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ORIGINAL RESEARCH

Testing the morphotype-based classification of two leaf shapes in *Ecclinusa guianensis* (Sapotaceae) from Central Amazonia

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Abstract
Ecclinusa guianensis (Sapotaceae, Chrysophylloideae) is a morpho-ecologically variable species and the second most widespread species of *Ecclinusa* in the Amazon basin. In Central Amazonia, two distinct leaf morphotypes can be field-recognized based on diagnostic morphology: elliptic-obovate and narrow-oblanceolate shapes. In this paper, we have explored the phenotypic differentiation between these two morphotypes of *E. guianensis* from Central Amazonia using morphometrics and near-infrared spectroscopy data. The results indicate the existence of two distinct groups that are sympatric. However, the narrow-oblanceolate leaf shape (subcanopy treelets or medium-sized trees) has never been collected with reproductive material, reinforcing the idea that it is a young phase of the elliptic-obovate leaf shape (large canopy trees). Therefore, we presume that *Ecclinusa guianensis* morphotypes probably correspond to a single species exhibiting phenotypic plasticity.

Keywords Amazonian tree flora · integrative taxonomy · leaf spectroscopy · morphometrics · phenotypic variation

Introduction

Ecclinusa Mart. is a small genus of the diverse family Sapotaceae (subfamily Chrysophylloideae) (Swenson & Anderberg, 2005) that shows remarkable vegetative and reproductive morphological variability. It comprises 12 accepted species distributed from Panama to South America in dry and wet tropical forests (Pennington, 1990; Terra-Araujo et al., 2015a). Members of *Ecclinusa* are shrubs, small subcanopy treelets to large canopy trees that are united by the following combination of characters: white latex, sessile flowers, and the absence of staminodes (Pennington, 1990, 1991; Terra-Araujo et al., 2015a; Faria et al., 2017). The genus has been recovered as monophyletic in several studies (Swenson et al., 2008; Terra-Araujo et al., 2015b; Faria et al., 2017) and is currently placed in a lineage of Chrysophylloideae that also encompasses *Elaeoluma* Baill., *Nemaluma* Baill., and *Ragala* Pierre (Swenson et al., 2023).

Brazil harbors at least seven known species of *Ecclinusa* (BFG, 2015, 2018; Pennington, 1990; Terra-Araujo et al., 2015a). Most of these are restricted to the Amazon basin, with the exception of the widespread species *Ecclinusa ramiflora* Mart., whose distribution extends to the Atlantic Forest in the northeast and southeast of Brazil (Pennington, 1990). In Amazonia, most *Ecclinusa* species grow in different forested landscapes such as non-flooded upland (*terra-firme*), floodplain (*igapó* and *várzea*), and white-sand (*campinarana*) forests (Pennington, 1990, 1991; Terra-Araujo et al., 2015a).

Ecclinusa guianensis Eyma was originally described by Eyma (1936). This species is morpho-ecologically variable and is the second most widespread species of *Ecclinusa* in the Amazon basin (Pennington, 1990), where it represents one of 227 dominant tree species in an assemblage estimated to contain around 16,000 tree species (ter Steege et al., 2013). In Central Amazonia, two distinct leaf morphotypes have been recognized in *Ecclinusa guianensis*, occurring along a rugged topographic gradient composed of plateaus, slopes, and valleys in dense *terra-firme* forests. The

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morphotype with elliptic-obovate leaves is found primarily on large canopy trees (> 12 m tall) having bark roughened by the presence of conspicuous conchoidal scars. On the other hand, the morphotype with narrow-oblanceolate leaves comprises subcanopy treelets or medium-sized trees (up to 12 m tall) having smooth or streaked bark (T.C.R. Carvalho & M.H. Terra-Araujo, pers. obs.). The former morphotype aligns more closely with the type material description based on overall foliage (Eyma, 1936) and field characteristics of the trees (Pennington, 1990). Both morphotypes are very abundant in the permanent plots of long-term biodiversity surveys and monitoring programs in the region, such as *Projeto Dinâmica Biológica de Fragmentos Florestais* (PDBFF hereafter) and *Programa de Pesquisa em Biodiversidade* (PPBio hereafter). They can be distinguished in the field based on the previously mentioned vegetative characteristics, but the presence of intermediates with high phenotypic overlap between the morphotypes can make their recognition difficult.

Accurate identification of species plays a critical role in most fields of biology (Bickford et al., 2007), but species complexes represent groups where the species number and circumscriptions remain unclear (Prata et al., 2018). In the highly diverse Amazonian flora (ter Steege et al., 2019; Cardoso et al., 2017), some researchers have investigated cryptic morphological variation in many species complexes (e.g., Terra-Araujo et al., 2012; Esteves & Vicentini, 2013; Prata et al., 2018; Damasco et al., 2019, 2021; Holanda et al., 2021). Clarifying the limits of each species within a species complex is one of the scientific tasks of taxonomy (Dayrat, 2005). It poses many challenges because some of the constituent species are often rare (with small population sizes and restricted geographic ranges) (Hubbell et al., 2008) and/or taxonomically poorly understood (Hopkins, 2019), several collections from local permanent plots are sterile (Gomes et al., 2013), and taxonomic studies and identification tools for regional flora are generally lacking (Prance, 2001). In addition, most undescribed species are concentrated in areas that are very diverse but have significant collection gaps due to poor sampling (Nelson et al., 1990; Hopkins, 2007; Schulman et al., 2007).

Species delimitation requires multiple sources of evidence (de Queiroz, 1998, 2007; Dayrat, 2005), and novel technologies that are both fast and inexpensive, along with analytical advances, have significantly enhanced the effectiveness and precision of species delimitation (Bik, 2017). Leaf spectra have been used to accurately discriminate taxa at different taxonomic levels (e.g., Durgante et al., 2013; Lang et al., 2017; Vasconcelos et al., 2020a, 2021; Gaem et al., 2022). This leaf spectral response is influenced by its internal chemical composition and anatomical structure (Asner & Martin, 2008; Feret & Asner, 2013). In addition to the traditional morphological approaches, scrutinizing the spectral signatures of near-infrared (NIR hereafter) using herbarium specimens can allow the discrimination of morphotypes even when there is a high phenotypic overlap among samples, especially when the reproductive material is unknown (Damasco et al., 2019; Gaem et al., 2022).

This study aims to explore the phenotypic differentiation between two morphotypes in *Ecclinusa guianensis* in an integrative taxonomic framework with morphometrics and NIR spectroscopy data.

