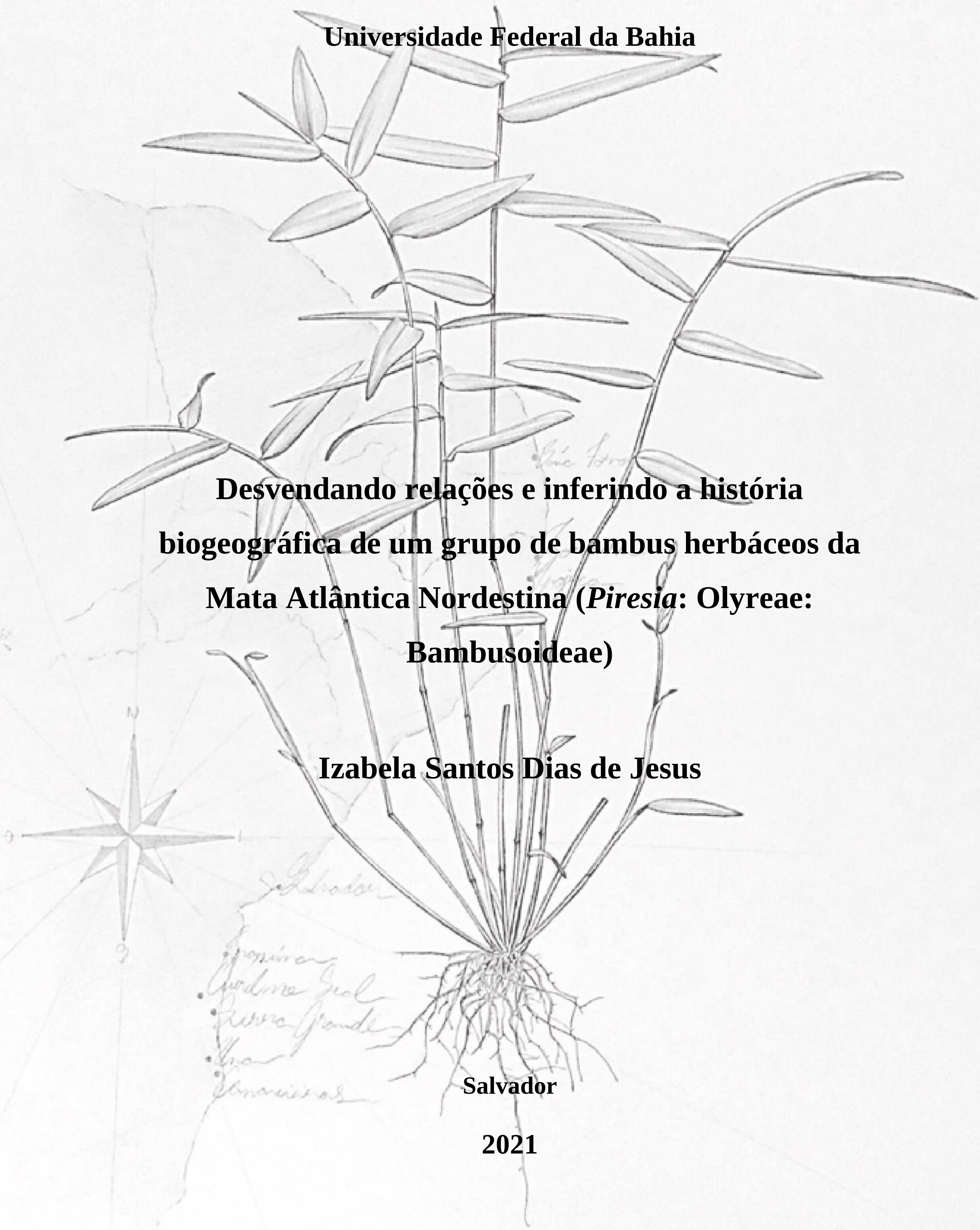

Programa de Pós-Graduação em Biodiversidade e Evolução

Universidade Federal da Bahia

Desvendando relações e inferindo a história biogeográfica de um grupo de bambus herbáceos da Mata Atlântica Nordestina (*Piresia*: Olyreae: Bambusoideae)

Izabela Santos Dias de Jesus

Salvador

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Dissertação apresentada ao Instituto de Biologia da Universidade Federal da Bahia para obtenção do Título de Mestre em Biodiversidade e Evolução pelo Programa de Pós-Graduação em Biodiversidade e Evolução.

Orientadora: Profa. Dra. Alessandra Selbach Schnadelbach

Coorientadora: Profa. Dra. Maria Luiza Silveira de Carvalho

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Comissão julgadora

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Dissertação de Mestrado submetida ao Programa de Pós-Graduação em Biodiversidade e Evolução da Universidade Federal da Bahia como parte dos requisitos necessários à obtenção do título de Mestre na área de Biodiversidade e Evolução.

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Índice de figuras e tabelas

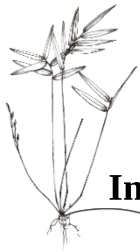
Introdução geral

Capítulo único

Índice

Apêndices

Anexos



Introdução Geral

1. Inconguências topológicas, suas causas e consequências.

O advento das técnicas utilizando dados de DNA no final da década de 70 propiciou um grande avanço nos estudos filogenéticos de diversos grupos de organismos (BRUNS; WHITE; TAYLOR, 1991; GOODMAN et al., 1998; WENDEL; DOYLE, 1998; SOLTIS; SOLTIS, 2000). No caso das plantas, cuja sistemática tinha por base o uso de dados morfológicos, a mudança ocorreu a partir da década de 90, com o aumento dos estudos envolvendo dados moleculares (BALDWIN et al., 1995; SOLTIS et al., 1997; APG, 1998).

Estudos filogenéticos em plantas têm, de maneira geral, se fundamentado na ampla utilização de dados do DNA plastidial, devido às suas características peculiares, como herança uniparental (na grande maioria dos grupos), baixas taxas de recombinação e taxas de evolução mais altas, quando comparadas com o DNA mitocondrial (OLMSTEAD; PALMER, 1994; JANSEN et al., 2005; SHAW et al., 2005). Dessa forma, é comum o uso dos dados do genoma plastidial como uma única região (TU et al., 2008; TRÖNDLE et al., 2010; LU et al., 2012; LIU et al., 2013; DONG et al., 2017; ATAEI et al., 2020). A prerrogativa de evolução do genoma plastidial como uma única unidade, no entanto, tem sido debatida nos últimos anos, porque diferentes estudos têm sugerido que determinados genes plastidiais podem não estar ligados, experienciando assim, diferentes forças evolutivas, como eventos de recombinação, bem como pressões seletivas e taxas de evolução variáveis (WOLFE; RANDLE, 2004; JANSEN; RUHLMAN, 2012; RUHLMAN et al., 2017; GONÇALVES et al., 2019). Marcadores nucleares, por sua vez, apresentam herança biparental, altas taxas de recombinação e taxas mais altas de evolução quando comparadas ao DNA plastidial, mas que diferem grandemente ao longo do genoma (WENDEL; SCHNABEL; SEELANAN, 1995; WENDEL; DOYLE, 1998; ZHANG; HEWITT, 2003).

Fatores como recombinação, diferentes taxas evolutivas e as diferentes forças evolutivas que os diferentes genomas experenciam podem, então, gerar reconstruções filogenéticas incongruentes entre si, ou diferentes árvores gênicas (WENDEL; DOYLE, 1998; WOLFE; RANDLE, 2004; GONÇALVES et al., 2019). Essas incongruências podem estar associadas a diferentes processos, tanto de natureza metodológica, como insuficiência de dados, atribuição incorreta dos modelos evolutivos ou erro na busca da árvore adequada

(JEFFROY et al., 2006), quanto por processos inerentes à biologia dos organismos, como seleção incompleta de linhagem (ILS), seleção natural, duplicação ou perda gênica, transferência horizontal de genes, hibridização e introgressão, esses dois últimos comumente encontrados em plantas (DEGNAN; ROSENBERG, 2006, 2009; CASTOE et al., 2009; KHALILI et al., 2020) (Figura 1).

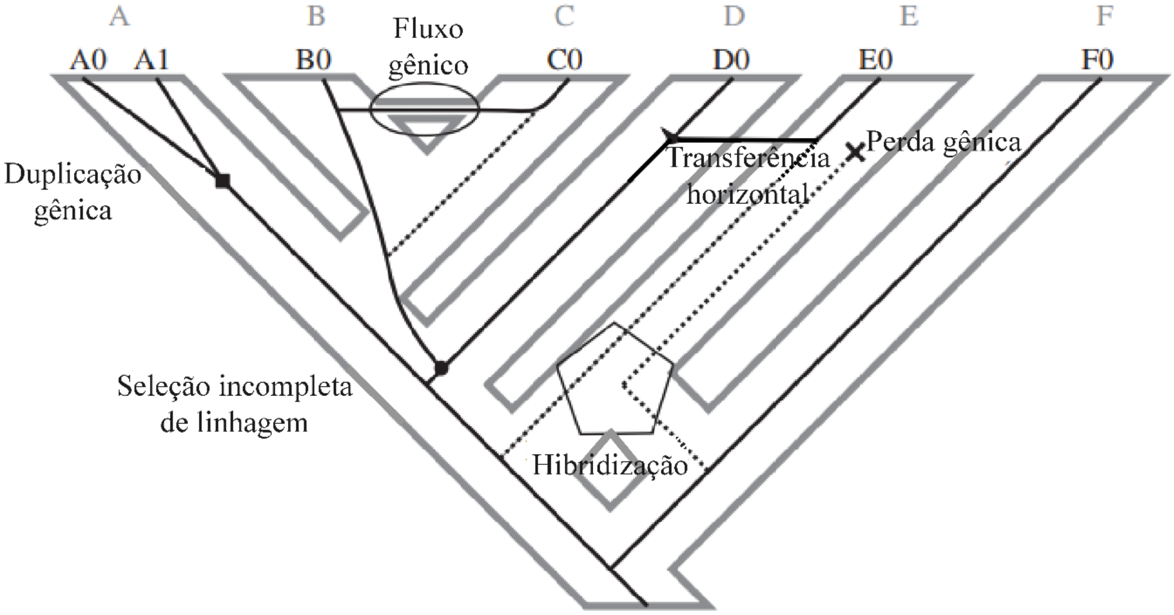


Figura 1. Processos evolutivos responsáveis por incongruências topológicas entre árvores de genes e de espécies. A topologia em cinza, ao fundo, representa a árvore de espécies e, em preto, a árvore gênica. As letras A, B, C, D, E e F correspondem às espécies, enquanto A0, A1, B0, C0, D0, E0 e F0 representam os indivíduos. Os processos evolutivos na história dessas espécies são indicados por nomes e símbolos: duplicação gênica (quadrado); perda gênica (cruz); transferência horizontal (seta); seleção incompleta de linhagem (círculo); hibridação (pentágono); e fluxo gênico (eclipse). As linhas tracejadas indicam linhagens extintas que não alcaçaram o presente devido à perda gênica. Figura retirada e adaptada de Mallo & Posada (2016).

Fatores relacionados aos processos metodológicos como o uso de dados incompletos, atribuição incorreta de modelos evolutivos e erro na busca das árvores mais adequadas podem incorrer em incongruências topológicas principalmente devido à presença de sinais não filogenéticos (JEFROY et al., 2006). Isso porque esses erros sistemáticos deixam de levar em conta fatores importantes na evolução das regiões analisadas, como heterogeneidade na composição dos nucleotídeos e variação nas taxas evolutivas tanto entre linhagens quanto dentro de um mesmo genoma (PHILLIPS; DELSUC; PENNY, 2004;

JEFROY et al., 2006). Desse modo, sinais filogenéticos verdadeiros são perdidos, levando a reconstruções filogenéticas inconsistentes, mas que podem apresentar altos valores de suporte (PHILLIPS; DELSUC; PENNY, 2004; JEFROY et al., 2006).

Já com relação aos processos relacionados à biologia dos organismos, podemos destacar o papel da ILS, por exemplo, que representa a retenção de linhagens gênicas na árvore de espécies devido à coalescência (evento estocástico em que uma amostra de genes atuais encontra o ancestral comum mais recente) (DEGNAN; ROSENBERG, 2009). A ILS é comumente observada em grupos que coalesceram recentemente (DEGNAN; ROSENBERG, 2009), pois esses grupos podem sofrer vários eventos de especiação em um curto período no tempo e, assim, a árvore de espécies pode apresentar ramos mais curtos (DEGNAN; ROSENBERG, 2009; RASMUSSEN; KELLIS, 2012). Dessa forma, várias linhagens de genes presentes nas populações podem ficar retidas em ramos mais profundos dessa árvore, levando à ocorrência de eventos coalescentes entre linhagens que não são as mais intimamente relacionadas (DEGNAN; ROSENBERG, 2009; RASMUSSEN; KELLIS, 2012). Assim, as árvores gênicas acabam sendo discordantes entre si e a topologia inferida pode estimar relações que não recuperam o monofiletismo das linhagens (AVISE, 2000; DEGNAN; ROSENBERG, 2009).

As duplicações ou perdas gênicas podem levar a incongruências topológicas devido à quebra de uma premissa básica das análises filogenéticas baseadas em dados moleculares, que é o uso de genes ortólogos (ou seja, que possuem uma origem comum por especiação) (GABALDÓN, 2008; KRISTENSEN et al., 2011; RASMUSSEM; KELLIS, 2012). Quando eventos de duplicação ou perda de genes ocorrem ao longo do genoma e esses eventos não são detectados, os genes utilizados nas análises filogenéticas podem se referir a cópias parálogas (que possuem uma origem por duplicação) e assim a árvore de espécies pode não refletir os reais eventos de especiação que ocorreram ao longo do tempo (GABALDÓN, 2008; KRISTENSEN et al., 2011; RASMUSSEM; KELLIS, 2012). A transferência horizontal de genes, por sua vez, pode ocorrer por meio de eventos de transformação, conjugação e trandução (SYVANEN, 1985; GALLARDO, 2017), sendo um fenômeno comumente associado a procariotos, mas podendo ocorrer de forma mais rara em organismos eucariotos (SYVANEN, 1985; GALLARDO, 2017; XIA et al., 2021). Esse fenômeno pode gerar incongruências topológicas por não ser representado por uma árvore de espécies estritamente bifucarda, ou seja, envolve uma história complexa de reticulação entre as espécies (RIESEBERG; SOLTIS, 1991; GE; WANG; KIM, 2005; GALLARDO, 2017).

Eventos de hibridação e introgressão, comumente regastadas através de análises do genoma nuclear, também podem contribuir para inferências errôneas sobre as relações entre as espécies, principalmente em plantas, nas quais esses eventos são comumente observados (ZHANG; HEWITT, 2003; SUN et al., 2015; MALLET; BESANSKY; HAHN, 2016). A ocorrência desses eventos confude os limites entre as espécies, especialmente naquelas intimamente relacionadas, onde as taxas de hibridação e introgressão podem ser maiores do que em espécies geneticamente distantes (MALLET, 2007; MALLET; BESANSKY; HAHN, 2016; PANAHI et al., 2018). Outra causa de incongruências filogenéticas pode advir de uma evolução convergente não neutra entre diferentes linhagens filogenéticas (ZHANG; KUMAR, 1997; CASTOE et al., 2008, 2009). Diferentes *loci* podem sofrer pressões seletivas similares e, dessa forma, evoluírem por convergência (ZHANG; KUMAR, 1997; CASTOE et al., 2008, 2009). O uso dessas regiões nas análises se torna problemático porque pode agrupar linhagens que não estão intimamente relacionadas e a árvore gênica pode, dessa forma, não coincidir com a árvore de espécies (CASTOE et al., 2009). Uma pressão de seleção diferencial em um mesmo genoma também pode gerar incongruências entre as árvores gênicas e, consequentemente, na árvore de espécies, pois ao longo do genoma os diferentes genes podem experienciar diferentes pressões de seleção, podendo evoluir sob uma seleção neutra ou sob uma seleção positiva ou negativa (GONÇALVES et al., 2019). Isso é particularmente problemático quando as regiões utilizadas são tidas como evoluindo em uma única unidade (GONÇALVES et al., 2019).

Ainda que diferentes processos possam atuar em diferentes loci, muitos estudos filogenéticos ainda têm se baseado no sequenciamento e concatenação de diferentes genes e na inferência da topologia mais comum como a árvore de espécies (DEGNAN; ROSENBERG, 2006, 2009; STIERANDOVÁ et al., 2016; CARVALHO-SOBRINHO et al., 2016; GÓNZALEZ et al., 2016; LI et al., 2016; LIAN et al., 2019; QIU et al., 2020). Essa prerrogativa pode fazer com que as inferências sejam estatisticamente inconsistentes, principalmente em grupos que apresentam uma história evolutiva recente e com processos biológicos complexos, como os eventos de reticulação mencionados acima (DEGNAN; ROSENBERG, 2006).

Além disso, a suposição de que a topologia mais comum é a mais provável para estimar as relações entre as espécies pode ser enganadora (FELSENSTEIN, 1978). Ao assumir que as regiões concatenadas evoluíram igualmente, ignora-se as diferentes pressões e/ou taxas evolutivas dos diferentes *loci*, o que pode resultar em uma árvore de espécies imprecisa, embora com altos valores de suporte (MOSSEL; VIGODA, 2005; EDWARDS; LIU; PEARL, 2007; KUBATKO; DEGNAN, 2007; DEGNAN; ROSENBERG, 2009). Degnan & Rosenberg (2006), por exemplo, discutem sobre as “árvores gênicas anômalas” (AGTs), que correspondem às topologias gênicas obtidas com mais frequência do que aquela que representaria a árvore de espécies. As AGTs são mais frequentes em grupos que especiaram rapidamente, a partir de eventos de coalescência recente (DEGNAN; ROSENBERG, 2006) e, por esse motivo, a inferência filogenética a partir de dados concatenados, principalmente em grupos com essas características, pode resultar em uma estimativa incorreta das relações entre as espécies (SOLTIS; KUZOFF, 1995; DEGNAN; ROSENBERG, 2006, 2009).

Nesse sentido, alguns métodos vêm sendo desenvolvidos para contornar esses processos de natureza biológica, como ILS, transferência e duplicação gênica, hibridação, introgressão e seleção diferencial. Um deles se baseia na quantificação da incongruência filogenética em quartetos (STRIMMER; VON HAESELER, 1997; STRIMMER; GOLDMAN; VON HAESELER, 1997; CHIFMAN; KUBATKO, 2014; MIRARAB; WARNOW, 2015; PEASE et al., 2018; ZHOU et al., 2020). Os quartetos se referem à unidade de informação mais básica em uma árvore filogenética, não enraizada, formada por conjuntos de quatro táxons (STRIMMER; GOLDMAN; VON HAESELER, 1997; STRIMMER; VON HAESELER, 1997; CHIFMAN; KUBATKO, 2014; MIRARAB; WARNOW, 2015). Os métodos baseados em quartetos propiciam uma maior consistência estatística das topologias obtidas devido à possibilidade de decomposição das árvores de referência em conjuntos de quartetos que podem ser comparados entre si (DEGNAN; SALTER, 2005; ZHOU et al., 2020), ao invés de bipartição, que se baseia na remoção de um ramo em uma filogenia e na separação dos táxons em dois subconjuntos disjuntos, e que são os mais comuns (RANWEZ; GASCUEL, 2001).

Um desses métodos consiste no *Accurate Species TRee ALgorithm* (ASTRAL-II), através do qual a árvore de espécies é estimada a partir da decomposição de quartetos induzidos de árvores gênicas não enraizadas, utilizando o modelo coalescente multiespécies (MIRARAB et al., 2014; MIRARAB; WARNOW, 2015). Quando comparado a outros métodos baseados em coalescência, como MP-EST e BUCKy-pop, o ASTRAL-II apresenta maior precisão na estimativa das árvores de espécies em condições em que há taxas baixas de erros nas estimativas das árvores gênicas e em árvores que apresentam taxas moderadas a altas de ILS (MIRARAB et al., 2014; MIRARAB; WARNOW, 2015). Além do ASTRAL-II, outro método baseado em quartetos é o SVDquartets, que, diferentemente, utiliza uma análise coalescente completa através da amostragem dos alinhamentos de nucleotídeos, ao invés de árvores gênicas (CHIFMAN; KUBATKO, 2014). Através de estatísticas algébricas e decomposição de valor singular, o SVDquartets permite o cálculo das melhores pontuações das topologias obtidas a partir dos quartetos (CHIFMAN; KUBATKO, 2014). Esse método tem apresentado estimativas mais precisas quando há menores taxas de ILS, quando comparado ao ASTRAL-II, mas que tendem a uma menor precisão quando as taxas de ILS são mais altas (acima de 85%) (CHOU et al., 2015).

2. Incongruências topológicas, história intricada de áreas e suas relações com o estabelecimento de padrões biogeográficos complexos: Mata Atlântica como exemplo

Incongruências topológicas decorridas da seleção incompleta de linhagem, seleção natural, duplicação gênica, transferência horizontal de genes, hibridação e introgressão, além de dificultarem o entendimento das relações filogenéticas entre os grupos, são fontes de incerteza para outros tipos de estudos, que têm como base as filogenias, como é o caso daqueles envolvendo biogeografia (ZHANG, FEILD; ANTONELLI, 2015), onde os efeitos das incongruências, são raramente considerados. O uso de topologias incertas, no entanto, pode ter como consequência a proposição de cenários biogeográficos enviesados, decorrentes da incerteza das próprias relações entre os táxons (BUERKI et al., 2011; ZHANG, FEILD; ANTONELLI, 2015). Esse cenário gera incertezas na própria reconstrução da história biogeográfica e nas estimativas de tempo de divergência, que podem variar a depender do conjunto de dados utilizado, influenciando assim os intervalos dos nós ancestrais e sua distribuição geográfica (NYLANDER et al., 2008; BUERKI et al., 2011; ZHANG, FEILD; ANTONELLI, 2015), como exemplificado na Figura 2 (abaixo).

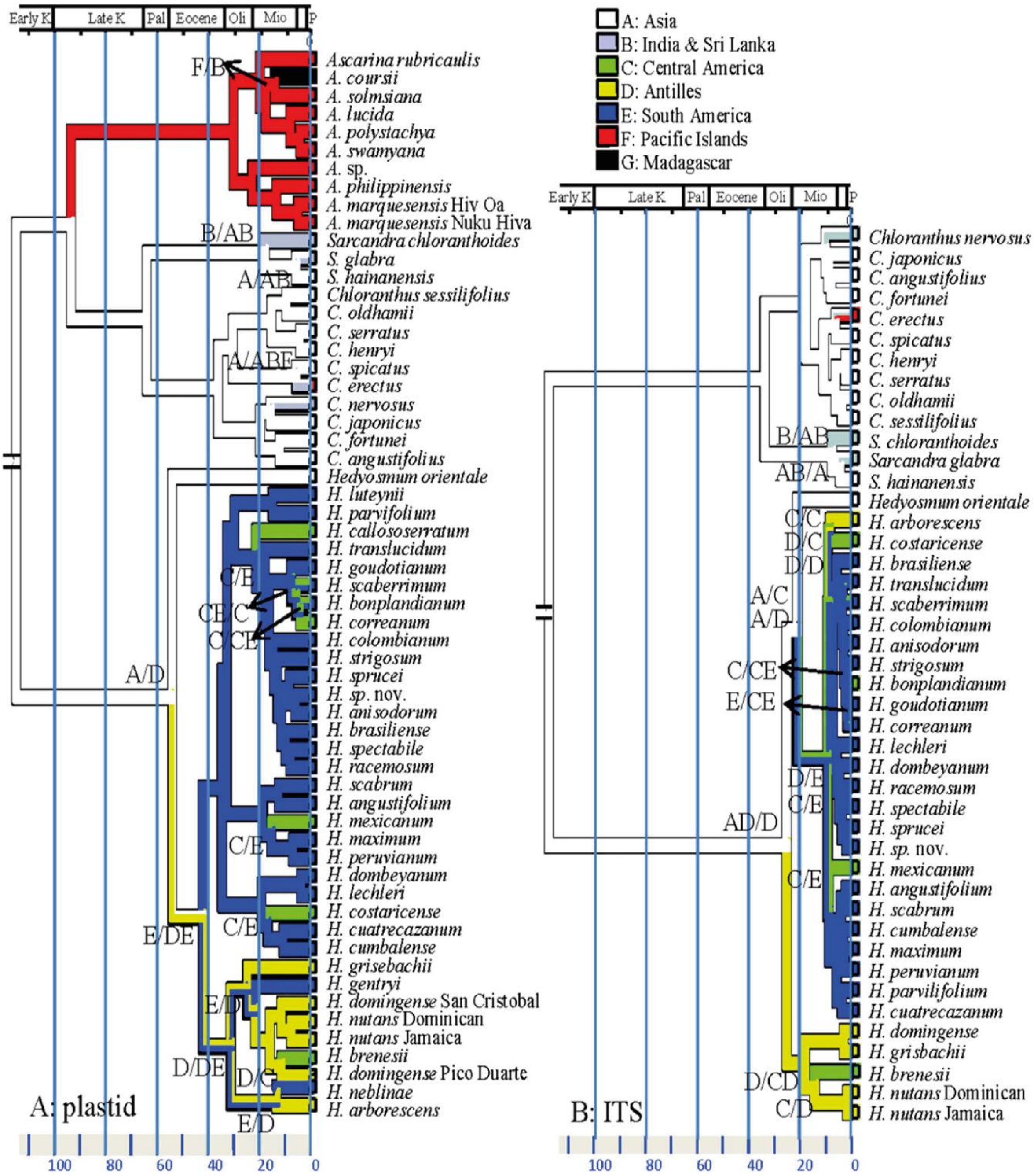


Figura 2. Mudanças nas estimativas das áreas ancestrais e dos tempos de divergência em Chloranthaceae com base nas árvores de plastídios (A) e núcleo (B). A reconstrução da área ancestral foi gerada pelo método Fich MP no programa Mesquite e foi inferida a partir do modelo de verossimilhança DEC no Lagrange. As cores e as letras representam as áreas. A, branco, Ásia; B, azul claro, Índia e Sri Lanka; C, verde, América Central; D, amarelo, Antilhas; E, azul escuro, América do Sul; F, vermelho, Ilhas do Pacífico; G, preto, Madagascar. As siglas na escala superior indicam os períodos. K inicial, Cretáceo inicial; K tardio, fim do Cretáceo; Pal, Paleoceno; Oli, Oligoceno; Mio, Mioceno; P, Pleistoceno. As escalas na parte inferior indicam o tempo em milhões de anos atrás. Figura retirada de Zhang, Feild & Antonelli (2015).

