Bacterial diversity and virus discovery in the invasive yellow crazy ant

Yellow crazy ant worker (left) and queen (right)

Meghan Cooling[1], Monica Gruber[1], Ben Hoffmann[2] and Phil Lester[1]
[1]School of Biological Sciences, Victoria University, New Zealand
[2]CSIRO Sustainable Ecosystems

Population Collapses of Invasive Species

- Uncommon, rarely documented or studied
- Giant African land snail disease syndrome
- Pathogens often invoked but rarely investigated

Biological Invasions **6:** 161–172, 2004.

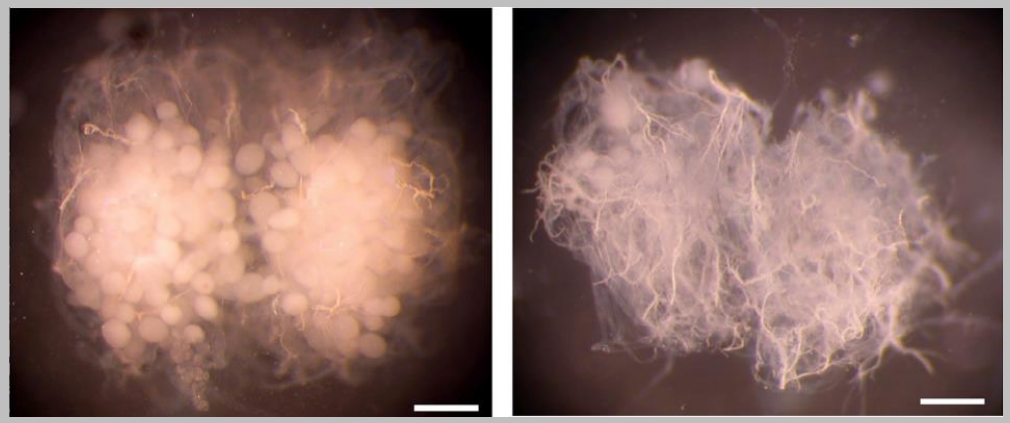
Now you see them, now you don't! – population crashes of established introduced species

Daniel Simberloff* & Leah Gibbons
*Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN 37996, USA; *Author for correspondence (e-mail: dsimberloff@utk.edu; fax:+1-865*

Giant African land snail

Pathogens in Ants

- Red imported fire ant (*Solenopsis invicta*)
  - Surveys in native range
  - Fungi, microsporidia, bacteria and viruses



Left: normal queen ovary with eggs Right: ovary from SINV-3 treated colony with very few eggs present Scale bar is 500um Figure from Valles *et al.* (2013)

Red imported fire ants

ELSEVIER

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

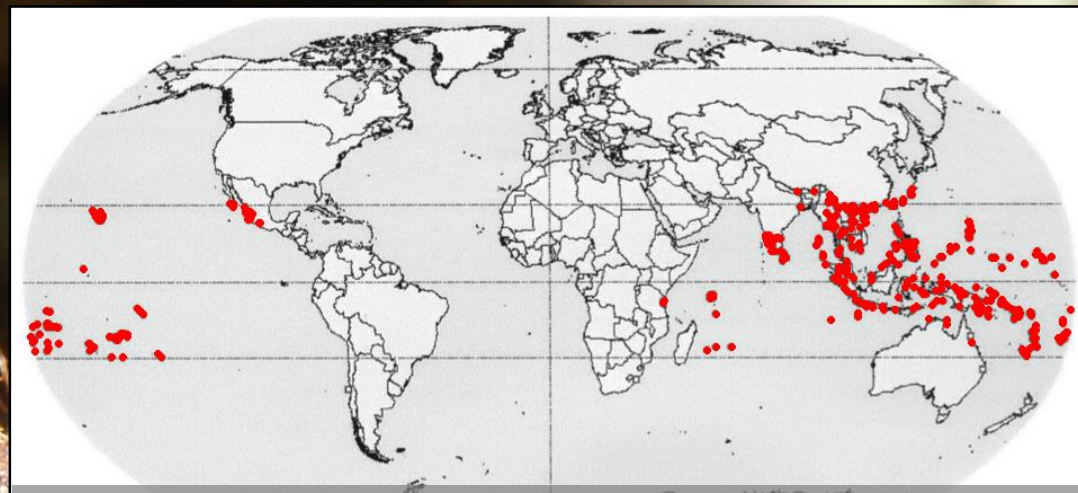
Successful transmission of Solenopsis invicta virus 3 to *Solenopsis invicta* fire ant colonies in oil, sugar, and cricket bait formulations

Steven M. Valles*, Sanford D. Porter, Man-Yeon Choi, David H. Oi

Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, 1600 SW 23rd Drive, Gainesville, FL 32608, USA

Yellow Crazy Ant (*Anoplolepis gracilipes*)

- Notorious invader worldwide
- Multiqueened colonies
- Can reach extraordinarily high densities
  - i.e. Christmas Island- invasional meltdown
- Boom and bust cycles
  - Seychelles (disappearance)
  - Tokelau (declines)
  - Arnhem Land (variable)



Worldwide distribution of the yellow crazy ant. Wetterer 2005

Background photo by Monica Gruber

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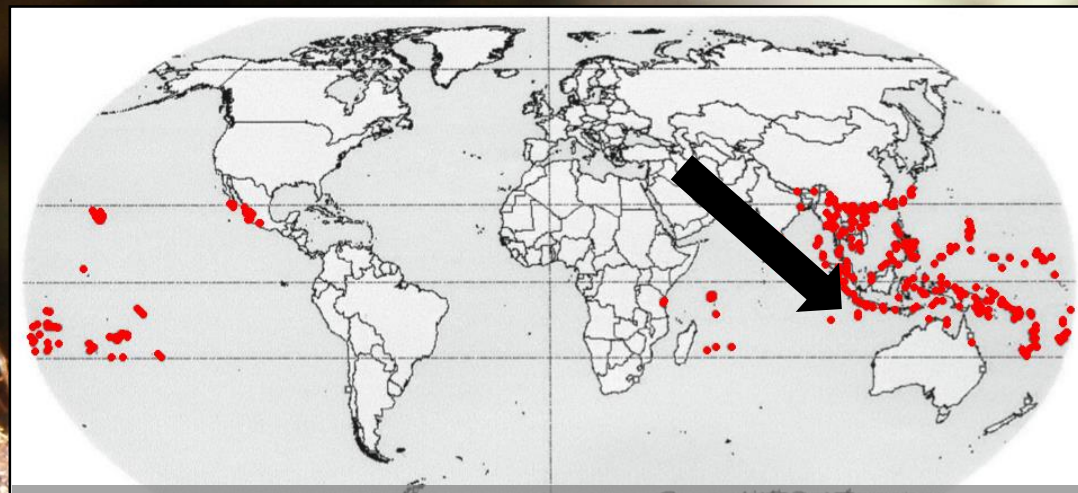

