Jamille Costa Veiga

História natural de abelhas nativas sem ferrão (Apidae, Meliponini):

biologia reprodutiva, tendências macroevolutivas e genética de populações

Natural history of native stingless bees (Apidae, Meliponini):

reproductive biology, macroevolutionary trends and population genetics

UNIVERSIDADE FEDERAL DO PARÁ
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

JAMILLE COSTA VEIGA

História natural de abelhas nativas sem ferrão (Apidae, Meliponini):

biologia reprodutiva, tendências macroevolutivas e genética de populações

Orientador: Prof. Dr. Felipe Andrés León Contrera
Co-orientador: Dr. Cristiano Menezes

Belém-Pará
2019

UNIVERSIDADE FEDERAL DO PARÁ
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

JAMILLE COSTA VEIGA

História natural de abelhas nativas sem ferrão (Apidae, Meliponini):

biologia reprodutiva, tendências macroevolutivas e genética de populações

Tese apresentada ao Programa de Pós-graduação em Ecologia, Universidade Federal do Pará e Embrapa Amazônia Oriental, como requisito parcial para a obtenção do título de Doutora em Ecologia.
Área de concentração: Ecologia.
Linha de Pesquisa: Ecologia de Organismos e Populações

Belém-Pará
2019

Ficha catalográfica

Dados Internacionais de Catalogação na Publicação (CIP) de acordo com ISBD
Sistema de Bibliotecas da Universidade Federal do Pará
Gerada automaticamente pelo módulo Ficat, mediante os dados fornecidos pelo(a) autor(a)

V426h Veiga, Jamille Costa
História natural de abelhas nativas sem ferrão (Apidae, Meliponini) : biologia reprodutiva, tendências macroevolutivas e genética de populações / Jamille Costa Veiga. — 2019.
170 f. : il. color.

Orientador(a): Prof. Dr. Felipe Andrés León Contrera
Coorientador(a): Prof. Dr. Cristiano Menezes
Tese (Doutorado) - Programa de Pós-Graduação em Ecologia, Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, 2019.

1. reprodução. 2. macroevolução. 3. genética de paisagem. 4. conservação. I. Título.

CDD 574.5248

JAMILLE COSTA VEIGA

História natural de abelhas nativas sem ferrão (Apidae, Meliponini):

biologia reprodutiva, tendências macroevolutivas e genética de populações

Tese apresentada ao Programa de Pós-Graduação em Ecologia do convênio da Universidade Federal do Pará e Embrapa Amazônia Oriental, como requisito parcial para obtenção do título de Doutora em Ecologia pela comissão julgadora composta pelos membros:

COMISSÃO JULGADORA

Prof. Dr. Felipe Andrés León Contrera

Universidade Federal do Pará (Presidente)

Profª. Drª. Astrid Peixoto Kleinert

Universidade de São Paulo

Profª. Drª. Batina Blochtein

Pontifícia Universidade Católica do Rio Grande do Sul

Dr. Dirk Louis P. Schorkopf

Swedish University of Agricultural Sceiences

Profª. Drª. Guiomar Nates Parra

Universidad Nacional de Colombia

Profª. Drª. Maria Auxiliadora Pantoja Ferreira

Universidade Federal do Pará

Dr. Sidnei Mateus

Universidade de São Paulo

Aprovada em: 29 de março de 2019.

Local da apresentação pública: Auditório Arlindo Pinto do Instituto de Ciências Biológicas da Universidade Federal do Pará.

Dedicatória

Às abelhas sem ferrão.

Às que tive a honra de conhecer, e àquelas que ainda terei a oportunidade de fazê-lo.

Com carinho,

Jamille

Epígrafe

“A única peça sólida de verdade científica sobre a qual me sinto totalmente confiante é que somos profundamente ignorantes sobre a natureza.”

Lewis Thomas,

The Medusa and the Snail, 1979.

“Aqui existe uma história, e ela precisa ser contada.”

V. L. I. F.

Agradecimentos

"Não há o que se construa sozinho ou do nada."

A presente tese constitui parte da minha formação acadêmica. Este documento representa o meu esforço de formação, mas também as inúmeras (sim, inúmeras) contribuições das pessoas que me auxiliaram direta ou indiretamente a alcançar esse momento. Por essa razão, optei por redigir a tese utilizando a primeira pessoa do plural, pois não sou capaz de me colocar como personagem única dessa construção, que foi coletiva. Embora eu a tenha protagonizado do início ao fim, é com orgulho que anuncio a todos que participaram desse processo: **essa tese é nossa!**

Em primeiríssimo lugar, agradeço à minha família: Auriel Costa, Jorge Veiga, Jéssica Veiga e Mabel(zinha). Reconheço ter uma atitude de isolamento em relação aos meus estudos e formação, mas foi através do incentivo, do apoio (emocional e financeiro) e da admiração de vocês pela minha atuação profissional, que eu reuni forças, a cada dia, para seguir adiante. À vocês, minha família: toda a minha gratidão! É clichê, mas *sem vocês eu não seria quem sou*. Amo muito vocês!

Ao meu companheiro de vida, Diógenis Assunção: MEUS MAIS PROFUNDOS AGRADECIMENTOS! Sem o seu apoio, paciência e cuidados, essa doutora e essa tese não seriam possíveis. Sou grata por cada abraço, acalento, sorriso, xícara de café, e tantas outras coisas, que você, com todo amor do mundo, nunca deixou faltar (nem mesmo nos momentos de crise!).

Aos meus orientadores, Felipe Contrera e Cristiano Menezes, agradeço por tê-los como mestres e amigos! Que o tempo nos mantenha trabalhando juntos, unindo forças em prol daquilo que acreditamos. As abelhas precisam de nós? Não. Mas nós precisamos das abelhas – sem dúvida! Muito obrigada por todos os ensinamentos!

Obrigada, Felipe, por ter me deixado trilhar um caminho que, mesmo tortuoso, me trouxe os maiores e melhores benefícios profissionais. Já são quase dez anos de parceria, conselhos e muita paciência. Que venham os próximos dez anos de colaboração!

Obrigada, Cristiano (Ju!), por ter sido essa pessoa cheia de energia e novas ideias. Isso é inspirador! Obrigada por me ensinar, dentre tantas coisas, a encantar as pessoas com as abelhas sem ferrão: essa é uma habilidade que não tem preço.

Aos meus também orientadores/colaboradores/supervisores – ao menos, foi assim que os enxerguei durante todo esse processo: Gustavo Ruiz, Gislene Carvalho Zilse, Rodolfo Jaffé e Christoph Grüter. A atuação profissional de cada um de vocês, bem como as conversas informais, me mostrou novas maneiras de olhar e indagar a natureza, de abordar perguntas e de ser cientista. Sou muito grata por terem aceitado colaborar comigo e me adotar temporariamente enquanto eu me aventurava em novas linhas de pesquisa. Cada um de vocês, à sua maneira, me ajudou nesse processo de empoderamento científico.

Obrigada, Gustavo, por me introduzir ao mundo da morfologia funcional e comparada! Com você, aprendi muito mais do que fui capaz de entender na época em que começamos a trabalhar juntos. Agradeço por dispor do seu tempo para me ajudar com as genitálias dos machos de abelhas sem ferrão. Sei que o fez com grande satisfação!

Obrigada, professora Gislene, muito obrigada mesmo! Tê-la conhecido foi uma honra. Nosso contato foi breve, mas resultou em solo fértil para o cultivo de uma amizade e de colaborações futuras. Eu a admiro tanto e, para mim, a senhora representa muito bem a atuação das mulheres na área científica. Mais que isso: sob o meu olhar, a senhora é símbolo de uma força humana muito potente, uma característica tão necessária ao fazer científico e aos grupos de pesquisa. Agradeço pela acolhida, enstusiasmo e ensinamentos.

Obrigada, Rodolfo! Agradeço por ter me apresentado um mundo novo: a genética da paisagem. Foi graças ao seu convite para participar de um grupo de estudos (lá em 2016...), que desenvolvi as minhas principais ferramentas de trabalho. Mais que isso: com você aprendi a me valorizar mais como profissional. Aprendi a relativizar onde estou, lembrar de onde eu vim, e então me reconhecer como alguém capaz, versátil, entusiasmada e comprometida. Muito obrigada pela cerpinha mais cara que já tomei na vida (R$ 20,00 uma long neck!) Hahahaha! Esse foi um momento super importante pra mim (não pela cerveja, mas pelas suas palavras - “melhor do que eu ficar falando aqui, é você chegar lá e ver por conta própria!” - nunca vou me esquecer), pois me ajudou a chegar de cabeça erguida *nas Alemanhas*! Muito obrigada!

Christoph, thank you very much! Vielen danke! MUITO OBRIGADA! Obrigada por aceitar me orientar durante o doutorado sanduíche. Que você era um cientista admirável, isso eu já sabia. O que eu não tinha conhecimento, era da sua habilidade para lidar com as pessoas: um talento inigualável. Você vai além da mera orientação: na minha leitura, é uma interação que parte do princípio de que somos (ou seremos) todos capazes de desenvolver as próprias ideias, e por isso, permite que todos brilhem. Para além disso, permite que cada um se responsabilize por aquilo que cria, entende e argumenta, e isso gera autoconfiança. Repito: seu talento com as pessoas é inigualável. Muito obrigada por tudo!

Aos colegas e amigos que fiz em todos os lugares que trabalhei:

Time d@s Abelhud@s: Kamila Leão, Elisângela Rêgo, Janete Gomes, Ana Carolina de Queiroz, Andrio Andrade, Hayron Cardoso, Álex Sousa, Janaína Brito, Thaliana Sousa, Yasmin Gomes, André Gomes, Beatriz Coelho, Alistair Campbell, e a todos que passaram pelo Laboratório de Botânica no tempo em que lá estive. Foi uma honra poder trabalhar com cada um de vocês! Agradeço também aos criadores de abelhas sem ferrão e grandes amigos, Belino e Elisângela (Eliii, você aqui de novo? Muito top, não?), e Rosa e Dukinha, por me permitirem coletar amostras de suas colônias. Muito obrigada, meus amigos!

Time da UFPA: Valéria Silva, Benedito Nunes e Sofia Camargo. Sem vocês eu não teria fotos de genitálias tão bonitas para apresentar! Agradeço também pelas conversas científicas. Nossas eventuais discussões no passado, muito me ajudaram no presente!

Time do ITV: Éder Lanes, Amanda Reis, Waléria Monteiro, Gilliana Rosa, Gleiciane Salvador – amigos queridos, unidos pelo amor e pela pressão dos prazos (Hahahahaha)! Luiza, Mariana, Karen, Wilson e Edilson – amigos queridos que fiz quando trabalhei com a lupa de automontagem do ITV (tirando tu, Edilson, que conheço de outros carnavais)! E minha querida Juliana Galaschi(nha) – grande conselheira e parceira!

Time do INPA: Tatiane Beker, Sr. Hélio Vilas-Boas, Douglas e Diego – pessoas incríveis e de grande coração que, assim como eu, se entusiasmaram com essa história de lesões de cópula!

Time da Johannes Gutenberg Universität: Anissa Kennedy, Marina Psalti, Matteo Negroni, Simone Glaser e Tian Fei Peng – amigos muito queridos, e que muito me ajudaram, durante os meses de doutorado sanduíche na Alemanha. Espero encontrá-los todos em breve! See you soon!

Aos meus eternos amores e irmãos, que a biologia me deu de presente, e cimentou na minha vida: André Sá, Chirla Miranda e Josyane Barros. Obrigada por tudo! Amo muito vocês!

À Cristiane Krug e Márcia Gabriel, por contribuírem para que eu pudesse estar nos lugares onde estive - Manaus (Brasil) e Mainz (Alemanha), respectivamente. A generosidade de vocês em dispor de tempo para me ajudar foi essencial. Muito obrigada!

Por fim, agradeço à Jamille de nove anos atrás que, sem me deixar a par dos seus planos, perseguiu esse título acadêmico – DOUTORA EM ECOLOGIA – como o mais precioso dos tesouros. Sei que ainda tenho muito a aprender, mas hoje julgo que posso fazê-lo com mais qualidade do que antes. Obrigada, Jamille! Afinal, o que seria de nós sem o nosso passado?

A todos e todas que me ajudaram de alguma maneira: SOU PROFUNDAMENTE GRATA!

História natural de abelhas nativas sem ferrão (Apidae, Meliponini): biologia reprodutiva, tendências macroevolutivas e genética de populações

Resumo

As abelhas sem ferrão (Tribo Meliponini) - importantes polinizadores nas regiões tropicais - estão sob ameaça devido à perda de hábitat e mudanças climáticas. O uso sustentável dessas abelhas pode ser uma potente ferramenta para conservá-las, porém grandes lacunas sobre a sua biologia básica ainda constituem entraves a essa estratégia. O objetivo da presente tese foi investigar a história natural de abelhas nativas sem ferrão, com ênfase em biologia reprodutiva, tendências macroevolutivas e genética de populações. Na primeira seção, estudamos o ciclo de vida dos machos adultos, a morfologia da sua cápsula genital, e as lesões de cópula em rainhas acasaladas. Nossos resultados lançam luz sobre os comportamentos pré-acasalamento de machos, e sobre fenômenos atípicos associados, a exemplo do dimorfismo de cor entre os machos de *Melipona flavolineata*; a ocorrência de lesões de cópula em seis dentre sete espécie de abelhas sem ferrão Neotropicais (*M. flavolineata*, *M. melanoventer, M. seminigra, Frieseomelitta longipes*, *Scaptotrigona* aff. *postica* e *Plebeia minima)*, a presença de plugues copulatórios permanentes em rainhas de *M. fasciculata;* e a possibilidade de acasalamentos múltiplos facultativos em *M. seminigra*. Na segunda seção, objetivamos reconstruir o estado ancestral do tamanho de colônias em Meliponini, e testar sua correlação evolutiva com outros atributos, como tamanho corporal e longevidade de operárias, dimorfismo de castas, arquitetura de ninho e hábitos de nidificação. Nossos resultados sugerem que a variabilidade no tamanho das colônias de Meliponini está relacionada a uma flexibilidade em perder e ganhar complexidade social, e que essa variação pode moldar sua organização social (*e. g.* o tamanho de colônias apresentou associação negativa com a qualidade de operárias, e relação positiva com o dimorfismo de castas). Nossas resultados também sugerem que a eussocialidade era um atributo já presente nos ancestrais de Meliponini. Na terceira seção, investigamos como as populações da abelha Jandaíra, *M. subnitida* - um polinizador que enfrenta condições extremas de seca e temperatura - respondem à perda de hábitat e mudanças climáticas, revelando uma redução no seu fluxo gênico associada à perda de habitat; e ainda, uma distribuição heterogênea da sua variabilidade genômica adaptativa, indicando adaptações locais. Considerados em conjunto, nossos estudos convergem para uma maior compreensão da história natural das abelhas nativas sem ferrão, avançando demandas de conhecimento para seu uso sustentável e conservação.

Palavras-chave: reprodução, macroevolução, genética de paisagem, conservação

Natural history of native stingless bees (Apidae, Meliponini): reproductive biology, macroevolutionary trends and population genetics

Abstract

The stingless bees (Tribe Meliponini) - important pollinators in tropical regions - are under threat due to habitat loss and climate change. The sustainable use of these bees can be a powerful tool to conserve them, but large gaps in their basic biology still constitute obstacles to this strategy. The aim of the present thesis was to investigate the natural history of native stingless bees, with emphasis on reproductive biology, macroevolutionary trends and population genetics. In the first section, we studied the life cycle of adult males, the morphology of their genital capsules, and the copulatory lesions in mated queens. Our results shed light on pre-mating behaviors of males, and on atypical phenomena, such as the color dimorphism between males of *Melipona flavolineata*; the occurrence of copulatory lesions in six out of seven Neotropical stingless bee species (*M. flavolineata*, *M. melanoventer, M. seminigra, Frieseomelitta longipes*, *Scaptotrigona* aff. *postica* and *Plebeia minima),* the presence of long-term copulatory plugs in queens of *M. fasciculata*; and the possibility of facultative multiple mating in *M. seminigra*. In the second section, we attempted to reconstruct the ancestral state of colony size in Meliponini, and to test its correlated evolution with other attributes such as worker quality, caste dimorphism, nest architecture, and nesting habits. Our results suggest that the variability in size of Meliponini colonies is related to a flexibility in losing and gaining social complexity, and that such variation may shape social organization (*e. g.* colony size was negatively associated to worker quality, while positively related to caste dimorphism). They also suggest that eusociality was a trait already present in the Meliponini ancestors. In the third section, we investigate how the populations of the Jandaíra bee, *M. subnitida* - a pollinator that experiences extreme conditions of drought and temperature - respond to habitat loss and climate change, suggesting a reduction in its gene flow associated with habitat loss; and also a heterogeneous distribution of its adaptive genomic variability, suggesting local adaptations. Taken together, our studies converge to a broader understanding of the natural history of native stingless bees, advancing knowledge demands for their sustainable use and conservation.

***Keywords*:** reproduction, macroevolution, landscape genetics, conservation

Sumário

INTRODUÇÃO GERAL

INTRODUÇÃO GERAL

Os polinizadores - grupo funcional chave em ambientes terrestres - garantem um **serviço ecossistêmico** vital para a manutenção das comunidades de plantas silvestres e cultivadas ao redor do mundo (Kevan & Baker, 1983; Aguilar et al., 2006; Klein et al., 2007). Os serviços de **polinização** dependem tanto de populações naturais, quanto manejadas (Garibaldi et al., 2013, 2014, 2016). Nesse quesito, destacam-se as abelhas: insetos polinizadores por excelência, os quais a humanidade aprendeu a manejar (Ollerton, 2017; Hung et al., 2018; Knapp et al., 2018). Estima-se em bilhões de dólares o valor econômico dos seus serviços de polinização (Gallai et al., 2016), visto que delas dependem ao menos 35% do volume de produção mundial de alimentos (Potts et al., 2016). Contudo, as populações de abelhas, e consequentemente seus serviços de polinização, estão sob ameaça (Vanbergen et al., 2013). Dentre as principais causas da perda de polinizadores, incluem-se a redução da cobertura de floresta, resultante do desmatamento e da expansão agrícola (Laurance et al., 2014; Brown et al., 2016); e as mudanças climáticas que, alterando as condições ambientais locais, impactam na sua amplitude de distribuição - em muitos casos limitando essas populações a refúgios (Martinet et al., 2015; Giannini, Costa, et al., 2017; Giannini, Maia-Silva, et al., 2017). O declínio generalizado nas populações desses insetos tem gerado preocupação global (Potts et al., 2010, 2016; Brown et al., 2016), alertando para os riscos generalizados na redução dos serviços de polinização (Vanbergen et al., 2013), e dando origem a iniciativas internacionais voltadas a sua conservação (Potts et al., 2016).

Por movimentarem uma grande quantidade de pólen no ambiente (Roubik, 1993), as abelhas sem ferrão (**Meliponini**) têm reconhecido papel na polinização (Heard, 1999; Giannini et al., 2015). Também conhecidas como meliponíneos, são um recurso valioso para a manutenção desse serviço nas áreas tropicias, onde têm ampla distribuição (Sakagami, 1982). Na região Neotropical, encontramos cerca de 85% das espécies, enquanto ao sul da região Australásia encontramos 10%, e na Africana, os demais 5% dessa diversidade (Michener, 2007; Vit et al., 2013). Os meliponíneos apresentam uma longa história de coevolução com as plantas nativas, e em cada uma dessas regiões, figuram como polinizadores abundantes e eficientes (Heard, 1999; Slaa et al., 2006; Pacheco-Filho et al., 2015; Roubik, 2018).

As abelhas sem ferrão, juntamente com as abelhas melíferas (**Apini**), formam os únicos grupos de **abelhas corbiculadas** a ter evoluído **eussocialidade avançada** (Michener, 2007). Logo,

os meliponíneos formam sociedades, compostas por fêmeas reprodutoras (as rainhas) e fêmeas estéreis (as operárias), havendo sobreposição entre as gerações, e cooperação no cuidado com a prole (Wilson, 1971; Lin & Michener, 1972). Porém, diferentemente das abelhas melíferas, as abelhas sem ferrão são o grupo mais diverso dentre todas as abelhas eussociais**,** formando um grande taxa, com cerca de 60 gêneros e aproxidamente 500 espécies (Michener, 2013; Ascher & Pickering, 2019). A tribo Meliponini reúne espécies com grande variação nas suas características biológicas, incluindo-se o tamanho corporal e a população dos ninhos, os hábitos de nidificação e a arquitetura dos favos de cria, bem como o comportamento de forrageio e os sistemas de comunicação (Roubik, 1992; Michener, 2007; Vit et al., 2013, 2018). A ampla variação morfológica e comportamental faz das abelhas sem ferrão um excelente modelo de estudo para pesquisa em evolução, ecologia e conservação.

Ao longo da sua área de distribuição geográfica, os meliponíneos vêm sendo manejados há séculos por povos tradicionais para obtenção de recursos como **mel** e **cerume** (Quezada-Euán et al., 2018; Posey, 1983; Villanueva-G et al., 2005). Essa prática antiga foi herdada pelas gerações seguintes, e desenvolveu-se no que ficou conhecido como meliponicultura – atividade baseada na criação e no manejo das abelhas nativas sem ferrão (Nogueira-Neto, 1997; Vit et al., 2013; Heard, 2016). Os crescentes avanços na meliponicultura a vêm transformando numa prática cada vez mais tecnificada (Venturieri et al., 2012), voltada tanto para a geração de produtos tradicionais, como o mel, quanto produtos alternativos, como o pólen, a própolis, as colônias e o seu serviço de polinização (Venturieri et al., 2006, 2012; Contrera et al., 2011).

Atualmente, a meliponicultura aparece como uma poderosa ferramenta para promover o uso sustentável e a conservação das abelhas sem ferrão, a fim de garantir a manutenção das suas populações naturais, e suportar a manutenção dos serviços de polinização nas regiões tropicais (Cortopassi-Laurino et al., 2006; Vit et al., 2013, 2018; Hill et al., 2019). Contudo, é uma atividade que ainda enfrenta muitos gargalos técnicos devido às lacunas no conhecimento básico sobre a biologia das diferentes espécies de abelhas sem ferrão (Venturieri et al., 2012). O estudo da história natural das abelhas sem ferrão permite entender um pouco mais sobre a sua ecologia, comportamento e evolução, e assim avançar demandas de conhecimento voltadas à conservação de suas populações e ao seu uso sustentável.

Objetivos e organização da tese

O objetivo da presente tese foi investigar a história natural das abelhas sem ferrão, com ênfase em biologia reprodutiva, tendências macroevolutivas e genética de populações.

A tese está organizada em três seções:

Seção I – A biologia reprodutiva de abelhas sem ferrão e fenômenos atípicos associados

[Nota científica: The Life Histories of "Uruçu Amarela" Males (Apidae: Meliponini: *Melipona flavolineata*)]

[Manuscrito: Do traumatic mating plugs prevent remating in stingless bees (Apidae: Meliponini)?]

Para entender um pouco mais sobre a biologia reprodutiva das abelhas sem ferrão, estudamos o ciclo de vida dos machos adultos, e a morfologia da sua cápsula genital. Em ambos os estudos, fomos confrontados com fenômenos atípicos, ainda não descritos para o grupo. Em *Melipona flavolineata*, observamos que os machos apresentam dimorfismo de cor; perdem suas cápsulas genitais permanentemente, tornando-se estéreis; e, mesmo estéries, podem permanecer vivos. No segundo estudo, investigando como as cápsulas genitais dos machos funcionam como plugues copulatórios, observamos que esses plugues geram lesões genitais nas fêmeas recém-fecundadas e, ao menos em uma espécie, esses plugues podem ser permanente. Nossos resultados mostram que os sistemas de acasalamento em abelhas sem ferrão não são uniformes.

Seção II – Tendências macroevolutivas: tamanho de colônias em abelhas sem ferrão

[Manuscrito: Macroevolutionary trends in colony size suggest advanced eusociality in stingless bee ancestors]

Nesse estudo, objetivamos reconstruir o estado ancestral do tamanho de colônias em Meliponini, e testar sua evolução correlacionada com outros atributos da colônia. Nossa abordagem comparativa sugere que colônias de tamanho médio é o estado ancestral mais provável para o grupo, de forma que colônias pequenas e grandes seriam estados derivados. Os resultados também sugerem que colônias grandes apresentam operárias com menor tamanho corporal e menos longevas, estando fortemente associadas com arquitetura de cria compacta (discos ou semi-discos) e com hábito de nidificação em sítios expostos (*i. e.* cupinzeiros ou ninhos

construídos pelas próprias abelhas). Argumentamos que o provável estado ancestral – colônias de tamanho médio - sugere eussocialidade avançada nos ancestrais de Meliponini, hipótese reforçada pela sua ancestralidade compartilhada com as abelhas eussociais da tribo Melikertini, um clado-irmão já extinto.

Seção III – Abelhas sem ferrão e paisagem: como suas populações respondem à perda de hábitat e mudanças climáticas?

[Artigo: Landscape genomics to the rescue of a tropical bee threatened by habitat loss and climate change]

Nosso objetivo foi investigar como as populações de um polinizador – a abelha Jandaíra (*Melipona subnitida*) - que habita uma das regiões mais quentes do mundo, e enfrenta longos períodos de seca, respondem à perda de hábitat e mudanças climáticas. Para tanto, utilizamos uma nova abordagem, a genética da paisagem. Nossos resultados sugerem que a diversidade genética das populações dessa abelha não é afetada pela redução na porcentagem de hábitat, mas afeta a sua conectividade genética. Demonstramos que o fluxo gênico é favorecido em áreas com maior cobertura de floresta, maior estabilidade na temperatura, menor elevação e menor irregularidade topográfica. Além disso, detectamos sinais de adaptação genômica a condições ambientais locais, sugerindo um padrão espacial associado à latitude e à altitude, ao longo da distribuição geográfica da Jandaíra. Nosso estudo levanta novas informações sobre a história natural da espécie *M. subnitida*, e sugere que regiões com grandes flutuações na temperatura, bem como áreas desmatadas e elevadas, atuam como barreiras à conectividade genética das populações naturais dessa espécie.

Referências

AGUILAR, R., ASHWORTH, L., GALETTO, L. & AIZEN, M.A. (2006) Plant reproductive susceptibility to habitat fragmentation : Review and synthesis through a meta-analysis. *Wiley Online Library*, 9, 968–980.

ASCHER, J.S. & PICKERING, J. (2019) Discover Life bee species guide and world checklist (Hymenoptera: Apoidea: Anthophila). Https://scholar.google.com/scholar?hl=pt-PT&as_sdt=0,5&cluster=10861599613270937375

BROWN, M.J.F., DICKS, L. V., PAXTON, R.J., BALDOCK, K.C.R., BARRON, A.B., CHAUZAT, M.-P., ET AL. (2016) A horizon scan of future threats and opportunities for pollinators and pollination. *PeerJ*, 4, e2249.

CONTRERA, F.A.L., MENEZES, C. & VENTURIERI, G.C. (2011) New horizons on stingless beekeeping (Apidae, Meliponini). *Revista Brasileira de Zootecnia*, 40, 48–51.

CORTOPASSI-LAURINO, M., IMPERATRIZ-FONSECA, V.L., ROUBIK, D.W., DOLLIN, A., HEARD, T., AGUILAR, I., ET AL. (2006) Global meliponiculture : challenges and opportunities. *Apidologie*, 37, 275–292.

GALLAI, N., GARIBALDI, L.A., LI, X. & BREEZE, T.D. (2016) Economic valuation of pollinator gains and losses. In *Thematic assessment on pollinators, pollination and food production* (eds S.G. Potts & V.L. Imperatriz-Fonseca), p. . IPBES.

GARIBALDI, L., CARVALHEIRO, L.G., VAISSIÈRE, B.E., GEMMILL-HERREN, B., HIPÓLITO, J., FREITAS, B.M. & NGO, H.T. (2016) Mutually beneficial pollinator diversity and crop yield outcomes in small and large farms. *Science*, 351, 388–391.

GARIBALDI, L.A., CARVALHEIRO, L.G., LEONHARDT, S.D., AIZEN, M.A., BLAAUW, B.R., ISAACS, R., ET AL. (2014) From research to action: Enhancing crop yield through wild pollinators. *Frontiers in Ecology and the Environment*, 12, 439–447.

GARIBALDI, L.A., STEFFAN-DEWENTER, I., WINFREE, R., AIZEN, M.A., BOMMARCO, R., CUNNINGHAM, S.A., ET AL. (2013) Wild Pollinators Enhance Fruit Set of Crops Regardless of Honey Bee Abundance. *Science*, 339, 1608–1611.

GIANNINI, T.C., BOFF, S., CORDEIRO, G.D., CARTOLANO, E.A., VEIGA, A.K., IMPERATRIZ-FONSECA, V.L. & SARAIVA, A.M. (2015) Crop pollinators in Brazil: a review of reported interactions. *Apidologie*, 46, 209–223.

GIANNINI, T.C., COSTA, W.F., CORDEIRO, G.D., IMPERATRIZ-FONSECA, V.L., SARAIVA, A.M., BIESMEIJER, J. & GARIBALDI, L.A. (2017) Projected climate change threatens pollinators and crop production in Brazil. *PLOS ONE*, 12, e0182274.

GIANNINI, T.C., MAIA-SILVA, C., ACOSTA, A.L., JAFFÉ, R., CARVALHO, A.T., MARTINS, C.F., ET AL. (2017) Protecting a managed bee pollinator against climate change: strategies for an area with extreme climatic conditions and socioeconomic vulnerability. *Apidologie*, 48, 784–794.

HEARD, T.A. (1999) The role of stingless bees in crop pollination. *Annual Review of Entomology*, 44, 183–206.

HEARD, T.A. (2016) The Australian native bee book: keeping stingless bee hives for pets, pollination, and sugarbag honey. In *Published by Sugarbag Bees, express print and mail* p. . Marrochydore, Australia.

HILL, R., NATES-PARRA, G., QUEZADA-EUÁN, J.J.G., BUCHORI, D., LEBUHN, G., MAUÉS, M.M., ET AL. (2019) Biocultural approaches to pollinator conservation. *Nature Sustainability*, 2, 214–222. Springer US.

HUNG, K.L.J., KINGSTON, J.M., ALBRECHT, M., HOLWAY, D.A. & KOHN, J.R. (2018) The worldwide importance of honey bees as pollinators in natural habitats. *Proceedings of the Royal Society B: Biological Sciences*, 285.

KEVAN, P.G. & BAKER, H.G. (1983) Insects as Flower Visitors and Pollinators. *Annual Review of Entomology*, 28, 407–453.

KLEIN, A.-M., VAISSIÈRE, B.E., CANE, J.H., STEFFAN-DEWENTER, I., CUNNINGHAM, S.A., KREMEN, C. & TSCHARNTKE, T. (2007) Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, 274, 303–313.

KNAPP, J.L., SHAW, R.F. & OSBORNE, J.L. (2018) Pollinator visitation to mass-flowering courgette and co-flowering wild flowers: Implications for pollination and bee conservation on farms. *Basic and Applied Ecology*, 34, 85–94. Elsevier GmbH.

LAURANCE, W.F., SAYER, J. & CASSMAN, K.G. (2014) Agricultural expansion and its impacts on tropical nature. *Trends in Ecology and Evolution*, 29, 107–116.

LIN, N. & MICHENER, C.D. (1972) Evolution of sociality in insects. *The Quartely Review of Biology*, 47, 131–159.

MARTINET, B., RASMONT, P., CEDERBERG, B., EVRARD, D., ØDEGAARD, F., PAUKKUNEN, J. & LECOCQ, T. (2015) Forward to the north: Two Euro-Mediterranean bumblebee species now cross the Arctic Circle. *Annales de la Societe Entomologique de France*, 51, 303–309.

MICHENER, C. (2013) The Meliponini. In *Pot-Honey: A legacy of the stingless bees* pp. 3–17. Springer Science & Business Media, New York.

MICHENER, C.D. (2007) The bees of the world, 2nd edition. Johns Hopkins University Press, Baltimore, Maryland, USA.

NOGUEIRA-NETO, P. (1997) Vida e criação de abelhas indígenas sem ferrão. Nogueirapis, São Paulo.

OLLERTON, J. (2017) Pollinator Diversity: Distribution, Ecological Function, and Conservation. *Annual Review of Ecology, Evolution, and Systematics*, 48, annurev-ecolsys-110316-022919.

PACHECO-FILHO, A.J. DE S., VEROLA, C.F., LIMA-VERDE, L.W. & FREITAS, B.M. (2015) Bee-flower association in the Neotropics: implications to bee conservation and plant pollination. *Apidologie*, 46, 530–541.

POSEY, D.A. (1983) Folk apiculture of the Kayapo Indians of Brazil. *Biotropica*, 15, 154–158.

POTTS, S.G., BIESMEIJER, J.C., KREMEN, C., NEUMANN, P., SCHWEIGER, O. & KUNIN, W.E. (2010) Global pollinator declines: Trends, impacts and drivers. *Trends in Ecology and Evolution*, 25, 345–353. Elsevier Ltd.

POTTS, S.G., FONSECA, V.L.I., NGO, H.T., BIESMEIJER, J.C., BREEZE, T.D., DICKS, L. V., ET AL. (2016) IPBES (2016): Summary for policymakers of the assessment report of the Intergovernmental Science - Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production. Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, Bonn, Germany.

QUEZADA-EUÁN, J.J.G., NATES-PARRA, G., MAUÉS, M.M., IMPERATRIZ-FONSECA, V.L. & ROUBIK, D.W. (2018) Economic and cultural values of stingless bees (Hymenoptera: Meliponini) among ethnic groups of tropical America. *Sociobiology*.

ROUBIK, D.W. (1992) Ecology and natural history of tropical bees, 1st edition. Cambridge University Press, Cambridge.

ROUBIK, D.W. (1993) Direct Costs of Forest Reproduction, Bee-Cycling and the Efficiency of Pollination Modes. *Journal of Biosciences*, 18, 537–552.

ROUBIK, D.W. (2018) 100 Species of Meliponines (Apidae: Meliponini) in a Parcel of Western Amazonian Forest at Yasuní Biosphere Reserve, Ecuador. In *Pot-Pollen in Stingless Bee Melittology* pp. 189–206. Springer International Publishing, Cham.

SAKAGAMI, S. (1982) Stingless Bees. In *Social Insects, v. 3* pp. 361–423.

SLAA, E.J., SÁNCHEZ CHAVES, L.A., MALAGODI-BRAGA, K.S. & HOFSTEDE, F.E. (2006) Stingless bees in applied pollination: practice and perspectives. *Apidologie*, 37, 293–315.

VANBERGEN, A.J., GARRATT, M.P., VANBERGEN, A.J., BAUDE, M., BIESMEIJER, J.C., BRITTON, N.F., ET AL. (2013) Threats to an ecosystem service: Pressures on pollinators. *Frontiers in Ecology and the Environment*, 11, 251–259.

VENTURIERI, G.C., ALVES, D.A., VILLAS-BÔAS, J.K., CARVALHO, C.A.L. DE, MENEZES, C., VOLLET-NETO, A., ET AL. (2012) Meliponicultura no Brasil: situação atual e perspectivas futuras para o uso na polinização agrícola. In *Polinizadores no Brasil: Contribuição e Perspectivas para a Biodiversidade, Uso Sustentável, Conservação e Serviços Ambientais* (eds V.L. Imperatriz-fonseca, D.A.L. Canhos, D. de A. Alves & A.M. Saraiva). Editora da Universidade de São Paulo, São Paulo.

VENTURIERI, G.C., SOUZA, M.S., PEREIRA, C.A.B. & RODRIGUES, S.T. (2006) Manejo de polinizadores autóctones de açaizeiro (Euterpe oleraceae Mart.) na Amazônia Oriental. In *VII Encontro sobre Abelhas* p. . Universidade de São Paulo, Ribeirão Preto.

VILLANUEVA-G, R., ROUBIK, D.W. & COLLI-UCÁN, W. (2005) Extinction of *Melipona beecheii* and traditional beekeeping in the Yucatán peninsula. *Bee World*, 86, 35–41.

VIT, P., PEDRO, S.R.M. & ROUBIK, D.W. (2018) Pot-Pollen in Stingless Bee Melittology. Springer International Publishing AG.

VIT, P., PEDRO, S.R.M. & ROUBIK, D.W. (2013) Pot-Honey: A legacy of stingless bees. Springer Science & Business Media, New York, NY.

WILSON, E..O. (1971) The Insect Societies. Harvard University Press, Cambridge.

SEÇÃO I

Biologia reprodutiva de abelhas sem ferrão
e fenômenos atípicos associados

Resumo

Para entender um pouco mais sobre a biologia reprodutiva das abelhas sem ferrão (Meliponini), investigamos o ciclo de vida dos machos adultos e sua interação com as fêmeas. No primeiro estudo desta seção, descrevemos as etapas do ciclo de vida dos machos da abelha sem ferrão *Melipona flavolineata*, o que nos revelou três fenômenos atípicos para os machos de Meliponini. Nessa espécie, observamos que (i) os machos apresentam dimorfismo de cor - encontramos machos claros e escuros; (ii) perdem suas cápsulas genitais, mesmo quando não copulam com sucesso, tornando-se estéreis; e, (iii) mesmo estéreis, podem permanecer vivos. No segundo estudo desta seção, investigamos a morfologia funcional da cápsula genital masculina em sete espécies de Meliponini Neotropicais. Nesse grupo, a cápsula masculina funciona como plugue copulatório, uma estratégia do macho para limitar o número de acasalamentos da fêmea. Comparamos a ativação da genitália masculina pré- e pós-ativação do plugue, bem como a câmara genital entre rainhas virgens e acasaladas. Mais uma vez, fomos confrontados com fenômenos ainda não descritos para Meliponini e, pela primeira vez, relatamos para o grupo a ocorrência de plugues permanentes e de lesões genitais causadas por estruturas do plugue. Em pelo menos uma espécie, *M. fasciculata*, o plugue é de longo prazo, permanecendo no interior das fêmeas por toda sua vida; enquanto nas demais espécies, é de curto prazo. Em *M. seminigra*, observamos repetidamente múltiplas lesões, indicando que o reacasalamento é possível nesta espécie. Nosso estudo mostra que os sistemas de acasalamento de abelhas sem ferrão não são uniformes. Por fim, propomos a investigação das lesões genitais como um método simples e de menor custo para inferir tentativas de acasalamento da rainha.

The Life Histories of the “Uruçu Amarela” Males (*Melipona flavolineata*, Apidae, Meliponini)

Jamille Veiga, Kamila Leão, Beatriz Coelho, Ana Carolina de Queiroz, Cristiano Menezes & Felipe Contrera

Publicado em **Sociobiology**, 65(4): 780-783 (2018), Edição Especial sobre Abelhas

As histórias de vida dos machos de “Uruçu Amarela” (*Melipona flavolineata*, Apidae, Meliponini)

Resumo

Neste trabalho descrevemos as etapas do ciclo de vida dos machos da abelha sem ferrão amazônica *Melipona flavolineata* Friese, popularmente conhecida como “Uruçu Amarela”. Os machos atingem a maturidade sexual dentro de seus ninhos de origem, apresentando vesículas seminais repletas de espermatozoides e tornando-se capazes de voar com uma média de idade de 10 e 15 dias, respectivamente. Eles formam agregações duas vezes durante suas vidas: uma vez antes de deixar o ninho, e outra em sítios externos de congregação. Sua capacidade de alcançar esses sítios depende de atributos morfológicos, como olhos grandes e tórax alongado. Além disso, descrevemos três fenômenos atípicos para machos de Meliponini: os machos de *M. flavolineata* apresentam dimorfismo de cor (encontramos machos claros e escuros); perdem suas cápsulas genitais, mesmo quando não copulam; e, ainda, os machos sem cápsula genital (estéreis) podem permanecer vivos por até dois dias. As estratégias de história de vida dos machos Meliponini apenas começaram a ser investigadas e fornecem muitas questões interessantes para estudos futuros.

The Life Histories of “Uruçu Amarela” Males (*Melipona flavolineata*, Apidae, Meliponini)

Abstract

Here we describe the life histories of adult males of the the Amazonian stingless bee *Melipona flavolineata*, commonly known as “Uruçu Amarela”. Males reach sexual maturity inside nests, presenting seminal vesicles full of sperm cells and bing able to fly at a mean age of 10 and 15 days, respectively. They aggregate twice in their life, once before leaving the nest, and another at external congregation sites; with their capacity to reach congregation sites dependent on morphological attributes, such as large eyes and elongated thorax. Furthermore, we describe three atypical phenomena for Meliponini males: *M. flavolineata* males have dimorphic color pattern; they lose their genital capsules, even when they fail to copulate; and penisless (sterile) males can stay alive for up to two days. The life history strategies of Meliponini males have only just started to be told and provide many interesting questions for future studies.

1. Introduction

Over the last 50 years, the life histories of male stingless bees (Apidae: Meliponini) have been documented in great detail (Nogueira-Neto, 1954; Kerr et al., 1962; Van Veen et al., 1997; Koffler et al., 2016; Schorkopf, 2016), showing that male behaviors and reproductive strategies are diverse and very different of Apini (Engels & Imperatriz-Fonseca, 1990; Paxton, 2005). Males leave the nests after they reach sexual maturity (Van Veen et al., 1997), before forming reproductive aggregations in specific sites (Paxton, 2005). During the copula, the male leaves his genital capsule inside the female, leaving the individual sterile (Kerr et al., 1962; Engels & Imperatriz-Fonseca, 1990).

This short note combines several field observations and experiments that, together, help to understand the life histories of *Melipona flavolineata* males. All observations took place at Embrapa Amazônia Oriental, Belém, Pará state, Brazil, between 2014 and 2017.

2. Inside the nests: intranidal behaviors, sexual maturity and the point of no return

To study sexual maturation in *M. flavolineata* males, we used the total number of sperm cells in seminal vesicles as a proxy of a male's sexual maturity, and evaluated its variation as a function of age (time since eclosure). Males were held in minicolonies under laboratory conditions and sampled at different ages - from 0 to 25 days after emergence (ESM 1 – Table 1). We also tested the flight ability of each male through light stimulus (details in ESM1). The sperm migration from the testicles to the seminal vesicles started between zero and five days, when we could observe a greater number of sperm bundles in the seminal vesicles, and a few free sperm cells. From 10 days, we could not find the bundles anymore, observing only the presence of individual sperm cells until 25 days (Figure 1a). We found that males reach sexual maturity between 10 and 15 days after emergence, following which, total sperm cells was similar to subsequent age categories (KW-H (5;55) = 36.425; $p < 0{,}001$), and the increment in the total number of sperm cells was followed by simultaneous improvement in flight ability (Figure 1a).

To investigate males' intranidal behaviors and the age that males would leave the nests we marked 160 individuals with numbered and colored tags (40 male bees from four different colonies). We observed three main activities performed by males inside the nests: walking-standing, self-grooming and nectar dehydration, as previously observed in other *Melipona* species (Van Veen et al., 1997). We also observed males forming groups of at least three individuals with a mean age of 14 days. Groups were observed near inner nest entrances, with a persistence of no longer than

48h. After this, these individuals were never observed again inside the nests. Outside of nests, we identified eight males at congregation sites, with a mean age of 20.5 days (± 1.41 s. d.).

3. Outside the nests: reaching the congregation sites

M. flavolineata reproductive aggregations can be formed at the entrance of conspecific nests, and sometimes are composed of hundreds of males (about 150 to 300 individuals; Figure 2a-b). Although male aggregations are only temporary, we observed that these aggregations remain present for several days or weeks between the months of July and December over three consecutive years (2013, 2014 and 2015). *M. flavolineata* seems to differ from other species of *Melipona*, whose males are known to aggregate distant from nests and in some cases in groups of few individuals (Sommeijer et al., 1995; Santos et al., 2014), but whether such pattern sustains out of a meliponary context, it still need to be confirmed.

Aggregations are known to be a source of pre-copulatory selection in eusocial bees, selecting more competitive males in *Apis mellifera* and *Scaptotrigona depillis* (Jaffé et al., 2010; Koffler et al., 2016). Based on this, we compared the body size of *M. flavolineata* males before and after the formation of congregation sites (corresponding to pre- and post-selection scenarios), as follows: (i) we first sampled males emerging as adults inside different nests (brood combs: pre-selection group; n=90 obtained from ten nests); and (ii) after about 20 days of this first step, males were sampled from congregation sites (congregation: post-selection group; n=90 collected at random in five different congregation sites); finally (iii) all bees were measured in order to compare head and thorax attributes (details in ESM1). We highlight that male origin was not controlled for the congregation group, which may have affected our results. However, we believe our sampling effort represented the variation in population once we obtained males from different congregation sites.

We found differences in body size among males sampled before and after congregation formations: males sampled from brood combs showed higher median values and variation of interocular distance and intertegular distance, and lower median values of thorax length in relation to males sampled from congregation sites (Figure 1b-e), despite not differing in other body attributes such as head width, head length and tibia length (ESM1 – Table 2). Our results show that although all males had similar head sizes, the males sampled from congregation sites had shorter interocular distances, meaning they have larger eyes; they also had shorter intertegular distances and more elongate thoraxes than males sampled from brood combs, suggesting their body shape may be more aerodynamic. We also suggest that extremes were negatively selected as variation in body size was much lower in congregations. Even though, the males collected inside colonies were

not compared to males from the same colonies that successfully reached an aggregation, which would be ideal, our sampled was not small and males were collect from different aggregations, and therefore we believe that probably represented the variation of population. Our results corroborate with previous findings that Meliponini males undergo pre-copulatory selection (Koffler et al., 2016).

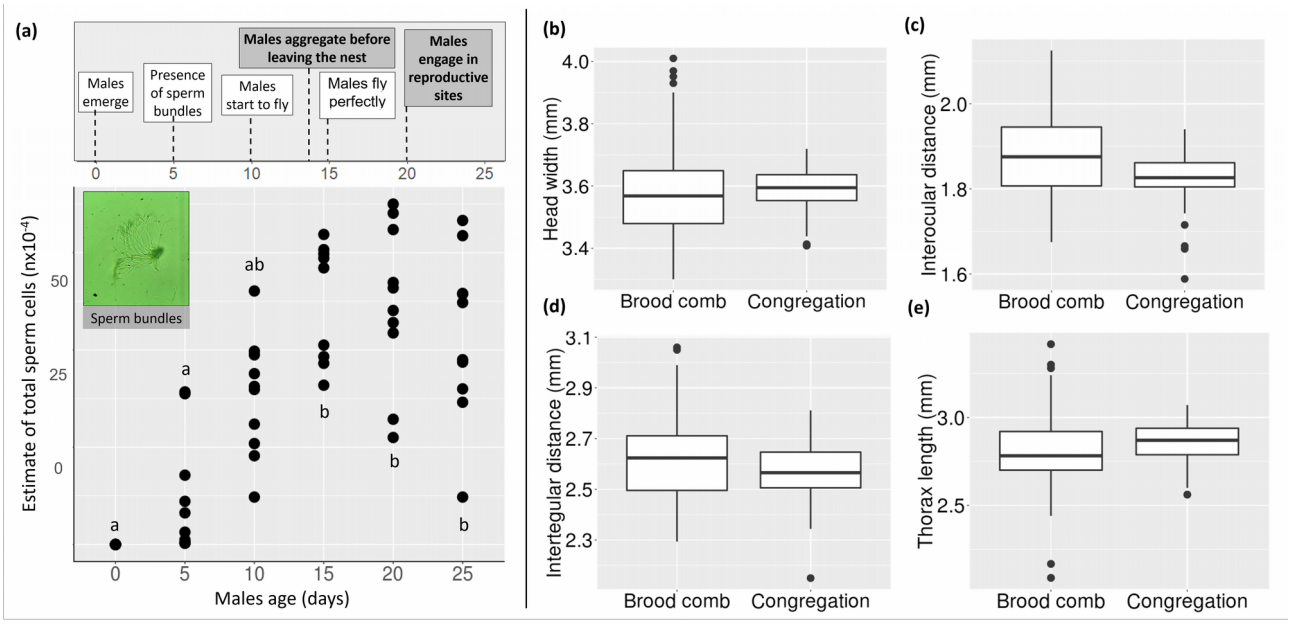


Figure 1: Development of sexual maturity of the "Uruçu Amarela" males, *Melipona flavolineata*. Here we show the phases of males' life history and the variation of estimated number of total sperm cells in seminal vesicles as a function of males' age in days; rank comparisons are indicated with letters (a); we also show the comparison of four body size attributes of *M. flavolineata* males sampled from brood combs (n = 90) and congregation sites (n = 90), showing similarities in head width (b), and differences in interocular distance (c), thorax length (d) and intertegular distance (e). Check for statistics in Table S2.

