

# Phylogenetics of phyllocladiferous *Phyllanthus* (Phyllanthaceae): a new section exclusive from the Atlantic Rain Forest, with morphological and molecular support

Priscila Orlandini (✉ [priscilaorlandini@gmail.com](mailto:priscilaorlandini@gmail.com))

State University of Campinas

Otávio Luis Marques da Silva

Instituto de Pesquisas Ambientais, Unidade Jardim Botânico

Inês Cordeiro

Instituto de Pesquisas Ambientais, Unidade Jardim Botânico

Vinicius Castro Souza

Universidade de São Paulo

---

## Research Article

**Keywords:** Neotropics, *Phyllanthus* sect. *Choretropsis*, *Phyllanthus* sect. *Xylophylla*, *Phylloclades*

**Posted Date:** March 16th, 2023

**DOI:** <https://doi.org/10.21203/rs.3.rs-2679315/v1>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

**Version of Record:** A version of this preprint was published at *Plant Systematics and Evolution* on September 13th, 2023. See the published version at <https://doi.org/10.1007/s00606-023-01871-1>.

# Abstract

The latest phylogenetic and taxonomic studies emphasizing *Phyllanthus* (Phyllanthaceae) have significantly altered the relationships between its taxa. In this work, we inferred a phylogeny for the phyllocladiferous species of *Phyllanthus*, traditionally included within *Phyllanthus* sects. *Choretropsis* and *Xylophylla* from eastern Brazil and Caribbean, respectively. Our analysis demonstrated that these two sections are clearly distinct, but sect. *Choretropsis*, as it currently stands, does not form a monophyletic group. Thus, phylloclades arose in *Phyllanthus* at least in five distinct clades. Most species of sect. *Choretropsis* are recovered among representatives of other groups (sects. *Antipodanthus* and *Phyllanthus* subsect. *Claussenianii*), but no macromorphological feature could be traced for the clades we recovered. One exception is a clade containing *P. dracenooides*, *P. gladiatus* and *P. pedicellatus*, species with dracenoid habit and exclusive to the humid dense forests along Atlantic Forest domain, for which we describe a new section.

## 1. Introduction

*Phyllanthus* is one of the most numerous genus of Phyllanthaceae, with species occupying different habitats and with great morphological diversity, notably the phyllanthoid ramification, which is present in several of its species (Webster, 1956). The species of *Phyllanthus* are predominantly monoecious (rarely dioecious), glabrous, with or without phylloclades, with cataphylls and stipules generally present; flowers sessile or pedicellate, with free or united stamens and entire or segmented nectaries in both flowers, without pistillodes or staminodes.

Wurdack et al. (2004), Samuel et al. (2005) and Kathriarachchi et al. (2005) recovered *Breynia*, *Glochidion*, *Reverchonina* and *Sauropus* within *Phyllanthus*, indicating the paraphyly of the later. This relationship was also recovered by Bouman et al. (2021), the most recent and comprehensive phylogenetic study proposed for the genus. This framework served as base for a new classification for the tribe Phyllantheae (Bouman et al. 2022) aiming to achieve the monophyly of *Phyllanthus*, splitting it in ten genera, considering basically molecular data. Some of them are very difficult to be morphologically recognized as belonging to different genera.

As it is currently circumscribed, *Phyllanthus* sensu stricto now comprises 213 species restricted to the American continent and the Caribbean (Bouman et al., 2022).

Among the *Phyllanthus* s.str., some species are remarkable for the presence of phylloclades and reduced leaves, present only in the early stages of the plant, or completely absent. These species were initially recognized as the distinct genus *Xylophylla* (Linnaeus 1771), which were later recognized as a subgenus or section within *Phyllanthus* (Persoon 1807 and Baillon 1858, respectively). Müller Argoviensis (1863) described *Phyllanthus choretroides* and placed it in a new section. *P.* sect. *Choretropsis*, distinguished from *P.* sect. *Xylophylla*, based on the shape of the phylloclades (cylindrical versus flattened, respectively).

The Brazilian *Phyllanthus gladiatus* and *P. klotzschianus*, which bear flattened phylloclades, were then transferred to *P. sect. Choretropsis* by Webster (1958a, b), who proposed significant changes to the delimitation of both phyllocladiferous groups within the genus, restricting *P. sect. Xylophylla* to the Caribbean and *P. sect. Choretropsis* to Brazil. This new classification was also based on morphology of pollen grains, type of anther opening (reticulate pollen grains and deeply emarginate anthers in *P. sect. Choretropsis* versus areolate pollen grains and anthers deiscing more or less horizontally in *P. sect. Xylophylla*), and branching pattern (pinnatifid in *P. sect. Choretropsis* versus bipinnatifid in *P. sect. Xylophylla*).

