

Nov 4-Nov 8      DNA barcoding  
Molecular systematics in plants (Les)  
Lab 8: likelihood (Garli and/or RAxML) and Bayesian analyses  
(Wagner in Austin)

Nov 11-15      Molecular systematics in animals (Simon) (Wagner in Austin)  
Lab 9: Find and analyze your own data set COI problem (Wagner  
in Austin)  
Nomenclature

Other matters:

\* Term paper: 11 (Monday) Nov → 15 (Friday), 16, 17?

Final

\* Wednesday, 11 December (8—11 AM)

# Identifying Life



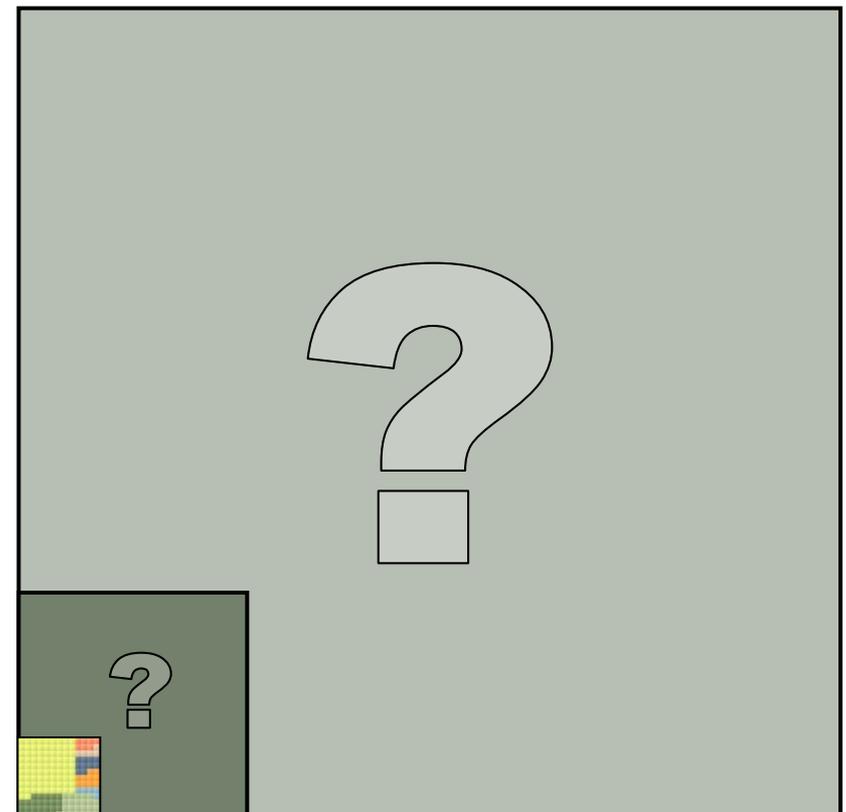
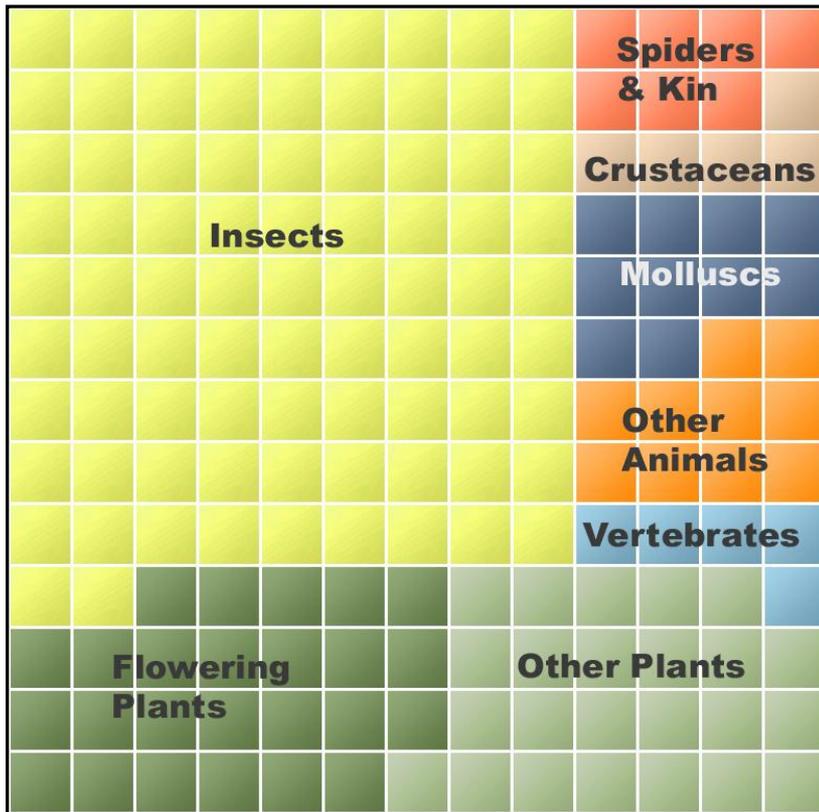
**OUR PLANET:**  
Home to approximately  
10 - 100 million species.



**OUR MIND:**  
Able to recall & recognize  
perhaps 1000 species.

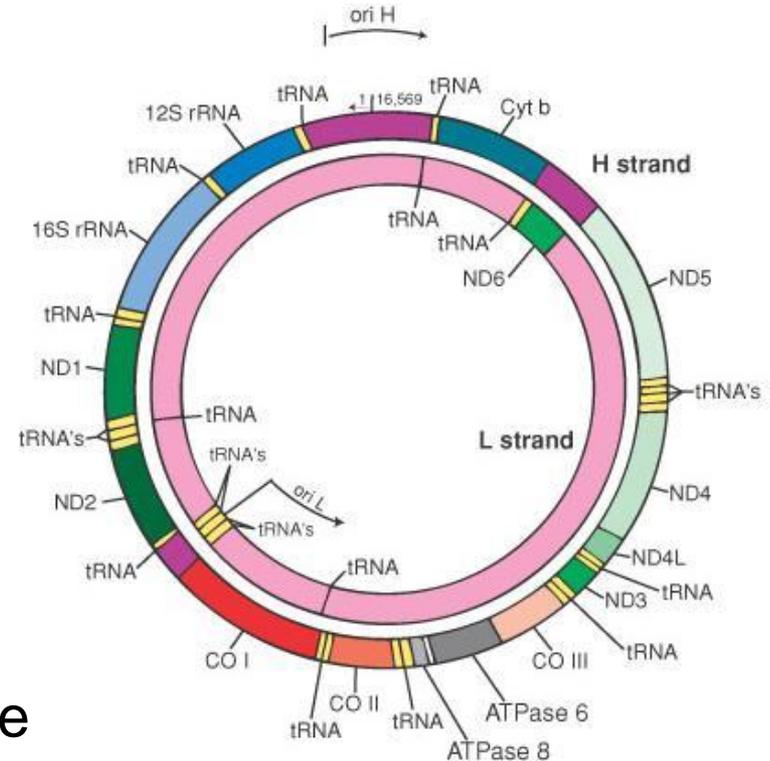
**Known biodiversity: 1.7 million species of plants and animals**