4. Atypical phenomena: color dimorphism and penisless males

Males of *M. flavolineata* exhibit dimorphic color-patterning, including both light and dark morphs (Figure 2). Light morphs can be diagnosed by their yellow metasoma, yellow-ferruginous legs with blackish marks, and vertex and mesosoma with corn yellow setae. The decumbent setae on third to fifth metasomal tergum are bright yellow. All these patterns being similar to those of worker bees. The dark morph differs from light morphs in having black legs and metasoma, vertex and mesosoma with brownish-yellow setae. The decumbent setae on third to fifth metasomal

tergum are brownish-yellow. We observed that the two morphs can emerge in the same colony. From a sample of twenty-one brood combs with pre-emergent pupae from twelve colonies, we observed of the 1.364 males emerging as adults, 78.15% were light morph and 21.85% were dark morph. The proportion of light and dark phenotypes produced in each brood comb varied and did not follow a pattern (ESM1 – Table 3). Abdominal coloration in stingless bees might be involved in the regulation of body temperature in extreme thermal conditions (Pereboom & Biesmeijer, 2003). This suggests that, it is possible that variation in color-patterns of males of *M. flavolineata* has also a role in thermoregulation. To our knowledge, this is the first report of color dimorphism in male stingless bees.

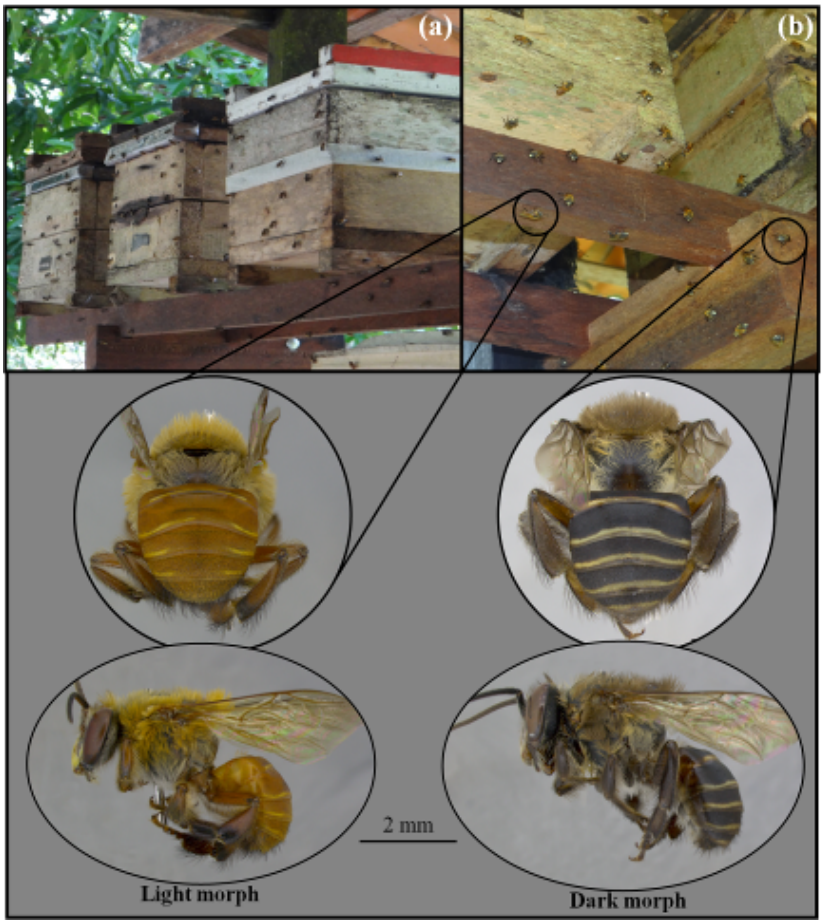


Figure 2: *Melipona flavolineata* reproductive aggregation placed at a congregation site near conspecific nests (a). Detail of the aggregation showing males with different body color-patterns (b). *M. flavolineata* dimorphic males, the light morph (left) and the dark morph (right).

Another atypical behavior was observed when we used males' mating attempts as a proxy of sexual attractiveness of virgin queens in *M. flavolineata* under laboratory conditions (Veiga et al., 2017). We observed two interesting points: (i) *M. flavolineata* males permanently lose their genital capsules when they copulate, also when they fail to do so (here failing in copulation means that males which attempted to copulate everted their genital capsule outside female's body); and (ii) these penisless (sterile) males can stay alive, reengage in congregation sites and behave similarly to virgin males. When males failed in copulation, they simply everted their genital capsules, losing it in the air (Figure 3a-b). We repeatedly observed such behavior under laboratory conditions, where 13.23% of 650 (in the first experiment), and 7.83% of 600 (in the second experiment) males permanently lost their genital capsules without a successful copulation. Additionally, when we sampled reproductive aggregations, we observed males lacking genital capsules (10% in the first congregation site, from a total of 50 sampled individuals; 5% in the second, from 60 samples, and 7.7% in the third, from 90 samples). Penisless males stay alive on average for a further 2.62 days (±1.41 s.d.) and can be easily identified by the absence of the genital capsule (Figure 3c-d).

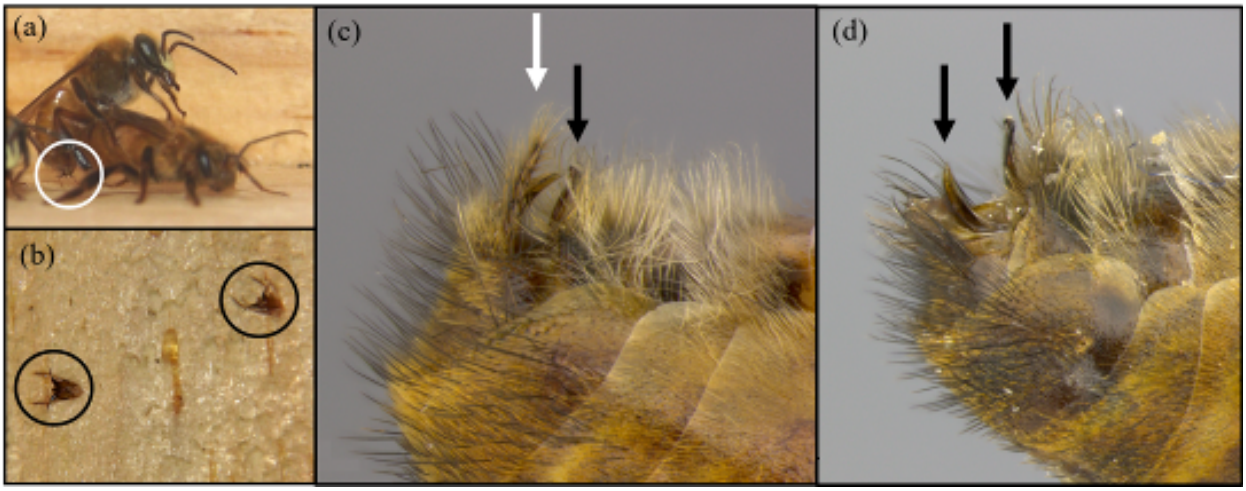


Figure 3: Atypical phenomena observed in *Melipona flavolineata* males: (a) loss of male genital capsule without a successful copulation, (b) male genital capsules lost after mating failures, (c) male with complete reproductive apparatus, including the genital capsule (white arrow) and genital sterna (black arrows), and (d) a penisless male found at congregation sites, presenting only genital sterna (black arrows) and with absent genital capsule.

The life history of males of stingless bees has just started to be told. The main message is that their life histories are much more diverse than previously thought, and many aspects are yet to be described and understood.

Acknowledgements

We would like to thank Denyse Cardoso da Silva and Rodriga de Andrade e Silva for their support in field work. We also thank Dr. Alistair Campbell for language review, and the anonymous referees for valuable suggestions. We thank CAPES/EMBRAPA (15/2014) for providing grants to JCV and KLL. This research was funded by CNPQ (400435/2014-4) through the PVE 2014 Project.

Literature cited

Bishop, G. H. (1920). Fertilization in the honey-bee. I. The male sexual organs: Their histological structure and physiological functioning. Journal of Experimental Zoology, 31:224–265. doi: 10.1002/jez.1400310203

Elias-Neto, M., Nascimento, A. L. O., Bonetti, A. M., Nascimento, F. S., Mateus, S., Garófalo, C. A., Bitondi, M. M. G. (2014). Heterochrony of cuticular differentiation in eusocial corbiculate bees. Apidologie, 45:397–408. doi: 10.1007/s13592-013-0254-1

Engels, W. & Imperatriz-Fonseca, V. L. (1990). Caste development, reproductive strategies, and control of fertility in honey bees and stingless bees. In: (Engels, W.), Social Insects (pp. 167–230). Springer Berlin Heidelberg.

Jaffé, R. & Moritz, R. F. A. (2010). Mating flights select for symmetry in honeybee drones (*Apis mellifera*). Naturwissenschaften, 97:337–343. doi: 10.1007/s00114-009-0638-2

Kerr, W. E., Zucchi, R., Nakadaira, J. T. & Butolo, J. E. (1962). Reproduction in the social bees (Hymenoptera: Apidae). Journal of the New York Entomological Society, 70:265–276.

Koffler, S., Meneses, H. M., Kleinert, A. de M. P. & Jaffé, R. (2016). Competitive males have higher quality sperm in a monogamous social bee. BMC Evolutionary Biology, 16:195. doi:10.1186/s12862-016-0765-2

Nogueira-Neto, P. (1954). Notas bionômicas sobre meliponíneos: III–Sobre a enxameagem. Arquivos do Museu Nacional, 42:419-451.

Paxton, R. J. (2005). Male mating behaviour and mating systems of bees: an overview. Apidologie, 36:145–156. doi: 10.1051/apido:2005007

Pereboom , J. J. M. & Biesmeijer, J. C. (2003). Thermal constraints for stingless bee foragers: the importance of body size and coloration. Oecologia, 42–50. doi: 10.1007/s00442-003-1324-2

Santos, C. F., Menezes, C., Vollet-Neto, A. & Imperatriz-Fonseca, V. L. (2014). Congregation sites and sleeping roost of male stingless bees (Hymenoptera: Apidae: Meliponini). Sociobiology, 61:115–118. doi: 10.13102/sociobiology.61i1.115-11

Schorkopf, D. L. (2016). Male meliponine bees (*Scaptotrigona* aff. *depilis*) produce alarm pheromones to which workers respond with fight and males with flight. Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology, 202:667–678. doi: 10.1007/s00359-016-1109-9

Sommeijer, M. J. & Bruijn, L. L. M. de (1995). Drone congregations apart from the nest in *Melipona favosa*. Insectes Sociaux, 42:123–127. doi: 10.1007/BF01242448

Van Veen, J. W., Sommeijer, M. J. & Meeuwsen, F. (1997). Behaviour of drones in *Melipona* (Apidae, Meliponinae). Insectes Sociaux, 44:435–447. doi: 10.1007/s000400050063

Veiga, J. C., Menezes, C. & Contrera, F. A. L. (2017). Insights into the role of age and social interactions on the sexual attractiveness of queens in an eusocial bee, *Melipona flavolineata* (Apidae, Meliponini). Naturwissenschaften, 104(3-4):31. doi: 10.1007/s00114-017-1450-z

Eletronic supplementary material

1.Inside the nest: intranidal behaviors, sexual maturity and the point of no return

Development of sexual maturity and flight ability methods

We kept males under laboratory conditions in order to follow the development of their sexual maturity. To do so, we built confined systems to keep males until pre-defined age categories until the moment we would sacrifice them and estimate the number of sperm cells present in their seminal vesicles (Table S1). Before sperm counts, we tested males' flight performance through light stimulus, as follows: (i) each male was positioned at a 1,5m distance of a transparent window, which worked as a light source; (ii) males were stimulated to move through a slight pressure in their thorax; (iii) if males flew towards the window, we classified it as "perfect flight", if they could not fly or were attempting to and failing, we classified it as "no flight" and "trying to fly", respectively, totalizing three categories of flight performance.

Table S1: Description of methods used in the study of *Melipona flavolineata* male's sexual maturity development.

Items	Description
Minicolonies set up details	The minicolonies comprised a total of eight males of uncontrolled origin, and about 40 workers of different ages, but unable to fly. Five female workers were added every day newly emerged, to simulate the constant replacement of individuals occurring within a colony. Minicolonies were formed in wooden boxes (dimensions: 14 cm x 14 cm x 6 cm), containing cerumen, a pot of pollen and honey (daily offer of 5 ml). The bees were confined inside the minicolony, therefore without access to the environment outside the wooden box. We formed a total of 12 minicolonies.
Sample size and sampled age categories	Males were sampled at the age categories of 0, 5, 10, 15, 20 and 25 days after emergence. We sampled a minimum of five, and a maximum of ten males per age category.
Estimative of sperm cells	By means of a slight pressure at the end of males' abdomen, the seminal vesicles were exposed and then extracted from the individuals and added to 50 µl of physiological solution (S1). After 15 minutes, the mixture was homogenized and 5 µl of S1 was added to 45 µl of physiological solution (S2), obtaining a dilution of 1:10. Finally, 10 µl of S2 were placed in a cell counting chamber (Neubauer chamber) to estimate the number of sperm cells in the external quadrants of the chamber. One count was performed for each sample, and only free sperm was included as units in the counts. The protocol for this estimation was adapted from Schlüns et al. (2003).*

* SCHLÜNS, H., SCHLÜNS, E.A., PRAAGH, J. VAN & MORITZ, R.F.A. (2003) Sperm numbers in drone honeybees (Apis mellifera) depend on body size. Apidologie, 34, 577–584.

In order to test at which age males differed in number of total sperm cells in the seminal vesicles, we used the non-parametric test Kruskal-Wallis, and the posterior comparison of ranks, the Nemenyi test. We considered a level of significance of 0.05.

2. Outside the nests: reaching the congregation sites

Body size comparisons

The experiment was conducted from August to September 2016. A total of 180 bees were used, of which 90 were sampled from brood combs (8 to 10 individuals per colony) and 90 were sampled from congregation sites. The sampling of congregation sites was performed about 20 days of the sampling of brood combs (what is the mean age males permanently left the nests), in order to guarantee comparisons of males in the same period. The head, thorax and right leg were dismembered and photographed using a stereomicroscope coupled to a camera using 0.8x magnification, and measurements were made using Motic ImagesPlus 2.0 software. The head, thorax and right leg were placed on a spongy surface and covered with a glass slide in order to standardize the surfaces for the measurements. The following measures were taken: head length, head width, interocular distance thorax length, intertegular distance, tibia length and tibia width.

To test differences on body size attributes between males sampled from brood combs and congregation sites, we used a t-test. If dataset could not meet parametric assumptions, we used the Wilcoxon-Mann-Whitney test instead. We considered a level of significance of 0.05.

Table S2: Statistical comparisons between males sampled from brood combs and congregation sites for seven morphometric attributes of *Melipona flavolineata* males. The differences indicated by the results of the t-test and Wilcoxon-Mann-Whitney were considered the level of significance less than or equal to 0.05.

Morphometric attributes	**Brood combs vs. Congregation**	
	W/t	P
Head width	4588	0.124
Head length	4256	0.556
Interocular distance	2780	0.0002811*
Intertegular distance	3325.5	0.0383*
Length tibia	t = -0.56993; df = 178	0.569
Thorax length	2998	0.002627*
Width tibia	3874	0.615

* p values considered significant

3. Atypical phenomena

Table S3: Number of light and black phenotypes of *Melipona flavolineata* males per sampled brood combs in four nests.

Nest of origin	**Brood comb**	**Proportion of male morphs**		Total individuals/comb
		Light morph (%)	Dark morph (%)	
1	I	21,54	78,46	65
	II	36,96	63,04	46
	III	73,81	26,19	42
2	I	100	0	22
	II	100	0	48
3	I	47,92	52,08	48
	II	56,58	43,42	76
4	I	56,82	43,18	44
	II	42,70	57,30	89

Do traumatic mating plugs prevent remating in stingless bees (Apidae: Meliponini)?

Jamille Veiga, Gustavo Ruiz, Gislene Carvalho Zilse, Cristiano Menezes & Felipe Contrera

A ser sbumetido**.**

Os plugues de cópula previnem contra o reacasalamento em abelhas sem ferrão (Apidae, Meliponini)?

Resumo

Plugues de cópula são adaptações dos machos para restringir o número de acasalamentos das fêmeas. Para entender melhor os mecanismos desses plugues em Meliponini, estudamos sua morfologia e investigamos se eles lesionam os órgãos genitais femininos. Em primeiro lugar, comparamos a ativação da cápsula genital pré e pós-acasalamento na espécie *Melipona fasciculata*, o que nos permitiu explorar sua morfologia funcional. Em segundo lugar, investigamos as lesões na câmara genital feminina, comparando rainhas virgens com acasaladas, em sete espécies de abelhas sem ferrão neotropicais. Mostramos que o plugue de cópula é um mecanismo irreversível, que causa uma perda permanente da genitália masculina, e também pode ser irreversível para as fêmeas em pelo menos uma espécie (*M. fasciculata*). Nas outras espécies, o plugue é de curto prazo. Pela primeira vez, relatamos lesões genitais causadas por plugues copulatórios neste grupo, sugerindo que estes resultam em custos para as rainhas. Em *M. seminigra*, observamos repetidamente múltiplas lesões, sugerindo que o reacasalamento pode ser comum nesta espécie, e corroborando relatos prévios sobre poliandria facultativa na espécie. Nosso estudo mostra que os sistemas de acasalamento de abelhas sem ferrão não são uniformes, variando entre monogamia entrita a algum nível de poliandria. Curiosamente, é pouco provável que os plugues de cópula impeçam totalmente o reacasalamento na maioria das espécies, não sendo, portanto, a única razão para a monogamia generalizada em abelhas sem ferrão. Por fim, propomos a investigação das lesões genitais como uma maneira simples e de menor custo para quantificar as tentativas de acasalamento da rainha.

Do traumatic mating plugs prevent remating in stingless bees (Apidae, Meliponini)?

Abstract

Mating plugs are adaptations used by males to restrict the number of females' copulations. To better understand the mechanisms of mating plugs in Meliponini, we studied their morphology and investigated whether they caused lesions in the female genital organs. First, we compared the male genital capsule pre- and post-mating (plug activation) in *Melipona fasciculata*, and compared it with descriptions of genitalia for most genera within the tribe. Second, we investigated the lesions in females' genital chamber by comparing virgin queens with mated queens in seven Neotropical stingless bees species. We show that male capsule always bears a pair of acute penis valves – the piercing and grasping structures of the plug –, and that activation of mating plug is irreversible for males, leading to a permanent loss of male genitalia. Plugging may also be irreversible for females, in at least in one species (*M. fasciculata*) the plug is long term; while in the other species, it is short-term. For the first time, we report copulatory lesions caused by traumatic mating plugs in this group, suggesting they injure queens. In *M. seminigra*, we repeatedly observed multiple multiple lesions, suggesting that remating may be common in the species. This corroborates previous reports on a facultative polyandry mating system in *M. seminigra*. Our study shows that stingless bee mating systems are not uniform, ranging from strict monogamy to some level of polyandry. Interestingly, mating plugs are unlikely to fully prevent queens from remating in most species, thus not being the unique reason for the widespread monogamy in stingless bees. We propose genital lesions as a simple, inexpensive way to quantify queen mating attempts.

1. Introduction

Antagonistic sexual interests between males and females may lead to endless conflicts and coevolutionary arms race between the sexes. Assuming male persistence to mate, and female resistance as a response (Eberhard, 1985; Rowe & Day, 2006), it is expected possible harmful interactions as a side effect (Rönn et al., 2007; Simmons, 2014). During copula, grasping structures in male genitalia, used for anchorage during mating, may inflict harm to females (Lange et al., 2013; Simmons, 2014). These possible harmful interactions, however, may provide fitness benefits to males, such as paternity certainty and efficient fertilization; to females, such as gain in offspring quality; or benefits to the couple, such as female fecundity stimulation (Lange et al., 2013). Known as traumatic mating (Lange et al., 2013), this particular phenomenon encloses male trauma-inducing devices and female counter-adaptations to reduce harm costs (Rönn et al., 2007; Lange et al., 2013; Kamimura, 2016).

Male harm-devices can be as diverse as spiky penises, calcareous love darts, and the teeth-like structures in male genital capsules (Crudgington & Siva-Jothy, 2000; Koene & Schulenburg, 2005; Baer & Boomsma, 2006; Rönn et al., 2007; Kamimura, 2008). Conversely, female counter-adaptations might include thickened genital plates, as found in seed beetles (Rönn et al., 2007; Dougherty et al., 2017), or reduced sclerotization in the genital area, such as the soft membranous pouches found in fruit flies (Kamimura, 2016). In both situations, male-induced harm in female genital tissues can be traced back through the melanization process developed where harm is inflicted (Laifook, 1966), an indicative of copulatory lesions (Rönn et al., 2007; Kamimura, 2008, 2016; Dougherty et al., 2017).

Widespread among male insects, mating plugs are genital adaptations to male-male competition, with the predicted function to prevent posterior matings by setting a physical barrier (Parker, 1970; Alcock, 1994; Wedell, 2005; Kamimura, 2016). In the social Hymenoptera (ants, bees and wasps), mating plugs evolved as a recurrent male strategy, apparently linked to monogamous systems (Boomsma et al., 2009). In the group, mating plugs may assume different forms (Colonello & Hartfelder, 2005): (i) they may consist of secretions produced by accessory glands in the male – the chemical plugs – as found in ants of the genus *Atta* (Baer & Boomsma, 2006), and in bees of the genera *Apis* and *Bombus* (Koeniger & Koeniger, 1991; Baer et al., 2000; Sauter et al., 2001); or (ii) may consist of the whole male genital capsule – the mechanical plugs – such as in *Vespula* wasps (Ross, 1983), and in the various species of stingless bees (Kerr et al., 1962; Da Silva et al., 1972; Michener, 1990, 2007).

In stingless bees, the male genital capsule turn into a mating plug after triggering the penis valves - a pair of sclerotized claw-like spines -, and its subsequent attachment into females' genital chamber (Camargo et al., 1967; Michener, 1990, 2007). From a behavioral point of view, plugs are known to be involved in the activation of the queens' ovaries in *Melipona quadrifasciata* (Melo et al., 2001), and some observations indicate they are removed by the newly mated queens as soon as they return from the nuptial flight (Da Silva et al., 1972; Van Veen & Sommeijer, 2000). The discovery that the whole male genital capsule works as a mating plug was important to establish the mating structure of stingless bees: if males invest their whole genitalia in a mating plug, and as consequence females return plugged to their nests, then monogamy was considered the most likely mating system in the group (Engels & Imperatriz-Fonseca, 1990). Monandry was also supported by sperm counts, since it was demonstrated that the number of sperm cells stored in the spermatheca of newly mated queens of *M. quadrifasciata* were similar to those sotored in the male seminal vesicles, implying females mated once (Kerr et al., 1962; Da Silva et al., 1972); and recently supported by molecular analysis of paternity, which demonstrates a high genetic relatedness among sisters ($r \geq 0.75$), thus implying a single patriline (Peters et al., 1999; Jaffé et al., 2014; Vollet-Neto et al., 2018).

However, controversy is still surrounding the mating system issue in the group: in *M. seminigra*, some queens mate multiply, including males of the same lineage (Francini, 2012, 2013), meaning multiple mating, and sometimes inbreeding, may not be costly to this species under certain circumstances. Because observations are rare (Kerr et al., 1962; Da Silva et al., 1972; Melo et al., 2001), the role of mating plugs in this large tribe remains poorly understood. A better comprehension of how mating plugs work in the stingless bees mating biology could provide new insights and broaden our understanding of mating systems in the group.

Our aim was to investigate how mating plugs work in stingless bees and if such mechanisms cause lesions in the female genital organs. Based on functional morphology approach, we outline a hypothetical mechanism for mating plugs in stingless bees in order to discuss whether mating plugs preclude females from remating in the group.

2. Material and methods

2.1. "*Corpus delicti"* in the royal caste: pattern and frequency of copulatory lesions in queens

To investigate the pattern and frequency of lesions caused by the mating plug, we examined the genital chamber of mated queens, searching for signs of previous mating events. To examine queens, we followed a "*corpus delicti*" method, which consisted in the inspection of the queens' genital chamber to identify possible lesions, and build a diagnose on possible correspondent mating

attempts. For inspection, each queen was individually inserted in a plastic tube with 4 mm length (Figure S1). The queen's head was positioned towards the bottom of the tube, leaving the terminal part of her abdomen at the other end. The queen was inspected under a stereomicroscope, with the help of common entomological tweezers used to hold the tergum T-VI and the sternum S-VI apart, and thus access her genital chamber (Figure S1).

Once we found melanized patches in the pair of membranous pouches below the gonopore (Figure S1), as an indication of copulatory lesions (based on Kamimura, 2008, 2016), we revised all the specimens and compared this genital area between virgin queens and mated queens. Comparisons were based on the occurrence and number of melanized patches, and their position in the genital chamber. We analyzed a total of 160 physogastric egg-laying queens - and an equal number of virgin queens for comparison - from seven stingless bee species (N = 15-35 physogastric queens per species): *M. fasciculata*, *M. flavolineata*, *M. melanoventer, M. seminigra*, *Frieseomelitta longipes*, *Scaptotrigona* aff. *postica* and *Plebeia minima* (Table 1). Here we do not consider *M. seminigra* subspecies, following Pedro (2014) review.

All specimens were obtained from colonies housed in wooden hives. The mated queens were collected after they were observed to lay eggs, to ensure they were active queens. The virgin queens were sampled directly from brood combs or hidden places inside the nest. After temporary accommodation in Petri dishes, queens were taken to the laboratory to be immediately inspected. All physogastric queens were classified by cumulative wing wear for age inference (Figure S3), and then returned to their original nests. Because virgin queens were sampled from queenright colonies, we kept them, thus they were frozen and stored for future reference.

To photograph lesion pattern and male genital capsule, we collected spare specimens of each species (except for *M. melanoventer*, due to limited availability of colonies, Table 1). We accessed the genital chamber of females and males through dissections. We first removed the final part of the abdomens of one individual per sex per species (from tergum T-IV and sternum S-IV in females; and from tergum T-VI and sternum S-VI in males). After removing, we manually exposed the lesioned areas in females by holding apart the terminal sternum and tergum using common entomological tweezers. For males, we exposed their genital capsule by fully removing it from the dissected part. These parts were immediately photographed after dissection to ensure the integrity of membranous tissues. High-resolution images were obtained with a stereomicroscope coupled with a camera, 1.0x lens and 50x magnification.

2.2. *Reconstructing the mating plug mechanism: functional morphology of the genital capsule, the plugging steps and the mating pair*

To reconstruct the mechanism of mating plug, we chose the species *M. fasciculata* because we verified the presence of a long-term mating plug in physogastric queens of this species during the "*corpus delicti"* diagnosis. This species allowed us to assess females presenting the mating plug in its most likely plugging position.

To describe and illustrate the functional morphology of the male genital capsule as a plug, we used three to five specimens belonging to the following categories: a) virgin queens; b) physogastric queens with mating plug attached; c) newly emerged adult males from brood combs; d) sterile penisless males obtained from reproductive aggregations (*i.e*. males that did not die after losing their genital capsules during mating attempts, Veiga et al., 2018). To access the genital region of females and males, we dissected their metasoma as previously described. The structures were exposed and then represented by means of illustrations, using a stereoscope (80x magnification) with a drawing tube.

We compared the structures of male genital capsule in its resting position in the male's body, and in its triggered state, as a mating plug, inside the female's genital chamber. After being able to identify the resting state and the triggered state, we outlined hypothetical intermediary positions of the pair of penis valves and the the pair of gonocoxites to represent changes in their positions during plug-triggering. After all, we visually compared our drawings to other illustrations of genital capsules for most genera within the tribe (Table S1).

To reconstruct the mating pair, we used *M. fasciculata* specimens (one male and one female), placed under a stereomicroscope coupled with drawing tube (20x magnification), and then we proceeded to the illustrations. Since we were not able to observe matings in *M. fasciculata*, one assumption had to be made: we considered behaviors during mating, and male and female body positions, to be similar among *Melipona* bees. For this reason, we used a previously recorded video of a mating pair of *M. flavolineata* as reference to adjust male and female positions in our drawings.

3. Results

3.1. "*Corpus delicti"* diagnose in the royal caste: signs of traumatic mating

Physogastric queens showed melanized patches on their membranous pouches in all species we examined, except for *M. fasciculata* (Figure 1, Table 1). These were the only genital area with lesion signs. No melanized patches were detected in virgin females examined. In each mated queen, the patches appeared as pairs, matching the inferred position of the pair of penis valves in the male genital capsule, with one patch located on each pouch, and symmetrically positioned (Figures 1 , 2).

Additionally, we found patches in queens with different ages (Figure S3) – for these reason we considered these lesion signs as permanent.

In *M. fasciculata*, we always found a single mating plug attached to the membranous pouches in mated queens, but no melanized patches. Inside queens, the mating plug was ventrally oriented in relation to the female's body, already lacking both gonostyli, and not showing any visible sign of a melanization process. We found plugs in all *M. fasciculata* queens, irrespective of their age (ESM1, Figure S3). For this reason, we considered such plugs as long-term plugs. In contrast, the remaining physogastric queens we analysed never contained a mating plug, only the melanized patches, irrespective of their age – for this reason, we considered such plugs as short-term plugs (Table 1).

However, in *M. seminigra*, we observed both single and multiple pairs of melanized patches, and we never found permanent mating plugs in this species. The distribution of multiple patches varied between three to seven individual melanized areas, with different types of arrangements. From the 33 *M. seminigra* specimens analyzed, 15 presented single paired patches pattern, 12 presented a single paired patches in combination with a central patch and six presented a multiple patch pattern (Figure S2). In the latter case, we were able to distinguish more than one patch on each side of the membranous tissue and central patches (Figure 2, Table 1). In these arrangements, the central melanized patch matched the inferred position of spatha, a central structure in the mating plug (Figure 3).

In addition, we ocasionally captured one extra female of *M. seminigra*, a newly-mated queen carrying two mating plugs: one typically attached inside her genital chamber, and another plug attached onto the external surface of her abdomen, between sterna IV and V (Figure S2). This newly-mated queen was not physogastric yet, thus not being included in our main dataset (Table 1).

Table 1: "*Corpus delicti*" diagnose of mated queens in seven Neotropical species of the tribe Meliponini. Predicted mating systems are given based on the diagnose. The symbol # means sample-size. *Equal number of virgin queens of eache species were sampled for comparison.

Species	***# of mated queens***	***"Corpus delicti" diagnose***				***Possibility of remating***	***Predicted mating system***
		Mating evidence	***# of patches***	***Patch pattern***	***Plug persistence***		
Melipona fasciculata	35	Mating plug	0	No patches	Long-term	No	Monogamy
Melipona flavolineata	30	Melanized patches	2	Single paired patches	Short-term	No	Monogamy
Melipona melanoventer	10	Melanized patches	2	Single paired patches	Short-term	No	Monogamy
Melipona seminigra	33	Melanized patches	2-7	Single paired patches & Multiple paired patches	Short-term	Yes	Monogamy & Facultative polyandry
Scaptotrigona aff. *postica*	15	Melanized patches	2	Single paired patches	Short-term	No	Monogamy
Frieseomelitta longipes	18	Melanized patches	2	Single paired patches	Short-term	No	Monogamy
Plebeia minima	19	Melanized patches	2	Single paired patches	Short-term	No	Monogamy
Total	160*						

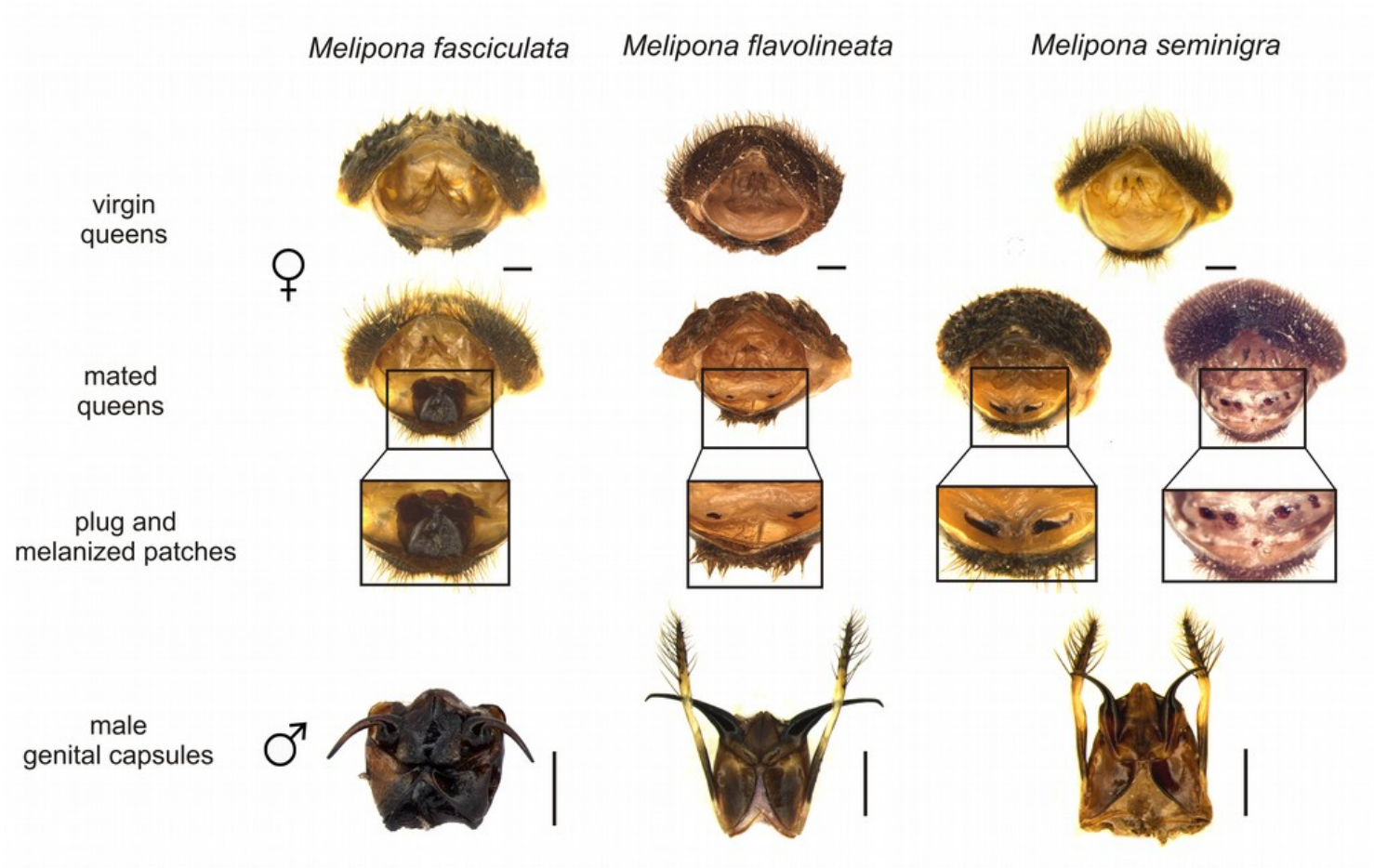


Figure 1: Female genital chamber, trauma pattern and ventral view of male genital capsules in mating plug state in three *Melipona* species: *M. fasciculata*, *M. flavolineata* and *M. seminigra*. High-resolution images were obtained with a stereomicroscope coupled with a camera, 1.0x lens and 50x magnification. The black bars represent a scale of 0,5mm.

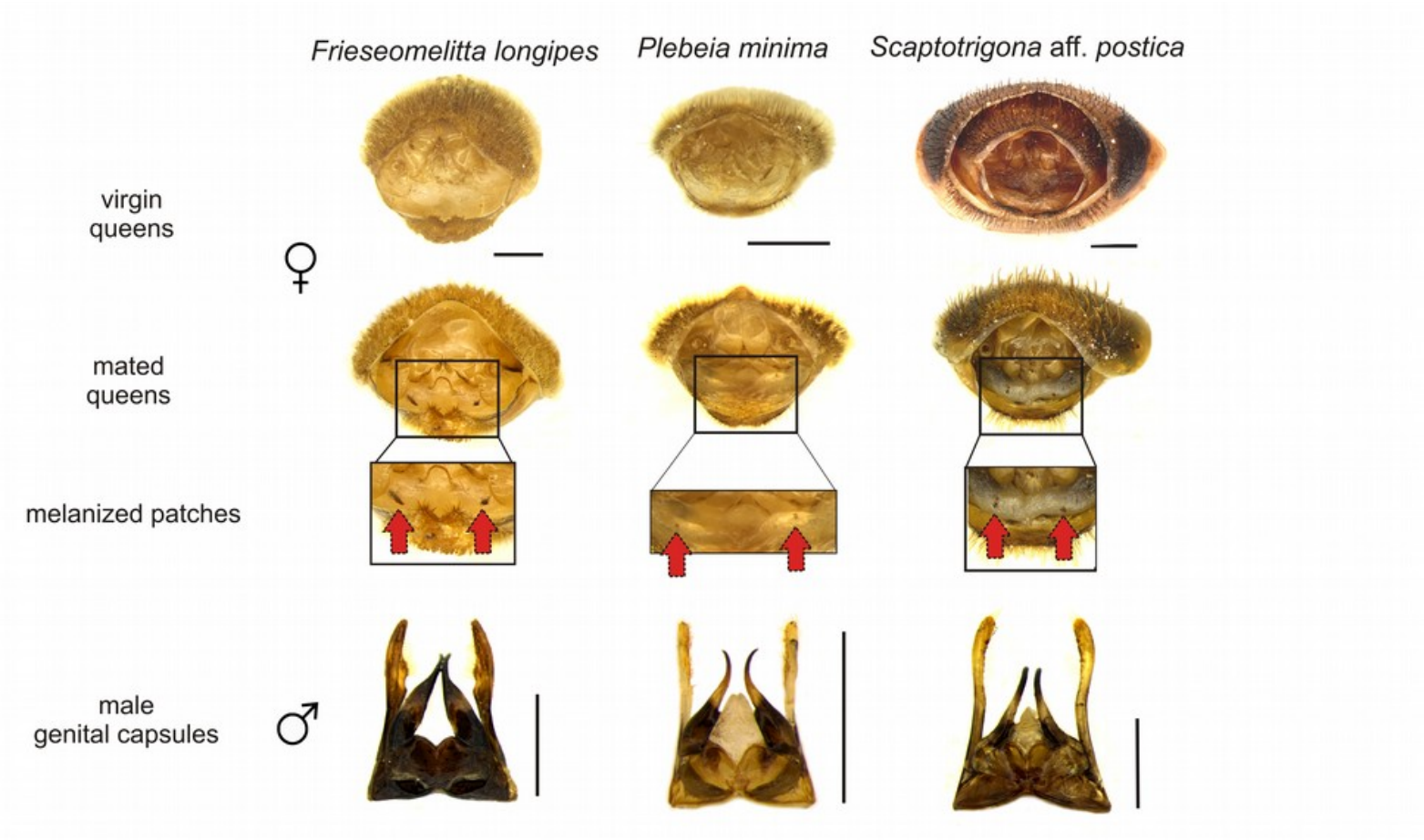


Figure 2: Female genital chamber, trauma pattern and ventral view of male genital capsules in mating plug state in three non-*Melipona* species: *Frieseomelitta longipes*, *Plebeia minima* and *Scaptotrigona* aff. *postica*. High-resolution images were obtained with a stereomicroscope coupled with a camera, 1.0x lens and 50x magnification. Red arrows indicate melanized patches. The black bars represent a scale of 0,5mm.

3.2. *The mating plug mechanism*

The male genital capsule morphology

The male genitalia of *M. fasciculata* has three components: two metasomal sterna (S-VIII and S-IX), and a genital capsule losely accomodated between them (Figure 3-A). The genital capsule has a pair of nearly triangular gonocoxites, in which a pair of long gonostyli and the pair of piercing penis valves are articulated; dorsally there is a narrow gonobase and a median spatha (Figures 3-E, 3-I). These sclerites are strongly held together by a series of membranes that allow their free articulation. All components are strongly sclerotized, smooth (except for the putatively sensitive setae on the distal third of gonostyli), and ventrally oriented in the male body.

The female genitalia of *M. fasciculata* is located inside a chamber formed by the tergum and sternum of the sixth abdominal segment. The final portion of the female body is membranous, poorly sclerotized, held by pairs of lateral hemitergites (T-VIII and T-IX). Conspicuously arising from the membranous rear end, we see a pair of short setose gonostyli, a central bulb and a pair of thin, lateral rami. Between these structures and the sternum S-VI beneath, there is a large membranous tissue pocket – the membranous pouches - onto which the male mating plug is fixed (Figure 5-A and 5-J).

Comparing the general morphology of penis valves abd spatha of *M. fasciculata* to other study species, we observed: both *M. flavolineata* and *M. semingra* also have slim penis valves, with low curvature. However, *M. semingra* has shorter valves, and its spatha seem rather developed when compared to the others (Figure 1). In the non-*Melipona* species (*Frieseomellita longipes*, *Plebeia minima* and *Scaptotrigona* aff. *postica*), we observed robust penis valves, with intermediary curvature, and a reduced spatha (Figure 2). Our visual comparison of male genital capsules across the Meliponini suggest dissimilarity in penis valves aspect and curvature, and in the development of spatha (Table S1).

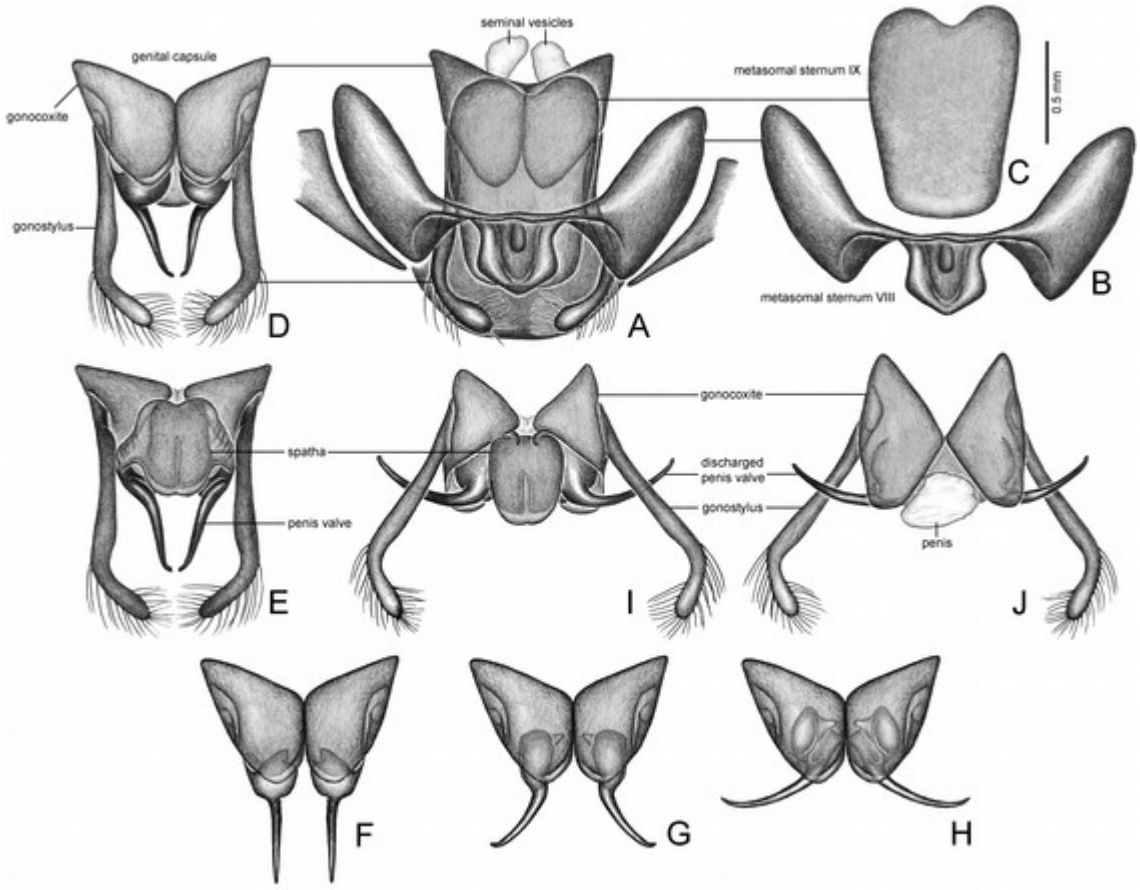


Figure 3: Male genital capsule and genital sternum of *Melipona fasciculata*. Ventral view of genital capsule, metasomal sternum S-VIII and metasomal sternum S-IX, as found inside virgin males (A); Ventral view of metasomal sternum S-VIII (B) and metasomal sternum S-IX (C); Genital capsule in ventral view, showing the gonocoxites and a pair of gonostyli (D); Genital capsule in dorsal view, presenting the spatha and a pair of penis valves (E); Genital capsule in ventral view, showing in sequence, the rotation of the penis valves, before gonocoxites lateral compression (F-H); Genital capsule with triggered penis valves and gonocoxites compressed laterally, in dorsal (I) and ventral view (J).

The mating pair

When a male in flight finds a female in the field (Figure 4-A), he mounts the female's body and start moving his wings uninterruptedly as the copulation begins (Figure 4-B). By positioning himself entirely over the female, the male firmly adjusts each pair of legs at specific locations of the virgin queen's body: i) his first pair of legs anchors under thighs of the first pair of legs of the female; ii) the second pair, under the trochanter of the third pair of legs of the female; iii) and his last pair of legs under the tip of female's abdomen, thereby lifting it (Figure 4-C). The male place the tip of his abdomen down the female's abdomen, forming a "J", moving it backward and upward (Figure 4-C).

Following genital protusion, and subsequent mating plug triggering, a successfully mating male leaves his genital capsule inside the female's genital chamber, which is indicated by the presence of an apparent pair of gonostyli (Figure 4-D).

