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SINZINANDO ALBUQUERQUE DE LIMA

ISOLAMENTO REPRODUTIVO ENTRE DUAS ESPÉCIES QUIROPTERÓFILAS SIMPÁTRICAS DE *BAUHINIA* L. (FABACEAE)

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Área de concentração: Ecologia e Conservação

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Orientador: Prof. Dr. Isabel Cristina Sobreira Machado

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“Nada na Biologia faz sentido exceto à luz da Evolução.”

Theodosius Dobzhansky

RESUMO

O isolamento reprodutivo entre espécies envolve a contribuição de múltiplas barreiras pré e pós-polinização, sendo essas barreiras cruciais para a formação e manutenção de espécies na natureza. Observações preliminares realizadas em uma área de Caatinga ofereceram uma situação interessante que serviu como um bom modelo para investigar as barreiras do isolamento reprodutivo: duas espécies filogeneticamente relacionadas e ecologicamente similares do gênero *Bauhinia* L. que ocorrem simpatricamente e compartilham os mesmos vetores de pólen. Portanto, neste estudo, investigamos o isolamento reprodutivo entre *B. acuruana* e *B. pentandra* no Parque Nacional do Catimbau. Nossas medições quantitativas dos componentes do isolamento reprodutivo mostraram que elas são completamente isoladas por uma combinação de barreiras temporais, espaciais, como também de isolamento interespecífico. Encontramos alto isolamento geográfico e, apesar da sobreposição na floração, há diferenças no pico de floração entre as duas espécies. Ambas as espécies apresentam atributos florais condizentes com quiropterofilia, e há partilha pelos mesmos morcegos polinizadores, porém a transferência interespecífica de pólen entre os dois táxons é evitada devido às diferenças no tamanho das flores, levando a uma deposição diferencial de pólen no corpo do polinizador. Além disso, encontramos forte incompatibilidade intertaxa. Propomos que vários fatores pré e pós-polinização contribuem para o isolamento dos dois taxas de *Bauhinia*, preservando a integridade deste par de espécies.

Palavras-chave: Distribuição geográfica. Glossophaginae. Isolamento mecânico. Morfologia floral. Polinização. Quiropterofilia.

ABSTRACT

The reproductive isolation between species involves the contribution of multiple pre- and post-zygotic barriers, being these barriers crucial to the formation and maintenance of species in nature. Preliminary observations carried out in a Caatinga area offer an interesting situation that served as a good model to investigate the barriers of reproductive isolation, two phylogenetically related and ecologically similar species of the genus *Bauhinia* L. that occur sympatrically, which shared the same pollen vectors. In this study, we investigated the reproductive isolation between *B. acuruana* and *B. pentandra* in the Catimbau National Park. Our quantitative measurements of the components of reproductive isolation have shown that they are completely isolated by a combination of temporal, spatial, as well as interspecific isolation barriers. We found high geographic isolation and, despite the overlap in flowering, there are differences in the peak of flowering between the two species. Both species have floral attributes consistent with chiroptera, and there is shared by the same pollinating bats, but the interspecific transfer of pollen between the two taxa is avoided due to differences in flower size, leading to a differential deposition of pollen in the pollinator body. In addition, we find strong intercept incompatibility. We propose that several pre and post-pollination factors contribute to the isolation of the two *Bauhinia* taxa, preserving the integrity of this pair of species.

Key-words: Chiropterophily. Floral morphology. Geographic distribution. Glossophaginae. Mechanical isolation. Pollination.

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1 INTRODUÇÃO

As barreiras reprodutivas são cruciais para a formação e manutenção de espécies na natureza (MAYR, 1942; COYNE & ORR, 2004) e o isolamento reprodutivo envolve a contribuição de múltiplas barreiras com diferentes importâncias para o isolamento total entre as espécies (RAMSEY et al., 2003; KAY, 2006; MARTIN & WILLIS, 2007). As barreiras reprodutivas podem ser classificadas como pré ou pós-polinização de acordo com o momento de sua ocorrência (BAACK et al., 2015).

Barreiras de pré-polinização incluem a separação espacial ou temporal da reprodução em espécies co-ocorrentes, tais como diferenciação de nicho (GROSSENBACHER et al., 2014), morfologias florais divergentes (GRANT & GRANT, 1964) ou assincronia de floração forte entre espécies co-ocorrentes (MARQUES et al., 2007). Barreiras pré-polinização são geralmente consideradas as barreiras mais importantes na manutenção da integridade das espécies, uma vez que agem no início do ciclo de vida de um organismo, impondo o mais forte impedimento ao fluxo gênico (LOWRY et al., 2008). No entanto, barreiras pós-polinização também são muito eficazes, reduzindo a viabilidade de híbridos ou seu potencial reprodutivo (BAACK et al., 2015), mesmo após a transferência de pólen heteroespecífico (YANG et al., 2007).

O gênero *Bauhinia* L. (Fabaceae - Cercidoideae) tem cerca de 125 espécies entre arbustos, árvores e cipós com distribuição pantropical (SINOU et al., 2009) e corresponde a aproximadamente 3,4% das Leguminosas. As flores das espécies de *Bauhinia* apresentam diversas formas, tamanhos, colorações, períodos e duração da antese (HOKCHE & RAMIREZ, 1990; ENDRESS, 1994). Muitas vezes, essa diversidade floral está associada a uma grande diversidade de polinizadores (HEITHAUS et al., 1974, RAMÍREZ et al., 1984, BERGALLO, 1990).

Observações preliminares realizadas em uma área de Caatinga ofereceram uma situação interessante que serviu como um bom modelo para investigar as barreiras do isolamento reprodutivo: duas espécies filogeneticamente relacionadas e ecologicamente similares do gênero *Bauhinia* L. que ocorrem simpatricamente e compartilham os mesmos vetores de pólen. No presente estudo, portanto, foram investigados aspectos do isolamento reprodutivo entre duas espécies simpátricas de *Bauhinia* polinizadas por morcegos, destacando o papel e as forças das barreiras reprodutivas que permitem essas espécies se manterem isoladas reprodutivamente.

Espera-se contribuir para o conhecimento da importância e do papel das barreiras reprodutivas para o isolamento reprodutivo entre espécies simpátricas e ecologicamente similares, especialmente no contexto de plantas polinizadas por morcegos. Esse estudo é o primeiro que mediu o isolamento reprodutivo em espécies simpátricas polinizadas por morcegos utilizando métodos para estimar a força do isolamento reprodutivo através de barreiras pré e pós-polinização.

2 FUNDAMENTAÇÃO TEÓRICA

2.1 POLINIZAÇÃO POR MORCEGOS (QUIROPTEROFILIA)

As Angiospermas são plantas que apresentam flores e majoritariamente tratam-se de organismos sésseis na fase adulta, que na sua maioria necessitam do auxílio de agentes externos para reprodução sexuada (PROCTOR, 1996; RECH et al., 2014). A função desses agentes externos é promover o transporte dos gametas masculinos, os grãos de pólen, até o estigma de outras flores em indivíduos da mesma espécie (FAEGRI & PIJL, 1979). Os estudos envolvendo os animais polinizadores e as plantas com flores são importantes para o entendimento da história evolutiva das Angiospermas, como também a compreensão dos processos evolutivos determinantes para diversidade biológica existente (ENDRESS, 1994; WILLMER, 2011). Além disso, são importantes para programas de conservação e gestão dos vários ecossistemas, bem como para a agricultura (WINFREE et al., 2011).

Dentre os vertebrados, existem vetores de pólen: mamíferos (voadores e não voadores), aves, e algumas espécies de répteis (lagartos). No entanto, aves e morcegos representam a mais expressiva parte das interações entre flores e vertebrados antófilos (RECH et al., 2014; WILLMER, 2011), sendo as aves vetores de pólen de pelo menos 65 famílias de angiospermas e os morcegos de 67 famílias de Angiospermas (DOBAT & PEIRKERT-HOLLE, 1985; CRONCK & OJEDA, 2008; FLEMING et al., 2009).

A polinização por esses vertebrados é bastante vantajosa, já que esses organismos possuem grande tamanho e elevada capacidade de deslocamento, transportando uma maior quantidade de grãos de pólen a uma maior distância (FLEMING et al., 2009). Além disso, o papel da polinização por vertebrados é mais substancial em locais onde as atividades dos insetos são limitadas por condições abióticas adversas (CRUDEN, 1972). Sendo assim, os vertebrados antófilos são excelentes em promover a polinização cruzada (ENDRESS, 1994; FLEMING et al., 2009; RECH et al., 2014).

Em regiões tropicais, o sistema de polinização por vertebrados compreende de 3,6 a 28% das espécies de angiospermas ocorrentes na comunidade (BAWA et al.,

1985; SILBERBAUER-GOTTSBERGER & GOTTSBERGER, 1988; ORMOND et al., 1993; KRESS & BEACH, 1994; OLIVEIRA & GIBBS, 2000; MACHADO & LOPES, 2004). Cerca de 880 espécies de vertebrados são especialistas em consumir néctar e no Brasil, cerca 338 espécies de vertebrados, pertencentes a 135 gêneros e 25 famílias são efetiva ou potencialmente vetores de pólen (FLEMING & MUCHHALA, 2008; BUZATO et al., 2012).

A quiropterofilia é o sistema de polinização no qual os polinizadores efetivos são morcegos e as plantas quiropterófilas geralmente possuem características comuns relacionadas à síndrome de polinização, como antese noturna ou crepuscular, cores claras, sombrias e opacas, emissão de um forte odor similar a frutos em fermentação e grande quantidade de néctar, com baixas concentrações de açúcares (FAEGRI & PIJL, 1979; MACHADO & LOPES, 1998, WILLMER, 2011).

As flores polinizadas por morcegos utilizam diferentes sinais para anunciar sua presença, entre elas é a presença de odores fétidos que é característico dessas flores, os quais são ricos em compostos contendo enxofre. Outro sinal são as cores das flores polinizadas por morcegos, que geralmente são brancas, marrons e verdes, podendo ser encontradas também flores de cores brilhantes como o rosa, fúcsia e amarelo (BAKER, 1960; FLEMING et al., 2009; TSCHAPKA & DRESSLER, 2002).

A posição das flores também é uma característica importante para esse sistema de polinização. No geral essas flores se posicionam longe da folhagem, podendo ou as flores ou inflorescências serem projetadas para cima ou para baixo (flageliflloria) das folhas, ou também essas flores e inflorescências podem sair diretamente dos ramos ou tronco (caulifloria), ou até mesmo, em alguns casos em algumas espécies de árvores decíduas, cujas plantas florescem apenas depois que as folhas caem (FLEMING et al., 2009; RECH et al., 2014; TSCHAPKA & DRESSLER, 2002).

A condição de nectarivoria na ordem Chiroptera evoluiu independentemente nos Neotrópicos (Microchiroptera – família Phyllostomidae) e Paleotrópicos (Macrochiroptera – família Pteropodidae). No entanto, os morcegos especializados em néctar das duas famílias compartilham características comuns associadas a este modo de alimentação. Estas incluem rostro alongado, dentição reduzida, tanto em tamanho como em número de dentes e uma longa língua que apresenta papilas filiformes, que auxilia na coleta de néctar nas rápidas visitas às flores (FLEMING et al., 2009; FLEMING & MUCHHALA, 2008).

Embora compartilhem esse conjunto de características comuns, cada uma das famílias com representantes quiropterófilos possui características e aspectos que variam na sua forma de interação com as flores. Os morcegos neotropicais nectarívoros são menores em tamanho, possuem línguas mais longas e pairam enquanto realizam as visitas às flores, de maneira que seus homólogos da região paleotropical são maiores, possuem línguas mais curtas e não pairam sobre as flores durante as visitas. Outra característica importante, que apenas os morcegos do novo mundo apresentam, é o uso de ultra-sons e ecolocalização para forrageamento do recurso (FLEMING et al., 2009; MUCHHALA & MUCHHALA, 2008; RECH et al., 2014).

