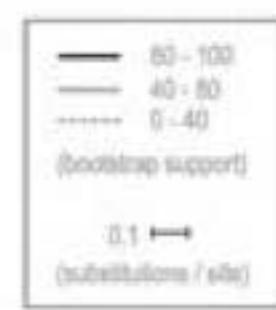


Ecological interactions

Myrmecophiles of the ants of
Vachellia (Acacia) drepanolobium

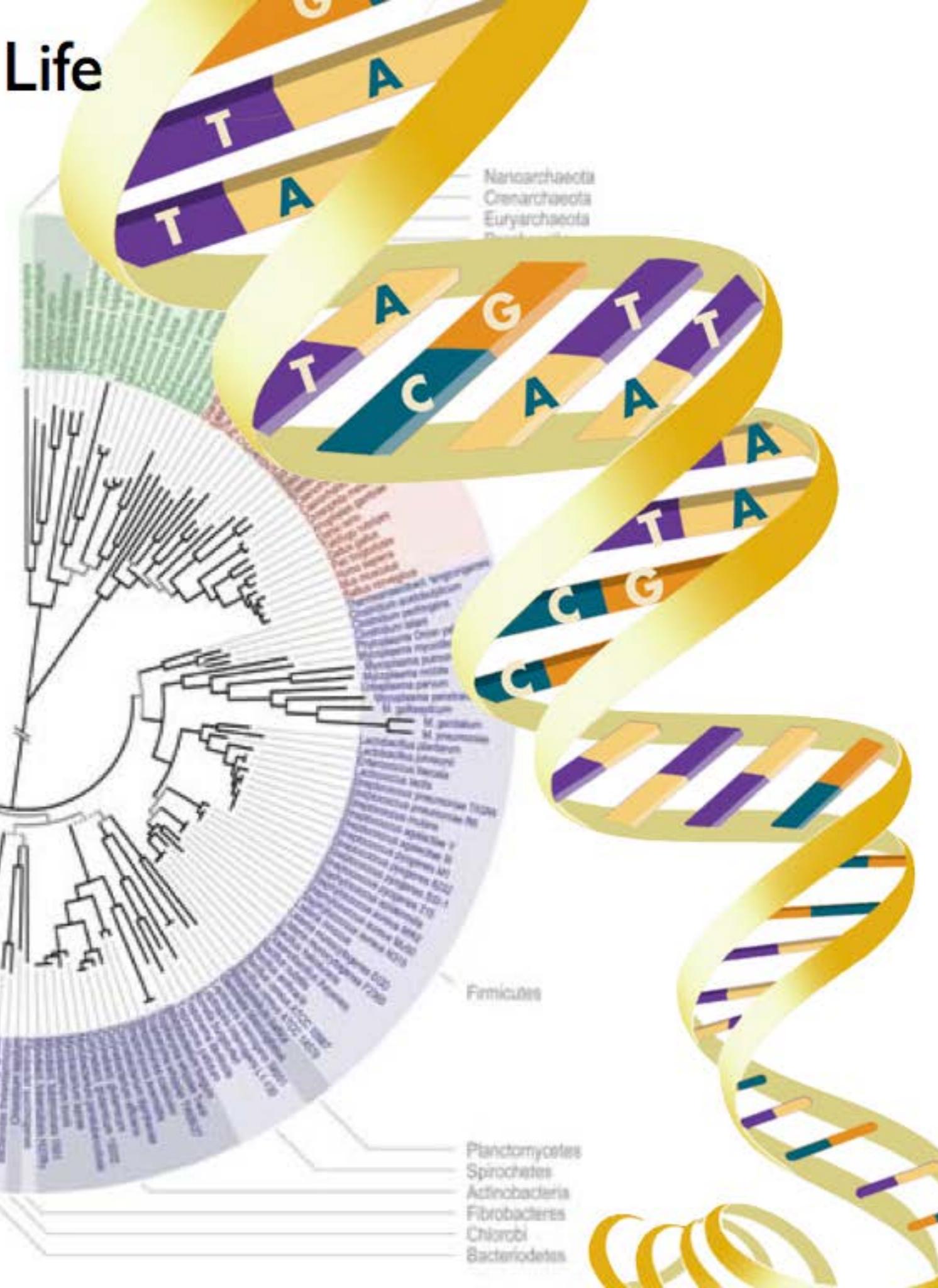
Naomi E. Pierce
Museum of Comparative Zoology
Harvard University

DNA and The Tree of Life



Nanoarchaeota
Crenarchaeota
Euryarchaeota
Diatomophytes

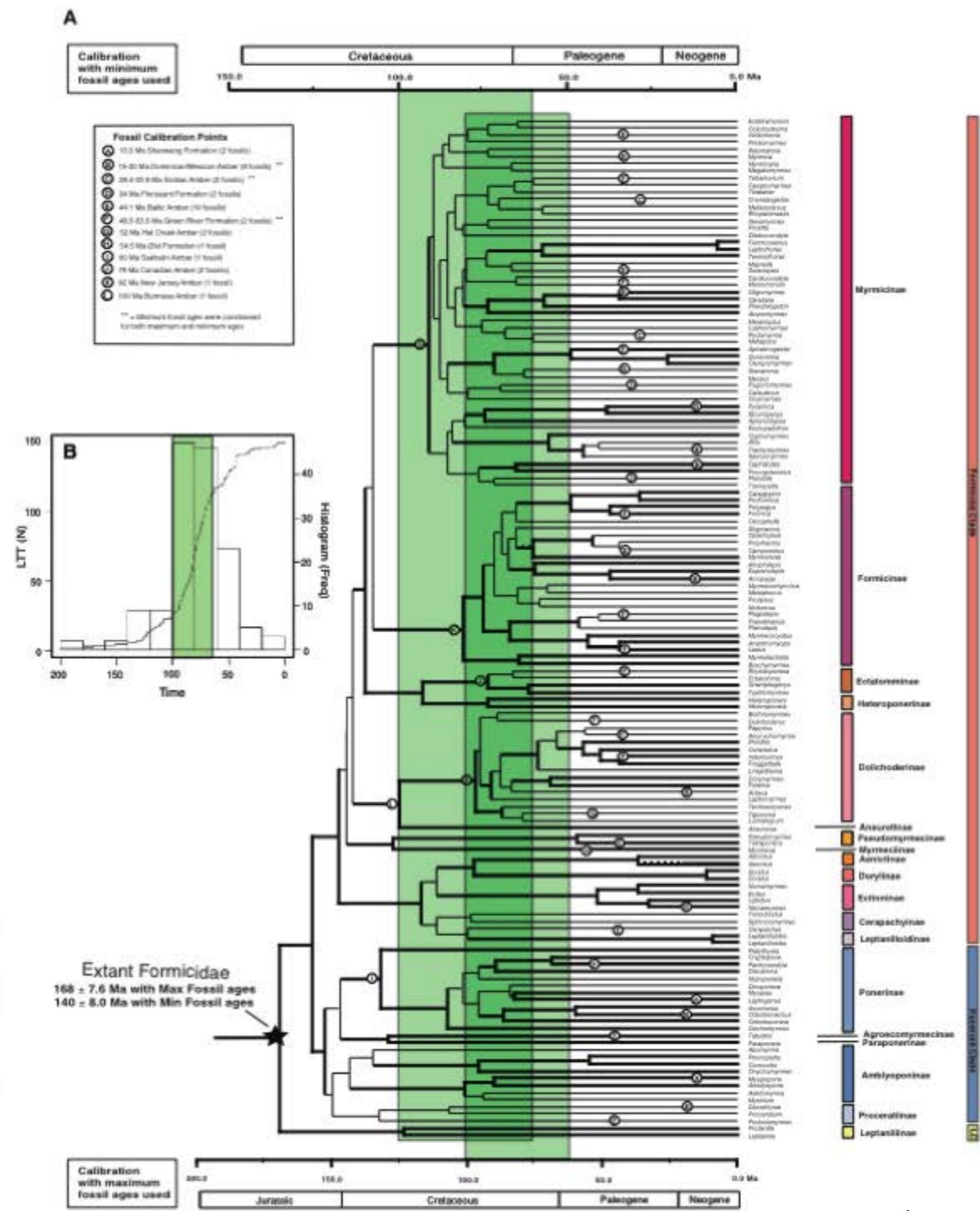
Planctomyces
Spirochete
Actinobacteria
Fibrobacteres
Chlorobi
Bacteroidetes



Evolutionary tree of the ants



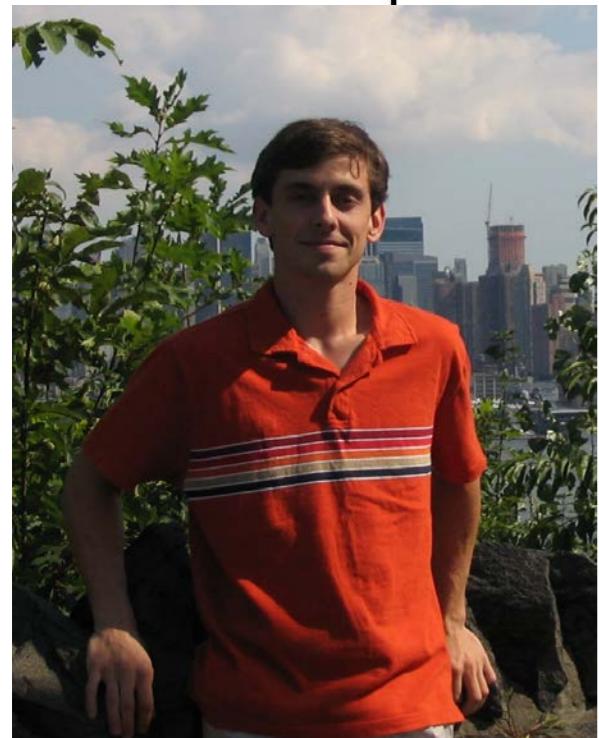
Corrie Moreau



Molecular prospecting: Ants and symbiotic bacteria



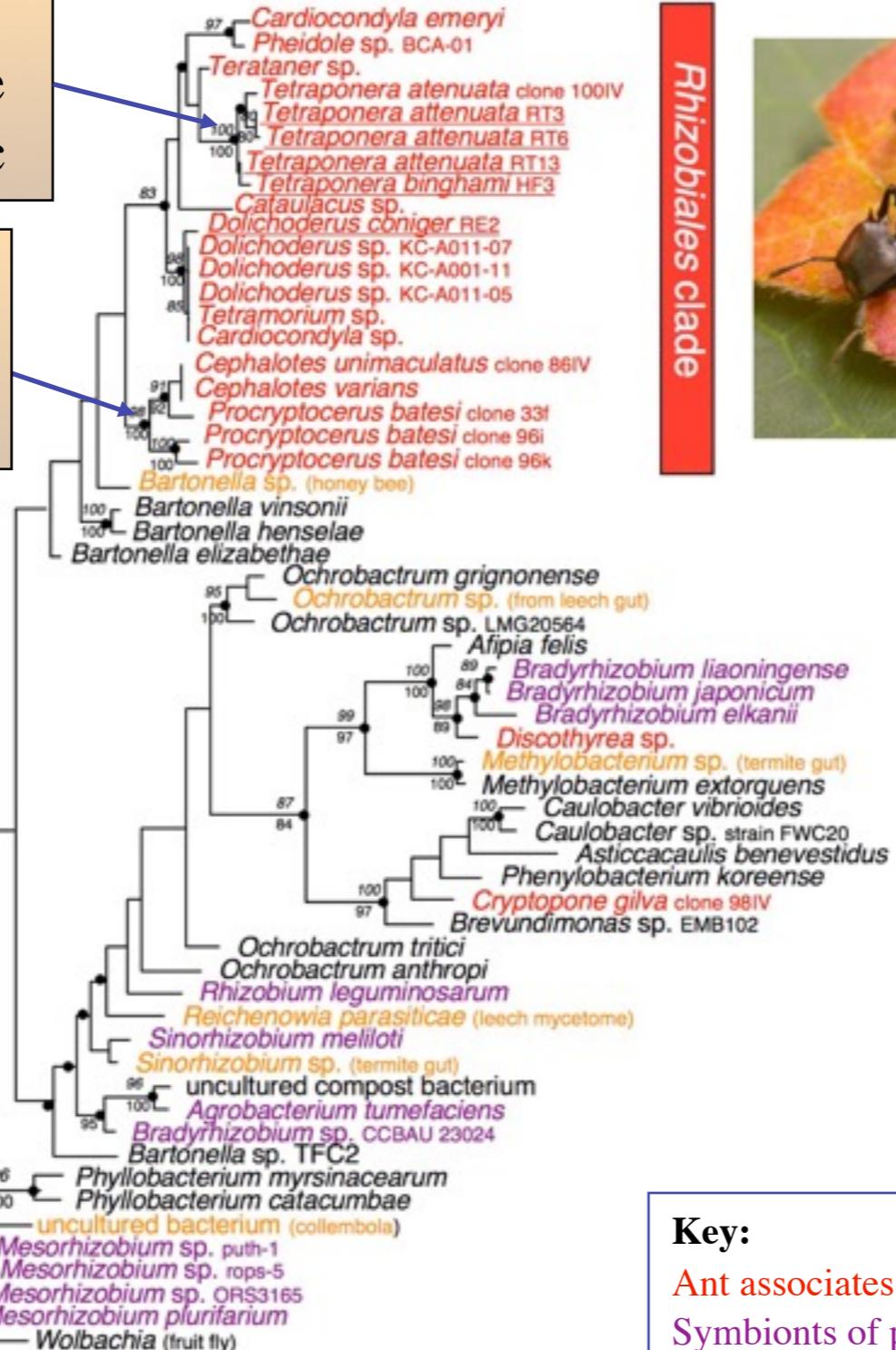
Bacteria from some ant groups are specialized and closely related



Jake
Russell

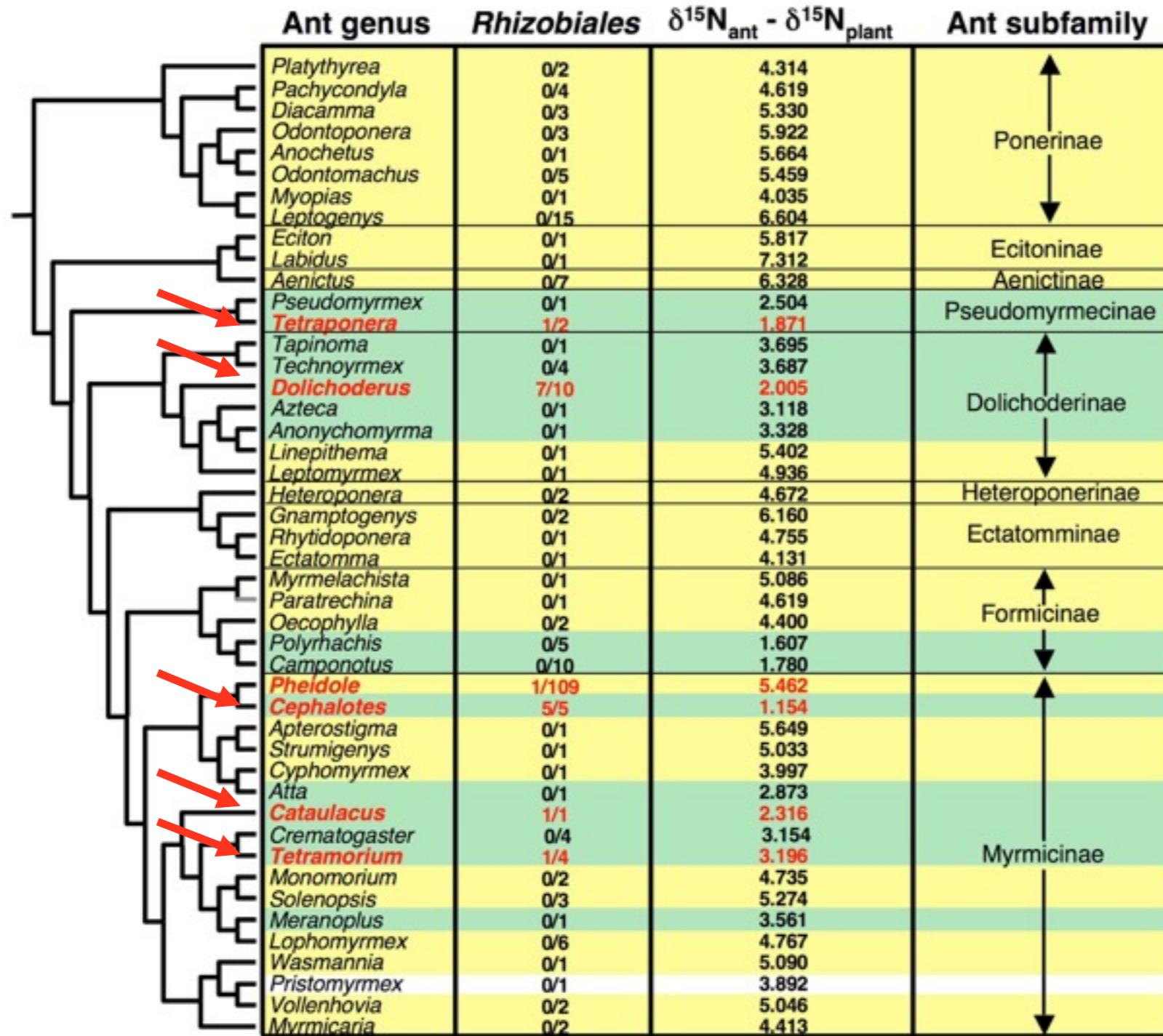
Tetraponera
associates are
monophyletic

Cephalotini
associates are
monophyletic



Key:
Ant associates
Symbionts of plants
Symbionts of invertebrates

Ants with *Rhizobiales* have evolved independently



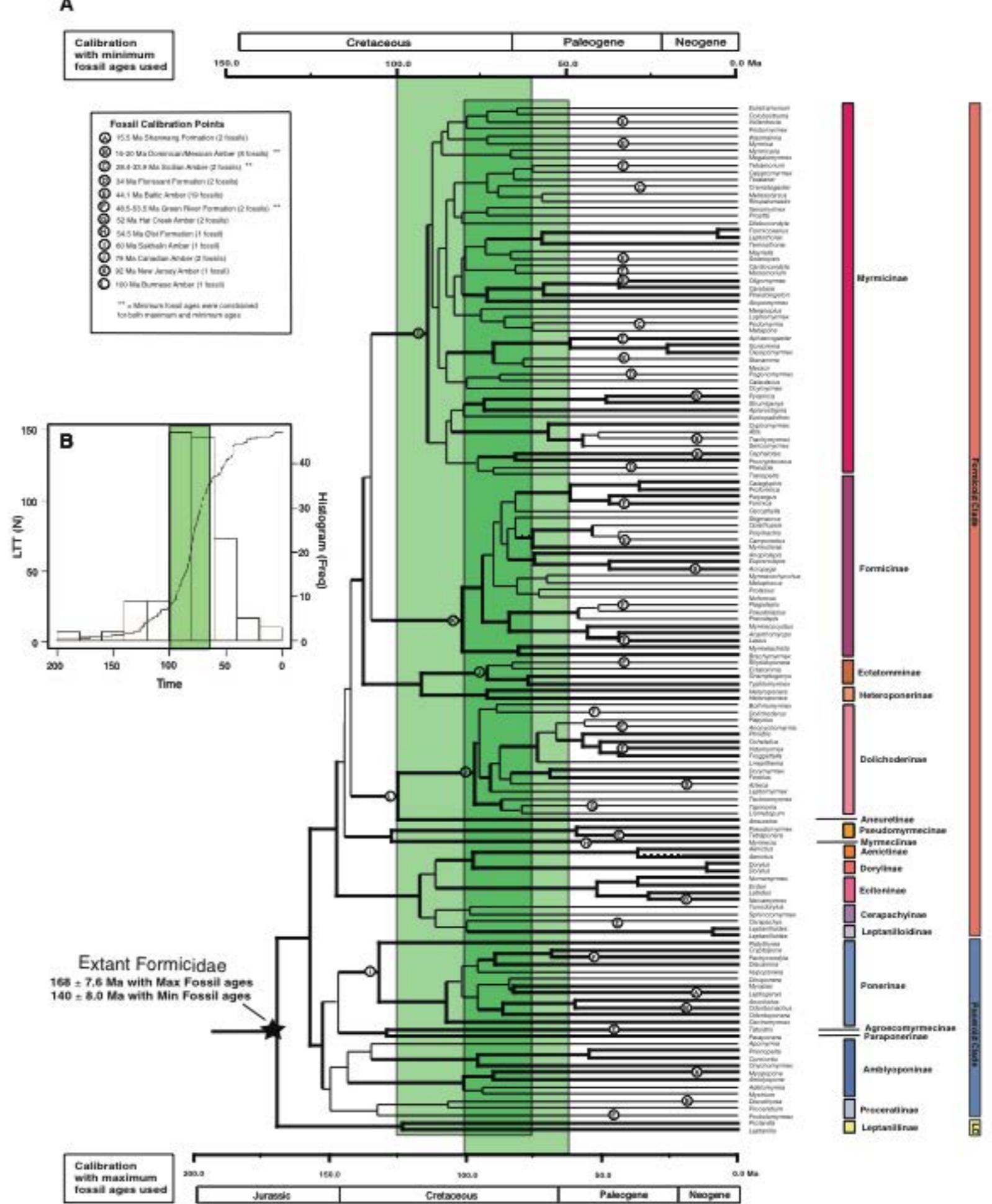
At least 5 separate origins of *Rhizobiales* associations with herbivorous ants

Key:

Herbivore
Predator
<i>Rhizobiales</i> host

Phylogeny from Moreau *et al.* 2006

Symbiotic gut bacteria are likely to have facilitated the evolution of herbivory in ants



Cephalotes



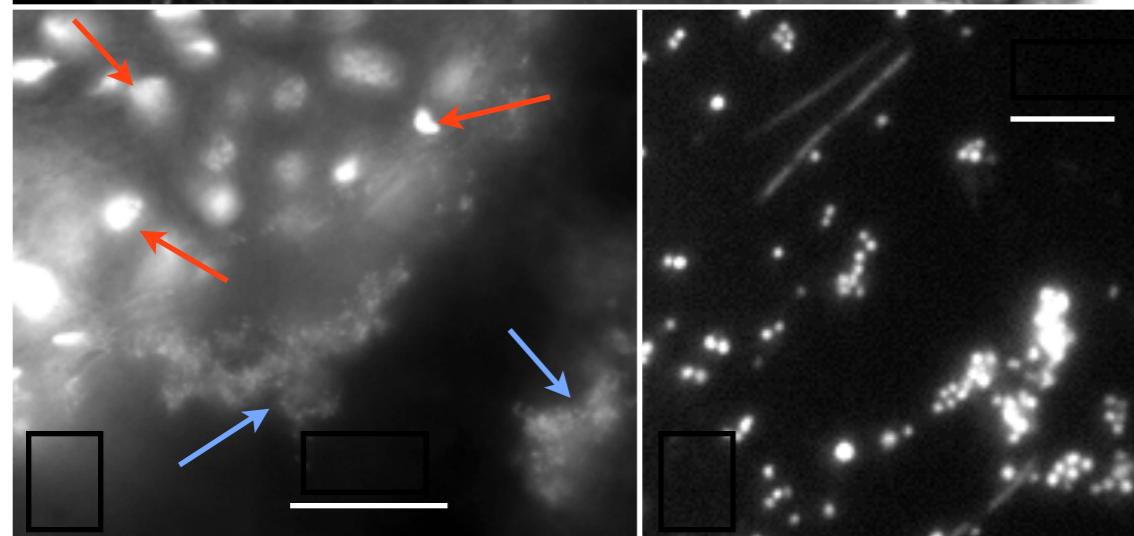
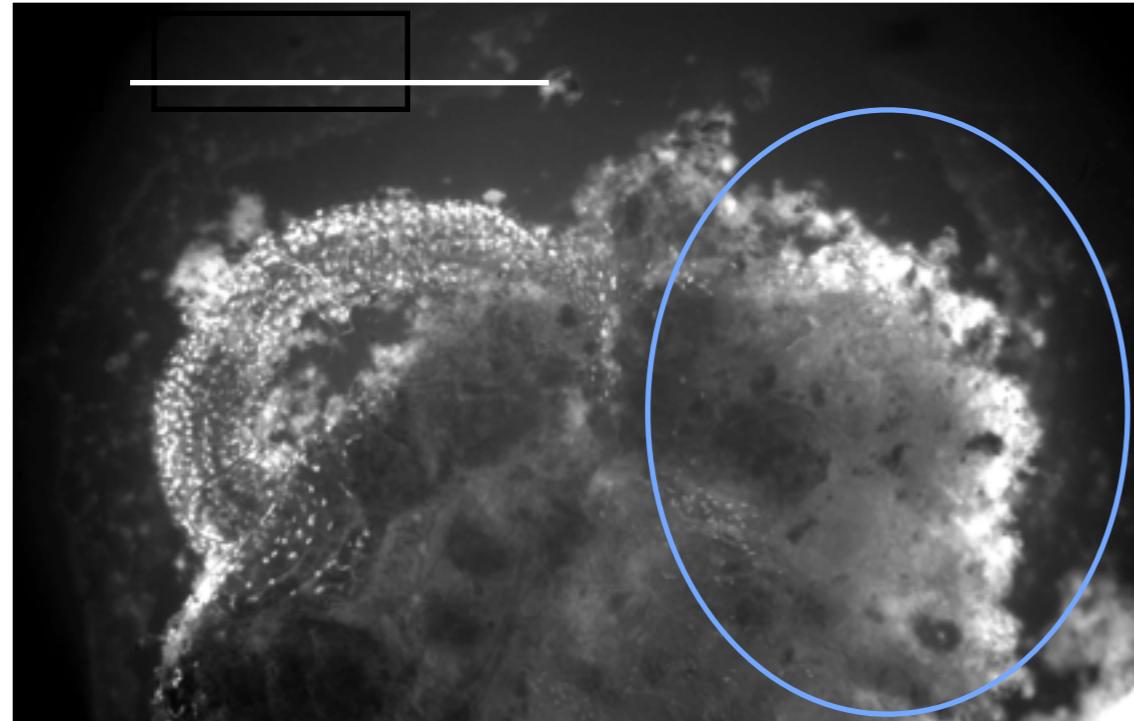
Jon Sanders



Coevolution of ants and their microbiota ...

