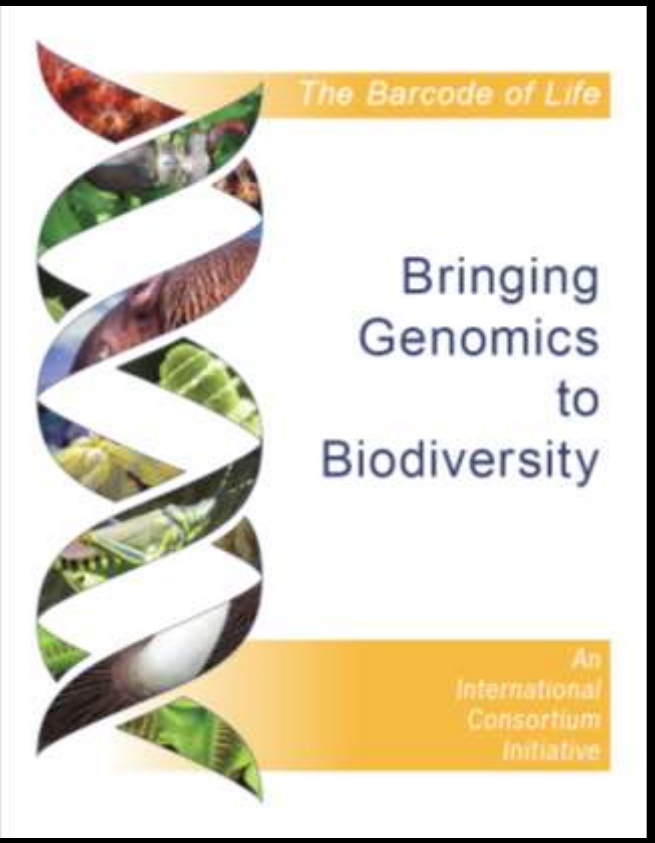
Applications of DNA barcoding to plant protection and biodiversity studies

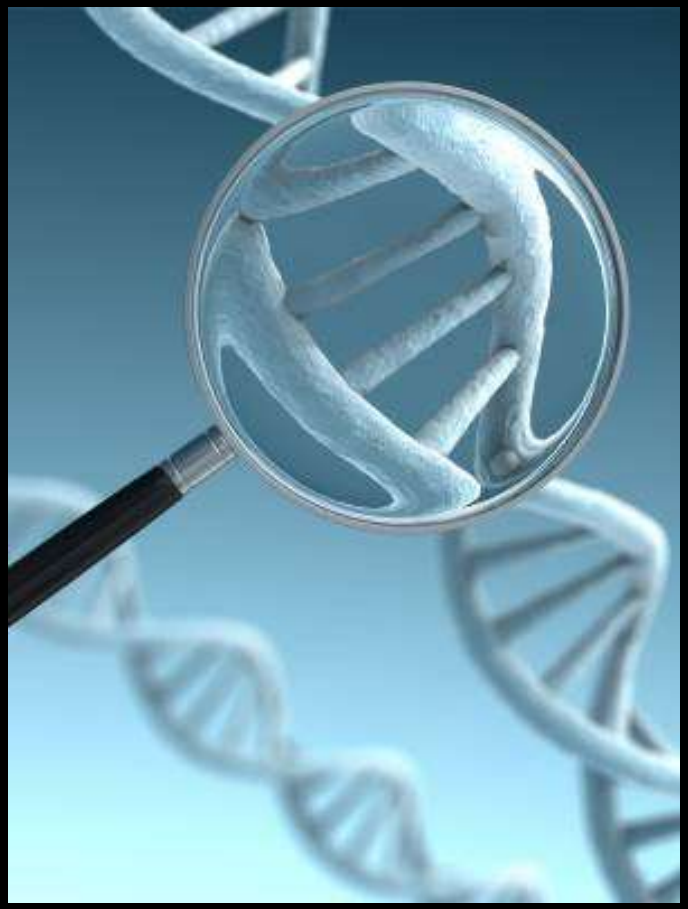


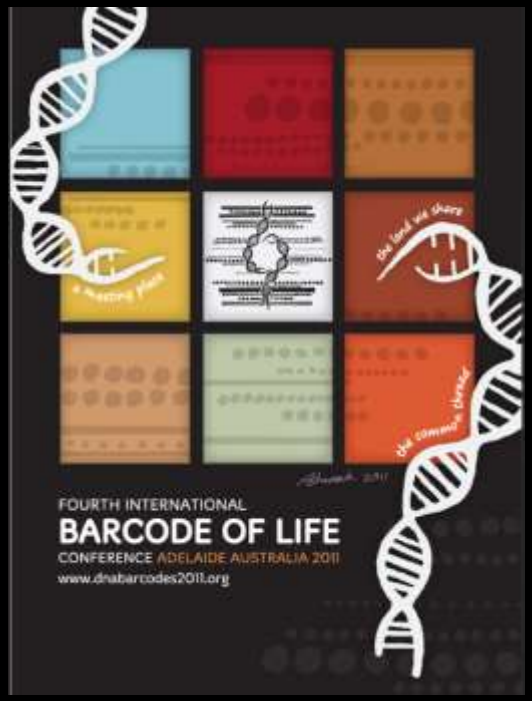
Carlos Lopez-Vaamonde & Rodolphe Rougerie

INRA, Orleans , France

Outline

- What is DNA barcoding?
- Rapid Biodiversity Assessments
- Plant Protection & Invasion biology
- Biocontrol
- Ecological networks and food webs





DNA barcoding

"The use of a short, standardized, DNA fragment for species identification and discovery"

THE ROYAL SOCIETY

Received 29 July 2002
Accepted 30 September 2002
Published online 8 January 2003

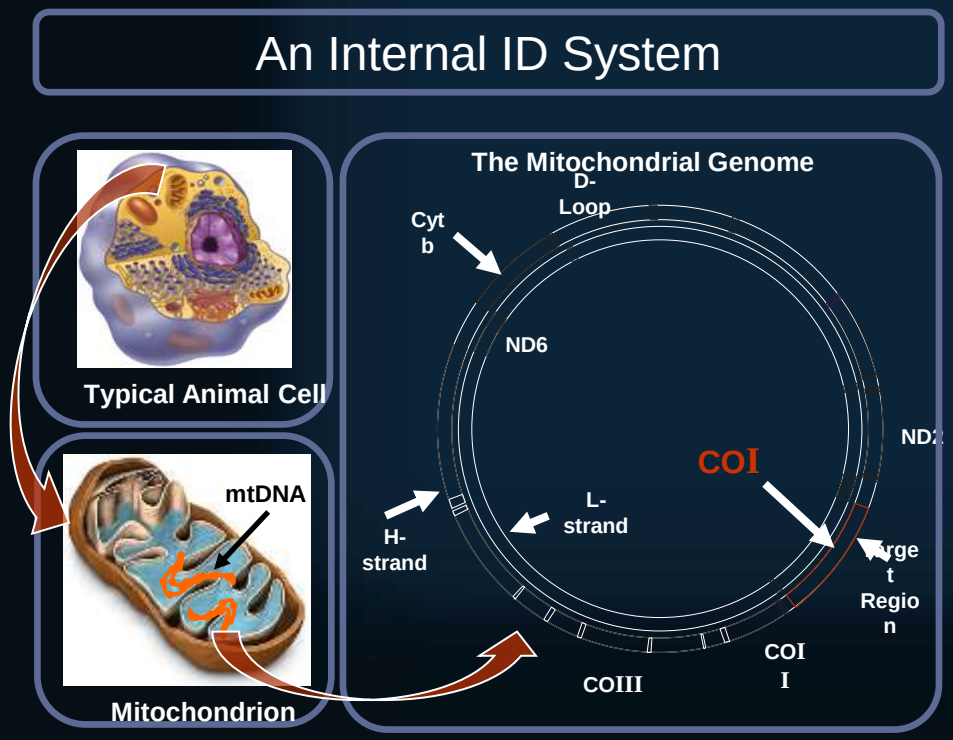
Biological identifications through DNA barcodes

Paul D. N. Hebert*, Alina Cywinska, Shelley L. Ball and Jeremy R. deWaard

Department of Zoology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

Background

DNA barcoding uses a short DNA fragment (the DNA barcode marker) to distinguish species



