Botany
An International Journal
for Plant Biology

Article

Contribution to the taxonomic elucidation of the *Geonoma maxima* complex (Arecaceae) in Central Amazonia, Brazil

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Abstract

Geonoma maxima (Poit.) Kunth is an example of a species complex, among many others restricted to Neotropical rain forests, which contribute to their high species diversity. Using environmental, morphological, karyological, and molecular data, we aim to test the taxonomic circumscription of 3 of the 11 *G. maxima* subspecies defined in the latest taxonomic treatment. We evaluated 217 samples of *G. maxima* complex from Ducke Reserve in the state of Amazonas, Brazil. Environmental preferences were significant at the 0.1% level. Subspecies *maxima* occurred in the slope, subsp. *chelidonura* in the floodplain, and subsp. *spixiana* in the plateau. Leaf morphology and height were different for each subspecies, but not leaf anatomy. The karyotypes of subspp. *chelidonura* and *maxima* were symmetrical with $2n = 28$ chromosomes, 16 metacentric and 12 submetacentric. Molecular analysis revealed two groups, one comprised subspp. *maxima* and *chelidonura*, and the other formed exclusively by subsp. *spixiana*. At Ducke Reserve, it is clear that the three subspecies are easily recognizable morphologically and ecologically, and it is likely that they do not interbreed locally. However, if these subspecies are analyzed on a larger geographic scale, it may not be possible to separate them.

Key words: Arecaceae, taxonomy, understory, Brazilian Amazon

Introduction

Geonoma Willd. (Arecaceae) is a genus restricted to Neotropical forests. It stands out not only because of the great number of species it comprises, but also and mainly because of the tremendous variation of its morphological characters. Some of the species are well defined, but many others are difficult to delineate due to the significant variation in leaf morphology, which was possibly the reason why there was an excess in species descriptions made between the 19th and 20th centuries (Souza 2006; Henderson 2011). Burret (1930) revised the genus and recognized 172 species, while Wessels Boer (1968) based on extensive field work recognized 75 species, considering the problem of intraspecific variations and synonymizing many of the valid names. Moore (1973), in turn, recorded 92 species. Later, Henderson et al. (1995) accepted 51 species, assigning 8 of them as "species complexes", in which many species were included in the category of varieties. However, the most recent taxonomic treatment recognized 68 species and the majority of the varieties considered in the previous study were reclassified as subspecies (Henderson 2011). The genus is monophyletic (Asmussen 1999*a*; Roncal et al. 2012; Loiseau et al. 2019) and many interspecific relationships were recovered using target capture sequence data (Loiseau et al. 2019). However, the large intraspecific variation, especially within species complexes, renders species delimitations difficult (Henderson 2011).

Studies using morphological and structural characters (Borchsenius 1999; Pintaud 1999; Henderson 2011; Bacon et al. 2021), karyotype (Roser 1994, 1997, 1999), ecological conditions (Chazdon 1991, 1992; Borchsenius 1999; Roncal 2006; Bacon et al. 2021), and reproductive biology (Knudsen 1999; Listabarth 1999; Borchsenius et al. 2016) and the use of molecular markers (Asmussen 1999*a*, 1999*b*; Roncal et al. 2010, 2012; Loiseau et al. 2019) are some of the evidence that has

provided important advances on the taxonomy and evolution of *Geonoma*. However, they are still insufficient mostly because they have not been widely used throughout the distribution of all species.

In the case of molecular markers, Loiseau et al. (2019) sequenced 3.988 genomic regions, including samples from 84% of the *Geonoma* species, which are the most informative genetic data, and detecting an important correlation between such data and the morphological characters of Henderson (2011).

This paper focuses on *Geonoma maxima* (Poit.) Kunth species complex. Henderson (2011) recognized 11 subspecies, distributed in two groups, differentiated by leaf division. Among these 11 subspecies, three—*Geonoma maxima* subsp. *maxima*, *Geonoma maxima* subsp. *spixiana* (Mart.) A.J. Hend., and *Geonoma maxima* subsp. *chelidonura* (Spruce) A.J. Hend.—are widely distributed in Amazonia, and are the subject of the present study. Souza (2002, 2006) suggested that they are distinct species due to the different environments they occupy and differences in their morphology and leaf texture.

The goal of this study is to contribute to the taxonomy of the *G. maxima* species complex using morphological, environmental, leaf anatomical, karyotype, and molecular data.

Materials and methods

Field study

The study was conducted in the period between 1998 and 2006 (Souza 2002, 2006) in the Adolpho Ducke Forest Reserve (2°57′42.0″S, 59°55′40.0″W), which is characterized as a primary forest in a protected area, with 100 km^2, 26 km from Manaus, capital of the state of Amazonas, and part of the Instituto Nacional de Pesquisas da Amazônia (INPA; National Institute of Amazonian Research) (Ribeiro et al. 1999; Hopkins 2005). Besides the Ducke Reserve, other nearby areas were visited (Table S1). National and international herbaria were consulted (CAY, R, RB, INPA, SP, SPF, VEN, IPA, PEUR, HGTP, HUAM, K, MG, NY, JBRJ, MNHN, IAN), whose abbreviations are presented in accordance with Holmgren et al. (1990).

In the field, three subspecies were identified (*G. maxima* subsp. *maxima*, *G. maxima* subsp. *spixiana*, *G. maxima* subsp. *chelidonura*), as stated in Henderson (2011). They were then characterized according to environment preferences and morphological, anatomical, karyological, and molecular analyses.

The total number of palm individuals analyzed from the field and herbaria was 586. Twenty-two of them were cataloged and incorporated into the herbarium collections of the INPA and the Universidade Federal do Amazonas (HUAM) (Table S1).

