



UNIVERSIDADE FEDERAL DE MINAS GERAIS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS

Departamento de Botânica

Programa de Pós-Graduação em Biologia Vegetal



UFMG

Samuel Siriani de Oliveira

**POLINIZAÇÃO DE ESPÉCIES DE *BLUMENBACHIA* SCHRAD. (LOASACEAE):
oferta de recursos florais e comportamento de forrageio de polinizadores**

Belo Horizonte

2020

Samuel Siriani de Oliveira

**POLINIZAÇÃO DE ESPÉCIES DE *BLUMENBACHIA* SCHRAD. (LOASACEAE):
oferta de recursos florais e comportamento de forrageio de polinizadores**

Tese apresentada ao Programa de Pós-Graduação em Biologia Vegetal da Universidade Federal de Minas Gerais como requisito parcial para a obtenção do título de doutor em Ciências Biológicas – Biologia Vegetal

Orientador: Clemens Schindwein

Belo Horizonte

2020

043

Oliveira, Samuel Siriani de.

Polinização de espécies de *Blumenbachia schrad.* (Loasaceae): oferta de recursos florais e comportamento de forrageio de polinizadores [manuscrito] / Samuel Siriani de Oliveira. – 2020.

126 f. : il. ; 29,5 cm.

Orientador: Clemens Schindwein.

Tese (doutorado) – Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas. Programa de Pós-Graduação em Biologia Vegetal.

1. Biologia. 2. Botânica. 3. Polinização. 4. Abelhas. 5. Especialização. I. Schindwein, Clemens. II. Universidade Federal de Minas Gerais. Instituto de Ciências Biológicas. III. Título.

CDU: 581



UFMG

Programa de Pós-Graduação em Biologia Vegetal
Universidade Federal de Minas Gerais
ICB - Departamento de Botânica

Tese defendida por Samuel Siriani de Oliveira em 31 de março de 2020 e aprovada pela Banca Examinadora constituída pelos professores:

Dr. Clemens Schindwein (UFMG)

Dra. Maria Cristina Gaglianone (Universidade Estadual do Norte Fluminense Darcy Ribeiro)

Dra. Rubia dos Santos Fonseca (Instituto de Ciências Agrárias- UFMG)

Dr. Élder Antônio Sousa e Paiva (UFMG)

Dr. Pietro Kiyoshi Maruyama Mendonça (UFMG)

Denise Mareda Figueiredo Monteiro
SECRETÁRIA EXECUTIVA
PROGRAMA DE PÓS-GRADUAÇÃO
EM BIOLOGIA VEGETAL
ICB - UFMG
08/04/2021

Agradecimentos

Agradeço principalmente à minha mãe Denise, ao meu pai Jorge e aos meus irmãos Daniel, Sacha e Bruno, pelo apoio incondicional à minha trajetória na vida. Sou profundamente grato ao Clemens pela orientação e por quase uma década de agradável convivência, à Reislá por todo apoio na construção dos trabalhos e pelo bom humor contagiante; à Isabelle por todo amor compartilhado e apoio integral no processo de produção dos trabalhos; aos membros do grupo Plebeia, principalmente à Ana Laura pela companhia nas nossas longas viagens de campo; aos professores do Programa de Pós-Graduação em Biologia Vegetal da UFMG que muito me ajudaram na minha formação profissional; à secretária Denise por toda ajuda com as questões burocráticas da pós-graduação; aos moradores de Minas do Camaquã, RS, pelo apoio e consideração; aos funcionários do Parque Nacional de São Joaquim, SC, pelo apoio logístico à realização da pesquisa no parque; ao ICMBio pelas licenças de coleta concedidas e à CAPES e FAPEMIG pelas bolsas de estudo.

Resumo

As interações entre as angiospermas e seus polinizadores são caracterizadas por atributos morfológicos, funcionais, fenológicos e comportamentais das espécies envolvidas, variando amplamente em níveis de especialização/generalização. Espécies de plantas com morfologia floral complexa, em que os recursos florais ficam ocultos ou são gradualmente disponibilizados aos visitantes florais, requerem uma manipulação floral especializada, que pode levar a uma restrição da guilda de polinizadores e resultar em interações planta-polinizador especializadas. Espécies melitófilas de Loasoideae (Loasaceae) possuem flores morfológicamente complexas. O androceu é organizado em cinco complexos de estaminódios, que se alternam com feixes de estames férteis. Cada complexo de estaminódios consiste em uma escama de néctar conspícua e côncava, oposta a dois estaminódios livres. O néctar é armazenado na base das escamas, entre a escama e os estaminódios livres. As flores são protândricas e liberam o pólen gradualmente por meio de movimentos sucessivos dos estames ao centro da flor a partir de uma posição inicial, encerrado em pétalas naviculadas. Os movimentos ocorrem de forma autônoma ou após estímulo mecânico nas escamas de néctar (tigmonastia). Após o movimento de todos os estames inicia a fase pistilada, o estigma cresce e se torna proeminente no centro da flor. Para aumentar o conhecimento sobre as interações entre espécies de Loasoideae e seus polinizadores, estudei a polinização de duas espécies de *Blumenbachia*, *B. insignis* e *B. catharinensis*, focando na dinâmica da apresentação de pólen e de néctar, no sistema reprodutivos das plantas e no comportamento de forrageio dos polinizadores. *Blumenbachia insignis* é polinizada exclusivamente por *Bicolletes indigoticus* (Colletidae) uma espécie de abelha oligolética. A oferta particionada de pólen e de néctar pelas flores, combinada à fidelidade floral das fêmeas de *B. indigoticus*, assegura a estas uma provisão exclusiva de recursos florais. O forrageio especializado das abelhas, por sua vez, garante fluxo polínico cruzado na população de *B. insignis*. Para entender a estratégia de forrageio das abelhas, manipulei experimentalmente a disponibilidade de néctar nas flores e realizei experimentos com extratos químicos de fêmeas coespecíficas. Os resultados revelaram que as fêmeas de *B. indigoticus* otimizam a busca por néctar, concentrando visitas em flores com néctar acumulado. Além disso, fêmeas rejeitaram flores recém visitadas e flores em que foram adicionados extratos químicos de fêmeas coespecíficas. Em *Blumenbachia catharinensis* encontramos uma nova espécie de abelha oligolética como principal visitante floral (*Rhophitulus ater*, Andrenidae), que foi descrita durante o doutoramento. A relação entre *B. catharinensis* e *R. ater* se revelou como um dos raros casos

em que uma abelha oligolética causa um efeito deletério na reprodução da sua espécie hospedeira. As fêmeas de *R. ater* constantemente inspecionam as flores de *B. catharinensis* pela presença de novos estames movidos no centro da flor e coletam, desta maneira, quase todo o pólen liberado. Além disso, removem pólen já depositado nos estigmas em flores na fase pistilada. Os resultados alcançados contribuem para o entendimento geral das interações entre plantas e polinizadores e reforçam que os sistemas de polinização de espécies de Loasoideae são predominantemente especializados.

Palavras-chave: Biologia. Botânica. Polinização. Abelhas. Especialização.

Abstract

The interactions between angiosperms and their pollinators are characterised by morphological, functional, phenological and behavioural attributes of the species involved, varying widely in degree of specialisation/generalisation. Plant species with complex floral morphology, in which floral resources are hidden or gradually offered to flower visitors, require specialised floral handling, which can restrict the guild of pollinators and result in specialised plant-pollinator interactions. Melittophilous species of Loasoideae (Loasaceae) share such exclusive and complex flower morphology. The androecium is organized in staminode complexes alternating with clusters of fertile stamens. Each staminode complex consists of a conspicuous concave nectar scale, opposite to two free staminodes. The nectar is stored at the base of the nectar scales and free staminodes. The flowers are protandrous and release pollen gradually through successive stamen movements to the center of the flower from an initial position, hidden in naviculate petals. The movements occur autonomously or after mechanical stimulation in the nectar scales (thigmonasty) by pollinators. After all the stamens have moved, the pistillate phase starts, the stigma grows and becomes prominent in the flower centre. To increase the knowledge about the interactions between species of Loasoideae and their pollinators, I studied the pollination of two species of *Blumenbachia*, *B. insignis* and *B. catharinensis*, focusing on the dynamics of pollen and nectar presentation, the reproductive systems of the plants and the foraging behaviours of the pollinators.

Blumenbachia insignis is pollinated exclusively by *Bicolletes indigoticus* (Colletidae) a species of oligolectic bee. The partitioned offer of pollen and nectar by the flowers, combined with the floral fidelity of the females of *B. indigoticus*, assures them an exclusive supply of floral resources. The specialized foraging of the bees, in turn, guarantees cross-pollen flow in the population of *Blumenbachia insignis*. To understand the foraging strategy of the bees, I have experimentally manipulated the availability of nectar in the flowers and carried out experiments with chemical extracts of conspecific females. The results revealed that the females of *B. indigoticus* optimize the search for nectar by concentrating the visits on flowers with accumulated nectar. In addition, females rejected recently visited flowers and flowers in which chemical extracts from conspecific females were added. In *Blumenbachia catharinensis* we found a new species of oligolectic bee as the main floral visitor (*Rhopitulus ater*, Andrenidae), which was described. The relationship between *B. catharinensis* and *R. ater* was revealed as one of the rare cases in which an oligolectic bee species causes a deleterious effect on the reproduction of its host plant species. Females of *R. ater* constantly

inspect the flowers of *B. catharinensis* whether they present a new moved stamen in the center of the flower and collect, in this way, almost all the released pollen. Moreover, they remove pollen from already deposited on the stigmas in flowers of the pistillate phase. The results achieved contribute to the general understanding of interactions between plants and pollinators and reinforce that the pollination systems of Loasoideae species are predominantly specialised.

Keywords: Biology. Botanic. Pollination. Bees. Specialisation.

SUMÁRIO

Introdução geral	10
Capítulo 1 – Specialised protagonists in a plant-pollinator interaction: the pollination of <i>Blumenbachia insignis</i> (Loasaceae)	25
Capítulo 2 – Fêmeas de <i>Bicolletes indigoticus</i> (Apoidea, Colletidae) identificam remotamente a presença de néctar ou pistas químicas indicativas da sua ausência em flores de <i>Blumenbachia insignis</i> (Loasaceae)	55
Capítulo 3 – A new oligolectic bee species of the genus <i>Rhophitulus</i> Ducke (Hymenoptera, Andrenidae) from South Brazil	76
Capítulo 4 – An oligolectic pollen thief through a specialized pollen foraging behaviour hinders effective pollination of its host plant, <i>Blumenbachia catharinensis</i> (Loasaceae)	93
Considerações finais	124

Introdução geral

Nas diversificadas interações entre as angiospermas e seus polinizadores, as abelhas são os visitantes forais predominantes (Kevan 1983, Raven *et al.* 2007). As interações entre plantas e abelhas são geralmente pautadas em benefícios mútuos para as espécies envolvidas. Enquanto as plantas dependem das abelhas no processo de polinização, as abelhas dependem dos recursos florais como fonte alimento (Knuth 1906, Proctor *et al.* 1996). O néctar é a principal fonte de energia para os adultos enquanto o pólen é coletado pelas fêmeas e destinado para a alimentação das larvas (Linsley 1958, Eickwort & Ginsberg 1980, Wcislo & Cane 1996). Como os grãos de pólen encerram os gametas masculinos das plantas e seu destino funcional é a superfície estigmática de coespecíficas, nas flores polinizadas por abelhas o destino pólen é conflitante visto que grande quantidade é transportado para ninhos dos visitantes florais (Westerkamp 1996). Em geral, menos de 4% do pólen produzido por uma flor melitófila é depositado em estigmas coespecíficos, enquanto a maior quantidade do pólen flui para a alimentação de abelhas (Harder & Thomson 1989, Schlindwein *et al.* 2005, Carvalho & Schlindwein 2011, Pick & Schlindwein 2011, Cerceau *et al.* 2019).

Abelhas são ótimas forrageadoras e possuem diversas estratégias para aumentar a eficiência de coleta de recursos. Já foi demonstrado que abelhas identificam flores com maior quantidade de recursos e abandonam de flores que demandem grande gasto energético durante o manuseio (Whitham 1977). Além disso, estes insetos podem adotar rotas de forrageio de acordo com o ritmo de apresentação de recursos pelas flores (Schlindwein & Wittmann 1997a) e sincronizar o forrageio com o início da antese (Araujo *et al.* 2020). A eficiência de forrageio é importante principalmente para abelhas solitárias, visto que a quantidade de recursos coletados está diretamente relacionada com o sucesso reprodutivo individual (Neff 2008).

Durante os voos de forrageio, as abelhas não visitam flores aleatoriamente. Elas tendem a restringir as visitas a flores de poucas espécies de plantas, mesmo tendo disponíveis flores de outras espécies no mesmo ambiente. A restrição, que pode ser entendida como um forrageio preferencial, pode ser temporária e individual, como no caso de abelhas generalistas que apresentam constância floral durante o período de floração de uma determinada espécie de planta (Aristóteles 350 a.C., Bannett 1883, Knuth 1906, Grant 1950, Linsley 1958, Free 1963, Waser 1986, Roubik 1989, Cane & Sipes 2006). Terminada a floração, elas passam a explorar flores de outras espécies disponíveis. Por outro lado, o forrageio preferencial em flores de poucas espécies de plantas pode ser um atributo inerente à espécie de abelha, como

no caso de abelhas que restringem a busca por pólen em plantas de um mesmo gênero ou família. Esse atributo é denominado oligoetia, termo cunhado por Robertson (1925) em um estudo sobre as abelhas nativas e suas plantas hospedeiras. As espécies de abelhas que exibem esse tipo de restrição inata são chamadas de abelhas oligoléticas, o que significa que possuem um hábito de forrageio especialista ou uma dieta polínica especializada. Em contrapartida, as espécies que exibem uma dieta polínica generalista são chamadas de abelhas poliléticas, pois exploraram o pólen de flores de diferentes famílias de plantas (Robertson 1925). A poliletia foi por muito tempo considerada como uma condição primitiva das abelhas, que evoluiu para a oligoetia independentemente em diversos clados (Linsley 1958, Moldenke 1979, Michener 2007). Entretanto, estudos filogenéticos mais recentes sustentam que oligoetia é a condição basal em algumas linhagens da qual as abelhas poliléticas evoluíram (Müller 1996, Larkin *et al.* 2008, Michez *et al.* 2008, Patiny *et al.* 2008, Litman *et al.* 2011, Danforth *et al.* 2013).

A oligoetia é mais recorrente em abelhas solitárias, principalmente em Neopasiphaeinae (Colletidae), Panurginae (Andrenidae), Rophitinae (Halictidae), Emphorini (Apidae) e Lithurgini (Megachilidae). Já a poliletia é comum em abelhas sociais como nas tribos Apini, Bombini e Meliponini (Apidae) (Schlindwein 2000, Silveira *et al.* 2002, Michener 2007). Dependendo da localização geográfica e do clima, o número de espécies de abelhas oligoléticas em uma comunidade varia. Por exemplo, as regiões subtropicais do continente americano com climas xéricos possuem alta riqueza e diversidade de abelhas oligoléticas, enquanto essas espécies são raras em regiões tropicais de clima úmido (Linsley 1958, Moldenke 1976, Michener 1979, Müller 1996, Schlindwein 1998, Minckley *et al.* 2000). Abelhas oligoléticas exibem menor variação genética e presume-se que existam em populações pequenas e isoladas em relação às poliléticas e, por esse motivo, apresentam maior sensibilidade às mudanças ambientais, tornando-as espécies com alta prioridade para conservação (Packer *et al.*, 2005, Zayed *et al.* 2005, De Palma *et al.* 2015).

Interação entre abelhas oligoléticas e suas plantas hospedeiras

Flores melitófilas podem ser visitadas por uma ampla gama de grupos taxonômicos de abelhas, incluindo abelhas oligoléticas e poliléticas, que podem variar quanto à eficiência na polinização (Faegri & van der Pijl 1979, Linsley 1985, Minkcley *et al.* 1994, Waser *et al.* 1996, Müller & Kuhlmann 2008, Brito *et al.* 2017, Rech *et al.* 2020). Nas interações envolvendo abelhas oligoléticas, estas são, muitas vezes, consideradas polinizadoras efetivas de suas plantas hospedeiras (Müller & Kuhlmann 2008, Tepedino *et al.* 2016, Cane 2018, Portman *et al.* 2018, Konzmann *et al.* 2019). Porém, em alguns casos, abelhas oligoléticas

podem não ser polinizadores eficientes (Schlindwein & Medeiros 2006) ou podem atuar como pilhadoras de pólen de suas plantas hospedeiras (Barrows 1976), o que demonstra que nem sempre essas interações são totalmente positivas para ambas as espécies envolvidas. Nas interações especializadas entre abelhas oligoléticas e plantas, as espécies que interagem podem apresentar características morfológicas, fisiológicas e comportamentais relacionadas à interação (Linsley 1958). Por exemplo, abelhas oligoléticas podem detectar voláteis florais específicos para encontrar suas plantas hospedeiras (Andrews 2007, Burger *et al.* 2010, Milet-Pinheiro *et al.* 2012, Carvalho *et al.* 2014), e podem exibir caracteres e/ou comportamentos que favoreçam a obtenção de pólen disposto em partes florais de difícil acesso, que exigem uma manipulação floral adequada (Alves-dos-Santos & Wittmann 1999, Milet-Pinheiro & Schlindwein 2010). Além disso, o período de floração das plantas pode ser sincronizado com o período de atividade das abelhas (Minckley *et al.* 1994, Schlindwein 1998, Carvalho & Schlindwein 2011, Cane 2018, Cerceau *et al.* 2019), o pólen de ser de difícil digestão por abelhas não especialistas (Praz *et al.* 2008) e movimentos de estames podem favorecer a obtenção de pólen por abelhas oligoléticas (Schlindwein & Wittmann 1997b, Siriani-Oliveira *et al.* 2018).

Loasoideae - Morfologia floral única entre as Angiospermas

A família Loasaceae Juss. é praticamente restrita ao continente americano e a maioria das espécies ocorre ao longo da Cordilheira dos Andes (Urban 1886, 1892, Urban & Gilg 1900, Weigend *et al.* 2004). Apenas ~5% das espécies da família ocorrem no Brasil, sendo a maioria delas localmente ou regionalmente endêmica. São 17 espécies de 5 gêneros de duas subfamílias monofiléticas, Loasoideae e Mentzelioideae, esta última restrita à somente uma espécie *Mentzelia aspera* L.. As Loasoideae brasileiras são divididas em duas tribos, Loaseae e Klapprothieae, sendo Loaseae a mais representativa, com 14 espécies de três gêneros: *Aosa* Weigend, *Blumenbachia* Schrad. e *Caiophora* C. Presl. As espécies desses gêneros apresentam morfologia floral bastante uniforme (Fig. 1) e ocorrem principalmente na porção leste do território brasileiro, desde o Rio Grande do Sul até o Rio Grande do Norte (Acuña *et al.* 2019).