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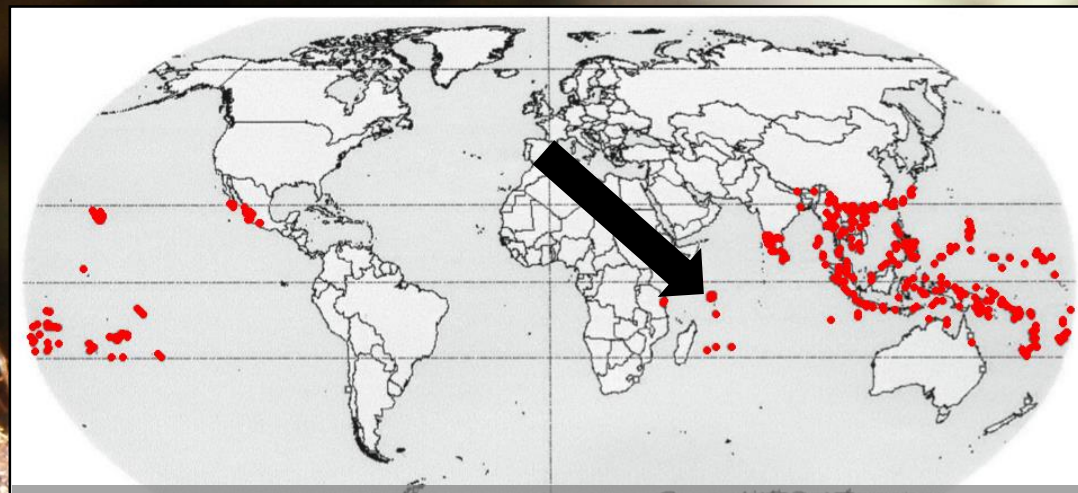

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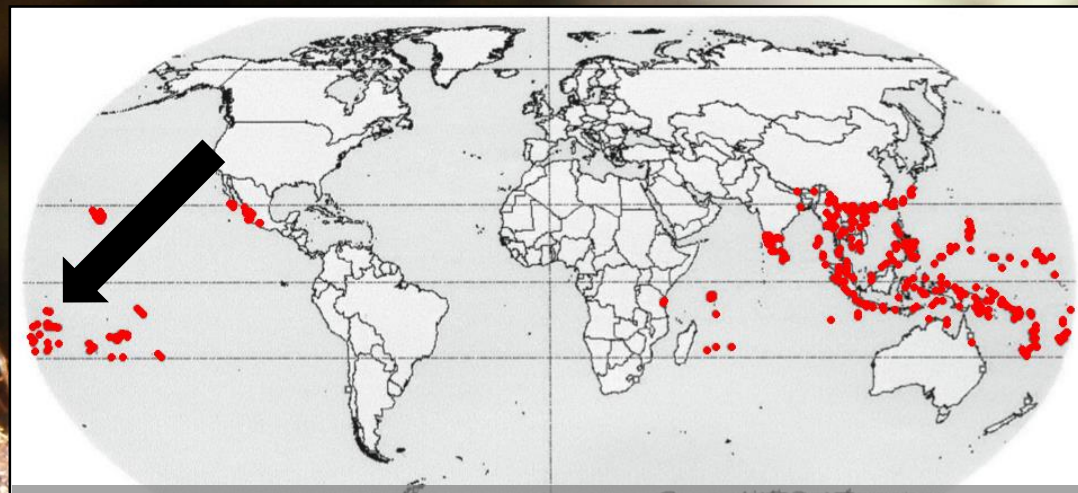

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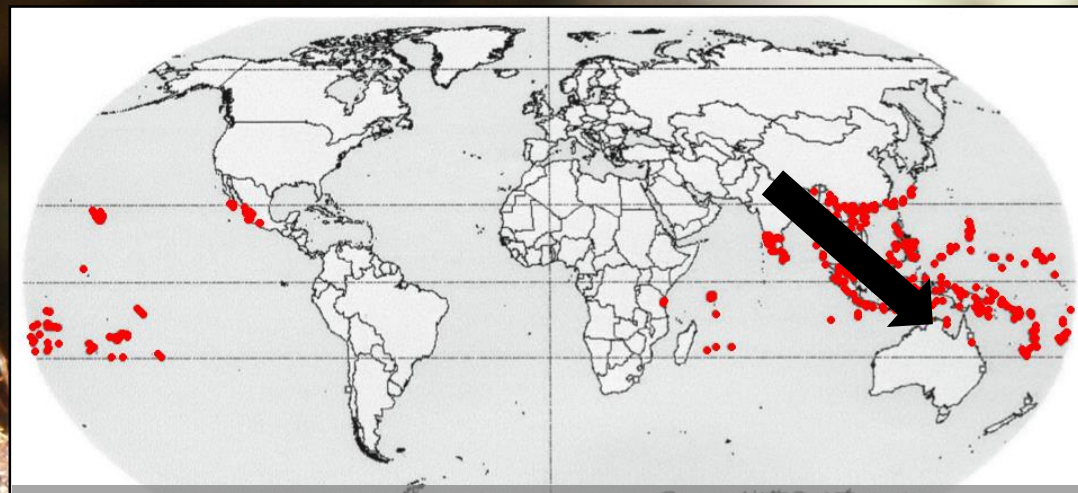

Worldwide distribution of the yellow crazy ant. Wetterer 2005

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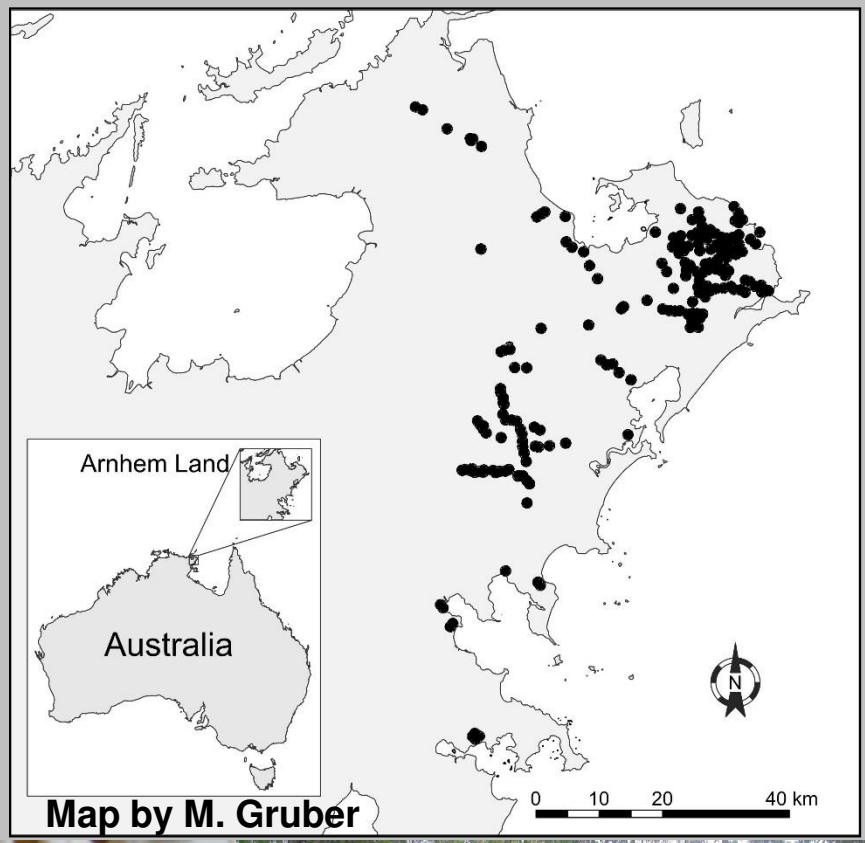
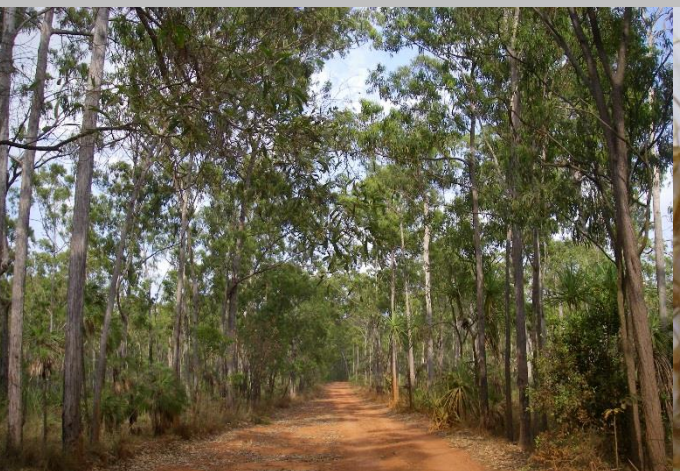
Yellow crazy ants in Arnhem Land