Morphology and environmental preferences

The criteria to determine the environmental preferences were based on Prance (1990) and Ribeiro et al. (1999). Baixio refers to occasionally flooded areas with predominantly sandy soils; vertente (slope) refers to places with "terra firme" and sandy–clayey soils; and platô (plateau) refers to raised areas, flat, with "terra firme" and predominant clayey soils. We recorded the preferred environment of 217 palm individuals and 3 morphological characters (stems clustered/solitary, individual height, and number of leaves). A χ^2 test (df = 216) was conducted for environmental preference and presence and/or absence of stems clustered, and a *t* test for individual height and number of leaves. To measure the angle between the rachis and the basal leaflet, distribution pits, type of inflorescence, and color and shape of the fruit, the minimum and maximum values of each marked individual were recorded.

Leaf anatomy

The anatomical study was conducted in the laboratory of the Faculdade de Ciências Agrárias of the Federal University of Amazonas. Leaf samples of nine individuals were collected, three representing each subspecies. Furthermore, for comparison purposes, samples of three individuals representing *Geonoma aspidiifolia* Spruce were collected. Then, the material was fixed in 70% ethanol/glycerin and subjected to common techniques in plant anatomy (Tomlinson 1961, 1990). Longitudinal and transverse sections were made in the epidermis of the leaf blade.

Dissociation of the epidermis

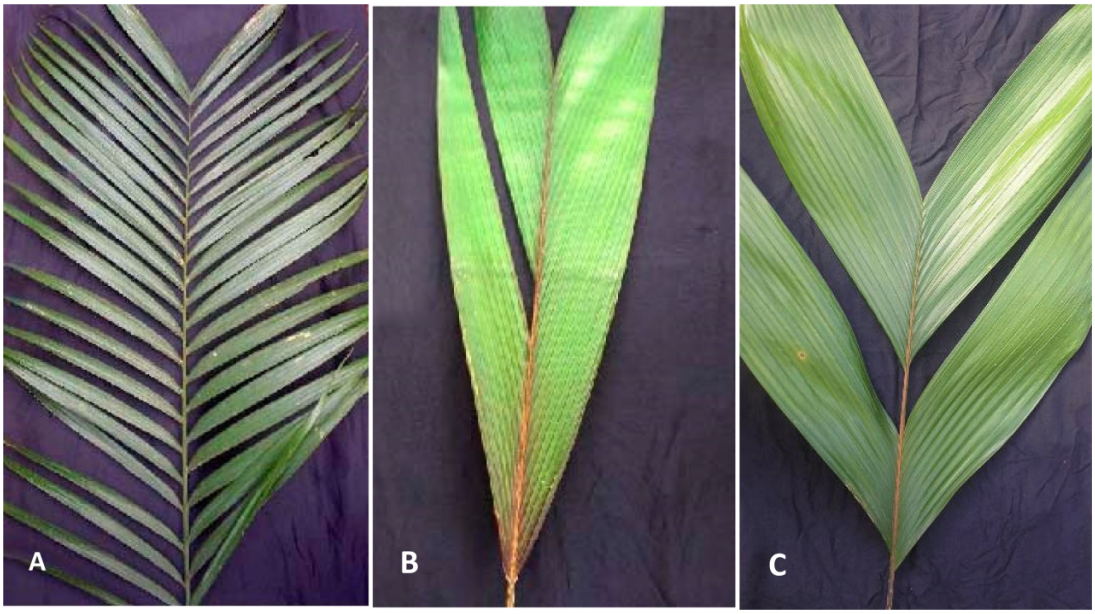
One sq. cm samples of the materials, stored in 70% ethanol/glycerin, of the veins and the median region of the pinnae of the three subspecies were subjected to the Jeffrey method (Johansen 1940). The material was boiled in a solution of non-diluted commercial hypochlorite, for approximately 40 min (adapted from Tomlinson 1961). Then, the samples were washed in distilled water, mounted on blades, and stained in aqueous safranin 1% and Astra blue. They were kept in each dye for approximately 30 s. Next, they were washed again in distilled water, dried, and mounted in glycerin on semi-permanent slides. Soon after, stomata and trichomes were counted in an optical microscope on the abaxial face of the leaf (10 × 40 slides) and measured (trichomes—100) for each subspecies studied (Tables S2 and S3). Subsequently, the values obtained were analyzed using the RStudio software, applying the Shapiro–Wilk, Kruskal–Wallis, and Dunnx's tests, at a significance level of 5% (Gotelli and Elisson 2011; Vieira 2018).

Histochemical tests were also performed in freehand sections with fixed material, using specific reagents: Lugol's solution for starch (Sass 1951); 80% sulfuric acid for crystals; and Sudan III for lipoprotein substances (Johansen 1940).

Histological sections

One sq. cm samples of the materials fixed in 70% ethanol/glycerin were subjected to crescent butyl series, included in paraffin, cut in rotation microtome with 10–11 mm, subjected for 30 min in butyl acetate, then 15 min in butyl acetate with ethanol 50%, and later subjected to the ethylic series (100%, 90%, 70%, 50%, and 30%) for 5 min each (Patiño 1986). Shortly after that, the preparations were stained in safranine and Astra blue for 10 min and then mounted in Canada balsam and observed with an optical microscope. The best slides, both for longitudinal and for transverse sections, were photographed with a Carl Zeiss photomicroscope (Ax-

Fig. 1. Leaf morphology of the *Geonoma maxima* complex at the Adolpho Ducke Forest Reserve, Amazonas, Brazil. (A) *Geonoma maxima* subsp. *maxima*; (B) *G. maxima* subsp. *spixiana*; and (C) *G. maxima* subsp. *chelidonura*.



ioskop, MC-80 camera). Electromicrographs were also made with a scanning electron microscope (Jeol, model JSM-5400 L).

