leaf specimens, floral bract specimens tended to group by species, with tightness of the grouping varying across different species (Fig. 11). Although there is a steep drop after the first principal coordinate axis in the scree plot of the eigenvalues of the principal coordinates involved in the analysis of floret

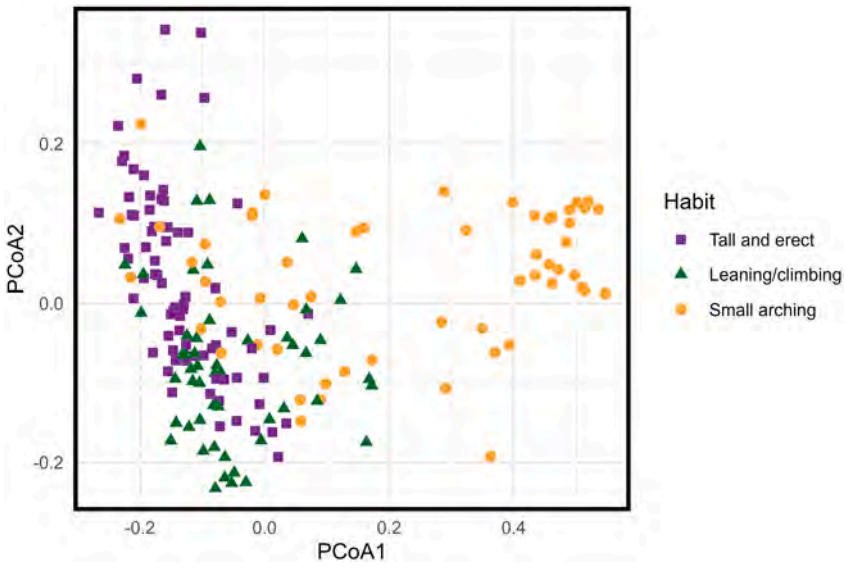


Fig. 7. Principal coordinates analysis scatterplot of 184 *Guadua* specimens belonging to 28 described species (13 tall and erect, seven leaning and/or climbing, and eight small and arching) and four putative species (one tall and erect, one leaning and/or climbing, and two small and arching) based on a distance matrix obtained using the simple matching coefficient for the presence or absence of 45 foliage leaf micromorphological structures. Specimens are categorized by habit: tall and erect, leaning/climbing, or small arching.

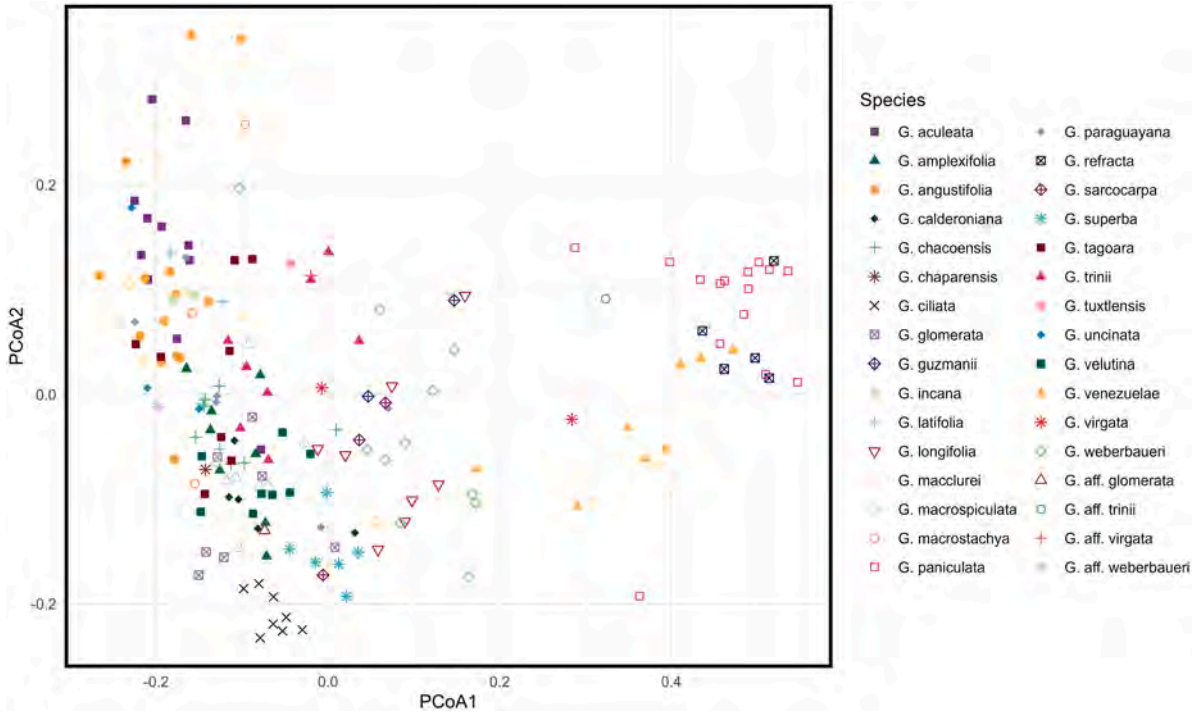


Fig. 8. Principal coordinates analysis scatterplot of 184 *Guadua* specimens belonging to 28 described species and four putative species based on a distance matrix obtained using the simple matching coefficient for the presence or absence of 45 foliage leaf micromorphological structures. Specimens are categorized by species identification.

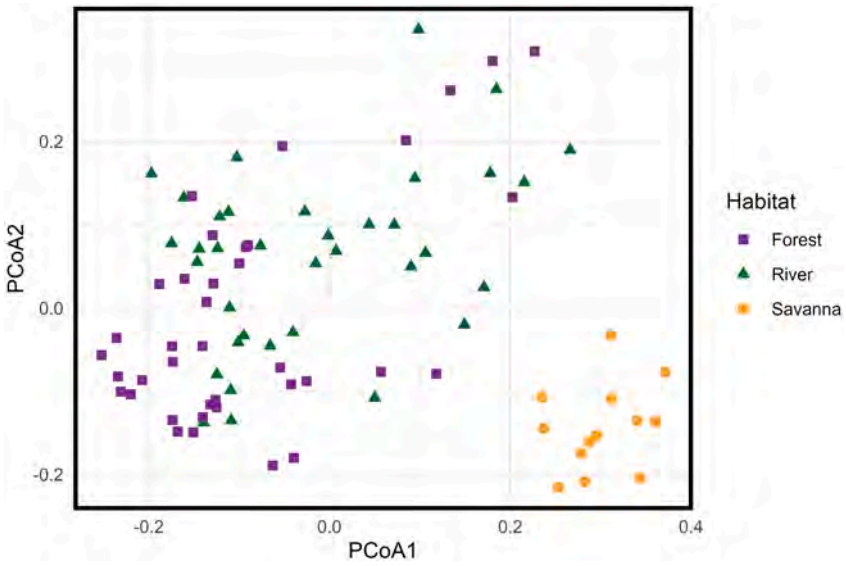


Fig. 9. Principal coordinates analysis scatterplot of 87 *Guadua* specimens belonging to 24 described species (12 primarily from forests, nine primarily river, and three savanna species) and two putative species (one from forests and one river) based on a distance matrix obtained using the simple matching coefficient for the presence or absence of 35 floral bract micromorphological structures. Specimens are categorized by habitat: forest, river, or savanna.

micromorphology, principal coordinate axes 1 and 2 are responsible for less than half of the variation in the PCoA (Fig. C.4). The "elbow" is seen at approximately the eighth coordinate axis, suggesting that at least the first seven principal coordinates are likely significant (Fig. C.4).

3.4. Factorial multivariate analyses of variance and model comparison

Model comparison using residual randomization indicated that for both foliage leaves and floral bracts, the model that best fit our data included habit, habitat, and the interaction between these two factors (AIC = −27,290 for foliage leaves and AIC = −8,492 for florets), followed by the model including habit and habitat without consideration of interaction (AIC = −27,229 for foliage leaves and AIC = −8,455 for florets). Models considering habitat alone (AIC = −27,078 for foliage leaves and AIC = −8,424 for florets) fit our data better than models that only considered habit (AIC = −26,916 for foliage leaves and AIC = −8,249 for florets).

