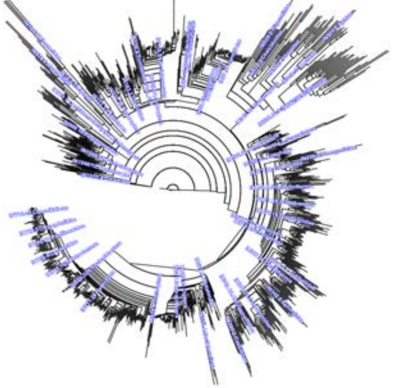

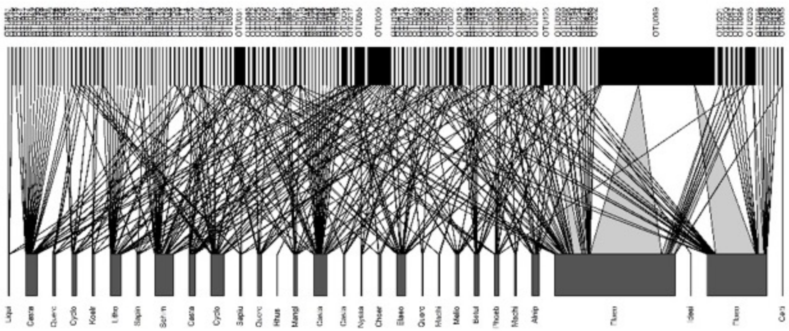

Insect systematics

+

species interaction

Chao-Dong Zhu

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*College of Biological Sciences, University of Chinese Academy of Sciences

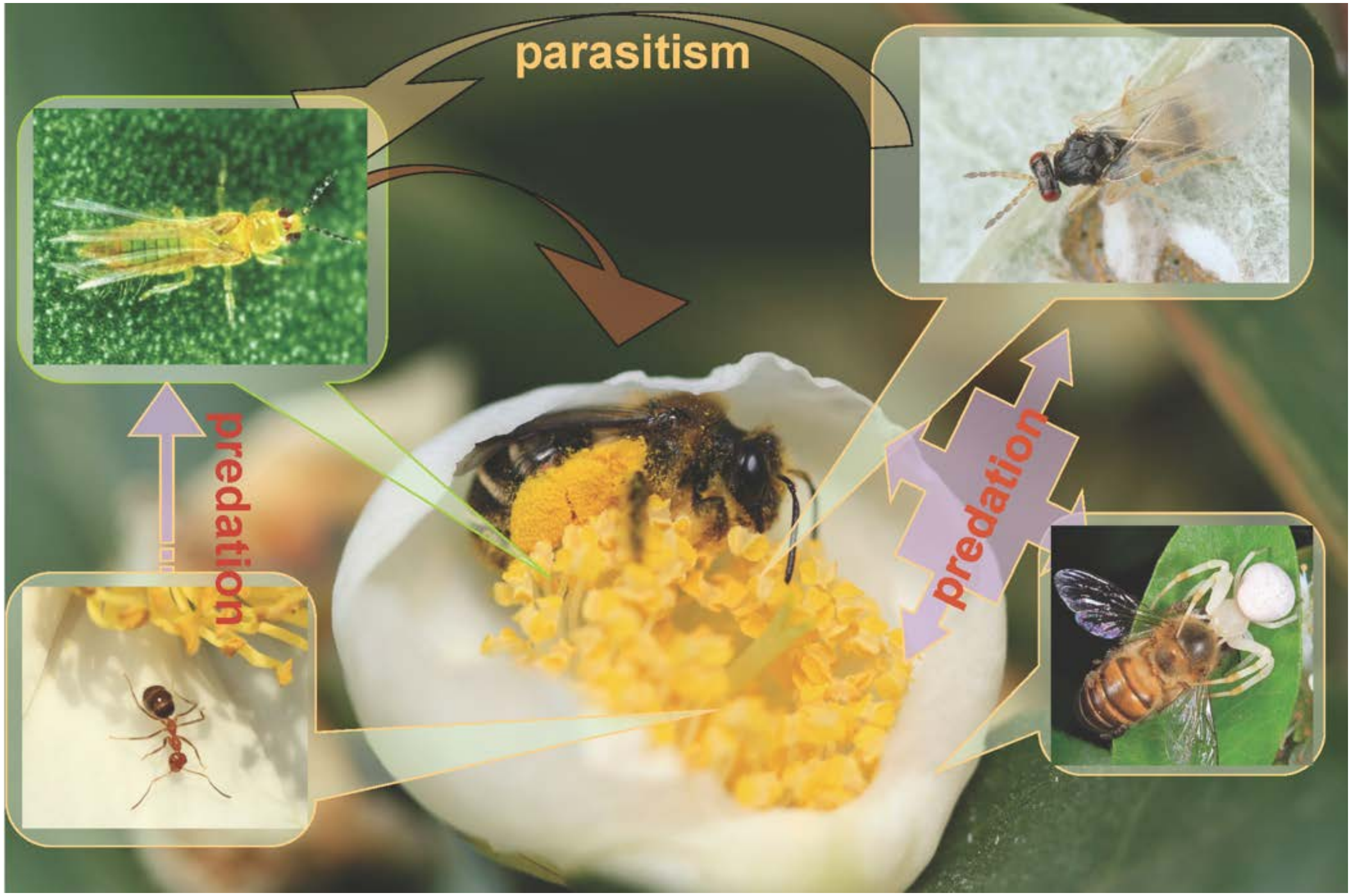
08.07.2021

Outline

1. Insect Systematics

2. Species Interactions

Functional Insect Groups



Pollinators

Predators

Parasitoids

Herbivores

Decomposers

Pollinator Insects

Parasitoid Wasps

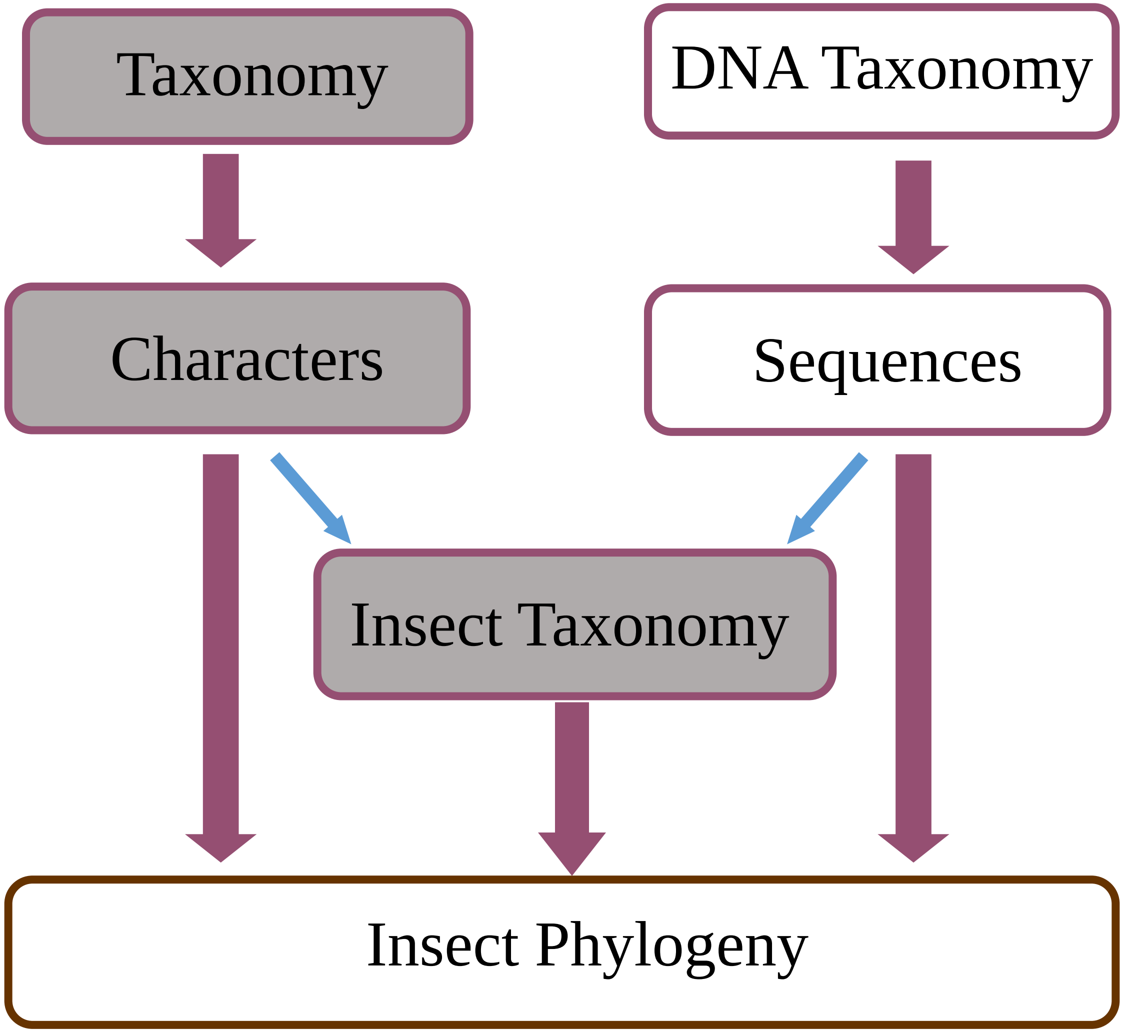
Scientific Questions

Prof. Robert May in Oxford:

"How many distinct life forms – species - does your planet have?"

（May 1986, 1992, 1993, *Nature*; May 1988, 2010, *Science*）

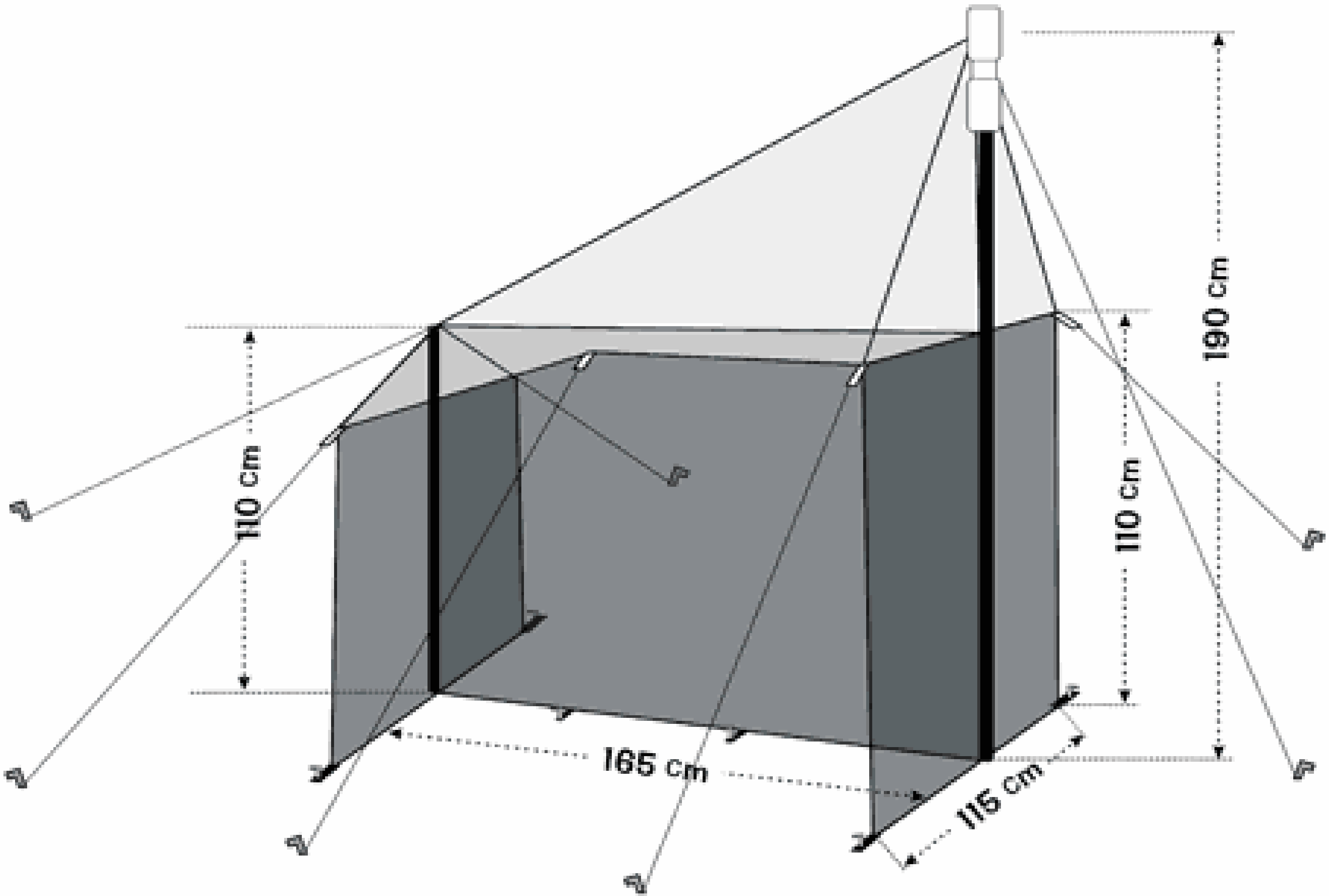
Morphology-based / 2003



Morphology-based/ 2003

Major Sampling Methods

Morphology-based/ 2003



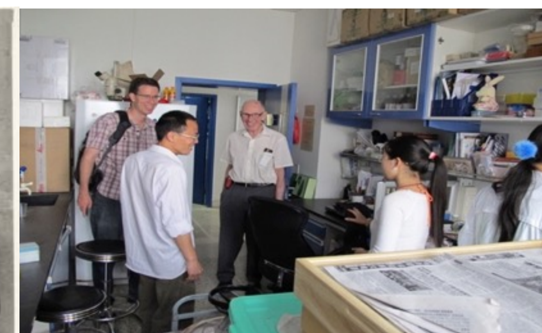
Morphology-based/ 2003

Speed Up Taxonomy?

Morphology-based/ 2003

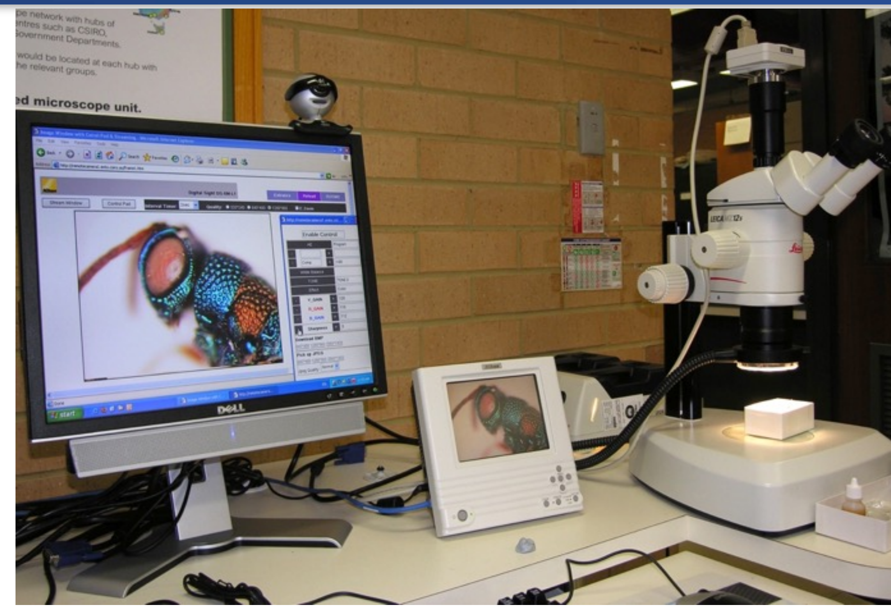
In China, we catalogued more than 1372 known bee species, published more than 70 papers after 2009, with many more unknown yet to sciences.






Morphology-based/ 2003

There are more than 1691 researchers and students joined us on insect identification via QQ. They are working on insect taxonomy, ecology, plant protection, quarantine and pollination biology.



