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VARIATION IN THE NUCLEOLAR ORGANISER REGIONS OF THE LONG-TAILED GIANT RATS (RODENTIA, MURIDAE, GENUS *LEOPOLDAMYS*) IN MALAYSIA

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ABSTRACT. — The nucleolar organiser regions of *Leopoldamys ciliatus* and *L. sabanus* from Peninsular Malaysia were studied by silver-staining. Three pairs of Ag-NORs were present in *L. ciliatus* while *L. sabanus* had four pairs of Ag-NORs. The two subacrocentric pairs were similar in these species. *L. ciliatus* had a metacentric pair while *L. sabanus* had two acrocentric pairs. Of the two acrocentric pairs in *L. sabanus*, the medium-sized autosome had the NOR located at the terminal part of the long arm. The complement of NORs in *L. ciliatus* and *L. sabanus* also differ from the published records of five pairs (two subacrocentric, two acrocentric, one metacentric) for *L. edwardsi* and three pairs (two subacrocentric, one acrocentric) for *L. neilli* from Thailand. Nucleolar organiser regions thus serve as an adjunct to delimit *L. ciliatus* from phenotypically similar species *L. edwardsi*, *L. neilli*, and *L. sabanus*.

KEY WORDS. — Ag-NOR, chromosomes, species differentiation, systematics, *Leopoldamys ciliatus*, *Leopoldamys edwardsi*, *Leopoldamys neilli*, *Leopoldamys sabanus*

INTRODUCTION

The murid genus *Leopoldamys* is represented by some seven species—*L. ciliatus* (Bonhote), *L. diwangkarai* Maryanto & Sinaga, *L. edwardsi* (Thomas), *L. milleti* (Robinson & Kloss), *L. neilli* (Marshall), *L. sabanus* (Thomas), and *L. siporanus* (Thomas) (see Musser & Carleton, 2005; Maryanto & Sinaga, 2008). They are giant rats with long tails, whence the common name 'long-tailed giant rats'. Two morphologically similar species (*L. ciliatus* and *L. sabanus*) are present in Malaysia. The Sundaic mountain leopoldamys *L. ciliatus* was previously referred to as a subspecies of Edward's leopoldamys *L. edwardsi* (see Yong, 1970; Medway, 1983; Corbet & Hill, 1992; Musser & Carleton, 2005).

The karyotypes of *L. ciliatus* (see Yong, 1968a), *L. edwardsi* (see Cao & Tran, 1984; Badenhorst et al., 2009), *L. neilli* (see Marshall Jr., 1977; Yosida, 1979; Badenhorst et al., 2009), and *L. sabanus* (see Yong, 1968a; Duncan & van Peenen, 1971; Markvong et al., 1973) have been reported. Excepting *L. ciliatus*, the nucleolar organiser regions (NORs) have been documented for *L. edwardsi* (see Badenhorst et al., 2011) and *L. neilli* and *L. sabanus* (Yosida, 1979).

We report here the variation in the NORs of *L. ciliatus* and *L. sabanus* from Peninsular Malaysia and the application of NORs as an adjunct to delimit *L. ciliatus* from phenotypically similar species *L. edwardsi*, *L. neilli*, and *L. sabanus*.

MATERIAL AND METHODS

The long-tailed giant rats were trapped in Peninsular Malaysia: *L. ciliatus* (two males) from the mountain (Gunung Bunga Buah, Selangor) and *L. sabanus* (3 males and 1 female) from lowland forest (Janda Baik, Pahang). The rats were treated with 0.01% (w/v) colchicine in RPMI for 1 h. Bone marrow of tibia and femur was used for chromosome preparation by the air drying technique (Yong, 1968a, 1969). Briefly, the colchicine-treated bone marrow cells were treated with 0.56%

KCl solution for 30 min, then fixed in 3:1 ethanol:acetic acid preservative (three changes). The final cell suspension was used for immediate chromosome preparation or stored in deep freezer until needed. The metaphase chromosomes were treated with seven parts 50% $AgNO_3$ and three parts of 0.02% formic acid for 2 h at 60°C, then stained with 4% Giemsa for 1 h (Yong, 1984). At least 20 well-spread metaphases of each specimen were photographed under oil immersion for Ag-NOR analysis.