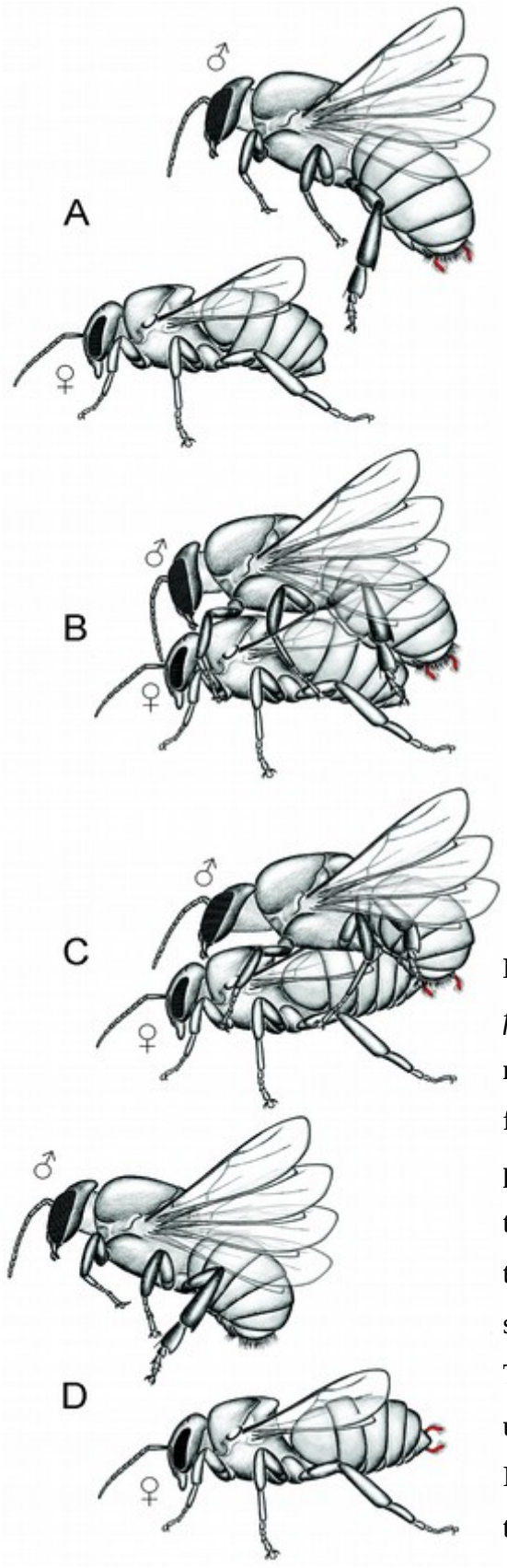


Figure 4: Hypothetical mating pair of *Melipona fasciculata*, based on a mating video record of a related species. From top to bottom: a) male in flight, seeking to position itself over the female; b) positioned male, showing pairs of legs fitted along the female's body; c) male raising the abdomen of the female; d) male leaving the female after successful mating, *i. e.* the mating plug attachment. The gonostyli are highlighted in red, and can be unambiguously observed in newly-mated queens. In all parts, male wings are illustrated to represent their movements during mating.

Mating plug attachment

The mating plug position found inside mated females matches the position of male's abdomen during copulation. In physogastric queens, the mating plug was attached to the membranous genital pouches by the penis valves, with spatha surface oriented towards the female ventral portion, placed under the gonopore (Figure 5-J). When the male genital capsule is irreversibly protuded from male body, the mating plug mechanism is triggered and results in modified positions of gonostyli, penis valves and gonocoxites.

Based on the comparison between resting state of genital capsules and triggered state of mating plugs, and in video records on mating behavior, we propose that triggering of mating plug occurs in five steps:

I) After immobilizing the female body, the male insets his metasomal sternum S-VIII under the female tergum T-VI, lifting it (Figure 4-C);

II) Once the female genital chamber is opened (Figure 5-A), the male gonostyli touch the female's genital region. The gonostyli are sensorial structures, which probably inform males the moment to trigger the mechanism. After positioning the sternum S-VIII, the male metasomal sternum S-IX protrudes out of the male's body towards the female's genital chamber along with the genital capsule, by a telescopic movement (Figure 5-F); the insertion of male genital capsule into the female genital chamber is not followed by the gonostyli, which move backwards during the process, remaining outside the female genital chamber (Figure 5-G);

III) In this moment, the rotation movements of penis valves starts (Figures 3-F, 3-G, 3-H), resulting in a position where the tapering and terminal portion of the valves, previously turned up (Figure 5-F), are now turned laterally, to the left and right (Figure 5-G), against the female genital membrane, without rupturing it in a first moment;

IV) Then the gonocoxites are laterally compressed, and the gonobase is retracted (Figure 5-I), increasing the angulation between the pair of penis valves and resulting in the perforation of female genital chamber membrane and a deep penetration into the membranous pouches, below the gonopore (Figure 5-J); Specifically in *M. fasciculata*, our model species, penis valves open widely enough to position themselves towards the oposite direction (compare penis valve open angles in Figure 1).

V) Once attached by the pair of penis valves, the genital capsule is irreversibly decoupled from male's body, lodged within the female genital chamber, and leaving only the gonostyli visible outside the female body (Figure 4-D). The mating plug is finally attached, the

male irreversibly loses his genital capsule, keeping intact in his body the sterna S-VIII and S-IX, and the female is finally “plugged in” (Figures 4-D, 5-J).

In newly-mated queens, the displaced gonostyli are still present in the queen and are visible outside of female body (ESM 1, Figure S2), and the gonocoxites harbor a large part of the seminal vesicles. In older physogastric queens, the gonostyli are absent. During mating attempts, it is not possible for males to protrude their genital capsule partially or twice: once triggered, the mechanism accomplishes all steps sequentially, resulting in an irreversible loss of male genital capsule (personal observations during previous mating assays in previous studies and during dissections).

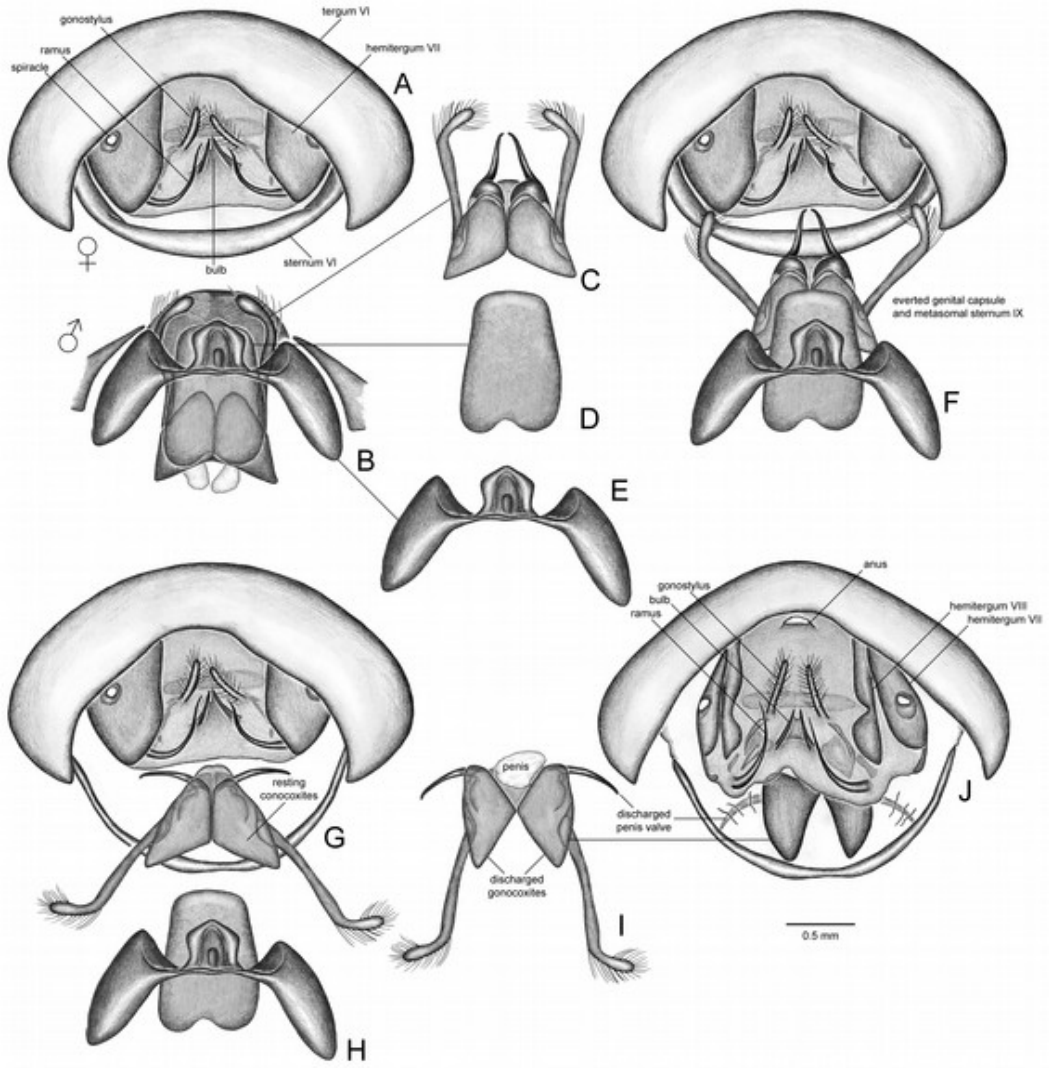


Figure 5: Female genitalia of *Melipona fasciculata* and hypothetical steps for mating plug coupling. A) Virgin female genitalia in frontal view; B) Male genitalia of pre-copula male, approaching the female genitalia; C-E) Genital capsule, metasomal sterna S-VIII and S-IX; F) Male genitalia starting the coupling of the plug: ventral view of genital capsule and metasomal sternum S-IX projecting out of the body of the male by a telescopic movement; G) Male genital capsule detached from male's body, with penis valves triggered, gonostyli facing the opposite position in relation to the initial; H) Metasomal sterna S-VIII and S-IX, where sternum S-IX assumes its original position inside the male's abdomen; I) Mating plug in detail, showing the lateral compression of gonocoxites; J) The genital chamber of a plugged female: the membranous region containing the mating plug attached.

4. Discussion

Here we show that mating in stingless bees involves females being wounded by specialized devices in the male genitalia. While males invest their whole genital capsules in a single mating event, mated queens show signs of traumatic mating – copulatory lesions indicated by melanized patches – except for *M. fasciculata*, in which we found a permaent mating plug, but no melanization process (genital tissues were disrupted by the mating plug as well, but maybe melanization starts only after plug removal). Armed with a pair of sclerotized genital appendages, the male genital capsules perforate the female genital tissue, and may cause lesions during copulation, as we observed in six out of seven species. In this species, we observed that patch number (a single pair) and position (in each side of membranous pouches below the gonopore) in female genital tissues matched male genital morphology – except for *M. seminigra*, whose mated queens presented multiple copulatory lesions. Based on (i) the hypothesized behavior of the mating pair, (ii) the male genital morphology and (iii) the "*corpus delicti*" diagnose of mated queens, we discuss that mating plugs are irreversible to males, being an efficient mechanism to prevent multiple mating in most cases. However, since *M. seminigra* show signs of remating, possibly being a facultatively polyandrous species, it suggests that mating plugs might not be enough to preclude remating under certain conditions.

In the Meliponini, the male harm device is a pair of penis valves, which are sclerotized claw-shaped structures, with rotation and perforation properties (Camargo et al., 1967; Michener, 1990, 2007); while the organ responsible for sperm delivery, the endophallus, consists in a soft membranous structure, unable to cause harm (Ferreira et al., 2004). Since penis valves are the structures involved in plug attachment and subsequent female harm in the Meliponini, their traumatic mating type matches the traumatic penetration subcategory, according to Lange et al. (2013). Beyond classifications, a harmful penetration that does not entail harmful insemination suggest the traumatic mating in stingless bees may be a collateral side-effect; it could result from a strong physical anchorage during copulation, as observed in bumblebees and ants (Brown & Baer, 2005; Baer & Boomsma, 2006). Even though such traumatic mechanism seems costly to both sexes – in one hand, males invest their full sperm content in a single mating event, while in the other, queens are lesioned –, the persistence of traumatic mating in the tribe may suggest fitness benefits. In the stingless bee *M. quadrifasciata*, the ovarioles of newly-mated queens develop under mechanical stimulation of plugs (Melo et al., 2001), suggesting both males and females might benefit from plugging behavior through female fecundity stimulation (Lange et al., 2013). However, considering queens might experience a life-history trade-off involving oviposition and wound repair

(Schwenke et al., 2016), harm effects to females should be quantified to test whether predicted mating benefits, under traumatic circumstances, outweigh possible costs in the female perspective.

In the Meliponini, females seem have developed a counter-adptive response to male harm – the membranous pouches that are lesioned when males fix their mating plugs. Instead of evolving hardened structures to resist male harm – like females of seed beetles (Rönn et al., 2007; Dougherty et al., 2017) -, the Meliponini queens may have evolved a strategy similar to *Drosophila* females (Kamimura, 2016): they posses soft membranous pouches, structures which seem to "lure" the male trauma-causing devices to areas where the resulting lesions will not interfere with subsequent insemination and oviposition. The case of permanently plugged queens in *M. fasciculata* corroborates this hypothesized function for the membranous pouches, because active queens are able to lay eggs even though they are long-term plugged. Future studies should examine whether variation in depth of such pouches reduce trauma effects, in order to test female resistance hypothesis (Holland & Rice, 1998; Kuijper et al., 2012; Dougherty et al., 2017).

Our "*corpus delicti*" diganose also show that time persistence of mating plugs vary among species. Here we highlight the *Melipona* bees, in which we found long-term plugged females (*M. fasciculata*), as well as short-term ones (*M. flavolineata*, *M. melanoventer* and *M. seminigra*). Long-term plugs are more likely to fully prevent females from remating, thus ensuring monogamy and paternity certainty, than short-term plugs. For species in which we found sings of single mating (*i. e.* single paired patches), short-term plugs seem efficient on precluding remating. However, its efficiency might depend on morpho-functional features, such as penis valve length, curvature and opening angle, which may co-vary in order to determine time persistence of plugs and female probability to remate. Differences in such morphological features may offer a mechanistic explanation about why some *M. seminigra* queens were probably remated, while others were not. Another striking observation was the central melanized patches in *M. seminigra*. It was clear that the pair of sclerotized penis valves are the primary source of trauma, but given we observed such patches, that did not match valve positions, we hypothesize that spatha may work as a secondary source of trauma in this species, reinforcing male genitalia as a trauma causing specialized device in this species.

Despite the control males try to exert over females, there is a tendency of females to escape from it when benefits of remating outweigh its related costs, as observed in the polyandrous *A. mellifera* and *B. hypnorum* (Tarpy & Page Jr., 2000; Brown et al., 2002; Boomsma et al., 2009). The same phenomeon might happen in stingless bees, since Meliponini queens could benefit from increased genetic diversity among their brood (Tarpy & Page Jr., 2000; Brown et al., 2002; Oldroyd & Fewell, 2007). In our study, some *M. seminigra* queens showed accumulated genital damage,

probably corresponding to remating. One could expect that multiple lesions would be explained by multiple mating attempts by the same male, however this is more likely to occurr in species in which male genital capsules are firmly fixed to the male reproductive tract, allowing the male to contact female genital tissues more than once in various positions, without losing it (*e. g.* the male genital capsule of Bombini and Euglossini bees, Michener, 2007) - but this is not the case in stingless bees.

In Meliponini, the male genital capsule posses a narrow, not-fixed gonobase, being losely accomodated between genital sterna in their reproductive tract (Figure 3-A). Additionally, males approach females with penis valves curvature oriented to the top, only being able to perforate female genital tissues after they rotate and open laterally, then completing the other steps sequentially (personal observations during mating assays in previous studies and during dissections). This implies that if the same male would be able to perforate the female more than once, the mating plug activation should be reversible before the final step: the mating plug release. However, this is less likely to happen based on the time for triggering the mechanism (less than a half second, personal observations), and the sequentially activation of each step before the mating plug release. If multiple attempts by the same male were more likely to occurr, we would expect a higher frequencey of multiple lesioned females than observed, also in other species – what was not the case among our samples. Based on these arguments, we consider remating as a good candidate explanation for multiple trauma pattern in the genital tissue of *M. seminigra* females. Finally, we argue that if multiple copulatory lesions were caused by different males, but only one (or a few) were able to successfully inseminate the queen, it can still be considered as remating, since female would interact with more than one male during the same mating event.

Previous data on *M. seminigra* colonies corroborate our suggestion of remating: it was found that nestmates are genetically less related than expected for a monogamous species, and that there was an average of eight patrilines among brood (Francini, 2013). Additionally, multiple mated queens were not eliminated by workers, even under excess (diploid) male production (Francini, 2012). Authors argued that such evolutionary strategy may maximize colony genetic diversity and such unusual level of polyandry in stingless bees minimizes the high costs and dangerous effects of diploid male production (Francini, 2013). Future studies should combine behavioral and molecular data to test the occurrence of remating in this and other stingless bee species, and understand the conditions under which such phenomenon is more likely to occur.

Our study raise two interesting questions when suggests long-term plugging and possible female remating. First, if plugs are long-term in some species – unumbigously resulting in monogamy – then why they are removed in other putatively monogamous species? In such cases,

short-term mating plugs might simply work during a narrow time-window, enough to ensure effective paternity of a single male, as observed in bumblebees (Duvoisin et al., 1999; Brown et al., 2002). Second, if queens can remove mating plugs in most species (Table 1; Da Silva et al., 1972; Kerr et al., 1962), and may mate with more than one male in some cases (Table 1, Figure 1; Francini 2012, 2013), then why stingless bees do not mate multiply in general like in honeybees? That multiple mating is rare in Meliponini suggest there are other reasons why females mate singly, for example a narrow time window for mating, lack of material gain from males and lack of male parental care (Boomsma & Ratnieks, 1996; Strassmann, 2001). Another reason could be the negative effects of multiple mating when they result in inbreeding (Boomsma et al., 1996; Camargo, 1976; Vollet-Neto et al., 2017). The hypothesis was recently explored on a study about the stingless bee *S. postica*, showing how diploid male production through inbreeding increase the chances of queen elimination (Vollet-Neto et al., 2017). However, it may not be the case for all stingless bees. For example, queens of *M. seminigra* that produced diploid males were not eliminated by workers (Francini, 2012). Those queens were allowed to live, as observed in *A. mellifera*, suggesting possible diploid male production costs may be reduced by other features, such as the benefits related to an increased genetic diversity among brood when females remate (Boomsma & Ratnieks, 1996). If persistence of plugs correlate with the likelihood of remating, being part of this explanation, it still need to be tested in Meliponini.

The functional morphology approach we used may be a reliable, low cost method to identify single and remated queens in stingless bees. Even though this approach does not allow us to infer the exact number of mating partners, it is possible to distinguish between singly and multiply mated queens. Since there are physogastric queens showing signs of remating while others only show signs of single mating (multiple trauma pattern versus single trauma pattern, or long-term plugs), these may be honest signs of mating attempts.

We conclude that the traumatic mating plug mechanism is a widespread strategy among Meliponini, towards which females show signs of counter-adaptations. The mechanistic differences we observed among species highlight that mating plugs may not fully prevent female remating; also the need to be cautious when generalizing mating systems across stingless bees. Finally, we suggest traumatic mating plugs are also good candidates as one of the proximate causes of mating system variation in stingless bees.

Acknowledgments

We thank this amazing team for their support in field work: Beatriz Coelho, Kamila Leão, Thaliana Souza, the Invertebrate Lab at UFPA (Valéria Silva, Benedito Nunes, Sofia Camargo), and

the Research Group on Bees at INPA (Tatiane Becker, Hélio Vilas-Boas, Diego, Douglas). We also thank Dr. Christoph Grüter for critical review of previous drafts. This research was funded by CAPES/EMBRAPA (15/2014), providing grants to JCV, and by CNPQ (400435/2014-4) through the PVE 2014 Project.

Literature cited

ALCOCK, J. (1994) Postinsemination associations between males and females in insects: the mate-guarding hypothesis. *Annual Review of Entomology*, 1–21.

ALCOCK, J. (2001) Animal Behavior: An Evolutionary Approach, 7th edition. Sinauer Associates, Sunderland, Massachusetts.

ARNQVIST, GÖRAN, ROWE, L. (2013) Sexual conflict in nature. *Journal of Chemical Information and Modeling*, 53, 1689–1699.

ARNQVIST, G. & ROWE, L. (2002) Antagonistic coevolution between the sexes in a group of insects. *Nature*, 415, 787–789.

BAER, B. & BOOMSMA, J.J. (2006) Mating biology of the leaf-cutting ants *Atta colombica* and *A. cephalotes*. *Journal of Morphology*, 267, 1165–1171.

BAER, B., MAILE, R. & SCHMID-HEMPEL, P. (2000) Chemistry of a mating plug in bumblebees. *Journal of Chemical Ecology*, 26, 1869–1875.

BOOMSMA, J. & RATNIEKS, F. (1996) Paternity in eusocial Hymenoptera. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 351, 947–975.

BOOMSMA, J.J., KRONAUER, D.J.C.D.. C. & PEDERSEN, J.S. (2009) The Evolution of Social Insect Mating Systems. In *Organization of insect societies: from genome to sociocomplexity* (eds J. Fewell & J. Gadau), p. 609. Harvard University Press.

BROWN, M.J.F. & BAER, B. (2005) The evolutionary significance of long copulation duration in bumble bees. *Apidologie*, 36, 157–167.

BROWN, M.J.F., BAER, B., SCHMID-HEMPEL, R. & SCHMID-HEMPEL, P. (2002) Dynamics of multiple-mating in the bumble bee *Bombus hypnorum*. *Insectes Sociaux*, 49, 315–319.

CAMARGO, C. A. (1976) Determinação do sexo e controle de reprodução em Melipona quadrifasciata anthidioides Lep. (Hymenoptera, Apoidea). Dissertation, University of Sao Paulo.

CAMARGO, J.M.F., KERR, W.E. & LOPES, C.R. (1967) Morfologia externa de *Melipona* (*Melipona*) *marginata* Lepeletier (Hymenoptera, Apoidea). *Papéis Avulsos de Zoologia*, 20, 229–258.

CHAPMAN, T., ARNQVIST, G., BANGHAM, J. & ROWE, L. (2003) Sexual conflict. *Trends in Ecology and Evolution*, 18, 41–47.

COLONELLO, N.A. & HARTFELDER, K. (2005) She's my girl - male accessory gland products and their function in the reproductive biology of social bees. *Apidologie*, 36, 231–244.

CRUDGINGTON, H.S. & SIVA-JOTHY, M.T. (2000) Genital damage, kicking and early death. *Nature*, 407, 855–856.

DA SILVA, D.N., ZUCCHI, R. & KERR, W. (1972) Biological and behavioural aspects of the reproduction in some species of *Melipona* (Hymenoptera, Apidae, Meliponinae). *Animal Behaviour*, 20, 123–132.

DOUGHERTY, L.R., VAN LIESHOUT, E., MCNAMARA, K.B., MOSCHILLA, J.A., ARNQVIST, G. & SIMMONS, L.W. (2017) Sexual conflict and correlated evolution between male persistence and female resistance traits in the seed beetle *Callosobruchus maculatus*. *Proceedings of the Royal Society B: Biological Sciences*, 284, 20170132.

DUVOISIN, N., BAER, B. & SCHMID-HEMPEL, P. (1999) Sperm transfer and male competition in a bumblebee. *Animal Behaviour*, 58, 743–749.

EBERHARD, W.G. (1985) Sexual selection and animal genitalia. Harvard University Press.

ENGELS, W. & IMPERATRIZ-FONSECA, V.L. (1990) Caste Development, Reproductive Strategies, and Control of Fertility in Honey Bees and Stingless Bees. In *Social Insects* pp. 167–230. Springer Berlin Heidelberg, Berlin, Heidelberg.

FERREIRA, A., ABDALLA, F.C., KERR, W.E. & CRUZ-LANDIM, C. (2004) Comparative anatomy of the male reproductive internal organs of 51 species of bees. *Neotropical Entomology*, 33, 569–576.

FRANCINI, I.B. (2013) Monandria e Poliandria como estratégia evolutiva no complexo de subespécies de *Melipona seminigra* Friese, 1903 (Apidae, Meliponini) na Amazônia. Instituto Nacional de Pesquisas da Amazônia.

FRANCINI, I.B., NUNES-SILVA, C.G. & CARVALHO-ZILSE, G.A. (2012) Diploid Male Production of Two Amazonian *Melipona* Bees (Hymenoptera: Apidae). *Psyche: A Journal of Entomology*, 2012, 1–7.

HOLLAND, B. & RICE, W.R. (1998) Perspective: Chase-Away Sexual Selection: Antagonistic Seduction Versus Resistance. *Evolution*, 52, 1–7.

HOTZY, C. & ARNQVIST, G. (2009) Sperm Competition Favors Harmful Males in Seed Beetles. *Current Biology*, 19, 404–407. Elsevier Ltd.

JAFFÉ, R., PIOKER-HARA, F.C., DOS SANTOS, C.F., SANTIAGO, L.R., ALVES, D. A, DE M P KLEINERT, A., ET AL. (2014) Monogamy in large bee societies: a stingless paradox. *Die Naturwissenschaften*, 101, 261–264.

KAMIMURA, Y. (2008) Copulatory wounds in the monandrous ant species *Formica japonica* (Hymenoptera, Formicidae). *Insectes Sociaux*, 55, 51–53.

KAMIMURA, Y. (2016) Significance of constraints on genital coevolution: Why do female *Drosophila* appear to cooperate with males by accepting harmful matings? *Evolution*, 70, 1674–1683.

KERR, W.E., ZUCCHI, R., NAKADAIRA, J.T. & BUTOLO, J.E. (1962) Reproduction in the Social Bees (Hymenoptera: Apidae). *Journal of the New York Entomological Society*, 70, 265–276.

KOENE, J.M. & SCHULENBURG, H. (2005) Shooting darts: Co-evolution and counter-adaptation in hermaphroditic snails. *BMC Evolutionary Biology*, 5.

KOENIGER, N. & KOENIGER, G. (1991) An evolutionary approach to mating behaviour and drone copulatory organs in *Apis*. *Apidologie*, 22, 581-590.

KUIJPER, B., PEN, I. & WEISSING, F.J. (2012) A Guide to Sexual Selection Theory. *Annual Review of Ecology, Evolution, and Systematics*, 43, 287–311.

LAIFOOK, J. (1966) The repair of wounds in the integument of insects. *Physical therapy*, 46, 195–226.

LANGE, R., REINHARDT, K., MICHIELS, N.K. & ANTHES, N. (2013) Functions, diversity, and evolution of traumatic mating. *Biological Reviews*, 88, 585–601.

MELO, G.A.R., BUSCHINI, M.L.T. & CAMPOS, L.A.O. (2001) Ovarian activation in *Melipona quadrifasciata* queens triggered by mating plug stimulation (Hymenoptera, Apidae). *Apidologie*, 32, 355–361.

MICHENER, C.D. (1990) Classification of the Apidae (Hymenoptera). *University of Kansas Science Bulletin*, 54, 75–164.

MICHENER, C.D. (2007) The bees of the world, 2nd edition. Johns Hopkins University Press, Baltimore, Maryland, USA.

OLDROYD, B.P. & FEWELL, J.H. (2007) Genetic diversity promotes homeostasis in insect colonies. *Trends in Ecology & Evolution*, 22, 408–413.

PARKER, G.A. (1970) Sperm competition and its evolutionary consequences in the insects. *Biological Reviews*, 45, 525–567.

PARKER, G.A. (2006) Sexual conflict over mating and fertilization: an overview. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 361, 235–259.

PEDRO, S.R.M. (2014) The stingless bee fauna in Brazil (Hymenoptera : Apidae). *Sociobiology*, 61, 348–354.

PETERS, J.M., QUELLER, D.C., IMPERATRIZ-FONSECA, V.L., ROUBIK, D.W. & STRASSMANN, J.E. (1999) Mate number, kin selection and social conflicts in stingless bees and honeybees. *Proceedings of the Royal Society B: Biological Sciences*, 266, 379–384.

PIZZARI, T. & WEDELL, N. (2013) The polyandry revolution. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368, 20120041–20120041.

RÖNN, J., KATVALA, M. & ARNQVIST, G. (2007) Coevolution between harmful male genitalia and female resistance in seed beetles. *Proceedings of the National Academy of Sciences*, 104, 10921–10925.

ROSS, K.G. (1983) Laboratory studies of the mating biology of the eastern yellowjacket, *Vespula maculifrons* (Hymenoptera: Vespidae). *Journal of the Kansas Entomological Society*, 56, 523–537.

ROWE, L. & DAY, T. (2006) Detecting sexual conflict and sexually antagonistic coevolution. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 361, 277–285.

SAUTER, A., BROWN, M.J.F. & BAER, B. (2001) Males of social insects can prevent queens from multiple mating. *Proceedings of the Royal Society B*, 268, 1449-1454.

SCHWENKE, R. A., LAZZARO B. P., WOLFNER, M. F. (2016) Reproduction-immunity trade-offs in insects. *Annual Review of Entomology,* 61, 239–256. (doi:10.1146/annurev- ento-010715-023924)

SIMMONS, L.W. (2014) Sexual selection and genital evolution. *Austral Entomology*, 53, 1–17.

STRASSMANN, J. (2001) The rarity of multiple mating by females in the social Hymenoptera. *Insectes Sociaux*, 48, 1–13.

TARPY, D.R. & PAGE JR., R.E. (2000) No Behavioral Control over Mating Frequency in Queen Honey Bees (*Apis mellifera* L.): Implications for the Evolution of Extreme Polyandry. *The American Naturalist*, 155, 820–827.

VAN VEEN, J.W. & SOMMEIJER, M.J. (2000) Observations on gynes and drones around nuptial flights in the stingless bees *Tetragonisca angustula* and *Melipona beecheii* (Hymenoptera, Apidae, Meliponinae). *Apidologie*, 31, 47–54.

VEIGA, J.C.., LEÃO, K.L.., COELHO, B.W.T.., QUEIROZ, A.C.M. DE;, MENEZES, C.. & CONTRERA, F.A.L. (2018) The Life Histories fo the 'Uruçu Amarela' Males (*Melipona flavolineata*, Apidae, Meliponini). *Sociobiology*, 65, 780–783.

VOLLET-NETO, A., KOFFLER, S., DOS SANTOS, C.F., MENEZES, C., NUNES, F.M.F., HARTFELDER, K., ET AL. (2018) Recent advances in reproductive biology of stingless bees. *Insectes Sociaux*, 0, 1–12.

VOLLET-NETO, A., OLIVEIRA, R.C., SCHILLEWAERT, S., ALVES, D.A., WENSELEERS, T., NASCIMENTO, F.S., ET AL. (2017) Diploid Male Production Results in Queen Death in the Stingless Bee *Scaptotrigona depilis*. *Journal of Chemical Ecology*, 43(4), 403–410.

WEDELL, N. (2005) Female receptivity in butterflies and moths. *Journal of Experimental Biology*, 208, 3433–3440.

Eletronic supplementary material

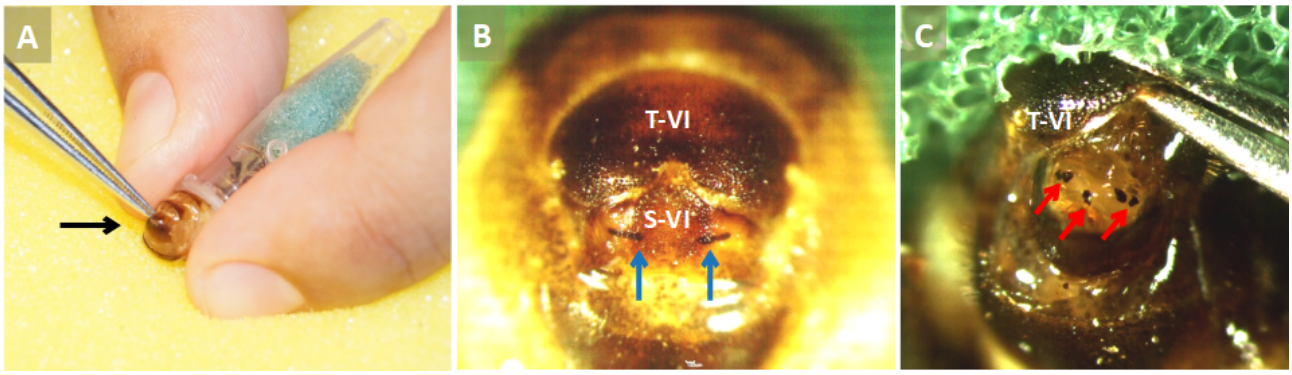


Figure S1: "*Corpus delicti*" in virgin and mated queens of stingless bees. A) Placement of the queen inside the tube, exposing the abdomen (indicated by a black arrow); B) Terminal portion of the queen's abdomen, where tergum T-VI and the sternum S-VI are found. In this position, it is possible to identify the melanized patches (indicated by blue arrows); C) Inside the genital chamber of the queen: here we show the melanized patches in the membranous tissue, below the gonopore area, as evidence of copulatory lesions (indicated by red arrows).

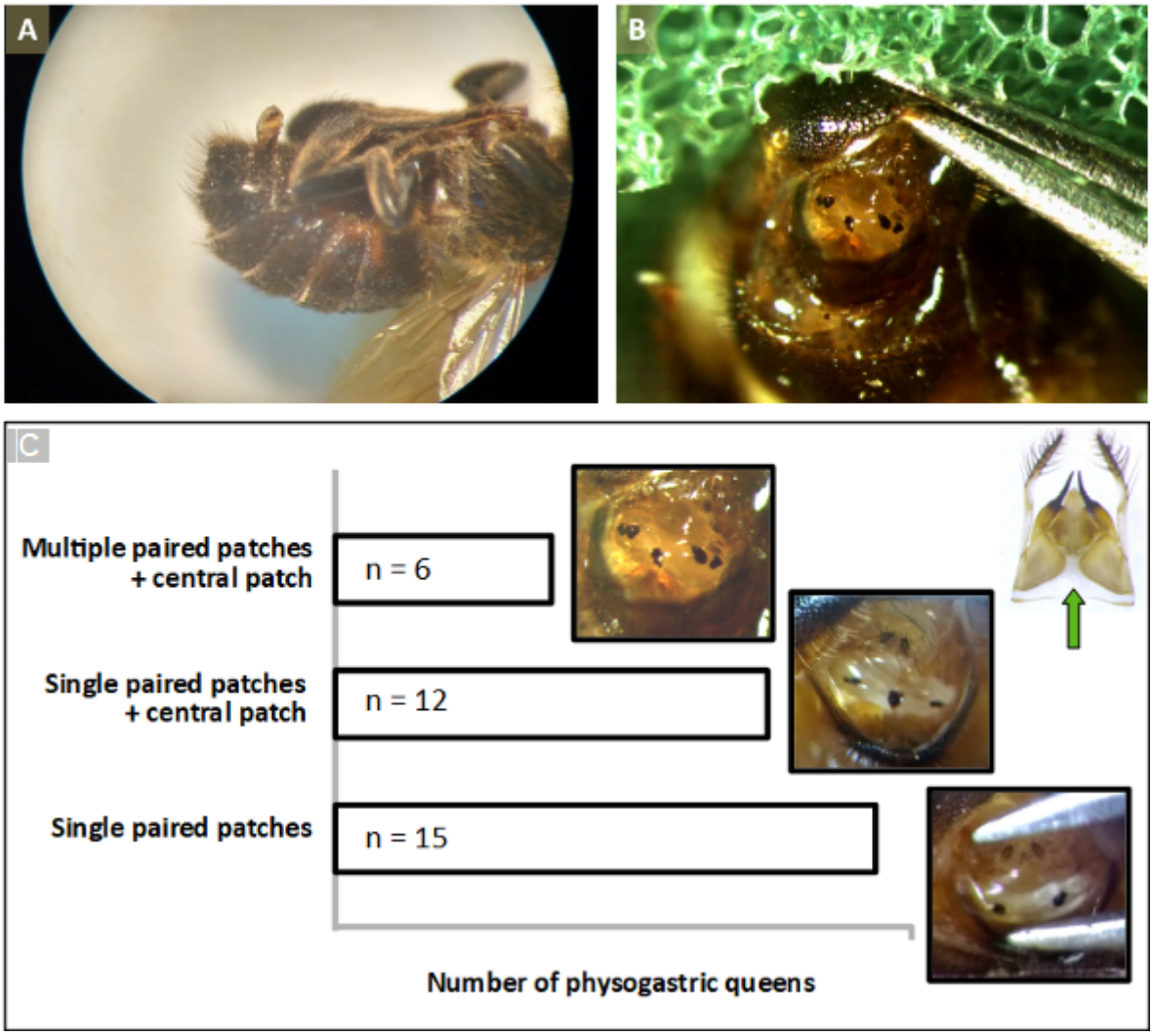


Figure S2: Signs of remating mating found *in Melipona seminigra*: A) newly-mated queen containing an exposed mating plug attached in the external surface of her abdomen. This same queen had another plug, typically attached inside her genital chamber (not visible in this picture); B) Trauma pattern found in a mated queen: in this image, there are five individualized melanized patches; C) Frequency of trauma pattern in *M. seminigra* physogastric queens, from top to bottom: multiple paired patches + central patch, single paired patches + central patch and singles paired patches. The *M. seminigra* male genital capsule is indicated by the green arrow.

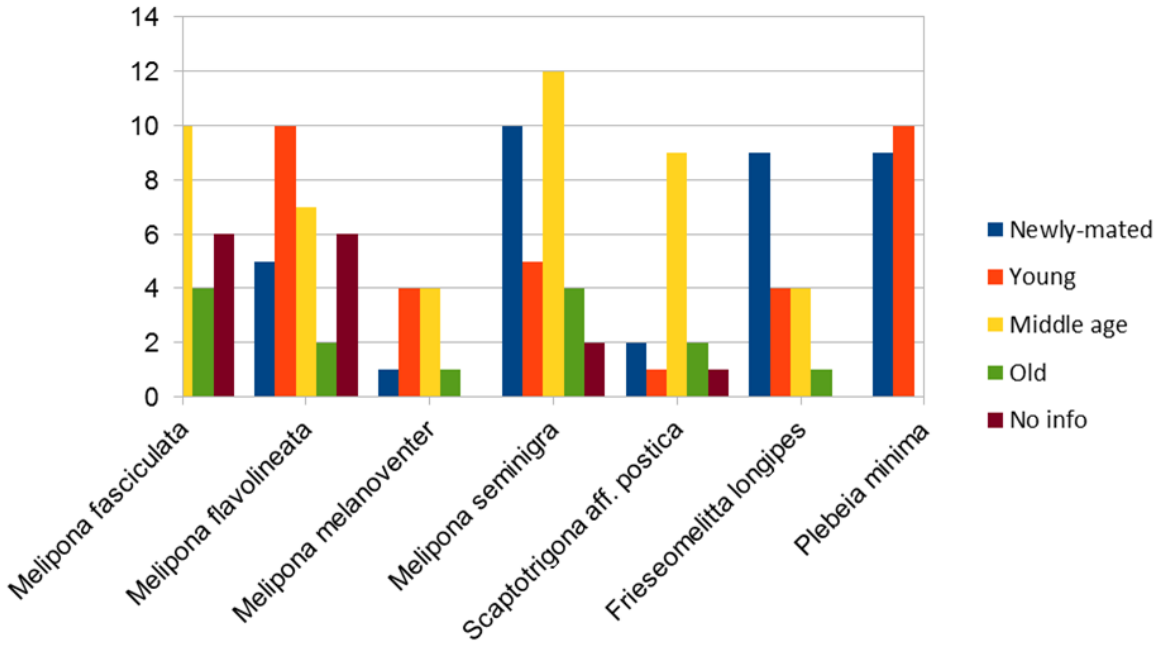


Figure S3: Females age inference based on frequency of wing wear among physogastric queens of seven species of Meliponini *Frieseomelitta longipes*, *M. fasciculata*, *M. flavolineata*, *M. melanoventer*, *M. seminigra*, *Plebeia minima* and *Scaptotrigona* aff. *postica*. For age inference, all queens were classified by cumulative wing wear into four categories: (i) intact wings (0-2 wear marks), with inferred age "newly-mated"; (ii) slightly worn wings (3-5 wear marks), with inferred age "Young"; iii) worn wings (6-12 wear marks), with inferred age "Middle ange"; and iv) very worn wings (above 12 wear marks), with inferred age "Old". The label "No info" means that data on wing wear was not collected from sampled queens.

Table S1: Across species comparisons of penis valve aspect and curvature, and development of spatha in Meliponini tribe. Classifications were made based on published male genital capsule representations.

Clade	Genus	Species	Penis valve aspect	Penis valve curvature	Development of spatha	Representation	Source
AT	*Dactylurina*	*Dactylurina schmidti*	Robust	Intermediary	Reduced	Illustration	Michener (2007)
AT	*Hypotrigona*	*Hypotrigona braunsi*	Slim	Weak	Reduced	Illustration	Michener (2007)
AT	*Liotrigona*	*Liotrigona mahafalya*	Slim	Weak	Reduced	Illustration	Michener (2007)
AT	*Meliponula (Meliplebeia)*	*Meliponula (Meliplebeia) beccarii*	Robust	Weak	Reduced	Illustration	Michener (2007)
AT	*Meliponula (Meliponula)*	*Meliponula (Meliponula) bocandei*	Robust	Strong	Absent	Illustration	Michener (2007)
AT	*Plebeina*	*Plebeina denoiti*	Robust	Weak	Reduced	Illustration	Michener (2007)
IM-AA	*Austroplebeia*	*Austroplebeia essingtoni*	Robust	Intermediary	Absent	Illustration	Dollin et al. (2015)
IM-AA	*Homotrigona*	*Homotrigona aliceae*	Robust	Intermediary	Reduced	Photo	Lee et al. (2016)
IM-AA	*Tetragonula*	*Tetragonula clypearis*	Robust	Weak	Absent	Photo	Dollin, Walker & Heard (2009)
NE	*Cephalotrigona*	*Cephalotrigona capitata*	Robust	Intermediary	Reduced	Illustration	Schwarz (1948)
NE	*Dolichotrigona*	*Dolichotrigona tavaresi*	Slim	Weak	Developed	Illustration	Camargo & Pedro (2005)
NE	*Duckeola*	*Duckeola ghilianii*	Robust	Intermediary	Reduced	Illustration	Camargo (1996)
NE	*Geotrigona*	*Geotrigona mombuca*	Robust	Strong	Reduced	Illustration	Camargo & Moure (1996)
NE	*Lestrimelitta*	*Lestrimelitta limao*	Slim	Strong	Reduced	Illustration	Schwarz (1948)
NE	*Melipona*	*Melipona (Eomelipona)*	Slim	Weak	Developed	Illustration	Schwarz (1932)

Clade	Genus	Species	Penis valve aspect	Penis valve curvature	Development of spatha	Representation	Source
	(Eomelipona)	*marginata*					
NE	*Melipona (Melikerria)*	*Melipona (Melikerria) fasciculata*	Slim	Intermediary	Reduced	Illustration	Rêgo (1990)
NE	*Melipona (Melipona)*	*Melipona (Melipona) favosa*	Slim	Weak	Reduced	Illustration	Schwarz (1932)
NE	*Melipona (Michmelia)*	*Melipona (Michmelia) fuliginosa*	Slim	Weak	Developed	Illustration	Camargo & Pedro (2008)
NE	*Oxytrigona*	*Oxytrigona tataira*	Robust	Intermediary	Reduced	Illustration	Schwarz (1948)
NE	*Paratrigona*	*Paratrigona opaca*	Robust	Intermediary	Reduced	Illustration	Camargo & Moure (1994)
NE	*Ptilotrigona*	*Ptilotrigona lurida*	Slim	Intermediary	Reduced	Illustration	Camargo & Pedro (2004)
NE	*Scaptotrigona*	*Scaptotrigona barrocoloradensis*	Slim	Intermediary	Reduced	Illustration	Michener (2007)
NE	*Scaura*	*Scaura latitarsis*	Robust	Strong	Reduced	Illustration	Schwarz (1948)
NE	*Schwarziana*	*Schwarziana quadripunctata*	Robust	Strong	Reduced	Illustration	Schwarz (1948)
NE	*Tetragona*	*Tetragona goettei*	Slim	Intermediary	Reduced	Illustration	Camargo (1996)
NE	*Trichotrigona*	*Trichotrigona camargoiana*	Robust	Intermediary	Reduced	Photo	Pedro & Cordeiro (2015)

Clade	Genus	Species	Penis valve aspect	Penis valve curvature	Development of spatha	Representation	Source
NE	*Trigona*	*Trigona amalthea*	Robust	Intermediary	Reduced	Illustration	Schwarz (1948)
NE	*Trigonisca*	*Trigonisca buyssoni*	Robust	Weak	Developed	Illustration	Michener (2007)

References

CAMARGO, J.M.F. (1996) Meliponini Neotropicais: o gênero *Camargoia* Moure, 1989 (Apinae, Apidae, Hymenoptera). *Arquivos de Zoologia*, 33, 71–92.

CAMARGO, J.M.F. & MOURE, J.S. (1994) Meliponinae Neotropicais: os gêneros *Paratrigona* Schwarz, 1938 e *Aparatrigona* Moure, 1951 (Hymenoptera, Apidae). *Arquivos de Zoologia*, 32, 33–109.

CAMARGO, J.M.F. & MOURE, J.S. (1996) O Gênero *Geotrigona* Moure, 1943 (Apinae, Apidae, Hymenoptera), com especial referência à Filogenia e Biogeografia. *Arquivos do Zoologia*. .

CAMARGO, J.M.F. & PEDRO, S.R.M. (2004) Meliponini neotropicais: o gênero *Ptilotrigona* Moure (Hymenoptera, Apidade, Apinae). *Revista Brasileira de Entomologia*, 48, 353–377.

CAMARGO, J.M.F. & PEDRO, S.R.M. (2005) Meliponini Neotropicais: o gênero *Dolichotrigona* Moure (Hymenoptera, Apidae, Apinae). *Revista Brasileira de Entomologia*, 49, 69–92.

CAMARGO, J.M.F. & PEDRO, S.R.M. (2008) Revisão das espécies de *Melipona* do grupo f*uliginosa* (Hymenoptera, Apoidea, Apidae, Meliponini). *Revista Brasileira de Entomologia*, 52, 411–427.

DOLLIN, A., WALKER, K. & HEARD, T. (2009) '*clypearis*' Sugarbag bee (*Tetragonula clypearis*). Updated on 12/9/2011 2:26:54 PM Available online: PaDIL - http://www.padil.gov.au.

DOLLIN, A.E., DOLLIN, L.J. & RASMUSSEN, C. (2015) Australian and New Guinean Stingless Bees of the Genus *Austroplebeia* Moure (Hymenoptera: Apidae)-a revision. *Zootaxa*, 4047(1), 1-73.

LEE, S., KESHARI, R. & WONHOON, L. (2016) Diversity of stingless bees (Hymenoptera, Apidae, Meliponini) from Cambodia and Laos. *Journal of Asia-Pacific Entomology*, 19, 947–961.

MICHENER, C.D. (2007) The bees of the world, 2nd edition. Johns Hopkins University Press, Baltimore, Maryland, USA.

