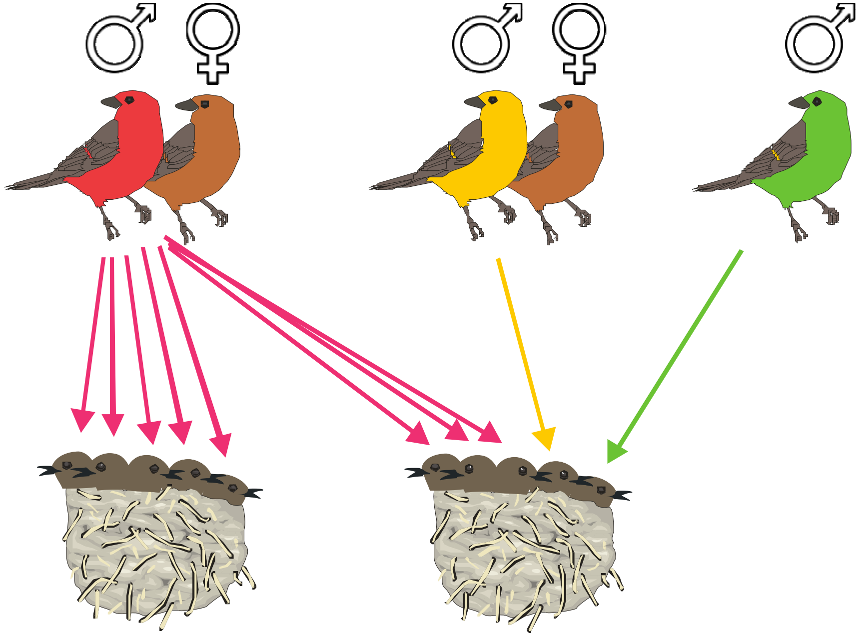
Molecular identification

Species, individual, sex



Species identification

DNA barcoding

Definition of species

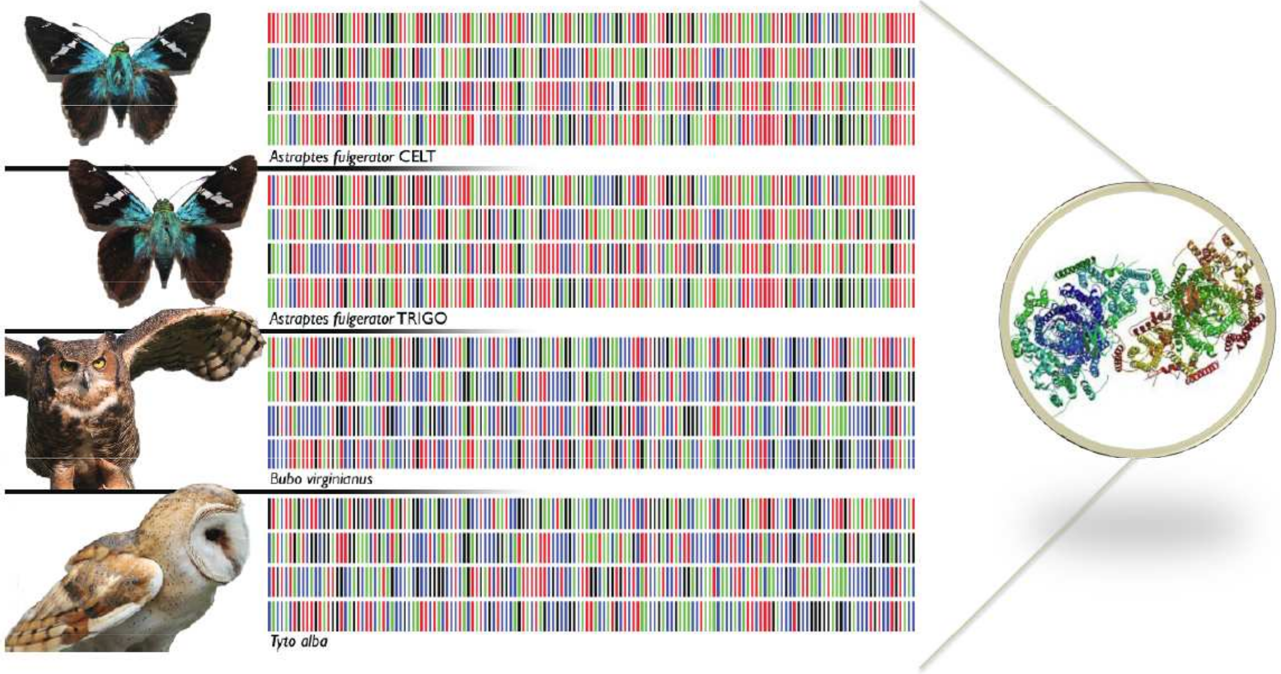
- **Biological species concept** (Mayr, 1942) – RIM = post- or prezygotic barriers of gene flow (not always so simple - hybrid zones, alopatric speciation, asexual species atd.)
- Other concepts (e.g. typological = morphological, genetic, phylogenetic aj.)

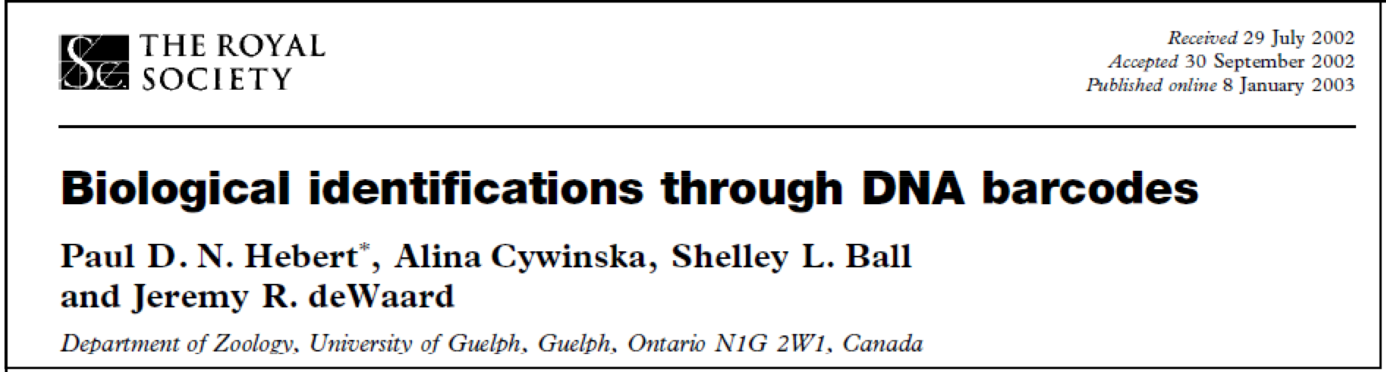
Why it is necessary to identify species (genetically)?

- Conservation biology needs names – it is necessary to decide which taxonomic unit (species) requires conservation attention
- Forensic genetics, developemental stages without identification traits, identification of prey items, etc.

Barcoding is a standardized approach to identifying plants and animals by minimal sequences of DNA, called DNA barcodes

DNA Barcode: A short DNA sequence, from a uniform locality on the genome, used for identifying species





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

first idea in 2003

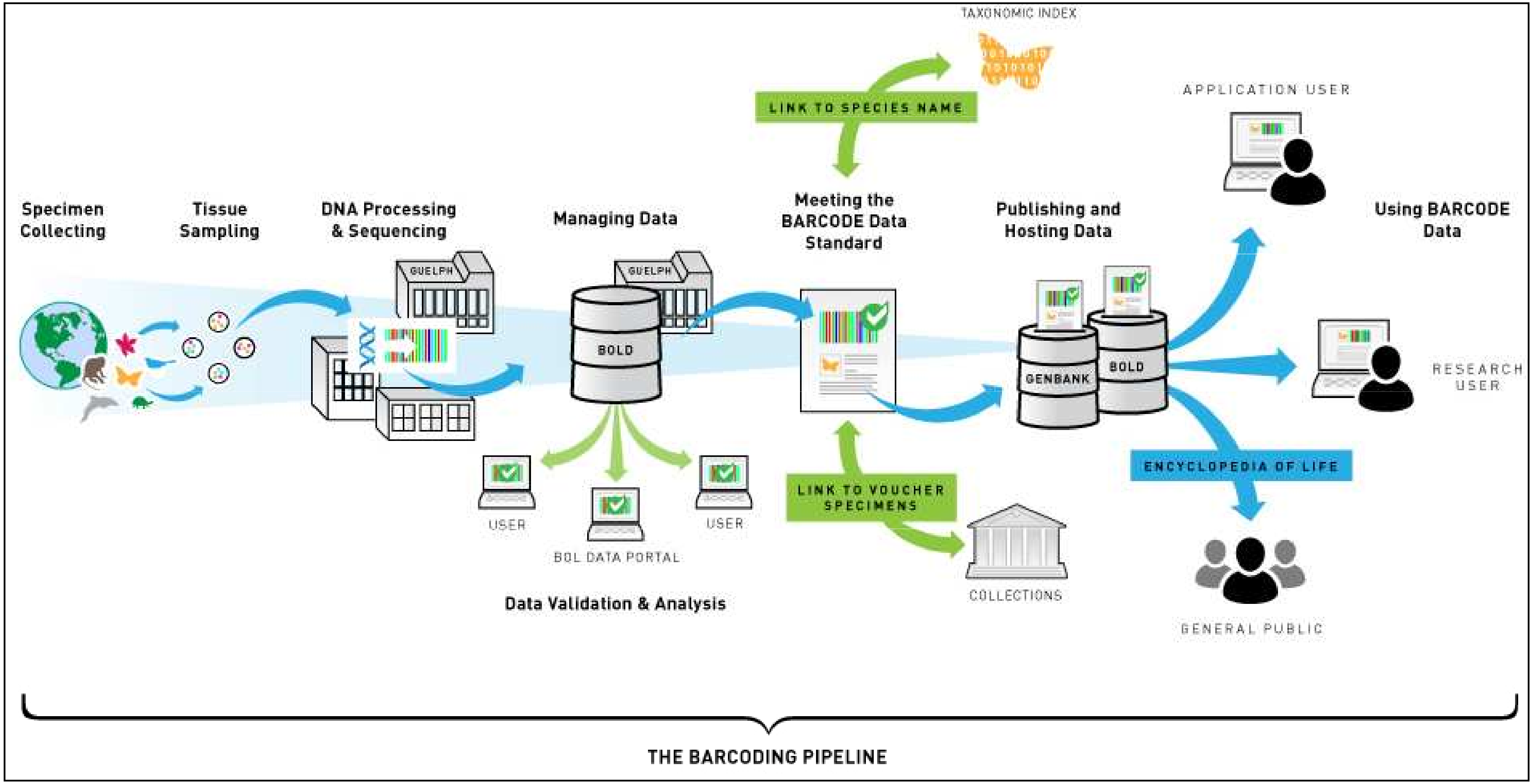
CONSORTIUM FOR THE BARCODE OF LIFE

CBOL in 2005

international BARCODE OF LIFE

iBOL 2010-2015

500 000 species barcoded in 2015

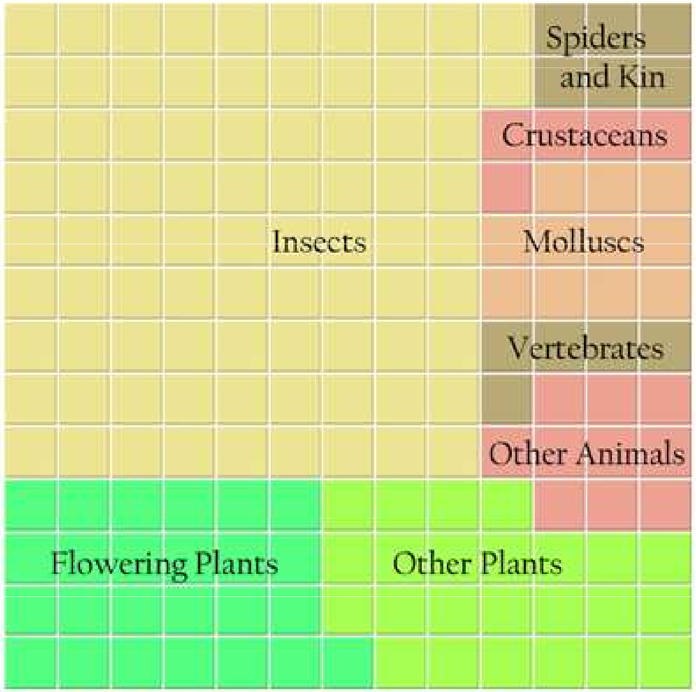


THE BARCODING PIPELINE

Why barcode animal and plant species?

Known Biodiversity

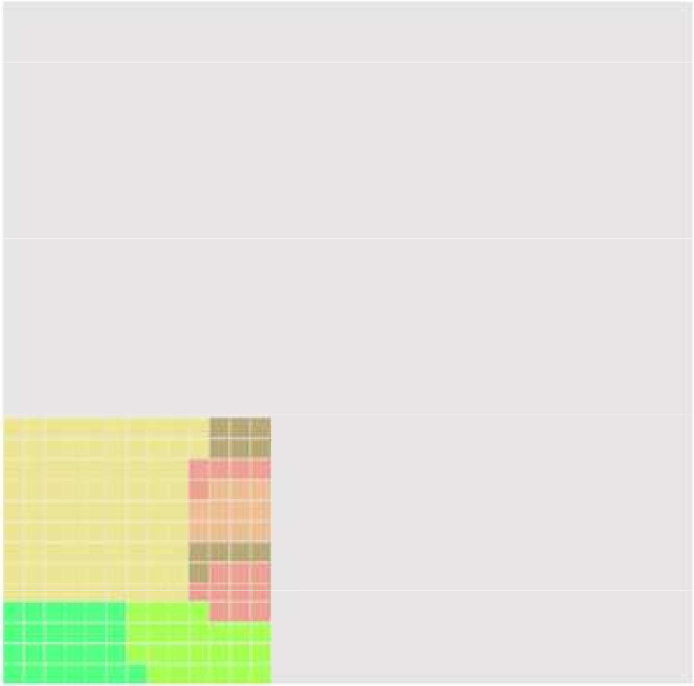
Approximately 1.7 million named species of plants and animals.



1 square = 10,000 species

Estimated Biodiversity

10 million species



Crisis of biodiversity and classical taxonomy

DNA barcoding is important part of **„integrative taxonomy“**

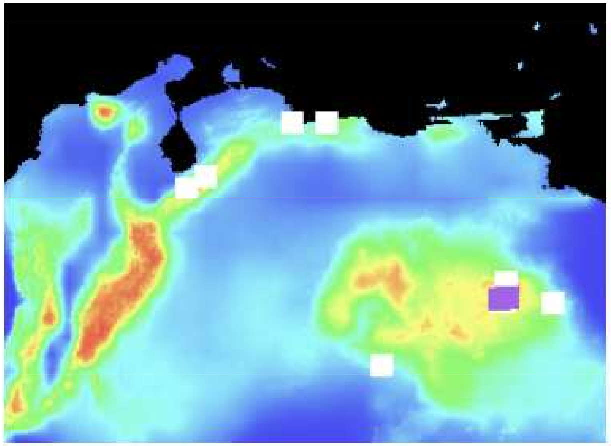
Integrative taxonomy

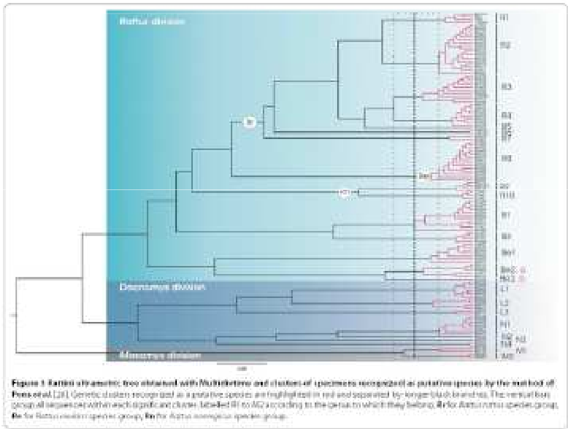
Integr. Taxonomy

Ecology

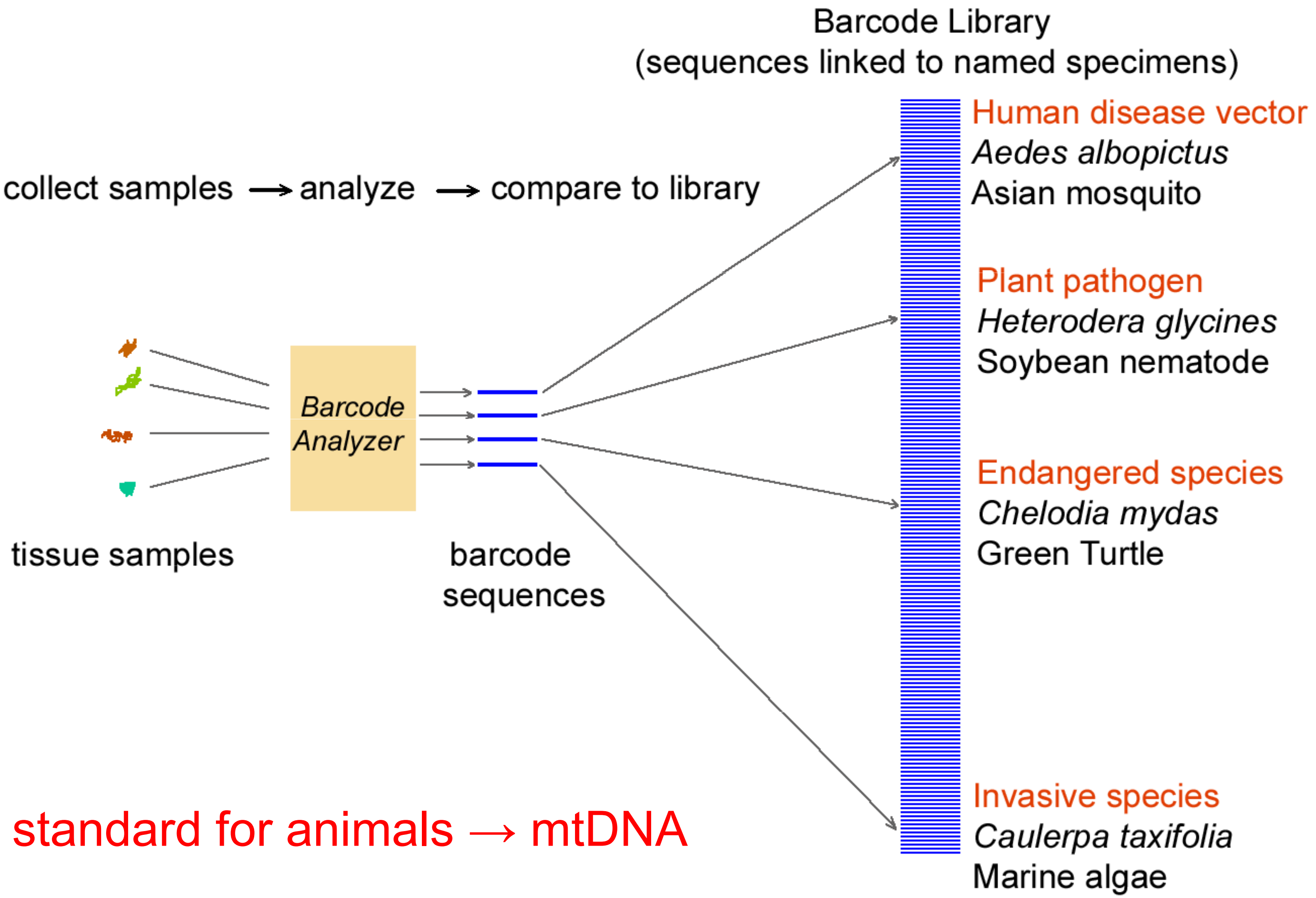
Genetics

Behavioural Biol.





What are the benefits of standardization?



collect samples → analyze → compare to library

Barcode Library
(sequences linked to named specimens)

tissue samples

Barcode Analyzer

barcode sequences

Human disease vector
Aedes albopictus
Asian mosquito

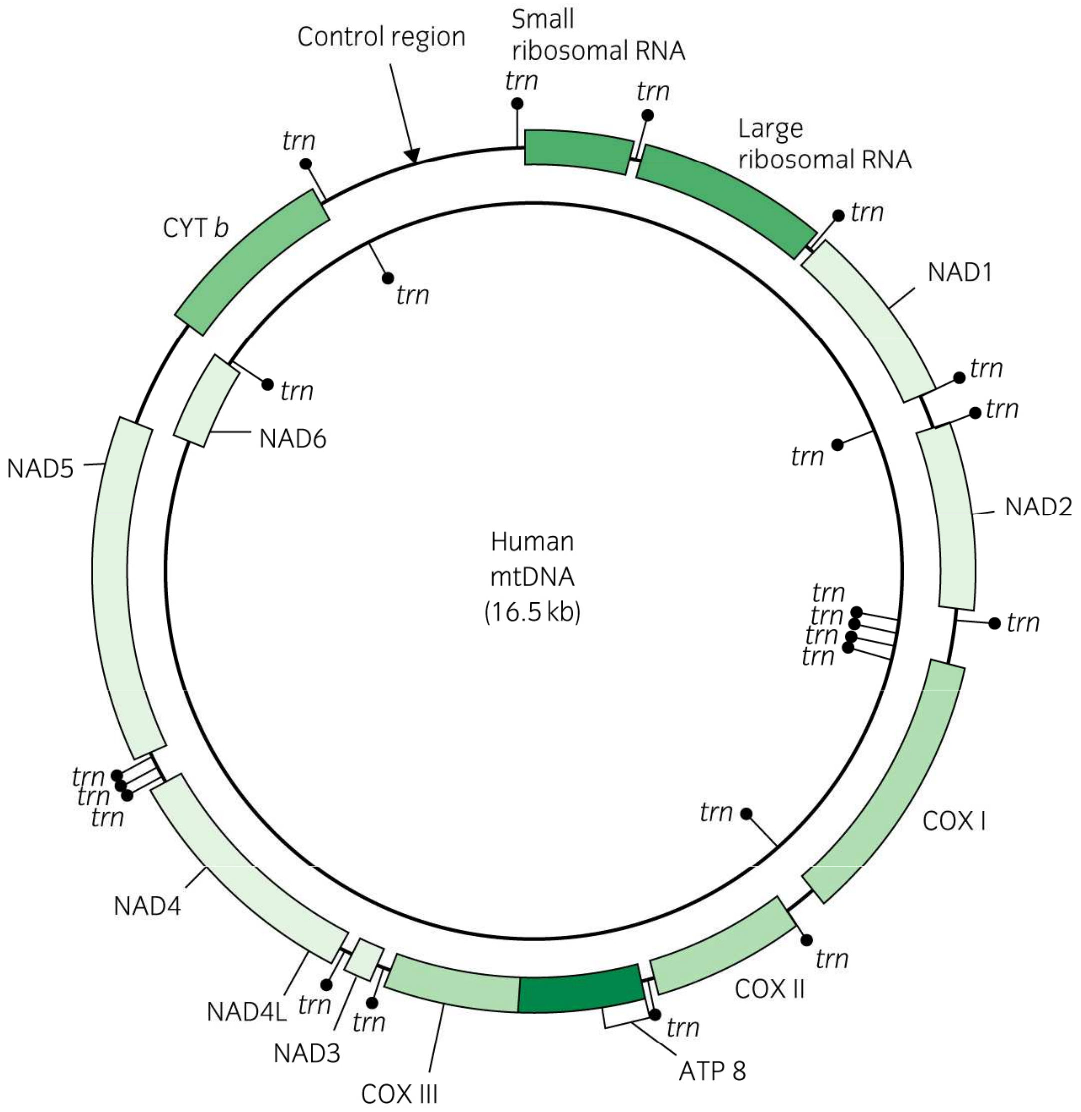
Plant pathogen
Heterodera glycines
Soybean nematode

Endangered species
Chelodia mydas
Green Turtle

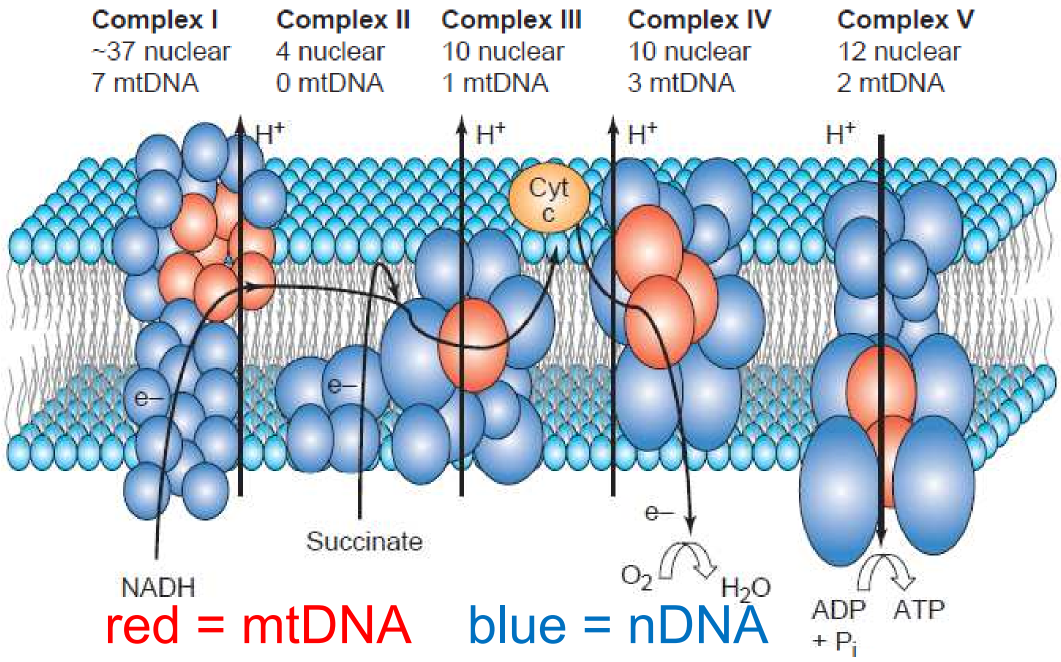
Invasive species
Caulerpa taxifolia
Marine algae

Suitable standard for animals → mtDNA

Why barcode animals with mitochondrial DNA?

