

Missing for almost 100 years: the rare and potentially threatened bee, *Pharohylaeus lactiferus* (Hymenoptera, Colletidae)

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Academic editor: Jack Neff | Received 6 October 2020 | Accepted 4 January 2021 | Published 25 February 2021

<http://zoobank.org/1D8CD33F-25C3-4B87-9F17-D9A151D8FE9B>

Citation: Dorey JB (2021) Missing for almost 100 years: the rare and potentially threatened bee, *Pharohylaeus lactiferus* (Hymenoptera, Colletidae). Journal of Hymenoptera Research 81: 165–180. <https://doi.org/10.3897/jhr.81.59365>

Abstract

The Australian endemic bee, *Pharohylaeus lactiferus* (Colletidae: Hylaeinae) is a rare species that requires conservation assessment. Prior to this study, the last published record of this bee species was from 1923 in Queensland, and nothing was known of its biology. Hence, I aimed to locate extant populations, provide biological information and undertake exploratory analyses relevant to its assessment. *Pharohylaeus lactiferus* was recently rediscovered as a result of extensive sampling of 225 general and 20 targeted sampling sites across New South Wales and Queensland. Collections indicate possible floral and habitat specialisation with specimens only found near Tropical or Sub-Tropical Rainforest and only visiting *Stenocarpus sinuatus* (Proteaceae) and *Brachychiton acerifolius* (Malvaceae), to the exclusion of other available floral resources. Three populations were found by sampling bees visiting these plant species along much of the Australian east coast, suggesting population isolation. GIS analyses used to explore habitat destruction in the Wet Tropics and Central Mackay Coast bioregions indicate susceptibility of Queensland rainforests and *P. lactiferus* populations to bushfires, particularly in the context of a fragmented landscape. Highly fragmented habitat and potential host specialisation might explain the rarity of *P. lactiferus*. Targeted sampling and demographic analyses are likely required to thoroughly assess the status of this species and others like it.

Keywords

Conservation, extinction risk, fragmentation, Hylaeinae, invertebrate conservation, Queensland, wildfire, rainforest

Introduction

The greatest threats to ecosystems and species worldwide are habitat loss, fragmentation and degradation (Vie et al. 2009). Australia has already cleared over 40% of its forests and woodlands since European colonisation, leaving much of the remainder fragmented and degraded (Bradshaw 2012). The vast majority of clearing has occurred on freehold and leasehold land and for animal agriculture (Evans 2016). In particular, Queensland is a contemporary land-clearing hotspot and is responsible for more than half of all land-clearing in Australia over the past four decades (Evans 2016). It is a failing of state and federal government policy and regulation that land clearing in Queensland continues at rates that should be of concern both nationally and internationally (Reside et al. 2017).

Despite the ecological importance of Australian native bees, we know very little about their biology (Batley and Hogendoorn 2009) or conservation status. North Queensland hosts high species richness and endemism (Crisp et al. 2001; Orme et al. 2005; Hurlbert and Jetz 2007) and several bee genera that are found nowhere else in Australia (Houston 2018; Smith 2018). These restricted bee genera include: *Ctenoplectra* Kirby (Apidae: Apinae), *Nomada* Scopoli (Apidae: Nomadinae), *Mellitidia* Guérin-Ménéville (Halictidae: Nomiinae), *Reepenia* Friese (Halictidae: Nomiinae), *Patellapis* Friese (Halictidae: Halictinae) and *Pharohylaeus* Michener (Colletidae: Hylaeinae).

Pharohylaeus has only two described species: *P. papuaensis* Hirashima & Roberts in Papua New Guinea and *P. lactiferus* (Cockerell) in Australia (Houston 1975; Hirashima and Roberts 1986). Both species are relatively large (9–11 mm), robust, mostly black with distinctive white facial and body markings, and have the first three tergal segments enlarged and enclosing the others. The former is known only from two females which were collected on *Syzygium aqueum* (Burm.f.) Alston (Myrtaceae) in 1982 (Hirashima and Roberts 1986). No published records of *P. lactiferus* have been made since the third of January 1923, when three males were collected in the Atherton Tablelands; in May of 1900 a male and a female were collected in Mackay while another female was collected in Kuranda prior to 1910 (Cockerell 1910; Houston 1975). However, the collection localities of these specimens are imprecise and no biological data were recorded.

