FERNANDA HURBATH PITA BRANDÃO

Sistemática, estrutura genética de populações e filogeografia de *Euphorbia* sect. *Brasilienses* V.W. Steinm. & Dorsey (Euphorbiaceae)

Tese apresentada ao Instituto de Botânica da Secretaria do Meio Ambiente, como parte dos requisitos exigidos para a obtenção do título de DOUTORA em BIODIVERSIDADE VEGETAL E MEIO AMBIENTE, na Área de Concentração de Plantas Vasculares em Análises Ambientais.

SÃO PAULO
2018

FERNANDA HURBATH PITA BRANDÃO

Sistemática, estrutura genética de populações e filogeografia de *Euphorbia* sect. *Brasilienses* V.W. Steinm. & Dorsey (Euphorbiaceae)

Tese apresentada ao Instituto de Botânica da Secretaria do Meio Ambiente, como parte dos requisitos exigidos para a obtenção do título de DOUTORA em BIODIVERSIDADE VEGETAL E MEIO AMBIENTE, na Área de Concentração de Plantas Vasculares em Análises Ambientais.

ORIENTADORA: DRA. INÊS CORDEIRO
CO-ORIENTADORA: DRA. CLARISSE PALMA DA SILVA

Ficha Catalográfica elaborada pelo **NÚCLEO DE BIBLIOTECA E MEMóRIA**

Brandão, Fernanda Hurbath Pita
B817s Sistemática, estrutura genética de populações e filogeografia de Euphorbia sect. Brasilienses V.W. Stein & Dorsey (Euphorbiaceae) / Fernanda Hurbath Pita Brandão -- São Paulo, 2018.
176p. il.

Tese (Doutorado) -- Instituto de Botânica da Secretaria de Estado do Meio Ambiente, 2018.
Bibliografia.

1. Euphorbia subg. Euphorbia. 2. Biogeografia. 3. Caatinga. I. Título.

CDU: 582.757.2

Comissão julgadora da Tese

Profa. Dra. Inês Cordeiro (Presidente)

Prof. Dr. Fabio Pinheiro

Profa. Dra. Lucia Rossi

Profa. Dra. Maria Beatriz Rossi Caruzo

Prof. Dr. Jefferson Prado

Shirlei Soares Dassi
(Secretária)

200
anos

MUSEU NACIONAL
UFRJ

Ao Museu Nacional, queimado em 02 de setembro de 2018, eu dedico.

“Somos o que fazemos, mas somos, principalmente, o que fazemos para mudar o que somos.”

(Eduardo Galeano)

Agradecimentos

Ao Programa de Pós-Gradução em Biodiversidade Vegetal e Meio Ambiente do Instituto de Botânica de São Paulo pelo apoio a esta tese.

Ao Instituto de Botânica e à Universidade Estadual Paulista de Rio Claro (UNESP-RC), em especial ao Departamento de Ecologia e ao Laboratório de Ecologia Molecular, por todo o auxílio e infraestrutura proporcionados para a realização deste trabalho.

À Fundação de Amparo à Pesquisa do Estado de São Paulo pelo financiamento concedido à este projeto (FAPESP 2015/11812-1).

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) pela concessão das bolsas de doutorado e doutorado sanduíche (PDSE 88881.134263/2016-01).

Ao Laboratório de Análise Genética Molecular e o Centro de Biologia Molecular e Engenharia Genética (CBMEG) da Universidade Estadual de Campinas (UNICAMP), funcionários, alunos e estagiários, em especial à professora Dra. Anete Pereira de Souza, que me possibilitaram desenvolver a biblioteca de marcadores microssatélites.

Ao laboratório FLORA da Universidade Federal da Bahia, que me proporcionou infraestrutura para uma importante etapa no desenvolvimento desta tese.

À Universidade da Flórida, ao Florida Museum of Natural History, e ao Cellinese Lab por terem me acolhido durante o meu doutorado sanduíche, proporcionando todas as condições para a realização do meu trabalho.

Aos curadores e funcionários dos herbários visitados durante este doutorado, por terem me recebido tão bem durante as visitas e consulta aos acervos.

Aos diretores e funcionários das unidades de conservação que nos receberam e nos prestaram apoio incomensurável às nossas coletas.

Aos guias que nos acompanharam nas coletas, sempre dispostos e interessados em nosso trabalho.

Aos meus amigos dos EUA! Meus roomies que me receberam e me acolheram tão bem, e seguraram minha mão em um dos momentos mais difíceis que vivi.

Aos meus colegas do laboratório de Ecologia Molecular da UNESP, meus companheiros de bancada, nas extrações e PCRs, tamo junto! De uma forma ou de outra, mesmo que não sendo uma ajuda direta em meu trabalho, mas sempre houve uma comunicação, uma troca de experiência, ou simplesmente uma companhia, compartilhando e movimentando o espaço de trabalho, o tornando acolhedor e confortável (Jordana, Carlinha, Felipe Aoki, Marcos, Vanessa, Giu, Tabatha, Ju, Mateus, Carol, Marília).

Aos professores das disciplinas que cursei durante este doutorado, que me ajudaram imensamente na execução deste trabalho!

Aos amigos da pós-graduação, galera do 3° Turno, apesar de pouco convívio, sempre me receberam calorosamente durante as disciplinas e idas ao Instituto de Botânica.

Ao Felipe Martello que me ajudou a identificar as formiguinhas!

À Vivi linda (Viviane Brito) que me ajudou na triagem do meu material.

À Marcelita Silva que apesar de tão pouco tempo que nos conhecemos, foi de grande ajuda no processo de fechamento desta tese.

À Rebecca Stubbs pela valiosa ajuda com o capítulo desenvolvido durante o doutorado sanduíche.

À Nico Cellinese que me orientou durante o doutorado sanduíche, me recebeu de braços abertos, sempre me proporcionando apoio e orientação quando necessária, por ter depositado tanta confiança em mim e em meu trabalho.

Ao Natanael pelas maravilhosas ilustrações, pela paciência com as correções, o vai e volta das pranchas, e pela dedicação que imprimiu em sua arte para deixar meu trabalho ainda mais belo.

Aos meus amigos do FLORA, eternos companheiros botânicos, desta vez foi menos tempo de convivência com vocês, mas sempre um laboratório de pessoas que me estimulam e de onde saio revigorada!

À minha co-orientadora Clarisse, que acreditou em mim! Obrigada pela confiança depositada. Sua orientação foi maravilhosa e eu só tenho a agradecer. Você soube estimular e valorizar o meu trabalho nas horas certas, em momentos em que parecia que a coisa não andava, você me manteve positiva! Me senti segura sendo orientada por você, aprendi muito, e espero ser, para meus futuros alunos, um pouco da orientadora que você foi para mim!

À minha orientadora Inês, em primeiro lugar por ter me aceitado como orientanda, em segundo, por ter sido uma orientadora tão prestativa e presente, mesmo estando longe! Amo o seu olhar para as plantas, eu sempre tentava estar por perto nas viagens de campo, para aprender um pouco mais! Infelizmente sinto que não aprendi nem metade do que você tinha para me ensinar. Sou muito grata por tudo, foram diversas formas de ajuda que obtive de você, não somente como orientadora! Obrigada, de verdade!

À Maria Beatriz, minha amiga linda que fiz no ballet e depois descobri que estávamos na mesma área de trabalho, e que depois viríamos a ir para a mesma cidade e quase o mesmo laboratório na Flórida!! Velhow...com você foram tantas coincidências que eu tenho certeza que é algo de vidas passadas, ou você é um anjinho que está aqui para me ajudar! E como me ajudou amiga! Obrigada!

À Maria Alves, minha amiga que sempre busca me animar, sempre me coloca para cima, me ajuda no que pode, me dando apoio emocional e profissional também! Te amo amiga linda!

À minha eterna orientadora e amiga Nádia Roque, minha querida próóóó...que ainda me inspira e muito, e acredito que para sempre a terei como modelo, principalmente pela paixão que imprime em tudo que faz. Obrigada por me fazer sentir eternamente do FLORA!

Aos meus amigos de Rio Claro (Bárbara e Cleber (Clebra, vcs tem agradecimentos especiais mais para frente!), meus companheiros de Rep Laje Erison, Léo e Rodrigo; Moara (companheira da bruschetta da madruga), Paolita (rainha do samba!), Ursinho, Mineiro, Bruna e Naty (vizinhas), Dani, Annia, Gambezito, Lelitos, Camila Eboli, Julia Oshima, Julia Assis, Raul e Sayuri (casal gastão), Fábio, Iha, Claudinha, Martello, Laurinha, Paty (craque do baba/pelada!), Pedrão (me apresentou o acarajé mineiro!), Empada, trio maravilha: Milene, Naty e Rê (madrinha de Fuste!), Flávia (feiticeira faceira!), Silvia, Carol Bello, Eveline, Paula Montagnan, Urucum, Kalinka (massagens maravilhosas!), Lucia, Bia, Tróia e Rafa (craques do baba/pelada tb! Rafa goleira oficial!), Camilinha, João e Aoki, Tavares (e o mágico?!), Xuleta, Talita, Gabi doida Damasceno (está amanhecendo!!!), Gabi Becca, Igor, Matheus, Pablo (cervejeiro dos afloramentos cársticos), Henrique, Betânia, Letícia, Lorena, Blanca, Swanni, Gabriel Marcusso, Fabis, Paula Caldas e galera da Éxciton, galera maravilhosa, que me trouxeram muitas alegrias, muitos deliciosos e precisadíssimos happy hour...Cada um de vocês fez a diferença nestes 4 anos de doutorado e eu agradeço muito, pois apesar de não terem sido contribuições diretas, foram contribuições para a minha alma e meu bem estar emocional, e que no final das contas, é o que mais importa para nossas vidas!!!

Ao meu amigo Cleberzito, meu Erison do laboratório! Kkkkkk...meu amigo maravilhoso! Agradeço imensamente pela ajuda com a tese, análises e idéias! Pela sua disposição e interesse em discutir resultados e insights! Obrigada pela companhia no laboratório, e principalmente fora dele, e muito obrigada pelos "vamo tomar uma?". Amizade que levarei para a vida todinha!

Ao Filipe, pela ajuda imensa com as viagens de coleta e pelo amor depositado em mim! Obrigada pela paciência com os longos períodos de afastamento, por compreender que era necessário para minha carreira, e por ter aguentado até quanto pôde! Não ser mais sua namorada foi um dos maiores custos que este doutorado me teve e uma das poucas coisas que me faz parar, refletir e pesar sobre as escolhas feitas em minha vida. Tudo o que ganhei e tudo o que perdi, todas as vantagens e todas as desvantagens, e com relação a você, esse doutorado distante teve uma grande desvantagem! Nós éramos o encaixe perfeito da tampa com a panela. Que sejamos amigos, para sempre! Te amo!

Ao Otávio, meu irmãozinho tricoca, meu grande amigo, literalmente! Uma pessoa maravilhosa e que eu sei que levarei em meu coração e pensamentos para o resto de minha vida. Você é um grande irmão e amigo, talvez o melhor título que eu possa ganhar é o de ser sua amiga! Obrigada pela maravilhosa ajuda, que foram tantas ao longo de todo este doutorado que me levaria o mesmo tanto de páginas que tem esta tese para enumerá-las...hahahahaha...mas principalmente neste final, o que teria sido de mim sem você?! Valeu mesmo! Você nunca mais vai se livrar de mim! Te amo!

À Barbarete, uma das amizades mais honestas e sinceras que fiz nesta minha vida! Você é completamente parte de mim agora, eu sinto muito amor por você minha amiga! Obrigada pela imensa ajuda que me proporcionou durante a execução desta tese. Você foi orientadora, amiga, irmã, conselheira, psicóloga e excelente companheira de copo! A você devo muito! Esta tese teria tomado outro rumo se não fosse por você, e talvez um rumo turbulento! Hahahaha... Nossa amizade é para a vida inteira, quero estar sempre por perto, pois você é iluminada e ilumina todos ao redor! Te amo muito e muito!

Aos meus pais que me ajudam em tudo que preciso! São tantas coisas que não daria para enumerar aqui. Obrigada pai e mãe, sem o apoio de vocês eu não teria conseguido finalizar mais esta trajetória! Obrigada por sempre apoiarem as minhas decisões!

A todos aqueles que contribuíram direta e indiretamente com esta tese e com a finalização dela! Acreditem, não esqueci de ninguém, só não tenho mais tempo e preciso finalizar por aqui! Hahahaha...

MUITO OBRIGADA!

Resumo

Euphorbia sect. *Brasilienses* V.W. Steinm. & Dorsey é um grupo exclusivamente brasileiro, que inclui *E. attastoma* Rizzini, *E. holochlorina* Rizzini, *E. phosphorea* Mart., *E. sipolisii* N.E. Br. e a recentemente descrita *E. tetrangularis* Hurbath & Cordeiro. Essas espécies possuem hábito arbustivo, caule fotossintetizante, suculento, sem espinhos, folhas inconspícuas e cedo caducas. *Euphorbia phosphorea* ocorre em praticamente toda a região da caatinga, enquanto *E. attastoma* restringe-se ao sudoeste da Bahia e nordeste de Minas Gerais, onde cresce em região de caatinga rupestre. *Euphorbia sipolisii* é exclusiva dos campos rupestres da região de Diamantina e *E. holochlorina* é endêmica de afloramentos rochosos no domínio da Mata Atlântica no leste de Minas Gerais e Espírito Santo e *E. tetrangularis* é microendêmica da Serra de Montevidéu, no Município de Monte Azul/MG. O objetivo desta tese foi investigar a história evolutiva da seção, utilizando-se da sistemática, taxonomia, biogeografia e filogeográfia, cujos resultados apresentamos na forma de quatro capítulos. No primeiro, apresentamos uma nova espécie para a seção, bem como uma análise filogenética baseada em inferência bayesiana, com seis marcadores plastidiais e um nuclear, onde a seção emergiu como um grupo monofilético, mas a relação de parentesco entre as espécies não foi claramente resolvida; no segundo, a revisão taxonômica da seção, incluindo chave para identificação das espécies, descrições, comentários e ilustrações; no terceiro, uma análise biogeográfica para o clado do novo mundo de *Euphorbia* subg. *Euphorbia*, onde a idade de *E*. sect. *Brasilienses* foi estimada em 3.15 Ma, com divergência de seu grupo irmão, *E*.sect. *Stachydium*, há mais de 16 Ma e diversificação in situ na Caatinga, que provavelmente tem idade acima de 16 Ma; e no quarto capítulo o estudo filogeográfico de *E. phosphorea*, cujas populações apresentam alta estruturação genética e idade estimada em 2.31 Ma.

Palavras-chave: *Euphorbia* subg. *Euphorbia*, Taxonomia, Biogeografia, Caatinga, SDTF.

Abstract

Euphorbia sect. *Brasilienses* V.W. Steinm. & Dorsey is an exclusively Brazilian group, and includes *E. attastoma* Rizzini, *E. holochlorina* Rizzini, *E. phosphorea* Mart., *E. sipolisii* N.E. Br. and the recently described *E. tetrangularis* Hurbath & Cordeiro. These species present a shrub habit, green photosynthetic succulent branches, without spines and leaves inconspicuous and deciduous. *Euphorbia phosphorea* is endemic to the Brazilian Caatinga, while *E. attastoma* is restricted to southwest Bahia and northeast of Minas Gerais, where it grows in a region of rocky caatinga. *Euphorbia sipolisii* is endemic to the *campos rupestres* of Diamantina region, *E. holochlorina* is endemic to rocky outcrops of Atlantic Forest Domain, eastern Minas Gerais and in Espírito Santo, and *E. tetrangularis* presents as a microendemic species from Serra de Montevideu in the Municipality of Monte Azul/MG. The aim of this work was to investigate the evolutionary history of the section, trough systematic, taxonomic, biogeographic and phylogeographic approches, whose results we present in four chapters. In the first we present a new species for the section, as well as a phylogenetic analysis based on Bayesian inference, with six plastial markers and one nuclear, where the section emerged as a monophyletic group, but the relationship between the species was not clearly resolved; in the second chapter, the taxonomic revision of the section, including key for species, descriptions, comments and illustrations; in the third, a biogeographic analysis for the new world clade of *Euphorbia* subg. *Euphorbia*, where the age of *E.* sect. *Brasilienses* was estimated at 3.15 Ma, with divergence from its sister group, *E.* sect. *Stachydium*, for more than 16 Ma. and diversification in situ in the Caatinga, whose age is probably greater than 16 Ma.; and in the fourth chapter the phylogeographic study of *E. phosphorea*, whose populations presented high genetic structure and age estimated at 2.31 Ma.

Keywords: *Euphorbia* subg. *Euphorbia*, Taxonomy, Biogeography, Caatinga, SDTF.

Sumário

Índice de Figuras e Tabelas

Introdução

Capítulo 1

Capítulo 2

Capítulo 3

Capítulo 4

Introdução Geral

1.1 ***Euphorbia*:** um gênero monofilético caracterizado pelo ciátio.

Euphorbia é um dos maiores gêneros dentre as angiospermas, com cerca de 2.000 espécies. O gênero é monofilético e apresenta uma ampla variedade de hábitos, desde ervas perenes ou anuais, subarbustos, arbustos e árvores. Muitas espécies são suculentas, cactiformes, áfilas, com orgãos modificados em espinhos. Diversas espécies são utilizadas como ornamentais: *Euphorbia pulcherrima* Willd. ex Klotzsch, conhecida como bico-de-papagaio, *Euphorbia milii* Des Moul., conhecida como coroa-de-cristo, além de outras cactiformes, como a *Euphorbia ingens* E. Mey. ex Boiss., *Euphorbia trigona* Mill. e *Euphorbia obesa* Hook. f. Algumas possuem potencial medicinal na fabricação de compostos anticancerígenos e anti-inflamatórios como a *Euphorbia tirucalli* L., *Euphorbia peplus* L., *Euphorbia prostrata* Aiton, *Euphorbia thymifolia* L. e *Euphorbia hirta* L. (Mwine and Van Damme, 2011; Oliveira and Evangelista-Coimbra, 2014; Santos et al., 2016), enquanto outras são invasoras como a agressiva *Euphorbia heterophylla* L. (amendoim-bravo).

No Brasil existem 62 espécies, sendo quase 50% delas endêmicas (*Euphorbia in* Flora do Brasil 2020 em construção). O gênero possui como sinapomorfia a inflorescência do tipo ciátio (Steinmann and Porter, 2002; Bruyns et al., 2006; Horn et al., 2012). O ciátio é um pseudanto, não encontrado em nenhuma outra família de angiospermas (Steinmann and Porter, 2002; Horn et al., 2012), sendo formado por 4-5 címulas de flores masculinas reduzidas a um único estame, circundando uma única flor feminina, também reduzida a um pistilo. As flores são protegidas por um invólucro de brácteas fundidas. Geralmente há glândulas nectaríferas intercaladas com os lobos do invólucro, que frequentemente apresentam apêndices morfologicamente muito variados.

O gênero atualmente está incluído na tribo Euphorbieae, subtribo Euphorbiinae, que na circunscrição original de Webster (1994) possuía mais seis gêneros: *Chamaesyce* Gray, *Endadenium* L.C.Leach, *Synadenium* Boiss., *Monadenium* Pax, *Pedilanthus* Neck. ex Poit. e *Cubanthus*, atualmente todos sinonimizados sob *Euphorbia.* A subtribo é caracterizada pelas flores aclamídeas (Webster, 1994). As outras duas subtribos, Anthosteminae, com os gêneros *Anthostema* A.Juss. e *Dichostemma* Pierre apresentando flores pistiladas e estaminadas monoclamídeas; e Neoguillauminiinae, com os gêneros *Calycopeplus* Planch. e *Neoguillauminia* Croizat, com cálice apenas nas flores pistiladas, além de glândulas entre as címulas de flores estaminadas.

A atual circunscrição de *Euphorbia* incluindo todas as espécies com ciátio foi estabelecida por Steinmann e Porter (2002) (Figura 01). Além disso, quatro clados foram reconhecidos pelos autores, posteriormente formalizados como subgêneros por Bruyns et al (2006), são eles:

Euphorbia subg. *Esula* Pers., *E.* subg. *Rhizanthium* (Boiss.) Wheeler, *E.* subg. *Chamaesyce* Raf. e *E.* subg. *Euphorbia*. *E.* subg. *Rhizanthium*, posteriormente renomeado como *E.* subg. *Athymalus* (Peirson et al., 2013). No estudo de Horn et al. (2012), as relações entre os subgêneros foram definidas, com o subgênero *Esula* sendo o primeiro clado a divergir, seguido por subg. *Athymalus*, e em seguida o clado formado pelos subgêneros *Chamaesyce* e *Euphorbia*.

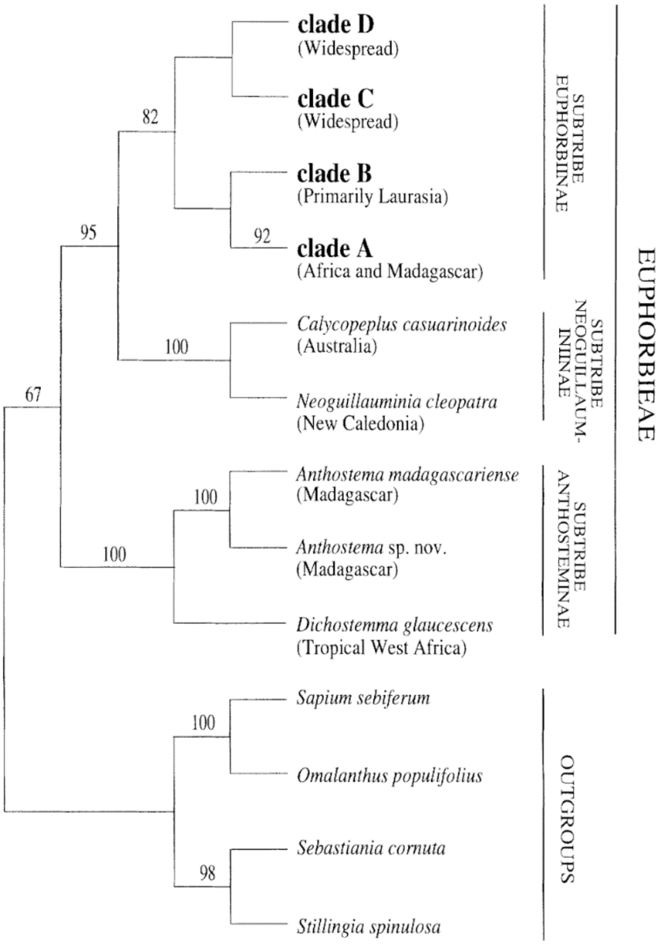


Figura 01: Filogenia da tribo Euphorbieae mostrando a subtribo Euphorbiinae e os clados que eram considerados gêneros e atualmente são circunscritos como subgêneros de *Euphorbia*: Clade A = subg. *Athymalus*; clade B = subg. *Esula*; clade C = subg. *Euphorbia;* clade D = subg. *Chamaesyce* (adaptado de Steinmann and Potter, 2002). As relações entre os subgêneros foram definidas posteriormente por Horn et al. (2012).

1.1.1 *Euphorbia* subg. *Euphorbia*: o subgênero mais diverso.

O subgênero *Euphorbia* apresenta cerca de 661 espécies (Dorsey et al., 2013) que ocorrem nos mais variados habitats (Horn et al., 2012), especialmente em regiões semiáridas, onde se destacam as espécies suculentas e xeromórficas, com órgãos modificados em espinhos (Horn et al., 2014). Cinco gêneros circunscritos por Webster (1994) encontram-se atualmente sinonimizados sob *Euphorbia* subg. *Euphorbia*: *Synadenium* Boiss., *Endadenium* L.C. Leach, *Pedilanthus* Necker, *Cubanthus* (Boiss.) Millspaugh e *Monadenium* Pax, sendo estes dois últimos atualmente considerados como *E.* sect. *Monadenium* [Pax] Bruyns e *E.* sect. *Cubanthus* [Boiss.] V.W. Steinm. & P.E. Berry. Em sua maioria os ciátios de *E.* subg. *Euphorbia* não apresentam apêndices nas glândulas.

Nos estudos filogenéticos de Horn et al. (2012) e Dorsey et al. (2013) foram reconhecidos 4 clados em *Euphorbia* subg. *Euphorbia*: Novo Mundo (América tropical), Velho Mundo I (Madagascar), Velho Mundo II (África, Arábia e Ásia) e Pacífico (Pacífico Sul). Dorsey et al. (2013) reconheceram 21 seções para esse subgênero, sendo nove delas novas. No Clado do Novo Mundo, com 99% de suporte, emergiram 11 linhagens com alto suporte, que foram reconhecidas como seções. (Figura 02).

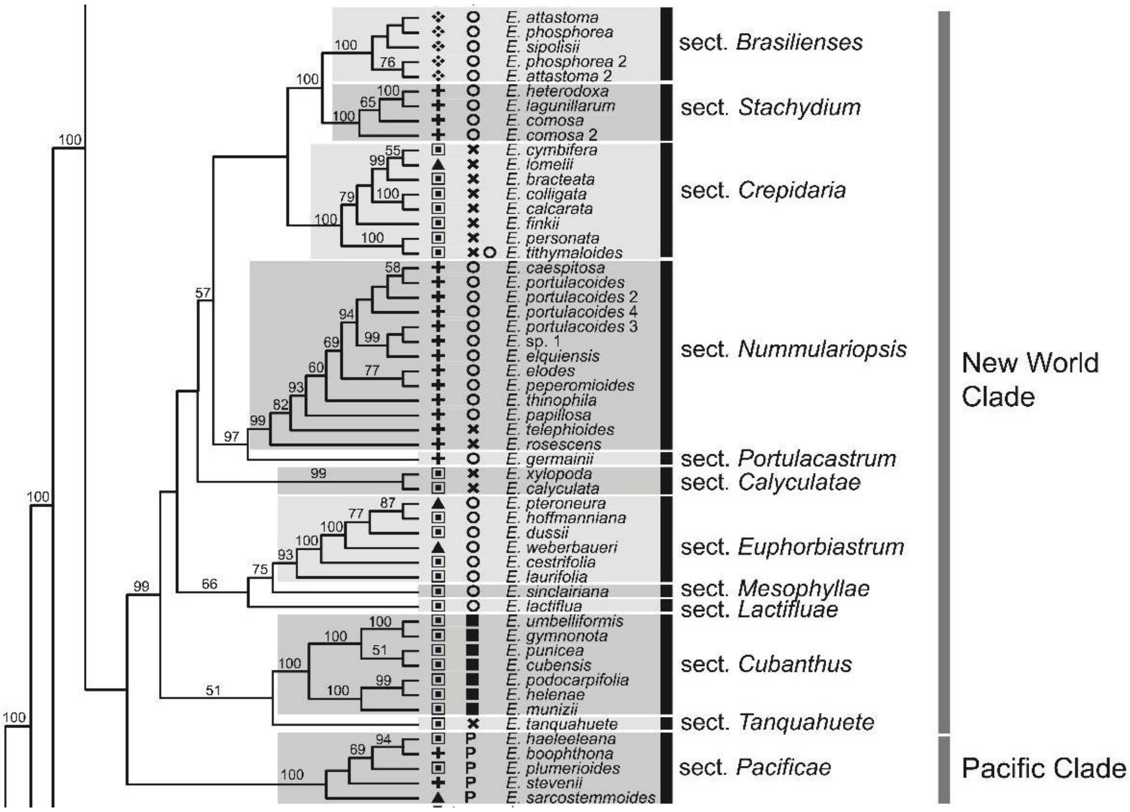


Figura 02: Filogenia do Clado do Novo Mundo adaptada de Dorsey et al. (2013). *Euphorbia* sect. *Brasilienses* com 100% de suporte e baixa resolução interna.

Uma dessas linhagens é composta exclusivamente por espécies brasileiras: *Euphorbia* sect. *Brasilienses* V.W.Steinm. & Dorsey, que na época possuía quatro espécies e duas variedades.

1.1.2 *Euphorbia* sect. *Brasilienses*: o clado das suculentas brasileiras.

Euphorbia holochlorina e as variedades de *E. attastoma* foram descritas por Rizzini (1989) em seu estudo das espécies cactiformes brasileiras de *Euphorbia*, onde ele incluiu *E. phosphorea* juntamente com outras duas espécies atualmente pertencentes a *Euphorbia* sect. *Crossadenia* Boiss.: *Euphorbia appariciana* Rizzini e *Euphorbia gymnoclada* Boiss.

Três das quatro espécies de *Euphorbia* sect. *Brasilienses* emergiram como um grupo monofilético no trabalho de Dorsey et al. (2013), entretanto sem clara afinidade estabelecida entre elas. Além de não incluir *Euphorbia holochlorina*, diferentes acessos de *E. attastoma* e *E. phosphorea* emergiram em clados separados nesta análise.

As espécies dessa seção, incluindo *Euphorbia tetrangularis* Hurbath & Cordeiro, recentemente descrita, são arbustos suculentos, com folhas inconspícuas e cedo caducas, caule costado e fotossintetizante e glândulas do ciátio com apêndices em formato de chifres (Figura 03), crescendo geralmente em afloramentos rochosos, inselbergs e solos arenosos, em vegetação de caatinga e campos rupestres, nos limites das Florestas Tropicais Sazonalmente Secas (Seasonally Dry Tropical Forests – SDTF) (Figura 04).

Figura 03: Ramo com ciátios de *Euphorbia phosphorea*, Brejo da Madre de Deus (PE).

Euphorbia phosphorea é a espécie de mais ampla distribuição geográfica da seção, ocorrendo em praticamente toda a porção nordestina da Caatinga, faltando, entretanto, na faixa mais meridional do bioma, no sudoeste da Bahia e norte de Minas Gerais, onde é substituída pelas variedades de *E. attastoma*. *Euphorbia sipolisii* N.E.Br. é endêmica de Minas Gerais, sendo restrita aos campos rupestres da região de Diamantina, enquanto *E. holochlorina* ocorre a leste de Minas Gerais e noroeste do Espírito Santo em afloramentos rochosos.

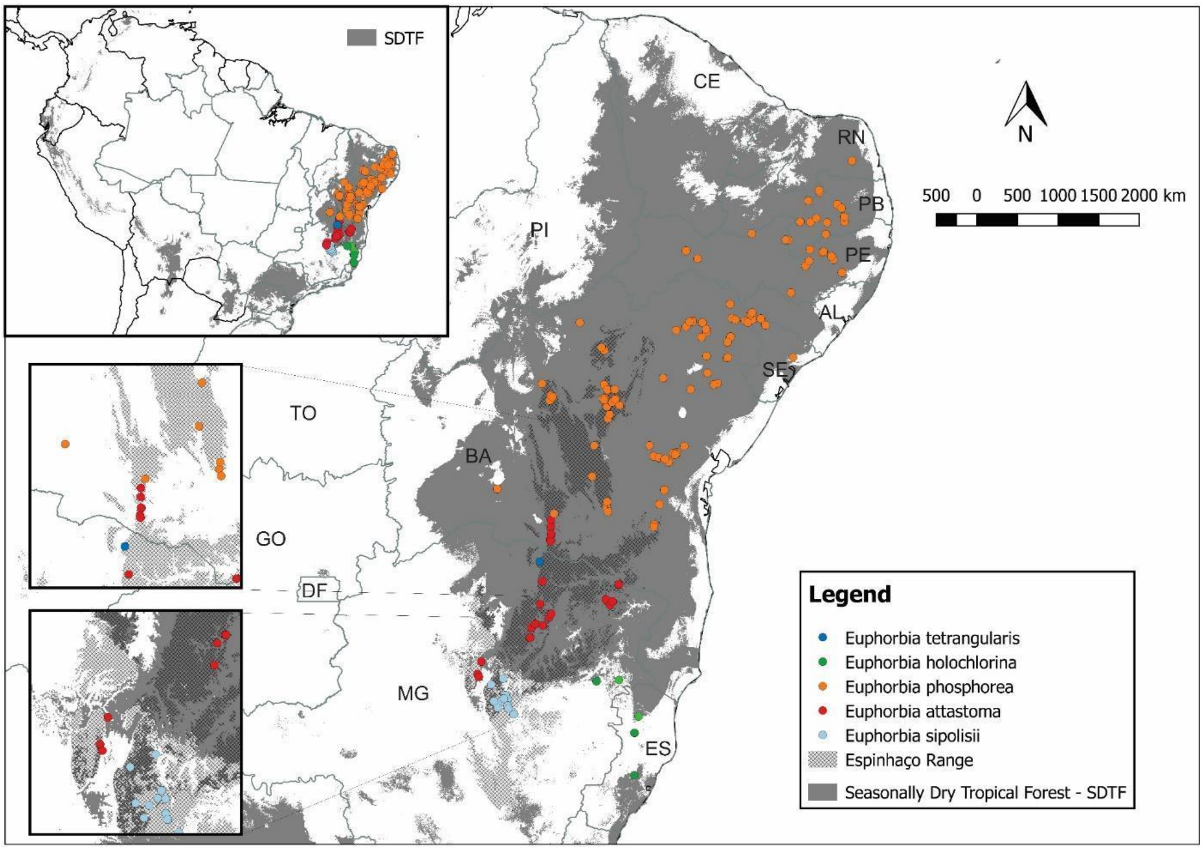


Figura 04: Mapa de distribuição de *Euphorbia* sect. *Brasilienses*

A distribuição geográfica do grupo é ideal para investigação de processos histórico-evolutivos em ambientes xéricos. No geral, poucos estudos biogeográficos e filogeográficos foram realizados com espécies de *Euphorbia* (Garrick et al., 2009; Dyer et al., 2010; Zecca et al., 2011; Cacho and Baum, 2012; Beatty et al., 2015; Geltman, 2015; Barres et al., 2017; Maya-Lastra et al., 2017; Yang et al., 2018), e mesmo com outros grupos de plantas de ambientes xéricos da América do Sul e Brasil (Turchetto-Zolet et al., 2013; Leal et al., 2016).

1.2 Florestas Tropicais Sazonalmente Secas (SDTF – Seasonally Dry Tropical Forest)

As SDTF ocorrem em núcleos disjuntos em toda a região neotropical, desde o México até o norte da Argentina e nordeste do Brasil, cobrindo mais de 20% da América do Sul (Pennington et al., 2000, 2006, 2009; Särkinen et al., 2011; Werneck et al., 2011). Essas formações recebem precipitação inferior a 1800 milímetros por ano, que pode chegar a menos de 100 mm, com um período de 5-6 meses de seca, sem geadas, em solos considerados férteis (nutrientes variando entre moderado a alto), com pH moderado a alto (Pennington et al., 2000), (Bullock, 1995; Gentry, 1995; Oliveira-Filho et al., 2006). A vegetação das SDTF é predominantemente lenhosa, com várias espécies suculentas, não tolerantes ao fogo e baixo número de espécies de gramíneas, sendo Fabaceae (Leguminosae) e Bignoniaceae as famílias mais representativas (Gentry, 1995; Pennington et al., 2009.

As maiores manchas ou núcleos das SDTF são encontradas no nordeste do Brasil (Caatinga), ao longo da bacia do rio Paraguai-Paraná (Misiones) e no sudoeste da Bolívia e noroeste da Argentina (Piemonte sub-andino); manchas menores, porém significativas, também ocorrem ao longo da costa caribenha da Colômbia e Venezuela, em vales nos Andes e espalhadas ao longo Cerrado (Prado 2000, Prado and Gibbs 1993). Semelhanças florísticas indicam possíveis ligações entre os fragmentos de florestas estacionais (Prado and Gibbs, 1993; Pennington et al.; 2000, 2006). Na América do Sul, as SDTF fazem parte da "diagonal seca" que inclui a Caatinga, o Cerrado e Chaco, contudo estas regiões são consideradas unidades fitogeográficas diferentes, com características edáficas, climáticas e florísticas distintas, além de que evidências filogenéticas de táxons dessas formações apontam idades diferentes para o Cerrado e Caatinga, com esta última sendo considerada mais antiga (de Queiroz et al., 2017).

As SDTFs estão entre os ecossistemas tropicais mais ameaçados, devido à exploração da madeira, agricultura, pecuária e fogo (Jazen, 1988; Geist e Lambin, 2002; Leal et al., 2005). Em uma escala global, as SDTF sul americanas experimentaram as maiores taxas de desmatamento e praticamente todos os remanescentes estão em risco de extinção, com prioridade urgente de conservação (Miles et al., 2006). Além disso, em comparação a outros ecossistemas, as SDTF são pouco conhecidas, inclusive em termos de biodiversidade, com poucos estudos biogeográficos e moleculares (Leal et al, 2016). A conservação dos fragmentos de SDTF é essencial, entre outras razões, pelo seu elevado número de espécies endêmicas (Collevatti et al., 2013).

A Hipótese do Arco Pleistocênico- PAH (Prado and Gibbs, 1993) propõe que a extensão máxima das SDTF ocorreu durante o último máximo glacial (LGM), durante o Pleistoceno. O clima mais frio e seco desse período, teria provocado a expansão das matas secas, responsável pela distribuição geográfica fragmentada de algumas de suas espécies (Prado e Gibbs, 1993; Pennington et al., 2000, 2004; Collevatti et al., 2013). Neste contexto, as espécies que melhor se desenvolvem em clima quente e úmido possivelmente se restringiram a áreas com estas condições (Teoria dos Refúgios – Haffer (1969)), até que as condições climáticas mudassem durante o período interglacial.

Estudos recentes tem corroborado (Zanella, 2000; Prado, 2003; Oliveira-Filho et al., 2006; Franco e Manfrin, 2013; Bonatelli et al., 2014) e refutado (Pennington et al., 2004; Mayle, 2006; Saslis-Lagoudakis et al., 2008; de Queiroz et al., 2017) a PAH como uma explicação para a história biogeográfica das SDTFs, entre estes últimos (Mayle, 2006) sugere que ao invés de relictuais, as SDTF possam ter surgido de formações adjacentes.

1.3 Caatinga: o maior núcleo de SDTF da América do Sul.

A Caatinga ocupa uma área aproximada de 850.000 Km^2 abrangendo parte dos estados do Piauí, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Sergipe, Bahia e Minas Gerais (Ab'Saber, 1977) (Figura 05). Caracterizado por um clima semi-árido, fortemente sazonal, com temperaturas entre 26 e 28 °C e baixa precipitação (menor que 1000 mm por ano), geralmente concentrada em três a cinco meses (Velloso et al., 2002).

A região apresenta diferentes unidades geomorfológicas, sendo as principais: as grandes depressões (Depressão Franciscana e Depressão Cearense); as chapadas altas com altitudes superiores a 800 m; áreas de solos com origem sedimentar (Chapada do Araripe, Serra do Ibiapaba, bacias sedimentares do Tucano-Jatobá); Chapada Diamantina (Cadeia do Espinhaço); Planalto da Borborema (Rio Grande do Norte, Paraíba, Pernambuco e Alagoas); afloramentos de calcários conhecidos como áreas cársticas; e as dunas de areias quartzosas da região do baixo e médio São Francisco (Queiroz, 2009).

Há diferentes tipos de solos como os de embasamento cristalino, rasos, argilosos, pedregosos e arenosos ocorrendo em superfícies sedimentares, tais como os litossolos, regossolos, latossolos, podzólicos e areias quartzosas (Queiroz, 2009).

Diferentes formações compõem o domínio da Caatinga: Formações com estrato arbóreo, geralmente descontínuo, com espécie predominantemente decíduas, suculentas, providas de espinhos ou acúleos, sem estrato herbáceo; florestas estacionais, com árvores de porte mais elevado e dossel contínuo, não completamente caducifólias e com sub bosque, com lianas e epífitas; florestas serranas ou 'brejos', podendo se apresentar como florestas perenifólias até estacionais deciduais; carrascos que apresentam estrato arbóreo-arbustivo muito denso e ausência de cactáceas e bromeliáceas terrestres (Queiroz, 2009); cerrados sobre solos rasos e distróficos, com baixa concentração de nutrientes e altos níveis de alumínio, sujeitos a queimadas periódicas (Ratter et al., 1997); campos rupestres em áreas da Chapada Diamantina, reconhecidos por uma vegetação herbáceo-arbustiva em altitudes acima dos 900m (Harley, 1995).

Com base nas diferenças geomorfológicas, edáficas e florísticas, oito ecorregiões foram reconhecidas para o Bioma Caatinga por Velloso et al. (2002). São elas:

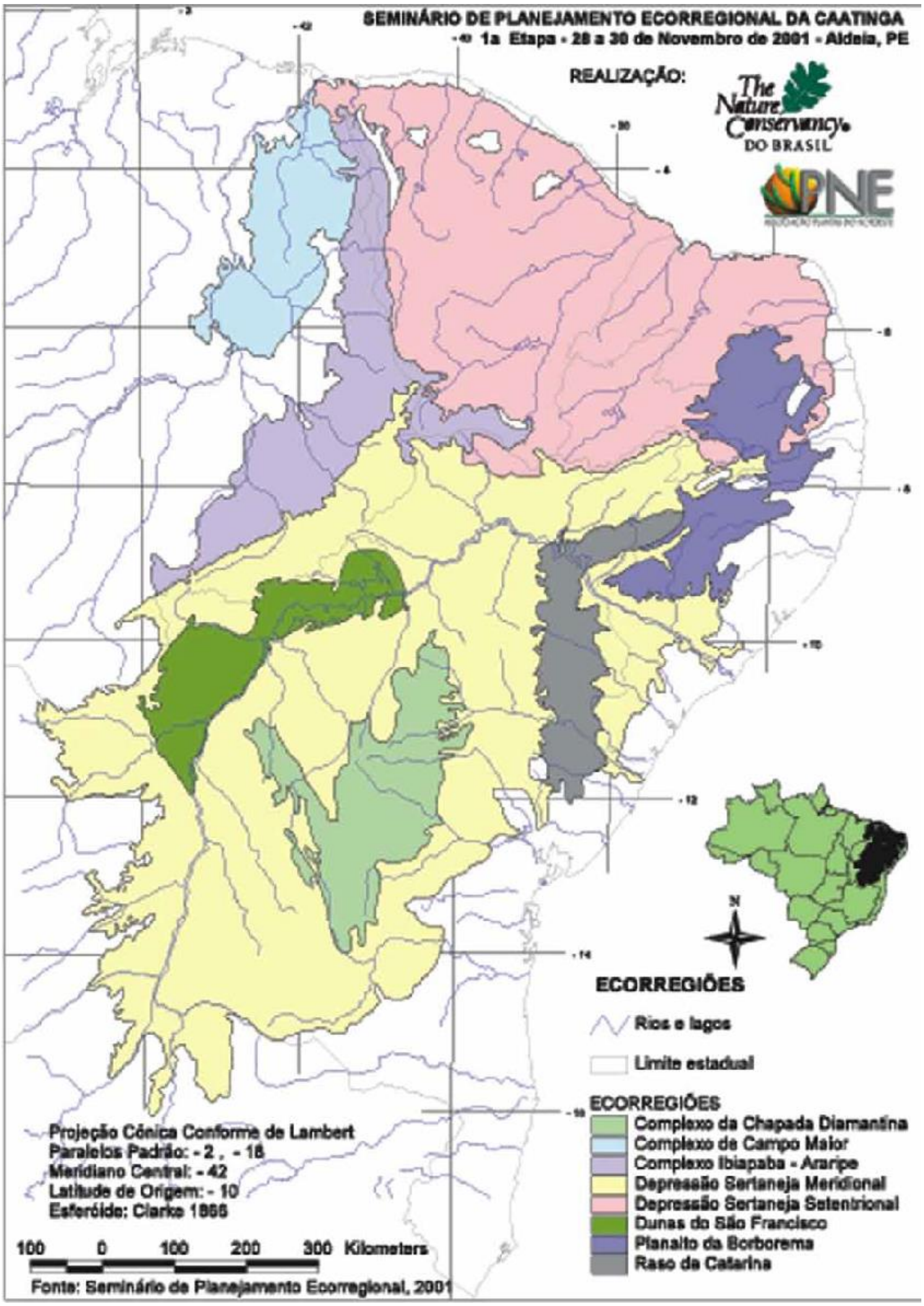


Figura 05: Ecorregiões da Caatinga (adaptado de Velloso et al., 2002)

1 – *Complexo do Campo Maior:* Solos sedimentares da Formação Longá, com baixa drenagem, que formam planícies inundáveis. Localiza-se em quase toda sua extensão no estado do Piauí, e uma pequena porção no Maranhão. Altitudes variando entre 50-200 m, com área total de 41.420 km^2. Predominância de vegetação herbácea com fisionomia de savana.

2 - *Depressão Sertaneja Setentrional:* Solos rasos e pedregosos de origem cristalina, férteis. Considerada a ecorregião mais seca da Caatinga. A altitude varia de 20-500m, podendo chegar a 800 m, sem rios permanentes (Velloso et al., 2002). Localiza-se na região norte, com cerca de 206.700 Km^2, abrangendo os estados da Paraíba, Rio Grande do Norte e Ceará e parte do Piauí. Predomínio de vegetação arbustiva a arbórea.

3 - *Depressão Sertaneja Meridional*: também apresenta condições climáticas típicas do semi-árido nordestino, contudo os solos são mais profundos e altitudes entre 100-500m. Localiza-se em sua maior extensão no estado da Bahia e Pernambuco, abrangendo também parte de Sergipe e Alagoas, e norte de Minas Gerais. É a maior das ecorregiões, com cerca de 374.000 km^2 (Velloso et al., 2002; Queiroz, 2009). A vegetação é um pouco mais alta que a setentrional, mas também predominantemente arbustiva a arbórea.

4 - *Raso da Catarina:* apresenta solos muito arenosos, profundos e pouco férteis. O relevo é predominantemente plano, com altitudes entre 350-700 m. Localiza-se na região centro-oeste, respeitando os limites geomorfológicos da bacia sedimentar, com cerca de 99.772 km^2. Caracterizada por uma vegetação arbustiva e densa.

5 - *Dunas do São Francisco*: predominância de solos profundos, de areias quartzosas e fertilidade muito baixa. Localiza-se especificamente a oeste do Rio São Francisco. As altitudes variam entre 150-700 m e abrange uma área de 36.160 km^2. Apresenta uma vegetação agrupada em densas moitas, geralmente arbustiva.

6 - *Planalto da Borborema:* formação granítica com solos de profundidade e fertilidade variadas, mas em geral férteis. Com altitudes que variam entre 150 a 650, podendo apresentar algumas elevações de até 1000 m. Abrange os estados do Rio Grande do Norte, Paraíba, Pernambuco e Alagoas, e uma área de 41.940 km^2. Vegetação que varia desde arbustiva e aberta, até arbórea, entremeada de matas secas e úmidas (brejos de altitude).

7 - *Complexo Ibiapaba-Araripe:* solos geralmente profundos e arenosos, com altitudes que variam de 650 a 950m, com as maiores na Chapada do Araripe. Localiza-se entre oeste do Ceará e nordeste do Piauí, e ao sul do Ceará e uma parte central do Piauí, com uma área de 69.511 km^2. Carrascos, florestas pluviais e cerradões são encontrados nesta ecorregião.

8 – *Complexo da Chapada Diamantina:* apresenta solos em geral rasos, pedregosos e pobres, e um relevo bastante acidentado, com grandes maciços de altitudes variando entre 500-2000 m, sendo a porção mais elevada da Caatinga. Localiza-se na região centro-sul da Caatinga, com cerca de 50.610 km^2. Composta por um verdadeiro mosaico vegetacional, com campos rupestres, cerrados, florestas secas e úmidas, decíduas e semi-decíduas.

A caatinga é um bioma ameaçado, com apenas 21,16%, de sua vegetação original, que foi substituída por pastagens e plantações (Leal et al., 2005). Em virtude da influência humana que altera as paisagens e a distribuição das populações naturais, estudos envolvendo ferramentas moleculares para identificação de padrões históricos de distribuição geográfica devem ser estimulados (Werneck et al, 2011).

Os estudos filogeográficos nos neotrópicos ainda são escassos, principalmente envolvendo espécies de ambientes xéricos (Werneck, 2011; Turchetto-Zolet et al., 2013) e no Brasil o quadro é ainda mais preocupante (Leal et al., 2016). Este quadro sugere urgência em ampliar os estudos em ambientes xéricos neotropicais, visto que serem considerados biomas altamente ameaçados, com poucos esforços de conservação e contínua fragmentação.

1.4 Filogeografia: compreender as forças que moldaram a grande biodiversidade da América do Sul.

Avise et al. (1987) definiram o termo Filogeografia para os estudos que buscam entender os princípios e processos históricos que promovem a distribuição geográfica das linhagens de organismos. A filogeografia faz uma ligação entre demais estudos evolutivos, como a filogenia, biogeografia e genética de populações, e relaciona a variação genética das linhagens e sua atual distribuição geográfica, de forma a desvendar quais processos influenciaram esta distribuição (Avise, 2009). Com os estudos filogeográficos é possível testar hipóteses biogeográficas, esclarecer padrões histórico-evolutivos, inferir processos demográficos históricos de uma espécie e até mesmo definir limites interespecíficos e estabelecer potenciais áreas para conservação (Avise et al., 1987; Avise, 2000). Com base na distribuição geográfica das populações de uma linhagem, a filogeografia busca entender quais forças atuaram em sua história evolutiva, que podem ser extrapolas para outras linhagens com padrão semelhante de distribuição.

Estudos sobre filogeografia nos biomas da América do Sul aumentaram considerávelmente desde 2006, segundo revisão feita por Turchetto-Zolet et al. (2013). A busca pela compreensão da existência de grande biodiversidade na região (Gentry, 1982; Antonelli and Sanmartín, 2011; Hughes, Pennington and Antonelli, 2013), tem estimulado a pesquisa no continente sul americano (Turchetto-Zolet *et al.*, 2013), e diversos fatores e teorias foram relacionados à essa riqueza de espécies da região, que possui cerca de 37% das espécies de angiospermas conhecidas até o momento (Antonelli and Sanmartín, 2011; Hughes, Pennington and Antonelli, 2013). Entre os fatores mais notáveis estão os de origem geomorfológica, visto que a América do Sul foi palco de consideráveis eventos, com destaque para o soerguimento dos Andes. Iniciado a 65 milhões de anos, desencadeou uma série de episódios não somente geomorfológicos, mas também climáticos, dando origem a novos ecossistemas (Antonelli *et al.*, 2009; Hoorn *et al.*, 2010; Garzione *et al.*, 2017).

Atualmente, a discussão sobre a origem da incrível biodiversidade na região sul neotropical gira em torno de múltiplos fatores que atuaram concomitantemente, abrangendo mais de uma hipótese (Antonelli and Sanmartín, 2011; Hughes, Pennington and Antonelli, 2013). Seguindo nesta linha, fatores intrínsecos de cada espécie, também podem ter ditado a forma como elas responderam a eventos como os de oscilações climáticas.

A maior parte dos estudos filogeográficos feitos com grupos da América do Sul foram realizados em florestas úmidas da Amazônia e Mata Atlântica (Turchetto-Zolet *et al.*, 2013). Para a Caatinga, o maior e mais biodiverso núcleo de SDTF (Queiroz, 2006), grande parte dos estudos envolve linhagens também relacionadas a outros biomas adjacentes, como o Cerrado e Mata Atlântica (Leal, Palma da Silva and Pinheiro, 2016). Com táxons mais ou menos exclusivos de caatinga, cerca de dez estudos foram realizados (Nascimento *et al.*, 2013; Magalhaes *et al.*, 2014; Pinheiro *et al.*, 2014; Werneck *et al.*, 2015; Vieira *et al.*, 2015; Thomé *et al.*, 2016; Gonçalves-Oliveira *et al.*, 2017; Miranda *et al.*, 2017; Balbino, Caetano and Almeida, 2018; Costa, Amorim and Mattos, 2018; Lanna *et al.*, 2018), e destes, apenas quatro com plantas (Pinheiro *et al.*, 2014; Vieira *et al.*, 2015; Gonçalves-Oliveira *et al.*, 2017; Balbino, Caetano and Almeida, 2018).

Em grande parte dos estudos filogeográficos com taxa de Caatinga, as idades foram estimadas para o Mioceno, com as populações apresentando-se altamente estruturadas (Nascimento *et al*., 2013; Magalhães *et al*., 2014; Pinheiro *et al*., 2014; Werneck *et al*, 2015; Vieira *et al*., 2015; Gonçalves-Oliveira *et al*., 2017), ou fracamente estruturadas (Thomé *et al*., 2016; Balbino *et al*., 2018). Limitação de fluxo gênico, deriva genética e ocorrência em possíveis refúgios foram alguns dos fatores apontados para grande parte dos taxa terem alta estruturação genética. Grupos que não apresentaram alta estruturação e ocorrem em populações disjuntas, inferiu-se contatos secundários e admixture que diminuiram a divergência genética entre populações. O único grupo que apresentou idade estimada para o pleistoceno foi um grupo de abelhas, contudo os autores não atribuem influencia climática na diversificação do mesmo, sem sinais de expansão demográfica. Lanna *et al*. (2018) estimaram a idade de um grupo de lagartos para o Plioceno, a partir do qual possíveis espécies crípticas diversificaram. Muitos destes estudos supracitados também inferiram a presença de espécies crípticas e a ocorrência de mudanças demográficas históricas nos respectivos grupos de estudo, a maioria apresentando expansão no LGM. Apesar da maior parte dos grupos ter idade estimada para o Mioceno, todos parecem ter sofrido algum tipo de influência durante as oscilações climáticas, e consequente instabilidade, do pleistoceno.

Em espécies de plantas, as diferenças encontrados entre os resultados das análises com marcadores plastidias e nucleares reflete diretamente nas síndromes de dispersão de semente e pólen (Pinheiro *et al.*, 2014; Gonçalves-Oliveira *et al.*, 2017). Os grupos estudados nestes trabalhos ocorrem em Inselbergs, e parecem não ter sofrido expansão ou retraçao durante o LGM o que pode estar relacionado aos inselbergs atuarem como microrefúgios para espécies de ambientes xéricos. Redução demográfica durante o LGM foi estimada para uma espécie de Moraceae, *Ficus bonijesulapensis* R.M.Castro (Vieira *et al.*, 2015), e Balbino et al. (2018) encontraram pouca estrutura genética em populações de umbu, *Spondias tuberosa* Arruda. Os autores inferiram um refúgio próximo ao Planalto da Borborema, onde eles acreditam ter havido expansão das florestas úmidas e instabilidade na porção leste da Caatinga.

Considerando os resultados contrastantes dos estudos filogeográficos desenvolvidos com taxa da caatinga, é indispensável a continuidade destes com linhagens exclusivas desse bioma, de maneira a isolar a influência dos biomas adjacentes, como Amazônia e Mata Atlântica.

OBJETIVOS

2.1 Objetivo geral:

Utilizar diferentes metodologias para investigar a história evolutiva e as relações ancestrais entre as espécies de *Euphorbia* sect. *Brasilienses.*

2.2 Objetivos específicos:

a) Propor uma filogenia para a seção incluindo todas as espécies de forma a elucidar as relações entre elas.

b) Realizar uma revisão taxonômica da seção.

c) Inferir a história evolutiva, padrões de endemismo e de diversificação do grupo no bioma Caatinga.

d) Avaliar o padrão filogeográfico da espécie de ampla distribuição (*Euphorbia phosphorea*).

REFERÊNCIAS BIBLIOGRÁFICAS

AB'SABER, A.N. 1977. Os domínios morfoclimáticos na América do Sul. *Geomorfologia*1–22.

AVISE, J.C. 2009. Phylogeography: Retrospect and prospect. *Journal of Biogeography* 36: 3–

15.

AVISE, J.C. 2000. Phylogeography: The History and Formation of Species. Harvard University Press, England, London.

AVISE, J.C. 1998. The history and purview of phylogeography.pdf. *Molecular Ecology*371–379.

AVISE, J.C., J. ARNOLD, R.M. BALL, E. BERMINGHAM, T. LAMB, J.E. NEIGEL, C. A REEB, and N.C. SAUNDERS. 1987. Intraspecific Phylogeography: The Mitochondrial DNA Bridge Between Population Genetics and Systematics. *Annual Review of Ecology and Systematics* 18: 489–522.

BANDELT, H.J., P. FORSTER, and A. ROHL. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16: 37–48. Available at: https://academic.oup.com/mbe/article-lookup/doi/10.1093/oxfordjournals.molbev.a026036.

BARRES, L., M. GALBANY-CASALS, A.L. HIPP, J. MOLERO, and R. VILATERSANA. 2017. Phylogeography and character evolution of euphorbia sect. Aphyllis subsect. Macaronesicae (Euphorbiaceae). *Taxon* 66: 324–342.

BEATTY, G.E., J.J. LENNON, C.J. O'SULLIVAN, and J. PROVAN. 2015. The not-so-Irish spurge: Euphorbia hyberna (Euphorbiaceae) and the Littletonian plant 'steeplechase.' *Biological Journal of the Linnean Society* 114: 249–259. Available at: https://academic.oup.com/biolinnean/article-lookup/doi/10.1111/bij.12435.

BONATELLI, I.A.S., M.F. PEREZ, A.T. PETERSON, N.P. TAYLOR, D.C. ZAPPI, M.C. MACHADO, I. KOCH, ET AL. 2014. Interglacial microrefugia and diversification of a cactus species complex: Phylogeography and palaeodistributional reconstructions for Pilosocereus aurisetus and allies. *Molecular Ecology* 23: 3044–3063.

BRUYNS, P. V., C. KLAK, and P. HANÁČEK. 2011. Age and diversity in old world succulent species of Euphorbia (Euphorbiaceae). *Taxon* 60: 1717–1733.

BRUYNS, P. V., R.J. MAPAYA, and T. HEDDERSON. 2006. A new subgeneric classification for Euphorbia (Euphorbiaceae) in southern Africa based on ITS and psbA-trnH sequence data. *Taxon* 55: 397–420.

BULLOCK, S.H. 1995. Plant reproduction in neotropical dry forests. *In* S. H. Bullock, H. A. Mooney, and E. Medina [eds.], Seasonally Dry Tropical Forests, 277–303. Cambridge University Press, Cambridge. Available at: http://ebooks.cambridge.org/ref/id/CBO9780511753398.

CACHO, N.I., and D.A. BAUM. 2012. The Caribbean slipper spurge Euphorbia tithymaloides: the first example of a ring species in plants. *Proceedings of the Royal Society B: Biological Sciences* 279: 3377–3383. Available at:

http://rspb.royalsocietypublishing.org/cgi/doi/10.1098/rspb.2012.0498.

COLLEVATTI, R.G., L.C. TERRIBILE, G. DE OLIVEIRA, M.S. LIMA-RIBEIRO, J.C. NABOUT, T.F. RANGEL, and J.A.F. DINIZ-FILHO. 2013. Drawbacks to palaeodistribution modelling: The case of South American seasonally dry forests. *Journal of Biogeography* 40: 345–358.

DORSEY, B.L., T. HAEVERMANS, X. AUBRIOT, J.J. MORAWETZ, R. RIINA, V.W. STEINMANN, and P.E. BERRY. 2013. Phylogenetics, morphological evolution, and classification of. *Taxon* 62: 291–315. Available at: https://www.google.fr/%5Cnpapers3://publication/uuid/D1ACB99C-785B-4DEB-A2B4-204889BE5F5D.

DYER, R.J., J.D. NASON, and R.C. GARRICK. 2010. Landscape modelling of gene flow: Improved power using conditional genetic distance derived from the topology of population networks. *Molecular Ecology* 19: 3746–3759.

FRANCO, F.F., and M.H. MANFRIN. 2013. Recent demographic history of cactophilic Drosophila species can be related to Quaternary palaeoclimatic changes in South America. *Journal of Biogeography* 40: 142–154.

FU, Y.X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147: 915–25. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9335623.

GARRICK, R.C., J.D. NASON, C.A. MEADOWS, and R.J. DYER. 2009. Not just vicariance: phylogeography of a Sonoran Desert euphorb indicates a major role of range expansion along the Baja peninsula. *Molecular Ecology* 18: 1916–1931. Available at: http://doi.wiley.com/10.1111/j.1365-294X.2009.04148.x.

GEIST, H.J., and E.F. LAMBIN. 2002. Proximate Causes and Underlying Driving Forces of Tropical Deforestation. *BioScience* 52: 143.

GELTMAN, D. V. 2015. Phytogeographical analysis of euphorbia subgenus esula (euphorbiaceae). *Polish Botanical Journal* 60: 147–161.

GENTRY, A.H. 1995. Diversity and floristic composition of neotropical dry forests. *In* S. H. Bullock, H. A. Mooney, and E. Medina [eds.], Seasonally Dry Tropical Forests, 146–194. Cambridge University Press, Cambridge. Available at: http://ebooks.cambridge.org/ref/id/CBO9780511753398A045.

GIELLY, L., and P. TABERLET. 1994. The use of chloroplast DNA to resolve plant phylogenies: noncoding versus rbcL sequences. *Molecular biology and evolution* 11: 769–777.