**Estimated biodiversity: 10 million to 100 million species**

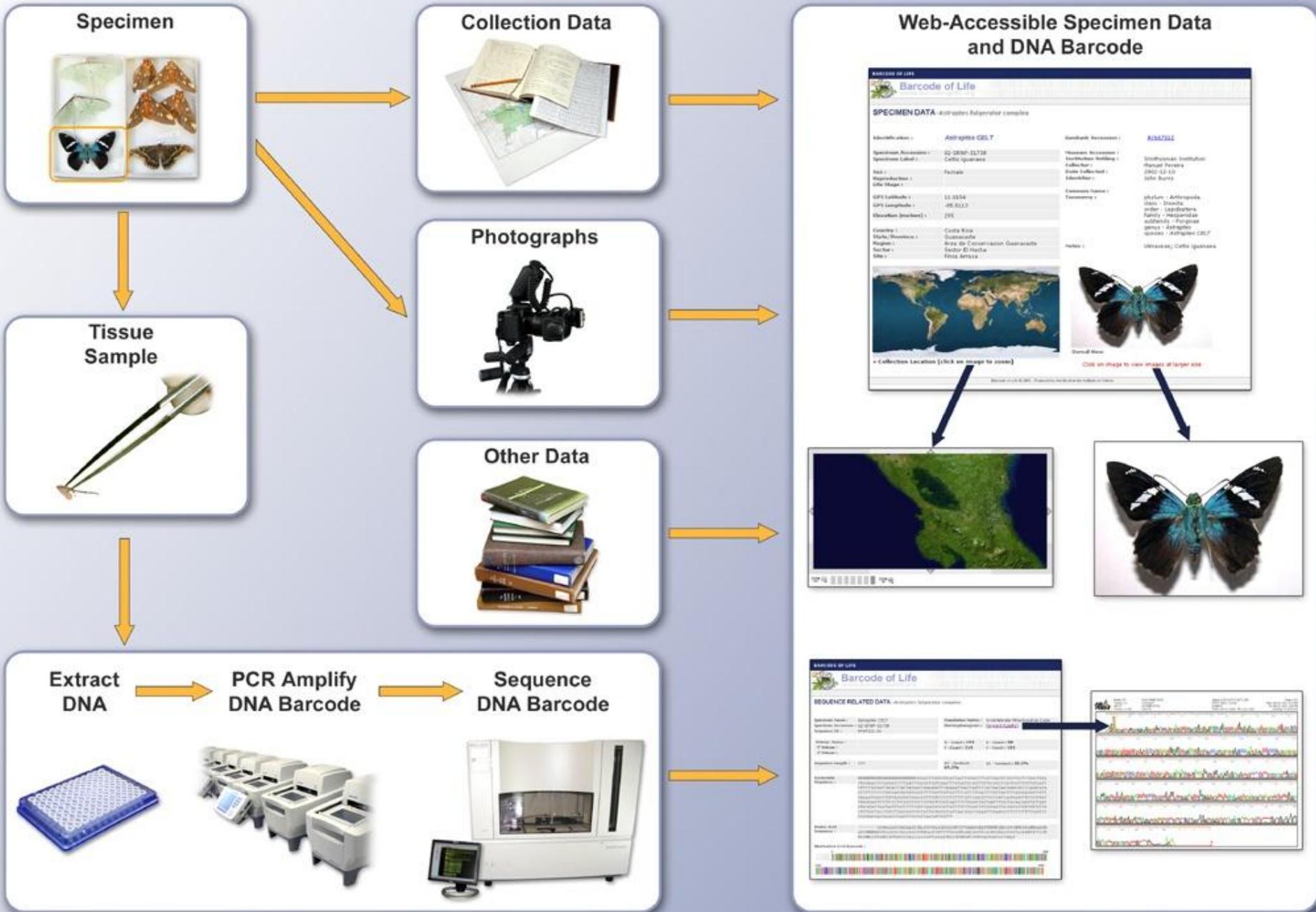


# DNA barcoding is based on the use of a short, standard region that enables cost-effective species identification

- \* Cytochrome c oxidase I (COI) has become standard for animals
- \* “Folmer region” at the 5' end of gene
- \* approx. 658 base pairs
- \* closely related species often differ by several percent
- \* smaller  $N_e$  means gene coalesces more quickly
- \* marker for both within species structure and among species structure



# DNA Barcoding: The Analytical Chain



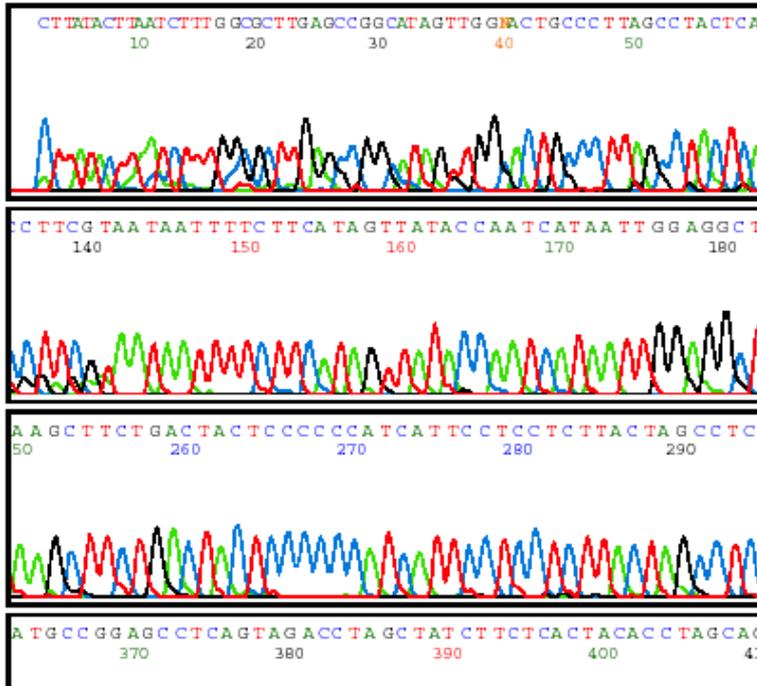
# Sequence Data



Model 377  
Version 3.3  
LR-377  
Version 3.3.1b2

13-TZBNA 238-03  
BF1  
TZBNA 238-03  
Lane 13

Signal G:117 A:154 T:91 C:178  
DT377\_BDv3\_v2.mob  
214BDv3  
Points 1380 to 15200 Pk 1 Loc:



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### SEQUENCE RELATED DATA - Birds of North America

Specimen Name :	Accipiter striatus	Translation Matrix :	Vertebrate Mitochondrial Code
Specimen Accession :	1B-3242	Electropherogram :	Forward (BF1)
Sequence ID :	TZBNA238-03		

Primer Name :		A - Count : 181	G - Count : 105
5' Primer :		T - Count : 192	C - Count : 215
3' Primer :			

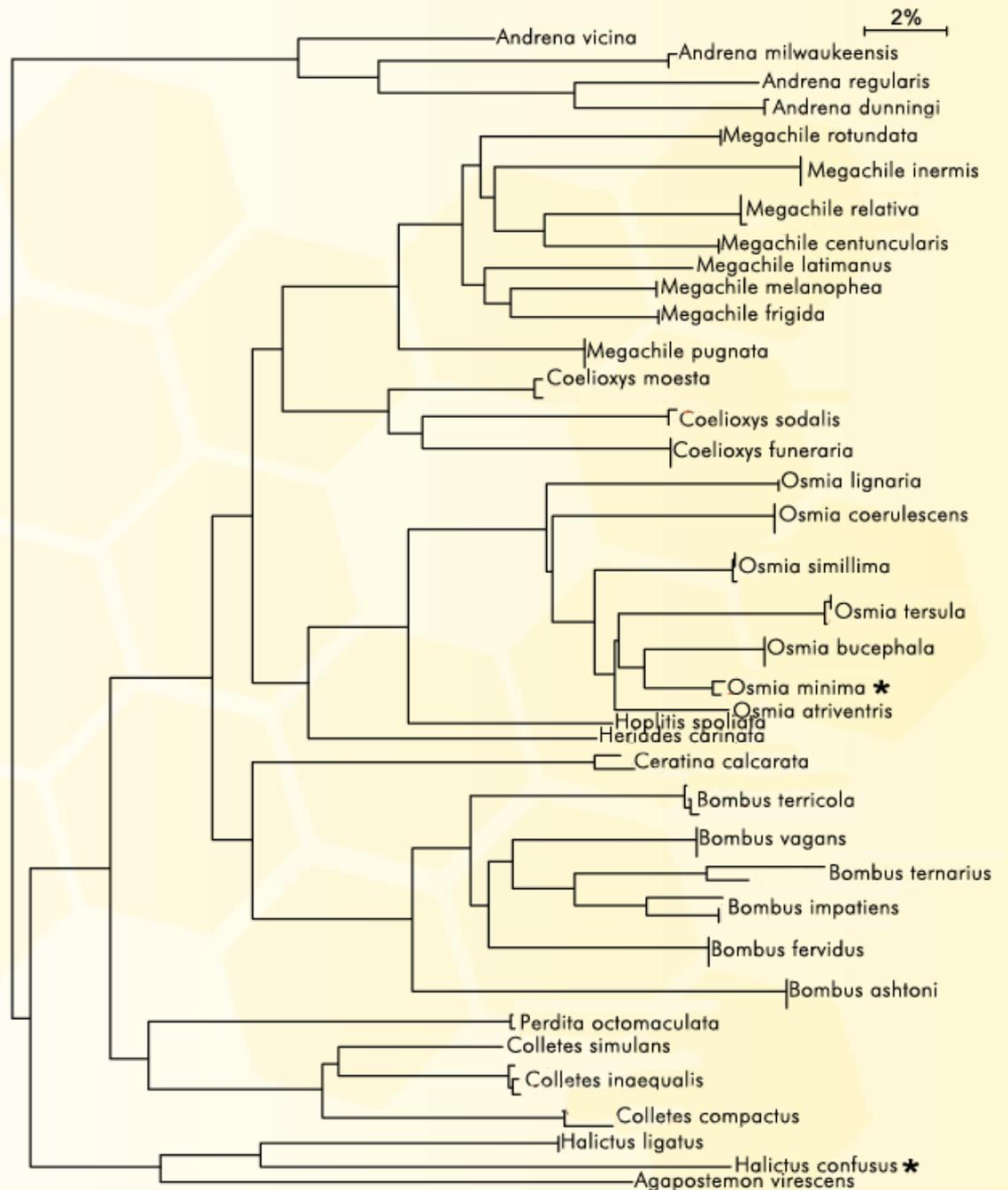
Sequence Length :	693	AT - Content :	53.8%	GC - Content :	46.2%
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Nucleotide Sequence :  
 NNNTTATACTTAATCTTTGGCCCTTGAGCCGGCATACTTGGCACTGCCCTTAGCCTACTCATTCCGCCAGAACTTGGC  
 CAACCAGCCACACTCCTAGCCGATGACCAAATCTATAAGTTATCGTCACCCGCACATGCCCTCGTAATAATTTCTTC  
 ATAGTTATACCAATCATAATTGGAGGCTTCGGAACTGACTCGTCCCGCTCATAATTGGCCGCTCGATATAGCTTTC  
 CCACGTATAAATAACATAAGCTTCTGACTACTCCCCCATCATTCCTCCTCTTACTAGCCTCTTCAACAGTAGAAGCA  
 GGAGCAGCAACCCGATGAACCTGCTACCCCTCCATTAGCTGGTAATATAGCCCATCCCGAGCCCTCAGTAGACCTAGCT  
 ATCTTCTCACTACACCTAGCAGGAATTTATCCATCCTAGGGCGCAATTAACCTTTATCACAACCCGCTATTAACATAAAA  
 CCCCCTGCCCTCTCCAATACCAACACCCTATTTCGTATGATCCGCTCCTCATCTACTGCTCTCCTACTACTCTCA  
 CTACCAGTCTTAGCTGCTGCCATTACTATACTACTAACAGATCGAAACCTCAATACACATTCTTCGATCCTGCTGCT  
 GGAGCCGCCCTATCCTATATCAACACCTCTTCTGATTCTTCGGACACCCAGAGTCTACATTCTCATTCTA

