The threatened Pookila (*Pseudomys novaehollandiae*) hosts a diverse macrobiome of arthropods at varying risks of co-extinction

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Abstract

The Pookila (*Pseudomys novaehollandiae*) is a threatened Australian mammal emblematic of the global decline of many vertebrate taxa. While conservationists are concerned with the survival of threatened vertebrates, few consider the rich diversity of symbiotic arthropods which often depend on these hosts for survival. Using *P. novaehollandiae* as a case study, we demonstrate that even opportunistic sampling can rapidly expand knowledge of the species richness of arthropod symbiont communities on poorly studied vertebrate hosts. We also demonstrate how DNA barcoding can be integrated to provide rapid and inexpensive identification capabilities for non-specialists. Finally, we show how various metrics and methods can be used to quantify and qualify extinction risk of poorly known arthropod symbionts to provide the foundations for conservation action.

Implications for insect conservation: this study highlights how opportunistic sampling coupled with DNA barcoding and extinction risk metrics can be used to rapidly and inexpensively characterise the arthropod macrobiomes of rare vertebrates and evaluate the risk of decline of their component taxa.

Keywords: parasite conservation; Australia; Laelaps; Siphonaptera; Ixodidae; New Holland mouse

Ethics statement

The authors declare no competing interests.

Disclosure statement

The authors declare that they have no financial or non-financial interest arise from the direct applications of this paper.

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Introduction

An extremely species-rich, though often overlooked, group of terrestrial arthropods are obligate symbionts of vertebrates. This includes ~5,000 lice (Phthiraptera) (Price et al. 2003), ~2,500 fleas (Siphonaptera) (Whiting et al. 2008), ~3,000 chigger mites (Trombiculidae) (Nielsen et al. 2021), almost 1,000 ticks (Ixodida) (Horak et al. 2003), and >6,000 dung beetles (Coleoptera: Trogidae; Geotrupidae; Scarabaeinae; Aphodiinae) (Schoolmeesters 2019), among many other taxa. Unsurprisingly, obligate symbionts are particularly vulnerable to co-extinction when one or more of their hosts vanish (Dunn, 2005). There are numerous examples of this, including the tick (*Ixodes nitens*) and the flea (*Xenopsylla nesiotes*) that both became extinct when their host, the Christmas Island rat (*Rattus macleari*), became vanished around 1903 (Kwak 2018; Mihalca et al. 2011). An even earlier example is the mammoth botfly (*Cobboldia russanovi*), described from frozen specimens taken from the stomach of a woolly mammoth (*Mammuthus primigenius*) exhumed from the Russian permafrost (Grunin 1973). When the woolly mammoth was lost to extinction, so too was its apparently obligate botfly species. Many vertebrates host speciose communities of symbiotic arthropods, and co-extinction can easily occur if the vertebrate host is lost. Despite this, only recently have calls to protect these unique symbiote species commenced (e.g., Carlson et al. 2020). Ecologists have also started trying to quantify the extinction risk in select groups. For example, it has been estimated that if the 114 primate species threatened with extinction were to vanish, 176 parasite species would become co-extinct with them (Herrera et al. 2021). A growing number of vertebrate species are declining across the globe and while increasing conservation attention is directed at them, little is known about the diversity of their arthropod symbiotes, let alone the population trends of those symbiotes (Brodie et al. 2021; Carlson et al. 2020). There is a dire need to identify the arthropod symbiotes of threatened vertebrates so that they can be characterised (morphologically and molecularly) and appropriate conservation actions enacted to prevent extinction of these invertebrates.

The Pookila (*Pseudomys novaehollandiae*) (sometimes called the New Holland mouse) is a threatened native Australian mammal with a fragmented distribution across the southeast of the continent (Burns 2019). Like so many threatened vertebrates, it has garnered increased attention from conservationists in recent years as its population has declined (Lazenby et al. 2018). The symbionts of *P. novaehollandiae* have only been studied in a haphazard way, and the community as a whole remains poorly characterised: the cosmopolitan parasite *Toxoplasma gondii* attracted some attention from Burns (2019); a single record of the invasive flea *Nosopsyllus londiniensis* was reported from *P. novaehollandiae* by Dunnet and Mardon (1974); Hastriter and Whiting (2002) described the host-specific flea *Macropsylla novaehollandiae* from *P. novaehollandiae*; and this work was later supplemented with expanded information by Kwak and Hastriter (2020). The symbiotic rove beetle *Myotyphlus newtoni* was also recorded from *P. novaehollandiae*, but its ecology and exact relationship with mammals remains poorly known (Jenkins Shaw et al. 2017; Solodovnikov and Jenkins Shaw, 2017; Jenkins Shaw 2021). Like so many other arthropod symbiont communities, that of *P. novaehollandiae* remains poorly studied, and the conservation needs of these unique arthropods largely unknown. Therefore, the aims of this study were to elucidate the diversity of arthropod symbionts of *P. novaehollandiae*, to facilitate their identification by non-specialists, and to assess their conservation needs. However, the intention of this work is not simply to provide more accurate information on the arthropod symbionts of *P. novaehollandiae* but to demonstrate a workflow which can be used to gather knowledge and conserve the arthropod symbionts of the speciose vertebrates at risk of extinction across the globe.

