INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA
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HISTÓRIA EVOLUTIVA DAS ÁRVORES DE CUIA (*Crescentia cujete*): UMA INTEGRAÇÃO ENTRE GENÓTIPO, AMBIENTE E CULTURA

PRISCILA AMBRÓSIO MOREIRA

Manaus, Amazonas
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Tese apresentada ao Instituto Nacional de Pesquisas da Amazônia como parte dos requisitos para obtenção do título de Doutor em Botânica.

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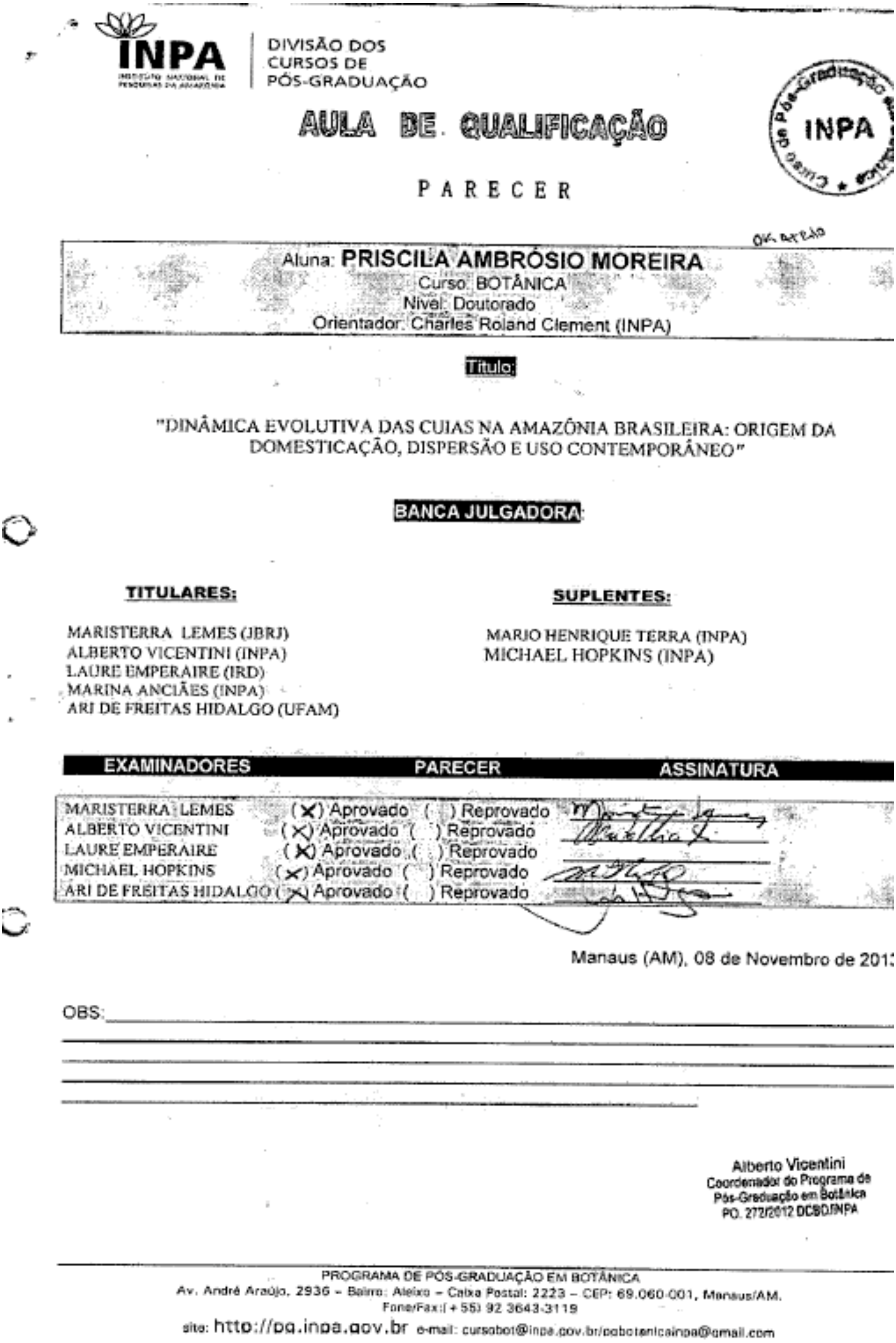

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"DINÂMICA EVOLUTIVA DAS CUIAS NA AMAZÔNIA BRASILEIRA: ORIGEM DA DOMESTICAÇÃO, DISPERSÃO E USO CONTEMPORÂNEO"

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ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DE DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA DO INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA

Aos quatorze dias do mês de agosto de 2017, às 14h00h, na sala de seminários da biblioteca do INPA-Campus I, reuniu-se a Comissão Examinadora da Defesa Pública, composta pelos seguintes membros: Dr. Michael John Gilbert Hopkins, do Instituto Nacional de Pesquisas da Amazônia – INPA, Dra. Patrícia Goulart Bustamante, da Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA, Dr. Rogério Gribel, do Instituto Nacional de Pesquisas da Amazônia - INPA Dr. Valdely Ferreira Kinupp, do Instituto Federal do Amazonas - IFAM, e Dra. Maria Teresa Fernandez Piedade, do Instituto Nacional de Pesquisas da Amazônia - INPA, tendo como suplentes: Dr. Adrian Paul Ashton Barnett, do Instituto Nacional de Pesquisas da Amazônia – INPA, e Dra. Izeni Pires Farias – da Universidade Federal do Amazonas - UFAM, sob a presidência do primeiro, a fim de proceder a arguição pública da TESE DOUTORADO da discente: **Priscila Ambrósio Moreira**, intitulada: "Genética e etnobotânica de cuias (*Crescentia* spp) na Amazônia para reconstruir sua história de uso e domesticação" sob a orientação: Dr. Charles Roland Clement e Coorientação do Dr. Yves Vigourox. Após a exposição, dentro do tempo regulamentar, a discente foi arguida oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

EXAMINADORES	PARECER		ASSINATURA
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IZENI PIRES FARIAS	( ) APROVADO	( ) REPROVADO	

Manaus (AM), 14 de Agosto de 2017.

OBS:____

Nada mais havendo, foi lavrado a presente ata, que, após lida e aprovada, foi assinada pelos membros da Comissão Examinadora

Dr. Michael John Gilbert Hopkins
Coordenador do Programa de
Pós Graduação em Botânica
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História evolutiva das árvores de cuia (*Crescentia cujete*):
uma integração entre genótipo, ambiente e cultura

Tese (doutorado em Botânica)--INPA, Manaus, 2017.

Orientador: Clement, Charles Roland

Área de concentração: Botânica

1. Cultura material 2. Domesticação de plantas 3. Ecologia histórica. 4. Filogeografia

CDDNNNNNNNN

Sinopse:

Estudou-se a reconstrução da história de domesticação, dispersão e diversificação das cuieiras (*Crescentia cujete*, Bignoniaceae) na Amazônia brasileira. Os frutos (cuias) possuem valores simbólicos e tecnológicos entre diferentes povos nativos da região Neotropical e, portanto, podem ser bons exemplos para integrar aspectos sociais e culturais à biologia da domesticação.

Palavras chave: Cultura material, Domesticação de plantas, Ecologia histórica, Filogeografia

Dedicatória

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"Tudo é escritura, ou seja, fábula. Mas para que nos serve a verdade que tranquiliza o honesto proprietário? A nossa verdade possível tem de ser invenção, ou seja, literatura, pintura, escultura, agricultura, piscicultura, todas as *turas* deste mundo. Os valores, *turas*, a santidade, uma *tura*, a sociedade, uma *tura*, o amor pura *tura*, a beleza, *tura* das *turas*."

(Julio Cortázar)

Resumo

Amazônia é um centro de domesticação de plantas, onde também se desenvolveu alta diversidade linguística, diferentes estilos artísticos e estratégias de subsistência. O papel dos humanos na distribuição geográfica da biodiversidade amazônica e as diferentes pressões que exercem na história de vida dos organismos tem sido amplamente debatido. A análise histórica do uso de uma planta ao longo de sua distribuição geográfica contribui com este debate, uma vez que busca identificar as modificações morfológicas e genéticas promovidas pelas atividades humanas, identificar áreas de diversidade biológica e cultural, bem como entender os fatores que influenciam o dinamismo no uso e manejo de suas variedades. Nesta perspectiva, o objetivo geral da presente tese foi contribuir com a reconstrução da história de domesticação, dispersão e diversificação das cuieiras (*Crescentia cujete*, Bignoniaceae) na Amazônia brasileira. Os frutos (cuias) possuem valores simbólicos e tecnológicos entre diferentes povos nativos da região Neotropical e, portanto, podem ser bons exemplos para integrar aspectos sociais e culturais à biologia da domesticação. A partir do desenvolvimento de uma biblioteca genômica de *C. cujete,* foram desenvolvidos marcadores moleculares, os quais serviram de base para avaliar a relação genética entre *C. cujete* e *C. amazonica* na Bacia Amazônica e testar as hipóteses de Gentry (1980) e Ducke (1946) sobre a origem da domesticação e diversificação morfológica dos frutos. A avaliação de como as pessoas percebem, usam e propagam os frutos de ambas as espécies na Amazônia e a integração de análises genéticas, morfológicas e etnobotânicas entre Amazônia e Mesoamérica possibilitou discutir hipóteses alternativas de origem da domesticação, inferir rotas de dispersão da planta e identificar fatores culturais e ecológicos de seleção e diversificação. Os resultados mostraram que hibridização é importante na diversificação fenotípica da planta. Variedades locais (*maracá, cuiupi, paranã*) estão intimamente associadas ao fluxo gênico entre quintais e florestas alagáveis, e seus usos específicos sugerem que o manejo da hibridização é uma prática antiga na Amazônia. Foi demonstrado que *C. cujete* foi introduzida na Bacia Amazônica e no México, mas sua origem geográfica permanece desconhecida. O padrão de diversificação genética entre Leste e Oeste na Bacia Amazônica permitiu inferir duas rotas de introdução na Amazônia. A dispersão concorda com rotas anteriormente propostas para grupos humanos e suas plantas. Mesoamérica e Amazônia são contrastantes em termos da diversidade morfológica de frutos cultivados de *C. cujete*. A diversidade e distribuição do formato dos frutos revelam que, apesar do amplo uso utilitário dos frutos, existiram diferentes preferências culturais ao longo da distribuição da planta. Mesmo após o colapso demográfico de populações humanas e suas plantas causado pela conquista europeia, os formatos e tamanhos de frutos manipulados atualmente pelos povos indígenas e famílias ribeirinhas são legados da ocupação pré-colombiana na Amazônia.

Abstract

Amazonia is a center of plant domestication, where high linguistic diversity, different artistic styles and subsistence strategies also developed. The role of humans in the geographical distribution of Amazonian biodiversity, and the different pressures they exerted in the life history of organisms are debated. The historical analyses of the use of a plant across its geographical distribution contributes to this debate, since it aims to identify the morphological and genetic modifications promoted by human activity, identify areas of biological and cultural diversity, as well as understand the factors that influence the dynamics of the use and management of plant varieties. In this context, the general objective of this thesis was to contribute to the reconstruction of the domestication history, dispersal and diversification of treegourds (*Crescentia cujete*, Bignoniaceae) in Brazilian Amazonia. The fruits (*cuia*) have symbolic and technological values for different native peoples across the Neotropics, and therefore, are good examples for the integration of the social and cultural aspects of the biology of domestication. From a genomic library of *C. cujete*, molecular markers were developed, which were the basis to analyze the genetic relationship between *C. cujete* and *C. amazonica* in the Amazon Basin and test Gentry's (1980) and Ducke's (1946) hypotheses about the origin of domestication and morphological diversification of treegourd fruits. The evaluation of how people perceive, use and propagate fruits of both species integrated with genetic, morphological and ethnobotanical analyses between Amazonia and Mesoamerica allowed discussion of alternative hypothesis of the origin of domestication, infer routes of the plant dispersal, and identify cultural and ecological factors of selection and diversification. The results showed that hybridization is important for the phenotypic diversity of the fruit. Local varieties (*maracá*, *cuiupi*, *paranã*) are intimately associated with gene flow between homegardens and flooded forests, and their specific uses suggest that management of hybridization is an ancient practice in Amazonia. It was demonstrated that domesticated *C. cujete* was introduced into the Amazon Basin and into Mexico, but its geographical origin of domestication remains unknown. The pattern of genetic diversification between Western and Eastern Amazonia allowed inferences of two routes of introduction. These agree with routes previously proposed for human groups and their plants. Mesoamerica and Amazonia have contrasting fruit morphological diversity, which suggests different cultural preferences along treegourd's dispersal routes. Even after the demographic collapse of human populations and their plants caused by European conquest, fruit shapes and fruit sizes manipulated by modern Native Amazonians and riverine families are legacies of the pre-Columbian occupation in Amazonia.

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Capítulo 1

Introdução Geral

A domesticação de plantas e animais permitiu o crescimento da população humana ao longo do Holoceno e o surgimento de sociedades estratificadas (Bellwood, 2005). Esta tese, fundamentada principalmente na associação entre a dispersão de línguas e a emergência da agricultura na Polinésia e Europa (Bellwood & Renfrew, 2002), ainda não foi confirmada na Amazônia (Blench, 2012; Neves, 2016). Embora evidências arqueológicas indiquem uma ampla rede econômica e política no fim do Holoceno na Amazônia (Hornborg, 2005; Heckenberger & Neves, 2009), tal complexidade social não está necessariamente associada ao desenvolvimento de agricultura na região (Moraes, 2015; Neves, 2016). Além disso, interpretar agricultura como sinônimo de cultivo de espécies domesticadas é uma visão bastante reduzida da diversidade de sistemas de produção de alimentos existentes ao redor do mundo (Leach, 1997; Michon et al., 2013). O comportamento e estratégias humanas de subsistência podem ser diversas, uma vez que acontecem em uma matriz interativa de espécies de interesse e manejo da paisagem em resposta às demandas sociais e ecológicas (Terrell et al., 2003; Balée & Erickson, 2006). É provável, portanto, que o balanço entre disponibilidade, necessidade e aguçado conhecimento sobre a flora e a fauna (Terrell et al., 2003) tenha resultado nos diferentes graus de domesticação de plantas e paisagens na Amazônia (Clement, 1999a; Clement et al., 2012). Agrícolas ou não, a capacidade de alterar o ambiente tornando-o mais habitável, favoreceu o crescimento da população humana, moldando a distribuição geográfica de espécies de plantas e animais no planeta (Boivin et al., 2016). Reconstruções arqueológicas mostraram duas fases de crescimento populacional humano na América do Sul: primeiro um crescimento logístico a partir de 13.000 anos AP (antes do presente), ampliando a dispersão no continente ao longo dos primeiros 8.000 anos de ocupação; seguido de um aumento exponencial na densidade populacional a partir dos 5.500 AP, quando provavelmente já havia maior interação entre as populações humanas, difusão de práticas e tecnologias, adaptação humana às paisagens e por consequência, aumento da capacidade de suporte (Goldberg et al., 2016). Em ambas as fases, alimentos e materiais foram importantes, mas a demanda provavelmente se tornou mais intensa conforme a população cresce. Os registros mais antigos de produção de cerâmica na Amazônia provém desta fase de aumento populacional, com 7.000 anos AP no baixo rio Amazonas em Santarém (Roosevelt et al., 1991). Neste período, a mandioca já havia sido dispersa do sudoeste da Amazônia para a costa do Pacífico no Peru (Pearsall, 1992) e as abóboras (*Cucurbita* sp.) da Mesoamérica para a região do rio Caquetá na Amazônia colombiana (Piperno, 2011). Para que a origem da domesticação e a dispersão subsequente da planta sejam propostas, tem sido necessário distinguir entre silvestre e domesticado em termos morfológicos e genéticos (Zeder et al., 2006; Clement et al., 2010; Meyer et al., 2012; Pickersgill, 2013; Larson et al., 2014, Boivin et al., 2016; Levis et al., 2017). Embora seja uma questão básica, nem sempre é fácil de reconhecê-la, pois estágios intermediários são comuns (Lévi-Strauss, 1950; Lins Neto et al., 2014). Além disso, a distinção depende de como diferentes sociedades humanas percebem e manejam a diversidade biológica (Caillon & Degeorges, 2007), uma vez que o mesmo recurso biológico acessível para diferentes grupos humanos é geralmente usado e valorizado de diferentes maneiras (Vandebroek, 2016). A domesticação de plantas depende, portanto, da investigação da dinâmica do uso e manejo da espécie a longo prazo em uma ampla escala geográfica. Nesta perspectiva, a reconstrução da história de uso das plantas pode ampliar o conhecimento sobre 1) como a história de ocupação humana na Amazônia foi influenciada pela domesticação de plantas, 2) como as plantas respondem à manipulação humana, e 3) como povos pré-colombianos influenciaram distribuição e diversificação de plantas usadas atualmente.

O objetivo geral da presente tese foi contribuir com a reconstrução da história de domesticação, dispersão e diversificação das cuieiras (*Crescentia cujete*, Bignoniaceae) na Amazônia brasileira. Os frutos (cuias) possuem valores simbólicos e tecnológicos entre diferentes povos nativos da região Neotropical e, portanto, podem ser bons exemplos para integrar aspectos sociais e culturais à biologia da domesticação. Os objetivos específicos foram:

(1) Analisar o papel da hibridização na diversidade morfológica e genética das árvores de cuias cultivadas;

(2) Examinar como famílias ribeirinhas percebem, usam e manejam a diversidade de cuias;

(3) Avaliar as hipóteses propostas sobre a origem da domesticação e inferir rotas de dispersão da planta;

(4) Identificar fatores genéticos, ecológicos e culturais associados à diversificação da planta.

1.1 *Uma breve história da tecnologia*

Tecnologia (do grego *tékhné* e *lógos*) significa a união entre ciência e arte. Uma das tecnologias mais antigas é o uso e manejo do fogo (Pyne, 1998), o qual está presente em diversas cosmologias e que remete ao tempo em que humanos conversavam com os animais (Mindlin, 2002; Lévi-Strauss, 2004). A partir do fogo, outras tecnologias foram desenvolvidas, como a domesticação de plantas e paisagens (Clement et al., 2012). Também, a partir do fogo foi possível o desenvolvimento de distintos centros de produção cerâmica pela Amazônia, os quais foram importantes para o processamento de alimentos (Neves, 2016).

A domesticacão de plantas pode ser definida como um processo coevolutivo no qual a seleção feita por humanos sobre os fenótipos de indivíduos tornam as futuras populações mais interessantes do ponto de vista do uso humano e mais adaptadas às paisagens modificadas pelos humanos (Clement, 1999a). A domesticação de plantas surgiu diversas vezes ao redor do mundo a partir de melhoristas desconhecidos e hábeis em perceber a diversidade, que - mesclando arte e ciência - inventaram modos de satisfazer as necessidades humanas (Vavilov, 1951). A partir do século XX, o conhecimento da variação biológica, com o advento da genética, uniu a prática antiga de agricultores tradicionais à teoria. Desta forma se adotou seleção metódica que serviu de base para o melhoramento genético moderno (Vavilov, 1951). Com o avanço do capitalismo no século XIX, o conhecimento e a prática se tornaram também produto de grandes corporações e especulação financeira (Shiva, 2012), com especial atenção a algumas poucas plantas alimentícias para exportação (FAO, 2015). Atualmente é crescente o interesse pelo resgate de imensa diversidade de plantas alimentares amazônicas, muitas certamente envolvidas em algum nível de domesticação no passado (Clement, 1999), e perfeitamente possíveis de serem consumidas (Kinupp & Lorenzi, 2014).

Contudo, a humanidade não vive só de calorias, mas também de necessidades materiais, relações estéticas e simbólicas (Emperaire et al., 2016). Tais plantas usadas para fins de ordem técnica e simbólica (ou simplesmente "plantas tecnológicas") estão associadas à cultura material (Ribeiro, 1995; Patiño, 1967; Albert & Milliken, 2009). O uso da maioria vem sendo substituído por materiais metálicos ou a base de petróleo, o que, de forma semelhante à situação alimentar, nos coloca dependentes de fontes restritas de recursos. Paralelamente, o uso de plantas tecnológicas tem se transformado em meros artigos de museu ou artesanatos turísticos. No entanto, um artefato não pode ser considerado fora de seu contexto social (van Velthem, 2012). Estas plantas estão intimamente relacionadas a diversos contextos do cotidiano, como à obtenção e processamento de alimentos, à qualidade da moradia e proteção, à mobilidade, à música, à espiritualidade (Ribeiro, 1995; Patiño, 1967; Albert & Milliken, 2009). A manutenção de determinadas práticas agrícolas, por exemplo, é determinante na existência da produção de artefatos como peneiras, cestos ou remos no médio rio Solimões (Sousa, 2009). Além do uso prático e funcional do artefato, a produção depende de habilidades manuais e percepção do ambiente e da planta, cujo aprendizado não faz sentido a não ser na experiência prática (Ingold, 2002).

Domesticação é, portanto, a interação entre sociedades humanas e a biodiversidade, e por definição, assume um caráter interdisciplinar. Assim, domesticação não se limita ao melhoramento genético (Clement et al., 2012), o qual promoveu a disseminação de variedades de rendimento máximo e transferência de tecnologia, ainda que no Brasil às custas do avanço da fronteira agrícola sobre a Amazônia (Carneiro da Cunha, 2012); mas também se constitui de outros sistemas de conhecimento locais pouco reconhecidos (Posey, 1987; Carneiro da Cunha, 2012). Há, portanto, centros "invisíveis" de geração de tecnologia na Amazônia, onde se produz conhecimento sobre como suprir a própria subsistência, mas que não oferecem necessariamente valor de mercado (Clement, 2007). Neste contexto, as reflexões sobre o "saber fazer" que as plantas de uso tecnológico despertam foram uma fonte primária de inspiração para a escolha da espécie a ser analisada nesta tese.

1.2 A ecologia histórica da Amazônia

Estudos em ecologia histórica buscam descrever as mudanças espaciais e temporais na relação entre humanos e ambiente, as quais exercem mútua influência (Balée & Erickson, 2006). Dentre as diferentes questões e disciplinas que permeiam esta abordagem (Armstrong et al., 2017), destaca-se a importância da análise histórica do uso de um recurso. A partir desta questão de base, é possível identificar as modificações morfológicas e genéticas promovidas pelas atividades humanas, mapear áreas de diversidade biológica e cultural, bem como entender os fatores que influenciam o dinamismo no uso e manejo de variedades de plantas ao longo de séculos e milênios. Na Amazônia, é crescente o número de estudos analisando a dinâmica a longo prazo dos sistemas de uso e manejo de plantas (Clement et al., 2009; 2010, 2016; Junqueira et al., 2010; Shepard & Ramirez, 2011; Fraser et al., 2012; Lins et al., 2015; Moreira et al., 2015; Smith & Fausto, 2016; Emperaire et al., 2016; Alves et al., 2016; Levis et al., 2017). O mapeamento da diversidade de plantas cultivadas e manejadas na bacia amazônica tem mostrado a importância de sítios arqueológicos como reservas de agrobiodiversidade, destacando a influência do manejo humano em períodos pré-colombianos nas paisagens atuais (Clement et al., 2003; Junqueira et al., 2010, Levis et al., 2012; 2017; Lins et al., 2015). O acúmulo de sementes e propágulos de diferentes origens promovido pela dispersão humana parece ter contribuído para a concentração de diversas plantas frutíferas ao longo da bacia amazônica (Clement, 1999ab; Clement et al., 2003; Junqueira et al., 2010; Levis et al., 2017). No entanto, ainda é difícil discernir quanto deste efeito foi motivado por sociedades pré-colombianas, manejo mais recente ou uma associação de ambos. Além disso, ao longo dos diferentes ecossistemas amazônicos e do longo tempo de ocupação humana, de pelo menos 13.000 anos (Roosevelt, 2014), as estratégias adaptativas podem ser muito variáveis (Moran, 1991), embora não necessariamente condicionadas por limitações ecológicas (Balée & Erickson, 2006). É provável que durante o início do Holoceno, o modo de produção de alimentos na Amazônia estivesse associado ao manejo *in situ* de espécies frutíferas e não a uma agricultura propriamente dita baseada no cultivo itinerante de roças (Clement, 2006; Neves, 2016). Mais tarde, a adoção do modo de vida sedentário é relacionado ao aparecimento de lixeiras com restos orgânicos e formação de solos antropogênicos (Neves et al., 2003). Solos antropogênicos (terras pretas de índio) marcam mudanças culturais e tecnológicas profundas na Amazônia, as quais estão sendo reconstruídas especialmente a partir da história de uso da mandioca (*Manihot esculenta*). Estes solos de alta fertilidade estão associados à origem da domesticação da mandioca e a adoção de cultivos de tubérculos como fontes mais estáveis de calorias (Arroyo-Kalin, 2010). Datações arqueológicas demonstraram que a fase sedentária se intensificou na América do Sul há 5.500-3.500 anos (Goldberg et al., 2016), enquanto que os registros mais antigos de solos antropogênicos datam de 7.000 anos no sudoeste da Amazônia (Neves, 2016). Ainda não é consenso se a domesticação de plantas tenha favorecido a expansão geográfica de populações humanas a partir do Sudoeste (Neves, 2016), especialmente porque as rotas de ocupação humana na Amazônia são complexas, caracterizada pela alta riqueza de famílias linguísticas e distintas formas e contextos de ocupação de assentamentos, como revelam as mudanças nos estilos cerâmicos ao longo da bacia amazônica (Neves, 2016; Barreto et al. 2016). É possível que o início da produção cerâmica na Amazônia não tenha relação com a domesticação inicial de plantas, e que, portanto, agricultura voltada para produção de alimentos não tivesse sido tão importante no desenvolvimento de sociedades complexas amazônicas (Neves, 2016). Nesta hipótese, a ampla rede de circulação de produtos e plantas seria oportunista e generalista, voltada ao consumo recreativo e religioso e não puramente alimentício, como sugere a ampla dispersão de milho e tabaco no período pré-Colombiano (1492) da Mesoamérica ao sul da América do Sul (Neves, 2016).

A perspectiva da ecologia histórica revela que, se por um lado, humanos ampliam a mobilidade das plantas e podem favorecer sua adaptação ecológica, o que finalmente influencia a dinâmica evolutiva das plantas (Rindos, 1984; Sodero Martins, 2005; Zeder, 2016), por outro, a história da domesticação de plantas pode ser indicador de comportamentos humanos e mudanças culturais, uma vez que a domesticação de plantas constitui uma das práticas que melhoram os assentamentos humanos e a qualidade do bem-viver (Smith, 2011; Zeder, 2016). A ecologia histórica, portanto, contribui para entender o contexto cultural no qual as plantas domesticadas expandiram sua distribuição geográfica.

1.3 Biogeografia das espécies vegetais com uso tecnológico e simbólico

Plantas tecnológicas fornecem matéria prima para suprir necessidades materiais e, portanto, é possível que tenham sido domesticadas antes mesmo das espécies alimentícias (Bellwood, 2005). A diversidade de artefatos tecnológicos (colheres, facões, arpões, flechas) feitos de pupunha (*Bactris gasipaes*) sugere que populações silvestres da planta foram selecionadas inicialmente para a qualidade da madeira (Clement et al., 2009). Os frutos de cabaças (*Lagenaria siceraria*), amplamente usados como recipientes, estão entre as plantas cultivadas mais antigas nos Neotrópicos (Piperno, 2011; Kistler et al., 2014). Embora as suas sementes sejam comestíveis (Heiser, 1993; Morimoto et al., 2005), a seleção de populações silvestres ancestrais na África teve como foco os frutos maiores, de diferentes formatos e não as sementes (Morimoto et al., 2005), o que sugere que o uso como recipiente precedeu o alimentício. Possivelmente, o uso das sementes seja um subproduto da diversificação dos frutos (Heiser, 1993), uma vez que populações silvestres possuem polpa tóxica (Morimoto et al., 2005). As abóboras (*Cucurbita* spp.), cujas populações silvestres apresentam cascas duras e polpa tóxica, parecem ter sido amplamente dispersas pelos grandes mamíferos do Pleistoceno (megafauna) na América atéo início do Holoceno (Kistler et al., 2015), o que não exclui a possibilidade de uso humano neste período, uma vez que várias espécies silvestres de *Cucurbita* no México têm registro de usos tecnológicos (Lira & Caballero, 2002). No entanto, a partir da extinção de seus dispersores, a adaptação de *Cucurbita* spp. aos assentamentos humanos favoreceu frutos não tóxicos com sementes maiores (Smith, 2006; Kistler et al., 2015), ampliando uso alimentício dos tipos domesticados.

Diversos centros de domesticação de plantas alimentícias foram postulados na América do Sul, em especial na periferia da bacia amazônica, onde populações silvestres de plantas domesticadas foram identificadas com análises genéticas e morfológicas (Clement, 1989; 1999a,b; Clement et al., 2010; 2016; Piperno, 2011). A distribuição e diversificação de espécies tecnológicas na bacia amazônica ainda não foram examinadas. A especialização na produção de artefatos na Amazônia pré-Colombiana (Ribeiro, 1995) aponta para a ocorrência de diferentes centros tecnológicos regionais. Nas águas pretas da bacia do rio Negro, os povos Tukano eram exímios artesãos de bancos de madeira, os Baniwa produziam as pimentas e ralos do processamento da mandioca, os Kubewa as máscaras, os Maku as flechas zarabatanas e cestos cargueiros aturá (Ribeiro, 1995; van Velthem, 2012). Nas águas barrentas e férteis do alto rio Solimões, as mulheres Cocama, Omagua, Yurimaguas eram referência na produção de cuias tingidas com cumatê (resina de diversas espécies botânicas), cuja prática também foi disseminada em Monte Alegre no baixo Amazonas (Rodrigues-Ferreira, 1933; Métraux, 1948). Esta especialização pode ter favorecido níveis diferenciados de seleção ao longo da distribuição da planta usada como matéria-prima.

O mapeamento de centros de domesticação de plantas na bacia amazônica ainda é incipiente. A concentração de centros de domesticação na periferia da bacia é apoiada por poucas espécies, não só pela escassez de estudos botânicos direcionados a esta questão, mas também porque possivelmente o manejo de espécies abundantes na floresta tenha sido mais importante na manutenção das populações humanas do que agricultura para produção de alimentos (Moraes, 2015; Neves, 2016; Levis et al., 2017), deixando, portanto, sinais incipientes de domesticação. Ao contrário, o estudo de plantas não alimentícias, mas com valores tecnológicos e simbólicos, pode ampliar o conhecimento sobre os contextos culturais e ecológicos onde a domesticação de plantas pode ter sido promovida na Amazônia.

1.4 Síndromes de domesticação: uma integração entre genótipo, ambiente e cultura

O conjunto de características que se modifica entre a população ancestral e a população submetida aos critérios de seleção humana se denomina síndrome de domesticação (Harlan, 1992; Meyer et al., 2012). A seleção tem consequências a partir da proteção e facilitação da planta em seu próprio ambiente natural, o que favorece a reprodução das plantas protegidas (Rindos, 1984). À medida que uma característica de interesse é valorizada, seleção se manifesta a partir do transplante intencional de propágulos para quintais, roçados, trilhas (Zeder et al., 2006). Além disso, as próprias condições do ambiente nos assentamentos humanos podem modificar a seleção à qual os organismos são submetidos, podendo favorecer fenótipos raros e pouco adaptativos na natureza, os quais serão propagados, se a característica for de interesse (Rindos, 1984; Milla et al., 2015).