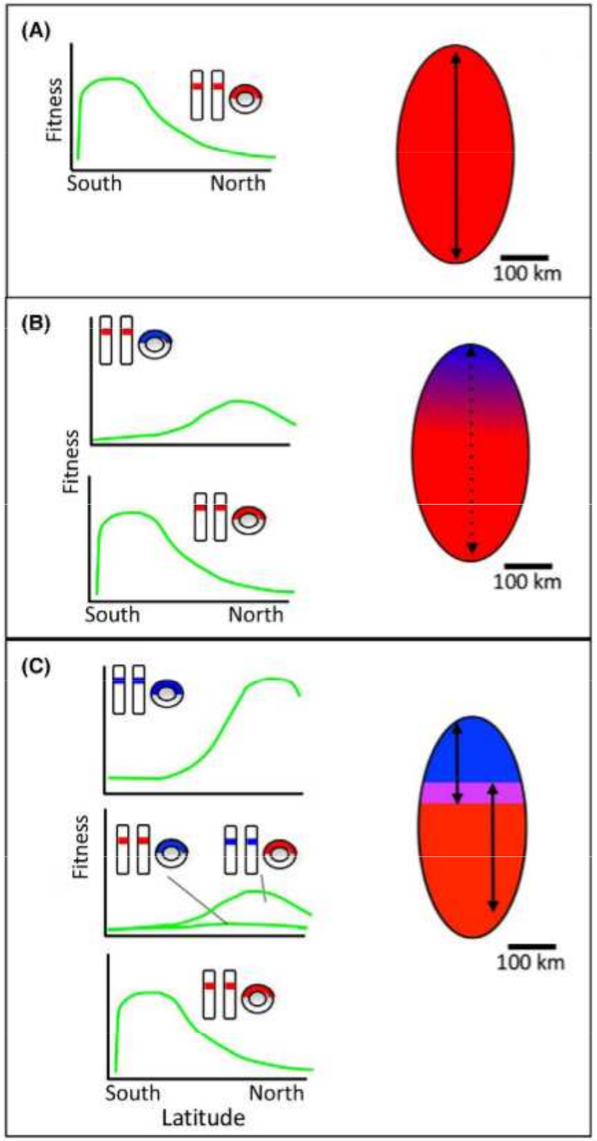


Mitonuclear coevolution as the genesis of speciation (?)



red = mtDNA blue = nDNA

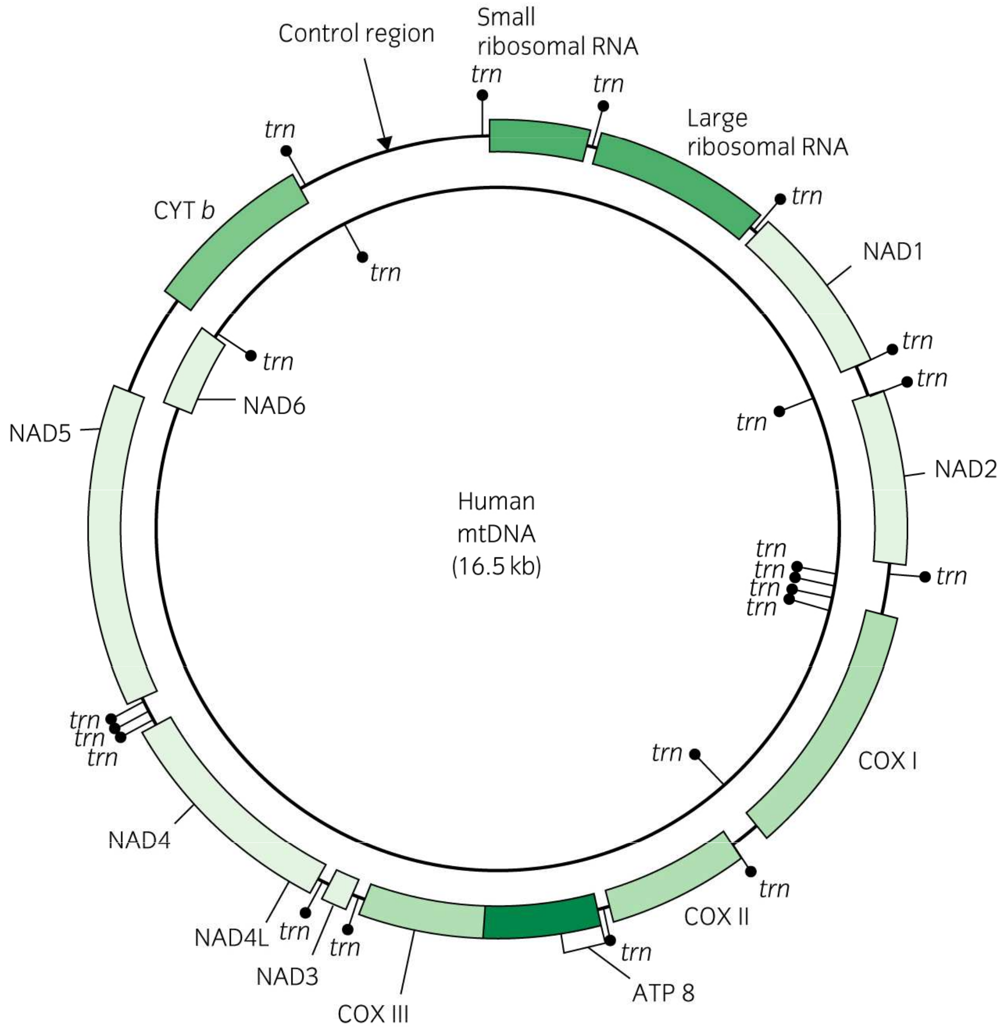
Rand et al. 2004



Hill 2016

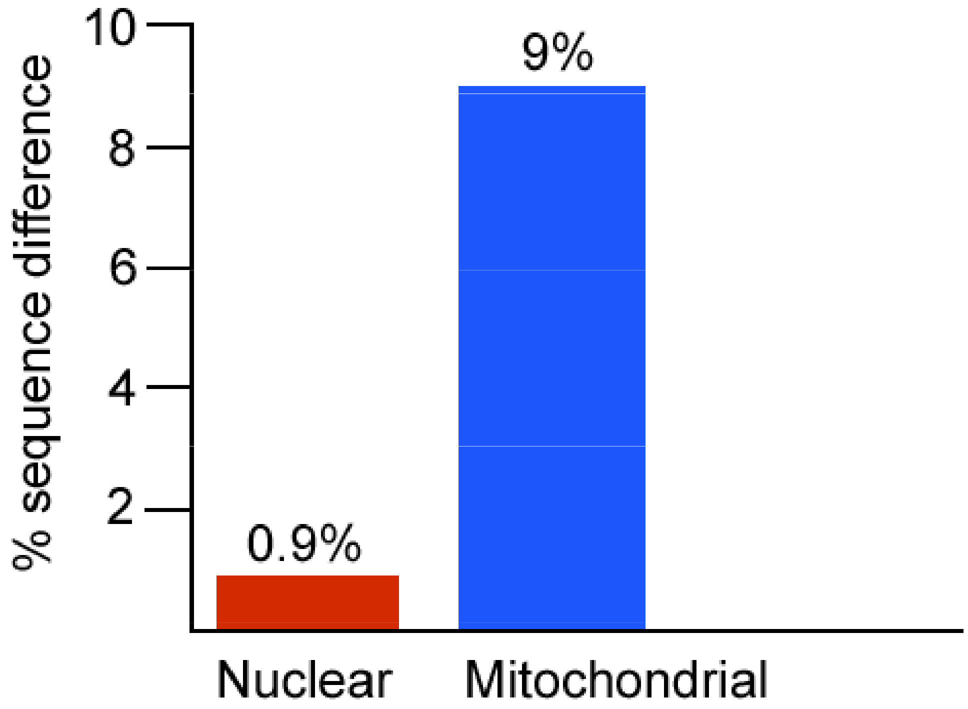
Why barcode animals with mitochondrial DNA?

Four properties make mitochondrial genomes especially suitable for identifying species



1. Greater differences among species, on average 5- to 10-fold higher in mitochondrial than in nuclear genes (lower N_e for mtDNA). Thus shorter segments distinguish among species, and because shorter, less expensively.

Average sequence differences in nuclear and mitochondrial DNA between human and chimp



- 2. Copy number There are 100-10,000 more copies of mitochondrial than nuclear DNA per cell, making recovery, especially from small or partially degraded samples, easier and cheaper.

- 3. Relatively few differences within species in most cases. Small intraspecific and large interspecific differences signal distinct genetic boundaries between most species, enabling precise identification with a barcode.

- 4. Introns, which are non-coding regions interspersed between coding regions of a gene, are absent from mitochondrial DNA of most animal species, making amplification straightforward. Nuclear genes are often interrupted by introns, making amplification difficult or unpredictable.

Focus to date

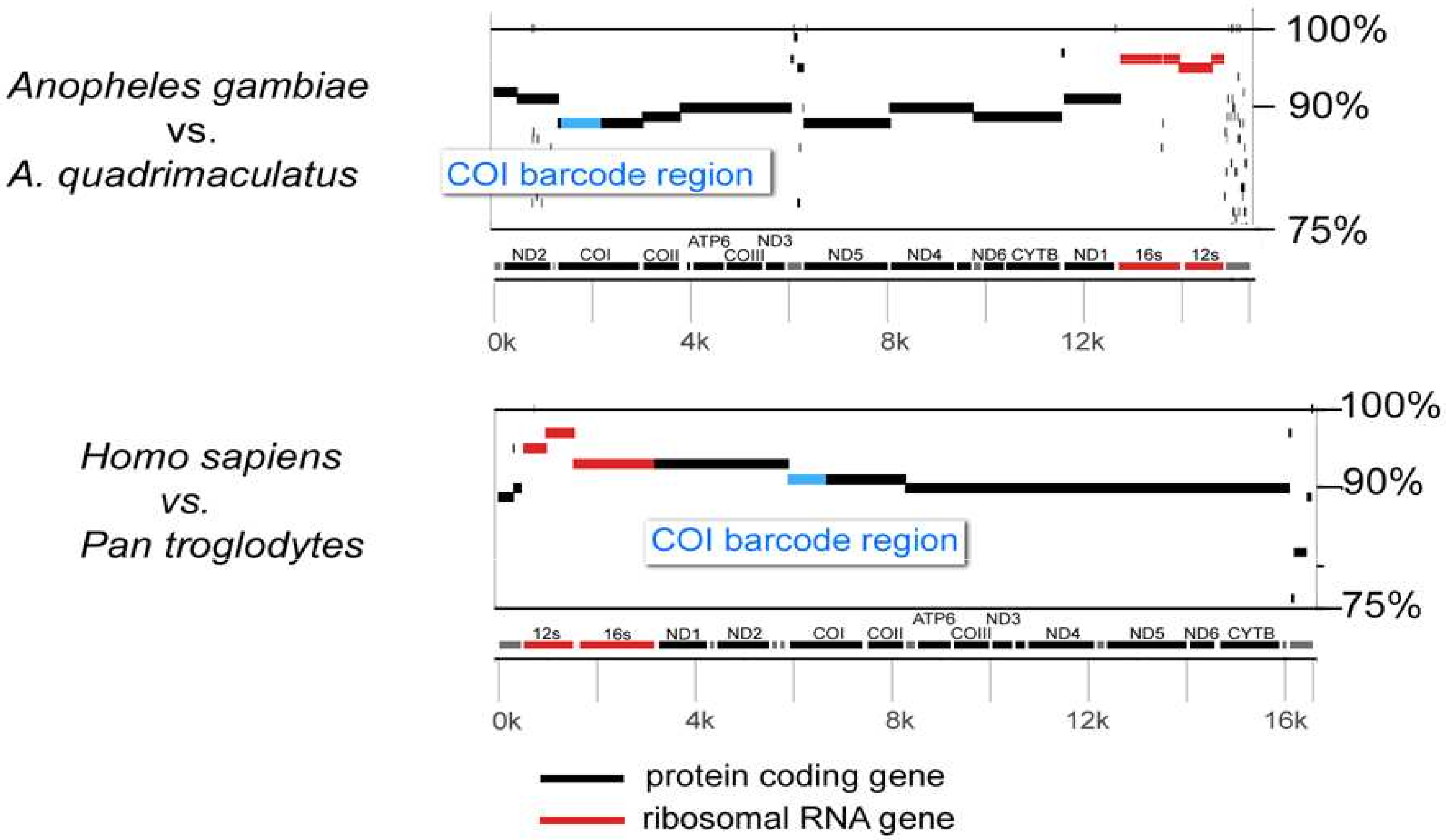
- For animals, a 658 base-pair fragment of the mitochondrial gene, **cytochrome oxidase subunit I** (mtCOI) – consensus for iBOL consortium; 18S rDNA – Nematoda etc.
- For plants, mitochondrial genes do not differ sufficiently to distinguish among closely related species. Promising markers are genes on cpDNA: matK and rbcL
- For bacteria, a 16S-rDNA emerges as very useful marker (especially when using next-generation sequencing)

- For particular taxonomic groups, also other barcodes are widely used, e.g. cytochrome *b* for mammals

Cytochrome *c* oxidase I (COI or CoxI) contains differences representative of those in other protein-coding genes

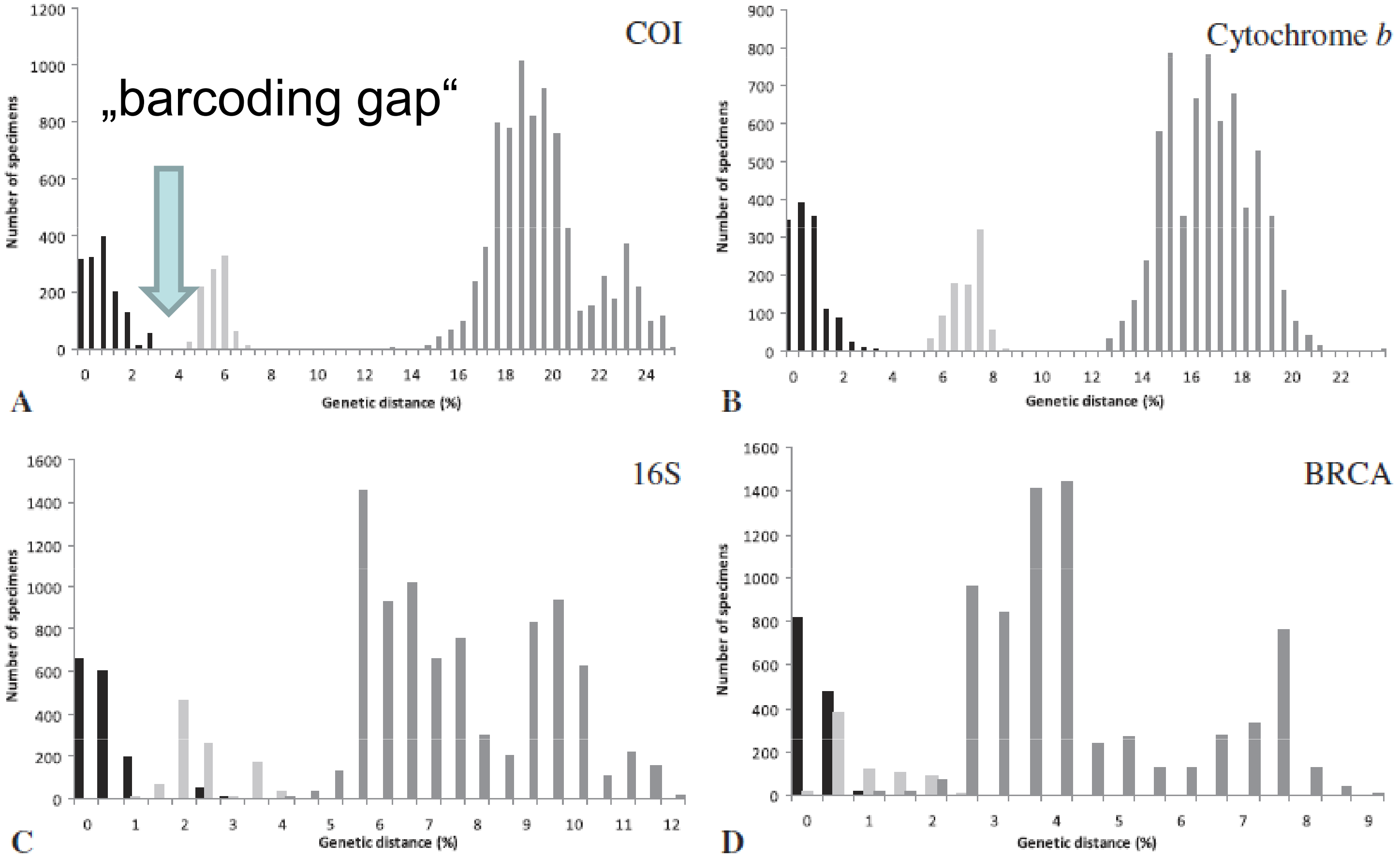
Possible gains in accuracy or cost using a different protein-coding gene would likely be small.

Percent identity plot (PIP) analysis of complete mitochondrial genomes



Barcoding, molecular taxonomy, and exploration of the diversity of shrews (Soricomorpha: Soricidae) on Mount Nimba (Guinea)

FRANÇOIS JACQUET[1]*, VIOLAINE NICOLAS[1], CELINE BONILLO[2],
CORINNE CRUAUD[3] and CHRISTIANE DENYS[1]



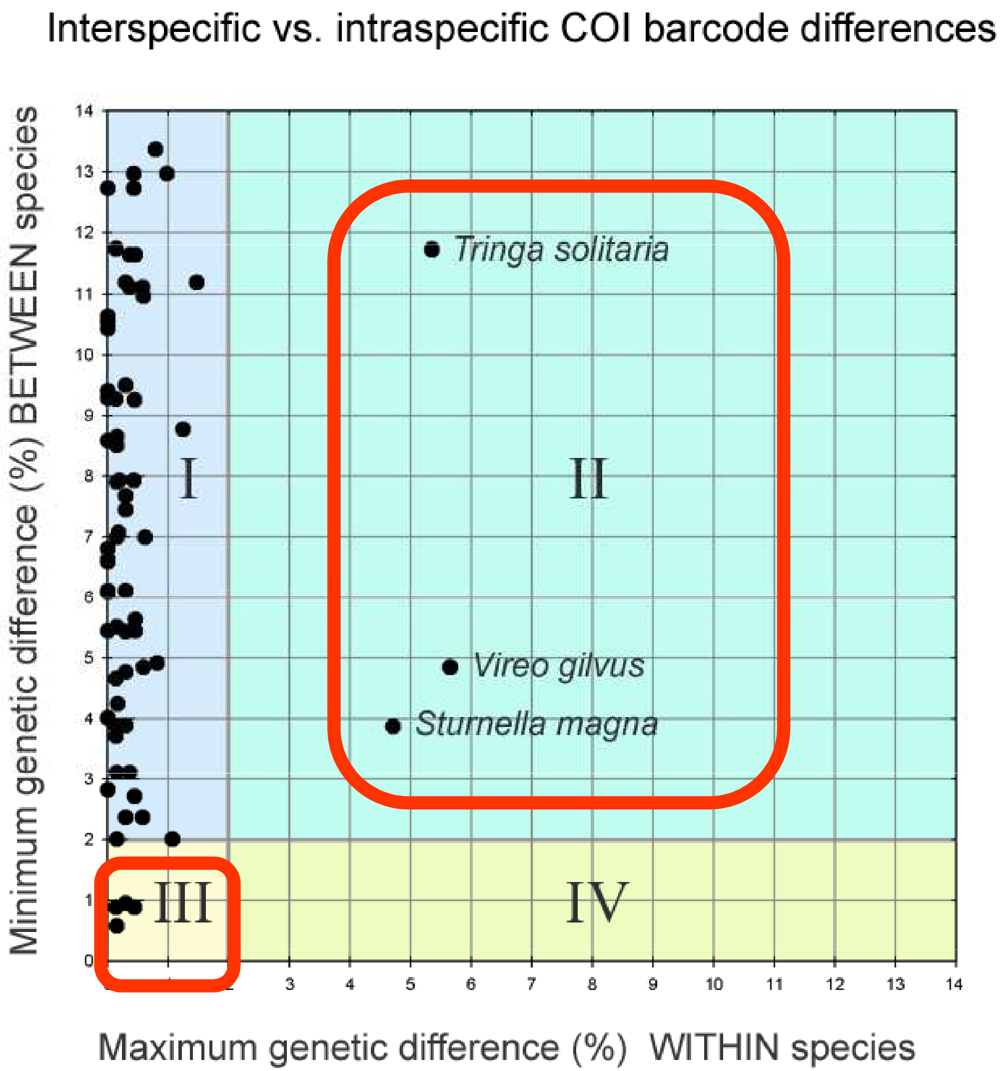
„barcoding gap“

Figure 3. Histograms of divergence percentage values between all sequences. Black, intraspecific distances; light grey, interspecific distances amongst clade C; dark grey, interspecific distances excluding clade C. All sequences were corrected using the Kimura two-parameter substitution model. Sequences corresponding to nuclear copies of cytochrome *b* were excluded from the analysis. BRCA, breast cancer gene; COI, cytochrome oxidase I.

What do barcode differences among and within animal species studied so far suggest?

- barcodes identify most animal species unambiguously
- approximately 2-5% of recognized species have shared barcodes with closely-related species - many of them hybridize regularly
- in all groups studied so far, distinct barcode clusters with biological co-variation suggest cryptic species

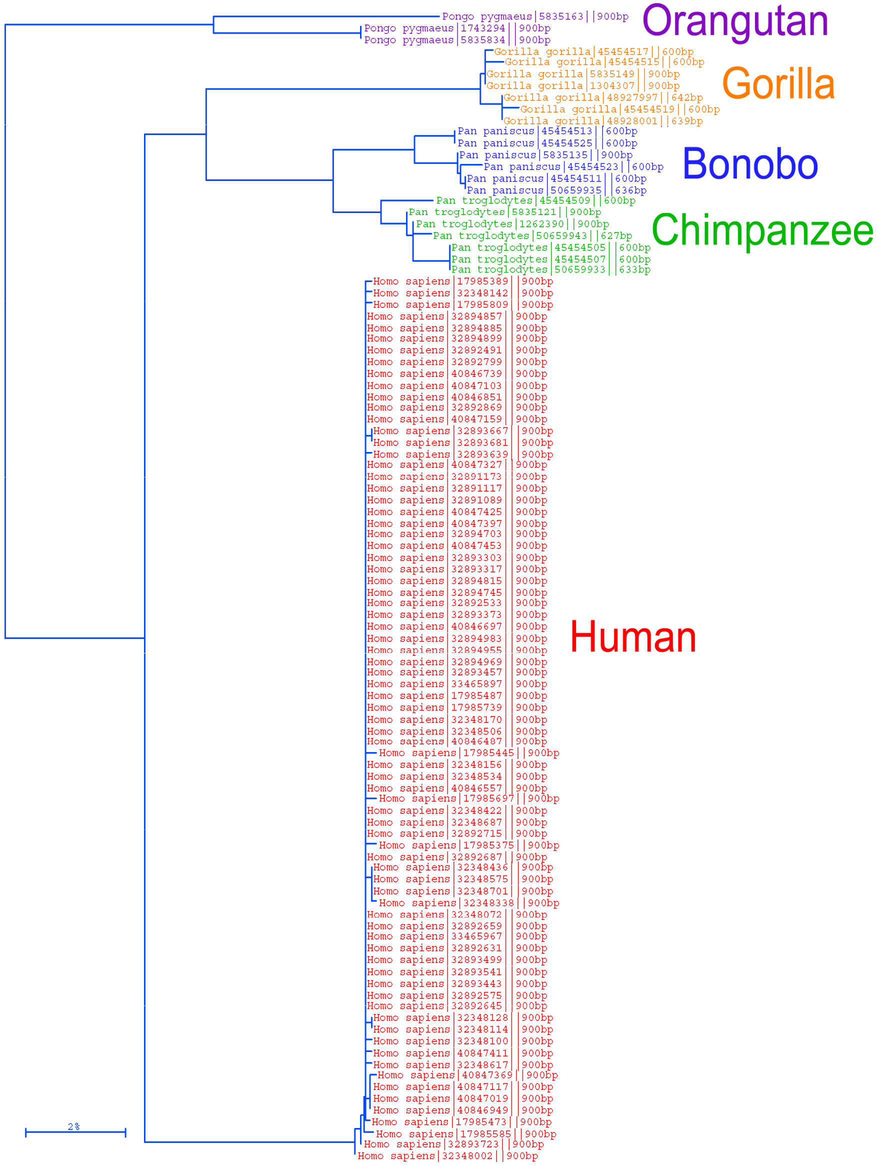
Barcoding North American birds



Tak co třeba znamená toto?

Barcodes affirm the unity of the species *Homo sapiens*

Comparisons show we differ from one another by only 1 or 2 nucleotides out of 648, while we differ from chimpanzees at 60 locations and gorillas at 70 locations.



A barcoder?