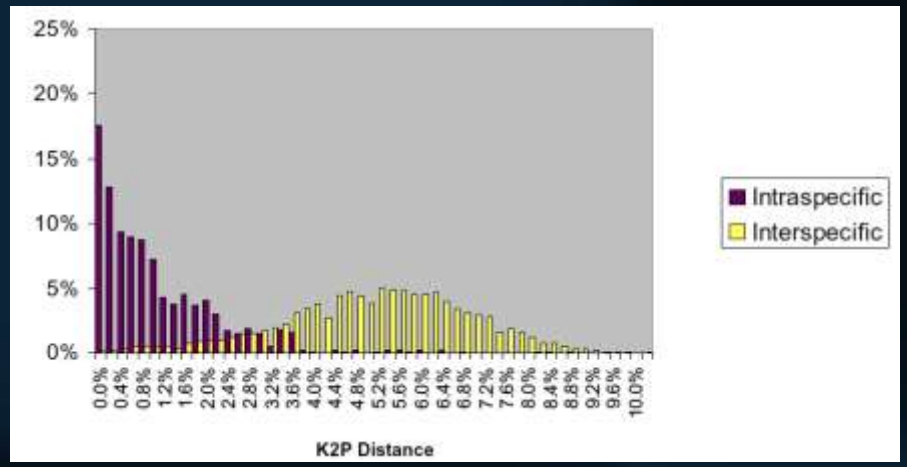
Marker choice:

- Animals: COI (658 bp)
- Prokaryotes: 16S rRNA
- Plants: rbcL and matK
- Fungi: ITS

DNA barcoding: Pros & Cons

- Semi-automated, fast, high-throughput
- Accuracy, high resolution power
- Applicable to any life-stage
- Cost effective
- Can discriminate operational units

- Introgression
- Incomplete lineage sorting
- Pseudogenes
- Heteroplasmy
- Recent radiations




Frontiers in Zoology

BioMed Central

Research

Open Access

Does the DNA barcoding gap exist? – a case study in blue butterflies (Lepidoptera: Lycaenidae)

Martin Wiemers* and Konrad Fiedler

Sampling Protocol

Specimen

Collection Data

Tissue Sample

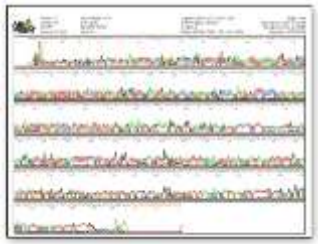
Photograph

DNA extraction

PCR Amplification

Sequencing

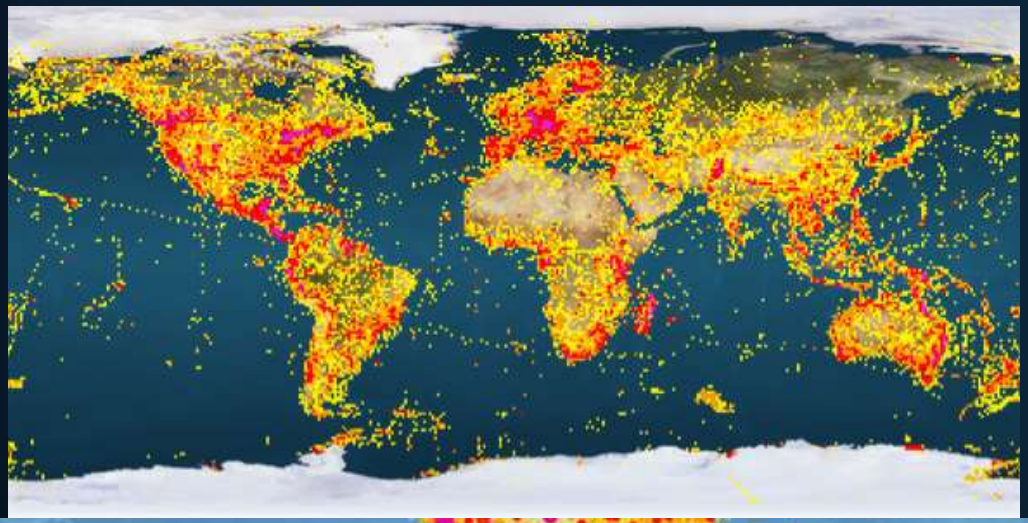
Web-Accessible Data and DNA Barcode



www.boldsystems.org: 2.5 Million barcodes

BOLDSYSTEMS Databases | Taxonomy | Identification | Workbench | Resources

Advancing species identification and discovery by providing an integrated environment for the assembly and application of DNA barcodes.



Barcodes: 2,510,307

Per Site: 1000 100 10 1

Formally described species		Sequence statistics	
Animals	137,972	Barcode clusters for animals	309,687
Plants	50,976	Sequences	2,877,559
Fungi & Other Life	3,402	Barcode Sequences	2,510,307

A New Taxonomy

DNA Barcoding

Traditional taxonomic work

Integrative Reference libraries
(BOLD)

Species IDs

New species

DNA extraction

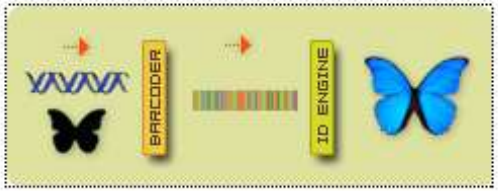
Automated Species Identification

Automated Species Identification

…anywhere, any stages.

IDENTIFICATION ENGINE

BOLD-IDS provides a species identification tool that accepts DNA sequences from the barcode region and returns a taxonomic assignment to the species level when possible.



A New Taxonomy

DNA Barcoding

Traditional taxonomic work

Integrative Reference libraries
(BOLD)