HAEVERMANS, T., P. HOFFMANN, P.P. LOWRY II, J.-N. LABAT, and E. RANDRIANJOHANY. 2004. Phylogenetic Analysis of the Madagascan Euphorbia Subgenus Lacanthis Based on ITS Sequence Data. *Annals of the Missouri Botanical Garden* 91: 247–259.

HAFFER, J. 1969. Speciation in Amazonian Forest Birds. *Science* 165: 131–137. Available at: http://www.sciencemag.org/cgi/doi/10.1126/science.165.3889.131.

HARLEY, R.M. 1995. Introdução. *In* Flora of the Pico das Almas, Chapada Diamantina, Bahia, 877.

HORN, J.W., B.W. VAN EE, J.J. MORAWETZ, R. RIINA, V.W. STEINMANN, P.E. BERRY, and K.J. WURDACK. 2012. Phylogenetics and the evolution of major structural characters in the giant genus Euphorbia L. (Euphorbiaceae). *Molecular Phylogenetics and Evolution* 63: 305–326. Available at: http://dx.doi.org/10.1016/j.ympev.2011.12.022.

HORN, J.W., Z. XI, R. RIINA, J.A. PEIRSON, Y. YANG, B.L. DORSEY, P.E. BERRY, ET AL. 2014. Evolutionary bursts in Euphorbia (Euphorbiaceae) are linked with photosynthetic pathway. *Evolution* 68: 3485–3504.

JAZEN, D.H. 1988. Tropical Dry Forests. *In* E. O. Wilson, and M. P. Frances [eds.], Biodiversity, 130–137. National Academy Press, Washington, D.C.

KUHNER, M.K. 2009. Coalescent genealogy samplers: windows into population history. *Trends in Ecology & Evolution* 24: 86–93. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0169534708003480.

LEAL, B.S.S., C. PALMA DA SILVA, and F. PINHEIRO. 2016. Phylogeographic Studies Depict the Role of Space and Time Scales of Plant Speciation in a Highly Diverse Neotropical Region. *Critical Reviews in Plant Sciences* 35: 215–230. Available at: http://dx.doi.org/10.1080/07352689.2016.1254494.

LEAL, I.R., J.M.C. DA SILVA, M. TABARELLI, and T.E. LACHER. 2005. Changing the Course of Biodiversity Conservation in the Caatinga of Northeastern Brazil\rCambiando el Curso de la Conservación de Biodiversidad en la Caatinga del Noreste de Brasil. *Conservation Biology* 19: 701–706. Available at: http://dx.doi.org/10.1111/j.1523-1739.2005.00703.x.

MAYA-LASTRA, C.A., J.M. PORTER, and V.W. STEINMANN. 2017. Evolutionary History and Taxonomy of the Euphorbia polycarpa Complex (Euphorbia subg. Chamaesyce sect. Anisophyllum, Euphorbiaceae). *Systematic Botany* 42: 257–270.

MAYLE, F. 2006. The late quaternary biogeographical history of South American seasonally dry tropical forests: Insights from Palaeo-ecological data. *In* T. R. Pennington, G. Lewis, and J. Ratter [eds.], Neotropical Savannas and Seasonally Dry Forests: Plant Diversity, Biogeography, and Conservation, 395–416. CRC press, New York.

MILES, L., A.C. NEWTON, R.S. DEFRIES, C. RAVILIOUS, I. MAY, S. BLYTH, V. KAPOS, and J.E. GORDON. 2006. A global overview of the conservation status of tropical dry forests. *Journal of Biogeography* 33: 491–505.

MWINE, T.J., and P. VAN DAMME. 2011. Why do Euphorbiaceae tick as medicinal plants?: a review of Euphorbiaceae family and its medicinal features. *Journal of Medicinal Plants Research* 5: 652–662. Available at: https://biblio.ugent.be/publication/1849112/file/2095574.pdf.

OLIVEIRA-FILHO, A., J.A. JARENKOW, and M.J.N. RODAL. 2006. Floristic Relationships of Seasonally Dry Forests of Eastern South America Based on Tree Species Distribution Patterns. *In* 159–192. Available at: http://www.crcnetbase.com/doi/abs/10.1201/9781420004496.ch7.

OLIVEIRA, B., and C. EVANGELISTA-COIMBRA. 2014. Euphorbia Tirucalli: No Tratamento Complementar do Cancêr. *Revista UNINGÁ Review* 20: 60–64. Available at: http://www.mastereditora.com.br/periodico/20141130_221543.pdf.

PARK, K.-R., and R.K. JANSEN. 2007. A phylogeny of Euphorbieae subtribe Euphorbiinae (Euphorbiaceae) based on molecular data. *Journal of Plant Biology* 50: 644–649.

PEIRSON, J.A., P. V. BRUYNS, R. RIINA, J.J. MORAWETZ, and P.E. BERRY. 2013. A molecular phylogeny and classification of the largely succulent and mainly African Euphorbia subg. Athymalus (Euphorbiaceae). *Taxon* 62: 1178–1199.

PENNINGTON, R.T., M. LAVIN, and A. OLIVEIRA-FILHO. 2009. Woody Plant Diversity, Evolution, and Ecology in the Tropics: Perspectives from Seasonally Dry Tropical Forests. *Annual Review of Ecology, Evolution, and Systematics* 40: 437–457. Available at: http://www.annualreviews.org/doi/10.1146/annurev.ecolsys.110308.120327.

PENNINGTON, R.T., M. LAVIN, D.E. PRADO, C.A. PENDRY, S.K. PELL, and C.A. BUTTERWORTH. 2004. Historical climate change and speciation: neotropical seasonally dry forest plants show patterns of both Tertiary and Quaternary diversification. *Philosophical Transactions of the Royal Society B: Biological Sciences* 359: 515–538. Available at: http://rstb.royalsocietypublishing.org/cgi/doi/10.1098/rstb.2003.1435.

PENNINGTON, R.T., D.E. PRADO, and C.A. PENDRY. 2000. Neotropical seasonally dry forests and Quaternary vegetation changes. *Journal of Biogeography* 27: 261–273. Available at: http://dx.doi.org/10.1046/j.1365-2699.2000.00397.x.

PENNINGTON, R.T., J.E. RICHARDSON, and M. LAVIN. 2006. Insights into the historical construction of species-rich biomes from dated plant phylogenies, neutral ecological theory and phylogenetic community structure. *New Phytologist* 172: 605–616.

PRADO, D.E. 2003. As Caatingas da América do Sul. *Ecologia e conservacão da Caatinga*3–73.

PRADO, D.E. 2000. Seasonally Dry Forests of Tropical South America: from forgotten ecosystems to a new phytogeographic unit. *Edinburgh Journal of Botany* 57: 3 437-461. Available at:

https://www.cambridge.org/core/product/5B9E9D41F0AFAC97D5D867596D9FF573.

PRADO, D.E., and P.E. GIBBS. 1993. Patterns of Species Distributions in the Dry Seasonal Forests of South America. *Annals of the Missouri Botanical Garden* 80: 902–927. Available at: http://www.jstor.org/stable/2399937.

DE QUEIROZ, L.P., D. CARDOSO, M.F. FERNANDES, and M.F. MORO. 2017. Diversity and Evolution of Flowering Plants of the Caatinga Domain. *In* J. M. Cardoso da Silva, I. R. Leal, and M. Tabarelli [eds.], Caatinga, 23–63. Springer International Publishing, Cham. Available at: http://link.springer.com/10.1007/978-3-319-68339-3_2.

RATTER, J.A., J.F. RIBEIRO, and S. BRIDGEWATER. 1997. The Brazilian cerrado vegetation and threats to its biodiversity. *Annals of Botany* 80: 223–230.

RIZZINI, C. 1989. Cactiform species of Euphorbia from Brazil (Euphorbiaceae). *Revista Brasileira de Biologia* 4: 979–997.

SANTOS, O.J. DOS, E.N. SAUAIA FILHO, F.R.F. DO NASCIMENTO, F.C.S. JÚNIOR, E.M.S. FIALHO, R.H.P. SANTOS, R.A.P. SANTOS, ET AL. 2016. Use of raw Euphorbia tirucalli extract for inhibition of ascitic Ehrlich tumor. *Revista do Colégio Brasileiro de Cirurgiões* 43: 18–21. Available at: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-69912016000100018&lng=en&tlng=en.

SÄRKINEN, T., J.R.V. IGANCI, R. LINARES-PALOMINO, M.F. SIMON, and D.E. PRADO. 2011. Forgotten forests - issues and prospects in biome mapping using Seasonally Dry Tropical Forests as a case study. *BMC Ecology* 11: 27. 1-15.

SASLIS-LAGOUDAKIS, C., M.W. CHASE, D.N. ROBINSON, S.J. RUSSELL, and B.B. KLITGAARD. 2008. Phylogenetics of neotropical Platymiscium (Leguminosae: Dalbergieae): Systematics, divergence times, and biogeography inferred from nuclear ribosomal and plastid DNA sequence data. *American Journal of Botany* 95: 1270–1286.

SCHNEIDER, S., and L. EXCOFFIER. 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. *Genetics* 152: 1079–89. Available at: http://link.springer.com/10.1007/BF02293108.

STEINMANN, V.W., and J.M. PORTER. 2002. Phylogenetic Relationships in Euphorbieae (Euphorbiaceae) Based on ITS and ndhF Sequence Data. *Annals of the Missouri Botanical Garden* 89: 453. Available at: http://www.jstor.org/stable/3298591?origin=crossref.

TAJIMA, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123: 585–595.

THE BRAZIL FLORA GROUP. 2015. Growing knowledge: an overview of Seed Plant diversity in Brazil. *Rodriguésia* 66: 1085–1113. Available at:

http://www.scielo.br/scielo.php?script=sci_arttext&pid=S2175-78602015000401085&lng=en&tlng=en.

THOMÉ, M.T.C., and B.C. CARSTENS. 2016. Phylogeographic model selection leads to insight into the evolutionary history of four-eyed frogs. *Proceedings of the National Academy of Sciences* 113: 8010–8017. Available at: http://www.pnas.org/lookup/doi/10.1073/pnas.1601064113.

TURCHETTO-ZOLET, A.C., F. PINHEIRO, F. SALGUEIRO, and C. PALMA-SILVA. 2013. Phylogeographical patterns shed light on evolutionary process in South America. *Molecular Ecology* 22: 1193–1213.

VELLOSO, A.L., A.L. VELLOSO, A.M. GIULIETTI, D.C. OREN, E.E. DE MIRANDA, and S. KEEL. 2002. Ecorregioes propostas para o bioma Caatinga. *In* A. L. Velloso, E. V. S. B. Sampaio, and F. G. C. Pareyn [eds.], 76. Instituto de Conservacao Ambiental The Nature Conservancy do Brasil, Recife.

WEBSTER, G.L. 1994. Synopsis of the Genera and Suprageneric Taxa of Euphorbiaceae. *Annals of the Missouri Botanical Garden* 81: 33–144.

WERNECK, F.P. 2011. The diversification of eastern South American open vegetation biomes: Historical biogeography and perspectives. *Quaternary Science Reviews* 30: 1630–1648. Available at: http://dx.doi.org/10.1016/j.quascirev.2011.03.009.

WERNECK, F.P., G.C. COSTA, G.R. COLLI, D.E. PRADO, and J.W. SITES. 2011. Revisiting the historical distribution of Seasonally Dry Tropical Forests: New insights based on palaeodistribution modelling and palynological evidencegeb. *Global Ecology and Biogeography* 20: 272–288.

YANG, Y., C.W. MORDEN, M.J. SPORCK-KOEHLER, L. SACK, W.L. WAGNER, and P.E. BERRY. 2018. Repeated range expansion and niche shift in a volcanic hotspot archipelago: Radiation of C 4 Hawaiian Euphorbia subgenus Chamaesyce (Euphorbiaceae). *Ecology and Evolution*1–14. Available at: http://doi.wiley.com/10.1002/ece3.4354.

YANG, Y., R. RIINA, J.J. MORAWETZ, T. HAEVERMANS, X. AUBRIOT, and P.E. BERRY. 2012. Molecular phylogenetics and classification of Euphorbia subgenus Chamaesyce: a group with high diversity in photosynthetic systems and growth forms. *Taxon* 61: 764–789.

ZANELLA, F.C. V. 2000. The bees of the Caatinga (Hymenoptera, Apoidea, Apiformes): a species list and comparative notes regarding their distribution. *Apidologie* 31: 579–592.

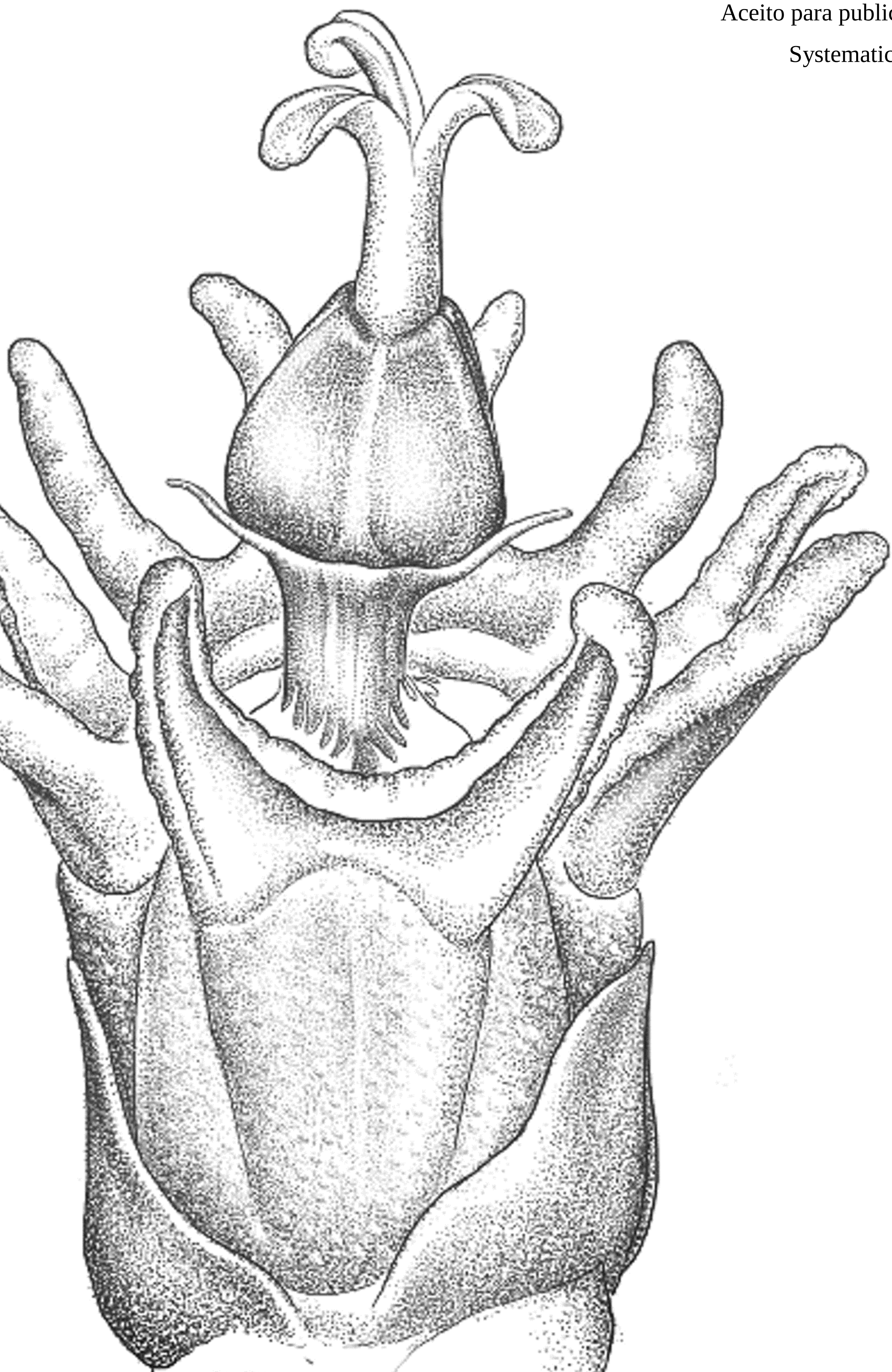
ZECCA, G., G. CASAZZA, L. MINUTO, M. LABRA, and F. GRASSI. 2011. Allopatric divergence and secondary contacts in Euphorbia spinosa L: Influence of climatic changes on the split of the species. *Organisms Diversity and Evolution*.

Capítulo 1

A new species and molecular phylogeny of Brazilian succulent *Euphorbia* sect. *Brasilienses*

Aceito para publicação no periódico

Systematics and Biodiversity



A new species and molecular phylogeny of Brazilian succulent *Euphorbia* sect. *Brasilienses*

FERNANDA HURBATH[1,4], BÁRBARA SIMÕES SANTOS LEAL[2], OTÁVIO LUIS MARQUES DA SILVA[1], CLARISSE PALMA-SILVA[2,3] & INÊS CORDEIRO[1]

[1] Núcleo de Pesquisa Curadoria do Herbário SP, Instituto de Botânica, Avenida Miguel Stéfano 3687, São Paulo, 04045-972, SP, Brazil. Phone: +55 11 50676086

[2] Laboratório de Ecologia Molecular, Departamento de Ecologia, Universidade Estadual Paulista – Rio Claro, Avenida 24A 1515, Rio Claro, 13506-900, SP, Brazil. Phone: +55 19 35269625

[3] Departamento de Biologia Vegetal, Universidade Estadual de Campinas, Cidade Universitária Zeferino Vaz - Barão Geraldo, Campinas, 13083-863, SP, Brazil.

[4] Correspondence to: Fernanda Hurbath. Email: fhurbath@gmail.com

This work was supported by the [Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP] under grant [2015/11812-1] and [Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq] under grant [309917/2015-8].

A new species of *Euphorbia* sect. *Brasilienses* V.W. Steinm. & Dorsey is described. *Euphorbia tetrangularis* Hurbath & Cordeiro is endemic to the Serra de Montevidéu, a part of the Espinhaço Range located in the state of Minas Gerais, Brazil. It differs from other species within the section based on the combination of following characters: 4-ribbed branches, green cyathia and green cyathyal glands with erect appendages. This new species would qualify as critically endangered (CR) according to IUCN criteria. An inferred phylogeny based on a combined data set of nuclear (ITS1) and plastid regions (*psbA-trnH, trnC-ycf6, matK, atpI-atpH, psbJ-petA, trnQ-rps16x1*) confirms the monophyly of *Euphorbia* sect. *Brasilienses* and supports the recognition of *E. tetrangularis*. The phylogeny also suggests that this group probably underwent a recent radiation.

Key words: *Campos Rupestres,* Espinhaço Range, *Euphorbia* subg. *Euphorbia, Inselbergs*, Rocky caatinga, Systematics.

Introduction

Euphorbia L. is the largest genus within Euphorbiaceae, and the fourth largest genus within angiosperms (Frodin, 2004). The genus is monophyletic and features a wide variety of growth forms, ranging from perennial to annual herbs, subshrubs and shrubs with often succulent stems or leaves, and trees (Bruyns, Mapaya, & Hedderson, 2006; Horn et al., 2012; Steinmann & Porter, 2002). In Brazil, there are 64 species of *Euphorbia*, and nearly 50% are endemic to the country (The Brazil Flora Group [BFG], 2015).

Four subgenera are currently recognized in *Euphorbia*: *E.* subg. *Athymalus* Neck. ex Rchb., *E.* subg. *Esula* Pers. ex Reich., *E.* subg. *Chamaesyce* Raf., and *E.* subg. *Euphorbia* (Dorsey et al., 2013; Peirson, Bruyns, Riina, Morawetz, & Berry, 2013; Riina et al., 2013; Yang et al., 2012). The subgenus *Euphorbia* is the largest and most diverse, including around 660 species occurring in various habitats (Dorsey et al., 2013), but especially semiarid regions. A vast number of them are succulent and xeromorphic species with organs modified into thorns (Carter, 1994). The phylogenetic study of Dorsey et al. (2013) recognizes four main clades for *Euphorbia* subg. *Euphorbia*: New World (tropical America), Old World I (Madagascar), Old World II (Africa, Arabia and Asia), and Pacific (South Pacific), and established 21 sections.

The New World clade consists of 11 sections, which comprises *Euphorbia* sect. *Brasilienses* V.W. Steinm. & Dorsey. This is a strongly supported monophyletic and exclusively Brazilian group, characterized by its succulent habit, early caducous leaves, ribbed stems, and cyathial glands with horn-shaped appendages (Dorsey et al., 2013). However, the relationship between the species has not been established since the different accessions of *E. attastoma* Rizzini and *E. phosphorea* Mart. emerged in separate clades. In addition, *E. holochlorina* Rizzini has not been sampled.

As part of an ongoing phylogeographic study and taxonomic revision, a new species endemic to Serra de Montevidéu (northern Minas Gerais) is here described. Relationships within the section are inferred based on a phylogenetic analysis with six plastid regions (*psbA-trnH*, *trnC-ycf6*, *matK*, *atpI-atpH*, *psbJ-petA*, *trnQ-rps16x1*) and the internal transcribed spacer ITS1. We also present a distribution map and illustrations for all species in sect. *Brasilienses* including the new taxon.

Materials and methods

Taxonomy

We examined collections of all species of *Euphorbia* sect. *Brasilienses* deposited in ALCB, BHCB, HRB, HRCB, HUEFS, R, RB, SP and SPF, as well as protologues and type collections. For the morphological description, we applied the terminology adopted in Beentje (2010), J. G. Harris and Harris (1994) and Radford (1986).

Phylogenetic analysis

Silica dried samples for DNA extraction were obtained from plants collected by the authors during field trips from 2014 to 2016. Sampling localities are represented on the map (Fig. 1). Vouchers were deposited at the herbarium of the Instituto de Botânica (SP), São Paulo, Brazil, and duplicates were sent to ALCB, BHCB, NMNH, RB and SPF.

For the phylogeny, we selected more than one sample per species. at different localities, with the exception of the two microendemic species *Euphorbia tetrangularis* and *E. sipolisii*, for which we included two samples of the same population, resulting in 16 accessions with 14 vouchers (voucher information in Table S1 – supplemental material). The outgroup includes two species from *Euphorbia* sect. *Stachydium* Boiss. (*E. comosa* Vell. and *E. heterodoxa* Müll. Arg.), which is the sister section of *E.* sect. *Brasilienses* according to the phylogeny of Dorsey et al. (2013). Trees were rooted with *E. potentilloides* Boiss. from *E.* subg. *Chamaesyce* Raf. (Yang et al., 2012).

DNA extraction was performed using epidermal tissue obtained from branches. This tissue then underwent a combination of five pre-washing steps using a sorbitol-buffer according to Tel-Zur, Abbo, Myslabodski, and Mizrahi (1999), and was prepared for analysis using the Dneasy Plant Mini Kit (Qiagen, California) following the manufacturer's protocol.

The chosen genetic regions are listed in Table S2 (see supplemental material online), along with the primers used for amplification. In the case of the entire ITS region, we calculated mean distances in PAUP* ver. 4.0b10 (Swofford, 2002) between sequences of *Euphorbia* sect. *Brasilienses* present in the dataset from Dorsey et al. (2013) but found very low divergence among them (less than 1%). This divergence was concentrated mainly in the first internal transcribed spacer ITS1, which we therefore decided to amplify instead of the entire region.

Amplifications were performed with a final volume of 30 µl, and final concentrations of: 1X buffer solution, 1.5-3 mM $MgCl_2$, 0.2-0.4 mM of dNTP (Promega), 0.33-0.5 µM of each primer,1U Taq polymerase (Biolase and Promega), DMSO 0.02%, 0.1-0.2 mM of BSA and ca. 5 ng of DNA. PCRs were performed using a Veriti 96-Well Thermal Cycler (Applied Biosystems) following the cycling conditions described in the original articles (Table S2), except for *psbA-trnH*, *trnC-ycf6* and ITS1. These were amplified as follows: 95°C for 4 min for initial denaturation; followed by 30 cycles at 95°C for denaturing during 35s, 45s for annealing (temperatures in Table S2 – supplemental material), 72°C for 1 min for extension; and a final 5 min extension at

72°C.

PCR products were examined in agarose gels (1%) by electrophoresis. All PCR products were sent to Macrogen (Seoul, South Korea) for purification and sequencing, using the same primers as for PCR, on a ABI3730XL DNA sequencer.

Consensus sequences were assembled using De Novo Assembly in the software Geneious 6.1.8. (Kearse et al., 2012), and checked base by base with the chromatogram. The alignment was done using MUSCLE (Edgar, 2004) also in Geneious, with posterior manual adjustment based on similarity criteria (Simmons, 2004).

Bayesian phylogenetic inferences were conducted using MrBayes v.3.2 (Ronquist et al., 2012). The analyses were performed over three different data sets: nuclear data (ITS1), plastid data (*psbA-trnH*, *trnC-ycf6*, *matK*, *trnQ-rps16x1, petA-psbJ*, *atpI-atpH*) and a combination of all regions (alignments for each region and for the combined plastid and nuclear matrix are available as supplemental material). The nucleotide substitution model for each individual alignment was selected based on the Bayesian Information Criterion (BIC) in jModelTest 2 (Darriba, Taboada, Doallo, & Posada, 2015; Guindon & Gascuel, 2003).

One run of four chains each (three heated, one cold) was performed for 10 million generations, starting from random trees with a default temperature of 0.2 and burn-in set to 25%. Trees were sampled every 1000 generations. The resulting probabilities and model parameters from all independent runs were visually inspected in Tracer (available at http://beast.bio.ed.ac.uk/Tracer), to verify run convergence and stationarity as indicated by ESS values. For clade support estimates we adopted 95-100% PP as high support and 85% -94% as moderate support.

Results

Taxonomic treatment

Euphorbia tetrangularis Hurbath & Cordeiro sp. nov. (Figs 2.1-2.6 and Figs 3.1-3.7)

TYPE: BRAZIL, Minas Gerais, Monte Azul, Serra Montevidéu, à leste da cidade, 15º08'36"S, 42º47'30"W, 23 Aug 2016, F. Hurbath, & I. Cordeiro 844 (holotype: SP!; isotypes: ALCB!, BHCB!, NMNH!, RB!, SPF!)

ETYMOLOGY: The specific epithet reflects the species four-ribbed branches, one of the main characteristics of this species (Fig. 3.2).

DIAGNOSIS*: Euphorbia tetrangularis* differs from other species of *Euphorbia* sect. *Brasilienses* based on the combination of 4-ribbed branches, a green cyathium and dark-green to yellowish cyathial glands with erect appendages (Figs. 2.1-2.3 and Figs. 3.4-3.6).

Description

Perennial, candelabriform shrub, 0.5-1.6 m high, latex abundant; branched from the base, older individuals developing a small trunk, branches erect, c. 0.9 cm diam, 4-ribbed, with prominent ribs, succulent, green, glabrous, wax abundant (easily removed in fragments when dried), older stems lignified close to the base with ribs becoming less prominent, almost cylindrical. Leaves early caducous, entire, sessile, 6.7-11 x 2.2-3 mm, conduplicate, lanceolate, fleshy, base truncate, margin entire, apex acute, venation inconspicuous, green to vinaceous, glabrous; stipules early caducous, not seen. Cyathia arranged in axillary fascicles, 1-5-cyathia. Cyathophylls c. 2-3 x 1.2-2.5 mm, widely-ovate to depressed ovate, base truncate, with colleters on inner face, margin entire, apex acute, vinaceous, fleshy, glabrous; involucre c. 4-5 x 3.5-4 mm, campanulate, green, glabrous; lobes 5, c. 1-1.5 x 1.5 mm, flabellate, apex lacerate, membranaceous, glabrous; glands 4-5, 1-1.5 x 1.5-2.5 mm, transversely elliptic, dark-green to yellowish, slightly verrucose, glabrous, with appendages bifurcate, 2.7-3 mm long, erect, green to yellowish, verrucose, glabrous. Staminate flowers in 5 cymules of 4-7 flowers, pedicels c. 1-3 mm long, filaments c. 1-1.5 mm long, anthers dorsifixed. Pistillate flowers with pedicels c. 1.3-7 mm long; perianth-like whorl with 3 filiform caducous lobes (Figs. 2.1-2.2 and Figs. 3.4 -3.5); ovary 1.5-3.5 x 1.3-3 mm, ovoid, carpels mostly green and often with a vinaceous strip on the septum between cocci and, in the middle of them, fleshy, glabrous; styles 3, 1.3-2.5 mm long, connate up to the middle, glabrous. Fruits c. 5-7 x 4-7 mm, oblate, mostly green and frequently with vinaceous strips on septum between cocci and along the keel, coriaceous, glabrous; columella 3-5 mm. Seeds 3-3.2 x 2.5-2.9 mm, widely ovoid, brown, coriaceous, testa smooth, glabrous; caruncle discoid.

ADDITIONAL MATERIAL EXAMINED: BRAZIL. Minas Gerais, Monte Azul, Serra Montevidéu, à leste da cidade, 15º09'7.44"S, 42º47'44.04"W, 12 Apr 2016, *F. Hurbath, O.L.M. Silva & I. Cordeiro 839* (SP!). Monte Azul. 7 km E of Monte Azul towards and beyond Vila Angical on road, then 3h on foot ascending the Serra above Vila Angical on path to the village of “Gerais”, at edge of sandstone cliff in open *campo rupestre* scrub on white to reddish hard sandstone. 1100 m, 28 Jan 1991, *N. Taylor, D. Zappi & U. Eggli 1472* (BHCB!, HRCB!, K).

Distribution and Habitat

Euphobria tetrangularis is endemic to rock outcrops of Serra de Montevidéu, in the Municipality of Monte Azul, northern Minas Gerais, Brazil (Fig. 1). Serra Montevidéu is part of the Espinhaço Septentrional, which is considered to be a transitional area between Cerrado and Caatinga domains. The climate is semi-arid, and the vegetation can be recognized as rocky caatinga (Campos, Guedes, Acevedo-Rodríguez, & Roque, 2016) or as *caatinga de altitude* - highland caatinga (Zappi & Taylor, 2008). *Euphorbia tetrangularis* is found in association with *Pilosocereus pachycladus* F. Ritter, *Micranthocereus albicephalus* (Buining & Brederoo) F. Ritter, *Wunderlichia mirabilis* Riedel ex. Baker and *Anteremanthus piranii* Roque & F.A. Santana, species which characterize the rocky caatinga according to Campos et al. (2016).

Despite the differences regarding geographic distribution, species of the section *Brasilienses* share similar habitats and life forms (Table 1). *Euphorbia phosphorea* is found throughout the Caatinga domain in north-eastern Brazil. *Euphorbia sipolisii* and *E. attastoma* are mostly found in *campos rupestres* within the Cerrado domain in the State of Minas Gerais, but *E. attastoma* also extends to southern Bahia, where it grows in rocky caatinga. *Euphorbia holochlorina* is restricted to rocky mountains (*Inselbergs*) within the Atlantic Forest domain, in the States of Minas Gerais e Espírito Santo, while *E. attastoma* var. *xanthochlora* is the only one found nearby Monte Azul, the type locality of *E. tetrangularis*.

Table 1**.** Information of species of *Euphorbia* sect. *Brasilienses* distribution, habitat and life form.

Species	Distribution	Habitat	Life form
E. phosphorea Mart.	The most widespread (northeastern Brazil – Caatinga Biome)	From 223 to 1148 m altitude. Caatinga vegetation, quartzitic *inselbergs*, sandy soils and flat slabs of rock (Figs S1-S5).	From candelabriform, up to 2 m high to a very robust and dense shrub up to 5 m, frequently leaning often supported by adjacent plants, with decumbent branches, resembling climbers or lianas (Figs S4-S5).
E. attastoma var. *attastoma* Rizzini and *E. attastoma* var. *xanthochlora* Rizzini	From southwestern Bahia to central-northern Minas Gerais	From 669 to 1020 m altitude. Rocky Caatinga and Campos rupestres vegetation (Fig. S6-S7)	Candelabriform shrub or densely caespitose shrub with decumbent branches, up to 2m (Fig. S8)
E. sipolisii N. E. Br.	Endemic to the region of Diamantina in the State of Minas Gerais.	From 900 to 1200 m altitude. Campos rupestres vegetation (Fig. S7)	Erect shrub, up to 1m
E. holochlorina Rizzini	Northeastern Minas Gerais and northern Espirito Santo.	From 157 to 372 m altitude. Endemic to granitic inselbergs in the Atlantic Forest (Fig. S9-S10)	Erect shrub, up to 1.5 m high, sometimes with decumbent branches.
E. tetrangularis Hurbath & Cordeiro	Endemic to Monte Azul in northern Minas Gerais	Over 1000 m altitude. Rupestrian Caatinga vegetation (Fig. S11).	Candelabriform erect shrub, up to 1.6 m

Conservation status

According to the International Union for Conservation of Nature (IUCN, 2001) criteria, *Euphorbia tetrangularis* is evaluated as Critically Endangered (CR) (B1b [i and ii]). This species is found only in Serra Montevidéu, which covers an area of less than 100 km^2, unprotected by law (roads are used as motorcycle tracks and the site is surrounded by mineral exploration occurring in neighbouring municipalities). Taylor and Zappi (2004) already deemed the area as a priority for conservation due to the occurrence of many endangered species of Cactaceae.

Identification Key for the species of *Euphorbia* sect. *Brasilienses*

1. Four-ribbed branches.
    2. Cyathium and glands red, with patent appendages; endemic to Diamantina (central region of Minas Gerais)..***Euphorbia sipolisii***
    2’ Cyathium and glands green, with erect appendages; endemic to Monte Azul (northern Minas Gerais).. ***Euphorbia tetrangularis***

1’. Six to eight-ribbed branches.
    3. Branches bright green; fascicles with one or two cyathia, involucre urceolate; appendages erect, north-eastern Minas Gerais and northern Espírito Santo .... ***Euphorbia holochlorina***
    3’. Branches dark green or yellowish and greyish; fascicles with more than 2 cyathia; involucre campanulate; appendages patent.
        4. Branches 6 to 8-ribbed; fascicles usually with more than 5 cyathia; north-eastern Brasil.. ***Euphorbia phosphorea***
        4’. Branches 6-ribbed; fascicles usually with 2-4 cyathium, northern State of Minas Gerais and southwestern Bahia.
            5. Cyathium red .... ***E. attastoma* var. *attastoma***
            5’. Cyathium green ....***E. attastoma* var. *xanthochlora***

Molecular phylogeny

The data matrix combining *psbA-trnH*, *trnC-ycf6*, *matK*, *trnQ-rps16x1*, *atpI-atpH*, *psbJ-petA* and ITS1 sequences included 3,689 base pairs (length for each region, informative sites and missing data are listed in Table S2). All sequences used in the analyses were newly generated, and GenBank accessions are given in Table S1.

In all (plastid, nuclear and concatenated) trees, *Euphorbia* sect. *Brasilienses* emerged as monophyletic with high support (PP = 99-1) (Figs. 4, 5). The ITS1 tree (Fig. 5) exhibited very poor resolution but *E. attastoma* var. *attastoma* from Grão Mogol (MG) arose nonetheless as a sister group to the remaining accessions, which were recovered in a polytomy. On the other hand, in the plastid tree (Fig. 5), two main clades were recovered with low support: one with 63 PP, contains all accessions of *E. phosphorea* in a clade with 55 PP, which is sister to the accessions of *E. holochlorina* from Teófilo Otoni (MG). The other main clade, with 55 PP, contains the samples of *E. attastoma*, *E. sipolisii* and *E. tetrangularis*, each species forming distinct clades with high support (1 PP), but the three species formed a polytomy with the accession of *E. holochlorina* from Águia Branca (ES). The accessions of *E. holochlorina* did not form a clade (the one from Teófilo Otoni (MG) arose as sister to *E. phosphorea* clade), while the ones from Águia Branca (ES) emerged in a polytomy with the remaining species. The samples of *E. attastoma* var. *attastoma* did not group together: the one from Grão Mogol (MG) was recovered as most closely related to the other variety (*E. attastoma* var. *xanthochlora*), instead of being sister to the other accession of *E. attastoma* var. *attastoma* from Joaquim Felício (MG).

In the concatenated tree, accessions of each species emerged as distinct clades, mostly with high support, confirming species identities through molecular data. The *E. phosphorea* clade had high support (PP=91), and accessions from Bahia were recovered as subsequent sisters to northernmost accessions from Pernambuco and Paraíba, concurring with the plastid tree. The *E. attastoma* clade was also highly supported (PP=1), and the two samples of *E. attastoma* var. *attastoma* arose -in contrast to the plastid tree- as a clade sister to *E. attastoma* var. *xanthochlora*, however with low support (PP=57). *Euphorbia holochlorina* was the only species recovered with low support (PP=53). *Euphorbia sipolisii* and *E. tetrangularis* were recovered in highly supported clades (PP=1), even though accessions were from individuals considered to be part of the same population.

Discussion

Euphorbia sect. *Brasilienses* emerged as a monophyletic group supported by morphological and molecular data, which corroborates results of Dorsey et al. (2013). However, even with an increased sampling effort and additional plastid markers, we could not establish a clear relationship among its species. Species clades emerged in a polytomy, due to the limited amount of sequence divergence present in the very short branch lengths of the tree. The low resolution pattern we found is similar to what Dorsey et al. (2013) also observed for this group. This could indicate recent speciation or incomplete lineage separation resulting from little accumulated genetic differences (Stephens et al., 2015; Sun, A. Wang, D. Wang, Wang, & Liu, 2012; Whitfield & Lockhart, 2007). Therefore, adding more molecular data in attempt to achieve better backbone resolution might not help (Whitfield & Lockhart, 2007).

Incongruence between plastid and nuclear markers could be explained by many factors, such as differential or incomplete lineage sorting or recent hybridization (Som, 2015), and by differences in seed and pollen dispersal. The low variability within ITS1 among the studied species compared to plastid sequences could suggest that pollen is more efficiently dispersed than seeds, a phenomenon reported for species of orchids and bromeliads from Brazilian rock outcrops (Palma-Silva et al., 2011; Pinheiro et al., 2011; Pinheiro et al., 2014).

Evans et al. (2014) pointed out that, for *Euphorbia* from Madagascar, similar environmental characteristics of the habitats are better related to niche conservatism instead of niche lability. Therefore, the presence of *E.* sect. *Brasilienses* in three different biomes would accordingly also be interpreted as a result of niche conservatism. This is supported by some traits characterizing all species in this section: growing under high light incidence, wind exposure and limited water availability; and sharing remarkable xeromorphic characteristics such as succulent and photosynthetic branches, a thick layer of epicuticular wax and early caducous succulent leaves (Figs. S12-S15 – supplemental material online). *Euphorbia* sect. *Brasilienses* is indeed endemic to north-eastern Brazil’s Seasonally Dry Tropical Forests (SDTF), according to the delimitation proposed by Särkinen, Iganci, Linares-Palomino, Simon, and Prado (2011). STDFs are fire-intolerant and succulent environments (Pennington, Lewis, & Ratter, 2006) characterized by low annual rainfall and a dry season of 5-6 months (Gentry, 1995).

Euphorbia tetrangularis occurs at less than 100 km from *E. attastoma* var. *xanthochlora,* with which it shares green cyathia, even though *E. holochlorina* is morphologically the most related species because of its combination of green cyathia with erect gland appendages. The 4-ribbed branches are shared only with *E. sipolisii*, but both *E. holochlorina* and *E. sipolisii* occur further away from *E. tetrangularis* than other species of the section, so the spatial distribution and distances do not fully correlate with morphological similarities between these species.

Even though the concatenated tree gives little information regarding relationships among species, in the plastid tree, *E. attastoma*, *E. holochlorina* (the sample from Águia Branca, ES), *E. sipolisii* and *E. tetrangularis* are recovered as a clade, distinguishable from *E. phosphorea.* The latter is characterized by its 8-ribbed branches, which is a putative autapomorphy of this species. Despite this unique trait, internal nodes inside the *E. phosphorea* clade arose with moderate to high support, possibly indicating the existence of cryptic species. This view is also supported by discrete differences in habit and in the morphology of cyathia observed among its populations. However, with our data we could not accurately correlate these differences with the accessions used in the phylogenetic analysis.

The varieties of *Euphorbia attastoma* emerged separated in the concatenated tree, although with low support (57 PP). Characteristics pointed out by Rizzini (1989) for *E. attastoma* var. *xanthochlora* (green cyathial glands and decumbent branches vs. red cyathial glands and erect branches for the typical variety) are not entirely coinciding with the sample from Serra Nova, which presents erect branches and green cyathia. Also, the plastid tree does not corroborate the recognition of the two varieties, since the sample of *E. attastoma* var. *attastoma* from Grão Mogol (MG) is recovered as most closely related to the sample of *E. attastoma* var. *xanthochlora* rather than as a sister taxon to the other accession of *E. attastoma* var. *attastoma* from Joaquim Felício (MG). Due to the low phylogenetic and morphological support, taxonomic decisions regarding these varieties need further evaluation.

Euphorbia holochlorina shares its characteristic 6-ribbed branches with *E. attastoma* and its green cyathia with erect gland appendages with *E. tetrangulari* and. is recovered as monophyletic in the concatenated tree, although with low support (53 PP). While the ITS1 topology is not informative, one of the accessions in the chloroplast dataset (from Águia Branca, ES) is recovered in a polytomy with *E. attastoma*, *E. sipolisii* and *E. tetrangularis*, while the other accession is recovered as a sister to the *E. phosphorea* clade, with which *E. holochlorina* does not share any diagnostic characteristics. Based on the chloroplast topology and shared morphological characteristics, we infer that *E. holochlorina* is more closely related to *E. attastoma* and *E. tetrangularis*. Previous studies showed how *inselbergs* can act as islands in terms of community geographical isolation, restricting gene flow (Barbará, Martinelli, Fay, Mayo, & Lexer, 2007; Collevatti, Rabelo, & Vieira, 2009; Pinheiro et al., 2014). The populations of the other species of the section also occur relatively isolated and emerged as very well supported clades. Therefore, the Atlantic Forest that surrounds the inselberg habitats of *E. holochlorina* may provide a more effective barrier to pollen and seed dispersion compared to open cerrado and caatinga vegetation.

Even though our results could not recover ancestral relationships among species, they highlighted the recent origin and ongoing diversification of this group. It was furthermore possible to distinguish species clades and assign morphological characteristics to recognize *Euphorbia* sect. *Brasilienses* species. *Euphorbia tetrangularis*, here described for the first time, is already evaluated as Critically Endangered (CR), which reinforces the importance of effective actions towards species conservation in the Espinhaço Septentrional, a neglected region already pointed as a priority target for implementing a conservation unit.

Acknowledgments

We are very thankful to Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP 2015/11812-1) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 309917/2015-8) for financial support, Alessandro Santana for help with fieldwork, Natanael dos Santos for the illustration and to the reviewers who helped to improve this manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

Supplemental data

Supplemental data for this article can be accessed here: https://doi.org/10.1080/14772000.2018.1473897

References

Barbará, T., Martinelli, G., Fay, M. F., Mayo, S. J., & Lexer, C. (2007): Population differentiation and species cohesion in two closely related plants adapted to neotropical high-altitude ‘inselbergs’, *Alcantarea imperialis* and *Alcantarea geniculate* (Bromeliaceae). *Molecular Ecology*, *16*, 1981–1992.

Beentje, H. (2010). *The kew plant glossary: an illustrated dictionary of plant terms*. Kew: Royal Botanical Gardens.

Bruyns, P. V., Mapaya, R. J., & Hedderson, T. (2006). A new subgeneric classification for *Euphorbia* (Euphorbiaceae) in Southern Africa based on ITS and *psb*A-*trn*H sequence data. *Taxon*, *55*, 397–420.

Campos, L., Guedes, M. L. S., Acevedo-Rodriguez, P., & Roque, N. (2016). Contributions to the floristic and vegetation knowledge of Espinhaço Septentrional, Bahia, Brazil. *Brazilian Journal of Botany*, *40*, 427–437.

Carter, S. A. (1994). Preliminary classification of *Euphorbia* subgenus *Euphorbia*. *Annals of the Missouri Botanical Garden*, *89*, 368–379.

Collevatti, R. G., Rabelo, S. G., &Vieira, R. F. (2009). Phylogeography and disjunct distribution in Lychnophora ericoides (Asteraceae), an endangered cerrado shrub species. *Annals of Botany*, *104*, 655–664.

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2015). Europe PMC Funders Group jModelTest 2 : more models , new heuristics and high- performance computing. *Nature Methods*, *9*(8), 6–9.

Dorsey, B. L., Haevermans, T., Aubriot, X., Morawetz, J. J., Riina, R., Steinmann, V. W., & Berry, P. E. (2013). Phylogenetics, morphological evolution, and classification of *Euphorbia* subgenus *Euphorbia*. *Taxon*, *62*, 291–315.

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*, 1792–1797.

Evans, M., Aubriot, X., Hearn, D., Lanciaux, M., Lavergne, S., Cruaud, C., … Haevermans, T. (2014). Insights on the evolution of plant succulence from a remarkable radiation in Madagascar (*Euphorbia*). *Systematics Biology*, *63*, 698–711.

Frodin, D. G. (2004). History and concepts of big plant genera. *Taxon*, *53*, 753–776.

Gentry, A. H. (1995). Diversity and floristic composition of neotropical dry forests. In S. H. Bullock, H. A. Mooney, & E. Medina (Eds.), *Seasonally Dry Tropical Forests* (pp. 146–194). Cambridge: Cambridge University Press.

Guindon, S., & Gascuel, O. (2003). A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood. *Systematic Biology*, *52*(5), 696–704.

Harris, J. G. & Harris, M. W. (1994). *Plant identification terminology: an illustrated glossary* (2nd ed.). Utah: Spring Lake Publishing.

Horn, J. W., Van Ee, B. W., Morawetz, J. J., Riina, R., Steinmann, V. W., Berry, P. E., & Wurdack, K. J. (2012). Phylogenetics and the evolution of major structural characters in the giant genus *Euphorbia* L. (Euphorbiaceae). *Molecular Phylogenetics and Evolution*, *63*, 305–326.

International Union for Conservation of Nature [IUCN]. (2001). *IUCN red list categories and criteria: version 3.1*. Switzerland: IUCN Species Survival Commission.

Kearse, M., R. Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*, 1647–1649.

Palma-Silva, C., Wendt, T., Pinheiro, F., Barbará, T., Fay, M. F., Cozzolino, S., & Lexer, C. (2011). Sympatric bromeliad species (*Pitcairnia* spp.) facilitate tests of mechanisms involved in species cohesion and reproductive isolation in Neotropical inselbergs. *Molecular Ecology*, *20*, 3185–3201.

Peirson, J.A., Bruyns, P.V., Riina, R., Morawetz, J.J., & Berry, P.E. (2013). A molecular phylogeny and classification of the largely succulent and mainly African *Euphorbia* subg. Athymalus (Euphorbiaceae). *Taxon, 62,* 1178–1199.

Pennington, R., Lewis, G., & Ratter, J. (2006). An Overview of the Plant Diversity, Biogeography and Conservation of Neotropical Savannas and Seasonally Dry Forests. In Pennington, R., Lewis, G., & Ratter, J. (Eds.) *Neotropical Savannas and Seasonally Dry Forests: Plant diversity, biogeography, and conservation* (Vol. 69, pp. 1–29). Boca Raton, FL: CRC press

Pinheiro, F., Barros, F., Palma-Silva, C., Fay, M. F., Lexer, C., & Cozzolino, S. (2011). Phylogeography and genetic differentiation along the distributional range of orchid Epidendrum fulgens: a neotropical coastal species not restricted to glacial refugia. *Journal of Biogeography*, *38*, 1923–1935.

Pinheiro, F., Cozzolino, S., Munt, D. D., Barros, F., Félix, L. P., Fay, M., & Palma-Silva, C. (2014). Rock outcrop orchids reveal the genetic connectivity and diversity of inselbergs of north-eastern Brazil. *BMC Evolutionary Biology*, *49,* 1–15.

Radford, A. E. (1986). *Fundamentals of plant systematics*. New York: Harper & Row.

Riina, R., Peirson, J.A., Geltman, D.V., Molero, J., Frajman, B., Pahlevani, A., … Berry, P.E. (2013). A worldwide molecular phylogeny and classification of the leafy spurges, *Euphorbia* subgenus Esula (Euphorbiaceae). *Taxon, 62,* 316–342.

Rizzini, C. T. (1989). Cactiform species of E*uphorbia* from Brazil (Euphorbiaceae). *Revista Brasileira de Biologia*, *49*, 979–997.

Ronquist, F., Teslenko, M., Van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Huelsenbeck, J. P. (2012). Mrbayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. *Systematics Biology*, *61*, 539–542.

Särkinen, T., Iganci, J. R. V., Linares-Palomino, R., Simon, M. F., & Prado, D. E. (2011). Forgotten forests - issues and prospects in biome mapping using Seasonally Dry Tropical Forests as a case study. *BMC Ecology*, *11*, 1–15.

Simmons, M.P. (2004). Independence of alignment and tree search. *Molecular Phylogenetics and Evolution*, *31*, 874–879.

Som, A. (2015). Causes, consequences and solutions of phylogenetic incongruence. *Briefings in Bioinformatics*, *16* (3), 536–548.

Steinmann, V.W., & Porter, J.M. (2002). Phylogenetic relationships in Euphorbieae (Ephorbiaceae) based on ITS and ndhF sequence data. *Annals of the Missouri Botanical Garden, 89,* 453–490.

Stephens, J. D., Rogers, W. L., Heyduk, K., Cruse-Sanders, J. M., Determann, R. O., Glenn, T. C., & Malmberg, R. L. (2015) Resolving phylogenetic relationships of the recently radiated carnivorous plant genus *Sarracenia* using target enrichment. *Molecular Phylogenetics and Evolution*, *85*, 76–87.

Sun, Y., Wang, A., Wang, D., Wang, Q., & Liu. J. (2012). Rapid radiation of *Rheum* (Polygonaceae) and parallel evolution of morphological traits. *Molecular phylogenetics and Evolution*., *63*(1), 150–158.

Swofford, D.L. (2002). *PAUP*: phylogenetics analysis using parsimony (*and other methods), version 4.0b10.* Suderland: Sinauer Associates Inc.

Taylor, N., & Zappi, D. C. (2004). *Cacti of Eastern Brazil*. Kew: Royal Botanical Gardens.

Tel-Zur, N., Abbo, S., Myslabodski, D., & Mizrahi, Y. (1999). Modified CTAB procedure for DNA isolation from epiphytic cacti of the genera *Hylocereus* and *Selenicereus* (Cactaceae). *Plant Molecular Biology Reporter*, *17*, 249–254.

The Brazil Flora Group [BFG]. (2015). Growing knowledge: an overview of seed plant diversity in Brazil. *Rodriguésia*, *66*, 1085–1113.

Whitfield, J. B., & Lockhart, P. J. (2007). Deciphering ancient rapid radiations. *Trends in Ecology and Evolution*, *22*, 258–265.

Yang, T., Riina, R., Morawetz, J.J., Haevermans, T., Aubriot, X., & Berry, P.E. (2012). Molecular phylogenetics and classification of *Euphorbia* subgenus Chamaesyce (Euphorbiaceae). *Taxon*, *61*, 764–789

Zappi, D. C., & Taylor, N. (2008). Diversidade e endemismo das Cactaceae na Cadeia do Espinhaço. *Megadiversidade*, *4*, 111–116.

Figures and Table legends

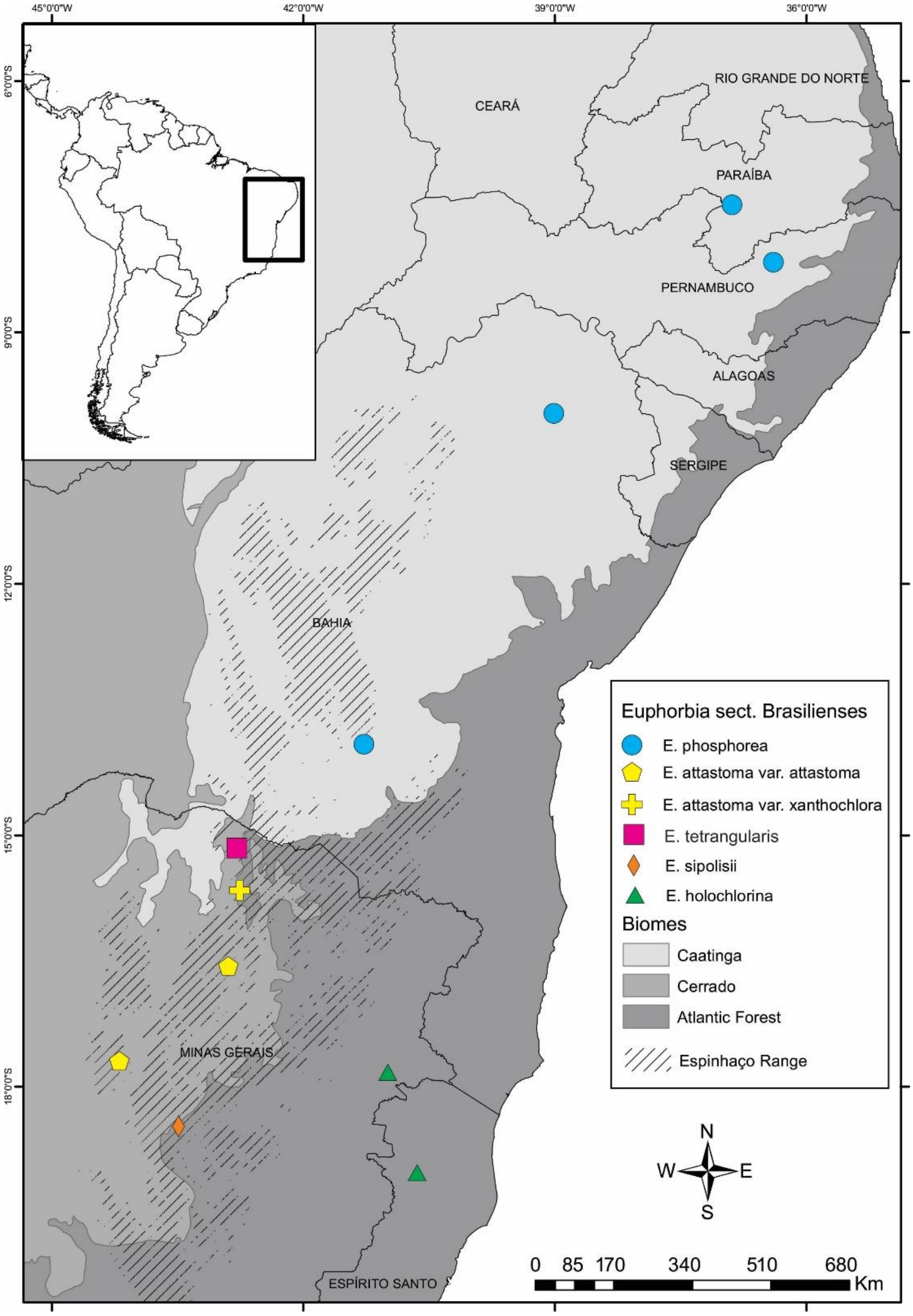


Fig. 1. Map showing the sampling localities of *Euphorbia* sect. *Brasilienses* used in the phylogeny. From north to south respectively: Pedra Lavrada-Paraíba; Brejo da Madre de Deus- Pernambuco; Canudos – Bahia; Tanhaçu – Bahia; Monte Azul – Minas Gerais; Serra Nova – Minas Gerais; Grão Mogol-Minas Gerais; Joaquim Felício – Minas Gerais; Teófilo Otoni-Minas Gerais; Diamantina/Serro- Minas Gerais; Águia Branca – Espírito Santo.

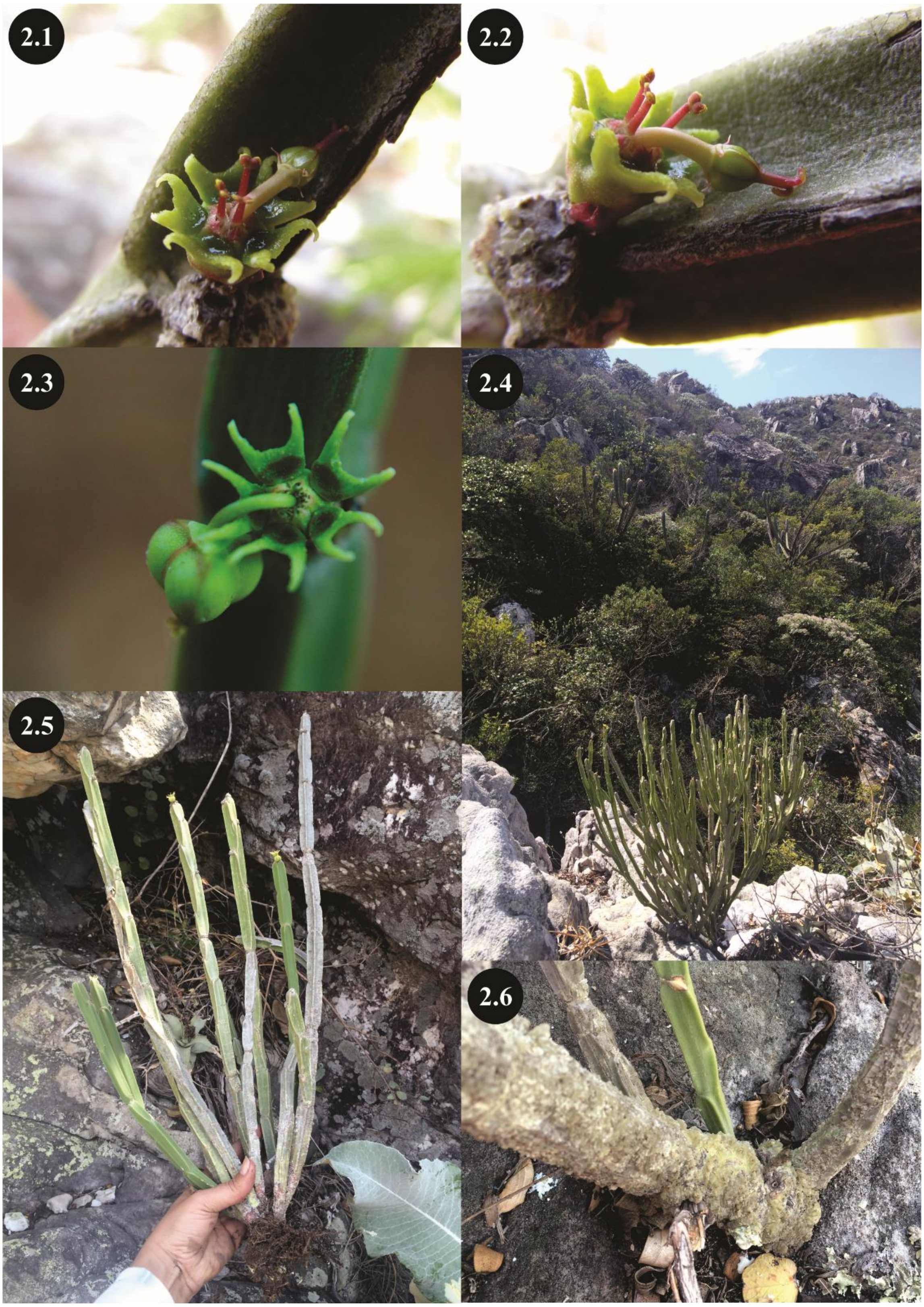


Fig 2. *Euphorbia tetrangularis.* (**2.1-2.6**). (**2.1**) Cyathium; (**2.2**) Cyathium with young fruit; (**2.3**) Cyathium with mature fruit; (**2.4**) Habitat of rocky caatinga; (**2.5**) Habit; (**2.6**) Base from an old individual showing the loss of ribs and a high accumulation of wax.