Alguns estudos discutem o uso de topologias incertas em abordagens biogeográficas. Zhang, Feild & Antonelli (2015), por exemplo, retratam como o conflito filogenético entre diferentes genomas (plastidial *versus* nuclear) pode influenciar as estimativas de tempo e de distribuição ancestral, gerando diferentes cenários para explicar a origem e diversificação do gênero *Hedyosmum* Sw. de plantas. Já Kramina e colaboradores (2016) e Scheunert & Heubl (2017) exemplificam como conflitos topológicos podem influenciar a calibração do relógio molecular em estimativas de tempo de divergência e inferência de distribuições ancestrais em *Lotus* L. e *Scrophularia* L., respectivamente, em decorrência do uso de marcadores moleculares altamente polimórficos e influenciados por eventos de reticulação, como hibridação, introgressão, transferência horizontal de genes e seleção incompleta de linhagem. Esses e outros exemplos refletem a importância do uso de inferências filogenéticas acuradas para a elaboração de estudos biogeográficos.

Além da problemática acima descrita, outro fator fundamental e que pode dificultar a reconstrução da história biogeográfica de um grupo é a própria história complexa das áreas que os organismos ocupam (CRACRAFT, 1988), como, por exemplo, a região Neotropical. Esta se estende desde o México até o sul da América do Sul e compreende diversos biomas e fitofisionomias, que vão desde zonas áridas até extensas florestas tropicais, sendo assim, uma das regiões mais diversas do planeta (ANTONELLI; SANMARTÍN, 2011; MORRONE, 2014).

A grande diversidade de habitats e de espécies abarcada pela região Neotropical é comumente associada a uma complexa interação de fatores bióticos e abióticos (HOORN et al., 2010; ANTONELLI; SANMARTÍN, 2011; RULL, 2011; HUGHES; PENNINGTON; ANTONELLI, 2013), especialmente grandes alterações geomorfológicas que ocorreram na região ao longo do tempo (GENTRY; 1982; ANTONELLI et al., 2009; HOORN et al., 2010; HUGHES; PENNINGTON; ANTONELLI, 2013). Estas alterações, por sua vez, decorrem principalmente de eventos tectônicos que promoveram a elevação de cadeias de montanhas, que resultaram em mudanças na conformação de rios e alterações na paisagem, no clima e na disponibilização de novos habitats (GENTRY; 1982; GREGORY-WODZICKI, 2000; HOORN et al., 2010). Mudanças climáticas também parecem ter influência na promoção de diversidade e endemismo nos Neotrópicos, principalmente aquelas relacionadas às flutuações do Pleistoceno (HAFFER, 1974; FJELDSÅ; LAMBIN; MERTENS, 2006; SOBRAL-SOUZA; LIMA-RIBEIRO; SOLFERINI, 2015), pois hipotetiza-se que as mesmas foram responsáveis por diversos ciclos de expansão e retração de florestas, que promoveram diversos eventos de especiação e radiação de organismos (HAFFER, 1974; HUBERT; RENNO, 2006; BRUMFIELD; EDWARDS, 2007; SOBRAL-SOUZA; LIMA-RIBEIRO; SOLFERINI, 2015).

Um exemplo clássico envolve os biomas da Amazônia (AM) e da Mata Atlântica (MA) (MORLEY, 2000), que estão entre os mais complexos e ricos do mundo (HUBBELL et al., 2008; RIBEIRO et al., 2011). Hipotetiza-se que, durante o Paleógeno, esses dois biomas constituíam uma floresta contínua e intermitente (MORLEY, 2000), mas que mudanças associadas à elevação dos Andes e ao clima ao longo do Neógeno levaram à disjunção dessa grande área florestada pela formação de uma área mais seca que hoje compreende os biomas Caatinga, Cerrado e Chaco (a "Diagonal Seca") (AB'SÁBER, 1977, 2003; COSTA, 2003; HOORN et al., 2010). Alguns estudos, no entanto, mostraram que, após essa separação, houve momentos menos pronunciados de isolamento entre esses dois biomas, principalmente durante o Plioceno e Pleistoceno, hipótese esta baseada em dados palinológicos, geológicos e filogeográficos, que demonstram a similaridade entre algumas regiões da AM e MA (POR, 1992; OLIVEIRA; BARRETO; SUGUIO, 1999; AULER; SMART, 2001; COSTA, 2003; AULER et al., 2004; PELLEGRINO et al., 2011).

Algumas rotas que parecem ter possibilitado o intercâmbio de espécies foram propostas por diferentes autores (BATALHA-FILHO et al., 2013; SOBRAL-SOUZA et al., 2015). Batalha-Filho e colaboradores (2013), por exemplo, propuseram duas rotas entre esses dois biomas a partir de sequências de DNA e dados de distribuição geográfica de aves suboscines. Segundo esses autores, uma primeira rota teria se estabelecido durante o final do Mioceno médio entre o sul do Cerrado no Mato Grosso e a região de transição entre o Chaco e as savanas de palmeiras da Bolívia e Paraguai, enquanto a segunda teria sido mais recente (entre o Plioceno e Pleistoceno), entre o nordeste do Brasil entre os biomas da Caatinga e Cerrado (BATALHA-FILHO et al., 2013). Sobral-Souza e colaboradores (2015), por sua vez, propuseram três possíveis rotas de conexão, utilizando modelagem de nicho ecológico: uma rota pela costa norte do Brasil, ligando a porção ocidental da AM e o norte da MA; uma rota central entre a porção ocidental da AM e a porção sul da MA; e outra rota conectando e limite sul da porção oriental da AM e o sul da MA.

Particularmente com relação a esse último bioma (MA), as alterações climáticas, principalmente do Pleistoceno, parecem ter provocado uma série de mudanças na conformação de áreas florestadas, tendo assim influência direta tanto sobre a história evolutiva, quanto sobre o padrão de distribuição dos organismos, provavelmente levando a um aumento nas taxas de diversificação das espécies (WHITMORE; PRANCE, 1987; CARNAVAL; MORTIZ, 2008; CABANNE et al., 2013; PORTO; CARNAVAL; DA ROCHA, 2013). Uma das hipóteses que explica essas mudanças é a “Teoria dos Refúgios Florestais”, que prediz que, durante os períodos glaciais, as florestas fragmentavam-se e ficavam isoladas por áreas de vegetação aberta, reconectando-se posteriormente durante os períodos interglaciais (HAFFER, 1969; VANZOLINI; WILLIAMS, 1970; BROWN; AB’SÁBER, 1979). Essa hipótese tem sido apoiada por diferentes estudos, como, por exemplo, o de Carnaval & Moritz (2008), que propõe a existência de duas áreas de refúgio para a MA nas regiões da Bahia e de Pernambuco durante o Pleistoceno, através da modelagem de distribuição ecológica de diferentes espécies.

Essas áreas, no entanto, ainda são objeto de discussão por diferentes autores, não apenas pela proposição de outros refúgios (como na região sudeste do Brasil) (CABANNE et al., 2013; PORTO; CARNAVAL; DA ROCHA, 2013), mas também pela estabilidade populacional de algumas espécies ocorrentes nessas áreas (BATALHA-FILHO; CABANNE; MIYAKI, 2012; THOMÉ et al., 2014). Entretanto, ainda existe concordância sobre a existência de áreas de refúgio, haja visto os registros fósseis de vegetação que indicam uma heterogeneidade no bioma MA durante períodos mais frios, como demonstrado no estudo de Costa e colaboradores (2018).

Além da hipótese de refúgios, outra hipótese provável para a diversidade de espécies da MA em associação às mudanças climáticas do Pleistoceno é a “Hipótese Atlântida”, proposta por Leite e colaboradores (2016). Estes autores mostraram a interação entre mudanças no nível do mar e a distribuição dos organismos ao longo da plataforma continental, sugerindo que, ao invés de retração das populações, algumas espécies podem ter expandido sua distribuição geográfica durante os períodos glaciais devido à exposição da plataforma continental, que permitiu a expansão das áreas florestadas e, consequentemente, dos organismos dependentes desses habitats (LEITE et al., 2016).

Diversos eventos de transgressões e regressões marinhas ao longo da costa brasileira foram documentados para o Terciário e Quaternário, períodos de grandes mudanças climáticas (VILAS BÔAS; SAMPAIO; PEREIRA, 1978; BITTENCOURT et al., 1979; SUGUIO et al., 2005). Esses eventos parecem ter sido a causa de remodelações nessa região, principalmente associada a mudanças na conformação de rios, levando à formação de diversas planícies litorâneas (VILAS BÔAS; SAMPAIO; PEREIRA, 1978; BITTENCOURT et al., 1979; SUGUIO et al., 2005). Todas essas alterações geomorfológicas parecem ter influenciado tanto a origem quanto a diversificação de organismos na região costeira do Brasil, como mostram, por exemplo, os estudos de Ramos-Fregonezi e colaboradores (2015) e Fazolato e colaboradores (2017) envolvendo a planta *Calibrachoa heterophylla* (Sendtn.) Wijsman (Solanaceae) e o lagarto *Tropidurus hygomi* Reinhardt & Luetken, respectivamente.

Apesar dessas hipóteses traçadas, outros estudos em paralelo demostraram que determinados grupos de espécies parecem apresentar histórias evolutivas muito mais complexas e que não se enquadram em um cenário histórico específico (TURCHETTO-ZOLET et al., 2013; LEAL; PALMA DA SILVA; PINHEIRO, 2016). De qualquer forma, é consenso que as mudanças climáticas e geomorfológicas que ocorreram nesses biomas ao longo do tempo tiveram um papel fundamental na evolução de muitos organismos, ajudando a moldar os padrões de distribuição principalmente daqueles que apresentam uma maior dependência de ambientes florestados (D'HORTA et al., 2011; MENEZES et al., 2016; PERES et al., 2019), como, por exemplo, os bambus herbáceos que habitam o subosque dessas florestas (SODERSTROM, 1984; JUDZIEWICZ et al., 1999; CLARK; JUDZIEWICZ, 2007; OLIVEIRA et al., 2014; CLARK; LONDOÑO; RUIZ-SANCHEZ, 2015).

3. *Piresia* Swallen, um bambu de história evolutiva enigmática, como modelo de estudo

Bambus representam um grupo de gramíneas de grande potencial econômico, sendo bastante utilizados na construção civil e na ornamentação, além de serem utilizados, em alguns locais, na alimentação humana e animal e na fabricação de papel (SODERSTROM; CALDERÓN, 1979; JUDZIEWICZ et al., 1999; EMAMVERDIAN et al., 2020). Seus representantes também apresentam grande importância na composição e estruturação de florestas tropicais e subtropicais, principalmente devido aos seus rizomas (caules subterrâneos modificados), que formam sistemas radiculares extensos e complexos e à biomassa de suas partes vegetativas (folhas e colmos) que, em conjunto, protegem o solo contra fortes chuvas, evitando sua erosão, além de fornecerem matéria orgânica (SODERSTROM; CALDERÓN, 1979; CLARK, 1990; JUDZIEWICZ et al., 1999; EMAMVERDIAN et al., 2020). Nesse sentido, alguns de seus representantes parecem possuir um importante papel na sucessão ecológica de ambientes florestados, devido à sua alta especificidade de habitat (CALDERÓN; SODERSTROM, 1980; CLARK, 1990; JUDZIEWICZ et al., 1999). O grupo apresenta ainda ampla distribuição geográfica, estando ausente apenas na Europa e Antártida (CLARK, 1990; JUDZIEWICZ, 1999; VORONTSOVA et al., 2016; SORENG et al., 2017) e a maior parte dos gêneros e espécies ocorre em regiões tropicais e subtropicais, sendo que a América Central e do Norte constituem um dos principais centros de diversidade do grupo (SODERSTROM; CALDERÓN, 1979; CLARK, 1990; JUDZIEWICZ, 1999; FILGUEIRAS; GONÇALVES, 2004; OLIVEIRA et al., 2014; VORONTSOVA et al., 2016; SORENG et al., 2017; CLARK; OLIVEIRA, 2018; RUIZ-SANCHEZ et al., 2021).

Taxonomicamente os bambus estão compreendidos na subfamília Bambusoideae, que atualmente abrange mais de 1680 espécies e aproximadamente 127 gêneros, divididos em três tribos: Arundinarieae, Bambuseae e Olyreae (CLARK, 1990; JUDZIEWICZ et al., 1999; SUNGKAEW et al., 2009; KELCHNER; BPG 2013; CLARK; LONDOÑO; RUIZ-SANCHEZ, 2015; KELLOGG, 2015; VORONTSOVA et al., 2016; SORENG et al., 2017). Arudinarieae e Bambuseae reunem os representantes lenhosos das regiões temperadas e tropicais e subtropicais, respectivamente, caracterizado pelos sistemas complexos de rizomas, colmos altamente lignificados e folhas bemdesenvolvidas, com grande ramificação e com lígulas externas (JUDZIEWICZ et al., 1999; KELCHNER; BPG 2013; CLARK; LONDOÑO; RUIZ -SANCHEZ, 2015; VORONTSOVA et al., 2016; SORENG et al., 2017). Já Olyreae é composta exclusivamente por representantes herbáceos, que se distribuem, quase que exclusivamente, na região Neotropical (exceto por *Olyra latifolia* L., considerada como Pantropical por ocorrer na África, e por *Buergersiochloa bambusoides* Pilg., da Nova Guiné, sendo caracterizada pela presença de colmos fracamente lignificados, folhas pouco diferenciadas, ausência de lígulas externas e uma ramificação vegetativa restrita (CLARK, 1990; JUDZIEWICZ et al., 1999; SUNGKAEW et al., 2009; KELCHNER; BPG 2013; CLARK; LONDOÑO; RUIZ-SANCHEZ, 2015; KELLOGG, 2015; VORONTSOVA et al., 2016; SORENG et al., 2017).

Os membros de Olyreae têm como centro de diversidade e endemismo as florestas neotropicais, ocorrendo comumente em regiões de sub-bosque, podendo ser encontrados também em habitats savânicos e em faces úmidas de penhascos (SODERSTROM, 1984; JUDZIEWICZ et al., 1999; JUDZIEWICZ; CLARK, 2007; KELCHNER; BPG 2013; OLIVEIRA et al., 2014; CLARK; LONDOÑO; RUIZ-SANCHEZ, 2015). As espécies dessa tribo se distribuem em faixas altitudinais que vão desde o nível do mar até ca. 1000m, com alguns grupos ocorrendo até 2200m de altitude (JUDZIEWICZ et al., 1999; JUDZIEWICZ; CLARK, 2007; CLARK; LONDOÑO; RUIZ-SANCHEZ, 2015).

Olyreae é reconhecidamente monofilética e atualmente composta por 24 gêneros e 136 espécies que se distribuem em três subtribos: Buergersiochloinae (que compreende 4 gêneros e 5 espécies), Parianinae (com 3 e 42, respectivamente) e Olyrinae, que compreende a maior parte dos gêneros e espécies (17 e 89, respectivamente), sendo então a maior e mais diversa subtribo e se distinguindo das demais pela ausência de fímbrias no ápice das bainhas das folhas (JUDZIEWICZ et al., 1999; CARVALHO, 2013; KELCHNER; BPG 2013; OLIVEIRA et al., 2014; CLARK; LONDOÑO; RUIZ-SANCHEZ, 2015; CLARK; OLIVEIRA, 2018; FERREIRA et al., 2019; I.L.C. OLIVEIRA et al., 2020; R.P. OLIVEIRA et al., 2020; RUIZ-SANCHEZ et al., 2019; CARVALHO et al., 2020; CARVALHO et al., 2021).

Atualmente são reconhecidas quatro linhagens principais em Olyrinae: uma delas inclui os gêneros *Diandrolyra* Stapf, *Parodiolyra* Soderstr., *Raddiella* Swallen e *Taquara* I.L.C.Oliveira & R.P.Oliveira; a segunda linhagem é formada pelos gêneros *Brasiolochloa* R.P.Oliveira & L.G.Clark, *Raddia* Bertol. e *Sucrea* Soderstr., relacionada a uma terceira linhagem composta pelos gêneros *Olyra* L., *Cryptochloa* Swallen, *Lithachne* P. Beauv. e *Arberella* C.E. Calderón & Soderstr.; e, por fim, uma quarta linhagem composta pelo gênero *Piresia* Swallen e pelo gênero monotípico *Reitzia* Swallen (OLIVEIRA et al. 2014; RUIZ-SANCHEZ et al., 2019; I.L.C. OLIVEIRA et al., 2020; R.P. OLIVEIRA et al., 2020; CARVALHO et al., 2020; CARVALHO et al., 2021). Entretanto, a diversidade e as relações dentro dessas linhagens ainda constituem objeto de investigação, tendo em vista a grande complexidade morfológica e evolutiva encontrada, que dificulta a delimitação dos táxons (sejam espécies ou até mesmo gêneros) e a compreensão das suas relações de parentesco, além do fato de alguns gêneros ainda estarem ausentes nas análises realizadas até o presente momento (OLIVEIRA et al., 2014; RUIZ-SANCHEZ et al., 2019; I.L.C. OLIVEIRA et al., 2020; R.P. OLIVEIRA et al., 2020; CARVALHO et al., 2020; CARVALHO et al., 2021).

Piresia se diferencia dos demais gêneros de Olyrinae pela presença de dois tipos de colmos, os folhosos, mais numerosos e que possuem muitas folhas em direção ao ápice, e os decumbentes, que apresentam folhas reduzidas e crescem escondidos sob a serrapilheira (SWALLEN, 1964; SODERSTROM, 1982; JUDZIEWICZ et al., 1999; CARVALHO et al., 2012, 2020). Esse gênero apresenta uma peculiar distribuição geográfica envolvendo uma disjunção, não coespecífica, entre as florestas secas e úmidas dos biomas da AM e da MA do Nordeste brasileiro (SWALLEN, 1964; SODERSTROM; CALDERÓN, 1974; JUDZIEWICZ et al., 1999; CARVALHO et al., 2012; CARVALHO, 2013), que refletem duas de suas linhagens evolutivas (CARVALHO et al., 2021) (vide Figura 3A-B). A história evolutiva e a taxonomia de *Piresia* foi bastante negligenciada durante muito tempo, sendo o grupo inconspicuamente representado nas filogenias de Olyreae (SUNGKAEW et al., 2009; OLIVEIRA et al., 2014; RUIZ-SANCHEZ et al., 2019; I.L.C. OLIVEIRA et al., 2020). Recentemente, no entanto, as relações dessas linhagens começaram a ser entendidas (CARVALHO, 2013; CARVALHO et al., 2021). No trabalho realizado por Carvalho e colaboradores (por exemplo), foram encontradas duas linhagens para *Piresia*, uma delas compostas exclusivamente por representantes da Amazônia e uma segunda composta por representantes da Mata Atlântica + *Reitzia* (Figura 3B). As relações internas entre as linhagens, no entanto, ainda pareçam duvidosas pela baixa resolução interna e presença de politomias, que dificultam não apenas o entendimento das relações, mas também a delimitação de possíveis novas espécies (Figura 3B) (CARVALHO et al., 2021).

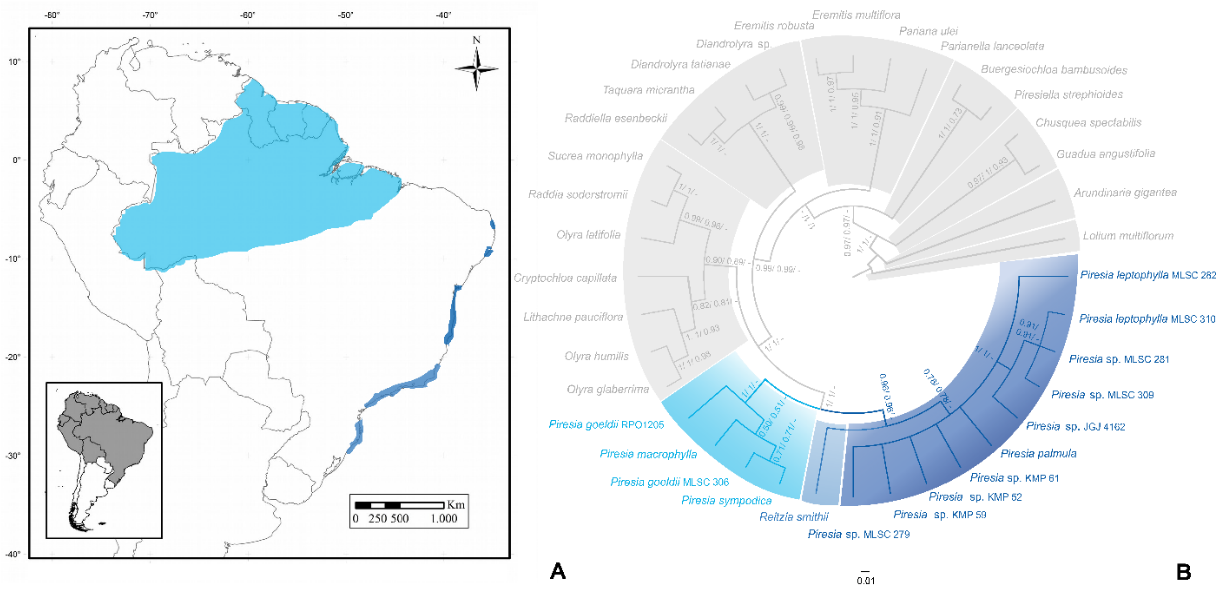


Figura 3. Distribuição geográfica e inferência filogenética para o clado *Piresia* (+ *Reitzia*). A. Mapa de distribuição das espécies de *Piresia* e *Reitzia* na Amazônia e Mata Atlântica do Nordeste do Brasil. B. Inferência Bayesiana para Olyreae com enfoque em *Piresia* utilizando marcadores plastidiais (*trnD-trnT*, *psbA-trnH*, *rpL32-trnL* e *trnS-trnG*) e mutações do tipo inserção e deleção (*indels*). As cores representam as respectivas distribuições geográficas dos clados: azul claro – espécies de *Piresia* da Amazônia; azul escuro -

espécies de *Piresia* da Mata Atlântica; e azul intermediário – *Reitzia smithii* Swallen da Mata Atlântica. Figura retirada e adaptada de Carvalho e colaboradores (2021).