- First recorded in 1984
- Discrete populations
- Formerly successful, large populations have been observed to shrink and/or disappear





Aims

- Identify pathogens and mutualists infecting yellow crazy ants
- Determine if expanding and declining populations differ in their viral and bacterial communities

Viruses and bacteria in yellow crazy ants

- Next generation sequencing to look for viruses and bacteria

- Viruses common in Hymenoptera (ants, bees, wasps, sawflies)
- Can be very host specific and highly pathogenic

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Research

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Community ecology

Invasive ants carry novel viruses in their new range and form reservoirs for a honeybee pathogen

Alexandra Sébastien[1,†], Philip J. Lester[1,†], Richard J. Hall[2], Jing Wang[2], Nicole E. Moore[2] and Monica A. M. Gruber[1]

[1]School of Biological Sciences, Victoria University of Wellington, PO Box 600, Wellington, New Zealand
[2]Institute of Environmental Science and Research, 66 Ward Street, Upper Hutt, New Zealand

Bacteria in yellow crazy ants

- Microbial community of an insect can have both positive and negative effects on host health, reproduction and longevity

- Mutualistic bacteria:
    - Provide essential nutrients
    - Mediate host thermal tolerance
    - Enhance pathogen/parasitoid resistance

- Gruber (2012) found positive correlation between yellow crazy ant abundance and bacterial diversity

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Ecology and Evolution

Open Access

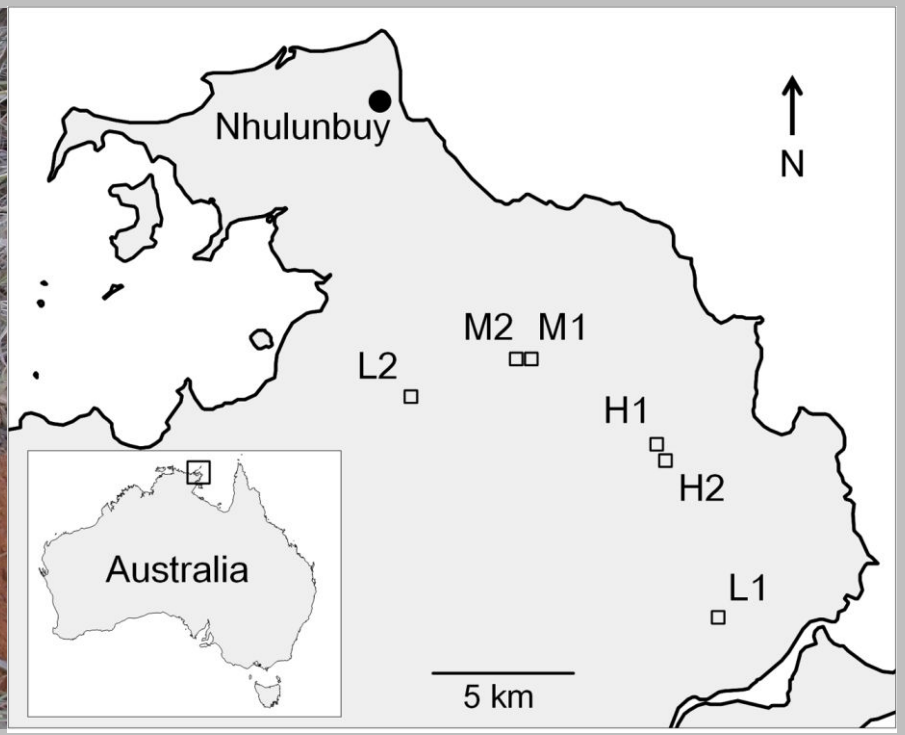
Genetic diversity is positively associated with fine-scale momentary abundance of an invasive ant

Monica A. M. Gruber[1], Benjamin D. Hoffmann[2], Peter A. Ritchie[1] & Philip J. Lester[1]

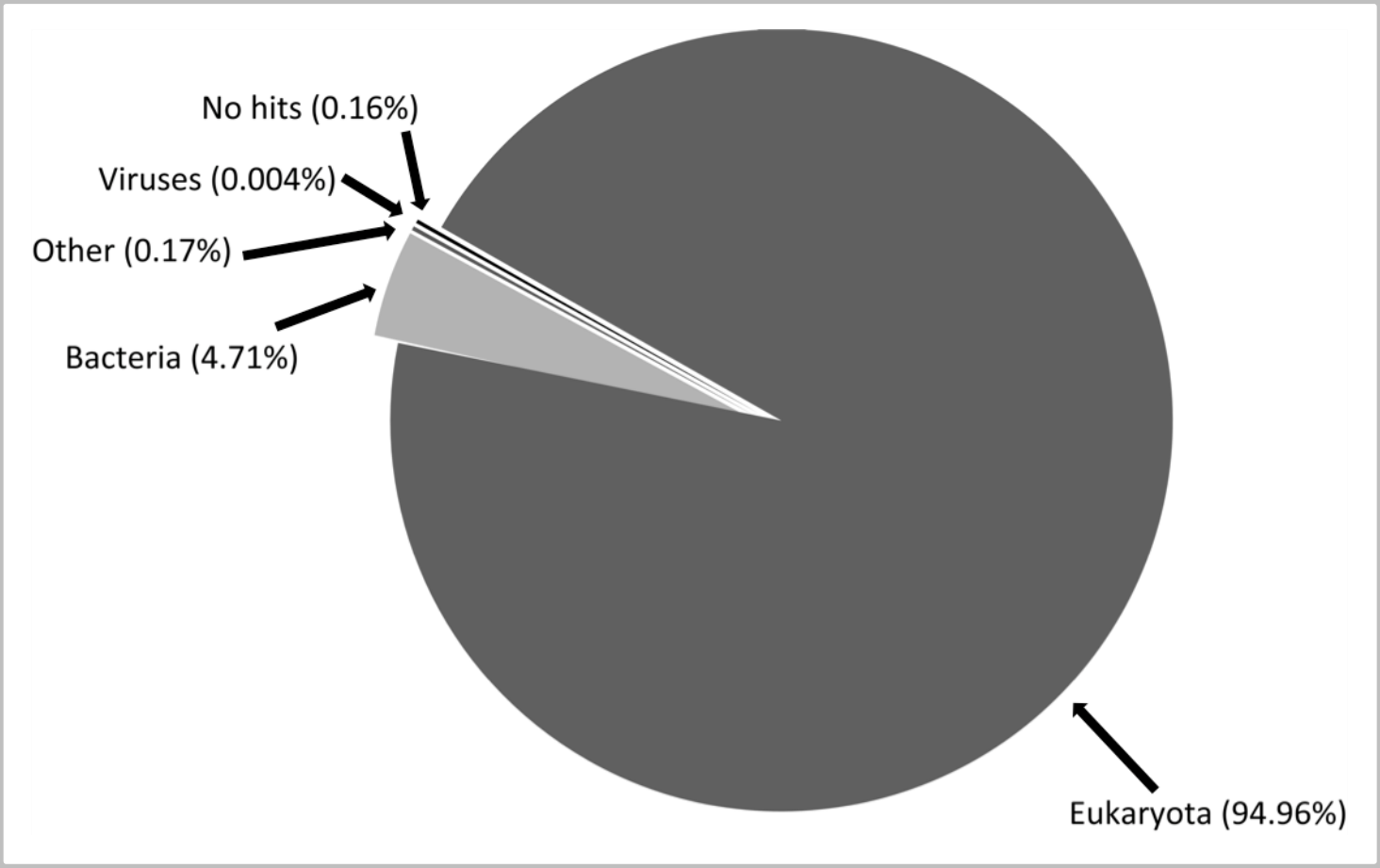
[1]School of Biological Sciences, Victoria University of Wellington, PO Box 600, Wellington, 6140, New Zealand
[2]CSIRO Ecosystem Sciences, Tropical Ecosystems Research Centre, PMB 44, Winnellie, Northern Territory, 0822, Australia

