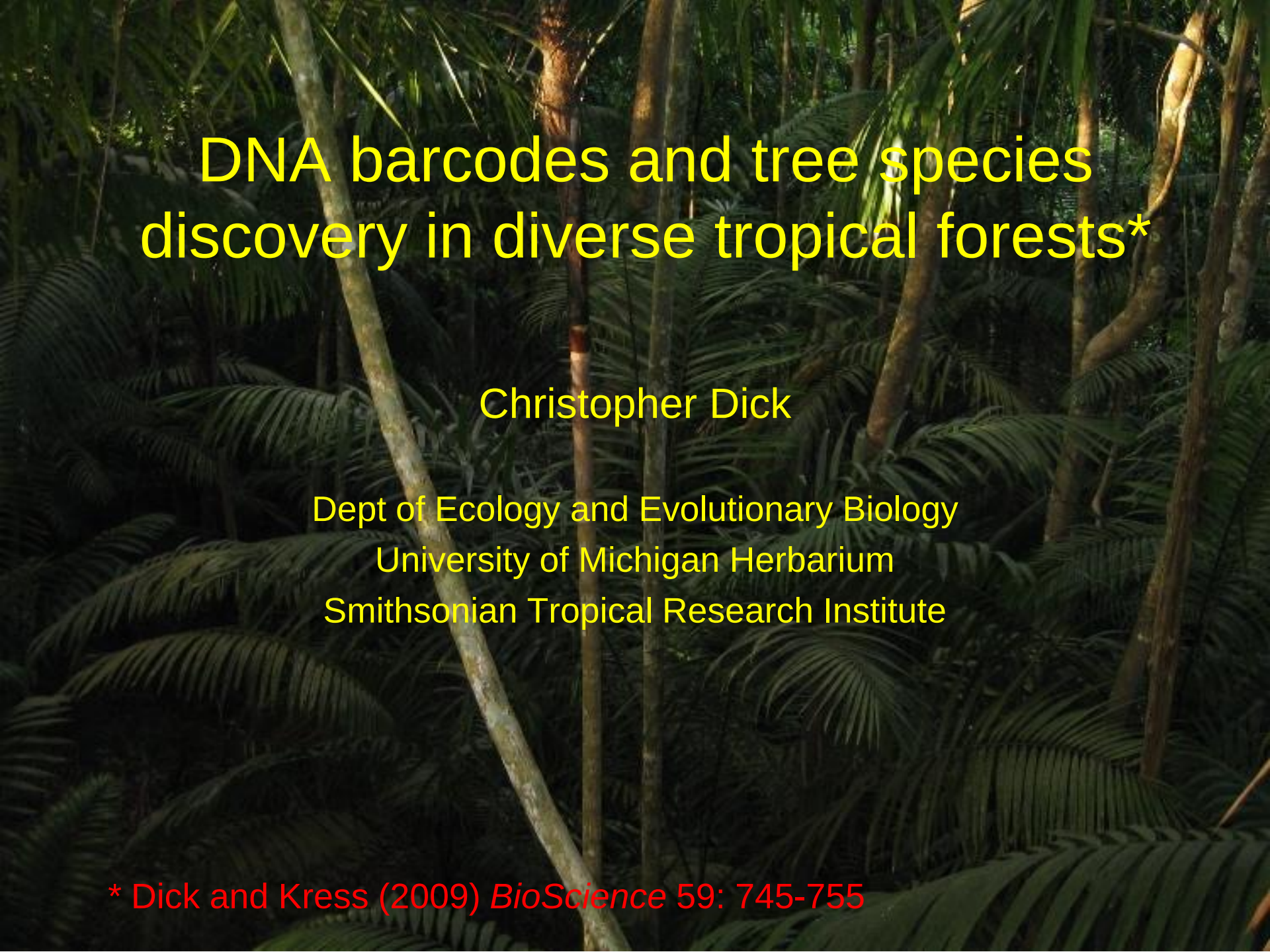
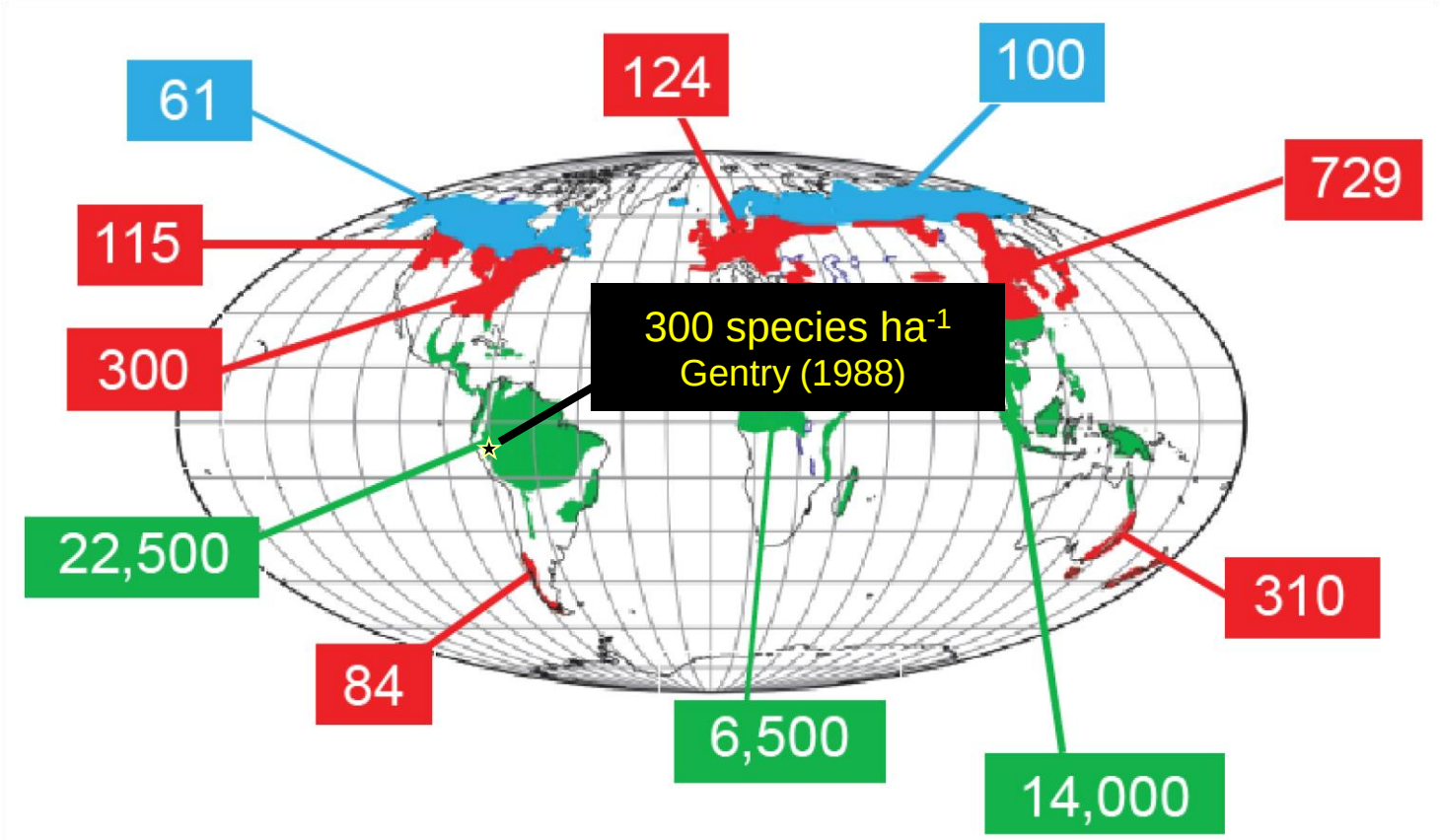