Atualmente são reconhecidas seis espécies para o gênero: *P. goeldii* Swallen, *P. sympodica* (Döll) Swallen, *P. macrophylla* Soderstr., *P. leptophylla* Soderstr., *P. palmula* M.L.S.Carvalho & R.P.Oliveira e *P. tenella* M.L.S.Carvalho & R.P.Oliveira (SWALLEN, 1964; SODERSTROM, 1982; CARVALHO et al., 2012, 2020) (Figura 4). Entretanto, evidências anatômicas, macromorfológicas, genéticas e ecológicas indicam que esse número pode estar subestimado (CARVALHO et al., 2012; CARVALHO, 2013; BEZERRA, 2017; JESUS, 2017; CARVALHO et al., 2020, 2021; CARVALHO et al. in prep.). Essa subestimativa pode estar relacionada a uma história evolutiva bastante intrincada envolvendo processos complexos de especiação, associados a eventos de hibridação, poliploidização e especiação simpátrica, que se refletem tanto na existência de linhagens parafiléticas (como possivelmente ocorre em *P. goeldii* e *P. leptophylla* (CARVALHO et al., 2021)), e espécies crípticas (*P. tenella* (CARVALHO et al., 2020)), quanto em uma inconspícua variação morfológica e baixa diferenciação genética entre os representantes ainda não descritos, o que dificulta a delimitação taxonômica e a compreensão das relações filogenéticas no grupo (CARVALHO et al., 2012, 2020; CARVALHO, 2013; OLIVEIRA et al., 2014; CARVALHO et al., 2021) (Figura 4).

Figura 4. Hábito dos representantes de *Piresia*. A. *P. goeldii*; B. *P. sympodica*; C. *P. leptophylla*; D. *P. palmula*; E. *P. tenella*; F. *Piresia* sp. KMP61; G. *Piresia* sp. MLSC322; H. *Piresia* sp. MLSC279; I. *Piresia* sp. MLSC281. Imagens A, B, D, F, G, H e I retirada e adaptada de Carvalho (2013), E retirada e adaptada de Carvalho e colaboradores (2020) e C cedida por Pinho, C.S.

A obtenção de estimativas do real número de espécies e das suas relações filogenéticas é particularmente problemática dentro do clado que abriga as espécies da Mata Atlântica. De acordo com o trabalho de Carvalho e colaboradores (2021), este clado apresenta relações fracamente resolvidas, além da presença de politomias entre seus representantes, que apresentam, como dito acima, baixa variação morfológica e molecular (CARVALHO et al., 2021) (Figuras 3B; 4). Além disso, foi recuperado o parafiletismo de uma espécie taxonomicamente reconhecida, *P. leptophylla* (CARVALHO et al., 2021) (FIGURA 3B). Essa espécie caracteriza-se por populações extensas com mais de mil indivíduos que formam grandes touceiras; seus indivíduos apresentam muitos colmos delgados e flexíveis, folhas estreitas e inflorescências longas dispostas tanto nos colmos aéreos quanto nos decumbentes (SODERSTROM, 1982; JUDZEWICZ et al., 1999; CARVALHO, 2013; CARVALHO et al., 2020). A mesma apresenta uma ampla distribuição geográfica pelas restingas da Mata Atlântica do nordeste brasileiro, que se estende desde o Rio Grande do Norte até o sul da Bahia, sendo a espécie com maior amplitude de distribuição desse bioma (SODERSTROM, 1982; JUDZIEWICZ et al., 1999; CARVALHO, 2013; CARVALHO et al., 2020). No estudo de Carvalho e colaboradores (2021), no entanto, foram utilizados acessos de apenas duas populações da espécie, o que pode dificultar o estabelecimento das suas relações filogenéticas. Nesse sentido, se faz real a necessidade de estudos focados na problemática desse clado da Mata Atlântica, principalmente no que se refere às relações de *P. leptophylla* entre os demais representantes do bioma.

Da mesma forma, a compreensão da história biogeográfica de *Piresia* é igualmente pouco compreendida e pouco tem sido feito para se entender quais processos podem ter influenciado o padrão de distribuição disjunto observado para o grupo e que se reflete diretamente na história de suas linhagens (CARVALHO et al., 2021). Soderstrom & Calderón (1974), há tempos hipotetizaram que a distribuição geográfica e a história evolutiva do grupo estariam associadas à evolução das inflorescências e de caracteres florais. Segundo esses autores, as espécies da MA apresentariam inflorescências com flores bissexuadas, mas funcionalmente femininas ou masculinas (pela presença de pistilódios ou estaminódios, respectivamente), enquanto as espécies da AM possuiríam inflorescências exclusivamente unissexuadas, tendo essas se originado a partir das primeiras (SODERSTROM; CALDERÓN, 1974). A partir dessa prerrogativa esses autores sugeriram uma provável origem de *Piresia* na MA, com uma posterior dispersão e diversificação na Amazônia (SODERSTROM; CALDERÓN, 1974).

Apesar disso, no trabalho preliminar de Mauri e colaboradores (2013) foi observado que tanto flores unissexuadas ocorriam em espécies da MA, quanto flores bissexuadas estavam presentes em espécies da AM, contrariando a hipótese de Soderstrom & Calderón (1974) sobre a associação entre esses caracteres e o padrão de disjunção geográfica, colocando assim em cheque a hipótese biogeográfica até então proposta para o grupo. A recente filogenia do grupo (CARVALHO et al., 2021) recuperou uma diferenciação genética associada à disjunção geográfica das espécies de *Piresia*, estabelecendo ainda uma possível relação de parentesco desse gênero com *Reitzia*, um ocorrente na MA do sudeste do Brasil (Santa Catarina, São Paulo, Rio de Janeiro e Espírito Santo) (Figura 3). Esses resultados levantam a hipótese sobre a origem e diversificação desse clado (*Piresia* + *Reitzia*) na MA do nordeste do Brasil, provavelmente associadas às dinâmicas florestais e climáticas que vêm se refletindo em respostas adaptativas ao clima (como mudanças na anatomia foliar) e na ocupação de nichos específicos entre os representantes da AM e da MA do nordeste do Brasil (SODERSTROM; CALDERÓN 1974, JUDZIEWICZ et al., 1999, CARVALHO et al., 2012, 2020; CARVALHO, 2013).

Entretanto, até o presente momento, não há nenhuma clareza sobre os processos que contribuíram para esses padrões e, por esse motivo, o presente trabalho se propõe a responder as seguintes perguntas:

1. Quais são os processos que estão atuando na evolução de *Piresia*, especificamente na linhagem ocorrente na MA?
2. Quais são as relações filogenéticas nessa linhagem, particularmente entre *P. leptophylla*?
3. A história biogeográfica da MA influenciou os padrões de distribuição e as relações filogenéticas?

Objetivos

Tendo em vista o exposto acima, essa dissertação foi estruturada em um capítulo único, que tem como objetivo entender as relações filogenéticas da linhagem da MA, os processos evolutivos que influenciaram sua história, assim como compreender se os padões biogeográficos estão relacionados às mudanças climáticas e dinamicidade das florestas. Para tanto, o presente trabalho se propõe a:

(i) Reconstruir árvores de genes e árvores de espécies a partir do uso de marcadores plastidiais e nucleares;

(ii) Inferir as relações filogenéticas em *Piresia*, focando na linhagem da MA, particurlamente em *P. leptophylla*;

(iii) Verificar se há incongruências entre as topologias e seus possíveis significados;

(iv) Estimar os tempos de divergência entre as linhagens de *Piresia*;

(v) Reconstruir as áreas de distribuição ancestral do gênero;

(vi) Tentativamente propor uma hipótese de origem e diversificação para o grupo;

(vii) Fornecer subsídios para os estudos filogenéticos, taxonômicos e biogeográficos futuros envolvendo os bambus herbáceos.

Capítulo Único

Título: Unraveling relationships and inferring the biogeographic history of a group of herbaceous bamboos from the Northeast Atlantic Forest (*Piresia*: Olyreae: Bambusoideae)

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Unraveling relationships and inferring the biogeographic history of a group of herbaceous bamboos from the Northeast Atlantic Forest (*Piresia*: Olyreae: Bambusoideae)

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Short title: Phylogeny and the biogeography of *Piresia* from Atlantic Forest

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ABSTRACT

Phylogenies with incongruent topologies commonly reflect different evolutionary processes that genes and species may undergo, especially groups with low internal resolution, such as *Piresia*, a South American herbaceous bamboo genus. Diversification in *Piresia* is likely recent and associated with hybridization, polyploidization, sympatric speciation and tropical forest dynamics, creating intricate phylogenetic relationships with paraphyletic/cryptic species. This study explored phylogenetic reconstructions, using gene and species trees from different molecular markers to better estimate relationships within the genus in the Atlantic Forest (AF), the most problematic lineage, particularly *P. leptophylla*, a paraphyletic species. We also applied a biogeographic approach to understand the processes influencing the evolutionary history of the genus. We recovered the disjunction between species from Amazonia (AM) and the AF, and low phylogenetic resolution within the latter. However, our analyses indicated that divergence between datasets may be associated with plastid DNA homoplasy. Therefore, we suggest the exclusion of certain markers in future studies. Moreover, incongruence between gene and species trees confirms the paraphyly of a well-defined species, *P. leptophylla*, probably by incomplete lineage sorting or differential selective pressures associated with climatic adaptation, suggesting this species needs recircumscription. The biogeographic analyses indicate that *Piresia* originated during the Pliocene and diversified in the Pleistocene, probably influenced by climatic fluctuations.

ADDITIONAL KEYWORDS: climatic changes – homoplasy – incomplete lineage sorting – Olyrinae – topological incongruence.

INTRODUCTION

Phylogenetic studies in plants frequently make use of plastid molecular markers to reconstruct evolutionary relationships (Chase *et al*., 1993; Olmstead & Palmer, 1994; Shaw *et al*., 2005, 2007; Ruhfel *et al*., 2014). The choice of the plastid genome is due to characteristics such as its easy extraction and amplification, small size (120-160 kpb), higher nucleotide substitution rates (compared to the mitochondrial genome), low recombination rates, mainly uniparental inheritance and evolution as a single unit (Olmstead & Palmer, 1994; Jansen *et al*., 2005; Shaw *et al*., 2005).

Some studies, however, have shown that plastid genes are not as intimately linked as previously hypothesized, and thus, may experience evolutionary pressures individually, which can lead to incongruent phylogenetic reconstructions when analyzed in concatenation. (Wolfe & Randle, 2004; Jansen & Ruhlman, 2012; Ruhlman *et al*., 2017; Gonçalves *et al*., 2019). Also, the use of a single data source (plastid genome) might result in incomplete reconstruction of phylogenetic relationships and the evolutionary history of the species, given the different processes that may exert influence on the genome (Soltis & Kuzoff, 1995).

Incongruences between the different plant genomes has been observed in several studies (e.g., Soltis & Kuzoff, 1995; Mason-Gamer & Kellogg, 1996; Kim & Donoghue, 2008; Pelser *et al*., 2010; Xu *et al*., 2012, Zhang, Zeng & Li, 2012), with both inter and intragenomic incongruities being particularly problematic in phylogenetic studies involving the construction of a combined matrix using multilocus data, and concatenated analyses (De Queiroz & Gatesy, 2006; Edwards, Liu & Pearl, 2007). This is due to the assumption that all loci in a given analysis are under the same evolutionary pressure, which frequently is not the case (De Queiroz & Gatesy, 2006; Edwards, Edwards *et al*., 2007). The divergences between gene and species trees caused by such incongruence, however, might reflect a reticulate evolutionary history with different intrinsic biological processes such as horizontal gene transfer, hybridization, introgression, natural selection or incomplete lineage sorting (ILS) (Maddison, 1997; Degnan & Rosenberg, 2006, 2009; Castoe *et al*., 2009).

Differing from horizontal gene transfer, the occurrence of hybridization and introgression is commonly reported in plants, directly influencing phylogenetic relationships (Mallet *et al*., 2007; Sun *et al*., 2015; Mallet, Besansky & Hahn, 2016; Xia *et al*., 2021). This influence emerges from the mixing of gene pools, which attenuates the interspecific borders, especially in closely related species, where hybridization and introgression rates are higher than in genetically distant groups (Mallet, 2007; Sun *et al*., 2015; Mallet *et al*., 2016; Panahi *et al.*, 2018). Alternatively, under natural selection, different evolutionary pressures on the genes can cause the erroneous grouping certain of lineages, and thus gene and species trees might not coincide (Zhang & Kumar, 1997; Slatkin & Pollack, 2008; Castoe *et al*., 2009). ILS, however, emerges from the retention of ancestral polymorphisms in certain branches of the species trees, possibly leading to the coalescence of lineages not intimately related, hindering the recovery of lineage relationships (Degnan & Rosenberg, 2009).

Despite topological incongruences between gene and species trees being attributed to different processes, they are also common in groups with low phylogenetic resolution (Degnan & Rosenberg, 2006, 2009; Townsend, 2007), such as the case of *Piresia* Swallen. This genus of herbaceous bamboos is currently formed by six species [*P. goeldii* Swallen, *P. sympodica* (Döll) Swallen, *P. macrophylla* Soderstr., *P. leptophylla* Soderstr., *P. palmula* M.L.S.Carvalho & R.P.Oliveira and *P. tenella* M.L.S.Carvalho & R.P.Oliveira], and it is characterized by the presence of two types of culms, the leafy ones, which are more abundant and bared well-developed and erect leaves, and the decumbent ones, which have reduced leaves, growing parallel to the ground, hidden under the litter (Swallen, 1964; Soderstrom, 1982; Judziewicz *et al*., 1999; Carvalho *et al*., 2012, 2020).

This genus has a disjunct and non-conspecific geographical distribution amongst dry and wet forest formations of the Amazonian (AM) and Atlantic Forest (AF) biomes, coinciding with the phylogenetic structure of the lineages (Soderstrom & Calderón, 1974; Judziewicz *et al.*, 1999, Carvalho *et al.*, 2012, 2020, 2021; Carvalho, 2013). It is possible, however, that the number of *Piresia* species is underestimated due to the low genetic and morphological differentiation between taxa not yet described for the genus, which exhibit a different morphology from the recognized species (Carvalho *et al.*, 2012, 2020, 2021; Carvalho, 2013). This is particularly observed within the AF, hindering taxonomic delimitation and phylogenetic relationship estimations in this lineage (Carvalho *et al.*, 2012, 2020, 2021; Carvalho, 2013).

Recent studies, and evidences such as the high genetic similarity and morphological superposition of certain species, supported hypotheses that the evolution of *Piresia* is recent and could involve complex evolutionary processes such as hybridization, polyploidization, disruptive selection and sympatric speciation, with the presence of cryptic (such as *P. tenella*) and paraphyletic (such as *P. goeldii* and *P. leptophylla*) species (Carvalho *et al.*, 2012, 2020, 2021; Carvalho, 2013; Bezerra, 2017; Jesus, 2017). *P. leptophylla*, specifically, is a species with wide geographic distribution and taxonomically recognized, if it differs from its other congeners by the presence of chartaceous–coriaceous leaves in the vegetative -culms (Soderstrom, 1982; Carvalho *et al*., 2012, 2020). It was recently dismembered from its cryptic pair, *P. tenella*, due to differences in plant size and leaf size and micromorphological characteristics, such as dimension, shape of the lodicules and fusoid cells (Carvalho *et al*., 2020) and was recovered as a paraphyletic species (Carvalho *et al*., 2021). The relationships between the populations of this species and the other representatives of the AF clade, however, have not been defined (Carvalho *et al*., 2021).

Furthermore, this only phylogenetic study focusing on the genus (Carvalho *et al.*, 2021) demonstrated that phylogenetic relationships within *Piresia* exhibit low internal resolution as well as polytomies (Carvalho *et al.*, 2021). Such issues are likely related to the non-phylogeographic approach of the study, without representation of populations from the problematic taxa, or even the use of molecular markers with lower evolutionary rates (such as those from the plastid genome) and/or with possible paralogous copies (such as those ITS from the nuclear genome), as well as the possible history of reticulate evolution within the group (Álvarez & Wendel, 2003; R.P. Oliveira *et al.*, 2014; I.L.C. Oliveira *et al.*, 2020; Carvalho *et al.*, 2021). Such factors hinder the reconstruction of phylogenetic relationships and the comprehension of the evolutionary processes affecting the group (Álvarez & Wendel, 2003; R.P. Oliveira *et al.*, 2014; Xi, Liu & Davis., 2015; I.L.C. Oliveira *et al.*, 2020; Carvalho *et al.*, 2021).

Regarding the evolutionary history of *Piresia*, it is likely associated to the dynamics of tropical forests and the adaptive responses to environmental changes (Carvalho *et al.*, 2012, 2020, 2021; Carvalho, 2013; Bezerra, 2017; Jesus, 2017), given the apparently high fidelity of herbaceous bamboos to their habitat (Soderstrom, 1984; Judziewicz *et al.*, 1999; Judziewicz & Clark, 2007; Oliveira *et al.*, 2014; Clark, Londoño & Ruiz-Sanchez, 2015). In that sense, several studies show that the AM and AF biomes underwent several changes throughout time due to environmental and geological changes that altered their configuration, especially during the Pliocene and Pleistocene, directly influencing the life history of its biota (Haffer, 1974; Whitmore & Prance, 1987; Pennington *et al.*, 2004; Hoorn *et al.*, 2010; Thomé *et al.*, 2010; Antonelli & Sanmartín, 2011; Batalha-Filho, Cabanne & Miyaki, 2012).

It is possible, for example, that during the Paleogene these biomes formed continuous and interconnected tropical forests (Morley, 2000). However, it has been proposed that with the elevation of the Andes and the drastic changes in planetary climate during the Neogene, a drier area (known as the Dry Diagonal) formed and was responsible for the disjunction of AF and AM. That the Dry Diagonal currently comprises the Caatinga, Cerrado and Chaco (Bigarella, Andrade-Lima & Riehs, 1975; Costa, 2003; Hoorn *et al*., 2010). Nonetheless, palynological, geological, phylogeographic and ecological distribution modelling studies suggest that the separation was less intense in certain periods after the formation of the Dry Diagonal (Thomé *et al*., 2016; Ledo & Colli *et al.*, 2017; Prates *et al*., 2017; Machado *et al.*, 2018).

Considering the AF, two main hypotheses exist to explain the influence of climate and geological changes on the evolutionary history of its species, one of them is based in the Refuge theory (Haffer, 1969; Vanzolini & Williams, 1970; Brown & Ab'Sáber, 1979). Using paleodistribution models, it was suggested that during colder periods of the Last Glacial Maximum (LGM) (21000 years ago), forest-dependent species had their geographic distribution reduced to the refugial areas in the regions of Bahia and Pernambuco, followed by expansion during the interglacial periods (Carnaval & Moritz, 2008). On the other hand, paleoclimatic models and genetic data suggest the Atlantis Forest hypothesis, in which these forests persisted and expanded their distribution during the LGM due to the exposed continental shelf, instead of retractions in the distribution area of forest formations (Leite *et al*., 2016). Some studies show, for example, that geomorphological changes in fluvial systems and the remodeling of the coastal region were responsible for phylogeographical breaks in different terrestrial species (Pellegrino *et al*., 2005; Amaral *et al*., 2013; Fazolato, Fernandes & Batalha-Filho, 2017; Paz *et al*., 2019).

Therefore, starting from expanded sampling, with the use of plastid molecular markers with higher evolutionary rates and a single-copy nuclear marker, we reconstruct gene and species trees and perform biogeographical analyses on *Piresia* with the following objectives:

(a) infer phylogenetic relationships in *Piresia* with a focus on the AF lineage; **(b)** evaluate possible incongruence between topologies and elucidate the cause(s); **(c)** test biogeographic models to assess the age of the genus and elucidate whether its evolution is related to climate and geological changes of a certain period; and **(d)** provide a starting point for future studies on the *Piresia* and on herbaceous bamboos in general.

MATERIAL AND METHODS

SAMPLING

Given the focus on the AF, especially on *P. leptophylla*, 15 specimens from seven populations of this species were selected, including its type population in Una - Bahia to compose the ingroup. Specimens of seven possible new taxa for the genus were also selected as the ingroup, with one sample from each being selected except for *Piresia* sp. MLSC 309, which was represented by two individuals from its population, given the positioning of an initial sample in a trichotomy by Carvalho *et al.* (2021) (Table 1). Two species from AM were also included in the ingroup, to test the monophyly of the AF lineage (Carvalho, 2013; Carvalho *et al*., 2021) (Table 1). The samples used were obtained in the study of Carvalho (2013).

As the outgroup, we used representatives of five genera of Olyreae from the three subtribes recognized: Buergersiochloinae (*Buergersiochloa bambusoides* Pilg.); Parianinae (*Pariana* sp.); and Olyrinae [*Cryptochloa strictiflora* (E.Fourn.) Swallen, *Diandrolyra* sp. and *Olyra latifolia* L.] (R.P. Oliveira *et al*., 2014; Ferreira *et al*., 2019; I.L.C. Oliveira *et al*., 2020; R.P. Oliveira *et al*., 2020; Carvalho *et al*., 2021), as well as two genera of woody bamboo from the tribe Bambuseae, sister group of Olyreae [*Guadua weberbaueri* Pilg and *Dendrocalamus farinosus* (Keng & Keng f.) L.C.Chia & H.L.Fung., the latter selected for presenting sequences available for the nuclear gene *LEAFY* and for enabling fossil dating (see topics below)] (Kelchner & BPG, 2013; Soreng *et al.*, 2017) (Table 1).

The vouchers of the *Piresia* samples are deposited in the Alexandre Leal Costa herbarium at the Universidade Federal da Bahia (ALCB) and at the herbarium of the Universidade Estadual de Feira de Santana (HUEFS) (acronyms following Thiers, 2021 continuously updated). All sequences for the outgroups were obtained from GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

DNA EXTRACTION, AMPLIFICATION, SEQUENCING AND ALIGNMENT

DNA extraction was performed using leaves that were dehydrated in silica gel, following the CTAB 2% (Cetyl-Trimethyl-Ammonium Bromide) protocol (Doyle & Doyle, 1987) adapted for microtubes. The genetic material obtained was analyzed qualitatively and quantitatively by 1% agarose gel electrophoresis and L-Quant spectrophotometer, respectively. DNA amplification was performed by Chain-Reaction Polymerase (PCR) for five plastid DNA regions (*ndhF* gene, *rpL16* intron, and *rpL32-trnL*, *trnD-trnT* and *trnL-trnF* spacers) and one nuclear DNA region (gene *LEAFY*) (Consistent with Clark, Zhang & Wendel, 1995; Zhang, 2000; Kelchner & BPG, 2013; Yang *et al.*, 2013; R. Oliveira *et al.*, 2014; Ferreira *et al.*, 2019; I.L.C. Oliveira *et al.*, 2020; R.P. Oliveira *et al.*, 2020; Carvalho *et al.*, 2021). Other regions previously used in phylogenetic studies with the group were not selected due to the low resolution, consequence of a low percentage of polymorphic sites (*trnS-trnG* and *trnH-psbA*) or high rates of homoplasy (*ITS*). We employed Top Taq Master Mix (Qiagen) kits, following the manufacturer’s recommendations, with addition of 0.2 μM of each primer and approximately 30ng of DNA, with a final volume of 15.4 μL. For the *LEAFY* gene, we used 0.2ng of BSA (bovine serum albumin), DMSO (dimethyl sulfoxide) at 10% and betaine solution at 5M. Primer sequences and amplification conditions for each region are described in Table 2.

The amplified fragments were purified in 11% polyethylene glycol (PEG) (Sambrook, Fritsch & Maniatis, 1989) and sequenced unidirectionally using BigDye Terminator kits on an automatic sequencer ABI 3500 XL (Applied Biosystems), available at the Instituto Gonçalo Moniz, Fundação Oswaldo Cruz (IGM-FIOCRUZ).

The electropherograms obtained were edited using Geneious 6.1.8 (Biometters Ltda.) and aligned using the MUSCLE algorithm (Edgar, 2004) with the software Mega 7 (Kumar, Stecher & Tamura, 2016). Insertion/deletion mutations were not considered due to their small influence on the resulting topologies and support values obtained in previous studies for the group (Duvall, Burke & Clark, 2019; Carvalho *et al*., 2021).

