Revisiting the taxonomy of the Rattini tribe: a phylogeny-based delimitation of species boundaries

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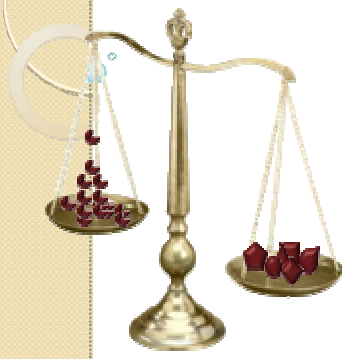
4th ICRBM, Bloemfontein (South Africa) 12th-16th April 2010

Context of the study

- Asia = center of diversification of rodents / complex community of rodents

rich community of pathogens

Biodiversity and disease risks



Global changes

biodiversity ↘ in community of hosts

changes in pathogen community

emergences of zoonoses

Risk of exposure ↗ for human, livestock and wild faunas

Necessary to understand host (rodents) and their pathogen communities = aim of the ANR project Ceropath

PREDICTION

What does it mean?

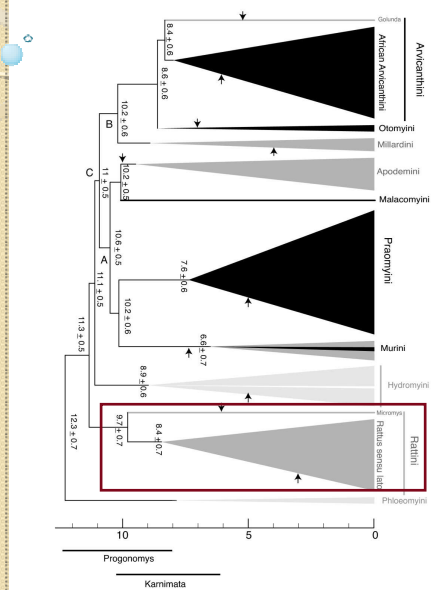
How many species in the community?

Which species are in interaction?

Specificity of the association rodent - pathogen?

Pre-requisite : to be able to recognize rodent species

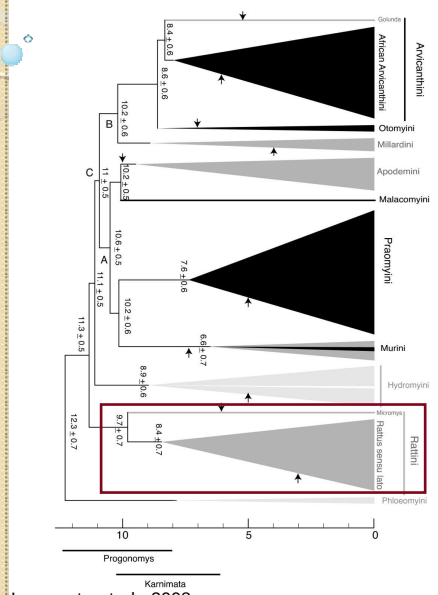
Rodents in Asia = Rattini



Rattini	Division	Genus	species / SEA	total species
	Crunomys division			
		Crunomys	0	4
		Sommeromys	0	1
	Dacnomys division			
		Leopoldamys	5	6
		Niviventer	7	17
		Anonymomys	0	1
		Chiromyscus	1	1
		Dacnomys	0	1
	Maxomys division			
		Maxomys	5	17
	Micromys division	*Micromys*	0	1
		~~*Chiropodomys*~~		
		Haeromys	0	3
		Hapalomys	2	2
		~~*Vandeleuria*~~		
		Vernaya	0	1
	Rattus division			
		Bandicota	2	3
		Berylmys	3	4
		Bunomys	0	6
		Diplothrix	0	1
		Rattus	11	66
		Sundamys	1	3
		Abditomys	0	1
		Bullimus	0	3
		Kadarsanomys	0	1
		Komodomys	0	1
		Limnomys	0	2
		Nesokia	0	2
		Nesoromys	0	1
		Palawanomys	0	1
		Papagomys	0	2
		Paruromys	0	1
		Paulamys	0	1
		Taeromys	0	7
		Tarsomys	0	2
		Tryphomys	0	1
	Melasmothrix division			
		Melasmothrix	0	1
		Tateomys	0	1

