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Phylogeny of *Virola* (Myristicaceae) and a reassessment of its taxonomy

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Abstract

Evolutionary relationships within the genus *Virola* (Myristicaceae) were explored with a set of 36 species, 14 of which were represented by a type specimen and 20 by more than one specimen in the phylogeny. Among these 20 species, 12 were resolved as monophyletic, with strong support. The status of species that are treated both as accepted names and as synonyms in the taxonomic literature was evaluated under the light of the new evidence provided by the molecular data. Furthermore, the placing of the monotypic genus *Bicuiba* in synonymy under *Virola*, as the older *V. bicuhyba*, was supported in this study by molecular and by morphological evidence. Previous classifications of the genus based on morphological characters showed a lack of congruency with the present phylogeny. A preliminary analysis of the geographical distribution of the members of the clades suggested that *Virola* might have originated in lowland Amazonia and repeatedly colonized other biomes, a hypothesis to be tested in the future. Finally, based on the results of our phylogeny combined with the taxonomic literature, we presented a curated list of accepted species to be used by the scientific community.

Key words: Amazonia, *Bicuiba*, *Virola bicuhyba*, plural inflorescences

Introduction

Historically, hypotheses on species delimitation and on the evolution of a group were mainly based on morphological, biogeographical and ecological data. The use of molecular data to test, or even to formulate these hypotheses, has advanced taxonomy substantially. After almost three hundred years of taxonomy and a few hundred thousand of accepted species names, testing species delimitation is an ongoing task. Most of the genera in the tree of life lack a modern revision using phylogeny as an instrument for taxonomic decisions, as well as for further understanding of evolution at the species level. The genus *Virola* Aubl., in Myristicaceae, is one of these cases (all names of *Virola* and authors are given in TABLE 1). Myristicaceae is a family of woody taxa distributed across the tropics. According to the WFO (2025), 537 species are currently accepted in 21 genera, with a debatable number of six genera occurring in the Neotropical region: *Bicuiba, Compsoneura*, *Iryanthera*, *Osteophloeum*, *Otoba* and *Virola*. The monotypic *Bicuiba* is one of the many examples in the Neotropical Myristicaceae taxa that raise conflict in the literature about its status.

Virola was the first genus described for the Neotropics, based on the appearance of the widespread Amazonian species *Virola sebifera* in "*Histoire des Plantes de la Guiane Françoise"* (Aublet, 1775). Still, for more than a century, practically all new Neotropical species were described under the genus *Myristica*. In the first attempt to recognize groups of related species, de Candolle (1856a) recognized 13 sections under *Myristica*, two of which, *Virola* and *Synchoneura*, included all taxa described for the Neotropics. Only after the first major taxonomic treatment of Myristicaceae by Warburg (1897), other genera were described and *Virola* Aublet became widely accepted. The genus, containing 27 species at that point, was divided in two sections, based on one unique androecium feature: *Oxyanthera* Warb. with 13 species; anthers longer than the column, and *Amblyanthera* Warb. with 14 species; anthers equal to or shorter than

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the column. In the 20th century, the increased number of species described and respective morphological variation led to the recognition of more groups of species, free of taxonomic ranking but based on a higher number of characters. Smith and Wodehouse (1937) recognized six groups among the 38 species studied, taking into account trichome type, degree of perianth connation, spacing of secondary leaf veins, column shape and anther apex shape. Roughly around the same time, Ducke (1936) published eight new species and highlighted fruit characteristics and venation, among other features, to facilitate species identification. Later on, taxonomic treatments of the genus that substantially contributed to the present knowledge include a revision of the species occurring in Brazil (W. A. Rodrigues, 1980), Flora of Ecuador (Jaramillo *et al.*, 2004) and the most recent and thorough revision of the Central American species by Santamaría-Aguilar *et al.* (2019). Finally, many new species were described for Central America and Northeastern South America (Jaramillo *et al.*, 2000; Santamaría-Aguilar *et al.*, 2019; Santamaría-Aguilar & Lagomarsino, 2022).

Among the Neotropical genera, *Virola* is the most species rich, with 71 accepted species, as well as the most widespread, occurring from Mexico to the Atlantic forest of Southeastern Brazil. The Amazonian lowlands concentrate the highest number of species, which also grow in different vegetation types.

Virola, as much as most Myristicaceae, shows rhythmic orthotropic growth and rhythmic plagiotropic branching, the presence of reddish exudate, leaves that are alternate and simple, with entire margins, a three lobed perianth and a dehiscent, unilocular fruit opening in two valves, with a single arillate seed with distinctly ruminate endosperm. Due to the relative uniformity of the flowers and fruits within the family, it is the combination of features that separates *Virola* from other Neotropical genera, with the easiest to observe being: the presence of stellate hairs, the inflorescence architecture and the fruit shape—either globose or longitudinally elliptical. Further, within *Virola*, morphological species delimitation is primarily based on leaf venation, indument patterns and on the ratio of anther/filament length, combined with other features.

