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Studies in Biogeography, Conservation, and Systematics of *Peperomia* (Piperaceae): Deciphering the historical biogeography, exploring Brazilian endemism and conservation, and bringing to light a new threatened Cerrado species

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Doctoral thesis submitted to the Graduate Program in Botany at the *Universidade de Brasília*, in fulfillment of the requisite conditions for the conferment of the Doctorate degree in Botany.

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Abstract

Peperomia, one of the most diverse genera among angiosperms, is abundantly present in the Neotropical region and displays a pantropical distribution. While nearly half of its species are epiphytic, the lineages that diverged earlier from the rest of the genus consist of terrestrial species. Despite its remarkable diversity, comprehensive investigations into the historical biogeography, chorological details, and conservation status of *Peperomia* species remain notably limited. Ranked fourth globally in *Peperomia* species richness, Brazil is home to 169 species, with approximately two-thirds of them being endemic, and having the Atlantic Forest Domain as the habitat for the majority of these species. To address knowledge gaps within the *Peperomia* genus, this thesis comprises four chapters, each adopting a scientific article format. Chapter 1 delves into historical biogeography using molecular and geographic data to elucidate the genus's origin and major clades, laying the foundational groundwork for numerous evolutionary studies. Chapter 2 analyzes occurrence data from open-access databases for all endemic *Peperomia* species in Brazil, conducting chorological analyses and preliminary assessments of extinction risk for each. In Chapter 3, using the same dataset, insights are provided into areas of endemism for the *Peperomia* genus within Brazil, including investigations into land use and land cover changes over a three-decade span. Finally, Chapter 4 introduces a new critically endangered *Peperomia* species endemic to the Brazilian Cerrado.

Keywords: Biogeography; Conservation; Neotropics; Piperoideae; Systematics.

Resumo

Título em português brasileiro:

Estudos em Biogeografia, Conservação e Sistemática de *Peperomia* (Piperaceae): Decifrando a biogeografia histórica, explorando a conservação e o endemismo brasileiro, com a descrição de uma nova espécie ameaçada do Cerrado

Peperomia, um dos gêneros mais ricos dentre as angiospermas, possui maior concentração de espécies na região Neotropical e exibe distribuição pantropical. Embora quase metade de suas espécies sejam epífitas, as linhagens que divergiram mais cedo do restante do gênero são compostas por espécies terrestres. Apesar de sua notável diversidade, investigações abrangentes sobre a biogeografia histórica, detalhes corológicos e o estado de conservação de suas espécies permanecem notadamente limitadas. Estando em quarto lugar em riqueza de espécies de *Peperomia* em nível global, o Brasil abriga 169 espécies, com aproximadamente dois terços delas sendo endêmicas, e tendo o domínio da Mata Atlântica como habitat para a maioria delas. Para preencher lacunas no conhecimento dentro do gênero *Peperomia*, esta tese é composta por quatro capítulos, cada um adotando o formato de artigo científico. O Capítulo 1 expõe aspectos de biogeografia histórica, utilizando dados moleculares e geográficos para elucidar a origem do gênero e seus principais clados, estabelecendo bases para diversos estudos evolutivos. O Capítulo 2 utiliza dados de ocorrência disponíveis em bases de dados de acesso aberto para todas as espécies endêmicas do Brasil, para realizar análises corológicas e avaliações preliminares do risco de extinção para cada uma delas. No Capítulo 3, utilizando o mesmo conjunto de dados do capítulo 2, são fornecidos insights sobre áreas de endemismo para o gênero no Brasil, incluindo investigações sobre mudanças no uso e cobertura da terra ao longo de três décadas. Por fim, o Capítulo 4 apresenta uma nova espécie de *Peperomia* criticamente ameaçada, endêmica do Cerrado brasileiro.

Palavras-chave: Biogeografia; Conservação; Neotrópico; Piperoideae; Sistemática.

Nomenclatural Clarification and Supplementary Resources

Chapter 4, which deals with the description of a new species, has no taxonomic (nomenclatural) effects, and the names used in this chapter will only be validly published when the scientific article reaches publication.

In the various chapters, designed in the format for scientific article submission, supplementary materials, such as Excel tables, are provided for additional support. These materials, not suitable for inclusion in the main text, can be found alongside the already published articles if not attached on the platform where you accessed this thesis. Alternatively, you may request them from the author via email (claptonmoura@gmail.com).

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"Calm. Kindness. Kinship. Love. I've given up all chance at inner peace. I've made my mind a sunless space. I share my dreams with ghosts. *[...]* My anger, my ego, my unwillingness to yield, my eagerness to fight, they've set me on a path from which there is no escape. I yearned to be a savior against injustice without contemplating the cost and by the time I looked down there was no longer any ground beneath my feet. *[...]* I burn my life to make a sunrise that I know I'll never see. And the ego that started this fight will never have a mirror or an audience or the light of gratitude. So what do I sacrifice? Everything!"

Luthen Rael's monologue
(Star Wars: Andor)

Chapter 1

The rise of *Peperomia* (Piperaceae): ancestral range estimates provide evidence for an upper Cretaceous origin in northwestern South America.

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Abstract

Peperomia is one of the richest genera among angiosperms. This genus exhibits a pantropical distribution and most of its species are found in the Neotropics. Although approximately half of the species in *Peperomia* grow as epiphytes, the early-divergent clades of the genus are composed mostly of species with a terrestrial habit. Despite its diversity, the historical biogeography of the genus still has not yet been fully investigated. In this study, we aim to estimate when and where *Peperomia* and its main clades originated. We utilized DNA sequences corresponding to the plastidial region *trnK/matK* from 171 species. Our results indicate that the genus originated in the upper Cretaceous in a range that corresponds to northwestern South America, with dispersal to other areas occurring only in the middle of the Paleogene. We assume that the climatic and environmental events involved in the Angiosperm Terrestrial Revolution, along with the ongoing process of formation of the northern portion of the Andes played a crucial role in the origin and divergence of the early lineages of *Peperomia*. The conquest of areas outside the Neotropical region, on the other hand, was probably driven by the ability of the species to disperse over long distances and occurred more than once.

Keywords

Introduction

Considered by Frodin (2004) as one of the largest genera of flowering plants, *Peperomia* Ruiz & Pav. is estimated to have 1600 accepted species (Frenzke *et al.* 2015). *Peperomia* species are distributed throughout the tropics and have their greatest diversity concentrated in the Neotropical region (Wanke *et al.* 2006, Frenzke *et al.* 2015, Morrone 2022a). The genus is now included in the subfamily Piperoideae Arn. (Samain *et al.* 2008), together with *Piper* L., the other big genus of family Piperaceae Giseke (Frodin, 2004).

Peperomia can be characterized by its inflorescences in a spadix that develops in the leaf axils, opposite to or at the apex of the plant (terminal) – that can be solitary or grouped (Tucker 1980, Mathieu *et al.* 2008); the flowers are achlamydeous and are protected by a bract, with only two stamens and one pistil (with a unilocular ovary bearing a single ovule). *Peperomia* is herbaceous, and stems and leaves are usually succulent. They can be epiphytic and/or terrestrial - the latter including all non-epiphytic forms such as geophytes and rupicolous (Wanke *et al.* 2006; Frenzke *et al.* 2015; Mathieu *et al.* 2015, Frenzke *et al.* 2016).

Despite being one of the largest plant genera (Frodin 2004), *Peperomia* holds significant importance in Neotropical ecosystems (Kreft *et al.* 2004, Krömer *et al.* 2007, Strutzenberger *et al.* 2010, Morales-Linares *et al.* 2021, Pereira *et al.*, 2021) and serves various human purposes (e.g., Krömer *et al.* 2018, Alam *et al.*, 2020; Moraes and Kato, 2021; Mostacero *et al.* 2021; Mathieu 2022). The study conducted by Wanke *et al.* (2006), which was the first to examine the monophyly of *Peperomia* infrageneric groups using molecular evidence, demonstrated that most of the groups recognized until then (based on Dahlstedt 1900) were unsupported. Their study also demonstrated that morphological characters used to segregate these groups, such as peltate leaves or paniculate inflorescences, evolved more than once in the evolutionary history of the genus. Approximately a decade later, Frenzke et al. (2015) proposed the division of *Peperomia* into 14 subgenera using

morphological, micromorphological, and molecular data. Subsequently, features such as epiphytism and fruit adaptations for epizoochorous dispersal were found to play a crucial role in the evolution of the genus's most diverse lineages (Frenzke *et al.* 2016).

To date, the most representative study of historical biogeography for *Peperomia* was conducted by Smith *et al.* (2008), showing a minimum age of 88.8 Ma for the crown node of the genus. However, their sample was small, with only 16 *Peperomia* species, as their focus was mainly on *Piper* biogeographic relationships. The Neotropical region was established as the probable origin for both *Peperomia* and *Piper*. Only six of the current 14 subgenera proposed for *Peperomia* (Frenzke *et al.* 2015) were represented in this analysis and the 16 species sampled were unevenly distributed among these clades - *P.* subg. *Micropiper* (Miq.) Miq. comprised half of the sampled species (8), followed by *P.* subg. *Leptorhynchum* (Dahlst.) Trel. ex Samain with 3 spp., *P.* subg. *Pseudocupula* Frenzke & Scheiris with 2 spp., and the last two with only one representative (*P.* subg. *Peperomia*, *P.* subg. *Pleurocarpidium* Dahlst. And *P.* subg. *Multipalmata* Scheiris & Frenzke).

Most species of *Peperomia* are circumscribed in subgenera that belong to lineages with epiphytic and epizoochorous dispersal adaptations (*P.* subg. *Erasmia* (Miq.) Dahlst., *P.* subg. *Leptorhynchum*, *P.* subg. *Micropiper*, *P.* subg. *Multipalmata*, *P.* subg. *Oxyrhynchum* (Dahlst.) Samain, *P.* subg. *Peperomia*, *P.* subg. *Pleurocarpidium* and *P.* subg. *Pseudocupula*) - including their terrestrial descendants, with some of these subgenera having a pantropical distribution. However, some lineages lack these adaptations (*P.* subg. *Hispidulae* Frenzke & Scheiris, *P.* subg. *Fenestratae* Pino, *P.* subg. *Panicularia* Miq., *P.* subg. *Phyllobryon* (Miq.) Scheiris & Frenzke and *P.* subg. *Tildenia* (Miq.) Miq. ex Dahlst.) and are restricted to the Neotropical region (Frenzke *et al.* 2015, 2016).

Peperomia lineages with adaptations to epiphytism and epizoochory (Frenzke *et al.* 2016) exhibit high capacity for long-distance dispersal (Valdebenito *et al.* 1990a, 1990b, 1992, Dantas *et al.* 2017, Lim *et al.* 2019). In investigations on the origin of *Peperomia* in the Pacific islands, Lim *et al.* (2019) showed that there were several colonization events on these islands by species included in two of

the main subgenera of the epiphytic group (*P.* subg. *Micropiper* and *P.* subg. *Pseudocupula*), and that these events occurred only from the late Neogene (ca. 10 Ma).

Without exhibiting apparent adaptations for epizoochory (Frenzke *et al.* 2015, 2016) and presenting at least two distinctive synapomorphies, such as subterranean (or partially subterranean) tubers with petioles and peduncles emerging directly from them (Wanke *et al.* 2006, Samain *et al.* 2009, 2011), the subgenus *Tildenia* is one of the only clades of *Peperomia* with its biogeographic history studied using molecular data (Symmank *et al.* 2011). Consisting of geophytic plants with a restricted ability to disperse over long distances, the group generally occurs in mountainous areas with distinct dry and rainy seasons - and the fertile period coinciding with the wet season (Hill, 1906; Mathieu *et al.*, 2011; Samain *et al.*, 2011). It is proposed that *Tildenia* originated in the mid to late Paleogene in a region corresponding to present-day northern/central Peru, and that orogenetic processes in the Andes, along with the reunion of the northern and southern portions of the American continent are strongly correlated with the origin and diversification of the clade (Samain *et al.* 2011). *Tildenia* is an interesting model, and its history may provide insights into the evolutionary patterns and processes of *Peperomia*, since Frenzke *et al.* (2016) point out that its ancestral lineages were probably terrestrial and lacked features such as epiphytic habit, adhesiveness, or fruit appendages (present in the more speciose groups today).

Fossils representing the stem lineages of Piperaceae have not been established so far, and fossils of taxa circumscribed within Piperaceae are commonly found in more recent geological strata (e.g., Horn *et al.* 2003, Friis *et al.* 2011), making it difficult to validate/invalidate estimates that indicate the origin of the clade as early as the lower Cretaceous (ca. 150-120 Ma in Bell *et al.* 2010 and Magallón 2010. But see Sauquet *et al.* 2022, for a discussion about the age of angiosperms). However, recently Martínez *et al.* (2015) described *Piper margaritae* Martínez-A., associating it to the Schilleria clade, for the upper Cretaceous (ca. 70 Ma) - thus establishing a minimum age of 111 Ma for the crown node of Piperoideae.

Thus, in this manuscript, we aim to find evidence of the spatio-temporal origin of *Peperomia* and its main clades, highlighting the main factors that influenced the macroevolution of the genus.

Materials and methods

Taxon sampling and sequence preparation

Molecular data from 171 taxa were used. Of these, 163 are from *Peperomia* species and eight from the outgroup (*Piper*). All subgenera of *Peperomia* (Frenzke *et al.* 2015) were represented. Sequences were obtained from GenBank® and accession numbers are available in supplementary material. Sequences from the plastidial *trn*K/*mat*K region, which has been widely used in studies with *Peperomia* (e.g. Wanke 2006, Samain *et al.*, 2011, Symmank *et al.* 2011, Frenzke *et al.* 2015, 2016) because of its high variability, low levels of homoplasmy and higher efficiency (resolution per nucleotide sequenced) when compared to other chloroplast regions (Wanke 2006), were used for the analyses. Sequences were aligned using the MUSCLE tool (Edgar 2004), implemented on the EMBL-EBI platform (Madeira *et al.* 2022), with adjustments performed in AliView software (Larsson 2014).

Divergence time estimation

Analyses to obtain divergence times were performed through the CIPRES Science Gateway (Miller *et al.* 2010), using BEAST v2.6.6 (Bouckaert *et al.* 2014). Selection of the nucleotide substitution model was performed using the bModelTest package (Bouckaert and Drummond 2017). Due to the limited fossil records of Piperaceae, and to avoid biases from uneven sampling between the genus *Peperomia* and the outgroup, we adopted the secondary calibration strategy (Sauquet 2013) for the calibration of the crown node of Piperoideae. The established date of 111 Ma was obtained by Martinez *et al.* (2015) through analysis using one of the oldest *Piper* fossils known so far (*Piper*

margaritae). In this analysis the following parameters were defined: relaxed lognormal clock (Drummond *et al.* 2006); birth-death model of speciation (Harmon 2019); and for the calibration of the crown node of Piperoideae - normal distribution (Ho 2007), with values of mean: 0.0, sigma: 1.5, and offset: 111.0. As the goal of this work was not to verify phylogenetic relationships, the clades obtained by Frenzke *et al.* (2015) were forced as monophyletic in the "priors" tab of BEAUTi 2. Five independent runs were performed, with chain length of 250 million and pre-burnin of one million each. In addition, tests were performed with alternative parameters to those described above, however these resulted in low ESS values. To check for convergence, we use Tracer v1.7.1 (Rambaut *et al.* 2018), and to combine and summarize the data obtained in the performed runs we use the applications LogCombiner v.2.6.7 (with 10% burn-in) and TreeAnnotator v2.6.6 (Bouckaert *et al.* 2014). Visualization and annotation of the trees were performed using FigTree v1.4.4 (Rambaut 2018).

Ancestral range estimation

The division of areas was carried out based on the bioregionalization work of Morrone *et al.* (2022a, 2022b), as well as established prior knowledge of the distribution of *Peperomia* species, and the correspondence of these areas with those used here is given in the supplementary material. Seven areas have been established: A - North America, Europe, northern portion of Africa, and Asia; B - Central America and the Caribbean; C - Northwestern South America and southern Central America; D - Amazon lowlands; E - Atlantic coast and dry diagonal of South America; F - Sub-Saharan Africa; G - Southeast Asia, Oceania, and the Pacific. An additional area "H" was included to house the species used in the preliminary analyses that do not have geographic distribution data, since they were described through herbarium material and/or living collections with no indication of locality. As was observed, the inclusion of this area did not significantly influence the results, so we decided to keep it in the final analyses. Data on species occurrence initially followed the data made available by

Mathieu (2022) and Flora e Funga do Brasil (2022), and thereafter open databases were checked (GBIF, SpeciesLink – Canhos 2022, and Tropicos.org) to verify occurrences in other areas not yet indicated in the initial source (supplementary material).

Ancestral range estimates were obtained using the BioGeoBEARS package (Matzke 2013) implemented in the R environment (R core Team 2022). The DEC/DEC+J, DIVALIKE/DIVALIKE+J and BAYAREALIKE/BAYAREALIKE+J models were tested. The best-fit model was chosen based on the lowest AIC values (supplementary material). The base script used is available on the PhyloWiki page (http://phylo.wikidot.com).

Summary Figure

Fig. 1 summarizes the most relevant information for the discussion of the results and was prepared using generic graphic design tools. Further information regarding the trees produced is available in the supplementary material.

Results and Discussion

We present here the first molecular historical biogeography work including representatives of all *Peperomia* subgenera proposed by Frenzke *et al.* (2015). We highlight the estimated age and most probable environmental events involved in the radiation episodes of the major lineages of the genus - establishing with Smith *et al.* (2008), Symmank *et al.* (2011), Frenzke *et al.* (2015, 2016) and Lim *et al.* (2019), the groundwork for better understanding the macroevolution of *Peperomia*. The model that showed the lowest AIC value was BAYAREALIKE+J and was therefore adopted as the best-fit model for our data. The BAYAREALIKE model implemented in BioGeoBears is a simplified likelihood interpretation of the "BayArea" of Landis *et al.* (2013, see Matzke 2013 and http://phylo.wikidot.com/biogeobears) and the +J parameter refers to the addition of "Founder-

event speciation" to the model (Matzke 2014). Tab. 1 provides the estimated divergence time for the crown node of the major *Peperomia* clades studied here.
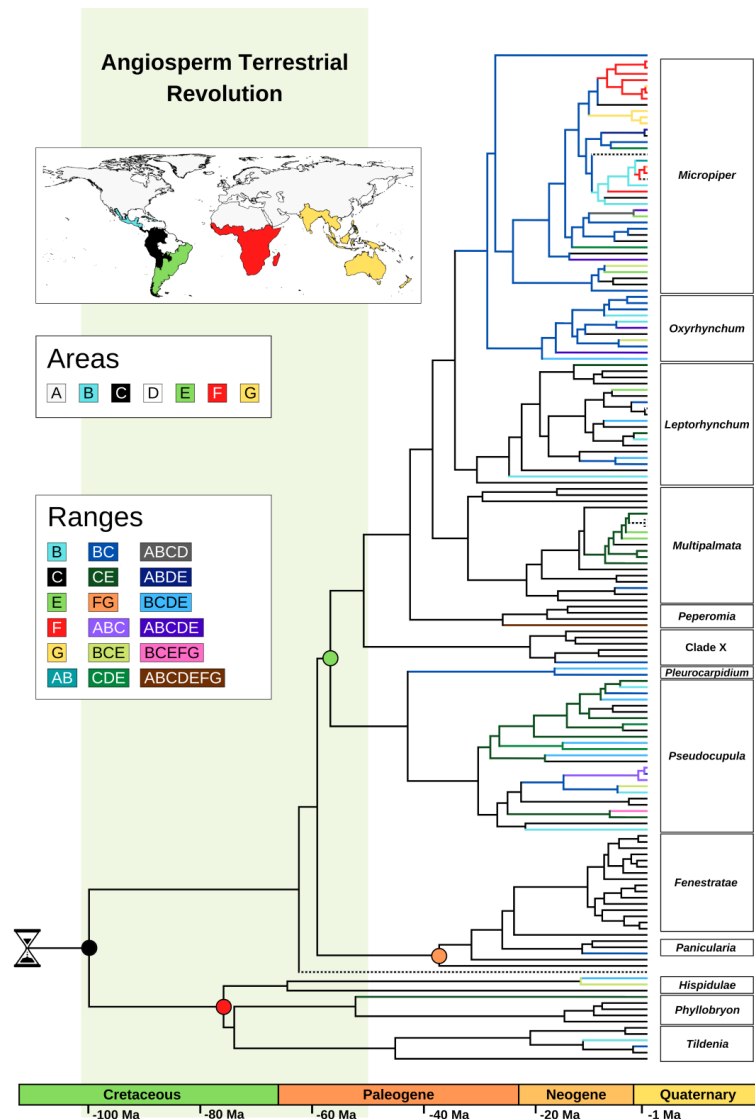

Figure 1. Area map and chronogram with estimated ancestral ranges. Hourglass: calibration point; black circle: *Peperomia* crown node; red circle: Cretaceous Terrestrial Clade crown node; green circle: Paleogene Epiphyte Clade crown node; orange circle: Paleogene Terrestrial Clade crown node; dotted lines: estimated ancestral range for species without geographic information.

Our estimates indicate that the most recent common ancestor (MRCA) of extant *Peperomia* lived about 99.88 Ma, in the early upper Cretaceous. This estimate is approximately ten million years older than that found by Smith *et al.* (2008), who established an age of approximately 88.89 Ma for the *Peperomia* crown node. However, it is important to note that Smith *et al.* (2008) set as the minimum age for the split between Piperaceae and Aristolochiaceae Juss. (calibration point), the

same age estimated for the oldest fossil of *Lactoris* Phil. – 91.2 Ma (Zavada and Benson 1987), and that this age is probably underestimated (as noted by Smith *et al.* 2008 and Symmank *et al.* 2011). Furthermore, the existence of *Piper margaritae* (Martínez *et al.* 2015) demonstrates that the major lineages of Piperaceae had already diverged by the end of the Cretaceous, during the so-called Angiosperm Terrestrial Revolution (Benton *et al.* 2022).

Table 1. Estimated ages for the main clades of *Peperomia*. The subgenera *P.* subg. *Erasmia* and *P.* subg. *Perlucida* are not shown, since they had only one representative, showing only estimates for the stem node (27.22 and 62.35 Ma, respectively).

Clade (crown node)	Age (Ma)	
	Mean	**95% HPD interval**
Peperomia	99.8846	83.4795 - 112.7384
Cretaceous Terrestrial Clade	75.8215	57.4189 - 93.4325
Paleogene Epiphytic Clade	56.7191	42.4093 - 71.5684
Paleogene Terrestrial Clade	37.2544	24.1375 - 51.4002
Subgenus		
P. subg. *Hispidulae*	64.4203	44.9362 - 83.6145
P. subg. *Phyllobryon*	52.2278	34.8312 - 70.5731
P. subg. *Tildenia*	45.1155	27.7900 - 63.0956
P. subg. *Multipalmata*	32.1067	21.3403 - 43.2477
P. subg. *Leptorhynchum*	30.5029	21.9426 - 39.6749
P. subg. *Pseudocupula*	30.2857	21.1729 - 39.5202
P. subg. *Peperomia*	25.8873	15.3442 - 36.6395
P. subg. *Micropiper*	24.0573	16.7674 - 31.4730
P. subg. *Oxyrhynchum*	18.8723	12.1389 - 26.0284
P. subg. *Pleurocarpidium*	16.6097	5.9717 - 28.9512
P. subg. *Panicularia*	11.6791	5.7133 - 18.4058
P. subg. *Fenestratae*	10.7135	6.6371 - 15.3032

The estimated ancestral range for the *Peperomia* crown node is equivalent to area C. This area has an intricate geological history, encompassing today the Northern Andes, the most species-rich portion of the Andes (Pérez-Escobar *et al.* 2022). In the upper Cretaceous, the planet had greenhouse environmental conditions (atmospheric CO_2 concentration about eight to ten times higher than today, high temperatures and high humidity), and part of the estimated range was submerged due to high sea level (Gale 2000, Scotese 2022, Friis *et al.* 2011). This region moved from

an arid climate zone in the lower Cretaceous to a climate zone considered tropical in the upper Cretaceous (Gale 2000, Scotese 2022, Friis *et al.* 2011), with evidence of the presence of araucarioid and podocarpoid conifers in local forests - typical of wetter environments, and which would have their populations gradually replaced by angiosperms (Peralta-Medina and Falcon-Lang 2012). At this age, the leading edge of the Caribbean plate collides with the northwest corner of the South American plate, in a process that would last about 20 million years (ca. 100 - 80 Ma) and would result in important environmental changes to the evolution of the local biota (Pérez-Escobar *et al.* 2022, e.g., Hoorn *et al.* 2022). In this context, the possibility of isolation of land ranges - due to high sea level, coupled with climate change and the knowledge that *Peperomia* lineages presented terrestrial habit and did not yet possess adaptations for long-distance dispersal (Frenzke *et al.* 2016), lead us to suggest that probable allopatric processes could be involved in the divergence event of the first two lineages of the genus. These assumptions can be extended to the divergence between the lineages that gave rise to the genera *Piper* and *Peperomia*, since this event occurred about 11 million years earlier (Smith *et al.* 2008), and regional conditions were relatively similar, including the connection of the northern and southern portions of the Atlantic - arising from the separation of Gondwana (Gale 2000).

The clade formed by the *P.* subg. *Hispidulae*, *P.* subg. *Phyllobryon* and *P.* subg. *Tildenia*, here referred to as the Cretaceous Terrestrial Clade, had its crown node estimated at 75.82 Ma. and its estimated ancestral range also equivalent to area C. These three subgenera are characterized by having a terrestrial life form, no adaptation to epizoochory and a geographic distribution restricted to the Neotropical region, with the greatest richness concentrated in the region equivalent to the estimated ancestral range (Frenzke *et al.* 2015, 2016). After the Cenomanian, when sea levels probably peaked in the Cretaceous, the planet went through a phase of regression that lasted until the Campanian, when sea levels rose again, almost reaching the previous peak (Hancock 1979, Gale 2000). The geomorphological processes, such as magmatism and orogenesis of the Andes have their activities intensified, providing the appearance and transformation of new environments, for

example the emergence of islands that would later form the Isthmus of Panama (Gale 2000, Jaillard *et al.* 2000, Friis *et al.* 2011). The environmental pressures at the time of the divergence event between the lineages that gave rise to the subgenera of the Cretaceous Terrestrial Clade are like those experienced by *Peperomia* MRCA. Therefore, we assume that allopatric processes may be the main explanation for the divergence of these lineages. However, it is important to note that *P.* subg. *Tildenia* species are commonly found in the same locality occupying distinct microhabitats - one occurring on top of rocks, with greater exposure to light, while another occupies the gap of these rocks with lower sun incidence and greater moisture maintenance (Samain *et al.* 2011, Symmank *et al.* 2011). Thus, peripatric, parapatric, and sympatric processes cannot be completely discarded.