PHYLOGENETIC ANALYSES AND TOPOLOGY TEST

The evolutionary models for each region selected were determined using MrModelTest v.2.3 (Nylander, 2004) by means of the Akaike Information Criteria (AIC, Akaike, 1974). The selected models were HKY+I for *trnD-tnrT* and *ndhF*, GTR for *rpL16* and *LEAFY*, GTR+Γ for *trnL-trnF* and HKY+Γ for *rpL32-trnL* (Table 2). To estimate the contribution of the informative data of each region used, we evaluated the total length in base pair (bp) and the number of substitutions for all taxa. Finally, to evaluate the existence of incongruence between partitions, we used the comparison of boostrap and posterior probability support values according to Cardoso et al. (2013). The incongruence length difference test (ILD test; Farris *et al*., 1994) was not used due to the criticisms made (eg, Cunningham, 1997; Barker & Lutzoni, 2002; Darlu & Lecointre, 2002).

Each data partition was analyzed individually using Maximum Likelihood (ML) and Bayesian Inference (BI). A combined dataset of the plastid regions, except the *rpL32-trnL* spacer (see results), and nuclear region was also analyzed using ML and BI. The ML analyses were performed in RAxML v8.2.8 (Stamatakis, 2014) implemented through CIPRES Science Gateway v. 3.3 (Miller, Pfeiffer & Schwartz, 2010) utilizing the GTR+Γ model for the total dataset with 1000 bootstrap repetitions (BS, Stamatakis, 2014). Branches with BS values ≥70% were considered supported (70–79% low, 80–89% moderate and 90–100%, high support). BI analyses were performed in MrBayes 3.2.7 (Ronquist *et al*., 2012), also available in CIPRES. Two independent races with 10 million generations and four Markov Monte Carlo Chains (MCMC) each were performed, with trees being saved every 1000 generations. Convergence between runs was analyzed using Tracer v.1.5 (Rambaut & Drummond, 2009), applying a 10% burn-in on the initial trees. Posterior probability (PP) values for each consensus tree were calculated, and groups with PP ≥ 0.95 were considered supported (Erixon *et al*., 2003). The topologies obtained in ML and BI were visualized in Figtree v.1.4.2 (Rambaut, 2014).

To evaluate the paraphyletic status of *P. leptophylla*, as recovered in the phylogenetic studies of Carvalho *et al*. (2021), a restricted alternative topology was generated forcing species monophyly (alternative hypothesis). The tree was posteriorly compared with the unrestricted tree obtained by ML for the combined dataset (null hypothesis), through the non-parametric Shimodaira-Hasegawa (SH) test (Shimodaira & Hasegawa, 1999). The test was performed in PAUP v.4.0b10 (Swofford, 2002) using the RELL optimization and likelihood criterion under the GTR+Γ model and 1000 bootstrap repetitions (Shimodaira & Hasegawa, 1999). Topologies with P≤0.05 were considered statistically significant.

SPECIES TREE RECONSTRUCTION

Considering the differences in topology obtained between the gene trees, species trees were estimated using the software ASTRAL-II v4.10.2 (Mirarab & Warnow, 2015) and

SVDquartets (Chifman & Kubatko, 2014), all analyses based in coalescence. ASTRAL-II infers the species tree based in quartet analysis obtained from individual unrooted gene trees (Mirarab & Warnow, 2015). Thus, the species tree was estimated based on individual gene trees inferred through RAxML v8.2.8 (Stamatakis, 2014) with 1000 bootstrap repetitions and support for the topology nodes evaluated through the Posterior Local Probabilities (LPP) of the method (Mirarab & Warnow, 2015).

SVDquartets was implemented through PAUP v.4.0b10 (Swofoord, 2002) and, using the quartets from the partitioned DNA sequence matrixes for the individuals (Chifman & Kubatko, 2014). For this, all quartets were sampled randomly, followed by a non-parametric bootstrap with 1000 repetitions to evaluate the node support for the estimated species tree.

DIVERGENCE TIME ESTIMATION

Divergence times were estimated using a Bayesian approach implemented in BEAST v.2.6.3. (Bouckaert *et al*., 2014) utilizing the Yule model of speciation and a relaxed molecular clock with lognormal distribution. We used a fossil calibration point for the tree root with a lognormal distribution (mean 3.55, SD 0.082) attributed to the age of a 35 Myr fossil (Strömberg, 2005) and two secondary calibration points with normal distribution for Bambuseae and Olyreae (average age 6.42 Mya and SD 3 Mya for Bambuseae; average age 36.25 Mya and SD 9.5 Mya for Olyreae) (Burke *et al*., 2014). For molecular calibration, we utilized a substitution rate of 1.1-1.6 x 10^{-4}/million years for plastid DNA and 5.8-8.1 x 10^{-4}/million years for nuclear DNA (Wolfe, Li & Sharp, 1987).

Two independent runs were performed with 100 million generations each, with a randomized initial tree, and parameter sampling each 5000 generation and burn-in at 10%. Convergence between runs was analyzed on Tracer v.1.6 (Rambaut & Drummond, 2009), where ESS ≥200 was considered adequate. Convergent trees were combined using

LogCombiner and the Maximum Clade Credibility tree (MCC) with median ages selected using TreeAnnotator. Final topology observed using Figtree v.1.4.2 (Rambaut, 2014).

ANCESTRAL AREA RECONSTRUCTIONS

Ancestral node distribution estimation was performed using BioGeoBEARS (Matzke, 2013) in R (R Core Team, 2018), utilizing the MCC tree obtained by the Yule speciation method in BEAST. We tested three general models: (1) DEC, a probability-based dispersion, extinction and cladogenesis model (Ree & Smith, 2008); (2) DIVALIKE, using dispersion and vicariance parameters based in parsimony (Ronquist, 1997); and (3) BAYAREALIKE, which adds parameters to evaluate sympatry by means of a Bayesian model (Landis *et al.*, 2013). DIVALIKE and BAYAREALIKE were implemented through BioGeoBEARS in a maximum likelihood structure, allowing comparisons between models (Matzke, 2013). All models allow for testing of dispersal events to a new area and extinction in another. Specifically, DEC was chosen to test whether speciation in this group occurred in sympatry in one of the areas, meaning that the ancestor could have been widely distributed in both AM and AF and one of the lineages speciated in sympatry (Matzke, 2013). DIVA was implemented to test whether the ancestor was widely distributed and speciation occurred by a vicariant event that separated the forest formations (Matzke, 2013). BAYAREA allowed a test of speciation by wide sympatry in the areas (Matze, 2013). Also, BioGeoBEARS allows the addition of a “j” parameter to all models, which adds the possibility of founding effect and “jump” speciation, meaning that it is possible to verify whether there were changes in the distribuition range per speciation event, with one of the derived lineages occupying the ancestral range, and the other occupying a new range (Matzke, 2014).

Five areas comprising AM and the AF were considered for the analysis: **(1)** Roraima, representing a portion of the states of Roraima and Amazonas; **(2)** Pará, representing part of the state territory (Morrone, 2014); **(3)** Ecoregion 1, a region comprised of the states of Rio Grande do Norte, Paraíba and Pernambuco; **(4)** Ecoregion 3, comprising the coastal region of Sergipe down to the city of Salvador, Bahia; and **(5)** Ecoregion 5, comprising the stretch of coast from southern Bahia to northern Rio de Janeiro (Cantidio & Souza, 2019). The terminals used were codified as present or absent for each area, and the models were compared using likelihood ratio tests and AIC values.

RESULTS

DATASET

The final alignment, excluding low-quality portions at the beginning and end of the sequences, constituted a group of six plastid regions with 3037 bp, with 626 for *ndhF*, 564 for *rpL16*, 564 for *rpL32-trnL*, 532 for *trnD-trnT* and 751 for *tnL-trnF* (Table 2). A 746 bp set was obtained for the nuclear region (*LEAFY*) (Table 2). The region with the highest percentage of Potentially Informative Characters (% PIC) was *trnL-trnF* with 20.11% PIC, followed by *rpL32-trnL* (19.07%), *rpL16* (18.12%), *ndhF* (11.7%), *trnD-trnT* (10.23%) and *LEAFY* (8.63%) (Table 2). The incongruence test revealed that the region *rpL32-trnL* was not congruent with the other sequences, and was then analyzed separately, as well as excluded from the combined ML and BI phylogenetic inferences, in which the remaining plastid (*ndhF*, *rpL16*, *trnD-trnT* and *trnL-trnF*) and nuclear DNA (*LEAFY*) were included.

PHYLOGENETIC ANALYSES AND TOPOLOGY TEST

Individual Analyses: the relationships obtained in the BI and ML analyses for the *rpL32* spacer were identical, with differences only in support values. Hence, the relationship will be described only once with posterior probability and bootstrap values for each branch in parenthesis, respectively. Tribe Olyreae was recovered as monophyletic (PP 1/BS 100), as well as subtribe Olyrinae (0.96/88) (Fig. 1A). In Olyrinae, *Olyra latifolia* was recovered as sister to clade comprising *Cryptochloa strictiflora* and *Piresia* (1/99) (Fig. 1A). *Piresia* was not be recovered as monophyletic due to the presence of *C. strictiflora* in a clade with no support in ML and high support in BI (0.98/68) (Fig. 1A). *Piresia* species from AM (*P. goeldii* and *P. sympodica*) emerged as sister to the remaining representatives of the clade (1/100), recovered in a polytomy with no support (0.70/55) (Table 3; Fig. 1A). This includes specimens of *P. leptophylla* from Salvador, Canavieiras and Una (BA) + *Piresia* sp. MLSC 308 + *Piresia* sp. MLSC 279 + *Piresia* sp. KMP 61 + *Piresia* sp. KMP 59 + *Piresia* sp. KMP 52 (0.99/63), a trichotomy formed by representatives of *P. leptophylla* from Canavieiras and Una (BA) (0.92/88) and a small polytomy with no support in ML and high support in BI (0.99/63) with individuals of *P. leptophylla* from Ipojuca (PE) and Baía Formosa (RN) + *Piresia* sp. MLSC 309 + *Piresia* sp. MLSC 281) and *C. strictiflora* (Table 3; Fig. 1A). The latter group contains a complementary inverted repeat that is six bases long (ATAAAA) in the *rpL32-trnL* spacer, when compared to the sequences of the remaining analyzed representatives (TTTTAT) (except *B. bambusoides*, which also has the inversion).

Among the remaining markers used, the topology for the *ndhF* gene recovered the same relationships between the outgroup taxa obtained in the combined analysis, except between *O. latifolia* and *C. strictiflora* which appear in a polytomy (0.99/74) formed by *Piresia* representatives (Fig. S1). In this polytomy, representatives of *P. leptophylla* (Una-BA) emerge in a polytomy with *P. goeldii* and *P. sympodica* (0.99/91) (Table 3; Fig. S1). The remaining representatives from AF, on the other hand, form a clade with moderate to high support (0.99/80), with two small subgroups (Fig. S1): one formed with the remaining populations of *P. leptophylla* (0.99/85) and other by representatives of possible new taxa in *Piresia* (1/93) (Table 3; Fig. S1).

The *rpL16* intron also resolve the same relationships between outgroups recovered in the combined analyses (Fig. S2). Regarding *Piresia*, all representatives appear in a polytomy with moderate support in ML and no support in BI (0.57/87), where it is possible to recover three small subgroups (Fig. S2): one formed by *P. goeldii* and *P. sympodica* (0.95/87), another by two subgroups of *P. leptophylla* (Canavieiras-BA) (1/98) and a third, with three individuals of *P. leptophylla* (Canavieiras-BA) (0.97/90) (Table 3; Fig. S2).

In the topology based the *trnD-trnT* spacer, the outgroup relationships differ, with *B. bambusoides* and *Pariana* sp. grouped (0.93/85), and *O. latifolia* and *C. strictiflora* forming a polytomy without support (0.78/65) (Fig. S3). *Piresia* remains monophyletic (1/100), but with *P. goeldii* and *P. sympodica* in a polytomy emerging as a sister group to the AF clade, forming a polytomy with all its representatives (0.95/77) (Table 3; Fig. S3).

The topology based the *trnL-trnF* spacer differs in the relationships between representatives of *P. leptophylla* (Ipojuca-PE), which emerge as sister to a clade formed by a polytomy between the remaining representatives of *Piresia* from AF (0.59/38), a small group formed by *O. latifolia* and *C. strictiflora* (0.99/91) and another formed by *P. goeldii* and *P. sympodica* (0.99/95) (Table 3; Fig. S4).

The relationships within *Piresia* estimated from the *LEAFY* gene presented a polytomy formed by individuals of *P. leptophylla* from Salvador-BA and a small group of individuals from Canavieiras-BA (1/95) (Fig. S5). Two other groups are presented: one with *P. goeldii* and *P. sympodica* (1/100), which emerges as sister to a non-supported polytomy in ML but with moderate support in BI containing the non-described taxa in *Piresia* (0.93/67) and the second group without support formed by the remaining *P. leptophylla* (0.59/67) (Table 3; Fig. S5). In the latter, two subgroups are formed, one composed by individuals from Una-BA (0.99/80) and the other by individuals from Canavieiras-BA (0.91/74) (Table 3; Fig. S5).

Combined analyses: the ML and BI topologies obtained through combined data analysis were identical, differing solely in the support values for some groups. We applied the same method of a single description with bootstrap and posterior probability values in parentheses for each branch. Tribe Olyreae, as an example, was recovered as monophyletic (1/100), as well as Olyrinae (0.99/93) and *Piresia* (1/100) (Fig. 1B). In this group, two clades were recovered (LI and LII), with moderate to high support, containing, respectively, species from AM (1/100) and AF (0.99/80) (Table 3; Fig. 1B).

Two main clades were found in the AF group with moderate to high support. These, however, present moderate to low support for their internal branches (Fig. 1B). The first clade (AFI) recovers the paraphyly of *P. leptophylla* with the population from Una (Bahia) emerging as sister to the clade composed of the remaining species of the biome (0.98/99). The second group subdivides into two clades with no support in ML and moderate support in BI (0.96/57) (Table 3; Fig. 1B), with the first (AFII) lacking support and comprising the remaining populations of *P. leptophylla* (60/0.89) and the second (AFIII), with potential new taxa for the genus (1/99) (Table 3; Fig. 1B). However, internal relationships within the groups were poorly recovered with low to moderate support.

In AFII, containing the remaining populations of *P. leptophylla*, three subgroups are recovered: one with no support in the analyses, containing individuals from Salvador and Canavieiras, emerging to the rest (0.67/40); the second containing only individuals from Canavieiras with no support in ML but high support in BI (0.99/48); and the third is a polytomy with no support in ML and moderate support in BI, comprising individuals from Ipojuca (Pernambuco) and Baía Formosa (Rio Grande do Norte) (0.97/64) (Fig. 1B). In AFIII, which contains the remaining AF taxa, *Piresia* sp. KMP 52 emerges as sister the other taxa, which form a polytomy (75/0.94) composed of *Piresia* sp. MLSC 308 + *Piresia* sp. MLSC 279 + *Piresia* sp. KMP 61 + *Piresia* sp. KMP 59 and a small group composed of *Piresia* sp. MLSC 281 and *Piresia* sp. MLSC 309 (85/0.99) (Fig. 1B).

Alternative Topology Test: results obtained from the SH test performed on the topology from the combined plastid (*ndhF, rpL16, trnD-trnT* and *trnL-trnF*) and nuclear (*LEAFY*) data consider the topology with no restrictions (*P. leptophylla* is paraphyletic) as the best tree, but do not refute the hypothesis of *P. leptophylla* as monophyletic (P = 0.394).

SPECIES TREE RECONSTRUCTION

Due to the non-rejection of the monophyly of *P. leptophylla*¸ species tree reconstruction was performed through ASTRAL-II and SVDquartets for the same dataset used in the phylogenetic analyses. Regarding the relationships of *P. leptophylla*, the topologies obtained by ASTRAL-II and SVDquartets were not congruent with the topologies obtained by ML and BI for the combined dataset (Figs 1B, 2).

In both analyses, *Piresia* was recovered as monophyletic (LPP 0.98/BS 98.9) and the AM species (*P. goeldii* and *P. sympodica*) form a clade with high support (0.99/93.9), recovered as sister to the AF species (Fig. 2A-B). The latter formed a clade with no support in ASTRAL-II and with moderate support in SVDquartets (0.49/92.1) (Fig. 2A-B).

In the topology generated by ASTRAL-II, representatives from AF were recovered as two groups with no support, the first composed of *Piresia* sp. MLSC 279 + *Piresia* sp. MLSC 281 + *Piresia* sp. MLSC 308 + *Piresia* sp. MLSC 309 + *Piresia* sp. MLSC KMP 52 + *Piresia* sp. MLSC KMP 59 + *Piresia* sp. MLSC KMP 61 (0.57) and the other by *P. leptophylla* which emerges as monophyletic, but without support (0.58) (Fig. 2A). *Piresia* MLSC 281 emerges as sister the other representatives which, in turn, form two subgroups. One is composed of *Piresia* sp. MLSC 308 + *Piresia* sp. MLSC KMP 59 + *Piresia* sp. MLSC 279 (0.57) and the second, is a polytomy between individuals of *Piresia* sp. MLSC 309 and a group with *Piresia* sp. MLSC KMP 61 + *Piresia* sp. MLSC KMP 52 (0.49) (Fig. 2A).

Regarding *P. leptophylla*, was recovered a group without support composed of individuals from Ipojuca (PE) and Baía Formosa (RN) (0.69) and a polytomy composed by the other individuals of the species, including individuals from Canavieiras and Salvador (BA), as well as a small group forming a polytomy composed of individuals from Canavieiras (BA) and Una (BA), which emerge as a group with low support (0.85) (Fig. 2A).

The topology recovered by SVDquartets lacked support in all relationships within groups. *Piresia* sp. KMP 52 was as sister to the other specimens from AF, which formed three groups, one comprising *Piresia* sp. MLSC 281 + *Piresia* sp. MLSC 309, another *Piresia* sp. KMP 61 + *Piresia* sp. MLSC 279 + *Piresia* sp. KMP 59 + *Piresia* sp. MLSC 308 and a third composed of all *P. leptophylla* in a polytomy containing the subgroup with all the individuals from Una (Fig. 2B).

DIVERGENCE TIME ESTIMATION

Divergence time estimation for *Piresia* gave relatively large 95% HPD (*Highest Posterior Density*) intervals, suggesting the possible origin for the group in the Pliocene (about 3.91 Mya), with internal divergences occurring mainly during Pleistocene (Fig. 3). For example, divergence between the AM and AF lineages (LI and LII) was estimated at around 2.52 Mya (Fig. 3), while internally, in AF, the separation of AFI [*P. leptophylla* from Una (BA)] dates from around 1.87 Mya, with AFII and AFIII diverging around 1.50 Mya (Fig. 3). Diversification in clades AFII and AFIII likely began in 1.09 and 0.88 Mya, respectively (Fig. 3).

For AFII, three main subgroups are recovered, with the group composed of individuals from Canavieiras and Salvador (BA) diverging from the other representatives around 0.58 Mya. Divergence between the second and third groups, respectively composed of individuals from Bahia and Northern AF (PE and RN), likely occurred around 0.84 Mya (Fig. 3). The second group, formed by populations from Canavieiras (BA), presented diversification around 0.51 Mya, and the third group, containing individuals from Ipojuca (PE) and Baía Formosa (RN), with diversification estimated around 0.37 and 0.20 Mya (Fig. 3).

For AFIII, we estimated first the diversification of *Piresia* sp. KMP 52 and the formation of two subgroups around 0.63 Mya (Fig. 3), the first diversifying around 0.44 Mya and composed exclusively of individuals from Bahia. The second group diversified around 0.15 Mya and is composed of individuals from Bahia and Pernambuco (Fig. 3).

ANCESTRAL AREA RECONSTRUCTIONS

Biogeographic model testing revealed that DIVALIKE+J gave the best adjustment to explain the evolutionary processes occurring within *Piresia* (Table 4). The model recovered an uncertain origin for the group, with equal probabilities in three regions (Roraima, Pará or Ecoregion 3) (Fig. 3, I), followed by a vicariant event between Roraima/Pará (AM) and Ecoregion 3 (AF) (Fig. 3). Regarding the AF group, the analysis indicates a possible origin in Ecoregion 3, followed by three dispersion events. Two of them correspond to a founder effect of Ecoregion 3 on Ecoregion 1, first around 0.9 Mya, a second around 0.2 Mya (Fig. 3, II and IV), while the third happened from Ecoregion 3 to Ecoregion 2 around 0.65 Mya (Fig. 3, III).

DISCUSSION

NOVELTIES IN THE PHYLOGENETIC RELATIONSHIPS OF *PIRESIA*

Based on the results obtained in this study, it was possible to elucidate part of the internal relationships in *Piresia* from the combined gene tree with better statistical support when compared to what was recently described for the group (Carvalho *et al*., 2021) (Table 3; Fig. 1B). The species trees will not be used to discuss the phylogenetic relationships in *Piresia* due to the low support found (see comments below) (Fig. 2).

Our results corroborate with the disjunction between the *Piresia* occurring in AM and AF, observed by Carvalho *et al*. (2021), which used a set of plastid DNA data. However, in addition better internal resolution, we also obtained results that contrast with Carvalho *et al*. (2021) for the internal relationships in the AF clade. While Carvalho *et al*. (2021) recovered a polytomy involving the AF representatives, with *P. leptophylla* as polyphyletic (with a representative from Salvador-BA and Ipojuca-PE), we recovered the paraphyletic status of *P. leptophylla* in a different configuration. Here, the populations from Una (species type population) emerged as sister (AFI) to all other AF representatives (AFII e AFIII), which includes the remaining populations of *P. leptophylla* and potentially new taxa, respectively (as indicated by Carvalho *et al.*, 2021) (Fig. 1B).

While AFI and AFII correspond to the *P. leptophylla* populations that inhabit the drier environments of the arboreal Restinga (Soderstrom, 1982; Judziewicz *et al.*, 1999; Carvalho, 2013; Carvalho *et al.*, 2020, 2021), with representatives that have a leaf morphology with narrower and elongated leaves, AFIII corresponds to the populations that inhabit more humid dense ombrophilous forests, presenting morphotypes with oval and oblong leaves (Judziewicz et al. 1999; Carvalho 2013; Carvalho *et al.* 2021). The latter clade (AFIII), was recovered with moderate support, with the formation of a small subclade, with high support, formed by *Piresia* sp. MLSC 281 and *Piresia* sp. MLSC 309 (Table 3; Fig. 1B), which in turn, correspond to the population from drier, more continental forests in localities from Bahia and Pernambuco (Carvalho, 2013). The emergence of these lineages could then be related to alterations in the *ndhF* gene related to responses to climatic stress, as described here and by Martín *et al*. (2009), and to the environmental adaptability already highlighted by Carvalho *et al*. (2020, 2021) (see coments bellow).

In relation to the higher internal resolution observed in this study, we can attribute it first to the use of a larger number of molecular markers with higher evolutionary rates, such as *rpL16* and *ndhF* (Wang, Chen & Zhang, 2018; Wang *et al*., 2020), while Carvalho *et al*. (2021) obtained lower internal resolution likely due to the low nucleotide substitution rate in the majority of the regions utilized (such as *psbA-trnH*, *trnS-trnG* and *trnD-trnT*) as well as recent speciation and possible occurrence of hybridization events, as highlighted by the authors.

Regions such as *rpL16* and *ndhF,* on the other hand, have been recognized as useful for inferring more internal phylogenetic relationships in closely related groups with recent evolutionary history (Kelchner & Clark, 1997; Tyrrell *et al*., 2012; I.L.C. Oliveira *et al*., 2020). Additionally, the use of single-copy nuclear markers such as *LEAFY* also have shown to be effective to estimate phylogenetic relationships in lower taxonomic levels, even with a lower nucleotide variation rate when compared to plastid genome (Yang *et al*., 2013).

In counterpoint, more variable regions such as the *trnL-trnF* and *rpL32-trnL* spacers, might influence directly the establishment of some phylogenetic relationships due to the high rates of homoplasy attributed (Dong *et al*., 2012; Kelchner & BPB, 2013; I.L.C. Oliveira *et al*., 2020). In spite of this fact, the same sequences are still being used to infer relationships in related groups, being widely applied in recent years in phylogenetic studies on herbaceous bamboos (Kelchner & BPG, 2013; Ferreira *et al*., 2019; I.L.C. Oliveira *et al*., 2020; R.P. Oliveira *et al*., 2020; Carvalho *et al*., 2021). Nonetheless, the use of these regions in phylogenetic studies must be approached with care or even avoided, given that the topologies recovered might reflect incorrect relationships (Bergsten, 2005; Dong *et al*., 2012; Kelchner & BPB, 2013; I.L.C. Oliveira *et al*., 2020).

