

NATURAL HISTORY NOTE

Important agricultural and cotton pests detected in the diet of two threatened insectivorous bats in a cotton agroecosystem: insights from a molecular study

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DOI: <https://doi.org/10.14709/BarbJ.15.1.2022.07>

Keywords: Australia, diet composition, ecosystem services, pest control, biocontrol, metabarcoding

received: February, 12th 2022
accepted: October, 25th 2022

ABSTRACT

Australian insectivorous bats are known to occupy and use resources in agroecosystems, yet little is known about their diets. We analysed a scat sample each from two individual bats, both threatened species (*Chalinolobus picatus* and *Vespadelus baverstocki*), in a major dryland cotton production zone. Both bats consumed economically important agricultural and cotton pests such as *Helicoverpa* sp. (bollworm). Our results suggest that these two bat species share around half of the insect prey resources available whilst consuming a wide range of prey items. This snapshot of dietary data provides further evidence that insectivorous bats consume a wide range of insect prey, including pests in cropping areas.

Bats are one of the most ubiquitous and diverse mammal groups in the world, with around 1456 species globally and 103 species in Australia (Armstrong et al. 2020, ASM 2022). However, habitat fragmentation and land clearing associated with broadacre cropping, urbanisation and mining threaten many Australian bat species (Lunney et al. 2002, Woinarski et al. 2015). The conservation of bats in agricultural landscapes is further hindered by negative public perceptions and a lack of understanding of their function and value to agricultural producers (Williams-Guillén et al. 2015, Hoffmaster et al. 2016). One way to change the public perception of bats and encourage bat habitat conservation is to emphasise the pest control service they provide in crop production systems (Lima & Bastos 2021). However, a lack of knowledge regarding the diet of many Australian insectivorous bats has made it difficult to link their potential pest consumption service with farm profits, thus impeding the conservation of bats and bat habitat in agroecosystems. *Chalinolobus picatus* (little pied bat, Vespertilionidae) and *Vespadelus baverstocki* (inland forest bat, Vespertilionidae) are both listed as vulnerable in New South Wales (NSW) under the *Biodiversity Conservation Act 2016* (BC Act), and their ecology, diet and distribution are poorly documented.

Both species are relatively common in inland and arid areas. *Chalinolobus picatus* occupies semi-arid woodland in eastern Australia, specifically coastal and inland Queensland and NSW as well as South Australia and Victoria (Van Dyck & Strahan 2008, OEH 2022a). In south-east Queensland, the species occurs in dry open woodland, dry sclerophyll

forest and vine forest. In NSW it occurs in dry open woodland, shrubland and riverine *Eucalyptus camaldulensis* (river red gum) dominated communities (Duncan et al. 1999). *V. baverstocki* occurs in inland arid and semi-arid environments over most of Australia in arid-zone shrubland (e.g. chenopods) (Bullen & Dunlop 2012), but its distribution is poorly known (OEH 2022b).

Both *C. picatus* (5.7 g) and *V. baverstocki* (4.6 g) are small insectivorous bats and emit high-frequency modulated calls in short pulses (<10 ms) (de Oliveira 1998, Pennay 2004). *Chalinolobus picatus* emit alternating pulses with a characteristic frequency of 38.5 to 43.0 kHz, whilst *V. baverstocki* calls are around 39.0 to 46.0 kHz and differentiated from those of *C. picatus* by the lack of pulse alternation (Pennay 2004). Both bat species echolocate at a peak frequency of approximately 40–45 kHz in inland northern NSW. Overlap in echolocation peak frequency and calls bandwidth may be suggestive of potential overlap in prey resources (e.g. in the type and size of insects detected and consumed) (Denzinger & Schnitzler 2013).