Using randomization of null model residuals with 1000 permutations to run factorial MANOVA on the binary presence/absence data, we found significant differences in the micromorphology of the foliage

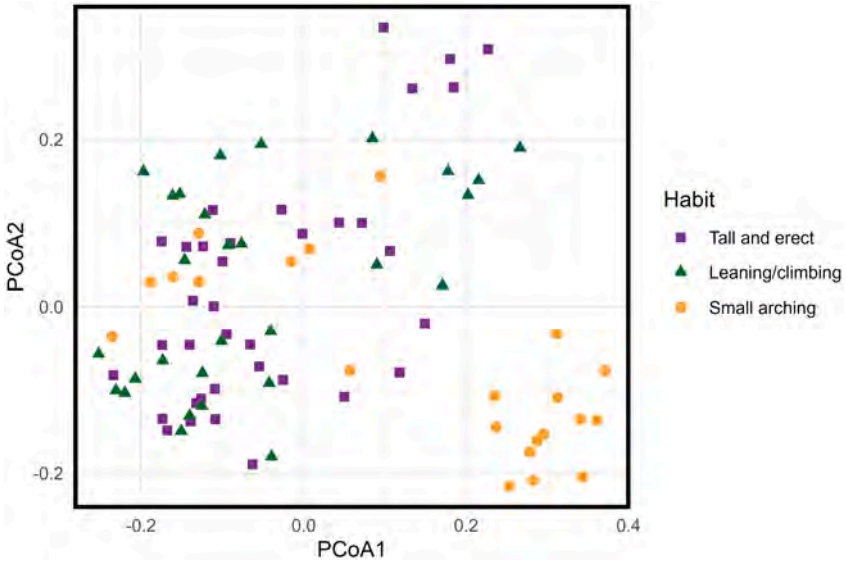


Fig. 10. Principal coordinates analysis scatterplot of 87 *Guadua* specimens belonging to 24 described species (11 tall and erect, seven leaning and/or climbing, and six small and arching) and two putative species (both leaning and/or climbing) based on a distance matrix obtained using the simple matching coefficient for the presence or absence of 35 floral bract micromorphological structures. Specimens are categorized by habit: tall and erect, leaning/climbing, or small arching.

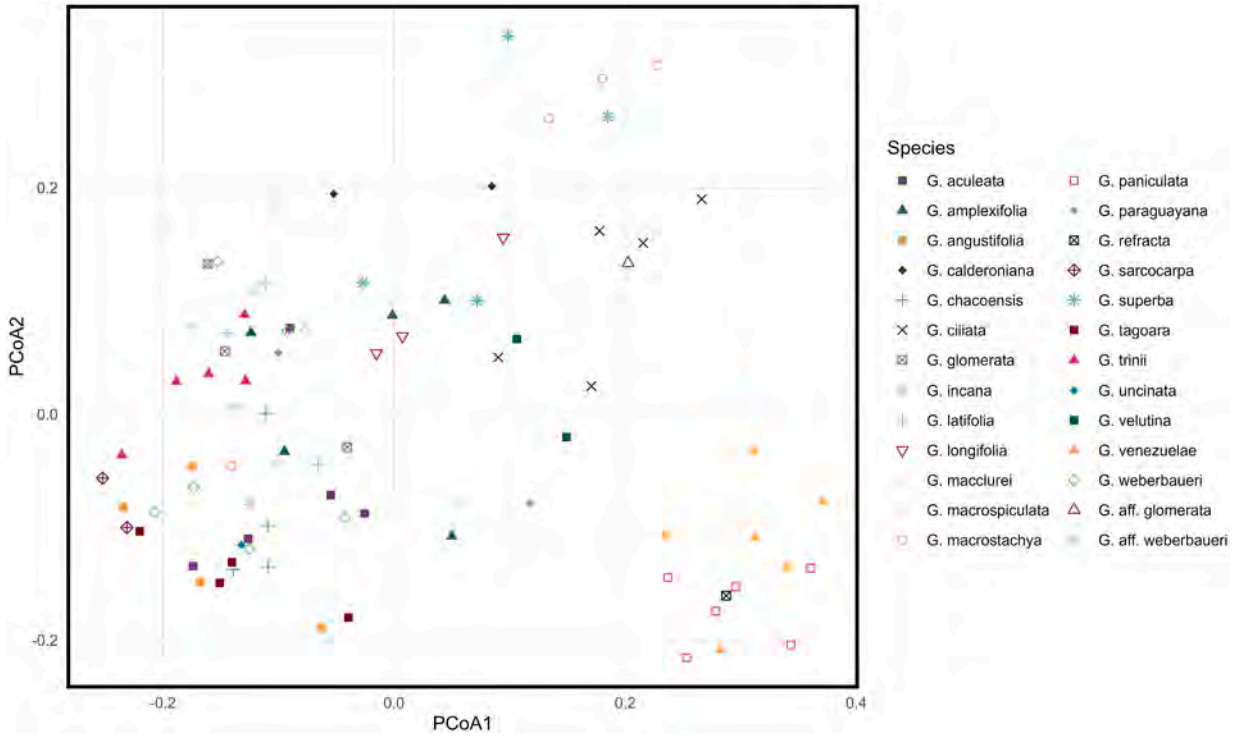


Fig. 11. Principal coordinates analysis scatterplot of 87 *Guadua* specimens belonging to 24 described species and two putative species based on a distance matrix obtained using the simple matching coefficient for the presence or absence of 35 floral bract micromorphological structures. Specimens are categorized species identification.

leaves of specimens belonging to species associated with different habitats, with large permutation-based effect sizes ($F = 17.103$, $Z = 9.330$, $p = 0.001$) and habits ($F = 22.065$, $Z = 7.339$, $p = 0.001$). The interaction term between habit and habitat was significant ($F = 4.104$, $Z = 8.165$, $p = 0.001$). Pairwise comparisons of micromorphology of foliage leaves of specimens from species associated with different habitat types revealed that there were significant differences between all studied habitats, with the smallest effect size in between specimens of forest and river species ($d = 0.162$, $Z = 4.062$, $p = 0.001$, where d is the difference between least-squares means), and the greatest effect size between specimens of forest and savanna species ($d = 0.539$, $Z = 5.674$, $p = 0.001$), closely followed by the effect size between specimens of river and savanna species ($d = 0.515$, $Z = 5.500$, $p = 0.001$). Similarly, pairwise comparisons of habits indicated that there were significant differences between all habit categories. The greatest effect size was between specimens of tall erect and small arching species ($d = 0.358$, $Z = 0.859$, $p = 0.001$), followed by the effect size between specimens of leaning and climbing species compared to small arching species ($d = 0.309$, $Z = 5.269$, $p =$

0.001), with the smallest effect size associated with the difference between specimens of leaning and climbing species ($d = 0.171$, $Z = 3.565$, $p = 0.001$).

When we explored the interaction between habitat and habit in micromorphology of foliage leaves using pairwise comparison of specimens for species of different habitat-habit groups, we found that there were significant differences between several habitat-habit groups. Of particular interest, we found that foliage leaf specimen groups showing significant pairwise differences in the distance between their means included forest-tall erect species compared to forest leaning-climbing species ($d = 0.250$, $Z = 3.091$, and $p = 0.001$), forest leaning/climbing species compared to river leaning/climbing species ($d = 0.227$, $Z = 2.096$, $p = 0.015$), forest small arching species compared to river small arching species ($d = 0.268$, $Z = 2.549$, $p = 0.006$), river tall erect compared to river small arching species ($d = 0.277$, $Z = 2.107$, $p = 0.019$), and river leaning/climbing species compared to river small arching species ($d = 0.298$, $Z = 3.262$, $p = 0.002$) (Table 1).