Quando propágulos são trazidos para assentamentos humanos, o fluxo gênico entre as populações dos diferentes ambientes pode se tornar mais restrito. Desta forma, espera-se que a diversidade genética diminua a partir de eventos fundadores e adaptação a novos habitats (Vavilov, 1951; Schaal & Olsen, 2000; Zeder et al., 2006). Como consequência, as práticas de seleção promovem gradativas divergências genéticas e morfológicas em relação a sua população silvestre ancestral (Harris, 1989; Rindos, 1984; Clement, 1999; Casas et al., 2007; Pickersgill, 2013).

Esta síndrome, como qualquer outra síndrome ecológica, pode ser diversa, uma vez que está relacionada a diferentes práticas humanas e a adaptação das espécies aos ambientes (Rindos, 1984; Meyer et al., 2012; Milla et al., 2015). Como resultado, a diversidade de fenótipos entre os tipos domesticados geralmente é mais ampla que nos silvestres (Tanksley et al., 2004; Morimoto et al., 2005). A diversidade entre as frutas cultivadas na Amazônia é significativa, apesar das perdas históricas pela colonização europeia (Clement, 1999, a, b). A síndrome de domesticação de plantas frutíferas envolve especialmente mudanças na qualidade nutricional dos frutos, como teor de óleo, amido, açúcar, compostos secundários ou quantidade de água (Clement, 2006; Meyer et al., 2012). A partir dos frutos pequenos originais de pupunha (*Bactris gasipaes*) foi desenvolvido um fruto maior e mais rico em amido, o que permitiu melhor fermentação e estocagem de alimento, mas também produção de álcool para consumo em festividades (Clement, 2006). No baixo rio Tapajós, as comunidades locais selecionam e promovem frutos não amargos de piquiá (*Caryocar villosum*) (Alves et al., 2016). A variação morfológica encontrada em populações silvestres de pequi (*Caryocar brasiliense*) no alto rio Xingu foi selecionada e concentrada em diferentes variedades, especialmente devido ao interesse do povo Kuikuro pela experimentação (Smith & Fausto 2016). O acúmulo de estudos com mandioca (*Manihot esculenta*) é um exemplo clássico de que cada contexto tem diferentes características ambientais e culturais que moldam a diversidade existente das plantas cultivadas (Elias et al., 2000; Peroni et al., 2007; Emperaire & Peroni, 2007; Rival & McKey, 2008; Fraser et al., 2012; Peña-Venegas et al., 2014) e, portanto, sua conservação não faz sentido fora das interações regionais entre as plantas e as pessoas. Desse modo, sua conservação nos acarreta uma responsabilidade adicional, uma vez que o alto grau de adaptação aos assentamentos humanos é acompanhado de maior dependência da planta à propagação humana (Harlan, 1992; Clement 1999a).

No caso das plantas tecnológicas, espera-se mudanças na resistência, durabilidade do material e especificidade funcional. Tais modificações podem influenciar o ciclo reprodutivo das plantas, como a capacidade de dispersão e a deiscência dos frutos, como pode ter sido o caso das cuias e urucum, uma vez que os frutos foram o foco de seleção (Aguirre-Dugua et al., 2012; Moreira et al., 2015). Assim como os recursos alimentícios, os tecnológicos também envolvem modificação quanto à disponibilidade e produtividade, como pode ter ocorrido ao longo da domesticação de urucum com o aumento de produção de pigmentos em suas sementes (Moreira et al., 2015). A especificidade significa que a planta pode apresentar variedades com diferentes funções. As cuias feitas de frutos de *Crescentia cujete* no Suriname, por exemplo, apresentam diferentes tamanhos, formas e resistência do exocarpo (casca), cujas árvores recebem nomes correspondentes à funcionalidade de seus frutos: árvores com cuias grandes e resistentes são usadas para lavar comida e corpos em rituais funerários (*gaán kúya*), para fazer colheres e conchas (*pempe kúya, kuyêè*), como recipientes cobertos para armazenar líquidos (*koómbu kiíki*), como tigelas pequenas para lavar as mãos (*bakáa kúya, mátu kúya*), como copos para beber (*bíngo kúya*) (Meulenberg, 2011). No entanto, a substituição por outra matéria-prima com a mesma finalidade é possível, como mostra a convergência de usos das cuias (*Crescentia cujete*) e das cabaças (*Lagenaria siceraria*) no norte da América do Sul, apesar de alguns usos serem mais específicos que outros, como, por exemplo, instrumentos musicais ou como remédios (Price, 1982; Meulenberg, 2011; Albert & Milliken, 2009). Os motivos associados a esta redundância funcional devem depender não só da disponibilidade do recurso no local, mas também dos valores simbólicos ligados à planta e ao objeto, o que pode variar entre sociedades humanas (Lévi-Strauss, 2004). Interessante notar que se as matérias-primas são facilmente substituídas, esperar-se-ia menor grau de seleção e domesticação (Palmer, 1994). No entanto, tanto as árvores (*Crescentia cujete*), quanto as herbáceas rasteiras (*Lagenaria siceraria*) são considerados cultivos altamente domesticados (Heiser, 1993; Clement, 1999a).

A síndrome de domesticação de plantas é o resultado, portanto, da integração entre a variação genética e morfológica da planta em um balanço entre seleção humana e ambiental ao longo da sua distribuição geográfica (Figura 1). Assim, práticas humanas e condições ambientais podem moldar o fenótipo de uma planta. O conhecimento da diversificação em diferentes contextos ecológicos e culturais é chave para reconstruir a história de domesticação da planta (Meyer & Purugganan, 2013).

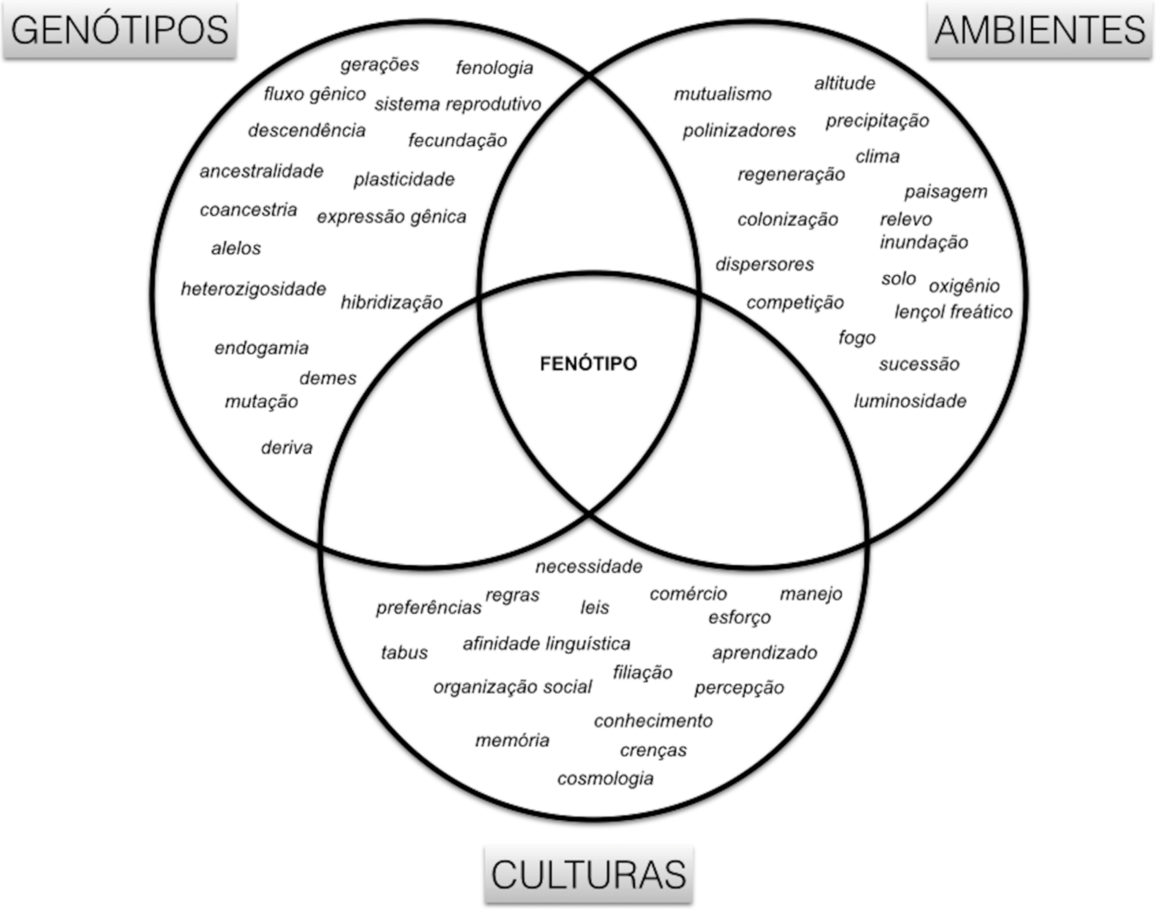

Figura 1. Representação esquemática das três dimensões que compõem os sistemas de domesticação de plantas, similar ao proposto por Leclerc & d'Eeckenbrugge (2012) e Milla et al. (2015). As áreas de sobreposição indicam a interação entre elas. A interação entre as dimensões revela o contínuo de variação que pode ser observado ao longo do processo de domesticação (Clement, 1999a). Uma espécie pode apresentar diferentes níveis de domesticação ao longo de sua distribuição.

1.5 Filogeografia aplicada à ecologia histórica da Amazônia

A filogeografia oferece a possibilidade de reconstruir a história evolutiva de uma espécie a partir da variação genética das populações atuais (Avise, 2004). A análise no contexto geográfico da espécie revela eventos demográficos de crescimento ou redução populacional que resultam de seleção, migração e deriva genética ocorridos no passado ao longo da sua distribuição (Avise, 2004). A variação encontrada no genoma cloroplastial e nuclear são as ferramentas básicas nos estudos de filogeografia. A análise de ambos os genomas fornece informações complementares. O genoma do cloroplasto possui taxas de mutação relativamente menores, o que implica em menor variação informativa; por outro lado, possui herança materna, o que facilita inferir rotas de dispersão. O genoma nuclear, herdado de ambos os parentais, permite detectar padrões mais completos da variabilidade genética. A variação no tipo de herança implica em diferentes tamanhos efetivos populacionais entre genoma nuclear e cloroplastial, o que é útil para diferenciar eventos de hibridização a longo ou curto prazo, uma vez que os marcadores inferem, respectivamente, eventos de divergência mais antigos e mais recentes (Schall & Olsen, 2000; Hare, 2001). Microssatélites (SSR) e SNPs (single nucleotide polymorphisms) são marcadores encontrados em ambos os tipos de genoma, amplamente aplicados para detectar variabilidade, dada a magnitude de suas taxas de mutação (Vieira et al., 2016).

Embora fatores ecológicos, geológicos e climáticos em diferentes escalas de tempo demonstram ser importantes para influenciar o padrão genético de espécies (Colevatti et al., 2009; Cavers & Dick, 2013; Baker et al., 2014), espera-se que o manejo humano no passado ao longo de várias gerações da planta também deixe assinaturas na diversidade genética das populações (Schaal & Olsen, 2000; Hanotte et al., 2002; Parker et al., 2010; Armstrong et al., 2017). A identificação das consequências genéticas devido à seleção é essencial para determinar a área de origem da domesticação. Espera-se alta estrutura genética entre população ancestral silvestre e populações selecionadas, especialmente em eventos fortes de seleção (Clement, 1999a; Clement et al., 2010; Meyer & Purugganan, 2013). A seleção pode ocorrer apenas uma vez na área de distribuição da população silvestre ou em múltiplos eventos de domesticação, quando as características selecionadas são fixadas independentemente em diferentes regiões (Allaby et al., 2008; Meyer & Purugganan, 2013). A domesticação pode ser mais efetiva quando populações selecionadas são removidas da distribuição geográfica do parente silvestre, diminuindo assim possíveis hibridizações com populações silvestres (Zeder et al., 2006; Allaby et al., 2008). A ampla dispersão da população silvestre pode favorecer múltiplas domesticações. Evidências sugerem múltiplos eventos de domesticação em várias espécies neotropicais [(*Spondias purpurea*: Miller & Schaal (2005); *Persea americana*: Chen et al. (2009); *Theobroma cacao*: Thomas et al. (2012); *Lagenaria siceraria*: Kistler et al. (2014); *Cucurbita pepo* (Smith, 2006; Kistler et al., 2015)]. Embora *Lagenaria siceraria* seja nativa da África, análises moleculares recentes mostraram que populações silvestres foram domesticadas em diferentes regiões neotropicais, provavelmente a partir de frutos silvestres trazidos no final do Pleistoceno pelas correntes do Oceano Atlântico até a costa norte da América do Sul ao Caribe (Kistler et al., 2014). Múltiplos eventos de domesticação podem ser promovidos a partir de diferentes usos da planta, motivados por diferentes preferências culturais ou especializações ambientais ao longo da área de distribuição das populações silvestres. Deste modo, cacau (*Theobroma cacao*) foi amplamente disperso a partir do oeste da Amazônia para América Central a partir da seleção inicial para a polpa dos frutos, sendo novamente selecionado para chocolate quando alcançou América Central (Clement et al., 2010; Thomas et al., 2012). De forma semelhante, populações ancestrais silvestres de abacaxi (*Ananas comosus* var. *ananassoides*) no Norte da América do Sul foram selecionadas para frutos grandes e suculentos (*A. comosus* var. *comosus*), mas também para fibras longas e facilmente extraídas (*A. comosus* var. *erectifolius*) (Coppens d'Eeckenbrugge & Duval, 2009). No entanto, a identificação de populações ancestrais não tem se mostrado uma tarefa fácil (Pickersgill, 2013; Parra & Casas, 2016). Dentre as razões, incluem: o ciclo de vida perene das árvores implica em gerações mais longas e os gargalos genéticos motivados pela seleção podem não ser marcantes (Gaut et al., 2015; Aguirre-Dugua & González-Rodríguez, 2016); as categorias silvestre e domesticado não são entidades necessariamente bem definidas, mas geralmente categorias ao longo de um contínuo (Clement, 1999a; Lins Neto et al., 2014); hibridização com espécies silvestres relacionadas pode mascarar os gargalos genéticos de seleção e ainda dar origem a outros fenótipos em áreas diferentes da origem, sendo selecionados se apresentam características de interesse (Jarvis & Hodgkin, 1999; Miller & Gross, 2011, Goldschmidt, 2013); centros de origem não são necessariamente locais com maior diversidade e abundância da planta, pois também podem ser centros secundários onde se acumula diversidade (Vavilov, 1951); e finalmente, as conclusões dependem muito de estratégias exaustivas de coletas nas possíveis áreas de origem, bem como disponibilidade e abundância de marcadores moleculares (Meyer et al., 2012).

Na ausência de evidências diretas sobre a origem da domesticação, o efeito humano na distribuição geográfica da planta pode ser inferido de acordo com o esperado em seu nicho ecológico. Deste modo, o padrão genético esperado de acordo com as habilidades dos dispersores naturais da planta é comparado ao padrão observado. Este tipo de inferência foi feita para avaliar o papel da extinção da megafauna na estrutura genética das plantas (Guimarães et al., 2008). No caso do efeito humano na dispersão, espera-se baixa estrutura genética ao longo da distribuição, uma vez que se a planta responde bem à seleção e propagação local, humanos ampliam a área de distribuição a partir da propagação de uma mesma subpopulacão, como proposto para castanheira (*Bertholletia excelsa*) (Shepard & Ramirez, 2011). Além disso, considerando antigos sistemas de troca e comércio na região Neotropical, rios, ambientes ripários e sítios arqueológicos são chave para reconstruir história da difusão e diversificação, uma vez que são marcas de assentamentos por onde houve rotas de dispersão, não só de pessoas e diásporas linguísticas (Denevan, 1996; Guix, 2009; Clement et al., 2010; Heckenberger, 2013; Levis et al., 2017), mas também de plantas (Godoy et al., 1999).

A filogeografia aplicada à ecologia histórica ainda foi pouco estudada na Amazônia. No entanto, tem um papel importante para ampliar o entendimento do uso e ocupação humana na Amazônia, no que se refere à identificação de centros de origem de domesticação de plantas, rotas de difusão e persistência dos centros de acúmulo de diversidade de plantas domesticadas na Amazônia.

1.6 O gênero *Crescentia*

O gênero *Crescentia* pertence à Tribo Crescentiae (Bignoniaceae) e se caracteriza por árvores de pequeno a médio porte, cujos galhos se ramificam formando uma copa aberta. Produz flores com cálice grande, as quais partem de nós dos troncos (caulifloria) e frutos indeiscentes do tipo peponídeo, que quando maduros desenvolvem cascas lenhosas (Gentry, 1980). *Crescentia* é composto por seis espécies de acordo com as referências taxonômicas mais completa até o momento (Gentry, 1980), e aceita pelos principais bancos de dados botânicos (The Plant List, Tropicos). A maioria das espécies (4) é encontrada apenas na América Central e o Caribe, das quais duas restritas às ilhas do Caribe (*Crescentia mirabilis* e *C. portoricensis*), uma amplamente distribuída no Caribe, mas também em Belize (*C. linearifolia*), e outra restrita ao continente (*C. alata*). Apenas uma ocorre na América do Sul (*Crescentia amazonica*), sendo restrita ao Norte do continente, em florestas alagáveis das bacias dos rios Ucayali-Solimões-Amazonas, do rio Orinoco e as terras baixas das Guianas. A distribuição da espécie que deu origem ao cultivo domesticado (*Crescentia cujete*) não é conhecida, mas suas populações domesticadas ocorrem em toda região Neotropical. De acordo com Gentry (1980), *Crescentia cujete* é provavelmente nativa da América Central, onde são encontradas populações aparentemente silvestres em savanas e florestas deciduais entre o sul do México e norte da América Central. Populações silvestres encontradas do sul de México (Figura 2) apresentam frutos pequenos, alongados e com exocarpo mais fino que os tipos cultivados nas áreas vizinhas (Aguirre-Dugua et al., 2012).

Figura 2. Populações silvestres de Crescentia cujete nas savanas alagáveis na Península de Yucatan, México. Crédito das fotos: Xitlali Aguirre-Dugua.

Entretanto, não é descartada uma distribuição original de *Crescentia cujete* até o norte da América do Sul, onde também foram registrados indivíduos aparentemente espontâneos em áreas de vegetação aberta usadas como pastagem na Colômbia (Arango-Ulloa et al., 2009). É provável que as populações que deram origem ao tipo domesticado de *Crescentia cujete* sejam tolerantes ao fogo e à seca (Bass, 2004), o que pode ter favorecido sua expansão, dado o histórico manejo com fogo em savanas (Bass, 2004; Pinter et al., 2011; Mistry et al., 2016). Ainda não foi avaliada a dispersão de populações silvestres entre América Central e América do Sul pela megafauna, os quais poderiam dispersar suas sementes a longas distâncias (Janzen & Martin, 1982). Neste caso, a ampla distribuição geográfica de populações silvestres de *Crescentia cujete* poderia ter promovido mais de um evento de domesticação, como sugerido em *Cucurbita* spp. (Kistler et al., 2015).

Embora o uso humano tenha sido registrado entre a maioria das espécies de *Crescentia*, apenas *C. cujete* é amplamente cultivada. Dentre as espécies com ocorrência caribenha, há registros de uso medicinal de *C. mirabilis* em Cuba (Melander, 2007). Não há uso registrado para a espécie *Crescentia portoricensis,* sendo considerada ameaçada de extinção, devido ao baixíssimo tamanho populacional e desflorestamento de seu ambiente natural (Silander, 1991). Na América Central, o uso de *Crescentia alata* está associado a confecção de instrumentos musicais (chocalhos) e utensílios domésticos a partir dos frutos. Suas sementes servem no preparo de uma bebida típica (um tipo de *orchata*) da Nicarágua (Gentry, 1980). Apesar dos usos tecnológicos serem semelhantes aos de *Crescentia cujete*, esta espécie tem distribuição restrita às áreas secas da costa do Pacífico, do Mexico à Costa Rica (Gentry, 1980). Embora seja considerada uma espécie de ocorrência natural, forma grandes adensamentos (*jíracales*) nas áreas de savana sempre ligados à ocupação humana na costa do Pacífico em Honduras, os quais são promovidos pelos sistema silvo-pastoril, que integra criação de gado, agricultura e manutenção da vegetação nativa (Bass, 2004). Possui folhas trifoliadas bastante marcantes e por isso foi considerada como uma espécie distinta de *Crescentia cujete* (Gentry, 1980). No entanto, híbridos com folhas simples de *Crescentia cujete* e folhas trifoliadas de *C. alata* foram encontrados no Mexico, El Salvador, Guatemala e Costa Rica (Gentry, 1980). Na América do Sul, a espécie *Crescentia amazonica* (Figura 3) foi considerada por Gentry (1980) como naturalizada na Amazônia a partir de *C. cujete*, pois a possível ausência de seleção para recipientes nas florestas de várzea poderia ter desfavorecido os frutos grandes e duros geralmente observados em *C. cujete* (Gentry, 1980). Por outro lado, Ducke (1946), o qual descreve *Crescentia amazonica* pela primeira vez, acreditava que a espécie seria um ancestral silvestre envolvido na domesticação de *C. cujete*. O uso dos frutos de *C. amazonica* serve como substituto ocasional de frutos de *C. cujete* na região da Guiana Inglesa (van Andel, 2000). No Brasil, os frutos de *C. amazonica* estão associados à confecção de instrumentos musicais (*maracás*) (Ducke, 1946) e uso comercial no artesanato (Wittmann & Wittmann, 2011).

Figura 3. *Crescentia amazonica* na planície de inundação ao longo dos rios Solimões e Amazonas, Brasil. Populações espontâneas ocorrem em florestas de várzea (c, d), mas também são toleradas ou intencionalmente cultivadas em quintais (g). Frutos são coletados para artesanato (a), como o tradicional chocalho maracá (h). Crédito das fotos: Juliana Lins (a,b); Bruno Garcia Luize (c, e); Priscila Ambrósio Moreira (d, f, g, h).

Análises moleculares baseadas em bandas polimórficas de marcadores AFLP apontaram que *C. cujete* é mais similar à *C. alata* do que à *C. amazonica* (Arango-Ulloa et al., 2009). A conclusão, no entanto, foi baseada em uma única amostra de cada espécie-irmã, sendo que o indivíduo de *C. alata* poderia ser um híbrido.

A origem de *Crescentia cujete* e sua distribuição natural permanecem incerta. A análise de populações de *C. cujete* indicaram que os propágulos foram introduzidos no México, apesar da ocorrência de possíveis populações silvestres de *C. cujete* na região (Aguirre-Dugua et al., 2012). Uma ampla amostragem na Bacia Amazônica, incluindo *C. amazonica*, é necessária para avaliar as hipóteses propostas por Ducke (1946) e Gentry (1980). A análise da relação genética entre amostras mesoamericanas e amazônicas contribuirá para esclarecer hipóteses sobre a origem da domesticação e inferir rotas de difusão da planta entre os continentes.

1.7 A etnobotânica de Crescentia cujete

As árvores cultivadas de *Crescentia cujete* (Figura 4) apresentam diferentes formatos, tamanhos e espessura dos frutos (Gentry, 1980; Arango-Ulloa et al., 2009; Aguirre-Dugua et al., 2012).

Figura 4. *Crescentia cujete* cultivada na Bacia Amazônica brasileira ao longo da planície de inundação e em terra-firme. Árvores produzem frutos de diferentes formatos e tamanhos. Crédito das fotos: Priscila Ambrósio Moreira (a, b, d, e, f, g); Leonardo Kumagai (c).

A casca dura dos frutos impede a dispersão espontânea das sementes; devido a isto são dispersados essencialmente por humanos a partir da propagação vegetativa (Aguirre-Dugua et al., 2012). O cultivo de sementes é evitado no México, pois gera indivíduos considerados "não genuínos" pelos agricultores locais, o que significa frutos menores (Aguirre-Dugua et al., 2012). A ampla variação ecotípica destacada por Gentry (1980) certamente está relacionada ao manejo humano, uma vez que é cultivada em diferentes condições ecológicas. Alta diversidade morfológica dos frutos foi registrada nas áreas caribenhas e da Bacia do rio Orinoco na Colômbia (Arango-Ulloa et al., 2009). Na Bacia do rio Orinoco é uma das espécies arbóreas mais importantes usadas pelos agricultores para identificar "solos bons" para estabelecer novos cultivos (Barrios & Trejo, 2003). Na Amazônia Central, é uma das espécies mais comuns nos quintais das planícies de inundação e áreas adjacentes (Santos, 1982; Lima & Saragoussi, 2000), onde é uma fonte complementar de renda para associações de artesãs, cujo modo de preparar as cuias foi reconhecido como patrimônio cultural brasileiro (Carvalho, 2011; IPHAN, 2015). Nos barrancos em terras altas (*bluffs*) ao longo do rio Madeira na Amazônia Central, *Crescentia cujete* geralmente é encontrada em manchas de terra preta de índio, solos de alta fertilidade associado a sítios arqueológicos e atualmente usados pelas famílias ribeirinhas (com. pess. André B. Junqueira). Os frutos de cuia estão presentes em diferentes culturas neotropicais e relacionados a usos simbólicos, utilitários e medicinais (Figura 5).

Figura 5. Diversidade do uso de frutos de *Crescentia cujete* cultivada na Bacia Amazônica brasileira. Tigelas e cestas para uso diário (a, c), em rituais de benzimento (b), bolsas (d), utensílios na torra da farinha (e), balde para carregar água (f), conchas e colheres para servir bebidas (g, m), utensílio para tirar água da canoa (h) e tigelas vendidas como artesanato e em restaurantes para consumo de tacacá (i, j, l). Crédito das fotos: Priscila Ambrósio Moreira (a, b, c, d, e, m); Juliana Lins (g, i, j, l) Leonardo Kumagai (f), Nigel Smith (h).

Histórias orais na Mesoamérica e na Amazônia mencionam seus frutos como um dos primeiros objetos que surgiram no mundo (Heiser, 1993; Ventura, 1996; Martin, 1999; Lévi-Strauss, 2004; Hugh-Jones, 2009). Na Amazônia, o uso das cuias está associado com outros objetos antigos como banco, a forquilha de cigarro e o consumo de ipadu (Ribeiro, 1995). Comparações arqueológicas nas Antilhas sugerem que as cuias tenham sido usadas como moldes para as primeiras cerâmicas (Gijn & Hofman, 2008). Na Mesoamérica, o uso de cuias foi associado como recipientes no método de cozimento usando pedras quentes (Mirambell, 1994), uma tecnologia importante para o consumo de feijão, o qual já era cultivado entre 4.500 e 3.500 anos AP na região (Piperno et al., 2009; Zizumbo-Villareal & GarcíaMarín, 2010). Além de utensílios domésticos, seus frutos têm sido usados como garrafas para transportar água, para tirar água da canoa, para construir armadilhas de pesca, como ornamentos corporais (*tapa-sexo*) ou instrumentos musicais (Steward, 1948; Patiño, 1967; Bennett, 1992; Heiser, 1993). No Suriname é uma espécie considerada mágica, devido ao valor simbólico de seus utensílios (van Andel et al., 2013). Restos arqueológicos indicam uso simbólico, provavelmente como oferendas em rituais funerários de 1035-1420 AD na República Dominicana (Conrad, 2001), mas também em ambientes domésticos como lixeiras do período clássico Maya na Mesoamérica (300-950 AD) (Lentz et al., 1996; Beaubien, 1993; Cavallaro, 2013). Aplicações medicinais similares foram documentadas na região Neotropical (Duke, 1929; Morton, 1968), que inclui neutralização de veneno de cobras e infecções intestinais (Otero et al., 2000; Volpato et al., 2009). Usos tradicionais sugerem, portanto, alto valor das cuias como recurso entre os povos mesoamericanos onde se desenvolveu a cultura Maya, mas também possivelmente entre grupos humanos com maior mobilidade e não essencialmente agrícolas. É, portanto, provável que as cuias tenham sido uma das primeiras plantas cultivadas na Amazônia, antes mesmo do advento da cerâmica, porém seus restos ainda não foram encontrados nas escavações em sítios arqueológicos amazônicos (Roosevelt, 2000; Erickson, 2006; Caromano et al., 2013).

Atualmente, apesar da introdução de produtos industrializados em ampla escala, aparentemente o cultivo de *Crescentia cujete* permanece ativo em diversas áreas dos Neotrópicos. Na Bacia Amazônica, as práticas de uso, manejo e percepção da diversidade de frutos ainda não foram avaliadas. O mapeamento da distribuição morfológica de cuias entre diferentes continentes e rios amazônicos contribui para entender o processo de diversificação da planta em seus diferentes contextos culturais e ecológicos.

Estrutura da tese

Esta tese teve como área de estudo amostras do México, Costa Rica e ao longo de cinco rios da Bacia Amazônica brasileira, o que inclui possíveis populações silvestres de *Crescentia cujete* e a coocorrência com a espécie silvestre *C. amazonica* (Figura 6).

A tese consiste em 5 capítulos: esta introdução geral com o embasamento teórico para os objetivos (Capítulo 1), três capítulos com resultados de pesquisa (Capítulos 2 a 4), e uma síntese (Capítulo 5).

No capítulo 2, eu desenvolvi marcadores moleculares específicos para *Crescentia cujete*, os quais serviram de base para as análises subsequentes. A partir do desenvolvimento de uma biblioteca genômica de *C. cujete*, foi feito sequenciamento completo do genoma cloroplastial de *C. cujete,* identificação de marcadores do tipo SNP (single nucleotide polymorphism) em *C. cujete* e *C. amazonica* e obtenção de marcadores microssatélites (SSR) nucleares específicos para *C. cujete*, os quais foram parcialmente transferidos para *C. amazonica*. Este capítulo foi publicado na revista Applications in Plant Sciences.

No capítulo 3, eu avaliei a relação genética entre *C. cujete* e *C. amazonica* na bacia amazônica, a qual serviu de base para testar as hipóteses de Gentry (1980) e Ducke (1946) sobre a origem da domesticação e diversificação morfológica dos frutos. Além disso, a partir da análise da influência da hibridização na diversificação das cuias, avaliei em ampla escala geográfica como as pessoas percebem, usam e propagam os frutos de ambas as espécies na Amazônia. Este capítulo foi publicado na revista Evolutionary Applications.

No capitulo 4, eu adicionei amostras da América Central a partir da colaboração com pesquisadores mexicanos. A integração de análises genéticas, morfológicas e etnobotânicas entre Mesoamérica e Amazônia possibilitou discutir hipóteses alternativas de origem da domesticação, inferir rotas de dispersão da planta e identificar fatores de seleção e diversificação em diferentes condições ambientais, históricas e culturais. Este capítulo foi submetido à revista Frontiers in Ecology and Evolution, na edição especial "Ecology and evolution of plants under domestication in the Neotropics".