The diet of *V. baverstocki* is poorly known, but is thought to include moths and other volant insects (OEH 2022b). *Chalinolobus picatus* consumes a range of arthropods including moths, beetles, wingless ants, cockroaches, stoneflies, katydids, crickets, cicadas, spiders, flies, termites and grasshoppers (Churchill 2009). However, the exact species composition of its diet and the proportion of prey items consumed remains unknown. *Chalinolobus*

picatus is an aerial forager, taking insects mid-flight close to vegetation, mainly in the low and middle strata of the canopy (Bullen & McKenzie 2001, Churchill 2009). Based on shared echolocation characteristics, *V. baverstocki* likely employs a similar foraging strategy, but the two species probably partition vertical and horizontal airspace when foraging together (Bullen & McKenzie 2001, Van Dyck & Strahan 2008).

Two individuals of both bat species were captured (sex not recorded) in December 2014, in a cotton crop on a dryland cotton farm near Bellata (29°49'28.7"S 149°39'10.8"E), in northern NSW, Australia. The identity of both species was confirmed by echolocation and by referring to Churchill (2009). Faecal pellets were collected from the two captured bats (one sample per bat, each consisting of more than two pellets). The 157 bp section of the cytochrome c oxidase subunit 1 (CO1) mitochondrial DNA barcoding region was amplified using arthropod-specific primers ZBJ-ArtF1c and ZBJ-ArtR2c (Zeale et al. 2011). DNA extraction, Polymerase Chain Reaction (PCR) and sequencing were completed by the Australian Genome Research Facility (AGRF) using the Illumina MiSeq platform. Full capture and sample processing methods are provided in Kolkert et al. (2019).

The resulting DNA sequences were trimmed, quality filtered and clustered into prey Operational Taxonomic Units (OTUs) with a minimum threshold identity of 97%, reflecting natural intraspecific divergence (Alberdi et al. 2018). OTUs were compared with reference sequences in the National Center for Biotechnology Information (NCBI) database using the nucleotide Basic Local Alignment Search Tool (BLAST) on 10 May 2020 and grouped into BLAST subjects. Unknown OTUs were removed from further analysis. Taxonomy was assigned to species level with an identity threshold of $\geq 98.5\%$ (Clarke et al. 2014) and $e\text{-value} < 1e^{-20}$. All identified species were manually checked against sequences held on the NCBI database, and species-level assignment was restricted to species known to be present in Australia (Ratnasingham & Hebert 2007, ALA 2022). For multiple best matches with sequences held in the database or when the best match was not an Australian arthropod, the next best match was identified, or the assignment was relegated to a higher taxonomic level. Based on the taxonomic identification of OTUs, the richness of prey species (counts of OTU read sequences) in each sample was assessed. Each taxonomically assigned prey species was classified as a pest or beneficial insect based on literature detailing their impact on natural and modified agricultural systems and cotton crops (Bailey 2007, Williams et al. 2011). Dietary overlap was calculated using Pianka's index (Pianka 1973) from the 'FSA/Misc' package in R (Ogle 2015). Dietary overlap indices range between 0 and 1: 0 indicates no overlap, and 1 indicates complete dietary overlap.

The richness of recovered OTUs in the scats of the two bats was similar – *V. baverstocki*, 282 OTUs, and *C. picatus*, 261 OTUs (Fig. 1). Overall, OTU dietary richness of prey was dominated by moths followed by beetles, flies, crickets and bugs (Fig. 1). *Chalinolobus picatus* also consumed one mayfly and one grasshopper not detected in the diet of *V. baverstocki*, whereas *V. baverstocki* consumed mosquitoes, not detected in the diet of *C. picatus*. Of the identified