The relatively low morphological variation has led to inconsistencies in species delimitation and a gap in the formulation of an evolutionary hypothesis. Even with the use of molecular data, the evolutionary relationships in Myristicaceae have seemed difficult to tackle until very recently. In a seminal work on the evolution of Magnoliales using molecular data, Sauquet *et al.* (2003) included 16 of the 20 genera, but found many polytomies, and, when some resolution was found, it had weak support. Furthermore, the authors highlighted that the low molecular variation of Myristicaceae, compared to the sister clade, could be interpreted as either a much younger age or an extreme slowdown in molecular evolution. At genus level, chloroplast and nuclear markers were used to elucidate the taxonomy of *Compsoneura* and *Virola* (Newmaster *et al.*, 2008; 2011). Steeves (2011) found two large subclades, informally called the "Multinervae" and "Sebiferae". Morphologically, the Multinervae group was composed by emergent canopy trees with numerous secondary leaf veins, relatively thicker pericaps and arils that are laciniate throughout their length, while Sebiferae included sub-canopy to canopy species that have fewer secondary veins, relatively thinner pericarps and arils that are only laciniate for half of their length. A more complete sampling of the genus remained, however, desirable. Here, using a new phylogeny, based on the nuclear ribosomal DNA, we unveil some of the phylogenetic relationships among *Virola* species to better inform decisions on the taxonomy of the genus and on the monophyly of species. By overlapping previous classifications as well as the geographical distribution of species on the phylogeny we provide insights for the formulation and future testing of evolutionary hypotheses. Finally, we present a curated list of accepted species based on our findings and on the taxonomic literature.

Methods

Collections of Neotropical Myristicaceae deposited in the National Herbarium of the Netherlands (U, L and WAG), and in other herbaria—A, BR, BBS, INPA, G, K, MO and P—were studied as part of ongoing taxonomic revisions of Myristicaceae for Flora do Brasil and for the Flora of the Guianas. Along with the morphological study, herbarium material samples were taken for DNA sequencing using genome skimming techniques. The low coverage of the skimming led us to recover only Nuclear Ribosomal DNA (NRb) and the plastome. All NRb was assembled based on denovo methodology, using the program 'GetOrganelle' (Jin *et al.*, 2020). We did not use plastome data here as the phylogenetic trees showed little resolution, and recent work has shown that plastomes may not always support species delimitation very well, as they may be shared among species in close proximity (Acosta & Premoli, 2010; Stegemann *et al.*, 2012; ter Steege *et al.*, 2023).

Nuclear Ribosomal DNA (NRb)

Leaf samples were taken from 86 specimens (19 corresponding to type specimen), including herbarium material and leaves that had been collected in silica in the field (DNA voucher specimen data in TABLE S1). We also added material from *Osteophleum platyspermum* and *Myristica fragrans* as outgroups. DNA was extracted using a modified CTAB protocol (Doyle & Doyle, 1987). DNA concentration was determined using the Dropsense spectrophotometer (Trinean NV, Gentbrugge, Belgium), and the quality of the DNA was checked using the QIAxcel system and DNA Screening kit (QIAGEN, Valencia, CA, USA). DNA was sonicated using the Covaris M220 (Covaris, Woburn, MA, USA) and microTUBE-59 AFA Fiber Screw-Cap according to the manufacturer's program for an insert size of 350 bp, followed by a Dual index library prep with the DNA NEBNext Ultra II Library Prep Kit, using a quarter of the recommended volumes with the Dual Index Primers Set, NEBNext Multiplex Oligos for Illumina (New England Biolabs). The libraries were checked for concentration with the QIAxcel and pooled equimolarly using the QIAgility instrument. Using a High Sensitivity chip, we did a final quantity and quality check with the Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Paired-end sequence reads of 150 bp were generated using the Illumina HiSeq 4000 and later the Illumina NovaSeq 6000 system (Illumina, San Diego, CA, USA).

We used GetOrganelle (Jin *et al.*, 2020) for de novo assembly of the nuclear ribosomal (NRb) DNA (5800–5900 bp: 26S-ITS1-5.8S-ITS2-18S), plus an offset of 300 bp before the 26S region that included the ETS region. Assembled contigs of the different specimens were imported to Geneious Prime (version 2021.2.2, Dotmatics, Bishop's Stortford, UK). When de novo assembly failed to produce a contig for a given specimen, we used the function Map to Reference in Geneious Prime, using one of the successfully de novo assembled specimens as reference. In total, we obtained 81 high-quality NRb sequences.

Sequences were aligned with MAFFT (Katoh & Standley, 2013), using the plug-in of Geneious Prime. We inferred phylogenetic trees using Bayesian inference (BI) (Huelsenbeck & Ronquist, 2001), as implemented in Geneious Prime, with standard settings, a chain length of 1,100,000, and burn-in of 110,000, and a subsampling frequency of 200. We used the GTR substitution model and rate variation as invgamma, as suggested by Abadi *et al.* (2019). We assessed convergence by analyzing the effective sample size (ESS) after the burn-in for all parameters.

We used the R package PlantR (de Lima *et al.*, 2023) to extract all Virola specimen occurrences from GBIF, SpeciesLink and JABOT databases (~34,000) and selected only those with proper coordinates, and medium to high identification, as flagged by PlantR and revised by the authors. We produced distribution maps for the species in our phylogeny based on these records (5,503 collections).

Results and discussion

Tree topology

We obtained sequences for a total of 81 specimens, 14 of which are from types, pertaining to 36 species. The whole nuclear ribosomal DNA phylogeny was based on a matrix of alignment length of 6363 base pairs (bp, minimum sequence length 6090bp, maximum sequence length 6395bp), with 87.2% identical and 8.08% parsimony-informative sites. On average, specimens assigned to the same species showed a difference of 41 bp, while the difference found for specimens assigned to different species was 245 bp (FIGURE S1). The result of the Bayesian Inference (BI) was already very stable before the end of the burn-in and remained so for the entire run, resulting in a very low deviation among the solutions. All ESS values were above 250 after the burn-in, suggesting good convergence among the two runs in the BI.