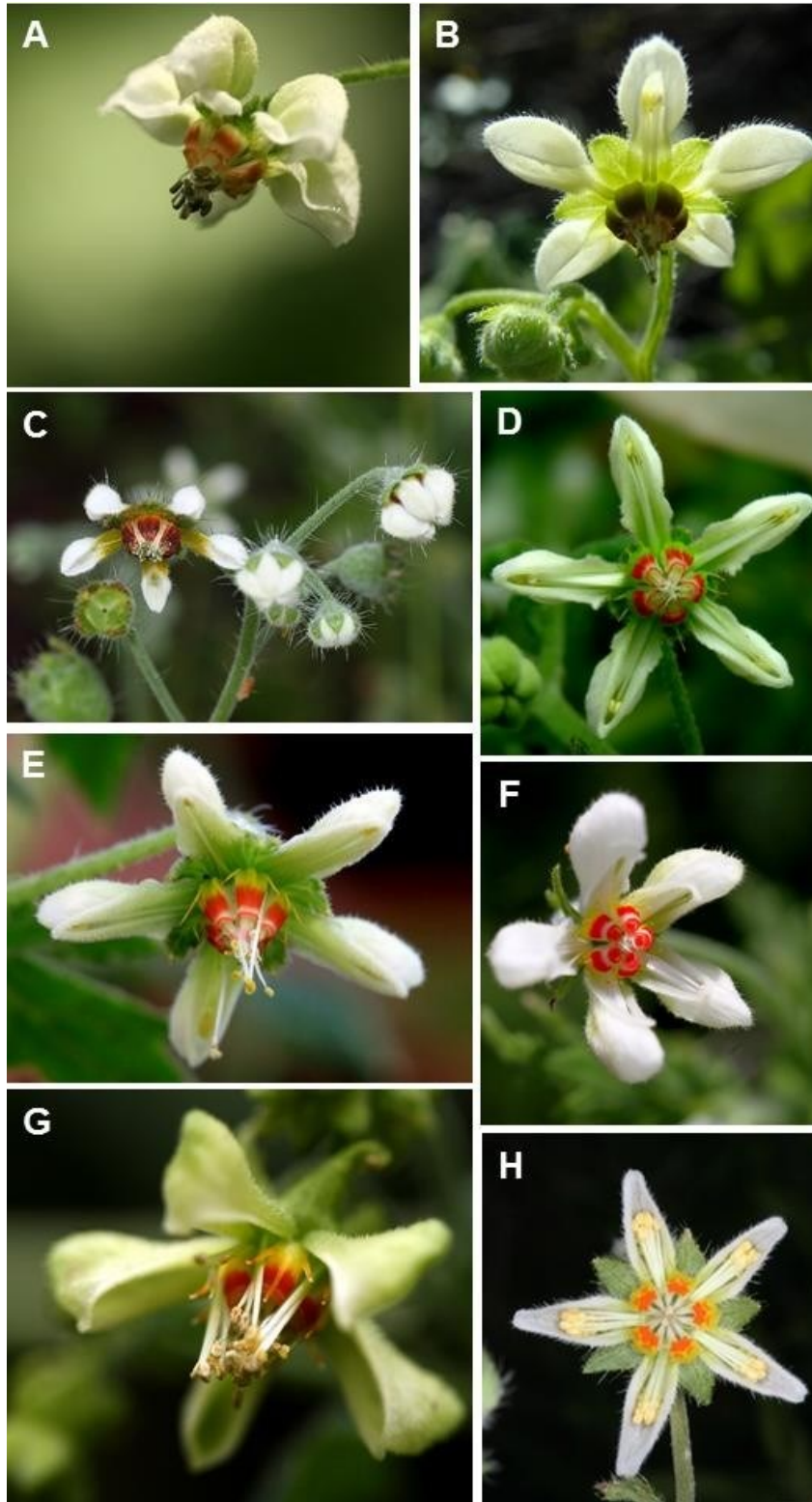


Figura 1. Flores de algumas espécies de três gêneros Loasoideae que ocorrem no Brasil.
 A - *Aosa uleana*, B - *Aosa parviflora*, C - *Aosa rupestris*, D - *Blumenbachia catharinensis*, E - *Blumenbachia amana*, F - *Blumenbachia insignis*, G - *Blumenbachia scabra*, H - *Caiophora arechavaletae*.

A morfologia floral de Loasoideae é única entre as angiospermas. As flores são protândricas com androceu estruturalmente complexo. Os estames são organizados em cinco feixes encerrados em pétalas naviculadas. Durante a fase estaminada, eles se movimentam, um a um, em direção ao centro da flor. O movimento pode ocorrer tanto de forma autônoma quanto pode ser desencadeado pelos visitantes florais. Após movimentação de todos os estames, inicia a fase pistilada com o alongamento do estilete e estigma até que este se torna proeminente no centro da flor. Alternando com os feixes de estames, encontram-se cinco conjuntos de estaminódios de coloração contrastante, denominadas escamas de néctar e dois estaminódios livres. Cada escama de néctar tem formato côncavo e ápice recurvado, sendo formada pela conação de três estaminódios. Opostos a cada escama de néctar ficam dois estaminódios livres de ápice filiforme (Urban 1886, 1892). O néctar é produzido em nectários no ápice do receptáculo floral e é liberado entre a base das escamas e os estaminódios livres (Brown & Kaul 1981, Weigend & Rodriguez 2003).

Para acessar o néctar, as abelhas precisam pousar de cabeça para baixo nas flores pêndulas, agarrar-se ao ápice recurvado das escamas e empurrar com a cabeça cada escama individualmente. Ao deslocar as escamas com a cabeça, é acionado um mecanismo que pode desencadear em alguns minutos o movimento de um estame, da pétala em direção ao centro da flor (Schlindwein & Wittmann 1997). Esses movimentos são tigmomásticos, que correspondem a respostas das plantas ao contato mecânico externo, desencadeando movimentos em estruturas vegetais (Jaffe 1985). Recentemente, foi demonstrado que a apresentação do pólen em resposta à estimulação mecânica do complexo escamas de néctar-estaminódios é uma apomorfia presente na maioria dos taxa de Loasoideae (Weigend *et al.* 2004, 2010, Henning & Weigend 2012, 2013, Henning *et al.* 2018, Siriani-Oliveira *et al.* 2018).

Polinização de espécies de Loasoideae

Os visitantes florais e polinizadores das espécies de Loasoideae são predominantemente fêmeas e machos de abelhas oligoléticas da família Colletidae, subfamília Neopasiphaeinae (Schlindwein 1998, Troncoso & Vargas 2004, Ackermann & Weigend 2006, Weigend & Gottschling 2006, Cares-Suárez *et al.* 2011, Leite *et al.* 2016, Siriani-Oliveira *et al.* 2018). Neopasiphaeinae é grupo monofilético de abelhas solitárias que ocorre principalmente na América do Sul e na Oceania (Michener 2007, Almeida *et al.* 2019). Além disso, é um clado que inclui muitas espécies de abelhas oligoléticas (Almeida *et al.* 2012, Wcislo & Cane 1996, Almeida & Gibran 2017, Carvalho & Schlindwein 2011, Gimenes

1991, Houston 1989, 1991, 2000, Laroca *et al.* 1989, Schlindwein 1998, Siriani-Oliveira *et al.* 2018).

Uma interpretação funcional dos movimentos tigmomásticos dos estames em Loasoideae, no contexto da ecologia da polinização, foi feita pela primeira vez para *Caiophora arechavaletae* (Schlindwein & Wittmann 1997). Análises do comportamento de forrageio individual de fêmeas *Bicolletes pampeana* Urban 1995 em ambiente natural revelaram uma estratégia de forrageio adaptada ao ritmo gradual de liberação do pólen pelas flores de *C. arechavaletae*. As fêmeas estabelecem curtas rotas de forrageio em manchas de flores que lhes permitem sincronizar sua chegada à flor quando o pólen é liberado após estímulos realizados em visita floral anterior. Um padrão de forrageio semelhante foi demonstrado recentemente para fêmeas de *Actenosigynes mantiqueirensis* Silveira 2009 em flores de *Blumenbachia amana* Henning & Weigend (Siriani-Oliveira *et al.* 2018). Em ambas as interações, as abelhas garantem a polinização cruzada de suas plantas hospedeiras devido à elevada constância floral e revisitas a flores individuais (Schlindwein & Wittmann 1997, Siriani-Oliveira *et al.* 2018).

Além dos dois casos de polinização citados, são conhecidos os visitantes florais de apenas três espécies de *Blumenbachia* na região sul do Brasil, *Blumenbachia eichleri* Urb. e *Blumenbachia catharinensis* Urban & Gilg. visitadas por *Actenosigynes fulvoniger* (Michener, 1989) (Schlindwein 2000), e *Blumenbachia insignis* Schrad. visitada por *Bicolletes indigoticus* Compagnucci & Roig-Alsina, 2008 (Schlindwein 1998). Registros de visitantes florais das espécies de *Aosa* são escassos. Entre as seis espécies conhecidas, apenas a interação entre *Aosa rupestris* (Gardner) Weigend e *Bicolletes nordestina* Urban 2006 foi estudada (Leite *et al.* 2016).

Diante do exposto, neste trabalho, objetivamos ampliar o conhecimento a respeito das interações entre espécies de Loasoideae brasileiras e seus polinizadores. Para isso, estudamos a polinização de duas espécies de *Blumenbachia*, *B. insignis* e *B. catharinensis*, nas suas respectivas áreas de ocorrência. Os estudos abordam a dinâmica de apresentação dos recursos florais e o sistema reprodutivo das plantas, além do comportamento de forrageio dos polinizadores.

Referências

Ackermann M., Weigend M. (2006) Nectar, floral morphology and pollination syndrome in Loasaceae subfam. Loasoideae (Cornales). *Annals of Botany*, **98**, 503 - 514.

- Acuña C.R., Luebert F., Henning T., Weigend M. (2019) Major lineages of Loasaceae subfam. Loasoideae diversified during the Andean uplift. *Molecular Phylogenetics and Evolution*, doi: <https://doi.org/10.1016/j.ympev.2019.106616>.
- Almeida E.A., Gibran N.S. (2017) Taxonomy of neopasiphaeine bees: review of *Tetraglossula* Ogloblin, 1948 (Hymenoptera: Colletidae). *Zootaxa*, **4303**(4), 521-544.
- Almeida E.A.B., Pie M.R., Brady S.G., Danforth B.N. (2012) Biogeography and diversification of colletid bees (Hymenoptera: Colletidae): Emerging patterns from the Southern End of the World. *Journal of Biogeography*, **39**, 526–544.
- Almeida E.A., Packer L., Melo G.A., Danforth B.N., Cardinal, S.C., Quinteiro F.B., Pie M.R. (2019) The diversification of neopasiphaeine bees during the Cenozoic (Hymenoptera: Colletidae). *Zoologica Scripta*, **48**(2), 226-242.
- Alves-dos-Santos I., Wittmann D. (1999) The proboscis of the long-tongued *Ancyloscelis* bees (Anthophoridae/Apoidea), with remarks on flower visits and pollen collecting with the mouthparts. *Journal of the Kansas Entomological Society*, 277-288.
- Andrews E.S., Theis N., Adler L.S. (2007) Pollinator and herbivore attraction to *Cucurbita* floral volatiles. *Journal of Chemical Ecology*. **33**(9), 1682–1691.
- Araujo F.F., Oliveira R., Mota T., Stehmann J.R., Schlindwein C. (2020). Solitary bee pollinators adjust pollen foraging to the unpredictable flower opening of a species of *Petunia* (Solanaceae). *Biological Journal of the Linnean Society*, **129**(2), 273-287.
- Aristotle (350 a.C.) *Historia animalium*. Bk. IX, Ch. 40. Traduzido por by d'Arcy Thompson (1910). Disponível em http://classics.mit.edu/Aristotle/history_anim.html.
- Barrows E.M., Chabot M.R., Michener C.D., Snyder T.P. (1976) Foraging and Mating Behavior in *Perdita texana* (Hymenoptera: Andrenidae). *Journal of the Kansas Entomological Society* **49** (2), 275-279.
- Bennett A.W. (1883) On the constancy of insects in their visits to flowers. *Zoological Journal of the Linnean Society*, **17**(100), 175-185.
- Brito V.L., Rech A.R., Ollerton J., Sazima M. (2017). Nectar production, reproductive success and the evolution of generalised pollination within a specialised pollen-rewarding plant family: a case study using *Miconia theizans*. *Plant Systematics and Evolution*, **303**(6), 709-718.

- Brown D.K., Kaul R.B. (1981) Floral structure and mechanisms in Loasaceae. *American Journal of Botany*, **68**, 361-72.
- Burger H., Dötterl S., Ayasse M. (2010) Host-plant finding and recognition by visual and olfactory floral cues in an oligolectic bee. *Functional Ecology* **24**, 1234–1240.
- Cane H.J. (2018) Co-dependency between a specialist *Andrena* bee and its death camas host, *Toxicoscordion paniculatum*. *Arthropod-Plant Interactions*, **12**, 657–662.
- Cane J.H., Sipes S. (2006) Characterizing floral specialization by bees: analytical methods and a revised lexicon for oligolecty. Pp. 99-122. In: Waser, N.M. & Ollerton, J. (eds.). *Plant-pollinator interactions: from specialization to generalization*. Chicago, The University of Chicago Press, 445pp.
- Cares-suárez R., Poch T., Acevedo R.F., Acosta-bravo I., Pimentel C., Espinoza C., Cares R.A., Muñoz P., González A.V., Botto-mahan C. (2011) Do pollinators respond in a dose-dependent manner to flower herbivory?: An experimental assessment in *Loasa tricolor* (Loasaceae). *Gayana Botanica*, **68** (2), 176-181.
- Carvalho A.T., Schlindwein C. (2011) Obligate association of an oligolectic bee and a seasonal aquatic herb in semi-arid north-eastern Brazil. *Biological Journal of the Linnean Society* **102**, 355–368.
- Carvalho A.T., Dötterl S., Schlindwein C. (2014) An aromatic volatile attracts oligolectic bee pollinators in an interdependent bee-plant relationship. *Journal of Chemical Ecology* **40**, 1126–1134.
- Cerceau I., Siriani-Oliveira S., Dutra A.L., Oliveira R., Schlindwein C. (2019) The cost of fidelity: foraging oligolectic bees gather huge amounts of pollen in a highly specialized cactus–pollinator association. *Biological Journal of the Linnean Society* **128** (1), 30-43.
- Danforth B.N., Cardinal S., Praz C., Almeida E.A., Michez D. (2013) The impact of molecular data on our understanding of bee phylogeny and evolution. *Annual review of Entomology*, **58**, 57-78.
- De Palma A., Kuhlmann M., Roberts S.P., Potts S.G., Börger L., Hudson L.N., Lysenko I., Newbold T., Purvis A. (2015) Ecological traits affect the sensitivity of bees to land-use pressures in European agricultural landscapes. *Journal of Applied Ecology* **52**(6), 1567-1577.

- Eickwort G.C., Ginsberg H.S. (1980) Foraging and mating behavior in Apoidea. *Annual Review of Entomology* **25**, 421-446.
- Faegri K., van der Pijl L. (1979) *The principles of pollination ecology*. 3rd ed. Pergamon Press, Oxford. pp. 244.
- Free J.B. (1963) The flower constancy of honeybees. *The Journal of Animal Ecology*, 119-131.
- Gimenes M. (1991) Some morphological adaptations in bees (Hymenoptera, Apoidea) for collecting pollen from *Ludwigia elegans* (Onagraceae). *Revista Brasileira de Entomologia*, **35**, 413–422.
- Grant V. (1950) The flower constancy of bees. *Botanical Review*, **16**, 379-398.
- Harder L.D., Thomson J.D. (1989) Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. *The American Naturalist*, **133**, 323–344.
- Henning T., Weigend M. (2012) Total control - pollen presentation and floral longevity in Loasaceae (blazing star family) are modulated by light, temperature and pollinator visitation rates. *PLoS ONE*, **7**(8), e41121. doi:10.1371/journal.pone.0041121.
- Henning T., Weigend M. (2013) Beautiful, complicated - and intelligent? Novel aspects of the thigmonastic stamen movement in Loasaceae. *Plant Signaling and Behavior*, **8**, 24605, <http://dx.doi.org/10.4161/psb.24605>.
- Henning T., Oliveira S., Schlindwein C., Weigend M. (2015) A new, narrowly endemic species of *Blumenbachia* (Loasaceae subfam. Loasoideae) from Brazil. *Phytotaxa*, **236** (2), 196–200.
- Henning T., Mittelbach M., Ismail S.A., Acuña-Castillo R.H., Weigend M. (2018) A case of behavioural diversification in male floral function – the evolution of thigmonastic pollen presentation. *Scientific reports*, **8**, 14018. doi:10.1038/s41598-018-32384-4.
- Houston T.F. (1989) *Leioproctus* bees associated with Western Australian smoke bushes (*Conospermum* spp.) and their adaptations for foraging and concealment (Hymenoptera: Colletidae: Paracolletini). *Records of the Western Australian Museum*, **14**(3), 275-292.
- Houston T.F. (1991) Two new and unusual species of the bee genus *Leioproctus* Smith (Hymenoptera: Colletidae), with notes on their behaviour. *Records of the Western*

- Australian Museum*, **15**(1), 83-96.
- Houston T.F. (2000) Native Bees on Wildflowers in Western Australia. A Synopsis of Native Bee Visitation of Wildflowers in Western Australia Based on the Bee Collection of the Western Australian Museum. *Special Publication No. 2 of the Western Australian Insect Study Society Inc.* Australia, Western Australian Museum, Perth, 235 pp.
- Jaffe M. J. (1985). Wind and other mechanical effects in the development and behavior of plants, with special emphasis on the role of hormones. In *Hormonal Regulation of Development III* (pp. 444-484). Springer, Berlin, Heidelberg.
- Kevan P.G. (1983) Insects as flower visitors and pollinators. *Annual Review of Entomology*, **28**, 407-53.
- Knuth, P. (1906) *Handbook of flower pollination*. Vol. 1. Translated by J. R. Ainsworth Davis. Oxford.
- Konzmann S., Kluth M., Karadana D., Lunau K. (2019) Pollinator effectiveness of a specialist bee exploiting a generalist plant—tracking pollen transfer by *Heriades truncorum* with quantum dots. *Apidologie*, 1-11.
- Laroca S., Michener C.D., Hoffmeister R.M. (1989) Long mouthparts among "short-tongued" bees and the fine structure of the labium in *Niltonia*. *Journal of the Kansas Entomological Society*, **62**, 400–410.
- Larkin L.L., Neff J.L., Simpson B.B. (2008) The evolution of a pollen diet: host choice and diet breadth of *Andrena* bees (Hymenoptera: Andrenidae). *Apidologie*, **39**(1), 133-145.
- Leite A.V., Nadia T., Machado I.C. (2016) Pollination of *Aosa rupestris* (Hook.) Weigend (Loasaceae): are stamen movements induced by pollinators? *Brazilian Journal of Botany*, **39**, 559–567.
- Linsley E.G. (1958) The ecology of solitary bees. *Hilgardia*, **27**, 543–599.
- Michener C.D. (1979). Biogeography of the bees. *Annals of the Missouri Botanical Garden*, 277-347.
- Litman J.R., Danforth B.N., Eardley C.D., Praz C.J. (2011) Why do leafcutter bees cut leaves? New insights into the early evolution of bees. *Proceedings of the Royal Society B: Biological Sciences*, **278**(1724), 3593-3600.

