20th February 2025

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Honey as a bioindicator: eDNA surveillance of pathogens in Australian Honey bees

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VICTORIAN APIARISTS' ASSOCIATION INC

AGRICULTURAL BIO-SOLUTIONS LAB

From Hive to Lab: Decoding Honey as a Bioindicator

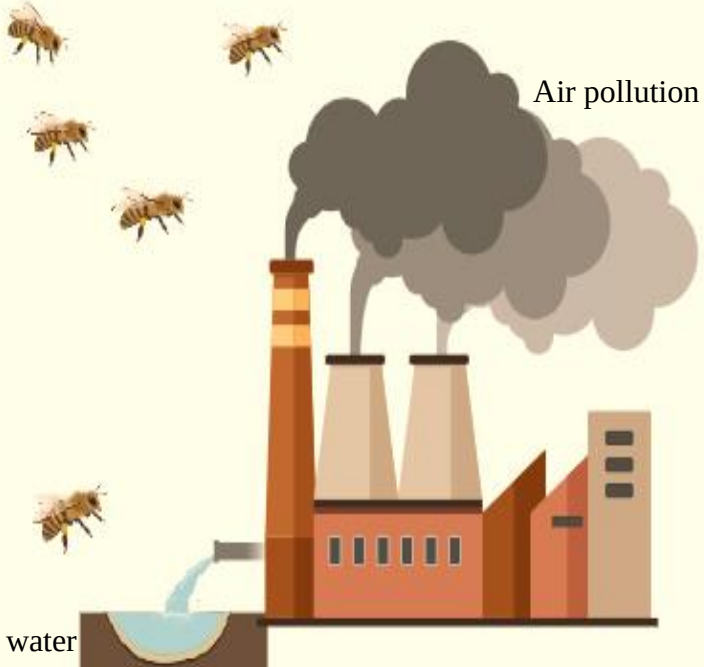
- Honey bees (*Apis mellifera*) - Important Livestock species.
- Indispensable for global agriculture, pollinating 70% of crops.
- Act as a complete Biosensor (Bioindicator and Bioaccumulator)
- From hive to jar carries countless information on
  - Biogeographic Origin
  - Microbial traces
  - Botanical origin

Environmental DNA: Tracing genetic footprints

Gather genetic materials derived from environmental samples

Agrochemical Pesticide

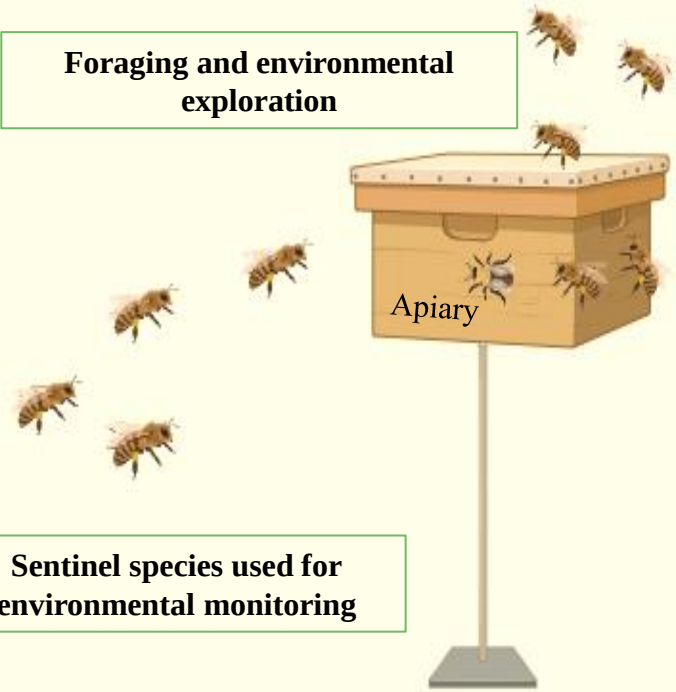
Plant pathogen



Air pollution

Dirty water

Foraging and environmental exploration



Apiary

Sentinel species used for environmental monitoring

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- Extensive environmental exploration.
- Foraging distance >3 km from hive.
- Hive products- Reservoir of eDNA .
- eDNA-genetic material from the environment.
- DNA reveals honeybee pathosphere information.