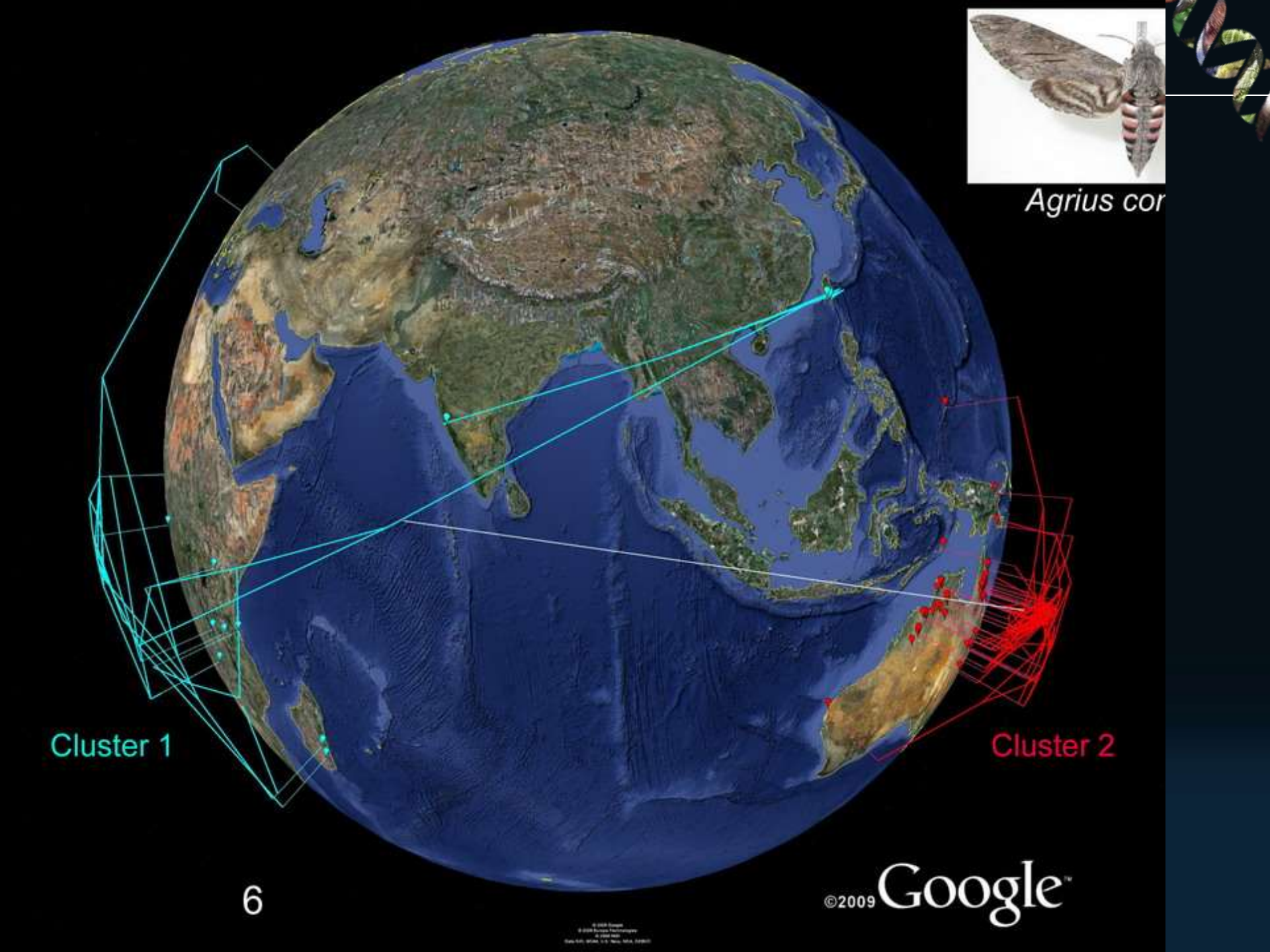
Species IDs

New species

Taxonomy users

Automated Species Identification

Taxonomy Using Barcodes

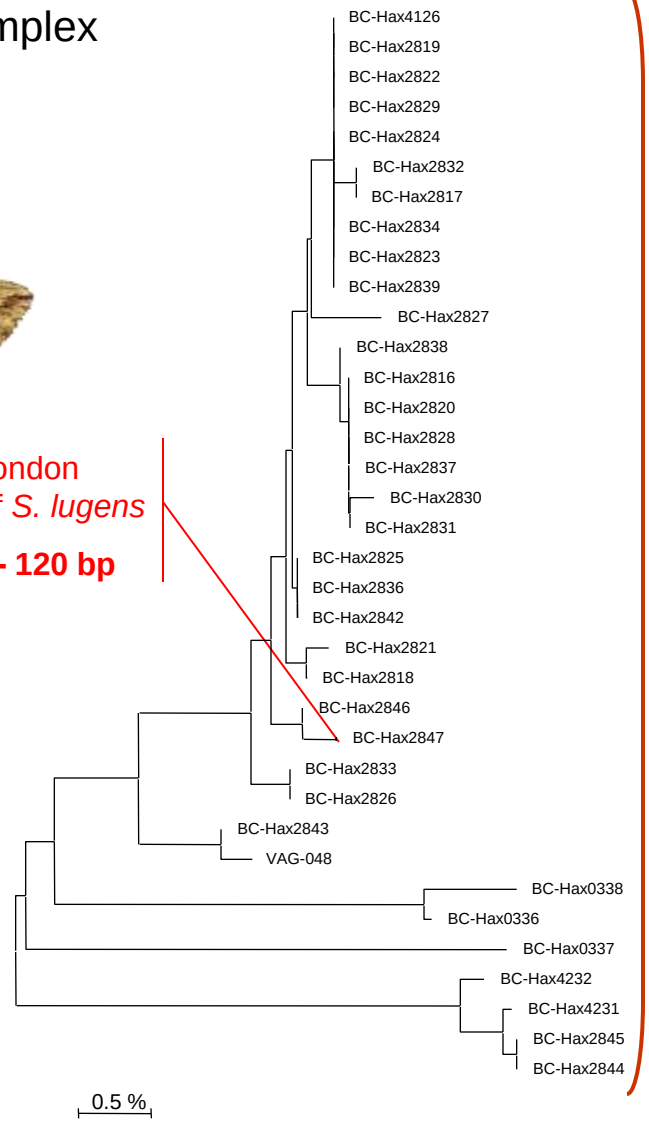

Agrius cor
Cluster 1
Cluster 2
©2009 Google

Taxonomy Using Barcodes

The *Sphinx lugens* complex

NHM, London
Lectotype of *S. lugens*

>150 y.o. – 120 bp



Several available names:

S. biolleyi (Schaus, 1912)

S. chisoya (Schaus, 1932)

S. geminus (R. & J., 1903)

S. lugens Walker, 1856

S. pitzahuac Mooser, 1948

S. smithi Cadiou, 1998

S. lugens

37 seq.

Mean dist. = 4.5%

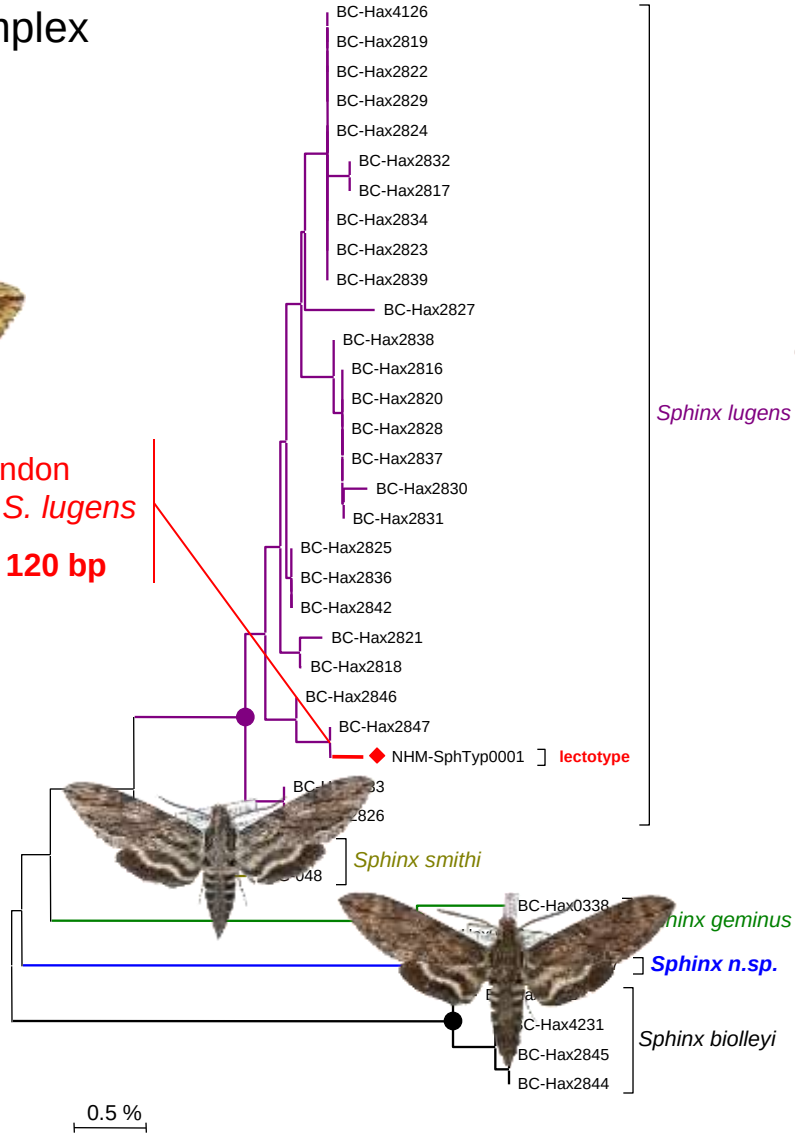
Max dist. = 6.7%

Taxonomy Using Barcodes

The *Sphinx lugens* complex

NHM, London
Lectotype of *S. lugens*

>150 y.o. – 120 bp



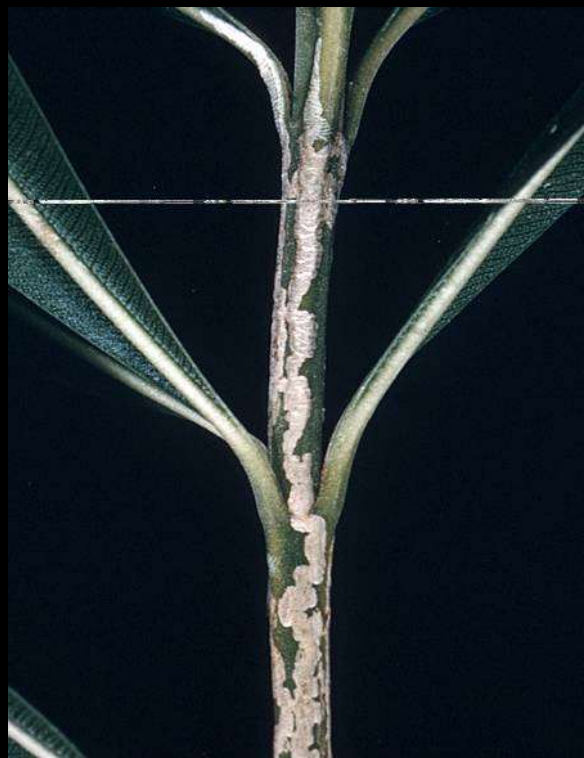
Incertae sedis:

S. chisoya (Schaus, 1932)

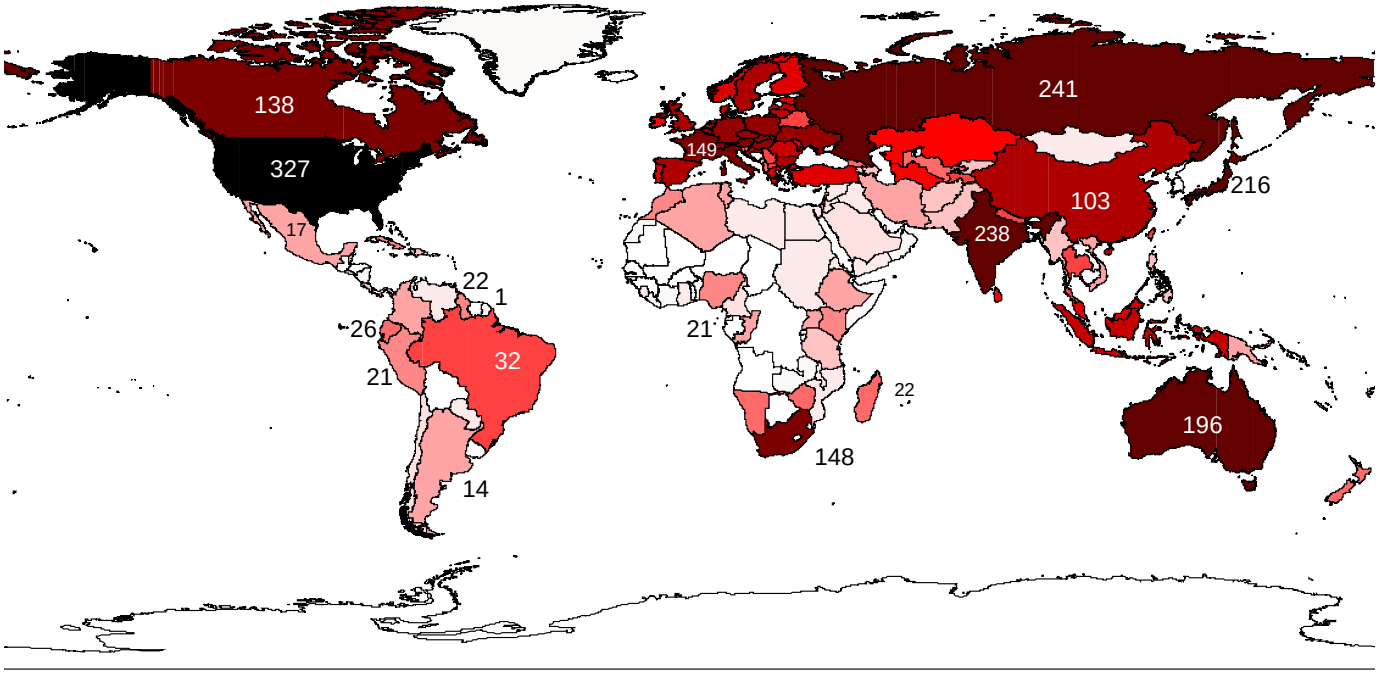
S. pitzahuac Mooser, 1948

Gracillariidae

- 99 genera, 1931 species described worldwide
- Agricultural pests and invasive species
- Leaf-miners, but also fruit mining, stem mining, leaf rolling, and galling



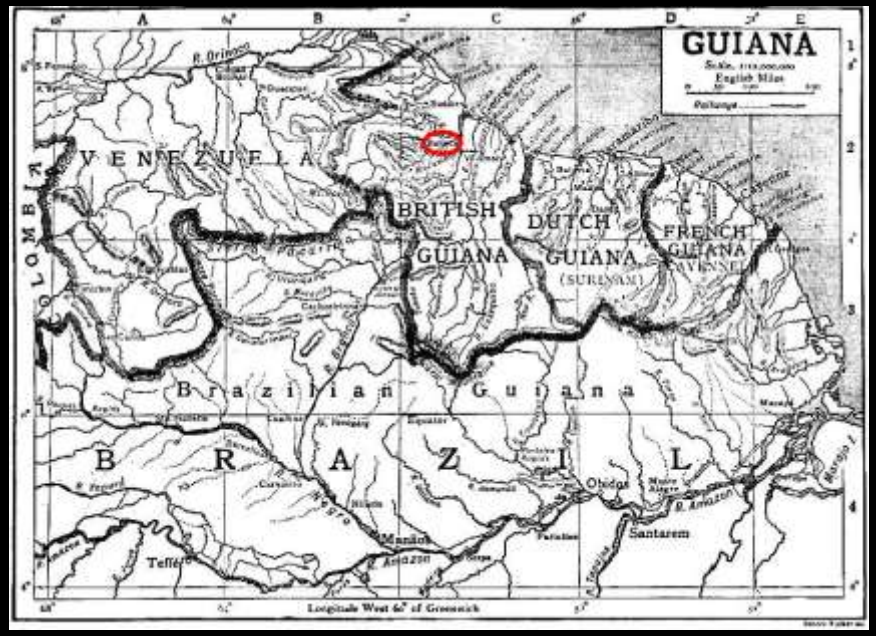
- Relatively low tropical species diversity
- is it genuine or caused by insufficient sampling?



Neotropical Diversity of Gracillariidae

- 183 gracillariid species recorded in the Neotropics
- 64% of species described by Meyrick before 1930's
- Only one species recorded from French Guiana

The late Edward Meyrick.



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

[Read May 5th, 1915.]

- 105 000 hectares
- Declared reserve 1986
- Two camps: 8 kms

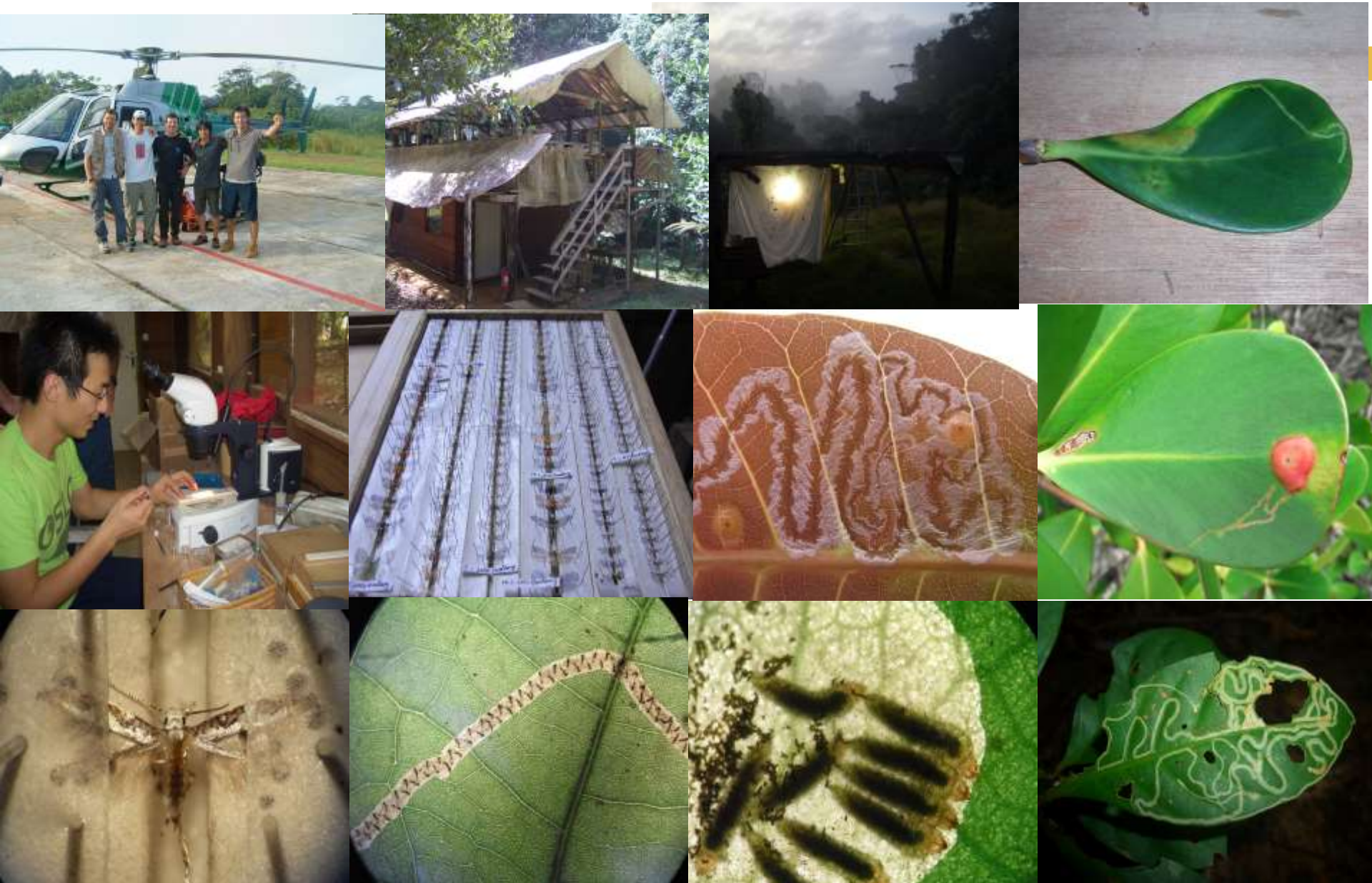
Molecular Ecology Resources (2013) doi: 10.1111/1755-0998.12178

DNA barcoding reveals a largely unknown fauna of Gracillariidae leaf-mining moths in the Neotropics

D. C. LEES,*[1] A. Y. KAWAHARA,†[1] R. ROUGERIE,‡ I. OHSHIMA,§ A. KAWAKITA,¶ O. BOUTELEUX,‡ J. DE PRINS** and C. LOPEZ-VAAMONDE‡

How to rapidly assess a local fauna using DNA barcoding when it is largely unknown?