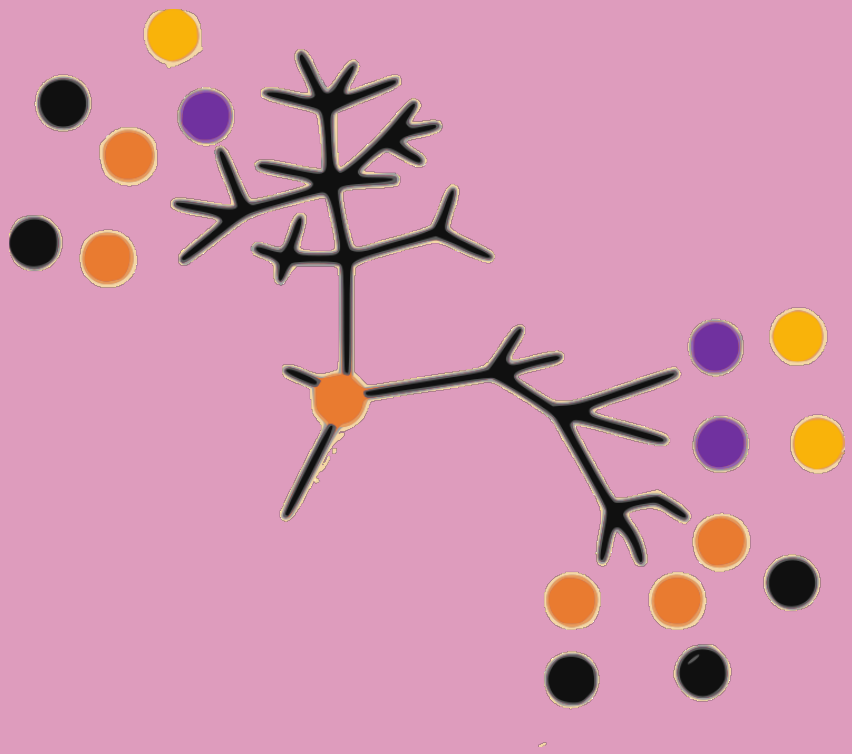
PEDRO, S.R.M. & CORDEIRO, G.D. (2015) A new species of the stingless bee *Trichotrigona* (Hymenoptera: Apidae, Meliponini). *Zootaxa*, 3956, 389.

RÊGO, M.C. (1990) Revisão do gênero *Melipona* Illiger, 1806: genitália e esternos pré-genitais de machos (Meliponinae, Apidae, Hymenoptera).

SCHWARZ, H.F. (1932) The genus *Melipona* the type genus of the Meliponidae or stingless bees. *Bulletin of the American Museum of Natural History*, 63, 231–460.

SEÇÃO II

Tendências macroevolutivas:
tamanho de colônias em abelhas sem ferrão



Macroevolutionary trends in colony size suggest advanced eusociality in stingless bee ancestors

Jamille Veiga & Christoph Grüter

Pesquisa realizada durante o doutorado sanduíche na Johannes Gutenberg Universität, entre Agosto/2018 e Janeiro/2019.

Tendências macroevolutivas no tamanho de colônias sugere eussocialidade avançada nos ancestrais das abelhas sem ferrão

Resumo

Nas sociedades de insetos, a complexidade social é amplamente explicada pelo tamanho das colônias. As abelhas sem ferrão (Meliponini), um grupo de insetos eussociais pantropicais, com mais de 500 espécies, são um bom exemplo de grande variação em ambos os atributos. Revisando a literatura, coletamos dados sobre o tamanho de colônias para 71 espécies de abelhas sem ferrão, e utilizamos uma abordagem filogenética para responder à seguinte questão: existe uma tendência geral na evolução do tamanho de colônias em abelhas sem ferrão? Além disso, considerando que o tamanho da colônia atue sobre diferentes aspectos da história de vida desses insetos, investigamos como o número de indivíduos em uma sociedade afeta atributos individuais e coloniais. Realizamos uma reconstrução de estado ancestral para estimar o estado ancestral mais provável entre os diferentes tamanhos de colônia: pequenas, médias e grandes; e testamos sua evolução correlacionada com as seguintes características: o tempo de vida e o tamanho corporal de operárias, o dimorfismo de tamanho corporal entre castas, a arquitetura de cria e o hábito de nidificação. Nossa abordagem comparativa para a evolução do tamanho de colônias sugere que o tamanho médio é o estado ancestral mais provável em Meliponini, enquanto os tamanhos pequenos e grandes são estados derivados, indicando flexibilidade na perda e no ganho de complexidade social ao longo da história evolutiva do grupo. Também sugere que a qualidade das operárias diminui com o aumento do número de indivíduos na colônia, ocorrendo o oposto com o dimorfismo de tamanho entre castas. Nossa abordagem sugere ainda que colônias grandes estão fortemente relacionadas a uma arquitetura compacta para acomodação da prole, e a um hábito de nidificação independente de cavidades – sugerindo que algumas espécies de Meliponini, como *Trigona* sp., superaram as limitações de espaço impostas pela ocupação de cavidades pré-existentes. Por fim, argumentamos que o tamanho ancestral (colônias médias) sugere eussocialidade avançada nos ancestrais das abelhas sem ferrão, uma hipótese suportada por sua ancestralidade compartilhada com as abelhas altamente eussociais da tribo Melikertini, um clado-irmão extinto.

Macroevolutionary trends in colony size suggest advanced eusociality in stingless bee ancestors

Abstract

Social complexity is largely explained by colony size in insect societies. Stingless bees (Meliponini) are a good example on wide variation in social complexity, a pantropical eusocial insect group with more than 500 species. We collected colony size data of 71 species from the literature and used a phylogenetic framework to address the following question: is there a general trend in colony size evolution in stingless bees? Also, since colony size is expected to affect different life-history aspects, impacting both individual and colony level traits, thus we predicted colony size to covary with individual and social features, such as workers lifespan and body size, queen-worker size dimorphism, brood architecture and nesting behavior. We performed an ancestral state reconstruction to trace back the most likely ancestral state among small, medium and large colony sizes; and tested its correlated evolution with individual, social and nesting traits. Our comparative approach to colony size evolution revealed medium colony size is the most likely ancestral state in Meliponini, whereas small and large colony sizes are derived states, meaning flexibility in the loss and gain of social complexity. We also show that colony size trades-off with worker quality – worker body size and worker lifespan –, while caste differences follow increments in colony size. Our correlative approach suggest large colony sizes are strongly related to a more compact design to accommodate brood and to cavity-independent nesting behavior, shedding light over how some species, like *Trigona* sp., overcame space limitation constrains to increase in colony population. Finally, we argue that medium size ancestral state of colony size suggest advanced eusociality in stingless bee ancestors, a hypothesis supported by their shared common ancestry with the Melikertini bees, an extinct highly eusocial sister clade.

1. Introduction

Social insects vary greatly in complexity and a key determinant of social complexity is colony size. Social groups are predicted to buffer against seasonality, predation, parasitism and diseases (Kaspari & Vargo, 1995; Moritz & Fuchs, 1998), thus different species may benefit more or less of a social structure, depending on how many individuals share a common living. Varying in size from a few dozens to millions of individuals, societies can be interpreted under a complexity spectrum, being less or more complex (Bourke, 1999). Societies with up to a hundred adult individuals are considered small (*e. g.* hover wasps, bumble bees and sphecid bees), while those reaching between a thousand to ten thousand times this size would be the large ones (*e. g*. in ants, termites, honey bees and stingless bees, Bourke, 1999). Even though the scale of colony size may vary according to the targeted system, thus leading to different categorizations of small, medium and large societies (see Bourke, 1999; Rodriguez-Serrano et al., 2012; Burchill & Moureau, 2016), in general, large societies may benefit from improved colony survival, reproductive output, food source exploitation and disease or stress resistance (Kaspari & Vargo, 1995; Rosengaus et al., 1998; Shik, 2008); while small group sizes are predicted to benefit from improved decision-making in complex environments (Kao & Couzin, 2014). The number of individuals in a society may also shapes other features, for example, caste dimorphism, division of labor and communication systems (Bourke, 1999; Dornhaus et al., 2012).

As any other trait, colony size has its own evolutionary history, being modeled by the evolutionary pathways each group had undergone (Hendry et al., 2011; Harmon, 2018). Macroevolutionary trends in social traits remain understudied in many groups of social insects, however, an improved understanding of phylogenetic relationships in some taxa have led to an increase in attention in recent years, specially represented by research on social Hymenoptera (Kramer & Schaible, 2013), specially on ants (Keller & Genoud, 1997; Burchill & Moureau 2016) and bees (Dew et al., 2012; Rodriguez-Serrano et al., 2012; Cueva-del-Castillo et al., 2015). In both groups, social complexity correlates with colony size, suggesting that species with large colonies are more likely to present a complex social organization in terms of communication, division of labor and source exploitation, than smaller ones. For example, honey bees (Apini) have large colonies (thousands to tens of thousands) and use complex communication and specialized division of labour (Von Frisch, 1967; Seeley, 2009), whereas bumble bees (Bombini) have much smaller colonies (a few hundred) and simpler communication (Dornhaus & Chittka, 1999) and less-specialized division of labour (*e.g.* Jandt et al., 2009).

Among corbiculate bees (Euglossini, Bombini, Apini and Meliponini), the stingless bees (Meliponini), a predominantly tropical eusocial group, are particularly interesting to study social evolution theory due to its diversity in natural history and social organization. The group comprises more than 500 species (Michener, 2013; Ascher & Pickering, 2018), and shows a wide variation in body size, shape, colors, nesting behavior and communication systems (Michener, 2007; Roubik, 1998, 2006) Stingless bees also vary greatly in colony size, including species with a few hundreds of individuals, such as some *Plebeia* and *Melipona*, and those with more than tens of thousands adult bees, like most *Scaptotrigona*, *Tetragona*, *Partamona* and *Trigona* (Roubik, 1979, 1983). Such great variation may tell us a story on how social features (*e. g.* compaction of brood and nesting space constraints), as well as individual features (*e. g.* workers size and lifespan), interacted during stingless bees evolution (starting around 81 and 96 Mya, Rasmussen & Cameron, 2010); and how colony size presumably mediated social complexity transitions in the group (Bourke, 1999). However, little is known about the evolutionary history of colony size and its correlates in the Meliponini (Rodriguez-Serrano et al., 2012).

We collected colony size data of 71 species from the literature and used a phylogenetic framework to address the following question: is there a general trend in colony size evolution in stingless bees? Based on their shared ancestry with bumblebees (Romiguier et al., 2015; Borsset et al., 2019), and on social complexity hypothesis (Bourke, 1999), we predicted that small colony size is the ancestral state in stingless bees. Thus, we expect transitions from small to medium and to large colony sizes, but not in the reverse direction. Since colony size is expected to affect different life-history aspects (Bourke, 1999), impacting both individual and colony level traits, thus we predicted colony size to covary negatively with individual features, such as workers lifespan and body size, but positively with queen-worker size dimorphism. Finally, we expected colony size to associate with brood architecture and nesting behavior.

2. Material and Methods

2.1. Character states: colony size and other traits

We found colony size estimates of 71 species in the literature (Table S1), considering colony size as the number of adult worker bees in the nest. In case we had different estimates, we calculated a weighted mean (Burchill & Moreau, 2016). In order to minimize the number of phylogenetic parameters to be estimated in our subsequent models, species were grouped into one of three categories according to the mean colony size estimate for each species: small (< 1,000 adult bees), medium (between 1,000 and 5,000 bees) and large (> 5,000 bees). We based our

classification on the available literature (Bourke 1999; Rodriguez-Serrano et al., 2012; Burchill & Moreau, 2016).

Colony size is likely to be linked to other traits, such as worker body size (body length), worker life span, caste dimorphism (queen-worker size ratio), brood architecture (clusters, semi-combs and combs) and nesting substrate (tree, ground, exposed nest) (Bourke, 1999; Michener, 1974). Therefore, we also collected these data from the available literature (see Supplementary Material). Since data on some life-history aspects is more readily available (*e.g.* nesting substrate) than on others (*e.g.* queen-worker size ratio), sample sizes for the different analyses differ.

2.2. Phylogenetic analysis

For phylogenetic relationships, we used the molecular phylogeny provided by Rasmussen & Cameron (2010). We pruned their tree to include only the species used in this study. For some relationships within the genera *Melipona* and *Plebeia*, more detailed phylogenies were used (Ramirez et al., 2010; Werneck, 2016; Grüter et al 2017). We also made assumptions on phylogenetic relationship of species that were not listed in the Rasmussen & Camaron (2010) tree, based on available biological information (Table S1) - *Tetragonula minangkabau*, *Austroplebeia cassiae, Austroplebeia australis* and *Scaptotrigona postica* -, in order to include them in our final tree. We built two main trees, one species-based, and another genus-based. The resulting tree was used as a framework to estimate ancestral states of discrete characters and test correlated evolution of both continuous and discrete traits.

2.3. Ancestral State Reconstruction (ASR)

For the estimation of ancestral states, we relied on two Bayesian inference methods, that use the likelihood of observed data to update the prior distribution and then calculate posterior distributions (Joy et al., 2016). First, we used a stochastic mapping approach for reconstructions of character states, assuming a continuous-time Markov model (Huelsenbeck et al., 2003). We ran 1000 simulations of a stochastic process of the character state changes across the tree branches, using empirical estimates for state frequencies for the prior distribution on the root node. The posterior probabilities were plotted on the phylogeny (Figure 2).

A second method for the estimation of ancestral character states for discretely valued traits under a threshold model provided similar results to the stochastic mapping approach (Figure S1). The phylogenetic threshold model assumes a quantitative character – the liability - which is an unobserved variable, determining the change on character state according to a threshold value. This approach assumes a discrete-state Markov process (the same process generally assumed for the

evolution of DNA sequences; Revell, 2013) to sample the liabilities of the different states of a trait both in ancestral and extant species, and the relative positions of thresholds, from their joint posterior probability distribution (Revell, 2014).

The main difference between the two approaches lies in the Markov chain model: the first uses a continuous-time process, while the second uses a discrete-state process. We ran the functions make.simmap and ancThresh and plotted results using the R-packages *ape* and *phytools* (Paradis, 2011; Revell, 2013).

2.4. Correlated evolution of traits

We used phylogenetic correlations to infer correlated evolution of traits, by testing associations between colony size and: (i) worker body size; (ii) worker life span; (iii) caste dimorphism; (iv) brood architecture, and (v) nesting behavior. For this purpose, we chose phylogenetic generalized least square models (PGLS) (Harmon, 2018). For categorical predictors, significance was corrected for multiple comparisons using the holm method (Holm, 1979).

Our data on colony sizes showed a slightly left-skew distribution (Figure 1). This indicated that characters might not have evolved under Brownian motion, violating the basic assumptions of Felsenstein's Method (Felsenstein, 1985). Thus, we chose Matin's Correlation Structure in the PGLS to test correlated evolution among traits (Martins et al., 1997; Paradis, 2011).

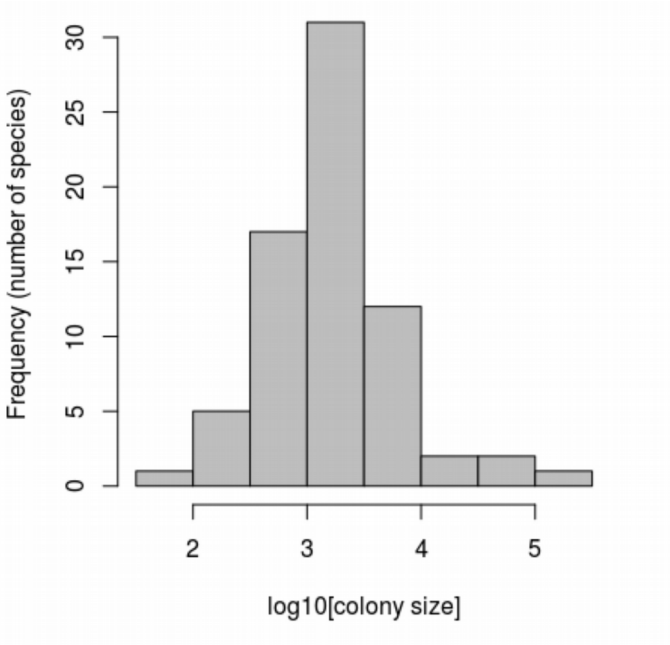


Figure 1: Colony size (log10 transformed) distribution of 71 stingless bee species. The correspondent non-transformed values of left and right extremes of $\log_{10}$[colony size] are 85 and 175,000 individuals, respectively. We observe a left-skew tendency, with most species having small to medium colony sizes (up to 5,000 adult bees).

3. Results

Small colonies showed a mean size of 471.44 (± 255.28 s.d.) adult bees, while medium size showed 2,294.69 (± 935.4 s.d.), and large colonies showed 32,488.86 (± 51,728.11). *M. micheneri* showed the smallest mean colony size, with 85 individuals, while *T. amazonensis* showed the largest mean size, containing 175,000 individuals - both genera represent extreme groups in the colony size dataset distribution (Figure 1). *Plebeia* was the only genus with at least one representant in each category of colony size, with six species classified as small (*P. franki*, *P. nigriceps*, *P. tobagoensis*, *P. jatiformis*, *P. frontalis* and *P. minima*), two as medium (*P. droryana* and *P. remota*), and one as large (*P. saiqui*, with a mean of 7,000 adult bees in their colonies).

The ancestral state estimation suggests that medium colony size is the ancestral condition in stingless bees with a probability of 59.1 ± 0.019% (± 95% confidence interval; N = 1000 simulations under continuous-time Markov model), against 15.5 ± 0.011% of being large, and 32.4 ± 0.03% of being small (Figure 2). Our results were similar when testing at a genus-level, since ancestral state estimation finds that medium sized colonies represents the ancestral state with a probability of 56.5 ± 0.00009% (± 95% confidence interval; N = 1000 simulations under continuous-time Markov model), against 12.3 ± 0.00001% of being large, and 3.11 ± 0.00002% of being small.

Medium size is the most likely ancestral state in stingless bees, suggesting that small and large sizes are derived states, and that transitions occur in both directions (small ↔ medium ↔ large). However, transitions did not seem to occur from small to large and *vice versa*. Small colony sizes were common in *Melipona* and *Plebeia* species, while large sizes were typical in the *Trigona* (Figure 2).

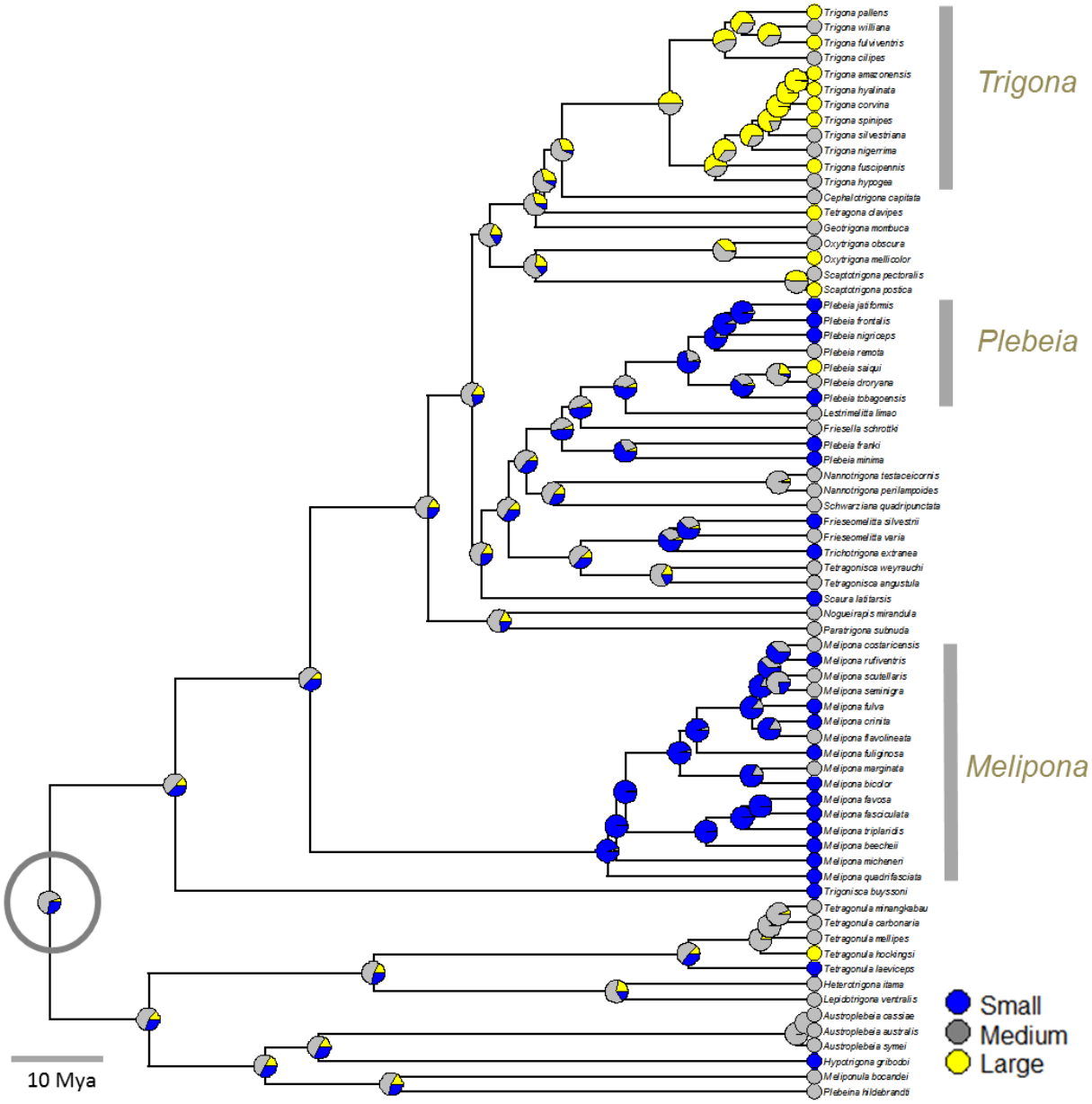


Figure 2: Reconstruction of the ancestral state of colony size in Meliponini. Filled circles in the terminal branches represent colony size states (small, medium or large) and the pie charts in the ancestral nodes indicate the probabilities of each state. The most likely ancestral condition of all stingless bees was estimated as medium colony size, with a probability of 59% (N = 1000 simulations), under continuous-time Markov model. The gray bar in the bottom left side indicates a 10 Mya time scale.

We found that colony size is correlated with all tested traits. Worker body length and lifespan correlated negatively with colony size (PGLS-body.length: r^2: - 0.129; p-value = 0.029; n = 32; PGLS-lifespan: r^2: - 0.292; p-value = 0.015; n = 17) (Figure 3), suggesting that worker quality decreases with increment in colony size. Queen-worker dimorphism, on the other hand, was positively correlated (PGLS-dimorphism: r^2: 0.192; p-value = 0.019; n = 25; Figure 4), showing that queen-worker morphological skew increases with colony size. Regarding nest traits, large colony sizes were found to be associated with comb/semi-comb brood cell architecture (PGLS-architecture: p = 0.025; n = 61), and with external nests (PGLS-nesting: p < 0.001 ; n = 33) (Figure 5).

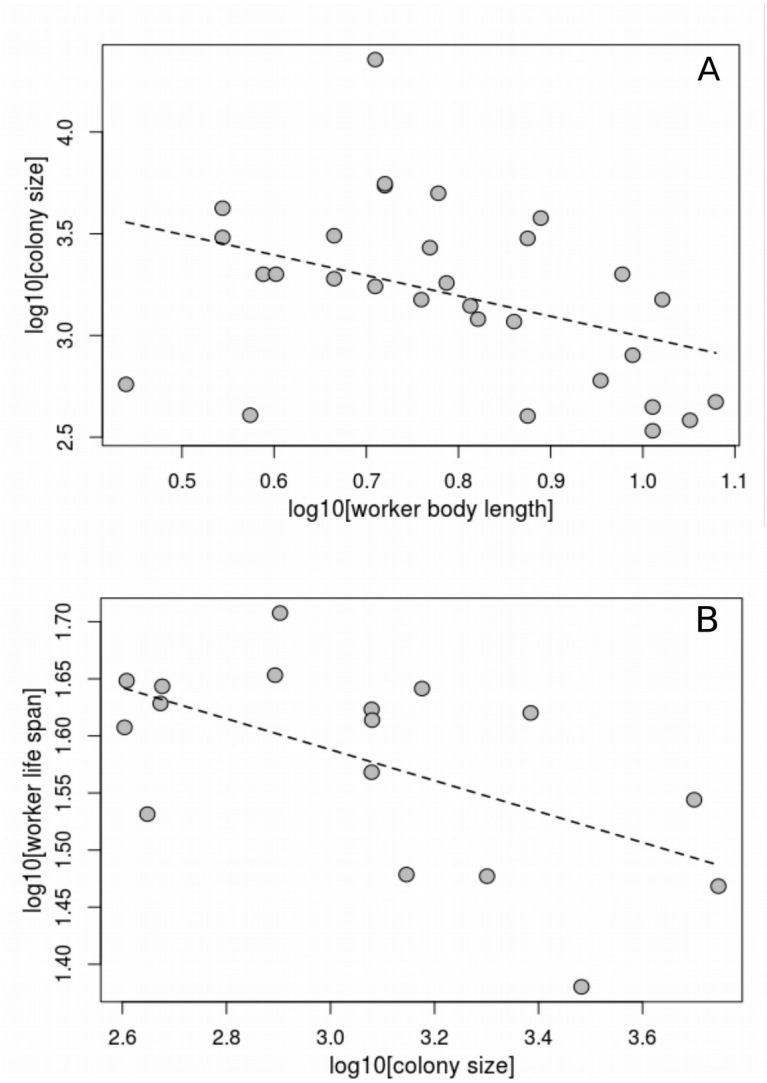


Figure 3: Relationship between colony size and (A) worker body length (PGLS-body.length: r^2: - 0.129; p-value = 0.029; n = 30), and (B) worker life span (PGLS-lifespan: r^2: - 0.292; p-value = 0.015; n = 17). Each dot represents one species from our pruned phylogeny of Meliponini.

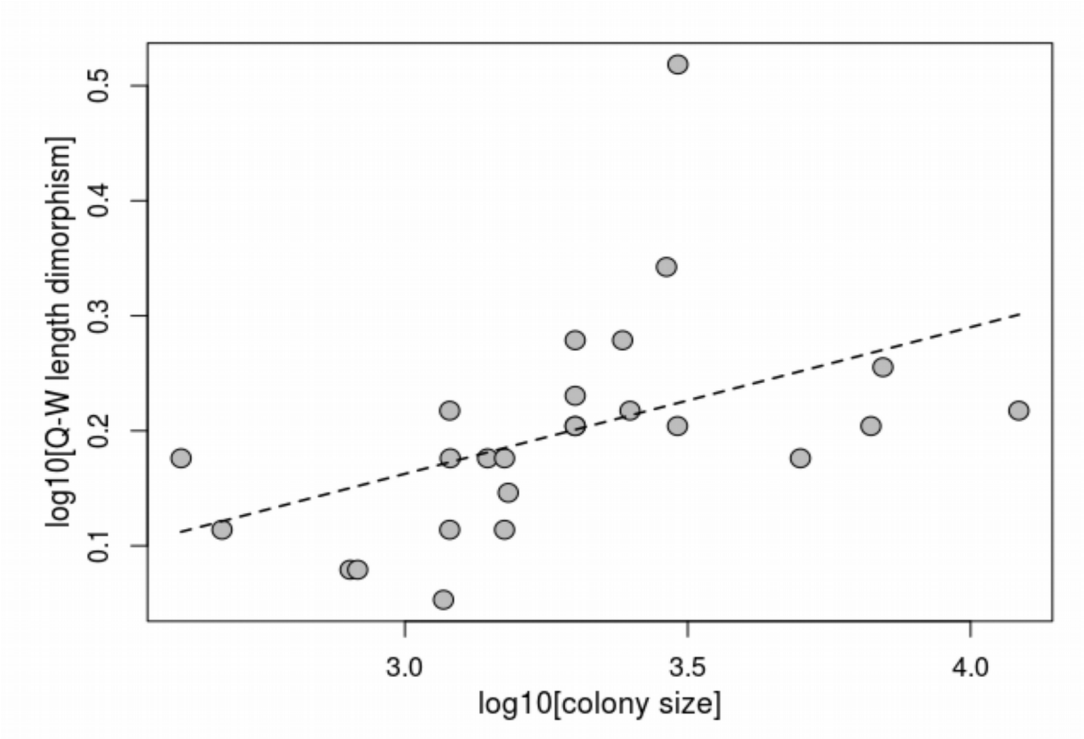


Figure 4: Relationship between colony size and caste dimorphism (PGLS-dimorphism: r^2: 0.192; p-value = 0.019; n = 25) in the Meliponini. Each dot represents one species from our Meliponini pruned phylogeny.

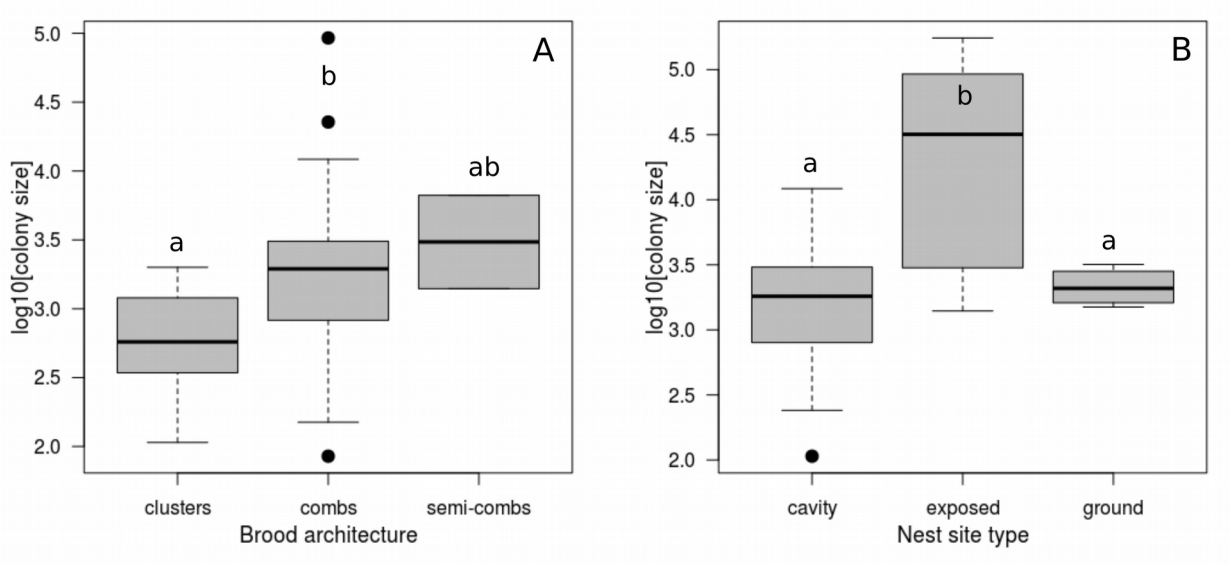


Figure 5: Relationship between colony size and (A) brood architecture (PGLS-architecuture: p = 0.025; n = 50) and (B) nest site type (PGLS-nesting: p < 0.001 ; n= 33). Lower case letters indicate significant differences. Significance was corrected for multiple comparisons.

4. Discussion

Our comparative approach suggests that medium colony size is the most likely ancestral state in stingless bees, whereas small and large colony sizes evolved later in the group. We also found that colony size predicts worker body size and worker lifespan, two measures of worker quality. Increments in colony size are associated with increased morphological skew between queens and workers. Finally, larger colony sizes are strongly linked to external nesting and the construction of brood combs, rather than clustered brood cells, possibly because combs represent a more compact design that can accommodate more brood. Although comparative analysis based on published data has limitations, roughly revealing functional relationships, we believe the amount and resolution of data used here may be enough for general hypothesis testing in a phylogenetic framework. All results presented were interpreted considering all possible methodological constraints linked to literature-based datasets.

Contrary to our expectations, medium colony size was inferred as the most likely ancestral state, meaning that small and large sizes are probably derived states. The ancestral condition seem common in both Old and New World species (Figure 2). Interestingly, a greater variation of colony size seems to occur in the Neotropics, where the origin of small colonies coincides with the early diversification of *Melipona* and *Plebeia* (around 24 to 28 Mya), while large colonies (in some cases between 60,000 to 175,000 individulas, Table S1) coincides with the diversification of *Trigona* (~ 19 Mya). Because intermediary colony sizes are the most likely ancestral state (Figure 2; Rodriguez-Serrano et al., 2012), transitions seem to have occurred in both directions, from medium to small or large size colonies, thus suggesting a flexible threshold underlying the gain and loss of complexity in the Meliponini. Interestingly, ants show a similar general pattern, with colonies transitioning in both directions from medium sized ancestors (Burchill & Moreau, 2016). Taken together, these results suggest intermediary colony sizes may have been advantageous to the diversification of highly eusocial lineages.

A common ancestor with a colony size between 1,000-5,000 workers sheds light over an interesting era of stingless bee evolutionary history. It suggests that this group was already highly eusocial, whereas the closest extant relative, the bumblebees, are primitively eusocial (annual colonies, weak queen-worker dimorphism, Michener, 2007). When did higher eusociality evolve in this branch of the corbiculate bees? A possible answer could be tied to their shared ancestry with an extinct sister clade - the tribe Melikertini (Engel, 2001). The Melikertini bees are considered the closest relatives of the Meliponini, showing a *Trigona*-like general habitus (Engel, 2001;

Rasmussen & Cameron, 2010), and morphologically specialized workers. The main differences with the stingless bees are the complete wing venation, the presence of a single metatibial spur and a functional sting (Engel, 2001). Thus, higher eusociality may not have evolved in stingless bees (*e. g.* Cardinal & Danforth 2011), but in the common ancestor of the Meliponini and the Melikertini, which most likely had a functional sting. Thus macroevolutionary trends in stingless bees colony size suggest eusociality in Meliponini+Melikertini ancestors.

We found that colony size is negatively linked to worker quality, represented by life span and body length in our analysis. To increase in numbers, a well known strategy is to invest less resources per unit, thus leading to the production and maintenance of more units (Oster & Wilson, 1964). In one hand, individual workers may be more valuable to the colony in small societies. On the other hand, they may be disposable in large societies, simply because losing a few units may cause low impact to the whole system. This argument is well developed by Oster & Wilson (1964) when discussing the adaptive value of castes in insect societies.

Our results suggest a negative relationship between queen-worker size dimorphism and colony size. Species with smaller colonies are predicted to show lower levels of caste differences, whereas large colonies tend to show higher caste divergence (Bourke, 1999). Also suggest some species of *Melipona* and *Plebeia*, for example, are less complex compared to the large societies of *Scaptotrigona*, *Tetragona*, and specially to the *Trigona*. Low caste polymorphism, found in most *Melipona* species, as well as increased worker reproduction, evolved secondarily in stingless bees (Grüter et al., 2017, 2018), indicating social conflict may be higher in such small societies, since workers may have more chances to reproduce, thus favoring selfish behaviors over cooperation (Keller & Chapuisat, 2006; Grüter, 2018). Maybe intense social conflicts help us to explain small societies: colony productivity may be constrained by such queen-worker competition for opportunities to lay eggs, and for brood investment (potentially queen's female eggs and worker's male eggs) – which is less likely to occur in large societies (Bourke, 1999).

Finally, our analyses of nesting traits suggest that larger colony sizes are associated with brood arranged in combs and external nests. This could represent a strategy to accommodate a larger worker force in a limited space, by building compact brood in combs or semi-combs, instead of clusters. An additional strategy to overcome space constraints in order to increase colony sizes was possibly the evolution of cavity-independent nesting behavior (Roubik, 1992, 2006), an evolutionary novelty that seems to have evolved recently, in both Afrotropical and Neotropical clades (~ 20 Mya). Cavity-independece is well represented by the exposed nests of the african *Dactylurina*, reaching its magnitude in some neotropical lineages (Roubik, 1992) - among them, the

Trigona show the largest stingless bee societies, with *T. spinipes* and *T. amazonica* reaching a mean of 92,500 amd 175,000 individuals, respectively (Table S1), almost 18 to 35 times the size of medium colonies in the tribe.

Taken together, our results suggest stingless bees followed different life-history strategies that met the demands of maintaining a successful society during their evolutionary history. Further research focused on testing such general trends in species level might offer new insights on stingless bee ecological flexibility, linking proximate (ecology) and ultimate (evolutionary) causes of colony size in Meliponini.

Acknowledgements

We thank M.Sc. Kamila L. Leão for providing unpublished data on the colony size of five amazonian. JC Veiga received CAPES/PDSE fellowship (88881.189098/2018-01).

Literature cited

BOSSERT, S., MURRAY, E.A., ALMEIDA, E.A.B., BRADY, S.G., BLAIMER, B.B. & DANFORTH, B.N. (2019) Combining transcriptomes and ultraconserved elements to illuminate the phylogeny of Apidae. *Molecular Phylogenetics and Evolution*, 130, 121–131.

BOURKE (1999) Colony size, social complexity and reproductive conflict in social insects. *Journal of Evolutionary Biology*, 12, 245–257.

BOURKE, A.F.G. (2007) Kin Selection and the Evolutionary Theory of Aging. *Annual Review of Entomology*, 38, 103–128.

BROWN, C.R. (2016) The ecology and evolution of colony-size variation. *Behavioral Ecology and Sociobiology*, 70, 1613–1632.

BURCHILL, A.T. & MOREAU, C.S. (2016) Colony size evolution in ants: macroevolutionary trends. *Insectes Sociaux*, 63, 291–298.

CUEVA DEL CASTILLO, R., SANABRIA-URBÁN, S. & SERRANO-MENESES, M.A. (2015) Trade-offs in the evolution of bumblebee colony and body size: A comparative analysis. *Ecology and Evolution*, 5, 3914–3926.

DEW, R., REHAN, S. & TIERNEY, S. (2012) A single origin of large colony size in allodapine bees suggests a threshold event among 50 million years of evolutionary tinkering. *Insectes Sociaux*, 59, 207–214.

DORNHAUS, A., POWELL, S. & BENGSTON, S. (2012) Group Size and Its Effects on Collective Organization. *Annual Review of Entomology*, 57, 123–141.

DORNHAUS, A. & CHITTKA, L. (1999) Evolutionary origins of bee dances. *Nature*, 401, 38.

ENGEL, M.S. (2001) A Monograph of the Baltic Amber Bees and Evolution of the Apoidea (Hymenoptera). *Bulletin of the American Museum of Natural History*, 1–192.

FELSENSTEIN, J. (2012) A Comparative Method for Both Discrete and Continuous Characters Using the Threshold Model. *The American Naturalist*, 179, 145–156.

GRÜTER, C. (2018) Repeated switches from cooperative to selfish worker oviposition during stingless bee evolution. *Journal of Evolutionary Biology*, 31, 1843–1851.

GRÜTER, C., SEGERS, F.H.I.D., MENEZES, C., VOLLET-NETO, A., FALCÓN, T., VON ZUBEN, L., ET AL. (2017) Repeated evolution of soldier sub-castes suggests parasitism drives social complexity in stingless bees. *Nature Communications*, 8, 4.

HARMON, L. J. (2018) Phylogenetic Comparative Methods. Version 1. Released under a CC-BY-4.0 license.

HOLM, S. (1979) A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*, 65–70.

HUELSENBECK, J.P., NIELSEN, R. & BOLLBACK, J.P. (2003) Stochastic mapping of morphological characters. *Systematic Biology*, 52, 131–158.

JANDT, J. M., HUANG, E. & DORNHAUS, A. (2009) Weak specialization of workers inside a bumble bee (*Bombus impatiens*) nest. *Behavioral Ecology and Sociobiology*, 63, 1829–1836.

JARAU, S., HRNCIR, M., ZUCCHI, R. & BARTH, F.G. (2004) A stingless bee uses labial gland secretions for scent trail communication (*Trigona recursa* Smith 1863), 233–239.

JOY, J.B., LIANG, R.H., MCCLOSKEY, R.M., NGUYEN, T. & POON, A.F.Y. (2016) Ancestral Reconstruction. *PLoS Computational Biology*, 12, 1–20.

KAO, A.B. & COUZIN, I.D. (2014) Decision accuracy in complex environments is often maximized by small group sizes. *Proceedings of the Royal Society B: Biological Sciences*, 281, 20133305–20133305.

KAPHEIM, K.M. (2018) Synthesis of Tinbergen's four questions and the future of sociogenomics. *Behavioral Ecology and Sociobiology* (https://doi.org/10.1007/s00265-018-2606-3).

KASPARI, M. & VARGO, E. (1995) Colony size as a buffer against sea- sonality: Bergmann's rule in social insects. *American Naturalist*, 146, 610– 632.

KELLER, L. & CHAPUISAT, M. (2006) Cooperation among Selfish Individuals in Insect Societies. *BioScience*, 49, 899–909.

KELLER, L. & GENOUD, M. (1997) Extraordinary lifespans in ants: A test of evolutionary theories of ageing. *Nature*, 389, 958–960.

KRAMER, B.H. & SCHAIBLE, R. (2013) Colony size explains the lifespan differences between queens and workers in eusocial Hymenoptera. *Biological Journal of the Linnean Society*, 109, 710–724.

MARTINS, E.P. & HANSEN, T.F. (2002) Phylogenies and the Comparative Method: A General Approach to Incorporating Phylogenetic Information into the Analysis of Interspecific Data. *The American Naturalist*, 149, 646–667.

MICHENER, C.D. (1974) The Social Behavior of the Bees: A comparative study. Harvard University Press, Cambridge, Massachusetts.

MICHENER, C.D. (2007) The bees of the world, 2nd edition. Johns Hopkins University Press, Baltimore, Maryland, USA.

MORITZ, R.F.A. & FUCHS, S. (1998) Organization of honeybee colonies: characteristics and consequences of a superorganism concept. *Apidologie*, 29, 7–21.

NIEH, J.C. (2004) Recruitment communication in stingless bees (Hymenoptera, Apidae, Meliponini). *Apidologie*, 35, 159–182.

OSTER, G.F. & WILSON, E.O. (1978) Caste and ecology in the social insects. Princeton University Press.

PARADIS, E. (2011) Analysis of Phylogenetics and Evolution With R.

RASMUSSEN, C. & CAMERON, S.A. (2010) Global stingless bee phylogeny supports ancient divergence, vicariance, and long distance dispersal. *Biological Journal of the Linnean Society*, 99, 206–232.

RATNIEKS, F.L.W., FOSTER, K.R. & WENSELEERS, T. (2005) Conflict Resolution in Insect Societies. *Annual Review of Entomology*, 51, 581–608.

REVELL, L.J. (2012) phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, 3, 217–223.

REVELL, L.J. (2014) Ancestral character estimation under the threshold model from quantitative genetics. *Evolution*, 68, 743–759.

RODRIGUEZ-SERRANO, E., INOSTROZA-MICHAEL, O., AVARIA-LLAUTUREO, J. & HERNANDEZ, C.E. (2012) Colony Size Evolution and the Origin of Eusociality in Corbiculate Bees (Hymenoptera: Apinae). *PLoS ONE*, 7, 1–8.

ROMIGUIER, J., CAMERON, S.A., WOODARD, S.H., FISCHMAN, B.J., KELLER, L. & PRAZ, C.J. (2016) Phylogenomics Controlling for Base Compositional Bias Reveals a Single Origin of Eusociality in Corbiculate Bees. *Molecular Biology and Evolution*, 33, 670–678.

ROSENGAUS, R.B., MAXMEN, A.B., COATES, L.E. & TRANIELLO, J.F.A. (1998) Diesease resistance: a benefit of sociality in the dampwood termite *Zootermopsis angusticollis* (Isoptera: Termospidae). *Behavioral Ecology and Sociobiology*, 44, 125–134.

ROUBIK, D.W. (1979) Nest and Colony Characteristics of Stingless Bees from French Guiana (Hymenoptera: Apidae). *Journal of the Kansas Entomological Society*, 52, 443–470.

ROUBIK, D.W. (1983) Nest and Colony Characteristics of Stingless Bees from Panamá (Hymenoptera: Apidae). *Journal of the Kansas Entomological Society*, 56, 327–355.

ROUBIK, D. (1992) Ecology and natural history of tropical bees, 1st edition. Cambridge University Press, Cambridge.

ROUBIK, D.W. (2006) Stingless bee nesting biology. *Apidologie*, 37, 124–143.

SCHMIDT, V.M., ZUCCHI, R. & BARTH, F.G. (2006) Recruitment in a scent trail laying stingless bee (Scaptotrigona aff. depilis): Changes with reduction but not with increase of the energy gain. *Apidologie*, 37, 487–500.

SEELEY, T.D. (2009) The Wisdom of the Hive: the social physiology of honey bee colonies. Harvard University Press.

SHIK, J.Z. (2008) Ant colony size and the scaling of reproductive effort. *Functional Ecology*, 22, 674–681.

TARPY, D.R., GILLEY, D.C. & SEELEY, T.D. (2004) Levels of selection in a social insect: a review of conflict and cooperation during honey bee (*Apis mellifera*) queen replacement. *Behavioral Ecology and Sociobiology*, 55, 513–523.

VON FRISCH, K. & CHADWICK, L.E. (1967) The dance language and orientation of bees. Belknap Press of Harvard University Press, Cambridge, MA.

ZUCCHI, R., SAKAGAMI, S.F., NOLL, F.B., MECHI, M.R., MATEUS, S., BAIO, M. V & SHIMA, S.N. (1995) *Agelaia vicina*, a swarm-founding polistine with the largest colony size among wasps and bees (Hymenoptera: Vespidae). *Journal of the New York Entomological Society*, 103, 129–137.

Eletronic Supplementary Material

Table S1: Dataset on colony population size of stingless bees (Meliponini) used to perform Ancestral State Reconstructions. Source of mean value: a) Mean value calculated using reported range (minimum and maximum number) by the study; b) Mean value reported in the study; c) Weighted mean calculated based on mean values reported in multiple studies.

