STANDARDIZED KARYOTYPE AND IDIOGRAM OF THE VARIABLE SQUIRREL, *Callosciurus finlaysoni bocourti* (MILNE-EDWARDS, 1867) BY CONVENTIONAL STAINING TECHNIQUE

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ABSTRACT

Standardized karyotype and idiogram of the variable squirrel (*Callosciurus finlaysoni bocourti*) was studied at Khon Kaen University, Thailand. Blood samples were taken from two male and two female variable squirrels. After lymphocyte culture, the mitotic chromosome preparation was accomplished by the hypotonic-air-drying method and conventional Giemsa's staining technique. The results showed that the number of diploid chromosomes was 2*n*=40 and the fundamental number (NF) was 78 in both male and female. The autosomes consisted of 4 large metacentric, 4 large submetacentric, 14 large acrocentric, 2 medium telocentric, 4 small metacentric, 6 small acrocentric and 4 small telocentric chromosomes. The X chromosome was a medium metacentric chromosome and the Y chromosome was a small metacentric chromosome. A pair of the short arm near centromere of chromosome pairs 14 showed clearly observable secondary constriction (satellite chromosomes). The chromosome marker of the variable squirrel is the chromosome pair 1 which is the largest submetacentric chromosome. The karyotype formula for the variable squirrel is as follows:

$2n\ (40) = L^{m}_{4}+L^{sm}_{4}+L^{a}_{14}+M^{t}_{2}+S^{m}_{4}+S^{a}_{6}+S^{t}_{4}+$ sex chromosomes

บทคัดย่อ

คารีโอไทป์ และอิดิโอแกรมมาตรฐานของกระรอกหลากสี (*Callosciurus finlaysoni bocourti*) จากมหาวิทยาลัยขอนแก่น ประเทศไทย ใช้ตัวอย่างเลือดกระรอกหลากสีเพศผู้ 2 ตัว และเพศเมีย 2 ตัว ทำการเพาะเลี้ยงเซลล์เม็ดเลือดขาว และเตรียมโครโมโซมด้วยเทคนิคโคลชิซิน-ไฮโปโทนิค-ฟิกเซชั่น-แอร์ดรายอิง ใช้วิธีย้อมสีแบบธรรมดา ผลการศึกษาพบว่ากระรอกหลากสีมีโครโมโซมดิพลอยด์ (2*n*) เท่ากับ 40 แท่ง มีจำนวนโครโมโซมพื้นฐาน (NF) เท่ากับ 78 ทั้งในเพศผู้และเพศเมีย โครโมโซมร่างกายประกอบด้วยโครโมโซมชนิดเมทาเซนทริกขนาดใหญ่ 4 แท่ง

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ซับเมทาเซนทริกขนาดใหญ่ 4 แท่ง อะโครเซนทริกขนาดใหญ่ 14 แท่ง เทโลเซนทริกขนาดกลาง 2 แท่ง เมทาเซนทริกขนาดเล็ก 4 แท่ง อะโครเซนทริกขนาดเล็ก 6 แท่ง และเทโลเซนทริกขนาดเล็ก 4 แท่ง โครโมโซมเพศมีโครโมโซมเอ็กซ์เป็นชนิดเมทาเซนทริกขนาดกลาง และโครโมโซมวายเป็นชนิดเมทาเซนทริกขนาดเล็ก ตรวจพบรอดคอดที่สองบนแขนข้างสั้นใกล้ เซนโทรเมียร์ในโครโมโซมร่างกายคู่ที่ 14 (เป็น satellite chromosome) โครโมโซมเครื่องหมายของกระรอกหลากสีพบว่าโครโมโซมร่างกายคู่ที่ 1 เป็นโครโมโซมชนิดซับเมทาเซนทริกขนาดใหญ่มากที่สุด กระรอกหลากสีมีสูตรคาริโอไทป์ ดังนี้