DNA barcodes and tree species discovery in diverse tropical forests*

Christopher Dick

Dept of Ecology and Evolutionary Biology
University of Michigan Herbarium
Smithsonian Tropical Research Institute

* Dick and Kress (2009) *BioScience* 59: 745-755

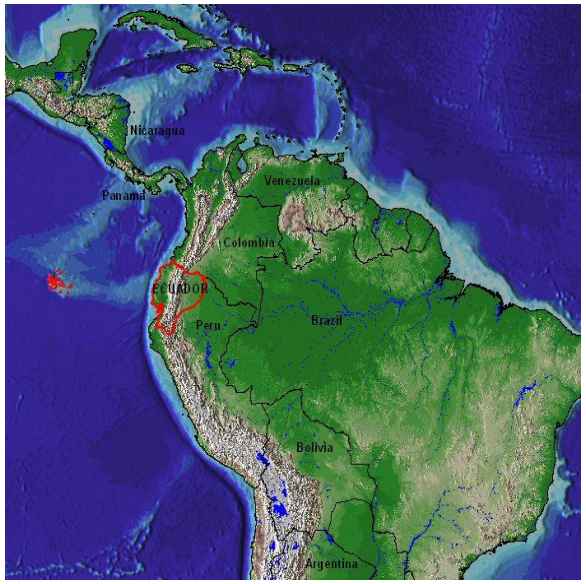


<u>Tree species per biome</u> (Fine & Ree 2006)

Boreal forest	**161**
Temperate broadleaf	**1,662**
Lowland tropical:	**43,000**

Underexplored diversity

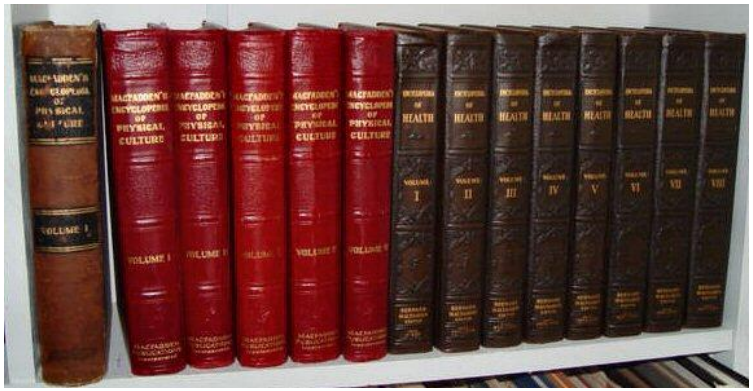
- 4,000 plant species are reported for Amazonian Ecuador (Jørgensen et al. 1999)
- An intensively surveyed 1 ha plot in Amazonian Ecuador yielded 900 vascular plant species (Balslev et al. 1998)



Does 1 ha contain ¼ of the species found in the surrounding 7 million ha? (Ruokolainen et al. 2005) **Probably not.**

Ecologist's dilemma

- Few field guides – none that are "handy"
- Diagnostic material (fruit, flowers) out of reach or unavailable for lianas, juvenile trees, tall trees, rare trees, infrequent flowering
- Ecologists don't have resources to invest in taxonomic inventory



It’s constraining research …

About 20% of tree species (≤ 10 cm dbh) in Amazon forest plots are never identified (Ruokolainen et al. 2005) limiting our description of beta and gamma diversity

Lianas, understory trees, juveniles, epiphytes too difficult and diverse to include in forest inventory

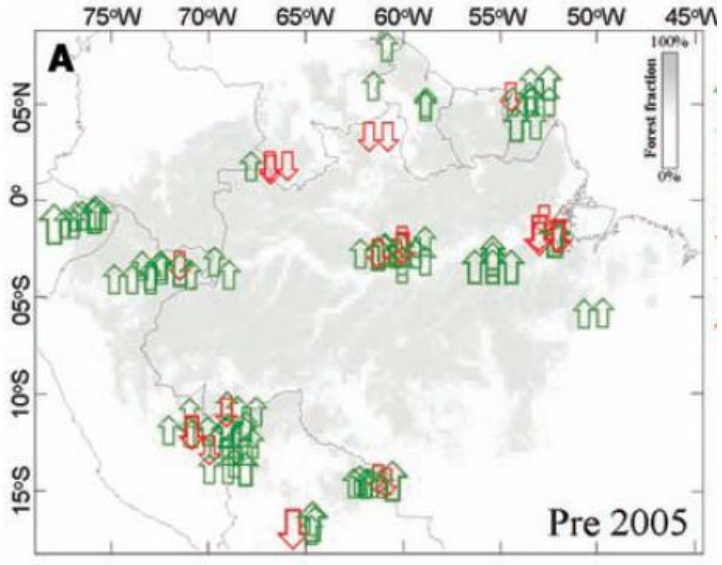
Plot networks used to evaluate global change impacts rely on genus level identifications (e.g. RAINFOR)

