Research Square

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The Role of Geography in Taxonomy and Conservation of Species Related to Banisteriopsis Malifolia (Malpighiaceae)

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Abstract

Banisteriopsis includes species that are difficult to define, and there is a hypothesis that sympatric species are more similar than those with wide distributions. The objective of this study was to test the circumscription of species related to *B. malifolia* to examine the relationship between geographical distribution, sympatry, and allopatry with the morphological variation of taxa, as well as to assess the degree of threat to the taxa. The results surprisingly point to greater morphological variation in allopatric species than in sympatric ones, the taxa are better explained within the current circumscription and no corroborate the new status for *Banisteriopsis malifolia* var, *appressa* recently proposed. We confirm the dramatic occurrence of threatened species in this group.

Introduction

Banisteriopsis C.B. Rob. ex Small was redefined by Davis & Anderson (2010) because the taxon splitted in three different clades. These subclades corresponded to the subgenera accepted by Gates (1982): the species of *B.* subg. *Pleiopterys* were transferred to the enlarged genus *Diplopterys* A.Juss., *B.* subg. *Hemiramma* was recognized as the genus *Bronwenia* W.R. Anderson & C. Davis, and *B.* subg. *Banisteriopsis* was retained in a restricted sense. *Banisteriopsis*, in the restricted sense, comprises 62 species (POWO 2024) or 65 (Almeida et al. 2024) distributed mainly in the tropical regions of the New World, with 48 of them occurring in Brazil (Francener and Almeida 2020).

Gates (1982) grouped complex species of *Banisteriopsis* based on evidence of hybridization, but without assigning any taxonomic category or phylogenetic connotation. Some of these groups have been recovered in phylogenetic studies, forming highly supportive clades (Davis and Anderson 2010). The difficulty in circumscribing species is evident in the significant number of unidentified *Banisteriopsis* specimens in herbaria.

The Malifolia group, informally created by Gates (1982), is characterized by species with samarids that have lateral wings parallel to the anterior, meaning the wings are directed to a single position, and the fruit locules are internally hairy (Gates 1982). Malifolia group comprises six species: *B. malifolia* (Nees & Mart.) B.Gates (including the varieties *B. malifolia* var. *malifolia* and *B. malifolia* var. *appressa* B.Gates), *B. variabilis* B.Gates, *B. confusa* B.Gates, *B. latifolia* (A.Juss.) B.Gates, which are endemic to the Cerrado, and two species endemic to Chapada dos Veadeiros, *B. hirsuta* B.Gates and *B. hatschbachii* B.Gates. Recently, *B. malifolia* var. *apressa* was recognised how a species *Banisteriopsis appressa* (B.Gates) R.F.Almeida & M.Pell (Almeida et al. 2024).

Malifolia has two subgroups that can be distinguished by the length and width of the petiole, leaf and pedicel (Gates 1982). The first subgroup, Malifolia (*B. malifolia* var. *malifolia*, *B. malifolia* var. *appressa*, and *B. confusa*), is characterized by a petiole that is (2–)3–8(–13) mm long and wider than 2 mm, leaf blades that are 2.5–7.5(–12) cm wide, and pedicels measuring 0.6–1 mm. The leaf blades of the second subgroup, Variabilis (*B. variabilis*, *B. latifolia*, *B. hirsuta*, and *B. hatschbachii*), measure 5.6–14.1 cm in

length, with petioles measuring 0.3(–4) mm long and more than 2 mm wide, while pedicels range from (0.8)1.0–2.2 mm in width.

In his analysis on the Malifolia group, Gates (1982) proposed several hypotheses for the pattern of morphological variation in the taxa, which have yet to be properly tested. Gates (1982) suggests that the subgroups (Malifolia and Variabilis) exhibit greater morphological coherence compared to individual species, as the latter are more challenging to delineate independently than when categorized into two subgroups. Moreover, Gates (1982) argues that Malifolia group species with broader geographical distributions demonstrate more pronounced morphological diversity than those with restricted ranges. Additionally, Gates (1982) proposes that geographical proximity may contribute to morphological disparities among Malifolia group species, noting that sympatric individuals from different species display greater morphological similarities than allopatric individuals from the same species. The verification of these hypotheses has the potential to elucidate the taxonomic boundaries within the complex formed by the Malifolia group.

Although these hypotheses were specifically established for the Malifolia group, the results can make a broader contribution beyond the delimitation of the species complex. For instance, species that exhibit morphological homogeneity pose challenging taxonomic problems (Stockman and Bond 2007) and can provide insight into the process of speciation and the acquisition of morphological novelties (Cadena et al. 2018). Therefore, testing the hypotheses proposed by Gates (1982) is important for understanding the geographical role of morphological variation and the importance of taxonomic complexes in species diversification and morphological innovations within the Malifolia group.

The aim of this study is to evaluate the circumscription of taxons related to Malifolia group and to examine the relationship between geographical distribution, sympatry and allopatry with the morphological variation of taxa and degree of conservation of species.

Materials and Methods

Morphological analysis

We compiled morphological descriptors into spreadsheets by conducting a thorough review of pertinent literature. Additionally, we analyzed herbarium collections to enhance the available information. Supplementary collections were acquired from Alexânia - GO, Alto Paraiso de Goiás - GO, Cavalcante - GO and Fazenda Água Limpa (FAL) - DF. Qualitative and quantitative characteristics were chosen as distinctive features.

Smaller structures were measured using graph paper, and measurements were taken using the ImageJ program to analyze available specimens on SpeciesLink.

A total of 22 quantitative and 15 qualitative characters were selected and organized by collector, collection number, locality, and geographical coordinates (refer to Appendix I). Geographical coordinates

were directly obtained from collection labels whenever accessible. When coordinates were not provided, they were estimated using the map application Google Maps based on the locality information provided by the collector.

The final database comprised 176 specimens (refer to Appendix II). These specimens were categorized according to two taxonomic proposals to determine the most efficient approach to explaining the morphological variability observed within the collections of the Malifolia group. The first proposal evaluated the delimitation of seven species, while the second explored the possibility of reducing these seven species to two taxa representing the subgroups Malifolia and Variabilis.

Statistical analysis

We performed statistical analyses using R v4.0.2. The morphological dataset underwent transformation into a morphological distance matrix utilizing a modified version of the script by Chartier et al. (2017). This script computes pairwise distances between each sample based on the average difference between each character. It calculates mean differences for continuous, categorical, and binary variables equally, thereby contributing to the final mean difference between each sample. Paired differences averages were calculated for each analyzed exsicate.

To identify the most effective delimitation proposal for explaining morphological variability in the dataset, we conducted PERMANOVA analysis on the morphological distance matrix. This analysis compared morphological variation with classification by seven species and with classification by two groups. The 'adonis' function in the vegan v2.5 package facilitated this statistical analysis, employing 999 permutations to calculate significance values. Additionally, we conducted a paired PERMANOVA analysis specifically for the seven-species delimitation proposal to identify species exhibiting significant morphological differences. This analysis also included 999 permutations, with a significance threshold set at $p \leq 0.05$ after Bonferroni correction. We executed this analysis using the 'adonisph' function provided by Chartier et al. (2017).