Mark Stoeckle	The Rockefeller University
Paul Waggoner	Connecticut Agricultural Experiment Station
Jesse Ausubel	Alfred P. Sloan Foundation

Next generation sequencing of amplicons

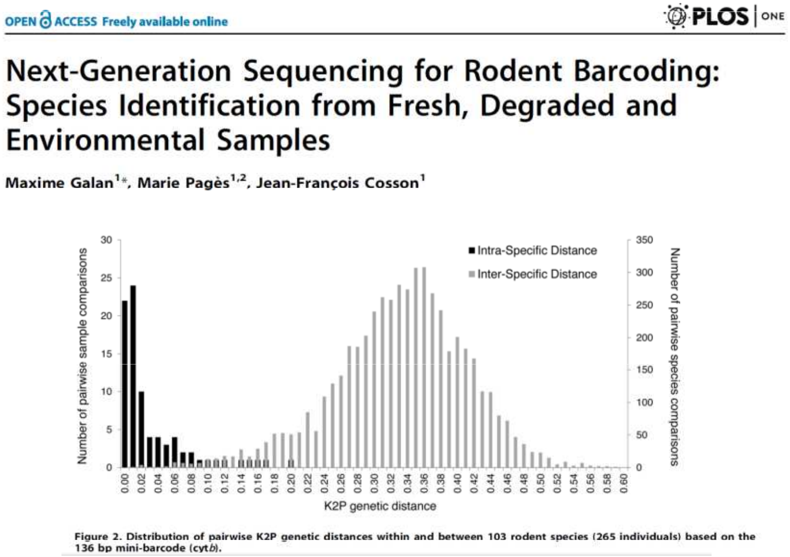
1. Metagenomics

- community of microorganisms
- PCR of 16S (18S) rRNA
- it is also possible to quantify (to some extent)

2. Diet composition

- COI barcoding (carnivores)
- chloroplast (cp)DNA (herbivores)

3. Analysis of contaminated samples



OPEN ACCESS Freely available online

PLOS | ONE

Next-Generation Sequencing for Rodent Barcoding: Species Identification from Fresh, Degraded and Environmental Samples

Maxime Galan[1*], Marie Pagès[1,2], Jean-François Cosson[1]

Figure 2. Distribution of pairwise K2P genetic distances within and between 103 rodent species (265 individuals) based on the 136 bp mini-barcode (cyt*b*).

|SE|S|AM|E| BARCODE

SEquences Seeker & AMplicons Explorer for Barcoding

Genotyping and barcoding based on
high-throughput multiplex amplicon sequencing

Illumina sequencers

Illumina MiSeq
4 millions reads/run
150 bp/read

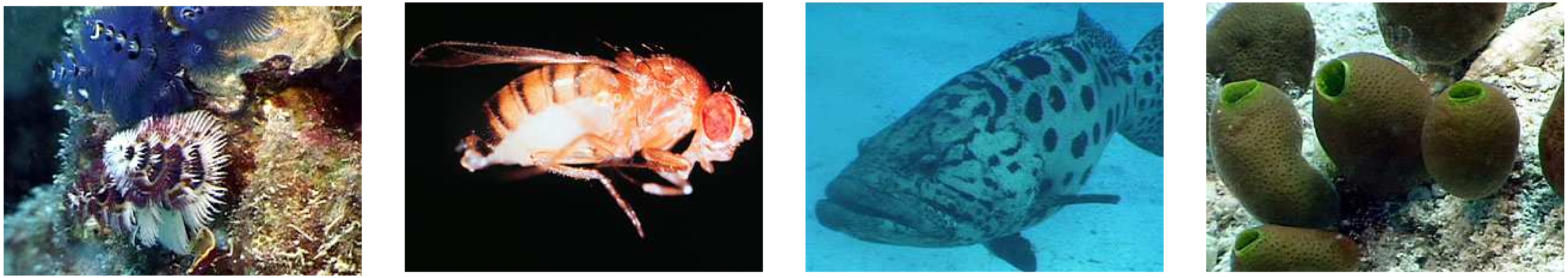
Illumina GAIIx
300 millions reads/run
150 bp/read

Illumina HighSeq
1500 – 3000 millions reads/run
100 bp/read

A barcoder? ... COMING SOON

Mark Stoeckle The Rockefeller University
Paul Waggoner Connecticut Agricultural Experiment Station
Jesse Ausubel Alfred P. Sloan Foundation

What *isn't* DNA Barcoding?



- it is not intended to, in any way, supplant or invalidate existing taxonomic practice
- it is not DNA taxonomy; it does not equate species identity, formally or informally, with a particular DNA sequence
- it is not intended to duplicate or compete with efforts to resolve deep phylogeny (e.g., Assembling the Tree of Life, ATOL)

What are the main limits to barcoding encountered so far?

- horizontal gene transfer
- gene tree vs. species tree
- nuclear pseudogenes
- hybrids – mtDNA introgression

1. Horizontal gene transfer

DNA barcoding cannot reliably identify species of the blowfly genus *Protocalliphora* (Diptera: Calliphoridae)

T.L Whitworth, R.D Dawson, H Magalon and E Baudry

Proc. R. Soc. B 2007 **274**, doi: 10.1098/rspb.2007.0062, published 22 July 2007

Wolbachia within an insect cell
(25-70% species of insects)

Results of nuclear and mitochondrial DNA do not match

AFLP

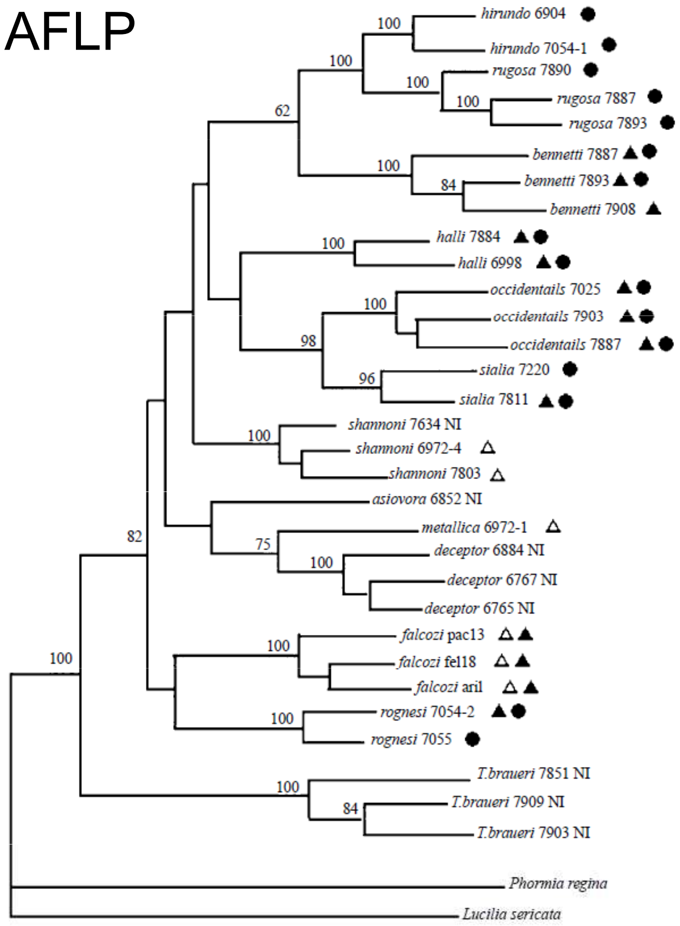
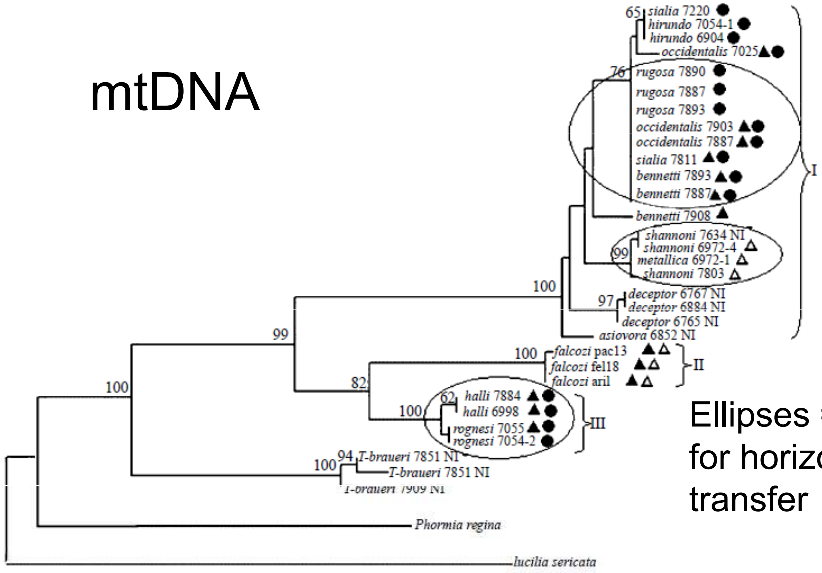


Figure 1. Phylogram of the *Protocalliphora* genus based upon AFLP data. The tree was generated by parsimony analysis using a heuristic search with tree bisection–reconnection. Bootstrap values are shown as percentage of 1000 replicates at each node only if they are 50% or greater. The *Wolbachia* infection status of each individual is shown on the tree. Individuals infected with *w*A1, *w*A2 or *w*B *Wolbachia* strains are respectively represented by an open triangle, a solid triangle and a circle. Non-infected individuals are symbolized by NI.

Symbols correspond to the type of *Wolbachia* infection

mtDNA

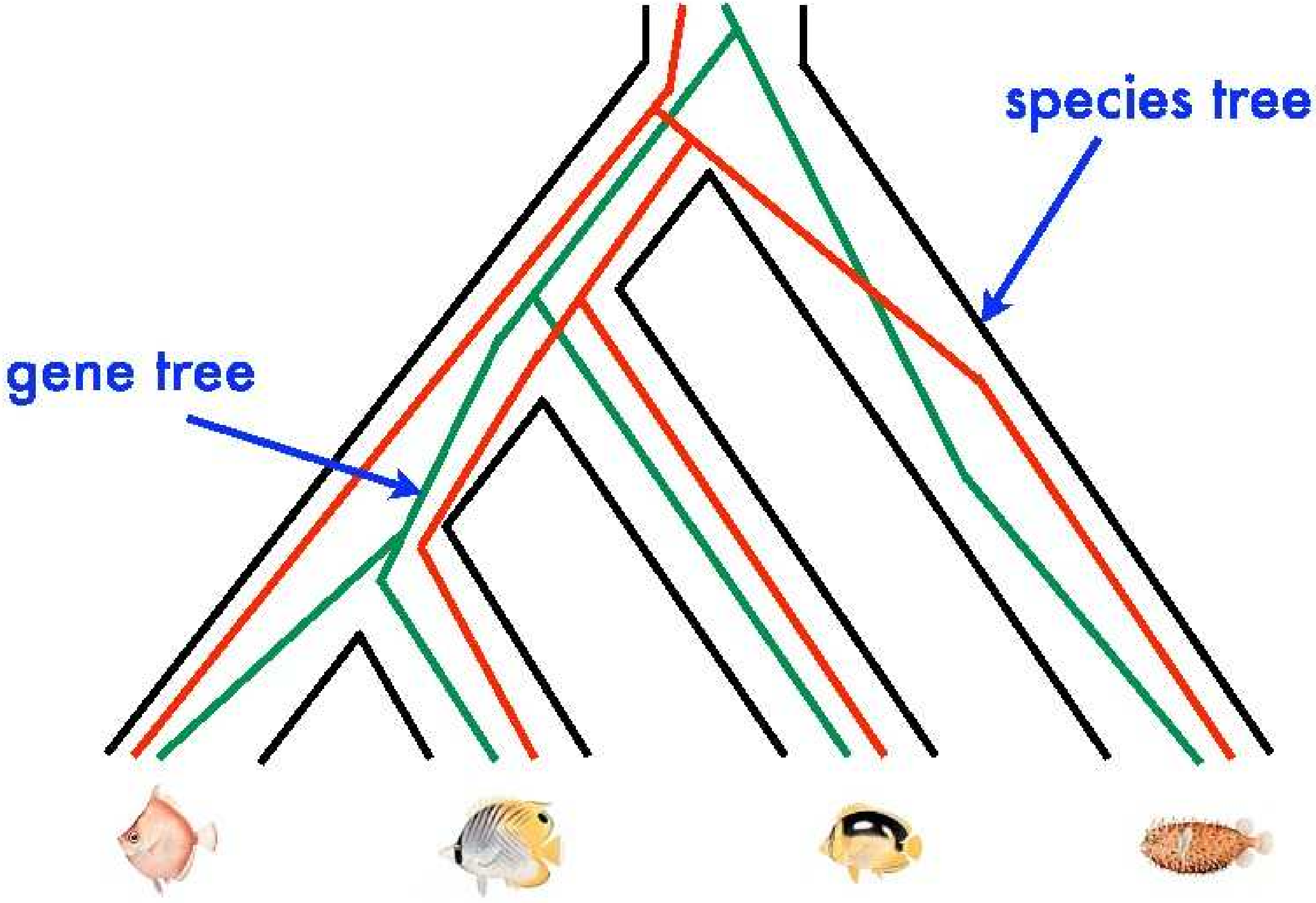


Ellipses = candidates for horizontal gene transfer

Figure 2. Phylogram of the *Protocalliphora* genus based upon COI and COII data (total of 953 bp). The tree was generated by maximum likelihood analysis using a heuristic search with tree bisection–reconnection. Bootstrap values are shown as percentage of 1000 replicates at each node only if they are 50% or greater. The *Wolbachia* infection status of each individual is shown on the tree. Individuals infected with *w*A1, *w*A2 or *w*B *Wolbachia* strains are respectively represented by an open triangle, a solid triangle and a circle. Non-infected individuals are symbolized by NI. Three clusters defined using 3 or 1.8% divergence as threshold values (§3) are shown on the figure. The three ellipses indicate cases where horizontal transfer of *Wolbachia* between species seems probable (§3).

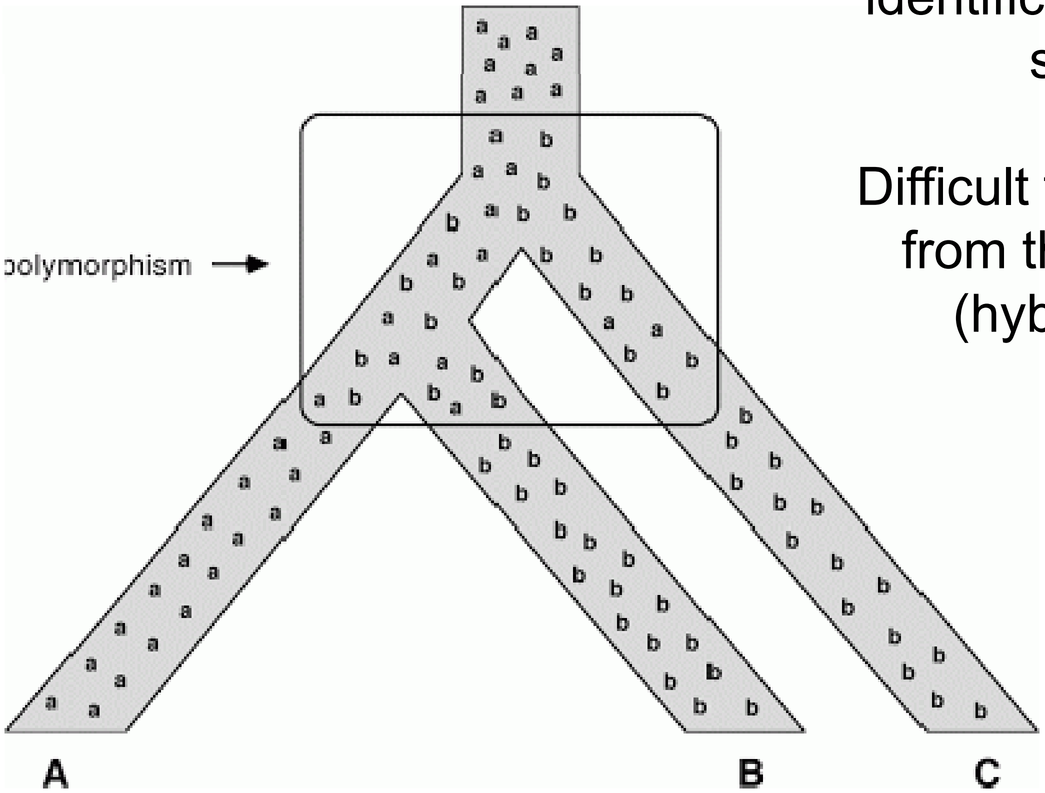
Horizontal transfer of mtDNA through *Wolbachia*
(among closely related species, at the level of genera the barcoding is OK)

2. Gene tree vs. species tree



One gene (mitochondrial) is not (sometimes) sufficient for species delimitation

Incomplete lineage sorting



Problems of species identification in young species

Difficult to distinghuish from the gene flow (hybridization)

Time to fix the polymorphisms = 2 N_e generations

Statistical species delimitation

Syst. Biol. 56(3):400–411, 2007

ISSN: 1063-5157 print / 1076-836X online
DOI: 10.1080/10635150701405560

Estimating Species Phylogeny from Gene-Tree Probabilities Despite Incomplete Lineage Sorting: An Example from *Melanoplus* Grasshoppers

BRYAN C. CARSTENS AND L. LACEY KNOWLES

Department of Ecology and Evolutionary Biology, 1109 Geddes Avenue, Museum of Zoology, University of Michigan, Ann Arbor, MI 48109-1079, USA; E-mail: knowlesl@umich.edu (L.L.K.)

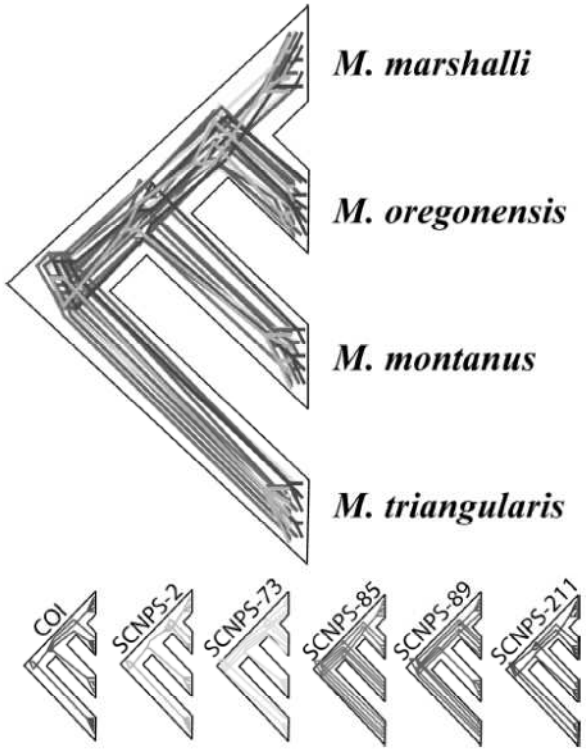


FIGURE 3. Estimate of the species phylogeny (ESP) with the highest likelihood score ($-\ln L = -10{,}487.1938$) calculated with the coalescent-based approach from the probabilities of the gene trees (see Fig. 2), with each gene tree shown within the species tree.

*BEAST
(species trees)

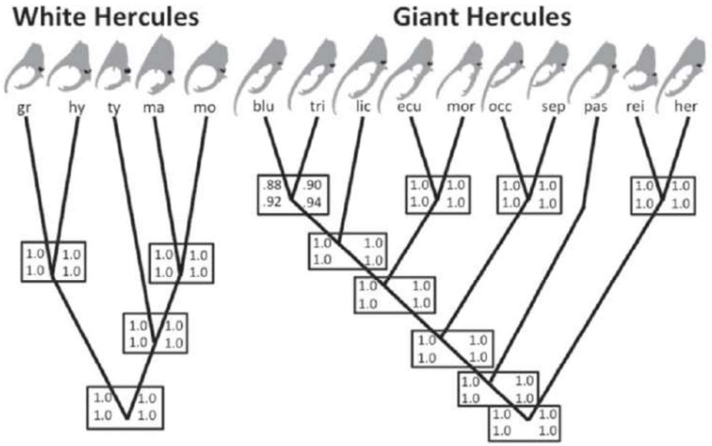


FIGURE 5. Results from iBPP analyses using combined genetic and morphological data sets; note that outlines of major male horn shape shown adjacent to the abbreviated taxonomic names are not drawn to scale (see Fig. 1 for relative body sizes). Support values reported for each node are based on the algorithm setting 0 for the rjMCMC, the algorithm setting 1 for the Brownian motion model, and for four different priors corresponding to large (vs small) ancestral population sizes with relatively deep (vs shallow) divergence times. Specifically, the support values in each box correspond to analyses with the following different priors: upper left, $\theta = G(1, 10)$ and $\tau = G(1, 10)$; lower left, $\theta = G(1, 10)$ and $\tau = G(2, 2000)$; upper right, $\theta = G(2, 2000)$ and $\tau = G(1, 10)$; lower right, $\theta = G(2, 2000)$ and $\tau = G(2, 2000)$. Taxonomic abbreviations and the corresponding names can be found in Figure 1.

BPP and iBPP

Syst. Biol. 0(0):1–15, 2016

DOI:10.1093/sysbio/syv119

The Species versus Subspecies Conundrum: Quantitative Delimitation from Integrating Multiple Data Types within a Single Bayesian Approach in Hercules Beetles

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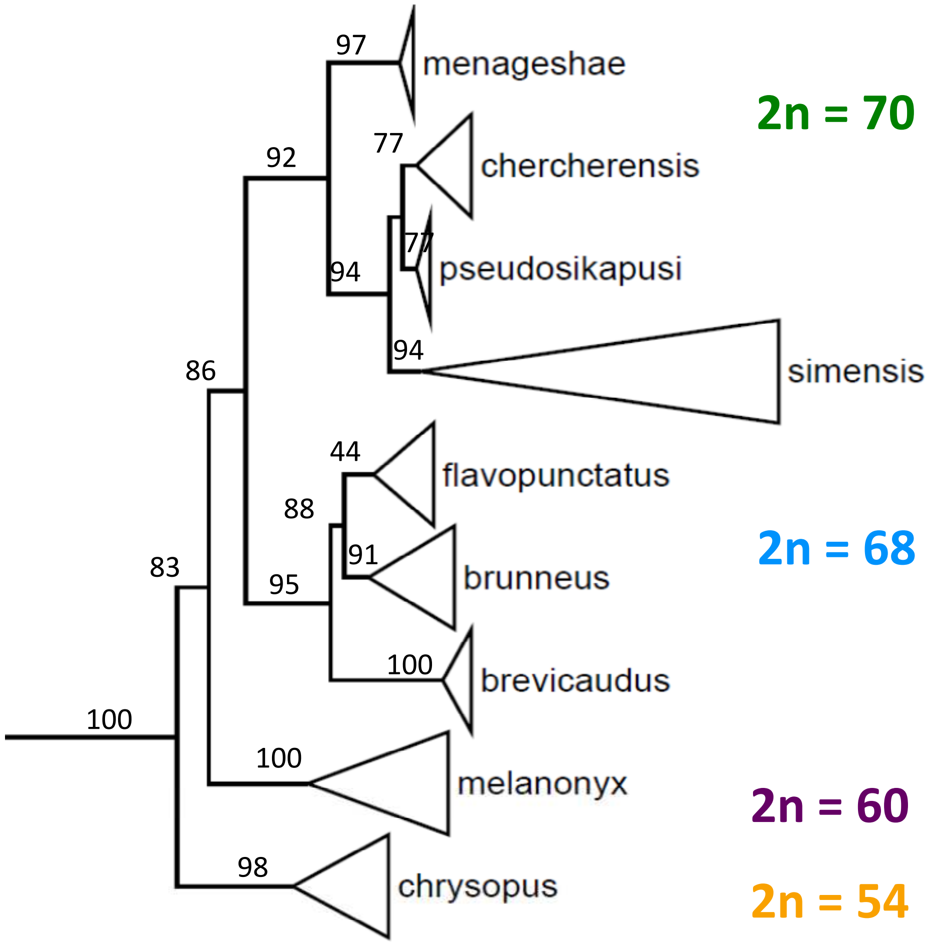
**Correspondence to be sent to: Department of Ecology and Evolutionary Biology, 1109 Geddes Avenue, Museum of Zoology, University of Michigan, Ann Arbor, MI 48109-1079, USA; E-mail: huangjp@umich.edu.*

Lophuromys flavopunctatus complex in Ethiopia

Sanger sequencing



2n = 70

menangeshae

chercherensis

pseudosikapusi

simensis

2n = 68

flavopunctatus

brunneus

brevicaudus

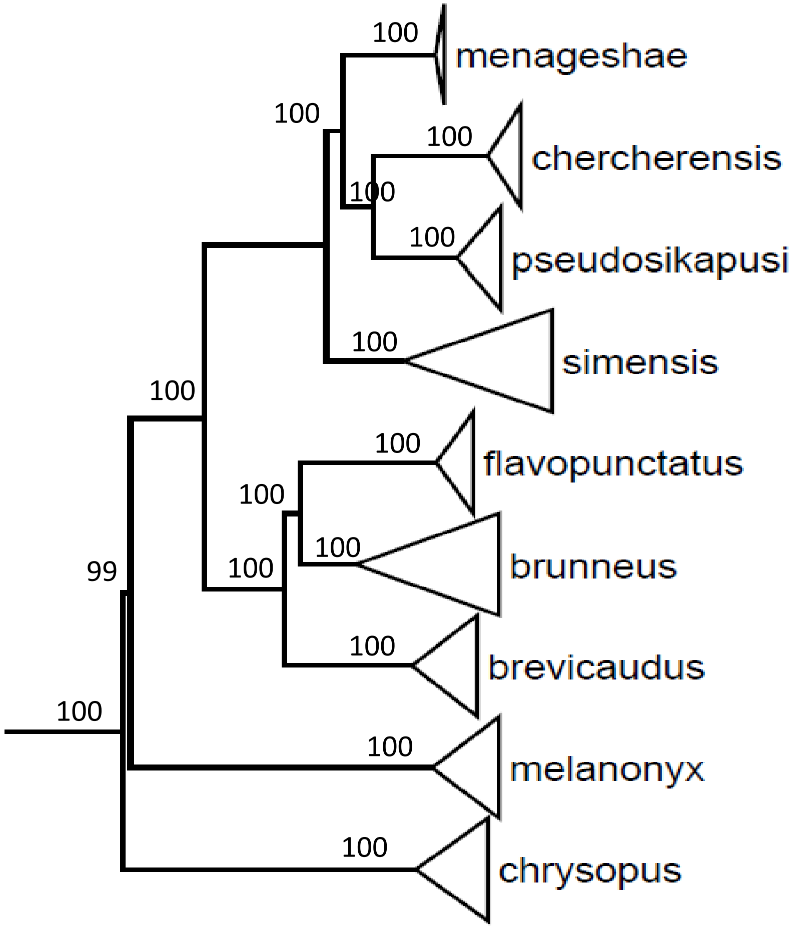
2n = 60

melanonyx

2n = 54

chrysopus

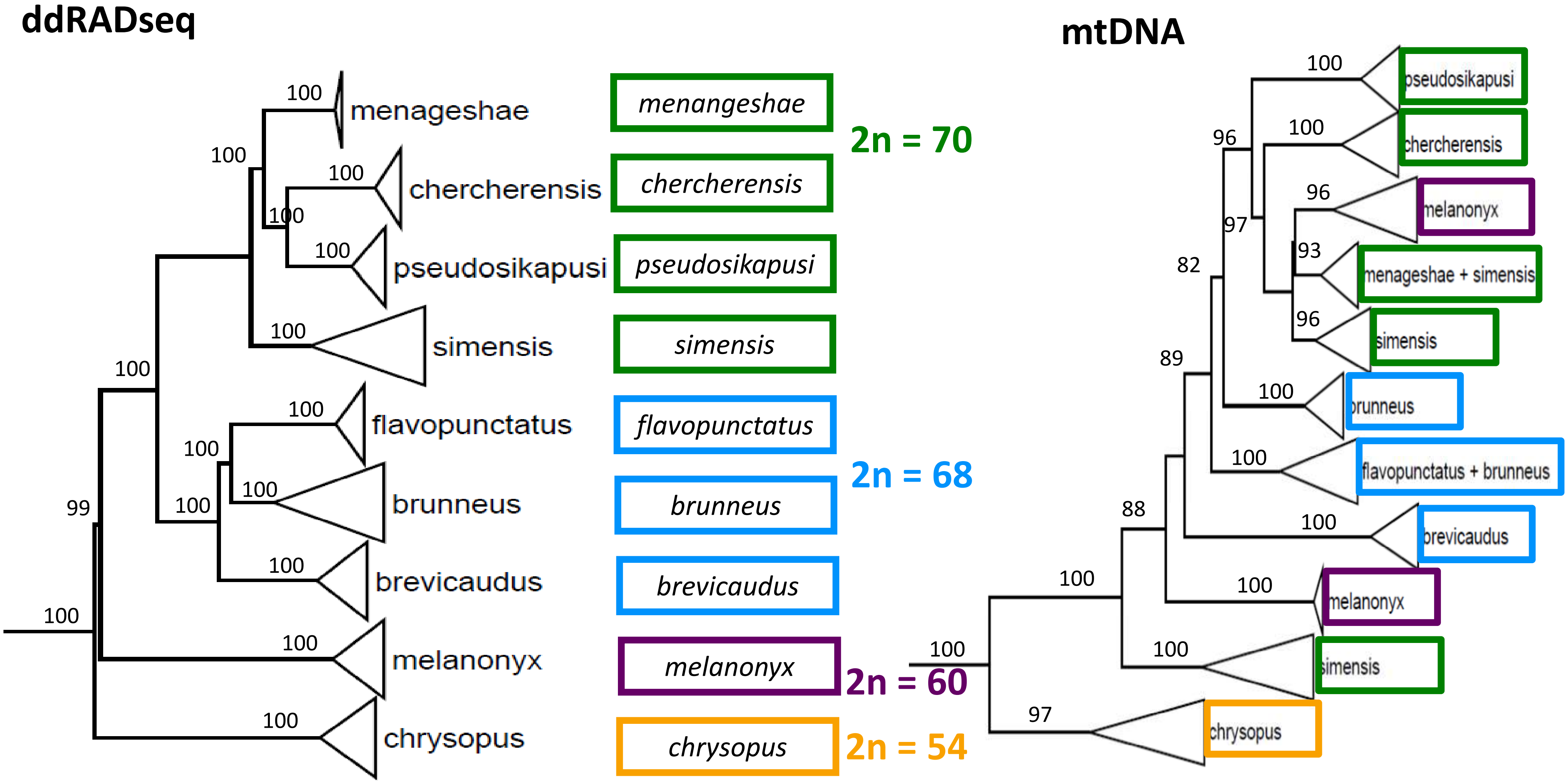
ddRADseq



4 nuclear markers (Komarova et al., submitted)
(2 604 bp concatenated dataset)

15 623 informative loci
(Mikula et al., in prep.)

