



Accreditation
No. 21138

Insect diversity assessment using air samples collected from approved arrangement facilities.

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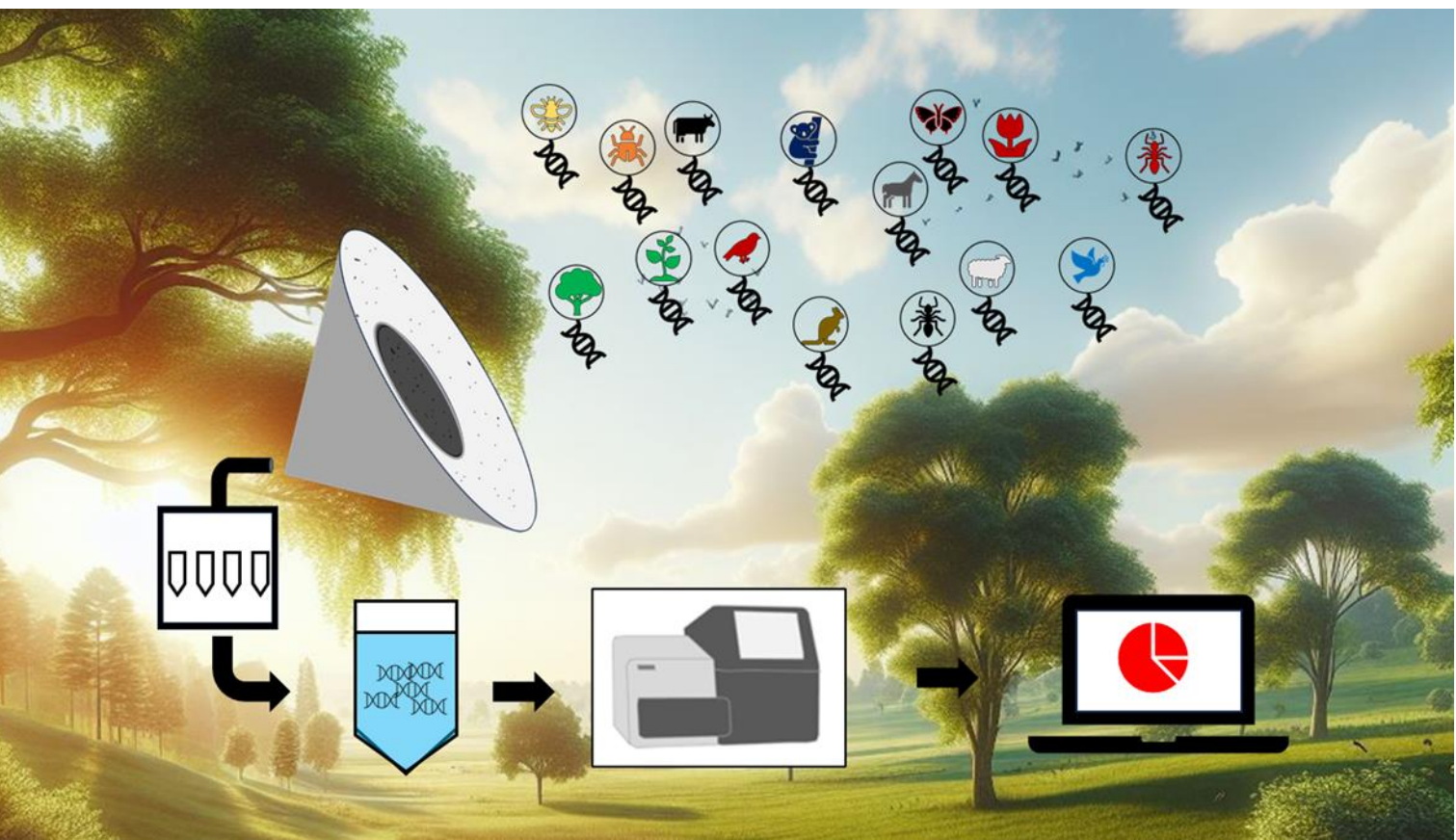
Airborne environmental DNA



what is airborne eDNA

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Airborne environmental DNA (eDNA) is an emerging field that allows for the detection of species from their genetic remnants in the air.



Fungi

nature > articles > article

Article | Open access | Published: 10 July 2024

Airborne DNA reveals predictable spatial and seasonal dynamics of fungi

Nerea Abrego, Brendan Furneaux, Bess Hardwick, Panu Somervuo, Isabella Palorinne, Carlos A. Aguilar-

Received: 12 November 2021 | Revised: 8 February 2022 | Accepted: 9 February 2022
DOI: 10.1002/edn3.290



Insect

ORIGINAL ARTICLE

Airborne environmental DNA metabarcoding for the monitoring of terrestrial insects—A proof of concept from the field

Fabian Roger, Hamid R. Ghanavi, Natalie Danielsson, Niklas Wahlberg

Received: 5 March 2024 | Revised: 4 June 2024 | Accepted: 9 July 2024
DOI: 10.1002/edn3.591



ORIGINAL ARTICLE

Continuous daily sampling of airborne eDNA detects all vertebrate species identified by camera traps

Marcel Polling, Ralph Buij, Ivo Laros, G. Arjen de Groot

Vertebrates

Research article | Open access | Published: 06 December 2021

Airborne environmental DNA metabarcoding detects more diversity, with less sampling effort, than a traditional plant community survey

Mark D. Johnson, Mohamed Fokar, Robert D. Cox & Matthew A. Barnes

BMC Ecology and Evolution 21, Article number: 218 (2021) | Cite this article



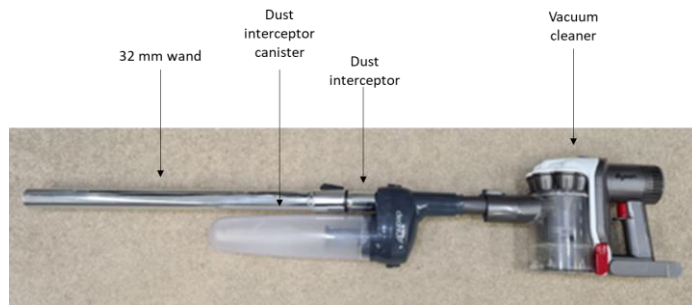
At border biosecurity and eDNA

The biosecurity sector in Australia is built around a need for rapid response

- Quick identification of pest species.
- Risk assessment.
- Actionable decision for biosecurity response.