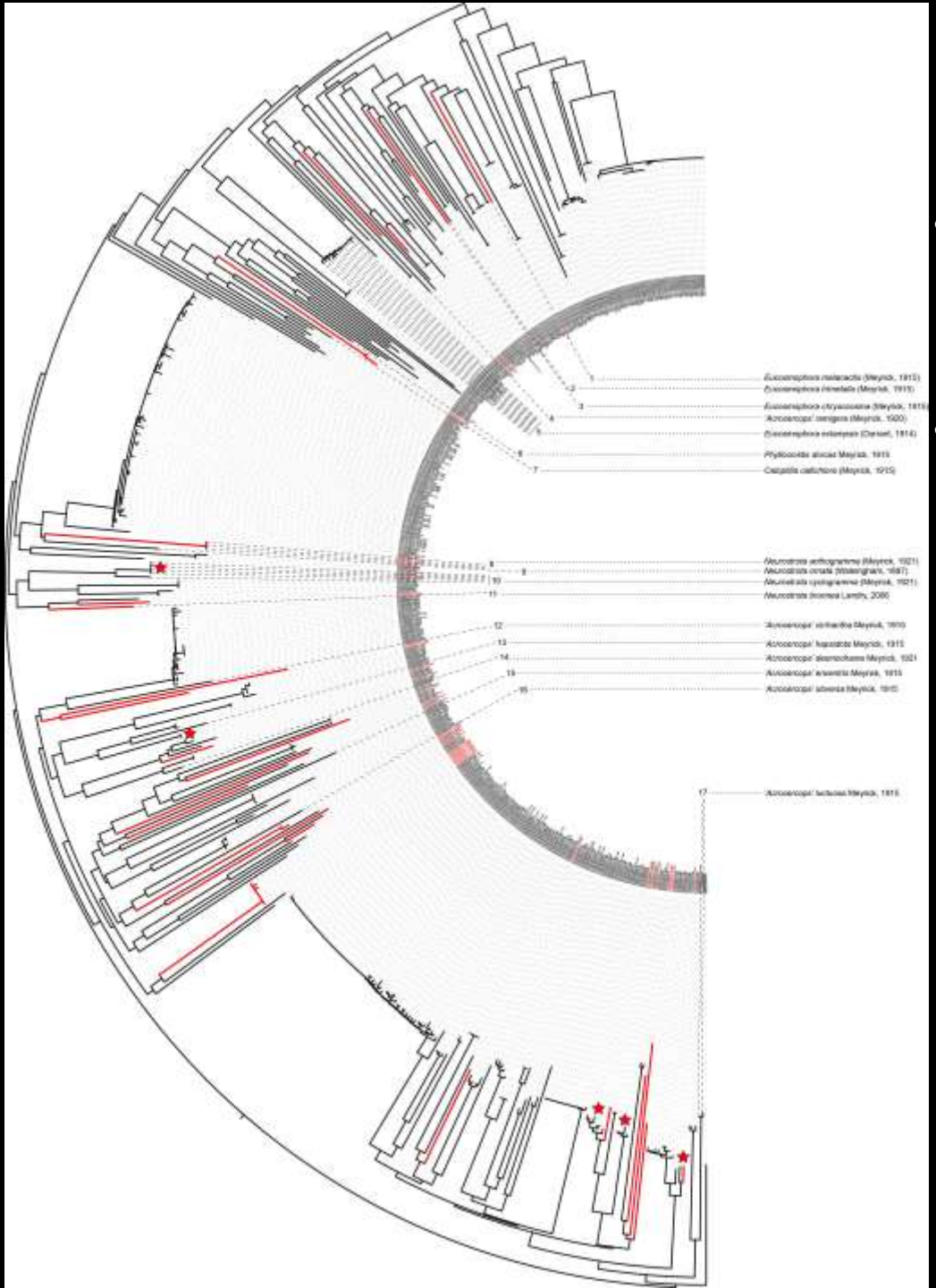
French Guiana: Two visits, January and September 2010
Sampling – 2 weeks, 30-50 person-nights per visit



- 485 barcodes:
  - 445 French Guiana
  - 40 Ecuador

- Species Delimitation:
  - Automatic Barcode Gap Discovery (ABGD)
  - Refined Single Linkage (RESL): Barcode Index Number (BIN) system

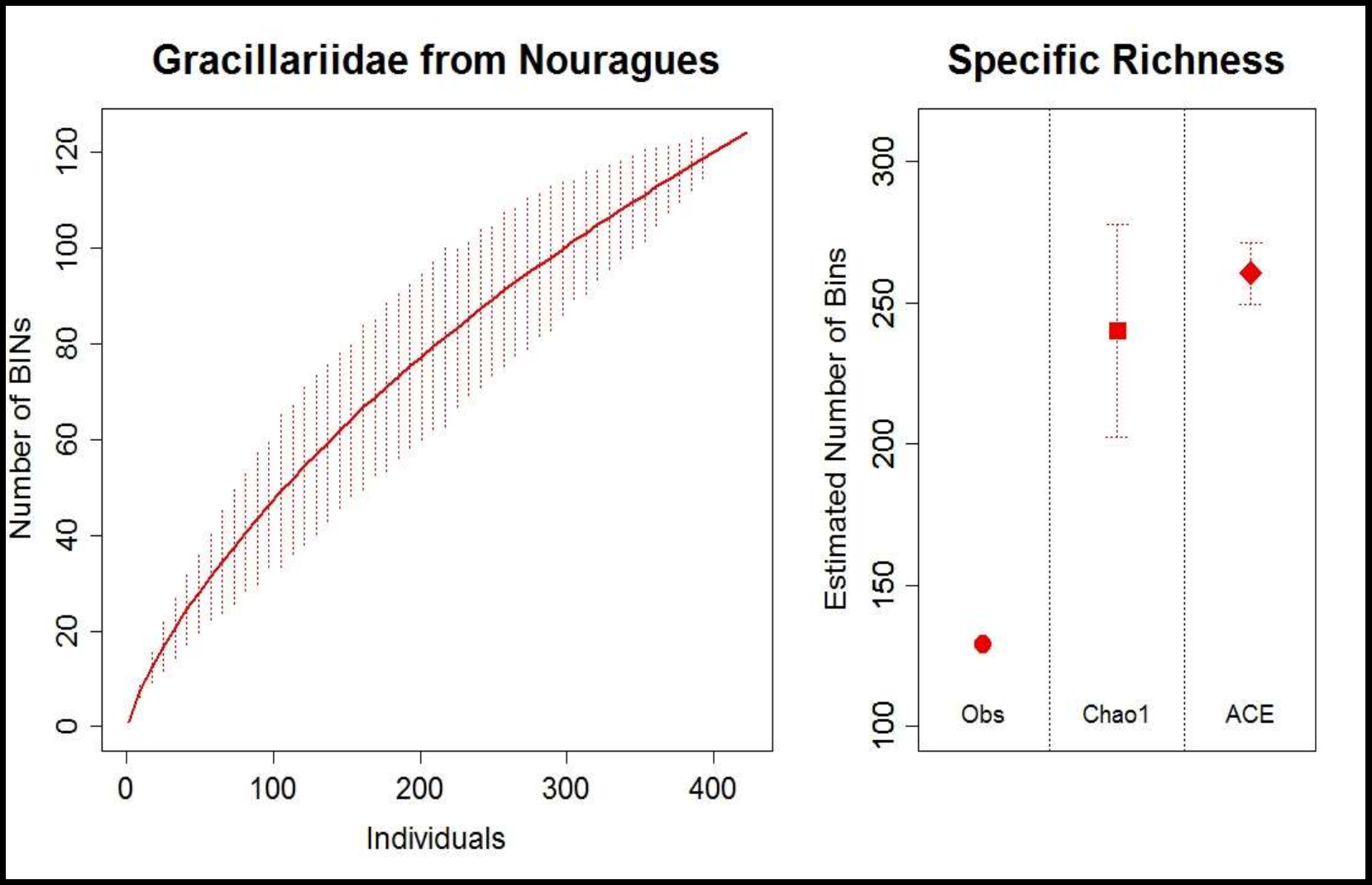
- Estimation of undescribed species: Meyrick's Types at BMNH London



- Species delimitation:
  - ABGD: 136 species
  - RESL: 151 species
- Identification using Types:
  - 17 tentative species names
  - 85% species undescribed
  - 5 species occur in both Ecuador and French Guiana