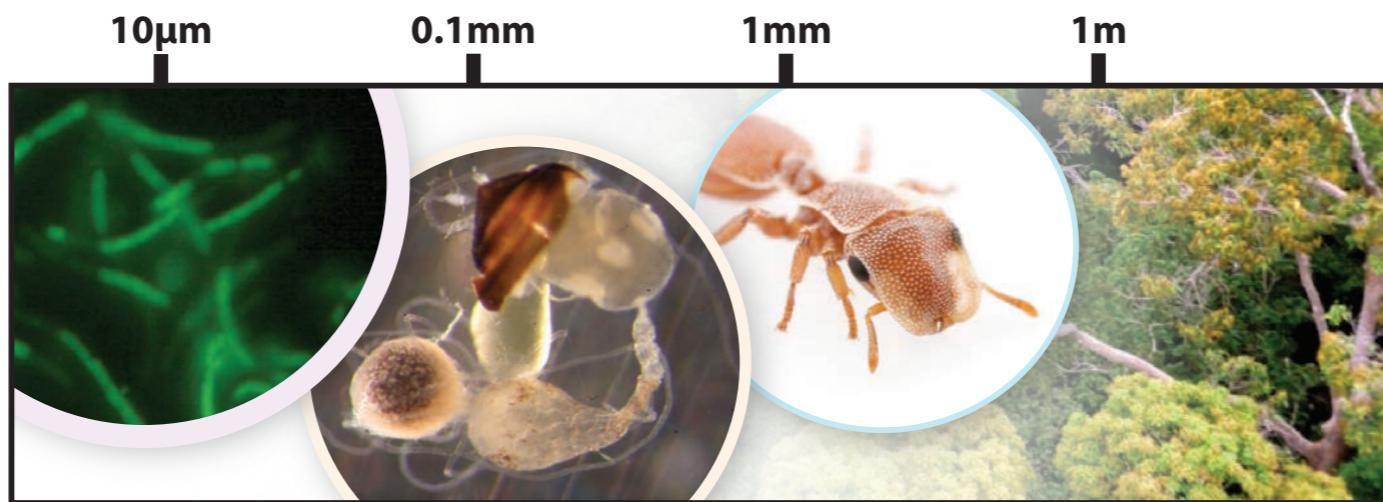


Characterizing gut bacteria for *Cephalotes*



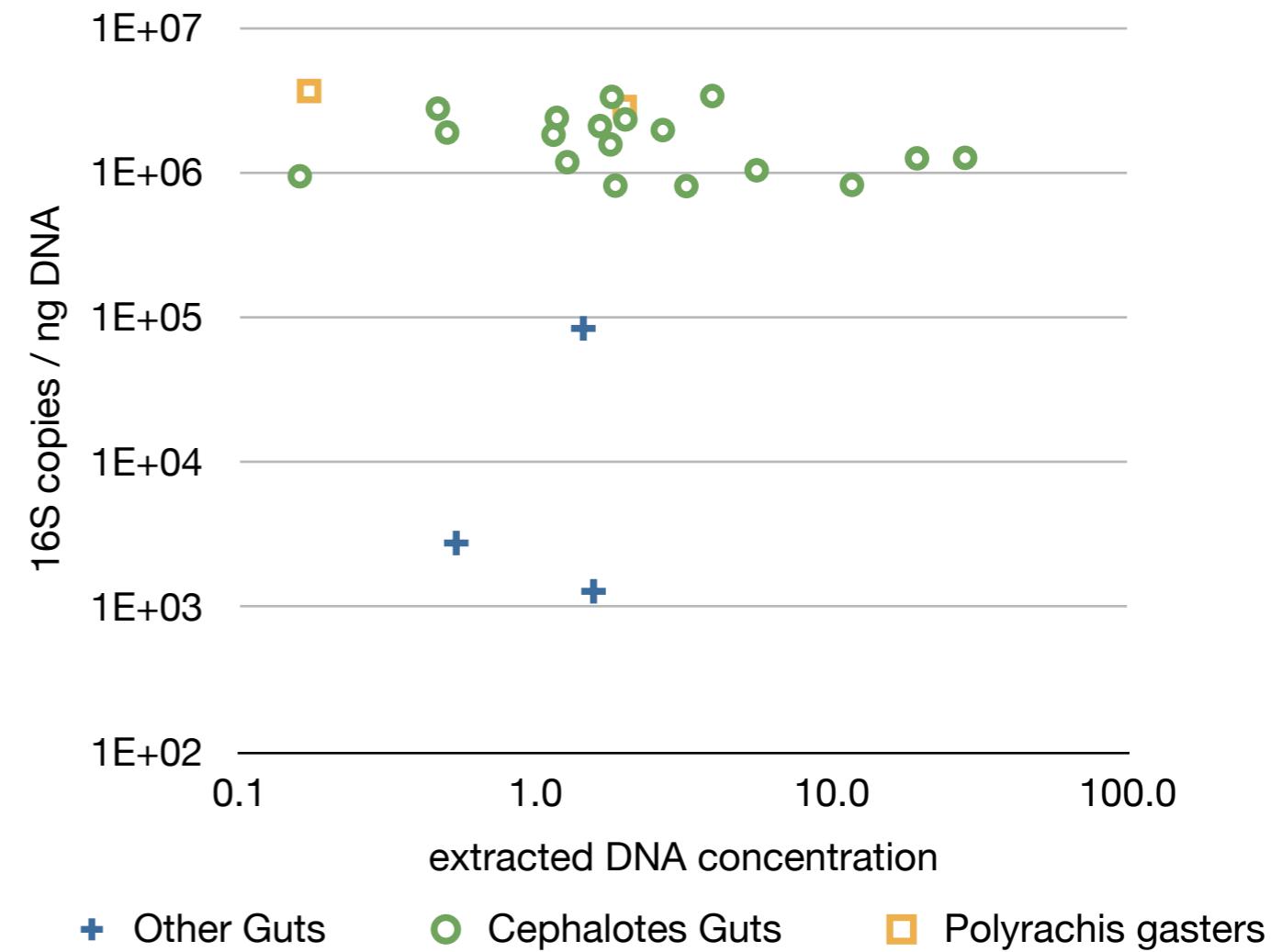
Cephalotes rohweri

- Host nuclei
- Bacteria

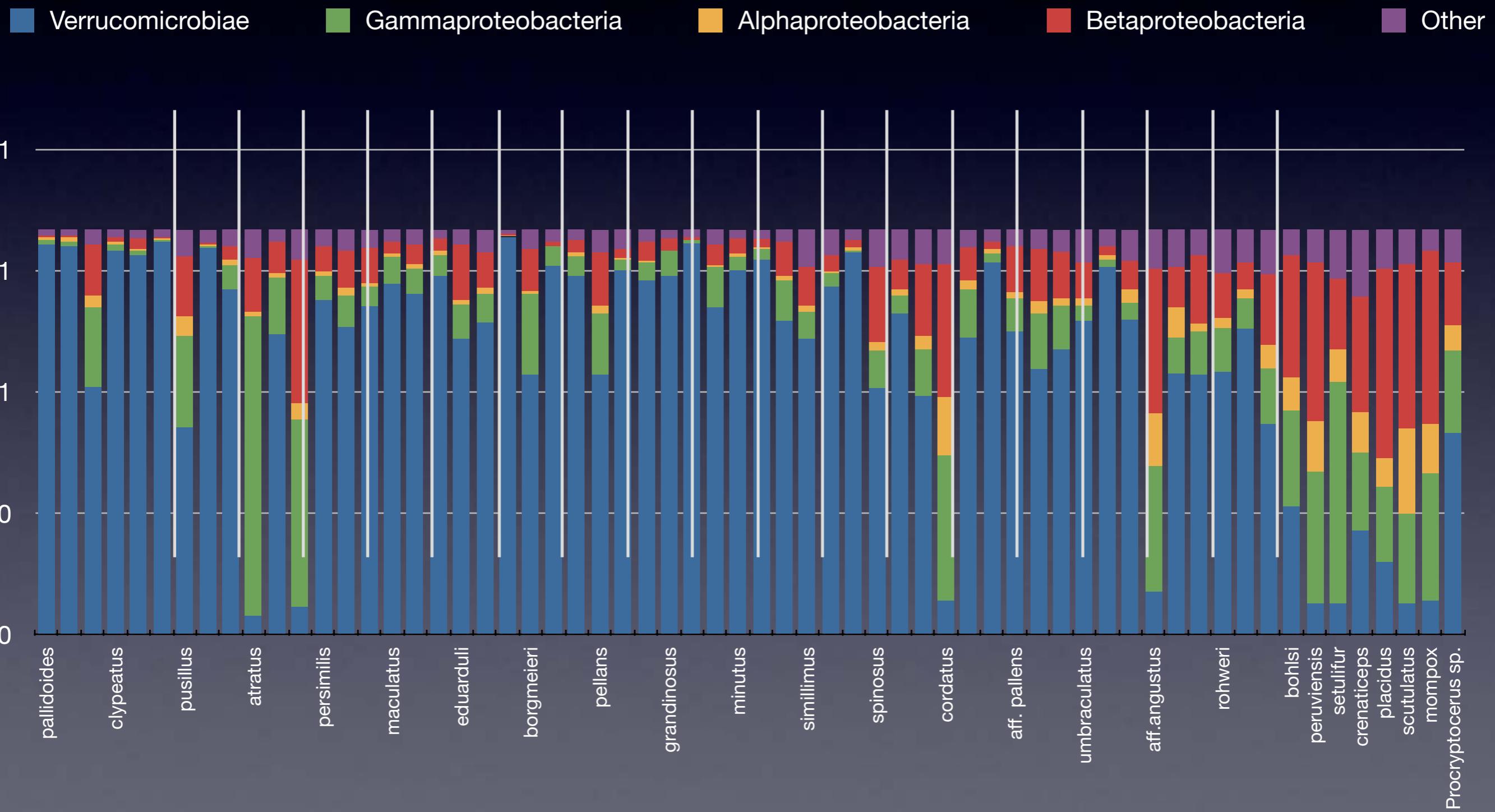


qPCR corroborates visual evidence

16S copies / ng DNA: ant guts



Abundances per sample



Many OTUs codiversify



pOTU width

Cephalotes

$p < 0.01$ (tested)

89

19 (83)

93

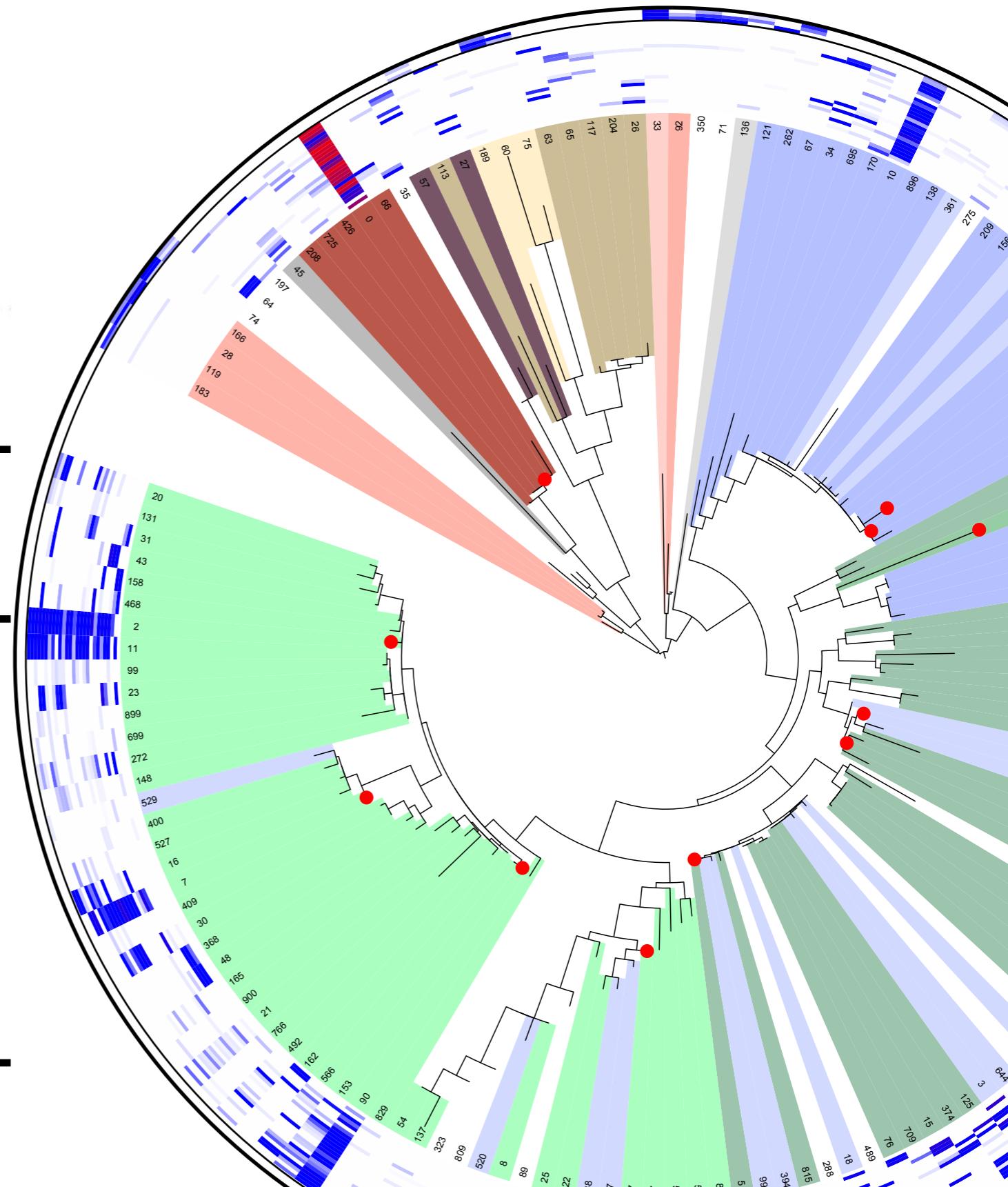
19 (80)

95

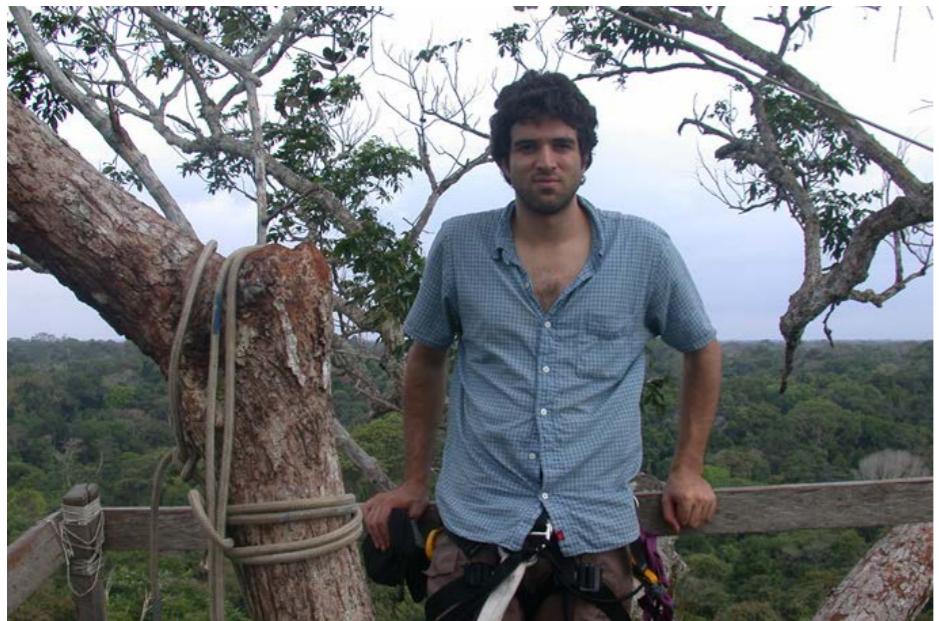
15 (123)

97

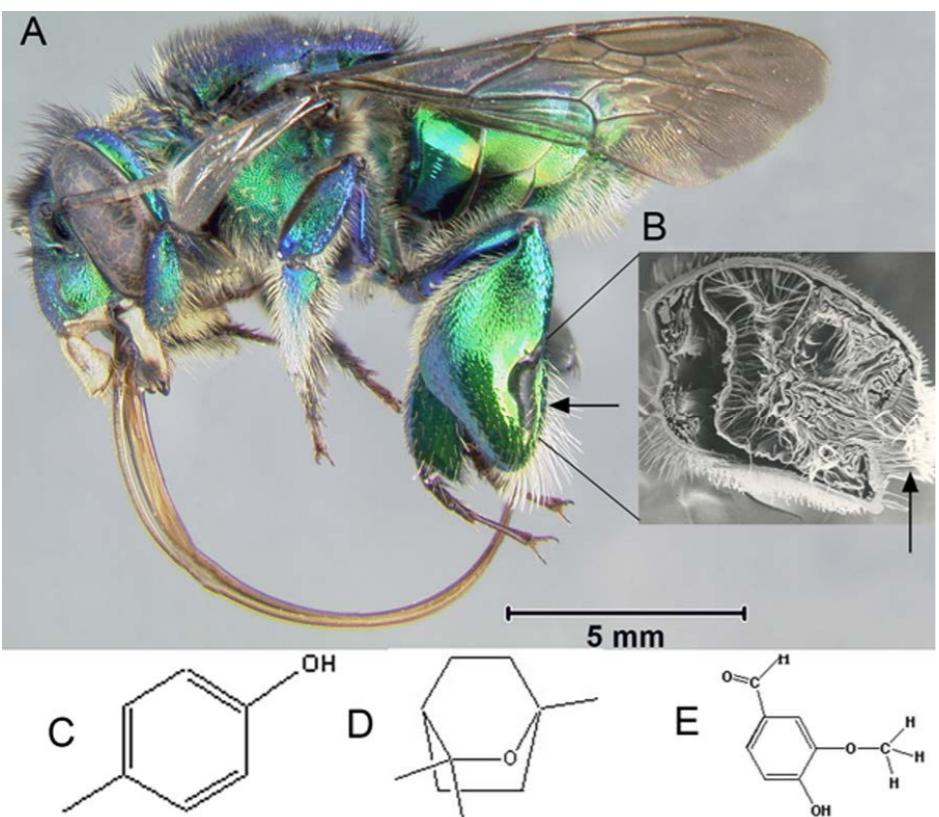
9 (166)



Pollination biology: the evolution of orchid bees

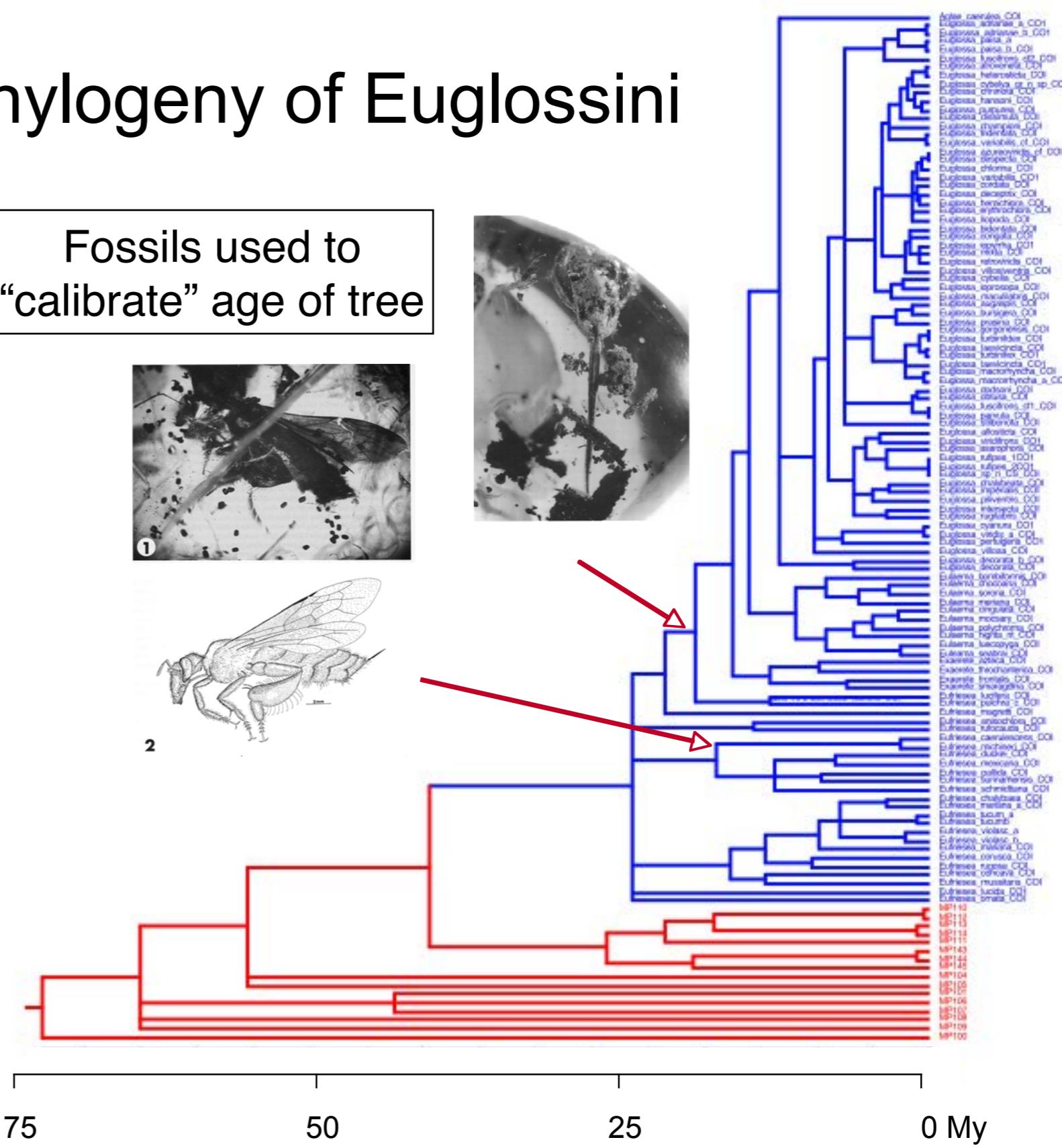


Santiago Ramírez

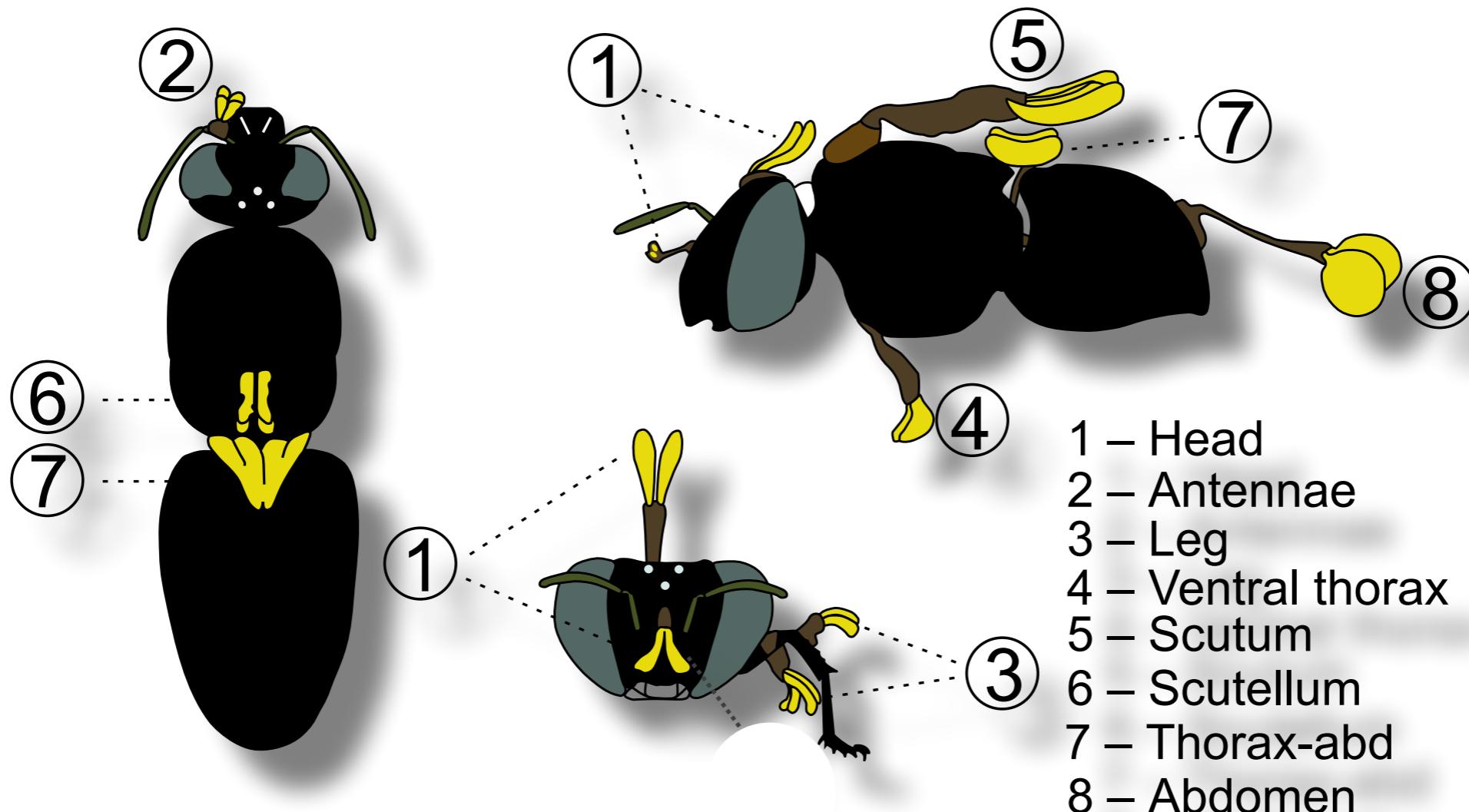


Phylogeny of Euglossini

Fossils used to
“calibrate” age of tree



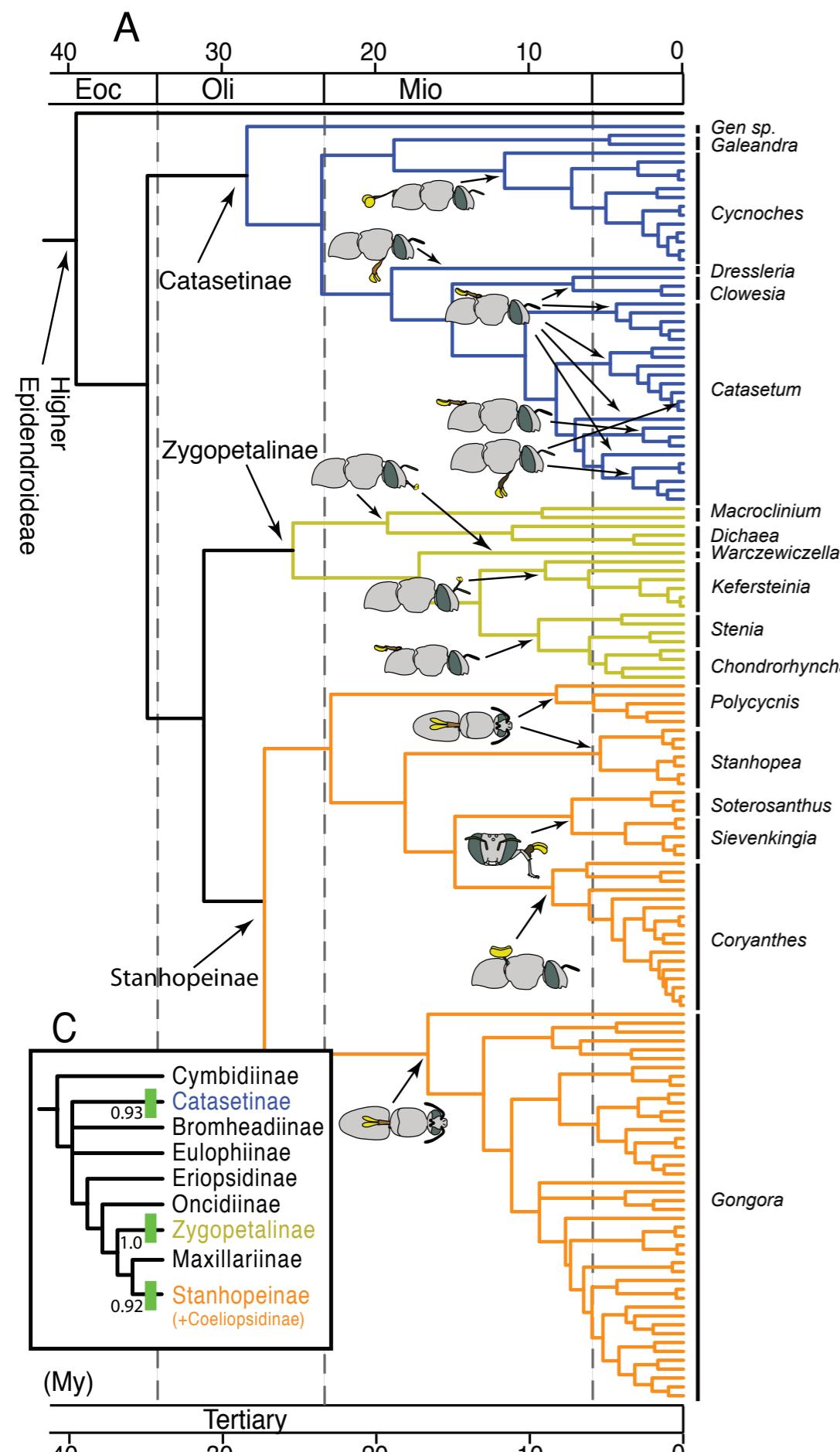
Mechanical floral isolation in euglossine-pollinated orchids