$2n$ (ดิพลอยด์) 40 = $L^{m}_{4}+L^{sm}_{4}+L^{a}_{14}+M^{t}_{2}+S^{m}_{4}+S^{a}_{6}+S^{t}_{4}+$ โครโมโซมเพศ

INTRODUCTION

Variable squirrel (*Callosciurus finlaysoni bocourti*) is member of the order Rodentia, family Sciuridae and subfamily Sciurinae. The subfamily Sciurinae represents only 17 species of 8 genera in Thailand, namely the Black giant squirrel (*Ratufa bicolor*), Cream-colored squirrel (*Ratufa affinis*), Plantain squirrel (*Callosciurus notatus*), Black-banded squirrel (*Callosciurus nigrovitatus*), Pallas's squirrel (*Callosciurus erythraeus*), Variable squirrel (*Callosciurus finlaysoni*), Grey-bellied squirrel (*Callosciurus caniceps*), Prevost's squirrel (*Callosciurus prevostii*), Horse-tailed squirrel (*Sundasciurus hippurus*), Slender squirrel (*Sundasciurus tenuis*), Low's squirrel (*Sundasciurus lowii*), Cambodian striped squirrel (*Tamiops rodolphii*), Burmese striped squirrel (*Tamiops mcclellandi*), Indochinese ground squirrel (*Menetes berdmorei*), Shrew-faced ground squirrel (*Rhinosciurus laticaudatus*), Three-striped ground squirrel (*Lariscus insignis*) and Red-cheeked squirrel (*Dremomys rufigenis*) (Lekagul and McNeely 1977, 1988; Parr, 2003).

The common color characteristics of the Variable squirrel ranges from completely creamy white to creamy white with a black back, nape, thighs, and upper side of the tail. Between these two extremes there are all degrees of intermediacy. The black may be restricted to the back and tail or only to the back. The back may be deep black or grayish black, dark or light gray or "dirty" white (when only the bases of the hairs on the back are black). Also, there may be white hairs scattered through the dark back (Lekagul and McNeely, 1977, 1988; Parr, 2003) (Figure 1).

There is only one report of a variable squirrel cytogenetic study according to the report of Nadler *et al*. (1975) that investigated this species' karyotype using the conventional staining technique. In addition, we also compare and validate make a confirmation and comparison for the

results with the previous report. Moreover, this study can provided important to similar basic research opportunities..

MATERIALS AND METHODS

Blood samples were collected from the caudal vein of two male and two female variable squirrels, which were kept at Khon Kaen University, Khon Kaen province, Thailand, using the aseptic technique. The samples were kept in 3 ml vacuum tubes containing heparin to prevent blood clotting and were cooled on ice until arriving at the laboratory.

Cell culture

The lymphocytes were cultured using the whole blood microculture technique adapted from Kampiranont (2003). The RPMI 1640 medium was prepared with 2% Phytohemagglutinin (PHA) as a mitogen and kept in blood culture bottles of 8 ml each. A blood sample of 0.5 ml was dropped into a medium bottle and mixed well. The culture bottle was loosely capped, incubated at 37°C under 5% of a carbondioxide environment and regularly shaken in the morning and evening. When reaching harvest time at the 96nd hour of incubation, colchicine was introduced and mixed well followed by further incubation for 20 minutes.

Cell harvest

The blood sample mixture was centrifuged at 3,000 rpm for 5 minutes and the supernatant was discarded. 13 ml of hypotonic solution (0.075 M KCl) was applied to the pellet and the mixture was incubated for 40 minutes. After centrifuging again and discarding the supernatant, the cells were fixed by a fresh cool fixative (3 methanol: 1 glacial acetic acid) gradually built added up to 7 ml before centrifuging and the discarding the supernatant. The fixation was repeated until the supernatant was clear and the pellet was mixed with a 1 ml fixative. The mixture was dropped onto a clean and cold slide using a micropipette followed by the air-dry technique.

