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Karyotype analysis of *Murina suilla* and *Phoniscus atrox* from Malaysia (Chiroptera: Murininae, Kerivoulinae)

Karyotypová analysa dvou netopýrů z Malajsie: trubkonosa vepřího (*Murina suilla*) a vlnouška vroubkozubého (*Phoniscus atrox*) (Chiroptera: Murininae, Kerivoulinae)

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Abstract. For the first time, chromosomal data are presented for *Murina suilla* (2n=44, FN=58) and *Phoniscus atrox* (2n=40). The karyotype of *Murina* is distinguished from that of *Myotis* by the presence of euchromatic short arms on chromosomes 12, 13 and 15. The chromosomal complement of *Phoniscus atrox* is characterized by extensive addition of heterochromatic short arms and a paracentric inversion in chromosome 8. The reduction in the diploid chromosome number is due to Robertsonian fusion of chromosomal arms 8+13 and 11+14, respectively.

INTRODUCTION

In recent years, progress in molecular genetic methods yielded fascinating new insights into bat phylogeny (for references see Eick et al. 2005, Teeling et al. 2005) and let, together with morphological and call frequency differences, to the discovery of cryptic species (e.g. *Pipistrellus pygmaeus*, Barrat et al. 1997, Mayer & von Helversen 2001; *Plecotus macrobullaris*, Kiefer & Veith 2001, Kiefer et al. 2002; *Myotis alcathoe* von Helversen et al. 2001, etc.).

The members of the vespertilionid subfamilies Murininae and Kerivoulinae, however, gained less interest than most other vespertilionid genera. This is not only true for molecular genetic investigations but also for cytogenetic studies.

Up to now, out of 15 recognized species of *Murina* Gray, 1842 (13 in Nowak 1994 plus two newly described species: Maeda & Matsumura 1998, Csorba & Bates 2005), chromosomal and fundamental numbers have been reported for six species only. Banded karyotypes have been studied only in *Murina ussuriensis* Ognev, 1913 and *M. hilgendorfi* Peters, 1880 (Harada et al. 1987).

Even less is known from the subfamily Kerivoulinae. Out of the 23 species recognized (Nowak 1994, Vanitharani et al. 2003, Bates et al. 2004), conventionally stained karyotypes have been presented only from *Kerivoula lanosa* (Smith, 1847) (Rautenbach et al. 1993) and *K. papillosa* (Temminck, 1840) (McBee et al. 1986).

In this paper we present detailed karyological information of *Murina suilla* and *Phoniscus atrox* from Malaysia together with a literature overview.

MATERIAL AND METHODS

Specimens examined

Murina suilla, 1 male, 1 female, Templer Park near Kuala Lumpur, SMF 69348, 69349, 1 male, Ulu Gombak Field Studies Centre, Selangor, Malaysia, SMF 69350.

Phoniscus atrox, 1 female, Ulu Gombak Field Studies Centre, Selangor, Malaysia, SMF 69316.
(SMF = catalogue number of the Senckenberg Museum, Frankfurt am Main, Germany)

Chromosome preparations and analysis

Cell culture and cytogenetic procedures were done as described in Volleth (1987) and Volleth et al. (2001). Chromosomal arms were numbered according to Bickham's scheme for American *Myotis* species (Bickham 1979). Karyotypes were compared with the basic karyotype of Vespertilionidae (Volleth & Heller 1994)

RESULTS

Murina suilla (Temminck, 1840)