Methods

Field sampling and identification

Arthropod symbionts were sampled opportunistically during small mammal live trapping surveys conducted between October 2015 and May 2019 at Wilsons Promontory National Park (Figure 1a) (the western most populations in the remnant range of the endangered southern evolutionarily significant unit (ESU) of *P. novaehollandiae*), which has been separated from the species northern ESU for over 200,000 years and is managed independently from a conservation perspective (Burns et al. 2023). Elliott small mammal traps (Elliott Scientific Co.: Upwey, Victoria; size 90 mm × 100 mm × 330 mm) were used across all surveys, baited with a mixture of peanut butter, golden syrup, and rolled oats, with non-absorbent nesting material for insulation. Between 20 and 50 traps were set per site for 3 – 5 nights. Traps were checked twice daily, shortly after sunrise and before sunset. In total the host sampling for this study comprised 6,239 trap nights (Figure 1b).

Small mammal captures were visually inspected for arthropod symbionts while morphometrics were taken for the host; ectoparasites observed moving freely across the hosts' fur were collected using forceps and placed in 70% ethanol. Visual inspection focused primarily on the head and around the base of the tail of the hosts. Following the release of the host at the site of capture, the cloth handling bag was inspected for any remaining ectoparasites, which were also collected and fixed in 70% ethanol. Not all observed ectoparasites were collected; many escaped by burrowing into host fur or jumping out of sight, and due to ethical requirements, efforts to collect ectoparasites were abandoned if collection would disturb the host or increase host handling time.

Specimens were examined using a M205C light microscope and identified to species level using keys and descriptions provided by Roberts (1970) for ticks, Dunnet and Mardon (1974) and Hastriter and Whiting (2002) for fleas, Domrow (1987) for mites, and Solodovnikov and Jenkins Shaw (2017) for beetles.

Molecular analysis

In total, 15 ectoparasite specimens were selected for DNA barcoding. All specimens were fixed in ethanol and were removed and air-dried for 1 minute. DNA extraction was performed using the non-destructive HotSHOT method developed by Truett et al. (2000). Each specimen was placed whole into 10µL of alkaline lysis buffer. The sample in the lysis buffer was then placed in a Bioer LifeECO thermocycler and incubated at 95°C for 30 minutes before being cooled to room temperature (~20°C). 10µL of acidic neutralising buffer was then added, to return solution to a neutral pH and prevent further lysis. DNA extractions obtained via HotSHOT were stored at -20°C and were be used for PCR within a week of extraction.

PCR was undertaken using 8µL of master mix (Taq DNA polymerase, buffer and dNTPs), 0.5µL of MgCl, 0.5µL of Bovine serum albumin (BSA), 1µL of forward primer, 1µL of reverse primer, and 5µL of DNA from the previous HotSHOT protocol. The m1COIintF and jgHCO2198 primers from Leray et al. (2013) and Geller et al. (2013) were used to amplify a 313bp mini-barcode for the cytochrome c oxidase I (COI) locus. Both forward and reverse primers were modified to each include a distinct 13bp tag (> 4 bp difference between any two tags) to facilitate the identification of the reads associated with each individual sample. The PCR was undertaken using a Bioer LifeECO thermocycler with initial denaturation at 94°C for 5 minutes, followed by 30 cycles of annealing at 45°C for 1 minute, extension at 72°C for 1 minute, and denaturation at 94°C for 30 seconds, with a final extension step at 72°C for 5 minutes after which the reaction was cooled to 20°C. Once all products had been pooled SPRI bead-based clean-up was undertaken using Ampure (Beckman Coulter). The products were then pooled and quantified with a Qubit™ dsDNA HS Assay Kit (Invitrogen).

Nanopore sequencing undertaken for this study was performed on a MinION Mk 1B sequencer using the same methods described in Srivathsan et al. (2021). MinION libraries were generated for our pooled DNA samples using a ligation-based kit (~200ng of DNA per flow cell). The kit instructions were used, except the FFPE DNA repair mix in the end-repair reaction. The reaction volumes for the R10.3 flow cell libraries consist of 45 µl of DNA, 7 µl of Ultra II End-prep reaction buffer (New England Biolabs), 3 µl of Ultra II End Prep enzyme mix (New England Biolabs), and 5 µl of molecular grade water. Data capture involved a MinIT or a Macintosh computer that meets the IT specifications recommended by ONT. The bases were called using Guppy: 4.2.3+f90bd04, under the high-accuracy model in MinIT taking advantage of its GPU. Sequences were then uploaded to MEGA X and trimmed (Kumar et al. 2018). Barcodes then clustered and intraspecific barcode divergence scores were calculated using SpeciesIdentifier (Meier et al. 2006).