Chromosomal checks karyotyping and idiograming

Chromosomal checks were performed on mitotic metaphase cells under a light microscope. Fifteen cells of female and male variable squirrel with clearly observable and well-spread chromosomes were selected and photographed. Metaphase chromosomes were used in karyotyping and idiograming from Nash and O'Brien (1987) and Wada *et al* (1991).

RESULTS AND DISCUSSION

After cell culturing at 37°C for 96 hours and conventional staining, we found that *C. finlaysoni bocourti* had 2*n* = 40 (Figures 2, 3 and 4). This result agrees with Nadler *et al*. (1975). Comparing with squirrel species in the genus *Callosciurus*, the chromosome numbers the same as reported by Nadler and Hoffmann (1970), who reported the chromosome numbers of *C. notatus* and *C. flavimanus* were 2*n*=40. Furthermore, Oshida and Yoshida (1999) and Li *et al*. (2004) also reported that the chromosome numbers of *C. erythraeus* and *C. prevostii* were 2*n*=40.

C. finlaysoni bocourti has the fundamental number (NF, number of chromosome arms) of 78 in both of male and female. Comparing with squirrel species in the genus *Callosciurus*, the NF was different according to Oshida and Yoshida (1999), who reported the NF of *C. erythraeus* and *C. prevostii* of NF=70. Furthermore, Nadler and Hoffmann (1970) also reported that the NF of *C. flavimanus* was NF=74.

C. finlaysoni bocourti has 4 large metacentric, 4 large submetacentric, 14 large acrocentric, 2 medium telocentric, 4 small metacentric, 6 small acrocentric and 4 small telocentric autosomes. Different chromosomal features were reported by Nadler *et al*. (1975), which indicated that *C. finlaysoni bocourti* had 12 metacentric, 20 submetacentric and 6 acrocentric autosomes.

The important chromosome marker of the variable squirrel is the asymmetrical karyotype, which is a karyotype found in all of 4 chromosomes types including metacentric, submetacentric, acrocentric and telocentric chromosomes. The largest and smallest chromosomes show large size differences (approximately 7 folds). The largest chromosomes was the metacentric chromosome, and the autosome pair 19 was the smallest telocentric chromosome (Figure 6).

The X chromosome of the *C. finlaysoni bocourti* is a medium metacentric chromosome and the Y chromosome is a small metacentric chromosome. These features are different to that reported by Nadler *et al*. (1975), indicating that the variable squirrel has a submetacentric X chromosome and a submetacentric Y chromosome. In comparison with other *Callosciurus*, the X chromosomes and the Y chromosomes of *C. notatus* and *C. flavimanus* are metacentric chromosomes (Nadler and Hoffmann, 1970).

In this investigation, the nucleolar organizer regions, NORs (satellite chromosomes), which represent the chromosome marker, were located only on the short arms near the centromere of the pair acrocentric autosome 14. This result agrees with Nadler *et al*. (1975) (Figure 5).

Measuring the length in centimeters of the chromosomes in mitotic metaphase cells for 15 cells in males and females resulted in the a mean length of the short arm chromosome (Ls), length long arm chromosome (Ll), length total arm chromosome (LT), relative length (RL), centromeric index (CI), standard deviation (SD) of RL, CI, size and type of chromosome in male and female of the variable squirrel shown in table 1. The idiogram of the variable squirrel shows gradually decreasing length of the autosomes and sex chromosomes (Figure 6). The karyotype formula for the variable squirrel is as follows:

$2n\ (40) = L^{m}_{4}+L^{sm}_{4}+L^{a}_{14}+M^{t}_{2}+S^{m}_{4}+S^{a}_{6}+S^{t}_{4}+$ sex chromosomes

In conclusion, this research establishes standardized karyotype and idiogram of *C. finlaysoni bocourti*, which is the first report of this subspecies. And the result shows novel cytogenetic information of *C. finlaysoni bourti*, which different from Naddler *et al.* (1975). In addition, this investigation shows genetic variation in subspecies level of *C. finlaysoni* that will use to clarify taxonomic ambiguity and show phylogenetic relationship of this species.