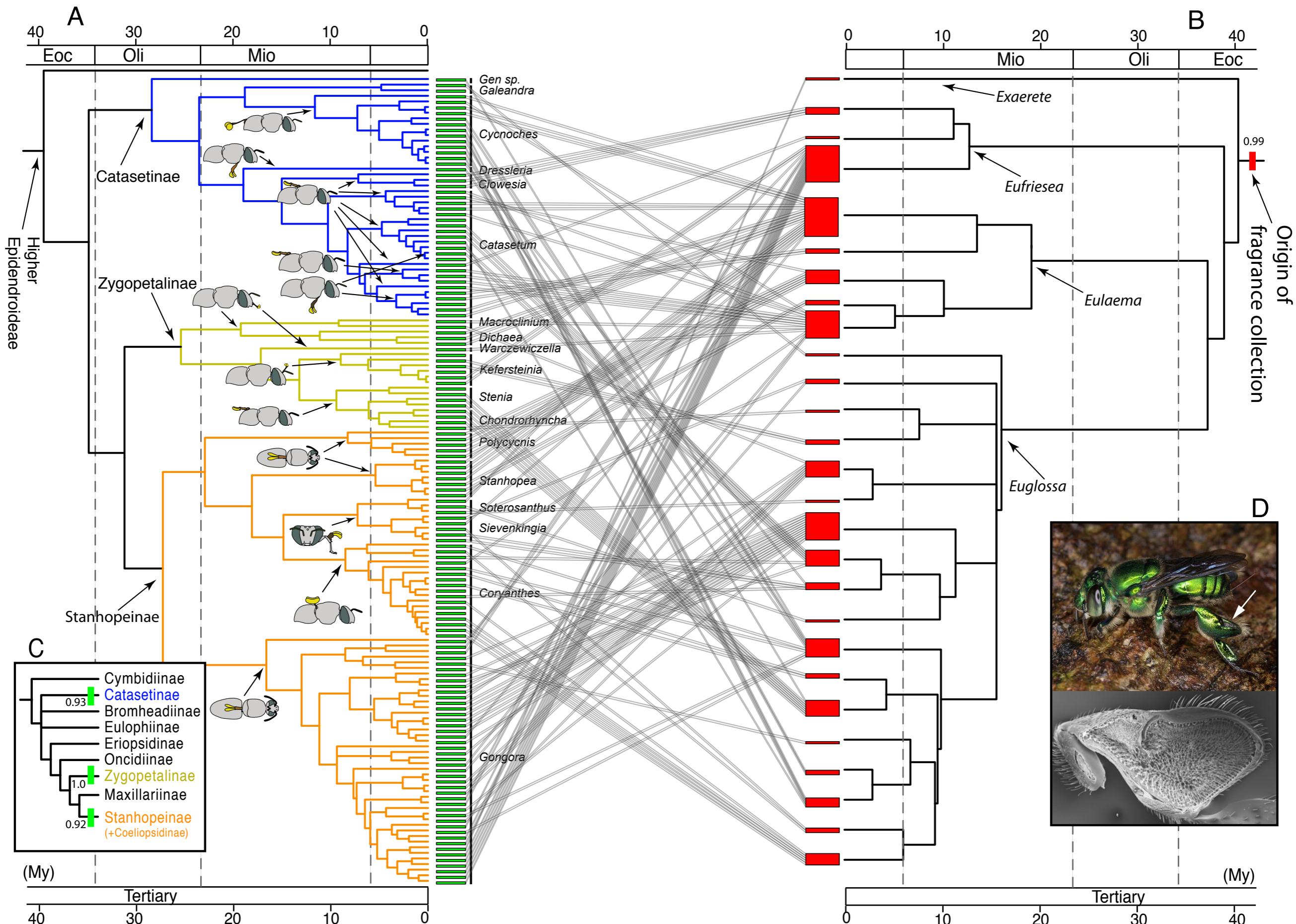
sequenced nrITS, ycf1
from ~150 pollinaria

Pollinaria sampling





Orchids

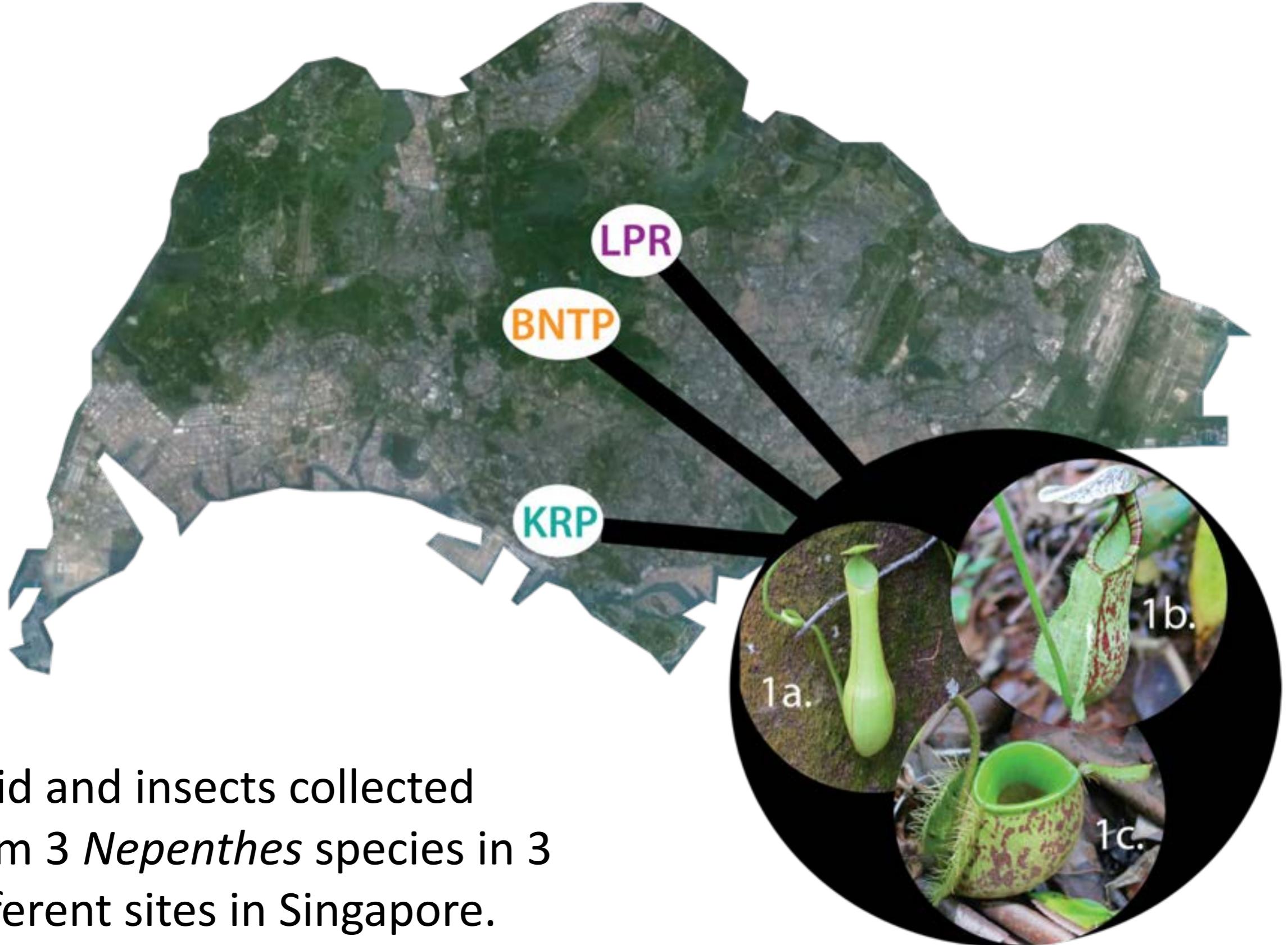




Leonora Bittleston







Fluid and insects collected
from 3 *Nepenthes* species in 3
different sites in Singapore.

Counts of macroscopic arthropods: 8 common ‘morphospecies’ including flies, mites and ants



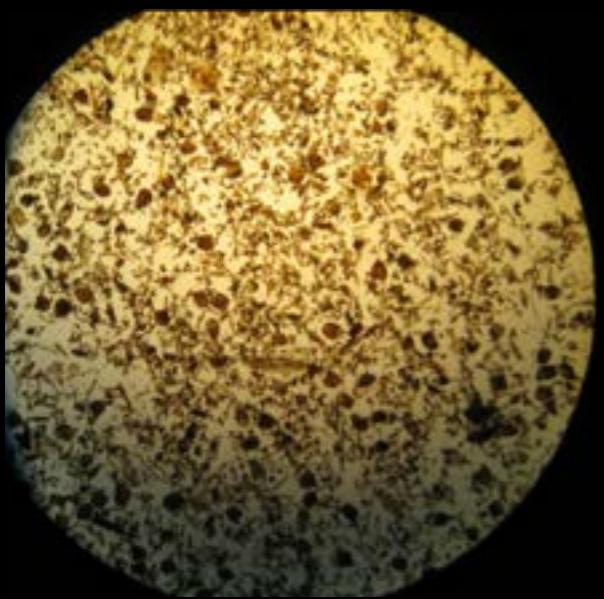
Mosquitoes: *Toxorhynchites*,
Culex, *Tripteroides*



Frog-biting midge: *Corethrella*



Scuttle fly: *Endonepentia*



Insect prey: mostly ant heads



Anoetid mites: *Zwickia*



Biting midge: *Dasyhelea*



Gall midge: *Lestodiplosis*

Counts by eye versus sequencing diversity

Counts

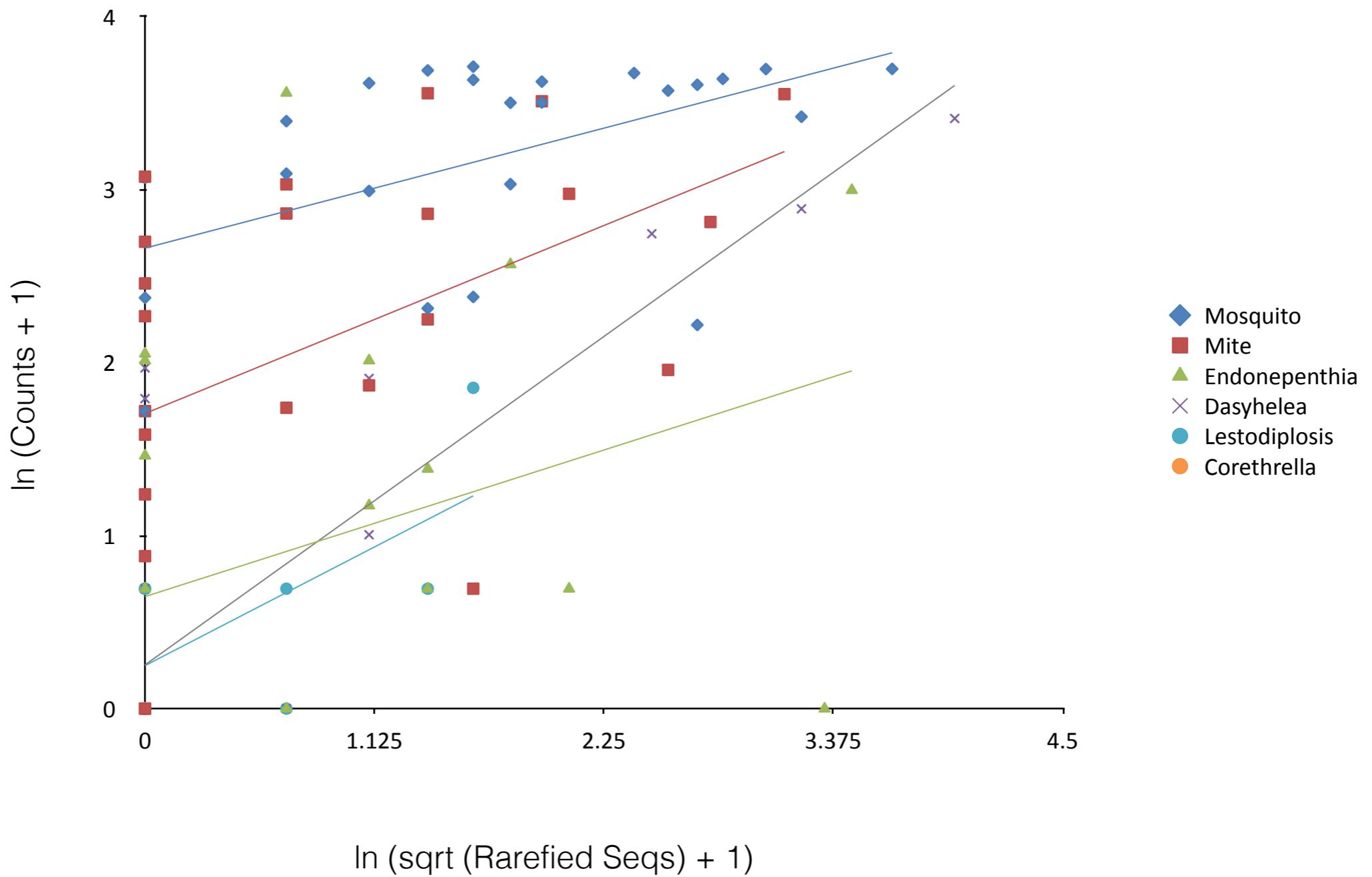
- 8 arthropod “morphospecies”

Metabarcoding

- 189 arthropod “OTUs” in rarefied table
 - Plus parasites, algae, protozoa, etc.

However, it's difficult to connect sequences to individuals, especially since reference sequences for most of these organisms are not yet in sequencing databases.

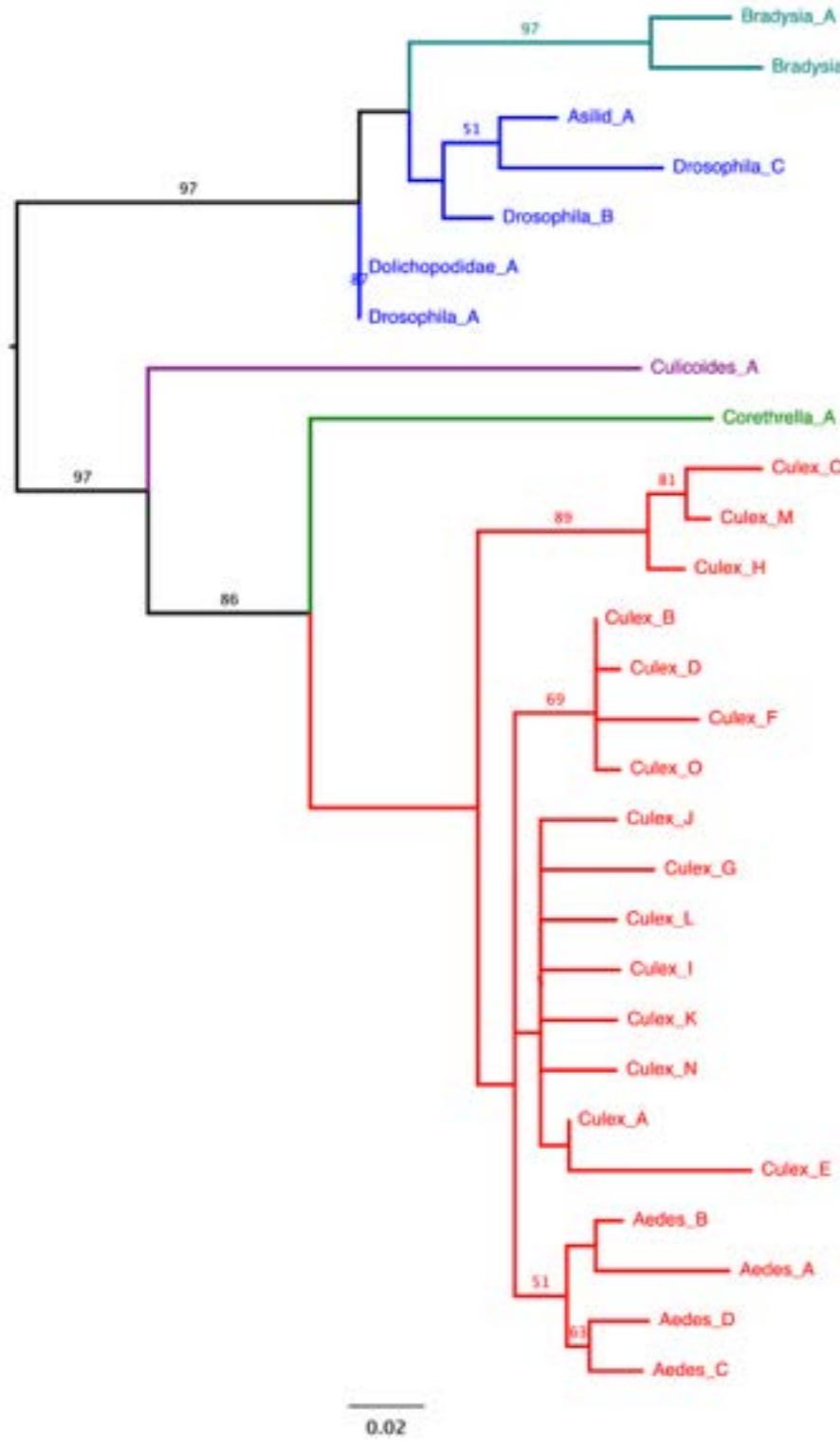
Inquiline counts and sequences generally match, but on different scales



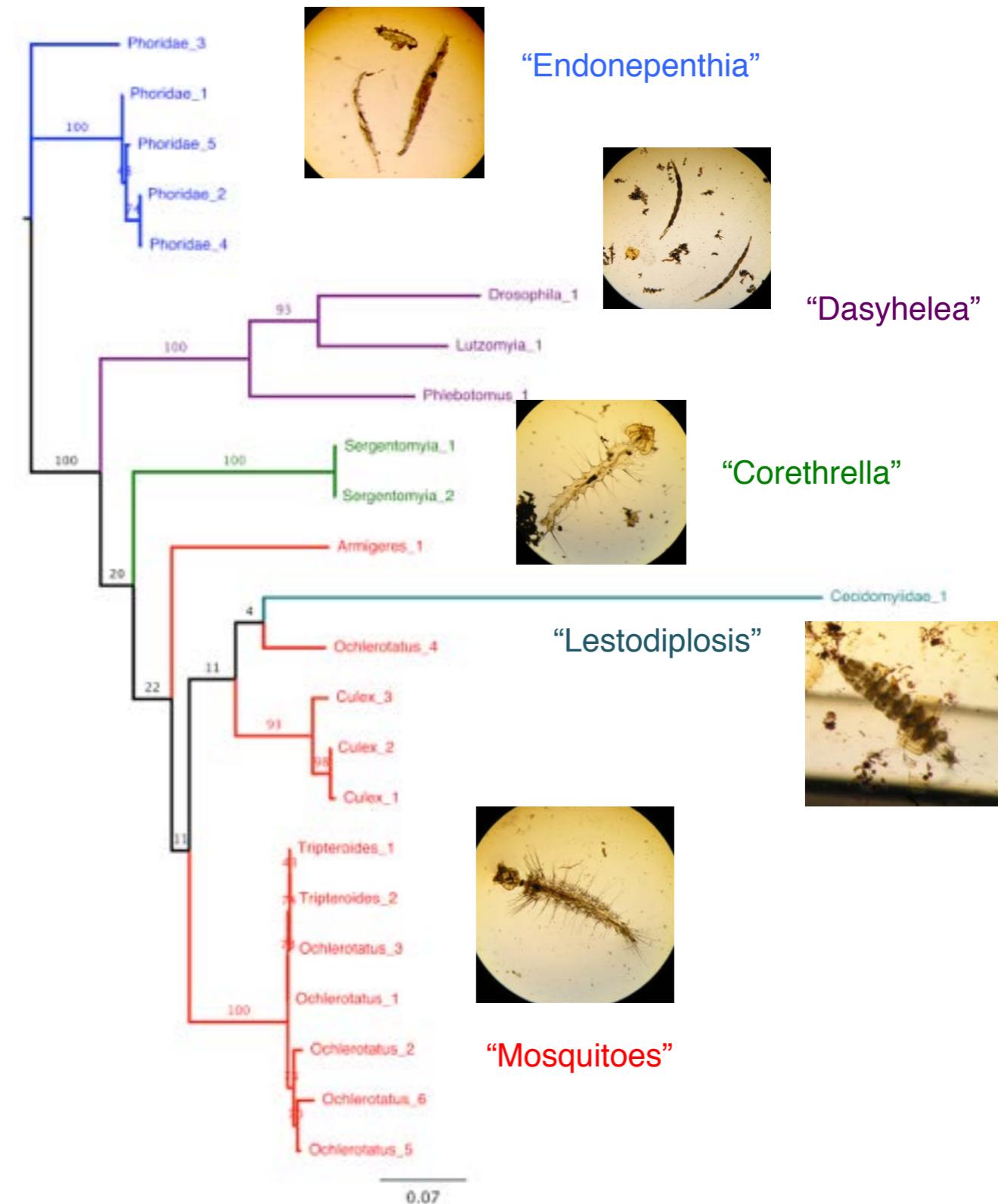
Mosquitoes and mites seem to be underrepresented in count data

Phylogenetic trees of dipteran inquilines

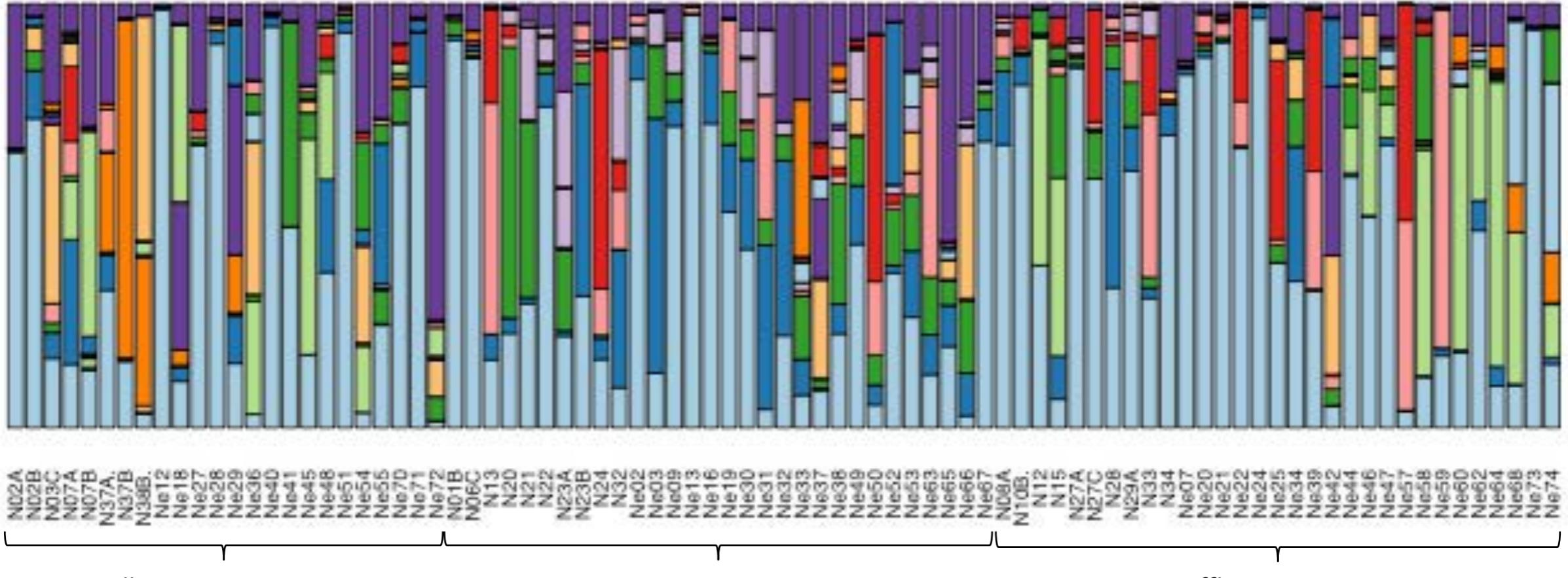
18S (ribosomal)



CO1 (mitochondrial)



Relative sequence abundance

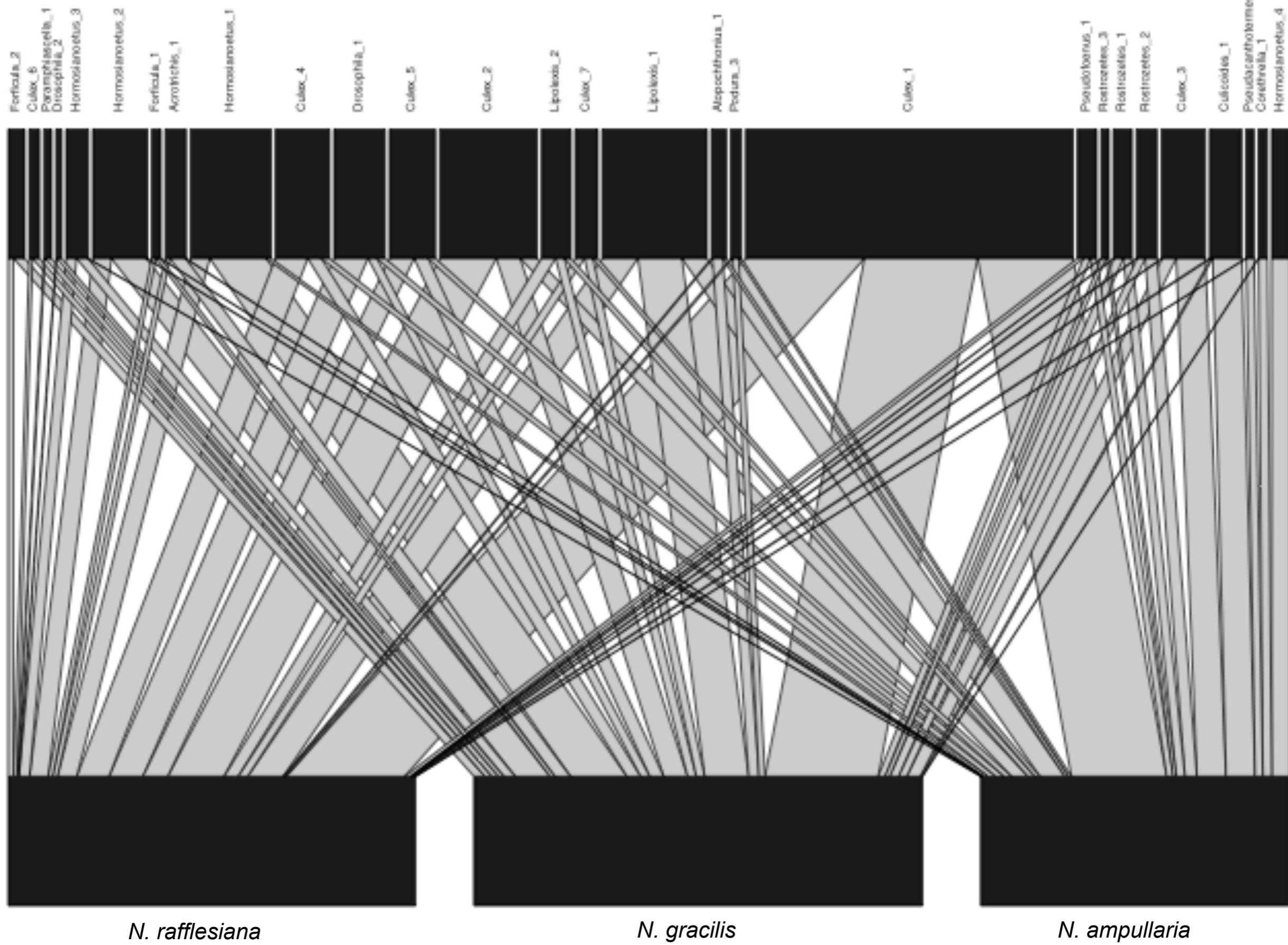
*N. ampullaria* communities*N. gracilis* communities*N. rafflesiana* communities

