



# An assessment of ectoparasites across highland and lowland populations of Leadbeater's possum (*Gymnobelideus leadbeateri*): Implications for genetic rescue translocations

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## ABSTRACT

Leadbeater's possum (*Gymnobelideus leadbeateri*) is a nocturnal arboreal marsupial with a restricted range centered on the Victorian Central Highlands, south-eastern Australia. Most populations inhabit wet montane ash forest and subalpine woodland, with one notable exception - a small, outlying and genetically-distinct lowland population inhabiting swamp forest at Yellingbo, Victoria. The species has been listed as critically endangered since 2015. Translocations are the mainstay of critical genetic rescue and this study explores the ectoparasites that are 'along for the ride' during translocation activities. Ectoparasites (133 fleas, 15 ticks and 76 mites) were collected opportunistically from 24 Leadbeater's possum colonies during population monitoring and genetic sampling across the lowland and highland populations. The composition of the flea assemblage varied by habitat type. Significantly greater numbers of the general marsupial fleas *Acanthopsylla r. rothschildii* and *Choristopsylla tristis* (as a proportion of total flea numbers) were detected in lowland habitats, compared to highland habitats (Fishers exact test,  $P < 0.0001$ ). Two host-specific flea species, *Stephanocircus domrowi* and *Wurunjerria warnekei* were detected only on possums in highland habitats. As a proportion of total fleas this was significantly different to possums in lowland habitats (Fisher's exact test,  $P = 0.0042$  and  $P < 0.0001$ , respectively). *Wurunjerria warnekei* was suspected to be extinct prior to this study. Ticks (*Ixodes tasmanii*,  $n = 15$ ) and mites (*Haemdoelaps cleptus*,  $n = 47$  and *H. anticlea*,  $n = 29$ ) have been identified in Leadbeater's possums historically. The possible causes of the different flea assemblages may be environmental/climatic, or due to the historic geographic division between highland and lowland animals. The planned translocations of highland individuals to lowland habitats will expose lowland individuals to novel species of previously exclusively highland fleas with unknown indirect consequences, thus careful monitoring will be required to manage any potential risks.

## 1. Introduction

Leadbeater's possum, *Gymnobelideus leadbeateri*, is a critically endangered arboreal marsupial that has an extremely restricted distribution in Victoria, south-eastern Australia (Harley, 2004a). Two genetically distinct populations are recognised, a highland population that includes the majority of surviving possums and inhabits montane ash forest and sub-alpine woodland, and one outlying lowland

population of less than 40 individuals, inhabiting a 680 ha remnant swamp forest at Yellingbo Nature Conservation Reserve (Smales, 1994; Hansen and Taylor, 2008; Hansen et al., 2008; Zilko et al., 2020).

The lowland population is the last remnant of a much broader distribution that occurred to the south of the species' current range prior to widespread clearing of suitable habitat for agriculture (Harley et al., 2004a; Greet et al., 2021). The separation between highland and lowland Leadbeater's possum populations is estimated to have occurred

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prior to the major environmental changes that followed European settlement of Australia in 1788 (Hansen et al., 2008). The major threats to both populations include habitat loss and degradation, particularly from fire and extensive logging (Blair et al., 2017; Australian Government, 2016).

Multiple conservation measures are underway to protect Leadbeater's possum populations, including habitat protection, revegetation, provision of nest boxes, and translocations to maintain breeding territories and enable population growth (Harley, 2016). Movement of ectoparasites between habitats directly via translocated animals, or in their nest boxes, has been identified via a disease risk analysis as a possible hazard, though with assumed low consequence to the host, new population, or any non-target species associated with the translocation strategy (Wicker et al., 2017).

Leadbeater's possums exhibit several behaviours that are likely to facilitate the spread of ectoparasites, especially fleas, between individuals within a colony and within local populations. Fleas alternate between being on the host and living in the non-host environment (Krasnov et al., 1998). Leadbeater's possums den communally in colonies consisting of a breeding pair and their young in nests of shredded bark in tree hollows (or artificial nest boxes) (Smith, 1984; Harley, 2004b). They have multiple diurnal dens in an exclusive territory between which they periodically shift (Smith and Lindenmayer, 1988; Lindenmayer and Meggs, 1996; Harley et al., 2005).