Santiago et al. (2006), organized the species of *P. sect. Choretropsis* in two subsections, according to phylloclade morphology: subsect. *Applanata* for the flattened phylloclades species, and subsect *Choretropsis*, for the species with cylindrical /subcylindrical phylloclades. Additionally, three new phyllocladiferous species have recently been described and placed in the section based solely on morphological characters (Orlandini et al., 2020, 2021, 2022), resulting in the recognition of 12 species in *P. sect. Choretropsis* that occupy both dry and humid habitats in eastern Brazil

In the work by Bouman et al. (2021), *Phyllanthus sect. Choretropsis* was recovered within a polytomy comprising the sects. *Antipodanthus*, *Loxopodium* and *Phyllanthus*. However, sect. *Choretropsis* is represented in the phylogenies published so far only by *P. klotzschianus*. Therefore, the sampling adopted in these works could not allow either testing the monophyly of *P. sect. Choretropsis* or any of the subsections proposed by Santiago et al. (2006). Here we present a phylogenetic framework for *Phyllanthus sect. Choretropsis* with a wider sampling effort, aiming to test the monophyly of *P. sect. Choretropsis* and the subsection proposed for it by Santiago et al. (2006) and bringing light to the evolution of phyllocladiferous species within *Phyllanthus* s.str. and building foundations for future works involving the evolutionary history of the group.

## 2. Material And Methods

### 2.1. Taxon sampling

We included nine species from *Phyllanthus sect. Choretropsis* for the ingroup, with more than one sample for those with wide distribution range, such as *P. klotzschianus*, following the recommendation by Naciri and Linder (2015). The only species of the group that were not sampled in this study are *P. edmundoi* and *P. goianensis*, which are known only by their types specimens.

To compose the outgroup, we included both representatives of the genera that compose *Phyllanthus* s.lat., with emphasis on *P. sect. Xylophylla*, as well as other genera from tribe *Phyllantheae* to allow a wider evaluation of the position of the species included in the ingroup given the uncertainties from the phylogenies published so far, which lack a sufficient representation covering the morphological variation found in the group. Finally, an accession of *Astrocasia neurocarpa* was used to root the trees, representing *Wielandieae*, the sister tribe of *Phyllantheae* according to other phylogenetic studies of the tribe (Kathriarachchi et al., 2006, Wurdack et al., 2004, Falcón et al., 2020, Bouman et al., 2021).

Thus, 22 accessions representing 14 from the total of 19 phyllocladiferous species of *Phyllanthus* are covered in this work, 11 of them newly generated. The complete list of samples, including those obtained from Genbank, as well as their vouchers and other informations, are presented in Appendix A.

## **2.2. DNA extraction to sequence assembling**

DNA was extracted from fresh material dried in silica-gel (Chase and Hills, 1991), or herborized material following the CTAB protocol by Tel-Zur et al. (1999). We selected the internal transcribed spacer 1, 5.8S intron and the internal transcribed spacer 2 (hereafter referred as ITS) and the maturase K gene (hereafter referred as matK) based on variability and to complement the data generated by Kathriarachchi et al. (2006), Falcón et al. (2020) and Bouman et al. (2021). Due to its length, for matK we further analyzed previous alignments provided in the works cited above with Noisy (Dress et al. 2008) to look for more variable and informative regions that would optimize our amplification efforts given most of our samples were not successful to get a complete sequence of this region.

The ITS sequences were generated employing primers 17SE and 26SE (Sun et al., 1994). with an annealing temperature of 60°C. For the partial matK, the primers used were 530R and 570F, and the primer sequences of matK, were GTTCCAATTCCAATACTCGTGAAG and TCCAAAATCAAAGAGCGATTGG, respectively (Samuel et al. 2005), with annealing temperatures of 48°C, resulting in a fragment of c. 706 bp. A detailed description of the volumes and amplification cycles for each region is presented in Table 1.

The length of the PCR fragments and inspection for multiple bands, especially in the case of ITS, were verified on a 1% agarose gel with electrophoresis. Sequencing was subsequently analyzed on either an ABI3500 and POP-7 polymer (Applied Biosystems, Forsters City, California, USA) using ABI BigDye Terminator V3.1 and the same primers employed for amplification at Helixxa Laboratory.

Forward and reverse sequences were then analyzed in the Phred/Phrap/Consed package (Ewing and Green, 1998, Ewing et al., 1998, Gordon et al., 1998), and contigs were assembled using phred 20 as the minimum quality standard.

## **2.3. Sequence alignment and datasets**

Sequences were aligned using MAFFT online (Kato et al., 2019, Kuraku et al., 2013) adopting the standard parameters. with posterior manual verification following the similarity criterion (Simmons, 2004). Two datasets (ITS and matK) were analyzed individually and then incongruence between them was evaluated visually prior to combining the datasets.

Maximum Likelihood (ML) analyzes were conducted using RAxML 1.5b2 software (Stamatakis et al., 2014), using the GTR+I+G nucleotide substitution model, and 10,000 replicates to evaluate clade support bootstraps. Bayesian inference (BI) analyzes were performed using MrBayes v. 3.2.5 (Ronquist and Huelsenbeck, 2003) with substitution models estimated through functions implemented since version 3.2 (Ronquist et al., 2012). Four independent runs were held, each with four simultaneous chains (one cold

and three heated), for ten million generations, sampling every 1,000th generation. Burn in was initially set to 25% in MrBayes and then checked through Tracer v.1.6 software (Rambaut and Drummond, 2007). For the Estimated Sample Size (ESS) we consider values above 200. The majority (50%) rule consensus was built after a burn in a set of 25% for Bayesian inference. The trees generated in the ML and Bayesian inference analysis were analyzed and edited in FigTree software (Rambaut, 2010).