- Michener C.D. (2007) *The Bees of the World*. 2nd Edition. John Hopkins University Press, Baltimore, 992 pp.
- Michez D., Patiny S., Rasmont P., Timmermann K., Vereecken N. J. (2008) Phylogeny and host-plant evolution in Melittidae sl (Hymenoptera: Apoidea). *Apidologie*, **39**(1), 146-162.
- Milet-Pinheiro P., Ayasse M., Schindwein C., Dobson H.E.M., Dötterl S. (2012) Host location by visual and olfactory floral cues in an oligolectic bee: innate and learned behavior. *Behavioral Ecology*, **23**: 531–538.
- Milet-Pinheiro P., Schindwein C. (2010) Mutual reproductive dependence of distylic *Cordia leucocephala* (Cordiaceae) and oligolectic *Ceblurgus longipalpis* (Halictidae, Rophitinae) in the Caatinga. *Annals of Botany*, **106**, 17–27.
- Minckley R.L., Wcislo W.T., Yanega D., Buchmann S.L. (1994) Behavior and phenology of a specialist bee (*Dieunomia*) and sunflower (*Helianthus*) pollen availability. *Ecology*, **75**(5), 1406-1419.
- Minckley R.L., Cane J.H., Kervin L. (2000) Origins and ecological consequences of pollen specialization among desert bees. *Proceedings of the Royal Society B: Biological Sciences* **267**, 265–271.
- Moldenke A.R. (1976) California pollination ecology and vegetation types. *Phytologia*, **34**(4), 305-361.
- Moldenke A.R. (1979) Host-plant coevolution and the diversity of bees in relation to the flora of North America. *Phytologia*, **43**(4), 357-420.
- Müller A., Kuhlmann M. (2008). Pollen hosts of western palaeartic bees of the genus *Colletes* (Hymenoptera: Colletidae): the Asteraceae paradox. *Biological Journal of the Linnean Society*, **95**(4), 719-733.
- Müller A. (1996) Host-plant specialization in western palearctic Anthidine bees (Hymenoptera: Apoidea: Megachilidae). *Ecological Monographs*, **66**(2), 235-257.
- Neff J.L. (2008). Components of nest provisioning behavior in solitary bees (Hymenoptera: Apoidea). *Apidologie*, **39**(1), 30-45.

- Packer L., Zayed A., Grixti J.C., Ruz L., Owen R.E., Vivallo F. and Toro H. (2005). Conservation genetics of potentially endangered mutualisms: reduced levels of genetic variation in specialist versus generalist bees. *Conservation Biology*, **19**(1), 195-202.
- Patiny S., Michez D., Danforth B.N. (2008) Phylogenetic relationships and host-plant evolution within the basal clade of Halictidae (Hymenoptera, Apoidea). *Cladistics*, **24**, 255–269.
- Pick R.A., Schlindwein C. (2011). Pollen partitioning of three species of Convolvulaceae among oligolectic bees in the Caatinga of Brazil. *Plant Systematics and Evolution*, **293**, 147–159.
- Praz C.J., Müller A., Dorn S. (2008). Specialized bees fail to develop on non-host pollen: do plants chemically protect their pollen. *Ecology*, **89**(3), 795-804.
- Portman Z.M., Tepedino V.J., Tripodi A.D. (2018) Persistence of an imperiled specialist bee and its rare host plant in a protected area. *Insect Conservation and Diversity*, doi: 10.1111/icad.12334.
- Proctor M., Yeo P., Lack A. (1996) *The natural history of pollination*. London, UK: Harper & Collins.
- Raven P.H., Evert R.F., Eichhorn S.E. (2007) *Biologia vegetal*. 7 ed. Rio de Janeiro: Guanabara Koogan, 2011. 728p.
- Rech A.R., Achkar M.T., Jorge L.R., Armbruster W.S., Almeida O.J.G. (2020) The functional roles of 3D heterostyly and floral visitors in the reproductive biology of *Turnera subulata* (Turneroideae: Passifloraceae). *Flora*, 151559.
- Robertson C. (1925) Heterotropic bees. *Ecology*, **6**, 412-436.
- Roubik D.W. (1989) *Ecology and natural history of tropical bees*. Cambridge, University Press.
- Ruan C.J., Silva J.A.T.D. (2011) Adaptive significance of floral movement. *Critical Reviews in Plant Sciences*, **30**, 293–328.
- Silveira F.A., Melo G.A.R., Almeida E.A.B. (2002) *Abelhas Brasileiras: Sistemática e Identificação*. Published by the authors, Belo Horizonte, 253 pp.
- Schlindwein C. (1998) Frequent oligolecty characterizing a diverse bee-plant community in a

- xerophytic bushland of subtropical Brazil. *Studies on Neotropical Fauna and Environment*, **33**, 46-59.
- Schlindwein C. (2000) Verhaltensanpassungen oligolektischer Bienen an synchrone und an kontinuierliche Pollenpräsentation. In: Breckle SW, Schweizer B, Arndt U, eds. *Results of worldwide ecological studies*. Stuttgart: Günter Heimbach Verlag, 235–250.
- Schlindwein C., Medeiros P.C. (2006). Pollination in *Turnera subulata* (Turneraceae): Unilateral reproductive dependence of the narrowly oligolectic bee *Protomeliturga turnerae* (Hymenoptera, Andrenidae). *Flora-Morphology, Distribution, Functional Ecology of Plants*, **201**(3), 178-188.
- Schlindwein C., Wittmann D., Martins C.F., Hamm A., Siqueira J.A., Schiffler D., Machado I.C. (2005) Pollination of *Campanula rapunculus* L. (Campanulaceae): How much pollen flows into pollination and into reproduction of oligolectic pollinators? *Plant Systematics and Evolution*, **250**, 147–156.
- Schlindwein C., Wittmann D. (1997a) Micro-foraging routes of *Bicolletes pampeana* (Colletidae) and bee-induced pollen presentation in *Cajophora arechavaletae*. *Botanica Acta*, **110**:177-83.
- Schlindwein C., Wittmann D. (1997b). Stamen movements in flowers of *Opuntia* (Cactaceae) favour oligolectic pollinators. *Plant Systematics and Evolution*, **204**, 179–193.
- Siriani-Oliveira S., Oliveira R., Schlindwein C. (2018). Pollination of *Blumenbachia amana* (Loasaceae): Flower morphology and partitioned pollen presentation guarantee a private reward to a specialist pollinator. *Biological Journal of the Linnean Society*, **124**, 479–491.
- Tepedino V.J., Arneson L.C., Durham S.L. (2016) Pollen removal and deposition by pollen- and nectar collecting specialist and generalist bee visitors to *Iliamna bakeri* (Malvaceae). *Journal of Pollination Ecology*, **19**, 50–56.
- Troncoso A.J, Vargas RR. (2004) Efecto del vecindario floral sobre la tasa de visitas por insectos a *Loasa triloba* Domb. ex A.J. Juss. y *Loasa tricolor* Ker-Gawl en la Reserva Nacional de Río Clarillo, Región Metropolitana, Chile. *Chloris Chilensis* **7** (1).
- Urban I. (1886) Die Bestäubungseinrichtungen der Loasaceen. *Jahrb Bot Gart Berlin* **4**, 364–3.
- Urban I. (1892) Blüten - und Fruchtbau der Loasaceen. *Berichte der Deutschen Botanischen*

- Gesellschaft*. **10**, 259–265.
- Urban I., Gilg W. (1900) Monographia Loasacearum. *Nova Acta Academiae Caesareae Leopoldo-Carolinae Germanicae Naturae*, **76**, 1–368.
- Waser N.M. (1986) Flower constancy: definition, cause, and measurement. *American Naturalist*, 593-603.
- Waser N.M., Chittka L., Price M.V., Williams N.M., Ollerton J. (1996) Generalization in pollination systems, and why it matters. *Ecology*, **77**, 1043–1060.
- Wcislo T.W., Cane H.J. (1996) Floral resource utilization by solitary bees (Hymenoptera: Apoidea) and exploitation of their stored foods by natural enemies. *Annual Review of Entomology*, **41**, 257-286.
- Weigend M., Rodriguez E. (2003) A revision of the *Nasa stuebeliana* group [*Nasa* ser. Saccatae (Urb. and Gilg) Weigend, Loasaceae] with notes on morphology, ecology, and distribution. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie*. **124**: 345–382.
- Weigend M., Gottschling M., Hoot S., Ackermann M. (2004) A preliminary phylogeny of Loasaceae subfam. Loasoideae (Angiospermae: Cornales) based on trnL (UAA) sequence data, with consequences for systematics and historical biogeography. *Organisms Diversity & Evolution*, **4**: 73–90.
- Weigend M., Gottschling M. (2006) Evolution of funnel-revolver flowers and ornithophily in *Nasa* (Loasaceae). *Plant Biology*, **8**: 120–142.
- Weigend M., Ackermann M., Henning T. (2010) Reloading the revolver – male fitness as simple explanation for complex reward partitioning in *Nasa macrothyrsa* (Cornales, Loasaceae). *Biological Journal of Linnean Society*, **100**: 124-31.
- Westerkamp C. (1996). Pollen in bee-flower relations: some considerations on melittophily. *Botanica Acta*, **109**, 325-332.
- Whitham, T. G. (1977). Coevolution of foraging in *Bombus* and nectar dispensing in *Chilopsis*: a last dreg theory. *Science*, **197**(4303), 593-596.
- Zayed A., Packer L., Grixti J.C., Ruz L., Owen R.E., Toro H. (2005) Increased genetic differentiation in a specialist versus a generalist bee: Implications for conservation.

Conservation Genetics, **6**: 1017–1026.

Capítulo 1

Specialised protagonists in a plant-pollinator interaction: the pollination of *Blumenbachia insignis* (Loasaceae)¹

¹ A publicação original está disponível em <https://onlinelibrary.wiley.com/doi/10.1111/plb.13072> Publicado como: Siriani-Oliveira S., Cerceau I., Schlindwein C. Specialised protagonists in a plant-pollinator interaction: the pollination of *Blumenbachia insignis* (Loasaceae). *Plant Biology*, **22**(2), 167-176.

Specialised protagonists in a plant-pollinator interaction: the pollination of *Blumenbachia insignis* (Loasaceae)

Abstract

Analyses of resource presentation, floral morphology and pollinator behaviour are essential for understanding specialised plant-pollinator systems. We investigated whether foraging by individual bee pollinators fits the floral morphology and functioning of *Blumenbachia insignis*, whose flowers are characterised by a nectar scale- staminode complex and pollen release by thigmonastic stamen movements. We described pollen and nectar presentation, analysed the breeding system and the foraging strategy of bee pollinators. We determined the nectar production pattern and documented variations in the longevity of floral phases and stigmatic pollen loads of pollinator-visited and unvisited flowers. *Bicolletes indigoticus* (Colletidae) was the sole pollinator with females revisiting flowers in staminate and pistillate phases at short intervals, guaranteeing cross-pollen flow. Nectar stored in the nectar scale-staminode complex had a high sugar concentration and was produced continuously in minute amounts ($\sim 0.09 \mu\text{l h}^{-1}$). Pushing the scales outward, bees took up nectar, triggering stamen movements and accelerating pollen presentation. Experimental simulation of this nectar uptake increased the number of moved stamens per hour by a factor of four. Flowers visited by pollinators received six-fold more pollen on the stigma than unvisited flowers, had shortened staminate and pistillate phases and increased fruit and seed set. Flower handling and foraging by *Bicolletes indigoticus* were consonant with the complex flower morphology and functioning of *Blumenbachia insignis*. Continuous nectar production in minute quantities but at high sugar concentration influences the pollen foraging of the bees. Partitioning of resources lead to absolute flower fidelity and stereotyped foraging behaviour by the sole effective oligolectic bee pollinator.

Keywords: Colletidae. Foraging behaviour. Loasoideae. Oligolectic bees. Tilt-revolver flowers.

Introduction

The complex relationship between flowering plants and their pollinators varies widely in degree of specialization /generalisation (Waser *et al.* 1996, Armbruster 2017). Species involved in specialised plant–pollinator interactions frequently exhibit physiological and morphological adaptations that characterise the interaction (Linsley 1958). Oligolectic bees,

for example, are specialised floral visitors that only feed their larvae with pollen from phylogenetically closely-related host plants of the same genus or family (Robertson 1925). The seasonal flight activity of oligolectic species is, in general, finely adjusted to the flowering season of the host plant, as is their daily foraging activity adjusted to the schedule of pollen presentation (Linsley 1958, Minckley *et al.* 1994, Wcislo & Cane 1996, Danforth 1999, Alves-dos-Santos & Wittmann 2000, Larsson 2005, Carvalho & Schlindwein 2011, Cane 2018). Due to the close relationship that oligolectic bees have with their host plants, a general prediction in plant–pollinator systems is that they are better adapted to manipulate host plant flowers than generalist bees. However, despite the specialised pollen diet of oligolectic bees and their high efficiency of resource collection, an open question is whether these pollen-specialist bees are good pollinators of their hosts plants and effectively contribute to fruit and seed set (Schlindwein 2004, Tepedino *et al.* 2016). Plant species that host oligolectic bees may exhibit adaptations that enhance the pollen transfer by its specialised floral visitors, including complex floral morphologies, the concealment of floral resources, requiring proper floral handling (Thorp 1979, Alves-dos-Santos & Wittmann 1999, Milet-Pinheiro & Schlindwein 2010) or the gradual release of minute quantities of both pollen and/or nectar, forcing the bees to repeatedly visit the flower (Harder & Thomson 1989, Morgan 2000). These adaptations will in turn contribute to narrow the spectrum of floral visitors, which can in theory result in highly specialised bee–flower relationships. Such systems are however rare and poorly investigated.

Melittophilous species of the subfamily Loasoideae (Loasaceae) share such unique and complex flower morphology. The androecium is composed of five groups of staminode complexes alternating with bundles of fertile stamens. Each staminode complex corresponds to a conspicuous concave nectar scale opposed by two free staminodes. The nectar, which is produced at the base of the flower, is stored at the base of the nectar scales, hidden between the scale and the free staminodes (Urban 1886, 1892, Brown & Kaul 1981, Weigend & Rodriguez 2003, Ackermann & Weigend 2006). The flowers are protandrous and release pollen gradually through individual movements of stamens from their initial position, concealed in the naviculate petals, to the centre of the flower. The movements either occur autonomously or thigmonastically *i.e.* when plant organs actively move in response to physical contact (Braam 2005), in this case, after mechanical stimuli of the nectar scales by pollinators (Schlindwein & Wittmann 1997). Once all the stamens have moved, the pistillate phase begins with the style stretching and the stigma becoming prominent in the centre of the flower. If

flower visitors do not remove the exposed pollen, the stigma contacts the pollen autonomously, thus assuring self-pollination (Henning & Weigend 2013).

Bee visitors must handle the flowers adequately to exploit floral resources. They must tilt each of the nectar scales separately with their head to collect nectar – thus the denomination ‘tilt-revolver flowers’ (Weigend & Gottschling 2006) – and adjust pollen foraging to the partitioned presentation of pollen in small packages. The main floral visitors of melittophilous species of Loasoideae are short-tongued bees of the family Colletidae (Schlindwein & Wittmann 1997, Schlindwein 1998, Troncoso & Vargas 2004, Ackermann & Weigend 2006, Weigend & Gottschling 2006, Cares-Suárez *et al.* 2011), with some species having narrow host plant preferences for pollen (oligolecty) (Schlindwein & Wittmann 1997, Leite *et al.* 2016, Siriani-Oliveira *et al.* 2018). Analysis of the foraging behaviour of oligolectic bees in flowers of species of Loasaceae has shown that they have foraging strategies adapted to the rhythm of pollen presentation, revisiting individual flowers of both floral phases at short intervals and contribute to a high rate of fruit and seed set (Schlindwein & Wittmann 1997, Siriani-Oliveira *et al.* 2018). The mechanism that drives such short revisits to individual flowers, however, has not yet been elucidated. Previous studies have suggested that pollen supply shaped the behaviour of pollinators, leaving the role of nectar in the background. It has been shown that melittophilous species of Loasaceae produce very small amounts of highly concentrated nectar (Ackermann & Weigend 2006), but the dynamics of nectar supply has not been measured in the field. The pattern of nectar production and presentation throughout anthesis is certainly important to attract bees, especially during the pistillate phase of the flowers and may provide an explanation for the foraging behaviour of these specialist bees.

We studied the pollination of the annual *Blumenbachia insignis* Schrad., a melittophilous species of Loasoideae. We focused on the dynamics of pollen and nectar presentation, its consequences for foraging behaviour of floral visitors and reproduction of the plant. Thus, we aimed to address the following questions: (i) what are the characteristics of floral resource presentation by *B. insignis*; (ii) how do pollinators handle complex flowers to collect nectar and pollen and behave considering the partitioned resource presentation; (iii) does longevity of individual flowers varies with regard to visits of pollinators; (iv) are oligolectic bees effective pollinators of *B. insignis* and how dependent is seed set from these specialised bees; and (v) is autonomous pollen deposition equivalent in number of pollen grains to the deposition by the pollinators?

Material and methods

Study area

The study took place throughout October–December, covering the flowering seasons of *B. insignis*, in 2016, 2017 and 2018. The study location was in the region of Guaritas, municipality of Caçapava do Sul, which is inserted in the Pampa domain of the state of Rio Grande do Sul, Brazil (30°53'41.0" S, 53°25'09.0" W; 226 m.a.s.l.). Guaritas are 30- to 100-m high steep hills formed by Cambrian-Ordovician sandstone and conglomerates, which are covered by patches of xeromorphic plants and surrounded by a matrix of open bushland with many herbs and scattered trees (Schlindwein 1998). The climate is humid, subtropical to temperate (Maluf 2000), with an average annual precipitation of 1509 mm. Mean monthly temperatures range from 23.5 to 13.4 °C (INMET- Instituto Nacional de Meteorologia 2018).

Study species

Blumenbachia insignis occurs from the state of Rio Grande do Sul, the southernmost state of Brazil, to Patagonia (Argentina) (Urban & Gilg 1900). In the study site, *B. insignis* grows on top of Guaritas, always in small isolated patches, usually leaning on terrestrial bromeliads of *Dyckia maritima* Baker. Together with four other species, it comprises the taxonomic core of the genus *Blumenbachia* sect. *Blumenbachia*. This section is morphologically quite homogeneous for vegetative characters, and all are annual soft-stemmed ascending herbs (Henning *et al.* 2015). As developed above, species of *Blumenbachia*, like most members of Loasaceae, have complex floral morphology and function (Fig. 1). A voucher of the studied species was deposited at BHCN herbarium (BHCN 185471).

Floral functioning

Stamen movements

In 2016 and 2017 we experimentally examined stamen movements of individual flowers in the field. We recorded and compared the number of moved stamens per hour in two groups of marked flowers: (i) hand-stimulated, and (ii) non-stimulated flowers (N = 10 flowers per group from five individual plants). In hand-stimulated flowers, we simulated flower visits by applying mechanical stimuli to nectar scales every 5 min for 1 h to evaluate whether flower visitors trigger stamen movements while taking up nectar. The interval for the

non-natural stimulation was established in concordance with published studies (Schlindwein & Wittmann 1997, Henning & Weigend 2012, Leite *et al.* 2016, Siriani-Oliveira *et al.* 2018). Stimuli consisted of the application of slight outward pressure to each of five nectar scales using a toothpick. When stamens moved, we measured the time interval between stimuli and the arrival of stamens in the centre of the flower. Non-stimulated flowers were bagged to prevent access of bee visitors. Bees nearing hand-stimulated flowers were dispelled to ensure that these flowers remained unvisited. All flowers used in this experiment were in the middle of the staminate phase (*i.e.* when about half of the stamens had already moved to the centre of the flower) and were evenly distributed among plants.