Flea species assemblages can differ across habitats, and differences are thought to be associated with environmental factors, host habitat use and host interspecific relationships (Krasnov et al., 1998, 2002). Dunnet and Mardon (1974) refer to four species of fleas in free-ranging Leadbeater's possums across highland habitat from 1966 to 1974, including *Choristopsylla tristis*, *Acanthopsylla r. rothschildii*, *Stephanocircus domrowi* and *Wurunjerria warnekei*. *Choristopsylla tristis* is commonly found on arboreal possums and gliders (including *Petaurus australis*, *P. norfolcensis*, *P. breviceps*) (Dunnet and Mardon, 1974). *Acanthopsylla rothschildii* is restricted in range to Australia and Papua New Guinea, and in Victoria has a broader host range encompassing possums, quolls, and native rodents (Domrow, 1972). *Wurunjerria warnekei* and *S. domrowi* (the goblin flea) appear to have host specificity for Leadbeater's possum (Dunnet and Mardon, 1974; Kwak, 2018). *Wurunjerria warnekei* was noted to have similarities with *Acanthopsylla* sp. which is known to occur on a range of marsupial and rodent hosts in Australia and New Guinea, but *W. warnekei* differs in some morphological features (e.g. lack of spiniforms on the frons), suggesting an adaptation to nest dwelling, and has so far only been found on the Leadbeater's possum (Dunnet and Mardon, 1974).

Since the 1970s, there has been only one description of ectoparasite assemblages for wild Leadbeater's possums. Lindenmayer et al. (1994) found ectoparasites on five out of 11 lowland individuals at Yellingbo and 20 out of 30 highland individuals captured across three locations in the Central Highlands of Victoria. Three flea species were detected: *Stephanocircus domrowi*, *Choristopsylla tristis*, and *Pygiopsylla rainbowi*. While this research sampled a limited number of individuals from a restricted number of locations, the work is of significance given past difficulties experienced in capturing highland Leadbeater's possums in the wild (D. Harley, pers. comm.). In addition to these flea species, Leadbeater's possums have also been noted to have the common marsupial flea *Pygiopsylla hoplia* (Baker and Beveridge, 2001), this occurrence likely originating in captivity (Healesville Sanctuary, Australia) as it was noted across other captive taxa at the same time point.

There are several references to Leadbeater's possum mites and ticks in the published literature. Mite species previously reported include *Haemolaelaps anticlea* sp. N. and *Haemolaelaps cleptusa* sp. N. (Domrow, 1972; Shaw, 2014), and one species of tick: *Ixodes tasmani* (Lindenmayer et al., 1994).

This study aimed to investigate ectoparasite assemblages of lowland and highland Leadbeater's possums, by opportunistically collecting

ectoparasites from a large sample of highland and lowland individuals captured for the purposes of population monitoring, genetic sampling and translocation.

## 2. Materials and methods

### 2.1. Study sites

Lowland Leadbeater's possums sampled for this study were located at Yellingbo Nature Conservation Reserve (37° 51' S, 145° 29' E; c. 110 m above sea level), approximately 50 km east of Melbourne, Australia. The reserve comprises 680 ha of remnant, riparian and swamp forest stretching along four watercourses, the Woori Yallock, Sheepstation, Macclesfield and Cockatoo Creeks. The mean daily ambient temperatures range from 8.0 °C ± 2.3 °C in winter to 19.4 °C ± 5.0 °C in summer. The area receives approximately 1100 mm of rainfall annually.

