DNA Barcodes for Soil Fauna Taxonomy

T. Decaëns,
D. Porco, R. Rougerie

OUTLINE

Introduction

➔ Soil biodiversity: the final frontier

➔ DNA barcoding for the sensus of soil biodiversity

Applications and perspectives

➔ Species id, biodiversity estimates,

taxonomy, biogeography, ecology

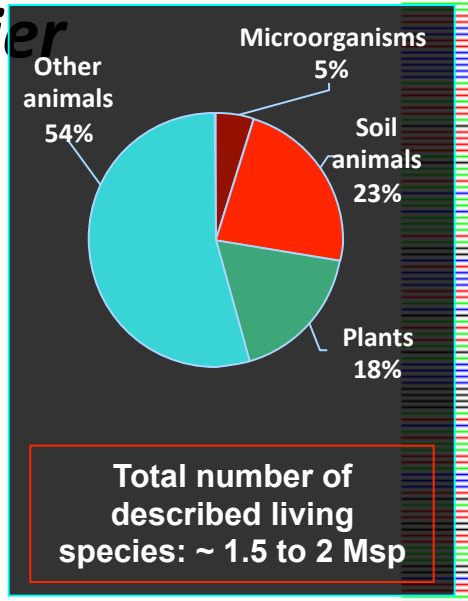
Introduction: Soil biodiversity, the final frontier

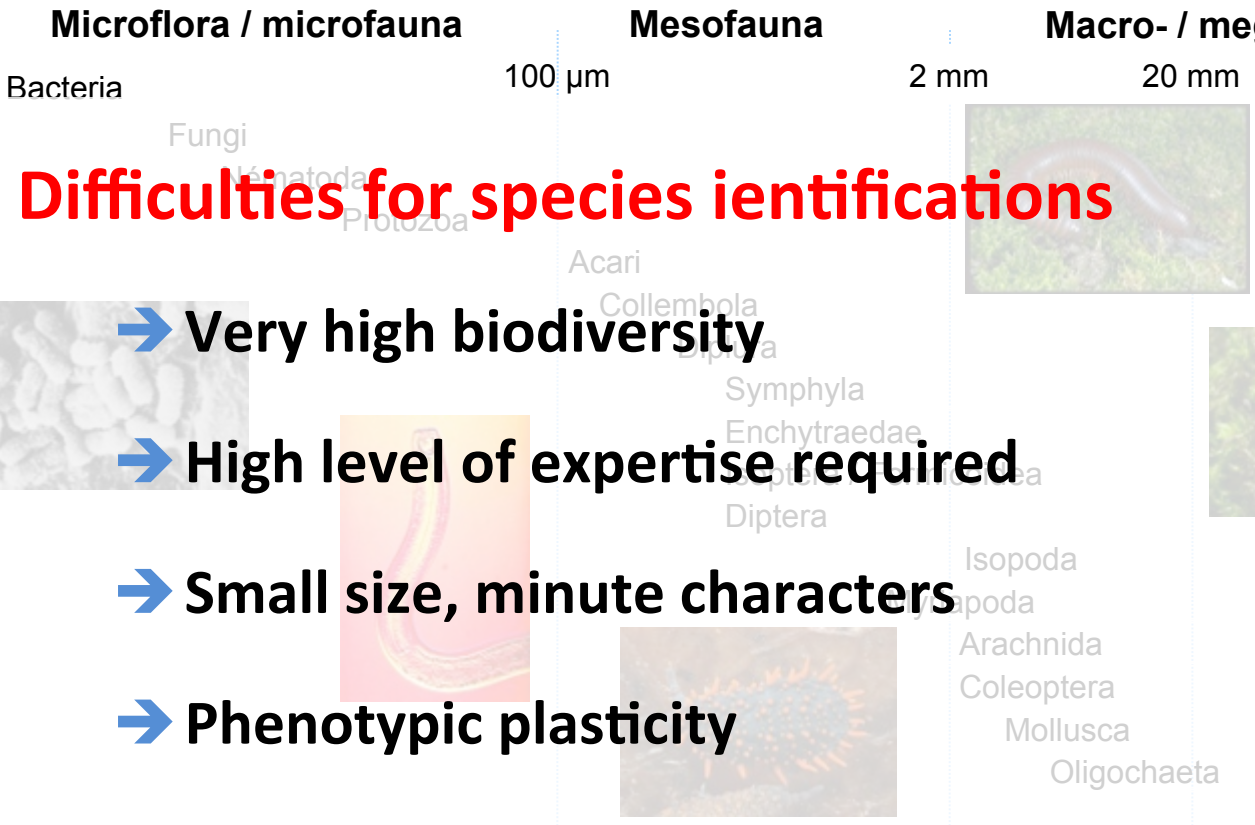
Importance of soil fauna

- **Ecological importance:** regulators of soil processes
- **Economic importance:** maintenance of soil fertility
- **Strategic importance:** highly diverse communities
- **Strategic importance:** invasive species
- **Practical importance:** used as bioindicators of soil quality or pollution

Introduction: Soil biodiversity, the final frontier

Problems with the study of soil biodiversity





Difficulties for species ientifications

- ➔ **Very high biodiversity**
- ➔ **High level of expertise required**
- ➔ **Small size, minute characters**
- ➔ **Phenotypic plasticity**
- ➔ **Non-diagnosable life stages**

Swift et al. (1979), Decaëns et al (2006), Brussaard (1997), Behan-Pelletier (1999), André (2001), Wall, André (2002), Decaëns (2010)

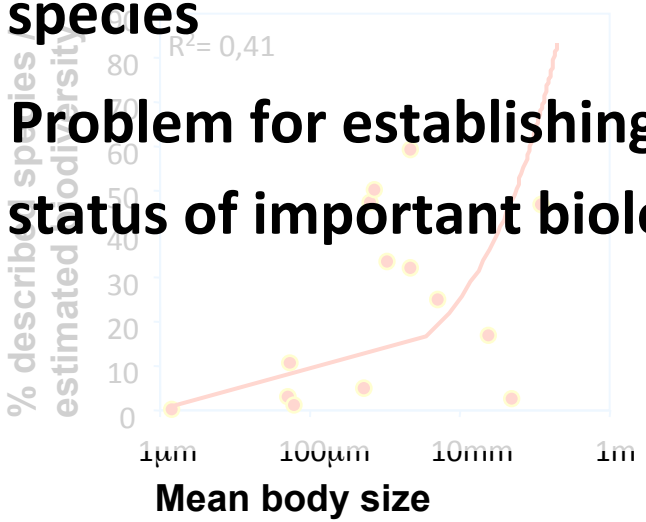
Introduction: Soil biodiversity, the final frontier

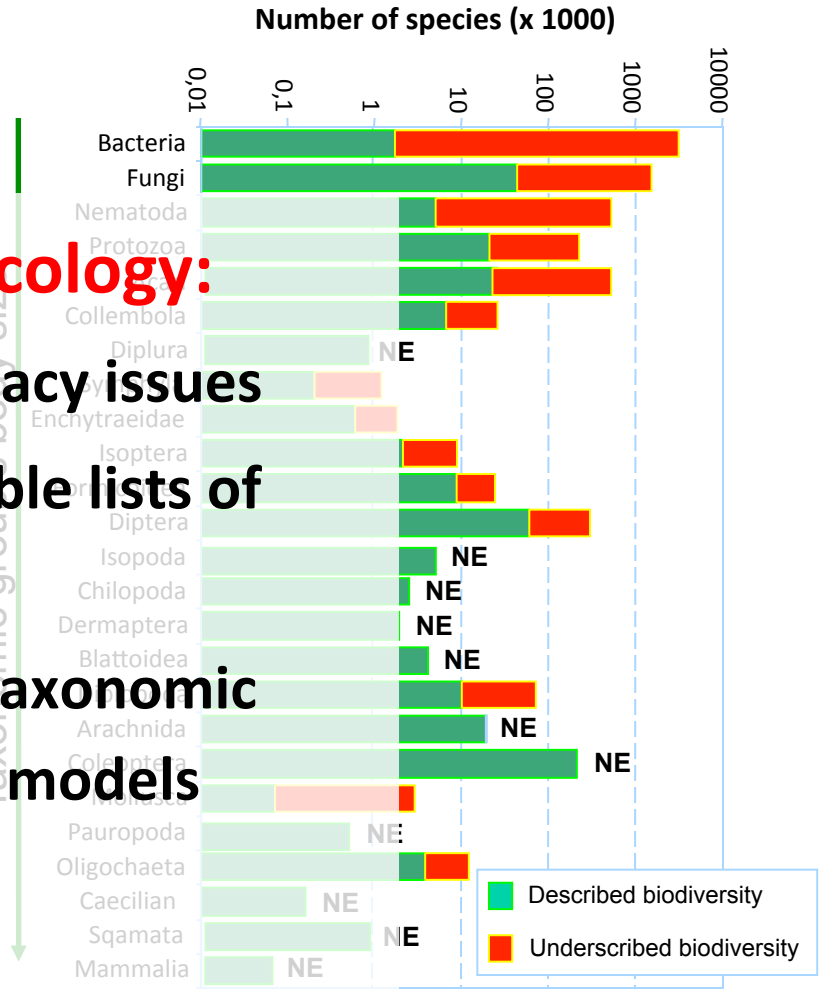
The taxonomic deficit

- Known biodiversity = small fraction of real diversity

Important limitations for soil ecology:

- **Deficit of taxonomists and legacy issues**
- **Problem for establishing reliable lists of species**
- **Problem for establishing the taxonomic status of important biological models**





Decaëns et al. (2008), Decaëns (2010)

Introduction: What is DNA barcoding?

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

DNA Barcoding = the use of a short standardized gene fragment for species identification and biodiversity exploration

Cytochrome c oxidase I (COI) is the selected standard 'barcode' in animals

➔ ***A standardized identification method for animals***

Hebert et al (2003)

Introduction: What is DNA b

Some controversy regarding the reliability of the method

- ➔ ***Potential false negatives***: identical barcodes in two actually different species (short divergence time, gene introgression);
- ➔ ***Potential false positives***: different barcodes within a single species (ancestral polymorphism, genetic introgression)

Now recognized as a reliable and straightforward tool for species discrimination

Hebert proposed the idea of a global barcode library for the animal kingdom

Rubinoff & Holland (2005), Trewick (2007), Wiemers & Fiedler (200
Will et al (2005), Hebert et al (2003, 2004, 2010)

biology letters
Biol. Lett. (2010) 6, 359–362
doi:10.1098/rsbl.2009.0848
Published online 16 December 2009
Evolutionary biology

DNA barcodes for 1/1000 of the animal kingdom

Paul D. N. Hebert[1,*], Jeremy R. deWaard[2,3] and Jean-François Landry[4]

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[2]*Department of Forestry Science, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4*
[3]*Entomology, Royal British Columbia Museum, Victoria, British Columbia, Canada V8W 9W2*
[4]*Research Centre, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada K1A 0C6*
**Author for correspondence (phebert@uoguelph.ca).*

This study reports DNA barcodes for more than 1300 Lepidoptera species from the eastern half of North America, establishing that 99.3 per cent of these species possess diagnostic barcode sequences. Intraspecific divergences averaged just 0.43 per cent among this assemblage, but most values were lower. The mean was elevated by deep barcode divergences (greater than 2%) in 5.1 per cent of the species, often involving the sympatric occurrence of two barcode clusters. A few of these cases have been analysed in detail, revealing species overlooked by the current taxonomic system. This study also provided a

Introduction: Building the reference library

The international Barcode of Life (iBOL)