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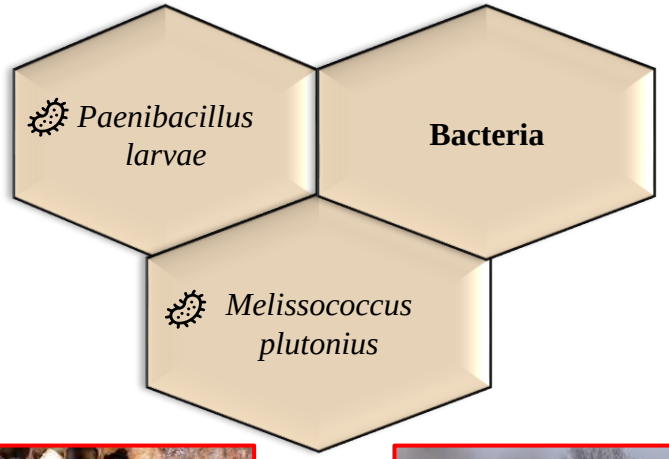
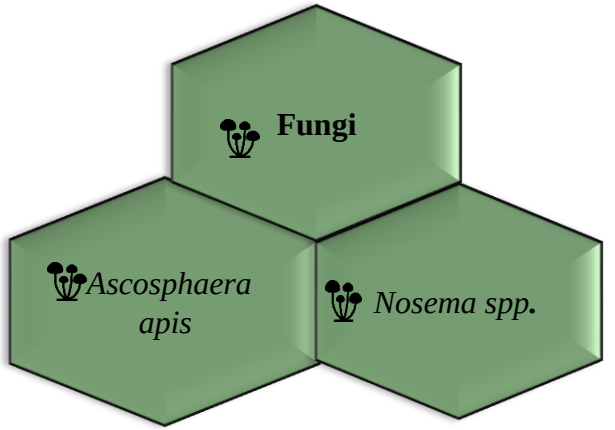
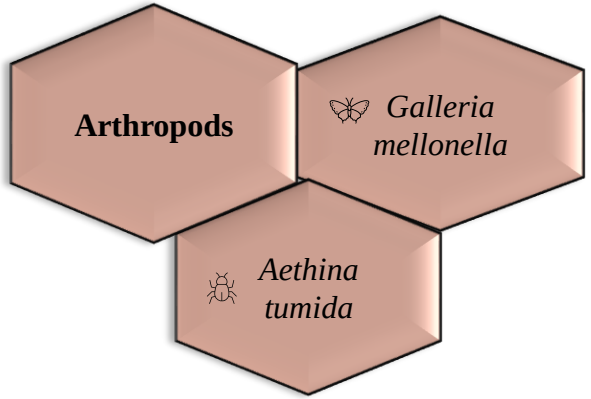
Honey Bee Pathogens: Insights into Colony Health