For support values, we adopted  $\geq 75$  to  $\leq 95$  BS (Bootstrap Support) or  $\geq 0.75$  to  $\leq 0.95$  PP (Bayesian Posterior Probability) as moderate support and  $\geq 95$  or  $\geq 0.95$  as strong support. The generated files (individual and combined matrices, plus individual and combined ML and Bayesian trees) are available as Supplementary Files.

### 3. Results

A summary with total sequences, length, missing data and evaluation of variable characters for each dataset, as well for the combined dataset, is presented in Table 2. All species from our ingroup were recovered within a monophyletic *Phyllanthus* s.str. for both datasets, with high support on BI trees (0.98 PP for *matK*, and 0.96 PP for ITS), but low support on ML trees (47 BS for *matK*, and 42 BS for ITS). Therefore, our focus will be on the relationships within this group, especially in those clades where phyllocladiferous species were recovered, as the combined tree has low resolution on its backbone (see ahead).

Regarding phylogenetic relationships, while the backbone of *Phyllanthus* s.str. is completely unresolved for the *matK* (Fig. 2), as well as the relationships within the clades recovered for that dataset, the ITS tree shows more resolved clades, also with relatively better support values in general. The phyllocladiferous species are recovered in distinct clades in the *matK* tree with variable support on the ML tree (57-99 BS) but are all highly supported (1 PP) on the BI tree, making both *P.* sects. *Xylophylla* and *Choretropsis* paraphyletic. On the other hand, although the ITS tree (Fig. 3) recovered phyllocladiferous species in distinct clades as well as in the *matK*, species from *P.* sect. *Xylophylla* are recovered as monophyletic in this dataset, instead of two in the *matK* tree. Also, the clades are recovered with mostly high support values (1 PP/100 BS) on both analyzes of the ITS dataset.

Both individual datasets recovered the species of *Phyllanthus* sect. *Choretropsis* in four distinct clades. The first is composed by *P. chapadensis* (sampled only on the ITS dataset), *P. choretroides*, *P. klotzchianus*, *P. sarothamnoides* and *P. spartioides*. These species are recovered either as moderately supported (0.75 PP/85 BS) sister to *P. dawsonii* (*P.* sect. *Antipodanthus*) in the ITS tree, forming a clade with high support (1 PP/100 BS), or as a polytomy in the *matK* tree, although the close relationship among all samples included in this polytomy are highly supported (1 PP/99 BS). A second clade, composed by *P. dracenooides*, *P. gladius* and *P. pedicellatus*, is recovered with high support (1PP/100BS) on the ITS tree, and as sister to *P. symphoricarpoides* (*P.* sect. *Pytorocladus*). On the other hand, the *matK* tree recovered all these species as a polytomy with high (1 PP) or low (57 BS) support.

*Phyllanthus flagelliformis* is recovered isolated from the other species of *P. sect. Choretropsis* in both datasets, and most closely related to the samples of *P. clausenii* (*P. sect. Phyllanthus* subsect. *Clausseniani*), either as a sister to these samples (ITS) or among them (*matK*). Lastly, *P. angustissimus* was sampled only for the ITS dataset, in which it was recovered as sister to *P. caparoensis* (*P. sect. Phyllanthus* subsect. *Clausseniani*).

Relationships within each of the clades containing species of *Phyllanthus* sect. *Choretropsis* are inconclusive on the *matK* tree due to polytomies, while species represented by two samples on the ITS tree are recovered together, with the exception of *P. klotzschianus* due to unresolved relationships.

Given the high number of polytomies found in the *matK*, many of them with low to moderate support on both ML and BI trees, we decided to combine the datasets for a total evidence tree (Fig. 4). In this tree, the monophyly of *P. sect. Xylophylla* is strongly supported (0.98 PP / 100 BS), while the paraphyly of *P. sect. Choretropsis* is also indicated, with the species of this section recovered in four distinct clades, largely following what was described for the ITS tree. It is noteworthy, however, that the backbone of this tree is poorly resolved.

## 4. Discussion

Our results corroborate what was proposed by Webster (1958b) based on morphological and distribution data, and confirmed with molecular data by Kathriarachchi et al., 2006; Falcón et al., 2020, Bouman et al., 2021 and Bouman et al. (2022), regarding the distinction of the species bearing phylloclades in two main lineages: *Phyllanthus* sect. *Xylophylla* in the Caribbean, and *P. sect. Choretropsis* in eastern Brazil. Also, this distinction between these two lineages is supported by the organization of phylloclades: bipinnate in the Caribbean species versus pinnate in the Brazilian species, although in rare cases we observed specimens of *P. klotzschianus* with bipinnate phylloclades.

