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Finding the origin of domestication of cupuaçu requires more than genomics.

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Theobroma subincanum; Botany; Biogeography; Archaeology; Linguistics; History;
Genetics

Colli-Silva et al.¹ recently proposed that cupuaçu (*Theobroma grandiflorum*) was domesticated in northwestern Amazonia as a selection from its sister species, cupuí (*T. subincanum*). This proposal ignores long-term research in taxonomy, botany, biogeography, and genetics about *Theobroma*, including cupuaçu. Our review of the research that was ignored and of Colli-Silva et al.'s results demonstrates that cupuaçu is a valid species, as they now agree², but cupuí may be paraphyletic, the distribution of wild cupuaçu was not included in their samples so the origin of domestication continues

unknown, precolonial archaeology lacks remains that can be attributed to either species, historical linguistics indicates that the term *cupuaçu* references the species' wild distribution and is a recent introduction in northwestern Amazonia, history suggests that cupuaçu started to be domesticated during the last 100-200 years, and the genomics results are not about the domestication of cupuaçu because it is a valid species and its wild distribution was not sampled.

This cupuaçu proposal¹ is based on a genomic analysis of the two species from four localities that were poorly selected to identify the origin of domestication of cupuaçu. Alphonse de Candolle³ observed that the origin of domestication is found where the species is known to occur spontaneously, i.e., where humans did not plant it. Wild populations of cupuaçu were reported, and can still be found today, in southern and eastern Pará and northern Maranhão⁴, cited by¹. De Candolle proposed that the search for origins is a multidisciplinary task, with botany, archaeology, biogeography, linguistics, and history contributing. In the early 20th century, Nikolai I. Vavilov⁵ added genetics to the list of disciplines. It follows that when the genetic results are correct, they will be supported by all other results. The Colli-Silva team includes botanists, geneticists, and archaeologists, but the study still exhibits problems that escaped peer review.

Curiously Colli-Silva et al. do not review previous work about domestication of cupuaçu, nor the phylogeny of the Glossopetalum section of *Theobroma* that contains their two species to provide context for readers and peer review. The review of the Glossopetalum section is especially important as they proposed in 2023 that cupuaçu is not a valid species; this is essential because domestication does not create new species, except via polyploidization⁶, which is not the case for these diploid species⁴. In 2024, they changed their opinion and now consider cupuaçu a valid species². In this Matters Arising, we introduce information that Colli-Silva et al. ignored (though cited in their references), as well as new information to show why their proposal is wrong.

Systematic botany provides information about the limits of and relationships among species. If cupuaçu was a domesticated form of cupuí, one would expect differences primarily in fruit characteristics, which is the part used and thus subject to human selection⁷. In Cuatrecasas'⁴ dichotomous key of *Theobroma*, the expectation from Colli-Silva et al.'s proposal is that cupuaçu and cupuí would be distinguished primarily by fruit characteristics. However, numerous vegetative characteristics also distinguish cupuaçu from cupuí. The key suggests that cupuaçu is not derived from cupuí and that both species are valid, as they now agree².

There is no molecular phylogenetic analysis of *Theobroma* that significantly questions Cuatrecasas'⁴ revision of the genus. Colli-Silva et al. cite four partial analyses, but only Sousa-Silva and Figueira⁸ include the two species of interest here. There is no indication that these species are not valid. Nonetheless, Colli-Silva et al. state that "Cupuaçu's closest relative is cupuí (*T. subincanum*), and in recent phylogenies the two species have consistently emerged as sister-groups." Such a relationship is not supported by the cited references, three of which do not include both species, but is now supported by the newest study from their group². If it is a sister species, as they now affirm, it cannot have arisen via domestication, which does not create species⁶.

The Amazonian flora is under sampled⁹. The few phylogeographical analyses of plant lineages in Amazonia suggest that some widespread 'species' contain independent

lineages with old divergences despite the lack of morphological differences. The widespread *Protium heptaphyllum* contains 9 lineages with most divergences before 750,000 years ago¹⁰. Similarly, there is abundant "cryptic" variation within a widespread *Pagamea* species complex of white-sand soils¹¹. Cuatrecasas⁴ subspecific classification of cacao (*T. cacao*) followed earlier hypotheses with two varieties (criollo and forastero), the latter with a pan-Amazonian distribution. Molecular analyses revealed 10 distinct genetic groups in Amazonia, one of which gave rise to criollo¹².

These studies reflect the poor knowledge about Amazonian species and the limits of morphology for revealing diversity. Hence, one should not assume that widespread Amazonian species like cupuí are well defined. The paraphyletic pattern of cupuí in relation to cupuaçu in Colli-Silva et al.'s Fig. 2a suggests that cupuí is poorly defined, not that cupuaçu was domesticated from cupuí.

Archaeobotany relies on plant remains to identify and date the species humans used. Although Colli-Silva et al. affirm that "Our dating somewhat precedes earliest archeological evidence for the use of cupuaçu that dates to the late Holocene (4200–500 yBP) and all known sites with archaeobotanical remains in the Middle-Upper Rio Negro Basin," there is no mention of cupuaçu remains in the citation. A recent review of the archaeobotany of edible Amazonian plants¹³ found three reports of *Theobroma*, none identified to species. In the many sites from the Carajás region (southern distribution of wild cupuaçu) carbonized wild plants exist in hearths¹⁴, but no cupuaçu or cupuí.

Biogeography draws on multiple disciplines to guide the search for origins³. Wild cupuaçu occurs from the eastern side of the middle Tapajós River in Pará state eastwards to Pre-Amazonian Maranhão state⁴. Curiously, Colli-Silva et al. disregarded this, although they cite⁴. Instead, they used this rationale: "(2) they (*sic*: the collection localities) encompass areas where *T. grandiflorum* is known to occur outside of botanical gardens, agronomic institutes, or intentionally established plantations, which would indicate potential cultivation on a larger scale" (Supplementary Methods). This rationale cannot distinguish between wild and cultivated; cupuaçu is generally cultivated in swiddens and home gardens, neither of which are classified as plantations, as well as in small plantations.