The best resolution for internal relationships obtained in this study might be attributed to the expanded sampling of AF representatives, mainly *P. leptophylla*, which have remodeled the relationships of this species with the remaining taxa in the biome. Some studies involving bamboos demonstrated the influence of expanded sampling in the better understanding of the phylogenetic relationships within the group. Zeng *et al*. (2010), for example, highlighted the importance of extensive sampling for the phylogenetic reconstruction of the tribe Arundinarieae, especially due to the existence of several paraphyletic and polyphyletic genera.

Zhou *et al*. (2017), in a study on palaeotropical woody bamboos, have also demonstrated that the inclusion of a wider array of taxa allows for better phylogenetic resolution for the groups, mainly due to the history of reticulate evolution, with introgression, hybridization and ILS associated to rapid radiation. Additionally, two other recent studies on the polyphyletic genus *Fargesia* Franch., a temperate woody bamboo, also exemplify the importance of representatively sampling the group and other closely related groups for better recovery of phylogenetic relationships (Zhang *et al*., 2019; Zhou *et al*., 2019).

In our study, despite using faster markers, wider sampling and the resulting better resolution of the internal relationships, some of the relationships, especially in clade AFIII, could not be completely elucidated. One of the factors that might be related is the low genetic differentiation between members of the group. Even though some of the markers here used present higher base substitution rates than others used previously (Wang *et al*., 2020; Carvalho *et al*., 2021), and that the evolutionary rates in herbaceous bamboos are higher when compared to woody bamboo (Gaut *et al*., 1997; Wang *et al*., 2020), studies indicate that several groups in Olyreae and, consequently, Olyrinae might have undergone speciation events recently with rapid divergence between them (Oliveira *et al*., 2014; Ferreira *et al*., 2019; I.L.C. Oliveira *et al*., 2020; R.P. Oliveira *et al*., 2020; Carvalho *et al*., 2021). This recent evolution might explain, for example, the low differentiation and the genetic and morphologic overlap found in AF populations, except in *P. leptophylla* (Carvalho, 2013; Carvalho *et al*., 2021). It is also likely that these species might have undergone hybridization and introgression (Carvalho *et al*., 2021; Carvalho *et al*., unpubl. data). All these processes can hinder the establishment of phylogenetic relationships, as pointed in general studies with plants (Funk, 1985), as well as bamboos, specifically (Zhang, Ma & Li, 2011; Zhang *et al*., 2012).

In this sense, the use of a larger number of single-copy nuclear markers should provide more robust phylogenetic estimations and better elucidation of evolutionary processes (such as hybridization) experienced by these species (Sang 2002; Small, Cronn & Wendel, 2004; Ness, Graham & Barrett, 2011; Zhang *et al*., 2012; Yang *et al*., 2013). This is due to the higher evolutionary rates compared to plastid DNA (Clegg *et al*., 1994; Small *et al*., 2004), the biparental nature of the nuclear gene heritage allows to better trace the evolutionary history of the species when compared to uniparental heritage molecular markers (Birky, 2001; Ness *et al*., 2011; Yang *et al*., 2013).

Hence, studies utilizing a larger dataset, for example, population genetics and phylogeographic studies and/or wider sampling of plastid genome might facilitate the resolution of phylogenetic relationships in recent groups and groups with complex evolution, as previously observed (Schaal *et al*., 1998; Knowles, 2003; Townsend, 2007; Xi *et al.*, 2015; Camacho *et al*., 2019; Niu *et al*., 2018; Wen *et al.*, 2018). Also, as previously indicated by Carvalho *et al*. (2021), the inclusion of other representatives that could not be sampled here, e.g., *P. palmula* for AF and *P. tenella* for AM, besides other taxa closely related to the genus such as *Maclurolyra* Calderón, *Rehia* Fijten and *Reitzia* Swallen, could help to better establish the relationships in the group.

TOPOLOGICAL INCONGRUENCE AND ITS SIGNIFICANCE IN THE EVOLUTIONARY HISTORY OF *PIRESIA*

The results here suggest topological incongruences both between datasets and analyses. In relation to datasets, the *rpL32-trnL* region is incongruent is the remaining markers. This is likely due to the relationships found between the representatives of *Piresia* and *C. strictiflora* (Fig. 1A). This intergenic spacer is recognized as highly polymorphic for several plant groups, presenting high substitution rates in Bambusoideae, especially in Olyreae (Dong *et al*., 2012; Wang *et al*., 2018, 2020). This has been the object of several phylogenetic studies in recent years, and in those the use of *rpL32-trnL* is common (Ferreira *et al*., 2019; I.L.C. Oliveira *et al*., 2020; R.P. Oliveira *et al*., 2020; Carvalho *et al*., 2021). However, only one of the studies pointed the region as incongruent in relation to the other regions employed in the study (I.L.C. Oliveira *et al*., 2020), indicating that the use of this marker might be problematic for the recovery of phylogenetic relationships in the group.

In the present study, however, we observed that a complementary inverted sequence of six bases (ATAAAA), in the *rpL32-trnL* spacer, was shared between individuals of *P. leptophylla* from Ipojuca (PE) and Baía Formosa (RN), *Piresia* sp. MLSC 309, *Piresia* sp. MLSC 281, *C. strictiflora* and *B. bambusoides*, while the remaining taxa analyzed presented another kind of sequence in the region (TTTTAT). This complementary inversion was previously reported by Zeng *et al*. (2010) and might explain the closest relationships found between these species (except *B. bambusoides*) in the topology recovered by *rpL32-trnL* (Fig. 1A).

Inversions of this nature are common in highly variable regions and also have been identified as problematic (Zeng *et al*., 2010; Dong *et al*., 2012). These inversions are associated with probable hairpin secondary structures and may influence sequence alignment and lead to erroneous conclusions regarding the phylogenetic relationships between species (Kelchner & Wendel, 1996; Kelchner & Clark, 1997; Zeng *et al*., 2010; Dong *et al*., 2012). In this sense, the results here indicate that the mutation is shared and might be associated to the attraction of long branches and homoplasies, due to the high substitution rates observed in non-coding plastid regions, as is the case of the intergenic spacer *rpL32-trnL* (Shaw *et al*., 2007; Dong *et al.*, 2012; I. Oliveira *et al*., 2020). This is why the genera *Piresia*, *Cryptochloa* Swallen and *Buergersiochloa* Pilg. have been recovered as distinct clades in all recent phylogenetic studies for the group based on a diversity of molecular markers (I.L.C. Oliveira *et al*., 2020; R.P. Oliveira *et al*., 2020; Carvalho *et al*., in 2021).

These findings are relevant for homoplasies in molecular datasets, which are rarely analyzed and considered in phylogenetic analyses, but some studies indicate that their levels might be similar to what is observed in morphological characters of plants and animals (Sanderson & Donoghue, 1989; Donoghue & Ree, 2000). If so, using highly variable DNA regions, commonly recommended for studies in lower taxonomic levels (Dong *et al.*, 2012), must be approached with care.

Regarding the topologies obtained with data from the combined regions and the species tree, the main incongruence observed concerns the relationships within *P. leptophylla*. While a gene tree recovers its paraphyletic status with the population from Una diverging as sister to the entire clade from AF, in the species trees *P. leptophylla* is recovered as monophyletic (Figs 1B, 2).

ASTRAL-II and SVDquartets analyses, however, gave low support for the internal branches of the group (Fig. 2), yet, despite the lower values, the alternative topology test did not reject the hypothesis of *P. leptophylla* as a monophyletic group. Thus, based on the analysis carried out in the present study, it is not possible to confirm the paraphyletic status of *P. leptophylla*, but micromorphological evidences suggests a paraphyletism (pers. comm), evidenced that more precise studies are needed (using an integrative approach, as an example) to help in the recirculation of the species Thus, based on the analysis performed in the present study, it is not possible to confirm the paraphyletic status of *P. leptophylla*, but micromorphological evidences suggesting a possible parafiletism, evidenciated that is making necessary more accurate studies (employing an integrative approach, as an example) to help in the recircumscription of the species.

This is due, in a general sense, to the fact that incongruence between gene and species trees is likely related to several factors, both methodological (low information content in the molecular markers chosen) (Saitou & Nei, 1986; Xi *et al*., 2015; Camacho *et al*., 2019), and intrinsic to the evolutionary history of the regions employed (Maddison, 1997; Jeffroy *et al*., 2006; Pollard *et al*., 2006; Castoe *et al.*, 2009; Degnan & Rosenberg, 2009; Kumar *et al*., 2012).

Hence, the separation of the *P. leptophylla* from Una from the remaining lineages in the group could be explained by the occurrence of ILS. This refers to the maintenance of genic lineages in deeper portions of the species tree, and it is commonly attributed to groups that went through fast radiation events or recent speciation (Pamilo & Nei, 1988; Rosenberg, 2002; Maddison & Knowles, 2006; Whitfield & Lockhart, 2007; Degnan & Rosenberg, 2009), as it seems to be the case with *Piresia*.

Groups with these characteristics might experience lower rates of genetic differentiation within group, and consequently, present shorter branches in phylogenetic trees (Maddison & Knowles, 2006; Degnan & Rosenberg, 2009). According to Maddison & Knowles (2006), species trees with shorter branches exhibit four times more ILS events when compared to trees with longer branches. Thus, coalescent events might occur below the divergence point between lineages that are not intimately related, resulting in discordances in topology between gene and species trees (Rosenberg, 2002; Maddison & Knowles, 2006; Degnan & Rosenberg, 2009).

Another explanation for the incongruence observed between gene and species trees in this study is the occurrence of different selective pressures, given the paraphyly recovered for *P. leptophylla* in the gene tree is likely related to the topology of the *ndhF* gene, which gave the best internal resolution (Fig. S1). Kim & Jansen (1995) indicated that over 50% of the changes in the *ndhF* sequence occur in the first and second positions on the codons, increasing the probability of changes in the amino acid sequence coding, and consequently, of its function. Saarela *et al*. (2018), in a study involving plastome within Poaceae, observed that this gene seems to be under selection, with 67 codons undergoing positive selection. These results indicated that the *ndhF* gene seems to experience a more relaxed selective pressure (Kim & Jansen, 1995).

This information is particular of interest given that the *ndhF* gene codes for a subunit of the Ndh complex in the thylakoid (Sazanov, Burrows & Nixon, 1998; Casano *et al.*, 2000; Rumeau *et al.*, 2005). This complex exerts a protective function against stress related to photo-oxidation such as extreme temperatures, fast changes in light intensity and hydric stress, as well as the transferal of electrons (Martín, Casano & Sabater, 1996; Endo *et al*., 1999; Martín *et al*., 2004, 2009). Hence, increases in activity in this subunit contribute to balance the redox level of the electron transports that are affected during the stress process (Casano *et al*., 2000; Joët *et al*., 2002; Martín *et al*., 2004). The subunit code for by *ndhF* is responsible specifically for the regulation of the Ndh complex activity by the phosphorylation of the polypeptide NDH-F (Lascano *et al*., 2003).

In other words, changes in the gene sequence of *ndhF* gene sequence can affect the responses of the ndh complex. In association with other genes related to climatic stress, the response of plants to the diversity of climate changes they may be subjected to can be altered

(Martín *et al*., 2009, 2015; Xu, Jiang & Zhou, 2015; Ivanova et al., 2017). In this sense, our findings indicate that the *P. leptophylla* population from Una (BA) might have experienced in the past different selective pressures related to climate adaptations, evolving as an independent lineage. This explanation agrees with hypotheses previously presented that the *Piresia* evolution might be associated to adaptive responses triggered by environmental change (Carvalho *et al*., 2020; Carvalho *et al*., 2021), as explored below.

However, in spite of this hypothesis, the influence of the remaining topologies in relation to the *ndhF* topology in the combined gene tree might be related to an anomalous genetic tree (AGT). These are the gene trees more commonly observed throughout the genome, but not correspondent to the species tree (Degnan & Rosenberg, 2006, 2009). AGTs are more likely to occur when the species trees present short branches (related to rapid radiation events, or in groups that are closely related) and might cause incongruences in the estimation of the species trees (Degnan & Rosenberg, 2006, 2009). In that sense, despite the *ndhF* not being predominant over the rest, it might represent a topology that better represents the phylogenetic relationships in *Piresia*.

FORESTS DYNAMICS DURING GLACIAL CYCLES AND THEIR INFLUENCE ON *PIRESIA* DISTRIBUTION

The biogeographical history of *Piresia* appears to be intimately related to the history of the dynamics of the forest environment it inhabits, given the probable dependence of its representatives on forest environments (Soderstrom & Calderón, 1974; Judziewicz *et al*., 1999; Carvalho *et al*., 2020, 2021). This hypothesis has a close connection with climatic and geological changes occurring mainly during the Pleistocene, a period marked by a sequence of glacial cycles that altered significantly solar incidence, temperature, sea and CO_2 levels, among other factors (Petit *et al*., 1999; Smith *et al*., 2005; Fritz *et al*., 2007; Cohen & Gibbard, 2019).

The origin of *Piresia* probably dates from the early Pliocene, with an ancestor equally likely in AM and AF (Fig. 3, I), with posterior geographical and phylogenetic disjunction between the biomes, likely due to a vicariant event (Fig. 3). These findings agree, to a degree, with the results from Carvalho *et al*. (2021), that despite recovering the phylogenetic disjunction between AM and AF for combined plastid data only, did not discard alternative biogeographic scenarios for the group, such as a possible origin in the north/west AM, with a posterior migration to AF. Neither their study nor ours supports the hypothesis posed by Soderstrom & Calderón (1974) of an AF origin for the group, with posterior dispersal to AM and diversification in both biomes.

In relation to the period, landscape alterations likely were influenced by the big changes resulting from the orogenic processes leading to the formation of the Andes, occurring in the mid- and late Miocene (Garzione *et al*., 2008; Hoorn *et al*., 2010). Beyond that, some studies indicate that during the Pliocene, there was an increase in precipitation and temperature, which favored the expansion of rainforests and allowed the connection between AM and AF (Morley, 2000; Zachos *et al*., 2001; Cheng *et al*., 2013) (Fig. 3). The expansion of both forests could indicate a wide origin for *Piresia* involving the AF and/or AM, or dispersal events between both biomes, a hypothesis raised in several studies involving distribution patterns and the origin of certain bird lineages (Batalha-Filho *et al*., 2013), as well as the age of certain woody plants, which indicate that organisms from forest environments are older than phylogenetically close organisms from open environments (Souza-Neto *et al*., 2016).

After the separation of the forests, contact routes such as continuous formations or series of forest patches were hypothesized to explain the expansion of forest environments and their connection between AM and AF: the northeastern route, where now the Caatinga biome is located; the center route, probably through the Cerrado; and the southern route, through intermittent forest formations throughout the hydrographic basin of the Paraná river (Bigarella *et al*., 1975; Andrade-Lima, 1982; Por, 1992; Oliveira-Filho & Ratter, 1995; Costa, 2003; Auler *et al*., 2004; Batalha-Filho *et al*., 2013).

Despite the different routes, studies have demonstrated similar disjunct species origin and diversification patterns between AM and AF, such as recent finding for the lizard genus *Anolis* and for plants from the genus *Ficus* L., whose life histories appear to be associated to the formation of forest patches that connected the biomes (Prates *et al*., 2017; Machado *et al*., 2018). In the case of *Piresia*, the northeastern route seems to be the most plausible to explain the contact between biomes, given the absence of representatives of the genus in the Brazilian South and Southeast regions (Fig. 3, I) (Soderstrom, 1982; Judziewicz *et al*., 1999; Carvalho, 2013; Carvalho *et al*., 2020) (Fig. 3).

The disjunction of AM and AF lineages of *Piresia* appears to have happened around 2.52 Mya, in a period marked by the beginning of a large global cooling followed by sequential glacial cycles (Cita, 2008; Cohen & Gibbard, 2019). These climate dynamics and their influence both on forest environments and on the sea level are consistent with the succession of diversification events that occurred internally in the AF group, influencing the formation of AFI, AFII and AFIII lineages found in our study.

The beginning of the diversification happened around 1.87 Mya, with the separation of the AFI lineage (including parti of the members of *P. leptophylla*), in southern Bahia (Una) (Fig. 3). According to inferences from marine isotopes (Cohen & Gibbard, 2019), this period coincides with the beginning of a glaciation during the Pleistocene, marked by global temperature and sea level drops, changes that could have influenced the isolation and diversification of AFI. This is likely because, according to geomorphological data, the region of Una was only lightly influenced by sea level oscillations during glacial cycles in the Pleistocene, which could explain its isolation in the region (Bittencourt *et al*., 1979).

In counterpoint, the regions north and south of Una were intensely remodeled during periods of lower sea level, resulting in the formation of well-developed coastal plains with a series of small islands, lakes and small bays (Bittencourt *et al*., 1979). This hypothesis is in accord to the hypothesis mentioned above that the MI divergent could be related to an adaptive response to the different climate changes the group experienced.

The diversification of the AFII and AFIII lineages coincide with periods of global cooling periods and sea level retraction (Cohen & Gibbard, 2019), that in turn presented relative climatic stability in the northern portion of AF, reflecting in a large refuge area in the biome (Carnaval & Moritz, 2008; Martins, 2011; Staggemeier *et al*., 2015; Menezes *et al*., 2016). Nonetheless, in spite of higher stability, two hypotheses suggest that the organisms might have been influenced differently during the expansion and retraction of the forests in the region, being these the Forest Refuge and Atlantis Forest hypothesis (see Carnaval & Moritz, 2008; Leite *et al*., 2016, respectively).

For the AFII lineage (with the remaining populations of *P. leptophylla*), its diversification is consistent with the Atlantis hypothesis by Leite *et al*. (2016), that postulates that, during glaciation periods, with the retraction of the sea level, and exposure of the continental shelf, forest habitats expanded to the new areas available. This is due to the representatives of the lineage presenting higher adaptability to drier environments (Soderstrom, 1982; Judziewicz *et al*., 1999; Carvalho *et al*., 2020) and thus, the populations could have expanded to drier Restinga areas, closer to the coast, which were more affected by glaciation events (Bittencourt *et al*., 1979; Vilas Bôas, Sampaio & Pereira, 2001; Ramos-Fregonezi *et al*., 2015; Fazolato *et al*., 2017), and then, diversified.

On lineage AFIII, composed by potentially new taxa, the Forest Refuge hypothesis (Carnaval & Moritz, 2008) appears to be adequate to explain its diversification, given their higher dependence for humid forest habitats (Judziewicz *et al*., 1999; Carvalho, 2013; Carvalho *et al*., 2020). This is likely due to global cooling and reduction of stable areas for the taxa to occur, the representatives of the group might have been restricted to forest fragments in a more stable area of the continent, and diversified in relation to the other representatives in the biome (Carvalho, 2013; Carvalho *et al*., 2021).

In that sense, *Piresia* appears to have an evolutionary history that involves both hypotheses, demonstrating that both can be applied to a single group. The occurrence of distinct evolutionary scenarios in phylogenetically related groups, however, is not unusual and has been related to several organisms in the neotropics. Examples include the Rufous-breasted leaftosser *Sclerurus scansor* (Ménétriès, 1835) (Scleruridae), several South American organisms (algae, plants, invertebrates and vertebrates), several groups of neotropical plants and of *Athenaea fasciculata* (Vell.) IMCRodrigues & Stehmann (Solanaceae) (e.g., D'Horta *et al.*, 2011; Turchetto-Zolet *et al*., 2013; Leal, Palma da Silva & Pinheiro, 2016; Mäder *et al.*, 2021, respectively), demonstrating the necessity of studies of this nature in different groups of organisms.

Besides the patterns here described, our study also detected internal dispersal and diversification events in AFII and AFIII for the northern most areas in AF (Fig. 3, II e IV). These two events coincide with drastic cooling periods occurring around 0.84 and 0.15 Mya during Mid Pleistocene (Cohen & Gibbard, 2019), inducing novel adaptive responses within lineages. In a general sense, the northernmost regions closer to the Equator tend to present tamer climate variations during global cooling events, which can influence the dispersal of organisms that occur in higher latitudes to lower latitude regions (Fisher, 1960; Williams *et al*., 1993; Hewitt, 2004).

In the case of AF, although the northern region, as a whole, is considered climatically more stable (Carnaval & Moritz, 2008; Martins, 2011; Staggemeier *et al*., 2015; Menezes *et al*., 2016), different portions could have experienced distinct conditions during global climate changes, mainly during periods with more drastic changes, such as what occurs in the mid Pleistocene (Pisias & Moore, 1981; Clapperton, 1990; Hewitt, 2004). Regions in Southern Bahia, as an example, appear to have undergone higher climate instability when compared to portions of the Northern coast of Bahia and regions further north, given the habitat heterogeneity caused by the larger area of coastal shelf present and exposed in the region (Martins & Coutinho, 1981; Leite *et al*., 2016).

In that sense, during peaks of temperature drop in the mid Pleistocene, representatives of AFII and AFIII could have dispersed to more climatically stable regions up north and further from the coast, as the case of the small group of *Piresia* sp. MLSC 281 and *Piresia* sp. MLSC 309, that are distributed in drier forest regions when compared to the remaining members of the clade (Carvalho, 2013; Carvalho *et al*., 2021) (Fig. 3, II and IV). These lineages could, posteriorly, have experienced isolation and diversification.

These ideas agree with the findings of studies with other taxa, such as a species of Passeriformes that presented an expansion movement to the Northern AF due to latitudinal differences, posteriorly undergoing population bottlenecks and isolation from the Southern lineages (D'Horta *et al*., 2011). In a study involving plants of the genus *Eugenia* sect. *Phyllocalyx* Nied., including ecological distribution modelling, it was also possible to observe changes in the geographic distribution ranges of the genus during the colder periods, with higher retraction on Southern populations, and expansion towards Northern areas (Bünger *et al*., 2016).

So, our data suggests a complex evolutionary history for the representatives of *Piresia* from AF, as hypothesized by Carvalho (2013) and Carvalho *et al*. (2020, 2021), taking into consideration abiotic factors related to climate and its influence in forest formation dynamics, as well as evolutionary pressures that marked the genetics and morphology of the group in a complex mode.

CONCLUSIONS AND PERSPECTIVES

Our results reveal a better resolution of phylogenetic relationships in *Piresia* in the lineage that represents the AF in the Brazilian northeastern region. Although some of the internal relationships still lack resolution, our data made evident how incongruences between datasets might influence the recovery of phylogenies and how the choice of adequate markers and methods can be crucial for the phylogenetic reconstruction.

Our findings appear to agree with the occurrence of homoplasies in regions of the plastid genome, incomplete lineage sorting events and occurrence of differential selective pressures possibly related to climatic adaptations, and for further elucidation, we consider necessary a wider coverage of plastidial genome, the use of single-copy nuclear regions and approaches that involve the study of populations (e.g., phylogeography).

In relation to the biogeographic history of *Piresia*, the evolution and distribution of its representatives appears to be directly associated to changes in climate and the forest dynamics in AM and AF, mainly those occurring during the Pleistocene. The ancestral distribution of the lineages in both biomes seems to involve a scenario with equal probabilities of origin in AM and AF, probably due to a wide origin during the Pliocene, with posterior geographic and genetic disjunction.

The AF lineage appears to have experienced a complex evolutionary history, with distinct distribution expansion and retraction for its populations, which influenced the patterns observed currently, and might be related to the groups occupying different regions of AF with different topologies. This interaction of distinct factors likely promoted the diversification of the lineage into three groups (AFI, AFII and AFIII), and could be responsible for its complex evolutionary history.

