Belg. J. Zool., 135 (1) : 21-25 January 2005

Description of the karyotype of *Heimyscus fumosus* and of several other murids from the Mount Doudou area (Gabon)

Emilie Lecompte[1], Violaine Nicolas[1,2], Marc Colyn[2], Christiane Denys[1] and Vitaly Volobouev[1]

[1] **Département Systématique et Evolution, UMR CNRS 5202 "Origine, structure et évolution de la Biodiversité", Mammifères et Oiseaux, Musée National d'Histoire Naturelle, Case Postale 51, 55 rue Buffon, 75 005 Paris, France. Tél : + 33 1 40 79 35 05. Fax : +33 1 40 79 30 63**

[2] **Station Biologique de Paimpont, Université de Rennes 1, CNRS UMR 6553, 35380 Paimpont, France**

Corresponding author : Emilie Lecompte, e-mail : lecompte@mnhn.fr

ABSTRACT. A first inventory of south-western Gabon forest rodent diversity was realised in the Mount Doudou AERF using cytotaxonomic analysis. The C-banded karyotypes of some *Heimyscus*, *Hylomyscus*, *Praomys*, *Hybomys* and *Malacomys* species are presented. Two karyotypes are here described for the first time : those of *Heimyscus fumosus* and *Hylomyscus sp*. The results of this chromosomal analysis increase the murine specific diversity recognized in central Africa : there probably exists a new species of *Hylomyscus* in the Mount Doudou area.

KEY WORDS : Rodentia, Murinae, karyotypes, C-banding, *Heimyscus fumosus.*

INTRODUCTION

One of the most striking features of tropical Africa is the diversity of its mammalian fauna, especially of the small mammals (DELANY & HAPPOLD, 1979). Since the 60's numerous studies were therefore conducted on small mammals, especially rodents, in central Africa which allowed : 1) to describe new species (e.g. BROSSET et al., 1965; DUBOST, 1965), 2) to identify sibling species, as in the genus *Hylomyscus* (ISKANDAR et al., 1988), and 3) to inventory many sites from Nigeria (e.g. HAPPOLD, 1987), Cameroon (HUTTERER et al., 1992; COLYN et al., 1996), Gabon (e.g. BROSSET et al., 1965; COLYN, et al., 1996), Republic of Congo (e.g. GRANJON, 1991; COLYN et al., 1996), Democratic Republic of Congo (e.g. COLYN & DUDU, 1986; DUDU, 1991; LEIRS et al., 1999), Central African Republic (PETTER & GENEST, 1970; BARRIÈRE & NICOLAS, 2000) and Uganda (e.g. DELANY, 1971; CLAUSNITZER & KITYO, 2001). However, despite this rather large number of studies, African small mammal diversity is not yet fully understood, and the biodiversity of some regions is still little known. In particular this is the case of south-western Gabon, where no precise inventory has been undertaken yet.

The cytotaxonomic analysis constitutes a powerful discriminating tool for screening faunistic diversity of small mammals in general, and rodents in particular (PETTER, 1971; ROBBINS & BAKER, 1978; ROBINSON, 2001); it has allowed taxonomists to reveal the presence of numerous sibling species (e.g., *Arvicanthis* : DUCROZ et al., 1997; *Mastomys* : GRANJON et al., 1997; VOLOBOUEV et al., 2001; *Otomys* : TAYLOR, 2000), which are morphologically similar but are showing sufficient chromosomal divergence to ensure reproductive isolation (see KING, 1993). Indeed, following early works of Matthey dating from the 60's (e.g. MATTHEY, 1959; 1963; 1965; 1967), ∝-systematics in African rodents has experienced a significant renewal greatly due to the input of cytogenetics (PETTER, 1971; ROBBINS & BAKER, 1978; TAYLOR, 2000; ROBINSON, 2001).

MATERIAL AND METHODS

An international project, funded by the WWF (project GA085300), was carried out between March 2000 and March 2001 in the Mount Doudou AERF ("Aire d'Exploitation Rationnelle de Faune"), South-western Gabon, to provide data on the small mammals diversity. The Mount Doudou AERF covers 332 000 hectares, and its altitude ranges from 110 to 700 m A.S.L. A one year study on small mammal community ecology was conducted in its eastern part (02°09S-10°30E), in mostly undisturbed lowland forest (110 m A.S.L.). Ten of the rodents captured during this study were subject to chromosomal analysis.

The chromosomal formula of these specimens (diploid number, 2n and autosomal fundamental number, NFa) was determined on the preparations obtained from fibroblast cell cultures using standard Giemsa staining and C-banding technique (SUMNER, 1972). The fibroblast cell lines are cryopreserved and available, as well as the ethanol preserved tissues at the cell and tissue collections of the Laboratoire Zoologie Mammifères et Oiseaux, Musée National d'Histoire Naturelle, Paris. The collection numbers indicated here refer to this "cell and tissue collection". Skulls were extracted and cleaned for species identification and bodies were preserved in 10% formalin. Both bodies and skulls are stored in the general collections of the Musée National d'Histoire Naturelle, Paris.

