

# Standardized karyotype and idiogram of Thai native swamp buffalo, *Bubalus bubalis* (Artiodactyla, Bovidae) by convention staining, G-banding, C-banding and NOR-banding techniques

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## ABSTRACT

Standardized karyotype and idiogram of the Thai native swamp buffalo (*Bubalus bubalis*) at Khon Kaen University, Thailand, were studied. Blood samples were taken from two male and two female swamp buffaloes. After lymphocyte culture, the mitotic chromosome preparation was accomplished by hypotonic-air-drying method. Conventional staining, G-banding, C-banding and NOR-banding techniques were applied to stain the chromosome. The results showed that diploid number of Thai native swamp buffalo was  $2n=48$ , the fundamental numbers (NF) were 58 in both male and female. The types of autosomes were 4 large metacentric, 4 large acrocentric, 2 small submetacentric and 36 small telocentric chromosomes. The X chromosome was large telocentric chromosome and the Y chromosome was the small telocentric chromosome. From G-banding, each chromosome pair appears with distinctively differentiated. C-banding shown C-positive (dark band) on centromere of all telocentric chromosomes but others appeared as C-negative (light band). NOR-banding exhibited 6 telocentric chromosome pairs.

The karyotype formula of Thai native swamp buffalo was as follows:

$2n$  (diploid)  $48 = L^m_{4+} + L^a_{4+} + S^{sm}_2 + S^t_{36} + \text{sex chromosomes}$

**Keywords:** chromosome, Thai native swamp buffalo (*Bubalus bubalis*), karyotype

## INTRODUCTION

Mammals in family Bovidae are large herbivores that have two hoofs (even-toed ungulates). They are ruminant and can be separated to 5 genera and 11 species in Thailand, namely gaur (*Bos gaurus* Smith, 1827), kouprey [*Bos sauveli* (Urbain, 1973)], banteng (*Bos javanicus* D'Alton, 1823), European cattle (*Bos taurus* Linnaeus, 1758), Indian cattle (*Bos indicus* Linnaeus, 1758), wild water buffalo [*Bubalus arnee* (Kerr 1972)] swamp buffalo (*Bubalus bubalis* Linnaeus, 1758), river buffalo (*Bubalus bubalis* Linnaeus, 1758), serow [*Naemorhedus sumatraensis* (Bechstein, 1799)], Chinese goral [*Naemorhedus caudatus* (Milne-Edwards, 1867)], goat (*Capra hircus* Linnaeus, 1758) and sheep (*Ovis aries* Linnaeus,

1758) (Lekagul and McNeely, 1977; 1988; Parr, 2003).

Before 1971 the Thai native swamp buffalo was a very important domestic animal in Thailand. Statistics from the Thailand agricultural statistical center showed that Thailand had about 5.5-6.5 million swamp buffalo during 1971 and 1981. The swamp buffalo population declined to 1.8 million in 1999. Swamp buffalo could be extinct from Thailand in 20 years, if no considerable public attention was received. Most of the swamp buffalo in Thailand are completely black in color (Fig. 1), only a few of them are white. The white buffalo are not albino but the color is due to uncertain genetic effects. On average, mature male buffalo weigh 450-600 kg and the mature females weigh 350-450 kg (Faarungsang, 2003).

As known, the *B. bubalis* includes 2 cytotypes commonly referred to as river ( $2n=50$ ) and swamp buffalo ( $2n=48$ ). Several cytogenetic studies have been carried out to define the conventional karyotype (Chandra, 1968; Fischer and Ulbrich, 1968; De Hondt and Ghanam, 1971) as well as the distribution of constitutive heterochromatin and G-banding pattern of this species (Cribiu and Obeidah, 1978; Gupta and Ray-Chaudhuri, 1978).