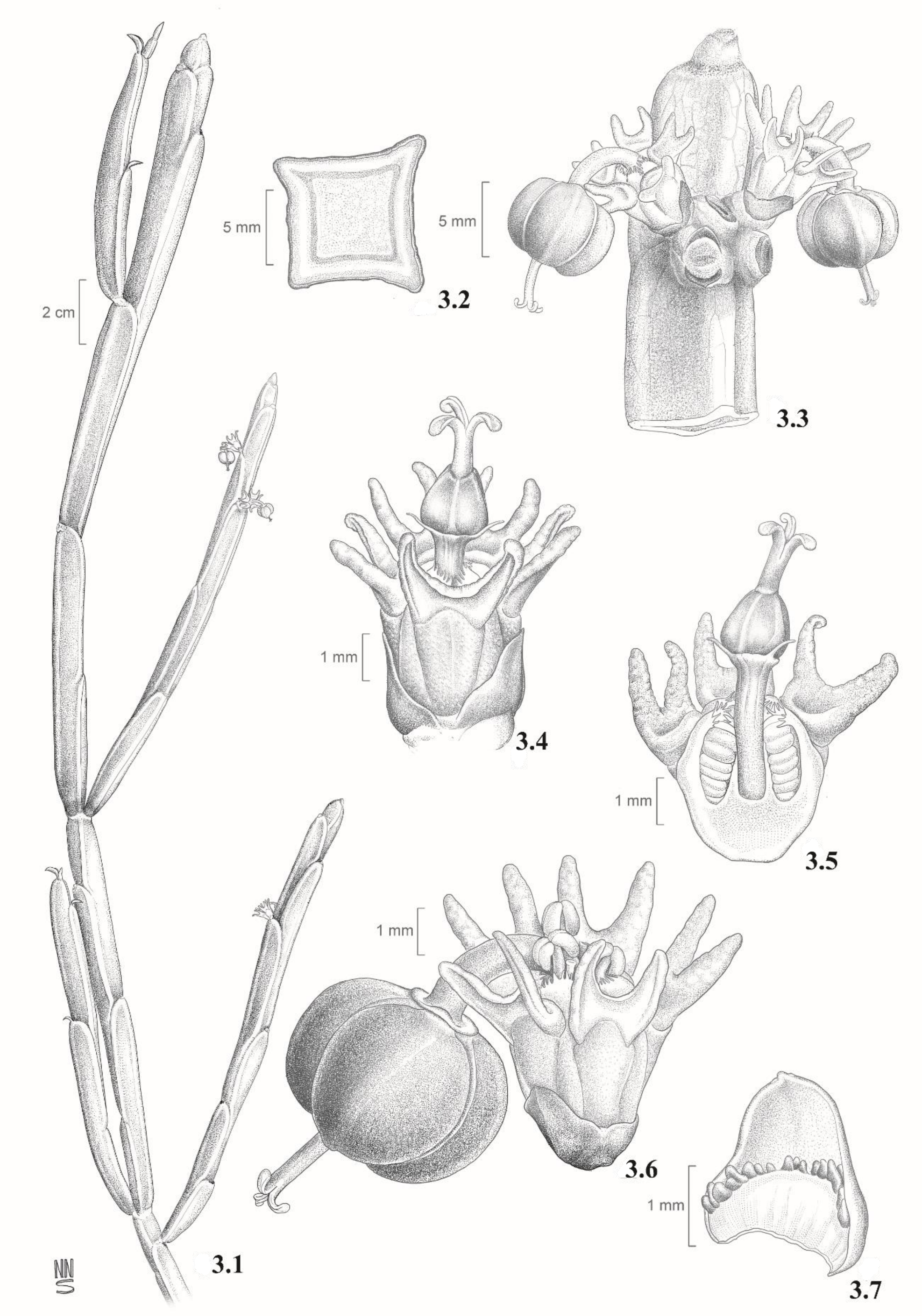


Fig 3. Illustration of *Euphorbia tetrangularis* Hurbath & Cordeiro (**3.1**-**3.7**). (**3.1**) Branches in reproductive state; (**3.2**) Cross section of a branch showing the four sides; (**3.3**) Fascicle of cyathia; (**3.4**) Cyathium with erect appendages; (**3.5**) Cyathium in longitudinal section; (**3.6**) Cyathium with fruit and male flowers exposed; (**3.7**) Cyathophyll with colleters on inner face.

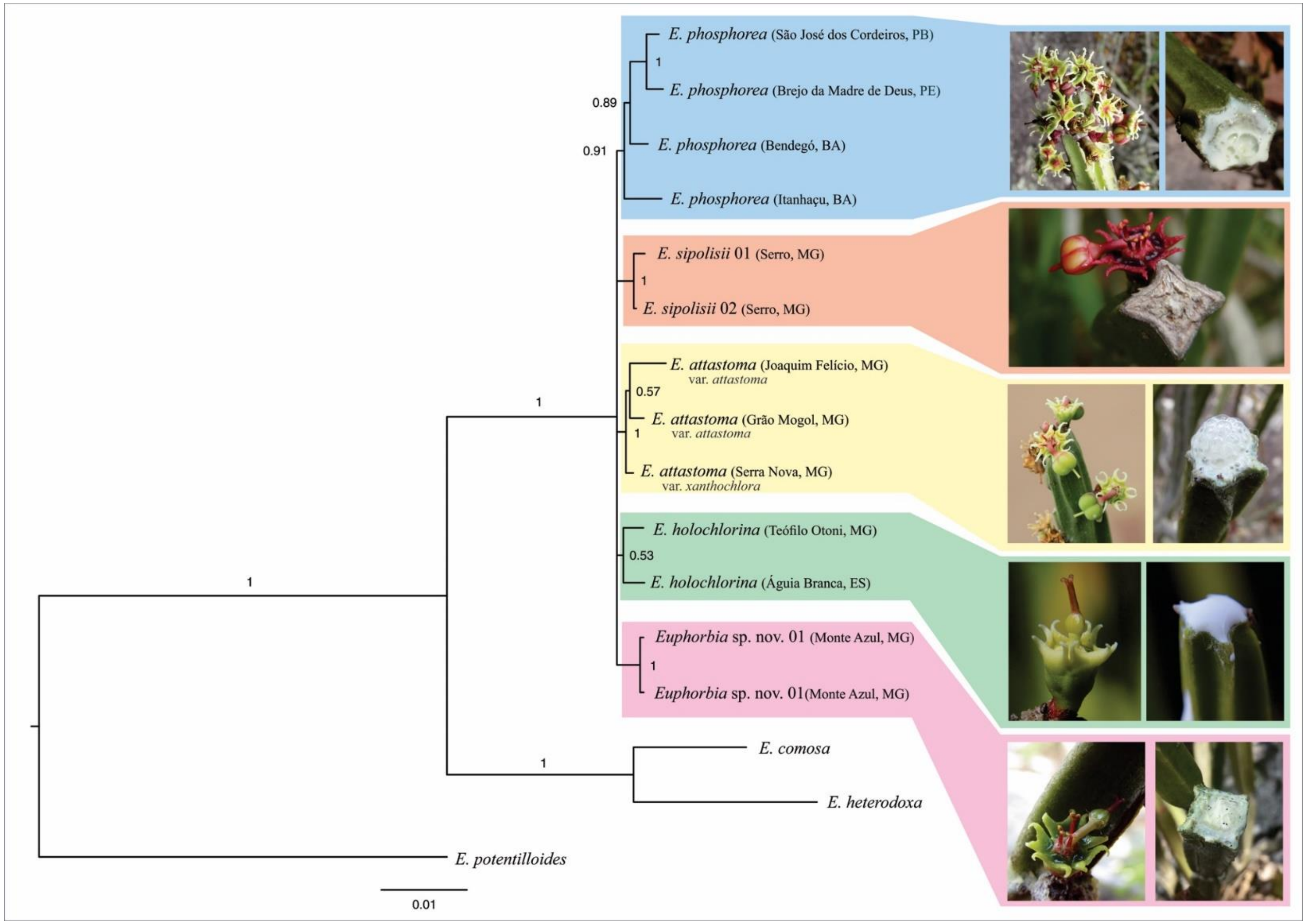


Fig. 4: Phylogenetic tree from combined plastid regions and nuclear (ITS1). Numbers next to nodes represent posterior probability of clades.

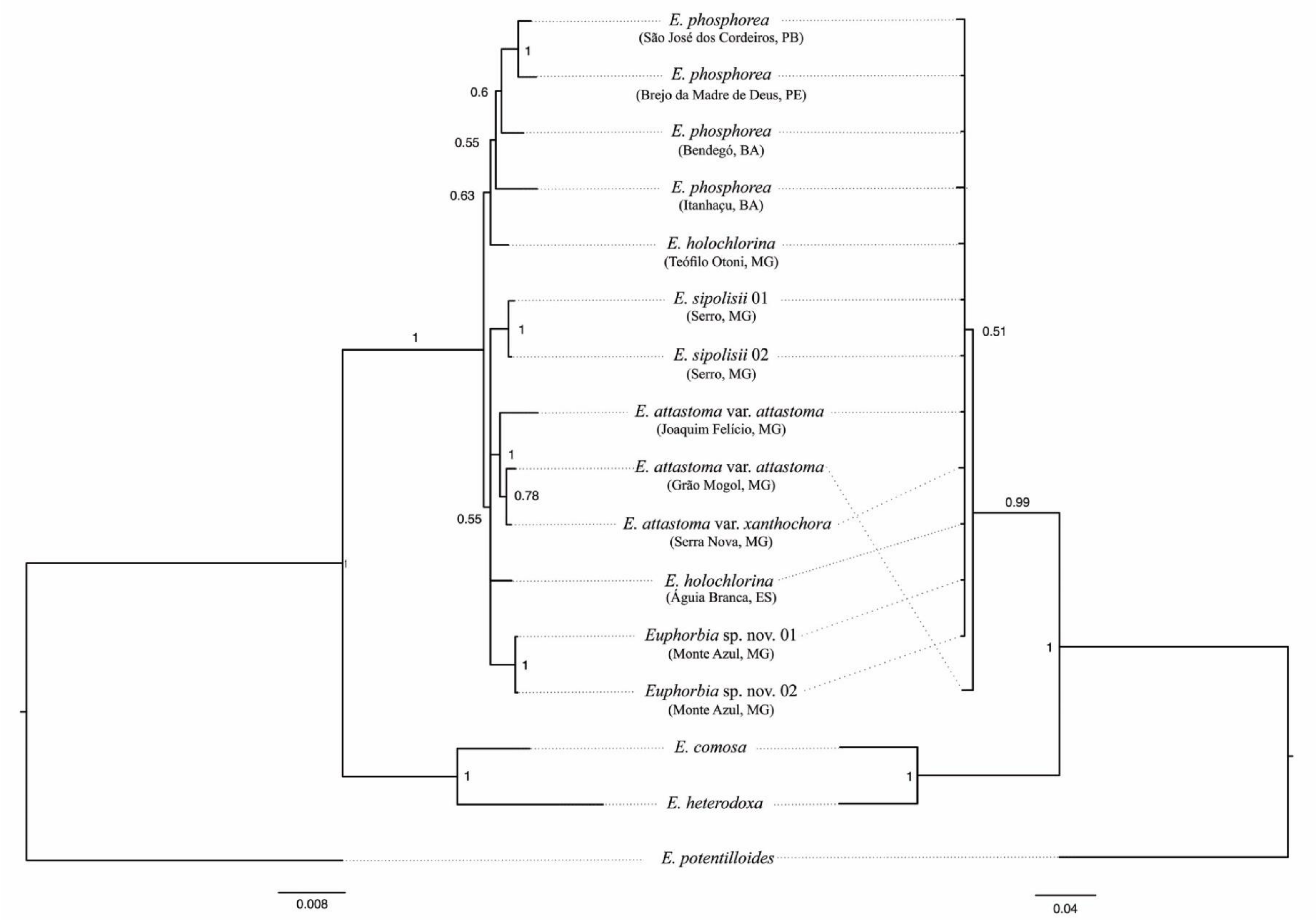
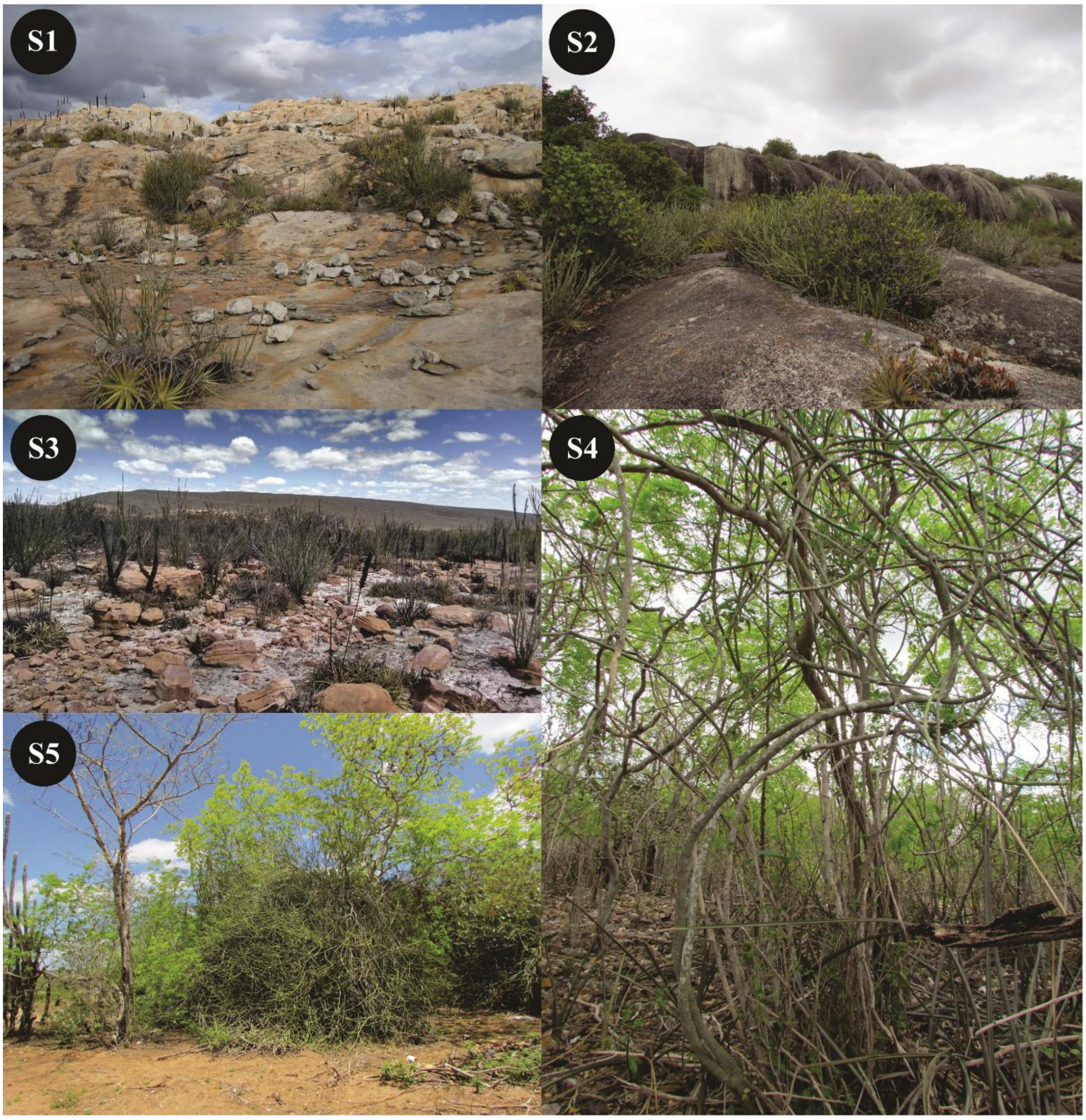
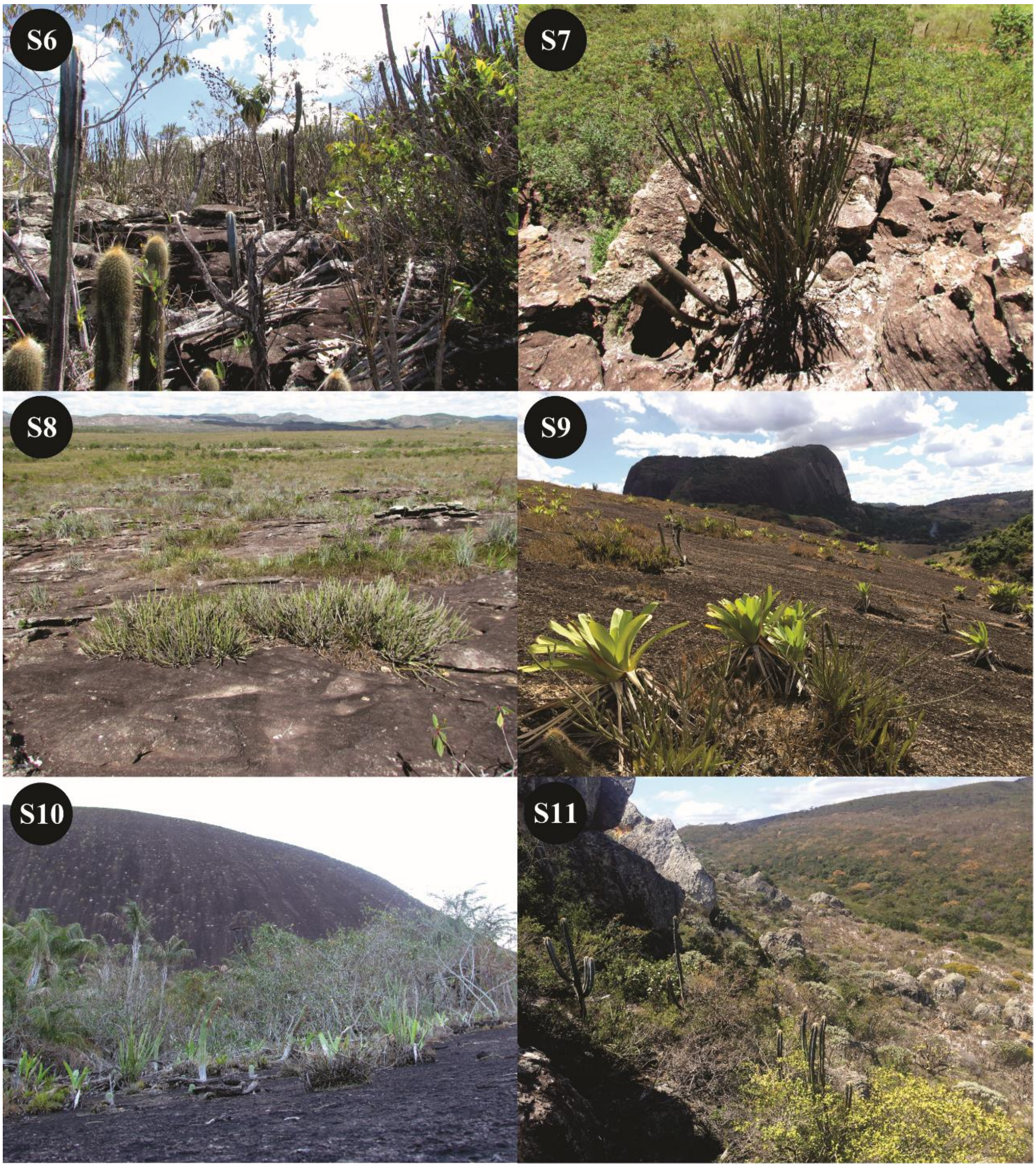


Fig. 5: Comparison between consensus trees from combined plastid regions (left) versus consensus trees from ITS1 (right).



Figs S1-S5: *Euphorbia phosphorea*: S1. Population on quarzitic *inselbergs* from Caatinga, in Pedra Lavrada (Paraíba), S2. Population on quarzitic *inselbergs* from Caatinga, in Brejo da Madre de Deus (Pernambuco); S3. Population of candelabriform individuals of *Euphorbia phosphorea* on flat slabs of rocks in Morro do Chapéu (Bahia); S4. Individual growing on sandy soils from Caatinga in Canudos (Bahia), leaning on other species; S5. Individual in rocky soils with sand from Caatinga, with habit resembling climbers or lianas.



Figs S6-S11: *Euphorbia* sect. *Brasilienses*: S6. *E. attastoma* var. *xanthochlora* – Serra Nova (Minas Gerais); S7. *E. attastoma* var. *attastoma* – Joaquim Felício/Serra do Cabral (Minas Gerais); S8. *E. sipolisii* – Serro (Minas Gerais); *E. holochlorina* S9-S10, S9. Teófilo Otoni (Minas Gerais), S10. Águia Branca (Espírito Santo); S11. *E. tetrangularis* – Monte Azul (Minas Gerais).

Fig. S12-S15: Xeromorphic characteristics of *Euphorbia* sect. *Brasilienses*. S12. Succulent branches with thick layer of wax, S13. Abundant latex, S14. Succulent leaves, but early caducous. S15. Stomata scars in wax layer of branches.

Table S1: Voucher information and Genbank accession numbers of the specimens of *Euphorbia* sect. *Brasilienses* and outgroups included in the molecular phylogenetic reconstructions

Taxon	Voucher (Herbarium)	Collection date	Municipality	Lat - Lon	Acc. no. *ITS*	Acc. no. *psbA-trnH*	Acc. no. *trnC-ycf6*	Acc. no. *matK*	Acc. no. *trnQ-rps16*	Acc. no. *atpI-atpH*	Acc. no. *petA-psbJ*
Euphorbia tetrangularis	Hurbath 844 (SP)	23-Aug-2016	Monte Azul	15.08 S 42.47 W	MH221171 MH221172	MH236544 MH236545	MH236528 MH236529	MH236570 MH236573	MH236514 MH236515	MH236499	MH236557 MH236558
Euphorbia sipolisii	Hurbath 830 (SP)	04-Apr-2016	Serro	18.28 S 43.29 W	MH221163 MH221164	MH236537 MH236538	MH236521 MH236522	MH236564	MH236509	X	MH236551
Euphorbia holochlorina	Hurbath 834 (SP)	06-Apr-2016	Teófilo Otoni	19.01 S 40.38 W	MH221167	MH236542	MH236526	MH236567	MH236512	MH236501	MH236555
Euphorbia holochlorina	Hurbath 635 (SP)	09-Jun-2014	Águia Branca	17.49 S 40.59 W	MH221170	MH236543	MH236527	MH236569	MH236513	MH236500	MH236556
Euphorbia attastoma var. attastoma	Hurbath 838 (SP)	11-Apr-2016	Grão Mogol	16.33 S 42.53 W	MH221166	MH236540	MH236524	MH236566	X	MH236503	MH236553
Euphorbia attastoma var. attastoma	Hurbath 824 (SP)	02-Apr-2016	Joaquim Felício	17.41 S 44.11 W	MH221165	MH236539	MH236523	MH236565	MH236510	MH236504	MH236552
Euphorbia attastoma var. xanthochlora	Hurbath 853 (SP)	25-Aug-2016	Serra Nova	15.36 S 42.44 W	MH221174	MH236541	MH236525	MH236572	MH236511	MH236497	MH236554
Euphorbia phosphorea	Hurbath 782 (SP)	15-Fev-2015	Brejo da Madre de deus	08.09 S 36.23 W	MH221162	MH236534	MH236518	MH236563	MH236508	MH236505	MH236550
Euphorbia phosphorea	Hurbath 768 (SP)	12-Fev-2015	São José dos Cordeiros	07.28 S 36.53 W	MH221161	MH236533	MH236517	MH248070	MH236507	MH236506	MH236549
Euphorbia phosphorea	Hurbath 755 (SP)	26-Nov-2014	Canudos	09.44 S 39.07 W	MH221160	MH236535	MH236519	X	X	X	X
Euphorbia phosphorea	Hurbath 727 (SP)	28-Aug-2014	Tanhaçu	13.54 S 41.16 W	MH221159	MH236536	MH236520	X	X	X	X
Euphorbia comosa	Silva O.L.M 245 (SP)	09-Mar-2016	Anastácio	29.44 S 55.39 W	MH221168	MH236546	MH236530	MH236568	X	MH236502	MH236559
Euphorbia heterodoxa	Amorim, V.O. 525 (ALCB)	13-Mar-2016	Feira de Santana	12.15 S 38.58 W	MH221169	MH236547	MH236531	MH236562	MH236516	X	MH236560
Euphorbia potentilloides	Hurbath 426 (SP)	21-Jan-2013	Licínio de Almeida	14.45 S 42.32 W	MH221173	MH236548	MH236532	MH236571	X	MH236498	MH236561

Table S2: Information of chosen regions for molecular phylogeny and primers used for amplification.

Region	**Annealing Temp (°C)**	**Number of base pairs in alignments/** ***informative sites: with outgroup – without outgroup***	**Percentages of missing data**	**Primer**	**Proposed by**
psbA (F)-*trnH* (R)	54	697 / *41-14*	0%	F: GTTATGCATGAACGTAATGCTC R: CGCGCATGGTGGATTCACAATCC	Shaw et al. 2005
trnC (F)-*ycf6* (R)	58	364 / *7-4*	0%	F: CCAGTTCRAATCYGGGTG R: GCCCAAGCRAGACTTACTATATCCAT	Shaw et al. 2005
matk	48	605 / *11-4*	18,75%	F: AGTATCTTCTTTAGAAAGGCC R: TAGCATTTGACTCCGTACTACC	Garrick et al. 2009
trnQ (F) - *rps16x1* (R)	48	624 / *4-4*	37,5%	F: GCGTGGCCAAGYGGTAAGGC R: GTTGCTTTYTACCACATCGTTT	Shaw et al. 2007
atpI(F) - *atpH* (R)	50	774 / *7-3*	37,5%	F: TATTTACAAGYGGTATTCAAGCT R: CCAAYCCAGCAGCAATAAC	Shaw et al. 2007
psbJ (F) -*petA* (R)	60	316 / *4-2*	18,75%	F: GTCCACTGAACCTTATCATTTAG R: GCTGCGTTCTTCATCGATGC	Shaw et al. 2014
ITS1	52	309 / *49-2*	0%	F: GTCCACTGAACCTTATCATTTAG R: GCTGCGTTCTTCATCGATGC	Urbatsch et al. 2000

References

Garrick, R. C., Nason, J. D., Meadows, C. A., & Dyer, R. J. (2009). Not just vicariance: phylogeography of a sonoran desert euphorb indicates a major role of range expansion along the Baja Peninsula. Molecular ecology, 18, 1916–1931.

Shaw, J., Lickey, E. B., Beck, J. T., Farmer, S. B., Liu, W., Miller, J., Siripun, K.C., Winder, C. T., Schilling, E. E. & Small., R. L. (2005). The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. American Journal of Botany, 92, 142–166.

Shaw, J., Lickey, E. B., Schilling, E. E., & Small, R. L. (2007). Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. American Journal of Botany, 94, 275–288.

Shaw J., Shafer H. L., Leonard O. R., Kovach M. J., Schorr M., Morris A. B. (2014). Chloroplast DNA sequence utility for the lowest phylogenetic and phylogeographic inferences in angiosperms: The tortoise and the hare IV. American Journal of Botany 101, 1987–2004.

Urbatsch, L. E., Baldwin, B. G., & Donoghue, J. M. (2000). Phylogeny of the Coneflowers and Relatives (Heliantheae: Asteraceae) Based on Nuclear rDNA Internal Transcribed Spacer (ITS) Sequences and Chlorplast DNA Restriction Site Data. Systematic Botany, 25, 539–565.

Capítulo 2

Taxonomic revision of *Euphorbia* sect. *Brasilienses* V.W.Steinm. & Dorsey (*Euphorbia* subg. *Euphorbia* – Euphorbiaceae)

A ser submetido ao periódico

Plant Systematics and Evolution.

Taxonomic revision of *Euphorbia* sect. *Brasilienses* V.W.Steinm. & Dorsey (*Euphorbia* subg. *Euphorbia* – Euphorbiaceae)

Fernanda Hurbath[1,2,3] & Inês Cordeiro[1]

[1] Nucleo de Pesquisa Curadoria do Herbário SP, Instituto de Botânica, Avenida Miguel Stéfano 3687, São Paulo, 04045-972, SP, Brazil.

[2]fhurbath@gmail.com, +55 71 992079666

[3]ORCID: 0000-0001-8857-775X

Euphorbia* sect. *Brasilienses

Abstract

Euphorbia sect. *Brasilienses* is an exclusively Brazilian taxon, which includes *Euphorbia attastoma*, *E. holochlorina*, *E. phosphorea*, *E. sipolisii* and *E. tetrangularis*. The species have a shrub habit with succulent branches, inconspicuous leaves, early caducous. *Euphorbia phosphorea* occurs in practically the entire Caatinga region, while *E. attastoma* is restricted to the southwest of Bahia, north and northeast of Minas Gerais, growing on rocky caatinga. *Euphorbia tetrangularis* also occurs on rocky caatinga in northern Minas Gerais. *Euphorbia sipolisii* is unique to campos rupestres of Diamantina region in the State of Minas Gerais, and *E. holochlorina* is endemic to the inselbergs eastern of Minas Gerais and Espírito Santo. The most relevant morphological characteristics to distinguish species are the number of ribs, morphology of cyathia involucre, color of cyathia and cyathial glands, and position of cyathial appendages. We present a taxonomic revision of the section, with an identification key for the species, a description and taxonomic updating of each species, with comments for morphological and ecological aspects of the species.

Keywords: Caatinga, *Euphorbia phosphorea*, *inselbergs* SDTF (Seasonally Dry Tropical Forests), succulent spurges.

Introduction

Euphorbia L. is one of the largest genera of angiosperms (Frodin 2004), with around 2000 species, almost distributed worldwide (Govaerts et al. 2000). The genus has been recognized as monophyletic in its present broad circumscription (Steinmann and Porter 2002; Bruyns et al. 2006). Its morphological synapomorphy is the cyathium (Steinmann and Porter 2002) a pseudanthial inflorescence not found in any other angiosperm's family, composed of 4-5 cymules of staminate achlamydeous flowers reduced to one stamen surrounding a single achlamydeous pistillate flower, which are enclosed within an involucre of fused bracts often with nectariferous glands, bearing morphologically diverse appendages (Prenner and Rudall 2007; Horn et al. 2012). The genus presents a great variety of habit, from herbs to trees and succulent shrubs, occurring in various habitats from forest to deserts. *Euphorbia* is an important element of arid ecosystems worldwide, especially in seasonally dry tropical areas (Horn et al. 2012) with many xerophytic species, largely characterized by its succulent stems (Horn et al. 2012, 2014; Dorsey et al. 2013).

The circumscription of the genus changed along the time since was published by Linnaeus in 1753. The first global revision including *Euphorbia* and other genera with cyathium, recognized at that time as part of Tribe Euphorbieae, was authored by Bossier (1862) in Candolle's Prodromus, where he recognized three genera (*Euphorbia, Pedilanthus* Neck. ex Poit., and *Synadenium* Boiss.), with *Euphorbia* housing 27 sections where most taxa were placed. Later, Pax and Hoffman (1931) kept the genera recognized by Bossier (1862) and created six others by raising some of the sections previously within *Euphorbia* to genus rank. Then, Wheeler (1943) published a taxonomic study for the entire tribe Euphorbieae, bringing the first time the category of subgenus within *Euphorbia* (subgenera: *Chamaesyce* Gray, *Agaloma* Raf., *Poinsettia* Graham, *Eremophyton* Bég., *Lyciopsis* Spach, *Tithymalus* Mill., *Rhizanthium* (Boiss.) Wheeler and *Esula* Pers.), some of which, later, (Webster 1967, 1975, 1994) raised to genus rank (*Chamaesyce*, *Endadenium* L.C.Leach, *Synadenium*, *Monadenium* Pax, *Pedilanthus* and *Cubanthus* (DC.) Millsp.

Till recently, Webster's classification was widely accepted, with the subtribe Euphorbiinae characterized by the presence of cyathium, including *Euphorbia* and the six genera cited above (Webster 1994; Radcliffe-smith 2001).

In the currently new circumscription, after phylogenetic studies (Steinmann and Porter 2002; Bruyns et al. 2006; Park and Jansen 2007; Horn et al. 2012; Yang et al. 2012) the other genera with cyathium recognized by Webster (1994) are now synonymized under *Euphorbia*. Therefore, subtribe Euphorbiineae is now composed only by *Euphorbia*.

Within *Euphorbia*, Steinmann and Porter (2002) stablished four major clades, that Bruyns et al. (2006) latter recognized as four subgenera: *Euphorbia*. subg. *Euphorbia*. *E.* subg. *Esula* Pers., *E.* subg. *Chamaesyce* Raf. and *E.* subg. *Rhizanthium* (Boiss.) Wheeler. The last one, was latter recognized as *E.* subg. *Athymalus* Neck. ex Rchb. by Peirson et al. (2013).

Euphorbia subg. *Euphorbia* is the largest and most diverse, with at least 660 species (Dorsey et al. 2013), occurring in various habitats (Horn et al. 2012), especially in semiarid regions, with great number of succulent and xeromorphic species with leaves and stipules modified in thorns. In the phylogenetic studies of Horn et al. (2012) and Dorsey et al. (2013), authors recognized four clades for *Euphorbia* subg. *Euphorbia*: New World (tropical America), Old

World I (Madagascar), Old World II (Africa and Asia) and Pacific (South Pacific). Five previous genera, *Monadenium* Pax*, Synadenium* Boiss.*, Endadenium* L.C. Leach, *Pedilanthus* Necker and *Cubanthus* (Boiss.) Millspaugh became synonyms of *Euphorbia* subg. *Euphorbia*, with 21 sections recognized by Dorsey et al. (2013), for the subgenus.

The New World clade consists of 11 sections, among which is *Euphorbia* sect. *Brasilienses* V.W. Steinm. & Dorsey. This is an exclusively Brazilian group, currently with six succulent taxa (*E. attastoma* var. *attastoma* Rizzini*, E. attastoma* var. *xanthochlora* Rizzini*, E. holochlorina* Rizzini*, E. phosphorea* Mart.*, E. sipolisii* N.E.Br. and *E. tetrangularis* Hurbath & Cordeiro), pencil-stemmed shrubs with inconspicuous early caducous leaves, ribbed stems and cyathia with horn-shaped appendages.

In Prodromus (Bossier 1862), *E. phosphorea* was not placed within any section, considered among the "Species imperfect notae". Later, in Flora Brasilienses, Muller Argoviensis (Argoviensis 1874) considered *E. phosphorea* part of section *Euphorbium* Boiss. by its succulent and costate stem with inconspicuous and deciduous leaves. Till then, this was the only Brazilian species belonging to this section, mostly comprising with succulent species of Africa. *Euphorbia sipolisii* N.E.Br. was described and placed by Brown (Royal Botanic Gardens 1893) also in the section *Euphorbium.* Berger (1906) placed *E. sipolisii* in a newly described section *Pteroneurae* A. Berger, together with *E. pteroneura* Berger from México. Both species are succulent pencil-stemmed shrubs with costate branches, but the author made no mention of *E. phosphorea.* Pax and Hoffmann (1931) classified *E. sipolisii* and *E. phosphorea* in different sections, respectively: section *Euphorbium,* subsection *Pteroneurae* A. Berger and section *Adenopetalum* (Klotzsch et Garcke) Benth, subsection *Crossadenia.* Just after Rizzini's (1989) publication of *E. attastoma* and *E. holochlorina,* (Eggli 1994) placed all South America succulent, ribbed-stem species, including *E. weberbaueri* Mansfield from Peru, in section *Pteroneurae* A. Berger. In the phylogeny of subgenus *Euphorbia* by Dorsey et al. (2013), the neotropical ribbed-stem species emerged in two different lineages: sect. *Brasilienses* (*E. attastoma, E. holochlorina, E. sipolisii, E. phosphorea*) and sect. *Euphorbiastrum* (*E. pteroneura* and *E.weberbaueri*) The succulent ribbed-stem habit is therefore homoplastic, occurring at least twice in the New World clade of *E*. subg. *Euphorbia* (Dorsey et al. 2013).

Euphorbia sect *Brasilienses* is a strongly supported clade (Hurbath & al., 2018), comprised only of Brazilian species sister to a clade of herbaceous species, *E.* sect. *Stachydium* Boiss, this last with species occurring North of South America and northeastern Brazil (Dorsey & al., 2013).

Overall, the species of *E.* sect. *Brasilienses* are very similar, so there were many misidentifications collections in the herbaria and authors (Eggli 1994; Machado 2000) have questioned species boundaries. Therefore, a taxonomic review of the section, based on previous phylogentic studies (Hurbath & al., 2018; Dorsey & al., 2013), was conducted to clarify species morphological singularities and better establishment of its boundaries, in addition to provide proper taxonomic treatment for the section.

Materials and methods

Field trips were carried out between 2014 and 2017 in thirty localities, four of them (Águia Branca – ES, Monte Azul – MG, Iaçu – BA, Morro do Chapéu – BA) were visited twice during different periods. Collected specimens were pressed and dried according to usual herbarium technique. Cyathia were put in 70% alcohol to keep original measurements. We examined collections of all species of *Euphorbia* sect. *Brasilienses* deposited in ALCB, BHCB, CEN, CEPEC, EAC, HRB, HRCB, HUEFS, JPB, MAC, MBM, PEUFR, R, RB, SP, SPF, UB, UEC, UFP and UFRN as well as digital images from G, K, M, NYBG, P, US and VIC. All type-collections were examined in herbaria or by digital photos as well as protologues. For the morphological description, we applied the terminology adopted in Radford et al. (1974), Harris and Harris (1994) and Beentje (2010). Qualitative characters were studied with the use of a microscope, measurements were made using a pachymetry and graph paper. In the list of exsiccatae, species are represented by acronyms: *Euphorbia attastoma* – EA, *E. holochlorina* – EH, *E. phosphorea* – EP, *E. sipolisii* – ES, *E. tetrangularis* – ET.

Taxonomic treatment

Euphorbia sect. ***Brasilienses*** V.W Steinm. & Dorsey, Taxon 62: 308-309. 2013. Type: ***Euphorbia phosphorea*** Mart.

Description. — Shrubs, phanerophyte, branched from the base, older individuals can develop a small trunk, erect to decumbent, sometimes scrambling, candelabriform to caespitose, frequently forming dense clumps, 0.5 to 4 m tall, pencil-like stems with latex abundant, white to beige. Branches succulent, green to yellowish or greyish, sometimes bright green, dark green, or vinaceous, usually red to cuprous when young, glabrous, angled, shallowly to deeply channeled, with 4-8-ribs, rarely 9, becoming cylindrical and lignified, wax abundant forming a thick layer (scar of stomates visible in magnifying glass) and providing an asper texture. Leaves early caducous, simple, alternate, distichous or spiral, entire, c. 1 cm long, green to vinaceous, narrowly lanceolate, fleshy, glabrous, sessile to subsessile, conduplicate, margin entire, apex acute, venation inconspicuous, green to vinaceous, glabrous, with many yellow colleters in the axils of the leaves. Synflorescences in monochasial cymes, glomeruliform to spiciform (circinnus) (Fig. 2a, c), axillary, succulent, with a thick layer of wax (Fig. 2b), 1-many cyathia. Cyathophylls depressed ovate or triangular (Fig. 2n), sessile, when young, navicular, conduplicate, involving completely the cyathium, usually vinaceous, frequently persistent on raquis, with colleters on axils and on the basis of margins, which are entire; apex acute, sometimes mucronulate. Cyathia with campanulate or cupuliform involucre, 2-4 mm long., subsessile, succulent, green to vinaceous, glabrous; lobes flabellate, membranaceous, glabrous; 5 glands located on the rim of the cyathia, transversely ellipsoid, concave, sometimes conduplicate, light-green, dark-green, yellowish or vinaceous (Fig. 3), usually with verrucose saliences, bearing 2 horn-shape (Fig. 2g, j-m), conspicuous to inconspicuous appendages (Fig. 2k), erect (Fig. 2f, i) or patent (Fig. 2e, h), frequently verrucose. Staminate flowers in 4-5 cymules of 3-7 flowers each, bracteoles fimbriated, translucent. Pistillate flowers sometimes presenting a perianth-like whorl with 1-3 filiform caducous lobes. Ovary ovoid, glabrous; styles 3, connate, glabrous. Fruits obloids with prominent keels (Fig. 2o) or rounded (Fig. 2p), vinaceous, green or yellowish frequently with vinaceous strips on septum and along the keel (Fig. 3), glabrous, becoming coriaceous and brown, with persistent columella (Fig. 2a). Seeds widely ovoid with base slightly truncate (Fig. 2q-r), brown, coriaceous, testa smooth to conspicuously papillose, with a discoid caruncle.

Notes. — *Euphorbia* sect. *Brasilienses* is endemic to Seasonally Dry Tropical Forest (SDTF) of Brazil as delimited by Särkinen et al. (2011) (Fig. 1). Species are characterized by a succulent habit with very reduced leaves (Fig. 4 and 6), stems performing photosynthesis (cladophyll), frequently part of a rock outcrop vegetation, usually under semi-arid climatic conditions of low precipitation and shallow soils with low water retention, high light incidence and temperatures. Species of the section *Brasilienses* is found on caatinga vegetation in depressions (Raso da Catarina), rock outcrops and inselbergs, also in campos rupestres of the Espinhaço Range and on inselbergs of the Rio Doce magmatic arc (Tedeschi et al. 2016). This last one is inserted in the Atlantic Rain Forest domain, however presents some of the xeric conditions cited above. The geographic distribution of the section covers an elevation range between 150 to 1300 m, and a latitudinal range between 6°S and 19°S (Rio Grande do Norte, Paraíba, Pernambuco, Sergipe, Bahia, Minas Gerais, Espírito Santo) (Fig. 1). The lowest altitudes were found in the caatinga central depression, Raso da Catarina, in the State of Bahia (223 m elevation), and on inselbergs from Espirito Santo (156 m elevation) and the highest ones in the Espinhaço Range (1300 m elevation).

Populations of species of *Euphorbia* sect. *Brasilienses* mostly presented more than 20 individuals, and at a first look of the landscape represents an important component of the vegetational community (Fig. 4). On inselbergs, it would always be growing together with bromeliads forming slightly dispersed patches or clumps (Fig. 4e). It was very common to see species of section *Brasilienses* together with species of Cactaceae (*Coleocephalocereus, Micranthocereus, Pilosocereus*) and Bromeliaceae (*Alcantharea, Dickia, Tillandisia, Vriesia),* besides others Euphorbiaceae (*Cnidoscolus, Jatropha, Stillingia*), among other species adapted to harsh environments (to cite some genus: *Hippeastrum, Acianthera, Vellozia, Epidendrum, Encyclia, Syagrus, Alamanda, Mandevilla, Clusia, Anteremanthus, Wunderlickia*).

In northeastern region of Brazil, most localities one can find only *Euphorbia phosphorea,* with exception of localities west and southwestern of State of Bahia, where one can find *E. attastoma* (Licínio de Almeida, Caetité, Serra do Ramalho, Iuiu). This last one, goes further south of the section's geographic distribution, occurring on campos rupestres and rocky caatinga of Espinhaço Range. *Euphorbia tetrangularis* is also found on rocky caatinga in Minas Gerais state, *E. holochlorina* on rocky vegetation of inselbergs in Mata Atlântica further east of the sections's distribution, and *E. sipolisii* on campos rupestres of Espinhaço Range, on the south limit of the group's distribution. Some species (*E. tetrangularis* and *E. attastoma*) occur at less than 100 km apart, but never recorded together in the same localities.

During field expeditions, it was very common to find species of ants (*Camponotus* spp.; *Cephalotes* sp.) foraging the cyathia, especially close to the glands (Fig. 5k, l), that offer nectar, apparently in great quantities in a way that we could see the exudate as if covered with a translucent film of exudate, secreted as drops forced from the glands and appendages (Fig. 5a-g). Besides ants, on the top of the cyathia, we also could observe flies and beetles (Fig. 5n). Other visitors were observed on the plants, close to cyathia but not on them: leaf footed-bugs (Fig 5j), wasp (Fig. 5 m), spiders forming nets under the sinflorescences (Fig. 5h-i), and bees scraping wax from branches' epidermis. We are not sure if these insects play a role as pollinators, however, the nectariferous glands are placed at the rim of the cyathium involucre, that is solid and promotes a great platform for landing and walking. Lizards where also reported foraging *Euphorbia phosphorea* during the dry periods of the caatinga (Aximoff and Felix 2017), seeking nectar.

The most remarkable characters to distinguish species are the number of ribs, shape of cyathia involucre (Fig. 2), color of cyathia and glands (Fig. 3), and position of glandular appendages (Fig. 2). We also found differences of phyllotaxis (Fig. 6), habit and habitat (Fig. 4). Overall the species are very homogeneous and therefore, species boundaries are based on sets of characteristics. Other differences are regarding how deep channeled the stems are (Fig. 6), if the synflorescences are congested or not (Fig. 2a, c), size of the involucre, presence of a perianth like whorl, level of connation of styles, and presence of prominent angles on fruit's cocci (Fig. 2o-p) are also useful for the circumscription of the taxa.

The synflorescences of *Euphorbia* sect. *Brasilienses* are monochasials, usually very congested and glomeruliform. However, we found individuals of *E. phosphorea* and *E. attastoma* with elongated, spiciform and sometimes ramified synflorescences (Fig. 2a, c). The rachis is succulent, and resembles the stem, sometimes also presenting ribs and producing a thick layer of wax that breaks and are easily removed when dried (Fig. 2b). The succulence of rachis is not uniform, and some internodes become larger and longer than others resulting as if it is strangled at the nodes, resembling a bead necklace (Fig. 2c). The zigzag branching synflorescence classify this cyme as cincinnus as Weberling (1989) described. Each cyathium is always subtended by one cyathophyll (Fig. 2d, n). Sometimes the synflorescence is reduced to one or two cyathia with apparently esterile cyathophylls on base of the rachis.

The cyathial glands usually presents a horn-shaped structure, with verrucose aspect (Fig. 2g, j-m), that we will refer as appendages. The tissue apparently is the same from the glands that is a nectariferous epidermis (Gagliardi et al. 2016), once is possible to see some nectar production on its surface with cup-like saliences. Anatomic studies would be necessary to identify the true nature of these appendages.

The phyllotaxis vary among the species (Fig. 6), with *E. phosphorea* having leaves arranged in spiral and *E. holochlorina*, *E. tetrangularis* and *E. sipolisii* distichous. *Euphorbia attastoma* presents both types of phyllotaxis. The spiral phyllotaxis was found only in the populations of *E. attastoma* in Grão-Mogol (Fig. 4b and 6a), in all other localities the phyllotaxis is distichous.

Identification key for species of *Euphorbia* sect. *Brasilienses*

1. Branches with 6-8(9)-ribs.. 2

1' Branches with 4 or 5 ribs. 3

2. Shrubs can develop a small trunk; branches shallowly channeled, with 8 ribs, only two ribs of the three that arise from one node reaches the upper node, leaves with 1.1-2 mm large, always spiral; monochasia usually with more than 4 cyathia, occurring on sandy gravelly soils or stone slabs of caatinga vegetation and quarzitic inselbergs. … .... ***E. phosphorea***

2’. Shrubs not developing a small trunk; branches deeply channeled, with 6 ribs, usually three ribs that arise from one node reaches the upper node; leaves with 2-3 mm large, usually distichous; monochasia usually with 1-4 cyathia; occurring on rocky caatinga or campos rupestres of the Espinhaço Range, on karstic rock outcrops and granitic inselbergs... 4

3. Cyathia with involucre and glands vinaceous; appendages patent, vinaceous; pistillate flowers with pedicels 3.1-3.5 mm long, no perianth-like whorl, styles connate up to 1/3; fruits 4.1-4.7 mm long, vinaceous to yellowish.....................................***E. sipolisii***

3’. Cyathia with involucre and glands green; appendages erect, whitish to light green; pistillate flowers with pedicels 1.3-3 mm long, with perianth-like whorl, styles connate up to 1/2; fruits 5-7 mm long, green.. ***E. tetrangularis***

4. Branches green to greyish or vinaceous to cuprous; monochasia with 3-4 cyathia; involucre campanulate, green to vinaceous; appendages patent, whitish to vinaceous; fruits with prominent keel, vinaceous or green frequently with vinaceous strips on septum and along the keel. ....***E. attastoma***

4’...........Branches bright green; monochasia with 1-2 cyathia; involucre cupuliforme, light green; appendages erect, whitish to green; fruits with rounded keel, green, frequently with vinaceous strips at the base. .... ***E. holochlorina***

Euphorbia attastoma Rizzini, Revista Brasil. Biol. 49: 987. 1989 [1990]. ≡ *Euphorbia attastoma* var. *attastoma* Rizzini. Type: Brazil, Minas Gerais: Serra de Grão Mogol, 03 Sept 1970, A.P. Duarte 13254 (Holotype: RB-00085142!).

= *Euphorbia attastoma* var. *xanthochlora* Rizzini, Revista Brasil. de Biol. 49: 992. 1989 [1990]. Type: Brazil, Minas Gerais: Pedra Azul, 21 Sept 1965, A.P. Duarte 9290 (Holotype: RB- 0008594!); **syn. nov.**

Shrub candelabriform to caespitose, erect to decumbent, rarely scrambling, individuals not developing trunk, 1-1.8 m high. Branches deeply channeled, with 6-ribs, very rarely 7-8-ribs, usually with the three ribs arising from each node reaching the upper node, green to greyish, vinaceous to cuprous when young (Fig. 6a). Leaves green to vinaceous, 5-10.6 x 2-3 mm, distichous, sometimes spiral. Monochasia 4.2-16.2 mm, glomeruliform to spiciform, usually with 3-4-cyathia. Cyathophylls 1.2-3.5 x 1.5-2.2 mm. Cyathia involucre 2.2-3.6 x 2.4-5.7 mm, campanulate, green to vinaceous; lobes 5-6 1.3-1.8 x 0.8-1.9 mm, glands 4-6, 0.8-1.9 x 1.9-2.9 mm, concave, light-green to vinaceous, appendages conspicous to rarely inconspicuous, patent, 0.7-3.1 mm long, whitish to vinaceous. Staminate flowers in 4-5 cymules of 4-5 flowers, pedicels with 0.9-5.1 mm long. Pistillate flowers with pedicels 1-1.9 mm long; very rarely with perianth-like whorl with 1 filiform lobe, caducous. Ovary 0.8-1.9 x 1-2.2 mm; styles 1.9-2.9 mm long, connate 1/4 to 2/3. Fruits with prominent keel, 3.3-4.6 x 3.9-6.1 mm, vinaceous or green, frequently with vinaceous strips on septum and along the keel; columella 2.5-3.5 mm. Seeds 2.5-3.5 x 1.7-2.7 mm.

Taxonomic notes **—** Rizzini (1989) described two varieties for this species that were characterized by the color of glands and appendages [red to vinaceous -var. *attastoma* (Fig. 3a, m) x green to yellowish -var. *xanthochlora* (Fig. 3b)], and the young stems (red var. *attastoma* x rose or cuprous -var. *xanthochlora*). We visited five localities were *E. attastoma* occurs, including the type localities of both varieties. We found what appear to be Rizzini’s *E. attastoma* var. *attastoma* in Joaquim Felício with red appendages, but with cuprous young stems. Color of appendages and young branches can vary in many populations of *E. phosphorea*, from whitish to vinaceous and young branches red to cuprous, therefore it seems fragile to recognize these varieties through these characteristics. To do so, it would mean that new varieties must be created to *E. phosphorea*, however, it would be very hard to identify an herbarium material if it does not come accompanied by a clear color description. Therefore, we have decided to synonymize Rizzini’s varieties.

This species is morphologically closer to *E. phosphorea* from which can be differentiated by number of ribs and how deeply channeled the stems are. Differences in the habit is also mentioned, with *E. phosphorea* many times presenting a small trunk (Fig. 4d, m) and *E. attastoma* always branched from the base (Fig. 4a, d, i-j). However, the habit seems to accompany the soil type and depth (see *E. phosphorea* notes and Fig. 4). Machado (2000) noticed that the arrange of the ribs can also distinguish *E. attastoma* from *E. phosphorea.* The first one presents three ribs arising from a node and reaching the upper node, and in *E. phosphorea* only two ribs reaches the upper node, while the third one continues to the next upper node. These patterns are apparently related to the phyllotaxis of each species: *E. attastoma* usually presents a distichous phyllotaxis and *E. phosphorea* spiral. However, we have notice that at Grão Mogol, many individuals of *E. attastoma* present a spiral phyllotaxis, consequently, this pattern of ribs is the same found in *E. phosphorea.* Also, Grão Mogol is the only locality where *E. attastoma* presents red young stems.

Euphorbia attastoma occurs on rocky caatinga and campos rupestres (Fig. 4a-b, i-j), on karstic rock outcrops and granitic inselbergs. It can be identified by the six-ribs branches, deeply channeled, usually the three ribs that arise from each node reaching the upper one, usually distichous phyllotaxy (Fig. 6a) and patent appendages (Fig. 2e, h).

Etymology. — The name for this species comes from the resemblance of the cyathial glands appendages to the chelicerae of *Atta* ants, popularly known as “saúva”.

Popular usage. — No use was mentioned, however the popular name “velinha” was written on the label of a collection from Grão Mogol. Velinha means small candle, maybe for the utilization of its waxy stems for lighting fire

Additional specimens examined. — BAHIA: Caetité, 28 Apr. 2008, *Guedes & al. 14332* (ALCB); Iuiu, 16 Jul. 2005, *Silva & al. 1440* (NY, RB); Licínio de Almeida, 4 Nov. 2013, *Hurbath & al. 730* (ALCB); Serra do Ramalho, 14 Apr. 2001, *Jardim & al. 3474* (CEPEC, HRB, HUEFS, NY, SP). MINAS GERAIS: Belo Horizonte, 25 Aug, 2011, *Ferreira-Junior s.n.* (ALCB 118780); Botumirim, 20 May 2001, *Tameirão Neto 3338* (BHCB); Cristália, 12 Mar. 1999, *Kawasaki & Rapini 1093* (RB, SP); Grão Mogol, 11 Apr. 2016, *Hurbath & al. 838* (SP); Joaquim Felício, 2 Apr. 2016, *Hurbath & al. 824* (SP); Pedra Azul, 10 Apr. 2016, *Hurbath & al. 837* (SP); Rio Pardo de Minas, 21 Mar. 2016, *Martinelli & al. 19230* (RB); Serra Nova, 25 Aug, 2016, *Hurbath & Cordeiro 853* (SP); Serranópolis de Minas, 17 Apr. 2007, *Ribas & Silva 7743* (MBM).

Euphorbia holochlorina Rizzini, Revista Brasil. de Biol. 49: 983. 1989 [1990]. Type: Brazil, Espírito Santo: Serra do Cristalino, Nova Venécia, 17 Nov 1953, *A.P. Duarte 3987* (Holotype: RB- 00538452!)

Shrub candelabriform to caespitose, erect, sometimes decumbent, rarely scrambling, individuals not developing trunk, 0.4-1.5 m high. Branches deeply channeled, with 6-ribs, with the three arising ribs from each node reaching the upper one, bright green, cuprous when young. Leaves green to vinaceous, 2-4 x 1.9-2.5 mm, distichous (Fig. 6b). Monochasia 2.1-9.6 mm, glomeruliform to spiciform, usually with 1-2-cyathia. Cyathophylls 1.4-2 x 1.3-1.9

mm. Cyathia involucre 3.6-5.1 x 3.2-4.6 mm, cupuliform, green; lobes 5, 1.4-1.8 x 1.4-1.7 mm, glands 5, 0.8-1.1 x 1.5-2.2 mm, concave to conduplicate, light-green, appendages conspicuous, erect, 0.9-2 mm long, whitish to light green. Staminate flowers in 5 cymules of 4-5 flowers, pedicels with 1.1-4.5 mm long. Pistillate flowers with pedicels 1.5-2.6 mm long; very rarely with perianth-like whorl with 1-3 filiform lobes, caducous. Ovary 1.1-1.7 x 1.3-1.7 mm; styles 2.2-2.6 mm long, connate 1/2 or up to the apex. Fruits with rounded keel, 3.7-5 x 4.8-5.7 mm, green, frequently with vinaceous strips at the base; columella 3.3-4 mm. Seeds 2.8-3.3 x 2.2-2.5 mm.

Taxonomic notes — This species also described by Rizzini was until recently only known by the type collection. In 2006 a collection from the municipality of Águia Branca guided new collections in the State of Espírito Santo. After we regained in Águia Branca, other two localities were found, one of them being a new occurrence for this species in the state of Minas Gerais, between municipalities of Teófilo Otoni and Carlos Chagas, since then this species was found only in Espírito Santo.

We could not find this species in the type locality, municipality of Nova Venécia (ES). We have tried looking in the most remarkable inselberg in the surround area, that is the Pedra do Elefante, with no success.

Euphorbia holochlorina presents less wax than the remaining species of the section, and when collected the branches shrivels faster, in less than a day, while cut branches of *E. phosphorea* can last for days without losing its turgor. The six-ribbed branches, with bright green color, erect appendages and cuplike involucre and monochasia usually with few cyathia and frequently on top of branches, differs this species from the others.

Etymology. — The name for this species comes from the green color of its branches and inflorescences.

Additional specimens examined. — ESPÍRITO SANTO: Águia Branca, 8 Apr. 2016, *Hurbath & al. 836* (SP); Vila Pavão, 7 Apr. 2016, *Hurbath & al. 835* (SP). MINAS GERAIS: Aimorés, 6 Aug, 2004, *Fontana & Vervloet 908* (SP); Carlos Chagas, 6 Apr. 2016, *Silva & al. 279* (SP); Teófilo Otoni, 6 Apr. 2016, *Hurbath & al. 834* (SP).

Euphorbia phosphorea Mart., Reise Bras. 2: 612, 726. 1828. **lectotype (designated here):** BRAZIL, Bahia: “deserto Bahiensi”, *Martius s. n.* (M-0241970 (digital image!))]

Description. — Shrub candelabriform to caespitose (Fig. 4l), erect to decumbent, or scrambling (Fig. 4g-h), individuals can develop a small trunk, 0.5-6 m high. Branches shallowly channeled, with 8-ribs, very rarely 6-9-ribs, from the three ribs arising from each node, only two reaching together one upper one, with the third reaching other upper node, green to yellowish, or greyish, red to cuprous when young. Leaves green to vinaceous, 4.2-10 x 1.1-2 mm, spiral. Monochasia 7.9-28.5 mm, glomeruliform to spiciform, usually with more than 4-cyathia. Cyathophylls 0.9-2 x 1.9-2.5 mm. Cyathia involucre 2.2-3.6 x 2.4-5.7 mm, campanulate, green to vinaceous; lobes 4-6 1-2.1 x 0.8-1.9 mm, glands 4-6 1.2-2 x 1.7-2.9 mm, concave, light-green to yellowish, frequentely vinaceous, appendages conspicuous, patent, 1.6-3.5 mm long, whitish to vinaceous. Staminate flowers in 4-6 cymules of 4-7 flowers, pedicels with 0.5-4.1 mm long. Pistillate flowers with pedicels 1.5-2.2 mm long; very rarely with perianth-like whorl with 1-3 filiform lobes, caducous. Ovary 0.9-1.8 x 0.9-1.7 mm; styles 1.3-3.2 mm long, connate 2/3 or up to the apex. Fruits with prominent keel, 3.2-4.3 x 4-5.8 mm, vinaceous or green, frequently with vinaceous strips on septum and along the keel; columella 2.3-5 mm. Seeds 1.7-3.2 x 1.7-2.9 mm.

Taxonomic notes. — The original collection of this species in M presents a label where it is written: “Habitat in apricis montanis prope Sebastianopolis”. The placement of this label was a clear mistake since the mountains of Rio de Janeiro are covered by rainforest and not even in their rocky outcrops shelter caatinga vegetation. Besides, In the second volume of Reise Brasilien (Spix and Martius 1828), the authors describe the Martius’s discovering of *E. phosphorea* in the state of Bahia, describing the unique vegetation found in Caatinga also mentioning several other typical species of this vegetation species. The diagnosis of *E. phosphorea* is on a footnote of this page. Also, in Systema materiae medicae vegetabilis brasiliensis (Martius 1843), Prodromus Systematis Naturalis (Bossier 1862) and Flora Brasiliensis (Argoviensis 1874) there are no mention of the locality Sebastianopolis, instead, all works mention “deserto Bahiensi”. Also, a collection from G that is probably a duplicate of the original collection doesn’t present this label, but one that also is written “desertum Bahia”. Martius´s report about the dense populations of *E. phosphorea* in the region of Serra do Sincorá, in Bahia, one of the driest regions of Brazil, and the great number of populations of *Euphorbia phosphorea* found in many localities of Bahia and other states encompassed by the Caatinga Biome during the development of this study, can support that *E. phosphorea* is a good indicator species of caatinga, since it is endemic and widely spread in this vegetation.

Euphorbia phosphorea is easily recognized by its eight ribs branches, shallowly channeled (Fig. 6c), with only two of the three ribs that arising from each node reaching the upper node, with spiral phyllotaxy and patent appendages (Fig. 2a, c and Fig. 3i-k, n-t).

Etymology. — The name of the species came from the phosphorescence of its latex that Martius (1828) described in Reise Brasilian. In the literary work: Os Sertões (Cunha 1984), the author also describes this phosphorescence, as well as some collectors in the labels. Rodrigues (1887) observed that the phosphorescence of *E. phosphorea* might be due to association with fungi. However, we could not see the phosphorescence in any of the 17 localities visited during the development of this work. Looking into publications about bioluminescent fungi, authors states that they are mainly restricted to humid habitat of evergreen forests, growing and extracting nutrients from litter and rotten wood (Desjardin et al. 2007; Fraga 2008).