Australian Department of Agriculture, Fisheries and Forestry funded eDNA application and research by EcoDNA for border biosecurity

eDNA and eRNA using dust samples from shipping container



Automated air sampling devices for remote surveillance inside shipping containers



Objective

Can we use airborne eDNA for border biosecurity?

Well, it's a question that is not that easy to answer. There are many more questions that need answering before we can answer this.

Can we detect insect diversity in an Approved Arrangement (AA) facility using airborne DNA?

Objectives:

- Develop an automated device capable of continuous airborne DNA collection.
- Uninterrupted sampling of airborne DNA inside an AA
- Downstream analyses for eDNA detection

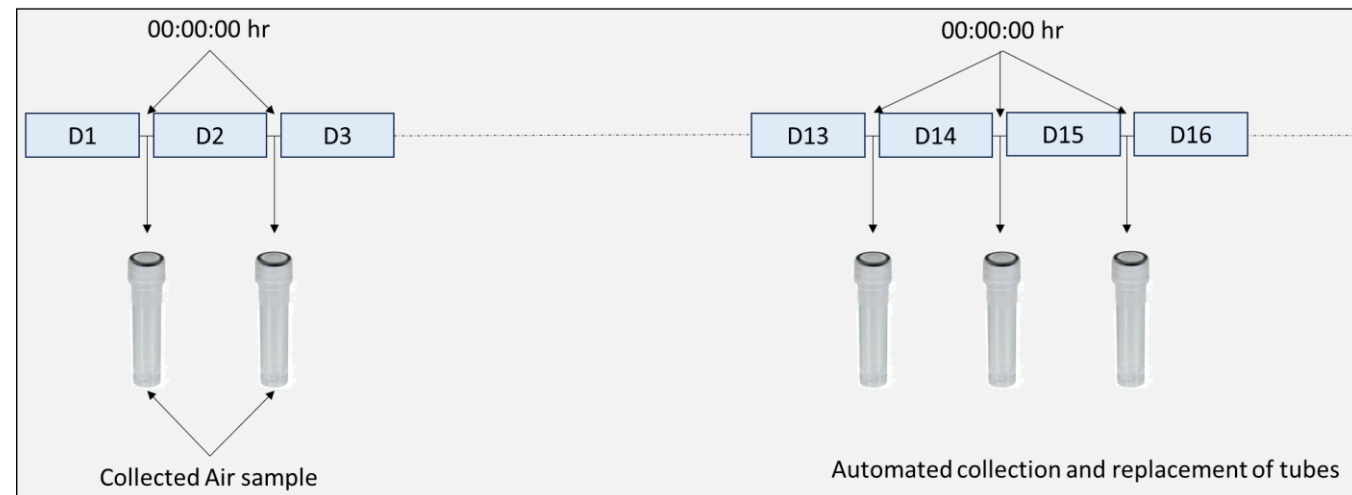
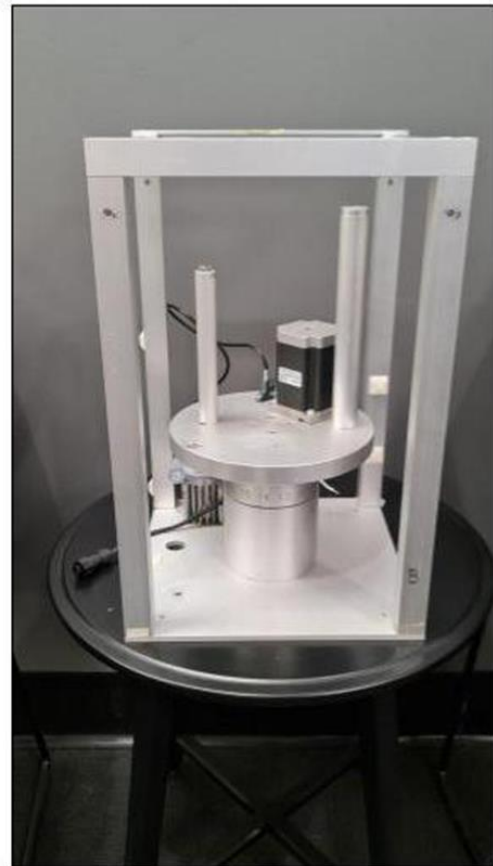


Automated air sampling sentinel units



Automated air sampling sentinel units designed by DATA EFFECTS

- Capture airborne particles
- Store in a 2 ml tube
- Automated change of tube every 24 hrs