The tree obtained showed two distinct clades A and B. Within clade B, *Bicuiba oleifera* assumed a basal position (B1) sister to the polytomy formed by three branches containing the rest of clade B (B2) (FIGURE 1).

Clade A is essentially Amazonian, but it also includes *Virola sebifera*, the species with the largest distribution range, from Central America to Central Brazil. It presents two well supported clades, one predominantly formed by *V. calophylla* and *V. macrocarpa,* with two type specimens (A1), and another clade with polytomies at different levels, and with a topology that hampers a clear interpretation (A2). The mix of *Virola* species in A2, which even includes the type specimen of *V. divergens*, might indicate either the synonymy of this species, as suggested in the literature, and/or the failure of NRb to resolve the relationships, as found in other branches of the tree. The species with the highest number of specimens in the polytomy is *V. elongata*, which is widespread and extremely variable in terms of morphology and requires further work.

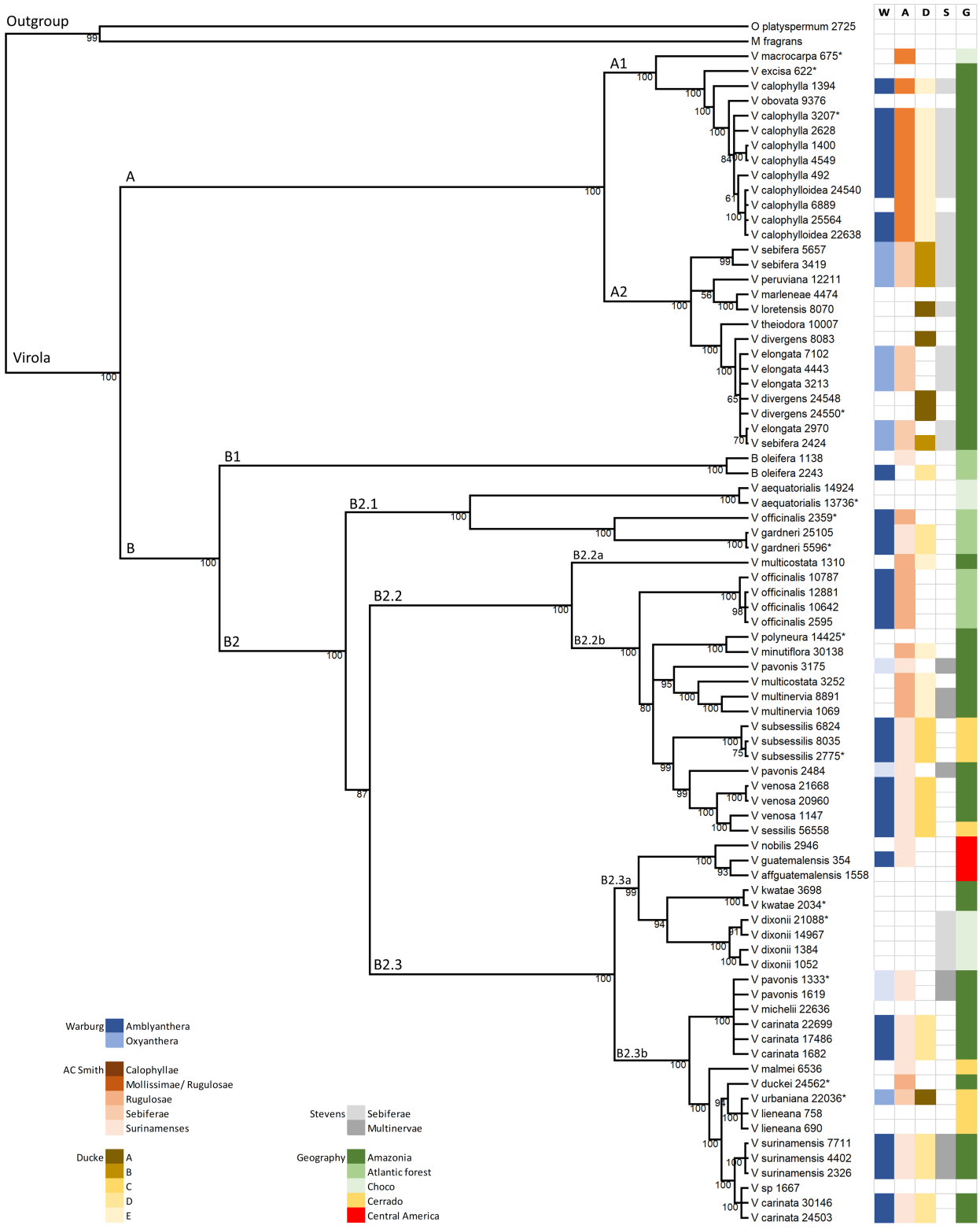


FIGURE 1. Phylogenetic tree of *Virola* spp, based on Bayesian inference of the full nuclear ribosomal DNA (alignment of 6,363 bp). Posterior probabilities in black. Color bars on the right indicate classifications by: Warburg (W); A.C.Smith (A); (D); Steevens (S); and geography the biome in which the species is mostly found (G). Color legend bottom left. The * indicate a type specimen; numbers are collector numbers.