Materials & Methods

Species concept. We adopted the unified species concept proposed by de Queiroz (1998, 2007), in which species are regarded as metapopulation lineages that evolve separately from one another. Under this definition, lineages that are morphologically distinct, but not necessarily monophyletic or reproductively isolated, may be regarded as species, depending on the criteria and methods used to make such inferences. Therefore, any information that provides evidence of the separation between lineages is relevant for inferring the boundaries and number of species.

Sampling. We examined a set of 204 specimens labeled as *Ecclinusa guianensis* from representative collections of INPA, RB, and MO herbaria (acronyms following Thiers, 2023 [continuously updated]). Additionally, our sampling incorporated specimens of local reference collections from permanent plots of PDBFF and PPBio in Central Amazonia. The JSTOR Global Plants database (https://plants.jstor.org/) were used to consult additional specimens (as digital images), including types. We separated these specimens through a morphotype-based classification into elliptic-obovate (Fig. 1A–C) and narrow-oblanceolate leaf types (Fig. 1D–F). Physical specimens examined in this study (see Supplemental Table S1) cover almost the entire distributional range of *Ecclinusa guianensis*, representing six countries (Brazil, Ecuador, French Guiana, Peru, Venezuela, and Suriname).

Geographic Distribution & Conservation Assessment. We also used web-based resources such as the Reflora Virtual Herbarium (https://floradobrasil.jbrj.gov.br/reflora/herbarioVirtual/), *species*Link (https://specieslink.net/search/), and Global Biodiversity Information Facility (GBIF, https://www.gbif.org/) to explore further these additional sources of occurrence data to supplement specimen-based distributional data for *E. guianensis*. The retrieved occurrence records were screened and flagged under the following criteria: missing coordinates, duplicates, points at sea, validity and zeros, and points whose latitude and longitude were identical. This filtering led to a processed dataset containing additional 118 unique occurrences. The distribution map was created using the QGIS v.3.28.1 (QGIS Development Team, 2022).

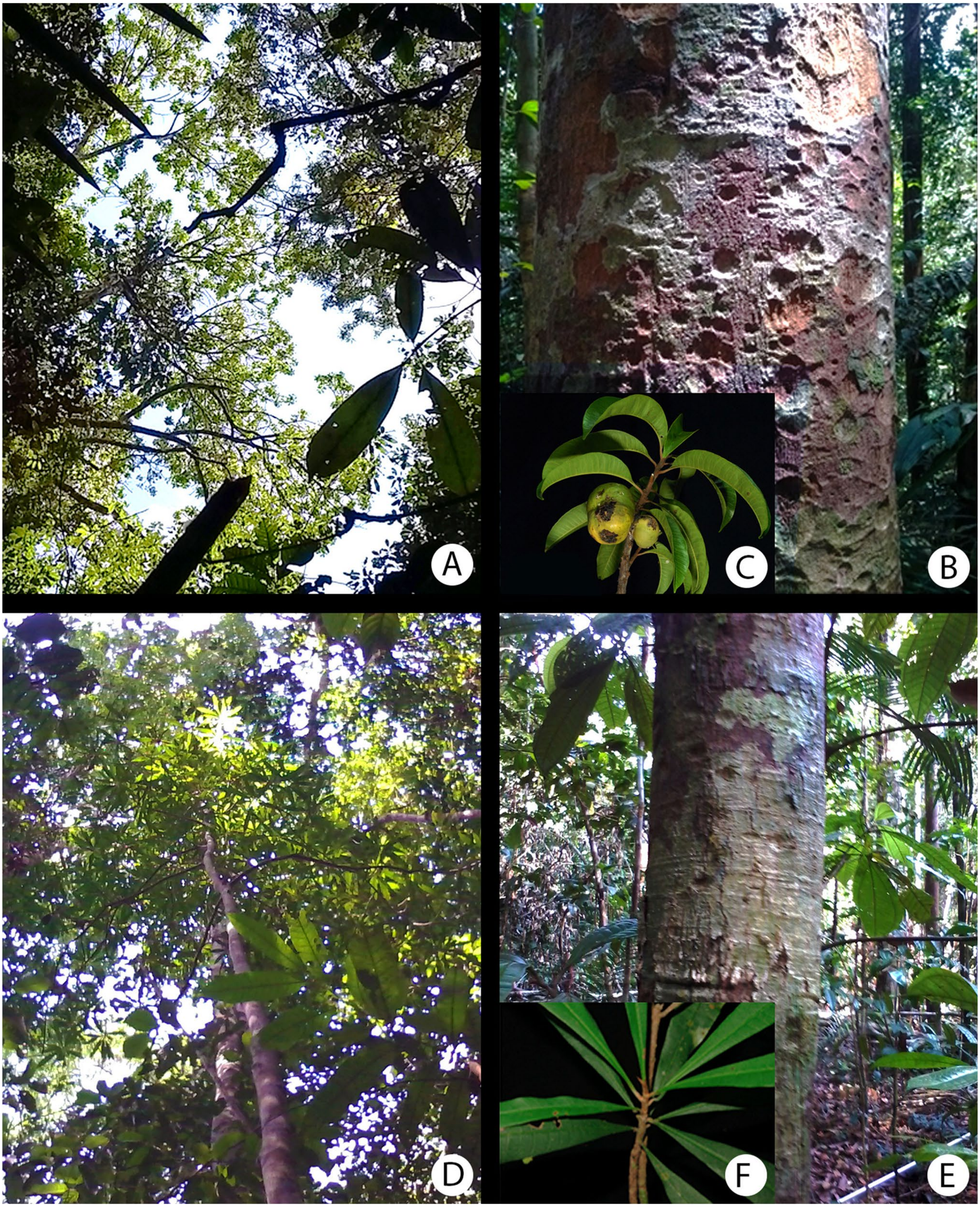

Fig. 1. Field images of two morphotypes assigned to *Ecclinusa guianensis* (Sapotaceae) in Central Amazonia. Elliptic-obovate leaf shape: **A.** Canopy tree view from below. **B.** Bark pattern. **C.** Fruiting branch. Narrow-oblanceolate leaf shape: **D.** Canopy tree view from below. **E.** Bark pattern. **F.** Sterile branch. (Photographs: A, B, D and E by C.C. Vasconcelos; C by I.D.K. Ferraz; F by T.C.R. Carvalho).