An even greater number of habitat-habit groups associated with foliage leaf specimens showed significant pairwise differences in within-group variation of their micromorphology. Significant pairwise differences of interest included those between forest tall erect species compared to forest leaning/climbing species ($d = 0.022$, $Z = 1.181$, $p = 0.027$), forest leaning/climbing species compared to river leaning/climbing species ($d = 0.056$, $Z = 3.673$, $p = 0.001$), forest small arching species compared to savanna small arching species ($d = 0.025$ $Z = 1.672$, $p = 0.040$), river tall and erect species compared to river leaning/climbing species ($d = 0.022$ $Z = 1.753$, $p = 0.036$), and river leaning/climbing species compared to river small arching species ($d = 0.042$, $Z = 2.515$, $p = 0.003$) (Table 2). Other pairwise comparisons of within-group variation of other habit-habitat groups did not show significant differences (Table 2).

We similarly ran factorial MANOVA using randomization of null model residuals with 1000 permutations for our binary presence/absence data of micromorphological features of floral bracts. As with foliage leaves, we found that there were significant differences in the micromorphology of floral bracts of species from different habitats ($F = 5.188$, $Z = 7.755$, $p = 0.001$) and habits ($F = 4.464$, $Z = 5.782$, $p = 0.001$), as well as in the interaction term between habit and habitat ($F = 2.0118$, $Z = 3.1181$, $p = 0.002$). Pairwise comparisons of habitat for floral bracts mirrored those of foliage leaves, indicating that there were significant differences between the micromorphology of floral bracts of specimens from all different habitat types. The greatest effect size was seen between specimens of species from river and savanna species ($d = 0.396$, $Z = 6.628$, $p = 0.001$), followed by specimens of forest and savanna species ($d = 0.4248$, $Z = 6.032$, $p = 0.001$). The smallest effect size was seen between floral bract specimens of forest and river species ($d = 0.149$, $Z = 2.535$, $p = 0.007$). Unlike in foliage leaves, pairwise comparisons revealed no significant difference in floral bract micromorphology of specimens from tall and erect species compared to leaning and climbing species ($d = 0.128$, $Z = 1.450$, $p = 0.082$). There were, however, significant differences in the floral bract micromorphology of specimens from small arching species compared to specimens of tall and erect species ($d = 0.248$, $Z = 4.869$, $p = 0.001$) and specimens of leaning and climbing species ($d = 0.273$, $Z = 5.179$, $p = 0.001$).

In contrast to foliage leaf specimens, when we investigated the effect of the interaction between habit and habitat on micromorphology of floral bracts by running pairwise comparisons of specimens from different habit-habitat groups, we found that only one comparison, forest small arching species compared to river small arching species showed significant differences in the distance between least-squares means ($d = 0.436$, $Z = 2.389$, $p = 0.011$) (Table 3). Far more significant differences were found in pairwise comparisons of floral bract specimens from different habit-habitat groups when comparing within-group variation. Within-group variation differed significantly between floral bract specimens of several groups, including between river tall and erect species compared to river small arching species ($d = 0.080$, $Z = 2.375$, $p = 0.007$) and river leaning/climbing species compared to river small arching species ($d = 0.065$, $Z = 1.862$, and $p = 0.019$), among

Table 1
Results of pairwise comparisons based on the distance between least-squares means of foliage leaf specimens belonging to species of *Guadua* from different habitat and habit categories. Distance, effect size, and p-value are bolded for comparisons found to have significant differences in distance between least-squares means.

d (distance between least-squares means)	Forest-tall erect	Forest-leaning/climbing	Forest-small arching	River-tall erect	River-leaning/climbing	River-small arching	Savanna- small arching
Forest-tall erect	–	–	–	–	–	–	–
Forest-leaning/climbing	**0.250**	–	–	–	–	–	–
Forest-small arching	0.186	0.605	–	–	–	–	–
River-tall erect	0.204	0.178	0.189	–	–	–	–
River-leaning/climbing	0.275	**0.227**	0.243	0.181	–	–	–
River-small arching	**0.357**	0.299	**0.268**	**0.277**	**0.298**	–	–
Savanna-small arching	0.605	0.484	0.520	0.542	**0.566**	0.427	–

Z (effect size)	Forest-tall erect	Forest-leaning/climbing	Forest-small arching	River-tall erect	River-leaning/climbing	River-small arching	Savanna- small arching
Forest-tall erect	–	–	–	–	–	–	–
Forest-leaning/climbing	**3.091**	–	–	–	–	–	–
Forest-small arching	−1.582	−1.316	–	–	–	–	–
River-tall erect	1.511	−0.951	−1.841	–	–	–	–
River-leaning/climbing	−0.946	**2.096**	−1.301	−0.507	–	–	–
River-small arching	**1.793**	1.482	**2.549**	**2.107**	**3.262**	–	–
Savanna-small arching	0.571	−2.023	1.236	−0.915	**2.143**	−1.603	–

p (p-value obtained with 1000 permutations)	Forest-tall erect	Forest-leaning/climbing	Forest-small arching	River-tall erect	River-leaning/climbing	River-small arching	Savanna- small arching
Forest-big erect	–	–	–	–	–	–	–
Forest-leaning/climbing	**0.001**	–	–	–	–	–	–
Forest-small arching	0.941	0.914	–	–	–	–	–
River-big erect	0.071	0.835	0.967	–	–	–	–
River-leaning/climbing	0.829	**0.015**	0.912	0.707	–	–	–
River-small arching	**0.033**	0.075	**0.006**	**0.019**	**0.002**	–	–
Savanna-small arching	0.275	0.976	0.115	0.821	**0.015**	0.947	–

Table 2
Results of pairwise comparisons based on within-group variation from foliage leaf specimens belonging to species of *Guadua* from different habitat and habit categories. Within-group variation, effect size, and p-value are bolded for comparisons found to have significant differences in distance between least-squares means.

d (within-group variation)	Forest-tall erect	Forest-leaning/ climbing	Forest-small arching	River-tall erect	River-leaning/ climbing	River-small arching	Savanna- small arching
Forest-tall erect	–	–	–	–	–	–	–
Forest-leaning/ climbing	**0.022**	–	–	–	–	–	–
Forest-small arching	0.015	0.007	–	–	–	–	–
River-tall erect	0.012	**0.033**	**0.026**	–	–	–	–
River-leaning/climbing	**0.034**	**0.056**	**0.049**	**0.022**	–	–	–
River-small arching	0.008	0.014	0.007	0.019	**0.042**	–	–
Savanna-small arching	0.011	**0.032**	**0.025**	0.001	**0.023**	0.018	–

Z (effect size)	Forest-tall erect	Forest-leaning/ climbing	Forest-small arching	River-tall erect	River-leaning/ climbing	River-small arching	Savanna- small arching
Forest-tall erect	–	–	–	–	–	–	–
Forest-leaning/ climbing	**1.181**	–	–	–	–	–	–
Forest-small arching	0.881	−0.167	–	–	–	–	–
River-tall erect	0.842	**2.572**	**1.791**	–	–	–	–
River-leaning/ climbing	**2.729**	**3.673**	**3.065**	**1.753**	–	–	–
River-small arching	−0.019	0.597	−0.424	1.128	**2.515**	–	–
Savanna-small arching	0.616	**2.564**	**1.672**	−1.436	**1.761**	1.001	–

p (p-value obtained with 1000 permutations)	Forest-tall erect	Forest-leaning/ climbing	Forest-small arching	River-tall erect	River-leaning/ climbing	River-small arching	Savanna- small arching
Forest-tall erect	–	–	–	–	–	–	–
Forest-leaning/climbing	**0.027**	–	–	–	–	–	–
Forest-small arching	0.221	0.565	–	–	–	–	–
River-tall erect	0.209	**0.002**	**0.028**	–	–	–	–
River-leaning/climbing	**0.001**	**0.001**	**0.001**	**0.036**	–	–	–
River-small arching	0.516	0.296	0.657	0.139	**0.003**	–	–
Savanna-small arching	0.297	**0.004**	**0.040**	0.916	**0.035**	0.168	–

Table 3
Results of pairwise comparisons based on the distance between least-squares means of floral bract specimens belonging to species of *Guadua* from different habitat and habit categories. Distance, effect size, and p-value are bolded for comparisons found to have significant differences in distance between least-squares means.