2. 2 POLINIZAÇÃO NOTURNA E INTERAÇÃO COM MORCEGOS ANTÓFILOS NA FAMÍLIA FABACEAE

A maioria das espécies vegetais são metabolicamente ativas durante o dia, fator esse, evidenciado pela maneira como as reações importantes da fotossíntese ocorrem durante o período diurno. Também se acredita que a maioria dos animais envolvidos na polinização são diurnos e desta maneira, se esta premissa for verdadeira, a maior parte da polinização ocorre durante o dia, sendo a polinização no período noturno uma exceção (BORGES et al., 2016).

A polinização no período noturno parece ser uma estratégia importante para espécies vegetais que sofrem com estresse hídrico, ocorrendo essencialmente em famílias com adaptações xerófitas, sugerindo que a floração noturna é principalmente uma adaptação a esse estresse hídrico (BORGES et al., 2016). Esses autores hipotetizaram que a antese noturna é uma vantagem seletiva pela redução da perda de água através da evapotranspiração, levando ao aumento do tamanho das flores. Por sua vez, as flores noturnas que oferecem mais recursos, como uma maior quantidade de néctar, apoiariam assim os polinizadores que demandam maiores recompensas (BORGES et al., 2016).

A polinização noturna é realizada por uma gama de grupos de animais, entre os quais, as mariposas (especialmente os esfingídeos) e os morcegos são os mais importantes (BAKER, 1961; BORGES et al., 2016). As espécies vegetais polinizadas por cada um desses grupos de animais polinizadores possuem atributos intimamente relacionados às características desses animais. Essa associação denomina-se síndrome de polinização, que no caso das mariposas denomina-se esfingofilia e nos morcegos a quiropterofilia (FAEGRI & PILJ, 1969, PROCTOR, 1996, WILMER, 2011, RECH, et al. 2014).

Em seu trabalho, BORGES et al. (2016) mostraram que 113 famílias de Angiospermas possuem polinização noturna perfazendo cerca de 30% de todas as famílias e 68% das ordens de angiospermas. Entre essas, temos a ordem Fabales, na qual temos apenas a família Fabaceae (= Leguminosae) com espécies de antese noturna.

Fabaceae é, depois das Asteraceae e Orchidaceae, o grupo mais diversificado de angiospermas (RODRÍGUEZ-RIAÑO et al., 1999). É uma família cosmopolita, sendo mais representativa nas regiões temperadas e tropicais, com 765 gêneros e 19500 espécies divididas em seis subfamílias a partir das análises filogenéticas baseadas na região plastidial (LPWG, 2017). De acordo com nova circunscrição, a família Fabaceae está dividida em seis subfamílias (Caesalpinioideae (incl. Clado mimosoid), Cercidoideae, Detarioideae, Dialioideae, Duparquetioideae, Papilionoideae) (LPWG, 2017) das quais quatro delas (Caesalpinioideae, Cercidoideae, Detarioideae, Papilionoideae) têm registros de espécies polinizadas por morcegos (Tabela 1). Dentre as espécies destes táxons, 2,3% têm registro de polinização por morcegos (FLEMING et al., 2009; LIMA & MACHADO, *dados não publicados*). A quiropterofilia ocorre principalmente nas regiões tropicais do mundo, sendo a região Neotropical a que apresenta o maior número de espécies de leguminosas quiropterófilas (FLEMING et al., 2009*).* Dentre as espécies de leguminosas quiropterófilas, os principais gêneros que possuem flores adaptadas à polinização por morcegos são *Bauhinia* L. (Cercidoideae), *Mucuna* Adans. (Papilionoideae) e *Parkia* R. Br. (Caesalpinioideae) (FLEMING et al., 2009).

2. 3 SÍNDROMES DE POLINIZAÇÃO E SISTEMA SEXUAL E REPRODUTIVO EM *BAUHINIA*

O gênero *Bauhinia* (Cercidoideae) possui cerca de 300 espécies entre arbustos, árvores e lianas com distribuição pantropical e distribuídas em oito seções (*AfroBauhinia, Alvesia, Amaria, Bauhinia, Micralvesia, Pauletia, Pseudophanera, Telestria*) (SINOU et al., 2009), que correspondem a aproximadamente 3,4% das leguminosas. As espécies desse gênero são caracterizadas por apresentar folhas com nervuras palmatinérvias com dois folíolos provindos do mesmo pulvino. Apresentam inflorescências racemosas do tipo pseudo-racemo, com flores inseridas aos pares no eixo principal (Vaz & Tozzi, 2003).

A maior parte dos estudos publicados sobre polinização refere-se a observações esporádicas em determinadas espécies ou inferência sobre os possíveis visitantes florais baseadas nos atributos florais (HEITHAUS et al., 1974; RAMÍREZ et al., 1984; BERGALLO, 1990; HOKCHE & RAMIREZ, 1990; ENDRESS, 1994; MUNIN et al., 2008; PAULINO-NETO, 2013). Considerando-se todas as espécies de *Bauhinia,* apenas 30% do total de espécies do gênero foram estudadas quanto aos tipos de polinizadores ou sistema reprodutivo.

As espécies de *Bauhinia* apresentam flores com diversificadas formas, tamanhos, colorações, períodos e durações de suas anteses (HOKCHE & RAMIREZ, 1990; ENDRESS, 1994). Muitas vezes essa diversidade floral está associada a uma grande diversidade de agentes polinizadores, a qual está em consonância com diversas síndromes específicas de polinização, tais como quiropterofilia, melitofilia e esfingofilia (Figura 1) (HEITHAUS et al., 1974; RAMÍREZ et al., 1984; BERGALLO, 1990).

De maneira geral, as espécies com antese diurna de *Bauhinia* são polinizadas por abelhas, borboletas, beija-flores e algumas espécies polinizadas por diversos pequenos insetos. As espécies que possuem esses vetores de pólen apresentam características como flores coloridas, baixo volume de néctar e alta concentração de açúcar, odor suave e agradável, com antese diurna e guias de néctar (ENDRESS, 1994).

Por sua vez, as espécies de *Bauhinia* com antese noturna são visitadas por esfingídeos e/ou morcegos (HOKCHE & RAMIREZ, 1990) e essas espécies reúnem características clássicas, como flores brancas, antese noturna, odor forte desagradável ou adocicado, com grande volume e baixa concentração de açúcar no néctar (HEITHAUS et al., 1974MUNIN et al., 2008).

Em sua revisão taxonômica do gênero *Bauhinia* para o Brasil, VAZ (2001) divide as espécies da seção *Pauletia* em dois sistemas de polinização, o primeiro grupo especializado na polinização por morcegos e um segundo sistema mais generalizado, com as flores não visitadas por morcegos, não apresentando polinizador efetivo característico, mas sendo visitadas e polinizadas por diferentes grupos de animais como esfingídeos, noctuídeos e beija-flores.

Essa alta taxa de espécies associadas a estes dois sistemas de polinização é resultado da alta proporção de espécies que possuem flores com antese noturna. O estudo realizado por VAZ (2001) aponta que todas as espécies da seção *Pauletia,* composta por 58 táxons possuem a abertura de seus botões durante a tarde e as flores duram uma noite, fenecendo na manhã seguinte.

Com relação ao sistema sexual e reprodutivo das espécies de *Bauhinia*, embora a autofecundação ocorra em algumas espécies, a xenogamia é a forma mais frequente de reprodução das espécies do gênero (SILVEIRA, 2005; MUNIN et al., 2008). As flores podem apresentar mecanismos morfológicos que evitam a autofecundação, tal como a hercogamia, ou seja, a separação espacial entre o estigma e as anteras. Normalmente, este mecanismo dificulta a autopolinização e o congestionamento da superfície estigmática por pólen da mesma flor. O gênero inclui exemplos de andromonoicia e ginomonoicia, sistemas sexuais que podem permitir um aumento da função masculina ou feminina, respectivamente (ENDRESS, 1994, SILVA et al., 1997).

Embora as espécies de *Bauhinia* estudadas possuam sistema de polinização bem definidos, estudos têm mostrado que o gênero apresenta indícios de baixa eficiência reprodutiva, um fenômeno comumente observado na família Fabaceae (AGOSTINI, 2004; LEITE & MACHADO, 2010). Esse baixo sucesso reprodutivo foi evidenciado em diferentes casos, como em situações de baixa taxa de produção de frutos em relação ao número de flores abertas, baixo número de frutos desenvolvidos em relação ao de frutos jovens e alta taxa de aborto de sementes dos frutos desenvolvidos (WEBB & BAWA, 1985; SILVEIRA, 2005; MUNIN et al., 2008; BERGAMINI, 2010). No entanto, as causas do baixo sucesso reprodutivo das espécies estudadas ainda não são bem conhecidas.

A maioria dos estudos de polinização com *Bauhinia* foram realizados na América com destaque para o Brasil, a Venezuela e o México, correspondendo a 75% de todas as espécies estudadas (Figura 2, 3). Para outras regiões tropicais podemos destacar a China com 15% das espécies estudadas (Figura 2, 3). O Brasil destaca-se com 41% de todos os trabalhos realizados, no entanto podemos observar que a maioria foi realizado nas regiões de Cerrado e na Caatinga, com um único estudo para a região Amazônica (Figura 3).

2. 4 BARREIRAS E ISOLAMENTO REPRODUTIVO EM ESPÉCIES POLINIZADAS POR MORCEGOS

Nos últimos anos foram feitos grandes avanços nos estudos que avaliam os componentes do isolamento reprodutivo das espécies vegetais (WIDMER et al., 2009). O isolamento reprodutivo tem grande importância na biologia evolutiva, é primordial para que ocorra o processo de especiação e ele ocorre através de múltiplas barreiras que restringem o fluxo gênico entre as populações (WIDMER et al., 2009; BAACK et al., 2015).

As barreiras reprodutivas geralmente são classificadas de acordo com o momento em que ocorrem e/ou se sua expressão é dependente do meio ambiente (BAACK et al., 2015). Em sua revisão, BAACK et al. (2015) apontam que a classificação das barreiras reprodutivas em pré- e pós-zigóticas é inadequada para as plantas, uma vez que a mesma barreira reprodutiva pode ter mecanismos pré ou pós-zigóticos envolvidos, e assim utilizam os termos de barreiras pré e pós-polinização. Aqui seguiremos a proposta feita por BAACK et al. (2015) para avaliar estudos que abordam o isolamento reprodutivo entre espécies polinizadas por morcegos.

Entre as barreiras do isolamento reprodutivo, às de pré-polinização têm sido consideradas as principais em promover fortemente a redução do fluxo gênico entre as espécies, sendo assim as principais em promover o isolamento reprodutivo (WIDMER et al., 2009). Em espécies polinizadas por beija-flores, estudos mostram que essas barreiras podem ser responsáveis por até 100% do isolamento reprodutivo em espécies intimamente relacionadas (KAY, 2006).

Dentre as barreiras pré-polinização, podemos encontrar diferentes mecanismos que permitem o isolamento reprodutivo, como, *I- Isolamento fenológico*, no qual as espécies apresentam épocas de floração diferenciadas, permitindo assim o compartilhamento dos mesmos polinizadores escalonado no tempo. *II-Especialização de polinizadores*, no qual as espécies diferem nos seus atributos florais (e.g. forma, cor, cheiro, etc) e consequentemente atraem uma gama de polinizadores diferentes, resultando em um menor compartilhamento ou mesmo na sua ausência e *III-Diferenciação de nicho*, no qual espécies relacionadas podem ser eco-geograficamente isoladas, não permitindo o fluxo gênico entre elas (RAMSEY et al., 2003; BAACK et al., 2015).