Historical linguistics uses lexical terms to trace the origin of words for cultigens. Terms for recently acquired plants cannot be reconstructed to an older protolanguage; these emerge in daughter languages via borrowing or shared innovation¹⁵. If Colli-Silva et al.'s proposal was valid, names for cupuaçu and cupuí would reconstruct in protolanguages of northwestern Amazonia.

This region is home to many language families and branches thereof, including Eastern Tukanoan, Naduhup (Makú), and Arawakan¹⁶. The diversification of these groupings began less than 5,000 years ago¹⁷, hence are close to the window of domestication proposed by Colli-Silva et al. Nheengatú, a colonial form of the coastal Tupí-Guaraní language Tupinambá¹⁸, arrived during the colonial period. The terms cupuí and cupuaçu are from Nheengatú (*cupu* + *-í* 'small' and *-açu* 'large'), not from a local language. Notably, terms for cupuaçu in languages of the Negro River region cannot be reconstructed to any of the relevant protolanguages, while terms for cupuí reconstruct.

Terms for cupuí and cupuaçu in the Tupí-Guaraní branch of Tupí offer an intriguing comparison. The likely point of dispersal for this widespread branch was the lower Xingu River basin in central Pará¹⁹, where wild cupuaçu occurs. While a form

**kupu* (Ramirez, personal reconstruction) or **kipi* (Balée, personal reconstruction) can be reconstructed to Proto-Tupí-Guaraní, it is not specified for one or the other species (cf. Nheengatú ‘little *cupu*’ and ‘big *cupu*’). Thus, historical linguistics suggests no difference in time-depth between cupuí and cupuaçu for Tupí-Guaraní – consistent with a scenario wherein both plants were familiar to speakers of the protolanguage.

The written history of Amazonia started in 1541-42 with Orellana’s voyage down the Amazon River. The first mention of these species dates from the mid-18th century; missionary João Daniel²⁰ acclaimed their flavors, but observed that neither was cultivated. In the 19th century, no European naturalists mention either species, although astute observers Richard Spruce and Alfred Wallace spent considerable time on the Negro River. Adolpho Ducke²¹ affirmed that cupuaçu was a pre-Columbian crop, but that cultivated plants are little different from wild ones. These historic citations provide conflicting observations: Ducke considered cupuaçu a crop; Daniel considered it wild; and other competent observers didn’t notice it.

In the mid-20th century, the discipline of ethnobotany became important and provides floristic inventories of Indigenous and traditional communities. A systematic review of the ethnobotany of the Negro River²² found the first mention of cupuaçu in 1985 in Indigenous communities along the upper river²³.

Genetics and now genomics are very popular methods for the study of domestication²⁴. When geneticists have access to an appropriate sample of living or preserved plants, they can study the diversity and structure of populations, estimate changes through time and propose areas that may be the origin of domestication, as Colli-Silva et al. purported to do. These authors, however, used an inappropriate sample of cupuaçu in their study, so their results are not about the domestication of cupuaçu. Please see Supplementary Information for a full botanical, archaeological, biographical, linguistic, historical, and genetic analysis.

Author contributions: Conceptualization, CRC; investigation, all authors; writing – original draft, all authors; writing – revision and editing, CRC; all authors agree with this manuscript and submission to Communications Earth & Environment.

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Finding the origin of domestication of cupuaçu requires more than genomics.

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Colli-Silva et al. (2023) recently proposed that cupuaçu (*Theobroma grandiflorum*) was domesticated in northwestern Amazonia as a selection from its sister species, cupuí (*T. subincanum*). This proposal ignores a century of scholarship on the taxonomy, botany, biogeography, and several decades of genetics about *Theobroma* and cupuaçu, which the Colli-Silva team is familiar with. Our review of the scholarship that was ignored and of Colli-Silva et al.'s results shows that cupuaçu is a valid species, as they now agree (Bossa-Castro *et al.* 2024), but cupuí may be paraphyletic, the distribution of wild cupuaçu was not included in their sample so the origin of domestication continues unknown, precolonial archaeology has no remains that can be attributed to either species, linguistics suggests that cupuaçu is a name from its wild distribution and a recent introduction in northwestern Amazonia, history suggests that cupuaçu started to be domesticated during the last 100-200 years, and the genomics results are not about the domestication of cupuaçu because cupuaçu is a valid species and its wild distribution was not sampled.

The Colli-Silva et al. proposal is based on a genomic analysis of the two species from four localities that were poorly selected to identify the origin of domestication of cupuaçu. Alphonse de Candolle (1883) observed that the search for the origin of domestication should start from where the species is known to occur spontaneously, i.e., where humans did not plant it. Wild populations of cupuaçu have been reported, and can still be found today, in southern and eastern Pará (Cuatrecasas 1964), cited by Colli-Silva *et al.* (2023). None of the localities used by Colli-Silva et al. for cupuaçu are in this area, although one is close. In addition, de Candolle proposed that the search for origins should be a multidisciplinary task, with botany, archaeology, biogeography, linguistics, and history contributing. In the early 20th century, Nikolai I. Vavilov (1992) added genetics to the list of disciplines. It follows that when the genetic results are correct, they will be supported by all other results. The Colli-Silva team includes

botanists, geneticists, and archaeologists, but the study has significant inconsistencies that escaped peer review.