Since the upper Cretaceous, the region corresponding to area C has had a warm and humid tropical climate with evidence of continued presence of forests in the region (Gale 2000, Scotese, 2022, Friis *et al.* 2011, Peralta-Medina and Falcon-Lang 2012, Carvalho *et al.* 2021). The local upper Cretaceous forests were dominated by gymnosperms (although angiosperms, and other groups such as ferns, also inhabited these environments) and had open canopy without significant vertical stratification (Peralta-Medina and Falcon-Lang 2012, Carvalho *et al.* 2021). By the end of the Cretaceous, the Earth had become less arid and with cooler temperatures, in this period the bolide impact at Chicxulub triggered the fifth major mass extinction, leading to irreversible environmental changes (Gale 2000, Peralta-Medina and Falcon-Lang 2012, Carvalho *et al.* 2021). As a result of these events, local forests once dominated by gymnosperms gradually shifted to a taxonomic composition more like current forests (Burnham and Johnson 2004, Graham *et al.* 2019). There are three possible explanations for this change (Carvalho *et al.* 2021): (1) absence of large herbivores, which triggered a "race for light"- given the emergence of habitats with wide variation in light; (2) differences in soil, which was nutrient-limited in the upper Cretaceous and more fertile in the early Paleogene; and (3) by selective extinction, since ecophysiological diversity among dominant gymnosperms was not high, these were more sensitive to mass extinctions. In contrast, the angiosperms were not dominant, but already ecophysiologically diverse, being more resistant to extinction.

With the absence of competition and new niches available, angiosperms that were previously discrete components of local forests gradually become dominant in these ecosystems (Burnham and Johnson 2004, Peralta-Medina and Falcon-Lang 2012, Graham *et al.* 2019, Carvalho *et al.* 2021). In this context, in about 56.71 Ma, the initial divergence of the Paleogene Epiphytic Clade takes place, a lineage with the highest richness of extant species and composed of the *P.* Subg. *Erasmia*, *P.* subg. *Leptorhynchum*, *P.* subg. *Micropiper*, *P.* subg. *Multipalmata*, *P.* subg. *Oxyrhynchum*, *P.* subg. *Peperomia*, *P.* subg. *Pleurocarpidium* and *P.* subg. *Pseudocupula*. Like the clades presented earlier, the Paleogene Epiphytic Clade has an estimated ancestral range equivalent to area C. Evidence presented by Frenzke *et al.* (2016) shows that epiphytism developed in the genus initially in this lineage, and this preceded an increase in diversification rates in the genus. Here, we infer that the ancestors of this group already inhabited the estimated ancestral range as terrestrial plants, and like the other angiosperms, the Paleogene Epiphytic Clade, acted as opportunists conquering the newly available niches.

An interesting point is that Piperaceae species are important components of Andean forests, being among the ten richest families in this mountain range (Pérez-Escobar *et al.* 2022), with the greatest diversity concentrated in the northern region. This, coupled with the fact that one of the oldest fossils of the family (*Piper margaritae*) was found in the Guaduas Formation, within the region equivalent to Area C, and the evidence produced here lead us to propose that ancestral lineages of *Peperomia* already inhabited the region at least since the upper Cretaceous, and that climate change and the process of Andean formation played a key role in the early diversification of the genus and in the distribution patterns of the species. Furthermore, all the events described so far, occurred during the so-called Angiosperm Terrestrial Revolution (100 - 50 Ma) - a phase in which angiosperms, given environmental conditions, moved from discrete and opportunistic lineages to central components of terrestrial ecosystems (Ramírez-Barahona *et al.* 2019, Benton *et al.* 2022, Sauquet *et al.* 2022).

The newest of the large *Peperomia* clades, the Paleogene Terrestrial Clade, had its crown node estimated at 37.25 Ma. The estimated ancestral range for this group is also equivalent to Area C. The clade is composed of the *P.* subg. *Fenestratae* and *P.* subg. *Panicularia*, both composed of terrestrial species and occurring mainly in tropical dry forests among the inter-Andean valleys, with some representatives in cloud forests at higher altitudes (Frenzke *et al.* 2015). The end of the Eocene marks the beginning of one of the major climate transitions of the Cenozoic era - with declining CO_2 concentrations and changes in ocean currents, the planet shifts to colder temperatures and a more arid climate, resulting in the emergence of ice sheets at high attitudes and latitudes (Berggren and Prothero 1992, Pearson *et al.* 2009). In the region corresponding to area C, evidence points to the existence of a Marine-lacustrine system, due to oceanic incursion over northwestern South America (Llanos basin and western Putumayo basin), in addition to the increase in rainfall seasonality - propitiating the establishment (in the Esmeraldas Formation) of one of the first tropical dry forests in the region (Martínez *et al.* 2021, De La Parra *et al.* 2021). This scenario indicates that the transition from humid to dry forests and the habitat fragmentation caused by oceanic incursion, in addition to the ongoing process of Andean formation, were determining factors that influenced the diversification event of the Paleogene Terrestrial Clade, and that this group emerged and has remained in these environments ever since.

The extension to other ranges occurs in the genus *Peperomia* only from the Paleogene, when lineages of *P.* subg. *Phyllobryon*, *P.* subg. *Pseudocupula* and *P.* subg. *Leptorhynchum* expand their distribution to adjacent areas (B, D and E), together with a lineage in *P.* subg. *Peperomia* (represented by P. pellucida (L.) Kunth) that shows its estimated ancestral range equivalent to the sum of all areas established in this study (ABCDEFG). Two other groups show an extension of the ancestral range of *Peperomia*, the *P.* subg. *Pleurocarpidium* and the clade formed by *P.* subg. *Erasmia*, *P.* subg. *Micropiper* and *P.* subg. *Oxyrhynchum*, with the union of areas B and C forming its estimated ancestral range. Except for *P. pellucida*, the conquest of regions outside the American continent does not begin until the Neogene, in lineages of *P.* subg. *Micropiper* and *P.* subg.

Pseudocupula (Paleogene Epiphytic Clade). These two subgenera are currently the richest and exhibit adaptations to epizoochorous dispersal (Frenzke *et al.* 2015, 2016). Long-distance dispersal, common in these lineages, is accomplished by the adhesion of the fruit to the feathers of migratory birds (Valdebenito *et al.* 1990a, 1990b, 1992, Dantas *et al.* 2017, Lim *et al.* 2019). Moreover, the interval in which diversification events occur in these groups is coincident with the radiation episodes of several Passeriform lineages, and with the change in the main dispersal form adopted by South American birds - from non-aerial to aerial from the Miocene to the present (Tambussi and Degrange 2013, Oliveros *et al.* 2019).

Excluding the Cretaceous Terrestrial Clade, all subgenera of *Peperomia* have their crown node estimated between the Oligocene and the late Miocene. These events occur concurrently with several significant environmental changes in the Neotropical region, such as: the closure of the Central American Seaway (Montes *et al.* 2015); changes in drainage patterns in northern South America (Hoorn *et al.* 1995); the continuing process of Andean formation (Gregory-Wodzicki 2000); and the establishment of Neotropical rainforests (Burnham and Johnson 2004).

Conclusions

Peperomia MRCA lived during the upper Cretaceous, had terrestrial habit and presumably occurred in open canopy gymnosperm-dominated forests in northwestern South America. The first two major lineages of the genus, Cretaceous Terrestrial Clade and Paleogene Epiphytic Clade, have their origin and diversification established during the events of the so-called Angiosperm Terrestrial Revolution, whereas the third major group, Paleogene Terrestrial Clade, has its crown node age estimated for after these events. We infer that five factors have significantly influenced the macroevolution of *Peperomia*: (1) the continuous and uneven formation of the Andes; (2) the marine incursions and transgressions over the ancestral range; (3) changes in temperature, humidity, and seasonality; (4)

the evolution and establishment of the Neotropical forests; and (5) the adaptations for epizoochorous dispersal.

References

Alam MdA, Nadirah TA, Mohsin GMd, Saleh M, Moneruzzaman KM, Aslani F, Juraimi AS, Alam MZ (2020) Antioxidant compounds, antioxidant activities, and mineral contents among underutilized vegetables. International Journal of Vegetable Science 27(2): 157–166. https://doi.org/10.1080/19315260.2020.1748785.

Bell CD, Soltis DE, Soltis PS (2010) The age and diversification of the angiosperms re-revisited. American Journal of Botany 97: 1296–1303. https://doi.org/10.3732/ajb.0900346

Benton MJ, Wilf P, Sauquet H (2022) The Angiosperm Terrestrial Revolution and the origins of modern biodiversity. New Phytologist 233: 2017–2035. https://doi.org/10.1111/nph.17822

Berggren WA, Prothero DR (2014) Eocene-oligocene climatic and biotic evolution: an overview. In: Prothero DR, Berggren WA (eds) Eocene-Oligocene Climatic and Biotic Evolution. Princeton University Press, Princeton, pp. 1–28. https://doi.org/10.1515/9781400862924.1

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D, Suchard MA, Rambaut A, Drummond AJ (2014) BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. PLoS Computational Biology 10(4): e1003537. https://doi.org/10.1371/journal.pcbi.1003537

Bouckaert R, Drummond A (2017) bModelTest: Bayesian phylogenetic site model averaging and model comparison. BMC Evolutionary Biology 17: 42. https://doi.org/10.1186/s12862-017-0890-6

Burnham RJ, Johnson KR (2004) South American palaeobotany and the origins of neotropical rainforests. Philosophical Transactions of the Royal Society B 359: 1595–1610. https://doi.org/10.1098/rstb.2004.1531

Canhos DAL, Almeida EAB, Assad AL, Bustamante MMC, Canhos VP, Chapman AD, Giovanni RD, Imperatriz-Fonseca VL, Lohmann LG, Maia LC, Miller JT, Nelson G, Peterson AT, Pirani JR, Souza S, Stehmann JR, Thiers B (2022) speciesLink: rich data and novel tools for digital assessments of biodiversity. Biota Neotropica 22(spe): e20221394. https://doi.org/10.1590/1676-0611-bn-2022-1394

Carvalho MR, Jaramillo C, de la Parra F, Caballero-Rodríguez D, Herrera F, Wing S, Turner BL, D'Apolito C, Romero-Baez M, Narvaez P, Martínez C, Gutierrez M, Labandeira C, Bayona G, Rueda M, Paez-Reyes M, Cárdenas D, Duque Á, Crowley JL, Santos C, Silvestro D (2021) Extinction at the end-Cretaceous and the origin of modern tropical rainforests. Science 372: 63–68. https://doi.org/10.1126/science.abf1969

Dahlstedt H (1900) Studien über süd- und central-amerikanische Peperomien mit besonderer Berücksichtigung der brasilianischen Sippen. Kongliga Svenska Vetenskaps – Akademiens handlingar 33.

Dantas TS, Câmara PEAS, Carvalho-Silva M (2017) *Peperomia* (Piperaceae) from Trindade Island: A New Species Based on Morphological and Molecular Data. Systematic Botany 42(4): 747–753. https://doi.org/10.1600/036364417X696366

De La Parra F, Pinzon D, Mantilla-Duran F, Rodriguez G, Caballero V (2021) Marine-lacustrine systems during the Eocene in northern South America – Palynological evidence from Colombia. Journal of South American Earth Sciences 108: 103188. https://doi.org/10.1016/j.jsames.2021.103188

Drummond AJ, Ho SYW, Phillips MJ, Rambaut A (2006) Relaxed Phylogenetics and Dating with Confidence. PLoS Biology 4(5): e88. https://doi.org/10.1371/journal.pbio.0040088

Edgar RC (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5: 113. https://doi.org/10.1186/1471-2105-5-113

Flora e Funga do Brasil (2022) Jardim Botânico do Rio de Janeiro. Avaliable at: http://floradobrasil.jbrj.gov.br/

Frenzke L, Scheiris E, Pino G, Symmank L, Goetghebeur P, Neinhuis C, Wanke S, Samain M-S (2015) A revised infrageneric classification of the genus *Peperomia* (Piperaceae). Taxon 64(3): 424–444. https://doi.org/10.12705/643.4

Frenzke L, Goetghebeur P, Neinhuis C, Samain M-S and Wanke S (2016) Evolution of Epiphytism and Fruit Traits Act Unevenly on the Diversification of the Species-Rich Genus *Peperomia* (Piperaceae). Frontiers in Plant Science 7:1145. https://doi.org/10.3389/fpls.2016.01145

Friis EM, Crane PR, Pedersen KR (2011) Early Flowers and Angiosperm Evolution. Cambridge University Press, Cambridge. https://doi.org/10.1017/CBO9780511980206

Frodin DG (2004) History and concepts of big plant genera. Taxon 53(3): 753–776. https://doi.org/10.2307/4135449

Gale A (2000) The Cretaceous world. In: Culver S, Rawson P (eds) Biotic Response to Global Change: The Last 145 Million Years, Cambridge University Press, Cambridge, pp 4–19. https://doi.org/10.1017/CBO9780511535505.003

GBIF.org (2022) Global Biodiversity Information Facility. Avaliable at: https://www.gbif.org

Graham HV, Herrera F, Jaramillo C, Wing SL, Freeman KH (2019) Canopy structure in Late Cretaceous and Paleocene forests as reconstructed from carbon isotope analyses of fossil leaves. Geology 47(10): 977–981. https://doi.org/10.1130/G46152.1

Gregory-Wodzicki KM (2000) Uplift history of the Central and Northern Andes: A review. GSA Bulletin 112(7): 1091–1105. https://doi.org/10.1130/0016-7606(2000)112<1091:UHOTCA>2.0.CO;2

Hancock JM, Kauffman EG (1979) The great transgressions of the Late Cretaceous. Journal of the Geological Society 136(2): 175–186. https://doi.org/10.1144/gsjgs.136.2.0175

Harmon LJ (2019) An introduction to phylogenetic comparative methods. Avaliable at: https://lukejharmon.github.io/pcm/

Hill AW (1906) The morphology and seedling structure of the geophilous species of *Peperomia*, together with some views on the origin of monocotyledons. Annals of Botany 20: 395–427. https://doi.org/10.1093/oxfordjournals.aob.a089110

Ho SYM (2007) Calibrating molecular estimates of substitution rates and divergence times in birds. Journal of Avian Biology 38: 409–414. https://doi.org/10.1111/j.0908-8857.2007.04168.x

Hoorn C, Guerrero J, Sarmiento GA, Lorente MA (1995) Andean tectonics as a cause for changing drainage patterns in Miocene northern South America. Geology 23(3): 237–240. https://doi.org/10.1130/0091-7613(1995)023<0237:ATAACF>2.3.CO;2

Horn SP, Sanford RL, Dilcher D, Lott TA, Renne PR, Wiemann MC, Cozadd D, Vargas O (2003) Pleistocene plant fossils in and near La Selva Biological Station, Costa Rica. Biotropica 35: 434–441. https://doi.org/10.1646/0006-3606(2003)035[0434:PPFIAN]2.0.CO;2

Hoorn C, Palazzesi L, Silvestro D (2022) Editorial Preface to Special Issue: Exploring the impact of Andean uplift and climate on life evolution and landscape modification: From Amazonia to Patagonia. Global and Planetary Change 211: 103759. https://doi.org/10.1016/j.gloplacha.2022.103759

Jaillard E, Hérail G, Monfret T, Díaz-Martínez E, Baby P, Lavenu A, Dumont JF (2000) Tectonic evolution of the Andes of Ecuador, Peru, Bolivia and northernmost Chile. In: Cordani UG, Milani EJ, Thomaz Filho A, Campos DA (eds) Tectonic evolution of South America. International Geological

Congress, Río de Janeiro, 31: 481–559. Avaliable at: https://horizon.documentation.ird.fr/exl-doc/pleins_textes/divers19-02/010074719.pdf

Kreft H, Köster N, Küper W, Nieder J, Barthlott W (2004) Diversity and biogeography of vascular epiphytes in Western Amazonia, Yasuní, Ecuador. Journal of Biogeography 31(9): 1463–1476. https://doi.org/10.1111/j.1365-2699.2004.01083.x

Krömer T, Kessler M, Gradstein SR (2007) Vertical stratification of vascular epiphytes in submontane and montane forest of the Bolivian Andes: the importance of the understory. Plant Ecology 189(2): 261–278. https://doi.org/10.1007/s11258-006-9182-8

Krömer T, Acebey A, Toledo-Aceves T (2018) Aprovechamiento de plantas epífitas: implicaciones para su conservación y manejo sustentable. In: Silva-Rivera E, Martínez-Valdéz V, Lascurain M, Rodríguez-Luna E (eds) De la recolección a los agroecosistemas: soberanía alimentaria y conservación dela biodiversidad, perteneciente a la serie: Hacia la sustentabilidad, Editorial de la Universidad Veracruzana, Xalapa, pp. 175–196. https://doi.org/10.25009/uv.2075.513

Landis MJ, Matzke NJ, Moore BR, Huelsenbeck JP (2013) Bayesian Analysis of Biogeography when the Number of Areas is Large. Systematic Biology 62(6): 789–804. https://doi.org/10.1093/sysbio/syt040

Larsson A (2014) AliView: a fast and lightweight alignment viewer and editor for large datasets. Bioinformatics 30(22): 3276–3278. https://doi.org/10.1093/bioinformatics/btu531

Lim JY, Marshall CR, Zimmer EA, Wagner WL (2019) Multiple colonizations of the Pacific by *Peperomia* (Piperaceae): Complex patterns of long-distance dispersal and parallel radiations on the Hawaiian Islands. Journal of Biogeography 46: 2651–2662. https://doi.org/10.1111/jbi.13717

Madeira F, Pearce M, Tivey ARN, Basutkar P, Lee J, Edbali O, Madhusoodanan N, Kolesnikov A, Lopez R (2022) Search and sequence analysis tools services from EMBL-EBI in 2022. Nucleic Acids Research 50(W1): W276–W279. https://doi.org/10.1093/nar/gkac240

Magallón S (2010) Using Fossils to Break Long Branches in Molecular Dating: A Comparison of Relaxed Clocks Applied to the Origin of Angiosperms. Systematic Biology 59(4): 384–399. https://doi.org/10.1093/sysbio/syq027

Martínez C, Carvalho MR, Madriñán S, Jaramillo CA (2015) A Late Cretaceous *Piper* (Piperaceae) from Colombia and diversification patterns for the genus. American Journal of Botany 102: 273–289. https://doi.org/10.3732/ajb.1400427

Martínez C, Jaramillo C, Martínez-Murcia J, Crepet W, Cárdenas A, Escobar J, Moreno F, Pardo-Trujillo A, Caballero-Rodríguez D (2021) Paleoclimatic and paleoecological reconstruction of a middle to late Eocene South American tropical dry forest. Global and Planetary Change 205: 103617. https://doi.org/10.1016/j.gloplacha.2021.103617

Mathieu G, Samain M-S, Reynders M, Goetghebeur P (2008) Taxonomy of the *Peperomia* species (Piperaceae) with pseudo-epiphyllous inflorescences, including four new species. Botanical Journal of the Linnean Society 157(2): 177–196. https://doi.org/10.1111/j.1095-8339.2008.00777.x

Mathieu G, Vergara-Rodriguez D, Krömer T, Karger DN (2015) *Peperomia* (Piperaceae) novelties from Veracruz State, Mexico. Phytotaxa 205(4): 268–276. https://doi.org/10.11646/phytotaxa.205.4.6

Mathieu G, Symmank L, Callejas R, Wanke S, Neinhuis C, Goetghebeur P, Samain MS (2011) New geophytic *Peperomia* (Piperaceae) species from Mexico, Belize and Costa Rica. Revista Mexicana de Biodiversidad 82: 357–382.

Mathieu G (2001-2022) the Internet *Peperomia* Reference. Avaliable at: peperomia.net

Matzke NJ (2013) Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. Frontiers of Biogeography 5(4): 242–248. http://dx.doi.org/10.21425/F5FBG19694

Matzke NJ (2014) Model Selection in Historical Biogeography Reveals that Founder-Event Speciation Is a Crucial Process in Island Clades. Systematic Biology 63(6): 951–970. https://doi.org/10.1093/sysbio/syu056

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. 2010 Gateway Computing Environments Workshop (GCE): 1–8. https://doi.org/10.1109/GCE.2010.5676129

Montes C, Cardona A, Jaramillo C, Pardo A, Silva JC, Valencia V, Ayala C, Pérez-Angel LC, Rodriguez-Parra LA, Ramirez V, Niño H (2015) Middle Miocene closure of the Central American seaway. Science 348(6231): 226–229. https://doi.org/10.1126/science.aaa2815

Morales-Linares J, Corona-López AM, Toledo-Hernández VH, Flores-Palacios A (2021) Ant-gardens: a specialized ant-epiphyte mutualism capable of facing the effects of climate change. Biodiversity and Conservation 30: 1165–1187. https://doi.org/10.1007/s10531-021-02138-2.

Moraes MM, Kato MJ (2021) Biosynthesis of Pellucidin A in *Peperomia pellucida* (L.) HBK. Frontiers in Plant Science 12: 641717. https://doi.org/10.3389/fpls.2021.641717.

Morrone JJ, Escalante T, Rodríguez-Tapia G, Carmona A, Arana M, Mercado-Gómez JD (2022a) Biogeographic regionalization of the Neotropical region: New map and shapefile. Anais da Academia Brasileira de Ciências 94(1): e20211167. https://doi.org/10.1590/0001-3765202220211167

Morrone JJ, Ebach MC (2022b) Toward a terrestrial biogeographical regionalisation of the world: historical notes, characterisation and area nomenclature. Australian Systematic Botany 35(3): 89–126. https://doi.org/10.1071/SB22002

Mostacero NR, Castelli MV, Barolo MI, Amigot SL, Fulgueira CL, López SN (2021) Fungal endophytes in *Peperomia obtusifolia* and their potential as inhibitors of chickpea fungal pathogens. World Journal of Microbiology and Biotechnology 37: 14. https://doi.org/10.1007/s11274-020-02954-8.

Oliveros CH, Field DJ, Ksepka DT, Barker FK, Aleixo A, Andersen MJ, Alström P, Benz BW, Braun EL, Braun MJ, Bravo GA, Brumfield RT, Chesser RT, Claramunt S, Cracraft J, Cuervo AM, Derryberry EP, Glenn TC, Harvey MG, Hosner PA, Joseph L, Kimball RT, Mack AL, Miskelly CM, Peterson AT, Robbins MB, Sheldon FH, Silveira LF, Smith BT, White ND, Moyle RG, Faircloth BC (2019) Earth history and the passerine superradiation. Proceedings of the National Academy of Sciences 116: 7916–7925. https://doi.org/10.1073/pnas.1813206116

Pearson P, Foster G, Wade B (2009) Atmospheric carbon dioxide through the Eocene–Oligocene climate transition. Nature 461: 1110–1113. https://doi.org/10.1038/nature08447

Peralta-Medina E, Falcon-Lang HJ (2012) Cretaceous forest composition and productivity inferred from a global fossil wood database. Geology 40(3): 219–222. https://doi.org/10.1130/G32733.1

Pereira AA, da Silva IV, Vicente RE (2021) Interaction between epiphytic chemical allelopathy and ant-pruning determining the composition of Amazonian ant-garden epiphytes. Arthropod-Plant Interactions 15: 399–407. https://doi.org/10.1007/s11829-021-09825-5

Pérez-Escobar OA, Zizka A, Bermúdez MA, Meseguer AS, Condamine FL, Hoorn C, Hooghiemstra H, Pu Y, Bogarín D, Boschman LM, Pennington RT, Antonelli A, Chomicki G (2022) The Andes through time: evolution and distribution of Andean floras. Trends in Plant Science 27(4): 364–378. https://doi.org/10.1016/j.tplants.2021.09.010

R Core Team (2022) R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing. Avaliable at: https://www.R-project.org

Rambaut A (2018) FigTree v1.4.4. Avaliable at: http://tree.bio.ed.ac.uk/software/figtree/

Rambaut A, Drummond AJ, Xie D, Baele G and Suchard MA (2018) Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67(5): 901–904. https://doi.org/10.1093/sysbio/syy032

Ramírez-Barahona S, Sauquet H, Magallón S (2020) The delayed and geographically heterogeneous diversification of flowering plant families. Nature Ecology & Evolution 4: 1232–1238. https://doi.org/10.1038/s41559-020-1241-3

Samain M-S, Mathieu G, Wanke S, Neinhuis C, Goetghebeur P (2008). Verhuellia revisited—unravelling its intricate taxonomic history and a new subfamilial classification of Piperaceae. Taxon 57(2): 583–587. https://doi.org/10.2307/25066024

Samain MS, Vanderschaeve L, Chaerle P, Goetghebeur P, Neinhuis C, Wanke S (2009) Is morphology telling the truth about the evolution of the species rich genus *Peperomia* (Piperaceae). Plant Systematic and Evolution 278: 1–21. https://doi.org/10.1007/s00606-008-0113-0

Samain M-S, Mathieu G, Pino G, Symmank L, Cieza N, Neinhuis C, Goetghebeur P, Wanke S (2011) The geophytic *Peperomia* subgenus *Tildenia* (Piperaceae) in the Andes with the description of new species in a phylogenetic framework. Plant Ecology and Evolution 144(2): 148–176(29). https://doi.org/10.5091/plecevo.2011.470

Sauquet H (2013) A practical guide to molecular dating. Comptes Rendus de l'Académie des Sciences 12(6): 355–367. https://doi.org/10.1016/j.crpv.2013.07.003

Sauquet H, Ramírez-Barahona S, Magallón S (2022) What is the age of flowering plants?. Journal of Experimental Botany 73(12): 3840–3853. https://doi.org/10.1093/jxb/erac130

Scotese C (2022) Paleomap Project. Avaliable at: http://www.scotese.com

Smith JF, Stevens AC, Tepe EJ, Davidson C (2008) Placing the origin of two species-rich genera in the late cretaceous with later species divergence in the tertiary: a phylogenetic, biogeographic and molecular dating analysis of *Piper* and *Peperomia* (Piperaceae). Plant Systematics and Evolution 275:9. https://doi.org/10.1007/s00606-008-0056-5

Strutzenberger P, Brehm G, Bodner F, Fiedler K (2010) Molecular phylogeny of Eois (Lepidoptera, Geometridae): evolution of wing patterns and host plant use in a species-rich group of Neotropical moths. Zoologica Scripta 39: 603–620. https://doi.org/10.1111/j.1463-6409.2010.00440.x

Symmank L, Samain M-S, Smith JF, Pino G, Stoll A, Goetghebeur P, Neinhuis C, Wanke S (2011) The extraordinary journey of *Peperomia* subgenus *Tildenia* (Piperaceae): insights into diversification and colonization patterns from its cradle in Peru to the Trans-Mexican Volcanic Belt. Journal of Biogeography 38: 2337–2349. https://doi.org/10.1111/j.1365-2699.2011.02586.x

Tambussi CP, Degrange FJ (2013) Bio-Connections Between Southern Continents: What is and What is Not Possible to Conclude. In: Tambussi CP, Degrange FJ (eds) South American and Antarctic Continental Cenozoic Birds, Springer, Dordrecht, pp 103–113. https://doi.org/10.1007/978-94-007-5467-6_9

Tropicos.org (2022) Missouri Botanical Garden. Avaliable at: https://tropicos.org

Tucker SC (1980) Inflorescence and Flower development in the Piperaceae. I. *Peperomia*. American Journal of Botany 67(5): 686–702. https://doi.org/10.1002/j.1537-2197.1980.tb07699.x

Valdebenito HA, Stuessy TF, Crawford DJ (1990a) Synonymy in *Peperomia* berteroana (Piperaceae) results in biological disjunction between Pacific and Atlantic oceans. Brittonia 42: 121–124. https://doi.org/10.2307/2807626

Valdebenito H, Stuessy TF, Crawford DJ (1990b) A new biogeographic connection between islands in the Atlantic and Pacific Oceans. Nature 347: 549–550. https://doi.org/10.1038/347549a0

Valdebenito H, Stuessy TF, Crawford DJ, Silva MO (1992) Evolution of *Peperomia* (Piperaceae) in the Juan Fernandez Islands, Chile. Plant Systematics and Evolution 182: 107–119. https://doi.org/10.1007/BF00941416

Wanke S, Samain M-S, Vanderschaeve L, Mathieu G, Goetghebeur P, Neinhuis C (2006) Phylogeny of the Genus *Peperomia* (Piperaceae) Inferred from the trnK/matK Region (cpDNA). Plant Biology 8(1): 93–102. https://doi.org/10.1055/s-2005-873060

Zavada MS, Benson JM (1987) First fossil evidence for the primitive angiosperm family Lactoridaceae. American Journal of Botany 74: 1590–1594. https://doi.org/10.1002/j.1537-2197.1987.tb12150.x

Chapter 2

Highlighted Student Research

Peperomia (Piperaceae) endemic to Brazil: Distribution, richness, and conservation status

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ABSTRACT

Among one of the largest genera of angiosperms, *Peperomia* has a Pantropical distribution. However, studies that analyze the chorological details and conservation status of its species still are scarce. Brazil is home to 169 species of *Peperomia* and approximately two thirds (111 spp.) are considered endemic in the Flora of Brazil. Due to this, the present study aims to answer: (1) Are the *Peperomia* cited in Flora do Brasil 2020 as endemic really endemic? (2) How are these species distributed? (3) What is the extinction risk of the *Peperomia* species endemic to Brazil? In order to extract and clean the occurrence data, we used records available in open databases then applied a workflow method developed by CNCFlora (National Center for the Conservation of Flora). Based on our data, at current 100 species can be considered endemic to Brazil. We found that almost half of the endemic species are threatened on some level and that the phytogeographical domain with the greatest richness and highest number of records is the Atlantic Forest.