The karyotype of *Murina suilla* shows a diploid chromosome number (2n) of 44 and a fundamental number of autosomal arms (FN) of 58. The G-banded karyotype of a male specimen is shown in Fig. 1. Chromosomes 7, 12, 13 and 15 show a small G-negative short arm. C-banding revealed that these short arms consist of euchromatic material (Fig. 2). Therefore these short arms arose very likely by pericentric inversions from the acrocentric homologues present in the basic karyotype of Vespertilionidae (Volleth & Heller 1994). AgNOR-staining detected active Nucleolus Organizing Regions (NORs) in the distal part of the euchromatic short arms of chromosomes 7, 12 and 15 and proximal to the centromere in minute short arms of chromosomes 8, 9, 14, 18–20, 22 and 24 or 25. These minute short arms are visible only in silver-stained preparations. The differences in the distribution of active NORs in two specimens are shown in Fig. 3 (35 analyzed cells from specimen 69350 and 20 from specimen 69349). In many cells only one homologue of a chromosomal pair was shown to bear an active NOR. The X is a submetacentric chromosome with a heterochromatic segment in the long arm adjacent to the centromere (see Fig. 2). The subtelocentric Y-chromosome has approximately the same size as chromosome 18 and consists largely of heterochromatic material.

Phoniscus atrox Miller, 1905

The karyotype of *P. atrox* consists of 40 chromosomes and shows a total number of autosomal arms of 76. However, quite a large number of autosomal arms consist of heterochromatin and therefore the fundamental number is presumed to be approximately 50 only. Fig. 4 shows a comparison of G-banded and C-banded chromosomes of the female studied.

The reduction in the diploid chromosome number from the 2n=44 vespertilionid basic karyotype to a 2n=40 in *Phoniscus atrox* is due to two Robertsonian fusion events, i.e. fusion of chromosomal arms 8+13 and 11+14, respectively. In addition, the karyotype is characterized by a paracentric inversion in arm 8.

In the *P. atrox* karyotype, extensive heterochromatin addition was found: first, thin interstitial heterochromatic bands are present in arm 2 (two bands) and in arm 6; second, heterochromatin