Methods

- Collected queens from 6 sites (2 high density/expanding, 2 medium density/declining, 2 low density/declined)
- Illumina sequencing done on 5 queens from each site
  - RNA, sequenced whole genome (HiSeq 2 x 100bp PE)
  - DNA, amplified with 16S universal bacterial primers (MiSeq 2 x 300bp PE)



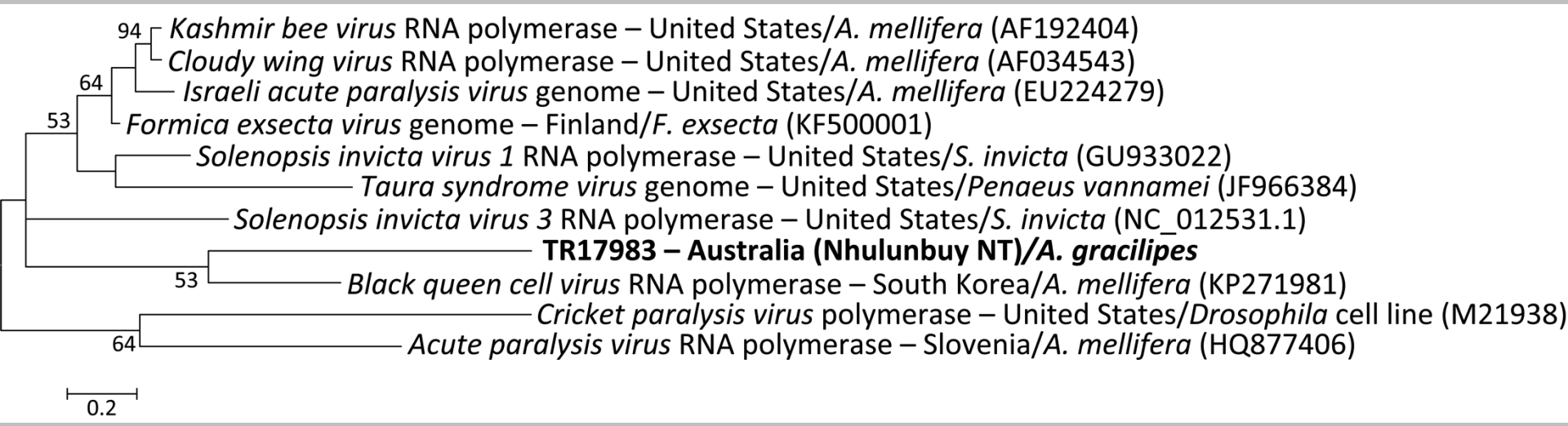
RNA results



- 20 hits to viral sequences found in low and medium-density sites
- BLASTx search against non-redundant nucleotide database in GenBank on viral sequences

Viruses in yellow crazy ants

- Three types of viral sequences, RNA polymerase gene (99% identity)
- Tentatively grouped with the Dicistroviridae
- Next step was PCR assays with viral primers



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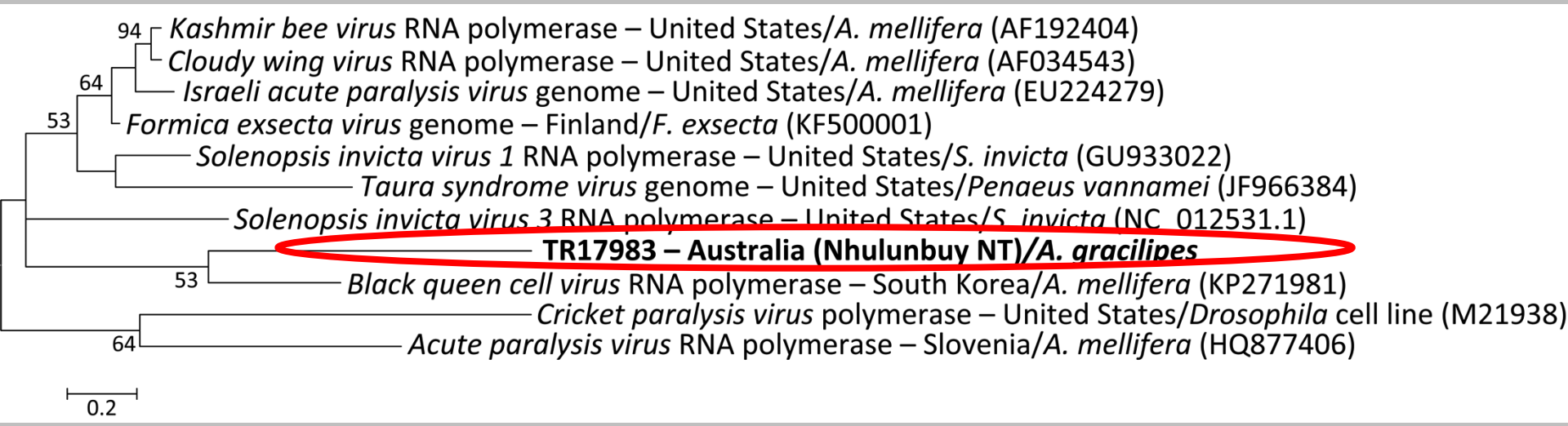


Viruses in yellow crazy ants

- Designed primers for unidentified viral sequence
- Surveyed for common honey bee viruses

- RT-PCR of 2013/2015 queens and 2013 workers for:
  - Unidentified viral sequence
  - KBV, BQCV, ABPV, IAPV, DWV, LHUV-1