RESULTS AND DISCUSSION

Heimyscus fumosus (2001-64 : male (M), and 2001-76 : female (F))

Although originally described as belonging to *Hylomyscus* (Brosset et al., 1965), it was later treated as a species belonging to a distinctive genus (Misonne, 1969). It seems to be present only in the central African lowland forests that are located between the Sanaga and the Oubangui-Congo rivers (Nicolas et al., 2003). The karyotype of *H. fumosus* is described here for the first time (Fig. 1). It is characterized by a diploid number (2n) of 40 and contains 5 pairs of meta- or submetacentric and 14 pairs of acrocentric autosomes thus giving NFa=48. Both sex chromosomes are metacentric, the X chromosome is the largest in the set and the Y chromosome is comparable in size with the largest pair of autosomes. The autosome heterochromatine is situated only in the pericentromeric region, the chromosomes short arms are all euchromatic (Fig. 1). The Y chromosome displays some heterochromatic regions.

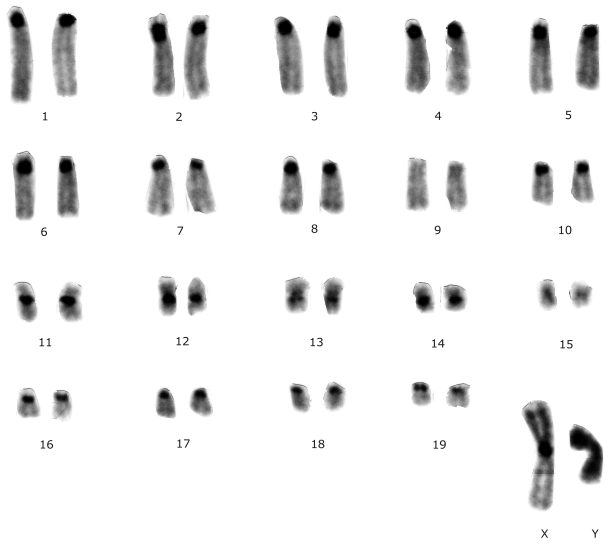

Fig. 1. – C-banded karyotype of *Heimyscus fumosus* (M : n°2001-064).

Hylomyscus

The systematics of this genus is very unclear due to the absence of clear diagnostic external morphological characters (review in Robbins et al., 1980). Since the first chromosomal studies (e.g. Matthey, 1963; 1967) it appeared that karyotype may be a useful discriminating character for at least some species. In the sample studied here two distinct karyotypes were found.

H. cf. stella (2001-98 : F, 2001-99 : F, 2001-100 : F)

These specimens are morphologically similar (skull and external characters) to *H. stella*, the species largely distributed in African rain forest (type locality in East Democratic Republic of Congo, Ituri forest). However the molecular data show that West central African specimens (Cameroon, Central African Republic, Gabon, Republic of Congo) are quite divergent from the East African specimens (Kenya, Democratic Republic of Congo : Lecompte, 2003; Nicolas, 2003). The karyotype of these three specimens has 2n = 46 and NFa = 68 (Fig. 2) and is similar to that earlier described for *H. stella* in Cameroon, Central African Republic and Gabon (Matthey, 1967; Viegas Péquignot et al., 1983; Iskandar et al., 1988; Robbins et al., 1980). The autosome heterochromatine is situated only in the centromeric region, the autosomes short arms are euchromatic whereas the X chromosome short arms are heterochromatic (Fig. 2).

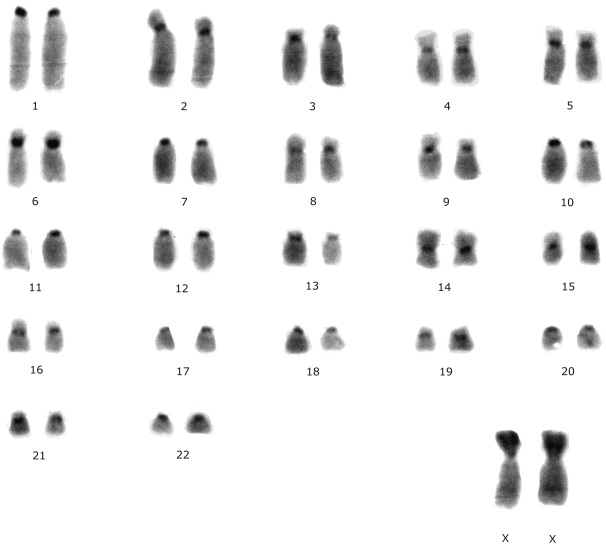

Fig. 2. – C-banded karyotype of *Hylomyscus stella* (F : n°2001-100).