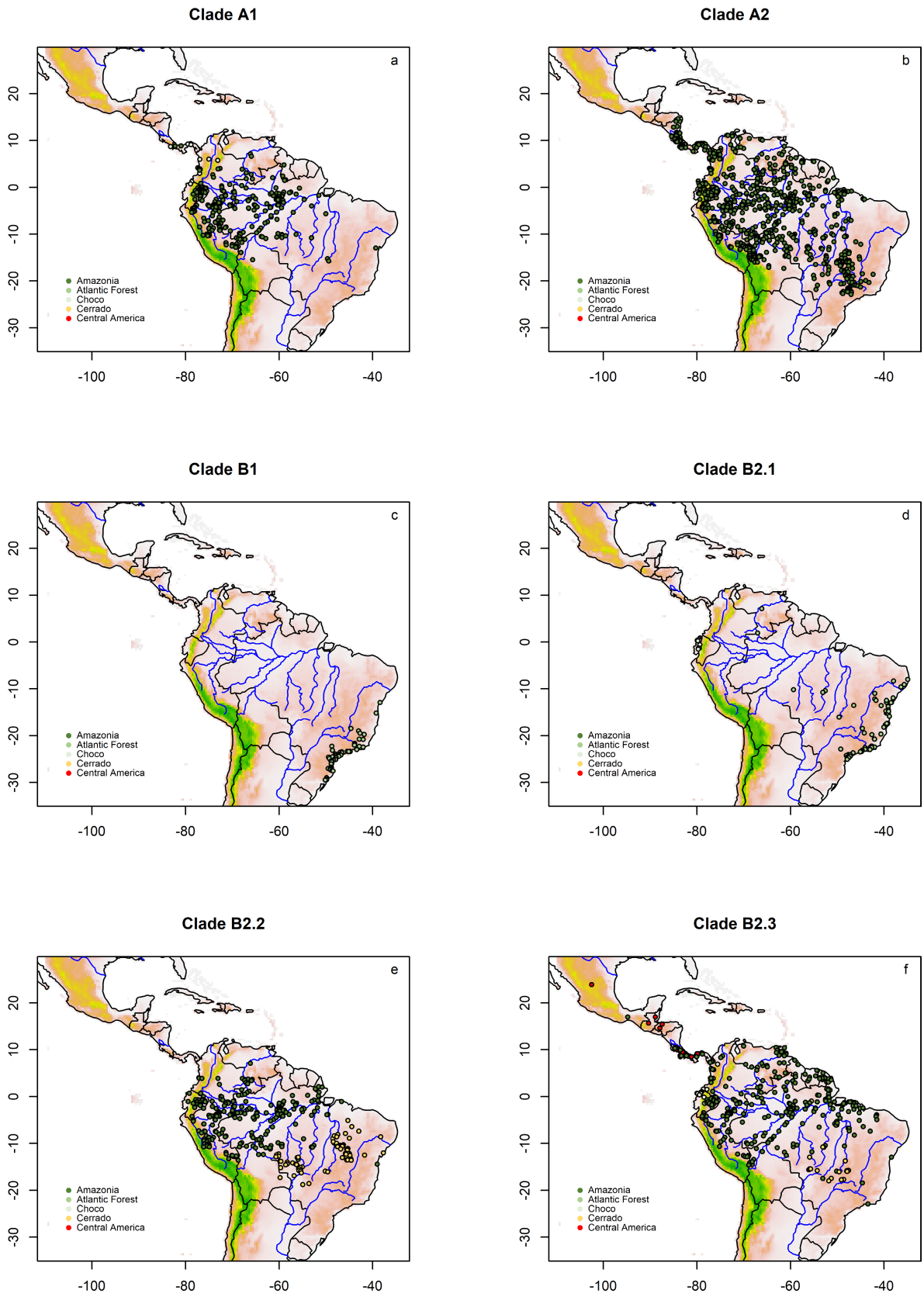


FIGURE. 2. Maps of *Virola* clades. **a**) Clade A1, *V. calophylla, V. macrocarpa, V. obovata*; **b**) Clade A2, *V. divergens, V. elongata, V. loretensis, V. peruviana, V. sebifera, V. theiodora*; **c**) Clade B1, V. bicuhyba; **d**) Clade B2.1, *V. aequatorialis, V. gardneri, V. officinalis*; **e**) Clade B2.*2, V. minutiflora, V. multicostata, V. multinervia, V. pavonis, V. polyneura, V. sessilis, V. subsessilis, V. venosa*; **f**) Clade B2.3*, V. carinata, V. dixonii, V. duckei, V. guatemalensis, V. kwatae, V. lieneana, V. malmei, V. michelii, V. nobilis, V. surinamensis, V. urbaniana.* Colors indicate major biome of species (see Fig 1, main text). Maps created with a custom R script. Base map source (country.shp, rivers. shp): ESRI (http://www.esri.com/data/basemaps, © Esri, DeLorme Publishing Company).