■ Other taxa
■ Acanthamoeba (amoebae)
■ Phaeodarea (protozoa)
■ Malacostraca (crustacea)
■ Cercomonas (protozoa)
■ Kinetoplastea (protozoa)
■ Microthamnion (algae)
■ Naididae (oligochaetes)
■ Bdelloid rotifer
■ Euglenida (protozoa)
■ Aeolosoma (annelids)
■ Heteromita (protozoa)
■ Lagenoeca (algae)
■ Conthreep (ciliates)
■ Saccharomycetes (yeast)
■ Goniomonas (algae)
■ Gregarininasina (protozoa)
■ Pseudomuriella (algae)
■ Arachnida (mostly mites)
■ Insecta (mostly dipterans)

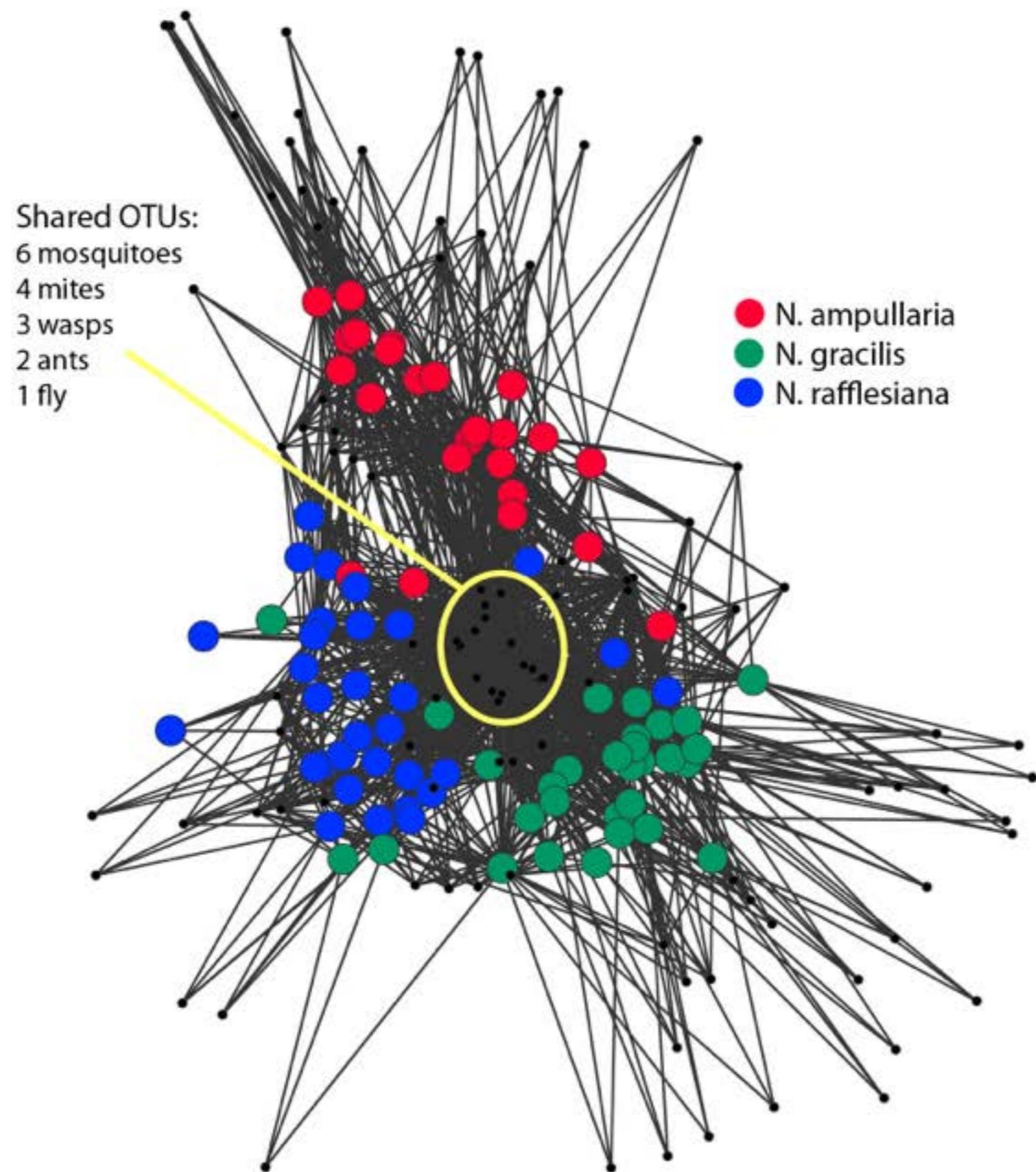
Taxon	% Of Total Seqs
Eukaryota; Opisthokonta; Metazoa; Arthropoda; Hexapoda; Insecta	41.90%
Eukaryota; Opisthokonta; Metazoa; Arthropoda; Chelicerata; Arachnida	8.10%
Eukaryota; Archaeplastida; Chloroplastida; Chlorophyta; Chlorophyceae; Pseudomuriella	6.40%
Eukaryota; SAR; Alveolata; Apicomplexa; Conoidasida; Gregarininasina	6.20%
Eukaryota; Cryptophyceae; Goniomonas; Goniomonas_sp._ATCC_50108;Other;Other	5.30%
Eukaryota; Opisthokonta; Fungi; Ascomycota; Saccharomycotina; Saccharomycetes	4.30%
Eukaryota; SAR; Alveolata; Ciliophora; Intramacronucleata; Conthreep	3.40%
Eukaryota; Opisthokonta; Holozoa; Choanomonada; Craspedida; Lagenoeca	2.10%
Eukaryota; SAR; Rhizaria; Cercozoa; Glissomonadida; Heteromita	2.10%
Eukaryota; Opisthokonta; Metazoa; Annelida; Family_Incertae_Sedis; Aeolosoma	1.60%
Eukaryota; Excavata; Discoba; Discicristata; Euglenozoa; Euglenida	1.50%
Eukaryota; Opisthokonta; Metazoa; Rotifera; Philodinidae; uncultured_bdelloid_rotifer	1.50%
Eukaryota; Opisthokonta; Metazoa; Annelida; Family_Incertae_Sedis; Naididae	1.20%
Eukaryota; Archaeplastida; Chloroplastida; Chlorophyta; Trebouxiophyceae; Microthamnion	1.00%
Eukaryota; Excavata; Discoba; Discicristata; Euglenozoa; Kinetoplastea	0.80%
Eukaryota; SAR; Rhizaria; Cercozoa; Cercomonadidae; Cercomonas	0.80%
Eukaryota; Opisthokonta; Metazoa; Arthropoda; Crustacea; Malacostraca	0.70%
Eukaryota; SAR; Rhizaria; Cercozoa; Thecofilosea; Phaeodarea	0.70%
Eukaryota; Amoebozoa; Discosea; Longamoebia; Centramoebida; Acanthamoeba	0.50%
Other Taxa	9.90%

Obligate insect
parasites!

Bipartite network of arthropods and pitcher plants



Spring-loaded
network of core
OTUs present in at
least 10% of the
samples from
each host species.



Networks can
reveal which
organisms are
shared across
different host
species and
samples.

Myrmecophiles of the ants of *Vachellia (Acacia) drepanolobium*





Chris Baker



Dino Martins

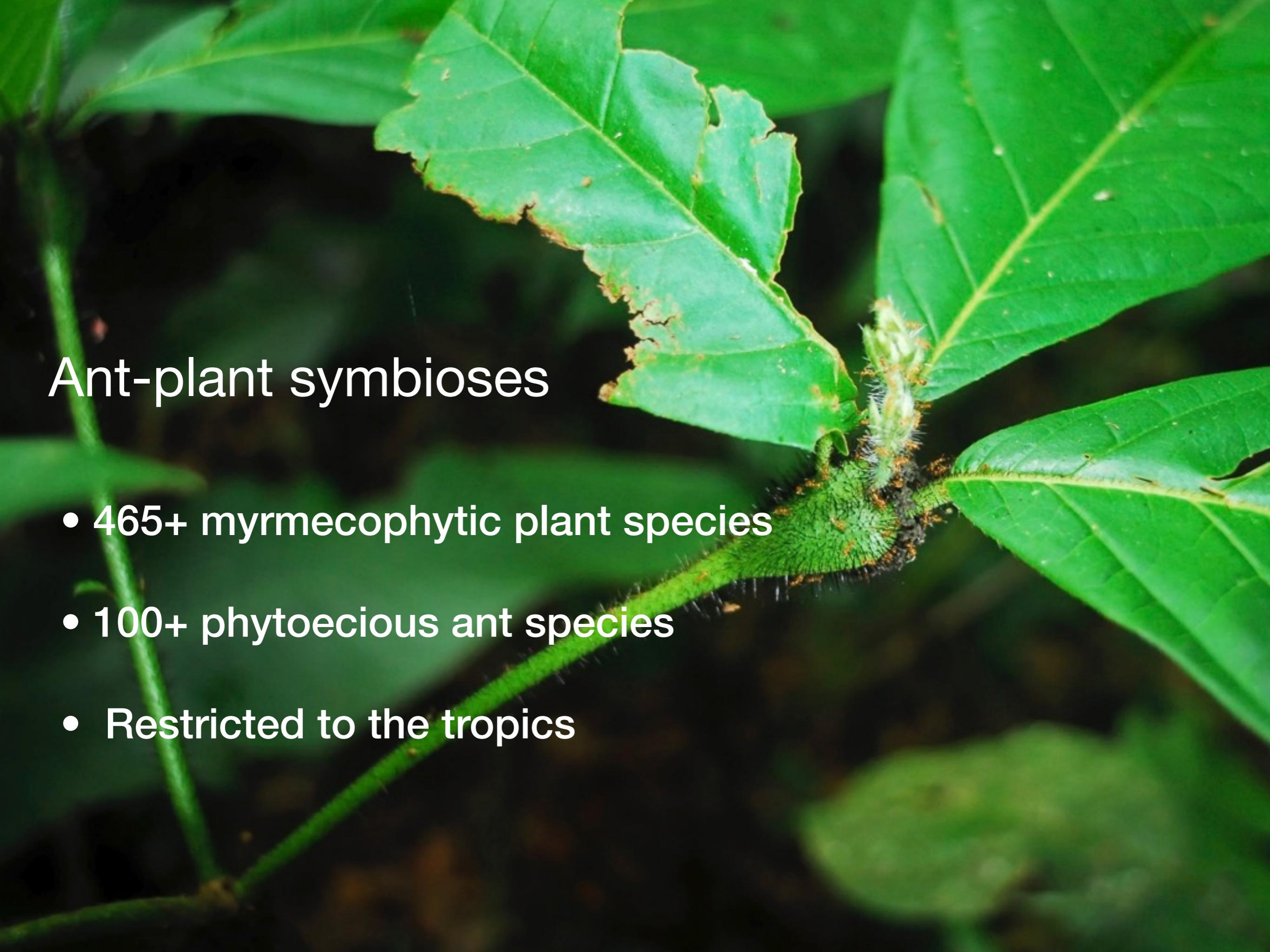


Jack Boyle



Julianne
Pelaez

Lori
Shapiro



Ant-plant symbioses

- 465+ myrmecophytic plant species
- 100+ phytoecious ant species
- Restricted to the tropics

An aerial photograph showing a vast, wetland landscape in East Africa. The terrain is a mix of green grassy areas and brown, dry, cracked earth. Numerous small, dark blue lakes and ponds are scattered across the land. A large, dark blue body of water, likely the Indian Ocean, is visible on the right side. The horizon shows a flat coastline under a clear sky.

East Africa...

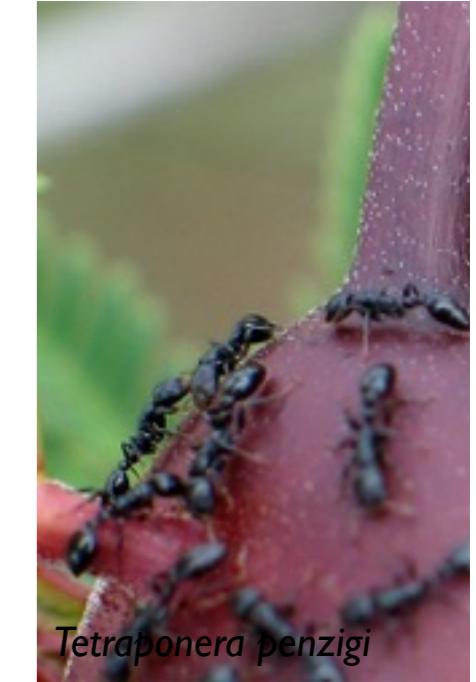
Vachellia drepanolobium is a dominant component of the flora of East Africa



Vachellia drepanolobium
with swollen thorn ant domatia



Up to 16 different ant species have been recorded inhabiting *Vachellia drepanolobium*:



Three primary ant species
occupy trees throughout
Kenya, typically one colony
per tree



Crematogaster mimosae

CM



Crematogaster nigriceps

CN



Tetraponera penzigi

TP



Two species of *Crematogaster*;
One *Tetraponera*

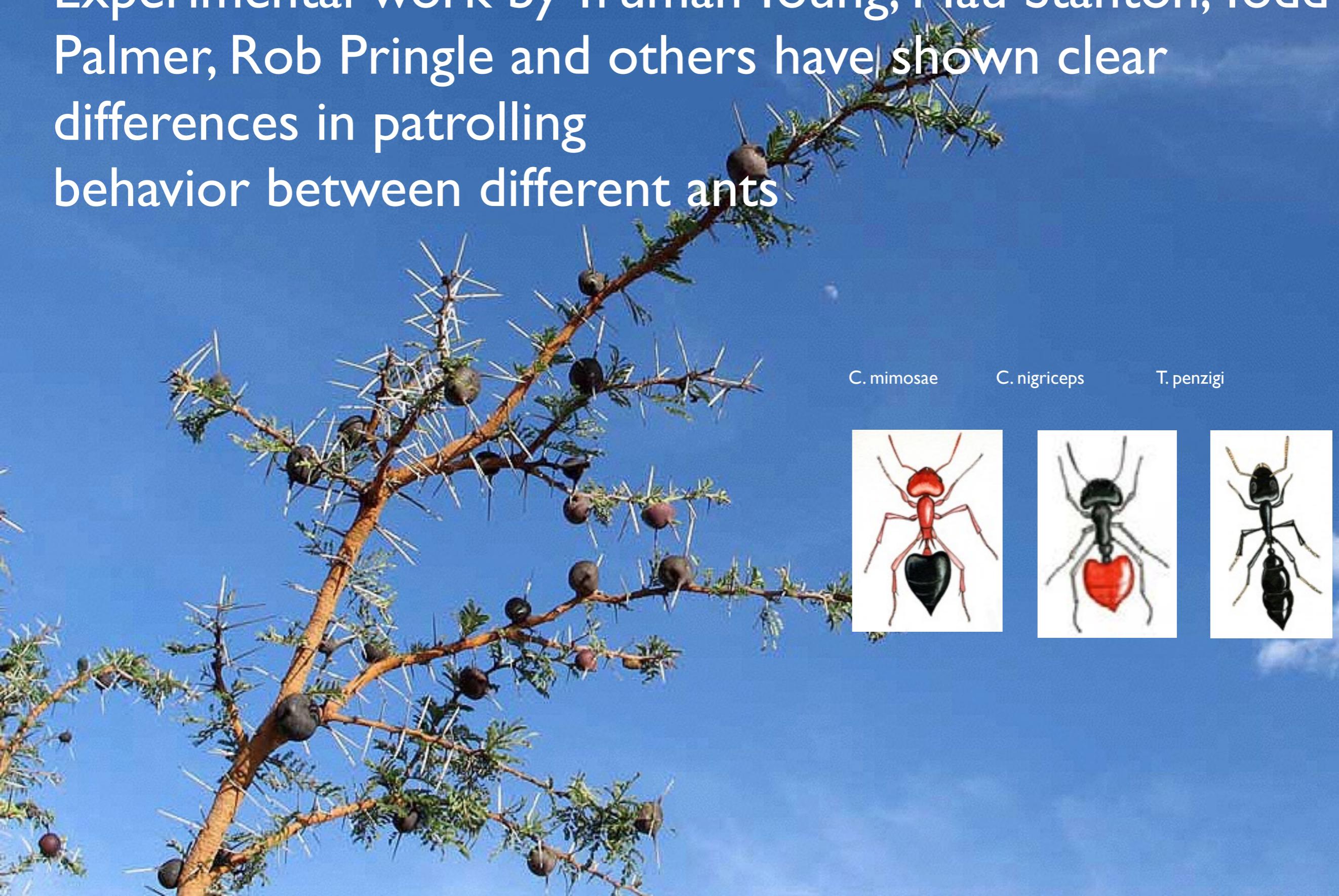
Illustrations by Dino Martins

The Plants
Domatia
Extra-floral
nectaries

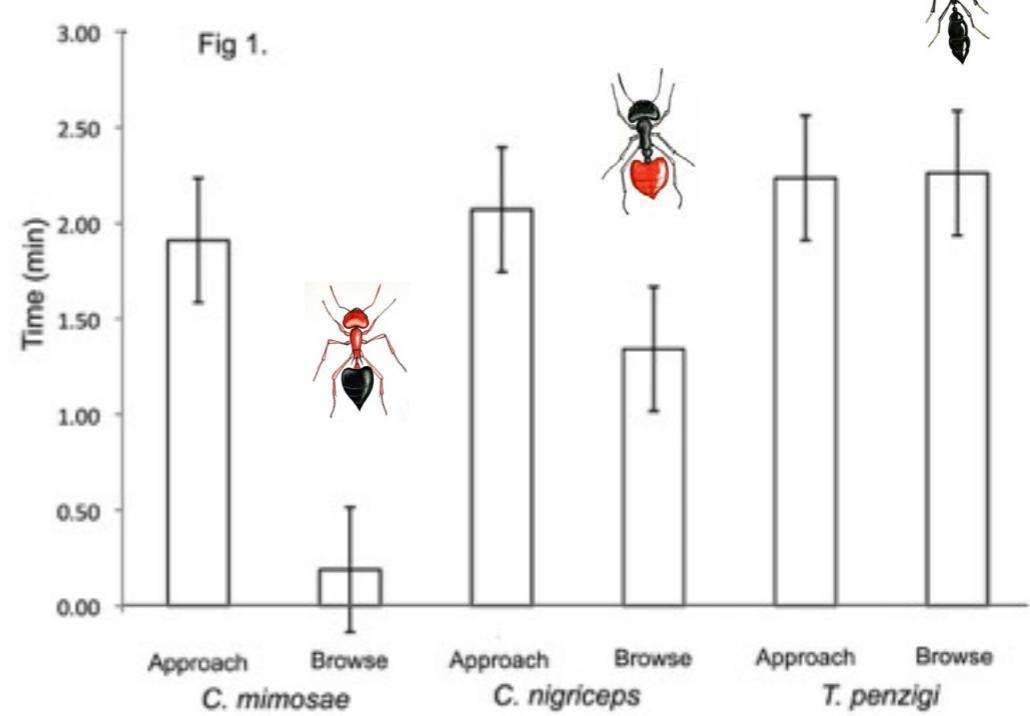
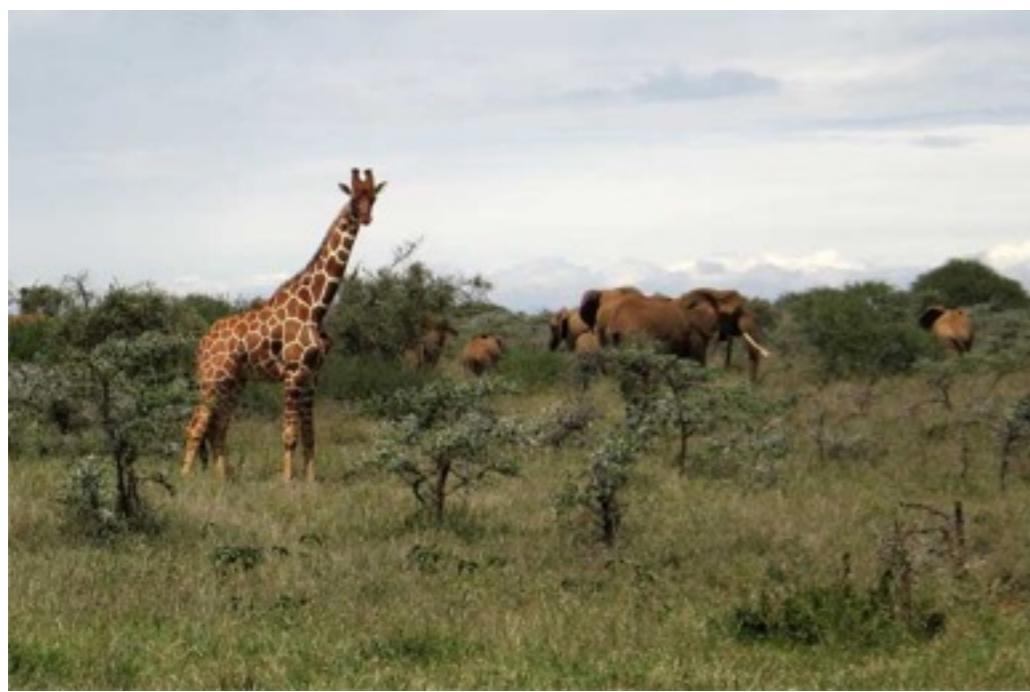


The Ants
Defensive
patrolling

Experimental work by Truman Young, Mau Stanton, Todd Palmer, Rob Pringle and others have shown clear differences in patrolling behavior between different ants



For example, Dino Martins showed that browsing giraffe spend less time feeding on plants inhabited by *C. mimosae*



This system has many third party associates



Expanding the network



Expanding the network



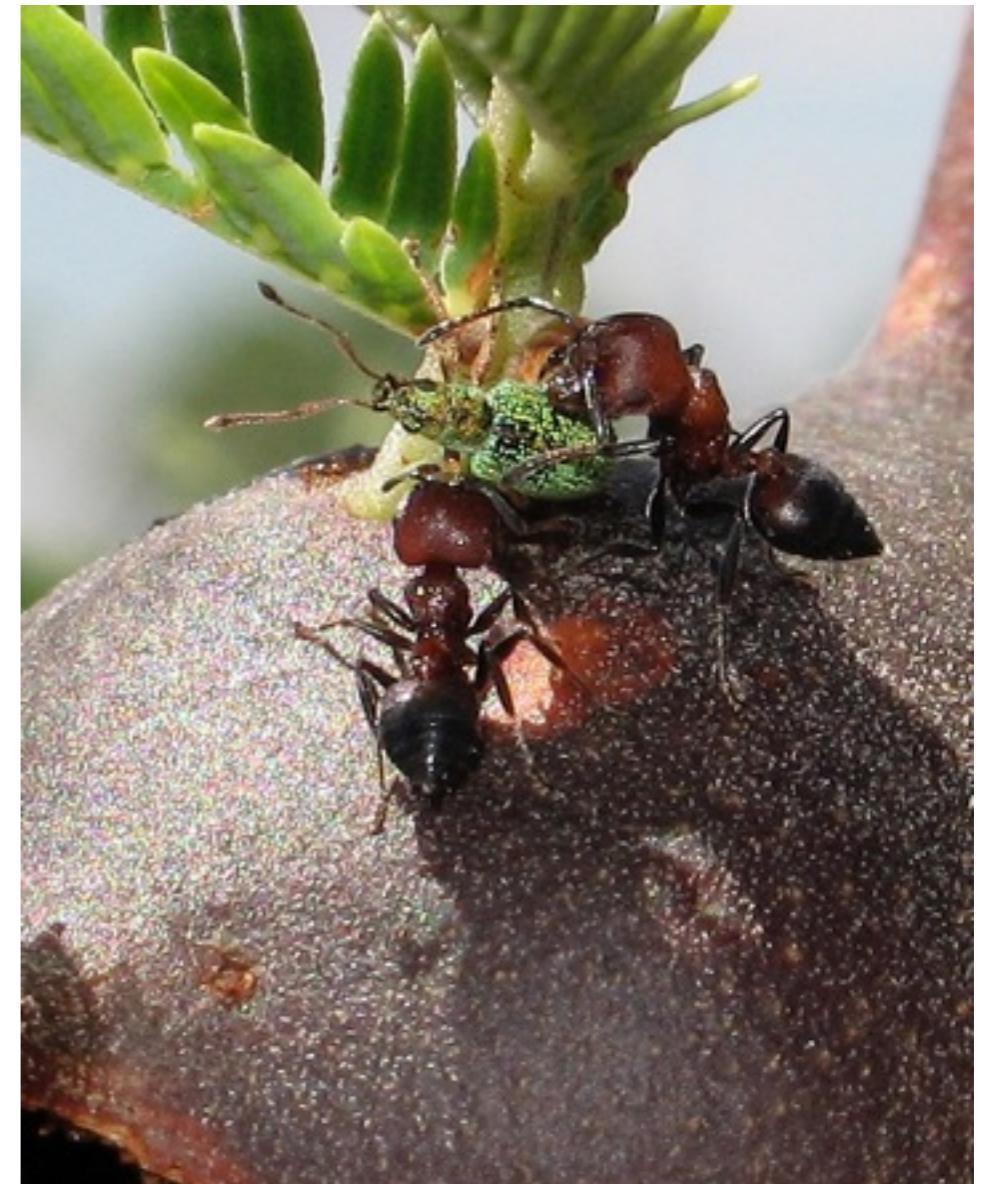
Microdon - a parasitic fly



Zoraptera in *Tetraponera domatium*
(feed on fungal spores and hyphae— < 3mm long)



Third parties in this system appear to interact at both the mutualistic and parasitic end of the spectrum



A systematic survey?

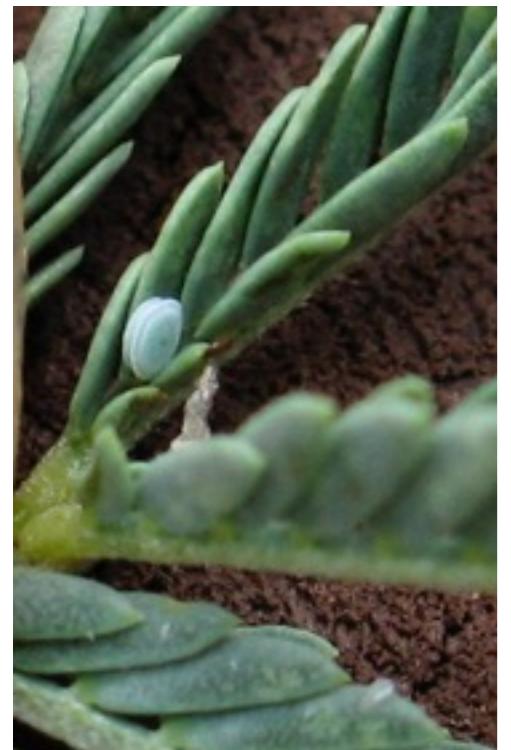
Lycaenid butterflies

Anthene usamba

Lycaenid butterfly that lays eggs on the acacias.

The larvae are chemically defended against ant attacks.

Do females prefer the more aggressive ant mutualist?