14 Days

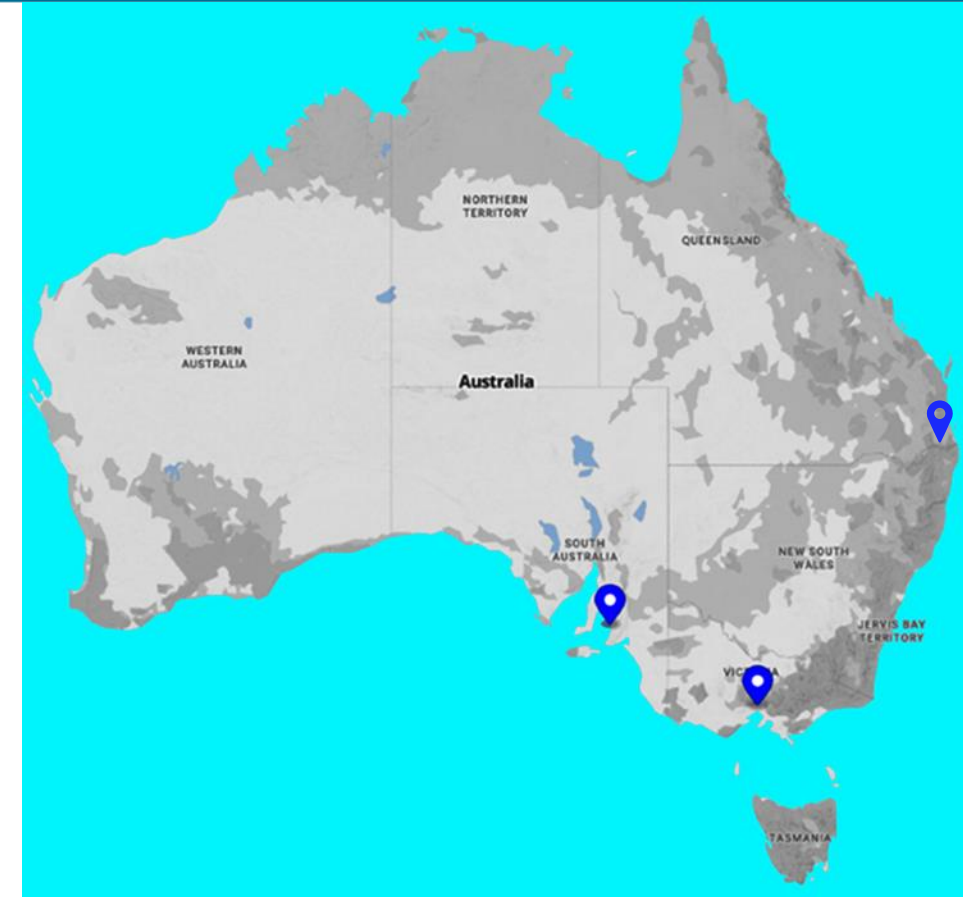
Visit by Data Effects
and DAFF personnel,
tube collection

Sampling regime and eDNA metabarcoding

A total of 462 samples were collected from three approved arrangement facilities in Australia.

Sampling sites	Duration	Number of samples*
Rapid Haulage in Adelaide	15/09/2023 – 30/04/2024	191
Mainfreight Perishables in Melbourne	15/09/2023 – 30/04/2024	226
Mainfreight Logistics Brisbane	15/03/2024 – 30/04/2024	45