Lecompte et al., 2008

Rodents in Asia = Rattini



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28 (Indochinese Region) / 166

Lecompte et al., 2008

Specific identification among the Rattini

high degree of similarity between sp.
high intra-specific polymorphism

Morpho. characters unsuitable for reliable ID

Other ID tools in need

Aim

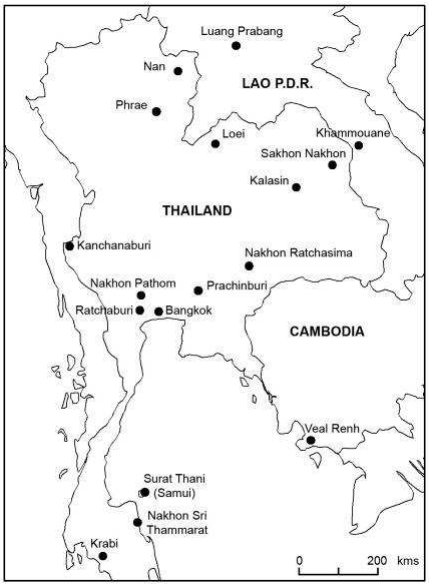
1/ To assess Rattini biodiversity in SEA with molecular markers

2/ then to design molecular ID tools

Assessing Rattini biodiversity in SE Asia with molecular tools

Sampling area

122 specimens / 2000 trapped in the fields



Assessing Rattini biodiversity in SE Asia with molecular tools

Methods 1/ Choice of targeted genes

- efficient markers to reconstruct robust rodents phylogenies (Jansa *et al.* 2004; Lecompte *et al.,* 2008; Rowe *et al.,* 2008; etc.)

1140 bp cytochrome *b*

670 bp Cytochrome c oxydase I

(DNA barcoding)

exon 1100-1200 bp IRBP

Assessing Rattini biodiversity in SE Asia with molecular tools

Methods 2/ Phylogenetic reconstructions

- outgroup: *Micromys minutus* (sisterships with *Rattus* sensu lato group; Michaux et al., 2007; Lecompte et al., 2008; Robins et al., 2008)

- model of evolution / AICc → MrAIC, Nylander, 2004

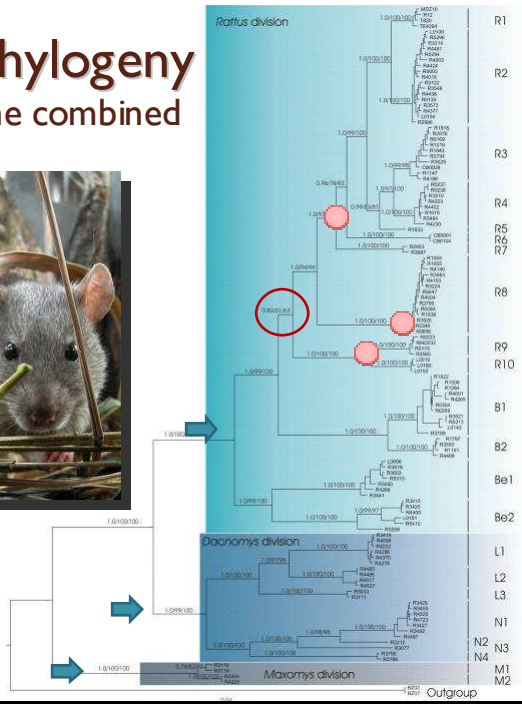
- ML reconstruction / PHYML, Guindon et Gascuel, 2003 on each independent genes → no conflict

- concatenation
  - → unpartitioned ML analysis: PHYML / 500 Bp resampling
  - → partitioned ML analysis : RAxML, Stamatakis, 2006
  - → partitioned BA: MrBayes, Ronquist and Huelsenbeck, 2003

Dataset: 3018 bp / 122 specimens

Indochinese rat phylogeny

based on the analysis of the combined dataset



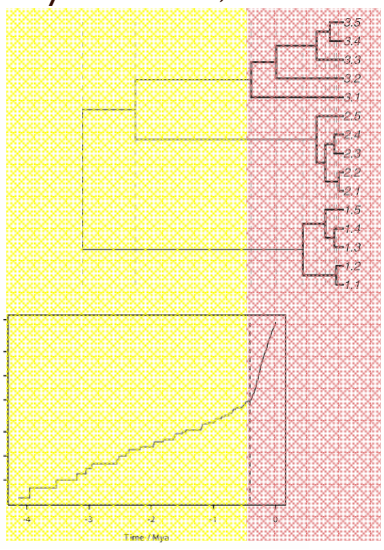
But how many species?