1. Introduction

The genus *Peperomia* Ruiz & Pav has a Pantropical distribution, with its greatest diversity concentrated in the Neotropical Region. It stands out for being one of the richest genera among all angiosperms, with more than 1160 species accepted – distributed in 14 subgenera (Frodin, 2004; Frenzke et al., 2015; Zotz et al., 2021). The genus is composed of herbaceous and, generally, succulent plants, with about 43% of the species growing as obligate or facultative epiphytes, but also growing on rupicolous or terrestrial substrates (Zotz, 2013; Frenzke et al., 2015). *Peperomia* is distinct from the other genera of Piperaceae Giseke mainly because its flowers have only two stamens and one pistil (with a unilocular ovary and harboring only one ovule). Its flowers are achlamydeous, arranged in a spadix (Mathieu et al., 2008) that develop in the axils of the leaves, opposite to or at the apex of the plant (terminal), being solitary or grouped (Tucker, 1980; Wanke et al., 2006; Frenzke et al., 2015).

In Brazil, the genus is comprised of 169 species, ranking fourth in *Peperomia* species richness in the world, of which approximately 65% are currently considered endemic (Flora do Brasil, 2020; Mathieu,

2021). Most species occur in forest formations, in the Amazon, the Atlantic Forest, the *Caatinga* and *Cerrado* domains, and are absent in the *Pampa* and *Pantanal* (Flora do Brasil, 2020). The Atlantic Forest stands out for being the richest domain, with about 75% of all species documented for Brazil, followed by the Amazon with about 32%, the *Cerrado* with 20% and the *Caatinga* with approximately 6.5% of Brazilian species existent in each territory (Flora do Brasil, 2020).

Peperomia species have a high potential for applications in the pharmaceutical industry (e.g., Alam et al., 2020; Moraes and Kato, 2021; Mostacero et al., 2021). They are widely used as ornamental plants (Mathieu, 2021), some species are edible (Krömer et al., 2018) and some participate in unique ecosystems so-called "Ant Gardens" - composed by interactions between ants and epiphytes (Morales-Linares et al. 2021; Pereira et al. 2021). Despite having a high richness and application potential, there is still a gap in systematics studies on *Peperomia*'s conservation status - worldwide, more than 1160 species are accepted (Zotz et al., 2021), and only 68 have been evaluated by the International Union for Conservation of Nature (IUCN, 2021). In Brazil, of the know species, the conservation status of only 39 (23%) have been evaluated by the National Center for the Conservation of Flora (CNCFlora, 2021).

Brazil is home to around 10% of all flowering plant species in the world (Christenhusz and Byng, 2016; BFG, 2018; 2021; Antonelli et al., 2020). More than half of these are considered endemic (BFG, 2018; 2021). Brazil also has two of the global biodiversity hotspots – the Atlantic Forest and *Cerrado* (Myers et al. 2000; Mittermeier et al. 2004). Despite being a shelter for this diversity, the country faces numerous threats due to human activities (e.g., Brandon et al., 2005). The Atlantic Forest has the oldest and most intense impacts, with only 11-28% of its natural coverage remaining (Ribeiro et al., 2009; Rezende et al., 2018; IBGE, 2021). The endemic angiosperms of Brazil are not homogeneous in their distribution, presenting a pattern in which the greatest concentration of species is found in the eastern portion of the country (where the Atlantic Forest predominates) and gradually decreases towards the interior of the continent (Gomes-da-Silva et al., 2021). Within the Atlantic Forest, which is the most highly studied and sampled domain in Brazil (Marques et al., 2021), mountainous regions have been highlighted as giving way to high values of richness for endemic angiosperm species (Werneck et al., 2011). This pattern is repeated for studies with other groups, such as vascular epiphytes (Menini Neto et al., 2016; Araujo and Ramos, 2021) and ferns (Suissa and Sundue, 2020). However, these data need to be observed with caution, since the areas with the highest concentration of species are close to major research centers, revealing a possible collection bias (Werneck et al., 2011; Menini Neto et al., 2016; Araujo and Ramos, 2021).

Comprehensive chorological studies of *Peperomia* have barely been carried out in the Neotropical Region (e.g., Vergara-Rodríguez et al., 2017), with information lacking on which areas are the richest, and consequently, which areas are priority for conservation, especially for endemic species. Analyzing these patterns helps to understand biogeographic patterns, supports conservation policies and provides a basis for directing new collection efforts (Gomes-da-Silva and Forzza, 2020). In recent years, the development of databases that compile biodiversity data, such as the Global Biodiversity Information Facility – GBIF (www.gbif.org), Reflora Virtual Herbarium – REFLORA (reflora.jbrj.gov.br) and *species*Link network (www.splink.org.br) have become increasingly useful for exploring information on geographic and conservation patterns (La Salle et al., 2016; Colli-Silva et al., 2020).

Given this, we used currently available databases (GBIF, REFLORA and speciesLink), with the genus *Peperomia* (Fig. 1) as a target group, to answer the following questions: **(1)** Are the *Peperomia* cited in Flora do Brasil (2020) as endemic really endemic? **(2)** How are these species distributed? **(3)** What is the extinction risk of the *Peperomia* species endemic to Brazil? Thereby we intended to test if the patterns found for the endemic *Peperomia* to Brazil were congruent or divergent with the results observed in literature. With these results, our final goal was to present, in an unprecedented way, an overview of the distribution and conservation status of the endemic *Peperomia* to Brazil.

2. Material and methods

2.1. Data collection

For the initial delimitation of the species, we used a list generated through the Flora of Brazil (2020) project, with a total of 111 species considered endemic to the Brazilian territory. Subsequently, aiming to obtain records for these species, we used the methodological workflow for pre-processing records of occurrences of specimens, developed by the Centro Nacional de Conservação da Flora/National Center for the Conservation of Flora (CNCFlora), of the Instituto de Pesquisas Jardim Botânico do Rio de Janeiro (JBRJ), whose steps were mechanized and consisted of: **(1)** Obtaining scientific names for *Peperomia* species given by Flora do Brasil (2020); **(2)** Searching for and compiling occurrence records in three databases: GBIF, REFLORA and *species*Link; **(3)** Standardizing occurrence record's attribute fields from different data sources according to the terms recommended by the Darwin Core standards (Wieczorek et al., 2012; Darwin Core Terms, 2021); **(4)** Validating
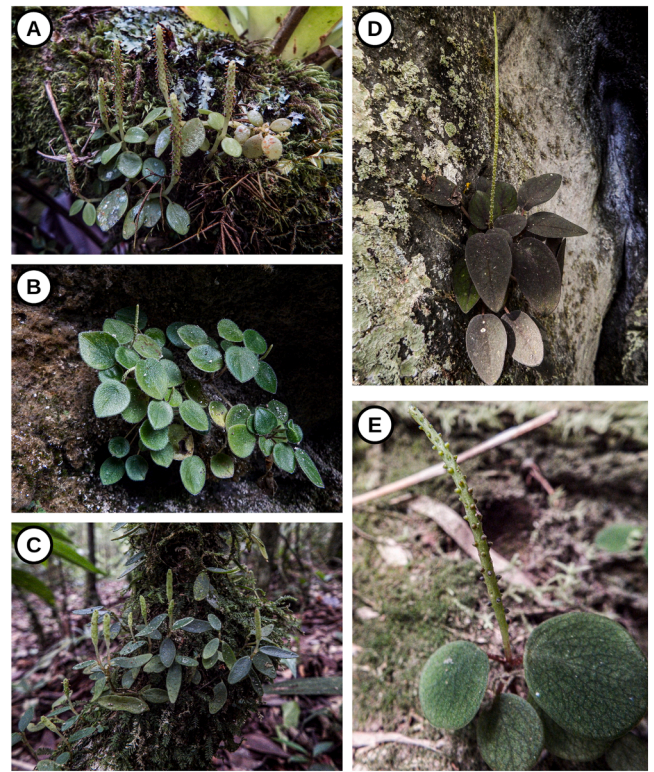

Fig. 1. – Specimens of endemic *Peperomia* to Brazil (photographed by G.M. Marcusso). (**A**): *Peperomia mandioccana*, (**B**): *P. calcicola*, (**C**): *P. pseudoestrellensis*, (**D**): *P. bernhardiana* and (**E**): *P. lyman-smithii*.

information in attribute fields (year, collectionCode, catalogNumber, recordedBy, recordNumber, country, stateProvince, municipality, locality, identifiedBy, dateIdentified, decimalLongitude and decimalLatitude); **(5)** Validating geographic coordinates with the municipality informed in the record. In the absence of coordinates, geographic coordinates were assigned according to the name of the municipality and state along with the database of CNCFlora's locations. In the cases that coordinates could not be provided, coordinates were assigned according to the centroids of the reported municipality. Occurrence records with coordinates projected outside of Brazil, projected on centroid coordinates of Brazil and records without information on geographic coordinates, municipality or locality (when necessary for validation) were flagged for exclusion; **(6)** Based on the recordedBy field, a list of main *Peperomia* collectors in Brazil was elaborated; **(7)** Subsequently, the sets of duplicates were grouped using a key composed of the fields of: family + recordedByStandardized + recordNumber; **(8)** Finally, the most informative record in the set of duplicates for each collection was selected. All data processing was performed in R environment (R Core Team, 2020) with the use of the packages CoordinateCleaner, downloader, dplyr, DT, geobr, jsonlite, lubridate, plyr, purrr, raster, readr, readxl, rgbif, rgdal, rnaturalearthdata, rvest, sp, sqldf, stringr, textclean and tibble; and with shapefile available in the database of the Instituto Brasileiro de Geografia e Estatística/Brazilian Institute of Geography and Statistics - IBGE (www.ibge.gov.br, 2020).

The data obtained from the three bases was then compiled into a single matrix with 8,885 records. Information on substrate type was obtained from Flora do Brasil (2020), from the protologues and type specimen of the species - consulted through the Taxonomic Repertory of the Genus *Peperomia* (peperomia.net - Mathieu, 2021) or from virtual herbaria. Subgeneric classification followed Frenzke et al. (2015).

2.2. Data refinement

Processing and validation of the list of *Peperomia* collectors in Brazil allowed for extracting, standardizing and relating the name of the main collector of the set of specimen collectors. Given that each collector has different practices for the inclusion of this information in the researched databases, standardization becomes useful by enabling, for example, the identification of duplicate records. With the standardized names, a unique code was then generated for each record, as in the example: "Piperaceae_CARVALHO-SILVA,M_421".

From grouping the set of duplicates for each collection in an automated approach was possible, selecting the most informative record among these, and eliminate duplicates. Thus, a matrix with 2554 records was obtained. Afterwards, the resulting matrix was manually checked for the correction or exclusion of records with suspect, incomplete, and/or incorrect information.

Once data preparation was concluded, 11 of the species did not have any record in the work matrix. Occurrence information for these species was then manually added, through individual searches in aforementioned bases, in Tropicos® (tropicos.org, 2021) and in the protologues of each species.

Previously, occurrence points had been overlaid on the map of Brazil, for a first visualization of species distribution. Suspicious coordinates were verified (possible misidentifications) along with those outside of the country's limits, which were later adapted or excluded.

Then the taxonomic refinement was applied, which consisted of keeping only those records that had enough information to guarantee that those specimens were identified by an expert within each group. For this purpose, a list was created (arbitrarily) with botanists who have made scientific contributions to Brazilian and Neotropical species of *Peperomia* (Supplementary Table S1). After this, a matrix with 1167 records was generated.

Finally, 281 records of epiphytic *Peperomia* species contained in the dataset of Atlantic Forest epiphytes (Ramos et al. 2019) were added. As it was not possible to access the data of the collector and identifier directly, the data with coordinates identical to those already obtained in the previous steps were considered repetitions and were excluded from the final matrix, totaling 1448 records used in the analyses (Table S1).

2.3. Geographic patterns analysis

Distribution maps, number of records and richness by grids were prepared in QGIS version 3.10 software (qgis.org - QGIS Association, 2021). The shapefiles were obtained from IBGE (www.ibge.gov.br, 2020) for the biomes map, here treated as phytogeographic domains (*sensu* BFG, 2018, 2021). For the Brazilian federation units (FU) map, the shapefile provided by the Núcleo de Economia Regional e Urbana/Center for Regional and Urban Economics of the University of São Paulo (www.usp.br/nereus, NEREUS/USP 2021) was used.

The Biological Records Tool of the FSC Plugin for QGIS version 3 (www.fscbiodiversity.uk - FSC BioLinks, 2021) was used for the number of records and richness analyses. Occurrence data was overlaid on the map of Brazil, divided into grids with 1°x1° cells (in the Supplementary Fig. S1 we provide a map with numbered grid cells). These cells were classified into seven (arbitrary) classes according to the number of records, and six classes according to richness. At total of 127 cells were generated. The classes of records were named: Minimum (1 record), Very Low (2-10 records), Low (11-19), Medium (21-33), High (38-49), Very High (63-83) and Maximum (128). The richness classes were: Minimum (1 species), Low (2-6 species), Medium (7-10), High (11-14), Very High (15-21) and Maximum (23).

2.4. Preliminary analysis of extinction risk

To obtain an overview of the conservation status of the studied species, an extinction risk analysis was performed using the GeoCAT Conservation Assessment Tool (geocat.kew.org - Bachman et al. 2011). Criterion B of the IUCN (2021) guidelines were adopted. Criterion B is used to delimit the Geographic range in the form of either B1 (extent of occurrence, EOO) AND/OR B2 (area of occupancy, AOO). We used the form B1. In addition to the two forms mentioned above, three conditions should be implemented for a final assessment of conservation status according to the IUCN guidelines (2021): (a) Severely fragmented populations; (b) Continuing decline observed, estimated, inferred or projected; (c) Extreme fluctuations in distribution. We assume here that the final conservation assessment of the species must be made on an individualized basis and with monitoring of populations over space and time, and the data obtained in the open databases alone are not sufficient to establish a definitive threat category, since they do not provide sufficient evidence to satisfy one of the three conditions established. Thus, we present here a preliminary analysis that should be complemented in the future by the competent entities.

3. Results

3.1. Are the Peperomia cited in Flora do Brasil (2020) as endemic really endemic?

We documented 100 *Peperomia* species endemic to Brazil (Tables 1 and 2), which is 59% of the country's species. Six species had no valid records after the cleaning process and were not included in the analyses. During the data preparation stage, it was possible to verify that 11 species could not be considered endemic to Brazil, since they occurred in other countries or were synonyms of species with known distribution in other countries. Table 3 gathers these species with brief comments, which justify their exclusion. Data for these species were then excluded from the analyses.

Although there is information on the occurrence of 94 of these 100 species, the distribution of the number of records per species is uneven, 74.4% of the studied species have 10 or less records, 23.4% have between 11-81 and only two species have more than 100 records (Tables 1, S1).

From a temporal point of view, 35.3% of the specimens were obtained in the 2000s and 2010s. Following these are the 1980s and 1990s with 11.4% and 12.2% of the samples, respectively. Of the other records, 20.2% are grouped between the 1840s and 1970s, with 1843 representing the oldest record (Fig. 2, Tables 1, S1). It was not possible to retrieve data from the year of collection for 20.6% of the records, most referring to additional data from Ramos et al. (2019). According to the last time each species was recorded, 39% were lastly recorded during the 2010s, 19% in the 2000s and more than one third (35%) of species were not recorded after the period between the 19th century and the 1990s (Fig. 3, Tables 1, S1). Seven species did not present data from the last collection.

Seven of the 14 subgenera of *Peperomia* are represented in the present study (Table 1), with 27% of the species belonging to *Micropiper* (Miq.) Miq., followed by *Pseudocupula* Frenzke & Scheiris with 18%, *Multipalmata* Scheiris & Frenzke with 9%, *Leptorhynchum* (Dahlst.) Trel. ex Samain with 7%, *Oxyrhynchum* (Dahlst.) Samain with 4%, *Peperomia* with 3% and *Hispidulae* Frenzke & Scheiris with only one species. A third of the species (31%) still do not have subgeneric circumscription. The subgenera *Erasmia* (Miq.) Dahlst., *Fenestratae* Pino, *Phyllobryon* (Miq.) Scheiris & Frenzke, and *Pleurocarpidium* Dahlst., present in Brazil, lack endemic representatives.

Considering the type of substrate, 50 species were found to be epiphytic, 39 rupicolous, 34 terrestrial, and 11 species could not be evaluated due to lack of information in the sources used. Still regarding the substrate, the species studied can be divided into restricted (60 spp.), those that occupy only one type of substrate, or unrestricted (29 spp.), occupying two or three substrates. Among the restricted substrate species, 29 are epiphytic, 17 terrestrial and 14 rupicolous. As for unrestricted substrate species, 12 are epiphytic and rupicolous; eight are

Table 1

Peperomia species endemic to Brazil. Substrate types - Epiphytic (E), Rupicolous (R), Terrestrial (T) and Data unavailable (-), NR = Number of records, LR = Latest record (Table S1).

Species	Subgenus	Substrate	NR	LR
Peperomia acreana C.DC.	*Oxyrhynchum*	E	1	1911
Peperomia adsurgens Yunck.	*Micropiper*	E, T	2	2011
Peperomia aggregata E.F. Guim. & M. Carv.-Silva	*Leptorhynchum*	R	5	2010
Peperomia albopilosa D. Monteiro	*Incertae sedis*	R	9	2016
Peperomia alegrensis Yunck.	*Incertae sedis*	-	1	1904
Peperomia apiahyensis Yunck.	*Micropiper*	T	1	1885
Peperomia arbuscula Yunck.	*Micropiper*	-	2	2010
Peperomia augescens Miq.	*Micropiper*	E, R, T	41	2016
Peperomia bernhardiana C.DC.	*Incertae sedis*	T	-	-
Peperomia blackii Yunck.	*Incertae sedis*	E	1	1948
Peperomia bradei Yunck.	*Micropiper*	E	3	1940
Peperomia brasiliensis (Miq.) Miq.	*Incertae sedis*	E, T	1	1999
Peperomia brevihirtella Yunck.	*Micropiper*	-	-	-
Peperomia calcicola Marcusso	*Multipalmata*	R	2	2019
Peperomia calophylla Yunck.	*Incertae sedis*	T	8	2014
Peperomia campinasana C.DC.	*Pseudocupula*	E, R	44	2014
Peperomia castelosensis Yunck.	*Pseudocupula*	E	45	2019
Peperomia ciliatocaespitosa Carv.-Silva & E.F.Guim.	*Micropiper*	R, T	3	2012
Peperomia claussenii Yunck.	*Incertae sedis*	T	1	1843
Peperomia clivicola Yunck.	*Micropiper*	E, R	37	2016
Peperomia cordigera Dahlst.	*Pseudocupula*	R	3	1979
Peperomia crinicaulis C.DC.	*Pseudocupula*	E	81	2018
Peperomia cruzeirensis M. Carvalho-Silva, E.F. Guim. & P. S. Câmara	*Incertae sedis*	E	2	1987
Peperomia crypticola C.DC.	*Oxyrhynchum*	R	1	1907
Peperomia decora Dahlst.	*Pseudocupula*	R	55	2017
Peperomia diamantinensis M. Carvalho-Silva, E.F. Guim. & P. S. Câmara	*Incertae sedis*	R, T	3	2005
Peperomia dichotoma Regel	*Incertae sedis*	E	6	2002
Peperomia duartei Yunck.	*Incertae sedis*	T	13	2015
Peperomia egleri Yunck.	*Incertae sedis*	E	2	1961
Peperomia epipremnifolia D. Monteiro & Leitman	*Incertae sedis*	T	8	2012
Peperomia flexicaulis Wawra	*Micropiper*	E	1	1859
Peperomia fluviatilis Yunck.	*Pseudocupula*	E	5	1986
Peperomia glazioui C.DC.	*Micropiper*	E, R	152	2016
Peperomia gracilicaulis Yunck.	*Incertae sedis*	E	2	1992
Peperomia gracilis Dahlst.	*Pseudocupula*	E	3	2016
Peperomia guarujana C.DC.	*Incertae sedis*	E	1	1907
Peperomia hemmendorffii Yunck.	*Micropiper*	T	1	2004
Peperomia hispidosa Dahlst.	*Pseudocupula*	E, R, T	3	1999
Peperomia huberi C.DC.	*Multipalmata*	T	4	2006
Peperomia humifusa Yunck.	*Leptorhynchum*	-	1	1891
Peperomia ibiramana Yunck.	*Multipalmata*	E, R, T	7	2009
Peperomia incana (Haw.) Hook.	*Leptorhynchum*	E, R	45	2018
Peperomia itatiaiana Yunck.	*Micropiper*	E, R	3	2012
Peperomia lindmaniana Dahlst.	*Oxyrhynchum*	T	1	1894
Peperomia loefgrenii Yunck.	*Incertae sedis*	T	1	1988
Peperomia lyman-smithii Yunck.	*Multipalmata*	T	28	2010
Peperomia mandioccana Miq.	*Micropiper*	E, R	53	2018
Peperomia marcoana C.DC.	*Incertae sedis*	R	1	1907
Peperomia megapotamica Dahlst.	*Pseudocupula*	R, T	42	2016
Peperomia menkeana Miq.	*Pseudocupula*	E	1	1953
Peperomia minensis Henschen	*Pseudocupula*	E	8	2003
Peperomia mosenii Dahlst.	*Micropiper*	-	2	1973
Peperomia nudifolia C.DC.	*Pseudocupula*	-	2	1985
Peperomia oreophila Henschen	*Pseudocupula*	R	58	2018
Peperomia papillispica C.DC.	*Micropiper*	-	4	-
Peperomia parcifolia C.DC.	*Micropiper*	E	1	1997
Peperomia parnassiifolia Miq.	*Multipalmata*	E	-	19th cent.
Peperomia pellucidoides Yunck.	*Peperomia*	R, T	3	1980
Peperomia pereirae Yunck.	*Pseudocupula*	E, R	6	1987
Peperomia perlongicaulis Yunck.	*Incertae sedis*	E	-	-
Peperomia polystachyoides Dahlst.	*Micropiper*	E, R	3	2002
Peperomia pseudobcordata Yunck.	*Incertae sedis*	E	8	2009
Peperomia pseudoestrellensis C.DC.	*Micropiper*	E, T	173	2018
	Incertae sedis	R, T	5	2012

Table 1 (*continued*)

Species	Subgenus	Substrate	NR	LR
Peperomia pseudoserratirhachis D. Monteiro				
Peperomia pubipeduncula Yunck.	*Incertae sedis*	E	3	2011
Peperomia punicea Dahlst.	*Micropiper*	R, T	11	2013
Peperomia ramboi Yunck.	*Micropiper*	T	2	1981
Peperomia regelii C.DC.	*Incertae sedis*	E, R	10	2000
Peperomia renifolia Dahlst.	*Incertae sedis*	T	7	1995
Peperomia retivenulosa Yunck.	*Micropiper*	T	2	1904
Peperomia riedeliana Regel	*Incertae sedis*	E	-	-
Peperomia riparia Yunck.	*Pseudocupula*	E	2	2003
Peperomia rizzinii Yunck.	*Incertae sedis*	E, R	26	2015
Peperomia rostulatiformis Yunck.	*Multipalmata*	R	1	2003
Peperomia rufispica Yunck.	*Pseudocupula*	E	1	1930
Peperomia schenkiana Dahlst.	*Leptorhynchum*	-	-	2005
Peperomia schwackei C.DC.	*Leptorhynchum*	E	8	2019
Peperomia segregata T.S.Dantas, Carv.-Silva & P.E.A.S.Câmara	*Micropiper*	T	2	2012
Peperomia serpentarioides Miq.	*Multipalmata*	T	9	2004
Peperomia serratirhachis Yuncker	*Hispidulae*	R, T	4	2002
Peperomia simulans C.DC.	*Peperomia*	-	1	1911
Peperomia sincorana C.DC.	*Micropiper*	R	70	2015
Peperomia spiritus-sancti E.F. Guim. & M. Carv.-Silva	*Leptorhynchum*	R	3	2011
Peperomia stenocarpa Regel	*Incertae sedis*	-	14	2001
Peperomia stroemfeltii Dahlst.	*Micropiper*	E, R	7	2010
Peperomia subemarginata Yunck.	*Incertae sedis*	E	8	2007
Peperomia subpilosa Yunck.	*Leptorhynchum*	E	11	2002
Peperomia subretusa Yunck.	*Pseudocupula*	E	32	2016
Peperomia subrubricaulis C.DC.	*Micropiper*	E, R	12	2011
Peperomia subrubrispica C.DC.	*Incertae sedis*	R	64	2018
Peperomia subsetifolia Yunck.	*Multipalmata*	-	1	1992
Peperomia subternifolia Yunck.	*Pseudocupula*	E, T	42	2016
Peperomia sulbahiensis D. Monteiro & M. Coelho	*Incertae sedis*	R	4	2011
Peperomia sulcata C.DC.	*Multipalmata*	R, T	3	1980
Peperomia sumidoriana C.DC.	*Incertae sedis*	E	1	1971
Peperomia tenuilimba C.DC.	*Peperomia*	T	5	2001
Peperomia trichocarpa Miq.	*Incertae sedis*	E	2	1983
Peperomia turbinata Dahlst.	*Oxyrhynchum*	E, R, T	7	2006
Peperomia velloziana Miq.	*Micropiper*	E, R, T	59	2018
Peperomia warmingii C.DC.	*Micropiper*	R	5	2016

rupicolous and terrestrial; five are epiphytic, rupicolous, and terrestrial; and four are epiphytic and terrestrial (Table 1).

3.2. How are these species distributed?

The endemic *Peperomia* species occur in practically all Brazilian phytogeographic domains, except in the *Pantanal* domain (Fig. 4, Tables 2, S1). With 73 species, the Atlantic Forest holds the greatest richness, followed by *Cerrado* (24 species), the Amazon (16), the *Caatinga* (14) and the *Pampa* (two) (Tables 2, S1). One species, *Peperomia segregata* T.S. Dantas, Carv.-Silva & P.E.A.S. Câmara occurs only on the island of Trindade (Espírito Santo state, Atlantic Ocean), with no occurrence in the Brazilian mainland (Tables 2, S1). Four domains have exclusive species to their area where again the Atlantic Forest stands out with 50 species endemic to its territory, followed by the Amazon with 13 species, the *Cerrado* with three, and the *Caatinga* with a single species.