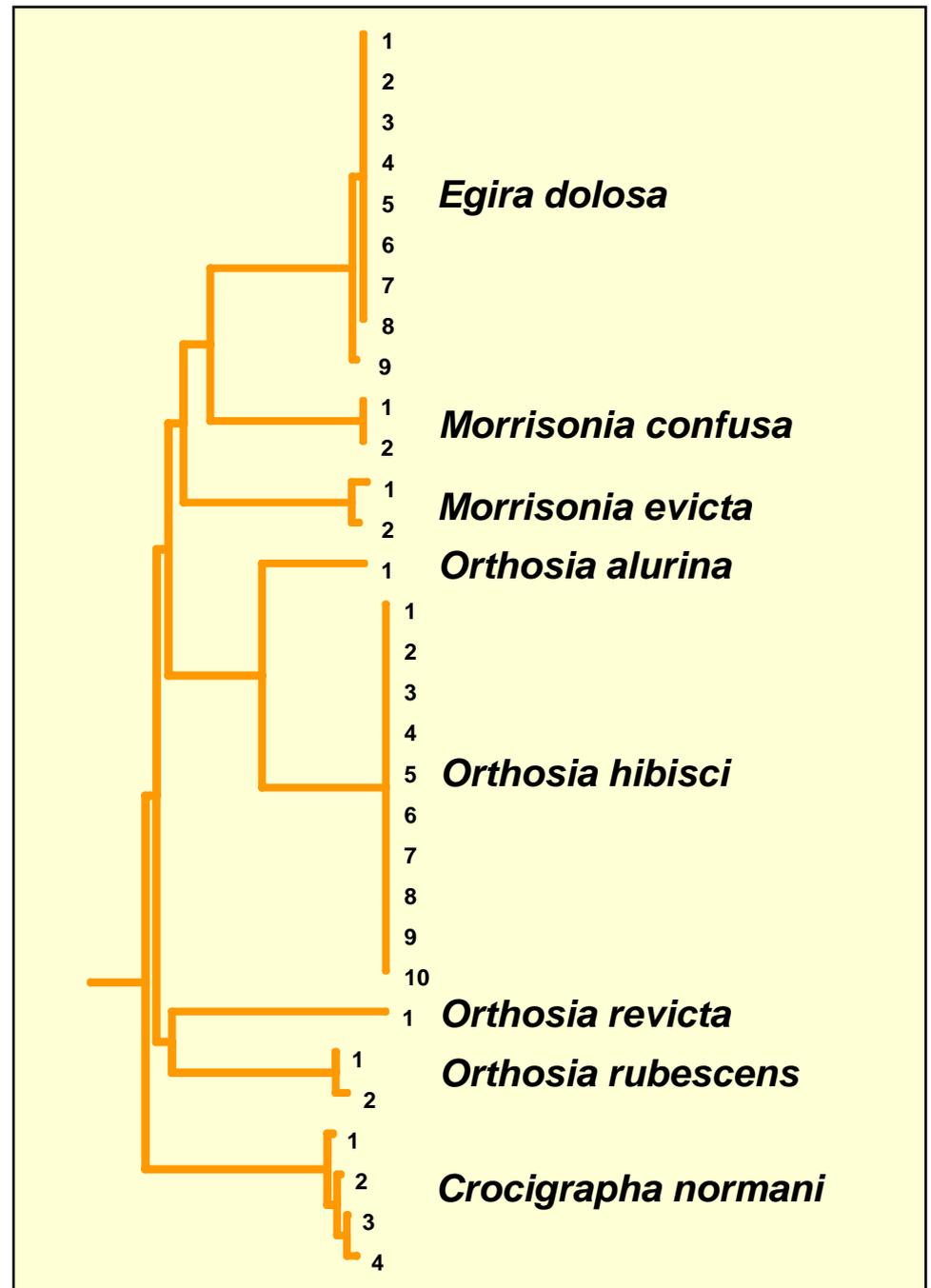
Amino Acid Sequence :  
 -LYLIFCAWAGMVCTALSLIRAE LQCPCTLLGDDQIYNVIVTAHAFVMIHFVNVMPIMICGFCGNLWVPLMICAPDMAF  
 PRMNNMSPWLLPPSFLLLASSTVRACACTGWVYVPLACNMAHAGASVDLAI FSLHLACISSILGAINFITTA INMK  
 PPALSQYQITPLFVWSVLITAVLLLLSLPVLAAGITMLLDRNLNTFFDPAGCCGDPILYQHLWFFGHPEVYVILL

Illustrative DNA barcode :