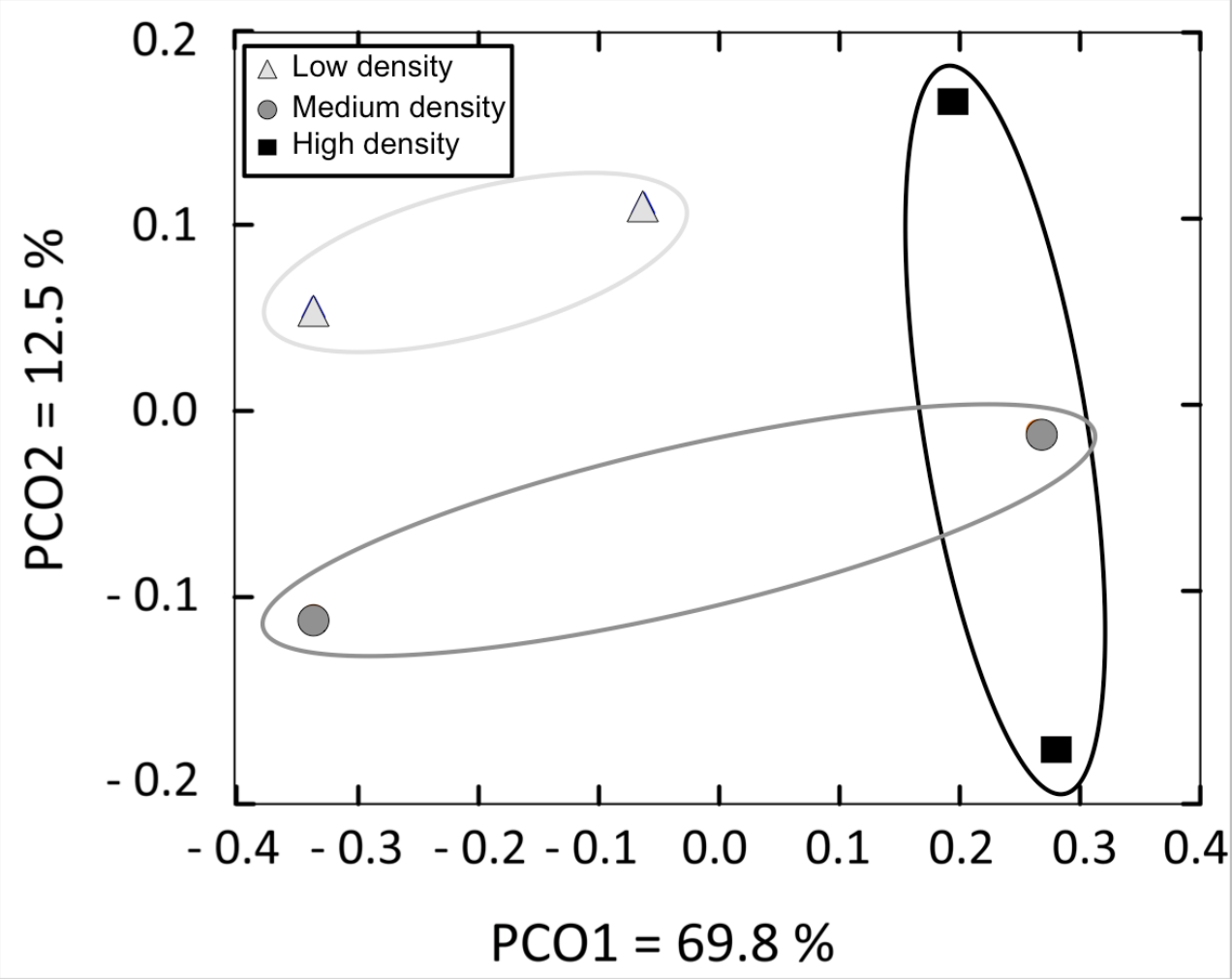
Found: All samples contained potential novel virus and black queen cell virus

- Viruses can be ingested

- Next step is to investigate replication

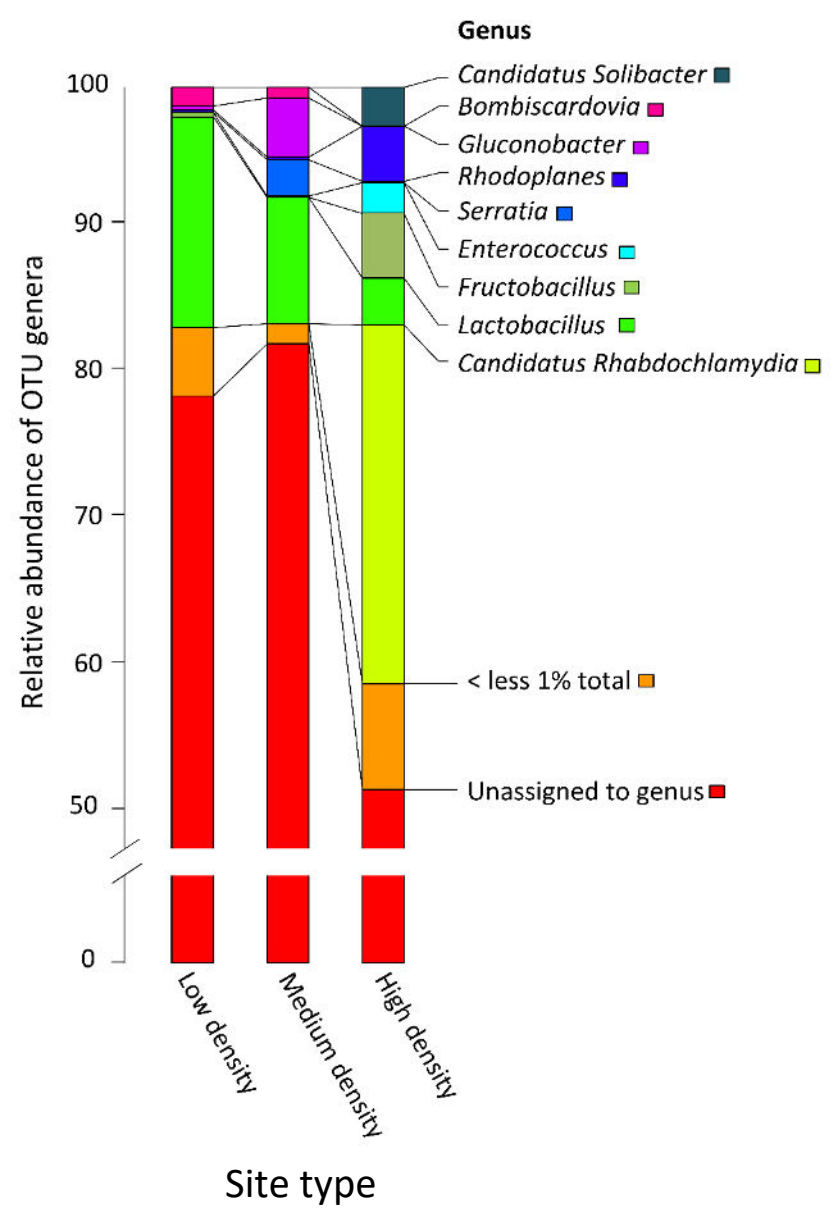
Honey bee pupa infected with black queen cell virus