Table 1. List of quantitative characters used in the multivariate analyses of morphotype-based classification of *Ecclinusa guianensis.*

Label	Description	Type
LLWB	Length between leaf widest part and leaf-blade base	mm
TLL	Total leaf length (LL + PL)	mm
LL	Leaf-blade length (excluding petiole)	mm
LW	Leaf-blade width	mm
PL	Petiole length	mm
PSV	Pairs of secondary veins	count
SMW	Stipule width measured at widest part	mm
SL	Average stipule length	mm
LS1	Leaf shape 1 (LLWB / LL)	ratio
LS2	Leaf shape 2 (LW / LL)	ratio
LS3	Leaf shape 3 (PL / TLL)	ratio
VD	Venation density (PSV / LL)	ratio
NIR	Near infrared spectroscopy data represented by a single NMDS axis	score

Geo-referenced data were imported into the *ConR* package (Dauby et al., 2017) to assess conservation status following the IUCN Red List Categories and Criteria (IUCN, 2012; IUCN Standards and Petitions Committee, 2022). We calculated the parameters of the Extent of Occurrence (EOO) and the number of subpopulations (5 km radius).

Morphometric & Near-infrared Spectroscopy Data. Only dried specimens were used for both measurement techniques. Based on initial observation, a total of 13 characters were selected as informative (eight quantitative, four ratios, and one a score; see Table 1). For individual-based morphometrics, each specimen was measured by a single person (T.C.R. Carvalho) to minimize biases, using a digital caliper set for millimeters (0.01 mm).

Additionally, leaf absorbance spectra were obtained using a Fourier Transform Near-Infrared (FT-NIR) Spectroscopy Analyzer from Thermo Fisher Scientific, model Antaris II employing the *Result*™ software. Whenever possible, four readings were obtained from at least two leaves per specimen (two readings on each of the upper and lower surfaces of each leaf). Each reading consists of 1,557 values of near-infrared leaf absorbance at 10,000 to 4,000 cm^{-1} wavenumbers. Each measurement produced by the instrument was an average of 16 scans at a resolution of 8 cm^{-1}. A black rubbery body of ethylene vinyl acetate copolymer (EVA) was used in the reading area of samples to avoid light scattering following Durgante et al. (2013). The background was calibrated automatically by the instrument every 4 hr.

Data Preparation & Multivariate Analyses. Because many floral characters were missing in most specimens, we removed such unavailable data from further analyses, focusing exclusively on vegetative characters. Morphometric variables (excluding ratios) were computed as the averages of two or more measurements taken from the same specimen. Similarly, for spectral variables, we utilized the average of readings per specimen. The spectral data underwent conversion from wavenumbers (cm^{-1}) to wavelengths (nm). Subsequently, we processed this data by removing erroneous scans with absorbance values exceeding 1.0 and regions of higher noise (2,401 to 2,500 nm; Cavender-Bares et al., 2016), as well as regions affected by water absorption (1,300 to 1,460 nm, 1,750 to 2,030 nm, 2,040 to 2,060 nm, 2,135 to 2,155 nm, and 2,153 to 2,173 nm; Thenkabail & Lyon, 2016; Wang & Gamon, 2019). The set of processed raw spectra was then reduced to a single axis of Non-metric Multidimensional Scaling (NMDS) (Kruskal, 1964) using Gower's distance (Gower, 1971). The NMDS-reduced axis was used to represent NIR spectra in the data matrix, alongside morphometric measurements and ratios. Before the formal analyses, we employed the K-means algorithm (MacQueen, 1967) with different approaches (Elbow and Silhouette methods) to assess clustering trends without a *priori* grouping. Both methods consistently identified k=2 as the optimal number of groups for our dataset (Supplemental Figure S1). Prior to the analyses, all characters were scaled to have unit variance, except for hierarchical clustering analysis.

We performed the Principal Component Analysis (PCA) (Pearson, 1901) to compare the relative contribution of the morphometric and spectral data, both separately and in combination. Identification of clusters and distances in morphospace was performed via the Discriminant Analysis of Principal Components (DAPC), which uses the transformation of variables by PCA before performing Discriminant Analysis (DA). This ensures that variables are uncorrelated, making it a suitable methodological approach for morphological data, which typically have a high degree of correlation (Jombart et al., 2010). Based on DAPC results, probabilities of group membership were also calculated for each individual, and the leave-one-out cross-validation was performed to assess the robustness of the morphotype-based classification using different models (datasets): (1) morphometrics, (2) raw NIR spectra, (3) morphometrics + raw NIR spectra, and (4) morphometrics + NMDS single-axis of NIR spectra. Finally, we defined the accuracy of the DAPC models as the ratio of the number of correctly predicted samples to the total number of samples, and the correct and incorrect predictions were summarized in confusion matrices. In addition, the t-test (Student, 1908) was applied to detect differences between groups using the first axis of the discriminant function. We also performed a Hierarchical Agglomerative Clustering (HAC) analysis with Ward's linkage (Ward, 1963) and Gower's distance to visualize the formation of progressive clusters through a dendrogram. All analyses were conducted using add-on packages (Supplemental Table S2) within the R environment v.4.2.2 (R Core Team, 2022) unless otherwise stated.