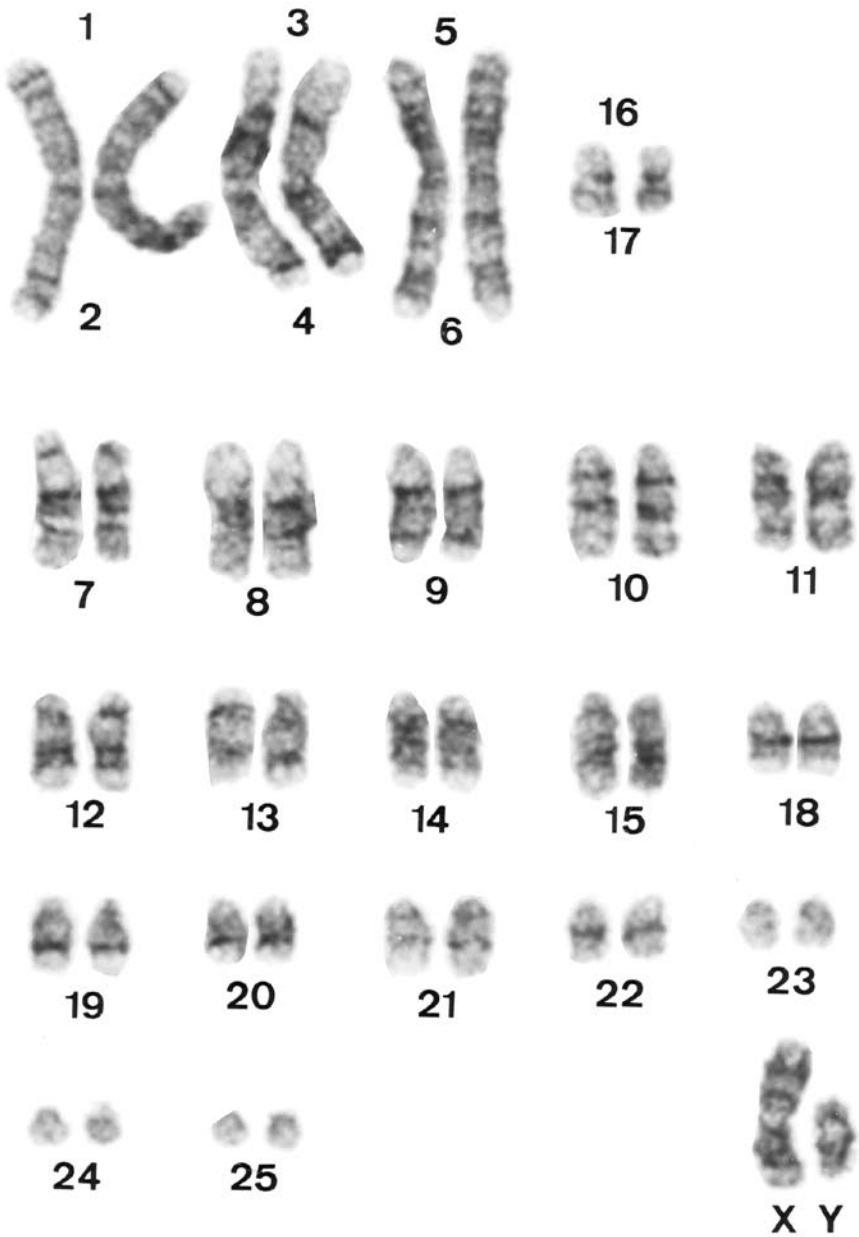


Fig. 1. G-banded karyotype of *Murina suilla*.
Obr. 1. Karyotyp trubkonosa vepřího (*Murina suilla*) barvený G-pruhováním.

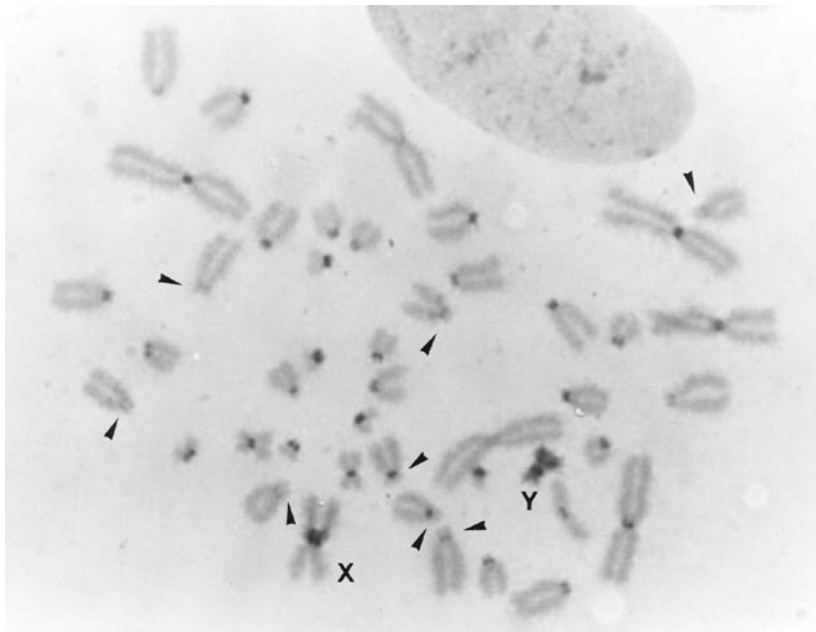


Fig. 2. C-banded metaphase spread of a *Murina suilla* male. The arrows point to the euchromatic short arms of chromosomes 7, 12, 13, and 15.
Obr. 2. Rozptyl C-pruhováním barvené metafase samce *Murina suilla*. Šipky ukazují euchromatická krátká ramena chromosomů 7, 12, 13 a 15.