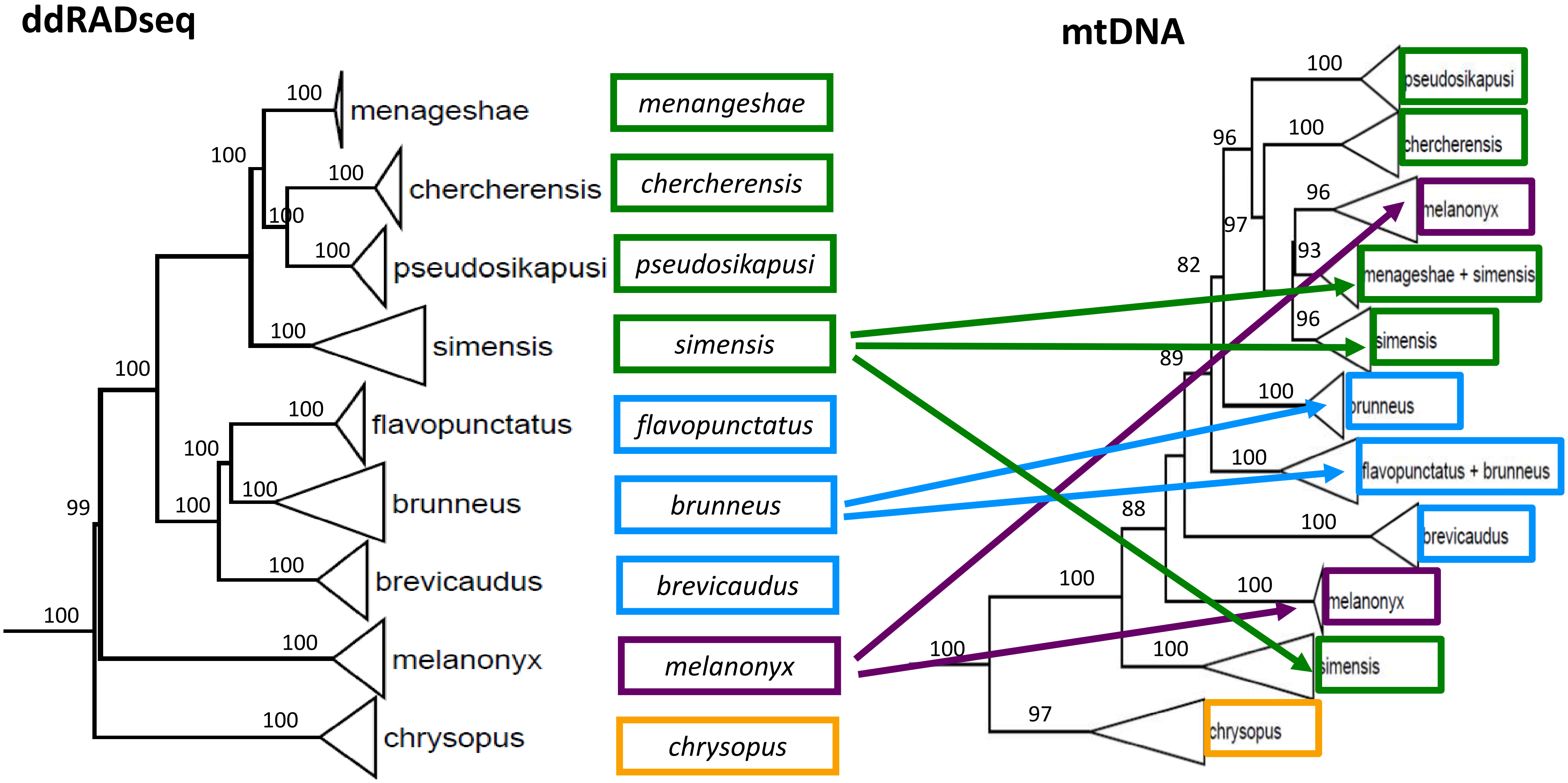
And what about mtDNA?



15 623 informative loci

cytochrome *b* (1140 bp)

And what about mtDNA?

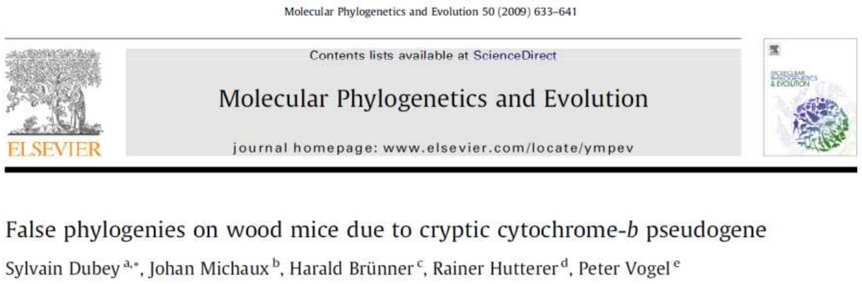


15 623 informative loci

cytochrome *b* (1140 bp)

„reticulate evolution“

3. Pseudogenes



Molecular Phylogenetics and Evolution 50 (2009) 633–641

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

False phylogenies on wood mice due to cryptic cytochrome-*b* pseudogene

Sylvain Dubey[a,*], Johan Michaux[b], Harald Brünner[c], Rainer Hutterer[d], Peter Vogel[e]

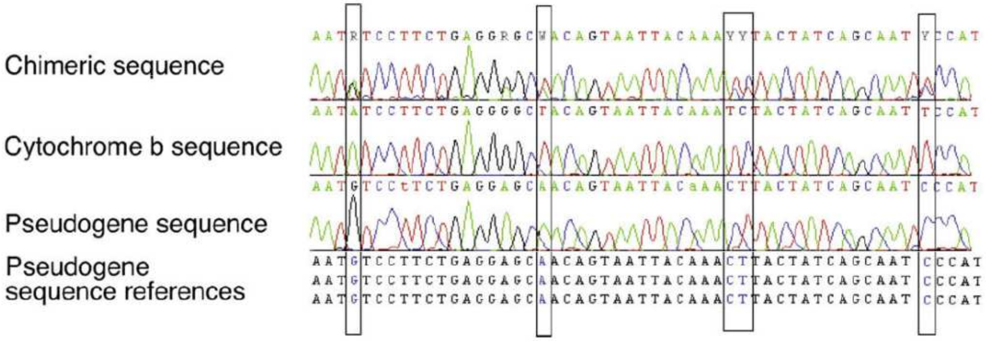
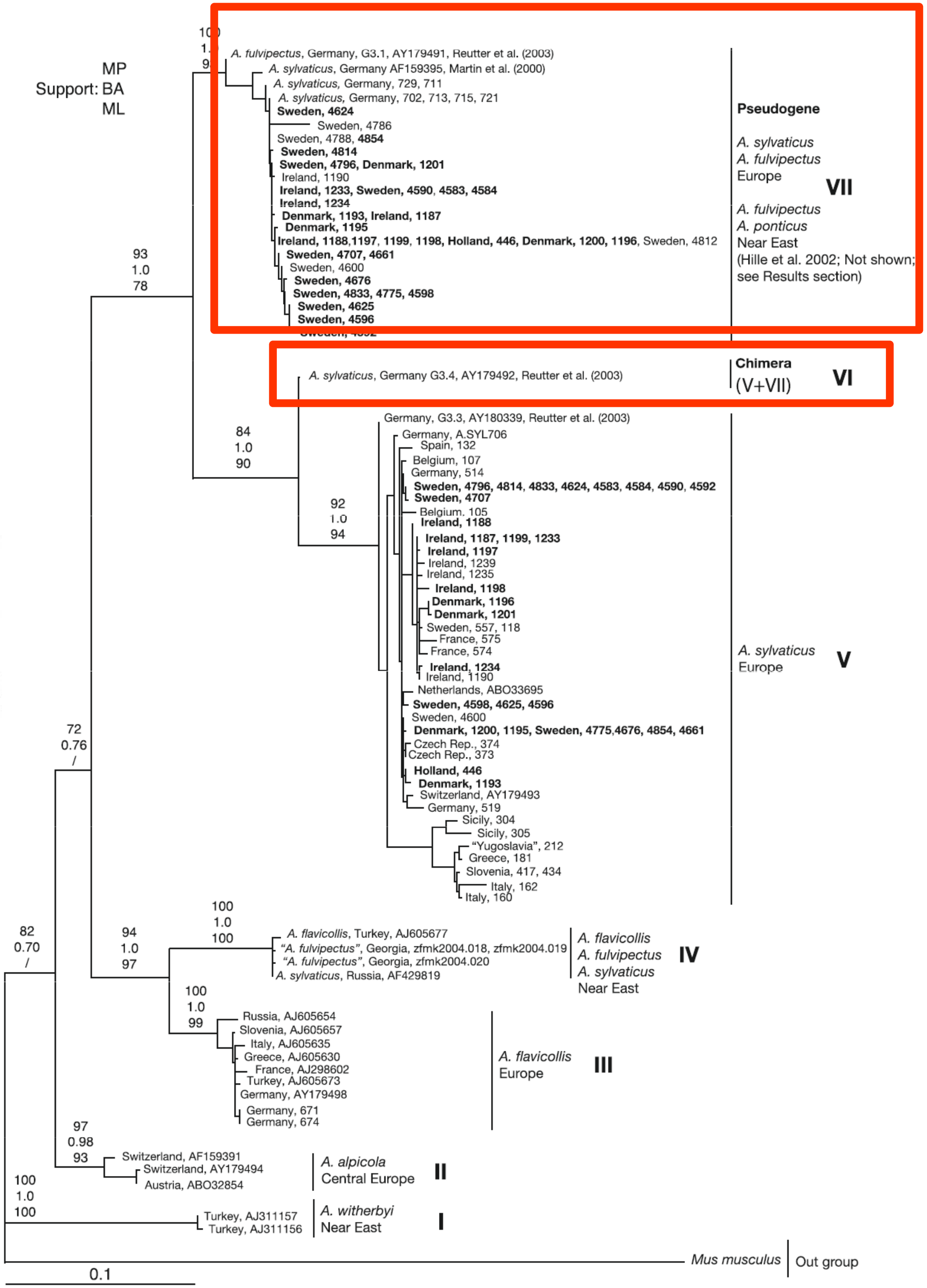


Fig. 2. Examples of electropherograms of a chimeric *cyt-b* sequence of lineage V/VII (VI) and of pure lineages V and VII.

Heterozygotes in mtDNA → be careful!

NUMTS = „**nu**clear copy of **mt**DNA **s**equences

Relatively often for cytochrome *b*



How to recognize numt?

- ultracentrifugation (fresh samples required)

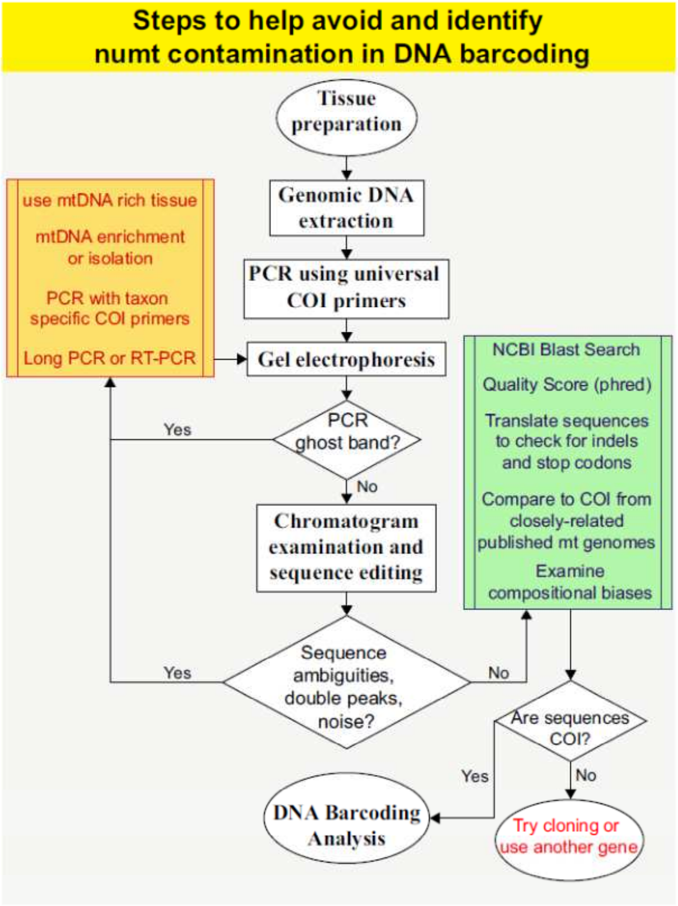
- the use of tissues with high proportion of mitochondria (e.g. muscles)

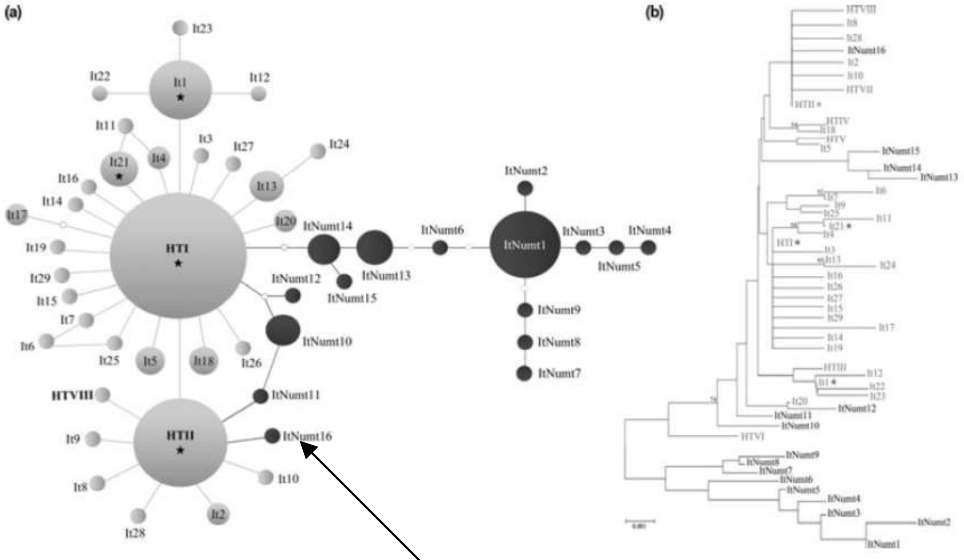
- *long-range PCR* (or sequence complete mtDNA)

- RT-PCR (pseudogenes are not transcribed)

- indels, stop codons

- cloning


Steps to help avoid and identify numt contamination in DNA barcoding



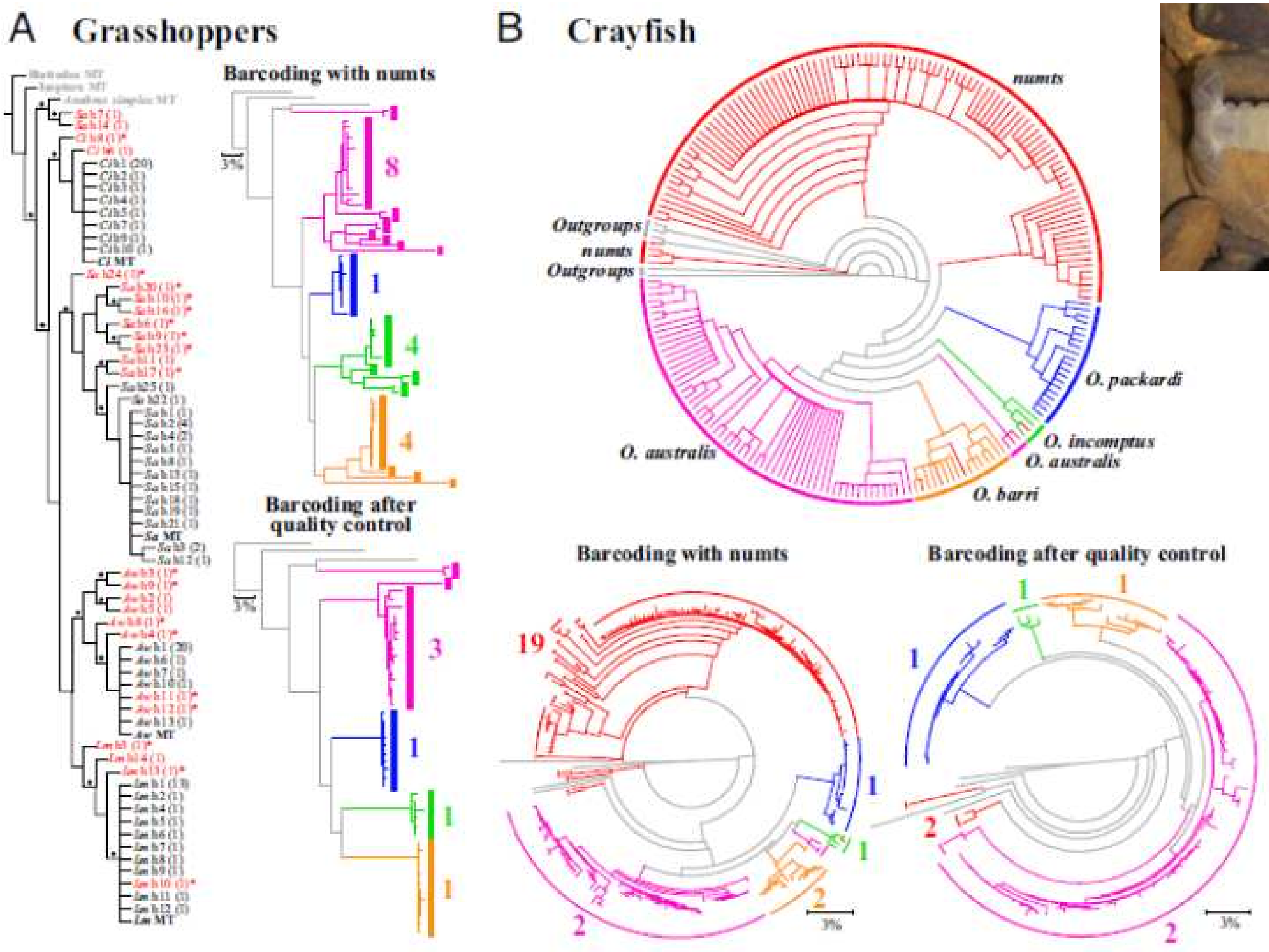
cryptic numts

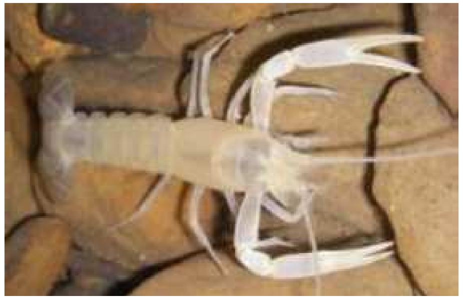
Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified

Hojun Song*†, Jennifer E. Buhay*‡, Michael F. Whiting*, and Keith A. Crandall*

*Department of Biology, Brigham Young University, Provo, UT 84602; and ‡Belle W. Baruch Institute for Marine Sciences, University of South Carolina, Columbia, SC 29208

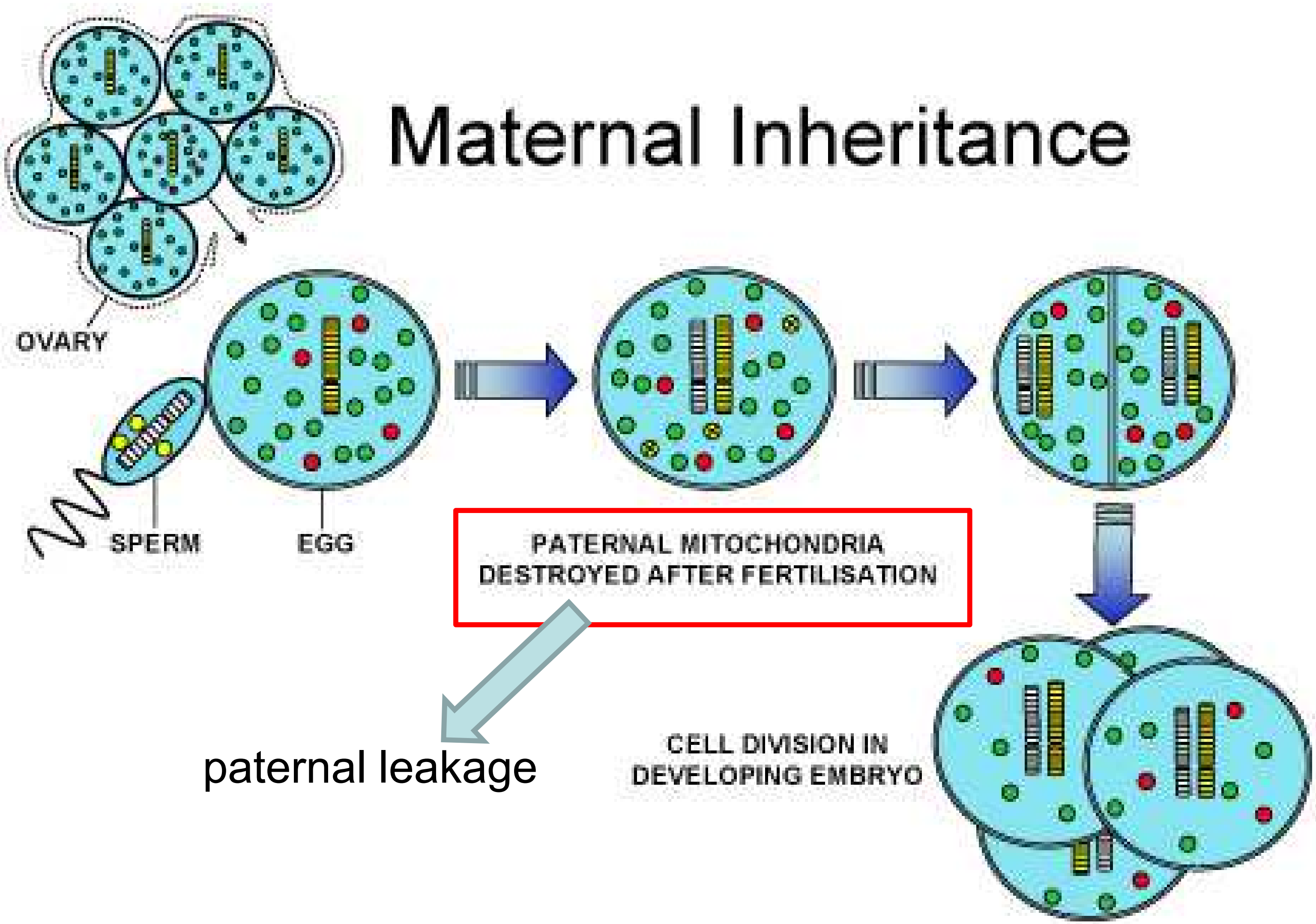
Edited by W. Ford Doolittle, Dalhousie University, Halifax, NS, Canada, and approved July 14, 2008 (received for review March 28, 2008)





number of suggested barcoded taxa based on 3% divergence on COI with/without numts (identified by stop-codons and indels)