Sampling of highland Leadbeater's possums encompassed the following three locations: i. high elevation alpine ash *Eucalyptus delegatensis* forest on the Toorongo Plateau (37° 75' S, 146° 09' E, approximately 1240 m above sea level), located 100 km east of Melbourne, with a mean ambient temperature range from 1.5 °C ± 1.9 °C in winter to 13.5 °C ± 3.4 °C in summer; ii. mountain ash *E. regnans* forest at Doweys spur (36° 43' S, 147° 18' E, approximately 700 m above sea level), located 75 km east of Melbourne, with a mean ambient temperature range from 5.7 °C ± 1.8 °C in winter and 19.3 °C ± 3.1 °C in summer; and iii. montane riparian thicket within subalpine woodland dominated by snow gum *E. pauciflora* on the Baw Baw plateau (37° 83' S, 146° 31' E, approximately 1500 m above sea level, including Mt St Gwinear, Mt Erica and the Baw Baw Resort Road), located 120 km east of Melbourne, with a mean ambient temperature range of 0.7 °C ± 2.1 °C in winter and 13.7 °C ± 5.2 °C in summer. The Victorian Central Highlands receives approximately 1600 mm of rainfall annually.

### 2.2. Sample collection and storage

Samples were collected from May 2018 to October 2019. Leadbeater's possums detected in nest boxes at each site were captured and restrained in dark cotton handling bags for examination. Any ectoparasites were collected directly from the animal or from the handling bags following the release of an individual back into its nest box. Ectoparasites were opportunistically collected from nine different possum colonies (two of these nine colonies were sampled twice) in lowland swamp habitat (Yellingbo Nature Conservation Reserve), five colonies in low elevation montane ash (one colony sampled twice) (Doweys Spur), four colonies in high elevation montane ash forest (Toorongo plateau), and six colonies in sub-alpine woodland (one colony sampled twice) (Mt St Gwinear, Baw Baw Resort Road, and Mt Erica). All ectoparasites collected from members of the same possum colony were placed into a single sterile 1.5 mL flip cap or screw cap tube containing 70% ethanol. All parasite samples were stored at ambient temperature prior to mounting and identification at the Melbourne Veterinary School, The University of Melbourne.

### 2.3. Mounting and identification of ectoparasites

Ectoparasites were removed from 70% ethanol and excess ethanol was removed using absorbent wipes (Kimwipe, Kimberly Clark). The ectoparasites were placed individually on a drop of Hoyer's mounting medium on the surface of a microscope slide. A dissection microscope (Nikon SM2 745, Tokyo, Japan) was used to position the parasite for identification before a coverslip was applied. Fleas were identified to species level using the taxonomic key prepared by Dunnet and Mardon (1974).

## 2.4. Statistical analysis

Species accumulation curves were created using iNEXT Online (Chao et al., 2016). Fisher's exact tests were performed using the GraphPad QuickCalcs website (<https://www.graphpad.com/quickcalcs/contingency1/>, accessed April 2022).

## 3. Results

Identifications were made for 133 individual fleas, 15 ticks and 76 mites. This comprised 46 fleas from nine lowland colonies occupying swamp forest at Yellingbo Nature Conservation Reserve, 41 fleas from five highland colonies at low elevation montane ash forest, 17 fleas from four highland colonies occupying high elevation montane ash forest and 29 fleas from six highland colonies occupying subalpine habitats. Four different flea species were identified as part of this study: *W. warnekei* (n = 71, 53.4% of the identified fleas), *S. domrowi* (n = 13, 9.8%), *C. tristis* (n = 32, 24.1%), and *A. rothschildii* sp. (n = 17, 12.8%) (Fig. 1).

The composition of the flea assemblage associated with Leadbeater's possum populations varied by habitat type (Table 1). Significantly greater numbers of the general marsupial fleas *A. r. rothschildii* sp. and *C. tristis* (as a proportion of total flea numbers) were detected in lowland habitats, compared to highland habitats (Fisher's exact test,  $P < 0.0001$ ). Two host-specific flea species, *S. domrowi* and *W. warnekei* were detected only on possums in highland habitats. As a proportion of total fleas this was significantly different to possums in lowland habitats (Fisher's exact test,  $P = 0.0042$  and  $P < 0.0001$ , respectively). Rarefaction curves (see supplementary material 1) indicated that adequate sampling had occurred to indicate that finding of a novel species was unlikely.