Curiously Colli-Silva et al. do not review previous work about domestication of cupuaçu, nor the phylogeny of the Glossopetalum section of *Theobroma* that contains their target species, which would provide context for their study to guide readers and peer review. The review of the Glossopetalum section is especially important as they proposed in 2023 that cupuaçu is not a valid species; this is essential because domestication does not create new species, except via polyploidization (Harlan 1992), which is not the case for these diploid species (Cuatrecasas 1964). They do not explain why cupuaçu is not valid, nor why they expected that cupuaçu was domesticated from cupuí, rather than from wild populations of cupuaçu itself, which is the logic that de Candolle (1883) highlighted. However, in their most recent study (Bossa-Castro *et al.* 2024) they accept cupuaçu as a valid species. In this essay, we introduce information that Colli-Silva et al. had access to and even cited the appropriate references, but did not use, as well as new information to show why their proposal of an origin of domestication of cupuaçu from cupuí in northwestern Amazonia is wrong.

Botany

Systematic botany provides information about the limits of and relationships among species, originally based on morphology and other traits and now including genetics. Cuatrecasas' (1964) revision of the genus is currently accepted in general. As was standard at that time, the revision is based on morphology and other botanical characteristics. If cupuaçu was a domesticated form of cupuí, one would expect differences primarily in fruit characteristics, which is the part used and thus subject to human selection (Darwin 1859). These selected characteristics are the basis of the domestication syndrome (Meyer *et al.* 2012). In a dichotomous key to differentiate species, the expectation from Colli-Silva et al.'s proposal is that cupuaçu and cupuí would be paired based on fruit characteristics and genetically correlated characteristics of the domestication syndrome, but that the trees and leaves of cupuaçu and cupuí would be indistinguishable. In fact, Colli-Silva et al. observe that "Morphologically, cupuí is very similar to cupuaçu, but with smaller fruits and seeds and more pubescent leaves."

However, Cuatrecasas' (1964) key to the Glossopetalum section highlights numerous other differences besides the pubescent leaves, including flower size and other flower characteristics, leaf texture and stipules, and other vegetative and reproductive characteristics. Most of these vegetative and even reproductive characteristics were not selected by humans, i.e., they are not part of the domestication syndrome. The key suggests that cupuaçu and cupuí are clearly morphologically distinct with some reproductive similarities and numerous vegetative differences, which is not what is expected in a domestication syndrome. The Colli-Silva team now agrees that cupuaçu is a valid species (Bossa-Castro *et al.* 2024).

There is no molecular phylogenetic analysis of *Theobroma* that significantly questions Cuatrecasas' (1964) revision of the genus. Colli-Silva et al. cite four partial analyses, but only Sousa-Silva and Figueira (2004) include the two species of interest here, as well as the closely related cupurana. They used a sequence of the trypsin inhibitor for their analysis, so they generated a single gene phylogeny that is highly congruent with Cuatrecasas' hypothesis for the phylogenetic relationships among

Theobroma species based on morphology. There is no indication in Sousa-Silva and Figueira (2004) that any of these three closely related species are not valid. Nonetheless, Colli-Silva et al. (2023) state that “Cupuaçu’s closest relative is cupuí (*T. subincanum*), and in recent phylogenies the two species have consistently emerged as sister-groups.”, but this relationship is not supported by any of the cited references, three of which do not include both species. A newly published phylogeny from the Colli-Silva team used five WRKY transcription factor loci and numerous morphological traits to analyze the relations among 19 of the 23 *Theobroma* species and found the relation they reported in 2023 (Bossa-Castro et al. 2024). If Colli-Silva et al.’s proposal that cupuaçu was domesticated directly from cupuí was valid, the new phylogeny would group the species together as a unit because the WRKY loci are highly conserved and unlikely to mutate much in 200 or even 5000 years. Instead, they found that cupuaçu and cupuí are sister species, with no indication that cupuassu is not valid. If it is a sister species, as they now affirm, it cannot have arisen via domestication, which does not create species (Harlan 1992).

Addison and Tavares (1952), cited by Colli-Silva et al. (2023), used controlled pollination and grafting compatibility to study the relationships among various *Theobroma* species, including cupuaçu, cupuí and cupurana. Since domestication is a recent phenomenon in the evolutionary histories of some plant species, domesticated and wild populations cross-pollinate freely (Harlan 1992; Ellstrand 2003) and should be fully graft compatible. When cupuaçu was the female parent and cupuí was the pollen parent, fruit set from 317 hand pollinations was 12 %; the reciprocal pollination gave fruit set of 22 % from 189 hand pollinations. Similarly, graft compatibility of cupuaçu on cupuí was 85 % and the reciprocal was 35 %. Neither of these methods of testing relationships suggest that cupuaçu is derived from cupuí.

The Amazonian flora is under sampled and poorly studied (Hopkins 2007), with some estimates suggesting that over 60 % of tree species have yet to be described (ter Steege et al. 2013). Most species have few samples in herbaria (Hopkins 2019), and well-known species, like Amazonian fruits, tend to be poorly represented in collections just because people know them. The few phylogeographical analyses of plant lineages in Amazonia suggest that many widespread 'species' may contain independent lineages with old divergences despite the lack of obvious morphological differences. The widespread ‘hyperdominant’ species (sensu ter Steege et al. 2013) *Protium heptaphyllum*, for example, contains 9 lineages after a large population and genomic sampling, with most divergences before 750,000 years ago (Damasco et al. 2021). Similarly, Prata et al. (2018) show abundant "cryptic" variation within a widespread *Pagamea* species complex of white-sand soils. Cacao (*T. cacao*) has similar subspecific variation, although not to the species level. Cuatrecasas’s (1964) subspecific classification of cacao followed earlier hypotheses with two varieties – criollo and foresteiro – the latter with a pan-amazonian distribution. Molecular analyses revealed 10 distinct genetic groups in Amazonia, one of which gave rise to criollo (Motamayor et al. 2008; Cornejo et al. 2018).

