

Coffee Berry Borer, *Hypothenemes hampei*
Ferrari (Coleoptera: Scolytidae)-
Microbial Interactions



Sayaka Aoki, Mark G Wright, Ania Wieczorek,
Russell Messing, Gordon Bennett and Fernando Vega

Department of Plant and Environmental protection Sciences
University of Hawaii at Manoa.

Functions of Mutualistic Bacterial Symbionts in insects

⊗ Host survival

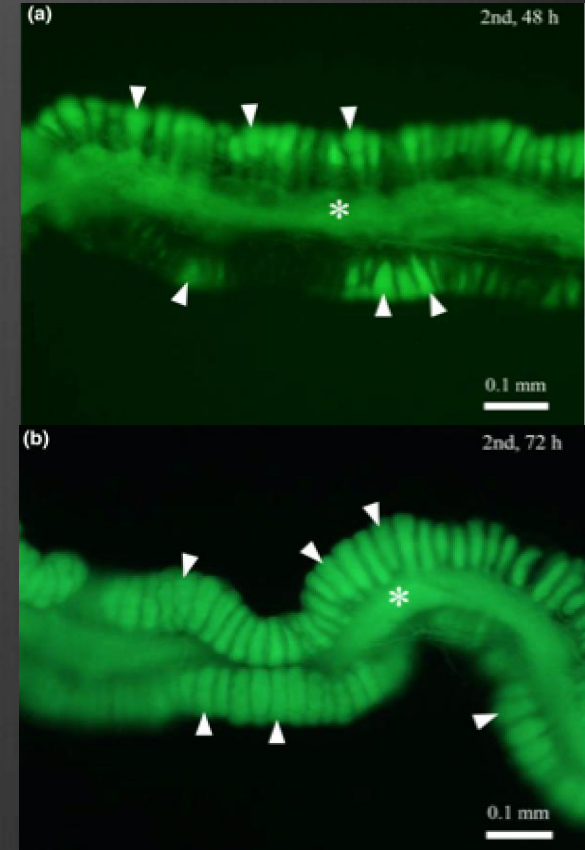
1. Providing vital nutrients
2. Breaking down food materials
3. Influencing host plant usage (by mediating detoxification of plant secondary metabolites)
4. Mediating interactions with natural enemies

⊗ Reproduction

- ⊗ Wolbachia (Alpha-Proteobacteria)
 - ⊗ Cytoplasmic incompatibility (CI)
 - ⊗ Feminization of genetic male
 - ⊗ Parthenogenesis induction (PI)
 - ⊗ Male killing

Bacterial Endosymbionts Example: *Burkholderia* (Beta-Proteobacteria)

- ⊗ Gut symbiont found in phytophagous insect hosts such as pentatomid stink bugs
 - ⊗ Prolong survival of adult
 - ⊗ Increased number of eggs
 - ⊗ Reduced time of oviposition (Kikuchi et al., 2014)
- ⊗ Confer insecticide resistance in pest insects
 - ⊗ Degrade fenitrothion insecticide in infected stinkbugs (Kikuchi, 2009)
- ⊗ Suppress *Beauveria bassiana* growth in Leaf cutter ants, *Atta sexdens rubropilosa* (Santos et al., 2004)



GFP labeled *Burkholderia* in *R. pedestris* mid gut visualized by epifluorescence microscopy (Kikuchi and Fukatsu, 2014)

Previous study: CBB-Microbial Symbionts

- ⊗ CBB associations with 22 families of bacterial species detected by pyrosequencing (Vega et al.,)
- ⊗ *Wolbachia* identified in CBB samples obtained from Benin, Brazil, Colombia, Ecuador, El Salvador, Honduras, India, Kenya, Mexico, Nicaragua, and Uganda (Vega et al., 2002)
- ⊗ Caffeine-degradation by *Pseudomonas* strain (Ceja-navarro, Vega et al., 2015)

Objective

- ④ Identification and characterization of bacterial endosymbionts associated with *Hypothenemus hampei* and their interaction with CBB

