

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



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Introduction

Among the Muridae rodents, the Rattini tribe encompasses 167 species inhabiting South East Asia, a hotspot of biodiversity facing with a growing economical development, affecting habitats, biodiversity and health but also a hotspot of emerging and re-emerging diseases. Rat species have been demonstrated as main hosts of pathogens but are still difficult to recognize at a specific level using morphological criteria. Because of global environmental changes and increasing mobility of humans and animals, contacts between pathogens and hosts and vectors are modified, amplifying the risk of disease emergence. An accurate identification of rats at a specific level is needed to understand their implications in the transmission of diseases. To provide a rigorous systematic framework for epidemiological surveys, we carried out a taxonomic revision of the Rattini tribe using molecular tools.

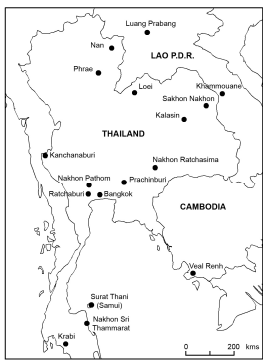
Methods and Results

Q1: How many lineages corresponding to how many rat species in Southeast Asia?

2 *mt* genes (COI and *cytb*) and the 1st exon of the IRBP gene from 122 rat samples from 17 localities in Thailand, Cambodia and Laos (Fig.1) were sequenced to perform phylogenetic reconstructions (ML and BI) (Fig.2). The method developed by Pons et al. [1] that determines with no a priori the locations of ancestral nodes defining putative species was then applied (Fig.3).

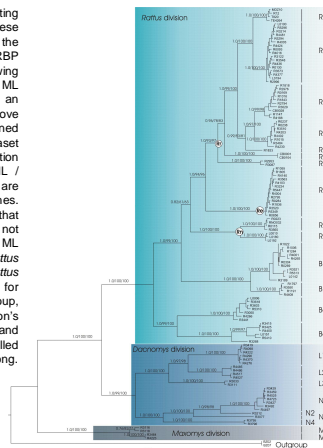
Sampling

Fig. 1: Sample locations of the Rattini specimens caught in the fields and included in this study.



Phylogenetic reconstructions

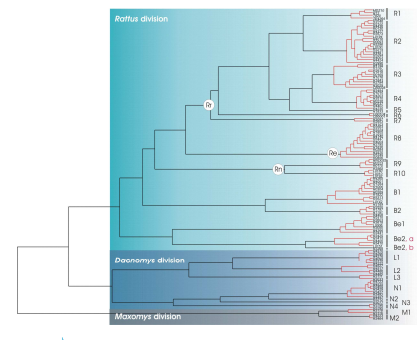
Fig.2: Phylogenetic tree depicting relationships of the Indo-Chinese Rattini based on the analyses of the combined *cytb*, COI and IRBP genes and reconstructed following Bayesian method. BI and ML analyses of the dataset gave an identical topology. Numbers above branches reflect supports obtained from the analysis of the dataset following 3 different reconstruction methods: BI / unpartitioned ML / partitioned ML. Support values are not shown for very short branches. The symbol \oplus indicates that phylogenetic relationships are not supported by the partitioned ML analysis. Rr stands for *Rattus rattus* species group, Re for *Rattus exulans* species group, Rn for *Rattus norvegicus* species group, following Musser and Carleton's denominations [2]. At the right hand of the tree, lineages are labelled according to the genus they belong.



23 lineages

Species delineation method

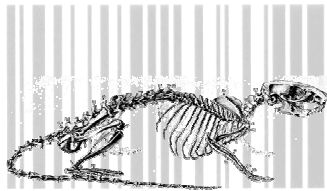
Fig.3: Rattini ultrametric tree obtained with Multidivtime [3], and clusters of specimens recognized as different species by the method of Pons et al. [1]. Genetic clusters recognized as a valid species are highlighted in red and separated by longer black branch.



24 putative species

These findings fit well with the number of species described in the literature at this place excepted for the *Berylmys* and the *Rattus* genera. 5 species belonging to the *Rattus rattus* species group have been described in this area (i.e. *R. andamanensis*, *argentiventer*, *tanezumii*, *losea*, *tiomanicus*). Our analysis reveals the presence of an additional and unexpected *Rattus* species (R3 in Fig. 2 and 3).

Q2: How to proceed for species assignment?



In this aim, we tested the relevance of DNA sequence obtained from the holotype specimen of the Neill's long-tailed giant rat. The holotype is by definition the element to which the name of a taxon is permanently attached. As museum samples contain tiny amounts of poorly preserved DNA, we targeted a 85 bp fragment of the *cytb* gene as a barcode following all the ancient DNA standards. This genuine sequence was assigned using the CAOS software [4] to the monophyletic cluster corresponding to the *Leopoldamys* species, L2, in our tree (Fig. 3). Consequently, this monophyletic cluster recognized as a valid species by the method of Pons et al. [1] could be without ambiguity named as *Leopoldamys neilli*.

Conclusion

Even if it is an ongoing project, our study revealed yet that at least 6 different species including a new and unsuspected one exist among the *Rattus rattus* species group, each probably having specific ecological traits and carrying its own set of diseases. This new element must now be absolutely taken into account by the medical science to understand the mechanisms underlying the emergence and the re-emergence of rodent-borne diseases in South East Asia. This fact illustrates how relevant could be evolutionary studies for health and medical science.

References

- [1] Pons et al., 2008
- [2] Musser and Carleton, 2005
- [3] Thorne and Kishino, 2002
- [4] Sarkar et al., 2008

Acknowledgments

We are particularly grateful to all the people that worked hard with us in the field to collect samples. Thanks to C. Tollenaere, JP Quéré, O. Verneau and F. Catzeflis who provided sequences or samples, and to PH. Fabre who offered us judicious advice to use the Multidivtime software. We would like to acknowledge the Ambrose Monell Cryo Collection (AMCC) at the American Museum of Natural History, New York, for their support in our research and also all the people of the ancient DNA platform PALGENE (Lyon, France). This study is part of the CERoPath project (ANR 07 BDIV 012) funded by the French National Agency for Research.