These studies reflect the current poor knowledge about Amazonian species, both their sampling and their definitions, and the limitations of morphology for revealing diversity. Therefore, one should not assume that Amazonian plant species are robustly defined, and better definition of the phylogenetic context beforehand is an important first step for population level analyses of any species, particularly widespread ones like

cupuí. The paraphyletic pattern of cupuí in relation to cupuaçu in Colli-Silva et al.'s Fig. 2a suggests that cupuí is currently poorly defined, not that cupuaçu was domesticated from cupuí. Including other closely related species in their analysis, particularly cupurana, might have permitted a better interpretation of the relations between cupuaçu and cupuí.

Archaeology

Archaeobotany uses several types of plant remains conserved in archaeological sites to identify and date the presence of species used by humans. Although Colli-Silva et al. affirm that “Our dating somewhat precedes earliest archeological evidence for the use of cupuaçu that dates to the late Holocene (4200–500 yBP) and all known sites with archaeobotanical remains in the Middle-Upper Rio Negro Basin.”, there is no mention of archaeological remains of cupuaçu in the citation given. A recent review of the archaeobotany of edible Amazonian plants (Cassino *et al.* 2021) found three reports of *Theobroma*, none identified to species. Maezumi et al. (2018) found archaeological pollen of *Theobroma* at Lake Caranã but no phytoliths in nearby plots in the Tapajós National Forest (just west of cupuaçu’s spontaneous distribution and where Colli-Silva et al. collected both species), although cupuaçu and cupuí are present in modern vegetation. The *Theobroma* pollen did not allow identification to species. Also, *Theobroma* does not produce diagnostic phytoliths (Piperno and McMichael 2020). The other two studies found carbonized pieces of *Theobroma* seeds but did not identify the species. In the dozens of sites studied in the Carajás region (adjacent to the southern part of the spontaneous distribution of cupuaçu), where modern inventories identify cupuí but not cupuaçu in the forest, from the most recent (+/- 500 years before present (BP)) to the oldest (+/- 11,000 BP) layers, mostly wild plant species were found carbonized around fireplaces, with some incipiently domesticated species (not dependent on humans), such as cashew (*Anacardium occidentale*) and açai-do-pará (*Euterpe oleracea*) (Magalhães *et al.* 2019; Santos *et al.* 2019), but no cupuaçu or cupuí. The scarcity of cupuaçu and cupuí remains may be explained by how they are used: cupuí is generally used as a snack while walking along trails in the forest, while cupuaçu is generally used to make a juice, especially when a sweetener is available.

Biogeography

De Candolle (1883) considered this discipline to be extremely important as it integrates information from botany, archaeology, linguistics across geographic space. In the case of cupuaçu, wild plants have been collected from the eastern side of the middle Tapajós River in Pará state eastwards to the Tocantins River and further eastwards into Pre-Amazonian Maranhão state (Ducke 1946; Cuatrecasas 1964); the map in Cuatrecasas (1964) is quite clear. Curiously, Colli-Silva et al. did not use this information to create their maps (Fig 1, Fig S1), even though they had the opportunity to study the “conditions of collection” of each GBIF data point included in Fig S1, which contains the collector’s description of the ecosystem where it was collected. This information generally allows clear discrimination of cultivated versus wild, although some botanists do not carefully discriminate between old second growth (i.e., successional swidden plots and abandoned home gardens) and mature forest. Rather than use this information, Colli-Silva et al. used this rationale: “2) they (*sic*: the collection localities) encompass areas where *T. grandiflorum* is known to occur outside

of botanical gardens, agronomic institutes, or intentionally established plantations, which would indicate potential cultivation on a larger scale” (Supplementary Methods). This rationale cannot distinguish between wild and cultivated; cupuaçu is generally cultivated in swiddens and home gardens, neither of which are classified as plantations, as well as in generally small plantations. Since the 2023 article, the Colli-Silva team has used remote sensing to analyze the distribution of cacao in Amazonia and found that outside of its region of origin in western Amazonia it is almost always found in cultivation or regrowth (Colli-Silva *et al.* 2024). If they had used the same methodology with their cupuaçu collections, they would have found that cupuaçu in Amazonas and Acre is in cultivation or regrowth.

In 1964, Cuatrecasas affirmed that cupuaçu is cultivated in eastern Amazonas state, as far as Manaus. Colli-Silva *et al.*'s Balbina locality is just north of Manaus, in the municipality of Presidente Figueiredo that specializes in cupuaçu production, including plantations, swiddens and home gardens (there is a yearly cupuaçu festival that elects a cupuaçu queen). Cuatrecasas' affirmation does not agree with Ducke (1946), however, who affirmed that cupuaçu is cultivated in all of Brazilian Amazonia, although it is less common in western Amazonas state. Colli-Silva *et al.*'s São Gabriel de Cachoeira is in northwestern Amazonas state and Xapuri (Acre) is immediately adjacent to southwestern Amazonas state. Observe that Cuatrecasas and Ducke agree that cupuaçu only occurs in cultivation in Amazonas and by extension Acre. Today there are numerous plantations in Amazonas, especially in Presidente Figueiredo, and in eastern Acre not far from Xapuri, and cupuaçu is an element in many swiddens and home gardens across the region.

Colli-Silva *et al.* have one locality close to the western margin of the spontaneous distribution in the lower Tapajós River, the Tapajós National Forest. This National Forest displays 4500 years of intensive anthropogenic management (Maezumi *et al.* 2018), so wild plants would be rarer there today than they might have been in the early Holocene. The other three localities are far from the region where cupuaçu is known to be wild, hence arrived by human dispersal sometime during the Holocene, most probably during the Portuguese and Brazilian colonial periods (see History below), as supported by Colli-Silva *et al.*'s stairway plot (Figure 2g).