Flower longevity

We measured the longevity of individual flowers while noting the duration of staminate and pistillate phases of anthesis three times a day [from 09:00 to 18:00 h – observed hours (o.h.)]. To evaluate whether floral visits influence flower longevity, we compared the duration of non-visited bagged flowers (N = 20 flowers) with bee-visited flowers (N = 54 flowers). We defined floral longevity as the length of the period that the flowers remained open and functional (according to Ashman & Schoen 1994, Primack 1995, Schlindwein *et al.* 2005, Henning & Weigend 2012). This period corresponds to the time from 09:00 to 18:00 h for *B. insignis*, since flowers close partially from late afternoon to the early morning and pause the movement of stamens. This time interval also corresponds to the period of pollinator activity. We considered the staminate phase to be the period from the beginning of flower opening until all stamens had moved. We considered the pistillate phase to be the period after the staminate phase when the stigma becomes prominent at the level of anthers of the moved stamens, until floral senescence. The flowers used in the experiment were evenly distributed among five individual plants.

Nectar production

Nectar production of *B. insignis* was evaluated by extracting and measuring the nectar content of 15 flowers from five different individual plants (three flowers per individual) three times a day (09:00 – 11:00, 12:00 – 14:00 and 15:00 – 17:00 h), for four consecutive days in 2018. All evaluated flowers were in the same stage of development (*i.e.* the beginning of the staminate phase) and were bagged the day before the measurements were taken to prevent the removal of nectar by flower visitors. We extracted the nectar of each flower by inserting

minicaps (1 μ l; Hirschmann Laborgeräte, Eberstadt, Germany) between the free staminodes and the nectar scale. To be certain that we removed the maximum amount of nectar in each staminode complex, we inserted capillaries twice at the base of the scales until the nectar column stopped moving upward. To compare the mean amount of nectar produced during the three-time intervals, we calculated an overall mean of the three intervals to estimate nectar production per hour and per minute.

We determined the average sugar concentration of nectar by pooling nectar extracted from five flowers from five different individual plants and calculated sugar concentrations from Brix measurements made (three times for each floral phase) with a handheld refractometer (Instrutherm, RT-82).

Number of ovules and stamens per flower

To determine the number of ovules and stamens per flower, we collected 25 flower buds from 15 individual plants (one to two flowers per individual) and fixed them in 70% ethanol. We then counted the number of ovules and stamens per flower in the laboratory using a stereomicroscope (Leica, WILD – M3Z).

Breeding system

To determine whether *B. insignis* is a facultative selfer we assessed whether its flowers set fruit and seeds when pollinators were excluded. For fruit set we considered the percentage of marked flowers with formed fruits, and for seed set we considered the mean number of seeds per fruit produced. We compared fruit and seed set of flowers available to pollinators (open/natural pollination; N = 38) to those that were simply bagged (autonomous self-pollination; N = 103) to those that were bagged and hand self-pollinated (hand self-pollination; N = 24). Autonomous self-pollinated and hand-pollinated flowers were bagged in the bud stage. When the latter reached the pistillate phase, we removed the bags and then the anthers of the stamens that moved in the staminate phase and used their pollen content to cover the stigma. Then, we re-bagged the flowers. The experiments were conducted during the three flowering seasons.

Pollinator foraging

Flower visitors and visitation frequency

We sampled flower visitors of *B. insignis* using entomological nets throughout the study period (~56 days, covering the entire flowering period of *B. insignis*). The specimens sampled were mounted with entomological pins, identified and deposited in the Entomological Collection of UFMG (Centro de Coleções Taxonômicas da UFMG, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil). We determined visitation frequency per flower throughout the day by making paired 30- min counts for 28 flowers of the staminate phase and 28 flowers of the pistillate phase during four time intervals (09:00 – 11:00, 11:00 – 13:00, 13:00 – 15:00 and 15:00 – 17:00 h), on ten non-consecutive days in the 3 years of the study. These established daytime intervals correspond to the flight activity of pollinators of *B. insignis*.

Pollen deposition on stigmas

To analyse pollen deposition on flowers, we quantified the amount of pollen grains that adhered to the stigmatic surfaces of styles collected from three sets of senescent flowers: (i) emasculated flowers – flowers in which pollen was deposited exclusively by pollinators (N = 18); (ii) unvisited, previously bagged flowers – flowers in which solely autonomous self-deposition occurred (N = 15); and (iii) control flowers – flowers in which both kinds of deposition occurred (autonomous self-deposition + deposition by pollinators) (N = 18). For flowers of all three sets we removed each style with preparation scissors, transferred it to a microscope slide with a small piece of glycerinated gelatine and covered it with a coverslip that was sealed with paraffin. We counted the pollen grains using a microscope (Zeiss – Axiolab A1) and compared the counts from the stigmas of the three sets of flowers. We also searched for heterospecific pollen grains while performing the pollen counts.

Foraging behaviour of pollinators

To describe the foraging behaviour of pollinators, in 2016 we captured individual female bees using entomological nets (N = 10 females), marked them on the mesoscutum with colour codes using Revell ink (Revell, Germany) and then released them. The procedure had no notable influence on their foraging behaviour. We numbered all open flowers in a flower patch of *B. insignis*, where it was possible to observe all flowers simultaneously, and recorded foraging bouts (sequence of flower visits) of individually marked bees. For each bout, we recorded: (a) duration, (b) total number of visits, (c) number of visits and revisits to individual flowers and (d) duration of the intervals between revisits. For each floral visit we

recorded: (i) whether nectar and/or pollen were collected, (ii) the number of nectar scales probed in search of nectar and (iii) the behaviour the female performed to remove pollen from anthers: *i.e.* ‘pollen brushing’ or ‘stamen pulling’ according to Siriani-Oliveira *et al.* (2018). Pollen brushing is when females legitimately brush pollen grains from moved or moving stamens using scopal hairs of the metasoma and hind legs. On the other hand, stamen pulling is when females illegitimately look for unmoved stamens that are still hidden in the naviculate petals, at which point they move to the petals, grasp a filament with the tarsal claws of the forelegs and the mandibles and then pull the stamens to the centre of the flower. We calculated mean flower-handling times for individually marked females by dividing the duration of consecutive flower visits by the number of flowers visited. Thus, the calculated handling time includes the duration of the flower visit plus the flight time to the next flower. We recorded 22 sequences of flower visits of ten individual bees (two to three foraging bouts per female). To obtain information on possible oligolecty of floral visitors, as demonstrated for pollinators of other Brazilian Loasaceae (Schlindwein & Wittmann 1997, Leite *et al.* 2016, Siriani-Oliveira *et al.* 2018), we analysed scopa pollen loads of ten females and sampled flower visitors of melittophilous plants in the extension of the Guaritas and the surrounding vegetation in the 3 years of the study.

Data analyses

We used Student’s *t*-test to compare the number of moved stamens in experimentally hand-stimulated flowers with the number moved in non-stimulated flowers. We used a Linear Mixed-Effects Model (LMM) to compare the frequency of flower visits among the four periods of the day and among flower phases. The frequency of flower visits was analysed as the response (dependent) variable, while periods of the day and flower phases were categorical predictor (independent) variables; ten non-consecutive days were also included as random variable. We used one-way repeated measures ANOVA to compare the duration of floral phases of non-visited flowers with the duration of the phases for bee-visited flowers. We used a Generalised Linear Mixed-Effects Model (GLMM) assuming gamma distribution to compare the mean volume of nectar produced by individual flowers among the three periods of the day throughout four consecutive days. Nectar volumes were analysed as the response (dependent) variable, while periods of the day and the four consecutive days were predictor (independent) variables; flower phase was also included as random variable. We used the non-parametric Kruskal-Wallis one-way ANOVA by ranks to compare: (i) average

seed set among the breeding systems treatments, and (ii) the mean number of pollen grains adhered to the stigmatic surfaces of styles from three sets of flowers. We conducted statistical analyses using SigmaStat 3.5 (Systat Software, Slough, UK) for Windows and R package lme4 (Bates *et al.* 2015) version 3.5.3.

Results

Floral functioning

Pollen presentation by stamen movements

Stamen movement for *B. insignis* could be triggered by pushing the nectar scales slightly outwards, thus characterizing thigmonasty. Stamens reached the centre of the flower in an average of 3.8 ± 1.7 min after experimental stimuli (N = 10 flowers). Approximately four times more stamens moved in hand-stimulated flowers (9.8 ± 2.9 , N = 10 flowers), than in non-stimulated flowers (2.3 ± 2.5 , N = 10 flowers) ($t = 6.153$, $df = 18$, $P < 0.001$, N = 20 flowers) (Fig. 2). Stamen movements occurred after 82% of the stimuli made in the 1-h stimulation experiments. After all stamens had moved, the styles elongated and became prominent in the centre of the flower, indicating the beginning of the pistillate phase (Fig. 3A).

Anthesis and flower longevity

The longevity of flowers was 3–5 days. Flower opening for individual plants of *B. insignis* was not simultaneous and occurred throughout the day, between 09:00 and 18:00 h. Flowers slightly reduced their opening angles at night, but reestablished full opening the next day. Flowers visited by bees remained open for an average of 3.6 ± 0.9 days (32.5 o.h., N = 54 flowers), which was shorter than the 5.0 ± 0.9 days (46.2 o.h., N = 20 flowers) of non-visited bagged flowers. Both staminate and pistillate phases of anthesis were shorter in bee-visited flowers. The duration of the staminate phase for bee-visited flowers was on average 2.6 ± 0.7 days (23.6 o.h., N = 54 flowers), 18.7% shorter than the 3.2 ± 0.6 days (29.7 o.h., N = 20 flowers) for non-visited flowers. The duration of the pistillate phase for bee-visited flowers was 1.0 ± 0.4 days (8.8 o.h., N = 54 flowers), 44.4% shorter than the 1.8 ± 0.4 days (16.5 o.h., N = 20 flowers) for non-visited flowers (one-way RM ANOVA, $F_{53,3,91} = 126.5$, $P < 0.001$, N = 74 flowers) (Fig. 4).

Nectar production

Floral nectar production by *B. insignis* was continuous throughout the day. Single flowers produced similar amounts of nectar every 3 h throughout four consecutive days (GLMM; $X^2 = 0.435$; $df = 2$; $P = 0.804$; $N = 15$ flowers, 180 measures, 12 measures per flower). In the first 2 days of anthesis (staminate phase), flowers produced on average $0.29 \pm 0.09 \mu\text{l}$ and $0.28 \pm 0.13 \mu\text{l}$ of nectar every 3 h ($N = 15$ flowers), respectively. In the last 2 days (pistillate phase), flowers produced on average $0.23 \pm 0.11 \mu\text{l}$ and $0.27 \pm 0.12 \mu\text{l}$ of nectar ($N = 15$ flowers), respectively. The overall average nectar production per flower was $0.27 \pm 0.11 \mu\text{l}$ ($N = 15$ flowers, 180 measurements) of nectar every 3 h. Using this measure, we estimated a mean secretion of $0.09 \mu\text{l h}^{-1}$ and $0.0015 \mu\text{l min}^{-1}$. The mean nectar concentration was $67.0 \pm 2.8\%$ ($N = 6$ measurements, three for each floral phase).

Number of ovules per flower and breeding system

Flowers of *B. insignis* contained on average 49.1 ± 13.0 ovules ($N = 25$ flowers). Fruits and seeds were produced by self-pollination, but at a lower rate than in the presence of pollinators. Hand self-pollinated flowers produced on average twice as many fruits with a similar number of seeds as those produced by autonomous self-pollinated flowers. All open-pollinated flowers formed fruits with three times more seeds than after self-pollination (Kruskal-Wallis = 6.075, $df = 2$, $P = < 0.001$, $N = 98$ flowers) (Table 1).

Pollinator foraging

Flower visitors and visitation frequency

Females and males of *Bicolletes indigoticus* (Compagnucci & Roig-Alsina, 2008) were the almost exclusive flower visitors of *B. insignis*. The bees visited flowers throughout the entire period of observation (~56 days), and females carried pollen loads exclusively from *B. insignis* in the scopa ($N = 10$). No individual of this species was sampled on flowers of any other plant species of the vegetation of the Guaritas and the surroundings. Males spent most of their time patrolling flower patches of *B. insignis* and visited the flowers only occasionally to take up nectar. During ~230 h of observation, only one visit by a female of *Colletes* sp. and five visits by males of *Bicolletes pampeana* Urban, 1995 were recorded. We recorded an overall average of 17.3 ± 7.3 ($N = 56$ flowers) flower visits 30-min^{-1} interval by females of *B. indigoticus*. The frequency of visits was similar between flowers of the staminate and

pistillate phases (17.2 ± 8.2 and 17.5 ± 6.5 visits 30 min^{-1} , respectively; $N = 28$ flowers per phase) and throughout the day, being just slightly lower only in the late afternoon [09:00 – 11:00 h = 16.6 ± 6.1 flower visits (f.v.); 11:00 – 13:00 h = 20.4 ± 5.9 f.v.; 13:00 – 15:00 h = 18.7 ± 9.9 f.v.; 15:00 – 17:00 h = 13.5 ± 5.2 f.v. per 30 min; $N = 56$, 14 flowers per interval, seven per flower phase; LMM; $X^2 = 7.430$; $df = 4$; $P = 0.115$; $N = 56$].

Flower handling and foraging behaviour

To land on the pendulous flowers of *B. insignis*, bees grasped the revolute collar-shaped apices of the nectar scales with their tarsal claws. The bees began to look for nectar immediately after landing in 90.9% of flower visits (471 of 518 visits), by inserting their head between nectar scales and free staminodes and pushing the scales outward (Fig. 3B). Females probed on average 3.5 ± 1.9 ($N = 471$ visits) nectar scales per nectar visit. Bees searched for nectar in all five scales during 18.0% (85 of 471 visits) of the nectar visits, pushing them in sequence in clockwise or counter-clockwise rotation. Most frequently, bees searched for nectar in only one scale 21.2% (100 of 471 visits). During nectar uptake in flowers in the pistillate phase, the bees continuously contacted the protuberant stigmas with the ventral surface of the mesosoma and metasoma (Fig. 3C), thus transferred allochthonous pollen to the stigmatic surface. During 53.3% of the flower visits (273 of 518 visits), female bees actively collected pollen after pushing the nectar scales, exhibiting the two pollen-collection behaviours: pollen brushing, which was used in 62.7% (173 of 273 visits) of the pollen-collection visits (Fig. 3D), and stamen pulling, which was used in 37.3% (103 of 273 visits) of the visits (Fig. 3E). The bees collected pollen from pulled stamens with already dehisced anthers by brushing the anthers with their hind legs. No pollen was removed from stamens that still had closed anthers.

Flower revisits

Observations of individually marked females of *B. indigoticus* in flower patches of *B. insignis* revealed that they maintained established foraging areas for up to 15 consecutive days in each year of the study. During the recorded foraging bouts, the marked females visited flowers at an average rate of 4.5 ± 1.7 visits min^{-1} ($N = 22$ foraging bouts). Handling time during the visits varied from 6.0 to 23.0 s (14.5 ± 4.2 s, $N = 22$ foraging bouts). Females continuously revisited the same flowers throughout foraging bouts, 47.1% (244 of 518) of all recorded visits were followed by revisits to previously visited flowers. Revisit intervals were

mostly short, with revisits occurring within 4 min in 81.6% of the cases (199 of 244 revisits), and most frequently in intervals of between 1 and 2 min (34.0%; 8 revisits).

Pollen deposition on stigmas

The number of pollen grains deposited on stigmatic surfaces varied among the three sets of flowers (Kruskal-Wallis = 25.896, $df = 2$, $P = < 0.001$, $N = 51$ flowers). The number of pollen grains was similar among bee-visited flowers, but differed from the number deposited on non-visited bagged flowers. Emasculated flowers contained on average 375 pollen grains ($N = 18$ flowers), the control 456 pollen grains ($N = 15$ flowers) and non-visited flowers only 83 pollen grains ($N = 18$ flowers) (Fig. 5). Only 13 heterospecific pollen grains (*Pinus* and Asteraceae) were found on stigma surfaces, which represented only 0.06% of the 19,990 pollen grains counted.

Discussion

The present study revealed a highly specialised plant–pollinator interaction between *Blumenbachia insignis* and the oligolectic bee *Bicolletes indigoticus*. The complex tilt-revolver flowers of *B. insignis* provide a plastic mechanism for floral resource presentation, which shapes the foraging behaviour of its specialised pollinators. The partitioning of pollen and nectar, allied with the expressive floral fidelity of these bees, guarantee the bees an almost exclusive provision of floral resources, which in turn promotes cross-pollen flow among conspecific plants.

Blumenbachia insignis has a wide geographic distribution in the Pampa domain, and its close interaction with *B. indigoticus* appears to be consistent over time and space. The type material for the bee species (described as *Leioproctus indigoticus*) was recorded in the same period of the year. Furthermore, flower visits of females exclusively to *B. insignis* were recorded at three localities in Argentina, including the southern boundary of the Pampa domain, ~1300 km distant from our study site in south Brazil (Compagnucci & Roig-Alsina 2008). Sporadic visits of *B. indigoticus* (cited as *Bicolletes franki* Friese, 1908) to flowers of the Loasoideae *Caiophora arechavaletae* (Urb.) Urb. and Gilg in the same region (Schlindwein & Wittmann 1997) confirm its close relationship to this subfamily.

Thigmonastic stamen movements and flower longevity

A functional interpretation of thigmonastic stamen movements in Loasaceae in the context of pollination ecology was first proposed for *Caiophora arechavaletae* (Schlindwein & Wittmann 1997). More recently it has been demonstrated that pollen presentation in response to mechanic stimulation of the nectar scale-staminode complex is an apomorphy present in several taxa of the lineage of Loasoideae (Weigend *et al.* 2004, 2010, Henning & Weigend 2012, 2013, Henning *et al.* 2018, Siriani-Oliveira *et al.* 2018). According to the theoretical framework proposed by the ‘Pollen Presentation Theory’ (Percival 1955, Harder & Thomson 1989, Harder & Wilson 1994), flowering plants evolved mechanisms that improve the efficiency of pollen export according to the availability of pollinators, or reduce the amount of pollen that a floral visitor can remove in a single visit, resulting in more movements among conspecific plants, thus improving reproductive success. Individual flowers of *B. insignis* are capable of regulating pollen supply in concordance to the given pollinator environment. By imitating pollinator behaviour, we were able to show that four times more stamens moved in stimulated flowers than in non-stimulated flowers. Under natural conditions with many flower visits, as is the case of the present study (a flower is visited approximately every 2 min), flowers offer pollen much faster, thus increasing the probability that the released pollen grains will reach receptive stigmas. When flower visitors are experimentally excluded, such as with bagged flowers, the release of pollen is delayed and fewer stamens move at a slower rate. This characteristic can be interpreted as a ‘standby mechanism’ during periods with low pollinator density, which may happen, for example, when there is temporary seasonal mismatch between flowering and emergence of specialist bees or momentary periods with bad weather when bees are not able to fly. These findings are congruent with those for the closely related *Blumenbachia amana* Henning and Weigend (Siriani-Oliveira *et al.* 2018) and those for species of Andean Loasoideae (Henning & Weigend 2012, 2013, Mittelbach *et al.* 2019).

With the accelerated rate of stamen movements under the high visitation rates by *B. indigoticus*, overall floral longevity is shortened by 30%. The capacity for variation in flower longevity is common among plant species and is interpreted as favouring outcrossing and ovule fertilisation (Primack 1985, Fung & Thomson 2017). Shortening in *B. insignis* occurs in both the pollen donation and the pollen reception phases in pollinator-visited flowers, thus enhancing male fitness due to accelerated pollen transfer onto effective pollinators, and female fitness by increasing fruit and seed set.