Na síntese, eu integrei os dados gerados nos capítulos 2, 3 e 4 para ampliar o conhecimento sobre a domesticação de plantas na Amazônia.

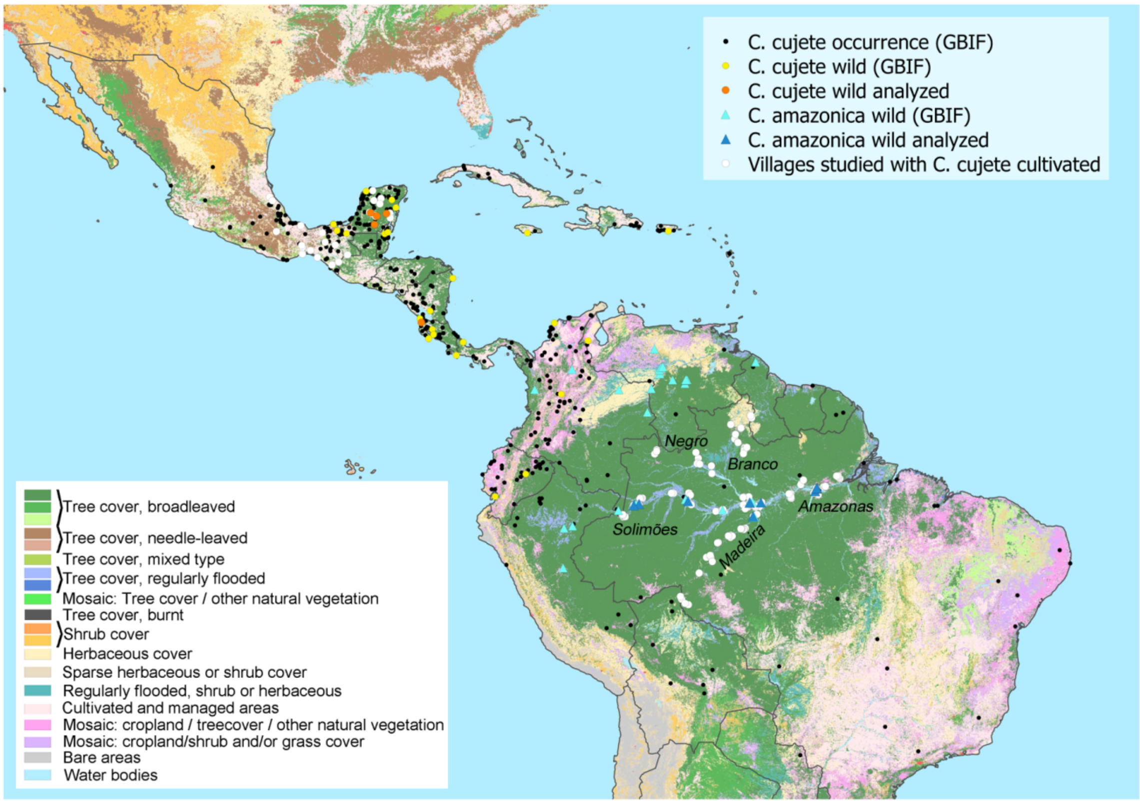

Figura 6. Área de estudo com a distribuição geográfica Neotropical de indivíduos cultivados de *Crescentia cujete*, possíveis populações silvestres de *C. cujete* e a coocorrência com a espécie silvestre *C. amazonica*. Análises genéticas ou morfológicas nesta tese incluem amostras do México, Costa Rica e ao longo de cinco rios da Bacia Amazônica brasileira. Registros em herbários disponíveis no Global biodiversity information facility (GBIF) indicam que indivíduos aparentemente espontâneos de *C. cujete* se sobrepõem ao mosaico de vegetação arbustiva e herbácea e áreas cultivadas entre o Caribe e noroeste da América do Sul, de acordo com Global Land Cover 2000 (Bartholomé & Belward, 2005); e portanto não descarta prévia dispersão humana.

Capítulo 2

Chloroplast sequence of treegourd (*Crescentia cujete*, Bignoniaceae) to study phylogeography and domestication

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Abstract

- *Premise of the study*: *Crescentia cujete* fruit rinds are traditionally used for storage vessels and handicrafts. We assembled its chloroplast genome and identified single nucleotide polymorphisms (SNPs).
- *Methods and Results*: Using a genome skimming approach, the whole chloroplast of *C. cujete* was assembled using 3,106,928 sequence reads of 150 bp. The chloroplast is 154,662 bp in length, structurally divided into LSC (84,788 bp), SSC (18,299 bp), and two IRs (51,575 bp) with 88 genes annotated. By resequencing the whole chloroplast, we identified 66 SNPs in *C. cujete* (N=30) and 68 SNPs in *C. amazonica* (N=6). Nucleotide diversity was estimated at 1.1×10^{-3} and 3.5×10^{-3}, for *C. cujete* and *C. amazonica*, respectively.
- *Conclusions*: This broadened *C. cujete* genetic toolkit will be important to study the origin, domestication, diversity and phylogeography of treegourds in the Neotropics.

Keywords: Bignoniaceae; calabash tree; *Crescentia amazonica*; cuia; next-generation sequencing; single-nucleotide polymorphism (SNP)

Introduction

Crescentia cujete L. (Bignoniaceae) is a diploid species (2n=40) that produces non-edible fruits that have been of great importance to many indigenous and traditional communities of tropical America since pre-Columbian times, especially as drinking cups and storage vessels. Its wild geographic distribution is unknown, but it is found in many areas in the Neotropics in close contact with wild relatives in quite different environments.

There are two hypotheses of its origin of domestication. Gentry (1980) hypothesized an origin in Mesoamerica, where wild populations are found in seasonally flooded savannas. This hypothesis was not confirmed with chloroplast microsatellites in the eastern Yucatan of Mexico (Aguirre-Dugua et al., 2012). Ducke (1946) hypothesized that *Crescentia amazonica* Ducke (described in 1937) gave rise to the cultivated *C. cujete*. This amazonian species is also found in the Orinoco Basin and elsewhere in northern South America (Gentry, 1980; Wittmann et al., 2006; Díaz, 2009), where it is common in floodplain forests. The distributions of the other four accepted species of *Crescentia* are restricted to Central America and Antilles, leading Gentry (1980) to comment on *C. amazonica*'s distribution outside of the distribution of the other species. Contrary to Ducke (1946), Gentry (1980) suggested that *C. amazonica* was derived from cultivated *C. cujete* "when human selection for large fruits is relaxed". However, using AFLP markers and a single accession of *C. amazonica* from the Orinoco Basin, Arango-Ulloa et al. (2009) found no relationship with *C. cujete* from Colombia.

Identification of the origin of domestication of treegourd and its routes of dispersal in the Neotropics remain unclear, and requires a molecular genetic analysis of a broader geographical sample. Using *C. amazonica* and *C. cujete* collections widely distributed along major rivers of the Brazilian Amazon Basin with the assembly of the chloroplast genome, we aim to identify single nucleotide polymorphisms (SNPs) to compare chloroplast diversity between *C. cujete* and *C. amazonica* in orderto evaluate the two hypotheses about the relationships between these species and better understand the domestication history of treegourd.

Methods and Results

DNA was extracted from dried leaves of 36 samples from the Brazilian Amazon Basin (Appendix 1). We used the cetyltrimethylammonium bromide (CTAB) 5% extraction protocol (Doyle & Doyle, 1990) with minor modifications; instead of cold isopropanol, NaCl was added to precipitate pellets. Barcoded libraries were constructed following the protocol of Mariac et al. (2014). Briefly, 0.5-1 µg of DNA were fragmented in a Bioruptor Pico sonicator (Diagenode, Liege, Belgium) using a standard protocol including six cycles and on/off conditions set to 30/90 s to reach a target size distribution of 300 bp. After sizing, end repair, ligation, and *Bacillus stearothermophilus* (Bst) DNA polymerase treatment, libraries were amplified with the KAPA HiFi HotStart Real-Time PCR Kit (KAPA Biosystems, Wilmington, Massachusetts, USA) with eightcycles to extend Illumina adapters and quantified by using the KAPA SYBR FAST LightCycler 480 qPCR Kit (KAPA Biosystem). Paired-end sequencing (2x150) was conducted on an Illumina MiSeq version 3 and HiSeq2500 (Illumina, San Diego, California, USA) at the CIRAD facilities (Montpellier, France) and at Genotoul (Toulouse, France), respectively. Twelve picomoles of the bulked libraries with 1 % phix were loaded in the flow-cell. Mean passing filter among the different runs was 84.3%, producing 13 million clusters. The percentage of bases having a quality score above Q30 was 93.7%.

Assembly was performed using the chloroplast of *Tanaecium tetragonolobum* (Jacq.) L. G. Lohmann (NC_027955) as a guide sequence for MITObim 1.7 (Hahn et al., 2013). First, MITObim mapped reads to the reference genome using MIRA version 4 (Chevreux et al., 1999) and an initial set of contigs was built (Appendix S1). Then, a second mapping was done on these contigs. Contigs were extended if there was at least a 31-bp overlap with a given read. This process was iterated until a complete *de novo* genome was achieved. For the assembly, we used the two-step strategy pioneered by Li et al. (2013), because the repetitive nature of the inverted repeat (IR) regions (i.e., IRA and IRB) was difficult to assemble (Li et al. 2013). We first performed an assembly using the sequence large single copy (LSC), IRA, and small single copy (SSC), followed by a second independent assembly using the sequence SSC, IRB and LSC from *Tanaecium tetragonolobum* (NC_027955). From the initial 3,106,862 shotgun reads, 268,499 reads were useful for the de novo chloroplast assembly. The SSC region showed a pairwise identity of 99.6% between the two assemblies, and LSC showed 99.7%. The slight differences observed are mainly locally close to repeat regions (mini- and microsatellite), and thus difficult to assemble. The IRs showed a 99.1% pairwise identity. The two fractions were manually aligned using the software Genious Pro 4.8.5 (Kearse et al., 2012), and a consensus *C. cujete* chloroplast sequence was built. The final assembly has a low number of N positions (46 Ns), and 96.7% of reads were properly paired, meaning that both read R1 and R2 were properly mapped. The mean depth of coverage of the sequence was 165x, meaning that for each position we have an average of 165 aligned reads. The final chloroplast genome size was 154,662 bp (Figure 1, Appendix 1).

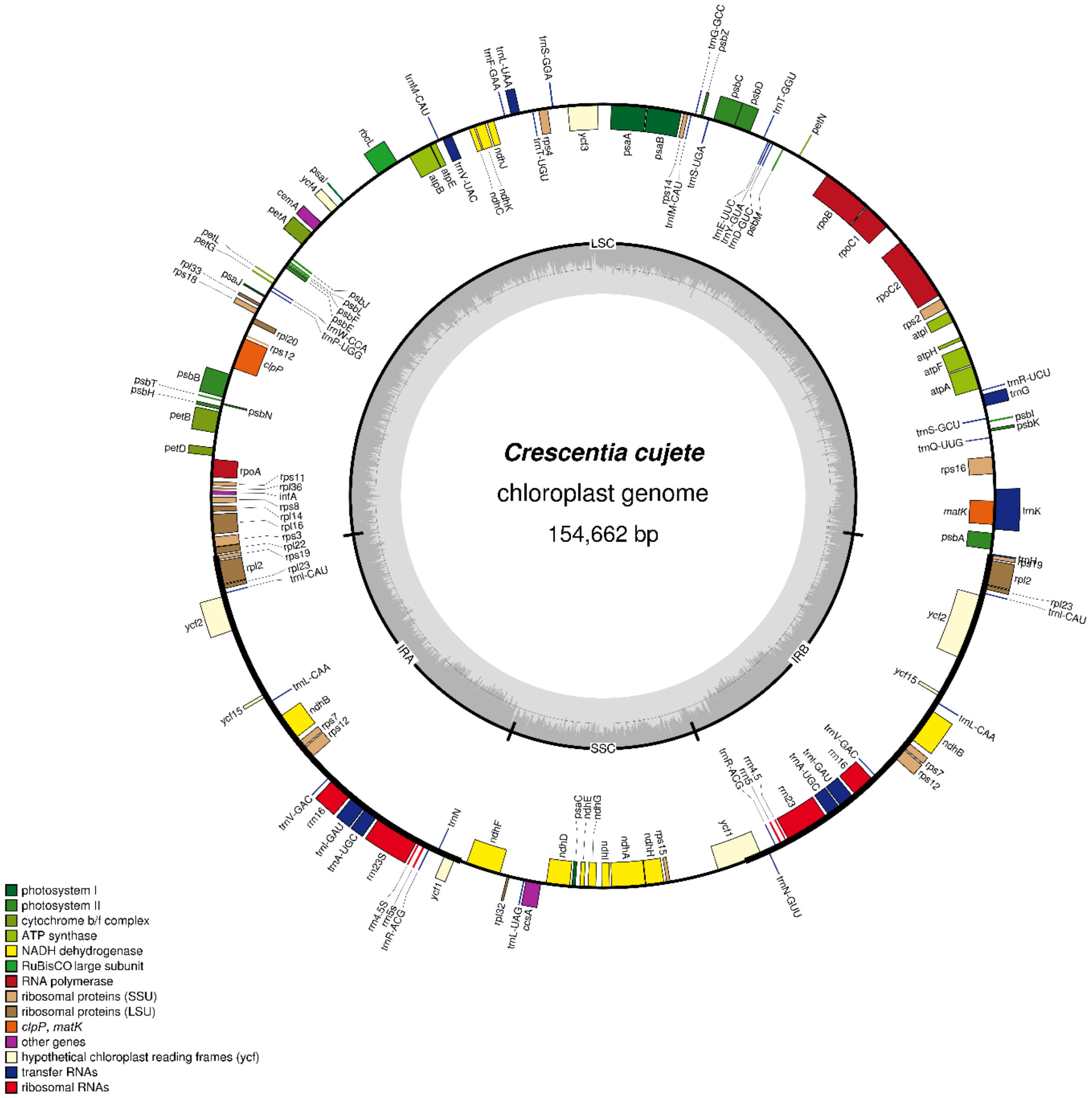


Figure 1. Circular map of the chloroplast genome of *Crescentia cujete* L. from Amazonas, Brazil (60°26' 24" W, 5°20'24" S) deposited in GenBank (accession number KT182634). Genes drawn within the circle are transcribed clockwise, while genes drawn outside are transcribed counterclockwise. Genes belonging to different functional groups are color-coded. Dark bold lines indicate inverted repeats (IRA and IRB) that separate the genome into large (LSC) and small (SSC) single copy regions. Drawn using OrganellarGenomeDraw (Lohse et al., 2013).

The *C. cujete* chloroplast genome was aligned with reference annotated genomes using the mauve algorithm implemented in Geneious Pro 4.8.5 (Kearse et al., 2012). For annotation, we used *Tanaecium tetragonolobum* (NC_027955; Bignoniaceae) as reference, and complemented it with *Olea europaea*L. (NC_013707; Oleaceae) and *Capsicum chinense* Jacq. (NC_KU041709; Solanaceae) to validate some tRNA orientations and add some introns lacking in the *Tanaecium* genome (*rpl*16, *rps*12). The correspondences of gene positions were identified and annotated manually. The *C. cujete* chloroplast sequence was deposited in GenBank (accession number KT182634). The assembled chloroplast genome of *C. cujete* was used to map another 30 *C. cujete* samples from homegardens and six *C. amazonica* samples from flooded forests with BWA 0.6.2 (Li & Durbin, 2009). Using SAMtools 0.1.7 (Li et al., 2009) and VarScan 2.3.7 (Koboldt et al., 2012), we generated and filtered the variant call format (VCF) files, following the Scarcelli et al. (2016) pipeline. The average number of chloroplast mapped reads was 54,747, equivalent to a 54x depth of coverage (Appendix 1). The minimum coverage was 15,324 reads, so even for this sample, each nucleotide was sequenced 15 times. We only have 0.7% of missing data in our 36 samples. Diversity analysis of the 30 *C. cujete* and 6 *C. amazonica* was done using DnaSP 5.10.1 (Librado & Rozas, 2009).

The size of the reconstructed chloroplast genome of *C. cujete* is 154,662 bp, structurally divided into four distinct regions: large single copy region (LSC: 84,788 bp), small single copy region (SSC:18,299 bp), and a pair of inverted repeat regions (IR: 51,575 bp) (Table 1, Figure 1). We identified 88 coding-genes, of which nine were duplicated within IR regions, four rRNAs duplicated in IRA and IRB, 30 tRNAs, of which six were duplicated within IR regions. The *C. cujete* chloroplast genome size (bp) and GC content are comparable to *Tanaecium tetragonolobum* (Table 1), and within the variation observed in the order Lamiales, where genome lengths vary from 153,493 to 155,889 bp and CG content from 37.6% to 38.3% (Nazareno et al., 2015). The *rps*19 and *rpl*2 gene positions duplicated in the boundaries of IR (Figure 1) agree with expectations from other angiosperms (Wang et al., 2008).

Table 1. Comparison of chloroplast genomes between two species of Bignoniaceae.

Characteristics	*Crescentia cujete*	*Tanaecium tetragonolobum*
Size (base pair; bp)	154,662	153,776
LSC length (bp)	84,788	84,612
SSC length (bp)	18,299	17,586
IRA, IRB combined length (bp)	51,575	51,578
GC content (%)	38.3	38.3
Number of genes	132	121
Protein-coding genes	88	85
Structure RNAs	30	35
Genes with intron(s)	24	13
Coding rRNAs genes (% bp)	5.85	5.85
Coding tRNAs genes (% bp)	1.78	1.81
Protein-coding genes (% bp)	43.78	51.21
Noncoding regions (% bp)	48.59	41.13
Reference	This study	Nazareno et al., 2015

Note: IRA = inverted repeat region A; IRB = inverted repeat region B; LSC = large single copy; SSC= small single copy.

We found 66 SNPs in 30 individuals of *C. cujete* with 24 haplotypes, and 68 SNPs in six individuals of *C. amazonica* with six haplotypes. Haplotype diversity (h) was 0.98 and 1.00, nucleotide diversity (π) was 1.1 x10^{-3} and 3.5 x10^{-3}, Watterson's estimator per site (θ_W) was 2.3 x10^{-3} and 4.1 x10^{-3} for *C. cujete* and *C. amazonica*, respectively. Diversity was about twiceas high in *C. amazonica* compared to *C. cujete.* If *C. amazonica* was simply derived from *C. cujete*, as suggested by Gentry (1980), diversity should be comparable or potentially even slightly lower. Consequently, we rule out the hypothesis that *C. amazonica* is derived from *C. cujete.* However, at this point we cannot rule out either that domestication of *C. amazonica* lead to *C. cujete* or that *C. cujete* is derived from other wild species from Central America.

Conclusions

Next-generation sequencing provided data to have a sufficient number of reads to perform a *de novo* assembly of the *C. cujete* chloroplast genome, the first assembled chloroplast in the *Crescentia* genus. The reconstructed *C. cujete* genome allowed the identification of SNPs in *C. amazonica* and *C. cujete* that produced diversity estimates that refuted the hypothesis that *C. amazonica* is derived from *C. cujete*, and will be useful in further studies about origin, diversity, and spread of treegourds in the Neotropics.

Appendix

Appendix 1. The number of mapped reads and geographical information for the 30 *Crescentia cujete* and six *C. amazonica* samples from the Brazilian Amazon Basin used to analyze chloroplast diversity in this study.

Species	Sample	No. of reads (bp)	Municipality, State	Geographical coordinates
Crescentia cujete L.	I2R26T92	23,923	Barcelos, Amazonas	62°55'12" W, 0°58'12" S
	I8R26T23	16,574	Barcelos, Amazonas	64°01'48" W, 0°06'36" S
	I10R26T58	64,404	Barcelos, Amazonas	62°55'48" W, 0°59'24" S
	R21T35	37,991	Caracaraí, Roraima	61°08'24" W, 1°44'24" N
	I2R26T102	46,813	Caracaraí, Roraima	60°53'24" W, 1°28'12" N
	I8R26T10	15,324	Fonte Boa, Amazonas	65°55'48" W, 2°31'12" S
	I8R26T24	25,859	Fonte Boa, Amazonas	65°58' 48" W, 2°28'48" S
	I11R26T37	268,499	Novo Aripuanã, Amazonas *	60°26' 24" W, 5°20'24" S
	I1R26T28	33,170	Manaus, Amazonas	60°02' 24" W, 2°47'24" S
	I2R26T87	195,654	Manaus, Amazonas	59°54' 36" W, 3°10'12" S
	I1R26T12	41,714	Manicoré, Amazonas	61°19' 12"W, 5°51'36" S
	I2R26T91	22,462	Manicoré, Amazonas	61°28' 12" W, 5°58'12" S
	I1R26T10	121,056	Novo Aripuanã, Amazonas	60°25' 48"W, 5°19'48" S
	I2R26T99	79,280	Parintins, Amazonas	56°53' 24" W, 2°33' 36" S
	I8R26T18	28,994	Parintins, Amazonas	56°54' W, 2°33' S
	I8R26T40	25,895	Parintins, Amazonas	56°53' 24"W, 2°33' 36" S
	R21T29	48,508	Santarém, Pará	54°44' 24" W, 2°08' 24" S
	I2R26T76	33,679	Santarém, Pará	54°43'12" W, 2°07'12" S
	I10R26T90	65,648	Santarém, Pará	54°46'48" W, 2°28'12" S

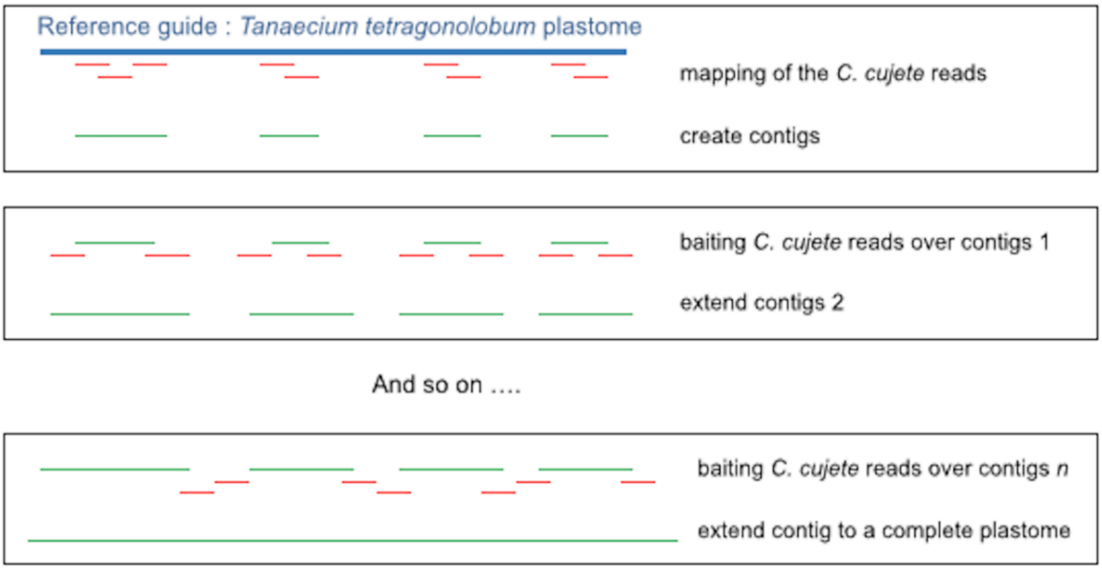
Species	Sample	No. of reads (bp)	Municipality, State	Geographical coordinates
	I8R26T7	46,862	São Gabriel da Cachoeira, Amazonas	67°14'24" W, 0°46'12" N
	I10R26T92	68,994	São Gabriel da Cachoeira, Amazonas	67°14'24"W, 0°46'12" N
	I2R26T101	56,728	São Luís do Anauá, Roraima	60°11'24" W, 1°04'48" N
	I1R26T35	36,852	São Paulo de Olivenca, Amazonas	68°39'36" W, 3°24' S
	I2R26T67	26,431	Tabatinga, Amazonas	69°54'36"W, 4°13'12" S
	I10R26T64	22,805	Tabatinga, Amazonas	69°54'36" W , 4°11'24" S
	I1R26T19	108,320	Tefé, Amazonas	64°33' W, 3°24' 36"S
	I1R26T42	45,478	Tefé, Amazonas	64°41' 24" W, 3°17'24" S
	I2R26T80	84,319	Tefé, Amazonas	64°32' 24"W, 3°24'36" S
	I10R26T82	28,696	Tefé, Amazonas	64°41'24" W, 3°17'24" S
	I10R26T61	81,698	Tefé, Amazonas	64°45' W, 2°28' 48" S
Crescentia amazonica Ducke[a]	I11R26T51	49,100	Borba, Amazonas	59°42'36" W, 4°19'48" S
	R18T23	33,401	Manaus, Amazonas	59°57' W, 3°14'24" S
	I1R26T45	17,428	Manaus, Amazonas	59°57' 36"W, 3°15' S
	I1R26T32	21,302	Santarém, Pará	54°43' 48" W, 2°07'12" S
	R21T13	24,231	São Paulo de Olivenca, Amazonas	68°37' 48"W, 3°21' S
	I1R26T44	20,820	São Paulo de Olivenca, Amazonas	69°02' 24" W, 3°27'36" S

Notes:* *Crescentia cujete* sample used to reconstruct the chloroplast sequence in this study.

[a] Vouchers of *Crescentia amazonica* from Borba and Santarém were deposited in the Instituto Nacional de Pesquisas da Amazônia (INPA) Herbarium (numbers 255.829 and 266.725, respectively).

Supplementary Material

Appendix S 1 Schematic MITObim workflow (Hahn et al., 2013). In the first step genomic reads of *Crescentia cujete* were mapped on the reference guide plastome of *Tanaecium tetragonolobum*. Contigs were created and used as a new reference in the second step. Baiting was performed with 31-bp overlap with the reference contigs. Extension of the contigs was done iteratively until a complete genome was achieved.



Capítulo 3

Human management and hybridization shape treegourd fruits in the Brazilian Amazon Basin

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Abstract

Local people's perceptions of cultivated and wild agrobiodiversity, as well as their management of hybridization are still understudied in Amazonia. Here we analyze domesticated treegourd (*Crescentia cujete*), whose versatile fruits have technological, symbolic and medicinal uses. A wild relative (*C. amazonica*) of the cultivated species grows spontaneously in Amazonian flooded forests. We demonstrated, using whole chloroplast sequences and nuclear microsatellites, that the two species are strongly differentiated. Nonetheless, they hybridize readily throughout Amazonia and the proportions of admixture correlate with fruit size variation of cultivated trees. New morphotypes arise from hybridization are recognized by people and named as local varieties. Small hybrid fruits are used to make the important symbolic rattle (*maracá*), suggesting that management of hybrid trees is an ancient human practice in Amazonia. Effective conservation of Amazonian agrobiodiversity needs to incorporate this interaction between wild and cultivated populations that is managed by smallholder families. Beyond treegourd, our study clearly shows that hybridization plays an important role in tree crop phenotypic diversification, and that the integration of molecular analyses and farmers'perceptions of diversity help disentangle crop domestication history.

Keywords: agrobiodiversity; *Crescentia amazonica*; *Crescentia cujete*, introgression, plant domestication, wetlands.

Introduction

Amazonia is an important center of plant domestication (Clement, 1999b; Meyer et al., 2012). Its great biological and cultural diversity (Balée, 2013) make it an especially interesting area to study the role of human societies in plant domestication and diversification (Clement et al., 2010; Balée, 2013). The distinction between wild and cultivated is one of the basic questions of plant domestication (Lévi-Strauss, 1950; Terrell et al., 2003; Pickersgill, 2013). The distinction, however, often goes unnoticed, given the lack of understanding of how local people perceive biological diversity in traditional societies (Caillon & Degeorges, 2007). People's perceptions of cultivated and wild diversity, as well as their management practices that deal with plant hybridization, are still understudied in Amazonia, especially for tree species (Moreira et al., 2015; Smith & Fausto, 2016; Rollo et al., 2016). Hybridization between related cultivated and wild plants may be favored or discouraged by local farmers (Jarvis & Hodgkin, 1999). It can promote domestication and diversification (Miller & Gross, 2011, Gompert & Buerkle, 2016), because hybrids often present interesting traits that can be selected and maintained (Zohary & Spiegel-Roy, 1975; Miller & Gross, 2011; Goldschmidt, 2013), but may not be adaptive in natural environments (Ellstrand, 2003). This is especially true of introgressive hybrids, since backcrossing to one parent maintains its useful characteristics with minor influence of the other parent (Ellstrand, 2003; Harrison & Larson, 2014). It follows that hybridization and introgression between cultivated and wild plants, as well as the human practices that maintain diversity, are important for effective agrobiodiversity conservation. The distinction between wild and cultivated and its linkages with natural ecosystems are essential for a broader understanding of agriculture (Aumeeruddy-Thomas et al., 2014). More efforts are necessary for its recognition and implementation by public agricultural and forestry policies (Michon et al., 2013).

Crescentia spp. (Bignoniaceae) are excellent candidates to study hybridization and domestication associated with floodplains in Amazonia. *Crescentia cujete* L. (1753), known as treegourd or calabash tree, is an important tree crop for Amazonian smallholders (Lima & Saragoussi, 2000; Wittmann & Wittmann, 2011). Its versatile fruits, called *cuia* in Portuguese, are traditionally used as storage vessels, drinking cups, scoops to bail water from canoes, traps for fishing, diving masks, bags, body ornaments, ritualistic musical instruments and, more recently, as "ecological" cups; they also have medicinal applications (Steward, 1948; Patiño, 1967; Morton, 1968; Bennett, 1992; Heiser, 1993; Bustamante et al., 2011; Acostupa et al., 2013). *C. cujete* presents an ample variation of fruit shapes and sizes (Gentry, 1980; Arango-Ulloa et al., 2009; Aguirre-Dugua et al., 2012) that support the wide range of uses. A wild relative (*C. amazonica* Ducke) occurs in flooded forests in the Orinoco and Amazon Basins, as well as smaller rivers of the Guianas (Gentry, 1980; Godoy et al., 1999; Wittmann et al., 2006; Díaz, 2009). *C. amazonica* fruits are smaller with thinner rinds that float in the water and are dispersed by fish (Waldhoff et al., 1996).

The relationship between the two species is largely speculative. *C. amazonica* was hypothesized to be the wild progenitor from which treegourd was domesticated (Ducke, 1946). Alternatively, treegourd was domesticated in Mesoamerica and later distributed to Amazonia (Gentry, 1980). In this case, it was hypothesized that *C. amazonica* was feralized *C. cujete* (Gentry, 1980). Their chloroplast genetic diversity does not support the possibility of *C. amazonica* being derived from *C. cujete* (Moreira et al*.*, 2016). Nonetheless, a close relationship between *C. cujete* and *C. amazonica* is recognized by local human populations. In Guyana, people "called spirits" when *C. amazonica* was found, because they recognized it is a sort of "shadow" of the domesticated *C. cujete* (van Andel, 2000). Since the two species co-occur in Amazonia and *Crescentia* species are hypothesized to be largely interfertile (Gentry, 1980), gene flow might be abundant, but has not yet been shown at the molecular level.