d (distance between least-squares means)	Forest-tall erect	Forest-leaning/ climbing	Forest-small arching	River-tall erect	River-leaning/ climbing	River-small arching	Savanna- small arching
Forest-tall erect	–	–	–	–	–	–	–
Forest-leaning/climbing	0.159	–	–	–	–	–	–
Forest-small arching	0.283	0.194	–	–	–	–	–
River-tall erect	0.159	0.173	0.270	–	–	–	–
River-leaning/climbing	0.229	0.236	0.340	0.201	–	–	–
River-small arching	0.362	0.361	**0.436**	0.330	0.341	–	–
Savanna-small arching	0.415	0.446	0.506	0.399	0.424	0.484	–

Z (effect size)	Forest-tall erect	Forest-leaning/ climbing	Forest-small arching	River-tall erect	River-leaning/ climbing	River-small arching	Savanna- small arching
Forest-tall erect	–	–	–	–	–	–	–
Forest-leaning/ climbing	−1.074	–	–	–	–	–	–
Forest-small arching	0.287	0.171	–	–	–	–	–
River-tall erect	−1.412	−1.343	−0.578	–	–	–	–
River-leaning/ climbing	−1.246	1.066	0.445	0.679	–	–	–
River-small arching	0.033	0.108	**2.389**	0.553	0.406	–	–
Savanna-small arching	−1.080	−0.318	0.155	−0.481	−0.541	−0.517	–

p (p-value obtained with 1000 permutations)	Forest-tall erect	Forest-leaning/ climbing	Forest-small arching	River-tall erect	River-leaning/ climbing	River-small arching	Savanna- small arching
Forest-tall erect	–	–	–	–	–	–	–
Forest-leaning/climbing	0.854	–	–	–	–	–	–
Forest-small arching	0.382	0.436	–	–	–	–	–
River-tall erect	0.922	0.915	0.707	–	–	–	–
River-leaning/climbing	0.899	0.137	0.324	0.258	–	–	–
River-small arching	0.489	0.459	**0.011**	0.286	0.338	–	–
Savanna-small arching	0.856	0.620	0.454	0.695	0.695	0.696	–

Table 4
Results of pairwise comparisons based on within-group variation from floral bract specimens belonging to species of *Guadua* from different habitat and habit categories. Within-group variation, effect size, and p-value are bolded for comparisons found to have significant differences in distance between least-squares means.

d (within-group variation)	Forest-tall erect	Forest-leaning/ climbing	Forest-small arching	River-tall erect	River-leaning/ climbing	River-small arching	Savanna- small arching
Forest-tall erect	–	–	–	–	–	–	–
Forest-leaning/ climbing	0.008	–	–	–	–	–	–
Forest-small arching	0.023	0.030	–	–	–	–	–
River-tall erect	0.022	0.015	**0.045**	–	–	–	–
River-leaning/climbing	0.007	0.001	0.030	0.015	–	–	–
River-small arching	**0.058**	**0.066**	0.035	**0.080**	**0.065**	–	–
Savanna-small arching	**0.050**	**0.057**	0.027	**0.072**	**0.057**	0.008	–

Z (effect size)	Forest-tall erect	Forest-leaning/ climbing	Forest-small arching	River-tall erect	River-leaning/ climbing	River-small arching	Savanna- small arching
Forest-tall erect	–	–	–	–	–	–	–
Forest-leaning/ climbing	−0282	–	–	–	–	–	–
Forest-small arching	0.567	1.030	–	–	–	–	–
River-tall erect	1.047	0.392	**1.627**	–	–	–	–
River-leaning/ climbing	−0.519	−1.904	0.936	0.353	–	–	–
River-small arching	**1.710**	**1.905**	0.676	**2.375**	**1.862**	–	–
Savanna-small arching	**2.328**	**2.650**	0.810	**3.273**	**2.538**	−0.836	–

p (p-value obtained with 1000 permutations)	Forest-tall erect	Forest-leaning/ climbing	Forest-small arching	River-tall erect	River-leaning/ climbing	River-small arching	Savanna- small arching
Forest-tall erect	–	–	–	–	–	–	–
Forest-leaning/climbing	0.611	–	–	–	–	–	–
Forest-small arching	0.302	0.169	–	–	–	–	–
River-tall erect	0.155	0.382	**0.046**	–	–	–	–
River-leaning/climbing	0.695	0.970	0.202	0.389	–	–	–
River-small arching	**0.035**	**0.019**	0.275	**0.007**	**0.019**	–	–
Savanna-small arching	**0.004**	**0.002**	0.221	**0.001**	**0.003**	0.779	–

several others (Table 4).

4. Discussion

Although both habit and habitat appeared to affect the micromorphology of *Guadua* foliage leaves, these factors did not act independently; for example, there was a significant difference in the least-squares means of specimens of forest species with tall and erect habits compared to those of leaning and climbing habits, a pattern that was repeated among river species. The leaves of leaning and climbing species are often partially shaded by the trees they climb, while tall and erect species raise their foliage leaves above surrounding trees, potentially resulting in a greater pressure to evolve micromorphological features that protect from UV radiation. Differences in variance between these two habits in specimens of forest and river species could be associated with the broader variation in habit in leaning and climbing species, which may be passive climbers (e.g., *G. ciliata*) or active tree-killers (e.g., *G. weberbaueri*), than tall and erect species (Griscom and Ashton, 2006; Londoño and Davidse, 1991). We also found that leaning and climbing specimens of river species differed significantly from those of small arching river species in both least-squares means and variance. These results might have been influenced by the relative homogeneity of habitats of river leaning and climbing species, which are limited to the Amazon, compared to river small arching species, which are found throughout the Neotropics (Cunha Santana, 2017; Londoño and Clark, 2002; Ruiz-Sanchez et al. 2024). Differences in micromorphology of specimens from forest leaning and climbing species compared to river leaning and climbing species as well as forest small arching species compared to river small arching species could potentially be attributable to differences in water availability and drought stress, which affect certain micromorphological features including silica bodies (Shakoor, 2014). Flowering has not been observed at all in *G. guzmanii* and only in the type collection of *G. virgata*, precluding study of floral bracts of these other small arching river species (Ruiz-Sanchez et al., 2024). Thus, significant differences in the variance of river specimens from small arching species compared to those of big and erect and leaning and climbing species were likely caused by low variance in micromorphology of small arching river specimens, which were exclusively collected from *G. longifolia*.

In addition to all savanna-adapted *Guadua*, small arching species include *G. guzmanii, G. longifolia, G. trinii, G. macclurei, G. virgata*, and *G. maculosa* E.G.Camus, which was not included in this study. Unlike other small arching *Guadua, G. macclurei* bears little resemblance to the rest of the *G. paniculata* species complex overall, with wider foliage leaves and culm leaf blades typically about half as long as sheath (Davidse and Pohl, 1992; Ruiz-Sanchez et al., 2024; Shirasuna et al., 2020). *Guadua guzmanii, G. longifolia, G. virgata,* and *G. trinii* share the narrow foliage leaves seen in the *G. paniculata* species complex, as well as culm leaf blades that are typically subequal to slightly more than half the length of culm leaf sheaths (Ruiz-Sanchez et al., 2024; Shirasuna et al., 2020). These three small arching species also share some micromorphological characteristics of the *G. paniculata* species complex, such as the presence of smooth saddle-shaped silica bodies in the intercostal zones of the abaxial leaf surface, although they typically lack the strongly correlated features of ridged saddle-shaped silica bodies in the costal zones and intercostal papillae on foliage leaves. *Guadua trinii* and *G. longifolia* typically bear papillae on their lemmas, a feature found in some forest species and all savanna species examined. However, *G. longifolia* has pseudospikelets far longer than typical of the *G. paniculata* species complex, with only three stamens per floret compared to the typical six in *Guadua*, while the culms of *G. virgata* and *G. trinii* are often more robust than those of the *G. paniculata* species complex and their pseudospikelets broader (Shirasuna et al., 2020; Swallen, 1938). *Guadua virgata* has been collected in gallery forests and disturbed areas near rivers in cerrado habitat, indicating that it might have some adaptations to savannas. However, this species showed

markedly different micromorphology between the two specimens examined, with *Clark & Morel 710* displaying abundant papillae on the foliage leaves and papillae absent from *Londoño & Filgueiras 768*; these two specimens fall relatively far apart from each other in the foliage leaf PCoA (Fig. 8). Cunha Santana (2017) reported a population of *G. virgata* with micromorphology like that of *Londoño & Filgueiras 768*, but further study is needed to confirm typical *G. virgata* foliage leaf micromorphology.