* Number of samples used for high throughput sequencing



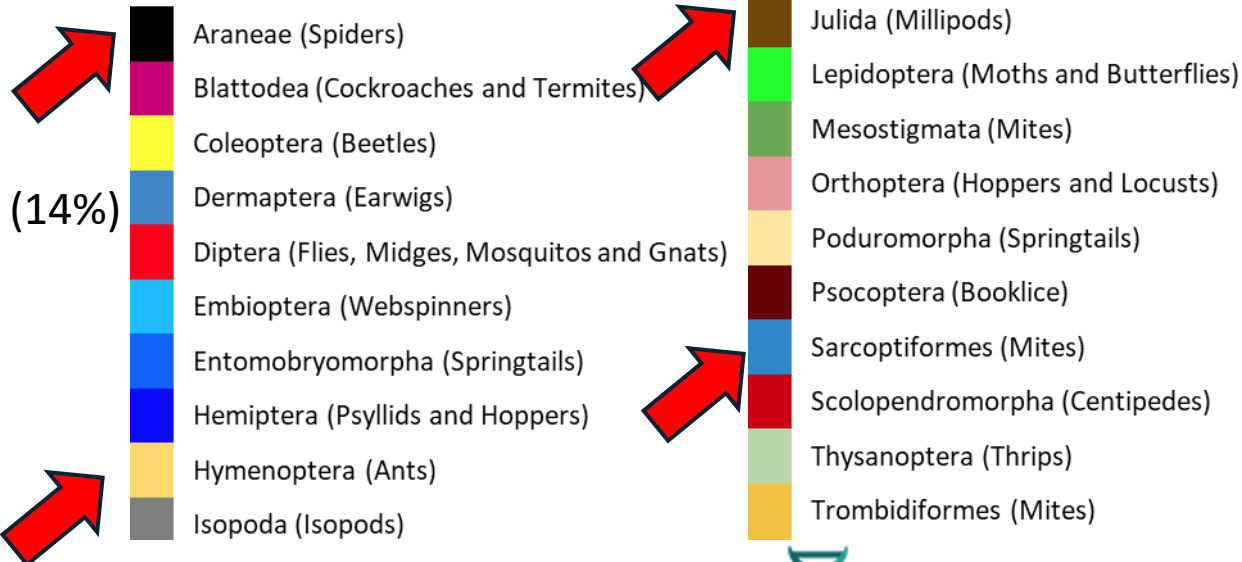
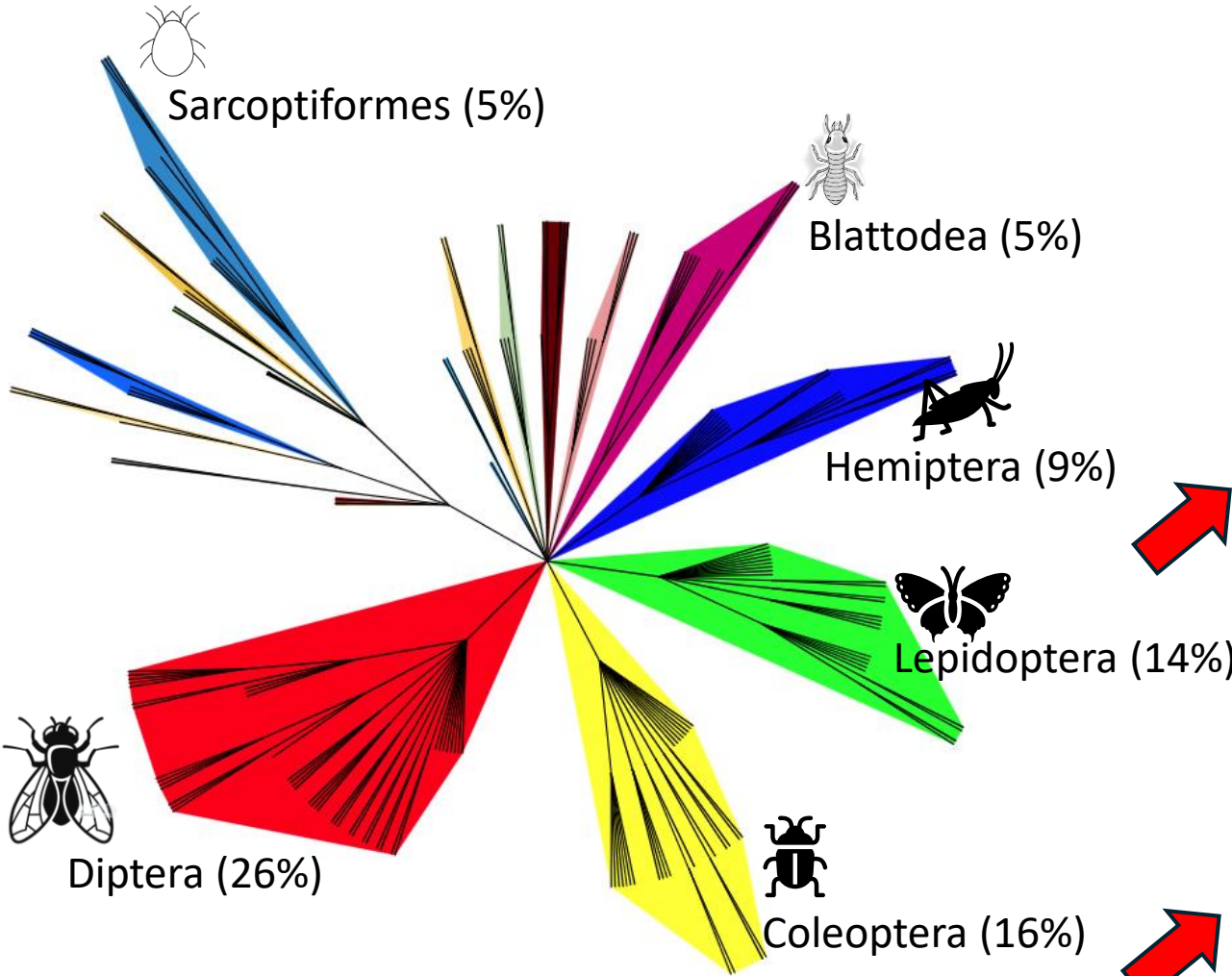
- Metabarcoding was performed following the SOP designed by the National eDNA Reference Centre (NRC)
- **Targeted gene:** COI (FwhF2a and Fdegen primer sets from Elbrecht et al. 2019 with slight modification)
- **Sequencing:** Illumina MiSeq V3 (2x300 Cycles)



Results (all three sites)

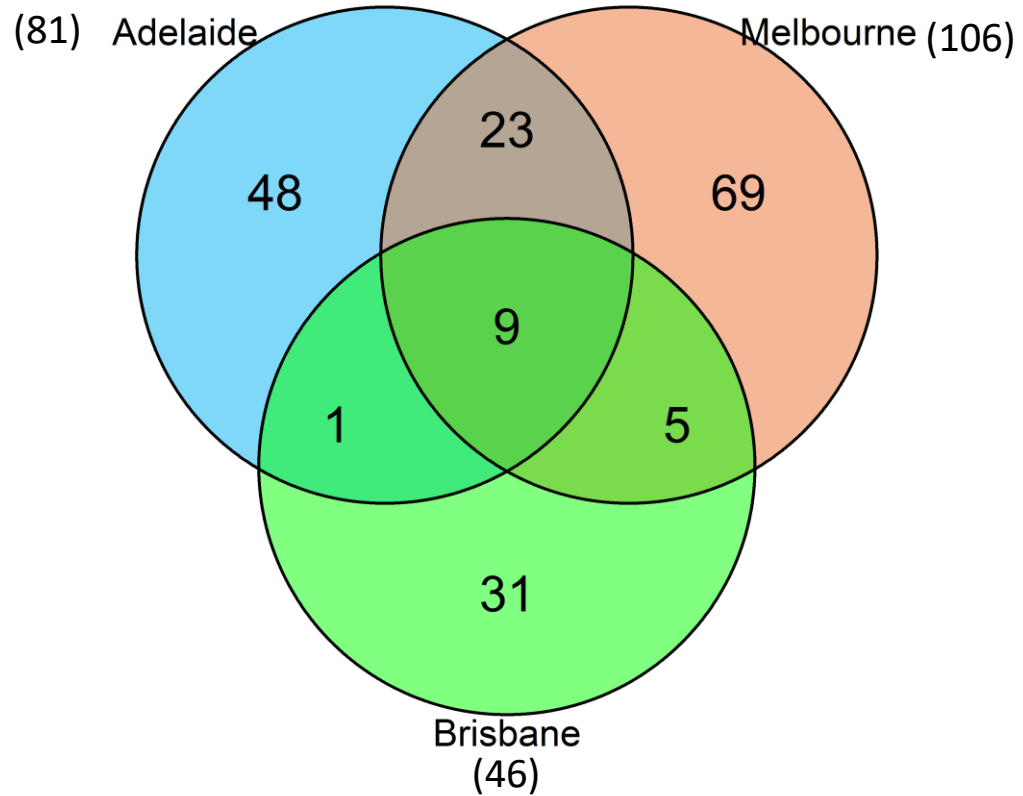
186 insect species belonging to 20 insect orders