There are several literature reviews about cytogenetic studies of *B. bubalis* including Dutt and Bhattacharya (1952); Chandra (1968); Fischer and Ulbrich (1968); De Hondt and Ghanam (1971); Bongso *et al.* (1977); Cribiu and Obeidah (1978); Gupta and Ray-Chaudhuri (1978); Di Berardino and Iannuzzi (1981, 1984); Miyake *et al.* (1981); Chavananikul (1989); Gallagher and Womack (1992); Iannuzzi (1994); Iannuzzi and Di Berardino (1985); Iannuzzi and Di Meo (1995); Prakash *et al.* (1997) and Iannuzzi *et al.* (1979, 1987, 1996, 1998, 2003). We can reach the basis cytogenetic knowledge of the Thai native swamp buffalo which accomplished with our report on the standardizations of chromosome including chromosome measuring to determine shape and size, karyotype formula, and idiogramming. In addition, we also make a confirmation and comparison for the results with the previous reports. Moreover, this study provides important basic knowledge which can apply to further advanced researches and conservation of family Bovidae in Thailand.

## METERIALS AND METHODS

The blood samples were collected from two males and two females of Thai native swamp buffalo,



**Figure 1** The male Thai native swamp buffalo, *Bubalus bubalis* (Artiodactyla, Bovidae).

raised at Khon Kaen University, Thailand. The samples were kept in 10 ml vacuum tubes containing heparin to prevent blood clotting and cooled on ice until arriving at the laboratory.

### Cell preparation

The lymphocytes were cultured using the whole blood microculture technique adapted from Rooney (2001) and Campiranon (2003). The growth medium was RPMI 1640 medium with 2% PHA (phytohemagglutinin) as a mitogen. Blood samples of 0.5 ml were grown in a medium bottle incubated at 37 °C for 72 hr. Cells were harvested and further performed by a 30 minute colchicine pretreatment in a hypotonic solution (0.075 M KCl) and fixation in methanol: acetic acid (3:1). Air-dried preparations were then made. The slide was conventionally stained with 20% Giemsa's solution.

### G-banding method

G-banding technique was adapted from Campiranon (2003). The slide was well dried and then soaked in working trypsin (0.025% trypsin EDTA) at 37 °C before the termination of trypsin activity by washing the slide with Sorensen's buffer. The slide was stained with 20% Giemsa's solution for 30 minutes.

### C-banding method

C-banding method was adapted from Verma and Babu (1995). Slides were heated at 60 °C for 2-3 days, soaked in 0.2 N HCl for 10-15 minutes, rinsed with distilled water then soaked in 0.05 N Ba(OH)<sub>2</sub> for 15 minutes at 37 °C, rinsed with distilled water at 60 °C. After that soaked in 2X SSC at 60 °C for 1-2 hours. The slide was stained with 20% Giemsa's solution for 30 minutes.

### NOR-banding method

NOR-banding method was adapted from Verma and Babu (1995). Add 2 drops of 50% silver nitrate and 2% gelatin on slides, respectively. Then sealed with cover glasses and incubated at 60 °C for

3 hours. After that soaked in distilled water until cover glasses were separated. The slide was stained with 20% Giemsa's solution for 1 minute.

### Chromosomal checks, karyotyping and idiogramming

Chromosome counting was performed on mitotic metaphase cells under light microscope. Twenty clearly observable and well-cells spread chromosomes of each male and female were selected and photographed. The length of short arm chromosome (Ls) and the length of long arm chromosome (Li) were measured and calculated to the length of total arm chromosome (LT,  $LT = Ls + Li$ ). The relative length (RL), the centromeric index (CI) and standard deviation (SD) of RL and CI were estimated. CI was also computed to classify the types of chromosomes according to Chaiyasut (1989). All parameters were used in karyotyping and idiogramming.