A maioria dos trabalhos com plantas polinizadas por morcegos investigou aspectos da biologia reprodutiva e ecologia da polinização de espécies alvo, envolvendo plantas pertencentes à diferentes famílias de Angiospermas (VOGEL, 1957, 1968, 1969a, 1969b; HEITHAUS et al., 1974; SAZIMA & SAZIMA, 1980; LOCATELLI et al., 1997; MACHADO et al., 1998; SAZIMA et al., 2003; MACHADO & VOGEL, 2004; ROCHA et al., 2007; FLEMING et al., 2009), como também estudos com enfoque nas interações ao nível de comunidade de plantas quiropterófilas (SAZIMA et al., 1999; MUCHHALA & JARRÍN, 2002; TSCHAPKA, 2004; PRIMO, 2008; TEIXEIRA, 2010; QUEIROZ, 2012). No entanto os estudos com uma abordagem relacionada às barreiras reprodutivas responsáveis pelo processo de especiação em plantas polinizadas por morcegos são incipientes e não medem a força das barreiras que determinam o isolamento reprodutivo (QUEIROZ et al. 2015).

O isolamento geográfico geralmente é considerado como a primeira oportunidade para limitar o fluxo gênico na sequência de barreiras que podem agir para separar as espécies (BARRACLOUGH & VOGLER, 2000). Para os vegetais, poucos são os estudos que mostram diferenças eco-geográficas como barreira que promove o isolamento reprodutivo entre espécies, além disso estes são direcionados para plantas polinizadas por insetos (BRISCOE-RUNQUIST et al. 2014).

A polinização por morcegos é um sistema encontrado em espécies de mais de 50 famílias de Angiospermas, podendo estas espécies estarem distribuídas em diferentes ecossistemas (DOBAT & PEIKERT-HOLLE, 1985; FLEMING & MUCHHALA, 2008; FLEMING et al., 2009). Em muitas regiões podemos observar a ocorrência de espécies de um mesmo gênero que são ecologicamente semelhantes, mas que não se cruzam pelo fato de ocuparem diferentes habitats. Em seu estudo que compara a guilda de plantas polinizadas por morcegos em dois sítios da Floresta Atlântica do sudeste do Brasil, SAZIMA et al. (1995, 1999) mostraram que embora os dois sítios compartilhem espécies do mesmo gênero, não existe similaridade florística entre as duas comunidades, evidenciando que a diferença de habitat relacionada a diferença de elevação entre essas áreas podem servir de barreira e manter o isolamento reprodutivo entre essas espécies quiropterófilas.

Diferenças na época de floração podem servir de barreira reprodutiva para muitas espécies relacionadas e que ocorrem simpatricamente (MOSSELER & PAPADOPOL, 1989). Como foi pontuado acima, a maioria dos trabalhos com espécies quiropterófilas/polinizadas por morcegos são estudos de caso individuais para diferentes espécies. Dados de como o isolamento fenológico pode servir de barreira para o isolamento reprodutivo em espécies polinizadas por morcegos podem ser analisados a partir de trabalhos que avaliaram comunidades de espécies quiropterófilas.

Neste contexto, avaliando-se a comunidade de espécies polinizadas por morcegos em dois locais de Mata Atlântica, no sudeste do Brasil, SAZIMA et al. (1995, 1999) encontraram diferentes espécies do gênero *Vriesea* Beer (Bromeliaceae) que ocorriam de maneira simpátrica nas áreas estudadas e que eram polinizadas pela mesma espécie de morcego. Em uma das áreas estudadas ocorriam três espécies de *Vriesea* que apresentavam floração sequencial sem sobreposição ao longo do ano, permitindo assim o compartilhamento do mesmo polinizador, e evitando o fluxo gênico entre elas.

Na mesma perspectiva, em um estudo que investigou a guilda de plantas esfingófilas e quiropterófilas em um remanescente de Mata Atlântica estacional semidecidual no nordeste brasileiro, PRIMO (2008), encontrou duas espécies de *Capparis* L. (Capparaceae) que eram polinizadas por morcegos. Analisando a fenologia reprodutiva dessas espécies, o autor observou que em três anos de acompanhamento fenológico as duas apresentavam florações em distintas épocas do ano sem sobreposição.

Os trabalhos acima citados não possuem o enfoque de avaliar as barreiras reprodutivas que permitem o isolamento reprodutivo entre essas espécies simpátricas. No entanto, eles descreveram o padrão fenológico dessas espécies e, a partir dos dados obtidos, observaram que as espécies relacionadas filogeneticamente possuem floração em épocas distintas não havendo sobreposição, e, portanto, evidenciando que nesses casos o isolamento fenológico pode estar funcionando como barreira reprodutiva pré polinização para as espécies envolvidas.

As diferenças nos traços florais de espécies próximas também podem servir de barreira para impedir o fluxo de pólen entre essas espécies, funcionando assim como uma barreira reprodutiva e permitindo o isolamento reprodutivo (RAMSEY et al., 2003). Estudos mostram que a mudança de coloração das flores é um traço que pode servir de barreira reprodutiva entre espécies próximas e relacionadas filogeneticamente. Essa mudança na coloração pode levar à uma diferenciação na guilda dos seus visitantes florais (RAMSEY et al., 2003). Em outros casos as espécies apresentam diferenças nas estruturas reprodutivas, e isso pode levar a deposição diferencial de grãos de pólen sobre o corpo do polinizador (MUCHHALA, 2008).

Um dos casos mais bem documentados sobre a diferenciação nas estruturas reprodutivas servindo de barreira reprodutiva em plantas polinizadas por morcegos foi observado nas florestas tropicais úmidas do Equador (MUCHHALA, 2008). Espécies do gênero *Burmeistera* H.Karst. & Triana (Campanulaceae) podem co-ocorrer em determinadas áreas, sobrepondo sua fenologia de floração e recebendo visitas dos mesmos polinizadores. No entanto as diferentes espécies desse gênero apresentam uma grande variação na disposição de estruturas reprodutivas (algumas longas e outras curtas) que são externalizadas para fora da corola. Os resultados desse estudo mostraram que a diferenciação nas dimensões das estruturas florais permitia a deposição de pólen em diferentes regiões da cabeça dos morcegos, atenuando a mistura de pólen heteroespecífico, contribuindo assim com o isolamento reprodutivo entre as diferentes espécies de *Burmeistera* (MUCHHALA, 2008).

Um outro caso bem interessante é descrito por QUEIROZ et al. (2015) com duas espécies de *Ipomoea* L. (Convolvulaceae) que apresentaram o mesmo período de floração, ocorrendo simpatricamente e partilhando os mesmos vertebrados polinizadores (beija-flores e morcegos), formando frutos e sementes em cruzamentos intertaxa. Foi constatado que variações em aspectos como morfologia, morfometria e orientação floral, além de diferenças na antese e deposição diferencial de pólen no corpo dos polinizadores são fatores determinantes para minimizar o fluxo de pólen entre os dois taxas envolvidos, favorecendo assim seu isolamento reprodutivo (QUEIROZ, 2014).

Diante do exposto, acreditamos que as informações aqui revisadas possam contribuir para embasar substancialmente a discussão teórica dos resultados deste trabalho de dissertação, que busca entender como duas espécies de *Bauhinia* com ocorrência simpátrica, filogeneticamente aparentadas (pertencentes ao mesmo gênero), ecologicamente similares e que compartilham os vetores de pólen se mantêm isoladas reprodutivamente e quais são as barreiras que impedem o fluxo gênico entre esse par de espécies.

***Tabela 1**: Gêneros de cada subfamília de Fabaceae com registro de polinização por morcegos. Em parênteses número de espécies em cada um dos gêneros com registro de polinização por morcegos.*

Subfamílias	***Gêneros***
Caesalpinioideae	*Acacia* (01); *Albizzia* (01); *Caesalpinia* (02); *Calliandra* (04); *Conzattia* (01); *Dichrostachys* (01); *Erythrophleum* (01); *Inga* (06); *Jacqueshuberia* (02); *Lysiloma* (01); *Mimosa* (01); *Parkia* (18); *Peltophorum* (01)
Cercidoideae	*Bauhinia* (08)
Dialioideae	–
Detarioideae	*Browneopsis* (04); *Cynometra* (01); *Daniellia* (01); *Elizabetha* (03); *Eperua* (03); *Hymenaea* (02); *Intsia* (01)
Duparquetioideae	–
Papilionoideae	*Alexa* (01); *Castanospermum* (01); *Erythrina* (01); *Inocarpus* (01); *Lonchocarpus* (01); *Mucuna* (13); *Ormosia* (01); *Sesbania* (01)

Fontes: Dobat & Peikert-Hole 1985; Fleming *et al.* 2009

***Figura 1**: Síntese do estado atual do conhecimento das síndromes de polinização das espécies do gênero Bauhinia* (Fabaceae).

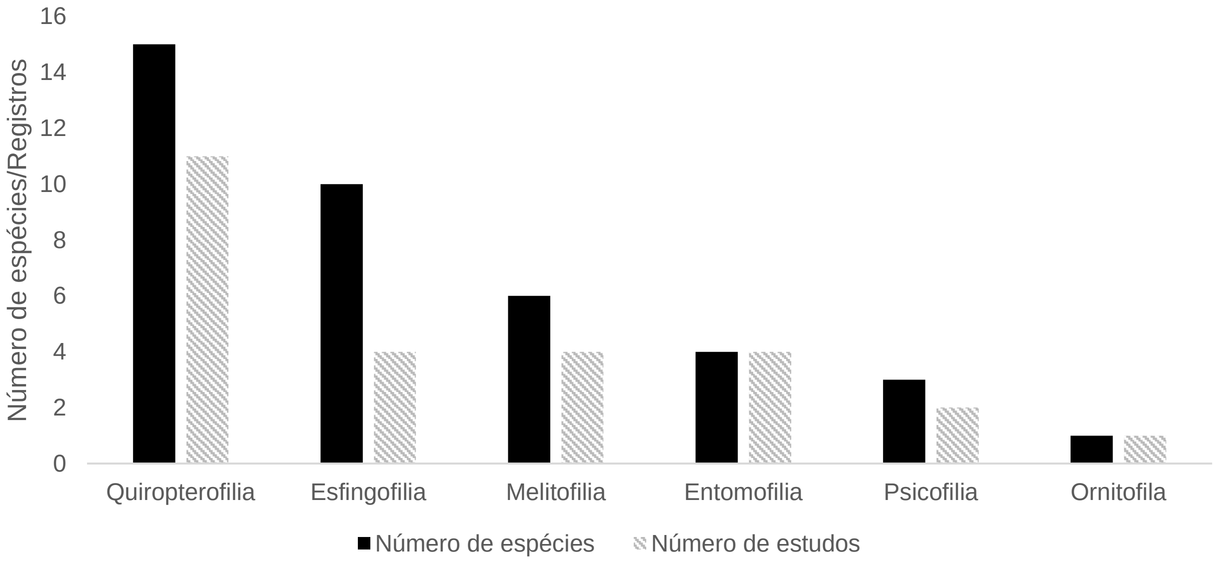


Figura 2: *Síntese do estado atual do conhecimento dos locais dos estudos dos sistemas de polinização para o gênero Bauhinia* (Fabaceae).