The strong similarities in the foliage leaf and floral bract micromorphology of the savanna-dwelling *G. paniculata* species complex and the difference between micromorphology of those species and other *Guadua* reinforce the need for a phylogenetic analysis of the genus. Despite the overall macromorphological and habitat similarities between species of the *G. paniculata* species complex, it is not known whether they form a clade. The newest, most complete phylogenetic analyses of *Guadua* were largely constrained by region. Afonso (2024) included primarily Brazilian and other Amazonian species, excluding *G. venezuelae*, specimens identified as *G. refracta*, and Central American and Mexican specimens of *G. paniculata.* Ruiz-Sanchez et al. (2025) had a roughly even mix of Mexican or Central American and South American *Guadua,* but only included one Mexican *G. paniculata* sample to represent the *G. paniculata* species complex. Determining the phylogenetic placement of members of the *G. paniculata* species complex could shed light on whether their macromorphological and micromorphological similarities are due to shared ancestry or represent convergent evolution related to their savanna habitat.

The papillae and ridged saddle-shaped silica bodies more commonly associated with *Guadua* species of open savanna habitats rather than forested or river habitats might protect the leaves of these species from higher exposure to UV radiation and damage by rain, as has been found in other taxa (Figs. 2, 3) (Haworth and McElwain, 2008; Shakoor, 2014). The association of papillae with adaxial stomatal complexes in several species of *Guadua* despite lack of papillae on the abaxial surface supports the hypothesis that these papillae provide protection to the stomatal complexes, whether by reducing water loss or sheltering them from precipitation. Papillae and macrohairs are both more common on foliage leaves of river species than forest species (Figs. 2, 3); if this pattern is related to variability of water availability in seasonally-inundated areas resulting in occasional drought stress in the dry season or because these structures more important for protection than prevention of water loss is unknown. Differing patterns in leaf trichomes between savanna and forest species have previously been demonstrated in non-bamboo plants, including *Strychnos* L. (Loganiaceae), where most savanna species have been observed to exhibit non-glandular trichomes, similar to the non-glandular macrohairs of *Guadua*; this hair type is lacking in forest species of *Strychnos* (Adebowale et al., 2014). Non-glandular trichomes in savanna species of *Strychnos* were hypothesized to be related to prevention of excessive evapotranspiration in the savanna habitat, suggesting that this pattern deserves further investigation across unrelated plant taxa (Adebowale et al., 2014).

Guadua found along rivers and in forests generally have narrower silica body types than the ridged saddle-shaped silica bodies of savanna-dwelling *Guadua.* Some of these relatively narrow silica body types, including bilobate silica bodies, were negatively correlated with the presence of papillae. Macrohairs and bicellular microhairs are also more frequently found on the foliage leaf surfaces of savanna species of *Guadua* than forest and river species (Figs. 2, 3); presence of bicellular microhairs is also positively correlated with that of ridged saddle-shaped silica bodies and papillae. As trichomes can potentially reduce water loss, this association could be an adaptation of savanna *Guadua* to prevent loss of water in a habitat where it is less readily available (Haworth and McElwain, 2008). Due to our reliance on herbarium specimens, for which habitat data are often not included, we were unable to assess whether factors such as distance from water sources or relative shading of individual plants affected presence of these features in individual plants. Further study of specimens for which accurate habitat information is available could shed light on potential causes of this variation that we observed and clarify how environment affects the presence or absence of various micromorphological features in individual species.

One drawback to analyzing the presence/absence of micromorphological features is that it excludes consideration of the amount or shape of these features. We did, however, incidentally observe some potential species-specific differences in density of some micromorphological features, including less dense papillae coverage of the adaxial surface of the foliage leaves in *G. venezuelae* than other members of the *G. paniculata* species complex. Interestingly, despite overlap between *G. paniculata, G. refracta*, and *G. venzuelae* foliage leaf specimens in the PCoA, the overall grouping of *G. venezuelae* was broader and differently placed than that of the other two species of the *G. paniculata* species complex, excluding one unusual Andean specimen of *G. paniculata* (Fig. 8). Such differences in foliage leaf micromorphology have the potential to be taxonomically significant (Ellis, 1979; Leandro et al., 2016), and should be considered when investigating the micromorphological variation seen within and between species.

Although floral bracts have largely been overlooked in comparative studies in *Guadua*, we found significant patterns in floral bract micromorphology based on differences in both habitat and habit. These differences were less pronounced than those seen in the foliage leaves. Additionally, based on the higher number of principal coordinates that accounted for most of the variation seen in the floret scree plot, the PCoAs used to visualize our data did not fit the floret data as well as they did the foliage leaf data. The differentiation seen between the savanna species and forest and river species may have seemed artificially strong, as it was based only the first two principal coordinate axes. Notably, only floral bracts specimens from species of small arching specimens differed significantly from other surveyed habits; specimens from species of leaning and climbing habits or tall and erect habits did not significantly differ in their micromorphology. The reasons for this pattern are unclear; surveyed small arching species are found in all habitat types, and the arching species *G. trinii* is unlikely to form a clade with other small arching species including *G. paniculata* and *G. longifolia* (Afonso, 2024). As floral bracts are only present on the plant for a brief time, the potentially protective effects of different silica body shapes, trichome types, and papillae may be less important for the plant on the floral bracts than the foliage leaves.

Differences in floral bract micromorphology seemed to largely mirror those of foliage leaf micromorphology, although some characteristics, such as the ridged saddle-shaped silica bodies that were commonly found in foliage leaves of savanna *Guadua*, were absent. Other characters, such as cross-shaped silica bodies, were present in floral bracts but not in foliage leaves. We also observed differences at the species level that support the usage of floral micromorphology in taxonomic studies. For example, lemmas of *G. amplexifolia* J.Presl and *G. velutina* Londoño & L.G.Clark, both of the *G. angustifolia* species complex (Young and Judd, 1992), bear papillae, while *G. angustifolia* lacks papillae on the lemmas.

Certain features that appear to be correlated with each other in *Guadua* are also found together in other Neotropical woody bamboos. This is exemplified by the frequently associated ridged saddle-shaped silica bodies and papillae on foliage leaves, which are also found together in several species of *Chusquea* sect. *Serpentes* L.G.Clark (McMurchie et al., 2022). While these features appear to be most common in *Guadua* from savanna habitats, members of *Chusquea* sect. *Serpentes* are restricted to forested, montane habitats (McMurchie et al., 2022). However, members of *Chusquea* sect. *Serpentes* are not understory bamboos, and frequently scramble over trees (McMurchie et al., 2022), potentially exposing them to sunlight and rain. The reasons behind the association between papillae and ridged saddle-shaped silica bodies remain unknown; further studies are needed to assess whether this association is found in other genera of Neotropical woody bamboos. Additional study of the possible protective functions of trichomes, broad

silica bodies, and papillae in *Guadua* is also needed to assess the reasons for the potential taxonomic patterns in these features.

5. Conclusions

Observation and statistical analysis of *Guadua* foliage leaves and floral bracts support the hypothesis that the micromorphology of species found in savanna habitats differs from that of species primarily found in more enclosed forest and river habitats. Trends in foliage leaf and floral bract micromorphology differentiating the savanna-associated *G. paniculata* species complex from its forest- and river-dwelling congeners accompany those in the macromorphology of these species. The foliage leaf micromorphology of species with typically small arching habits, including the *G. paniculata* species complex but also several forest and river species, also differed significantly from that of species with larger habits, including tall and erect species and species that lean on or climb up trees. Presence of certain micromorphological features on foliage leaves and floral bracts, such as presence of ridged saddle-shaped silica bodies in the costal zones and papillae, were correlated with each other; these two features are most common in species of *Guadua* from savannas, and might be advantageous in this habitat. The effects of habit and habitat on foliage leaf and floral bract micromorphology were not independent from each other, however, as indicated by the significant interaction that we found between these factors. Rather, an interplay between these two factors seems to be involved in the patterns of micromorphology of *Guadua* foliage leaves and, to a lesser extent, floral bracts.