SPECIMEN INTERNET EXPERT

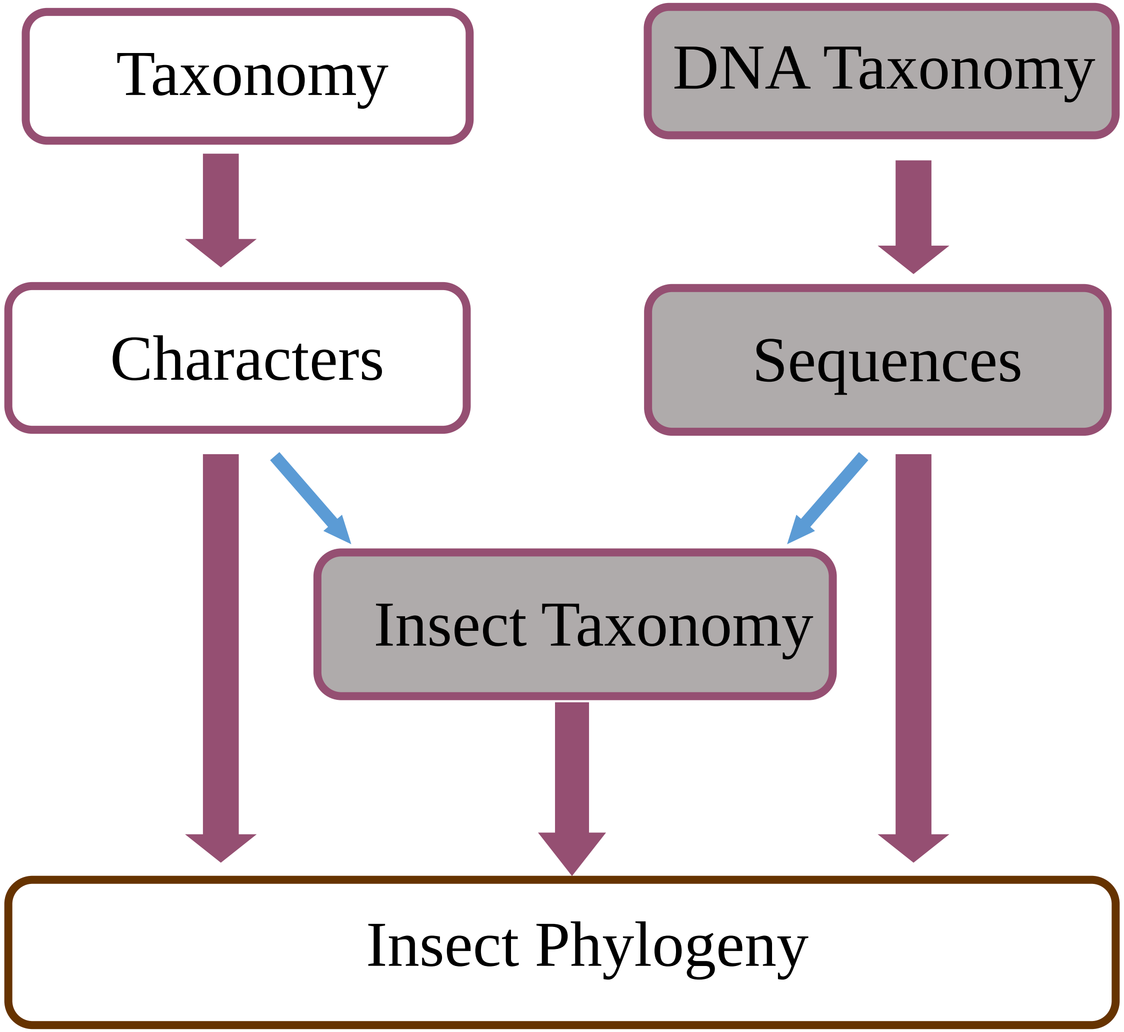
LIVE!

Morphology-based/ 2003

Morphology based taxonomists have been in decline into rare specialists. However, more demands for accurate identifications from agriculture, ecology. How can we fill in the gap?

- With a lot of unkown species, we need to speed up taxonomy;
- With very large molecular data, we need to provide taxonomy supports

DNA Taxonomy / 2006



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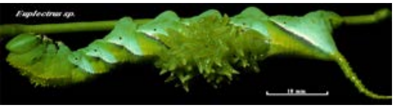
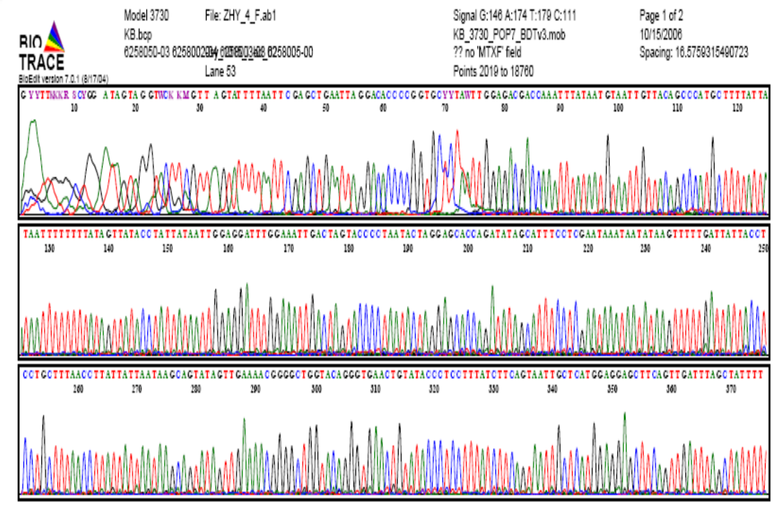
DNA Taxonomy / 2006

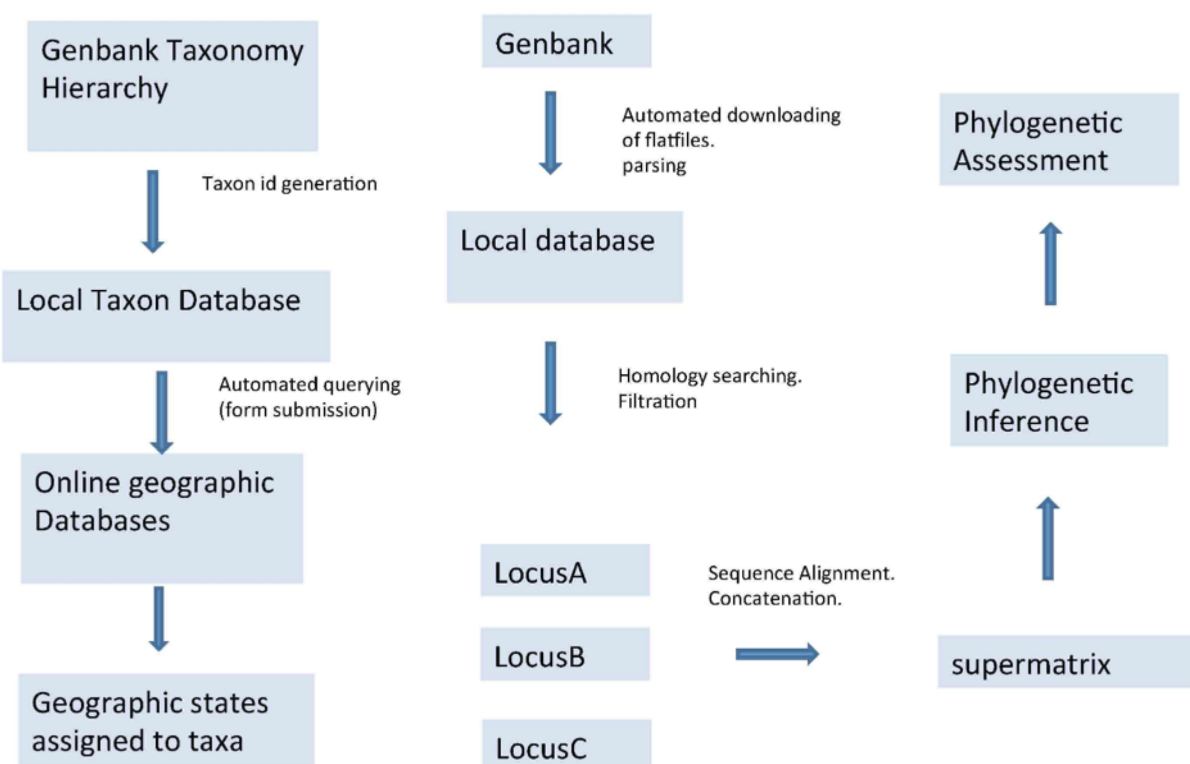


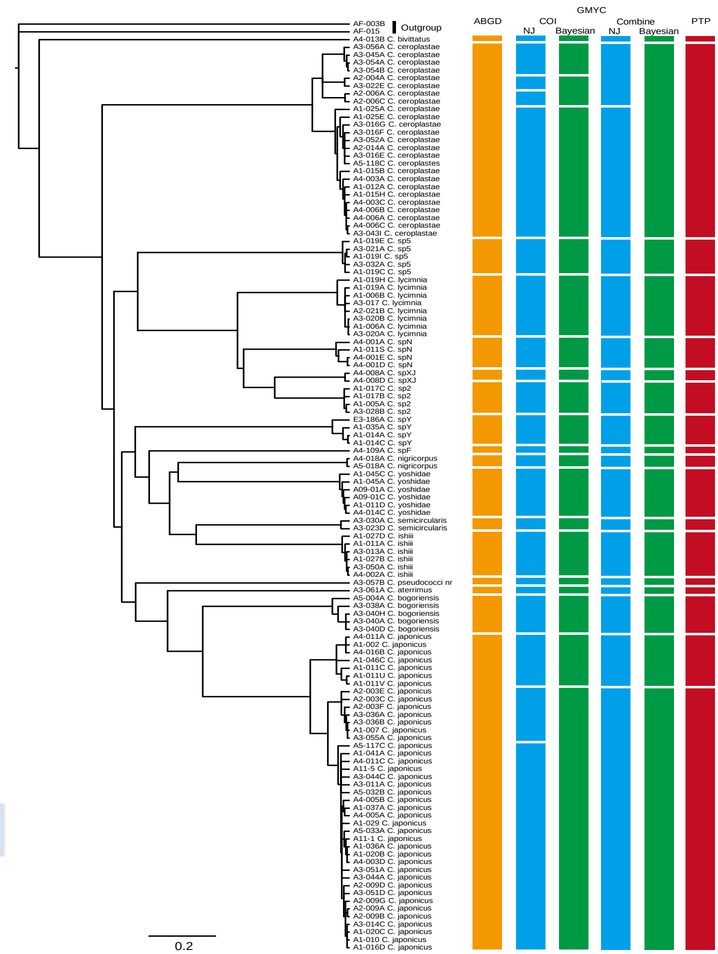


DNA Taxonomy / 2006

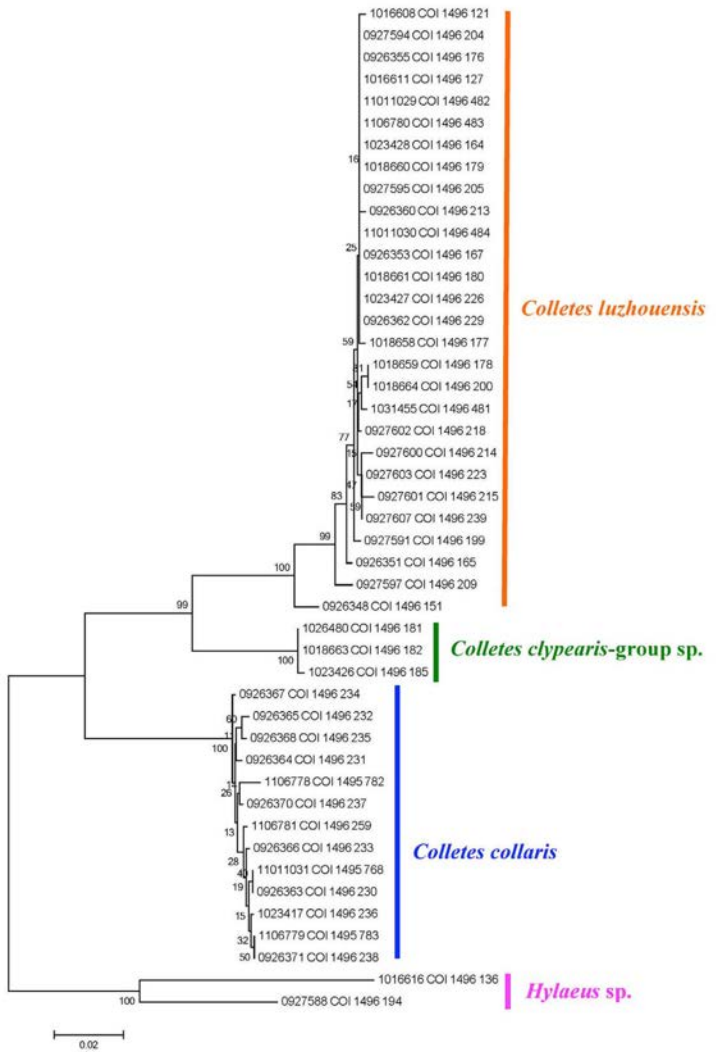
1. Quick and Efficient DNA based identification system

Methods. phyloinformatic pipeline.





DNA Taxonomy / 2006



DNA Taxonomy / 2006

2. Single Locus Species Delimitation

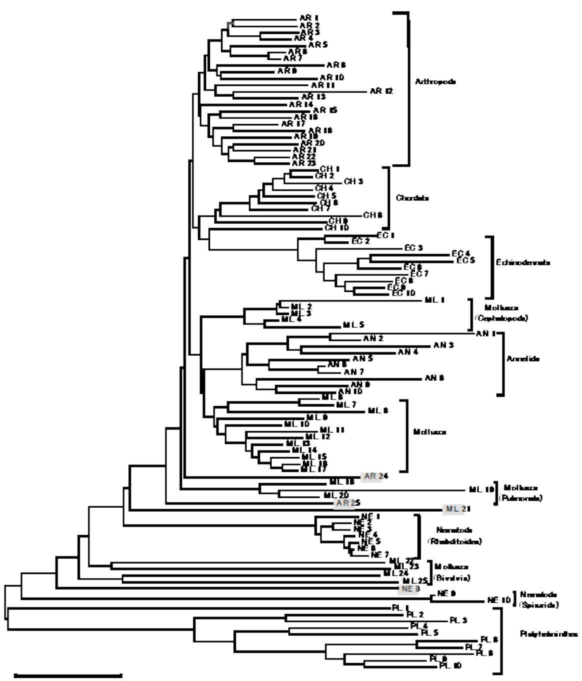


Table 2. Percentage success in classifying species to membership of a particular taxonomic group based upon sequence variation at COI.
(*n* indicates the number of taxa that were classified using each taxon 'profile'.)

taxon	target group	*n*	% success
kingdom Animalia	7 phyla	55	96.4
class Hexapoda	8 orders	50	100
order Lepidoptera	200 species	150	100