In this study, we asked whether (i) hybridization plays a significant role in shaping genetic and morphologic diversity in *Crescentia* species, and whether (ii) hybrid and introgressed individuals are managed by Amazonian smallholders. To address these questions, we combined a genetic study based on chloroplast (single nucleotide polymorphisms – SNP) and nuclear (simple sequence repeats – SSR) markers with local farmer interviews. Using these datasets, we analyzed (i) the genetic differences between *C. amazonica* and *C. cujete* in the Amazon Basin, and gene flow between them; and (ii) the relationship between genetic and morphological diversity, and how people use and perceive this diversity.

Methods

Field sampling and interviews

We visited rural and peri-urban villages in 36 municipalities distributed along the major rivers of the Brazilian Amazon Basin (Figure 1). The broad geographical sampling followed two criteria for village selection: dependence on river resources and treegourd use in daily life. Data were collected after an informed consent invitation that was read collectively in each village and signed by a local representative. This research followed the International Society for Ethnobiology's code of ethics (International Society of Ethnobiology, 2006) and was approved by the Committee for Ethics in Research with Human Beings of the National Research Institute for Amazonia (CEP INPA, proc. no. 408.611, 2013). We collected leaves for genetic analyses of each treegourd (N = 469) found in domestic areas in the villages (Table S1). We considered domestic areas to include homegardens, swiddens, ports near the river, football fields, trails and old homestead sites. Clones propagated from the same individual, identified based on farmers information, were avoided in order to better assess available diversity. Fruits were measured, photographed and their shape categorized according to Arango-Ulloa *et al.* (2009). We performed semi-structured interviews about use, management and history of most trees collected, and in nine municipalities we practiced participant observation of daily activities and urban farmers'markets. To map wild treegourd distribution, we surveyed herbarium records of *C. amazonica*, and photographs of its fruit were presented to farmers in all villages visited to stimulate their memory of its presence in local flooded forests. We collected leaves of *C. amazonica* (N = 32) growing spontaneously in seven areas of flooded forests in six municipalities along the Solimões-Amazonas and lower Madeira Rivers (Figure 1). Collection was authorized by the Brazilian System for Authorization and Information in Biodiversity, Chico Mendes Institute for Biodiversity Conservation, proc. no. 25052-1, 2012, and transportation by the Brazilian Institute for the Environment and Renewable Natural Resources, proc. no. 14BR015576/DF, 2014.

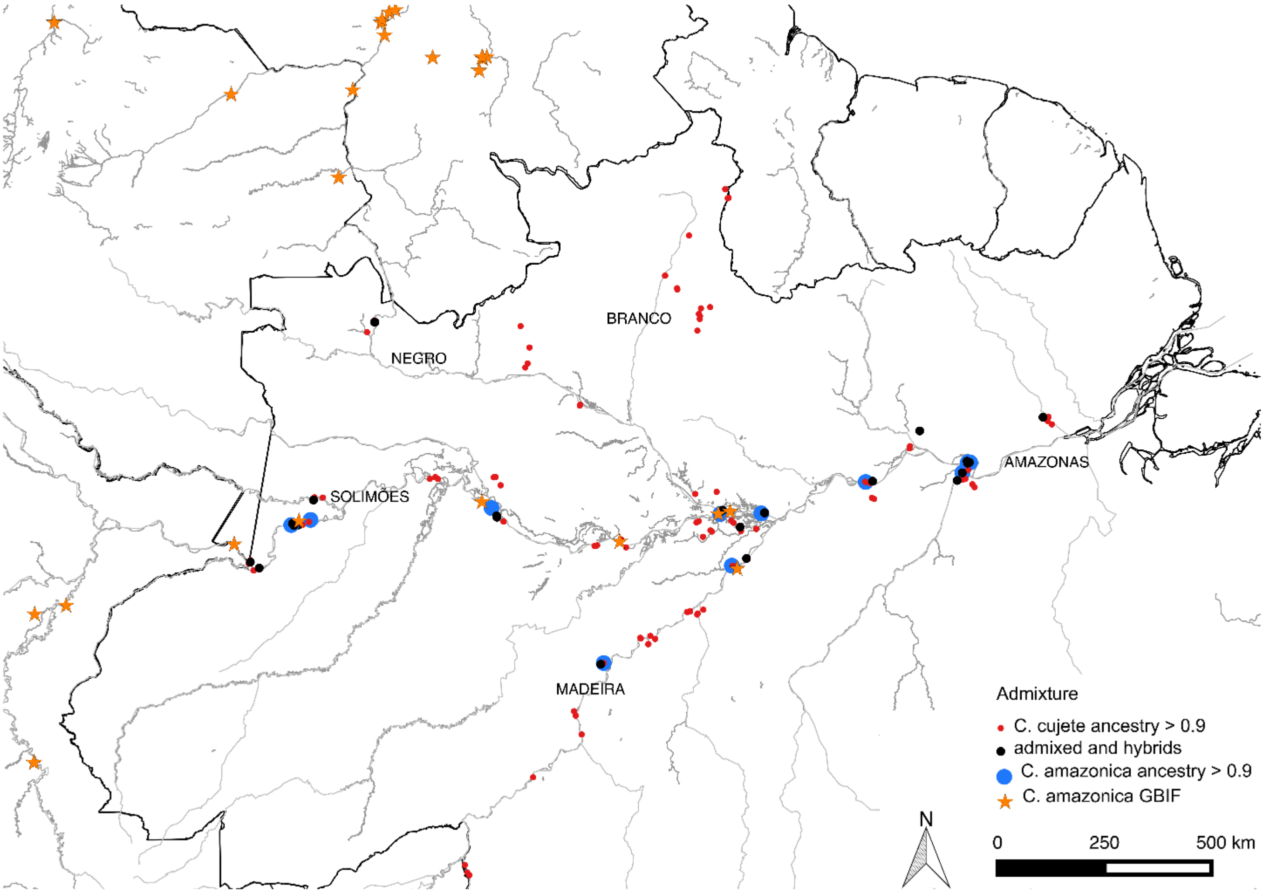

Figure 1. Geographical distribution of *Crescentia cujete* and *C. amazonica* treegourds collected for this study along major rivers of Brazilian Amazonia (N = 234). Proportions of admixture identified by Structure at K = 2 are indicated. Proportions above 0.9 were considered pure and below 0.9 were classified as admixed. *Crescentia amazonica* records in northern South America are from the Global Biodiversity Information Facility.

Genetic analysis

DNA was extracted from dried leaves (N = 234) using the CTAB 5% protocol (Doyle & Doyle, 1990) with minor modifications. For nuclear SSR analysis, we genotyped all 234 samples, among which 184 were also analyzed for chloroplast SNPs (Table S1). Collected leaves without enough DNA or with low quality were excluded. To develop the nuclear SSRs, a barcoded library of *C. cujete* genomic DNA was sequenced (Moreira et al*.,* 2016) using an Illumina MiSeqv.3 (San Diego, California, USA). QDD software 3.1.2 (Meglécz et al., 2009) was used to identify nuclear SSR motifs and design primers from 113,865 merged reads. The parameters used to select the nSSR primers were as follows: one primer pair for each read, to avoid repeated regions of the genome; avoidance of mononucleotide microsatellites; preference for perfect microsatellites with ≥8 repeats; and avoidance of primers that are very close (≤20 bp) to the target SSR. A total of 1,436 SSR were identified, of which 1,068 were perfect SSR motifs with 819 di-, 191 tri-, 47 tetra-, 10 penta-, and 1 hexa-repeat motifs, and 368 compound motifs. The primers were designed for the perfect SSRs (Table S2). We performed preliminary tests of amplification of 15 SSRs using *C. cujete* samples (N = 3). Five of the primer pairs failed to amplify, even using different temperatures and DNA concentrations, and were discarded. The remaining 10 SSR primers were labeled with fluorescence (FAM, NED, HEX; Applied Biosystems, Foster City, California, USA) and genotyped in an ABI 3130xLGenetic Analyzer (Applied Biosystems) using GS-500 LIZ as the size standard (Applied Biosystems). Although all 10 SSR were polymorphic, at least for cultivated samples (N = 221), two loci were excluded (SSR2 and SSR9) because they failed to amplify in 60% of the samples. We kept the remaining eight SSRs (Table S3) for hybridization analysis, since they also amplified successfully for *C. amazonica*. Locus amplifications were made in simplex and multiplexed for fragment analysis, using the PCR kit (Qiagen, n.206143, Hilden, Germany) with the following program: 95 ^{o}C for 15 min; 38 cycles, each of 94 ^{o}C for 30 s, T_a ^{o}C for 1.30 min and 72 ^{o}C for 1 min; and a final step of 60 ^{o}C for 30 min. Fragment size and allele identification were determined using GeneMapper (Applied Biosystems). We also obtained SNPs observed in the whole chloroplast using previously described approaches (Moreira et al., 2016; Scarcelli et al*.,* 2016).

Diversity analyses

The eight nuclear SSRs were used to genotype 234 treegourds, among which 221 were from domestic areas and 13 from flooded forests (Table 1). Relationships among individuals were assessed with Structure 2.3, using the admixture model (Pritchard et al., 2000). We varied the number of genetic clusters (K) from K = 1 to 20, with 100.000 burn-in, 100.000 iterations, and 5 different runs for each K value. The ad hoc ΔK (Evanno et al., 2005) was used to identify the most likely number of clusters in the matrix. We considered an individual to belong to a given cluster if its proportion of admixture was less than 0.10, i.e., with more than 0.90 of the individual's SSR profile attributable to the given cluster. In this study, we used the term "admixed"for ancestry between 0.90 to 0.60, and the term "hybrid"for ancestry from 0.60 to 0.40 (Table S1). We also calculated a hybridization index using Introgress 1.2.3 (Gompert & Buerkle, 2010) and compared it with the Structure admixture proportions. The whole chloroplast sequences of 174 domestic treegourds and 10 from flooded forests were analyzed (Table S1). We built a haplotype network based on 250 SNPs using the median joining algorithm (Bandelt et al., 1999). The network was visualized using the samples with less than 4 % of missing data, according to software requirements in POPART 1.7 (Leigh & Bryant, 2015). The nuclear and chloroplast comparison defined paternal and maternal introgression, respectively, and determined the final botanical identification (170 *C. cujete* and 14 *C. amazonica*). To assess the impact of the uneven number of samples of the two species, we performed a complementary Structure analysis using the same sample size for both species (14 *C. amazonica*, 14 *C. cujete*; Figure S1), with the *C. cujete* samples chosen at random. Finally, we assessed the potential impact of null alleles on admixture inferences (Figure S2), by coding any missing data as a homozygote recessive allele (Falush et al., 2007).

Table 1. Summary of nuclear (SSR) admixture proportions (N = 234) and chloroplast (SNPs) haplotypes (N = 184) of the *Crescentia cujete* and *Crescentia amazonica* collections analyzed in this study and the habitats they were collected in. A. Admixture proportions for *C. cujete* and *C. amazonica* in columns and chloroplast haplotypes in lines. Not confirmed means the chloroplast was not analyzed in plants that were genotyped with nSSR. B. Habitats in which pure and admixed treegourds were collected.

			pure cujete	cujete admixed	hybrids	amazonica admixed	pure amazonica
nSSR (N = 234)		***N***	**175**	**25**	**11**	**5**	**18**
A)							
Haplotypes (N = 184)	*C. cujete*	170	142	16	8	1	3
	C. amazonica	14	0	0	1	2	11(a)
	not confirmed	50	33	9	2	2	4
B)							
Domestic areas (N = 221)			175	25	11	5	5
Flooded forest (N = 13)			0	0	0	0	13(b)

[a] *C. amazonica* with "pure amazonica" assignment in Structure at K = 2 is predominant in flooded forests (N = 10), but one was found cultivated (N = 1). Admixed *C. amazonica* were only found in domestic areas.

[b] All of these are likely to be *C. amazonica*, but three were not analyzed for their chloroplast haplotypes.

Nuclear genetic diversity of *C. cujete* and *C. amazonica* was explored with Principal Components Analysis (PCA) executed with stats R package (R Core Team, 2015). Nuclear genetic diversity and species differentiation were estimated using hierfstat (Goudet, 2005). Pairwise F_{ST} were calculated and statistically assessed using1000 bootstraps (Nei, 1987). The significance of F_{IS} was measured as deviation from Hardy-Weinberg equilibrium using pegas R (Paradis, 2010). Chloroplast diversity was estimated using DNAsp 5.10.1 (Librado & Rozas, 2009) and Arlequin 3.5 (Excoffier & Fischer, 2010). We examined the relationship between *C. amazonica* admixture proportions and *C. cujete* fruit diameters (N = 61) with simple regression in R package (R Core Team, 2015).

Results

Genetic structure revealed with nuclear SSRs

The Structure analyses identified two clusters as the most likely structure (K = 2, Figure 2a), based on the ad hoc ΔK approach (Evanno et al., 2005; Figure S1). The two clusters correspond to domesticated *C. cujete* and wild *C. amazonica*, the two accepted botanical species already described in the Amazon Basin. We also found a significant amount of admixture between them (Figure 2a, Table 1), especially along the Solimões-Amazonas River (Figures 1, 2b). The frequency of individuals with admixture proportions suggesting hybridization and introgression was similar in both groups. Among the 234 samples, 200 were assigned primarily to *C. cujete*, of which 24 (12%) were admixed to some degree, and 23 were assigned to *C. amazonica*, of which five (21%) were admixed (Table 1). Eleven plants presented admixture proportions between 40 and 60% and were classified as hybrids. These proportions were similar when the *C. cujete* sample size was reduced at random to be equal to the *C. amazonica* sample size ($r^2 = 0.99$, $p < 10^{-15}$, Figure S1). At the second possible grouping (K = 3, Figure S3), similar admixture proportions were also found ($r^2 = 0.98$, $p < 10^{-15}$), while the original *C. cujete* group was subdivided without relation to geography or fruit morphology (Figure S3). The hybridization index calculated with Introgress was highly correlated with the admixture proportions calculated with Structure (Figure S4, $r^2 = 0.83$, $p < 10^{-15}$). Finally, coding all missing data as homozygous recessive alleles did not have an impact on admixture inferences (Figure S2, $r^2 = 0.96$, $p < 10^{-15}$).

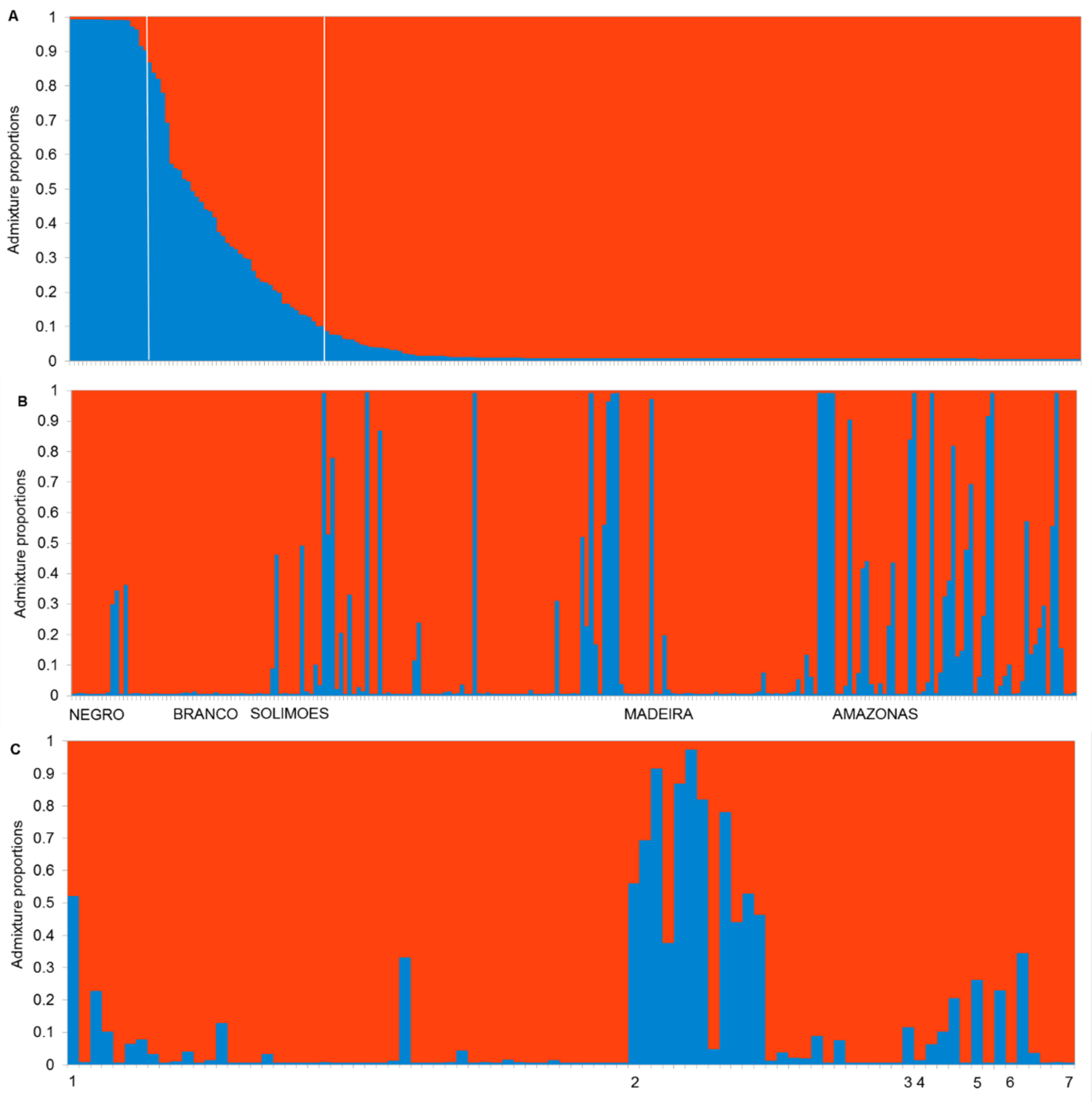

Figure 2. Structure analysis of 234 treegourd samples collected in Brazilian Amazonia. The y-axis shows the proportion of assignment to the groups at K = 2 (red - *Crescentia cujete* and blue - *Crescentia amazonica*). (a) Samples are ordered by their proportion of admixture: admixed if > 0.1, hybrids if 0.4 to 0.6, pure if > 0.9 of assignment to the group. (b) Samples were ordered by their geographical location along the main rivers: the Negro, Solimões and Amazonas Rivers are ordered west to east; the Branco River is ordered north to south; the Madeira River is ordered south to north. (C) Samples are ordered by seven fruit shapes (see Figure 4) and fruit size, with size increasing from left to right.

Comparison between chloroplast SNPs and nuclear SSRs diversity

The chloroplast analyses also clearly identified the two botanical species (Figure 3), with 63 mutational differences between the *C. cujete* and *C. amazonica* chloroplast sequences. In the haplotype network, samples with *C. cujete* nuclear assignments showed exclusively *C. cujete* chloroplast haplotypes (Figure 3, Table 1). For individuals with *C. amazonica* nuclear assignments (N = 23), 77 % had *C. amazonica* haplotypes (N = 13) and 17 % had *C. cujete* haplotypes (N = 4). Among the hybrids, most had *C. cujete* haplotypes (88 %, N = 8), and one had a *C. amazonica* haplotype. The flooded forests harbored exclusively pure *C. amazonica* samples (N = 10), while domestic areas harbored pure and admixed samples of both species (Table 1, Figure 5c). The genetic diversities of the pure samples of both species were lower than their admixed samples (Table S4). The rarified allele count (A_r) varied from 1.1 to 5.4, with the lowest value in *C. amazonica*, slightly higher within its admixed samples and the highest value in admixed *C. cujete*. The mean expected heterozygosity (H_s) of pure *C. cujete* was 0.31 and that of pure *C. amazonica* was 0.09, while their admixed samples showed mean H_s values of 0.58 and 0.33, respectively (Table S4). *C. amazonica* showed extremely low diversity, with fixed alleles at 6 loci. These low diversity values for *C. amazonica* are probably due to marker development from *C. cujete* with consequent poor transferal due to the significant divergence between species. Although such bias might lead to imprecise estimation of *C. amazonica* diversity, it does not have an impact on the identification of hybrids, since admixture is based on allele frequency differences and not diversity *per se* (Pritchard et al., 2000).

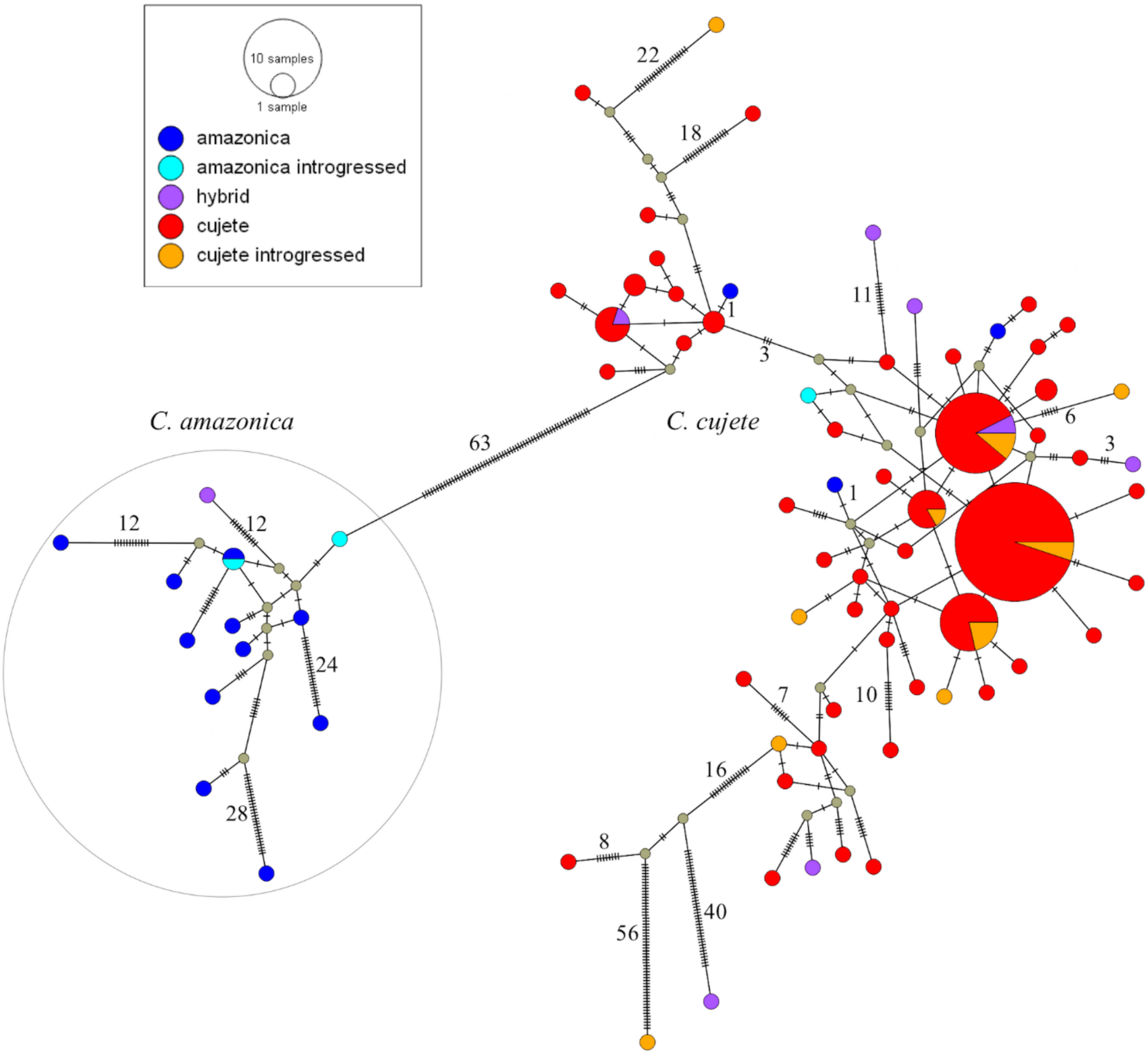

Figure 3. Chloroplast haplotype median joining network of *Crescentia cujete* and *C. amazonica* from Brazilian Amazonia based on 250 chloroplast SNPs. Nuclear ancestry of each sample (N = 184) was evaluated using Structure (Table 1). The size of the circle reflects the number of individuals presenting the same haplotype. Numbers of mutations are indicated as hatch marks and numbers between haplotypes. Colors represent percentage of nuclear admixture, where: *C. amazonica*> 0.9 (dark blue); admixed *C. amazonica*< 0.9 and > 0.6 (light blue); hybrids < 0.6 and > 0.4 (violet); admixed *C. cujete*< 0.9 and > 0.6 (orange) and *C. cujete*> 0.9 (red). A clear chloroplast difference is observed between *C. amazonica* and *C. cujete*, although 17 % (N = 4) of the *C. amazonica* ancestry samples (N = 23, Table 1) have *C. cujete* haplotypes.

Differentiation between *C. cujete* and *C. amazonica* was very high ($F_{ST} = 0.74$, $IC_{95\%} = 0.59 - 0.80$ excluding admixed samples; $F_{ST} = 0.56$, $IC_{95\%} = 0.41 - 0.66$ with all samples). F_{IS} (Table S4) was not significant for *C. cujete*, but significant for *C. amazonica* ($F_{IS} = 0.44$, $p < 0.05$). In the chloroplasts, we found 92 SNPs in 14 individuals of *C. amazonica* with 14 haplotypes, and 178 SNPs in 170 individuals of *C. cujete* with 71 haplotypes. Nucleotide diversity (π) was 3×10^{-3} and 1.2×10^{-3}, for *C. amazonica* and *C. cujete*, respectively. Chloroplast differentiation between species was high ($F_{ST} = 0.89$, $p < 0.05$).

Admixture proportions correlated with fruit size

There is ample morphological diversity of treegourd fruits in the Brazilian Amazon Basin (Figure 4). We recorded seven types of *C. cujete* fruit shapes in domestic areas (N = 167) and one fruit type of *C. amazonica* in flooded forests (N = 10). In domestic areas, two of them account for 86.4% of the plants: 63% flattened (type 1) and 23.4% oblong (type 2). The other 5 types were rare: 0.6% was cuneate (type 3), 3.6% elongated (type 4), 1.8% globular (type 5), 4.8% rounded-drop (type 6) and 1.2% oblong-drop (type 7). The two *C. amazonica* samples found in domestic areas showed type 2 fruits. While the fruits of *C. amazonica* in the flooded forest have small diameters ranging from 2.5 to 5.4 cm (median: 4.85 cm), in domestic areas diameters were slightly larger, ranging from 4 to 8.4 cm (median: 6.2 cm) (Figure 4). Among *C. cujete*, there is great size variation, especially within types 1, 2 and 5, with variation from 5.2 to 29 cm. Smaller fruits of *C. cujete* were correlated with *C. amazonica* admixture ($r^2 = 0.34$, $p = 1.26 \times 10^{-6}$, Figure 5), especially the oblong (type 2) fruit ($r^2 = 0.62$, $p = 4.81 \times 10^{-6}$). This relation remains even if we exclude the most extreme admixed samples (wild ancestry higher than 0.8, $r^2 = 0.23$, $p = 1.3 \times 10^{-4}$). The smaller *C. cujete* fruits, between 3 and 10 cm in diameter, are those with the highest admixture proportions (Figure 5). Some *C. amazonica* ancestry could be observed in all shapes found in domestic areas, except in the rare type 7 (Figure 2c).

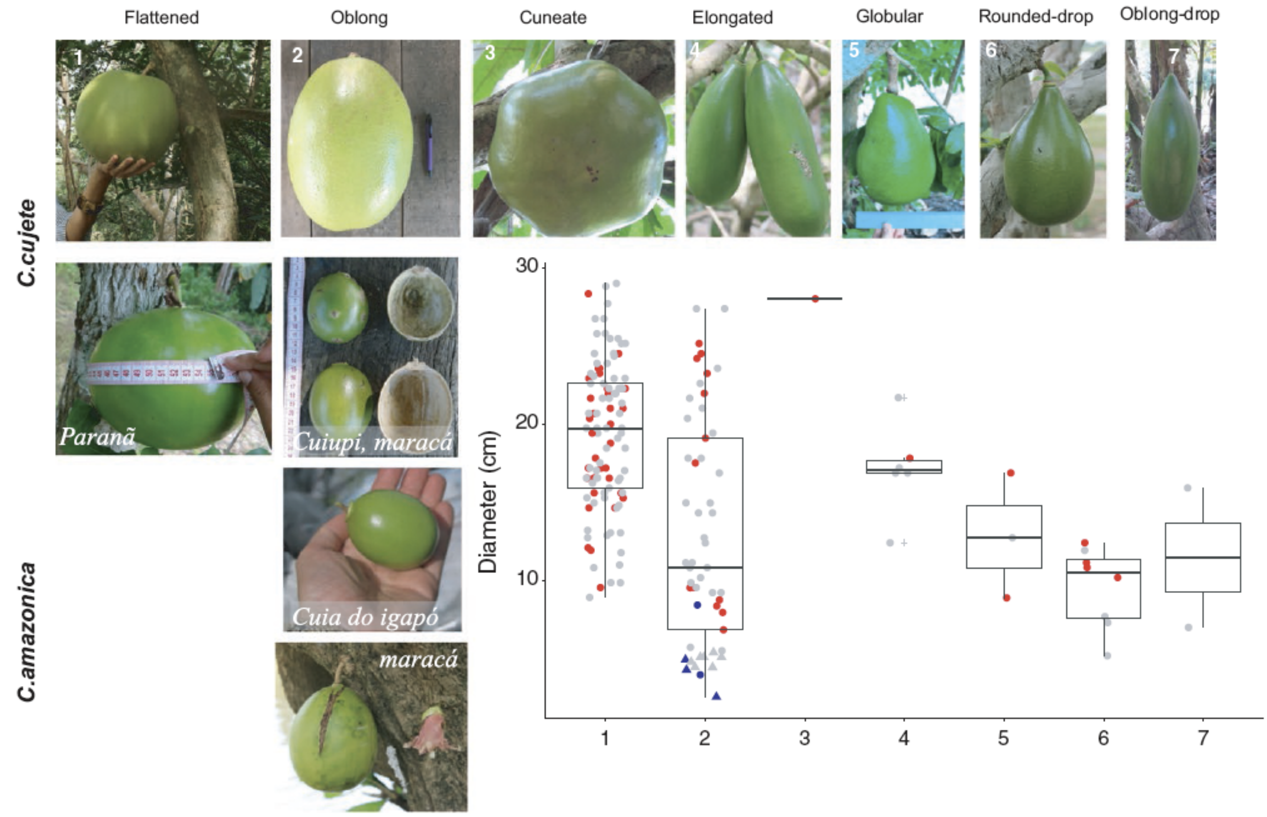

Figure 4. Diversity of fruit shapes and sizes in Amazonian treegourds. Seven fruit shapes of *Crescentia cujete* and one shape of *Crescentia amazonica* were found in the Brazilian Amazon Basin. Shape classification follows Arango-Ulloa et al. (2009). Smaller fruits of type 1 and 2 have local names that are shown in italics in the corresponding photo. Box plots of the variation in diameter (cm) of each fruit shape, with domestic (●) and wild individuals (▲). The colors represent chloroplast haplotypes (red - *C. cujete*; blue - *C. amazonica*; gray - not confirmed). In fruit shape 2, fruits smaller than 10 cm can be *C. amazonica* or admixed *C. cujete*, in which case they correspond to the *maracá* fruit type.

