Latitudinal biodiversity gradients of leaf miners and DNA barcoding

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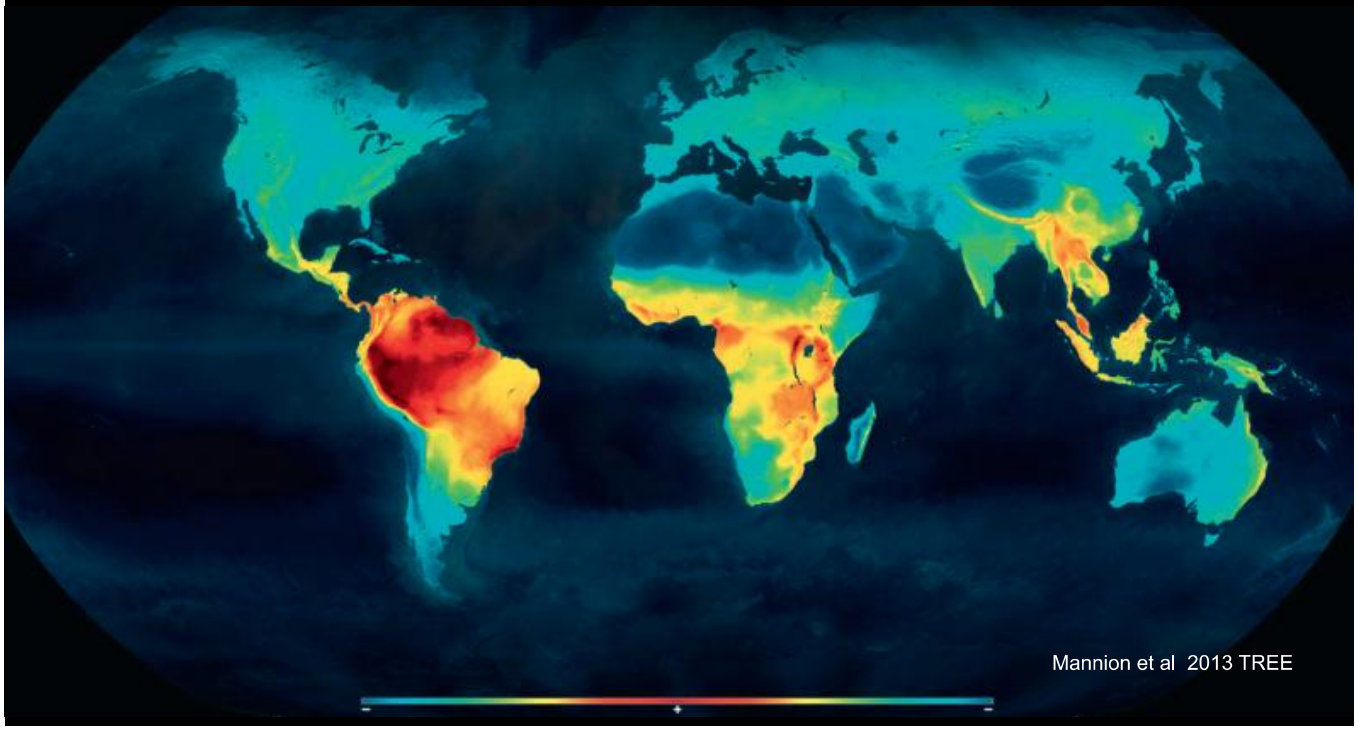
INRA, Orleans , France

Latitudinal Biodiversity Gradient (LBG)

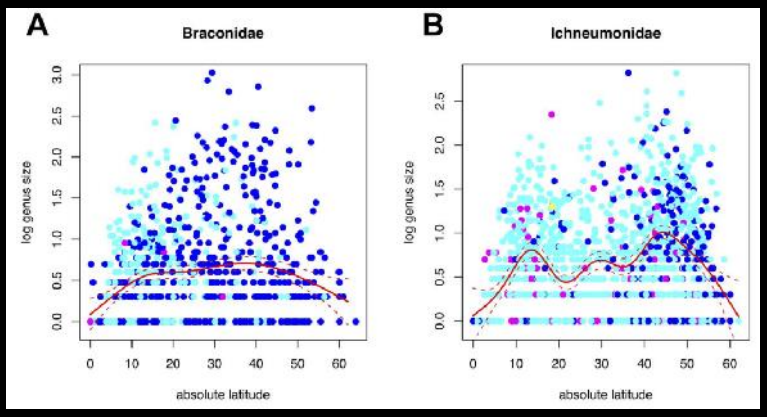
- Species Richness tends to increase towards the equator
- Distribution of extant terrestrial vertebrate species



Mannion et al 2013 TREE

Reversed Latitudinal Biodiversity Gradients or sampling artifact?