Both flying and crawling insects



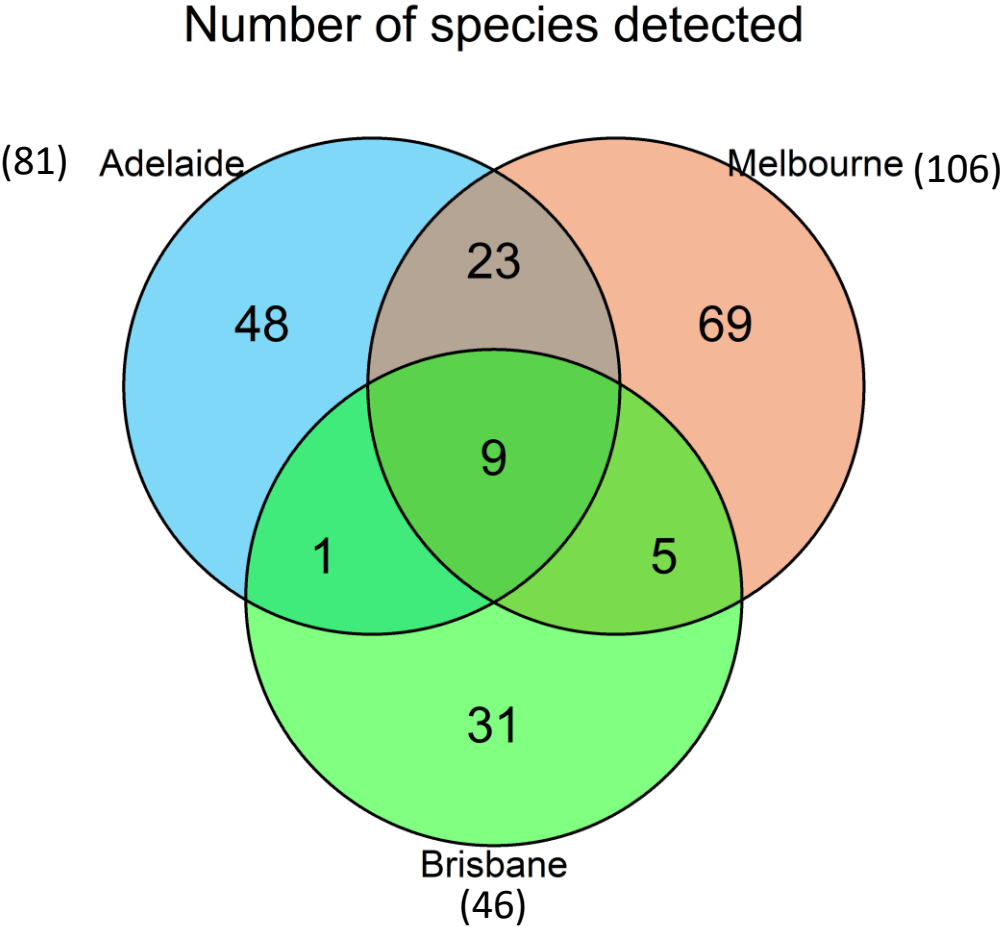
Results (all three sites)

Number of species detected

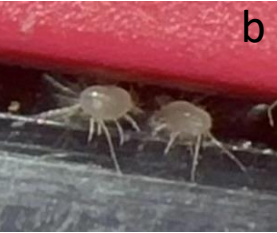


Results (all three sites)

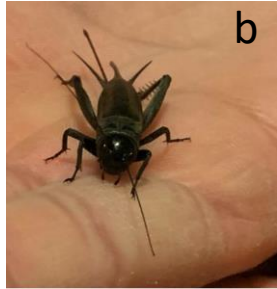
Frequently detected species:



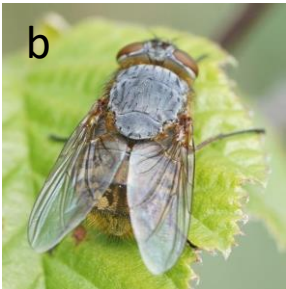
Aleuroglyphus ovatus
(brown-legged mite)



Tyrophagus putrescentiae
(a cosmopolitan mite species)



Teleogryllus commodus
(black field cricket)



Calliphora stygia
(brown blowfly)



Coptotermes acinaciformis
(subterranean termite)