Ticks were identified as *Ixodes tasmani* (n = 15) (in both highland and lowland habitats), and mites were identified as *Haemolaelaps cleptusa* (n = 47) and *H. anticlea* (n = 29) (in all highland habitats). In addition, one suspected *Ornithonyssus* sp. and nine mites/ticks were unable to be identified by the authors due to damage to internal structures or opaque blood meals.

**Table 1**

Number of fleas detected opportunistically on highland (three different vegetation types) and lowland Leadbeater's possum (*Gymnobelideus leadbeateri*). Lowland possums inhabit swamp forest, and highland possums are found in high elevation montane ash, low elevation montane ash, and sub-alpine woodland.

Flea species	Lowland swamp forest	Low elevation montane ash	High elevation montane ash	Sub-alpine woodland	Total
<i>Choristopsylla tristis</i>	29	0	0	3	32
<i>Acanthopsylla rothschildii</i>	17	0	0	0	17
<i>Wurunjerria warnekei</i>	0	32	14	25	71
<i>Stephanocircus domrowi</i>	0	9	3	1	13
Total	46	41	17	29	133

## 4. Discussion

Each of the four flea species detected in this study have previously been observed in association with the possum, with *S. domrowi* and *W. warnekei* thought to be host-specific to Leadbeater's possum (Dunnet and Mardon, 1974). Dunnet and Mardon (1974) suggest that *S. domrowi*'s unusually large number of spines on the ctenidia (rows of combs on the dorsal cuticle) are an adaptation to an arboreal host; making it the only known species of *Stephanocircus* to be host specific/host adapted. The last recorded detection of *W. warnekei* was in 1966, and it had since been suggested that the species could be extinct (Kwak, 2018). *Wurunjerria warnekei* was found to be the most abundant species in the present study, and the dearth of recent records presumably reflects the limited capture and sampling of wild Leadbeater's possums in the Victorian Central Highlands.

Ticks and mites identified during this study had all been recorded previously on Leadbeater's possums (Lindenmayer et al., 1994). The flea sampling was more comprehensive in this study; tick samples often had



**Fig. 1.** Fleas detected on the Leadbeater's possum (*Gymnobelideus leadbeateri*); *Stephanocircus domrowi* (panel A), *Choristopsylla tristis* (panel B), *Wurunjerria warnekei* (panel C), *Acanthopsylla rothschildii rothschildii* (panel D).

some structural damage from removal and had full blood meals impeding identification. Similarly, opportunistic sampling and the small size of mites resulted in restricted sampling for this group. While this study represents the most extensive ectoparasite sampling conducted across Leadbeater's possums' range to date and provides a strong basis to compare differences in ectoparasite assemblages among populations and habitats, future studies could incorporate more structured sampling design which would allow for regression analysis of ectoparasite abundance, or presence against habitat parameters.

A clear demarcation of flea species on Leadbeater's possums by habitat type was identified. Previous studies have recorded a similar delineation of flea species by habitat type and elevation; lowland possums were previously observed to carry *C. tristis*, whereas highland possums from Cambarville (low elevation montane ash), Mt Margaret (mid elevation/high elevation montane ash) and Lake Mountain (subalpine woodland) were observed to carry *S. domrowi* (Lindenmayer et al., 1994). Two flea species recorded in this study, *A. rothschildii* and *W. warnekei* were not reported by Lindenmayer et al. (1994). The single finding of one *P. rainbowi* observed by Lindenmayer et al. (1994) may have been incidental as its common host, the agile antechinus *Antechinus agilis*, is known to cohabitate den trees used by Leadbeater's possums (Lindenmayer et al., 1990; Wicker et al., 2017).