Due to the dearth of biological information on *P. lactiferus* prior to this study, I aimed to locate extant populations and contribute biological information as part of a broader bee survey. Because of this, much of what follows are exploratory analyses of the potential risks for *P. lactiferus* and suggestions for future research. Hence, I undertook a series of post-hoc analyses in order to provide insights into the biology, ecology and potential extinction risks associated with *P. lactiferus*. I provide insights into the circumstances of the rediscovery of *P. lactiferus* and what is now known of its floral and habitat associations. I also explore spatial data relating to *P. lactiferus* (vegetation association, potential fire risks and occurrences) and my sampling methods (for potential biases). The possible floral and habitat specialisation along with the rarity of *P. lactiferus* raises concerns about its conservation status. I further highlight the need for preservation of remnant vegetation and better arthropod-diversity monitoring, particularly for at-risk and phylogenetically important species.

Methods

Sample locations and methods

I undertook general collections in parts of Queensland and New South Wales in a variety of habitats with most collections made across two sampling periods from December to February 2018–19 and November to December of 2019 (summer). I chose sampling sites by the presence of flowering vegetation on the side of roads and trails. I caught specimens by sweep-netting (up to 13 m from the ground using an 11 m Lito net) off flowering plants, vegetation or potential bee-nesting sites. After collection, I transferred specimens to 99% ethanol and stored them at $-2\text{ }^{\circ}\text{C}$. For each collection event I recorded latitude, longitude and elevation in metres above sea level (m asl) and later checked these to ensure accuracy. I estimated the number of bees for each vial while in the field. Other data that I collected included date, time, collector, sampling effort (in minutes), sampling notes and, where possible, flower species visited and the resource that I used to identify that plant. I undertook general collections between 0525 and 2200 (Suppl. material 2: Table S1). I did not collect any plant vouchers. I identified *Pharohylaeus* using the keys by Houston (1975) and Smith (2018).

I used two initial collection events of *P. lactiferus* to inform targeted sampling (see results). However, I undertook general collections and observations at every site where I found *P. lactiferus* in an attempt to find additional associated plant species. I chose targeted sampling sites haphazardly as target plant species were encountered, generally near the side of roads or hiking trails. I undertook observations of the target plant species, *Stenocarpus sinuatus* (Loudon) Endl. (Proteaceae) and *Brachychiton acerifolius* (A.Cunn. ex G.Don) F.Muell. (Malvaceae), for a minimum of five minutes (maximum of 67 minutes) for each collection event (Suppl. material 2: Table S1). Where I increased sampling time, I did so to collect *P. lactiferus* specimens and to determine their distribution or activity times. I did not undertake a systematic temporal sampling regime; however, I made targeted collections throughout the day (between 0730 and 1751) (Suppl. material 2: Table S1).

Representative materials are stored at the South Australian Museum (SAMA 32-37949, SAMA 32-37950, SAMA 32-40838, SAMA 32-40846, SAMA 32-40847, SAMA 32-40848, SAMA 32-40849).

Data sources and terminology

Historic bee records

I sourced general bee collection data for Australian bioregions from the Atlas of Living Australia (ALA 2019) and overlaid them with Australian bioregion data using *QGIS* version 3.8 (QGIS Development Team 2020). I analysed all data using *R* version 3.6.1 (R Development Core Team 2019) and produced plots using the *R* packages *graphics* and *ggplot2* (Wickham 2016). Because I sampled flowering plants as I encountered them, I also examined potential collection biases (Suppl. material 1: Appendix).