（Hebert 2003, *Proc. R. Soc. Lond. B*）

DNA Taxonomy / 2006

All PNGs have similar efficiency to distinguish species

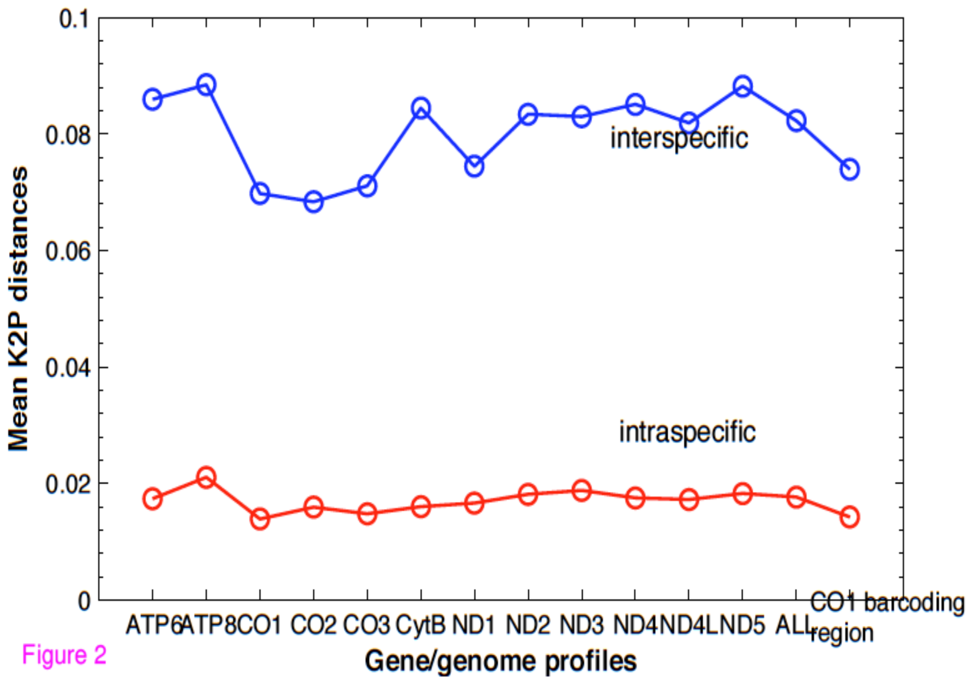
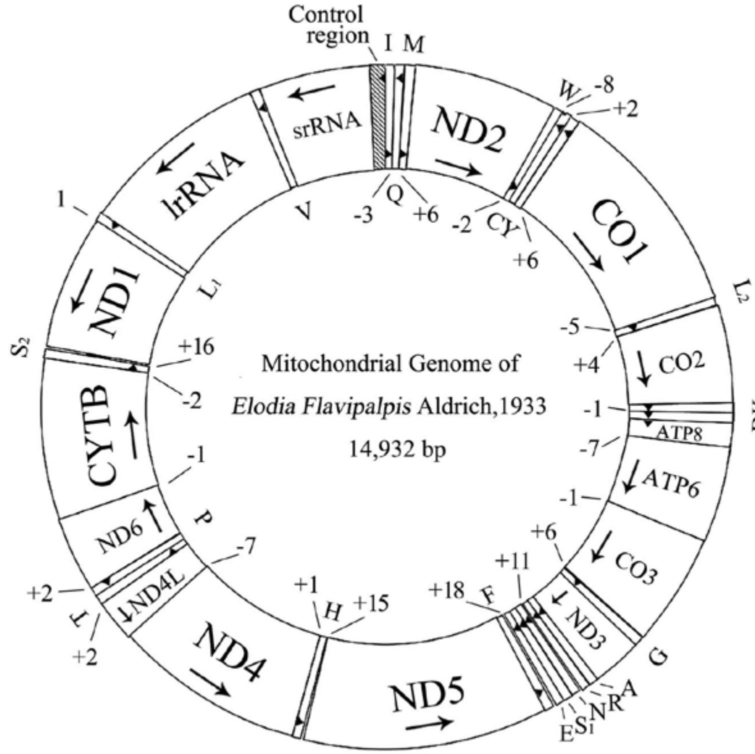


Figure 2



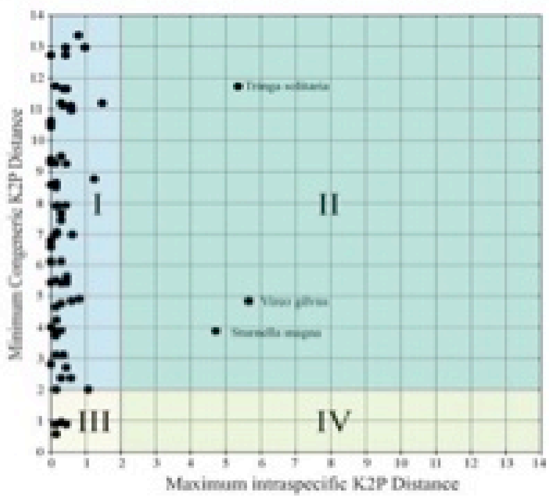
（Luo …, Cameron*, Zhu* 2011, *BMC Genomics*）

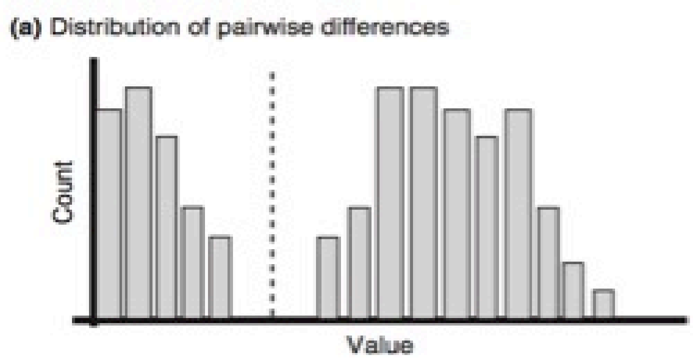
DNA Taxonomy / 2006

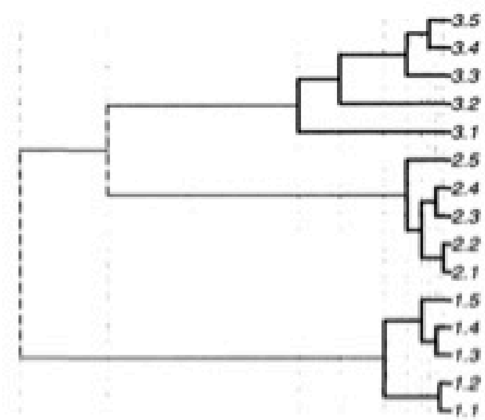
1

Sample size is important for DNA barcoding

- **DNA barcoding can be complicated by varying levels of genetic polymorphism.**
- **Detailed understanding of intraspecific polymorphism forms the basis, is important for constructing reference databases.**
- **In practice, there is the compromise between sampling degree and taxonomic coverage.**





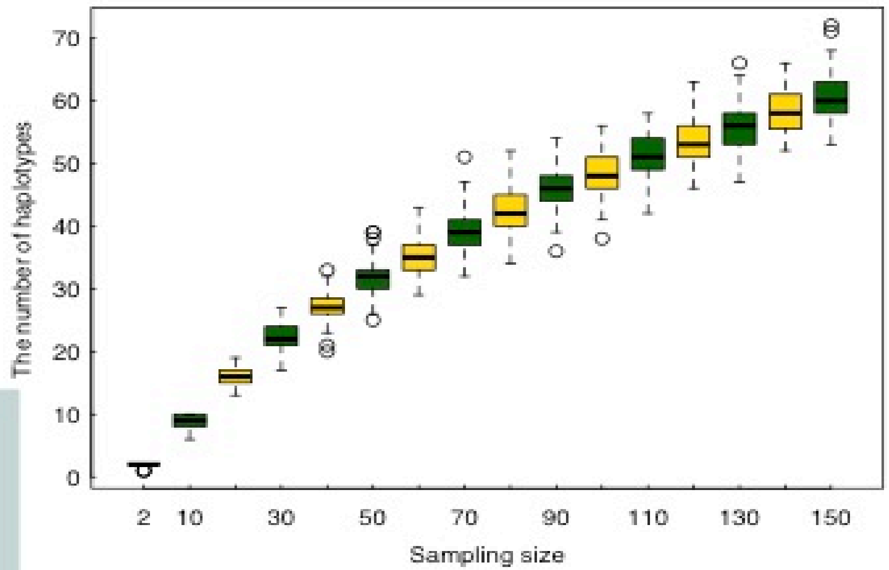


Hebert et al., 2004; Pons et al., 2006; Puillandre et al., 2012

DNA Taxonomy / 2006

Effect on the number of haplotypes

- The number of different haplotypes is an important indicator of genetic diversity in studies of population.
- Random samples of 2, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, and 150, each with 100 replicates



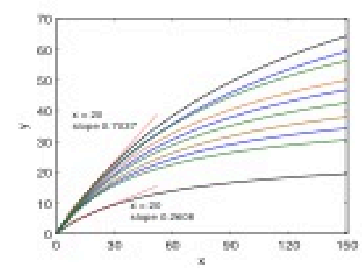
Conclusion:

The benefit of increasing sample size is confirmed.

A sample size of 20 is able to provide a reasonable reflection of the polymorphism of the entire population.

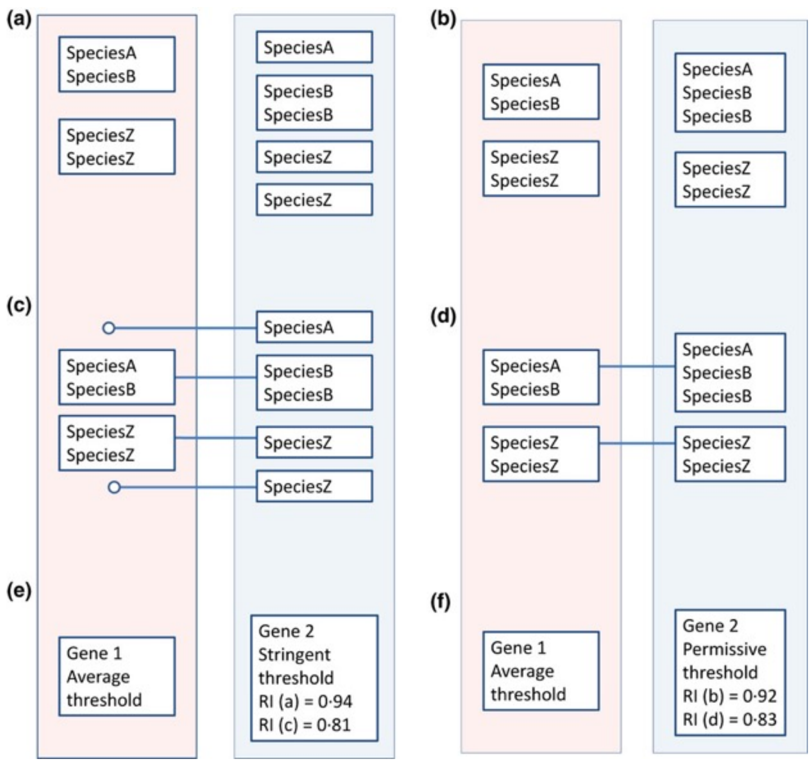
Stratified sampling would be involved if more populations are considered.

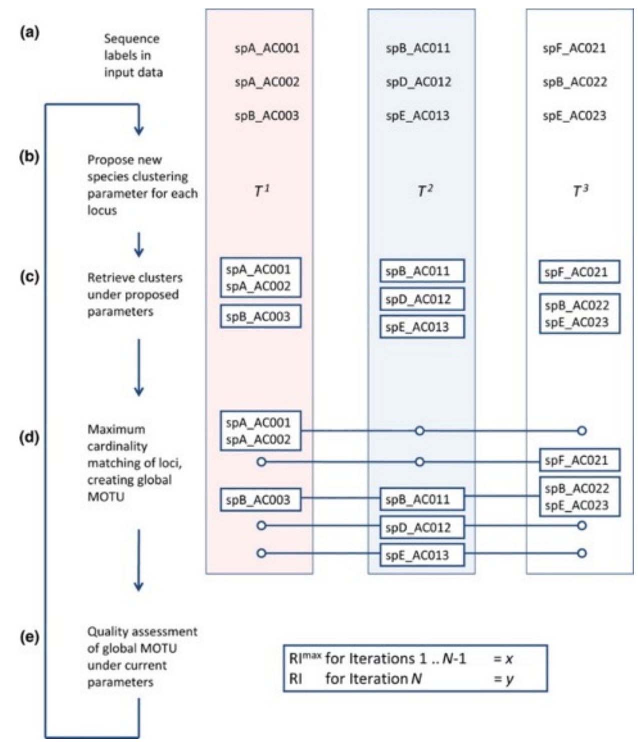
$$F(x) = \frac{ax}{1+bx}$$



DNA Taxonomy / 2006

3. Multiple Loci Taxonomy

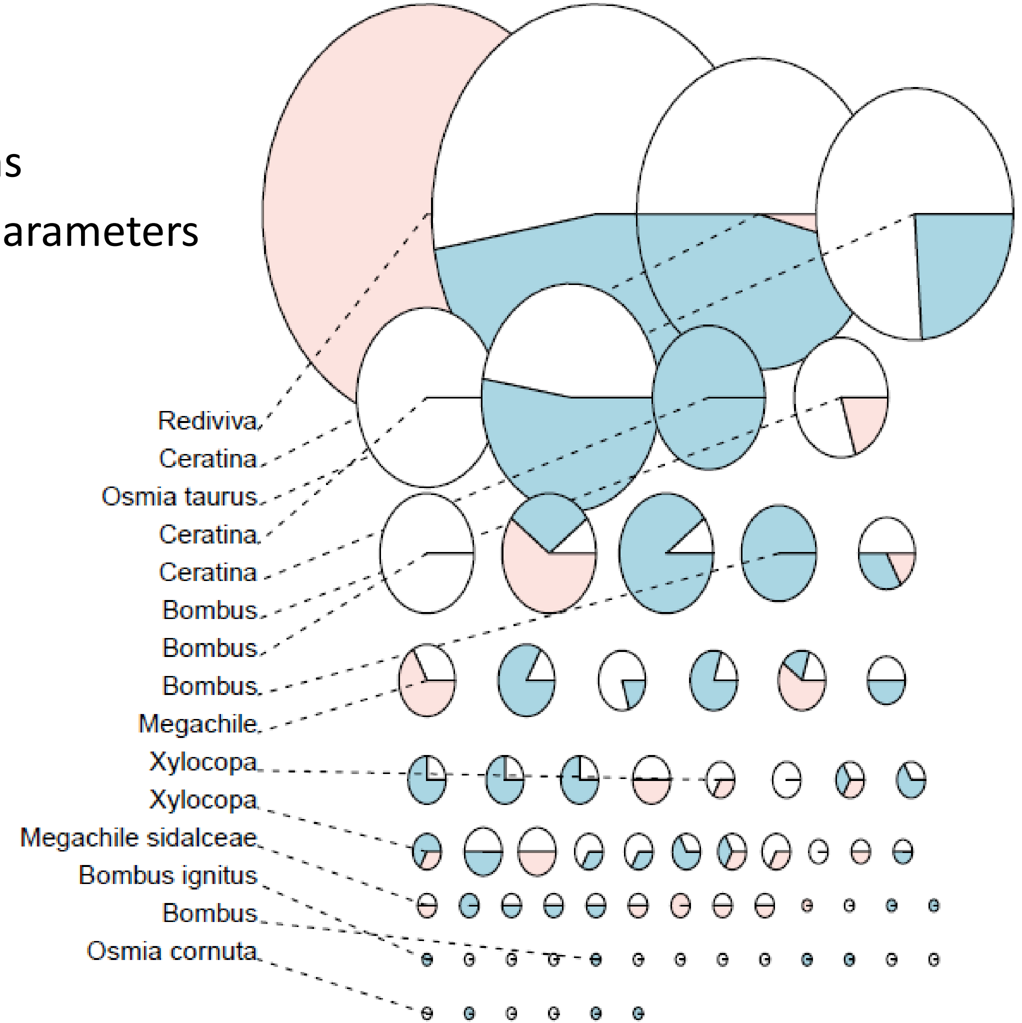




（Chesters…, Zhu*, 2015, *Methods in Ecology and Evolution*）

DNA Taxonomy / 2006

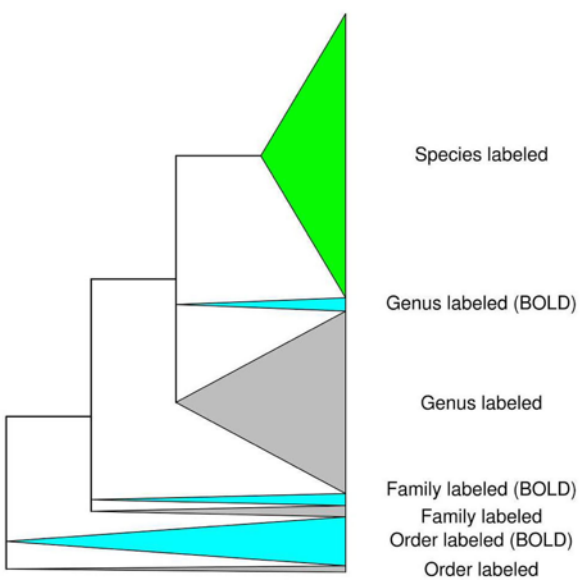
- 3 gene dataset for 250 bee specimens
- Novel heuristic search of clustering parameters
- Figure shows 70 global species units



DNA Taxonomy / 2006

4. Species delimitation in HUGE database

About 80000 insect species were automatically
and quickly identified based on 24 gene loci