Breeding system

In contrast to other annual species of Loasoideae that guarantee high fruit and seed set even in the absence of pollinators (Schlindwein & Wittmann 1997, Henning & Weigend 2013, Siriani-Oliveira *et al.* 2018), flowers of *B. insignis* produced unexpected rates of fruit and seed set. Autonomous self-pollinated flowers produced less fruits, with only about one-third the quantity of seeds. To guarantee high levels of fruit and seed formation, flowers thus require repeated arrival of pollen *via* pollinators after stigma maturation. The stigmas of autonomously self-pollinated flowers undergo only a single deposition event, when the style, during elongation, passes through the bundle of anthers of mature stamens in the centre of the flower. This phenomenon was referred to as ‘mid-anthetic self-pollination in the absence of pollinators’ by Henning & Weigend (2013). We showed that only a few pollen grains adhered to the stigmatic surfaces in autonomous self-pollinated flowers, indicating the need for contact between stigmas and the ventral scopas of the specialised *B. indigoticus* bees, which press their body forward to reach nectar in the nectar scales.

Nectar production

Our measurements of nectar production revealed that the flowers of *B. insignis* produce highly concentrated nectar in very small quantities ($\sim 0.09 \mu\text{l h}^{-1}$ and $0.0015 \mu\text{l min}^{-1}$) continuously throughout anthesis regardless of the floral phase. This amount is minimal when compared to other pollination systems of melittophilous species with continuous nectar replenishment. For example, flowers visited by several taxonomical groups of bees produce eight to 73 times more nectar per hour when compared to flowers of *B. insignis* (Galetto & Bernardello 2004, Lu *et al.* 2015, Ye *et al.* 2017). Moreover, species pollinated exclusively by long-tongued bees produce 170 and 333 times more nectar than *B. insignis*, but with lower sugar concentration, varying from 22% to 37% (Ashworth & Galetto 2002, Varassin *et al.* 2018). Considering the lineage to which Loasoideae belongs, nectar production of *B. insignis* corresponds to Loasoideae Group I of Ackermann & Weigend (2006): a group of low-elevation melittophilous plants with small, white, star-shaped flowers and low nectar quantity with very high sugar concentration. Continuous nectar production was also found for *Nasa macrothyrsa* (Urb. and Gilg) Weigend, which have flowers that are structurally similar to those of *B. insignis* (Weigend *et al.* 2010), but belong to Loasoideae Group III (high-elevation plants with large flowers, high nectar quantity with low sugar concentration). Nectar production of *N. macrothyrsa* ($4.2\text{--}9 \mu\text{l h}^{-1}$) is by far larger than that found for *B. insignis*, and indeed has a lower sugar concentration (Weigend *et al.*, 2010). Flowers of *N.*

macrothyrsa are pollinated by large carpenter bees (*Xylocopa lachnea* Moure, 1951) that visit the flowers exclusively to take up nectar. Therefore, foraging by these bees is exclusively motivated and influenced by the dynamics of nectar production. This contrasts with Loasoideae Group I, which especially attract pollen-seeking oligolectic bees for which the pollen presentation schedule is of great importance for their foraging strategy (Schlindwein & Wittmann 1997, Siriani-Oliveira *et al.* 2018).

Foraging behaviour of *Bicolletes indigoticus*

The continuously produced small quantities of nectar might explain the high frequency at which bees insert their mouthparts into the nectar scales. The nectar standing crop – *i.e.* amount of nectar that floral visitors can encounter while foraging (Zimmerman 1988) – in flower patches of *B. insignis* must be quite variable and unpredictable to pollinators of single flowers, since various bee individuals forage at the same time, constantly removing the small amounts of nectar secreted. Each flower receives an average of 17 visits per 30 min, which is about one visit every 2 min throughout the lifespan of the flower. Given that bees searched for nectar during almost all visits, and taking into account an average production rate of $0.0015 \mu\text{l min}^{-1}$, a bee would receive around $\sim 0.003 \mu\text{l}$ of nectar per visit in a single flower. Considering that each of the five nectar scales produce an equivalent amount of nectar, and that bees mostly probe only one nectar scale per visit, we can estimate that a bee only receives an uncertain quantity of $\sim 0.0006 \mu\text{l}$ of nectar per probed scale every 2 min.

We hypothesise that this minute energy uptake per insertion of mouthparts in a nectar scale induces bees of *B. indigoticus* to visit flowers at frequencies high enough to obtain sufficient energetic profit during foraging flights. Because nectar is continuously replenished, bees might be encouraged to search for nectar throughout the day, as occurs in other plant–pollinator interactions (Thomson *et al.* 1982, Varassin *et al.* 2018). Flowers in staminate and pistillate phases produce similar quantities of nectar, and bees visit both of them equally. Consequently, the dynamics of nectar supply may directly contribute to male and female fitness, since nectar foraging decisions affect pollen movement within conspecific flowers (Thomson 1986, Real & Rathcke 1991, Mitchell & Waser 1992, Fischer & Leal 2006).

When floral visitors stimulate stamen movements during nectar uptake, pollen becomes available in only a matter of a few minutes. Revisit intervals to flowers within the first 4 min accounted for over 80% of all revisits by females of *B. indigoticus*. Thus, pollen grains of newly migrated stamens are soon exported to conspecific flowers. The dynamics of

both pollen and nectar presentation, associated with the foraging strategy of *B. indigoticus*, results in efficient export and receipt of pollen exclusively by this specialised bee species. Dehisced anthers in the staminate phase and the receptive stigma in the pistillate phase are correspondingly positioned in the centre of the flowers. During stereotypical nectar uptake by females of *B. indigoticus*, a fraction of the pollen content, which is passively or actively incorporated into their ventral scopa during visits to staminate phase flowers, is accurately deposited on receptive stigmas of pistillate phase flowers. According to our data, the number of bee-deposited xenogamous pollen grains on the stigma of emasculated flowers was seven times higher than the number of ovules, and thus adequate to fertilise all of them. Seed set in naturally pollinated flowers is maximum, which reflects the efficiency of this bee–plant relationship, as also observed in several other specialised pollination systems (Linsley 1958, Alves-dos-Santos & Wittmann 2000, Milet-Pinheiro & Schlindwein 2010, Cane 2018, Cerceau *et al.* 2019).

The consonance between resource presentation of *B. insignis* and foraging behaviour of *B. indigoticus* is similar to that of *Caiophora arechavaletae* with *Bicolletes pampeana* and *Blumenbachia amana* with *Actenosigynes mantiqueirensis* Silveira 2009 (Schlindwein & Wittmann 1997, Siriani-Oliveira *et al.* 2018). In all three cases, the bees continuously stimulate nectar scales and trigger stamen movements, with pollen being presented just a few minutes later. Females of these three bee species adopt areas for constant foraging for a number of days, returning to previously visited flowers at short intervals, always in search of nectar in the nectar scale-staminode complex and removing pollen from pollen-presenting anthers. While females of *B. pampeana* collect pollen exclusively by pollen brushing, females of *B. indigoticus* and *A. mantiqueirensis* use pollen brushing and stamen pulling. It is interesting that species of two different genera exhibit illegitimate stamen pulling despite being evolutionary distant within Neotropical Neopasiphaeinae (Almeida *et al.* 2019). A comparative study of associations between Neopasiphaeinae and Loasaceae could provide insights into the evolutionary history of this still little studied bee clade.

These new findings lead us to conclude that there is a surprisingly high degree of similarity with other studied cases of the close relationships between species of Loasoideae and neopasiphaeine bees. They also lead us to conclude that analyses of both flower morphology and functioning and pollinator foraging behaviour are essential for characterising such specialised interactions. The complex flower morphology and continuous pollen and nectar removal by specialised bee pollinators empty the flowers of resources and make them

unattractive to any opportunistic floral visitors, as observed in other systems that involve oligolectic bees and their host plants (Schlindwein *et al.* 2005, Milet-Pinheiro & Schlindwein 2010, Cerceau *et al.* 2019).

The dynamics of continuous nectar production and the magnitude of energy supplied per unit time, however, may be a key factor mediating the interaction between flowers of *B. insignis* and their pollinators. Studies on how nectar replenishment influences pollinator foraging behaviour and flower attractiveness might explain whether these small quantities of extremely concentrated nectar mediate this close plant–pollinator interaction.

Acknowledgements

We thank Ana Laura Dutra and Reislá Oliveira for help with fieldwork and statistical analyses and for providing constructive comments; João Renato Stehmann, Stefan Dötterl and Vinícius Brito for comments on an earlier version of the manuscript and three anonymous reviewers for their comments, which improved the manuscript; ICMBio for collection license (Nº 55425-2); and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES - Finance Code 001) for a research grant to S.S.O., and CNPq for a research grant to C.S. (311935/2018-4).

References

- Ackermann M., Weigend M. (2006) Nectar, floral morphology and pollination syndrome in Loasaceae sub-fam. Loasoideae (Cornales). *Annals of Botany*, **98**, 503–514.
- Almeida E.A.B., Packer L., Melo G.A.R., Danforth B.N., Cardinal S.C., Quinteiro F.B., Pie M.R. (2019) The diversification of neopasiphaeine bees during the Cenozoic (Hymenoptera: Colletidae). *Zoologica. Scripta*, **46**, 226–242.
- Alves-dos-Santos I., Wittmann D. (1999) The proboscis of the long-tongued *Ancyloscelis* bees (Anthophoridae/Apoidea), with remarks on flower visits and pollen collecting with the mouthparts. *Journal of Kansas Entomological Society*, **72**, 277–288.
- Alves-dos-Santos I., Wittmann D. (2000) Legitimate pollination of the tristylous flowers of *Eichhornia azurea* (Pontederiaceae) by *Ancyloscelis gigas* bees (Anthophoridae, Apoidea). *Plant Systematics and Evolution*, **223**, 127–137.
- Armbruster W.S. (2017) The specialization continuum in pollination systems: diversity of concepts and implications for ecology, evolution and conservation. *Functional Ecology*,

31, 88–100.

- Ashman T.L., Schoen D.J. (1994) How long should flowers live? *Nature*, **371**, 788.
- Ashworth L., Galetto L. (2002) Differential nectar production between male and female flowers in a wild cucurbit: *Cucurbita maxima* ssp. *andreaana* (Cucurbitaceae). *Canadian Journal of Botany*, **80**, 1203–1208.
- Bates D., Maechler M., Bolker B., Walker S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, **67**, 1–48.
- Braam J. (2005) In touch: plant responses to mechanical stimuli. *New Phytologist*, **165**, 373–389.
- Brown D.K., Kaul R.B. (1981) Floral structure and mechanisms in Loasaceae. *American Journal of Botany*, **68**, 361–72.
- Cane H.J. (2018) Co-dependency between a specialist *Andrena* bee and its death camas host, *Toxicoscordion paniculatum*. *Arthropod-Plant Interactions*, **12**, 657–662.
- Cares-suárez R., Poch T., Acevedo R.F., Acosta-Bravo I., Pimentel C., Espinoza C., Cares R.A., Muñoz González A.V., Botto-Mahan C. (2011) Do pollinators respond in a dose-dependent manner to flower herbivory? An experimental assessment in *Loasa tricolor* (Loasaceae). *Gayana Botanica*, **68**, 176–181.
- Carvalho A.T., Schlindwein C. (2011) Obligate association of an oligolectic bee and a seasonal aquatic herb in semi-arid north-eastern Brazil. *Biological Journal of the Linnean Society*, **102**, 355–368.
- Cerceau I., Siriani-Oliveira S., Dutra A.L., Oliveira R., Schlindwein C. (2019) The cost of fidelity: foraging oligolectic bees gather huge amounts of pollen in a highly specialized cactus–pollinator association. *Biological Journal of the Linnean Society*, **128**, 30–43.
- Compagnucci L.A., Roig-Alsina A. (2008) Nuevos *Leioproctus* Smith de la Argentina correspondientes a los subgéneros *Spinolapis* Moure y *Perditomorpha* Ashmead (Hymenoptera, Colletidae). *Revista del Museo Argentino de Ciencias Naturales*, **10**, 319–327.
- Danforth B.N. (1999) Emergence dynamics and bet-hedging in a desert bee, *Perdita portalis*. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **266**, 1985–

1994.

- Fischer E., Leal I. (2006) Effect of nectar secretion rate on pollination success of *Passiflora coccinea* (Passifloraceae) in the Central Amazon. *Brazilian Journal of Biology*, **66**, 747–754.
- Fung H.F., Thomson J.D. (2017) Does lack of pollination extend flower life? *Journal of Pollination Ecology*, **21**, 86–91.
- Galetto L., Bernardello G. (2004) Floral nectaries, nectar production dynamics and chemical composition in six *Ipomoea* species (Convolvulaceae) in relation to pollinator. *Annals of Botany*, **94**, 269–280.
- Harder L.D., Thomson J.D. (1989) Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. *The American Naturalist*, **133**, 323–344.
- Harder L.D., Wilson W.G. (1994) Floral evolution and male reproductive success: optimal dispensing schedules for pollen dispersal by animal-pollinated plants. *Evolutionary Ecology*, **8**, 542–559.
- Henning T., Weigend M. (2012) Total control – pollen presentation and floral longevity in Loasaceae (blazing star family) are modulated by light, temperature and pollinator visitation rates. *PLoS ONE*, **7**, e41121.
- Henning T., Weigend M. (2013) Beautiful, complicated – and intelligent? Novel aspects of the thigmonastic stamen movement in Loasaceae. *Plant Signaling and Behavior*, **8**, 24605.
- Henning T., Oliveira S., Schlindwein C., Weigend M. (2015) A new, narrowly endemic species of *Blumenbachia* (Loasaceae subfam. Loasoideae) from Brazil. *Phytotaxa*, **236**, 196–200.
- Henning T., Mittelbach M., Ismail S.A., Acuña-Castillo R.H., Weigend M. (2018) A case of behavioural diversification in male floral function – the evolution of thigmonastic pollen presentation. *Scientific Reports*, **8**, 14018.
- INMET- Instituto Nacional de Meteorologia (2018) Banco de Dados Meteorológicos para Ensino e Pesquisa - BDMEP. Dados históricos da estação meteorológica 83980 - Bagé RS entre 1988 e 2018. Brasília, DF, Brasil. Available from <http://www.inmet.gov.br/portal/index.php?r=bdmep/bdmep> (accessed 14 August 2018).

- Larsson M. (2005) Higher pollinator effectiveness by specialist than generalist flower-visitors of unspecialized *Knautia arvensis* (Dipsacaceae). *Oecologia*, **146**, 394–403.
- Leite A.V., Nadia T., Machado I.C. (2016) Pollination of *Aosa rupestris* (Hook.) Weigend (Loasaceae): are stamen movements induced by pollinators? *Brazilian Journal of Botany*, **39**, 559–567.
- Linsley E.G. (1958) The ecology of solitary bees. *Hilgardia*, **27**, 543–599.
- Lu N.-N., Li X.-H., Li L., Zhao Z.-G. (2015) Variation of nectar production in relation to plant characteristics in protandrous *Aconitum gymnandrum*. *Journal of Plant Ecology*, **8**, 122–129.
- Maluf J.R.T. (2000) Nova classificação climática do estado do Rio Grande do Sul. *Revista Brasileira de Agrometeorologia*, **8**, 141–150.
- Milet-Pinheiro P., Schlindwein C. (2010) Mutual reproductive dependence of distylic *Cordia leucocephala* (Cordiaceae) and oligolectic *Ceblurgus longipalpis* (Halictidae, Rophitinae) in the Caatinga. *Annals of Botany*, **106**, 17–27.
- Minckley R.L., Wcislo W.T., Yanega D., Buchmann S.L. (1994) Behavior and phenology of a specialist bee (*Dieunomia*) and sunflower (*Helianthus*) pollen availability. *Ecology*, **73**, 1406–19.
- Mitchell R.J., Waser N.M. (1992) Adaptive significance of *Ipomopsis aggregata* nectar production: pollination success of single flowers. *Ecology*, **73**, 633–638.
- Mittelbach M., Kolbaia S., Weigend M., Henning T. (2019) Flowers anticipate revisits of pollinators by learning from previously experienced visitation intervals. *Plant Signaling & Behavior*, **14**(6), 1595320.
- Morgan M.T. (2000) Evolution of interactions between plants and their pollinators. *Plant Species Biology*, **15**, 249–259.
- Percival M.S. (1955) The presentation of pollen in certain angiosperms and its collection by *Apis mellifera*. *New Phytologist*, **54**, 353–368.
- Primack R.B. (1985) Longevity of individual flowers. *Annual Review of Ecology, Evolution, and Systematics*, **16**, 15–37.
- Real L.A., Rathcke B.J. (1991) Individual variation in nectar production and its effect on

- fitness in *Kalmia latifolia*. *Ecology*, **72**, 149–155.
- Robertson C. (1925) Heterotropic bees. *Ecology*, **6**, 412–436.
- Schlindwein C. (1998) Frequent oligolecty characterizing a diverse bee–plant community in a xerophytic bushland of subtropical Brazil. *Studies on Neotropical Fauna and Environment*, **33**, 46–59.
- Schlindwein C. (2004) Are oligolectic bees always the most effective pollinators? In Freitas B. M., Pereira J.O.P. (Eds) *Solitary bees. Conservation, rearing and management for pollinators*. Imprensa Universitária Fortaleza, Brazil, pp 285.
- Schlindwein C., Wittmann D. (1997) Micro-foraging routes of *Bicolletes pampeana* (Colletidae) and bee- induced pollen presentation in *Cajophora arechavaletae*. *Botanica Acta*, **110**, 177–83.
- Schlindwein C., Wittmann D., Martins C.F., Hamm A., Siqueira J.A., Schiffler D., Machado I.C. (2005) Pollination of *Campanula rapunculus* L. (Campanulaceae): how much pollen flows into pollination and into reproduction of oligolectic pollinators? *Plant Systematics and Evolution*, **250**, 147–156.
- Siriani-Oliveira S., Oliveira R., Schlindwein C. (2018) Pollination of *Blumenbachia amana* (Loasaceae): flower morphology and partitioned pollen presentation guarantee a private reward to a specialist pollinator. *Biological Journal of the Linnean Society.*, **124**, 479–491.
- Tepedino V.J., Arneson L.C., Durham S.L. (2016) Pollen removal and deposition by pollen- and nectar- collecting specialist and generalist bee visitors to *Iliamna bakeri* (Malvaceae). *Journal of Pollination Ecology*, **19**, 50–56.
- Thomson J.D. (1986) Pollen transport and deposition by Bumblebees in *Erythronium*: influences of floral nectar and bee grooming. *Journal of Ecology*, **74**, 329–341.
- Thomson J.D., Maddison W.P., Plowright R.C. (1982) Behavior of bumble bee pollinators of *Aralia hispida* Vent. (Araliaceae). *Oecologia*, **54**, 326–336.
- Thorp L.W. (1979) Structural, behavioral and physiological adaptations of bees (Apoidea) for collecting pollen. *Annals of the Missouri Botanical Garden*, **66**, 788–812.
- Troncoso A.J., Vargas R.R. (2004) Efecto del vecindario floral sobre la tasa de visitas por insectos a *Loasa triloba* Domb. ex A.J. Juss. y *Loasa tricolor* Ker-Gawl en la Reserva

- Nacional de Río Clarillo, Región Metropolitana, Chile. *Chloris Chilensis*, **7**. Available from <http://www.chlorischile.cl/loasa/Loasaalejandra.htm> (accessed 29 January 2019).
- Urban I. (1886) Die Bestäubungseinrichtungen der Loasaceen. *Jahrbuch des Botanischen Gartens, Berlin*, **4**, 364–388.
- Urban I. (1892) Blüten – und Fruchtbau der Loasaceen. *Berichte der Deutschen Botanischen Gesellschaft*, **10**, 259–265.
- Urban I., Gilg W. (1900) Monographia Loasacearum. *Nova Acta Academiae Caesareae Leopoldo-Carolinae Germanicae Naturae*. **76**, 1–384.
- Varassin I.G., Baggio A.C., Guimarães P.C., Prazeres L.C., Cervi A.C., Bueno R.O. (2018) Nectar dynamics and reproductive biology of *Passiflora actinia* Hook. (Passifloraceae) in Araucaria Forest. *Acta Botanica Brasilica*, **32**, 426–433.
- Waser N.M., Chittka L., Price M.V., Williams N.M., Ollerton J. (1996) Generalization in pollination systems, and why it matters. *Ecology*, **77**, 1043–1060.
- Wcislo T.W., Cane H.J. (1996) Floral resource utilization by solitary bees (Hymenoptera: Apoidea) and exploitation of their stored foods by natural enemies. *Annual Review of Entomology*, **41**, 257–286.
- Weigend M., Gottschling M. (2006) Evolution of funnel-revolver flowers and ornithophily in *Nasa* (Loasaceae). *Plant Biology*, **8**, 120–142.
- Weigend M., Rodriguez E. (2003) A revision of the *Nasa stuebeliana* group [*Nasa* ser. Saccatae (Urb. and Gilg) Weigend, Loasaceae] with notes on morphology, ecology, and distribution. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie*, **124**, 345–382.
- Weigend M., Gottschling M., Hoot S., Ackermann M. (2004) A preliminary phylogeny of Loasaceae subfam. Loasoideae (Angiospermae: Cornales) based on trnL (UAA) sequence data, with consequences for systematics and historical biogeography. *Organisms Diversity & Evolution*, **4**, 73–90.
- Weigend M., Ackermann M., Henning T. (2010) Reloading the revolver – male fitness as simple explanation for complex reward partitioning in *Nasa macrothyrsa* (Cornales, Loasaceae). *Biological Journal of Linnean Society*, **100**, 124–131.