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Data, material, and code availability

Code and original data files are available at Elizabeth McMurchie's personal GitHub repository: https://github.com/ekmcmurchie/guadua_leaf. Scanning electron microscopy images are available for viewing at https://doi.org/10.25380/iastate.28635959. Herbarium vouchers of specimens used in this study area available at A, ISC, MO, and US. Additional information about some of the specimens used in this study, as detailed in Supplemental File 1, can be found online by searching their respective digitized herbarium records. Specimen search is available for the Missouri Botanical Garden (MO) at https://www.tropicos.org/specimen/Search, for the Smithsonian Herbarium (US) at https://collections.nmnh.si.edu/search/botany/, and for the Harvard University Herbaria (including A) at https://kiki.huh.harvard.edu/databases/specimen_index.html. Specimens at the Ada Hayden Herbarium at Iowa State University (ISC) are not digitized; please contact the corresponding author or ISC curator for further specimen information.

CRediT authorship contribution statement

Elizabeth K. McMurchie: Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Josephine A. Crock:** Investigation. **Devin Molnau:** Validation, Methodology, Formal analysis. **Haldre S. Rogers:** Writing – review & editing, Supervision, Methodology. **Dean C. Adams:** Writing – review & editing, Supervision, Software, Methodology. **Lynn G. Clark:** Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.flora.2025.152767.

Appendix A. Data dictionary for foliage leaves

Associated with raw data file guadua_leaf_raw_3.xlsx
The following variables are to be used:

- Specimen: Specimen collector and number, with species name included
- Country: Country in which the specimen was collected
- Region: Region in which specimen was collected: Mexico, Central America, Andes, or Eastern South America
- Species: Species name
- Habit: Habit typical of species: Small arching, tall and erect, or leaning and climbing
- Habitat: Habitat typical of species: Forest, river, or savanna
- General_habitat: Habitat typical of species, with river and forest categories combined to include savanna or general forest habitats.

- Ab_papillae_long_cells_stomatal: Abaxial: Papillae on the long cells in the stomatal zone 0 = absent 1 = present
- Ab_papillae_long_cells_interstomatal: Abaxial: Papillae on the long cells in the interstomatal zone 0 = absent 1 = present
- Ab_pickle: Abaxial: Prickle hairs: 0 = absent; 1 = present
- Ab_bimicro: Abaxial: Bicellular microhairs: 0 = absent; 1 = present
- Ab_macro: Abaxial: Macrohairs: 0 = absent; 1 = present
- Ab_bilobate_intercostal: Abaxial: Bilobate silica bodies in the intercostal zone: 0 = absent; 1 = present
- Ab_ridged_saddle_intercostal: Abaxial: Ridged saddle-shaped silica bodies in the intercostal zone: 0 = absent; 1 = present
- Ab_smooth_saddle_intercostal: Abaxial: Smooth saddle-shaped silica bodies in the intercostal zone: 0 = absent; 1 = present
- Ab_vertically_elongated_rounded_intercostal: Abaxial: Vertically elongated (rounded) silica bodies in the intercostal zone: 0 = absent; 1 = present
- Ab_reniform_intercostal: Abaxial: Reniform silica bodies in the intercostal zone: 0 = absent; 1 = present
- Ab_nodular_intercostal: Abaxial: Nodular silica bodies in the intercostal zone: 0 = absent; 1 = present
- Ab_bilobate_costal: Abaxial: Bilobate silica bodies in the costal zone: 0 = absent; 1 = present
- Ab_ridged_saddle_costal: Abaxial: Ridged saddle-shaped silica bodies in the costal zone: 0 = absent; 1 = present
- Ab_smooth_saddle_costal: Abaxial: Smooth saddle-shaped silica bodies in the costal zone: 0 = absent; 1 = present
- Ab_vertically_elongated_rounded_costal: Abaxial: Vertically elongated (rounded) silica bodies in the costal zone: 0 = absent; 1 = present
- Ab_reniform_costal: Abaxial: Reniform silica bodies in the costal zone: 0 = absent; 1 = present
- Ab_nodular_costal: Abaxial: Nodular silica bodies in the costal zone: 0 = absent; 1 = present
- Ab_triangular_sub_cells: Abaxial: Triangular stomata subsidiary cells: 0 = absent; 1 = present
- Ab_dome_sub_cells: Abaxial: Dome-shaped stomata subsidiary cells: 0 = absent; 1 = present
- Ab_parallel_sub_cells: Abaxial: Parallel-sided stomata subsidiary cells: 0 = absent; 1 = present
- Ad_stomata: Adaxial: Stomata on the adaxial surface of foliage leaf blades: 0 = absent; 1 = present.
- Ad_stomata_freq: Adaxial: Frequency of stomata if present on the adaxial surface of foliage leaf blades: 0 = common; 1 = infrequent.
- Ad_papillae_long_cells_stomatal: Adaxial: Papillae on the long cells in the stomatal zone exclusive of bulliform cells 0 = absent 1 = present
- Ad_papillae_long_cells_interstomatal: Adaxial: Papillae on the long cells in the interstomatal zone: 0 = absent; 1 = present.
- Ad_papillae_overarch: Adaxial: Papillae on long cells of the intercostal zone adjacent to the stomata: 0 = not overarching the stomata; 1 = overarching the stomata.
- Ad_papillae_bulliform: Adaxial: Papillae on the bulliform cells: 0 = absent; 1 = present.
- Ad_pickle: Adaxial: Prickle hairs: 0 = absent; 1 = present
- Ad_bimicro: Adaxial: Bicellular microhairs: 0 = absent; 1 = present
- Ad_macro: Adaxial: Macrohairs: 0 = absent; 1 = present
- Ad_bilobate_intercostal: Adaxial: Bilobate silica bodies in the intercostal zone: 0 = absent; 1 = present
- Ad_ridged_saddle_intercostal: Adaxial: Ridged saddle-shaped silica bodies in the intercostal zone: 0 = absent; 1 = present
- Ad_smooth_saddle_intercostal: Adaxial: Smooth saddle-shaped silica bodies in the intercostal zone: 0 = absent; 1 = present
- Ad_vertically_elongated_rounded_intercostal: Adaxial: Vertically elongated (rounded) silica bodies in the intercostal zone: 0 = absent; 1 = present
- Ad_reniform_intercostal: Adaxial: Reniform silica bodies in the intercostal zone: 0 = absent; 1 = present
- Ad_nodular_intercostal: Adaxial: Nodular silica bodies in the intercostal zone: 0 = absent; 1 = present
- Ad_bilobate_costal: Adaxial: Bilobate silica bodies in the costal zone: 0 = absent; 1 = present
- Ad_ridged_saddle_costal: Adaxial: Ridged saddle-shaped silica bodies in the costal zone: 0 = absent; 1 = present
- Ad_smooth_saddle_costal: Adaxial: Smooth saddle-shaped silica bodies in the costal zone: 0 = absent; 1 = present
- Ad_vertically_elongated_rounded_costal: Adaxial: Vertically elongated (rounded) silica bodies in the costal zone: 0 = absent; 1 = present
- Ad_reniform_costal: Adaxial: Reniform silica bodies in the costal zone: 0 = absent; 1 = present
- Ad_nodular_costal: Adaxial: Nodular silica bodies in the costal zone: 0 = absent; 1 = present
- Ad_triangular_sub_cells: Adaxial: Triangular stomata subsidiary cells: 0 = absent; 1 = present
- Ad_dome_sub_cells: Adaxial: Dome-shaped stomata subsidiary cells: 0 = absent; 1 = present
- Ad_parallel_sub_cells: Adaxial: Parallel-sided stomata subsidiary cells: 0 = absent; 1 = present