A total of 127 grid cells were generated in the analyses of number of records and richness (Fig. S1). Of these, considering the classes for numbers of records, 45 cells fell into the Minimum class, 48 in the Very Low class, 11 in Low, nine in Medium, nine in High, four in Very High, and only one in the Maximum class (Fig. 5). For richness, 53 cells belong to the Minimum class, 48 to Low, 16 to Medium, six to High, three to Very High and again, only one to the Maximum class (Fig. 6).

The cell that presented the highest results, both in terms of the number of records and the number of species (Figs. 5 and 6), occurs in the city of Rio de Janeiro and its surroundings, with 128 records of 23 species (cell 86 - Fig. S1). Three other cells stand out in terms of richness and number of records, being in the Very High class in both analyses.

Table 2

Distribution and conservation status of the endemic *Peperomia* of Brazil. Phytogeographical domains: Amazon Rain Forest (AMA), Atlantic Rain Forest (ATL), *Caatinga* (CAA), *Cerrado* (CER), *Pampa* (PAM). Federative Units of Brazil = FU (Table S1), GeoCAT conservation status = IUCN.

Species	Domain	FU	IUCN
Peperomia acreana	AMA	AC	CR
Peperomia adsurgens	ATL	RJ	CR
Peperomia aggregata	ATL	ES, MG	VU
Peperomia albopilosa	AMA	PA	EN
Peperomia alegrensis	AMA	AM	CR
Peperomia apiahyensis	ATL	SP	CR
Peperomia arbuscula	ATL	RJ	CR
Peperomia augescens	ATL, CER, PAM	GO, MG, RJ, RS, SC, SP	LC
Peperomia bernhardiana	-	-	DD
Peperomia blackii	AMA	PA	CR
Peperomia bradei	ATL	RJ	CR
Peperomia brasiliensis	ATL	PR	CR
Peperomia brevihirtella	-	-	DD
Peperomia calcicola	ATL	SP	CR
Peperomia calophylla	ATL, CER	MS, PR	NT
Peperomia campinasana	ATL, CAA, CER	BA, DF, MG, PE, PR, SP	LC
Peperomia castelosensis	ATL	ES, MG, PR, RJ, RS, SC, SP	LC
Peperomia ciliatocaespitosa	ATL	ES	EN
Peperomia claussenii	ATL	MG	CR
Peperomia clivicola	ATL	ES, PR, RJ, RS, SC, SP	LC
Peperomia cordigera	ATL, CER	MG, RJ	EN
Peperomia crinicaulis	ATL, CAA, CER	BA, ES, MG, PR, RJ, SP	LC
Peperomia cruzeirensis	AMA	AC, AM	CR
Peperomia crypticola	ATL	MG	CR
Peperomia decora	ATL, CAA, CER	BA, MG	LC
Peperomia diamantinensis	CAA	BA	CR
Peperomia dichotoma	ATL	RJ, SP	VU
Peperomia duartei	ATL, CER	PR, SP	LC
Peperomia egleri	AMA	PA	CR
Peperomia epipremnifolia	ATL	BA	EN
Peperomia flexicaulis	ATL	BA	CR
Peperomia fluviatilis	AMA	AC, RO	LC
Peperomia glazioui	ATL, CAA, CER	BA, ES, MG, PR, RJ, RS, SC, SP	LC
Peperomia gracilicaulis	ATL	PR, SC	CR
Peperomia gracilis	ATL	MG, RJ	VU
Peperomia guarujana	ATL	SP	CR
Peperomia hemmendorffii	CER	MG	CR
Peperomia hispidosa	ATL	RJ, SP	EN
Peperomia huberi	AMA	AC, AM, PA	LC
Peperomia humifusa	ATL	RJ	CR
Peperomia ibiramana	ATL	PR, SC	NT
Peperomia incana	ATL	BA, ES, MG, RJ	LC
Peperomia itatiaiana	ATL	ES, MG, RJ	EN
Peperomia lindmaniana	AMA	MT	CR
Peperomia loefgrenii	ATL	RJ	CR
Peperomia lyman-smithii	ATL, CER	ES, MT, PR, RS, SC	LC
Peperomia mandioccana	ATL, CER	ES, MG, PR, RJ, SC, SP	LC
Peperomia marcoana	ATL	MG	CR
Peperomia megapotamica	AMA, ATL	AM, ES, RJ, RS, SC	LC
Peperomia menkeana	ATL	RJ	CR
Peperomia minensis	ATL, CER	GO, MG, SP	NT
Peperomia mosenii	CER	MG	CR
Peperomia nudifolia	ATL	PR	CR
Peperomia oreophila	ATL, CAA, CER	BA, GO, MG, SP	LC
Peperomia papillispica	ATL	SP	VU
Peperomia parcifolia	ATL	BA	CR
Peperomia parnassiifolia	-	-	DD
Peperomia pellucidoides	AMA, CER	PA	NT
Peperomia pereirae	ATL	ES, PR, RJ	LC
Peperomia perlongicaulis	-	-	DD
Peperomia polystachyoides	ATL	ES, MG, RJ	NT
Peperomia pseudobcordata	ATL	PR, SC	VU
Peperomia pseudoestrellensis	ATL, CAA	BA, ES, MG, PR, RJ, SC, SP	LC
Peperomia pseudoserratirhachis	AMA	PA	EN
Peperomia pubipeduncula	ATL	RJ	CR
Peperomia punicea	ATL, CER	ES, MG, PR, RJ	LC

Table 2 (*continued*)

Species	Domain	FU	IUCN
Peperomia ramboi	ATL	PR, SC	CR
Peperomia regelii	ATL, CER	ES, GO, MG, PR, RJ, SP	LC
Peperomia renifolia	ATL	MG, PR, RS, SC	LC
Peperomia retivenulosa	ATL	PR	CR
Peperomia riedeliana	-	-	DD
Peperomia riparia	ATL	BA, MG	CR
Peperomia rizzinii	ATL	PR, RJ, RS, SC	LC
Peperomia rostulatiformis	CER	MG	CR
Peperomia rufispica	ATL	MG	CR
Peperomia schenkiana	-	SP	DD
Peperomia schwackei	ATL	ES, MG, PR, RJ, SP	LC
Peperomia segregata	Trindade Island	ES	CR
Peperomia serpentarioides	ATL	BA	VU
Peperomia serratirhachis	ATL, CER	BA, GO SC, TO	LC
Peperomia simulans	AMA	AC	CR
Peperomia sincorana	CAA, CER	BA, MG	NT
Peperomia spiritus-sancti	ATL	ES	EN
Peperomia stenocarpa	ATL, CAA	BA, MG, PE, RJ	LC
Peperomia stroemfeltii	ATL	BA, SC	LC
Peperomia subemarginata	ATL	PR, SC	NT
Peperomia subpilosa	ATL	BA, ES, MG, RJ	LC
Peperomia subretusa	ATL	MG, PR, RJ, RS, SP	LC
Peperomia subrubricaulis	ATL, CAA, CER	BA, MG, PR	LC
Peperomia subrubrispica	ATL, CAA, CER	BA, ES, MG, PR, SP	LC
Peperomia subsetifolia	ATL	SP	CR
Peperomia subternifolia	ATL	MG, PR, RJ, SP	LC
Peperomia sulbahiensis	ATL	BA	EN
Peperomia sulcata	AMA	AC, PA	EN
Peperomia sumidoriana	AMA	AC	CR
Peperomia tenuilimba	AMA, CER	AC, MS	NT
Peperomia trichocarpa	ATL	BA, PR	CR
Peperomia turbinata	ATL, CAA	BA, ES, RJ, SP	LC
Peperomia velloziana	ATL, CAA, CER, PAM	BA, DF, ES, GO, MG, PR, RJ, RS, SC, SP	LC
Peperomia warmingii	ATL	ES, MG, PR	LC

Table 3

Species excluded from the analyses.

Excluded species	Notes
Peperomia cooperi C.DC.	Synonym* of *Peperomia sancarlosiana* C.DC. It does not occur in Brazil.
Peperomia cowanii Yunck.	Occurs outside Brazil, selected material: "MORI, S.A. 25665 (NY 806965)", French Guiana.
Peperomia dahlstedtii C. DC.	Occurs outside Brazil, selected material: "CABALLERO, I. G.V. 3479 (NY 559030)", Bolivia.
Peperomia decipiens C.DC.	Synonym* of *Peperomia blanda* (Jacq.) Kunth. Species with wide distribution.
Peperomia fimbriata Miq.	*Peperomia fimbriata* f. *pilosior* Miq. which occurs in Brazil, is synonymous* with *Peperomia subrubrispica* C. DC.
Peperomia hilariana Miq.	Occurs outside Brazil, selected material: "RUSBY, H.H. 224 (MO 2859769)", Bolivia.
Peperomia hydrocotyloides Miq.	Occurs outside Brazil, selected material: "MELLO-SILVA, R. 2030 (SPF 156931)", Bolivia.
Peperomia morungavana Yunck.	Synonym* of *Peperomia pereskiifolia* (Jacq.) Kunth. It occurs from Mexico to Brazil.
Peperomia ripicola C.DC.	Occurs outside Brazil, selected material: "BETANCUR, J. 5474 (COAH 9930)", Colombia.
Peperomia spruceana Benth.	Occurs outside Brazil, selected material: "PLOWMAN, T 6861 (F 1823978)", Peru.
Peperomia suboppositifolia Yunck.	Synonym* of *Peperomia unduavina* C.DC. It occurs in Brazil and Bolivia.

* Synonym status follows Mathieu (2021) and the Flora do Brasil (2020).

These are: the Mantiqueira mountains in Rio de Janeiro state (cell 85 – Fig. S1), with 83 records and 15 spp.; the coastal Paraná state region (cell 108 – Fig. S1), with 76 records and 18 spp.; and the mountainous region of central Rio de Janeiro (cell 87 – Fig. S1), with 63 records and 21 spp. Another area that stands out is the region of central Minas Gerais state, appearing in the Very High class of records and High of richness, with 72 records and 12 spp., respectively (cell 59 – Fig. S1).
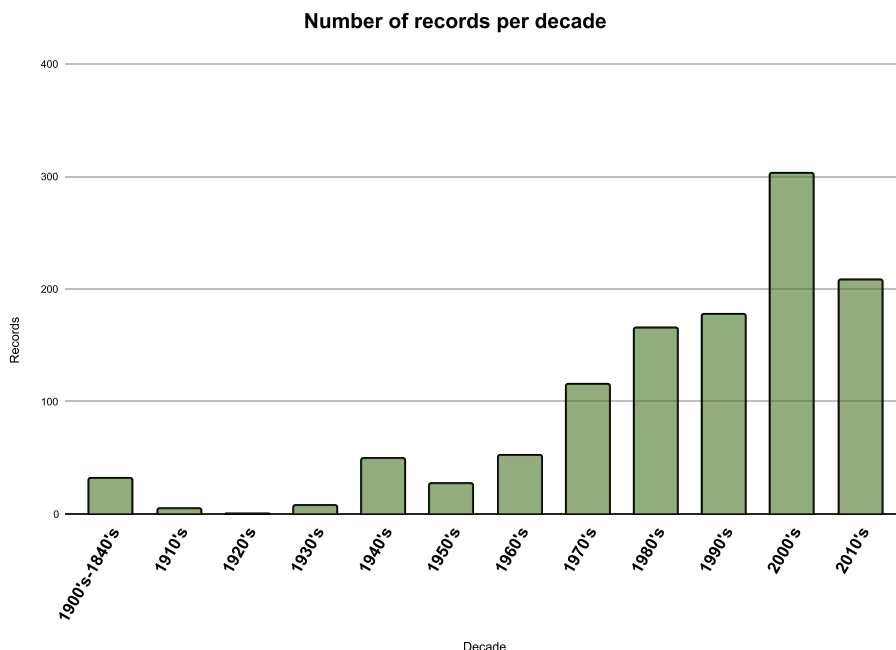
Number of records per decade

Fig. 2. – Number of records per decade.
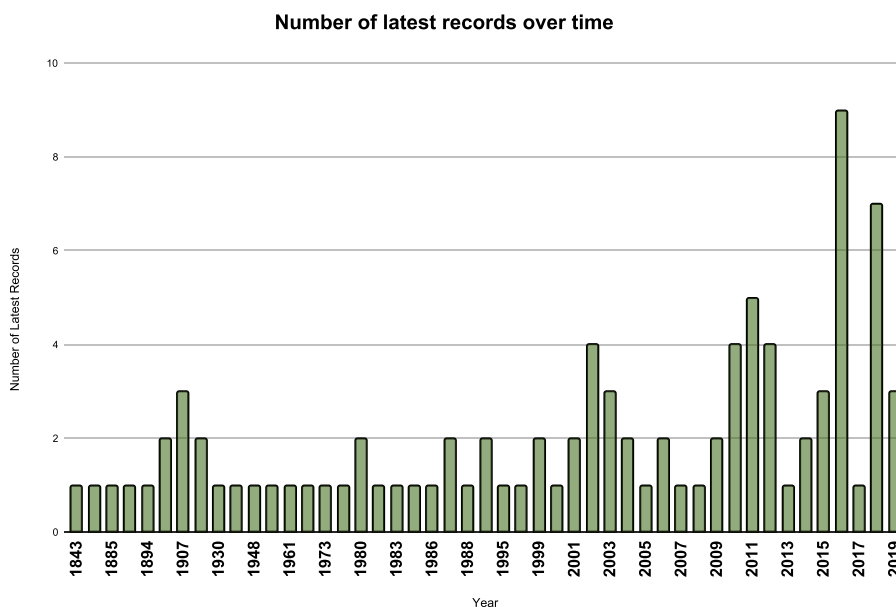
Number of latest records over time

Fig. 3. – Number of latest records over time.

3.3. What is the extinction risk of the Peperomia species endemic to Brazil?

Extinction risk analysis (Tables 2, S1) revealed that 33 species are in the Least Concern (LC) category, eight species in the Near Threatened (NT), six in Vulnerable (VU), ten in Endangered (EN), 37 in the Critically Endangered (CR) and six were circumscribed in the Deficient Data (DD) category, because there is no data on their locality of occurrence in the sources used here. It was possible to demonstrate that approximately half of the species (53) can be classified in some category of threat (CR, EN or VU).

4. Discussion

This is the first study involving a comprehensive analysis of endemic

Peperomia species in Brazil from a biogeographic and conservationist approach. Initially, we worked with a list of 111 species considered to be endemic by the Flora do Brasil (2020). However, during the analysis, it was possible to verify that 11 species did not meet the criteria of endemism for the country. This data reinforces the need for continuous reviews to refine the data presented in this herculean Brazilian project (BFG, 2018; 2021).

We started with a matrix of 8885 records and reduced them to an end matrix with 1448 records, which was used in the analyses. This reduction of approximately four fifths is reasonable considering similar results for this reduction process in other studies such as in Colli-Silva et al. (2020) with 75% and about 79% in Amorim et al. (2021). In addition, the results of records by period, obtained by Colli-Silva et al. (2020) for flowering plants, reinforce the pattern found here, of a greater number of records obtained starting in the mid-1990s, probably due to the
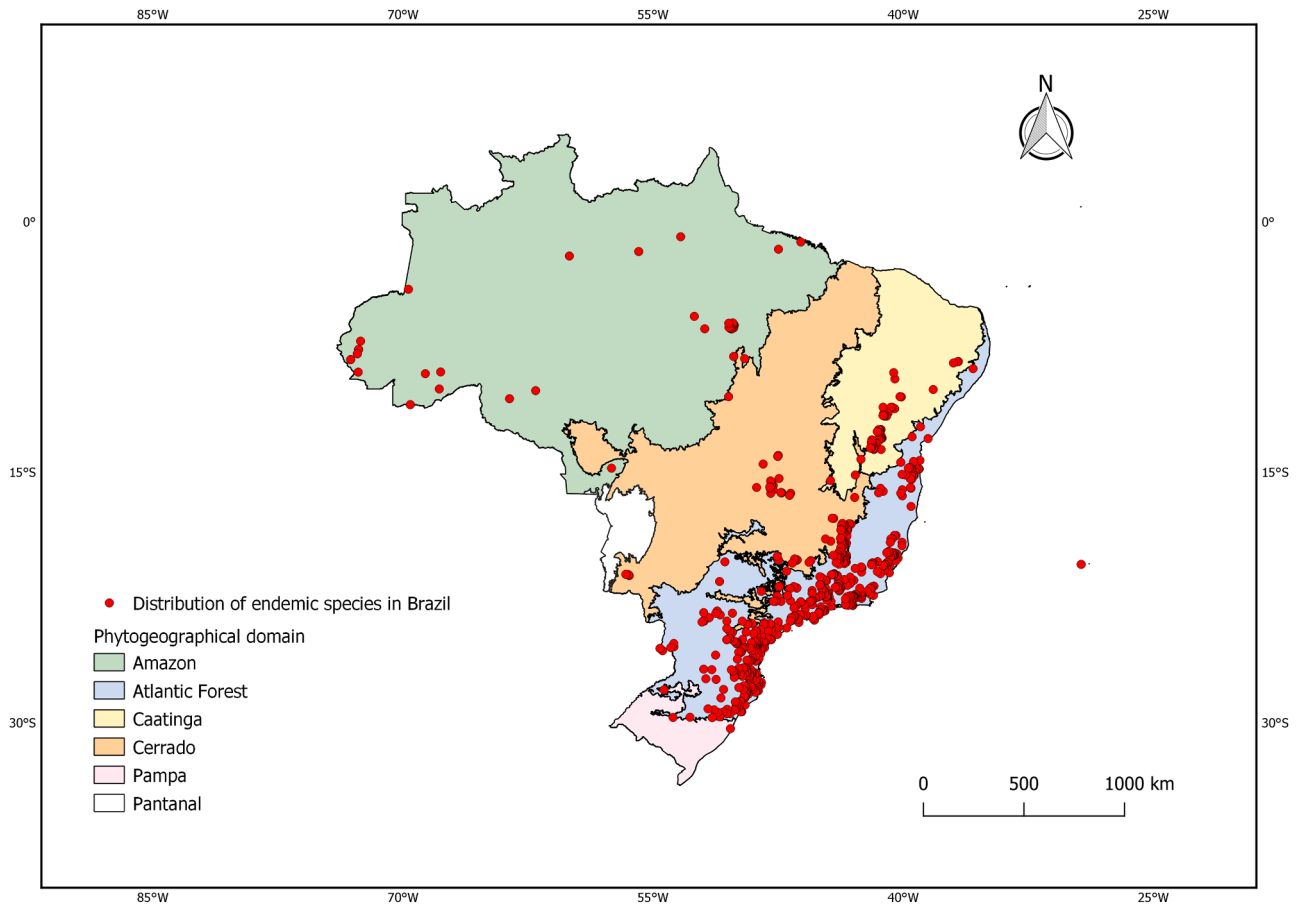
Fig. 4. – Distribution of endemic *Peperomia* in Brazilian phytogeographic domains.

popularization of technologies such as the Global Positioning System (GPS) and systems for digitizing collections and specimens (Graham et al., 2004; Kaplan and Hegarty, 2017).

The highest values of records and richness in the Atlantic Forest repeat similar patterns found in studies carried out with angiosperms endemic to Brazil (Gomes-da-Silva and Forzza, 2020), Brazilian mosses (Amorim et al., 2021), Neotropical ferns (Suissa and Sundue, 2020), and Neotropical vascular epiphytes (Marcusso et al., 2022). These results confirm that *Peperomia* are commonly found co-occurring with bryophytes (Zoghbi et al., 2005). Considering the repetition of patterns found for different groups of land plants, we can extend this hypothesis by the fact that environmental factors such as high water availability, elevational and latitudinal gradients and the variety of microhabitats may be associated with the establishment of the studied species in the Atlantic Forest (Costa and Peralta, 2015; Batista and Santos, 2016; Freitas et al., 2016). Furthermore, the Atlantic Forest is also known for harboring a great diversity of birds, and evidence shows that several *Peperomia* have adaptations for epizoochoric dispersal (sticky secretions and hook-shaped appendages on the fruits) by these animals, which are associated with higher rates of diversification and, consequently, can act as a speciation driver (Frenzke et al., 2016; Tonetti et al., 2017; Vale et al., 2018).

The biogeographic history of the Atlantic Forest biota is associated with the heterogeneous landscape where it occurs, in the most mountainous region of Brazil (Guedes et al., 2020), and by its broad latitudinal gradient (Oliveira-Filho and Fontes, 2000; Stehmann et al., 2009). Historically, the emergence of the dry diagonal in South America during the Oligocene/Miocene, isolated the Atlantic Forest from the Amazon, acting as vicariant barrier, and, consequently, driving the diversification in both isolated forest patches (Prado and Gibbs, 1993; Zanella, 2011). It

was during this period that the diversification of various plant groups occurred in eastern Brazil (e.g., Perret et al., 2006; Moreno et al., 2015), with subsequent intensification in the Pliocene (e.g., Morales et al., 2021). Furthermore, the geomorphological dynamics of the mountainous ranges of eastern Brazil was contemporary to these events (Almeida and Carneiro, 1998) - a consequence of the new environments arising (Ribeiro, 2006). This fact highlights the importance of mountains for biotic evolution in the Atlantic Forest, considered as one of the main generators of biodiversity (Gentry, 1982; Antonelli et al., 2018). The climatic stability - since the Miocene - in the Brazilian shore also may have resulted in the high accumulation of species and endemism found today (Prance, 1982; Fiaschi et al., 2016).

Being predominantly an epiphytic genus (Zotz, 2013), *Peperomia* species show great dependence on forest vegetation. This is concerning given that the phytogeographic domain of the Atlantic Forest has had about 64% of the domain's territory converted into areas for agricultural activities, and less than 30% of the Atlantic Forest has forested areas today, according to MapBiomas (mapbiomas.org, 2021). The Atlantic Forest has been suffering from the impacts of human actions for centuries (Dean, 1995). Among these, the conversion of natural systems into agricultural areas and exotic tree plantations, with large areas destinated to eucalypt (*Eucalyptus* L'Hér) and sugarcane (*Saccharum* L.), and the associated use of herbicides and pesticides, as well as the expansion of urban areas affect the region (Ribeiro et al., 2009; Leão et al., 2021). Even with these issues, recently, new and endemic species of *Peperomia* have been described in the remnants of the Atlantic Forest (e.g., Monteiro et al., 2016; Dantas et al., 2017; Carvalho-Silva et al., 2019; Marcusso et al., 2020), meaning that taxonomic and field efforts still are needed, even in this most studied floristic domain of Brazil (Marques et al., 2020).
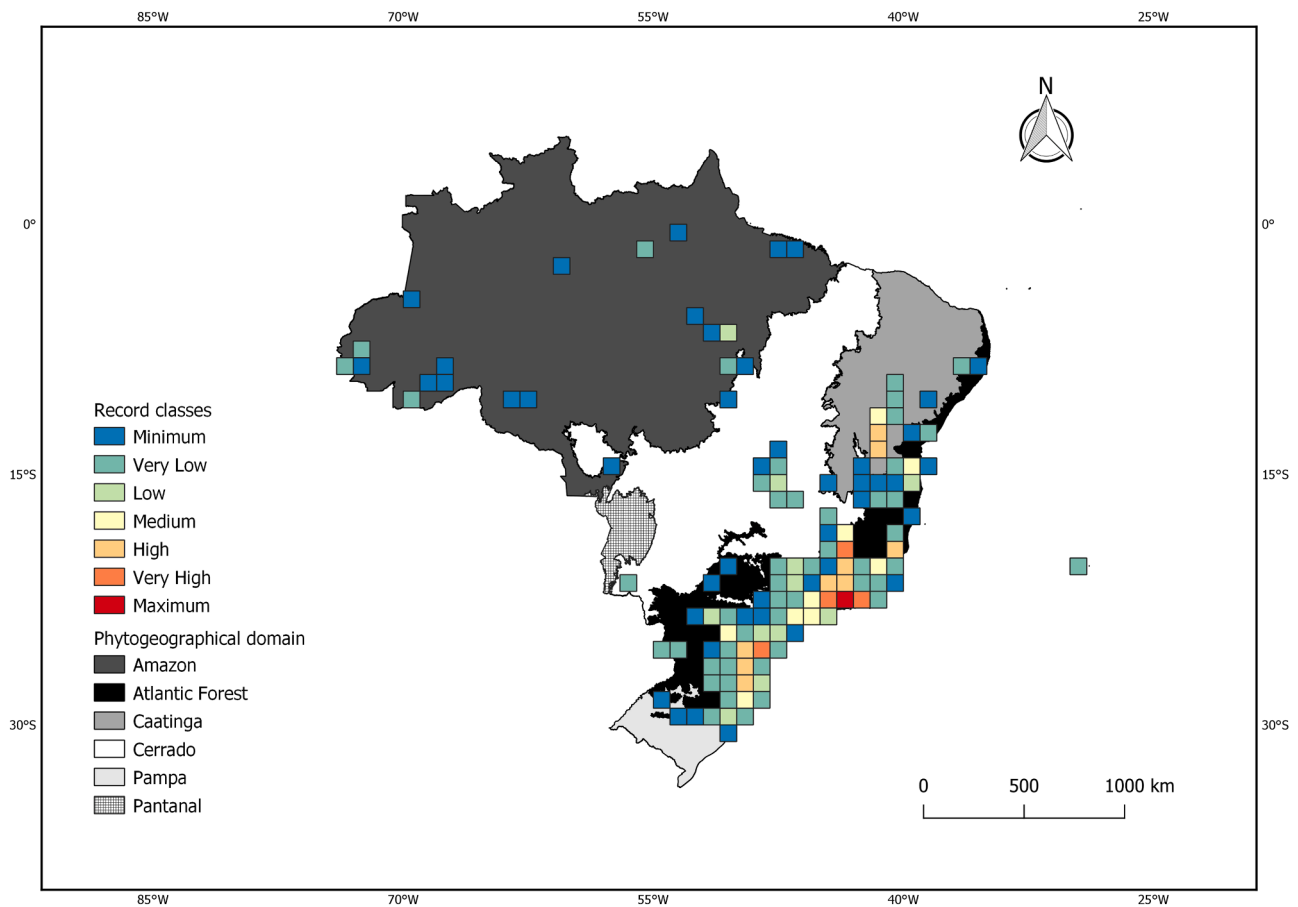
Fig. 5. – Map of Brazilian phytogeographic domains showing the grid cells presence of endemic *Peperomia* species and their classes, from the record analysis.