Karyological analyses

The karyological study was conducted at the Laboratório de Citogenética e Evolução Vegetal of the Department of Botany of the Federal University of Pernambuco. The root tips of 23 samples were collected from adult individuals of the subspecies *maxima*, *spixiana*, and *chelidonura*, from different regions in the state of Amazonas (Table S1). The root tips were kept in a solution of 8-hydroxyquinoline (2 mmol/L) at 5 °C for approximately 20 h, fixed in Carnoy solution 3:1 (three parts of absolute ethanol and one part of glacial acetic acid (P.A); *v*/*v*) at room temperature (~25 °C) for 24 h, and then stored in a freezer (−20 °C). For the chromosomic preparations, the root tips were washed twice in distilled water for 5 min and submitted to an enzymatic digestion (2% cellulase and 20% pectinase) for 30 min at 37 °C in a humid chamber. Then, the root tips were hydrolyzed in HCI 5 N for 20 min and washed again in distilled water. The meristems were macerated in acetic acid at 45% and later crushed between slide and coverslip and frozen in liquid nitrogen. The preparations were stained in Giemsa 2% for approximately 20 min and then mounted in Entellan (Merck) for the chromosome counting. A double base-specific stain was also conducted with the fluorochromes: chromomycin A3 (CMA; 0.5 mg/mL) for 60 min and 4′,6-diamidino-2-fenilindol (DAPI, 2 μg/mL) for 30 min, which showed rich areas in GC and AT, respectively, according to the protocol recommended by Schweizer and Ambros (1994).

Molecular analyses

The genetic diversity study was carried out with the logistical support of the Institute of Biosciences of the University of São Paulo. Leaf samples of 33 individuals belonging to the subspecies in question (Figs. 1A–1C) were collected, growing in occasionally or never flooded forests, from different localities in the state of Amazonas, Brazil (Table S1). Leaf samples from these specimens were cut in small fragments, which were kept in silica gel prior to DNA extraction. DNA extraction followed the protocol by Ferreira and Grattapaglia (1995) and AFLP (amplified fragment length polymorphism) analysis followed the procedures of the AFLP Plant Mapping Protocol (Applied Biosystems 2000). After digesting total DNA with *Eco*RI and MseI (Invitrogen™), the resulting fragments were attached to adapters and submitted to the pre-selective and selective amplifications, using reagents of the AFLP Plant Mapping Kit for small genome (Applied Biosystems). The primer combinations used for the selective amplification were *Eco*RI-AC/MseI-CAG and *Eco*RI-AG/MseI-CTG. The selectively amplified fragments of each sample were analyzed with the ABI Prism 310 automated DNA sequencing (Applied Biosystems), following the procedures of Applied Biosystems (2000) and Pinheiro (2005). Electropherograms were analyzed with the aid of the software GeneScan and Genotyper (Applied Biosystems), to obtain a binary matrix containing fragments (characterized by numbers of nucleotides) and corresponding plant samples. Analyses of the electropherograms were optimized by hand. The electropherograms of each sampled set were analyzed individually, since the number of polymorphisms can be influenced by the number of individuals and their relationships.

Two multivariate analyses were used for assessing the taxonomic implication of AFLP data: the unweighted pair group method with arithmetic average (UPGMA) with Dice coefficient and principal coordinate analysis (PCO) with Dice coefficient. The combined use of methods with different strategies aims at improving the interpretation of the data:

UPGMA is a cluster method, suitable for discontinuous data variation (clusters), whereas PCO is an ordination method that detects patterns within the continuous variation among samples. The software FITOPAC (Shepherd 1996) was used to conduct all multivariate analyses.

Results

Morphology and environmental preference

Environmental preferences among subspecies were significant at the 0.1% level. Subspecies *maxima* occurred with greater frequency in the slope, subsp. *chelidonura* in the floodplain, and subsp. *spixiana* in the plateau (Table S4). The subsp. *maxima* represented 29% (62) of the records, whereas *spixiana* represented 51% (111) and *chelidonura* represented 20% (44), as shown in Table S4. The occurrence of subsp. *spixiana* was noteworthy at Madereira Itacoatiara, due to the number of individuals observed—148. This number was higher than the total subsp. *spixiana* occurring in the three environments at Ducke Reserve. The environmental preference for the plateau was the same.

As for the habit, the number of clustered individuals was higher (82.5%) than the number of solitary individuals (17.5%) for the three subspecies (Table S5). The χ^2 test confirmed the values above, according to which *spixiana* was more clustered than the other subspecies—*spixiana* versus *maxima* with $\chi^2 = 17.4$ ($p < 0.001$; df = 172) and *spixiana* versus *chelidonura* with $\chi^2 = 8.3$ ($p < 0.01$; df = 154). The difference between *maxima* and *spixiana* was significant at the 5% level with $t = 8.7$ ($p < 0.001$). The difference between *maxima* and *chelidonura* was also significant, with $t = 7.4$ ($p < 0.001$). The number of leaves also varied significantly at the 5% level between *maxima* and *chelidonura*—$t = 4.6$ ($p < 0.001$). It was not significant when *maxima* and *spixiana* were compared—$t = 0.1$ ($p > 0.005$), or when *chelidonura* and *spixiana* were compared—$t = 0.28$ ($p > 0.05$) (Table S5).