Principal component analysis was utilized to create morphological spaces for mixed Gaussian model analysis (MGS) based on the covariance matrix of log-transformed data. Dimensionality reduction involved selecting axes with the greatest power to discriminate groups for use in MGS, without prior knowledge of groupings. MGS analysis, without prior knowledge of groups, evaluated a range of possible classifications from a single group explaining all morphological variation to up to 10 possible groups. The Bayesian information criterion (BIC) assessed taxonomic arrangement models. A script adapted from Cadena et al. (2018) executed the analysis with the mclust 5.4.8 and clustvarsel (2.3.4) packages.

To determine if morphological variability of widely distributed species surpassed that of species with constrained distributions and to evaluate if groups exhibited greater morphological cohesion than individual species, an ANOSIM analysis was conducted. This analysis compared observed variability within the morphological distance matrix by proposed taxonomic delimitation involving seven species or

by grouping taxa into two distinct categories. Significance was determined through 999 permutations using the 'anosim' function within the vegan v2.5 package.

To investigate potential influence of geographical proximity on morphological similarity, a geographical distance matrix was generated and compared with the morphological distance matrix. Geographical coordinates of each specimen were utilized to compute paired geodesic distances between samples, facilitated by the 'geodist' function within the geodist v0.0.7 package. Subsequently, a distance correlation analysis was conducted on the two matrices using the Mantel Test and the 'mantel' function from the vegan v2.5 package. Significance was determined at a threshold of ≤ 0.05 following 999 permutations.

Following determination of the most suitable classification proposal, an examination of both qualitative and quantitative attributes was undertaken to identify those exhibiting the most discriminatory capacity among taxa. Independence of qualitative attributes across variables and taxa was assessed utilizing the Chi-square contingency table. Concurrently, for continuous attributes, Kruskal-Wallis analyses were conducted, followed by pairwise discrimination among taxa using the Wilcoxon paired test. Non-parametric tests were selected due to taxonomic data not meeting normal distribution and homogeneity of variance requirements. A significance threshold of $p \leq 0.05$ was applied for these analyses, conducted using functions within the basic statistical environment of R v4.0.2.

Distribution and degree of conservation of species

We analyzed the distribution of the species utilizing data sourced from virtual herbaria websites and newly collected data. The conservation status of the species was evaluated employing the Geospatial Conservation Assessment Tool (GeoCAT) to ascertain the extent of occurrence (EOO) and area of occupancy (AOO) (Bachman et al. 2011). These calculations adhered to guidelines set forth by the International Union for Conservation of Nature (IUCN) (IUCN Standards and Petitions Committee 2022) and utilized 2x2 km grid cells (equivalent to a 4 km² area).

Results

Species Delimitation

The results of the PERMANOVA (Table 1) analysis indicate that the taxonomic scheme comprising seven species marginally outperforms the alternative proposal, which would condense the taxa into two groups based on subgroup classification. The taxonomic hypothesis of seven species provided a better explanation for the variability of the data found in the morphological distance matrix ($F = 31.606$, $R^2 = 0.614$, $p < 0.0001$) than the proposed reduction to two groups ($F = 27.829$, $R^2 = 0.183$, $p < 0.0001$). However, both proposals were equally significant in explaining morphological variation in this taxonomic complex.

Table 1
Results of the PERMANOVA analysis using a morphological distance matrix of species related to *Banisteriopsis malifolia* to determine the significance between species. No significant morphological difference (ns), significant morphological difference *(*).*

	B. confusa	***B. hatschbachii***	***B. hirsuta***	***B. latifolia***	***B. malifolia* var. *appressa***	***B. malifolia* var. *malifolia***	***B. variabilis***
B. confusa	<NA>	8.911	0.993	5.996	17.775	70.329	16.029
B. hatschbachii	*	<NA>	4.478	2.928	17.575	28.788	7.951
B. hirsuta	ns	ns	<NA>	2.123	9.673	25.119	4.853
B. latifolia	ns	ns	ns	<NA>	22.155	79.465	20.408
B. malifolia var. *appressa*	*	*	ns	*	<NA>	49.815	21.618
B. malifolia var. *malifolia*	*	*	*	*	*	<NA>	65.555
B. variabilis	*	*	ns	*	*	*	<NA>

The paired PERMANOVA analysis showed partial confirmation of the observed pattern, with significant differences among taxa in the Malifolia subgroup, while some species within the Variabilis subgroup did not exhibit significant differentiation. Specifically, *B. hirsuta* and *B. hatschbachii* within *B. variabilis*, as well as *B. latifolia*, *B. hirsuta*, and *B. hatschbachii*, did not show significant differences. Moreover, *B. variabilis* and *B. hirsuta* were not significantly different. *Banisteriopsis malifolia* var. *malifolia* was distinct from all others, while *B. malifolia* var. *apressa* did not differ significantly from *B. hirsuta*. In contrast, *B. confusa* did not differ significantly from *B. hirsuta* and *B. latifolia*. In the other subgroup, *B. variabilis* differs significantly from the other species, except for *B. hirsuta*, which differs significantly from *B. malifolia* var. *apressa* and *B. latifolia*. Finally, *B. hatschbachii* does not differ significantly from *B. latifolia* and *B. hirsuta*.

The analysis of mixed Gaussian models revealed that the seven-species hypothesis was superior to the two-group hypothesis. The best models indicated the formation of five or six groups, with the five-group hypothesis being slightly superior to the six-group hypothesis (see Fig. 1). This analysis not only disproves our hypothesis but also suggests a new classification possibility for this group based on Gaussian models.

Geographical distribution and morphological variability

The results of the ANOSIM analysis indicate that species with widespread distribution demonstrate low morphological variability, whereas more constrained species display higher morphological variability ($r = 0.730$, $p = 0.001$). *Banisteriopsis malifolia* var. *malifolia*, *B. malifolia* var. *appressa*, and *B. variabilis*,

characterized by wide distribution, do not exhibit greater morphological variability compared to more restricted species such as *B. hirsuta* and *B. hatschbachii*, endemic to the Chapada dos Veadeiros (refer to Fig. 2).

The association between geographical distance and morphological diversity of *Banisteriopsis* species within the Malifolia group is depicted in Fig. 2 (r = 0.730, significance = 0.001).

Geographical proximity and morphological similarity

The Mantel test results did not support our third hypothesis that individuals who are geographically closer are more morphologically similar. The correlation between geographical distance and morphological variation was low (r = 0.13, significance = 0.04). The low correlation indicates that different species sharing a geographical region may not have shared morphological characters, and there is morphological variation among them.