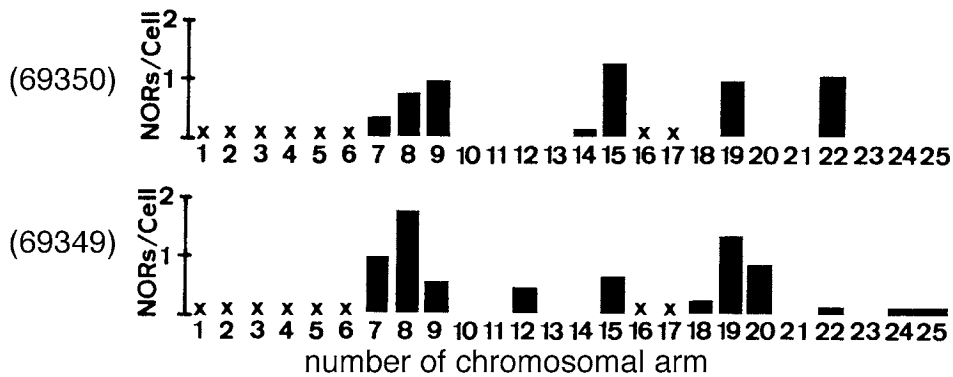


Fig. 3. NOR distribution in two specimens of *Murina suilla* (SMF catalogue number in parentheses). Chromosomal arms of metacentric chromosomes are marked by x.
Obr. 3. Distribuce oblastí organisujících jadérko (NOR) u dvou jedinců *Murina suilla* (v závorkách jsou sbírková čísla exemplářů Senckenbergského musea, Frankfurt nad Mohanem, Německo). Chromosomální ramena metacentrických chromosomů jsou označena x.

is found in the short arms of chromosomes 7, 9, 10, 12, 15, 18–25. In the specimen studied, the size of these heterochromatic arms differs between the homologues of several pairs. This is especially evident for chromosomes 9 and 18 (Fig. 4). In the heterochromatin blocks, G-positive segments are followed by G-negative bands. According to the staining properties, three different entities can be distinghuished: C-positive, CMA brightly fluorescing, G-negative bands are found only adjacent to centromeres of chromosomes 9, 10, 12, 18, 19, 22 and 24. Second, G-positive, C-positive, and DAPI-positive heterochromatin is found in the proximal parts of the short arms of chromosomes 10, 15, 18, 22 and 24. Applying Distamycin A/DAPI double staining, these regions show a weak but clearly positive fluorescence, rarely found in other bat species. The third class is C-negative non-euchromatin, staining either G-positive or G-negative. It is found in the distal parts of the short arms of chromosome 7, 10, 12, 19–21, 23–25. Silver staining revealed active NORs in the short arms of chromosomes 7, 12, 18, 20 and 23. In the case of chromosomes 12, 18 and 23, however, only one homologue each showed an active NOR. These NOR-bearing chromosomes were clearly distinguishable from the respective homologues by the presence of a secondary constriction in the short arm. The X chromosome is a medium-sized submetacentric with a banding pattern similar to that of *Myotis* Kaup, 1829.

Karyotype comparison of vespertilionid genera have revealed that some of the chromosomal arms exist in two states (I, II) differing by inversions (Volleth & Heller 1994). In *Phoniscus*, chromosomal arms 11, 13, 15 and the X, all show state I, as in the genus *Myotis*. The state of chromosome 7 is difficult to determine. The short arm is clearly different from that of *Myotis* due to the terminal addition of two G-positive bands. According to the banding pattern of the long arm, however, chromosome 7 of *Phoniscus* Miller, 1905 evolved very likely from state I.