With regard to the morphology of the leaves, in the forest the three subspecies were very distinguishable (Figs. 1A–1C). All the individuals identified as *maxima* presented leaves with numerous pinnae, 9–33 per side. In general, these pinnae were opposite or subopposite, with soft texture, narrow, 0.6–3 cm wide. The apical pair may be a little wider than the others, 3–7.5 cm wide. The seedlings of subsp. *maxima* were also observed, and the first leaf (eophyll) was whole, undivided, bifid, and of soft texture. When seedlings were 18 cm tall, they presented two or three pairs of narrow pinnae, 1–1.5 cm wide. When seedlings were 23–48 cm tall, four pairs of narrow pinnae (0.5–1 cm wide) were seen. Subspecies *spixiana,* in turn, generally, presented entire long leaves, of rigid texture, plicate, bifurcated, undivided, and in greater quantity in the leaf crown. Few individuals were seen with two pinnae per side. They stand out in the understory, especially in relation to *chelidonura*, due to their much darker tone of green (Fig. 1B). Seedlings of this subspecies were not found. Subspecies *chelidonura* had two large, sigmoid pinnae, 4–10 cm wide. It can present one more intermediate pinna, narrow, 1 cm wide. It may be confused with subsp. *spixiana,* especially if the evaluation is restricted to herbarium specimens, where many samples are incomplete. Subspecies *spixiana* has very rarely been found with two pinnae per side, and subsp. *chelidonura* does not have plicate leaves (Fig. 1C). Seedlings of *chelidonura* were also observed. The first leaf (eophyll) was undivided, bifid, and 2–3 cm wide. It is later differentiated with two pinnae per side, relatively large, and separated, albeit not always by another narrow pinna.

The measurement of the angle between the pinna and the rachis of the 217 studied samples revealed that *maxima* varied from 20 to 60° ($N = 62$), with only one individual presenting 20°. *Spixiana* varied from 5 to 30° ($N = 111$), in which the most common angle was 10°, whereas *chelidonura* varied from 20 to 43° ($N = 44$). It can be noticed that the angle measurements presented by *maxima* and *chelidonura* almost overlap.

With regard to the type of inflorescence: subsps. *maxima* and *chelidonura* presented second- and third-order branching, while subsp. *spixiana* presented first- and second-order branching. The number of rachillae, their thickness, and the distribution of pits also revealed some differences among the subspecies. The greatest number of rachillae was observed in subsp. *maxima*, varying from 9 to 59, followed by subsp. *chelidonura,* which varied from 4 to 51. Subspecies *spixiana* had a smaller variation, from 5 to 32 (Table S6).

Leaf anatomy

With the optical microscope in frontal view, on the abaxial surface of the blade, it was possible to visualize, in the three subspecies, slightly wavy, hexagonal, rectangular, oval, and squared cells, with pits. The vein region revealed rectangular cells, evenly distributed in parallel to it. On the adaxial surface, the cells are longer and diagonally disposed in relation to the length of the blade.

The leaf epidermis is amphistomatic in the three subspecies, present in greater number on the abaxial surface and restricted to the intercostal region, and on the adaxial surface, they are in a much smaller number and restricted to the costal region. They are disposed at the same level in relation to the other epidermal cells, with four subsidiary cells—two smaller polar, rounded cells and two larger lateral cells. *Geonoma aspidiifolia*, included in the present study for comparison purposes, had similar leaf blade with amphistomatic stomata, also surrounded by four subsidiary cells, taking diverse shapes and very dispersed in the blade (Figs. S1–S3).

With regard to the quantification of these stomata, the Shapiro–Wilk test indicated that the distribution is normal (p value = 0.1292); however, the test for homogeneity of variances (Bartlett's test) revealed a p value = 3.262–5, showing heterogeneity of variances. Thus, the requirements for the analysis of variance were not completely met. The Kruskal–Wallis test was then applied and revealed a p value = 5.197–16, indicating that there was a significant difference between the groups. The Dunn's test revealed no significant difference in the number of stomata among the three subspecies. However, the number of stomata in *G. aspidiifolia* was significantly lower than in any of the three *G. maxima* subspecies (Fig. 2). Subspecies *maxima* showed a lower number of trichomes than any of the other taxa (Fig. 3).

Fig. 2. Boxplots of stomata number observed on the abaxial leaf epidermis of the *Geonoma maxima* complex and *Geonoma aspidiifolia*. (A) Subspecies *maxima*; (B) subsp. *spixiana*; (C) subsp. *chelidonura*; and (D) *G. aspidiifolia*.

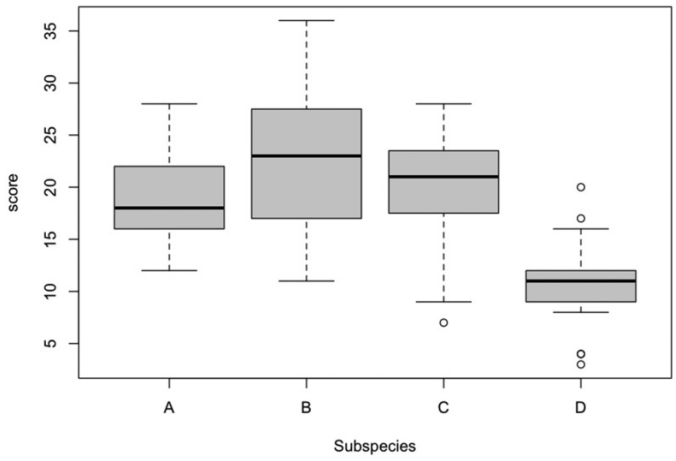
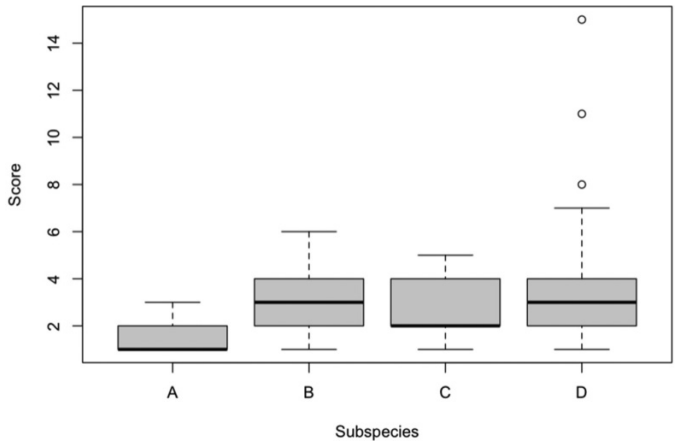