➔ **Involves a network of taxonomists for a range of taxonomic groups**

Introduction: Building the reference library

DNA barcoding workflow

Specimen

Collection Data

Tissue Sample

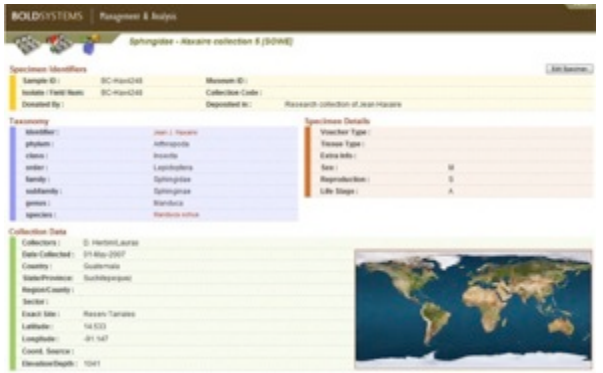
Photograph

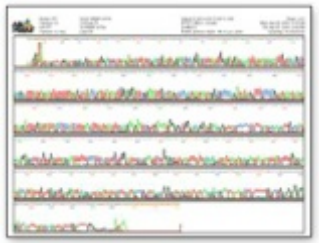
DNA extraction

PCR Amplification

Sequencing

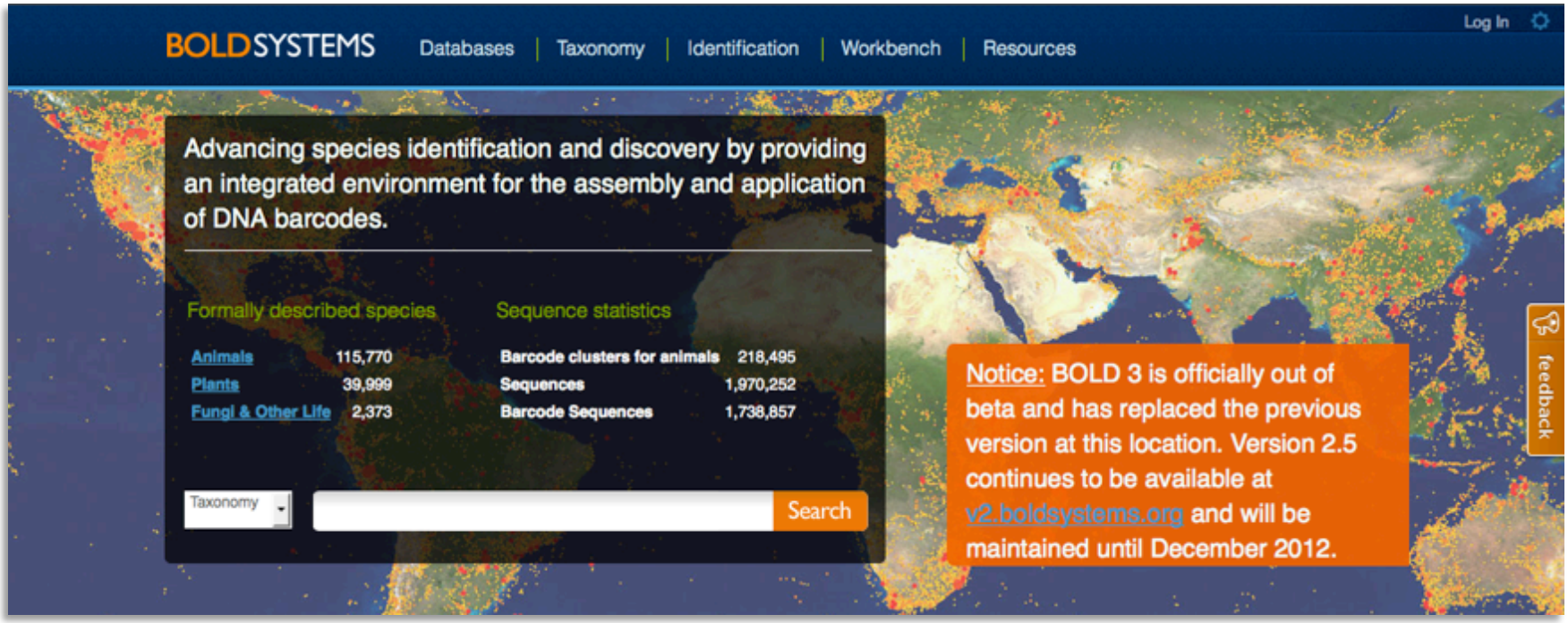
Web-Accessible Data and DNA Barcode





Introduction: Building the reference library

A dedicated bioinformatics platform (BOLD)



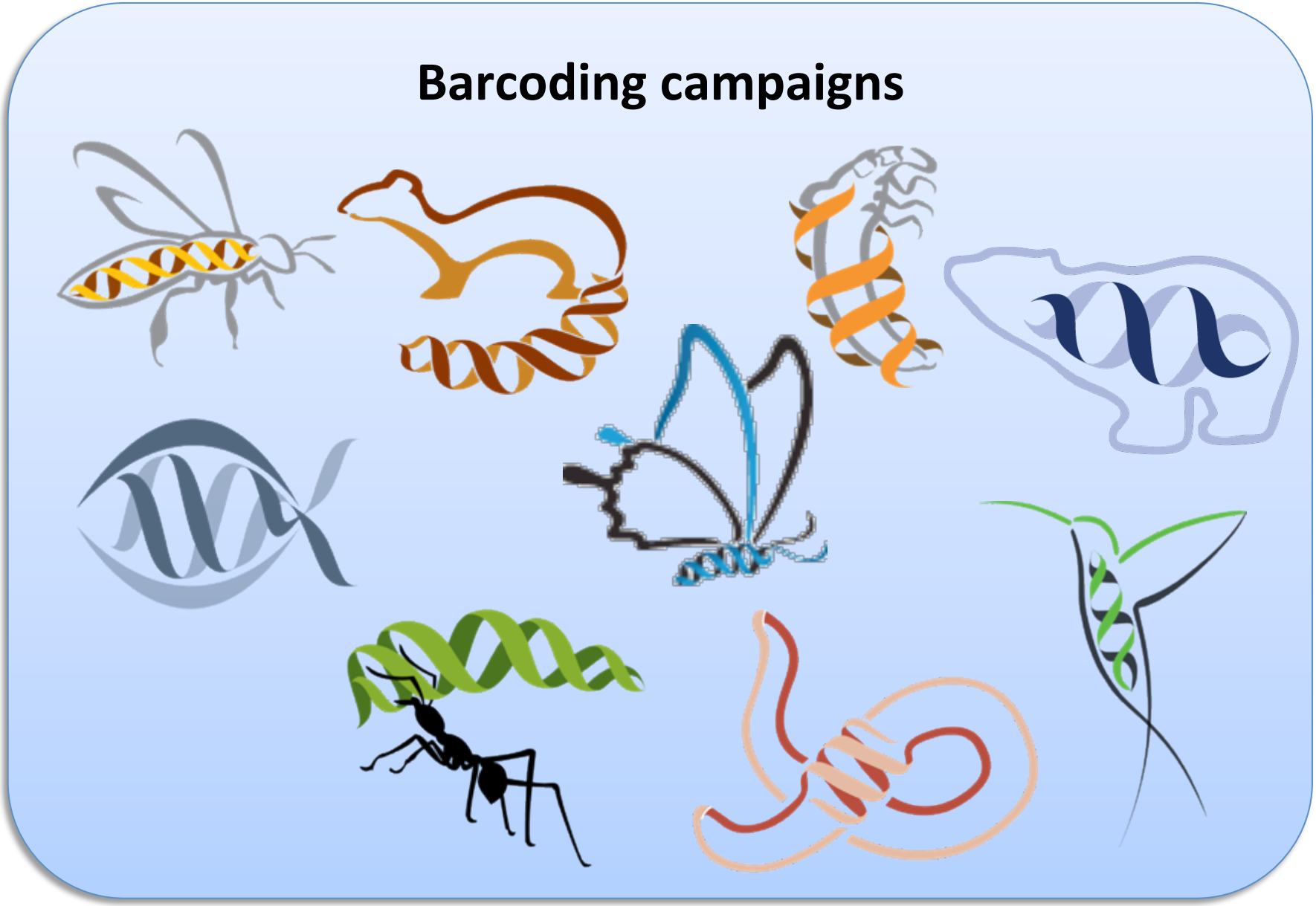
- BOLD is used for most sequence manipulations, analyses, comparisons, mapping
- Confidentiality requirements can be managed with BOLD

Ratnasingham & Hebert (2007)

Introduction: Building the reference library

Current coverage :
≈ 2200K Barcode Records; > 250K Species

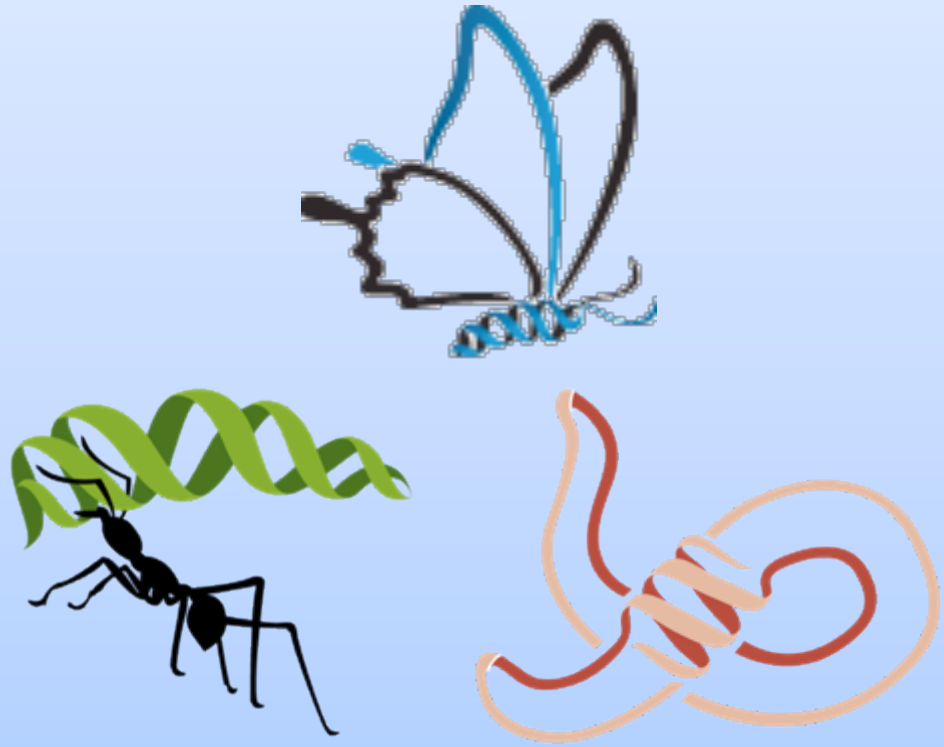
Barcoding campaigns of soil invertebrates