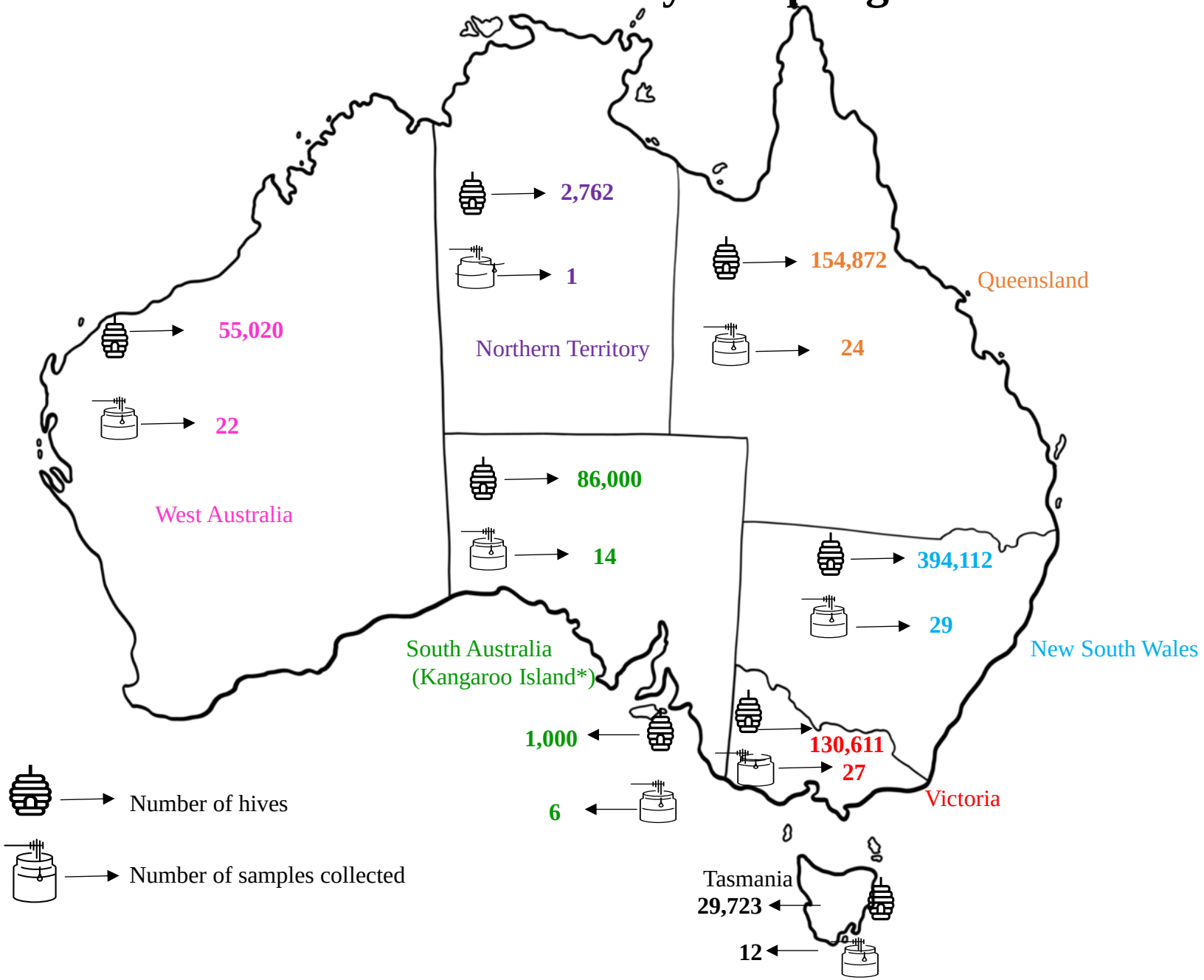


AFB infection

Burning infected hive



Honey Sampling Across Australia's Hives



The Australian Honey bee industry

- 855,300 hives.
- Annual honey production ~ 30,000 tonnes
- Commercial beekeeping is migratory
- Pollination services- $4-6 billion annually

Data source: Honey Bee & Pollination, AgriFutures Australia Annual Report 2022-2023

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Challenges in DNA extraction

Challenging substances

Components of Honey

- Carbohydrates-Glucose, Fructose
- Phenolic compounds
- Plant Material-Xylan, Pectin
- Proteins & Amino acids
- Vitamins, Minerals & Organic acids

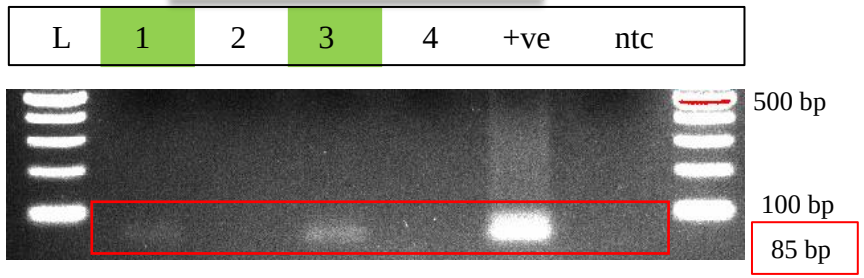
Challenges

- Inhibitory effect Plant material Pectin, Xylan
- Complex Matrix
- Viscosity
- Low DNA Concentration