Colli-Silva *et al.*'s Fig 2f suggests that the origin of cupuaçu is in northwestern Amazonia. The evidence behind this figure is weak. The ancestral area reconstruction (Sup Fig 6) has no probabilities associated with any ancestral area used, so they are all likely, and estimation of ancestral states is extremely sensitive to sampling (number of terminals, where Balbina is over-represented) and to the definition of geographical areas (Holland *et al.* 2020). Thus, the reasoning that the "earliest diverging cupuaçu specimens are found to be most closely related to cupuí samples from São Gabriel da Cachoeira and Balbina, in the Middle-Upper Rio Negro Basin (...), suggesting that initial domestication occurred somewhere around these localities" is probably a sampling artifact due to their use of single specimens to represent monophyletic groups in the phylogeny (Sup Fig 4; Supplementary Methods).

In the case of cupuí it is much more difficult to infer where the species originated because it is hyperdominant (number 32 on the list in (ter Steege *et al.* 2013)) and widely distributed in Amazonia (Fig S1 in Colli-Silva *et al.* (2023)), and, as discussed above, the species limits are not clear, i.e., how many lineages of cupuí exist and their geographic distributions. The paraphyletic cupuí reported in Colli-Silva *et al.*'s

Fig S2, does not necessarily indicate that there are many lineages of cupuí, as paraphyly does not reject a good biological species (*sensu* Mayr) per se, but also does not reject the hypothesis that the different lineages of cupuí are different species, or at least lineages (a western and an eastern) that have been in isolation for some time.

Linguistics

Historical linguistics uses lexical terms (e.g., names for plants) to trace the acquisition and identify the cultural relevance of the concepts they represent (i.e., the plants). In general, terms for recently acquired concepts cannot be reconstructed to an older protolanguage and generally emerged in the respective daughter languages via borrowing or language-internal innovation (e.g., derivation using a pre-existing term as the base form) (Epps 2015). When concepts do persist over time, the names that designate them tend to be more stable for culturally salient referents than for non-salient ones; accordingly, names for domesticates are replaced less quickly than names for wild species (Berlin *et al.* 1973; Balée and Moore 1991).

Northwestern Amazonia is home to many languages, including the eastern branch of the Tukanoan family and the Naduhup (Makú) family (Epps and Bolaños 2017; Cayón and Chacon 2022). Several branches of the Arawakan family (and possibly the family as a whole) probably originated in northwestern Amazonia (Ramirez 2020: vol 2., 15, 25). The diversification of each of these language families probably began less than 5,000 years ago (Chacon 2013; Michael 2021), so they fall just outside the 5,000-8,000 BP window of domestication proposed by Colli-Silva *et al.* for cupuaçu. Hence, terms for cupuaçu would probably reconstruct to the respective protolanguages and should be the oldest in Amazonia. A much more recent arrival to the Negro River was Nheengatú or Lingua Geral, a colonial form of the coastal Tupi-Guarani language Tupinambá favored by Jesuit missionaries in the 17th-18th centuries. Nheengatú continued to expand in the region through the 19th century and even into the 20th, acting as a lingua franca within local economic networks (Moore 2014). The terms *cupuí* and *cupuaçu* are themselves from Lingua Geral (*cupu* + *-í* ‘small’ and *-açu* ‘large’), not from a local language.

Notably, terms for cupuaçu in languages of the Negro River region cannot be reconstructed to any of the relevant protolanguages. They also show evidence of being highly innovative, indicating that they are quite recent additions to these languages, via either borrowing or language-internal innovation. In contrast, terms for cupuí are considerably less innovative – the opposite of what we would expect if both plants were of similar antiquity in the region, given the general tendency for relative stability among terms for domesticates. In regional Arawakan languages, for example, cupuí terms show evidence of similarity across related languages (e.g., Baniwa-Kurripako *maawi-Jó-da*, Yukuna *maʔawe-ré* ‘cacao’, Piapoco *mawi-ri* ‘cacao’ and Tariana *mawílo* (Cuatrecasas 1964: 566; Aikhenvald *et al.* 2001: 105; Ramirez 2001: 202), and Ramirez (2020: vol.4, 55) reconstructs **maʔawe* / **maʔawi* (‘a kind of wild cocoa tree’) for the Proto-Japura-Colombia branch of Arawakan. On the other hand, terms for cupuaçu are loans from Nheengatú in Baniwa-Kurripako and Tariana (*kopowaso*, *kupuasú* (Aikhenvald *et al.* 2001: 104; Silva 2013: 91).

Similarly, in most of the eastern Tukanoan languages the forms for cupuaçu are transparently derived from the cupuí terms in the respective languages, and most are literally ‘big cupuí’ (e.g., Siriano *babe kara paga* (Cabalar 2016: 118)). Evidence of

dialectal variation in cupuaçu terms also suggests recent innovation; e.g., the Tuyuka term recorded in Brazil is *susu paka* ('big cacao/cupuí' (Cabalzar 2016: 118)), while the term recorded in Colombia is *cocowasu* (an apparent adaptation of a Nheengatú loan (Barnes 2012: 103)). The Tukanoan cupuí terms themselves (Tukano *wapê-kara*, Wanano *pe-kara*, Desano *wabékará*, Makuna *abekara*, Siriano *babe kara* (Ramirez 1997: 213; Cabalzar 2016: 122)) are etymologically complex but consistent and very likely reconstruct to the protolanguage.