Conservation analysis

The vulnerability of symbionts of *P. novaehollandiae* to co-extinction events was assessed using the 'second.extinct' function in the R package 'Bipartite'(Dormann et al., 2009; Dormann et al., 2014; R core team, 2022). Weighted host-parasite interaction matrices were constructed using 745 interaction records from Domrow (1961), Dunnet and Mardon (1974), Solodovnikov and Jenkins Shaw (2017), Jenkins Shaw et al. (2017), Jenkins Shaw (2021) and records from the Australian National Insect Collection (ANIC). One matrix was constructed for insect symbionts of *P. novaehollandiae* while the other matrix was constructed for arachnid symbionts of *P. novaehollandiae*. Non-native symbionts were excluded from the analysis. Using the 'second.extinct' function in the R package 'Bipartite', host species were removed one by one from the matrix and the co-extinctions this caused in the symbiont community were plotted. This simulation was replicated 1000 times and the mean co-extinction slope was plotted and the mean robustness score was calculated to compare the vulnerability to co-extinction of insect and arachnid symbiont communities, respectively.

Host specificity for each symbiont species recorded from *P. novaehollandiae* was quantified using the equation by Poulin and Mouillot (2003) by analysing the aforementioned interaction compiled from Domrow (1961), Dunnet and Mardon (1974), Solodovnikov and Jenkins Shaw (2017), Jenkins Shaw et al. (2017), and records from the Australian National Insect Collection (ANIC).

Conservation assessments were undertaken for all the native symbionts of *P. novaehollandiae* using the IUCN Red List categories and criteria in conjunction with the guidelines for using the IUCN Red list categories and criteria (IUCN Standards and Petitions Committee 2022). Conservation assessments were also undertaken using the conservation assessment methodology for animal parasites (CAMAP) developed by Kwak et al. (2020). These assessments were supplemented with IUCN assessments and data for *P. novaehollandiae* and *P. fumeus* (IUCN redlist 2022a,b).

Results

In total, 332 *P. novaehollandiae* were sampled during this study. We opportunistically collected a total of 48 arthropod symbionts, representing 8 species, from 39 *P. novaehollandiae* over the course of the sampling period (Figure 1c). Of these, 6 represented new species records for *P. novaehollandiae*, including two new undescribed mite species (Table 1, Figure 2). DNA barcodes for the cytochrome c oxidase I (COI) locus were amplified and sequenced for *Ixodes tasmani*, *Laelaps calabyi*, *Laelaps* sp. 1, *Laelaps* sp. 2, *Metastivalius rectus*, and *Stephanocircus pectinipes* (Table 2). Barcodes were already available for *Macropsylla novaehollandiae* (EU336001) by Whiting et al. (2008) and *Myotyphlus newtoni* (MN513061) by Jenkins Shaw et al. (2020), and therefore we did not produce DNA barcodes for these two species.

The analyses to assess the co-extinction threat faced by the two taxonomic groups of arthropod symbionts (insects and arachnids) of *P. novaehollandiae* indicated different levels of risk (Figure 3). The insect community was more insulated against co-extinctions with a robustness score of 0.62 (Figure 3A) compared to the arachnid community which had a co-extinction robustness score of 0.48 (Figure 3B). Although both communities each had one monoxenic host specialist species each, the difference in robustness scores was largely due to the higher level of host redundancy among the insects which had a lower mean host specificities than the arachnid community.

Host specificity was quantified for the native symbionts of *P. novaehollandiae* (Figure 4). Although *Nosopsyllus londiniensis* has been recorded on *P. novaehollandiae,* it was excluded because it is not native to Australia (Dunnet and Mardon 1974). Two species in the symbiont community, *M. novaehollandiae* and *Laelaps* sp. 2, were host specialists of *P. novaehollandiae* and had host specificity value of 0. While others species in the community showed lower host specificity levels ranging from 1 (e.g., in the two other *Laelaps* spp.) to 3.54 in the highly polyxenic tick *I. tasmani*.