Discriminating characters

The results of the chi-squared test delineate the proportion of characters present in the groups and identify the most discriminating qualitative characters for species identification. This analysis focused on five qualitative characteristics that demonstrated the greatest variation among species. However, only two of these characteristics exhibited a significant difference between the groups: adaxial pilosity ($p < 0.001$) and abaxial pilosity ($p < 0.001$) of the leaf (refer to Fig. 3). These characteristics yielded significantly low p-values. Accordingly, leaf hairiness on both the abaxial and adaxial surfaces emerged as distinguishing features between species within the groups, as evidenced by the significant disparity in color depicted in the graph for the two groups (see Fig. 3).

The Kruskal-Wallis analysis identified seven quantitative characters that exhibited the most notable differences between species and groups. Petiole length ($p < 0.01$) and petiole diameter ($p < 0.001$) emerged as the most distinctive characteristics between the groups (refer to Fig. 4).

Conservation status

Banisteriopsis latifolia (Fig. 5) is a species that is characteristic of the Cerrado due to its arboreal habit with developed cork and cauliflory. It has the widest distribution among the species of the Malifolia group and a high number of collections, being mainly recorded in the states of Goiás and the Federal District. Although well-documented, *B. latifolia* is considered a Vulnerable species (VU) due to the destruction of its natural habitat in areas of Cerrado restricted sense and campo sujo. It is included in the list of threatened species. The EOO and AOO assessments were 12,185,254 km^2 and 96,000 km^2, respectively (see Fig. 6).

Banisteriopsis hatschbachii (Fig. 7) is classified as an Endangered species (EN) and exhibits endemism to Chapada dos Veadeiros - GO, with a limited number of collections. Presently, there are 17 records available on SpeciesLink, one of which is our collection, being the most recent. The assessments of its

EOO and AOO were 576,596 km^2 and 36,000 km^2, respectively (see Fig. 5). Three populations were identified during this investigation. Similarly, *B. hirsuta* is considered Endangered (EN), also endemic to Chapada dos Veadeiros - GO, with only 8 collection records documented on SpeciesLink. Its EOO and AOO assessments were 1,225,365 km^2 and 24,000 km^2, respectively (see Fig. 5). However, it was not rediscovered during the present study.

Due to their widespread distribution, high population abundance, and low risk of extinction, the following species are categorized as Least Concern (LC): *B. confusa*, *B. malifolia* var. *malifolia*, and *B. malifolia* var. *appressa* (Fig. 9) and *B. variabilis* (Fig. 10). EOO assessments for each species are 738,254,273 km^2, 1,450,813,069 km^2, 330,036,238 km^2 and 1,265,283,101 km^2, in that order. The AOO for these species is 40,000 km^2, 140,000 km^2, 56,000 km^2, and 140,000 km^2, respectively (see Fig. 8).

Discussion

The PERMANOVA data support the seven-species hypothesis as a more appropriate classification. Similarly, the Gaussian mixed model analysis also supports the seven-species hypothesis but suggests a new classification into five groups.

Debates over the definition of species are common, but little attention has been paid to the methods used to delimit them (Sites and Marshall 2004; Cadena et al. 2018). Therefore, works such as Sites & Marshall (2003, 2004) and Cadena (2018) aim to test different methods for species delimitation. Understanding evolutionary processes is crucial, as species are the fundamental units in biology (Hausdorf and Hennig 2010). The delimitation of the groups proposed by Gates and the species can also be investigated beyond morphological characters, including molecular analyses.

The Mantel test results indicate that individuals of different species sharing the same geographical space are not morphologically similar, contrary to Gates (1982) expectations. Studies demonstrate that species similarity decreases with increasing geographical distance; that is, the closer they are, the more morphologically similar they are, as stated in the Neutrality Theory (Hubbell 2001). The similarity of species in a community decreases with increasing geographical distance, regardless of environmental differences (Hubbell 2001, 2006; Ferreira et al. 2011). Therefore, it is believed that differences between species are linked to dispersal (Scudeller et al. 2001).

Various environmental factors, such as climate, soil, altitude, relief, and phytophysionomies found in the Cerrado biome, can interfere with morphological variability. Leaf hairiness, which showed the greatest difference between the Malifolia and Variabilis subgroups, is a qualitative character related to habitats. Some reports suggest that hairiness protects against the sun's rays and herbivory (Brewer et al. 1991; Lara 1996). Trichomes are epidermal cell-derived structures that vary in shape, position on leaves, and surface density (Jeffree 1986). Barônio conducted a study on *Qualea multiflora* Mart. leaf hairiness and herbivory in a Cerrado area, demonstrating that increasing trichome density reduced the proportion of leaf area affected by herbivores.

Petiole length and thickness were the only quantitative characters that differentiated the groups, as highlighted by Gates (1982). The petiole is a supporting structure that connects the leaf to the stem and can present many anatomical differences, as investigated by Araújo (2010). In their study of leaf and petiole anatomical characters of three genera of Malpighiaceae, including *Banisteriopsis*, Araújo (2010) found differences in the accessory bundles of the petiole.

The status of the two endemic species of Chapada dos Veadeiros - GO, *B. hirsuta* and *B. hatschbachii*, which are on the red list of threatened species, classified as Endangered (EN) (CNCF 2012) has been confirmed. The populations of these species are strongly threatened by the degradation of the biome and uncontrolled fires that consume large areas of the Cerrado annually (Klink and Machado 2005; Melo and Martins 2020) The Cerrado biome is home to a diverse array of species, many of which are endemic. Unfortunately, the reduction in the biome's original area has led to an increase in threats to these populations, making it challenging to conduct studies (Klink and Machado 2005). In conclusion, preserving the Cerrado is crucial for the conservation of its unique biodiversity.

Conclusion

The hypotheses proposed by Gates (1982) were tested, and the delimitation of the Malifolia group and its two subgroups, Malifolia and Variabilis, as treated by Gates, was confirmed. The species as circumscribed by the author were also corroborated, including the non-recognition of *B. malifolia* var. *appressa* as a distinct species, as suggested. No new characters exclusive to the group or contributing to the formation of new subgroups of the studied species were found.

The study confirmed the discriminatory characters essential for species identification. It reaffirmed the endangered status of *B. hirsuta* and *B. hatschbachii*, underscoring their degree of threat. Furthermore, the research successfully assessed the threat level for other species, particularly highlighting the Vulnerable status of *B. latifolia*. Urgent conservation strategies are imperative to protect these species.

Declarations

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Taxonomic Treatment

We recognized *B. malifolia* var. *appressa* as a not different specie of *B. malifolia* var. *malifolia* as proposed by Almeida (2024).