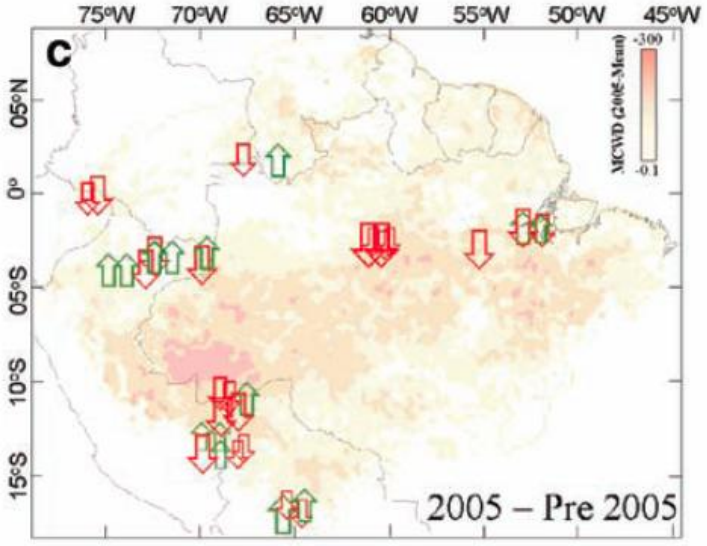
http://www.geog.leeds.ac.uk/projects/rainfor/

Drought Sensitivity of the Amazon Rainforest

SCIENCE VOL 323 6 MARCH 2009

Oliver L. Phillips,[1]* Luiz E. O. C. Aragão,[2] Simon L. Lewis,[1] Josh





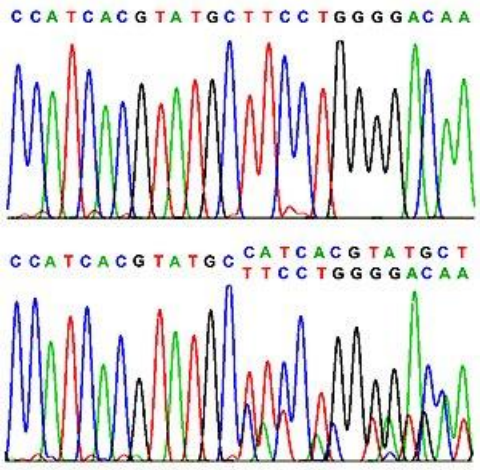
"Amazon drought kills selectively and therefore may also alter species composition, pointing to potential consequences of future drought events on the biodiversity in the Amazon region."

We know Amazon forests are changing, but not at the species level…

Innovative tools for species ID

Can advance botanical exploration by distinguishing described and undescribed or poorly collected species

- Digital image recognition technologies
- Web based interactive identification keys
- **DNA “barcodes”**



DNA barcodes

Short gene sequences (<700 bp) used to identify species through reference to DNA libraries or database

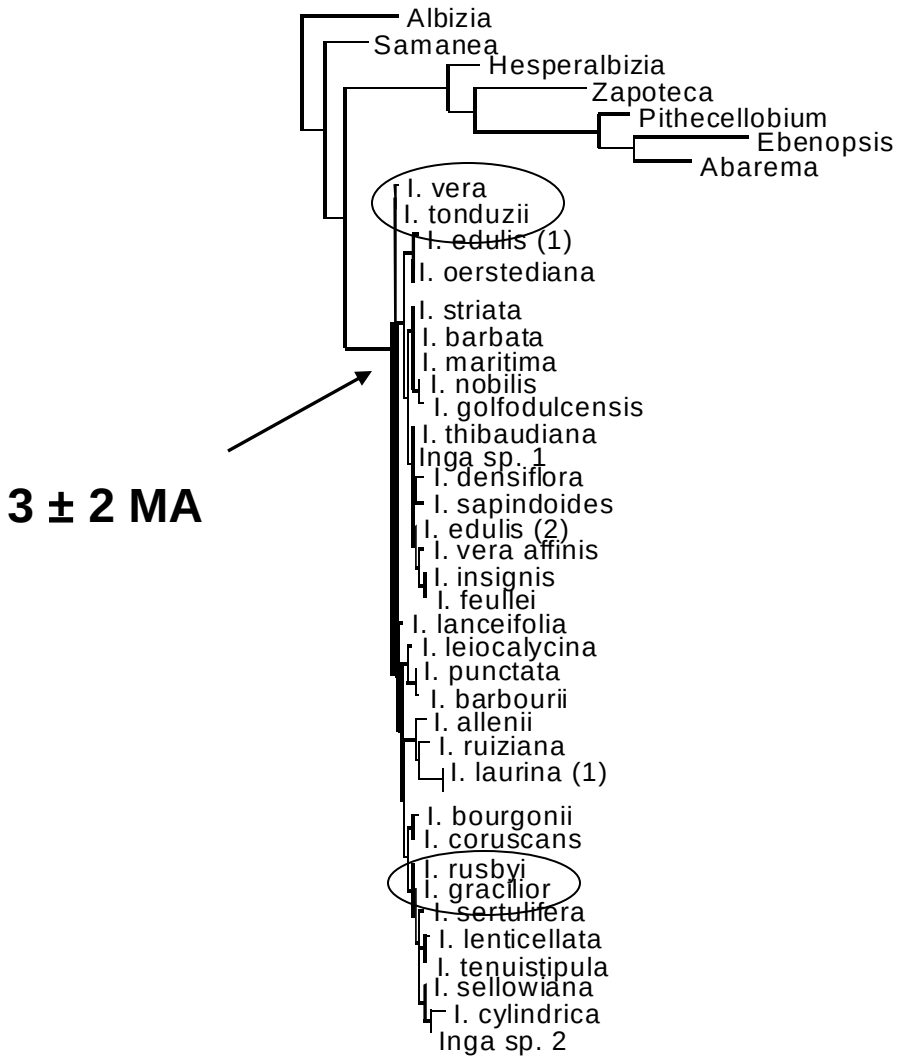
Limitations of DNA barcodes

DNA barcodes have approximately 30% failure to accurately identify plant species…