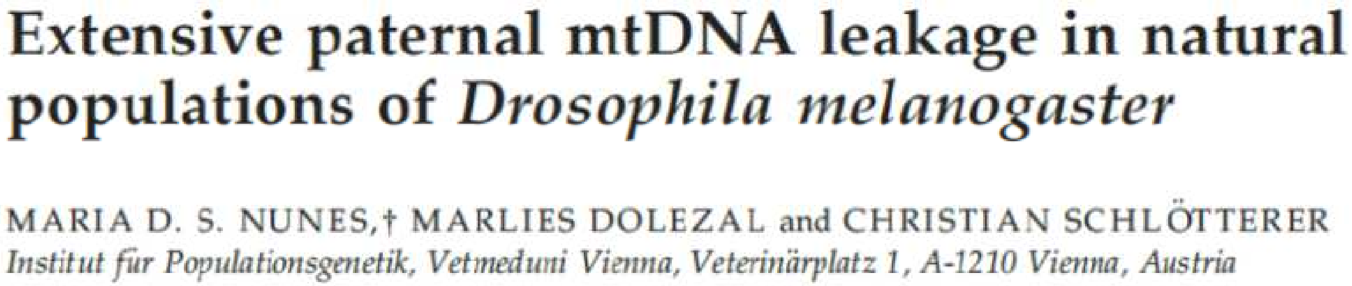
Heteroplasmy



paternal leakage

- well studied mitochondrial disorders in human
- low N_e of mtDNA → usually fast fixation of new mutations – mitochondrial bottleneck

Paternal leakage

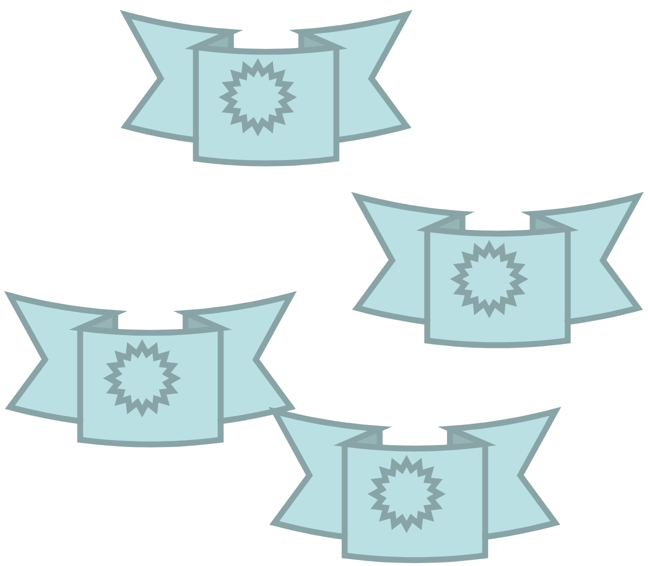


Extensive paternal mtDNA leakage in natural populations of *Drosophila melanogaster*

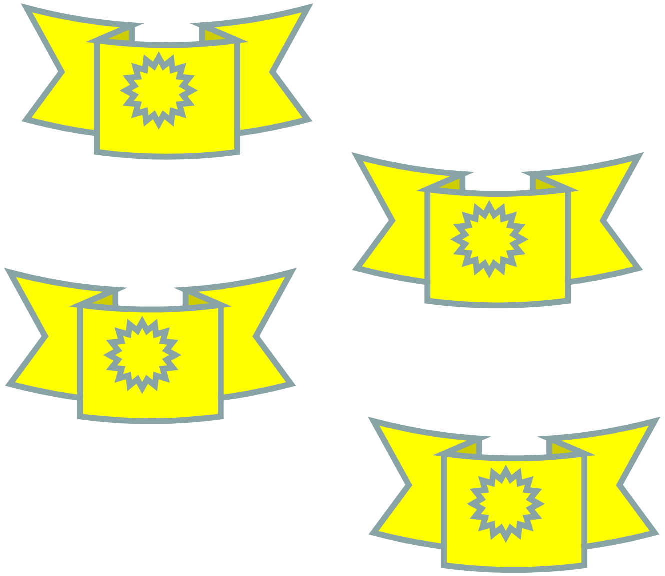
MARIA D. S. NUNES,† MARLIES DOLEZAL and CHRISTIAN SCHLÖTTERER
Institut für Populationsgenetik, Vetmeduni Vienna, Veterinärplatz 1, A-1210 Vienna, Austria

- allele-specific real-time quantitative PCR (RT-qPCR) → heteroplasmie je asi častý jev
- 14 % jedinců, ale velmi nízká frekvence druhého haplotypu
- paternal leakage 6 %

4. Introgression/replacement of mtDNA



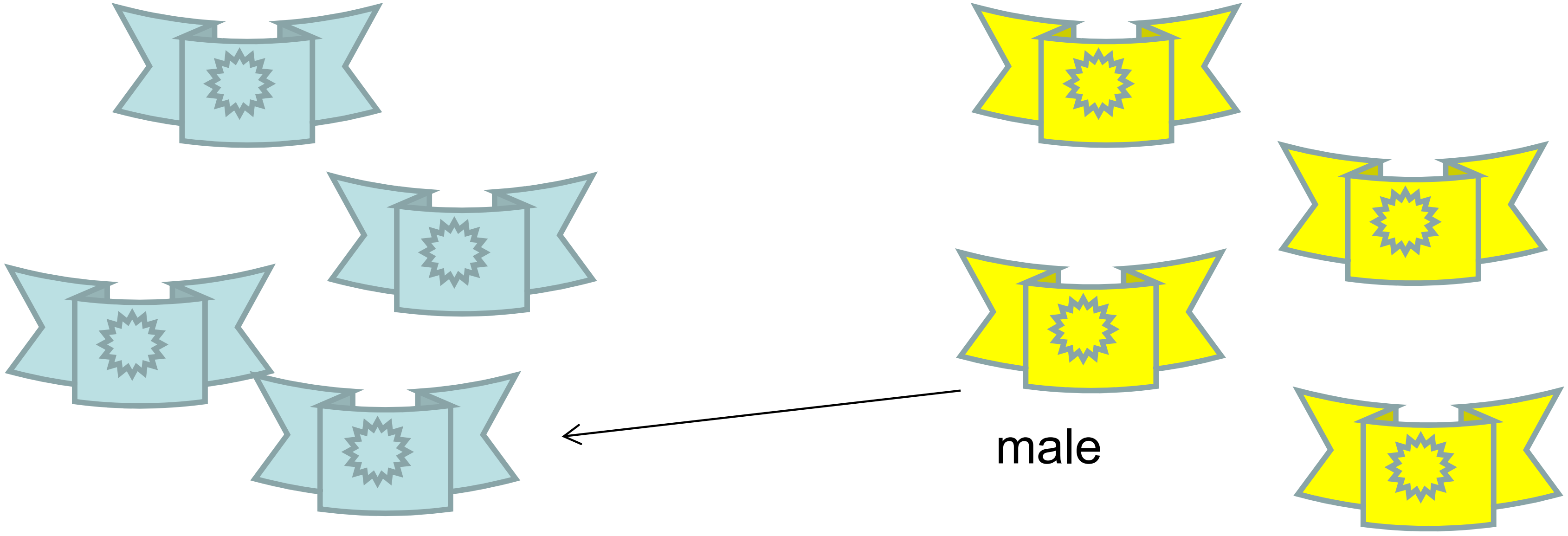
Myotis myotis - Europe



Myotis blythii - Asia

Berthier et al. 2006

Myotis blythii vs. *Myotis myotis* - mtDNA replacement

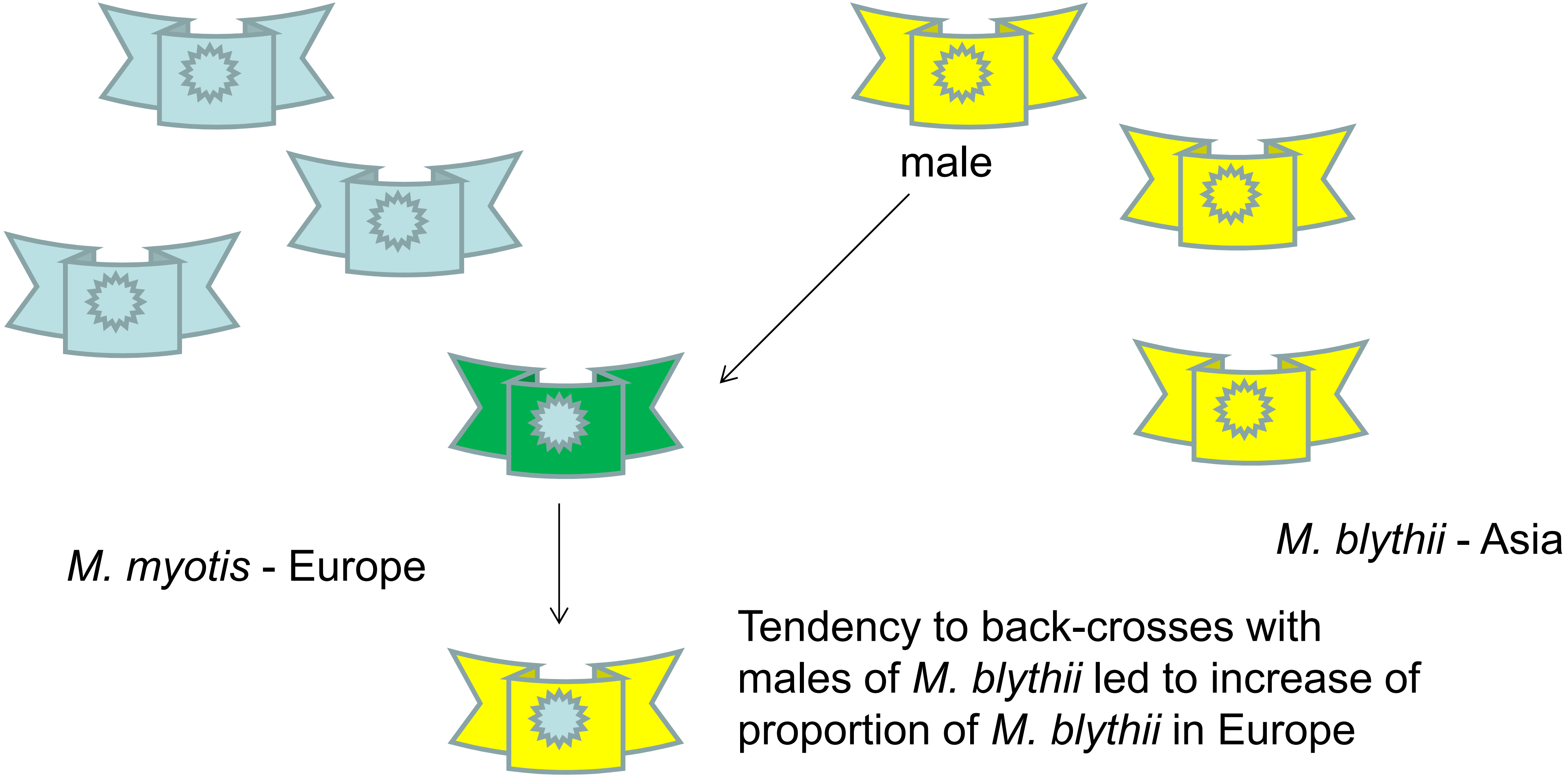


male

M. myotis - Europe

M. blythii - Asia

Myotis blythii vs. *Myotis myotis* - mtDNA replacement



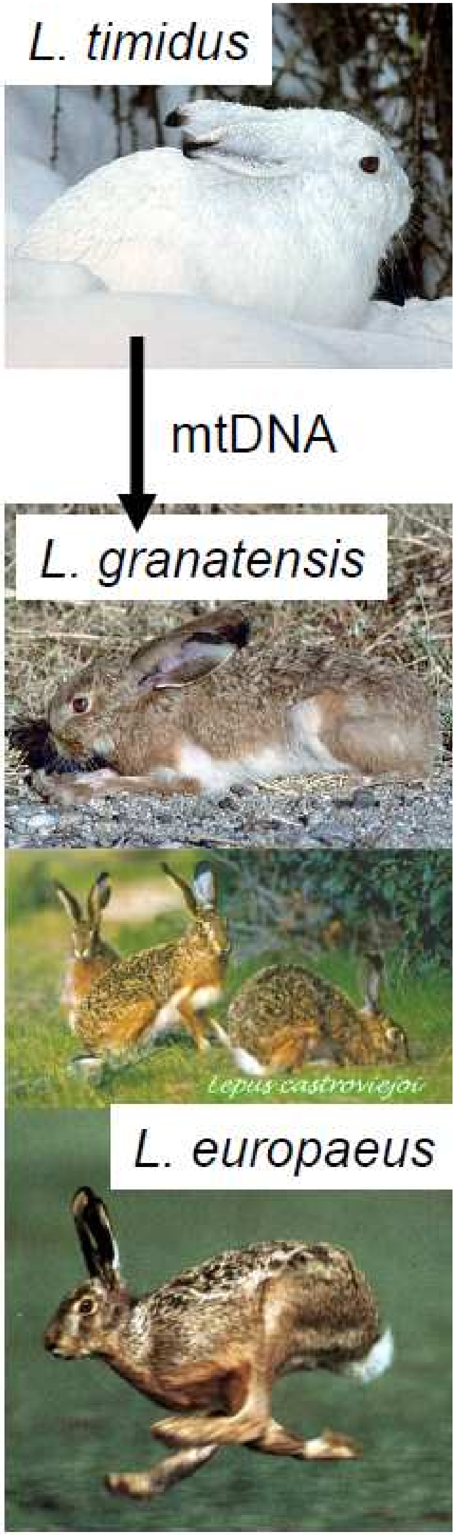
Tendency to back-crosses with males of *M. blythii* led to increase of proportion of *M. blythii* in Europe

Colonizing (invasive) species often adopt mtDNA of original speices *(Currat et al. 2008)*

Zajíci ve Španělsku a Portugalsku

L. timidus mtDNA

- U zajíců *Lepus granatensis, L. castroviejoi* a *L. europaeus* je často mtDNA z *L. timidus*
- *L. timidus* odtud však vymizel na konci doby ledové
- Známo u řady jiných druhů: netopýři, čolci, siveni
- Neutrální proces v důsledku expanze



Molecular Ecology (2010) 19, 4783–4799 doi: 10.1111/j.1365-294X.2010.04847.x

Plio-Pleistocene history of West African Sudanian savanna and the phylogeography of the *Praomys daltoni* complex (Rodentia): the environment/geography/genetic interplay

J. BRYJA,* L. GRANJON,†[1] G. DOBIGNY,†[2] H. PATZENHAUEROVÁ,* A. KONEČNÝ,*†
J. M. DUPLANTIER,† P. GAUTHIER,† M. COLYN,‡ L. DURNEZ,§ A. LALIS¶ and V. NICOLAS¶

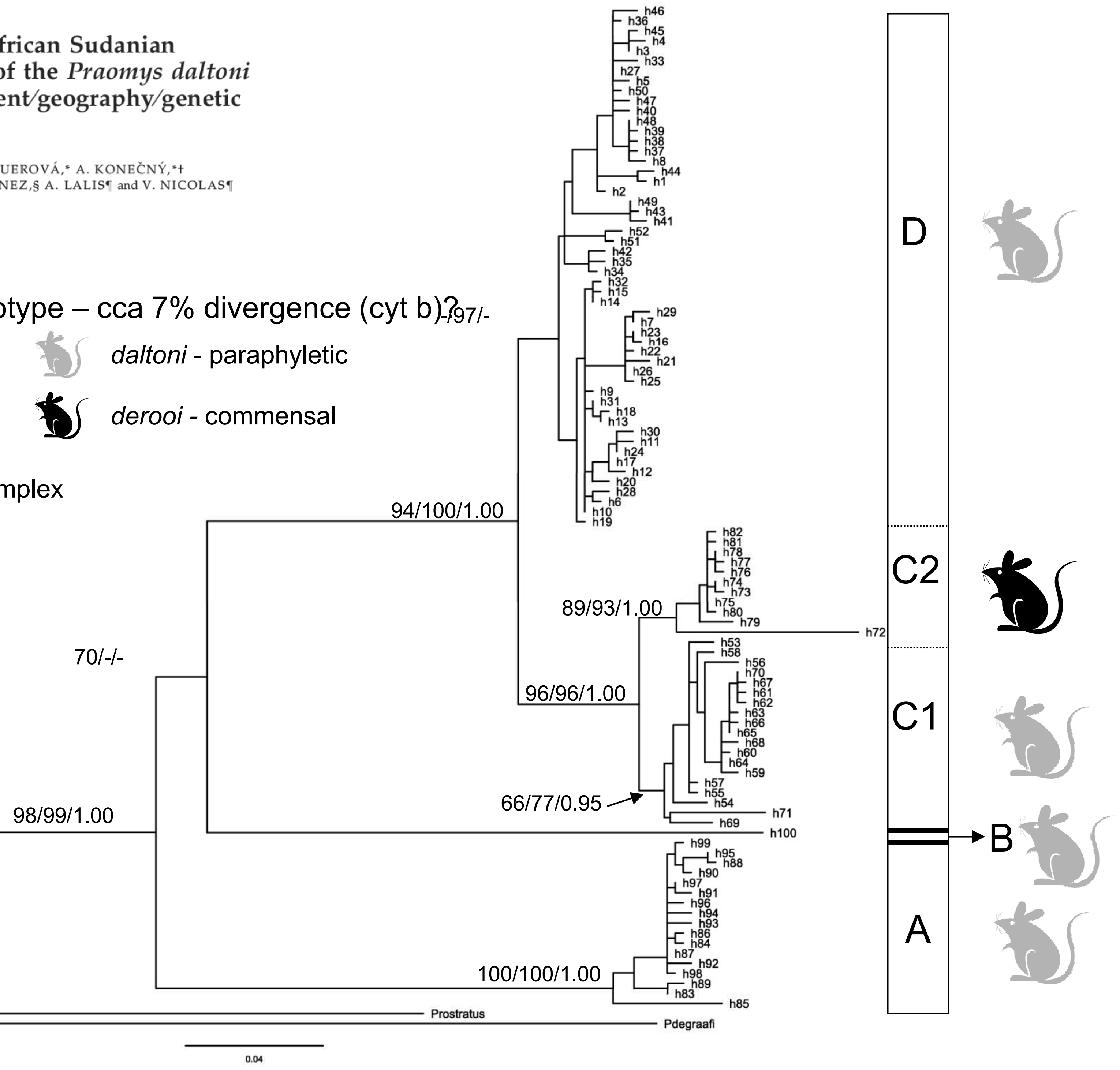
How many species?

Four based on mtDNA genotype – cca 7% divergence (cyt b)?
Two based on phenotype?