Geographic information system data

I sourced current and pre-European National Vegetation Information System maps from the National Mapping Division (NMD 2003b, a), which defines 85 Major Vegetation Subgroups for Australia (e.g., Tropical or Sub-Tropical Rainforests (TSTRs); Major Vegetation Subgroup 2). NMD (2003b) compiled pre-European maps using the best-available data collected at varying scales, on varying dates and by several organisations. I sourced Interim Biogeographic Regionalisation for Australia maps from the Department of Environment and Energy (DEE 2017), which defines 89 large and distinct bioregions (e.g., the Wet Tropics and Central Mackay Coast).

Results

Sampling

Of the ~3,585 bee specimens that I collected in Queensland over 3,446 sampling-minutes, I collected 694 (19%) in the Wet Tropics and 153 (4%) in the Central Mackay Coast bioregions (Suppl. material 5: Fig. S3; Suppl. material 2: Table S3). In the adjacent bioregions of Cape York Peninsula, Einasleigh Uplands and the Brigalow Belt North, I collected 260 (7%), 453 (13%) and 271 (8%) bee specimens, respectively (Suppl. material 5: Fig. S3). In New South Wales, I collected 2,141 bee specimens over 2,441 sampling-minutes (Suppl. material 2: Table S3). In total I sampled 225 general collection sites; 130 in Queensland and 95 in New South Wales (Suppl. material 2: Table S3).

Following my initial collection of a *P. lactiferus* female on foliage adjacent to Hallorans Hill Conservation Park, Queensland (Atherton; Wet Tropics), I intensified my sampling of flowering plants around the park for a period of three days between the 3rd and 5th of February 2019 (Fig. 1). Subsequently, I collected five males patrolling the flowers of an ~8 m high *S. sinuatus* tree (flowering times range from February to June (Floyd 1978; Foreman 2020)). *Pharohylaeus lactiferus* males patrolled all flowers (5–8 m high). I undertook further sampling of *S. sinuatus* (and other plants) in nearby rainforest patches between the 5th and 19th of February 2019 but I did not collect any further *P. lactiferus* during this period (Fig. 1).

I resampled Hallorans Hill Conservation Park on the 13th of November 2019 and collected *P. lactiferus* foraging on *B. acerifolius* (flowers November to January (Guymer 1988)). I then undertook further sampling on *B. acerifolius* in Queensland and New South Wales between the 13th and 28th of November (Suppl. material 5: Fig. S3). From these collections, I collected four (2♂2♀) *P. lactiferus* near Hallorans Hill Conservation park on *B. acerifolius*. In Kuranda (Wet Tropics) and Eungella (Central Mackay Coast), I collected five (4♂1♀) and seven (3♂4♀) specimens, respectively, on *B. acerifolius* (Fig. 1).