Preferences tested in bush house

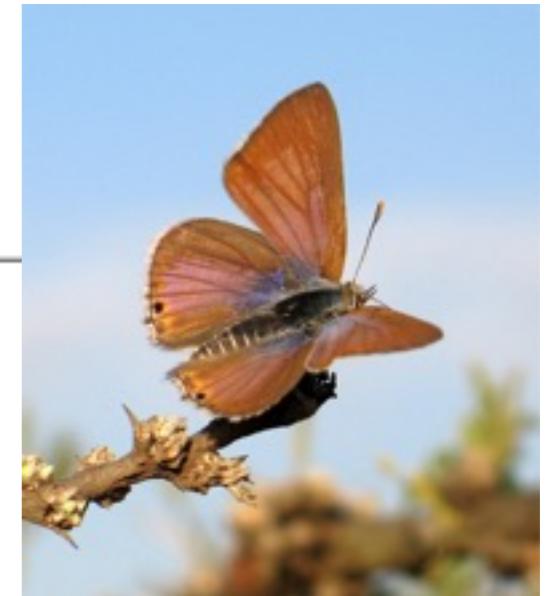
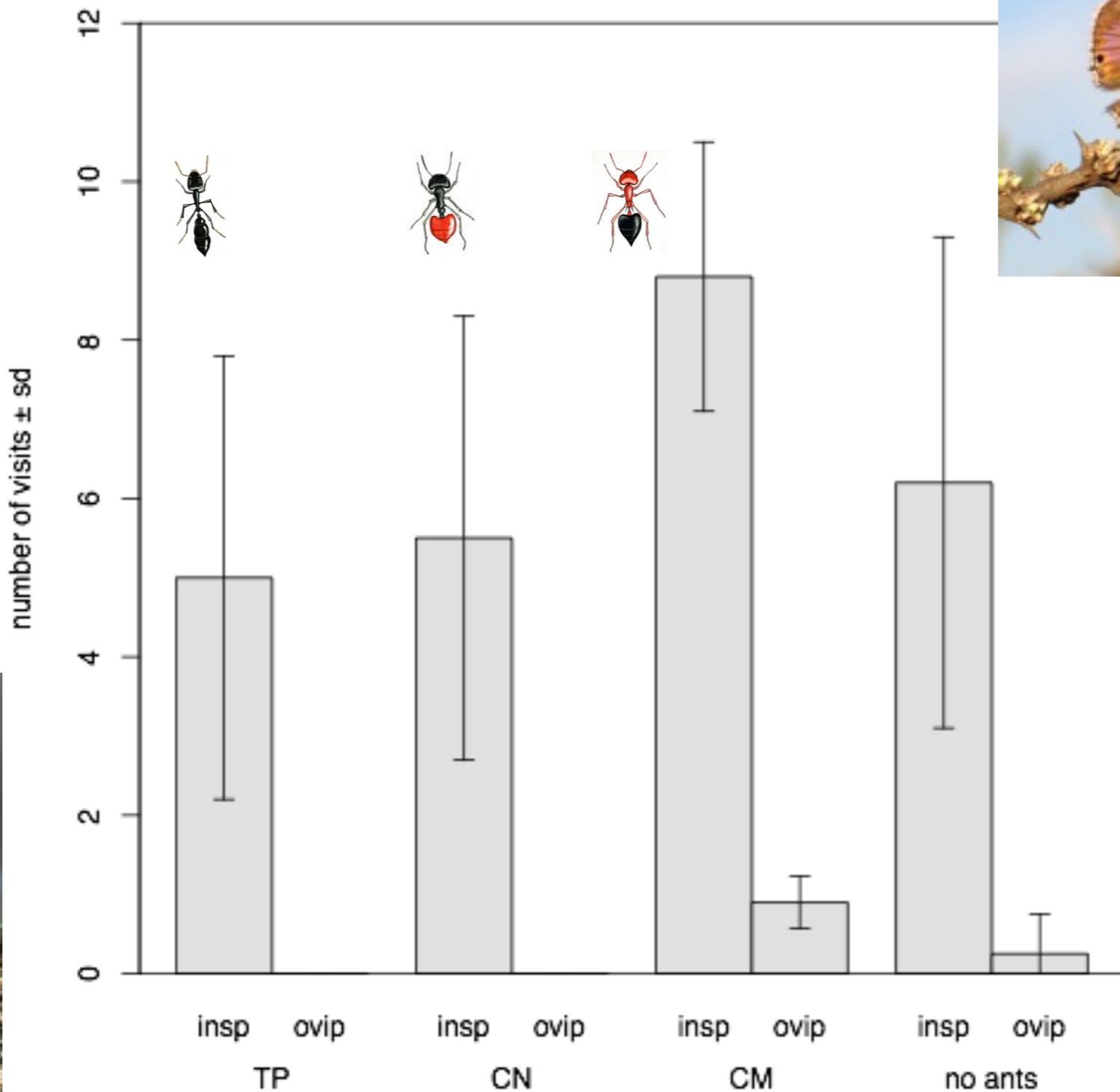
Oviposition Experiment

Females were given a choice of saplings inhabited by 3 different ant species, or no ants:

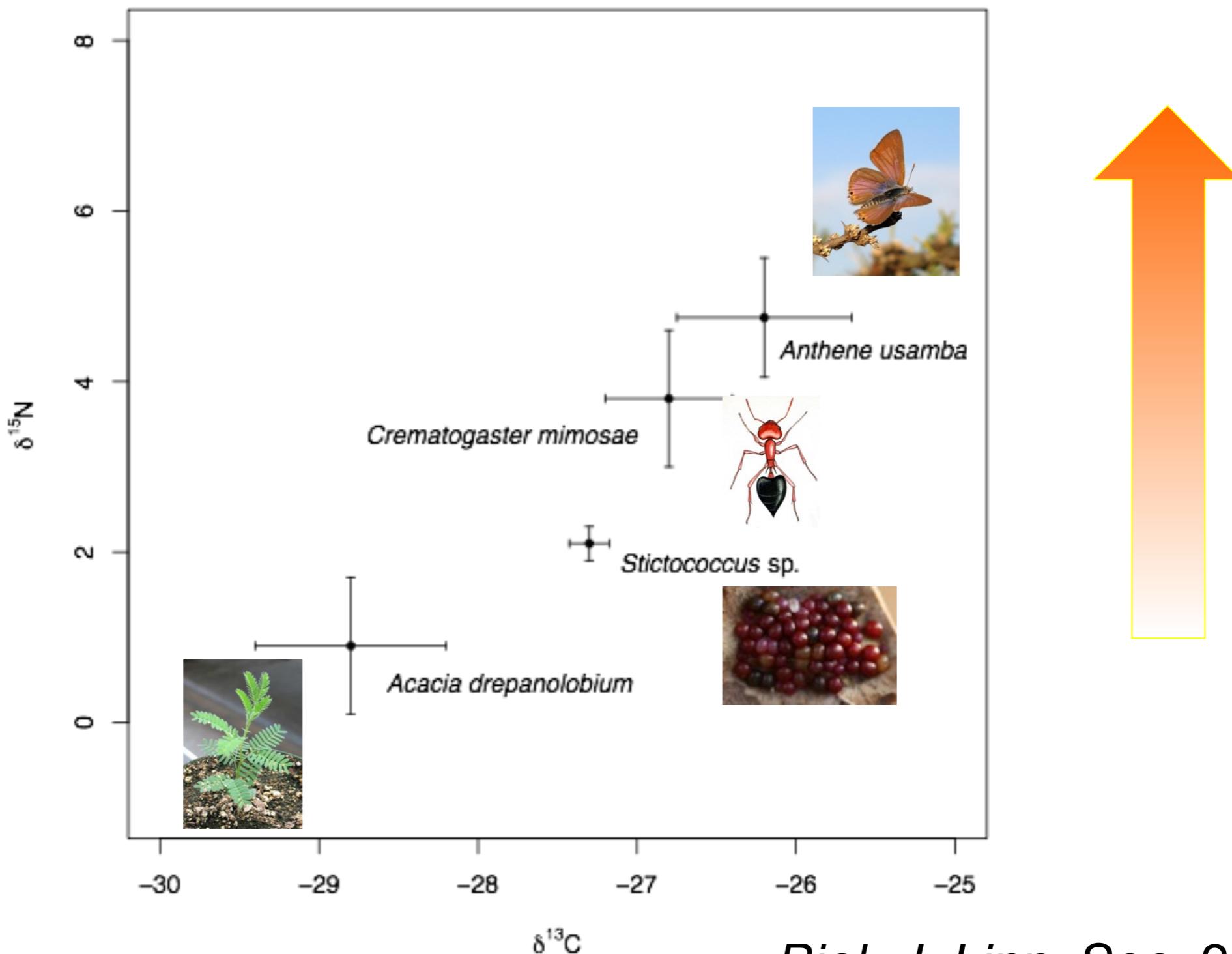
- *C. mimosae*
- *C. nigriceps*
- *T. penzigi*
- No ants



Females of *Anthene usamba* laid eggs on plants with *C. mimosae* or no ants



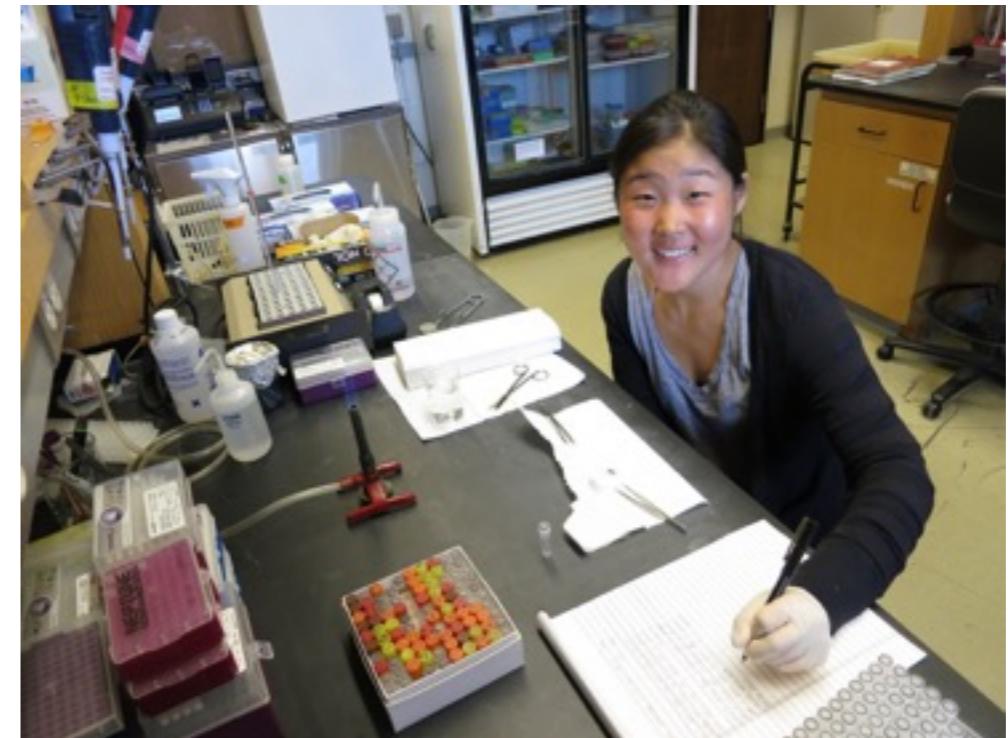
Stable isotope analysis indicates that *Anthene usamba* is carnivorous, and is likely to be eating ants inside the domatia.



45 species of lycaenids parasitize acacia-ant mutualisms



Our survey has involved collecting and processing many individual ant colonies from each of the three species throughout their range



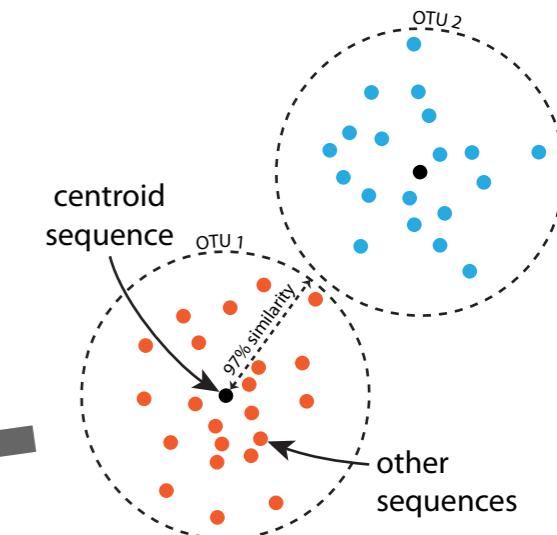
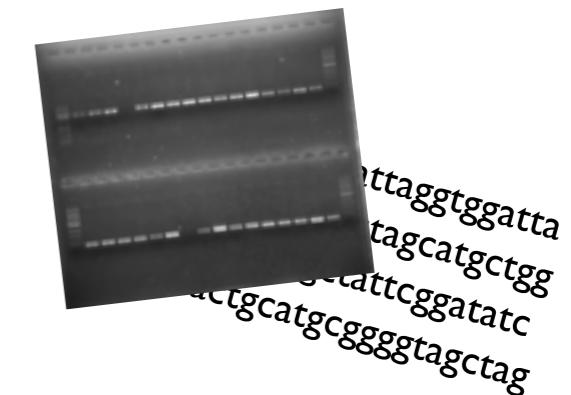
Workflow



DNA extraction



PCR + sequencing
COI primers



statistical and
genetic analyses



assign taxonomy
where possible

treat each OTU
as a distinct taxon

group similar
sequences
into 'OTU's

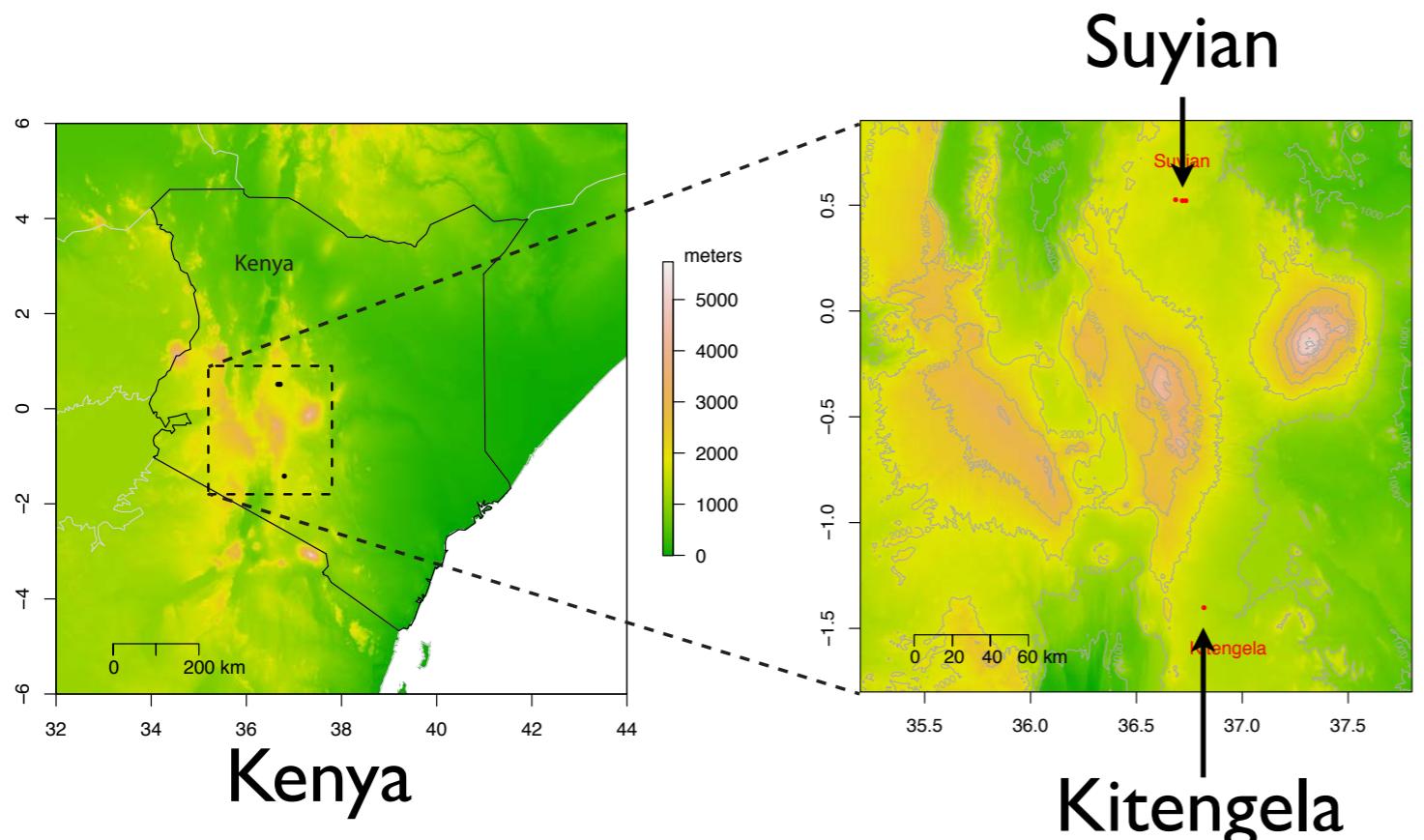
Numbers summary for myrmecophile data

480 trees sorted
(162 CM, 163 CN, 140 TP)

22,229 domatia

176 trees without myrmecophiles

304 trees with myrmecophiles



2361 myrmecophiles
(median = 3 per tree)

1091 with
sequence data

82 different OTUs

1270 without
sequence data
(6 high abundance taxa)

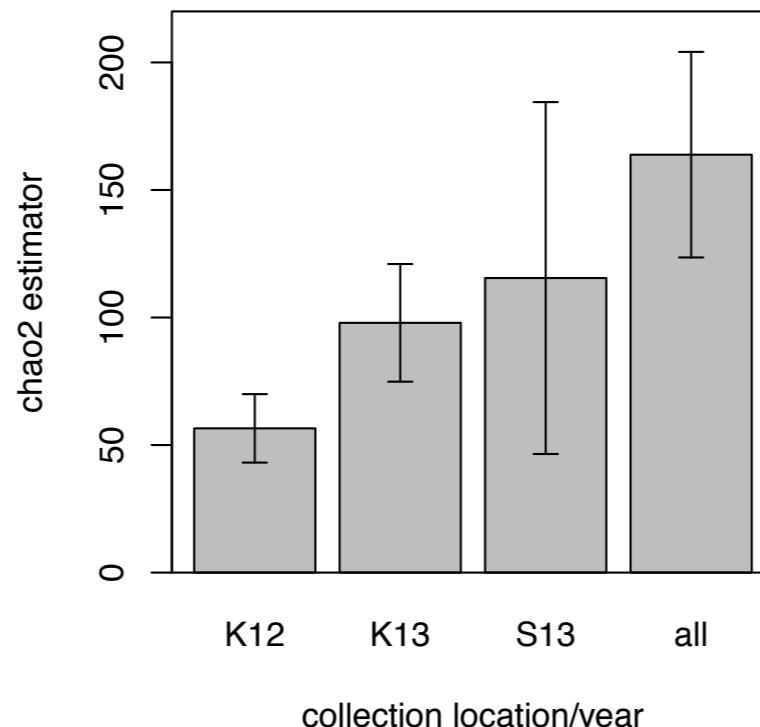
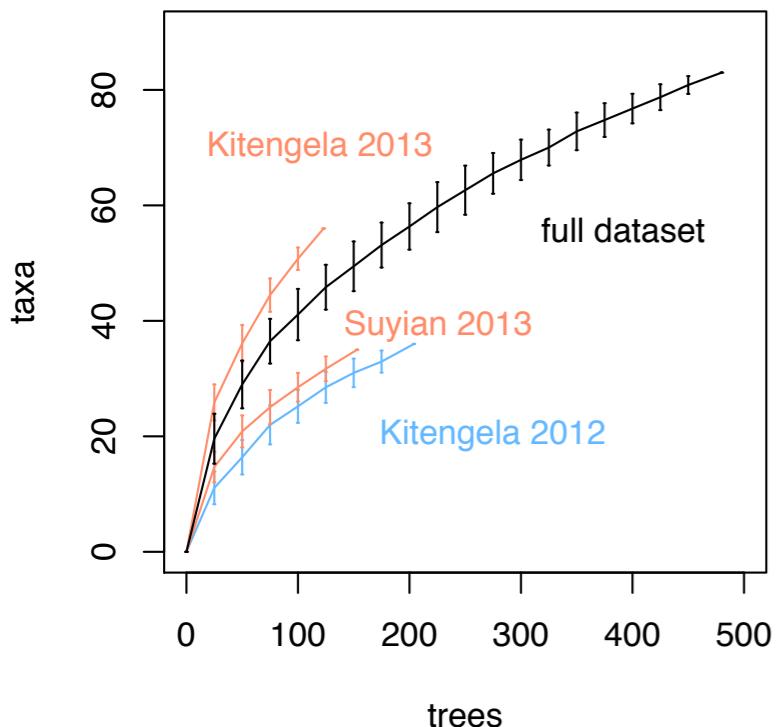
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Pelaez Aug 2013
K13.019A.004



Pelaez Aug 2013
K13.046A.003



Pelaez Aug 2013
S13.209.010



Pelaez Aug 2013
S13.221.002



Pelaez Aug 2013
K13.056A.003



Pelaez Aug 2013
K13.019A.003



Pelaez Aug 2013
K13.131A.002



Pelaez Aug 2013
K13.158A.002



Pelaez Aug 2013
K13.003A.002



Pelaez Aug 2013
K13.046A.005



Pelaez Aug 2013
S13.222.001



Pelaez Aug 2013
S13.224.003



Pelaez Aug 2013
K13.003A.001



Pelaez Aug 2013
K13.056A.002



Pelaez Aug 2013
S13.222.008



Pelaez Aug 2013
S13.222.012



Pelaez Aug 2013
K13.087A.001



Pelaez Aug 2013
S13.222.010



Pelaez Aug 2013
K13.004A.008



Pelaez Aug 2013
K13.009A.005



Pelaez Aug 2013
K13.019A.001



Pelaez Aug 2013
S13.204.026



Pelaez Aug 2013
K13.056A.001

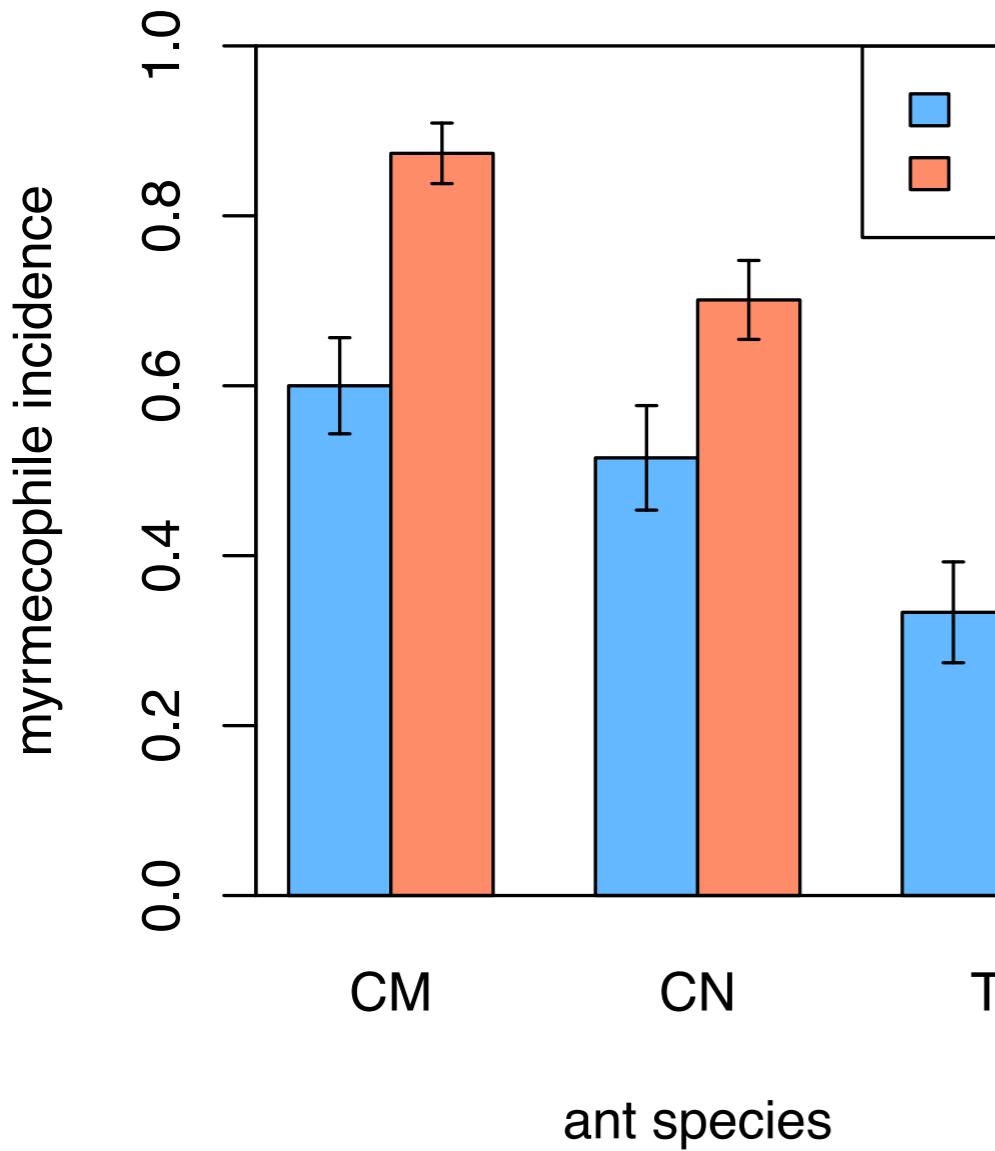


Pelaez Aug 2013
K13.017A.002

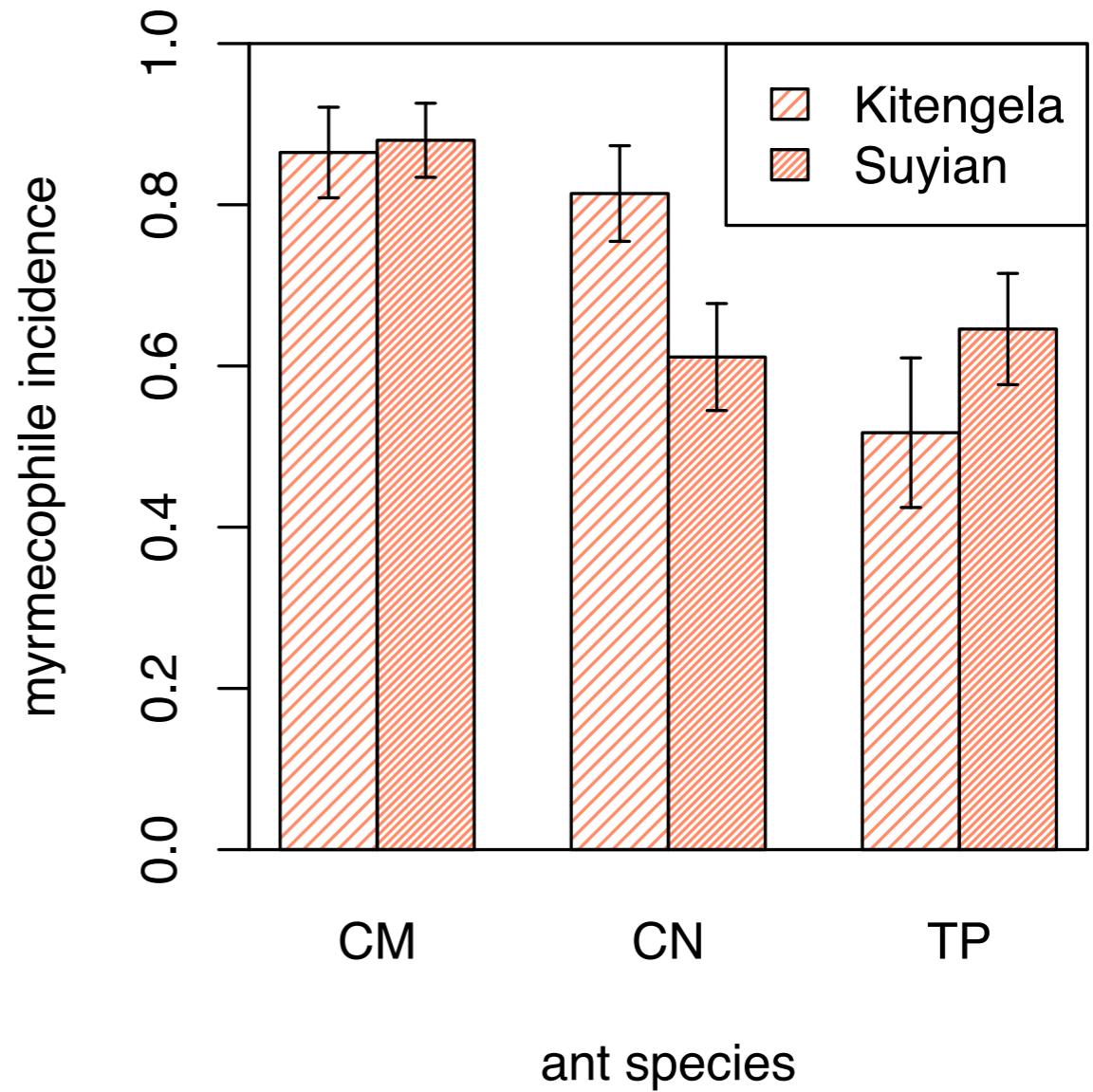


Trees occupied by *C. mimosae* have more myrmecophiles than those inhabited by *C. nigriceps* or *T. penzigi*