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Figures

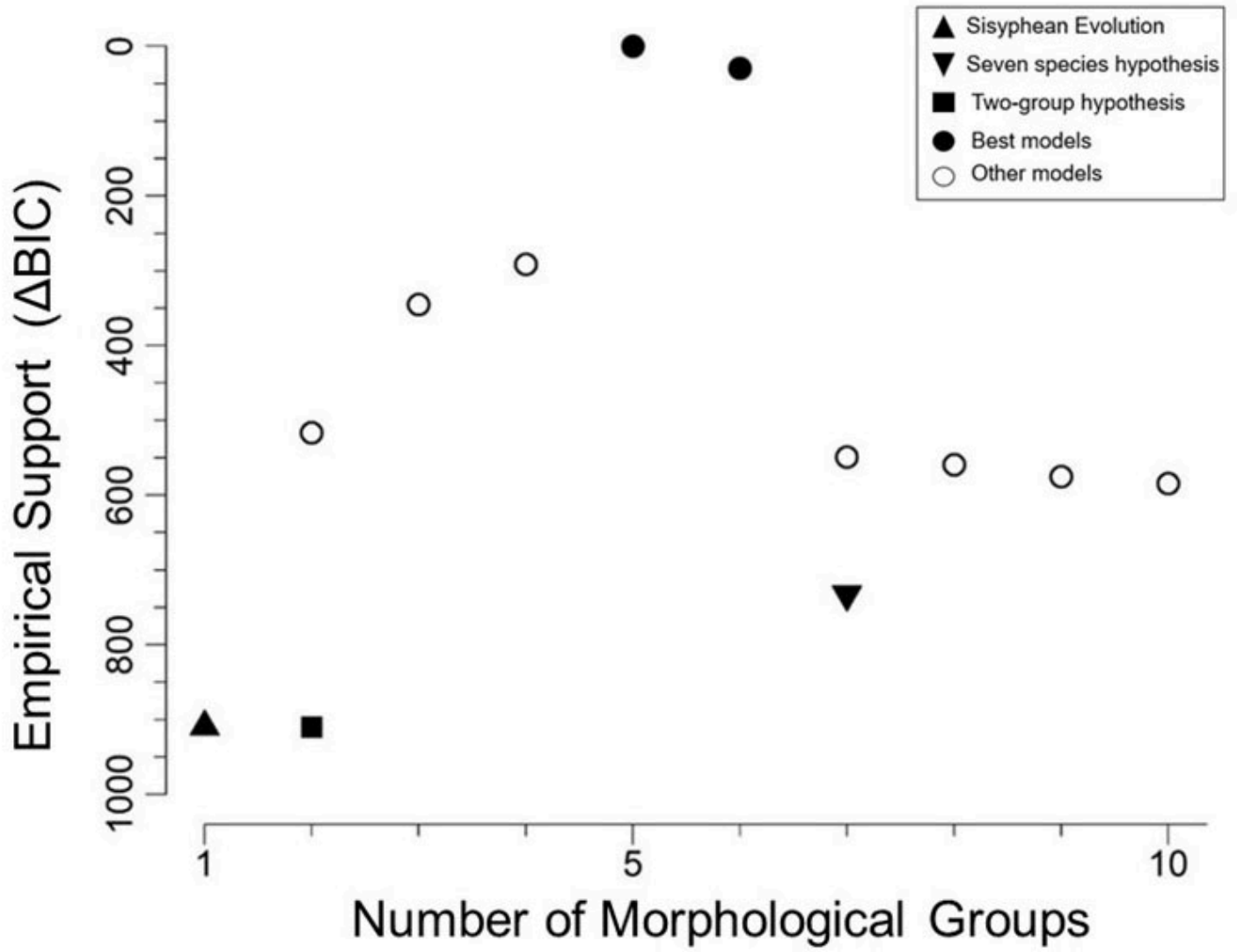


Figure 1

Graph showing the hypotheses of two or seven species of Malifolia group and the best models proposed by MGS.

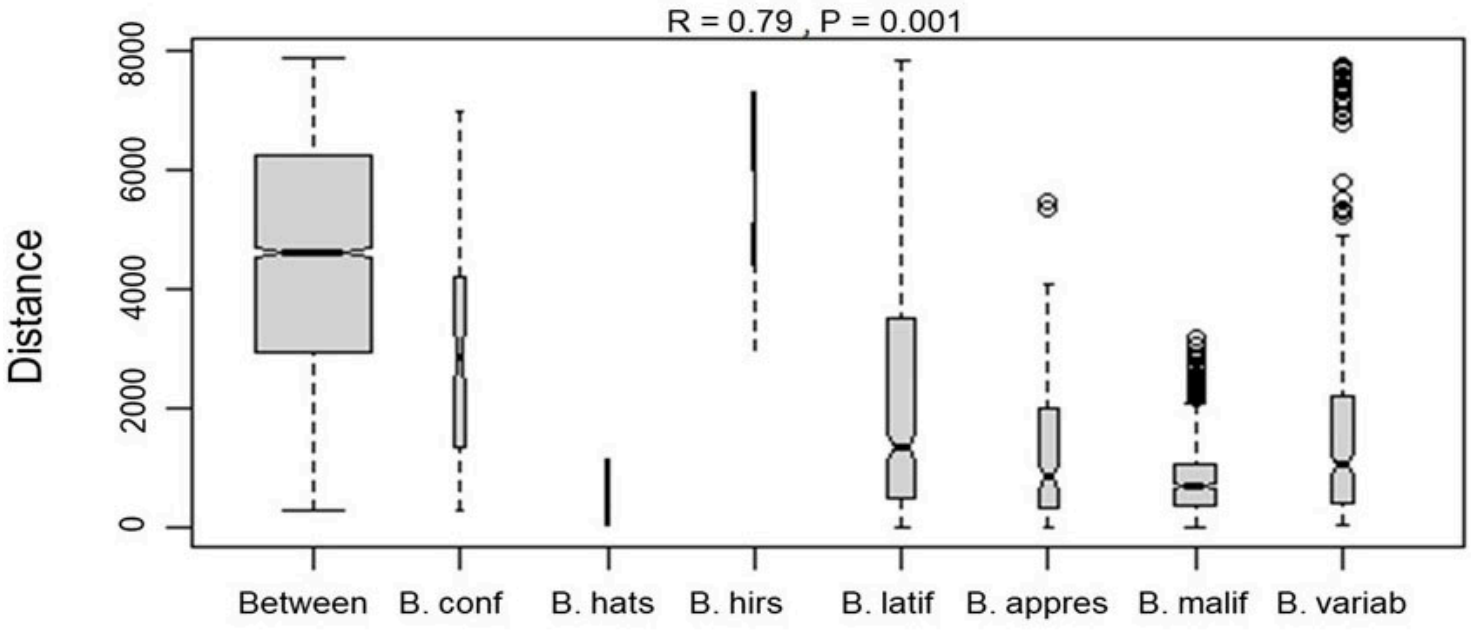


Figure 2

Relationship between geographical distance and morphological variety of species in the Malifolia group.