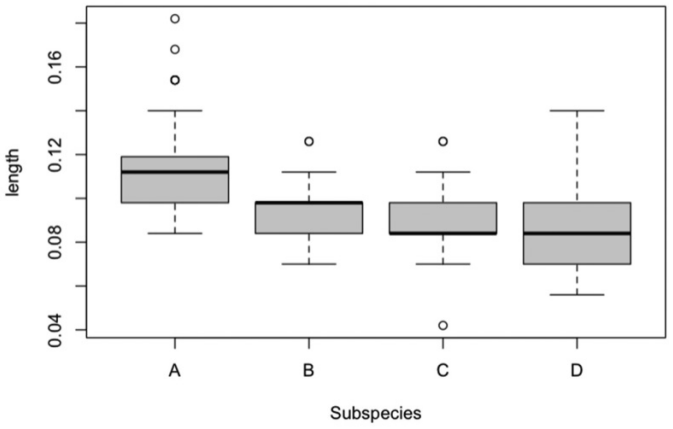
Fig. 3. Boxplots of number of trichomes observed in the abaxial leaf epidermis of the *Geonoma maxima* complex and *Geonoma aspidiifolia*. (A) Subspecies *maxima*; (B) subsp. *spixiana*; (C) subsp. *chelidonura*; and (D) *G. aspidiifolia*.



Tector trichomes, with sizes varying from 0.08 to 0.18 mm, were observed on both surfaces of the leaf blade, but in smaller number on the adaxial surface. However, these trichomes do not vary morphologically. They are oval-shaped, tapering in the extremities, and alternatively distributed, sometimes in pairs or in triads along the vein region. They are rarely seen out of such region. They are multicellular, with cells transversely arranged. Trichomes were also present in the pinnae of *G. aspidiifolia*, and their morphology was similar to that observed in the three subspecies of *G. maxima* (Figs. S1–S3).

Trichome length was significantly larger in the subspecies *maxima* than in any of the other taxa (Fig. 4). Subspecies *spixiana* also showed larger trichome length than subspp. *chelidonura* and *G. aspidiifolia*. Only between the subsp. *chelidonura* and the species *G. aspidiifolia* there was no significant difference in trichome length (Fig. 4).

Fig. 4. Boxplots of trichome length observed in the abaxial leaf epidermis of the *Geonoma maxima* complex and *Geonoma aspidiifolia*. (A) Subspecies *maxima*; (B) subsp. *spixiana*; (C) subsp. *chelidonura*; and (D) *G. aspidiifolia*.



The transverse section revealed homogeneous mesophyll in the three subspecies, constituted of rounded cells with thick walls, interspersed with vascular bundles, surrounded by schlerenchyma cells, containing isolated crystals, confirmed by the histochemical tests, and numerous chloroplasts. The scanning electron microscopy confirmed what was seen with the optical microscope: cells with thick walls, stomata surrounded by four cells at the same level as the others, and multicellular trichomes alternating in the vein region (Fig. S3).