## RESULTS AND DISCUSSION

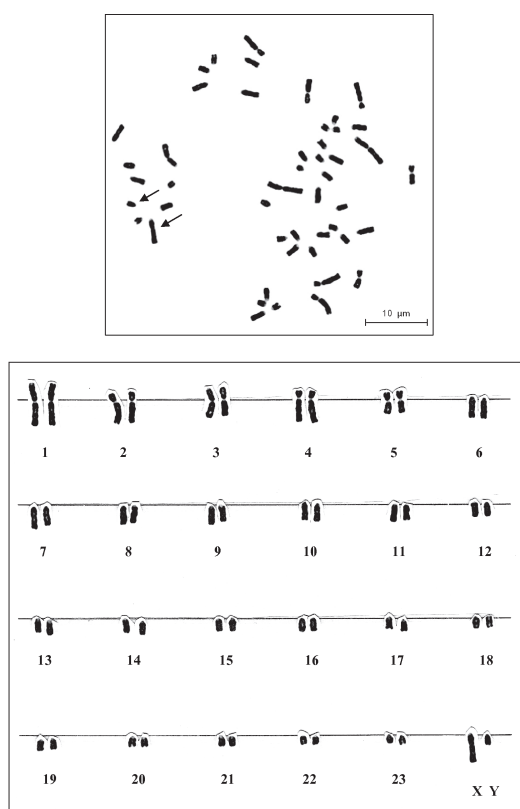
We found that the Thai native swamp buffalo has  $2n=48$  (Figs. 2 and 3). This result agrees with Di Berardino and Iannuzzi (1981); Chavananikul (1989) and Iannuzzi (1994). The swamp buffalo has difference diploid number when compare to the others species in same genus including river buffalo (*B. bubalis*) and wild water buffalo (*B. arnee*) which have  $2n=50$  which reported by Dutt and Bhattacharya (1952), Bongso *et al.* (1977), Cribiu and Obeidah (1978), Di Berardino and Iannuzzi (1981, 1984), Chavananikul (1989), Iannuzzi (1994), Iannuzzi and Di Meo (1995), Iannuzzi *et al.* (1987, 1998, 2003) and Flamand *et al.* (2003).

The domestic buffalo (*B. bubalis*) has been classified into two general types according to geographical distribution: one is the river type buffalo, raised in most areas from India to Egypt and some south and eastern European countries; the other is the swamp type buffalo of Southeast Asia (Mason, 1974). The karyotypes differ in the two types of buffalo, and their diploid numbers are 48 and 50 in the swamp type buffalo and the river type buffalo,

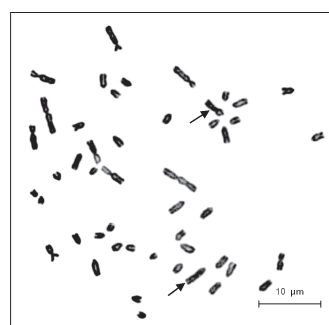
respectively (Fischer and Ulbrich 1968). The karyotypes of the two types of buffalo differ due to the tandem fusion translocation: the swamp type chromosome 1 resulted from a telomere-centromere tandem fusion between the river type chromosome 4p and 9, with a loss of the centromere of river type chromosome 9 (Di Berardino and Iannuzzi 1981; Bongso and Hilmi 1982; Tanaka *et al.*, 1999) (Fig. 4).

This examination also revealed that the fundamental number (NF, number of chromosome arms) of the Thai native swamp buffalo was 58 in both male and female. This is the same NF for the swamp buffalo as reported by Di Berardino and Iannuzzi (1981), Iannuzzi and Di Berardino (1985)

and Chavananikul (1989). The family Bovidae includes several species demonstrating variable diploid numbers but having similar fundamental numbers (NF=60), with the exception of a few cases that vary between 58 and 62. The karyotype contains various numbers of centric fusions, which have change the diploid number but not change the NF (Wurster and Benirschke, 1968). These rearrangements of a basic karyotype consisting of one-armed chromosomes have later been confirmed by studies using banding techniques in various species of Bovidae (Evans *et al.*, 1973; Buckland and Evans, 1978; Bunch and Nadler, 1980; Di Berardino and Iannuzzi, 1981; 1984).



**Figure 2** Metaphase chromosome plate (top) and karyotype (bottom) of male Thai native swamp buffalo (*Bubalus bubalis*)  $2n = 48$  by conventional staining technique, showing sex chromosomes (arrows).