Of the 8 native symbiotic arthropods recorded from *P. novaehollandiae*, three were found to be vulnerable, while a further two were data deficient (Table 3). The sole host of *Laelaps* sp. 2 and *M. novaehollandiae* is *P. novaehollandiae* which is presently classified on the IUCN redlist as vulnerable based on assessment criteria B2ab(ii,iii,iv,v). Both species have, however, only been detected on individuals in Victoria and Tasmania (*M. novaehollandiae* only), where *P. novaehollandiae* is classified as Endangered using the Common Assessment Method (i.e. IUCN criteria) (Department of Natural Resources and Environment 2015; Department of Energy, Environment and Climate Action 2023). Given the more than 200k years of genetic isolation between Victorian *P. novaehollandiae* and their less threatened northern counterparts (Burns et al. 2023), it is plausible that *Laelaps* sp. 2 and *M. novaehollandiae* are only found in the endangered southern ESU of *P. novaehollandiae*. As *Laelaps* sp. 2 and *M. novaehollandiae* are host specialists of *P. novaehollandiae*, their geographic range is nested within the range of their host, they can also be classified as vulnerable based on criteria B2ab(ii,iii,iv,v) (IUCN redlist 2022a). However, it is likely that their range is significantly more limited than their host and as such they may warrant a higher threat classification as greater field data accumulates on their distribution. *Laelaps* sp. 1 was recorded from both *P. novaehollandiae* and its congeneric species *Pseudomys fumeus* during our field work. *Pseudomys fumeus* is classified as vulnerable based on criteria A2bce (IUCN redlist 2022a,b). As reduced geographic range of the host and host population reductions both can be inferred to produce similar patterns in host-specific parasites. Therefore, we classify *Laelaps* sp. 1 as vulnerable based on these same criteria as its two hosts. The CAMAP criteria are far simpler and using criteria 6 of this assessment rubric we classify *M. novaehollandiae*, *Laelaps* sp. 1, and *Laelaps* sp. 2 as vulnerable based on the fact that their primary host(s) are also classified as vulnerable and as such, loss of the hosts would result in loss of the symbionts. *Metastivalius rectus* was classified as data deficient because very few records of this species exist. Before this study, only 14 specimens of *M. rectus* were known, all of which were collected before 1960 (Dunnet and Mardon 1974). Therefore, although this enigmatic flea species has been recorded from a number of non-threatened host species, the geographic range, number of populations, and trends within those populations are poorly understood. Likewise, *Myotyphlus newtoni* is poorly studied and known from a small number of specimens, and while it has been recorded on several non-threatened hosts, its geographic range, number of populations, and trends within those populations are also poorly understood. Therefore, *M. newtoni* was also classified as data deficient. The remaining species, *I. tasmani* and *S. pectinipes* are widespread, relatively common, and utilise a large number of non-threatened host species (Roberts 1970; Dunnet and Mardon 1974). Consequently, these two species were classified as species of least concern.

Discussion

Pseudomys novaehollandiae has declined across much of its range due to a variety of threats, including invasive predators such as cats (*Felis catus*) and foxes (*Vulpes vulpes*), as well as habitat degradation/fragmentation (Lazenby et al 2018; Burns 2019; Burns et al. 2020). Inbreeding and pathogens (e.g., *Toxoplasma gondii*) are also suspected of playing a role in the decline (Burns 2019; Burns et al. 2023). While these factors indirectly contribute to the decline of the host-dependent arthropod symbionts of *P. novaehollandiae*, a range of other factors may also impact these invertebrates. A significant factor for co-threatened symbionts is their host's population density. In symbionts such as parasites, host population densities must remain above a specific threshold during at least part of the lifetime of the parasite for transmission to occur from infested hosts to naïve hosts. In fact, MacKenzie and Pert (2018) provided evidence that in cases where host densities drop below this threshold for critical periods of time, symbionts can become extinct. However, it remains unclear what the host density requirements are for each of the arthropod symbionts dependent on *P. novaehollandiae*. Competition and competitive exclusion may also play a role in the decline of certain taxa within communities of symbionts. Fellin and Schulte-Hostedde (2022) showed that the presence of particular symbiont species may act to reduce the numbers of other symbiont species on certain hosts (Fellin and Schulte-Hostedde 2022). One of the symbionts recorded in this study, the rove beetle *Myotyphlus newtoni*, has been suggested to have a predatory ecology and probably hunts other symbionts on the body of the host or in the burrow/nest material (Jenkins Shaw 2021). Therefore, the presence of *M. newtoni*, though natural, may result in a decline in abundance of other symbionts on individual *P. novaehollandiae*. However, the dataset in our study was too small for statistical analysis to be undertaken to assess the relationship between the presence of *M. newtoni* and other symbionts on *P. novaehollandiae*.