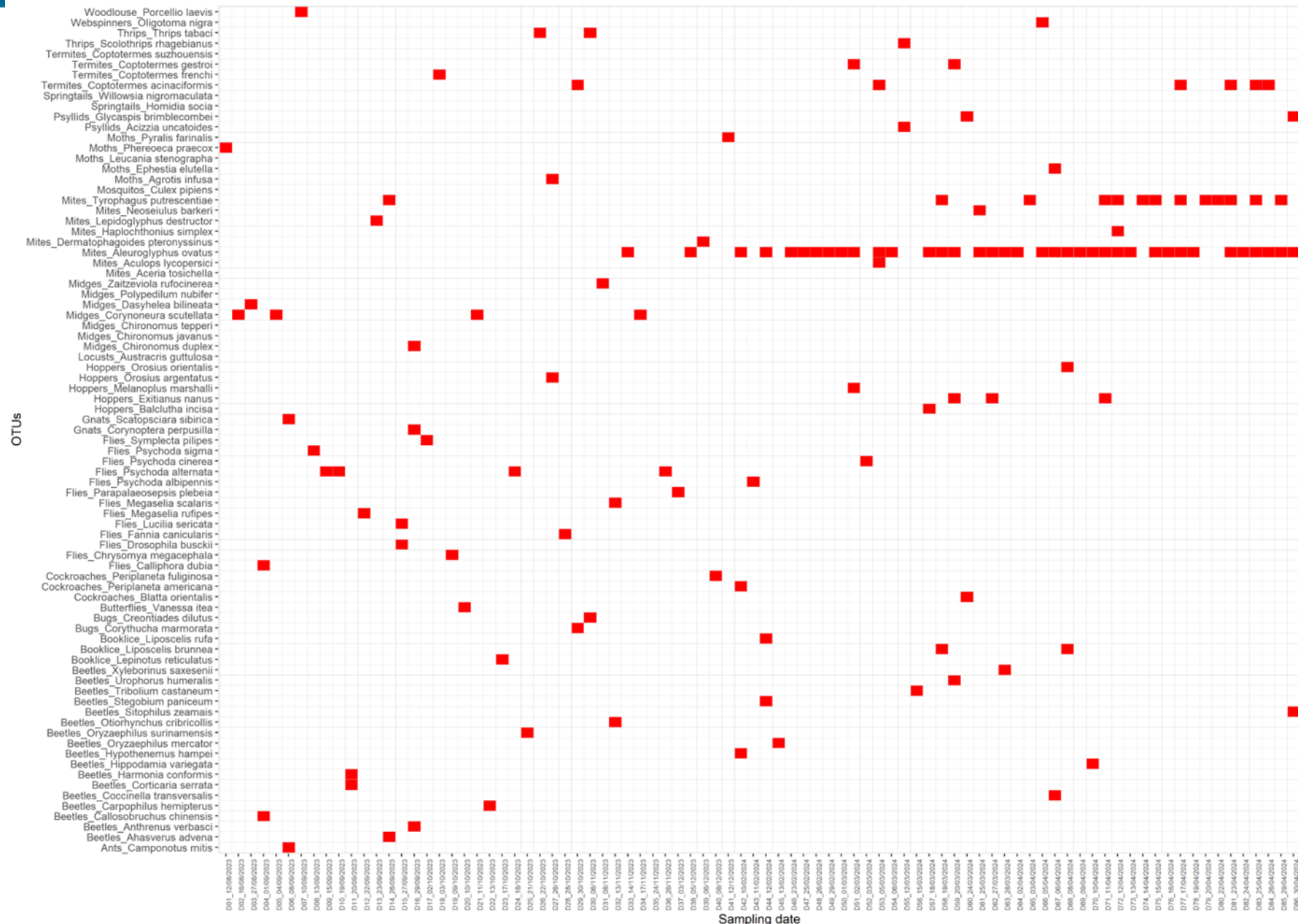
Image source:
a) Zou et al. 2023 (DOI: 10.1007/s10493-023-00861-9)
b) Atlas of Living Australia (<https://www.ala.org.au/>)

Results (Adelaide)

Detection No Yes

Adelaide:

Number of samples with no detection	105
Number of samples with at least one positive detection	86
Total	191



Results (Melbourne)

Detection No Yes

Melbourne:

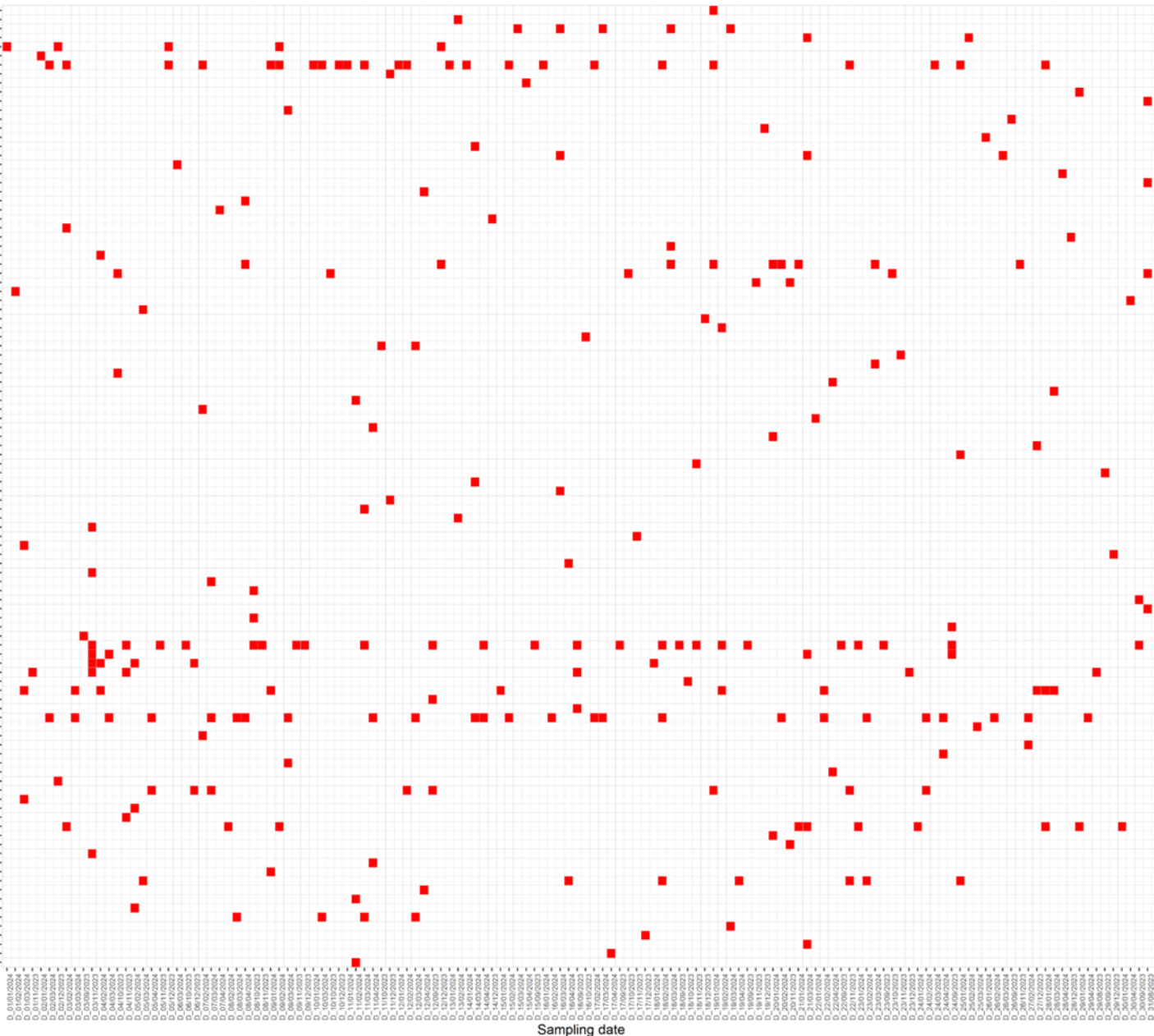
Number of samples with no detection	90
Number of samples with at least one positive detection	136
Total	226