Regarding sect. *Choretropsis*, with our wider sampling we found that although most samples of the group are concentrated in a clade sister to *P. dawsonii* (sect. *Antipodanthus*), the section is paraphyletic (Fig. 4) in their current circumscription. Therefore, phylloclades would have evolved in at least four lineages among the Brazilian species. In front of this, relationships among these clades need further evaluation, with a wider sampling of the other sections, to determine changes to the delimitation of infrageneric groups in *Phyllanthus* s.str.

Two species (*P. angustissimus* and *P. flagelliformis*), sampled for the first time, are surprisingly most closely related to species of sect. *Phyllanthus* subsect. *Clausseniani*, but in two distinct clades, and both species apparently share no morphological similarities with species from the subsection to which they are sisters. Santiago et al. (2008) evaluated the anatomy of the phylloclades in *P. sect. Choretropsis* and found that *P. flagelliformis* is unique by having a combination of an open ring of vascular bundles following phylloclade shape and isodiametric photosynthetic cells. This same vascular bundle arrangement is also present in *P. edmundoi*, but we could not sample it in our phylogeny. *Phyllanthus flagelliformis* is also noteworthy among sect. *Choretropsis* for its more developed leaves when in

comparison with other species of the section. On the other hand, no anatomical feature of interest is indicated for *P. angustissimus*, and we could not sample it in the *matK* dataset. In the absence of anatomical data for the species of sect. *Phyllanthus* subsect. *Clausseniani* to which *P. angustissimus* and *P. flagelliformis* are sisters, no further suggestions may be addressed.

Our phylogenetic hypothesis does not support the division in subsections within sect. *Choretropsis* as proposed by Santiago et al. (2006). Such division is based mainly in the shape of the phylloclades in transversal section (terete or subterete in subsect. *Choretropsis* and flattened in subsect. *Applanata*) is not sustained since, although all species with terete/subterete phylloclades (*P. chapadensis*, *P. choretroides*, *P. sarothamnoides* and *P. spartioides*) were recovered within a same clade, which also contains species with flattened phylloclades, not forming any grouping within it.

*Phyllanthus dracaenoides*, *P. gladius* and *P. pedicellatus* are noteworthy species with dracenoid habit, i.e. presenting a monopodial main stem, in addition to lanceolate and flattened phylloclades concentrated at the apex. These species also share a common habitat (humid dense forests along the Atlantic Forest domain), in which other species of sect. *Choretropsis* are usually not found (only *P. flagelliformis* and *P. klotzschianus* in seashore open vegetation as exceptions). Among these species, only *P. gladius* were analyzed by Santiago et al. (2006), but they indicated a distinct arrangement of the vascular tissue in the phylloclades of this species, with the presence of a central cylinder plus lateral bundles distinguishing it from the other representatives of sect. *Choretropsis*, who have a single, open or closed ring of vascular bundles. In addition to these features, they were recovered in a clade with high support in our phylogeny (1 PP/97 BS, Figure 4), allowing its segregation as a new section of *Phyllanthus* described here – *Phyllanthus* sect. *Gladius* (see formal taxonomy below). Our analyzes indicate *P. symphoricarpoides*, a species of sect. *Pityrocladus* from high elevations in Colombia and Peru, as sister to sect. *Gladius*. This species, however, does not share any apparent morphological feature with species of sect. *Gladius*, demanding further exploration.

As the remaining species of *Phyllanthus* sect. *Choretropsis* were recovered amongst species from *Phyllanthus* sects. *Antipodanthus*, *Choretropsis*, *Loxopodium* and *Phyllanthus* in a poorly resolved topology, we recommend that future works should focus on the reorganization of the taxonomy of these groups. These studies would benefit from a wider sampling of species in phylogenies, as well as investigation of micromorphological features. Such efforts could help elucidate relationships among these groups, which could not be better resolved with the more consistent dataset of Bouman et al. (2022) composed by five molecular markers. Improvements on the taxonomy of these groups that help their delimitation would also be crucial, as all these sections were recognized in the phylogenetic classification of tribe Phyllantheae by Bouman et al. (2022) in spite of their clear paraphyletic nature in previous works.

### ***Nomenclatural changes***

*Phyllanthus* sect. *Gladius* P. Orlandini, I. Cordeiro & V.C. Souza, sect. nov.

*Phyllanthus* sect. *Choretropsis* Müll.Arg. (1863: 4, 52). Santiago et al. (2006: 138), *pro parte*, excluding the type.

Type: *Phyllanthus gladius* Müll.Arg.

*Phyllanthus* sect. *Gladius* differs from the *Phyllanthus* sect. *Choretropsis* for having plants with a monopodial main stem (palm like plants), phylloclades always lanceolate to gladiate, and concentrated at the apex of the main stem (versus plants with sympodial growth without an elongated axis, phylloclades never lanceolate or gladiate, and not concentrated at the apex of the main stem).

Included species (3) - *P. dracaenoides* P. Orlandini & I. Cordeiro; *P. gladius* Müll.Arg; *P. pedicellatus* P. Orlandini, I. Cordeiro & V.C. Souza.