Clade B is formed by species across the complete geographical distribution of the genus—from Central America to Southeast Brazil. Clade B1 places *Bicuiba oleifera* as a separate lineage, which on one hand agrees with the hypothesis that the monotypic genus is a synonym of *Virola bicuhyba*, and on the other hand accommodates its peculiar inflorescence morphology (see discussion below). Clade B2 splits in one Chocó (B2.1a) and one Cerrado / Atlantic Forest clade (B2.1b), and in a larger clade (B2.2 + B2.3) with species from the whole geographical range of the genus (FIGURE 2). Both clades B2.2 and B2.3 present a mix of well supported monophyletic species as well as paraphyly in cases that are difficult to interpret, such as the specimens of *V. multicostata* in B2.2a and in B2.2b. Guided by this result, a reevaluation of the specimen sister to the clade of *V. multinervia* (B2.2b) showed that one of the features differentiating the two species—petiole length—would place the specimen in *V. multinervia*, while other important diagnostic features are lacking. In short, in order to distinguish between true paraphyly of the species studied and a failure of proper specimen identification, due to the morphological overlap of the specimens examined, a broader sampling is needed.

Within B2.2b, one well supported clade with four specimens of *V. officinalis* is separated from a *V. officinalis* specimen, which belongs to B2.1b and forms a clade with two specimens of *V. gardneri*, one of them the Holotype. It is important to notice that the *V. officinalis* specimen Blanchet 2359 was cited by Warburg (1897) when he published *V. officinalis*, a new combination of the basyonym *Myristica officinalis*. The basyonym *Myristica officinalis* was treated by Bentham (1853), who cited two collections: Martius 650 and Gardner 5596. The latter would become the type specimen of *Myristica gardneri* A.DC. (De Candolle, 1856b). That would make Martius 650 the type specimen for the new combination *Virola officinalis* made by Warburg (1897). But at the time that Warburg published the new combinations, he cited several collections of Blanchet (including the number 2359) and of Martius, but without referring to any of them as types. W. A. Rodrigues (1980) designated Martius 650 as lectotype, making our sampled specimen a syntype. The fact that *M. officinalis* Mart. was an illegitimate later homonym of *M. officinalis* L.f. (1781) does not seem to have any influence in this conundrum. What does matter is that Warburg might have cited specimens of Blanchet that were previously determined as *M. officinalis*, but not necessarily corresponding well with the delimitation of the new combination, since *V. gardneri* was also previously within the delimitation of *M. officinalis*. Back to our sampled specimens, although the collection Blanchet 2359 does not show the typical attenuate leaf base of *V. gardneri*, the racemose fasciculate inflorescences are closer to this species than to *V. officinalis*. The two species must remain accepted, but ideally: the lectotype Martius 650 should be sampled for molecular work; the syntype designation of the collections of Blanchet should be reevaluated.

Although it is too early to identify geographical signals, the only two species from Central America—*V. guatemalensis* and *V. nobilis* form a separated clade from a node within one of the two clades of B2.3 (B2.3aa). This position of *V. nobilis*, refutes, once more, its previous synonymizing under *V. surinamensis*, and supports the decision of Santamaría-Aguilar *et al.* (2019) to recognize them as two distinct species. All three specimens of *V. surinamensis*, on their turn, are solved as monophyletic within the other clade of B2.3 (B2.3bb), i.e. quite a distant position.

The inclusion of more than one specimen per species, in order to verify whether these specimens are resolved as monophyletic, helps to access the strength of previous species delimitation. From the 36 species, 20 had more than one representative specimen in the phylogeny. From these 20 species, 12 were resolved as monophyletic in clades with strong support. Five of those 12 species—*V. aequatorialis*, *V. dixonii*, *V. gardneri*, *V. kwatae* and *V. subsessilis*—included the type specimen in their respective clades.

On the other and, the absence of monophyly in nine species could have a number of causes, to be analyzed one by one. They can increase the evidence of a taxonomic decision or be subject of further exploration with an extended dataset and targeted sequencing of multiple regions. The latter is certainly the case of the polytomies involving multiple species, such as in clade A2, *V. pavonis* / *V. michellii* / *V. carinata* or *V. elongata* / *V. divergens*. For instance, *V. carinata* and *V. pavonis* are notoriously difficult to identify, so the next step in this case is to separate the limitations of the specimen identification from the proposed species delimitations, which should reflect biologically different entities. Furthermore, there is the possibility of hybridization, due to their overlapping ranges, as shown by the analysis of inter and intraspecific molecular variation of three species of *Virola* in French Guiana (Binelli *et al.*, 2020).

Classification

Previous classifications of the genus do not completely match the main clades we found. Oxyanthera Warb. is nested within Amblyanthera Warb., which makes sense, since the variation in the rate of column / anther is less clear-cut

than proposed by Warburg (1897) . The groups of Ducke (1936) and Smith and Wodehouse (1937) are quite helpful for species identification, but do not reflect the evolutionary history of the group. Finally, the clades Sebiferae and Multnervia of Steeves (Steeves, 2011) seem to hold, corresponding to our clades A and B, except for the position of *Virola dixonii*.

There is a strong need of more specimens, a broader coverage of the species represented, and the use of target sequencing in order to strengthen the molecular evidence by adding more DNA regions and single copy genes to the analysis. At a first glance, geography may have played a more important role than morphology in the evolution of the group. Clade A is made of mainly Amazonian species, while clade B includes Amazonia + Cerrado and Atlantic forest as well as Amazonian + Choco, and Central America. If lowland Amazonia is the origin of the genus, different events of colonization of other biomes may have underlined speciation in *Virola*. The role of adaptation to a different environment following migration event was already highlighted in *Otoba*, another Neotropical Myristicaceae (Frost *et al.*, 2022).