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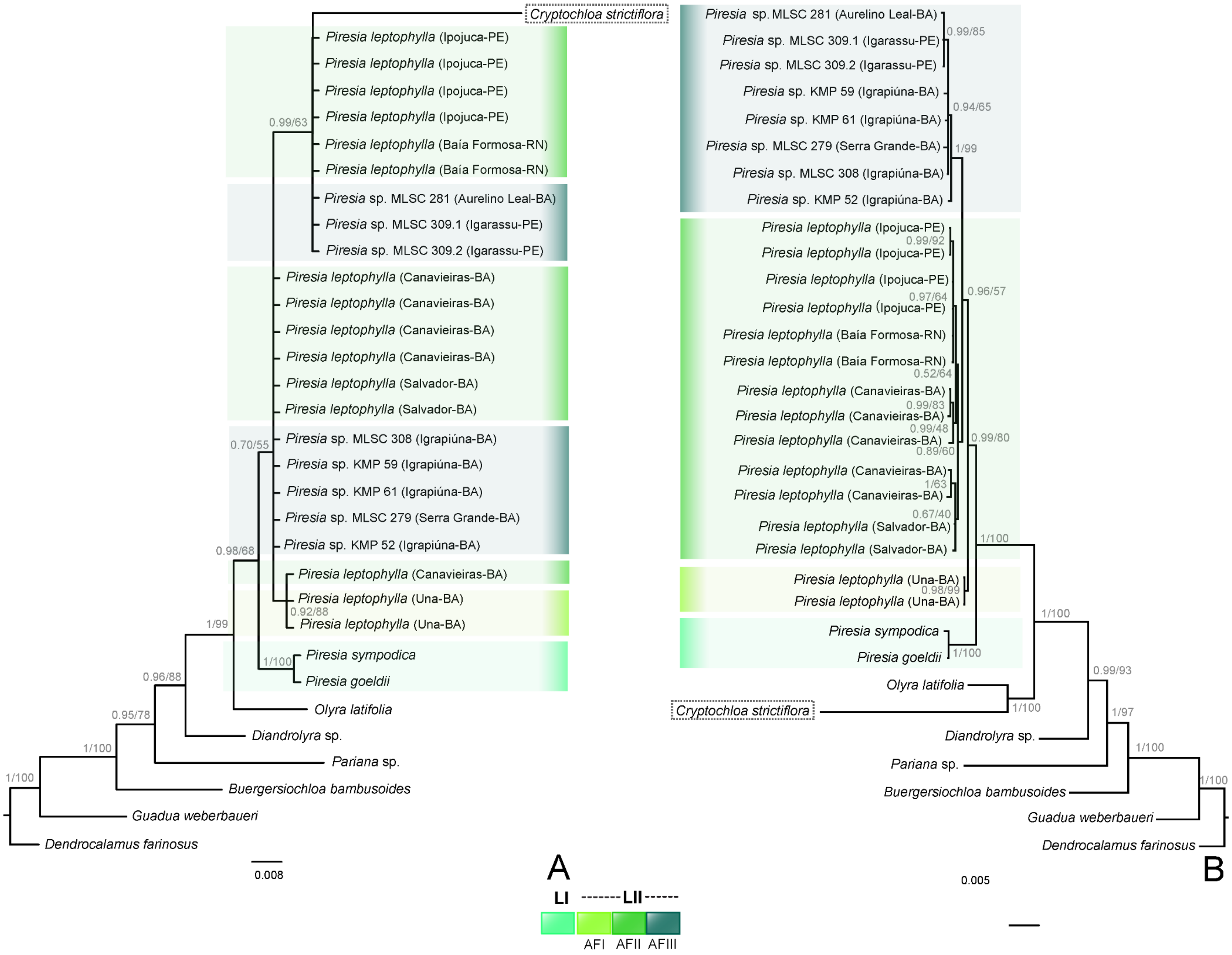
Figure legends

Figure 1. Comparison of the topologies obtained through Maximum Likelihood (ML) and Bayesian Inference (BI) for the spacer *rpL32-trnL* and the combined dataset. A, Data from the plastid spacer *rpL32-trnL*. B, Data from combined plastid (*nhdF, rpL16, trnD-trnT, trnL-trnF*) and nuclear (*LEAFY*) DNA regions. Values presented at each node represent the posterior probability of BI and bootstrap values for ML, respectively separated by a slash ("/"). Acronyms and different colors represent the recovered lineages: LI, lineage from AM and LII lineage from AF (*sensu* Carvalho *et al*., 2020); AFI, AFII and AFIII, lineages of LII.

Figure 2. Comparison of the species trees reconstructed through ASTRAL-II and SVDquartets for the combined plastid (*nhdF, rpL16, trnD-trnT, trnL-trnF*) and nuclear (*LEAFY*) DNA data. A, topology obtained through ASTRAL-II with Local Posterior Probability (LPP) values on each node. B, Topology obtained through SVDquartets with bootstrap values in each node. Different acronyms and colors represent the recovered lineages: LI, lineage from AM and LII lineage from AF (*sensu* Carvalho *et al*., 2020); AFI, AFII and AFIII, lineages of LII.

Figure 3. Divergence time estimation based on nuclear and plastid DNA regions, secondary calibrations and estimation of ancestral areas. Ancestral areas were inferred using the DIVALIKE+J model. The bars on each branch represent the 95% HPD (*Highest Posterior Density*) confidence interval for divergence times. Map with the areas utilized for the inference of ancestor nodes. The colors in the map and nodes represent the areas *sensu* Morrone (2014) and Cantidio & Souza (2019): light yellow, Roraima; darker yellow, Pará; light blue, Ecoregion 1; blue, Ecoregion 2; deep blue, Ecoregion 3. Arrows, full and dotted lines and number represent the events: double arrow with continuous line (I), wide origin between biomes; single arrow with dotted line (II, III e IV), wide dispersal events. Plei, Pleistocene. The tracing besides the lineages and their different colors and acronyms represents the lineages found in LII: AFI, AFII and AFIII.


Cryptochloa strictiflora
Piresia leptophylla (Ipojuca-PE)
Piresia leptophylla (Ipojuca-PE)
Piresia leptophylla (Ipojuca-PE)
Piresia leptophylla (Ipojuca-PE)
Piresia leptophylla (Baía Formosa-RN)
Piresia leptophylla (Baía Formosa-RN)
0.99/63
Piresia sp. MLSC 281 (Aurelino Leal-BA)
Piresia sp. MLSC 309.1 (Igarassu-PE)
Piresia sp. MLSC 309.2 (Igarassu-PE)
Piresia leptophylla (Canavieiras-BA)
Piresia leptophylla (Canavieiras-BA)
Piresia leptophylla (Canavieiras-BA)
Piresia leptophylla (Canavieiras-BA)
Piresia leptophylla (Salvador-BA)
Piresia leptophylla (Salvador-BA)
0.70/55
Piresia sp. MLSC 308 (Igrapiúna-BA)
Piresia sp. KMP 59 (Igrapiúna-BA)
Piresia sp. KMP 61 (Igrapiúna-BA)
Piresia sp. MLSC 279 (Serra Grande-BA)
Piresia sp. KMP 52 (Igrapiúna-BA)
0.98/68
Piresia leptophylla (Canavieiras-BA)
Piresia leptophylla (Una-BA)
0.92/88
Piresia leptophylla (Una-BA)
1/99
1/100
Piresia sympodica
Piresia goeldii
0.96/88
Olyra latifolia
0.95/78
Diandrolyra sp.
1/100
Pariana sp.
1/100
Buergersiochloa bambusoides
Guadua weberbaueri
Dendrocalamus farinosus
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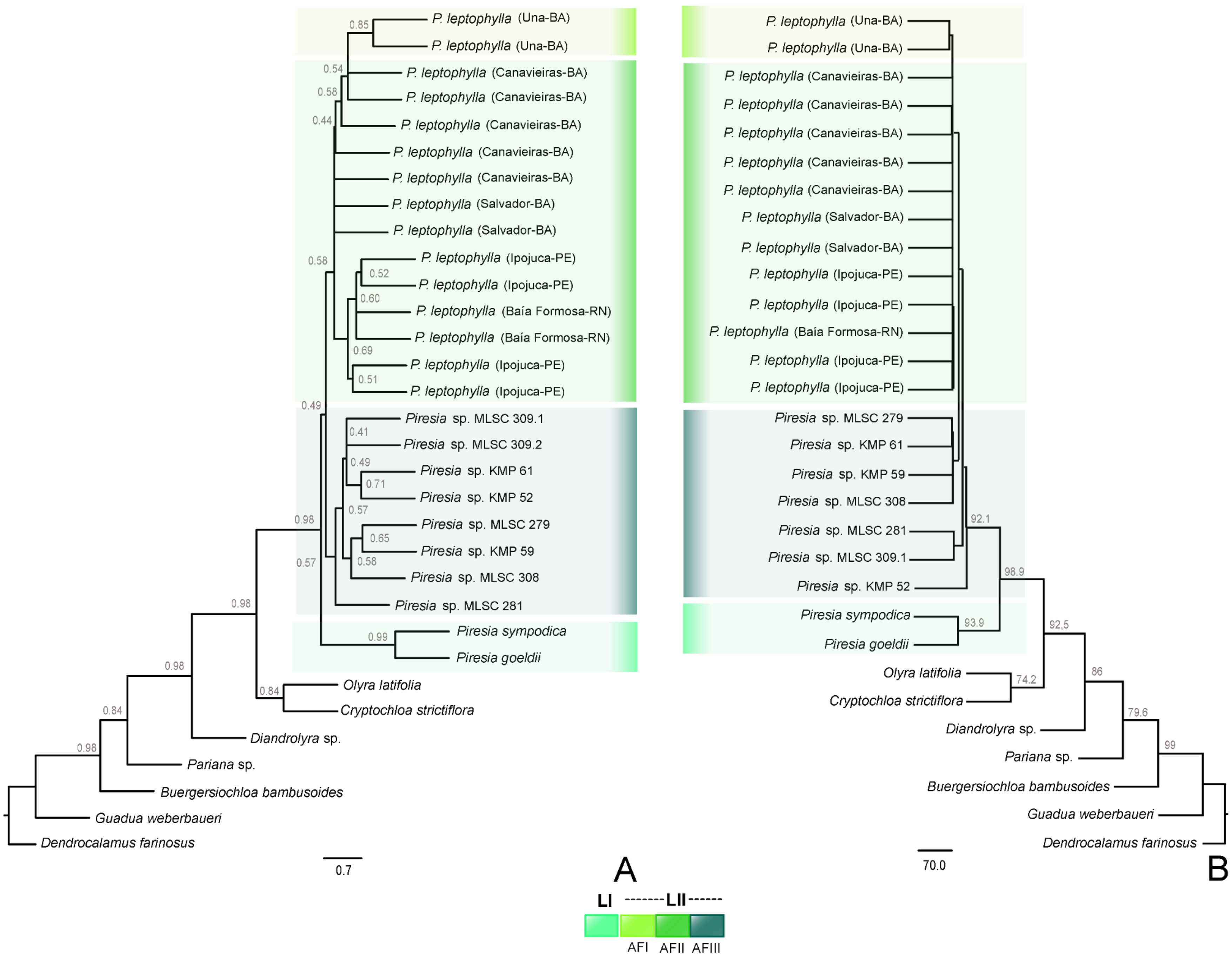
Piresia sp. MLSC 281 (Aurelino Leal-BA)
0.99/85
Piresia sp. MLSC 309.1 (Igarassu-PE)
Piresia sp. MLSC 309.2 (Igarassu-PE)
Piresia sp. KMP 59 (Igrapiúna-BA)
0.94/65
Piresia sp. KMP 61 (Igrapiúna-BA)
Piresia sp. MLSC 279 (Serra Grande-BA)
1/99
Piresia sp. MLSC 308 (Igrapiúna-BA)
Piresia sp. KMP 52 (Igrapiúna-BA)
Piresia leptophylla (Ipojuca-PE)
0.99/92
Piresia leptophylla (Ipojuca-PE)
Piresia leptophylla (Ipojuca-PE)
0.96/57
0.97/64
Piresia leptophylla (Ipojuca-PE)
Piresia leptophylla (Baía Formosa-RN)
Piresia leptophylla (Baía Formosa-RN)
0.52/64
Piresia leptophylla (Canavieiras-BA)
0.99/83
Piresia leptophylla (Canavieiras-BA)
0.99/48
Piresia leptophylla (Canavieiras-BA)
0.99/80
0.89/60
Piresia leptophylla (Canavieiras-BA)
1/63
Piresia leptophylla (Canavieiras-BA)
0.67/40
Piresia leptophylla (Salvador-BA)
1/100
Piresia leptophylla (Salvador-BA)
Piresia leptophylla (Una-BA)
0.98/99
Piresia leptophylla (Una-BA)
1/100
Piresia sympodica
Piresia goeldii
1/100
0.99/93
Olyra latifolia
Cryptochloa strictiflora
1/100
1/97
Diandrolyra sp.
1/100
Pariana sp.
1/100
Buergersiochloa bambusoides
Guadua weberbaueri
Dendrocalamus farinosus
0.005
B
LI
-------LII------
AFI
AFII
AFIII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P. leptophylla (Una-BA)
P. leptophylla (Canavieiras-BA)
P. leptophylla (Canavieiras-BA)
P. leptophylla (Canavieiras-BA)
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P. leptophylla (Canavieiras-BA)
P. leptophylla (Salvador-BA)
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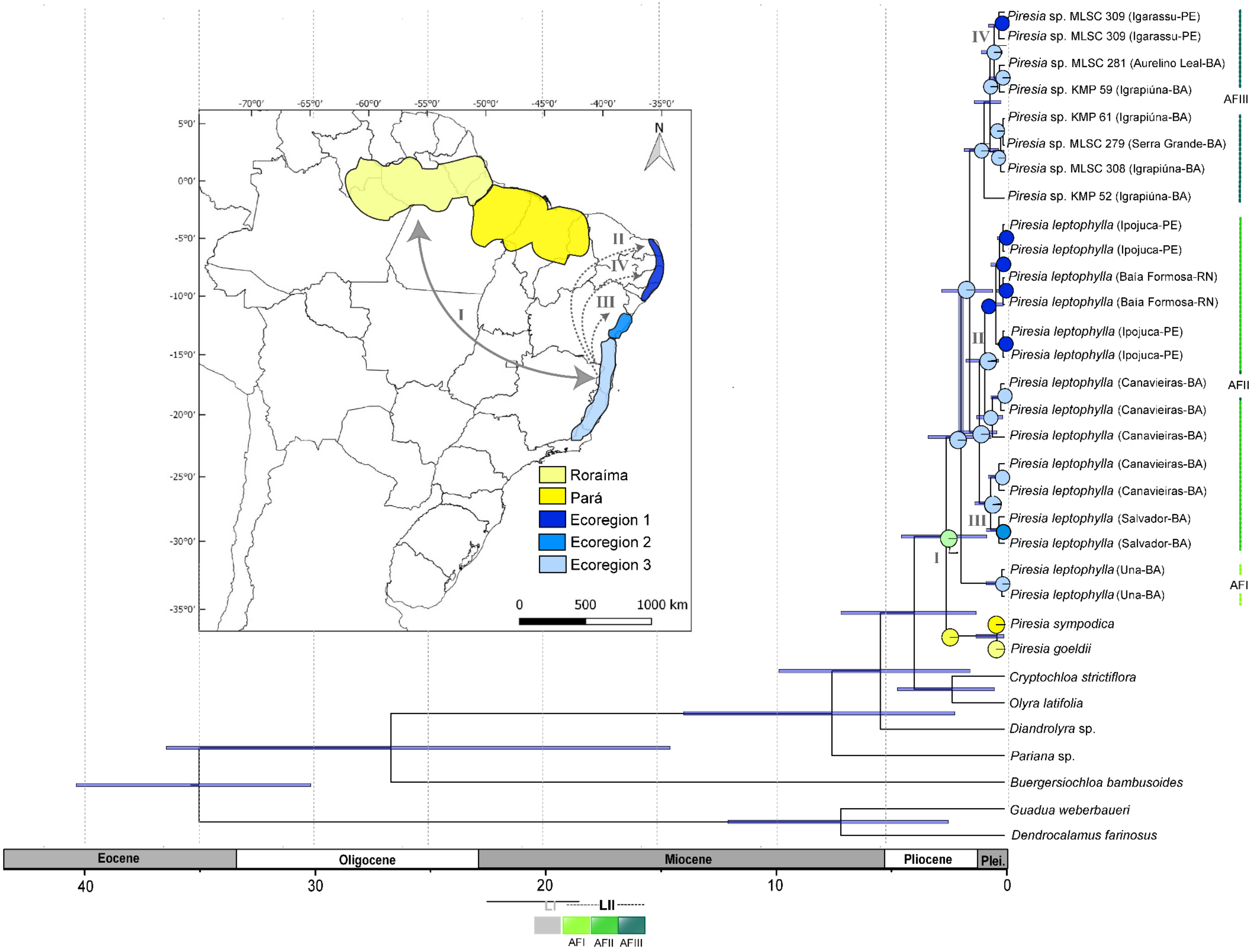
P. leptophylla (Ipojuca-PE)
P. leptophylla (Ipojuca-PE)
P. leptophylla (Baía Formosa-RN)
P. leptophylla (Baía Formosa-RN)
P. leptophylla (Ipojuca-PE)
P. leptophylla (Ipojuca-PE)
Piresia sp. MLSC 309.1
Piresia sp. MLSC 309.2
Piresia sp. KMP 61
Piresia sp. KMP 52
Piresia sp. MLSC 279
Piresia sp. KMP 59
Piresia sp. MLSC 308
Piresia sp. MLSC 281
Piresia sympodica
Piresia goeldii
Olyra latifolia
Cryptochloa strictiflora
Diandrolyra sp.
Pariana sp.
Buergersiochloa bambusoides
Guadua weberbaueri
Dendrocalamus farinosus
0.85
0.54
0.58
0.44
0.58
0.52
0.60
0.69
0.51
0.49
0.41
0.49
0.71
0.57
0.65
0.58
0.57
0.98
0.99
0.98
0.98
0.84
0.84
0.98
0.7
A
P. leptophylla (Una-BA)
P. leptophylla (Una-BA)
P. leptophylla (Canavieiras-BA)
P. leptophylla (Canavieiras-BA)
P. leptophylla (Canavieiras-BA)
P. leptophylla (Canavieiras-BA)
P. leptophylla (Canavieiras-BA)
P. leptophylla (Salvador-BA)
P. leptophylla (Salvador-BA)
P. leptophylla (Ipojuca-PE)
P. leptophylla (Ipojuca-PE)
P. leptophylla (Baía Formosa-RN)
P. leptophylla (Ipojuca-PE)
P. leptophylla (Ipojuca-PE)
Piresia sp. MLSC 279
Piresia sp. KMP 61
Piresia sp. KMP 59
Piresia sp. MLSC 308
Piresia sp. MLSC 281
Piresia sp. MLSC 309.1
Piresia sp. KMP 52
Piresia sympodica
Piresia goeldii
Olyra latifolia
Cryptochloa strictiflora
Diandrolyra sp.
Pariana sp.
Buergersiochloa bambusoides
Guadua weberbaueri
Dendrocalamus farinosus
92.1
98.9
93.9
92,5
74.2
86
79.6
99
70.0
B
LI
--------LII------
AFI
AFII
AFIII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I
II
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IV
Roraíma
Pará
Ecoregion 1
Ecoregion 2
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500
1000 km
Piresia sp. MLSC 309 (Igarassu-PE)
Piresia sp. MLSC 309 (Igarassu-PE)
Piresia sp. MLSC 281 (Aurelino Leal-BA)
Piresia sp. KMP 59 (Igrapiúna-BA)
Piresia sp. KMP 61 (Igrapiúna-BA)
Piresia sp. MLSC 279 (Serra Grande-BA)
Piresia sp. MLSC 308 (Igrapiúna-BA)
Piresia sp. KMP 52 (Igrapiúna-BA)
Piresia leptophylla (Ipojuca-PE)
Piresia leptophylla (Ipojuca-PE)
Piresia leptophylla (Baia Formosa-RN)
Piresia leptophylla (Baia Formosa-RN)
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Piresia leptophylla (Canavieiras-BA)
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Piresia leptophylla (Canavieiras-BA)
Piresia leptophylla (Canavieiras-BA)
Piresia leptophylla (Salvador-BA)
Piresia leptophylla (Salvador-BA)
Piresia leptophylla (Una-BA)
Piresia leptophylla (Una-BA)
Piresia sympodica
Piresia goeldii
Cryptochloa strictiflora
Olyra latifolia
Diandrolyra sp.
Pariana sp.
Buergersiochloa bambusoides
Guadua weberbaueri
Dendrocalamus farinosus
IV
II
III
I
AFIII
AFII
AFI
Eocene
Oligocene
Miocene
Pliocene
Plei.
40
30
20
10
0
LI
LII
AFI
AFII
AFIII

Table 1. Taxa utilized in this study (classified *sensu* Soreng *et al*., 2017). Country, State, Municipality, Vouchers and GenBank access numbers for the DNA sequences for plastid (*rpl32-trnL*, *trnD-trnT*, *trnF-trnL*, *rpL16* e *ndhF*) and nuclear (*LEAFY*). -----: Without sequence; X0X0: new sequences for GenBank.

Taxa	**Country**	**State**	**Municipality**	**Voucher**	***rpL32-trnL***	***trnD-trnT***	***trnL-trnF***	***rpL16***	***ndhF***	***LEAFY***
BAMBUSOIDEAE – Bambuseae										
Dendrocalamus farinosus (Keng & Keng f.) L.C.Chia & H.L.Fung	China	Yunnan	-	Zhang 12291	KT227946.1	GU354837.1	KT226899.1	KT228069.1	KT227356.1	KM264758.1
Guadua weberbaueri Pilg.	Colômbia	Letícia	-	Londoño & Kobayashi 582	KP793062.1	KP793062.1	KP793062.1	KP793062.1	KP793062.1	--------
BAMBUSOIDEAE – Olyreae										
Buergersiochloinae										
Buergersiochloa bambusoides Pilg.	Papua Nova Guiné	East Sepik	-	Dransfield 1365	KJ871000.1	KJ871000.1	KJ871000.1	KJ871000.1	KJ871000.1	------------
Parianineae										
Pariana sp.	-	-	-	ZMY 026	KP319246.1	KP319246.1	KP319246.1	KP319246.1	KP319246.1	-------------
Olyrinae										
Cryptochloa strictiflora (E.Fourn.) Swallen	Belize	Toledo	-	Davidse 35629	JX235348.1	JX235348.1	JX235348.1	JX235348.1	JX235348.1	-------------
Diandrolyra sp.	-	-	-	Clark 1301	KJ870991.1	KJ870991.1	KJ870991.1	KJ870991.1	KJ870991.1	KJ870991.1
Olyra latifolia L.	-	-	-	Fisher 97	KF515509.1	KF515509.1	KF515509.1	KF515509.1	KF515509.1	-------------
Piresia goeldii Swallen	Brasil	Amazonas	Manaus	Carvalho 289	X0X0	X0X0	X0X0	X0X0	X0X0	X0X0
Piresia leptophylla Soderstr.	Brasil	Bahia	Una	Carvalho 317	X0X0	X0X0	X0X0	X0X0	X0X0	X0X0
Piresia leptophylla	Brasil	Bahia	Canavieiras	Carvalho 277	X0X0	X0X0	X0X0	X0X0	X0X0	X0X0
Piresia leptophylla	Brasil	Bahia	Canavieiras	Carvalho 278	X0X0	X0X0	X0X0	X0X0	X0X0	X0X0
Piresia leptophylla	Brasil	Bahia	Salvador	Carvalho 282	X0X0	X0X0	X0X0	X0X0	X0X0	X0X0
Piresia leptophylla	Brasil	Pernambuco	Ipojuca	Carvalho 310	X0X0	X0X0	X0X0	X0X0	X0X0	X0X0
Piresia leptophylla	Brasil	Pernambuco	Ipojuca	Carvalho 313	X0X0	X0X0	X0X0	X0X0	X0X0	X0X0
Piresia leptophylla	Brasil	Rio Grande do Norte	Baía Formosa	Carvalho 417	X0X0	X0X0	X0X0	X0X0	X0X0	X0X0

Piresia sympodica (Döll) Swallen	Brasil	Pará	Belém	Carvalho 302	X0X0	X0X0	X0X0	X0X0	X0X0	X0X0
Piresia sp. MLSC 279	Brasil	Bahia	Serra Grande	Carvalho 279	X0X0	X0X0	X0X0	X0X0	X0X0	X0X0
Piresia sp. MLSC 281	Brasil	Bahia	Aurelino Leal	Carvalho 281	X0X0	X0X0	X0X0	X0X0	X0X0	X0X0
Piresia sp. MLSC 308	Brasil	Bahia	Igrapiúna	Carvalho 308	X0X0	X0X0	X0X0	X0X0	X0X0	X0X0
Piresia sp. KMP 52	Brasil	Bahia	Igrapiúna	Pimenta 52	X0X0	X0X0	X0X0	X0X0	X0X0	X0X0
Piresia sp. KMP 59	Brasil	Bahia	Igrapiúna	Pimenta 59	X0X0	X0X0	X0X0	X0X0	X0X0	X0X0
Piresia sp. KMP 61	Brasil	Bahia	Igrapiúna	Pimenta 61	X0X0	X0X0	X0X0	X0X0	X0X0	X0X0
Piresia sp. MLSC 309	Brasil	Pernambuco	Igarassu	Carvalho 309	X0X0	X0X0	X0X0	X0X0	X0X0	X0X0

Table 2. Plastidial and nuclear DNA regions utilized in this study. Primer, sequences, amplification conditions, number of samples used (N), original size of the sequence (Sz)/size of the part of the sequence used, evolutionary models, percentage of potential informative characters (% PIC) and references.