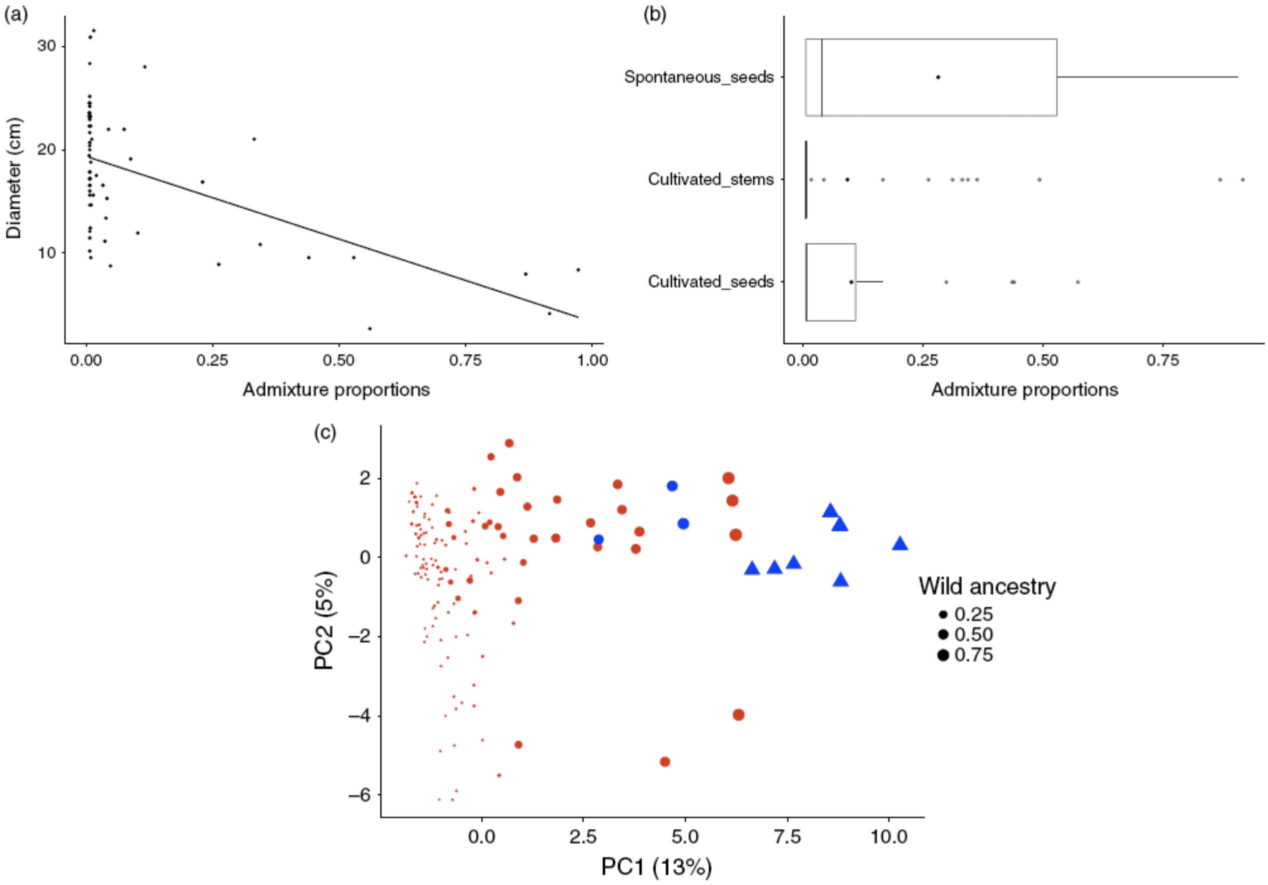

Figure 5. Admixture proportions of treegourds based on 8 nSSR and its relationship with fruit size and propagation method, and the dispersal of diversity in the principal component analysis. (a) Fruit diameter of genetically confirmed *Crescentia cujete* samples (N = 61) as a function of the proportion of admixture with *C. amazonica*, where diameter = 17.9 - 2.07*admixture (r^2 = 0.34, p = 1.26 x 10-6). (b) Admixture proportions of *C. cujete* (N = 80) samples propagated spontaneously by seeds and cultivated by stems and by seeds. Black dots indicate the average and white circles are outliers that highlight the active maintenance of admixed individuals by people. (c) Principal components analysis of the genetic relationships among *Crescentia cujete* (N = 170; red (●)) and *C. amazonica* (N = 14; blue (▲)) individuals. The proportion of the variance explained by each PC is shown in parentheses along each axis. The gradient of admixture is visible along PC1 and the admixed individuals correspond to the smaller fruit size varieties *paranã* (flattened type), *maracá* and *cuiupi* (oblong types) found exclusively in domestic areas.

Use, management and perception of fruit diversity

We recorded 11 current domestic uses of *C. cujete* fruits that are related to fruit morphology and were categorized in levels of specialization. Five uses are related with fruit size, without restriction of shape. The small gourds that are mainly used for food consumption (*xibé*, a meal of water and manioc flour), *açaí* (juice from *Euterpe oleracea* or *E. precatoria*), water or some particular medicine were reported in 31% of the municipalities. In the upper Negro River these are called *cuiupi*. Large gourds are preferred for use as baskets to store seeds, seedlings, cleaning products or to serve food (fish, *tapioca*), and were reported in 66% of the municipalities. Large gourds are also used as a unit of measurement of manioc during the preparation of flour and were reported in 47% of the municipalities, for the daily bath in the river (39%), to bail water from inside the canoe (39%), and pulp of broken fallen fruits as fodder for livestock (16%).

Four uses require greater specialization because they need a combination of size and fruit shape. Larger (20-24 cm) oblong fruits (type 2) are used to make a kind of bag with fiber handles (called *coió*, *comboró*, *mocó*), recorded in 30% of the municipalities, and used to transport things to the swiddens or to fish. Flattened fruits (type 1) with relatively smaller sizes (13-15 cm) are used in different contexts. They are involved in *tacacá* commerce, a typical Amazonian soup served in treegourd bowls (called *paranã*), whose manufacture was recorded in 11% of the municipalities. They are also used to make bowls to prepare blessings (14 cm) during religious events, recorded in 8% of the municipalities, and more rarely as parts of clothing sewn for regional festivals (2.8% of the municipalities).

Finally, there is an opportunistic use of fruit diversity, recorded in 16% of the municipalities, which incorporates different fruit shapes and sizes into the repertoire of manufactured objects sold as handicrafts. In this repertoire, even the wild species, *C. amazonica,* with its small fruits, is included, as recorded in four municipalities along the Solimões-Amazonas River. Its fruits are designed as small cups, painted and sold to cosmetic and food companies as "ecological cups", and are worth U$ 6 per 100 cups for handicrafters (price in 2014). Other wild fruit type handicraft designs include the reproduction of ancient musical instruments called *maracá*, and also as toys for children (*carrapeta*). The use of these artifacts was recorded in domestic contexts in 8% of the municipalities, all along the Solimões-Amazonas River, where admixture is relatively frequent and with higher levels (Figure 2b).

While large fruits have a general name (*cuia* and *coité*) with variations that include shape information (e.g., long gourd), the smaller fruits are distinguished with different local names that are related to flooded environments: *cuia-do-igapó*, *cuiupi*, *paranã* and *maracá. "Cuia-do-igapó"* is the wild tree that grows spontaneously in the *igapó,* a local word for a forest environment that is periodically flooded by black or clear water (Irion et al., 1997), and has smaller size fruit (*"it is the same as cuia, but is small, is from the forest, is not planted"*). The other smaller gourds mentioned depend on human intervention to survive and are found exclusively in domestic contexts. *Cuiupi* was cited along the Madeira River as similar to the wild type ("*there is cuiupi in the lakes, near the river, but I prefer the big one"*), while along the upper Negro River it is the general term for small gourds. *Paranã* is a local word to describe secondary river channels, often between the edge of the forest and the white-water floodplain system rich in nutrients and sediments (Irion et al., 1997), the habitat of *C. amazonica*. *Maracá* develops increased fruit size in domestic contexts ("*maracá is the cuia-do-igapó, but it is bigger"*) and its fruit production is restricted to flooding season ("*the fruit fails, does not give all the time*"). Based on genetic analyses and people's designation of varieties, *cuia-do-igapó* refers to *C. amazonica,* while *cuiupi* and *paranã* are *C. cujete* with moderate admixture proportions. *Maracá* is a special case, since it is applied not only to admixed *C. cujete*, but also to *C. amazonica*.

Cultivation by seeds, but also by stems, maintains highly admixed samples (Figure 5b), since many desirable gourds have smaller sizes, which is partly due to admixture effects (Figure 2c). Most treegourds are propagated by stem cuttings (63% of the sampled trees), and less frequently by seeds (37%). Stem cuttings have a purpose: they ensure a fast way to produce fruits ("*by seeds takes more time*") and the maintenance of fruit morphology, avoiding those fruits that break easily ("*by stems it is better for the cuiupi not to become soft and break*"). This is also the preferred method among traders who cultivate large clonal areas in upland environments for *tacacá* bowls, the flattened smaller gourd (type 1) with moderate levels of admixture called *paranã.* Seed propagation can be a spontaneous event when flood water brings seeds (28%) or spontaneously propagated through discarded fruit pulp near the house (71%). Seedlings, despite the lack of guarantee in fruit morphology, can survive floods better than cuttings in floodplain landscapes. People stated that: *"it is not correct to plant seeds, but stems do not take here*", *"when it grows by seeds, from the pulp, it grows smaller".* In the floodplain, stem cuttings require greater effort ("*to make big cuia takes much work*"), and success is not guaranteed ("*it is not all the stems that work, here I've tried hard*"). Thus, seed propagation is a way to deal with the high annual flooding events in the floodplain.

Another motivation is that seedlings are more suitable to produce smaller fruit sizes that are also useful, especially for handicraft purposes, as observed in the social movement of craftwomen along the middle Amazonas River.

Discussion

Identification of geographically widespread admixture

The tenuous nature of reproductive barriers among *Crescentia* species (Gentry, 1980) was confirmed by the large amount of admixture observed between *C. cujete* and *C. amazonica.* Our inference of admixture proportions was very robust even with unequal sample sizes and was correlated with the hybridization index of Gompert & Buerkle (2010), even with our small set of nuclear makers. The main reason is certainly the very high differentiation between the two species (F_{ST} = 0.74 with nSSR; F_{ST} = 0.89 with cSNP). Although we could not rule out the existence of null nSSR alleles in *C. amazonica*, they do not influence the high differentiation observed between species. Different histories of gene flow might result in similar patterns of admixture proportions (Barton & Hewitt, 1985; Gompert & Buerkle, 2016). In this case, one question is if these admixture proportions reveal hybridization after secondary contact or a long-term divergence. The high number of substitutions between *C. cujete* and *C. amazonica* chloroplast sequences suggests ancient divergence between the two species. As *C. cujete* is a species with cultivated populations, the origin of this domestication is unlikely to be older than other domestications in the Americas, which started by 11.000 years (Piperno, 2011). The chloroplast sequence divergence that we found suggests that the two species diverged earlier. Therefore, *C. cujete* was likely introduced by humans into South America (Gentry, 1980), and the admixture observed is secondary contact.

The hybrids are concentrated along the east-west axis of the Amazon Basin, the Solimões and Amazonas Rivers (Figure 1). Although proximity to floodplains is an important parameter for the occurrence of hybrids, admixed individuals are also found beyond *C. amazonica*'s known distribution. One example is the occurrence of admixture along the Negro River, where no *C. amazonica* has been collected to date (Figures 1 and 2b). This pattern might result from social networks and propagule exchange of admixed plants by humans between rivers, such as between the Orinoco and Negro basins (Hornborg, 2005; Lathrap, 2010).

Flooded forests are a source for cultivated treegourd phenotypic diversity

Fruit size variation of domesticated *Crescentia cujete* in Amazonia is partly shaped by admixture between wild and cultivated plants. Note here that we do not have a common garden experiment to evaluate the fruit phenotype. Establishing such a common garden will be difficult because treegourd is of minor economic importance outside local communities and consequently there is no conservation or breeding in a research institution to allow working in already available common gardens. However, most of the cultivated plants measured shared a cultivated environment in homegardens. If size was simply associated with variability of the environment, we would not detect a significant association with introgression. The absence of a common environment certainly adds more variability in size, but consequently also renders significant correlations with admixture more difficult to detect.

As larger fruit size is an expected feature of tree domestication syndromes (Miller & Gross, 2011; Meyer et al., 2012), hybridization and introgression create variation in fruit size (Cornille et al., 2014) that can be managed (García-Marin et al., 1986; Zerega et al., 2004; Hughes et al., 2007; Cornille et al., 2012; Aumeeruddy-Thomas et al., 2014). The great diversity of fruit sizes in Amazonian homegardens was also observed in the floodplains of the Orinoco River and the Caribbean regions of Colombia (Arango-Ulloa et al., 2009). Similarly, in the Yucatan Peninsula of Mexico, large propagated fruits and spontaneous smaller fruits were reported in homegardens (Aguirre-Dugua et al., 2012). This suggests that perceptions of hybridization are used to manage fruit size and shape across the Neotropics.

Our results showed that pollen gene flow occurs in both directions between these *Crescentia* species. Bat pollination observed in both species (Fleming et al., 2009) certainly favors this pollen flow between villages and flooded forests. However, the hybrid plants were restricted to human managed areas. Hybrid and introgressed *C. cujete*/*C. amazonica* are certainly selected against in the flooded forest and favored in human areas, as adaptation to natural environments is likely to be reduced by hybridization with domesticated populations (Ellstrand, 2003).

Traditional communities manage hybridization

Use of larger treegourds is widely distributed throughout the Amazon Basin, while the use of smaller ones is reported in less than half of the municipalities visited. Small fruits are much appreciated for the manufacture of handicrafts. However, the record of domestic artifacts made of small fruits suggests ancient use and not only contemporary commercial handicraft demand. The historical use of smaller treegourd fruits was mentioned by many ethnographers, especially in rituals and for medicinal purposes. *Maracá* is a symbol in spiritual practices of different Native Amazonian cultures, as well as an ancient rattle, and *paranã*, the flattened round type, was traditionally used for bowls in ceremonial rituals (Steward, 1948; Ribeiro, 1995; Lévi-Strauss, 2004). Both of these objects made from smaller treegourds are considered by oral histories as the first things to be in the world in different cultures of Mesoamerica, the Antilles and South America (Heiser, 1993), such as the Taíno from the Dominican Republic (Martin, 1999), the Guarani from southern Brazil (Montardo, 2002) and the Tukano from Amazonian Brazil and Colombia (Hugh-Jones, 2009). These smaller fruits come from admixed individuals (Figures 4 and 5), which are maintained and dispersed by people. In the Brazilian Amazon Basin, they are recognized as local varieties (*cuiupi*, *paranã*, *maracá* and *cuia-do-igapó*), whose names and associated traditional ecological knowledge are explicitly related to *C. amazonica* habitat, or wild-cultivated hybridization and its morphological consequences. The association with flooded environments is also present in the Tupi origin of the name *cuiupi* (from *kuy' y*) that refers to gourds of the water (Ferreira, 2004). The name *maracá* (*mbara'ka*) refers to the small rattles played in order to talk to lakes and heal sick people (Andía, 2015). *Maracá* was also used by Ducke (1946) when he described *C. amazonica* collected in flooded forests of the Solimões River in 1937. All of this confirms that the small fruited varieties used to manufacture important objects in the Amazon Basin, such as *maracás*, are the result of human selection of hybrid and admixed trees, and highlight that treegourd diversity is partly dependent on hybridization between homegarden and flooded forest genepools.

Although there is an historical use of these admixed treegourds, there is no evidence of ancient cultivation of *C. amazonica.* Our documentation of cultivation of *C. amazonica* along the Solimões-Amazonas River, especially in Santarém handicrafter villages, appears to be a recent practice. Treegourd handicrafts have been famous since before the Colonial period (Rodrigues Ferreira, 1933; Medina, 1934; Patiño, 1967) and gained prominence recently as a Brazilian Cultural Heritage (IPHAN, 2015). Handicrafts are motivated not only by social and economic demands, as highlighted by Santos (1982) and Carvalho (2011), but also ecological pressures, since severe flooding in these areas influenced people to use seedlings as an alternative way to produce treegourds for sale. As a result, we observed a high frequency of admixture in these handicrafter villages located in the middle Amazonas River (Figure 2b). The most common cultivation practice of *C. cujete,* however, is vegetative propagation, not only in Amazonia, but also in Mexico (Aguirre-Dugua et al., 2012). This is the traditional way to maintain useful fruit phenotypes, a practice that allows management of hybridization also (Miller & Gross, 2011). The admixed treegourds have been dispersed by humans along Amazonian rivers and potentially over larger geographical areas, which might create a complex pattern of geographic admixture, as observed for several other Neotropical fruit trees and their wild populations [*Spondias* (Miller, 2008); *Inga* (Dawson et al., 2008); *Chrysophyllum* (Petersen et al., 2014)], as well as in the Old World genus *Prunus* (Delplancke et al., 2012). Therefore, human activity not only maintains, but promotes congener interaction, as expected with other crops (Riesenberg & Wendel, 1993; Anderson, 2005). Hybrids are perceived and propagated in a dynamic way, so that hybridization is managed according to people's needs. This is in agreement with local farmers' practices and experimentation observed worldwide (García-Marin et al., 1986; Jarvis & Hodgkin, 1999; Brush, 2000; Hughes et al., 2007).

Conclusions & Perspectives

We provided evidence that variation of fruit size of the two *Crescentia* species found in the Brazilian Amazon Basin is related to their admixture proportions. New morphotypes that arise from hybridization are clearly recognized by people and named as local varieties (*maracá, cuiupi, paranã*), whose symbolism is emblematic for Amazonian cultures. Beyond treegourd, our study clearly shows that hybridization plays an important role in crop phenotypic diversification. We also showed that the integration of molecular analyses and farmers' perceptions of diversity can help disentangle crop domestication history. The specific traditional uses suggest that admixture management is an ancient human practice, also used in current traditional communities. We found that treegourd phenotype diversity depends partially on gene flow between homegardens and flooded forests. These results highlight the linkages between agriculture and forest ecosystems necessary for effective conservation of Amazonian agrobiodiversity. This is especially important since traditional ecological knowledge and floodplain conservation are neglected by development models for Amazonia (Posey & Balick, 2006; Castello et al., 2013).

Data sharing

1. Geographical coordinates are available in Table S1.

2. Vouchers of *C. amazonica* (Lat -4.32/ Lon -59.71 and Lat -2.11/Lon -54.72) were deposited in the National Research Institute of Amazonia Herbarium (INPA numbers 255.829 and 266.725).

3. The sequence alignments and microsatellite genotypes are available in Dryad (doi:10.5061/dryad.t84p3)

Supplementary Materials

Table S1. Passport data of all *Crescentia cujete* and *C. amazonica* collections made in Brazilian Amazonia, with additional information about genetic analyses and morphology. Column A = Collection code; B = Sequencing code; C = Species field identification; D = Local name; E = Latitude; F = Longitude; G = Municipality; H = State; I = Local landscape; J = River basin; K = if analysed for nSSR; L = if analysed for cpSNP; M = Mapped reads from chloroplast sequencing; N = % of missing data in sequence; O = Fruit diameter; P = Fruit shape; Q = Propagation method; R = Nuclear cluster; S = Q1 (%) from Structure analysis; T = Q2 (%) from Structure analysis; U = Chloroplast cluster.

(available on http://onlinelibrary.wiley.com/doi/10.1111/eva.12474/abstract)

Table S2. List of the 1068 perfect nuclear SSR developed in this study for use with *Crescentia cujete*. The name of the loci, the SSR motif, the number of repeats, the forward and reverse primer sequences, the left primer distance from the SSR (bp), the QDD design and the merged read where they were located, with microsatellites indicated in lowercase font.

(available on http://onlinelibrary.wiley.com/doi/10.1111/eva.12474/abstract)

Table S3. Eight nuclear SSR primers used to analyze genetic diversity of *Crescentia cujete* and *C. amazonica*. The type and estimated number of SSR repeat motifs, the total number of alleles found in each locus, their size by base pairs (bp) and the primer annealing temperature (T_a), all determined for *C. cujete*.

Locus	Primer sequence (5'-3')	Repeat motif	N. alleles	Allele size (bp)	T_a (^oC)
Ccuj3	F:GACACTTGAGAGGGATACAGGG R:TGTTTGGGTATGGAGTTGTCAGA	$(TA)^{11}$	9	234-270	59
Ccuj4	F:GGAGAAATAAGGGATACAGATCTACC R:TGAAGTGTAAGCCCGAATGACT	$(TAT)^{8}$	3	193-208	59
Ccuj7	F:GGGCTTGGGAAGGAACACAT R:ACAACCATGACTCTAGCCTATTACA	$(ATA)^{10}$	6	165-174	59
Ccuj8	F:AACACAACAACGATAATCAAGGG R:ACAACCATGACTCTAGCCTATTACA	$(AT)^{10}$	11	170-190	55
Ccuj11	F:ACGTGGCACTGTCCCTATCT R:AGTGGAAGATGGGCAGTAGC	$(TAT)^{8}$	3	149-160	59
Ccuj13	F:ACTTGGCTTCTAGCTGTTAGAACT R:ACAACCATGACTCTAGCCTATTACA	$(TA)^{12}$	8	185-203	58
Ccuj14	F:GGGAATAATTGGTGGGATTTCAGT R:ACAACCATGACTCTAGCCTATTACA	$(TC)^{11}$	5	153-169	55
Ccuj15	F:TAAAGCTCGCAACGACTCGT R:ACAACCATGACTCTAGCCTATTACA	$(TA)^{11}$	10	137-171	58

Table S4. Genetic diversity of *Crescentia cujete* and *C. amazonica* based on eight nSSR. Samples were classified by their levels of admixture according to Structure simulations: pure (ancestry > 0.9), admixed (ancestry < 0.9). *N* = number of samples, A_r= rarefied allele counts, H_o = observed heterozigosity, H_s= expected gene diversity, and mean F_{IS} (* significant at $p < 0.05$).

Species	*C. cujete*						*C. amazonica*					
Admixture	pure			admixed			admixed			pure		
N	142			28			3			11		
	A_r	H_o	H_s	A_r	H_o	H_s	A_r	H_o	H_s	A_r	H_o	H_s
SSR3	6.7	0.48	0.53	7.7	0.44	0.74	2.0	1.00	0.5	1.0	0.00	0.00
SSR4	2.0	0.54	0.43	3.0	0.67	0.56	1.5	0.50	0.38	1.0	0.00	0.00
SSR7	4.0	0.18	0.2	4.6	0.12	0.23	1.0	0.00	0.00	1.3	0.33	0.29
SSR8	8.9	0.55	0.58	8.7	0.67	0.76	1.6	1.00	0.5	1.0	0.00	0.00
SSR11	3.0	0.03	0.06	2.0	0.33	0.49	1.3	0.33	0.28	1.0	0.00	0.00
SSR13	6.0	0.08	0.37	7.0	0.18	0.68	1.0	0.00	0.00	1.0	0.00	0.00
SSR14	2.0	0.01	0.02	4.0	0.21	0.60	1.6	1.00	0.5	1.5	0.10	0.48
SSR15	6.6	0.17	0.27	6.4	0.54	0.59	1.6	1.00	0.5	1.0	0.00	0.00
Mean	4.9	0.25	0.31	5.4	0.39	0.58	1.4	0.60	0.33	1.1	0.05	0.09
F_{IS}		0.19									0.44^*	

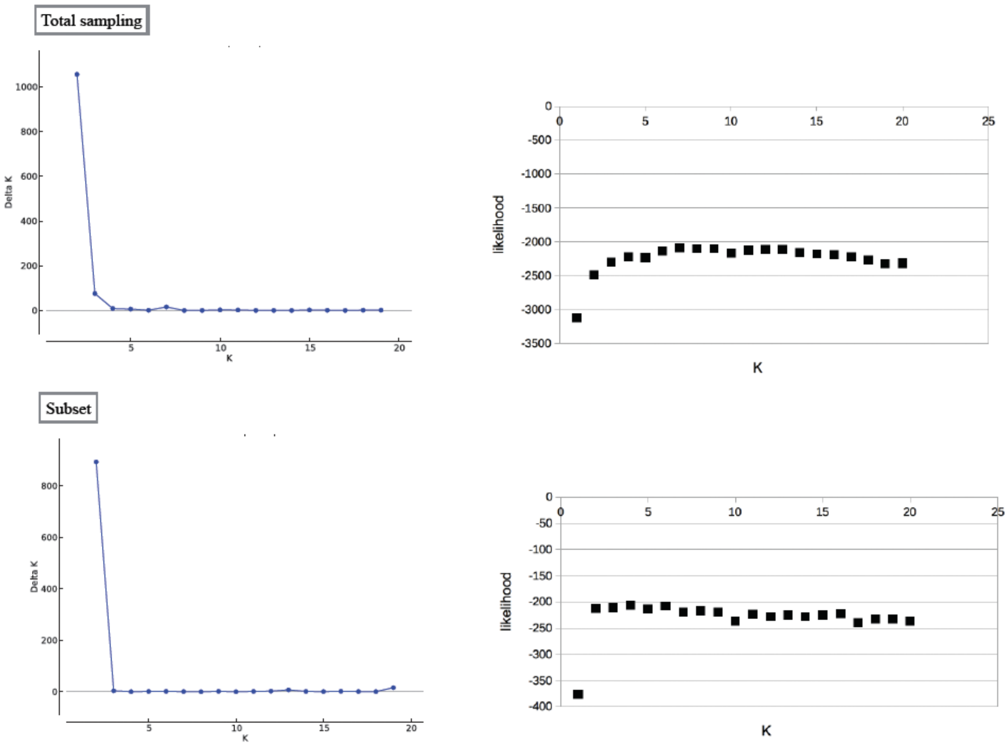

Figure S1. Estimation of the number of *Crescentia cujete* and *C. amazonica* population clusters identified by Structure simulations based on 8 nSSR and the ad hoc ΔK of Evanno et al. (2005). Above. The most likely number of groups (K) using the total sample (N = 234). Below. A randomly chosen subset of the *C. cujete* sample (N = 14) equivalent to the *C. amazonica* sample (N = 14). For each individual, the proportion of admixture obtained from the subset run was compared with the proportion obtained from whole dataset ($r^2 = 0.99$, $p < 10^{-15}$).

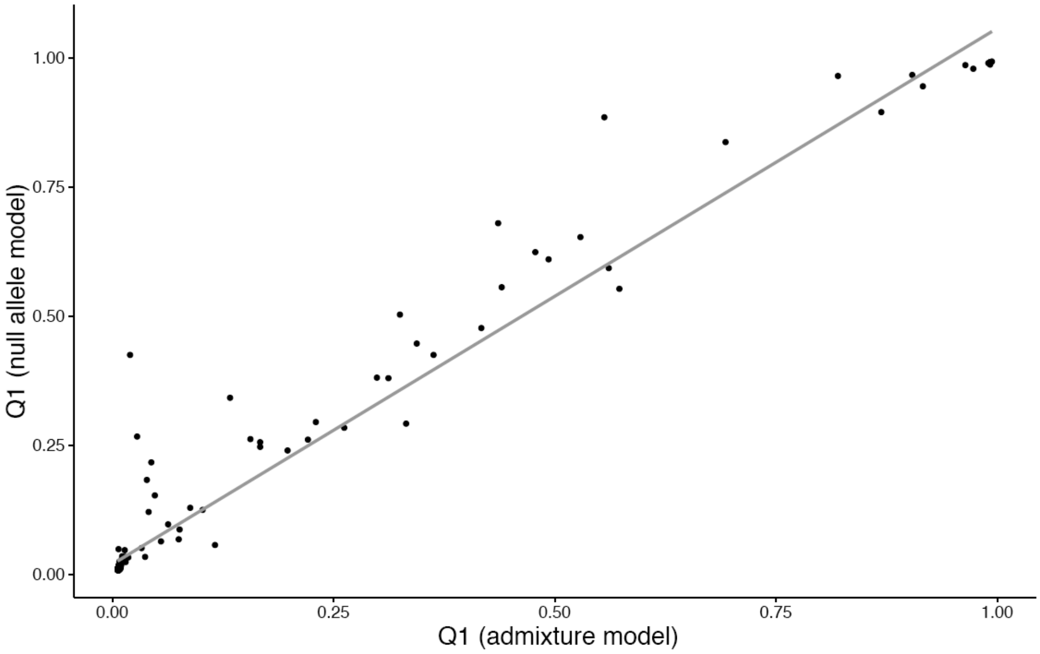

Figure S2. Impact of null alleles on admixture inference following Falush et al. (2007). For each individual of *Crescentia cujete* and *Crescentia amazonica* with missing data, these were substituted with null homozygotes and the admixture proportions obtained using the Structure specifications for the admixture model were compared to those using the null allele model (r^2= 0.96, p < 10-15).