Reassessment of species' taxonomic status

The first consequence of the obtained phylogenetic relationships in *Virola* taxonomy is in the clade of *V. calophylla* and *V. calophylloidea* in A1.2. When *V. calophylloidea* was published, its diagnosis portrayed a species similar to *V. calophylla*, but in many aspects smaller and having "more" anthers (Markgraf, 1923). Later, these differences were described with more detail, with measurement of inflorescences and leaves and reinforcing the idea that *V. calophylloidea* was a "smaller" *V. calophylla* (Santamaría-Aguilar *et al.*, 2019; Smith & Wodehouse, 1937). That they should be accepted as two different species was contested by W. A. Rodrigues (1980), who synonymized them, based on the argument that *V. calophylla* has a large phenotypic plasticity caused by its wide distribution range. The synonymizing was accepted in some later publications (Jaramillo *et al.*, 2004; Ribeiro, 1999), but not by all. Interestingly, specimens with more attenuate leaves are often identified as *V. calophylloidea* in different herbaria, even though this was not a feature highlighted by previous authors. Morphological differences are noticeable, but apparently not conclusive. In the present study, clade A1.2 offers additional evidence for their combination: a polytomy with a few specimens of *V. calophylloidea,* as well as specimens of *V. calophylla* (well-fitting the original description), is nested within a clade to which the type of *V. calophylla* belongs. The recommendation is to treat *V. calophylloidea* as a synonym of *V. calophylla*, and to consider that the morphological variation is a result of consecutive identification, in different herbaria, of specimens of *V. calophylla* that fall within a specific morphological variation.

As mentioned in the description of clade B, the placement of *Bicuiba oleifera* within *Virola* support the recommendation of William A. Rodrigues (1998) and is in line with the results obtained recently with the phylogenomics of the Magnoliids (Helmstetter *et al.*, 2025). We abolish the genus *Bicuiba* and reinstate *Virola bicuhyba*.

The names *Bicuiba oleifera, Virola bicuhyba* and *Virola oleifera* are used intermingled in the literature. In some cases, two of the three names are listed as different accepted species, as it is the case of POWO, where both *B. oleifera* and *V. bicuhyba* appear as accepted (POWO, 2025). *Myristica oleifera* was invalidly published by H.W. Schott (1823), while the name *Myristica bicuhyba* was validly published by the same author in 1827 (H.W. Schott, 1827). That is why the new combination *Virola bicuhyba* published by Warburg was also valid. Smith and Wodehouse (1937) retook the epithet "*oleifera*" and published this binomial inside the genus *Virola*, based on the invalidly published name. Both names were reduced to synonymy under *Bicuiba oleifera* (Wilde, 1991). The publication of the new genus by de Wilde (1991) was based on the classification of the inflorescence as plural, a state that, according to the author, was also found in other Neotropical genera, but not in *Virola*. While in other Neotropical genera plural inflorescences are visibly branched from the base and develop concomitantly, the condition in *Bicuiba* is only spotted due to the presence of (scars of) prophylls on the base of the peduncle, the signal of a previous development. Another diagnostic character was the aril of *Bicuiba*: laciniate for a third to a quarter of the length of the seed, much less than in most *Virola* species. A closer inspection of these features showed that they are probably an extreme of a variation already present in *Virola*. For instance, species such as *V. gardneri* and *V. sebifera* have laciniation less than half and half the length of the fruit, respectively. Plural inflorescences, although rare, were recorded in some individuals belonging to *V. minutiflora*, *V. venosa, V. michelii, V. carinata* and *V. calophylla* (FIGURE 3), and even in the isolectotype of *V. officinalis* deposited in P, as well as collections P02031894, P02031893.

The polytomy formed by the two specimens of *V. lieneana* and the type specimen of *V. urbaniana* reinforces the suggestion of Rodrigues (1980) that the former is a synonym of the later. The difference in bp among the three specimens is 8 and 10, which falls within the variation of species showing monophyly in this study.