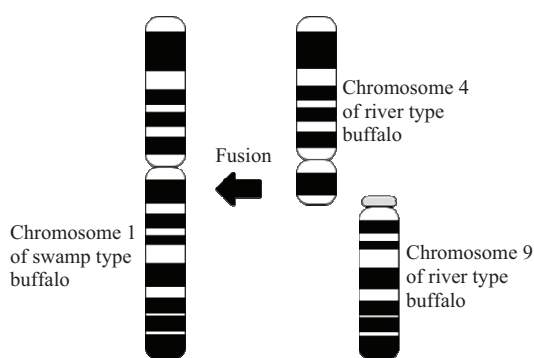
**Figure 3** Metaphase chromosome plate (top) and karyotype (bottom) of female Thai native swamp buffalo (*Bubalus bubalis*)  $2n = 48$  by conventional staining technique, showing sex chromosomes (arrows).

The types of swamp buffalo autosomes were 4 large metacentric, 4 large acrocentric, 2 small submetacentric and 36 small telocentric chromosomes. These features are similar to the report of Wurster and Benirschke (1968) and Popescu *et al.* (1996). The X chromosome is a large telocentric chromosome and the Y chromosome is the small telocentric chromosome. These features are similar to the report of Di Berardino and Iannuzzi (1981) and Chavananikul (1989). In comparison with the other ruminant species in the genus *Bos* in Thailand, the X chromosomes of gaur (*B. gaurus*), banteng (*B. javanicus*), cattle (*Bos taurus*) and cattle (*Bos indicus*) are submetacentric chromosomes and the Y chromosomes of all those species are metacentric, submetacentric, submetacentric and acrocentric chromosome, respectively (Wurster and Benirschke, 1968).

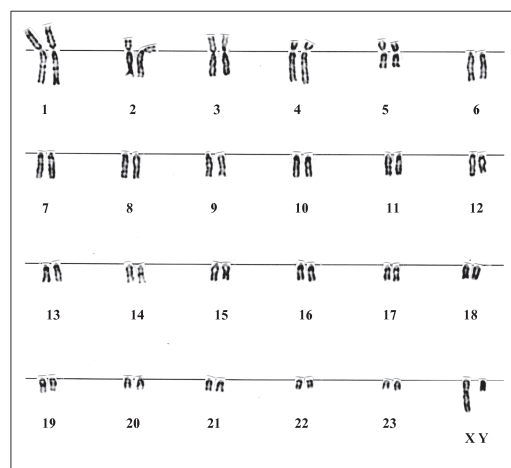
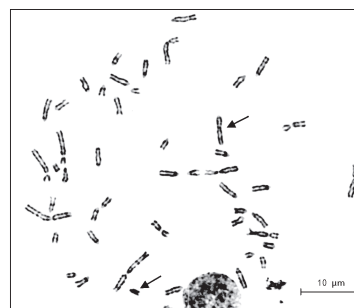
From G-banding technique, each chromosome pair appears with distinctively differentiated. The G-banded revealed that the number of bands on 1 set of haploid chromosomes, which includes autosomes, X and Y chromosomes, are 226 bands (Figs. 5, 6 and 7). The number of chromosome bands is defined by a visible band on a haploid set which compose of

autosomes, X and Y chromosome. Thus, the haploid set of the Thai buffalo consist of 23 autosomes include X and Y chromosome. However, some chromosomes are not obvious G-band. As above, the chromosome band counting represent by only clearly band that appeared.

C-banding technique demonstrated dark bands (C-positive) on centromeres of all telocentric chromosomes (18 pair autosomes), the representative of constitutive heterochromatin. However, there is no dark band (light or C-negative) on the Y chromosome and autosome pair 1 to 4 (Figs. 8, 9 and 10). The C-banding can provide a positive band on constitutive heterochromatin which contains the highly repetitive DNA sequences. The C-bands can be found at all