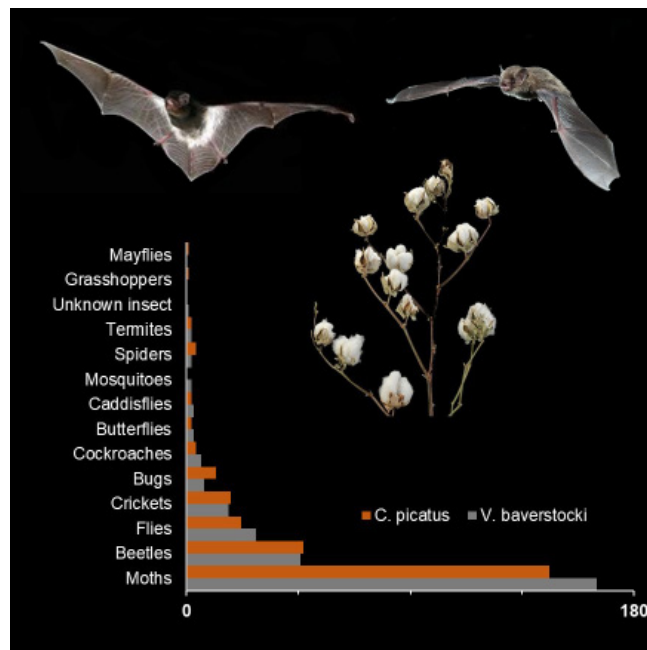


Fig. 1 - The number of Operational Taxonomic Units (OTUs) detected in the diet of *Chalinolobus picatus* (left) (photo by Michael Pennay) and *Vespadelus baverstocki* (right) (photo by Dennis Matthews). Bats were captured in cotton crops in northern New South Wales, Australia.

OTUs in the diet of *V. baverstocki*, 124 (44%) sequences were matched to order and assigned to Lepidoptera ($n = 123$) and one Coleoptera (a 'false wireworm' isopteron beetle), 99 were identified to genus and assigned to 82 taxa, and 54 were assigned to 49 different species (SM1). The most frequently consumed species of lepidopteran prey were Noctuidae (18 species) and Pyralidae (7 species). Of the 49 prey species consumed by *V. baverstocki*, 11 were lepidopteran agricultural pests (Table 1). *Endotricha puncticostalis*, a pest of peanut (*Arachis hypogaea*), had the greatest frequency of matched OTUs in samples ($n = 3$). *Vespadelus baverstocki* also consumed two major cotton pests, *Helicoverpa punctigera* (Australian bollworm, 2) and *H. armigera* (cotton bollworm, 1). Bollworms are globally significant agricultural pests, with *H. armigera* a major pest of cotton and the most destructive pest insect globally (RBG 2017).

Of the identified OTUs in the diet of *C. picatus*, 78 (30%) could be confidently identified to order and assigned to Lepidoptera ($n = 76$), Coleoptera (a false wireworm beetle, *Isopteron*) and Orthoptera (*Teleogryllus oceanicus*, black field cricket) (SM1). Sixty-six OTUs could be assigned to 56 genera, and 39 were identified to 33 different species (SM1). *Faveria* was the most common genus of prey ($n = 5$). *Faveria tritali* (couchgrass webworm), a pest of turf, was the most frequently identified prey species ($n = 4$), followed by *Endotricha puncticostalis* ($n = 3$). Nine agricultural pests were consumed including nine species of Lepidoptera and one orthopteran (black field cricket). *Chalinolobus picatus* consumed three major cotton pests, including *H. punctigera*, *Agrotis munda* (brown cutworm) and *Spodoptera exigua* (lesser armyworm) (Table 1).

Table 1 - The number of Operational Taxonomic Units (OTUs) matched to agricultural pests in one faecal sample (consisting of several pellets) each from *Chalinolobus picatus* (Chpi) and *Vespadelus baverstocki* (Veba), collected in a cotton crop in northern New South Wales, Australia. Crop type indicates the crops that are susceptible to the insect pest. Major cotton pests are indicated with an asterisk (*).