Barcoding campaigns of soil invertebrates

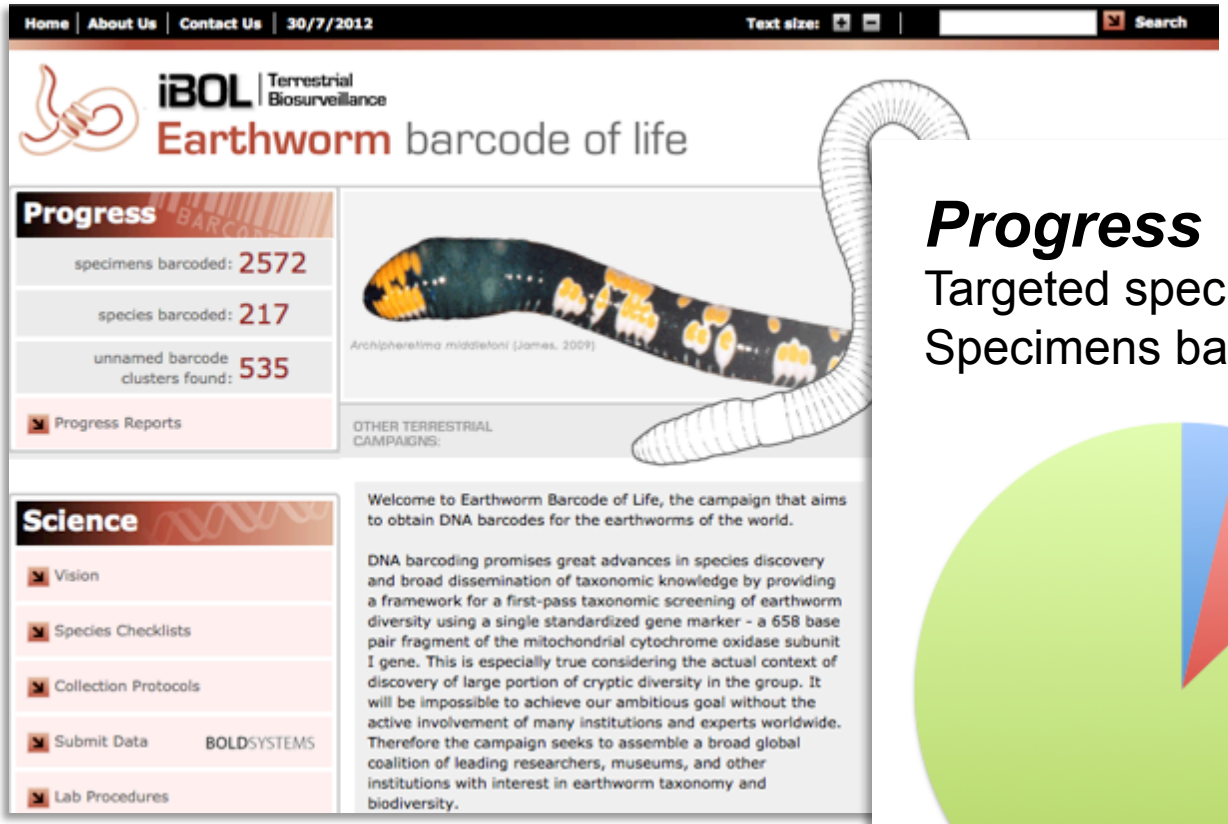
Barcoding campaigns

WG 1.9 – Terrestrial Bio-Surveillance



Soil invertebrate barcoding campaigns

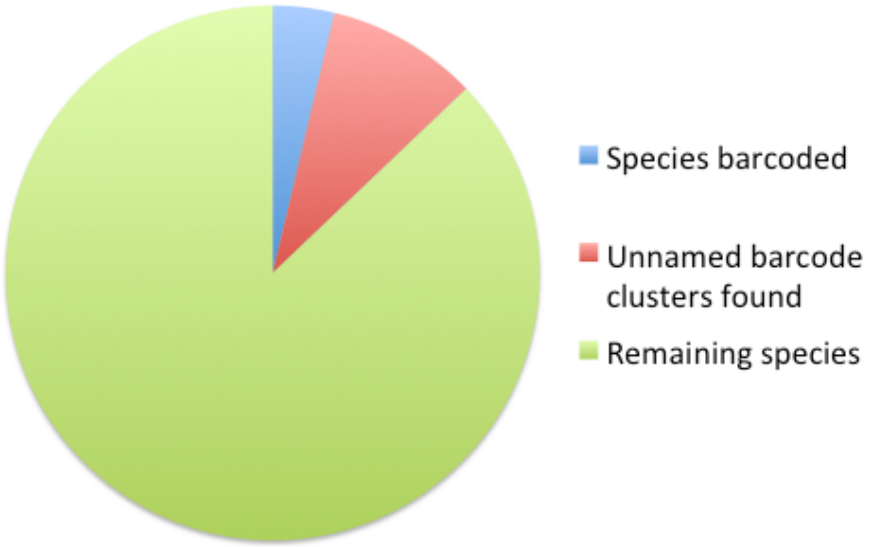
The Earthworm barcode of life



Progress

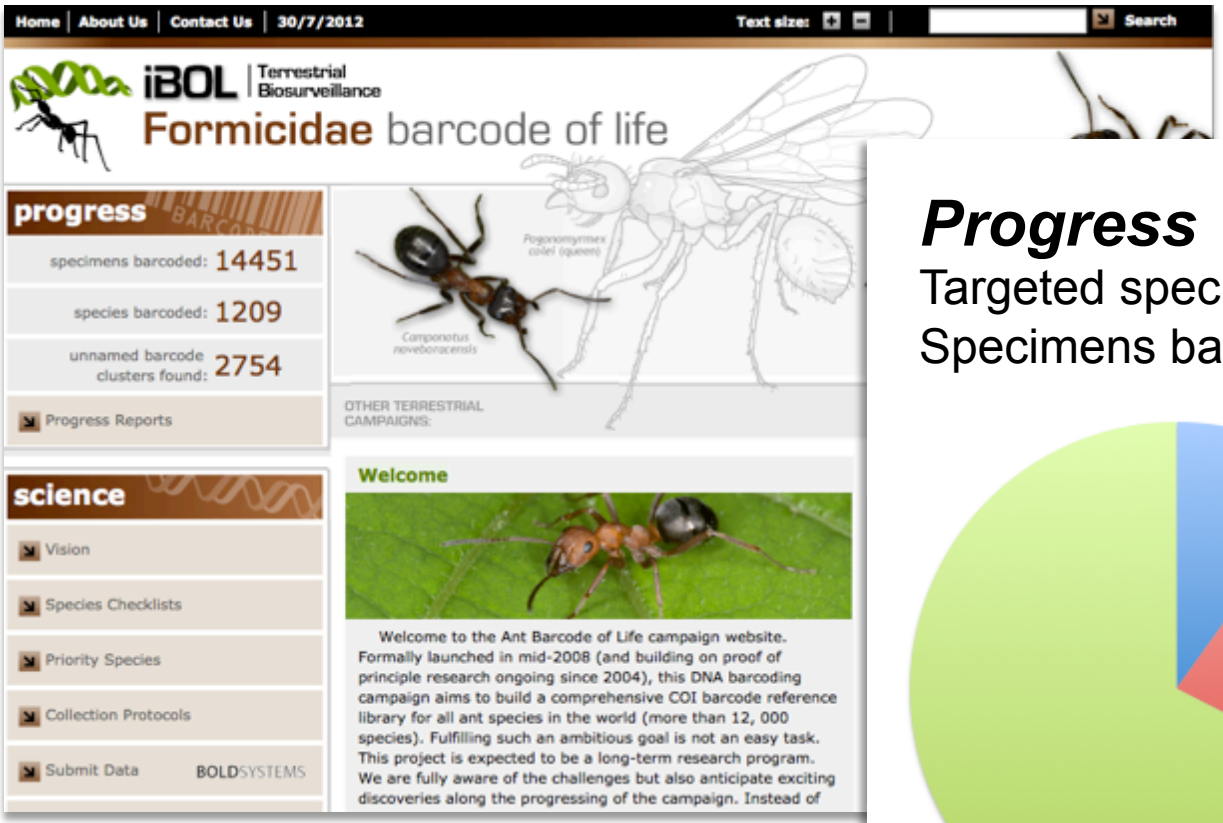
Targeted species: 5829

Specimens barcoded: 4000



Soil invertebrate barcoding campaigns

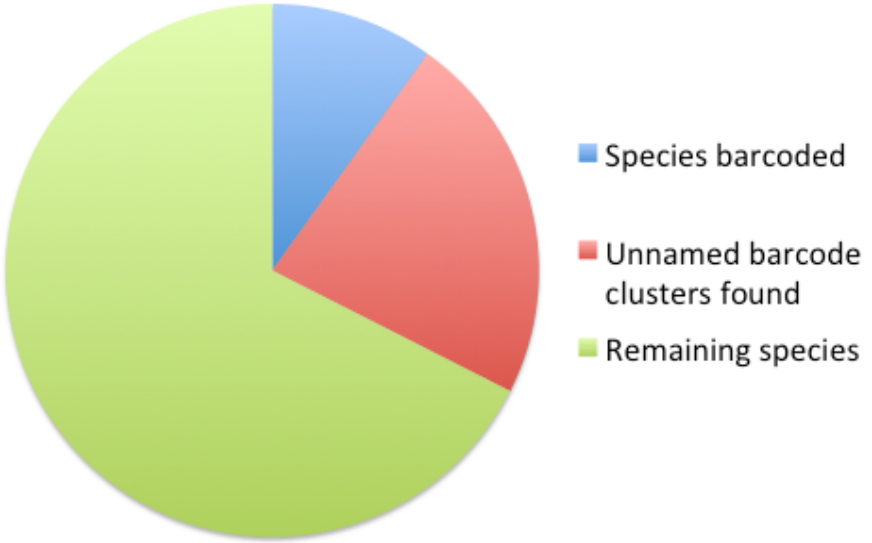
The Formicidae barcode of life



Progress

Targeted species: 12 205

Specimens barcoded: 14 451

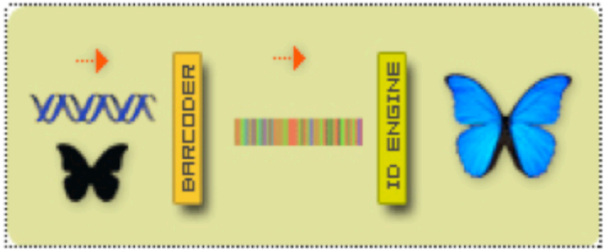


Applications: Species identification

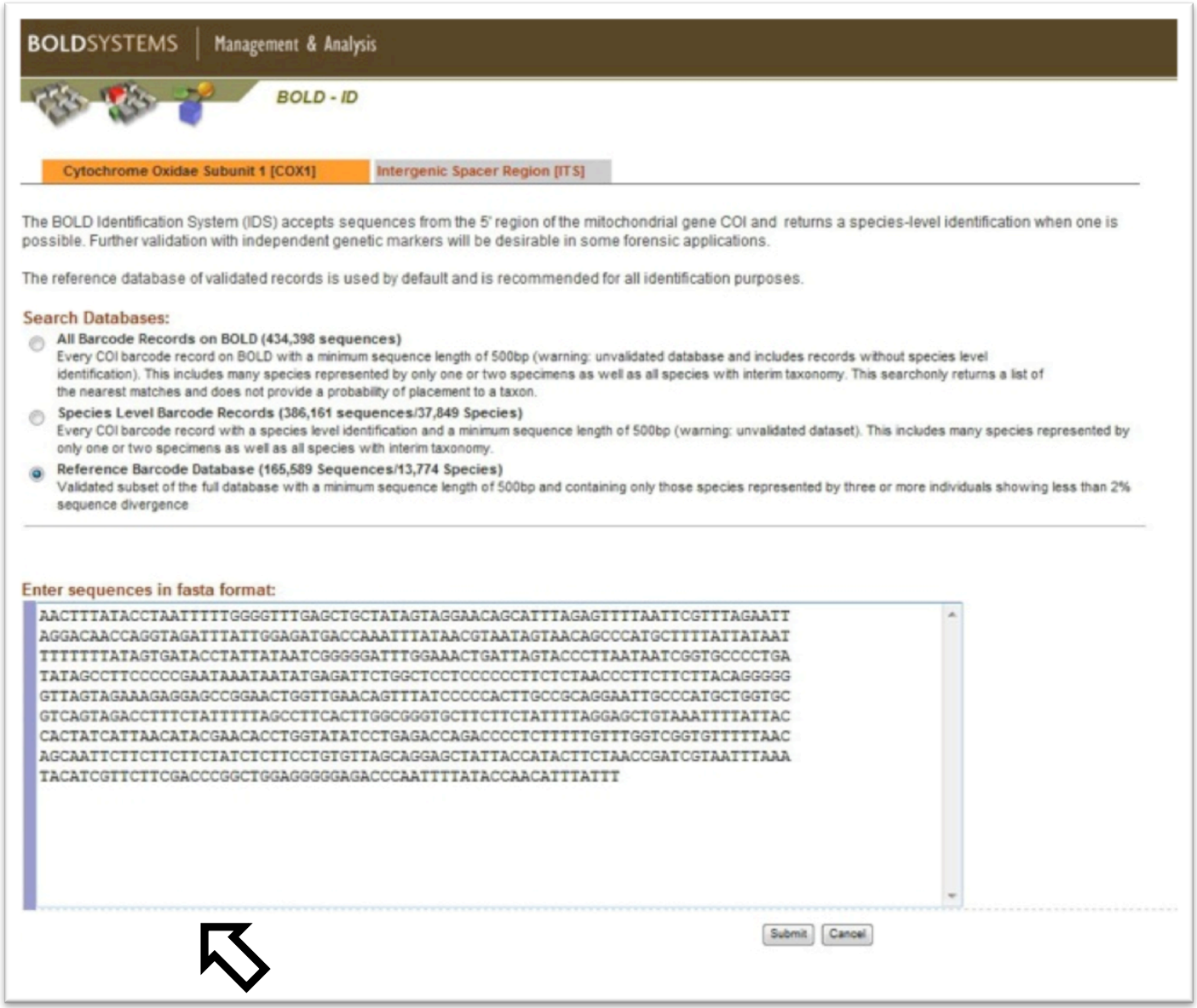
Identification through closest match in a curated reference library

IDENTIFICATION ENGINE

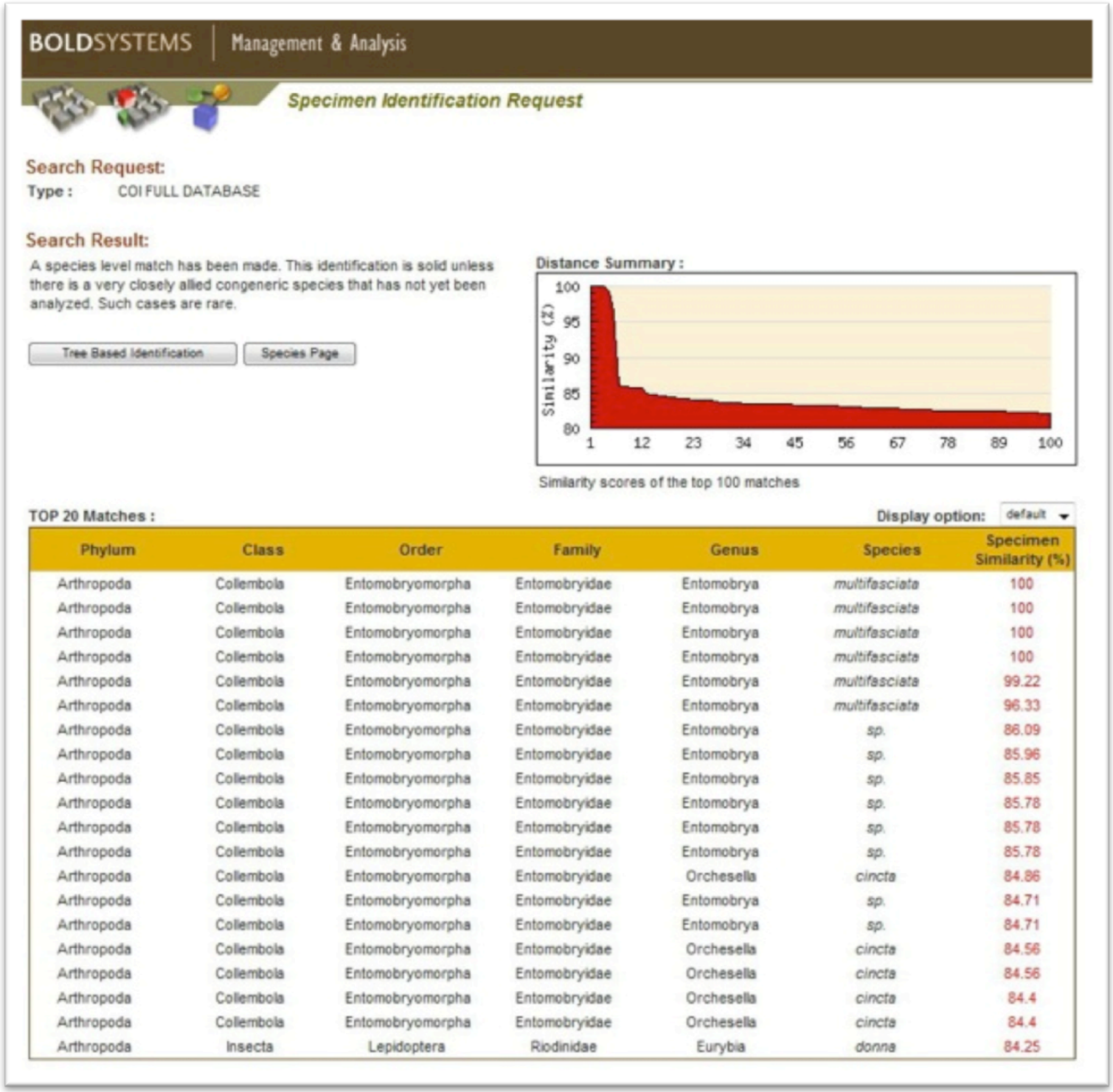
BOLD-IDS provides a species identification tool that accepts DNA sequences from the barcode region and returns a taxonomic assignment to the species level when possible.



Applications: Species identification



Applications: Species identification



BOLDSYSTEMS | Management & Analysis

Specimen Identification Request

Search Request:

Type : COI FULL DATABASE

Search Result:

A species level match has been made. This identification is solid unless there is a very closely allied congeneric species that has not yet been analyzed. Such cases are rare.

Tree Based Identification | Species Page

Distance Summary :

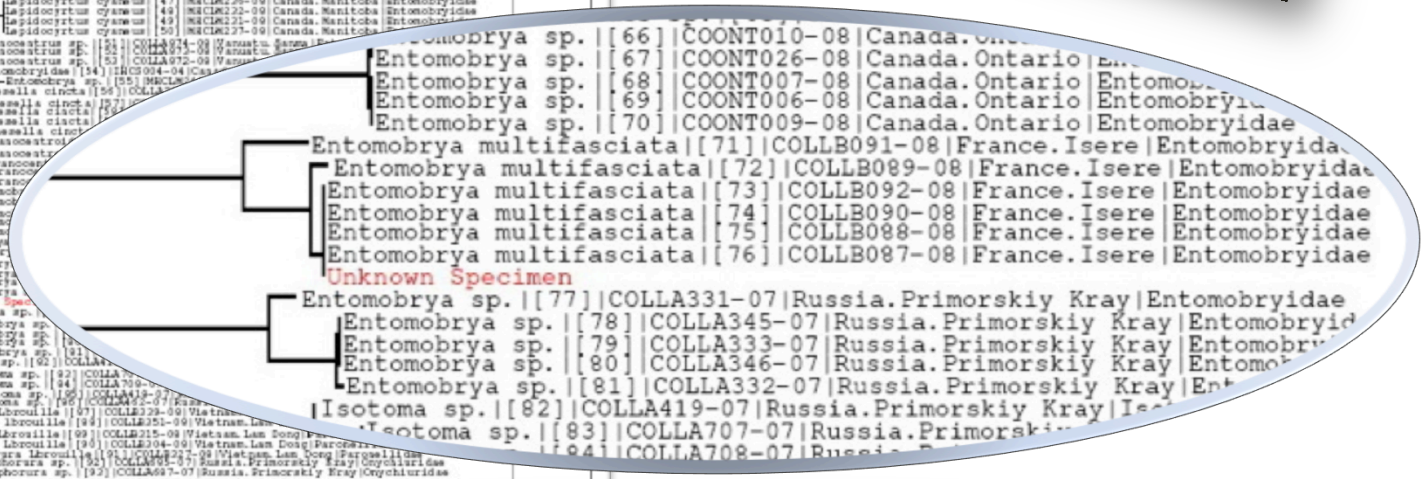
Similarity scores of the top 100 matches

TOP 20 Matches :

Display option: default

Phylum	Class	Order	Family	Genus	Species	Specimen Similarity (%)
Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Entomobrya	*multifasciata*	100
Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Entomobrya	*multifasciata*	100
Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Entomobrya	*multifasciata*	100
Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Entomobrya	*multifasciata*	100
Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Entomobrya	*multifasciata*	99.22
Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Entomobrya	*multifasciata*	96.33
Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Entomobrya	*sp.*	86.09
Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Entomobrya	*sp.*	85.96
Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Entomobrya	*sp.*	85.85
Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Entomobrya	*sp.*	85.78
Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Entomobrya	*sp.*	85.78
Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Entomobrya	*sp.*	85.78
Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Orchesella	*cincta*	84.86
Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Entomobrya	*sp.*	84.71
Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Entomobrya	*sp.*	84.71
Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Orchesella	*cincta*	84.56
Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Orchesella	*cincta*	84.56
Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Orchesella	*cincta*	84.4
Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Orchesella	*cincta*	84.4
Arthropoda	Insecta	Lepidoptera	Riodinidae	Eurybia	*donna*	84.25

Applications: Species identification



Applications: Species identification

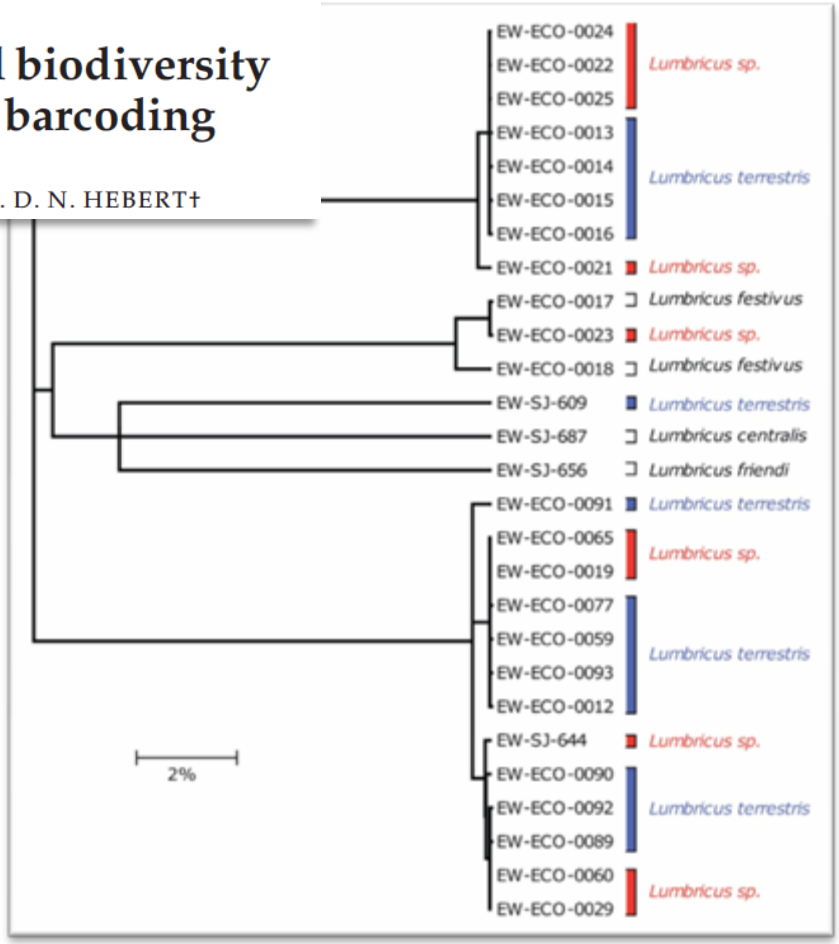
… anywhere, any stages

MOLECULAR ECOLOGY
RESOURCES
Molecular Ecology Resources (2010) 10, 606–614 doi: 10.1111/j.1755-0998.2009.02822.x