# Bees of Nova Scotia



# A Dearth of Deep Intraspecific Diversity

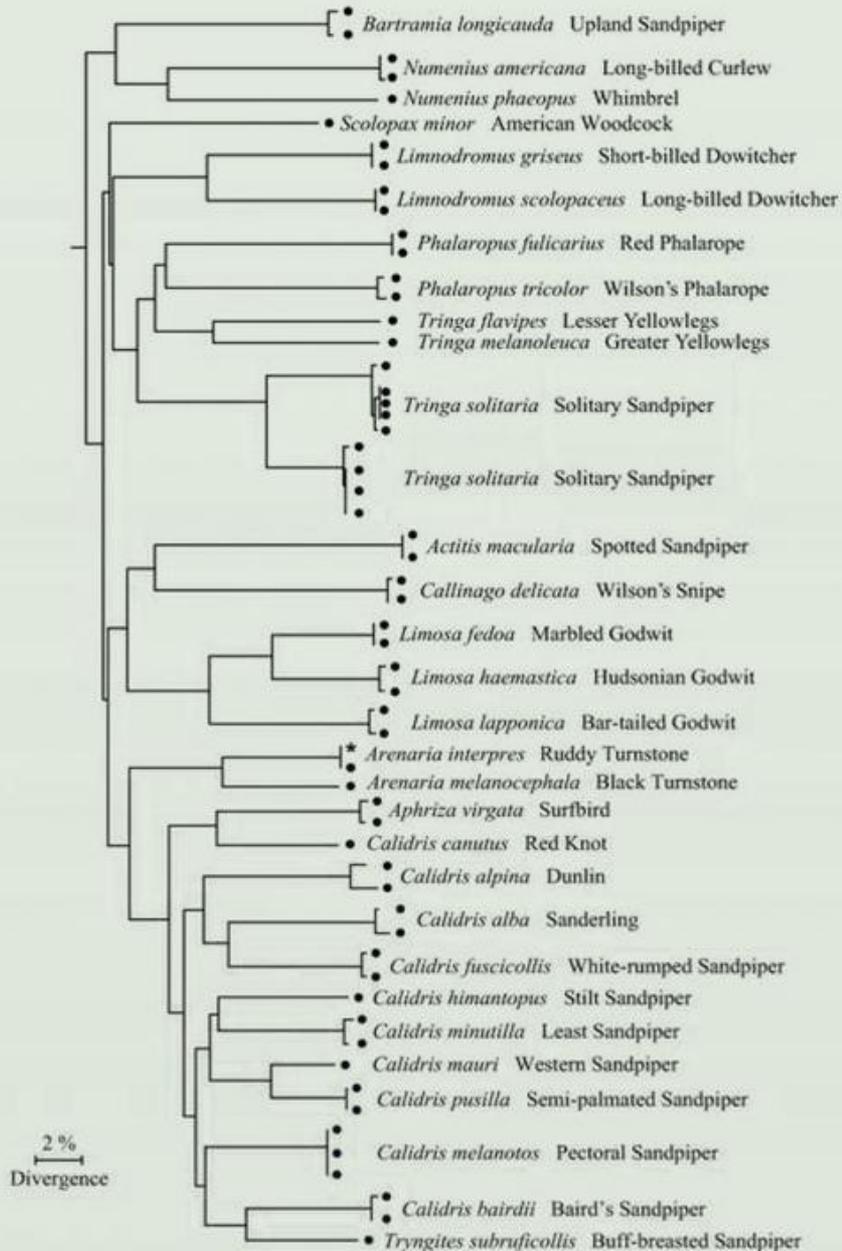




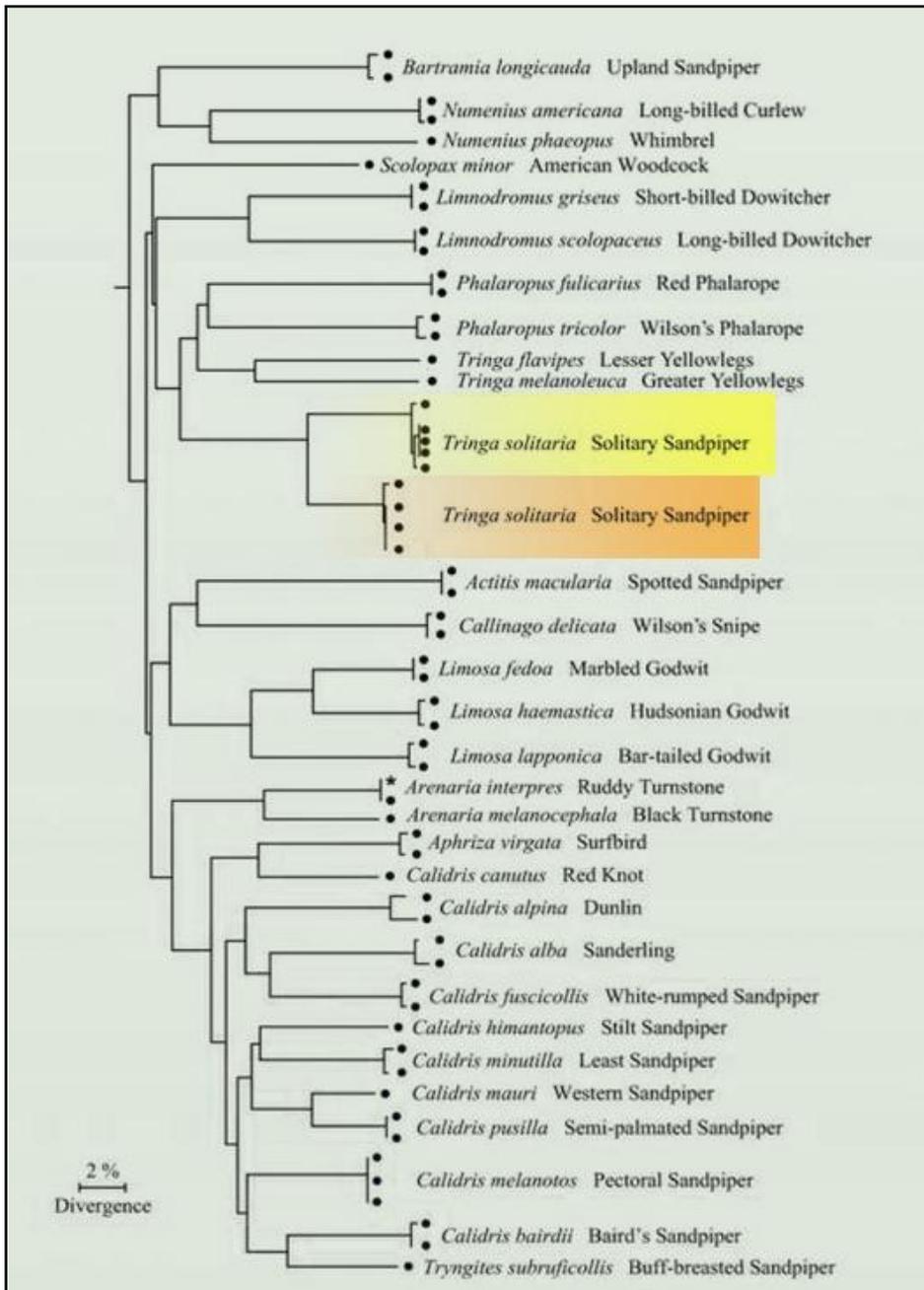
# Fish Identification through DNA barcodes



# Bird Identification through DNA barcodes



# Bird Identification through DNA barcodes



# Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*

Paul D. N. Hebert<sup>\*†</sup>, Erin H. Penton<sup>\*</sup>, John M. Burns<sup>‡</sup>, Daniel H. Janzen<sup>§</sup>, and Winnie Hallwachs<sup>§</sup>

<sup>\*</sup>Department of Zoology, University of Guelph, Guelph, ON, Canada N1G 2W1; <sup>‡</sup>Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560-0127; and <sup>§</sup>Department of Biology, University of Pennsylvania, Philadelphia, PA 19104

Contributed by Daniel H. Janzen, August 20, 2004

*Astraptes fulgerator*, first described in 1775, is a common and widely distributed neotropical skipper butterfly (Lepidoptera: Hesperiidae). We combine 25 years of natural history observations in northwestern Costa Rica with morphological study and DNA barcoding of museum specimens to show that *A. fulgerator* is a complex of at least 10 species in this region. Largely sympatric, these taxa have mostly different caterpillar food plants, mostly distinctive caterpillars, and somewhat different ecosystem preferences but only subtly differing adults with no genitalic divergence. Our results add to the evidence that cryptic species are prevalent in tropical regions, a critical issue in efforts to document global species richness. They also illustrate the value of DNA barcoding, especially when coupled with traditional taxonomic tools, in disclosing hidden diversity.

mitochondrial gene cytochrome *c* oxidase I (COI), whereas divergences among conspecific individuals average only 0.25% (11). Similar values were obtained in birds, with intraspecific divergences at COI averaging 0.27%, whereas congener divergences averaged 7.93% (14).

In this study, the addition of DNA barcodes to data on food plants, ecological distributions, caterpillar color patterns, and adult facies indicates that *A. fulgerator* consists of 10 largely sympatric species in the ACG. This result raises the prospect that, over its huge neotropical range, *A. fulgerator* may comprise many more hidden species. Imagine the biodiversity implications of this result for other wide-ranging, common, and “somewhat variable” species of neotropical animals and plants.



**YESENN**



**SENNOV**



**TRIGO**



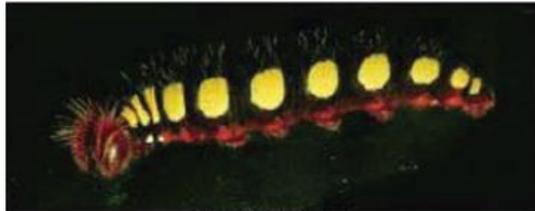
**CELT**



**LONCHO**



**INGCUP**



**LOHAMP**



**HIHAMP**



**BYTTNER**



**FABOV**



**YESENN**



**SENNOV**

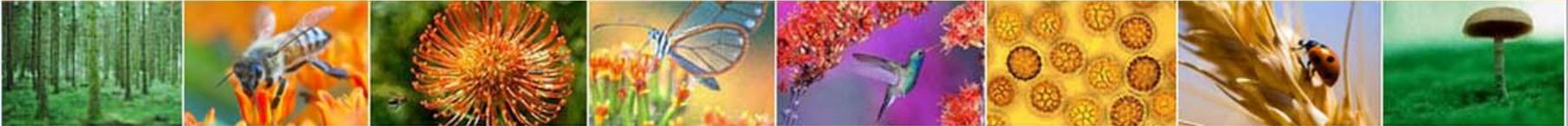
Fig. 2. Last-instar caterpillars of 10 species in the *A. fulgerator* complex from the ACG. Interim names reflect the primary larval food plant and, in some cases, a color character of the adult.

# BARCODE OF LIFE DATA SYSTEMS v 2.5

Advancing species identification and discovery through the analysis of short, standardized gene regions

SEARCH

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The Barcode of Life Data Systems (BOLD) is an online workbench that aids collection, management, analysis, and use of DNA barcodes. It consists of 3 components (MAS, IDS, and ECS) that each address the needs of various groups in the barcoding community.

## MANAGEMENT & ANALYSIS

**BOLD-MAS** provides a repository for barcode records coupled with analytical tools. It serves as an online workbench for the DNA barcode community.