Clade	Genus	Species	Mean colony size	Source of mean value	Reference
AT	*Hypotrigona*	*Hypotrigona gribodoi*	575	a	Wille & Michener (1973)
AT	*Meliponula*	*Meliponula bocandei*	1170	b	Kajobe (2006)
AT	*Plebeina*	*Plebeina hildebrandti*	3091	b	Namu & Wittmann (2016)
IM-AA	*Austroplebeia*	*Austroplebeia australis*	2000	b	Hammond & Keller (2004)
IM-AA	*Austroplebeia*	*Austroplebeia cassiae*	3000	b	Grüter unpublished
IM-AA	*Austroplebeia*	*Austroplebeia symei*	2000	b	Hammond & Keller (2004)
IM-AA	*Heterotrigona*	*Heterotrigona itama*	5000	b	Kwong et al. (2017)
IM-AA	*Lepidotrigona*	*Lepidotrigona ventralis*	4221	b	Chinh et al. (2005)
IM-AA	*Tetragonula*	*Tetragonula carbonaria*	3033	c	Halcroft et al. (2013) in Vit et al. (2013), Kwong et al. (2017)
IM-AA	*Tetragonula*	*Tetragonula hockingsi*	6666	c	Palmer et al. (2002) in Jaffé et al. (2014)
IM-AA	*Tetragonula*	*Tetragonula laeviceps*	782.5	c	Chinh et al. (2005), Sakagami et al. (1983), Kwong et al. (2017)
IM-AA	*Tetragonula*	*Tetragonula mellipes*	2000	b	Hammond & Keller (2004)
IM-AA	*Tetragonula*	*Tetragonula minangkabau*	1200	c	Sakagami et al. (1983); Inoue et al. (1996); Inoue et al., (1985)
NE	*Cephalotrigona*	*Cephalotrigona capitata*	1250	a	Michener (1974)
NE	*Friesella*	*Friesella schrottki*	1400	b	Sakagami et al. (1973); Michener (1974); Tóth et al. (2004)
NE	*Frieseomelitta*	*Frieseomelitta silvestrii*	500	a	Michener (1974)

Clade	Genus	Species	Mean colony size	Source of mean value	Reference
NE	*Frieseomelitta*	*Frieseomelitta varia*	1200	a	Tóth et al. (2004)
NE	*Geotrigona*	*Geotrigona mombuca*	2500	b	Tóth et al. (2004)
NE	*Lestrimelitta*	*Lestrimelitta limao*	2700	c	C. Menezes and unpublished estimates in Grüter et al. (2016), von Zuber & Grüter unpublished estimates in Grüter et al. (2016)
NE	*Melipona (Eomelipona)*	*Melipona bicolor*	475	a	Tóth et al. (2004)
NE	*Melipona (Eomelipona)*	*Melipona marginata*	1130	b	Tóth et al. (2004)
NE	*Melipona (Eomelipona)*	*Melipona micheneri*	85	a	Roubik (1983)
NE	*Melipona (Melikerria)*	*Melipona beecheii*	800	b	van Veen et al. (2004)
NE	*Melipona (Melikerria)*	*Melipona fasciculata*	4713.75	c	Leão et al. unpublished; Kerr et al. (2001) in Correa Gomes et al. (2015)
NE	*Melipona (Melikerria)*	*Melipona triplaridis*	340	b	Roubik (1983)
NE	*Melipona (Melipona)*	*Melipona favosa*	402	c	Sommeijer (1984); Roubik (1982); Roubik (1979); Bijlsma et al. (2006)
NE	*Melipona (Melipona)*	*Melipona quadrifasciata*	825	c	Michener (1974); Tóth et al. (2004)
NE	*Melipona (Michmelia)*	*Melipona costaricensis*	2000	b	Wille & Michener (1973)
NE	*Melipona (Michmelia)*	*Melipona crinita*	315	a	Roubik (1983)
NE	*Melipona (Michmelia)*	*Melipona flavolineata*	1523	c	Leão et al. unpublished; C. Menezes unpublished estimates in Grüter et al. (2016)
NE	*Melipona (Michmelia)*	*Melipona fuliginosa*	383	b	Roubik (1983)
NE	*Melipona (Michmelia)*	*Melipona fulva*	444.75	c	Roubik (1982); Roubik (1979)
NE	*Melipona (Michmelia)*	*Melipona rufiventris*	600	b	Nieh et al. (2005)
NE	*Melipona (Michmelia)*	*Melipona scutellaris*	1500	b	Tóth et al. 2004

Clade	Genus	Species	Mean colony size	Source of mean value	Reference
NE	*Melipona (Michmelia)*	*Melipona seminigra*	2000	a	C. Menezes unpublished estimates in Grüter et al. (2016)
NE	*Nannotrigona*	*Nannotrigona perilampoides*	1350	a	Quezada-Euán & Gonzales-Acereto (2002); Quezada-Euán et al. (2011)
NE	*Nannotrigona*	*Nannotrigona testaceicornis*	2500	a	Michener (1974)
NE	*Nogueirapis*	*Nogueirapis mirandula*	3178.5	a	Wille (1966)
NE	*Oxytrigona*	*Oxytrigona mellicolor*	5442	b	Roubik (1983)
NE	*Oxytrigona*	*Oxytrigona obscura*	1900	b	Roubik (1983)
NE	*Paratrigona*	*Paratrigona subnuda*	2000	c	Roubik (1983)
NE	*Plebeia*	*Plebeia droryana*	2425.5	c	Tóth et al. (2004); Roldão et al. (2018); Kwong et al. (2017)
NE	*Plebeia*	*Plebeia franki*	113	b	Roubik (1983)
NE	*Plebeia*	*Plebeia frontalis*	1000	c	Wille & Michener (1973)
NE	*Plebeia*	*Plebeia jatiformis*	800	a	Roubik (1983)
NE	*Plebeia*	*Plebeia minima*	240.835	c	Leão et al. unpublished; Roubik (1983)
NE	*Plebeia*	*Plebeia nigriceps*	150	a	Witter et al. (2007)
NE	*Plebeia*	*Plebeia remota*	2900	c	van Benthem et al. (1995)
NE	*Plebeia*	*Plebeia saiqui*	7000	b	Witter et al. (2007)
NE	*Plebeia*	*Plebeia tobagoensis*	685	c	Hofstede & Sommeijer (2006); Hofstede (2006); Bijlsma et al. (2006)
NE	*Scaptotrigona*	*Scaptotrigona pectoralis*	3600	a	Quezada-Euan & Gonzalez-Acereto(2002), Roubik (1983)
NE	*Scaptotrigona*	*Scaptotrigona postica*	5002	a	Tóth et al. (2004), Michener (1974)
NE	*Scaura*	*Scaura latitarsis*	405.75	c	Roubik (1983); Wille & Michener (1973)
NE	*Schwarziana*	*Schwarziana quadripunctata*	1640	b	Hammond & Keller (2004)

Clade	Genus	Species	Mean colony size	Source of mean value	Reference
NE	*Tetragona*	*Tetragona clavipes*	12171.875	c	Duarte et al. (2016)
NE	*Tetragonisca*	*Tetragonisca angustula*	3037.5	c	Tóth et al. (2004); Roubik (1983)
NE	*Tetragonisca*	*Tetragonisca weyrauchi*	2500	a	Cortopassi-Laurino & Nogueira-Neto (2003)
NE	*Trichotrigona*	*Trichotrigona extranea*	107	b	Camargo & Pedro (2007)
NE	*Trigona*	*Trigona amazonensis*	175000	b	J. M. F. Camargo in Zucchi et al., (1995)
NE	*Trigona*	*Trigona cilipes*	1812.33	c	Roubik (1979)
NE	*Trigona*	*Trigona corvina*	22680	a	Roubik (1983)
NE	*Trigona*	*Trigona fulviventris*	5750	b	Roubik (1983)
NE	*Trigona*	*Trigona fuscipennis*	7500	a	Roubik (1983)
NE	*Trigona*	*Trigona hyalinata*	44586	c	Nieh et al. (2003); Kwong et al. 2017; Roubik (1979)
NE	*Trigona*	*Trigona hypogea*	1741.5	c	Camargo & Roubik (1991)
NE	*Trigona*	*Trigona nigerrima*	1398	b	Roubik (1983)
NE	*Trigona*	*Trigona pallens*	5568.5	c	Roubik, (1979)
NE	*Trigona*	*Trigona silvestriana*	3000	b	Roubik (1983)
NE	*Trigona*	*Trigona spinipes*	92500	a	Michener (1974)
NE	*Trigona*	*Trigona williana*	3775	c	Roubik (1979); Wille & Michener (1973)
NE	*Trigonisca*	*Trigonisca buyssoni*	343	c	Roubik (1983)

Table S2: Workers meand body length dataset of stingless bees. All values retrieved from Schwarz (1948).

Clade	Genus	Species	Mean body length (mm)
AT	*Hypotrigona*	*Hypotrigona gribodoi*	2.75
AT	*Meliponula*	*Meliponula bocandei*	7.25
AT	*Plebeina*	*Plebeina hildebrandti*	4.625
IM-AA	*Austroplebeia*	*Austroplebeia australis*	3.875
IM-AA	*Heterotrigona*	*Heterotrigona itama*	6.0
IM-AA	*Lepidotrigona*	*Lepidotrigona ventralis*	3.5
IM-AA	*Tetragonula*	*Tetragonula carbonaria*	3.5
NE	*Lestrimelitta*	*Lestrimelitta limao*	5.875
NE	*Melipona (Eomelipona)*	*Melipona marginata*	6.625
NE	*Melipona (Melikerria)*	*Melipona beecheii*	9.75
NE	*Melipona (Melikerria)*	*Melipona fasciculata*	12.0
NE	*Melipona (Melikerria)*	*Melipona triplaridis*	10.25
NE	*Melipona (Melipona)*	*Melipona favosa*	7.5
NE	*Melipona (Michmelia)*	*Melipona costaricensis*	9.5
NE	*Melipona (Michmelia)*	*Melipona fuliginosa*	11.25
NE	*Melipona (Michmelia)*	*Melipona fulva*	10.25
NE	*Melipona (Michmelia)*	*Melipona rufiventris*	9.0
NE	*Melipona (Michmelia)*	*Melipona scutellaris*	10.5
NE	*Oxytrigona*	*Oxytrigona mellicolor*	5.25
NE	*Oxytrigona*	*Oxytrigona obscura*	4.625
NE	*Paratrigona*	*Paratrigona subnuda*	4.0
NE	*Scaura*	*Scaura latitarsis*	3.75
NE	*Schwarziana*	*Schwarziana*	5.75

Clade	Genus	Species	Mean body length (mm)
		quadripunctata	
NE	*Trigona*	*Trigona amazonensis*	6.875
NE	*Trigona*	*Trigona cilipes*	6.125
NE	*Trigona*	*Trigona corvina*	5.125
NE	*Trigona*	*Trigona hyalinata*	5.75
NE	*Trigona*	*Trigona hypogea*	5.125
NE	*Trigona*	*Trigona nigerrima*	6.5
NE	*Trigona*	*Trigona pallens*	5.25
NE	*Trigona*	*Trigona silvestriana*	7.5
NE	*Trigona*	*Trigona williana*	7.75

Table S3: Workers mean life span dataset of stingless bees retrieved from literature.

Clade	Genus	Species	Mean longevity (days)	Source
AT	*Meliplebeia*	*Meliplebeia becarrii*	52.7	Moses et al. (2013)
IM-AA	*Tetragonula*	*Tetragonula minangkabau*	37.0	Inoue et al. (1996)
NE	*Melipona (Eomelipona)*	*Melipona bicolor*	44.0	Giannini (1997)
NE	*Melipona (Eomelipona)*	*Melipona marginata*	41.1	Mateus, unpublished
NE	*Friesella*	*Friesella schrottki*	30.1	Giannini (1997)
NE	*Frieseomelitta*	*Frieseomelitta languida*	33.3	Giannini (1997)
NE	*Frieseomelitta*	*Frieseomelitta paupera*	40.0	Bijlsma et al. (2006)
NE	*Frieseomelitta*	*Frieseomelitta varia*	42.0	Cardoso (2010)
NE	*Melipona (Melikerria)*	*Melipona beecheii*	51.0	Biesmeijer & Tóth (1998)
NE	*Melipona (Melikerria)*	*Melipona fasciculata*	42.5	Giannini (1997); Gomes et al. (2015)
NE	*Melipona (Melipona)*	*Melipona favosa*	40.5	Sommeijer (1984); Roubik (1982)
NE	*Melipona (Melikerria)*	*Melipona eburnea*	39.5	Bustamante (2006)
NE	*Melipona (Melikerria)*	*Melipona fulva*	34.0	Roubik (1982)
NE	*Melipona (Melikerria)*	*Melipona scutellaris*	43.8	Santos (2013)
NE	*Melipona (Melikerria)*	*Melipona seminigra*	30	Bustamante (2006)
NE	*Plebeia*	*Plebeia droryana*	41.7	Terada et al. (1975)
NE	*Scaptotrigona*	*Scaptotrigona postica*	35.0	Simoes & Bego (1972, 1991)
NE	*Scaura*	*Scaura latitarsis*	44.5	Bustamante (2006)
NE	*Tetragonisca*	*Tetragonisca angustula*	24.0	Grosso & Bego (2002)
NE	*Tetragonula*	*Tetragonula laeviceps*	45.0	Inoue et al. (1985)
NE	*Trigona*	*Trigona pallens*	29.4	Cardoso (2010)
NE	*Trigona*	*Trigona pellucida*	41.5	Cardoso (2010); Mateus & Zucchi (2008)

Table S4: Queen-worker (Q-W) size dimorphism dataset of stingless bees retrieved from literature.

Clade	Genus	Species	Q-W length ratio	Reference
AT	*Meliponula*	*Meliponula bocandei*	1.13	Sakagami & Portugal-Araujo (1977)
IM-AA	*Austroplebeia*	*Austroplebeia australis*	1.6	Tóth et al. (2004)
IM-AA	*Austroplebeia*	*Austroplebeia symei*	1.9	Tóth et al. (2004)
IM-AA	*Tetragonula*	*Tetragonula carbonaria*	1.6	Tóth et al. (2004)
IM-AA	*Tetragonula*	*Tetragonula hockingsi*	1.6	Tóth et al. (2004)
IM-AA	*Tetragonula*	*Tetragonula mellipes*	1.6	Tóth et al. (2004)
IM-AA	*Tetragonula*	*Tetragonula minangkabau*	1.65	Tóth et al. (2004)
NE	*Melipona (Eomelipona)*	*Melipona bicolor*	1.3	Tóth et al. (2004)
NE	*Melipona (Eomelipona)*	*Melipona marginata*	1.5	Tóth et al. (2004)
NE	*Friesella*	*Friesella schrottki*	1.5	Tóth et al. (2004)
NE	*Frieseomelitta*	*Frieseomelitta varia*	1.3	Tóth et al. (2004)
NE	*Geotrigona*	*Geotrigona mombuca*	1.65	Tóth et al. (2004)
NE	*Melipona (Melipona)*	*Melipona beecheii*	1.2	Tóth et al. (2004)
NE	*Melipona (Melipona)*	*Melipona favosa*	1.5	Tóth et al. (2004)
NE	*Melipona (Melipona)*	*Melipona flavolineata*	1.4	Our data
NE	*Melipona (Melipona)*	*Melipona quadrifasciata*	1.2	Tóth et al. (2004)
NE	*Melipona (Melipona)*	*Melipona scutellaris*	1.3	Tóth et al. (2004)
NE	*Paratrigona*	*Paratrigona subnuda*	1.7	Tóth et al. (2004)
NE	*Plebeia*	*Plebeia droryana*	1.9	Tóth et al. (2004)
NE	*Plebeia*	*Plebeia remota*	2.2	Tóth et al. (2004)
NE	*Plebeia*	*Plebeia saiqui*	1.8	Tóth et al. (2004)
NE	*Scaptotrigona*	*Scaptotrigona postica*	1.5	Tóth et al. (2004)
NE	*Schwarziana*	*Schwarziana quadripunctata*	1.5	Tóth et al. (2004)
NE	*Tetragona*	*Tetragona clavipes*	1.65	Tóth et al. (2004)
NE	*Tetragonisca*	*Tetragonisca angustula*	3.3	Tóth et al. (2004)

Tabela S5: Brood architecture dataset of stingless bees retrieved from literature (reviewed by Grüter et al., unpubhished).

Clade	Genus	Species	Brood architecutre type
AT	*Hypotrigona*	*Hypotrigona gribodoi*	clusters
AT	*Meliponula*	*Meliponula bocandei*	combs
AT	*Plebeina*	*Plebeina hildebrandti*	combs
IM-AA	*Austroplebeia*	*Austroplebeia australis*	clusters
IM-AA	*Austroplebeia*	*Austroplebeia symei*	clusters
IM-AA	*Lepidotrigona*	*Lepidotrigona ventralis*	combs
IM-AA	*Tetragonula*	*Tetragonula carbonaria*	combs
IM-AA	*Tetragonula*	*Tetragonula hockingsi*	semi-combs
IM-AA	*Tetragonula*	*Tetragonula laeviceps*	clusters
IM-AA	*Tetragonula*	*Tetragonula mellipes*	semi-combs
IM-AA	*Tetragonula*	*Tetragonula minangkabau*	combs
NE	*Cephalotrigona*	*Cephalotrigona capitata*	combs
NE	*Friesella*	*Friesella schrottki*	semi-combs
NE	*Frieseomelitta*	*Frieseomelitta silvestrii*	clusters
NE	*Frieseomelitta*	*Frieseomelitta varia*	clusters
NE	*Lestrimelitta*	*Lestrimelitta limao*	combs
NE	*Melipona (Eomelipona)*	*Melipona bicolor*	combs
NE	*Melipona (Eomelipona)*	*Melipona marginata*	combs
NE	*Melipona (Eomelipona)*	*Melipona micheneri*	combs
NE	*Melipona (Melikerria)*	*Melipona fasciculata*	combs
NE	*Melipona (Melikerria)*	*Melipona triplaridis*	combs
NE	*Melipona (Melipona)*	*Melipona favosa*	combs
NE	*Melipona (Melipona)*	*Melipona quadrifasciata*	combs
NE	*Melipona (Michmelia)*	*Melipona costaricensis*	combs
NE	*Melipona (Michmelia)*	*Melipona flavolineata*	combs
NE	*Melipona (Michmelia)*	*Melipona fuliginosa*	combs
NE	*Melipona (Michmelia)*	*Melipona fulva*	combs
NE	*Melipona (Michmelia)*	*Melipona rufiventris*	combs
NE	*Melipona (Michmelia)*	*Melipona scutellaris*	combs
NE	*Melipona (Michmelia)*	*Melipona seminigra*	combs

Clade	Genus	Species	Brood architecutre type
NE	*Melipona (Melikerria)*	*Melipona beecheii*	combs
NE	*Nannotrigona*	*Nannotrigona perilampoides*	combs
NE	*Nannotrigona*	*Nannotrigona testaceicornis*	combs
NE	*Oxytrigona*	*Oxytrigona mellicolor*	combs
NE	*Oxytrigona*	*Oxytrigona obscura*	combs
NE	*Paratrigona*	*Paratrigona subnuda*	combs
NE	*Plebeia*	*Plebeia droryana*	combs
NE	*Plebeia*	*Plebeia franki*	clusters
NE	*Plebeia*	*Plebeia frontalis*	combs
NE	*Plebeia*	*Plebeia jatiformis*	combs
NE	*Plebeia*	*Plebeia nigriceps*	combs
NE	*Plebeia*	*Plebeia remota*	combs
NE	*Plebeia*	*Plebeia saiqui*	combs
NE	*Scaptotrigona*	*Scaptotrigona pectoralis*	combs
NE	*Scaptotrigona*	*Scaptotrigona postica*	combs
NE	*Scaura*	*Scaura latitarsis*	combs
NE	*Tetragona*	*Tetragona clavipes*	combs
NE	*Tetragonisca*	*Tetragonisca angustula*	combs
NE	*Tetragonisca*	*Tetragonisca weyrauchi*	combs
NE	*Trichotrigona*	*Trichotrigona extranea*	clusters
NE	*Trigona*	*Trigona cilipes*	combs
NE	*Trigona*	*Trigona corvina*	combs
NE	*Trigona*	*Trigona fulviventris*	combs
NE	*Trigona*	*Trigona fuscipennis*	combs
NE	*Trigona*	*Trigona hypogea*	combs
NE	*Trigona*	*Trigona nigerrima*	combs
NE	*Trigona*	*Trigona pallens*	combs
NE	*Trigona*	*Trigona silvestriana*	combs
NE	*Trigona*	*Trigona spinipes*	combs
NE	*Trigona*	*Trigona williana*	combs
NE	*Trigonisca*	*Trigonisca buyssoni*	clusters

Tabela S6: Nest site type of stingless bees dataset retrieved from literature (reviewed by Grüter et al., unpubhished).

Clade	Genus	Species	Nest site classification
AT	*Hypotrigona*	*Hypotrigona gribodoi*	cavity
AT	*Meliponula*	*Meliponula bocandei*	cavity
AT	*Plebeina*	*Plebeina hildebrandti*	cavity
IM-AA	*Austroplebeia*	*Austroplebeia symei*	cavity
IM-AA	*Lepidotrigona*	*Lepidotrigona ventralis*	cavity
IM-AA	*Tetragonula*	*Tetragonula carbonaria*	cavity
IM-AA	*Tetragonula*	*Tetragonula hockingsi*	cavity
IM-AA	*Tetragonula*	*Tetragonula laeviceps*	cavity
IM-AA	*Tetragonula*	*Tetragonula mellipes*	cavity
NE	*Cephalotrigona*	*Cephalotrigona capitata*	cavity
NE	*Frieseomelitta*	*Frieseomelitta silvestrii*	cavity
NE	*Frieseomelitta*	*Frieseomelitta varia*	cavity
NE	*Geotrigona*	*Geotrigona mombuca*	ground
NE	*Lestrimelitta*	*Lestrimelitta limao*	cavity
NE	*Melipona (Melikerria)*	*Melipona beecheii*	cavity
NE	*Melipona (Melikerria)*	*Melipona fasciculata*	cavity
NE	*Melipona (Michmelia)*	*Melipona scutellaris*	cavity
NE	*Melipona (Michmelia)*	*Melipona seminigra*	cavity
NE	*Melipona (Eomelipona)*	*Melipona bicolor*	cavity
NE	*Melipona (Eomelipona)*	*Melipona marginata*	cavity
NE	*Nannotrigona*	*Nannotrigona perilampoides*	cavity
NE	*Nannotrigona*	*Nannotrigona testaceicornis*	cavity
NE	*Nogueirapis*	*Nogueirapis mirandula*	ground
NE	*Plebeia*	*Plebeia droryana*	cavity
NE	*Plebeia*	*Plebeia frontalis*	cavity
NE	*Plebeia*	*Plebeia minima*	cavity
NE	*Scaura*	*Scaura latitarsis*	cavity
NE	*Schwarziana*	*Schwarziana quadripunctata*	ground
NE	*Tetragona*	*Tetragona clavipes*	cavity
NE	*Tetragonisca*	*Tetragonisca angustula*	cavity

Clade	Genus	Species	Nest site classification
NE	*Trichotrigona*	*Trichotrigona extranea*	cavity
NE	*Trigona*	*Trigona amazonensis*	exposed
NE	*Trigona*	*Trigona cilipes*	cavity
NE	*Trigona*	*Trigona corvina*	exposed
NE	*Trigona*	*Trigona fulviventris*	cavity
NE	*Trigona*	*Trigona fuscipennis*	cavity
NE	*Trigona*	*Trigona hyalinata*	exposed
NE	*Trigona*	*Trigona hypogea*	ground
NE	*Trigona*	*Trigona nigerrima*	exposed
NE	*Trigona*	*Trigona pallens*	cavity
NE	*Trigona*	*Trigona silvestriana*	exposed
NE	*Trigona*	*Trigona spinipes*	exposed
NE	*Trigona*	*Trigona williana*	cavity

References

BIESMEIJER, J.C. & TÓTH, E. (1998) Individual foraging, activity level and longevity in the stingless bee *Melipona beecheii* in Costa Rica (Hymenoptera, Apidae, Meliponinae). *Insectes Sociaux*, 45, 427–443.

BIJLSMA, L., DE BRUIJN, L.L.M., MARTENS, E.P. & SOMMEIJER, M.J. (2006) Water content of stingless bee honeys (*Apidae, Meliponini*): interspecific variation and comparison with honey of *Apis mellifera* . *Apidologie*, 37, 480–486.

CAMARGO, J.M.F. & PEDRO, S.R.M. (2007) Notas sobre a bionomia de *Trichtrigona extranea* Camargo & Moure (Hymenoptera, Apidae, Meliponini). *Revista Brasileira de Entomologia*, 51, 72–81.

CHINH, T.X., SOOMMEIJER, M.J., BOOT, W.J. & MICHENER, C.D. (2005) Nest and Colony Characteristics of Three Stingless Bee Species in Vietnam with the First Description of the Nest of Lisotrigona carpenteri (Hymenoptera: Apidae: Meliponini). *Journal of the Kansas Entomological Society*, 78, 363–372.

CORTOPASSI-LAURINO, M. & NOGUEIRA-NETO, P. (2003) Notes on the bionomy of The stingless bee Tetragonisca weyrauchi Schwarz, 1943 (Apidae, Meliponini). *Acta Amazonica*. .

DUARTE, R.S., SOUZA, J. & SOARES, A.E.E. (2016) Nest architecture of Tetragona clavipes (Fabricius) (Hymenoptera: Apidae: Meliponini). *Sociobiology*, 63, 813–818.

GIANNINI, K. (1997) Labor Division in Melipona compressipes fasciculata Smith (Hymenoptera: Apidae: Meliponinae). *Anais da Sociedade de Entomologia do Brasil*, 26, 153–162.

GOMES, R.L.C., MENEZES, C. & CONTRERA, F.A.L. (2014) Worker longevity in an Amazonian Melipona (Apidae, Meliponini) species: effects of season and age at foraging onset. *Apidologie*, 46, 133–143.

GROSSO, A.F. & BEGO, L.R. (2002) Labor division, average life span, survival curve, and nest architecture of Tetragonisca angustula angustula (Hymenoptera, Apinae, Meliponini). *Sociobiology*, 40, 615–637.

GRÜTER, C., VON ZUBEN, L.G., SEGERS, F.H.I.D. & CUNNINGHAM, J.P. (2016) Warfare in stingless bees. *Insectes Sociaux*, 63, 223–236.

HAMMOND, R.L. & KELLER, L. (2004) Conflict over male parentage in social insects. *PLoS Biology*, 2.

HOFSTEDE, F.E. & SOMMEIJER, M.J. (2006) Influence of environmental and colony factors on the initial commodity choice of foragers of the stingless bee Plebeia tobagoensis (Hymenoptera, Meliponini). *Insectes Sociaux*, 53, 258–264.

INOUE, T. (1996) Individual variation in worker polyethism of the sumatran stingless bee, Trigona (Tetragonula) minangkabau (Apidae, Meliponinae). *Japanese Journal of Entomology*, 64, 641–668.

INOUE, T., SALMAH, S., ABBAS, I. & YUSUF, E. (1985) Foraging behavior of individual workers and foraging dynamics of colonies of three sumatran stingless bees. *Research in Population Ecology*, 27, 373–392.

JAFFÉ, R., PIOKER-HARA, F.C., DOS SANTOS, C.F., SANTIAGO, L.R., ALVES, D. A, DE M P KLEINERT, A., ET AL. (2014) Monogamy in large bee societies: a stingless paradox. *Die Naturwissenschaften*, 101, 261–264.

KAJOBE, R. (2007) Pollen foraging by Apis mellifera and stingless bees Meliponula bocandei and Meliponula nebulata in Bwindi Impenetrable National Park, Uganda. *African Journal of Ecology*, 45, 265–274.

KWONG, W.K., MEDINA, L.A., SOH, E.J.Y., JAFFÉ, R., ASCHER, J.S., KOCH, H., ET AL. (2017) Dynamic microbiome evolution in social bees. *Science Advances*, 3, e1600513.

MATEUS, S. & ZUCCHI, R. (2008) Biologia comportamental de Trigona pellucida cockerell, 1912 (Hymenoptera, Apidae, Meliponini). *Anais Encontro Sobre Abelhas*.

MICHENER, C.D. (1974) The Social Behavior of the Bees: A comparative study. Harvard University Press, Cambridge, Massachusetts.

MOSES, N., MOGHO, T. & WITTMANN, D. (2013) Tasks partitioning among workers of Meliponula (Meliplebeia) Becarrii (Meliponini) in Cameroon. *International Journal of Environmental Sciences*, 3, 1796–1805.

NAMU, F.N. & WITTMANN, D. (2017) An African stingless bee Plebeina hildebrandti Friese nest size and design (Apidae, Meliponini). *African Journal of Ecology*, 55, 111–114.

NIEH, J.C., CONTRERA, F.A.L. & NOGUEIRA-NETO, P. (2003) Pulsed mass recruitment by a stingless bee, Trigona hyalinata. *Proceedings of the Royal Society B: Biological Sciences*, 270, 2191–2196.

NIEH, J.C., KRUIZINGA, K., BARRETO, L.S., CONTRERA, F.A.L. & IMPERATRIZ-FONSECA, V.L. (2005) Effect of group size on the aggression strategy of an extirpating stingless bee, Trigona spinipes. *Insectes Sociaux*, 52, 147–154.

QUEZADA-EUAN, J.J. & GONZALEZ-ACERETO, J.A. (2002) Notes on the nest habits and host range of cleptobiotic Lestrimelitta niitkib (Ayala 1999) (Hymenoptera: Meliponini) from the Yucatan peninsula, Mexico. *Acta Zoologica Mexicana*, 86, 245–249.

QUEZADA-EUÁN, J.J.G., LÓPEZ-VELASCO, A., PÉREZ-BALAM, J., MOO-VALLE, H., VELAZQUEZ-MADRAZO, A. & PAXTON, R.J. (2011) Body size differs in workers produced across time and is associated with variation in the quantity and composition of larval food in Nannotrigona perilampoides (Hymenoptera, Meliponini). *Insectes Sociaux*, 58, 31–38.

ROUBIK, D.W. (1979) Nest and Colony Characteristics of Stingless Bees from Panamá (Hymenoptera: Apidae). *Journal of the Kansas Entomological Society*, 52, 443–470.

ROUBIK, D.W. (1982) Seasonality in colony food storage, brood production and adult survivorship: Studies of Melipona in tropical forest (Hymenoptera: Apidae). *Journal of the Kansas Entomological Society*, 55, 789–800.

ROUBIK, D.W. (1983) Nest and Colony Characteristics of Stingless Bees from Panamá (Hymenoptera : Apidae). *Journal of the Kansas Entomological Society*, 56, 327–355.

SAKAGAMI, S.F., CAMILO, C. & ZUCCHI, R. (1973) Oviposition Behavior of a Brazilian Stingless Bee , Plebeia (Friesella) schrottkyi , with Some Remarks on the Behavioral Evolution in Stingless Bees. *Jour. Fac. Sci. Hollaido Univ. Ser.*, 19, 163–189.

SAKAGAMI, S.F., INOUE, T., YAMANE, S. & SALMAH, S. (1983) Nest architecture and colony composition of the Sumatran stingless bee Trigona (Tetragonula) laeviceps. *Kontyu Tokyo*, 51, 100–111.

SAKAGAMI, S.F., ZUCCHI, R. & ARAÚJO, V. DE P. (1977) Oviposition behavior of an aberrant african stingless bee Meliponula bocandei, with notes on themechanims and evolution of oviposition behavior in stingless bees. *Journal of the Faculty of Science, Hokkaido University Serie VI Zoology*, 20, 647–690.

SCHWARZ, H.F. (1948) Stingless bees of the western hemisphere. *Bulletin of the American Museum of Natural History*, 90, 1–546.

SIMÕES, D. & BEGO, L. (1991) Division of labor, average life span and life table in Nannotrigona (Scaptotrigona) postica Latreille (Hymenoptera, Apidae, Meliponinae). *Naturalia*, 16, 81–97.

SOMMEIJER, M.J. (1984) Distribution of labor among workers of Melipona favosa F.: age-polyethism and worker oviposition. *Insectes Sociaux*, 31, 171–184.

TERADA, Y., GAROFALO, C.A. & SAKAGAMI, S.F. (1975) Age-Survival Curves for Workers of Two Eusocial Bees (Apis Mellifera and Plebeia Droryana) in a Subtropical Climate, with Notes on Worker Polyethism in P. Droryana. *Journal of Apicultural Research*, 14, 161–170.

TÓTH, E., QUELLER, D.C., DOLLIN, A. & STRASSMANN, J.E. (2004) Conflict over male parentage in stingless bees. *Insectes Sociaux*, 51, 1–11.

VAN BENTHEM, F. D. J., IMPERATRIZ-FONSECA, V.L. & VELTHUIS, H.H.W. (1995) Biology of the stingless bee *Plebeia remota* (Holmberg): observations and evolutionary implications. *Insectes Sociaux*, 42, 71–87.

VAN VEEN, J.W., ARCE, H.G.A. & SOMMEIJER, M.J. (2004) Production of queens and drones in Melipona beecheii (Meliponini) in relation to colony development and resource availability. *Proceedings of the Netherlands Entomological Society Meeting*, 15, 35–39.

VIT, P., PEDRO, S.R.M. & ROUBIK, D.W. (2013) Pot-Honey: A legacy of stingless bees. Springer Science & Business Media, New York, NY.

WILLE, A. (1966) Notes on two Species of Ground Nesting Stingless bees (Trigona mirandula and Trigona buchwaldi) from the Pacific Rain Forest of Costa Rica. *Revista de Biologia Tropical*, 14, 251–277.

WILLE, A. & MICHENER, C.D. (1973) The nest architecture of stingless bees with special reference to those of Costa Rica (Hymenoptera, Apidae). *Revista de Biologia Tropical*, 21, 278.

WITTER, S., BLOCHTEIN, B., ANDRADE, F., WOLFF, L.F. & IMPERATRIZ-FONSECA, V.L. (2007) Meliponiculture in Rio Grande do Sul: contribution to the biology and conservation of Plebeia nigriceps (Friese, 1901) (Apidae, Meliponini). *Bioscience Journal*, 23, 134–140.

ZUCCHI, R., SAKAGAMI, S.F., NOLL, F.B., MECHI, M.R., MATEUS, S., BAIO, M. V & SHIMA, S.N. (1995) Agelaia vicina, a swarm-founding polistine with the largest colony size among wasps and bees (Hymenoptera: Vespidae). *Journal of the New York Entomological Society*, 103, 129–137.

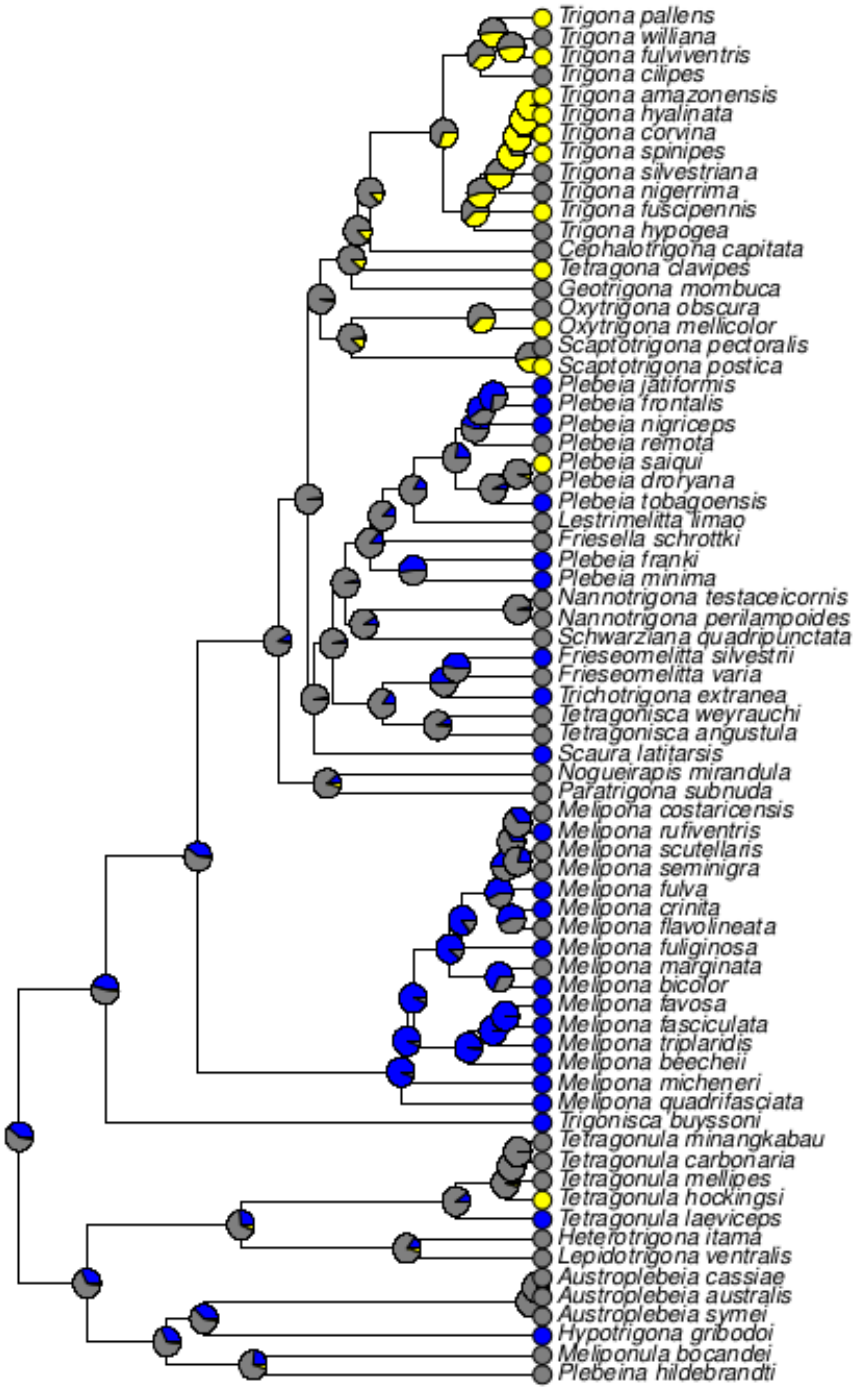


Figure S1: Ancestral State Reconstructions our second approach, under a derivate-state Markov Chain Model (ancThresh function). Medium colony size is estimated as the ancestral state with a probability of 65.5% (0.655 ± 0.077, probability ± 95% confidence interval).

SEÇÃO III

Abelhas sem ferrão e paisagem:
como as populações respondem à perda de hábitat e mudanças climáticas?

Landscape genomics to the rescue of a tropical bee threatened by habitat loss and climate change

Rodolfo Jaffé* & Jamille Veiga*, Nathaniel Pope, Éder Lanes, Carolina Carvalho, Ronnie Alves, Sónia Andrade, Maria Arias, Vanessa Bonatti, Airton Carvalho, Marina de Castro, Felipe Contrera, Tiago Francoy, Breno Freitas, Tereza Giannini, Michael Hrncir, Celso Martins, Guilherme Oliveira, Antonio Saraiva, Bruno Souza, Vera Imperatriz-Fonseca

Pesquisa realizada em colaboração com grupo de pesquisa em Genética da Paisagem do Instituto Tecnológico Vale.

Publicado em **Evolutionary Applications** (DOI: 10.1111/eva.12794)

*Estes autores contribuíram igualmente para o artigo.

Genômica da paisagem para o resgate de uma abelha tropical ameaçada por perda de hábitat e mudanças climáticas

Resumo

A degradação de hábitat e as mudança climáticas ameaçam os polinizadores nativos, comprometendo sua capacidade de fornecer serviços de polinização para plantas silvestres e cultivadas. A genômica da paisagem oferece ferramentas poderosas para avaliar a influência das modificações da paisagem sobre a diversidade genética e conectividade funcional das populações, e identificar adaptações às condições ambientais locais que poderiam facilitar a sobrevivência futura das abelhas. Nesse estudo, avaliamos padrões gerais de estrutura e diversidade genética, fluxo gênico e adaptação local na abelha sem ferrão *Melipona subnitida*, um polinizador tropical de importância ecológica e econômica, que habita uma das regiões mais quentes e secas da América do Sul. Nossos resultados revelam quatro grupos genéticos em toda a distribuição da espécie. Todas as populações apresentaram-se sob equilíbrio de mutação-deriva e a diversidade genética não foi influenciada pela quantidade de hábitats naturais remanescentes. No entanto, a relação genética apresentou padrão de autocorrelação espacial, e o isolamento pela resistência da paisagem explicou melhor os padrões de parentesco entre os indivíduos em comparação ao isolamento pela distância geográfica, contradizendo resultados anteriores para as abelhas sem ferrão. Especificamente, o fluxo gênico foi reforçado pelo aumento da estabilidade térmica, maior cobertura florestal, menores elevações e terrenos de topografia menos irregulares. Por fim, detectamos assinaturas genômicas de adaptação à temperatura, precipitação e cobertura florestal, espacialmente distribuídas em padrões latitudinais e altitudinais. Em conjunto, nossas descobertas trazem informações sobre a história natural de *M. subnitida* e destacam o papel de regiões com grandes flutuações térmicas, áreas desmatadas e cadeias de montanhas como barreiras de dispersão. Ações de conservação, como restringir o transporte de colônias de longa distância, preservar as adaptações locais e melhorar a conectividade entre áreas altas e baixas (áreas de chapada e de sertão, respectivamente), podem garantir serviços futuros de polinização.

Landscape genomics to the rescue of a tropical bee threatened by habitat loss and climate change

Abstract

Habitat degradation and climate change are currently threatening wild pollinators, compromising their ability to provide pollination services to wild and cultivated plants. Landscape genomics offers powerful tools to assess the influence of landscape modifications on genetic diversity and functional connectivity, and identify adaptations to local environmental conditions that could facilitate future bee survival. Here we assessed range-wide patterns of genetic structure, genetic diversity, gene flow and local adaptation in the stingless bee *Melipona subnitida*, a tropical pollinator of key biological and economic importance inhabiting one of the driest and hottest regions of South America. Our results reveal four genetic clusters across the species' full distribution range. All populations were found to be under a mutation-drift equilibrium and genetic diversity was not influenced by the amount of reminiscent natural habitats. However, genetic relatedness was spatially autocorrelated and isolation by landscape resistance explained range-wide relatedness patterns better than isolation by geographic distance, contradicting earlier findings for stingless bees. Specifically, gene flow was enhanced by increased thermal stability, higher forest cover, lower elevations, and less corrugated terrains. Finally, we detected genomic signatures of adaptation to temperature, precipitation and forest cover, spatially distributed in latitudinal and altitudinal patterns. Taken together, our findings shed important light on the life history of *M. subnitida*, and highlight the role of regions with large thermal fluctuations, deforested areas and mountain ranges as dispersal barriers. Conservation actions such as restricting long-distance colony transportation, preserving local adaptations, and improving the connectivity between highlands and lowlands, are likely to assure future pollination services.

1. Introduction

Although bees are now widely acknowledged as key pollinators of wild and cultivated plants, as well as important income sources for beekeepers around the globe (Potts et al., 2016), the joint impact of habitat degradation and climate change is currently threatening their wild populations (Brown & Paxton, 2009; Hadley & Betts, 2011; S. G. . Potts et al., 2010; Viana et al., 2012; Wratten, Gillespie, Decourtye, Mader, & Desneux, 2012). Habitat loss has been related to reductions in native bee abundance and richness (Kennedy et al., 2013), whereas land use changes have fragmented populations in some species (Jha, 2015; Jha & Kremen, 2013). Additionally, climate change is expected to modify the availability of floral and nesting resources and affect bee physiology, thereby resulting in distribution range shifts and reductions in many species (Faleiro, Nemésio, & Loyola, 2018; Giannini et al., 2017; Kerr et al., 2015; Le Conte & Navajas, 2008; Pyke, Thomson, Inouye, & Miller, 2016; Willmer, 2014).

Landscape genomics offers powerful tools to assess the influence of habitat loss on genetic diversity and functional connectivity, and to identify adaptations to local environmental conditions that could facilitate future bee survival (N. Balkenhol et al., 2017; J. Lozier & Zayed, 2017). For instance, landscape resistance to gene flow has been assessed in both temperate and tropical species (Davis, Murray, Fitzpatrick, Brown, & Paxton, 2010; Jackson et al., 2018; Jaffé, Castilla, et al., 2016), and genomic signatures of adaptations to environmental conditions have been identified in the honeybee *Apis mellifera* (Chávez-Galarza et al., 2013; Henriques et al., 2018) and the bumblebee *Bombus lapidarius* (Theodorou et al., 2018). Nonetheless, while most studies assessing landscape effects on gene flow have employed microsatellite markers (Niko Balkenhol, Cushman, Waits, & Storfer, 2016; Monteiro et al., 2019), none has thus far employed genomic data to assess both isolation by landscape resistance and local adaptation in bees (Storfer, Patton, & Fraik, 2018).

Dispersal is believed to be particularly restricted in stingless bees (Apidae: Meliponini), because daughter colonies rely on resources from their maternal colonies during their initial establishment, and consequently do not establish far from each other (Roubik, 2006; Van Veen & Sommeijer, 2000; P. Vit, Pedro, & Roubik, 2013). As restricted dispersal implies a diminished ability to move to high-quality habitats and maintain gene flow across fragmented landscapes, habitat loss and fragmentation are expected to reduce and isolate stingless bee populations, making them extremely susceptible to genetic erosion through the action of genetic drift (F. Allendorf, Luikart, & Aitken, 2013; J. Lozier & Zayed, 2017). However, previous studies using microsatellite markers were not able to detect an effect of forest or land cover on stingless bee gene flow, suggesting that these bees have a remarkable ability to maintain high gene flow across heterogeneous and human-altered

landscapes (Jaffé, Castilla, et al., 2016; Jaffé, Pope, et al., 2016; Landaverde-González et al., 2017). Since estimates of genetic diversity and gene flow are strongly influenced by the type and number of genetic markers employed (F. W. Allendorf, 2017; Leroy et al., 2018; J. D. Lozier, 2014), genomic studies employing thousands of single nucleotide polymorphisms (SNPs) are needed to confirm if stingless bees are really resilient to habitat loss and fragmentation, or whether the lack of significant isolation by resistance effects in previous studies is due to the resolution of the genetic markers employed (Alvarado-Serrano, Van Etten, Chang, & Baucom, 2019; McCartney-Melstad, Vu, & Shaffer, 2018).

Here we employ novel landscape genomic tools to assess the joint influence of habitat degradation and climate change on an economically important tropical stingless bee inhabiting one of the most deforested, driest and hottest regions of the Americas. Distributed across Northeastern Brazil, *Melipona subnitida* Ducke, 1911 (known as the Jandaíra bee) is a key pollinator of native plants and local crops, and one of the most widely used stingless bee species for honey production (Imperatriz-Fonseca, Koedam, & Hrncir, 2017), contributing to the household income of many rural families (Giannini et al., 2017; Jaffé et al., 2015). The species natural range spans four different biomes (Giannini et al., 2017; Imperatriz-Fonseca et al., 2017), namely Tropical Dry Forest (Caatinga), Savanna (Cerrado), the Atlantic Rain Forest, and Mangrove Forests, thus encompassing important climatic and altitudinal gradients, which are expected to drive local adaptations (Koffler et al., 2015; Maia-Silva, Hrncir, Silva, & Imperatriz-Fonseca, 2015). Species distribution models suggest the species could respond to climate change by seeking refuge in higher elevations, where both the bees and their plant resources are more likely to find suitable climatic conditions in the future (Giannini et al., 2017). However, such response depends on the bee's ability to relocate to high-quality habitats, which could be hindered by the region's increasing human-led desertification (Marengo, Torres, & Alves, 2017; Vieira et al., 2015). Given *M. subnitida's* key biological and economic importance, efforts are urgently needed to safeguard this key pollinator by facilitating its migration towards higher lands. Such conservation actions will nevertheless require the prior identification of barriers to gene flow and the spatial distribution of adaptive genetic variation.

Relying on thousands of SNPs, we explicitly tested if genetic diversity and gene flow in *M. subnitida* are affected by the amount of reminiscent natural habitats, and identified genomic signatures of adaptations to local environmental conditions. Considering the life history characteristics of our study species and the high statistical power granted by the large number of genetic markers employed, we formulated the following predictions: i) Since natural habitats harbor floral resources and nesting sites (Vit et al., 2013) we expected to find a positive association between genetic diversity and the amount of reminiscent natural habitats surrounding sampling sites

(DiLeo & Wagner, 2016; Fahrig, 2013), as found in bumble bees (Jackson et al., 2018); ii) As restricted dispersal implies reduced gene flow across deforested areas, we expected to find significant isolation by landscape resistance (McRae, 2006), and predicted that gene flow would be influenced by habitat amount as well as environmental correlates of genetic connectivity in other bee species, including elevation, terrain roughness, temperature and precipitation (El-Niweiri & Moritz, 2011; Jackson et al., 2018; Jaffé, Pope, et al., 2016; Jha, 2015); iii) Based on the documented tolerance of our study species to extreme heat and water scarcity (Maia-Silva et al., 2015), and previous candidate genes found associated with precipitation and latitude in honeybees (Chávez-Galarza et al., 2013; Henriques et al., 2018) and urban land cover in bumblebees (Theodorou et al., 2018), we expected to find genomic signatures of adaptation related to temperature, precipitation and forest cover. Ours constitutes the first genomic study assessing both isolation by landscape resistance and local adaptation across the full distribution range of a bee pollinator.