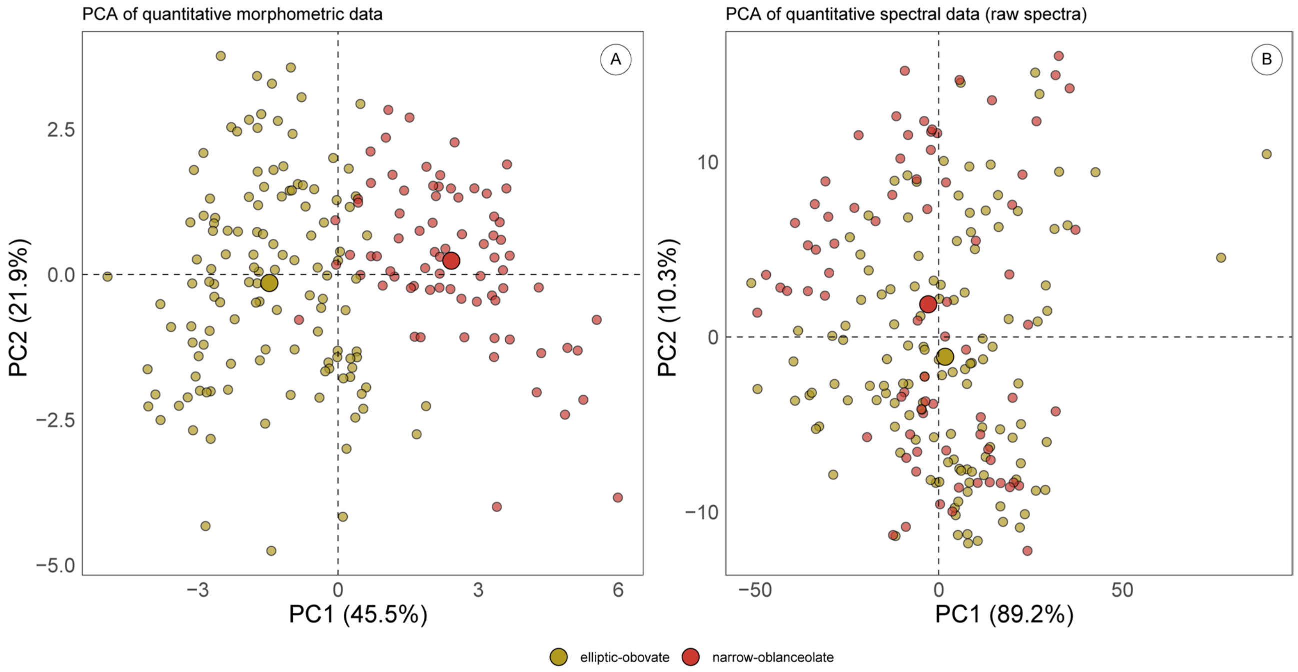

Fig. 2. Principal Component Analysis (PCA) results. **A**. Scatterplot of the first two principal components for quantitative morphometric data. **B**. Scatterplot of the first two principal components for quantitative spectral data. The points were color-coded by groups, considering the complete set of *Ecclinusa guianensis* specimens.

Results

Morphometric & Spectral Variability. Regarding the relative contribution of different datasets, the first two PCA axes captured 67.4% of the total variation in morphometric data and 99.5% in spectral data (Fig. 2). The first axis alone explains about half of the total variation in morphometric data and almost 90% in spectral data. When evaluating the inferred morphotypes in two-dimensional space, we detected a clear pattern: morphometric data show little overlap between individuals of different morphotypes, revealing a clear separation between them. On the other hand, despite the highly explained variance in the first two PCA axes, spectral data alone showed considerable overlap, with no apparent separation pattern among morphotypes (Fig. 2).

When considering the 'Morphometrics + NMDS single-axis NIR spectra' dataset, the first two PCA axes accounted for 62.6% of the total variation observed, highlighting a minimal overlap between morphotypes (Fig. 3). The first axis is primarily defined by five variables related to blade shape (LS2, LS3, TLL, LL) and venation (PSV). The second axis is defined jointly by five variables related to venation (VD), leaf blade (LLWB, TLL, LW), and petiole (PL). The third axis is also defined by five variables related to stipule (SMW), leaf blade (LS1, LS3, LW), and petiole (PL). The fourth axis is defined only by two variables related to the stipule (SMW, SL). The fifth PCA axis is defined exclusively by the variable representing the NIR spectral response, which shows the highest correlation compared to the other obtained PCA axes (Table 2). The explained proportions by all PCA axes using the 'Morphometrics + NMDS single-axis NIR spectra' dataset are also available in Supplemental Table S3.

Discriminant Models & Hierarchical Clustering for Predicting Groups. Our hypothesis of two groups in *Ecclinusa guianensis* from Central Amazonia was well supported in all models tested with leave-one-out DAPC (accuracies $>$ 97%) using vegetative variables, except when considering spectral data alone (Table 3). Contrary to our expectations, the model using morphometrics alone performed best in discriminating the inferred groups. However, we included the 'Morphometrics + NMDS single-axis NIR spectra' model in all analyses because of its high accuracy and its ability to include both spectral and morphometric variation. Confusion matrices for all models tested are provided in Supplemental Figure S2.

Most individuals were confirmed in their originally assigned morphotypes with the combined 'Morphometrics + NMDS single-axis NIR spectra' dataset (Fig. 4A), showing low incongruence between posterior association assignments. Comparing the morphotypes on the first axis of the discriminant function (LD1) using the t-test revealed