- Ye Z.M., Jin X.F., Wang Q.F., Yang C.F., Inouye D.W. (2017) Nectar replenishment maintains the neutral effects of nectar robbing on female reproductive success of *Salvia przewalskii* (Lamiaceae), a plant pollinated and robbed by bumble bees. *Annals of Botany*, **119**, 1053–1059.
- Zimmerman M. (1988) Nectar production, flowering phenology, and strategies for pollination. In: Doust J. L., Doust L. L. (Eds), *Plant reproductive ecology – patterns and strategies*. Oxford University Press, New York, USA, pp 157–178.

Table Captions

Table 1: Fruit and seed set for autonomous self-pollination, hand self-pollination and open/natural pollination treatments with 15 individual plants of *Blumenbachia insignis* each; (Kruskal-Wallis = 6.075, df = 2, P = < 0.001, N = 98 flowers). Different letters indicate significant differences in average seed set.

Treatment	N (flowers)	Fruit set and (%)	Seed set, Median
Autonomous self-pollination	103	40 (38.8)	15.0 ^a
Hand self-pollination	24	20 (83.3)	15.0 ^a
Open/natural pollination	38	38 (100)	50.5 ^b

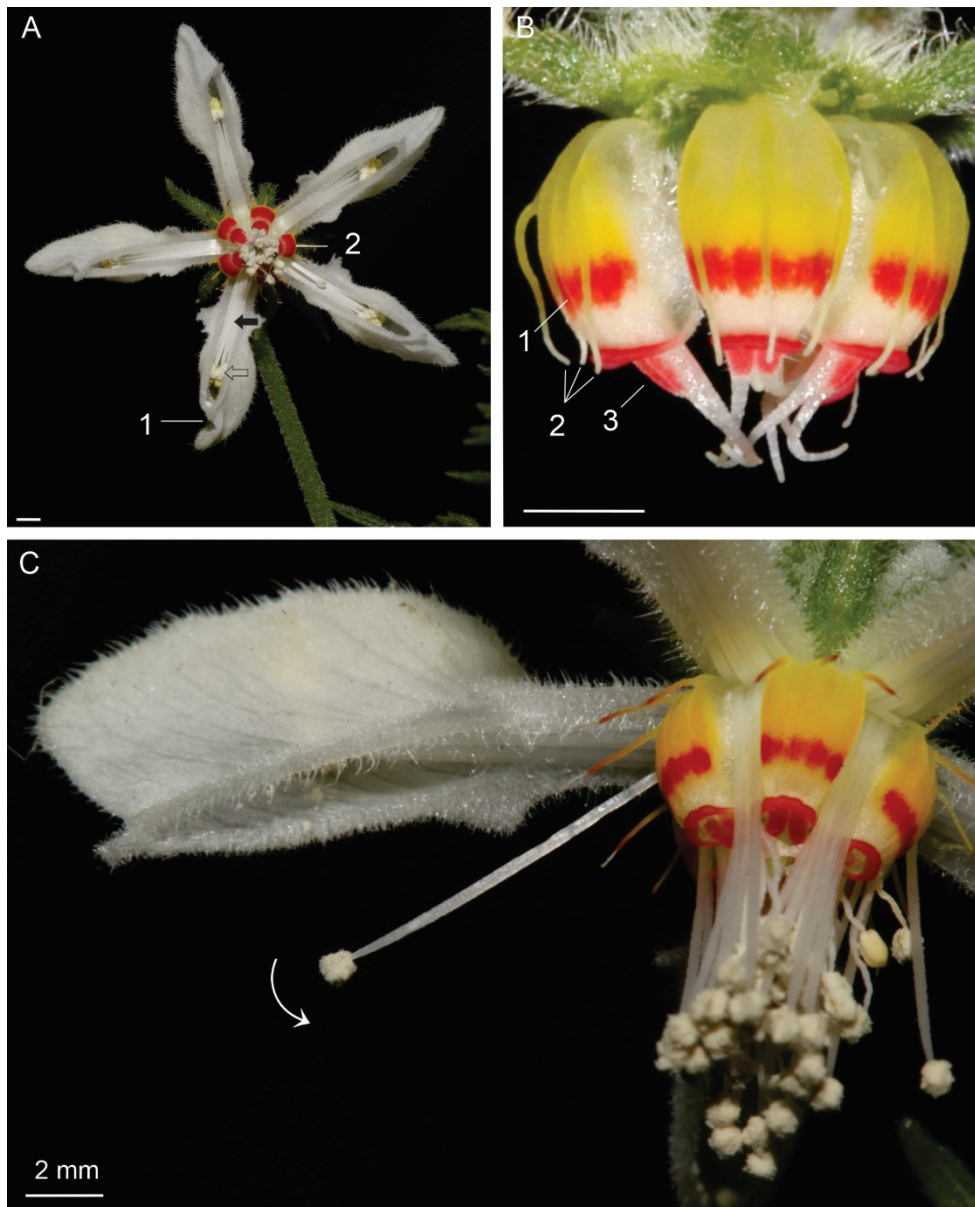


Fig. 1. Flower structure of *Blumenbachia insignis*. (A) Front view of the flower, 1 – naviculate petal hiding a fascicle of stamens before movements (solid arrow); note that the uppermost anthers are already dehiscent and present pollen grains (empty arrow with black outline); 2 – nectar scale-staminode complex. (B) Nectar scale-staminode complex in detail, 1 – nectar scale; 2 – three filiform appendices of a nectar scale; 3 – free staminodes. C – Stamen moving to the centre of the flower; the arrow indicates the direction of movement. Scale bars 2 mm unless indicated otherwise.

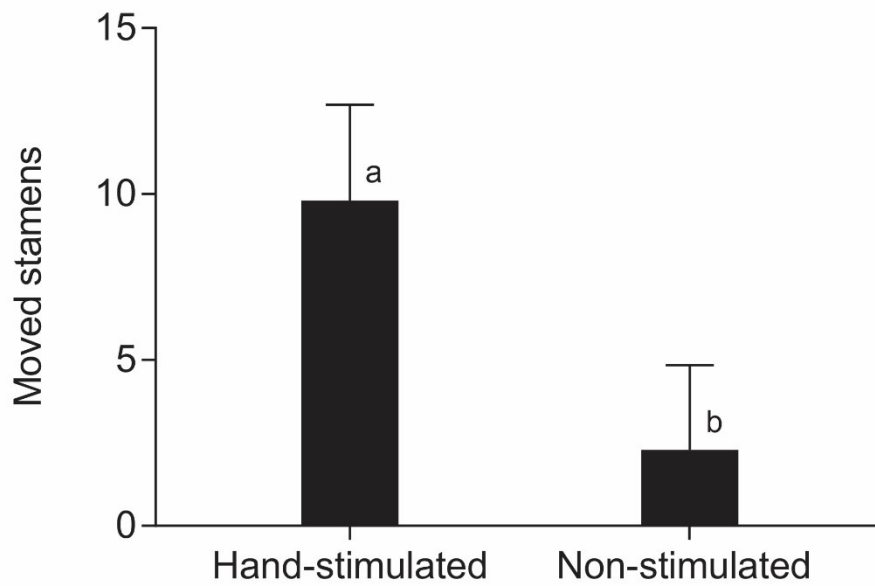


Fig. 2. Number of moved stamens in flowers of *Blumenbachia insignis*. Number of moved stamens per hour in non-stimulated and hand-stimulated flowers. Values are means \pm SD. Different letters represent significant differences between means ($t = 6.153$, $df = 18$, $P < 0.001$, $N = 20$ flowers).



Fig. 3. Visits of *Bicolletes indigoticus* to flowers of *Blumenbachia insignis*. (A) A female approaching a flower in the pistillate phase. Long style with the prominent stigma in the centre of the flower (empty arrow with white outline); moved withered stamens in the flower centre with most of the anthers empty, having been harvested by females of *B. indigoticus* (white solid arrow); (B) Nectar uptake – a red marked female bends a nectar scale outward with her head and inserts mouthparts to take up nectar (black solid arrow), while clinching to the foot hold provided by the apex of the nectar scale- staminode complex (empty arrow with black outline). (C) A female contacts the long stigma with her ventral scopa filled with pollen during nectar uptake in a visit to a flower during the pistillate phase (white arrow). (D) An orange marked female collecting pollen from a moved stamen in the centre of the flower – ‘pollen brushing’ (white arrow). (E) Female pulling a non-moved stamen with still closed anther downward with her fore legs and

mandibles – ‘stamen pulling’ (white arrow). Scale bars represent 5 mm unless indicated otherwise.

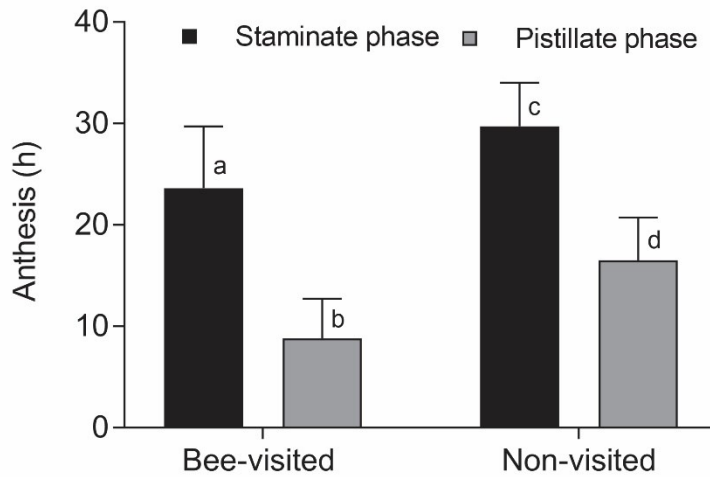


Fig. 4. Flower longevity of *Blumenbachia insignis*. Duration of staminate and pistillate phases of non-visited and bee visited flowers. Only daylight hours of open flowers were considered. Values are means \pm SD. Different letters represent significant differences between means (one-way RM ANOVA, $F_{53,3,91} = 126.5$, $P < 0.001$, $N = 74$ flowers).

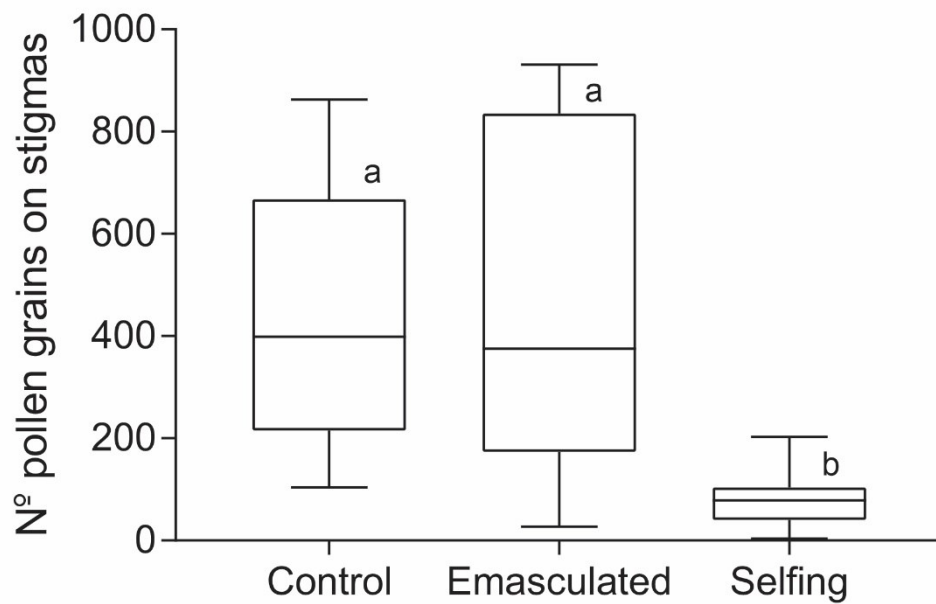


Fig. 5. Number of pollen grains adhering to the stigma of individual flowers of *Blumenbachia insignis*. Control flowers – pollen is deposited by pollinators and autonomously; emasculated flowers – pollen is deposited exclusively by pollinators; autonomous selfing – pollen is solely deposited by autonomous self-deposition. Different letters represent significant differences between means (Kruskal-Wallis = 25.896, $df = 2$, $P \ll 0.001$, $N = 51$ flowers).

Capítulo 3

A new oligolectic bee species of the genus *Rhopitulus* Ducke (Hymenoptera, Andrenidae) from South Brazil²

² A publicação original está disponível em <https://www.rbentomologia.com/en-a-new-oligolectic-bee-species-articulo-S0085562619300688?referer=buscador>. Publicado como: Ramos K.S., Siriani-Oliveira S., Schlindwein C. (2019) A new oligolectic bee species of the genus *Rhopitulus* Ducke (Hymenoptera, Andrenidae) from South Brazil. *Revista Brasileira de Entomologia* **63**: 349-355.

A new oligolectic bee species of the genus *Rhophitulus* Ducke (Hymenoptera, Andrenidae) from South Brazil

Abstract

The genus *Rhophitulus* Ducke, 1907 is a large and complex group of bees of the tribe Protandrenini comprising small, slender, mostly black ground-nesting species that are restricted to South America. We describe a new species of *Rhophitulus* from Parque Nacional São Joaquim, Urubici, state of Santa Catarina, Brazil. *Rhophitulus ater* sp. nov. is distinctive and easily distinguished from other species of the genus by a unique combination of morphological characters in both sexes, but especially by the following: dull blackbody, coarsely and densely punctate integument, basal area of metapostnotum depressed and areolate rugose, posterior margin of hind tibia and pygidial fimbria of female with blackish pilosity, and characters of the male genitalia with hidden sterna. The new species is closely associated with *Blumenbachia catharinensis* (Loasaceae), which is restricted to cloud forest of the southeastern rim of Serra Geral. Flowers of *B. catharinensis* are pollen and nectar resources and mating sites for the new species.

Keywords: *Blumenbachia*. Loasaceae. Protandrenini. South America. Taxonomy.

Introduction

Rhophitulus Ducke, 1907 is a bee genus of the tribe Protandrenini, and is exclusively distributed in South America (Schlindwein and Moure 1998, 1999, Michener 2007, Moure *et al.* 2007, 2012). Among the genera of Protandrenini, *Rhophitulus* is phylogenetically related to the South American genera *Cephalurgus* Moure & Lucas de Oliveira, *Chaeturginus* Lucas de Oliveira & Moure and *Psaenythisca* Ramos (Ruz and Melo 1999, Michener 2007, Ramos and Rozen 2014, Ramos 2014). Moure (in Schlindwein and Moure, 1998) provides a new genus name – *Panurgillus* – for species morphologically similar to *Rhophitulus*. This new genus, however, is a paraphyletic group from which *Rhophitulus* s. str. evolved (Michener 2007, K.S. Ramos personal observations). In this paper, *Panurgillus* is employed as junior synonym of *Rhophitulus*.

The genus is defined by the following combination of characters present in both sexes: forewing with two submarginal cells, stigma wider than prestigma, head commonly narrower than mesosoma, lower face convex, tentorial pit at intersection of outer subantennal and epistomal sutures, metapostnotum striate basally, and S2 to S5 with fine pilosity on premarginal areas. In addition, males have the inner orbits parallel or slightly converging

below, antennal flagellum longer than head width, metasomal terga with depressed postgradular area compared to their discs, and S8 with slender *lateral* apodeme. Despite these diagnostic characteristics, the genus has no evident morphological synapomorphies, and thus a phylogenetic study is needed to verify its monophyly in relation to other closely related genera such as *Cephalurgus* (Silveira *et al.* 2002, Michener 2007, Ramos 2014). Males of the genus also have dorsal sclerotization of the membrane in the genital capsule (see Ruz and Melo 1999: 231, Ascher 2003). This especially interesting structure is only found among other Protandrenini of the genera *Chaeturginus*, *Cephalurgus* and *Psaenythisca* (Moure and Lucas de Oliveira 1962, Ruz and Melo 1999, Michener 2007, Ramos and Rozen 2014).

Rhophitulus currently comprises 32 species that are frequently collected in xeric and temperate areas of Argentina, Brazil, and Paraguay (Schlindwein and Moure 1998, 1999, Moure *et al.* 2012, Ramos 2014). Nevertheless, virtually nothing is known about their natural history. Nesting biology and immature stages are known only for *Rhophitulus xenopalpus* Ramos, 2014 and *R. mimus* Ramos, 2014 which are communal, ground nesting and bi- or multi- voltine, and provision their nests with pollen from *Heliotropium* (Boraginaceae, *Rhophitulus xenopalpus*) and Asteraceae (*Rhophitulus mimus*) (Rozen 2014). Species of *Rhophitulus* seem to be oligolectic (*sensu* Robertson, 1925) — females provision their nests with pollen from flowers of the same plant family, including Apiaceae, Onagraceae, Oxalidaceae, Cactaceae, and Verbenaceae (Sakagami *et al.* 1967, Schlindwein and Moure, 1998, 1999, Gimenes 2003, Gonçalves and Melo 2005, Martins and Freitas 2018). Detailed information on their behavior in the host plants and their effectiveness as pollinators, however, is not available.