## 5. Conclusions

The species of *Phyllanthus* s.str. bearing phylloclades, circumscribed to two sections – *P.* sect. *Xylophylla* and *P.* sect. *Choretropsis*, emerged as five distinct lineages in our work. Consequently, the phylloclades evolved more times than previously thought. Although the distinction of the Caribbean (sect. *Xylophylla*) and the Brazilian phyllocladiferous species (sect. *Choretropsis*) is clear in our phylogeny and widely supported by morphological features, sect. *Choretropsis*, as currently delimited, is not monophyletic, and further studies involving sects. *Antipodanthus* and *Phyllanthus* subsect. *Claussenianii* are needed for understanding the limits of these groups, as all of them were recovered as paraphyletic in our work, as well as in previous ones. Exploration of micromorphological characters is valuable for this purpose.

As *Phyllanthus gladius* and two other recently described species (*P. dracaenoides* and *P. pedicellatus*) with dracenoid habit clearly do not belong to sect. *Choretropsis*, we described a new section (sect. *Gladius*) that contains these three species that are confined to humid dense forest along the Atlantic Forest.

## Declarations

## Acknowledgements

We would like to thank the Universidade Estadual de Campinas, in the figure of Professor Dr. Samantha Koehler, for the infrastructure of the Molecular Biology Laboratory, used for the extractions and amplifications performed in this study. This study was financed in part by the Coordination for the Improvement of Higher Education Personnel – Brazil (CAPES) – Finance Code 001, and the National Council for Scientific and Technological Development [processes 141913/2020-6 and 442162/2020-0]. PO also thanks The Mohamed bin Zayed Species Conservation Fund [grant 210528056] and IDEA WILD for support [grant number ORLABRAZ0121].



# References

1. Baillon, M.H. 1858. Étude Générale du Groupe des Euphorbiacée. Victor Masson, Paris, 684p.
2. Bouman, R.W., Keßler, P.J.A., Telford, I.R.H., Bruhl, J.J., Strijk, J.S., Souders, R.M.K., Welzen, P.C. van. 2021. Molecular phylogenetics of *Phyllanthus* sensu lato (Phyllanthaceae): Towards coherent monophyletic taxa. *Taxon* 70(1), 72-98.
3. Bouman, R.W., Keßler, P.J.A., Telford, I.R.H., Bruhl, J.J., Strijk, J.S., Souders, R.M.K., Esser, H.J., Hidalgo, B.F., and Welzen, P.C. van. 2022. A revised phylogenetic classification of tribe Phyllantheae (Phyllanthaceae). *Phytotaxa*, 540: 1-100.
4. Chase, M.W., Hills, H.H. 1991. Silica-gel: an ideal material for field preservation of leaf samples for DNA. *Taxon* 40, 215-220.
5. Dress, A.W.M., Flamm, C., Fritzschn, G., Grünewald, S., Kruspe, M., Prohaska, S.J., and Stadler, D.F. 2008. Noisy: identification of problematic columns in multiple sequence alignments. *Algorithms for Molecular Biology* 3: 7.
6. Ewing, B., Green, P. 1998. Base-calling of automated sequencer traces using phred II. Error probabilities. *Genome Res.* 8, 186-194.
7. Ewing, B., Hiller, L., Wendl, M.C., Green, P. 1998. Base-calling of automated sequencer traces using phred I. Accuracy assessment. *Genome Res.* 8, 175-185.
8. Hidalgo, B.F., Bazán, S.F. 2020. Phylogenetic relationships and character evolution in Neotropical *Phyllanthus* (Phyllanthaceae), with a focus on the Cuban and Caribbean taxa. *International Journal of Plant Sciences*, 181 (3), 284-305.
9. Gordon, D., Abajian, C., Green, P. 1998. Consed: a graphical tool for sequence finishing. *Genome Res.* 8, 195-202.
10. Kathriarachchi, H., Samuel, R., Hoffmann, P., Wurdack, K.J., Chase, M.W. 2005. Molecular phylogenetics of Phyllanthaceae inferred from five genes (plastid *atpB*, *matK*, *3'ndhF*, *rbcl*, and nuclear *PHYC*). *Molecular Phylogenetics and Evolution*, 36: 112-134.
11. Kathriarachchi, H., Samuel, R., Hoffmann, P., Mlinarec, J., Wurdack, K.J., Ralimanana, H., Stuessy, T.F., Chase, M.W. 2006. Phylogenetics of tribe Phyllantheae (Phyllanthaceae; Euphorbiaceae sensu lato) based on nrITS and plastid *matK* DNA sequence data. *American Journal of Botany* 93 (4), 637-655.
12. Katoh, K., Rosewick, J., Yamada, K.D. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief. Bioinform.* 19,20(4): 1160-1166.
13. Kuraku, S., Zmasek, C.M., Nishimura, O., Katoh, K. 2013. aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. *Nucleic Acids Res.* 41. DOI 10.1093/nar/gkt389.
14. Linnaeus, C. 1771. *Mantissa Plantarum*. Holmiae (Estocolmo): Impensis Laurentii Salvii.
15. Müller Argoviensis, J. 1863. Euphorbiaceae. *Vouläufige Mittheifungen aus dem für De Candolle's Prodrömus bestimanten Manuscript über dieses Familiae*. *Linnaea*, 32, 1-786.