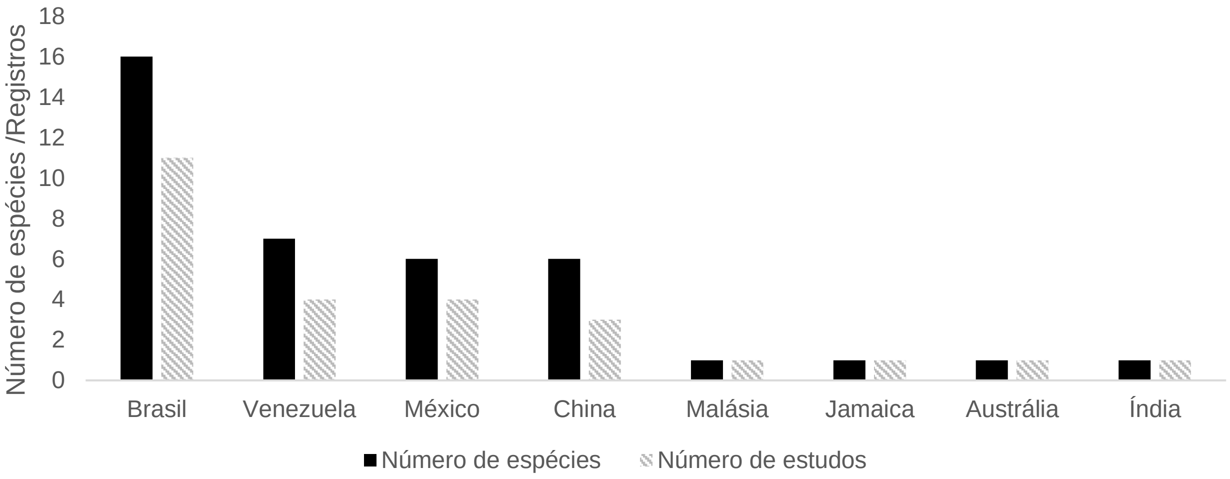
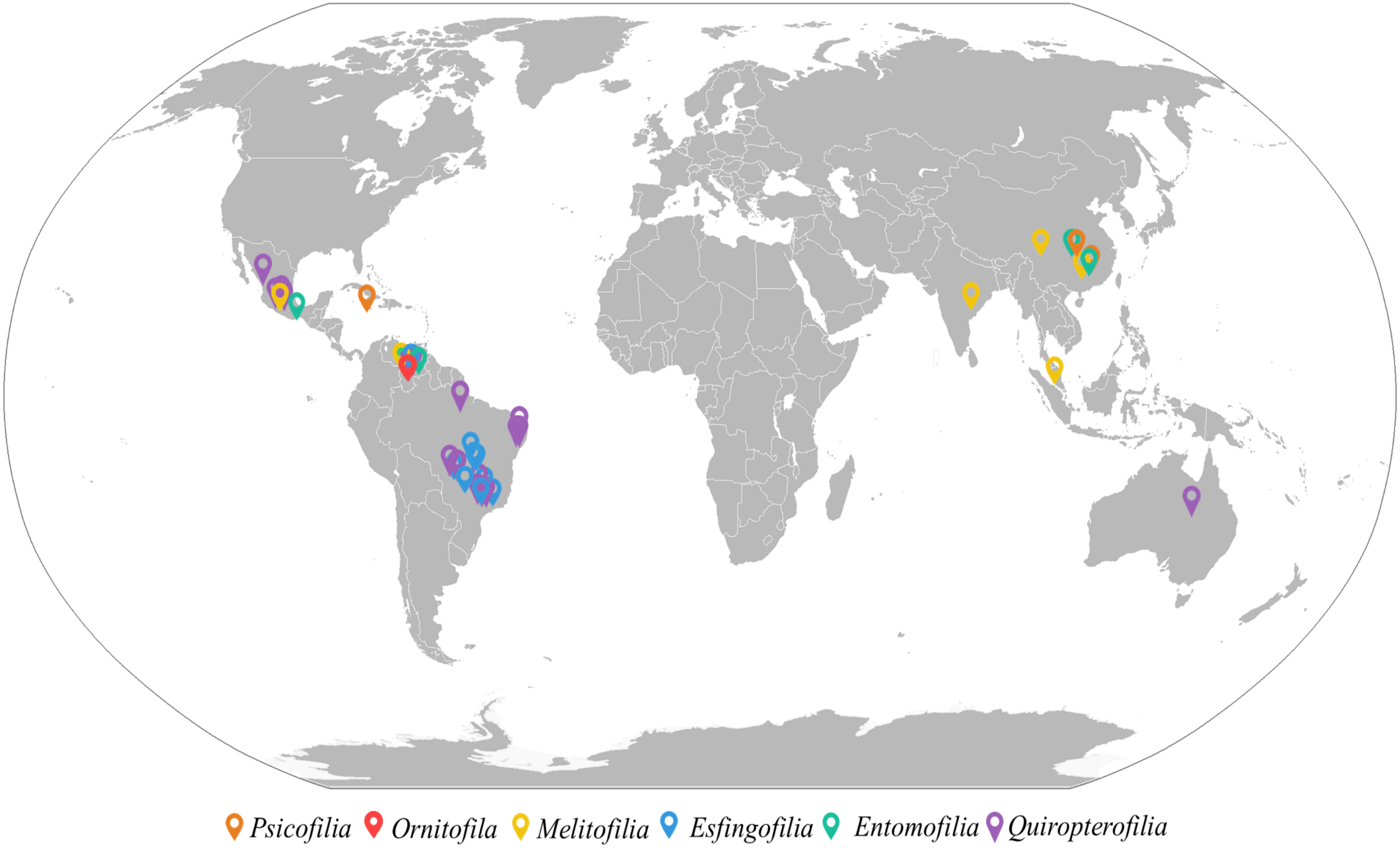


Figura 3: *Distribuição dos estudos de polinização para o gênero Bauhinia* (Fabaceae) *nas diferentes áreas tropicais do globo.*



Fontes: Hokche & Ramirez 1990; Wunderlin 1984; Silberbauer-Gottsberger & Gottsberger 1975; Fleming et al. 2009; Ramirez et al. 1984; Lau 2001, 2009; Quirino & Machado 2014; Leite & Machado 2010; Munin et al. 2008; Percival 1974; Neto 2013; Texeira 2000; Karmakar et al. 2010; Lim et al. 2018; Endress 1994; Dobat & Peikert-Hole 1985.

Reproductive isolation between two sympatric bat-pollinated *Bauhinia* L. (Fabaceae)

Short title: Reproductive isolation of two species of *Bauhinia*

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Abstract

Reproductive barriers are crucial for the formation and maintenance of species in nature, and the reproductive isolation (RI) involves the contribution of multiple barriers of different importance for total isolation between species. These barriers can be classified as pre- or post-pollination according to the timing of their occurrence and understanding the strength of these barriers can illuminate the factors that maintain the reproductive isolation integrity of species. Two bat-pollinated *Bauhinia* (Fabaceae) with similar floral morphology that fits the chiropterophilous syndrome, occur sympatrically in an area of Caatinga, a dry forest in northeastern Brazil. Our quantitative measurements of the components of reproductive isolation show that they are, in fact, completely isolated by a combination of pre- and post-pollination barriers. We found high geographic isolation and a flowering overlap, however, there is a substantial difference in the flowering peak between the two species. Both species share the same bats as pollinators, but the interspecific transfer of pollen between the two taxa is diminished due to the differences in the size of the flowers, leading to differential pollen deposition in the bat bodies. Furthermore, we found total incompatibility intertaxa. This work is the first study that measured the RI between sympatric plant species pollinated by bats. We propose that several factors contribute to the isolation of two sympatric and congeneric species of *Bauhinia*, thus preserving the integrity of this pair of species. Our results showed that different pre and post-pollination barriers contribute and maintaining the reproductive isolation of this coexisting Bauhinia species.

Keywords: Character displacement, chiropterophily, floral morphology, geographic distribution, Glossophaginae, mechanical isolation

Introduction

Speciation is a key process responsible for high biodiversity, and reproductive isolation is an important factor in its explanation (Coyne and Orr 2004). Reproductive barriers are crucial for the formation and maintenance of species in nature (Mayr, 1942; Coyne e Orr, 2004), and the reproductive isolation involves the contribution of multiple barriers of different importance for total isolation between species (Ramsey et al. 2003; Kay 2006; Martin and Willis 2007).

Reproductive barriers can be classified as pre- or post-pollination according to the timing of their occurrence (Baack et al. 2015). Pre-pollination barriers include spatial or temporal separation of reproduction in co-occurring species, such as niche differentiation (Grossenbacher et al. 2014), divergent floral morphologies (Grant and Grant 1964) or strong flowering asynchrony among co-occurring species (Marques et al. 2007). As most angiosperms depend on biotic pollination for breeding (Ollerton et al. 2011), the preferences and fidelity of their pollinators strongly affect the degree of isolation between species (Schemske et al. 1999; Martin et al. 2008; Paudel et al 2018). Pre-pollination barriers are generally considered to be the most important barriers in maintaining the integrity of species, since they act early in the life cycle of an organism, imposing the strongest impediment to gene flow (Lowry et al. 2008). However, post-pollination barriers can also be effective, reducing the viability of hybrids or their reproductive potential (Baack et al. 2015), even after heterospecific pollen transfer (Yang et al. 2007).

The floral characteristics play an important role in pre-pollination barriers (Grant 1994; Lowry et al. 2008; Kay and Sargent 2009; Muchhala 2008; Schiestl and Schlüter 2009). Characteristics such as flower size, flower orientation, length in reproductive structures, color of flowers provide mechanical isolation, in which floral morphologies are adapted to different rates of pollinators with different shapes or sizes of body, or behavioral fidelity. (Grant 1994, Schemske and Bradshaw 1999, Xu et al., 2011, Queiroz et al., 2015, Sun et al., 2015, Paudel et al. 2018).

Bauhinia L. is a pantropical genus, with about 300 species, belonging to the Fabaceae, subfamily Cercidoideae. The species of this genus are shrubs, trees and lianas and corresponds to approximately 1.5% of the Fabaceae (Sinou et al. 2009). They present racemic inflorescences type, with flowers inserted in pairs on the main axis (Vaz and Tozzi 2003). The flowers of the *Bauhinia* species present diverse forms, sizes, colorations, periods and anthesis duration (Hokche and Ramirez 1990; Endress 1994). Often, this floral diversity is associated with a large diversity of pollinators (Heithaus et al., 1974, Ramírez et al., 1984, Bergallo 1990). In general, diurnal *Bauhinia* species are pollinated by bees and hummingbirds, while nocturnal species are visited by moths or bats (Hokche and Ramirez 1990).

While a large number of pollination ecology studies on *Bauhinia* spp. have been conducted (Heithaus et al. 1974; Hokche and Ramirez 1990; Munin et al. 2008; Paulino-Neto 2013; Ramírez et al. 1984), none has quantified the efficiency of reproductive isolation barriers in avoiding gene flow between co-occurring bat-pollinated species. Preliminary observations carried out in a Caatinga area offer an interesting situation that can serve as a good model for these studies. In the present study, we aimed to measure the strength of multiple pre-pollination and post-pollination components separating two sympatric *Bauhinia* species in a Caatinga area, possibly sharing the same pollen vectors, and quantified their relative contribution in chronological order to total reproductive isolation. We tackled the following main questions concerning the reproductive isolation between two species: (1) Are there differences in floral morphologies and flowering phenology between the two species? (2) What is the relative importance of different barriers of isolation in causing reproductive isolation between the two species? (3) How is the species integrity of these sympatric and closely related *Bauhinia* maintained?

Materials and methods

Study area

The field activities were conducted monthly from January 2017 to July 2018 in the National Park of Catimbau (PARNA Catimbau), Pernambuco, Northeastern Brazil, a natural reserve covering nearly 64,000 ha (8°24’00” and 8°36’25” S; 37°09’30” and 37°09’30” W). The area has a semiarid climate, BSh according to the Köppen scale (Peel et al. 2007). The annual average temperature is 23°C and annual rainfall varies from 480 to 1100 mm. The Catimbau Park contains typical Caatinga taxa, i.e. shrubs and trees mostly belonging to the families Fabaceae, Poaceae, Euphorbiaceae, Asteraceae, and Convolvulaceae, while the herbaceous understory plants are largely from the Cactaceae and Bromeliacae (Rito et al. 2017; Athiê-Souza et al. 2019).