Bacterial community

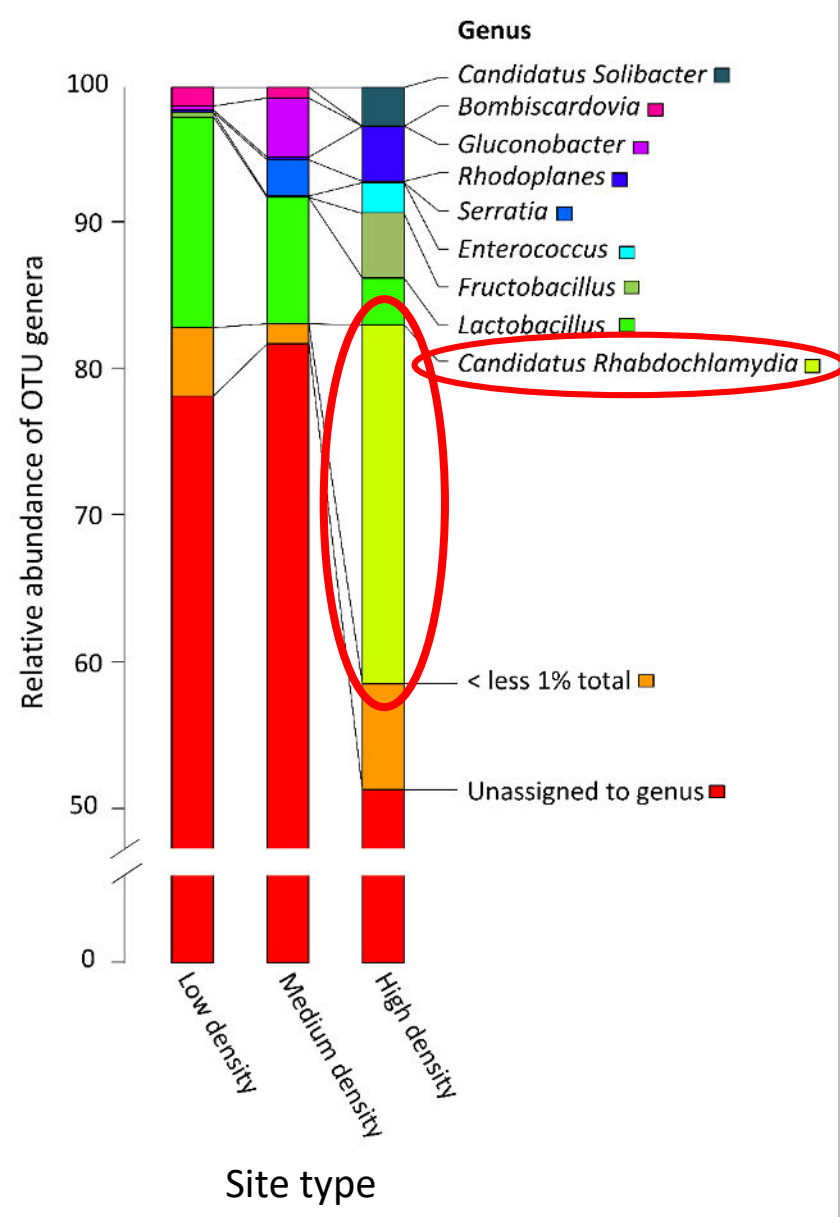


- No significant differences in bacterial community structure at order level between site types
- ANOSIM (R= -0.056, *p*= 0.533)