Username

Password

[Request a new user account](#)

[Forgot your username or password?](#)

## IDENTIFICATION ENGINE

**BOLD-IDS** provides a species identification tool that accepts DNA sequences from the



### BARCODE COUNTS

<b>Formally Described Species With Barcodes</b>	<b>117,429</b>
<b>Total Barcode Records</b>	<b>1,412,505</b>
<b>Source</b>	<b>Breakdown</b>
GenBank	111,828
Canadian Centre	1,186,126
Others	114,551

## BOLDSYSTEMS BOLD 2.5 Release

Version 2.5, unveiled on Nov 11th 2009 at the Third International Barcoding of Life conference in Mexico City, provides new core functionality including support for multiple sequence markers per specimen and more complex workflows. Features include identification services for ITS, matK, and rbcL markers, comparative analytics, web services and a variety of convenience upgrades. A few are highlighted here :

**Accumulation curves** Explore diversity of species and sequences by site or higher level taxonomy.

- \* 1.8 million barcode records
- \* 175,000 species
- \* 10,000 barcodes added per week

# BOLD – Specimen Data

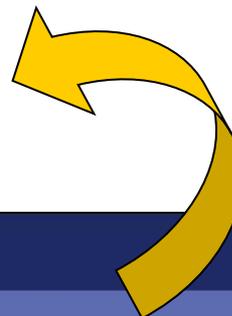
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Name : *Adhemarius ypsilon* Image :02-SRNP-18079-DHJ35550.jpg - Dorsal View



Name : *Adhemarius ypsilon* Image :02-SRNP-18079-DHJ35551.jpg - Ventral View



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## SPECIMEN DATA -Sphingidae of Costa Rica

[Edit](#)

Identification : *Adhemarius ypsilon*

Specimen Accession : 02-SRNP-9473  
Specimen Label : *Adhemarius ypsilon*

Sex : Male  
Reproduction : Sexual  
Life Stage : Adult

GPS Latitude : 10.9255  
GPS Longitude : -85.4716

Elevation (meters) :

Country : Costa Rica  
State/Province : Guanacaste  
Region :  
Sector :  
Site :

Museum Accession : 02-SRNP-9473  
Institution Holding : Smithsonian Institution  
Collector : Unknown  
Date Collected : 2002-07-21  
Identifier : Dan Janzen

Common Name :  
Taxonomy :  
phylum - Arthropoda  
class - Insecta  
order - Lepidoptera  
family - Sphingidae  
genus - *Adhemarius*  
species - *Adhemarius ypsilon*

Notes : 43



▲ Collection Location [click on image to zoom]



Dorsal View

[Click on image to view images at larger size](#)

Arthropoda; Insecta; Lepidoptera; Nymphalidae; Danainae; Danaus;

Print

Search Taxonomy

Taxon Description (Wikipedia)

The **Monarch butterfly** (*Danaus plexippus*) is a milkweed butterfly (subfamily Danainae) in the family Nymphalidae. It is perhaps the best known of all North American butterflies. Since the 19th century, it has been found in New Zealand, and in Australia since 1871, where it is called the **wanderer**. It is resident in the Canary Islands, the Azores, and Madeira, and is found as an occasional migrant in Western Europe and a rare migrant in the United Kingdom. Its wings feature an easily recognizable orange and black pattern, with a wingspan of 8.9–10.2 cm (3½–4 in). (The viceroy butterfly is similar in color and pattern, but is markedly smaller, and has an extra black stripe across the hind wing.) Female monarchs have darker veins on their wings, and the males have a spot called the androconium in the center of each hind wing. Males are also slightly larger than female monarchs. [Full article at Wikipedia](#)

BOLD Stats

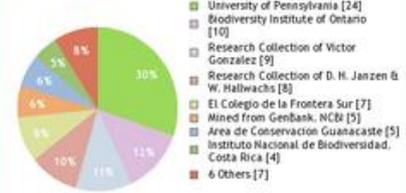
Specimen Records:	93	Public Records:	46
Specimens With Sequences:	101	Public Species:	1
Specimens With Barcodes:	88	Public BINs:	1
Species:	1		
Species With Barcodes:	1		

Species List - Progress

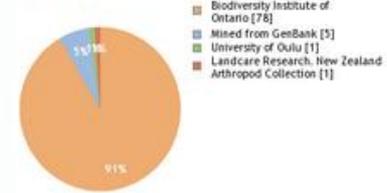
Access Published & Released Data

Contributors (Specimens & Sequencing)

Specimen Depositories:



Sequencing Labs:

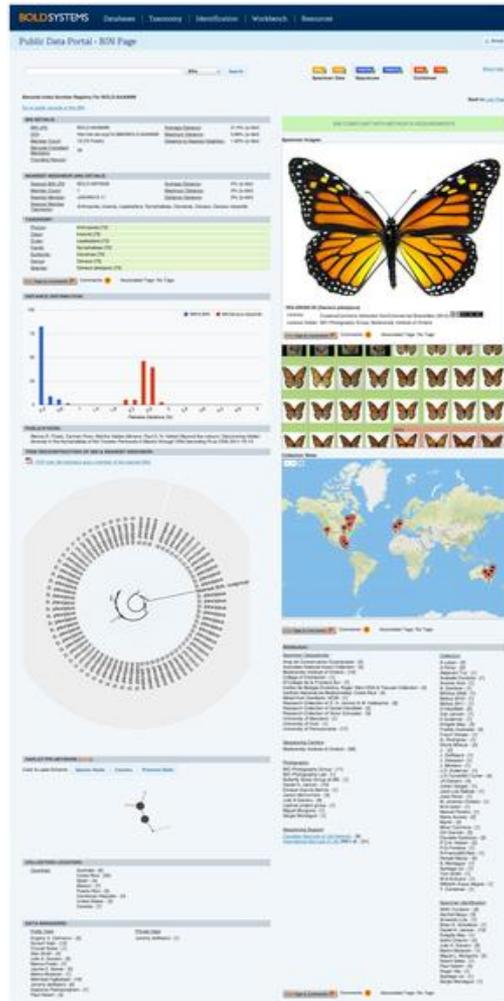


Imagery

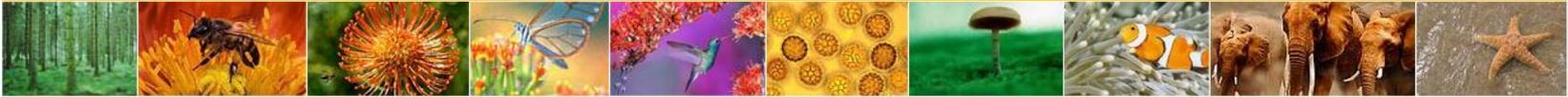
Images representing specimens of *Danaus plexippus*



Figure 11. BIN page for *Danaus plexippus* (Linnaeus, 1758), the monarch butterfly.



Ratnasingham S, Hebert PDN (2013) A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System. PLoS ONE 8(7): e66213. doi:10.1371/journal.pone.0066213  
<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0066213>

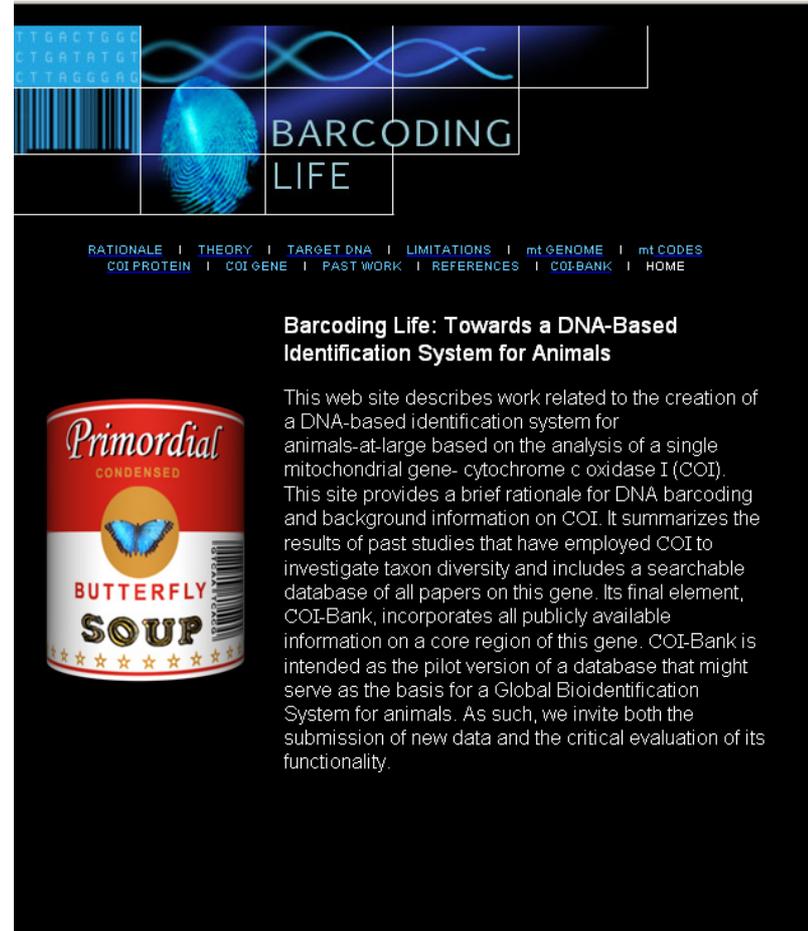


## Goals

- \* Build, over the next 20 years, a barcode library for all eukaryotic life
- \* Add alignment browser for visualizing nucleotide and amino acid alignments
- \* Add capacity for multiple sequence markers
- \* Add nuclear markers
- \* Include plant markers: ITS, matK, and rbcL markers
- \* Develop algorithms for discovering new leaves on the “Tree of Life”
- \* Consider character-based identifications
- \* Develop probabilities and certainty values
- \* Develop algorithms for dealing with hybridization and lineage sorting (coalescence issues)

# DNA Barcoding

- \* Is it just a new tool for taxonomy? Might it also be a threat to traditional taxonomy?
- \* Why is its use generating controversy among practitioners, users, funders, etc.
- \* When does it work?
- \* When does it fail?
- \* Why and how can claims about its utility and accuracy be so different?