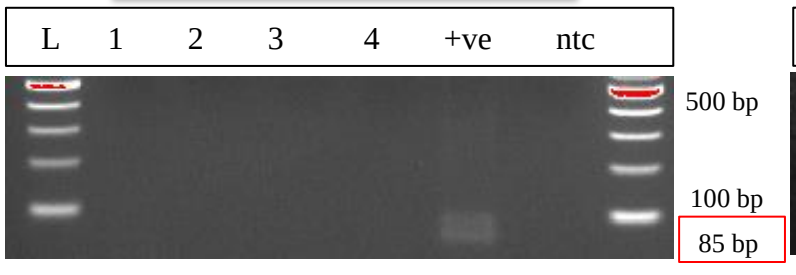
Honey: Diverse DNA Extraction Methods

Contemporary extraction methods - *Apis mellifera* MtDNA COI-COII Polymerase chain reaction

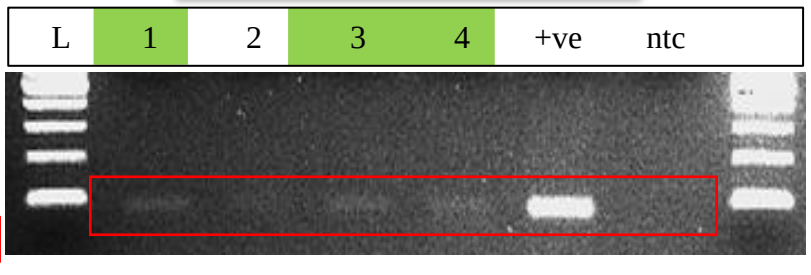
1. CTAB* Extraction



2. CTAB + SDS' Extraction



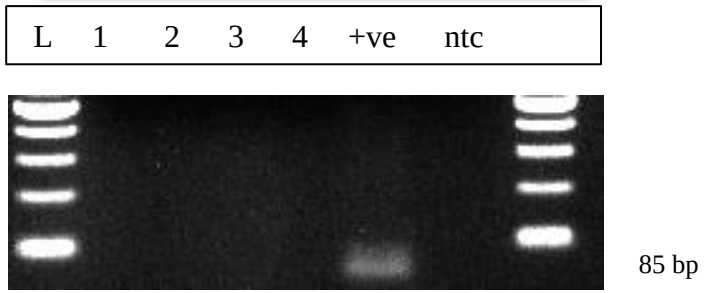
3. CTAB + LN^ Extraction



Commercial extraction kits - *Apis mellifera* MtDNA COI-COII Polymerase chain reaction