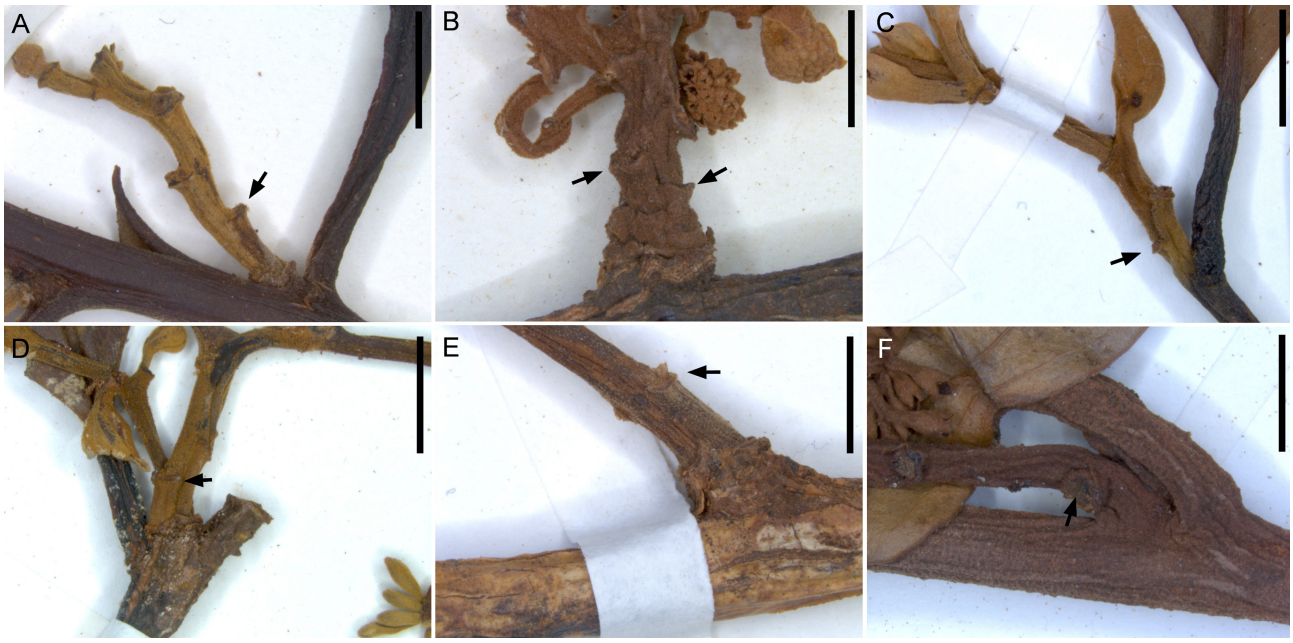


FIGURE 3. Leaf scars found in various species of Virola. (A) Scars typical of Bicuiba; (B) V. minutiflora; (C) V. venosa; (D) V. michelii; (E) V. carinata; (F) V. calophylla. Arrows point to (scars of) prophylls. Scale bar = 5mm.

An overview of the currently accepted species and synonyms in Virola, based on the current literature, WFO, observations of species morphology for Flora do Brasil (Oliveira 2020) and the results of this study, is given in TABLE 1.

TABLE 1. List of species names in the genus *Virola* Aublet (Myristicaceae) with status and *adjustments recommended for WFO.

Name	Status
**Bicuiba oleifera* (Schott) J.J.de Wilde	= *Virola bicuhyba* (Schott) Warb.
***Virola aequatorialis* Muriel & Balslev**	Accepted
***Virola aguarunana* D.Santam.**	Accepted
***Virola albidiflora* Ducke**	Accepted
***Virola allenii* D.Santam. & Aguilar**	Accepted
***Virola alvaroperezii* D.Santam.**	Accepted
***Virola amistadensis* D.Santam.**	Accepted
Virola araujovii Warb.	= *Virola oleifera* (Schott) A.C.Sm.
***Virola bicuhyba* (Schott) Warb.**	Accepted
Virola bicuhyba var. *schenckii* Warb.	= *Virola bicuhyba* (Schott) Warb.
Virola boliviensis Warb.	= *Virola sebifera* Aubl.
***Virola bombuscaroensis* D.Santam.**	Accepted
Virola brachycarpa Standl.	= *Virola multiflora* (Standl.) A.C.Sm.
***Virola caducifolia* W.A.Rodrigues**	Accepted
***Virola calimensis* D.Santam.**	Accepted
***Virola calophylla* (Spruce) Warb.**	Accepted
**Virola calophylloidea* Markgr.	= *Virola calophylla* (Spruce) Warb.
***Virola carinata* (Spruce ex Benth.) Warb.**	Accepted
Virola carinata var. gracilis (A.DC.) Warb.	= *Virola carinata* (Spruce ex Benth.) Warb.
Virola carinita var. *gracilis* Warb.	= *Virola carinata* (Spruce ex Benth.) Warb.
***Virola chrysocarpa* D.Santam. & Aguilar**	Accepted

.....continued on the next page

TABLE 1. (Continued)

Name	Status
***Virola coelhoi* W.A.Rodrigues**	Accepted
***Virola cogolloi* D.Santam.**	Accepted
***Virola crebrinervia* Ducke**	Accepted
***Virola cumala* D.Santam.**	Accepted
***Virola cuspidata* (Benth.) Warb.**	Accepted
Virola cuspidata var. *membranacea* (Poepp. ex A.DC.) Warb.	= *Virola cuspidata* (Benth.) Warb.
***Virola decorticans* Ducke**	Accepted
***Virola divergens* Ducke**	Accepted
***Virola dixonii* Little**	Accepted
***Virola duckei* A.C.Sm.**	Accepted
Virola elliptica A.C.Sm.	= *Virola pavonis* (A.DC.) A.C.Sm.
***Virola elongata* (Benth.) Warb.**	Accepted
Virola elongata var. *longicuspis* Warb.	= *Virola cuspidata* (Benth.) Warb.
Virola elongata var. punctata (Spruce ex Benth.) Warb.	= *Virola cuspidata* (Benth.) Warb.
Virola elongata var. subcordata Warb.	= *Virola cuspidata* (Benth.) Warb.
***Virola excisa* D.Santam.**	Accepted
***Virola flexuosa* A.C.Sm.**	Accepted
***Virola fosteri* D.Santam.**	Accepted
***Virola gardneri* (A.DC.) Warb.**	Accepted
Virola glaziovii var. *latifolia* Malme	= *Virola surinamensis* (Rol. ex Rottb.) Warb.
Virola glaziovii Warb.	= *Virola surinamensis* (Rol. ex Rottb.) Warb.
***Virola guatemalensis* (Hemsl.) Warb.**	Accepted
***Virola guggenheimii* W.A.Rodrigues**	Accepted
Virola incolor Warb.	= *Virola calophylla* (Spruce) Warb.
***Virola koschnyi* Warb.**	Accepted
Virola kukachkana L.O.Williams	= *Virola albidiflora* Ducke
***Virola kwatae* Sabatier**	Accepted
***Virola laevigata* Standl.**	Accepted
Virola lepidota A.C.Sm.	= *Virola calophylla* (Spruce) Warb.
**Virola lieneana* Paula & E.P.Heringer	= *Virola urbaniana* Warb.
***Virola loretensis* A.C.Sm.**	Accepted
***Virola macrocarpa* A.C.Sm.**	Accepted
***Virola malmei* A.C.Sm.**	Accepted
***Virola marleneae* W.A.Rodrigues**	Accepted
***Virola megacarpa* A.H.Gentry**	Accepted
Virola melinonii (Benoist) A.C.Sm.	= *Virola michelii* Heckel
Virola merendonis Pittier	= *Virola koschnyi* Warb.
***Virola michelii* Heckel**	Accepted
***Virola micrantha* A.C.Sm.**	Accepted
***Virola minutiflora* Ducke**	Accepted
Virola mocoa (Poepp. ex A.DC.) Warb.	= *Virola sebifera* Aubl.
***Virola mollissima* (Poepp. ex A.DC.) Warb.**	Accepted
***Virola montana* D.Santam.**	Accepted
***Virola multicostata* Ducke**	Accepted
***Virola multiflora* (Standl.) A.C.Sm.**	Accepted