TTGACTGCC  
CTGATATGT  
CTTAGGGR

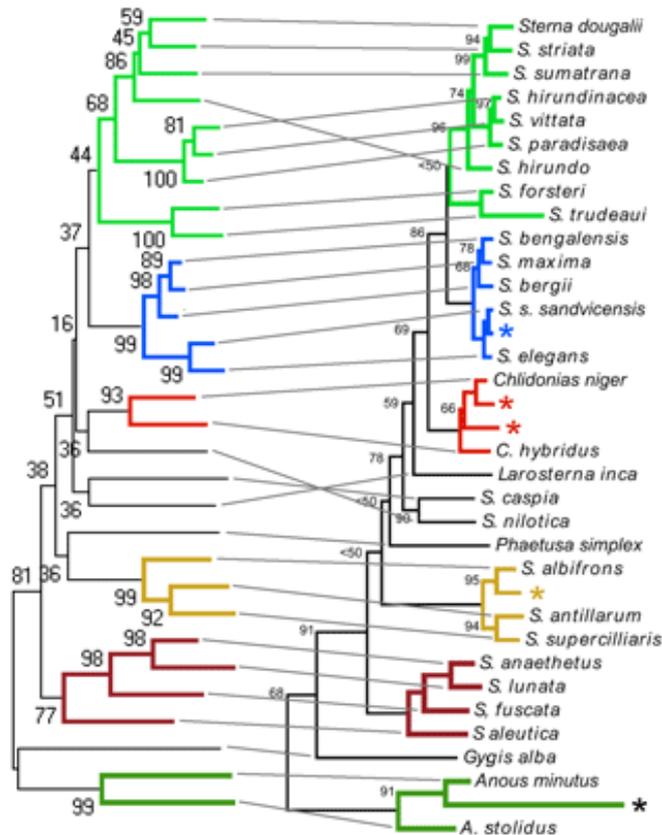
BARCODING  
LIFE

[RATIONALE](#) | [THEORY](#) | [TARGET DNA](#) | [LIMITATIONS](#) | [mtGENOME](#) | [mtCODES](#)  
[COLPROTEIN](#) | [COI GENE](#) | [PAST WORK](#) | [REFERENCES](#) | [COI-BANK](#) | [HOME](#)

### Barcoding Life: Towards a DNA-Based Identification System for Animals

This web site describes work related to the creation of a DNA-based identification system for animals-at-large based on the analysis of a single mitochondrial gene- cytochrome c oxidase I (COI). This site provides a brief rationale for DNA barcoding and background information on COI. It summarizes the results of past studies that have employed COI to investigate taxon diversity and includes a searchable database of all papers on this gene. Its final element, COI-Bank, incorporates all publicly available information on a core region of this gene. COI-Bank is intended as the pilot version of a database that might serve as the basis for a Global Bioidentification System for animals. As such, we invite both the submission of new data and the critical evaluation of its functionality.





gray lines show relative positions of each species on the 2 trees (asterisks mark species missing from NJ tree); colored and black terminal branches of tree indicate polytypic and monotypic genera respectively, in revised phylogeny (AOU Check-list (2006))

NJ tree 648 bp COI (from BOLD) vs Bayesian tree 2800 bp mtDNA (Bridge 2005)

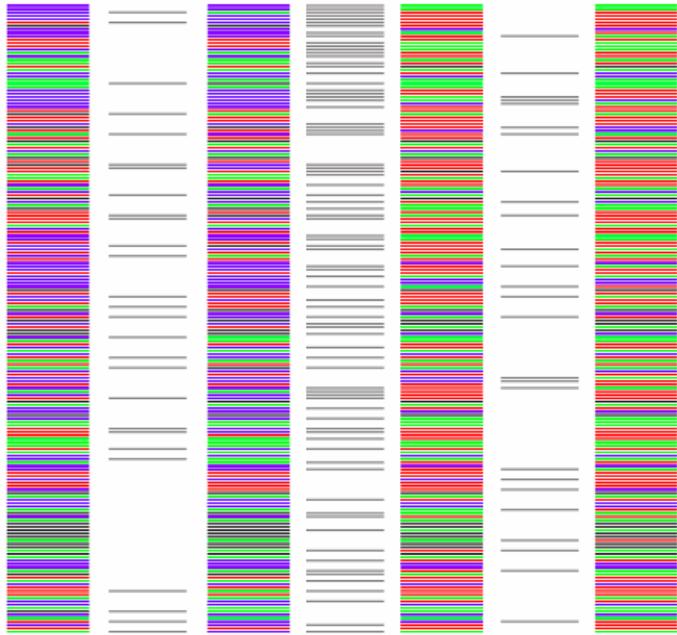
COI barcodes may give clues to relationships among species

Here I look at one example from birds, comparing differences among COI barcodes to a recently revised phylogeny of terns (subfamily Sternini). According to [American Ornithologists' Union Check-list of North American Birds Supplement 47 \(2006\)](#), "the data show that the genus *Sterna* as currently defined...is paraphyletic."... "[W]e follow the recommendation of [Bridge et al 2005](#) to resurrect four generic names currently placed in synonymy with *Sterna*." The figure at left, taken from the 2005 paper by Bridge, Jones, and Baker, shows phylogenetic relationships based on 2800 bp of mtDNA from 33 species of terns (Bayesian tree with ML distances and ML bootstrap support indices), and is juxtaposed to an NJ tree of COI barcodes from 29 of the same 33 taxa. The figure is colored according to the revised generic assignments (AOU 2006).

The topology of the COI NJ tree is similar to the larger data set tree, including that all currently recognized genera are reciprocally monophyletic, and most show similarly high bootstrap values as in the Bayesian/ML analysis based on the larger data set.

Of course mitochondrial DNA is widely used in analyzing relationships among animal species, including birds. Most of these studies are focused on relatively small groups of species, such as the tern study cited here. With growing DNA barcode libraries it will be increasingly possible to get at least a preliminary look at genetic relationships for large numbers of species (so far 2,393 avian species (24% of world birds) have barcode records in BOLD). This could be exciting!

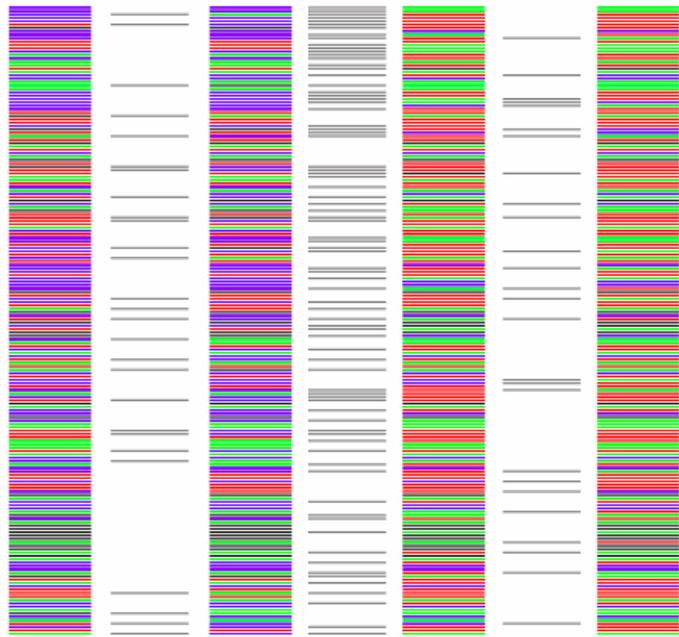
# BARCODING LIFE: TEN REASONS TO IDENTIFY SPECIES BY DNA



Hermit Thrush American Robin Bumblebee Honey Bee

1. Works with fragments
2. Works with all life stages
3. Unmasks look-alikes
4. Reduces ambiguity
5. Makes expertise go further
6. Democratizes access
7. Open way for electronic handheld guide
8. Sprouts new leaves on the tree of life
9. Demonstrates value of collections
10. Speeds writing the encyclopedia of life

# BARCODING LIFE: MORE REASONS TO IDENTIFY SPECIES BY DNA



11. Phylogenetic signal → a research agenda for taxonomists
12. A higher benchmark for type series
13. Enables research on minute taxa (microlepidoptera, nematodes, algae)
14. Mitochondrial genes coalesce faster