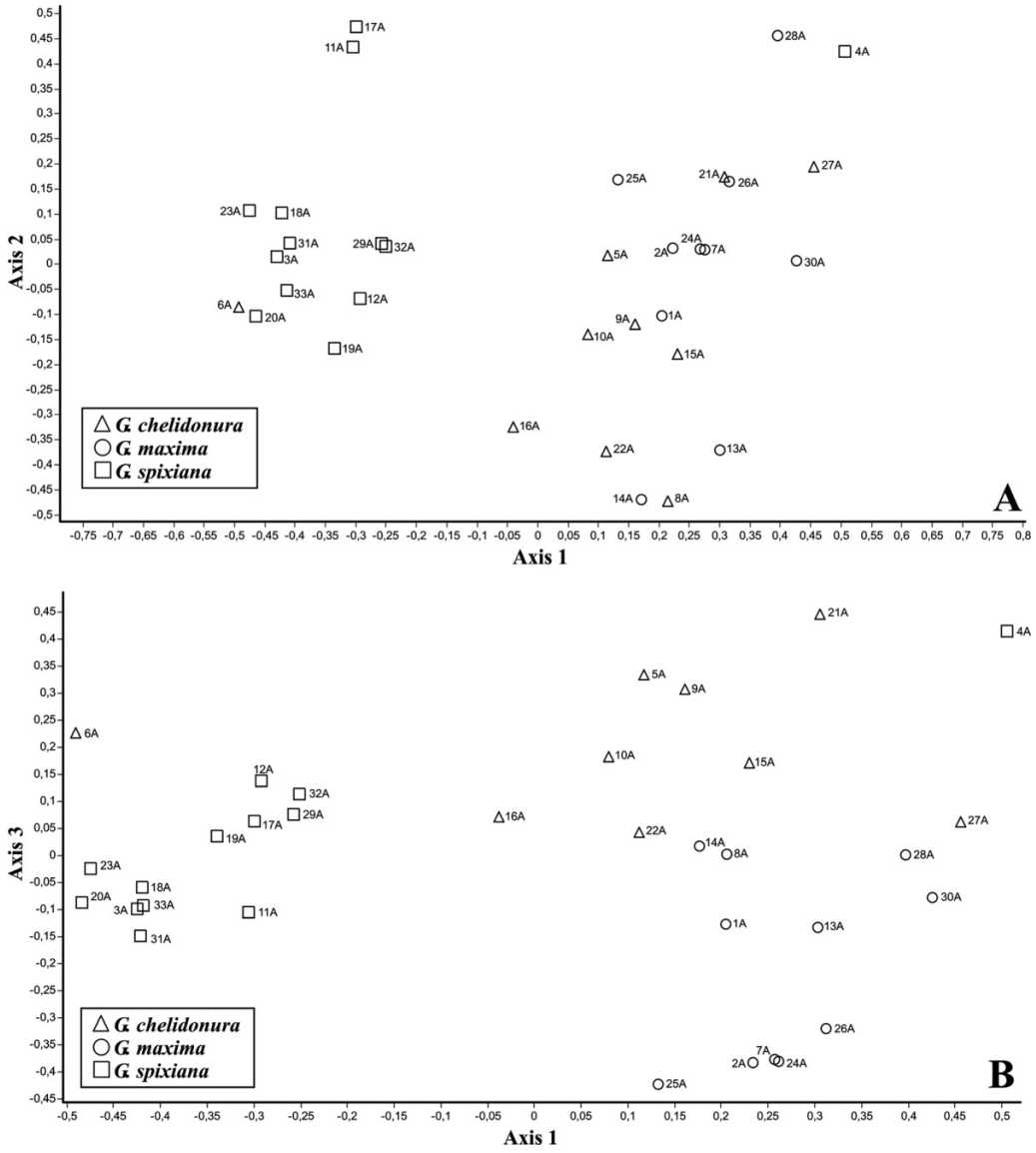
Karyological analyses

The karyological analyses presented results only for *G. maxima* subsp. *maxima* and *G. maxima* subsp. *chelidonura*. The interphasic nuclei of both of them were characterized as semi-reticulated, with some differences in relation to the chromocenters and to the euchromatic reticulum (Figs. S4A and S4B). In *maxima*, the chromosomes were big, dispersed, and irregularly delimited, with the euchromatic reticulum slightly stained (Fig. S4A). On the other hand, the chromocenters of *chelidonura* were grouped in a denser form and the euchromatin was more strongly stained (Fig. S4B).

A pattern of heterogeneous condensation was observed in late prometaphases in cells of both subspecies (Fig. S5C). Some chromosomes were already completely condensed, whereas others presented arms with partial condensation. The number of chromosomes was defined only for the subspp. *maxima* and *chelidonura*, both with $2n = 28$ (Figs. S4A and S4B). Both karyotypes belonged to the gradual type, constituted of 16 metacentric (m) and 12 submetacentric (sm) chromosomes (Figs. S4C and S4F), according to the nomenclature described in Guerra (1986).

Additionally, the findings of the analyses with base-specific fluorochromes were obtained only for subsp. *chelidonura*, which revealed a pair of regions CMA+/DAPI− (Figs. S4E and S4F). For *G. maxima* subsp. *spixiana*, even after exhausting repetitions of chromosomic preparations with various protocol adjustments, it was not possible to obtain cells in metaphases

Fig. 5. Principal coordinate analysis of the AFLP (amplified fragment length polymorphism) data using the Dice coefficient for 33 individuals of the subspp. *chelidonura*, *maxima*, and *spixiana*. (A) First and second axes explain 19.99% and 11.18% of the total variation, respectively. (B) First and third axes explain 19.99% and 9.26% of the total variation, respectively.



with enough quality for analysis. The few cells in division that were observed presented chromosome overlapping. Therefore, it was not possible to count them or to describe their karyotype.

Molecular analyses

The AFLP analysis of the 33 samples representing the subspp. *maxima*, *chelidonura*, and *spixiana* yielded 86 fragments, among which 61 (71%) were polymorphic. The number of fragments reproduced by primer pairs varied from 42 to 44, with 31 (73.8%) and 30 (68.2%) polymorphic fragments, respectively (Table S7). The multivariate analyses UPGMA (Fig. S5) and PCO (Fig. 5) had similar results: two groups were identified, one formed by subspp. *maxima* and *chelidonura* and the other by subsp. *spixiana*. In the PCO, axis 3 separated slightly subspp. *maxima* and *chelidonura*.

Discussion

Morphology and environmental preference

In the case of the *G. maxima* complex, although none of the subspecies were exclusive of a specific environment, the preference of each one was different. Two other studies, conducted in the *Geonoma macrostachys* Mart. complex, had similar results. One took place in Peru (Roncal 2006) and the other one in Ecuador (Borchsenius et al. 2016). In both studies, two morphotypes were identified—one of them was restricted to occasionally flooded environments and the other one occurred predominantly in "terra firme". However, in both cases, the preferences were defined only on a local scale. On a continental scale, however, environmental preferences of morphotypes were not clear (Bacon et al. 2021). Bacon et al. (2021) concluded that the environmental heterogeneity in Amazonia and the morpholog-

ical forms specific to local ecological conditions are leading to the formation of new lineages and speciation in the *G. macrostachys* complex. In the Amazonia, these events do not occur only in the Arecaceae. Prance (1988), for example, already drew attention to species complexes in the family Caryocaraceae, where he verified that three subspecies of *Caryocar glabrum* (Aubl.) Pers. were adapted to distinct environments. The author called them geographic subspecies.

Regarding the morphology in *G. maxima*, the presence of pinnae above 18 was common in subsp. *maxima* (Souza 2002, 2006), which corroborates Henderson's (2011) classification that places subsp. *maxima* within a group of plants with regularly distributed pinnate leaves. As reported in Wessels Boer (1968) for a population in Suriname, subsp. *maxima* in our study site had pinnate-shaped leaves from the seedling stage until the adult phase.

Most individuals of subsp. *spixiana* had whole, bifid leaves and very seldom had two pairs of pinnae. This is in agreement with Spruce (1871) and Burret (1930), who referred to *spixiana* as a "plant with whole leaves and of rigid texture". Similarly, Wessels Boer (1968) defended the plicate character of the *spixiana* leaf (though as a distinct taxon—*Geonoma spixiana*) as a determining feature (Fig. 1B). In the subspecies *chelidonura*, the presence of two pairs of sigmoid pinnae, wide, predominated, from the seedling stage, agreeing with Spruce (1871), Burret (1930), and Wessels Boer (1968), but defended the subspecies as a distinct taxon.

Our data on the angle between the pinnae and rachis were very similar to Henderson's (2011). According to Henderson, subsp. *maxima* varied from 34 to 80°, subsp. *spixiana* varied from 4 to 20°, and subsp. *chelidonura* varied from 10 to 87°.