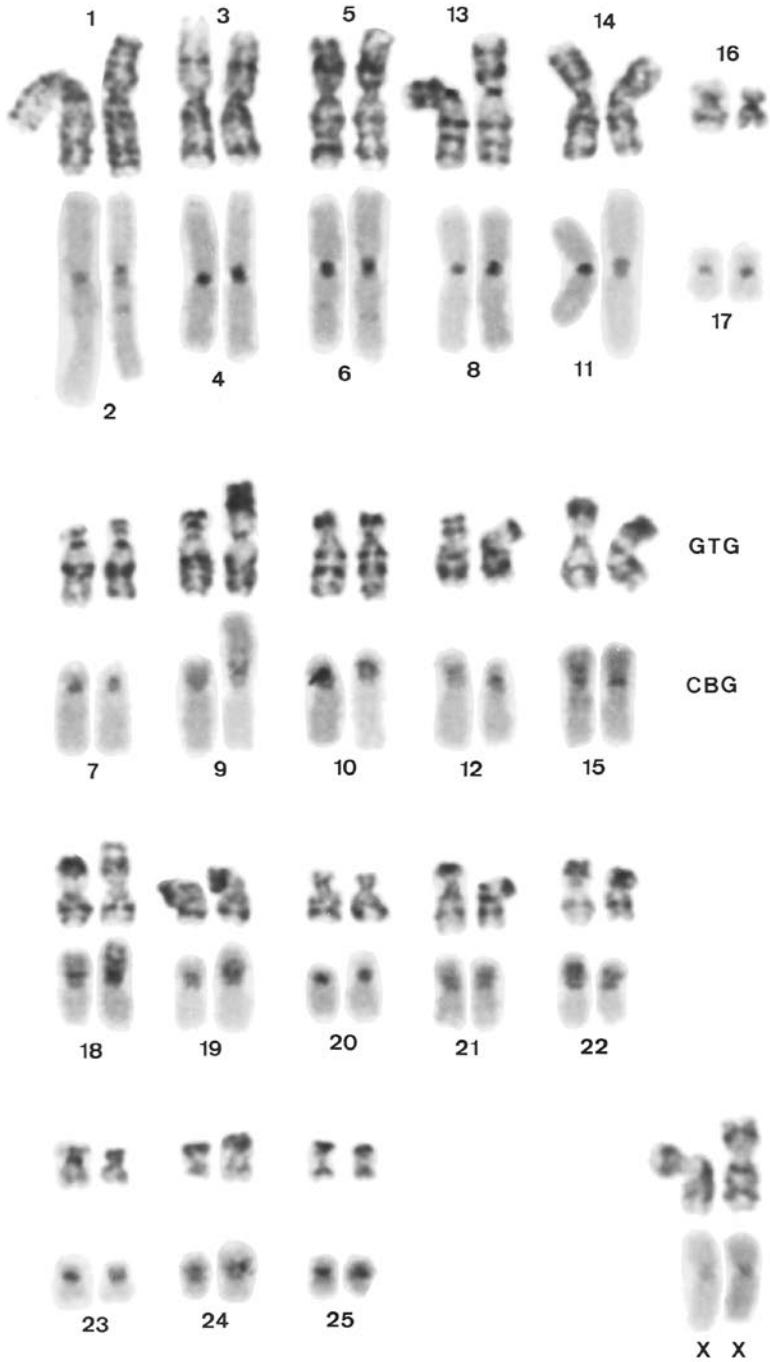
DISCUSSION

As *Myotis*, the members of the subfamily Kerivoulinae show the highest tooth number found in Vespertilionidae, i.e. 38. Apart from this primitive character, a wide range of morphological features characterizes this subfamily (see Miller 1907, Koopman 1994). There are 23 recognized species in two genera, *Kerivoula* Gray, 1842 and *Phoniscus*. According to Miller (1907), "the greatly increased size and peculiar shape of the upper canine and the four-cusped inner mandibular incisors distinguish this genus (*Phoniscus*) sufficiently from *Kerivoula*". Together with Hill (1965) and Corbet & Hill (1992) we follow Miller (1907) in treating *Phoniscus* as a separate genus and not as a subgenus of *Kerivoula* as Koopman (1994).