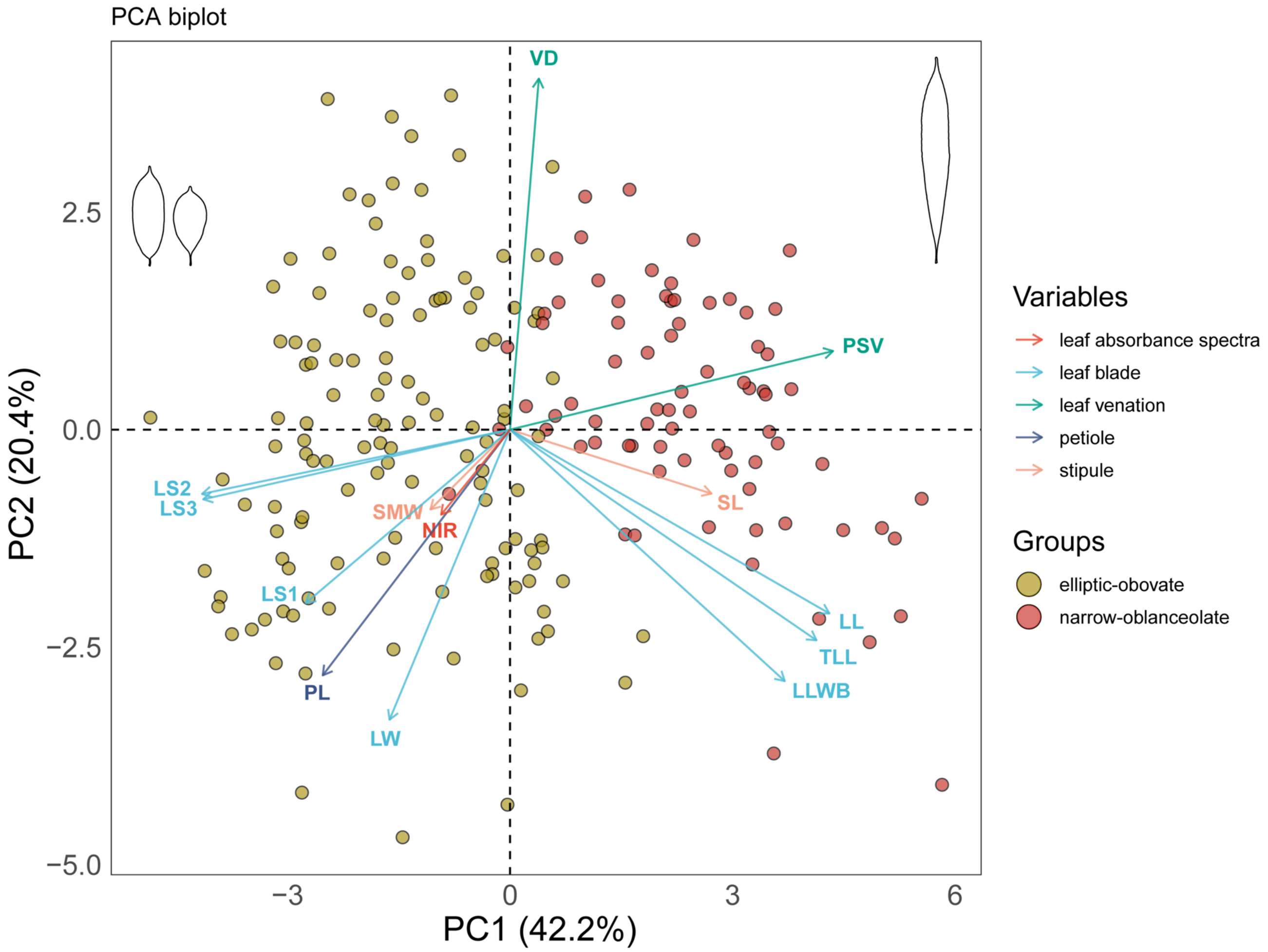

Fig. 3. Principal Component Analysis (PCA) plot of the first two PC axes for all individuals of *Ecclinusa guianensis* based on the phenotypic variation (morphometric and spectral data). Colored shapes refer to groups and vectors to quantitative variables. The acronyms correspond to the quantitative variables listed in Table 1.

significant differences (p-value < 0.0001) (Fig. 4B). The morpho-spectral variation of each morphotype to the LD1 axis can be visualized in Fig. 4C and concerning the original variables in Fig. 5.

The dendrogram-based clustering analysis (Fig. 6), built with the 'Morphometrics + NMDS single-axis NIR spectra' model, shows a visually clear division of *Ecclinusa guianensis* specimens into two broad groups that mostly correspond to the originally assigned morphotypes. Group 1 consists of specimens of the narrow-oblanceolate morphotype, while Group 2 consists of specimens of the elliptic-obovate morphotype mixed with some narrow-oblanceolate specimens, comprising a much larger number of specimens than Group 1. This separation demonstrates a strong correspondence between the morphometric and spectral characteristics of the specimens and the initially assigned morphotypes.

Only about 8% of the specimens from the narrow-oblanceolate morphotype were included in the elliptic-obovate group, and no specimens from the latter morphotype were included in the narrow-oblanceolate group in the dendrogram (Fig. 6), supporting the validity and consistency of the morphotype-based classification.

Distribution and informal conservation status. The geographic distribution of *Ecclinusa guianensis* is shown in Fig. 7, with the distribution primarily concentrated in the northeastern (NE), northwestern (NW), and central subregions of Pan-Amazonia. Overall, the species has a wide ecological range and can occur in a variety of habitats such as highland, upland, and lowland landscapes, in both moist and dry forests, ranging from open areas (e.g., savanna) to densely forested regions (e.g., *terra-firme*), with an elevational range from near sea level to 1,400 m.

Table 2. Quantitative characters (Morphometrics + NMDS single-axis NIR spectra dataset) used in the multivariate analyses of morphotype-based classification of *Ecclinusa guianensis*. PC1 to PC5: correlation between the original variables and the first five principal components, respectively. Correlation (r) ≥ 30% is highlighted in bold.

Label	PC1	PC2	PC3	PC4	PC5
SMW	-0.08945079	0.1104934	**-0.47530779**	**0.64462165**	-0.007831701
SL	0.24002641	0.08975549	-0.26839514	**0.47289437**	-0.28556547
LS1	-0.23788669	0.25272685	**-0.36450337**	-0.2689772	0.023050288
LS2	**-0.36537371**	0.10088754	0.24866902	0.23753	-0.005592354
LS3	**-0.3615067**	0.10310905	**-0.31273043**	-0.1617929	0.133248005
VD	0.02901737	**-0.51544217**	-0.2837362	-0.03453431	0.033774606
LLWB	0.33195804	**0.36028565**	-0.08548748	-0.1345793	0.069615648
TLL	**0.36877942**	**0.30076224**	-0.01175467	-0.07610658	0.056687799
LL	**0.38343134**	0.26189542	0.01653029	-0.0590524	0.057498267
LW	-0.13864179	**0.42787702**	**0.39068104**	0.26338368	0.031691278
PL	-0.21550893	**0.35652696**	**-0.34856343**	-0.20585555	0.213120123
PSV	**0.38430743**	-0.1238126	-0.20536711	-0.05883677	0.080178637
NIR	-0.07872048	0.12247951	-0.07083571	-0.24918833	**-0.913626646**

Table 3. Performance of morphotype-based classification of *Ecclinusa guianensis* using DAPC models with leave-one-out cross-validation built using different datasets.

Model	Accuracy (%)	CI (95%)	Misclassifications
Morphometrics	99.5	97.3–99.9	1
Raw NIR spectra	76.9	70.5–82.5	43
Morphometrics + Raw NIR spectra	97.5	94.3–99.2	4
Morphometrics + NMDS single-axis NIR spectra	99.0	96.5–99.8	2

Ecclinusa guianensis is known from 76 subpopulations, giving an EOO of 5,484,097 km^2. The species is widespread (Fig. 7), frequently abundant in the localities where it occurs (Zent & Zent, 2004; ter Steege et. al., 2013; Nascimento et al., 2014), and seedlings are often seen in the field (C.C. Vasconcelos, pers. obs.). Due to its large EOO, its presence in several protected areas, and the absence of any direct threats, *E. guianensis* is assigned a preliminary IUCN status of Least Concern (LC).

Discussion

Overall, the scarcity of detailed studies on the conditions of species coexistence within species complexes, coupled with significant effort gaps in botanical collections, helps to account for the limited knowledge of plant diversity in the Amazonian flora, where many species marked by polymorphic morphologies and wide distributions may in fact represent complexes encompassing two or more cryptic species (Esteves & Vicentini, 2013). Our findings support the existence of two morphologically and spectrally distinct groups within *Ecclinusa guianensis* (Figs. 2–6), which co-occur in Central Amazonia. If reproductive characters were available for both morphotypes, the two groups could be tested as separate taxa. However, since this is not the case, it is more likely that there is phenotypic plasticity within the same species. Both groups occupy different niches within the same environment, with individuals from the elliptic-obovate group inhabiting the canopy and those from the narrow-oblanceolate group inhabiting the subcanopy (Fig. 1A