Termites

- Webspinner_Oligotoma saundersii
- Thrips_Thrips trehernei
- Thrips_Thrips tenochrips inc
- Thrips_Frankliniella occidentalis
- Termites_Coptotermes lacteus
- Termites_Coptotermes gestroi
- Mites_Coptotermes acinaciformis
- Springtails_Hypogastrura viatica
- Springtails_Entomobrya atocincta
- Springtails_Entomobrya albocincta
- Springtails_Ceratophysella gibbosa
- Springtails_Ceratophysella derliculata
- Spiders_Steatoda grossa
- Psyllids_Arioecoribossa unicomuta
- Psyllids_Acuzia uncatoides
- Moths_Plochosia asaphes
- Moths_Persectania ewingii
- Moths_Pararguda tephropsis
- Moths_Mythimna corveta
- Moths_Merophyas divulsana
- Moths_Leucania uda
- Moths_Hyalocora eucalypti
- Moths_Hellula hydralis
- Moths_Helicoverpa punctigera
- Moths_Helicoverpa armigera
- Moths_Chrysodeixis argentifera
- Moths_Capua dura
- Moths_Ardzyga eumela
- Moths_Agrodis infusa
- Mites_Tyrophagus putrescentiae
- Mites_Tyrophagus longior
- Mites_Haplochthonius simplex
- Mites_Dermatophagoides pteronyssinus
- Mites_Dermatophagoides farinae
- Mites_Aleuroglyphus ovatus
- Mites_Aculops lycopersici
- Mites_Abacarus loli
- Mites_Abacarus hystrix
- Millipods_Choneilius palmatus
- Midges_Zaitzeviola rufocinerea
- Midges_Tanytarsus formosanus
- Midges_Riethia stictoptera
- Midges_Polypedilum nubifer
- Midges_Lepidocyrtus fimetarius
- Midges_Kiefferulus martini
- Midges_Kiefferulus intertinctus
- Midges_Culicoides victoriae
- Midges_Chironomus tepperi
- Midges_Chironomus oppositus
- Midges_Chironomus duplex
- Midges_Chironomus australis
- Isopods_Porcellio laevis
- Hoppers_Orosius argentatus
- Hoppers_Maestas knighti
- Hoppers_Exitianus nanus
- Hoppers_Balclutha incisa
- Hoppers_Acrida conica
- Gnats_Corynoptera perpallida
- Flies_Symplecta pilipes
- Flies_Sarcophaga lota
- Flies_Parapalaosepsis plebeia
- Flies_Megaselia scalaris
- Flies_Megaselia rufipes
- Flies_Lucilia sericata
- Flies_Hydrellia tritici
- Flies_Fahnia canicularis
- Flies_Drosophila simulans
- Flies_Drosophila immigrans
- Flies_Drosophila busckii
- Flies_Chrysomya rufifacies
- Flies_Calliphora stygia
- Flies_Calliphora dubia
- Flies_Calliphora augur
- Flies_Calliphora albifrontalis
- Flies_Abstrosimulium furiosum
- Flies_Australsepsis niveipennis
- Flies_Anax papuensis
- Earwigs_Nala lividipes
- Crickets_Teleogryllus commodus
- Cockroaches_Platyzosteria alternans
- Centipedes_Cryptops hortensis
- Butterflies_Pieris rapae
- Bugs_Xylocoris palacianus
- Bugs_Nysius plebeius
- Bugs_Armadillidium vulgare
- Booklice_Liposcelis rufa
- Booklice_Liposcelis brunnea
- Booklice_Dorypteryx longipennis
- Beetles_Tribolium castaneum
- Beetles_Rhantus suturalis
- Beetles_Phoracantha semipunctata
- Beetles_Paropsisterna varicollis
- Beetles_Orzyaophilus mercator
- Beetles_Necrobia rufipes
- Beetles_Naupactus leucoloma
- Beetles_Eistrotius bonariensis
- Beetles_Hippodamia variegata
- Beetles_Gabrieus nigrifolus
- Beetles_Eurhopalus vespaluae
- Beetles_Eurhopalus australis
- Beetles_Coptocercus rubripes
- Beetles_Anthrenus verbasci
- Beetles_Apoclyonathus olivieri
- Aphids_Therioaphis trifolii
- Ants_Hypononea opacior
- Ants_Camponotus claripes