The extinction risk faced by host-dependent arthropod communities and species is often assumed to be identical to that of their threatened host; however, this is often an oversimplification. Host specificity can vary markedly within symbiont communities. The utilisation of multiple host species by polyxenic symbionts creates host redundancy which can act as a buffer against extinction. While the utilisation of a single host by monoxenic symbiont species can render that species particularly vulnerable to co-extinction. Within the two subcommunities on *P. novaehollandiae*, insects were significantly more buffered against extinction than arachnids due to their overall lower host specificities. Among symbionts detected in this study, only the New Holland flea (*M. novaehollandiae*) and *Laelaps* sp. 2 were monoxenic and therefore vulnerable to co-extinction with *P. novaehollandiae*. However, *Laelaps* sp. 1 is only known from *P. novaehollandiae* and *P. fumeus*, giving it a similarly precarious existence given that both rodent species are threatened (IUCN redlist 2022a, b). Although we elected to base our assessment on host conservation assessments from the IUCN redlist, it is likely that the true extinction risk is underestimated as *P. novaehollandiae* is classified as vulnerable under the EPBC Act nationally (Date effective: 11-Aug-2010) , endangered in Victoria (Flora and Fauna Guarantee Act 1988 (Victoria): June 2023 list), vulnerable in the Australian Capital Territory (Nature Conservation Act 2014 (Australian Capital Territory): April 2023 list), endangered in Tasmania (Threatened Species Protection Act 1995 (Tasmania): December 2022 list). It is likely that when *P. novaehollandiae* is reassessed by the IUCN that its conservation status will be upgraded to a higher risk category. A reassessment of the conservation status of *P. novaehollandiae* at a national level in Australia (under the EPBC Act) is due to be released in April 2024.

Among the myriad challenges associated with conserving arthropods is the vast number of undescribed species (Cardoso et al. 2011). Among the arthropod species identified on *P. novaehollandiae* in this study, two represented distinct undescribed species (*Laelaps* sp. 1, *Laelaps* sp.

2). This was based on molecular divergence at the COI locus and a range of morphological characters. However, due to the small number of samples of each at the time of writing, we elected not to describe these until more material is collected. While the lack of a formal binomial does somewhat limit conservation action, it does not completely prevent it. For example, in Australia, the undescribed ant *Myrmecia* sp. 17 has been afforded protected status as a threatened species (New et al. 2012). The DNA barcodes presented in this study will facilitate identification for future monitoring of all the symbionts reported in this study, including the undescribed species, as has been done for other biodiverse groups (e.g., Ardura et al. 2010).

Unfortunately, while threatened vertebrate hosts frequently attract considerable attention from conservationists, their symbionts (particularly their parasites) are just as frequently ignored. Carlson et al. (2020) lamented this point and highlighted the need for greater conservation attention for symbionts such as parasites. In response to this, the IUCN established a parasite specialist group to assess the conservation status of parasites, add threatened parasite species to the IUCN redlist, and to develop recovery plans for those species in decline (Hopkins and Kwak 2023). As international support for parasite conservation grows, the opportunities to engage with governments, zoos, land managers and conservation organisations to protect the threatened symbionts of *P. novaehollandiae* increase. Already, the government in the Australian state of Tasmania lists the Tasmanian devil tapeworm (*Dasyurotaenia robusta*) as 'Rare' in the Threatened Species Protection Act 1995 (Threatened Species Section 2023). Therefore, formal recognition of other threatened parasites (including those of *P. novaehollandiae*) in government legislation may also be achieved to spur conservation action.

Kwak and Hastriter (2020) noted the need for conservation action to support the recovery of the flea *M. novaehollandiae*; and many of these strategies could also be employed to protect the threatened *Laelaps* species detected on *P. novaehollandiae*. In the past, zoos have been responsible for causing the deliberate extinction of co-endangered parasites (Rózsa and Vas 2015). However, Zoos Victoria operate a conservation breeding program for *P. novaehollandiae*, which specifically aims to also conserve the species' symbionts. Founders collected for the program have intentionally not been treated for parasites so they can pass their natural symbiont communities on to their offspring. Eventual reintroductions of *P. novaehollandiae* in Victoria will, by design, involve a simultaneous reintroduction of their threatened arthropod symbionts, thereby reducing extinction risk of multiple species. Ectoparasite sampling of the *P. novaehollandiae* breeding program is required to determine whether *M. novaehollandiae* and the threatened *Laelaps* species are currently integrated into this ex-situ conservation strategy (Kwak and Hastriter 2020). Finally, actions taken to support wild populations of *P. novaehollandiae* from threats such as habitat degradation/fragmentation, invasive predators, and inbreeding will likely also have positive impacts on the aforementioned threatened symbionts too. Clearly there are significant opportunities for collaboration between vertebrate biologists and parasitologists/entomologists/acarologists to not only elucidate the macrobiomes of threatened vertebrates, but also to conserve the diverse communities of host-specific arthropods which rely or partially rely on these vertebrates for their survival.

Conclusion

This study demonstrates the significant benefits that opportunistic sampling can have for expanding knowledge of arthropod symbionts of poorly studied threatened vertebrates. Within this study, we tripled the number of arthropod symbiotes known from *P. novaehollandiae*, including two threatened undescribed mite species. However, more relevant to broader conservation objectives is the

305 demonstration that the symbionts of the same threatened host species often have markedly different
306 extinction risks and as such require different conservation actions to ensure their survival.