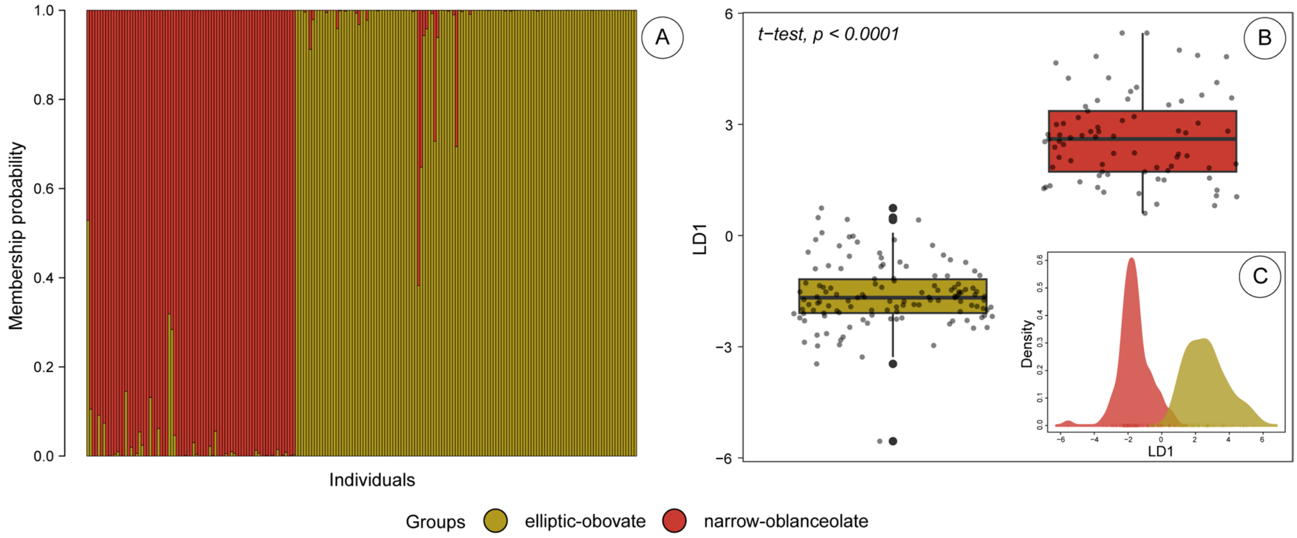

Fig. 4. **A** Membership probability obtained from DAPC, where each vertical-colored line represents an individual specimen of *Ecclinusa guianensis* and mixture clusters refer to the incongruity between membership posterior assignments. **B.** Boxplot of the t-test result comparing the two morphotypes by the first linear discriminant axis (LD1). **C.** Density plot of LD1 by groups.

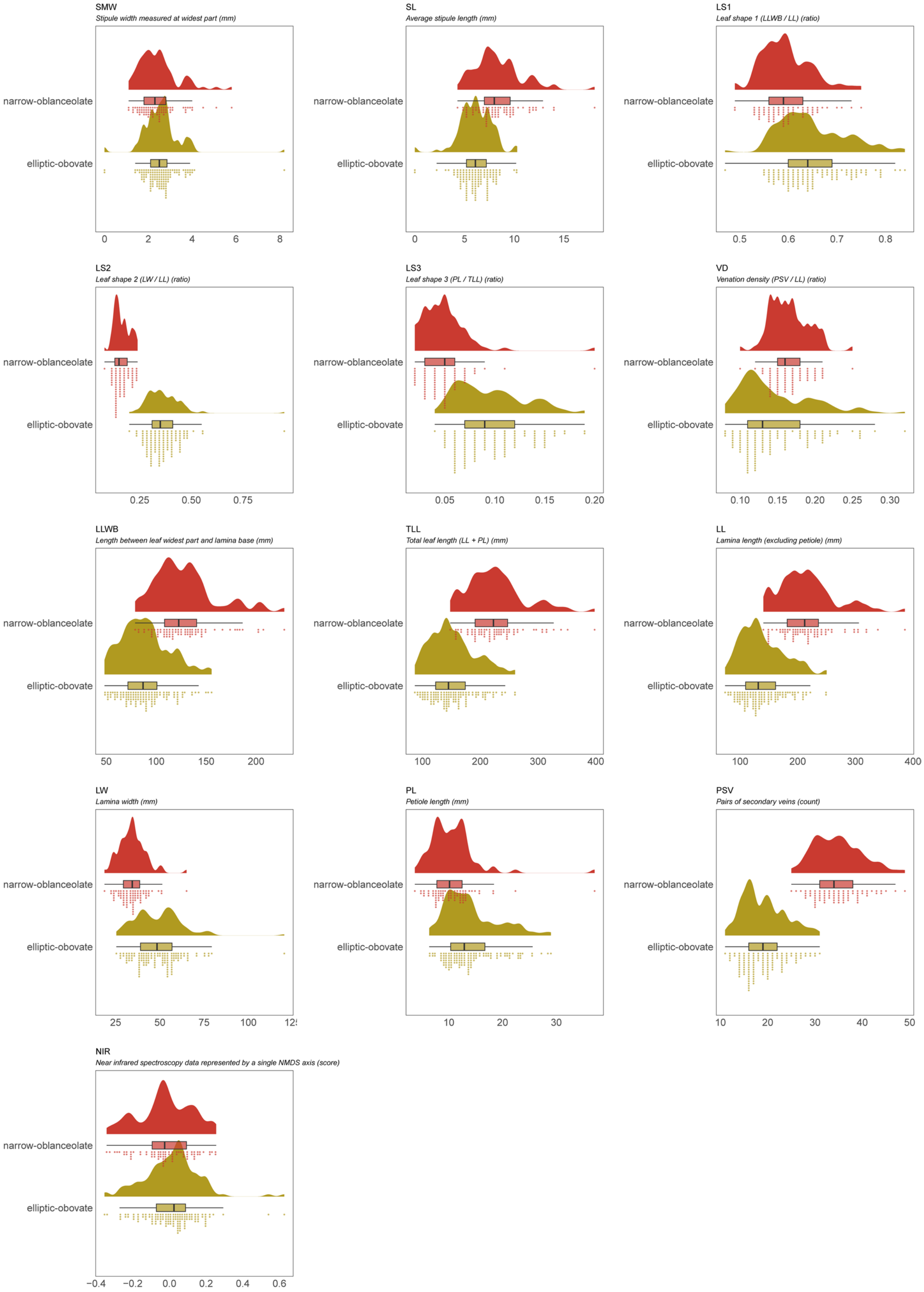

Fig. 5. Raincloud plots for the 13 quantitative characters used in the multivariate analyses of morphotype-based classification of *Ecclinusa guianensis.*