2. Material and methods

Sampling and DNA extraction

We collected samples of *M. subnitida* across its entire distribution range (Pedro, 2014), aiming to maximize temperature, precipitation, elevation and forest cover gradients (Figure 1). Samples were collected between 2013 and 2014 (SISBIO collection permits 38000-1 and 10393-1). We sampled one bee per colony from a total of 160 nests of beekeepers who could certify their local origin. We only collected samples from local colonies, and not from colonies of unknown origin or from nests brought from different locations. We also registered any information on previous introductions of bee colonies that beekeepers could provide, as long-distance colony transportation is common and has been shown to have profound effects on stingless bee gene flow (Jaffé, Pope, et al., 2016). In cases when beekeepers had a few weak colonies we did not collect any samples, or collected from a single colony. When beekeepers had many strong colonies we collected samples from more than one colony. Distance separating sampling locations therefore ranged between 0 Km (samples from the same beekeeper) and 947Km. Only freshly emerged (callow) workers were collected from the nest's interior, to minimize the sampling of drifters. All individuals were stored in absolute ethanol, and then frozen at -20ºC until DNA extractions. The geographic location of all samples was recorded by GPS (see Table S1 in Supporting Information).

Total genomic DNA was extracted from the whole insects (discarding only the heads) using Quiagen's DNeasy Blood and Tissue Kit, according to the manufacturer's protocols. DNA integrity was then verified on 1% agarose gels, and its concentration was quantified with a Qubit 2.0

Fluorometer (Invitrogen). Only samples containing non-degraded DNA, concentrations of at least 20 ng/µl and a total DNA amount greater than 2µg were used for subsequent RAD sequencing.

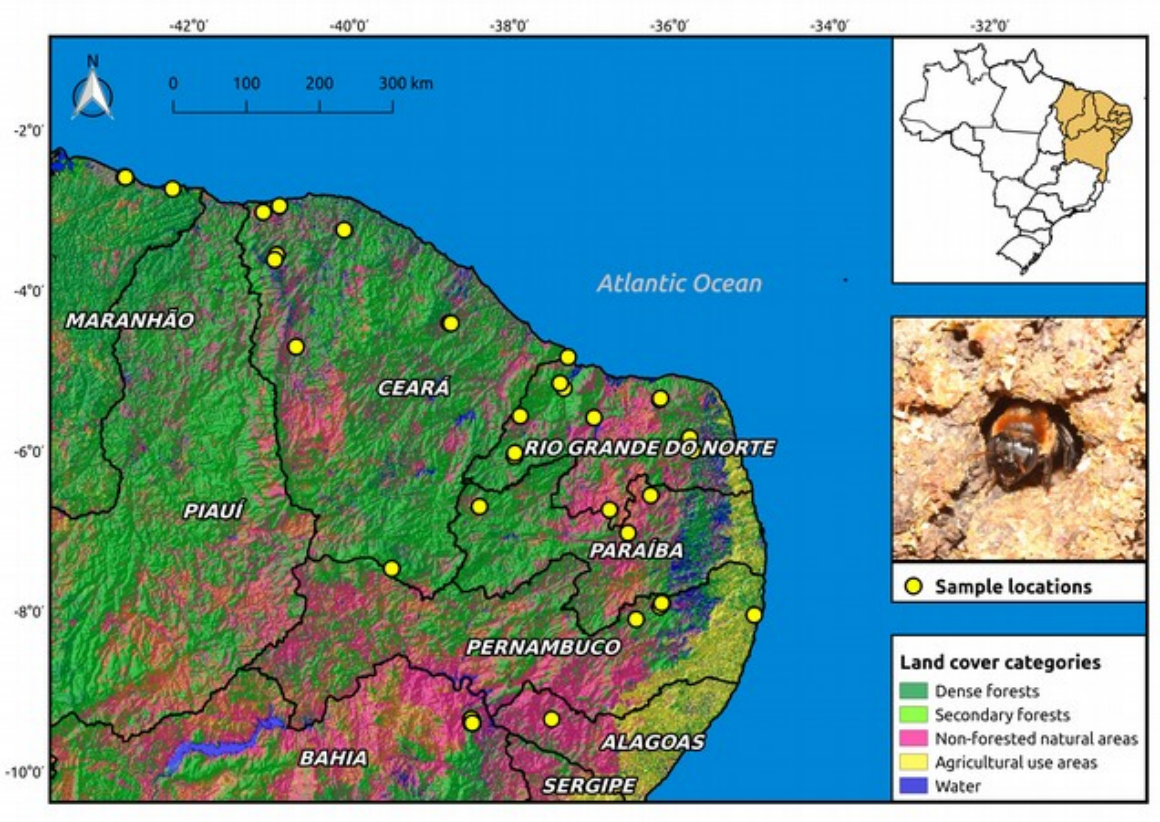


Figure 1: *Melipona subnitida* sampling locations across Northeastern Brazil over a land cover map (source: http://mapbiomas.org/).

RAD sequencing and SNP discovery

DNA samples were then shipped to Floragenex, Inc. (Eugene, OR, USA) for RAD library preparation, Illumina sequencing, and bioinformatic processing. Briefly, libraries were prepared based on the genome size of this species (Tavares, Carvalho, Aparecida, & Soares, 2010), digesting genomic DNA with the *SgrAI* restriction enzyme. The resulting fragments were tagged with individual barcodes, which were then multiplexed and sequenced using 100-bp single-end methodology on the HiSeq 2000 platform (Illumina). Libraries were created at the same time and ran on the same sequencing run on two different lanes. Total number of generated reads per individual ranged from ~550000 to 2.3 million. Samples were then demultiplexed and barcode sequences trimmed to result in final fragment lengths of 92 bp. Quality filtering was done in SAMtools during the genotyping stages, and coverage was limited to loci having less than 500x to

limit the incidence of possible contaminants. Stacks was then used to cluster loci from a single individual and generate a RAD reference, allowing two haplotypes from each locus. The *de novo* clustering of sequences into RAD tags was performed using VELVET (version 1.2.10), considering a minimum cluster depth of 5 and maximum of 1500, a maximum number of two haplotypes per cluster and a maximum of three variants per cluster. SNP calling was performed using SAMTOOLS (version 0.1.16), and loci harboring SNPs were included in the final genotype (VCF) table if they had at least 6x individual sequence coverage over at least 75% of the population, had individual per locus genotype quality scores of at least 10, a minimum FASTQ quality score of 20, and a minimum distance to other SNPs of 50bp (average individual Phred-score was 60.8, while average individual sequencing coverage was 36.3 x). A variant was cataloged when it was present in a single sample (only 3% of loci had minor allele frequencies – MAF- below 0.5, 2% of loci had MAF below 0.3 and 1% of loci had MAF below 0.1).

Population structure and genetic diversity

The R package *r2vcftools* (https://github.com/nspope/r2vcftools), a wrapper for VCFtools (Danecek et al., 2011), was used to perform final filtering and quality control on the genotype data. To assess genetic diversity and population structure across the distribution range of our study species, we first filtered loci for quality (Phred score 30 - 80), read depth (20 - 50), linkage disequilibrium (LD, $r2 < 0.4$), and strong deviations from the Hardy Weinberg Equilibrium (HWE, $p < 0.0001$). Additionally, we removed any potential loci under selection detected through genome scans. *Fst* Outlier tests were applied after adjusting *p*-values using the genomic inflation factor (λ), and setting false discovery rates to q=0.05, using the Benjamini-Hochberg algorithm (Benjamini & Hochberg, 1995; François, Martins, Caye, & Schoville, 2016).

Two complementary genetic clustering software were employed to assess population structure using the resulting set of neutral and independent loci: The *snmf* function of the LEA (v2.0) package (Frichot & François, 2015; Frichot, Mathieu, Trouillon, Bouchard, & François, 2014) and Admixture (Alexander, Novembre, & Lange, 2009). The number ancestral populations (k) was allowed to vary between 1 and 10, with 10 replicate runs for each k-value, and the best k was chosen based on cross-entropy and cross-validation errors (Frichot et al., 2014). Individuals were then assigned to genetic clusters based on the ancestry coefficients retrieved from LEA (Q-matrix), identifying the cluster with the highest ancestry.

We then calculated genetic diversity metrics for each one of the identified genetic clusters. These included observed heterozygosity (*Ho*), expected heterozygosity (*H*E), nucleotide diversity (π) and inbreeding coefficient (*F*, see VCFTools manual for details). Effective population size (*N*e)

was also estimated employing the linkage disequilibrium method implemented in NeEstimator 2.0.1 (Do et al., 2014), using a threshold lowest allele frequency value of 0.05 and assuming a monogamy model (Jaffé et al., 2014). Additionally, we calculated Tajima's *D*, representing the difference between the mean number of pairwise differences and the number of segregating sites. In a population of constant size evolving under mutation-drift equilibrium Tajima's *D* is expected to be zero; negative values result from an excess of rare alleles and thus indicate a recent selective sweep or a population expansion after a recent bottleneck; and positive values appear when rare alleles are lacking, therefore suggesting balancing selection or a sudden population contraction (Tajima, 1989). We used *r2vcftools* to compute a genome-wide estimate of Tajima's D and perform a simulation from the neutral model to correct for bias due to a minor-allele-frequency filter.

Landscape genetic analyses

Aiming to assess the influence of habitat amount on genetic diversity, we reclassified a high-resolution land cover/land use map for 2013 (http://mapbiomas.org/) into habitat (*i.e.* all types of natural forest and non-forest formations) and non-habitat (farming, non-vegetated areas and water bodies). The percentage of habitat cover in 2 km radius buffers surrounding our sampling locations was then calculated, as such radius comprises the estimated foraging distance for this species (Silva & Ramalho, 2016). Because more than one colony was sampled in some some locations (Table S1), we computed mean percentage of habitat cover and mean genetic diversity (*Ho*, H_E and *F*) for each location (N = 56 locations). We then used the *nlme* package (Pinheiro, Bates, DebRoy, Sarkar, & Team, 2018) to fit generalized least squares models (gls) containing genetic diversity metrics as response variables, percentage of habitat cover as predictor, and different correlation structures (no autocorrelation, linear, exponential, Gaussian, spherical, and rational quadratics) to account for spatial autocorrelation. Logit-transformations were used to normalize/linearize heterozygosities. The sample-size corrected Akaike Information Criterion (AICc) was then used to compare models with different correlation structures, fitted with restricted maximum-likelihood. The best models (ΔAICc $\leq$ 2) were finally selected, fitted once more using maximum-likelihood, and compared to reduced models without predictor variables using likelihood ratio tests (LRT, $\alpha = 0.05$). All models were validated by plotting residual vs. fitted values and by checking for residual autocorrelation.

Prior to assessing isolation by landscape resistance (IBR) we evaluated fine-scale spatial genetic structure by quantifying spatial autocorrelation in genetic relatedness. To do so we used local polynomial fitting (LOESS) of pairwise relatedness to pairwise geographic distance (https://github.com/rojaff/Lplot; Bruno et al. 2008). Yang's Relatedness between pairs of individuals (Yang et al., 2010) was used, since similar measures of relatedness have been found to be highly

accurate as individual-based genetic distance metrics for landscape genetic studies (Shirk, Landguth, & Cushman, 2017). We then evaluated the contribution of habitat amount, elevation, terrain roughness, temperature and precipitation in explaining patterns of gene flow (Niko Balkenhol et al., 2016). We created a first resistance surface using the reclassified land cover/land use map for 2013 described above, attributing low resistance (0.1) to habitat pixels and high resistance (0.9) to non-habitat pixels. This allowed us to test if gene flow is enhanced by natural habitats or hindered by habitat-deployed environments (Jaffé, Castilla, et al., 2016). A second resistance surface was then created using an inverted forest cover map from the University of Maryland (http://earthenginepartners.appspot.com/science-2013-global-forest/download.html), to test for higher gene flow across forested areas. To test for a reduced gene flow across highlands and corrugated terrains we created elevation and terrain roughness surfaces, using raw elevation and terrain roughness maps. While elevation was retrieved from WorldClim (http://www.worldclim.org/), terrain roughness was created from this elevation layer using the Terrain Analysis plug-in in QGIS V2.14. To evaluate resistance due to environmental conditions we created three additional resistance surfaces containing the raw values from those WorldClim bioclimatic variables explaining most variation across our study region (mean temperature of coldest quarter, temperature annual range and precipitation of driest quarter, see details below). We thereby tested for reduced gene flow across areas with higher temperatures, higher temperature range and higher precipitation. To assess isolation by geographic distance (IBD) we created a last resistance surface replacing all pixel values in our elevation map with 0.5. Using the program Circuitscape V4.0 (McRae, 2006) we then calculated pairwise resistance distances between all samples, employing all the resistance surfaces described above. Due to Circuitscape's computing limitations, all rasters were cropped to the extent of sample locations plus a buffer area of one decimal degree to minimize border effects (Jaffé, Castilla, et al., 2016), and all pixels containing zero values were replaced with 0.001.

To assess IBR, we fit mixed-effects regression models using penalized least squares and a novel correlation structure designed to account for the non-independence of pairwise distances across individuals and spatial locations (based on the maximum-likelihood population effects or MLPE model: https://github.com/nspope/corMLPE; Clarke et al. 2002). Because more than one colony was sampled in some some locations (Table S1), there exists a spatial dependence structure in pairwise comparisons between individuals that is not captured by the MLPE model. This is because the MLPE correlation structure only models dependence across pairwise comparisons that overlap in the individuals being compared, with no reference to spatial location. The unmodeled spatial dependence has the net effect of making inference anti-conservative, and can be diagnosed by

calculating serial autocorrelation in the normalized residuals of the MLPE model, after sorting the data by spatial locations. To combat this, we introduce a novel modification of the MLPE model that incorporates correlation between pairwise measurements due to comparison of both individuals and spatial locations (Nested MLPE or NMLPE). Briefly, the original MLPE model has a random-effects representation where the expected value for each pairwise observation includes a pair of iid random effects (one for each of the individuals being compared in the observation); whereas our extension (NMLPE) additionally incorporates iid random effects for pairs of spatial locations.

Yang's Relatedness between pairs of individuals was used as response variable and the different resistance distances (geographic distance, forest cover, elevation, roughness, temperature annual range, mean temperature of coldest quarter, and precipitation of driest quarter) as predictors in our MLPE models. We used the Akaike Information Criterion (AIC) to compare models containing all possible combinations of non-collinear predictors ($r < 0.6$), created with the *dredge* function from the MuMIn (v1.4) package (https://github.com/rojaff/dredge_mc; Barton 2018). Likelihood ratio tests were then performed to assess the influence of the inclusion of each predictor variable on the best-fitting model's log-likelihood. Finally, we re-fitted the best models using NMLPE models to obtain parameter estimates unbiased by spatial dependence. To evaluate the impact of excluding samples from our IBR analyses, we also ran a sensitivity analysis, generating one hundred data subsets (randomly excluding different numbers of samples) and performing one hundred independent model selection protocols for each subset (using MLPE models). We report the number of times predictor variables were included in the set of best-fitting MLPE regression models ($\Delta AIC \leq 2$), after randomly excluding different numbers of samples (Figure S5).

Identification of putative adaptive loci

To identify genomic signatures of adaptations to local environmental conditions, we employed environmental association tests. To this end we used our original dataset filtered by quality and depth only (as described above), but not for LD or HWE. We first ran a principal component analysis using all 19 WorldClim bioclimatic variables plus altitude and forest cover (all scaled), to select a set of orthogonal variables explaining most environmental variation across our study area. Since the first four principal components accounted for 92.08% of total variance, we selected the four variables that were most strongly correlated with these axes. The selected variables (mean temperature of coldest quarter, forest cover, temperature annual range and precipitation of driest quarter) were then used to run latent factor mixed models (LFMM), aiming to identify possible associations between SNPs and environmental variables, while accounting for the underlying population structure (De Kort et al., 2014; Frichot, Schoville, Bouchard, & François,

2013; Rellstab, Gugerli, Eckert, Hancock, & Holderegger, 2015). LFMMs have been used extensively and are currently one of the most commonly used Environmental Association Analysis approaches (Ahrens et al., 2018), given they provide a good compromise between detection power and error rates, and are robust to a variety of sampling designs and underlying demographic models (Rellstab et al., 2015). LFMM were implemented in R through the LEA package, using 1,000 iterations, a burn-in of 5,000 and five runs per environmental variable (Frichot & François, 2015). The *p*-values were adjusted using the genomic inflation factor ($\lambda = 1$) and false discovery rates were set using the Benjamini-Hochberg algorithm at a rate of $q = 0.05$ (Benjamini & Hochberg, 1995). Since incorrect assumptions about underlying demographic structure can increase both Type I and Type II errors (Cushman & Landguth, 2010; Storfer et al., 2018), we ran LFMM using $k \pm 1$ latent factors (where k was the optimum number of ancestral populations detected), and only considered as candidate loci those shared between all runs for each environmental variable. Full R scripts of LFMM can be found in the LEA website (http://membres-timc.imag.fr/Olivier.Francois/LEA/index.htm). In order to map adaptive genetic variability we used the *adegenet* package (Jombart, 2008) to run a spatial principal component analysis (sPCA) on all identified candidate SNPs, and interpolated the first two principal components on a grid covering our study area.

3. Results

Genetic diversity and population structure

We identified 29,349 SNPs from which 3,454 loci remained after filtering for quality, depth, HWE, LD and F_{ST} outlier loci. We detected four genetic clusters (K = 4) using two different clustering approaches (Figure 2, Figure S1), and excluded four samples from subsequent analyses (final sample size was 156 individuals) because they were likely introduced bees from a distant location (Figure S2, Table S1).

Cluster assignments were generally unambiguous as ancestry coefficients for the assigned clusters were usually above 0.50 (lower 25% quantile = 0.50, median = 0.60, upper 75% quantile = 0.86). Clusters 1 and 4, located at the northern and southern extremes of the species distribution range, showed the lowest *N*e and π. All genetic clusters showed small but significant inbreeding and values of Tajima's D overlapping zero (Table 1).

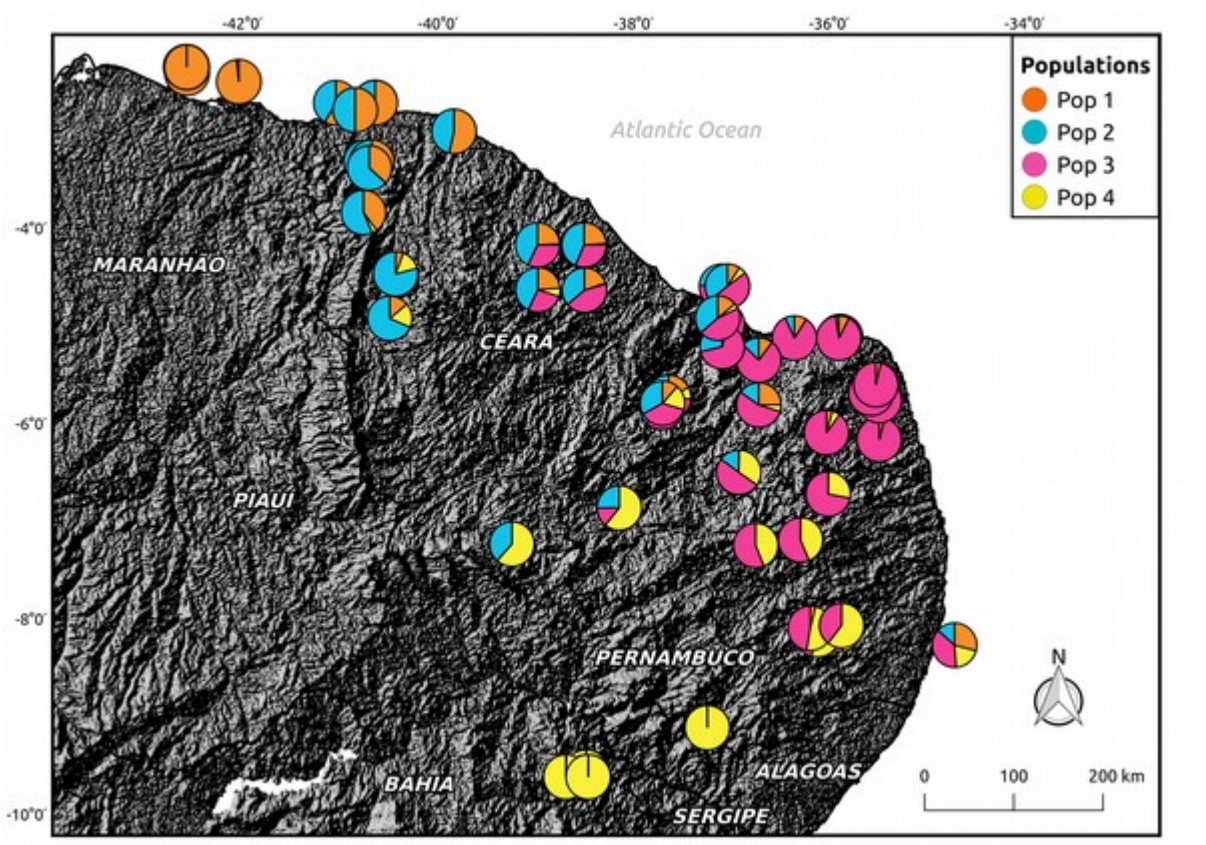


Figure 2: Map showing *Melipona subnitida* assignments to four genetic clusters against an elevation map (from USGS Earth Explorer). Pie charts represent ancestry coefficients determined using the LEA package.

Table 1: Genetic diversity estimates for *Melipona subnitida* by genetic cluster. Sample sizes (N) are shown followed effective population size (N_e), observed heterozygosity (H_o), expected heterozygosity (H_E), inbreeding coefficient (F), nucleotide diversity (π) and Tajima's D. Lower and upper 95% confidence intervals are shown for each estimate.

Genetic cluster	***N***	***N_e***	***H_O***	***H_E***	***F***	***π***	***Tajima's D***
Pop1	19	62.3 / 64.2	0.21 / 0.25	0.25 / 0.25	0.01 / 0.15	0.17 / 0.19	-0.09 / 0.36
Pop2	32	211.9 / 221.0	0.22 / 0.22	0.23 / 0.23	0.03 / 0.05	0.21 / 0.22	-0.09 / 0.44
Pop3	66	285.1 / 292.3	0.20 / 0.21	0.22 / 0.22	0.05 / 0.09	0.21 / 0.22	-0.06 / 0.54
Pop4	39	107.5 / 109.7	0.19 / 0.20	0.22 / 0.22	0.08 / 0.15	0.19 / 0.21	-0.04 / 0.54

Landscape genetic analyses

Habitat amount was not found associated with heterozygosity or inbreeding (Table 2). We found positive spatial autocorrelation in pairwise genetic relatedness for up to 300 Km, after which spatial autocorrelation became negative (Figure 3). However, IBR was better able to explain genetic relatedness patterns than IBD (Table 3). Temperature annual range (defined as the difference between maximum temperature of warmest month and minimum temperature of coldest month), was the best predictor of relatedness patterns across the full distribution range of *M. subnitida*. The second best IBR model was nearly 18 AIC units apart from the model containing temperature annual range (Table 3), suggesting that forest cover, altitude and terrain roughness combined, did not explain relatedness patterns as well as temperature fluctuations alone.

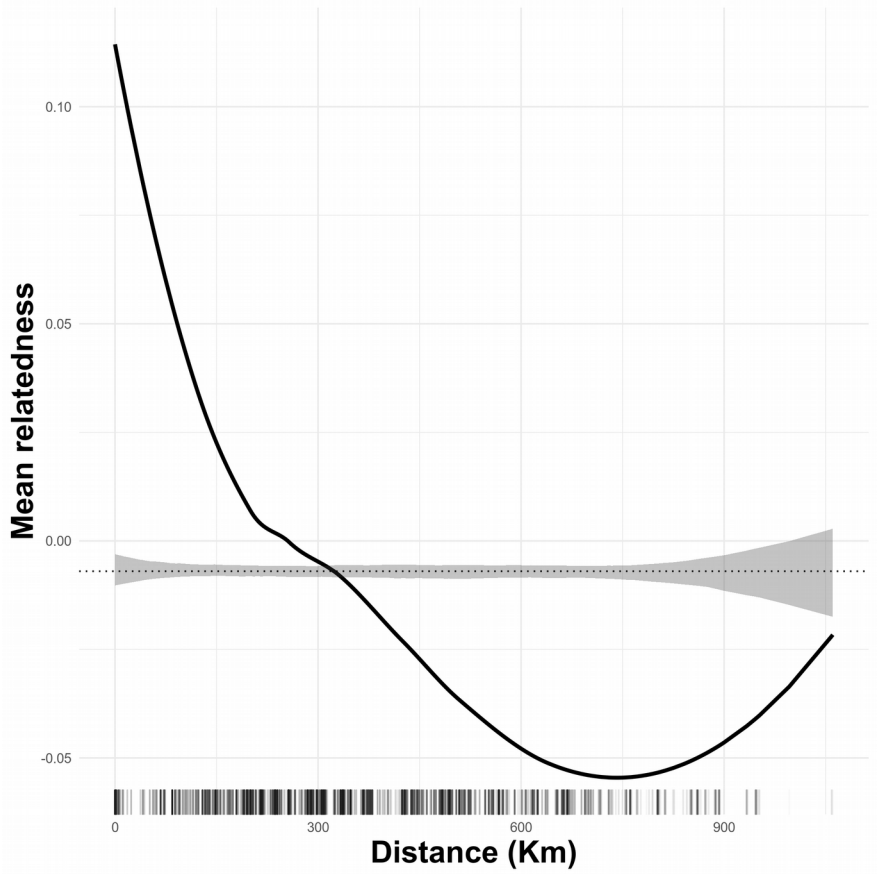


Figure 3: Spatial autocorrelation in genetic relatedness. The black solid line is the LOESS fit to the observed genetic relatedness, while the gray shaded regions are 95% confidence bounds around the null expectation (black dotted line). Short vertical lines at the bottom of the figure are observed pairwise distances.

Table 2: Effect of habitat amount on observed heterozygosity (H_O), expected heterozygosity (H_E) and inbreeding (F). Generalized least squares models contained genetic diversity metrics as response variables, percentage of habitat cover as predictor, and different correlation structures to account for spatial autocorrelation. Logit-transformations were used to normalize/linearize heterozygosities. The table shows X^2 and p-values values from likelihood ratio tests applied on best-fitting models.

Response variable	***Correlation strucutre***	***X^2***	***p-value***
H_O	Exponential	0.24	0.63
H_E	None	0.22	0.64
F	Exponential	0.25	0.62

Table 3: Summary statistics for the top MLPE regression models. All models contained inter-individual genetic relatedness as response variable and the different landscape resistance distances as predictors. Log-likelihoods are followed by the Akaike Information Criterium (AIC), ΔAIC, model weight and the MLPE correlation coefficient rho (***ρ).*** Isolation by geographic distance was included here for comparison.

Predictors	**logLik**	**AIC**	**ΔAIC**	**Weight**	***ρ***
Temperature Annual Range***	22813.91	-45619.8	0.00	1	0.27
Inverted Forest Cover***, Altitude***, Terrain Roughness*	22807.03	-45602.1	17.76	0	0.23
Inverted Forest Cover***	22576.63	-45145.3	474.56	0	0.25
Geographic Distance	22521.73	-45035.5	584.37	0	0.27

*Likelihood ration tests: * p < 0.05, ** p < 0.01, * p < 0.001*

All these predictors showed a significantly negative association with genetic relatedness (Table 4, Figure 4). Although temperature annual range did not show a larger variation than the other predictors used to assess IBR (Figure S3), it was highly correlated with forest cover (r = 0.94; Figure S4), so the effect of temperature annual range is likely confounded by forest cover to some extent. Nested MLPE models (accounting for spatial dependence) substantially improved fit and reduced evident autocorrelation. Although parameter estimates from NMLPE models had slightly larger standard errors (implying these were more conservative estimates) all effects continued to be significantly different from zero (Table 4). Our sensitivity analysis on IBR models revealed that temperature annual range, altitude and terrain roughness were the most frequent variables included in the set of best models (Figure S5).

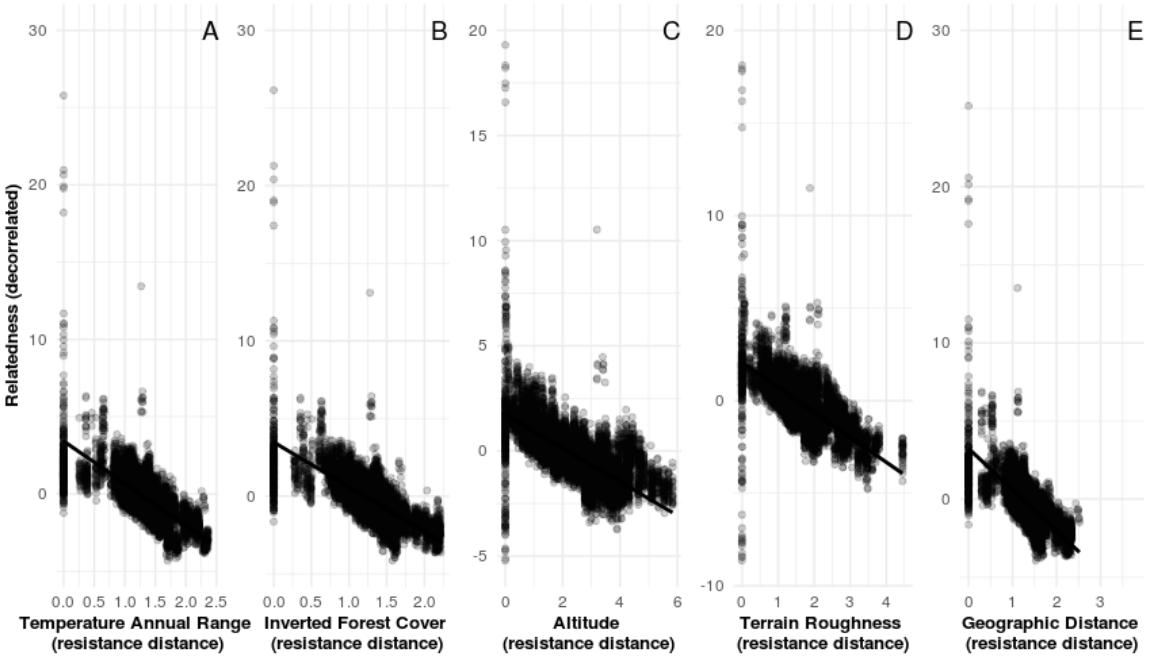


Figure 4: Isolation by resistance effects across the entire distribution range of *Melipona subnitida*. Plots show the relationship between genetic relatedness and temperature annual range (A), inverted forest cover (B), altitude (C), terrain roughness (D), and geographic distance (E). Although the isolation by geographic distance was not among the top models, we include it here for comparison. Relatedness is de-correlated for the MLPE correlation structure.

Table 4: Parameter estimates for the best-fitting MLPE regression models (ΔAIC<20, see Table 3), and NMLPE regression models (unbiased by spatial dependence; in parentheses). Estimates are followed by standard errors (SE), and 95% confidence intervals (CI). Although the isolation by geographic distance was not among the top models, we include it here for comparison.

Predictors	*Estimate*	*SE*	*CI*
Temperature Annual Range	-0.14 (-0.09)	0.001 (0.003)	-0.14 / -0.14 (-0.1 / -0.09)
Inverted Forest Cover	-0.11 (-0.07)	0.002 (0.005)	-0.12 / -0.11 (-0.08 / -0.06)
Altitude	-0.02 (-0.01)	0.001 (0.003)	-0.02 / -0.02 (-0.01 / -0.001)
Terrain Roughness	-0.01 (-0.02)	0.002 (0.005)	-0.01 / -0.001 (-0.03 / -0.01)
Geographic Distance	-0.14 (-0.15)	0.001 (0.001)	-0.14 / -0.14 (-0.1 / -0.08)

Identification of putative adaptive loci

About ten percent of all analyzed sequences contained signatures of selection (Table 5), and most of the identified candidate loci were associated with temperature (Figure 5). The spatial distribution of adaptive genetic variability revealed latitudinal and altitudinal gradients (Figure 6).

Table 5: Summary of the number of adaptive signals detected employing environmental association tests. Both the number of candidate SNPs and the number of contigs (RAD tags) containing candidate SNPs are presented for each environmental predictor by the number of independent (non-overlapping) detections in parentheses.

Signal type	*Total analyzed*	*Total under selection*	*Environmental Association Tests**			
			Mean Temp CoQ	*Forest Cover*	*Temp AnR*	*Prec DrQ*
SNPs	27,799	1,798	997 (444)	718 (281)	700 (261)	478 (67)
Contigs	15,924	1,356	768 (334)	532 (195)	535 (203)	371 (45)

*Environmental variables: Mean Temperature of Coldest Quarter (Mean Temp CoQ), Forest Cover, Temperature Annual Range (Temp AnR), Precipitation of Driest Quarter (Prec DrQ).

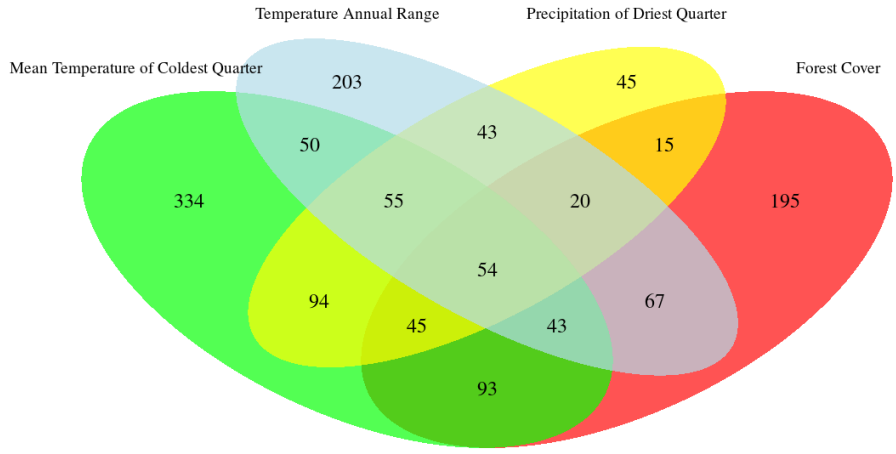


Figure 5: Venn diagram showing the intersection of sequences (contigs) containing candidate SNPs for *Melipona subnitida.* Putative adaptive loci were identified using environmental association tests, employing Mean Temperature of Coldest Quarter, Temperature Annual Range, Precipitation of Driest Quarter and Forest Cover.

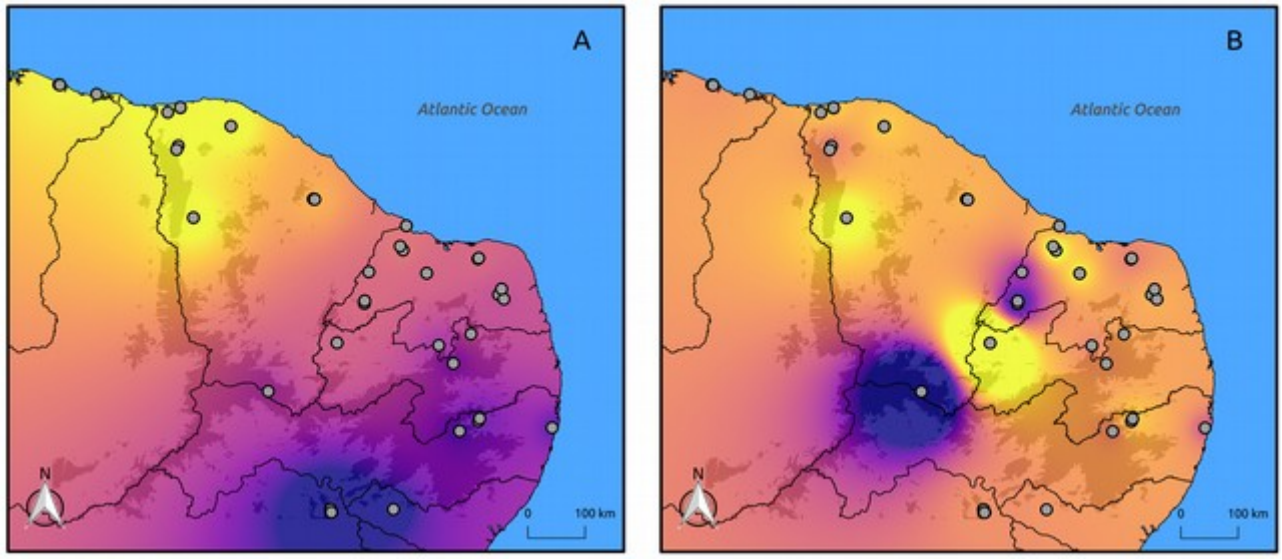


Figure 6: Spatial distribution of adaptive genetic variability in *Melipona subnitida*. Colors represent interpolated Spatial Principal Components (sPCA), and suggest a latitudinal pattern associated to sPC1 (A) and an altitudinal pattern associated to sPC2 (B). Shaded areas represent elevations of at least 500 masl.

Discussion

Our study reveals a clinal change in genetic structure across the distribution range of *M. subnitida*, with four identifiable genetic clusters. Genetic diversity was not influenced by habitat amount, pairwise relatedness showed spatial autocorrelation, and isolation by resistance explained range-wide relatedness patterns better than isolation by geographic distance. Specifically, gene flow was enhanced by low annual temperature variation, more forest cover, lower elevations, and flatter terrains. Finally, we detected genomic signatures of adaptation to temperature, precipitation and forest cover, and found latitudinal and altitudinal patterns in the spatial distribution of adaptive genetic variation.

Although previous genetic studies found intra-specific variation and signals of population structure in *M. subnitida* (Bonatti, Simões, Franco, & Francoy, 2014; Cruz et al., 2006; Silva et al., 2014), we here present the first assessment of spatial genetic structure based on thousands of independent genetic markers and performed across the full distribution range of this species. Our results reveal four genetic clusters, confirmed by two complementary methods, and extensive admixture (Figure 2). Genetic clusters located at the northernmost and southernmost extremes of the species' distribution range (Pop 1 and Pop 4) showed lower nucleotide diversity and lower effective population size (*Ne*) but similar heterozygosity and inbreeding as the genetic clusters located at the distribution's core (Pop 2 and Pop 3). This finding suggests that *M. subnitida* colonized these peripheral regions more recently, whereas clusters Pop 2 and Pop 3 had more time to accumulate a higher genetic variability, as stated by the central-peripheral hypothesis (Diniz-Filho et al., 2009).

Genetic diversity was slightly higher than that reported in other genomic bee studies (Jackson et al., 2018; Romiguier et al., 2014), and in all genetic clusters we found Tajima's D values overlapping with zero, which indicates mutation-drift equilibrium. Additionally, we did not find a significant association between habitat amount and genetic diversity. These results suggest that genetic variation has not been influenced by habitat loss yet, and that the observed levels of inbreeding are presumably related to the reproductive biology of this species (DiLeo & Wagner, 2016). Monogamy (Jaffé et al., 2014), low population densities and sharp seasonal variations in the production of reproductive individuals (Ferreira, Blochtein, & Serrão, 2013; Roubik, 2006; Santos-Filho, Alves, Eterovic, Imperatriz-Fonseca, & Kleinert, 2006), may result in mating between related individuals. We nevertheless caution that longer time-lags may be necessary to detect an effect of habitat loss on genetic diversity (Schlaepfer, Braschler, Rusterholz, & Baur, 2018).

We detected significant spatial autocorrelation in genetic relatedness, contradicting earlier microsatellite-based studies for other stingless bee species (Jaffé, Castilla, et al., 2016; Landaverde-

González et al., 2017). Interestingly, spatial autocorrelation was positive for up to 300 Km (Figure 3), indicating that related colonies can be found across large areas and that gene flow did not erase the genetic signals left by limited colony dispersal. Above 300 Km, spatial autocorrelation became negative, suggesting that population differentiation caused lower than random relatedness between individuals from different clusters (Figure 2). These results suggest it would be safe to transport colonies no further than 300 Km to avoid altering the genetic composition of wild populations (Jaffé, Pope, et al., 2016).

Isolation by landscape resistance explained range-wide relatedness patterns better than isolation by geographic distance alone. For instance, temperature fluctuations were found to be the most important factor explaining relatedness patterns in *M. subnitida*, followed by forest cover, elevation and terrain roughness. Our IBR results hold when accounting for spatial dependence and when excluding different numbers of samples, although forest cover looses importance when more samples are excluded (Figure S5). Significant isolation by resistance (altitude) was only found in one other stingless bee (*Partamona helleri* Friese, 1900), out of 18 analyzed species to date (Jaffé, Pope, et al., 2016; Landaverde-González et al., 2017), suggesting that the resolution of these microsatellite-based studies only allowed the detection of very strong IBR effects. Our findings thus imply that fine-scale genetic structure and IBR may be more common in this group of bees than previously acknowledged, and that studies employing thousands of genetic markers and large sample sizes are needed to identify or rule out weak, but significant IBR effects.

Our results reveal that thermal stability and forest cover (which were highly correlated) are key mediators of genetic connectivity in this stingless bee species, and support earlier findings stressing out the role of elevation as a bee dispersal barrier (Jackson et al., 2018; Jaffé, Pope, et al., 2016). Although thermal range is known to have a profound influence on insect physiology (Dixon et al., 2009), ours is the first study to report an effect on dispersal behaviors. Interestingly, temperature annual range was found to be the main abiotic predictor of bee richness and diversity in the eastern Neotropics, suggesting environmental variability may have led to higher speciation (Faria & Gonçalves, 2013). Our results imply that a similar mechanism may be operating at the species level, with increased thermal variability hindering gene flow, and thus facilitating local adaptation (F. Allendorf et al., 2013).

Even though thermal stability was the main factor explaining gene flow patterns in *M. subnitida*, temperature annual range and forest cover were highly correlated, so we could not disentangle the relative contribution of each. Our work is nevertheless the first to reveal a significant effect of forest cover on stingless bee gene flow, a long standing expectation for this group of bees with restricted dispersal (Jaffé, Pope, et al., 2016). Reduced gene flow across deforested areas was only found in

one other tropical organism so far (Monteiro et al., 2019), namely the army ant *Eciton burchelli* Westwood, 1842 (Pérez-Espona, McLeod, & Franks, 2012). Interestingly, these army ants exhibit striking life history similarities with stingless bees, as queens are permanently wingless and thus show a restricted dispersal (Jaffé, Moritz, & Kraus, 2009). Forested areas thus seem important dispersal corridors for this stingless bee, which could facilitate the migration towards higher elevations predicted under climate change (Figure S6).

Our environmental association tests can be considered robust to deviations from the underlying demographic structure, as candidate loci were intersected across different k-values. Additionally, LFMM were calibrated based on the distribution of adjusted *p*-values, so the incidence of false discovery rates was low (François et al., 2016). Interestingly, we found latitudinal and altitudinal gradients in the distribution of adaptive genetic variation. While the former gradient was also found in the honeybee *A. mellifera* (Chávez-Galarza et al., 2013; Henriques et al., 2018), and is probably related to climatic conditions; the altitudinal gradient suggests different adaptations to current highland and lowland areas. Our findings thus reveal the presence of locally-adapted bees, which should be preserved to maintain evolutionary potential (Hoffmann & Sgrò, 2011; Sgrò, Lowe, & Hoffmann, 2011). While lowland populations of *M. subnitida* are expected to shift to higher elevations by 2050 (Figure S6), current highland populations are at risk, since highlands will have no climate analogs in the future (Colwell, Brehm, Cardelús, Gilman, & Longino, 2008; Giannini et al., 2017). Conservation actions should thus prioritize the protection of current highland populations while improving the connectivity between highlands and lowlands, preserving or restoring foothill and mountain forests (Giannini et al., 2017).

Taken together, our findings shed important light on the life history of *M. subnitida*, and highlight the role of regions with large thermal fluctuations, deforested areas and mountain ranges as dispersal barriers. Moreover, our works unravels previously unknown patterns of local adaptation in these bees. This knowledge could help guide future conservation actions such as avoiding the transportation of colonies beyond 300 Km, preserving highland and lowland populations separately, and conserving or restoring foothill and mountain forests. Considering the high biological and economic importance of this native pollinator, such conservation efforts will be easily offset if honey production and pollination services are maintained in the future.

Acknowledgements

We thank Marcela de Matos Barbosa, Dirk Koedam, Jânio Ângelo Félix, Ulyses Madureira Maia and Márcia Rêgo for help with the collection of specimens. R Jaffé was supported by a research grant from CNPq (478982/2013-5), JC Veiga by a CAPES/EMBRAPA PhD scholarship (15/2014), NS Pope by an NSF predoctoral fellowship, CS Carvalho by a CAPES postdoctoral fellowship (88887.156652/2017-00) and ECM Lanes by a CNPq postdoctoral fellowship (300714/2017-3). R Jaffé, BM Freitas, CF Martins, MC Arias and VL Imperatriz-Fonseca received CNPq productivity research fellowship (301616/2017-5, 305126/2013-0, 302976/2015-9, 306932/2016-4 and 311531/2014-8 respectively). Funding was also provided by the Research Center on Biodiversity and Computing (BioComp), Empresa Brasileira de Pesquisa Agropecuária (02.11.01.029.00.00) and Instituto Tecnológico Vale.

Literature Cited

Ahrens, C. W., Rymer, P. D., Stow, A., Bragg, J., Dillon, S., Umbers, K. D. L., & Dudaniec, R. Y. (2018). The search for loci under selection: trends, biases and progress. *Molecular Ecology*, *27*(6), 1342–1356. https://doi.org/10.1111/mec.14549

Alexander, D., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, *19*, 1655–1664.

Allendorf, F., Luikart, G., & Aitken, S. (2013). *Conservation and the genetics of populations*. Oxford: Wiley-Blackwell.

Allendorf, F. W. (2017). Genetics and the conservation of natural populations: allozymes to genomes. *Molecular Ecology*, *26*(2), 420–430. https://doi.org/10.1111/mec.13948

Alvarado-Serrano, D. F., Van Etten, M. L., Chang, S.-M., & Baucom, R. S. (2019). The relative contribution of natural landscapes and human-mediated factors on the connectivity of a noxious invasive weed. *Heredity*, *122*(1), 29–40. https://doi.org/10.1038/s41437-018-0106-x

Balkenhol, N., Cushman, S. A., Waits, L. P., & Storfer, A. (2016). *Landscape Genetics: Concepts, Methods, Applications*. (N. Balkenhol, S. Cushman, A. Storfer, & L. Waits, Eds.). Hoboken, NJ: John Wiley & Sons.