In addition to the karyotype with NFa = 68, Matthey (1963) found NFa = 70 in the same locality (Pointe Noire, Congo). The same value of NFa was found in one specimen from Cameroon (Robbins et al., 1980). Interestingly, morphologically similar animals from Burundi showed a karyotype with 2N = 48 and NFa = 82, which is rather distinct from that described here and in earlier studies (Maddalena et al., 1989). However, considering that the specimens of our study possess only NFa = 68, the nature of this variation remains unknown: The possibility of heterochromatic arms as a source of NFa variation is possible, while no heterochromatic arms have been identified in our sample, representing the lowest autosomal fundamental number known. In order to identify the nature of this karyotypic variation, it is necessary to study specimens from all the distribution area using C- and G-banding pattern. Such study will allow clarifying the taxonomic status of the karyotypic (and molecular) variants of *H. stella*.

Hylomyscus sp (2001-65 : F)

This specimen is morphologically close (skull and external characters) to *H. aeta*, however the interorbital constriction shape as well as palatal foramina are slightly different, and the mammary formula is 2+2 = 8 instead of

1+2 = 6. Its karyotype is characterized by 2n = 56 and NFa = 86 (Fig. 3). The autosome heterochromatine is only situated in the centromeric region (Fig. 3). The X chromosomes show a large band of heterochromatine, including most of the short arms of the X chromosome.

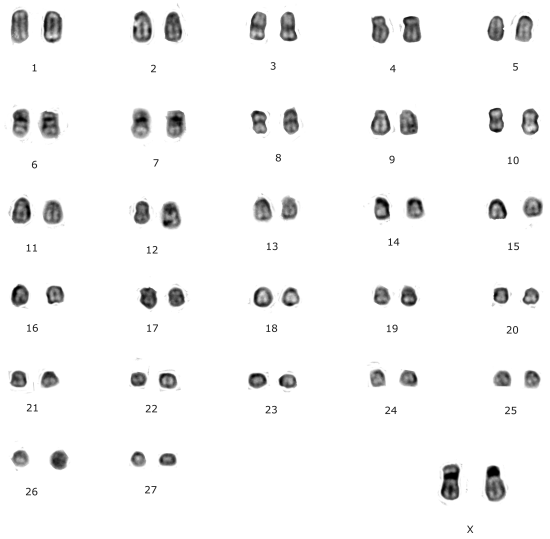

Fig. 3. – C-banded karyotype of *Hylomyscus sp*. (F : n°2001-065).

This karyotype is closer to a series of karyotypes ascribed to *H. aeta* (MATTHEY, 1967; ROBBINS et al., 1980), and differs from the chromosomal formulae classically found in *Hylomyscus* species with 2n ranging between 44 and 48 (MATTHEY, 1963; ROBBINS et al., 1980; VIEGAS-PÉQUIGNOT et al., 1983; ISKANDAR et al., 1988; MADDALENA et al., 1989). The karyotype of *H. aeta* was described for the first time by MATTHEY (1967) for a male specimen from Fernando Pô (Bioko) as having 2n = 52 and NFa = 78, with a metacentric X and an acrocentric Y chromosomes being the largest in the set. However ROBBINS et al. (1980) found two specimens in Cameroon identified as *H. aeta* possessing 2n = 54 and NFa = 86. As a result at least three rather distinct karyotypes have been ascribed to *H. aeta*. Although it is difficult in the absence of chromosome banding data to characterize the exact nature of the chromosomal differences it is clear that this karyotypic diversity most probably hides an unknown taxonomic diversity thus urging for new cytogenetic and molecular studies.

Praomys sp
(2001-63 : M and 2001-77 : M)

These *Praomys* specimens are morphologically identical (skull and external characters) to *P. petteri* (VAN DER STRAETEN et al., 2003). They are characterized by 2n = 42, NFa = 40. Both sex chromosomes are metacentric, the X chromosome is comparable in size with the largest pair of autosomes. This karyotype was already described by MATTHEY (1963) after the analysis of a single specimen from Pointe Noire (Congo). The C-bands allow to identify six autosomes (pairs 1, 2, 3, 6 7 and 8) showing large heterochromatine blocks with a distribution pattern that is highly specific (Fig. 4). These heterochromatine blocks are situated right under the centromeric region and covers between ¼ to ½ of the chromosome length. The heterochromatine pattern is pericentromeric for all the other chromosomes.