Subspecies *spixiana* was the most different in relation to the thickness of the rachillae and the distribution of pits. It presented thicker rachillae and pits were very close to each other in a row, confirming what Burret (1930) and Wessels Boer (1968) had already reported as a distinct feature in this subspecies. Subspecies *maxima* and *chelidonura*, in turn, have thinner rachillae and their pits are spirally arranged. Another character related to reproductive biology, which could help to understand the variation in the three subspecies, is the phenology of the flowering period. Kütcheimester (1997) monitored the flowering times of the three *G. maxima* subspecies at Ducke Reserve and concluded that there is no evidence of prezygotic reproductive isolation based on differences in flowering times. Subspecies *maxima* presented four flowering periods during the year—from March until May, from the end of June until the beginning of July, from August until September, and from October until November. Subspecies *spixiana*, in turn, had two flowering periods, from April until June and from October until November. Subspecies *chelidonura* also had two flowering periods—from January until February and from September until October. Therefore, there is a flowering time overlap for subspp. *maxima* and *spixiana*, from October until November, and quite close to the flowering time of subsp. *chelidonura* from September until October.

Leaf anatomy

The presence of stomata on both leaf surfaces of the three subspp. contradicts Tomlinson (1961), who pointed to their presence in *Geonoma* only in the intercostal region of the abaxial surface. The findings by Pereira and Potiguara (1995), in turn, were similar to those of this study. They also registered for *Geonoma baculifera* (Poit.) Kunth, an amphistomatic epidermis, besides the cells diagonally organized in the blade, which are square and rectangular in the veins. Similar to our studied subspecies, *G. baculifera* has also a homogeneous mesophyll in transversal section. Pereira and Potiguara (1995) had already affirmed the genus homogeneity from the structural point of view, which Tomlinson (1961) corroborated at the tribal level. Similarly, amphistomatic stomata were also noticed in other palms, such as *Mauritia flexuosa* L. f. (Passos and Mendonça 2006), the genus *Oenocarpus* (Silva and Potiguara 2008), and *Socratea exorrhiza* (Mart.) H. Wendl. (Kikuchi et al. 2016).

In the three subspecies, stomata are in greater quantity on the abaxial surface and restricted to the intercostal region. On the adaxial face, however, they are much smaller and restricted to the costal region. Stomata are arranged on the same plane in relation to the other epidermal cells, with four subsidiary cells, two smaller and rounded polar ones, and two larger lateral ones, sometimes the opposite, making it difficult to fit into any classification, but very similar to the tetracytic type. Several authors have also recorded tetracytic stomata for other Amazonian palms (Passos and Mendonça 2006; Silva and Potiguara 2008; Kikuchi et al. 2016).

We registered the presence of tector trichomes in the three subspecies, which are also found in *G. aspidiifolia*, a species external to the *G. maxima* complex. Therefore, the trichomes were taxonomically uninformative. Pereira and Potiguara (1995) also registered trichomes in the pinnae of *G. baculifera*, with the same morphology as seen in the *G. maxima* subspecies. Despite a similar trichome morphology in the *G. maxima* complex, *G. aspidiifolia*, and other *Geonoma* species, we still do not discard their potential use for the taxonomy of the genus. Passos and Mendonça (2006) report, for the *M. flexuosa* leaf, the presence of long unicellular trichomes with an enlarged base, especially in the costal region of the adaxial surface. In *S. exorrhiza* (Kikuchi et al. 2016), the trichomes vary in shape and size and are present in the costal and intercostal regions of the adaxial surface.

The little significance in the structural differences observed in the three subspecies reinforce the homogeneity of anatomical characters in the genus *Geonoma* (Tomlinson 1961; Pereira and Potiguara 1995).

Karyological analyses

According to Röser (1994), the type of chromatin organization in the interphasic nucleus, observed in both subspp. *maxima* and *chelidonura*, is very common in members of subfamilies Arecoideae (where *Geonoma* in included) and Ceroxyloideae, like the pattern of heterogeneous condensation presented by both subspecies. Röser (1994) states that it is a type of chromosomic behavior seen in some species of subfamilies Coryphoideae s.l. and Ceroxyloideae, e.g., some species of

Chamaedorea. He also affirms that such species of *Chamaedorea* present geographic distribution and morphological variations that are similar to those presented in *Geonoma*. For Röser, the subfamilies Arecoideae and Ceroxyloideae have some karyological characters in common, such as the organization of chromatin in interphasic nuclei and the condensation behavior of prophase chromosomes.

The number of chromosomes, $2n = 28$ (Figs. S4C–S4D), registered in both subspp. *maxima* and *chelidonura* in the present study, was the same as found by Röser (1997) for *Geonoma interrupta* (Ruiz & Pav.) Mart.

Röser (1994) associates more evolved members in Arecaceae, whether from the floral, morphological, or ecological point of view, with a low number of chromosomes. The author states that "some of these advanced taxa show indications of active evolutionary radiation at present", such as *Chamaedorea* in subfamily Ceroxyloideae and *Geonoma* in subfam. Arecoideae, among others.

On the other hand, the number of chromosomes differs from the one observed in *Geonoma gracilis* André and *Geonoma vaga* Griseb. & H. Wendl., both with $2n = 32$ (Sharma 1970; Goldblat 1981). The same chromosome number was obtained for *Butia* (Correia et al. 2009), which is considered as the most frequent chromosome number in subfamily Arecoideae. These latter authors found that the few studies on the karyological characterization in the group limit the interpretations of the mechanisms involved in the chromosomic evolution of Arecaceae as a whole. The record of gradual-type karyotypes for the three subspecies studied here, consisting of 16 metacentric (m) and 12 submetacentric (sm) chromosomes (Figs. S4C–S4F), seems to be common in the subfamily Arecoideae, since this number was recorded for three *Euterpe* species. However, the chromosome number recorded for *Euterpe* species was $2n = 36$ (Oliveira et al. 2016), being one of the highest within Arecoideae (Röser 1994, 1999).