Fazekas et al. (2008) PlosOne

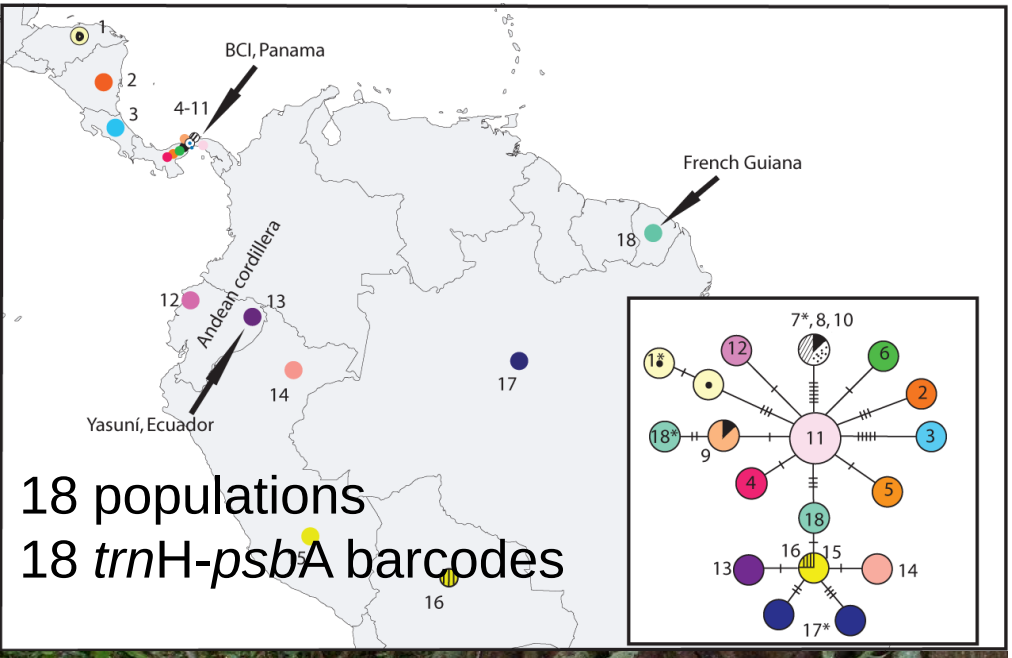
Low variation in young genera

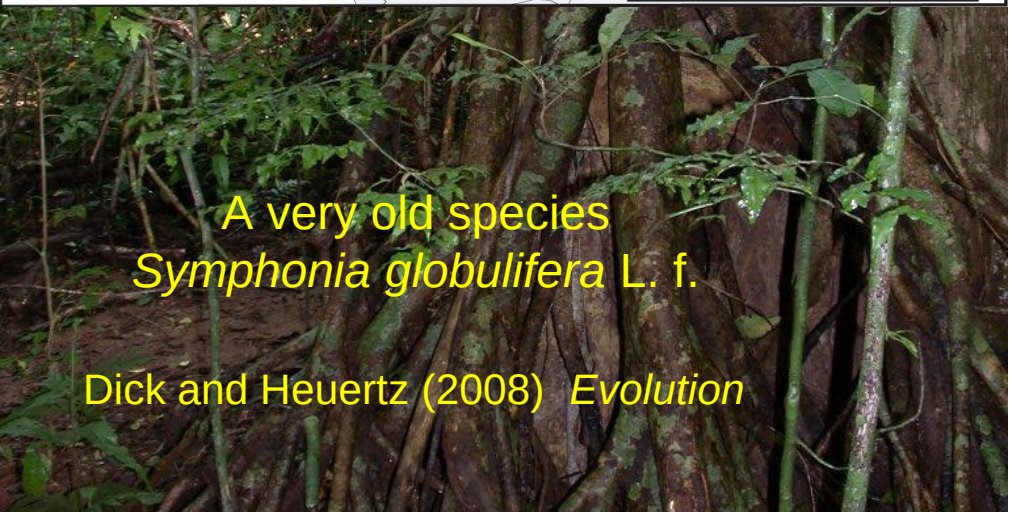
Inga (~300 young species)



Richardson et al. (2001) ***Science***

Genetic variation within species





More than one DNA barcode per species if:

(1) Species retain shared ancestral DNA

(2) Monophyletic species has accumulated variation over time

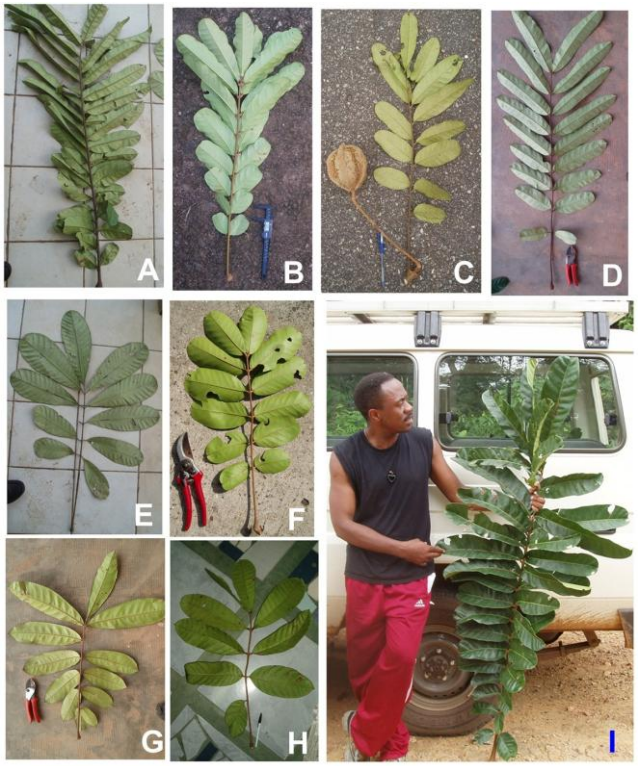
Variation in old species can be used to barcode populations

Introgression

cpDNA introgression is frequent in major temperate zone tree genera: *Quercus*, *Salix*, *Populus*, *Betula*, *Acer* …

The extent of hybridization and introgression in tropical trees is poorly characterized

Taxonomic uncertainty



Carapa – 3 or 27 species?