DNA BARCODING

Re-integrating earthworm juveniles into soil biodiversity studies: species identification through DNA barcoding

B. RICHARD,* T. DECAËNS,* R. ROUGERIE,† S. W. JAMES,‡ D. PORCO† and P. D. N. HEBERT†



Richard et al (2010)

Applications: Cryptic diversity

Cryptic species = species morphologically identical but well discriminated by their barcodes

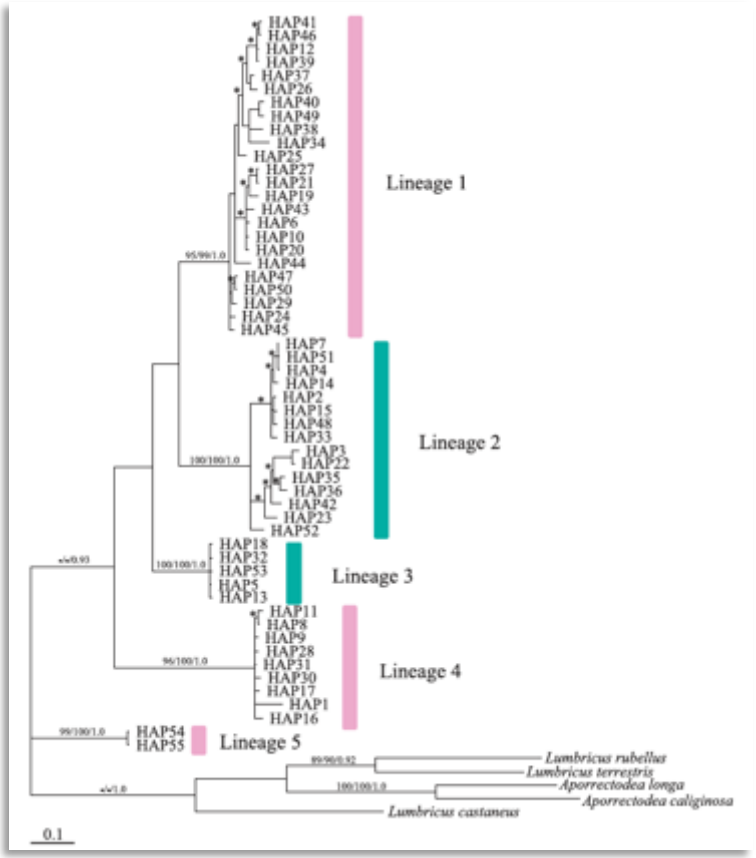
Extensive survey of earthworms from deferent British localities:

- ➔ *A. chlorotica*, *A. longa*, *A. rosea* and *L. rubellus* all comprise highly divergent lineages with species-level divergence
- ➔ AFLP markers showed that 14% of divergence at COI might not be enough to consider two lineages as distinct species

Molecular Ecology (2008) 17, 4684–4698 doi: 10.1111/j.1365-294X.2008.03931.x

Opening a can of worms: unprecedented sympatric cryptic diversity within British lumbricid earthworms

R. ANDREW KING, AMY L. TIBBLE and WILLIAM O. C. SYMONDSON



Pedobiologia 54 (2011) 217–224

New insight into the genetic structure of the *Allolobophora chlorotica* aggregate in Europe using microsatellite and mitochondrial data

L. Dupont[a,*], F. Lazrek[a], D. Porco[b,d], R.A. King[c,g], R. Rougerie[b,d], W.O.C. Symondson[c], A. Livet[a], B. Richard[d,h], T. Decaëns[d], K.R. Butt[e], J. Mathieu[f]

King et al (2008), Dupont et al. (2011), Porco et al. (in prep)

Applications: Cryp

The *L. terrestris / herculeus* complex

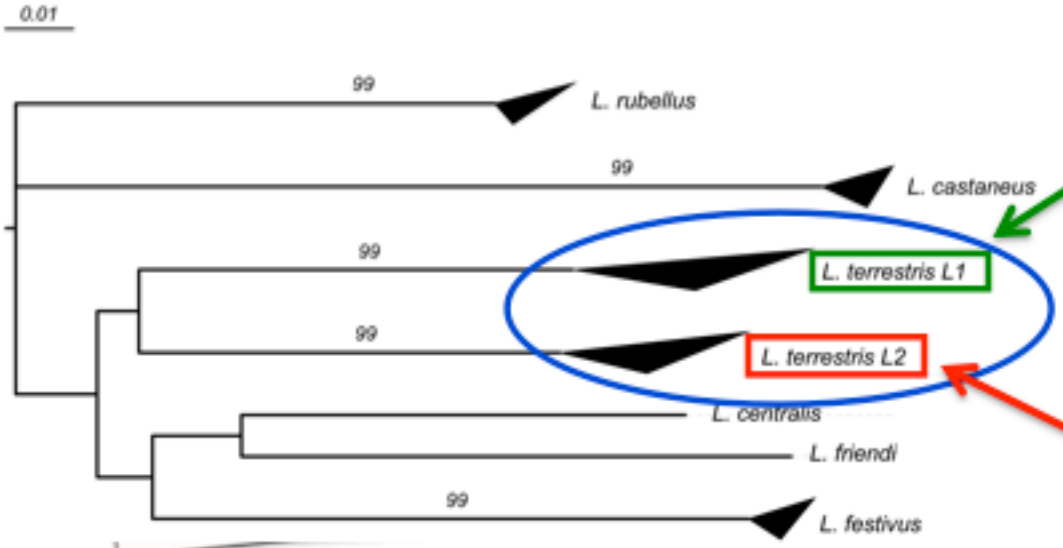
OPEN ACCESS Freely available online

PLoS one

DNA Barcoding Reveals Cryptic Diversity in *Lumbricus terrestris* L., 1758 (Clitellata): Resurrection of *L. herculeus* (Savigny, 1826)

Samuel W. James[1,3]*, David Porco[2,3], Thibaud Decaëns[3], Benoit Richard[3], Rodolphe Rougerie[2,3], Christer Erséus[4]

200 specimens from:
France, Scandinavia, Canada and USA



L1 = *Lumbricus terrestris*

L2 = *Lumbricus herculeus*

Risk of « chimerical biology »

Richard et al (2010), James et al (2010)

Applications: Alpha taxonomy

Zootaxa 2639: 59–68 (2010)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Description and DNA barcoding assessment of the new species *Deutonura gibbosa* (Collembola: Neanuridae: Neanurinae), a common springtail of Alps and Jura

DAVID PORCO[1], ANNE BEDOS[2] & LOUIS DEHARVENG[2]

ELSEVIER

Available online at www.sciencedirect.com
ScienceDirect
Organisms, Diversity & Evolution 7 (2007) 231–240

ORGANISMS DIVERSITY & EVOLUTION
www.elsevier.de/ode

Taxonomic re-evaluation of the Taiwanese montane earthworm *Amynthas wulinensis* Tsai, Shen & Tsai, 2001 (Oligochaeta: Megascolecidae): Polytypic species or species complex?

Chih-Han Chang[a], Yu-Hsung Lin[b], I.-Han Chen[b], Shu-Chun Chuang[b], Jiun-Hong Chen[a,b,*]

OPEN ACCESS Freely available online
PLoS one

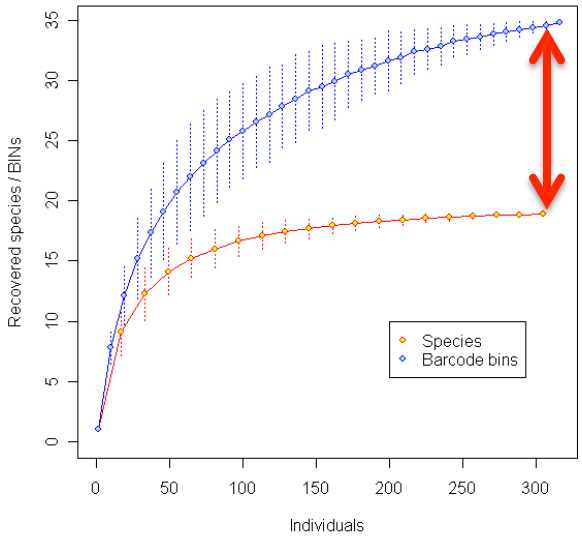
A Revision of Malagasy Species of *Anochetus* Mayr and *Odontomachus* Latreille (Hymenoptera: Formicidae)

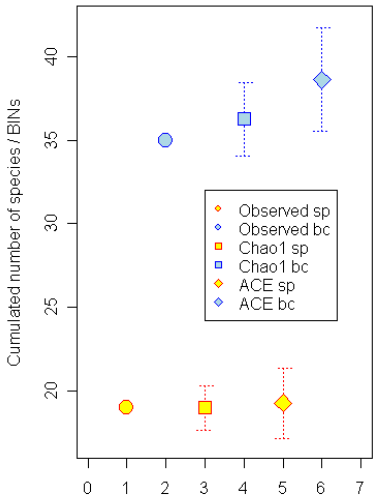
Brian L. Fisher[1*], M. Alex Smith[2*]

Applications: Biodiversity estimates

- "Earthworms of Normandy" sub-project
- "Earthworms of Canada" sub-project

➔ About 800 sequences





Molecular Ecology Resources (2009) 9 (Suppl. 1), 208–216 doi: 10.1111/j.1755-0998.2009.02646.x

BARCODING ARTHROPODS

DNA barcode accumulation curves for understudied taxa and areas

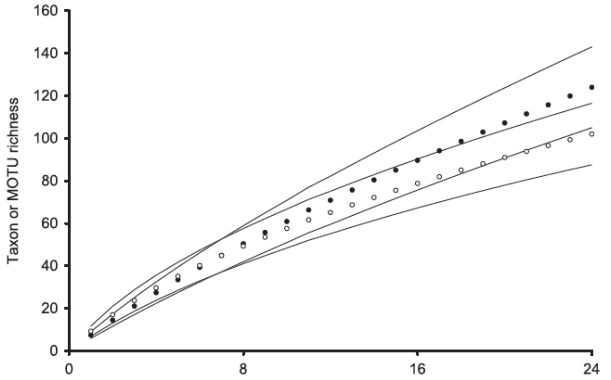
M. ALEX SMITH,* JOSE FERNANDEZ-TRIANA,* ROB ROUGHLEY† and PAUL D. N. HEBERT*

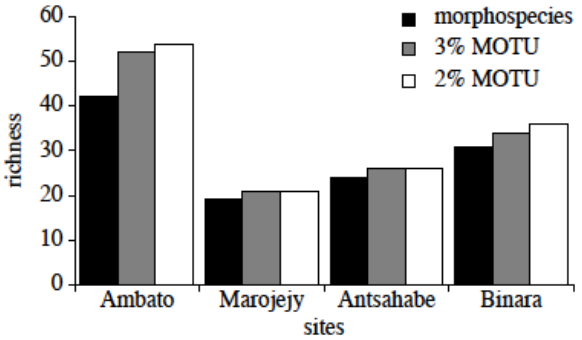
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