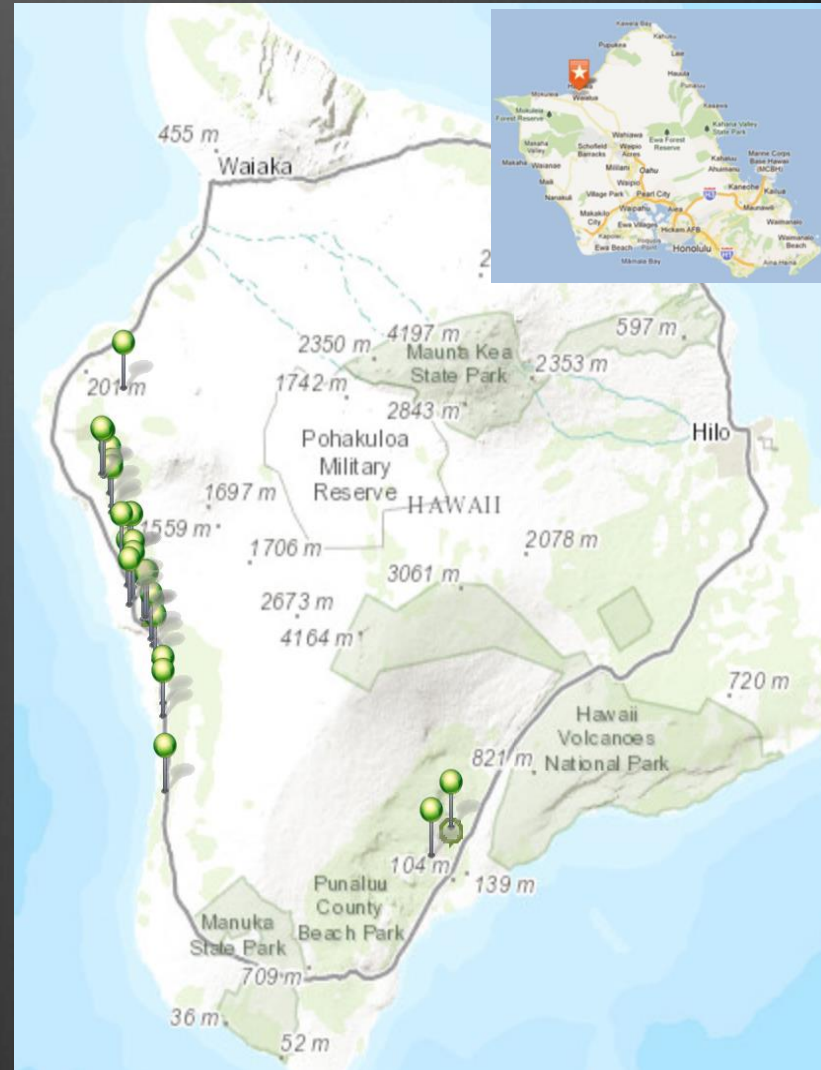
Bacterial Screening

Sampling

33 sites

- 32 farms from Kona and Ka'u, (Hawai'i)
- 1 farm from Waialua (Oahu)

Treatment	# sites
Botanigard®	12 (2 sites <i>B. bassiana</i> but not GHA strain)
No treatment	16
Botanigard®+Kaolin	2
Pyrethrin +Botanigard	1
Lactic Acid Bacteria	1
No info	1
Total	33



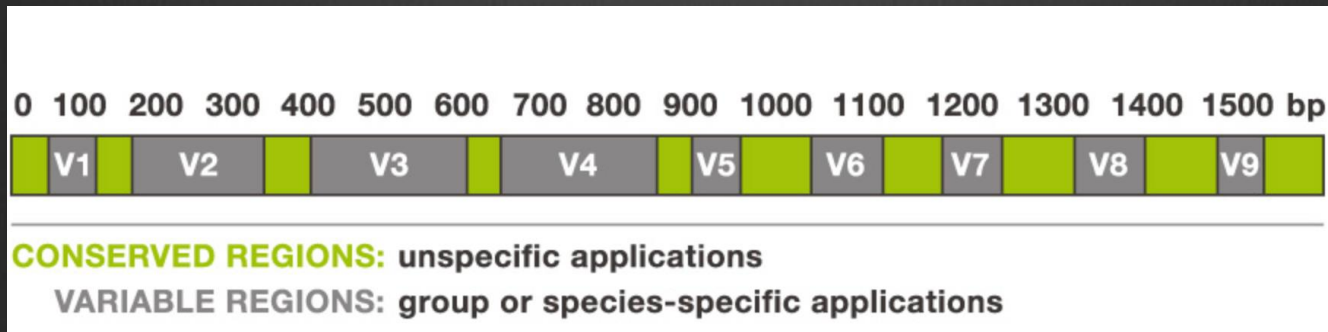
Bacterial Screening:

Sample collection and extraction

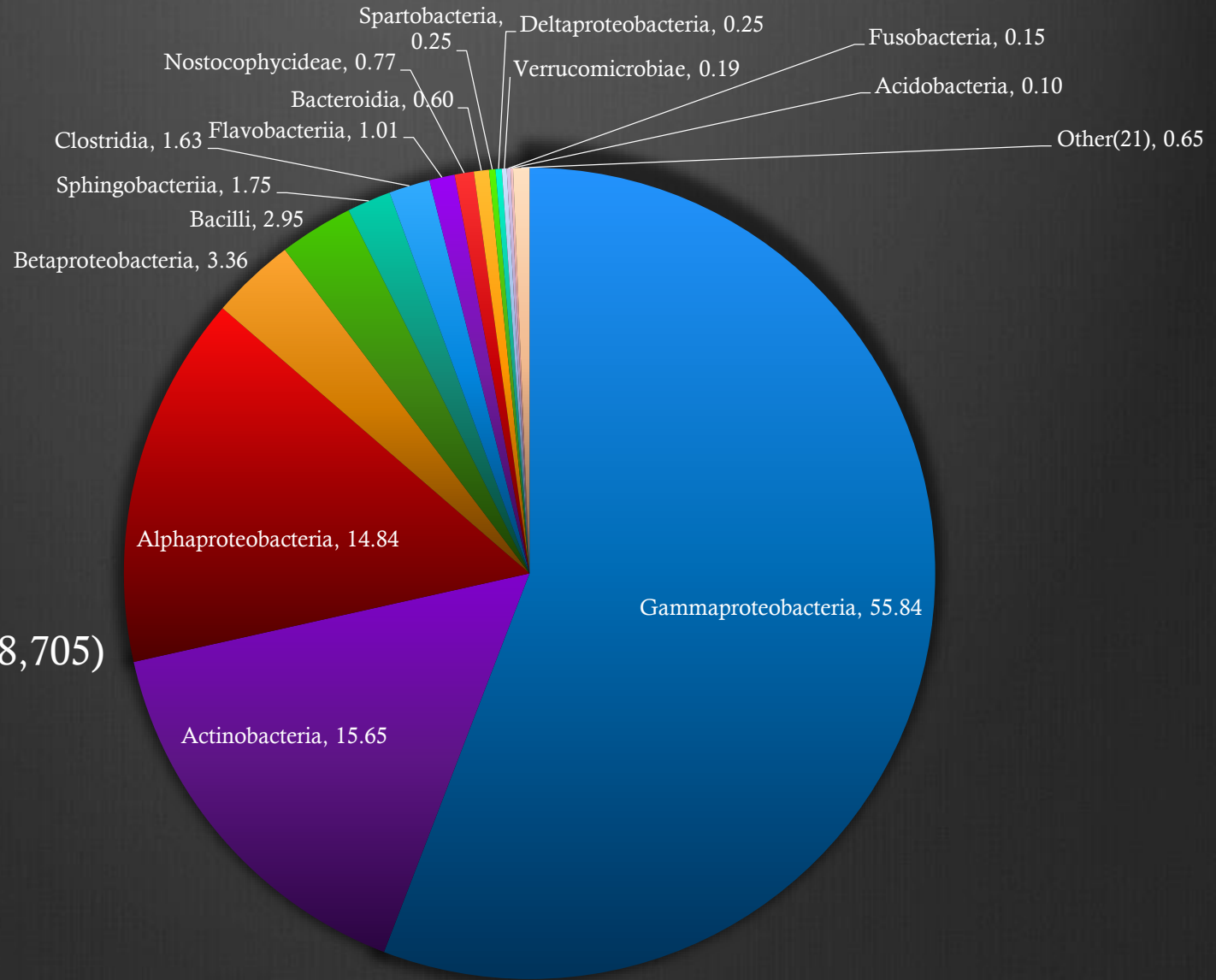
- ⊗ Red cherries/raisins are collected from sites
 - ⊗ Frozen at -5°C
 - ⊗ Quarantined for at least 48 hours at Kona HDoA
 - ⊗ Beetles are extracted at UH lab
 - ⊗ Female CBB from all sites (33)
 - ⊗ Male CBB from Waialua location (1)
 - ⊗ Larvae (1) and Pupae (1)
- Total 36 samples
- ⊗ Kept in 70% ETOH at -5°C