Flies

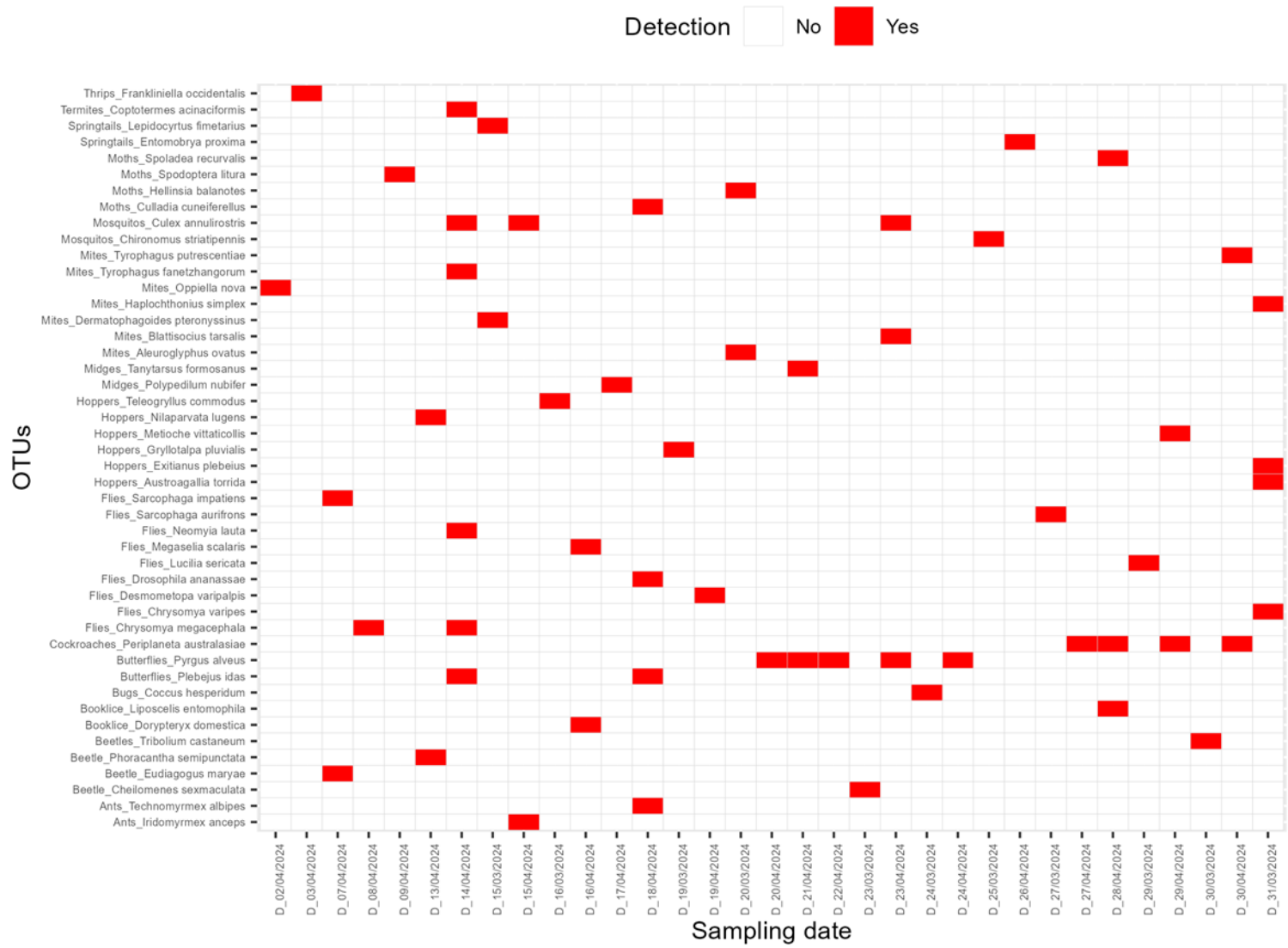
Beetles



Results (Brisbane)

Brisbane:

Number of samples with no detection	12
Number of samples with at least one positive detection	33
Total	45



Conclusion and Future Direction

What we have learned:

- ✓ Automated air sampling sentinel units can capture airborne eDNA at the approved arrangement facilities.
- ✓ Airborne eDNA is efficient in detecting both flying and crawling insects.

Can this help the government in actionable decision-making

Conclusion and Future Direction

What we have learned:

- ✓ Automated air sampling sentinel units can capture airborne eDNA at the approved arrangement facilities.
- ✓ Airborne eDNA is efficient in detecting both flying and crawling insects.

Can this help the government in actionable decision-making

- ✓ It is unclear if some of the non-frequent detections were from live/active insects within the facility or historical traces from external sources.
- ✓ To overcome this, we are currently performing environmental RNA (eRNA) based experiments.

Acknowledgement

Thavamanikumar Saro (DAFF)

Andrew Baker (Data Effects)

Paul Coldrey (Data Effects)

The EcoDNA technical support team: Jenn,
Kym, Henriette and Brennan



Funding:



Australian Government
**Department of Agriculture,
Fisheries and Forestry**