The Amazon, although it also is comprised of humid (but hotter and mainly lowland) forests, did not present similar results to those of the Atlantic Forest, corroborating the low richness values found for the genus in the Brazilian Amazon territory (e.g., Obermuller et al., 2014; Melo et al., 2016). Suissa and Sundue (2020) emphasize that topographical differences (more homogeneous) may be responsible for the manifestation of this pattern, since areas with greater topographical heterogeneity (as in the Atlantic Forest) present greater availability of habitats, favoring biological diversity (Freitas et al., 2016). In addition, the genus *Peperomia* is mostly composed of epiphytic species (Tucker, 1980; Wanke et al., 2006; Frenzke et al., 2015), and data for other areas in the Neotropical region show that vascular epiphytes have higher richness in mountainous ranges (Krömer et al., 2005; Marcusso et al., 2022), a different condition from that found in the Brazilian Amazon. Still, it is possible to perceive through our results that extensive areas in the Amazon region do not have any occupied grid cells, while the cells with the highest values of records and richness are in locations close to large botanical collections (or areas where their researchers collect), suggesting the possible existence of the sampling bias known as the 'museum effect' (Ponder et al., 2002; Gasper et al., 2020). Solutions to these types of gaps include the installation of research centers in under-sampled locations, guided collection efforts aiming to fulfill these gaps, and greater investments in research and development, which has been the exact opposite of what has been happening in Brazil in recent years (Moerman and Estabrook, 2006; Hallal, 2021). For example, field efforts in the Amazon ironstone have brought up new and endemic species of *Peperomia* (Monteiro, 2018). In addition, little is known about the diversity of *Peperomia* in the Brazilian Amazon, which is facing more threats to its biodiversity every year (Silva Junior et al., 2021), thus compromising the future of these organisms.

Despite data suggesting a possible 'museum effect' (Ponder et al., 2002), we believe that for the Atlantic Rain Forest the high values both in records and in richness are jointly explained by the unique environmental characteristics mentioned above (Zoghbi et al., 2005; Costa and Peralta, 2015; Batista and Santos, 2016; Frenzke et al., 2016; Tonetti et al., 2017; Vale et al., 2018), and because it is the most studied domain in Brazil (Marques et al., 2021), differing from others analyzed here, which showed extensive collections gaps in their territories.

According to our preliminary extinction risk assessment, approximately half of Brazil's endemic *Peperomia* species fall into one of the threat categories (CR, EN or VU) proposed by the IUCN (2021), and therefore need to be cited in the IUCN Red List of Threatened Species. These results coincide with those presented by Vergara-Rodríguez et al. (2017), where about 45% of the *Peperomia* species from Veracruz State (Mexico) belong to a threat category. When we compare these numbers with data provided by institutions responsible for carrying out analyses of extinction risk, we find a very large variation in the percentage of species that fall into any of the threat categories. According to data obtained from CNCFlora (2021), 39 species of *Peperomia* that occur in Brazil were evaluated for their risk of extinction, in which nine (ca. 25%) are in some category of threat. According to information from the IUCN (2021), 68 species of *Peperomia* have been evaluated worldwide, with 57 being in some category of threat - more than 80% of all evaluated species. The low number of evaluated species reinforces the need for more studies that include *taxa* that are little studied. According to Martinelli and Moraes (2013), all previous red lists made in Brazil had evaluated only a small part of the native flora, resulting in a low number of analyzed species and, in many cases, including only ornamental species well known by scientists. This scenario was confirmed by conservation assessments on Araceae (Krömer et al., 2019) and Bromeliaceae (Zizka
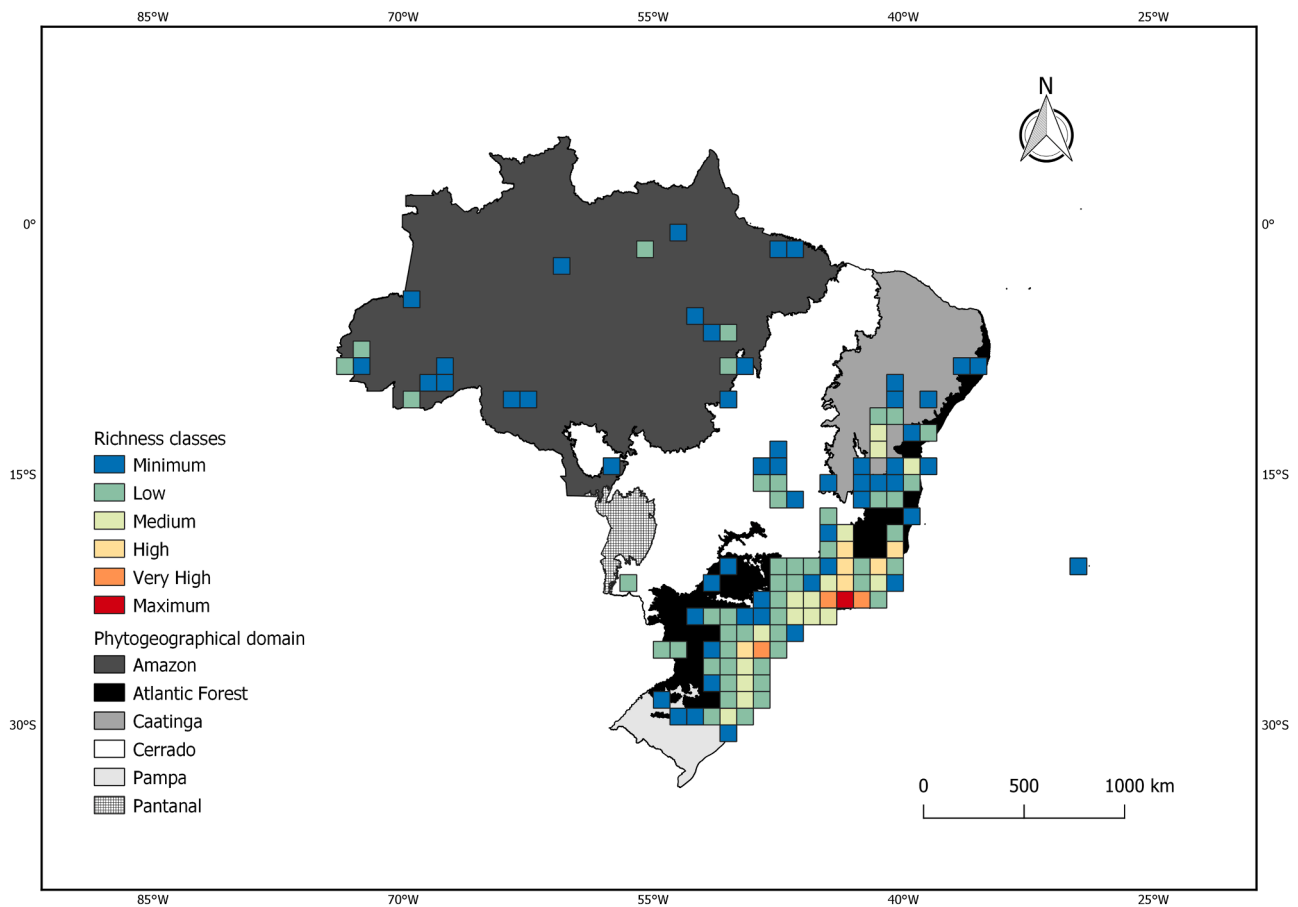
Fig. 6. – Map of Brazilian phytogeographic domains showing the grid cells presence of endemic *Peperomia* species and their classes, from the richness analysis.

et al., 2020). Many threatened *taxa* and other species of conservation interest end up being neglected when only selective assessments are carried out. Thus, it can be inferred that about half of the endemic *Peperomia* species to Brazil may be threatened in some way, especially considering that less than 15% of the natural vegetation of the Atlantic Forest, which shelters most species of the genus in the country, still resists (Myers et al., 2000; Mittermeier et al., 2004; Scarano and Ceotto, 2015). This reinforces the demand for continuous assessments and strategies for the conservation of these *taxa*.

Another factor that needs to be taken into account is intrinsic to the data reduction process, where part of the records were excluded because they did not fit the established criteria (to ensure reliability and correct identification of species) - directly affecting the results of conservation analyses. This reinforces the importance of continuous updating of data from the original collections, including the addition of relevant information about the identification and collection location of these samples.

In addition, due to their high ornamental potential, several species of *Peperomia* are cultivated and used in decorations and landscaping (Mathieu, 2021). However, there are no data in the literature that assess the potential risk of this interaction with these species, as is well reported for species of Cactaceae Juss. (e.g., Novoa et al., 2017), and for several other Mexican epiphytes (e.g. Bromeliaceae and Orchidaceae), as demonstrated by Flores-Palacios and Valencia-Díaz (2007), for example.

In summary, we demonstrate that the available databases are a relevant source for biogeographical and conservation studies, and *Peperomia* demonstrated to be a good model for this approach – a rich genus with a great number of collections, which is perhaps due its small size and great time span of fertility. We found a lower number of Brazilian endemic species than previously reported, however, more than

half are threatened at some level. The Atlantic Forest stands out as the richest domain, its heterogeneity and biogeographic and climatic history are reasonable explanations to support our results. On the other hand, biased sampling still compromises the exploration of areas that may harbor more endemic species, such as in the Amazon. Efforts to collect and establish research institutions in sub-sampled areas are essential for a better understanding Brazilian biodiversity and promoting its conservation. Finally, we hope that our results of extinction risk of endemic Brazilian *Peperomia* can be incorporated further in the Official National List of Brazilian Flora Species Threatened of Extinction and can provide a basis for conservation of priority areas, such as the creation of protected areas in the hotspots.

Funding

Supplementary material

Supplementary Fig. S1 - Map of Brazilian phytogeographic domains showing the numbered grid cells.

Supplementary Table S1 - A. Clean data used in analyses; B. Records, occurrences, and GeoCAT analysis; C. Federative Units of Brazil; D. List of specialists.

CRediT authorship contribution statement

Clapton Olimpio de Moura: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Pablo Hendrigo Alves de Melo:** Conceptualization, Methodology, Data curation, Writing – review & editing. **Eduardo Toledo de Amorim:** Conceptualization, Methodology, Writing – review & editing. **Gabriel Mendes Marcusso:** Conceptualization, Writing – review & editing. **Micheline Carvalho-Silva:** Conceptualization, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest that might influence this work.

Data Availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.flora.2022.152170.

References

Alam, Md.A., Nadirah, T.A., Mohsin, G.Md., Saleh, M., Moneruzzaman, K.M., Aslani, F., Juraimi, A.S., Alam, M.Z., 2020. Antioxidant compounds, antioxidant activities, and mineral contents among underutilized vegetables. J. Veg. Sci. 27 (2), 157–166. https://doi.org/10.1080/19315260.2020.1748785.

Almeida, F.F.M., Carneiro, C.D.R., 1998. Origem e evolução da Serra do Mar. Revista Brasileira de Geociências 28 (2), 135–150.

Amorim, E., Menini Neto, L., Luizi-Ponzo, A., 2021. An overview of richness and distribution of mosses in Brazil. Plant Ecol. Evolut. 154 (2), 183–191. https://doi.org/10.5091/plecevo.2021.1635.

Antonelli, A., Kissling, W.D., Flantua, S.G.A., Bermúdez, M.A., Mulch, A., Muellner-Riehl, A.N., Hoorn, C., 2018. Geological and climatic influences on mountain biodiversity. Nat. Geosci. 11, 718–725. https://doi.org/10.1038/s41561-018-0236-z.

Antonelli, A., Fry, C., Smith, R.J., Simmonds, M.S.J., Kersey, P.J., Pritchard, H.W., et al., 2020. State of the world's plants and fungi. R. Botan. Garden Kew. https://doi.org/10.34885/172.

Araujo, M.L., Ramos, F.N., 2021. Targeting the survey efforts: gaps and biases in epiphyte sampling in a biodiversity hotspot. Forest Ecol. Manag. 498, 119544 https://doi.org/10.1016/j.foreco.2021.119544.

Bachman, S., Moat, J., Hill, A., de la Torre, J., Scott, B., 2011. Supporting Red List threat assessments with GeoCAT: geospatial conservation assessment tool. Zookeys 150, 117–126. https://doi.org/10.3897/zookeys.150.2109.

Batista, W.V.S.M., Santos, N.D., 2016. Can regional and local filters explain epiphytic bryophyte distributions in the Atlantic Forest of southeastern Brazil? Acta Botanica Brasilica 30 (3), 462–472. https://doi.org/10.1590/0102-33062016abb0179.

BFG - The Brazil Flora Group, 2018. Brazilian Flora 2020: innovation and collaboration to meet Target 1 of the global strategy for plant conservation (GSPC). Rodriguesia 69 (4), 1513–1527. https://doi.org/10.1590/2175-7860201869402.

BFG - The Brazil Flora Group, 2021. Brazilian Flora 2020. Jardim Botânico do Rio de Janeiro, Rio de Janeiro. https://doi.org/10.47871/jbrj2021001.

Brandon, K., Fonseca, G.A.B., Rylands, A.B., SILVA, J.M.C., 2005. Conservação brasileira: desafios e oportunidades. Megadiversidade 1 (1), 7–13.

Carvalho-Silva, M., Guimarães, E.F., Sarnaglia Junior, V.B., 2019. Two new species of *Peperomia* Ruiz & Pavon (Piperaceae) from southeastern Brazil and four new synonymies. Phytotaxa 422 (3), 225–232. https://doi.org/10.11646/phytotaxa.422.3.2.

Christenhusz, M.J.M., Byng, J.W., 2016. The number of known plants species in the world and its annual increase. Phytotaxa 261 (3), 201–217. https://doi.org/10.11646/phytotaxa.261.3.1.

CNCFlora - Centro Nacional de Conservação da Flora (Instituto de Pesquisas Jardim Botânico do Rio de Janeiro): Piperaceae, 2021. http://cncflora.jbrj.gov.br/portal/pt-br/listavermelha/PIPERACEAE. accessed 01 July.

Colli-Silva, M., Reginato, M., Cabral, A., Forzza, R.C., Pirani, J.R., Vasconcelos, T.N.C., 2020. Evaluating shortfalls and spatial accuracy of biodiversity documentation in the Atlantic Forest, the most diverse and threatened Brazilian phytogeographic domain. Taxon 69 (3), 567–577. https://doi.org/10.1002/tax.12239.

Costa, D.P., Peralta, D.F., 2015. Bryophytes diversity in Brazil. Rodriguesia 66 (4), 1063–1071. https://doi.org/10.1590/2175-7860201566409.

Dantas, T.S., Câmara, P.E.A.S., Carvalho-Silva, M., 2017. *Peperomia* (Piperaceae) from Trindade Island: a new species based on morphological and molecular data. Syst. Botany 42 (4), 747–753. https://doi.org/10.1600/036364417X696366.

Darwin Core Maintenance Group, 2021. List of Darwin Core terms. http://rs.tdwg.org/dwc/doc/list/2021-07-15.

Dean, W., 1995. A ferro e fogo: a história e a devastação da Mata Atlântica Brasileira. Companhia das Letras, São Paulo.

Fiaschi, P., Pirani, J.R., Heiden, G., Antonelli, A., 2016. Biogeografia da Flora da América do Sul. In: Carvalho, C.J.B., Almeida, E.A.B. (Eds.), Biogeografia da América do Sul: análise de tempo, espaço e forma, 2nd ed. Roca, Rio de Janeiro, pp. 215–226.

Flora do Brasil, 2020. Jardim Botânico do Rio de Janeiro. http://floradobrasil.jbrj.gov.br. accessed 17 August.

Frenzke, L., Scheiris, E., Pino, G., Symmank, L., Goetghebeur, P., Neinhuis, C., Wanke, S., Samain, M.-S., 2015. A revised infrageneric classification of the genus *Peperomia* (Piperaceae). Taxon 64 (3), 424–444. https://doi.org/10.12705/643.4.

Frenzke, L., Goetghebeur, P., Neinhuis, C., Samain, M.-S., Wanke, S., 2016. Evolution of Epiphytism and fruit traits act unevenly on the diversification of the species-Rich Genus *Peperomia* (Piperaceae). Front. Plant Sci. 7, 1145. https://doi.org/10.3389/fpls.2016.01145.

Frodin, D.G., 2004. History and concepts of big plant genera. Taxon 53 (3), 753–776. https://doi.org/10.2307/4135449.

Gasper, A.L., Stehmann, J.R., Roque, N., Bigio, N.C., Sartori, Â.L.B., Grittz, G.S., 2020. Brazilian herbaria: an overview. Acta Botanica Brasilica 34 (2), 352–359. https://doi.org/10.1590/0102-33062019abb0390.

GBIF - The Global Biodiversity Information Facility, 2020. https://www.gbif.org. accessed 17 August.

Gentry, A.H., 1982. Neotropical floristic diversity: phytogeographical connections between central and south America, Pleistocene climatic fluctuations, or an accident of the Andean orogeny? Ann Mo Bot Gard 69 (3), 557–593. https://doi.org/10.2307/2399084.

Gomes-da-Silva, J., Forzza, R.C., 2020. Two centuries of distribution data: detection of areas of endemism for the Brazilian angiosperms. Cladistics 37 (4), 442–458. https://doi.org/10.1111/cla.12445.

Gomes-da-Silva, J., Lanna, J., Forzza, R.C., 2021. Distribution of endemic angiosperm species in Brazil on a municipality level. Biodivers. Data J 9, e66043. https://doi.org/10.3897/BDJ.9.e66043.

Graham, C.H., Ferrier, S., Huettman, F., Moritz, C., Peterson, A.T., 2004. New developments in museum-based informatics and applications in biodiversity analysis. Trends Ecol. Evol. 19 (9), 497–503. https://doi.org/10.1016/j.tree.2004.07.006.

Guedes, T.B., Azevedo, J.A.R., Bacon, C.D., Provete, D.B., Antonelli, A., 2020. Diversity, endemism, and evolutionary history of Montane biotas outside the Andean region. In: Rull, V., Carnaval, A. (Eds.), Neotropical Diversification: Patterns and Processes. Fascinating Life Sciences. Springer, Cham, pp. 299–328. https://doi.org/10.1007/978-3-030-31167-4_13.

Hallal, P.C., 2021. SOS Brazil: science under attack. Lancet North Am. Ed. 397 (10272), 373–374. https://doi.org/10.1016/S0140-6736(21)00141-0.

IBGE - Instituto Brasileiro de Geografia e Estatística, 2020. https://www.ibge.gov.br. accessed 17 August.

IBGE - Instituto Brasileiro de Geografia e Estatística: IBGE Retrata Cobertura Natural dos Biomas do país de 2000 a 2018, 2021. https://agenciadenoticias.ibge.gov.br. accessed 1 August.

IUCN - The IUCN Red List of Threatened Species, 2021. https://www.iucnredlist.org/search/stats?taxonomies=102395. accessed 01 Julyand. https://www.iucnredlist.org.

Kaplan, E.D., Hegarty, C.J., 2017. Understanding GPS/GNSS: Principles and Applications, 3d ed. Artech House, London.

Krömer, T., Kessler, M., Gradstein, S.R., Acebey, A., 2005. Diversity patterns of vascular epiphytes along an elevational gradient in the Andes. J. Biogeogr. 32 (10), 1799–1809. https://doi.org/10.1111/j.1365-2699.2005.01318.x.

Krömer, T., Acebey, A.R., Toledo-Aceves, T., 2018. Aprovechamiento de plantas epífitas: Implicaciones para su conservación y manejo sustentable en México. In: Silva-Rivera, E., Martínez-Valdéz, V., Lascurain, M., Rodríguez-Luna, E. (Eds.), De la Recolección a los Agroecosistemas: Soberanía Alimentaria y Conservación de la Biodiversidad. Universidad Veracruzana, Xalapa, pp. 175–196.

Krömer, T., Acebey, A.R., Armenta-Montero, S., Croat, T.B., 2019. Diversity, distribution, and conservation status of araceae in the State of Veracruz, Mexico. Ann Mo Bot Gard 104 (1), 10–32. https://doi.org/10.3417/2018214.

La Salle, J., Williams, K.J., Moritz, C., 2016. Biodiversity analysis in the digital era. Philosoph. Trans. R. Soc. B 371, 20150337. https://doi.org/10.1098/rstb.2015.0337.

Leão, T.C.C., Reinhardt, J.R., Lughadha, E.N., Reich, P.B., 2021. Projected impacts of climate and land use changes on the habitat of Atlantic Forest plants in Brazil. Global Ecol. Biogeogr. 30 (10), 2016–2028. https://doi.org/10.1111/geb.13365.

MapBiomas - Projeto de Mapeamento Anual do Uso e Cobertura da Terra no Brasil, 2021. https://mapbiomas.org. accessed 15 December.

Marcusso, G.M., Melo, P.H.A., Lombardi, J.A., 2020. *Peperomia calcicola* (Piperaceae), a new species from limestone outcrops of the southeastern Brazilian Atlantic Forest. Phytotaxa 446 (4), 245–252. https://doi.org/10.11646/phytotaxa.446.4.5.

Marcusso, G.M., Kamimura, V.A., Borgiani, R., Menini Neto, L., Lombardi, J.A., 2022. Phytogeographic meta-analysis of the vascular epiphytes in the neotropical region. Botan. Rev. https://doi.org/10.1007/s12229-021-09270-2.

Marques, M.C.M., Trindade, W., Bohn, A., Grelle, C.E.V., 2021. The Atlantic forest: an introduction to the Megadiverse Forest of South America. In: Marques, M.C.M., Grelle, C.E.V. (Eds.), The Atlantic Forest. Springer, Cham, pp. 3–23. https://doi.org/10.1007/978-3-030-55322-7_1.

Martinelli, G., Moraes, M.A., 2013. Livro vermelho da flora do Brasil, 1st ed. Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Rio de Janeiro.

Mathieu, G., Samain, M.-S., Reynders, M., Goetghebeur, P., 2008. Taxonomy of the *Peperomia* species (Piperaceae) with pseudo-epiphyllous inflorescences, including four new species. Bot. J. Linn Soc. 157 (2), 177–196. https://doi.org/10.1111/j.1095-8339.2008.00777.x.

Mathieu, G., 2021. The Internet *Peperomia* Reference. https://www.peperomia.net. routinely accessed 2021.

Melo, A., Guimarães, E.F., Alves, M., 2016. Synopsis of the genus *Peperomia* Ruiz & Pav. (Piperaceae) in Roraima State, Brazil. Hoehnea 43 (1), 119–134. https://doi.org/10.1590/2236-8906-75/2015.

Menini Neto, L., Furtado, S.G., Zappi, D.C., Oliveira Filho, A.T., Forzza, R.C., 2016. Biogeography of epiphytic Angiosperms in the Brazilian Atlantic Forest, a world biodiversity hotspot. Rev. Bras Bot. 39, 261–273. https://doi.org/10.1007/s40415-015-0238-7.

Mittermeier, R.A., Gil, P.R., Hoffman, M., Pilgrim, J., Brooks, T., Mittermeier, C.G., Lamoreux, J., Fonseca, G.A.B., 2004. Hotspots Revisited. Earth's Biologically Richest and Most Endangered Terrestrial Ecoregions. Cemex, Mexico City.

Moerman, D.E., Estabrook, G.F., 2006. The botanist effect: counties with maximal species richness tend to be home to universities and botanists. J. Biogeogr. 33 (11), 1969–1974. https://doi.org/10.1111/j.1365-2699.2006.01549.x.

Monteiro, D., Leitman, P., Coelho, M.M., 2016. Two new *Peperomia* (Piperaceae) species from Southern Bahia, Brazil. Phytotaxa 258 (3), 287–294. https://doi.org/10.11646/phytotaxa.258.3.4.

Monteiro, D., 2018. Flora of the Canga of the Serra dos Carajás, Pará, Brazil: piperaceae. Rodriguesia 69 (3), 1285–1309. https://doi.org/10.1590/2175-7860201869329.

Moraes, M.M., Kato, M.J., 2021. Biosynthesis of Pellucidin A in *Peperomia pellucida* (L.) HBK. Front. Plant Sci. 12, 641717 https://doi.org/10.3389/fpls.2021.641717.

Morales, N.G., Brito, A.L.V.T., Mauad, A.V.S.R., Smidt, E.C., 2021. Molecular phylogeny and biogeography of *Pabstiella* (Pleurothallidinae: Orchidaceae) highlight the importance of the Atlantic Rainforest for speciation in the genus. Bot. J. Linn Soc. 195 (4), 568–587. https://doi.org/10.1093/botlinnean/boaa092.

Morales-Linares, J., Corona-López, A.M., Toledo-Hernández, V.H., Flores-Palacios, A., 2021. Ant-gardens: a specialized ant-epiphyte mutualism capable of facing the effects of climate change. Biodivers. Conserv. 30, 1165–1187. https://doi.org/10.1007/s10531-021-02138-2.

Moreno, N.C., Amarilla, L.D., Las Peñas, M.L., Bernardello, G., 2015. Molecular cytogenetic insights into the evolution of the epiphytic genus *Lepismium* (Cactaceae) and related genera. Bot. J. Linn Soc. 177, 263–277. https://doi.org/10.1111/boj.12242.

Mostacero, N.R., Castelli, M.V., Barolo, M.I., Amigot, S.L., Fulgueira, C.L., López, S.N., 2021. Fungal endophytes in *Peperomia obtusifolia* and their potential as inhibitors of chickpea fungal pathogens. World J. Microbiol. Biotechnol. 37, 14. https://doi.org/10.1007/s11274-020-02954-8.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858. https://doi.org/10.1038/35002501.

NEREUS/USP - Núcleo de Economia Regional e Urbana/Universidade de São Paulo, 2021. http://www.usp.br/nereus/?dados=brasil. accessed 01 July.

Novoa, A., Le Roux, J.J., Richardson, D.M., Wilson, J.R.U., 2017. Level of environmental threat posed by horticultural trade in Cactaceae. Conserv. Biol. 31 (5), 1066–1075. https://doi.org/10.1111/cobi.12892.

Obermuller, F.A., Freitas, L., Daly, D.C., Silveira, M., 2014. Patterns of diversity and gaps in vascular (hemi-) epiphyte flora of Southwestern Amazonia. Phytotaxa 166 (4), 259–272. https://doi.org/10.11646/phytotaxa.166.4.2.

Oliveira-Filho, A.T., Fontes, M.A.L., 2000. Patterns of floristic differentiation among Atlantic forests in southeastern Brazil and the influence of climate. Biotropica 32, 793–810. https://doi.org/10.1111/j.1744-7429.2000.tb00619.x.

Pereira, A.A., da Silva, I.V., Vicente, R.E., 2021. Interaction between epiphytic chemical allelopathy and ant-pruning determining the composition of Amazonian ant-garden epiphytes. Arthropod-Plant Interact. 15, 399–407. https://doi.org/10.1007/s11829-021-09825-5.

Perret, M., Chautems, A., Spichiger, R., 2006. Dispersal-vicariance analyses in the tribe Sinningieae (Gesneriaceae): a clue to understanding biogeographical history of the Brazilian Atlantic Forest. Ann. Mo Bot. Gard. 93 (2), 340–358. https://doi.org/10.3417/0026-6493(2006)93[340:DAITTS]2.0.CO;2.

Ponder, W.F., Carter, G.A., Flemons, P., Chapman, R.R., 2002. Evaluation of museum collection data for use in biodiversity assessment. Conserv. Biol. 15 (3), 648–657. https://doi.org/10.1046/j.1523-1739.2001.015003648.x.

Prado, D.E., Gibbs, P.E., 1993. Patterns of species distributions in the dry seasonal forests of South America. Ann. Mo Bot. Gard. 80 (4), 902–927. https://doi.org/10.2307/2399937.

Prance, G.T., 1982. Forest refuges: evidence from woody angiosperms. Biological Diversification in the Tropics. Columbia University Press, New York, pp. 137–157.

QGIS Association, 2021. QGIS Geographic Information System Version 3.10. https://qgis.org.

FSC BioLinks, 2021. FSC Plugin for QGIS Version 3. https://www.fscbiodiversity.uk/fsc-plugin-qgis-v3.

R Core Team, 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. https://www.R-project.org. with plugins available at. https://cran.r-project.org.