Research of D.K. Kenfack

DNA barcodes can only identify species that have been named…

Many species are undescribed

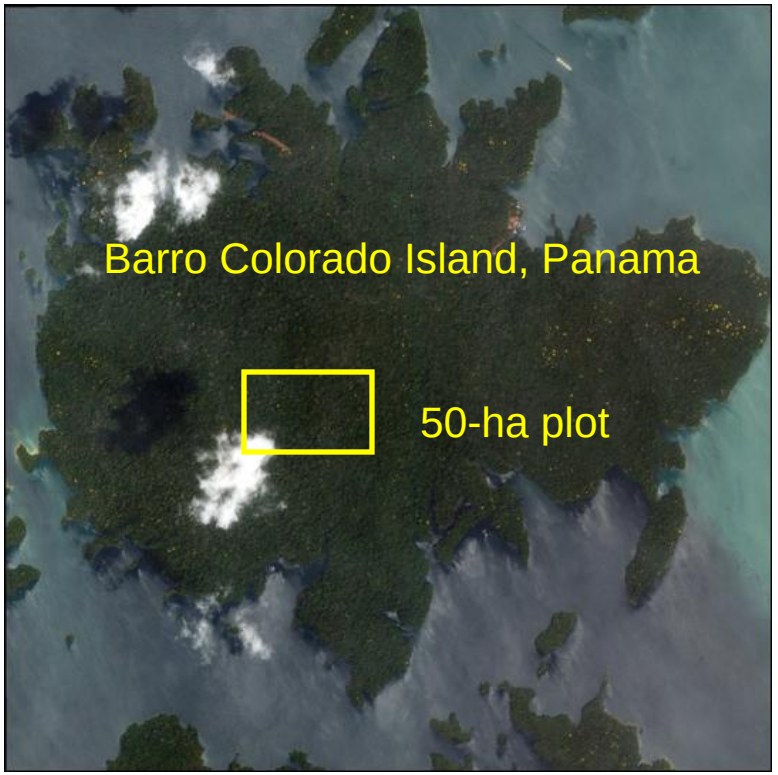
- 1/100 specimens from tropical regions are new to science (Prance & Campbell 1988)
- Not enough herbarium material to describe many new species
- Declining pool of specialists to describe new species

Case study of Barro Colorado Island (BCI), Panama

*psb*A-*trn*H, *rbc*L and *mat*K amplified from tree species in the 50 ha plot

95% resolution of species (274 out of 282)

8 unresolved species from species-rich genera (*Ficus*, *Inga*, *Piper*)