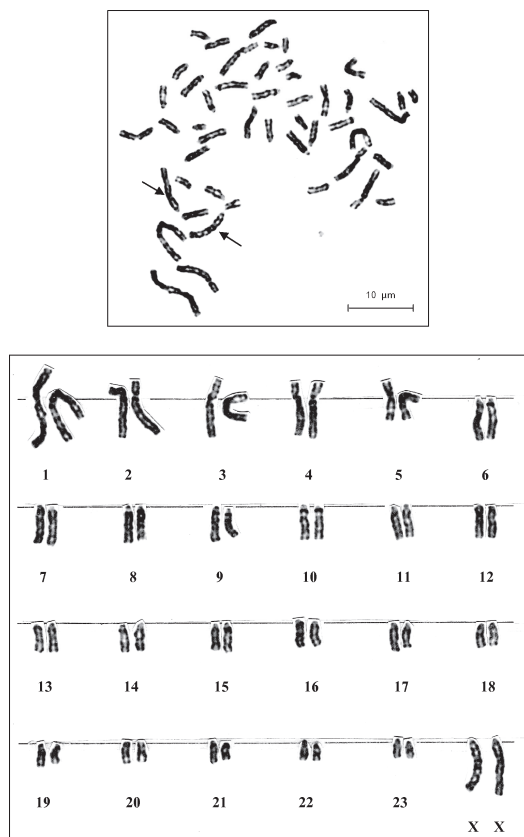
**Figure 4** The karyotype of the two type of buffaloes differ due to the tandem fusion translocation: the swamp-type chromosome 1 resulted from a telomere-centromere tandem fusion between the river-type chromosome 4p and 9, with a loss of centromere of river-type chromosome 9.



**Figure 5** Metaphase chromosome plate (top) and karyotype (bottom) of male Thai native swamp buffalo (*Bubalus bubalis*)  $2n = 48$  by G-banding technique, showing sex chromosomes (arrows).

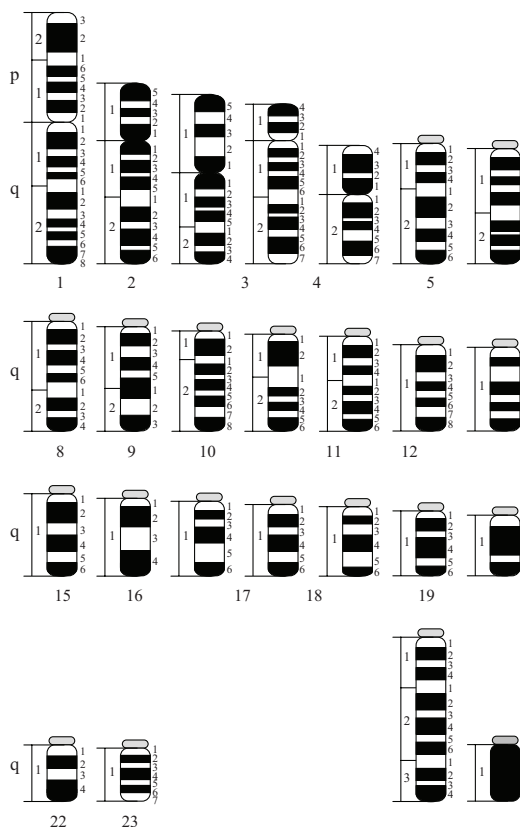
centromeres and some telomere of chromosomes. C-banding is an acceptable technique for the sex chromosome studying, especially for the identification of human centromere 1, 9, 16 and Yq chromosome because of its individual characteristics that normally cannot provide a dark region on the centromere (Campiranon, 2003).

In this investigation, the six nucleolar organizer regions, NORs (satellite chromosomes), are located on the long arm near centromere of 3 pairs of telocentric autosomes (6 positions) (Fig. 11). In contrast, Di Berardino and Iannuzzi (1981) indicated that NORs of the swamp buffalo and river buffalo appear on the long arm near centromere of the pair



**Figure 6** Metaphase chromosome plate (top) and karyotype (bottom) of female Thai native swamp buffalo (*Bubalus bubalis*)  $2n = 48$  by G-banding technique, showing sex chromosomes.