No a priori method proposed by Pons et al., 2008

analyses of branch lengths based on the difference in branching rates at the level of species and populations.

✓ Branch lengths between species = speciation and extinction rates
✓ Branch lengths within a species = coalescence processes at the level of populations

→ locations of ancestral nodes that define putative species



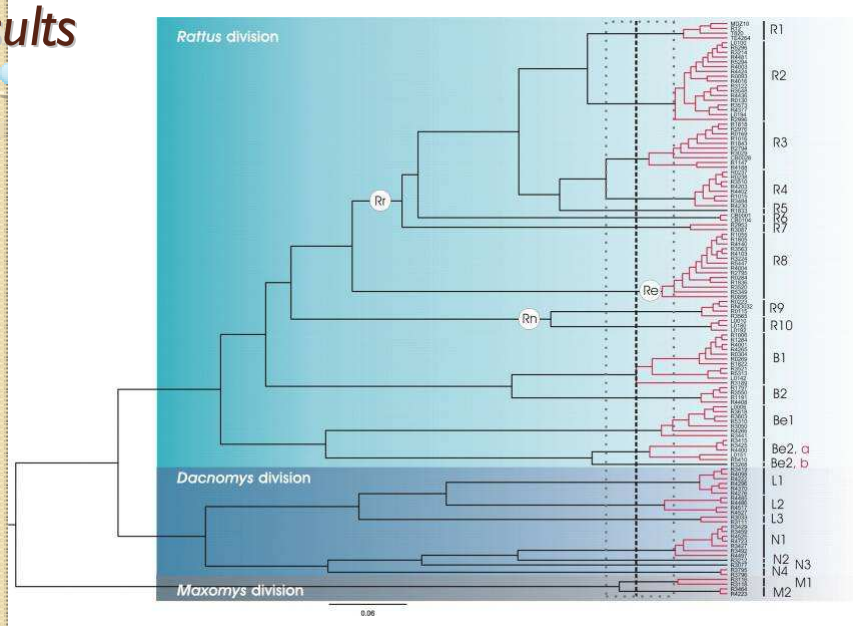
Prerequisite = Ultrametric tree

→ Multidivtime, Thorne and Kishino, 2002

→ Thanks to Pierre-Henri Fabre

How many species?

Results

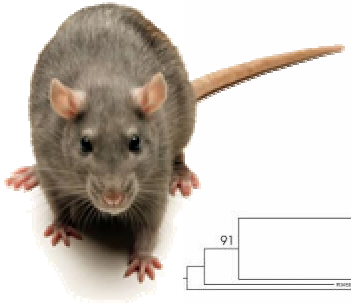


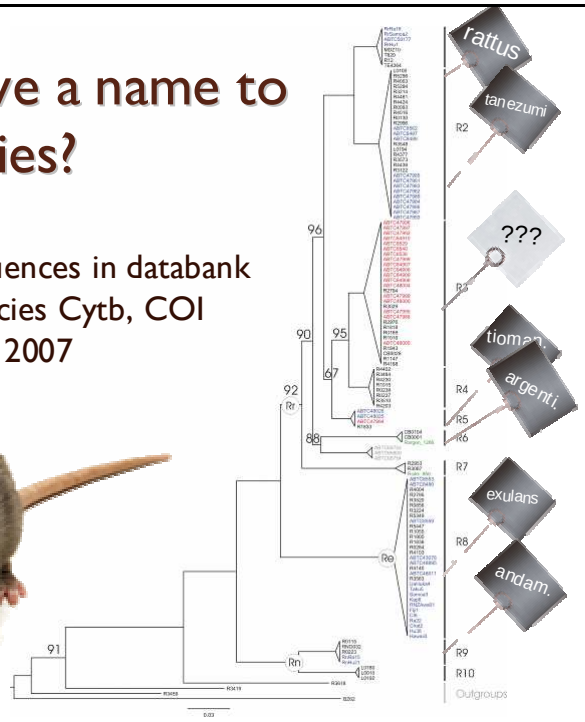
How to give a name to these species?