The fluorochromes were analyzed and a pair of regions was detected—CMA+/DAPI− in the subsp. *chelidonura* (Figs. S4E and 4SF), which Röser (1994) suggests is a common character among members of the Arecoideae. This contrasts with the results of other Arecaceae subfamilies.

The failure in obtaining cells in metaphase for subsp. *spixiana* can be due to the presence of secondary metabolites in plant tissue (Röser 1997).

Molecular analyses

The clustering and ordination analysis gave similar results (Fig. S5; Fig. 5). Thus, it was possible to verify the predominant formation of two groups—one with individuals of *spixiana* and the other with individuals of *maxima* and *chelidonura*.

This genetic affinity between *maxima* and *chelidonura* strengthens what is observed in the field. Although subsp. *maxima* has numerous regularly distributed pinnae and subsp. *chelidonura* has no more than three pinnae per side, the characters in common are in greater proportion, such as the pinnae texture, the number of ramifications in the inflorescence, the value of the angle between the rachis and the pinna, and fruit size, among others. In turn, *spixiana* differs from the other subspecies by their bifurcated leaves, almost always whole and plicate, which makes it easier to identify herbarium specimens. Furthermore, rachillae thickness and pit arrangement are morphological characters, useful for distinguishing the studied subspecies. Thick, erect rachillae, with ramifications of second order at the most, characterize *spixiana* individuals and distinguish them from *maxima* and *chelidonura*. Another difference is that in subspecies *spixiana*, pit "swellings" are absent on the rachillae during flowering or fruiting, but present in subspecies *maxima* and *chelidonura*. The pits are also deeper in *spixiana*, in six to eight series or rows, with a distance of only 1.0–1.5 mm among them. Wessels Boer (1968) drew attention to pit arrangements in representatives of Geonomateae.

Our two genetic groups formed, one, by *maxima* and *chelidonura* individuals, and other, only by *spixiana* individuals, contradicts the morphometric analysis of Henderson (2011) who, when classifying *G. maxima* subspecies into two subgroups, isolated the subspecies *maxima* of *chelidonura,* joining this latter with *spixiana* within the second subgroup. Our multivariate analysis revealed three individuals (6A, 16A, and 28A) grouping outside of their corresponding morphologies. These outliers could be explained by errors in sample manipulation in the field or laboratory. The last robust molecular phylogenetic analysis of the tribe Geonomateae, conducted by Loiseau et al. (2019) with a taxonomic sampling of 84% of the genus *Geonoma*, revealed that *G. maxima* is monophyletic, and together with the subspecies *chelidonura* and *camptoneura* are exclusive to a single clade (clade I), which differs from the classification made by Henderson (2011), who included *G. maxima* within the clade of *G. macrostachys* (clade III in Loiseau et al. 2019). Furthermore, two of the subspecies of *G. maxima* (*camptoneura* and *chelidonura*), recognized by Henderson (2011), are not monophyletic for Loiseau et al. (2019). Roncal et al. (2012) sampled 63% of the genus *Geonoma* and found a clade of two *G. maxima* samples. We, therefore, raise the question of whether these subspecies are reproductively isolated.

The molecular phylogenetic tree from Loiseau et al. (2019) while being the most informative from a molecular point of view opens further questions since several congruencies with the morphometric data of Henderson (2011) were revealed, but also differences, both within the *G. maxima* complex and in other species complexes such as *Geonoma pohliana* Mart., *Geonoma stricta* (Poit.) Kunth, *G. macrostachys*, and *G. cuneata* H. Wendl. ex Spruce, where some intraspecific taxa were not confirmed as monophyletic.

We acknowledge that the present study is restricted to one location for a widely distributed Amazonian species. As in previous local studies with the *G. macrostachys* complex (Roncal et al. 2007; Borchsenius et al. 2016), which showed a local environmental, morphological, and genetic distinction that disappeared when the observation area was expanded to a regional/continental scale (Bacon et al. 2021), the same could hold true for the *G. maxima* complex.

At Ducke Reserve, it is clear that the three subspecies are easily recognizable morphologically and ecologically, and thus, it may seem that the three subspecies do not interbreed. However, if these subspecies are analyzed on a larger geographic scale, it may not be possible to separate them.

Acknowledgements

The authors thank the National Council of Scientific and Technological Development (Conselho Nacional do Desenvolvimento Científico e Tecnológico of Brazil—CNPq) for funding this project, the International Palm Society for the financial incentive of the present field work, the logistical support given by the Instituto Nacional de Pesquisas da Amazonia—INPA, the Laboratory of the Faculdade de Ciências Agrárias of the Federal University of Amazonas, the Bioscience Laboratory of the University of Sao Paulo, the Laboratory of Plant Cytogenetics of the Federal University of Pernambuco, the curators of the national Herbaria (INPA, MG, SP, JBRJ, IPA, PEUR, HUAM, R), and foreign Herbaria (VEN, MNHN, BOL, K, NYBG, MOBOT) for permission to study the specimens. The authors also thank Professors Raquel Nunes for correcting the manuscript in the English language and Rogério Craveiro for conducting the statistical tests.

Article information

History dates

Received: 25 November 2022
Accepted: 17 August 2023
Accepted manuscript online: 25 August 2023
Version of record online: 31 October 2023

Data availability

The data analyzed during this study are available in the INPA repository: https://repositorio.inpa.gov.br/handle/1/12828.

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Competing interests

The authors declare there are no competing interests.

Funding information

Conselho Nacional de Desenvolvimento Científico e Tecnológico (Grant/Award Number: "(scholarship)") and International Palm Society (Grant/Award Number: "(scholarship)"). Preprint deposit: Repositório do INPA: https://repositorio.inpa.gov.br/handle/1/12828.

Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/cjb-2022-0130.

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