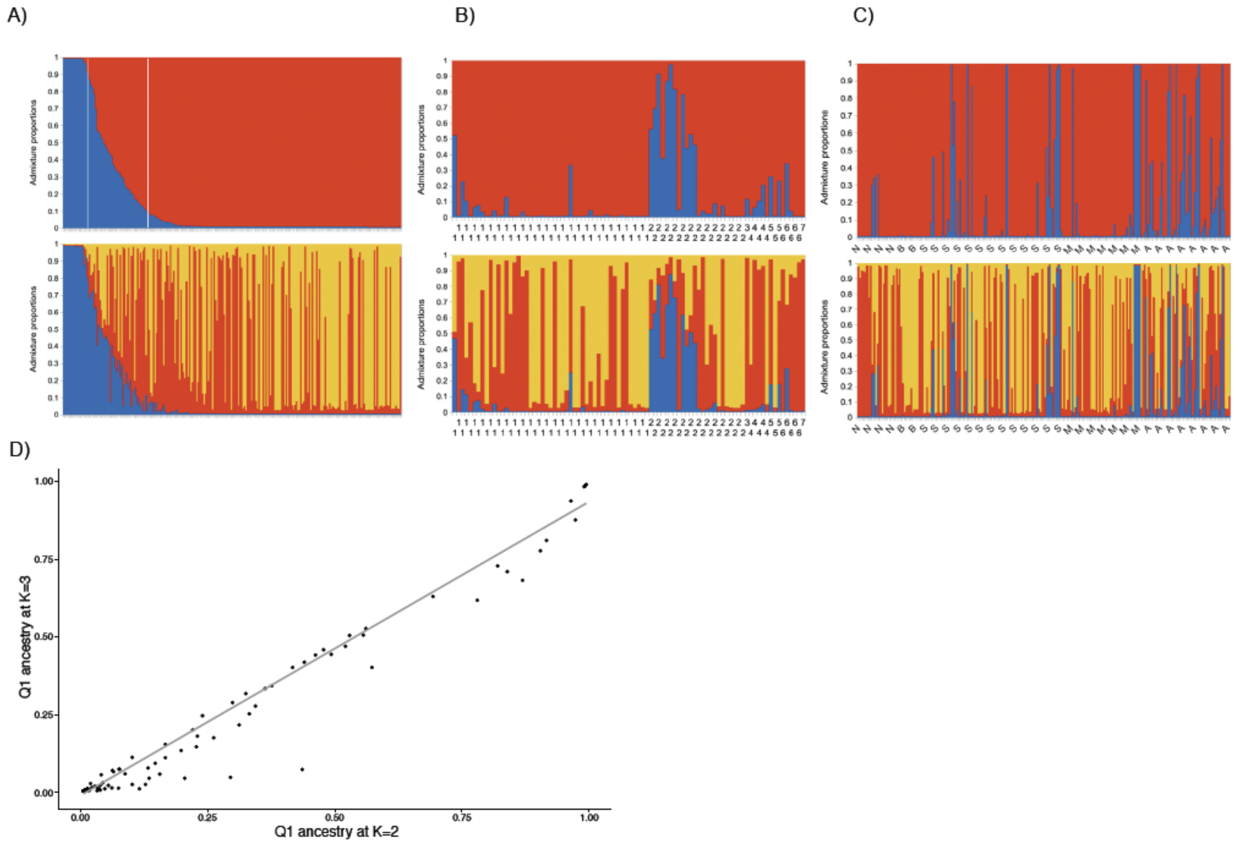

Figure S3. Comparison of Structure analysis at K = 2 and K = 3 of treegourd samples collected in Brazilian Amazonia using 8 nSSR. (a) Above. The full data set (N = 234) at K = 2. The y-axis shows the % of assignment to the groups (red - *Crescentia cujete* and blue - *C. amazonica*). Samples were ordered according to their level of admixture: admixed if > 0.1, hybrids if 0.4 to 0.6, pure if > 0.9 of assignment to the group. Below. The full data set at K = 3. *Crescentia cujete* is divided into red and yellow groups. Middle. (b) Within each fruit type (from 1 to 7), samples were arranged by increasing fruit size from left to right (N = 88). Above – K = 2; below – K = 3. (c) Samples were arranged according to their geographical location along the main rivers: The Negro (N), Solimões (S) and Amazonas Rivers (A) are ordered west to east; the Branco (B) River is ordered north to south; the Madeira (M) River is ordered south to north. Above – K = 2; Below – K = 3. (d) The proportion of the blue assignment (*C. amazonica*) (Q1) is similar using K = 2 or K = 3 ($r^2 = 0.98$, $p < 10^{-15}$).

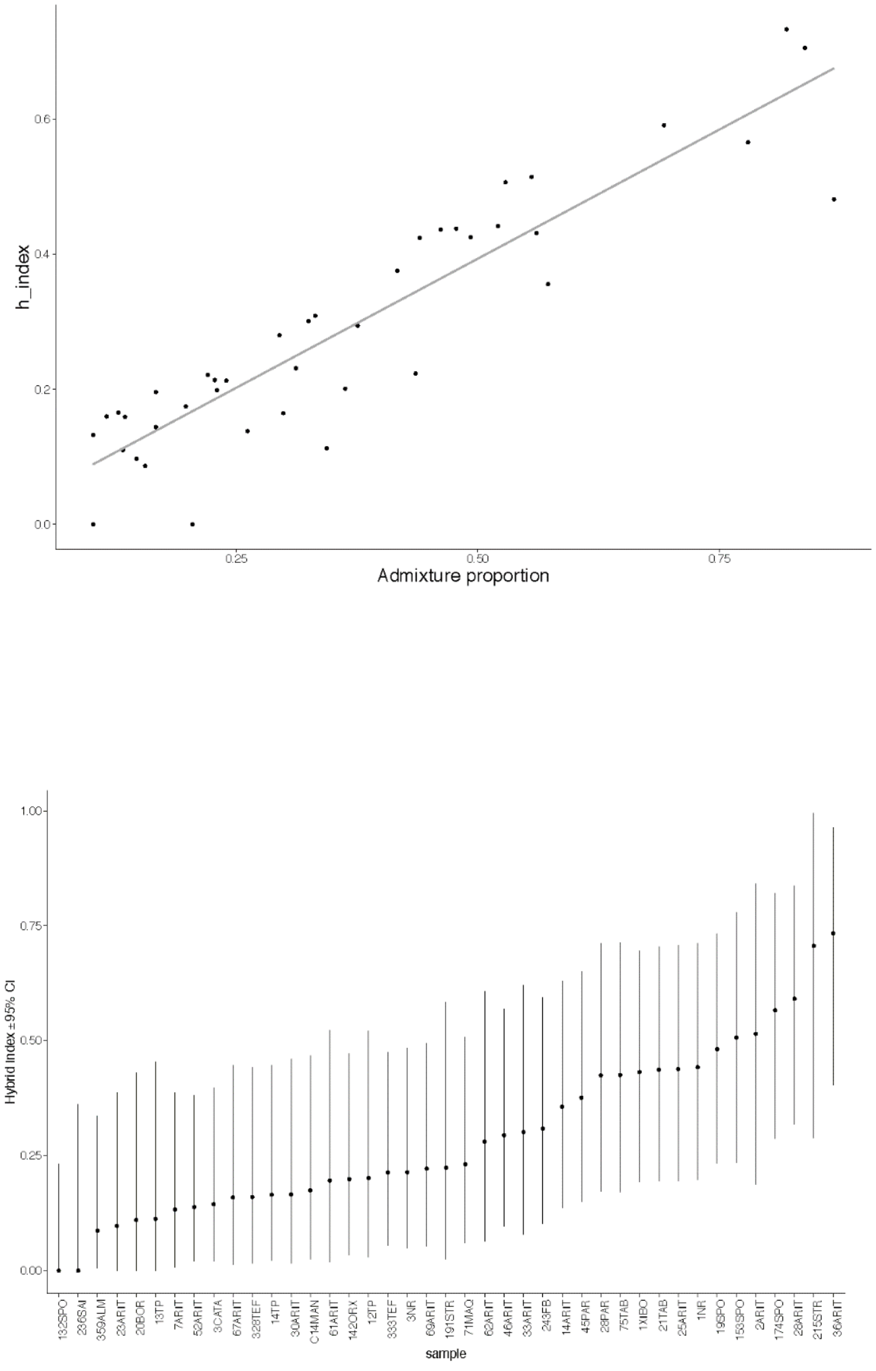

Figure S4. Hybridization index (h_index) estimated by Introgress 1.2.3 (Gompert & Buerkle, 2010). Above - relationship between the hybridization index and the proportion of *Crescentia amazonica* ancestry (Q1 < 0.9) of the admixed and hybrid samples (N = 41, see Table 1) obtained by Structure based on eight nSSR at K = 2 ($r^2 = 0.83$, p < 10-15). Below - the 95 % confidence intervals of each hybrid sample. The names of samples along the x-axis are equivalent to their passport (Table S1).

Capítulo 4

Diversity of treegourd (*Crescentia cujete*) suggests introduction and prehistoric dispersal routes into Amazonia

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Special edition: Ecology and evolution of plants under domestication in the Neotropics

Abstract

The use and dispersal of domesticated plants may reflect patterns of early human diffusion of technologies and lifestyles. Treegourd (*Crescentia cujete*) has fruits with ancient utilitarian and symbolic value in the Neotropics. We assessed its diversity based on chloroplast (SNPs), nuclear (SSR) markers and fruit shapes, since few studies have demonstrated the genetic signatures of plants' geographic dispersals mediated by humans in Amazonia. The haplotype network showed three distinct groups: *Crescentia amazonica*, wild Mesoamerican *C.cujete*, and cultivated *C. cujete* from Brazilian Amazonia and Mexico. Mexico and Brazil shared two haplotypes, with slightly different distributions in Amazonia. The most divergent haplotype is well represented in Eastern Amazonia. Nuclear differentiation between Mesoamerican wild and cultivated *C. cujete* is relatively low (F_{ST} = 0.35), compared with Amazonian cultivated (F_{ST} = 0.45 to 0.61). Differentiation is also higher between wild *C. amazonica* and cultivated *C. cujete* (F_{ST}= 0.57), but modest within cultivated *C. cujete* from Amazonia and Mexico (F_{ST}= 0.04), with higher genetic similarity in northwestern Amazonia. Mexico and Amazonia showed similar chloroplast nucleotide diversity (4.66 x 10^{-2} and 5.31 x 10^{-2}, respectively), although sample sizes are very different. Except in Northwestern and Eastern Amazonia, we found ample genetic homogeneity of cultivated *C. cujete* across Amazonia, but highest morphological diversity in the Northwest, with fruit shapes that are absent in Mexico. We conclude that treegourds introduced into the Amazon Basin and Mexico share a common ancestry with a currently unknown origin. The patterns of genetic diversity across Amazonia allow two hypotheses of the routes of introduction: a northwestern introduction into the Negro and Solimões Rivers, and an eastern introduction from the coastal Guianas into the Amazonas River. The dispersal into Amazonia followed previously proposed routes of human and plant migrations. The fruit shape diversity reveals different utilitarian demands for fruits. Mexico and Amazonia contrast on their fruit morphological diversity, which suggests different cultural preferences along treegourd's dispersal routes. More comparative studies of its different uses with a broader genetic and phenotypic distribution would be useful to better understand the dispersal and diversification of *C. cujete* in the Americas.

Keywords: Amazonia, historical ecology, domestication, phylogeography, ethnobotany

Introduction

The use and dispersal of domesticated plants may reflect the patterns of diffusion of human technologies and lifestyles since prehistoric times (Bellwood, 2005; Blench, 2012). Humans greatly expand plants' geographical distributions, which ultimately exerts different ecological and cultural pressures on the evolutionary pathways of plants (Rindos, 1984; Sodero Martins, 2005; Leclerc & Coppens d'Eeckenbrugge, 2012; Meyer & Purugganan, 2013). Various centers of domestication have been proposed in the Americas (Meyer et al., 2012) from where people exchanged plants (Schultes, 1984; Heiser, 1965; GarcíaMarín & Zizumbo-Villarreal, 2004). Amazonia is one of them (Clement, 1999), and also encompasses great linguistic diversity (Blench, 2012), ceramic styles (Barreto et al., 2016) and landscape management strategies (Eriksen and Danielsen, 2014), whose geography and chronology are being disentangled (Mayle & Iriarte, 2014; Clement et al., 2015; Neves, 2016; Levis et al., 2017). The Amazonian routes of dispersal of plants and people have been associated with rivers and riparian environments (Schultes, 1984; Godoy, 1999; Guix, 2009). However, few studies have demonstrated the genetic signatures of the plants' geographical dispersal mediated by humans in Amazonia (Clement et al., 2010; Shepard & Ramirez, 2011; Thomas et al., 2012; Freitas & Bustamante, 2013), even though they are persistent markers of the long-term use and management of resources (Hanotte et al., 2002; Parker et al., 2010; Armstrong et al., 2017).

Treegourd (*Crescentia cujete*) is a good case study, since its trees produce fruits with ancient utilitarian and symbolic value widely dispersed across the Neotropics (Gentry, 1980; Arango-Ulloa et al., 2009; Aguirre-Dugua et al., 2013; Meulenberg, 2011; Medeiros & Albuquerque, 2014; Moreira et al., 2017). It currently is one of the most common species in homegardens of the floodplains and adjacent communities of Amazonia (Santos, 1982; Lima & Saragoussi, 2000). Its fruits have different shapes and sizes that are used as bowls, vessels or bottles for drinking or transporting water, bags for provisions, utensils for cooking and eating, bailing water from canoes, construction of fish traps, manufacture of body ornaments, and musical instruments (Steward, 1948; Patiño, 1967; Morton, 1968; Price, 1982; Bennett, 1992; Heiser, 1993; Meulenberg, 2011). Medicinal uses are also similar across its distribution (Duke, 1929; Morton, 1968), which include neutralization of snake venom and intestinal parasites (Otero et al., 2000; Volpato et al., 2009).

While the great phenotypic variability of cultivated treegourd is a distinctive feature among *Crescentia* species (Gentry, 1980), its wild populations from Mexican savannahs in the Yucatan Peninsula have smaller, elongated fruits with thinner exocarps (Aguirre-Dugua et al., 2012). The indehiscent and thicker exocarp of cultivated treegourd fruits makes the spontaneous dispersal of seeds impossible (Aguirre-Dugua et al., 2012). Its oldest remains found to date come from a Peruvian archaeological site dating to 5,000-3,800 years BP (Solis, 2006). This pattern contrasts to the bottle gourd, collected from a vine (*Lagenaria siceraria*), one of the ancient crops similarly used for technological purposes in the Americas (Heiser, 1993). Bottle gourd has been managed at least since the Late Pleistocene (Kistler et al., 2014) and was found in Colombian Amazon by 8,000 BP (Piperno, 2011). The wild progenitor of the cultivated *C. cujete* remains elusive (Gentry, 1980; Arango-Ulloa et al., 2009; Aguirre-Dugua et al., 2012; Moreira et al., 2017). Gentry (1980) pointed out that *C. cujete* was certainly native to Mesoamerica, where putative wild populations are found in savannahs and semi-evergreen forests of southern Mexico and northern Central America (Figure 1). However, northern South America cannot be ruled out as part of the original distribution area of wild *C. cujete*, given the occurrence of apparently spontaneous *C. cujete* in grazed savannahs of Andean and Caribbean regions of Colombia (Arango-Ulloa et al., 2009). Historical anthropogenic fire management in savannahs (Pinter et al., 2011) may have been advantageous for its early dispersal (Bass, 2004) in these regions. Recently, the wild species native to Amazonian and Orinocan floodplains (*C. amazonica*) was ruled out as the wild progenitor of cultivated *C. cujete* (Ducke, 1946; Moreira et al., 2017). Likewise, the wild *C. cujete* populations found in the Yucatan Peninsula of Mexico are not the wild progenitor either (Aguirre-Dugua et al. submitted). In this study, we infer treegourd dispersal and diversification across two pivotal regions of the Neotropics: Amazonia and Mesoamerica. We (1) identify genetic relationships among Mesoamerican and Amazonian cultivated *C. cujete*; (2) infer routes of introduction into and dispersal within the Amazon Basin; and (3) identify centers of morphological and genetic diversity. We discuss whether this genetic/morphological diversity is linked to 1) introgression with local wild parents, 2) ecological diversification, or 3) cultural diversification, since all three of them are possible along the dispersal routes.

Material & Methods

Sampling

We performed molecular analyses using full chloroplast (SNPs) and nuclear (SSR) markers. We also analyzed fruit morphology along the major rivers of Brazilian Amazonia and in parts of Mesoamerica. We used a previously published genetic and morphological dataset (Moreira et al., 2017) of cultivated *C. cujete* (N = 372) distributed in 122 localities along the five major rivers of the Brazilian Amazon basin, as well as wild Brazilian treegourds (*C. amazonica*) (N = 20) distributed in three of the rivers mentioned (Figure 1).

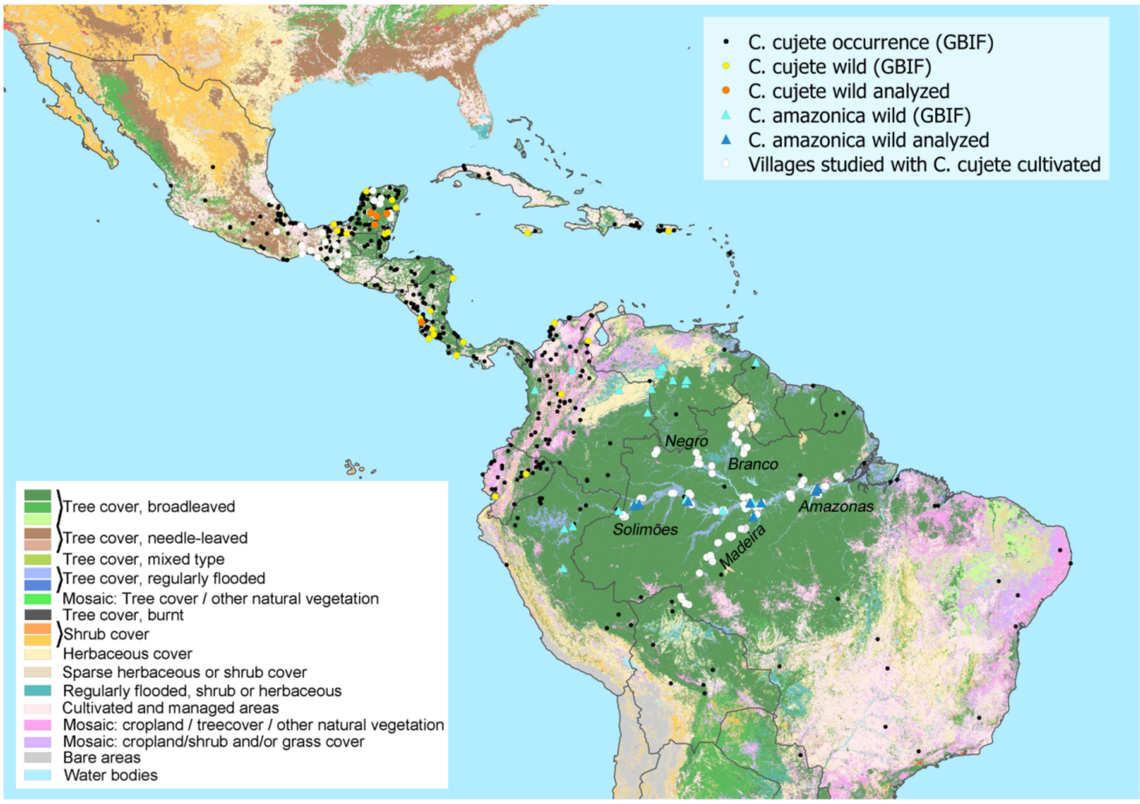


Figure 1. The geographical distribution of cultivated *Crescentia cujete*, putative wild populations of *C. cujete* and wild *C. amazonica* in the Neotropics. Genetic or morphological analyses include samples from Mexico, Costa Rica and five rivers in Brazilian Amazonia (Negro, Branco, Solimões, Madeira, Amazonas). Their distributions were complemented with records from the Global Biodiversity Information Facility (GBIF) and plotted over the vegetation cover (Bartholomé & Belward, 2005). The wild *C. cujete* distribution was hypothesized based on apparently spontaneous individuals growing in a mosaic of shrub and grass cover, which does not rule out previous human dispersion, since areas might include abandoned or burned croplands.

From Mexico, we add new genetic data of cultivated *C. cujete* from the Yucatan Peninsula, Oaxaca and Chiapas, wild samples from the Yucatan savannahs and a putative wild sample from Costa Rica (Figure 1). We also integrate morphological data from Mesoamerican samples (N = 188), part of which (N = 124) was published previously (Aguirre-Dugua et al., 2013). All Mesoamerican wild samples were identified as *C. cujete* Linnaeus 1753. In order to depict the putative geographical distribution of wild *C. cujete*, we searched for individuals of *C. cujete* described as spontaneous in savannahs on herbarium descriptions found in GBIF (Global Biodiversity Information Facility) (Figure 1).

This research followed the International Society for Ethnobiology's code of ethics (International Society of Ethnobiology, 2006) and was approved by the Committee for Ethics in Research with Human Beings of the National Research Institute for Amazonia (CEP INPA, proc. no. 408.611, 2013). Collection in Brazil was authorized by the Brazilian System for Authorization and Information in Biodiversity, Chico Mendes Institute for Biodiversity Conservation, proc. no. 25052-1, 2012, and transportation by the Brazilian Institute for the Environment and Renewable Natural Resources, proc. no. 14BR015576/DF, 2014. Collection in Mexico and Costa Rica was authorized by proc. no. SGPA/DGGFS/712/3691/10.

Genetic analysis

We used a previously described protocol for genotyping nuclear microsatellites and the detection of single nucleotide polymorphisms along the entire sequence of the maternally inherited chloroplast genome (Moreira et al., 2016; Moreira et al., 2017). In total, 250 samples were genotyped for eight nuclear microsatellites (SSR): 234 from Brazilian Amazonia (215 cultivated *C. cujete* and 19 wild *C. amazonica*), and 16 from Mesoamerica (7 cultivated *C. cujete* from Mexico, 8 wild *C. cujete* from Mexico, one wild *C. cujete* from Costa Rica). Data from the chloroplast genome was obtained from a total of 215 samples: 191 *C. cujete* and 16 *C. amazonica* from Amazonia, 5 cultivated *C. cujete* from Mexico, 2 wild *C. cujete* from Mexico, one wild *C. cujete* from Costa Rica. Among the total sample (N = 250), 80% were genotyped and sequenced for both kinds of markers.

The nuclear SSR dataset was used to assess population structure with a Bayesian approach (Structure 2.3, Pritchard *et al.* 2000). We applied the admixture model in order to identify ancestral population proportions for each individual and their probable populations of origin. Using total sampling and assuming independent allele frequencies in each population, which reduces the risk of overestimating the number of clusters (Pritchard et al., 2000), we assessed the number of clusters K varying from 1 to 20, with 100.000 burn-in, 100.000 iterations, and 5 different runs for each K value. To attempt to identify different genetic pools within the cultivated cluster, we performed an additional analysis on a subset including only cultivated *C. cujete* samples, whose membership probability was higher than 0.6 in the cultivated cluster (N = 200). Using the admixture model, we experimented with two allele frequency assumptions (Pritchard et al., 2000): the independent model as default; and the correlated (assuming lambda = 1), since it is likely that cultivated populations share ancestry due to migration and vegetative propagation. Evanno et al.'s (2005) ΔK was used to guide our choice of the most likely number of groups. Additionally, we performed a Principal Components Analysis (PCA) with stats R package (R Core Team, 2015) in order to uncover additional genetic structure in our data (Jombart et al., 2009). The PCA was non-centered, but scaled in order to compensate for differences in polymorphism and missing data among the loci analyzed. The spatial interpolation of the clusters obtained in Structure was analyzed using the kriging method in the *fields* R package (Nychka et al., 2015). Based on geostatistics and maximum likelihood, the krig function estimates the covariance in a grid (we used the scale parameter theta =50) and infers the fitted surface between geographical coordinates and genetic relationship among samples (Nychka et al., 2015). Nuclear genetic diversity of *C. cujete* [allelic richness (A_r), private alleles (A_p), observed heterozygosity (H_o), expected heterozygosity (H_s)] was estimated for the five Amazonian rivers considered and the Mexican samples using *hierfstat* (Goudet 2005) and *poppr* (Kamvar, 2014) R packages. Pairwise F_{ST} between regions were estimated and statistically evaluated using 1000 bootstraps (Nei, 1987). A neighbor-joining dendrogram of regions was constructed based on Nei's distance and 1000 bootstraps (Saitou & Nei, 1987). The inbreeding coefficient F_{IS} for each regionwas estimated and its significance evaluated (considering a Bonferroni corrected p value of 0.006) using *pegas* R package (Paradis, 2010).

For the identification of chloroplast SNPs, we used a bioinformatic pipeline previously validated for the sequencing of the entire chloroplast genome (Scarcelli et al., 2016). Briefly, SAMTOOLS 0.1.7 with option -B (Li et al., 2009) was used to generate an mpileup file. VARSCAN 2.3.7 (Koboldt et al., 2012) was used to call SNPs from this mpileup file. The variant call format file (VCF) generated was filtered following Scarcelli et al. (2016) and resulted in a total of 334 cpSNPs detected in our dataset. The final vcf file was exported as a fasta file using VCFtools 1.14 (Danecek et al., 2011) and haplotypes identified with DNAsp 5.10.1 (Librado & Rozas 2009). An haplotype network was constructed using the median joining algorithm (Bandelt et al., 1999) and samples with up to 6.5 % of missing data using POPART 1.7 (Leigh & Bryant, 2015). The geographical distribution of the shared haplotypes of *C. cujete* samples was plotted using GenGIS 2.5 (Parks et al., 2009). The chloroplast diversity of *C. cujete* [total number of polymorphic sites (S), number of haplotypes (h) and nucleotide diversity (π)] were estimated according to Nei (1987) using DNAsp 5.10.1. The presence of singleton samples and their contribution with unique alleles were identified by VCFtools 1.14. Paired F_{ST} among the Amazonian rivers and Mexico were estimated using the distance method of Tajima and Nei (1984), and their significance was evaluated with 1000 permutations at a significance level of 0.05 using Arlequin 3.5 (Excoffier & Lischer, 2010).

Morphological analysis

Fruit shapes of cultivated *C. cujete* were registered in 286 individuals and fruit diameter was measured in 175 individuals in the Amazon Basin. For Mesoamerican samples, we analyzed 117 cultivated individuals from Mexico, among which 64 were from 9 localities in the Yucatan Peninsula (Aguirre-Dugua et al., 2013) and 53 were from 19 localities representing the Gulf of Mexico coast, Tehuacan Valley, and Pacific Ocean coast from the states of Michoacan, Oaxaca and Chiapas (Figure 1).

The shape of the mature fruits of each individual was classified visually into nine categories: spherical, flattened, oblong, cuneate, elongated, globular, rounded-drop-shaped, oblong-drop-shaped, and kidney-shaped. All of these categories, except spherical, followed the classification created for Colombian fruits (Arango-Ulloa et al. 2009). The spherical fruit was added as a new category, since it is a remarkable shape found in Mexico, which has a higher index of roundness than flattened fruits (Aguirre-Dugua et al., 2012, 2013). For Brazilian samples, the flattened type was sub-divided in order to discriminate these perfectly spherical fruits from flattened ones based on visual comparison of photographs. The Shannon index was adapted to estimate fruit shape diversity using $H' = -\sum_i p_i \log p_i$, from Pielou (1975), where p_i is the relative frequency of each fruit shape. The Shannon index was calculated for each Amazonian river, and for Amazonia and Mexico.

Results

Geographic patterns of nuclear diversity

Evanno et al.'s (2005) ΔK suggested that two clusters are the most likely structure in the dataset (K = 2, Figure 2a, Appendix Figure S1). At K = 2, a clear distinction among wild and cultivated samples was observed (clusters shown in blue and red in Fig. 2a, respectively), regardless of their geographical origin. Mexican cultivated *C. cujete* samples showed an admixed pattern (membership probability to wild cluster from 0.16 to 0.87), as did some of the cultivated *C. cujete* from the Amazon Basin (membership probability to wild cluster from 0.01 to 0.98). The wild admixture within cultivated *C. cujete* in the Amazon Basin had higher proportions along the Amazonas River, decreasing values along the Solimões, Madeira and Negro rivers, and was absent along the Branco River (Figure 2a). The wild Costa Rican sample displayed a membership probability of 0.25 to the cultivated cluster, a larger proportion than the membership shown by the Mexican wild samples (0.01-0.02). In the Principal Component Analysis (PCA), the first two principal components explained 16.7 % of the total variance found in the dataset (Figure 2b). Principal component one separated wild from cultivated samples, while principal component two separated the Brazilian wild *C. amazonica* from the Mesoamerican wild *C. cujete* samples. The wild sample from Costa Rica was intermediate between wild and cultivated Mexican samples, which agree with its ancestry pattern observed in the clustering analysis performed by Structure. One Brazilian sample from Amazonas River was relatively closer to the Costa Rican sample (Figure 2b). To assess to what extent the intermediate ancestry of cultivated Mexican samples between wild Mesoamerican and Brazilian cultivated samples (Figure 2b) was associated with hybridization or divergence, we performed a Structure analysis among only Mesoamerican samples. This analysis clearly differentiates two groups of wild and cultivated Mesoamerican *C. cujete* (Appendix Figure S2). However, we still observed the Costa Rican sample as having intermediate ancestry among these Mesoamerican samples (Appendix Figure S2). Consequently, the intermediate ancestry detected in cultivated Mesoamerican samples may reflect divergence rather than hybridization. The differentiation between wild *C. cujete* and cultivated samples was also evident in the neighbor-joining dendrogram (Figure 2c). The level of differentiation between Mesoamerican wild and cultivated *C. cujete* was relatively low (F_{ST} = 0.35, $IC_{95\%}$ = 0.13-0.60).The differentiation between Mesoamerican wild and Amazonian cultivated *C. cujete* samples was lowest with the Negro River (F_{ST} = 0.45,$IC_{95\%}$ =

0.27- 0.65), followed by the Amazonas (F_{ST}= 0.50, $IC_{95\%}$ = 0.37- 0.64), the Solimões (F_{ST}= 0.52, $IC_{95\%}$ = 0.40- 0.68), Madeira (F_{ST} = 0.57, $IC_{95\%}$ =0.39- 0.81) and Branco (F_{ST} = 0.61,$IC_{95\%}$ =0.45- 0.82). The wild *C. amazonica* samples showed high differentiation compared with cultivated *C. cujete* samples (F_{ST}= 0.57, $IC_{95\%}$ = 0.37-0.64).