Lees et al 2013 Molecular Ecology Resources

Species richness estimates for Nouragues ranged from 240 species (Chao1) to 260 species (ACE)



Lees et al 2013 Molecular Ecology Resources

Rapid Biodiversity Assessment

- Traditional Sanger-sequencing can only sequence specimens individually.
- Inadequate for processing complex environmental samples: Thousands of specimens

Biomonitoring made easier: Metabarcoding

- Massively Parallel Pyrosequencing:
- Species identifications from ‘slurries’

Doug Yu

ECOLOGY LETTERS

Ecology Letters, (2013) 16: 1245–1257 doi: 10.1111/ele.12162

Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding

Methods in Ecology and Evolution

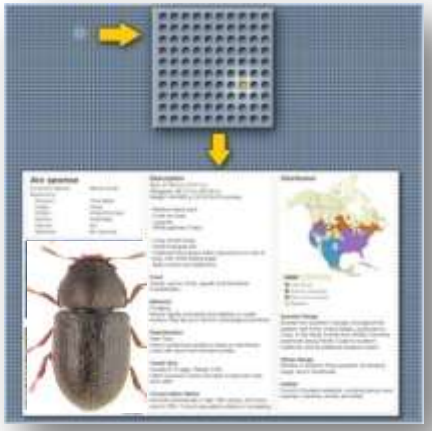
Methods in Ecology and Evolution doi: 10.1111/j.2041-210X.2012.00198.x

Biodiversity soup: metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring

Douglas W. Yu[1,2*†], Yinqiu Ji[1†], Brent C. Emerson[2‡], Xiaoyang Wang[1], Chengxi Ye[1], Chunyan Yang[1] and Zhaoli Ding[3]

Le projet PASSIFOR

Propositions d'Amélioration du Système de Suivi de la blodiversité FORestière (PASSIFOR) Coord. Guy Landmann, GIP ECOFOR



Librairie de référence

Séquençage environnemental
Suivis de biodiversité à large échelle

ETUDE DE FAISABILITÉ D'UN SUIVI QUANTITATIF SIMPLIFIÉ DIRECT DES **COLÉOPTÈRES SAPROXYLIQUES** SUR UN RÉSEAU NATIONAL DE PLACETTES FORESTIÈRES

Identifying areas of origin

Out of 1514 exotic invertebrate species established in Europe , 221 (14.6%) have an **unknown origin** (Roques et al 2010)

Determining the area of origin, key step of biocontrol

Cameraria ohridella: Alien or native ?

- New species first described from Macedonia in 1984.
- Invaded Europe in 20 years
- Larvae attack Horse-chestnut, ornamental tree

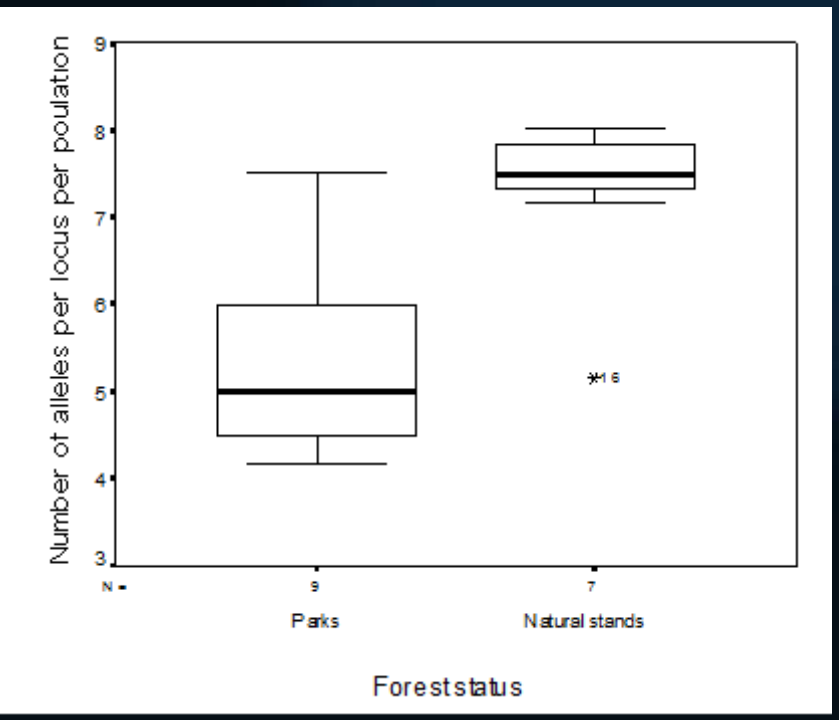
- **Balkan Origin?**
  - Origin of the host tree
  - First found in this region

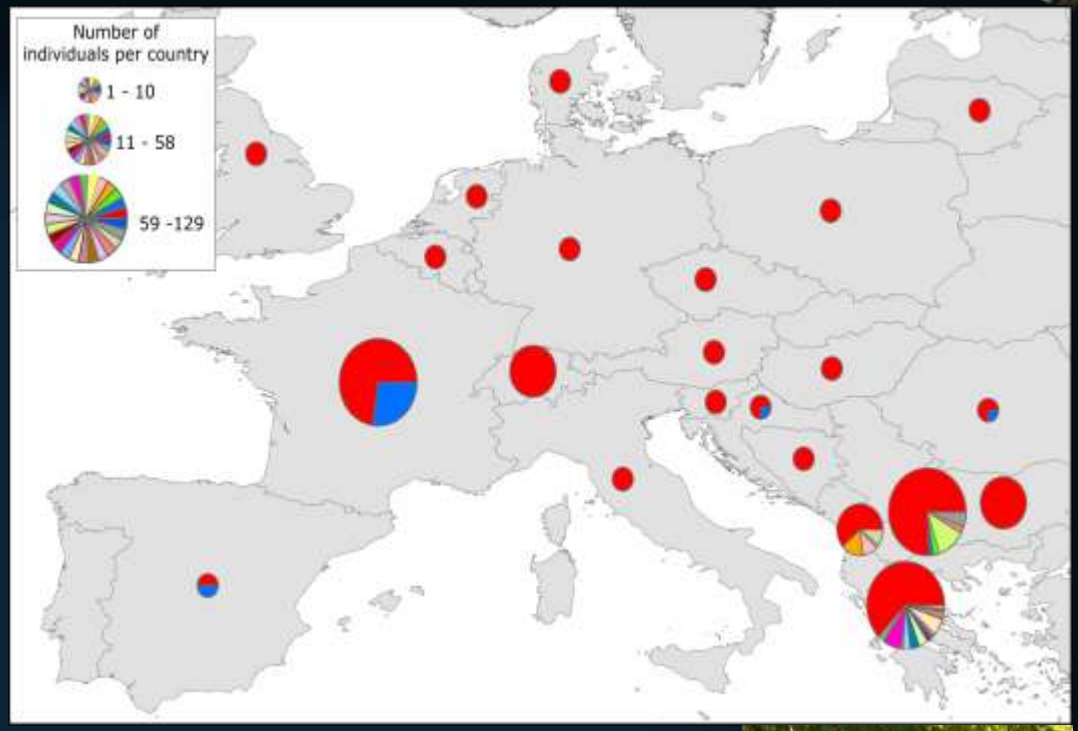
BUT
- Why spreading only in the 80s?
- No other *Cameraria* in Europe
- Only polyphagous parasitoids reared
- Still at outbreak density after 20 years

Higher Genetic diversity in Balkans

Neus Mari MSc

Microsatellites





Mitochondrial DNA (COI)

Romain Valade MSc

Valade et al (2009) Molecular Ecology

Mari Mena et al., 2008 **European Journal of Entomology**

Historical Data: Herbaria

Looking for mines of *C ohridella* in herbarium collections of *Aesculus hippocastanum*.

David Lees (Studium Fellow, Natural History Museum London)

Walter Lack (Botanical Museum, Freie Universität Berlin)

Historic herbaria with mines

- 32 sheets had leaf mines with larvae/pupae inside date back to **1879**
- COI minibarcodes confirm ID

Mine densities similar to late-summer damage in 21st century European parks natural phenomenon?

Major oversight of herbaria by entomologists

Lees et al (2011) **Frontiers in Ecology and Evolu**

Looking for natural enemies in the Balkans: *Pediobius saulius* as potential biocontrol agent?

Antonio Hernandez Lopez (Postdoc EFPA INRA)

Pediobius saulius (Walker, 1839)

- Pupal generalist parasitoid:

76 espèces Lepidoptera: Gracillariidae (59 espèces)
20 espèces de Hymenoptera et 11 Coleoptera

Principal parasitoid of *C. ohridella* in the Balkans

Host Race specific to *C. ohridella* in the Balkans?

Phyllonorycter spp.