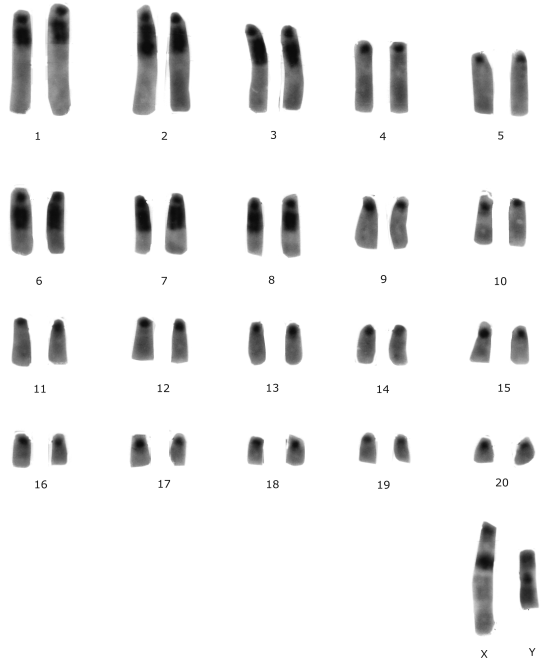

Fig. 4. – C-banded karyotype of *Praomys sp*. (M : n°2001-063).

In the western part of central Africa, three *Praomys* species are identified : *P. jacksoni* (2n = 28; NFa = 26), *P. tullbergi* (2n = 34; NFa = 32) and *P. petteri* (2n = 42; NFa = 62). As our specimens, the karyotype of *P. petteri* is characterized by a diploid number of 42 but contains 11 pairs of meta- or submetacentrics and 9 pairs of acrocentric autosomes thus giving NFa = 62 (MATTHEY, 1965). It would be very interesting to compare specimens of *P. petteri* with those from Gabon, morphologically similar, to investigate the heterochromatine pattern and the presence of such heterochromatine blocks using C-banding. This will allow to verify if the short arms of *P. petteri* metacentric chromosomes are heterochromatic or not and thus to identify the cause of the NFa variation. This could allow a conclusion about the taxonomic status of these new specimens from Gabon. But, as it is for the moment, we know that 1/ the X chromosome is much bigger than the X in *P. petteri*, which is metacentric; 2/ that the Y in our Gabon specimens is metacentric, whereas it is acrocentric in *P. petteri*. These differences seem sufficient to suggest that the specimens from Gabon could be a distinct species. A revision of the genus *Praomys*, combining both molecular and morphometrical data, is presently being investigated in order to solve this problem.

Hybomys univittatus
(2001-72 : F)

The karyotype of the specimen trapped in Mount Doudou is characterized by 2n = 44, NFa = 46. All the chromosomes are acrocentric except for a small pair of metacentric ones. This karyotype is the same as the one described by VERHEYEN & VAN DER STRAETEN (1985) for

H. univittatus from Cameroon. The type locality of *H. univittatus* is in Gabon (Dongila), i.e. in the same faunal region as our study area.

VERHEYEN & VAN DER STRAETEN (1985) analysed three species in the genus *Hybomys* : *H. trivirgatus* (Côte d'Ivoire) 2n = 40 NFa = 38 ; *H. univittatus* (Cameroon) 2n = 44 NFa = 46 ; *H. lunaris* (Rwanda) 2n = 48 NFa = 48. MATTHEY (1959) studied *Hybomys* species labelled *univittattus* in his paper, following VIEGAS-PÉQUIGNOT et al. (1983 ; 1986), characterized by 2n = 48 NFa = 48. This specimen was trapped by Misonne in the Democratic Republic of Congo (F. PETTER, pers. comm.). The karyotype described by MATTHEY (1959) is similar to the *lunaris* karyotype from VERHEYEN & VAN DER STRAETEN (1985) and this specimen from RDC may be a *lunaris* and not a *univittatus* as initially proposed.

Malacomys longipes (2001-70 : F)

The karyotype of this species is characterized by 2n = 48, NFa = 48. All the autosomes are acrocentric except the smallest pair which is metacentric. The X chromosome is a large metacentric one. The karyotype of our study corresponds to that described earlier by VIEGAS-PÉQUIGNOT et al. (1983) from Ivory Coast. The type locality of *M. longipes* is in Gabon (Gaboon river, vicinity of Ogooué), i.e. in the same faunal region as the Mount Doudou area.

CONCLUSION

This study allowed realising a first inventory of forest rodent diversity of the south-western Gabon. Two karyotypes are here described for the first time : those of *Heimyscus fumosus* and *Hylomyscus sp*. The result of this chromosomal analysis increases the murine specific diversity recognised in central Africa : there is probably a new species of *Hylomyscus* in the Mount Doudou area (*Hylomyscus sp* with 2n = 56). Moreover, this study also revealed some systematic problems within *Hylomyscus* and *Praomys* genera.

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Received: November 17, 2003
Accepted: January 18, 2005