Field observations. — The habit of this species seems to change under different edaphic conditions (Fig. 4c-h, k-n). When on sandy-soils with gravels, apparently deeper than stone slabs and other rocky soils, individuals formed dense clamps and bushes, also leaning on shrub and tree individuals that were close to, scrambling towards a greater height. This habit was perfectly described in Os Sertões (Cunha 1984) and also reported by Machado (2000). Sometimes, when *E. phosphorea* presents a scrambling habit, looks like a woody climbing plant, pending from other supporting shrubs or trees as a liana or woody vine. Frequently we could see decumbent stems reaching the floor, growing roots, and young new branches arising and forming a new individual shrub, losing connection to the mother plant, comparable to vegetative propagation (Fig. 4n). This habit was not seen in other species of section *Brasilienses*, with exception of *E. attastoma* var. *xanthochlora* that can also be found as a decumbent shrub (see comments of this species). However, only *E. phosphorea* grows in sandy soils (Fig. 4e), all others are restricted to rocky outcrops of campos rupestres, rocky caatinga and inselbergs. When found on stone slopes and inselbergs, *E. phosphorea* presents as an erect shrub, usually candelabriform, frequently with a small trunk, reaching lower heights. The tallest individuals of *E. phosphorea* were those leaning on others. Intriguing, Machado (2000) reports that when on rocks the individuals could not form a small trunk always branching from the ground level, and when growing on sandy soils of caatinga the author comment about the small trunks. During our field collections many individuals growing on inselbergs presented small trunks (Fig. 4d, m), and the ones found in sandy soil of the caatinga were very much branched from the base. Machado (2000) also infer that rock soils are might not be the preferable habitat for this species, and we believe the author was talking about the campos rupestres of the rocky outcrops of Chapada Diamantina (Espinhaço Range). Indeed, *Euphorbia phosphorea* is not found in campos rupestres, however, it is found on many inselbergs from four different states where caatinga is the predominant physiognomy (Rio Grande do Norte, Paraíba, Pernambuco and Bahia).

Popular usage. — *Euphorbia phosphorea* is widely used for creating and enhance fire by rural residents (personal communication) that cut and let dry its stems. Possibly, the abundant wax of the stems is responsible to enhance the fire. Most common popular names are: cunanã, cipó-de-cunanã, avelós, avelós-de-pedra, cipó-de-leite, bilô, cachorro-pelado, pau-de-leite, pinga-leite.

Additional specimens examined. — BAHIA: Bendegó, 26 Nov. 2014, *Hurbath & al. 755* (SP); Boa Nova, 23 Mar. 2013, *Machado & al. 1221* (HUEFS); Cafarnaum, 11 Aug, 2001, *Aona & al. 819* (UEC); Campo Formoso, 8 Mar. 1974, *Harley 16960* (CEPEC, NY, P, RB, US); Canudos, 26 Nov. 2014, *Hurbath & al. 760* (SP); Euclides da Cunha, 5 Jan. 1991, *Taylor & al. 1359* (CEPEC, HRCB); Gentio do Ouro, 24 Aug, 2014, *Hurbath & al. 657* (SP); Iaçu, 21 Aug, 2014, *Hurbath & al. 629* (SP); Itaberaba, 14 Sep. 1984, *Hatschbach 48193* (CEPEC, MBM, US); Itatim, 21 Aug, 2014, *Hurbath & al. 622* (SP); Itatim, 21 Aug, 2014, *Hurbath & al. 623* (SP); Jaguaquara, 6 Oct. 1972, *Pinheiro 1987* (CEPEC); Jeremoabo, 25 Nov. 2014, *Hurbath & al. 753* (SP); Juazeiro, 16 Fev. 1986, *Fernandes s.n.* (RB 366783); Maracás, 23 Jun. 2010, *Queiroz 4606* (HRB); Milagres, 21 Aug, 2014, *Hurbath & al. 628* (SP); Morro do Chapéu, 23 Aug, 2014, *Hurbath & al. 654* (SP); Mucugê, 26 Aug, 2014, *Hurbath & al. 671* (SP); Paulo Afonso, 24 Nov. 2014, *Hurbath & al. 747* (SP); Queimadas, 9 Jun. 1915, *Rose & Russel 19848* (NY, US); Santa Brígida, 27 Nov. 2014, *Hurbath & al. 763* (SP); Santana, 13 Jul. 2007, *Silva & Ratusniak 1556* (RB); Sento Sé, 1 Apr. 2002, *Leite & al. 184* (HUEFS); Serrolandia, 22 Aug, 1980, *Fonseca 322* (HRB); Sertão, 20 Sep. 1965, *Duarte 9685* (RB); Tamburuí, 25 Jan. 1965, *Pabst & Pereira 8622* ((vazio)); Tanhaçu, 28 Aug, 2014, *Hurbath & al. 727* (SP); Tucano, 23 Mar. 1993, *Queiroz & Sena 3110* (CEPEC, HUEFS, MBM, UB); Uauá, 30 Mar. 2000, *Saar & al. 12* (ALCB, HUEFS, SPF); Urucuri, 12 Sep. 1956, *Pereira 2155* (RB). PARAÍBA: Cabaceiras, 22 Mar. 2007, *Lucena & al. 1856* (UFP); Cabrobó, 17 Fev. 2015, *Hurbath & al. 795* (SP); Esperança, 19 Jan. 1977, *Barbosa 563* (RB); Junco do Seridó, 21 Fev. 1999, *Schlindwein 925* (JPB); Pedra Lavrada, 12 Fev. 2015, *Hurbath & al. 773* (SP); Pocinhos, 31 Oct. 2007, *Gadelha Neto & Lima 1936* (JPB); Princesa, 12 Jan. 1912, *Xavier s.n.* (JPB 574); Puxinãnã, 29 Aug, 2008, *Brasileiro 17* (UEC); São João do Cariri, 25 Fev. 2005, *Lacerda & Barbosa 376* (JPB, SP); São João do Tigre, 13 Mar. 2010, *Gadelha Neto & al. 2732* (HUEFS, JPB); São José dos Cordeiros, 12 Fev. 2015, *Hurbath &*

al. 768 (SP); Serra Branca, 31 Mar. 2009, *Amorim & Costa 463* (UFP). PERNAMBUCO: Alagoinha, 22 Sep. 1999, *Krause & Liebig 151* (PEUFR, RB); Brejo da Madre de Deus, 15 Fev. 2015, *Hurbath & al. 782* (SP); Pesqueira, 19 Jun. 2005, *Oliveira 1836* (SPF, UFP); São Caetano, 20 Mar. 2010, *Mendes & al. 425* (UFP). RIO GRANDE DO NORTE: Equador, 12 Aug, 2015, *Moura & al. 404* (UFRN); Monte Gameleiras, 5 Nov. 2016, *Garcia & Gonçalves 425* (UFRN); Serra Caiada, 28 Jun. 2016, *Francener & al. 1462* (UFRN). SERGIPE: Canindé de São Francisco, 27 Nov. 2014, *Hurbath & al. 766* (SP); Poço Redondo, 25 Jan. 1985, *Fotius 3930* (HUEFS).

Euphorbia sipolisii N.E. Br., Bull. Misc. Inform. Kew 1893 (79): 158. 1893. Type: Brazil, Minas Gerais, 1992, *Glaziou s. n.* (Holotype: K- 000253928 (digital image!))

Shrub candelabriform to caespitose, erect, individuals not developing trunk, 0.6-1 m high. Branches shallowly channeled, with 4-ribs, rarely 5-ribs, with three ribs arising from one node and only one reaching the upper one, dark green, red to cuprous when young. Leaves green, 3-7 x 2.8-3 mm, distichous (Fig. 6d). Monochasial 3.5-4 mm, glomeruliform, usually with 2-3-cyathia. Cyathophylls 1.4-2.1 x 1.5-2.3 mm. Cyathia involucre 3-4.7 x 2.9-5.8 mm, campanulate, vinaceous; lobes 4-6, 1-2 x 1-1.7 mm, glands 4-6, 1.1-1.6 x 1.2-2.9 mm, concave, vinaceous, appendages conspicuous, patent, 1-2.4 mm long, vinaceous. Staminate flowers in 5 cymules of 3-5 flowers, pedicels with 1.2-4.4 mm long. Pistillate flowers with pedicels 3.1-3.5 mm long; no perianth-like whorl. Ovary 2.5-3.3 x 2.7-3.1 mm; styles 1.3-1.8 mm long, connate 1/3. Fruits with prominent keel, 4.1-4.7 x 4.9-5.6 mm, vinaceous, or yellow with vinaceous strips on septum and along the keel; columella 3.4-3.9 mm. Seeds 3.2-3.4 x 2.2-2.9 mm.

Taxonomic notes. — This species was described based on a Glaziou collection from 1892, without number and precise location, only mentioning the state of Minas Gerais. We believe that Diamantina is probably where the type specimen was collected, since during the present work it was only encountered in localities in this municipality or very near, and the species name is in honor to the director of Seminary of Diamantina.

This species only occurs in campos rupestres reaching the highest altitudes (1300 m) in the section. Machado (2000) mentioned he encountered many individuals of this species with five ribs, while we found rare individuals bearing five-ribs branches, and yet only few branches of a plant. Till recently this was the only species with four ribbed branches, however, it was recently discovered a new species in the municipality of Monte Azul (Hurbath et al., 2018).

Euphorbia sipolisii can form dense clumps of shrub, like *E. phosphorea,* however usually less than 1 meter high. The dark green color of the stems gives an aspect of older individuals, also, in comparison to *E. tetrangularis,* the branches of *E. sipolisii* are thicker. This species can be recognized as a lower height shrub, caespitose, frequently forming dense clumps, four-ribs branches, red to vinaceous involucre and glands (Fig. 3u-v), and patent appendages, also vinaceous.

Etymology. — The species was named after Abbé Michel Marie Sipolis, director of the Seminary of Diamantina (Minas Gerais).

Popular usage. — No use was mentioned, however the popular name vela-de-garimpeiro was written on the label of a collection from a mineral extraction site in Boa vista locality (Diamantina).

Additional specimens examined. — MINAS GERAIS: Datas, 5 Fev. 1972, *Anderson & al. 35515* (NY, HRB); Diamantina, 3 Apr. 2016, *Hurbath & al. 828* (SP); Gouveia, 10 Aug, 1998, *Stehmann 2386* (BHCB); São Gonçalo do Rio das Pedras, 18 Fev. 2009, *Cordeiro & al. 3039* (SP); São Gonçalo do Rio Preto, Jun. 1999, *Salino 4844* (BHCB); Serro, 4 Apr. 2016, *Hurbath & al. 830* (SP).

Euphorbia tetrangularis Hurbath & Cordeiro, Systematics and Biodiversity 0(0): 1–10. 2018. Type: Monte Azul: Serra Montevideu, à leste da cidade, 15°08'36S, 42°47'30W, 23 Aug 2016, *F. Hurbath* 844. (Holotype: SP- 497680!)

Shrub candelabriform, erect, individuals can develop a small trunk, 0.5-1.6 m high. Branches shallowly channeled, with 4-ribs, with only one of the three ribes arising from each node and reaching the upper node, green, cuprous when young. Leaves green, 6.7-11 x 2.2-3 mm, distichous. Monochasia 2.3-5 mm, glomeruliform, usually with 2-3(5)cyathia. Cyathophylls 2–3 x 1.2–2.5 mm. Cyathia involucre 4–5 x 3.5 mm, campanulate, green; lobes 4-5, 1–1.5 x 1.5 mm, glands 4-5, 1–1.5 x 1.5–2.5 mm, concave, usually dark-green becoming yellowish, appendages conspicous, erect, 2.7-3 mm long, whitish to light green. Staminate flowers in 5 cymules of 4-7 flowers, pedicels with 2-4.5 mm long. Pistillate flowers with pedicels 1.3-3 mm long; usually with perianth-like whorl with 3 filiform lobes, caducous. Ovary 1.5–3.5 x 1.3–3 mm; styles 1.3–2.5 mm long, connate up to 1/2. Fruits with prominent keel, 5–7 x 4–7 mm, green frequently with vinaceous strips on septum and along the keel; columella 3-5 mm. Seeds 3–3.2 x 2.5–2.9 mm.

Taxonomic notes. — Recently described, already classified as Critically Endangered (CR) according to criteria of the International Union for Conservation of Nature (IUCN 2001), this is a rare species, micro endemic to the rocky caatingas of Monte Azul municipality (MG), between altitudes of 1098 and 1147 m. It is very closely morphologically to *E. sipolisii,* also presenting four-ribs branches (Fig. 6e). However, its cyathia are like those of *E. holochlorina*, with green involucre and glands with erect appendages (Fig. 2i and Fig. 3w). Known from one single locality, occurs near the northern limits of *E. attastoma.* Four ribbed branches, green involucre and glands, erect appendages, and perianth-like whorl in the pistillate flowers characterize this species.

Etymology. — The name for this species comes from the four ribbed branches.

Additional specimens examined. — MINAS GERAIS: Monte Azul, 28 Jan. 1991, *Taylor & Eggli 1472* (BHCB, HRCB).

List of Exsiccatae. — Agra, M.F.: 4145 (EP). Almeida, T.E.: 1442 (ES). Amorim, A.M.: 726 (EP), 8286 (EP). Amorim, B.S.: 463 (EP). Anderson, W.R.: 35515 (ES). Andrade-Lima, D.: 1063 (EP), 70-6146 (EP), s.n.(ALCB 2167) (EP). Aona, L.Y.S.: 819 (EP), 2167 (EP). Araujo, A.P.: 150 (EP). Arbo, M.M.: 5159 (ES). Atui, J.P.: 41 (ES). Barbosa, V.P.: 499 (EP), 563 (EP). Barbosa-Silva, R.G.: 586 (EP), 942 (ES). Barreto, M.: 9645 (ES). Bastos, B.C.: 200 (EP). Belém, R.P.: 1623 (EH). Benko-Iseppon, A.M.: 336 (ES). Bondar, G.: s.n.(V0194601F) (EP), s.n.(V0194603F) (EP). Borba, E.L.: 2042 (EP), 2217 (EA). Brasileiro, J.C.B.: 17 (EP). Cardoso, D.: 885 (EP). Carneiro-Torres, D.S.: 513 (EP), 980 (EP). Carrión, J.F.: 1735 (EP). Caruzo, M.B.R.: 145 (EP). Carvalho, A.M.: 2428 (EP). Carvalho, M.G.: 411 (EA), 415 (EA). Cavalcanti, T.B.: CFCR 10297 (ES), CFCR 8086 (EA), CFCR 9691 (EA). Cerati, T.M.: 219 (EA). Coelho, D.M.: 208 (EP). Coradin, L.: 6235 (EP). Cordeiro, I.: 2204 (EP), 3039 (ES), 3748 (EP), CFCR 9286 (ES). Costa, A.L.: s.n.(ALCB 2166) (EP). Costa, J.M.: 944 (EA). Costa, R.M.T.: 21 (EP), 40 (EP). da Silva, S.I.: 40 (EP). Davis, P.H.: 61118 (EP). Delfini, C.: 379 (ES). Duarte, A.P.: 3987 (EH), 9290 (EA), 9685 (EP), 9701 (EA), s.n.(RB 233466) (EA). Eggli, U.: 1190 (EP). Equipe Projeto Abelhas: 273 (EP). Farinaccio, M.A.: 534 (ES). Fernandes, A.: s.n.(EAC 14032) (EP). Fernandes, A.M.: s.n.(RB 366783) (EP). Ferreira, M.C.: 1292 (EP). Ferreira-Junior, C.A.: s.n.(ALCB 118780) (EA). Fonseca, W.: 322 (EP). Fontana, A.P.: 908 (EH). Forzza, R.C.: 934 (EH), 4857 (ES). Fotius, G.: 3930 (EP). França, F.: 1401 (EP), 1597 (EP), 2865 (EP), 4094 (EP), 4369 (EA), 5137 (EP). Francener, A.: 1462 (EP). Francisco, S.F.S.: 1 (EA). Furlan, A.: CFCR 310 (EP). Gadelha Neto, P.C.: 1688 (EP), 1936 (EP), 2732 (EP). Garcia, G.S.: 425 (EP). Gibbs, P.: 2282 (EA). Giulietti, A.M.: CFCR

2239 (ES), CFCR 3546 (EA), PCD 3268 (EP). Glaziou, A.F.M.: s.n.(K 000253928) (ES). Gomes, F.S.: 118 (EP). Gottsberger, I.: 21 (EP). Guedes, M.L.: 9883 (EP), 10875 (EP), 14331 (EA), 14332 (EA), 17091 (EP), 22681 (EA). Harley, R.M.: 16960 (EP), 19423 (EP), 25192 (EA), 54310 (EP), CFCR 6253 (ES). Hatschbach, G.: 28963 (ES), 48193 (EP), 52023 (EA), 67976 (EA), 69419 (EA), 75814 (EP). Hurbath, F.: 222 (EA), 410 (EA), 510 (EA), 580 (EA), 622 (EP), 623 (EP), 628 (EP), 629 (EP), 635 (EH), 645 (EP), 654 (EP), 655 (EP), 657 (EP), 670 (EP), 671 (EP), 727 (EP), 728 (EA), 729 (EA), 730 (EA), 747 (EP), 752 (EP), 753 (EP), 755 (EP), 760 (EP), 763 (EP), 766 (EP), 768 (EP), 773 (EP), 782 (EP), 795 (EP), 824 (EA), 828 (ES), 830 (ES), 834 (EH), 835 (EH), 836 (EH), 837 (EA), 838 (EA), 839 (ET), 844 (ET), 853 (EA). Irwin, H.S.: 23359 (EA), 27091 (EA), 27922 (ES), 30676 (EP), 32576 (EP), 23453a (EA). Isejima, E.M.: CFCR 5601 (ES). Jardim, J.G.: 3474 (EA). Kawasaki, M.L.: 1093 (EA), CFCR 8301 (EA). Krause, L.: 111 (EP), 126 (EP), 151 (EP). Kuhlmann, H.G.: s.n.(RB 17934) (EP). Lacerda, A.V.: 376 (EP). Leite, K.R.B.: 184 (EP). Leoni, R.A.: 37 (EP). Lima, A.A.A.: 5 (ES). Lombardi, J.A.: 9048 (EA). Lucena, M.F.A.: 1856 (EP). Machado, A.F.P.: 1221 (EP). Machado, R.F.: 503 (EP). Magalhães, G.M.: 18361 (EA). Mamede, M.C.H.: CFCR 6348 (EA). Marinho, L.C.: 402 (EP). Martinelli, G.: 5836 (EA), 11234 (EA), 18190 (ES), 19230 (EA). Martius, C.F.P. von: s.n.(G 00441558) (EP), s.n.(G 00441559) (EP), s.n.(M 0241970) (EP). Meireles, L.D.: 1140 (EA). Mello-Silva, R.: 3195 (EA). Melo, E.: 1173 (EP), 4585 (EP), 5782 (EP), 6020 (EP), 7247 (EP), 7256 (EP). Mendes, K.: 425 (EP). Mendes, M.S.: 381 (EA). Miranda, A.M.: 5525 (EP). Miranda-Silva, E.: 224 (EP). Moonlight, P.W.: 338 (EP). Moura, D.: 706 (EP). Moura, E.O.: 404 (EP), 461 (EP). Munhoz, C.B.R.: 7229 (ES). Nascimento, L.M.: 518 (EP). Ogasawara, H.A.: 369 (EP). Oliveira, A.: 89 (EP). Oliveira, J.O.S.: s.n.(EAC 49887) (EP). Oliveira, M.: 1836 (EP), 2589 (EP). Pabst, G.: 8622 (EP). Paula-Souza, J.: 5505 (EP). Pereira, E.: 2155 (EP). Pignal, M.: 3332 (EP). Pinheiro, R.S.: 1987 (EP). Pinto-Junior, H.V.: 189 (EH). Pirani, J.R.: CFCR 12916 (EA), CFCR 912 (EA). Pscheidt, A.C.: 162 (EA). Queiroz, L.P.: 739 (EP), 1636 (EP), 3110 (EP), 4606 (EP), 7615 (ES), 13191 (EP). Ribas, O.S.: 7743 (EA). Rizzini, C.T.: 37 (EA). Rocha, D.: 336 (EP), 357 (EP). Rodrigues, L.: 10 (EP). Romero, R.: 5463 (EA). Roque, A.A.: 1834 (EP). Roque, N.: 1168 (EP), CFCR 15035 (EA), PCD 2262 (EP). Rose, J.N.: 19848 (EP). Saar, E.: 12 (EP). Saavedra, M.M.: 952 (EP). Saint-Hilaire, A.F.C.P.: s.n.(P 05506773) (EP). Salino, A.: 4844 (ES). Santos, A.K.A.: 375 (EP). Scatigna, A.V.: 168 (EA). Schlindwein, C.: 925 (EP). Sessegolo: 163 (EP). Shepherd, G.J.: 3990 (ES). Silva, B.R.: 1440 (EA), 1556 (EP). Silva, O.L.M.: 134 (EH), 144 (EP), 279 (EH), 282 (EH). Silva, R.A.: 243 (EP). Silva, T.T.S.: 6 (EP). Simão, R.: CFCR 8801 (ES). Simões, A.O.: 1169 (EA), 1297 (EA). Souza, V.C.: 5187 (EA), 22573 (EA), 25551 (EA), 26545 (EP). Stehmann, J.R.: 2386 (ES). Tameirão Neto, E.:

1865 (ES), 2376 (EH), 3338 (EA). Tavares, P.: 3 (EP). Taylor, N.P.: 1359 (EP), 1472 (ET), 1511 (EA), 1581 (EP). Vasconcelos, M.F. de: s.n.(BHCB 39966) (EH), s.n.(BHCB 40161) (ES). Verdi, M.: 6933 (ES). Wanderley, M.G.L.: 1384 (EA). Xavier, L.P.: s.n.(JPB 574) (EP). Zappi, D.C.: CFCR 11105 (ES).

Acknowledgments

We are very thankful to Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP 2015/11812-1) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 309917/2015-8) for financial support, Natanael dos Santos for the illustration and Otávio Luis Marques da Silva for the production of photo plates and providing some photos from his personal archive.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Argoviensis JM (1874) Euphorbia. In: Martius CFP von, August Wilhelm E, Urban I (eds) Flora Brasiliensis, Fasc. 64. Centro de Referência em Informação Ambiental-CRIA, pp 665–702

Aximoff I, Felix E (2017) Lygodactylus klugei (Kluge's Dwarf Gecko). DIET. Herpetol Rev 48:439

Beentje H (2010) The Kew Plant Glossary: an illustrated dictionary of plant terms. Royal Botanic Gardens, Kew

Berger A (1906) Sukkulente Euphorbien. Eugen Ulmer, Stuttgart

Bossier E (1862) suborbo I. Euphorbieae. In: de Candolle AP (ed) Prodromus systematis naturalis regni vegetabilis. Parisiis, Paris, pp 3–186

Bruyns P V., Mapaya RJ, Hedderson T (2006) A new subgeneric classification for Euphorbia (Euphorbiaceae) in southern Africa based on ITS and psbA-trnH sequence data. Taxon 55:397–420. doi: 10.2307/25065587

Cunha E da (1984) Os Sertões. Três, São Paulo

Desjardin DE, Capelari M, Stevani C (2007) Bioluminescent Mycena species from São Paulo, Brazil. Mycologia 99:317–331. doi: 10.1080/15572536.2007.11832592

Dorsey BL, Haevermans T, Aubriot X, et al (2013) Phylogenetics, morphological evolution, and classification of. Taxon 62:291–315. doi: https://doi.org/10.12705/622.1

Eggli U (1994) Xerophytic euphorbias from Brazil. Euphorbia J 2–23

Fraga H (2008) Firefly luminescence: A historical perspective and recent developments. Photochem Photobiol Sci 7:146. doi: 10.1039/b719181b

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

Gagliardi KB, Cordeiro I, Demarco D (2016) Protection and attraction: Bracts and secretory structures in reduced inflorescences of Malpighiales. Flora Morphol Distrib Funct Ecol Plants 220:52–62. doi: 10.1016/j.flora.2016.02.003

Govaerts R, Frodin DG, Radcliffe-smith A (2000) World Checklist and Bibliography of Euphorbiaceae (and Pandaceae). Royal Botanic Gardens, Kew

Harris JG, Harris MW (1994) Plant identification terminology: an illustrated glossary, 2nd edn. Spring Lake Publishing, Utah

Horn JW, van Ee BW, Morawetz JJ, et al (2012) Phylogenetics and the evolution of major structural characters in the giant genus Euphorbia L. (Euphorbiaceae). Mol Phylogenet Evol 63:305–326. doi: 10.1016/j.ympev.2011.12.022

Horn JW, Xi Z, Riina R, et al (2014) Evolutionary bursts in Euphorbia (Euphorbiaceae) are linked with photosynthetic pathway. Evolution (N Y) 68:3485–3504. doi: 10.1111/evo.12534

International Union for Conservation of Nature [IUCN] (2001) IUCN red list categories and criteria: version 3.1

Machado M (2000) Euphorbia phosphorea - the largest growing Brazilian euphorbia. Br Cactus Succul J 18:151–158

Martius KFP von (1843) Systema materiae medicae vegetabilis brasiliensis. apud F. Fleischer; apud F. Beck in comm., Lipsiae,Vindobonae, Park K-R, Jansen RK (2007) A phylogeny of Euphorbieae subtribe Euphorbiinae (Euphorbiaceae) based on molecular data. J Plant Biol 50:644–649. doi: 10.1007/BF03030608

Pax F, Hoffman K (1931) Euphorbiaceae. In: Engler A (ed) Das Pflanzenreich IV, Heft 52. Engelmann, Leipzig, pp 210–214

Peirson JA, Bruyns P V., Riina R, et al (2013) A molecular phylogeny and classification of the largely succulent and mainly African Euphorbia subg. Athymalus (Euphorbiaceae). Taxon 62:1178–1199. doi: 10.12705/626.12

Prenner G, Rudall PJ (2007) Comparative ontogeny of the cyathium in Euphorbia (Euphorbiaceae) and its allies: Exploring the organ-flower-inflorescence boundary. Am J Bot 94:1612–1629. doi: 10.3732/ajb.94.10.1612

Radcliffe-smith A (2001) Genera Euphorbiaceaerum. Royal Botanic Gardens, Kew

Radford AE, Dickison WC, Massey JR, Bell CR (1974) Vascular Plant Systematics. Harper & Row, New York, San Francisco, Evanston, London

Rizzini C (1989) Cactiform species of Euphorbia from Brazil (Euphorbiaceae). Rev Bras Biol 4:979–997

Rodrigues B (1887) O tamakoaré: espécies novas da ordem das Ternstroemiaceas. J do Amaz

Royal Botanic Gardens K (1893) Bulletin of miscellaneous information /. H.M. Stationery Office, London :

Särkinen T, Iganci JRV, Linares-Palomino R, et al (2011) Forgotten forests - issues and prospects in biome mapping using Seasonally Dry Tropical Forests as a case study. BMC Ecol 11:. doi: 10.1186/1472-6785-11-27

Spix JB, Martius CFP (1828) Reise in Brasilien auf Befehl Sr. Majestät Maximilian Joseph I. König von Baiern in den Jahren 1817-1820 gemacht und beschrieben. Teil 2. 415–884

Steinmann VW, Porter JM (2002) Phylogenetic Relationships in Euphorbieae (Euphorbiaceae) Based on ITS and ndhF Sequence Data. Ann Missouri Bot Gard 89:453. doi: 10.2307/3298591

Tedeschi M, Novo T, Pedrosa-Soares A, et al (2016) The Ediacaran Rio Doce magmatic arc revisited (Araçuaí-Ribeira orogenic system, SE Brazil). J South Am Earth Sci 68:167–

186. doi: 10.1016/j.jsames.2015.11.011

Weberling F (1989) Morphology of Flowers and Inflorescences. Press Syndicate of the University of Cambridge, Cambridge

Webster G (1975) Conspectus of a new classification of the Euphorbiaceae. Taxon 24:593–601

Webster GL (1967) The Genera of Euphorbiaceae in the southeastern United States. J Arnold Arbor 48:303–361

Webster GL (1994) Synopsis of the Genera and Suprageneric Taxa of Euphorbiaceae. Ann Missouri Bot Gard 81:33–144. doi: 10.2307/2399909

Wheeler LC (1943) The genera of the living Euphorbieae. Am Midl Nat 30:456–503

Yang Y, Riina R, Morawetz JJ, et al (2012) Molecular phylogenetics and classification of Euphorbia subgenus Chamaesyce: a group with high diversity in photosynthetic systems and growth forms. Taxon 61:764–789.

Figure legends

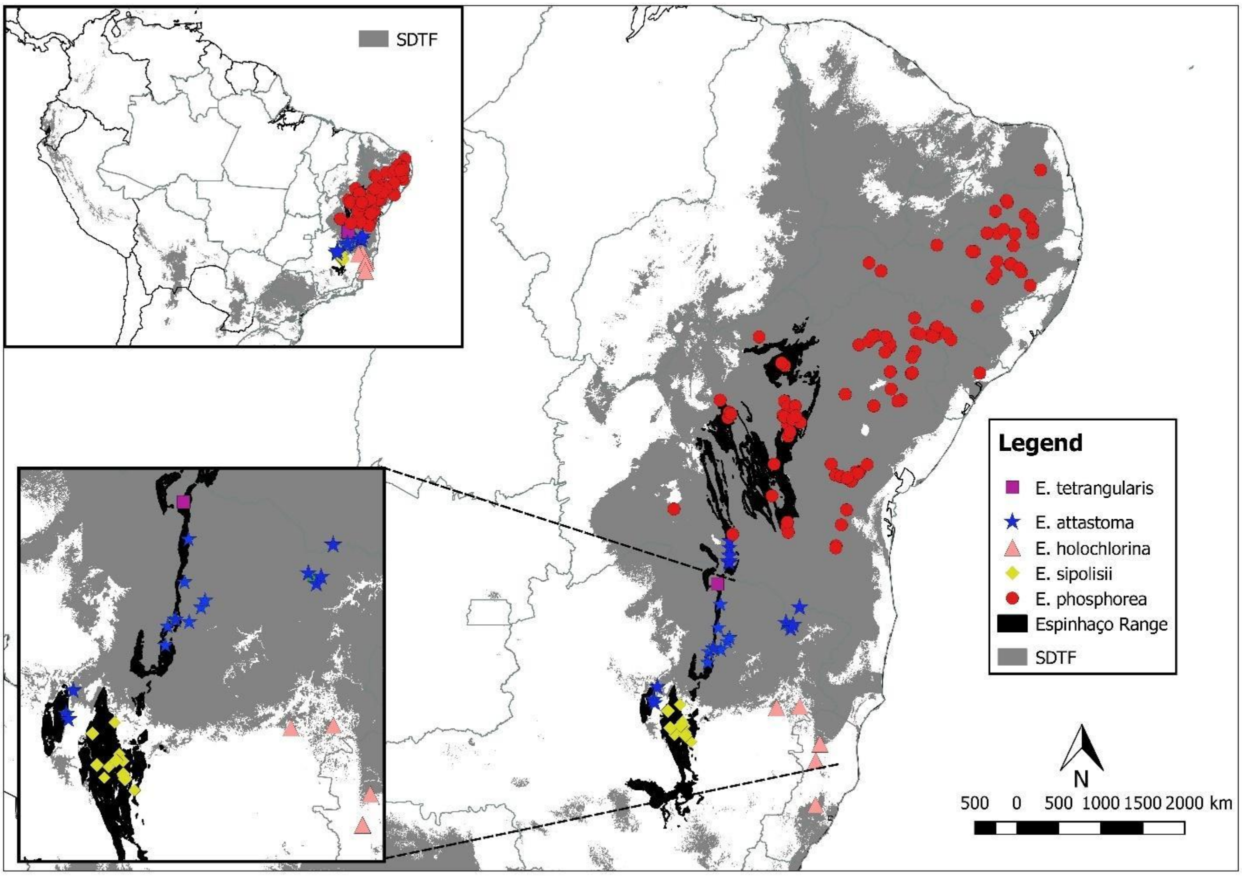


Fig. 1 Geographic distribution of *Euphorbia* sect. *Brasilienses.*

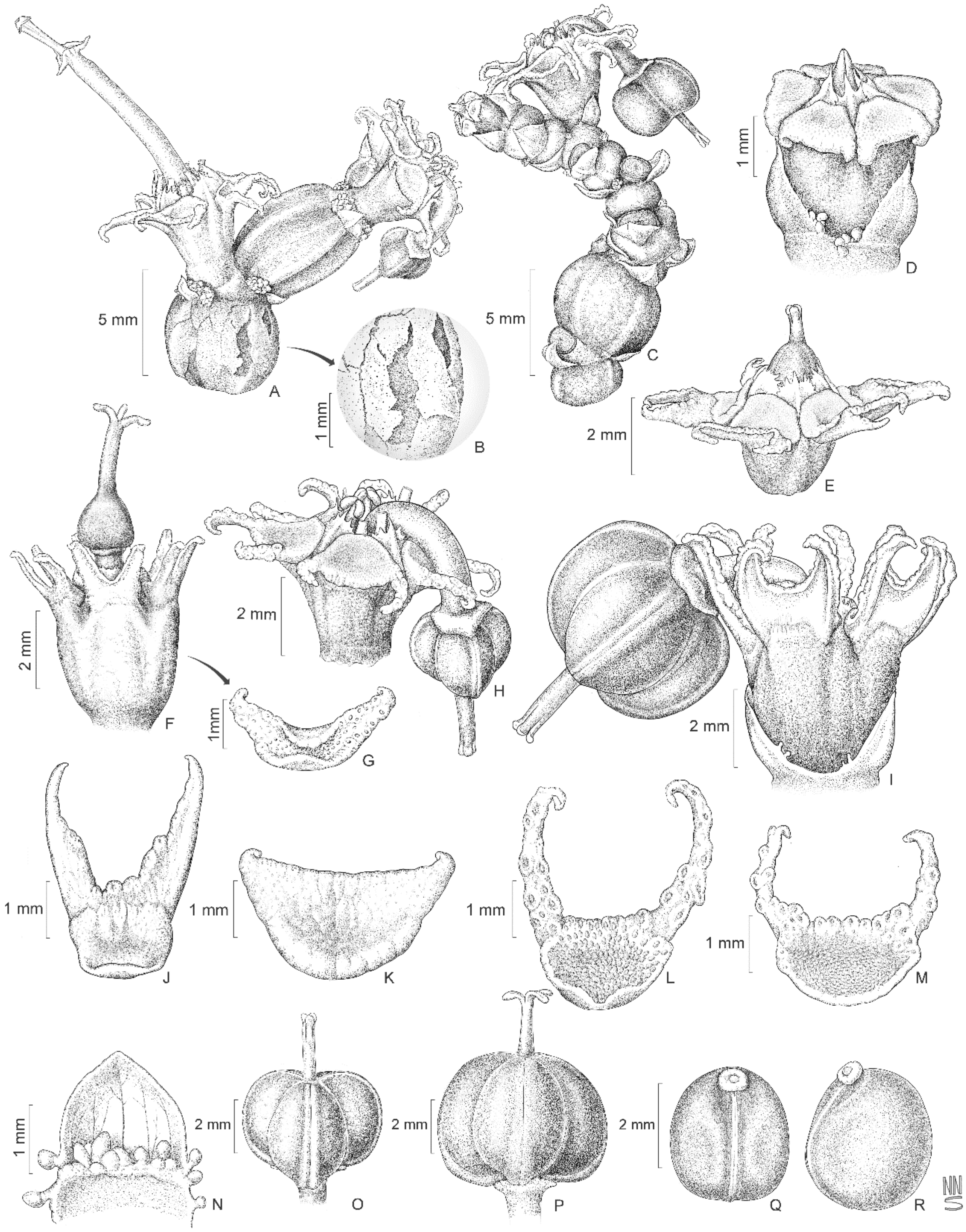


Fig. 2 Floral characters of *Euphorbia* sect. *Brasilienses* species. (a-c) ***E. phosphorea***:detail of a monochasium; (b) Detail of tick layer of wax present on axis; (c) spiciform monochasium (d-e, h) ***E. attastoma***: (d) young cyathium; (e) cyathium with young fruit; (h) cyathium with fruit; (f-g) ***E. holochlorina***: (f) cyathium with erect gland appendages; (g) cyathia gland conduplicate; (i) ***E. tetrangularis***: cyathium with a fruit; (j-m) cyathia glands : (j-k) ***E. attastoma***: (j) Grão Mogol, (k) Rio Pardo de Minas; (l-m) ***E. phosphorea***; (n) Cyathophyll with coleteres; (o-p) fruits: (o) ***E. phosphorea***; (p) ***E. holochlorina***; (q-r) seeds of ***E. tetrangularis***: (q) ventral view; (r) side view.

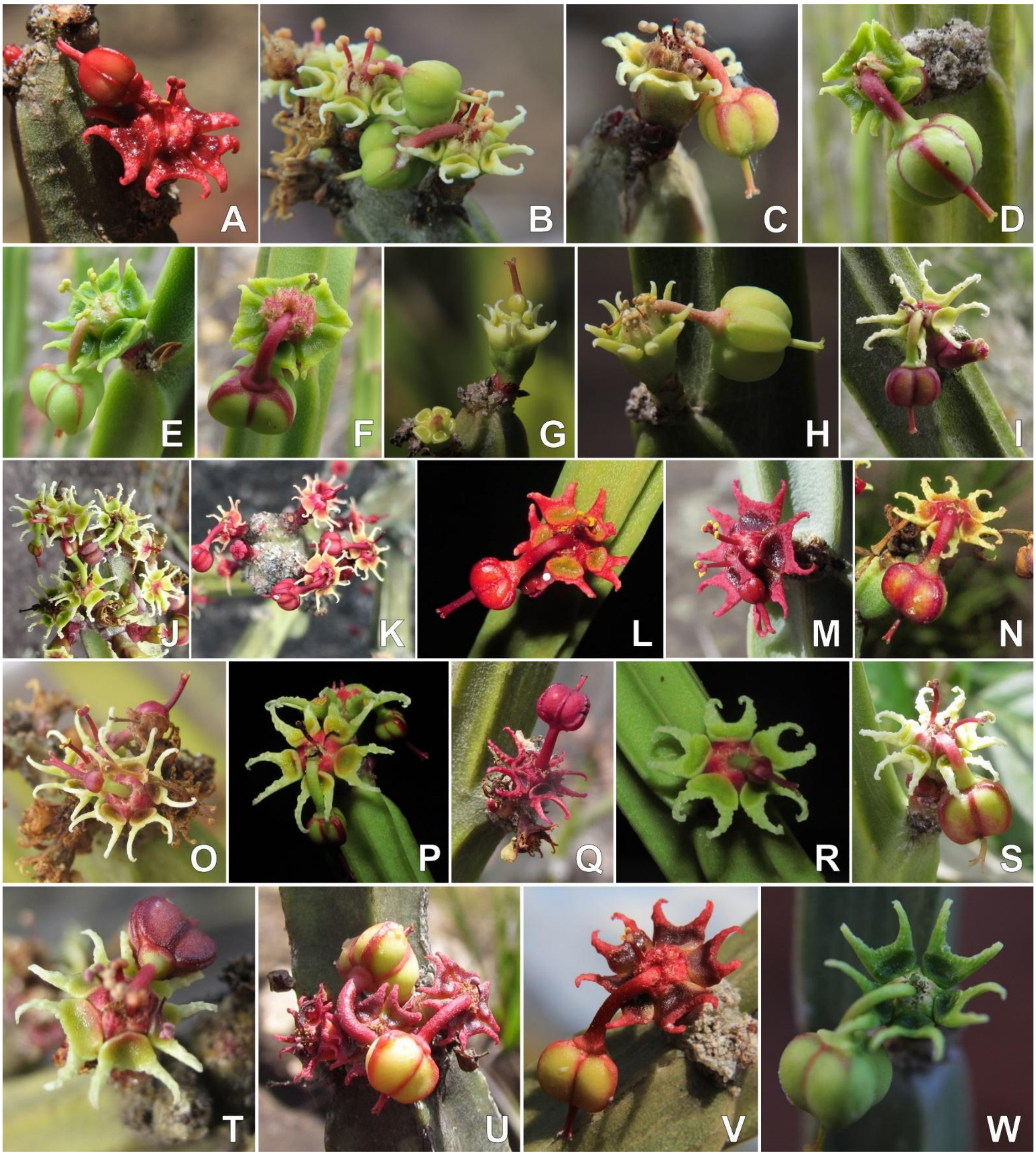


Fig. 3 Cyathia of *Euphorbia* sect. *Brasilienses* species. (a-f) ***E. attastoma***: (a) Joaquim Felício/MG; (b-c) Pedra Azul/MG; (d-f) Rio Pardo de Minas/MG; (g-h) ***E. holochlorina***: (g) Pedra da Baleia/MG; (h) Vila Pavão, Pedra Trigêmeas/ES; (i-l) ***E. phosphorea***: (i-k) Brejo Madre de Deus/PE; (l) Gentio do Ouro/BA; (m) ***E. attastoma*** - Grão Mogol/MG; (n-t) **E. phosphorea**: (n) Itatim/BA; (o) Ituaçu/BA; (p) Milagres/BA; (q) Morro do Chapéu/BA; (r) Mucugê/BA; (s) Paulo Afonso/BA; (t) São José Cordeiros/PB; (u-v) ***E. sipolisii***: (u) Diamantina/MG; (v) Serro/MG; (w) ***E. tetrangularis*** - Monte Azul/MG.

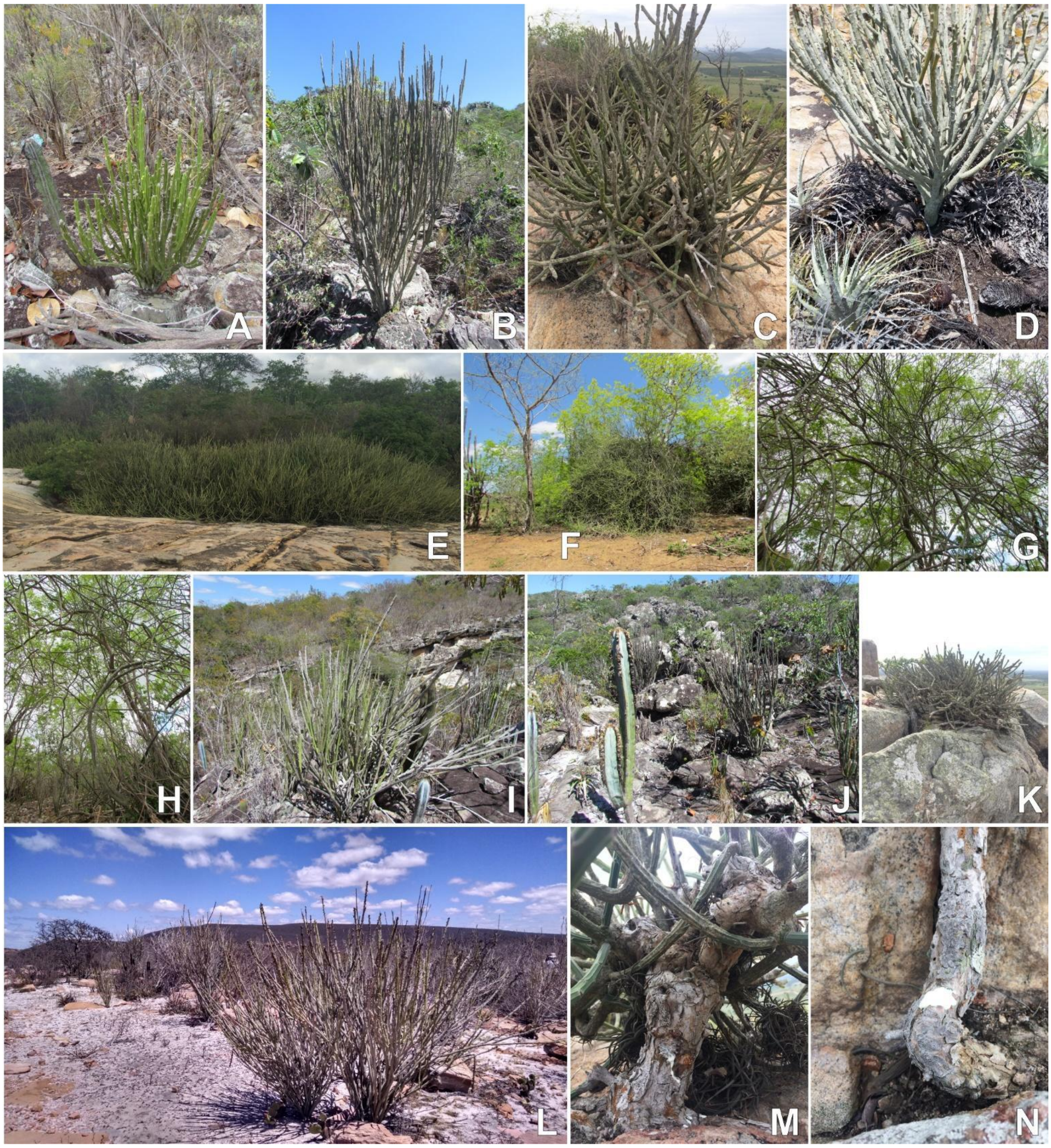


Fig. 4 Variety of habit and habitat of *Euphorbia* sect. *Brasilienses* species, with locality and estimated time of observation. (a-b) ***E. attastoma*** - Shrub - rocky caatinga – (a) Rio Pardo de Minas/MG, (b) Grão Mogol/MG; (c-d) ***E. phosphorea*** - inselberg - (c) Serra Caiada/RN, (d) Cabrobó/PE (shrub with small trunk); (e) ***E. phosphorea*** - Dense clumps of shrubs – inselberg – São José dos Cordeiros/PB**;** (f-h) ***E. phosphorea*** - shrub with decumbent to scrambling branches - sandy soils of caatinga - Raso da Catarina/BA; (i-j) ***E. attastoma*** - (i) caespitose shrub - rocky caatinga - Rio Pardo de Minas/MG, (j) candelabriform shrub – rocky caatinga - Grão Mogol/MG; (k-l) ***E. phosphorea*** - (k) caespitose shrub - inselberg - Serra Caiada/RN; (l) candelabriform - stone slabs - Morro do Chapéu/BA; (m-n) ***E. phosphorea*** – (m) small trunk, (n) rooted branch.

Fig. 5 Nectar and visitors. (a) ***E. attastoma*** – Grão Mogol – 11am; (b) ***E. phosphorea*** – Milagres/BA – 2pm; (c) ***E. attastoma*** – Joaquim Felício – 11am; (d) ***E. phosphorea*** – Itatim/BA – 3pm; (e) ***E. sipolisii*** – Diamantina – 3pm, (f) Milho verde – 1pm; (g) ***E. phosphorea*** – Iaçu/BA – 10am; (h) ***E. attastoma*** – Pedra Azul/MG – 8am; (i) ***E. holochlorina*** – Pedra da Baleia, Teófilo Otoni/MG - 1pm; (j) ***E. sipolisii*** - Diamantina – 11am; (k, m-n) ***E. attastoma*** - Pedra Azul/MG - 8-9am; (l) ***E. attastoma*** - Rio Pardo de Minas/MG - 11am-12pm.

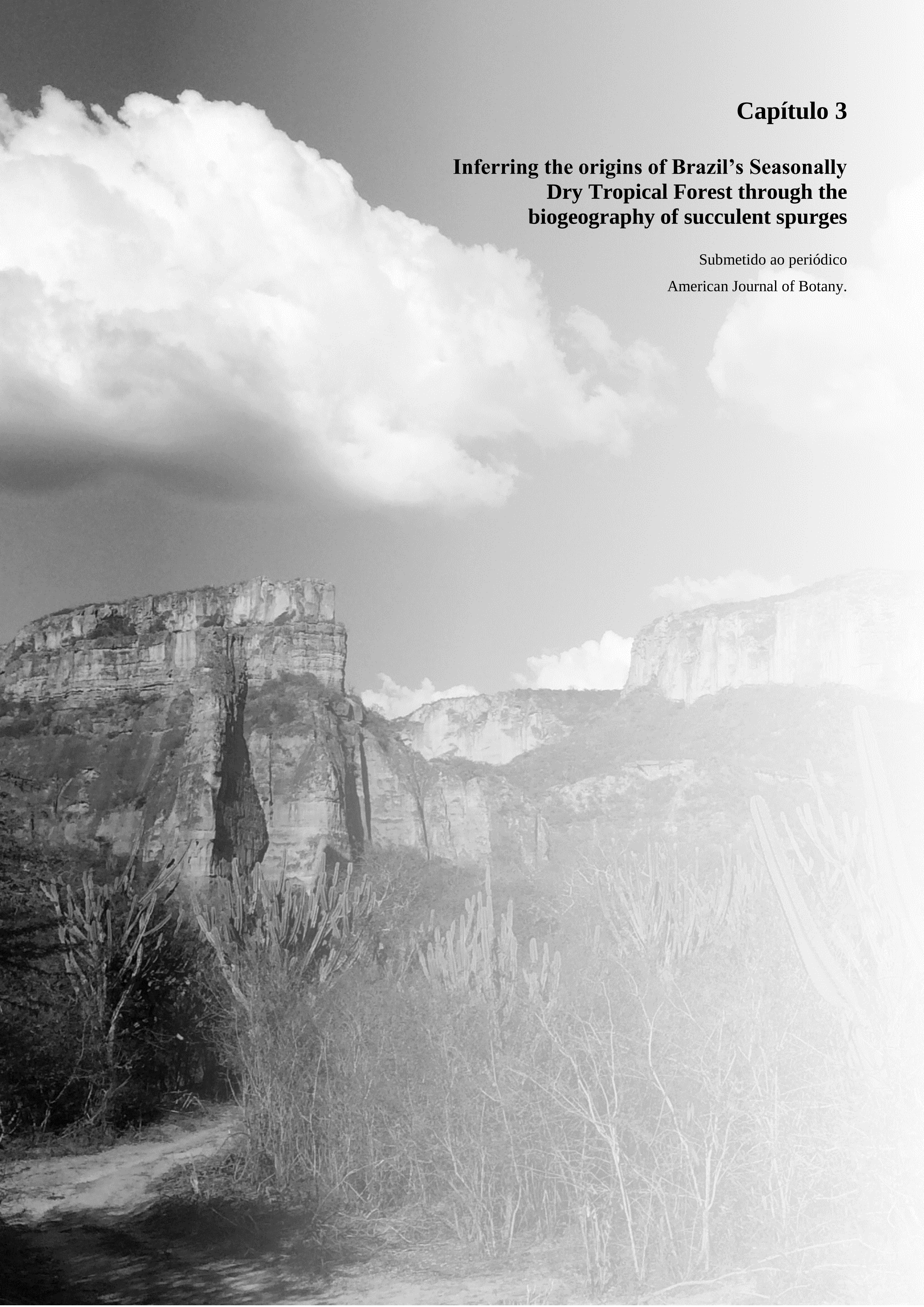
Fig. 6 Phyllotaxis of *Euphorbia* sect. *Brasilienses* species. (a) *E. attastoma* - spiral; (b) *E. holochlorina* - distichous; (c) *E. phosphorea* - spiral; (d) *E. sipolisii* – disticous; (e) *E. tetrangularis* – distichous.

Capítulo 3

Inferring the origins of Brazil's Seasonally Dry Tropical Forest through the biogeography of succulent spurges

Submetido ao periódico

American Journal of Botany.



Inferring the origins of Brazil's Seasonally Dry Tropical Forest through the biogeography of succulent spurges

Fernanda Hurbath[2,5], Rebecca L. Stubbs[3,4], Inês Cordeiro[2] and Nico Cellinese[3]

PREMISE OF THE STUDY: Seasonally Dry Tropical Forests (SDTF) are one of the most threatened and least studied of South America's ecosystems, and eastern Brazil has the largest and most isolated fragment. Yet, despite this fact, the biogeographic history of SDTF, both within and outside Brazil, is still poorly known. We investigated the biogeographic history of *Euphorbia* sect. *Brasilienses,* a group of cactiform spurges endemic to Brazilian SDTF. Understanding the origin and evolution of this group enables insights into the history of the eastern Brazil's SDTF.

METHODS: A concatenated matrix was built for 126 accessions with four markers: one nuclear (ITS1) and three plastid (*matK, ndhF,* trnL (UAA)-*trn*F (GAA) loci. We inferred the phylogenetic relationships using Maximum Likelihood. Clade ages and ancestral ranges were estimated.

KEY RESULTS: *Brasilienses* clade and its sister group *Stachydium* diverged from a common ancestor during the Miocene around 16.52 Ma in eastern Brazil SDTF. Clade *Stachydium* was recovered with a mean age of 7.72 Ma, while *Brasilienses* was recovered as a relatively young group, having diversified 3.15 Ma.

CONCLUSIONS: Our findings corroborate previous studies and suggest that the origin of eastern Brazil's SDTF pre-dates the Pleistocene, and most likely originated in the mid-Miocene. Long distance dispersal can be invoked to explain the distribution of clade *Stachydium.* This aligns with two of the hypotheses explaining distribution of taxa in SDTF and rejects the Pleistocene Arc Hypotheses. Our results point to an in-situ diversification, attesting the strong niche conservatism of *Brasilienses* and *Stachydium* clades.

KEW WORDS: Caatinga; Diversification; Euphorbiaceae; *Euphorbia* sect. *Brasilienses*; *Euphorbia* sect. *Stachydium*; Neotropics; South America.

[1]Manuscript received_________; revision accepted _________.

[2]Nucleo de Pesquisa Curadoria do Herbário São Paulo, Instituto de Botânica, Avenida Miguel Stéfano 3687, São Paulo, 04045-972, São Paulo, Brazil;

[3]Florida Museum of Natural History, University of Florida, Gainesville, Florida 32611 USA;

[4]Department of Biology, University of Florida, Gainesville, Florida 32611, USA

[5]Author for correspondence (e-mail: fhurbath@gmail.com); ORCID id 0000-0001-8857-775X. http://orcid.org/0000-0001-8857-775X

Biogeography of Brazil's SDTF

Seasonally Dry Tropical Forests (SDTF) are one of the most threatened and least studied of South America's ecosystems (Jazen, 1988; Miles et al., 2006). This is due, in part, to SDTF being heavily fragmented by both anthropogenic and non-anthropogenic causes. All areas of SDTF are exposed to a variety of threats and there have been multiple pleas for greater conservation efforts for these regions (Jazen, 1988; Geist and Lambin, 2002; Miles et al., 2006; Pennington et al., 2009). Therefore, investigations into the evolution and biogeography of the biota in these regions are essential for furthering conservation efforts.

Seasonally Dry Tropical Forests are found scattered throughout Central and South America, from northwestern Mexico to northern Argentina, and southwestern and eastern Brazil (Fig. 1). Notably, eastern Brazil has the largest and most isolated fragment of SDTF (Prado and Gibbs, 1993; de Queiroz, 2006; de Queiroz et al., 2017). This is considered the second most degraded ecosystem in Brazil with almost 50% loss of its original vegetation and what remains is largely fragmented (Castelletti et al., 2003; Leal et al., 2005). Additionally, this region has the highest level of biodiversity and endemism compared to other SDTF in South America (de Queiroz, 2006; de Queiroz et al., 2017). The landscape comprises a diversity of geological formations—including valleys, depressions, mountains (Espinhaço Range), *Inselbergs* (dome-shaped mountains commonly isolated and rising above erosional lowlands)—and offers a mosaic of vegetation types (Velloso et al., 2002). Surprisingly, there have been a limited number of studies that investigated the evolution of taxa occurring in the SDTF (Pennington et al., 2009; Turchetto-Zolet et al., 2013; Leal et al., 2016; de Queiroz et al., 2017). Up until 2004, only 14 percent of studies in tropical forest focused on dry forest, and most of these occurred in Mexico and Costa Rica (Sánchez-Azofeifa et al., 2005).

Seasonally Dry Tropical Forests are characterized by very low precipitation levels (< 1600 mm annually), extremely fertile soils, highly deciduous vegetation, and a pronounced five to six months dry season (Gentry, 1995; Dirzo et al., 2011). Due to this remarkable seasonal change, much of the flora is thought to have adapted to survive the prolonged water deficit that is characteristic of this ecosystem (Bullock, 1995). These adaptations include deciduousness, spines, waxy coatings, photosynthetic stems, and succulence. Due to both the specialized adaptations, vital for the survival of plants in the SDTF, and the fragmentation of these forests, many SDTF taxa belong to clades endemic to these areas and are often nested into much larger groups with extensive distributions. These clades, therefore, exemplify phylogenetic niche conservatism (de Queiroz, 2006; Pennington et al., 2009). One of such clades represents the *Euphorbia* sect. *Brasilienses* (Euphorbiaceae) and it will be referred as the *Brasilienses* clade.

Euphorbia with around 2000 species, is one of the largest genera of angiosperms (Govaerts et al., 2000) with a worldwide distribution and a multitude of growth forms (Bruyns et al., 2011; Horn et al., 2012). A great number of *Euphorbia* species occurs in semi-arid environments of tropics. Many of its clades have worldwide distributions and remarkable disjunctions (Bruyns et al., 2011; Horn et al., 2012; Dorsey et al., 2013). Yet, despite the complex and intriguing distribution of this group, *Euphorbia* has been neglected as a candidate for biogeographical studies in South America.

The *Euphorbia* sect. *Brasilienses* includes taxa morphologically adapted to harsh environmental conditions, such as extreme drought (Fig. 2). It comprises non-spiny pencil-like succulent shrubs, with photosynthetic cladodes and early caducous leaves (Horn et al., 2012) (Fig. 2). This group belongs to *Euphorbia* subg. *Euphorbia* (it will be referred as the *Euphorbia/Euphorbia* clade), which is the most diverse of the four subgenera in terms of morphology and habitats (Horn et al., 2012). Endemic to the SDTF of eastern Brazil (Fig. 1), the *Brasilienses* clade only occurs on rocky outcrops or shallow soils in caatingas, campos rupestres and *Inselbergues* (Fig. 2). Though the clade has been strongly supported, relationships within have been poorly resolved, in part due to their putative recent, rapid diversification (Hurbath et al., in press.; Dorsey et al., 2013). Comprised of five species, this clade contains only succulent taxa. Therefore, due to the high level of endemism (100%), in conjunction with the distinct adaptations to harsh climates, this group is ideal for investigating the origins of SDTF.

There are three main hypotheses regarding the evolution of SDTF. Prado and Gibbs (1993) proposed that the SDTF was once a single, continuous area, with its maximum extension during Last Glacial Maximum (LGM), therefore, the present distribution of SDTF would represent vestiges of a bigger and more continuous formation; this is referred to as the Pleistocene Arc Hypothesis (PAH). According to PAH, disjunctions in the SDTF would consist of many related lineages produced by vicariance events during the interglacial periods in the Pleistocene (Prado and Gibbs, 1993). This theory has been corroborated by floristic, biogeographic, and phylogeographic studies (Zanella, 2000; Prado, 2003; Oliveira-Filho et al., 2006; Franco and Manfrin, 2013; Bonatelli et al., 2014). A second hypothesis argues that SDTF disjunctions and the diversification of lineages from these areas are much older than the Pleistocene. Therefore, this hypothesis suggests that most taxa in SDTF would have originated during the Miocene and Pliocene (Pennington et al., 2004; Saslis-Lagoudakis et al., 2008; de Queiroz et al., 2017). A long-distance dispersal hypothesis has also been invoked to explain floristic similarities between the disjunct SDTF areas (Gentry, 1982; Mayle, 2006; Naciri et al., 2006). This hypothesis suggests an allopatric diversification of lineages in SDTF. Therefore, our aim is to yield insights into the evolution of the Brazilian SDTF by investigating the biogeography of the *Brasilienses* clade. By adding to the understanding of the origin and diversification of this group of cactiform spurges endemic to Brazilian SDTF, our study contributes to the knowledge of SDTF in South America.

MATERIAL AND METHODS

***Taxonomic sampling* —** Specimens of *Euphorbia* sect. *Brasilienses* and Brazilian species of *Euphorbia* sect. *Stachydium* were collected during fieldwork conducted in Brazil from 2014–2017. Vouchers were deposited at the Herbarium of the Instituto de Botânica (SP) in Sao Paulo, Brazil. Additionally, Genbank accessions of other Euphorbiaceae species were added to our dataset to enable our dating and biogeographic analyses, since the available fossils are related to *Acalypha* and *Hippomaneae*. At least one species was selected to represent each major group within Euphorbiaceae and *Euphorbia*, following the classifications of Wurdack et al. (2005), Horn et al. (2012) and Dorsey et al. (2013). Due to our focus on *Euphorbia*, this group was the most sampled—represented by 86 accessions of the four Euphorbia subgenera. These accessions were primarily generated by phylogenetic studies in *Euphorbia* (Yang and Berry, 2011; Yang et al., 2012; Dorsey et al., 2013; Riina et al., 2013). *Pera* was selected as the most distant outgroup. In total, the final dataset comprised 126 accessions, representing 27 genera of Euphorbiaceae (Table S1).