Study species

We chose a pair of species belong to *Bauhinia* genus, *Bauhinia acuruana* Moric. and *B. pentandra* (Bong.) D. Dietr. Both species are native shrub or trees (Figure 1) distributed in dry vegetation areas of the Brazilian Cerrado and Caatinga (Figure 2) (Vaz and Tozzi 2003; 2005). *Bauhinia acuruana* is more narrowly distributed and endemic to northeastern of Brazil and is usually found in mountainous areas with altitudes of 600 to 1100 meters, while *Bauhinia pentandra* has the wider range distribution, occurring in Brazil, Bolivia and Paraguay (Vaz and Tozzi 2005). According to the published revision of this genus, *B. pentandra* and *B. acuruana* have phylogenetic proximity, belonging to the same sect. *Pauletia* (Cav.) DC., the most widely distributed in the Neotropical Region (Vaz 2001).

Floral traits

To investigate the differences in floral attributes between the two *Bauhinia* taxa, we collected data on the lengths of entire flowers, hypanthium, styles and pistils. We monitored the anthesis in different individuals (n = 15 flowers per taxon) and recorded the opening time of floral buds, anthers dehiscence, stigmatic receptivity and flower senescence. To test for stigmatic receptivity, one flower per individual (n = 24 individuals per taxon) of each species had the pistil dipped in hydrogen peroxide, and the stigma was considered receptive when bubbles were released on the stigmatic surface (Zeisler 1938). The measurements of stigmatic receptivity were carried from the pre-anthesis bud stage to flower senescence, at intervals ow two hours (n = 3 flowers per time per taxon).

We recorded the total volume (μL) and concentration (%) of nectar produced during anthesis in both *Bauhinia* taxa. We measured sugar volume using graduated microsyringes (Hamilton, NV, USA) and concentration measurements with a portable refractometer (0-50%; Atago, Tokyo, Japan). The time and number of measurements differed according to the daily anthesis period of each taxon: seven measures (volume and concentration) from 06:00 p.m. to 06:00 a.m. in both species. In each period, we measured the accumulated volume of nectar in a group of ten flowers of different individuals previously bagged that could not be accessed by floral visitors (Galetto and Bernardello 2005).

We tested the differences in floral morphometry and nectar characteristics between the two *Bauhinia* species with a series of t-tests - Welch. For outcrossing treatments (Outcrossing × Natural pollination), we compared the fruit and seed set among treatments with a G test. All statistical analysis was performed in the BioEstat 5.3 software. All tests followed Sokal and Rohlf (1995).

Geographic isolation

We estimated the geographic isolation between *B. acuruana* and *B. pentandra* by comparing the overlap in their distribution ranges in Brazil. Here we included all the records for the state of Pernambuco, which encompasses a high distribution proportion of both species. Records from the locations of the occurrences of the two species were determined by examining the voucher of these species deposited in the national public database Specieslink (splink.org.br). This database contains the main herbarium specimen's collection deposited in the most important herbaria of Brazil for both species (ASE, FCAB, HST, HUEFS, HUNEB, HVASF, IPA, JPB, MAC, PEUFR, R and UFP). Some records had incomplete data, thus we decided to include only herbarium records which were taxonomically verified and that had the location of the collection with geographic coordinates. In order to avoid data redundancy, records of the same species that were collected in the same locality were excluded. To estimate the degree of geographic overlap among species, we quantified the number of sympatric and allopatric populations, the latter including cases that only one species was recorded. Reproductive isolation due to geographic separation was calculated from equation 4C of Sobel and Chen (2014),

$$RI_{geogr} = 1 - (S / S + U) \qquad \textit{Equation 1}$$

in which, S = the fraction of space that is shared by both species; and U = the unshared fraction.

Microhabitat isolation

We examined the fine-scale isolation of the micro-habitat between species by quantifying the degree of co-occurrence at the study site, from 35 predefined and already existing PARNA Catimbau plots of 20 m × 30 m. In the field, we counted the number of plots containing only *B. acuruana*, only *B. pentandra*, or both species. We determined the proportion of shared and unshared plots for each species. From these proportions, we calculated the isolation of the microhabitat using equation (1).

Phenological isolation

To estimate the degree of interspecific flowering overlap, we performed a total of 18 censuses from 1 January to 15 July 2018. We tagged 30 to 45 plants of each species along the PARNA Catimbau and recorded the vegetative and/or reproductive phenophases of each plant monthly, creating a flowering profile for each species in each during the follow up. The floral life spans of individual flowers were recorded from the day on which the buds opened until the day the corolla wilted. We adopted the Sobel and Chen method (2014) to calculate the strength of a phenological asynchronous insolation barrier using the 4S1 equation:

$$RI_{phenol} = 1 - 2 \sum_i ((A_i / A_{total}) \text{ x } (B_i / (A_i + B_i))) \qquad \textit{Equation 2}$$

in which, the temporal isolation, was calculated by estimating the proportion of species *A* available for mating on day *i* to its total abundance (A_i/A_{total}) throughout the breeding season and multiplying by the relative abundance of the heterospecific species on day *i* (B*i*/(A*i*+B*i*)).

Pollinator isolation

To estimate the strength of pollinator isolation, we directly observed floral visitors of *B. acuruana* and *B. pentandra* in the field during the flowering period of both species. We made 6-10 focal observations from crepuscular and nocturnal visitors from 5:30 pm to 5:00 am, for a total of 45 hours (20 for *B. acuruana* and 25h for *B. pentandra*). We also use a camera attached to tripod in some flowers per plant. All visitor species were photographed during observation periods. We determined the identity of pollinators through photographs and specialized field guides (Reis et al. 2013). We calculated pollinator-mediated reproductive isolation (RI) using equation 4A from Sobel and Chen (2014),

$$RI_{pollinator} = 1 - 2 \times (H / H + C) \qquad \textit{Equation 3}$$

in which, H = the number of pollinators that is shared by both species; and C = total number of pollinators species.

Post-pollination isolation and breeding system

To estimate the post-pollination isolation, we compared the success of intertaxa pollen crossing to intrataxa crossing in terms of fruit and seed set. We performed experimental crossings during the flowering season of 2017 and 2018. To analyze the reproductive system of the two studied *Bauhinia,* flower buds of different individuals were covered with voil bags, and the following procedures were performed after the beginning of anthesis: (i) agamospermy (*n* = 45 for each species)— emasculation of flowers (ii) spontaneous self-pollination (*n* = 45 for each species)—without hand pollen transfer to the stigma; (iii) manual self-pollination (*n* = 45 for each species)—with hand pollen transfer to the stigma of the same flower; (iv) outcrossing intrataxa (*n* = 30 for each species)—with hand pollen transfer among individuals of the same taxon; and (v) outcrossing intertaxa (*B. acuruana*, *n* = 10; *B. pentandra*, *n* = 15)—flowers of both species was used as donor and receptors of interspecific pollen; (vi) control (*n* = 50 both species) – natural pollination. Matured fruits, when formed, were collected after 5 weeks. From these proportions, we calculated the post-pollination isolation using equation (3), in which, H = the number of fruits formed by outcrossing intertaxa; and C = total number of outcrossing intertaxa experiments.

Total reproductive isolation

Reproductive barriers may show asymmetry between species, this way we quantified the degree of total reproductive isolation separately for *B. acuruana* and *B. pentandra*. We compute total reproductive isolation between two species as a multiplicative function of the individual components of reproductive isolation (RI) at sequential stages in the life history, and for two stages of isolation, we quantified the absolute contribution (AC) of a component of reproductive isolation (RI) at stage n in the life history, in accordance to Ramsey et al. (2003),

$$AC_n = RI_n \text{ x } (1 - \sum_{i=1}^{n-1} AC_i) \qquad \textit{Equation 4}$$

hence, a given reproductive barrier eliminates gene flow that has not already been prevented by previous stages of reproductive isolation. The total reproductive isolation is calculated in the following manner,

$$T = \sum_{i=1}^{n-1} AC_{I.} \qquad \textit{Equation 5}$$

Finally, we also quantified total reproductive isolation without ecogeographic isolation, the latter providing an estimate of the strength of reproductive isolation in sympatry in accordance to Ramsey et al. (2003).

Results

Floral biology and floral traits

The two *Bauhinia* species produce terminal inflorescences, with one or two opened flowers per night per inflorescence (Figure 1). The flowers of both species are zygomorphic, but present different total lengths (Table 1). The flowers of *B. acuruana* have five green, thick sepals that in anthesis are united in 2-3 free lobes. The five petals are white, linear and free. The androecium is formed by 10 equal stamens, with greenish filaments and versatile yellow anthers. *Bauhinia pentandra* have also five green sepals, but they open forming 2 reflexed lobes. The five petals have a greenish shade and are also strongly reflexed. Stamens are slightly dimorphic (five long and five short) with greenish filaments and versatile yellow anthers (Figure 1). The flower, hypanthium, and pistil lengths of *B. acuruana* were significantly lower than those of *B. pentandra* (Table 3). The stamens of the two species show a divergence in their length, so that *B. pentandra* has stamens significantly larger than *B. acuruana* (Table 3).In both species, flower opening begins with a slow separation of the sepals from the base towards the apex. When the sepals are completely curled back, the stamens are already fully extended and the petals facing up. Flowers opened between 4:30pm and 06:30pm, but most flowers opened between 5:00pm and 6:00 and were receptive for only one night. Anther dehiscence began in the bud, but pollen was not fully exposed until approximately 30 min after full flower opening. Stigmas are receptive prior to anthesis and remain functional until senescence.

Nectar was produced and accumulated in the hypanthium, sometimes exceeding the hypanthium volume. Small amounts of nectar moved up the basal portion of the stamen filaments by capillarity without pollinator visits, causing it to shed. Nectar secretion began after flower opening around 6pm and continued throughout the night until 4:00am for both species.

Geographic isolation

According to the herbarium collections deposited on the specieslink we recorded 442 allopatric populations of *B. acuruana* and 562 allopatric populations of *B. pentandra*. Both species co-occurred in only 23 populations. Geographic isolation between two species was high, indicated by a $RI_{geogr,B.a} = 0{,}95$ for *B. acuruana* and $RI_{geogr,B.p} = 0{,}96$ for *B. pentandra*. The entire distribution of the allopatric and sympatric populations of both *Bauhinia* species in Brazil is shown in figure 2.

Microhabitat isolation

At the studied site, the two species co-occurred in 12 of the 35 plots. At this spatial scale, microhabitat separation showed middle reproductive isolation, where the microhabitat isolation for *B. acuruana* was $RI_{microhabitat,B.a} = 0{,}66$, and *B. pentandra* was $RI_{microhabitat,B.p} = 0$, that is, no microhabitat isolation from *B. acuruana*.

Phenological isolation

During 18 months of phenological monitoring, the two *Bauhinia* species showed a long flowering period, blooming for 8-12 months per year. We did not find a separation between flowering of the pairs of species, however, the two species showed distinct flowering peaks. *Bauhinia acuruana* showed a lower flowering period throughout the year than *B. pentadra*, which in turn presents flowers throughout all 18 months of follow-up (Figure 3). In 2017 *B. acuruana* presented a short flowering phase between the months of January and February and between the months of May and October showed its greater flowering, being its peak between the months of June and July. In 2018, *B. acuruana* flowered between the months of January and May, showing a variation when compared to the year 2017. *Bauhinia pentandra*, in turn, bloomed during all 18 months, with flowering peaks between January and February 2017 and December 2017 until February 2018. Temporal isolation between the species due to flowering phenology was therefore low for *B. acuruana* ($RI_{temporal,\ B.a}$ = 0.32), and moderate for *B. pentandra* ($RI_{temporal,\ B.p}$ = 0.48).