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Figure 1 General characteristics of variable squirrel, *Callosciurus finlaysoni bocourti* (Rodentia, Sciuridae).

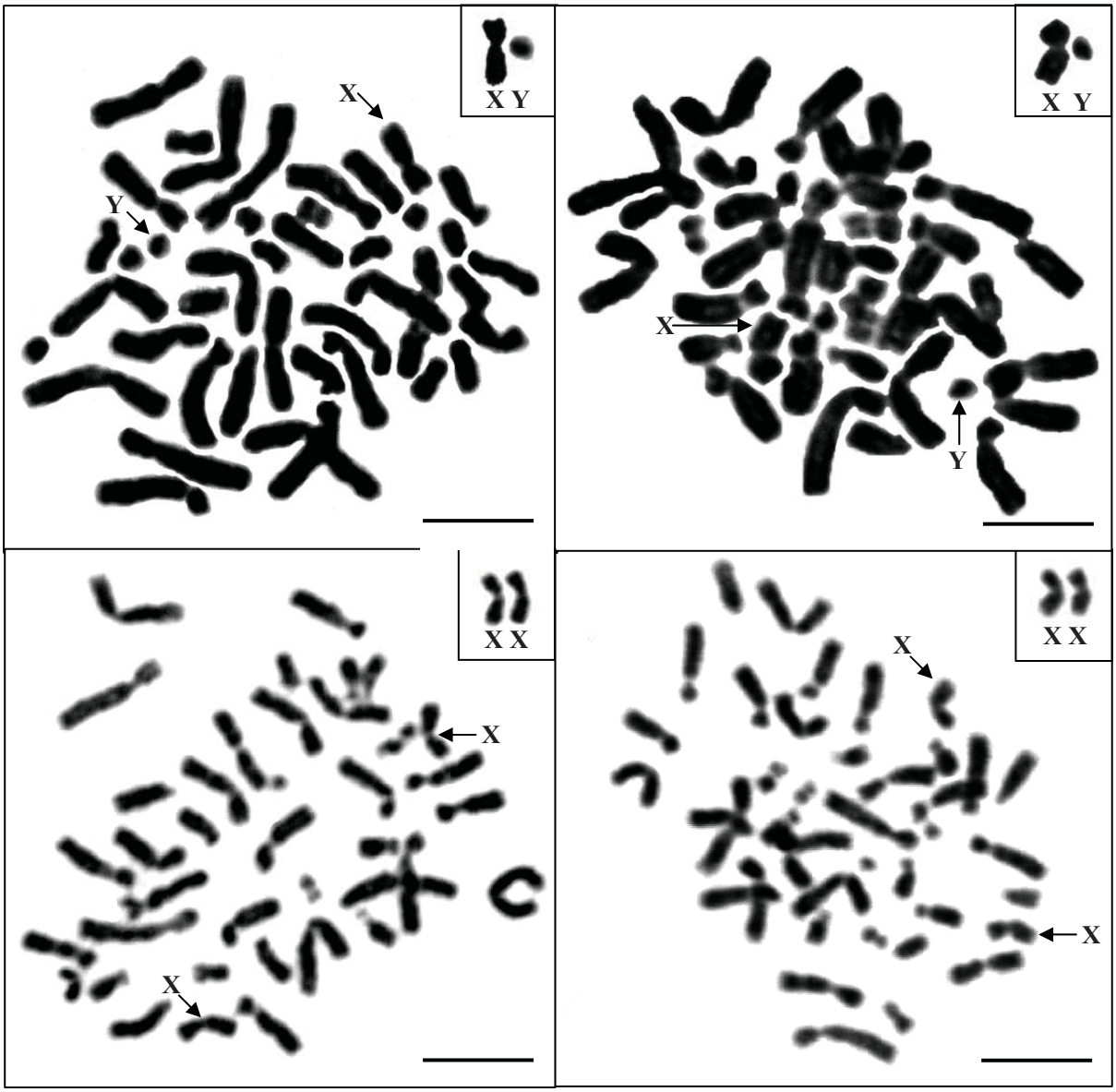

Figure 2 Metaphase chromosome plates of male (A. and B.) and female (C. and D.) the variable squirrel, (*Callosciurus finlaysoni bocourti*) 2*n* (diploid) = 40, by conventional staining technique, arrows indicate sex chromosomes (scale bars 10 micrometer).