REFLORA - Herbário Virtual, 2020. Jardim Botânico do Rio de Janeiro. http://reflora.jbrj.gov.br. accessed 17 August.

Rezende, C.L., Scarano, F.R., Assad, E.D., Joly, C.A., Metzger, J.P., Strassburg, B.B.N., Tabarelli, M., Fonseca, G.A., Mittermeier, R.A., 2018. From hotspot to hopespot: an opportunity for the Brazilian Atlantic Forest. Perspect. Ecol. Conservat. 16 (4), 208–214. https://doi.org/10.1016/j.pecon.2018.10.002.

Ribeiro, A.C., 2006. Tectonic history and the biogeography of the freshwater fishes from the coastal drainages of eastern Brazil: an example of faunal evolution associated with a divergent continental margin. Neotropical. Ichthyol. 4, 225–246. https://doi.org/10.1590/S1679-62252006000200009.

Ribeiro, M.C., Metzger, J.P., Martensen, A.C., Ponzoni, F.J., Hirota, M.M., 2009. The Brazilian Atlantic Forest: how much is left, and how is the remaining forest distributed? Implications for conservation. Biol. Conserv. 142 (6), 1141–1153. https://doi.org/10.1016/j.biocon.2009.02.021.

Scarano, F.R., Ceotto, P., 2015. Brazilian Atlantic Forest: impact, vulnerability, and adaptation to climate change. Biodivers. Conserv. 24, 2319–2331. https://doi.org/10.1007/s10531-015-0972-y.

Silva Junior, C.H.L., Pessôa, A.C.M., Carvalho, N.S., Reis, J.B.C., Anderson, L.O., Aragão, L.E.O.C., 2021. The Brazilian Amazon deforestation rate in 2020 is the greatest of the decade. Nat. Ecol. Evol. 5, 144–145. https://doi.org/10.1038/s41559-020-01368-x.

SpeciesLink Network, 2020. https://specieslink.net. accessed 17 August.

Stehmann, J.R., Forzza, R.C., Salino, A., Sobral, M., Costa, D.P., Kamino, L.H.Y., 2009. Plantas da Floresta Atlântica. Jardim Botânico do Rio de Janeiro, Rio de Janeiro.

Suissa, J.S., Sundue, M.A., 2020. Diversity patterns of neotropical ferns: revisiting tryon's centers of richness and endemism. Am. Fern J. 110 (4), 211–232. https://doi.org/10.1640/0002-8444-110.4.211.

Tonetti, V.R., Rego, M.A., De Luca, A.C., Develey, P.F., Schunck, F., Silveira, L.F., 2017. Historical knowledge, richness and relative representativeness of the avifauna of the largest native urban rainforest in the world. Zoologia 34, 1–18. https://doi.org/10.3897/zoologia.34.e13728.

Tropicos® - Missouri Botanical Garden, 2021. Tropicos®. https://www.tropicos.org. routinely accessed.

Tucker, S.C., 1980. Inflorescence and flower development in the Piperaceae. I. *Peperomia*. Am. J. Bot. 67 (5), 686–702. https://doi.org/10.1002/j.1537-2197.1980.tb07699.x.

Vale, M.M., Tourinho, L., Lorini, M.L., Rajão, H., Figueiredo, M.S.L., 2018. Endemic birds of the Atlantic forest: traits, conservation status, and patterns of biodiversity. J. Field. Ornithol. 89 (3), 193–206. https://doi.org/10.1111/jofo.12256.

Vergara-Rodríguez, D., Mathieu, G., Samain, M.S., Armenta-Montero, S., Krömer, T., 2017. Diversity, distribution, and conservation status of *peperomia* (Piperaceae) in the State of Veracruz, Mexico. Trop. Conservat. Sci. 10, 1–28. https://doi.org/10.1177/1940082917702383.

Wanke, S., Samain, M.-S., Vanderschaeve, L., Mathieu, G., Goetghebeur, P., Neinhuis, C., 2006. Phylogeny of the Genus *Peperomia* (Piperaceae) inferred from the trnK/matK region (cpDNA). Plant Biol. 8 (1), 93–102. https://doi.org/10.1055/s-2005-873060.

Werneck, M.S., Sobral, M.E.G., Rocha, C.T.V., Landau, E.C., Stehmann, J.R., 2011. Distribution and endemism of angiosperms in the atlantic forest. Natureza Conservação 9 (2), 188–193. https://doi.org/10.4322/natcon.2011.024.

Wieczorek, J., Bloom, D., Guralnick, R., Blum, S., Döring, M., Giovanni, R., Robertson, T., Vieglais, D., 2012. Darwin core: an evolving community-developed biodiversity data standard. PLoS One 7 (1), e29715. https://doi.org/10.1371/journal.pone.0029715.

Zanella, F.C.V., 2011. Evolução da Biota da Diagonal de Formações Abertas Secas da América do Sul. In: Carvalho, C.B., Almeida, E. (Eds.). Biogeografia da América do Sul: padrões e processos, v. 1. Roca, São Paulo, pp. 198–220.

Zizka, A., Azevedo, J., Leme, E., Neves, B., da Costa, A.F., Caceres, D., Zizka, G., 2020. Biogeography and conservation status of the pineapple family (Bromeliaceae). Divers Distrib. 26 (2), 183–195. https://doi.org/10.1111/ddi.13004.

Zoghbi, M.G.B., Andrade, E.H.A., Lobato, R.C.L., Tavares, A.C.C., Souza, A.P.S., Conceição, C.C.C., Guimarães, E.F., 2005. *Peperomia circinnata* Link and *Peperomia rotundifolia* (L.) Kunth growing on different host-trees in Amazon: volatiles and relationship with bryophytes. Biochem. Syst. Ecol. 33 (3), 269–274. https://doi.org/10.1016/j.bse.2004.09.006.

Zotz, G., 2013. The systematic distribution of vascular epiphytes - a critical update. Bot J. Linn Soc. 171, 453–481. https://doi.org/10.1111/boj.12010.

Zotz, G., Weigelt, P., Kessler, M., Kreft, H., Taylor, A., 2021. EpiList 1.0: a global checklist of vascular epiphytes. Ecology 102 (6), e03326. https://doi.org/10.1002/ecy.3326.

Chapter 3

Peperomia (Piperaceae) endemic to Brazil: Areas of endemism and landscape changes over 35 years

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Abstract

Peperomia is one of the richest genera among angiosperms, with its highest richness concentrated in the Neotropical region. Brazil ranks fourth in the world in terms of species richness within this genus, with over half of them being endemic. The Atlantic Forest, which harbors most of the endemic _Peperomia_ species in Brazil, has also been heavily impacted by human activities. In this context, endemic species take center stage because locating areas with a high richness of these species is essential for preserving unique lineages and evolutionary histories. Our study aims to identify endemic areas for the genus in Brazil and assess land use and land cover quality in these areas over 35 years. We identified a total of six areas of endemism, with two in the Amazon and four in the Atlantic Forest. The Amazon areas are primarily affected by recent pasture activities, while the Atlantic Forest areas have endured centuries of exploitation.

Study Implications

About half of Brazil's endemic *Peperomia* species can be categorized as threatened. The evidence presented here highlights the importance of focusing on the chorological aspects of these species in the Amazon, where two areas of endemism were discovered, bordering Peru, a country with the highest species diversity in the genus. Not surprisingly, the Atlantic Forest exhibited four areas of endemism within its boundaries, emphasizing the need for establishing new conservation units to protect the biological richness of this domain, recognized as one of the global biodiversity hotspots.

Keywords

Amazon Forest, Atlantic Forest, Conservation, Piperoideae.

Estimates indicate that plants currently exhibit extinction rates 500 times higher than the normal background rate of extinction (Humphreys et al. 2019). Furthermore, Brown et al. (2023) emphasize that three out of every four newly described plant species may be under threat. Up to this point, 39.75% of the plant species assessed by the International Union for Conservation of Nature (IUCN 2023) are categorized under some form of threat. Moreover, this decline in species diversity is not exclusive to plants (Gao et al. 2020), and there is already an extensive body of literature pointing to the onset of the 'sixth mass extinction' – a crisis and subsequent collapse of biodiversity as a result of human actions and impacts (Cowie et al. 2022).

Considered a megadiverse country, Brazil encompasses over 35,000 angiosperm species within its territory, with well over half of them considered endemic (Flora e Funga do Brasil 2023). The *Centro Nacional de Conservação da Flora*'s data (CNCFlora 2023) indicates that, within Brazil, approximately 48.84% of evaluated species are considered threatened, a figure approximately 10% higher than the ratio reported by the IUCN (2023) for the global assessment. With a significant diversity of habitats, the Atlantic Forest distinguishes itself in Brazil by harboring areas of endemism for basal

angiosperms, a pattern that similarly recurs among eudicots and monocots (Gomes-da-Silva and Forzza 2021), within a region that encompasses portions of both the Espinhaço and Serra do Mar mountain ranges. Despite being a refuge for vast biodiversity, the Amazon forest did not exhibit areas of endemism for major angiosperm groups when considered together (Gomes-da-Silva and Forzza 2021).

Peperomia is a genus with a pantropical distribution, with the highest species diversity concentrated in the Neotropical region. The genus comprises approximately 1600 accepted species (Frodin 2004; Frenzke 2015). Countries harboring the largest number of *Peperomia* species (Mathieu 2023, Flora e Funga do Brasil 2023) include Peru (with 405 spp.), Colombia (259), Ecuador (237), Brazil (172), Costa Rica (155), and Mexico (139). Moura et al. (2022) have provided evidence that 100 *Peperomia* species can be considered endemic to the Brazilian territory, with roughly half of these species qualifying for classification under one of the IUCN (2023) threat categories.

Geographical areas characterized by the co-occurrence of multiple endemic taxa are commonly termed Areas of Endemism (AoE - Cox and Moore 2009; Morrone 1994, 2014). Recognizing these areas holds paramount significance for conservation efforts, given that these territories safeguard unique lineages and evolutionary histories (Carvalho 2009). Therefore, the objectives of the present study are as follows: (**1**) to determine the areas of endemism for the genus *Peperomia* in Brazil; (**2**) to ascertain whether these areas encompass federal conservation units (CUs) categorized under the "Strict Protection" category; and (**3**) to infer the quality of land use and land cover changes within these areas over a 35-year period (1985-2020). This research aims to generate valuable evidence that, in conjunction with data from other taxa, can inform the planning and execution of conservation initiatives.

2. Methods

Data collection

To conduct the analyses, we relied upon the list of *Peperomia* species that are endemic to Brazil, along with occurrence data, as published by Moura et al. (2022). This study employed a workflow developed by CNCFlora for the extraction, cleaning, and preprocessing of occurrence data (sourced from open-access databases, including GBIF, *species*Link, and REFLORA) for spatial analyses.

Parsimony analysis of endemicity

The Parsimony Analysis of Endemicity (PAE) procedure was employed for the demarcation of AoEs. This technique is grounded on the principle that an area qualifies as an area of endemism when it features the non-random co-occurrence of two or more endemic species (Morrone 1994; 2014). The inference of AoEs was conducted based on the grid cells map produced by Moura et al. (2022), with each grid cell covering an area of 1°, approximately 111 km² (Supplement 1 includes a map that lists all the grid cells and their respective identification numbers - IDs). A matrix reflecting species presence (1) or absence (0) within each grid cell was generated and utilized for analyses. The matrix was created and analyzed using the Nona 2.0 software (Goloboff 1993) within the WinClada 1.00.08 interface (Nixon 2002). This process resulted in the production of a cladogram via the parsimony algorithm. Areas of endemism were recognized as monophyletic clades formed by one or more grid cells that harbor two or more exclusive species (synapomorphies).

Landscape metrics

We performed landscape metric analyses for all identified AoEs using the LecoS - Landscape ecology analysis (Jung 2016) plugin, which was implemented in QGIS v3.10 (QGIS Development Team 2023). Land use and land cover data for the years 1985 and 2020 were obtained from MapBiomas *Collection 7* (MapBiomas 2023; Souza Jr. et al. 2020). The raster resolution was set at 0.00003 km² per pixel. We applied a set of landscape metrics (including Land cover in km² and percentage,

number of patches, mean patch area in km², and greatest patch area in km²) to all the identified AoEs, considering each MapBiomas class.

Additionally, we conducted an evaluation to ascertain whether Conservation Units (CUs) categorized under the "Strict Protection" designation were encompassed within the boundaries of the AoEs. CUs polygons were acquired from the database of the *Instituto Chico Mendes de Conservação da Biodiversidade* (ICMBio 2023). In alignment with Brazilian legislation (Federal Law No. 9985, Brazil 2000), CUs under the "Strict Protection" category are established with the objective of "preserving nature, allowing only indirect use of natural resources, and hence the rules and norms are restrictive". We considered CUs with polygons that partially or wholly overlapped with the AoE polygons.

3. Results

We retrieved six AoEs (Figure 1 and Supplement 2) and a total of 15 indicator species (synapomorphies) associated with these areas through the PAE (Table 1). Among these AoEs, two (1 and 2) are situated within the Amazonian domain, whereas the remaining four (3, 4, 5, and 6) have their boundaries defined within the Atlantic Forest domain. Furthermore, we present an overview of land use and land cover quality and changes for the period from 1985 to 2020. The complete dataset for all metrics is available in Supplement 3. It was observed that only AoEs 3, 4, and 6 had CUs within their boundaries, with no CUs in areas 1, 2, and 5. Additionally, in Supplement 4, we provide images of all AoEs with their land use and land cover classes for the years 1985 and 2020. We include polygons of the overlapping CUs on the AoEs (depicted in the 2020 images) and a document containing the codes used by MapBiomas for the classes and colors in Collection 7.
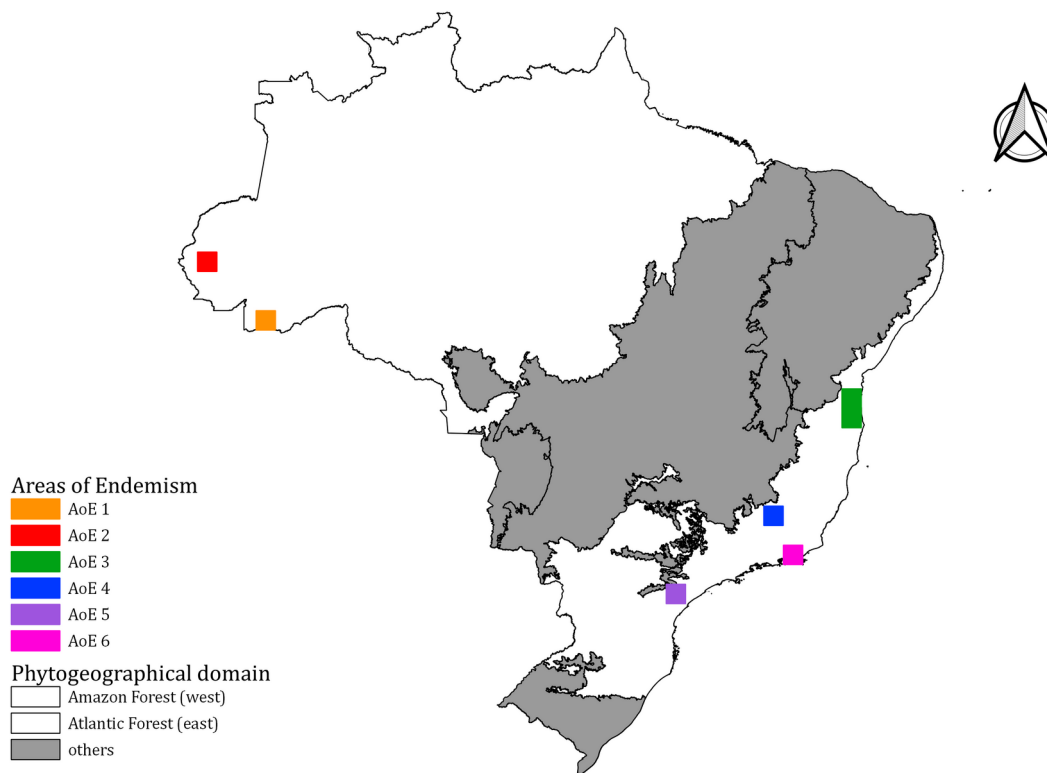
Figure 1. Areas of endemism (AoE) for the genus *Peperomia* in Brazil, on the left the AoEs recovered for the Amazon domain, and on the right the AoEs found in the Atlantic Forest domain.

Table 1. Areas of endemism along with their respective grid cell IDs, synapomorphic species, and other co-occurring Brazilian endemic species in these areas.

AoE id	Grid cell ID	Synapomorphic species	Co-occurring species
AoE 1	21	*P. acreana and P. simulans.*	—
AoE 2	10	*P. cruzeirensis and P. sumidoriana.*	*P. tenuilimba.*
AoE 3	39 + 47	*P. epipremnifolia, P. flexicaulis, P. parcifolia and P. sulbahiensis.*	*P. pseudoestrellensis, P. riparia, P. serpentarioides, P. stenocarpa, P. subpilosa, P. trichocarpa and P. velloziana.*
AoE 4	66	*P. crypticola and P. marcoana.*	*P. augescens, P. campinasana, P. cordigera, P. crinicaulis, P. decora, P. glazioui, P. mandioccana, P. oreophila, P. subrubrispica, P. velloziana and P. warmingii.*
AoE 5	100	*P. apiahyensis and P. calcicola.*	*P. castelosensis, P. glazioui, P. mandioccana, P. pereirae, P. pseudoestrellensis.*
AoE 6	87	*P. arbuscula, P. bradei and P. menkeana.*	*P. adsurgens, , P. augescens, P. castelosensis, P. clivicola, P. crinicaulis, P. dichotoma, P. glazioui, P. gracilis, P. lyman-smithii, P. mandioccana, P. megapotamica, P. pereirae, P. pseudoestrellensis, P. pubipeduncula, P. rizzinii, P. subretusa, P. subternifolia and P. velloziana.*

Area of endemism 1

AoE 1 (grid cell 21) is characterized by the presence of the species *Peperomia acreana* and *P. simulans*, with no other species of *Peperomia* endemic to Brazil recorded within this AoE. Landscape metrics indicate that in 1985, 97.31% of the landscape was covered by Forest Formation, while 2.09% was designated as Pasture, with the remaining land cover classes each constituting less than 1% of the total land cover. By 2020, the area covered by Forest Formation had declined to 88.18%, while Pasture had expanded to occupy 11.27% of the landscape, approximately five times its initial coverage. The other land cover classes remained below 1% land cover.

In 1985, the total terrestrial natural coverage for AoE 1 was 97.64%, while human utilization covered 2.10% of the area. Over a span of 35 years, natural coverage decreased to 88.52%, whereas human use coverage increased to 11.28%. In 1985, the number of patches within the Forest Formation class totaled 460, and this number had increased to 2094 patches by 2020. The mean patch area for this class was 25.1822 km² in 1985 and had reduced to only 5.0127 km² in 2020, with the other land cover classes represented by values less than 1 km². Furthermore, the largest patch area for the Forest Formation class measured 7935.1848 km² in 1985 and expanded to 9993.9528 km² in 2020.

Area of endemism 2

Delimited by the presence of *Peperomia cruzeirensis* and *P. sumidoriana*, AoE 2 (grid cell 10) also records *P. tenuilimba*, another Brazilian endemic species, although not exclusive to AoE 2. Similar to the pattern observed for AoE 1, the coverage of Forest Formation in the year 1985 was 94.77%, reducing to an occupancy of 86.43% by 2020. The primary class of human use and occupation, once again, was Pasture, which occupied 4.10% of AoE 2 in 1985 and, 35 years later, extended to 12.19%, tripling its extent during this period. All other classes exhibited values below 1% for both analyzed periods.

The total area occupied by terrestrial natural formations decreased from 95.21% in 1985 to 86.97% in 2020. In contrast, the coverage of human use classes increased from 4.17% in 1985 to 12.39% in 2020. The number of patches for the Forest Formation class increased from 1239 patches in 1985 to 3443 patches in 2020. The mean patch area values were 9.8147 km² in 1985 and 3.2213 km² in 2020. Meanwhile, the results for the greatest patch area varied from 6650.5995 km² in 1985 to 5063.2677 km² in 2020.

Area of endemism 3

Situated at the boundaries of the Atlantic Forest, AoE 3 is delimited by the exclusive presence of *Peperomia epipremnifolia*, *P. flexicaulis*, *P. parcifolia*, and *P. sulbahiensis*. This AoE comprises two grid cells (39 + 47), with grid cell 39 characterized by the endemism of *P. flexicaulis* and *P. parcifolia*, while also sharing the aforementioned two species with grid cell 47. Additionally, seven other Brazilian endemic species co-occur in this AoE: *P. pseudoestrellensis*, *P. riparia*, *P. serpentarioides*, *P. stenocarpa*, *P. subpilosa*, *P. trichocarpa*, and *P. velloziana*. Significantly, there is an overlap of CUs polygons (*Reserva Biológica De Una/Refugio De Vida Silvestre De Una* and the *Parque Nacional Da Serra Das Lontras*) at the boundaries of grid cell 47. AoEs within the Atlantic Forest exhibit greater heterogeneity in the number of classes, encompassing both natural formations and anthropogenic ecosystems.

The Forest Formation class covered 52.10% of the AoE in 1985, representing the primary type of natural formation, followed by the Savanna Formation class at 1.41% and the Wetland class at 1.01%. All other natural vegetation classes each occupied less than 1% of the AoE area. By 2020, only the Forest Formation and Savanna Formation classes occupied more than 1% of the total AoE area, with 45.47% and 2.71%, respectively. In 1985, the predominant non-natural class was Pasture, covering 33.39% of the total area, followed by Mosaic of Uses at 10.78%, with all other classes

having less than 1% coverage. In 2020, the Pasture class covered 38.16%, and Mosaic of Uses was at 10.63%, both with coverage exceeding 1%.

The total coverage of terrestrial natural areas in this AoE was 55.03% in 1985, decreasing to 49.46% in 2020. Meanwhile, the coverage of anthropogenic classes increased from 44.33% in 1985 to 49.91% in 2020. In 1985, the Forest Formation class had 11,295 patches, the Savanna Formation class had 15,116 patches, and the Wetland class had 3,808 patches. In 2020, these classes had 18,612, 17,842, and 2,739 patches, respectively. The primary natural coverage class, Forest Formation, exhibited a mean patch area of 1.1178 km² and a greatest patch area of 10,229.1705 km² in 1985. In 2020, the values for these metrics were 0.5919 km² and 7,645.4325 km², respectively. The Savanna Formation and Wetland classes had mean patch area values of 0.0226 km² and 0.0646 km², and greatest patch area values of 3.7566 km² and 31.1382 km², respectively, in 1985. In 2020, these values were 0.0369 km² (mean patch area) and 12.5226 km² (greatest patch area) for the Savanna Formation class, and 0.0752 km² (mean patch area) and 32.0238 km² (greatest patch area) for the Wetland class.

Area of endemism 4

Delineated by the endemism of *Peperomia crypticola* and *P. marcoana*, AoE 4 (grid cell 66) also encompasses 12 other endemic species from Brazil: *P. augescens*, *P. campinasana*, *P. cordigera*, *P. crinicaulis*, *P. decora*, *P. glazioui*, *P. mandioccana*, *P. marcoana*, *P. oreophila*, *P. subrubrispica*, *P. velloziana*, and *P. warmingii*. Within this area, there is an overlap with a CU (P*arque Nacional Serra do Gandarela*). In 1985, the primary anthropogenic coverage classes were Pasture and Mosaic of Uses, covering 37.33% and 15.77% of the area, respectively. The three natural coverage classes that occupied more than 1% of the total area were Forest Formation (35.49%), Grassland (7.04%), and Rocky Outcrop (1.83%). The data for the year 2020 highlight five non-natural coverage classes with over 1% coverage: Pasture (29.36% of the area), Mosaic of Uses (15.34%), Forest Plantation (3.16%),

Mining (2.01%), and Urban Area (1.52%). Prominent among the natural coverage classes are Forest Formation (36.97%), Grassland (7.92%), and Rocky Outcrop (1.87%).

The total coverage of terrestrial natural areas was 44.84% in 1985 and increased to 46.94% in 2020. Conversely, the values for human use classes were 54.74% in the initial year and 52.79% in the final year. In 1985, the primary natural coverage classes displayed the following numbers of patches: 16,739 patches (Forest Formation), 9,058 (Grassland), and 1,804 (Rocky Outcrop). By 2020, these values had risen to 19,165 patches for Forest Formation, 8,232 for Grassland, and 1,623 for Rocky Outcrop. The mean patch area for these classes in 1985 was 0.2484 km², 0.0911 km², and 0.1190 km², respectively. In 2020, Forest Formation recorded a mean patch area of 0.2259 km², followed by Grassland with 0.1127 km², and Rocky Outcrop with 0.1352 km². The greatest patch area for these classes in 1985 was 1,677.6477 km² (Forest Formation), 301.3002 km² (Grassland), and 34.4376 km² (Rocky Outcrop). In 2020, these values were 1,318.9986 km² (Forest Formation), 366.5727 km² (Grassland), and 58.7538 km² (Rocky Outcrop).

Area of endemism 5

Characterized by the presence of *Peperomia apiahyensis* and *P. calcicola*, AoE 5 (grid cell 100) also records five other Brazilian endemic species: *P. castelosensis*, *P. glazioui*, *P. mandioccana*, *P. pereirae*, and *P. pseudoestrellensis*. The only natural coverage class that exceeded 1% in 1985 was Forest Formation, occupying 64.62% of AoE. Human use coverage classes exhibited values of 18.19% for Mosaic of Uses, 11.81% for Pasture, 2.19% for Forest Plantation, and 1.95% for Other Temporary Crops. In 2020, values above 1% included 66.96% (Forest Formation), 12.48% (Mosaic of Uses), 7.71% (Forest Plantation), 5.59% (Pasture), 3.30% (Soybean), and 2.67% (Other Temporary Crops).

Terrestrial natural coverage increased during the analyzed period, starting from 65.09% coverage in 1985 to 67.44% in 2020, while anthropogenic classes decreased from 34.62% to 32.29% over this

interval. The number of patches in the Forest Formation class in 1985 was 9,056. In 2020, this value increased to 9,110. The mean patch area was 0.8037 km² in 1985 and 0.8278 km² in 2020. The values for the greatest patch area for this class were 3,516.7176 km² in 1985 and 3,722.2299 km² in 2020.

Area of endemism 6

Characterized by the exclusive presence of *Peperomia arbuscula*, *P. bradei*, and *P. menkeana*, AoE 6 has four CUs crossing its polygon (*Reserva Biológica União, Reserva Biológica De Poço Das Antas, Parque Nacional Da Serra Dos Orgãos and Estação Ecológica Da Guanabara*). Additionally, 18 other endemic species from Brazil co-occur in this area (*P. adsurgens*, *P. augescens*, *P. castelosensis*, *P. clivicola*, *P. crinicaulis*, *P. dichotoma*, *P. glazioui*, *P. gracilis*, *P. lyman-smithii*, *P. mandioccana*, *P. megapotamica*, *P. pereirae*, *P. pseudoestrellensis*, *P. pubipeduncula*, *P. rizzinii*, *P. subretusa*, *P. subternifolia*, and *P. velloziana*). The two terrestrial natural coverage classes that achieved coverage values above 1% were Forest Formation (36.71% in 1985 and 38.94% in 2020) and Wetland (1.65% in 1985 and 1.63% in 2020). Human use coverage was represented by Pasture with 34.70% and Mosaic of Uses with 20.61% in 1985, and by Pasture (34.12%), Mosaic of Uses (15.65%), and Urban Area (4.08%).