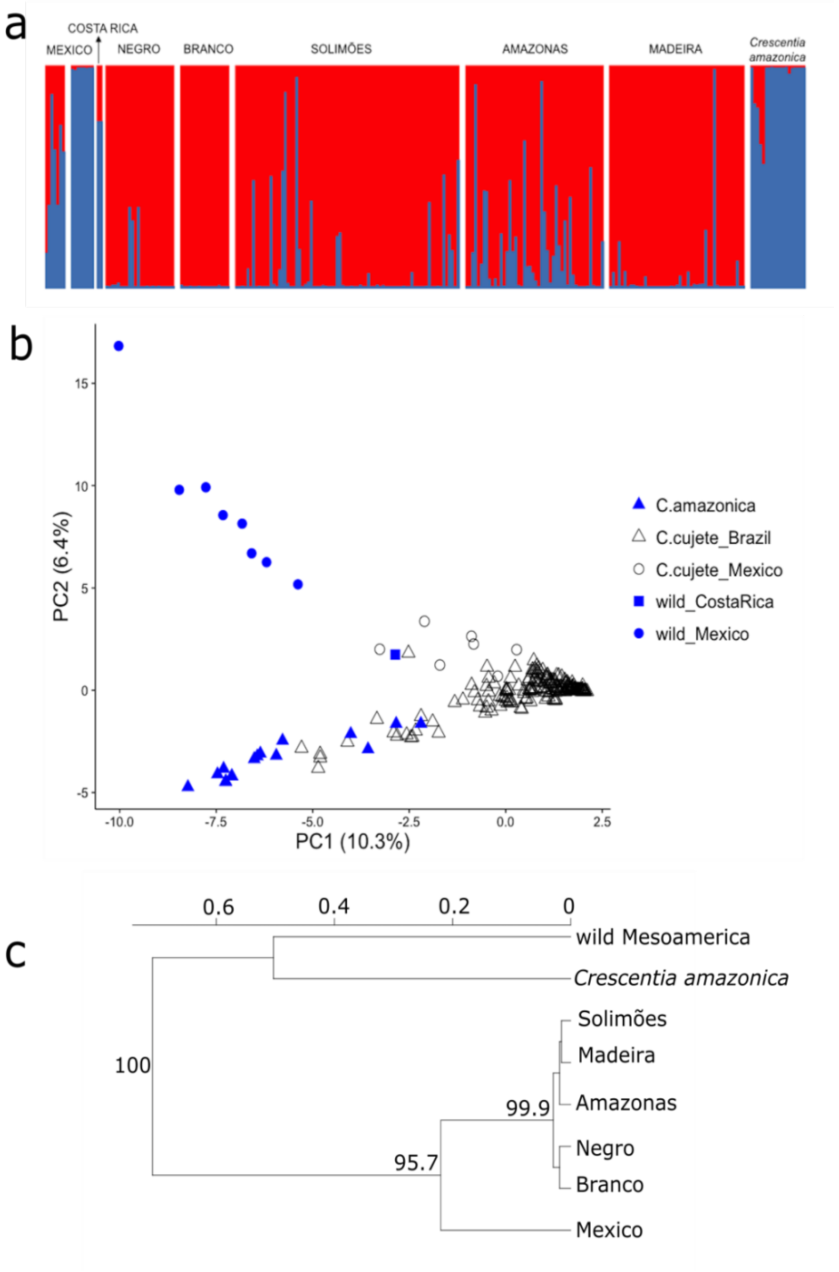


Figure 2. Genetic structure of 250 cultivated and wild treegourds (*Crescentia cujete*; *C. amazonica*) from Brazilian Amazonia and Mesoamerica based on 8 nSSR. Wild C. cujete were from the Yucatan Peninsula in Mexico and the Pacific coast of Costa Rica. (a) Structure plots at K=2. The y-axis shows the proportion of assignment to the cluster and each vertical bar represents a single plant. The geographic locations and river basins are separated by white vertical columns; in Mexico, the first group is cultivated and the second is wild. (b) Principal components analysis (PCA) of nuclear genetic structure. The solid symbols represent the two species in Brazil, while the gray refers to wild and cultivated C. cujete in Mesoamerica. Numbers in parenthesis show the percentage of the allelic variation explained by each axis. (c) Neighbor-joining tree of the geographic relationships between wild and cultivated samples based on Nei's genetic distance with 1000 bootstraps supports indicated on the nodes.

We performed another Structure analysis with the cultivated samples, using only plants whose membership probability was higher than 0.6 in the cultivated cluster (Figure 2a). The two allele frequency models showed similar patterns, with better defined clusters using the correlated model (Appendix Figures S3 and S4). Again, Evanno et al.'s (2005) ΔK suggested that two clusters are the most likely structure (K = 2, Appendix Figure S3); these distinguished Mexican from Brazilian samples, with considerable admixture widely distributed in the Amazon Basin (Figure 3a). Evanno et al.'s ΔK suggested decreasing likelihood of structure up to four clusters (K = 3 and K = 4), although the fourth cluster did not show a pattern that was clearly different from K = 3. At K = 3, Mexican and Brazilian samples showed strong admixture (green and yellow clusters in Fig. 3a). The green cluster membership was found in Mexico, but was higher along the Negro River and upper sections of the Branco River, with decreasing membership along the Solimões, Amazonas and Madeira rivers (Figure 3a). In contrast, the third yellow cluster, also found in Mexico, was predominant along the Amazonas and Madeira rivers, scattered along the Solimões, but also high in the middle Negro River (Figure 3a). The neighbor-joining tree differentiated two groups within Amazonia that are both genetically different from Mexico (Figure 3b). However, the differentiation between Amazonia and Mexico is modest (F_{ST}= 0.04, IC $_{95\%}$ = 0.006-0.08). Spatial interpolation of the Structure clusters highlights that, although the admixture between Mexico and Amazonia (Figure 3a), genetic similarity is higher between Mexican samples and northwestern Amazonia (Figure 3c). The spatial interpolation also reveals the wide genetic homogeneity of cultivated *C. cujete* across Amazonia, except for the genetic differentiation in the Northwest and East, which is free from local wild-admixture effect in this data set (Figure 3c). The Northwestern and Eastern regions are relatively similar (Figure 3c), which agrees with the distribution of the Eastern yellow cluster up to the middle Negro River (Figure 3a). As expected, the Structure clusters in Amazonia without the Mexican samples show similar spatial interpolation pattern (Appendix Figure S5).

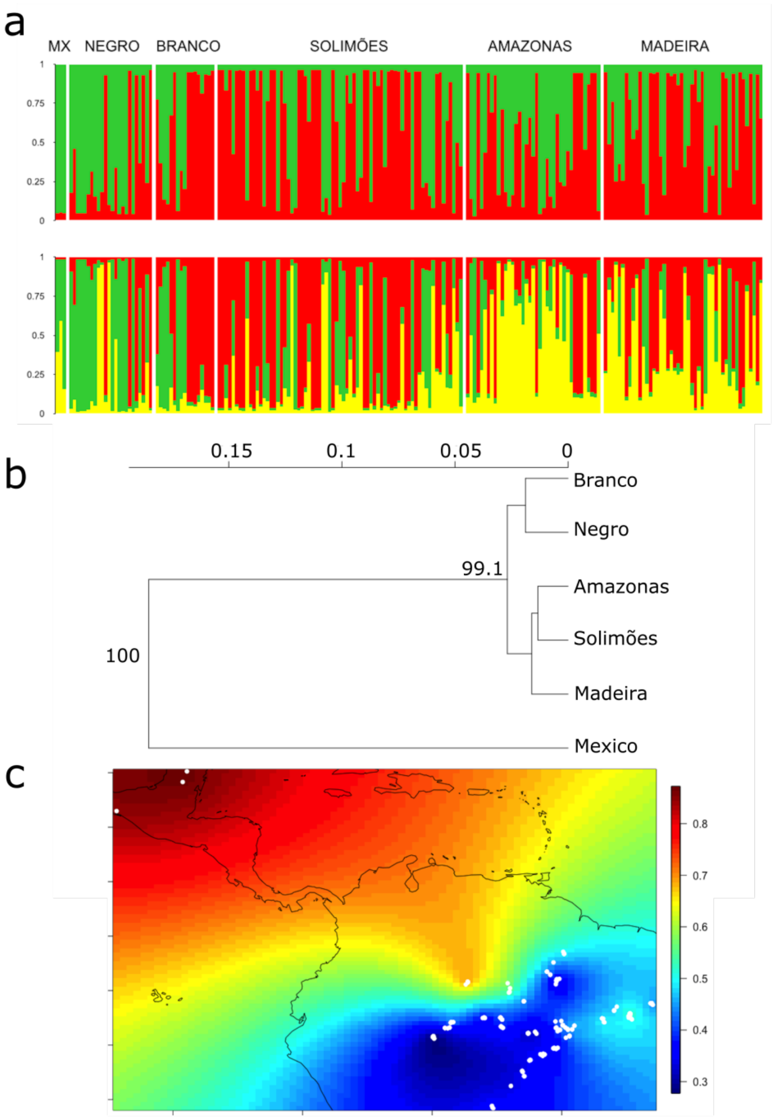


Figure 3. Nuclear genetic differentiation between cultivated *C. cujete* samples (N = 200) from Mexico and Brazilian Amazonia using 8 nSSR. Only samples with high cultivated membership (> 0.6) from Figure 2 were included. (a) Structure analysis based on correlated allele frequency model. Plots show the two likely groupings (K = 2 and K = 3). The y-axis shows the proportion of assignment to the cluster and each vertical bar represents a single plant. Samples were ordered by their geographical location along the main rivers/country: the Negro, Solimões and Amazonas Rivers are ordered west to east; the Branco River and Mexico are ordered north to south; the Madeira River is ordered south to north. (b) Neighbor-joining tree of the geographic relationships based on Nei's genetic distance with 1000 bootstraps supports indicated on the nodes. (c) Spatial interpolation of the Structure clusters (Q) at K = 2 indicated above (Figure 3a). The colored bar on the right indicates the probability of assignment to the green cluster (Figure 3a) between samples (white dots). Although the admixture between Mexico and Amazonia (Figure 3a), genetic similarity is higher between Mexican samples and northwestern Amazonia. Within Amazonia, cultivated *C. cujete* is genetically homogeneous, except by the differentiation in the Northwest and in the Eastern, which agrees with K=3 (Figure 3a).

Geographic patterns of chloroplast diversity

The haplotype network showed three distinct groups: *C. amazonica*, wild Mesoamerican *C. cujete*, and cultivated *C. cujete* from Brazil and Mexico (Figure 4a).

The wild Mexican *C. cujete* lineage is more distant from cultivated *C. cujete* (55 substitutions + 12 substitutions) than is wild *C. amazonica* (39 + 12 substitutions). In the cultivated *C. cujete* group, five common haplotypes were identified, among which four are very close to each other (1 and 2 substitutions) at the core of the cultivated haplogroup (H1, H2, H3, H4). Haplotype H5 is differentiated by at least four substitutions from the core of the network. Divergent cultivated *C. cujete* samples from the Amazon basin were arranged in the extreme branches of the *C. cujete* group in the haplotype network (Figure 4a); the highest number of substitutions (36 and 75) was comparable to the differentiation between the wild and cultivated groups.

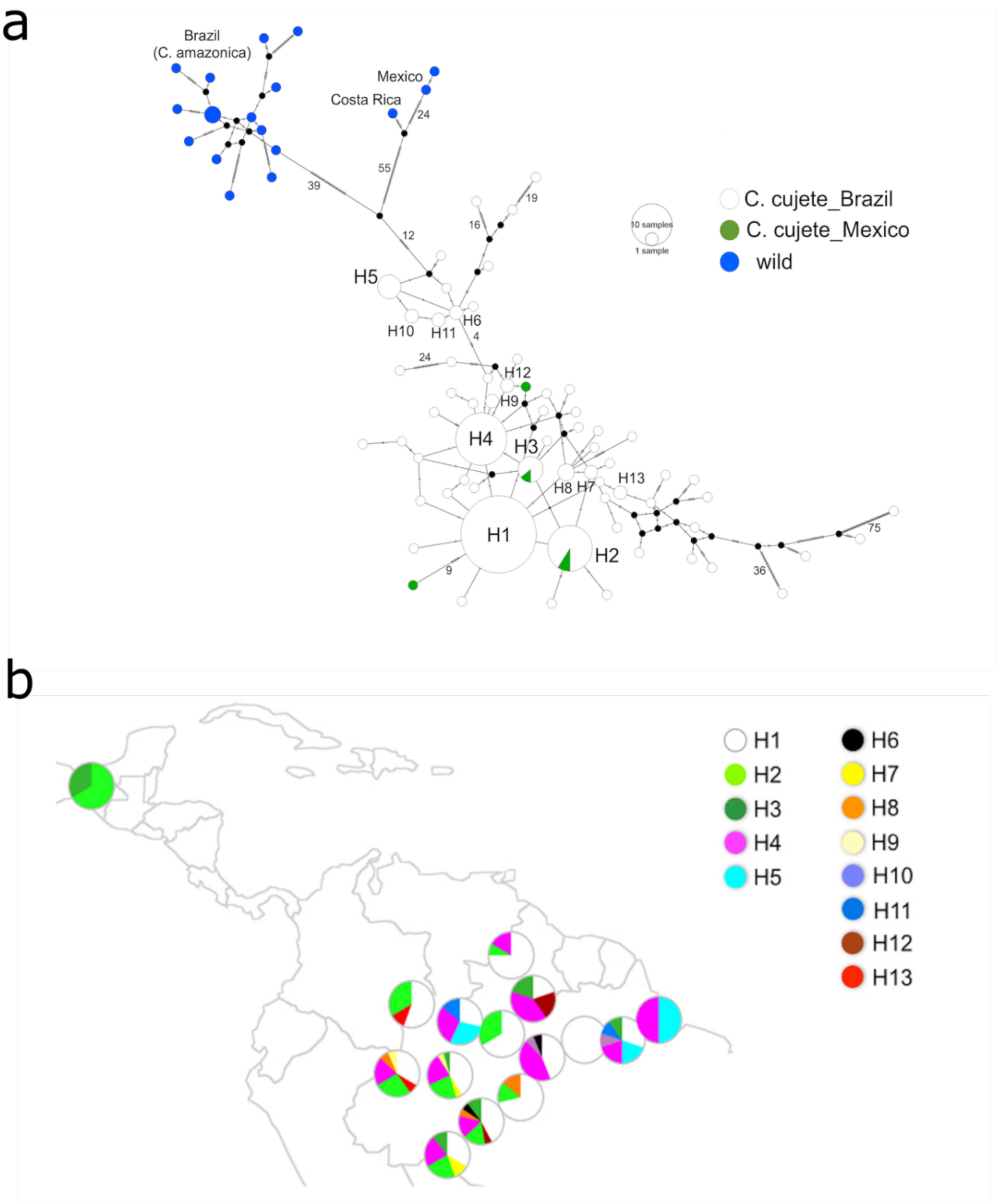


Figure 4. Chloroplast diversity of 215 cultivated and wild treegourds (*Crescentia cujete*; *C. amazonica*) from Brazilian Amazonia and Mesoamerica based on 334 SNPs. (a) Haplotype median-joining network of wild samples from Mexico and Costa Rica (*C. cujete*) and Brazil (*C. amazonica*), and cultivated C. cujete from Brazil and Mexico. The most abundant haplotypes are H1-H5 and the rare haplotypes, which are shared between at least two samples, are H6-H13. Number of substitutions between haplotypes is indicated on the branches. Black dots represent hypothetical intermediate haplotypes (b) Geographic distribution of 13 haplotypes of cultivated *C. cujete* depicted in the network (H1-H13). The area of the pie-slice is proportional to relative frequency of each haplotype in the section (upper, middle, lower) of Amazonian rivers and in Mexico.

The most common haplotype in the Amazon basin (H1) was widely dispersed, but not found in Mexico. Mexico and Brazil shared haplotypes H2 and H3, which, although different by only one substitution, showed slightly different distributions in the Amazon Basin (Figure 4b). Haplotype H2, the most common in Mexico, is restricted to the western half of Brazilian Amazonia, with higher frequency in the Northwest. Haplotype H3 is unevenly distributed in the Amazon Basin, but absent in the Northwest. Haplotype H4 is widely distributed, whereas haplotype H5, the most divergent haplotype (Figure 4a), is less abundant and found at low frequencies along the middle Negro River, but is well represented in Eastern Amazonia. The most divergent rare haplotypes (H6, H10, H11) agree with the geographical distribution of the haplotype H5. The other rare haplotypes (H7, H8, H9) were sparsely distributed along the Solimões and Madeira rivers, except the haplotype H12 shared between Madeira and Branco River and the haplotype H13, restricted to the upper sections of Negro and Solimões Rivers (Figure 4b). None of the Amazonian rivers were significantly divergent from Mexico (Table 1), certainly because of the small sample size from Mexico. Within the Amazon Basin, the Amazonas River is the most differentiated from all other rivers (Table 1).

Table 1. Paired F_{ST} distance matrix between cultivated *C. cujete* chloroplast sequences (N = 181) based on 93 SNPs from Mexico and five Amazonian rivers. The F_{ST} values are below the diagonal; in italics above the diagonal the significance evaluated using 1000 bootstraps at $p \leq 0.05$. Significant F_{ST} are indicated with bold script. Samples with singletons (see text) were not included.

	Mexico	Negro	Branco	Solimões	Amazonas	Madeira
Mexico		*0.09*	*0.06*	*0.32*	*0.18*	*0.29*
Negro	0.11		*0.14*	*0.41*	*0.05*	*0.13*
Branco	0.14	0.03		*0.87*	*0.00*	*0.80*
Solimões	-0.02	0.00	-0.01		*0.00*	*0.27*
Amazonas	0.07	**0.05**	**0.11**	**0.08**		*0.00*
Madeira	0.03	0.02	-0.01	0.00	**0.14**	

Genetic diversity in cultivated C. cujete

Based on 8 nSSR of cultivated samples, there were 31 alleles in Mexico and 55 in the Amazon Basin (Table 2), although the sample sizes of the two regions are very different. The number of private alleles among cultivated samples showed that seven alleles were only found in cultivated Mexican samples and 31 alleles in cultivated Amazonian samples (Table 2), among which six are also found in wild Mesoamerican samples. Among Amazonian samples, the Amazonas River concentrated private alleles (5) not found in local wild *C. amazonica*. The Negro, Solimões and Madeira rivers had fewer private alleles, while none was found in the Branco River (Table 2). Mexico presented the highest expected heterozygosity (H_s). In the Amazon Basin, heterozygosity was highest along the Negro River, followed by the Solimões, Amazonas, Madeira rivers, and was lowest along the Branco River (Table 2). Mexico presented significant inbreeding, while in the Amazon Basin inbreeding was significant along the Branco and Madeira rivers (Table 2).

Table 2. Genetic diversity of cultivated *Crescentia cujete* in Mexico and along major rivers of the Brazilian Amazonia, based on 8 nuclear SSR, 93 chloroplast SNPs and eight fruit shapes. N = number of samples, A_t = total number of alleles, A_r = rarefied allele counts, A_p = number of private alleles, H_o = observed heterozigosity, H_s= expected gene diversity, mean F_{IS} (* significant at $p < 0.05$ at least at 50 % of loci), S = number of polymorphic sites, h = number of haplotypes, π = nucleotide diversity, H'shape = Shannon index of fruit shape diversity estimated for each region and D = fruit diameter (average ± sd).

	Nuclear diversity							Chloroplast diversity **				Fruit morphology			
Regions	N	A_t	A_r	A_p	H_o	H_s	F_{IS}	N	S	h	π $(x10^{-2})$	N	H'_{shape}	N	D (cm)
Mexico	7	31	3.84	7	0.43	0.63	0.29*	4	8	3	4.66	117	0.49	71	15.4±3.1
Amazon Basin	215	55	6.75	31	0.27	0.38	0.28	177	93	68	5.31	286	0.60	175	18±6
Negro	24	28	3.43	2	0.37	0.47	0.21	20	14	10	3.34	30	0.65	13	13±7.3
Branco	17	17	2.05	0	0.21	0.24	0.12*	19	12	9	2.40	14	0.45	NA	NA
Solimões	79	41	5.07	2	0.30	0.37	0.18	65	64	28	5.63	118	0.56	72	17.8±5.8
Amazonas	48	41	5.02	5	0.23	0.39	0.40	27	46	20	8.77	87	0.52	60	17.5±5.3
Madeira	47	32	3.89	1	0.25	0.31	0.20*	46	49	25	4.17	37	0.47	30	21±5.8
Overall	222	62	7.6	-	0.28	0.40	0.29	181	93	69	5.31	403	0.61	246	17.2±5.4

(**) samples with singletons were not included (N = 15).

Among the 334 SNPs found in chloroplast sequences, 206 were found in cultivated *C. cujete*. Mexico and the Amazon Basin showed similar nucleotide diversity (π), 3.78×10^{-2} and 3.83×10^{-2}, respectively, although sample sizes are very different and the Amazon Basin harbors highly divergent samples (Figure 4a). Among cultivated *C. cujete*, 15 samples produced 119 unique SNP alleles, of which 66 % were from only two samples collected along the Amazonas River, which thus produced an extremely high nucleotide diversity estimate for this river ($\pi = 9.31 \times 10^{-2}$). When these 15 singleton samples were discarded, there were 93 SNPs and nucleotide diversity in Mexico was still similar to the Amazon Basin (Table 2). The highest nucleotide diversity was still along the Amazonas River, with decreasing values along the Solimões, Madeira, Negro and Branco rivers (Table 2).

Morphological diversity of cultivated C. cujete

We identified a total of eight fruit shapes in the Amazon Basin and five in Mexico (Figure 5a). Fruit shapes shared among these regions were spherical, flattened, oblong, elongated and cuneate, with higher frequencies of spherical, flattened and oblong shapes in both regions. Three types (globular, rounded-drop and oblong-drop) were only recorded in the Amazon Basin. The kidney-shaped fruit found in Colombia was not found in Mexico or Brazilian Amazonia. The absence of drop-shaped fruits in Mexico, which are types clearly distinguished from the others, indicate higher morphological diversity along Amazonian rivers than in Mexico. The Solimões River harbors all the eight fruit shapes described (Figure 5b). The spherical shape, the most frequent in Mexico, is relatively rare in the Amazon Basin, with a higher frequency along the Amazonas River (Figure 5b). The fruit types absent in Mexico were rare in the Amazon Basin as well, except the rounded-drop shape. This fruit type showed relatively high frequency along the Negro River, more than the more common flattened and oblong shapes (Figure 5b). The fruit shape diversity index was higher along the Negro River, with decreasing values along the Solimões and Amazonas rivers, followed by Mexico, and lowest along the Madeira and Branco rivers (Table 2). The fruit shape diversity index was not correlated with any of the genetic estimators ($p > 0.05$). The fruit diameters showed the lowest average along the Negro River and in Mexico, and the highest along the Madeira River (Table 2). Mexico and the Negro River also showed the extremes of size variation, with Mexico least variable and the Negro most variable (Table 2).

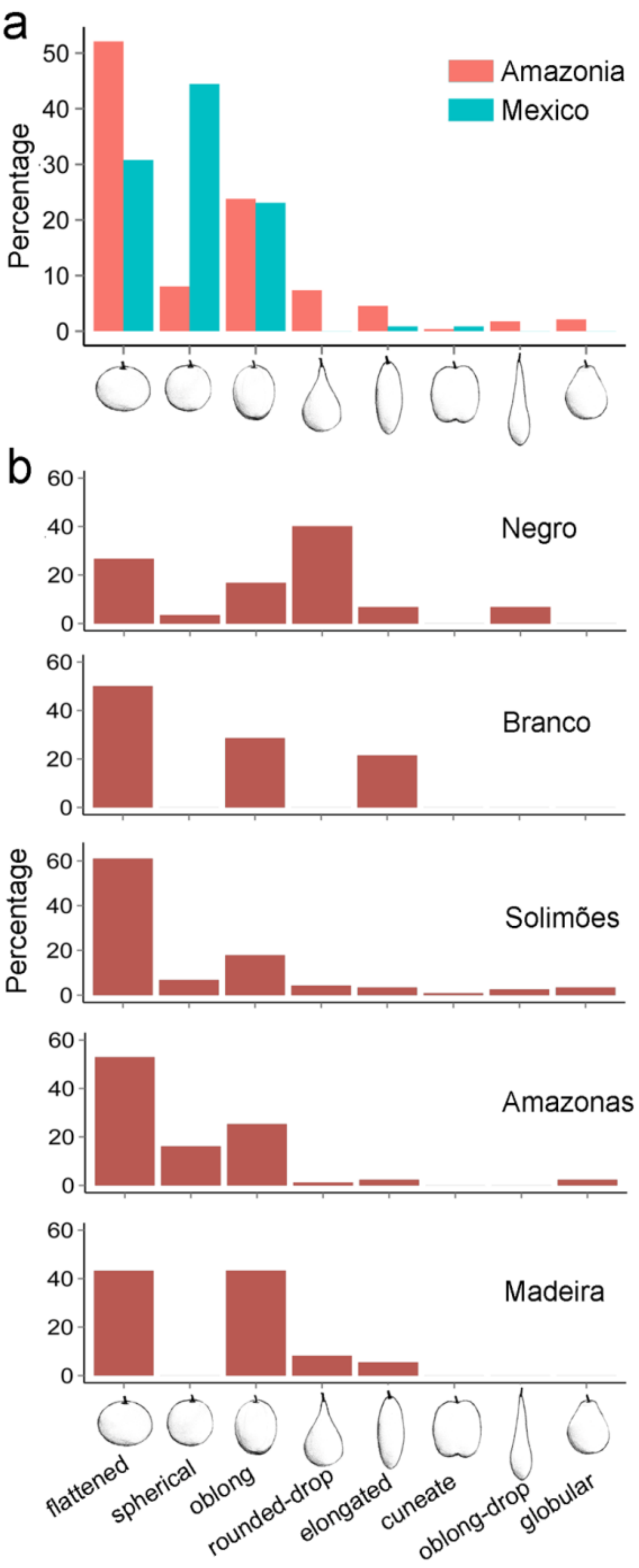


Figure 5. Morphological diversity of cultivated *Crescentia cujete* in (a) the two domestication centers studied (Mexico N= 117 and Amazonia N=286) and (b) along Amazonian rivers. Fruit shapes correspond to those described by Arango-Ulloa et al (2009), except the spherical shape.

Discussion

Cultivated *C. cujete* are quite similar from Mexico to Brazil, suggesting a common genetic origin. But these cultivated types are strongly differentiated from wild types, both from Mexico and Amazonia, suggesting these wild populations are not the direct ancestors of cultivated *C. cujete*. The geographical origin of the domestication of this species is still uncertain. However, the high diversity of cultivated *C. cujete* from Mexico, compared to Amazonia, suggests that its origin may be in Central America. Diversity analyses allowed discussion of the different routes of introduction into Amazonia and subsequent dispersal. More than one route may have been used: a northwestern introduction into the Negro and Solimões Rivers; and an eastern introduction from the coastal Guianas into the Amazonas River. Finally, fruit shape diversity suggests distinct selection pressures across the crop's distribution.

Relationships among Mesoamerican and Amazonian treegourd populations

The wild samples from Mexico (taxonomically identified as *C. cujete*) and the Amazon Basin (identified as *C. amazonica*) were strongly differentiated from the cultivated samples, given their F_{ST} values based on nuclear SSR and number of substitutions in the chloroplast genome. The high number of substitutions in the chloroplast sequences between these wild taxa suggests ancient divergence. The differentiation between wild and cultivated in Mexico (Aguirre-Dugua et al., 2012; Aguirre-Dugua et al. submitted) and between wild and cultivated in Amazonia was already noted (Moreira et al., 2017). These results suggest that neither of these wild relatives are the direct ancestor of cultivated *C. cujete,* although Mexican wild samples present clear morphological identification as *C. cujete* based on Gentry (1980) description.

The Costa Rican sample showed an intermediate admixed nuclear pattern, but high chloroplast differentiation from the cultivated samples (Figure 2, Figure 4a). Consequently, it could be a wild individual pollinated by cultivated *C. cujete*. However, because ancestry could also reflect divergence, increased sampling in Central America is of interest. Although our results rule out the possibility that cultivated *C. cujete* was derived from the wild samples from the Yucatan Peninsula, we cannot rule out an origin somewhere between Central America and northern South America, where other potentially wild *C. cujete* populations occur in savannahs (Figure 1). Nevertheless, our results provide evidence that introduction of domesticated *C. cujete* in Mexico and Amazonia originated from the same source, given the Mexican relationship with Amazonian samples (Figure 3a, yellow and green clusters) and occurrence of wild Mesoamerican alleles in cultivated Amazonian *C. cujete* samples.

Hypotheses of treegourd introduction into Amazonia

The patterns of treegourd genetic diversity across the Amazon Basin allow two, not mutually exclusive, hypotheses of introduction: a Northwestern route and an Eastern route. A Northwestern route into the upper Negro River is supported by the relatively high levels of heterozygosity and fruit shape diversity (Table 2), higher proportions of Mexican ancestry (Figure 3a, green cluster) and higher frequency of the most common haplotype in Mexico (Figure 4b, haplotype H2). This route into Negro River is possible from the Orinoco River, given the fluvial connections via de Cassiquiare canal. This route was part of an extensive social trading network (Hornborg, 2005), based at least in part on the Arawak network (Eriksen and Danielsen, 2014). This route has also been suggested for various crop dispersals (Schultes, 1984), such as cocona (*Solanum sessiliflorum*), whose populations were domesticated in the upper Orinoco River (Volpato et al., 2004) and which was widely cultivated in Northwestern Amazonia (Schultes, 1957). Similarly, people from the upper Negro River reported intentional collection of treegourd propagules from the Cassiquiare, where treegourd is considered a spontaneous tree in the floodplains, while along the Negro River cultivation demands more effort (P.A.M., personal observation).

A possible Western route into the upper Solimões River is partially supported by heterozygosity and fruit diversity (Table 2); the presence of all fruit shapes described enhances the possibility (Figure 5b). Moderately high nucleotide diversity with the highest number of haplotypes are the strongest evidence (Table 2), especially because hybridization with wild populations was not reported (Moreira et al., 2017), suggesting that this is *C. cujete* diversity. This route might reflect introduction from the Pacific coast and crossing of the Andes mountains via the Napo and Putumayo rivers (Schultes, 1984), as might be the case of cacao (*Theobroma cacao*) (Thomas et al., 2012) and peach palm (*Bactris gasipaes*) (Rodrigues et al., 2005) demonstrated by molecular evidence. However, it is also possible that this is a continuation of the Negro River route across interfluvial areas, as suggested by the distribution of abundant haplotype H2 and the rare haplotype H13 (Figure 4b).

The Eastern route into the Amazonas River is supported by high heterozygosity and fruit diversity (Table 2), with high Mexican ancestry not found in Western Amazonia (Figure 3a, yellow cluster). The highest levels of nucleotide diversity (Table 2) and the particular distribution of haplotypes not found in Western Amazonia (Figure 4b, haplotype H5), which include one of the Mexican haplotypes (Figure 4b, H3), agree with the nuclear pattern. This route is linked to the coastal Guianas, an ancient area of exchange of Amazonian crops with Mesoamerica (Schultes, 1984). Molecular data of early maize (*Zea mays*) introduction into South America support dispersal from Mesoamerica through the Caribbean, spreading along the lowlands of the northeastern coast of South America to finally reach Amazon Basin through river systems (Freitas et al., 2003; Bedoya et al., 2017), although the oldest archaeological remains of maize are western (Bush et al., 2016). This route also agrees with pineapple dispersal from the Guianas, where it was domesticated and introduced into Mexico (Coppens d'Eeckenbrugge & Duval, 2009).

The extremely high chloroplast nucleotide diversity along the Amazonas River, almost twice that along the Solimões River (Table 2), is an unexpected result. Such high diversity was also observed with nuclear markers, given the relatively higher number of exclusive cultivated alleles along the Amazonas River (Table 2), which might not be related to local hybridization, since they were not found in *C. amazonica* (Moreira et al., 2017). While nuclear information is limited by the small number of loci analyzed, the chloroplast pattern is robust and they are in agreement. Therefore, we do not rule out that diversity along the Amazonas River might have been promoted by inter-specific hybridization between Mesoamerica and northern South America, where most diversity of *Crescentia* species is found (Gentry, 1980) and hybrid samples might have been introduced into Amazonia. Another process that is complementary and also deserves future investigations is the role of seed cultivation to deal with high flooding described along the Amazonas River (Moreira et al., 2017), since seeds might show diversity not found among cuttings as usually practiced (Aguirre-Dugua et al., 2012; Arango-Ulloa et al., 2009; Moreira et al., 2017). This hypothesis follows that of manioc (*Manihot esculenta*), where cuttings are usually practiced, but seed propagation is important to maintain diversity (Peroni & Martins, 2000; Elias et al., 2001; Duputié et al., 2009; McKey et al., 2010).