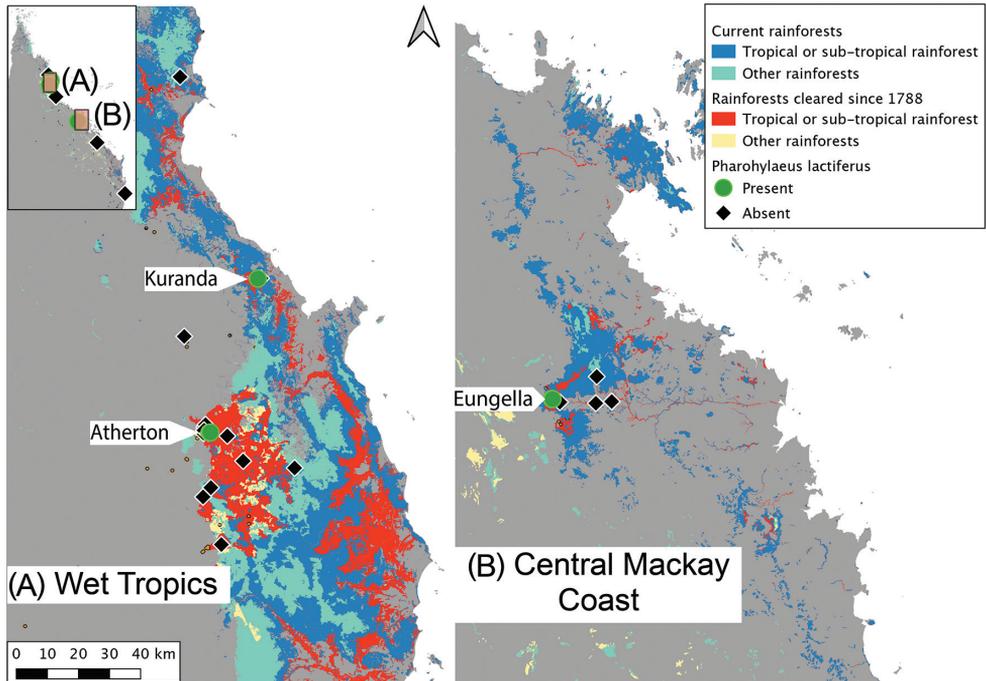


Figure 1. (blues) Current rainforests and (reds) rainforests cleared since European arrival (1788) in the **A** Wet Tropics and the **B** Central Mackay Coast (NMD 2003b, a). Black markers indicate flowering *Stenocarpus sinuatus* or *Brachychiton acerifolius*, sites where no *Pharohylaeus lactiferus* were detected, and green markers (Kuranda, Hallorans Hill C.P. (Atherton), and Eungella) indicate those where they were present. Inset shows Queensland and northern New South Wales as well as sample locations.

From the sites where I successfully collected *P. lactiferus*, I sampled most plant species that were flowering at the time (and with flowers at or below ~13 m). The plants on which I collected bees – other than *P. lactiferus* – at these sites included: *Alpinia* sp. (Zingiberaceae), Asteraceae sp., *Callicarpa pedunculata* R.Br. (Lamiaceae), *Duranta repens* L. (Verbenaceae), *Leptospermum* sp. (Myrtaceae), *Melicope rubra* (Lauterb. & K.Schum.) T.G.Hartley (Rutaceae), *Parsonsia straminea* (R.Br.) F.Muell. (Apocynaceae), *Senna* sp. (Fabaceae), *Solanum seaforthianum* Andrews (Solanaceae), and *Syzygium* sp. (Myrtaceae) (Suppl. material 2: Table S3). I only identified plants on which I caught bee specimens; thus, this represents a subset of those examined.

I undertook a total of 42 observation events on either *S. sinuatus* or *B. acerifolius*. Ten of my observation events resulted in *P. lactiferus* collections across three sites and 32 of my observation events returned no *P. lactiferus* across 20 sites (Fig. 1). I was only successful in collecting *P. lactiferus* between 351 and 877 m asl and only at three sites in the Atherton, Kuranda and Eungella regions of Queensland (Fig. 1; Suppl. material 2: Table S1). Successful collections were made between 0855 and 1637 (Suppl. material 2: Table S1). Of the 20 sites examined, 15 were within 1 km of TSTRs and

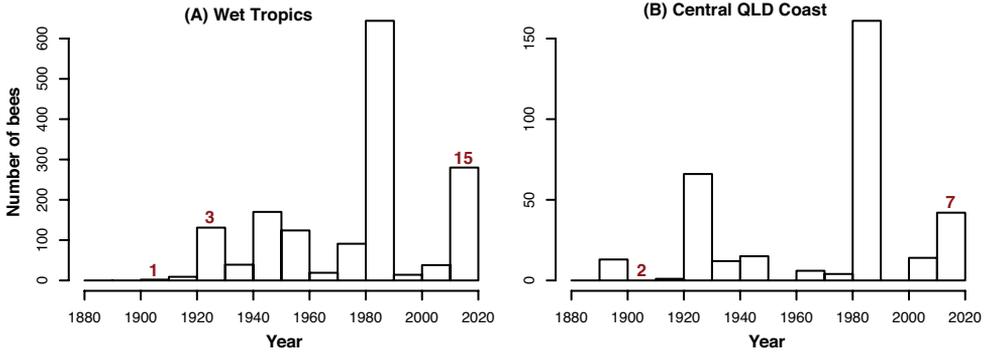


Figure 2. The number of bee specimens recorded on the Atlas of Living Australia for the **A** Wet Tropics and **B** Central Mackay Coast bioregions in ten-year intervals. Numbers above bars indicate *Pharoehylaeus lactiferus* specimen collections.