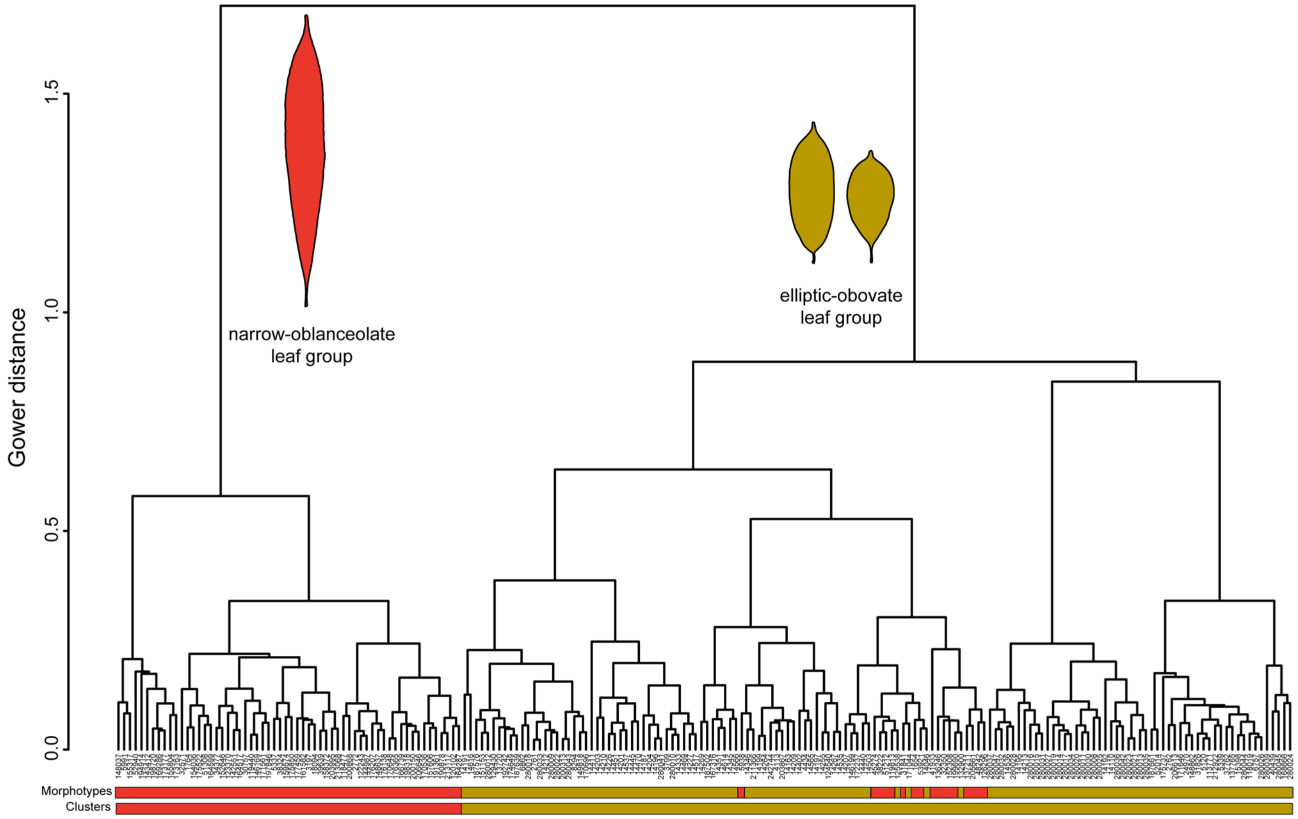

Fig. 6. Ward hierarchical clustering applied to the complete set of *Ecclinusa guianensis* specimens and 13 quantitative variables (see Table 1) using Gower distance.

and D), in addition to other morphological characteristics traditionally used to recognize these morphotypes.

Classical methods commonly used for taxonomic studies involve examining and measuring morphological characters. In this study, specimens were satisfactorily classified solely based on vegetative morphometric data (Table 3 and Fig. 2), which were then used to differentiate between the two morphotypes, since the variables most strongly correlated with the ordination axes primarily correspond to leaf shape and size (Fig. 3). By integrating morphological data with spectral data, the separation of the species into two groups remained highly consistent (Fig. 3 and Table 3). A few samples exhibited discrepancies in classifications, suggesting the presence of intermediate individuals between the morphotypes, and thereby generating uncertainties regarding their assigned group (Fig. 4 and Supplemental Figure S2).

Phenotypic plasticity denotes the ability of individual genotypes to produce different phenotypes in response to different environmental conditions (Schlichting, 1986), such as herbivory, light, water, nutrients, CO_2 and predation (Arnold et al., 2019). This response can include adaptive or non-adaptive changes to individual biochemistry, physiology, morphology, behavior, or life history (Whitman & Agrawal, 2009). For *Ecclinusa guianensis*, the explanation of phenotypic plasticity is plausible, given the complete lack of reproductive data in samples from the narrow-oblanceolate group, in contrast to the approximately 24% of samples from the elliptic-obovate group that contain reproductive data. This suggests that these individuals might not have reached reproductive maturity and were either juveniles mixed with adult plants (Gomes et al., 2013) or were collected outside the reproductive period — a scenario that would be unlikely to remain undetected during monitoring in these plots. Consequently, no differentiation in terms of reproductive characters could be observed between the morphotypes.

Recent studies in Central Amazonia have shown that young and adult individuals of the same species can have different spectral signatures, with an accuracy of over 80% when young samples represent at least 20% of the total samples analyzed (Lang et al., 2015). For *Ecclinusa guianensis*, around 37% of the analyzed specimens are samples of the narrow-oblanceolate morphotype, which we hypothesize could be from young individuals. This would explain the high accuracy of the adopted classification (Table 3). Such a separation in the spectral signature between young and adult specimens would be expected because leaves are composed of complex organic compounds that can exhibit