***DNA extraction, amplification and sequencing* —** For the field-collected samples, DNA extractions, PCRs, and sequencing were performed following Hurbath et al. (in press). Briefly, the workflow consists of the following steps. DNA was extracted from silica dried epidermal tissue, followed by a combination of five pre-washing steps using sorbitol-buffer (Tel-Zur et al. 1999) and completed with Dneasy Plant Mini Kit (Qiagen, California), following manufacturer's protocol. The 20–30 µl reaction amplification volume included 1-2µl DNA, 0.33-0.5 µM of forward and reverse primers, and 1 U Taq DNA polymerase. All PCR amplifications followed the protocols cited from original papers (Taberlet et al., 1991; Urbatsch et al., 2000; Garrick et al., 2009), with the exception of ITS1. ITS1 was amplified as follows: 95°C for 4 min for initial denaturation, followed by 32 cycles of denaturation at 95°C for 35 s, 45 s for annealing (temperatures in Table S2). The final extension at 72°C for 6 mins. All PCR products were sent to Macrogen (Seoul, South Korea) for purification and sequencing.

***Phylogenetic Analysis* —** A concatenated matrix was built with 126 accessions for four markers consisting of one nuclear (ITS1) and three plastid (*matK*, *ndhF*, *trn*L (UAA)-*trn*F (GAA) loci (alignment and original tree files in TreeBase – ID 22787). We decided to combine nuclear and plastid markers in order to diminish noise due to missing data (Table S1). Sequences were aligned using MAFFT (Katoh and Standley, 2016) in CIPRES Science Gateway. All alignments were reviewed and evaluated for quality. Maximum likelihood tree searches were performed using RAxML 8.2.10 (Stamatakis, 2014), with 1000 bootstrap replicates and the GTR + Gamma model, selected as the best model based on the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) in jModelTest 2 (Darriba et al., 2015). Bayesian phylogenetic inferences were run in MrBayes (Ronquist et al., 2012) on XSEDE (3.2.6) available in CIPRES Science Gateway with parameters set to two runs of four chains each (three heated, one cold), running for 100 million generations, starting from random trees, using the default temperature of 0.2, and sampling trees every 1000 generations with the burn-in set to 25%. Outputs of all phylogenetic analyses were read using Figtree v1.4.2 (Rambaut, 2014).

***Fossil calibration and Dating* —** Dating analyses were conducted in BEAST v.1.8.3. (Drummond et al., 2012). Two analyses were run under a relaxed uncorrelated lognormal clock model, but one assumed the Yule process and the other assumed the Birth-Death process. Each was run for 400,000,000 generations. Two analyses were run for each approach, results were checked for convergence in Tracer v1.7 (Rambaut et al., 2018), burn-in was selected as 10% of total trees, and the final chronograms were visualized in Figtree v1.4.2 (Rambaut, 2014).

We modeled all fossil constraints as a lognormal distribution with a mean of 2.0 and standard deviation of 1.0. The fossil of *Hippomanoidea warmanensis* Crepet & Daghlian, from the mid-Eocene deposits of the Claiborne Formation (Crepet and Daghlian, 1982), was assigned to the clade including *Sebastiania*, *Hippomane* and *Bonania* (Fig. 3). This taxon is closely comparable to the modern *Gymnanthes* from the Hippomaneae clade, and it was constrained to a minimum age of 41 Ma. Another fossil, morphologically comparable to *Acalypha* pollen type from the Early-Paleocene (as cited in Xi et al., 2012, supplemental appendix, p. 32), was assigned to the crown node of Acalyphoideae, and constrained to a minimum of 61 Ma. Secondary calibration points for Euphorbiaceae were applied following the results of Xi et al. (2012); we restricted the crown of Euphorbiaceae to the mean age of 89.9 Ma with a standard deviation of 4.5 Ma.

***Historical Biogeography Analyses* —** Ancestral ranges were estimated using the BioGeoBEARS package as implemented in R (Matzke, 2013). For this analysis we used the dated tree constructed under the Birth-Death process because this branching process prior has been shown to perform better in older clades with putative high extinction rates (Condamine et al., 2015). We tested a total of six models (Matzke, 2013): Dispersal-Extinction-Cladogenesis (DEC; Ree and Smith, 2008), Dispersal-Vicariance Analysis (DIVA; Ronquist, 1997), BayArea model (Landis et al., 2013), and, for each model, an additional free parameter (+ J), controlling relative probability of founder-event speciation events at cladogenesis. We used likelihood-ratio tests and AIC values to compare the fit of these models to the data.

Only New World Clade of *Euphorbia*/*Euphorbia* were used for the BioGeoBears analyses, since the aim of this study was to elucidate the evolution of the *Brasilienses* clade. Additionally, for correct input in BioGeoBears, we pruned our matrix to a single representative of each species, as a representative of all areas where that species occurs. Species distributions and habitat data were compiled from fieldwork, consulting herbaria collections (ALCB, BHCB, HRB, HRCB, HUEFS, R, RB, SP, SPF and FLAS), Global Plants Database (Jstor Global Plants, 2018), Tropicos.org (Missouri Botanical Garden, 2018), The Brazil Flora Group (2015), Specieslink (CRIA - Centro de Referência e Informação Ambiental, 2018), EUPHORBIA PBI data portal (Riina and Berry, 2018) and we vetted records from Global Biodiversity Information Facility (Gbif.org, 2017). Range areas per terminal in the phylogeny were coded in relation to each species total distribution. The biogeographic regions follow the World Wild Life ecoregions classification (Olson et al., 2001), in combination with previous biogeographic studies in South America (Pennington et al., 2000; Särkinen et al., 2011). The nine areas chosen were: Western North America, Eastern North America, Central America, Andes, South America Grasslands, Atlantic Forest, Cerrado, Southern South America, and Eastern Brazil SDTF.

RESULTS

***Phylogenetics* —** Our final aligned total evidence dataset consisted of 126 taxa, 2089 characters (297 ITS1, 357 *matK*, *ndhF*, *trn*L (UAA)-*trn*F (GAA), and 35% of total missing data (Table S1). The Bayesian and ML analyses (Fig. S1) recovered almost identical results for major clades, and differences were evident only in branches with low support values.

All major clades (Acalyphoideae s.s., Crotonoideae s.s. and Euphorbioideae) were recovered with high bootstrap support (BS >95% in the ML analysis), except for Crotonoideae (BS = 80%) (Fig. 3); however, backbone relationships, including among all the above clades, were not resolved and arose with very low resolution. The ML analysis yielded strong support throughout *Euphorbia* and all four traditional subgenera, but the deepest nodes along the backbone had low resolution.

All major clades in *Euphorbia*/*Euphorbia* (Pacificae clade, New World clade, and Old World clades I and II) were recovered with high support, but some relationships were not strongly supported. For instance, the backbone for the New World Clade had low resolution (BS<74%). Notably, our focal clade *Brasilienses* and its sister clade, were recovered with high support.

***Dating Inference* —** The divergence time estimated for the *Euphorbia* indicated a mean crown age of 49.9 Ma (95% highest posterior density; HPD: 41.79–57.9 Ma) (Table 1, Fig. S2). *Euphorbia*/*Euphorbia* arose 33.06 Ma (HPD: 28.13–39.56 Ma) with the New World Clade diversification around 28.6 Ma (HPD: 23.28- 33.91 Ma) (Fig. 4). *Brasilienses* and its sister group *Stachydium* diverged 16.52 Ma (95% HPD; 11.3–21.8 Ma) (Fig. 4). The *Stachydium* clade arose 7.72 Ma (95% HPD: 4–11.51), while *Brasilienses* was recovered as a relatively young group, having diversified 3.15 Ma (95% HPD; 1.52–5.06 Ma).

***Ancestral range estimation* —** Of the six historical biogeographic models compared using BioGeoBEARS, the Dispersal-Extinction-Cladogenesis model with founder events (DEC + J) was the best fit for the data (Figs. S3 and S4). Results suggest that *Stachydium* + *Brasilienses*

(PP = 1) originated in SDTF in eastern Brazil, and subclade *Brasilienses* also arose in SDTF in eastern Brazil (Fig. 5). In contrast, the *Stachydium* subclade was recovered with a broader ancestral range, including South America grasslands, Cerrado, Atlantic Forest and eastern Brazil SDTF. Finally, the clade *E. heterodoxa* + *E. lagunillarum* (PP = 1) shows a remarkable disjunction given *E. heterodoxa* is endemic to Brazilian SDTF, while *E. lagunillarum* is from Venezuelan (South America Grassland) SDTF.

The most probable ancestral distribution for the New World Clade of *Euphorbia*/*Euphorbia,* diverging 28.6 Ma, was a broad range encompassing western North America, Andes, Central America and southern South America (Fig. 5). The two major clades within New World Clade, *Euphorbiastrum* + *Calyculatae* + *Cubanthus* and *Brasilienses* + *Stachydium* + *Nummulariopsis* +*Portulacastrum* + *Crepidaria* (~26.48 Ma and ~27.48 Ma, respectively), each had a restricted ancestral range estimation to the Andes. That said, both clades, had a low Posterior Probability (PP = 0.5), and results are interpreted cautiously. However, most subclades had high support (PP>0.95).

DISCUSSION

Our results suggest that Brazil’s SDTF has been isolated for an extended period of time, pre-dating the mid-Miocene (~20 Ma) (Fig. 4 and 5). We also detected an in-situ diversification pre-dating the Pleistocene, and our results support strong niche conservatism for the *Brasilienses* and *Stachydium* clades. First, we will briefly discuss the phylogenetic tree generated and used in our biogeographic analyses. Second, we will discuss past geological and climatic events that occurred during the putative divergence and diversification of our clades of interest. Finally, we will address hypotheses regarding the distribution of lineages of eastern Brazil’s SDTF and conclude with insights into groups for the New World Clade.

***Phylogenetic hypothesis* —** Our phylogenetic results did not recover strong support for the deeper nodes in Euphorbiaceae (Fig. 3). However, this was expected since Wurdack et al. (2005) included greater sampling than our study and still recovered low backbone resolution. In contrast to our findings, Wurdack et al. (2005) recovered the Acalyphoideae as sister to a clade including Adenoclineae, Euphorbioideae and Crotonoideae, whereas our results had a polytomy at the backbone and recovered Adenoclineae clade (*Omphalea* and *Endospermum* taxa) as sister to Acalyphoideae. However, low support recovered by both studies and our lower sampling likely explains this incongruence.

Additionally, the topology that we recovered for the major clades in *Euphorbia* was in concordance with previous studies (e.g., Horn et al., 2012, Dorsey et al., 2013). A notable difference is seen within *Euphorbia/ Euphorbia*, in which our results did not recover the Old World Clade I as sister to Old World clade II as previously suggested (Dorsey et al., 2013). Instead, the latter was recovered with closer ancestral relationship with the Pacific clade (e.g., *Euphorbia haeleeleana*, *E. boophthona*, *E. plumerioides*, *E. stevenii* and *E. sarcostemoides*) (Fig. 3).

***Late Pliocene diversification of Brasilienses clade* —** Our study suggests that *Brasilienses* diversified in the late Pliocene (~3.15 Ma), which agrees with the timing of divergence of many plant taxa endemic of eastern Brazil SDTF (de Queiroz et al., 2017). The age of clade *Brasilienses* precedes the period of great climatic variations that marked the Quaternary, and this subsequent climatic fluctuation might have accelerated diversification of lineages inside the group. Notably, the sister clade to *Brasilienses*, *Stachydium*, which includes *E. heterodoxa,* a taxon endemic to eastern Brazilian SDTF, also emerged during the late Miocene (~7.72 Ma).

Most subclades in the New World Clade diversified during the Miocene, and this is in concordance with the divergence of most modern Neotropical lineages (Antonelli et al., 2015). During the Miocene, much of the present-day geographic features of the Neotropics was defined. This includes the occurrence of most geomorphological events, such as the Andes uplift (~12–4.5 Ma) and the genesis of the Amazon River (~11.8 Ma to present). Additionally, during this time the world's climate fluctuated as a result of cooling and aridification (Zachos et al., 2001), which co-occurred with an abrupt decrease of atmospheric CO_2 concentrations (Beerling and Royer, 2011).

Our study recovered an origin for *Euphorbia* between the mid-Eocene and beginning of the Paleocene (Table 1), very close to the beginning of Andes uplift ~65 Ma (Hoorn et al., 2010). Major changes in South America's landscapes occurred as a result of this orogeny (Hoorn et al., 2010; Antonelli and Sanmartín, 2011). In fact, some authors argue that the Andean uplift is responsible for the remarkable evolutionary diversifications in South America, enabling its great biodiversity (Gentry, 1982; Antonelli et al., 2009; Hoorn et al., 2010). However, Antonelli and Sanmartin (2011) concluded that many evolutionary processes besides geotectonic events—such as speciation and extinction rates, niche conservatism, and dispersal abilities—also played major roles in the diversification of Neotropical lineages. The crown age of the New World Clade of *Euphorbia*/*Euphorbia* suggests that this group diversified during Oligocene (~28.6 Ma). During this period, the northern part of the Andean uplift was occurring, resulting in the Andes first high peaks. Horn et al. (2012) recovered similar ages for *Euphorbia* and major clades within (Table 1).

TABLE 1. Estimated age for major clades within *Euphorbia* and comparison with previous study.

Clade	**Median Crown Age (Ma)**	**95% HPD**	**Previous study findings (Horn et al., 2014)**
Euphorbia	49.9	41.38 – 57.9	47.8 [95% HPD: 41.0 – 54.7]
Esula	41.44	31.38 – 51.7	40.5 [95% HPD: 33.2 – 47.7]
Athymalus	17.41	9.16 – 26.22	24.6 [95% HPD: 16.3 – 33.5]
Chamaesyce	33.04	26.64 – 39.48	36 [95% HPD: 30.1 – 42.1]
Euphorbia/Euphobia	33.06	28.13 – 39.56	30.8 [95% HPD: 24.6 – 36.4]
New World Clade (*Euphorbia/Euphorbia*)	28.6	23.28 – 33.91	24.92 [95% HPD: ≈18-32]

***Origin of eastern Brazil's SDTF* —** Our biogeographic results suggest an evolutionary history for eastern Brazil's SDTF beginning before the mid-Miocene. The most recent common ancestor of *Brasilienses* and *Stachydium* was recovered as being endemic to eastern Brazil's SDTF (~16.52 Ma), indicating an in-situ evolutionary history that pre-dates the Pleistocene by millions of years. A meta-analysis involving taxa from different groups endemic to eastern Brazil's SDTF also concluded that most taxa present a pre-Pleistocene in-situ diversification, and therefore, a connection with other patches of SDTF would have to pre-date the mid-Miocene (de Queiroz et al., 2017).

In-situ diversification is reinforced by niche conservatism and limited dispersal abilities commonly seen in taxa from seasonally dry biomes (Pennington et al., 2006; Pennington et al., 2009; Särkinen et al., 2011). Long distance seed dispersals for taxa restricted to uncommon habitats might be evolutionarily hazardous since it would be risky to find similar habitat conditions (Olivieri et al., 2016). This low dispersal ability and niche conservatism leads to geographically structured lineages, generally with allopatric distribution (Pinheiro et al., 2011; Pinheiro et al., 2014), resulting in speciation events. For instance, in *Euphorbia*, many taxa have an explosive, short-distance seed dispersal followed by secondary dispersal by ants (myrmecochory) (Berg, 1990; Webster, 1994; Griz and Machado, 2001). Both types of dispersal do not reach great distances (Leal et al. 2007). In fact, myrmecochory is also a common dispersal syndrome in other taxa endemic to eastern Brazil's SDTF (Leal et al., 2007, Leal et al., 2017). However, our analyses points to one long dispersal event that might have occurred during the early Pleistocene (~2.53 Ma), when the most recent common ancestor of *E. heterodoxa* and *E. lagunillarum* diverged. The former is restricted to eastern Brazil's SDTF, and the latter is endemic to Venezuela.

Therefore, our results support a combination of two hypotheses mentioned previously: connection between SDTF patches pre-dating the mid-Miocene and long distance dispersal events for younger taxa during Pleistocene. The hypotheses of an earlier Pleistocene connection followed by disjunction caused by climatic fluctuations (Pleistocene Arc Hypotheses), was not corroborated since there were no broad ancestral range distributions recovered during this period. In agreement with Werneck et al. (2011), our results suggest that Quaternary climatic fluctuations might have further increased the fragmentation of SDTF and expansion would have started during the Holocene and continued into the present.

Therefore, in contrast to the PAH, our results indicate a broader distribution of Eastern Brazil's SDTF pre-dating the Pleistocene, which is supported by the recovery of *Stachydium*'s ancestral range reaching South America grasslands, Atlantic Forest, Cerrado, and Eastern Brazil's SDTF around 7.53 Ma.

The in-situ diversification of *Brasilienses* + *Stachydium* clade might have been enhanced since the Miocene by geographic isolation of eastern Brazil's SDTF, with formation of Brazilian savannas inferred to occur around 10 Ma (Simon and Pennington, 2012). Savannas occupy a central portion of Brazil and act as a barrier to non-fire adapted taxa that are predominant in SDTF. Additionally, the dates suggested by our analyses for the origin of vegetation in eastern Brazil's SDTF are concurrent with the development of modern arid ecosystems and grasslands as a result of climatic changes worldwide (Zachos et al., 2001) and higher CO_2 concentrations (Beerling and Royer, 2011). Further, these dates and climatic events are consistent with the dates proposed for the evolution of the CAM and C4 photosynthetic pathways in many *Euphorbia* taxa as an adaptive response to aridity (Horn et al., 2014).

With more than 1,000,000 km^2, Brazil's SDTF is the largest patch of SDTF and it harbors a diversity of geological formations and vegetation. Therefore, it is not surprising that the evolutionary histories recovered for distinct groups in eastern Brazil's SDTF are varied. As mentioned, the evolution of *Brasilienses* during the Miocene coincides with a period marked by great changes in environmental and geological conditions in South America (Stebbins, 1981; Webb, 1985; Pascual and Jaureguizar, 1990). In contrast, research in Cactaceae recovered a Pleistocene origin for taxa occurring in Brazil's SDTF (Collevatti et al., 2013; Bonatelli et al., 2014; Calvente et al., 2016), and an ancestral radiation from other biomes (Menezes et al., 2016). Due to this variation, there is an increased need for research about this overlooked biome and, specifically, in SDTF.

External to eastern Brazil's SDTF: ancestral distribution range shifts between Andean, Central America and North America — The ancestral distribution of the *Nummulariopsis* + *Portulacastrum* clade was recovered in the Andes, however, the backbone of the New World Clade is not strongly supported. Therefore, we are not able to draw definitive conclusions about the common ancestor of *Brasilienses* + *Stachydium* and the *Nummulariopsis* + *Portulacastrum* clades, but our analyses suggested an origin in eastern Brazil's SDTF. That said, the biogeographical analysis also suggested a high probability of an ancestral range in the Andes (Fig. S3), so it is possible that the ancestral distribution of these clades during the late Oligocene (~26.34 Ma) was much wider.

The *Nummulariopsis* + *Portulacastrum* clade presents a much more complex evolutionary history, with both vicariance and dispersal being invoked to explain distributions from North America into the Andes and southern South America during the Miocene and Pliocene. Disjunctions between North and South America have received much attention in the literature (Lia et al., 2001; Lavin et al., 2004; Bessega et al., 2006; Moore et al., 2006; Majure et al., 2012; Willis et al., 2014) and long distance dispersal has been the principal explanation for this notable distribution.

The other major clades *Cubanthus*, *Calyculatae* and *Euphorbiastrum* were reconstructed as arising in the Andes, albeit with low backbone support. That said, our results suggest that *Cubanthus* and *Euphorbiastrum* diversified in-situ, the first within Central America and the second in the Andes, followed by dispersal to Central America. Notably, taxa from *Euphorbiastrum* were recovered as occurring in SDTF areas in the Andes and Central America. This further supports the pattern of limited dispersal, niche conservatisms, and in-situ diversification seen in taxa found in the SDTF.

CONCLUSIONS

Our findings corroborate previous studies based on other taxa endemic to SDTF and eastern Brazil. Connections between SDTF patches most likely pre-dates the Pleistocene, and therefore the Pleistocene Arc Hypotheses is rejected. Our results showed one SDTF lineage with an origin in Pleistocene, but this lineage is geographically disparate, and subsequently, dispersal is the most plausible explanation for this event. Therefore, our results combine two hypotheses: connections pre-dating the mid-Miocene and long-distance dispersal during the Pleistocene. Studies show that SDTF taxa present limited dispersal abilities, and most lineages of eastern Brazil’s SDTF likely diversified in-situ, which is reinforced by biome fragmentation. As a result, taxa endemic of SDTF, like *Brasilienses* clade, include geographically structured lineages, generally with allopatric distributions. The complexity of the evolutionary history of SDTF is evident throughout the literature and many hypotheses have been put forth to explain the origin of SDTF lineages. The SDTF is considered a highly endangered biome, with few conservation efforts and ongoing fragmentation by deforestation. Added to this, eastern Brazil's SDTF is one of the most degraded ecosystems in Brazil, and nearly half of the original vegetation is destroyed and fragmented. Therefore, there is an urgent need for research into the evolutionary history of South America SDTF, with the goal of increasing the knowledge about these areas and addressing how these ecosystems will respond to future climate changes.

ACKNOWLEDGEMENTS

The authors thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2015/11812-1) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES PDSE2016).

REFERENCES

ANTONELLI, A., J.A.A. NYLANDER, C. PERSSON, and I. SANMARTIN. 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution. *Proceedings of the National Academy of Sciences* 106: 9749–9754. Available at: http://www.pnas.org/cgi/doi/10.1073/pnas.0811421106.

ANTONELLI, A., and I. SANMARTÍN. 2011. Why are there so many plant species in the Neotropics? *Taxon* 60: 403–414.

ANTONELLI, A., A. ZIZKA, D. SILVESTRO, R. SCHARN, B. CASCALES-MIÑANA, and C.D. BACON. 2015. An engine for global plant diversity: Highest evolutionary turnover and emigration in the American tropics. *Frontiers in Genetics* 6: 1–14.

BEERLING, D.J., and D.L. ROYER. 2011. Convergent Cenozoic CO 2 history. *Nature Geoscience* 4: 418–420. Available at: http://dx.doi.org/10.1038/ngeo1186.

BESSEGA, C., J.C. VILARDI, and B.O. SAIDMAN. 2006. Genetic relationships among American species of the genus Prosopis (Mimosoideae, Leguminosae) inferred from ITS sequences: Evidence for long-distance dispersal. *Journal of Biogeography* 33: 1905–1915.

BONATELLI, I.A.S., M.F. PEREZ, A.T. PETERSON, N.P. TAYLOR, D.C. ZAPPI, M.C. MACHADO, I. KOCH, ET AL. 2014. Interglacial microrefugia and diversification of a cactus species complex: Phylogeography and palaeodistributional reconstructions for Pilosocereus aurisetus and allies. *Molecular Ecology* 23: 3044–3063.

BRUYNS, P. V., C. KLAK, and P. HANÁČEK. 2011. Age and diversity in old world succulent species of Euphorbia (Euphorbiaceae). *Taxon* 60: 1717–1733.

BULLOCK, S.H. 1995. Plant reproduction in neotropical dry forests. *In* S. H. Bullock, H. A. Mooney, and E. Medina [eds.], Seasonally Dry Tropical Forests, 277–303. Cambridge University Press, Cambridge. Available at: http://ebooks.cambridge.org/ref/id/CBO9780511753398.

CALVENTE, A., E.M. MORAES, P. LAVOR, I.A.S. BONATELLI, P. NACAGUMA, L.M. VERSIEUX, N.P. TAYLOR, and D.C. ZAPPI. 2016. Phylogenetic analyses of Pilosocereus (Cactaceae) inferred from plastid and nuclear sequences. *Botanical Journal of the Linnean Society* 183: 25–38. Available at: https://academic.oup.com/botlinnean/article-lookup/doi/10.1111/boj.12491.

CASTELLETTI, C.H.M., J.M.C. DA SILVA, M. TABARELLI, and A.M.M. SANTOS. 2003. Quanto ainda resta da Caatinga? Uma estimativa preliminar. *Ecologia e Conservação da Caatinga*777–796. Available at: http://www.infoteca.cnptia.embrapa.br/bitstream/item/18266/1/Caatinga.pdf.

COLLEVATTI, R.G., L.C. TERRIBILE, G. DE OLIVEIRA, M.S. LIMA-RIBEIRO, J.C. NABOUT, T.F. RANGEL, and J.A.F. DINIZ-FILHO. 2013. Drawbacks to palaeodistribution modelling: The case of South American seasonally dry forests. *Journal of Biogeography* 40: 345–358.

CONDAMINE, F.L., N.S. NAGALINGUM, C.R. MARSHALL, and H. MORLON. 2015. Origin and diversification of living cycads: A cautionary tale on the impact of the branching process prior in Bayesian molecular dating. *BMC Evolutionary Biology* 15: 1–18. Available at: ???

CREPET, W., and C. DAGHLIAN. 1982. Euphorbioid inflorescences from the Middle Eocene Claiborne formation. *American journal of botany* 69: 258–266. Available at: http://www.jstor.org/stable/2443013.

CRIA - CENTRO DE REFERÊNCIA E INFORMAÇÃO AMBIENTAL. Specieslink - Sistema de Informação Distribuído para Coleções Biológicas: a Integração do Species Analyst e do SinBiota (FAPESP). Available at: http://splink.cria.org.br/index?criaLANG=pt.

DARRIBA, D., G.L. TABOADA, R. DOALLO, and D. POSADA. 2015. Europe PMC Funders Group jModelTest 2 : more models , new heuristics and high- performance computing. *Nature Methods* 9: 6–9.

DIRZO, R., H.S. YOUNG, M. HAROLD A, and G. CEBALLOS. 2011. Seasonally Dry Tropical Forest: Ecology and Conservation.

DORSEY, B.L., T. HAEVERMANS, X. AUBRIOT, J.J. MORAWETZ, R. RIINA, V.W. STEINMANN, and P.E. BERRY. 2013. Phylogenetics, morphological evolution, and classification of Euphorbia subgenus Euphorbia. *Taxon*.

DRUMMOND, A.J., M.A. SUCHARD, D. XIE, and A. RAMBAUT. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29: 1969–1973.

FRANCO, F.F., and M.H. MANFRIN. 2013. Recent demographic history of cactophilic

Drosophila species can be related to Quaternary palaeoclimatic changes in South America. *Journal of Biogeography* 40: 142–154.

GARRICK, R.C., J.D. NASON, C.A. MEADOWS, and R.J. DYER. 2009. Not just vicariance: phylogeography of a Sonoran Desert euphorb indicates a major role of range expansion along the Baja peninsula. *Molecular Ecology* 18: 1916–1931. Available at: http://doi.wiley.com/10.1111/j.1365-294X.2009.04148.x.

GBIF.ORG. GBIF: The Global Biodiversity Information Facility. Available at: https://www.gbif.org/.

GEIST, H.J., and E.F. LAMBIN. 2002. Proximate Causes and Underlying Driving Forces of Tropical Deforestation. *BioScience* 52: 143.

GENTRY, A.H. 1995. Diversity and floristic composition of neotropical dry forests. *In* S. H. Bullock, H. A. Mooney, and E. Medina [eds.], Seasonally Dry Tropical Forests, 146–194. Cambridge University Press, Cambridge. Available at: http://ebooks.cambridge.org/ref/id/CBO9780511753398A045.

GENTRY, A.H. 1982. Neotropical Floristic Diversity : Phytogeographical Connections Between Central and South America , Pleistocene Climatic Fluctuations , or an Accident of the Andean Orogeny ? Author (s): Alwyn H . Gentry Source : Annals of the Missouri Botanical Garden. *Annals of the Missouri Botanical Garden* 69: 557–593.

GOVAERTS, R., D.G. FRODIN, and A. RADCLIFFE-SMITH. 2000. World Checklist and Bibliography of Euphorbiaceae (and Pandaceae). Royal Botanic Gardens, Kew.

HOORN, C., F.P. WESSELINGH, H. TER STEEGE, M.A. BERMUDEZ, A. MORA, J. SEVINK, I. SANMARTÍN, ET AL. 2010. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science* 330: 927–931.

HORN, J.W., B.W. VAN EE, J.J. MORAWETZ, R. RIINA, V.W. STEINMANN, P.E. BERRY, and K.J. WURDACK. 2012. Phylogenetics and the evolution of major structural characters in the giant genus Euphorbia L. (Euphorbiaceae). *Molecular Phylogenetics and Evolution* 63: 305–326. Available at: http://dx.doi.org/10.1016/j.ympev.2011.12.022.

HORN, J.W., Z. XI, R. RIINA, J.A. PEIRSON, Y. YANG, B.L. DORSEY, P.E. BERRY, ET AL. 2014. Evolutionary bursts in Euphorbia (Euphorbiaceae) are linked with photosynthetic pathway. *Evolution* 68: 3485–3504.

HURBATH, F., B.S.S. LEAL, O.L.M. SILVA, C. PALMA-SILVA, and I. CORDEIRO. A new species and molecular phylogeny of Brazilian succulent Euphorbia sect. Brasilienses. *Systematic and Biodiversity*. Available at: http://mc.manuscriptcentral.com/tsab.

JAZEN, D.H. 1988. Tropical Dry Forests. *In* E. O. Wilson, and M. P. Frances [eds.], Biodiversity, 130–137. National Academy Press, Washington, D.C.

JSTOR GLOBAL PLANTS. Global Plants Database. Available at: https://plants.jstor.org/.

KATOH, K., and D.M. STANDLEY. 2016. A simple method to control over-alignment in the MAFFT multiple sequence alignment program. *Bioinformatics* 32: 1933–1942.

LANDIS, M.J., N.J. MATZKE, B.R. MOORE, and J.P. HUELSENBECK. 2013. Bayesian analysis of biogeography when the number of areas is large. *Systematic Biology* 62: 789–804.

LAVIN, M., B.P. SCHRIRE, G. LEWIS, R.T. PENNINGTON, A. DELGADO-SALINAS, M. THULIN, C.E. HUGHES, ET AL. 2004. Metacommunity process rather than continental tectonic history better explains geographically structured phylogenies in legumes. *Philosophical Transactions of the Royal Society B: Biological Sciences* 359: 1509–1522. Available at: http://rstb.royalsocietypublishing.org/cgi/doi/10.1098/rstb.2004.1536.

LEAL, B.S.S., C. PALMA DA SILVA, and F. PINHEIRO. 2016. Phylogeographic Studies Depict the Role of Space and Time Scales of Plant Speciation in a Highly Diverse Neotropical Region. *Critical Reviews in Plant Sciences* 35: 215–230. Available at: http://dx.doi.org/10.1080/07352689.2016.1254494.

LEAL, I.R., J.M.C. DA SILVA, M. TABARELLI, and T.E. LACHER. 2005. Changing the Course of Biodiversity Conservation in the Caatinga of Northeastern Brazil\rCambiando el Curso de la Conservación de Biodiversidad en la Caatinga del Noreste de Brasil. *Conservation Biology* 19: 701–706. Available at: http://dx.doi.org/10.1111/j.1523-1739.2005.00703.x.

LIA, V. V., V.A. CONFALONIERI, C.I. COMAS, and J.H. HUNZIKER. 2001. Molecular phylogeny of Larrea and its allies (Zygophyllaceae): Reticulate evolution and the probable time of creosote bush arrival to North America. *Molecular Phylogenetics and Evolution* 21: 309–320.

MAJURE, L.C., R. PUENTE, M. PATRICK GRIFFITH, W.S. JUDD, P.S. SOLTIS, and D.E. SOLTIS. 2012. Phylogeny of Opuntia s.s. (Cactaceae): Clade delineation, geographic origins, reticulate evolution. *American Journal of Botany* 99: 847–864.

MATZKE, N.J. 2013. Probabilistic historical biogeography : new models for founder - event speciation , imperfect detection , and fossils allow im - proved accuracy and model - testing. *Frontiers in Biogeography* 5: 242–248.

MAYLE, F. 2006. The late quaternary biogeographical history of South American seasonally dry tropical forests: Insights from Palaeo-ecological data. *In* T. R. Pennington, G. Lewis, and J. Ratter [eds.], Neotropical Savannas and Seasonally Dry Forests: Plant Diversity, Biogeography, and Conservation, 395–416. CRC press, New York.

MENEZES, M.O.T., D.C. ZAPPI, E.M. MORAES, F.F. FRANCO, N.P. TAYLOR, I.R. COSTA, and M.I.B. LOIOLA. 2016. Pleistocene radiation of coastal species of Pilosocereus (Cactaceae) in eastern Brazil. *Journal of Arid Environments*.

MILES, L., A.C. NEWTON, R.S. DEFRIES, C. RAVILIOUS, I. MAY, S. BLYTH, V. KAPOS, and J.E. GORDON. 2006. A global overview of the conservation status of tropical dry forests. *Journal of Biogeography* 33: 491–505.

MISSOURI BOTANICAL GARDEN. Tropicos.org. Available at: http://www.tropicos.org/.

MOORE, M.J., A. TYE, and R.K. JANSEN. 2006. Patterns of long-distance dispersal in Tiquilia subg. Tiquilia (Boraginaceae): Implications for the origins of amphitropical disjuncts and Galápagos Islands endemics. *American Journal of Botany* 93: 1163–1177.

NACIRI, Y., S. CAETANO, T.R. PENNINGTON, D. PRADO, and R. SPICHIGER. 2006. Population genetics and inference of ecosystem history: an example using two Neotropical seasonally dry forest species. *In* T. R. Pennington, G. Lewis, and J. Ratter [eds.], Neotropical Savannas & Dry Forests: Plant diversity, Biogeography and Conservation, 417–432. CRC press, New York.

OLIVEIRA-FILHO, A., J.A. JARENKOW, and M.J.N. RODAL. 2006. Floristic Relationships of Seasonally Dry Forests of Eastern South America Based on Tree Species Distribution Patterns. *In* 159–192. Available at: http://www.crcnetbase.com/doi/abs/10.1201/9781420004496.ch7.

OLSON, D.M., E. DINERSTEIN, E.D. WIKRAMANAYAKE, N.D. BURGESS, G.V.N. POWELL, E.C. UNDERWOOD, J.A. D'AMICO, ET AL. 2001. Terrestrial Ecoregions of the World: A New Map of Life on Earth. *BioScience* 51: 933. Available at: https://academic.oup.com/bioscience/article/51/11/933-938/227116.

PASCUAL, R., and E.O. JAUREGUIZAR. 1990. Evolving climates and mammal faunas in cenozoic South America. *Journal of Human Evolution* 19: 23–60.

PENNINGTON, R.T., M. LAVIN, and A. OLIVEIRA-FILHO. 2009. Woody Plant Diversity, Evolution, and Ecology in the Tropics: Perspectives from Seasonally Dry Tropical Forests. *Annual Review of Ecology, Evolution, and Systematics* 40: 437–457. Available at: http://www.annualreviews.org/doi/10.1146/annurev.ecolsys.110308.120327.

PENNINGTON, R.T., M. LAVIN, D.E. PRADO, C.A. PENDRY, S.K. PELL, and C.A. BUTTERWORTH. 2004. Historical climate change and speciation: neotropical seasonally dry forest plants show patterns of both Tertiary and Quaternary diversification.

Philosophical Transactions of the Royal Society B: Biological Sciences 359: 515–538. Available at: http://rstb.royalsocietypublishing.org/cgi/doi/10.1098/rstb.2003.1435.

PENNINGTON, R.T., D.E. PRADO, and C.A. PENDRY. 2000. Neotropical seasonally dry forests and Quaternary vegetation changes. *Journal of Biogeography* 27: 261–273. Available at: http://dx.doi.org/10.1046/j.1365-2699.2000.00397.x.

PRADO, D.E. 2003. As Caatingas da América do Sul. *Ecologia e conservacão da Caatinga*3–73.

PRADO, D.E., and P.E. GIBBS. 1993. Patterns of Species Distributions in the Dry Seasonal Forests of South America. *Annals of the Missouri Botanical Garden* 80: 902–927. Available at: http://www.jstor.org/stable/2399937.

QUEIROZ, L.P. 2006. The Brazilian Caatinga: Phytogeographical Patterns Inferred from Distribution Data of the Leguminosae. *Neotropical Savannas and Seasonally Dry Forests: Plant Diversity, Biogeography, and Conservation*121–157.

DE QUEIROZ, L.P., D. CARDOSO, M.F. FERNANDES, and M.F. MORO. 2017. Diversity and Evolution of Flowering Plants of the Caatinga Domain. *In* J. M. Cardoso da Silva, I. R. Leal, and M. Tabarelli [eds.], Caatinga, 23–63. Springer International Publishing, Cham. Available at: http://link.springer.com/10.1007/978-3-319-68339-3_2.

RAMBAUT, A. 2014. Figtree, a graphical viewer of phylogenetic tree. Available at: http://tree.bio.ed.ac.uk/software/figtree/.

RAMBAUT, A., A.J. DRUMMOND, D. XIE, G. BAELE, and M.A. SUCHARD. 2018. Tracer v1.7. Available at: http://tree.bio.ed.ac.uk/software/tracer/.

REE, R.H., and S.A. SMITH. 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology* 57: 4–14.

RIINA, R., and P.E. BERRY. Euphorbia Planetary Biodiversity Inventory database. Available at: http://app.tolkin.org/projects/72/taxa.

RIINA, R., J.A. PEIRSON, D. V. GELTMAN, J. MOLERO, B. FRAJMAN, A. PAHLEVANI, L. BARRES, ET AL. 2013. A worldwide molecular phylogeny and classification of the leafy spurges, Euphorbia subgenus Esula (Euphorbiaceae). *Taxon* 62: 316–342.

RONQUIST, F. 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeogrpahy. *Syst. Biol* 46: 195–203.

RONQUIST, F., M. TESLENKO, P. VAN DER MARK, D.L. AYRES, A. DARLING, S. HÖHNA, B. LARGET, ET AL. 2012. Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.

SÁNCHEZ-AZOFEIFA, G.A., M. QUESADA, J.P. RODRÍGUEZ, J.M. NASSAR, K.E. STONER, A. CASTILLO, T. GARVIN, ET AL. 2005. Research priorities for neotropical dry forests. *Biotropica* 37: 477–485.

SÄRKINEN, T., J.R.V. IGANCI, R. LINARES-PALOMINO, M.F. SIMON, and D.E. PRADO. 2011. Forgotten forests - issues and prospects in biome mapping using Seasonally Dry Tropical Forests as a case study. *BMC Ecology* 11: .

SASLIS-LAGOUDAKIS, C., M.W. CHASE, D.N. ROBINSON, S.J. RUSSELL, and B.B. KLITGAARD. 2008. Phylogenetics of neotropical Platymiscium (Leguminosae: Dalbergieae): Systematics, divergence times, and biogeography inferred from nuclear ribosomal and plastid DNA sequence data. *American Journal of Botany* 95: 1270–1286.

SIMON, M.F., and T. PENNINGTON. 2012. Evidence for Adaptation to Fire Regimes in the Tropical Savannas of the Brazilian Cerrado. *International Journal of Plant Sciences* 173: 711–723. Available at: http://www.journals.uchicago.edu/doi/10.1086/665973.

STAMATAKIS, A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.

STEBBINS, G.L. 1981. Coevolution of Grasses and Herbivores. *Annals of the Missouri Botanical Garden* 68: 75–86.

TABERLET, P., L. GIELLY, P. G., J. BOUVET, and G. PAUTOU. 1991. Universal primer for amplification of three non-coding regions of chloroplast DNA\n. *Plant Molecular Biology* 17: 1105–1109. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1932684.

TEL-ZUR, N., S. ABBO, D. MYSLABODSKI, and Y. MIZRAHI. 1999. Modified CTAB procedure for DNA isolation from epiphytic cacti of the genera Hylocereus and Selenicereus (Cactaceae). *Plant Molecular Biology Reporter* 17: 249–254.

THE BRAZIL FLORA GROUP. 2015. Growing knowledge: an overview of Seed Plant diversity in Brazil. *Rodriguésia* 66: 1085–1113. Available at: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S2175-78602015000401085&lng=en&tlng=en.

TURCHETTO-ZOLET, A.C., F. PINHEIRO, F. SALGUEIRO, and C. PALMA-SILVA. 2013. Phylogeographical patterns shed light on evolutionary process in South America. *Molecular Ecology* 22: 1193–1213.

URBATSCH, L.E., B.G. BALDWIN, and M.J. DONOGHUE. 2000. Phylogeny of the Coneflowers and Relatives (Heliantheae: Asteraceae) Based on Nuclear rDNA Internal Transcribed Spacer (ITS) Sequences and Chlorplast DNA Restriction Site Data. *Systematic Botany* 25: 539. Available at: http://www.jstor.org/stable/2666695?origin=crossref.

VELLOSO, A.L., A.L. VELLOSO, A.M. GIULIETTI, D.C. OREN, E.E. DE MIRANDA, and S. KEEL. 2002. Ecorregioes propostas para o bioma Caatinga. *In* A. L. Velloso, E. V. S. B. Sampaio, and F. G. C. Pareyn [eds.], 76. Instituto de Conservacao Ambiental The Nature Conservancy do Brasil, Recife.

WEBB, S.D. 1985. Late Cenozoic mammal dispersal between the Americas. *The Great American Biotic Interchange*357–386.

WILLIS, C.G., B. FRANZONE, Z. XI, and C.C. DAVIS. 2014. Community assembly meets biogeography: Malpighiaceae and the origin of seasonally dry tropical forests of

Mexico. *Frontiers in Genetics* 5: 1–14.

WURDACK, K.J., P. HOFFMANN, and M.W. CHASE. 2005. Molecular phylogenetic analysis of uniovulate Euphorbiaceae (Euphorbiaceae sensu stricto) using plastid rbcL and trnL-F DNA sequences. *American Journal of Botany* 92: 1397–1420.

XI, Z., B.R. RUHFEL, H. SCHAEFER, A.M. AMORIM, M. SUGUMARAN, K.J. WURDACK, P.K. ENDRESS, ET AL. 2012. Phylogenomics and a posteriori data partitioning resolve the Cretaceous angiosperm radiation Malpighiales. *Proceedings of the National Academy of Sciences* 109: 17519–17524. Available at: http://www.pnas.org/cgi/doi/10.1073/pnas.1205818109.

YANG, Y., and P.E. BERRY. 2011. Phylogenetics of the Chamaesyce clade (Euphorbia, Euphorbiaceae): Reticulate evolution and long-distance dispersal in a prominent C4 lineage. *American Journal of Botany* 98: 1486–1503.

YANG, Y., R. RIINA, J.J. MORAWETZ, T. HAEVERMANS, X. AUBRIOT, and P.E. BERRY. 2012. Molecular phylogenetics and classification of Euphorbia subgenus Chamaesyce: a group with high diversity in photosynthetic systems and growth forms. *Taxon* 61: 764–789.

ZACHOS, J., M. PAGANI, L. SLOAN, E. THOMAS, and K. BILLUPS. 2001. Trends, Global Rhythms, Aberrations in Global Climate 65Ma to Present. *Science* 292: 686–693. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11326091%5Cn%3CGo to ISI%3E://000168478300041.

ZANELLA, F.C. V. 2000. The bees of the Caatinga (Hymenoptera, Apoidea, Apiformes): a species list and comparative notes regarding their distribution. *Apidologie* 31: 579–592.

FIGURE LEGENDS

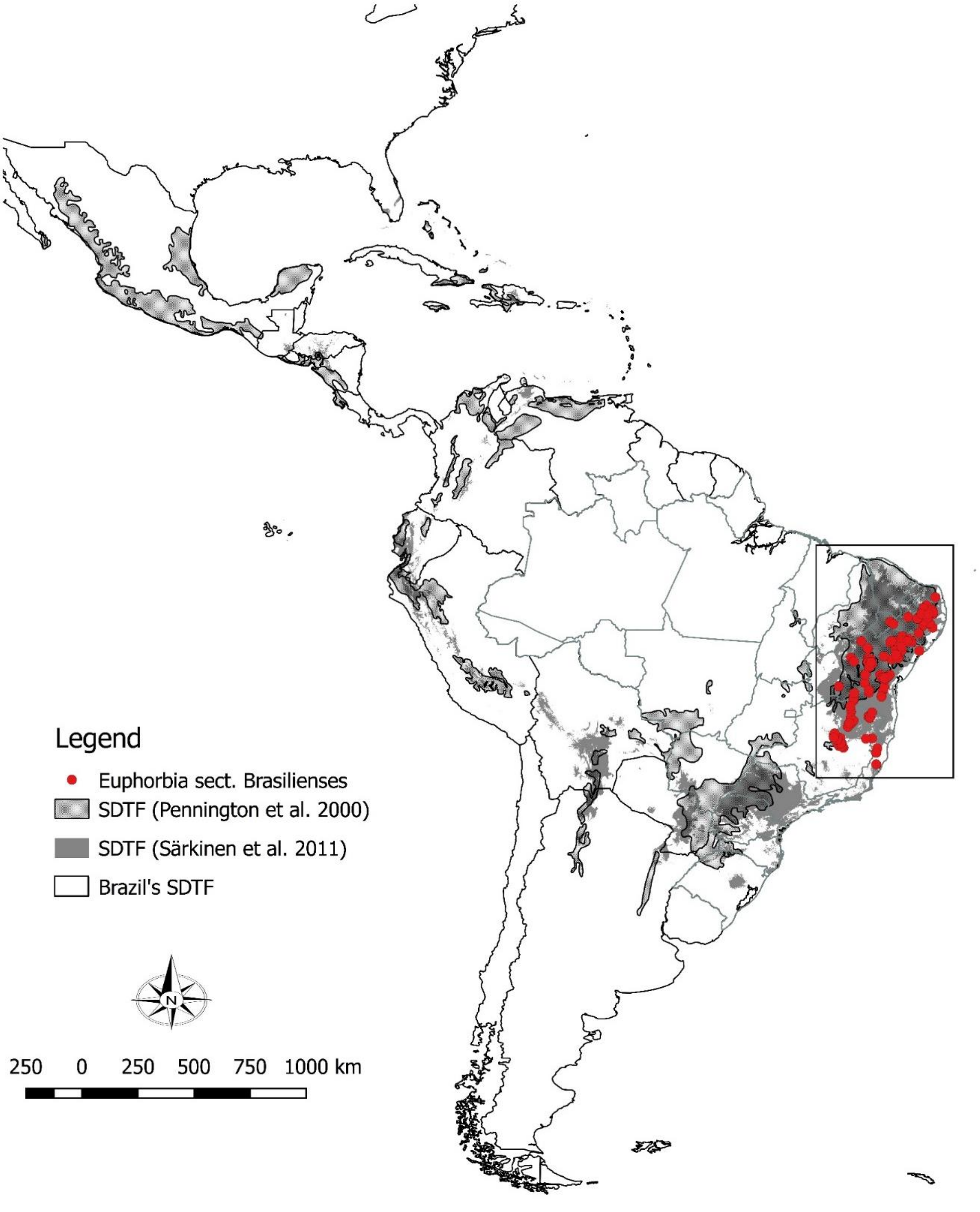


FIGURE 1. Map with distribution of *Euphorbia* sect. *Brasilienses* (red dots) in eastern Brazil’s SDTF. Two delimitations for SDTF (Pennington et al., 2000; Särkinen et al, 2011) are represented with different shades of grey.

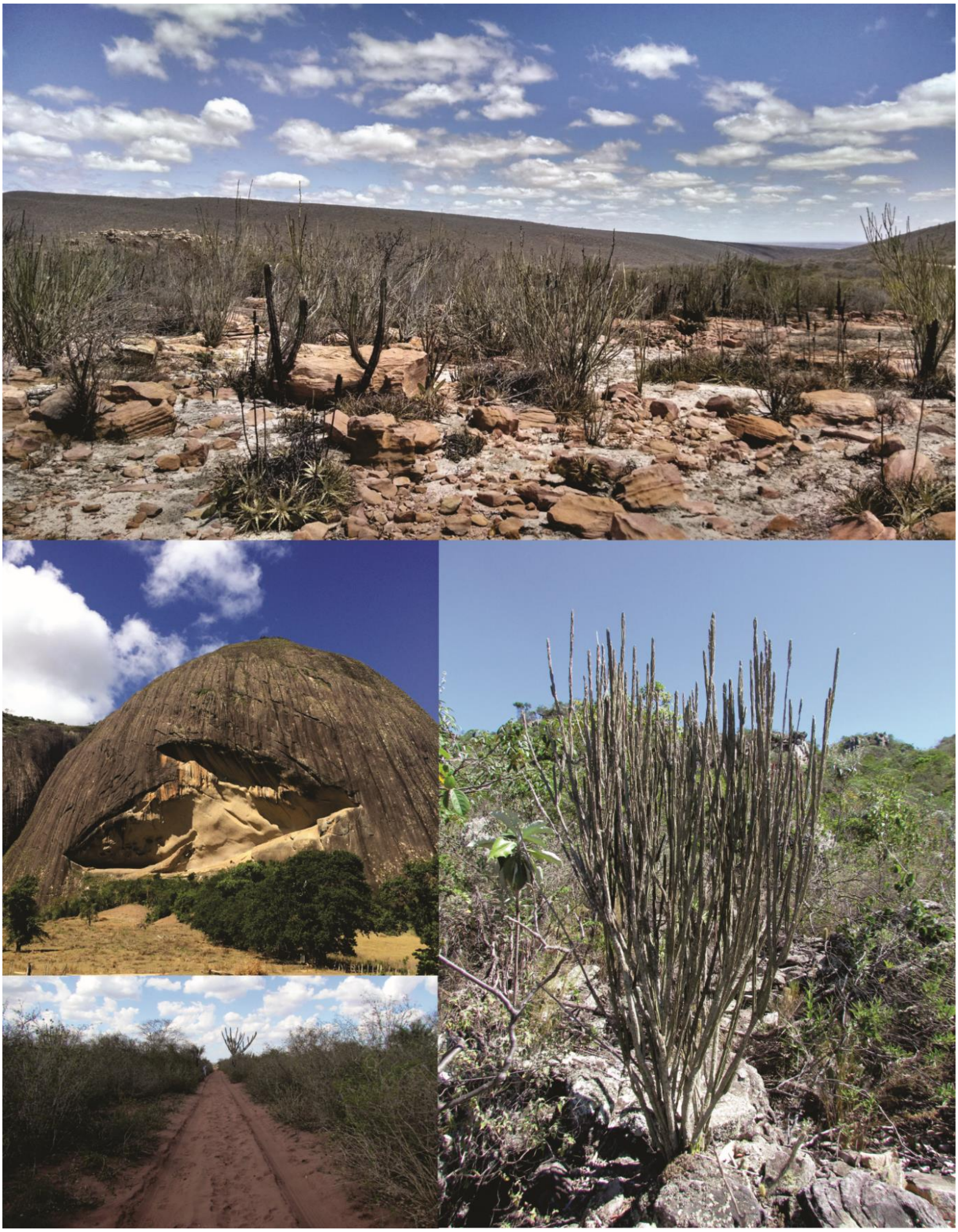


FIGURE 2. Habitat and life form of species of *Euphorbia* sect. *Brasilienses.* A. Caatinga vegetation on rock slopes, B. *Inselberg*, C. Caatinga vegetation on sandy soils, D. Individual of *Euphorbia attastoma*, showing erect branches, candelabrifom shrub and cactiform habit.

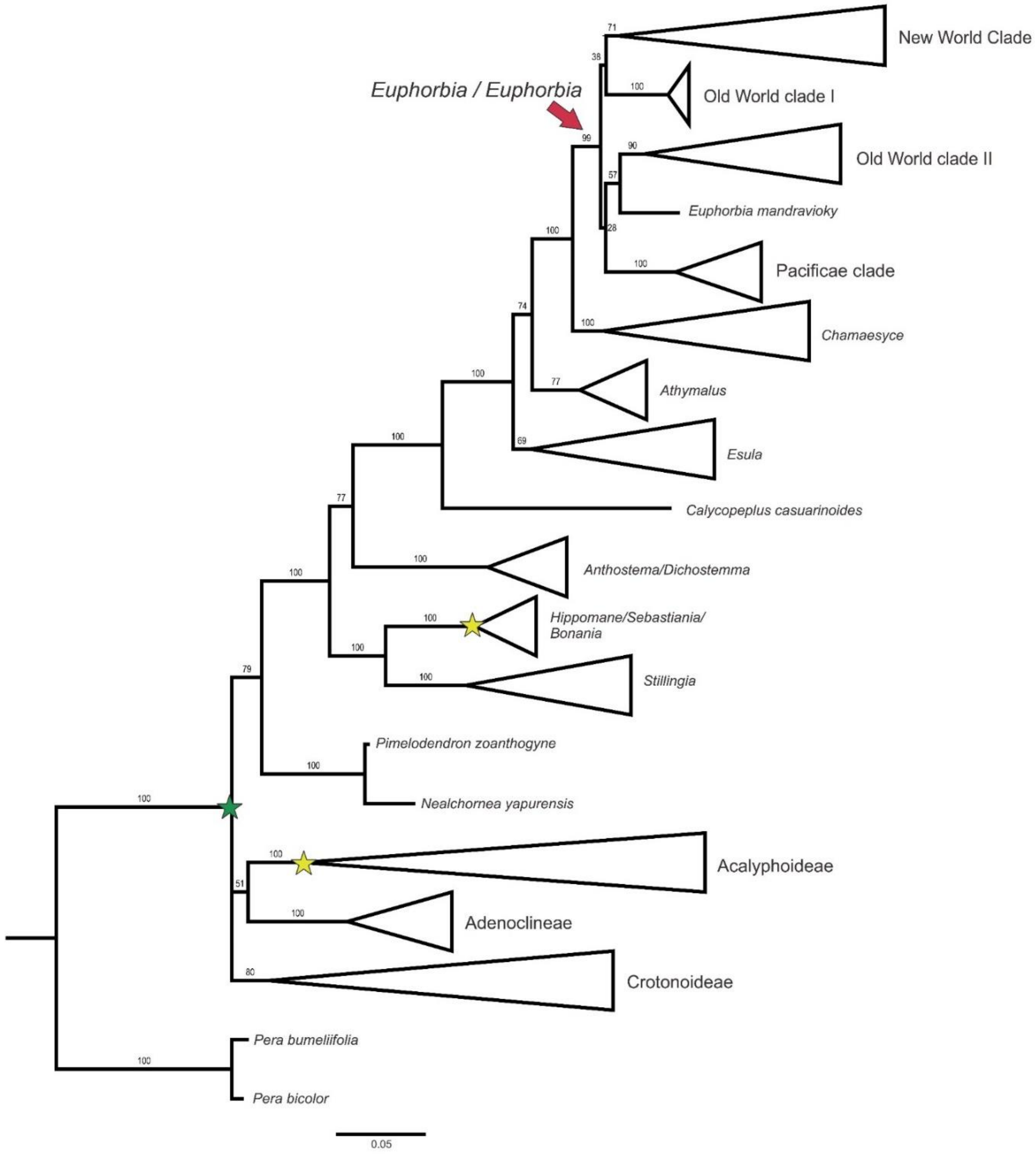


FIGURE 3. Phylogeny of Euphorbiaceae generated in maximum likelihood analyses. Green star represents secondary calibration and yellow ones represent fossil calibration. Number above branches represent Posterior Probabilities.

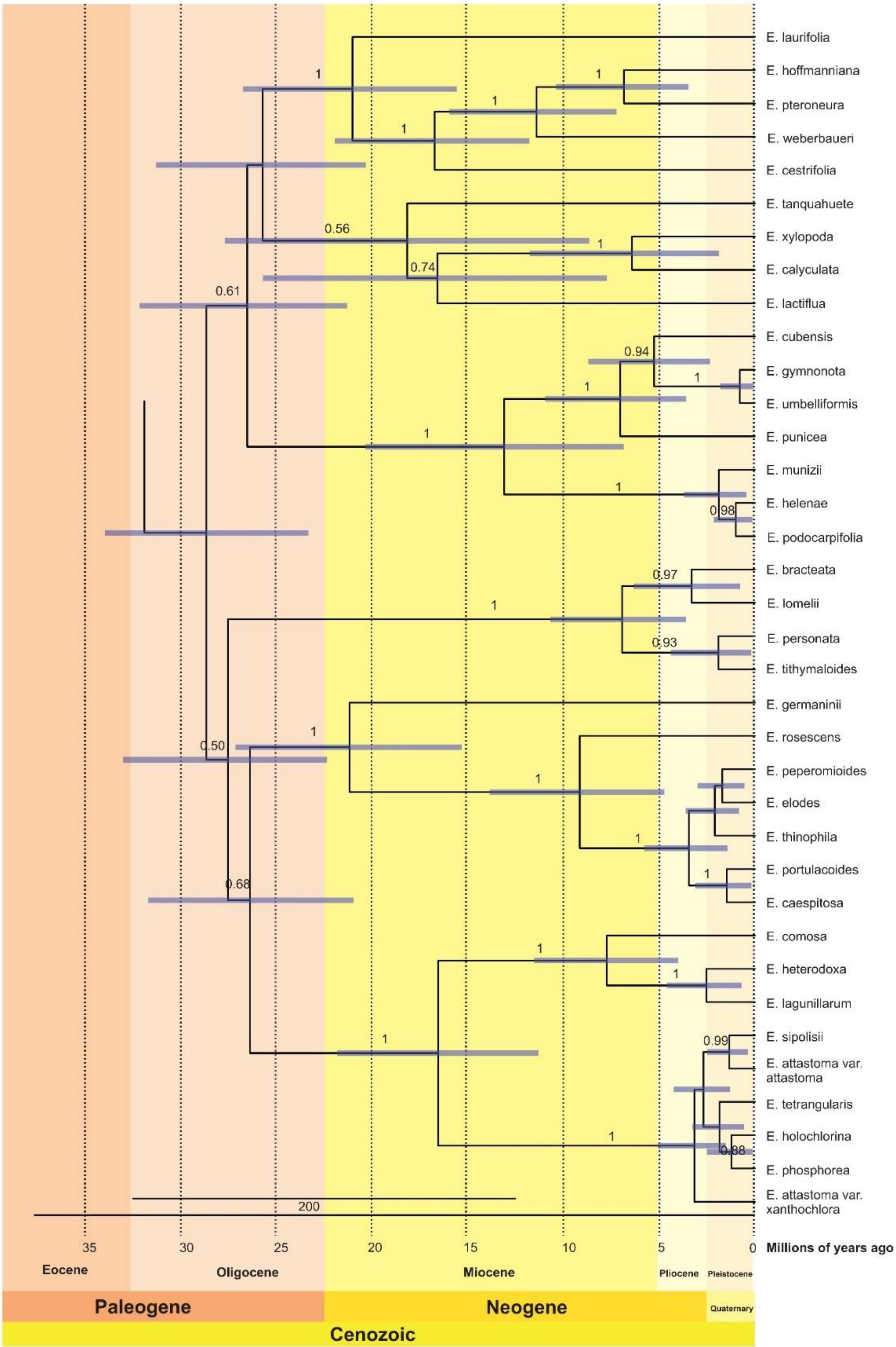


FIGURE 4. Dated phylogeny of New World Clade (*Euphorbia / Euphorbia*) with Time scale and chronostratigraphic for Era, Period and Epoch, the latter represented with colored columns. Number above branches represent Posterior Probabilities.