1. Comm

3. Commercial Kit 3

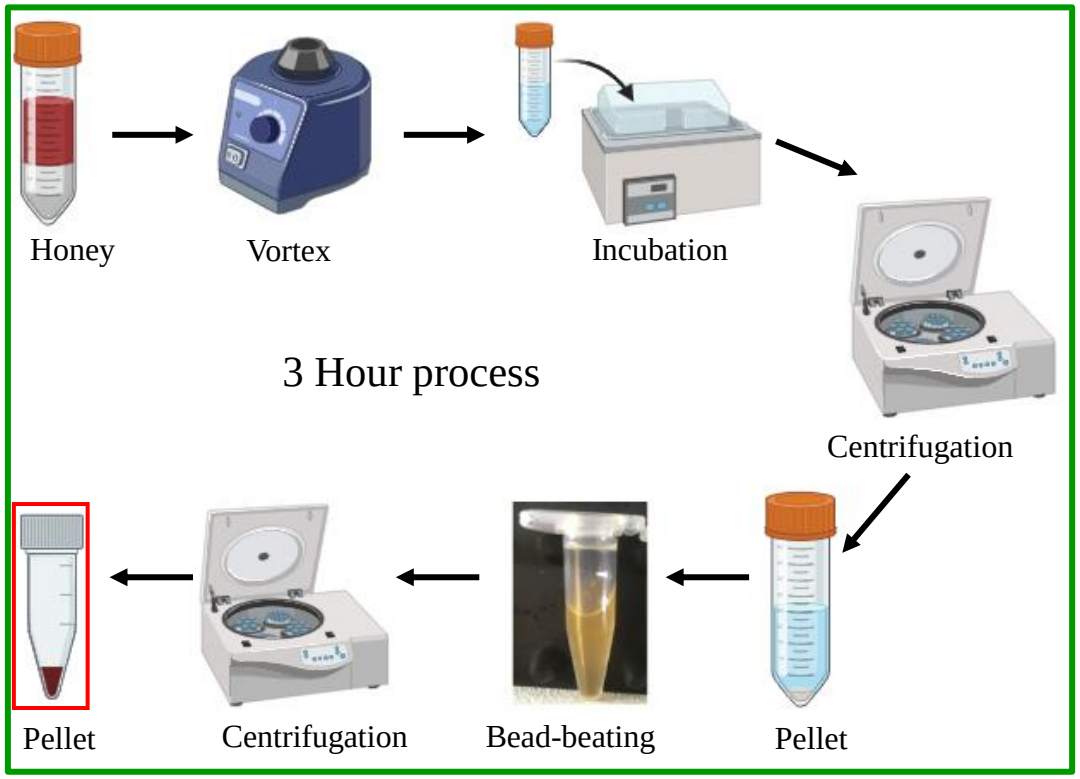


Abbreviations

* CTAB - Cetyltrimethylammonium bromide
^ LN - Liquid Nitrogen
' SDS - sodium dodecyl sulphate
ntc - No template control
+ve - Positive Control (Genomic DNA)

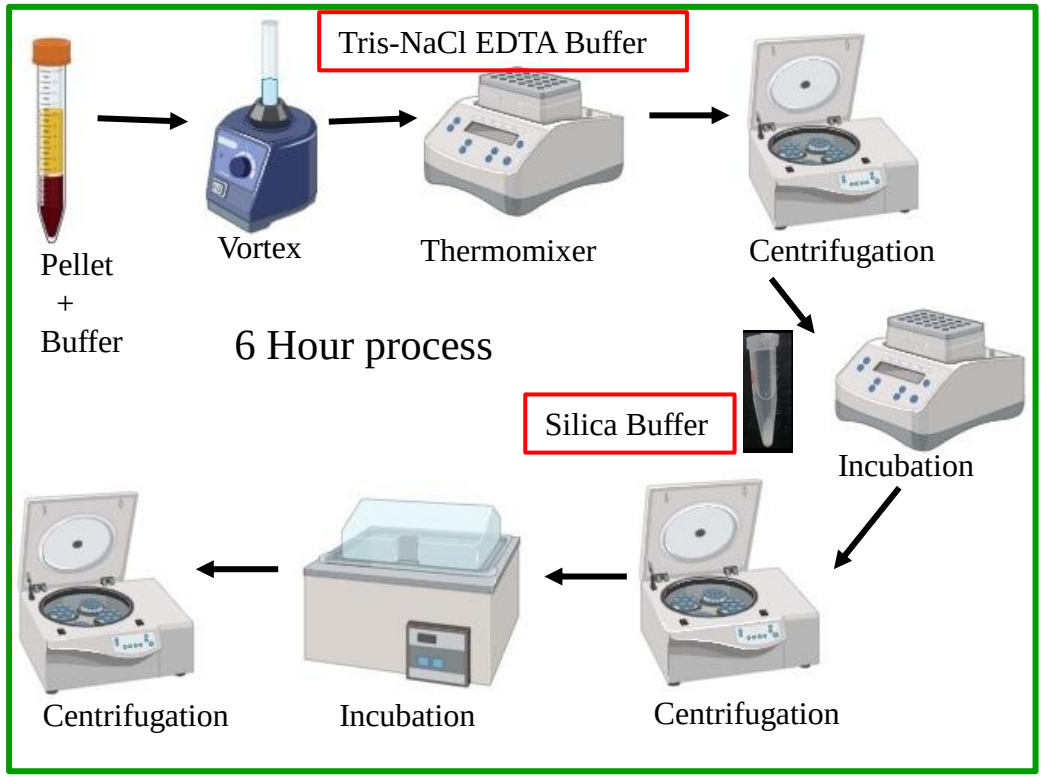
Optimised in-house eDNA Extraction protocol