.....continued on the next page

TABLE 1. (Continued)

Name	Status
***Virola multinervia* Ducke**	Accepted
Virola mycetis Pulle	= *Virola sebifera* Aubl.
***Virola nobilis* A.C.Sm.**	Accepted
***Virola obovata* Ducke**	Accepted
***Virola officinalis* Warb.**	Accepted
Virola oleifera (Schott) A.C.Sm.	= Virola bicuhyba (Schott) Warb.
***Virola otobifolia* D.Santam.**	Accepted
Virola panamensis (Hemsl.) Warb.	= *Virola sebifera* Aubl.
***Virola parkeri* D.Santam. & Lagom.**	Accepted
***Virola parvifolia* Ducke**	Accepted
***Virola parvusligna* Vásquez & L.Valenz.**	Accepted
***Virola pavonis* (A.DC.) A.C.Sm.**	Accepted
***Virola peruviana* (A.DC.) Warb.**	Accepted
Virola peruviana var. *tomentosa* Warb.	= *Virola sebifera* Aubl.
***Virola polyneura* W.A.Rodrigues**	Accepted
***Virola reidii* Little**	Accepted
Virola rufula Warb.	= *Virola theiodora* (Spruce ex Benth.) Warb.
***Virola rugulosa* (Spruce) Warb.**	Accepted
***Virola sanguinea* D.Santam.**	Accepted
***Virola schultesii* A.C.Sm.**	Accepted
Virola schwackei Warb.	= *Virola gardneri* (A.DC.) Warb.
***Virola sebifera* Aubl.**	Accepted
Virola sebifera var. *curvinervia* Warb.	= *Virola sebifera* Aubl.
***Virola sessilis* (A.DC.) Warb.**	Accepted
***Virola steyermarkii* W.A.Rodrigues**	Accepted
***Virola subsessilis* (Benth.) Warb.**	Accepted
***Virola surinamensis* (Rol. ex Rottb.) Warb.**	Accepted
***Virola theiodora* (Spruce ex Benth.) Warb.**	Accepted
***Virola tuckerae* D.Santam. & Lagom.**	Accepted
***Virola urbaniana* Warb.**	Accepted

Conclusions

The present study offered a first assessment of the taxonomy of the genus *Virola* with the support of a newly generated phylogeny. Based on the sampling of multiple specimens, as well as of type specimens, for a number of species, we offered molecular support for the monophyly and presumably robust species delimitation of 12 species. Species with a disputed status in the literature were re-evaluated under the light of the molecular data and morphology. Among those, we recommend the synonymy of *V. lieneana* under *V. urbaniana* and *V. calophylloidea* under *V. calophylla*. Further, the monotypic genus *Bicuiba* (Schott) J.J.de Wilde is concluded to be a synonym of *Virola* Aublet, and consequently *Bicuiba oleifera* a synonym of *Virola bicuhyba.* The conclusion is based on the genus' position in the phylogeny but also by our morphological study of the inflorescence in other species of *Virola*, since this was the most important diagnostic character of *Bicuiba.*

Previous infra-generic classifications or informal groupings do not match the clades recovered in our phylogeny. Apparently, the variation in morphological features, including the morphology of the androecium, is not related to species evolution in the genus. In terms of geography, one predominantly Amazonian clade is sister to all remaining. Based on the geographical distribution of the species in the clades, we suggest future testing of the hypothesis that *Virola* have originated in lowland Amazonia and repeatedly colonized other biomes.

Author contributions

according to the the CRediT model: Conceptualization: SMdO; Methodology: SMdO, HtS, ME, CH, SdB; Software: does not apply / Validation: does not apply; Formal analysis: SMdO, HtS; Investigation: SMdO; Resources: SMdO, HtS; Data Curation: SMdO; Writing—original draft preparation: SMdO; Writing—review and editing: all authors; Visualization: HtS, CH; Supervision: does not apply; Project administration: SMdO; Funding acquisition: HtS.

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