Using other sequences in databank

→ *Rattus* species Cytb, COI

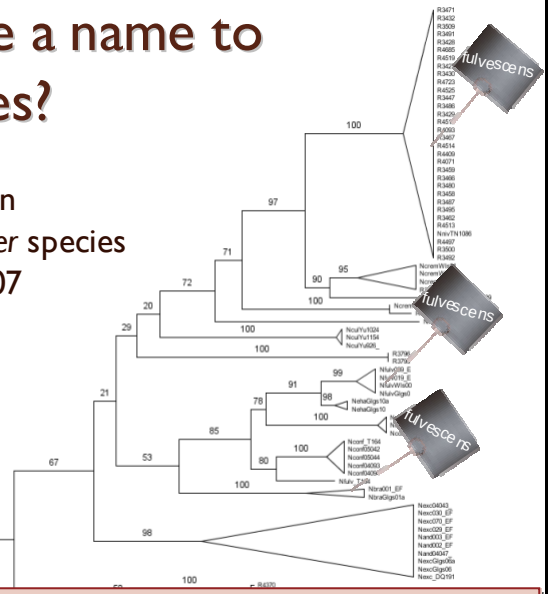
Robins et al., 2007





How to give a name to these species?

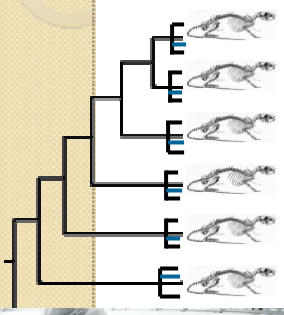
Using other sequences in databank → *Niviventer* species Cytb / Jing et al., 2007



RELIABLE MORPHO. ID OF SPECIMENS IN THE BANK?

How to give a name to these entities?

Sequencing the reference = the holotype specimen



ANCIENT DNA

degraded and chemically modified

- ✓ Very prone to contaminations by exogenous DNA
- ✓ Work in rooms dedicated to aDNA handling
- ✓ Strict manipulation protocols to authenticate results
- ✓ Small PCR targeted fragment of DNA / fragmentation

Molecular work on holotype

Choice of the marker:
130 bp cyt*b* fragment

→Télétchéa *et al.*, 2008, Molecular identification of **vertebrate** species by oligonucleotide microarray in **food** and **forensic** samples

→used in Pagès *et al.* , 2008 to identify sturgeon species based on **archeological** specimens