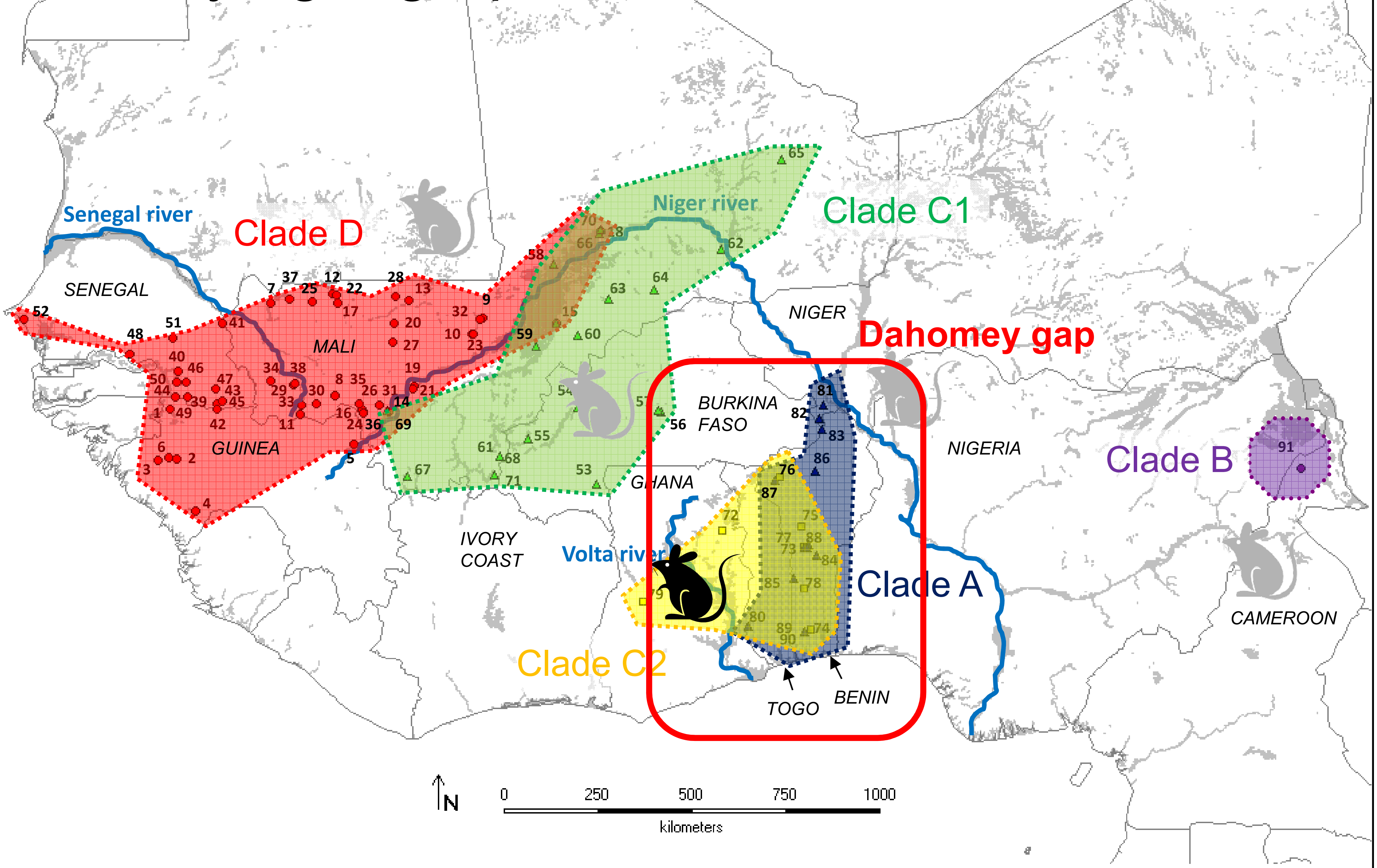
daltoni - paraphyletic

derooi - commensal

Příklad: *Praomys* cf. *daltoni* complex



Phylogeographic structure at mtDNA



Partial mtDNA introgression in Dahomey gap

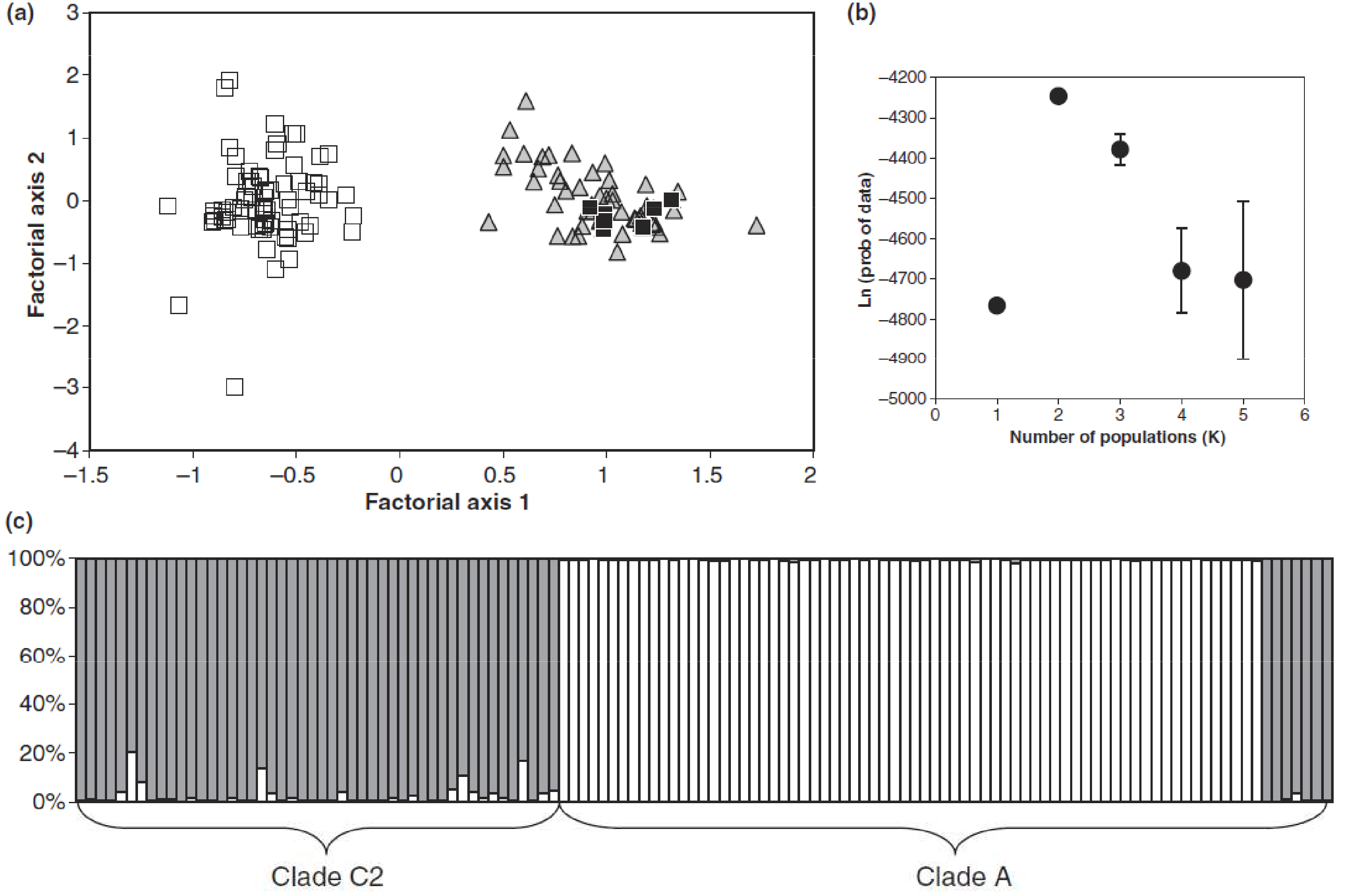
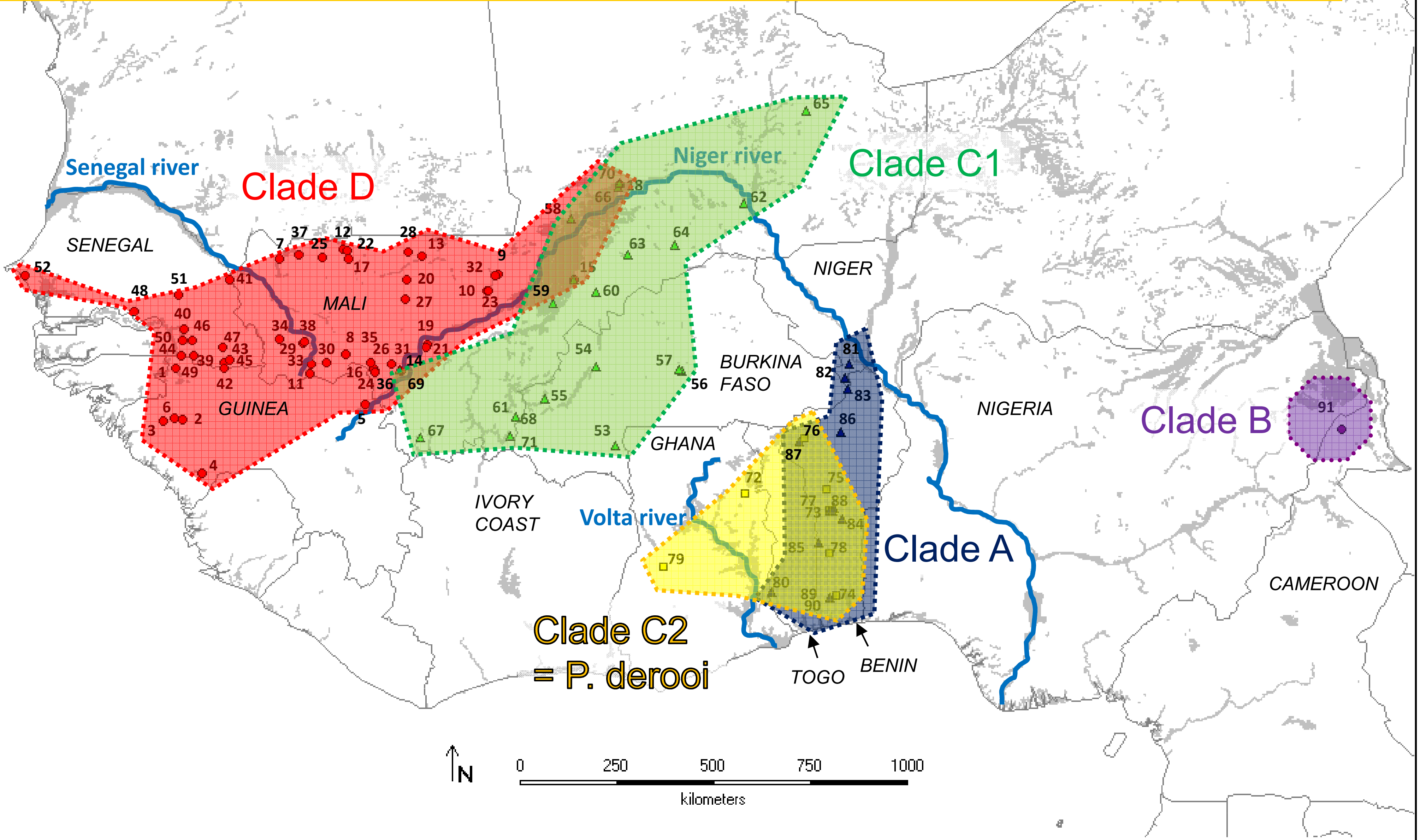
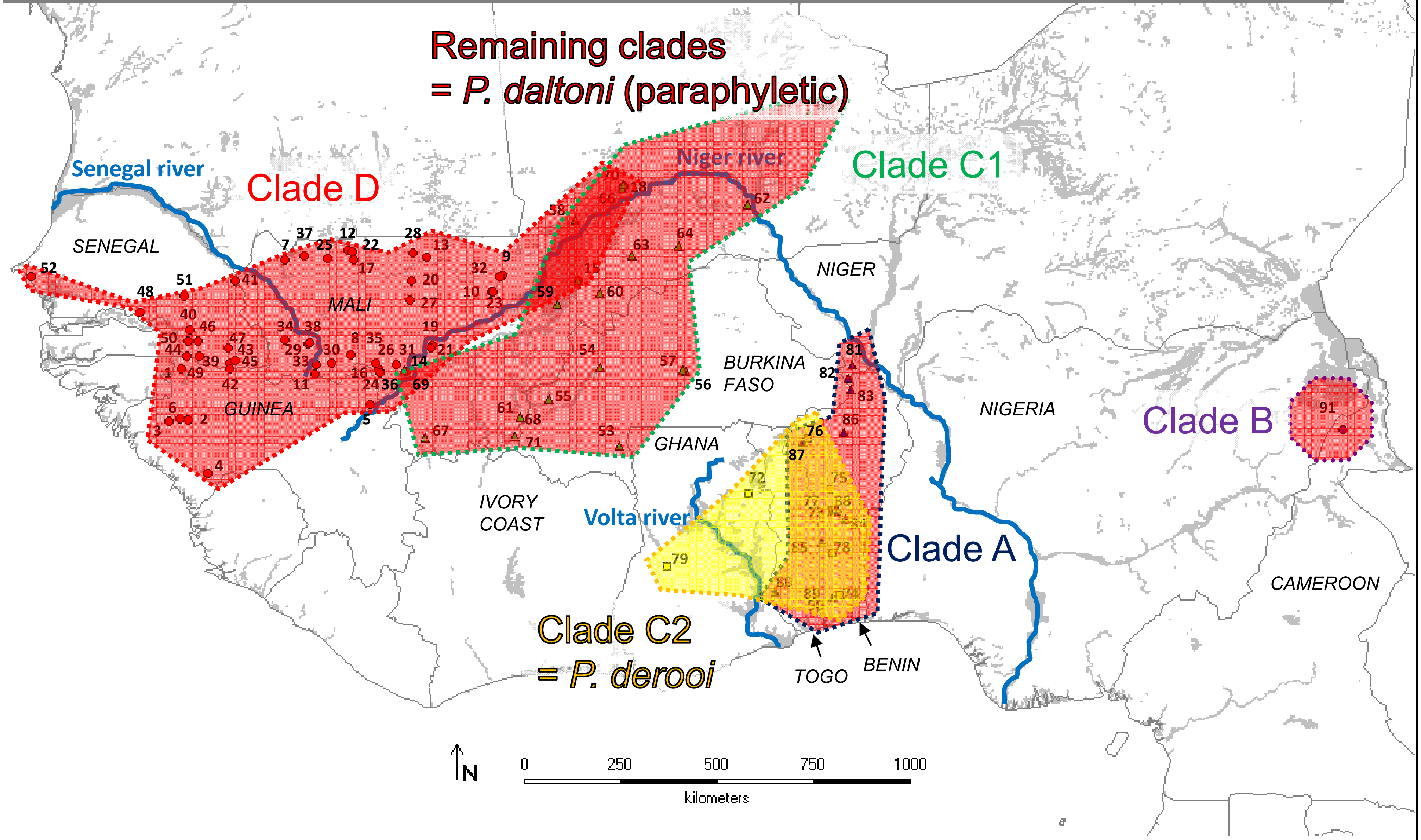


Fig. 6 Results of microsatellite analysis of individuals from Benin. (a) Factorial correspondence analysis in Genetix; *squares*—individuals with mtDNA from clade A, *triangles*—from clade C2. Note the seven individuals with mtDNA from clade A, but clustered within individuals with mtDNA from clade C2 (marked by *black squares*). (b) Likelihood of models in STRUCTURE for increasing number of populations (K); the highest likelihood is observed for $K = 2$. (c) Assignment of individuals to particular populations using the best model (i.e. $K = 2$; the run with the highest likelihood) in STRUCTURE.

What is species???



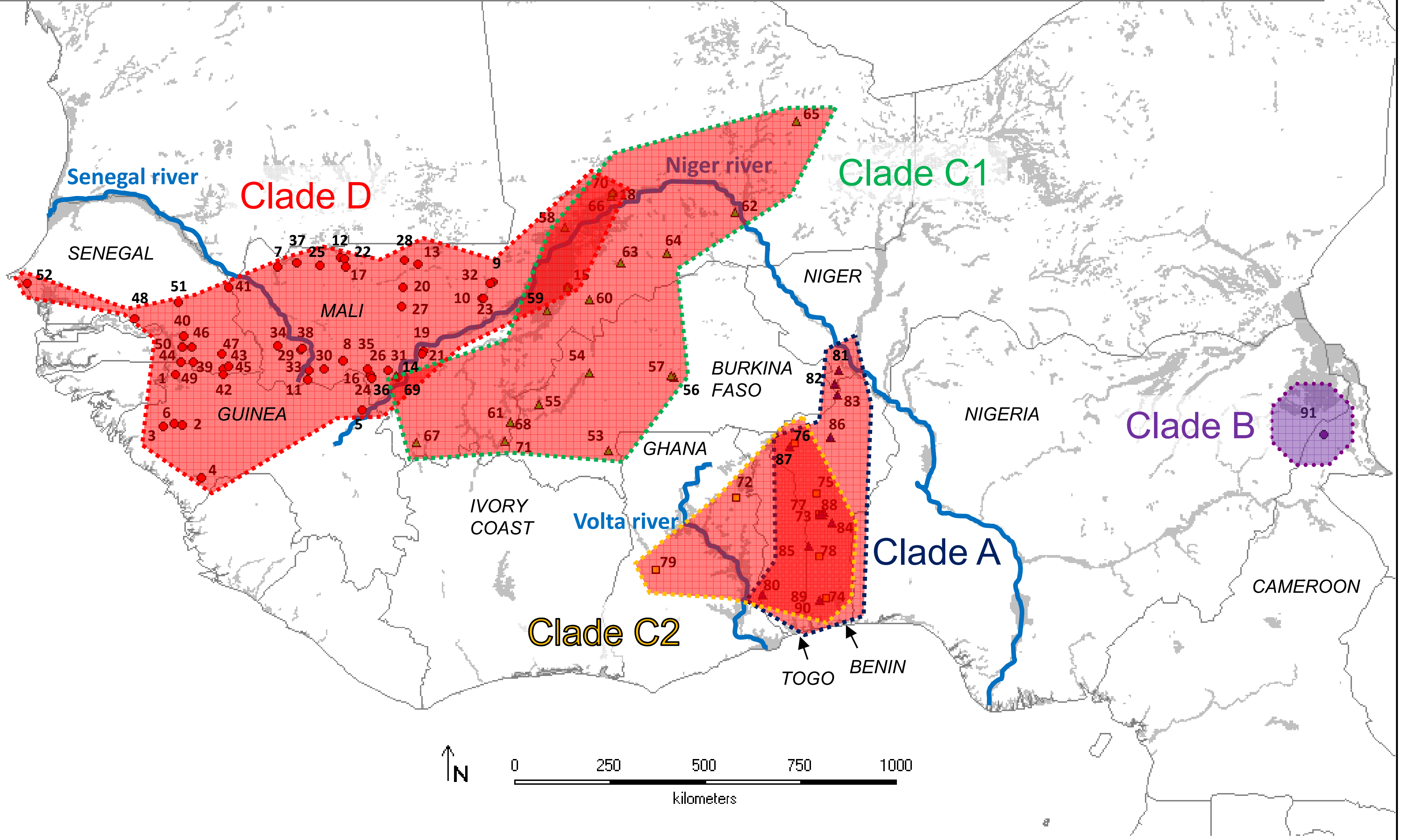
Morphology and ecology



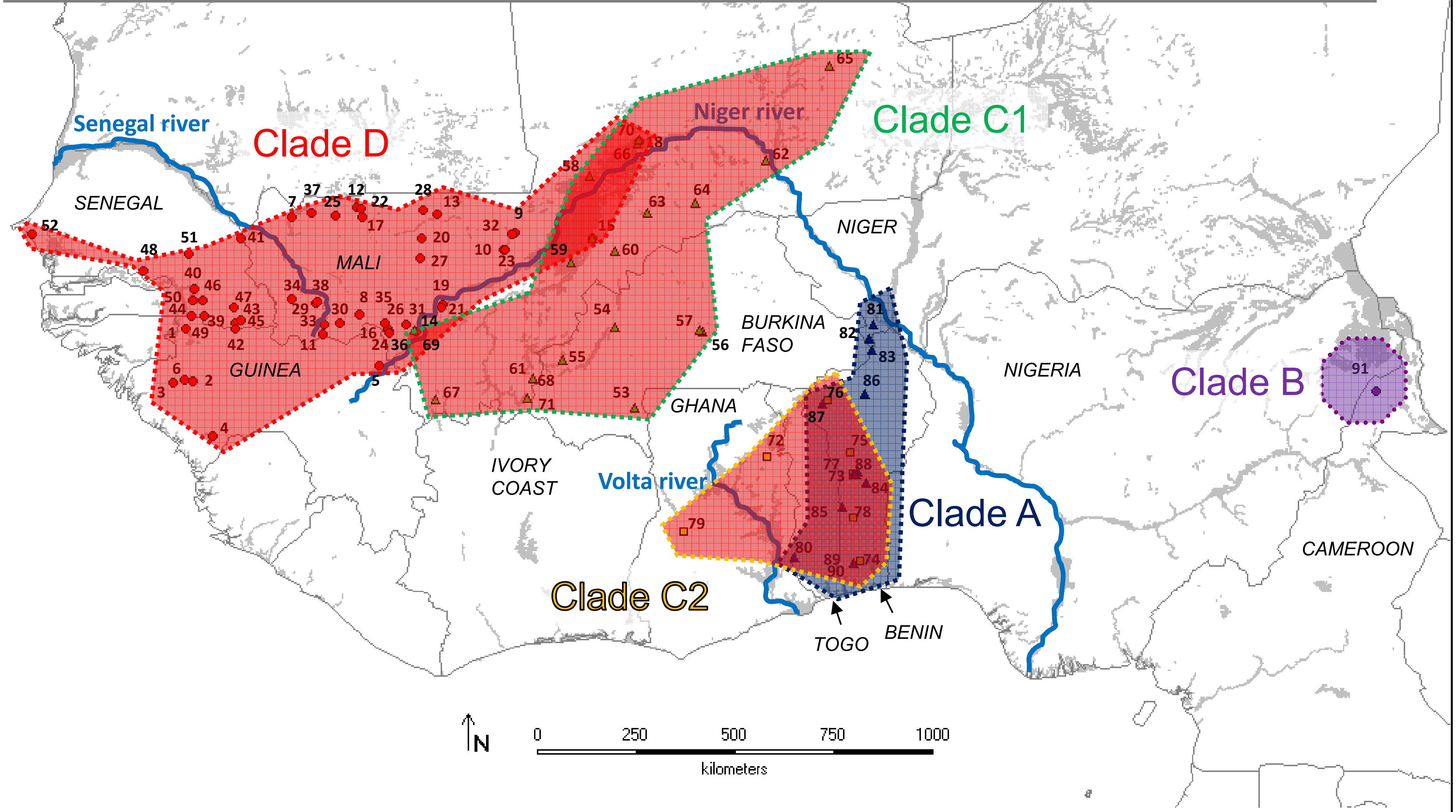
Remaining clades
= *P. daltoni* (paraphyletic)

Clade C2
= *P. derooi*

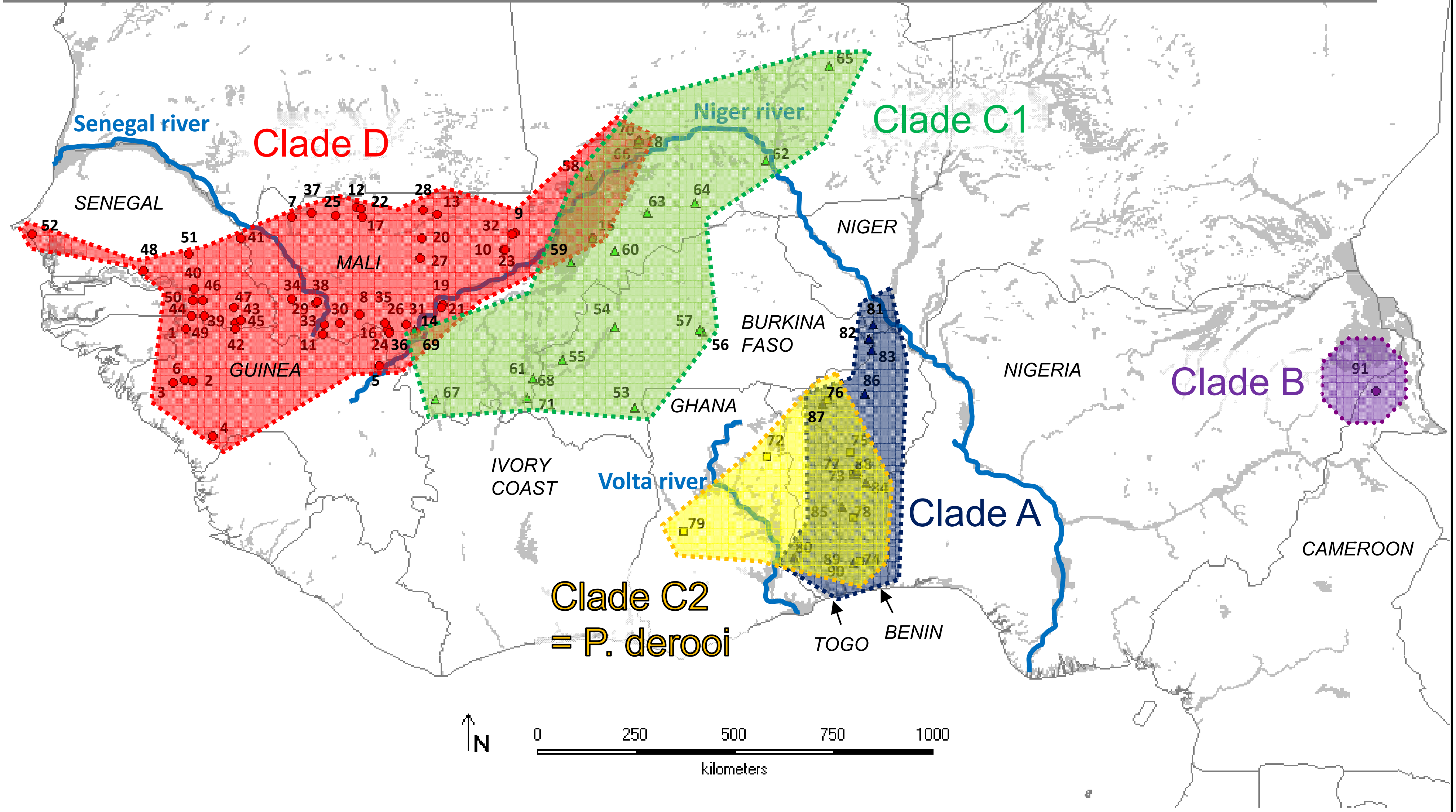
Karyotypes


Senegal river
Clade D
Niger river
Clade C1
SENEGAL
MALI
NIGER
BURKINA FASO
NIGERIA
Clade B
GUINEA
GHANA
IVORY COAST
Volta river
Clade A
Clade C2
CAMEROON
TOGO
BENIN
65
70
18
66
62
58
37
12
28
7
25
22
13
64
63
9
52
17
32
15
51
41
20
48
10
59
60
27
23
40
MALI
34
38
19
46
8
35
50
47
26
31
21
54
44
43
29
30
14
57
81
1
39
45
33
16
82
49
42
11
24
36
69
56
55
83
6
61
68
86
2
3
67
71
53
76
4
5
87
72
75
77
88
73
84
85
78
79
80
89
74
90
91
N
0
250
500
750
1000
kilometers

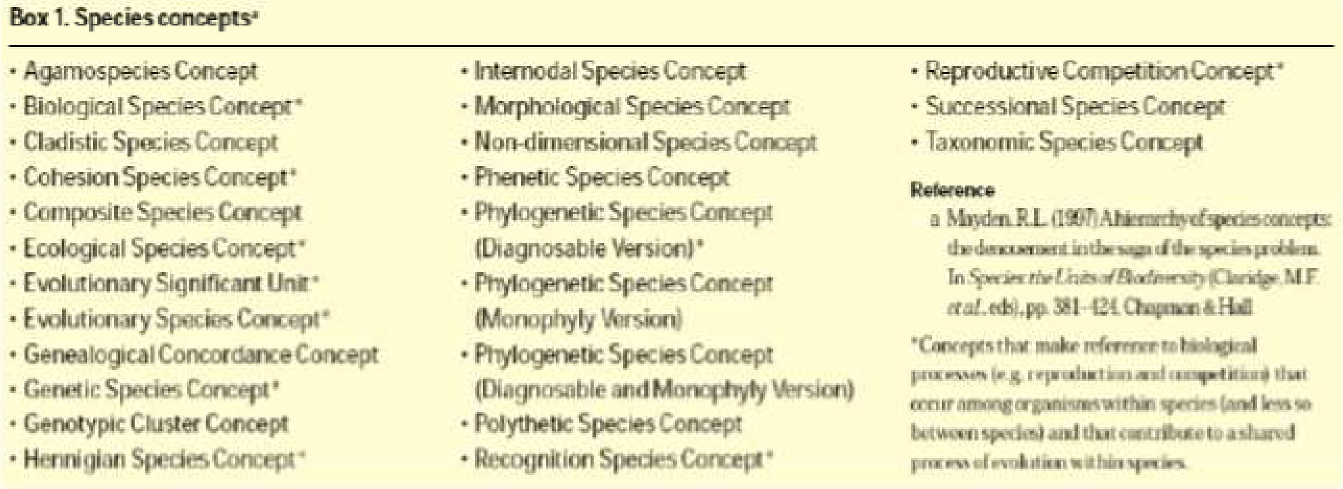
Mitochondrial DNA + microsatellites in Benin + karyotypes



Splitting approach taking morphology and ecology into account – the reproductive barriers between clades A, B, C1, and D remains to be identified



Existují vůbec druhy? Pokud ano, tak jak je potom definovat?



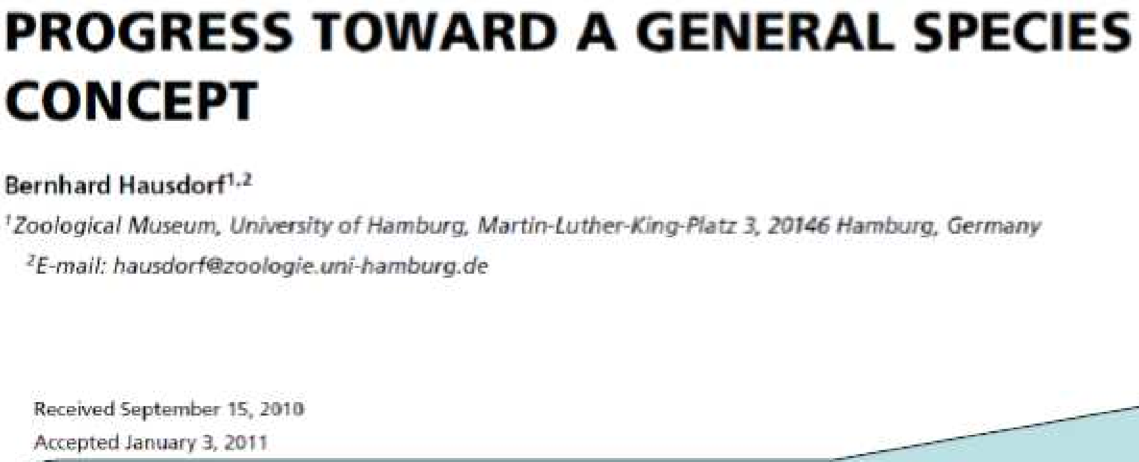
Box 1. Species concepts[a]

- Agamospecies Concept
- Biological Species Concept*
- Cladistic Species Concept
- Cohesion Species Concept*
- Composite Species Concept
- Ecological Species Concept*
- Evolutionary Significant Unit*
- Evolutionary Species Concept*
- Genealogical Concordance Concept
- Genetic Species Concept*
- Genotypic Cluster Concept
- Hennigian Species Concept*
- Internodal Species Concept
- Morphological Species Concept
- Non-dimensional Species Concept
- Phenetic Species Concept
- Phylogenetic Species Concept (Diagnosable Version)*
- Phylogenetic Species Concept (Monophyly Version)
- Phylogenetic Species Concept (Diagnosable and Monophyly Version)
- Polythetic Species Concept
- Recognition Species Concept*
- Reproductive Competition Concept*
- Successional Species Concept
- Taxonomic Species Concept

Reference

a Mayden, R.L. (1997) A hierarchy of species concepts: the denouement in the saga of the species problem. In *Species: the Units of Biodiversity* (Claridge, M.F. *et al.*, eds), pp. 381–424, Chapman & Hall

*Concepts that make reference to biological processes (e.g. reproduction and competition) that occur among organisms within species (and less so between species) and that contribute to a shared process of evolution within species.