Phil. Trans. R. Soc. B (2005) **360**, 1825–1834
doi:10.1098/rstb.2005.1714
Published online 8 September 2005

DNA barcoding for effective biodiversity assessment of a hyperdiverse arthropod group: the ants of Madagascar

M. Alex Smith[1,*,†], Brian L. Fisher[2,*,†] and Paul D. N. Hebert[1]





Decaëns et al (accepted), Porco et al. (in prep), Smith et al (2005, 2009)

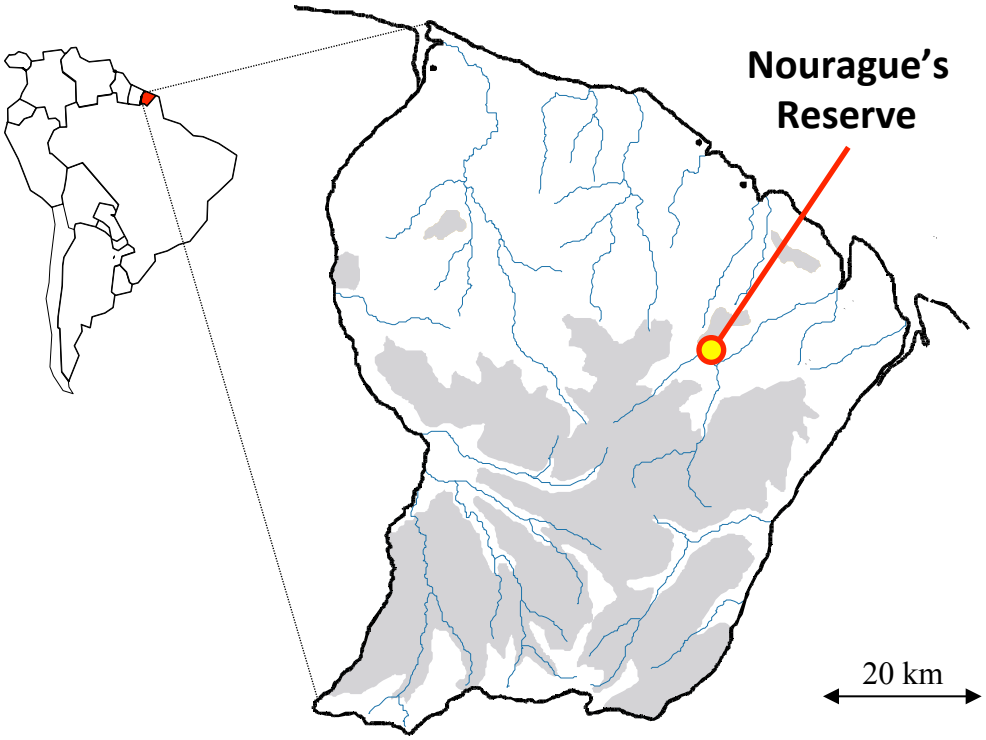
Applications: Ecology

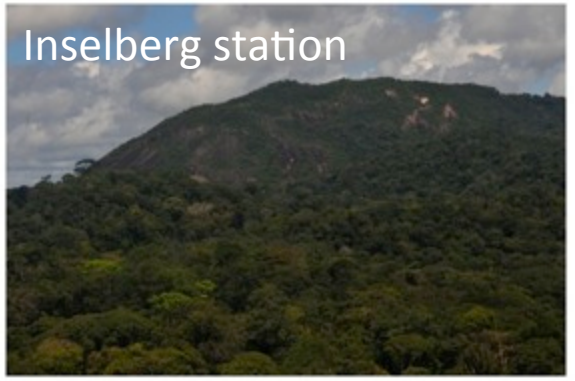
DNA barcoding to estimate underground biodiversity - a case study with earthworms in the Nouragues Research Station (French Guyana)

T. Decaëns[1], S.W. James[2], V. Chassany[3], L. Dupont[4], E. Lapied[5], R. Rougerie[1], V. Roy[4], J.-P. Rossi[6], D. Porco[1]

Objective:

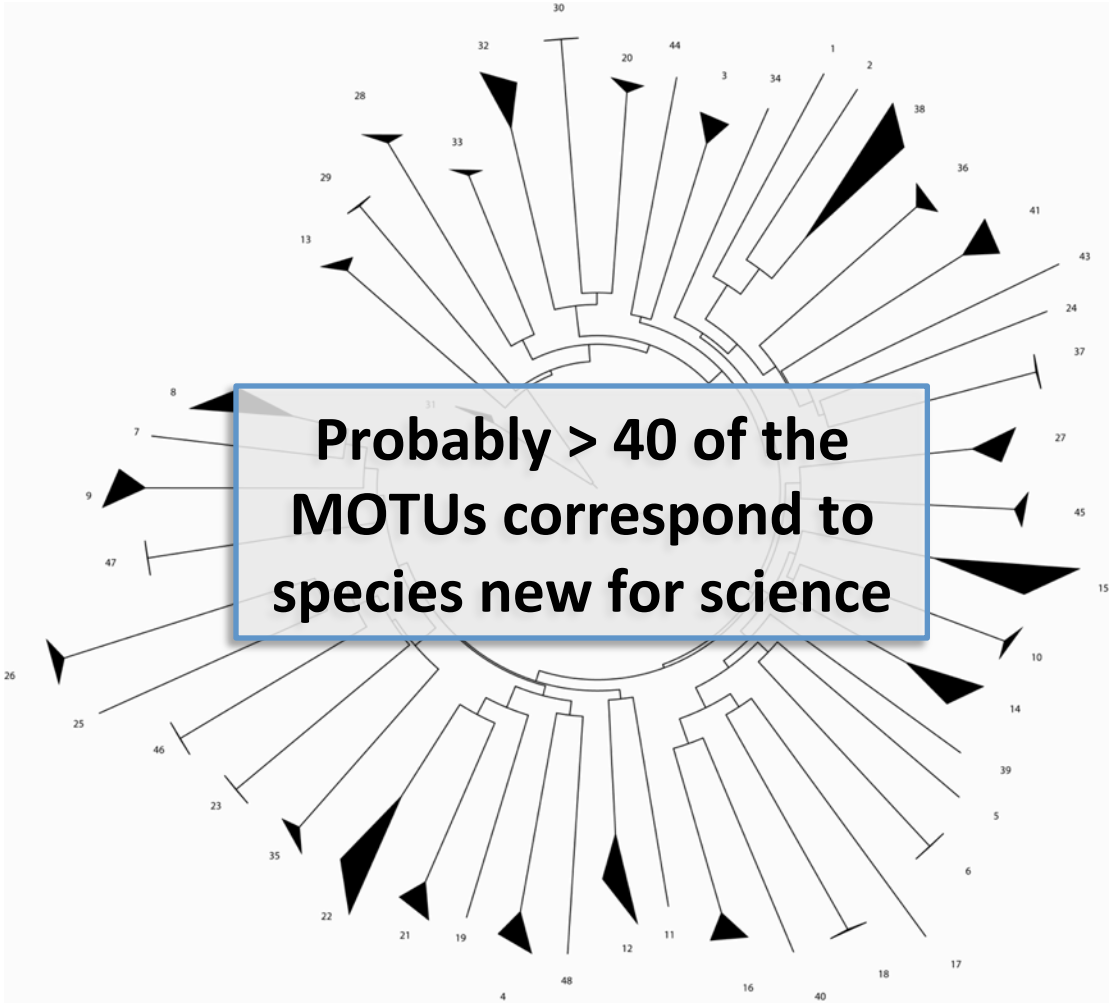
To describe earthworm biodiversity patterns in the hyperdiverse ecosystems of the Nouragues natural reserve, French Guayana





Decaëns et al (in prep), poster at ICSZ XVI

Applications: Ecology



- 748 earthworms
- 4 families: Glossoscolecidae, Octochaetidae, Ocnerodrilidae, Haplotaxidae
- 605 sequences

→ > 80% success

15% threshold

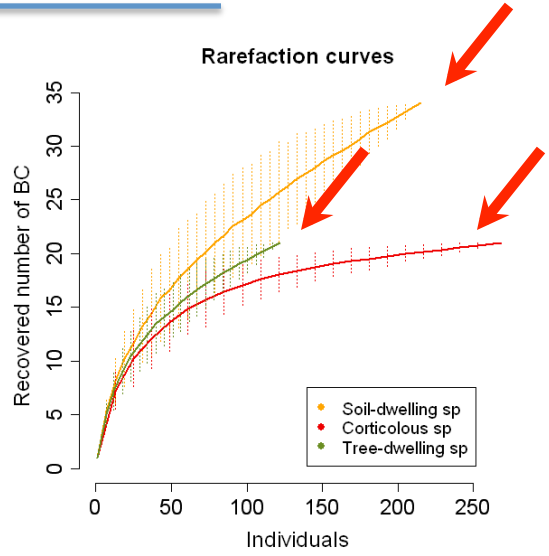
➔ ***50 MOTUs***

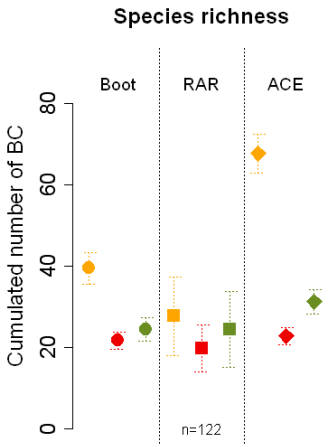
- ~ 30% of singletons

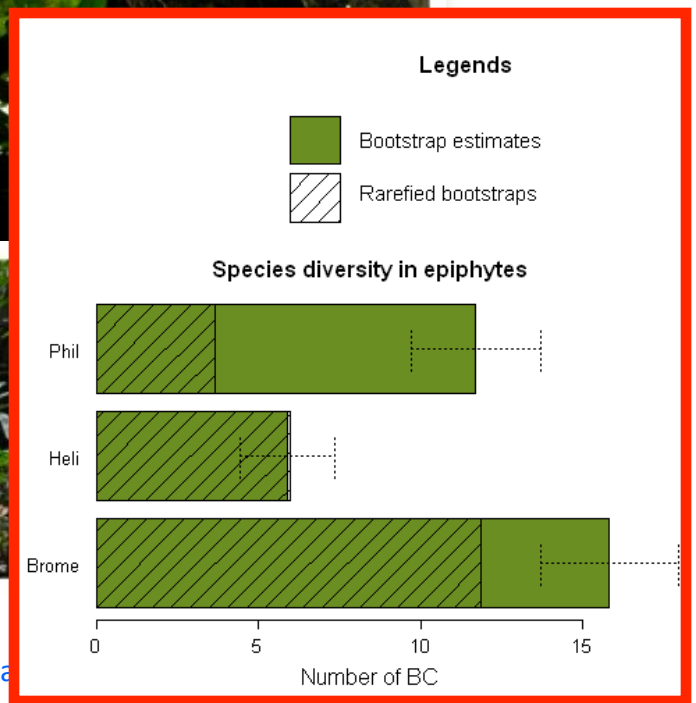
Locality with the highest species richness worldwide !