Majority of ichneumonid and braconid species described from temperate regions



Quicke 2012 PLoSOne

Discovery of over 200 undescribed orthocentrine species from Ecuador
Massive under-description of tropical parasitoid faunas

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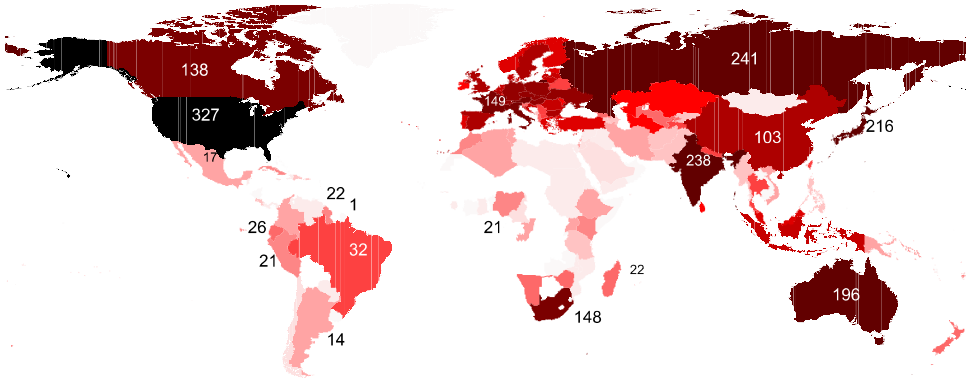
Unprecedented ichneumonid parasitoid wasp diversity in tropical forests

Anu Veijalainen[1,2,*], Niklas Wahlberg[1], Gavin R. Broad[3], Terry L. Erwin[4], John T. Longino[5] and Ilari E. Sääksjärvi[1]

Gracillariidae

- 99 genera, 1931 species described worldwide
- Agricultural pests and invasive species
- Leaf-miners, but also fruit mining, stem mining,
- High levels of host specificity

- Higher species richness in temperate areas
- Research on gracillariid systematics mainly in Europe, Asia, and North America
- Only one species recorded from French Guiana



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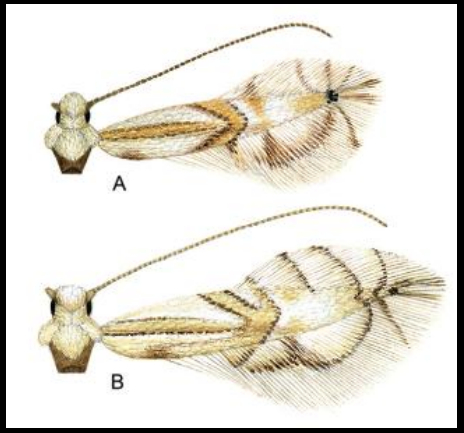
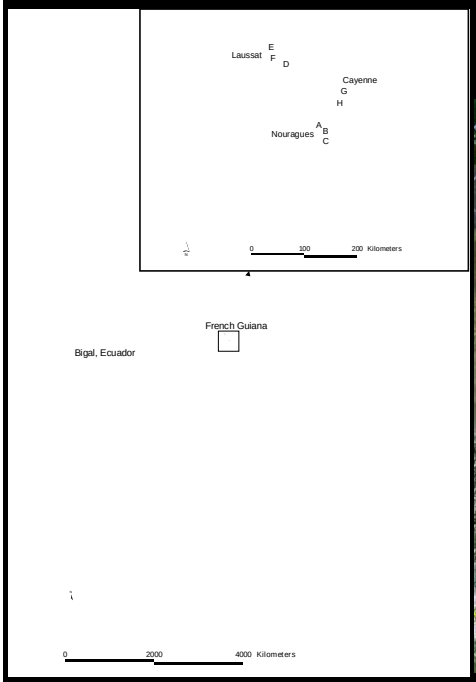
Low diversity in Neotropics: artefact of undersampling?

- 184 gracillariid species recorded in the Neotropics
- 1996 – 1998, preliminary survey by Davis & Wagner (ALAS) Project
- La Selva Biological Station a lowland rain forest site in northwestern Costa Rica
- Based on mine morphology and host plant information: 200 species estimated
- Most specious genus *Phyllocnistis* with an estimated 60 species, (only a single previously known species)



- Hypothesis: Low diversity of Neotropical gracillariids is due to insufficient sampling and strong description deficit

- Problem: how to rapidly assess a local fauna when it may be partly or largely unknown