autosomes 4p, 8, 20, 22, 23 (10 positions) and 3p, 4p, 8, 21, 23, 24 (12 positions), respectively. By comparing the two types of buffalo it was concluded that: all of the chromosomes are similar in banding patterns; chromosome 1 of swamp results from a telomere-centromere tandem fusion between two chromosomes identified as 4p and 9, respectively, in the river buffalo karyotype, thus accounting for the reduced diploid number of swamp buffalo; the fusion causes the loss of NORs on the telomeres of chromosome 4, thus accounting for the reduced number of NOR chromosome pairs of swamp; the presence of a pale C-banded are in the region of the junction between chromosome 4 and 9 involved in the fusion suggests that the centromeric region of the later is retained and altered (Gallagher and Womack, 1992; Tanaka et al., 1999).



**Figure 7** Idiogram of Thai native swamp buffalo (*Bubalus bubalis*)  $2n = 48$  by G-banding technique.



The mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), total length of arm chromosome (LT), relative length (RL), centromeric index (CI), standard deviation (SD) of RL, CI, size and shape of chromosomes presented in Table 1. The idiogram of Thai native swamp buffalo shows gradually decreasing length of the autosomes and sex chromosomes (Figs. 7, 10 and 12).

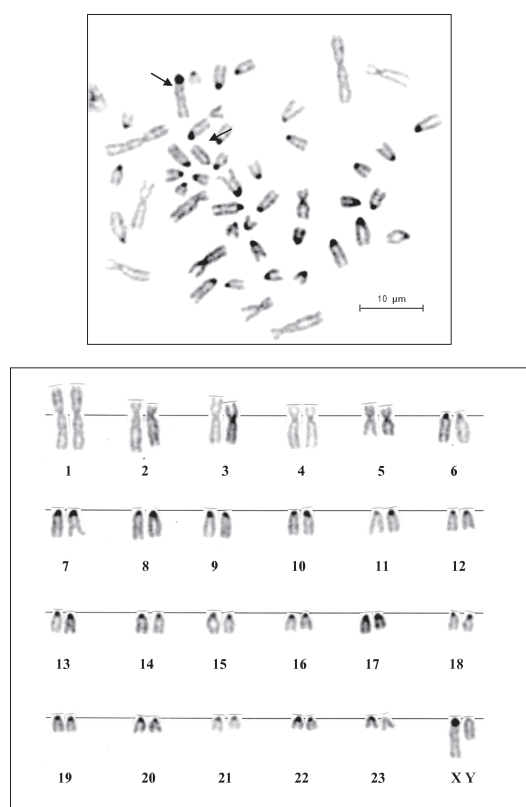
The Thai's native swamp buffalo revealed that the chromosome marker is the chromosome pair 1, which is the largest metacentric chromosome. The karyotype is the asymmetrical type, which is all 4 types of chromosomes were found (metacentric, submetacentric, acrocentric and telocentric

chromosome). The largest and smallest chromosomes show difference size (approximately 5 folds). The karyotype formula of Thai native swamp buffalo was as follows:

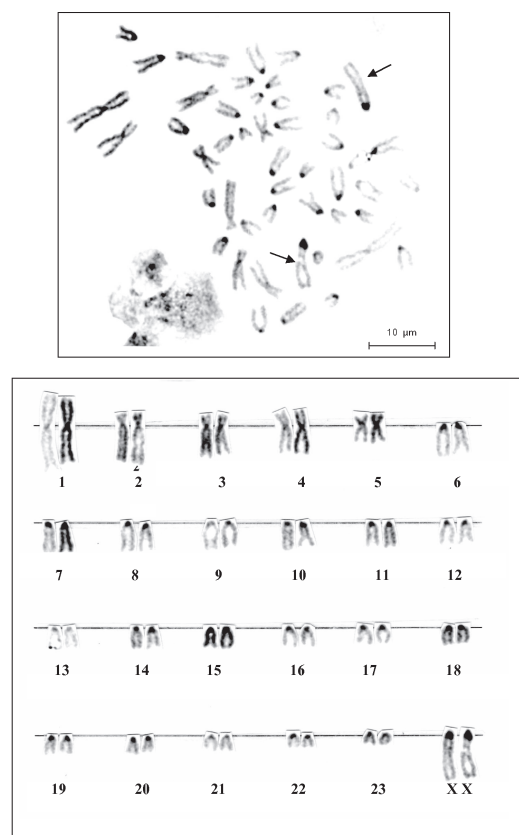
$2n$  (diploid)  $48 = L^m_4 + L^a_4 + S^{sm}_2 + S^t_{36} + \text{sex chromosomes}$