Molecular analysis

- DNA extraction (50mg/sample:~136beetles/sample)
- PCR: 16SrRNA
- Sequencing by Miseq ®
 - V3 and V4 regions of 16SrRNA (~300bp) were targeted for library construction (SeqMatic LLC)
- OTU clustering and analysis by The Illumina BaseSpace 16S pipeline (SeqMatic LLC)

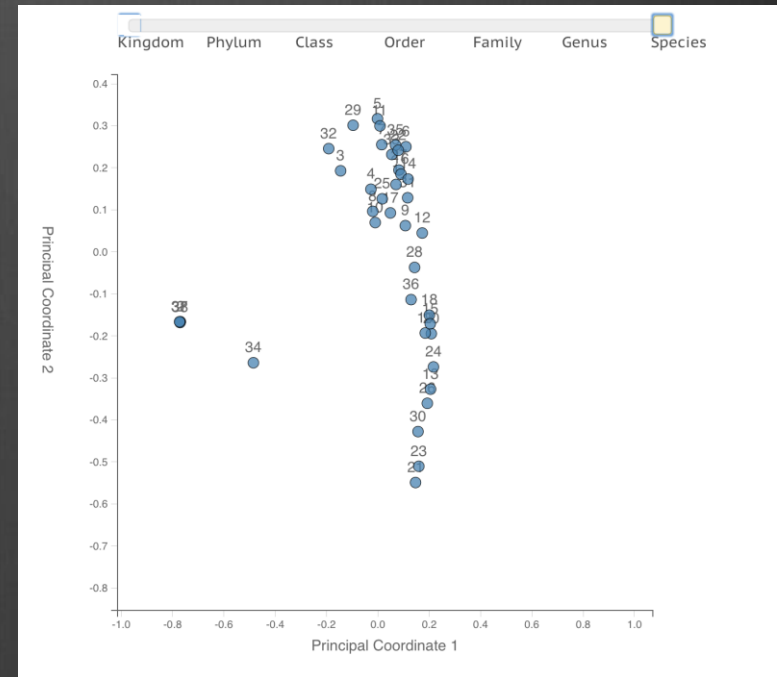
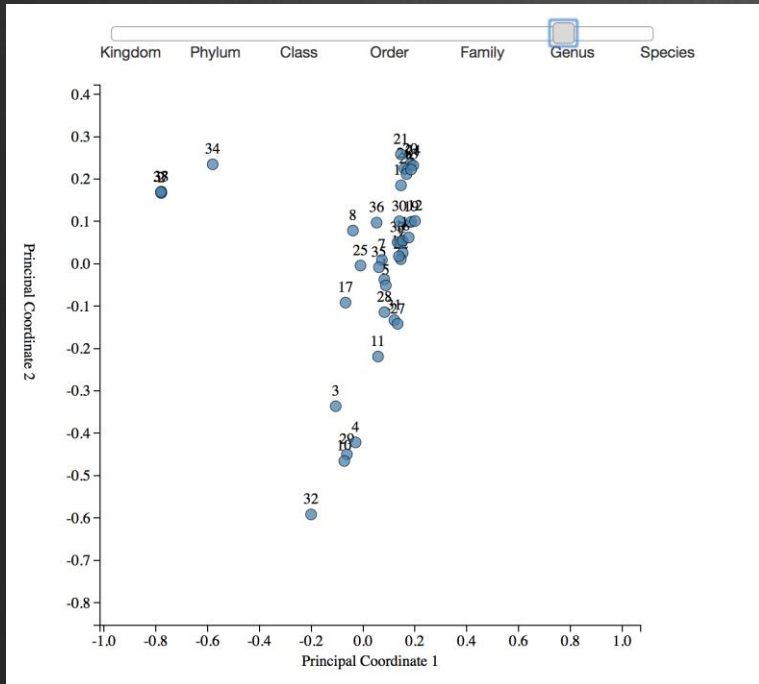