1. •Sample DB
2. •Blast search sample against complete DB
3. •Calculate overlap (Hit Span Fraction) for each hit
4. •Partitioning of loci (with MCL) using overlap scores
5. •(optional) Select locus partition parameters according to species returned and annotation congruence
6. •(optional) Test suitability of each homolog for MSA, then discard where appropriate
7. •(optional) Isolate reference dataset (species labeled sequences), then taxon_blast
8. •(optional) Clustering (in esprit) of reference dataset using pairwise similarities from taxon_blast
9. •(optional) Select optimal species clustering parameters using reference dataset
10. •Obtain subject dataset (exemplars for each named species, combined with all incompletely labeled data)
11. •taxon_blast subject dataset, then cluster (esprit) under optimal parameters determined earlier
12. •Combine single locus MOTU using multi-partite matching
13. •Assign names to global MOTU using labeled exemplars

（Chesters and Zhu*, 2015, *Systematic Biology*)

DNA Taxonomy / 2006

5. Large insect phylogenies

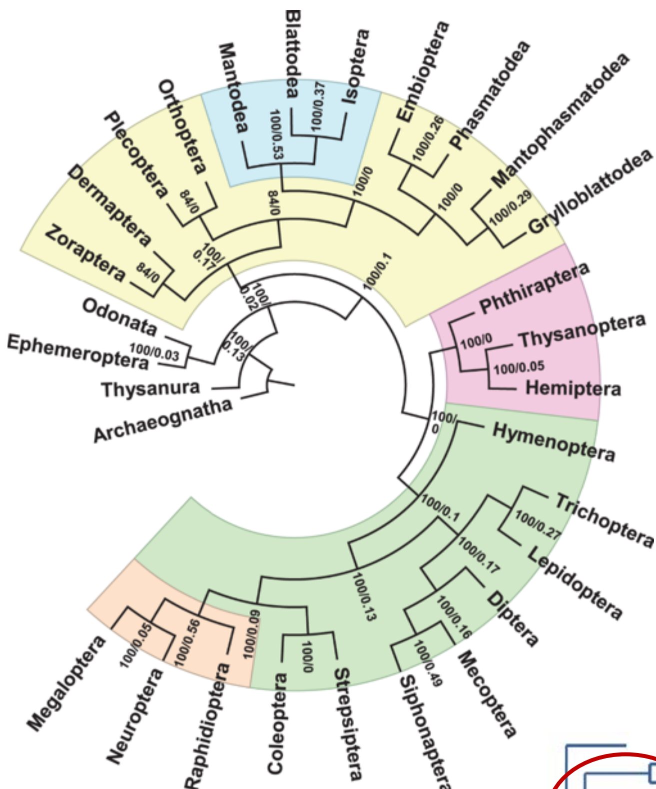
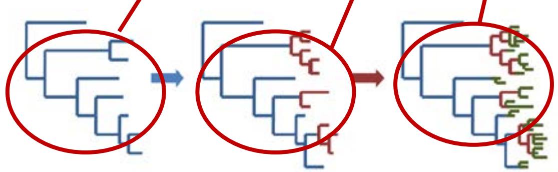


TABLE 1. Description of data-type, filtering treatment and topological constraints of the 11 phylogenies inferred

Label	Description
Nucl0	Nuclear orthologs unfiltered
Nucl1	Nuclear orthologs reduced for compositional heterogeneity
Nucl2	Nuclear orthologs reduced for branch-length heterogeneity
Nucl3	Nuclear orthologs reduced for information content
Nucl4	Nuclear orthologs reduced for alignment quality
Nucl5	Nuclear orthologs reduced for compositional and branch-length heterogeneity, and information content
Nucl6	Nuclear ortholog *bins* reduced for compositional and branch-length heterogeneity, and information content
Unconstr-Mt	Mitogenomes unconstrained
Nucl-Mt	Mitogenomes constrained to nuclear ortholog results.
Unconstr-Sp	Species-rich partition unconstrained
Nucl-Sp	Species-rich partition constrained to a consensus of trees from Nucl1-Nucl4
Nucl-Mt-Sp	Species-rich partition constrained to tree Nucl-Mt, as per Figure 1



Chesters, 2017, *Systematic Biology*

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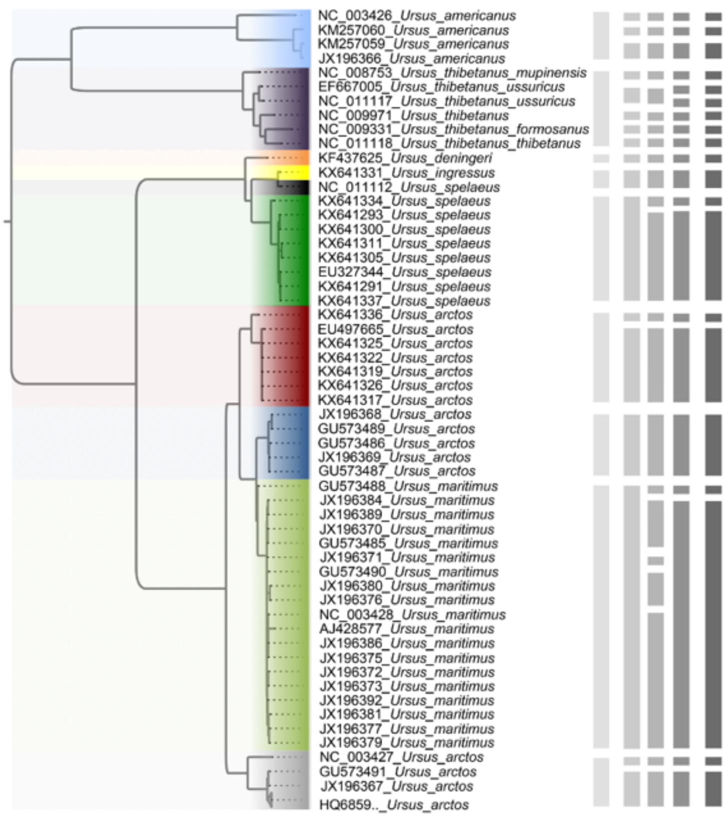
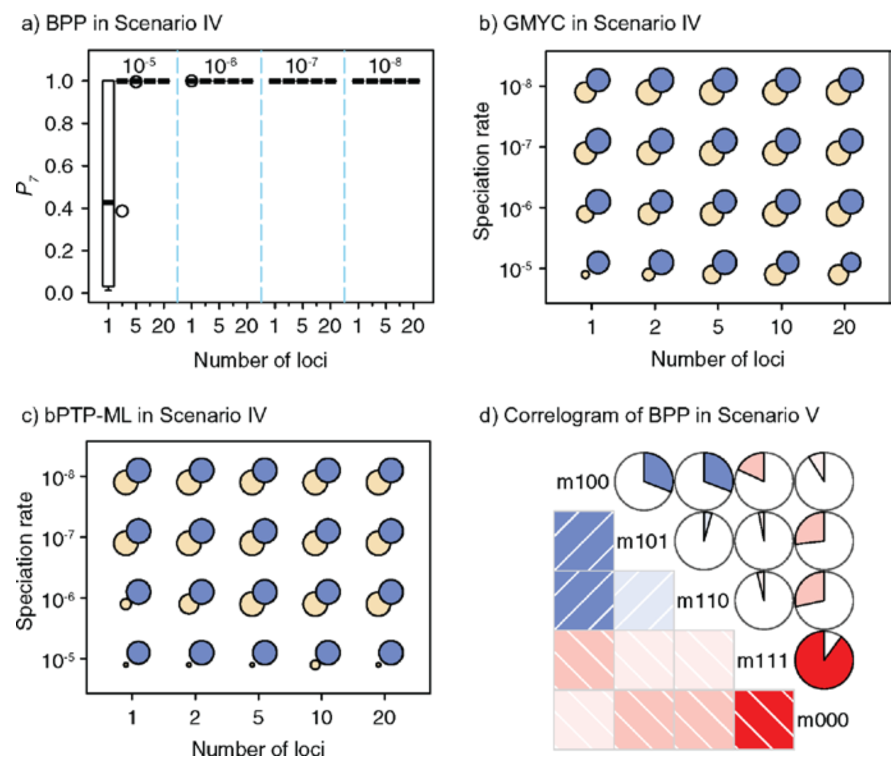
DNA Taxonomy / 2006

6. Species delimitation across a range of speciation scenarios





Luo…, Zhu*, 2018, *Systematic Biology*

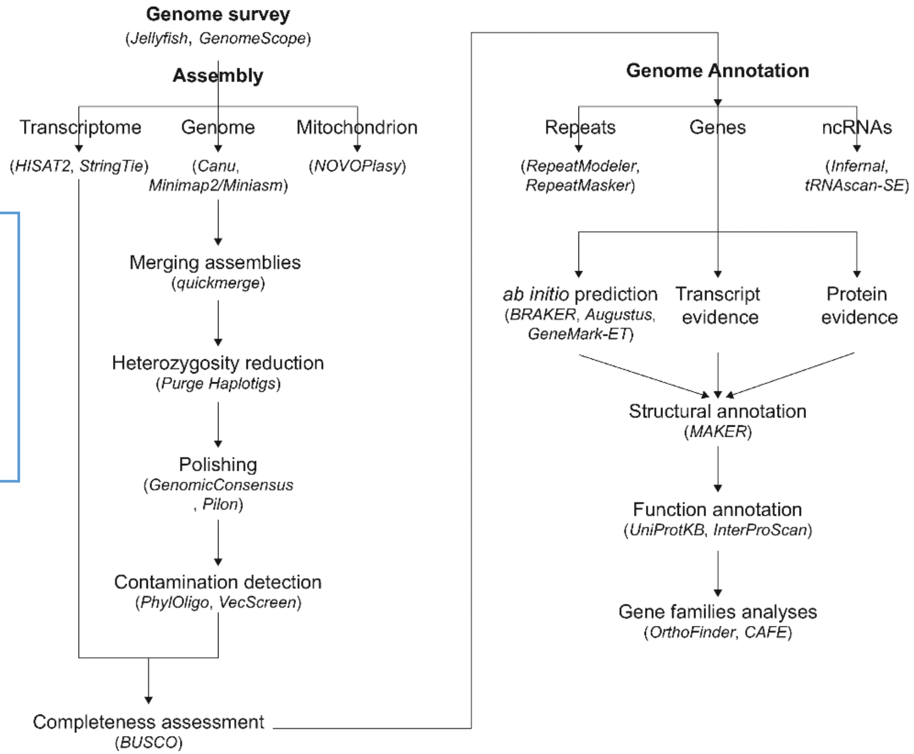
NGS Taxonomy / 2016

Developing a new pipeline

High-quality Assemble Genome

Detecting species with very low abundance

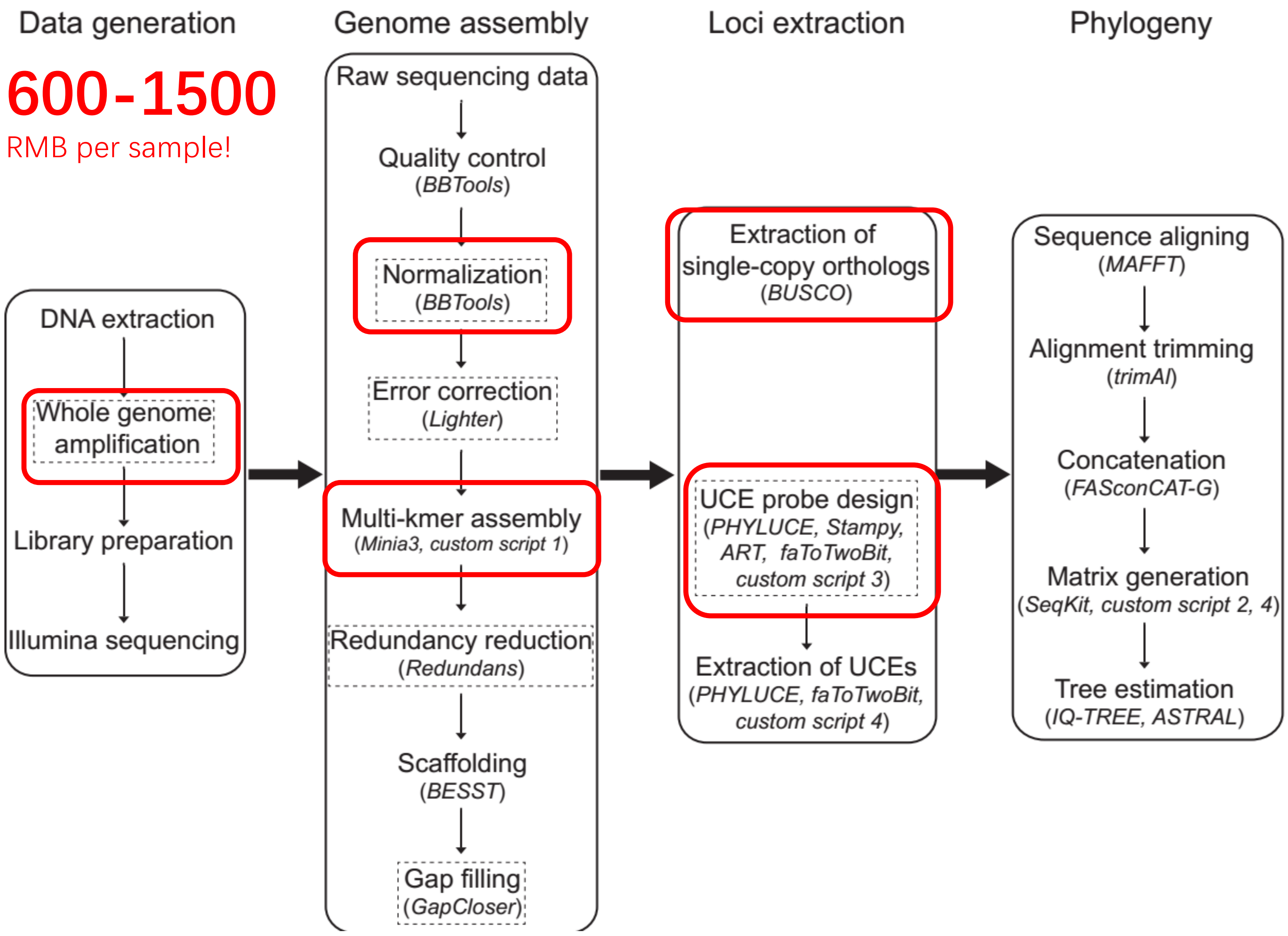
More accurately on abundance



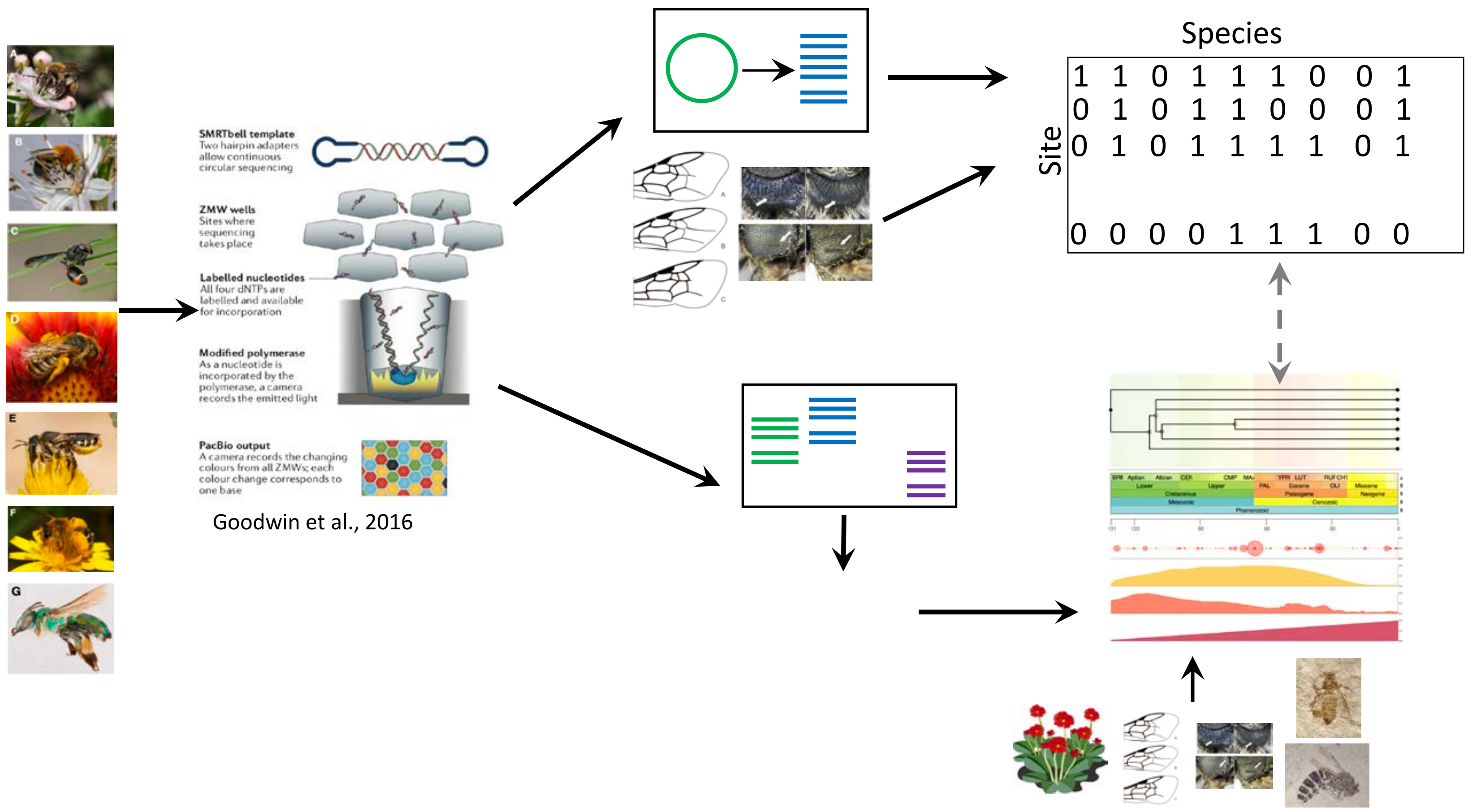
Zhang*…, Zhu, 2019, *Genome Biology & Evolution*

NGS Taxonomy / 2016

600-1500
RMB per sample!