Pollinator isolation

The two *Bauhinia* species are pollinated by the same nectarivorous bats: the Pallas long-tongued bat *Glossophaga soricina* and the Goldman's Nectar Bat *Lonchophylla mordax* (Glossophaginae) (Figure 4). The bats did not show a species-specific preference and accessed the flowers of both *Bauhinia* species for a long period after beginning of the anthesis (06:00pm–02:00am hours). In 45 hours of observation (20 for *B. acuruana* and 25h for *B. pentandra*) we recorded 95 bat visits (4,75 visits h^{-1}) to *B. acuruana*, in which the pollen was deposited on the neck of the bats. We recorded 171 visits (6,84 visits h^{-1}) to *B. pentandra*, in which the pollen was deposited in the region between the neck and abdomen of the bat (Figure 4).

The bats made fast approaches to the inflorescences and made short flights in front of flowers. In this position, they threw their heads forward, placing their tongues inside the flower (Figure 4). They made several quick bouts (<1s) on the same flower or in the other open flowers in the plant, always describing the same route, foraging mostly following a trapline rout.

Sporadically, the Glittering-bellied Emerald hummingbird *Chlorostilbon lucidus* and hawkmoths were also seen collecting nectar but they did not contact the reproductive structures. *C. lucidus* visited the recently opened flowers at dusk when the flowers already contained small amounts of nectar. They collected nectar by inserting their beak between the base of the stamens, thus avoiding contact with the anthers. Other behavior was to drill the base of the hypanthium and collected the nectar, also not contacting the reproductive structures. The hawkmoths visited were less frequent in the flowers of the two *Bauhinia* species. They landed on the flowers and clunged to the hypanthium, inserting their proboscis among the stamens in search of nectar. The RI index of pollinator isolation ($RI_{pollinator}$) was zero for both species.

Post-pollination isolation and breeding system

The hand pollination experiments showed that both *Bauhinia* taxa were obligate xenogamous. Fruit and seed were set exclusively in the treatment of hand cross-pollination (Table 1). Natural seed set in both *Bauhinia* taxa did not differ from outcrossing treatments (Table 2). The outcrossing inter-taxa experiments yielded no fruit formation for both species, indicating complete incompatibility between species. Therefore, based on no seed output, the strength of reproductive barriers was found as: $RI_{postpollination,\ B.a\ and\ B.p} = 1$.

Total reproductive isolation

The total RI was 100% between *B. acuruana* ($RI_{total,\ B.a} = 1$) e *B. pentandra* ($RI_{total,\ B.p} = 1$), the individual effects of the four pre-pollination barriers and one post-pollination barrier are summarized for each species in Table 2. Although the intertaxa incompatibility was absolute, this post-pollination barrier made a small relative contribution to the total isolation of the two species, considering the cumulative effect of pre-pollination barriers, mainly due to the spatial and temporal separation of the two species. Even excluding the effect of geographic distribution, the total isolation value would still be complete, $RI_{\text{total-geog}, \, B.a} = 1$ for *B. acuruana* and $RI_{\text{total-geog}, \, B.p} = 1$ for *B. pentandra*.

Discussion

In this study, we describe two new cases of bat-pollination in *Bauhinia,* and we quantified five pre- and post-pollination components of RI between two synchronopatric species in the Caatinga. The reproductive isolation between *B. acuruana* and *B. pentandra* was practically total and was the result of a combination of multiple barriers. We brought evidence that both pre- and post-pollination barriers are important to maintain reproductive isolation among *Bauhinia* species, however the pre-pollination barriers had a prominent role for the isolation of both species. In addition, we have demonstrated the role of character displacement as a critical mechanism for the evolution of reproductive barriers contributing to the reproductive isolation between these two Bauhinia species, as well as the importance of this divergence as an implement to avoid pollen loss.

Bat pollination on Bauhinia species

The two *Bauhinia* species have a typical set of attributes associated with pollination by bats, i.e. flowers exposed above foliage, crepuscular anthesis, white and greenish flowers, large nectar volumes with low sugar concentration and brush-type flowers with narrow access to nectar (Faegri and van der Pijl 1979; Dobat and Peikert-Holle 1985; Tschapka and Dressler 2002; Willmer 2011). The anthesis of both is synchronic as indicated by Hokche and Ramirez (1990) for a nocturnal *Bauhinia* species. However, the authors reported that *B. aculeata* anthesis was asynchronous. Other species like *B. ungulata* also have a certain asynchrony in their anthesis process (Hokche and Ramirez 1990).

Pollination by bats has been reported for the genus (Heithaus et al. 1974; Wunderlin 1983; Ramirez et al. 1984; Hokche and Ramirez 1990; Fisher 1992). *Bauhinia cheilantha* is another species that occurs in the Caatinga and is also pollinated by bats (Quirino and Machado 2014), although authors say that this species presents morphology that allows interaction with both bats and hawkmoths.

In general, studies have shown that most of the studied *Bauhinia* species are pollinated by bats, followed by hawkmoths. This high species rate with these pollination systems may be associated with a high proportion of species that have nocturnal flowers. In the study conducted by Vaz (2001) it points out that all species of *sect. Pauletia* (including *B. acuruana* and *B. pentandra*), composed of 58 taxa, open their buds between late afternoon and nightfall, showing a strong relationship with nocturnal pollinators.

In both species, nectar was produced in large quantities and at low concentrations in the hypanthium. the similarity in the volume and concentration of nectar offered by *B. acuruana* and *B. pentandra* may indicate that both tend to secrete volume efficient to attract the same pollinators. Species that secrete higher amounts of nectar can receive more pollinator visits, increasing the chances of pollination (Lemke 1984; Fischer and Leal 2006). Large amounts of nectar seem to be a feature of several *Bauhinia* species (Heithaus et al. 1974; Wunderlin 1983; Ramirez et al. 1984; Hokche and Ramirez 1990), mainly those in which pollination by vertebrates occurs, because these pollinators have a higher body mass than other pollinators and they are endotherms (Stiles 1977; Faegri and van der Pijl 1979).

Geographic isolation

We found only 23 sympatric populations, indicating that the geographic isolation constitutes an important barrier for the two *Bauhinia* species. In order to estimate reproductive isolation, the reproductive barriers are recognized sequentially and geographic isolation is the first barrier taken into account in the studies which measure reproductive isolation (Ramsey et al. 2003; Kay 2006; Ma et al. 2016; Cuevas et al. 2018; Paudel et al. 2018). Geographic isolation may contribute more to total isolation than reproductive barriers that act later since a barrier can only prevent gene flow that has not been eliminated by previous stages of reproductive isolation (Schemske 2000; Martin and Willis 2007; Cahenzli et al. 2018). This can be seen in our study, where geographic isolation was highly important as a reproductive barrier and was responsible for an almost complete isolation between *B. acuruana* and *B. pentandra.* Geographic isolation functioning as significant isolating mechanisms, are reported in some plant taxa, including two coexisting species of *Roscoea* in the Himalaya (Paudel et al. 2018), *Rhododendron delavayi* and *R. cyanocarpum* (Ma et al. 2016) and in two *Salvia* species pollinated by hummingbirds (Cuevas et al. 2018).

The importance of geographic isolation in the speciation process has long been recognized (Mayr 1959) and little overlap between related species may indicate that geographic isolation was important to initiate speciation (Barraclough and Vogler 2000; Kay 2006). In our study, we observed that the two species of *Bauhinia* have a broad distribution and a smaller number of populations occurring sympatrically in some regions. In this way, the current geographic isolation between this pair of species may be a remnant of an allopatric historical distribution with expansion of the distribution band connecting some of the populations (Mayr 1947; Kay 2006).

Some of the studies of reproductive isolation concentrate only on regions where the species are sympatric, disregarding the total distribution of populations and the geographic factor as an important measure for reproductive isolation (Lowry et al. 2008). However, our results show that the geographic isolation constitutes a relevant barrier between the *Bauhinia* species, supporting the need to incorporate this factor in reproductive isolation studies, mainly in the cases of bat-pollinated species. Taking into account that bats are pollinators with a high energy demand and that travel long distances between feeding locations (Fleming et al. 2009), they can promote pollen flow between geographically distant populations, thus reducing the spatial isolation between species.

At local level in the PARNA Catimbau, *B. acuruana* and *B. pentandra* exhibit moderate spatial isolation. In the study site *B. acuruana* has a wider distribution and occurs in 35% of the plots, while *B. pentandra* occurs in 11% of the plots and in all of them in sympatry with *B. acuruana*. This sympatric distribution in some areas is probably the result of the ecological similarities between this pair of species, as well as the association of these species with the habitat where they occur. The two *Bauhinia* species occur in Brazilian dry forests and are concentrated in the Cerrado and mainly in the Caatinga (Vaz and Tozzi 2003, 2005).

Phenological isolation

Divergent flowering events may work with as a significant reproductive isolation mechanism (Husband and Schemske 2000; Martin and Willis 2007; Paudel et al. 2018). In communities of bat-pollinated species, studies have shown that sympatric and congeneric species show sequential flowering or at different times of the year (Sazima et al. 1999; Primo 2008). Studying species in the Atlantic Forest of southeastern Brazil, Sazima et al. (1995, 1999) found different sympatric species of the genus *Vriesea* (Bromeliaceae) that showed sequential flowering throughout the year, allowing pollinator partitioning. Sequential flowering seems to be a strong pattern found in sympatric *Vriesea* species and is also observed for *V. incurvate*, *V.*

ensiformis e *V. carinata,* both hummingbird pollinated species of southeast Brazil (Araujo et al. 1994).

In our study, no strong RI was detected in the phenology of both species of *Bauhinia*, showing that the phenological isolation was not a strong reproductive barrier between these species. During 18 months we found a flowering overlap between B. acuruana and B. pentandra similar to that found for other groups of plants (Kay 2006; Queiroz et al. 2015; Zhang and Gao 2017; Cuevas et al. 2018; Liang et al. 2018), however, there is a substantial difference in the peak flowering between the two species. The separation of flowering peaks can be an important mechanism as a barrier to reproductive isolation between species and can avoid plant competition by using pollen vectors in the same periods. However, in our study, the pair of studied species showed mechanical isolation and complete post-pollination isolation (see below), so that the overlap can act positively, contributing to the attraction of pollinators (Moeller 2004).

The overlap has a positive effect when coexisting species collectively share the same pollinators, especially when attracting highly mobile pollinators that feed in large areas (Moeller 2004). In this study, we showed that B. acuruana and B. pentandra attract pollinating bats, which in turn are vectors that travel large areas to obtain resources, and can cover about 50 km to meet their energy needs (Fleming et al. 2009).

In addition, the segregation in the flowering period obtained by the separation in the flowering peaks between the two species of *Bauhinia* studied here, can contribute to the facilitation allowing the floral resources to be available to the pollinating bats during a prolonged period of time, thus increasing the growth or maintenance of the pollinator population (Moeller 2004). Throughout 18 months of measurements, we observed 11 months of overlapping flowering and separation in flowering peaks showing that these species are important resources in the area occurring for the assemblage of pollinating bats.

The flowers of both species studied here have a long period of flowering, throughout the year at least one species of *Bauhinia* carrying flowers, which is especially relevant to a community of long-lived pollen vectors, such as bats (Stiles 1975). The supply of flowers and fruits throughout the year is common in tropical regions, where the climate is more uniform compared to regions with higher latitudes in the globe, which allows vertebrates to act as vectors for the pollination and dispersion of angiosperms (Tschapka and Dressler 2002).