Primer	**Sequences**	**Amplification condistions**	**N**	**Sz/Used**	**Models**	**% PIC**	**References**
ndhF 536F	TTG TAA CTA ATC GTG TAG GGG A	94°C, 1 min; 35x (94°C, 40 sec; 52°C, 45 sec; 72°C, 1 min and 30 sec); 72°C, 5 min	27	1075/626	HKY+I	11,7%	Olmstead & Sweere, 1994; Aliscioni *et al*., 2003
ndhF 1660 R	ATC CAA TGA ACA AAG TAA AAA G						
rpL16 F	GCT ATG CTT AGT GTG TGT GTC TC	94°C, 1 min; 35x (94°C, 45 sec; 55°C, 40 sec; 72°C, 2 min); 72°C, 5 min	29	1,181/564	GTR	18,12%	Giussani *et al*., 2009; Donadío *et al*., 2009
rpL16 R	CCA KAT TTT TCC ACC ACG AC						
rpL32	CAG TTC CCA AAA AAACGT ACT TC	80°C, 5 min; 30x (95°C, 1min; 50°C, 1 min; 65°C, 4 min); 65°C, 5min	29	952/564	HKY+Γ	19,07%	Shaw *et al*., 2007
trnL	CTG CTT CCT AAG AGC AGC GT						
trnD	ACC AAT TGA ACT ACA ATC CC	94°C, 1 min; 35x (94°C, 45 seg; 54°C 40 sec; 72°C, 1 min e 10 sec); 72°C, 5 min	29	1,155/532	HKY+I	10,23%	Fisher *et al*., 2009
trnT	CCC TTT TAA CTC AGT GGT A						
trnL	CGA ATT CGG TAG ACG CTA CG	94°C, 4 min; 30x (94°C, 1 min; 55°C, 1 min; 72°C, 2 min); 72°C, 10 min	29	983/751	GTR+Γ	20,11%	Taberlet *et al*., 1991; Soumaya *et al*., 2014
trnF	ATT TGA ACT GGT GAC ACG AG						
LEAFY F	CCA ACG ACG CCT TCT CGG	95°C, 2 min; 35x (95°C, 1 min; 51°C, 1 min; 72°C, 1 min e 30 sec); 72°C 20 min	24	846/746	GTR	8,63%	Yang *et al*., 2013
LEAFY R	GGC ACT GCT CGT ACA GAT GG						

Table 3. Summary of support values for the clades of gene and species trees reconstructions from multiple DNA regions. Values separated by a slash ("/") represent the posterior probability and the bootstrap for gene trees (BI and ML, respectively) and the Local Posterior Probability and the bootstrap for species trees (ASTRAL-II and SVDquartets, respectively). Different acronyms represent the recovered lineages: LI, lineage from AM and LII lineage from AF (sensu Carvalho et al., 2020); AFI, AFII and AFIII, lineages of LII.

Analysis	DNA regions	LI	LII	AFI	AFII	AFIII
Gene trees	*ndhF*	0.99/91	-/-	-/-	0.99/85	1/93
	rpL16	0.95/87	-/-	-/-	-/-	-/-
	rpL32-trnL	1/100	-/-	-/-	-/-	-/-
	trnD-trnT	-/-	0.95/77	-/-	-/-	-/-
	trnL-trnF	0.99/95	-/-	-/-	-/-	-/-
	LEAFY	1/100	-/-	0.99/88	-/-	0.93/67
	Combined	1/100	0.99/80	0.98/99	0.89/60	1/99
Species tree	Combined	0.99/93.9	0.49/92.1	0.85/-	-/-	0.57/-

Table 4. Comparison between results from different models implemented on BioGeoBEARS. Models utilized, likelihood ratio tests, number of parameters (n. params), estimations for dispersion (d), extinction (e) and founder effect/jump speciation, AIC and P-values. In highlight, the most probable model.

Model	LnL	n. params	d	e	J	AIC	P value
DEC	-23,90386	2	4,84E-02	1,00E-12	0	51,8	
DEC+J	-18,75983	3	1,00E-12	1,00E-12	0,022	43,52	0,0013
DIVA	-19,22746	2	5,11E-02	4,90E-09	0	42,44	
DIVALIKE+J	**-17,24254**	**3**	**1,00E-12**	**1,00E-12**	**0,021**	**40,49**	**0,047**
BAYAREA	-39,95262	2	9,48E-02	2,09E-01	0	79,91	
BAYAREALIKE+J	-21,18978	3	1,00E-07	1,00E-07	0,032	48,38	7,40E-09

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Apêndices

Material suplementar do capítulo único

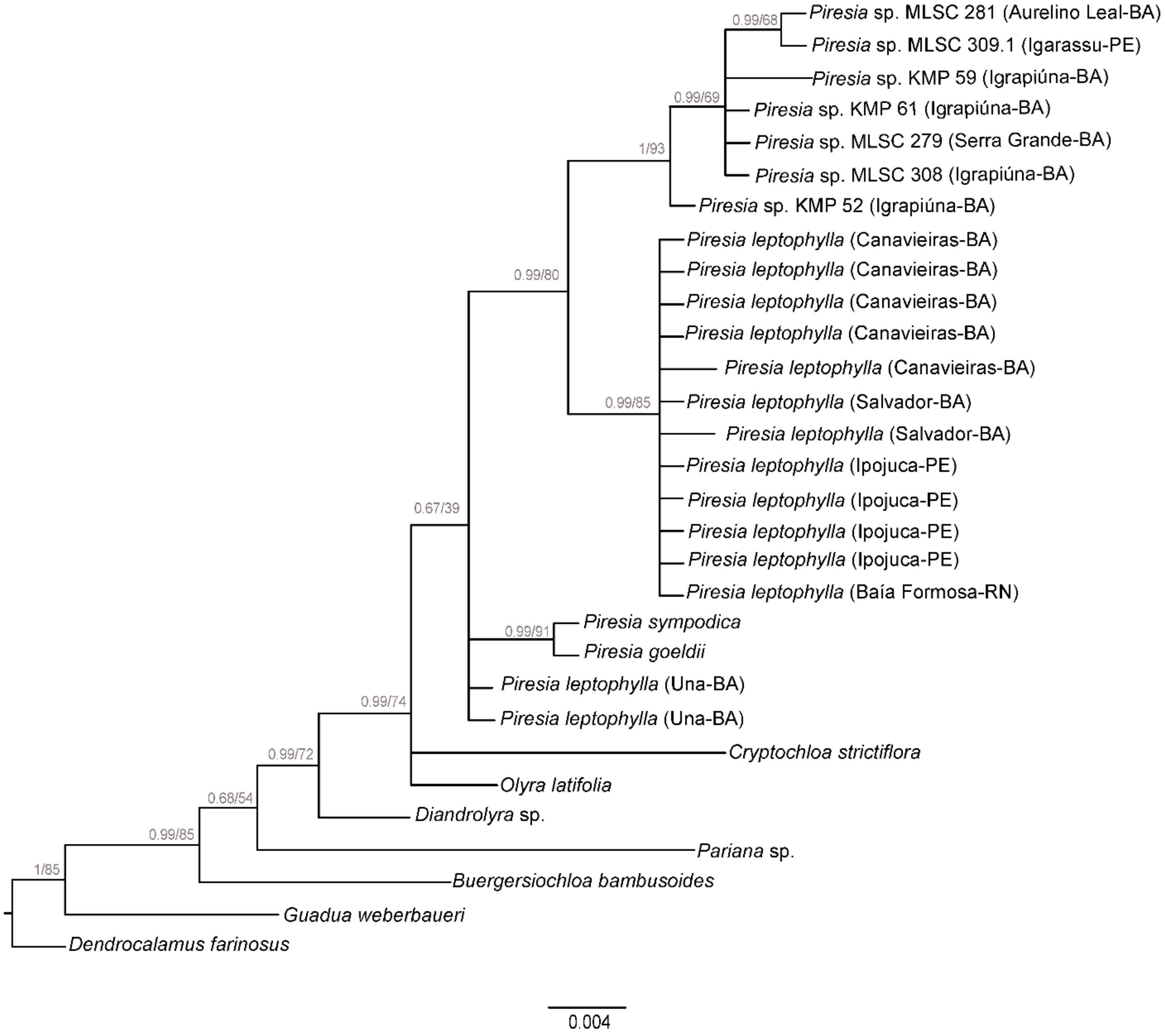


Figure S1. Topology obtained through Maximum Likelihood (ML) Bayesian Inference (BI) for the plastidial DNA gene *ndhF*. Values presented in each node the posterior probability of BI and the bootstrap values for ML, respectively separated by a slash ("/").

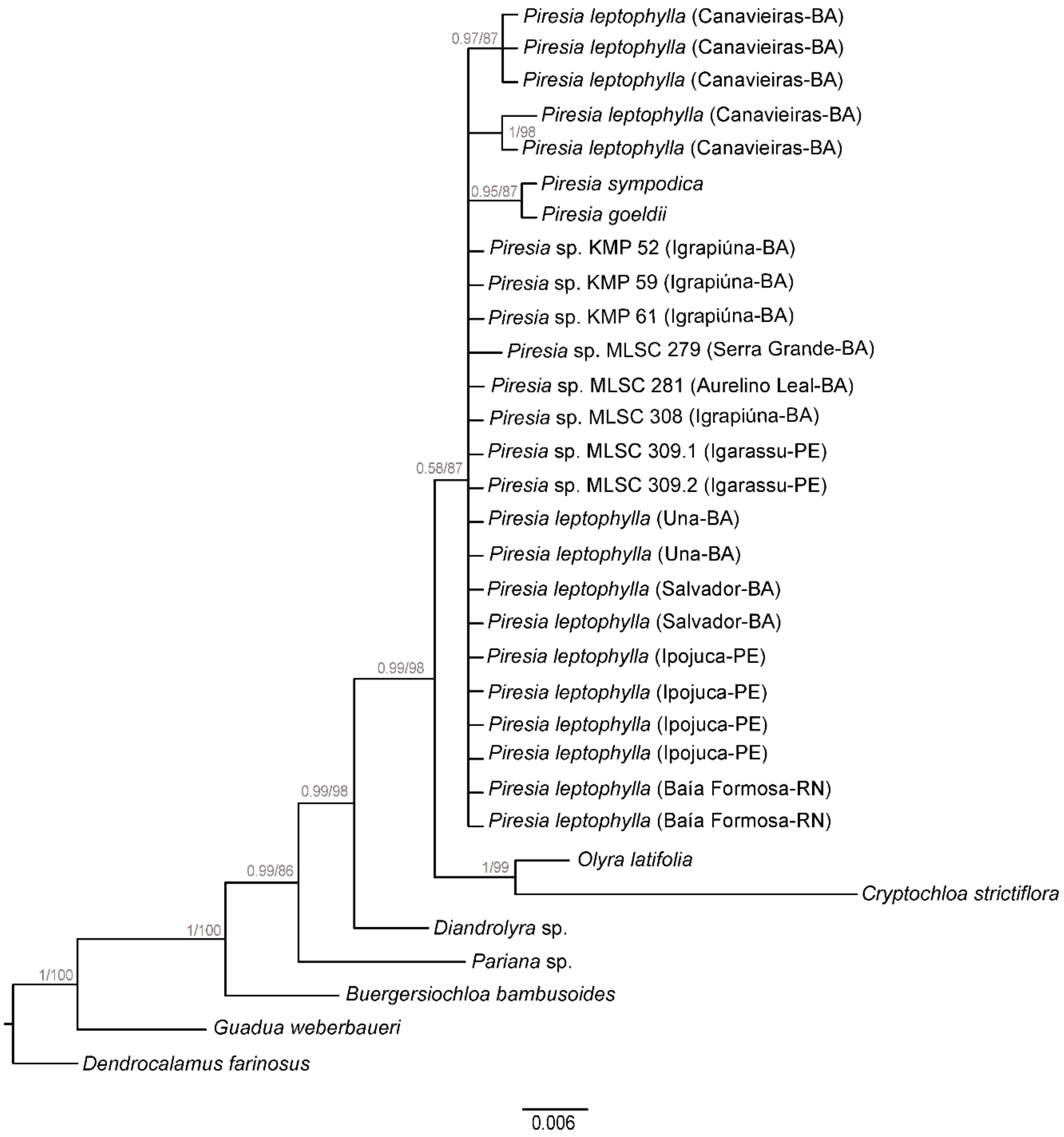


Figura S2. Topology obtained through Maximum Likelihood (ML) Bayesian Inference (BI) for the plastidial DNA intron *rpL16*. Values presented in each node the posterior probability of BI and the bootstrap values for ML, respectively separated by a slash (“/”).

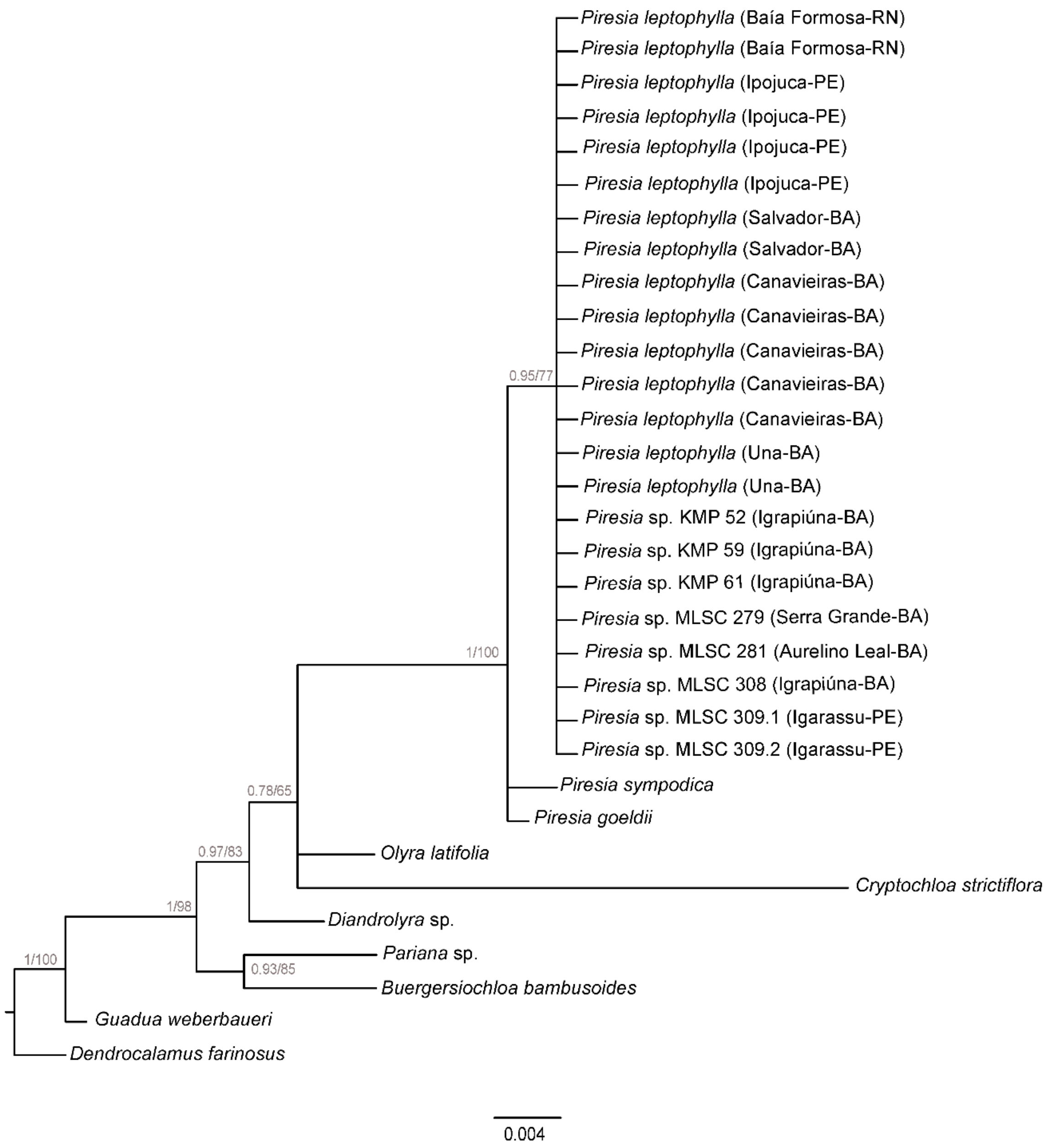


Figure S3. Topology obtained through Maximum Likelihood (ML) Bayesian Inference (BI) for the plastidial DNA spacer *trnD-trnT*. Values presented in each node the posterior probability of BI and the bootstrap values for ML, respectively separated by a slash (“/”).

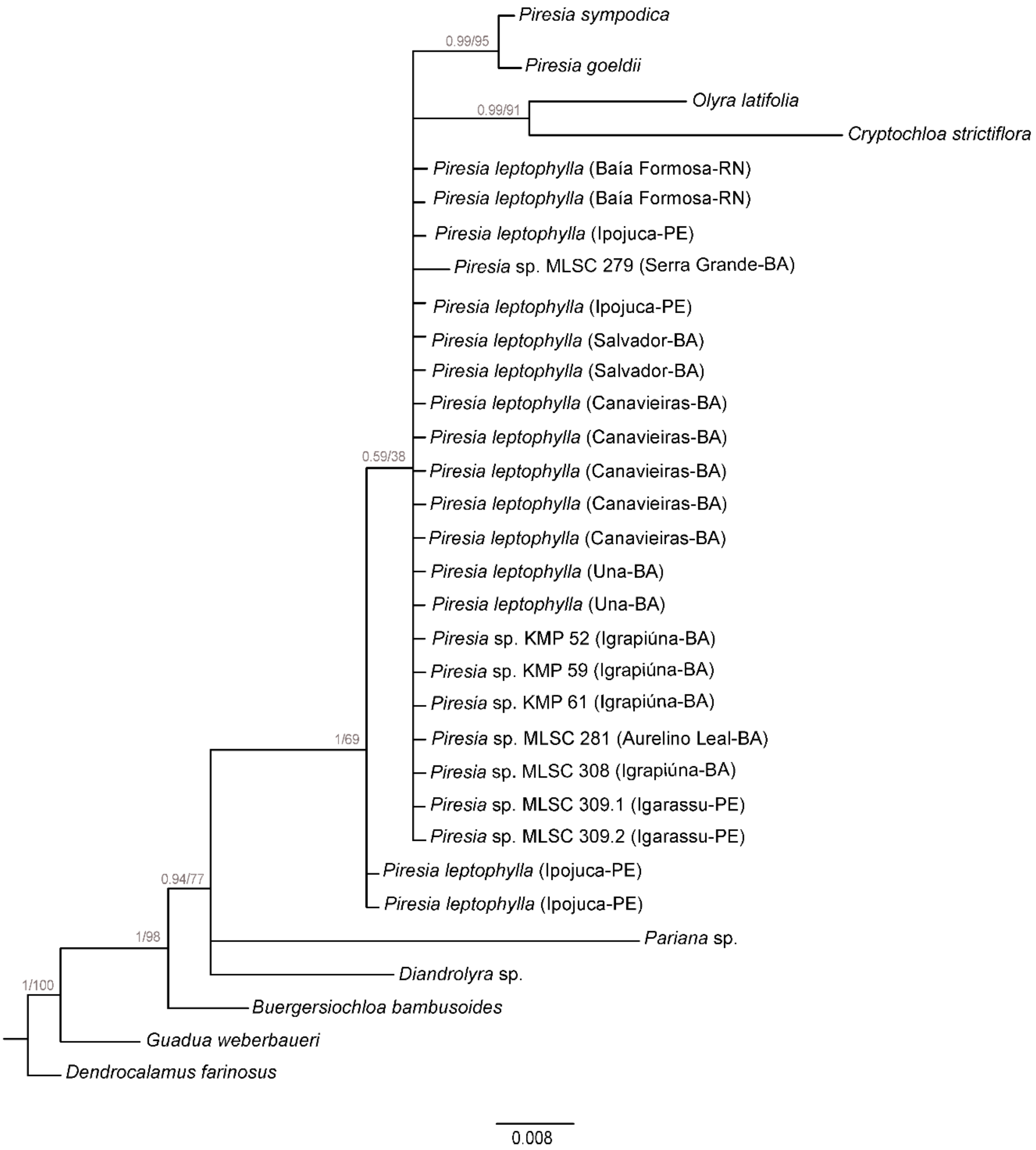


Figura S4. Topology obtained through Maximum Likelihood (ML) Bayesian Inference (BI) for the plastidial DNA spacer *trnL-trnF*. Values presented in each node the posterior probability of BI and the bootstrap values for ML, respectively separated by a slash ("/").