Field Sampling

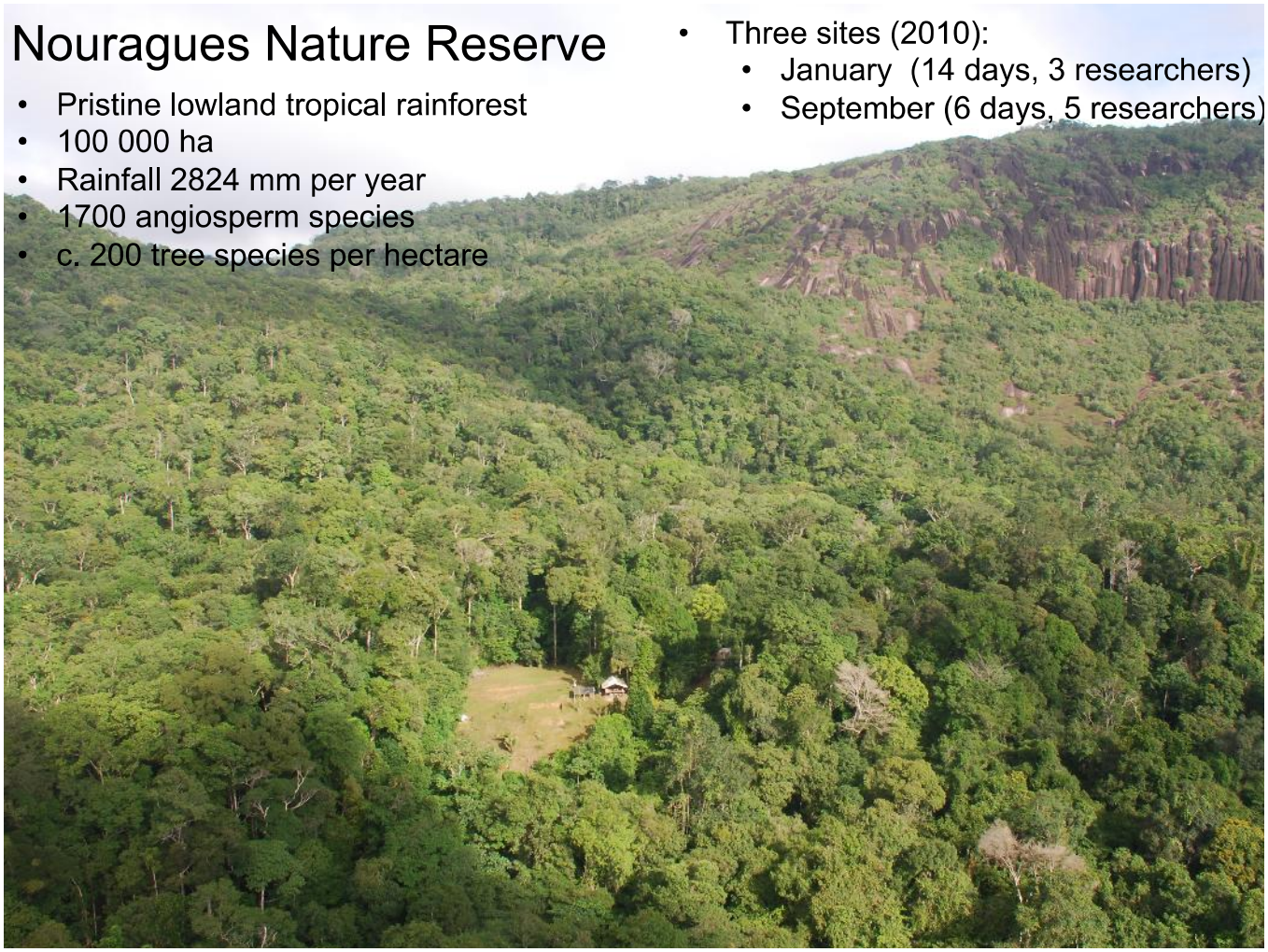
- French Guiana:
  - Nouragues (3 sites 20 days)
  - 5 other sites (1 day each)

- Ecuador: Bigal (1 researcher 6 days)

Nouragues Nature Reserve

- Pristine lowland tropical rainforest
- 100 000 ha
- Rainfall 2824 mm per year
- 1700 angiosperm species
- c. 200 tree species per hectare

- Three sites (2010):
  - January (14 days, 3 researchers)
  - September (6 days, 5 researchers)



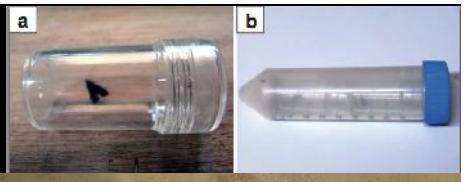
Inselberg Camp (150 m alt)

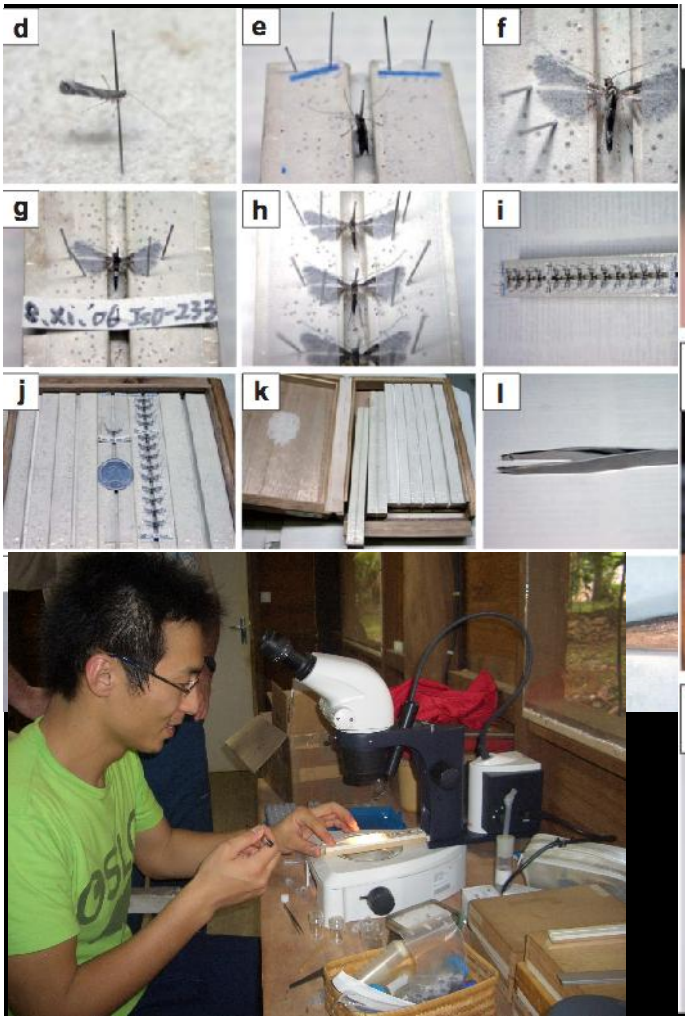
Summit of Inselberg (475 m alt)