RESULTS

Ag-NORs (silver-stained nucleolar organiser regions) were present in three pairs of chromosomes in *L. ciliatus*, comprising the longest subacrocentric (sa_1) autosome, the third longest subacrocentric (sa_3) autosome, and the small metacentric (m) autosome (Fig. 1). In contrast, *L. sabanus* had four pairs of autosomes with Ag-NORs, viz. longest subacrocentric (sa_1), third longest subacrocentric (sa_3), longest acrocentric (a_l), and medium acrocentric (a_m) (Fig. 2). Of the two Ag-NOR acrocentric autosomes in *L. sabanus*, the NOR in the shorter pair (a_m, medium-sized acrocentric) is located at the terminal end of the long arm.

DISCUSSION

The type locality of the Sundaic mountain leopoldamys is Gunung Inas, Perak, Peninsular Malaysia, and was first described as *Mus ciliata* (see Bonhote, 1900). Bonhote (1903) categorically allied *ciliatus* with *edwardsi*. The taxon *ciliatus* was subsequently treated as a subspecies of *edwardsi* under the genus *Rattus* or *Leopoldamys* (Chasen, 1940; Ellerman, 1947; Yong 1970; Medway & Yong, 1976; Medway, 1983; Corbet & Hill, 1992). At present, it is regarded as a distinct species *L. ciliatus*, distributed in Peninsular Malaysia and Sumatra (Musser, 1981; Musser & Carleton, 2005).

Yong (1970) stated that "although the systematic status of *R. e. ciliatus* and *R. s. vociferans* are without doubt valid, the question whether the Malayan taxa are actually conspecific with allopatric taxa *edwardsi* and *sabanus* still remains." The taxon *ciliatus* has been subsequently separated from *edwardsi* and accorded specific status as *L. ciliatus* (Musser, 1981).

Morphologically, Malaysian specimens of *L. sabanus* (Fig. 3) are easily distinguished from *L. ciliatus* (Fig. 4) by the contrasting orange stripe on the flank separating the dark dorsum and pale venter, and possession of a bicoloured tail (Fig. 5). Variation in body and tail colouration, including albinism (Yong, 1967), would however pose identification problems (Yong, 1970). Nonetheless, karyotype and serology provide distinct genetic discrimination of these two taxa (Yong, 1968a, 1970). The body dimensions (head and body length, tail length, and hind foot length) of these and other *Leopoldamys* species exhibit considerable variation and overlaps (Table 1).

The Ag-NORs show distinct differences between *L. ciliatus* and *L. edwardsi* from Thailand: three pairs (two subacrocentric and one metacentric) for *L. ciliatus* (Fig. 1) and five pairs (two subacrocentric, two acrocentric, and one metacentric) for *L. edwardsi* (Badenhorst et al., 2011). Whether the two subacrocentric pairs are identical in the two taxa remain to be validated, particularly the longer pair. In addition to Ag-NORs, there also appear to be slight differences in the karyotypes of *L. ciliatus* and *L. edwardsi* (Table 2). The Y-chromosome in *L. ciliatus* is a small acrocentric (Yong, 1968a, 1968b) while that of *L. edwardsi* is dot-like (Badenhorst et al., 2009).

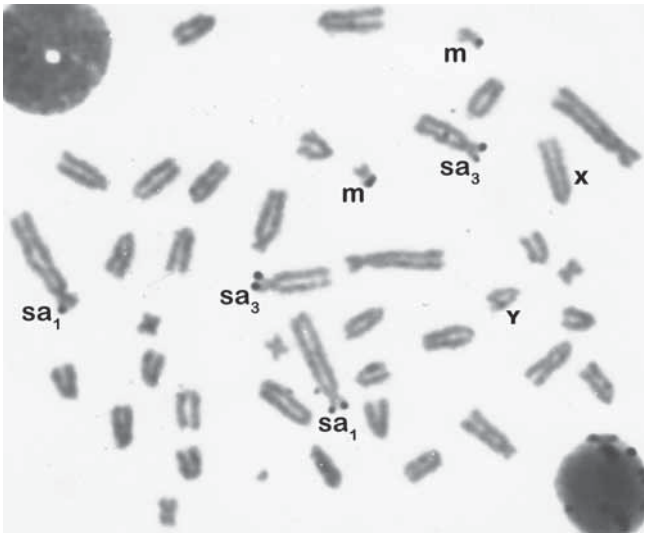

Fig. 1. Metaphase of male *Leopoldamys ciliatus* with three pairs of NORs (sa_1, sa_3 and m) stained with silver nitrate. X-chromosome is the longest acrocentric and Y the smallest acrocentric in the complement.