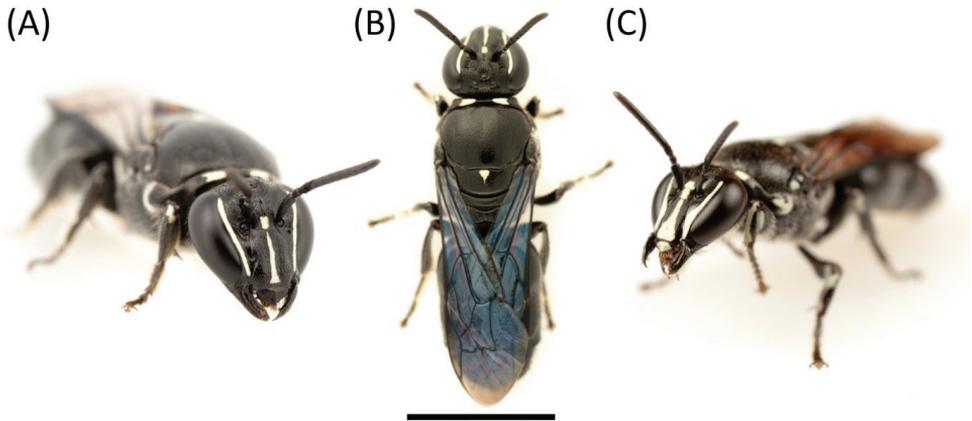


Figure 3. An **A** oblique and **B** dorsal photo of a female and an **C** oblique photo of a male *Pharoehylaeus lactiferus*. Scale bar: 5 mm (**B**). Photos are not to scale.

12 were within 213 m (Suppl. material 2: Table S1). According to Beck et al. (2018)'s Köppen-Geiger climate classification map, *P. lactiferus* occurs in both tropical and sub-tropical climates.

Historic collection data

Historic bee records. The Atlas of Living Australia has a total of 2,198 bee records for the Wet Tropics and 584 for the Central Mackay Coast. Of these records, 637 (29%) in the Wet Tropics and 250 (43%) in the Central Mackay Coast do not include year of collection. Of the records that included year of collection, the Atlas of Living Australia only had 11 of 1,561 and 15 of 334 records that pre-dated 1924 for the Wet Tropics and Central Mackay Coast, respectively (Fig. 2).

Supplementary results summary

I caught significantly more *P. lactiferus* and other bees and spent more time sampling near TSTRs (Suppl. material 1: Appendix; Suppl. material 6: Fig. S4). However, the sum of sampling time was not significantly correlated with the number of *P. lactiferus* caught, suggesting some resilience of data interpretation to bias (Suppl. material 1: Appendix; Suppl. material 6: Fig. S4). *Pharohylaeus lactiferus* was only collected within 213 m of TSTR (Suppl. material 1: Appendix). Tropical or Sub-Tropical Rainforests have undergone habitat destruction and fragmentation since European colonisation and are susceptible to fire (Suppl. material 1: Appendix). In New South Wales and Queensland, most *B. acerifolius* and *S. sinuatus* records occur in rainforests.