Balkenhol, N., Dudaniec, R. Y., Krutovsky, K. V, Johnson, J. S., Cairns, D. M., Segelbacher, G., … Selmoni, O. (2017). Landscape Genomics: Understanding Relationships Between Environmental Heterogeneity and Genomic Characteristics of Populations. In O. P. Rajora

(Ed.), *Population Genomics: Concepts, Approaches and Applications*. Springer International Publishing. https://doi.org/10.1007/13836_2017_2

Barton, K. (2018). MuMIn: multi-model inference. R package version 1.13. 4.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Source Journal of the Royal Statistical Society. Series B (Methodological)*, *57*(1), 289–300. https://doi.org/10.2307/2346101

Bonatti, V., Simões, Z. L. P., Franco, F. F., & Francoy, T. M. (2014). Evidence of at least two evolutionary lineages in Melipona subnitida (Apidae, Meliponini) suggested by mtDNA variability and geometric morphometrics of forewings. *Naturwissenschaften*, *101*(1), 17–24. https://doi.org/10.1007/s00114-013-1123-5

Brown, M. J. F. F., & Paxton, R. J. (2009). The conservation of bees: a global perspective. *Apidologie*, *40*(3), 410–416. https://doi.org/10.1051/apido/2009019

Bruno, C., Macchiavelli, R., & Balzarini, M. (2008). Non-parametric smoothing of multivariate genetic distances in the analysis of spatial population structure at fine scale. *Theoretical and Applied Genetics*, *117*(3), 435–447. https://doi.org/10.1007/s00122-008-0788-1

Chávez-Galarza, J., Henriques, D., Johnston, J. S., Azevedo, J. C., Patton, J. C., Muñoz, I., … Pinto, M. A. (2013). Signatures of selection in the Iberian honey bee (Apis mellifera iberiensis) revealed by a genome scan analysis of single nucleotide polymorphisms. *Molecular Ecology*, *22*(23), 5890–5907. https://doi.org/10.1111/mec.12537

Clarke, R. T., Rothery, P., & Raybould, A. F. (2002). Confidence limits for regression relationships between distance matrices: Estimating gene flow with distance. *Journal of Agricultural, Biological, and Environmental Statistics*, *7*(3), 361–372. https://doi.org/10.1198/108571102320

Colwell, R. K., Brehm, G., Cardelús, C. L., Gilman, A. C., & Longino, J. T. (2008). Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. *Science*. https://doi.org/10.1126/science.1162547

Cruz, D. de O., Jorge, D. M. de M., Pereira, J. O. P., Torres, D. C., Soares, C. E. A., Freitas, B. M., & Grangeiro, T. B. (2006). Intraspecific variation in the first internal transcribed spacer (ITS1) of the nuclear ribosomal DNA in Melipona subnitida (Hymenoptera, Apidae), an endemic stingless bee from northeastern Brazil. *Apidologie*, *37*, 376–386. https://doi.org/10.1051/apido

Cushman, S. A., & Landguth, E. L. (2010). Spurious correlations and inference in landscape genetics. *Molecular Ecology*, *19*(17), 3592–3602. https://doi.org/10.1111/j.1365-294X.2010.04656.x

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., … Durbin, R. (2011). Variant Call Format , Binary Variant Call Format and VCFtools. *Bioinformatics*, *27*(15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Davis, E., Murray, T., Fitzpatrick, U., Brown, M., & Paxton, R. (2010). Landscape effects on extremely fragmented populations of a rare solitary bee, Colletes floralis. *Molecular Ecology*, *19*, 4922–4935.

De Kort, H., Vandepitte, K., Bruun, H. H., Closset-Kopp, D., Honnay, O., & Mergeay, J. (2014). Landscape genomics and a common garden trial reveal adaptive differentiation to temperature across Europe in the tree species Alnus glutinosa. *Molecular Ecology*, *23*(19), 4709–4721. https://doi.org/10.1111/mec.12813

DiLeo, M. F., & Wagner, H. H. (2016). A Landscape Ecologist's Agenda for Landscape Genetics. *Current Landscape Ecology Reports*, *1*(3), 115–126. https://doi.org/10.1007/s40823-016-0013-x

Diniz-Filho, J. A. F., Nabout, J. C., Bini, L. M., Soares, T. N., de Campus Telles, M. P., de Marco, P., & Collevatti, R. G. (2009). Niche modelling and landscape genetics of Caryocar brasiliense ("Pequi" tree: Caryocaraceae) in Brazilian Cerrado: An integrative approach for evaluating central-peripheral population patterns. *Tree Genetics and Genomes*. https://doi.org/10.1007/s11295-009-0214-0

Dixon, A. F. G., Honěk, A., Keil, P., Kotela, M. A. A., Šizling, A. L., & Jarošík, V. (2009). Relationship between the minimum and maximum temperature thresholds for development in insects. *Functional Ecology*, *23*(2), 257–264. https://doi.org/10.1111/j.1365-2435.2008.01489.x

Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., & Ovenden, J. R. (2014). NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size from genetic data. *Molecular Ecology Resources*, *14*(1), 209–214. https://doi.org/10.1111/1755-0998.12157

El-Niweiri, M. A. A., & Moritz, R. F. A. (2011). Mating in the rain? Climatic variance for polyandry in the honeybee (Apis mellifera jemenitica). *Population Ecology*, *53*(3), 421–427. https://doi.org/10.1007/s10144-011-0271-8

Fahrig, L. (2013). Rethinking patch size and isolation effects: The habitat amount hypothesis. *Journal of Biogeography*. https://doi.org/10.1111/jbi.12130

Faleiro, F. V., Nemésio, A., & Loyola, R. (2018). Climate change likely to reduce orchid bee abundance even in climatic suitable sites. *Global Change Biology*, *24*(6), 2272–2283. https://doi.org/10.1111/gcb.14112

Faria, L. R. R., & Gonçalves, R. B. (2013). Abiotic correlates of bee diversity and composition along eastern Neotropics. *Apidologie*, *44*(5), 547–562. https://doi.org/10.1007/s13592-013-0205-x

Ferreira, N. T., Blochtein, B., & Serrão, J. E. (2013). Seasonal production and spatial distribution of Melipona bicolor schencki (Apidae; Meliponini) castes in brood combs in southern Brazil. *Apidologie*, *44*(2), 176–187. https://doi.org/10.1007/s13592-012-0169-2

François, O., Martins, H., Caye, K., & Schoville, S. D. (2016). Controlling false discoveries in genome scans for selection. *Molecular Ecology*. https://doi.org/10.1111/mec.13513

Frichot, E., & François, O. (2015). LEA: An R package for landscape and ecological association studies. *Methods in Ecology and Evolution*, *6*(8), 925–929. https://doi.org/10.1111/2041-210X.12382

Frichot, E., Mathieu, F., Trouillon, T., Bouchard, G., & François, O. (2014). Fast and efficient estimation of individual ancestry coefficients. *Genetics*, *196*(4), 973–983. https://doi.org/10.1534/genetics.113.160572

Frichot, E., Schoville, S. D., Bouchard, G., & François, O. (2013). Testing for Association Between Loci and Environmental Gradients Using Latent Factor Mixed Models. *Molecular Biology and Evolution*, *30*(7), 1687–1699. https://doi.org/10.1093/molbev/mst063

Giannini, T. C., Maia-Silva, C., Acosta, A. L., Jaffé, R., Carvalho, A. T., Martins, C. F., … Imperatriz-Fonseca, V. L. (2017). Protecting a managed bee pollinator against climate change: strategies for an area with extreme climatic conditions and socioeconomic vulnerability. *Apidologie*, *48*(6), 784–794. https://doi.org/10.1007/s13592-017-0523-5

Hadley, A. S., & Betts, M. G. (2011). The effects of landscape fragmentation on pollination dynamics: absence of evidence not evidence of absence. *Biological Reviews*, *87*, 526–544.

Henriques, D., Wallberg, A., Chávez-Galarza, J., Johnston, J. S., Webster, M. T., & Pinto, M. A. (2018). Whole genome SNP-associated signatures of local adaptation in honeybees of the Iberian Peninsula. *Scientific Reports*, *8*(1), 1–14. https://doi.org/10.1038/s41598-018-29469-5

Hoffmann, A. A., & Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, *470*(7335), 479–485. https://doi.org/10.1038/nature09670

Imperatriz-Fonseca, V., Koedam, D., & Hrncir, M. (2017). *A abelha jandaíra: no passado, no presente e no futuro*. EdUFERSA. Retrieved from https://www.researchgate.net/profile/Breno_Freitas/publication/321278052_Meliponineos_e_polinizacao_a_abelha_jandaira_e_outros_meliponineos_na_polinizacao_agricola_no_semiarido/links/5a1868bfaca272df080a8388/Meliponineos-e-polinizacao-a-abelha-jandaira-e-

Jackson, J. M., Pimsler, M. L., Oyen, K. J., Koch-Uhuad, J. B., Herndon, J. D., Strange, J. P., … Lozier, J. D. (2018). Distance, elevation and environment as drivers of diversity and divergence in bumble bees across latitude and altitude. *Molecular Ecology*, *27*(14), 2926–2942. https://doi.org/10.1111/mec.14735

Jaffé, R., Castilla, A., Pope, N., Imperatriz-Fonseca, V. L., Metzger, J. P., Arias, M. C., & Jha, S. (2016). Landscape genetics of a tropical rescue pollinator. *Conservation Genetics*, *17*(2), 267–278. https://doi.org/10.1007/s10592-015-0779-0

Jaffé, R., Moritz, R. F. A., & Kraus, F. B. (2009). Gene flow is maintained by polyandry and male dispersal in the army ant Eciton burchellii. *Population Ecology*, *51*(2), 227–236. https://doi.org/10.1007/s10144-008-0133-1

Jaffé, R., Pioker-Hara, F. C., Dos Santos, C. F., Santiago, L. R., Alves, D. A., Kleinert, A. de M. P., … Imperatriz-Fonseca, V. L. (2014). Monogamy in large bee societies: a stingless paradox. *Die Naturwissenschaften*, *101*(3), 261–264. https://doi.org/10.1007/s00114-014-1149-3

Jaffé, R., Pope, N., Acosta, A. L., Alves, D. A., Arias, M. C., De la Rúa, P., … Carvalheiro, L. G. (2016). Beekeeping practices and geographic distance, not land use, drive gene flow across tropical bees. *Molecular Ecology*, *25*(21), 5345–5358. https://doi.org/10.1111/mec.13852

Jaffé, R., Pope, N., Carvalho, A. T., Maia, U. M., Blochtein, B., de Carvalho, C. A. L., … Imperatriz-Fonseca, V. L. (2015). Bees for development: brazilian survey reveals how to

optimize stingless beekeeping. *PLOS ONE*, *10*(3), 1–21. https://doi.org/10.1371/journal.pone.0121157

Jha, S. (2015). Contemporary human-altered landscapes and oceanic barriers reduce bumble bee gene flow. *Molecular Ecology*, *24*(5), 993–1006. https://doi.org/10.1111/mec.13090

Jha, S., & Kremen, C. (2013). Urban land use limits regional bumble bee gene flow. *Molecular Ecology*, *22*, 2483–2495.

Jombart, T. (2008). adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*(11), 1403–5. https://doi.org/10.1093/bioinformatics/btn129

Kennedy, C. M., Lonsdorf, E., Neel, M. C., Williams, N. M., Ricketts, T. H., Winfree, R., … Kremen, C. (2013). A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. *Ecology Letters*, *16*(5), 584–599. https://doi.org/10.1111/ele.12082

Kerr, J. T., Pindar, A., Galpern, P., Packer, L., Potts, S. G., Roberts, S. M., … Pantoja, A. (2015). Climate change impacts on bumblebees converge across continents. *Science*, *349*(6244), 177–180. https://doi.org/10.1126/science.aaa7031

Koffler, S., Menezes, C., Menezes, P. R., Kleinert, A. D. M. P., Imperatriz-Fonseca, V. L., Pope, N., & Jaffé, R. (2015). Temporal Variation in Honey Production by the Stingless Bee Melipona subnitida (Hymenoptera: Apidae): Long-Term Management Reveals Its Potential as a Commercial Species in Northeastern Brazil. *Journal of Economic Entomology*, *108*(3), 858–867. https://doi.org/10.1093/jee/tov055

Landaverde-González, P., Enríquez, E., Ariza, M. A., Murray, T., Paxton, R. J., & Husemann, M. (2017). Fragmentation in the clouds? The population genetics of the native bee Partamona bilineata (Hymenoptera: Apidae: Meliponini) in the cloud forests of Guatemala. *Conservation Genetics*, *18*(3), 631–643. https://doi.org/10.1007/s10592-017-0950-x

Le Conte, Y., & Navajas, M. (2008). Climate change: impact on honey bee populations and diseases. *Rev. Sci. Tech. Off. Int. Epizoot*, *27*, 499–510.

Leroy, G., Carroll, E. L., Bruford, M. W., DeWoody, J. A., Strand, A., Waits, L., & Wang, J. (2018). Next-generation metrics for monitoring genetic erosion within populations of conservation concern. *Evolutionary Applications*, *11*(7), 1066–1083. https://doi.org/10.1111/eva.12564

Lozier, J. D. (2014). Revisiting comparisons of genetic diversity in stable and declining species: assessing genome-wide polymorphism in North American bumble bees using RAD sequencing. *Molecular Ecology*, *23*(4), 788–801. https://doi.org/10.1111/mec.12636

Lozier, J., & Zayed, A. (2017). Bee conservation in the age of genomics. *Conservation Genetics*, *18*(3), 713–729. https://doi.org/10.1007/s10592-016-0893-7

Maia-Silva, C., Hrncir, M., Silva, C. I., & Imperatriz-Fonseca, V. L. (2015). Survival strategies of stingless bees (Melipona subnitida) in an unpredictable environment, the Brazilian tropical dry forest. *Apidologie*, *46*(5), 631–643. https://doi.org/10.1007/s13592-015-0354-1

Marengo, J. A., Torres, R. R., & Alves, L. M. (2017). Drought in Northeast Brazil—past, present, and future. *Theoretical and Applied Climatology*, *129*(3–4), 1189–1200. https://doi.org/10.1007/s00704-016-1840-8

McCartney-Melstad, E., Vu, J. K., & Shaffer, H. B. (2018). Genomic data recover previously undetectable fragmentation effects in an endangered amphibian. *Molecular Ecology*, *27*(22), 4430–4443. https://doi.org/10.1111/mec.14892

McRae, B. H. (2006). Isolation by resistance. *Evolution*, *60*(8), 1551. https://doi.org/10.1554/05-321.1

Monteiro, W. P., Veiga, J. C., Silva, A. R., Carvalho, C. da S., Lanes, É. C. M., Rico, Y., & Jaffé, R. (2019). Everything you always wanted to know about gene flow in tropical landscapes (but were afraid to ask). *PeerJ*, *7*, e6446. https://doi.org/10.7717/peerj.6446

Pedro, S. R. M. (2014). The stingless bee fauna in Brazil (Hymenoptera : Apidae). *Sociobiology*, *61*(December), 348–354. https://doi.org/10.13102/sociobiology.v61i4.348-354

Pérez-Espona, S., McLeod, J. E., & Franks, N. R. (2012). Landscape genetics of a top neotropical predator. *Molecular Ecology*, *21*(24), 5969–5985. https://doi.org/10.1111/mec.12088

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & Team, R. C. (2018). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-137. Retrieved from https://cran.r-project.org/package=nlme

Potts, S. G. ., Biesmeijer, J. C. ., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. . (2010). Global pollinator declines: Trends, impacts and drivers. *Trends in Ecology and Evolution*, *25*(6), 345–353. https://doi.org/10.1016/j.tree.2010.01.007

Potts, S. G., Imperatriz-Fonseca, V. L., Ngo, H. T., Biesmeijer, J. C., Breeze, T. D., Dicks, L. V., … Viana, B. F. (2016). *IPBES (2016): Summary for policymakers of the assessment report of the Intergovernmental Science - Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production*. Bonn, Germany: Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services.

Pyke, G. H., Thomson, J. D., Inouye, D. W., & Miller, T. J. (2016). Effects of climate change on phenologies and distributions of bumble bees and the plants they visit. *Ecosphere*, *7*(3), e01267. https://doi.org/10.1002/ecs2.1267

Rellstab, C., Gugerli, F., Eckert, A. J., Hancock, A. M., & Holderegger, R. (2015). A practical guide to environmental association analysis in landscape genomics. *Molecular Ecology*, *24*(17), 4348–4370. https://doi.org/10.1111/mec.13322

Romiguier, J., Gayral, P., Ballenghien, M., Bernard, A., Cahais, V., Chenuil, A., … Galtier, N. (2014). Comparative population genomics in animals uncovers the determinants of genetic diversity. *Nature*, *515*(7526), 261–263. https://doi.org/10.1038/nature13685

Roubik, D. W. (2006). Stingless bee nesting biology. *Apidologie*, *37*(2), 124–143. https://doi.org/10.1051/apido

Santos-Filho, P. S., Alves, D. a., Eterovic, a., Imperatriz-Fonseca, V. L., & Kleinert, a. M. P. (2006). Numerical investment in sex and caste by stingless bees (Apidae: Meliponini): a comparative analysis. *Apidologie*, *37*, 207–221. https://doi.org/10.1051/apido:2006015

Schlaepfer, D. R., Braschler, B., Rusterholz, H.-P., & Baur, B. (2018). Genetic effects of anthropogenic habitat fragmentation on remnant animal and plant populations: a meta-analysis. *Ecosphere*, *9*(10), e02488. https://doi.org/10.1002/ecs2.2488

Sgrò, C. M., Lowe, A. J., & Hoffmann, A. A. (2011). Building evolutionary resilience for conserving biodiversity under climate change. *Evolutionary Applications*, *4*(2), 326–337. https://doi.org/10.1111/j.1752-4571.2010.00157.x

Shirk, A. J., Landguth, E. L., & Cushman, S. A. (2017). A comparison of individual-based genetic distance metrics for landscape genetics. *Molecular Ecology Resources*, *17*(6), 1308–1317. https://doi.org/10.1111/1755-0998.12684

Silva, G. R., Souza, B. A., Pereira, F. M., Lopes, M. T. R., Valente, S. E., & Diniz, F. (2014). New molecular evidence for fragmentation between two distant populations of the threatened

stingless bee Melipona subnitida Ducke (Hymenoptera, Apidae, Meliponini). *Journal of Hymenoptera Research*, *38*, 1–9. https://doi.org/10.3897/jhr.38.7302

Silva, M. D., & Ramalho, M. (2016). The influence of habitat and species attributes on the density and nest spacing of a stingless bee (Meliponini) in the Atlantic Rainforest. *Sociobiology*, *63*(3), 991. https://doi.org/10.13102/sociobiology.v63i3.1037

Storfer, A., Patton, A., & Fraik, A. K. (2018). Navigating the interface between landscape genetics and landscape genomics. *Frontiers in Genetics*, *9*. https://doi.org/10.3389/fgene.2018.00068

Tajima, F. (1989). Statistical Method for Testing the Neutral Mutation. *Genetics Society of America*, *123*, 585–595. Retrieved from http://www.mathstat.concordia.ca/faculty/lpopovic/Tajima1989.pdf

Tavares, M. G., Carvalho, C. R., Aparecida, F., & Soares, F. (2010). Genome size variation in Melipona species (Hymenoptera: Apidae) and sub-grouping by their DNA content. *Apidologie*, *41*, 636–642.

Theodorou, P., Radzevičiūtė, R., Kahnt, B., Soro, A., Grosse, I., & Paxton, R. J. (2018). Genome-wide single nucleotide polymorphism scan suggests adaptation to urbanization in an important pollinator, the red-tailed bumblebee (Bombus lapidarius L.). *Proceedings of the Royal Society B: Biological Sciences*. https://doi.org/10.1098/rspb.2017.2806

Van Veen, J. W., & Sommeijer, M. J. (2000). Colony reproduction in Tetragonisca angustula (Apidae, Meliponini). *Insectes Sociaux*, *47*(1), 70–75. https://doi.org/10.1007/s000400050011

Viana, B. F., Boscolo, D., Mariano-Neto, E., Lopes, L. E., Lopes, A. V., Ferreira, P. A., … Primo, L. M. (2012). How well do we understand landscape effects on pollinators and pollination services? *Journal of Pollination Ecology*, *7*, 31–41.

Vieira, R. M. S. P., Tomasella, J., Alvala, R. C. S., Sestini, M. F., Affonso, A. G., Rodriguez, D. A., … Santana, M. O. (2015). Identifying areas susceptible to desertification in the Brazilian northeast. *Solid Earth*, *6*, 347–360. https://doi.org/10.5194/se-6-347-2015

Vit, P., Pedro, S. R. M., & Roubik, D. W. (2013). *Pot-Honey: A legacy of stingless bees*. (P. Vit, S. R. M. Pedro, & D. Roubik, Eds.). New York, NY: Springer Science & Business Media. https://doi.org/10.1007/978-1-4614-4960-7

Willmer, P. (2014). Climate change: Bees and orchids lose touch. *Current Biology*, *24*(23), R1133–R1135. https://doi.org/10.1016/j.cub.2014.10.061

Wratten, S., Gillespie, M., Decourtye, A., Mader, E., & Desneux, N. (2012). Pollinator habitat enhancement: benefits to other ecosystem services. *Agriculture, Ecosystems and Environment*, *159*, 112–122.

Yang, J., Benyamin, B., Mcevoy, B. P., Gordon, S., Henders, A. K., Dale, R., … Visscher, P. M. (2010). Common SNPs explain a large proportion of heritability for human height. *Nature Genetics*, *42*(7), 565–569. https://doi.org/10.1038/ng.608

Eletronic Supplementary Material

Figures

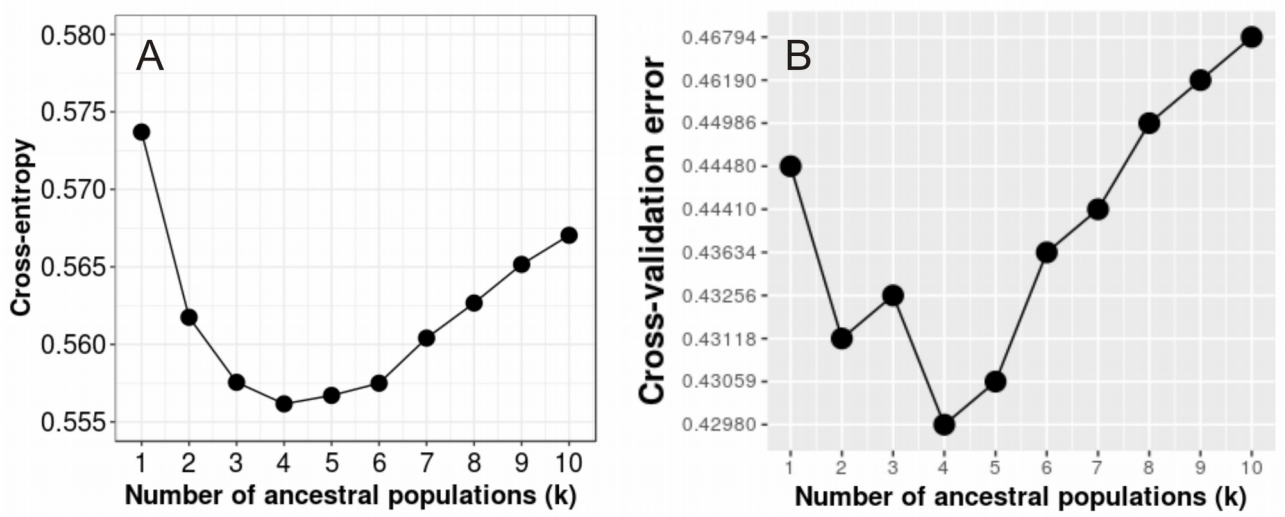


Figure S1: Plots showing the optimal number of genetic clusters. Optimal k choice is based on mean ± sd cross-entropy (LEA, A) and cross-validation errors (Admixture, B).

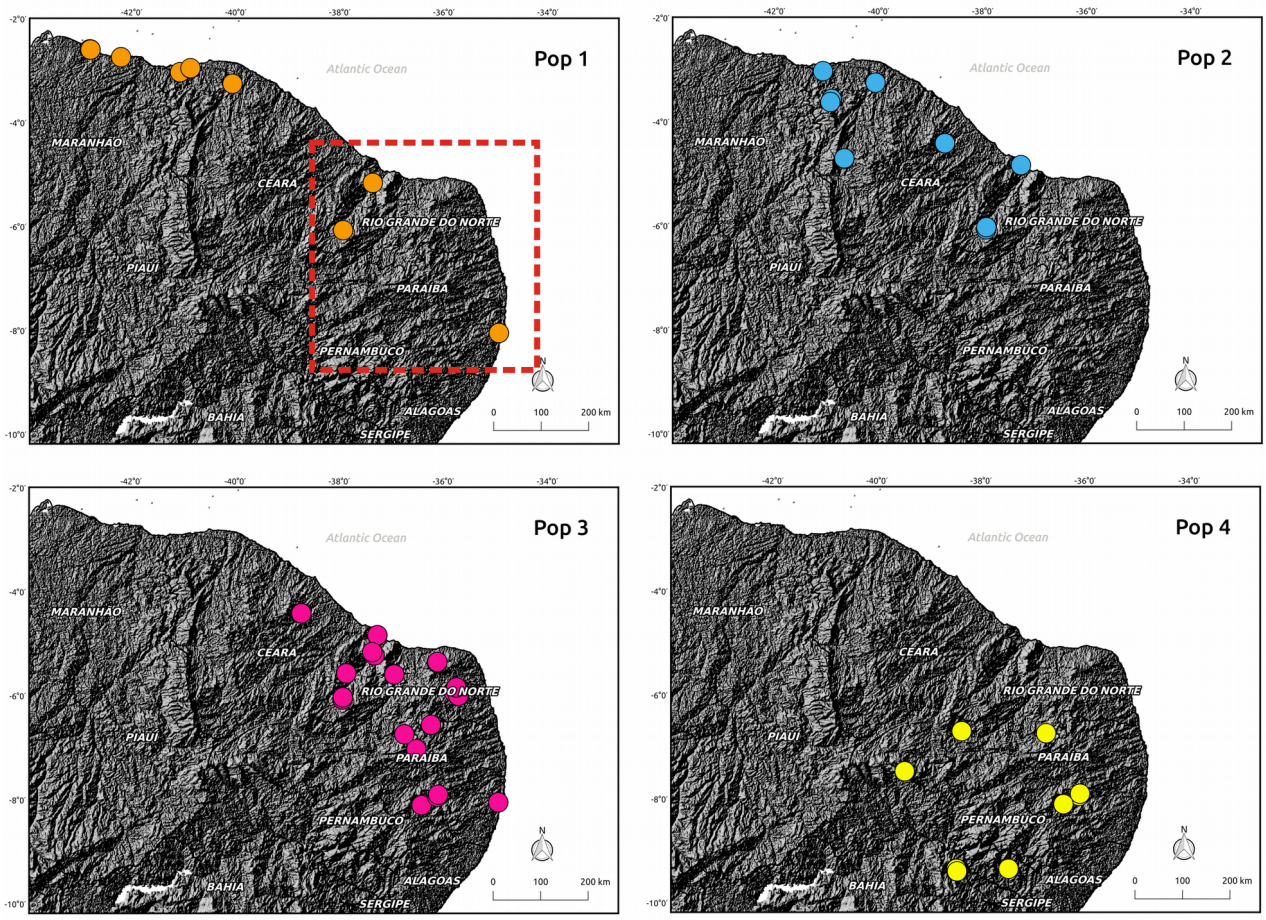


Figure S2: Maps showing *Melipona subnitida* assignments to four genetic clusters against an elevation map (from USGS Earth Explorer). The red square shows the excluded samples from Pop 1 (orange cluster), given they were likely introduced bees.

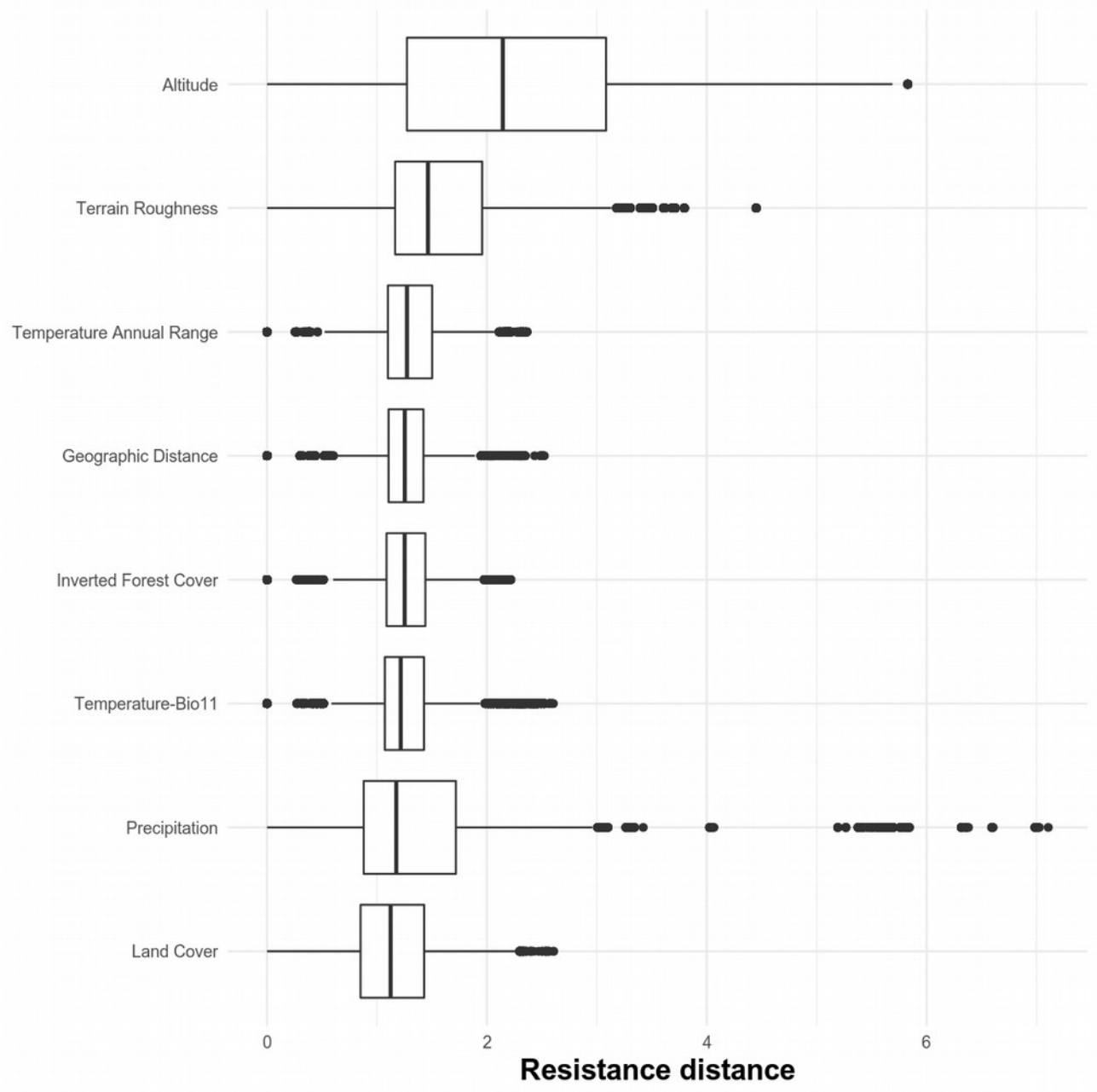


Figure S3: Variable ranges for all pairwise resistance distances used to run MLPE regression models.

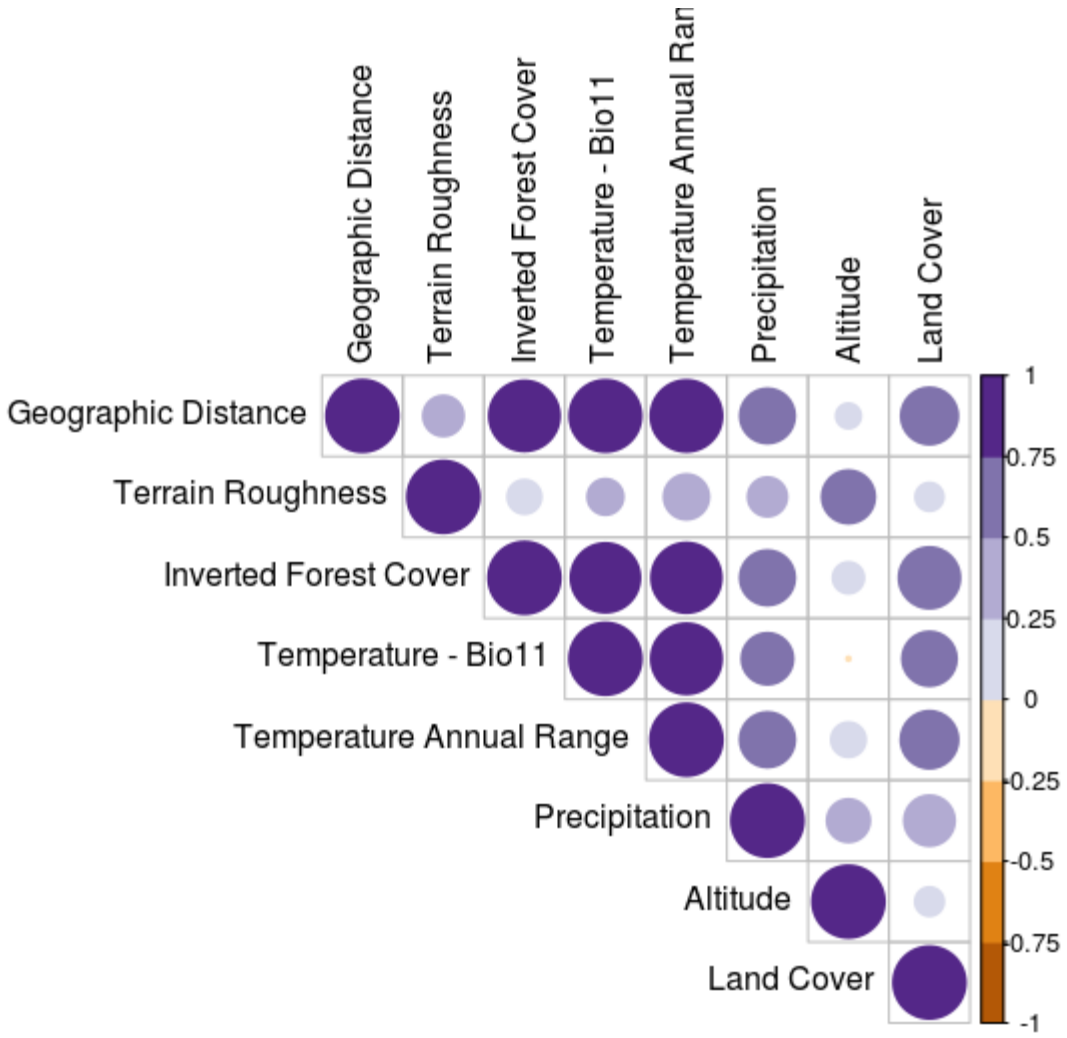


Figure S4: Correlogram showing the correlation between all resistance distances included as predictors in MLPE regression models. Pearson's correlation coefficients (*r*) are depicted by colors and circle sizes.

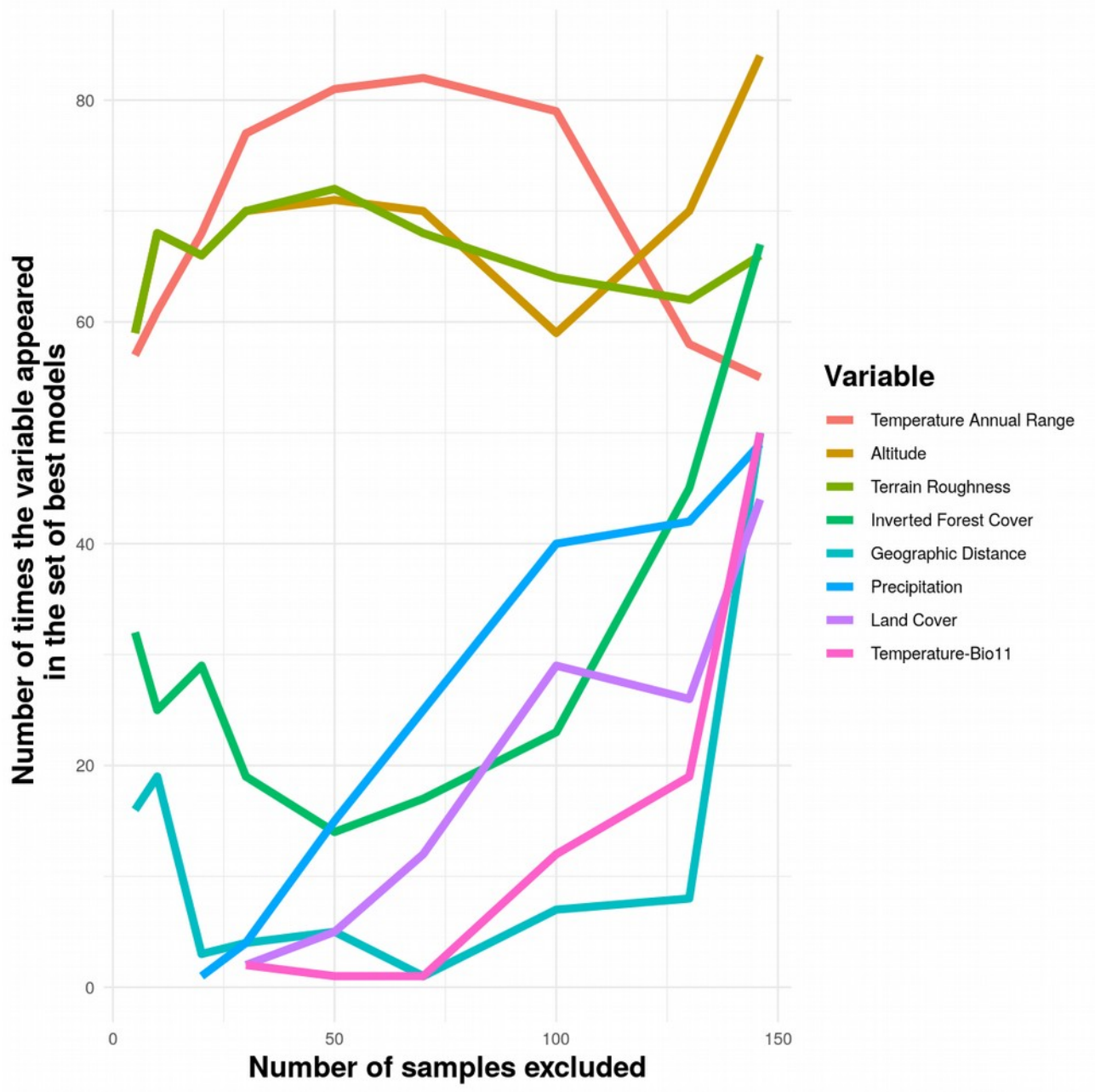


Figure S5: Sensitivity analyses showing the number of times predictor variables were included in the set of best-fitting MLPE regression models (ΔAIC ≤ 2), after randomly excluding different numbers of samples. One hundred data subsets were generated and one hundred independent model selection protocols were performed for each treatment (number of excluded samples: 5, 10, 20, 30, 50, 70, 100, 130, and 146).

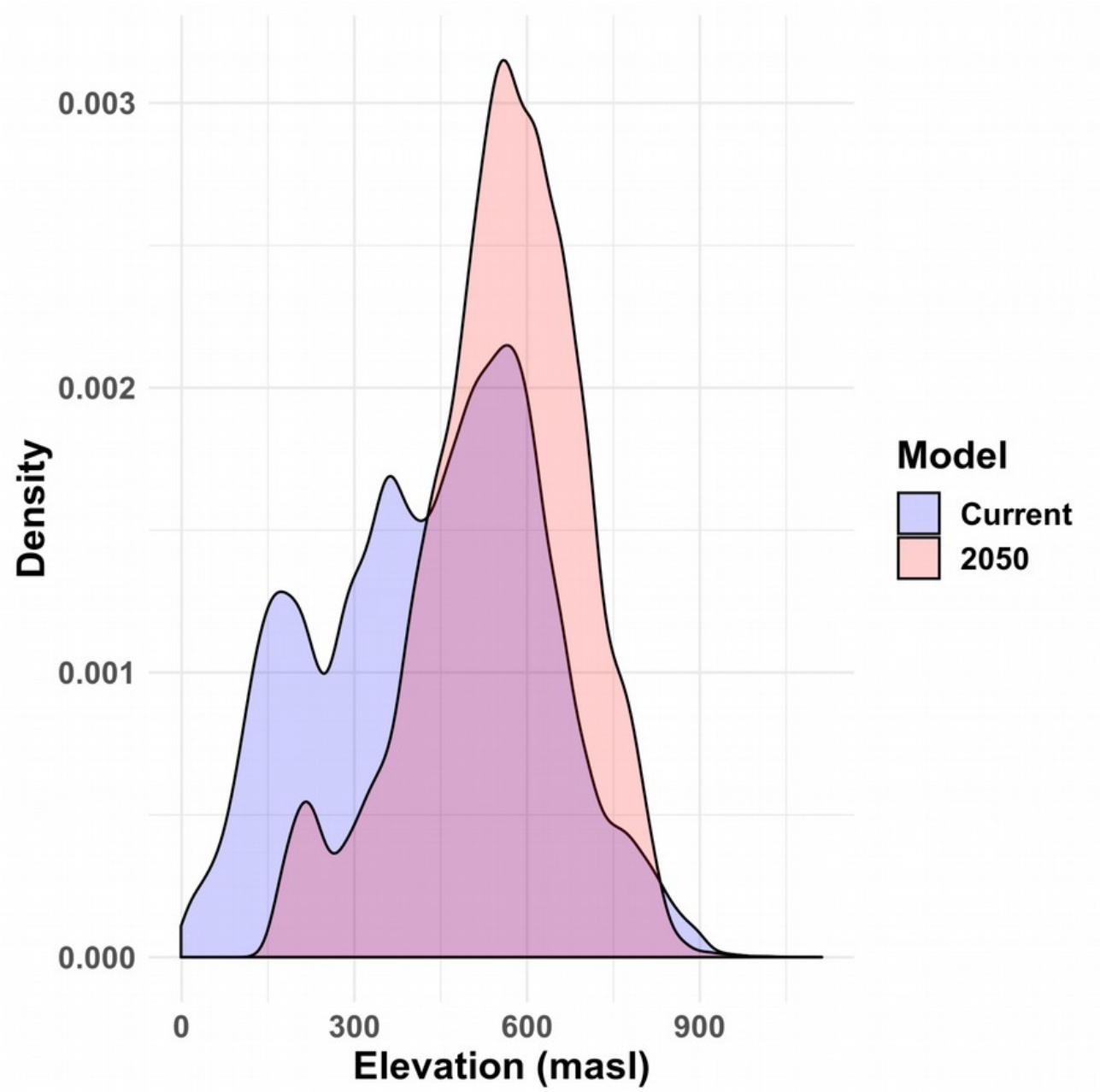


Figure S6: Density plots showing the current and future (2050) distribution of elevations where *M. subnitida* is most likely to occur across Northeastern Brazil (probability of occurrence ≥ 50%), according to the species distribution models (SDM) by Giannini et al. (2017). SDM were cropped to the extent of our sampling locations and a Wilcoxon test was employed to compare both distributions ($W = 6.7_x10^8$, $p < 0.001$). The maximum altitude were samples were collected in our study was 872.3 masl.

Tables

Table S1: Information on the collected *Melipona subnitida* samples, including sample ID, location name, State, and geographic coordinates (in decimal degrees).