16. Müller Argoviensis, J. Euphorbiaceae. In: *Prodromus Systematics Universalis Regni Vegetabilis* 15(2), ed. A.L.P.P. de Candolle. Paris: Victor Masson. pp. 1–1286, 1866.
17. Naciri, Y. & Linder, H. 2015. Species delimitation and relationships: the dance of the seven veils. *Taxon*: 3-16.
18. Orlandini, P., Cordeiro, I., Campos-Rocha, A., Souza, V.C. 2020. A new species of *Phyllanthus* L. (Phyllanthaceae) with phylloclades from Minas Gerais, Brazil. *Phytotaxa* 455 (2), 167-172.
19. Orlandini, P.; Cordeiro, I.; Mendes, J.C.R.; Campos-Rocha, A. & Souza, V.C. A new species of phyllocladiferous *Phyllanthus* (Phyllanthaceae) from the Atlantic Rain Forest. *Systematic Botany*, 46(4): 1011-1015, 2021.
20. Orlandini, P.; Cordeiro, I.; Silva, M.J. & Souza, V.C. *Phyllanthus chapadensis*, a new phyllocladiferous *Phyllanthus* (Phyllanthaceae) from the Chapada dos Veadeiros, Goiás, Brazil. *Phytotaxa*, 532 (2): 169-175, 2022.
21. Persoon, C.H. 1807. Pars Secunda. *Synopsis plantarum*. Parisiis Lutetiorum, Apud Bibliopolas Treuttel et Würtz et Tubingæ, Apud J.G. Cottam.
22. Rambaut, A. 2010. FigTree. Version 1.4. <http://tree.bio.ed.ac.uk/software/figtree/> (Accessed on 25 August 2021).
23. Rambaut, A., Drummond, A.J. 2007. Tracer. Version 1.4. <http://tree.bio.ed.ac.uk/software/tracer/> (Accessed on 25 August 2021).
24. Ronquist, F., Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinform* 17, 1572-1574.
25. Ronquist, F., Teslenko, M., Van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61, 539-542.
26. Samuel, R., Kathriarachchi, H., Hoffmann, P., Barfuss, M.H., Wurdack, K.J., Davis, C.C., Chase, M.W. 2005. Molecular Phylogenetics of Phyllanthaceae: evidence from plastid *matK* and nuclear *PHYC* sequences. *American Journal of Botany* 92(1), 132-141.
27. Santiago, L.J.M., Louro, R.P. & Emmerich, M. 2008. Phylloclade anatomy in *Phyllanthus* section *Choretropsis* (Phyllanthaceae). *Botanical Journal of the Linnean Society*, 157: 81-102.
28. Santiago, L.J.M., Louro, R.P. & Emmerich, M. 2006. *Phyllanthus* section *Choretropsis* (Euphorbiaceae) in Brazil. *Botanical Journal of the Linnean Society*, 150, 131-164.
29. Simmons, M.P., 2004. Independence of alignment and tree search. *Mol. Phyl. Evol.* 31, 874–879.
30. Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetics analysis and post-analysis of large phylogenies. *Bioinform* 30, 1312-1313.
31. Sun, Y., Skinner, G., Liang, H., Hulbert, H. 1994. Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacer of nuclear ribosomal DNA. *Theoretical and Applied Genetics* 89, 26-32.

32. Tel-Zur, N., Abbo, S., Myslabodoski, D., Mizrahi, Y. 1999. Modified CTAB procedure for DNA isolation from epiphytic cacti of genera *Hylocereus* and *Selenicereus* (Cactaceae). *Plant Molecular Biology Reporter* 17, 249-254.
33. Webster, G.L. 1956. Studies on the Euphorbiaceae, Phyllanthoideae II. The American species of *Phyllanthus* described by Linnaeus. *Journal of The Arnold Arboretum*, 37 (1), 1-14.
34. Webster, G.L. 1958a. A monographic study of the West Indian species of *Phyllanthus*. *Journal of The Arnold Arboretum*, 39, 49-100.
35. Webster, G.L. 1958b. A monographic study of the West Indian species of *Phyllanthus*. *Journal of The Arnold Arboretum*, 39, 111-212.
36. Wurdack, K.J., Hoffmann, P., Samuel, R., de Bruijn, A., van der Bank, M. and Chase, M.W. 2004. Molecular phylogenetic analysis of Phyllanthaceae (Phyllanthoideae pro parte, Euphorbiaceae sensu lato) using plastid *rbcL* DNA sequences. *American Journal of Botany* 91: 1882–1900.

## Tables

Table 1. Detailed description of the volumes and amplification cycles for each region

Primer	Foward or reverse primer	Sequence (5'-3')	Annealing temperature (°C)	Volume (μ)	Source
<b>tmK 570F</b>	Foward	TCCAAAATCAAAGAGCGATTGG	48	0,75	Samuel et al. (2005)
<b>530R</b>	Reverse	GTTCCAATTCCAATACTCGTGAAG	48	0,75	Samuel et al. (2005)
<b>17SE</b>	Foward	CGAATTCATGGTCCGGTGAAGTGTTTC	58	0,75	Sun et al. (1994)
<b>26SE</b>	Reverse	TAGAATTCCCCGGTTCGCTCGCCGTTAC	58	0,75	Sun et al. (1994)

Table 2. Summary of individual marker datasets used in the analyses.