Appendix B. Data dictionary for floral bracts

Associated with raw data file guadua_lemmas_palesa_raw_3.xlsx
The following variables are to be used:

- Specimen: Specimen collector and number, with species name included
- Country: Country in which the specimen was collected
- Region: Region in which specimen was collected: Mexico, Central America, Andes, or Eastern South America
- Species: Species name
- Habit: Habit typical of species: Small arching, tall and erect, or leaning and climbing
- Habitat: Habitat typical of species: Forest, river, or savanna
- General_habitat: Habitat typical of species, with river and forest categories combined to include savanna or general forest habitats.
- Recorder: Primary researcher observing presence/absence data: JAC (Josephine A. Crock) or EKM (Elizabeth Kathleen McMurchie)
- Notes_lemma: Basic introductory notes taken for lemmas of some specimens
- Notes_palea: Basic introductory notes taken for paleas of some specimens
- Lemma_papillae_simple: Lemma: Simple papillae present on long cells: 0 = absent, 1 = present
- Lemma_papillae_bifurcated: Lemma: Bifurcated papillae present on long cells: 0 = absent, 1 = present
- Lemma_papillae_branch: Lemma: Branching papillae present on long cells: 0 = absent, 1 = present
- Lemma_prickle: Lemma: Prickle hairs: absent = 0, present = 1

- Lemma_bicellular_microhair: Lemma: Bicellular microhairs: absent = 0, present = 1
- Lemma_macrohair: Lemma: Macrohairs: absent = 0, present = 1
- Lemma_rondel: Lemma: Rondel shaped silica bodies: 0 = absent, 1 = present
- Lemma_cross: Lemma: Cross-shaped silica bodies: 0 = absent, 1 = present
- Lemma_bilobate_vertical: Lemma: Bilobate vertically elongated silica bodies: 0 = absent, 1 = present
- Lemma_bilobate_horizontal: Lemma: Bilobate horizontally elongated silica bodies: 0 = absent, 1 = present
- Lemma_saddle: Lemma: Saddle-shaped silica bodies: 0 = absent, 1 = present
- Lemma_vertical_elongated: Lemma: Vertically elongated (rounded but not bilobate) silica bodies: 0 = absent, 1 = present
- Lemma_horizontal_elongated: Lemma: Horizontally elongated (rounded but not bilobate) silica bodies: 0 = absent, 1 = present
- Lemma_reniform_vertical: Lemma: Reniform, vertically elongated silica bodies: 0 = absent, 1 = present
- Lemma_reniform_horizontal: Lemma: Reniform, horizontally elongated silica bodies: 0 = absent, 1 = present
- Lemma_stomata: Lemma stomata: 0 = absent, 1 = present
- Lemma_stomata_freq: Lemma stomata: 0 = frequent, 1 = infrequent
- Lemma_stomata_subsidiary_cells_triangular: Lemma stomata subsidiary cells triangular: 0 = absent, 1 = present
- Lemma_stomata_subsidiary_cells_domed: Lemma stomata subsidiary cells dome-shaped: 0 = absent, 1 = present
- Lemma_stomata_subsidiary_cells_parallel: Lemma stomata subsidiary cells parallel sided: 0 = absent, 1 = present
- Palea_papillae_simple: Palea: Simple papillae present on long cells: 0 = absent, 1 = present
- Palea_papillae_bifurcated: Palea: Bifurcated papillae present on long cells: 0 = absent, 1 = present
- Palea_papillae_branch: Palea: Branching papillae present on long cells: 0 = absent, 1 = present
- Palea_mar_prickle: Palea margin: Prickle hairs: absent = 0, present = 1
- Palea_mar_bicellular_microhair: Palea margin: Bicellular microhairs: absent = 0, present = 1
- Palea_mar_macrohair: Palea margin: Macrohairs: absent = 0, present = 1
- Palea_sul_prickle: Palea sulcus: Prickle hairs: absent = 0, present = 1
- Palea_sul_bicellular_microhair: Palea sulcus: Bicellular microhairs: absent = 0, present = 1
- Palea_sul_macrohair: Palea sulcus: Macrohairs: absent = 0, present = 1
- Palea_rondel: Palea: Rondel shaped silica bodies: 0 = absent, 1 = present
- Palea_cross: Palea: Cross-shaped silica bodies: 0 = absent, 1 = present
- Palea_bilobate_vertical: Palea: Bilobate vertically elongated silica bodies: 0 = absent, 1 = present
- Palea_bilobate_horizontal: Palea: Bilobate horizontally elongated silica bodies: 0 = absent, 1 = present
- Palea_saddle: Palea: Saddle-shaped silica bodies: 0 = absent, 1 = present
- Palea_vertical_elongated: Palea: Vertically elongated (rounded but not bilobate) silica bodies: 0 = absent, 1 = present
- Palea_horizontal_elongated: Palea: Horizontally elongated (rounded but not bilobate) silica bodies: 0 = absent, 1 = present
- Palea_reniform_vertical: Palea: Reniform, vertically elongated silica bodies: 0 = absent, 1 = present
- Palea_reniform_horizontal: Palea: Reniform, horizontally elongated silica bodies: 0 = absent, 1 = present
- Palea_stomata: Palea stomata: 0 = absent, 1 = present
- Palea_stomata_freq: Palea stomata: 0 = frequent, 1 = infrequent
- Palea_subsidiary_cells_triangular: Palea stomata subsidiary cells triangular: 0 = absent, 1 = present
- Palea_subsidiary_cells_domed: Palea stomata subsidiary cells dome-shaped: 0 = absent, 1 = present
- Palea_subsidiary_cells_parallel: Palea stomata subsidiary cells parallel sided: 0 = absent, 1 = present