The subfamily Murininae, comprising three genera, *Murina*, *Harpiola* Thomas, 1915 (Bhattacharyya 2002, Kuo et al. 2006) and *Harpiocephalus* Gray, 1842, is characterized by tubular nostrils. It has been regarded by Miller (1907) "as a specialized offshoot from some low, *Myotis*-like Vespertilioninae form".

Up to now, only few molecular genetic studies have dealt with phylogenetic relationships of Murininae and Kerivoulinae. Consistently all studies revealed a sister relationship between *Myotis* and a clade containing Murininae and Kerivoulinae (Van Den Bussche & Hoofer 2001, Hoofer & Van Den Bussche 2003, Stadelman et al. 2004, Kawai et al. 2002). As a result, Hoofer & Van Den Bussche (2003) proposed subfamiliar rank for Myotini.

Our cytogenetic results revealed one common feature for *Myotis*, *Murina* and probably also *Phoniscus*, i.e. an euchromatic short arm on chromosome 7 (see Volleth & Heller 1994). From karyological reasons, it cannot be decided at the moment whether this character is ancestral or derived. Remarkably, in the Australian genus *Vespadelus* Troughton, 1943, belonging to the


1
3
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16
2
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11
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GTG
CBG
7
9
10
12
15
18
19
20
21
22
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24
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X X

←

Fig. 4. Karyotype of *Phoniscus atrox* after G-banding (upper rows) and C-banding (lower rows). Note size polymorphism of heterochromatic short arms.
Obr. 4. Karyotyp vlnouška vroubkozubého (*Phoniscus atrox*) po G-pruhovém barvení (horní řady) a C-pruhovém barvení (dolní řady). Nápadný je polymorfismus velikosti heterochromatických krátkých ramen.

Vespertilionini, euchromatic short arms are also present on chromosomes 7 and 13 (Volleth & Tidemann 1989). As in *Myotis* and *Murina*, these short arms occurred probably by pericentric inversions. In the case of *Vespadelus*, however, these characters are clearly derived, as closely related genera lack euchromatic short arms. In the case of *Murina* and *Vespadelus*, we are clearly faced with a re-use of identical or similar inversion break-points on two chromosomes, 7 and 13. It could be suspected that these break-points have occurred within relatively short fragile regions, being hot-spots of rearrangements. Recently, this model has been proposed to explain break-point clustering in chromosome evolution by Pevzner & Tesler (2003). The authors concluded that "mammalian genomes are mosaics of fragile regions with high propensity for rearrangements and solid regions with low propensity for rearrangements". This break-point clustering in fragile regions reveals limitations of the widely accepted random breakage theory.

Comparison with published karyological data

From the subfamily Kerivoulinae, only two out of 23 species have been studied karyologically up to now. The examined female specimen of *Kerivoula papillosa* showed a diplod chromosome number of 38 and a fundamental number (including the X chromosomes) of 52 (McBee et al. 1986). The karyotype of the second species studied, *K. lanosa*, consists of 28 chromosomes

Table 1. Chromosomal data for *Murina*. Abbreviations: M – metacentric; SM – submetacentric; ST – subtelocentric; A – acrocentric; conv – conventional staining; G – G-banding; C – C-banding; AgNOR – silver-staining of NORs
Tab. 1. Chromosomální údaje o rodu *Murina*. Zkratky: M – metacentrický; SM – submetacentrický; ST – subtelocentrický; A – akrocentrický; conv – konvenční barvení; G – G-pruhování; C – C-pruhování; AgNOR – barvení oblastí organisujících jadérko (NOR) stříbrem

species	2n	FN	M-SM	ST	A	X	Y	method	source
aurata[1]	44	60	5	4	12	SM	A	conv	Ando et al. 1977
cyclotis	44	50?	4	–	17	SM	–	conv	Rickart et al. 1999
hilgendorfi[2]	44		4	–	17	SM	–	conv	Harada 1973
hilgendorfi	44	56	4	3	14	SM	A	G	Harada et al. 1987
leucogaster	44	58	4	4	13	SM	A	conv	Ando et al. 1977
leucogaster	44	50	4*	–	17	SM	A	conv	McBee et al. 1986
puta	44	50	4	–	17	M	A	conv	Lin et al. 2002
silvatica	44	56	3	4	14	M	A	conv, AgNOR	Ono & Obara 1994
suilla	44	58	4	4	13	SM	ST	G, C, AgNOR	this study
ussuriensis	44	56	4	3	14	SM	A	G	Harada et al 1987