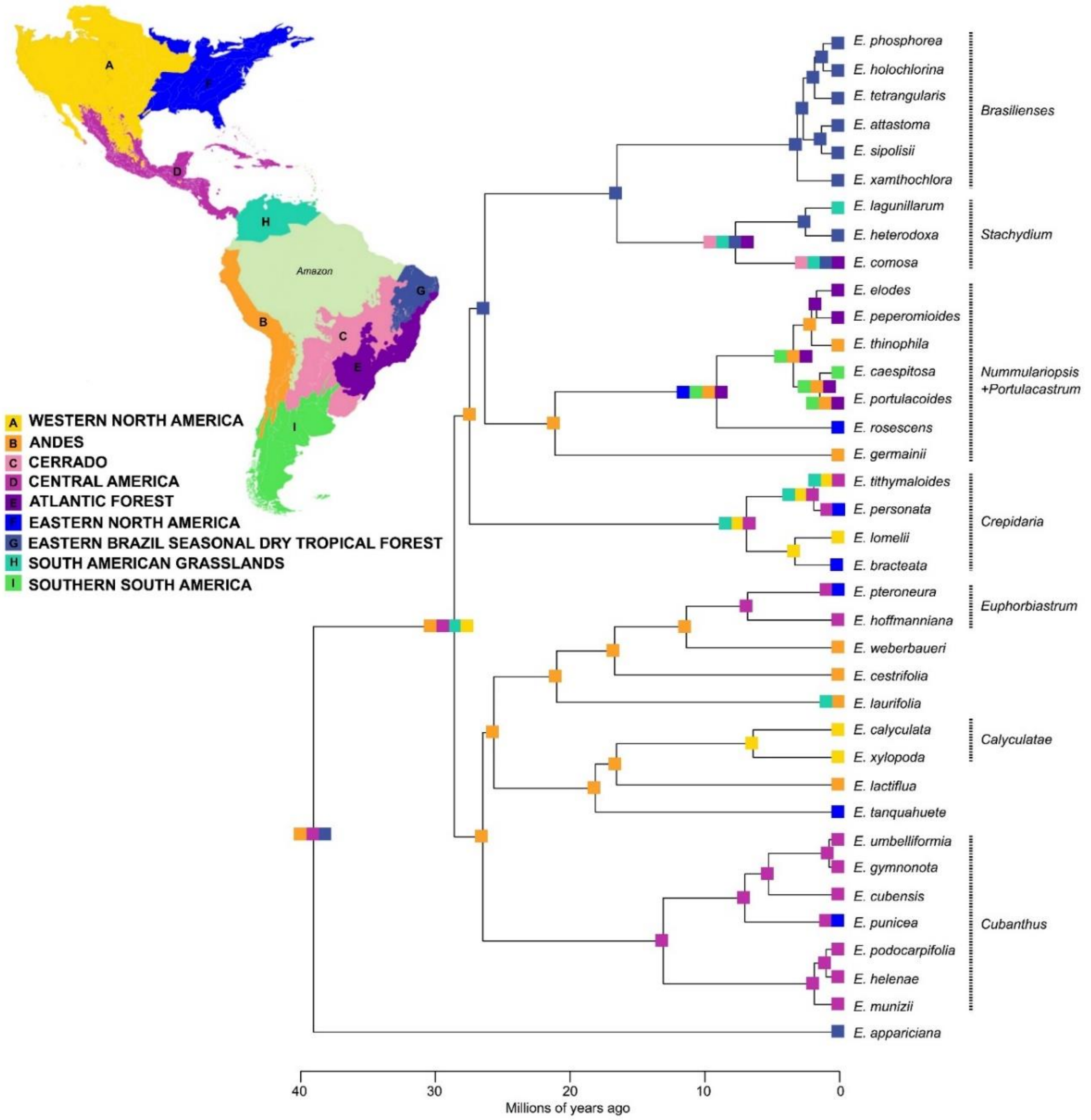


FIGURE 5. Results for the biogeographic analyses with map representing chosen areas.

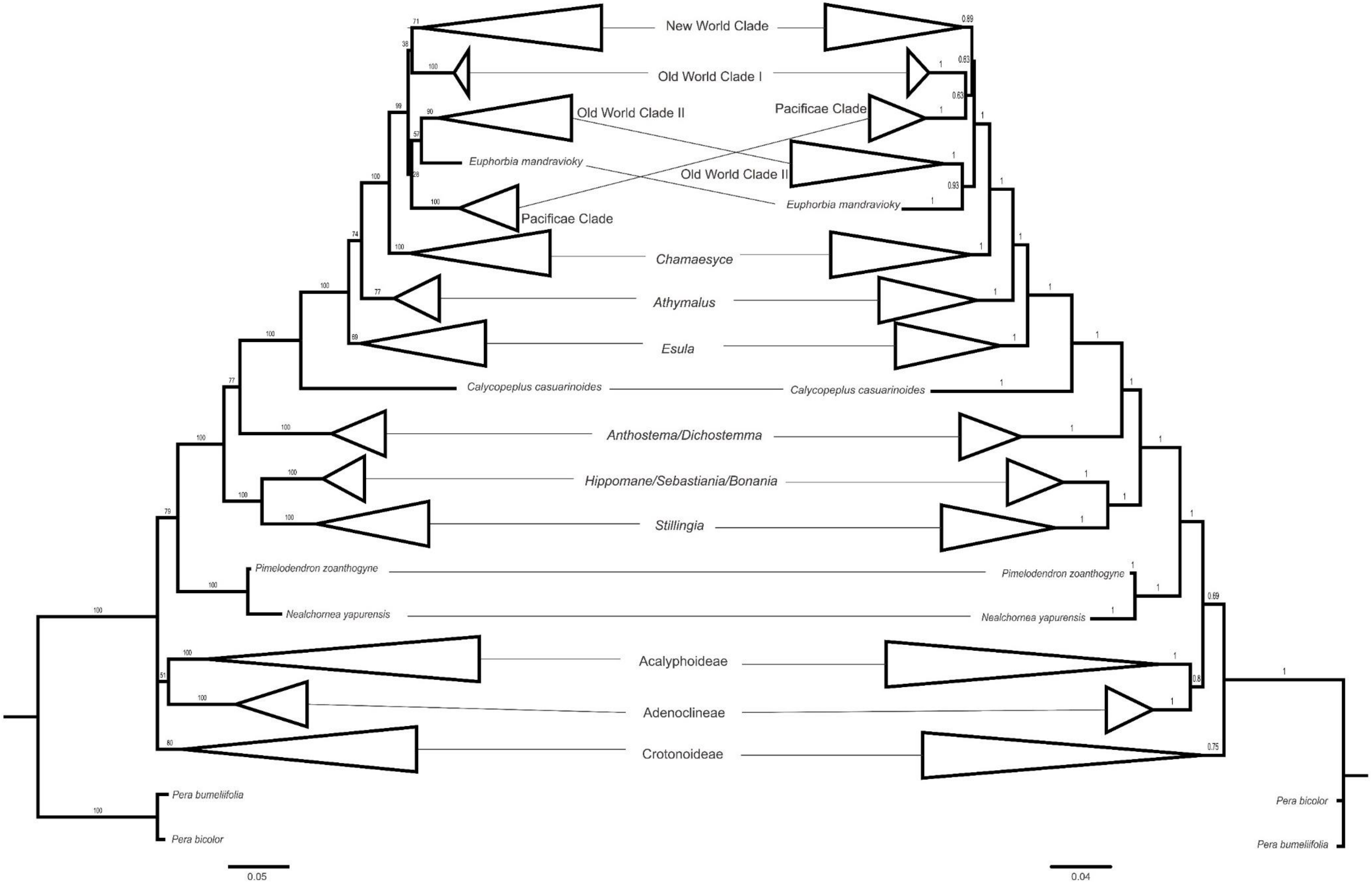


FIGURE S1. Comparison between maximum likelihood analyses tree (left) and bayesian analyses tree (right).

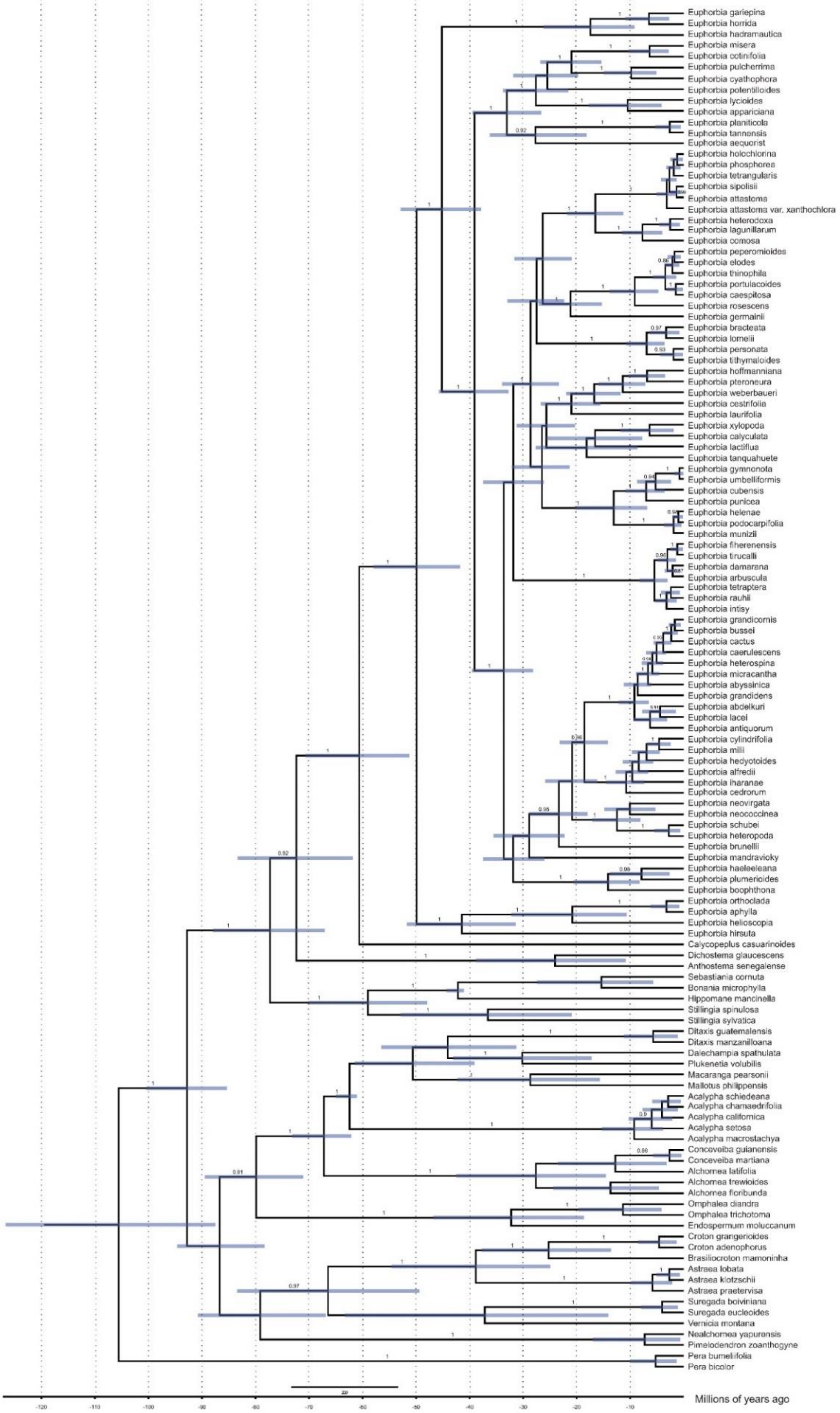


FIGURE S2. Chronogram and estimated divergence times of *Euphorbia* generated by molecular clock analysis using BEAST v. 1.8.3.

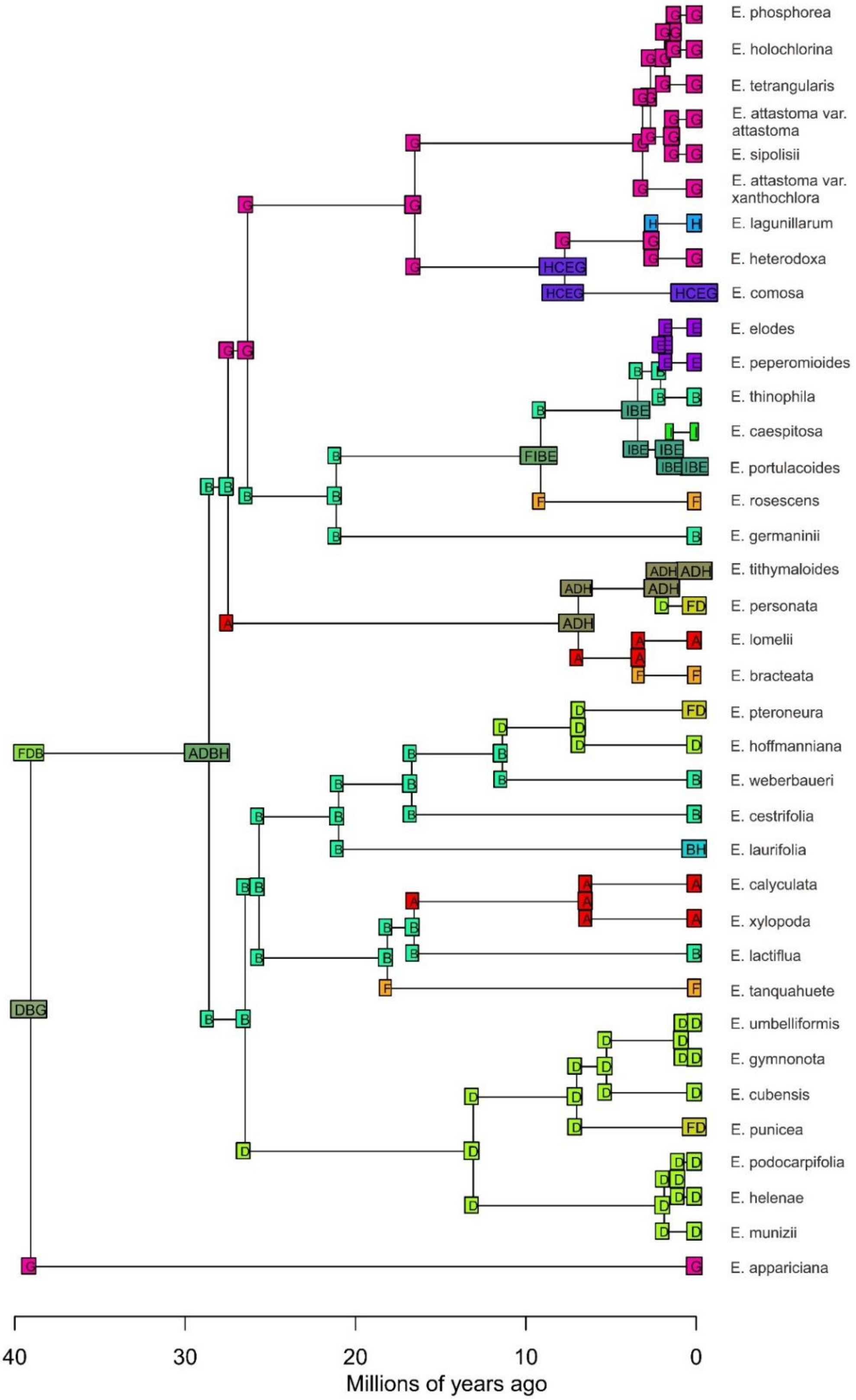


FIGURE S3: BioGeoBears result for *Euphorbia* subg. *Euphorbia* New World Clade. DEC + j: d = 0.003; e = 0; j = 0.026

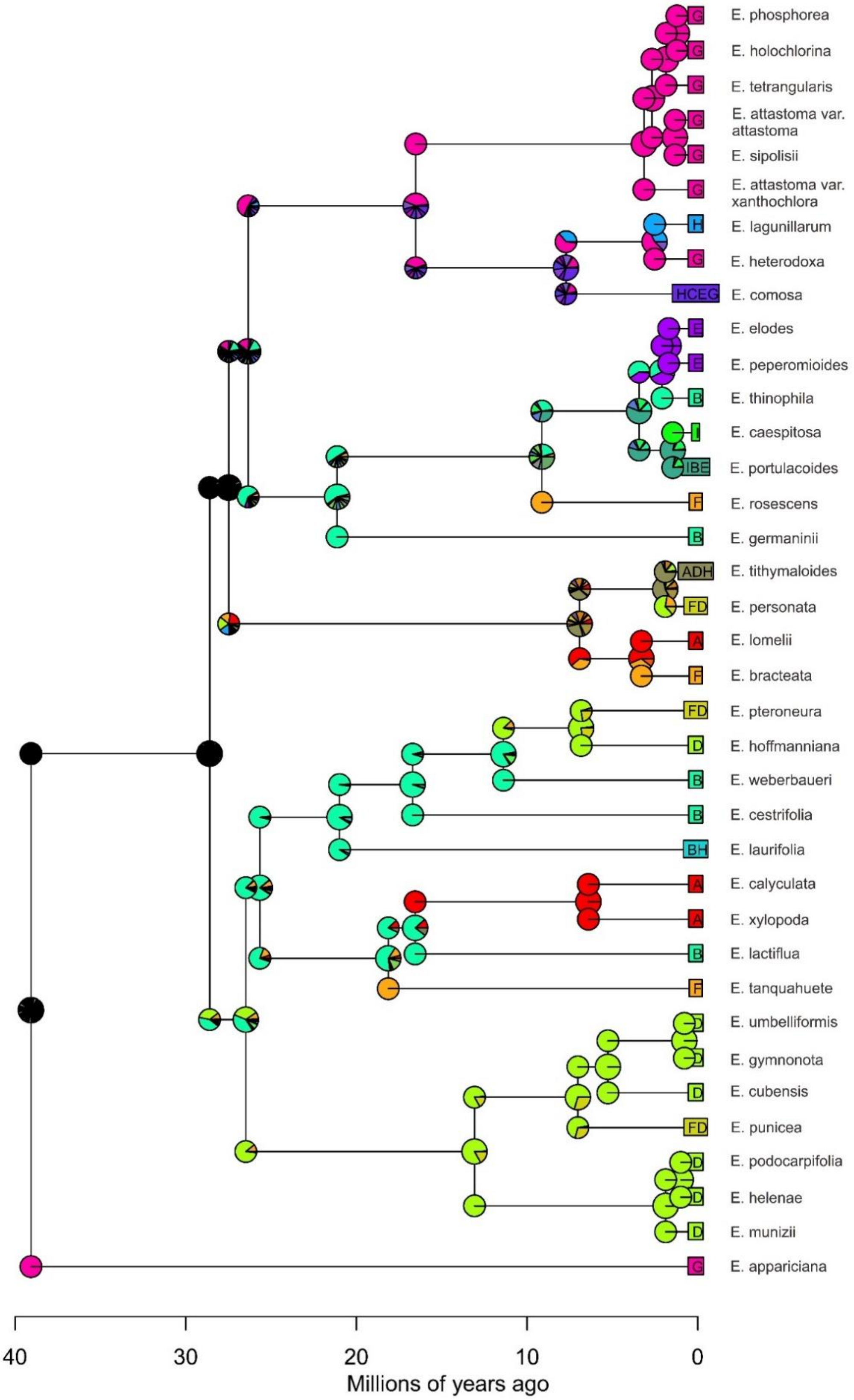
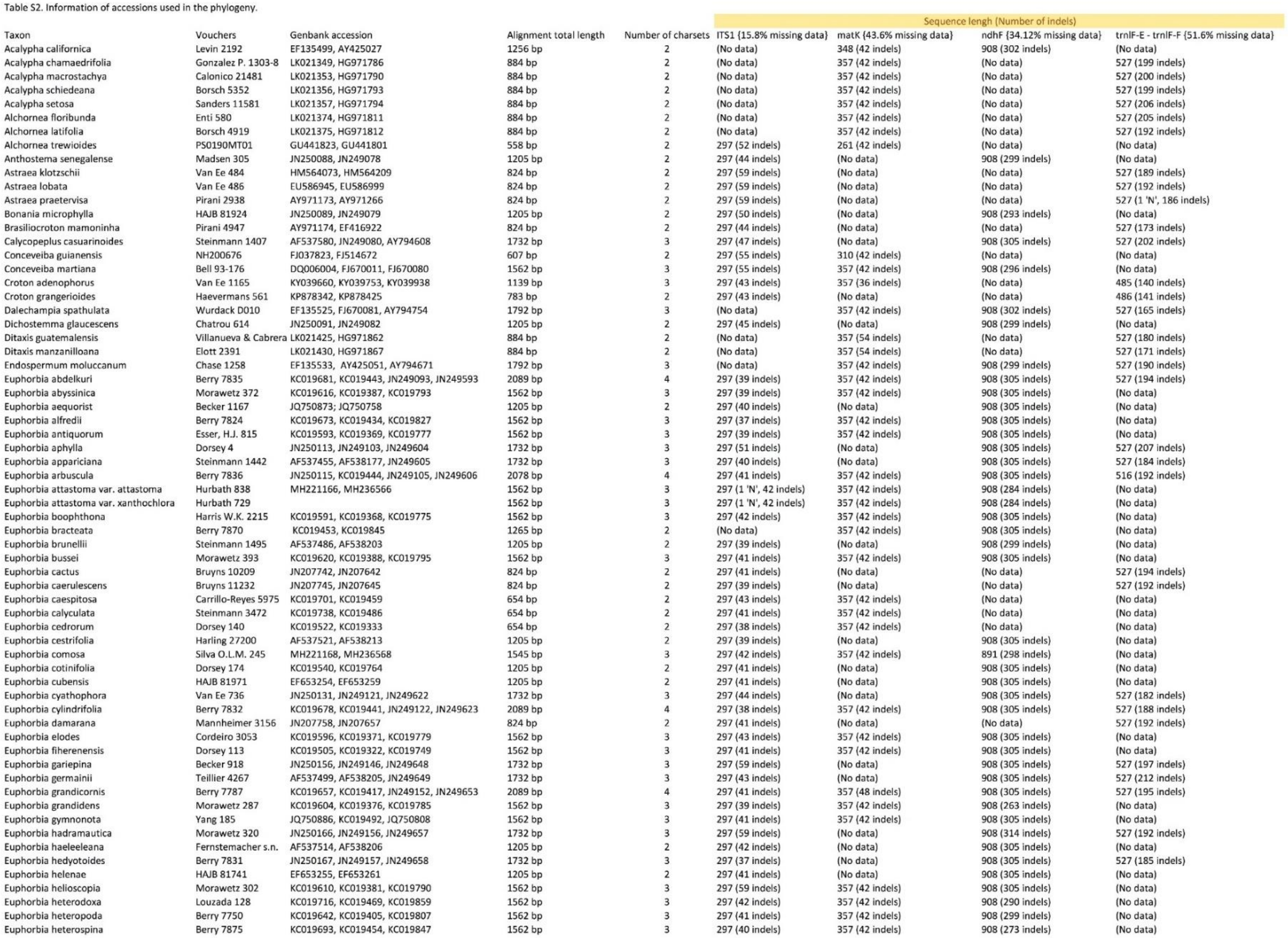


FIGURE S4: Piecharts of BioGeoBears results for *Euphorbia* subg. *Euphorbia* New World Clade. DEC + j: d = 0.003; e = 0; j = 0.026

Table S2. Information of accessions used in the phylogeny.



					Sequence lengh (Number of indels)			
Taxon	Vouchers	Genbank accession	Alignment total length	Number of charsets	ITS1 {15.8% missing data}	matK {43.6% missing data}	ndhF {34.12% missing data}	trnlF-E - trnlF-F {51.6% missing data}
Acalypha californica	Levin 2192	EF135499, AY425027	1256 bp	2	(No data)	348 (42 indels)	908 (302 indels)	(No data)
Acalypha chamaedrifolia	Gonzalez P. 1303-8	LK021349, HG971786	884 bp	2	(No data)	357 (42 indels)	(No data)	527 (199 indels)
Acalypha macrostachya	Calonico 21481	LK021353, HG971790	884 bp	2	(No data)	357 (42 indels)	(No data)	527 (200 indels)
Acalypha schiedeana	Borsch 5352	LK021356, HG971793	884 bp	2	(No data)	357 (42 indels)	(No data)	527 (199 indels)
Acalypha setosa	Sanders 11581	LK021357, HG971794	884 bp	2	(No data)	357 (42 indels)	(No data)	527 (206 indels)
Alchornea floribunda	Enti 580	LK021374, HG971811	884 bp	2	(No data)	357 (42 indels)	(No data)	527 (205 indels)
Alchornea latifolia	Borsch 4919	LK021375, HG971812	884 bp	2	(No data)	357 (42 indels)	(No data)	527 (192 indels)
Alchornea trewioides	PS0190MT01	GU441823, GU441801	558 bp	2	297 (52 indels)	261 (42 indels)	(No data)	(No data)
Anthostema senegalense	Madsen 305	JN250088, JN249078	1205 bp	2	297 (44 indels)	(No data)	908 (299 indels)	(No data)
Astraea klotzschii	Van Ee 484	HM564073, HM564209	824 bp	2	297 (59 indels)	(No data)	(No data)	527 (189 indels)
Astraea lobata	Van Ee 486	EU586945, EU586999	824 bp	2	297 (59 indels)	(No data)	(No data)	527 (192 indels)
Astraea praetervisa	Pirani 2938	AY971173, AY971266	824 bp	2	297 (59 indels)	(No data)	(No data)	527 (1 'N', 186 indels)
Bonania microphylla	HAJB 81924	JN250089, JN249079	1205 bp	2	297 (50 indels)	(No data)	908 (293 indels)	(No data)
Brasiliocroton mamoninha	Pirani 4947	AY971174, EF416922	824 bp	2	297 (44 indels)	(No data)	(No data)	527 (173 indels)
Calycopeplus casuarinoides	Steinmann 1407	AF537580, JN249080, AY794608	1732 bp	3	297 (47 indels)	(No data)	908 (305 indels)	527 (202 indels)
Conceveiba guianensis	NH200676	FJ037823, FJ514672	607 bp	2	297 (55 indels)	310 (42 indels)	(No data)	(No data)
Conceveiba martiana	Bell 93-176	DQ006004, FJ670011, FJ670080	1562 bp	3	297 (55 indels)	357 (42 indels)	908 (296 indels)	(No data)
Croton adenophorus	Van Ee 1165	KY039660, KY039753, KY039938	1139 bp	3	297 (43 indels)	357 (36 indels)	(No data)	485 (140 indels)
Croton grangerioides	Haevermans 561	KP878342, KP878425	783 bp	2	297 (43 indels)	(No data)	(No data)	486 (141 indels)
Dalechampia spathulata	Wurdack D010	EF135525, FJ670081, AY794754	1792 bp	3	(No data)	357 (42 indels)	908 (302 indels)	527 (165 indels)
Dichostemma glaucescens	Chatrou 614	JN250091, JN249082	1205 bp	2	297 (45 indels)	(No data)	908 (299 indels)	(No data)
Ditaxis guatemalensis	Villanueva & Cabrera	LK021425, HG971862	884 bp	2	(No data)	357 (54 indels)	(No data)	527 (180 indels)
Ditaxis manzanilloana	Elott 2391	LK021430, HG971867	884 bp	2	(No data)	357 (54 indels)	(No data)	527 (171 indels)
Endospermum moluccanum	Chase 1258	EF135533, AY425051, AY794671	1792 bp	3	(No data)	357 (42 indels)	908 (299 indels)	527 (190 indels)
Euphorbia abdelkuri	Berry 7835	KC019681, KC019443, JN249093, JN249593	2089 bp	4	297 (39 indels)	357 (42 indels)	908 (305 indels)	527 (194 indels)
Euphorbia abyssinica	Morawetz 372	KC019616, KC019387, KC019793	1562 bp	3	297 (39 indels)	357 (42 indels)	908 (305 indels)	(No data)
Euphorbia aequorist	Becker 1167	JQ750873; JQ750758	1205 bp	2	297 (40 indels)	(No data)	908 (305 indels)	(No data)
Euphorbia alfredii	Berry 7824	KC019673, KC019434, KC019827	1562 bp	3	297 (37 indels)	357 (42 indels)	908 (305 indels)	(No data)
Euphorbia antiquorum	Esser, H.J. 815	KC019593, KC019369, KC019777	1562 bp	3	297 (39 indels)	357 (42 indels)	908 (305 indels)	(No data)
Euphorbia aphylla	Dorsey 4	JN250113, JN249103, JN249604	1732 bp	3	297 (51 indels)	(No data)	908 (305 indels)	527 (207 indels)
Euphorbia appariciana	Steinmann 1442	AF537455, AF538177, JN249605	1732 bp	3	297 (40 indels)	(No data)	908 (305 indels)	527 (184 indels)
Euphorbia arbuscula	Berry 7836	JN250115, KC019444, JN249105, JN249606	2078 bp	4	297 (41 indels)	357 (42 indels)	908 (305 indels)	516 (192 indels)
Euphorbia attastoma var. attastoma	Hurbath 838	MH221166, MH236566	1562 bp	3	297 (1 'N', 42 indels)	357 (42 indels)	908 (284 indels)	(No data)
Euphorbia attastoma var. xanthochlora	Hurbath 729		1562 bp	3	297 (1 'N', 42 indels)	357 (42 indels)	908 (284 indels)	(No data)
Euphorbia boophthona	Harris W.K. 2215	KC019591, KC019368, KC019775	1562 bp	3	297 (42 indels)	357 (42 indels)	908 (305 indels)	(No data)
Euphorbia bracteata	Berry 7870	KC019453, KC019845	1265 bp	2	(No data)	357 (42 indels)	908 (305 indels)	(No data)
Euphorbia brunellii	Steinmann 1495	AF537486, AF538203	1205 bp	2	297 (39 indels)	(No data)	908 (299 indels)	(No data)
Euphorbia bussei	Morawetz 393	KC019620, KC019388, KC019795	1562 bp	3	297 (41 indels)	357 (42 indels)	908 (305 indels)	(No data)
Euphorbia cactus	Bruyns 10209	JN207742, JN207642	824 bp	2	297 (41 indels)	(No data)	(No data)	527 (194 indels)
Euphorbia caerulescens	Bruyns 11232	JN207745, JN207645	824 bp	2	297 (39 indels)	(No data)	(No data)	527 (192 indels)
Euphorbia caespitosa	Carrillo-Reyes 5975	KC019701, KC019459	654 bp	2	297 (43 indels)	357 (42 indels)	(No data)	(No data)
Euphorbia calyculata	Steinmann 3472	KC019738, KC019486	654 bp	2	297 (41 indels)	357 (42 indels)	(No data)	(No data)
Euphorbia cedrorum	Dorsey 140	KC019522, KC019333	654 bp	2	297 (38 indels)	357 (42 indels)	(No data)	(No data)
Euphorbia cestrifolia	Harling 27200	AF537521, AF538213	1205 bp	2	297 (39 indels)	(No data)	908 (305 indels)	(No data)
Euphorbia comosa	Silva O.L.M. 245	MH221168, MH236568	1545 bp	3	297 (42 indels)	357 (42 indels)	891 (298 indels)	(No data)
Euphorbia cotinifolia	Dorsey 174	KC019540, KC019764	1205 bp	2	297 (41 indels)	(No data)	908 (305 indels)	(No data)
Euphorbia cubensis	HAJB 81971	EF653254, EF653259	1205 bp	2	297 (41 indels)	(No data)	908 (305 indels)	(No data)
Euphorbia cyathophora	Van Ee 736	JN250131, JN249121, JN249622	1732 bp	3	297 (44 indels)	(No data)	908 (305 indels)	527 (182 indels)
Euphorbia cylindrifolia	Berry 7832	KC019678, KC019441, JN249122, JN249623	2089 bp	4	297 (38 indels)	357 (42 indels)	908 (305 indels)	527 (188 indels)
Euphorbia damarana	Mannheimer 3156	JN207758, JN207657	824 bp	2	297 (41 indels)	(No data)	(No data)	527 (192 indels)
Euphorbia elodes	Cordeiro 3053	KC019596, KC019371, KC019779	1562 bp	3	297 (43 indels)	357 (42 indels)	908 (305 indels)	(No data)
Euphorbia fiherenensis	Dorsey 113	KC019505, KC019322, KC019749	1562 bp	3	297 (41 indels)	357 (42 indels)	908 (305 indels)	(No data)
Euphorbia gariepina	Becker 918	JN250156, JN249146, JN249648	1732 bp	3	297 (59 indels)	(No data)	908 (305 indels)	527 (197 indels)
Euphorbia germainii	Teillier 4267	AF537499, AF538205, JN249649	1732 bp	3	297 (43 indels)	(No data)	908 (305 indels)	527 (212 indels)
Euphorbia grandicornis	Berry 7787	KC019657, KC019417, JN249152, JN249653	2089 bp	4	297 (41 indels)	357 (48 indels)	908 (305 indels)	527 (195 indels)
Euphorbia grandidens	Morawetz 287	KC019604, KC019376, KC019785	1562 bp	3	297 (39 indels)	357 (42 indels)	908 (263 indels)	(No data)
Euphorbia gymnonota	Yang 185	JQ750886, KC019492, JQ750808	1562 bp	3	297 (41 indels)	357 (42 indels)	908 (305 indels)	(No data)
Euphorbia hadramautica	Morawetz 320	JN250166, JN249156, JN249657	1732 bp	3	297 (59 indels)	(No data)	908 (314 indels)	527 (192 indels)
Euphorbia haeleeleana	Fernstemacher s.n.	AF537514, AF538206	1205 bp	2	297 (42 indels)	(No data)	908 (305 indels)	(No data)
Euphorbia hedyotoides	Berry 7831	JN250167, JN249157, JN249658	1732 bp	3	297 (37 indels)	(No data)	908 (305 indels)	527 (185 indels)
Euphorbia helenae	HAJB 81741	EF653255, EF653261	1205 bp	2	297 (41 indels)	(No data)	908 (305 indels)	(No data)
Euphorbia helioscopia	Morawetz 302	KC019610, KC019381, KC019790	1562 bp	3	297 (59 indels)	357 (42 indels)	908 (305 indels)	(No data)
Euphorbia heterodoxa	Louzada 128	KC019716, KC019469, KC019859	1562 bp	3	297 (42 indels)	357 (42 indels)	908 (290 indels)	(No data)
Euphorbia heteropoda	Berry 7750	KC019642, KC019405, KC019807	1562 bp	3	297 (41 indels)	357 (42 indels)	908 (299 indels)	(No data)
Euphorbia heterospina	Berry 7875	KC019693, KC019454, KC019847	1562 bp	3	297 (40 indels)	357 (42 indels)	908 (273 indels)	(No data)

Euphorbia hirsuta	Riina 1769	JN250171, JN249161, JN249662	1732 bp	3	297 (41 indels)	(No data)	908 (302 indels)	527 (213 indels)
Euphorbia hoffmanniana	Haber 10501	AF537508, AF538211	1205 bp	2	297 (41 indels)	(No data)	908 (305 indels)	(No data)
Euphorbia holochlorina	Hurbath 635	MH221170, MH236569	654 bp	2	297 (1 'N', 42 indels)	357 (42 indels)	(No data)	(No data)
Euphorbia horrida	Berry 7783	KC019655, JN249162, JN249663	1728 bp	3	297 (54 indels)	(No data)	908 (305 indels)	523 (192 indels)
Euphorbia iharanae	Berry 7854	KC019685, KC019479, JN249164, JN249665	2089 bp	4	297 (37 indels)	357 (42 indels)	908 (305 indels)	527 (187 indels)
Euphorbia intisy	Dorsey 127	KC019504, KC019325, KC019748	1562 bp	3	297 (1 'N', 41 indels)	357 (42 indels)	908 (305 indels)	(No data)
Euphorbia lacei	Esser, H.J. 810	KC019592, KC019776	1205 bp	2	297 (39 indels)	(No data)	908 (305 indels)	(No data)
Euphorbia lactiflua	Carrillo-Reyes 5928	KC019700, KC019457	654 bp	2	297 (42 indels)	357 (42 indels)	(No data)	(No data)
Euphorbia lagunillarum	Riina 1836	KC019731, KC019874	1205 bp	2	297 (1 'N', 42 indels)	(No data)	908 (290 indels)	(No data)
Euphorbia laurifolia	Riina 1601	KC019718, KC019470	654 bp	2	297 (42 indels)	357 (42 indels)	(No data)	(No data)
Euphorbia lomelii	Van Ee 703	JN249174, JN249674	1326 bp	2	(No data)	(No data)	908 (305 indels)	418 (174 indels)
Euphorbia lycioides			1562 bp	3	297 (40 indels)	357 (42 indels)	908 (2 'N', 305 indels)	(No data)
Euphorbia mandravioky	Rauh 22994	JQ952365, JQ952069,	608 bp	2	297 (41 indels)	311 (42 indels)	(No data)	(No data)
Euphorbia micracantha	Berry 7802	KC019662, KC019422, KC019817, JN249680	2089 bp	4	297 (42 indels)	357 (42 indels)	908 (305 indels)	527 (195 indels)
Euphorbia milii	Berry 7826	JN250191, KC019436, JN249180, JN249681	2089 bp	4	297 (36 indels)	357 (42 indels)	908 (305 indels)	527 (188 indels)
Euphorbia misera	Van Ee 711	JN250192, JN249181, JN249682	1732 bp	3	297 (41 indels)	(No data)	908 (305 indels)	527 (183 indels)
Euphorbia munizii	HAJB 81796	EF653256, EF653262	1205 bp	2	297 (41 indels)	(No data)	908 (293 indels)	(No data)
Euphorbia neococcinea	Berry 7749	KC019641, KC019404, KC019806	1562 bp	3	297 (41 indels)	357 (42 indels)	908 (302 indels)	(No data)
Euphorbia neovirgata	Dorsey 215	KC019564, KC019358	654 bp	2	297 (41 indels)	357 (42 indels)	(No data)	(No data)
Euphorbia orthoclada	Rauh 70626	AJ534819, FN423752	824 bp	2	297 (52 indels)	(No data)	(No data)	527 (207 indels)
Euphorbia peperomioides	Cordeiro 3057	KC019597, KC019372, KC019780	1562 bp	3	297 (43 indels)	357 (42 indels)	908 (305 indels)	(No data)
Euphorbia personata	MEO 955	GU214939, GU214832	654 bp	2	297 (66 indels)	357 (44 indels)	(No data)	(No data)
Euphorbia phosphorea	Hurbath 655		1562 bp	3	297 (1 'N', 42 indels)	357 (42 indels)	908 (293 indels)	(No data)
Euphorbia planiticola	Gillespie L.J. 7324	KC019629, KC019394, JQ750839	1562 bp	3	297 (40 indels)	357 (42 indels)	908 (305 indels)	(No data)
Euphorbia plumerioides	Berry 7884	JN250214, KC019456, JN249201, JN249704	2033 bp	4	241 (32 indels)	357 (42 indels)	908 (305 indels)	527 (248 indels)
Euphorbia podocarpifolia	HAJB 81712	EF653257, EF653263	1205 bp	2	297 (41 indels)	(No data)	908 (305 indels)	(No data)
Euphorbia portulacoides	Van Ee 582	JN250217, JN249707	824 bp	2	297 (43 indels)	(No data)	(No data)	527 (188 indels)
Euphorbia potentilloides	Hurbath 426	MH221173, MH236571	1562 bp	3	297 (49 indels)	357 (42 indels)	908 (314 indels)	(No data)
Euphorbia pteroneura	Pirani 6621		1562 bp	3	297 (41 indels)	357 (42 indels)	908 (305 indels)	(No data)
Euphorbia pulcherrima	Steinmann 1070	AF537432, AF538168	1205 bp	2	297 (42 indels)	(No data)	908 (299 indels)	(No data)
Euphorbia punicea	Berry 7848	JN250221, JN249208, JN249710	1732 bp	3	297 (41 indels)	(No data)	908 (305 indels)	527 (193 indels)
Euphorbia rauhii	Dorsey 181	KC019545, KC019345, KC019765	1562 bp	3	297 (1 'N', 41 indels)	357 (42 indels)	908 (305 indels)	(No data)
Euphorbia rosescens	Weekley sn	KC019587, KC019774	1205 bp	2	297 (40 indels)	(No data)	908 (305 indels)	(No data)
Euphorbia schubei	Dorsey 200	KC019551, KC019350	654 bp	2	297 (41 indels)	357 (42 indels)	(No data)	(No data)
Euphorbia sipolisii	Hurbath 828		1562 bp	3	297 (2 'N', 42 indels)	357 (42 indels)	908 (284 indels)	(No data)
Euphorbia tannensis	Fryxell 4475	JN250245, JN249231, JN249731	1732 bp	3	297 (40 indels)	(No data)	908 (305 indels)	527 (192 indels)
Euphorbia tanquahuete	Steinmann 1620	AF537525, AF538224	1205 bp	2	297 (41 indels)	(No data)	908 (305 indels)	(No data)
Euphorbia tetrangularis	Hurbath 844	MH221171, MH236570	1181 bp	3	297 (1 'N', 42 indels)	357 (42 indels)	(No data)	527 (3 'N', 191 indels)
Euphorbia tetraptera	Randrianasolo 1113	KC019495, KC019317	654 bp	2	297 (41 indels)	357 (42 indels)	(No data)	(No data)
Euphorbia thinophila	Dillon-Teillier 5127	AF537530, AF538218	1205 bp	2	297 (43 indels)	(No data)	908 (305 indels)	(No data)
Euphorbia tirucalli	Berry 7772	KC019650, JN249234, JN249734	1732 bp	3	297 (40 indels)	(No data)	908 (305 indels)	527 (192 indels)
Euphorbia tithymaloides	Wurdack D034	JN250249, JN249235, AY794604	1623 bp	3	297 (65 indels)	(No data)	908 (299 indels)	418 (172 indels)
Euphorbia umbelliformis	HAJB 81901	JN250252, JN249238, JN249737	1732 bp	3	297 (41 indels)	(No data)	908 (305 indels)	527 (187 indels)
Euphorbia weberbaueri	Berry 7879	JN250256, JN249242, JN249741	1732 bp	3	297 (40 indels)	(No data)	908 (305 indels)	527 (180 indels)
Euphorbia xylopoda	Steinmann 6752	KC019739, KC019487	654 bp	2	297 (41 indels)	357 (42 indels)	(No data)	(No data)
Hippomane mancinella	Fuentes 673	LK021451, HG971888	884 bp	2	(No data)	357 (42 indels)	(No data)	527 (205 indels)
Macaranga pearsonii	Slik M104	DQ866573, DQ899231	824 bp	2	297 (56 indels)	(No data)	(No data)	527 (205 indels)
Mallotus philippensis	Kathriarachchi HK64	DQ866614, DQ899273	824 bp	2	297 (55 indels)	(No data)	(No data)	527 (192 indels)
Nealchornea yapurensis	Fine s.n.	JN250098, JN249089, AY794662	1732 bp	3	297 (2 'N', 77 indels)	(No data)	908 (305 indels)	527 (203 indels)
Omphalea diandra	Chase 570	FJ670016, FJ670087, AY794672	1792 bp	3	(No data)	357 (42 indels)	908 (299 indels)	527 (167 indels)
Omphalea trichotoma	Borsch 4572	LK021475, HG971912	884 bp	2	(No data)	357 (42 indels)	(No data)	527 (158 indels)
Pera bicolor	Gillespie 4300	EF135578, AY674747, AY794808	1772 bp	3	(No data)	357 (48 indels)	888 (275 indels)	527 (201 indels)
Pera bumeliifolia	Xi et al., 2010	JX661957, JX662766	1265 bp	2	(No data)	357 (30 'N', 18 indels)	908 (162 'N', 113 indels)	(No data)
Pimelodendron zoanthogyne	Chase 1268	EF135582, AY794661	884 bp	2	(No data)	357 (42 indels)	(No data)	527 (205 indels)
Plukenetia volubilis	Ibarra 3608	LK021490, HG971927	884 bp	2	(No data)	357 (42 indels)	(No data)	527 (203 indels)
Sebastiania cornuta	Steinmann 589	AF537587, AF538263, AY794611	1732 bp	3	297 (49 indels)	(No data)	908 (293 indels)	527 (225 indels)
Stillingia spinulosa	Felger 92-381	AF537588, AF538264	1205 bp	2	297 (75 indels)	(No data)	908 (314 indels)	(No data)
Stillingia sylvatica	Wurdack D117	JN250101, JN249092, AY794631	1695 bp	3	297 (75 indels)	(No data)	908 (299 indels)	490 (200 indels)
Suregada boiviniana	Rakotomalaza 1292	DQ006006, AY794663	824 bp	2	297 (42 indels)	(No data)	(No data)	527 (201 indels)
Suregada eucleoides	Harder 1569	DQ006007, AY794664	824 bp	2	297 (42 indels)	(No data)	(No data)	527 (201 indels)
Vernicia montana	SCBGP481_1	KP092944, KP094072	565 bp	2	297 (45 indels)	268 (30 indels)	(No data)	(No data)

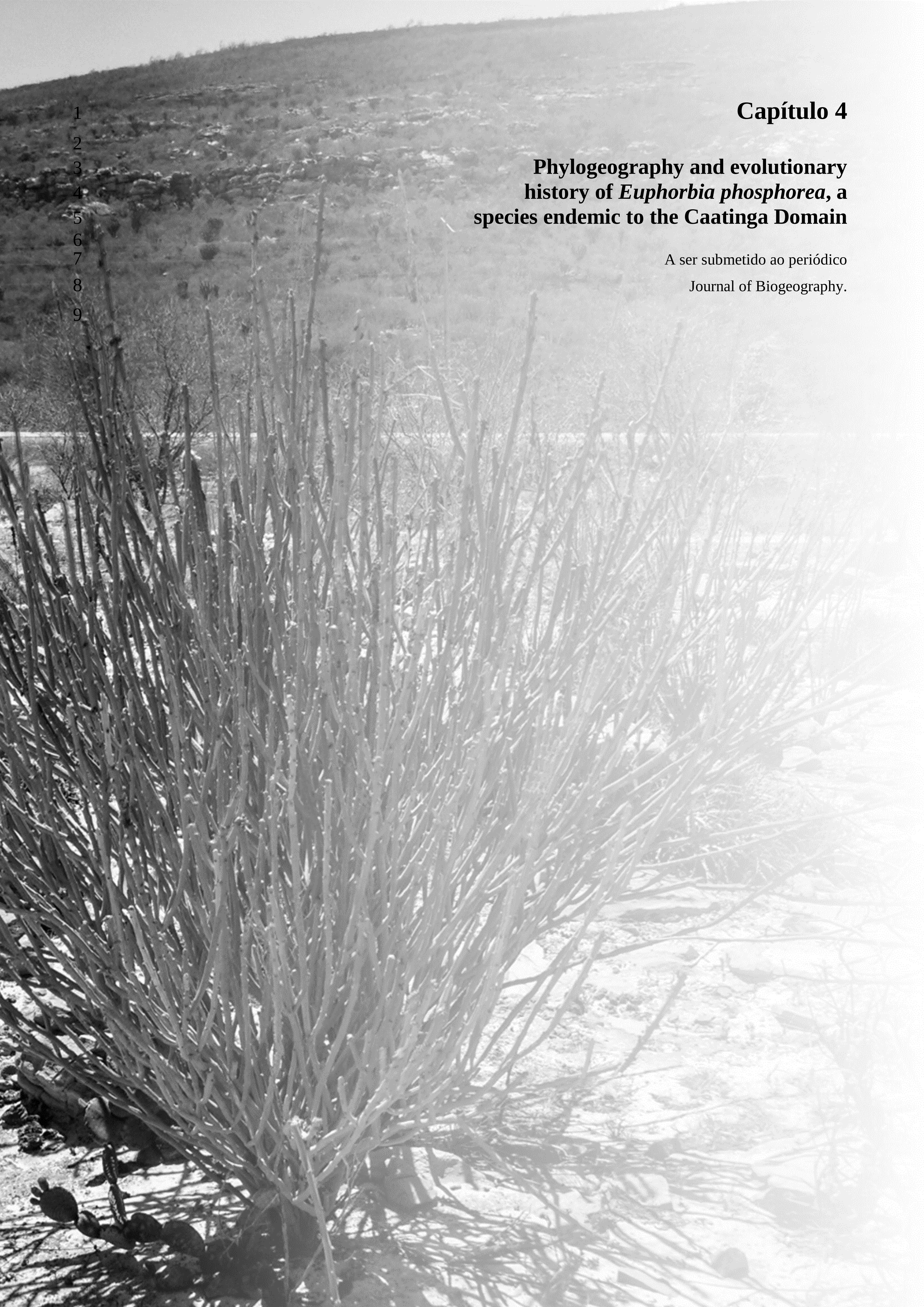
Table S2: Information of primers used for amplification and sequencing of *Euphorbia* sect. *Brasilienses.*

Region	**Annealing Temp (℃)**	**Proposed by**	**Primer**
matk	48	Garrick et al., (2009)	F: AGTATCTTCTTTAGAAAGGCC R: TAGCATTTGACTCCGTACTACC
ndhF	46	Garrick et al., (2009)	F: TAATAGCTTGGTTGACTGCGG R: TGTAACCTCGATTATAGGAC
*trn*L (UAA) *trn*F (GAA)	54	Tarbelet et al., (1991)	F: GGTTCAAGTCCCTCTATCCC R: ATTTGAACTGGTGACACGAG
ITS1	52	Urbatsch et al., (2000)	F: GTCCACTGAACCTTATCATTTAG R: GCTGCGTTCTTCATCGATGC

Capítulo 4

Phylogeography and evolutionary history of *Euphorbia phosphorea*, a species endemic to the Caatinga Domain

A ser submetido ao periódico
Journal of Biogeography.



Phylogeography and evolutionary history of *Euphorbia phosphorea*, a species endemic to the Caatinga Domain

Fernanda Hurbath[1,4], Bárbara Simões Santos Leal[2], Inês Cordeiro[1], Clarisse Palma-Silva[2,3]

Abstract

Stage for a remarkable amount of geomorphological processes and climate changes, studies have established that many processes acted concomitantly in shaping the great biodiversity in South America. However, studies acquired different outcomes, due to intrinsic factors of biomes and taxa, and therefore urges the necessity for more studies using phylogeographic approaches. Here, we provide another missing piece of the puzzle that is the evolution of most biodiverse yet poorly studied nucleus of SDTF, the Caatinga Domain (CD).

Aim

Our aim was to conduct a phylogeographic study with *E. phosphorea* using this endemic species as model for inferring the evolutionary history of the CD, combining molecular analyses from sequence markers and species distribution models to infer *E. phosphorea* evolutionary history and phylogeographic structure.

Methods

We sampled 129 individuals comprising 10 localities of *E. phosphorea.* We sequenced two plastid regions and one nuclear and conducted analyses to infer genetic diversity and structure, as well as phylogenetic relationships between populations. In addition, we conducted a distribution modeling to infer historical connectivity during LGM and Holocene.

Results

Our results recovered a stronger genetic structure for cpDNA than for nrDNA, and low levels of genetic diversity within populations. Although our modeling analyses suggests connectivity during LGM, our demographic analyses from molecular markers detected no signs of demographic expansion. Finally, our phylogenetic analyses estimated that *E. phosphorea* has diverged in the early Pleistocene.

Main conclusions

Differences between plastid and nuclear patterns could be strongly related to species capability to seed and pollen dispersal. Species originated and diversified before main climatic instability reported for upper Pleistocene, however our modelling results attest that the species may have been influenced by LGM driest climate.

Keywords

Phylogeography, SDTF, microrefugia, Euphorbiaceae, rock outcrops, inselbergs, Raso da Catarina, Planalto da Borborema.

[1] Nucleo de Pesquisa Curadoria do Herbário SP, Instituto de Botânica, São Paulo, São Paulo, Brazil.
[2] Laboratório de Ecologia Molecular, Departamento de Ecologia, Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil.
[3] Departamento de Biologia Vegetal, Universidade Estadual de Campinas, Barão Geraldo, Campinas, São Paulo, Brazil.

Correspondence

Fernanda Hurbath, Instituto de Botânica, Nucleo de Pesquisa Curadoria do Herbário SP, Avenida Miguel Stéfano 3687, São Paulo, 04045-972, SP, Brazil. Email: fhurbath@gmail.com

Funding information

Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (2015/11812-1); Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (309917/2015-8).

INTRODUCTION

South America possesses great biodiversity, estimated to hold 37% of known species of seed plants in the globe (Antonelli & Sanmartín, 2011). Studies have tried to answer questions related to South America evolutionary history and why it harbors so much biodiversity (Haffer, 1969; Gentry, 1982; Antonelli & Sanmartín, 2011; Hughes et al., 2013a; Rull, 2013; Antonelli et al., 2018). South America was the stage for an impressive amount of geomorphological processes (Hoorn et al., 2010), and for some authors it would explain the amazing biodiversity found within (Gentry, 1982; Hoorn et al., 2010; Antonelli & Sanmartín, 2011). Historical climatic changes have also been pointed as a major factor underlying the Neotropical diversification (Haffer, 1969; Pennington et al., 2004; Antonelli et al., 2010; Silva et al., 2018). In fact, many processes must have acting concomitantly, shaping South America's current biodiversity (Hughes et al., 2013b; Rull, 2013). However, the same abiotic events could have incited different outcomes between biomes and taxa, due to intrinsic factors, and therefore, each biome or region in South America needs further investigation of its patterns of diversification (Antonelli & Sanmartín, 2011; Hughes et al., 2013b).

Phylogeographic studies use fine scale filters to elucidate how broad geological and climate events have shaped demographic history of populations. It can provide knowledge of aspects involved in the origin and distribution of biodiversity, since It can detect evolutionary forces acting under effect of landscape elements. Great effort has been made to understand what molded currently disjunct patched distribution of Seasonally Dry Tropical Forests (SDTF). Studies have agreed that present distribution of SDTF represents vestiges of a larger and continuous formation during Pleistocene climatic fluctuations (Pleistocene Arc Hypothesis - PAH) (Zanella, 2000; Prado, 2003; Oliveira-Filho et al., 2006; Collevatti et al., 2012; Franco & Manfrin, 2013; Bonatelli et al., 2014) and others agree with a pre-Pleistocene vicariance (Pennington et al., 2004; Saslis-Lagoudakis et al., 2008; Garcia et al., 2011; Werneck et al., 2012b; Magalhaes et al., 2014; Vieira et al., 2015; de Queiroz et al., 2017; Camps et al., 2018; Silva et al., 2018; Costa et al., 2018; Lanna et al., 2018). However, each SDTF patch or nucleous presents high endemism and few species are shared between them, therefore, even more inherent investigations, in a microevolutionary scale, should be made, involving each patch and its endemic species.

The Caatinga Domain (CD) is the largest continuously nucleus of Seasonally Dry Tropical Forests (SDTF), corresponding to 10% of the Brazilian territory, presenting the highest level of biodiversity and endemism compared to any other SDTF nucleus in South America (Queiroz, 2006). Such domain is characterized by a semi-arid and strongly seasonal climate, and presents a mosaic of geological formations and vegetational types (Velloso et al., 2002). One can find typical caatinga vegetation growing in depressions on crystalline origin soils, resultant of a process of planation during the Neogene and lower Quaternary (Ab'Saber, 1974; Valadão, 2009). Queiroz (2006) inferred that species distribution patterns along the CD permitted interpret that vegetation associated to soils with crystalline basement (crystalline caatinga), differs from the ones associated to sedimentary origin soils from São Francisco basin (sedimentary caatinga), with the first showing closest floristic relation with other SDTF nucleus (Queiroz, 2006; de Queiroz et al., 2017). Inselbergs can be found scattered along the depressions, representing remnants of the older surface that once covered most of the region until lower Tertiary (Ab'Saber, 1974; Valadão, 2009). Enclaves of humid forest vegetation can be found in river valleys, that some authors consider as vestiges of a past connection between Atlantic Forest and Amazonia, also considered refuges of once expanded moist forests (Andrade et al., 2007; Batalha-Filho et al., 2013; Thomé et al., 2016). Plateaus and chain of mountains, achieving up to 1.900 m altitude, can also be found, like Planalto da Borborema in northeast portion of the CD, and Chapada Diamantina, in the meridional region, occurring typical vegetation of Campos Rupestres (Velloso et al., 2002; Queiroz, 2006). Such high heterogeneity may determine distinct responses to climatic oscillations according to organism specific features.

The heterogenous landscapes characteristic of CD could allow lineages to survive trough environmental changes, enabling microrefugia (Turchetto-Zolet et al., 2013). Paleoclimatic evidences pointed to a predominance of semi-arid climate in northeastern Brazil's since Miocene (Ab'Saber, 1974), and many studies reported the evolution of arid biomes during Miocene-Pleistocene all over the world (Pascual & Jaureguizar, 1990; Zachos et al., 2001; Guo et al., 2004; Potter & Szatmari, 2009; Tang & Ding, 2013), adding to that, divergence times for most endemic lineages were estimated for Miocene and Pliocene (de Queiroz et al., 2017). Therefore, most lineages in Caatinga Domain probably went through an *in situ* diversification, congruent with an old and isolated habitat (Losos & Glor, 2003) coupled with low dispersal abilities, characteristic of taxa from xeric formations, due to niche conservatism (Pennington et al., 2009). In fact, recent study testified that Caatinga works as a sink of biodiversity, receiving lineages more than giving, which corroborates to niche conservatism of its endemic lineages. However, CD evolutionary history is characterized by humid forest recurrent invasions (Ab'Saber, 1974; Thomé et al., 2016), contraction and expansion of its limits (Werneck, 2011; Vieira et al., 2015), orogenic changes with uplifts and planation (Valadão, 2009; Maia et al., 2010), and changes of São Francisco river course, the largest river in the region (Potter, 1997).

In South America, a greater effort was given to humid forests than dry vegetations, specially the semi-arid CD in Northeastern Brazil (Leal et al., 2016). The few phylogeographic studies performed with taxa endemic of CD show that species present contradicting responses to quaternary climatic events. This imply a complex evolutionary history possibly explained by more than one aspect, including individual taxon adaptations to climatic oscillations. For instance, Vieira et al. (2015) inferred that *Ficus bonijesulapensis* R.M. Castro suffered a demographic expansion southward of its range, during Holocene, similar to what was found for another Caatinga endemic tree, *Spondias tuberosa* Arruda. Contrastinly high population structure with no apparent demographic changes were reported for herbaceous plant species found in inselbergs in the CD (*Epidedrum* spp – Pinheiro et al 2014; *Encholirium spectabile* Mart. ex Schult.f. Gonçalves-Oliveira et al. 2017). Moreover, although most lineages divergence occurred in pre-Pleistocene periods (Nascimento et al., 2013; Magalhaes et al., 2014; Werneck et al., 2015; Thomé et al., 2016; Costa et al., 2018; Lanna et al., 2018), few studies inferred that quaternary climatic changes were important in shaping population genetic structure of Caatinga species(Bonatelli et al., 2014; Miranda et al., 2017).

Here, we conducted a phylogeographic study with *Euphorbia phosphorea* Mart., using this endemic species as model for inferring the evolutionary history of the Caatinga Domain. Such species is a xeric environment specialist, with succulent shrub habit, usually associated with rock outcrops, stone slabs and inselbergs, but also occurring on sandy gravelly soils from CD. The naturally fragmented distribution and ecological features make *E. phosphorea* an interesting model to fill gaps on the response of diversification of Neotropical xeric organisms to Quarternary climatic oscilations. We combined molecular analyses from sequence markers and species distribution models to infer *E. phosphorea* evolutionary history and phylogeographic structure. We proposed three hypotheses to guide our study: (1) Populations of *E. phosphorea* are highly structured as found for other species of xeric environments associated with rocky formations; (2) since this species is highly adapted to xeric environment, *E. phosphorea* presents a historical demographic variation and expansion during LGM, when climate was drier and colder; (3) Current distribution of genetic diversity is associated to stable CD areas during Pleistocene climatic oscillations. The present work provides another missing piece of the puzzle that is the evolution of the largest and most biodiverse nucleus of SDTF, yet poorly studied.

MATERIAL AND METHODS

Studied species and sampling

Euphorbia phosphorea is one of the fewest succulent *Euphorbia* species in Brazil (Dorsey et al., 2013). It is endemic to CD, being considered a targeting element of caatinga vegetation (Hurbath and Cordeiro, in prep). Its distribution is naturally fragmented since this species is usually found on rock outcrops and inselbergs. *Euphorbia phosphorea* presents as an erect candelabriform shrub, 1.5 to 4 meters high but also forming slightly dispersed patches or clumps when growing on inselbergs, frequently associated with bromeliads and other matting forming species (Porembski et al., 1998). Nevertheless, *E. phosphorea* can also be found growing on sandy soils, of sedimentary origin, with great amount of gravels and low fertility, in Raso da Catarina (Bahia). Individuals in this area were found growing as scrambling shrubs, leaning on other species, and forming great bushes. The species presents a self-disperse syndrome, with explosion of its capsules, and probably a secondary mymermochoric dispersion due to caruncle presence on seeds, as already documented for *Euphorbia* and other species from Caatinga (Leal et al., 2017). Field observations led us to infer that pollination could also be played by ants (*Camponotus* spp.; *Cephalotes* sp. - Felipe Martello Ribeiro, personal communication, June 7, 2016).) and some other winged insects like flies, beetles and wasps (field observations). The plants offers nectar that can attract even lizards during dry seasonal periods as documented by Aximoff & Felix (2017).

We collected 10 populations of *E. phosphorea* covering most of its distribution, from 6° to 13° latitudinal range (Tab. 1, Fig. 1). Populations presented altitudinal range from 228 to 1121 m alt. We collect individuals at least 5 meters apart to avoid clones, since this species can present vegetative propagation. Voucher specimens are deposited in the herbarium SP, Instituto de Botânica, São Paulo, São Paulo, Brazil.

Table 1 Sample information and haplotypes of 10 populations of **Euphorbia phosphorea** Mart. (Euphorbiaceae).