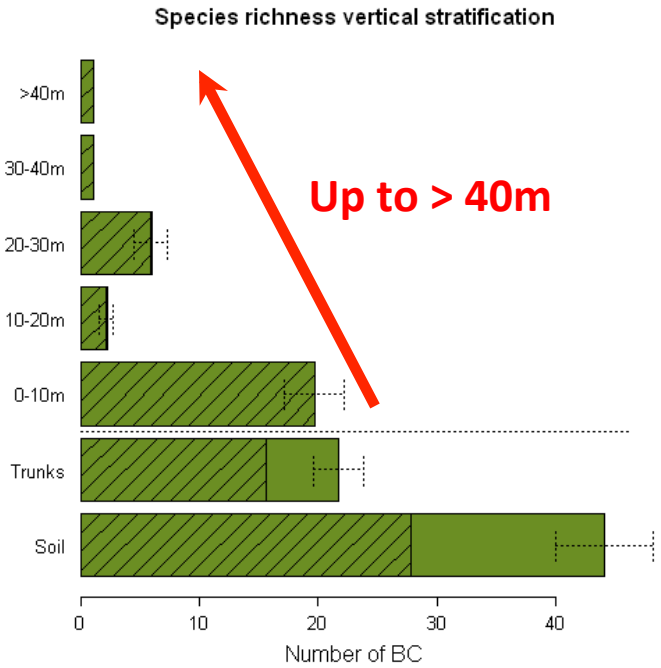
Decaëns et al (in prep), poster at ICSZ XVI

Applications: Ecology









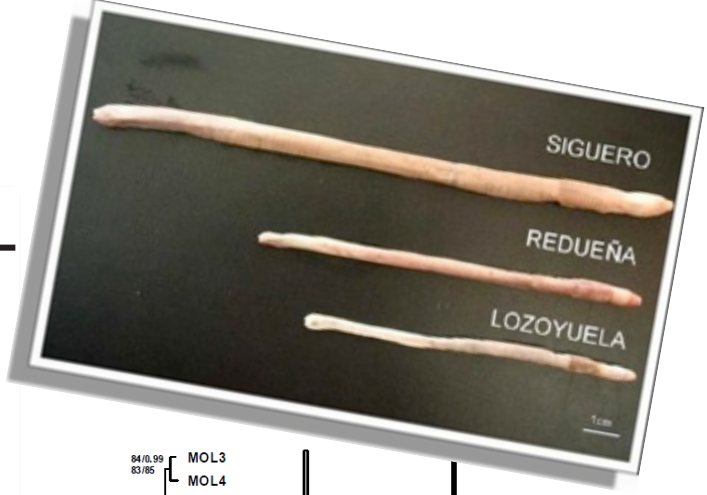
Deca

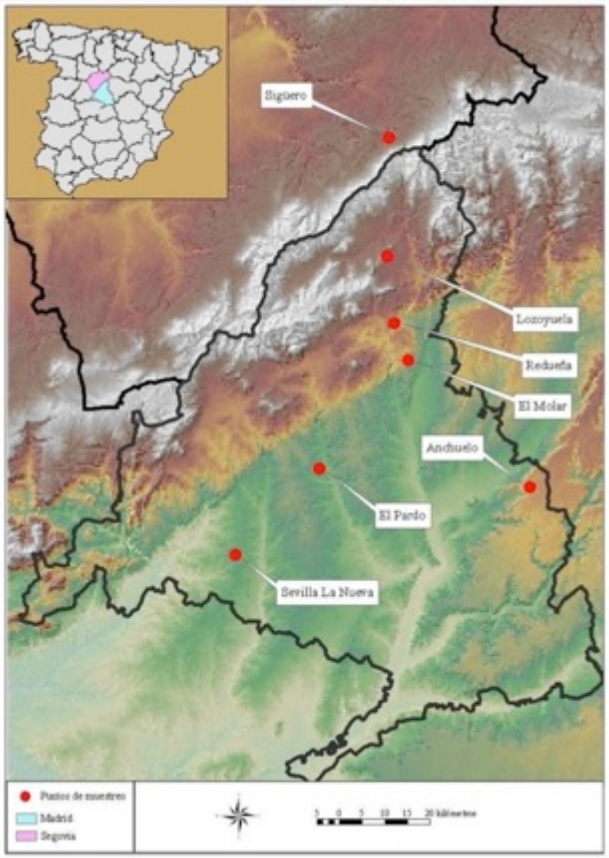
Applications: Phylogeography

Zoologica Scripta, **38**, 5, September 2009, pp 537–552

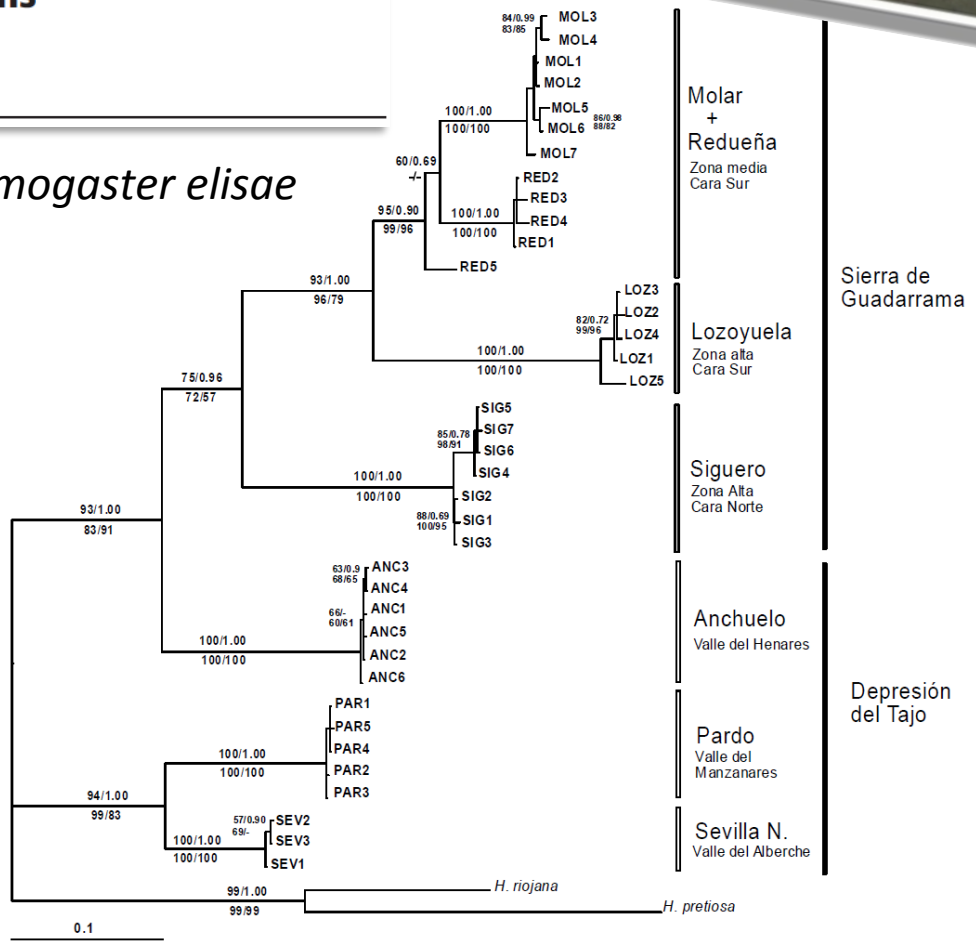
High genetic divergence of hormogastrid earthworms (Annelida, Oligochaeta) in the central Iberian Peninsula: evolutionary and demographic implications

MARTA NOVO, ANA ALMODÓVAR & DARÍO J. DÍAZ-COSÍN





Hormogaster elisae



Novo et al (2009)

Applications: Phylo

Inferring Ant Reproductive Dispersal Through Phylogeographic Comparisons of COI

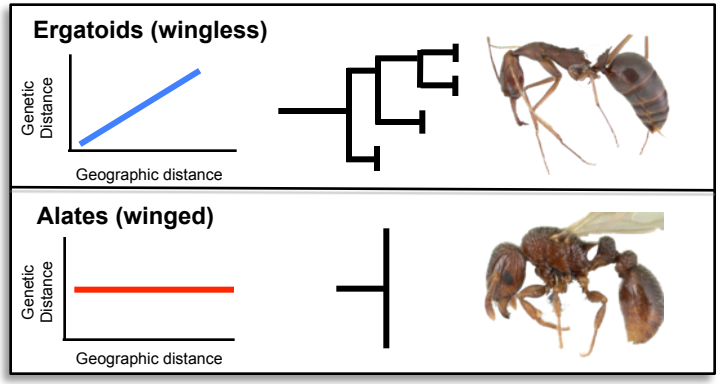
Fiona Tsoi [1], Claudia Bertrand[1], Brian L. Fisher [2] and M. Alex Smith [1]
[1] Department of Integrative Biology, University of Guelph, [2] California Academy of Sciences, San Francisco

Objectives:

To predict female reproductive dispersal patterns using phylogeographic comparisons of DNA barcodes

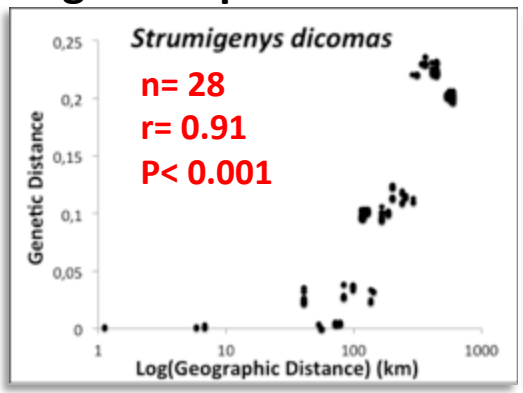
Study region: Madagascar

Predictions:

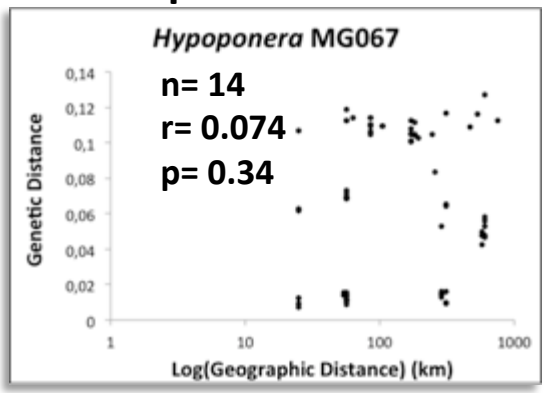


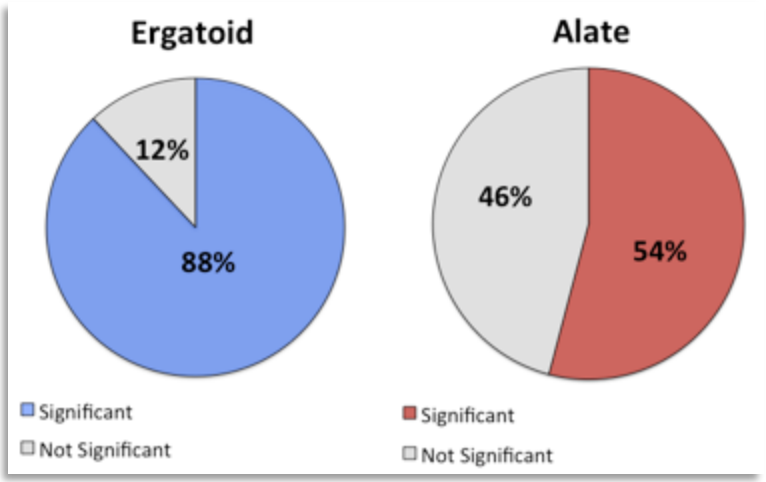
Results:

Ergatoid species



Alate species





Tsoi et al (2010) poster at iBOL Conference

Applications: Monitoring of invasive species

Frontiers in Zoology

BioMed Central

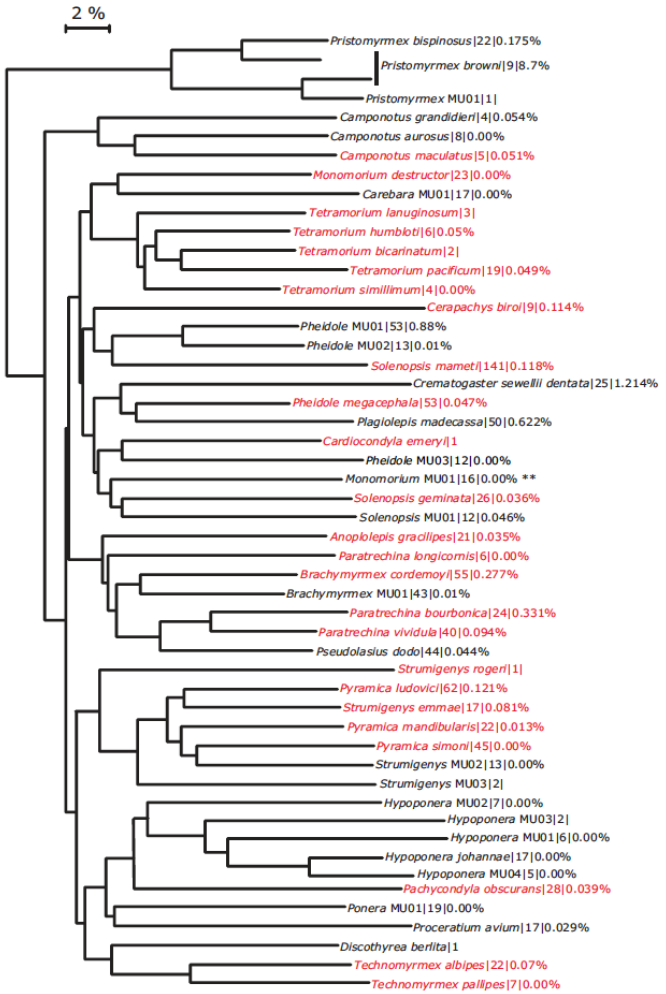
Research

Open Access

Invasions, DNA barcodes, and rapid biodiversity assessment using ants of Mauritius

M Alex Smith*[1] and Brian L Fisher*[2]

- **10 sites and > 1000 sequenced specimens**
- **52 species / 25 introduced**
  - ➔ Among which the “biological Attila”s *Pheidole megacephala, Anoplolepis gracilipes, Paratrechina longicornis…*



Smith & Fisher (2009)

Applications: Monitoring of invasive species

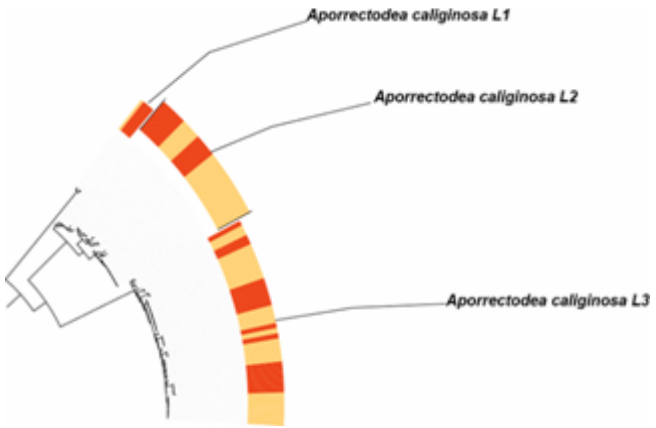
Invasion process (origin, expansion)

Biol Invasions (2013) 15:899–910
DOI 10.1007/s10530-012-0338-2

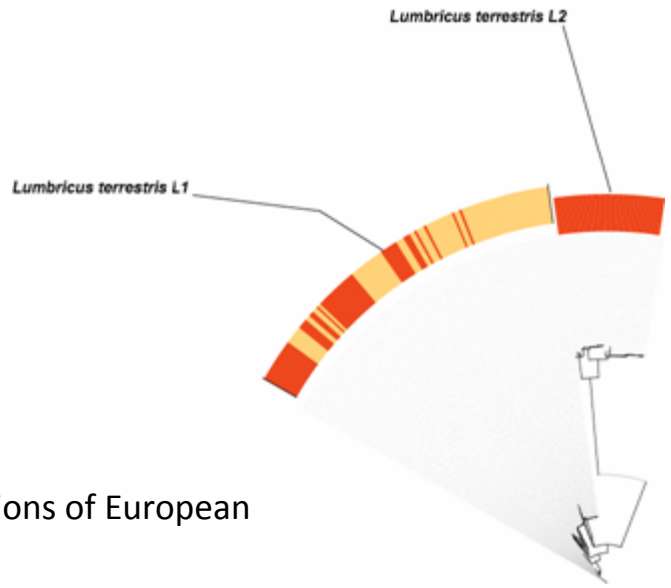
Biological invasions in soil: DNA barcoding as a monitoring tool in a multiple taxa survey targeting European earthworms and springtails in North America

David Porco · Thibaud Decaëns · Louis Deharveng · Samuel W. James · Dariusz Skarżyński · Christer Erséus · Kevin R. Butt · Benoit Richard · Paul D. N. Hebert

Aporrectodea caliginosa



Lumbricus terrestris / herculeus



- Comparing COI variation in autochthonous versus invasive populations of European Lumbricidae and Collembola
- Genetic structure of invasive population reveals invasion history
- Most species have been introduced in North America repeatedly and massively

Porco et al (2013)

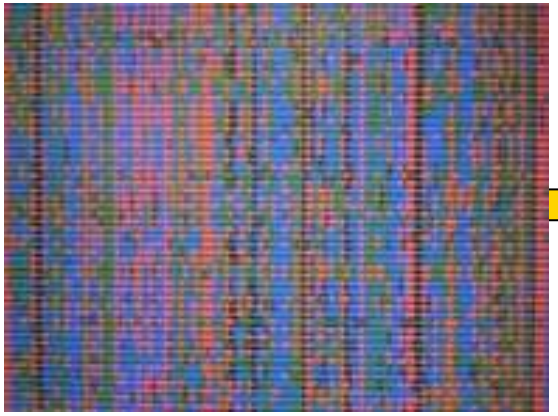
Perspectives : Environmental barcoding

Environmental Sample

Target Barcode Library

454 FLX Pyrosequencer

Pyrosequencing data



Species identities and counts

Decaëns, Sebag, Porco et al (2011-2013) GENOSOIL-Fr project, poster at ICSZ XVI

Perspectives : Environmental barcoding

MOLECULAR ECOLOGY

Molecular Ecology (2012) **21**, 2017–2030 doi: 10.1111/j.1365-294X.2011.05407.x

Tracking earthworm communities from soil DNA

FRIEDERIKE BIENERT,* SÉBASTIEN DE DANIELI,† CHRISTIAN MIQUEL,* ERIC COISSAC,* CAROLE POILLOT,* JEAN-JACQUES BRUN† and PIERRE TABERLET*

A high-throughput genomics approach for comprehensive biological assessment of environmental change

Project leader: M. Hajibabaei

The GENOSOIL-Fr project : insights from metagenomics into the study of total biodiversity of soils

T. Decaëns[1], D. Porco[1], D. Sebag[1], J. Oswald[2], R. Rougerie[1], A. Husté1, L. Dupont[3], S. Doledec[4], J.-P. Rossi[5], M. Hajibabaei[6]

(1) University of Rouen, France; (2) University of Rennes, France; (3) University Paris 6, France; (4) University of Lyon, France; (5) University of Montpellier, France; (6) University of Guelph, Canada; cor. auth.: thibaud.decaens@univ-rouen.fr

GENOSOIL

Bienert et al (2012), Hajibabaei et al (unpublished),
Decaëns, Sebag, Porco et al (2011-2013) GENOSOIL-Fr project, poster at ICSZ XVI

Perspectives: Education

Hands-on DNA barcoding: Engaging students of the ECO–TROP field class for rapid invertebrate diversity assessment in Lopé National Park, Gabon

D. Porco[1], N. Lepengue[2], D. Sebag[3], R. Rougerie[1], M. Andeime Eyene[4], L. Bremond[5], S. Dufour[6], P. Le Gall[7], J. Lebamba[2], G. Moussavou[8,9], Makaya Mvoubou[2], R. Oslisly[7], J. Oszwald[6], C. Paupy[7,9], O. Scholtz[10], M. Tindo[11], M. Veuille[12], T. Decaëns[1]

- In February 2011 and March 2012-2013
- In the National Park of La Lopé (Gabon)
- Invertebrate diversity assessment using DNA barcoding

Decaëns et al (in prep), poster at ICSZ XVI

Perspectives: Education

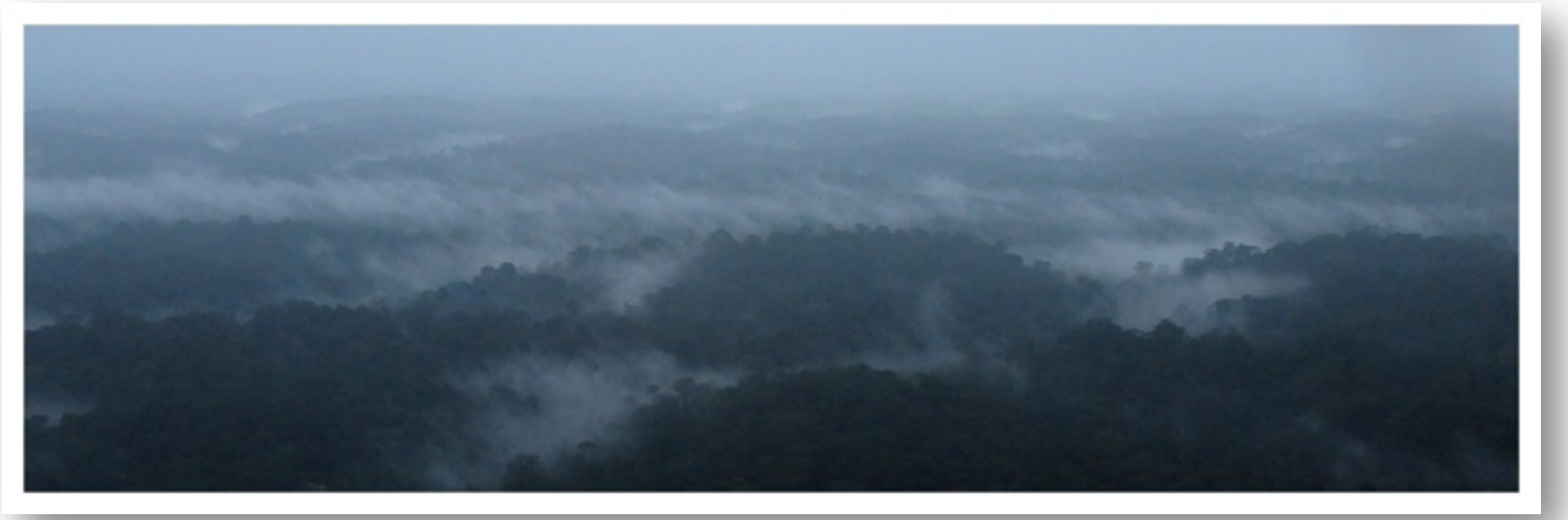
DNA barcoding: para quê e para quem?

Improve our taxonomic knowledge of soil fauna:

- ➔ Taxonomy and systematics
- ➔ Community ecology
- ➔ Functional ecology
- ➔ Conservation biology

Perspectives: Education

DNA barcoding: para quê e para quem?



In the context of Brazil:

- Describe soil invertebrate biodiversity in the main natural ecosystem
- Provide new insights for the monitoring of this biodiversity in deforested and agricultural landscapes
- Participate to high level education in biodiversity research and environmental sciences

Obrigado pela atenção