All samples

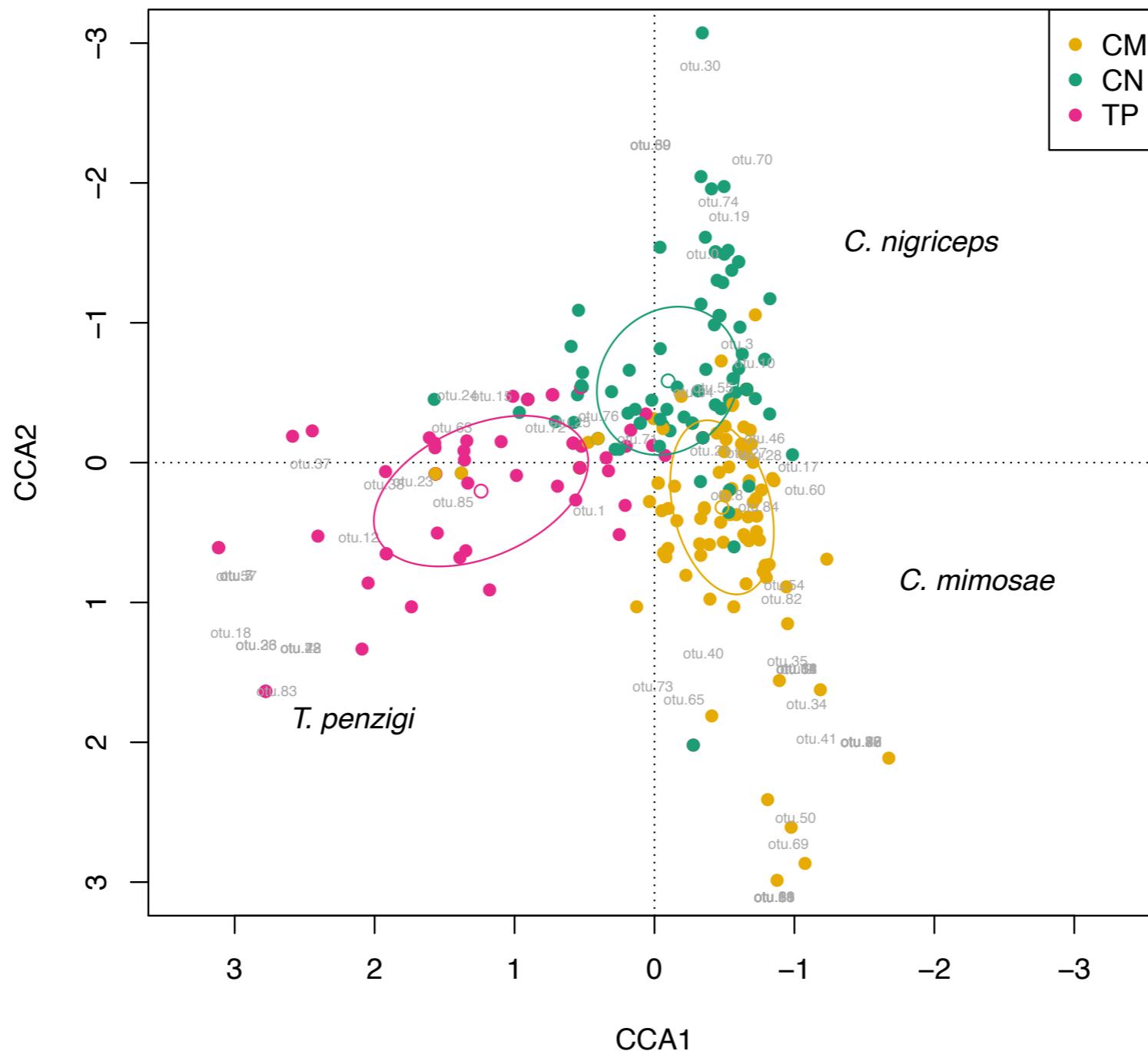


2013 samples only



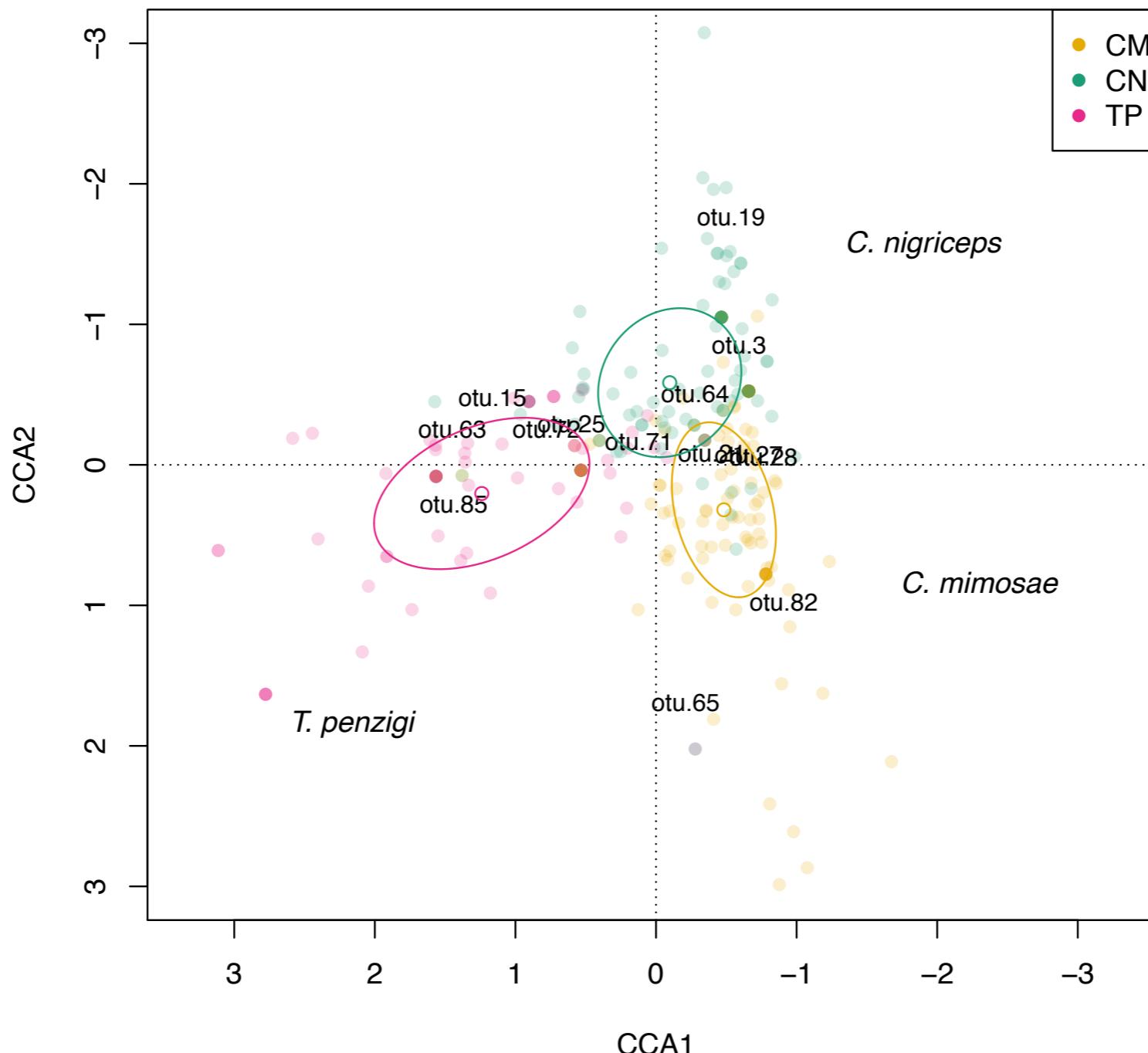
Different ants have different myrmecophile communities

2012–13 samples / all myrmecophiles



Different ants have different myrmecophile communities

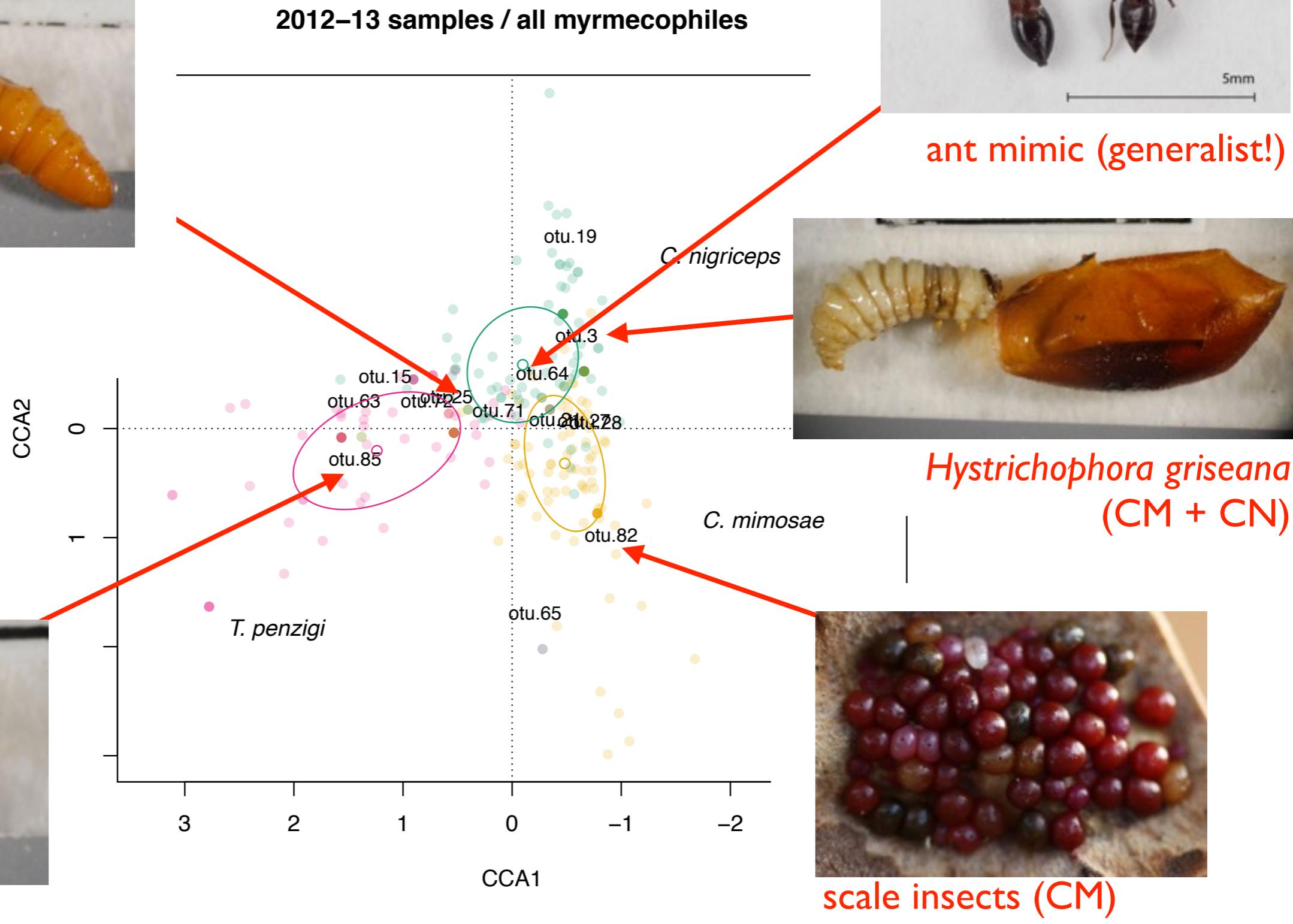
2012–13 samples / all myrmecophiles



Specialists and generalists



Dichomeris sp.
(generalist)



Photographs and barcodes can be used to establish life histories



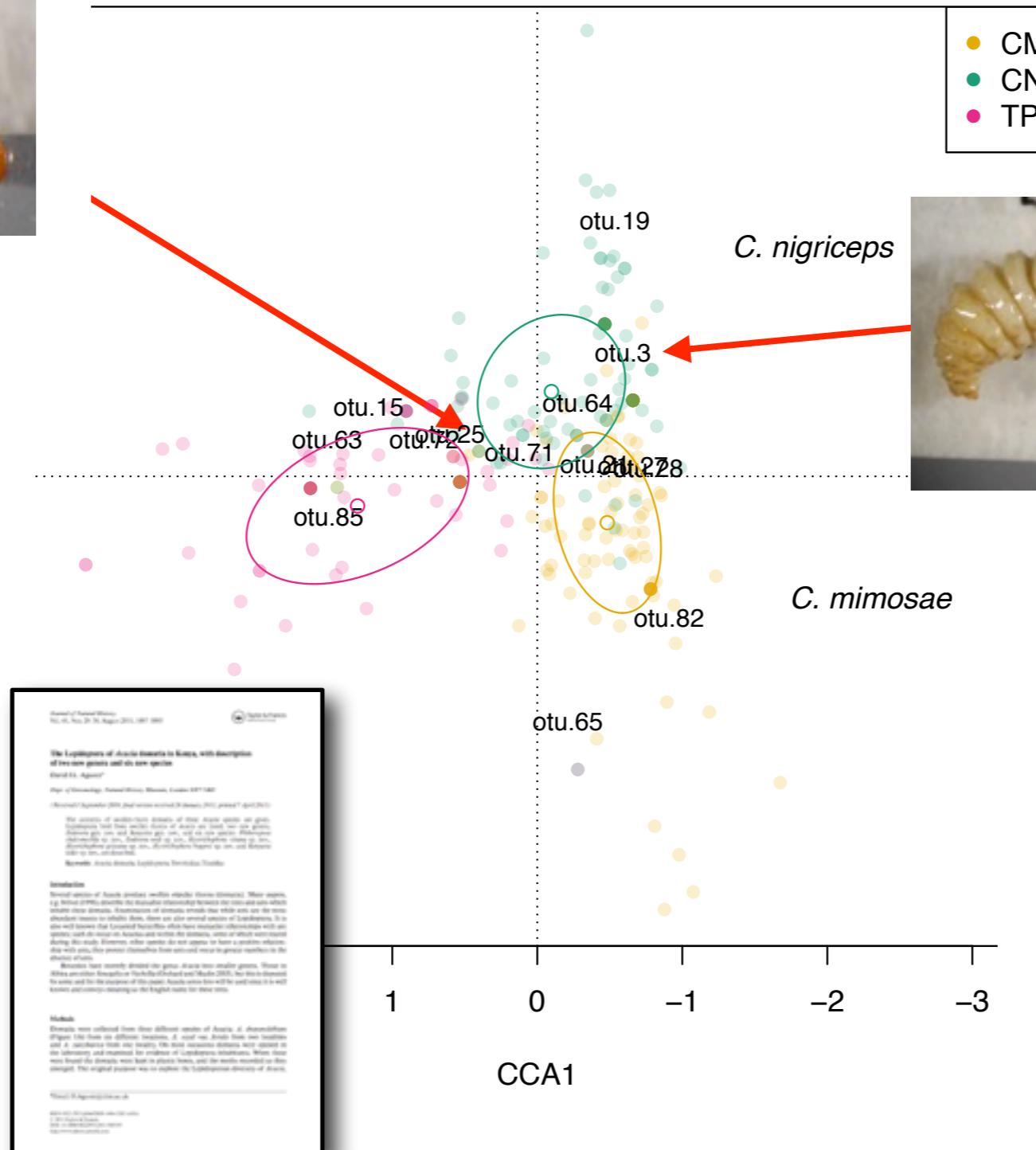
Dichomeris sp.
(generalist)

K13.067.002



Agassiz 2011

2012–13 samples / all myrmecophiles



Hystrichophora griseana
(CM + CN)



Myrmecophiles

Overall incidence

- Varies strongly with year and location
- *C. mimosae* more likely to have myrmecophiles

Taxonomic breakdown

- Lepidoptera dominate, then spiders
- Domatium communities distinct from canopy communities

Community composition

- Strong effect of location, year, and ant species
- Strong ant specificity not common, but does occur

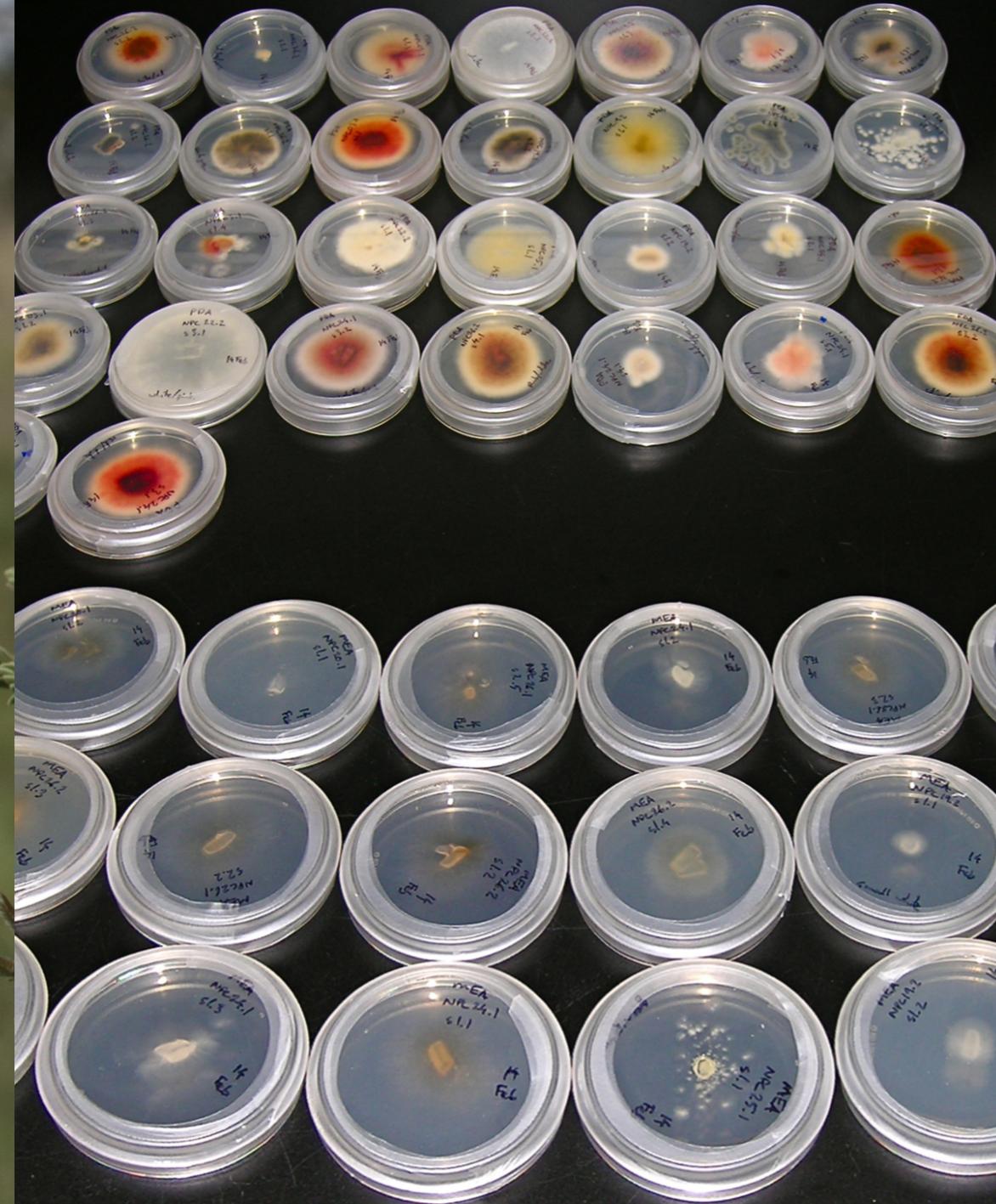
Additional potential for barcoding

- Allows analysis of broad communities by non-taxonomists
- Link up life history information
- Population genetics analysis

Expanding the network



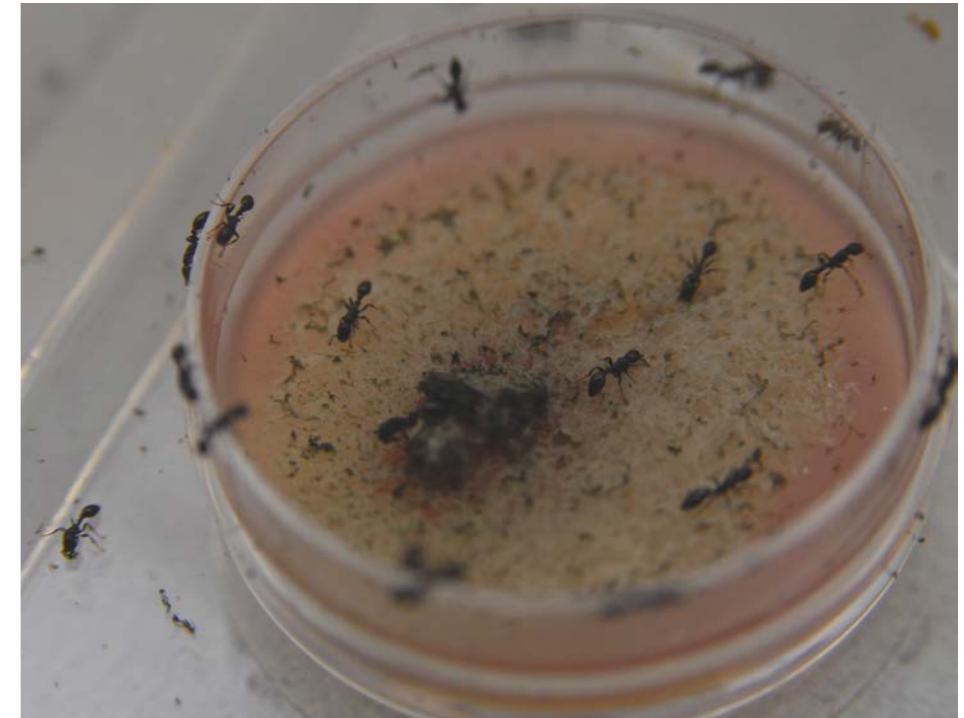
Expanding the network



Fungal communities

- How many species?
- What species?
- What governs diversity?
- What role in system?

Why fungal communities?

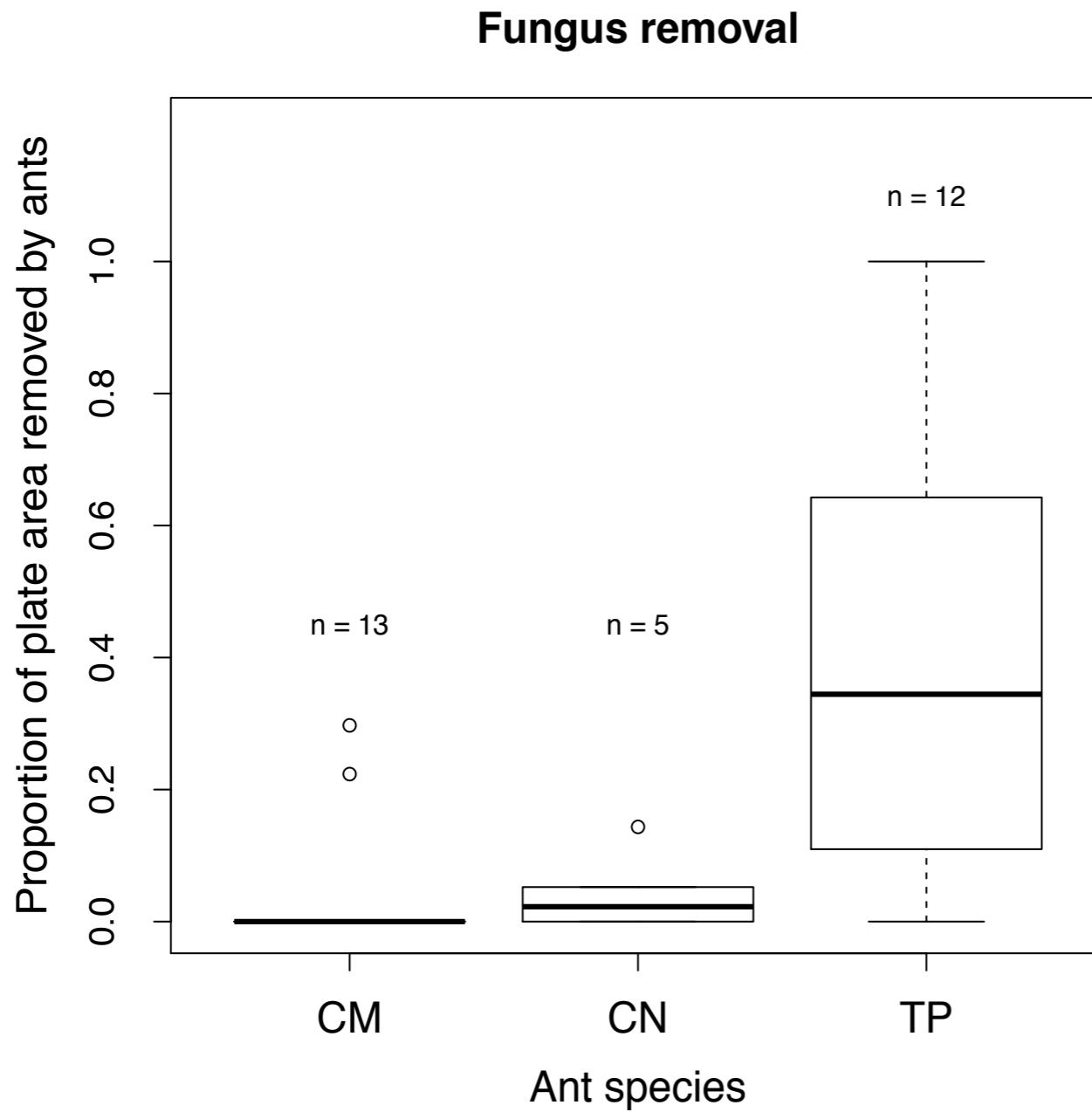


Dino Martins



Showed that workers of *Tetraponera penzigi* seemed to be cultivating and harvesting white fungus in nest tubes in the lab

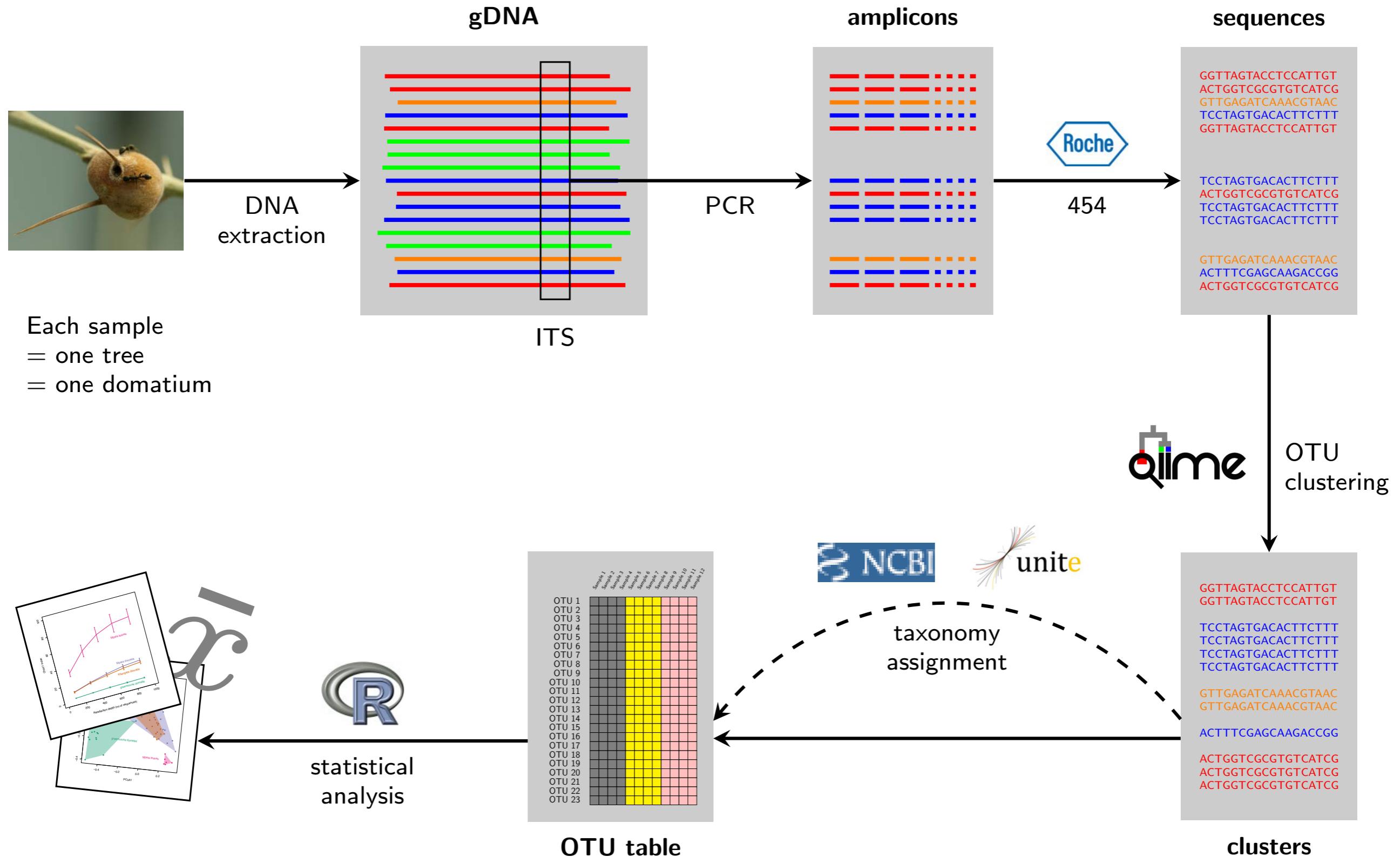
Why fungal communities?