Population code	**Municipality**	**Coordinates**	**Alt.**	**N**
GEN	Gentio do Ouro-BA	42W 31' 34.1", 11S 24' 33.1"	1121	15
CHA	Morro do Chapéu-BA	41W 20' 04.5", 11S 29' 23.6"	877	15
TAN	Tanhaçu-BA	41W 16' 46.11", 13S 54' 44.25"	506	7
BE	Bendegó-BA	39W 07' 20", 9S 44' 29.8"	484	8
CAN	Canudos-BA	39W 01' 02.3", 9S 57' 42.1"	493	8
CABRO	Cabrobó-PE	39W 12' 59.3", 8S 18' 12"	663	14
BRE	Brejo da Madre de Deus-PE	36W 23' 39.9", 8S 09' 28.4"	1161	16
COR	São José dos Cordeiros-PB	36W 53' 20.2", 7S 28' 23.5"	650	16
LAVR	Pedra Lavrada-PB	36W 27' 13.4", 6S 48' 10.6"	651	15
RN	Serra Caiada-RN	35W 43' 23.16", 6S 5' 33.51"	228	15

DNA extraction and sequencing

DNA extraction was conducted using epidermal tissue from branches. We used (Tel-Zur et al., 1999) first steps protocol combining five pre-washing steps using a sorbitol-buffer and then proceeded with the use of Plant Mini Kit (Qiagen, California), following the manufacturer's protocol.

From a total of 44 tested markers, we chose two plastid (cpDNA) markers (*psbA-trnH*, *trnC-ycf6*) and one nuclear (nrDNA) (ITS1) (Tab. S1) according to polymorphism levels. We calculated mean distances in PAUP* ver. 4.0b10 (Swofford, 2002) between sequences of *Euphorbia* sect. *Brasilienses* for the entire ITS region and found almost no divergence among them (less than 1%). This divergence was concentrated mainly in the first internal transcribed spacer ITS1. Therefore, we've decided to amplify only the first region. Amplifications were performed with a final volume of 30 µl, and final concentrations of: 1X buffer solution, 1.5-3 mM $MgCl_2$, 0.2-0.4 mM of dNTP (Promega), 0.33-0.5 µM of each primer,1U Taq polymerase (Biolase and Promega), DMSO 0.02%, 0.1-0.2 mM of BSA and ca. 5 ng of DNA. PCRs were performed using a Veriti 96-Well Thermal Cycler (Applied Biosystems) following the cycling conditions as follows: 95°C for 4 min for initial denaturation; followed by 30 cycles at 95°C for denaturing during 35s, 45s for annealing (Tab. S1), 72°C for 1 min for extension; and a final 5 min extension at 72°C. PCR products were examined in agarose gels (1%) by electrophoresis. Sequencing and purification were performed by Macrogen (Seoul, South Korea) using the same primers as for PCR, on an ABI3730XL DNA sequencer.

Sequences were de novo assembled in the software Geneious 6.1.8. (Kearse et al., 2012), and checked manually, base by base, with the chromatogram. The alignment was performed using MUSCLE (Edgar, 2004) also in Geneious, no manual adjustment was necessary. We used the algorithm PHASE implemented in DnaSP 5.10.01 (Librado & Rozas, 2009) to infer sequence phase at heterozygous sites for the nuclear marker, under a no recombination model.

Genetic diversity and structure analyses

Molecular diversity indices were calculated based on number of polymorphic sites (S), number of haplotypes (h), haplotype diversity (Hd), and nucleotide diversity (p) using the software DnaSP (Librado & Rozas, 2009) for populations, for both genetic clusters (see BAPS results) and for the species. Population pairwise F_{ST} and linearized F_{ST} comparisons were calculated using Arlequin version 3.5 (Excoffier et al., 2005). A Median joining network analysis (Bandelt et al., 1999) implemented in Network 4.6.1.0 (fluxus-engineering.com) was used to infer the historical relationships among haplotypes.

We examined the population genetic structure using the 'Clustering with linked loci' module of BAPS 6 software (Corander & Tang, 2007). Plastid markers were concatenated, while nuclear marker was tested separately. We performed an analysis of molecular variance (AMOVA) using Arlequin version 3.5 With to obtain partition of genetic diversity among groups (obtained from BAPS, for plastid markers), among populations within groups, and within populations. As we found no BAPS group with nuclear sequences (see Results), AMOVAwere performed to calculate the partition of diversity among and within populations. We estimated the significance of the variance components using 10,000 permutations of the data.

Phylogenetic reconstruction

Estimation of time for the most recent common ancestor (TMRCA) was inferred by BEAST 1.8.4 (Drummond et al., 2012), using a combined matrix of nrDNA and cpDNA. Phylogeny with only plastid sequences were also performed and included in supplemental material (Fig. S1). Substitution models for each gene were selected prior to phylogenetic reconstructions using jModelTest 2.1.7 (Darriba et al., 2015), under the Bayesian information criterion (BIC): ITS1 - K80 (HKY, all equal base frequency in BEAUTI settings), *psb*A-*trn*H - F81 (GTR, turning off operators for bases), *trn*C-*ycf6* - TPM1uf (K81). In data matrix, only partition tree was linked. All calibration points were secondary, and therefore we applied a normal distribution corresponding to the split between *E.* sect.

Stachydium (the most distant outgroup used in this analyses) at 16.56 Ma, taken from Hurbath et al (in prep), another corresponding the node age *E.* sect. *Brasilienses* of 3.15 Ma and another using tree prior, as stem calibration of *E. tetrangularis*. We performed two independent runs with 400,000,000 iterations and trees sampled every 40,000 generations, under an uncorrelated lognormal relaxed molecular clock, and a coalescent constant size prior. Convergence were checked with Tracer 1.7 (Rambaut et al., 2018) and combined runs with LogCombiner discarding a burn-in of 10%.

Demographic analyses

In order to detect evidences of population expansion/retraction for each inferred clade, we calculated Fu's Fs, Tajima's D and Roza's R2 statistics based on segregating sites and performed neutrality tests based on 10,000 coalescent simulations with DnaSP. We also performed an Extended Bayesian Skyline Plot (EBSP) analysis (Drummond et al., 2005) to estimate changes in population size over time for the Northern Clade from Beast phylogenetic analyses, combining markers as independent partitions in Beast 2 (Bouckaert et al., 2014). We set a strict clock prior using substitutions rates intervals for each marker inferred by our Beast species tree as priors (1.101E-3±0.786 for plastid markers; 4.4741E-2±1.45 for nuclear). We then adjusted the weights of the analysis' operators according to the recommendations of the EBSP tutorial (Drummond et al., 2012). Three independent runs of 400,000,000 generations, with a thinning interval of 20,000, results were not combined since only one run achieved more than 200 value for ESS (402) in Tracer 1.6.

Species distribution models

Species distribution models (SDMs) were performed for *Euphorbia phosphorea* using 123 spatially unique occurrence points across the whole range of distribution (Tab. S2) Such occurrences points were obtained from GPS measurements during field trips and visited herbarium collections deposited in ALCB, BHCB, CEN, CEPEC, EAC, HRB, HRCB, HUEFS, JPB, MAC, MBM, PEUFR, R, RB, SP, SPF, UB, UEC, UFP, UFRN as well as from digital images from G, K, M, NYBG, P, US, VIC collections. After excluding the highly correlated variables, five bioclimatic variables (Appendix 1) available from the WorldClim database (Fick & Hijmans, 2017), were selected to characterize the background environmental conditions: Annual Precipitation, Annual Mean Temperature, Precipitation Seasonality, Temperature Annual Range, and Precipitation of Coldest Quarter (Appendix 1).

We used a maximum entropy algorithm as implemented in MAXENT 3.3.3e (Phillips & Dudík, 2008) to perform SDMs under the current climatic scenario. Then, projections of models were made on the paleoclimatic dataset simulated by the Community Climate System Model – CCSM4 (Gent et al., 2011) for Holecene (6 kya) and Last Glacial Maximum (LGM, 21 kya) in order to infer the outcome of past climatic oscillations on the species niche. We set 50000 as the maximum number of iterations, with 20 replicates of each model generated by random seed (25% - training, 75% - testing). Average values of the replicates were used as final logistic outputs. Furthermore, we applied the 'maximum training sensitivity plus specificity logistic' threshold, that maximizes cases where the model erroneously assigns unsuitable habitat (true negative) and misses suitable habitat (false positive).

We conducted a jackknife test to address the relative importance of each climatic variable to the models. The algorithm performance was assessed by the threshold independent statistics areas under the receiver operating characteristic curves (AUC; Phillips et al., 2006). Since AUC values trend to be higher for species with narrow ranges, we further evaluated model precision with the true skill statistics (TSS; Allouche et al., 2006). We used R custom scripts and R-packages raster (Hijmans & Elith, 2013) and rgdal (Bivand et al., 2013) to prepare raster layers for modeling and mapping results.

RESULTS

Higher genetic structure for cpDNA than nrDNA

Consensus sequences were obtained from 129 individuals of *Euphorbia phosphorea* and three outgroups, for each marker (Tab. S1). The concatenated alignment presented 1,371 base pairs (pb) and 103 informative sites, including outgroups, and 1,239 pb and 27 informative sites for the ingroup. Individual alignments for plastid and nuclear, without outgroup, presented respectively, 918 pb (25 variable sites and 14 haplotypes) and 321 pb (7 variable sites and 17 haplotypes) (Fig. 1, Tab. 2).

Table 2 Genetic diversity indices of *Euphorbia phosphorea* based on nrDNA and cpDNA data. S = Number of polymorphic sites, h = number of haplotypes, Hd = haplotype diversity, p = nucleotide diversity. *p<0.01

	nrDNA				**cpDNA**			
Population code	***S***	***h***	***H*d(sd)**	***p***	***S***	***h***	***H*d(sd)**	***p***
GEN	0	1	0.0000	0.000000	1	2	0.5143	0.019780
CHA	0	1	0.0000	0.000000	0	1	0.0000	0.000000
TAN	1	2	0.3626	0.001130	0	1	0.0000	0.000000
BE	0	1	0.0000	0.000000	9	2	0.4286	0.148352
CAN	2	3	0.7000	0.003115	0	1	0.0000	0.000000
CABRO	6	7	0.7804	0.007401	0	1	0.0000	0.000000
BRE	5	7	0.7218	0.007016	8	2	0.2333	0.071795
COR	1	2	0.2722	0.000848	0	1	0.0000	0.000000
LAVR	4	4	0.4506	0.002292	0	1	0.0000	0.000000
RN	5	5	0.6299	0.006610	2	3	0.3619	0.018315
E. phosphorea	**7**	**17**	**0.727**	**0.00474**	**25**	**14**	**0.887**	**0.00674**

We found lower within-population genetic diversity in *Euphorbia phosphorea* for the cpDNA than nrDNA (Tab. 2). Most population presented a single cpDNA haplotype, while populations presented one to seven haplotypes for the nuclear marker (Tab. 1, Fig. 2). All sequenced individuals, for nrDNA and cpDNA, exhibited overall haplotype diversity of 0.727 and 0.887, and overall nucleotide diversity of 0.0047 and 0.0067, respectively (Tab. 2).

Nuclear haplotypes were well shared among populations, with a total of eight shared haplotypes between two and six populations (Fig. 2), showing less structuring than plastid markers. Shared plastid haplotypes were only found between COR and LAVR populations (Fig. 1 A), with all others being exclusive to a single population (Fig. 1 A-C).

The cpDNA haplotype network shows deep differentiation among clusters (Fig. 1), with a lack of a central haplotype and the presence of many median vectors (mv). We detected four clusters, grouping the most related haplotypes (dashed outlined squares - Fig. 1): one comprising most northern populations (RN, LAVR, COR and BRE), a second with geographically distant populations in central and south region of species distribution (CABRO, BE, GEN and TAN), third cluster with only one population from Morro do Chapéu (CHA) and a forth cluster with the population from Canudos (CAN), which is geographically close to BE, in Raso da Catarina. Similar clusters were recovered as clades for phylogenetic analyses (see in next section).

Nuclear network presented a different configuration with low genetic structure (Fig. 2). Two central haplotypes (Hap 1 and 3) are shared by 114 individuals and 69 individuals, respectively. The other haplotypes are shared by less than 13 individuals, including two singletons haplotypes. However, two groups can be cautiously inferred: one comprising the further north populations of species distribution, in Rio Grande do Norte, Paraíba and Pernambuco and in north of Bahia Raso da Catarina in Bahia (RN, COR, LAVR, BRE, CABRO, BE), and a further south group, with populations from Chapada Diamantina (CHA, GEN and TAN). CAN features haplotypes from both groups, and it is located in the middle of the geographic distribution of the species.

Bayesian clustering analysis (BAPS) for cpDNA confirms the high genetic structure found in *Euphorbia phosphorea.* Individuals were gathered in seven genetic groups (Fig. 1 C), each practically corresponding to a single population, with exception of COR, LAVR and BRE that formed a single group, also similar to Northern Clade from phylogenetic analyses (Fig.1). BAPS results for nuclear sequences recovered no clear genetic structure (Fig. 2 C). AMOVA showed a significant genetic structure when contrasting the genetic groups indicated by BAPS for the cpDNA ($F_{CT} = 0.88294$, $P < 0.001$; Tab. 3). Differentiation among populations is higher for cpDNA than nrDNA ($F_{ST}=0.92064$ and $F_{ST}=0.37592$, respectively).

Table 3 AMOVA results for nuclear and plastid sequences. Plastid DNA groups are deffined according to results of BAPS analysis.

		N samples	**% variation**	**Fixation index**	**p-value**
cpDNA	Among groups	7	88.29%	F_{CT}: 0.883	<0.001
	Among pop. within groups	2	3.77%	F_{SC}: 0.322	<0.0001
	Within pop.	119	7.94%	**F_{ST}: 0.921**	<0.0001
nrDNA	Among pop.	9	37.59%	**F_{ST}: 0.376**	<0.0001
	Within pop.	248	62.41%		

Northern populations recovered as a single lineage

BEAST bayesian phylogenetic analysis recovered two main clades: most populations from northern distribution arose in a clade with high support (0.95 PP; Northern Clade) and estimated age of ~1.13 Ma; and another clade, with low support (0.36 PP) and estimated age of ~1.26 Ma, comprised by CAN, TAN and CABRO populations (Fig. 3). Besides TAN, the other two populations from Chapada Diamantina (GEN and CHA), were recovered in older divergences constituting tree's backbone. GEN was recovered as sister clade to all others, with divergence around ~2.31 Ma, and CHA as sister to the remaining clades, with divergence around ~1.87 Ma. Divergences and clades diversification occurred during early Pleistocene. TAN and BE were the youngest clades to diversify, around 0.13 and 0.17 Ma, respectively.

Past connections with no demographic changes

Most neutrality tests were not significant for both cpDNA and nrDNA suggesting no historical demographic changes, except for Fu's Fs statistics for nrDNA (Fs=-6.3205; $p<0.05$). Accordingly, ESBP analysis (Drummond et al. 2005) found no past demographic changes for the Northern Clade (Fig. 4).

The results for AUC and TSS, two metrics commonly used to evaluate SDM accuracy, showed that maxent algorithm presented good prediction for all models and replicates (AUC: 0.943 | TSS: 0.615 – Current; AUC: 0.942 | TSS: 0.627 – Holocene; AUC: 0.946 | TSS médio: 0.635 – LGM). The bioclimatic variables that mostly contributed to the models were: annual precipitation, followed by annual mean temperature with very close values of precipitation seasonality (Appendices 3). Our paleodistributional modeling shows that *Euphorbia phosphorea* distribution was larger during the glacial period, mainly along the meridian CD region. Therefore, it presented a potentially connected distribution during LGM and a fragmented distribution in warmer periods (Fig. 5). No expansion further CD's limits was detected by SDMs.

DISCUSSION

Our study uses a highly endemic species from Caatinga to provide insights on the evolutionary history of this biome. We used a phylogeography approach and species distribution modeling to access how species responded to historical events. Our results detected a strong genetic structure for cpDNA, likely consequence of limited seed-dispersal, and low levels of genetic diversity. Our SDMs suggest that Caatinga was more suitable to the species during LGM, showing a higher connectivity among populations. However, we didn't detect any signs of past demographic expansion in the last 21,000 yrs., and species divergence occurred in earlier ages (~2.31 Ma), with emergent lineages originating from middle to upper Pleistocene. Further, we will discuss our main results based on the patterns recorded from studies with taxa from Caatinga.

Seed and pollen dispersal shaping haplotypes spatial patterns

Our genetic patterns indicate that gene flow via pollen is likely more effective than seed-mediated gene flow in *Euphorbia phosphorea*. The network analysis highlights a strong cpDNA genetic structure, with many exclusive haplotypes and almost no-sharing, even between neighbor populations. Contrasting our cpDNA patterns, we found a lower nrDNA genetic structure, as illustrated by the BAPS and AMOVA results. Although we lack specific information on *E. phosphorea* seed-dispersers, field observations and studies on related species point that dispersal may explain the limited gene flow in this species. Studies have shown, for instance, that many Euphorbiaceae species are primarily self-dispersed via explosive dehiscent capsules, and that ants can provide secondary dispersion (Webster, 1994; Leal et al., 2007). Ants possess very low dispersal abilities (Leal et al., 2007), and high levels of local endemism, that could directly reflect on the low dispersal and patch distribution of myrmecochory plants (Leal et al., 2017). On the other hand, *E. phosphorea* pollination may be more effective in homogenizing populations, once we have seen many winged insects, besides ants, foraging the species nectar (personal observation). Moreover, the high nectar production during dry seasons, when food resources are scarce, can even attract lizards (Aximoff & Felix, 2017). As postulated for other inselberg specialists from CD (Pinheiro et al., 2014; Gonçalves-Oliveira et al., 2017; Hmeljevski et al., 2017), genetic drift may have been the main evolutionary force shaping the species evolution, but pollen mediated gene flow could have contributed to maintain species cohesion in face of extremely low seed dispersal.

Despite the low structure for nrDNA, we detected two incipient groups of populations: a northern group, comprising populations that mainly share Hap 3, and Chapada Diamantina group, with Hap 1 as almost the only haplotype shared by populations. In nuclear network, these haplotypes appear as dominant, in a central position linked to many rare haplotypes, differing by few mutations. CAN population seems to be geographically connected these groups, bearing both haplotypes. This population occurs in sedimentary Caatinga known as Raso da Catarina/BA, a very singular area with excessively drained sandy soils, of low fertility (Velloso et al., 2002). This area comprise many dry canyons, and it has being speculated that São Francisco river once crossed this region and drained to

Atlantic, in a different location from current (Werneck et al., 2015). The non-sharing nuclear haplotypes between northern group and Chapada Diamantina group might be related to pollinators limitation in crossing São Francisco river and Raso da Catarina, especially during historical climatic oscillations, since this region was presented as historical unstable according to predictions made by Werneck et al (2011). This discontinuity between Chapada Diamantina and Borborema Plateau, possible related to positioning of Raso da Catarina, was also attested by Pinheiro et al. (2014) and Gonçaves-Oliveira et al. (2017).

Divergence timing and demographic stability contrast to connectivity during LGM

Our phylogenetic tree indicates an early Pleistocene origin (2.31 Ma) for *Euphorbia phosphorea*, and middle to upper Pleistocene origins for emergent clades within the species, suggesting that earlier events than LGM were responsible for species populations divergences. Our phylogenetic results indicate that Chapada Diamantina populations GEN and CHA were the first to diverge within *E. phosphorea*. Many other species and lineages from Caatinga presents a Miocene to Plio-Pleistocene divergence (de Queiroz et al., 2017), and patterns of genetic variation and structure reflect earlier influences, possibly from quaternary first glaciation, without major demographic changes during LGM and LIG (Werneck et al., 2012a; Silva et al., 2018). In fact, our Bayesian Skyline and neutrality tests results indicate no demographic change for *E. phosphorea* despite the higher connectivityin the LGM inferred by SDMs. Perhaps it wasn’t enough time to stablish gene flow connectivity trough seed dispersal, accentuated by species low vagility and niche conservatism as highly adapted to rocky soils (Olivieri et al., 2016). As postulated by Thomé et al. (2016), humid forest invasion was recurrent and caused repeated vicariance events, and rock outcrops and inselbergs could be associated to long-term local stability, as also detected in other organisms (Pinheiro et al., 2014; Werneck et al., 2015; Gonçalves-Oliveira et al., 2017) and probably remained uncovered during these forest invasions. While seed dispersion is limited due to high suitability of climate and edaphic conditions, pollen dispersal is busted by physiognomies connectivity. Seed dispersal limitations characterize many other species endemics to xeric environments (Pennington et al., 2009), since investing in long distance seed dispersals for taxa highly adapted to harsh environments might be evolutionarily hazardous and risky to find similar habitat conditions (Olivieri et al., 2016).Therefore, last climatic oscillations might not have been long enough to affect highly specialized lineages that have long invested in its survival in such harsh environments.

Our findings from historical distribution analyses indicate a connectivity through viability of suitable places for xeric adapted vegetation, during LGM. Persistent patches since Holocene were inferred by models in part of Chapada Diamantina, at southeast Caatinga, and especially in Borborema Plateau. Accordingly, for both cpDNA and nrDNA results, we infered that Borborema Plateau presents some areas of stability, specially associated with inselbergs, where populations might have persisted long time despite climate changes. For northern populations in such region (COR, LAVR, BRE and RN), our results show a greater genetic and haplotypic diversity in comparison to all other populations.

Also, these populations showed closer network connectivity for cpDNA, and less structuring in BAPs results, besides emerging as high supported clade (0.92 PP) in phylogeny (part of Northern clade) with estimated age of 0.89 Ma. Our findings on persistence of *E. phosphorea* in the Borborema Plateau contrast results found by Werneck et al. (2011), that predicted Borborema Plateau region as highly unstable. However, *E. phosphorea* is highly restricted to inselbergs in Borborema Plateau, that could, therefore, be considered as microrefugia, harboring older communities, enduring past climatic oscillations.

CONCLUSIONS

Recent environmental instability does not appear to be the primary factor influencing genetic outcomes of diversification process in *Euphorbia phosphorea.* This species originated and diversified before main climatic instability reported for upper Pleistocene. However, the species evolution could be related to older and less studied climatic instabilities from early Pleistocene. The contrasting patterns of plastid and nuclear structure found in *E. phosphorea* are also postulated for other organisms of Caatinga and could be strongly related to distinct species ability to dispersal seed and pollen. We addressed our discussion aware of our data limitations, since we found very low polymorphism in nuclear marker that may provide a limited view of population history. Increase number of markers or invest in genome-scale datasets might be our next steps, besides investing in more novel analyses of time and space like Approximate Bayesian Computation (ABC). From our three previously formulated hypothesis, our findings corroborate strongly with the first, since populations of *E. phosphorea* are highly structured for cpDNA markers. Our results partially corroborate the second hypothesis that *E. phosphorea* presents a historical demographic variation and expansion during LGM, as attested by our distribution modelling analyses, but not recovered by our demographic analyses. Regarding our third hypotheses, Borborema Plateau area probably harbors an area of stability, since we found greater cohesion among its populations, and higher levels of genetic diversity.

ACKNOWLEDGEMENTS

We are very thankful to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2015/11812-1) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 309917/2015-8) for financial support.

REFERENCES

Ab'Saber, A.N. (1974) O domínio morfoclimático semi-árido das caatingas brasileiras. *Geomorfologia*, **43**, 37.

Allouche, O., Tsoar, A., & Kadmon, R. (2006) Assessing the accuracy of species distribution models: prevalence, kappa and the true skill statistic (TSS). *Journal of Applied Ecology*, **43**, 1223–1232.

Andrade, I.M., Mayo, S.J., van den Berg, C., Fay, M.F., Chester, M., Lexer, C., & Kirkup, D. (2007) A Preliminary Study of Genetic Variation in Populations of Monstera adansonii var. klotzschiana (Araceae) from North-East Brazil, Estimated with AFLP Molecular Markers. *Annals of Botany*, **100**, 1143–1154.

Antonelli, A. & Sanmartín, I. (2011) Why are there so many plant species in the Neotropics? Author(s): Alexandre Antonelli and Isabel Sanmartín Source: *Taxon*, **60**, 403–414.

Antonelli, A., Verola, C.F., Parisod, C., & Gustafsson, A. Lovisa, S. (2010) Climate cooling promoted the expansion and radiation of a threatened group of South American orchids (Epidendroideae: Laeliinae). *Biological Journal of the Linnean Society*, **100**, 597–607.

Antonelli, A., Zizka, A., Carvalho, F.A., Scharn, R., Bacon, C.D., Silvestro, D., & Condamine, F.L. (2018) Amazonia is the primary source of Neotropical biodiversity. *Proceedings of the National Academy of Sciences*, **115**, 6034–6039.

Aximoff, I. & Felix, E. (2017) Lygodactylus klugei (Kluge’s Dwarf Gecko). DIET. *Herpetological Review*, **48**, 439.

Bandelt, H.J., Forster, P., & Rohl, A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.

Batalha-Filho, H., Fjeldså, J., Fabre, P.-H., & Miyaki, C.Y. (2013) Connections between the Atlantic and the Amazonian forest avifaunas represent distinct historical events. *Journal of Ornithology*, **154**, 41–50.

Bivand, R., Keitt, T., & Rowlingson, B. (2013) rgdal: bindings for the geospatial data abstraction library. .

Bonatelli, I.A.S., Perez, M.F., Peterson, A.T., Taylor, N.P., Zappi, D.C., Machado, M.C., Koch, I., Pires, A.H.C., & Moraes, E.M. (2014) Interglacial microrefugia and diversification of a cactus species complex: Phylogeography and palaeodistributional reconstructions for Pilosocereus aurisetus and allies. *Molecular Ecology*, **23**, 3044–3063.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.H., Xie, D., Suchard, M.A., Rambaut, A., & Drummond, A.J. (2014) BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. *PLoS Computational Biology*, **10**, 1–6.

Camps, G.A., Martínez-meyer, E., Verga, A.R., Sérsic, A.N., & Cosacov, A. (2018) Genetic and climatic approaches reveal effects of Pleistocene refugia and climatic stability in an old giant of the Neotropical Dry Forest. 1–20.

Collevatti, R.G., Terribile, L.C., Lima-Ribeiro, M.S., Nabout, J.C., De Oliveira, G., Rangel, T.F., Rabelo, S.G., & Diniz-Filho, J.A.F. (2012) A coupled phylogeographical and species distribution modelling approach recovers the demographical history of a Neotropical seasonally dry forest tree species. *Molecular Ecology*, **21**, 5845–5863.

Corander, J. & Tang, J. (2007) Bayesian analysis of population structure based on linked molecular information. *Mathematical Biosciences*, **205**, 19–31.

Costa, W.J.E.M., Amorim, P.F., & Mattos, J.L.O. (2018) Synchronic historical patterns of species diversification in seasonal aplocheiloid killifishes of the semi-arid Brazilian Caatinga. *Plos One*, **13**, 1–10.

Darriba, D., Taboada, G.L., Doallo, R., & Posada, D. (2015) Europe PMC Funders Group jModelTest 2 : more models , new heuristics and high- performance computing. *Nature Methods*, **9**, 6–9.

Dorsey, B.L., Haevermans, T., Aubriot, X., Morawetz, J.J., Riina, R., Steinmann, V.W., & Berry, P.E. (2013) Phylogenetics, morphological evolution, and classification of. *Taxon*, **62**, 291–315.

Drummond, a. J., Rambaut, a., Shapiro, B., & Pybus, O.G. (2005) Bayesian coalescent inference of past population dynamics from molecular sequences. *Molecular Biology and Evolution*, **22**, 1185–1192.

Drummond, A.J., Suchard, M.A., Xie, D., & Rambaut, A. (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, **29**, 1969–1973.

Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with improved accuracy and speed. 689–690.

Excoffier, L., Laval, G., & Schneider, S. (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.

Fick, S.E. & Hijmans, R.J. (2017) Worldclim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, .

Franco, F.F. & Manfrin, M.H. (2013) Recent demographic history of cactophilic Drosophila species can be related to Quaternary palaeoclimatic changes in South America. *Journal of Biogeography*, **40**, 142–154.

Garcia, M.G., Silva, R.S., Carniello, M.A., Veldman, J.W., Rossi, A.A.B., & de Oliveira, L.O. (2011) Molecular evidence of cryptic speciation, historical range expansion, and recent intraspecific hybridization in the Neotropical seasonal forest tree Cedrela fissilis (Meliaceae). *Molecular Phylogenetics and Evolution*, **61**, 639–649.

Gent, P.R., Danabasoglu, G., Donner, L.J., Holland, M.M., Hunke, E.C., Jayne, S.R., Lawrence, D.M., Neale, R.B., Rasch, P.J., Vertenstein, M., Worley, P.H., Yang, Z.-L., & Zhang, M. (2011) The Community Climate System Model Version 4. *Journal of Climate*, **24**, 4973–4991.

Gentry, A.H. (1982) Neotropical Floristic Diversity : Phytogeographical Connections Between Central and South America , Pleistocene Climatic Fluctuations , or an Accident of the Andean Orogeny ? Author (s): Alwyn H . Gentry Source : Annals of the Missouri Botanical Garden. *Annals of the Missouri Botanical Garden*, **69**, 557–593.

Gonçalves-Oliveira, R.C., Wöhrmann, T., Benko-Iseppon, A.M., Krapp, F., Alves, M., Wanderley, M. das G.L., & Weising, K. (2017) Population genetic structure of the rock outcrop species Encholirium spectabile (Bromeliaceae): The role of pollination vs. seed dispersal and evolutionary implications. *American Journal of Botany*, **104**, 868–878.

Guo, Z., Peng, S., Hao, Q., Biscaye, P.E., An, Z., & Liu, T. (2004) Late Miocene-Pliocene development of Asian aridification as recorded in the Red-Earth Formation in northern China. *Global and Planetary Change*, **41**, 135–145.

Haffer, J. (1969) Speciation in Amazonian Forest Birds. *Science*, **165**, 131–137.

Hijmans, R.J. & Elith, J. (2013) Species distribution modeling with R. *CRAN.R-project.org/package = raster*, 87.

Hmeljevski, K.V., Nazareno, A.G., Leandro Bueno, M., dos Reis, M.S., & Forzza, R.C. (2017) Do plant populations on distinct inselbergs talk to each other? A case study of genetic connectivity of a bromeliad species in an Ocbil landscape. *Ecology and Evolution*, **7**, 4704–4716.

Hoorn, C., Wesselingh, F.P., Ter Steege, H., Bermudez, M.A., Mora, A., Sevink, J., Sanmartín, I., Sanchez-Meseguer, A., Anderson, C.L., Figueiredo, J.P., Jaramillo, C., Riff, D., Negri, F.R., Hooghiemstra, H., Lundberg, J., Stadler, T., Särkinen, T., & Antonelli, A. (2010) Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science*, **330**, 927–931.

Hughes, C.E., Pennington, R.T., & Antonelli, A. (2013a) Neotropical Plant Evolution: Assembling the Big Picture. *Botanical Journal of the Linnean Society*, **171**, 1–18.

Hughes, C.E., Pennington, R.T., & Antonelli, A. (2013b) Neotropical Plant Evolution: Assembling the Big Picture. *Botanical Journal of the Linnean Society*, **171**, 1–18.

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

Lanna, F.M., Werneck, F.P., Gehara, M., Fonseca, E.M., Colli, G.R., Sites, J.W., Rodrigues, M.T., &

Garda, A.A. (2018) The evolutionary history of Lygodactylus lizards in the South American open diagonal. *Molecular Phylogenetics and Evolution*, **127**, 638–645.

Leal, B.S.S., Palma da Silva, C., & Pinheiro, F. (2016) Phylogeographic Studies Depict the Role of Space and Time Scales of Plant Speciation in a Highly Diverse Neotropical Region. *Critical Reviews in Plant Sciences*, **35**, 215–230.

Leal, I.R., Ribeiro-Neto, J.D., Arnan, X., Oliveira, F.M.P., Arcoverde, G.B., Feitosa, R.M., & Andersen, A.N. (2017) Ants of the Caatinga: Diversity, Biogeography, and Functional Responses to Anthropogenic Disturbance and Climate Change. *Caatinga* pp. 65–95. Springer International Publishing, Cham.

Leal, I.R., Wirth, R., & Tabarelli, M. (2007) Seed dispersal by ants in the semi-arid caatinga of north-east Brazil. *Annals of Botany*, **99**, 885–894.

Librado, P. & Rozas, J. (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451–1452.

Losos, J.B. & Glor, R.E. (2003) Phylogenetic comparative methods and the geography of speciation. *Trends in Ecology and Evolution*, **18**, 220–227.

Magalhaes, I.L.F., Oliveira, U., Santos, F.R., Vidigal, T.H.D.A., Brescovit, A.D., & Santos, A.J. (2014) Strong spatial structure, Pliocene diversification and cryptic diversity in the Neotropical dry forest spider Sicarius cariri. *Molecular Ecology*, **23**, 5323–5336.

Maia, R.P., Bezerra, R.H.F., & Claudino-sales, V. (2010) Geomorfologia do Nordeste: concepções clássics e atuais acerca das superfícies de aplainamento nordestinas. *Revista de Geografia*, **especial V**, 6–19.

Miranda, E.A., Ferreira, K.M., Carvalho, A.T., Martins, C.F., Fernandes, C.R., & Del Lama, M.A. (2017) Pleistocene climate changes shaped the population structure of Partamona seridoensis (Apidae, Meliponini), an endemic stingless bee from the Neotropical dry forest. *Plos One*, **12**, 1–13.

Nascimento, F.F., Lazar, A., Menezes, A.N., Durans, A. da M., Moreira, J.C., Salazar-Bravo, J., DAndrea, P.S., & Bonvicino, C.R. (2013) The Role of Historical Barriers in the Diversification Processes in Open Vegetation Formations during the Miocene/Pliocene Using an Ancient Rodent Lineage as a Model. *PLoS ONE*, **8**, .

Oliveira-Filho, A., Jarenkow, J.A., & Rodal, M.J.N. (2006) Floristic Relationships of Seasonally Dry Forests of Eastern South America Based on Tree Species Distribution Patterns. pp. 159–192.

Olivieri, I., Tonnabel, J., Ronce, O., & Mignot, A. (2016) Why evolution matters for species conservation: Perspectives from three case studies of plant metapopulations. *Evolutionary Applications*, **9**, 196–211.

Pascual, R. & Jaureguizar, E.O. (1990) Evolving climates and mammal faunas in cenozoic South America. *Journal of Human Evolution*, **19**, 23–60.

Pennington, R.T., Lavin, M., & Oliveira-Filho, A. (2009) Woody Plant Diversity, Evolution, and Ecology in the Tropics: Perspectives from Seasonally Dry Tropical Forests. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 437–457.

Pennington, R.T., Lavin, M., Prado, D.E., Pendry, C.A., Pell, S.K., & Butterworth, C.A. (2004) Historical climate change and speciation: neotropical seasonally dry forest plants show patterns of both Tertiary and Quaternary diversification. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **359**, 515–538.

Phillips, S.J., Anderson, R.P., & Schapire, R.E. (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, **190**, 231–259.

Phillips, S.J. & Dudík, M. (2008) Modeling of species distribution with Maxent: new extensions and a comprehensive evalutation. *Ecograpy*, **31**, 161–175.

Pinheiro, F., Cozzolino, S., Draper, D., De Barros, F., Félix, L.P., Fay, M.F., & Palma-Silva, C. (2014) Rock outcrop orchids reveal the genetic connectivity and diversity of inselbergs of northeastern Brazil. *BMC Evolutionary Biology*, **14**, .

Porembski, S., Martinelli, G., Ohlemuller, R., & Barthlott, W. (1998) Diversity and ecology of saxicolous vegetation mats on inselbergs in the Brazilian Atlantic rainforest. *Diversity and Distributions*, **4**, 107–119.

Potter, P.E. (1997) The Mesozoic and Cenozoic paleodrainage of South America: a natural history. *Journal of South American Earth Sciences*, **10**, 331–344.

Potter, P.E. & Szatmari, P. (2009) Global Miocene tectonics and the modern world. *Earth-Science Reviews*, **96**, 279–295.

Prado, D.E. (2003) As Caatingas da América do Sul. *Ecologia e conservacão da Caatinga*, 3–73.

Queiroz, L.P. (2006) The Brazilian Caatinga: Phytogeographical Patterns Inferred from Distribution Data of the Leguminosae. *Neotropical Savannas and Seasonally Dry Forests: Plant Diversity, Biogeography, and Conservation*, 121–157.

de Queiroz, L.P., Cardoso, D., Fernandes, M.F., & Moro, M.F. (2017) Diversity and Evolution of Flowering Plants of the Caatinga Domain. *Caatinga* (ed. by J.M. Cardoso da Silva, I.R. Leal, and M. Tabarelli), pp. 23–63. Springer International Publishing, Cham.

Rambaut, A., Drummond, A.J., Xie, D., Baele, G., & Suchard, M.A. (2018) Tracer v1.7.

Rull, V. (2013) Some problems in the study of the origin of neotropical biodiversity using palaeoecological and molecular phylogenetic evidence. *Systematics and Biodiversity*, **11**, 415–423.

Saslis-Lagoudakis, C., Chase, M.W., Robinson, D.N., Russell, S.J., & Klitgaard, B.B. (2008) Phylogenetics of neotropical Platymiscium (Leguminosae: Dalbergieae): Systematics, divergence times, and biogeography inferred from nuclear ribosomal and plastid DNA sequence data. *American Journal of Botany*, **95**, 1270–1286.

Silva, G.A.R., Antonelli, A., Lendel, A., Moraes, E. de M., & Manfrin, M.H. (2018) The impact of early Quaternary climate change on the diversification and population dynamics of a South American cactus species. *Journal of Biogeography*, **45**, 76–88.

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

Tang, Z.H. & Ding, Z.L. (2013) A palynological insight into the Miocene aridification in the Eurasian interior. *Palaeoworld*, **22**, 77–85.

Tel-Zur, N., Abbo, S., Myslabodski, D., & Mizrahi, Y. (1999) Modified CTAB procedure for DNA isolation from epiphytic cacti of the genera Hylocereus and Selenicereus (Cactaceae). *Plant Molecular Biology Reporter*, **17**, 249–254.

Thomé, M.T.C., Sequeira, F., Brusquetti, F., Carstens, B., Haddad, C.F.B., Rodrigues, M.T., & Alexandrino, J. (2016) Recurrent connections between Amazon and Atlantic forests shaped diversity in Caatinga four-eyed frogs. *Journal of Biogeography*, **43**, 1045–1056.

Turchetto-Zolet, A.C., Pinheiro, F., Salgueiro, F., & Palma-Silva, C. (2013) Phylogeographical patterns shed light on evolutionary process in South America. *Molecular Ecology*, **22**, 1193–1213.

Valadão, R.C. (2009) Geodinâmica De Superfícies De Aplanamento, Desnudação Continental E Tectônica Ativa Como Condicionantes Da Megageomorfologia Do Brasil Oriental. **10**, 9157–6962.

Velloso, A.L., Velloso, A.L., Giulietti, A.M., Oren, D.C., Miranda, E.E. De, & Keel, S. (2002) Ecorregioes propostas para o bioma Caatinga. 76.

Vieira, F. de A., Novaes, R.M.L., Fajardo, C.G., Santos, R.M. dos, Almeida, H. de S., Carvalho, D. de, & Lovato, M.B. (2015) Holocene southward expansion in seasonally dry tropical forests in South

America: phylogeography of Ficus bonijesulapensis (Moraceae). *Botanical Journal of the Linnean Society*, **177**, 189–201.

Webster, G.L. (1994) Synopsis of the Genera and Suprageneric Taxa of Euphorbiaceae. *Annals of the Missouri Botanical Garden*, **81**, 33–144.

Werneck, F.P. (2011) The diversification of eastern South American open vegetation biomes: Historical biogeography and perspectives. *Quaternary Science Reviews*, **30**, 1630–1648.

Werneck, F.P., Gamble, T., Colli, G.R., Rodrigues, M.T., & Sites, J.W. (2012a) Deep diversification and long-term persistence in the south american “dry diagonal”: Integrating continent-wide phylogeography and distribution modeling of geckos. *Evolution*, **66**, 3014–3034.

Werneck, F.P., Leite, R.N., Geurgas, S.R., & Rodrigues, M.T. (2015) Biogeographic history and cryptic diversity of saxicolous Tropiduridae lizards endemic to the semiarid Caatinga. *BMC Evolutionary Biology*, **15**, 1–24.

Werneck, F.P., Nogueira, C., Colli, G.R., Sites, J.W., & Costa, G.C. (2012b) Climatic stability in the Brazilian Cerrado: Implications for biogeographical connections of South American savannas, species richness and conservation in a biodiversity hotspot. *Journal of Biogeography*, **39**, 1695–1706.

Zachos, J., Pagani, M., Sloan, L., Thomas, E., & Billups, K. (2001) Trends, Global Rhythms, Aberrations in Global Climate 65Ma to Present. *Science*, **292**, 686–693.

Zanella, F.C. V. (2000) The bees of the Caatinga (Hymenoptera, Apoidea, Apiformes): a species list and comparative notes regarding their distribution. *Apidologie*, **31**, 579–592.

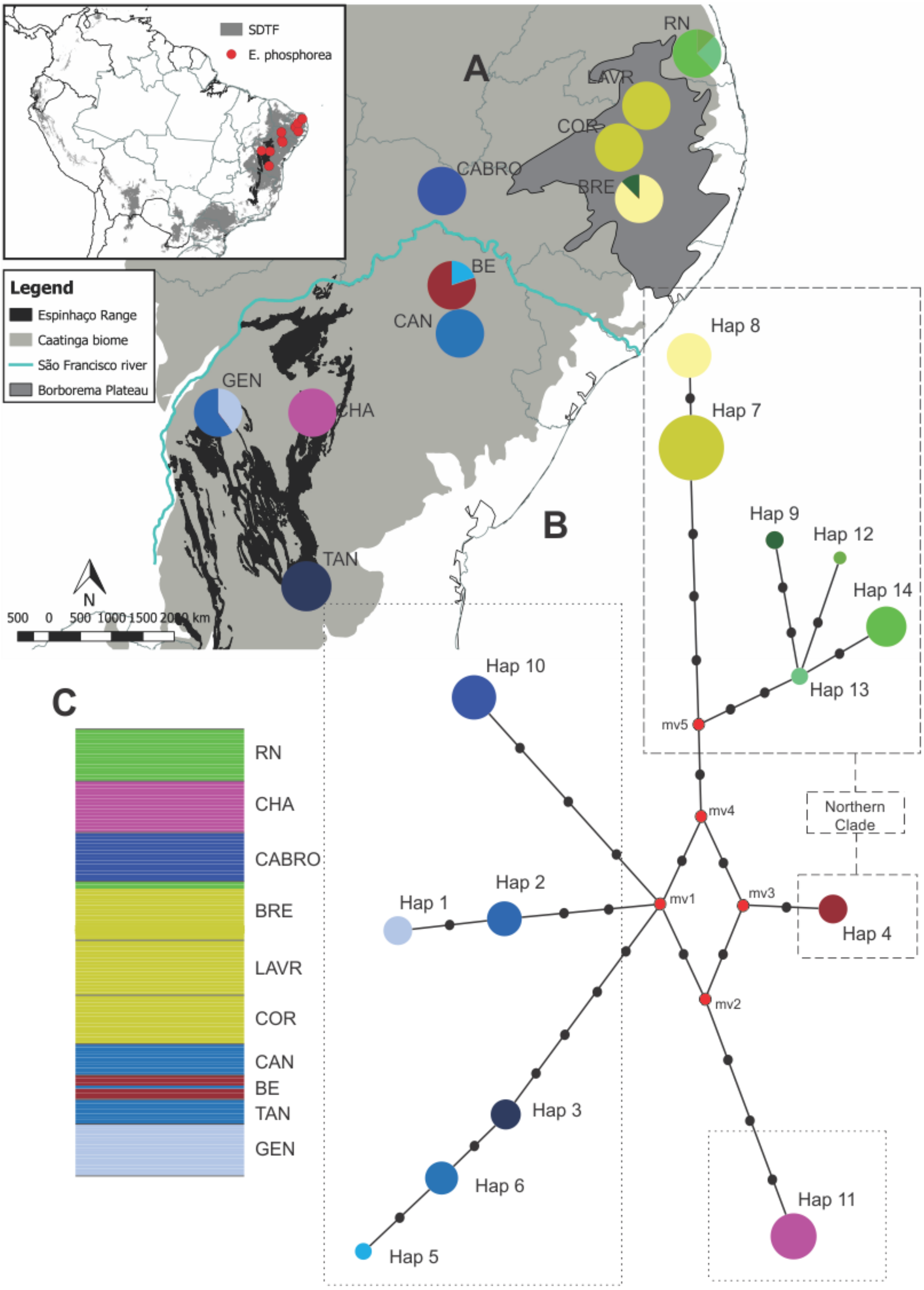


Figure 1 Map of distribution for populations of *Euphorbia phosphorea*, showing haplotype geographic arrangement. **A.** Map showing Caatinga Domain's limits and SDTF scattered across South America in smaller map to the left, and haplotypes where each population occur; **B.** Haplotype network for plastid sequences; **C.** BAPS results showing seven genetic groups.

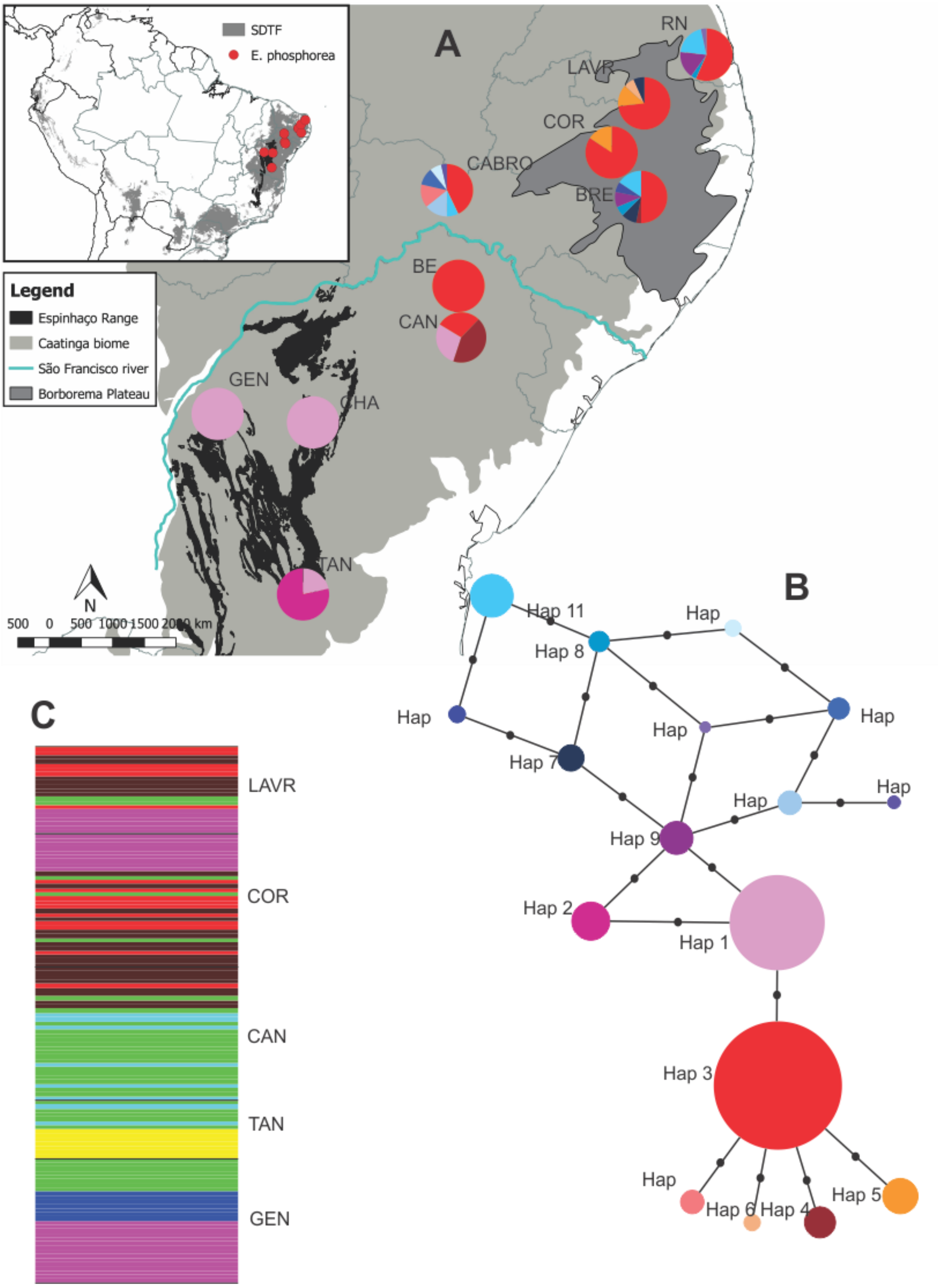


Figure 2 Map with nuclear haplotype geographic arrangement. **A.** Haplotypes plotted on population occurence; B. Haplotype network for nuclear sequences; **C.** BAPS results showing no structure among populations.

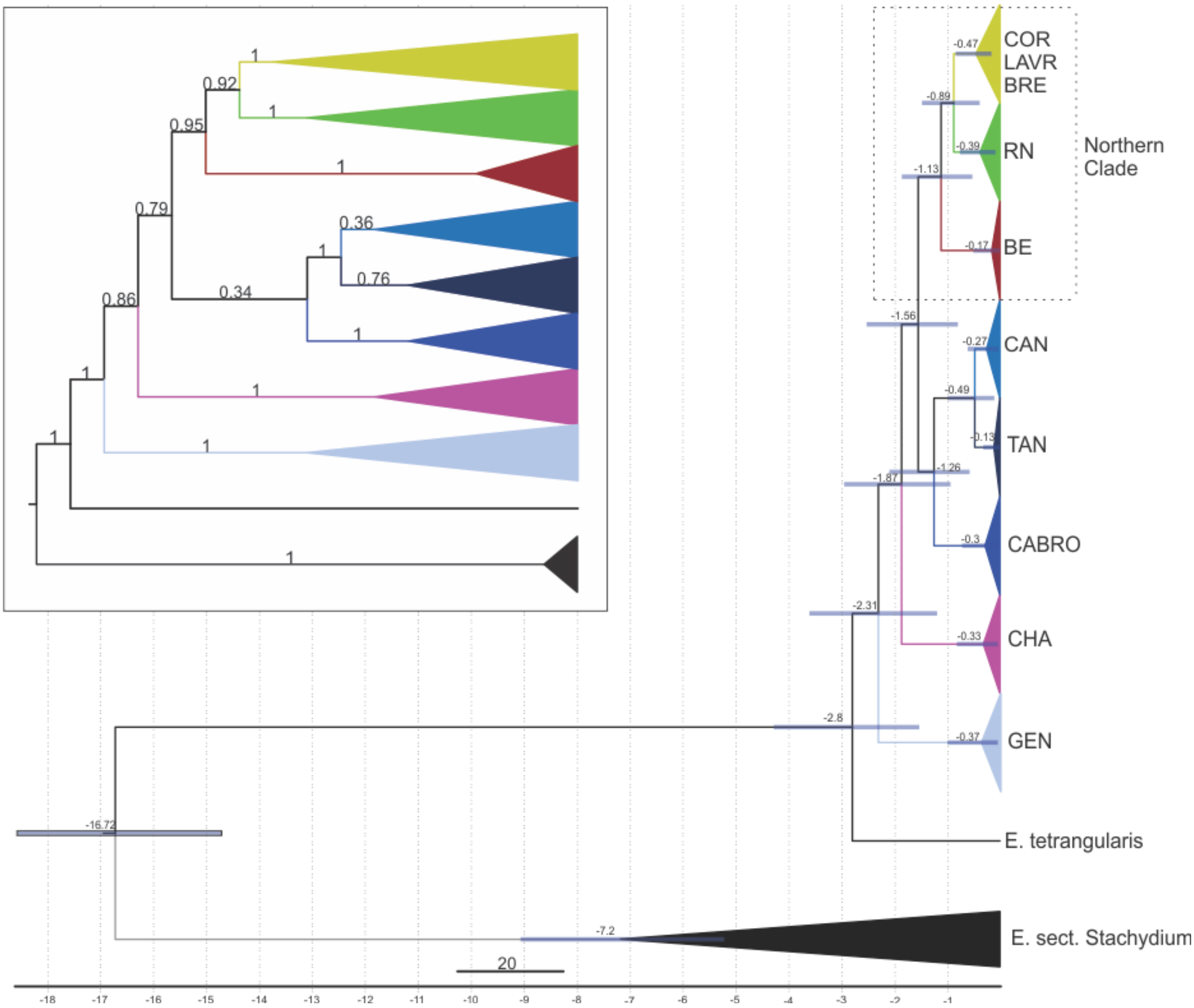


Figure 3 Phylogenetic tree generated by BEAST. Negative numbers represent estimated ages of clades, and positive number (smaller representation of the tree) represent posterior probabilities.

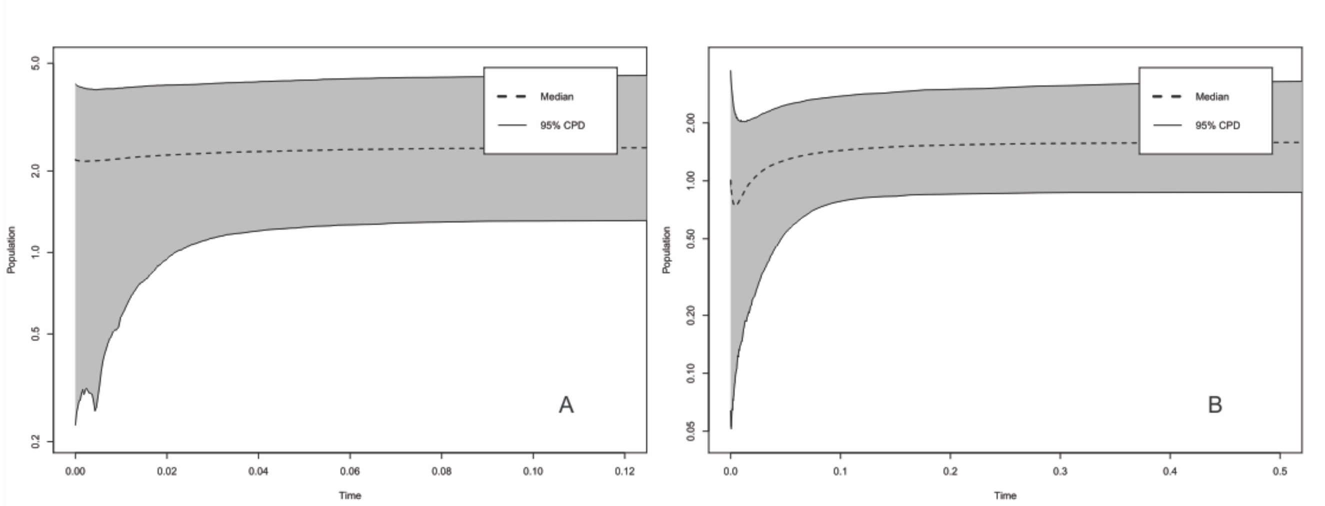


Figure 4 Bayesian skyline plotting results for Northern Clade (see figure 3). Population size X Time in million years (x 1000000). **A.** Shorter time scale, up to 120,000 years; **B.** Longer time scale, up to 500,000 years.

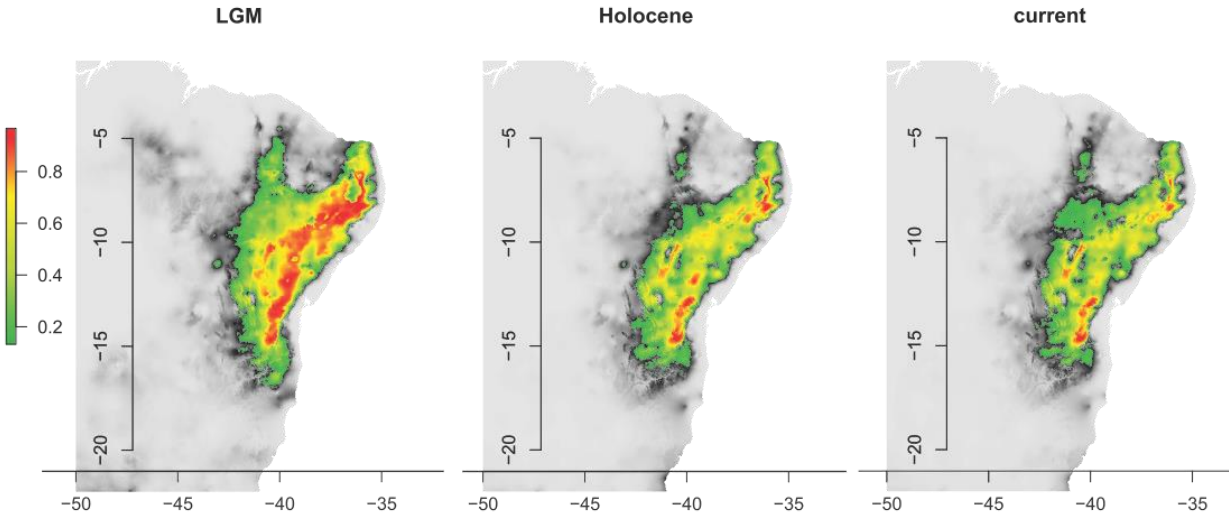


Figure 5 Results of analyses of distribution modelling for *Euphorbia phosphorea* for LGM, Holocene and current distribution. Colour bar represents values of threshold, and scales represents latitudinal and longitudinal ranges.

Table S1 Information of chosen regions for molecular phylogeny and primers used for amplification.

Region	**Annealing Temp (°C)**	**Primer**	**Proposed by**
psbA (F)-*trnH* (R)	54	F: GTTATGCATGAACGTAATGCTC R: CGCGCATGGTGGATTCACAATCC	Shaw et al. 2005
trnC (F)-*ycf6* (R)	58	F: CCAGTTCRAATCYGGGTG R: GCCCAAGCRAGACTTACTATATCCAT	Shaw et al. 2005
ITS1	52	F: GTCCACTGAACCTTATCATTTAG R: GCTGCGTTCTTCATCGATGC	Urbatsch et al. 2000

Shaw, J., Lickey, E. B., Beck, J. T., Farmer, S. B., Liu, W., Miller, J., Siripun, K.C., Winder, C. T., Schilling, E. E. & Small., R. L. (2005). The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany*, **92**, 142–166.

Urbatsch, L. E., Baldwin, B. G., & Donoghue, J. M. (2000). Phylogeny of the Coneflowers and Relatives (Heliantheae: Asteraceae) Based on Nuclear rDNA Internal Transcribed Spacer (ITS) Sequences and Chlorplast DNA Restriction Site Data. *Systematic Botany*, **25**, 539–565.