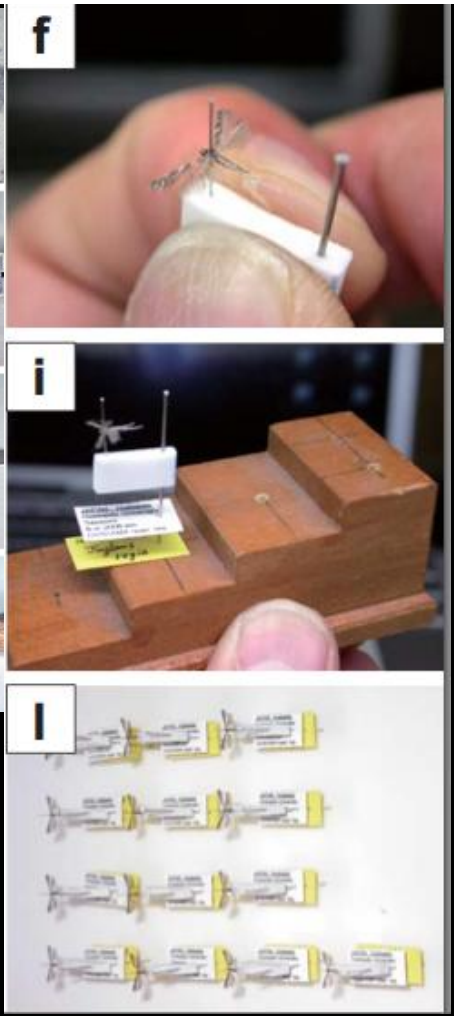
le **site Pararé**

Field Sampling

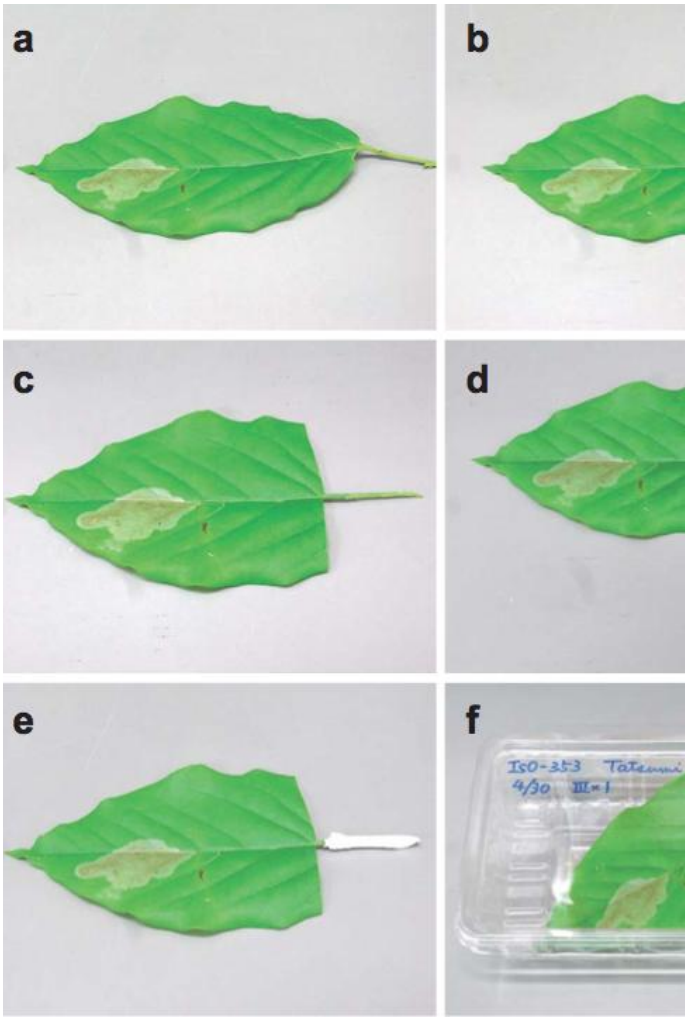
- 125-250 W MV lamps for sheet use at two close points (camp 150 m, inselberg 475 m)
- Leaf mine collecting