Order / scientific name	Common name	Wingspan (mm)	Crop type	Chpi	Veba
Lepidoptera				14	16
<i>Agrotis munda</i> *	Brown cutworm	40	Cotton, lucerne, corn & tobacco	1	0
<i>Athetis tenuis</i>	None	25	Peanut & soybean	1	0
<i>Diarsia intermixta</i>	Chevron cutworm		Brassica, beet & corn	1	1
<i>Endotricha puncticostalis</i>	Snout moth	30	Peanut	3	3
<i>Etiella behrii</i>	Lucerne seed web moth	10–22	Legumes (i.e. peanut, soybean & mungbean)	0	2
<i>Faveria tritalis</i>	Couch-grass web moth	20–30	Turf	4	1
<i>Helicoverpa armigera</i> *	Cotton bollworm	30–40	Cotton, corn, legumes & other crops	0	1
<i>Helicoverpa punctigera</i> *	Australian bollworm	40	Cotton, corn, legumes & other crops	1	2
<i>Hellula hydralis</i>	Cabbage centre grub	20	Brassica	0	1
<i>Hippotion celerio</i>	Vine hawk-moth	60–80	Taro, sweet potato, grape & ornamentals	1	0
<i>Leucania loreyi</i>	Sugarcane armyworm	34–44	Cereal crops (wheat barley, corn, sugar, barley & sorghum)	0	2
<i>Merophyas divulsana</i>	Lucerne leaf roller	15	Lucerne, carrot, lettuce & other herbaceous plants	0	1
<i>Philobota chionopectera</i>		25	Pastures and turf	0	1
<i>Philobota orescoa</i>	Pasture tunnel moths	20	Pastures	0	1
<i>Spodoptera exigua</i> *	Beet armyworm	30	Crops and pastures (i.e. cotton, tomato, corn, lettuce, brussels sprout, sugar beet & soybean)	1	0
<i>Theretra oldenlandiae</i>	Impatiens hawk moth	60	Ornamentals	1	0
Orthoptera				1	0
<i>Teleogryllus oceanicus</i>	Black field cricket	28–42 (length)	Sugarcane, pineapple, pastures, soybean, pulses, cereals & other crops in swarms	1	0
Total				15	16

Of the species identified (49 and 33 species, respectively), 16 species were detected in the diet of both bats. Pianka's index of dietary niche overlap may indicate (although our sample size is clearly not enough to test any competition or sharing behaviour between these species) some resource sharing (Pianka's index = 0.46 at species-level and 0.47 at genus-level). Both bats consumed many small to medium-sized moths, but the differences in diet suggest that they may partition foraging space vertically or employ different foraging strategies, allowing them to co-exist. This could be verified in the future with larger sample sizes and by sampling prey abundance along vertical gradients. Both bats consumed most prey species once (as identified by singular OTU read sequences), although agricultural pests and particular genera and families of arthropods dominated diets (SM1). Several reads of *E. puncticostalis*, *H. punctigera* and *F. tritalis* were obtained, which may indicate the abundance of these pests in the cotton landscape. Hymenopterans were

not detected in either diet, despite the previous records of *C. picatus* consuming wingless ants (Churchill 2009). No beneficial species were detected in either diet, but some OTUs were assigned to flies and spiders, and some were attributed to unknown prey (SM1). This highlights the benefit of bats foraging in crops but also may suggest limitations of Australian insect DNA sequences held in global DNA databases (Burgar et al. 2014), and the possibility of further unexplored diversity in bat diets. Globally, bats in cotton and other agricultural systems are known to have diverse diets, yet target pest irruptions (Federico et al. 2008, McCracken et al. 2012, Kolkert et al. 2019, Baroja et al. 2021, Maslo et al. 2022).

Whilst our data does not allow dietary inferences to be made between sexes, seasons, locations or at different physiologically demanding life stages due to unitary sample sizes, this snapshot of dietary information is

critical to the preparation of recovery and conservation plans for these species. Uncovering a benefit in terms of a natural pest control service may enhance farm profits and justify incorporating insectivorous bat activity into agri-environmental schemes, thus assisting the recovery of these species in the semi-arid interior of NSW.

ACKNOWLEDGEMENTS

We would like to thank the Kirkby family who allowed us to work on their property. This project was funded by the Holsworth Wildlife Research Endowment – Equity Trustees Charitable Foundation, the NSW North West Local Land Services (LLS) and the Brigalow-Nandewar Biolinks Project (Australia Government Biodiversity Fund – Projects LSP-991865-1429 and LSP-944752-1076) managed by the North West and Northern Tablelands LLS.

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