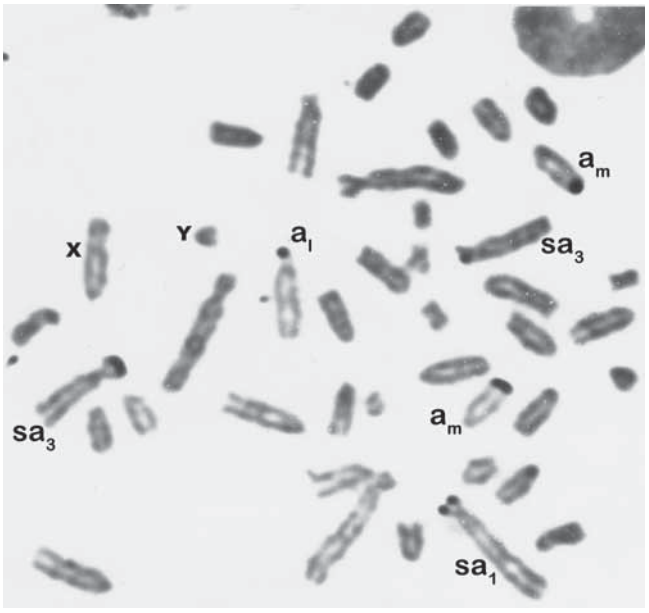

Fig. 2. Ag-NOR stained metaphase of male *Leopoldamys sabanus*: NORs located on two subacrocentric autosomes (sa_1 and sa_3), a large acrocentric (a_l), and a medium-sized acrocrocentri (a_m with NOR at the terminal end of the long arm). X-chromosome is the longest acrocentric and Y the smallest acrocentric in the complement.

Table 1. Range of body dimensions (in mm) of *Leopoldamys* species. [a]data from Yong (1968b); [b]data from Maryanto & Sinaga (2008); [c]data from Francis (2008).

Species	Head & body length	Tail length	Hind foot length
L. ciliatus[a]	220–275	310–355	51–56
L. diwangkarai[b]	197–225	293–317	42.73–49
L. edwardsi[c]	210–280	290–360	46–54
L. milleti[c]	210–280	290–360	46–54
L. neilli[c]	200–235	240–300	39–45
L. sabanus[a]	210–260	315–420	44–52
L. siporanus[b]	178–287	220–335	45–52

Table 2. Karyotypes of *Leopoldamys* species. 2N, diploid number; M, metacentric; S, subacrocentric/subterminal; A, acrocentric/telocentric. [a] data from present study and Yong (1968a); [b] data from Badenhorst et al. (2009); [c] data from Marshall Jr. (1977) and Yosida (1979).

Species	2N	Autosomes			Allosomes	
		M	S	A	X	Y
L. ciliatus [a]	42	6	8	26	A	A
L. edwardsi[b]	42	6	8	26	A	dot
L. neilli[c]	44	4	4	34	A	?
L. sabanus [a]	42	4	8	28	A	A

Fig. 3. *Leopoldamys sabanus* (from Janda Baik, Pahang) with a contrasting orange stripe on the flank demarcationg the dark dorsum and pale venter.

The present results on Ag-NORs provide further evidence of distinct differences between *L. sabanus* and *L. ciliatus* as well as *L. edwardsi*. Of the four pairs of NORs (sa_1, sa_3, a_l, a_m) in *L. sabanus*, the two subacrocentric pairs are similar, if not identical, to those in *L. ciliatus*. The metacentric autosome with NOR in *L. ciliatus* and *L. edwardsi* is not present in *L. sabanus*: *L. sabanus* has two pairs of metacentric autosomes while *L. ciliatus* and *L. edwardsi* have three pairs, hence the metacentric autosome with NOR is not represented in *L. sabanus*.

The Ag-NOR constitution of *L. sabanus* from Malaysia is identical to that reported by Yosida (1979), with a_l corresponding to pair 5 and a_m corresponding to pair 9. The a_m element (pair 9 of Yosida) with the NOR at the terminal end of the long arm is also present in *L. neilli* (Yosida,

Fig. 4. *Leopoldamys ciliatus* (from Gunung Bunga Buah, Selangor) without an orange stripe on the flank demarcationg the dark dorsum and pale venter.