Mounting all ‘micros’ in field

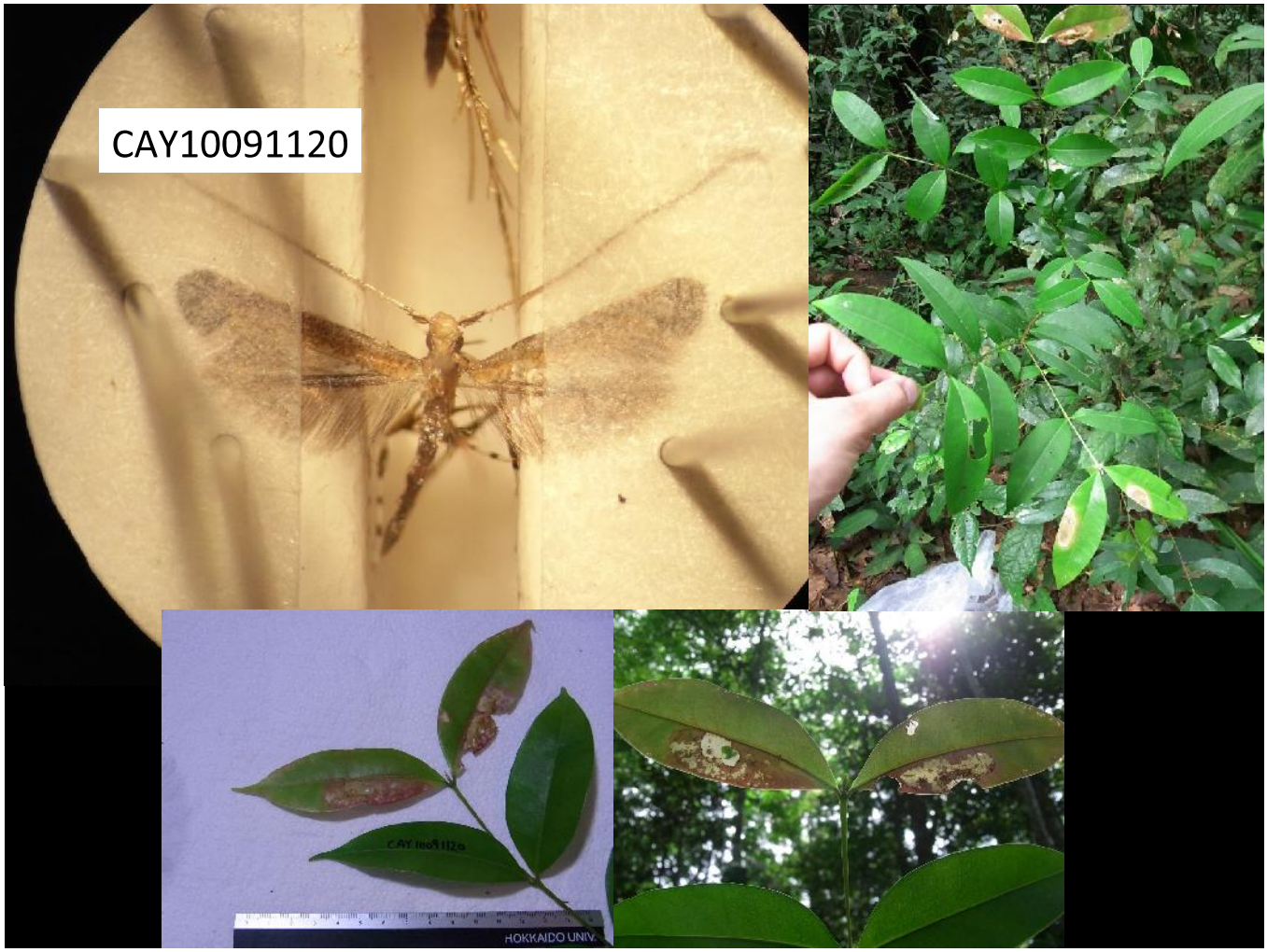


Rearing Protocol

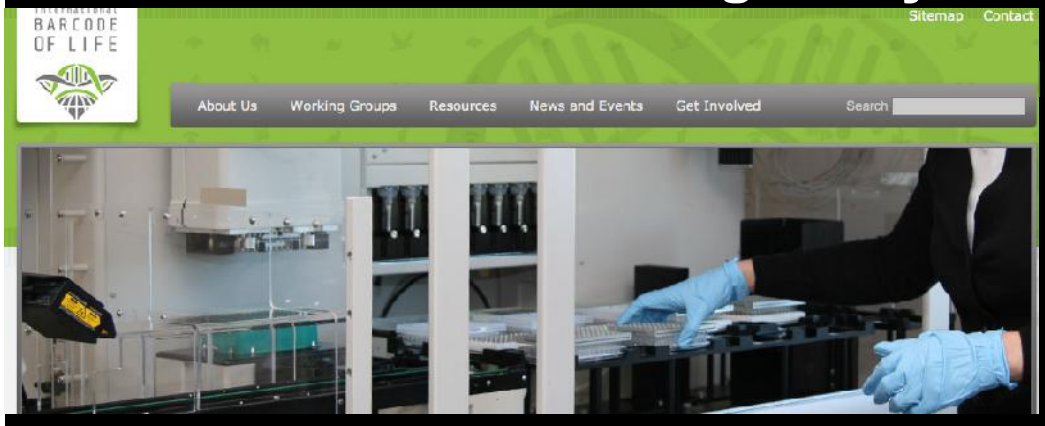
- 1% sugar solution
- Keep tissue wet

Back to the lab from the field

Lab Rearing



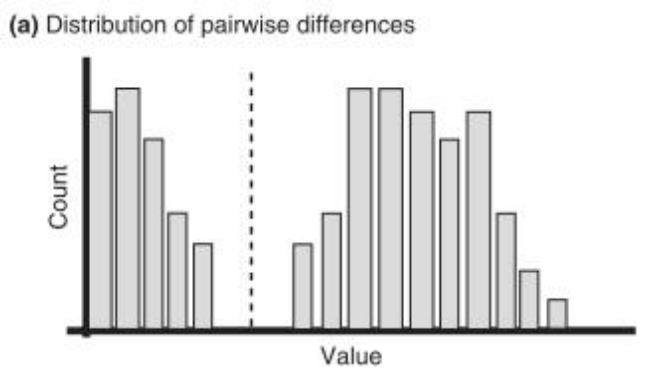
DNA barcoding Analysis



- One hind leg per specimen (adults)
- Barcoding at Guelph, Canada.
- 485 barcodes (94% success per plate):
  - 445 French Guiana
  - 40 Ecuador
- 372 adults, 104 larvae and nine pupae
- Nearly all barcodes novel to BOLD: only three *Eucosmophora* had close matches

Species delimitation

- Automatic Barcode Gap Discovery



Intraspecific divergence (organisms belonging to the same species) < interspecific divergence (organisms from different species)

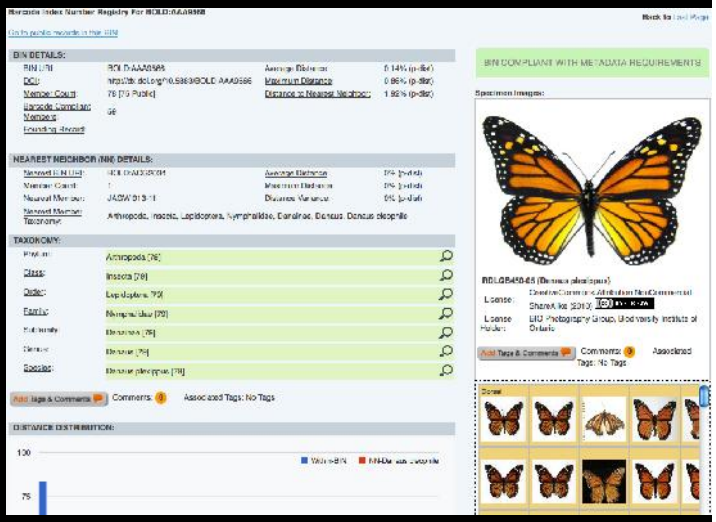
MOLECULAR ECOLOGY
Molecular Ecology (2012) 21, 1864–1877 doi: 10.1111/j.1365-294X.2011.0

ABGD, Automatic Barcode Gap Discovery for primary species delimitation

N. PUILLANDRE,* A. LAMBERT,† S. BROUILLET‡§ and G. ACHAZ‡§

Species delimitation: Refined Single Linkage algorithm

- Rapid automated Operational Taxonomic Units (OTUs) recognition system
- Each OTU is assigned a Barcode Index Number (BIN)
- Persistent registry : DOI number
- 274 000 BIN web pages available: http://www.boldsystems.org/bin



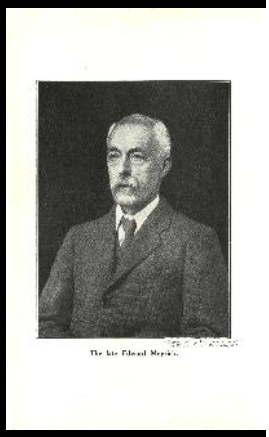
OPEN ACCESS Freely available online
PLOS ONE

A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System

Sujeevan Ratnasingham[1]*, Paul D. N. Hebert[1,2]
1 Biodiversity Institute of Ontario, University of Guelph, Guelph, Ontario, Canada, 2 Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada

Morphological Analysis

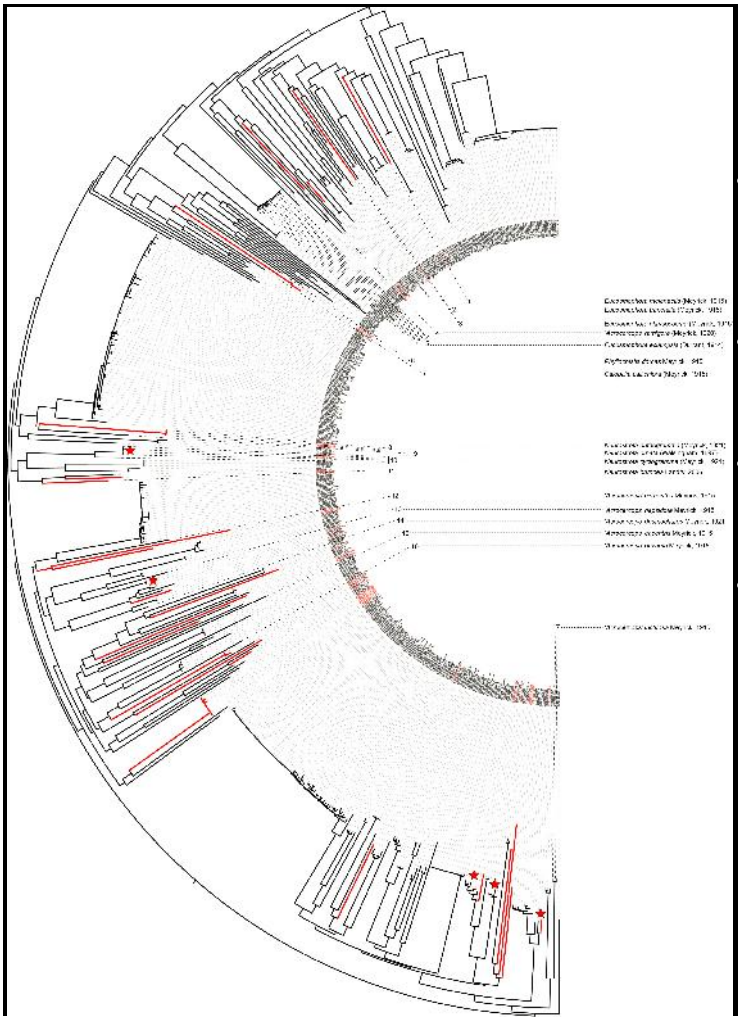
Eucosmophora_echinulata



- Identification of species based on wing patterns (monographic revisions and taxonomic literature): morphospecies
- Meyrick's Types at BMNH London
- 64% of 184 Neotropical species described by Meyrick before 1930's
- 150 species examined from types/literature.

VII. *Descriptions of South American* Micro-Lepidoptera.
By E. Meyrick, B.A., F.R.S.

[Read May 5th, 1915.]



- Species delimitation:
  - ABGD: 136
  - BINs: 151
- Identification using morphology:
  - 17 tentative species names
- Nouragues: 108 species 64 (59.3%) are represented by singletons
- 85% of the species collected as adults do not fit any of the 150 species we examined from types/ literature.

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Spatial and Temporal Turnover

Table 1 Local species richness for the nine sampling sites

Collecting sites	Number of barcodes	Number of BINS	Number of ABGD
A: Nouragues, Inselberg Camp	132	72	67
B: summit of Inselberg	65	20	20
C: Nouragues Pararé Camp	166	41	39
D: Laussat 'terra firme'	21	10	9
E: Laussat 'white sand'	6	5	5
F: Carbet Montagne de Fer	2	2	2
G: Grand Matoury	30	12	12
H: Cayenne	24	10	11
I: Bigal	40	27	27

- Groupings by the two delimitation methods were remarkably congruent

• Five BINs occur in both Ecuador and French Guiana: vast distribution ranges

- High temporal turnover:
  - Out of 72 BINs at Nouragues camp (site A) only eight BINs (11.1%) in common between January and September

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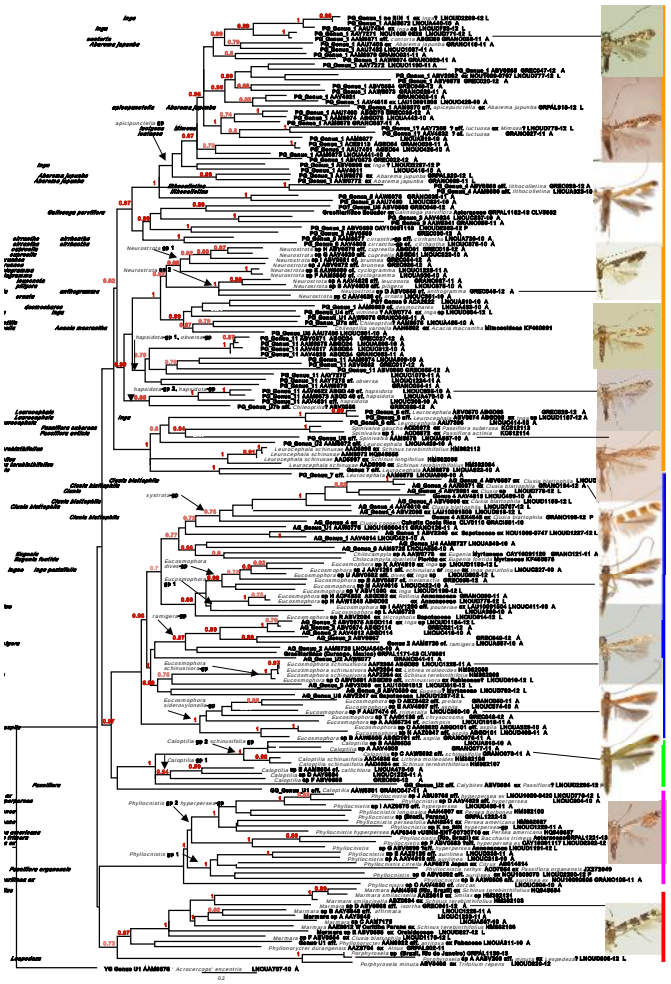