	ITS	<i>matK</i>	Combined dataset
<b>Number of samples</b>	101	99	105
<b>Aligned length (bp)</b>	708	769	1477
<b>Variable characters (%)</b>	89 (12,57)	120 (15,60)	209 (14,15)
<b>Parsimony-informative characters (%)</b>	342 (48,30)	165 (21,45)	507 (34,32)
<b>Missing data (%)</b>	1,61	1,83	6,49

## Figures



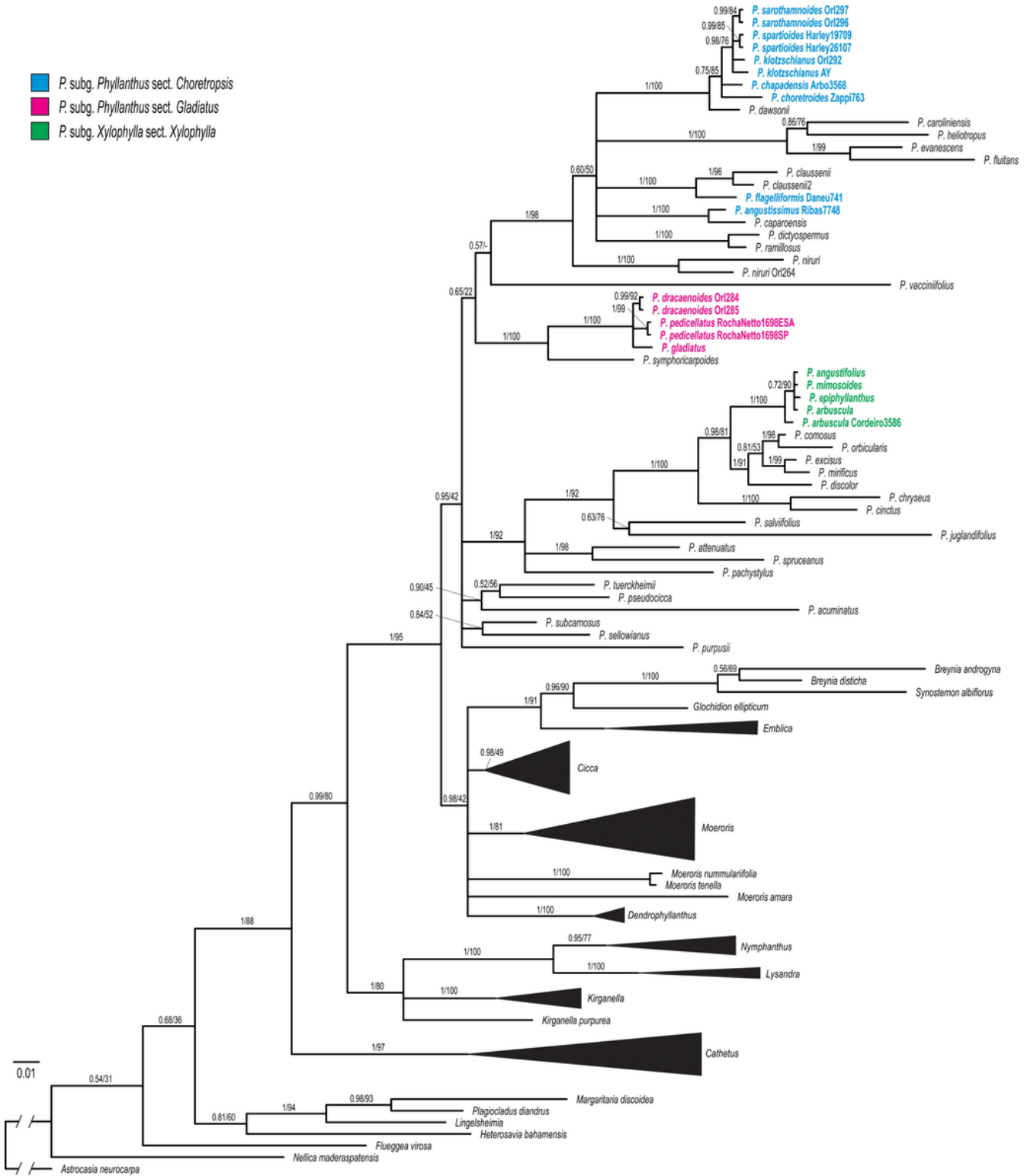
**Figure 1**

Morphological characters featured in this study. A. *Phyllanthus tenellus*. Herb with phyllanthoid branching. B. *Phyllanthus dawsonii*. Shrub with non-phyllanthoid branching. C. *Phyllanthus dracaenoides*. Dracenoïd herb with phyllanthoid ramification and phylloclades. D. *Phyllanthus klotzschianus*. Shrub with phyllanthoid ramification and phylloclades. E. *Phyllanthus gladiatus*. Dracenoïd herb with phyllanthoid ramification and phylloclades.