Total-evidence Taxonomy / 2021



Scientific Questions

The effects of tree diversity on plant-insect interaction network

Schuldt et al. 2010, Journal of Ecology

The effects of climate change on plant, herbivores and predators.

Jamieson et al., 2012, Plant Physiology

Species Interactions

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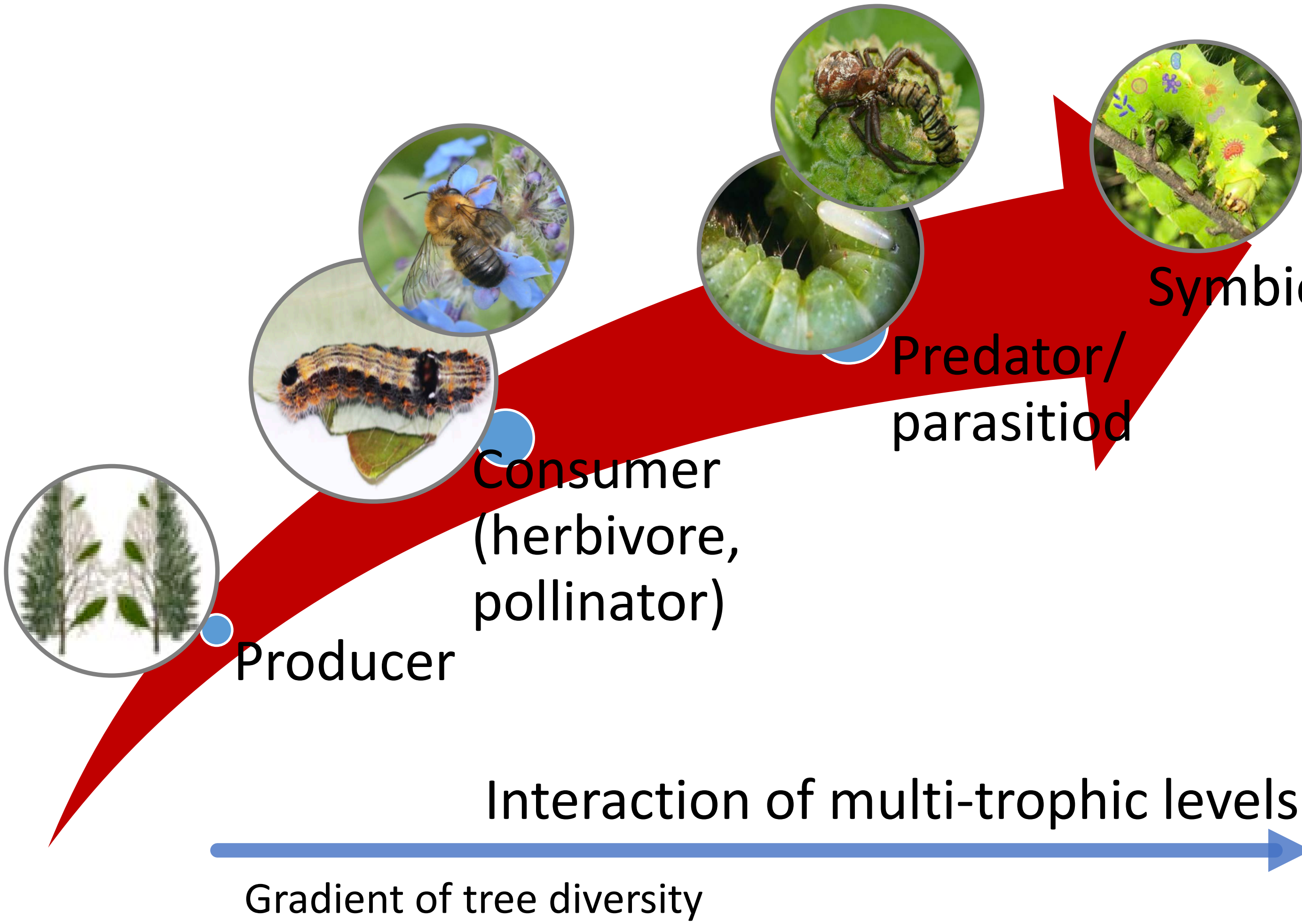
Scientific Questions



Biodiversity?

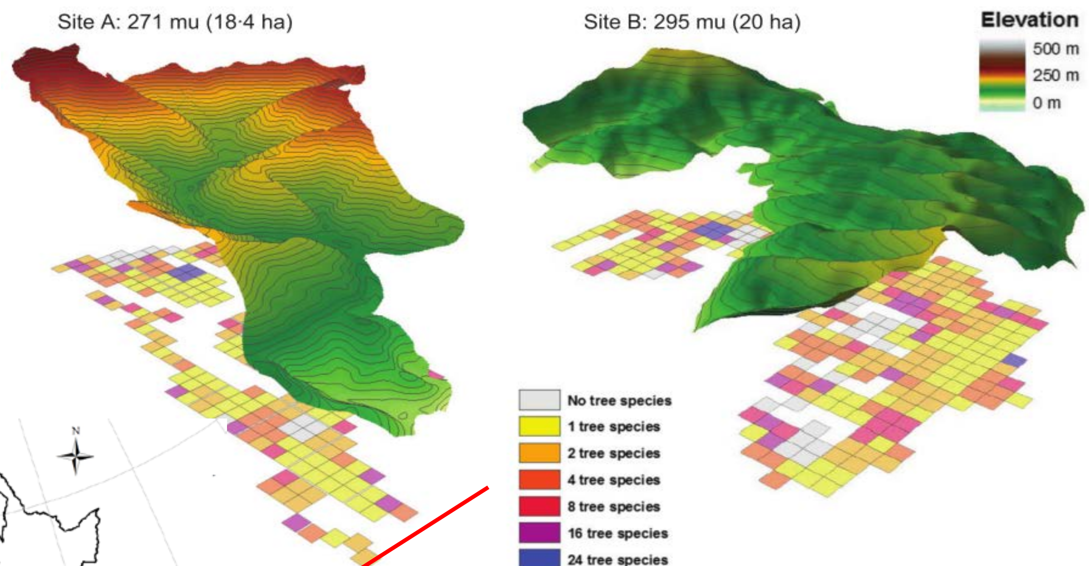
Network?

Co-existence?

Stability?

……

BEF-China Field Site



DNA Approaches

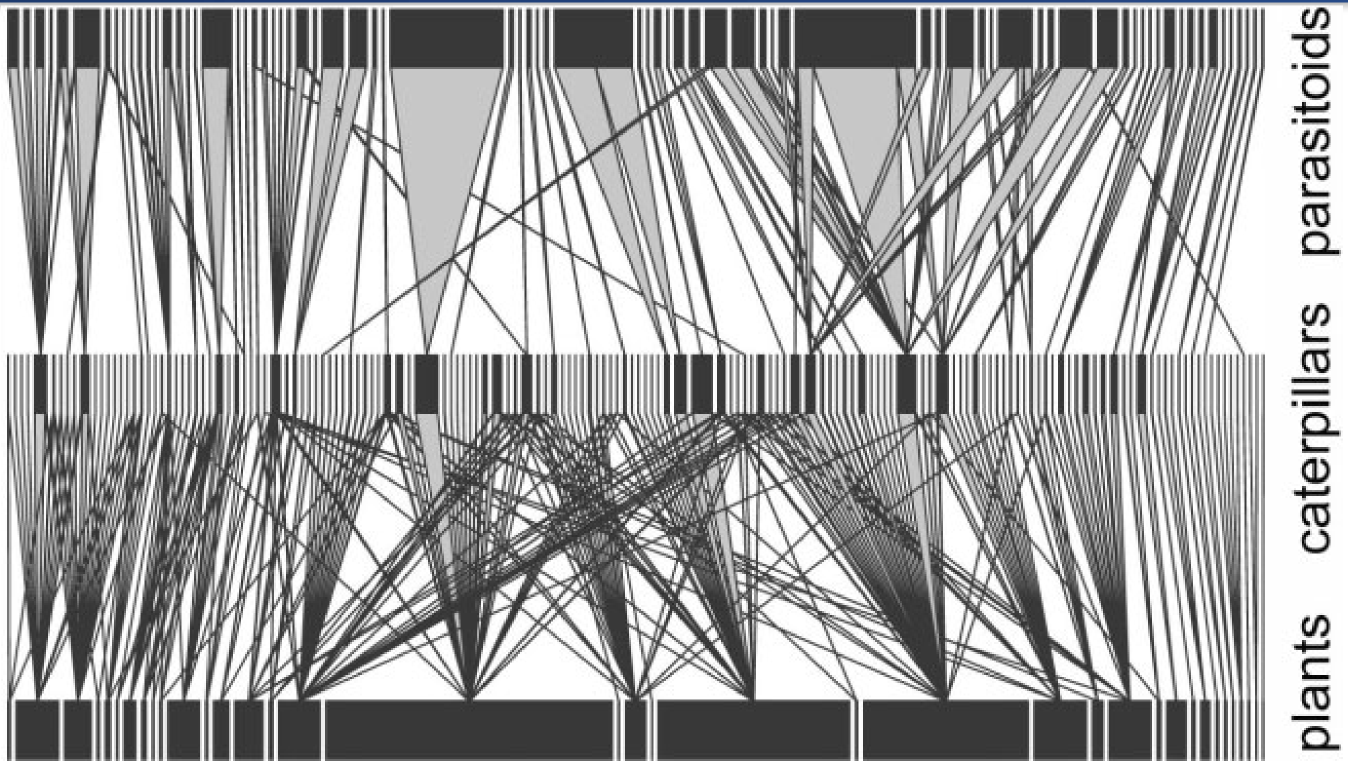


Fig. 4. Quantitative tri-trophic food web for secondary rainforest vegetation in PNG comprising 37 plant species, 4803 caterpillars from 154 species (exposed and semi-concealed guilds) and 643 parasitoids from 76 species (mostly Braconidae, Tachinidae and Ichneumonidae).

Morphology or DNA?

An example from plant-caterpillars-parasitoids

Hrcek et al. 2013, Oecologia

DNA Approaches

Monitoring biodiversity via metabarcoding

Ji et al. Ecology Letters, 2013

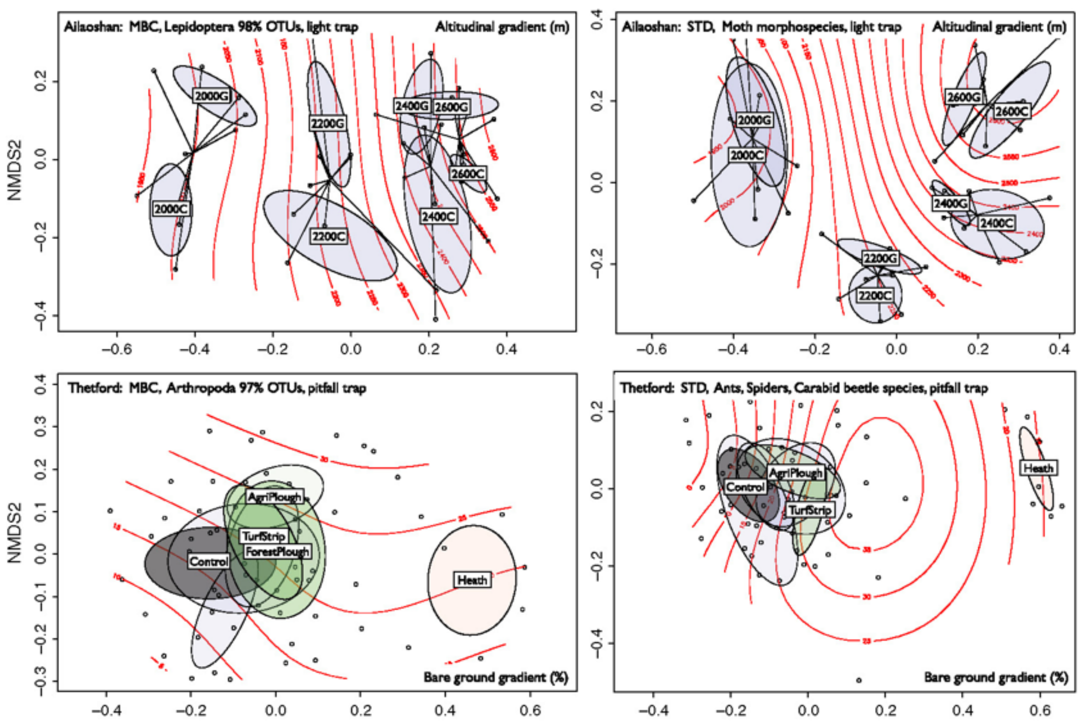
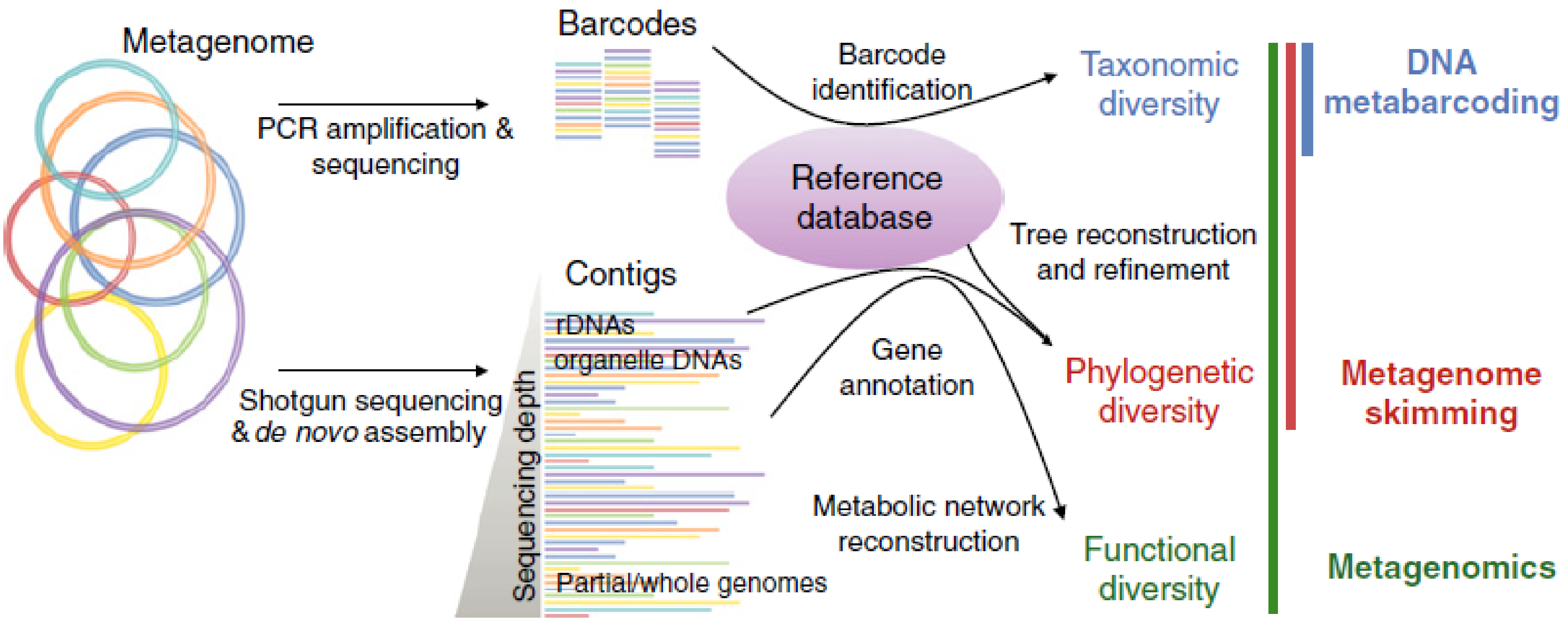