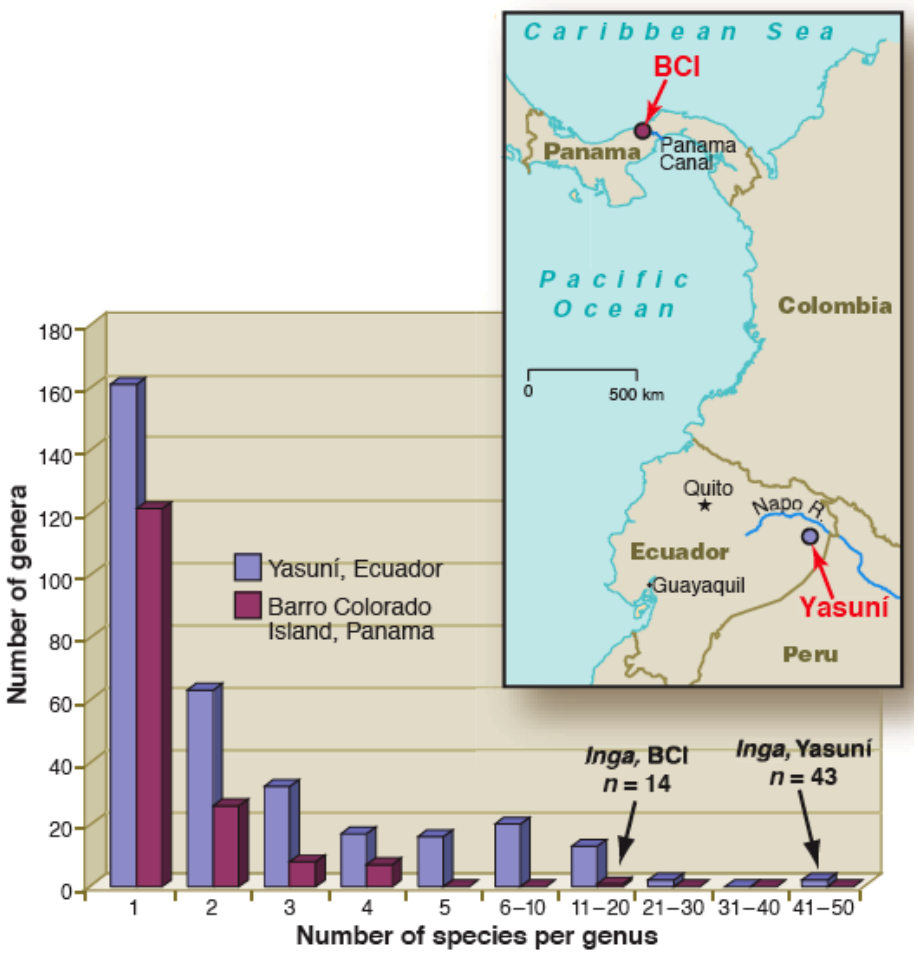


Kress et al. 2009 PNAS

Barcode ID success rate in local scales

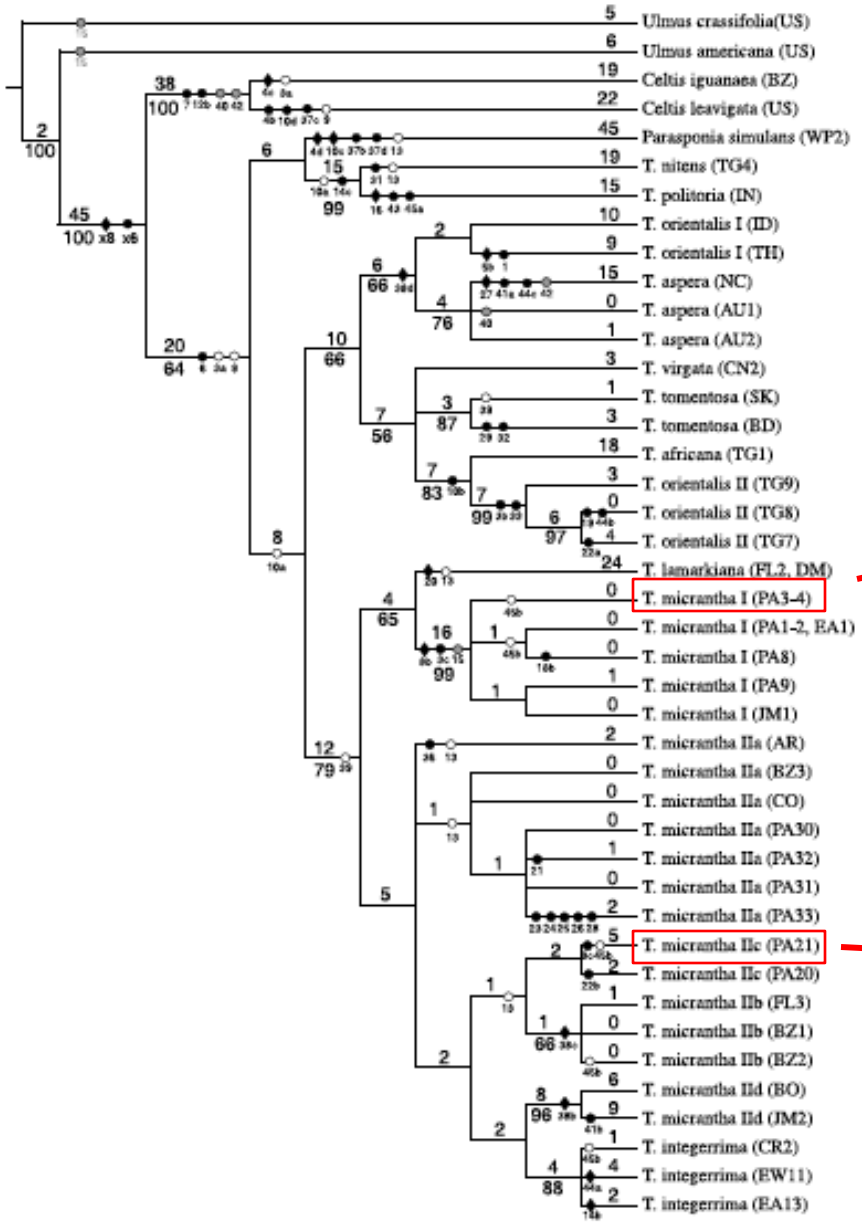
The BCI flora is phylogenetically diverse (174 genera) with few closely related species

Taxonomic certainty (e.g. Croat 1978 *Flora of Barro Colorado Island*)



Species discovery

DNA barcoding detected two morphotypes of *Trema micrantha*, which differ only in the endocarp structure but occupy different ecological niches (Silvera et al. 2003)



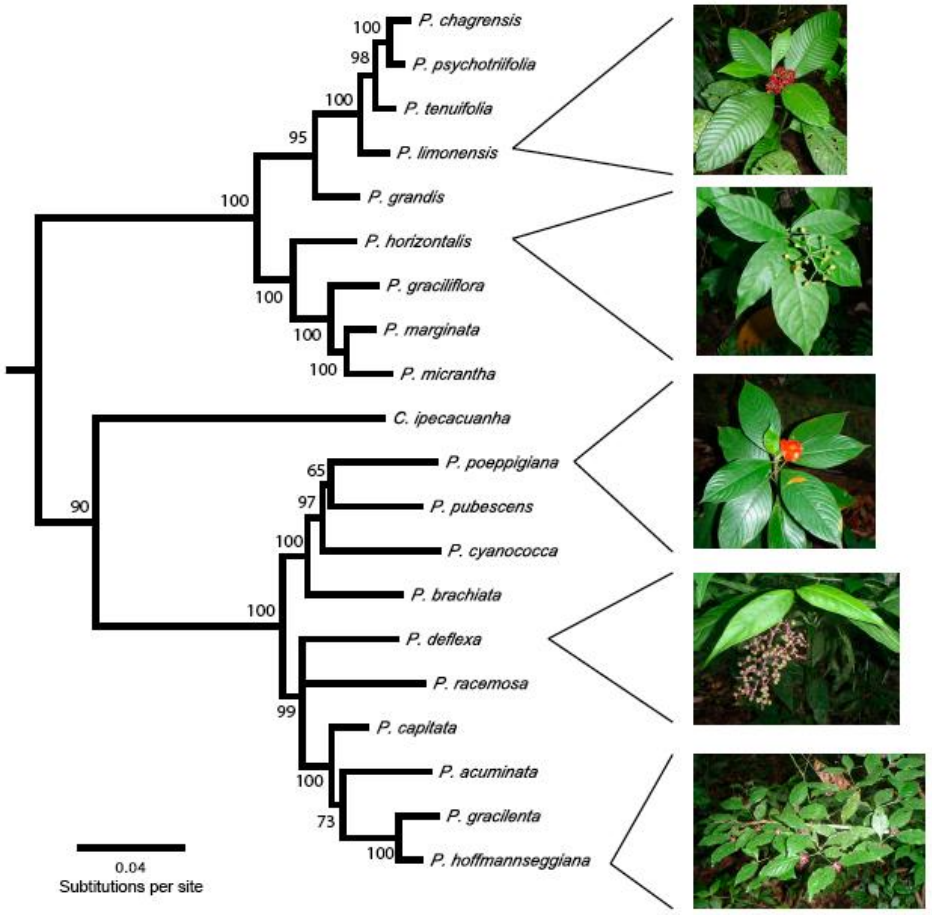
Yesson et al. (2004) *Plant Syst & Evol*.