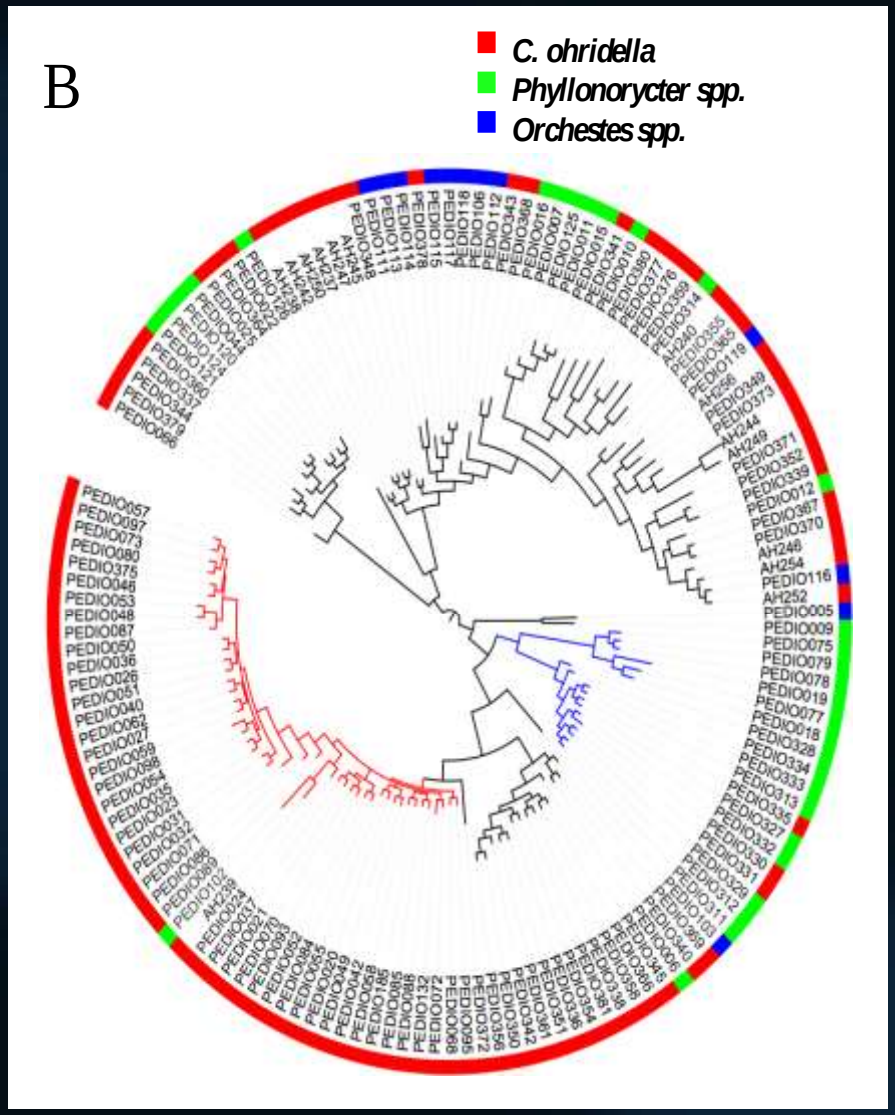
Orchestes spp.

C. ohridella

Potential biocontrol agent?

→ One Haplogroup associated mainly to *C. ohridella* in the Balkans

→ **potential biocontrol candidate**?
  → Testing host specificity
  → Documenting **host range** of *P. saulius* in the Balkans



Hernandez et al (2011) **Evolutionary Applications**

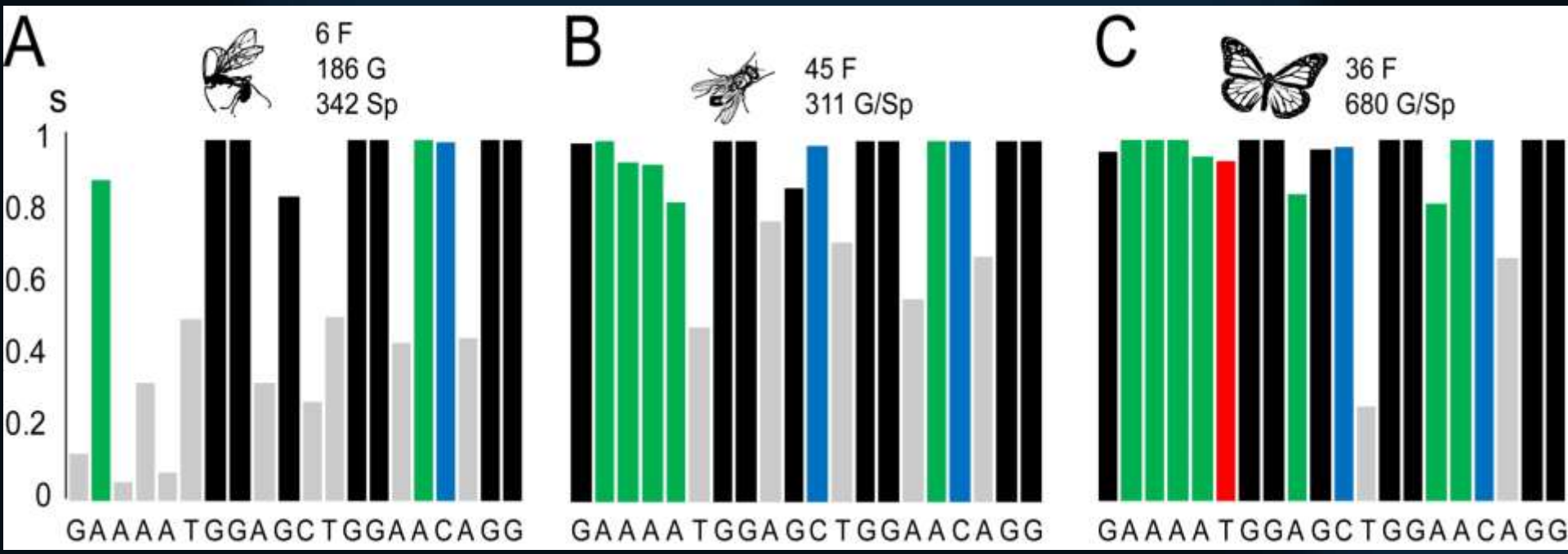
Host-parasitoid relationships through DNA barcoding

- Documenting host range is time-consuming: rearing experiments or repeated field observations
- Reverse primer MLepR1 binds 307 bp inside the standard barcode region