Figure 1 Non-metric multidimensional scaling (NMDS) ordinations. Points are census sites, and coloured ellipses are 95% confidence intervals of species centroids for each treatment level ['ordiellipses' (Oksanen *et al.* 2012)]. These ordinations are for visualisation; all statistical tests of treatment effects are conducted using *mvabund* (see text for details). (a, b). Ailaoshan. Altitude and Strata (canopy, ground) effects on moth communities. Sites within the same altitude are connected by line segments, with ellipses drawn for each combination of altitude and stratum. An NMDS ordination of the metabarcoding (MBC) Arthropoda 97% operational taxonomic units (OTUs) data set is in Supporting Information section S2. (c, d). Thetford. Restoration treatment effects on MBC (Arthropoda 97% OTUs) and STD (ants, spiders, carabid beetles) communities. Green ellipses indicate treatments that significantly shifted community composition away from the undisturbed Control (black) sites towards the target Heathland habitat (red). Blue ellipses indicate treatments that are not significantly different from Control sites (left unlabelled for clarity).

More bioinformatics required for large data analyses

DNA Approaches

Overview of NGS-based molecular methods for biodiversity assessment.



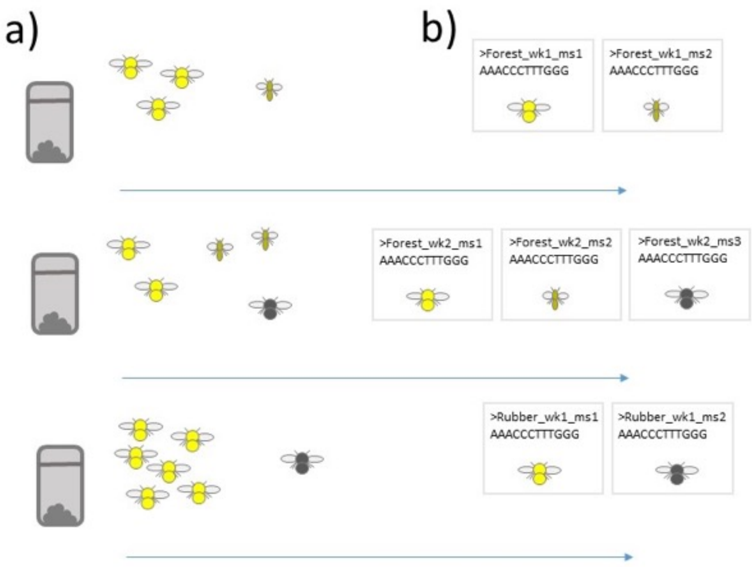
DNA Approaches

Building on previous work on bee diversity in and around rubber plantations

Pollen obtained from common bees such as the eastern honey bee

Plant library constructed for local species

2 pollen barcodes sequenced using Illumina platform



Liu et al. (2017). *Scientific Reports*.

Project 1. Nest Trap System

- **Limited but representative number of species （pollinator、predator、parasitoid）**
- **Cheap, standardized, readily available**
- **Quantifying trophic interactions （pollen - bee， prey – wasp， host - parasitoid）**
- **Assessment of species’ entire life history**

Project 1. Nest Trap System

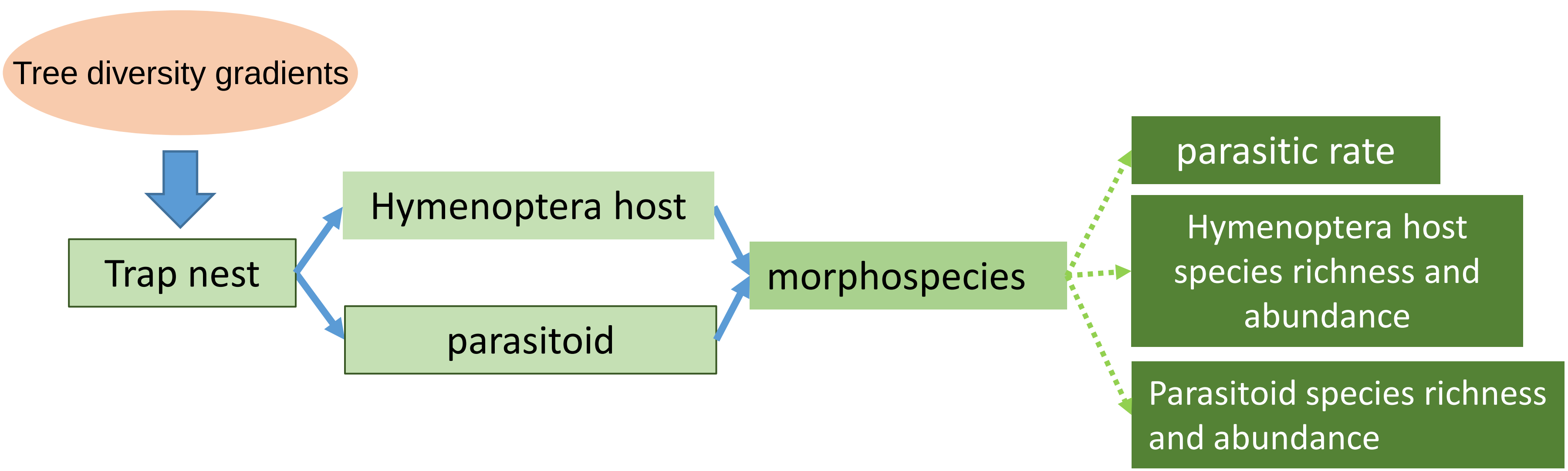
- parasitism
  - cleptoparasitism
    - Adult broke the seal
    - Larva broke the seal
  - parasitoid
    - Monoparasitoid
    - Hyperparasitoid

Melittobia sp.

Project 1. Nest Trap System

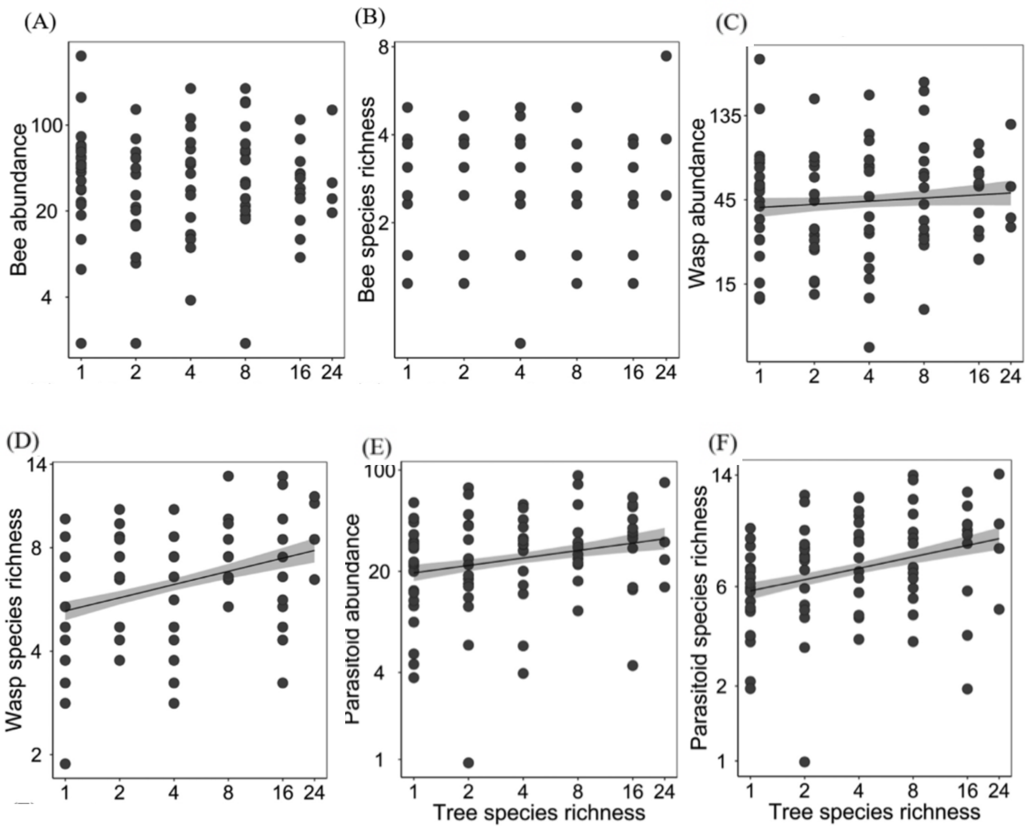


Year	2015-2016	2018
Number of tubes	5321	2312
Number of cells	13790	5164
Number of parasitoids	1598	493



Project 1. Nest Trap System

The ecological effect of tree diversity on cavity-nesting Hymenoptera and associated parasitoids



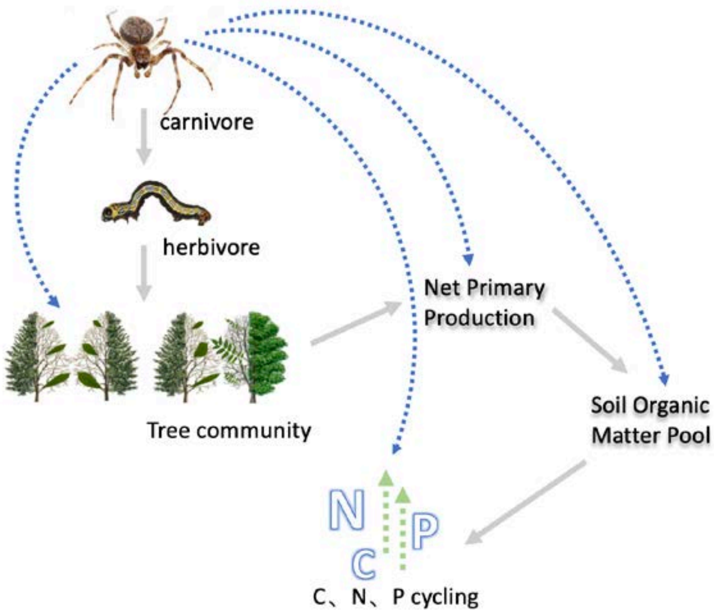
- Abundance and species richness of predatory wasps and parasitoids were positively correlated with tree species richness, while bee abundance and bee species richness were unrelated to tree species richness.

- tree species richness increases the abundance and species richness of important communities such as predators and parasitoids.

Guo,…Li, … Zhu. 2020. Basic and applied Ecology*

Project 2. Predator System

The ecological effect of tree diversity on predator (spider) diversity.



Natural enemy hypothesis: Predators tend to be **polyphagous** and have **broad habitat requirements**, so they would be expected to encounter **a greater array of alternative prey** and **microhabitats** in a heterogeneous environment

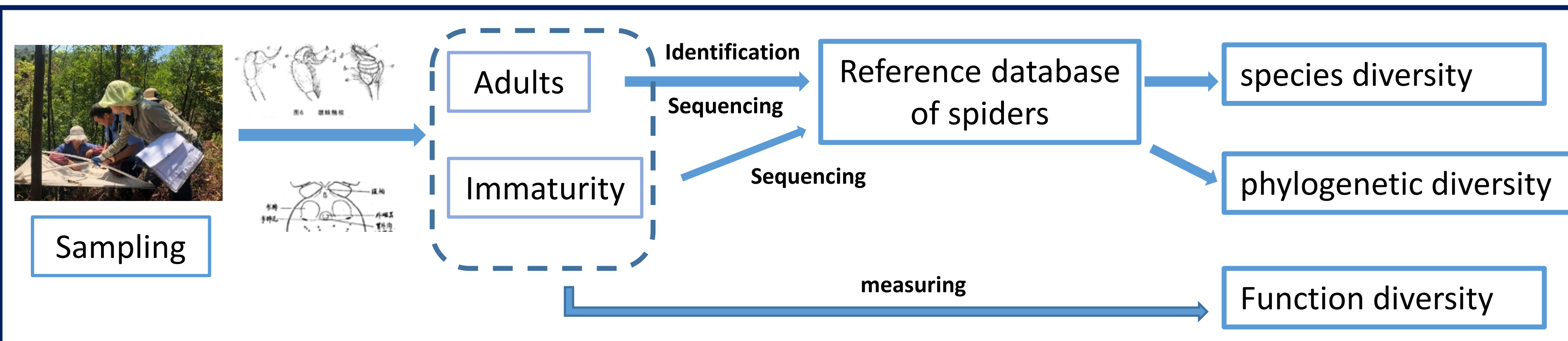
Most related studies were conducted in farmland and grassland, not forest.

Q1: How do predators' species diversity respond to tree diversity?

Q2: How do predators' functional traits respond to tree diversity?