PRE-TREATMENT



Pre-treatment pellets serve as starting material for post-treatment

POST-TREATMENT



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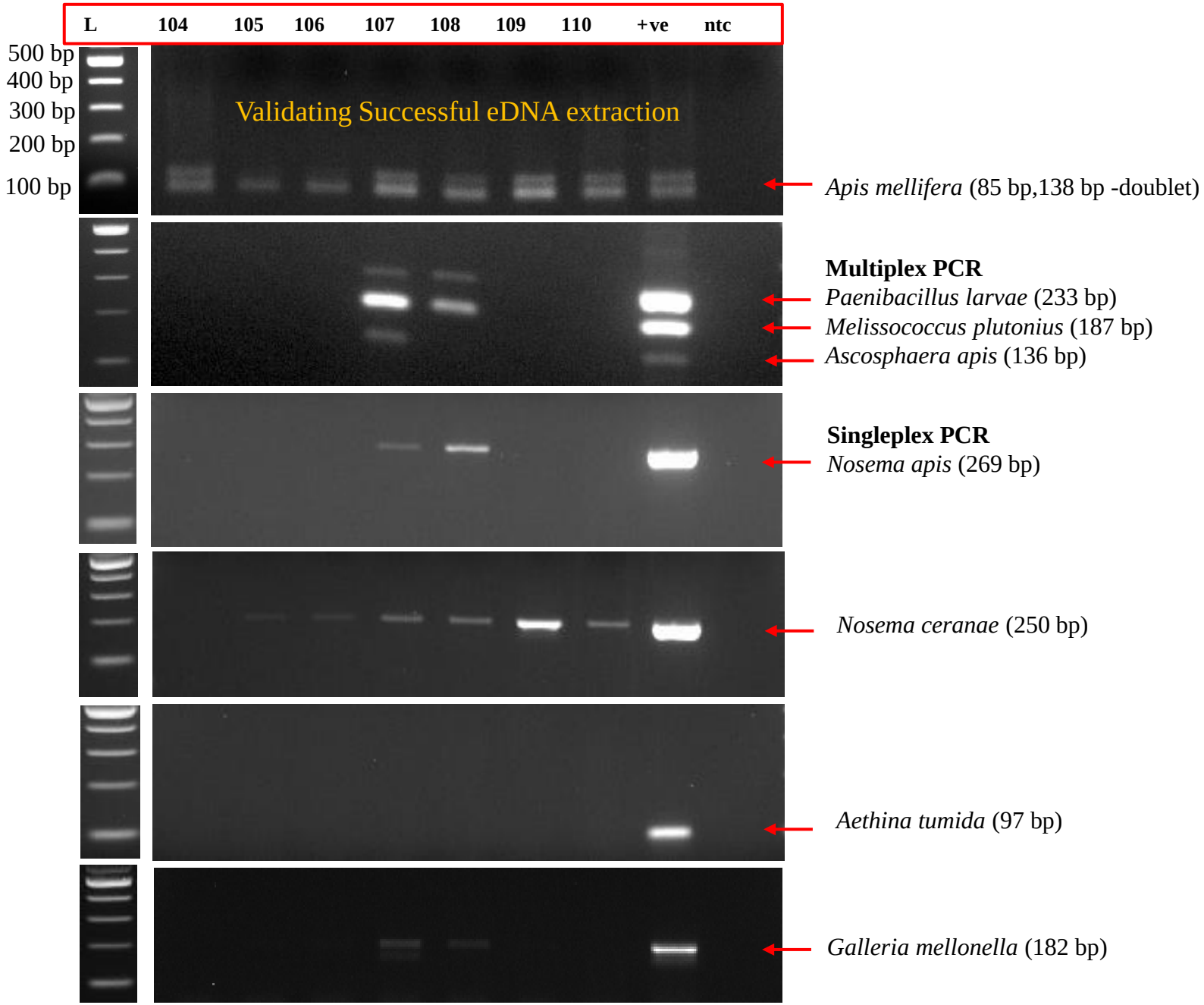
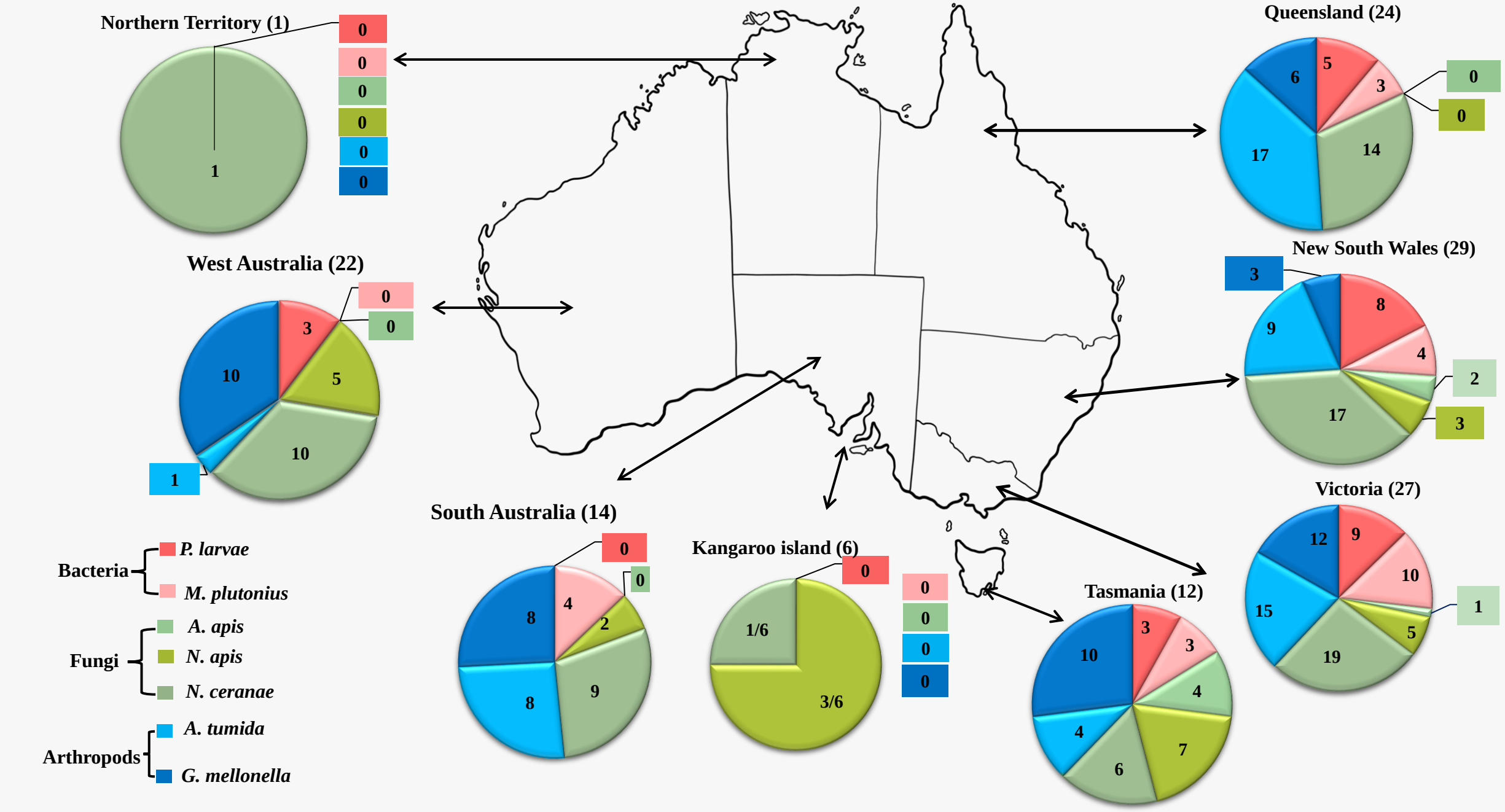
PCR analysis and gel electrophoresis of known pathogens of Honey bees

- Gold standard method for pathogens detection.
- Precise identification of pathogens.
- High sensitivity and specificity.
- Multiplex PCR-Multiple target in single reaction.
- PCR as a routine surveillance tool.