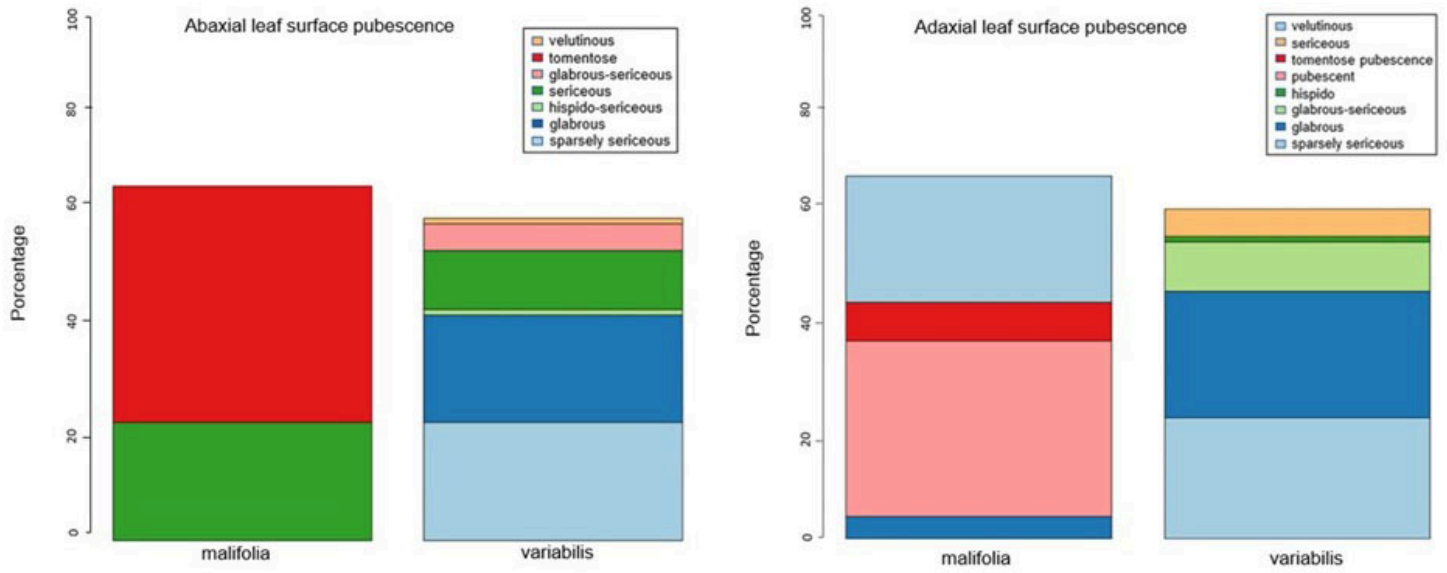


Figure 3

Percentage of varieties with leaf hairiness in the Malifolia and Variabilis subgroups, showing which characters are most significant in each subgroup.

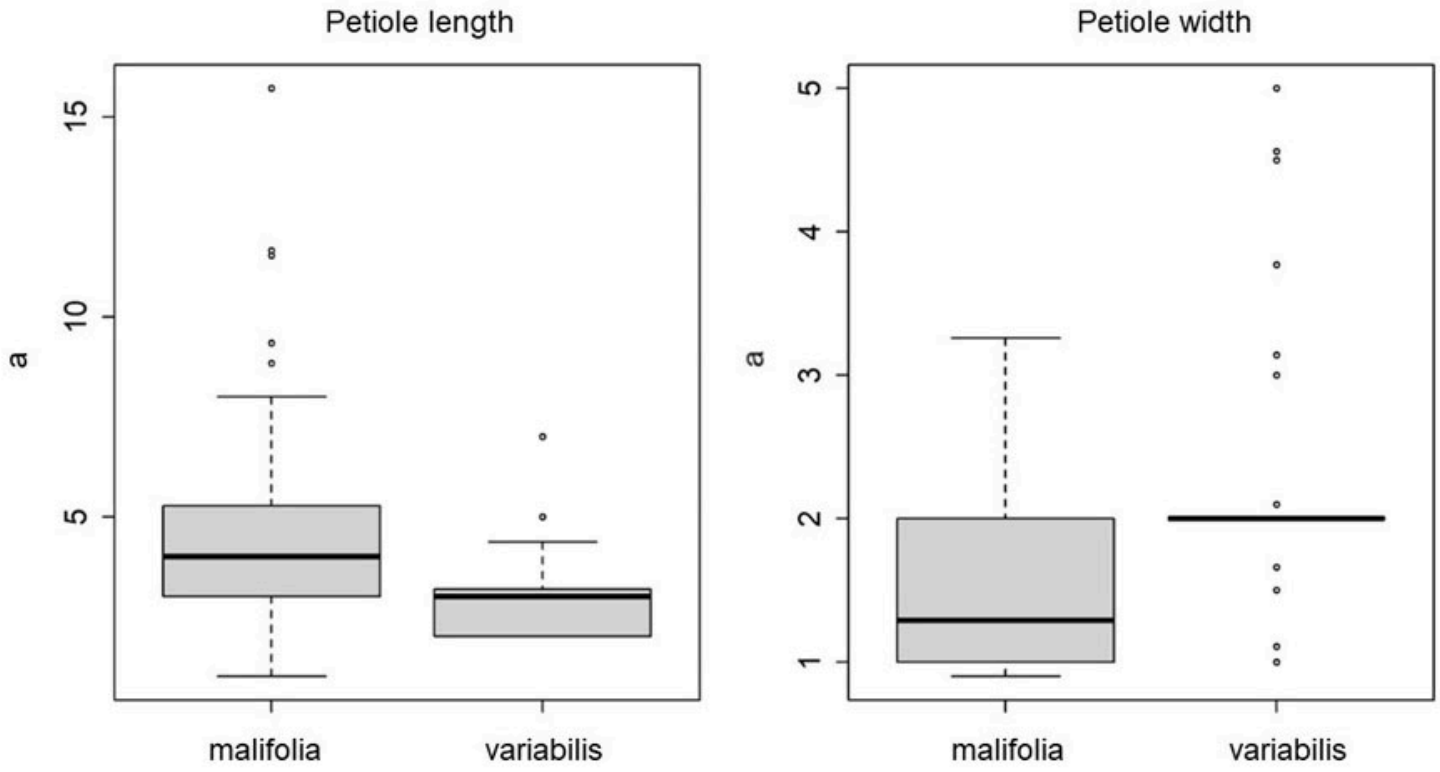


Figure 4

Boxplot illustrating Kruskal-Wallis analysis results for significant characters and subgroup variance within the Malifolia group. Outliers are denoted by black circles.