Relative Abundance (%) of Bacteria by Class



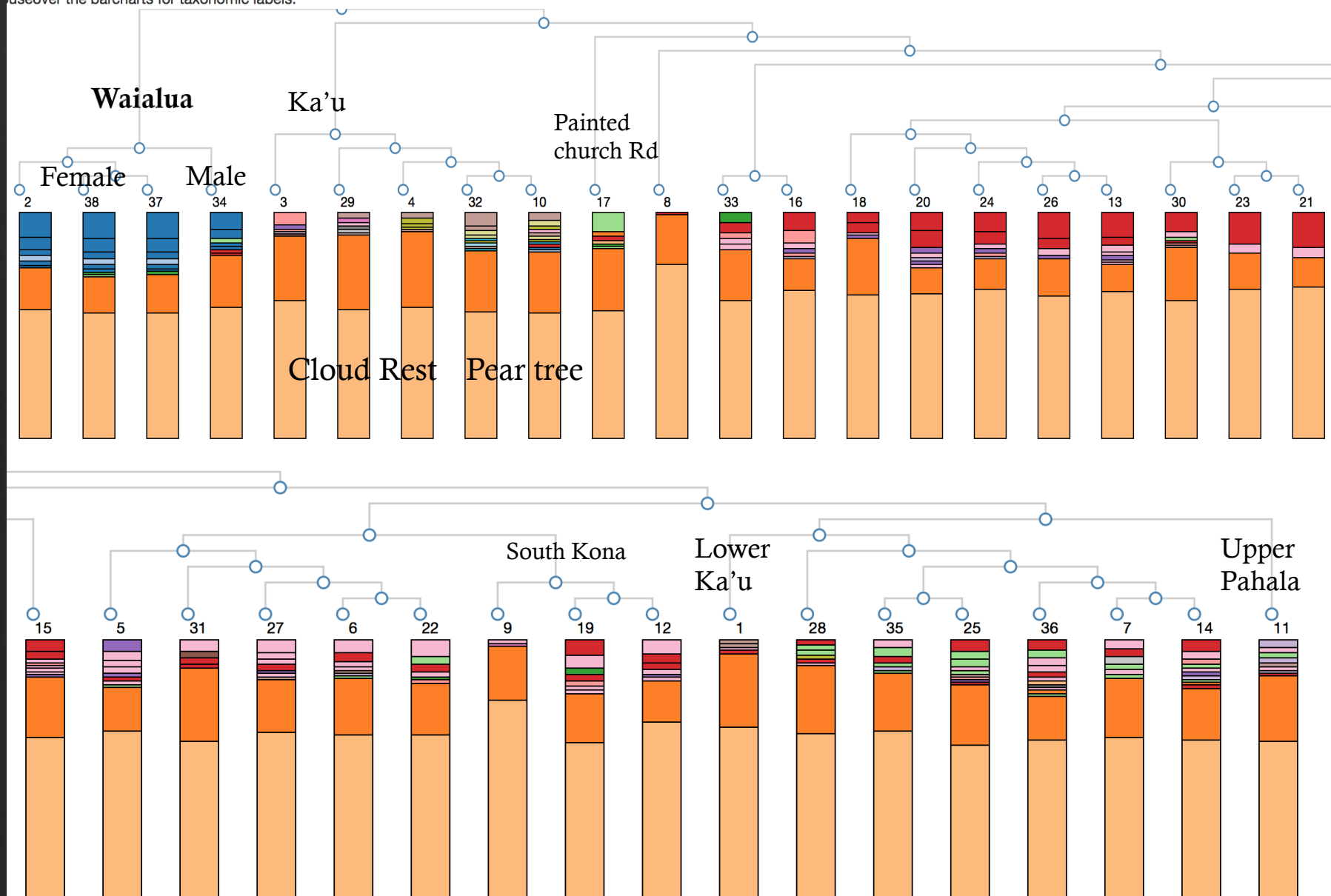
- 57 Class (11,348,705)
- 114 Order
- Family: 258
- Genus: 761
- Species: 2337

Principal Coordinate Analysis by Sites (genus and species level)



Relative abundance and hierarchical clustering by genus-level

discover the bar charts for taxonomic labels.



Interesting bacteria

- ⊗ *Trabulsiella odototermis*: Gut symbiont of fungus-growing termites
- ⊗ *Wolbachia pipensis*: 13 locations
- ⊗ *Serratia entomophil*: found in gut of grass grub, *Costelytra zealandica*, in New Zealand causes amber disease
- ⊗ *Burkholderia cepacia* complex (7 species)

Further Analysis

- ⊗ Comparison of Bacterial community compositions
 1. Abundance of bacteria of interests by treatment with *Beauveria bassiana* sites vs. Non-treatment
 2. Analysis of bacterial compositions on/in CBB by female vs. male CBB
 3. Analysis of bacterial compositions on/in CBB by different developmental stage: larvae, pupae, and adult
 4. Geographical pattern of bacterial abundance

Thank you