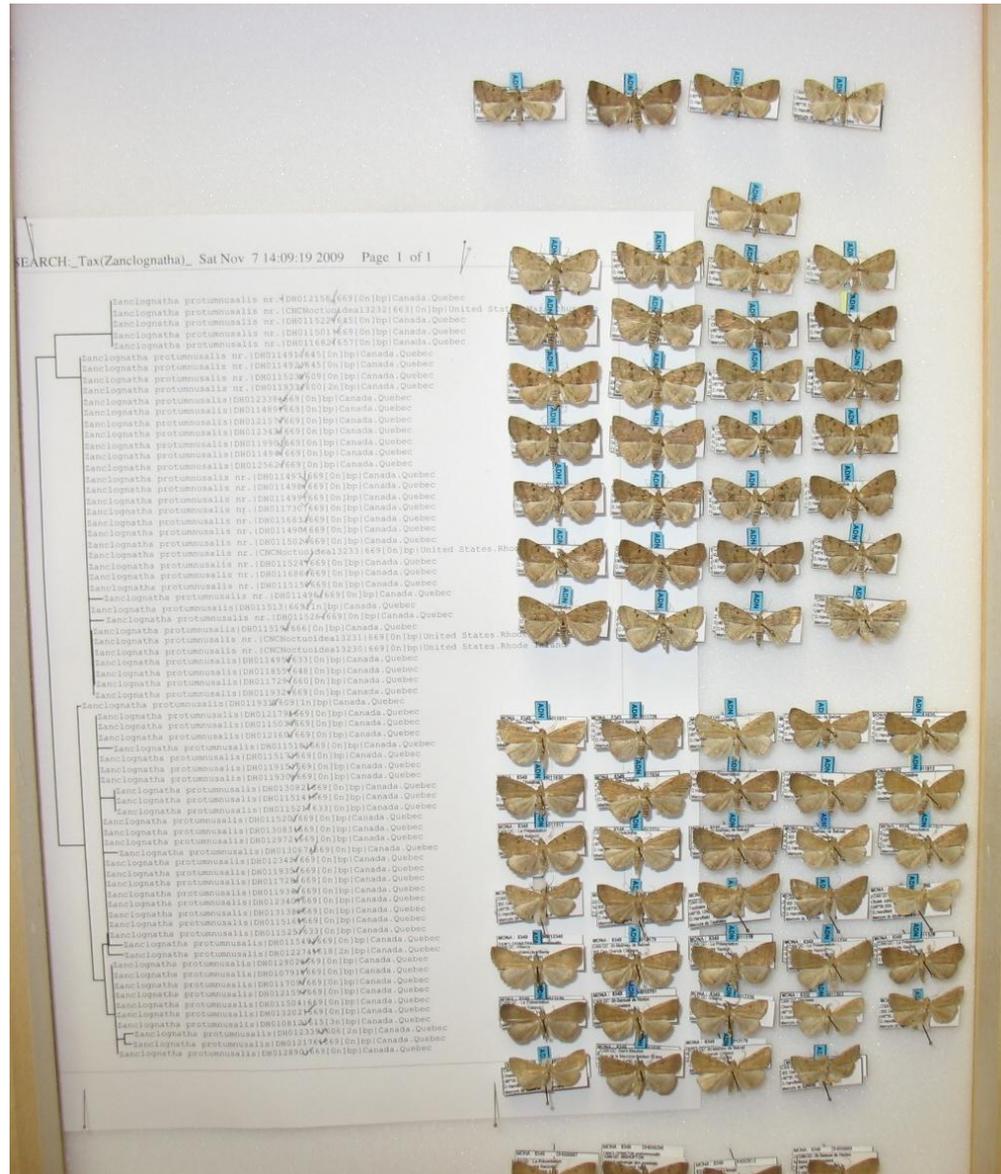
Hermit Thrush   American Robin   Bumblebee   Honey Bee

## **7. Opens the way for an electronic handheld field guide, the Life Barcoder.**

Barcoding links biological identification to advancing frontiers in DNA sequencing, miniaturization in electronics, and computerized information storage. Integrating those links will lead to portable desktop devices and ultimately to hand-held barcoders. Imagine the promise of a schoolchild with a barcoder in hand learning to read wild biodiversity, the power granted to a field ecologist surveying with a barcoder and global positioning system, or the security imparted by a port inspector with a barcoder linked to a central computer!



# Zanclognatha protumnusalis



# Some Problems

- \* ideally daughter species should be reciprocally monophyletic
  - Funk and Omland (2003) claimed only 70% of species in their review were reciprocally monophyletic.
- \* **hybridization** is especially problematic:
  - e.g., one haplotype is shared by 5 species of New England damselflies
    - important shortfall for many large genera
    - sloppy sex matters...many animals with external fertilization have evidence of hybridization



# Some Problems

- \* ancestral polymorphism may be the most pervasive problem among closely related taxa
- \* an issue with recently speciating a taxa
- \* deep within-species splits possible:  
because haplotypes do not recombine,  
any biogeographic (phylogeographic) splits  
will linger (until haplotype extinction)  
e.g., sky-island populations from southern  
Appalachians usually different from  
Canadian haplotypes





*Colias interior*

**COI samples from Ottawa have a 6% divergence**

# More Problems

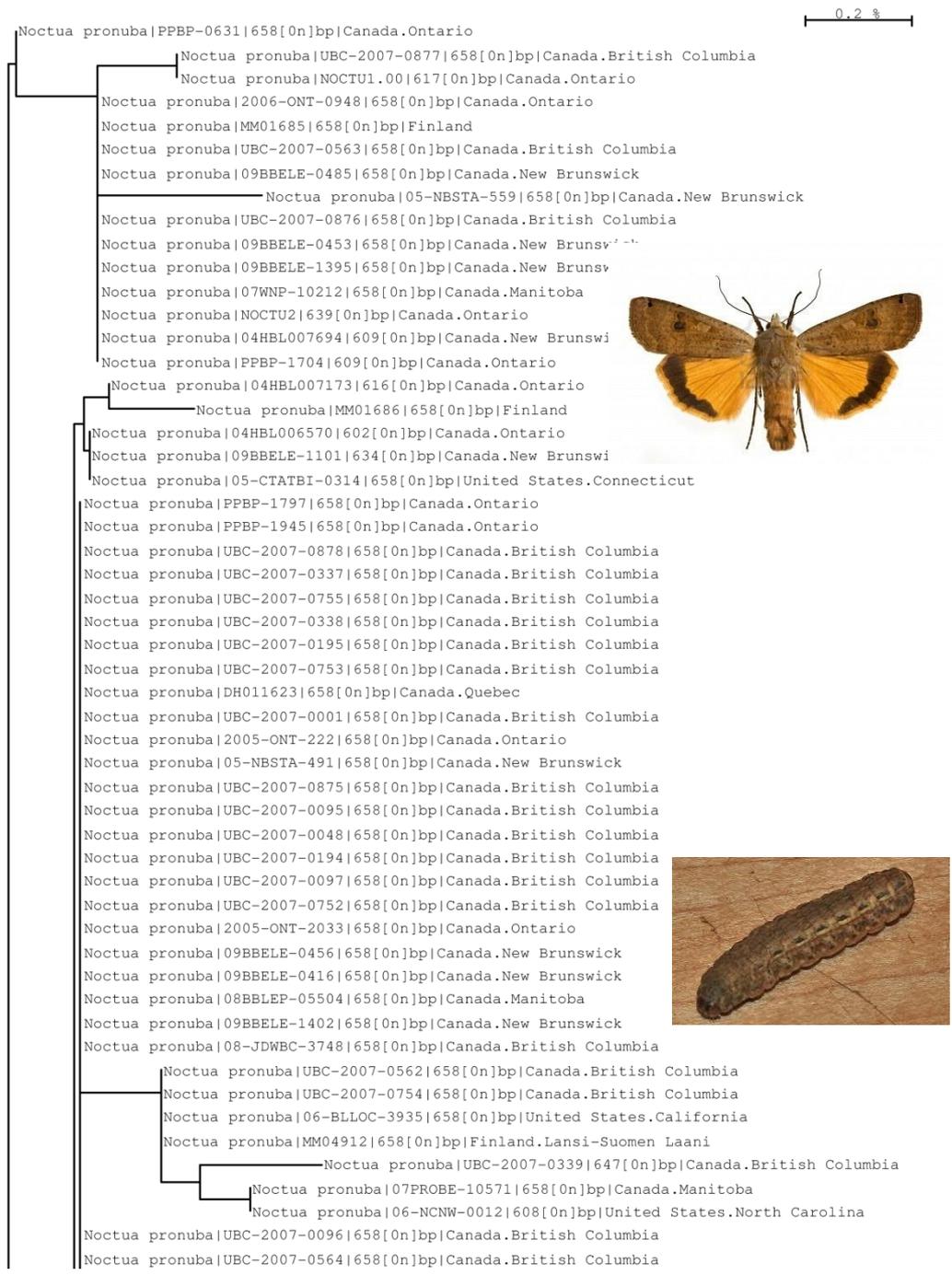
- \* do barcodes work for asexual taxa?
- \* distances between taxa mean...exactly what?
- \* sometimes too little resolution (recent splits/taxa: e.g., lacewing song morphs) may not show up in COI
- \* sometimes too many haplotypes to make much sense (*Noctua pronuba*)



# Mitochondrial Haplotypes in *Noctua pronuba*

My thought with the barcodes that that recently introduced species would most likely have only a single barcode, or maybe a few if multiple individuals were introduced, and maybe these would even tie them to a source like China, England, etc. We're not sure with some species, like *Scoliopteryx libatrix*, *Lycaena phlaeas*, *Pyrhhaia exprimens*, whether they are naturally holarctic or are introductions by man. So, I thought a really good test of this premise would be *Noctua pronuba* because we know the year (about 1979) when a single individual was found at Halifax, a port where many European insects have arrived by ship. It's systematic spread through the maritime provinces, then west and south in 200 to 300 mile increments until reaching Alaska, Florida, and Mexico in about 25 years. However, when I do the barcodes, I see there are - so far - 14 different haplotypes for it, several as divergent as 0.5 %. We are told that a divergence of 2 % usually is indicative of full species and takes likely millions of years. No one has adequately explained to me how a species with apparently a single introduction can have so many haplotypes, unless hundreds of individuals flew off the boat carrying many haplotypes.