PROGRESS TOWARD A GENERAL SPECIES CONCEPT

Bernhard Hausdorf[1,2]

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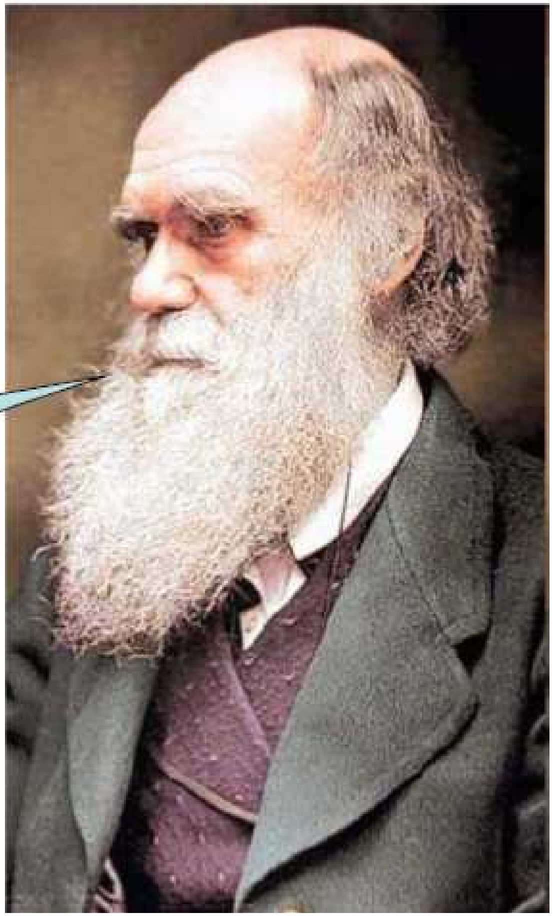
Accepted January 3, 2011

> It is really laughable to see what different ideas are prominent in various naturalists minds, when they speak of “species”; … It all comes, I believe, from trying to define the indefinable.
>
> *Darwin 1856*



Identification of individuals

DNA fingerprinting
(DNA profiling)

Identification of individuals – why?

- if we do not see the individual
- non-invasive genetics – elusive animals, samples from faeces, urines, hairs – can be joined with individual variation of their diet
- forensic genetics – identification of DNA in animal products, poachers, etc.

- species conservation – e.g. in falconary (confirmation of parentage)

Microsatellites

- Tandem repetitions of short motifs

CTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTT

CTTTCTTTCTTTCTTTC

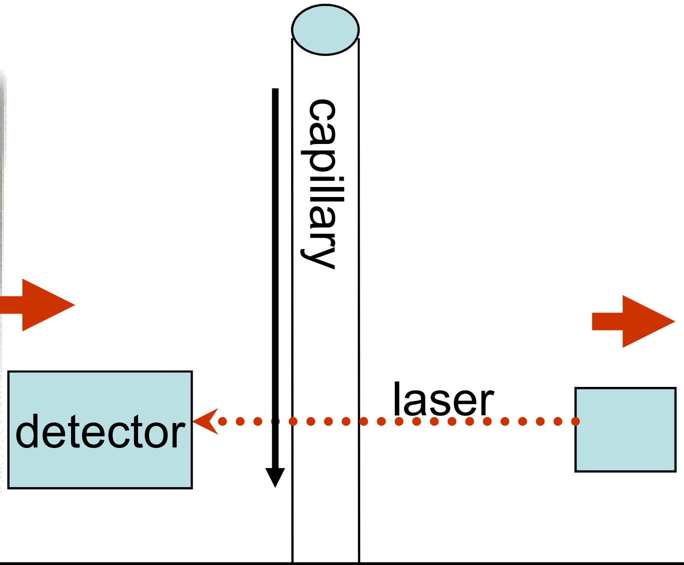
- DNA extraction

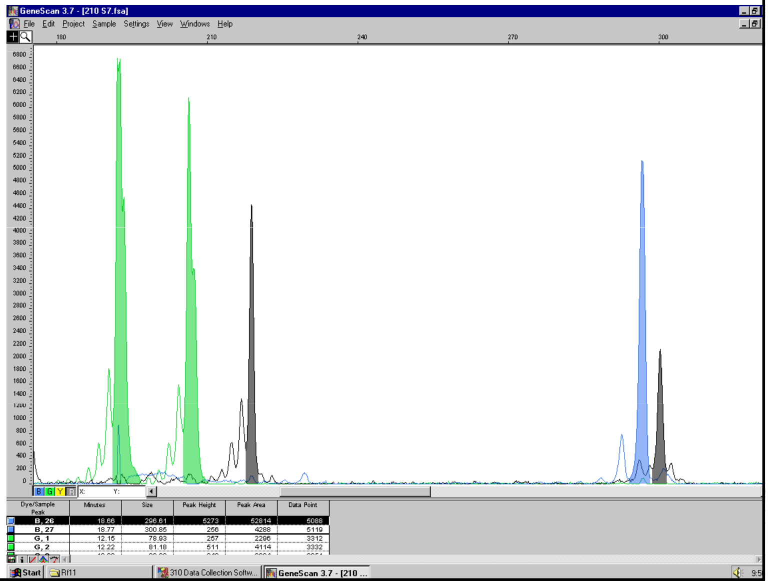
- PCR

CTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTT

CTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTT

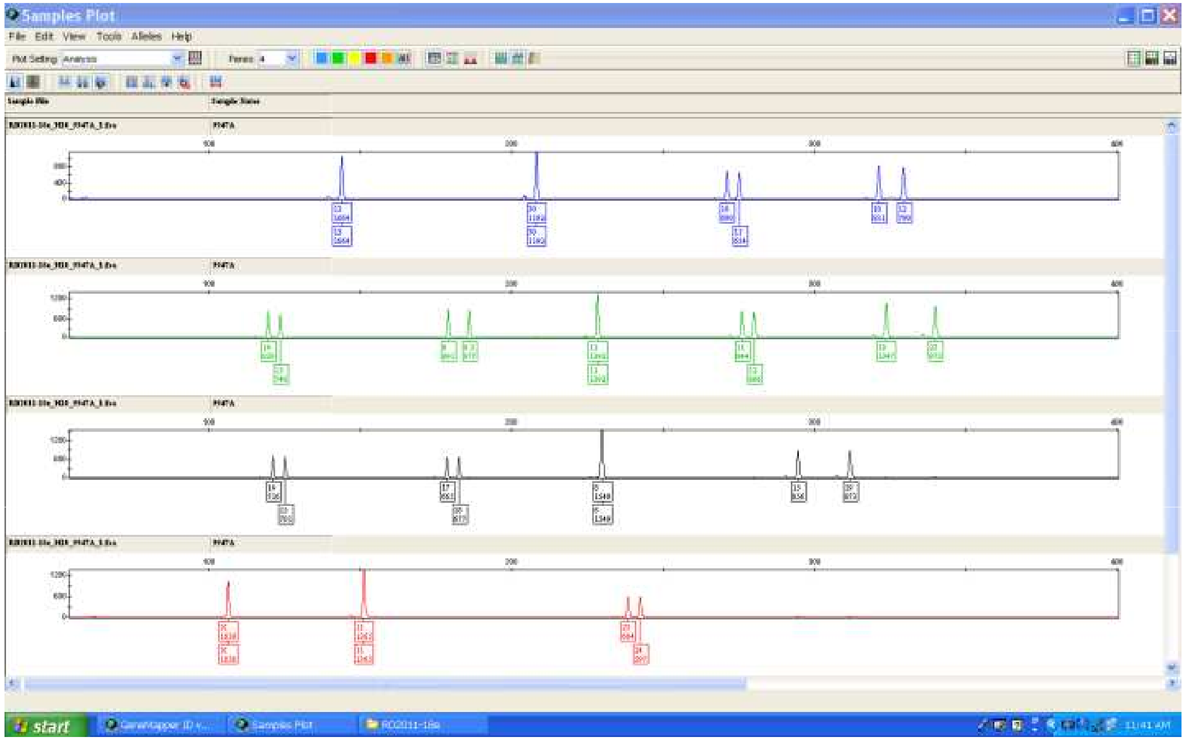
- Detection of alleles
  → sequencer, fragment analysis

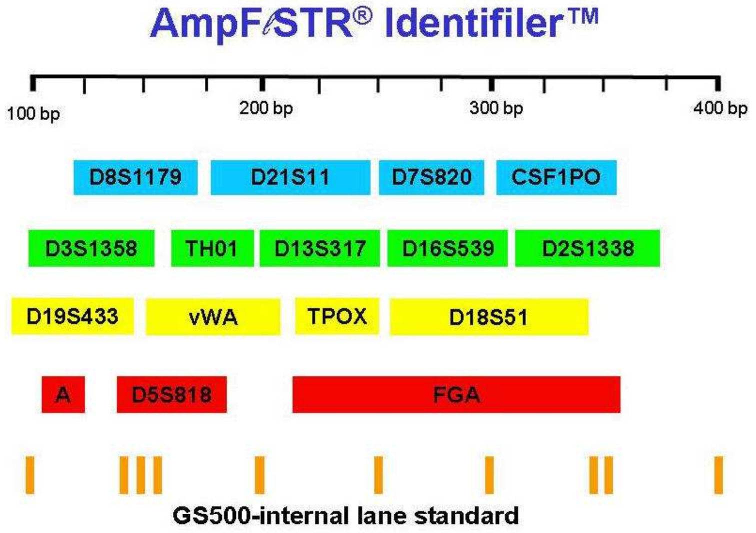




Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
B, 26	18.66	296.61	5273	52814	5088
B, 27	18.77	300.85	256	4288	5119
G, 1	12.15	78.93	257	2296	3312
G, 2	12.22	81.18	511	4114	3332

Individual human identification




AmpFℓSTR® Identifiler™

100 bp
200 bp
300 bp
400 bp

D8S1179 D21S11 D7S820 CSF1PO

D3S1358 TH01 D13S317 D16S539 D2S1338

D19S433 vWA TPOX D18S51

A D5S818 FGA

GS500-internal lane standard

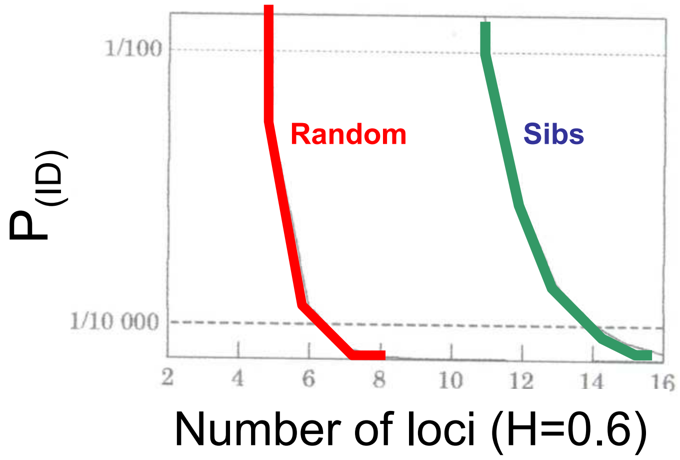
16 loci = reliable individual identification
(Euro-American population)

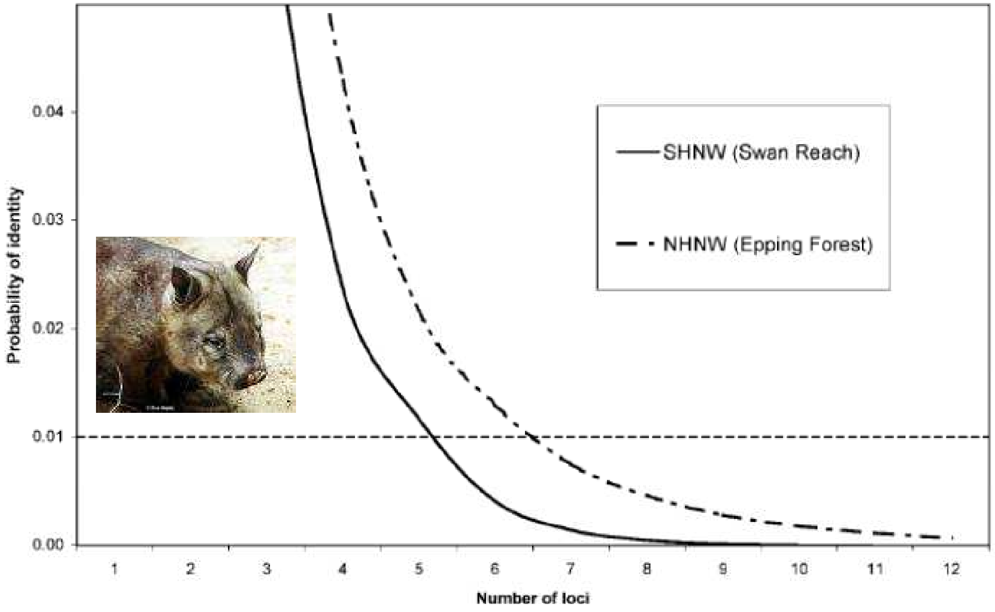
Identification of individuals depends on level of polymorphism

- multilocus microsatellite fingerprinting – power estimated as „probability of identity“ ($P_{(ID)}$) (Waits et al. 2001) – e.g. GenAlex program

$$P_{(ID)} = \sum p_i^4 + \sum \sum (2p_i p_j)^2$$

$$P_{(ID)sib} = 0.25 + (0.5\sum p_i^2) + \left[0.5(\sum p_i^2)^2\right] - (0.25\sum p_i^4)$$





- pilot studies with tissue samples are required to identify $P_{(ID)}$ in a population studied by e.g. non-invasive methods

Brown bears in Pyrenees

Taberlet et al. 1997

- Faeces and hairs
- 24 microsatellites
- 4 males and 1 female with unique multilocus genotypes (more than according footprints and photos)
- Multiple-tube approach

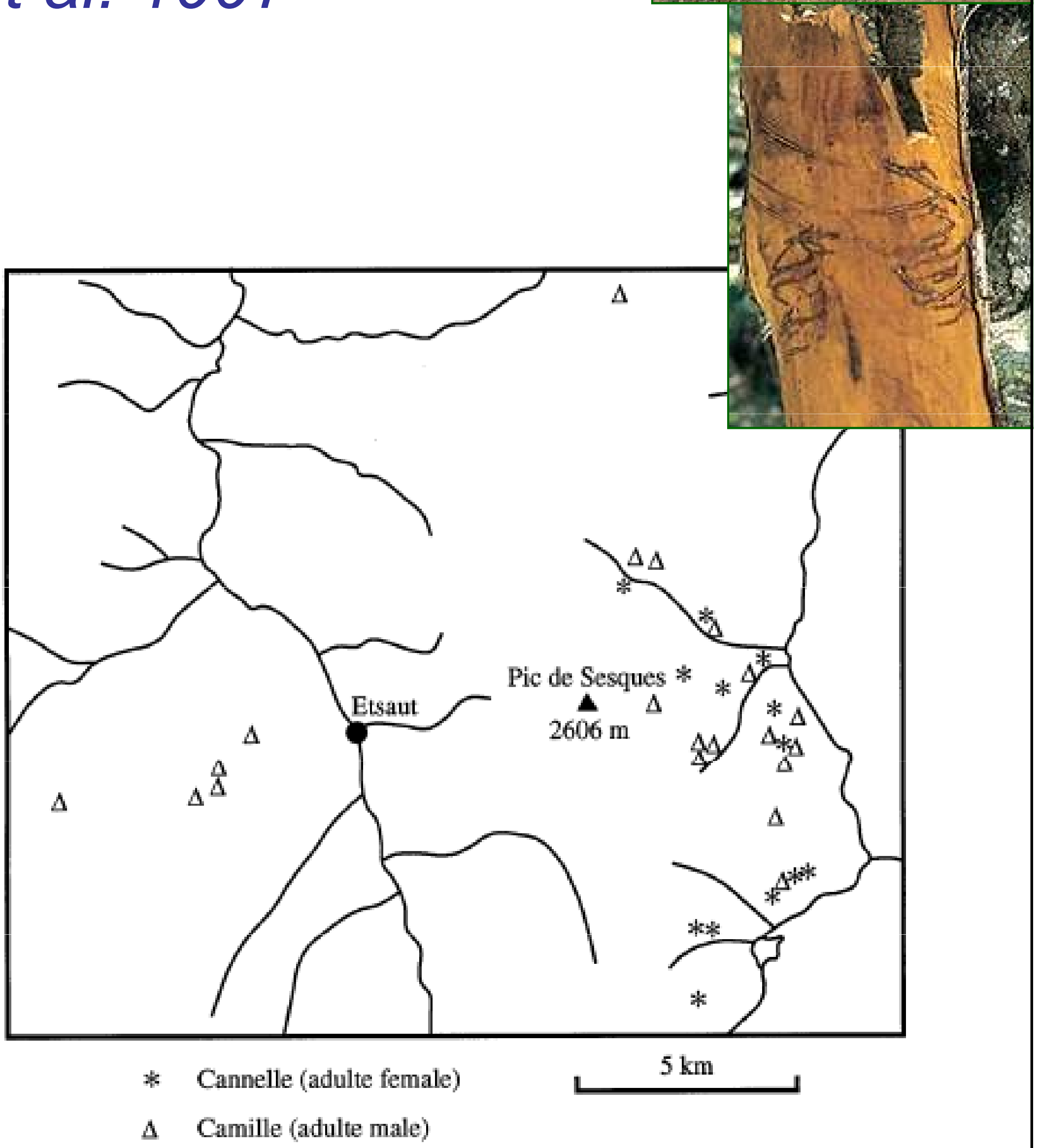
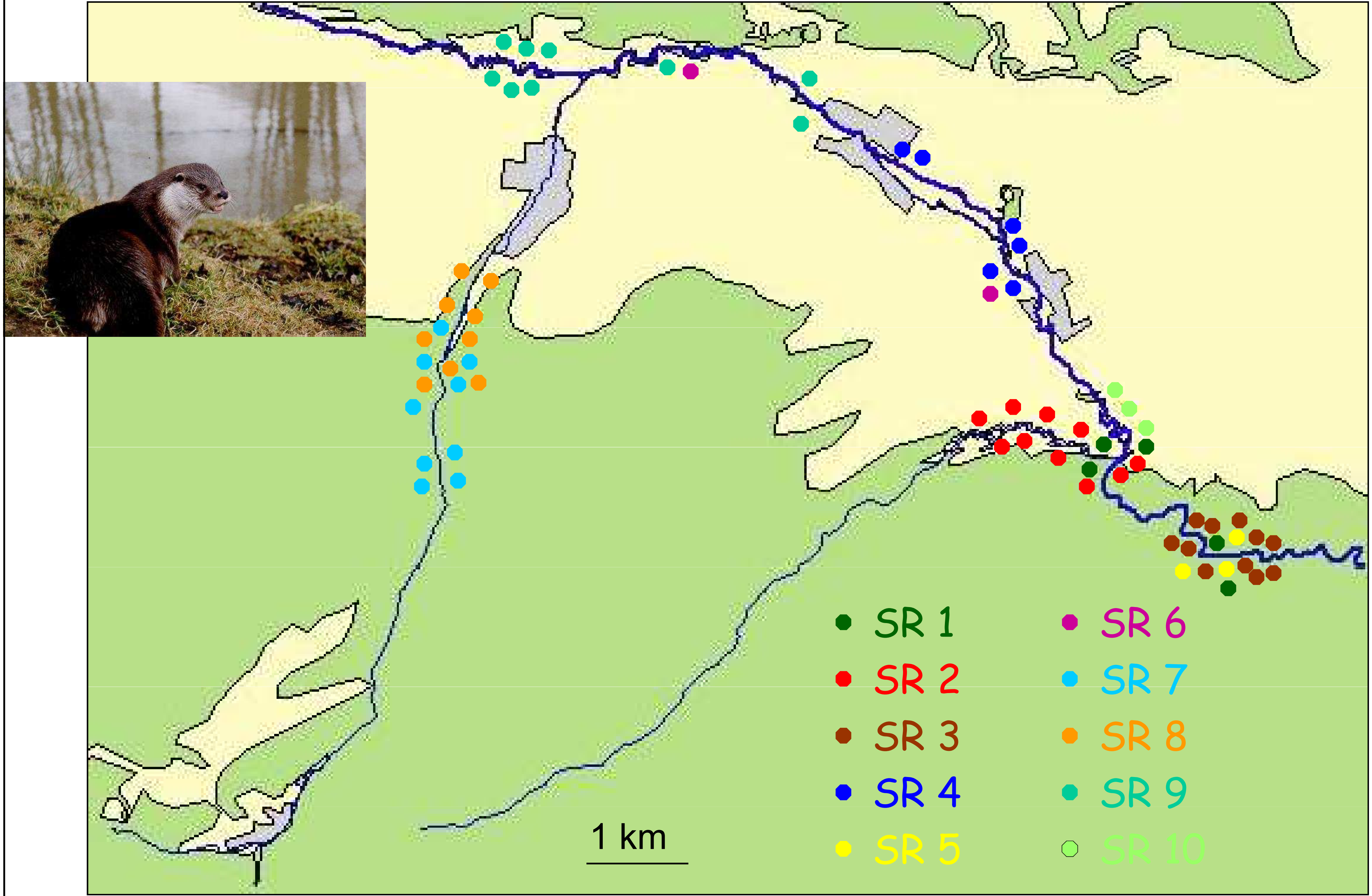


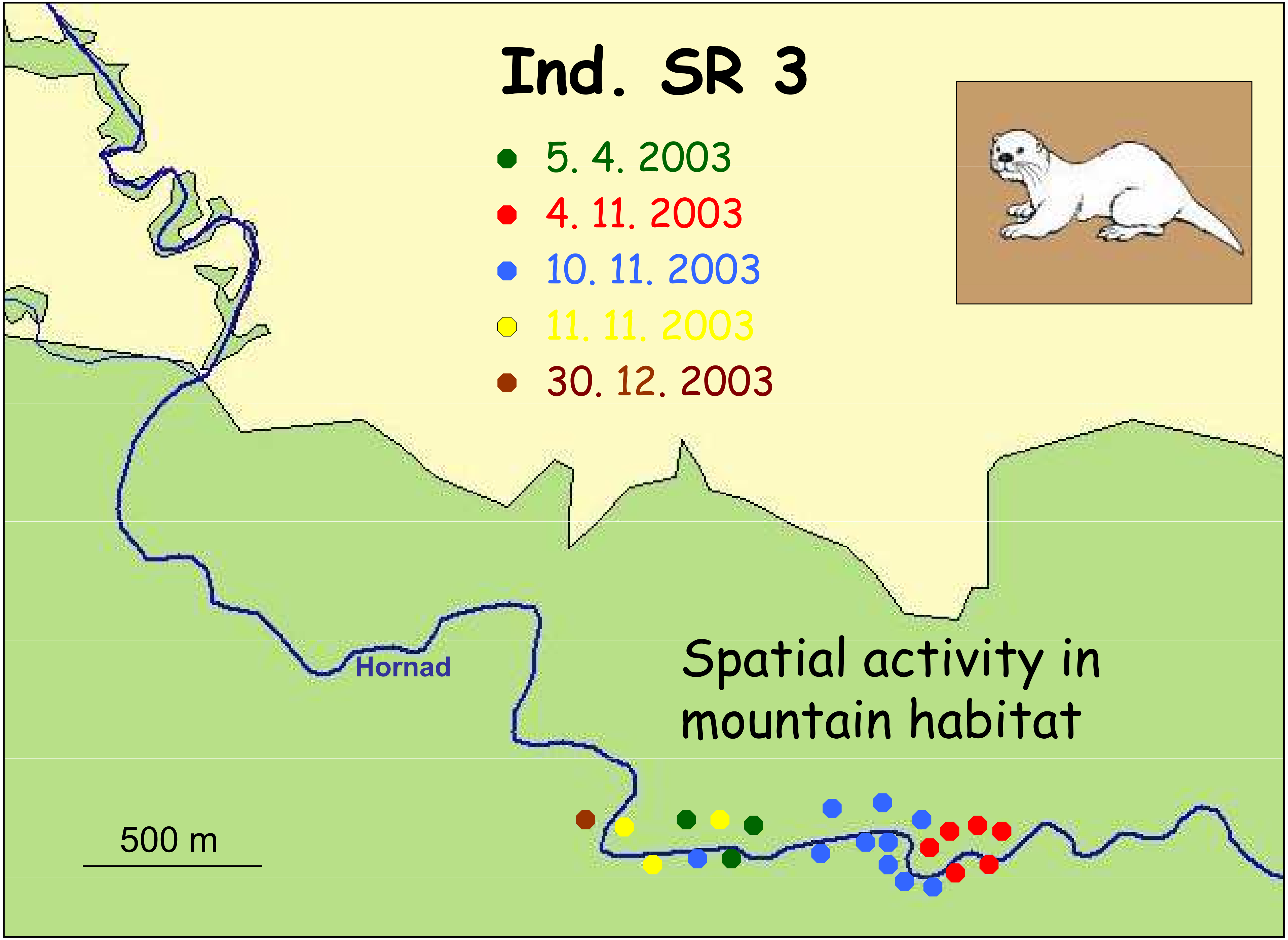
Fig. 3 Home range of two Pyrenean brown bears obtained by noninvasive genetic sampling and genotyping.