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Figure 1: A) Distribution (in green) of the Pookila (*Pseudomys novaehollandiae*) in Australia; Points with EX next to them denote extinct populations; B) *Pseudomys novaehollandiae* trap nights per month during the sampling period; C) *Pseudomys novaehollandiae* trapped per month during the sampling period.

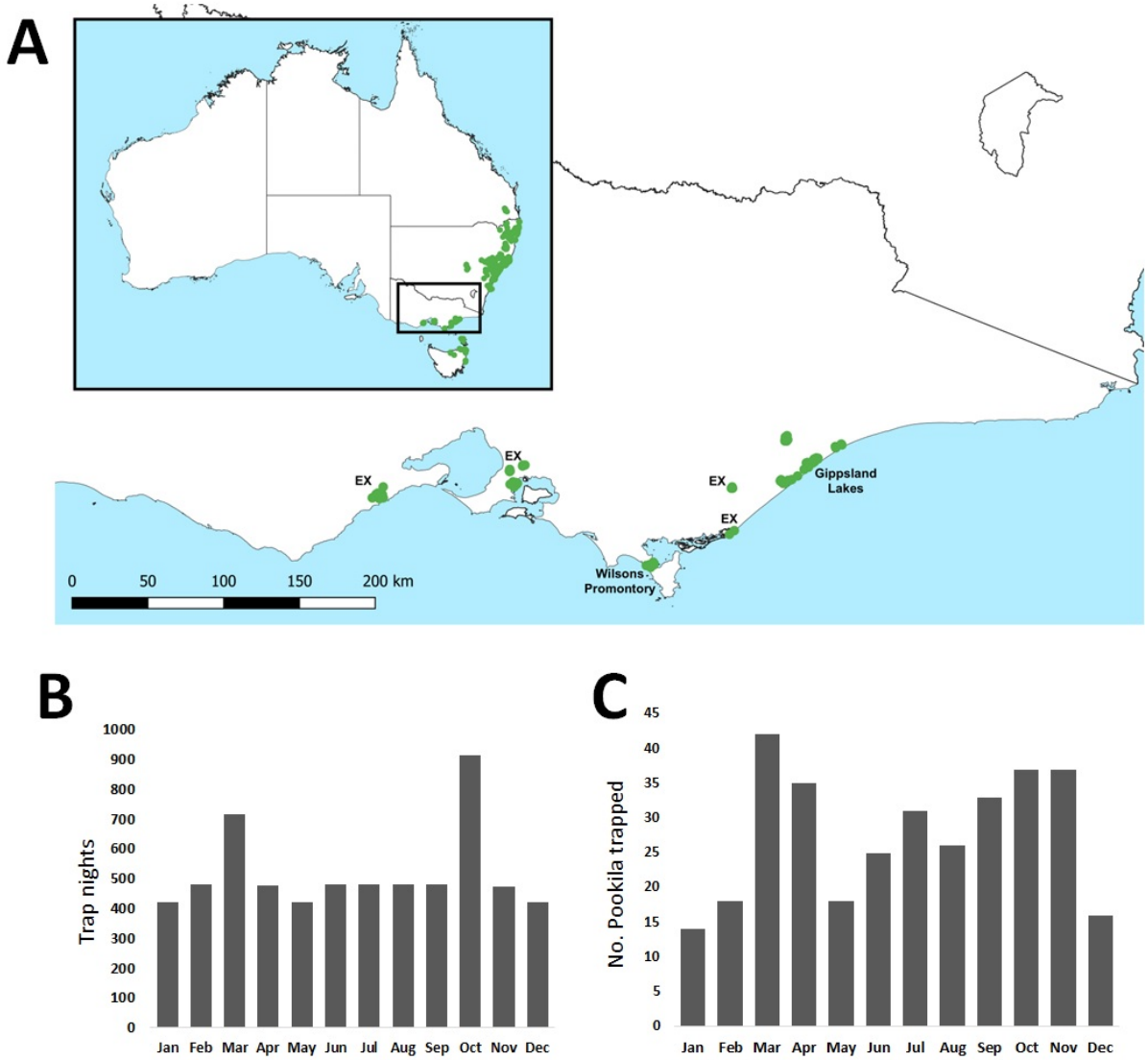

Figure 2: Native arthropods symbionts associated with *Pseudomys novaehollandiae*; A) *Laelaps* sp. 1, B) *Laelaps* sp. 2, C) *Laelaps* calabyi, D) *Ixodes tasmani*, E) *Myotyphlus newtoni*, F*) Macropsylla novahollandiae*; G) *Metastivalius rectus*, H) *Stephanocircus pectinipes*.