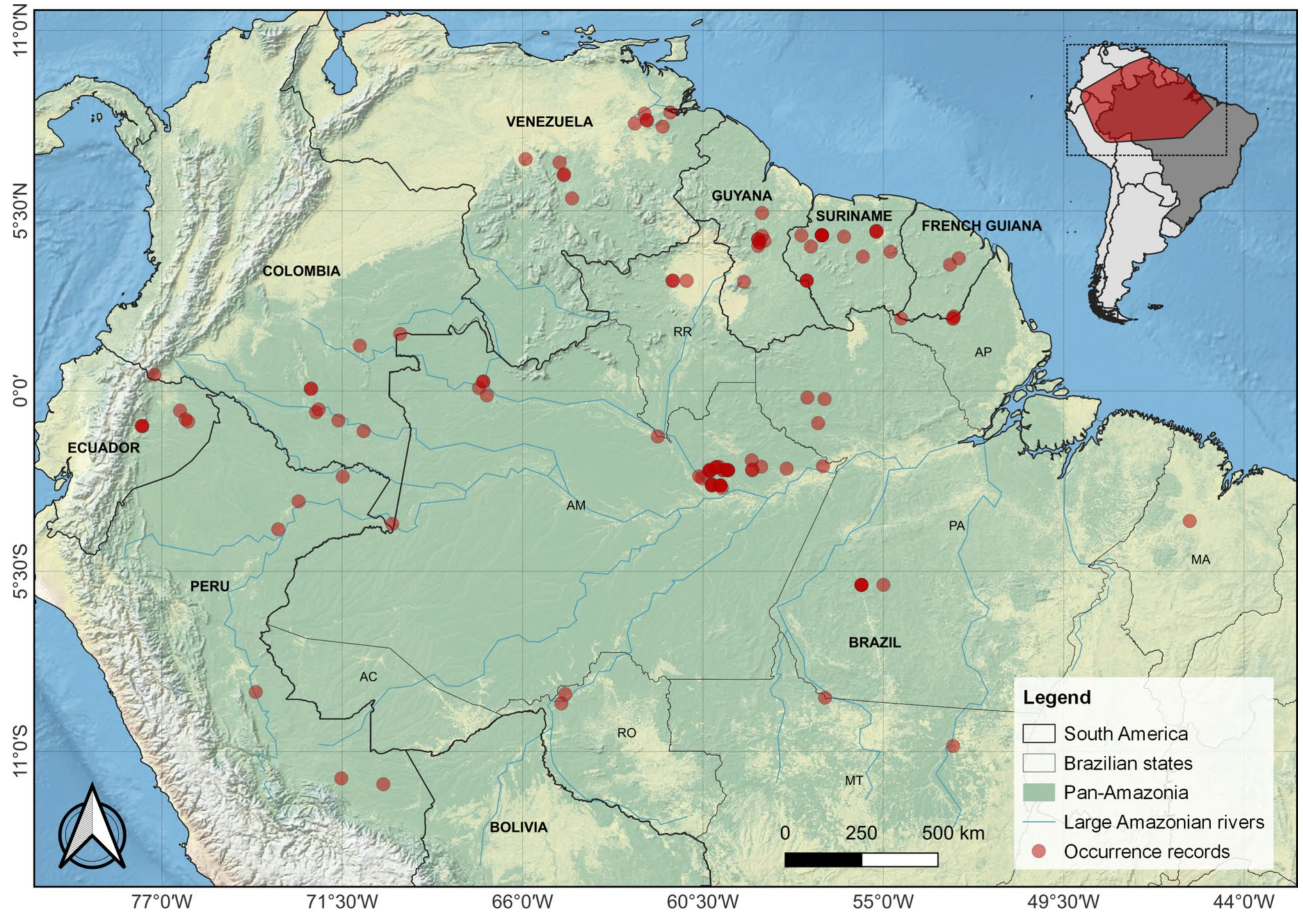

Fig. 7. Known geographic distribution of *Ecclinusa guianensis* in Pan-Amazonia. The base map and country borders are sourced from Natural Earth (https://www.naturalearthdata.com/) and the Brazilian state borders from IBGE (https://www.ibge.gov.br/).

different responses when exposed to infrared light (da Luz, 2006; Lang et al., 2015), as observed in young and mature leaves of *Eschweilera amazoniciformis* S.A.Mori (Lecythidaceae) by Durgante et al. (2013) and *E. guianensis* in our study.

Leaves from different stages of the growing season (e.g., young vs. mature leaves), differences between adaxial and abaxial leaf surfaces (e.g., presence of trichomes and waxes), and sun vs. shade leaves (e.g., canopy vs. subcanopy or understory) are factors that can introduce intraspecific spectral variability (da Luz, 2006). This is because tree leaves undergo chemical, morphological, and structural changes during development that may reveal distinct spectral signatures between young and adult individuals (Lang et al., 2015), as well known for leaf shape and size (Dang-Le et al., 2013), and phenology (Mediavilla & Escudero, 2009), for example. In addition, these changes include functional and ecological modifications in photosynthetic capacity and water usage (Cavender-Bares & Bazzaz, 2000; Mediavilla & Escudero, 2004), as well as biomass distribution and carbon-rate balance (Lusk, 2004).

Although the morphological and spectral evidence indicated the presence of two groups in *Ecclinusa guianensis*, the current data do not allow testing of these morphotypes as separate species due to the absence of flowers and fruits for the narrow-oblanceolate morphotype. Even though the two morphotypes are frequently found in in permanent plots with continuous monitoring in Central Amazonia, but the narrow-oblanceolate morphotype has never been collected or observed in a reproductive state, unlike the elliptic-obovate morphotype (about 24% of samples with reproductive material). Therefore, we assume that the two morphotypes of *E. guianensis* represent a single species that shows phenotypic plasticity. To date, the relationship of the evident leaf differentiation between young and adult individuals has not been directly tested with environmental factors, and further field observations and long-term monitoring of growing individuals may help to confirm details of leaf development.

The restricted occurrence of these morphotypes in Central Amazonia, rather than across the entire climatically favorable distributional area of *Ecclinusa guianensis* (Fig. 7; Vasconcelos et al., 2020b), may also reflect a sampling issue resulting from the limited botanical exploration that has occurred in many areas in the Amazonian region (Hopkins, 2019). Nonetheless, the detection of these two morphotypes underscores the crucial role of relatively well sampled and collected permanent plots in this region.

Conclusion

The two morphotypes of *Ecclinusa guianensis* investigated in this study showed remarkable phenotypic differentiation when combining morphometric and spectral data, but the size class of each morphotype and the lack of reproductive structures associated with the smaller morphotype (with narrow-oblanceolate leaves) suggests that morphotype represents an immature stage in the development of the same species that goes on to produce elliptic-obovate leaves. This explanation seems to be more plausible for the classification based on leaf shape variation. For the time being, we presume that the two morphotypes of *E. guianensis* coexisting in the Central Amazonia probably correspond to a single species in this region. Integrating morphometric and spectral approaches with molecular data could permit further explorations of species boundaries in *Ecclinusa*, thereby adding robustness to results.

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Author's Contributions

TCRC conceived the study, collected and compiled the morphological and spectral datasets, contributed to data analyses, and wrote the earlier draft of the manuscript; CCV contributed to data analyses, interpreting and discussion, and writing the manuscript; MHTA conceived the study, supervised the first author and contributed to the data interpretation and discussion. All authors provided critical reviews to improve the manuscript.

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Declarations

Competing Interests

The authors have no competing interests to declare that are relevant to the content of this article.

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