Following the pattern found for the AoEs in the Atlantic Forest, this AoE showed an increase from 39.78% (1985) to 41.92% (2020) for terrestrial natural coverage classes and a decrease from 56.86% (1985) to 54.57% (2020) for human use classes. In the initial year (1985), the number of patches was 12,207 for Forest Formation and 2,731 for Wetland. In the final year (2020), both classes had increased values, with Forest Formation having 12,478 and Wetland with 3,379 patches. The mean patch area and greatest patch area for Forest Formation in 1985 were 0.3302 km² and 2,003.9751 km², respectively. For Wetland, these values were 0.0664 km² (mean patch area) and 15,9471 km² (greatest patch area). In 2020, the Forest Formation class obtained values of 0.3426 km² (mean

patch area) and 2,136.0438 km² (greatest patch area), while the Wetland class had values of 0.0531 km² (mean patch area) and 20,4363 km² (greatest patch area).

4. Discussion

The Atlantic Forest stands out as the domain with the highest number of areas of endemism, with four areas identified in this study. Surprisingly, the Amazon domain presents two AoEs within its boundaries. The Flora e Funga do Brasil (2023) and Moura et al. (2022) demonstrate that the Atlantic Forest is the Brazilian domain with the highest number of records and species richness of endemic *Peperomia*, while the Amazonian domain exhibits lower species richness, including significant collection gaps.

In general, for major clades of angiosperms, there are no areas of endemism (consensus) within the Amazon domain (Gomes-da-Silva and Forzza 2021). Therefore, the AoEs found within the boundaries of the Amazon Forest become relevant focal points for future studies involving the *Peperomia* genus in Brazil. Despite the absence of CUs protecting the species residing within these AoEs, the *Estação Ecológica Rio Acre* and the *Parque Nacional Da Serra Do Divisor* are two CUs located within 50 km of the boundaries of AoE 1 and AoE 2 (at their closest edges), respectively. Moreover, these CUs are of particular interest due to their potential to serve as habitats for various *Peperomia* species, as they border Peru, a country with the highest number of *Peperomia* species in the world (Mathieu 2023).

Furthermore, landscape metrics reveal that the primary stressor in these AoEs is the activity represented by the Pasture class, which at a minimum tripled its extent over the period. Meanwhile, the principal natural formation class, Forest Formation, exhibited a negative difference of approximately 10% from its original coverage. Additionally, the number of patches in the Forest Formation class increased during the analyzed period (for both AoEs), indicating habitat degradation through the fragmentation of natural formations (Murcia 1995, Haila 2002). Although the data used

in the analyses only cover the period from 1985 to 2020, more recent evidence points to the ongoing pressure from agricultural activities. For instance, the state of Acre, where both Amazonian AoEs are located, lost approximately 2000 km² of native areas in the cumulative years 2021 and 2022 (INPE 2023).

On the other hand, AoE 3 is located in the northern part of the *Hileia Baiana* (southwest of the state of Bahia) within the territory of the Atlantic Forest. Although this region was one of the last to experience large-scale deforestation, the peak of deforestation of its native forests occurred recently, between the 1960s and 1980s (Albuquerque and Torresan 2022, Faria et al. 2021). Additionally, Faria et al. (2021) emphasize that despite large-scale deforestation occurring later compared to other parts of the Atlantic Forest, the range corresponding to AoE 3 has been intensively exploited since the 19th century for timber extraction (e.g., brazilwood - *Paubrasilia echinata* (Lam.) Gagnon, H.C.Lima & G.P.Lewis) and various types of crops (e.g., cacao tree - *Theobroma cacao* L., cassava - *Manihot esculenta* Crantz, sugarcane – *Saccharum* spp., etc.)— resulting in even fragments of native forest formations being de facto secondary forests. During the period analyzed here, the primary anthropogenic land use class was Pasture, which occupied one-third of AoE in 1985 and increased by 5% in 2020. This demonstrates that the region, despite experiencing late extensive occupation, has been subject to various types of human-induced impacts, even though it is considered a high biodiversity and conservation priority area (Faria et al. 2021, JBRJ 2023).

Although not identified in the analyses conducted by Gomes-da-Silva and Forzza (2021) for major angiosperm groups, the *Hileia Baiana* is recognized as a center of endemism and biodiversity richness for various other organism groups (Faria et al. 2021, Gomes-da-Silva and Forzza 2021, Albuquerque and Torresan 2022, de Souza et al. 2021). This AoE stands out for having the highest number of exclusive species (four) and is composed of two grid cells, setting it apart from the others. In grid cell 47, there was an overlap of CUs, with one of them (*Parque Nacional Da Serra Das Lontras*)

recording *P. epipremnifolia*, one of the synapomorphies of this AoE. The other species in this area did not have records within CUs. Despite harboring four endemic species, two of them (*P. flexicaulis* and *P. parcifolia*) are found exclusively in grid cell 39. Notably, grid cell 39 boasts the highest number of exclusive species yet lacks CUs within its boundaries. Provisionally classified as Critically Endangered (Moura et al. 2022), *P. flexicaulis* and *P. parcifolia* are species that demand increased attention to address the Wallacean and Prestonian shortfalls (Hortal et al. 2015), ultimately providing evidence for a more precise extinction risk assessment and conservation initiatives.

Unlike the pattern observed in the previously discussed areas, AoEs 4, 5, and 6 have shown an approximately 2% increase in natural land cover classes. This is partly attributed to the enactment of national laws aimed at protecting the Atlantic Forest (e.g., Federal Law No. 11.428, Brasil 2006). Despite the change appearing ostensibly positive, the domain of the Atlantic Forest has endured more intensive impacts in periods prior to our analysis (Solórzano et al. 2021). Currently, the remnants of this domain collectively constitute only 11-32% of their original coverage (Ribeiro et al. 2009; Rezende et al. 2018, MapBiomas 2023). These three AoEs are located in sectors of the Atlantic Forest that are often associated with areas/centers of endemism for more inclusive groups of angiosperms (Gomes-da-Silva and Forzza 2021), as well as specific groups such as epiphytic angiosperms (Menini Neto et al. 2016), ferns, and lycophytes (de Souza et al. 2021), for example. The Atlantic Forest is the domain that harbors the highest number of endemic *Peperomia* species in Brazil (Moura et al. 2022), and forest environments (Forest Formation class), the primary ecosystem type accommodating these species, are undergoing a process of fragmentation, despite a proportional expansion in total area.

Located within one of the world's prime regions of species endemism (Tietje et al. 2023), AoE 4 is situated in the Southern Mountains Complex (Echternacht et al. 2011) of the *Espinhaço* Range in the state of Minas Gerais, within the region locally referred to as the *Quadrilátero Ferrífero* (Iron Quadrangle). In the northwest segment of the grid cell, there is an overlap with the *Parque Nacional*

da Serra do Gandarela, the sole CU within this AoE, although none of the records of the indicator species (*P. crypticola* and *P. marcoana*) occur within its boundaries. Positioned in a transitional zone between two global biodiversity hotspots (Cerrado and Atlantic Forest) and situated in one of the world's leading regions for metallic mineral production, AoE 4 has witnessed an approximately fourfold increase in areas occupied by the Mining class, which, according to Jacobi and Carmo (2008), has been a significant source of biodiversity impacts.

The other two AoEs (5 and 6) situated within the Atlantic Forest are established in the mountain complex of the *Serra do Mar* (Almeida and Carneiro 1998), a system renowned for its high richness and endemism indices for angiosperms in the neotropical region (Raedig et al. 2010, Morawetz and Raedig 2007). Furthermore, Moura et al. (2022) emphasize that this region constitutes the epicenter of richness for endemic *Peperomia* species in Brazil. AoE 5 is located in a border region, spanning the southern part of the state of São Paulo and the coastal region of the state of Paraná. Represented by grid cell 100, this AoE shares its southern boundary with grid cells 107 and 108, as identified by Moura et al. (2022) as one of the areas with high richness values for endemic *Peperomia* species in Brazil, even though the synapomorphic species (*P. apiahyensis* and *P. calcicola*) do not occur in these neighboring areas. The nearest CUs to this AoE are approximately 25 km from its southern border; thus, there are no records of indicator species within federal CUs. On the other hand, AoE 6 (grid cell 87) is situated in the northern portion of the *Serra do Mar* mountain complex, within the *Serra dos Órgãos* region, northwest of the city of Rio de Janeiro. This AoE is encompassed within a range (grid cells: 59, 66, 77, 85, 86, and 87) that hosts the highest richness indices and records for endemic *Peperomia* species in Brazil (Moura et al. 2022). Although four CUs overlap its boundaries, only *P. bradei* is protected within one of them (the *Parque Nacional da Serra dos Órgãos*), whereas *P. arbuscula* and *P. menkeana*, the other two synapomorphies of AoE, lack records within UCs. While we can enumerate the primary anthropogenic land cover classes currently affecting these areas (Mosaic of Uses, Forest Plantation, and Pasture for AoE 5; and Pasture, Mosaic of Uses, and Urban Area for AoE 6), we can consider the long history of use and occupation by human populations

(Carlucci et al. 2021) as a key stressor for the natural ecosystems in this region, along with its inevitable consequences.

Unexpectedly, two AoEs were discovered within the Amazonian domain, and both lack CUs within their boundaries. The four synapomorphic species within these AoEs are classified as threatened, with their most recent records dating back more than three decades. This underscores the critical need for further research addressing the genus within the Amazonian domain (Moura et al. 2022). Conversely, the AoEs within the Atlantic Forest domain consistently overlap or border areas of high species richness found among the genus's endemic species in Brazil (Moura et al. 2022), except for AoE 3, located to the north of the *Hileia Baiana*. Remarkably, AoE 3 stands out for hosting the highest number of exclusive species (four) within its boundaries, followed closely by AoE 6, which harbors more than two such species.

It is widely recognized that, due to the impacts of human activities, we are facing one of the greatest global biodiversity crises (Rinawati et al. 2013). One of the initial expected responses for terrestrial species in the face of environmental changes is to move to cooler and higher elevations within their potential distribution range (Pecl et al. 2017). As discussed by Moura et al. (2022), the predominant presence of these species in high-altitude moist forest formations is supposedly linked to the diversity of habitats (and resulting environmental conditions) found in these ecosystems. Therefore, migration of these species to other environments becomes practically unfeasible in the short term (Corlett and Westcott 2013), given that their primary areas of richness and endemism are situated within one of the world's biodiversity hotspots and the most devastated domain in the Brazilian territory (Ribeiro et al. 2009; Rezende et al. 2018, MapBiomas 2023).

The data generated within this study, in conjunction with research conducted on other taxa, has the potential to inform discussions regarding conservation efforts. Such efforts may encompass the establishment of new CUs designed to safeguard these organisms and provide a window of opportunity for species to either adapt to changing conditions or confront the risk of extinction. It is

also evident that research on Amazonian *Peperomia* species is essential, given the discovery of two endemic areas in this study and the existing knowledge gap concerning this genus within the Amazonian domain (Moura et al. 2022). Furthermore, it is crucial to emphasize the need for ongoing updates, incorporating new occurrence data for the species studied here. This ongoing effort will, over time, help solidify our understanding of the biogeographic patterns and processes associated with these species, enabling the effective implementation of conservation strategies.

References

Albuquerque, N.C.B., and F.E. Torresan. 2022. A floresta da Hileia Baiana. In Albuquerque, N.C.B. et al. (eds.). *Sementes Florestais da Mata Atlântica: Um guia para manejo de espécies da Hileia Baiana* . Programa Arboretum de Conservação e Restauração da Diversidade, Teixeira de Freitas. p. 27–33.

Almeida, F.F.M., and C.D.R. Carneiro. 1998. Origem e evolução da Serra do Mar. *Revista Brasileira de Geociencias* 28(2):135–150. https://doi.org/10.25249/0375-7536.1998135150

Brazil. 2000. *Federal Law No. 9985 of July 18* - Regulates Article 225, § 1°, items I, II, III, and VII of the Federal Constitution, establishes the National System of Nature Conservation Units, and provides other provisions. https://www.planalto.gov.br/ccivil_03/leis/l9985.htm

Brazil. 2006. *Federal Law No. 11428 of December 22* - Regulates the use and protection of native vegetation in the Atlantic Forest biome and establishes other provisions. https://www.planalto.gov.br/ccivil_03/_ato2004-2006/2006/lei/l11428.htm

Brown, M.J.M., S.P. Bachman, and E. Nic Lughadha. 2023. Three in four undescribed plant species are threatened with extinction. *New Phytologist* 19214. https://doi.org/10.1111/nph.19214

Carlucci, M.B., V. Marcilio-Silva, and J.M. Torezan. 2021. The Southern Atlantic Forest: Use, Degradation, and Perspectives for Conservation. In Marques, M.C.M., and C.E.V. Grelle (eds.) *The Atlantic Forest*. Springer, Cham. p. 91–111. https://doi.org/10.1007/978-3-030-55322-7_5

Carvalho, C.J.B. 2009. Padrões de endemismos e a conservação da biodiversidade. *Megadiversidade* 5(1-2): 77–86.

CNCFlora - *Centro Nacional de Conservação da Flora*, Instituto de Pesquisas Jardim Botânico do Rio de Janeiro. 2023. *Piperaceae*. http://cncflora.jbrj.gov.br/portal/pt-br/listavermelha/PIPERACEAE. accessed 01 Feb.

Corlett, R.T., and D.A. Westcott (2013) Will plant movements keep up with climate change? *Trends in Ecology and Evolution* 28(8):482–488. https://doi.org/10.1016/j.tree.2013.04.003

Cowie, R.H., P. Bouchet, and B. Fontaine. 2022. The Sixth Mass Extinction: fact, fiction or speculation?. *Biological Reviews* 97:640–663. https://doi.org/10.1111/brv.12816

Cox, C.B., and P.D. Moore. 2009. *Biogeography: An Ecological and Evolutionary Approach*, 7th Edition. Wiley-Blackwell, Hoboken. 440 p.

Echternacht, L., M. Trovó, C.T. Oliveira, and J.R. Pirani. 2011. Areas of endemism in the Espinhaço Range in Minas Gerais, Brazil. *Flora - Morphology, Distribution, Functional Ecology of Plants* 206(9):782–791. https://doi.org/10.1016/j.flora.2011.04.003

Faria, D., J.H.C. Delabie, M.H. Dias. 2021. The Hileia Baiana: An Assessment of Natural and Historical Aspects of the Land Use and Degradation of the Central Corridor of the Brazilian Atlantic Forest. In Marques, M.C.M., and C.E.V. Grelle (eds.). *The Atlantic Forest*. Springer, Cham. p. 60–90. https://doi.org/10.1007/978-3-030-55322-7_4

Flora e Funga do Brasil. 2023. Jardim Botânico do Rio de Janeiro. http://floradobrasil.jbrj.gov.br. accessed 01 Feb.

Frenzke, L., E. Scheiris, G. Pino, L. Symmank, P. Goetghebeur, C. Neinhuis, S. Wanke, and M.-S. Samain. 2015. A revised infrageneric classification of the genus *Peperomia* (Piperaceae). *Taxon* 64(3):424–444. https://doi.org/10.12705/643.4

Frodin, D.G. 2004. History and concepts of big plant genera. *Taxon* 53(3):753–776. https://doi.org/10.2307/4135449

Gomes-da-Silva, J. and R.C. Forzza. 2021. Two centuries of distribution data: detection of areas of endemism for the Brazilian angiosperms. *Cladistics* 37:442–458. https://doi.org/10.1111/cla.12445

Gao, JG., H. Liu, N. Wang, J. Yang, and X.-L. Zhang. 2020. Plant extinction excels plant speciation in the Anthropocene. *BMC Plant Biology* 20:430. https://doi.org/10.1186/s12870-020-02646-3

Goloboff, P.A. 1993. *NONA ver. 2.0*. INSUE Fundación y Instituto Miguel Lillo, Tucumán.

Haila, Y. 2002. A Conceptual Genealogy of Fragmentation Research: From Island Biogeography to Landscape Ecology. *Ecological Applications* 12(2):321–334. https://doi.org/10.2307/3060944

Jacobi, C.M., and F.F. Carmo. 2008. The Contribution of Ironstone Outcrops to Plant Diversity in the Iron Quadrangle, a Threatened Brazilian Landscape. *AMBIO: A Journal of the Human Environment* 37(4): 324–326. https://doi.org/10.1579/0044-7447(2008)37[324:TCOIOT]2.0.CO;2

Hortal, J., F. Bello, J.A.F. Diniz-Filho, T.M. Lewinsohn, J.M. Lobo, and R.J. Ladle. 2015. Seven Shortfalls that Beset Large-Scale Knowledge of Biodiversity. *Annual Review of Ecology, Evolution, and Systematics* 46:523–549. https://doi.org/10.1146/annurev-ecolsys-112414-054400

Humphreys, A.M., R. Govaerts, S.Z. Ficinski, E.N. Lughadha, and M.S. Vorontsova. 2019. Global dataset shows geography and life form predict modern plant extinction and rediscovery. *Nature Ecology & Evolution* 3:1043–1047. https://doi.org/10.1038/s41559-019-0906-2

ICMBio - Instituto Chico Mendes de Conservação da Biodiversidade. 2023. https://www.gov.br/icmbio/pt-br/assuntos/dados_geoespaciais/mapa-tematico-e-dados-geoestatisticos-das-unidades-de-conservacao-federais. accessed 01 Feb.

INPE - *Instituto Nacional de Pesquisas Espaciais*. 2023. http://terrabrasilis.dpi.inpe.br/app/dashboard/deforestation. accessed 01 Jul.

IUCN - International Union for Conservation of Nature. 2023. *The IUCN Red List of Threatened Species*. https://www.iucnredlist.org. accessed 01 Feb.

JBRJ - *Instituto de Pesquisas Jardim Botânico do Rio de Janeiro*. 2023. *Administrative Ruling No. 23, dated August 16th* - Approves the National Action Plan for the Conservation of Endangered Trees in Southern Bahia - PAN Hileia Baiana, encompassing 221 threatened taxa. It delineates its overall objective, specific goals, covered species, execution timeline, implementation methods, supervision, and review. https://in.gov.br/en/web/dou/-/portaria-n-23-de-16-de-agosto-de-2023-504725812

Jung, M. 2016. LecoS — A python plugin for automated landscape ecology analysis. *Ecological Informatics* 31:18–21. https://doi.org/10.1016/j.ecoinf.2015.11.006

Mathieu, G. 2023. *The Internet Peperomia Reference*. https://www.peperomia.net. accessed 01 Feb.

Menini Neto, L., S.G. Furtado, D.C. Zappi, A.T. Oliveira Filho, and R.C. Forzza. 2016. Biogeography of epiphytic Angiosperms in the Brazilian Atlantic forest, a world biodiversity hotspot. *Brazilian Journal of Botany* 39:261–273. https://doi.org/10.1007/s40415-015-0238-7

Morawetz, W., and C. Raedig. 2007. Angiosperm biodiversity, endemism and conservation in the Neotropics. *Taxon* 56:1245–1254. https://doi.org/10.2307/25065916

Morrone, J.J. 2014. Parsimony analysis of endemicity (PAE) revisited. *Journal of Biogeography* 41(5): 842–854. https://doi.org/10.1111/jbi.12251

Morrone, J.J. 1994. On the Identification of Areas of Endemism. *Systematic Biology* 43(3):438–441. https://doi.org/10.1093/sysbio/43.3.438

Moura, C.O., P.H.A. de Melo, E.T. de Amorim, G.M. Marcusso, and M. Carvalho-Silva. 2022. Peperomia (Piperaceae) endemic to Brazil: Distribution, richness, and conservation status. Flora 297:152170. https://doi.org/10.1016/j.flora.2022.152170

Murcia, C. 1995. Edge effects in fragmented forests: implications for conservation. *Trends in Ecology & Evolution* 10(2):58–62. https://doi.org/10.1016/S0169-5347(00)88977-6

Nixon, K.C. 2002. *WinClada ver. 1.00.08*. Published by the author, Ithaca.

Pecl, G.T., M.B. Araújo, J.D. Bell, J. Blanchard, T.C. Bonebrake, I-C. Chen, T.D. Clark, R.K. Colwell, F. Danielsen, B. Evengård, L. Falconi, S. Ferrier, S. Frusher, R.A. Garcia, R.B. Griffis, A.J. Hobday, C. Janion-Scheepers, M.A. Jarzyna, S. Jennings, J. Lenoir, H.I. Linnetved, V.Y. Martin, P.C. McCormack, J. McDonald, N.J. Mitchell, T. Mustonen, J.M. Pandolfi, N. Pettorelli, E. Popova, S.A. Robinson, B.R. Scheffers, J.D. Shaw, C.J.B. Sorte, J.M. Strugnell, J.M. Sunday, M.-N. Tuanmu, A. Vergés, C. Villanueva, T. Wernberg, E. Wapstra, and S.E. Williams. 2017. Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being. *Science* 355:eaai9214. https://doi.org/10.1126/science.aai9214

QGIS Development Team. 2023. *QGIS Geographic Information System version 3.10*. The Open Source Geospatial Foundation (OSGeo). https://qgis.org

Raedig, C., C.F. Dormann, A. Hildebrandt, and S. Lautenbach. 2010. Reassessing Neotropical angiosperm distribution patterns based on monographic data: a geometric interpolation approach. *Biodiversity and Conservation* 19:1523–1546. https://doi.org/10.1007/s10531-010-9785-1

Rezende, C.L., F.R. Scarano, E.D. Assad, C.A. Joly, J.P. Metzger, B.B.N. Strassburg, M. Tabarelli, G.A. Fonseca, and R.A. Mittermeier. 2018. From hotspot to hopespot: an opportunity for the Brazilian Atlantic Forest. *Perspectives in Ecology and Conservation* 16(4):208–214. https://doi.org/10.1016/j.pecon.2018.10.002

Ribeiro, M.C., J.P. Metzger, A.C. Martensen, F.J. Ponzoni, M.M. Hirota. 2009. The Brazilian Atlantic Forest: how much is left, and how is the remaining forest distributed? Implications for conservation. *Biological Conservation* 142(6):1141–1153. https://doi.org/10.1016/j.biocon.2009.02.021

Rinawati, F., K. Stein, and A. Lindner. 2013. Climate Change Impacts on Biodiversity—The Setting of a Lingering Global Crisis. *Diversity* 5(1):114–123. https://doi.org/10.3390/d5010114

de Souza, J.F., M.L. Bueno, and A. Salino. 2021. Atlantic Forest: centres of diversity and endemism for ferns and lycophytes and conservation status. *Biodiversity and Conservation* 30:2207–2222. https://doi.org/10.1007/s10531-021-02194-8

Solórzano, A., L.S.C.d.A. Brasil, and R.R. de Oliveira. 2021. The Atlantic Forest Ecological History: From Pre-colonial Times to the Anthropocene. In Marques, M.C.M., and C.E.V. Grelle (eds.). *The Atlantic Forest*. Springer, Cham. p. 23–44 https://doi.org/10.1007/978-3-030-55322-7_2

Souza Jr., C.M., J.Z. Shimbo, M.R. Rosa, L.L. Parente, A.A. Alencar, B.F.T. Rudorff, H. Hasenack, M. Matsumoto, L.G. Ferreira, P.W.M. Souza-Filho, S.W. de Oliveira, W.F. Rocha, A.V. Fonseca, C.B. Marques, C.G. Diniz, D. Costa, D. Monteiro, E.R. Rosa, E. Vélez-Martin, E.J. Weber, F.E.B. Lenti, F.F. Paternost , F.G.C. Pareyn, J.V. Siqueira, J.L. Viera, L.C. Ferreira Neto, M.M. Saraiva, M.H. Sales, M.P.G. Salgado, R. Vasconcelos, S. Galano, V.V. Mesquita, and T. Azevedo. 2020. Reconstructing Three Decades of Land Use and Land Cover Changes in Brazilian Biomes with Landsat Archive and Earth Engine. *Remote Sensing* 12(17):2735. https://doi.org/10.3390/rs1217273

Tietje, M., A. Antonelli, F. Forest, R. Govaerts, S.A. Smith, M. Sun, W.J. Baker, and W.L. Eiserhardt. 2023. Global hotspots of plant phylogenetic diversity. *New Phytologist* 19151. https://doi.org/10.1111/nph.19151

MapBiomas. 2023. *MapBiomas Collection 7*. https://brasil.mapbiomas.org/colecoes-mapbiomas

Chapter 4

***Peperomia* sp. nov. (Piperaceae): a new critically endangered and endemic epiphytic species from the Brazilian Cerrado**

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Abstract

Peperomia sp. nov., a newly discovered species of *Peperomia* subgenus *Pseudocupula*, is described and illustrated. This novel species is distinguished by morphological characteristics, such as a long, upwardly curved peduncle in the inflorescence, and shares morphological affinity with *Peperomia minensis*. Its classification within the subgenus *Pseudocupula* is supported by both morphological traits and molecular analyses utilizing the *trnK/matk* region. The species, found in the border region between the states of Minas Gerais and Goiás, Brazil, produces flowers from February to August and fruits from May to August and has been classified as "Critically Endangered" according to IUCN criteria.

Keywords: Goiás, Minas Gerais, Piperoideae, Pseudocupula, Threatened.

Introduction

The genus *Peperomia* Ruiz López & Pavón (1794: 8) comprises about 1600 species (Wanke *et al.* 2006, Wanke *et al.* 2007, Frenzke *et al.* 2015), pantropically distributed, with areas of diversification in the Neotropics and Southeast Asia (Wanke *et al.* 2006). The genus is among the richest in number of species among flowering plants (Frodin 2004). Brazil harbors approximately 173 species, more than half of them endemic (Flora e Funga do Brasil 2022, Moura *et al.* 2022). Features characterizing *Peperomia* include its herbaceous habit, fleshy leaves, a spadix or (rarely) raceme inflorescence type, and flowers with two stamens and a single stigma (Wanke *et al.* 2006, Frenzke *et al.* 2015).

The infrageneric classification of *Peperomia* was initially established by Miquel (1843), but the classification proposed by Dahlstedt (1900) was the most widely used. The latter author divided the genus into six subgenera and nine sections, primarily based on fruit characteristics. Yuncker (1974) employed this classification and recognized Brazilian species in five of these sections.

The earliest phylogenetic studies have demonstrated *Peperomia* as a monophyletic group (Qiu *et al.* 2000, Jaramillo *et al.* 2004, Neinhuis *et al.* 2005, Wanke *et al.* 2006, Smith *et al.* 2008, Samain *et al.* 2009). Frenzke *et al.* (2015) revised the infrageneric classification of *Peperomia* and recognized 14 subgenera based on the phylogenetic results and morphological characters. The subgenus *Micropiper* (Miquel 1843: 76) Dahlstedt (1900: 138) was reclassified by Frenzke *et al.* (2015: 436) as the subgenus *Pseudocupula* (Frenzke & Scheiris). The subgenus *Pseudocupula* is characterized by the

presence of a pseudocupula, a cup-shaped sticky structure at the base of its fruits, an apical stigma, prostrate to semi-erect stems, thickened and rooted nodes, and verticillate or opposite leaves.

During the early 2000s and 2010s, specimens of an epiphytic plant were collected in the influence area of the Queimado Hydroelectric Plant (UHE Queimado), located on the border between the states of Goiás and Minas Gerais in Brazil. Initially identified as *Peperomia minensis* Henschen (1873: 29), these samples underwent analyses, encompassing both morphological and molecular characteristics. These analyses resulted in the description of a new species, discovered within the gallery forests of the Brazilian Cerrado domain. This novel species is described and illustrated, supplemented with additional insights on its conservation status, phenology, and distribution.