Bacterial community



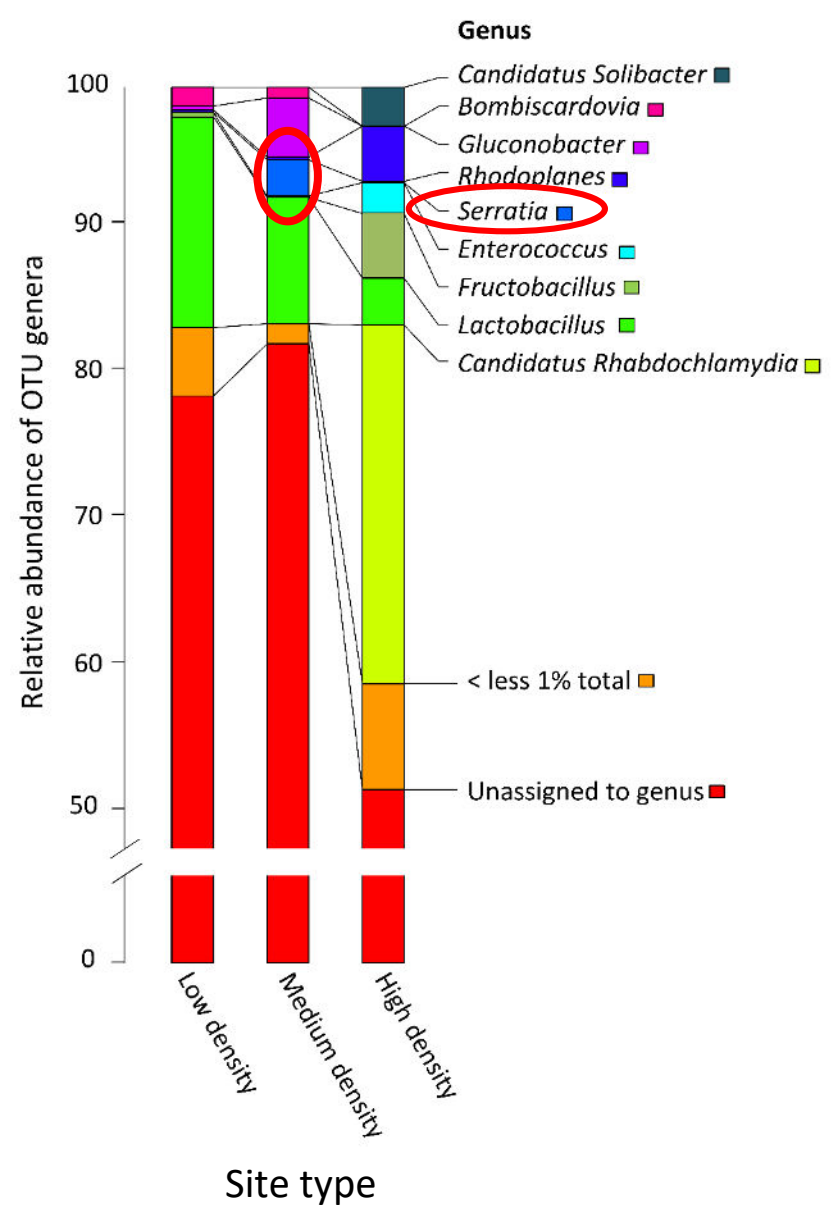
Bacterial community



Pathogenic bacteria

- *Rhabdochlamydia*

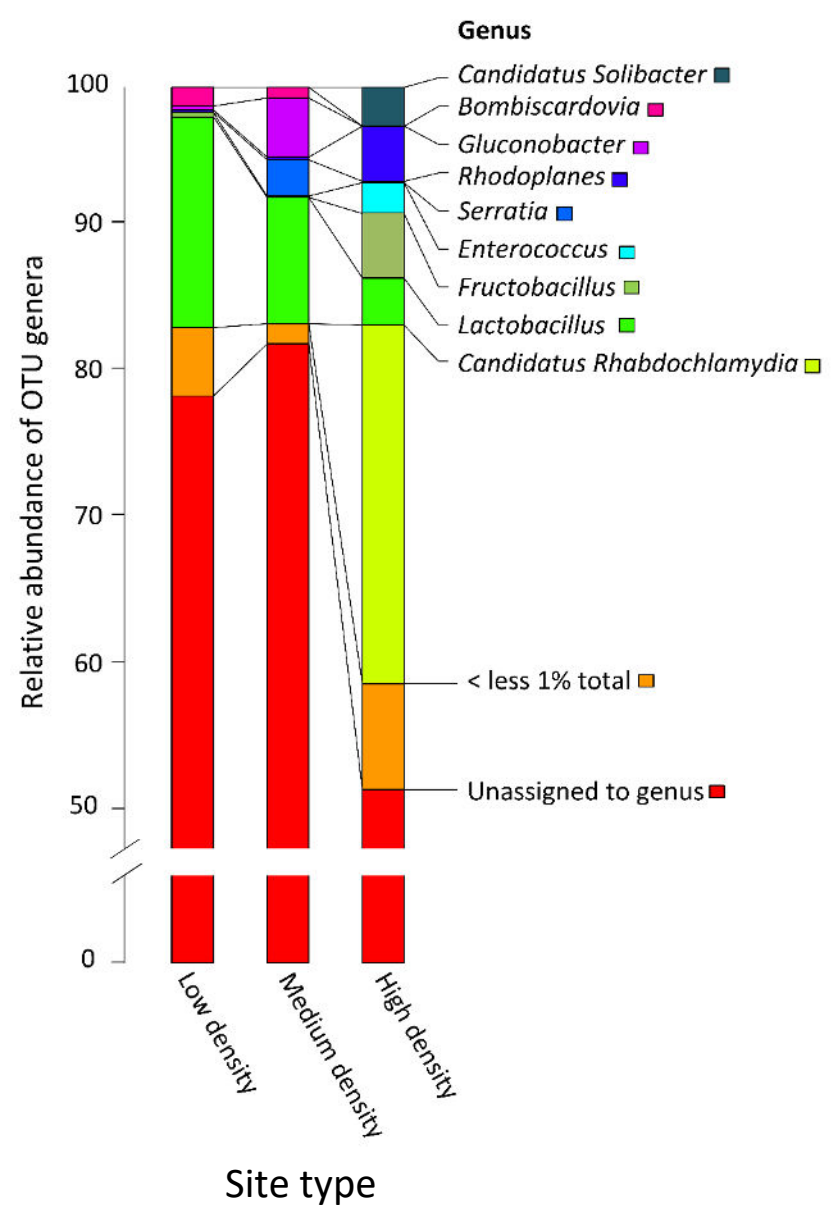
Bacterial community



Pathogenic bacteria

- *Rhabdochlamydia*
- *Serratia marcescens*

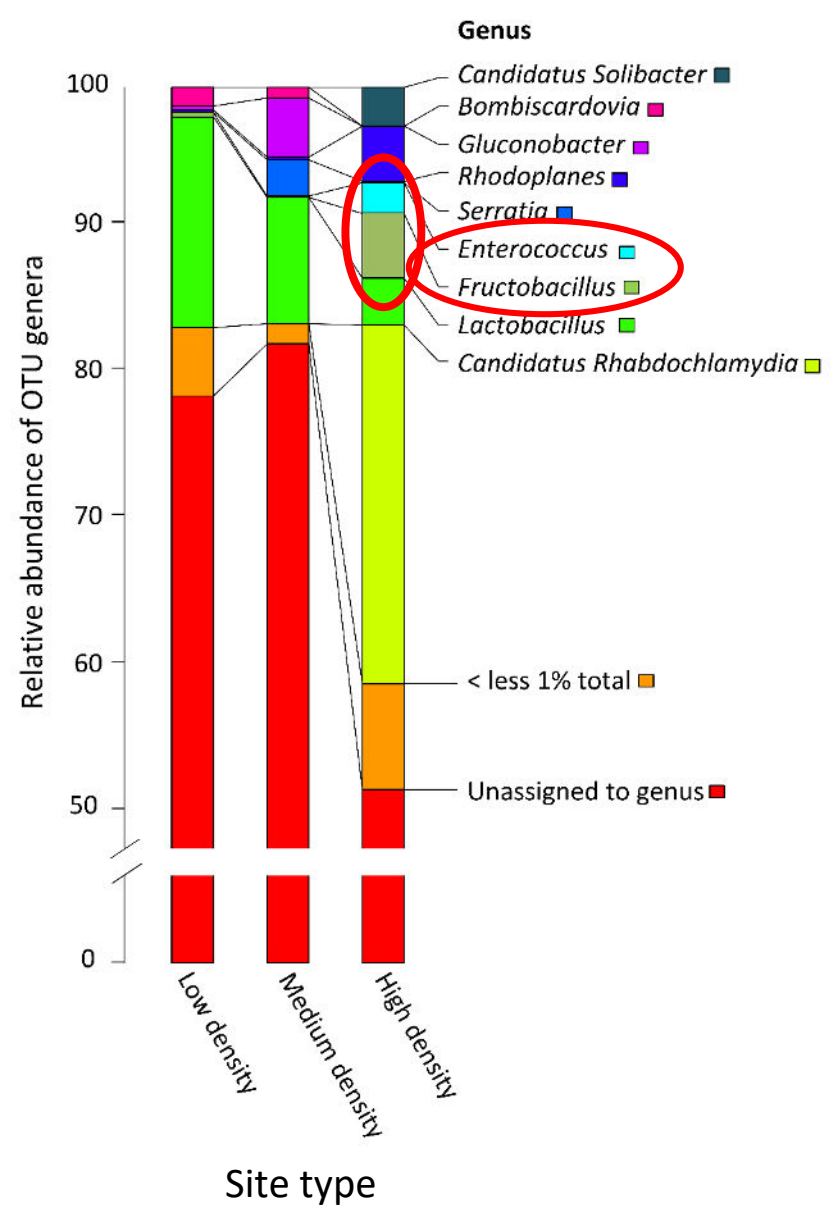
Bacterial community



Potential pathogens

- *Rhabdochlamydia*
- *Serratia marcescens*
- *Cardinium*

Bacterial community



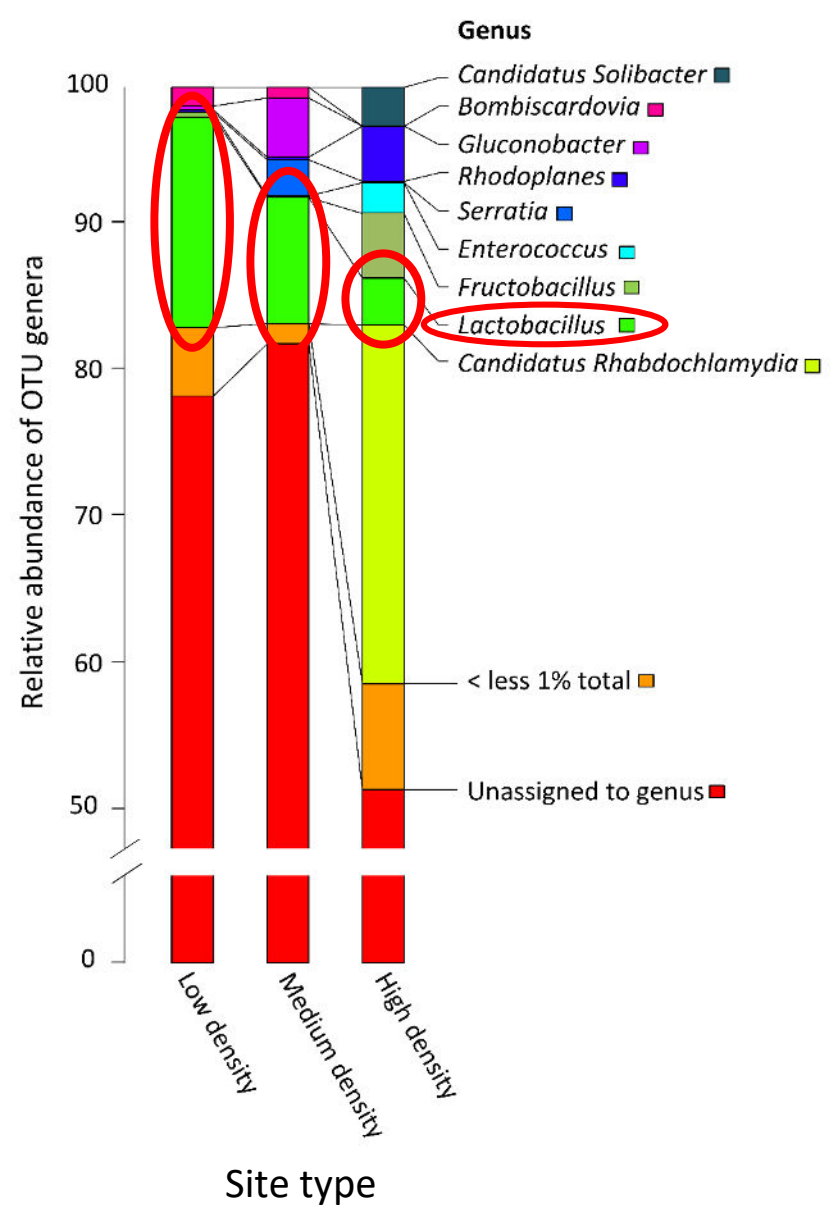
Potential pathogens

- *Rhabdochlamydia*
- *Serratia marcescens*
- *Cardinium*

Potential mutualists

- *Enterococcus*
- *Fructobacillus*

Bacterial community



Potential pathogens

- *Rhabdochlamydia*
- *Serratia marcescens*
- *Cardinium*

Potential mutualists

- *Enterococcus*
- *Fructobacillus*
- *Lactobacillus*

Conclusions

- Identify pathogens and mutualists infecting yellow crazy ants
    - **Possible Dicistrovirus, BQCV**
    - ***Canadidatus Rhabdochylamydia, Serratia marcescens, Canadidatus Cardinium***
    - **Assortment of potential mutualists, lactic acid bacteria**