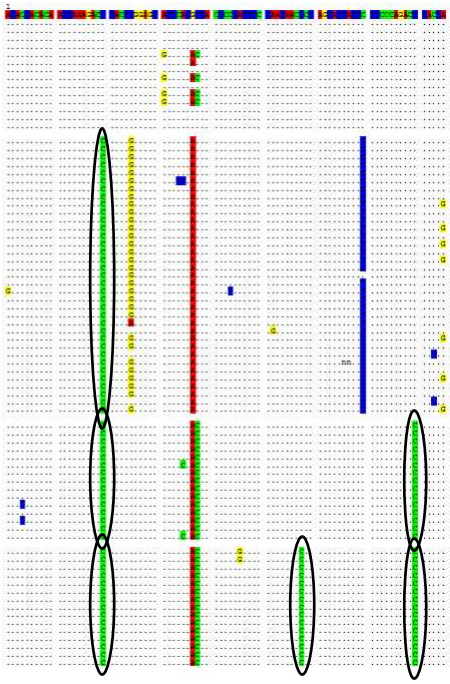
→ relevant for SEA rats

R1: *rattus*

R2: *tanezumi*

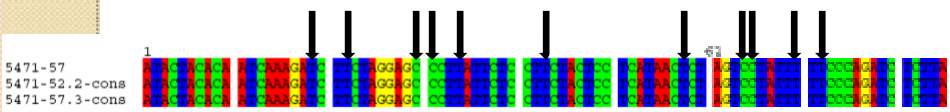
R3

R4: *losea*



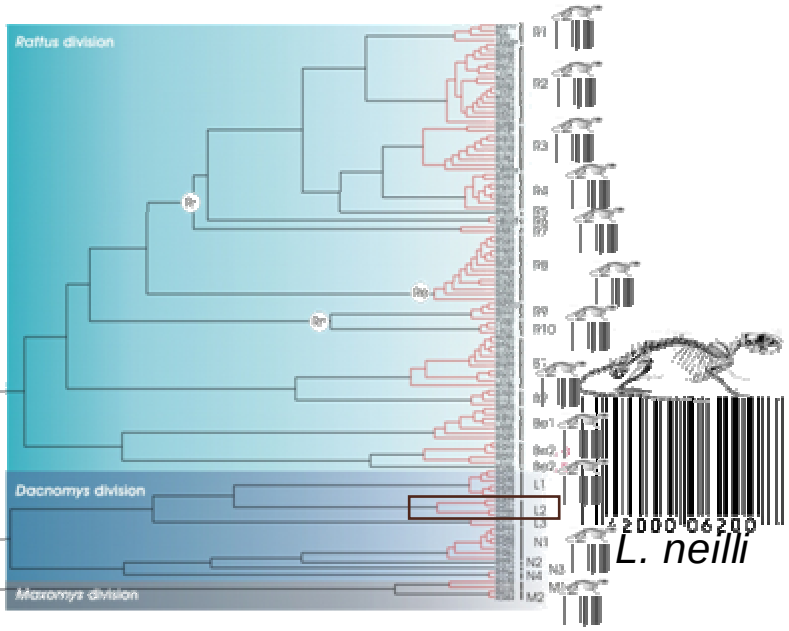
First test on Holotype

Leopoldamys neilli holotype →
PALGENE plateform of palaeogenetics
(Lyon, France)



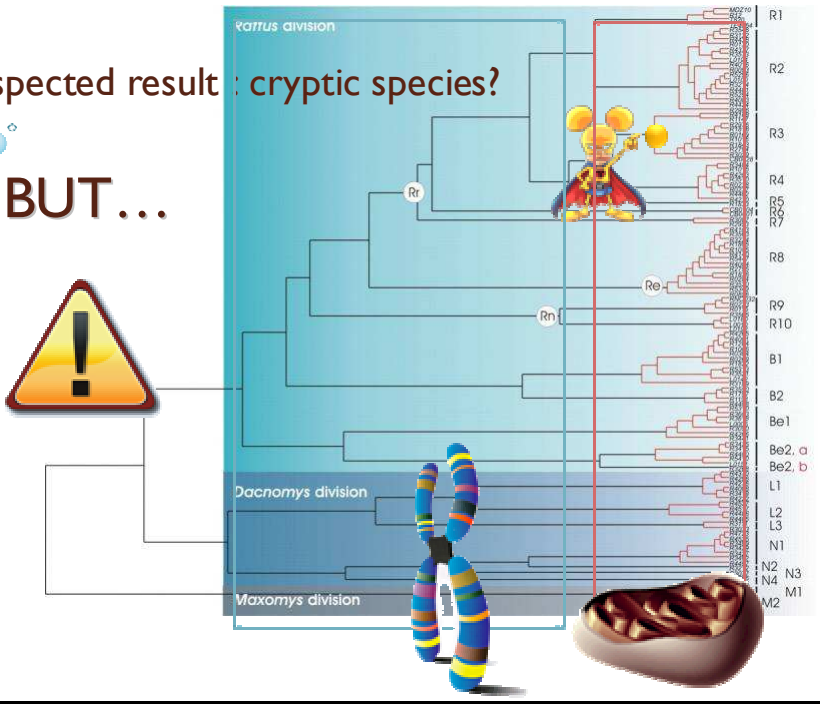
L. neilli

position	19	22	30	31	34	43	58	64	65	79	83
base	T	T	C	C	T	T	T	C	C	T	T
probability	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000



Unsuspected result : cryptic species?

BUT…



To conclude

ACURRATE SPECIES IDENTIFICATION

Characterization of SEA rat and their pathogen communities

Acknowledgments

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Thank you for your attention!