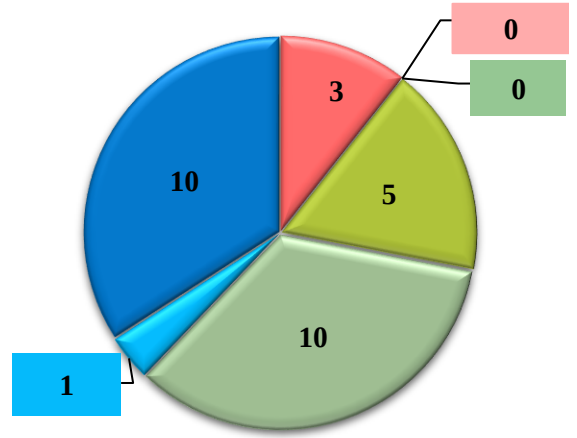
Prevalence of bacterial, fungal, and arthropod pests in Australian honey



Regional Variation in Pathogen Prevalence

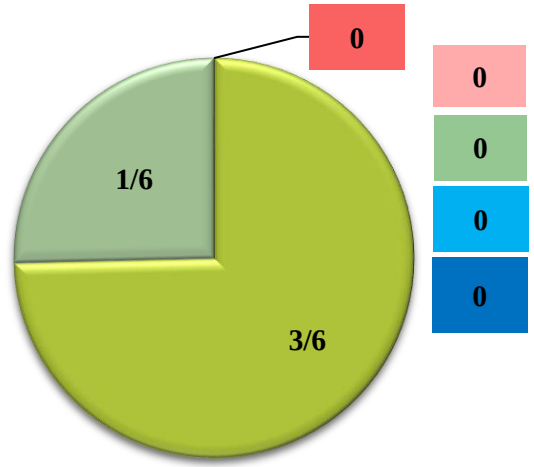
- European foulbrood (EFB) first isolated in the 1970s in Australia.
- Widely distributed in all states and territories except West Australia (WA).

West Australia (22)



- Last pure strain of Ligurian bee (*Apis mellifera ligustica).*
- Low incidence of honey bee diseases.
- *Nosema apis* and *Nosema ceranae* detected from honey samples.

Kangaroo island (6)



Bacteria: *P. larvae*, *M. plutonius*

Fungi: *A. apis*, *N. apis*, *N. ceranae*

Arthropods: *A. tumida*, *G. mellonella*

Future of Honey eDNA in Pathogen Detection and Bee Health

Victoria

Eastern Australia

Queensland

- Non-invasive surveillance tool.

Practical application

- Monitor a single pathogen or an entire community.

Monitor endemic pests and pathogens regularly

- Early detection of emerging pathogens.

Tracking the regional distribution and spread

- Management Strategies and sustainable beekeeping practices.

Viability of tested pathogen-Direct culturing from honey

High frequency of all pathogens

Risk assessment for colony health to guide treatment strategies

Pollination services
Migratory beekeeping

Almonds, watermelons, avocado, macadamia

ACKNOWLEDGEMNET

AGRICULTURAL BIO-SOLUTIONS LAB

Professor Travis Beddoe

- Dr. Gemma Zerna
- Dr. Bhuvana Shanbhag
- Dr. Tim Cameron
- Dr. Alexandra Knox
- Hoda Salah Mohamed Abbas
- Dr. Meysam Khodaparast Afarmajani
- Danielle Ackerly
- Olivia Ducommun-Dit-Verron
- Luke Mckay
- Danielle Wiles
- Chloe Sherbourne
- Chloe Van Der Linden
- Lilly Tran

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Thank you

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