Chris Baker

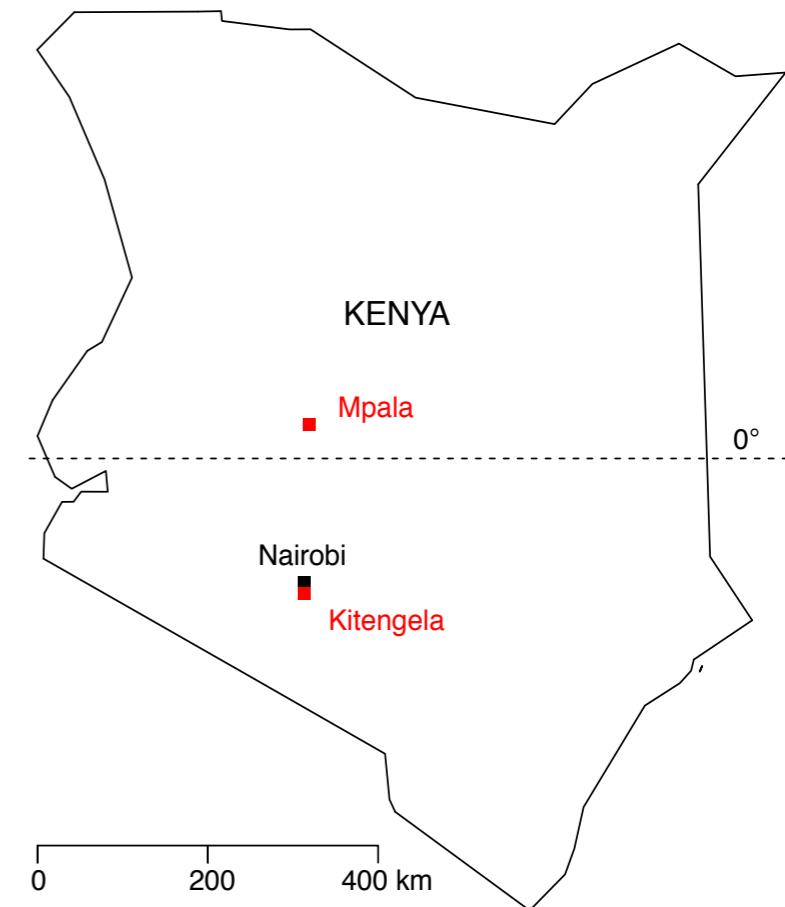
Showed that when offered fungi cultured from the domatia, workers of *T. penzigi* harvested them, whereas *C. mimosae* and *C. nigriceps* were not as interested

Pyrosequencing workflow



Fungal sampling

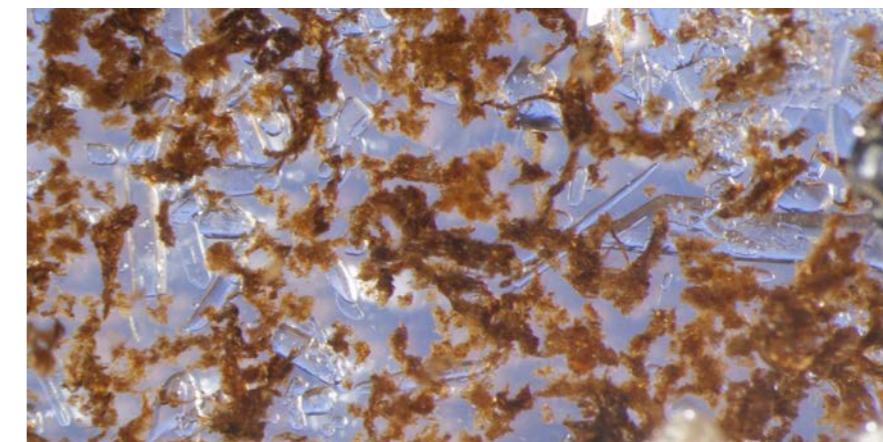
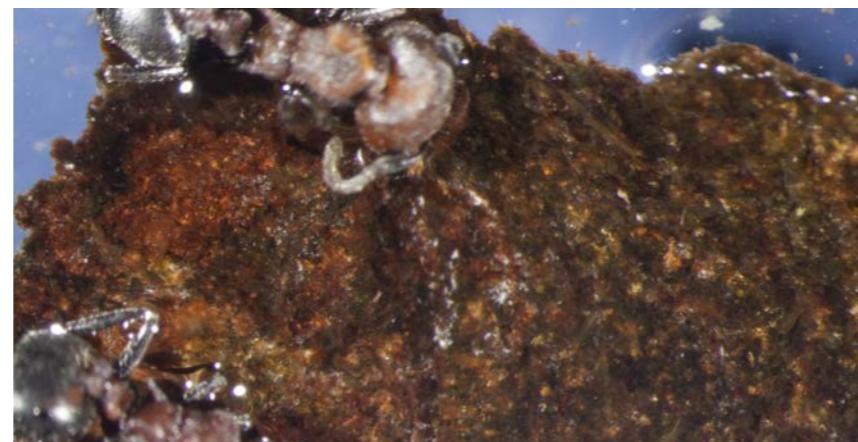
	Kitengela	Mpala
CN	8	9
CM	7	12
TP	7	13



CN

CM

TP



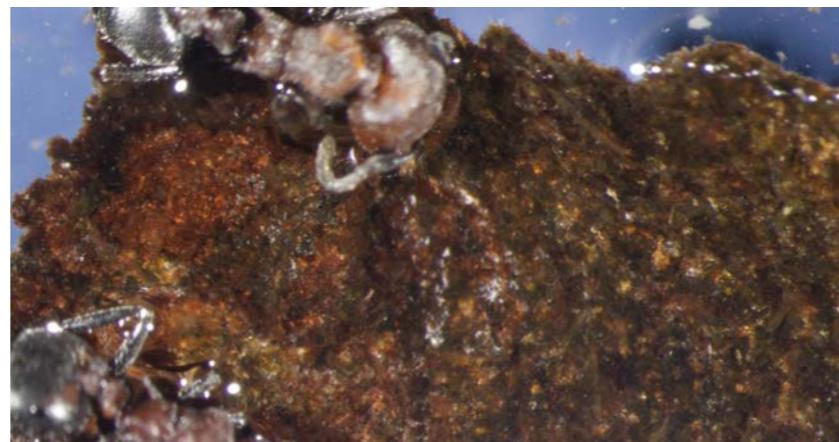
Fungal sampling

	Kitengela	Mpala	greenhouse	
CN	8	9	8	
CM	7	12	3	domatia
TP	7	13	2	

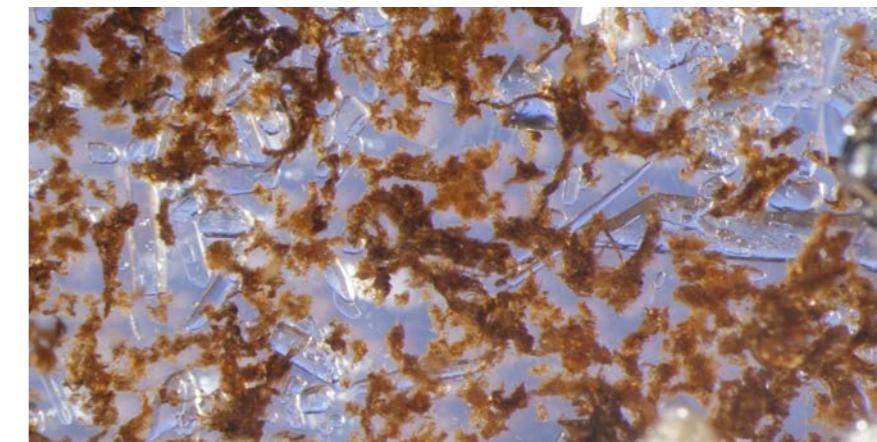
CN



CM



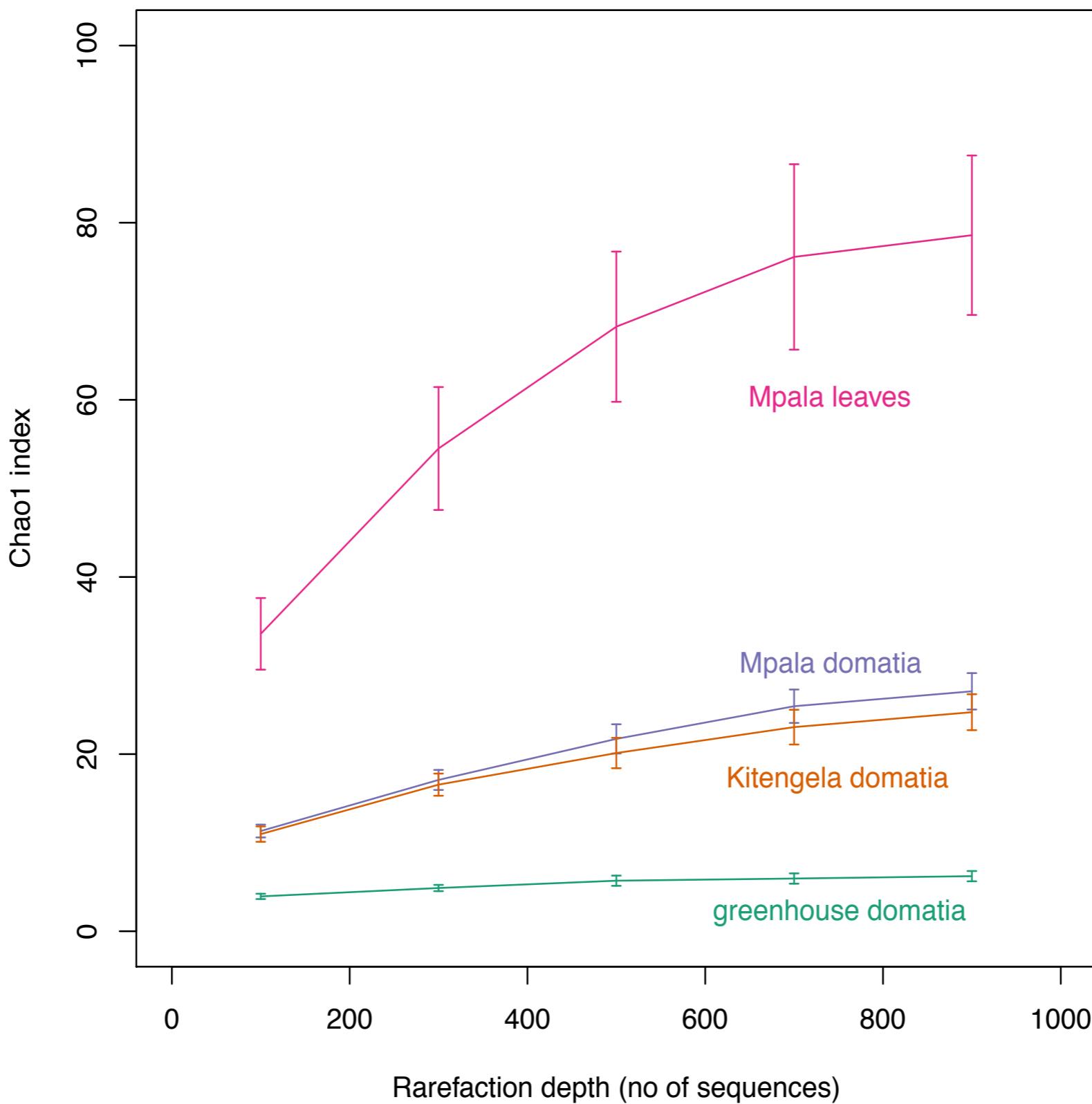
TP



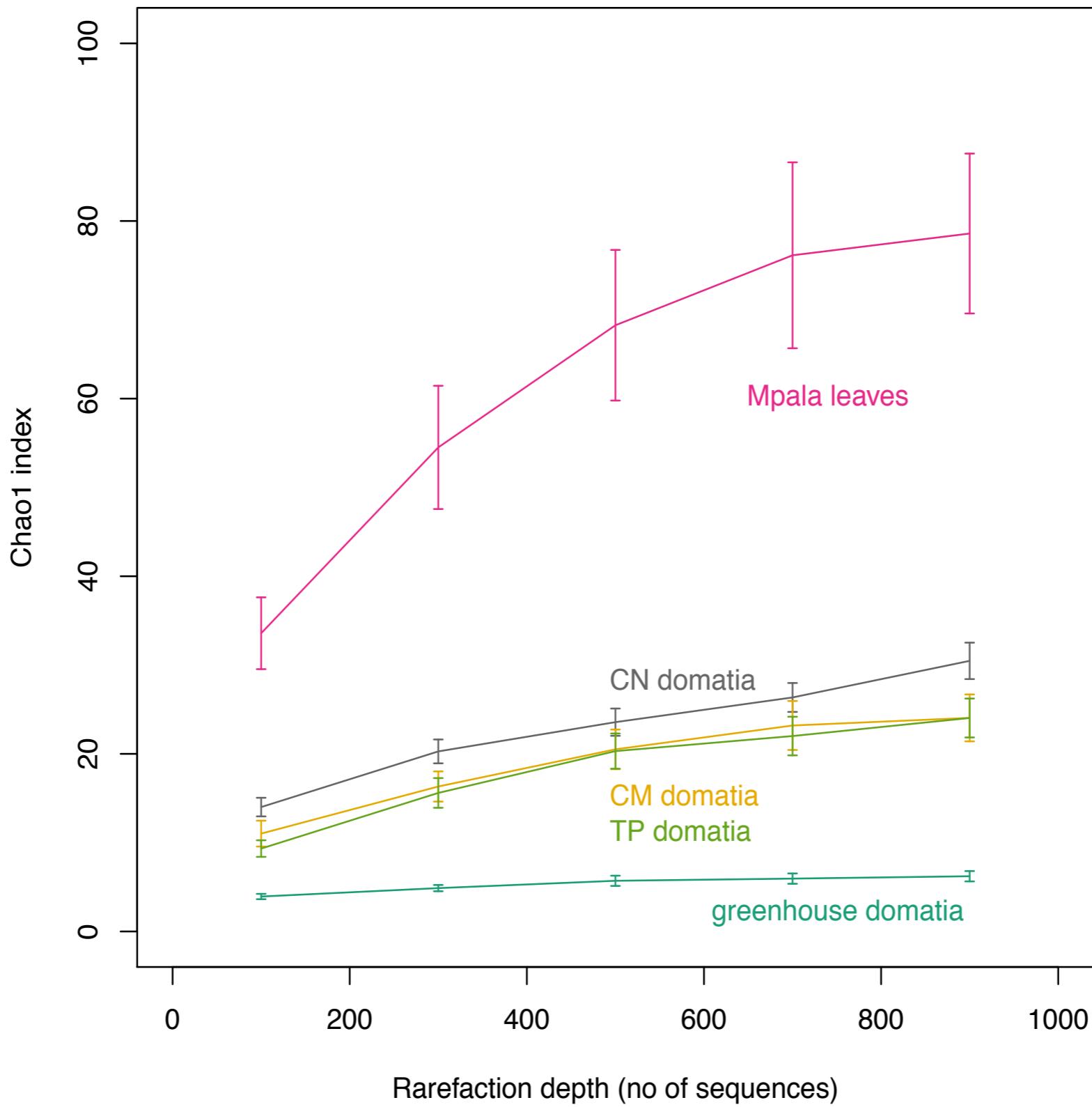
Fungal sampling

	Kitengela	Mpala	greenhouse	
CN	8	9	8	domatia
CM	7	12	3	
TP	7	13	2	
CN		—		leaves
CM		3		
TP		3		

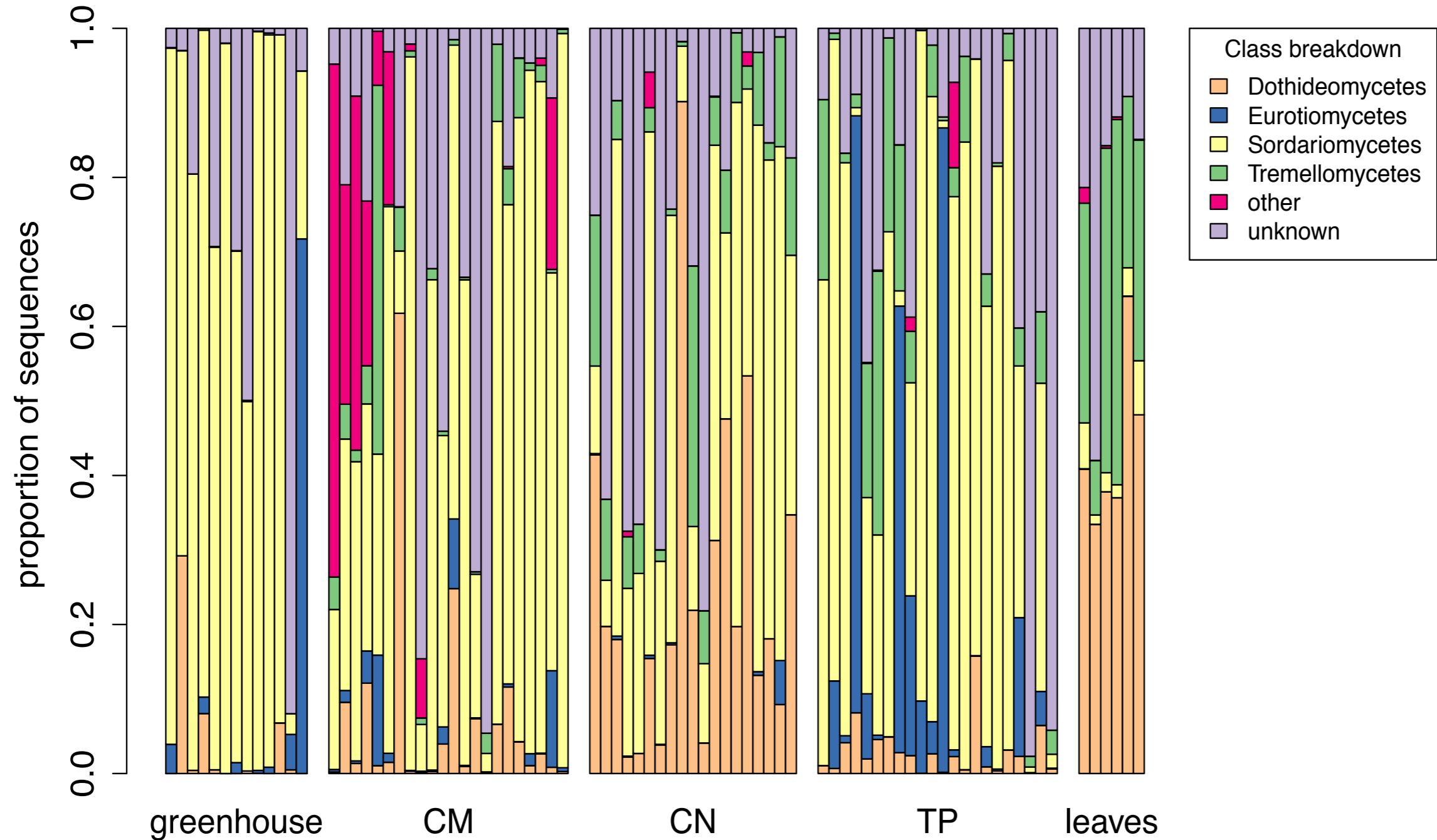
Location and sample type affect diversity



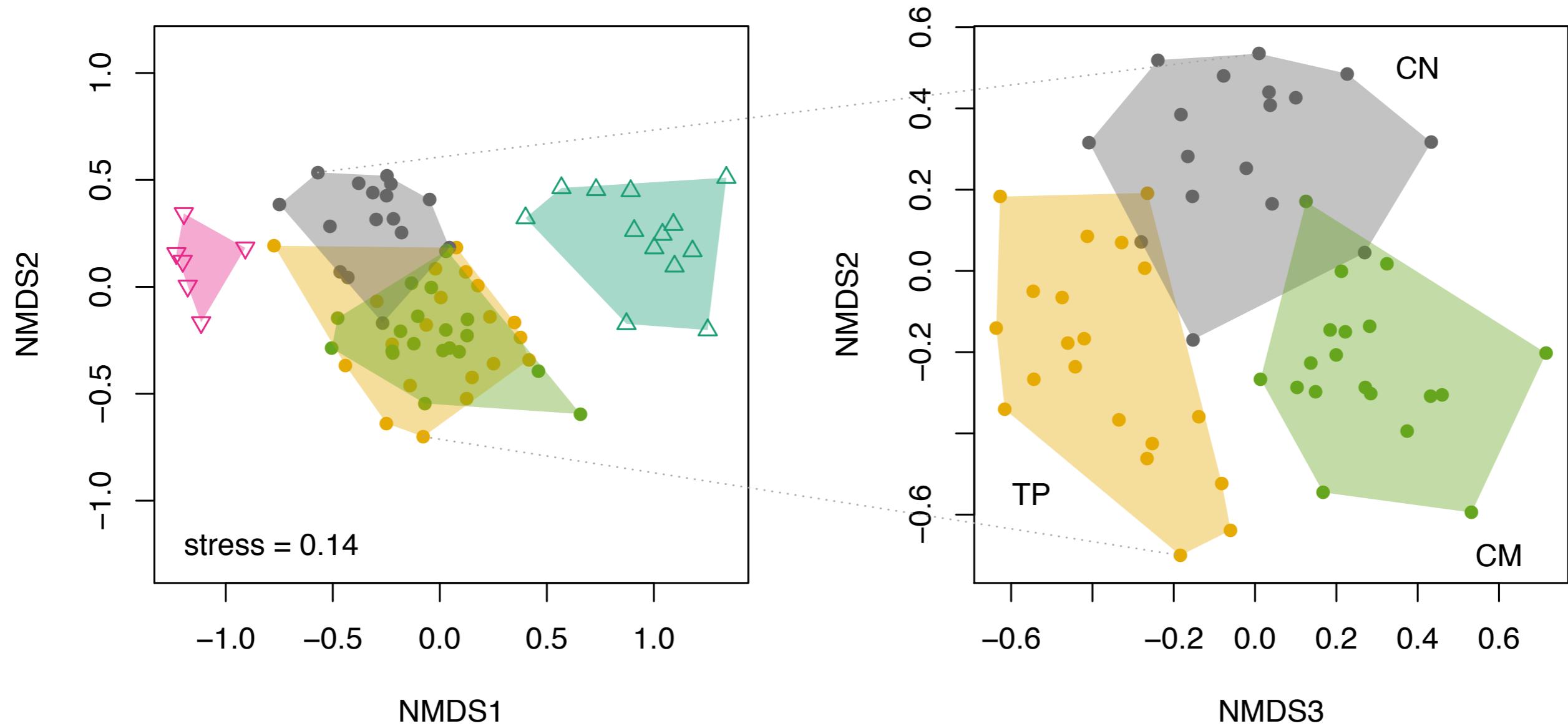
Ants have limited effect on diversity in domatia