Species status confirmed by further phylogenetic analyses

DNA barcodes can help to characterize ecological processes that maintain diversity at local scales

- Phylogenetic effects on competition
- Trophic interactions
- Belowground competition

Community phylogenetics



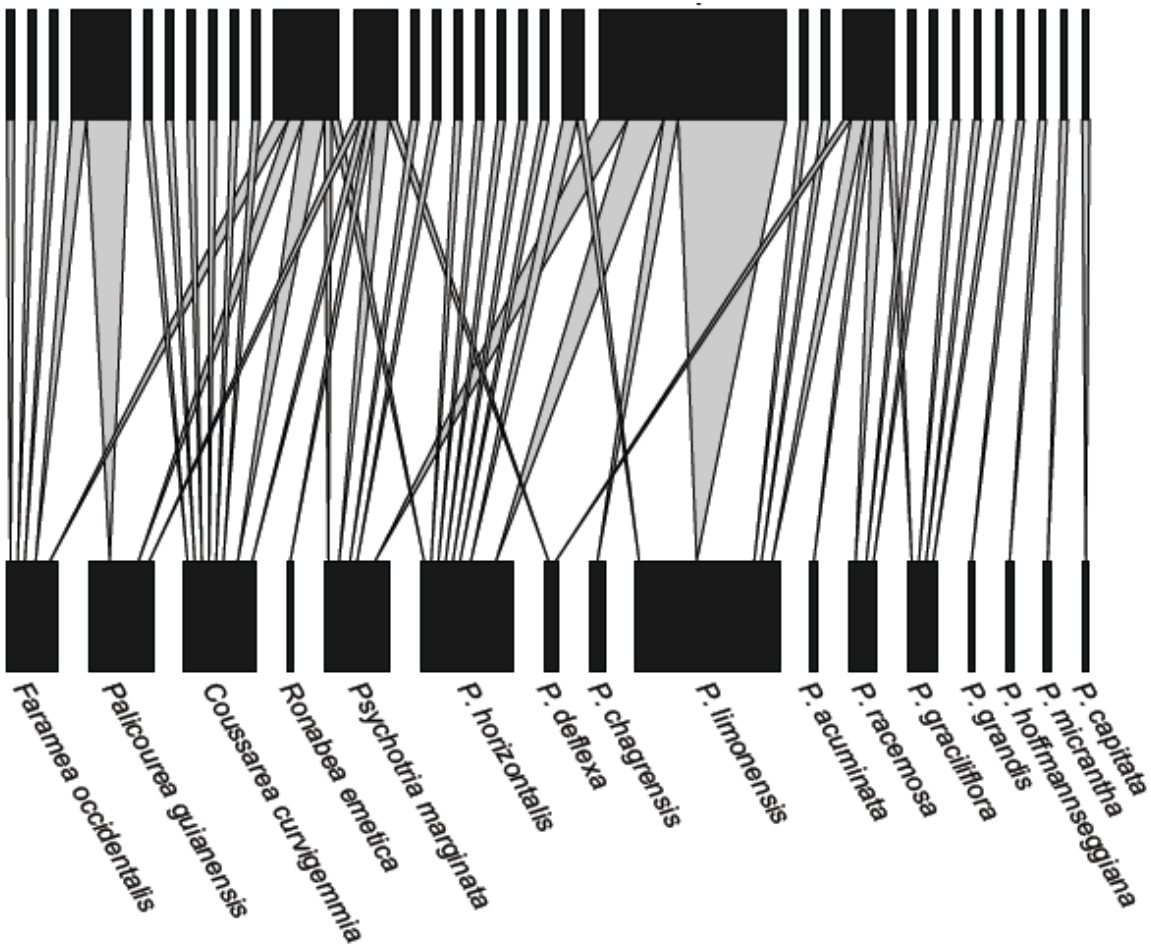
Closely related *Psychotria* species are most likely to coexist at small spatial scales (3 m^2), evidence of phylogenetic clustering

Kress *et al.* (2009) performed whole tree community phylogeny

(ITS and psbA-psbB phylogeny of *Psychotria*, B. Sedio, unpublished)

Plant insect interactions

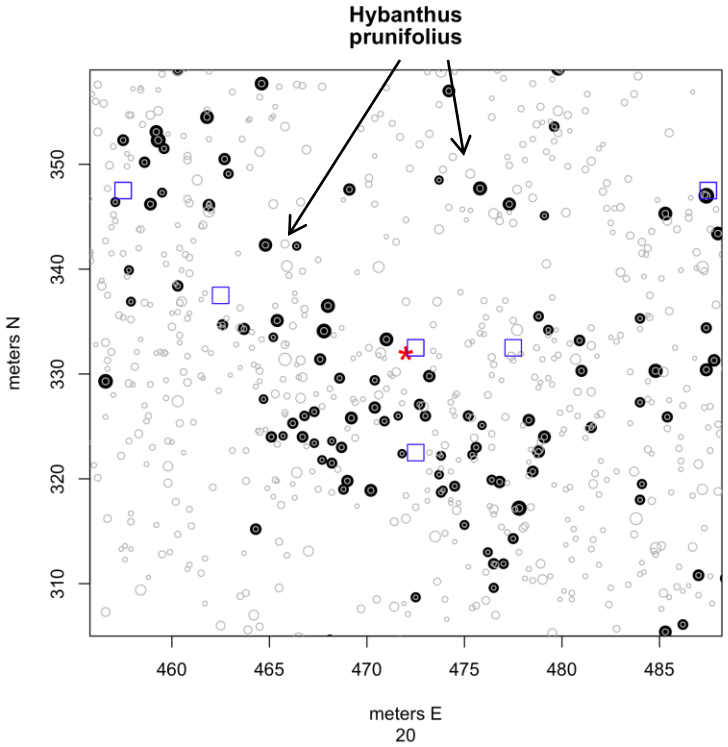
Beetles collected on *Psychotria* leaves



Brian Sedio (in prep)

Psychotria herbivores appear specialized to the level of subgenus

Root level dynamics



Roots from soil cores can be identified to species using DNA barcodes; mapped trees give minimal root lengths

F. A. Jones *et al.* (unpublished)

Root level dynamics

- 12 soil cores in 1 ha
  - *rbc*L and *trn*H-*psb*A
  - 130 sequences, 35 species
  - 15% of total species in 0.064 m^2
- Rooting versus canopy area:
  - Canopy area ~ 60 m2
  - Root area ~ 120 m