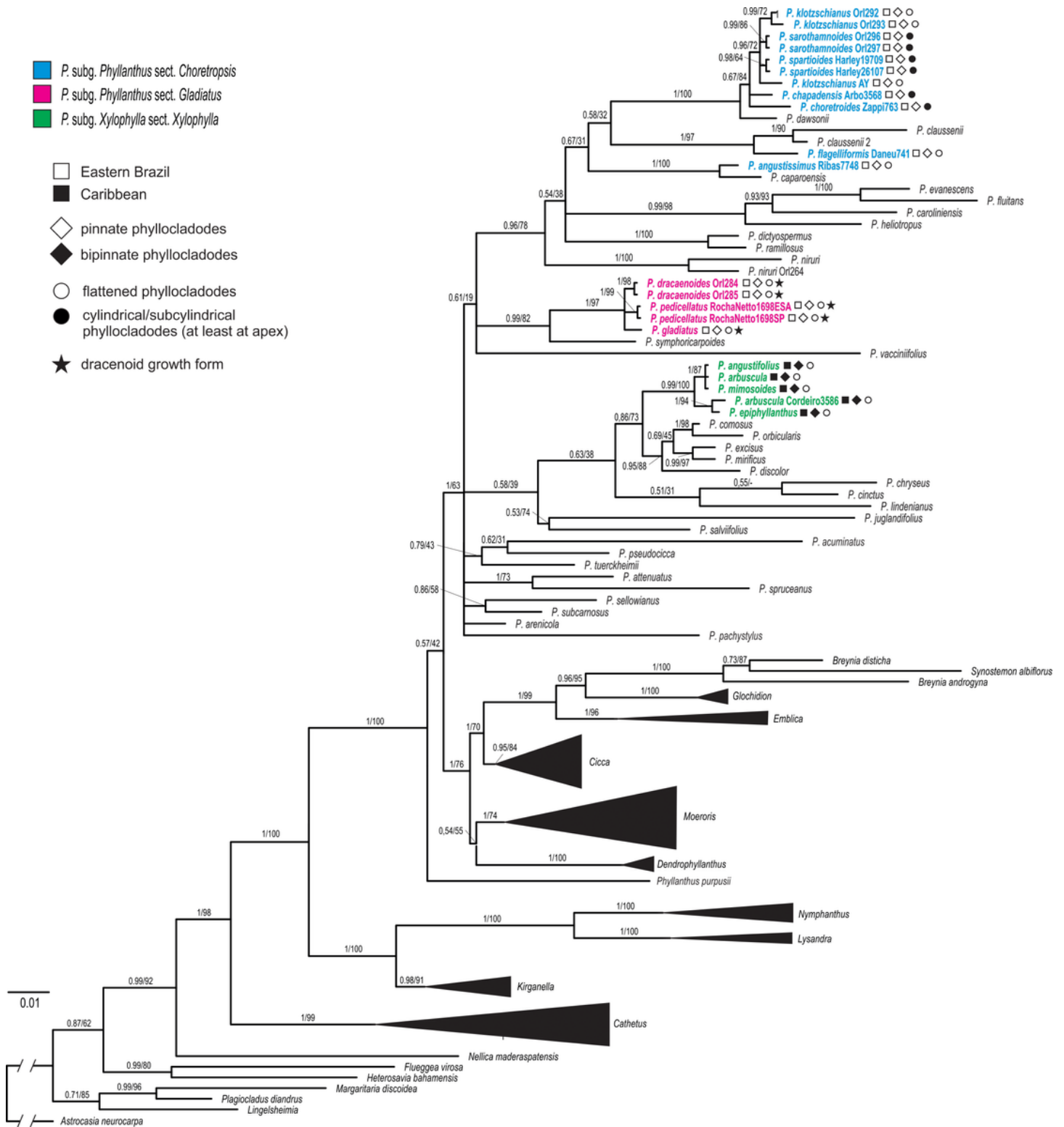
**Figure 2**

Majority rule (50%) consensus tree obtained from Bayesian analysis of *matK* dataset. Values above branches represent Bayesian posterior probabilities / maximum likelihood bootstrap percentages. Samples of other genera from tribe Phyllantheae are shown as collapsed clades. Species with phylloclades and their respective sections within *Phyllanthus* s.str. are coloured following the legend on the upper left corner.



**Figure 3**

Majority rule (50%) consensus tree obtained from Bayesian analysis of ITS dataset. Values above branches represent Bayesian posterior probabilities / maximum likelihood bootstrap percentages. Samples of other genera from tribe Phyllanthae are show as collapsed clades. Species with phylloclades and their respective sections within *Phyllanthus* s.str. are coloured according to the legend on the upper left corner.



**Figure 4**

Majority rule (50%) consensus tree obtained from Bayesian analysis of *matK* dataset. Values above branches represent Bayesian posterior probabilities / maximum likelihood bootstrap percentages. Samples of other genera from tribe Phyllanthaeae are show as collapsed clades. Species with phylloclades, their respective sections within *Phyllanthus* s.str., and morphological features are indicated following the legend on the upper left corner.



## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [AppendixA.Supplementarymaterial.docx](#)