## ACKNOWLEDGEMENTS

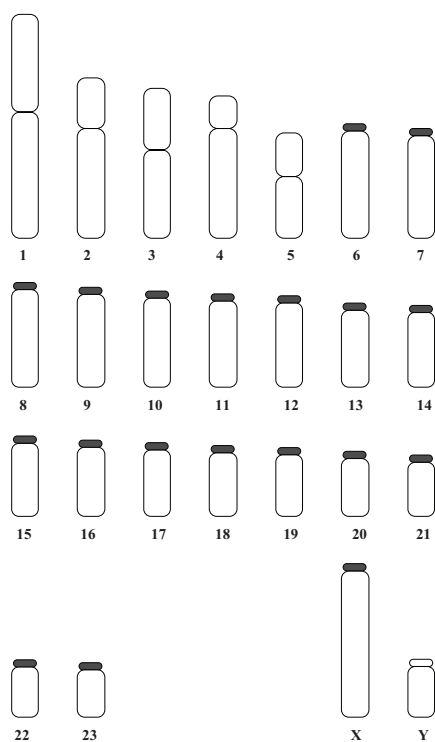
The financial support from The Zoological Park Organization under the Royal Patronage of H.M. the King is gratefully acknowledged. We also thank Dr. Sopon Dumnui, Director of the organization, Dr. Sumat Kamolnaranath, Chief of the Educational Division, for valuable help.



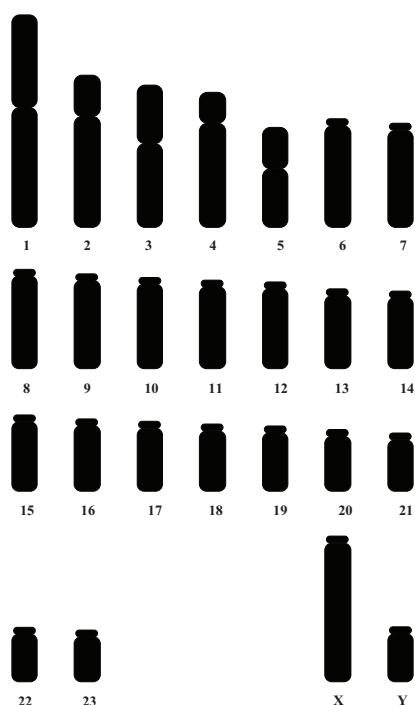
**Figure 8** Metaphase chromosome plate (top) and karyotype (bottom) of male Thai native swamp buffalo (*Bubalus bubalis*)  $2n = 48$  by C-banding technique, showing sex chromosomes.



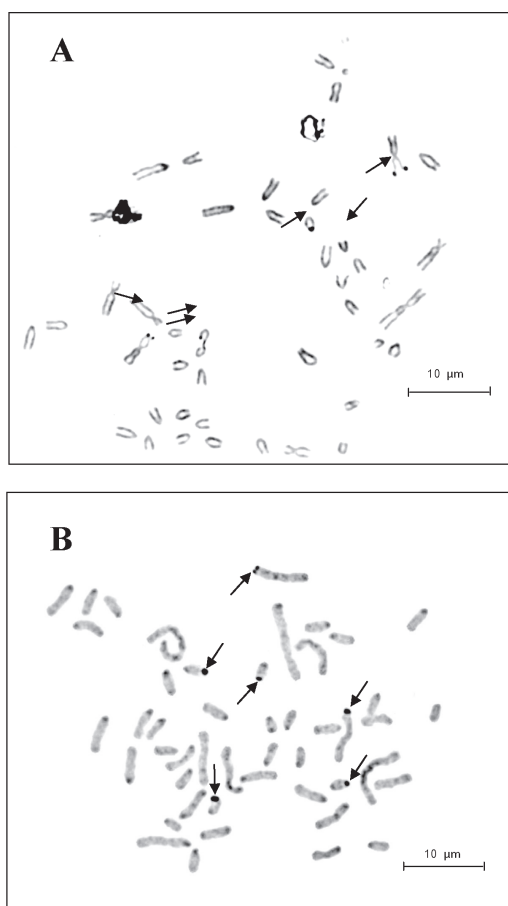
**Figure 9** Metaphase chromosome plate (top) and karyotype (bottom) of female Thai native swamp buffalo (*Bubalus bubalis*)  $2n = 48$  by C-banding technique, showing sex chromosomes.