Discussion

Despite my extensive non-targeted and targeted sampling as well as bee collection records on the Atlas of Living Australia, *P. lactiferus* records remain rare. Apparent habitat specialisation to TSTRs and few associated floral taxa (*S. sinuatus* and *B. acerifolius*) might explain the rarity of *P. lactiferus*. However, in many cases I found *P. lactiferus* difficult to catch due to the height of the associated plant species (of the trees that I sampled, flowers were between 1 m and 13 m high) and the bees' quick flight (Suppl. material 1: Appendix). It is possible that *P. lactiferus* is a naturally rare species that is not threatened. But, why at least two early collectors sampled *P. lactiferus* on three separate occasions prior to 1924 (Houston 1975) and no published records have been made in the years since, despite a greater sampling effort (Fig. 2), is both unclear and of concern.

The occurrence of host plant species could limit suitable habitat for *P. lactiferus*. For example, the persistence of a *P. lactiferus* population in any one rainforest could require several host plant species to provide food throughout their activity period. From current and historical collections, we know that *P. lactiferus* is active at least between November and May. This could indicate a long flight period, bivoltinism or, like many other tropical bee species (e.g., (ALA 2019; Dorey et al. 2019)), activity could be year-round. Additionally, as many hylaeines nest in preformed holes (Almeida 2008; Houston 2018), *P. lactiferus* might require very specific nesting substrates (Hearn et al. 2019). Nesting substrate could further be limited to certain plant species, and by certain stem-borers that pre-excavate potential nests (Dew and Schwarz 2013; Houston 2018). Habitat destruction and fragmentation might also limit the persistence of the required species in fragments (Suppl. material 1: Appendix). These factors might be particularly relevant to *P. lactiferus*, which was only found within ~200 m of TSTR, suggesting a low foraging and dispersal distance (Suppl. material 1: Appendix).

That bees use *S. sinuatus* and *B. acerifolius* might be unexpected for two primary reasons. Firstly, both plant species exhibit a pollination syndrome that is associated

with birds (e.g., they are bright red) (Nicolson and Van Wyk 1998; Williams and Adam 2010; Shrestha et al. 2013). Bee vision is shifted towards ultraviolet wavelengths and they are thought to not perceive red wavelengths (Dyer et al. 2015); although, this is not always the case (Peitsch et al. 1992) and insect visual perception is complex (Horridge 1998). It is possible that the flowers of *S. sinuatus* and *B. acerifolius* have ultraviolet, or similar, markings or produce olfactory cues that attract bees. That at least ten bee species across eight genera were foraging on *B. acerifolius* could indicate that this plant is not exclusively bird-pollinated (Suppl. material 2: Table S1). Hylaeinae bees were the primary visitors of *B. acerifolius* during observations (Suppl. material 2: Table S1) which could indicate phylogenetically conserved traits that allow the use of flowers that exhibit bird-pollination syndromes (e.g., pollen specialisation or red-shifted vision). Secondly, Guymer (1988) reported that *B. acerifolius* lacks nectaries. While I did not observe bees inside flowers due to the height of trees, I did observe bees ‘drinking’ from flowers of the related *B. populneus* (Schott & Endl.) R.Br., which Guymer (1988) also reports as lacking nectaries. Melittologists might avoid sampling plants that exhibit bird-pollination syndromes and this could bias their collections. The foraging preferences of *P. lactiferus* require further study, likely with a particular focus on plants exhibiting bird-pollination syndromes (e.g., *Alloxylon pinnatum* (Maiden & Betche) P.H. Weston & Crisp, *Castanospermum australe* A.Cunn. ex Mudie or *Erythrina vespertilio* Benth.) or even on canopy-flowering plants in general (Roubik 1993).

In the bioregions that *P. lactiferus* has been found, this major vegetation subgroup has undergone habitat destruction and fragmentation since European colonisation (Suppl. material 1: Appendix) (Bradshaw 2012). Although Queensland’s Wet Tropics have largely been protected from clearing in contemporary times, like much of the state, habitat fragmentation remains a major conservation concern (Tucker 2000). Additionally, three of four rainforest vegetation types (including TSTR) burnt every year between 1988 and 2020 (for which data are available; Suppl. material 1: Appendix). While there was no significant change over time in the area of rainforest burnt during that period, the 2019–2020 bushfire season burnt a greater area than in any year prior for each rainforest type (Suppl. material 1: Appendix).