Turning to Naduhup (Makú), a small family with four members, terms for cupuí in the closely related languages Hup and Yuhup are similar (*bəh-ʔuk*, *bo-ʔuk*), while the terms in Dâw and Nadëb are distinct (*húlʔ pif*, *jakaro*) (Epps fieldnotes, Silva and Silva (2012: 115)). Naduhup terms for cupuaçu are once again more diverse and more innovative: Hup has a Nheengatú loan (*kupuwasú*), and both Yuhup and Nadëb have derived complex terms meaning 'big cupuí' (*bo-ʔuk pog*, *jakaro ib*). (Dâw, on the other hand, is unique among all the languages considered here in that the cupuí term is the derived counterpart, 'small cupuaçu'; this may be the outcome of a markedness shift).

In summary, terms for cupuí among Negro River languages are consistently more uniform and more morphologically simplex, while terms for cupuaçu are almost without exception more variable and more complex. The cupuaçu terms tend to be either loanwords from Nheengatu or are transparently derived from the corresponding cupuí terms, typically as 'big cupuí', which itself may well be a Nheengatú calque (i.e., a loan translation from *cupu-açu* 'cupu-big'). These facts provide robust evidence that the cupuí terms are older throughout the region, while the cupuaçu terms are recent innovations – a scenario that is directly consistent with a recent introduction of cupuaçu, likely associated with the same colonial presence that brought Nheengatú.

Terms for cupuí and cupuaçu in the Tupi-Guarani family offer an intriguing comparison. The most likely point of dispersal for this widespread family was from the lower Xingu River basin in central Pará (O'Hagan *et al.* 2019), where wild cupuaçu occurs. Two possible forms can be reconstructed to Proto-Tupi-Guarani: **kupu(a)* (Ramirez, personal reconstruction); **kîpi* (Balée, personal reconstruction). Importantly, neither is specified for one or the other species (cf. Nheengatú 'little *cupu*' and 'big *cupu*'; cf. also *cupurana* 'false *cupu*' for *T. obovatum*). Thus, historical linguistics does not imply any difference in time-depth between cupuí and cupuaçu for the Tupi-Guarani family – consistent with a scenario in which both plants were familiar to speakers of the protolanguage.

History

The written history of Amazonia started in 1541 with the expedition of Francisco de Orellana from Quito, Ecuador, down the Napo, Solimões and Amazonas Rivers and north into the Caribbean (Medina 1934). Neither cupuaçu nor cupuí were mentioned. The first Portuguese colony was the Capitania de Maranhão e Grão Pará, with its capital in São Luiz, Maranhão, just south of the eastern limits of the spontaneous distribution of cupuaçu. In the 1600s, the missionaries Claude d'Abbeville (1975) and Cristóvão de Lisboa (1967) inventoried the natural resources of the eastern part of the colony; neither species was mentioned. In the mid-1700s, the missionary João Daniel (2004) mentioned both species and was quite enthusiastic about their flavors and aromas, but observed that neither was cultivated. At the end of the century,

the first European naturalist, Alexandre Rodrigues Ferreira (2008), mentioned that both are domesticated Indigenous fruits, but gave no details about quality, cultivation or localities where he saw them. At nearly the same time, Lobo D'Almada (1861) only observed cupuí along the Branco River, the main tributary of the Negro and commented that it is not cultivated. In the 1800s, neither Avé-Lallemant (1980), Bates (1962), Spix and Martius (2017), Spruce (2014), nor Wallace (2004) mention either species. Spruce and Wallace spent considerable time on the Negro River. Agassiz & Agassiz (2000) did mention cupuaçu, but with no details; they called it a type of wild cacao. The economic botanist Adolpho Ducke (1946) affirmed that cupuaçu was a pre-Colombian crop, but observed that the cultivated plants are little different from wild plants, except for stature – they grow less in full sun than in the shade of the forest. These historic citations provide conflicting observations: Ferreira and Ducke considered cupuaçu an Indigenous crop, Daniel and Agassiz and Agassiz considered it to be wild, and other competent observers didn't notice it, even along the Negro River, where Colli-Silva et al. propose that it was domesticated.

In the late 19th century references to cultivation appear and become more numerous. In 1869, just south of Santarém, Para, the confederate immigrant from the USA, Richard Hemington, reported that he had 30 trees of cupuaçu in his new orchard (Guilhon 1987). Shortly thereafter, in 1886, José Veríssimo (2014) reported planted cupuaçu in a home garden, probably in Óbidos, Para, northwest of Santarém.

In the mid-20th century, the discipline of ethnobotany became important and provides floristic inventories of Indigenous and traditional communities. A systematic review of the ethnobotany of the Negro River (Assis 2022) found the first mention of cupuaçu in 1985 in Indigenous communities along the upper river (Ribeiro 1995). However, by this time the agricultural extension service of the State of Amazonas was actively distributing cupuaçu seedlings to Indigenous and traditional communities across the state, including the Negro River, encouraged by Embrapa and INPA (e.g., Clement and Venturieri (1990), cited by Colli-Silva *et al.* (2023); Alves et al. (2014)). As research in ethnobotany expanded in the 1990s and later, the number of citations expanded rapidly, but this review suggests that cupuaçu is a recent arrival to the Negro River.

Genetics

Genetics and now genomics are very popular methods for the study of domestication, especially since it was discovered that DNA can be extracted from archaeological remains (McMichael *et al.* 2023). When geneticists have access to an appropriate sample of living or preserved plants, they can study the diversity and structure of populations, estimate changes through time and propose areas that may be the origin of domestication, as Colli-Silva et al. attempted.