Appendix C. Additional figures

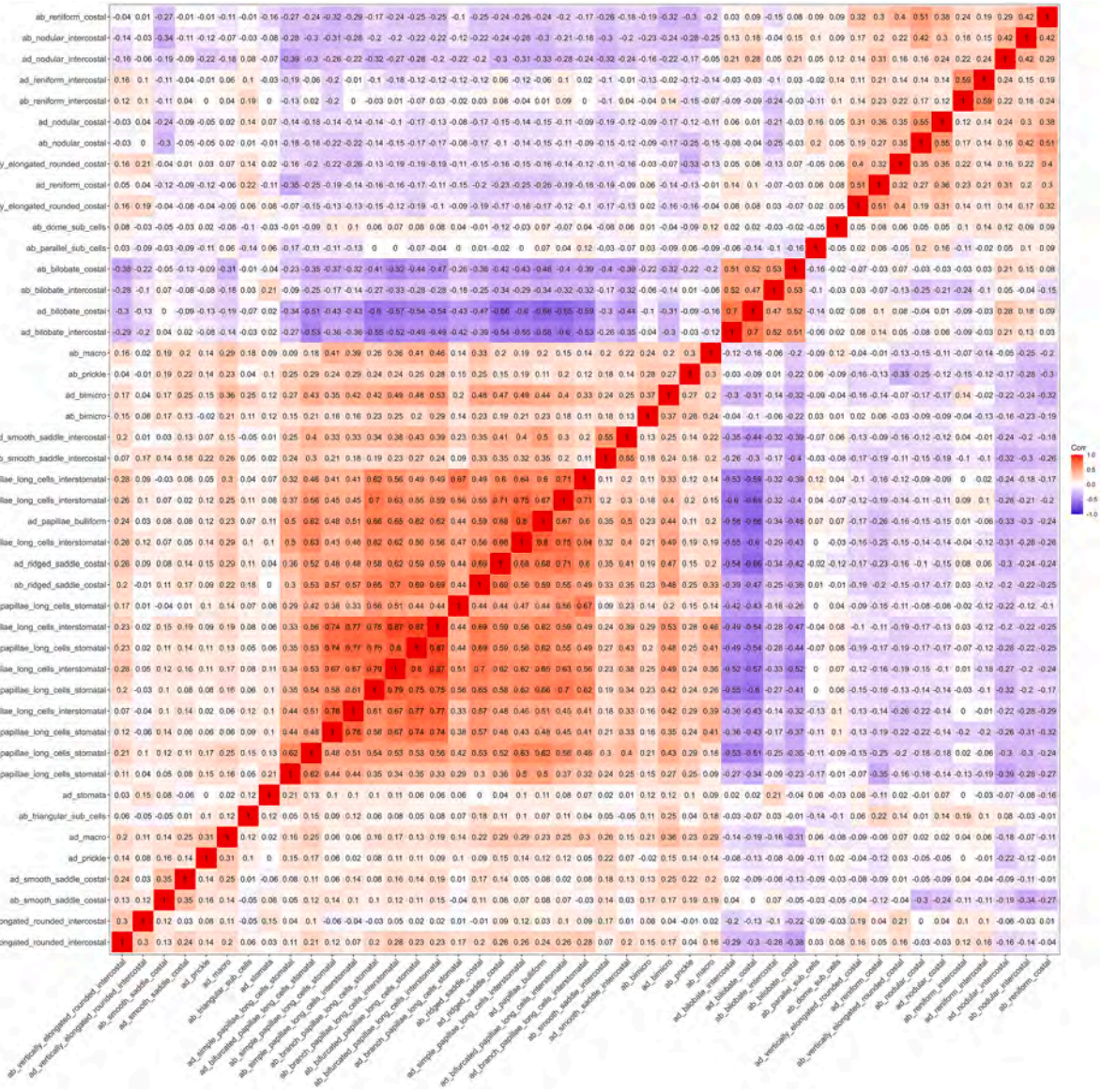


Fig. C.1. Correlation matrix showing the strength of the correlation between presence of various *Guadua* foliage leaf micromorphological features. Refer to Appendix A, the data dictionary vfor foliage leaf micromorphology, for the definition of each feature.

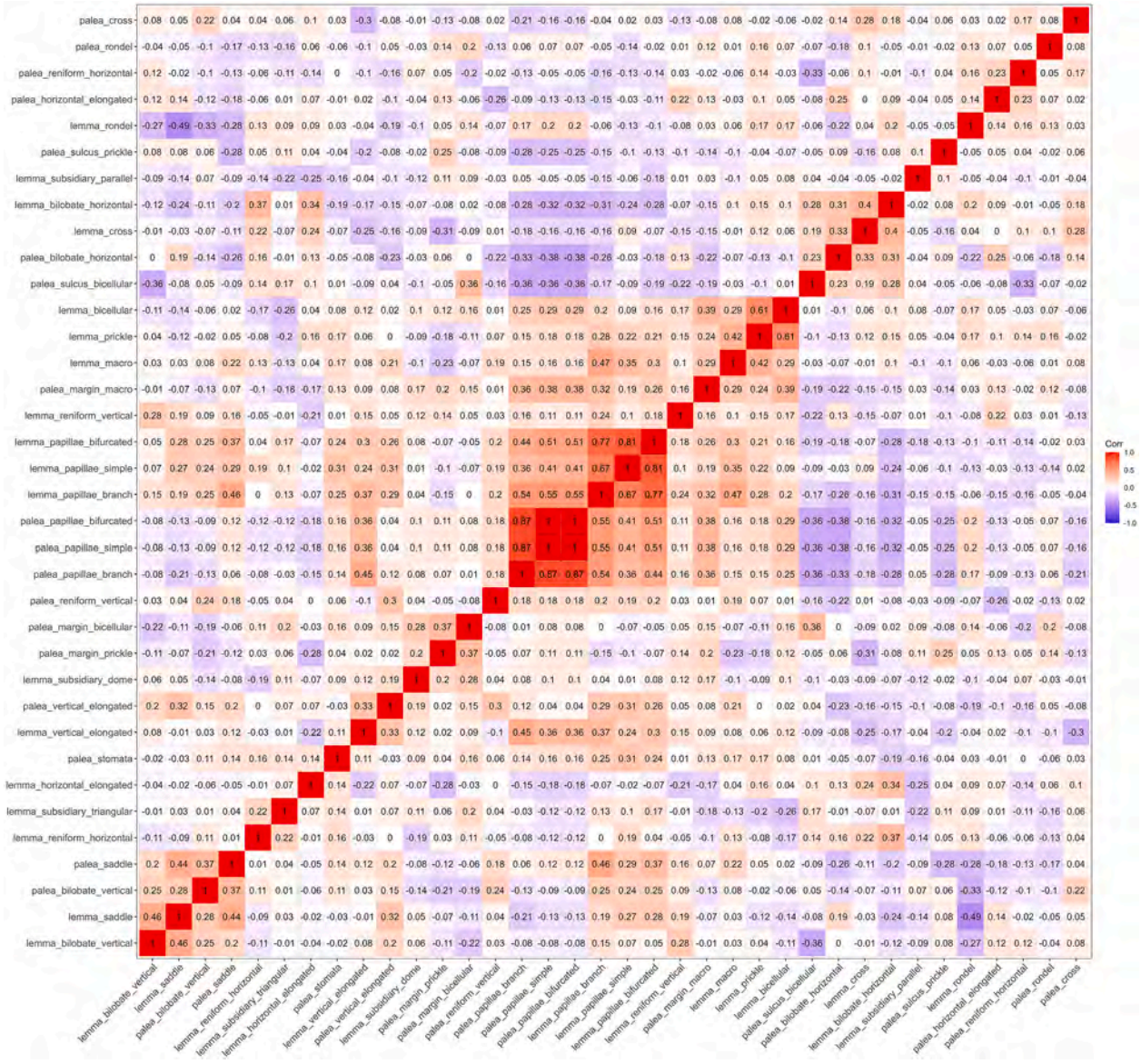


Fig. C.2. Correlation matrix showing the strength of the correlation between presence of various *Guadua* floral bract micromorphological features. Refer to Appendix B, the data dictionary for floral bract micromorphology, for the definition of each feature.

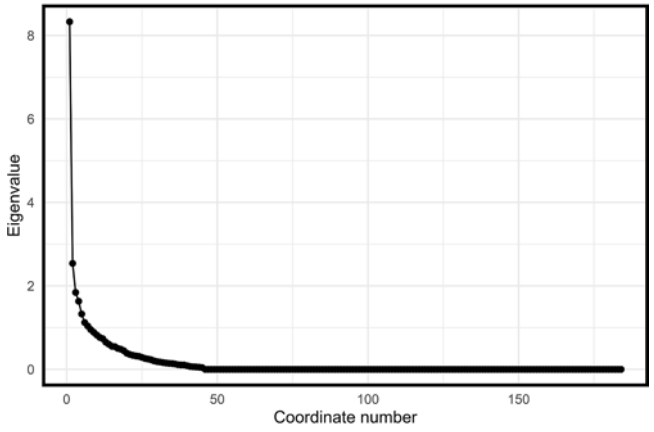


Fig. C.3. Scree plot of eigenvalues of principal coordinates involved in analysis of *Guadua* foliage leaf micromorphology.

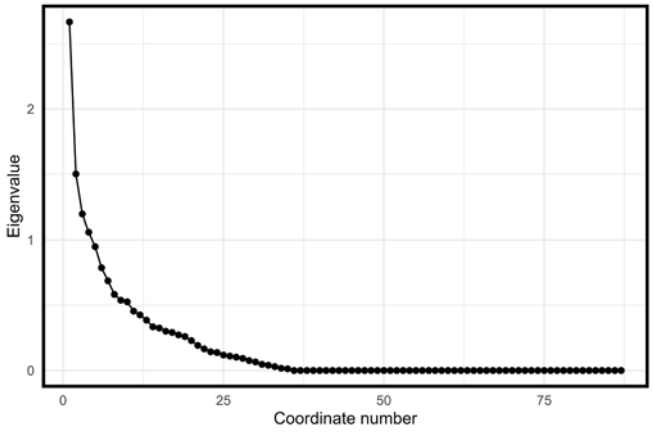


Fig. C.4. Scree plot of eigenvalues of principal coordinates involved in analysis of *Guadua* floret micromorphology.

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