To monitor and assess the conservation status of each species we require an understanding of their biology and targeted sampling. Data deficiency for rare species raises concerns that other rare or specialist species could become extinct before being discovered, leaving no opportunity to conserve those taxa. We must increase biomonitoring, particularly of diverse invertebrate fauna to assess and protect such taxa worldwide. Additionally, increasing institutional investment to digitise collections would vastly increase the research utility of online databases and potentially allow us to differentiate rare from threatened taxa.

Future research should aim to increase our understanding of the biology, ecology and population genetics of *P. lactiferus*. This work could use targeted seasonal sampling throughout the year at sites where *P. lactiferus* is known to occur, providing insights into phenology and host plant species. Future studies could also use trap-nests at various heights from the ground and targeted searches to uncover nesting requirements and inform conservation management (Roubik 1993; Sutherland et al. 2010).

These data, along with an expanded *a priori* sampling regime, should allow accurate implementation of species distribution models to uncover other potential populations or translocation sites. To determine if *P. lactiferus* is threatened (undergone population declines in the recent past) or simply rare (stable population in the recent past), genetic data could be used to examine past demographies. Additionally, genetic data for each population could allow examination of population isolation. Such research will be invaluable to assess the conservation status of *P. lactiferus* and provide an exemplar for the assessment of other poorly-studied and threatened bee taxa.

Conclusions

Despite extensive sampling undertaken during this study and from publicly available records, *P. lactiferus* remains poorly collected and little is known of its biology. *Pharohylaeus lactiferus* has only been collected on two plant species (*S. sinuatus* and *B. acerifolius*), to the exclusion of other available resources. Thus far, only males have been collected on *S. sinuatus*. These collections might indicate floral specialisation, potentially on plants that exhibit bird-pollination syndromes.

Many of the analyses undertaken here are exploratory and this must be considered when making conclusions. However, it is important for likely issues to be raised in order to inform future research and conservation efforts. To these ends, I make the following remarks. *Pharohylaeus lactiferus* could be a floral- and habitat-specialist bee. The absence of *P. lactiferus* collections since 1923, despite far-greater sampling effort prior to this study, raises concerns about its conservation status. Habitat destruction and fragmentation might have acted synergistically with the floral- and habitat-specialisation of *P. lactiferus* to explain its rarity. However, collection habits of melittologist (e.g., possible avoidance of plants with bird-pollination syndromes) and the height of known associated plants might make possible declines difficult to confirm. Regardless, known populations of *P. lactiferus* remain rare and susceptible to habitat destruction (e.g., from changed land use or stochastic events such as fires; Suppl. material 1: Appendix).

Acknowledgements

I would like to thank Olivia K. Davies, Michael P. Schwarz and Mark I. Stevens for proofing early versions of the manuscript. I further thank Lori Lach, Jack Neff, Terry Houston and four anonymous reviewers for their insightful comments on the manuscript that led to its improvement. I would also like to thank Erinn P. Fagan-Jeffries and Peter Rühr for their company during field work. I would finally like to thank the funders of this research (Holsworth Wildlife Research Endowment, Playford Trust PhD Scholarship and AJ and IM Naylor PhD Scholarship) that support and promote the research of many young scientists. This work was carried out under the Queensland national parks research permit number PTU18-001277-1 and the New South Wales National Parks & Wildlife Service scientific licence number SL102137.

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Supplementary material 1

Appendix

Authors: James B. Dorey

Data type: pdf file

Explanation note: Bee flight observations, collection bias analysis, geographical information systems, and historic associated plant records.

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Link: <https://doi.org/10.3897/jhr.81.59365.suppl1>

Supplementary material 2

Tables S1–S5

Authors: James B. Dorey

Data type: Collection, site, plant and GIS data

Explanation note: **Table S1.** Collection data and notes for both successful and unsuccessful searches for *Pharohylaeus lactiferus* in QLD and north-east NSW, Australia.