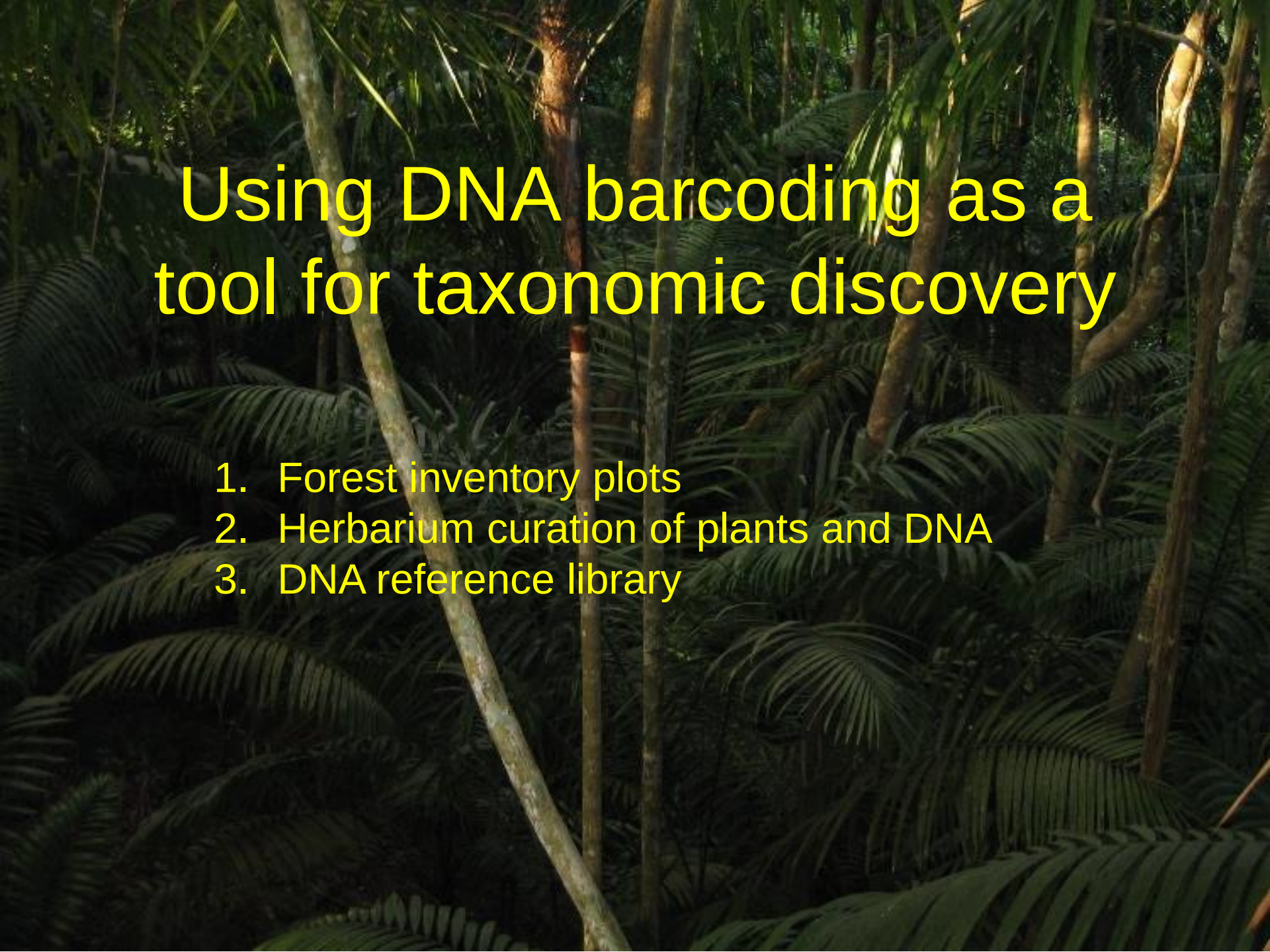
F. A. Jones *et al.* (unpublished)

And more diverse forests?

After 10 years of effort, 584 of 1104 morphospecies have been identified in Yasuní 25 ha plot (Valencia et al. 2004)

Challenge for building DNA reference library

Potential for discovery of species new to Ecuador and new to science



Using DNA barcoding as a tool for taxonomic discovery

1. Forest inventory plots
2. Herbarium curation of plants and DNA
3. DNA reference library

Forest plots

Permanent forest plots are living museums

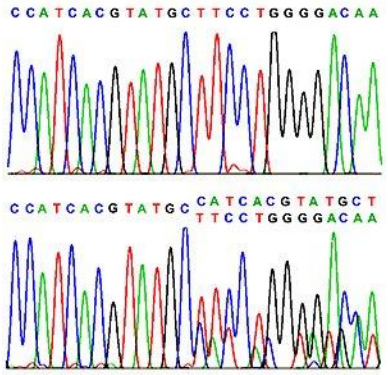
Can return to mapped trees for flowers, fruits, field characters needed by systematists and ID resulting in description of rare species

Example: intensive survey of Ducke reserve in Manaus, Brazil resulted in discovery of 55 new species (Prance et al. 2000)

Future prospects

22 CTFS plots, 3 million trees, estimated 6500 species

DNA Barcode library



Bioinformatics database that integrates ecological data, digital images, DNA sequences and trace files, and provides algorithms for species identification

Consortium for DNA Barcode of Life (COBOL) database does this for animal DNA barcodes

Herbarium curation

Storage of the physical specimen associated with the DNA barcode library

Available for systematists to identify, describe as new, or revise as classifications change

Broader evolutionary applications require long term curation of DNA

Partnership of taxonomy and DNA barcoding

DNA barcoding projects will reach full potential for scientific discovery by engaging systematists and ecologists

Likewise, systematics should benefit from the informatics, plant collections and funding provided by DNA barcode initiatives

Conclusions

- The fullest benefits of DNA barcoding in tropical forests will be realized through:
  - Focus on large tropical forest inventory plots
  - Links between ecologists and systematists
  - Vouchered DNA barcode library
  - Co-funding for curation and training opportunities in systematics

Acknowledgements

- Organizers Lúcia Lohmann, Mariana Cabral de Oliveira and Cristina Miyaki
- Brian Sedio (University of Michigan) and Andy Jones (Imperial College, UK) for sharing unpublished research