Here we describe a new species of *Rhophitulus* from South Brazil. The species was discovered in the mountainous region of the state of Santa Catarina, located at the eastern rim of the Serra Geral within the Atlantic Forest domain, during a study of the pollination biology of *Blumenbachia catharinensis* Urb. & Gilg (Loasaceae). The vegetation of the area is characterized as cloud forest (“*matinha nebular*”, Rambo 1956) surrounded by mixed ombrophilous forest dominated by *Araucaria angustifolia* (Bertol) Kuntze (Araucariaceae).

Material and methods

The material examined is deposited in the collection of Departamento de Zoologia, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil (DZMG). Paratypes were also deposited in the American Museum of Natural History, New York, United States (AMNH),

Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil (MZSP), Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ), and Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Brazil (DZUP). Morphological terminology mainly follows that of Michener (2007), except for the term labral plate to refer to the central part of the labrum characterized by an elevated and glabrous area. The surface-sculpture nomenclature follows Harris (1979). Antennal flagellomeres are referred to as F1 to F11, and metasomal terga and sterna as T1 to T7 and S1 to S8, respectively. Punctuation density and the intervals between punctures are indicated in relation to puncture diameter (pd). The labels of the type specimens were transcribed in the Type material section in the following way: one inverted bar (\) indicates different lines on the label and quotation marks indicate different labels for the same specimen. All measurements are given in millimeters (mm) and are the maximum width/length of the measured structure. For the study of the male genitalia, terminalia were detached from the metasoma, cleared in a 10% KOH solution for 24 h, neutralized in acetic acid and stored in glycerin. Photographs were taken with a Canon EOS Rebel T3i camera equipped with a Canon MP-E 65 mm macro lens connected to a StackShot macro-rail and a Leica videocamera DFC 295 attached to a Leica stereomicroscope M205C employing Leica Application Suite software (LAS V3.6.0). Multi-focal images were produced using the software CombineZP and ZereneStacker version 1.04, and processed with Adobe Photoshop©.

Results

Rhophitulus Ducke, 1907

Rhophitulus ater new species Ramos, Siriani-Oliveira & Schlindwein

(Figs. 1–13 and 15–17)

Diagnosis

The new species has the following diagnostic characteristics in both sexes: integument of body predominantly reticulate between coarse and dense punctures (Figs. 1–4), basal area of metapostnotum depressed and areolate rugose (Fig. 5), pronotal lobe black (Figs. 2–4), marginal zone of T1–T2 densely punctate (Figs. 6, 7), mesoscutum with short pilosity (about half the diameter of the scape), and labral plate sub-rectangular. In addition, the posterior margin of hind tibia and pygidial fimbria of the female with blackish hairs (Fig. 6), female with basal area of fore- and mid tibia black, and clypeus of male with a longitudinal yellow

mark (Fig. 3) are features that distinguish the new species among other species of *Rhophitulus*.

Rhophitulus ater sp. nov. is similar to *R. aeneiventris* (Friese, 1908), *R. malvacearum* (Schlindwein & Moure 1998) (Figs. 22–23), *R. ogloblini* (Cockerell, 1930), *R. pygidialis* (Vachal, 1909) and *R. reticulatus* (Schlindwein & Moure, 1998) (Figs. 18–21) by the integument surface of head predominantly reticulate between punctures. Despite this, it can be easily distinguished from these species by the predominantly coarsely punctate integument of head and metasomal terga in both sexes. The new species runs to couplet 9 for females of *R. reticulatus* and *R. malvacearum*, and couplet 11 for males with *R. hamatus* (Schlindwein & Moure, 1998) in Schlindwein and Moure's (1999) key. In addition to the features already mentioned, females of *Rhophitulus ater* sp. nov. differs from *R. malvacearum* (males are unknown) (Figs. 22–23) mainly by the ventral portion of mesepisternum with hooked hairs, labral plate as wide as long and rugulose, and scutellum predominantly smooth between punctures. The new species can be distinguished from *R. reticulatus* (Figs. 18–21) by the following characters in either sex: marginal zone of metasomal terga punctate, basal area of metapostnotum shorter than metanotum length, first labial palpomere as long as the combined length of the three distal palpomeres, and pilosity of mesoscutum shorter than the maximum diameter of the scape. The clypeus of the males of *R. ater* sp. nov. is partly yellow while in *R. reticulatus* it is wholly black (Fig. 20). *Rhophitulus ater* sp. nov. differs from *R. hamatus* (Figs. 24–25) mainly by the following characters: for either sex – face with dense punctures and reticulate integument, basal area of metapostnotum glabrous, wings with veins and pterostigma blackish; for a female – base of hind and mid tibiae without yellow marks, prepygidial and pygidial fimbria black, and marginal zones of metasomal terga not translucent; for males – mandible and pronotal lobe black, mid tibia and hind femur without yellow marks.

Comments

The new species fits well within the diagnosis of *Rhophitulus* (see Introduction) based on external morphology and hidden sterna. However, the following morphological characteristics of male genitalia differ from what is known for the genus: base of genital capsule without small dorsal sclerite, gonocoxite without deep oblique impression, gonostylus partly fused to gonocoxite, volsella denticulate only on opposable surfaces of digitus and cuspis, and cuspis slightly longer than digitus (Figs. 12, 13). Further studies involving taxonomic revision, phylogenetic analysis and comparative morphological analysis, including

the male genitalia, of *Rhopitulus* are needed to provide comprehensive information about morphological variation within the genus.

Description

Holotype female

Approximate body length: 6.7 mm; maximum head width: 2.0 mm; intertegular distance: 1.5 mm; forewing length: 5.5 mm; T2 maximum width: 1.9 mm. *Color*. Body predominantly black except as follows: mandible apex dark brown; tegula dark brown, translucent; forewing membrane light brown, translucent, slightly infumated at distal third; veins and pterostigma dark brown; tibial spurs light brown; marginal zone not translucent (Fig. 6). *Pubescence*. Mostly white; ventral portion of basitarsus and tarsi light yellow; basitibial plate, posterior margin of hind tibia, prepygidial and pygidial fimbria blackish. Compound eyes with minute setae, almost inconspicuous; ventral portion of gena and lateral surface of mesepisternum with relatively long (about 0.45 mm), erect and plumose pubescence; tegula with anterior half with decumbent branched hairs and posterior half glabrous; mesoscutum and scutellum with tiny pilosity intermixed with sparse, long and erect branched hairs; pilosity shorter and fine on metanotum; metasomal terga with shorter and fine pilosity on disc, more dense and long on the sides; ventral surface of mesepisternum with simple hooked hairs; dorsolateral portion of propodeum with dense erect plumose hairs (Fig. 5); metapostnotum glabrous. Scopa on hind tibia with sparse and simple hairs, longer than maximum tibia width (Fig. 2); hairs on hind basitarsus shorter than those on tibia. Disc of T1–T4 with tiny decumbent hairs, except for glabrous declivous portion of T1 (Fig. 6); premarginal line of T4 with loose fringe of finely branched hairs (Fig. 6); T5 and T6 with prepygidial and pygidial fimbria of plumose hairs (Fig. 6); marginal zone of metasomal terga and sterna glabrous; disc of S1–S5 with long, erect and finely branched pilosity. *Integumental surface*. Predominantly coarsely punctate and reticulate between punctures, except for smooth and shiny surface between punctures on supraclypeal area, posteriorly on disc area of mesoscutum, disc of scutellum, and posterior half of tegula. Labral plate rugulose with one fine median longitudinal carina; clypeus coarsely punctate (Fig. 1); inferior paraocular area moderately densely punctate (about ≥ 1 pd); frons, vertex and genae densely punctate (< 0.5 pd). Mesoscutum, metanotum and dorsolateral portion of propodeum densely punctate, reticulate between punctures (< 1 pd); disc of scutellum with sparse punctures (> 1 pd); posterior surface of propodeum impunctate, strongly reticulate (Fig. 5); basal area of

metapostnotum coarsely areolate rugose (Fig. 5). Metasomal terga densely punctate (<0.5 pd) and lightly reticulate between punctures, except for completely impunctate and shiny declivous portion of T1; marginal zone finely and densely punctate (<0.5 pd) with smooth, shiny, non-translucent apical margin (Fig. 6); pygidial plate reticulate. *Structure and measurements.* Head approximately $1.2\times$ wider than long (2.0:1.6); first labial palpomere as long as the combined length of the three distal palpomeres; labral plate $1.2\times$ wider than long (0.28:0.26), distal margin weakly emarginate; compound eyes $2\times$ longer than wide (1.2:0.6), inner orbits slightly convergent below (upper distance 1.33, lower distance 1.21) (Fig. 1); clypeus $1.8\times$ wider than long (1.07:0.6); subantennal sutures subparallel; frontal line slightly cariniform in the interalveolar area and grooved to the median ocellus; upper paraocular area slightly inflated; facial fovea narrow and long, $4.7\times$ longer than wide (0.33:0.07); length of the first three flagellomeres 0.21, 0.13, 0.13, respectively; gena in lateral view $0.8\times$ as wide as eye width; parapsidal line impressed and linear, as long as tegula length; median mesoscutal line deeply impressed; first submarginal cell slightly longer than second; 1m-cu reaching second submarginal cell at basal third; hind wing with 9 hamuli; ventral margin of mid femur with pronounced angle but not forming tooth; mid tibial spur finely serrate, $0.8\times$ as long as basitarsus (0.5:0.6); mid basitarsus $3\times$ longer than wide (0.6:0.2); hind tibial spurs similar in length with apex straight; tarsal claws bifid, teeth of similar sizes; basal area of metapostnotum depressed, shorter than scutellum (Fig. 5); anterior portion of T1 strongly declivous; discs of T2–T4 almost flat; T1 and T2 with lateral line; lateral fovea of T2 oval and slightly depressed; marginal zone of metasomal terga slightly depressed in comparison to disc (Fig. 6); pygidial plate V-shaped, slightly rounded at apex.

Paratype male

Approximate body length: 5.7 mm; maximum head width: 1.5 mm; intertegular distance: 1.2 mm; forewing length: 4.7 mm; maximum T2 width: 1.35 mm. Very similar to female in coloration, pubescence and integumental surface. Body predominantly black except for yellow longitudinal area on central portion of clypeus (Fig. 3) and small yellow spot on basal portion of fore tibia; basal half of anterior surface of fore tibia and distitarsi light brown. Pubescence mostly white, except for brown hairs on T7; ventral surface of mesepisternum with plumose hairs, apex straight (without hooked hairs); hind tibia with long, sparse and branched hairs, shorter than maximum tibia width (Fig. 4); premarginal line of T4 and T5 with loose fringe of simple or finely branched hairs (Fig. 7); T7 with loose fimbria of plumose hairs; discs of S1–S5 with sparse semidecumbent and finely branched pilosity. Body surface

coarsely punctate and reticulate between punctures (Figs. 3, 4); labral plate smooth and shiny on distal half, without longitudinal carina; premarginal line of T1–T2 with very sparse punctures (≥ 3 pd); marginal zone of T1–T2 densely punctate (< 1 pd) (Fig. 7); marginal zone of T3 with dense punctures on basal half (Fig. 7); marginal zone of T4–T7 smooth and shiny (Fig. 7). Structure and measurements. Head approximately 1.2 \times longer than wide (1.8:1.5); labral plate 1.4 \times wider than long (0.2:0.14), distal margin weakly emarginate; compound eyes 1.8 \times longer than wide (1.1:0.6), inner orbits convergent below (upper distance 0.73, lower distance 0.61); clypeus 1.2 \times broader than long (0.6:0.5); subantennal sutures subparallel; frontal line cariniform in the interalveolar area, becoming a weak line up to the median ocellus; facial fovea elliptic, 2 \times longer than wide (0.14:0.07); length of the first three flagellomeres 0.15, 0.10, 0.13, respectively; gena in lateral view 0.8 \times as wide as eye width; hind wing with 8 hamuli; ventral margin of mid femur without pronounced angle; mid tibial spur finely serrate, 0.5 \times as long as basitarsus (0.28:0.52); mid basitarsus about 4 \times longer than wide (0.52:0.15); hind tibia with toothed posterior margin; anterior portion of T1 declivous; pygidial plate absent; distal margin of T7 slightly emarginate (Fig. 8); S6 with shallow V-shaped emargination distally (Fig. 9); S7 with apical lobes attached to small discal area, constricted basally, with similar width from base to apex and few coarse hairs at apex (Fig. 10); S8 with long apical process, broadly-rounded apically, and basal portion slender compared to distal (Fig. 11); lateral apodeme of S8 basally directed (Fig. 11); genital capsule longer than broad, small dorsal sclerite absent; gonostylus about one half as long as gonocoxite, pilose apically, partly fused to gonocoxite, not reaching apex of penis valve (Figs. 12, 13); penis membranous and not beyond the apex of penis valve; cuspis of volsella slightly longer than digitus (Figs. 12, 13); volsella denticulate only on opposable surfaces of the digitus and cuspis (Figs. 12, 13); apodeme of penis valve hidden by gonocoxite, not surpassing genital capsule opening (Fig. 12).

Variation

The number of hamuli can vary from 7 to 10 in the same individual and in both sexes. The surface between punctures in the supraclypeal area and disc of scutellum can vary from smooth to microreticulate. The frontal line of some males is shorter, not reaching the median ocellus.

Distribution

Brazil, Santa Catarina, known only from the type locality. The species was discovered within the limits of the Parque Nacional São Joaquim (São Joaquim National Park) (28°08'30" S, 49°38'07" W), between 1300 and 1500 m elevation. The surrounding vegetation is dominated by mixed Araucaria forest and tropical rainforest (Atlantic Forest). Individuals were collected while foraging on flowers of *Blumenbachia catharinensis* growing on humid soil at the edge of the forest or over old fences called "Taipa", which are built with blocks of stones and mainly used to delimit pasture areas (Fig. 14).

Type material

Holotype female (DZMG) (UFMG-IHY-1803416) "PARNA [Parque Nacional] São Joaquim\ Urubici [Urubici], SC [Santa Catarina]\Brasil 13/12/2016\Samuel Oliveira leg." "L.320 P.706\Blumenbachia\catharinensis". Paratypes: 1 female (DZMG) (UFMG-IHY-1901612) and 3 males (DZMG) (UFMG-IHY-1901605, UFMG-IHY-1901606 and UFMG-IHY-1901607) same data as holotype; 1 female (MZSP 62272) same data as holotype; 1 female (DZMG) (UFMG-IHY-1901610) and 1 male (DZMG) (UFMG-IHY-1901608) same data as holotype except 12/12/2016; 1 male (MZSP 62273), same data except 02/12/2016; 2 females (DZMG) (UFMG-IHY-1901609 and UFMG-IHY-1901611) same data as holotype except 11/11/2016; 1 female (MZSP 62274) and 1 male (one with terminalia dissected) (MZSP 62275), same data; 1 female and 1 male (DZUP), same data; 1 female and 1 male (AMNH), same data; 1 female and 1 male (terminalia dissected) (MNRJ), same data.

Visited flowers

Blumenbachia catharinensis Urb. & Gilg (Loasaceae). The genus *Blumenbachia* Schrad. is a morphologically quite homogeneous species group of annual stinging herbs (Henning *et al.* 2015). *Blumenbachia catharinensis* is a rare species with discontinuous occurrence throughout the southeastern border of the Serra Geral Plateau in the states of Santa Catarina and Rio Grande do Sul (Santos and Trinta 1985). Like most species of Loasaceae, *B. catharinensis* possesses complex floral morphology and a narrow relationship with oligolectic pollinators (Schlindwein and Wittmann 1997, Siriani-Oliveira *et al.* 2018). *Rhopitulus ater* sp. nov. was the main floral visitor of *B. catharinensis* during fieldwork for a pollination study carried out between November and December of 2016 to 2018 (Siriani-Oliveira and Schlindwein not published). A forthcoming study will provide information on the foraging and reproductive behavior of this species and its relationship with its host plant. Females and males rely exclusively on plants of *B. catharinensis* as a food source (pollen and nectar) (Figs.

16, 17), which also provide sleeping places for males (Fig. 17) and mating sites (Fig. 15). No male or female bees of *R. ater* sp. nov. were sampled on other co-flowering plant species in the vegetation surrounding individuals of *B. catharinensis*.

Flight activity

Specimens were collected in November and December.

Etymology

The specific epithet is derived from the Latin ‘ater’ (= dark, black, gloomy), in reference to the black body of both sexes of this species.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

We thank Gabriel Biffi for assistance with photographing collected specimens; Isabelle Cerceau for help with fieldwork; and ICMBio for the collection license (No55425-2). The authors also thank anonymous reviewers for their helpful comments and corrections. This work was financed by Conselho Nacional de Desenvolvimento Científico e Tecnológico – Brazil (CNPq) to CS (Universal 436095/2018-1 and PQ 311935/2018-4); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES)– Finance Code 001 to KSR and SSO (88882.184391/2018-01); and PROTAX - Programa de Capacitação em Taxonomia to KSR (CNPq 440574/2015-3 and FAPESP 2016/50378-8).

References

- Ascher, J.S., 2003. Appendix: Evidence for the phylogenetic position of *Nolanomelissa* from nuclear EF-1a sequence data. In: Melo, G.A.R., Alves-dos-Santos, I (Eds.), *Apoidea Neotropica: Homenagem aos 90 anos de Jesus Santiago Moure*. UNESCO, Criciúma, pp. 107–108.
- Ducke, A., 1907. Beitrag zur Kenntnis der Solitärbiene Brasiliens. (Hym.). *Z. Syst. Hymenopterol. Dipterol.* 7, 361–368.
- Gimenes, M., 2003. Interaction between visiting bees (Hymenoptera, Apoidea) and flowers of *Ludwigia elegans* (Camb.) hara (Onagraceae) during the year in two different areas in São Paulo. Brazil. *Braz. J. Biol.* 63, 617–625.