Table S2. Atlas of Living Australia data for *Brachychiton acerifolius* and *Stenocarpus sinuatus* by major vegetation subgroup (MVS) number in New South Wales and Queensland. Green highlighted rows indicate rainforest major vegetation subgroups.

Table S3. Collection data from New South Wales and Queensland. **Table S4.** Major vegetation subgroup (MVS) data including MVS number, MVS name. Measurements include total MVS area (km²) and proportions, and sampled MVS area (km²) and proportions. Sum of the number of bees, sample time and the total area of NSW and QLD for each MVS are also included. **Table S5.** Summary of fragmentation statistics for the two bioregions, the *Wet Tropics* and *Central Mackay Coast*.

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Link: <https://doi.org/10.3897/jhr.81.59365.suppl2>

Supplementary material 3

Figure S1

Authors: James B. Dorey

Data type: Figure relating to GIS analyses of plant taxa (*Brachychiton acerifolius* and *Stenocarpus sinuatus*) associated with *Pharohylaeus lactiferus*

Explanation note: The number of **A** *Brachychiton acerifolius* and **B** *Stenocarpus sinuatus* in New South Wales (NSW; blue) and Queensland (QLD; maroon) by each major vegetation subgroup (MVS).

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Link: <https://doi.org/10.3897/jhr.81.59365.suppl3>

Supplementary material 4

Figure S2

Authors: James B. Dorey

Data type: The number of each plant taxa (*Brachychiton acerifolius* and *Stenocarpus sinuatus*) associated with *Pharohylaeus lactiferus* by each MVS region and by year of collection.

Explanation note: The number of **A** & **B** *Brachychiton acerifolius* and **C** & **D** *Stenocarpus sinuatus* in New South Wales (NSW; blue) and Queensland (QLD; maroon) by each major vegetation subgroup (MVS).

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Link: <https://doi.org/10.3897/jhr.81.59365.suppl4>

Supplementary material 5

Figure S3

Authors: James B. Dorey

Data type: GIS data and map of bee collections across New South Wales and Queensland

Explanation note: Heatmap of bee collections by Australian bioregion with the focus bioregions bolded. Points indicate the location of bee samples.

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Link: <https://doi.org/10.3897/jhr.81.59365.suppl5>

Supplementary material 6

Figure S4

Authors: James B. Dorey

Data type: Results of collection bias analyses

Explanation note: The sum of **A** number of bees, **B** sample time (mins) and **C** *P. lactiferus* by distance from major vegetations subgroup (MVS) 2 – tropical or sub-tropical rainforest – in 10 km bins. The sum of **E** number of bees, **F** sample time (mins) and **G** *P. lactiferus* by distance from MVS 2 in the first 10 km bin of a, b and c split in 200 m bins. The sum of *P. lactiferus* in **D** 10 km bins and **H** 200 m bins over sampling time (mins) where bin width is 100 minutes.

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Link: <https://doi.org/10.3897/jhr.81.59365.suppl6>

Supplementary material 7

Figure S5

Authors: James B. Dorey

Data type: Sampling effort

Explanation note: Bars show the cumulative **A** number of insects and **B** sampling time by the major vegetation subgroups (MVS) that were sampled in New South Wales (NSW) and Queensland (QLD) (left-most Y-axes).

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Link: <https://doi.org/10.3897/jhr.81.59365.suppl7>

Supplementary material 8

Figure S6

Authors: James B. Dorey

Data type: Wildfire data for Australian rainforests

Explanation note: The area of Major Vegetation Subgroups (MVS) **A** one (cool temperate rainforest), **B** two (tropical or sub-tropical rainforest), **C** six (warm temperate rainforest) and **D** 62 (dry rainforest or vine thickets) burnt by year from 1988 to 2016 and the 2019-20 fire season (red).

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