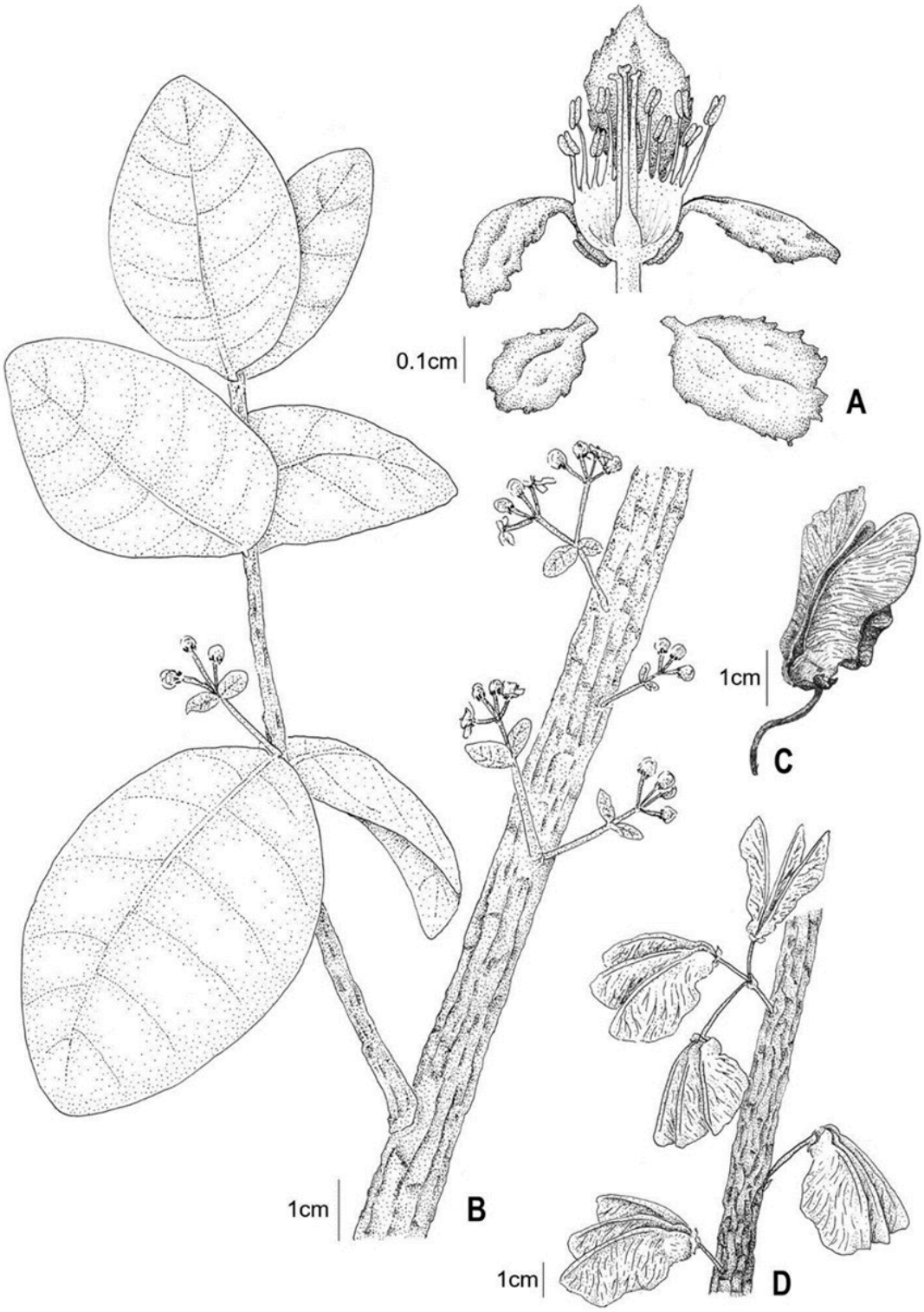


Figure 5

Banisteriopsis latifolia. A, Flower; B, Cauliflorous steem; C and D, Samarids showing the wings rotated to lie paralels. Illustrator: Gabriela Hirata.