* chromosomes showing different morphology compared to the other species.
[1] these Japanese specimens would probably be classified with *M. ussuriensis* today.
[2] the subspecies *leucogaster hilgendorfi* has been elevated to species rank by Yoshiyuki (1989).

with a FN of 50 (Rautenbach et al. 1993). The *Phoniscus atrox* female presented here is the first Kerivoulinae species where banding methods have been applied. Comparing the three species studied, it becomes clear that the karyological variability is much greater in the subfamily Kerivoulinae than in the Murininae and Myotinae. Chromosomal data from other Kerivoulinae species are therefore urgently needed for a karyological characterization of this subfamily.

More chromosomal data are available from the subfamily Murininae. Conventionally stained karyotypes of 6 *Murina* species have been published (references see Table 1), all with a diploid chromosome number of 44. Concerning the number of subtelocentric chromosomal pairs, the data differ, in some cases even within the same species. To my opinion, this is more likely a matter of interpretation than of real differences, as the minute short arms are clearly visible only in high quality metaphases. On the other hand, chromosomal differences could probably point to hidden species. One example could be the *M. leucogaster* Milne-Edwards, 1872 specimen of McBee et al. (1986), showing a clearly different morphology of the metacentric pairs. An indication for cryptic species in the genus Murina was also revealed by cytochrome b sequence comparison of *Murina* cf. *cyclotis* Dobson, 1872 specimens from Laos (Stadelman et al. 2004). From the two other Murininae genera, *Harpiola* and *Harpiocephalus*, chromosome description has only be given for a single female specimen of *Harpiocephalus mordax* Thomas, 1923 with a 2n=40 (McBee et al. 1986). According to the 5 subtelocentric chromosomal pairs observed in this specimen, it could be suspected that there are also some pairs with euchromatic short arms as in *Murina*.

From the genus *Murina*, G-banding patterns of two species, *Murina ussuriensis* and *Murina hilgendorfi*, have been published previously (Harada et al. 1987). The results are in good agreement with the data published here. Only the euchromatic short arm on chromosome 7, present in *Murina* and the genus *Myotis*, has not been mentioned by Harada et al. (1987).

The distributional pattern of Nucleolus Organizing Regions has only been studied in *M. silvatica* (Ono & Obara 1994). As in the genus *Myotis* and in *M. suilla*, multiple NORs on minute short arms of acrocentric chromosomes have been found in this species.

As multiple NORs are also present in *Phoniscus atrox*, the distributional pattern of NORs is very similar in Myotinae, Kerivoulinae and Murininae. However, the ancestral location of the NORs for Vespertilionidae is not yet ascertained and therefore this feature is not suited for judging phylogenetic relationships amongst these subfamilies.

SOUHRN

Poprvé jsou uvedeny popisy chromosomů u trubkonosa vepřího (*Murina suilla*) (2n=44, FN=58) a vlnouška vroubkozubého (*Phoniscus atrox*) (2n=40). Karyotyp rodu *Murina* se liší od karyotypu rodu *Myotis* přítomností euchromatických krátkých ramen na chromosomech 12, 13 a 15. Chromosomová sada druhu *Phoniscus atrox* je typická extensivním přídavkem heterochromatických krátkých ramen a paracentrickou inversí na chromosomu 8. Redukce diploidního počtu chromosomů je způsobena Robertsonskými fusemi chromosomálních ramen 8+13 a 11+14.

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