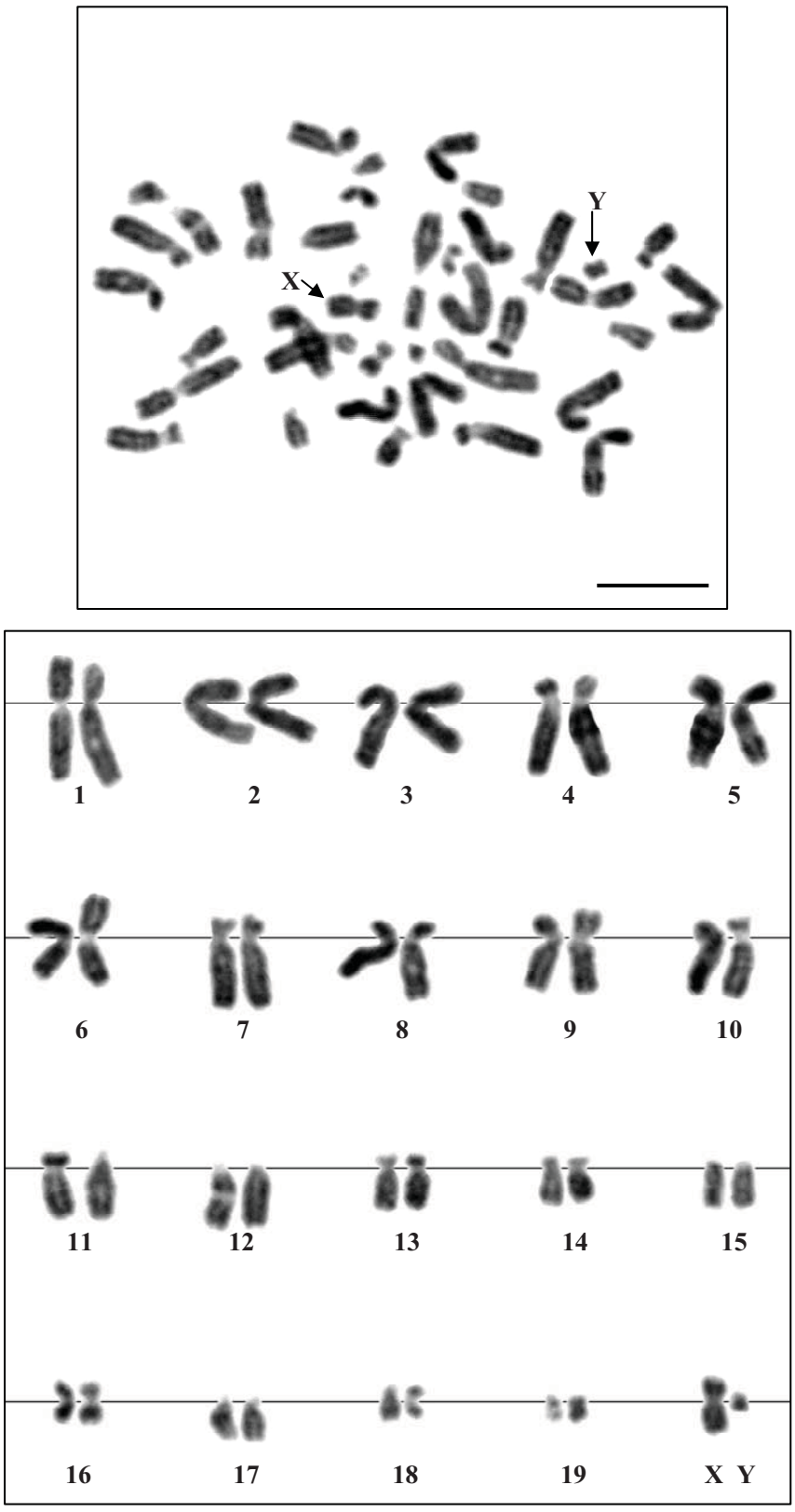

Figure 3 Metaphase chromosome plate and karyotype of the male variable squirrel, (*Callosciurus finlaysoni bocourti*) 2*n* (diploid) = 40, by conventional staining technique, arrows indicate sex chromosomes (scale bars 10 micrometer).