ID	*Location name*	*State*	*Latitude*	*Longitude*
J03*	Martins	RN	-6.06348300352692	-37.9369529988616
J04	Martins	RN	-6.06348300352692	-37.9369529988616
J06	Martins	RN	-6.06348300352692	-37.9369529988616
J07	Martins	RN	-6.06348300352692	-37.9369529988616
J08	Martins	RN	-6.06348300352692	-37.9369529988616
J09	Martins	RN	-6.06348300352692	-37.9369529988616
J10	Martins	RN	-6.06348300352692	-37.9369529988616
J12	Pé da Serra de Martins	RN	-6.02193902246654	-37.9327550064772
J13	Pé da Serra de Martins	RN	-6.02193902246654	-37.9327550064772
J14	Pé da Serra de Martins	RN	-6.02193902246654	-37.9327550064772
J15	Pé da Serra de Martins	RN	-6.02193902246654	-37.9327550064772
J16	Pé da Serra de Martins	RN	-6.02193902246654	-37.9327550064772
J17	Açu	RN	-5.5826364364475	-36.9447094202041
J18	Açu	RN	-5.5826364364475	-36.9447094202041
J19	Açu	RN	-5.5826364364475	-36.9447094202041
J20	Açu	RN	-5.5826364364475	-36.9447094202041
J21	Açu	RN	-5.5826364364475	-36.9447094202041
J24	Aroeiras, Jandaira	RN	-5.3541820	-36.1265490
J25	Aroeiras, Jandaira	RN	-5.3541820	-36.1265490
J26	Aroeiras, Jandaira	RN	-5.3541820	-36.1265490

ID	*Location name*	*State*	*Latitude*	*Longitude*
J27	Aroeiras, Jandaira	RN	-5.3541820	-36.1265490
J28	Aroeiras, Jandaira	RN	-5.3541820	-36.1265490
J30	Jandaíra	RN	-5.3543428145349	-36.1266305577009
J31	Jandaíra	RN	-5.34169116988778	-36.1143727786839
J32	Jandaíra	RN	-5.34169116988778	-36.1143727786839
J33	Jandaíra	RN	-5.34169116988778	-36.1143727786839
J34	Jandaíra	RN	-5.34169116988778	-36.1143727786839
J35	Jandaíra	RN	-5.34169116988778	-36.1143727786839
J36	Aroeiras, Jandaira	RN	-5.3541820	-36.1265490
J40	S. Paulo Potengi	RN	-5.9219220	-35.7980690
J41	S. Paulo Potengi	RN	-5.9219220	-35.7980690
J42	S. Paulo Potengi	RN	-5.9219220	-35.7980690
J43	S. Paulo Potengi	RN	-5.9219220	-35.7980690
J44	S. Paulo Potengi	RN	-5.9219220	-35.7980690
J45	Sta. Maria	RN	-5.83328191190958	-35.75014882721
J46	Sta. Maria	RN	-5.83328191190958	-35.75014882721
J47	Sta. Maria	RN	-5.83328191190958	-35.75014882721
J48	Sta. Maria	RN	-5.83328191190958	-35.75014882721
J49	Eloy de Souza	RN	-5.99348857998848	-35.7126027625054
J50	Eloy de Souza	RN	-5.99348857998848	-35.7126027625054
J51	Eloy de Souza	RN	-5.99348857998848	-35.7126027625054
J54	Jardim do Seridó	RN	-6.73099103383719	-36.7511167936027
J55	Jardim do Seridó	RN	-6.73099103383719	-36.7511167936027

ID	*Location name*	*State*	*Latitude*	*Longitude*
J56	Jardim do Seridó	RN	-6.73315666615963	-36.7505053337663
J57	Jardim do Seridó	RN	-6.73315666615963	-36.7505053337663
J58	Joazerinho	RN	-7.02022436074912	-36.5198833029717
J59	Joazerinho	RN	-7.02022436074912	-36.5198833029717
J60	Joazerinho	RN	-7.02022436074912	-36.5198833029717
J61	Joazerinho	RN	-7.02022436074912	-36.5198833029717
J62	Joazerinho	RN	-7.02022436074912	-36.5198833029717
J65	Aurora da Serra, Apodi	RN	-5.56177144870162	-37.8667800407856
J66	Aurora da Serra, Apodi	RN	-5.56177144870162	-37.8667800407856
J67	Aurora da Serra, Apodi	RN	-5.56177144870162	-37.8667800407856
J68	Aurora da Serra, Apodi	RN	-5.56177144870162	-37.8667800407856
J70	Mossoró	RN	-5.21579196676611	-37.3215300310403
J71	Mossoró	RN	-5.21579196676611	-37.3215300310403
J72	Mossoró	RN	-5.21579196676611	-37.3215300310403
J73	Barrinha, Icapuí	CE	-4.82721707783639	-37.268912801519
J74	Barrinha, Icapuí	CE	-4.82721707783639	-37.268912801519
J75	Barrinha, Icapuí	CE	-4.82721707783639	-37.268912801519
J76	Barrinha, Icapuí	CE	-4.82721707783639	-37.268912801519
J78	Arapá, Tianguá	CE	-3.6133560	-40.9317650
J79	Arapá, Tianguá	CE	-3.6133560	-40.9317650
J80	Arapá, Tianguá	CE	-3.6133560	-40.9317650
J81	Arapá, Tianguá	CE	-3.6133560	-40.9317650
J82	Sitio Letreiro, Tianguá	CE	-3.5538220	-40.9067260

ID	*Location name*	*State*	*Latitude*	*Longitude*
J83	Sitio Letreiro, Tianguá	CE	-3.5538220	-40.9067260
J84	Sitio Letreiro, Tianguá	CE	-3.5538220	-40.9067260
J85	Sitio Letreiro, Tianguá	CE	-3.5538220	-40.9067260
J86	Sitio Letreiro, Tianguá	CE	-3.5538220	-40.9067260
J87	Sitio Letreiro, Tianguá	CE	-3.5538220	-40.9067260
J88	Lagoa Sto. Antonio, Aurora, Ararendá	CE	-4.698096960783	-40.6634870264679
J89	Lagoa Sto. Antonio, Aurora, Ararendá	CE	-4.698096960783	-40.6634870264679
J90	Lagoa Sto. Antonio, Aurora, Ararendá	CE	-4.698096960783	-40.6634870264679
J91	Lagoa Sto. Antonio, Aurora, Ararendá	CE	-4.698096960783	-40.6634870264679
J92	Lagoa Sto. Antonio, Aurora, Ararendá	CE	-4.698096960783	-40.6634870264679
J93	Lagoa Sto. Antonio, Aurora, Ararendá	CE	-4.698096960783	-40.6634870264679
J94	Lagoa Sto. Antonio, Aurora, Ararendá	CE	-4.698096960783	-40.6634870264679
J95	Camocim	CE	-3.020333	-41.073278
J98	Camocim	CE	-2.946417	-40.871417
J99	Camocim	CE	-3.020333	-41.073278
J100	Camocim	CE	-3.020389	-41.072944
J101	Camocim	CE	-2.946417	-40.871417

ID	*Location name*	*State*	*Latitude*	*Longitude*
J102	Camocim	CE	-3.020333	-41.073278
J104	Camocim	CE	-2.946417	-40.871417
J105	Morrinhos	CE	-3.241444	-40.064528
J107	Morrinhos	CE	-3.241444	-40.064528
J112	Camocim	CE	-2.942528	-40.871417
J116	Morrinhos	CE	-3.241444	-40.064528
J117	Morrinhos	CE	-3.241444	-40.064528
J118	Camaragibe	PE	-8.0433	-34.9438
J120	Taquaritinga do Norte	PE	-7.895722	-36.099611
J121	Taquaritinga do Norte	PE	-7.895722	-36.099611
J123	Taquaritinga do Norte	PE	-7.895722	-36.099611
J124	Taquaritinga do Norte	PE	-7.895722	-36.099611
J128	Taquaritinga do Norte	PE	-7.93725	-36.11825
J131	Taquaritinga do Norte	PE	-7.93725	-36.11825
J132*	Camaragibe	PE	-8.04329599253833	-34.9438010249286
J133*	Camaragibe	PE	-8.04329599253833	-34.9438010249286
J157	Aracoiaba	CE	-4.40326795913279	-38.7486160174012
J158	Aracoiaba	CE	-4.40326795913279	-38.7486160174012
J161	Aracoiaba	CE	-4.40095799043774	-38.7514920160174
J162	Aracoiaba	CE	-4.40095799043774	-38.7514920160174
J164	Aracoiaba	CE	-4.40095799043774	-38.7514920160174
J165	Aracoiaba	CE	-4.40095799043774	-38.7514920160174
J171	Aracoiaba	CE	-4.40717300400137	-38.7294359598308
J172	Aracoiaba	CE	-4.40717300400137	-38.7294359598308

ID	*Location name*	*State*	*Latitude*	*Longitude*
J173	Aracoiaba	CE	-4.40717300400137	-38.7294359598308
J178	Aracoiaba	CE	-4.40228501334786	-38.7326169759035
J179	Aracoiaba	CE	-4.40228501334786	-38.7326169759035
J180	Aracoiaba	CE	-4.40228501334786	-38.7326169759035
J220	São João do Rio do Peixe	PB	-6.69141403399407	-38.3740939758718
J221	São João do Rio do Peixe	PB	-6.69141403399407	-38.3740939758718
J222	São João do Rio do Peixe	PB	-6.69141403399407	-38.3740939758718
J223	São João do Rio do Peixe	PB	-6.69141403399407	-38.3740939758718
J224	São João do Rio do Peixe	PB	-6.69141403399407	-38.3740939758718
J225	São João do Rio do Peixe	PB	-6.69141403399407	-38.3740939758718
J226	São João do Rio do Peixe	PB	-6.69141403399407	-38.3740939758718
J227	São João do Rio do Peixe	PB	-6.69141403399407	-38.3740939758718
J228	São João do Rio do Peixe	PB	-6.69141403399407	-38.3740939758718
J230	São João do Rio do Peixe	PB	-6.69141403399407	-38.3740939758718
J231	São João do Rio do Peixe	PB	-6.69141403399407	-38.3740939758718
J232	São João do Rio do Peixe	PB	-6.69141403399407	-38.3740939758718
J244	Ararendá	CE	-4.69809402711689	-40.6635489687323
J245	Ararendá	CE	-4.69809402711689	-40.6635489687323
J246	Brejo da Madre de Deus	PE	-8.09712499380111	-36.41880299896
J247	Brejo da Madre de Deus	PE	-8.09712499380111	-36.41880299896
J250	Brejo da Madre de Deus	PE	-8.09712499380111	-36.41880299896
J251	Mourelandia	PE	-7.464116960763930	-39.470182964578200
J252	Mourelandia	PE	-7.464116960763930	-39.470182964578200

ID	*Location name*	*State*	*Latitude*	*Longitude*
J255	Mourelandia	PE	-7.464116960763930	-39.470182964578200
J256	Mourelandia	PE	-7.464116960763930	-39.470182964578200
J257	Mourelandia	PE	-7.464116960763930	-39.470182964578200
J259	Mourelandia	PE	-7.464116960763930	-39.470182964578200
J261	Mourelandia	PE	-7.464116960763930	-39.470182964578200
J262*	Tibau	RN	-5.152516979724160	-37.367801992222600
J263	Tibau	RN	-5.152516979724160	-37.367801992222600
J264	PNLM	MA	-2.584867	-42.796867
J265	PNLM	MA	-2.585267	-42.793383
J266	PNLM	MA	-2.585233	-42.793383
J269	PNLM	MA	-2.58175	-42.795367
J270	PNLM	MA	-2.580633	-42.79435
J272	PNLM	MA	-2.583283	-42.797883
J274	PNLM	MA	-2.5793333	-42.8062667
J275	PNLM	MA	-2.5795833	-42.8103667
J276	Tutóia	MA	-2.730692	-42.206304
J277	Brejo do Brugo	BA	-9.3428333	-38.4743889
J278	Brejo do Brugo	BA	-9.3413889	-38.4828611
J279	Brejo do Brugo	BA	-9.3413889	-38.4828611
J282	Brejo do Brugo	BA	-9.3426667	-38.47375
J283	Brejo do Brugo	BA	-9.3426667	-37.47375
J284	Brejo do Brugo	BA	-9.3425556	-38.4739167
J289	Brejo do Brugo	BA	-9.3891111	-38.4563333

ID	*Location name*	*State*	*Latitude*	*Longitude*
J290	Brejo do Brugo	BA	-9.3891389	-38.4563056
J292	Brejo do Brugo	BA	-9.3911111	-38.4636111
J293	Brejo do Brugo	BA	-9.3911111	-38.4636111
J294	Brejo do Brugo	BA	-9.3911111	-38.4636111
J296	Brejo do Brugo	BA	-9.391	-38.4786944
J297	Picuí	PB	-6.551574972	-36.23790904
J298	Picuí	PB	-6.551574972	-36.23790904

* Beekeepers reported previous introductions of bee colonies in these locations, and genetic clustering analyses revealed that these samples were attributed to distant populations. We therefore excluded these samples from subsequent analyses.

DISCUSSÃO GERAL

Na presente tese, foram desenvolvidos quatro estudos inéditos sobre a ecologia e a evolução das abelhas sem ferrão. Através deles, avançamos o entendimento da história natural do grupo, e fornecemos conhecimento-base voltado a sua conservação, através da (o):

(1) Descrição de fenômenos atípicos associados à biologia reprodutiva dos machos de abelhas sem ferrão, com destaque para as variações intraespecíficas.

(2) Reconstrução da história evolutiva do tamanho de colônias em Meliponini, acessando tendências macroevolutivas para entender a diversificação desse atributo no grupo.

(3) Uso de novas ferramentas - a genômica da paisagem - para avaliar que elementos favorecem a conectividade genética das populações naturais em uma espécie de abelha sem ferrão, e identificar adaptações às condições locais em nível genômico.

Considerados em conjunto (Figura 1), nossos estudos convergem para o maior entendedimento da **variabilidade intraespecífica** e das **adaptações** morfológicas, comportamentais e genômicas em Meliponini. Essas são informações valiosas para compreender as fontes do **isolamento reprodutivo** nas populações de abelhas sem ferrão, e contextualizar esse isolamento num cenário de rápidas transformações ambientais.

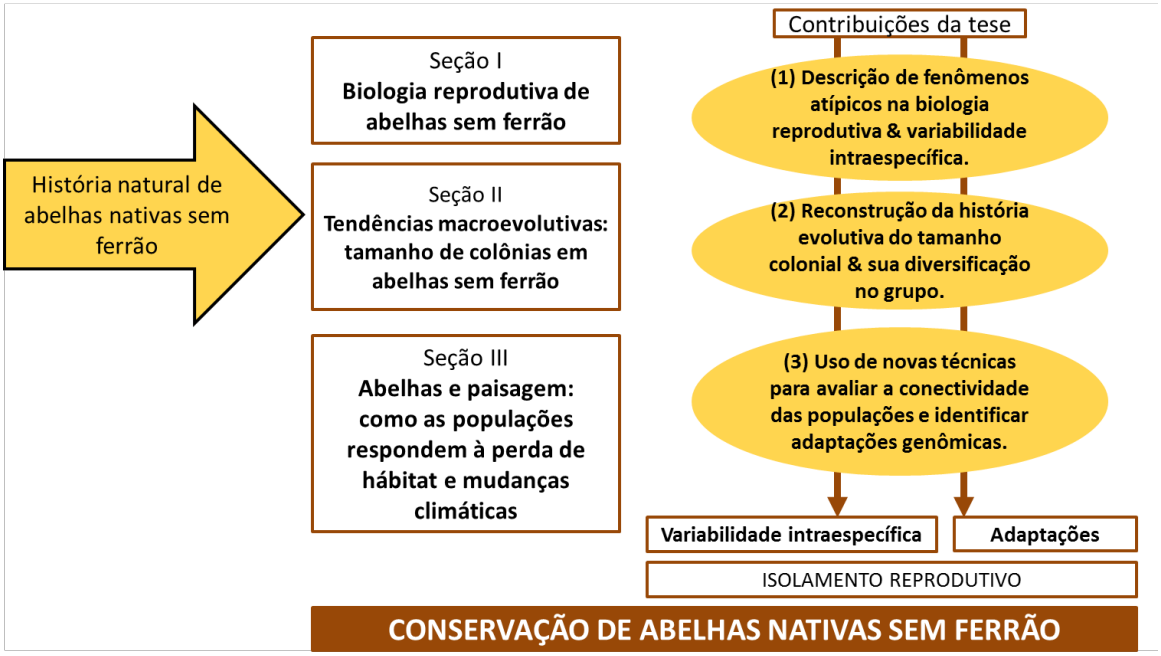


Figura 1: Representação das contribuições da tese. A seta em amarelo inclui o título, representando o objetivo geral da tese. Os retângulos vazados incluem os títulos de cada seção

do documento; as elipses descrevem as principais contribuições. As setas em coloração marrom indicam os temas transversais, convergindo para a questão central: a conservação das abelhas nativas sem ferrão.

Na Seção I, ao explorarmos a biologia reprodutiva das abelhas sem ferrão em dois estudos separados, fomos confrontados com fenômenos atípicos nunca antes relatados em maiores detalhes para o grupo. Em nosso primeiro estudo, observamos que os machos apresentam dimorfismo de coloração corporal; perdem suas cápsulas genitais ao tentar copular, tornando-se estéreis; e, mesmo nessa condição, podem permanecer vivos.

O dimorfismo de cor nos machos pode ter impactos sobre seu **fitness**. Nas abelhas sem ferrão, a cor do tegumento está ligada ao controle da temperatura corporal e à perda de água (Pereboom & Biesmeijer, 2003). Sabe-se que a coloração clara é vantajosa em ambientes quentes, abertos e de baixa altitude, enquanto a cor escura, em ambientes úmidos e montanhosos (Pereboom & Biesmeijer, 2003). Descrevemos em maiores detalhes a variação na coloração corporal em machos de *M. flavolineata* (previamente observado por Maués-Venturieri, 1991), mostrando que essa variação manifesta-se facultativamente numa espécie de integumento claro (Schwarz, 1932). O fenômeno levanta questionamentos sobre as possíveis vantagens de um macho apresentar coloração escura nas diferentes paisagens tropicais, em comparação com aqueles de coloração clara. Considerando que os machos são o veículo de dispersão de genes por longas distâncias (Mueller et al., 2012; Beani et al., 2014), o dimorfismo de cor também pode impactar o fitness da rainha. Se os benefícios se restringem à sobrevivência dos machos – a coloração do macho afeta sua sobrevivência em diferentes ambientes? -, ou se estendem-se ao seu sucesso reprodutivo – há diferença na qualidade reprodutiva entre machos claros e escuros? -, são questões que permanecem em aberto. Para responder tais perguntas, *M. flavolineata* é um excelente modelo de estudo, seja pelo dimorfismo de cor entre os machos (Seção I), seja pela sua ampla distribuição em diferentes biomas brasileiros (Pedro, 2014).

Com relação aos machos estéreis, era inesperado que permanecessem vivos após a perda da sua cápsula genital (em comparação com machos de *Apis* spp., Koeniger & Koeniger, 1991), e que, mesmo estéreis, ainda fossem capazes de frequentar agregações reprodutivas por até dois dias. A presença de machos estéreis em agregações reprodutivas levanta questões sobre como isso impacta as populações em termos de razão sexual, uma vez que a probabilidade de um determinado macho acasalar, nesse caso, dependerá em grande

parte da densidade de indivíduos compondo a agregação (Alcock et al., 1978; Alexander et al., 1997). Além disso, abre questionamentos sobre a quantidade de machos estéreis ecnontrada nas agregações (5% - 10% do total que amostramos): investigando que parte dessa porcentagem se deve a cópulas efetivas, ou a tentativas mal-sucedidas (que também os fazem perder permanentemente suas cápsulas) e, combinando essa informação com a caracterização morfológica e comportamental desses machos, permitirá testar como os episódios de seleção sexual eliminam ou favorecem determinados indivíduos, numa perspectiva populacional (Rhainds, 2010, 2019; Harpur et al., 2013). As variações intraespecíficas e intracoloniais, como por exemplo o dimorfismo de cor dos machos, e a capacidade individual dos machos de realizarem acasalamentos efetivos, podem ser fonte de isolamento reprodutivo nas populações de abelhas sem ferrão.

Em nosso segundo estudo (Seção I), investigamos a morfologia funcional da cápsula genital masculina em sete espécies de Meliponini Neotropicais. Nesse grupo, a cápsula masculina funciona como plugue copulatório (Kerr et al., 1962), uma estratégia do macho para limitar o número de acasalamentos da fêmea (Parker, 1970; Alcock, 1994). Novamente, fomos confrontados com fenômenos ainda não descritos para Meliponini: pela primeira vez, relatamos a ocorrência de plugues permanentes e de lesões genitais causadas por estruturas do plugue, as valvas do pênis. Em pelo menos uma espécie, *M. fasciculata*, o plugue é de longo prazo, ou seja, permanece fixado no interior das rainhas fisogástricas por toda sua vida (que podem alcançar até sete anos de idade, como observado em algumas espécies de *Melipona*, Carvalho-Zilse & Kerr, 2004); enquanto nas demais espécies, este é de curto prazo (tempo de permanência ainda indeterminado). Por fim, em *M. seminigra*, observamos repetidamente múltiplas lesões, indicando a ocorrência de **reacasalamento** nessa espécie.

Nosso estudo sugere que os sistemas de acasalamento de abelhas sem ferrão não são uniformes, desafiando a hipótese vigente de monogamia generalizada em Meliponini (Strassmann, 2001; Jaffé et al., 2015). A possibilidade de uma rainha copular com mais de um macho, como é o caso de *M. seminigra*, abre novos questionamentos sobre as consequências do conflito sexual nas abelhas sem ferrão – considerando que os plugues de cópula causam lesões na fêmea, quais os custos de acasalar com mais de um macho? Embora os acasalamentos múltiplos possam resultar em custos para as fêmeas (Parker et al., 2013), deve-se considerar seu principal benefício: o aumento da variabilidade genética entre a prole (Jennions & Petrie, 2000; Barth et al., 2014). Dessa maneira, investigar sob quais condições ecológicas os benefícios de um acasalamento múltiplo superam os seus custos em abelhas sem

ferrão (*i. e.* resistência a patógenos e otimização da divisão de tarefas na colônia, como em *A. mellifera*, Jennions & Petrie, 2000), é importante para entender a dinâmica das suas populações frente a possíveis gargalos genéticos (Alves et al., 2011), e ao fluxo gênico restrito resultante de sua baixa capacidade de dispersão (Van Veen & Sommeijer, 2000; Roubik, 2006; Vit et al., 2013). Por fim, propomos a investigação das lesões genitais como uma maneira simples e de menor custo para quantificar as tentativas de cópula das rainhas em abelhas sem ferrão. A partir da sua aplicação, poderemos investigar a possível ocorrência de poliandria em outras espécies de meliponíneos; e em combinação com técnicas moleculares para determinação de paternidade, o diganóstico de acasalamento múltiplo poderá ser confirmado.

Até o momento, exploramos as variações dentro das espécies. Contudo, é por meio de uma perspectiva comparativa entre as espécies que podemos entender um pouco mais sobre como as abelhas sem ferrão diferem na sua capacidade de lidar com o ambiente. Essas diferenças se devem em especial ao número de abelhas que compõem cada sociedade – tamanho da colônia -, que é de fato a característica mais importante para insetos sociais. Na Seção II, usamos uma abordagem comparativa a fim de reconstruir a história evolutiva do tamanho das colônias em Meliponini, nos revelando um processo evolutivo marcado por progressões e regressões entre os seus diferentes estados – pequenas, médias e grandes colônias. Nossa abordagem mostrou que o estado ancestral mais provável para o grupo são colônias de tamanho médio (entre 1.000 e 5.000 indivíduos adultos). A partir do estado ancestral, *i. e.* colônias de tamanho médio, observamos a derivação de outros dois, colônias pequenas e grandes, indicando flexibilidade na transição entre os estados ancestral e derivados. Além disso, nossos resultados sobre correlação evolutiva entre tamanho de colônias e características sociais (a qualidade da casta operária e o dimorfismo entre rainhas e operárias), e do ninho (arquitetura dos favos e o hábito de nidificação), evidenciam a complexidade social subjacente à diversidade de espécies em Meliponini (Bourke, 1999; Kapheim, 2018). Em termos práticos, as tendências macroevolutivas no tamanho e colônias de Meliponini, variando entre colônias pequenas, médias e grandes, revelam sua ampla capacidade de lidar com o ambiente ao longo da sua faixa de ocorrência no globo, durante sua história evolutiva (estima-se seu início entre 81 e 96 milhões de anos atrás, Rasmussen & Cameron, 2010). Além de abrir novas questões sobre a história evolutiva do grupo, a variabilidade nas características sociais das diferentes espécies de abelhas sem ferrão reforça seu papel como insetos polinizadores, oferecendo às diversas plantas tropicais, cultivadas ou

não, um amplo e variado serviço de polinização (Slaa et al., 2006; Giannini et al., 2015; Roubik et al., 2018).

Na Seção III, utilizamos uma nova abordagem, a genômica de paisagem, a fim de avaliar como as populações de uma espécie de abelha sem ferrão respondem às alterações do ambiente, buscando compreender que elementos da paisagem favorecem a conectividade ecológica e genética das populações naturais da abelha Jandaíra (*M. subnitida*) ao longo da sua distribuição geográfica. Pela primeira, vez foi realizado um estudo que combinou alta cobertura do genoma de uma espécie de abelha tropical não-modelo, com amostragem ao longo de toda sua distribuição geográfica. Nossos resultados apontam que a espécie *M. subnitida* estrutura-se em quatro populações. Mostram ainda, que a redução de áreas favoráveis à ocorrência da espécie (hábitat) não afeta a diversidade genética das diferentes populações, porém reduz o seu fluxo gênico. Além disso, mostram que o genoma dessa espécie apresenta regiões fortemente associadas às condições locais de temperatura, precipitação e cobertura de floresta, indicando que essas condições favorecem a conectividade genética em determinadas áreas, e o isolamento reprodutivo em outras.

Embora seja um processo subjacente à diferenciação das populações e à especiação (Allendorf et al., 2013; Balkenhol et al., 2016; Allendorf, 2017), o isolamento reprodutivo torna-se uma questão sensível diante de entraves como a perda de hábitat e as mudanças climáticas. Apesar de *M. subnitida* ser uma espécie tolerante ao calor extremo (Hrncir et al., 2019) e a longos períodos de seca (Maia-Silva et al., 2014, 2015), a perda de áreas florestadas afeta negativamente o seu fluxo gênico, processo microevolutivo fundamental para resiliência das populações, naturais ou manejadas (Balkenhol et al., 2016, 2017; Jaffé et al., 2016). Ocorrendo ruptura desse fluxo entre as diferentes áreas de ocorrência da espécie, as populações desconectadas estarão mais vulneráveis à erosão de sua variabilidade genética (Allendorf et al., 2013; Lozier & Zayed, 2017).

Por fim, o deslocamento de colônias de abelhas sem ferrão consiste num terceiro agravante (Byatt et al., 2016; Chapman et al., 2018), cujo maior impacto é a homogeneização genética das populações manejadas (Jaffé et al., 2016). A movimentação antrópica de colônias pode resultar em diluição ou perda de adaptações em nível genômico, como aquelas que encontramos em *M. subnitida*. Por exemplo, deslocar colônias de Jandaíra dos brejos de altitude (Porto et al., 2004; Albuquerque et al., 2012) para regiões ao nível do mar, significa remover esses **alelos** do seu local de origem, e introduzí-los em condições totalmente adversas

de temperatura, precipitação e estabilidade termal, podendo alterar sua susceptibilidade a doenças e parasitas (Vollet-Neto et al., 2018). Portanto, para conservação futura desses polinizadores tropicais, sugere-se regulação das práticas de meliponicultura, como priorizar a criação de espécies locais (Jaffé et al., 2015) e evitar o deslocamento de ninhos para regiões muito distantes ou ambientalmente muito diferentes (Jaffé et al., 2016). Para Jandaíra, por exemplo, sugerimos que seu transporte associado à meliponicultura seja restrito à distribuição das suas quatro populações, com deslocamentos a menos de 300 km da sua origem; e ainda, que os gradientes de latitude e altitude sejam também respeitados como critério para decidir de onde e para onde transportar essas colônias. Com essas recomendações, esperamos contribuir para manutenção do fluxo gênico natural e da variabilidade adaptativa associada a condições locais. Por fim, quais os limites desse deslocamento para outras espécies, são informações que ainda precisam ser levantadas (Vollet-Neto et al., 2018).

Sobre as condições climáticas no futuro, há uma corrente discussão sobre quais espécies serão favorecidas e quais serão simplesmente eliminadas, cujo objetivo é traçar estratégias voltadas a manutenção dos níveis globais atuais de diversidade no futuro (Vanbergen et al., 2013; Samways, 2015; Potts et al., 2016; Brown et al., 2016; Hill et al., 2019); e pela sua relevância ecológica, econômica e cultural, essa discussão certamente inclui as abelhas nativas sem ferrão (Potts et al., 2016; Giannini, Maia-Silva, et al., 2017; Hill et al., 2019). Sabemos que muitas espécies terão suas áreas hábitat deslocadas, alterando a sua probabilidade de ocorrência futura, e em alguns casos restando-lhe apenas refúgios climáticos (Wennersten & Robbins, 2017; Giannini, Costa, et al., 2017; Giannini, Maia-Silva, et al., 2017). Levantamos essa breve discussão a fim de lembrar que não é possível discutir o futuro, menos ainda conservação, sem considerá-los à luz do presente. Entender que por traz dessas poderosas ferramentas prediditivas, o dado fundamental é o simples, porém desafiador, registro de ocorrência das espécies, bem como o conhecimento aprofundando de sua história natural (Pearson et al., 2007; Cassini, 2011; Guillera-Arroita et al., 2017; Reside et al., 2019), ressalta o quão importante é ir a campo, encontrar as abelhas, descrever seus hábitos e comportamentos e, na tentativa de entendê-las um pouco mais, buscar conservá-las usando as melhores e mais eficientes estratégias que pudermos desenvolver.

Nossas contribuições avançam o conhecimento básico e aplicado sobre a história natural das abelhas nativas sem ferrão, fornecendo informações para ampliar as possibilidades de conservação dessas abelhas. Contudo, mais esforços de pesquisa são necessários para atingir

práticas de conservação através do seu uso sustentável. Com base nos estudos apresentados nessa tese, sugerimos esforços de pesquisa nas seguintes áreas: (i) **sistemas de acasalamento em abelhas sem ferrão e sua interação com o ambiente** – entender sob quais condições os sistemas de acasalamento alternam entre monândrico e poliândrico em diferentes espécies de abelhas sem ferrão pode fornecer pistas sobre como as populações naturais e manejadas sobrevivem a possíveis gargalos genéticos, a exemplo daqueles originados de intensas alterações na paisagem; (ii) **distribuição espacial da variabilidade genética (neutral e adaptativa), com a finalidade de projetar ações conservacionistas baseadas na manutenção dos processos naturais de fluxo gênico e adaptação local** – entender como a paisagem modela a distribuição da variabilidade genética das populações de diferentes espécies de abelhas sem ferrão pode fornecer direcionamentos claros sobre os limites a serem respeitados no deslocamento do material genético que as colônias de abelhas representam, assim favorecendo seus processos naturais de fluxo gênico e adaptação local. Além disso, informa sobre que elementos da paisagem devem ser priorizados – a exemplo da vegetação nativa no sopé das chapadas - para manter a conectividade entre as populações de abelhas separadas por gradientes de altitude.

Concentrar esforços nesses dois tópicos poderá favorecer a continuidade espaço-temporal das populações naturais e manejadas, visando a manutenção de todo o seu potencial evolutivo. Dessa maneira, esperamos contribuir para que as abelhas sem ferrão, um grupo de polinizadores tão antigo e especializado, siga sua história evolutiva ao longo da sua distribuição geográfica, mantendo serviços ecosssitêmicos de polinização em todos os ambientes onde ocorrem.

Referências

ALBUQUERQUE, U.P. DE, DE LIMA ARAÚJO, E., EL-DEIR, A.C.A., DE LIMA, A.L.A., SOUTO, A., BEZERRA, B.M., ET AL. (2012) Caatinga Revisited: Ecology and Conservation of an Important Seasonal Dry Forest. *The Scientific World Journal*, 2012, 1–18.

ALCOCK, J. (1994) Postinsemination associations between males and females in insects: the mate-guarding hypothesis. *Annual Review of Entomology*, 1–21.

ALCOCK, J., BARROWS, E.M., GORDH, G., HUBBARD, L.J., KIRKENDALL, L., PYLE, D.W., ET AL. (1978) The ecology and evolution of male reproductive behaviour in the bees and wasps. *Zoological Journal of the Linnean Society*, 64, 293–326.

ALEXANDER, R.R.D., MARSHALL, D.D. & COOLEY, J. (1997) Evolutionary perspectives on insect mating. *The evolution of mating system in insects and arachnids*, 4–31.

ALLENDORF, F.W. (2017) Genetics and the conservation of natural populations: allozymes to genomes. *Molecular Ecology*, 26, 420–430.

ALLENDORF, F.W., LUIKART, G. & AITKEN, S.N. (2013) Conservation and the genetics of populations, 2nd edition. Wiley-Blackwell.

ALVES, D.A., IMPERATRIZ-FONSECA, V.L., FRANCOY, T.M., SANTOS-FILHO, P.S., BILLEN, J. & WENSELEERS, T. (2011) Successful maintenance of a stingless bee population despite a severe genetic bottleneck. *Conservation Genetics*, 12, 647–658.

BALKENHOL, N., CUSHMAN, S.A., WAITS, L.P. & STORFER, A. (2016) Landscape Genetics: Concepts, Methods, Applications. John Wiley & Sons, Hoboken, NJ.

BALKENHOL, N., DUDANIEC, R.Y., KRUTOVSKY, K. V, JOHNSON, J.S., CAIRNS, D.M., SEGELBACHER, G., ET AL. (2017) Landscape Genomics: Understanding Relationships Between Environmental Heterogeneity and Genomic Characteristics of Populations. In *Population Genomics: Concepts, Approaches and Applications* (ed O.P. Rajora), p. . Springer International Publishing.

BARTH, M.B., MORITZ, R.F.A. & KRAUS, F.B. (2014) The evolution of extreme polyandry in social insects: Insights from army ants. *PLoS ONE*, 9.

BEANI, L., DESSÌ-FULGHERI, F., CAPPA, F. & TOTH, A. (2014) The trap of sex in social insects: From the female to the male perspective. *Neuroscience & Biobehavioral Reviews*, 46, 519–533. Elsevier Ltd.

BOURKE (1999) Colony size, social complexity and reproductive conflict in social insects. *Journal of Evolutionary Biology*, 12, 245–257.

BROWN, M.J.F., DICKS, L. V., PAXTON, R.J., BALDOCK, K.C.R., BARRON, A.B., CHAUZAT, M.-P., ET AL. (2016) A horizon scan of future threats and opportunities for pollinators and pollination. *PeerJ*, 4, e2249.

BYATT, M.A., CHAPMAN, N.C., LATTY, T. & OLDROYD, B.P. (2016) The genetic consequences of the anthropogenic movement of social bees. Springer Basel. *Insectes Sociaux*.

CARVALHO-ZILSE, G.A. & KERR, W.E. (2004) Substituição natural de rainhas fisogástricas e distância de vôo dos machos em Tiuba (*Melipona compressipes fasciculata* Smith, 1854) e Uruçu (*Melipona scutellaris* Latreille, 1811) (Apidae, Meliponini). *Acta Amazonica*, 34, 649–652.

CASSINI, M.H. (2011) Ecological principles of species distribution models: the habitat matching rule. *Journal of Biogeography*, 38, 2057–2065.

CHAPMAN, N.C., BYATT, M., COCENZA, R.D.S., NGUYEN, L.M., HEARD, T.A., LATTY, T. & OLDROYD, B.P. (2018) Anthropogenic hive movements are changing the genetic structure of a stingless bee (Tetragonula carbonaria) population along the east coast of Australia. *Conservation Genetics*, 19, 619–627. Springer Netherlands.

CORTOPASSI-LAURINO, M., IMPERATRIZ-FONSECA, V.L., ROUBIK, D.W., DOLLIN, A., HEARD, T., AGUILAR, I., ET AL. (2006) Global meliponiculture : challenges and opportunities. *Apidologie*, 37, 275–292.

FRANCINI, I.B. (2013) Monandria e Poliandria como estratégia evolutiva no complexo de subespécies de Melipona seminigra Friese, 1903 (Apidae, Meliponini) na Amazônia. Instituto Nacional de Pesquisas da Amazônia.

GIANNINI, T.C., BOFF, S., CORDEIRO, G.D., CARTOLANO, E.A., VEIGA, A.K., IMPERATRIZ-FONSECA, V.L. & SARAIVA, A.M. (2015) Crop pollinators in Brazil: a review of reported interactions. *Apidologie*, 46, 209–223.

GIANNINI, T.C., COSTA, W.F., CORDEIRO, G.D., IMPERATRIZ-FONSECA, V.L., SARAIVA, A.M., BIESMEIJER, J. & GARIBALDI, L.A. (2017) Projected climate change threatens pollinators and crop production in Brazil. *PLOS ONE*, 12, e0182274.

GIANNINI, T.C., MAIA-SILVA, C., ACOSTA, A.L., JAFFÉ, R., CARVALHO, A.T., MARTINS, C.F., ET AL. (2017) Protecting a managed bee pollinator against climate change: strategies for an area with extreme climatic conditions and socioeconomic vulnerability. *Apidologie*, 48, 784–794.

GUILLERA-ARROITA, G., LAHOZ-MONFORT, J.J., VAN ROOYEN, A.R., WEEKS, A.R. & TINGLEY, R. (2017) Dealing with false-positive and false-negative errors about species occurrence at multiple levels. *Methods in Ecology and Evolution*, 8, 1081–1091.

HARPUR, B.A., SOBHANI, M. & ZAYED, A. (2013) A review of the consequences of complementary sex determination and diploid male production on mating failures in the Hymenoptera. *Entomologia Experimentalis et Applicata*, 146, 156–164. John Wiley & Sons, Ltd (10.1111).

HILL, R., NATES-PARRA, G., QUEZADA-EUÁN, J.J.G., BUCHORI, D., LEBUHN, G., MAUÉS, M.M., ET AL. (2019) Biocultural approaches to pollinator conservation. *Nature Sustainability*, 2, 214–222. Springer US.

HRNCIR, M., MAIA-SILVA, C., DA SILVA TEIXEIRA-SOUZA, V.H. & IMPERATRIZ-FONSECA, V.L. (2019) Stingless bees and their adaptations to extreme environments. *Journal of Comparative Physiology A*, 1–12. Springer Berlin Heidelberg.

JAFFÉ, R., POPE, N., ACOSTA, A.L., ALVES, D.A., ARIAS, M.C., DE LA RÚA, P., ET AL. (2016) Beekeeping practices and geographic distance, not land use, drive gene flow across tropical bees. *Molecular Ecology*, 25, 5345–5358.

JAFFÉ, R., POPE, N., CARVALHO, A.T., MAIA, U.M., BLOCHTEIN, B., DE CARVALHO, C.A.L., ET AL. (2015) Bees for development: brazilian survey reveals how to optimize stingless beekeeping. *PLOS ONE*, 10, 1–21.

JENNIONS, M.D. & PETRIE, M. (2000) Why do females mate multiply? A review of the genetic benefits. *Biological Reviews*, 75, 21–64.

KAPHEIM, K.M. (2018) Synthesis of Tinbergen's four questions and the future of sociogenomics. Behavioral Ecology and Sociobiology. *Behavioral Ecology and Sociobiology*. .

KERR, W.E., ZUCCHI, R., NAKADAIRA, J.T. & BUTOLO, J.E. (1962) Reproduction in the Social Bees (Hymenoptera: Apidae). *Journal of the New York Entomological Society*, 70, 265–276.

KOENIGER, N. & KOENIGER, G. (1991) An evolutionary approach to mating behaviour and drone copulatory organs in *Apis*. *Apidologie*, 22, 581–590.

LOZIER, J.D. & ZAYED, A. (2017) Bee conservation in the age of genomics. *Conservation Genetics*, 18, 713–729. Springer Netherlands.

MAIA-SILVA, C., HRNCIR, M., SILVA, C.I. & IMPERATRIZ-FONSECA, V.L. (2015) Survival strategies of stingless bees (Melipona subnitida) in an unpredictable environment, the Brazilian tropical dry forest. *Apidologie*, 46, 631–643.

MAIA-SILVA, C., IMPERATRIZ-FONSECA, V.L., SILVA, C.I. & HRNCIR, M. (2014) Environmental windows for foraging activity in stingless bees, Melipona subnitida Ducke and Melipona quadrifasciata lepeletier (Hymenoptera: Apidae: Meliponini). *Sociobiology*, 61, 378–385.

MAUÉS-VENTURIERI, M.M. (1991) Aspectos etológicos do processo de oviposição em *Melipona rufiventris flavolineata* Friese, 1900 (Hymenoptera, Apidae, Meliponinae). Dissertação de Mestrado, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da Universidade de São Paulo - Ribeirão Preto, SP.

MUELLER, M.Y., MORITZ, R.F. & KRAUS, F.B. (2012) Outbreeding and lack of temporal genetic structure in a drone congregation of the neotropical stingless bee Scaptotrigona mexicana. *Ecology and Evolution*, 2, 1304–1311.

PALMER, K.A., OLDROYD, B.P., QUEZADA-EUÁN, J.J.G., PAXTON, R.J. & MAY-ITZÁ, W. DE J. (2002) Paternity frequency and maternity of males in some stingless bee species. *Molecular Ecology*, 11, 2107–2113.

PARKER, G.A. (1970) Sperm competition and its evolutionary consequences in the insects. *Biological Reviews*, 45, 525–567.

PARKER, G.A., BIRKHEAD, T.R. & PARKER, G.A. (2013) Polyandry: the history of a revolution. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368, 20120335–20120335.

PEARSON, R.G., RAXWORTHY, C.J., NAKAMURA, M. & TOWNSEND PETERSON, A. (2007) Predicting species distributions from small numbers of occurrence records: A test case using cryptic geckos in Madagascar. *Journal of Biogeography*, 34, 102–117.

PEDRO, S.R.M. (2014) The stingless bee fauna in Brazil (Hymenoptera : Apidae). *Sociobiology*, 61, 348–354.

PEREBOOM, J.J.M. & BIESMEIJER, J.C. (2003) Thermal constraints for stingless bee foragers: The importance of body size and coloration. *Oecologia*, 137, 42–50.

PETERS, J.M., QUELLER, D.C., IMPERATRIZ-FONSECA, V.L., ROUBIK, D.W. & STRASSMANN, J.E. (1999) Mate number, kin selection and social conflicts in stingless bees and honeybees. *Proceedings of the Royal Society B: Biological Sciences*, 266, 379–384.

PORTO, KJ.C., CABRAL, J.J.P. & TABARELLI, M. (2004) Brejos de Altitude em Pernambuco e Paraíba. In *Brejos de Altitude em Pernambuco e Paraíba: História Natural, Ecologia e Conservação* p. .

POTTS, S.G., FONSECA, V.L.I.-, NGO, H.T., BIESMEIJER, J.C., BREEZE, T.D., DICKS, L. V., ET AL. (2016) IPBES (2016): Summary for policymakers of the assessment report of the Intergovernmental Science - Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production. Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, Bonn, Germany.

RASMUSSEN, C. & CAMERON, S.A. (2010) Global stingless bee phylogeny supports ancient divergence, vicariance, and long distance dispersal. *Biological Journal of the Linnean Society*, 99, 206–232.

RESIDE, A.E., CRITCHELL, K., CRAYN, D.M., GOOSEM, M., GOOSEM, S., HOSKIN, C.J., ET AL. (2019) Beyond the model: expert knowledge improves predictions of species' fates under climate change. *Ecological Applications*, 29, e01824.

RHAINDS, M. (2010) Female mating failures in insects. *The Netherlands Entomological Society*, 136, 211–226.

RHAINDS, M. (2019) Ecology of female mating failure/lifelong virginity: a review of causal mechanisms in insects and arachnids. *Entomologia Experimentalis et Applicata*, 167, 73–84.

ROUBIK, D.W. (2006) Stingless bee nesting biology. *Apidologie*, 37, 124–143.

ROUBIK, D.W., HEARD, T. & KWAPONG, P. (2018) Stingless bee colonies and pollination. In *The pollination of cultivated plants: A compendium for practitioners* pp. 39–64.

SAMWAYS, M.J. (2015) Future-proofing insect diversity. *Current Opinion in Insect Science*, 12, 71–78. Elsevier Inc.

SLAA, E.J., SÁNCHEZ CHAVES, L.A., MALAGODI-BRAGA, K.S. & HOFSTEDE, F.E. (2006) Stingless bees in applied pollination: practice and perspectives. *Apidologie*, 37, 293–315.

SCHWARZ, H.F. (1932) The genus Melipona the type genus of the Meliponidae or stingless bees. *Bulletin of the American Museum of Natural History*, 63, 231–460.

VANBERGEN, A.J., GARRATT, M.P., VANBERGEN, A.J., BAUDE, M., BIESMEIJER, J.C., BRITTON, N.F., ET AL. (2013) Threats to an ecosystem service: Pressures on pollinators. *Frontiers in Ecology and the Environment*, 11, 251–259.

VAN VEEN, J.W. & SOMMEIJER, M.J. (2000) Colony reproduction in Tetragonisca angustula (Apidae, Meliponini). *Insectes Sociaux*, 47, 70–75.

VIT, P., PEDRO, S.R.M. & ROUBIK, D.W. (2013) Pot-Honey: A legacy of stingless bees. Springer Science & Business Media, New York, NY.

VOLLET-NETO, A., BLOCHTEIN, B., VIANA, B.F., DOS SANTOS, C.F., MENEZES, C., NUNES-SILVA, P., ET AL. (2018) Desafios e recomendações para o manejo e transporte de polinizadores. A.B.E.L.H.A., São Paulo.

VOLLET-NETO, A., KOFFLER, S., DOS SANTOS, C.F., MENEZES, C., NUNES, F.M.F., HARTFELDER, K., ET AL. (2018) Recent advances in reproductive biology of stingless bees. *Insectes Sociaux*, 0, 1–12. Springer International Publishing.

VOLLET-NETO, A., OLIVEIRA, R.C., SCHILLEWAERT, S., ALVES, D.A., WENSELEERS, T., NASCIMENTO, F.S., ET AL. (2017) Diploid Male Production Results in Queen Death in the Stingless Bee Scaptotrigona depilis. *Journal of Chemical Ecology*, 43, 403–410. Journal of Chemical Ecology.

WENNERSTEN, J. & ROBBINS, D. (2017) Rising Tides: Climate Refugees in the Twenty-first Century.

Glossário*

*Definições construídas a partir de consulta bibliográfica indicada ao final do glossário.

Abelhas corbiculadas: clado monofilético de abelhas cuja característica principal é a presença da corbícula (ou cesta de pólen), uma modificação no último par de pernas das fêmeas. Compreende as tribos Apini, Meliponini, Bombini e Euglossini. Dentre elas, as três primeiras são eussociais.

Adaptação: processo que ocorre em uma população - sob forças seletivas, ao longo do tempo e de muitas gerações - gerando diferenciação genética nas linhagens futuras. Também entendida como mudanças em uma população que aumentam o seu fitness.

Alelo: forma alternativa, ou variante, de um gene, situado em um mesmo loco em cromossomos homológos; responsável pelas diferentes manifestações fenotípicas de um caráter, apresentando segregação monogênica.

Apini: tribo das abelhas melíferas (*Apis* sp.), de morfologia apiforme, e comportamento eusocial avançado. Sua distribuição geográfica se dá em todas as regiões africanas e orientais, estendendo-se pelas regiões (i) paleártico norte, (ii) sul da Noruega e (iii) provínciais marítimas do pacífico da Rússia.

Bombini: tribo das abelhas mamangavas de morfologia bombiforme e comportamento eusocial primitivo. Sua distribuição geográfica se dá principalmente em áreas de clima temperado, alcançando altitudes e latitudes mais altas que outras abelhas. Algumas poucas espécies ocorrem em climas tropicais.

Cerume: mistura de cera produzida pelas abelhas e resinas vegetais coletadas em plantas. Material produzido com exclusividade pelas abelhas sem ferrão.

Eussocialidade: comportamento social caracterizado por sobreposição de gerações, cuidado reprodutivo com a prole e divisão reprodutiva de trabalho (geralmente resultando em castas morfo-funcionalmente distintas). Nas sociedades com eussocialidade avançada, a diferenciação de castas é irreverssível, não podendo a rainha tornar-se uma operária, e vice-versa.

Favos: uma camada, ou camadas, de células de cria. Nas abelhas sem ferrão, podem assumir diferentes arquiteturas: discos, semi-discos, cachos, formato irregular e verticais.

Fitness: medida teórica do sucesso reprodutivo de um indivíduo. Representa sua aptidão direta por meio do número de descendentes, e da persistência de seus genes nas gerações seguintes.

Isolamento reprodutivo: ausência ou redução do fluxo gênico entre populações devido a diferenças fenotípicas relacionadas ao processo de seleção natural ou sexual.

Macroevolução: consiste na escala de um processo evolutivo que ocorre em nível de espécie, ou acima. A definição de macroevolução faz contraponto com a microevolução, a qual se refere a mudanças evolutivas ao nível das frequências alélicas dentro das populações de uma espécie. Ambas descrevem o mesmo processo – evolução –, porém em diferentes escalas temporais.

Mel: substância produzida pelas abelhas (Apini e Meliponini), a partir do néctar coletado nas plantas.

Meliponini: tribo das abelhas sem ferrão, com individuos de corpo trigoniforme a apiforme, e que apresentam comportamento eusocial avançado. Sua distribuição geográfica é pantropical, ocorrendo nas regiões tropicais e subtropicais do globo.

Polimorfismo: é um fenômeno ao nível de indivíduos ou genes, consistindo na ocorrência de duas ou mais formas de uma unidade em uma população. Em insetos eussociais, a ocorrência de diferentes tipos de operárias, como observado em formigas, é um exemplo de polimorfismo ao nível de indivíduos. Em populações, quando os genes apresentam variantes com frequência > 1%, portanto mais de um alelo, diz-se que são genes polimórficos.

Polinização: transferência do pólen do órgão masculino da flor para os órgãos femininos de flores da mesma espécie, resultando na fertilização de seus óvulos.

Reacasalamento: comportamento de acasalar mais de uma vez. O termo foi traduzido livremente do inglês “remating”.

Serviços ecossistêmicos: consituem os muitos e variados benefícios que os humanos obtêm do ambiente natural e dos ecossistemas quando estes se encontram em equilíbrio.

Bibliografia consultada

ALLENDORF, F.W. (2017) Genetics and the conservation of natural populations: allozymes to genomes. *Molecular Ecology*, 26, 420–430.

CGIAR RESEARCH PROGRAM ON WATER LAND AND ECOSYSTEMS, W. (2014) Ecosystem services and resilience framework. Colombo, Sri Lanka.

EBERHARD, W.G. (1985) Sexual selection and animal genitalia. Harvard University Press.

MICHENER, C.D. (2007) The bees of the world, 2nd edition. Johns Hopkins University Press, Baltimore, Maryland, USA.

NOGUEIRA-NETO, P. (1997) Vida e criação de abelhas indígenas sem ferrão. Nogueirapis, São Paulo.

VIT, P., PEDRO, S.R.M. & ROUBIK, D.W. (2013) Pot-Honey: A legacy of stingless bees. Springer Science & Business Media, New York, NY.

VIT, P., PEDRO, S.R.M. & ROUBIK, D.W. (2018) Pot-pollen in stingless bee melittology. In *Pot-Pollen in Stingless Bee Melittology* p. . Springer, Cham, CH.