Sharing pollinators and the role of floral morphology for reproductive isolation

Both *B. acuruana* and *B. pentandra* were pollinated by the same species of bats, *Glossophaga soricina* and *Lonchophylla mordax*. This indicate that the pollinators no represent a reproductive barrier between the pair of *Bauhinia* species. The Glossophagine bats are a group of bats highly specialized in collecting nectar, due to morphological specialization in terms of rostral length and reduction of the dentition (Tschapka and Dressler 2002; Fleming et al. 2009).These bat group occur from humid places to dry forests, being responsible for the pollination of different species of several flowering families (Vogel et al 2004, 2005; Machado et al. 2006; Rocha et al. 2007; Fleming et al. 2009; Queiroz et al. 2015, 2016). In the Caatinga, bats *G. soricina* and *L. mordax* are responsible for pollination of different species, such as *Bauhinia cheilantha* (Fabaceae) (Quirino and Machado 2014), *Encholirium spectabile* (Bromeliaceae) (Queiroz et al. 2016), *Harpochilus neesianus* (Acanthaceae) (Vogel et al. 2004), *Ipomoea marcellia* and *Ipomoea vespertilia* (Convolvulaceae) (Queiroz et al. 2015), *Mimosa lewisii* (Fabaceae) (Vogel et al. 2005), *Pilosocereus tuberculatus* (Cactaceae) (Rocha et al. 2007) and *Tarenaya spinosa* (Cleomaceae) (Machado et al. 2006).

Even when pollinators can perform hetero-specific visits, the floral morphologies prevent the transfer of heterospecific pollen (mechanical isolation) (Grant and Grant 1964). Although the two species of *Bauhinia* have overlapping flowering for several months, occur in some areas sympatrically and have the same pollen vectors, we find a degree of isolation associated with floral morphology. Our floral morphometry confirmed a degree of mechanical isolation (Figure 4). The interspecific transfer of pollen between the two taxa of *Bauhinia* is diminished due to the differences between the floral morphs causing differences in the size of the flowers, leading to differential deposition of pollen. Both species deposit the pollen grains in the ventral region of the body of the bats. In this case, the flowers of *B. pentandra* are significantly larger than those of *B. acuruana* and can act with a factor that avoids the mixing of pollen in the body of the bats, where *B. acuruana* deposits its pollen grains on the neck of the bats, while *B. pentandra* deposits in the region between the neck and breast of the bat.

In one night nectarivorous bats can visit flowers of various species increasing the chance of transporting heterospecific pollen (Tschapka and Dressler 2002; Fleming et al. 2009). Thus, selecting the specific space for pollen deposition in the bat body is an important means of avoiding the heterospecific pollen mixture between species (see below) (Howell 1977; Muchhala 2008).

As we have seen here, the interspecific transfer of pollen by bats can be avoided by the morphology of flowers, which is also seen in other studies with species pollinated by bats (Howell 1977; Muchhala 2008; Queiroz et al. 2015). Studies with chiropterophilous species have shown the relevance of floral morphology as an important tool to avoid the mixture of heteroespecific pollen, as seen by Muchhala (2008) in the humid tropical forests of Ecuador. Species of the genus *Burmeistera* (Campanulaceae) can often co-occur in certain areas with overlaps in their flowering phenology. However, species of this genus present a large interspecific variation in their reproductive structures, resulting in pollen deposition in different regions of the bats' head, attenuating the mixture of heterospecific pollen. This differentiation can, in turn, contribute with reproductive isolation between the different *Burmeistera* species (Muchhala 2008). In the Caatinga, another case study was performed by Queiroz et al. (2015)

with two species of *Ipomoea* (Convolvulaceae), which have a similar flowering phase, occur sympatrically and shared the same pollinating vertebrates (hummingbirds and bats), where they saw variations in aspects of floral morphology and floral orientation, as well as differences in the anthesis and differential deposition of pollen in the body of the pollinators.

Character displacement and heterospecific pollen deposition

In areas where related species are sympatric, these may diverge in some characteristics to avoid interspecific competition, a process known as character displacement, which may be physiological, behavioral, ecological or morphological (Brown and Wilson 1956). From the results obtained in this study we can observe that *B. acuruana* and *B. pentandra* present character displacement associated with the morphology of their stamens, which, due to their different size, the pollen is deposited on different parts of the bats' body, thus avoiding direct competition between this pair of species, as well as the pollen wastage.

Character displacement can play a critical role in the speciation process by finalizing or initiating reproductive isolation (Pfenning and Pfenning 2009). In addition, the divergence of traits may accentuate the differences already existing between species in sympatry, which contributes to better reproductive isolation between the groups involved (Pfenning and Pfenning 2009). In the system studied here, we have seen that *B. acuruana* and *B. pentandra* have character displacement, and it is reasonable to think that this factor was important for the process of reproductive isolation and may have been the initial step for the isolation between this pair of species.

Such divergence may indirectly have promoted reproductive isolation by the evolution of a post-pollination barrier (Pfenning and Pfenning 2009). The fact that *B. acuruana* and *B. pentandra* deposited pollen in different regions of the body of the bats allowed no pollen mixture throughout the speciation, strengthening the intertaxa self-incompatibility existing between the two species.

The character displacement process seems to be an important factor for congeneric plants pollinated by bats, both as an important mechanism in the reproductive isolation, as reported here, as a strategy to avoid competition (Muchhala and Potts 2007). Mucchala and Potts (2007), found strong evidence that competition for pollinators shaped the community structure of *Burmeistera*, and showed that the divergence in the length of exertion of the reproductive parts reduced the interspecific transfer of pollen among pairs of *Burmeistera* species.

In addition to this, it is important to emphasize that this character displacement, along with geographic and temporal separation, can contribute as a mechanism to avoid the loss of pollen through interspecific transfer (Morales and Traveset 2008), which could be led to a high impact on fitness. The lack of displacement between the two *Bauhinia* could reduce seed set as a result of the interspecific pollen transfer that could be obtained by means of two non-exclusive routes, (I) co-specific pollen loss and (II) heterospecific pollen deposition (Waser 1978). In the first case, the shared pollen vector would carry pollen to individuals of both species, resulting in pollen wastage on foreign stigmas, and in the second case, the interspecific transfer of pollen would lead to a loss of the surface area of the receptive stigma for both species (Waser 1978).

The strength of post-pollination barriers for total isolation

The complete lack of fruit set after intertaxa outcrossings, regardless of whether the interspecific pollen receptor was *B. acuruana* or *B. pentandra*, points to a strong isolation between the two *Bauhinia* species. Although post-pollination isolation was complete, the absolute individual contribution of this barrier to total RI was low. This is related to the sequential importance of the previous barriers (Rieseberg 2007). It is evident here that, regardless of flower architecture, flowering overlapping, and sharing of pollen vectors by the two *Bauhinia* species, the post-pollination barrier in both species completely blocked the gene flow between them. Therefore, if these species experienced changes in some of its pre-pollination barriers, the reproductive isolation would be maintained thanks to the incompatibility between this pair of species.

In our study we saw that both species of *Bauhinia* are self-incompatible, which is evidence to support the intertaxa isolation, since interspecific crosses and hybridization are usually more common in lineages in which the species are self-compatible (Abdalla 1972). In contrast, Queiroz et al. (2015) in their study with two species of *Ipomoea* reported that these species are self-incompatible but there were no hybrids in the study area, but they observed fruit and seed production by intertaxa outcrossing.

The complete isolation obtained between these species was maintained even after the elimination of the geographic component of the total isolation (Table 2), showing that the barriers measured are sufficient to cause complete isolation between the two *Bauhinia* species. Similar results were found for *Pedicularis* (Liang et al. 2018), *Mimulus* (Ramsey et al. 2003) and *Costus* (Kay 2006).

Conclusions

The floral characteristics of *B. acuruana* and *B. pentandra* and the pollination by Glossophagine bats show two classic examples of chiropterophilous species. We found that several factors contribute to the isolation of this two sympatric *Bauhinia* in the Caatinga, thus preserving the integrity of this pair of species. Our results showed an interaction between pre and post pollination barriers, thus maintaining the reproductive isolation of coexisting species, and that character displacement may have been an important factor in the evolution of these barriers, as well as avoiding pollen loss.

This work is the first study that measured the reproductive isolation between sympatric species pollinated by bats that used methods to estimate the strength of the reproductive isolation through pre- and post-pollination barriers. Further studies, including phylogenetic analyzes, could contribute to responses in a more evolutionary context to the reproductive isolation between *B. acuruana* and *B. pentandra*, taking into account the proximity and phylogenetic relationships between these species.

We highlight that studies with reproductive isolation in the neotropical region are incipient, mainly in a context of chiropterophilous species and that to understand the speciation process through reproductive isolation, it is important to estimate the strengths of pre and post-pollination barriers acting simultaneously.

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Tables

Table 1. Floral traits (measured in cm) and nectar attributes in two co-flowering *Bauhinia* taxa (Fabaceae) in the Caatinga, northeastern Brazil. A P < 0.05 was considered statistically significant.

Traits	*Bauhinia acuruana*		*Bauhinia pentandra*		Comparisons
	n	mean±SD	n	mean±SD	
Flower Length	30	3.89±0.38	30	5.86±0.20	t=-16.03; df=30; p=0.0001
Hypantium Lenght	30	1.35±0.10	30	2.02±0.42	t=-5.87; df=17.18; p=0.0001
Stamens Length	30	2.83±0.19	30	3.42±0.08 (Long) 2.99±0.35 (Short)	t=-9.09; df=19.13; p=0.0001 t=-1.3; df=30; p=0.004
Pistil Lenght	30	4.05±0.44	30	5.30±0.10	t=-9.33; df=16.75; p=0.0001
Nectar Volume (µl)	30	67.8±40.86	30	79.8±46.48	t=0.39; df=12; p=0.10
Nectar Concentration (%)	30	20.16±5.7	30	19.11±5.46	t=0.48; df=12; p=0.12

Table 2. Fruit and seed set in two co-flowering *Bauhinia* taxa (Fabaceae) under different pollination conditions in the Caatinga, northeastern Brazil. Fruit set: (I) Outcrossing and natural pollination —values within the same column followed by the same subscribed letter were not statistically different (P > 0.05); Seed set: (II) Outcrossing and pollination natural—values within the same column followed by the same subscribed letter were not statistically different (P > 0.05); *Flowers used as receptors of interspecific pollen.