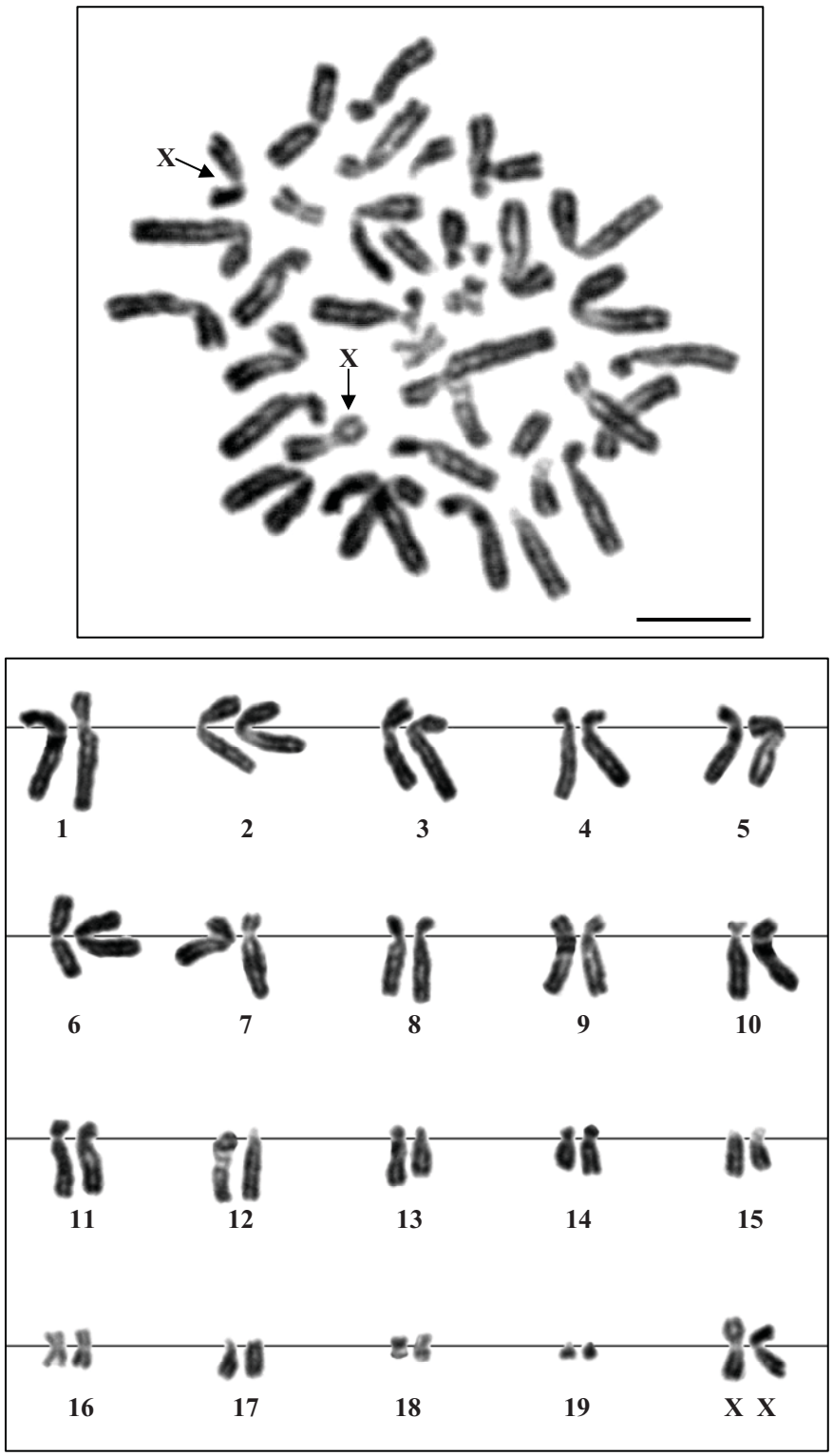

Figure 4 Metaphase chromosome plate and karyotype of the female variable squirrel, (*Callosciurus finlaysoni bocourti*) 2*n* (diploid) = 40, by conventional staining technique, arrows