Project 2. Predator System

	2017	2018	2019	2020
Total	4449	2685	1707	2751
April	1862	961	385	NA
June	1577	782	370	973
September	1010	942	952	1779



Species Interactions

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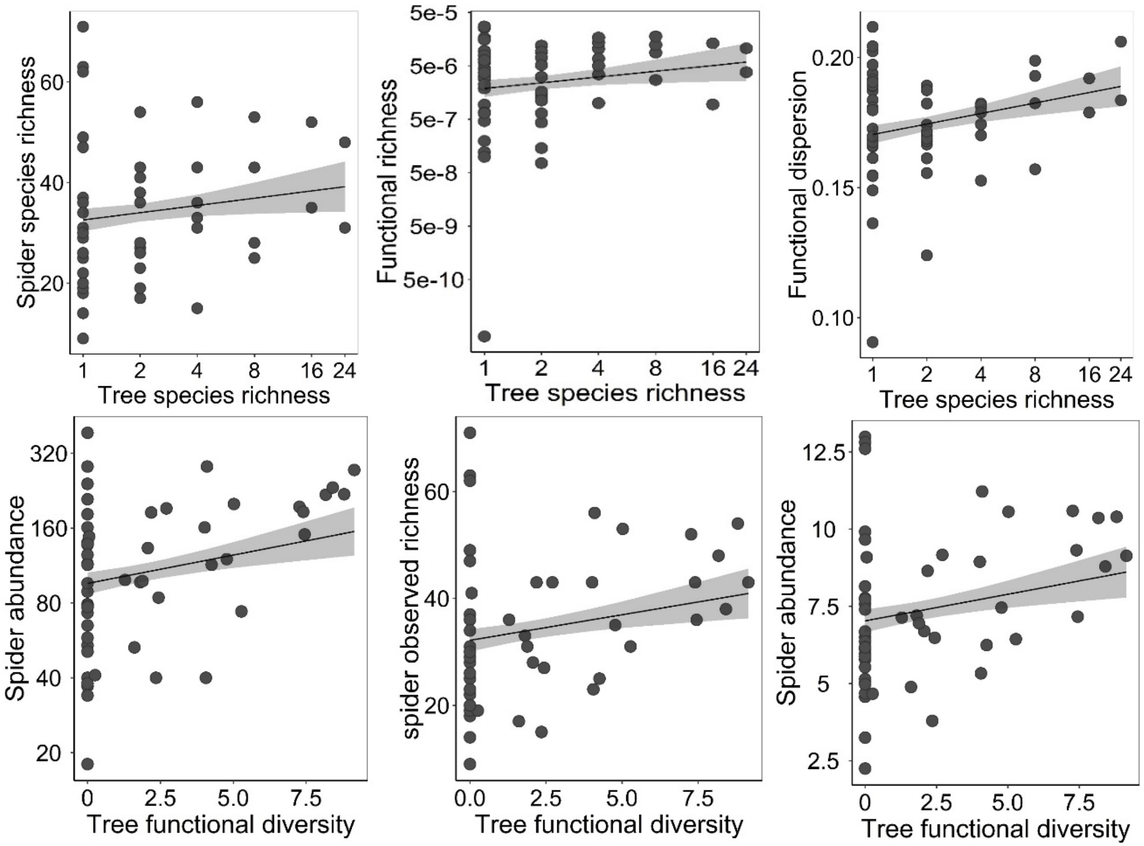
Project 2. Predator System



- Abundance and species richness of spiders were positively correlated with tree functional richness

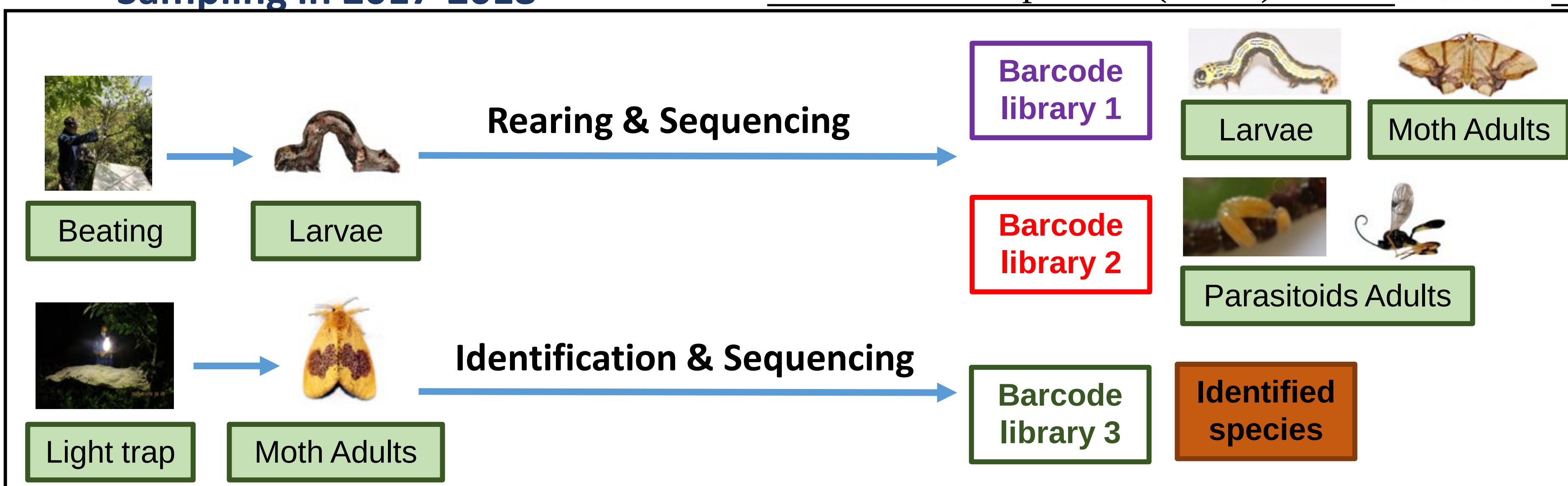
Chen,…Zhu. In preparing

Institute of Zoology, Chinese Academy of Sciences

Project 3. Herbivore System

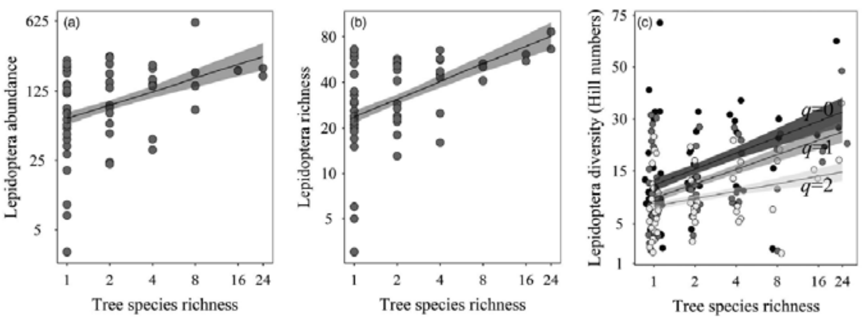
Sampling in 2017-2018

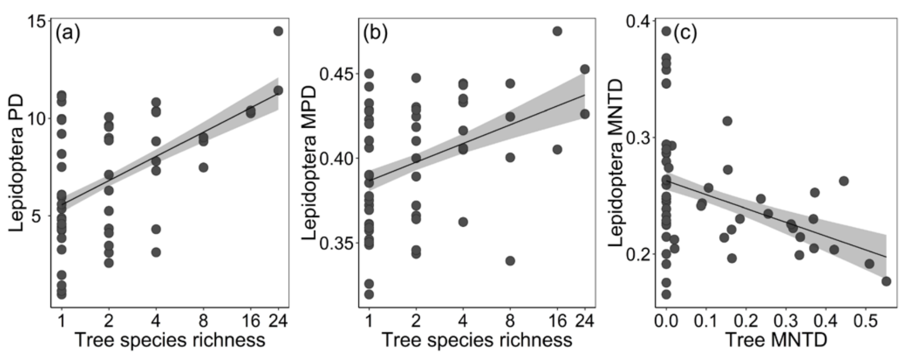
Year	Month	Number of larvae
2017	April(7-24)	718
	June(7-7.6)	2053
	September(1-15)	472
2018	April(7-20)	1162
	June(6-17)	3488
	September(3-15)	574

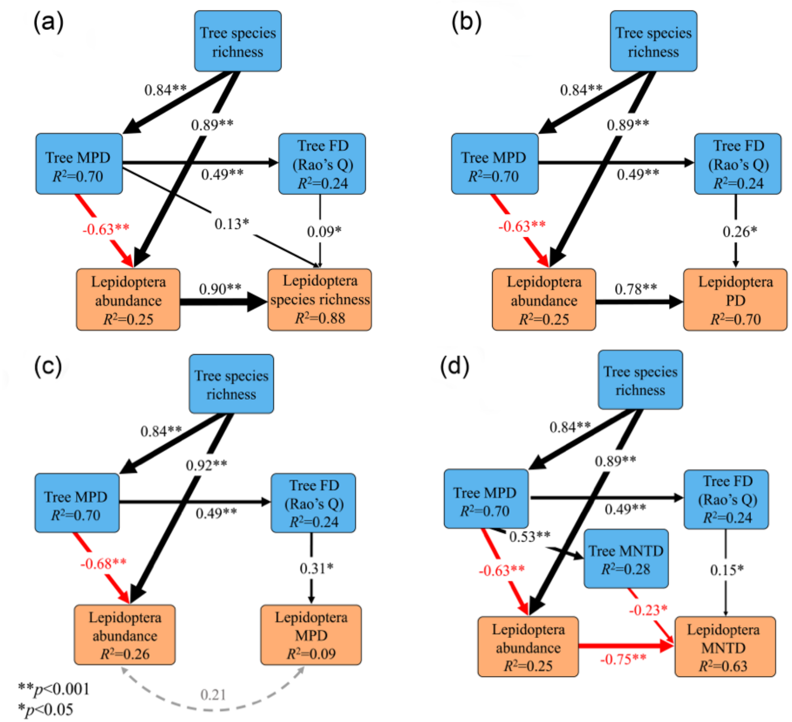


Project 3. Herbivore System

3.1 The ecological effect of tree diversity on herbivore diversity





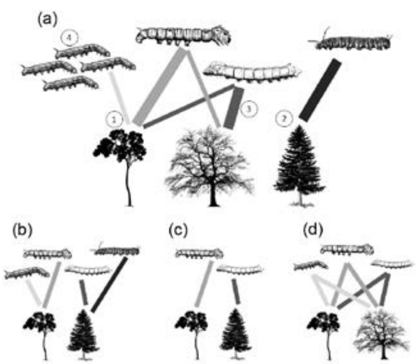


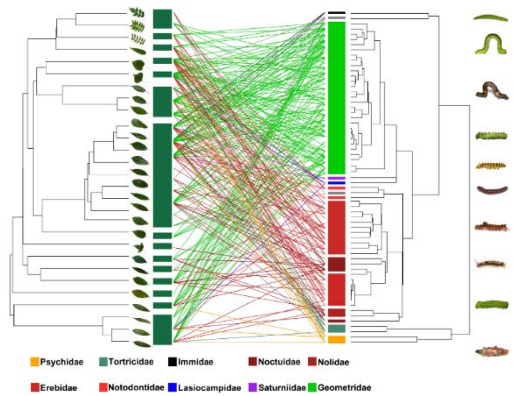
- Lepidopteran abundance, the effective number of species and Faith's PD and MPD ,but not MNTD, significantly increased with experimentally manipulated tree species richness.

Wang, Li, … Zhu. 2019. Journal of Ecology*

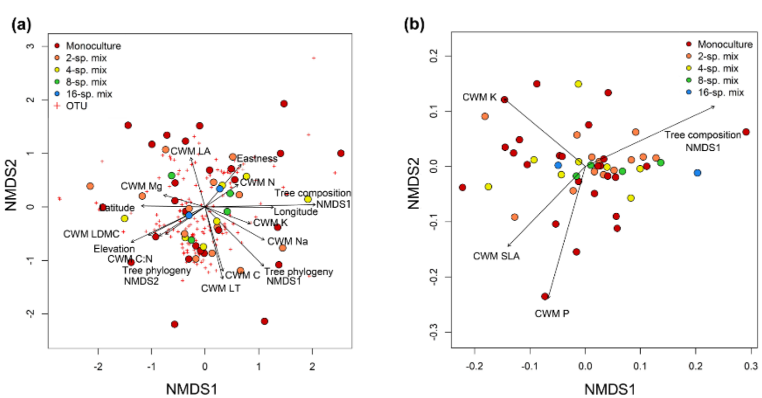
Project 3. Herbivore System

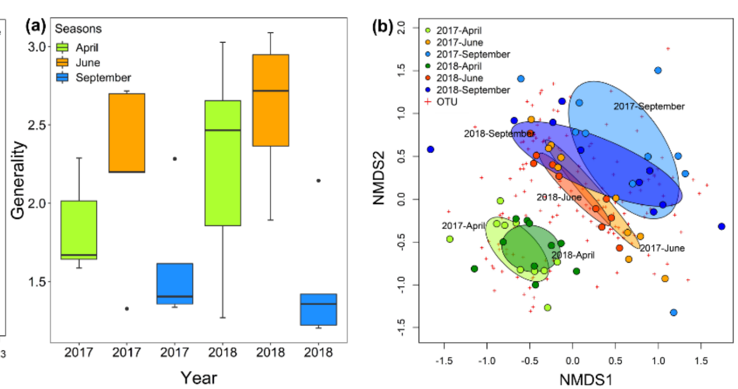
3.2 Host functional and phylogenetic composition structure plant–herbivore networks





- phylogenetic host composition and related palatability/defence traits but not tree species richness significantly affected herbivore communities and interaction network complexity at both the species and community levels





Wang, Li, … Zhu. 2020. Molecular Ecology*

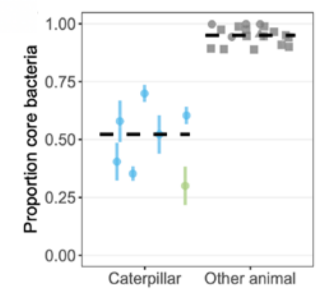
Project 3. Herbivore System

3.3 Tree species richness and leaf traits shaped/driven caterpillar-associated microbiomes

Caterpillars lack a resident gut microbiome PNAS

Tobin J. Hammer[a,b,1], Daniel H. Janzen[c], Winnie Hallwachs[c], Samuel P. Jaffe[d], and Noah Fierer[a,b]

[a]Department of Ecology and Evolutionary Biology, University of Colorado Boulder, Boulder, CO 80309; [b]Cooperative Institute for Research in Environmental Sciences, University of Colorado Boulder, Boulder, CO 80309; [c]Department of Biology, University of Pennsylvania, Philadelphia, PA 19104; and [d]The Caterpillar Lab, Keene, NH 03431

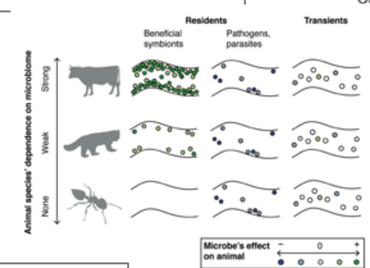


MINIREVIEWS – Environmental Microbiology

OXFORD FEMS JOURNALS investing in science

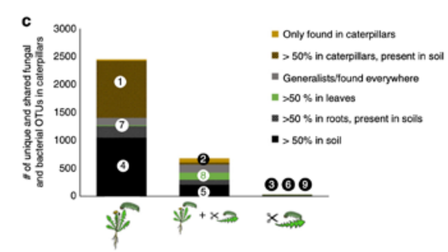
Not all animals need a microbiome

Tobin J. Hammer[1,*,†], Jon G. Sanders[2,‡] and Noah Fierer[3,4]



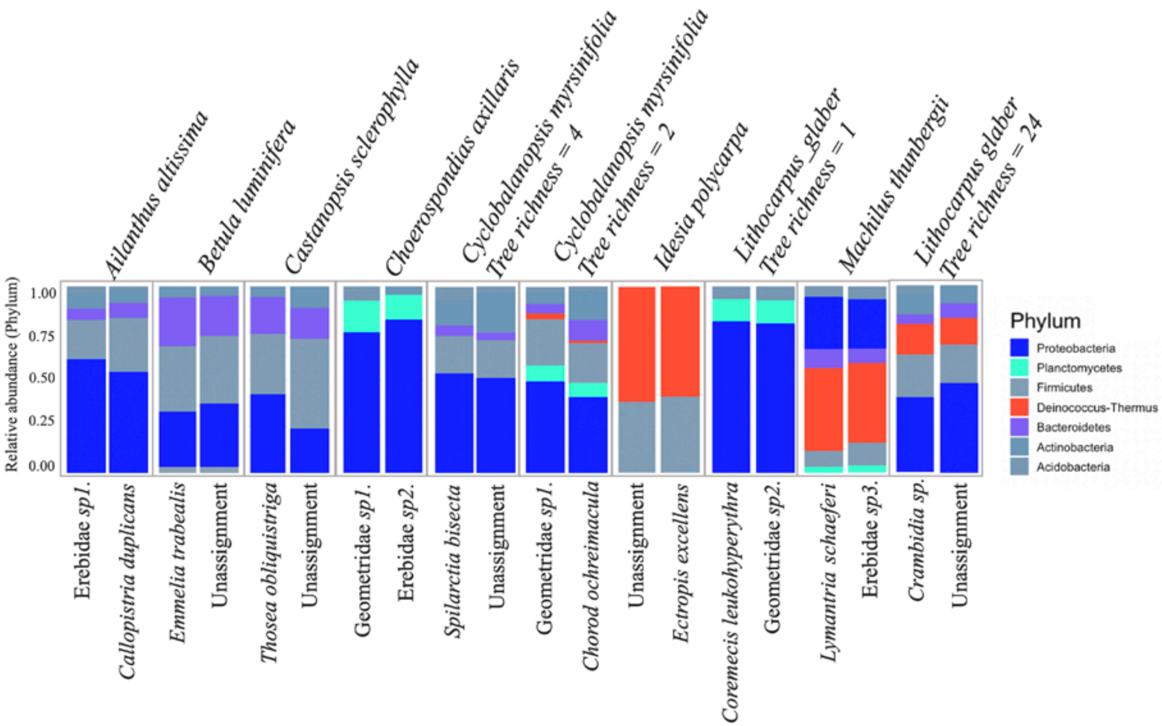
NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-019-09284-w