Hypothesis of fruit dispersal and diversification

Domesticated varieties often present greater fruit shape diversity than their wild relatives, as observed in bottle gourd (*Lagenaria siceraria*), whose fruits have similar technological uses (Morimoto et al., 2005; Heiser, 1993). Across its distribution, the pattern of treegourd fruit shape diversity (Figure 5) suggests different cultural preferences affecting diversification. The highest shape diversity was found along the Negro and Solimões rivers (Figure 5b, Table 2). Similar high diversity was also observed in the Orinoco and Caribbean regions of Colombia (Arango-Ulloa et al., 2009), suggesting northwestern South America is an area of treegourd diversification. This pattern of diversity agrees with Amazonian ethnographies that underscore the cultural value of morphotype diversity cultivated for its own sake, such as in manioc (Rival & McKey, 2008) and pequi (*Caryocar brasiliense*) (Smith & Fausto, 2016). Nevertheless, the greater local frequency of the spherical type in Mexico and rounded-drop shape along the Negro River (Figure 5) suggests distinct selection pressures, as also described for popcorn in Peru (Grobman et al. 2012) and the differential selection of bitter and sweet manioc between Amazonia and the Atlantic Forest in Brazil (Emperaire & Peroni, 2007). Modern Maya people in Mexico and Guatemala have a long history of strong selection of spherical fruits of *C. cujete* for bowls (*jícaras*) to use with traditional beverages in rituals and also daily life situations (Ventura 1996; Aguirre-Dugua et al. 2012, 2013). The spherical and drop-shaped fruits of *C. cujete* have different symbolic importance and are recognized with distinct names by Tukano Oriental speakers (Pieter van der Veld, pers. communication), a linguistic family found in Northwestern Amazonia. The spherical fruit is called *wahatowê*, and is used as bowls to prepare ipadu powder (*Erythroxylum coca* var. *ipadu*) in rituals. In contrast, the rounded-drop, called *ñahsãwaha*, is common in daily life as a spoon and cup for collective food consumption (*xibé*, a meal of water and manioc flour, and *açaí*, the juice from *Euterpe precatoria*). Local people along the upper Negro River reported that the spherical type was also used as an ashtray by healers (*pajé*) in blessing rituals with tobacco smoke. Ethnographies also reported different treegourd fruits for each type of use, such as *cuia-de-tapioca* and *cuia-de-ipadu* (Ribeiro, 1995), although shape differences were not mentioned. In Northwestern South America, these bowls are cultural markers for the traditional use of coca introduced from the Andean foothills (Plowman, 1984). Interestingly, the spherical fruit shape selected in Mexico was the same as the one used in special rituals in Negro River Basin.

This suggests that the wide dispersal of plants between South America and Mesoamerica in pre-Columbian times was motivated not essentially by food consumption, as would be expected for agrarian societies, but mainly for recreative and religious purposes (Neves, 2016). Indeed, archaeological remains of *Crescentia cujete* in Central America and the Antilles were found in ritualistic contexts, such as offerings in funerary rituals (Beaubien, 1993; Conrad, 2001). This hypothesis of recreative and religious exchanges is also supported by the ancient dispersal of maize (*Zea* spp.) for beer preparation and tobacco (*Nicotiana* spp.) for magic and therapeutic uses, both widely exchanged between these continents (Heiser, 1965; Smalley et al., 2003), possibly as sacred gifts (Norton, 2008).

The relatively high morphological diversity found along the Solimões and Amazonas rivers, where most of rare fruit shapes were found (Figure 5b), suggests different demands for fruit shapes since pre-historic times, as expected among plants with technological uses (Blench, 2012). The upper Solimões River and middle Amazonas River were ancient treegourd handicraft centers that were regarded by both Europeans and Native Amazonians as one of the best expressions of their arts and an important article of trade (Métraux, 1948; Rodrigues-Ferreira, 1933). During the colonial period, villages along the Amazonas River produced 5000-6000 bowls a year that were exchanged for food (Rodrigues-Ferreira, 1933). This handicraft tradition extends until today, especially for the production of *tacacá* bowls (a kind of soup), which are made with the rounded (spherical and flattened) fruits (Moreira et al., 2017).

Although there is similarly high biological and cultural complexity in Mesoamerica and Amazonia (Casas et al., 2017; Blench, 2012; Clement et al., 2015), these two plant domestication centers contrast in terms of the morphological diversity of cultivated *C. cujete* fruits. Curiously, although Mexico pre-history is especially rich in complex societies, such as the Maya (Willey, 1956), morphological fruit diversity is lower and particular fruit shapes are absent, which also reinforces different cultural selection pressures between these regions. It follows that, although the introduction of the cultivated germplasm into both Mexico and Amazonia should lead to a bottleneck (i.e., through founder effect), it might be less severe in Amazonia due to a more diverse array of usages. Moreover, although the spread of a phenotype during dispersal might also be influenced by wild introgression/hybridization (Meyer & Purugganan, 2013), this effect was remarkable only on treegourd fruit size and not on shape diversity in Amazonia (Moreira et al., 2017). Within Mexico, elongated and smaller shapes spontaneously grown in homegardens, resulted possibly from gene flow with wild populations, are not appreciated in Yucatan Peninsula (Aguirre-Dugua et al., 2012), but are selected in the Pacific Coast as spoons (X.A.D, personal observation), although at low frequencies (Figure 5a). Therefore, cultural selection influences the bottleneck during introduction and afterwards the management of hybridization with local wild congeners. Whereas distribution of shape diversity reflects different culture preferences, size is more influenced by local wild introgression effects.

Conclusions

We demonstrated with molecular evidence that *Crescentia cujete* introduced into the Amazon Basin and Mexico share a common ancestry with a currently unknown origin. The dispersal followed previously proposed routes of human and plant migrations into Amazonia. The patterns of genetic diversity across Amazonia allow two, not mutually exclusive, hypotheses of the routes of introduction: a Northwestern introduction into the Negro and Solimões rivers, and an Eastern introduction from the coastal Guianas into the Amazonas River. The fruit shape diversity reveals different ancient utilitarian demands for the fruits. Mesoamerica and Amazonia have contrasting fruit morphological diversity, which suggests different cultural preferences along treegourd's dispersal routes. More comparative studies of its different uses, with a broader genetic and phenotypic distribution, would be useful to better understand the dispersal and diversification of *C.cujete* in the Americas.

Supplementary Materials

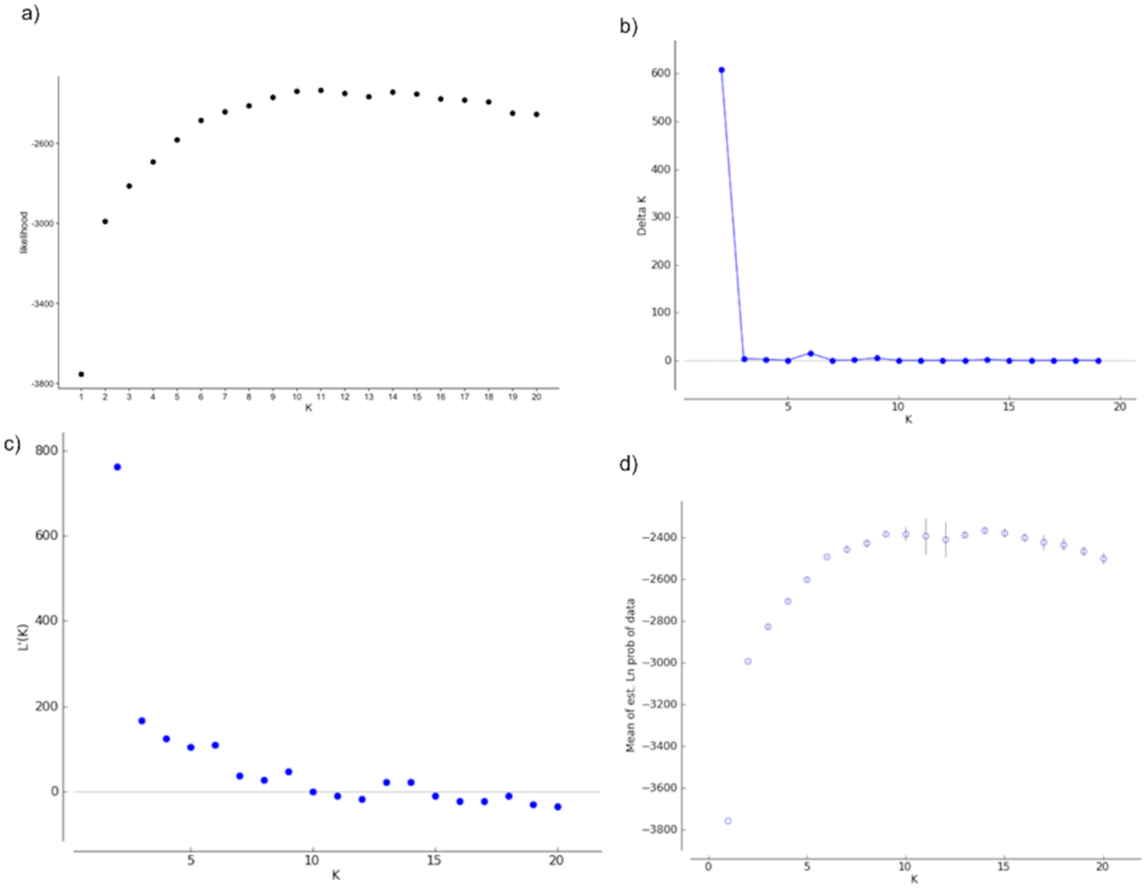


Figure S1. Log likelihoods estimated by Structure (a) and evaluation of the ad hoc ΔK of Evanno et al. (2005) (b, c, d). These estimators allow evaluation of the most likely number of population clusters of wild and cultivated treegourds in Mesoamerica and Brazilian Amazonia based on 8 nSSR as shown in Figure 2. Variation stabilized at K = 2 (b). Larger Ks showed an increase of the rate of change of the likelihood (c), but had higher variance (d) between runs, which suggests that the most likely K had already been attained.

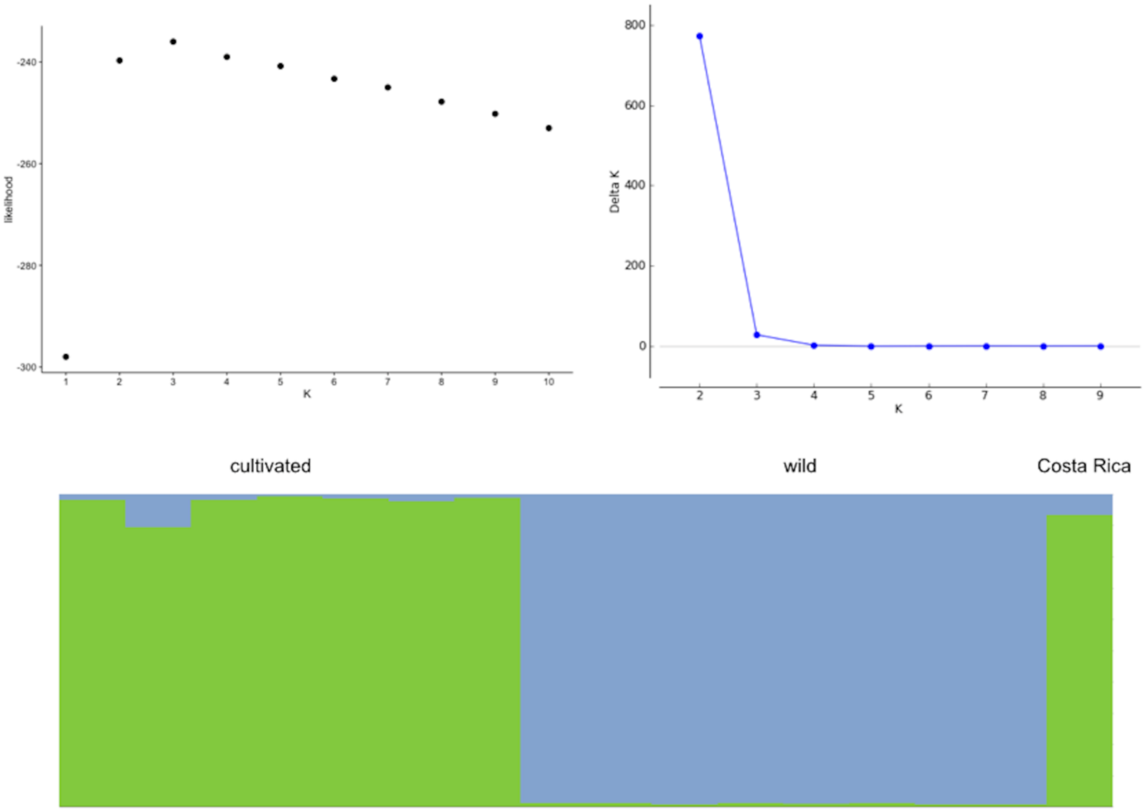
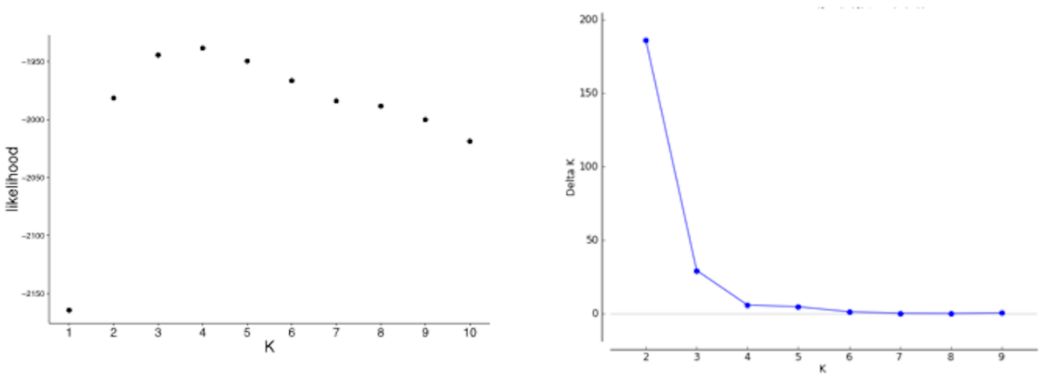


Figure S2. Structure assignments of cultivated and wild *C. cujete* samples (N = 16) from Mesoamerica based on the independent allele frequency model using 8 nSSR. Log likelihoods estimated by Structure and evaluation of the ad hoc ΔK of Evanno et al. (2005).

a) independent frequency model



b) correlated frequency model

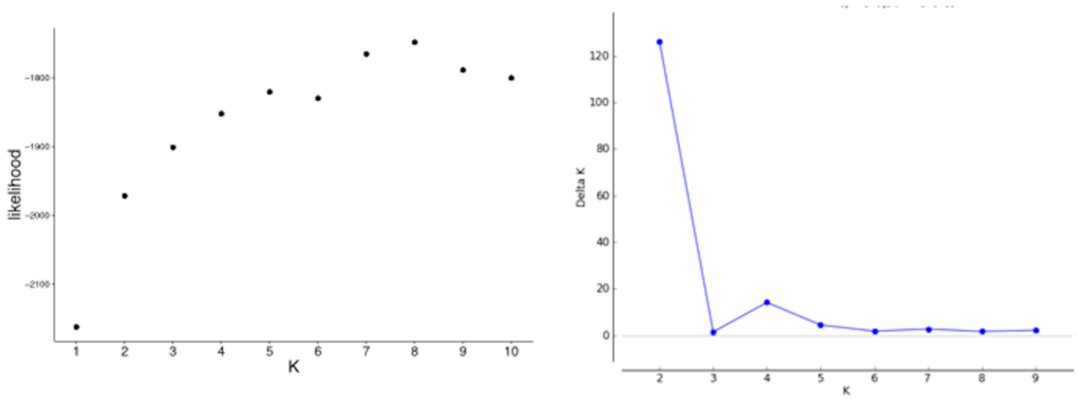


Figure S3. Log likelihoods estimated by Structure and evaluation of the ad hoc ΔK of Evanno et al. (2005) performed with different allele frequency models. These are estimators that allow evaluation of the most likely number of population clusters of cultivated *C. cujete* in Mesoamerica and Brazilian Amazonia based on 8 nuclear SSR as shown in Figure 3.

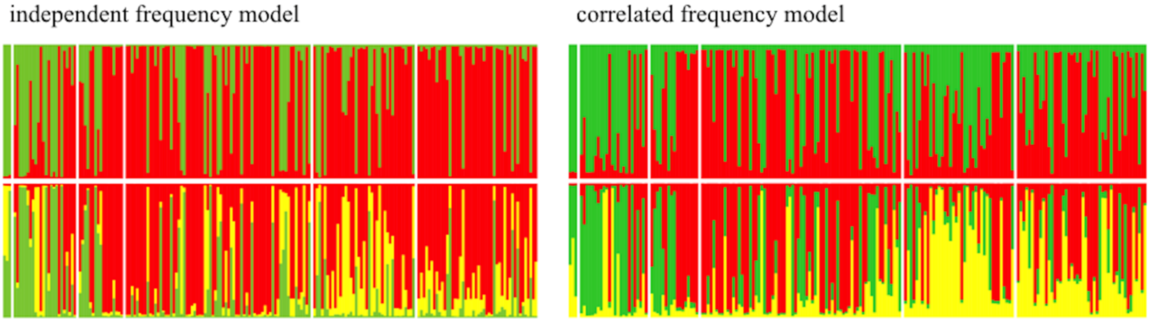


Figure S4. Structure plots based on independent and correlated allele frequency models of cultivated *C. cujete* samples (N = 200) from Mexico (extreme left) and Brazilian Amazonia (from left to right: Negro, Branco, Solimões, Amazonas, Madeira rivers) using 8 nSSR. Better definition at K = 3 was indicated for the correlated model (Figure S3).

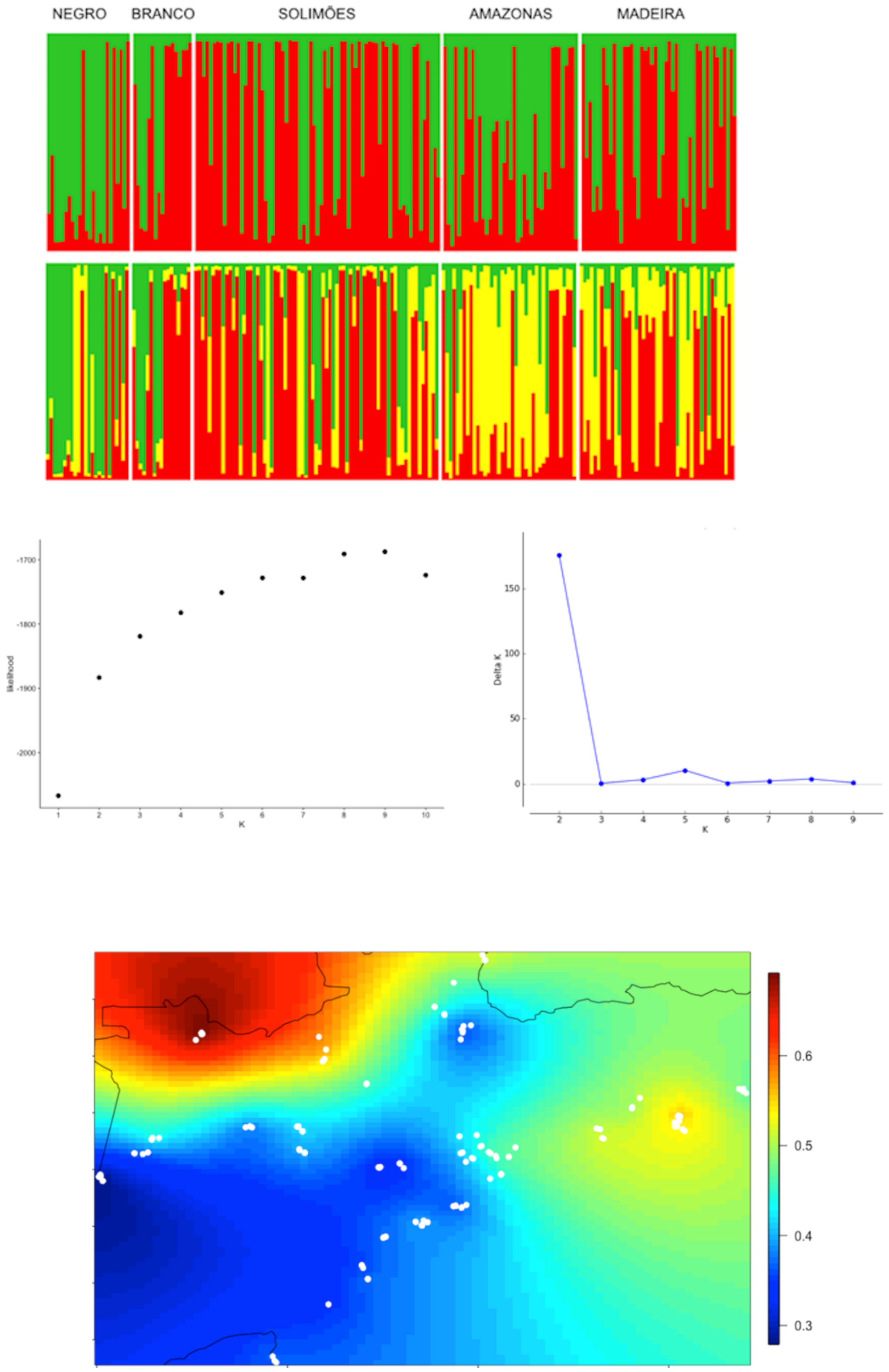


Figure S5. Structure assignments of cultivated *C. cujete* samples (N = 197) from Brazilian Amazonia only based on the correlated allele frequency model using 8 nSSR. Log likelihoods estimated by Structure and evaluation of the ad hoc ΔK of Evanno et al. (2005). As expected, Structure results were similar to Figure S4, where the three cultivated Mexican samples were included. The spatial interpolation of the green Structure cluster also showed the same pattern as Figure 3c.

Table S1. Passport data of all *Crescentia cujete* and *C. amazonica* collections made in Brazilian Amazonia and Mesoamerica, with additional information about genetic analysis and morphology. Column A = Collection code; B = Sequencing code; C = Species field identification; D = Latitude; E = Longitude; F = Country; G = Municipality; H = Village; I = river/region; J = if analyzed for nSSR; K = if analyzed for cpSNP; L = % of missing data in sequence; M = if singleton detected; N = Management (if cultivated or wild); O = Fruit shape; P = Fruit diameter (cm) and Q = source of data.

(attached)

Capítulo 5

Síntese

O conhecimento sobre a dinâmica do uso e manejo de uma espécie, a longo prazo em uma ampla escala geográfica, contribui para ampliar o conhecimento sobre 1) como a história de ocupação humana na Amazônia foi influenciada pela domesticação de plantas, 2) como as plantas respondem à manipulação humana, e 3) como povos pré-colombianos influenciaram a distribuição e diversificação de plantas usadas atualmente. Em relação à questão 1, a história evolutiva de uma espécie representa uma pequena parte do conjunto de domesticações de paisagens e plantas típicos da construção de nichos que influenciam a história humana de ocupação (Piperno et al., 2017; Zeder et al., 2016). Estudos de caso usando plantas de uso tecnológico, como as cuias, são modelos interessantes que contrastam com plantas alimentícias geralmente estudadas (Clement, 1989; 1999; Clement et al., 2010; 2016; Piperno, 2011; Levis et al., 2017). Plantas de uso tecnológico refletem padrões de difusão de tecnologia e demandas materiais, que podem ser chave para aumentar a eficiência humana na obtenção e uso de alimentos e na construção de nichos nos ambientes. Espera-se, portanto, que plantas de usos utilitários sejam bons modelos para descrever organização social, modos de subsistência e preferências culturais, aspectos ainda pouco conhecidos na história da Amazônia com base nas fases arqueológicas da produção de cerâmica (Schann, 2007). Em relação à questão 2, genética e etnobotânica ajudam a identificar as modificações morfológicas e genéticas que caracterizam o processo de domesticação de plantas. Em relação à questão 3, a localização de centros de diversidade refletem preferências culturais por suas variedades e as redes sociais de propagação da planta.

No segundo capítulo apresentei as ferramentas genéticas que usei neste estudo. O sequenciamento completo do cloroplasto, o primeiro para o gênero *Crescentia* e um dos poucos disponíveis para Amazônia, mostrou ser eficiente para discriminar espécies do gênero e mapear a dispersão dos haplótipos com base em uma amostragem molecular robusta. O desenvolvimento de microssatélites nucleares específicos para *C. cujete* foi fundamental para identificar processos de hibridização, as relações de coancestralidade e centros de diversidade; e ainda disponibiliza centenas de microssatélites potencialmente informativos para futuros estudos com espécies de *Crescentia*.

No terceiro capítulo demonstrei que a expansão geográfica de *Crescentia cujete* promovida por humanos favoreceu processos de hibridização com uma espécie silvestre do gênero, *C. amazonica*, influenciando a diversificação fenotípica das plantas. O uso tradicional de tipos híbridos identificados como variedades locais (*maracá, cuiupi, paranã*) sugere que o manejo da hibridização seja uma prática antiga na Amazônia. O manejo da hibridização de cuias demonstra que a manipulação tradicional de plantas na Amazônia também se vale de espécies silvestres relacionadas que proporcionam diversidade morfológica e genética às plantas cultivadas. Devido ao fluxo gênico entre *C. amazonica* e *C. cujete,* a distinção precisa entre populações silvestres de *C. amazonica* e populações espontâneas e cultivadas de *C. amazonica* e *C. cujete* só foi possível com o uso de marcadores moleculares associados à percepção local da diversidade de cuias.

No quarto capítulo demonstrei que *Crescentia cujete* foi introduzida na Bacia Amazônica e no México, embora a origem geográfica de sua domesticação permaneça desconhecida. O padrão de diversificação genética entre Leste e Oeste na Bacia Amazônica permitiu inferir duas rotas de introdução na Amazônia, as quais concordam com rotas anteriormente propostas em estudos etnobotânicos entre grupos humanos e suas plantas. Embora Mesoamérica e Amazônia sejam similares em termos de alta riqueza e complexidade biológica e cultural (Casas et al., 2017; Blench, 2012; Clement et al., 2015), são contrastantes em termos da diversidade morfológica de frutos cultivados de *C. cujete*. A diversidade e distribuição do formato dos frutos revelam que, apesar do amplo uso utilitário dos frutos, existiram diferentes preferências culturais ao longo das rotas de dispersão da planta. A distribuição e diversidade de cuieiras cultivadas atualmente são legados da manipulação da planta em períodos pré-colombianos da ocupação da Amazônia, como indicado pela persistência de centros de diversidade morfológica e genética. Mesmo após o colapso demográfico das populações dos povos nativos e suas plantas, causado pela conquista europeia, restaram formas e tamanhos de frutos que atestam este legado do passado que é usado hoje.

Ésurpreendente constatar que *C. cujete* tenha sido introduzida e amplamente dispersada na Amazônia, mesmo diante de uma ampla diversidade de espécies na Amazônia potencialmente disponíveis para uso como recipientes (Albert & Milliken, 2009), tais como *Lagenaria siceraria* (oriunda da África), frutos (pixídios) de Lecythidaceae, espatas de palmeiras (*Socratea* sp., *Attalea* sp.), ou bambus (*Guadua* spp.), e ainda o uso amplamente difundido da cerâmica (Barreto et al., 2016). Os múltiplos usos das cuias, os quais abarcam dimensões utilitárias, mas também simbólicas e medicinais, certamente influenciaram a ampla expansão das populações domesticadas de *C. cujete*. No entanto, a reconstrução da rotas de intercâmbio de plantas e migração humana nos Neotrópicos com base na distribuição geográfica das plantas domesticadas, como as cuias, permanece uma questão em aberto. No caso das cuias, possivelmente dados linguísticos serão informativos, uma vez que há nomes particulares em línguas nativas para tipos de cuias e seus diferentes usos, como comentado no capítulo 4. Por outro lado, dados arqueológicos podem ser limitados, uma vez que a degradação da casca dos frutos diminui a chance de encontrar vestígios e *Crescentia* não produz fitólitos diagnósticos (Jennifer Watling, com. pess.). Considerando que a diversidade genética pode ser influenciada pela hibridização entre espécies relacionadas, como descrito nos Capítulos 3 e 4, são necessárias análises filogeográficas com as diferentes espécies propostas para o gênero *Crescentia*, incluindo na América Central, especialmente Costa Rica, como mencionado no Capítulo 4, mas também no litoral norte da América do Sul e o Caribe. Modelos de coalescência com cuias contribuirão para inferir os períodos de expansão da distribuição de *C. cujete* e as rotas das redes sociais de dispersão dos propágulos nos Neotrópicos. Plantas com usos tecnológicos complementariam os modelos de distribuição geralmente analisados a partir de plantas alimentícias, como mandioca (Arroyo-Kalin, 2010; Rival & McKey et al., 2008) e milho (Vigouroux et al., 2008; Freitas et al., 2003; Bedoya et al., 2017), uma vez que produtos tecnológicos podem ter favorecido a expansão populacional e otimizado o forrageamento humano, sem que a domesticação estivesse necessariamente associada à pressão demográfica humana e ao desenvolvimento de agricultura (Blench, 2012). Filogenias datadas também permitirão avaliar a escala temporal da diversificação de *Crescentia cujete*. A diversificação recente no Holoceno concordaria com a hipótese de introdução na Amazônia proposta nesta tese. Ao contrário, se mais antiga, será um indício do papel da megafauna na dispersão dos frutos desta espécie anteriormente aos humanos.

O uso e cultivo de cuias têm sido historicamente uma alternativa econômica nas áreas ribeirinhas da Amazônia (Santos, 1982; Carvalho, 2003; Capítulo 3 e 4). Atualmente, o sistema artesanal das cuias, reconhecido como patrimônio cultural brasileiro (IPHAN, 2015), envolve não só diferentes variedades da planta (Capítulo 3), mas outras espécies ligadas à extração de tintas naturais em áreas de terra-firme (Ferreira-Rodrigues, 1933; Patiño, 1967; Ribeiro, 1995; Carvalho, 2011; Almeida et al., 2013). Este sistema ainda foi pouco estudado quanto à diversidade das plantas utilizadas, história da domesticação e arranjos sociais do manejo comunitário. Além do cultivo comercial, o uso essencialmente doméstico das cuias, registrado em 36 municípios ao longo dos principais rios da Amazônia nesta tese, em especial o cultivo das cuias grandes e redondas (66% dos municípios, Capítulo 3), demonstra seu valor cultural, apesar da introdução de produtos industrializados, como alumínio, e principalmente, plástico. Mais estudos comparativos ao longo de sua distribuição geográfica contribuirão no mapeamento das consequências da seleção humana associada aos seus múltiplos usos e que moldam a diversidade fenotípica desta planta. Por fim, esta tese destaca a histórica vocação biotecnológica dos povos nativos na Amazônia, transmitida às gerações seguintes a partir do uso e domesticação de plantas com fins utilitários, simbólicos e medicinais, como as cuias. Esta experiência do saber fazer, embora nem sempre atraente para o mercado global ou políticas de desenvolvimento, persiste até os dias atuais, desafiando nossos modos de valorizar a Amazônia a partir da arte e sua diversidade biocultural.

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