Community composition varies at class level

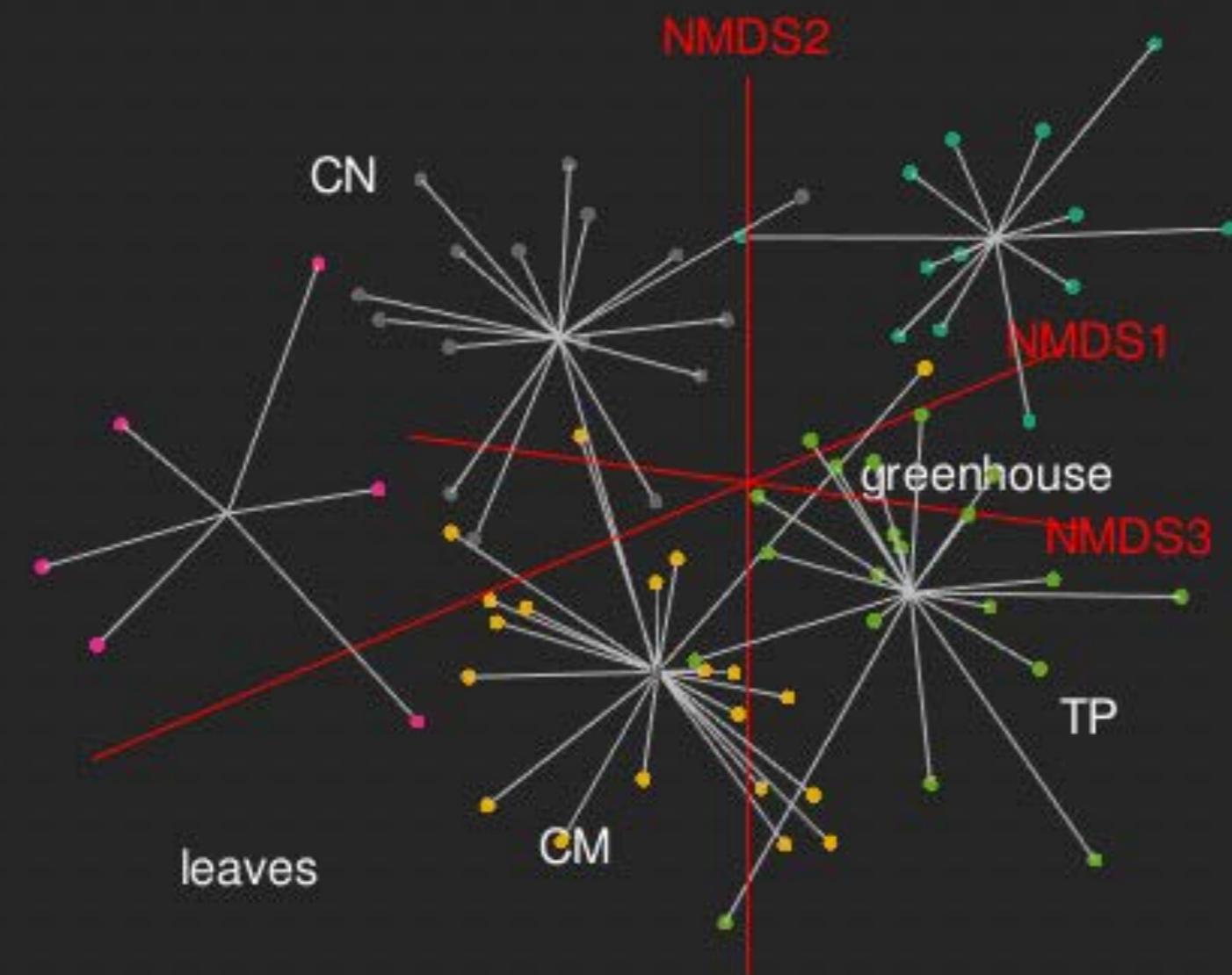


Community composition varies at class level

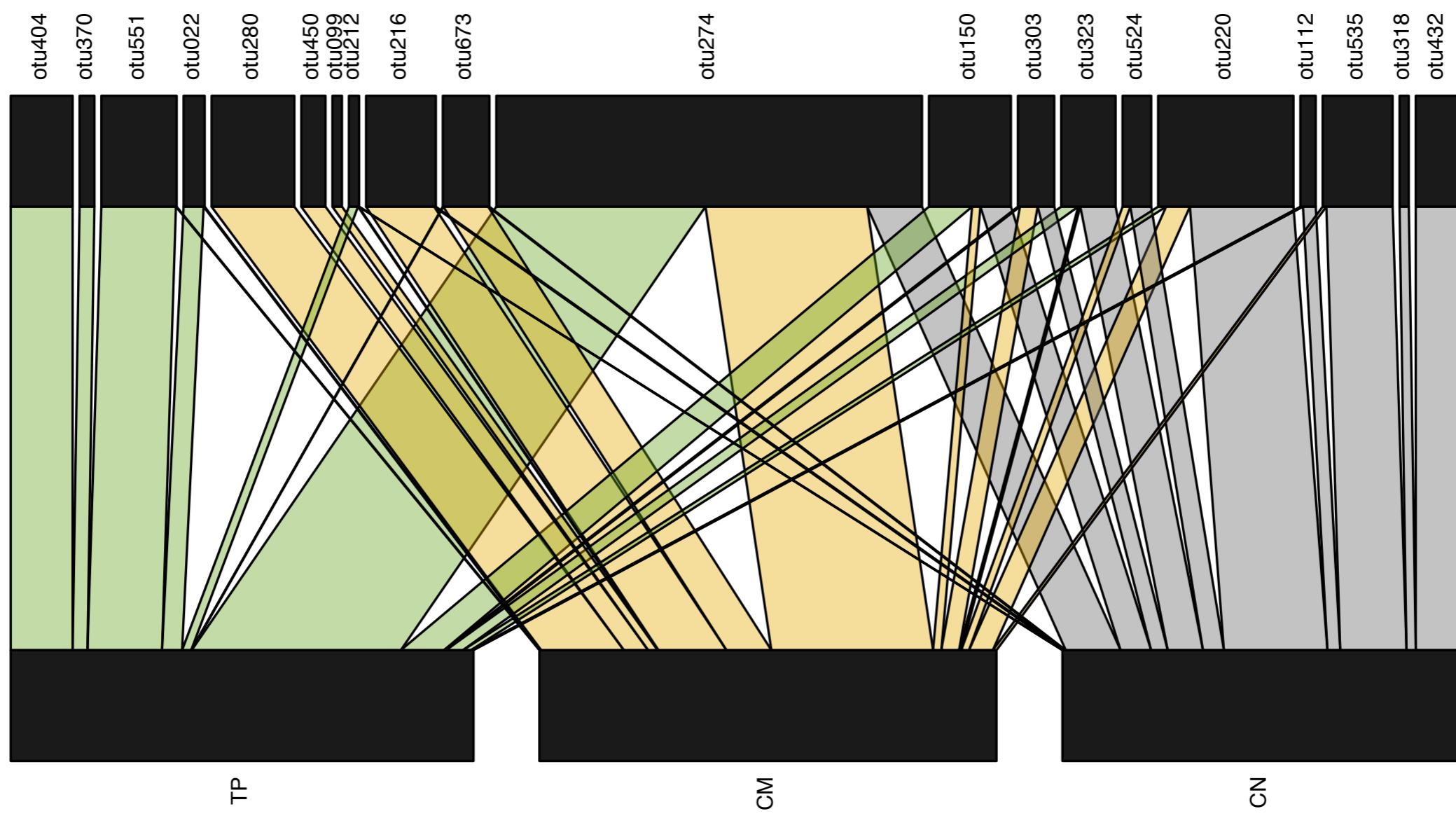


Community composition varies by ant species

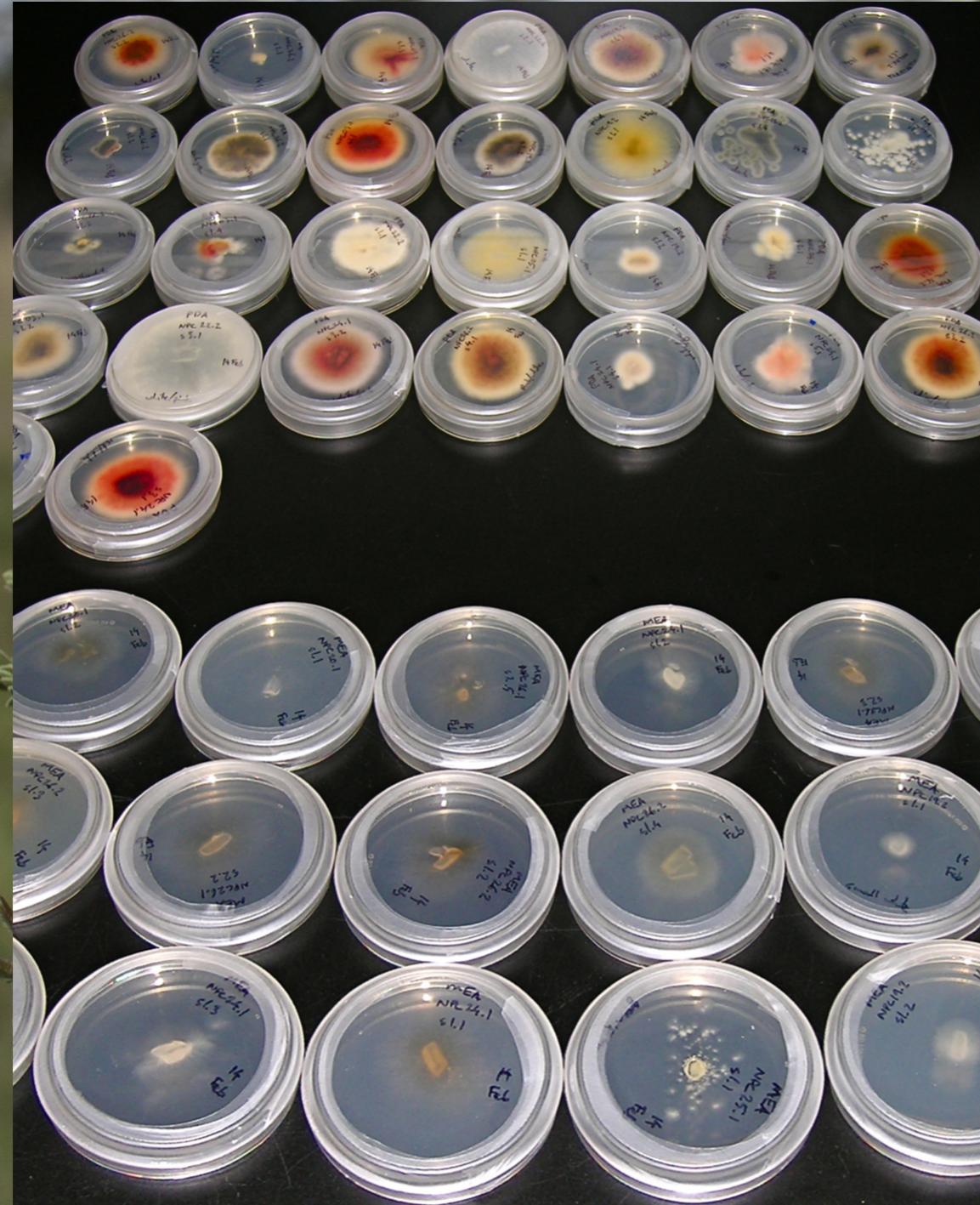
Location and sample type affect community composition



Ant-specific fungal partners?



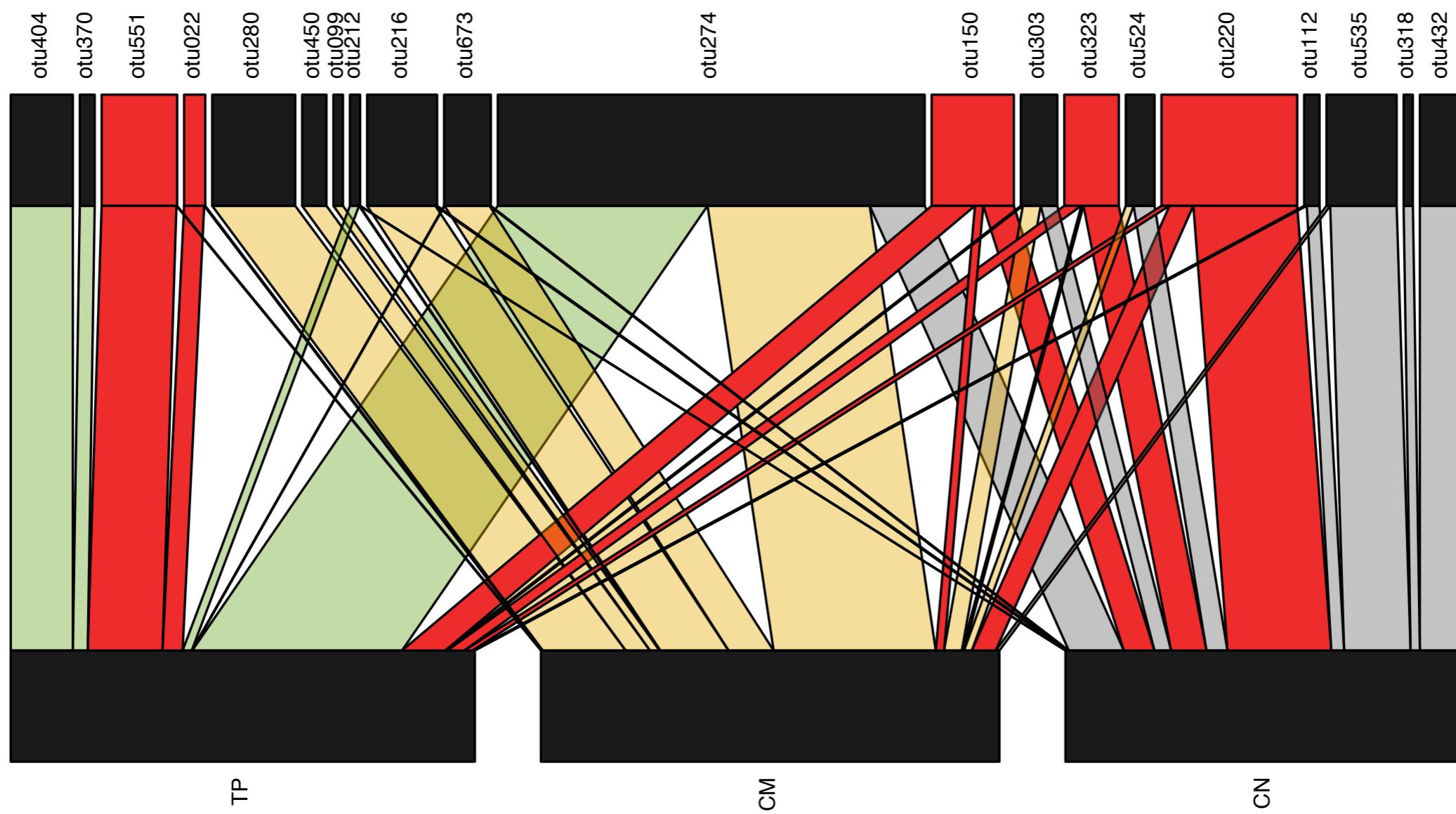
Summary



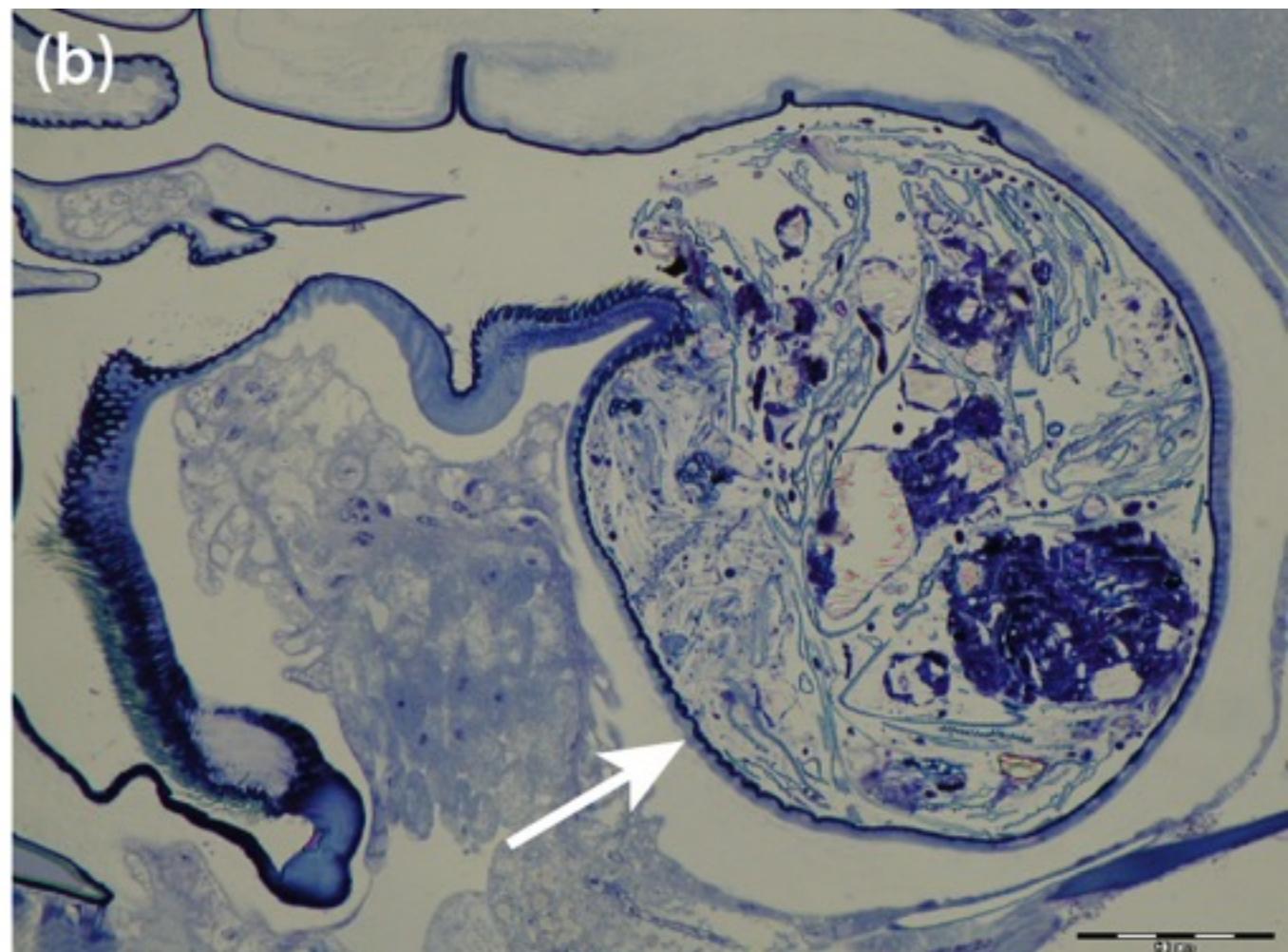
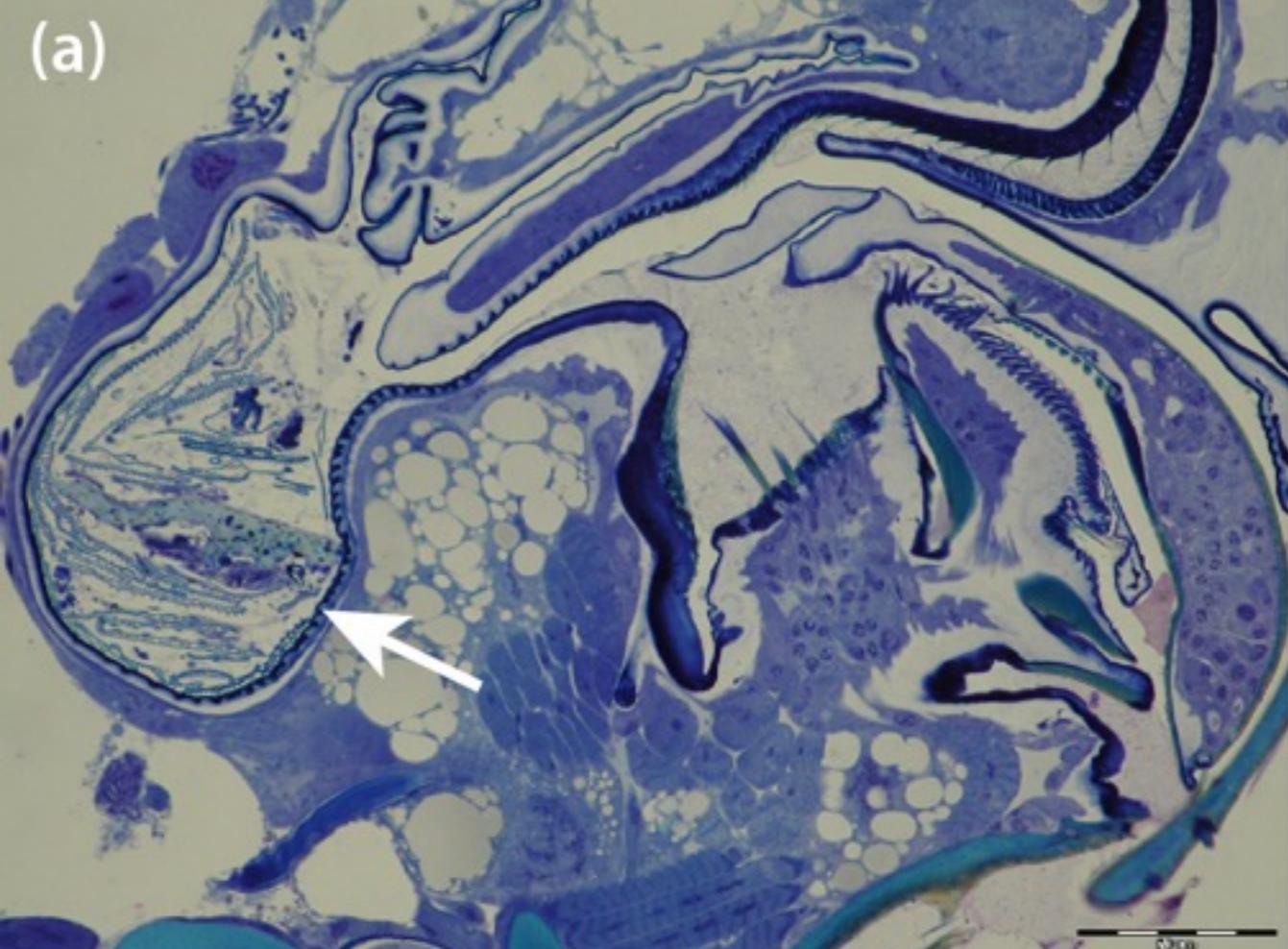
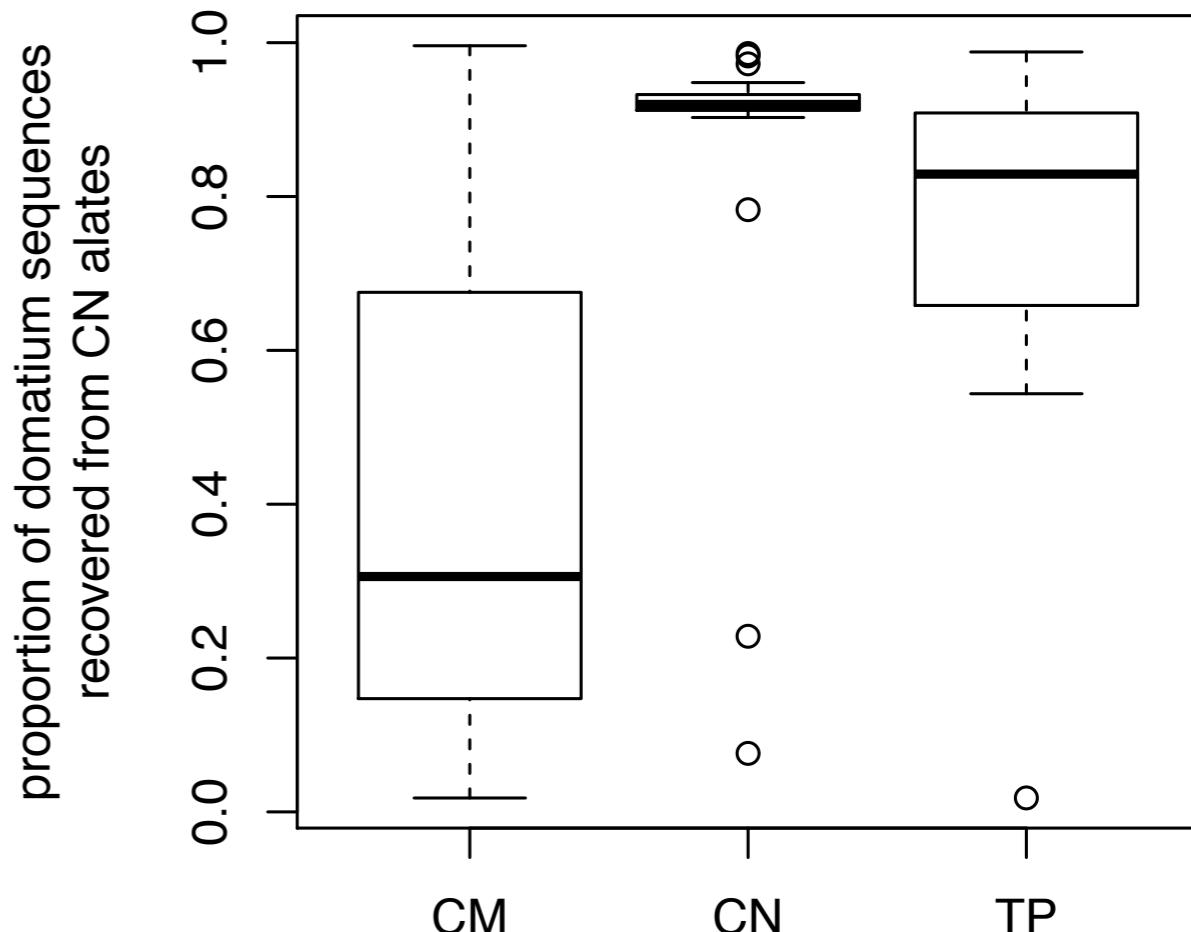
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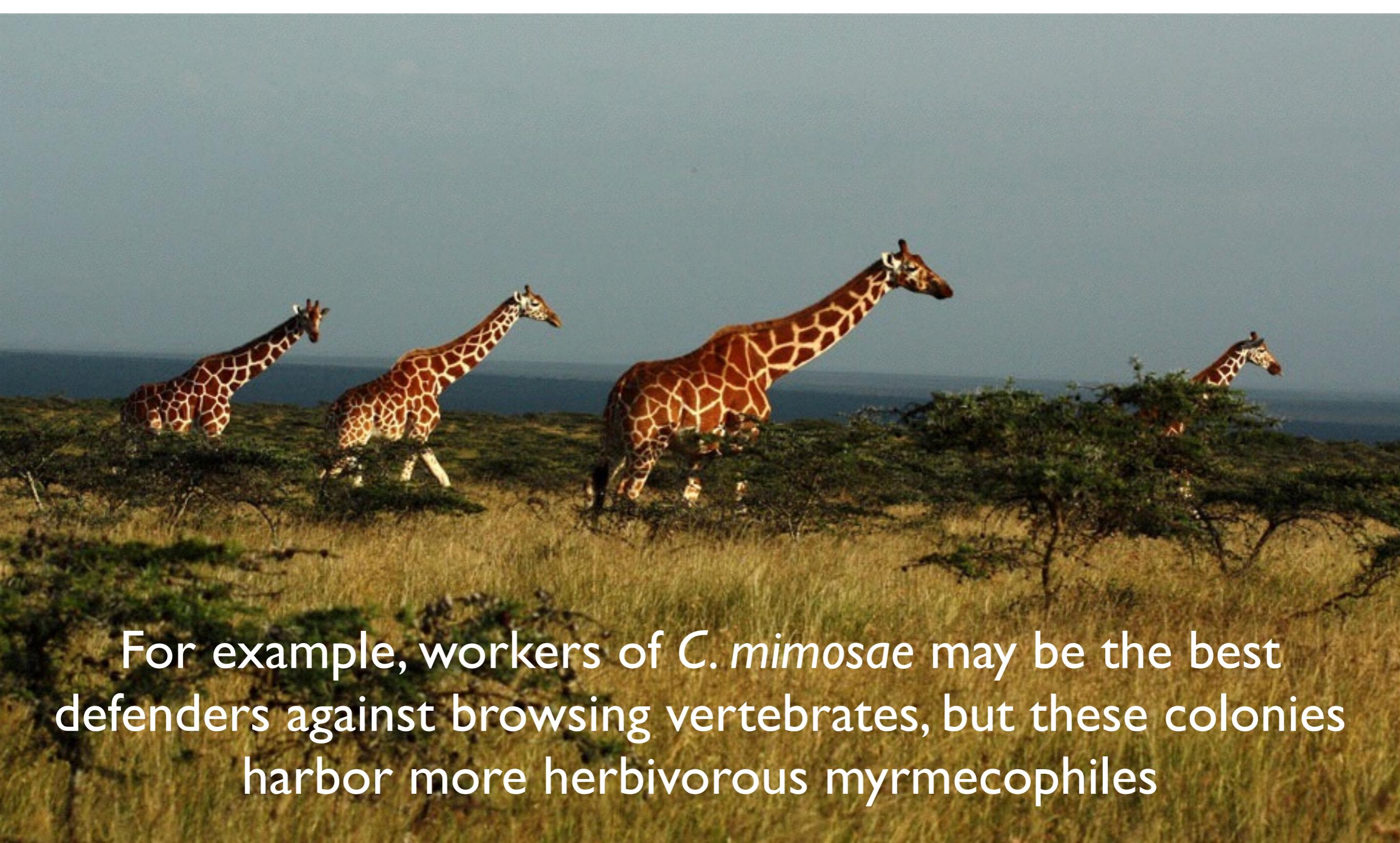
Ant-specific fungal partners?



Alates of *C. nigriceps*
carry most of the
fungal diversity from
the domatia in their
infrabuccal pockets



Take home message: it's important to consider ant-plant mutualisms in the context of third party interactions and the 'extended phenotype' of different ant species



For example, workers of *C. mimosae* may be the best defenders against browsing vertebrates, but these colonies harbor more herbivorous myrmecophiles

Perhaps the true mutualism is between the plant and the fungi, and ants are simply a vector for fungal colonization?



Summary

‘Barcodes’ as identifiers can uncover pattern and process because they provide both identification tools in biological prospecting and historical markers to analyze population processes, phylogeography and phylogeny

- “Ants as legumes” Bacteria may have played a key role in the evolution of herbivorous ants, enabling them to live in the canopy and other nitrogen-poor environments
- Mutualisms are often assymmetrical, with one partner providing the ecological template against which the other can radiate
- Cospeciation between bacterial symbionts and their hosts may be an indicator of mutualism
- Symbiosis is a source of evolutionary novelty



Thanks!

Templeton Foundation
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Putnam Expeditionary Fund
Baker Foundation





Army ant myrmecophile data from Rettenmeyer (2011)

Eciton burchellii 300 obligate myrmecophiles: beetles, mites, spiders, bristletails, snails, flies, wasps, millipedes



Ant-plant myrmecophiles: *Vachellia drepanolobium*: Lepidoptera, spiders, wasps, flies, beetles



