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ScienceDirect

C. R. Biologies 332 (2009) 480–488

Comptes Rendus Biologies

http://france.elsevier.com/direct/CRASS3/

Evolution / Évolution

Morphological identification of sibling species: the case of West African *Mastomys* (Rodentia: Muridae) in sympatry

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Received 9 May 2008; accepted after revision 20 November 2008

Available online 9 January 2009

Presented by Pierre Buser

Abstract

In this study, we investigate skull size and shape differentiation between sibling species of *Mastomys* with the aim to characterize and discriminate three sympatric species found in West Africa: *M. huberti, M. erythroleucus* and *M. natalensis*. A total of 133 genetically determined specimens were used for the morphometric analyses. Statistical analyses clearly demonstrated that the three species largely overlapped in centroid size (*M. erythroleucus* tends to be larger on average than the *M. huberti* and *M. natalensis*) but they exhibited large differences in skull shape. The current study focused on skull shape, and allowed us to discriminate three morphological groups that are congruent with the three species suggested by molecular identification (90% of the individuals are correctly assigned by cross-validated classifications). In the *Mastomys*, the evolution of cranial length and shape may be influenced by competitive pressure between closely related species separated by ecological segregation. This source of variability could possibly induce character displacement between species of *Mastomys*. ***To cite this article: A. Lalis et al., C. R. Biologies 332 (2009).***

Résumé

Identification morphologique d'espèces jumelles : le cas de *Mastomys* (Rodentia : Muridae) en sympatrie. La différenciation morphométrique entre trois espèces jumelles de *Mastomys* d'Afrique de l'Ouest a été étudiée à partir de 133 spécimens, en recherchant les caractères diagnostiques crâniens chez *M. huberti, M. erythroleucus* et *M. natalensis* en sympatrie. L'étude de morphométrie géométrique a permis de mettre en évidence des différenciations associées à des phénomènes d'adaptation à différents biotopes. La légère différence observée dans la taille du crâne entre les espèces (*M. erythroleucus* présentant une taille crânienne supérieure à celle observée chez *M. natalensis* ou *M. huberti*) ne constitue pas un critère fiable d'identification. En revanche, les principales différences de formes observées entre les espèces révèlent une variabilité dans le processus de mastication, qui pourrait résulter d'une compétition dans la disponibilité des ressources et qui aurait favorisé l'émergence de traits différents par déplacement de caractères et spécialisation pour un nouveau type d'habitat. L'analyse des composantes de formes a donc permis de confirmer la présence d'unités morphologiques partiellement distinctes pour les trois espèces retrouvées en sympatrie. ***Pour citer cet article : A. Lalis et al., C. R. Biologies 332 (2009).***

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1631-0691/$ – see front matter
doi:10.1016/j.crvi.2008.11.004

Keywords: Sibling species; Interspecific variability; Skull shape; Geometric morphometrics; *Mastomys*

Mots-clés : Espèces jumelles ; Variabilité interspécifique ; Conformation crânienne ; Morphométrie géométrique ; *Mastomys*

1. Introduction

In taxonomy, species identification may rely on the morphospecies, or typological, concept which has been used since the beginning of systematics [1]. This concept has been commonly used in palaeontology and zoology for a lot of species described only on the basis of diagnostical morpho-anatomical characters. However, the recent development of various other systematic techniques (DNA molecular sequencing and morphometrics) and new diagnostic characters like karyotypes or chromosome banding patterns have allowed the description of new species [2–4]. It has been reported that karyotyped data can provide good evidence of probable reproductive isolation at specific level, without any sign of morphological discrimination even by more sophisticated methods of morphometrics [5,6]. In other cases, analyses of size and shape by conventional or geometric morphometrics have suggested specific separation [7–10]. In West Africa, the multimammate rat *Mastomys* is the dominant genus from the rodent communities [11–13]. Using its exceptional ability to adapt and reproduce, this rodent is generally found in all habitats from houses to forests. The taxonomy of the genus has been problematic due to the high morphological convergence between the species which prevents correct assignation to a specific level by traditional systematic analyses [14]. The determination key of *Mastomys* is based on various morphological features like the mammae number (between 5 to 12 pairs); sperm and penis morphology and dimensions [15,16]; karyotype characters such as the diploid number (2N); and the fundamental number (FN). The use of cytogenetic and molecular tools has recently highlighted presence of many sibling species within the genus, especially in West Africa [14]. There is now a consensus to consider *M. natalensis* ($2n = 32$, NFa = 52–54), *M. huberti* ($2n = 32$, NFa = 44) and *M. erythroleucus* ($2n = 38$, NFa = 54–56). Few phylogenetic analyses have been devoted to this genus. Karyological analyses by Britton-Davidian et al. [17] found *M. natalensis* to be closer *M. huberti*, while *M. erythroleucus* was the basal taxon of this clade. Protein electrophoresis data placed *M. erythroleucus* closer to *M. huberti* and both well separated from *M. natalensis*, while immunological data and DNA/DNA hybridization did not provide species discrimination [18]. A cytochrome-b phylogeny found *Mastomys* genus paraphyletic with a well supported monophyletic group including *M. huberti*, *M. erythroleucus* and *M. natalensis* [19]. All these results are incongruent, emphasizing the difficulty of classification among these taxa. Duplantier [20] tried to find other morphometric and genetic characteristics, but was unable to completely separate the three different species in Senegal in his analyses of mandibular and craniometrical distances. For instance, in a discriminant analysis of 15 cranial distance measurements [20], three species groups were shown with percentages of well classified individuals ranging from 75 to 92%. Said study was not able to provide significant criteria to allow for reliable identification. At lower taxonomic levels, morphological divergence can be subtle and traditional morphometrics is sometimes insufficient to differentiate groups, while geometric morphometrics performs better [21,22]. Thus, geometric morphometrics is especially well suited when investigating the evolution of forms among and within species [e.g. [23–26]] as well as to address problems of functional morphology and ecological divergence [27,28]. In this study, we investigate skull size and shape differentiation between closely related species of *Mastomys*. The goal of the present geometric morphometrics study is to characterize and discriminate three sympatric species of West African *Mastomys*: *M. huberti, M. erythroleucus* and *M. natalensis*.

2. Material and methods

2.1. Specimens

A total of 133 genetically determined (DNA sequencing for cytochrome b) specimens were used for the morphometric analyses. They were classified into three taxa: *M. huberti* ($N = 43$), *M. erythroleucus* ($N = 60$), and *M. natalensis* ($N = 30$). They were trapped at 2 sites in Guinea (Mankountan and Bantou) separated by 426 km. *M. huberti* and *M. natalensis* were only trapped in Mankountan and Bantou respectively whereas *M. erythroleucus* was trapped in both sites (Mankountan $N = 30$; Bantou $N = 30$). As in preliminary analyses, it was found that the two populations of *M. erythroleucus* present a significant difference in size (student's t-test, $F = 4.21$, $p < 0.01$) and in shape