Spatial activity of otters

- **P. Hájková – PhD thesis**

Identified individuals - Hornád, NP Slovenský Raj



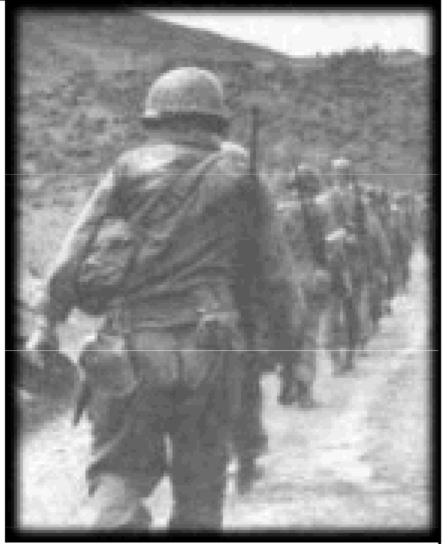


Ind. SR 3

- ● 5. 4. 2003
- ● 4. 11. 2003
- ● 10. 11. 2003
- ● 11. 11. 2003
- ● 30. 12. 2003

Spatial activity in mountain habitat

Human forensic genetics



- **Pozůstatky vojáků z války** Vietnam a Korea

  Identifikace na základě mtDNA příbuzných osob
  (lze jen někdy)
  V současnosti: vzorek DNA (krve) při odvodu, jiné markery
  Armed Forces Repository of Specimen Samples for the Identification of Remains

- **Soudní pře**
  Clinton-Lewinská
  Pozůstatky ruského cara Nikolaje II
- **Kriminalistika**
- **Oběti tragických událostí**

Klony

Bambus *Sasa senanensis*

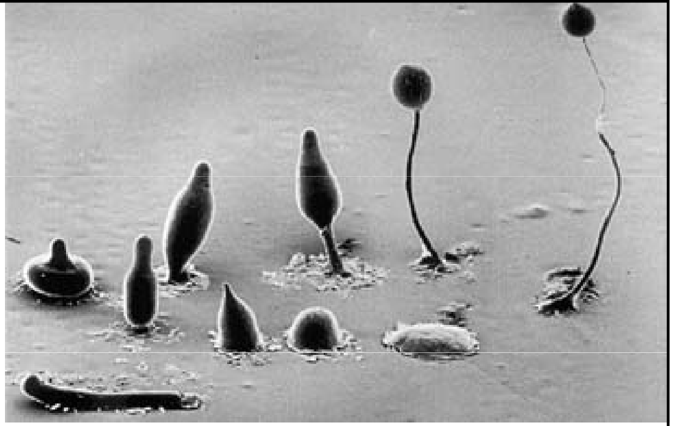
- Suyama et al. 2000
- Plocha 10 hektarů
- AFLP
- 22 klonů
- Klon na ploše 300 m v průměru

Slavní klonální bezobratlí

- Rotifera – Bdelloidea
- Ostracoda (*Darwinula*)
- Partenogenetické klony vysokého stáří (milióny let)

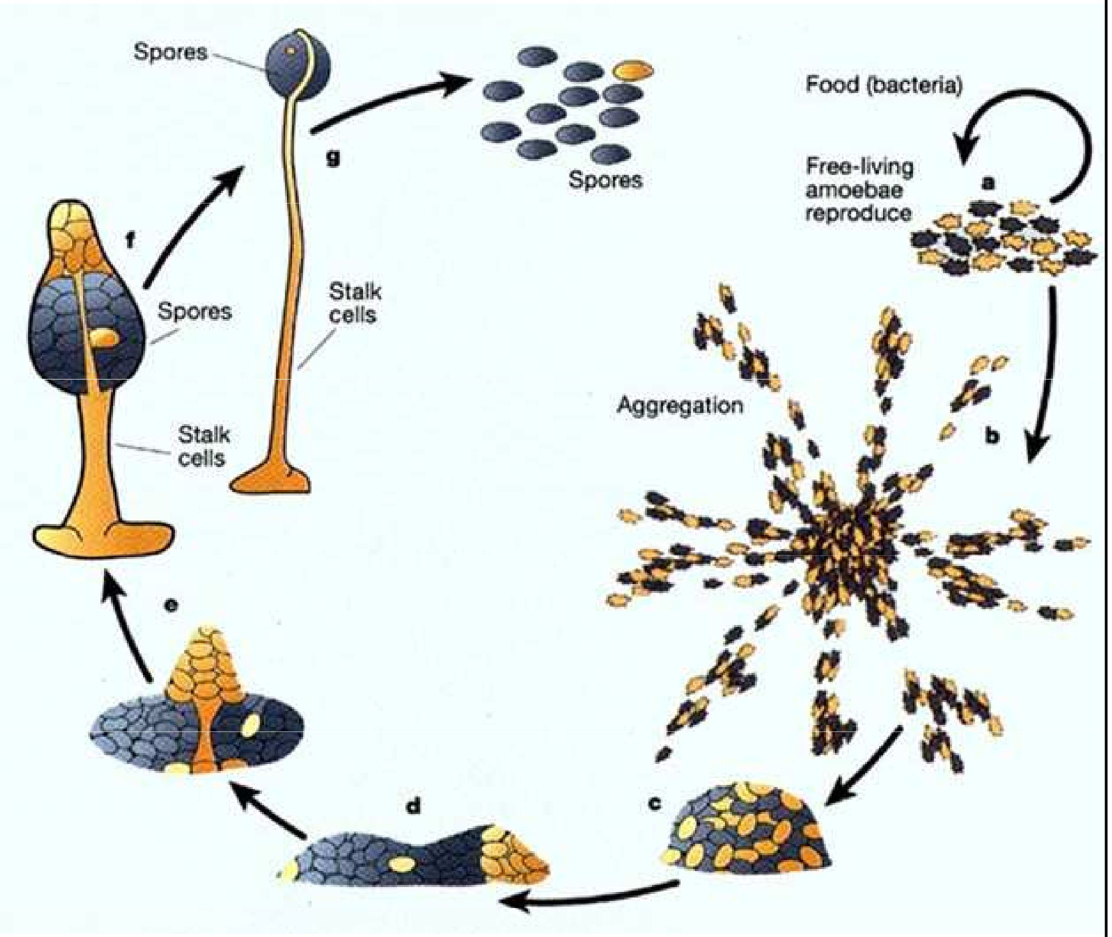
Darwinula stevensoni

Genetické chiméry



- organismy složené z buněk s různými genotypy

- *Dictyostelium discoideum*
  chimérismus je pravidelná součást života



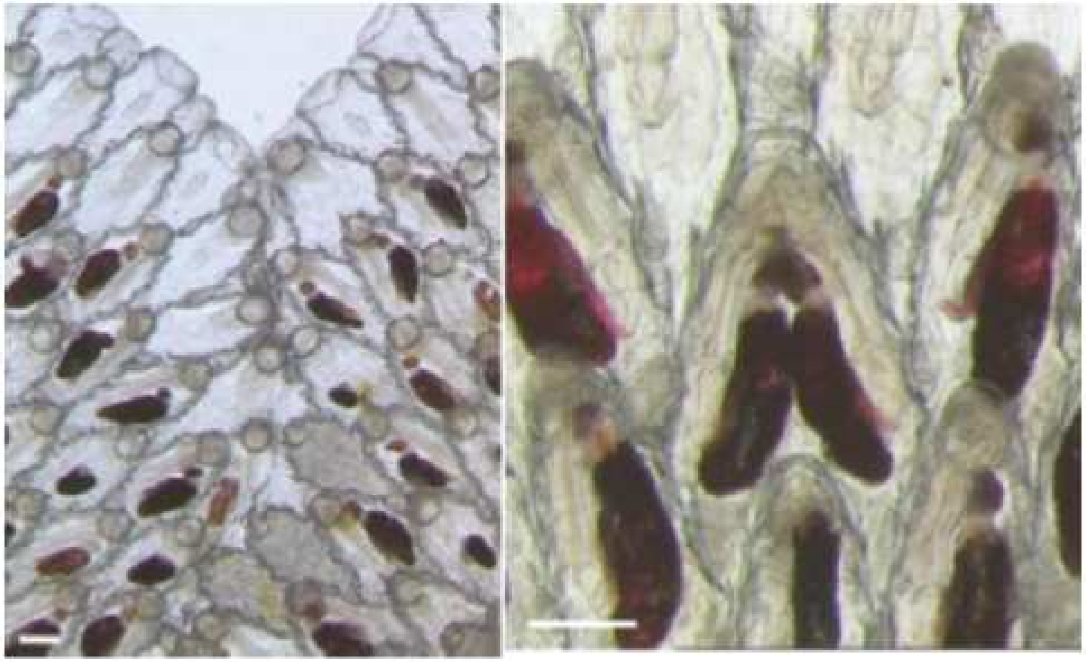
Genetické chiméry

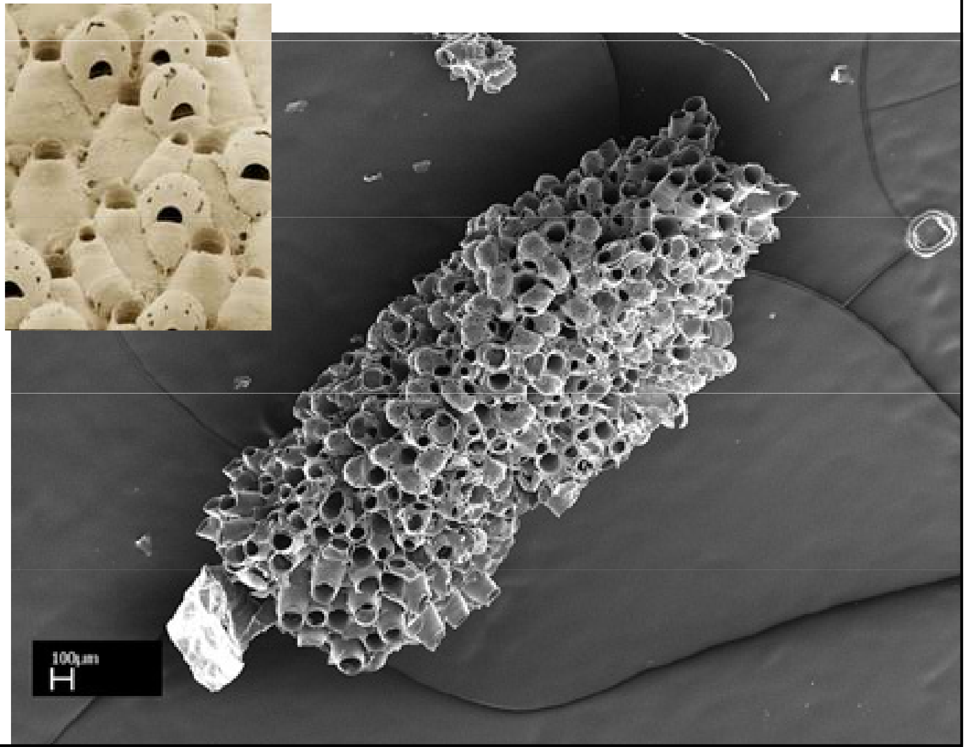
- *Ficus* srůst kořenů různých jedinců
- sumky *Botryllus schlosseri* chimérické kolonie příbuzní jedinci
- *Diplosoma listerianum* i nepříbuzní

Celleporella hyalina (Bryozoa)

Hughes et al. 2004

- Pravděpodobnost fůze koreluje s příbuzností
- Histokompatibilita
- Lepší rozpoznávání v pokročilejších fázích
  → dozrávání imunokompetence
- Speciální proteiny (spongikany...)

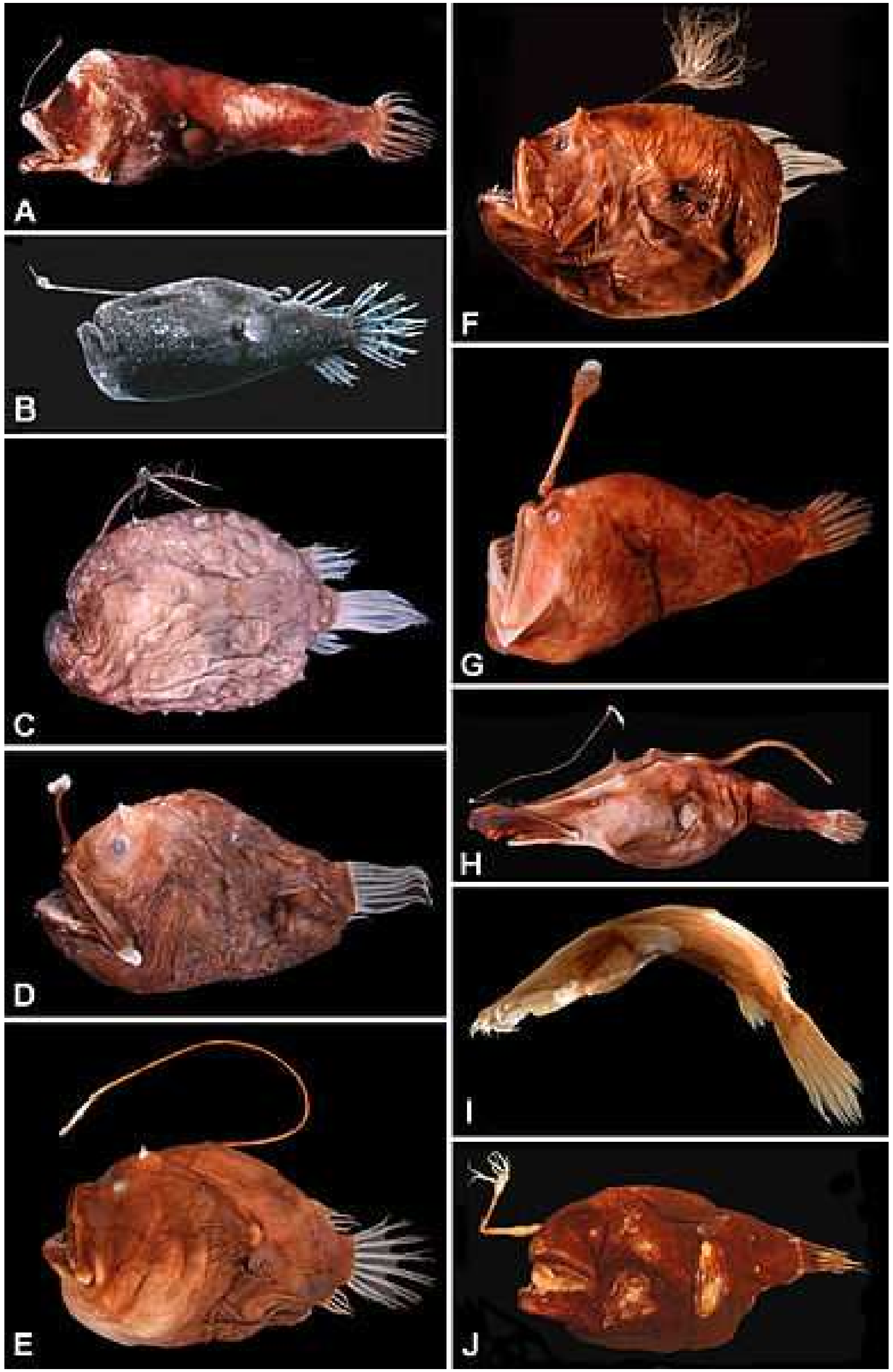




Ceratioid anglerfish

- miniaturní samec po narození vyhledá samici, její kůže vyloučí hydrolytický enzym a samec přiroste

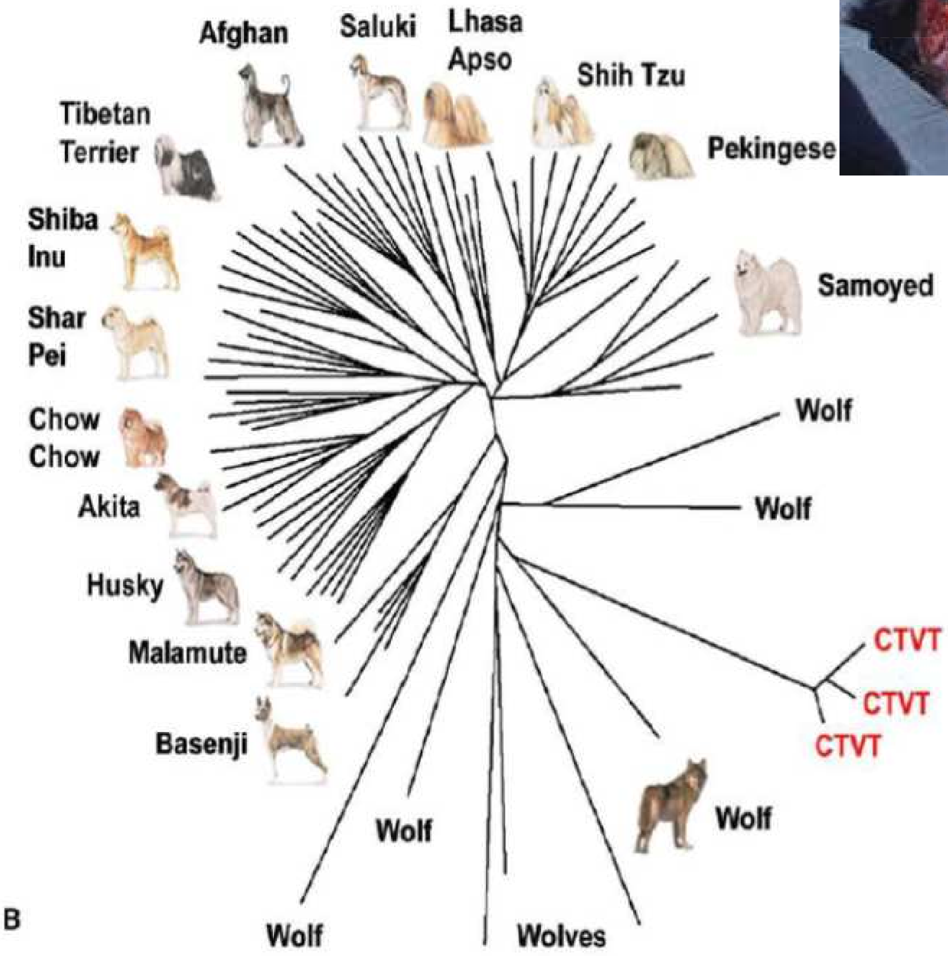
- vzniká hermafroditická chiméra



Genetické chiméry – „microchimerism“

- kosman bělovousý *Callithrix jacchus* (asi i rod *Saguinus*)
- dizygotická dvojčata
- DNA fingerprinting krve - hematopoietické chiméry
- během embryonálního vývoje vzájemná výměna buněk kostní dřeně
- týká se to asi jen krve (neinvazivní metody – chlupy, trus → jeden genotyp)
- průnik embryonálních erytroblastů a volné DNA přes placentu i u člověka
- (pohlaví dítěte před narozením lze určit i pomocí PCR sekvencí typických pro Chr Y, jako templát je periferní krev matky)

Canine transmissible venereal tumor (CTVT)



Devil facial tumour disease

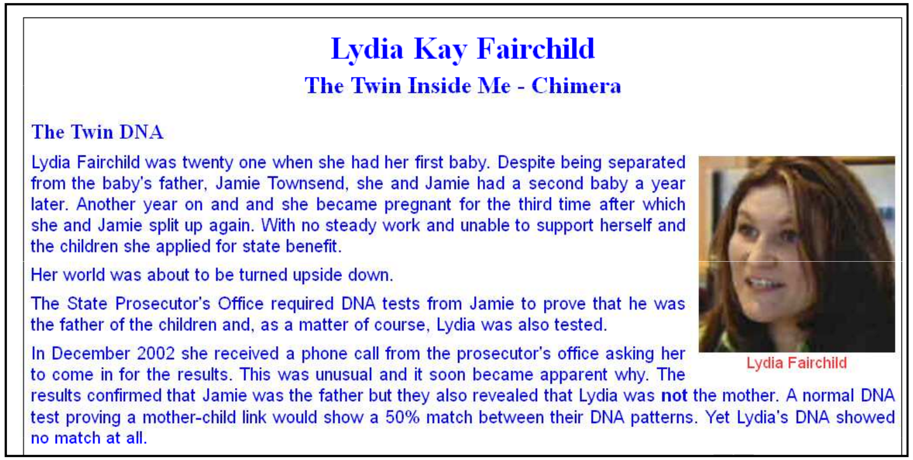
- **parasitic cancer**
- **„single cell parasitic wolf“**

Známé „lidské chiméry“

Foekje Dillema

46XX/46XY woman

- holandská atletka, mistryně na 100 a 200 m
- odmítla test na pohlaví
- mozaika zjištěna až posmrtně (v r. 2007)



Lydia Kay Fairchild

The Twin Inside Me - Chimera

The Twin DNA

Lydia Fairchild was twenty one when she had her first baby. Despite being separated from the baby's father, Jamie Townsend, she and Jamie had a second baby a year later. Another year on and and she became pregnant for the third time after which she and Jamie split up again. With no steady work and unable to support herself and the children she applied for state benefit.

Her world was about to be turned upside down.

The State Prosecutor's Office required DNA tests from Jamie to prove that he was the father of the children and, as a matter of course, Lydia was also tested.

In December 2002 she received a phone call from the prosecutor's office asking her to come in for the results. This was unusual and it soon became apparent why. The results confirmed that Jamie was the father but they also revealed that Lydia was **not** the mother. A normal DNA test proving a mother-child link would show a 50% match between their DNA patterns. Yet Lydia's DNA showed no match at all.

Lydia Fairchild

- geneticky nepotvrzené mateřství
- chiméra matky

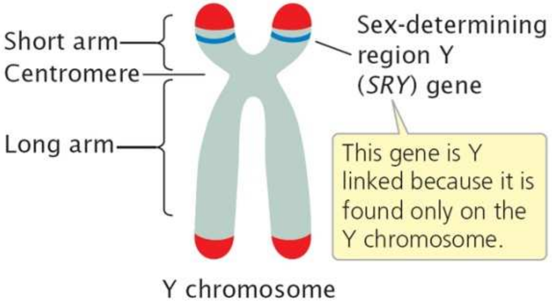
Identification of sex

DNA sexing

Why?

1) species without sex dimorphism (birds, but also many mammals)
2) embryos, larvae
3) non-invasive methods

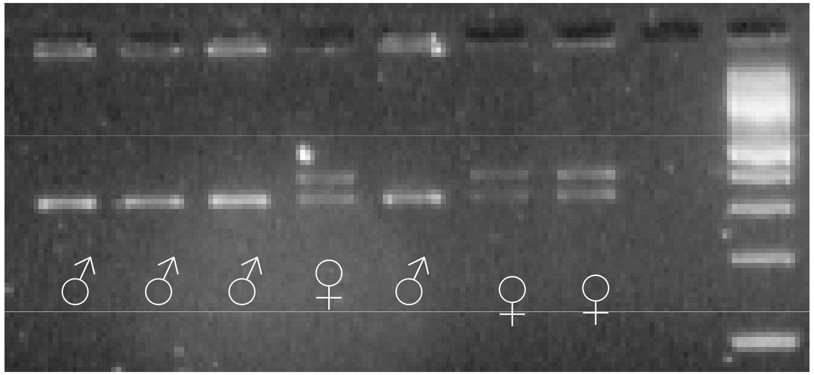
Genetic sex identification



- genetic sex determination
- birds (♂=ZZ, ♀=ZW)
- mammals (♂=XY, ♀=XX)
- DNA amplification of W/Y chromosome
- W, Y – small chromosomes

Sex identification – birds

Griffith et al. 1998



- *CHD1W* and *CHD1Z*, genes at sex chromosomes (chromobox-helicase-DNA-binding gene (CHD) – Griffiths & Tiwari 1995)
- Primers amplifying introns of both genes
- Introns differ by their length
- Up to three primer combinations
- Problematic species, e.g. Struthioniformes

Manorina melanocephala

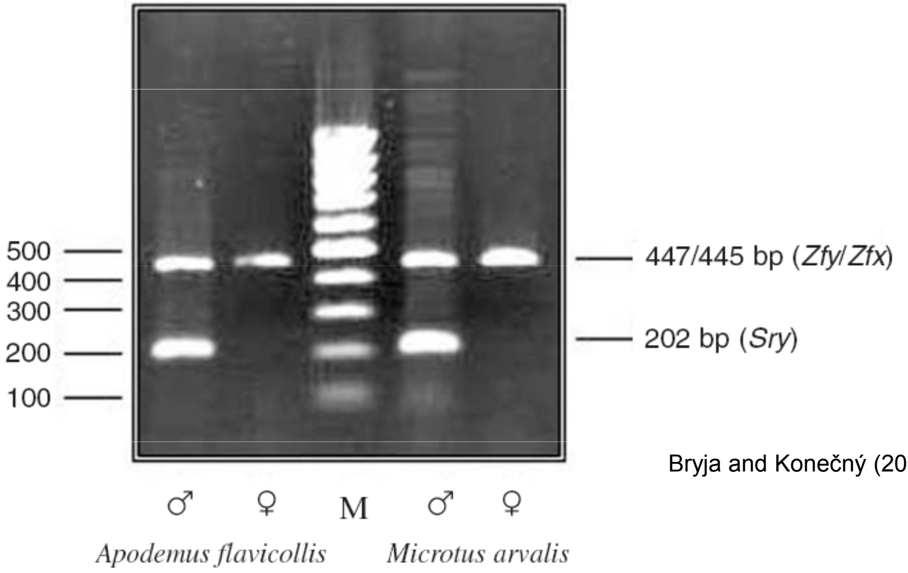
(Meliphagidae) *Arnold et al. 2001*

medosavka hlučná

- Sons = „helpers“
- In adults
  2,31 males vs. 1 female
- Offspring in nests
  sex ratio 1:1 (57:57)
- Males are hatching first
  (in 17 out of 18 nests)
  they are bigger and heavier when leaving the nest

Sex identification - mammals

- Amplification of a gene at Y-chromosome (*Sry*)
  (in duplex PCR with X-linked or autosomal fragment)



Bryja and Konečný (2003)

- *Microtus cabrerae*
  Sry at Chr X
  Ellobius, Tokudaia
  Sry completely missing
- *Nannomys*
  Large variability

Nannomys

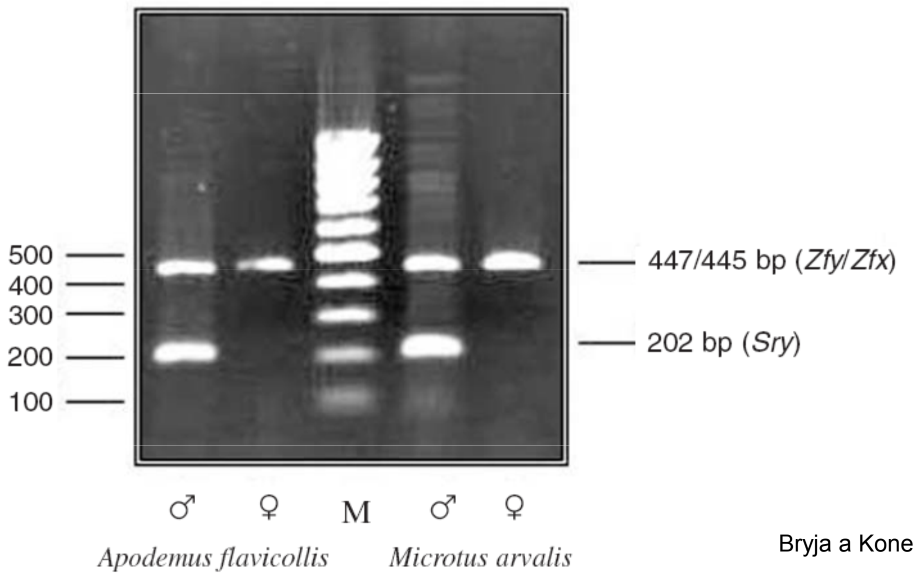
Ellobius

M. cabrerae

Tokudaia osimensis

Sex identification - mammals

- Amplification of a gene at Y-chromosome (*Sry*)
  (in duplex PCR with X-linked or autosomal fragment)



Bryja a Konečný 2003

- Faecal analyses: species-specific primers are required to avoid a cross amplification with species in the diet

x

Murphy et al. 2003