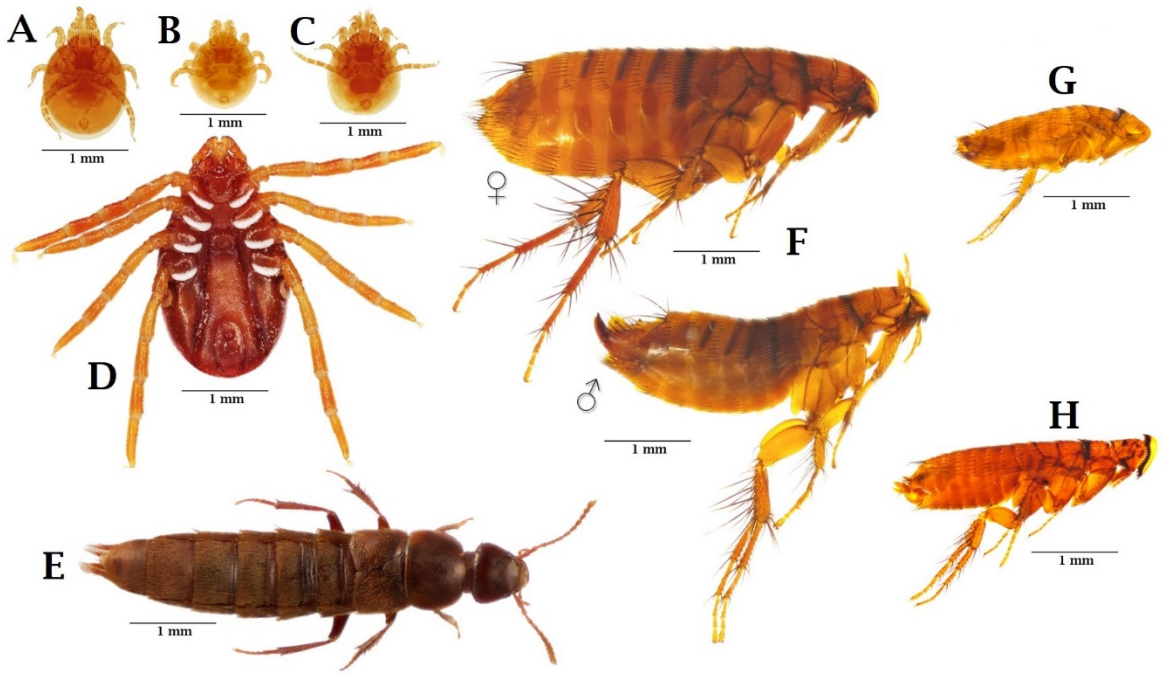

Figure 3: Co-extinction rates of native arthropod symbionts recorded from *Pseudomys novaehollandiae*; A) insects (robustness = 0.62), B) arachnids (robustness = 0.48). (1000 replicates).

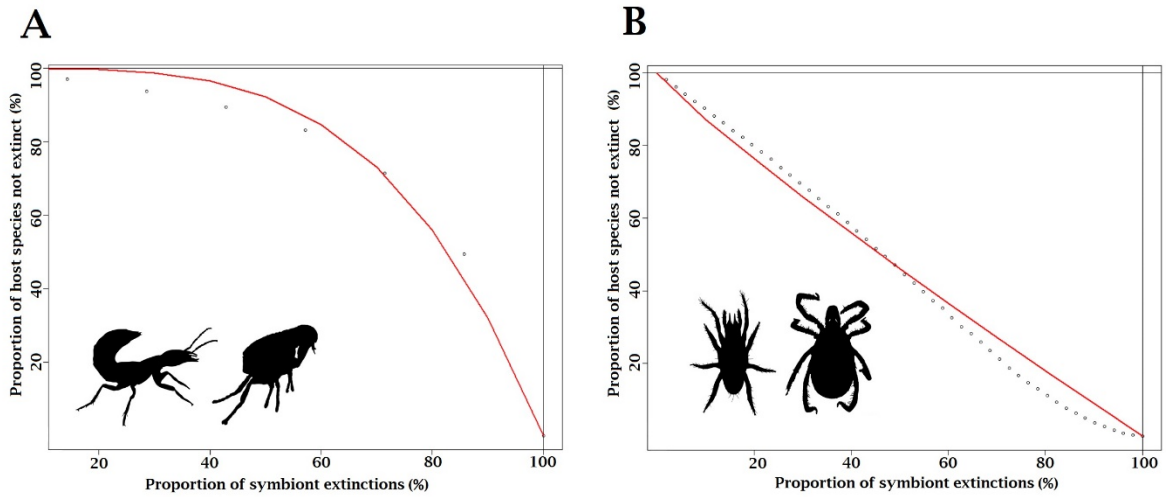

Figure 4: Host specificity of native arthropod symbionts recorded from *Pseudomys novaehollandiae*.