indicate sex chromosomes (scale bars 10 micrometer).

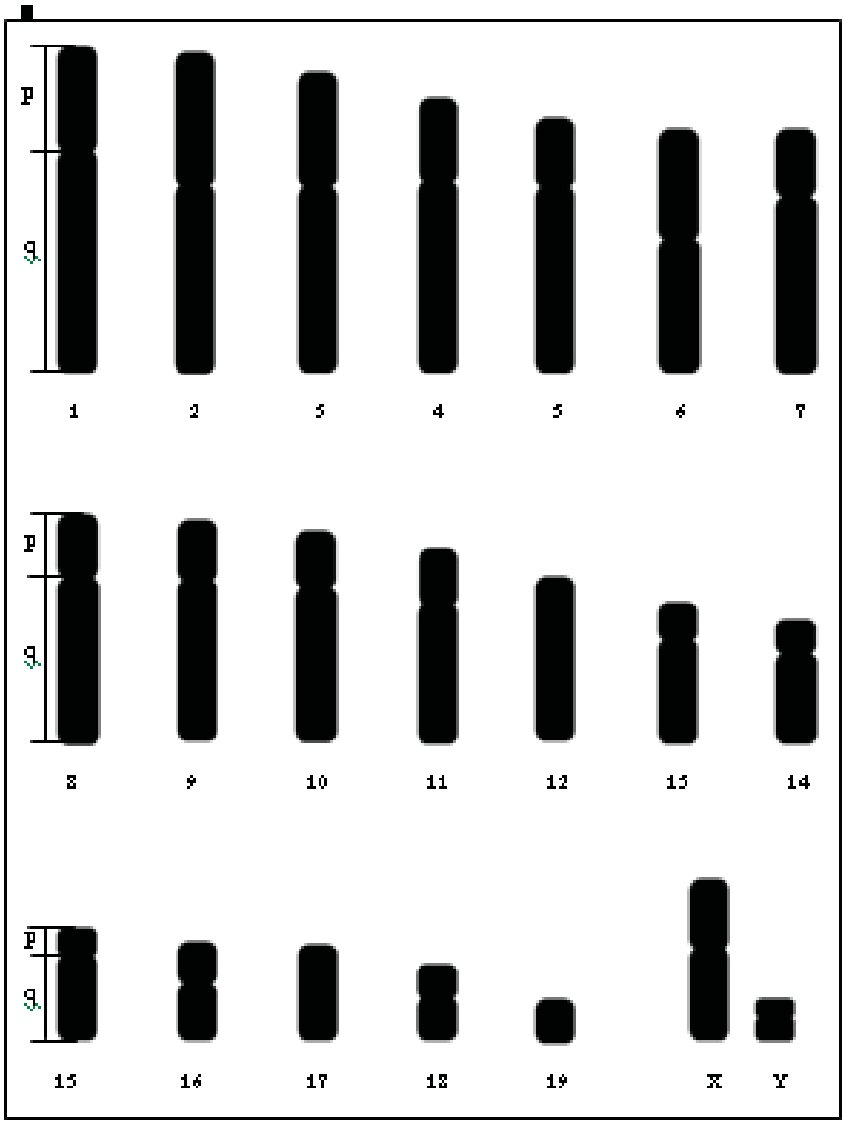

Figure 5 Idiogram of the variable squirrel, (*Callosciurus finlaysoni bocourti*) 2*n* (diploid) = 40, by conventional staining technique.