Treatments	*Bauhinia acuruana*			*Bauhinia pentandra*		
	n	Fruits (%)	Seeds (Mean±SD)	n	Fruits (%)	Seeds (Mean±SD)
Agamospermy	45	0 (0)	0	45	0	0
Spontaneous self-pollination	45	0 (0)	0	45	0	0
Hand self-pollination	45	0 (0)	0	45	0	0
Outcrossing (intrataxa)	30	6(20)^{a}	21±1,67^{a}	30	8(26,6)^{a}	14,5±1,5^{a}
Outcrossing (intertaxa)	10*	0 (0)	0	15*	0	0 (0)
Natural pollination	50	8(16)^{a}	22,4±1,13^{a}	50	12(24)^{a}	16,8±1,17^{b}

Table 3. Effectiveness of pre-pollination and post-pollination reproductive isolation barriers and their absolute contribution (AC) to reproductive isolation (RI) between *Bauhinia acuruana* (Ba) and *B. pentandra* (Bp) in the Caatinga, northeastern Brazil. AC.Ba and AC.Bp included geographic isolation while *-geog* excluded geographic isolation and only considered isolation components estimated in sympatry. Isolation components vary from zero (no barriers) to one (complete isolation)

Isolation barrier	RI. *Ba*	RI.*Bp*	AC.*Ba*	AC.*Bp*	AC.*Ba* - geog	AC.*Bp* - geog
Geographic	0.95	0.96	0.95	0.96	-	-
Microhabitat	0.66	0	0.033	0	0.66	0
Phenology	0.32	0.48	0.0054	0.0192	0.309	0.48
Pollinator	0	0	0	0	0	0
Pos-pollination	1	1	0.0116	0.0208	0.031	0.52
TOTAL			1	1	1	1

Figures

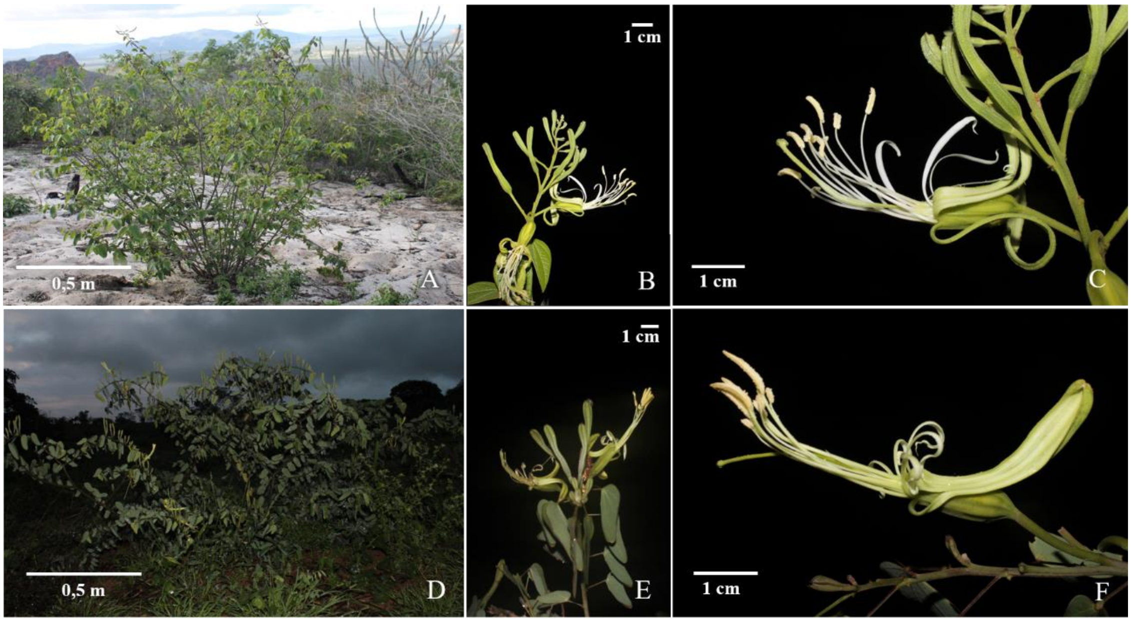

Figure 1. Individuals, inflorescences and flowers of two *Bauhinia* species in the Caatinga, northeastern Brazil. Flowering individual (A), terminal inflorescence (B) and flower (C) of *Bauhinia acuruana*. Flowering individual (D), terminal inflorescence (E) and flower (F) of *Bauhinia pentandra*. Both species show similar floral morphology, but have differences in the size of their flowers.

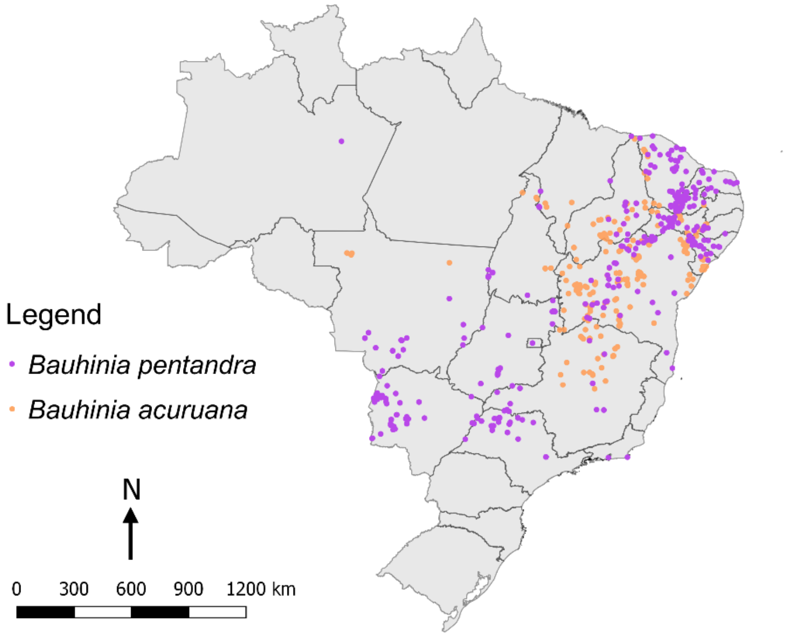

Figure 2. The distribution range of *Bauhinia acuruana* and *B. pentandra* in Brazil. The distribution range of the two species was based on localities in herbarium collections deposited on the SpeciesLink.

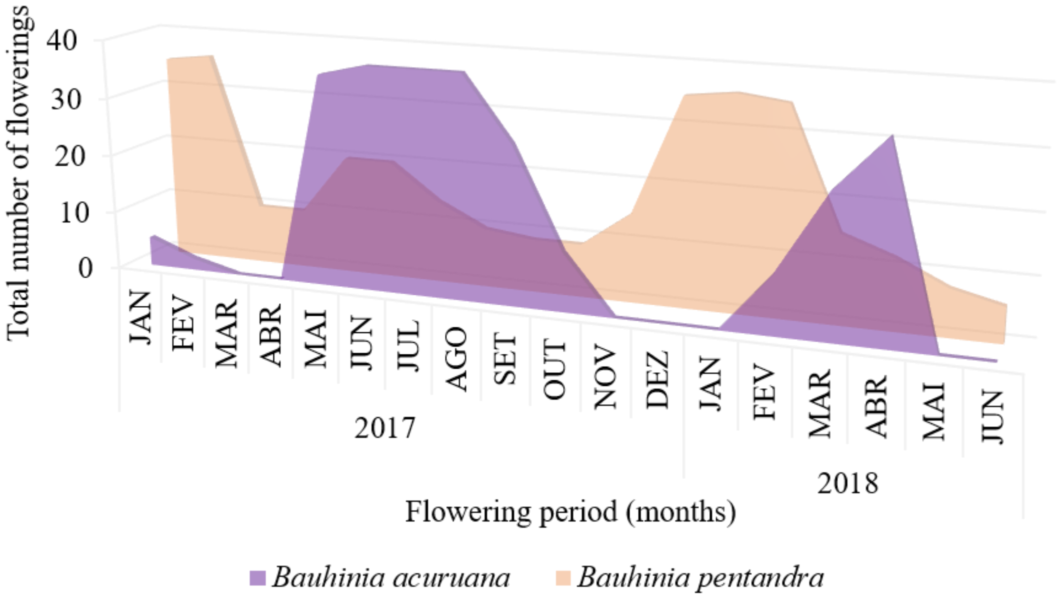

Figure 3. Flowering phenology of two *Bauhinia* species in the Caatinga, northeastern Brazil.

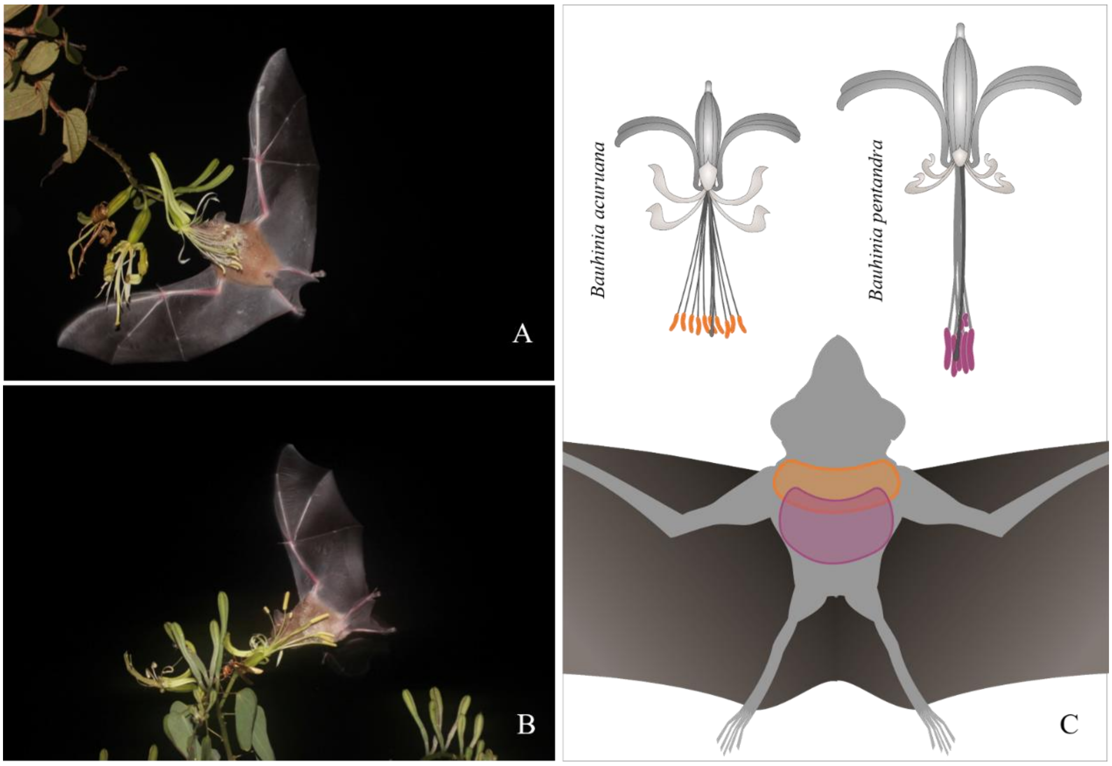

Figure 4. Pollination by glossophagine bats in two co-flowering *Bauhinia* in the PARNA Catimbau, a Caatinga area, northeastern Brazil. Glossophagine bats accessing the flower of *Bauhinia acuruana* with sternotribic pollen deposition on the neck (A and C). and accessing a flower of *Bauhinia pentandra* with sternotribic pollen deposition on the neck and abdomen (B and C).

4 CONCLUSÕES

As características florais das duas espécies de *Bauhinia* e a polinização por morcegos Glossophagine evidenciam dois exemplos clássicos de quiropterofilia. Constatamos que vários fatores como diferenças no tamanho e estruturas florais, além de diferenças nos picos de floração, contribuem para o isolamento de duas espécies simpátricas de *Bauhinia* na Caatinga, preservando a integridade desse par de espécies. Nossos resultados mostraram uma interação entre as barreiras pré e pós polinização, mantendo o isolamento reprodutivo das espécies coexistentes, e que o deslocamento de caracteres pode ter sido um fator importante na evolução dessas barreiras, bem como evitar a perda de pólen.

Este trabalho é o primeiro estudo que mediu o isolamento reprodutivo entre espécies simpátricas polinizadas por morcegos usando métodos para estimar a força do isolamento reprodutivo através de barreiras pré e pós-polinização. Outros estudos, incluindo análises filogenéticas, poderiam contribuir para respostas em um contexto evolutivo, levando em consideração a proximidade e as relações filogenéticas entre estas espécies, evidenciando as origens do isolamento entre essas espécies.

Por fim, destacamos que os estudos com isolamento reprodutivo na região neotropical são incipientes, principalmente em um contexto de espécies quiropterófilas e que para entender o processo de especiação através do isolamento reprodutivo, é importante estimar as forças de barreiras pré e pós-polinização que atuam simultaneamente.

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