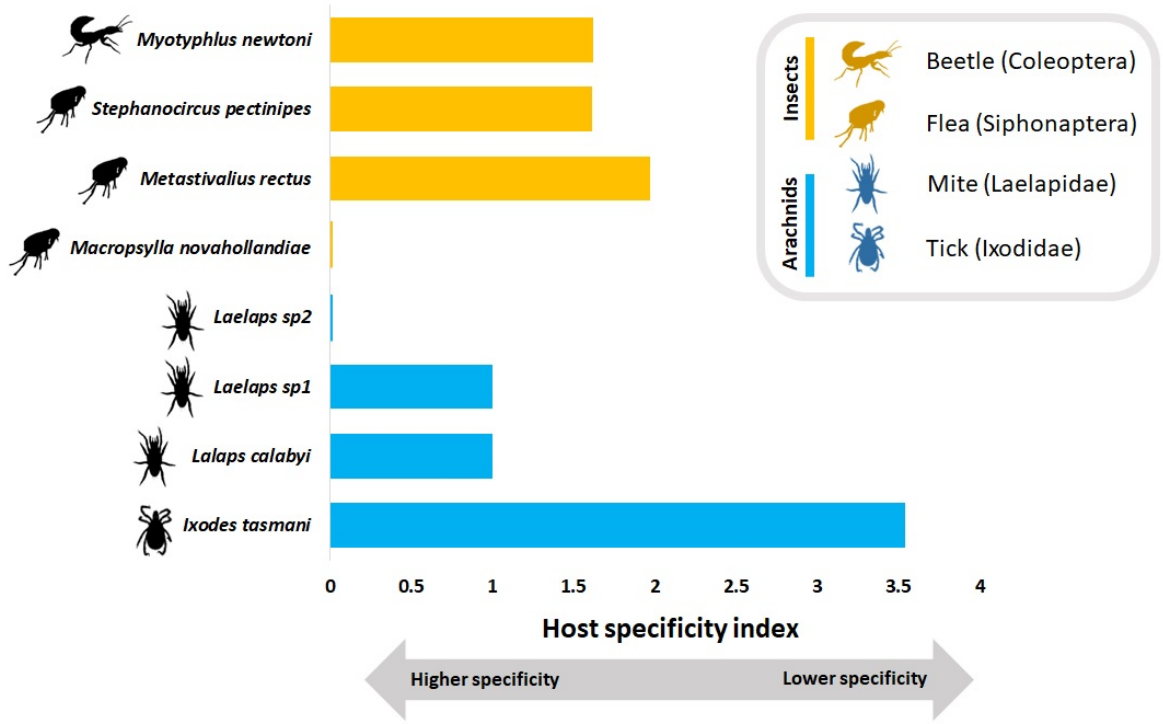

Table 1: arthropod symbionts recorded from the New Holland mouse (*Pseudomys novaehollandiae*)

Order	Family	Species	Natural host	Number recorded in this study	First reco
Ixodida	Ixodidae	*Ixodes tasmani*	Yes	1	This publi
Mesostigmata	Laelapidae	*Laelaps calabyi*	Yes	2	This publi
		Laelaps sp1	Yes	4	This publi
		Laelaps sp2	Yes	1	This publi
Siphonaptera	Macropsyllidae	*Macropsylla novaehollandiae*	Yes	7	Hastriter a
	Stivaliidae	*Metastivalius rectus*	Yes	28	This publi
	Ceratophyllidae	*Nosopsyllus londiniensis*	No	0	Dunnet ar
	Stephanocircidae	*Stephanocircus pectinipes*	Yes	5	This publi
Coleoptera	Staphylinidae	*Myotyphlus newtoni*	Yes	1	Jenkins Sl

Table 2: Cytochrome c oxidase DNA barcodes for the natural symbionts of the New Holland mouse (*Pseudomys novaehollandiae*)

Species	Genbank ID	Reference
Ixodes tasmani	OR689302	This publication
Laelaps calabyi	OR689299	This publication
Laelaps sp1	OR689300	This publication
Laelaps sp2	OR689298	This publication
Macropsylla novaehollandiae	EU336001	Whiting et al. (2008)
Metastivalius rectus	OR689303	This publication
Stephanocircus pectinipes	OR689301	This publication
Myotyphlus newtoni	MN513061	Jenkins Shaw et al (2020)

Table 3: conservation assessment of the natural arthropod symbionts of the New Holland mouse (*Pseudomys novaehollandiae*)

Species	IUCN status	IUCN criteria	CAMAP status	CAMAP criteria
Ixodes tasmani	Least concern	None	Least concern	None
Laelaps calabyi	None	None	None	None
Laelaps sp1	Vulnerable	A2bce **&**	Vulnerable	Criterion 6
Laelaps sp2	Vulnerable	B2ab(ii,iii,iv,v)	Vulnerable	Criterion 6
Macropsylla novaehollandiae	Vulnerable	B2ab(ii,iii,iv,v)	Vulnerable	Criterion 6
Metastivalius rectus	Data deficient	B2ab(ii,iii,iv,v)	Data deficient	None
Stephanocircus pectinipes	Least concern	None	Least concern	None
Myotyphlus newtoni	Data deficient	None	Data deficient	None