Materials and methods

Specimens preparations:—The delimitation of the new species was based on morphological characters observed on herbarium sheets (CEN, NY, RB, and UB), all personally inspected, as well as through field observations (conducted in the years 2003 and 2012). In addition, comparisons were made with the type of *P. minensis* and specimens determined by T.G. Yuncker as *P. minensis*. The assignment to a subgenus was determined through morphological and molecular analyses. The description was based on freshly collected plants and herbarium specimens. The specimens collected were deposited in the CEN and UB herbaria (acronyms according to Thiers, continuously updated). Some leaves were preserved in silica gel for DNA analysis.

DNA Extraction, Amplification, Sequencing, and Phylogenetic Analyses:—Total genomic DNA was extracted from silica gel-dried leaves, following the method of Doyle & Doyle (1987), with modifications (Borsch *et al.* 2003). The *trnK/matk* region was amplified in two parts using the primers MG15 and Pe-matK-2500R, and Pi-matK-2030F and MG1 (Liang & Hilu 1996, Wanke *et al.* 2006). Sequences from three individuals of this new species were used in this study. The polymerase chain reaction (PCR) reaction mixture, with a total volume of 50 µl, contained 5 µl of Mg buffer, 5 µl of MgCl2, 2 µl of BSA, 4 µl of dNTP mix, 0.5 µl of Taq polymerase, 2.5 µl of each primer, 2 µl of DNA, and 26.5 µl of Milli-Q water. The extractions and amplifications were conducted at the Plant Molecular Biology Laboratory of the University of Brasília. The PCR amplifications were performed in a thermocycler using an initial cycle at 95 °C for 1 min, followed by 35 cycles at 95 °C for 30 s, 51 or 52 °C for 45 s, and 72 °C for 1 min, and a final extension at 72 °C for 5 min. Purification and Sanger sequencing were carried out by BPI Biotecnologia, Pesquisa e Inovação Ltda., Brazil. Sequences were edited using Geneious v. 6.0.6 (www.geneious.com, Kearse *et al.* 2012), and aligned using CLUSTALX v. 2.1 (Higgins & Sharp 1988), with additional visual adjustments made in PhyDE v. 0.9971 (Müller *et al.* 2010).

Maximum parsimony (MP) analyses were conducted using PAUP v. 4.0b10 for Mac (Swofford 2002), maximum likelihood (ML) using RAxML v. 8 (Stamatakis 2006, Stamatakis *et al.* 2008), and Bayesian inference (BI) using Mr. Bayes v. 3.2.6 (Ronquist & Huelsenbeck 2003). The best evolutionary model for likelihood analyses was determined with JModelTest 2.1.1 (Guindon & Gascuel 2003, Darriba *et al.* 2012). Insertions/deletions were incorporated into the analysis and coded as necessary using the simple indel coding method by Simmons & Ochoterena (2000). For Bayesian inference, four Markov Chain

Monte Carlo chains were run for 5,000,000 generations, with samples taken every 1,000 generations, in two parallel runs. The first 25% of the trees were discarded as burn-in. Clade support was assessed using non-parametric bootstrap (BS, Felsenstein 1985), with 1,000 replicates for MP and 100 replicates for ML. The BI support was evaluated using posterior probabilities (PP). We utilized 56 sequences of *Peperomia*, representing all subgenera (according to Frenzke *et al.* 2015), two sequences of *Piper* Linnaeus (1753: 28) as the outgroup, and incorporated the three new sequences of the new species. The sequences were obtained from GenBank, and the accession numbers are available in Suppl. 1A. The cladogram depicted in Fig. 1 was created using general graphic design tools and FigTree v. 1.4.4 (Rambaut 2018) and used the BI tree as a template.

Conservation assessment:—The conservation status was assessed using criterion B, established by the IUCN (2022), and adopting the GeoCAT Conservation Assessment Tool (geocat.kew.org; Bachman *et al.*, 2011). To meet the conditions outlined by criterion B and assess the quality of the habitat where the new species occurs, a landscape analysis was conducted using the LecoS - Landscape Ecology Analysis plugin (Jung 2016) implemented in QGIS v3.10 (QGIS Development Team 2023). Rasters of land use and land cover from MapBiomas - Collection 8 (MapBiomas 2023; Souza Jr. *et al.* 2020) were utilized for this analysis. In this stage, (1) the minimum convex polygon was delineated based on the occurrence points; (2) the centroid of this polygon was then calculated; (3) subsequently, a buffer/polygon of 0.35° (with an area of 4552.8937 km²) was generated through this centroid; (4) within the area of this new polygon, where all occurrences are included, the following landscape metrics were calculated (for the years 2000 and 2022): land cover, number of patches, greatest patch area, and mean patch area. The land use and land cover

classes were categorized as Natural Formation, Anthropogenic Formation, and Water Bodies.

Results

The aligned sequences yielded 3428 bp for the *trnK/matk* region, with 513 variable characters, 2156 conserved characters, and 759 parsimony-informative characters. The inferred phylogenetic reconstruction supported the monophyly of *Peperomia* subgenus *Pseudocupula*, in agreement with Frenzke *et al.* (2015), and indicated that the new species is monophyletic with high support (1 PP, 96% BS, and 59% BS) based on BI, ML, and MP, respectively (Fig. 1, Suppl. 2, 3, and 4). *Peperomia minensis* was not included in the phylogenetic studies because these specimens were collected prior to 1950, and it was not possible to obtain their DNA from herbarium collections.

Taxonomic treatment

***Peperomia* sp. nov.** M. Carvalho-Silva & C.O. Moura *sp. nov.* (Fig. 2, Fig. 4).

Similar to Peperomia minensis Henschen *but differs in the long, upwardly curved peduncle, 55–70 mm long (vs. short erect peduncle, 15–25 mm long).*

Type:—BRAZIL. Minas Gerais: Unaí, forest below the Spillway, area of direct influence of the AHE Queimado, 16°13'13''S, 47°19'29''W, 25 Jun 2002 (fl, fr), *A.A. Santos et al. 1270* (holotype CEN00047328!).

Epiphytic herbs, creeping, terminal branches decumbent; **stems** succulent, hirtellous, vinaceous to dark green, drying slightly glossy and peeling off, internodes 20–60 mm long. **Leaves** verticillate, 3–(4–5) at a node, succulent, slightly concave, drying papyraceous, elliptic, obovate-elliptic, rarely rhombic, acrodromous; lamina 1.5–3 cm long, 0.7–1.7 cm wide, hirtellous to glabrescent, vinaceous abaxial surface and dark-green adaxial surface with brown glands on both sides, abaxial surface with vinaceous spot, mainly next to the veins; apex obtuse; plane margin; acute base; nerves 3, inconspicuous; petiole 2–3(–6) mm long, cylindrical, hirtellous, vinaceous. **Inflorescence** 1, terminal, green in flower; peduncle 55–70 mm long, upwardly curved, hirtellous; peduncle bract absent; rachis erect, 20–45 mm long × 1.8–2 mm wide, 30–50 mm long × 2 mm wide in fruit, fleshy, foveolate, glabrous, verrucose, fovea 0.5–1 mm long × 0.5–0.7 mm wide; **flower bract** 0.5–0.9 mm long × 0.5–0.7 mm wide, orbicular, elliptic, peltate, glabrous. **Fruit** ca. 0.7–1.2 mm long × 0.5–0.7 mm wide, ellipsoid, glabrous, smooth or slightly papillate, longitudinal ribs minute, apex with oblique, terminal stigma, 0.3–0.5 mm long; pseudocopula basal, sticky, covering the fruit irregularly; pseudopedicel minute, glabrous.
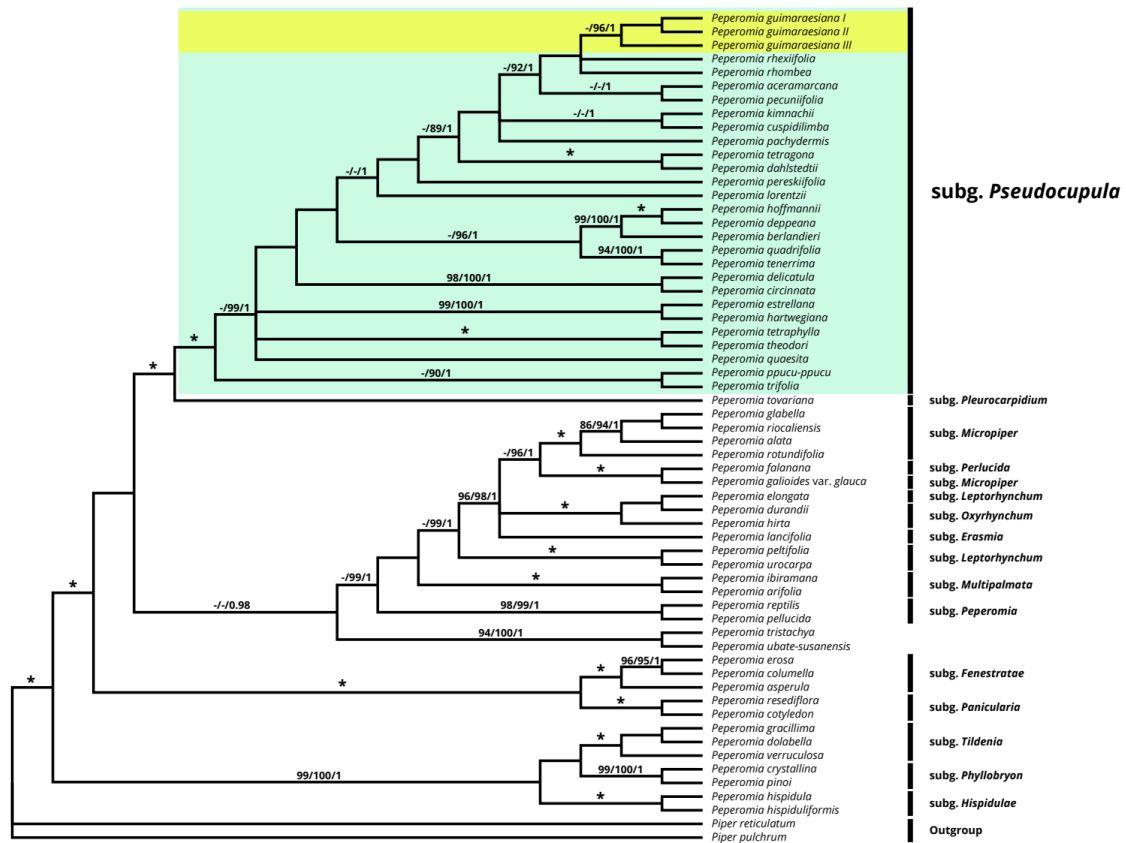
Figure 1. Cladogram depicting the new species included in *Peperomia* subg. *Pseudocopula*. Asterisks designate the clades that achieved maximum support in all analyses, while for the remaining ones, support is indicated when it exceeded 85% for Parsimony and Maximum Likelihood, and 0.95 for Bayesian inference. *Peperomia* sp. nov. is highlighted in yellow, while *P.* subg. *Pseudocupula* is highlighted in blue.

Etymology:—The specific epithet honors Dr. Elsie Franklin Guimarães, a botanist and expert in Brazilian Piperaceae from the Instituto de Pesquisas Jardim Botânico do Rio de Janeiro.
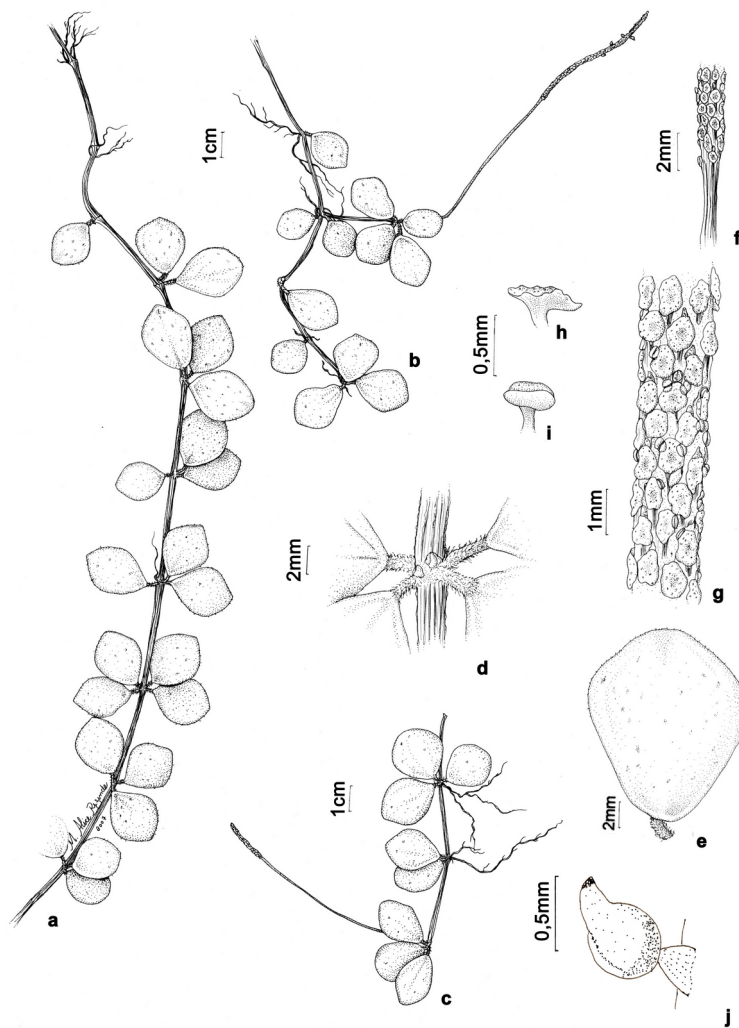
Figure 2. Illustration of *Peperomia* sp. nov. M. Carvalho-Silva & C.O. Moura. (a) habit, (b) plant showing the upwardly curved peduncle, (c) plant with leaves and inflorescence, (d) detailed view of the hirtellous petioles, (e) leaf in detail, (f) spadix base, (g) spadix detail - showing the flower arrangement (h) lateral view of a bract, (i) stamens, (j) fruits showing the pseudocupula at the base.

Distribution, habitat, and conservation assessment:—Growing on trees in the gallery forests of the Cerrado, at the border between the states of Goiás and Minas Gerais, this species thrives near small streams at elevations of about 820 m (Fig. 3, Suppl. 1B). The landscape analysis (Suppl. 1C, 5, and 6) revealed that the natural formation class decreased from an initial coverage of 61.60% in 2000 to 32.62% in 2022. Meanwhile, the water bodies

class increased more than sixfold due to the impacts of the UHE Queimado, starting at 0.22% in 2000 and ending with a total coverage of 1.43% in 2022. The anthropogenic formation class nearly doubled its coverage area, going from 38.18% in 2000 to 65.95% in 2022. Furthermore, the metrics of the patches demonstrate that the reduction in the coverage of the natural formation class resulted not only from the shrinking size of the existing fragments in the initial year but also from the extinction of larger fragments, as evidenced by the decline in the values of the greatest and mean patch area. In addition, the extent of occurrence was 77,779 km², and the area of occupancy was 16,000 km². However, despite the existence of fragments of natural formations, all known collection locations are currently under direct or indirect anthropogenic impact, such as agricultural activities and the hydroelectric power plant. Unfortunately, there are no active protection measures for the species. As a result, we have classified this species as Critically Endangered (CR), B1ab(iii), according to the IUCN guidelines (2022) using criterion B.

Notes:—*Peperomia* sp. nov. is a stoloniferous herb, with a pseudopedicel frequently observed in freshly collected plants. It was observed with flowers from February to August and fruits from May to August. The verticillate leaves, short petiole, and ellipsoid fruit with a sticky basal pseudocupula are morphological features that support the classification of this new species within the *Peperomia* subg. *Pseudocupula*. It distinguishes itself from the closely related *Peperomia minensis* (Fig. 4), which is also found in Minas Gerais, by its long, upwardly curved peduncle measuring 55–70 mm in length. In contrast, *Peperomia minensis* features a short, erect peduncle, typically spanning 15–25 mm.
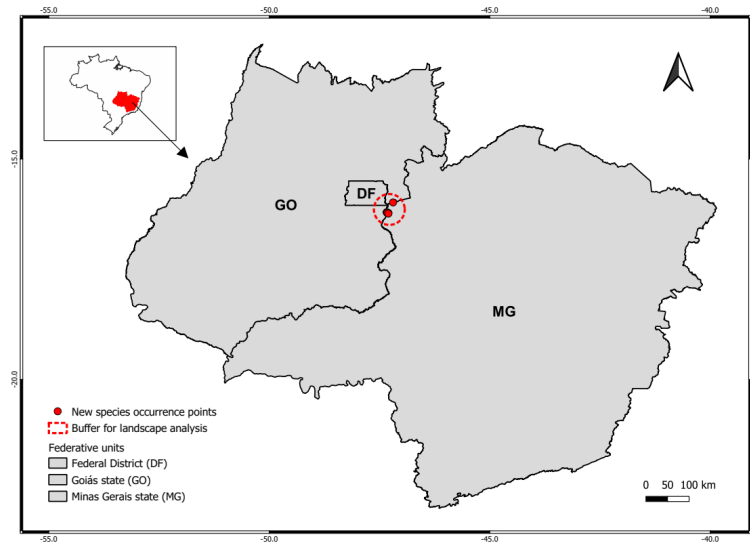
Figure 3. Map showing the currently known distribution of *Peperomia* sp. nov. (red dots). The box in the upper left corner shows in red the Brazilian states of Goiás, Minas Gerais, and the Federal District. The dashed line highlights the area used for landscape analysis.

Additional material examined:—BRAZIL. Goiás: Cristalina, forest to the left of the bridge (right margin) that gives access to the sentry-box at the exit to Palmital, area of direct influence of the AHE Queimado, 16°12'35''S, 47°20'24''W, 15 May 2002, *A.A. Santos et al. 1165* (CEN00047234!); right bank of the Preto river, above the wooden bridge, access to Palmital, direct influence area of the AHE Queimado, upstream of the construction site, 16°12'19''S, 47°19'59''W, 12 Aug 2002, *A.A. Santos et al. 1366* (CEN00047408!). Minas Gerais: Cabeceira Grande, right bank of the Bezerra river, ca. 1 km east of the Perta-Pé Lagoon (Army area), area of indirect influence of the UHE Queimado, 15°59'06''S, 47°11'35''W, 5 Mar 2002, *G. Pereira-Silva et al. 5982* (CEN00043162!). Unaí, escape tunnel waterfall forest, area of indirect influence of the AHE Queimado, 16°13'26''S, 47°17'49''W, 18 Feb 2003, *A.A. Santos & J.B. Pereira 1843* (CEN00047601!); road that gives access to the escape tunnel, downstream of the construction site, area of direct influence of AHE Queimado, 16°13'14''S, 47°19'27''W, 27 Jun 2002, *A.A. Santos et al. 1326* (CEN00047383!);

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farm near the dam of UHE Queimado, 16°14'15''S, 47°18'00''W, 1 Mar 2012 (fl), *M. Carvalho-Silva et al. 1168* (UB189565!).

Additional materials examined from *P. minensis*:—BRAZIL. Minas Gerais: Caldas, Jan 1869, *A.F. Regnell III 1631* (NY00956981!, S14-40236 photo!, S-R-4258 photo!, S14-40240 photo!); 15 May 1874, *C.W. Mosén 1649* (NY00559491!). Passa Quatro, 03 May 1948, *A.C. Brade 18931* (RB00274029!). Pomba, 16 May 1947, *E.P. Heringer 2517* (RB00274034!). Espírito Santo: Cachoeiro de Itapemirim, 31 May 1949, *A.C. Brade 19931* (RB00274025!). São Paulo: Serra do Caracol, 01 Apr 1874, *C.W. Mosén 1650* (NY00559490!).

Figure 4. Type material of *P.* sp. nov. – *A.A. Santos* 1270 (a) and *P. minensis* - *A.F. Regnell* III 1631 (b).

References

Bachman, S., Moat, J., Hill, A., de la Torre, J. & Scott, B. (2011) Supporting Red List threat assessments with GeoCAT: geospatial conservation assessment tool. *ZooKeys* 150: 117–126. https://doi.org/10.3897/zookeys.150.2109

Borsch, T., Hilu, K.W., Quandt, D., Wilde, V., Neinhuis, C. & Barthlott, W. (2003) Noncoding plastid *trnT-trnF* sequences reveal a well resolved phylogeny of basal angiosperms. *Journal of Evolutionary Biology* 16: 558–576. https://doi.org/10.1046/j.1420-9101.2003.00577.x

Dahlstedt, H. (1900) *Studien über süd- und central-Amerikanische Peperomien: mit besonderer Berücksichtigung der Brasilianischen Sippen*. *Kongliga Svenska Vetenskaps-Akademiens Handlingar* 33 (2): 1–218.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods* 9: 772. https://doi.org/10.1038/nmeth.2109

Doyle, J.J. & Doyle, J.L. (1987) A rapid isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 779–783. https://doi.org/10.2307/2408678

Flora e Funga do Brasil (2022) Jardim Botânico do Rio de Janeiro. Available from: http://floradobrasil.jbrj.gov.br/

Frenzke, L., Scheiris, E., Pino, G., Symmank, L., Goetghebeur, P., Neinhuis, C., Wanke, S. & Samain, M. (2015) A revised infrageneric classification of the genus *Peperomia* (Piperaceae). *Taxon* 64: 424–444. https://doi.org/10.12705/643.4

Frodin, D.G. (2004) History and concepts of big plant genera. *Taxon* 53: 753–776. http://dx.doi.org/10.2307/4135449

Guindon, S. & Gascuel, O. (2003) A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology* 52: 696–704.

Henschen, S.E. (1873) Études sur le genre *Peperomia*, comprenant les espèces de Caldas, Brésil. *Nova acta Regiae Societatis Scientiarum Upsaliensis* 3 (4): 29.

Higgins, D.G. & Sharp, P.M. (1988) CLUSTAL: A package for performing multiple sequence alignment on a microcomputer. *Gene* 73: 237–244.

IUCN. (2022). Guidelines for Using the IUCN Red List Categories and Criteria. Version 15.1. Available from: https://www.iucnredlist.org/resources/redlistguidelines.

Jaramillo, M.A., Manos, P.S. & Zimmer, E.A. (2004) Phylogenetic Relationships of the Perianthless Piperales: Reconstructing the Evolution of Floral Development. *International Journal of Plant Sciences* 165 (3): 403–416. https://doi.org/10.1086/382803

Jung, M. (2016) LecoS — A python plugin for automated landscape ecology analysis. *Ecological Informatics* 31: 18–21. https://doi.org/10.1016/j.ecoinf.2015.11.006

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P. & Drummond, A. (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28 (12): 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Liang, H. & Hilu, K.W. (1996) Application of the *matK* gene sequences to grass systematics. *Canadian Journal of Botany* 74: 125–134. https://doi.org/10.1139/b96-017

Linnaeus, C. von (1753) *Species Plantarum*. Holmiae, Impensis Laurentii Salvii. 1: 28. https://doi.org/10.5962/bhl.title.669

MapBiomas (2023) *MapBiomas Collection 8*. Available from: https://brasil.mapbiomas.org/colecoes-mapbiomas

Miquel, F.A.W. (1843) *Systema Piperacearum*. H.A. Kramers, Rotterdam, 1–571.

Moura, C.O., Melo, P.H.A., Amorim, E.T., Marcusso, G.M. & Carvalho-Silva, M. (2022) *Peperomia* (Piperaceae) endemic to Brazil: distribution, richness, and conservation status. *Flora* 152170. https://doi.org/10.1016/j.flora.2022.152170.

Müller, K., Quandt, D., Müller, J. & Neinhuis, C. (2010) PhyDE®: Phylogenetic data editor, version 0.9971. Available from: www.phyde.de

Neinhuis, C., Wanke, S., Hilu, K., Müller, K. & Borsch, T. (2005) Phylogeny of Aristolochiaceae based on parsimony, likelihood, and Bayesian analyses of *trnL-trnF*

sequences. *Plant Systematics and Evolution* 250: 7–26. https://doi.org/10.1007/s00606-004-0217-0

QGIS Development Team (2023) *QGIS Geographic Information System version 3.10*. The Open Source Geospatial Foundation (OSGeo). Available from: https://qgis.org

Qiu, Y., Lee, J., Bernasconi-Quadroni, F., Soltis, D.E., Soltis, P.S., Zanis, M., Zimmer, E.A., Chen, Z., Savolainen, V. & Chase, M.W. (2000) Phylogeny of Basal Angiosperms: Analyses of Five Genes from Three Genomes. *International Journal of Plant Sciences* 161 (S6): S3–S27. https://doi.org/10.1086/317584

Rambaut. A. (2018) FigTree v1.4.4. Avaliable at: http://tree.bio.ed.ac.uk/software/figtree/

Ronquist, F. & Huelsenbeck, J.P. (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Ruiz López, H. & Pavón, J.A. (1794) *Florae Peruvianae, et Chilensis Prodromus*. Imprenta de Sancha, Madrid, p. 8.

Samain, M.S., Vanderschaeve, L., Chaerle, P., Goetghebeur, P., Neinhuis, C. & Wanke, S. (2009) Is morphology telling the truth about the evolution of the species rich genus *Peperomia* (Piperaceae)? *Plant Systematics and Evolution* 278: 1–21. https://doi.org/10.1007/s00606-008-0113-0

Simmons, M.P. & Ochoterena, H. (2000) Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369–381. https://doi.org/10.1093/sysbio/49.2.369

Smith, J.F., Stevens, A.C., Tepe, E.J. & Davidson, C. (2008) Placing the origin of two species-rich genera in the late cretaceous with later species divergence in the tertiary: a phylogenetic, biogeographic and molecular dating analysis of *Piper* and *Peperomia* (Piperaceae). *Plant Systematics and Evolution* 275: 9. https://doi.org/10.1007/s00606-008-0056-5

Stamatakis, A. (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Stamatakis, A., Hoover, P. & Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* 57: 758–771. https://doi.org/10.1080/10635150802429642

Swofford, D.L. (2002). *PAUP*. Phylogenetic analyses using parsimony (*and other methods)*, version 4.0. Sinauer Associates, Sunderland (Massachusetts).

Thiers, B. Index herbariorum: a global directory of public herbaria and associated staff. [continuously updated]. New York Botanical Garden's Virtual Herbarium, New York. Website http://sweetgum. nybg. org/ih/[accessed 10 February 2019], 2019.

Wanke, S., Jaramillo, M.A., Borsch, T., Samain, M.S., Quandt, D. & Neinhuis, C. (2007) Evolution of the Piperales—*matK* and *trnK* intron sequence data reveal lineage specific resolution contrast. *Molecular Phylogenetics and Evolution* 42: 477–497. https://doi.org/10.1016/j.ympev.2006.07.007

Wanke, S., Samain, M.S., Vanderschaeve, L., Mathieu, G., Goetghebeur, P. & Neinhuis, C. (2006) Phylogeny of the genus *Peperomia* (Piperaceae) inferred from the *trnk/matk* region (cpDNA). *Plant Biology* 8: 93–102. https://doi.org/10.1055/s-2005-873060

Yuncker, T.G. (1974) The Piperaceae of Brazil-III: *Peperomia*; Taxa of uncertain status. *Hoehnea* 4: 71–413.