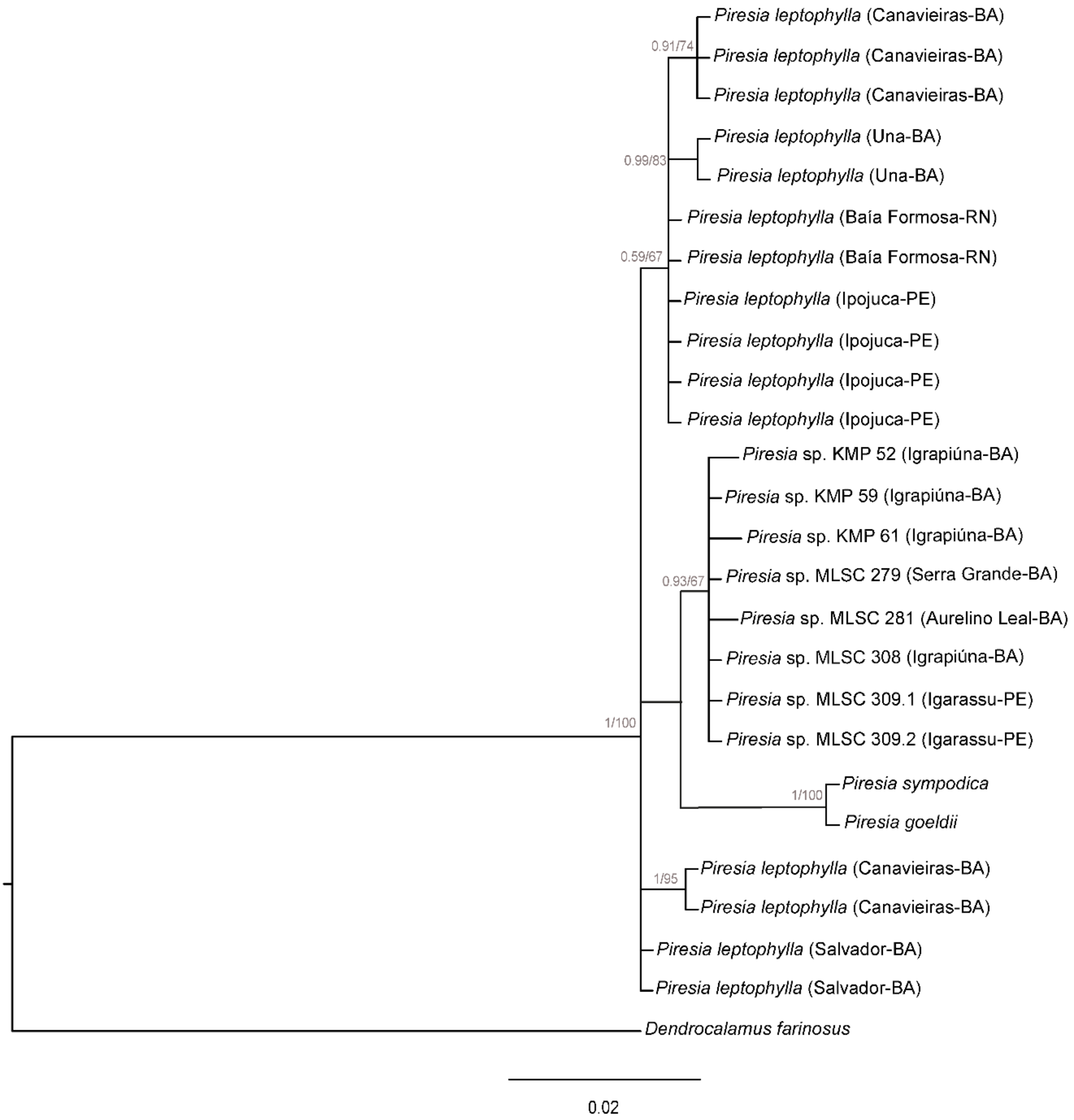
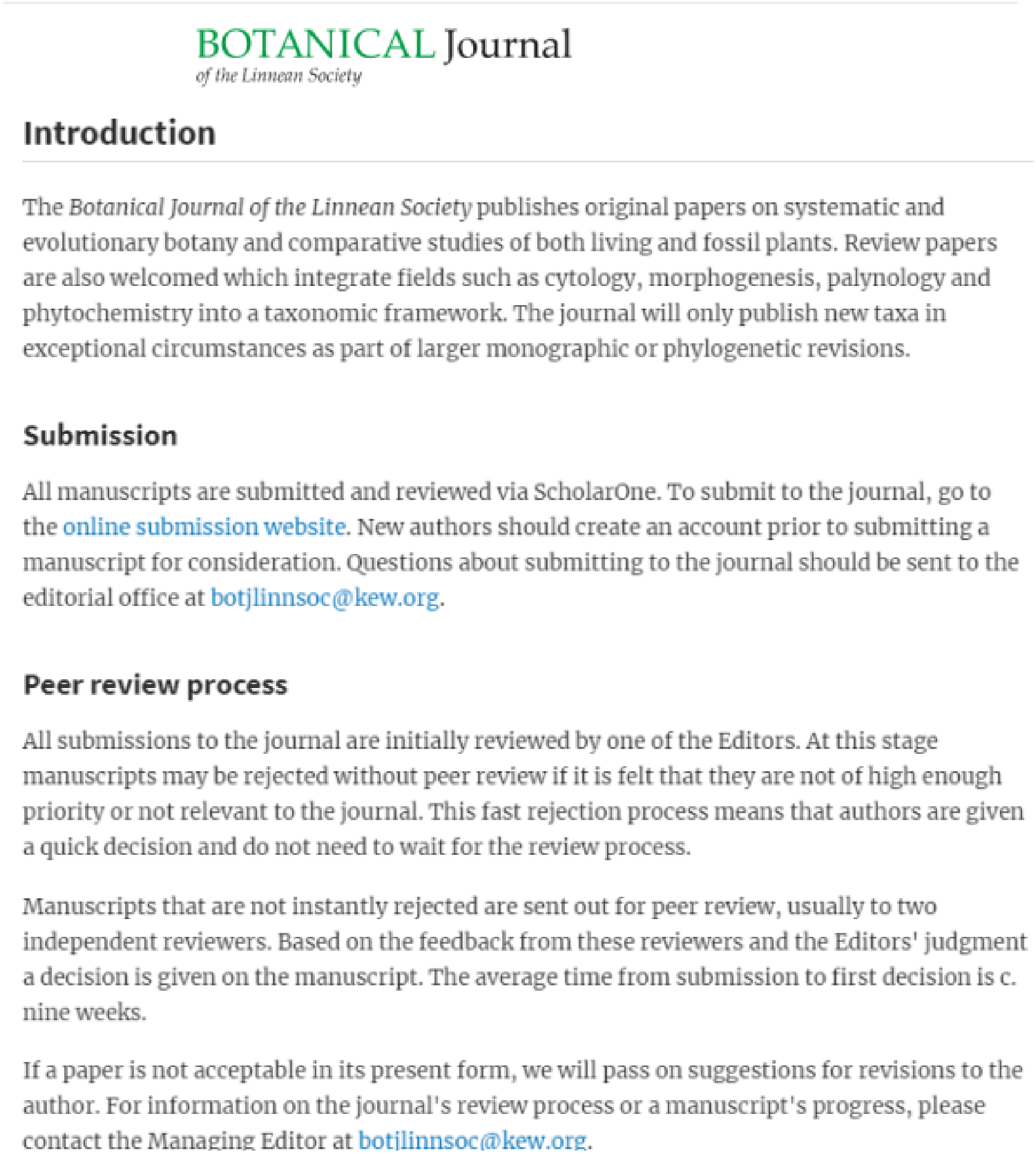


Figura S5. Topology obtained through Maximum Likelihood (ML) Bayesian Inference (BI) for the nuclear DNA gene *LEAFY*. Values presented in each node the posterior probability of BI and the bootstrap values for ML, respectively separated by a slash (“/”).

Anexos

Normas do periódico



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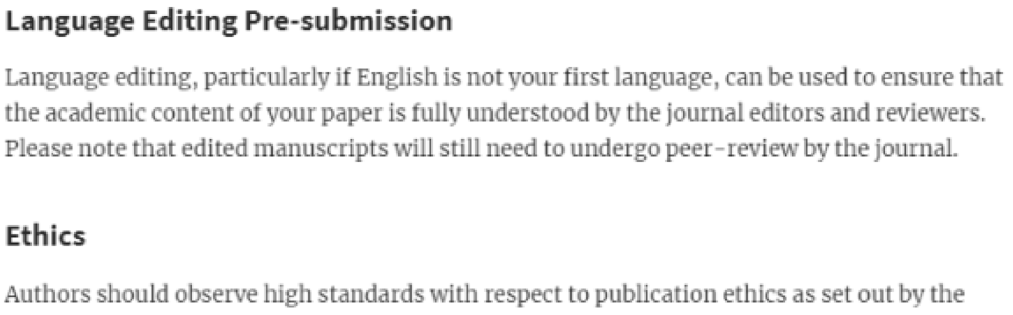

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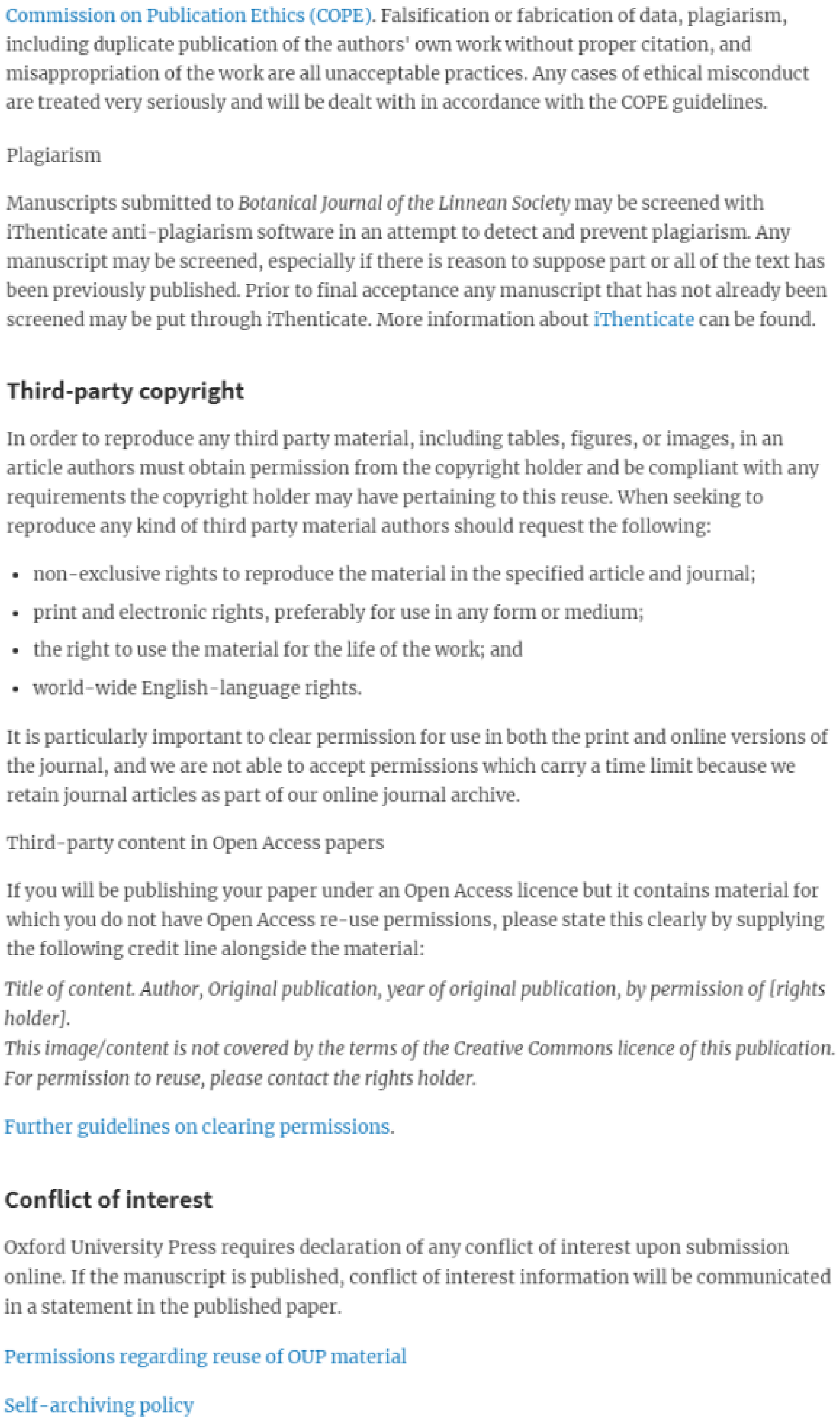

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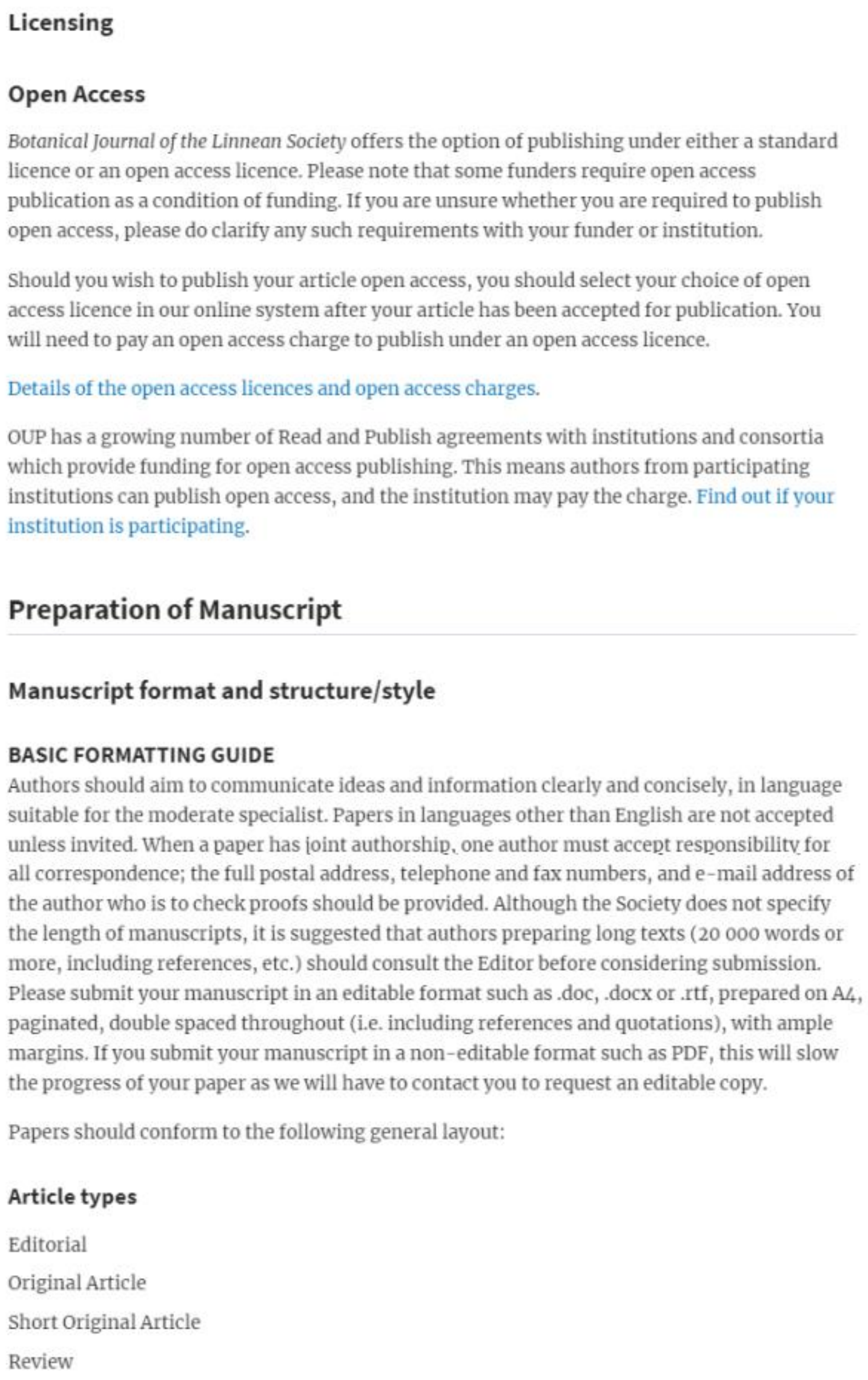

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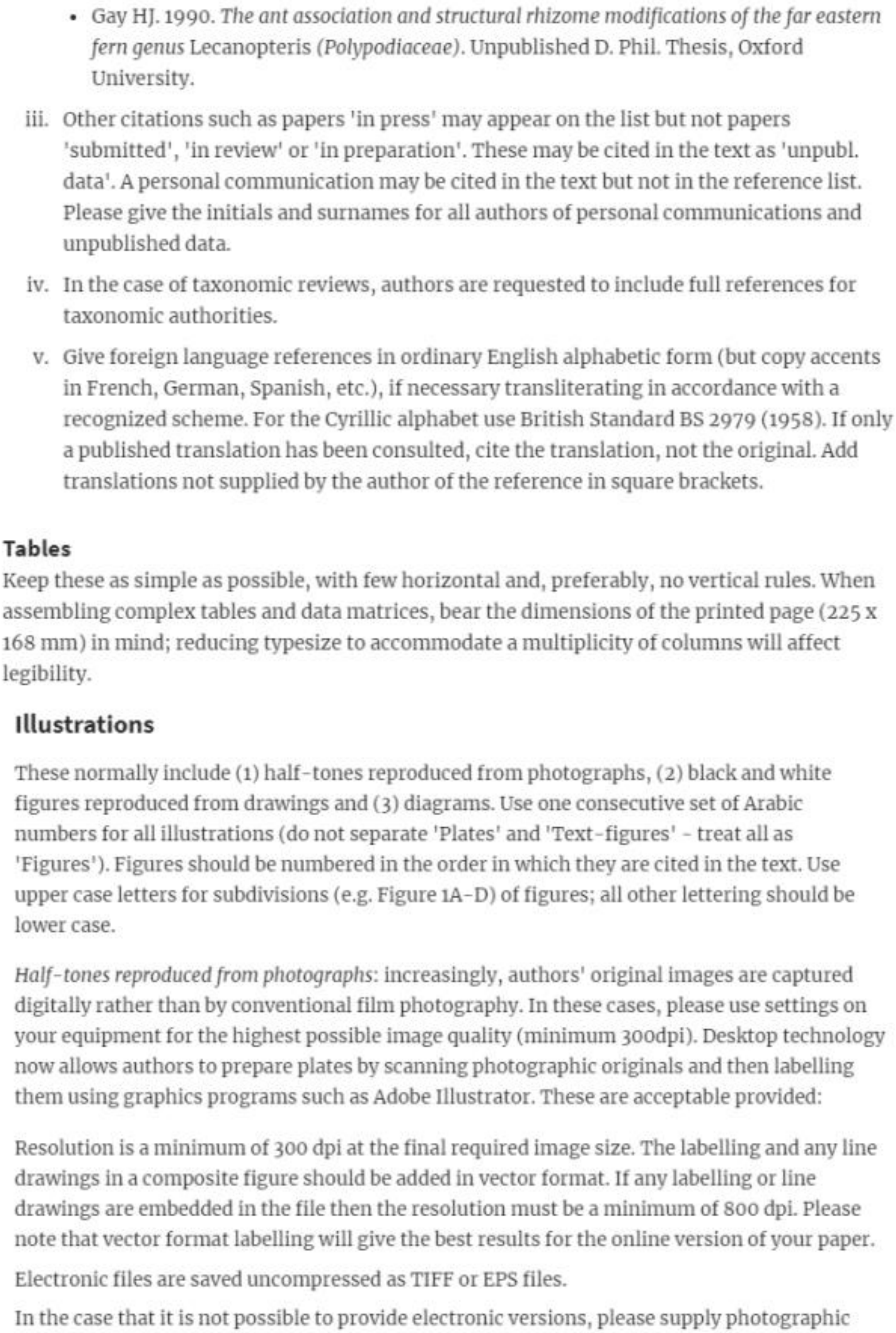
- Dow MM, Cheverud JM, Rhoads J, Friedlaender J. 1987b. Statistical comparison of biological and cultural/history variation. In: Friedlaender J, Howells WW, Rhoads J, eds. *Solomon Islands project: health, human biology, and cultural change*. New York: Oxford University Press, 265-281.



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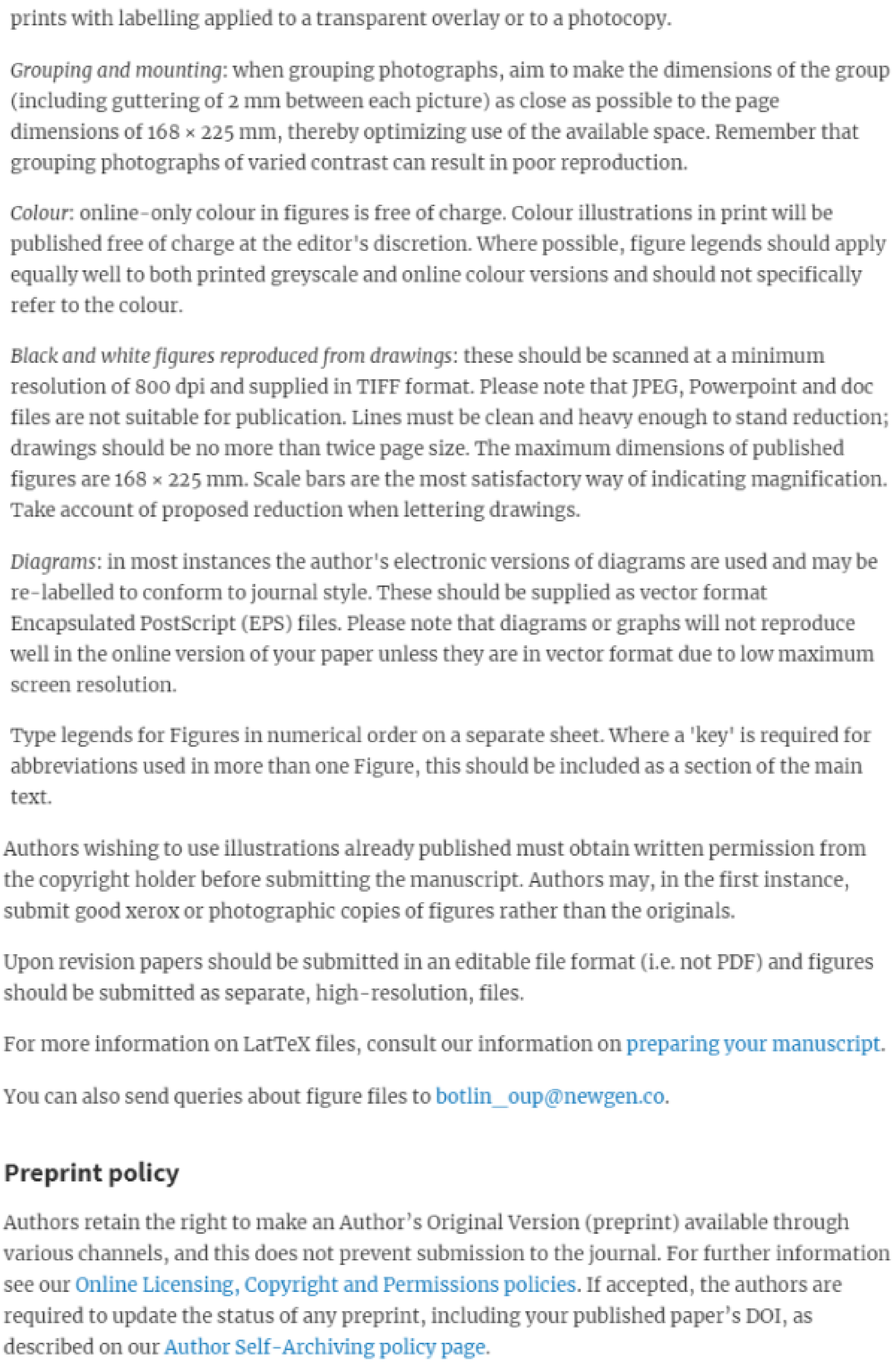

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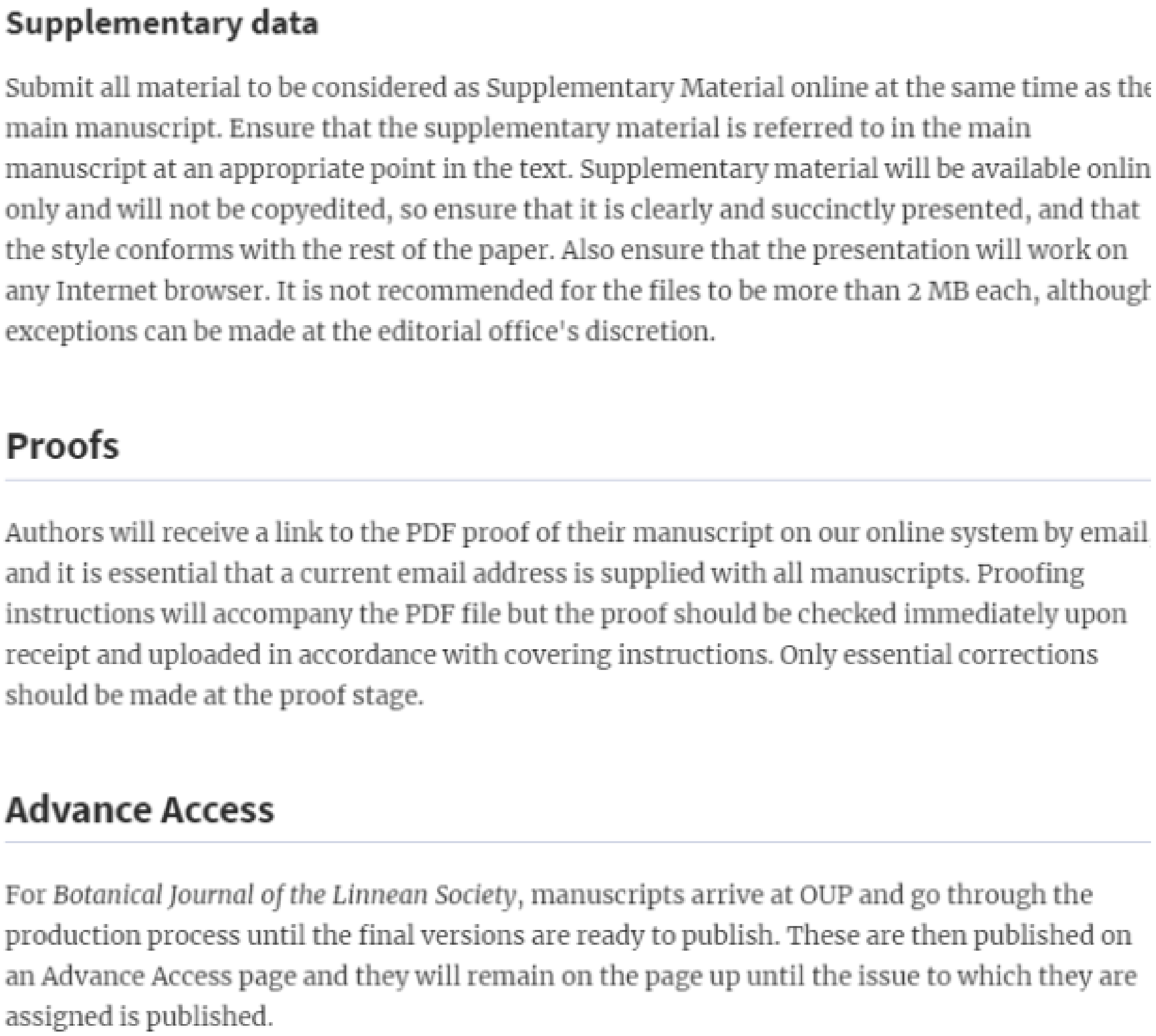

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