Rougerie et al (2010) Molecular Ecology

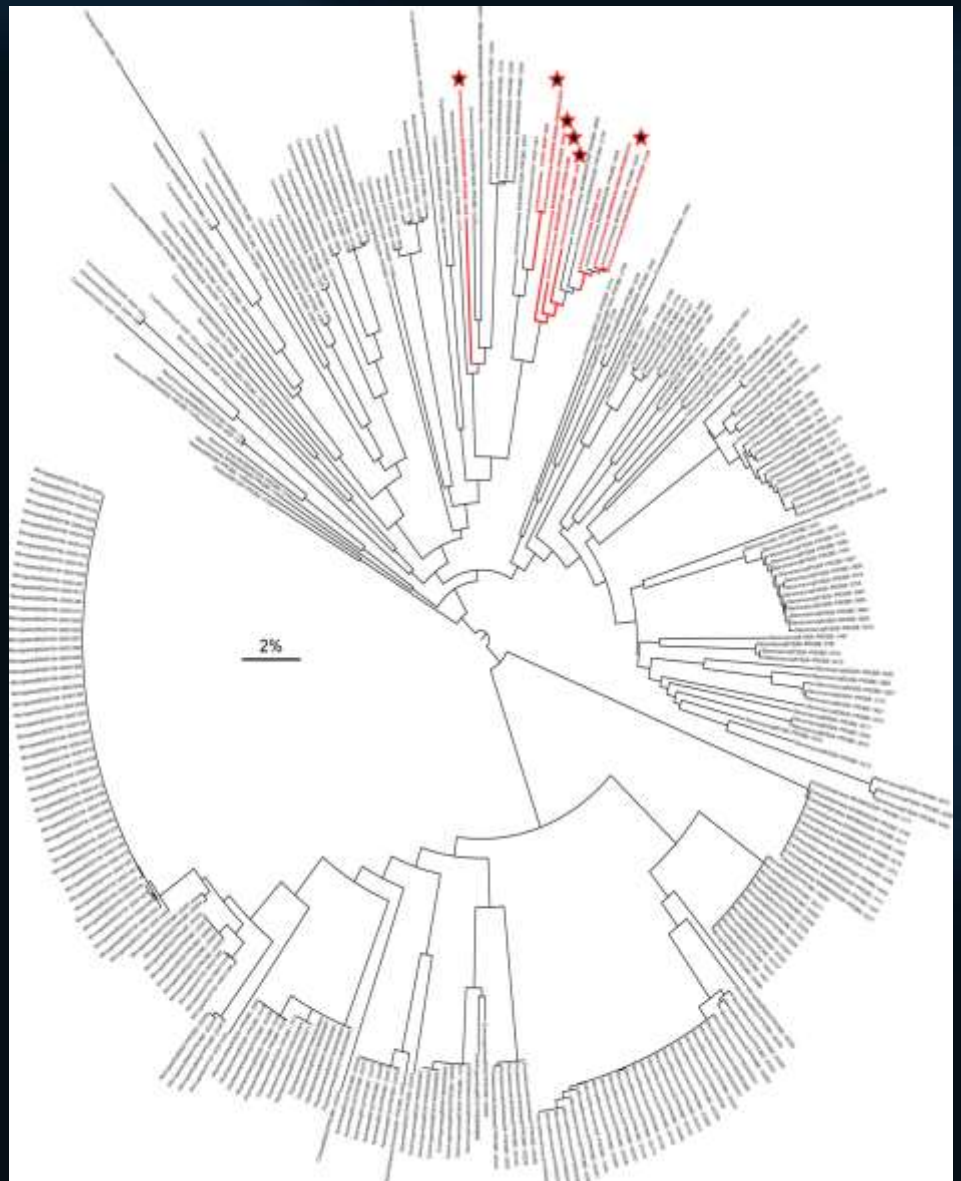
In Silico analysis of Primer match

- Close match and high conservation of the binding site of MLepR1 in **Diptera and Lepidoptera**
- Poor binding affinities of MLepR1 in Hymenoptera



Rougerie et al (2010) Molecular Ecology

Amplification success of MLepR1 in parasitoids



Only 9 (highlighted terminals) out of 297 samples of parasitoid wasps were successfully amplified and sequenced using primer MLepR1 associated to a universal forward primer

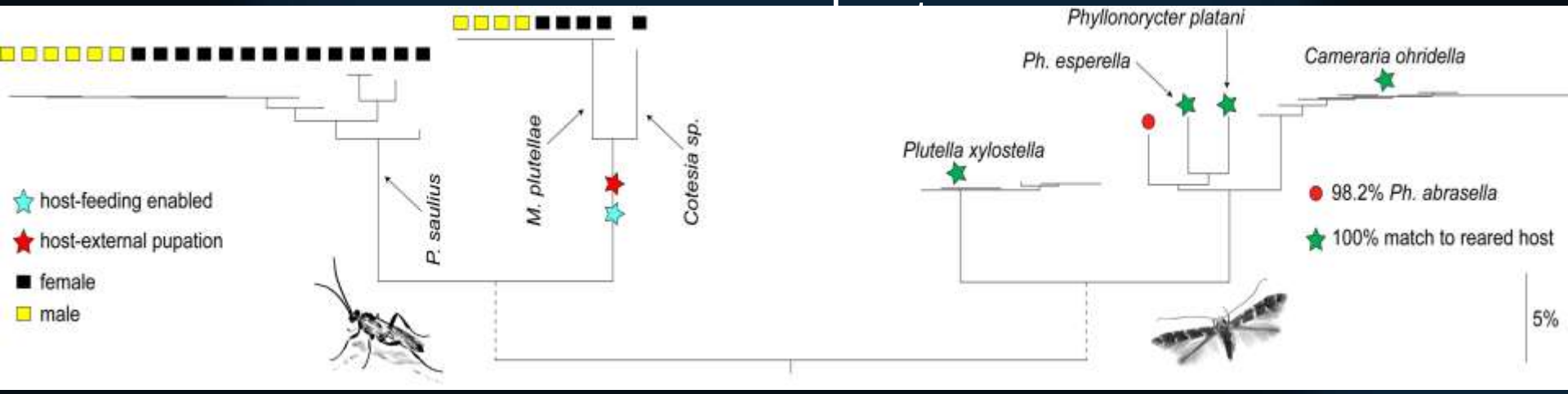
Rougerie et al (2010) Molecular Ecology

Barcoding of gut contents of adult parasitoids reveal larval ho

29 Parasitoid wasps were sequenced

Wasp DNA: primer pairs LepF1/LepR1, or LepF1/C_ANTMR1D

Same DNA extractions primer pair LepF1/MLepR1 : all match known microlep

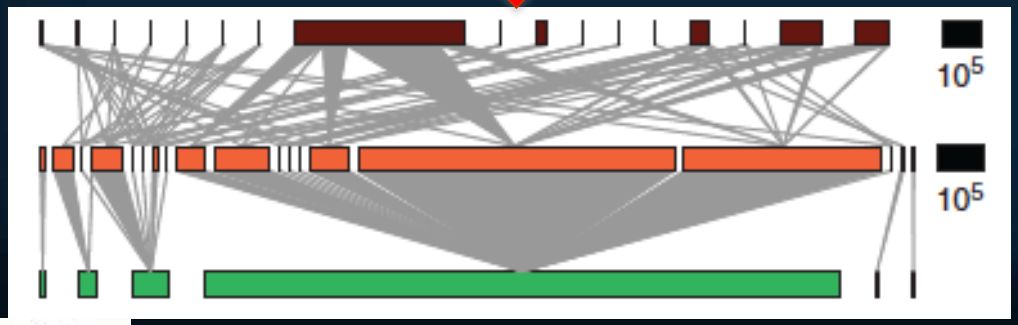


Rougerie et al (2010) Molecular Ecology

Ecological networks, Food webs

- Sequencing from the abdomen of the adult wasp a 307 bp fragment of the DNA barcode allowing unambiguous identification of the host

- Ingested host DNA does persist through metamorphosis in the gut-contents of the adult wasp.



Mutualistic Pollinators & Seed Dispersers

Herbivores-Parasitoid-Pathogens

Acknowledgements





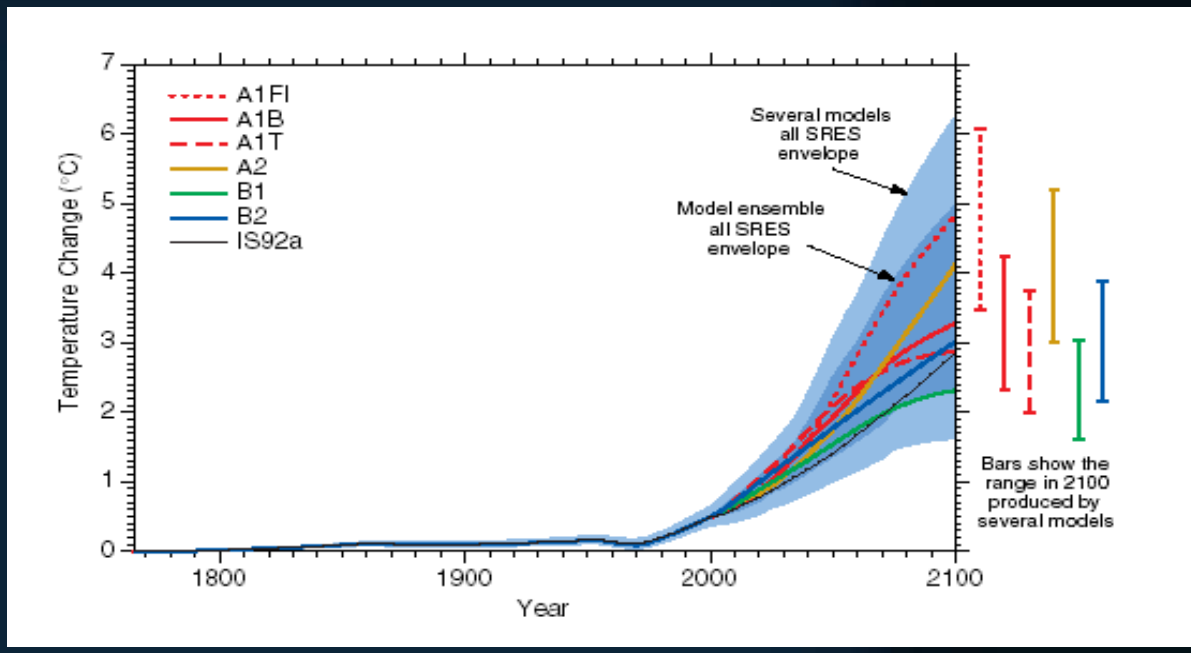




Funding:
Studium fellowship to David Lees & Natalia Kirichenko
Region centre. Project ENDOFEED
EFPA INRA
SEE-ERA.NET
EU Projet Alarm

Biodiversity Crisis

Unprecedented transformation of Landscapes: Rapid land-use and climate changes



- To manage and conserve biodiversity, one must know what is being lost, where, and why
- Urgent Need for Rapid Biodiversity Assessments