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Original Investigation

Increased geographic sampling reveals considerable new genetic diversity in the morphologically conservative African Pygmy Mice (Genus *Mus*; Subgenus *Nannomys*)

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ABSTRACT

African endemic pygmy mice (Genus *Mus*; sub-genus *Nannomys*) have considerable economic and public health significance, and some species exhibit novel sex determination systems, making accurate knowledge of their phylogenetics and distribution limits important. This phylogenetic study was based on the mitochondrial control region and cytochrome *b* gene, for which a substantial body of published data was available. Study specimens were sourced from eight previously unsampled or poorly sampled countries, and include samples morphologically identified as *Mus bufo*, *M. indutus*, *M. callewaerti*, *M. triton* and *M. neavei*. These analyses increase the known genetic diversity of *Nannomys* from 65 to 102 haplotypes; at least 5 unassigned haplotypes are distinguished by potentially species-level cytochrome *b* genetic distances. The monophyly of *Nannomys* is supported. *Mus musculoides*, *M. callewaerti*, *M. indutus*, *M. bufo*, *M. haussa*, *M. mattheyi*, *M. baoulei* and *M. sorella* are supported as discrete species. The range of *M. indutus* is extended to include Botswana. *M. setulosus* and *M. minutoides* appear to be species complexes. A south and east African *M. minutoides* clade was defined and includes 8 new haplotypes out of 15. *M. setulosus* sensu lato includes *M. setulosus* sensu stricto and a strongly-supported *M. bufo* clade. Two samples, morphologically identified as *M. triton* and *M. neavei*, fall within the *M. minutoides* clade.

Introduction

Mice have an enormous impact on the health and well-being of humans. In addition to their role as agronomic pests, African pygmy mice (subgenus *Nannomys*; genus *Mus*; Rodentia; Muridae) are known to be host reservoirs for Arenaviruses (Lecompte et al., 2007). Two *Nannomys* species, *Mus minutoides* and *M. triton*, have been shown to exhibit novel systems of sex determination (Jotterand-Bellomo, 1988; Veyrunes et al., 2010a). Britton-Davidian et al. (2012) point to the need for more comprehensive sampling throughout Africa to predict the limits and distribution of populations of interest by molecular typing, including cytochrome *b*. They predict that this may result in African pygmy mice becoming one of the most important biological models for the study of the evolution of sex chromosomes and sex determination in mammals.

This study focuses on the molecular systematics and evolution of *Nannomys*. This subgenus of *Mus* is thought to have originated 6.7–7.8 Mya and to have colonized Africa *via* Arabia through Miocene-era land bridges, subsequently radiating throughout the continent (Chevret et al., 2005; Lecompte et al., 2008a; Veyrunes et al., 2005). This is consistent with the earliest *Nannomys* remains found in east Africa and dated at 4.5 Mya (Winkler, 2002). The taxonomy of this group, representing 50% of the species richness of *Mus* (Veyrunes et al., 2005), is still a work in progress. Inference of phylogeny in this group is complicated by considerable morphological homoplasy (Michaux and Catzeflis, 2000), relatively high rates of molecular evolution and a fossil record which is not well-enough sampled to give accurate divergence times (Jansa et al., 2006; Honeycutt et al., 2007). The combination of morphological homoplasy and karyotypic diversity (Castiglia et al., 2002; Veyrunes et al., 2004) suggests that this sub-genus might harbor considerable cryptic genetic diversity.

We aimed to resolve phylogenetic relationships and species identities in a sample of *Nannomys* obtained opportunistically from eight African countries; Botswana, Namibia, Angola, Mozambique, South Africa, Swaziland, Tanzania and the DRC. This sample complements those used in previous molecular phylogenetic studies of *Nannomys*, most of which have been based on mitochondrial

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cytochrome *b* sequences (Kan Kouassi et al., 2008; Veyrunes et al., 2005, 2010b; Mboumba et al., 2011; Britton-Davidian et al., 2012), as it covers previously unsampled or poorly sampled areas. It also includes samples morphologically identified as *Mus bufo*, *M. callewaerti*, *M. triton* and *M. neavei*, which have not been clearly identified in molecular phylogenies. We provide a phylogenetic update on the most recent review of *Nannomys* (Britton-Davidian et al., 2012) by including all currently available sequences in our analysis. We note that while phylogenies based on mitochondrial data can be suggestive of species level differences, such hypotheses require verification from nuclear sequences and other data types (Britton-Davidian et al., 2012; Zachos et al., 2013).

Britton-Davidian et al. (2012) recognize 18 species of *Nannomys* and Happold and Veyrunes, 2013 recognize 20 species. Kan Kouassi et al. (2008) detected five *Nannomys* species in Guinea and four in Cote d'Ivoire, inclusive of *M. mattheyi*, *M. minutoides*, *M. setulosus*, *M. baoulei* and *M. musculoides*, and postulated that the *M. setulosus* clade may be a species complex. In addition, the *Nannomys* phylogenies presented by Mboumba et al. (2011) and Britton-Davidian et al. (2012) contain *M. indutus, M. haussa, M. sorella*, and an implied additional unknown species from Chad (associated with Genbank sequence AJ875085). Based on a combined cytochrome *b* and nuclear IRBP dataset, Veyrunes et al. (2005) recover a monophyletic *Nannomys* clade; they find support for *M. setulosus*, *M. mattheyi* sister to *M. haussa*, *M. indutus* basal to sister species *M. minutoides* and *M. musculoides*, and the abovementioned previously-unknown species from Chad.