**Figure 10** Idiogram of Thai native swamp buffalo (*Bubalus bubalis*)  $2n=48$  by C-banding technique.



**Figure 12** Idiogram of Thai native swamp buffalo (*Bubalus bubalis*)  $2n = 48$  by conventional staining technique.



**Figure 11** Metaphase chromosome plate of male (A) and female (B) Thai native swamp buffalo (*Bubalus bubalis*)  $2n = 48$  by NOR-banding technique, showing satellite chromosomes (arrows).



**Table 1** Mean of the short arm chromosome length (Ls), the long arm chromosome length (Ll), total arm chromosome length (LT), relative length (RL), centromeric index (CI), standard deviation of RL and CI, chromosome size and chromosome shape of metaphase chromosomes of 20 cells in male and female the Thai native swamp buffalo (*Bubalus bubalis*),  $2n = 48$ .

Chromosome pair	Ls	Ll	LT	RL±SD	CI±SD	Size	Shape
1	1.28	1.77	3.05	0.095±0.006	0.582±0.029	L	m
2	0.59	1.59	2.18	0.068±0.004	0.730±0.019	L	a
3	0.85	1.19	2.04	0.063±0.004	0.582±0.011	L	a
4	0.57	1.37	1.94	0.060±0.002	0.705±0.007	L	m
5	0.57	0.87	1.44	0.044±0.002	0.603±0.024	S	sm
6	0.00	1.46	1.46	0.045±0.003	1.000±0.000	S	t
7	0.00	1.39	1.39	0.043±0.001	1.000±0.000	S	t
8	0.00	1.33	1.33	0.041±0.007	1.000±0.000	S	t
9	0.00	1.27	1.27	0.039±0.003	1.000±0.000	S	t
10	0.00	1.22	1.22	0.038±0.002	1.000±0.000	S	t
11	0.00	1.17	1.17	0.036±0.003	1.000±0.000	S	t
12	0.00	1.15	1.15	0.035±0.004	1.000±0.000	S	t
13	0.00	1.05	1.05	0.032±0.002	1.000±0.000	S	t
14	0.00	1.02	1.02	0.031±0.001	1.000±0.000	S	t
15	0.00	0.99	0.99	0.030±0.002	1.000±0.000	S	t
16	0.00	0.94	0.94	0.029±0.001	1.000±0.000	S	t
17	0.00	0.90	0.90	0.028±0.001	1.000±0.000	S	t
18	0.00	0.87	0.87	0.027±0.002	1.000±0.000	S	t
19	0.00	0.84	0.84	0.026±0.004	1.000±0.000	S	t
20	0.00	0.79	0.79	0.024±0.003	1.000±0.000	S	t
21	0.00	0.74	0.74	0.023±0.002	1.000±0.000	S	t
22	0.00	0.69	0.69	0.021±0.003	1.000±0.000	S	t
23	0.00	0.64	0.64	0.020±0.001	1.000±0.000	S	t
X	0.00	1.99	1.99	0.062±0.004	1.000±0.000	L	t
Y	0.06	0.91	0.97	0.030±0.001	0.938±0.010	S	t

**Abbreviation:** L=large chromosome, S=small chromosome, m=metacentric chromosome, sm=submetacentric chromosome, a=acrocentric chromosome and t=telocentric chromosome

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