In the late 20th century, the Brazilian Enterprise for Agricultural Research (Embrapa) initiated a plant breeding program for cupuaçu (Souza *et al.* 2009; Alves *et al.* 2014). The two principal Embrapa centers in Amazonia, Embrapa Eastern Amazonia in Belém and Embrapa Western Amazonia in Manaus, created germplasm collections, as did the National Research Institute for Amazonia (INPA, Manaus) and, curiously, EletroNorte, the government holding company for generation and distribution of electricity. By law, EletroNorte was obliged to support research institutions to collect samples of biodiversity that would be destroyed when the Tucuruí Hydroelectric Dam

was flooded in the late 1980s. Tucuruí is in the region where cupuaçu occurs spontaneously and these wild plants were a priority. A few years later, INPA researchers collected in pre-Amazonian Maranhão and adjacent eastern Pará, again concentrating on wild cupuaçu. Shortly thereafter, Embrapa Eastern Amazonia researchers collected wild cupuaçu in Nova Ipixuna, Pará, and included it in the germplasm collection in Belém.

These three samples of wild cupuaçu were included in the first and only relatively wide-ranging genetic analysis of cupuaçu (Alves *et al.* 2007), cited by Colli-Silva *et al.* (2023). The Embrapa-led group used 21 nuclear microsatellites (simple sequence repeats) transferred from cacao (Alves *et al.* 2006) that they had also used to characterize cupuaçu's mating system (Alves *et al.* 2003). In the mating system analysis of the Nova Ipixuna wild cupuaçu, they found that cupuaçu is highly outbreeding, but has some biparental inbreeding (Alves *et al.* 2003). This raised the question of the extent of inbreeding in other populations, both spontaneous and cultivated, which was one of the objectives of the 2007 study. In that study, Alves *et al.* (2007) used three population samples (40 plants each) of wild cupuaçu (identified above), three groups of cultivated cupuaçu from Amapá, central Pará (Belém to Santarém along the Amazon River), and Amazonas (along the Amazon and Solimões Rivers), as well as from a commercial plantation in Tomé-Açu, Pará (in the region of wild cupuaçu). They found that the three populations of wild cupuaçu were highly divergent ($\theta_p = 0.301$), both among themselves and with the cultivated samples, and that Tomé-Açu grouped with the wild samples, rather than the cultivated samples, suggesting that it is a recent local selection. Compared to the wild cupuaçu, the cultivated groups were only slightly divergent (θ_p not estimated), as found by Colli-Silva *et al.* As expected from the mating system analysis, they found significant inbreeding in the wild populations (mean 0.192), which suggests limited gene flow among these plants that are generally relatively rare in the landscape (Cuatrecasas 1964; Alves *et al.* 2014). Alves *et al.* (2007) also concluded that the cultivated cupuaçu in Amapá, Pará and Amazonas were not derived from any of these three wild populations. Since no further analyses of wild and cultivated cupuaçu have been done, the population(s) that gave origin to cultivated cupuaçu along the Amazon River remain unknown.

What can Colli-Silva *et al.*'s analysis tell us about cupuaçu and cupuí? Taken at face value, their phylogenetic analyses (Fig. 2a; Fig. S4) suggest that cupuí is at least two lineages rather than a single monophyletic species, as discussed above. However, cupuí from Balbina is paraphyletic, with three plants grouping with Acre and three with Pará (Fig. 2a). In the Balbina-Acre group, Acre is derived from Balbina (Fig S4). In the Balbina-Pará-São Gabriel group, São Gabriel is the sister of the Balbina-Pará group (Fig S4), and there is no plant that groups with São Gabriel as in Fig. 2a. Because Balbina is paraphyletic, its mean nucleotide diversity is higher than any other location in either species (Table S3) and its genetic diversity is structured (Figs. 2c and 2d). It is not surprising that cupuaçu in Balbina is paraphyletic given its importance in the local economy, where different farmers might introduce seeds from different sources. The same is not true for cupuí since there is no current market interest in the species.

These curious relationships may be because Colli-Silva *et al.* called their SNPs from a published cacao genome, and cacao is quite distant from cupuaçu and cupuí in the trypsin phylogeny (Sousa Silva and Figueira 2004) and the new WRKY phylogeny (Bossa-Castro *et al.* 2024), and Cuatrecasas (1964) considers it the most derived

Theobroma. Using short-read sequences to identify SNPs has several technical difficulties that may influence the data set obtained (Mastretta-Yanes *et al.* 2015). Among these, using a reference genome from another species is known to introduce biases towards regions that evolve slowly (Leigh *et al.* 2018) and may decrease the probability of mapping alleles that are different from the reference genome (Pool *et al.* 2010). This might explain the low levels of nucleotide diversity observed by the authors (Table S3), although SNPs are known to have lower mutation rates and lower within-locus variation than SSR (Freeland *et al.* 2011).

In Fig 2a and Fig S4 cupuaçu is monophyletic, with three of the four localities consistent in Fig 2a. Again, Balbina mixes with another locality, in this case São Gabriel. However, in Fig S4 there is more mixture with Balbina dispersed in various parts of the phylogeny. If cupuaçu was derived from cupuí in northwestern Amazonia, as Colli-Silva *et al.* propose, one would expect a phylogeny that mixes some cupuí plants with the cupuaçu plants from the same region, because the latter is derived from the former. There is no such mixture in either figure.

Disappointingly, Colli-Silva *et al.* did not take the opportunity to date their Bayesian phylogeny (Fig S4). However, phylogenetic divergence estimates between cacao (the outgroup in Colli-Silva *et al.*'s Fig S4) and the *Glossopetalum* clade that includes cupuí and cupuaçu have a mean value of 10.96 Ma and the crown node of *Glossopetalum*+*Andropetalum* is about 4 Ma (Richardson *et al.* 2015). Although Richardson *et al.*'s dating does not include cupuí, considering the phylogenetic position and branch lengths in Fig. 4 of Sousa-Silva and Figueira (2004), the common ancestor of cupuí and cupuaçu may be much older than 720,000 years ago, dramatically older than the 8000 years estimated by Colli-Silva *et al.* in Fig 2g for the domestication of cupuaçu from cupuí.