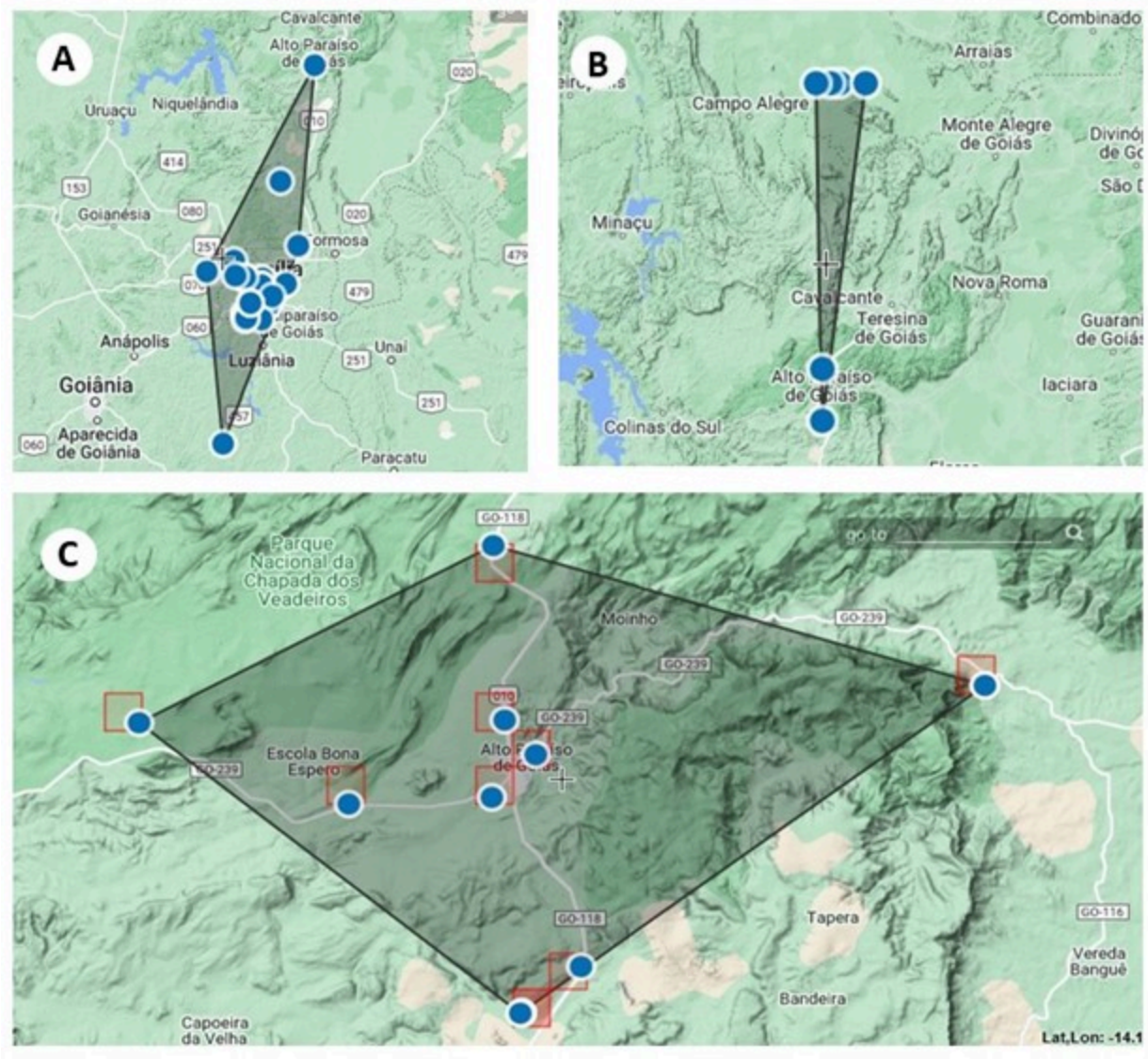


Figure 6

Area of occurrence of the following species: A, *B. latifolia*; B, *B. hirsuta*; C, *B. hatschbachii*.

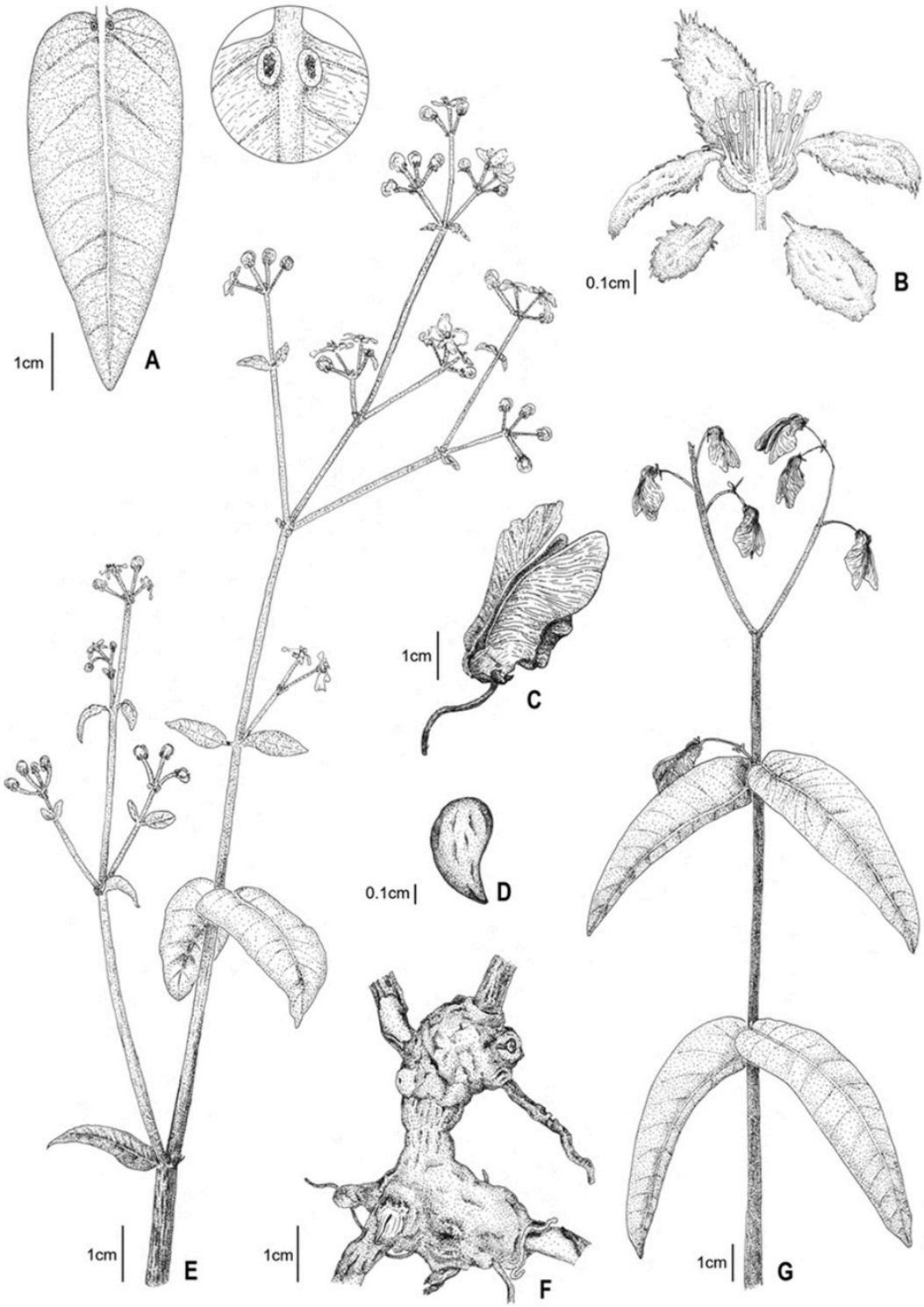


Figure 7

Banisteriopsis hatschbachii. A, Leaf highlighting the pair of proximal glands of leaf surface; B, Flower; C, Samarids; D, Seed; E, Steem with inflorescence; F, Xylopodium; G, Steem with samarids, showing the typical falcate leaves. Illustrator: Gabriela Hirata.