M. minutoides, one of the smallest rodents, is found throughout southern Africa (de Graaff, 1997; Skinner and Chimimba, 2005; Musser and Carleton, 2005; Monadjem, 2008) extending to northwestern and northeastern Africa (Veyrunes et al., 2010a,b). It appears to be a well-differentiated monophyletic species that separated from other *Nannomys* species 1.17 Mya (Mboumba et al., 2011). This species is subdivided into three clades, from south and east Africa, west-central Africa and west Africa respectively (Britton-Davidian et al., 2012). The type locality of *M. minutoides* is Cape Town (Musser and Carleton, 2005), close to where the karyotype is $2n = 18$ (de Graaff, 1997), representative of *M. minutoides sensu stricto*. A karyotype of $2n = 34$ (Rb X.1) (de Graaff, 1997) has been reported for this species in KwaZulu-Natal (Veyrunes et al., 2010a,b).

Mus indutus (Thomas, 1910), the Desert pygmy mouse, is typically found in the drier northwestern parts of South Africa, Botswana, Namibia and western Zimbabwe (de Graaff, 1997; Skinner and Smithers, 1990; Chimimba and Bennett, 2005). This species may also be found in southern Angola (Crawford-Cabral, 1998 in Musser and Carleton, 2005), however this range could be limited to the morphologically similar *M. sybilla* (Thomas, 1918), which was previously included as a sub-species of *M. indutus* by Meester et al. (1986).

The Gray-bellied Pygmy Mouse, *Mus triton* (Thomas, 1909), is larger than most other *Nannomys* (except for *M. callewaerti*) and is typically found in highlands, grasslands, swamps and cultivated areas in central and eastern Africa (de Graaff, 1997; Chimimba and Bennett, 2005; Dieterlen and Agwanda, 2008). The taxonomy of *M. triton* is problematic (Chimimba and Bennett, 2005) as this species is morphologically similar to wild populations of *Mus musculus* and shares dental features with Indian *Mus cervicolor* (Chimimba and Bennett, 2005). *M. triton*, which displays novel sex determination systems (Jotterand-Bellomo, 1988), has not thus-far been included in published molecular phylogenies, but is distinguished by a chromosomal translocation, Rb (X.12) (Veyrunes et al., 2004).

The Toad Mouse, *Mus bufo* (Thomas, 1906), is endemic to the montane western Rift of Africa (Musser and Carleton, 2005). Morphologically similar to *M. triton*, it is distinguished by dentition and tail length (Petter and Matthey, 1975) and is also chromosomally distinct (Robbins and Baker, 1978). *M. bufo* is more closely related to *M. minutoides* than *M. triton* (Chimimba and Bennett, 2005). There is uncertainty regarding the identity of a sample from Burundi, lodged on Genbank as *M. bufo* (DQ789905), but regarded by Britton-Davidian et al. (2012) as potentially incorrectly assigned, as it nests within *M. setulosus*.

Although de Graaff (1997) regarded Neaves mouse, *M. neavei* (Thomas, 1910), as a sub-species of *M. sorella* (Thomas, 1909), it is also recognized as a valid species (Petter and Matthey, 1975; Meester et al., 1986; Musser and Carleton 1993; Bronner et al., 2003; Chimimba and Bennett, 2005). This species is thought to occur in southeast Zambia, the type locality, eastern DRC, southern Zimbabwe, northeast South Africa, southwestern Mozambique and southern Tanzania (Chimimba and Bennett, 2005).

Callewaert's pygmy mouse, *Mus callewaerti* (Thomas 1925), is a poorly known but distinctive species occurring in central and northern Angola and southern DRC. It is the largest member of the genus, having a head and body length of 84–97 mm (Crawford-Cabral, 1998; Happold, 2013).

Aims

The aim of this study, based on mitochondrial cytochrome *b* and control region sequence data, was to resolve phylogenetic relationships and species identities in a *Nannomys* sample obtained opportunistically from eight poorly sampled or unsampled African countries, and including morphologically-identified *Mus bufo*, *M. callewaerti*, *M. triton* and *M. neavei*, previously equivocal or absent in molecular phylogenies of *Nannomys*. We include all currently available sequences from DNA sequence databases and provide an updated cytochrome *b* based phylogeny for *Nannomys*, as well as estimates of the age of the major *Nannomys* clades.

We hypothesize that the following are discrete monophyletic species: *Mus minutoides*, *M. musculoides, M. indutus, M. haussa, M. mattheyi, M. bufo, M. callewaerti, M. baoulei, M. setulosus* and *M. sorella*.

We aimed to test the following hypotheses: (1) the monophyly of *Nannomys* is retained in an enlarged sample set derived from previously unsampled localities in Africa; (2) improved geographic sampling of *Nannomys*, which spans the African continent, will reveal new mitochondrial lineages which may be confirmed as cryptic species pending additional studies.

Material and methods

Sampling

This study was based on 36 *Nannomys* specimens obtained opportunistically from 14 localities within South Africa, Tanzania, Namibia, Botswana, Angola, Mozambique, Swaziland, and the DRC (Table 1). Reference samples downloaded from the NCBI GenBank website were also included in the dataset (Table 2). Specimens were obtained from PJT (South Africa), P. Kaleme (DRC), SE (Angola), A. Monadjem (Mozambique), M. McDonough (Botswana) and from the EcoRatProject (www.nri.org/ecorat/) (Namibia, Swaziland and Tanzania) under relevant collecting permits. Specimens were provisionally identified in the field and then confirmed to genus and species level where possible at the Durban Natural Science Museum (DNSM) and the National Museums of Namibia (NMN) by analysis of external characters or skull morphology. Skins and skulls of specimens, or whole bodies in 70% Ethanol (EtOH), are housed at the DNSM, BNM, NSRL, FMNH or the NMN for further reference (Table 1). Tissue samples of liver, kidney, muscle or skin were dissected and stored in 90% EtOH.