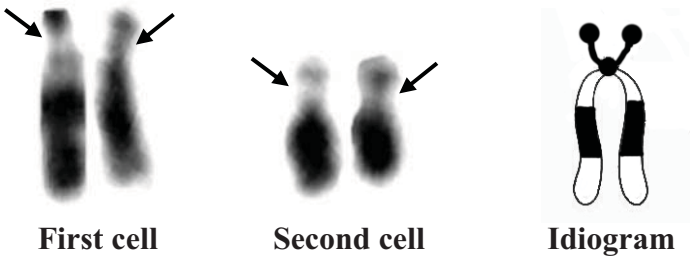

Figure 6 The nucleolar organizer regions, NORs (satellite chromosomes) which represents the chromosome marker, locates only on the short arms near centromere of the pair acrocentric autosomes 14, arrows indicate secondary constrictions.

Table 1 The mean value of the short arm chromosome length (Ls), of the long arm chromosome length (Ll), of the total arm length of the chromosome (LT), of the relative length (RL) and of the centromeric index (CI). The standard deviation (SD) of RL and CI, the size and type of the chromosomes of 15 cells in male and female variable squirrel, (*Callosciurus finlaysoni bocourti*)) 2*n* (diploid) = 40.

Chromosome pairs	Ls	Ll	LT	CI±SD	RL±SD	Size of Chromosome	Type of Chromosome
1	0.331	0.702	1.033	0.683±0.095	0.042±0.004	L	sm
2	0.421	0.596	1.016	0.586±0.095	0.041±0.004	L	m
3	0.360	0.596	0.956	0.624±0.107	0.039±0.005	L	sm
4	0.264	0.608	0.873	0.702±0.082	0.035±0.004	L	a
5	0.217	0.598	0.814	0.724±0.110	0.033±0.003	L	a
6	0.351	0.424	0.775	0.548±0.054	0.032±0.003	L	m
7	0.211	0.561	0.772	0.726±0.063	0.031±0.004	L	a
8	0.191	0.534	0.725	0.735±0.051	0.029±0.002	L	a
9	0.192	0.519	0.710	0.728±0.089	0.029±0.002	L	a
10	0.177	0.494	0.671	0.736±0.065	0.027±0.002	L	a
11	0.175	0.446	0.621	0.721±0.068	0.025±0.004	L	a
12	0.000	0.530	0.530	1.000±0.000	0.022±0.003	M	t
13	0.117	0.338	0.455	0.744±0.074	0.019±0.002	S	a
14	0.103	0.297	0.400	0.740±0.043	0.016±0.002	S	a
15	0.090	0.282	0.372	0.761±0.051	0.015±0.002	S	a
16	0.137	0.186	0.324	0.576±0.071	0.013±0.003	S	m
17	0.000	0.314	0.314	1.000±0.000	0.013±0.003	S	t
18	0.107	0.144	0.251	0.575±0.051	0.010±0.003	S	m
19	0.000	0.148	0.148	1.000±0.000	0.006±0.002	S	t
X	0.223	0.296	0.518	0.570±0.273	0.021±0.011	M	m
Y	0.071	0.097	0.168	0.574±0.028	0.007±0.001	S	m

Notes: L = large chromosome, M = medium chromosome, S = small chromosome, m = metacentric chromosome, sm = submetacentric chromosome and t = telocentric chromosome