Phylogenetic signal

Parectopa group

Maximum likelihood analysis segregated our specimens among five of the six major lineages of Gracillariidae as defined by Kawahara et al. (2011) based on 21 nuclear protein-coding genes

Acrocercops group

Gracillariinae

Phyllocnistinae

Lithocolletinae + *Marmara*

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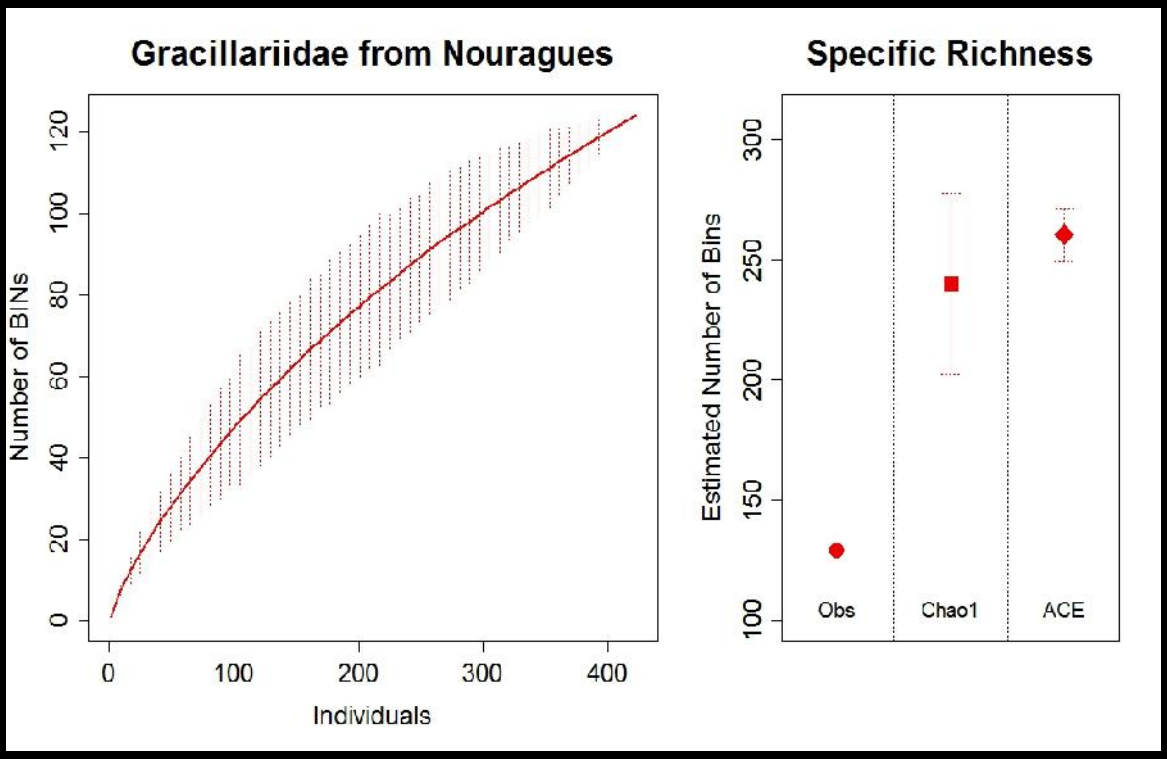
Species richness estimates for Nouragues: 240 species (Chao1) - 260 species (ACE). As many as in the whole of Europe



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Conclusions

- High alpha diversity: 108 species at one site (Nouragues).
- Frequency of singletons (59.3%) at Nouragues twice as high as average for tropics (32%):
  - Very incomplete Inventory: 240–260 species at Nouragues are likely underestimates
- we found as many species as described on the whole continent within the past 150 years.
- DNA barcoding allows researchers to overcome the taxonomic impediment: rapid biodiversity assessments in poorly documented regions
- The reverse LBG found in Gracillariidae is an artefact of insufficient sampling and underdescription

Perspectives

- Formal taxonomic treatment could take years using traditional methods (i.e. genitalia dissections,wing and SEM preparations).
  - Species description pipelines
- Improve sampling effort: Too many singletons
- 16 000 tree species in Amazonia: 16 000 Gracillariid species?

Acknowledgements

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