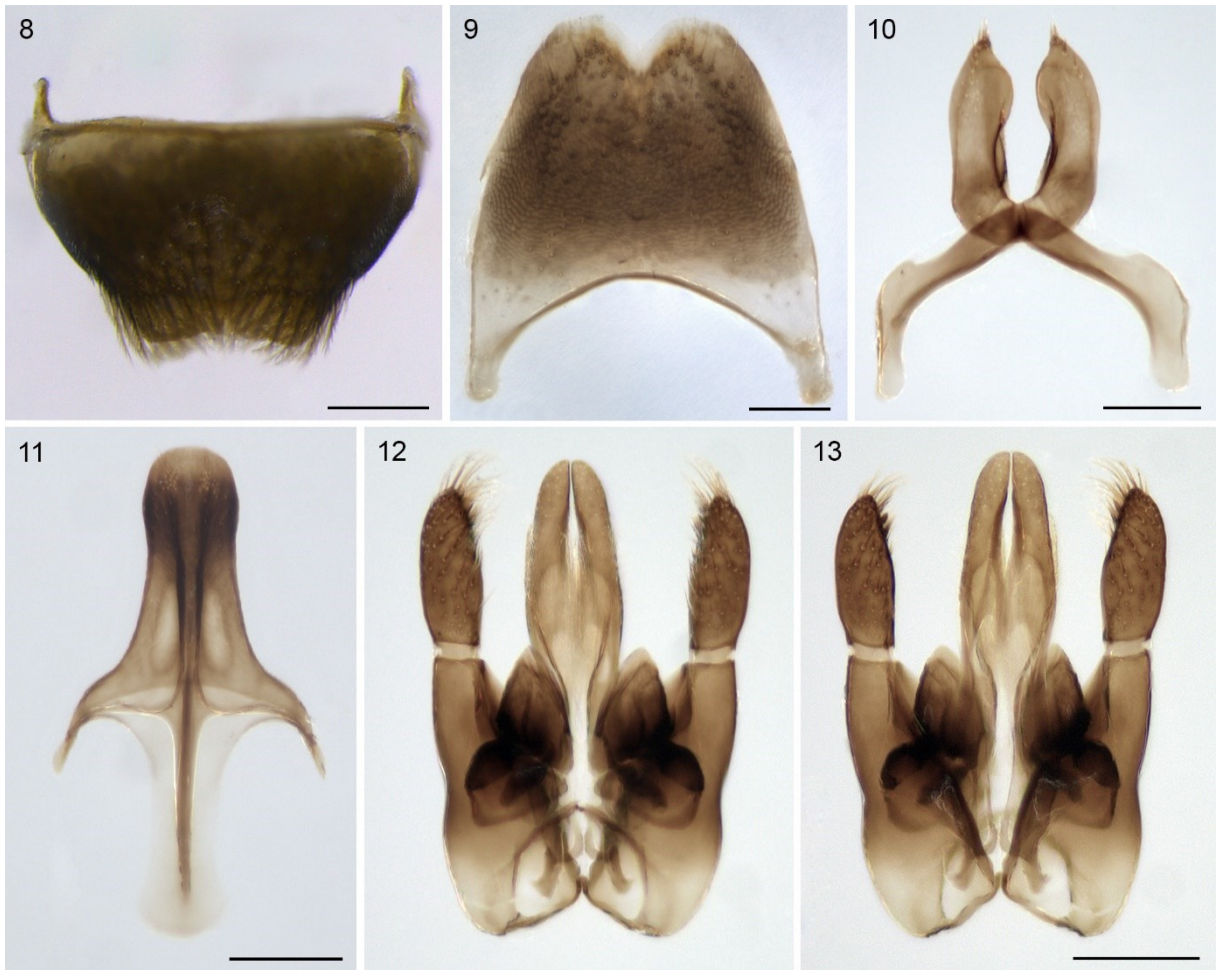
- Gonçalves, R.B., Melo, G.A.R., 2005. A comunidade de abelhas (Hymenoptera, Apidae s. l.) em uma área restrita de campo natural no Parque Estadual de Vila Velha, Paraná: diversidade, fenologia e fontes florais de alimento. *Rev. Bras. Entomol.* 49, 557–571.
- Harris, R.A., 1979. A glossary of surface sculpturing. *Occ. Pap. Entomol.*, 28, 1–31, State of California.
- Henning, T., Oliveira, S., Schlindwein, C., Weigend, M., 2015. A new, narrowly endemic species of *Blumenbachia* (Loasaceae subfam. Loasoideae) from Brazil. *Phytotaxa* 236, 196–200.
- Martins, C., Freitas, L., 2018. Functional specialization and phenotypic generalization in the pollination system of an epiphytic cactus. *Acta Bot. Bras.* 32, 359–366.
- Michener, C.D., 2007. *The Bees of the World*, second ed. Johns Hopkins, Baltimore, Maryland.
- Moure, J.S., Lucas de Oliveira, B., 1962. Novo gênero de Panurginae para a América do Sul (Hymenoptera: Apoidea). *Bol. Univ. Fed. Paraná, Zool.* 15, 1–14.
- Moure, J.S., Urban, D., Melo, G.A.R., 2007. Catalogue of bees (Hymenoptera, Apoidea) in the Neotropical Region. *Sociedade Brasileira de Entomologia*, Curitiba.
- Moure, J.S., Urban, D., Dal Molin, A., 2012. Protandrenini Robertson, 1904. In: Moure, J.S., Urban, D., Melo, G.A.R. (Eds.), *Catalogue of bees (Hymenoptera, Apoidea) in the Neotropical region*. <http://www.moure.cria.org.br/catalogue> (Accessed 30 January 2019).
- Rambo, B., 1956. A flora fanerogâmica dos Aparados riograndenses. *Sellowia* 7/8, 235–298.
- Ramos, K.S., 2014. Three new bee species of *Rhophitulus* Ducke (Hymenoptera, Apidae, Protandrenini) from Argentina and Brazil. *Zootaxa* 3847, 545–556.
- Ramos, K.S., Rozen Jr., J.G., 2014. *Psaenythisca*, a new genus of bees from South America (Apoidea: Andrenidae: Protandrenini) with a description of the nesting biology and immature stages of one species. *Am. Mus. Novit.* 3800, 1–32.
- Robertson, C., 1925. Heterotropic bees. *Ecology* 6, 412–436.
- Rozen Jr., J.G., 2014. Nesting biology and immature stages of the panurgine bee genera *Rhophitulus* and *Cephalurgus* (Apoidea: Andrenidae: Protandrenini). *Am. Mus. Novit.* 3814, 1–16.

- Ruz, L., Melo, G.A.R., 1999. Reassessment of the bee genus *Chaeturginus* (Apoidea: Andrenidae, Panurginae), with the description of a new species from southern Brazil. *Univ. Kansas Mus. Nat. Hist. Spec. Publ.* 24, 231–236.
- Sakagami, S.F., Laroca, S., Moure, J.S., 1967. Wild bee biocoenotics in São José dos Pinhais (PR), south Brazil. Preliminary Report. *J. Fac. Sci. Hokkaido Univ. Ser. VI, Zoo.* 16, 253–291.
- Santos, E., Trinta, E.F., 1985. Loasáceas. In: Reitz, R (Ed.), *Flora Ilustrada Catarinense*. Herbário Barbosa Rodrigues, Itajaí, pp. 1–20.
- Schlindwein, C., Moure, J.S., 1998. *Panurgillus* gênero novo de Panurginae, com a descrição de quatorze espécies do sul do Brasil (Hymenoptera, Andrenidae). *Rev. Bras. Zool.* 15, 397–439.
- Schlindwein, C., Moure, J.S., 1999. Espécies de *Panurgillus* Schlindwein & Moure (Hymenoptera, Andrenidae) depositados no Naturkunde Museum. Berlin. *Rev. Bras. Zool.* 16, 113–133.
- Schlindwein, C., Wittmann, D., 1997. Micro-foraging routes of *Bicolletes pampeana* (Colletidae) and bee-induced pollen presentation in *Cajophora arechavaletae*. *Bot. Acta* 110, 177–183.
- Silveira, F.A., Melo, G.A.R., Almeida, E.A.B., 2002. *Abelhas Brasileiras: Sistemática e Identificação*. Fernando Silveira, Belo Horizonte.
- Siriani-Oliveira, S., Oliveira, R., Schlindwein, C., 2018. Pollination of *Blumenbachia amana* (Loasaceae): flower morphology and partitioned pollen presentation guarantee a private reward to a specialist pollinator. *Bio. J. Linn. Soc.* 124, 479–491.

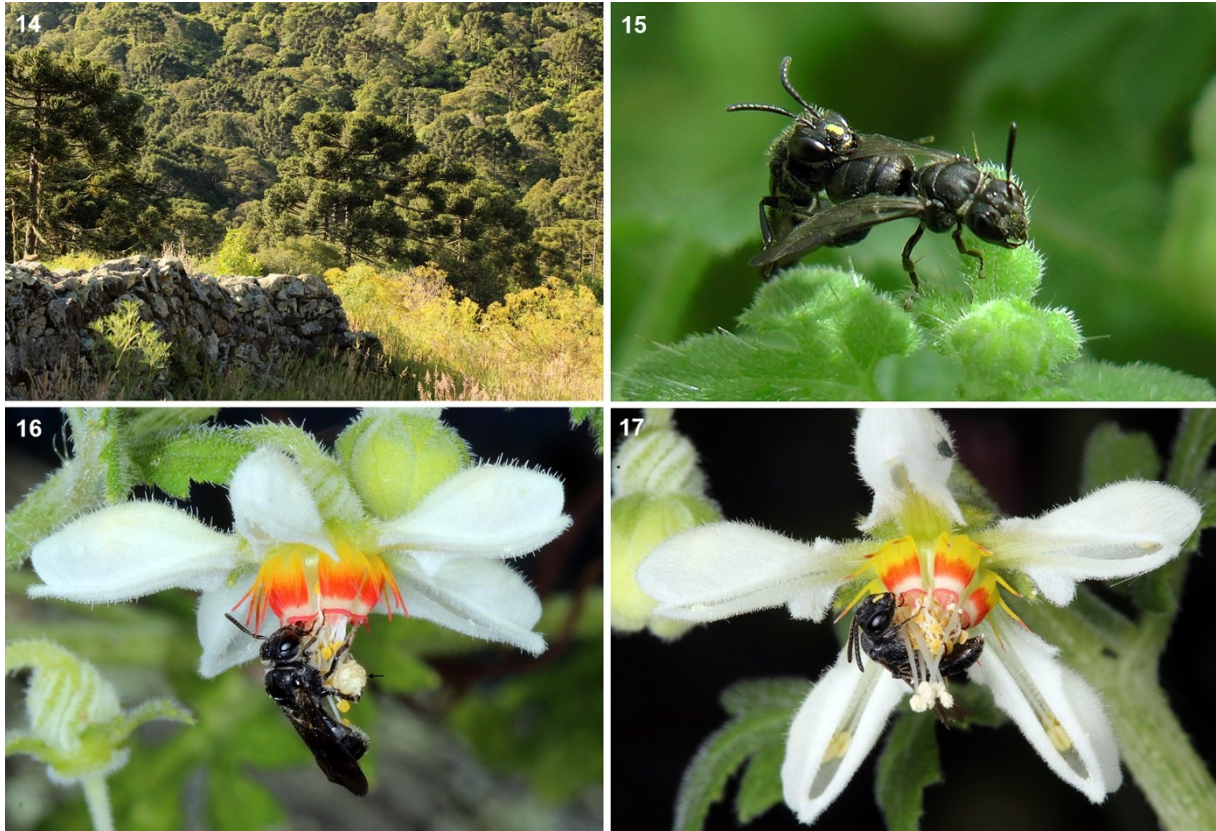
Figures



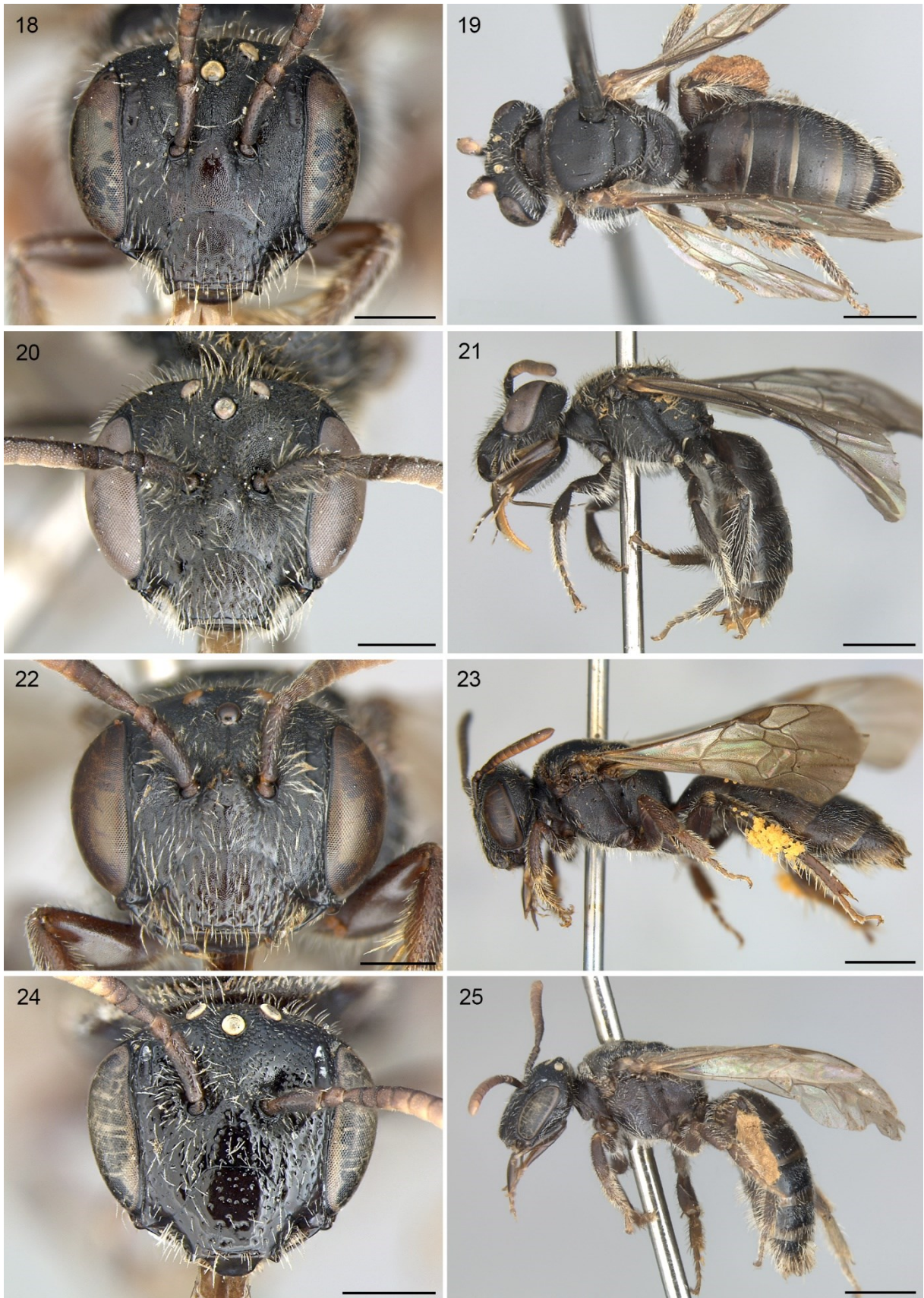
Figs. 1–7. *Rhopitulus ater* sp. nov.: (1) female (holotype), head in frontal view; (2) female (holotype), lateral view; (3) male (paratype), head in frontal view; (4) male (paratype), lateral view; (5) female (paratype), mesosoma in dorsal view; (6) female (paratype), metasoma in dorsal view; and (7) male (paratype), metasoma in dorsal view. Scale bar for figures 1–4 = 1 mm, figures 5–7 = 0.5 mm.



Figs. 8–13. Male of *Rhopitulus ater* sp. nov. (paratype): (8) T7 in dorsal view; (9) S6 in ventral view; (10) S7 in ventral view; (11) S8 in ventral view; (12) genitalia in ventral view; and (13) genitalia in dorsal view. Scale bar = 0.2 mm.



Figs. 14–17. (14) Type locality of *Rhopitulus ater* sp. nov. in Parque Nacional São Joaquim, Santa Catarina, Brazil. The bees were collected on flowers of *Blumenbachia catharinensis* growing over “Taipas” (old fences built with stones to delimit pasture areas); mixed Araucaria forest in background. (15–17) *Rhopitulus ater* sp. nov. in *Blumenbachia catharinensis*. (15) Male and female in mating position on young leaves. (16) Female foraging on a pendulous flower; the black arrow indicates a hind tibia filled with pollen of *B. catharinensis*. (17) Male sleeping in a flower.



Figs. 18–25. *Rhophitulus* species. (18–19) *R. reticulatus* female paratype, Caçapava do Sul (RS, Brazil): (18) Head in frontal view. (19) Body in dorsal view. (20–21) *R. reticulatus* male,

Guarani das Missões (RS, Brazil): (20) Head in frontal view. (21). Body in lateral view. (22–23) *R. malvacearum* female paratype, Caçapava do Sul (RS, Brazil): (22) Head in frontal view. (23) Body in lateral view. (24–25) *R. hamatus* female paratype, Capão da Canoa (RS, Brazil): (24) Head in frontal view. (25) Body in lateral view. Scale bar for figures 18, 20, 22, 24 = 0.5 mm, figures 19, 21, 23, 25 = 0.5 mm.

Considerações finais

Nos estudos desenvolvidos nesta tese, as interações foram analisadas tanto na perspectiva das plantas quanto dos polinizadores. O primeiro capítulo trouxe a descrição da interação entre *Blumenbachia insignis* e seu polinizador oligolético *Bicolletes indigoticus*, e revelou um relacionamento planta-abelha muito similar ao conhecido para outras espécies relacionadas, tanto do ponto de vista da planta como dos polinizadores. Este trabalho trouxe algumas novidades como a mensuração da produção de néctar ao longo da antese e a quantificação da deposição de pólen nos estigmas, medidas que nunca haviam sido feitas em campo em uma espécie de Loasoideae. Estes dados são de difícil obtenção, trazem um maior nível de detalhamento ao estudo e proporcionam uma descrição mais acurada da interação.

O segundo capítulo foi um desdobramento do primeiro e investigou o comportamento de forrageio de néctar dos polinizadores. As análises realizadas no estudo focaram na tomada de decisão das abelhas frente a flores manipuladas experimentalmente. Esse tipo de investigação é pouco explorada em sistemas de polinização. Estudos sobre forrageio de abelhas normalmente enfocam em espécies sociais domesticadas, como *Apis mellifera* e espécies do gênero *Bombus*, que podem ser criadas em cativeiro e os experimentos podem ser executados em ambientes controlados. Estudos sobre o comportamento de forrageio de abelhas solitárias em campo são desafiadores devido a condições adversas que podem se impor ao estudo. Como por exemplo, a imprevisibilidade de encontrar as abelhas ou a possibilidade de outros visitantes florais interferirem no forrageio das espécies alvo do estudo. Entretanto, as interações entre espécies de Loasoideae e seus polinizadores descritas até o momento demonstram que apenas uma espécie de planta interage quase que exclusivamente com uma espécie de abelha. Além disso, as plantas normalmente ocorrem em pequenas agregações de indivíduos na paisagem, o que possibilita ambientes limitados espacialmente para execução de experimentos. Isso faz com que esses sistemas de polinização sejam ótimos para trabalhos sobre o comportamento de forrageio das abelhas.

O terceiro capítulo trouxe a descrição de *Rhopitulus ater*, uma nova espécie de abelha oligolética pertencente a uma linhagem de abelhas que nunca havia sido reportada interagindo com uma espécie de Loasoideae. Essa nova descrição se une às várias novas espécies de abelhas que nos últimos anos foram descritas a partir de estudos de polinização de espécies de Loasoideae.

O quarto capítulo trouxe a descrição do sistema de polinização de *Blumenbachia catharinensis*. Do ponto de vista da planta, o sistema é muito semelhante aos de espécies relacionadas. Do ponto de vista dos polinizadores, o estudo trouxe tanto novidades específicas para os sistemas de polinização de Loasoideae quanto para estudos de polinização como um todo. O visitante floral predominante *Rhophitulus ater*, como mencionado anteriormente, é a primeira espécie de abelha fora da linhagem de Neopasiphaeinae a demonstrar oligoetia em uma Loasoideae. Além disso, essas abelhas coletam pólen diretamente dos estigmas, um comportamento de coleta de pólen pouco relatado para abelhas, principalmente para abelhas oligoléticas. *Blumenbachia catharinensis* tem baixa produção de sementes na presença de *R. ater*, demonstrando que nem sempre abelhas oligoléticas são boas polinizadoras de suas plantas hospedeiras.

Em síntese, esta tese descreveu dois sistemas de polinização especializados. Enquanto as flores de Loasoideae apresentam adaptações que maximizam a polinização cruzada, como a oferta particionada de pólen por movimentos de estames e néctar dividido em estaminódios, as abelhas oligoléticas possuem adaptações comportamentais que as tornam forrageadoras eficientes diante do padrão de oferta de recursos. Os resultados apresentados reforçam a premissa de que as interações entre espécies Loasoideae e seus polinizadores são predominantemente especializadas.