Don Lafontaine Nov 2011



0.2 %

Noctua pronuba|PPBP-0631|658[0n]bp|Canada.Ontario  
Noctua pronuba|UBC-2007-0877|658[0n]bp|Canada.British Columbia  
Noctua pronuba|NOCTU1.00|617[0n]bp|Canada.Ontario  
Noctua pronuba|2006-ONT-0948|658[0n]bp|Canada.Ontario  
Noctua pronuba|MM01685|658[0n]bp|Finland  
Noctua pronuba|UBC-2007-0563|658[0n]bp|Canada.British Columbia  
Noctua pronuba|09BBELE-0485|658[0n]bp|Canada.New Brunswick  
Noctua pronuba|05-NBSTA-559|658[0n]bp|Canada.New Brunswick  
Noctua pronuba|UBC-2007-0876|658[0n]bp|Canada.British Columbia  
Noctua pronuba|09BBELE-0453|658[0n]bp|Canada.New Brunswick  
Noctua pronuba|09BBELE-1395|658[0n]bp|Canada.New Brunswick  
Noctua pronuba|07WNP-10212|658[0n]bp|Canada.Manitoba  
Noctua pronuba|NOCTU2|639[0n]bp|Canada.Ontario  
Noctua pronuba|04HBL007694|609[0n]bp|Canada.New Brunswick  
Noctua pronuba|PPBP-1704|609[0n]bp|Canada.Ontario  
Noctua pronuba|04HBL007173|616[0n]bp|Canada.Ontario  
Noctua pronuba|MM01686|658[0n]bp|Finland  
Noctua pronuba|04HBL006570|602[0n]bp|Canada.Ontario  
Noctua pronuba|09BBELE-1101|634[0n]bp|Canada.New Brunswick  
Noctua pronuba|05-CTATBI-0314|658[0n]bp|United States.Connecticut  
Noctua pronuba|PPBP-1797|658[0n]bp|Canada.Ontario  
Noctua pronuba|PPBP-1945|658[0n]bp|Canada.Ontario  
Noctua pronuba|UBC-2007-0878|658[0n]bp|Canada.British Columbia  
Noctua pronuba|UBC-2007-0337|658[0n]bp|Canada.British Columbia  
Noctua pronuba|UBC-2007-0755|658[0n]bp|Canada.British Columbia  
Noctua pronuba|UBC-2007-0338|658[0n]bp|Canada.British Columbia  
Noctua pronuba|UBC-2007-0195|658[0n]bp|Canada.British Columbia  
Noctua pronuba|UBC-2007-0753|658[0n]bp|Canada.British Columbia  
Noctua pronuba|DH011623|658[0n]bp|Canada.Quebec



*Noctua pronuba*

# More Problems

- \* although mitochondria are excellent markers for phylogeographic and species-level taxa we know of examples where the mt haplotypes are poor proxies for known ESUs, e.g., when females are philopatric and males are vagile
- \* pseudogenes in nucleus (numts)
- \* heteroplasmy
- \* in an application of DNA barcoding to the identification of species of *Protocalliphora* flies, it was found that several distinct morphospecies had identical cytochrome c oxidase I gene sequences, most likely through horizontal gene transfer by *Wolbachia* species as they jump across host species (Wikipedia: <http://en.wikipedia.org/wiki/Wolbachia>)
- \* and ?



**Table 2. Properties of the eight datasets used in testing performance of algorithms for OTU delineation.**

**Table 2.** Properties of the eight datasets used in testing performance of algorithms for OTU delineation.

Datasets	Species	Sequences	Sequences per Species	Mean Max-Intraspecific Distance	Mean N-N Distance
Birds (Argentina)	497	1589	3.2	0.39	8.20
Birds (North America)	575	1936	3.4	0.43	6.70
Bees (Ireland)	56	231	4.1	0.48	8.87
Fishes (Australia)	212	753	3.6	0.50	8.73
Fishes (Canada)	190	1359	7.2	0.40	7.68
Geometrid Moths (Bavaria)	298	649	2.2	0.36	7.11
Moths and Butterflies (North America)	1327	11144	8.4	0.77	5.96
Plusiinae Moths (North America)	69	1182	17.3	0.52	3.53

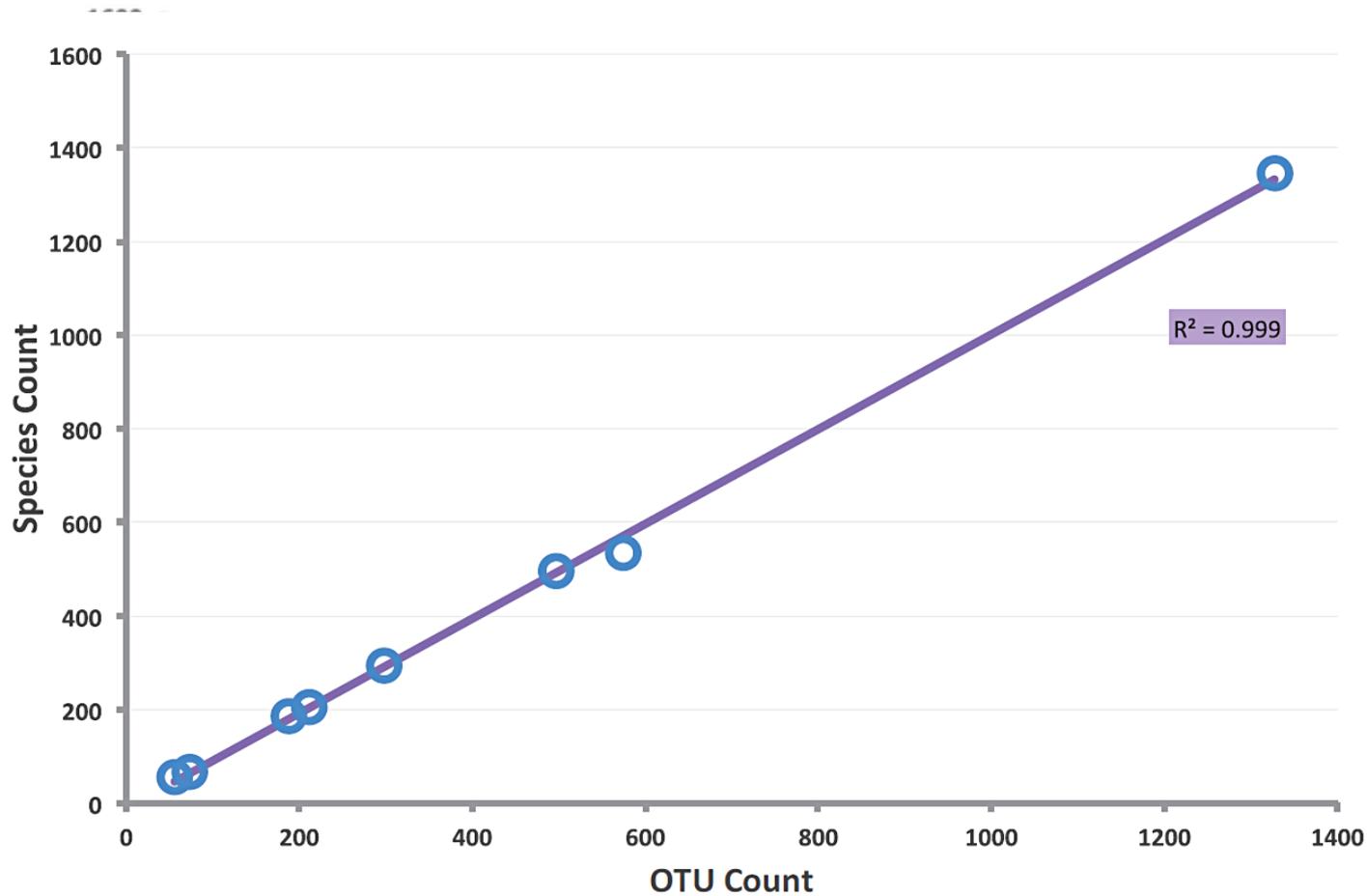
doi:10.1371/journal.pone.0066213.t002

Ratnasingham S, Hebert PDN (2013) A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System.

PLoS ONE 8(7): e66213. doi:10.1371/journal.pone.0066213

<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0066213>

**Figure 4. Correspondence between OTUs generated by RESL and the number of species in eight datasets.**



Ratnasingham S, Hebert PDN (2013) A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System. PLoS ONE 8(7): e66213. doi:10.1371/journal.pone.0066213  
<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0066213>

**Figure 5. Comparison of BIN and species boundaries in six datasets.**

The Barcode Index Number (BIN) System



Ratnasingham S, Hebert PDN (2013) A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System. PLoS ONE 8(7): e66213. doi:10.1371/journal.pone.0066213

<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0066213>