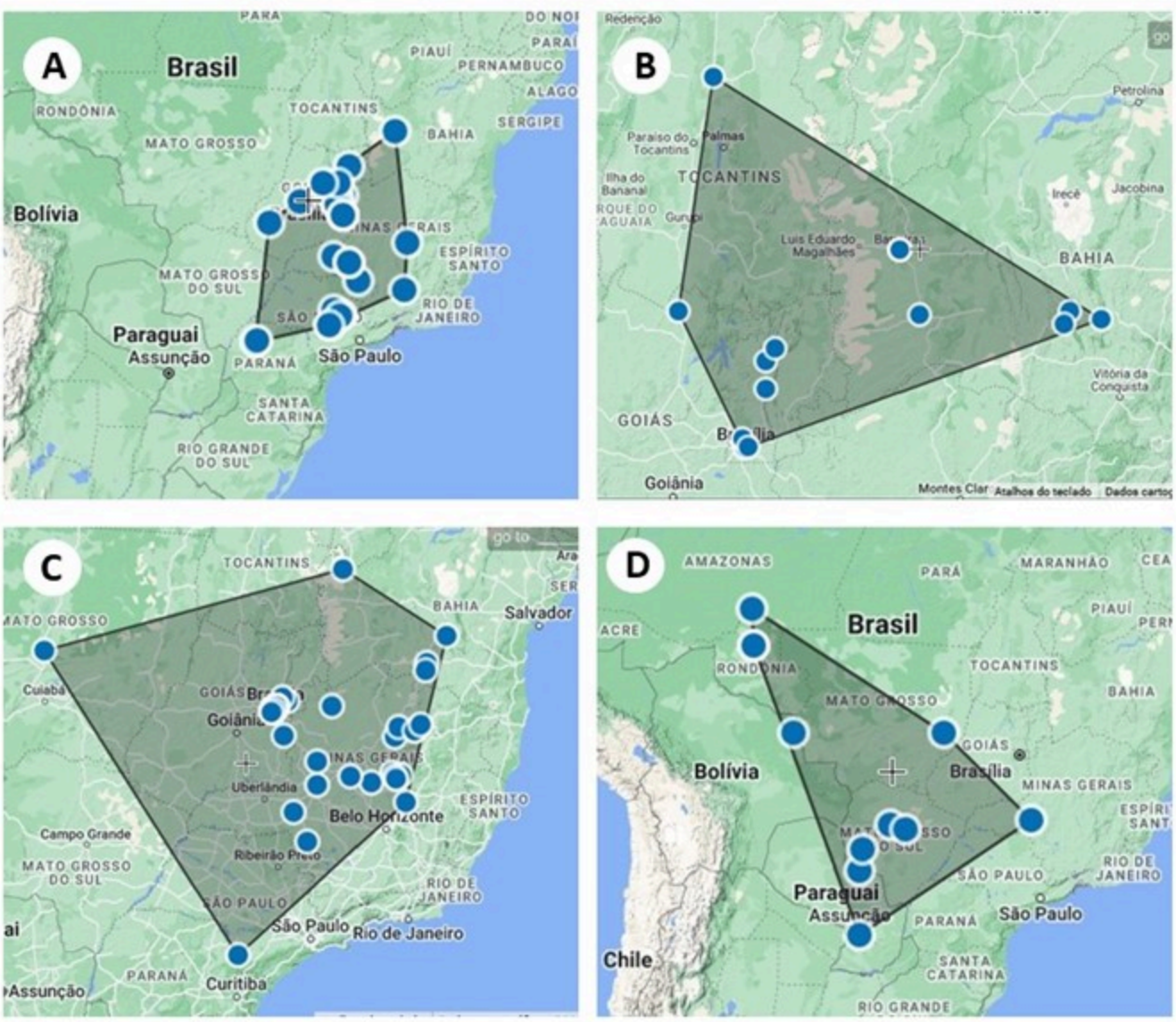


Figure 8

Area of occurrence of four species: A, *B. variabilis;* B, *B. malifolia var. appressa;* C, *B. malifolia var. malifolia;* D, *B. confusa.*

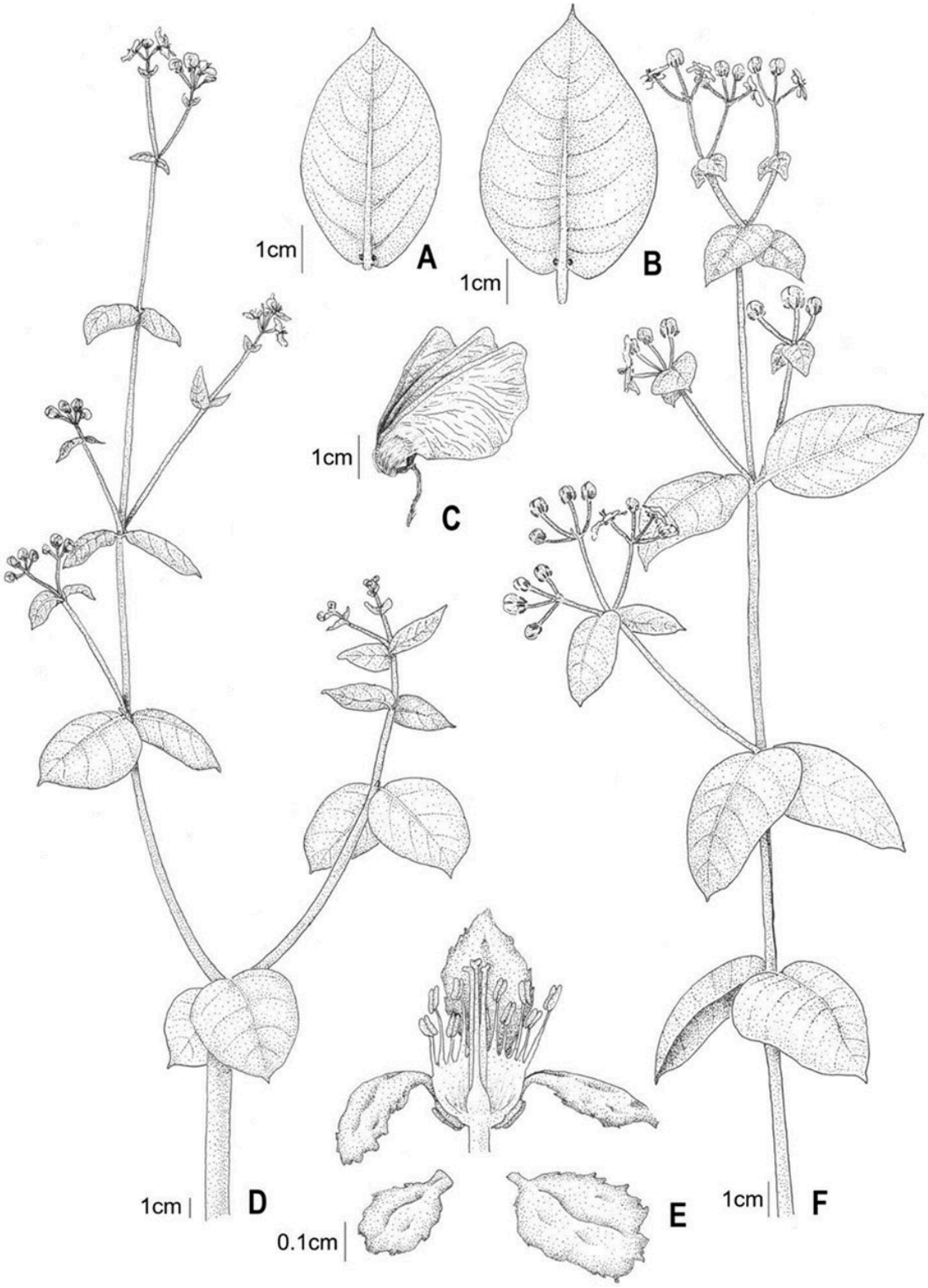


Figure 9

Banisteriopsis malifolia var. malifolia (A and D) and Banisteriopsis malifolia var. appressa (B and F). A and B, Leaves highlighted the pair of proximal glands on leaf surface; C, Samarids; E, Flower representative of two varieties. Illustrator: Gabriela Hirata.



Figure 10

Banisteriopsis variabilis; A, Habit; B, leaf with a pair of glands at base of central vein; C. Samarids with characteristic wings rotated to lie paralels of Malifolia group.

Supplementary Files

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- Supplementarymaterial.docx