There are three potential factors that could explain the difference in flea assemblages: flea sensitivity to the environment, changes to the environment, or indirect founder effect. Flea assemblages have been found to differ within a species depending on habitat type through a range of multifaceted factors including environmental effect (temperature, humidity and material in the nest), and host interspecific relations (including burrow sharing with other species) (Krasnov et al., 1998; Beveridge, 2020). The fleas in this study were collected primarily in winter across a two-year period. Other studies have found changes in winter temperatures to affect assemblages within one host species (Krasnov et al., 2002); there is an environmental temperature difference in this study with lowland average ambient winter temperatures on average 2.3–7.3 °C warmer than highland habitats (montane ash or subalpine woodland) (McCombe et al., 2021). Flea species assemblages can also vary by time of year and season (Krasnov et al., 2002) and while the species assemblage differences here are marked, there could be a variation noted if future samples were collected throughout the year.

Habitat degradation has reduced the prevalence of >190-year-old 'stag' trees containing tree hollows in which possums would normally build nests. This is most marked in lowland populations which have an increasing reliance on artificial nest boxes. At least three have been installed per territory, with an up to 75–85% occupancy from 1995 to 2003, likely higher still today (Harley and Lill, 2006; Harley, 2016). The lowland nest boxes have been known to shelter a range of species; the agile antechinus (*Antechinus agilis*) and sugar gliders (*Petaurus breviceps*) are a common nest box interloper in this area, with black rats (*Rattus rattus*) and common ringtail possums (*Pseudocheirus peregrinus*) infrequently, and feathertail gliders (*Acrobates pygmaeus*) rarely, inhabiting nest boxes (Wicker et al., 2017). A similar spread of inhabitants is known to share tree hollows in the highland range, but it is possible that artificial nest boxes allow for higher flea egg or larval loads to be maintained than tree hollows (Wesolowski and Stanska, 2001). While the highland animals surveyed in this study do use nest boxes (and were captured out of the same for the sampling in this study), they generally have far less access to this conservation measure and primarily rely on tree hollows.

The historic separation of lowland and highland populations occurred several hundred to thousands of years ago and there is no recent contact or gene flow among these populations, causing an 'indirect' founder effect (Hansen and Taylor, 2008). Highland Leadbeater's possums have recently been detected at three sites with lowland swamp forest habitat within the possum's core highland range (D. Harley, pers comm). No fleas have been collected from these localities to date but doing so in these areas could provide information about whether the

host-specific *W. warnekei* and *S. domrowi* are also specific to montane and subalpine habitats or elevation.

Conservation efforts for the Leadbeater's possum are focused on translocations (Wicker et al., 2017; Portas, 2018). In a recent disease risk analysis, ectoparasites being translocated with possums was classified as having an 'almost certain' likelihood of occurrence but was categorized as minor consequence and low risk (Wicker et al., 2017; Portas, 2018). This assessment is based on the limited research into pathogens carried by Australian fleas with no records of disease transmission associated with *S. domrowi* or *W. warnekei*. Fleas are one of many suspected vectors for a newly identified *Breintlia* sp. haemoparasite, albeit with low suspected pathogenicity (Steventon et al., 2020). While fleas can transmit tapeworms, or viral diseases in humans and domesticated species, Harvey et al. (2019) found that the virome of some Australian fleas didn't appear to carry viral pathogens that could be transmitted to mammals. Nonetheless, flea infestation is not entirely benign. Fleas, particularly in young animals, can cause pruritis, skin trauma and anaemia (Vogelnest, 2019). Captive Leadbeater's possums requiring medical treatment for seemingly unrelated illness, anecdotally have a high flea burden when compared to other, non-medical, admissions (e.g. microchip placement), suggesting that fleas can become more abundant in debilitated animals (Pers. Comm. Wicker, 2021).

The introduction of highland individuals to lowland habitats is recommended to preserve the remaining lowland population (Zilko et al., 2021). This will expose lowland individuals to novel species of previously exclusively highland fleas with unknown consequences, although the anticipated outcomes have been predicted to have low with unknown indirect consequences. Any perceived consequence of these measures, including the spread of haemoparasites, will be critical to inform disease risk assessments associated with ongoing translocations to conserve this critically endangered species.

#### Declaration of competing interest

None.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijppaw.2022.05.002>.

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