Foliar-feeding insects acquire microbiomes from the soil rather than the host plant



Project 3. Herbivore System

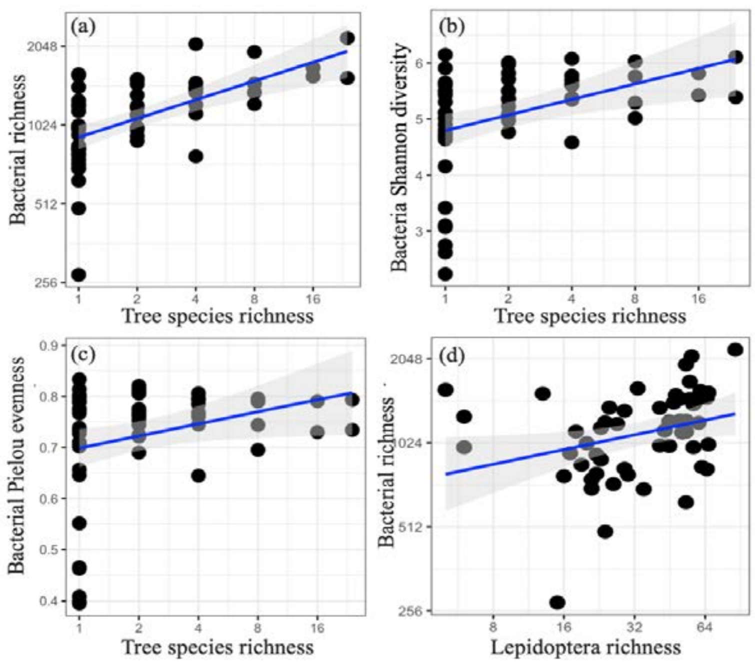
3.3 Tree species richness and leaf traits shaped/driven caterpillar-associated microbiomes



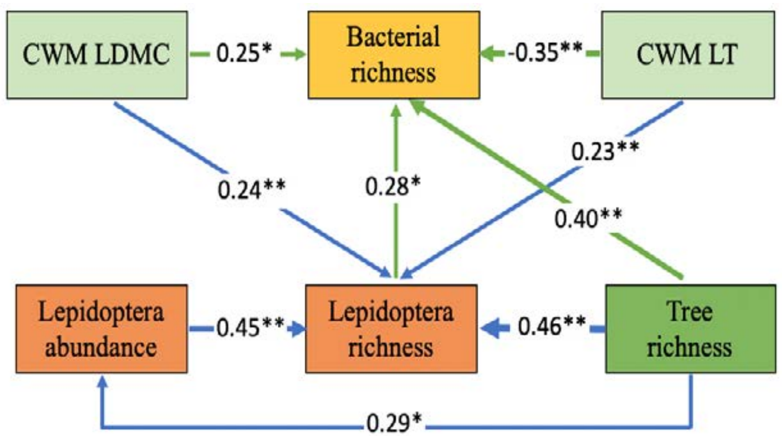
- 7,909 bacterial OTUs from 634 caterpillar individuals
- Different caterpillar species feeding on the same tree species showing the similar pattern of microbial composition.

Project 3. Herbivore System

3.3 Tree species richness and leaf traits shaped/driven caterpillar-associated microbiomes



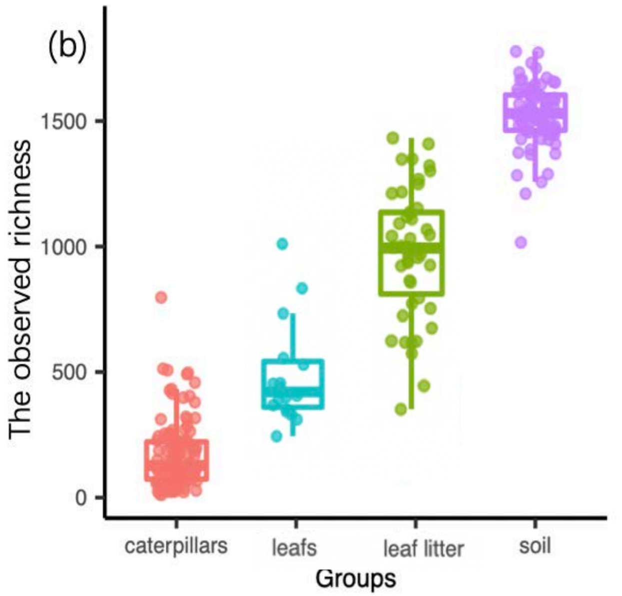
- Tree diversity and leaf traits (Leaf toughness and dry matter content) was found to drive the diversity of caterpillar-associated bacteria both directly and indirectly via effects on caterpillar communities

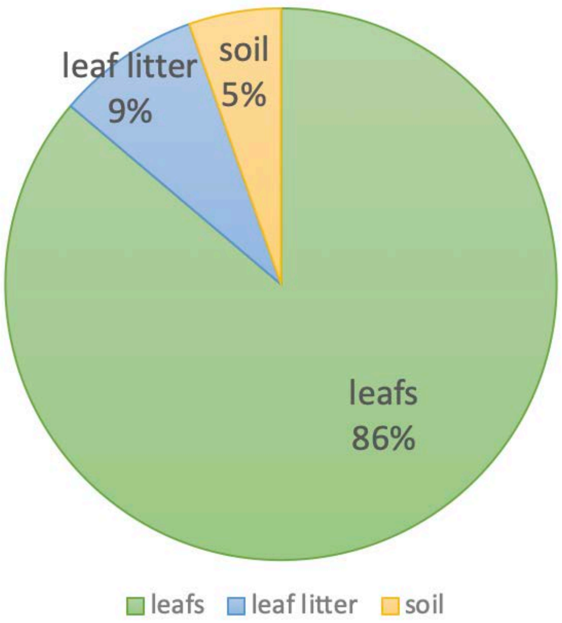


Li,…,Zhu. 2020. Ecology and Evolution*

Project 3. Herbivore System

3.3 Tree species richness and leaf traits shaped/driven caterpillar-associated microbiomes



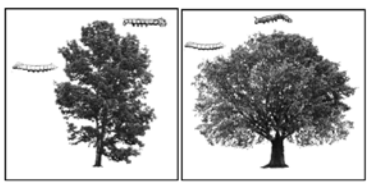


- Microbial α-diversity: soil > leaf litter > leaves > caterpillar

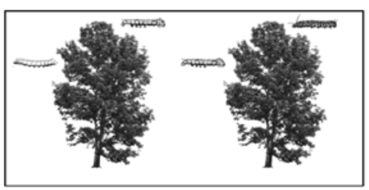
- Phyllosphere is one of the main sources of the herbivore microbiome

Project 3. Herbivore System

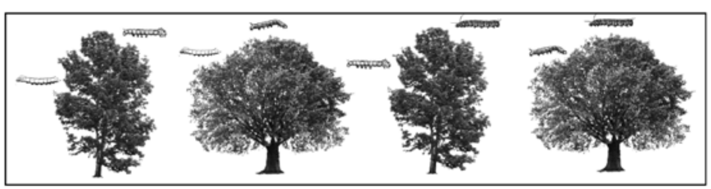
3.4 Phylogenetic relatedness, functional traits and spatial scale determine herbivore co-occurrence



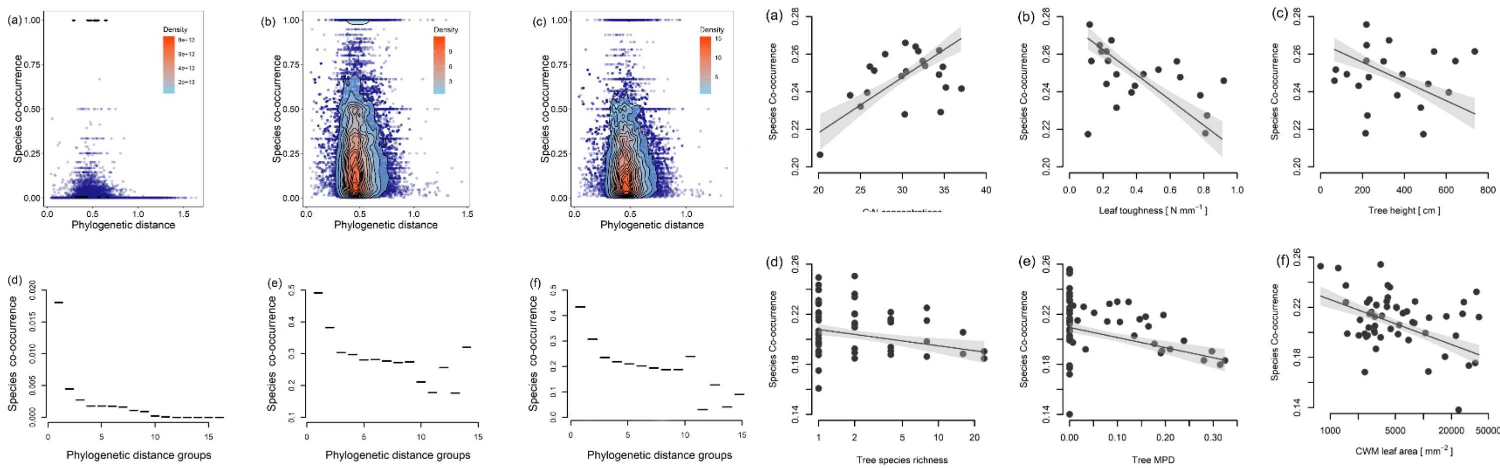
(a) Tree individual level



(b) Tree species level



(c) Plot level



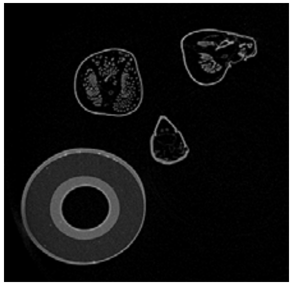
- Lepidoptera co-occurrence was negatively associated with their phylogenetic relatedness, tree diversity and defensive traits, but positively associated with nutritional functional traits. Further, species co-occurrence was higher at larger spatial scales (tree species or plot) than at smaller scale (individual trees).

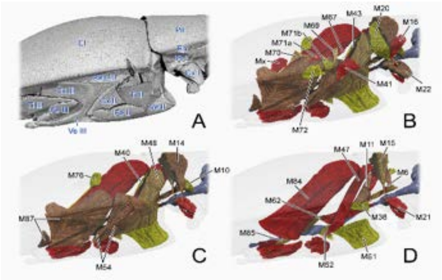
Wang, Li, … Zhu. in revision*

Project 4. Functional Traits

Specimen

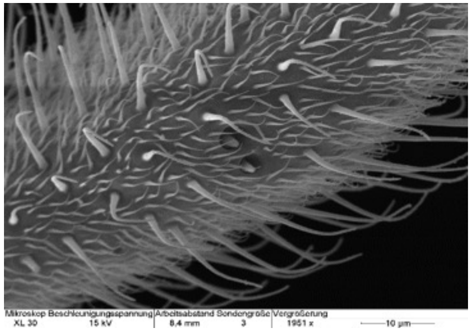
μ-Computer Tomography (μ-CT)





3D Reconstruction

Critical Point Dryer



Scanning Electron Microscope (SEM)

Project 4. Functional Traits

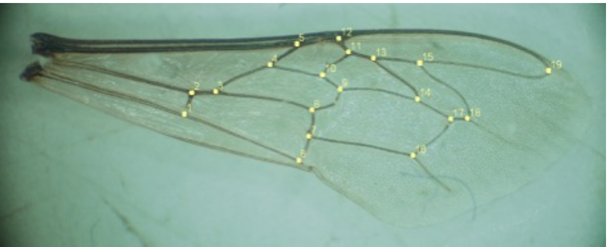
Transfer descriptive morphology into digital bioinformatic pipeline:

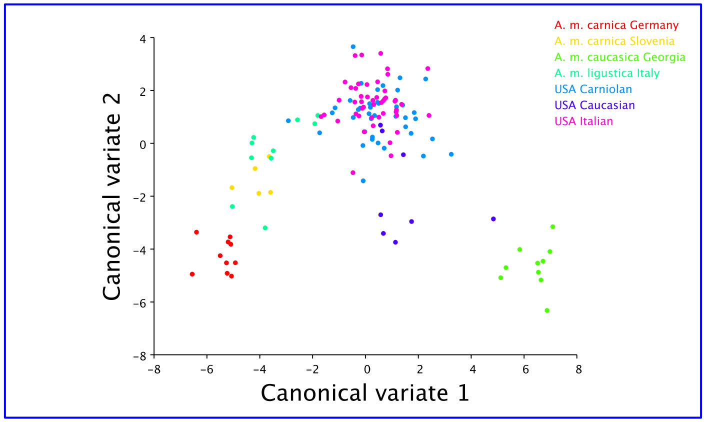
(1) Undamaged anatomy and more detailed characters

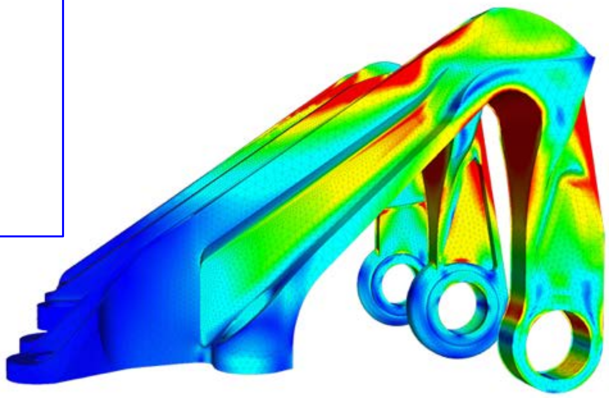
(2) Phylogenetic and geometric morphometrics

(3) Functional morphological data for ecological comparison and modeling

(4) Finite element analysis for forces and material properties







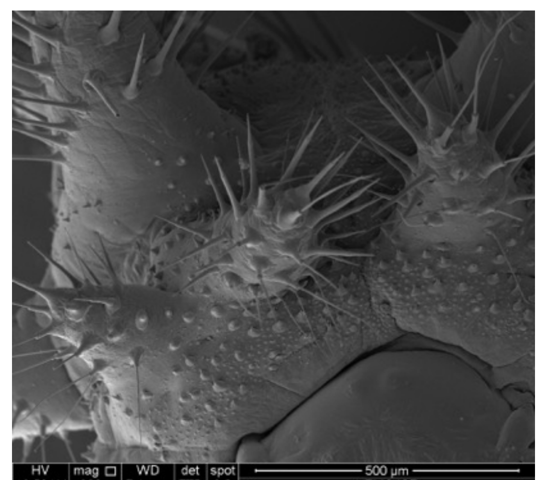
Project 4. Functional Traits

Question: What kinds of morphological characters assist the larvae have such a powerful mouthpart crossing phylogenetic barriers?

Experiment:
(1) Carefully examine the eating processes of phylogenetically multiple lepidopteran larvae
(2) Make 3D reconstruction for their mouthparts
(3) Referring to the corresponding plant morphological characters, summarize the important mouthpart characters of larvae

Additional experiment:
3D reconstruction of mouthpart of special feeding habits (e.g. leaf mining)

Mouthpart of larvae



Labial palp

Acknowledgements

Institute of Zoology, Chinese Academy of Sciences

Team Members

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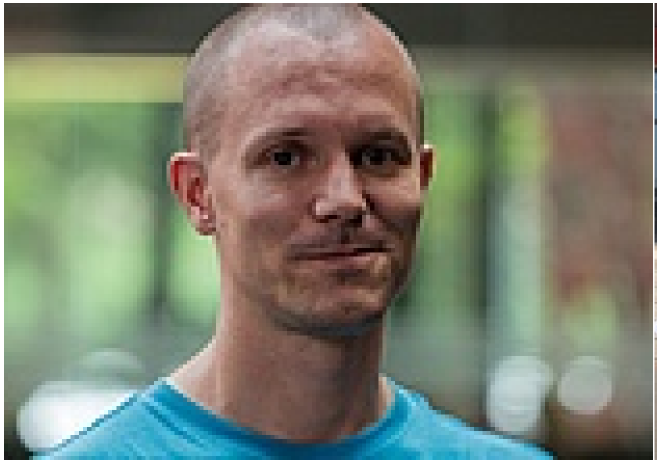

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Thank You !