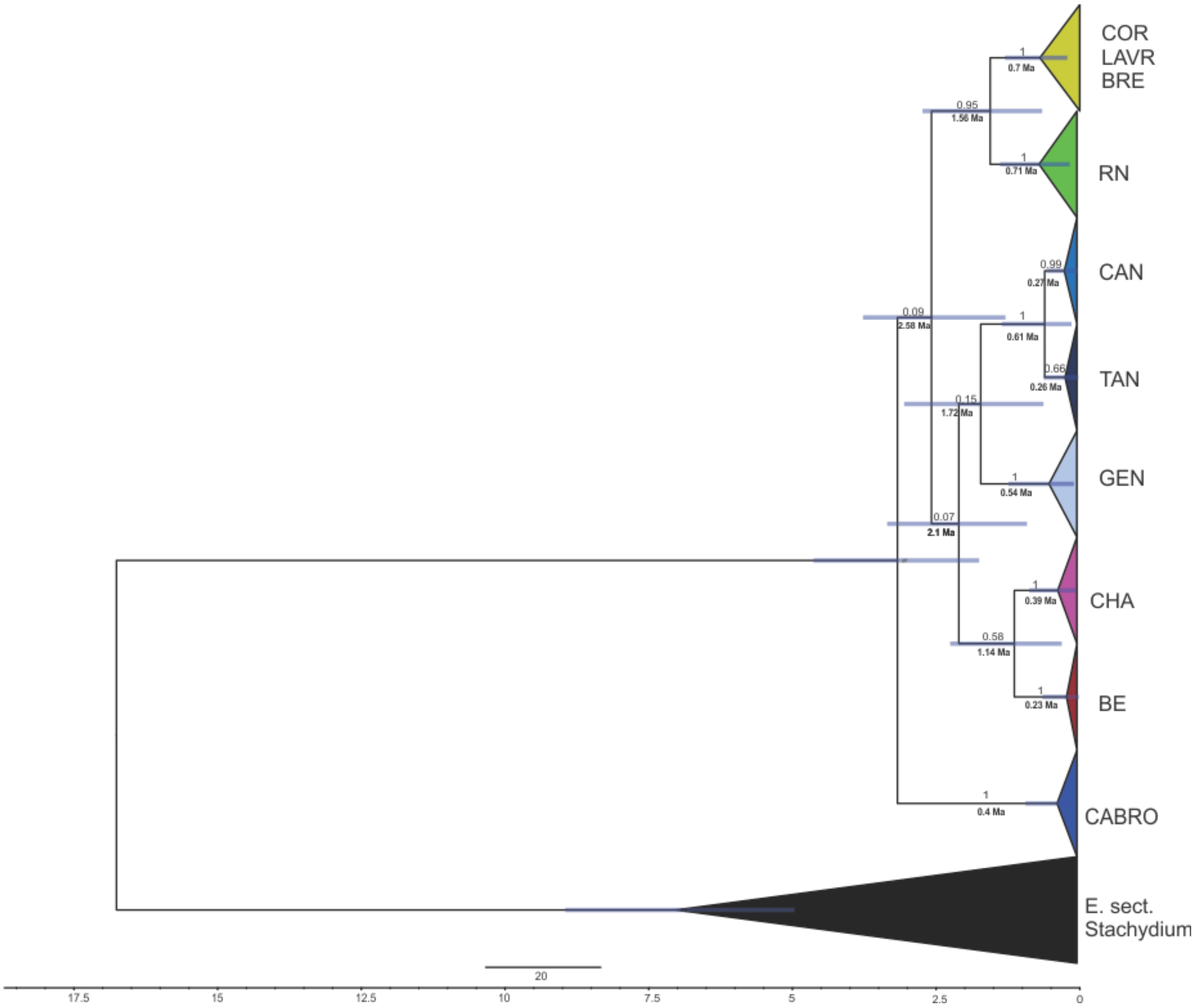
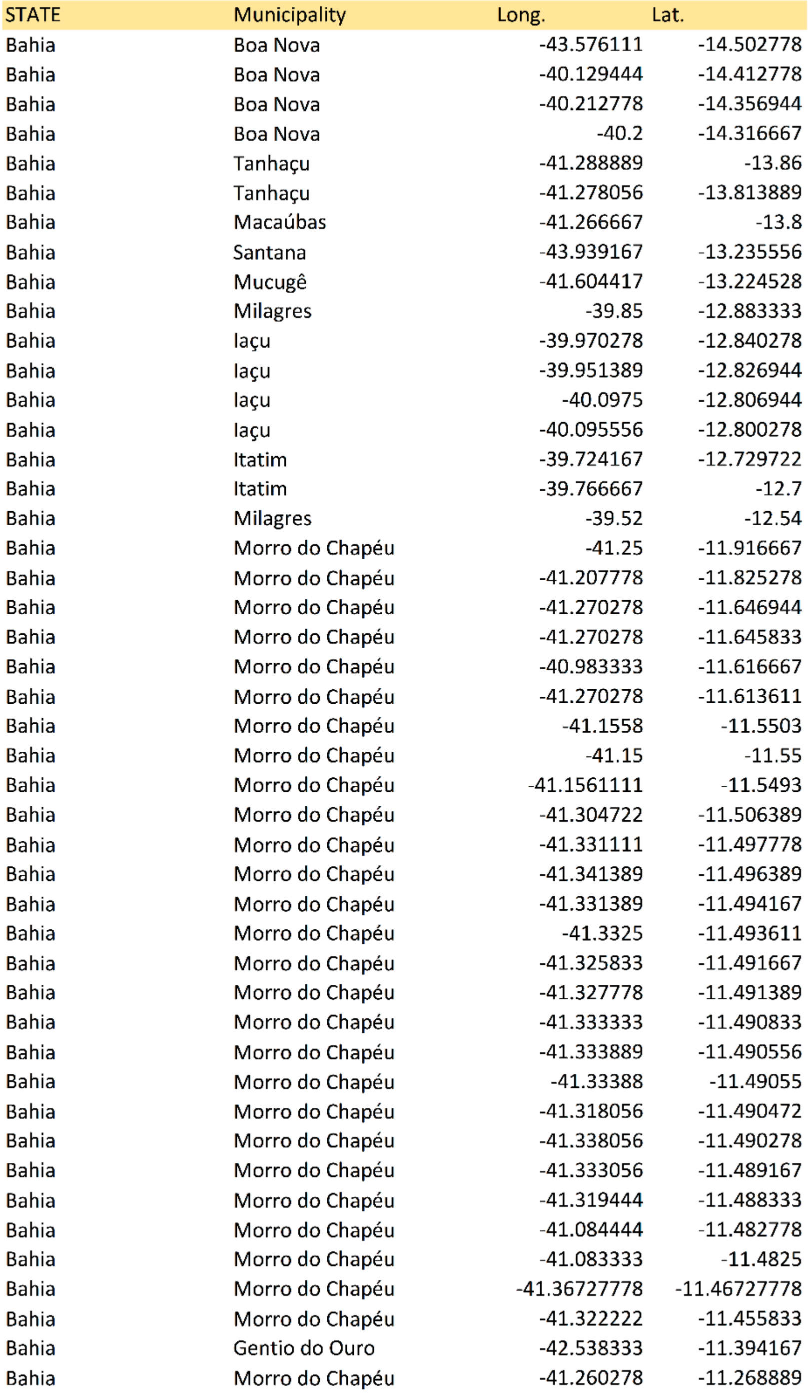


Figure S1 Phylogenetic analyses only with plastid sequences run in BEAST. Posterior probabilities (PP) above branches and estimated ages below.

Table S2 Coordinates points, with indication of municipality and State, used in analyses of *Euphorbia phosphorea* distribution models.



STATE	Municipality	Long.	Lat.
Bahia	Boa Nova	-43.576111	-14.502778
Bahia	Boa Nova	-40.129444	-14.412778
Bahia	Boa Nova	-40.212778	-14.356944
Bahia	Boa Nova	-40.2	-14.316667
Bahia	Tanhaçu	-41.288889	-13.86
Bahia	Tanhaçu	-41.278056	-13.813889
Bahia	Macaúbas	-41.266667	-13.8
Bahia	Santana	-43.939167	-13.235556
Bahia	Mucugê	-41.604417	-13.224528
Bahia	Milagres	-39.85	-12.883333
Bahia	Iaçu	-39.970278	-12.840278
Bahia	Iaçu	-39.951389	-12.826944
Bahia	Iaçu	-40.0975	-12.806944
Bahia	Iaçu	-40.095556	-12.800278
Bahia	Itatim	-39.724167	-12.729722
Bahia	Itatim	-39.766667	-12.7
Bahia	Milagres	-39.52	-12.54
Bahia	Morro do Chapéu	-41.25	-11.916667
Bahia	Morro do Chapéu	-41.207778	-11.825278
Bahia	Morro do Chapéu	-41.270278	-11.646944
Bahia	Morro do Chapéu	-41.270278	-11.645833
Bahia	Morro do Chapéu	-40.983333	-11.616667
Bahia	Morro do Chapéu	-41.270278	-11.613611
Bahia	Morro do Chapéu	-41.1558	-11.5503
Bahia	Morro do Chapéu	-41.15	-11.55
Bahia	Morro do Chapéu	-41.1561111	-11.5493
Bahia	Morro do Chapéu	-41.304722	-11.506389
Bahia	Morro do Chapéu	-41.331111	-11.497778
Bahia	Morro do Chapéu	-41.341389	-11.496389
Bahia	Morro do Chapéu	-41.331389	-11.494167
Bahia	Morro do Chapéu	-41.3325	-11.493611
Bahia	Morro do Chapéu	-41.325833	-11.491667
Bahia	Morro do Chapéu	-41.327778	-11.491389
Bahia	Morro do Chapéu	-41.333333	-11.490833
Bahia	Morro do Chapéu	-41.333889	-11.490556
Bahia	Morro do Chapéu	-41.33388	-11.49055
Bahia	Morro do Chapéu	-41.318056	-11.490472
Bahia	Morro do Chapéu	-41.338056	-11.490278
Bahia	Morro do Chapéu	-41.333056	-11.489167
Bahia	Morro do Chapéu	-41.319444	-11.488333
Bahia	Morro do Chapéu	-41.084444	-11.482778
Bahia	Morro do Chapéu	-41.083333	-11.4825
Bahia	Morro do Chapéu	-41.36727778	-11.46727778
Bahia	Morro do Chapéu	-41.322222	-11.455833
Bahia	Gentio do Ouro	-42.538333	-11.394167
Bahia	Morro do Chapéu	-41.260278	-11.268889

Bahia	Morro do Chapéu	-41.095556	-11.258333
Bahia	Santaluz	-39.3747	-11.2558
Bahia	Morro do Chapéu	-41.36727778	-11.46727778
Bahia	Morro do Chapéu	-41.322222	-11.455833
Bahia	Gentio do Ouro	-42.538333	-11.394167
Bahia	Jeremoabo	-38.4725	-10.068611
Bahia	Tucano	-38.7736	-11.1233
Bahia	Tucano	-38.995833	-10.886944
Bahia	Tucano	-38.85	-11.15
Bahia	Gentio do Ouro	-42.73880556	-11.12922222
Bahia	Tucano	-38.773611	-11.123333
Bahia	Tucano	-38.7736	-11.1233
Bahia	Tucano	-38.995833	-10.886944
Sergipe	Poço Redondo	-38.555	-10.547222
Sergipe	Capela	-37.054667	-10.536861
Bahia		-41.33333	-10.36666
Bahia	Jeremoabo	-38.540556	-10.192222
Bahia	Bendegó	-39.129444	-10.078333
Bahia	Jeremoabo	-38.4725	-10.068611
Sergipe	Poço Redondo	-37.684167	-9.805278
Sergipe	Poço Redondo	-37.690283	-9.798472
Bahia	Canudos	-39.109444	-9.745
Bahia	Santa Brígida	-38.082778	-9.742778
Bahia	Uauá	-39.4	-9.733333
Bahia	Uauá	-39.332222	-9.723056
Bahia	Santa Brígida	-38.145556	-9.714167
Bahia	Uauá	-39.332222	-9.706389
Sergipe	Canindé do São Francisco	-37.980139	-9.684222
Sergipe	Canindé do São Francisco	-37.98	-9.684167
Bahia	Paulo Afonso	-38.363056	-9.683611
Sergipe	Canindé de São Francisco	-37.789167	-9.66
Sergipe	Canindé do São Francisco	-38.0175	-9.563333
Sergipe	Canindé do São Francisco	-38.016667	-9.55
Sergipe	Canindé de São Francisco	-37.985556	-9.525042
Bahia	Santa Brígida	-38.483333	-9.333333
Sergipe	Canindé de São Francisco	-37.10744444	-9.075041667
Pernambuco	Cabrobó	-39.21652778	-8.303
Pernambuco	São Caetano	-36.19041667	-8.25
Pernambuco	Brejo da Madre de Deus	-36.197778	-8.238056
Pernambuco	São Caitano	-36.189806	-8.235167
Pernambuco	Parnamirim	-39.47913889	-8.126333333
Paraíba	São João do Tigre	-36.678056	-8.095833
Paraíba	Monteiro	-37.17122222	-7.878694444
Paraíba	Monteiro	-37.22894444	-7.875027778
Paraíba	Serra Branca	-36.665	-7.483333
Paraíba	São José dos Cordeiros	-36.898333	-7.470556
Paraíba	São José dos Cordeiros	-35.893778	-7.469361

Paraíba	Queimadas	-35.898333	-7.358333
Paraíba	Pocinhos	-36.061111	-7.076944
Rio Grande do Norte	Equador	-36.723611	-6.919444
Rio Grande do Norte	Monte Gameleiras	-35.806389	-6.451389
Rio Grande do Norte	Monte Gameleiras	-35.807222	-6.450278
Rio Grande do Norte	Monte Gameleiras	-35.778333	-6.4425
Rio Grande do Norte	Serra Caiada	-35.723333	-6.094167
Bahia	Itatim	-39.684	-12.68583333
Bahia	Itatim	-39.70177778	-12.71908333
Bahia	Milagres	-39.85480556	-12.87052778
Bahia	Iaçu	-40.09814167	-12.80689444
Bahia	Morro do Chapéu	-41.33458333	-11.48988889
Bahia	Morro do Chapéu	-41.10826944	-11.49292778
Bahia	Gentio do Ouro	-42.52613889	-11.40919444
Bahia	Gentio do Ouro	-42.56580556	-11.5105
Bahia	Mucugê	-41.60441667	-13.22452778
Bahia	Tanhaçu	-41.279475	-13.91229167
Bahia	Paulo Afonso	-38.44416667	-9.651583333
Bahia	Jeremoabo	-38.47483333	-10.06655556
Bahia	Jeremoabo	-39.70102778	-9.920611111
Bahia	Bendegó	-39.12222222	-9.741611111
Bahia	Canudos	-39.01730556	-9.961694444
Bahia	Santa Brígida	-38.14513889	-9.715166667
Paraíba	São José dos Cordeiros	-37.97638889	-9.647583333
Paraíba	São José dos Cordeiros	-36.88894444	-7.473194444
Pernambuco	Brejo da Madre de Deus	-36.39441667	-8.157888889
Pernambuco	Cabrobó	-39.21647222	-8.303333333
Paraíba	Pedra Lavrada	-36.45372222	-6.802944444
Rio Grande do Norte	Serra Caiada	-35.723288	-6.092593

Appendix 1: information of distribution models analyses

- Percentage of each Worldclim variable contribution.

CURRENT (AUC: 0.943 | TSS médio: 0.615)

Variable	**% contribution**	**Permutation importance**
Bio12	66	86.2
Bio1	14.3	6.6
Bio15	13.7	4.6
Bio7	4.7	2.2
Bio19	1.3	0.4

HOLOCENE (AUC: 0.942 | TSS médio: 0.627)

Variable	**% contribution**	**Permutation importance**
Bio12	68	87.5
Bio1	14.2	4.6
Bio15	12.7	4.7
Bio7	4.2	2.2
Bio19	0.9	0.9

LGM (AUC: 0.946 | TSS médio: 0.635)

Variable	**% contribution**	**Permutation importance**
Bio12	67.6	84.8
Bio1	14.8	3.5
Bio15	12.3	6.1
Bio7	4.7	4.6
Bio19	0.6	0.9

Table S3 TSS values indicate good prediction, according to Kappa's scale (Monserud & Leemans 1992) *

CURRENT			
	Replica	**TSS**	**Bin.Prob**
1	phosphorea_0	0.6447	2.22E-11
2	phosphorea_1	0.6324	5.00E-11
3	phosphorea_10	0.6447	2.28E-11
4	phosphorea_11	0.6051	2.70E-10
5	phosphorea_12	0.639	3.29E-11
6	phosphorea_13	0.6461	2.01E-11
7	phosphorea_14	0.618933333	3.53E-10
8	phosphorea_15	0.6272	6.87E-11
9	phosphorea_16	0.6242	8.45E-11
10	phosphorea_17	0.6537	1.23E-11
11	phosphorea_18	0.588566667	3.95E-09
12	phosphorea_19	0.5808	1.14E-09
13	phosphorea_2	0.572633333	6.32E-09
14	phosphorea_3	0.571433333	6.50E-09
15	phosphorea_4	0.592133333	1.91E-09
16	phosphorea_5	0.6154	1.50E-10
17	phosphorea_6	0.600233333	1.17E-09
18	phosphorea_7	0.643	2.53E-11
19	phosphorea_8	0.6294	5.94E-11
20	phosphorea_9	0.568133333	7.79E-09
Média		0.614888333	1.50E-09
HOLOCENE			
1	phosphorea_0	0.641	2.83E-11
2	phosphorea_1	0.6832	1.51E-12
3	phosphorea_10	0.632733333	1.46E-10
4	phosphorea_11	0.6777	3.10E-12
5	phosphorea_12	0.6311	5.43E-11
6	phosphorea_13	0.616	1.42E-10
7	phosphorea_14	0.629	6.25E-11
8	phosphorea_15	0.6192	1.15E-10
9	phosphorea_16	0.589633333	2.19E-09
10	phosphorea_17	0.572566667	1.10E-08
11	phosphorea_18	0.6178	1.23E-10
12	phosphorea_19	0.629	6.45E-11
13	phosphorea_2	0.6976	3.92E-13
14	phosphorea_3	0.6134	1.56E-10
15	phosphorea_4	0.617233333	3.94E-10
16	phosphorea_5	0.616633333	4.10E-10
17	phosphorea_6	0.607466667	1.18E-09
18	phosphorea_7	0.6203	1.15E-10
19	phosphorea_8	0.624	8.72E-11
20	phosphorea_9	0.607533333	7.69E-10
Média		0.627155	8.51E-10
LGM			
1	phosphorea_0	0.6432	2.53E-11

2	phosphorea_1	0.6496	1.58E-11
3	phosphorea_10	0.6159	1.44E-10
4	phosphorea_11	0.6569	9.81E-12
5	phosphorea_12	0.696	5.91E-13
6	phosphorea_13	0.6462	2.03E-11
7	phosphorea_14	0.6205	1.03E-10
8	phosphorea_15	0.664633333	3.64E-12
9	phosphorea_16	0.6598	8.15E-12
10	phosphorea_17	0.630533333	1.67E-10
11	phosphorea_18	0.6365	3.79E-11
12	phosphorea_19	0.616733333	4.18E-10
13	phosphorea_2	0.6674	4.83E-12
14	phosphorea_3	0.638733333	9.32E-11
15	phosphorea_4	0.6019	3.46E-10
16	phosphorea_5	0.6241	8.25E-11
17	phosphorea_6	0.585133333	2.89E-09
18	phosphorea_7	0.617233333	4.02E-10
19	phosphorea_8	0.585766667	4.73E-09
20	phosphorea_9	0.6488	1.65E-11
Média		0.635278333	4.76E-10

*Monserud, Robert A., & Rik Leemans (1992) Comparing global vegetation maps with the Kappa statistic. *Ecological modelling*, **62**, 275-293.

Considerações Finais

Durante esta tese, foram analisados aspectos taxonômicos, morfológicos, moleculares e evolutivos de *Euphorbia* sect. *Brasilienses*.

No primeiro capítulo, realizamos a análise filogenética da seção para entender as relações entre suas espécies. Em nossa inferência filogenética, o grupo emergiu como monofilético, com 100% de suporte. Apesar de usarmos sete marcadores, um nuclear (ITS1) e seis plastidiais (*psb*A-*trn*H, *trn*C-*ycf*6, *mat*K, *atp*I-*atp*H, *psb*J-*pet*A, *trn*Q-*rps*16x1), a filogenia não recuperou as relações entre as espécies, levando-nos a concluír que provavelmente trata-se de um grupo com origem recente, cujas linhagens ainda não são completamente divergentes. Neste primeiro capítulo, também descrevemos uma nova espécie para a seção, com base em caracteres morfológicos, e sustentada pela filogenia, que apesar de não resolver o relacionamento entre as espécies, agrupou separadamente os acessos de cada uma delas. Segundo os critérios da IUCN, esta espécie deve ser considerada como Criticamente em perigo (CR).

Na revisão taxonômica da seção apresentada no segundo capítulo, sinonimizamos *Euphorbia attastoma* var. *xanthochlora* Rizzini em *E. attastoma* e lectotipificamos *E. phosphorea*, além de reunir as informações sobre a taxonomia, distribuição geográfica, ecologia e fenologia de cada uma das espécies, com base em estudos de campo (30 localidades visitadas) e de acervos de 20 herbários.

A análise biogeográfica do clado do novo mundo de *Euphorbia* subg. *Euphorbia* apresentada no terceiro capítulo estimou uma idade de 3.15 milhões de anos para E. sect. Brasilienses, demonstrando que esta teria divergido de seu ancestral ainda no Plioceno, com conservação de nicho ao longo de sua história evolutiva, corroborando outros estudos com espécies das SDTF, estimando a idade da Caatinga em mais de 16 milhões de anos, visto que o ancestral de *E.* sect. *Brasilienses* e de seu grupo irmão *E.* sect. *Stachydium* já encontrava-se na região atualmente ocupada por esse bioma, e o clado *Brasilienses* + *Stachydium* manteve-se restrito à Caatinga durante quase toda a diversificação das linhagens, com exceção de uma aparente dispersão à longa distância evidenciada pela relação entre a espécie venezuelana *E. lagunillarum* e a espécie brasileira *E. heterodoxa*.

No estudo filogeográfico com *E. phosphorea*, a instabilidade climática da última glaciação não parece ter influenciado fortemente os processos evolutivos da espécie, cuja origem foi estimada para o início do Pleistoceno (ca. 2.31 Ma). Encontramos diferenças entre os resultados dos marcadores plastidiais e nucleares, com os primeiros exibindo alta estruturação e diferenciação entre as populações e baixa diversidade genética, e o segundo apresentando baixa estruturação e valores mais altos de diversidade. As análises demográficas demostraram estabilidade para as populações mais ao norte da distribuição da espécie, que emergem em um clado com alto suporte na filogenia, nos permitindo inferir que a região norte da Caatinga deva ser uma área histórica de estabilidade climática. Os resultados da modelagem nos indicam que houve uma conectividade maior para o LGM, contudo, a ausência de variação demográfica apresentada pelo Bayesian Skyline, indica que *E. phosphorea* apresenta-se restrita à formações rochosas há muito tempo e que provavelmente as limitações de sua dispersão impediram sua expansão para os novos ambientes que se tornaram disponíveis com a contração das florestas úmidas, durante a última glaciação.

ANEXO I

Primers testados durante a execução deste trabalho

ANEXO I: Tabelas com todos os primers testados durante a execução deste trabalho.

Tabela 1: Pares de primers nucleares testados.

PARES DE PRIMERS NUCLEARES	**REFERÊNCIA BIBLIOGRÁFICA**	**AMPLIFICAÇÃO**
EIWxy (F) - *EIWxy* (R)	Garrick et al. (2008)	Não
EIPi (F) - *EIPi* (R)	Garrick et al. (2008)	Não
EIMs (F) - *EIMs* (R)	Garrick et al. (2008)	Não
EIAdh (F) – *EIAdh* (R)	Garrick et al. (2008)	Não
EILfy (F) – *EILfy* (R)	Garrick et al. (2008)	Não
EIRpb2 (F) - *EIRpb2* (R)	Garrick et al. (2008)	Não
EIG3pdh (F) - *EIG3pdh* (R)	Garrick et al. (2008)	Não
Eh-E04 (F) - *Eh-E04* (R)	Beatty et al. (2015)	Não
ITS I (F) - *ITSII* (R)	Urbatsch et al. (2000)	Sim
Pe4474III_F1 (F) - *Pe4474V_R3* (R)	Naumann et al (2012)	Não
By2AGT1 (F) - *By2AGT1* (R)	Vasconcelos (comunicação pessoal)	Não
G3pdh (F) - *G3pdh* (R)	Sass e Specht (2010)	Não
By1ncpGS (F) - *By1ncpGS* (R)	Vasconcelos (comunicação pessoal)	Não
rpb2 (F) - *rpd2* (R)	Denton et al. (1998) e Roncal et al. (2005)	Não
Lfslx-3 (F) - *Lftxr* (R)	Frohlich and Meyerowitz (1997)	Não
EMB2765_9F (F) - *EMB2765_9R* (R)	Wurdack e Davis (2009)	Não
G5cp687 (F) – *G5cp994* (R)	Emshwiller and Doyle (1999)	Não
phyC515f-br (F) - *phyC1690r-br* (R)	Barfuss (2012)	Não

Tabela 2: Pares de primers cloroplastidiais testados.

PARES DE PRIMERS PLASTIDIAIS	**REFERÊNCIA BIBLIOGRÁFICA**	**AMPLIFICAÇÃO**
trnS (F) - *trnG* (R)	Demesure et al. (1995)	Sim (parte das amostras)
matK (F) - *matK* (R)	Garrick et al. (2009)	Sim

trnK3914 (F) - *matK1168* (R)	Johnson and Soltis (1994, 1995)	Não
Eh_trnS (F) – *Eh_trnG* (R)	Beatty et al. (2015)	Sim
ndhF (F) – *ndhf* (R)	Garrick et al. (2009)	Sim (parte das amostras)
rps16 (F) - *trnK* (R)	Shaw et al. (2007)	Sim (parte das amostras)
trnC (F) - *ycf6* (R)	Shaw et al. (2005)	Sim
rpl32 (F) – *trnL* (R)	Shaw et al. (2007)	Não
trnL-Fe (F) – *trnL-Ff* (R)	Taberlet et al. (2007)	Não
trnL-Fc (F) – *trnL-Fd* (R)	Taberlet et al. (2007)	Não
trnT-a (F) – *trnL-b* (R)	Taberlet et al. (2007)	Não
psbA (F) – *trnH* (R)	Shaw et al. (2005)	Sim
trnK19F (F) – *matK1326* (R)	Gravendeel et al. (2001)	Não
matK390F (F) – *trnK1710* (R)	Cuénoud et al. (2002) e Barfuss et al. (2005)	Sim (parte das amostras)
ndhF (F) - *rpL32* (R)	Shaw et al. (2007)	Não
trnV (F) – *ndhC* (R)	Shaw et al. (2007)	Não
trnS (GCU) (F) – *SGRev2* (R)	Shaw et al. (2005) e Bonatelli et al. (2013)	Não
SGFWd2 (F) - *5trnG2S* (R)	Shaw et al. (2005) e Bonatelli et al. (2013)	Não
rpL16F71 (F) – *rpL16R1516* (R)	Small et al. (1998)	Sim(duas amostras)
trnQ (F) – *rps16x1* (R)	Shaw et al (2007)	Sim
atpL (F) – *atpH* (R)	Shaw et al (2007)	Sim
ndhJ (F) – *trnF* (R)	Taberlet et al. (1991) e Shaw et al. (2007)	Não
rps16 (F) – *trnK* (R)	Shaw et al (2007)	Não
F71 (F) – *R1661* (R)	Jordan et al (1996)	Não
petA (F) – *psbJ* (R)	Shaw et al (2014)	Sim
SP43122 (F) – *SP44097* (R)	Hershkovitz (2006)	Não

Tabela 3: Regiões selecionadas pela quantidade de polimorfismos e qualidade dos sequenciamentos e amplificações das amostras.

Região sequenciada	Annealing Temp (℃)	Primer	Número de indivíduos	Utilização no trabalho	Pares de base
psbA-trnH	54	F: GTTATGCATGAACGTAATGCTC R: CGCGCATGGTGGATTCACAATCC	288 ind.	Filogeografia e Filogenia	ca. 900pb
ycf6-trnC	58	F: CCAGTTCRAATCYGGGTG R: GCCCAAGCRAGACTTACTATATCCAT	288 ind.	Filogeografia e Filogenia	ca. 400pb
ITS1	48	F: AGTATCTTCTTTAGAAAGGCC R: TAGCATTTGACTCCGTACTACC	288 ind.	Filogeografia e Filogenia	ca. 400pb
matK	46	F: TAATAGCTTGGTTGACTGCGG R: TGTAACCTCGATTATAGGAC	23 ind.	Filogenia	ca. 700pb
trnQ-rps16x1	48	F: ATAGGTACTGTARCYGGTATT R: AACARTTYGARAAGGTTCAATT	23 ind.	Filogenia	ca. 800pb
atpL-atpH	50	F: TATTTACAAGYGGTATTCAAGCT R: CCAAYCCAGCAGCAATAAC	23 ind.	Filogenia	ca. 800pb
psbJ-petA	60	F: GTCCACTGAACCTTATCATTTAG R: GCTGCGTTCTTCATCGATGC	23 ind.	Filogenia	ca. 600pb

REFERÊNCIAS BIBLIOGRÁFICAS

Barfuss, M.H.J. 2012. **Molecular studies in Bromeliaceae: Implications of plastid and nuclear DNA markers for phylogeny, biogeography, and character evolution with emphasis on a new classification of Tillandsioideae**. University of Vienna, Vienna, 244 pp.

Barfuss, M.H.J., Samuel, R., Till, W., Stuessy, T.F. 2005. Phylogenetic relationships in subfamily Tillandsioideae (Bromeliaceae) based on DNA sequence data from seven plastid regions. **American Journal of Botany**, n. 92, p. 337–351.

Beatty, G. E., Lennon, J. J., O’Sullivan, C. J., Provan, J. 2015. The not-so-Irish spurge: Euphorbia hyberna (Euphorbiaceae) and the Littletonian plant ‘steeplechase’. **Biological Journal of the Linnean Society**, v. 114, n. 2, p. 249-259.

Bonatelli, I.A.S., Perez, M.F., Townsend, P.A., Taylor, N.P., Zappi, D.C., Machado, M.C., Koch, I., Pires, A.H.C., Moraes, E.M. 2013. Interglacial microrefugia And diversification of a cactus species complex: phylogeography and palaeodistributional Reconstructions for *Pilosocereus aurisetus* and allies. **Molecular Ecology**, Oxford, v. 23, p. 3044-3063.

Cuénoud, P., Savolainen, V., Chatrou, L. W., Powell, M., Grayer, R. J., Chase, M. W. 2002.

Molecular phylogenetics of Caryophyllales based on nuclear 18S rdna and plastid *rbcl*, *atpb*, and *matk* DNA sequences. **American Journal of Botany**, n. 89, p. 132–144.

Demesure, B.; Sodzi, T. N.; Petit, R. J.A. 1995. Set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. **Molecular Ecology**. n. 4, p. 129-131.

Denton, A. L., Mcconaughy, B. L., Hall, B. J. 1998. Usefulness of RNA polymerase II coding sequences for estimation of green plant phylogeny. **Molecular Biology and Evolution,** n. 15, p. 1082–1085.

Emshwiller, E., Doyle, J. J. 1999. Chloroplast-expressed glutamine synthetase (ncpgs): Potential utility for phylogenetic studies with an example from *Oxalis* (Oxalidaceae). **Molecular Phylogenetics and Evolution**. n. 12, p. 310–319.

Frohlich, M. W., Meyerowitz, E. M. 1997. The Search for Flower Homeotic Gene Homologs in Basal Angiosperms and Gnetales: A Potential New Source of Data on the Evolutionary Origin of Flowers. **International Journal of Plant Sciences.** V. 158, n. 6, p. 131-142.

Garrick R. C., Nason, J. D., Meadows, C. A. And Dyer, R. J. 2009. Not just vicariance: phylogeography of a sonoran desert euphorb indicates a major role of range expansion along the Baja Peninsula. **Molecular ecology**, n. 18, p. 1916–1931.

Garrick, R. C., Meadows, C. A., Nicolas, A. N., Nason, J. D., Dyer, R. J. 2008. A set of polymorphic nuclear intron markers for conservation genetics and phylogeography of Euphorbia species (Pedilanthus clade). Conservation Genetics, v. 9, n. 6, p. 1673–1676

Gravendeel, B., Chase, M. W., de Vogel, E. F., Roos, M. C., Mes, T. H., Bachmann, K. 2001. Molecular phylogeny of Coelogyne (Epidendroideae; Orchidaceae) based on plastid RFLPS, matk, and nuclear ribosomal ITS sequences: evidence for polyphyly. **American Journal of Botany**, v. 88, n. 10, p. 1915-1927.

Johnson, L. A., Soltis, D. E. 1994. *Matk* DNA sequences and phy- logenetic reconstruction in Saxifragaceae s. Str. **Systematic Botany**, n. 19, p. 143–156.

Johnson, L. A., Soltis, D. E. 1995. Phylogenetic inference in Saxifragaceae sensu stricto and *Gilia* (Polemoniaceae) using *matk* sequences. **Annals of the Missouri Botanical Garden**, n. 82, p. 149–175.

Jordan, W. C., Courtney, M. W., Neigel, J. E. 1996. Low levels of intraspecific genetic variation at a rapidly evolving chloroplast DNA locus in North American duckweeds (Lemnaceae). **American Journal of Botany**, n. 83, p. 430–439.

Roncal, J., Francisco-Ortega, J., C. B. Asmussen, C. B., C. E. Lewis, C. E. 2005. Molecular phylogenetics of tribe Geonomeae (Arecaceae) using nuclear DNA sequences of phosphoribulokinase and RNA polymerase II. **Systematic Botany,** n. 30, p. 275–283.

Sass, C., Specht, C. D. 2010. Phylogenetic estimation of the core Bromelioids with an emphasis on the genus Aechmea (Bromeliaceae). **Molecular Phylogenetics and Evolution**, n. 55, p. 559–571.

Shaw, J., E. B. Lickey, E. E. Schilling and R. L. Small. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. **American Journal of Botany**. v. 94, n. 3, p. 275–288.

Shaw, J., E. B. Lickey, J. T. Beck, S. B. Farmer, W. Liu, J. Miller, K.C. Siripun, C. T. Winder, E. E. Schilling and R. L. Small. 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. **American Journal of Botany,** n. 92, p. 142–166.

Small, R. L.; Ryburn, J. A.; Cronn, R. C.; Seelanan, T.; Wendel, J. F. 1998. The tortoise and the hare: choosing between noncoding plastome and nuclear ADH sequences for phylogeny reconstruction in a recently diverged plant group. **American Journal of Botany**. n. 9, v. 85, p. 1301–1315.

Taberlet P, Coissac E, Pompanon F, et al. 2007. Power and limitations of the chloroplast trnl (UAA) intron for plant DNA barcoding. **Nucleic Acids Research** n. 35, p. 14.

Urbatsch, L. E., B. G. Baldwin and J. M. Donoghue. 2000. Phylogeny of the Coneflowers and Relatives (Heliantheae: Asteraceae) Based on Nuclear rdna Internal Transcribed Spacer (ITS) Sequences and Chlorplast DNA Restriction Site Data. **Systematic Botany**, n. 25, p. 539–565.

Wurdack, K. J.; Davis, C. C. 2009. Malpighiales phylogenetics: Gaining ground on one of the most recalcitrant clades in the angiosperm tree of life. **American Journal of Botany.** V. 96, n. 8, p. 1551-1570.

ANEXO II

Bibliotecas de marcadores microssatélites, desenho dos primers e testes de polimorfismo e transferabilidade

ANEXO II: Desenvolvimento de bibliotecas de marcadores microssatélites, desenho dos primers e testes de polimorfismo e transferabilidade.

Durante o desenvolvimento desta tese, buscamos desenvolver uma biblioteca de marcadores microssatélites. Infelizmente não obtivemos muito sucesso, e por uma questão de logística, abortamos esta metodologia neste momento, para possível retomada em trabalhos futuros além desta tese. Neste anexo trouxemos resumidamente os resultados das tentativas de desenvolver as bibliotecas de marcadores microssatélites.

Duas placas foram desenvolvidas para *E. phosphorea*, durante a disciplina "Bibliotecas Enriquecidas em Microssatélites de Eucariotos" na UNICAMP, ministrada pela Dra. Anete Pereira de Souza, em fevereiro e março de 2016.

Duas placas com 96 clones foram sequenciadas utilizando o serviço do Laboratório Multiusuário de Genotipagem e Sequenciamento LAGM – CBMEG da UNICAMP.

Foram desenhados um total de 19 pares de primers para marcadores microssatélites (10 da primeira placa e 9 da segunda) (Tabela 01). Somente testamos os 10 primers que foram desenvolvidos na primeira placa quanto a polimorfismo e transferibilidade com as demais espécies de *Euphorbia* sect. *Brasilienses.*

A segunda placa apresentou microssatélites muito curtos, a maioria com 5 a 8 repetições de Di-nucleotídeos, e, portanto, não foram, ainda, enviados para serem sintetizados e posteriormente testados. Imaginamos que o resultado da segunda placa não seria diferente do da primeira, pois para realizarmos um trabalho de genética de populações são necessários no mínimo 10 marcadores microssatélites polimórficos. Optamos por não testar o polimorfismo dos novos primers desenhados, pois a chance de conseguirmos a quantidade necessária de marcadores microssatélites polimórficos é bastante reduzida devido aos poucos primers desenhados e o tamanho dos marcadores.

Resolvemos também testar outros 18 pares de primers que foram desenhados para *Jatropha curcas* L. (Bressan et al. 2012; Prumichai et al. 2011) e dois para *Euphorbia kansui* T.N. Liou e *Euphorbia palustris* L. (Yan et al. 2014; Durka 2009). Estes testes foram realizados em oito indivíduos de diferentes populações do grupo de estudo.

PCRs foram realizados para um volume final de 10 µl contendo: 10 ng deDNA, 2,5X GoTaq Master Mix (Promega), 0,5 µM de *primer forward* e 1 µM de *reverse,* e 1 µM de *primer* universal M13 marcado com florescência FAM, VIC, PET ou NED. Reações foram realizadas com programa Touchdown descrito por Palma-Silva et al. (2007). Os sequenciamentos foram realizados em sequenciador automático ABI PRISM 3500 (Applied Biosystems) e genotipados de acordo com GeneScan 500 LIZ (Biosystems) utilizando software GENEMARKER v1.95 (Softgenetics, Statecollege, Pennysylvania, USA). A classificação dos alelos em categorias de tamanho de fragmento foi feita usando o programa FLEXIBIN (Amos et al., 2007) e depois checados manualmente. O programa Micro-Checker 2.2.3 (Van Oosterhout et al. 2004) foi utilizado para genotipar erros devido à presença de alelos nulos e *stuttering*.

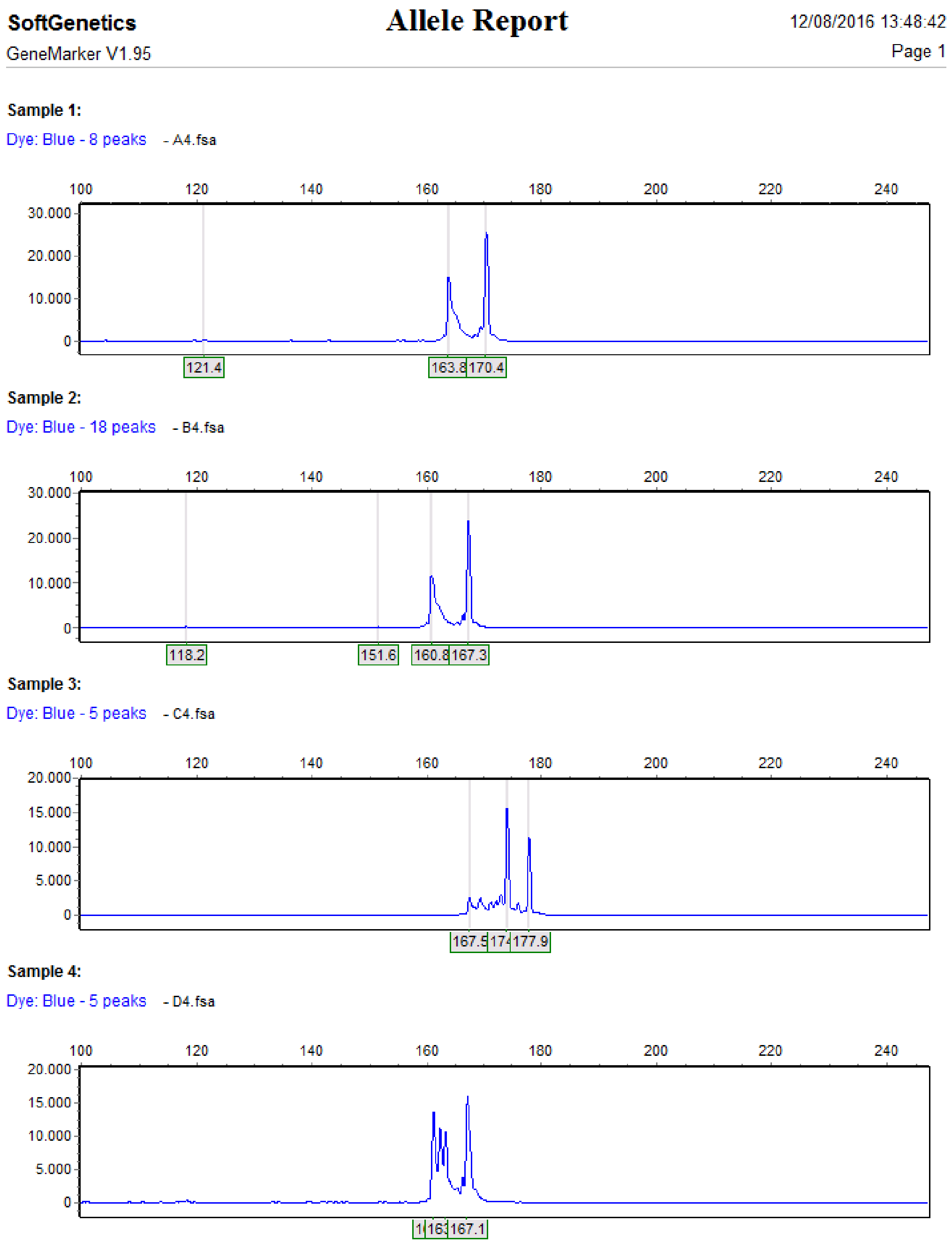
Quase todos os pares de primers desenhados para *Euphorbia phosphorea* amplificaram nas demais espécies da seção, e dois pares desenhados para *Jatropha curcas*, entretanto, apenas um microssatélite apresentou polimorfismo (Figura 01).

Tabela 1: Pares de primers para marcadores microssatélites. Em amarelo foram desenhados para *Jatropha curcas* por Prumichai et a. (2011), de azul os desenhados por Bressan et al. (2012), em laranja os desenhados para *Euphorbia kansui* por Yan et al. (2014), de verde os desenhados para *Euphorbia palustris* por Durka (2009), e em tons de cinza os desenhados durante a execução deste projeto para *Euphorbia phosphorea*.(cinza mais claro são os desenhados da primeira biblioteca). *transferabilidade positiva, **polimorfismo positivo.

*JCT10-F	CATATCGAACCATGAACGA
JCT10-R	AGCCGTTTATCATTACGA
JCT12-F	CTCATAGCCGCAGATCACA
JCT12-R	GAGATCGGACGTGGTGA
*JCT18-F	CGGAGGAATCAATGAAAGGACA
JCT18-R	TGCTTGTTGAACCCTGTGAA
JCT23-F	CACCAGACCCAGGAAAAGGAC
JCT23-R	TGTTGCTGTTGCGATTTCTC
JCT28-F	CCGCAGCCATCTTGAAGGTTA
JCT28-R	CAAAATTTCAAGCCATGCTC
JCT35-F	CGGAAAACAGTCCTTCACTTG
JCT35-R	GACTAAGGGCAACACTGAAC
JCT16-F	CGCCTCCAGCATCTTTCAATC
JCT16-R	AACAATCCCCATTCCTCCTC
JCT45-F	CAGTCGATTGGTACCCTTTCT
JCT45-R	AGACGCTTCTTTTTCCTCTT
JCT50-F	CTCCCAAGTCATGATTCAATA
JCT50-R	AAGGCCGTTAGAATCTCATT
JCT59-F	CGGTGACTCCTGAATGCTTGG
JCT59-R	GGTGACTCCTGAATGCTTGG
JCT76-F	CATTTGTTTGCTGTGTGACTG
JCT76-R	TCTCAGCTCTCATTTCAGGT
mJCENA41-F	CCTTTCTTACCCCTCATCCTT
mJCENA41-R	AAAGCCAGGACATACTTGAA

mJCENA87-F	CATCTGGAGTGAAACCAAAGA
mJCENA87-R	CACATGGTAAGCATTACAAGC
mJCENA108-F	CGTGTGGTGCTTACCCCTATTTT
mJCENA108-R	GCCTCCTTTTCTTTTCCTGTTT
mJCENA110-F	CGCGTAGAAACACAGGAACATCA
mJCENA110-R	ACTCTCAATGGTTGTTATGGGC
mJCENA111-F	CAAGCCCAGTTGCTCATATTCAG
mJCENA111-R	CAAGGCTCAAAGATAGAAGGGA
Ek23-F	CCTTCCTCTGCTCTGGTTCTC
Ek23-R	TCCTTACGCTCTTAGGCTTT
Ep05-F	CAAAGCCCACTACGCAACAAG
Ep05-R	AAAACACTCCGACGGTCAAG
*Ephos01-F	CAGTCTGTGGTCCGATTCGTT
*Ephos01-R	GCCCTAGTTTTTAGGCATTGG
*Ephos09-F	CGGAATAGCATCTAAGGGCAGA
**Ephos09-R	GCAACTGAGTAACAAGTCTAGGA
**Ephos14-F	CCCTGGTGGATCTACCTCGAA
*Ephos14-R	TCAAATGAGGGACCGAAGTC
*Ephos22-F	CCCACAAGCACTAAACCATTCC
*Ephos22-R	GAGGATGCCCATGTTCAAAG
*Ephos50-F	CTGCATAACATTATGTCACACACA
*Ephos50-R	TCTTTGTAACAAGTTGGCATTTT
Ephos60-F	CGCGTATGAAATCAGAAGTTGGA
Ephos60-R	TGATGTGACGCAAGAGGAAC
Ephos80-F	CTCCAAACTCAACCCGAAAAC
Ephos80-R	CTCGTGGAGTCAGTCGTGAA
*Ephos85-F	CGGTGTGATGCAAGAGGAACA
*Ephos85-R	GCGTATGAAATCAGAAGTTGGA
*Ephos94-F	CAACCCGAGATGGTATTCGTG
*Ephos94-R	TTAAAGGCGGTTGAGACCAC
*Ephos95-F	CTTCCTCATTGCGTGGAGCTA
*Ephos95-R	TGGTTGGATTAGGCAACAGA
EphosH04_01-F	ACTCACGAACATCCCAAACT
EphosH04_01-R	CGGTTATGTGTAAGCGTACTG
EphosH04_02-F	AATCATCATGCGACACAAAG
EphosH04_02-R	CACCCATCCGTCTAGTTTTT
EphosG01-F	AATATGGCCAACTTCAAGGA
EphosG01-R	CCCCTACAATAGTTGGGTTC
EphosF09-F	TTTACGGTTTCGATGCCTAT

EphosF09-R	CGCGTGGACTAACAATTACA
EphosF01-F	GCTGTGTGGAGCTACAAATG
EphosF01-R	CGGTTATCAGAGAGATGGTGT
EphosE06-F	TCCTCGCTTCGTGGAACTAC
EphosE06-R	TTGGGAGACATGTGCAGAAA
EphosC09-F	TTCGTGTCATCCGATTTCAA
EphosC09-R	GGGCAGTTTAAAAGCAGTGG
EphosC03-F	TTTTCTGCGATGAGTTGTGT
EphosC03-R	CTTACGCGTGGACTAACAAG
EphosB02-F	ATGCTTCAAAGGCTGAAAGC
EphosB02-R	CGCGTGGACTAACTAGCAGA



SoftGenetics
GeneMarker V1.95

Allele Report

12/08/2016 13:48:42
Page 1

Sample 1:
Dye: Blue - 8 peaks - A4.fsa

Sample 2:
Dye: Blue - 18 peaks - B4.fsa

Sample 3:
Dye: Blue - 5 peaks - C4.fsa

Sample 4:
Dye: Blue - 5 peaks - D4.fsa

Figura 01: Resultado da genotipagem do único marcador microssatélite que apresentou polimorfismo.

REFERÊNCIAS BIBLIOGRÁFICAS

Amos, W.; Hoffman, J.I.; Frodsham, A.; Zhang, L.; Best, S.; Hill, A.V.S. 2007. Automated binning of microsatellite alleles: problems and solutions, **Molecular Ecology Notes**, v. 7, p. 10-14.

Bressan, E.A., Scotton, D. C., Ferreira, R. R., Jorge, E.C., Sebbenn, A.M., Gerald, L.T.S., Figueira, A. 2012. Development of microsatellite primers for *Jatropha curcas* (Euphorbiaceae) and transferability to congeners, **American Journal of Botany**, n. 99, p. 237-239.

Durka, W. Isolationcharacterization of microsatellite loci for Euphorbia palustris (Euphorbiaceae). 2009. **Genome.** n. 52, p. 1037-1039.

Palma-Silva, C., Cavallari, M.M., Barbará, T., et al. 2007. A set of polymorphic microsatellite loci for *Vriesea gigantea* and *Alcantarea imperialis* (Bromeliaceae) and cross-amplification in other bromeliad species. **Molecular Ecology Notes**, n. 7, p. 654–657.

Phumichai C, Phumichai T, Kongsiri N, Wongkaew A, Sripichit P, Kaveeta R. Isolation of 55 microsatellite markers for *Jatropha curcas* and its closely related species. 2011. **Biologia Plantarum**, n. 55, p. 387–390.

Van-Oosterhout, C.; Hutchinson, W. F.; Wills, D. P. M.; Shirley, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. **Molecular Ecology Notes**, n. 4, p. 535–538.

Yan, X.-H.; Fang, M.-F.; Qian, Z.-Q.; Liu, Z.-L.; Wang, M.-L.; Tian, C.-P.; Du, M.-H.; Li, Y.-J; Li, Z.-H; Zhao, G.-F. 2014. Isolation and characterization of polymorphic microssatelites in perennial herb *Euphorbia kansui* using paired-end Illumina shotgun sequencing. **Conservation Genetics Resources**, n. 6, p. 841-843.

Anexo III

Euphorbia from Brazil: the succulent section *Brasilienses*

Euphorbia from Brazil: the succulent section *Brasilienses*

By Ricarda Riina, Otávio Marques & Fernanda Hurbath

We selected the succulent *Euphorbia* section *Brasilienses* to start our series about *Euphorbia* species with distribution in Brazilian biomes. For this and future contributions, we will present groups of species according to their relatedness based on the current taxonomic classification of the genus *Euphorbia*. This classification system is based on a phylogenetic hypothesis, i.e. an evolutionary tree built with sequences of selected DNA regions, that depicts relationships among species. In this new classification *Euphorbia* is subdivided in four major groups (subgenera *Athymalus, Chamaesyce, Esula* and *Euphorbia*) and 65 sections (Yang et al., 2012; Riina et al., 2013; Dorsey et al., 2013; Peirson et al., 2013).

Section *Brasilienses* belongs to subgenus *Euphorbia*, which is the largest of the four subgenera with around 700 species and includes the majority of the cactiform plants. Although cactiform groups of *Euphorbia* reach their highest diversity in the Old World, particularly in Africa, there are a few representatives of spineless cactiform species in the New World (e.g. section *Brasilienses*).

Fig. 1 (above): Individual of Euphorbia attastoma *from Grão Mogol, Minas Gerais (photo R. Louzada).*

Fig. 2: Individual of Euphorbia attastoma *showing decumbent branches.*

Fig. 3:Typical habitat of Euphorbia attastoma *in Licínio de Almeida, Bahia (photo N. Roque)*

One of the most interesting findings derived from phylogenetic analyses (Horn et al., 2012) is the fact that the New World cactiform lineages of *Euphorbia* are distantly related to the Old World ones, showing what is known as convergent morphological evolution. Another example of this phenomenon are the pencil-stem *Euphorbia*, which have also arisen several times in separate branches of the *Euphorbia* phylogeny and in different geographic regions.

Section *Brasilienses* is a small group composed of four species restricted to rocky habitats of eastern Brazil, namely *Euphorbia attastoma* Rizzini, *Euphorbia holochlorina* Rizzini, *Euphorbia phosphorea* Mart., and *Euphorbia sipolisii* N.E.Br. Although these species have been treated in previous publications, here we update their taxonomy, ecology, and distribution.

Fig. 4:Variation of cyathium colour in Euphorbia attastoma

A little bit of history

Euphorbia phosphorea, the oldest name within the section, was published in "Reise in Brasilien" (1828) by Carl von Martius. It was later illustrated in Flora Brasiliensis (1874), where it was presented as the only Brazilian representative of section *Euphorbium*; however the author already called attention to the glands, bearing two horns that were lacking in all other species within that section.

Still in the 19th century, *Euphorbia sipolisii* was described based on a Glaziou specimen from the state of Minas Gerais (without specific location) in honor to Abbé Michel Marie Sipolis, director of the Seminary of Diamantina (Minas Gerais), who supported many researchers in his region.

In the late 1980's, two others succulent species (*Euphorbia attastoma* and *Euphorbia holochlorina*) were described by Carlos T. Rizzini, a renowned Brazilian scientist who worked on ecogeographical studies all over the country. Rizzini presented a review of cactiform species of *Euphorbia* from Brazil, which he recognized as members of section *Euphorbium*. In this group he included two more species, *E. appariciana* Rizzini and *E. gymnoclada* Boiss., which are now placed in section *Crossadenia* of subgenus *Chamaesyce*.

Fourteen years later, in 1994, Urs Eggli published his work "Xerophytic Euphorbias from Brazil" in The Euphorbia Journal, and placed all Brazilian succulent species in three different sections following Pax and Hoffman (1931). Unlike Rizzini, Eggli excluded *Euphorbia appariciana* and *Euphorbia gymnoclada* from sect. *Euphorbium*, however he emphasized that the placement of succulent South American taxa was still in need of revision. Lastly, Machado (2000) provided a detailed account of *Euphorbia phosphorea* and its three related species. This author also hypothesized that they were closely related to two other succulent species: *Euphorbia pteroneura* A.Berger from Mexico and *Euphorbia weberbaueri* Mansf. from Peru and Ecuador. However, we can point out important morphological differences. *Euphorbia pteroneura* has well developed leaves and its branches are not angular. *Euphorbia weberbaueri* has angular branches like the Brazilian cactiform species, but it lacks glands that bear horn-like appendages.

Fig. 5: Habit of Euphorbia holochlorina; *plant found in Águia Branca, Espírito Santo*

Fig. 6. Habitat of Euphorbia holochlorina

Fig. 7: Detail of the cyathium of Euphorbia holochlorina

Currently, the four Brazilian cactiform taxa are the only members of *Euphorbia* sect. *Brasilienses*, a section proposed by Dorsey et al. (2013) based on molecular, morphological and geographic evidence. In the phylogeny of subgenus *Euphorbia*, these species form a monophyletic group most closely related to section *Stachydium*, and distantly related to the Old World clades of succulent *Euphorbia*. Section *Brasilienses* is also distantly related to the New World section *Euphorbiastrum* (Klotzsch & Garcke) Boiss., where *Euphorbia weberbaueri* and *E. pteroneura* belong (Dorsey et al. 2013).

Morphology of sect. *Brasilienses*

Species in this section are typically shrubs adapted to xerophytic environments (Figs 2, 3, 6, 8, 10). They have stems that are succulent, green (sometimes with a waxy appearance), erect or decumbent (Fig. 2), with abundant latex. Plants are commonly about 1-2 m tall, but some individuals grow up to 5 m (rarely 6 m) and can have numerous ascending angular (ribbed) branches (Figs 1, 4, 5, 8, 10). The leaves are highly reduced, fleshy, conduplicate and quickly deciduous. The showy cyathia, usually red, are axillary with four to five, rarely more, oblong, two-horned glands placed on the rim of the involucre (Figs 4, 7, 9, 11). The stalk subtending the ovary, the gynophore, terminates in a reduced 3-lobed perianth-like structure. Capsules are exerted from the cyathial involucre and could be dark red, yellow, green or bicolor (e.g., red and yellow). This group is the only New World section of *Euphorbia* where all the members are cactiform plants.

Table 1. Main differences among the four species of Euphorbia *section* Brasilienses

	E. attastoma	***E. holochlorina***	***E. phosphorea***	***E. sipolisii***
stems max. length (m)	1-1.5	1.5-2	2-6	0.5-1.5
branches	6 ribs, faces concave	6 ribs, faces concave	8-9 ribs, faces slightly concave	4 ribs, faces almost flat
pattern of ribs (see Machado, 2000)	The 3 ribs arising from a given node reach the same next node up	The 3 ribs arising from a given node reach the same next node up	2 of the 3 ribs arising from a given node reach a subsequent node; the third rib converges to a different node	2 of the 3 ribs arising from a given node reach a subsequent node; the third rib converges to a different node
lignification	in old branches	absent	main trunk and old branches	absent
involucre	campanulate	urceolate	campanulate	campanulate
gland horns	patent	erect	patent	patent
ovary/ capsule	red or red/yellow/ green	mostly green	red, red/yellow or red/ green	red or red/yellow
distribution (states)	Minas Gerais, SW Bahia	Espírito Santo	Bahia, Sergipe, Pernambuco, Paraíba	Minas Gerais

The number of stem angles or ribs is one of the main features used to distinguish species in this group. *Euphorbia phosphorea* usually presents 8 to 10 ribs, which are less prominent than in the other species. *Euphorbia attastoma* and *E. holochlorina* have stems with six prominent ribs and the stems of *Euphorbia sipolisii* present only four ribs (Fig. 11). Machado (2000) has described in detail the pattern in which ribs and nodes are connected in different species, which seems to be useful for distinguishing two groups within the section (see Table 1).

Differences in the cyathium are not evident, except in *Euphorbia holochlorina* (Fig. 7), which has glands with erect horns and a more closed involucre (urceolate), whereas in the other species the horns are reflexed, and the involucre is more open or campanulate (Figs 4, 9, 11). Regarding the plant habit, only *Euphorbia phosphorea* exhibits a small trunk (tree-like) and has taller individuals (Fig. 8) than the other species. The main features distinguishing the four species are summarized in Table 1.

Fig. 8: Habit, habitat of Euphorbia phosphorea. *Note the old woody stem of an old individual from Bahia (upper right).*

Habitat, distribution and conservation

Members of this section are distributed in eastern Brazil in the states of Espírito Santo, Bahia, Minas Gerais, Paraíba, Pernambuco and Sergipe, at elevations of 220-1250 m.

These plants share a preference for open areas with rocky substrates, however different species are found in different biomes. *Euphorbia phosphorea* occurs throughout the Caatinga biome in northeastern Brazil, where it usually grows in deciduous forests or thorny scrubs over exposed rocky soils, gravelly sandy substrates, or on inselbergs. *Euphorbia attastoma* and *E. sipolisii* are found in the Cerrado biome, where they grow in campos rupestres, i.e. rocky outcrops located along the Espinhaço range in central-eastern Brazil. *Euphorbia attastoma* has also been reported from southeastern Bahia, in deciduous forests on rocky outcrops. *Euphorbia holochlorina* occurs in the Atlantic Forest biome in the state of Espírito Santo, where it grows in open areas on granite inselbergs.

Having the widest geographic distribution within section Brasilienses, *Euphorbia phosphorea* is of little conservation concern. It is easily found in suitable habitats along roadsides in Bahia and Sergipe states, however in the states of Pernambuco and Paraíba its distribution is more restricted to granitic inselbergs.

Fig. 9: Variation found in the cyathia of individuals of Euphorbia phosphorea

Fig. 10: Habitat of Euphorbia sipolisii *with several individuals of this species in São Gonçalo do Rio das Pedras, Minas Gerais*

Euphorbia attastoma and *E. sipolisii*, although not widely distributed, have most of their populations within protected areas in Minas Gerais (Parque Estadual de Grão Mogol and Parque Estadual de Biribiri). Lastly, *Euphorbia holochlorina* is only known from three localities, including the type one, all of them very near each other and outside of protected areas in the state of Espírito Santo. In general, habitats of section *Brasilienses* are not suitable for agriculture or cattle raising, however these plants are often used as fuel for fire.

Fig. 11:Detail of the cyathium of Euphorbia sipolisii *and a cross section of its distinctive quadrangular stem.*

References

Dorsey, B. L., Haevermans, T., Aubriot, X., Morawetz, J. J., Riina, R., Steinmann, V. W. & Berry, P. E. (2013): Phylogenetics, morphological evolution, and classification of Euphorbia subgenus Euphorbia. Taxon 62: 291-315

Eggli, U. (1994): Xerophytic Euphorbias from Brazil. Euphorbia J. 9: 11-23

Horn, J. W., van Ee, B. W., Morawetz, J. J., Riina, R., Steinmann, V. W., Berry, P. E. & Wurdack, K. J. (2012): Phylogenetics and the evolution of major structural characters in the giant genus Euphorbia L. (Euphorbiaceae). Mol. Phylogenet. Evol. 63: 305-326

Machado, M. (2000): Euphorbia phosphorea – the largest growing Brazilian Euphorbia. Cact. Succ. J. Gr. Brit. 18(3): 151-158

Pax, F. & Hoffman, K. (1931): Euphorbiaceae. In: Engler, A. & Prantl, K. (eds): Die Natürlichen Pflanzenfamilien vol. 19c. 2nd ed. Leipzig: 210-214

Peirson, J. A., Bruyns, P. V., Riina, R., Morawetz, J. J. & Berry, P. E. (2013): A molecular phylogeny and classification of the largely succulent and mainly African Euphorbia subg. Athymalus (Euphorbiaceae). Taxon 62: 1178-1199

Riina, R., Peirson, J. A., Geltman, D. V., Molero, J., Frajman, B., Pahlevani, A., Barres, L., Morawetz, J. J., Salmaki, Y., Zarre, S., Kryukov, A., Bruyns, P. V. & Berry, P. E. (2013): A worldwide molecular phylogeny and classification of the leafy spurges, Euphorbia subgenus Esula (Euphorbiaceae). Taxon 62: 316-342

Yang, Y., Riina, R., Morawetz, J. J., Haevermans, T., Aubriot, X. & Berry, P. E. (2012): Molecular phylogenetics and classification of Euphorbia subgenus Chamaesyce (Euphorbiaceae). Taxon 61: 764-789

Authors' contact:
rgriina@gmail.com
otaviolmarques@gmail.com
fhurbath@gmail.com