Fig. 5. Ventral side of tail of *Leopoldamys sabanus* from Janda Baik, Pahang (top, bicoloured with pale venter) and *L. ciliatus* from Gunung Bunga Buah, Selangor (bottom, uniformly coloured).

1979). *L. neilli* however has 2n = 44 with three pairs of NORs; the other two pairs are subacrocentric pairs 1 and 3, corresponding to sa_1 and sa_3 respectively.

In the present study, not all the metaphases of an individual revealed the full complement of active NORs (Figs. 6,7). The non-active NORs may involve any members of the homologous pairs. As silver staining detect only the active NOR, and because of the occurrence of intraspecific variation, adequate sample sizes and geographically representative sampling are needed for unequivocal coclusion on the number and location of the NORs (Badenhorst et al., 2011). The present findings on *L. ciliatus* and *L. sabanus* are beyond reasonable doubt as more than one individual and many metaphases were studied.

It is evident from the present study and published data that chromosomal characters will be a useful adjunct for systematic and phylogenetic studies. For *L. ciliatus*, information on the Sumatran taxon *setiger* is needed: "Uniting Sumatran populations with those on Malay Peninsula within the same species requires testing by analyses of multi-trait morphological data and molecular sequences." (Musser & Carleton, 2005). Likewise, systematic resolution is needed for *L. edwardsi* from various geographical regions (Musser & Carleton, 2005), and perhaps all other taxa in the genus.

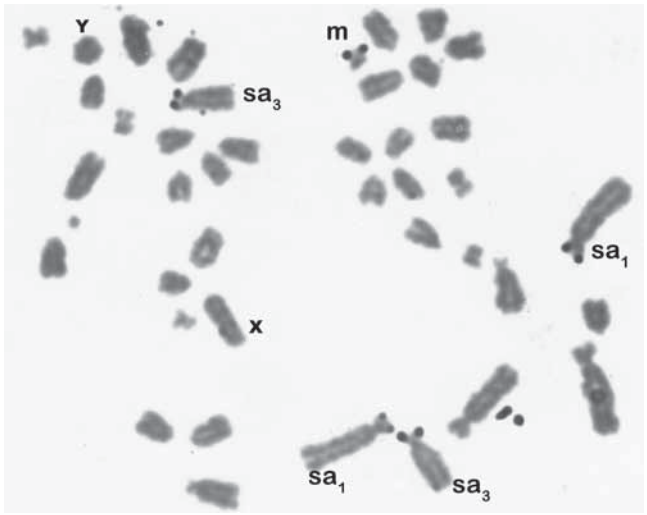

Fig. 6. Ag-NOR metaphase of male *Leopoldamys ciliatus*: NOR not expressed in one member of the metacentric pair.

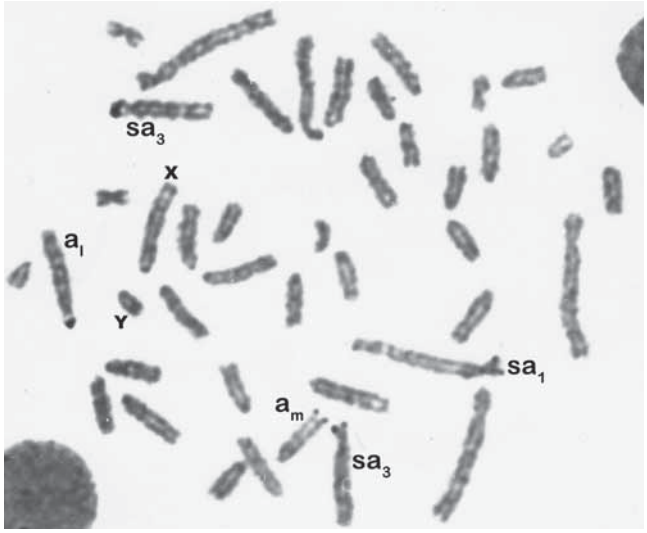

Fig. 7. Ag-NOR metaphase of male *Leopoldamys sabanus* with one member each of three pairs (sa_1, a_l. a_m) being expressed.

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