In Fig 2b, the haplotype network shows that all cupuaçu are minor variations on a single theme, while cupuí appears to be two lineages with much more variation, as discussed above. As in Fig 2a, Balbina is an anomaly as it is present on both sides of cupuaçu. Nowhere do Colli-Silva *et al.* mention the Balbina anomalies.

In Fig 2c, there is remarkably little variation in any of the four groups in the PCA, which is not surprising for cupuaçu, but is for the others. Disappointingly, Colli-Silva *et al.* did not explore the structure of each of these four groups by isolating them in individual analyses. Nonetheless, the distribution of genomic diversity in the PCA space does not support the hypothesis that cupuaçu is derived directly from cupuí.

A Structure analysis has the potential to display admixture between a domesticate and its putative wild ancestor, since the domesticated individuals should display significant proportions of genetic diversity of the wild population from which they were derived. At $K = 3$, the most likely number of groups, Colli-Silva *et al.* did find one plant of cupuí with a signal of admixture (Fig 2d) and that plant is from Pará, not Balbina or São Gabriel de Cachoeira, the putative origin of domestication in their hypothesis. There is no admixture in cupuaçu, where it should be more abundant if it were selected from cupuí. What $K = 3$ does suggest is that cupuí is paraphyletic, as mentioned above and shown in Fig 2a, 2b and Fig S4.

Colli-Silva *et al.* suggest that the reduced genetic diversity (Table S3) and the lower number of loci under selection associated with a greater mutation load (Fig. 2e) in cupuaçu when compared to cupuí may be direct evidence of a domestication bottleneck in cupuaçu. This is expected during domestication, whether from wild cupuaçu or cupuí.

Additionally, the reduction of genetic diversity and increased mutation load may result from post-domestication processes also (Allaby *et al.* 2019), such as dispersal (genetic drift) and subsequent local selections. Furthermore, the tests for selection and annotation recovery were based on the coding sequences of cacao, which assume complete synteny across the three species' genomes. Given the difficulties mentioned above (Pool *et al.* 2010; Leigh *et al.* 2018) this assumption requires careful evaluation, which Colli-Silva *et al.* do not provide.

The demographic history of cupuaçu (Fig 2g) suggests a remarkable loss of nucleotide diversity between 8000 and 5000 years ago, conventionally called a bottleneck, as well as a gradual and expected decline in the last 100-200 years of dispersal (see History above). If cupuaçu was selected from cupuí, the period before 8000 years ago should represent cupuí, but this is not clear in Methods. If it does represent cupuí and the authors then adopted cupuaçu nucleotide diversity, the 8000-5000 year ago loss may be an artifact of the poorly selected collecting localities that combine domestication and a long history of dispersal. Assuming that the loss is real requires an examination of domestication theory. Rindos (1984) suggested that domestication starts in the landscape, i.e., humans select individual plants, protect and tend them, and these then have greater probability of reproduction, with or without humans dispersing them. This kind of domestication of plant populations in the landscape has been reported for cupuí in the upper Negro River (Franco-Moraes *et al.* 2019) and eastern Pará and northeastern Maranhão (Balée 1994). Recently, Allaby and colleagues (2022) restated Rindos' proposal and suggested that one of the implications is that genetic bottlenecks are less common than previously imagined (Allaby *et al.* 2019). Another of the implications is that human selection is only slightly stronger than natural selection, especially at the beginning of the process (Allaby *et al.* 2015). This kind of selection in the landscape is incompatible with the 8000-5000-year bottleneck. If the loss in Fig 2g is real, a different type of domestication is necessary: vegetative propagation. As Harlan (1992) pointed out, this is instant domestication, and the loss of genetic variability is dramatic – all alleles not present in the cloned plant are excluded from the domesticate. Although Amazonian Indigenous Peoples practiced vegetative propagation with numerous crops, this was all via cuttings, such as with manioc (*Manihot esculenta*) and other herbaceous annuals. There is no record of the use of cuttings with woody perennials, such as cupuí and cupuaçu, nor is there a record of grafting (Patiño 1963; Patiño 2002), which is common today with cupuaçu (Alves *et al.* 2014). Since the 8000-5000 BP loss of diversity cannot be attributed to domestication, it may be due to the extinction of Pleistocene megafauna who dispersed *Theobroma* spp before humans arrived (Guimaraes *et al.* 2008).

Conclusions

Our review of the available information about cupuaçu and cupuí suggests that Colli-Silva *et al.*'s proposal that cupuaçu was domesticated from cupuí in northwestern Amazonia is wrong. In terms of botany, there is no evidence in the literature before Colli-Silva *et al.*'s publication that cupuaçu is not a valid species and these authors do not provide such evidence. In fact, their new phylogeny shows that cupuaçu is valid. They do show that there is a strong possibility that cupuí contains multiple lineages, rather than being a monophyletic species. In terms of precolonial archaeology, there are currently no macro or micro remains that have been attributed to cupuaçu or cupuí. In

terms of biogeography, ignoring the region where cupuaçu is spontaneous eliminated the opportunity of finding the real origin of domestication of cupuaçu. In terms of linguistics, words for cupuaçu in languages of the Negro River are consistently more innovative than are terms for cupuí, and the majority have a plausible source in the Nheengatú term that would have accompanied cupuaçu's arrival in the region in the colonial period. In terms of history, although there are conflicting reports from the 18th century, it appears that cupuaçu started to be domesticated during the last 100-200 years, not 8000-5000 years ago, and was dispersed into the Negro River basin in the last 100 years. In terms of genetics, the genomic analysis says nothing about the domestication of cupuaçu from cupuí because cupuaçu is a valid species and the wild distribution was not sampled, although it provides some information about cupuí, i.e., it is not monophyletic.

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