- Determine if expanding and declining populations differ in their viral and bacterial communities
    - **though differences, no obvious patterns indicating observed pathogens play a role in observed population declines**
    - **unidentified virus could be promising biocontrol candidate**

Acknowledgements

- VUW Ant Club
- Dhimurru Aboriginal Corporation
- Conservation Volunteers Australia
- Funding
  - Victoria University Research Trust
  - CSIRO
  - Rio Tinto Alcan

Victoria
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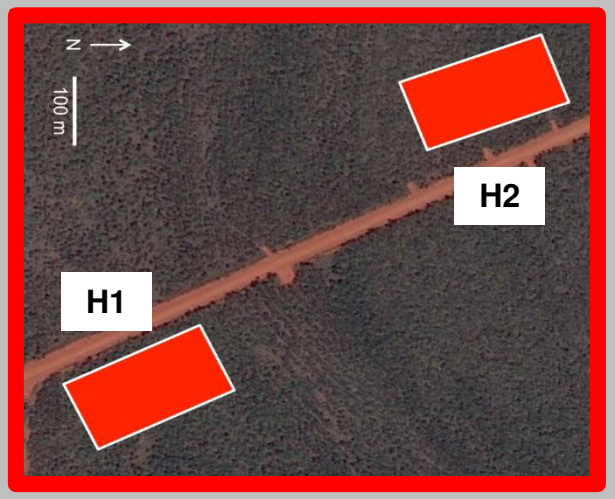
Site types


N
100 m
H2
H1


M2
M1
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N


L1
100m
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L2
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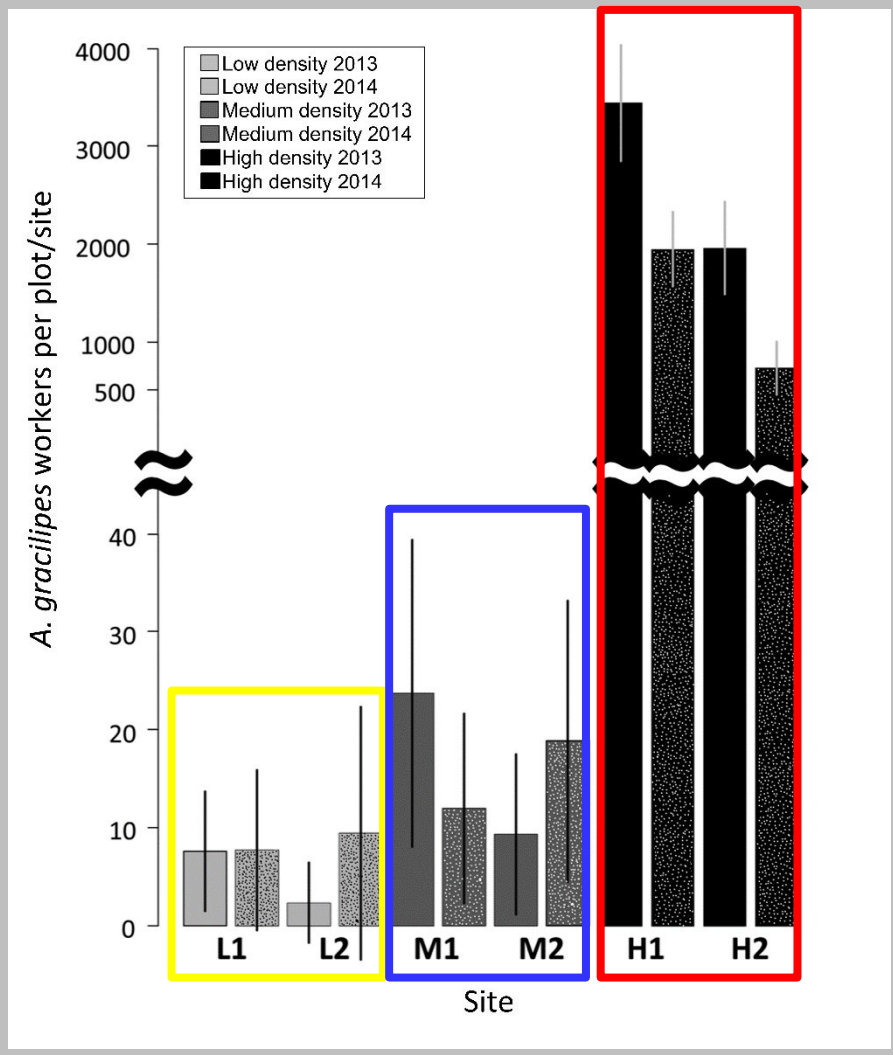
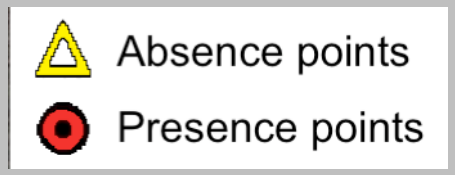

Low density 2013
Low density 2014
Medium density 2013
Medium density 2014
High density 2013
High density 2014
A. gracilipes workers per plot/site
4000
3000
2000
1000
500
40
30
20
10
0
L1
L2
M1
M2
H1
H2
Site


Absence points
Presence points

Bacterial community