Standardized Karyotype and Idiogram of Thai Native Goat, *Capra hircus* (Artiodactyla, Bovidae) by Conventional Staining, GTG-banding, CBG-banding and Ag-NOR Banding Techniques

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Abstract

Cytogenetics of Thai native goat (Capra hircus) at Surindra Rajabhat University was studied. Blood samples were taken from two males and two females goats. After standard whole blood lymphocytes were cultured at 37 ˚C for 72 hours in presence of colchicine, the metaphase spreads were performed on microscopic slide and air-dried. Conventional staining, GTG-banding, CBG-banding and Ag-NOR banding techniques were applied to stain the chromosome. The results showed that diploid chromosomes number of Thai native goat was 2*n*=60, the fundamental numbers (NF) were 62 in both male and female. The types of autosomes were 22 large acrocentric, 14 medium acrocentric and 22 small acrocentric chromosomes. The X chromosome was largest acrocentric chromosome and the Y chromosome was the smallest submetacentric chromosome. From GTG-banding, each chromosome pair appears with clearly differentiated. CBG-banding shown C-positive (Dark band) on centromere of all telocentric autosomes but C-negative (Light band) on X and Y chromosomes.

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NOR-banding exhibited 6 satellite chromosomes (Nucleolar organizer regions, NORs) as acrocentric autosomes in Thai native goat. The karyotype formula of Thai native goat was as follows: 2*n* (diploid) 60 = L^{a}_{22} + M^{a}_{14} + S^{a}_{22} + sex chromosomes

Keywords : Cytogenetics, Thai native goat (Capra hircus), karyotype, ideogram

Introduction

In many countries, goats are found in the arid zone, but in Thailand, most of the Thai native goat population is in the southern humid tropical zone. They are exclusively raised for meat by Thai Muslims in mixed small-scale faming. FAO has reported that the Thai native goat population in Thailand in 1994 was 141,000 head, but it was only 78,000 head in 1995 and 1996, respectively (Pakawanit and Pralomkarn, 1998).

Although the first important step in arranging standard banded karyotypes of domestic animals was taken at the Reading Conference (1976), the goat standard karyotype was represented only by GTG-banded contracted chromosomes. Later both G-banded and R-banded karyotypes were reported, and in recent studies GTG-banded (Mensher et al., 1989), RBA-banded (Di Berardino et al., 1987) and RBG-banded (Hayes et al., 1991) banding techniques were used.

Autosomes in domestic bovids (Family bovidae) are all acrocentric chromosomes (Cattle and goat) with the exception of three metacentric chromosomes in sheep (*Ovis aries*) and five submetacentric chromosomes in river buffalo (*Bubalus bubalis*) which have originated from centric fusion events (Di Berardino et al., 1981; Hayes et al., 1991; Gallagher & Womack, 1992; Iannuzzi & Di Meo, 1995). The acrocentric chromosomes nature of the majority of the bovid chromosomes is a source of problems for the construction of karyotypes, especially when using contracted chromosome preparations (Di Berardino et al., 2001).

There are several reports on cytogenetic studies of goats including Evan et al. (1973); Buckland & Evans (1978); Cribiu & Matejka (1987); Di Berardino et al. (1987, 1993, 2001); Hondt et al. (1988); Mensher et al. (1989); ISCNDA 1989 (1990); Di Meo et al. (1991, 2005); Hayes et al. (1991); Gallagher and Womack (1992); Leopoldo et al. (1994); Iannuzzi & Di Meo (1995), and Di Berardino & Burghete (1998). No cytogenetic study on the native goat

from Thailand has yet been undertaken. The present study were therefore undertaken to elucidate conventional, GTG-banded, CBG-banded and Ag-NOR banded staining to provide fine karyotypic details vis-à-vis its comparison with previous published account.

Materials and methods

Blood samples and cell cultures

Blood samples from two males and two females of Thai native goat maintained in Surindra Rajabhat University, Surin province were collected from the jugular vein using aseptic technique, in 10 ml vacuum tubes coated with heparin to prevent blood clotting and kept on ice. 0.5 ml of whole blood were cultured in 5 ml RPMI 1640 medium supplemented with 2% phytohemagglutinin (PHA) as a mitogen at 37˚C, 5% CO_2 (Rooney, 2001; Campiranont, 2003). The cultured bottle was loosely capped and regularly shaken two times a day, in the morning and evening. After 72 hours of incubation, colchicine was introduced and mixed for further incubation for 30 minutes.

Cell harvest and chromosome staining after colchicine incubation, the blood mixture was centrifuged at 1,200 rpm for 10 minutes. After discarding the supernatant, the cells were treated with 10 ml of hypotonic solution (0.075 M KCl) and incubated at 37˚C for 30 minutes. Then the cells were centrifuged and the supernatant was discarded. Fresh cool fixative (3: 1, methanol: acetic acid) was used to fix the cells by gradually adding up to 8 ml. After centrifugation, the fixation was repeatedly done until supernatant was clear. Finally 1 ml fixative was added to cells and the cell solution was dropped onto a clean cold slide and then dried by air-dry technique.

GTG-banding technique

GTG-banding technique was adapted from Campiranont (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 buffer. The slide was stained with 20% Giemsa's solution for 30 minutes.

CBG-banding technique

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 $Ba(OH)_2$ 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.

Ag-NORs banding technique

Add 2 drops of 50% silver nitrate and 50% 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 idiograming

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 (Ll) were measured and calculated to the length of total arm chromosome (LT, LT = Ls+Ll). The relative length (RL) and the centromeric index (CI) were estimated. CI was also computed to classify the types of chromosomes according to Chaiyasut (1989). All parameters were used in karyotyping and idiograming.

Results and Discussion

According to the results, this is the first report on Thai native goat cytogenetics knowledge. Our result that show $2n=60$ of diploid number is consistent to the report of Evan *et al*. (1973); Buckland & Evans (1978); Cribiu & Matejka (1987); Di Berardino et al. (1987, 1993, 2001); Hondt et al. (1988); Mensher et al. (1989); ISCNDA 1989 (1990); Di Meo et al. (1991, 2005); Hayes et al. (1991); Gallagher & Womack (1992); Leopoldo et al. (1994); Iannuzzi & Di Meo (1995), and Di Berardino and Burghete (1998) that showed $2n=60$. We found that the fundamental number (NF, number of chromosome arms) of Thai native goat were 62 in both male and female. This is the same NF for the goat as reported in previous studies (Buckland & Evans, 1978; Di Berardino et al., 1987; Mensher et al., 1989; ISCNDA 1989, 1990).

The autosomes of Thai native goat consist of 22 large acrocentric, 14 medium acrocentric and 22 small acrocentric chromosomes (58 acrocentric autosomes). These features are similar to the report of Buckland and Evans (1978); Di Berardino et al. (1987); Mensher et al. (1989); ISCNDA 1989 (1990); Di Meo *et al*. (1991); Hayes et al. (1991); Gallagher & Womack (1992); Iannuzzi and Di Meo (1995), & Di Berardino and Burghete (1998). Animals in family Bovidae has many acrocentric

chromosome lead to easily chromosome fusion at centromere or Robertsonian translocation (Popescu & Pech, 1991).

The X chromosome of Thai native goat is a largest acrocentric chromosome and the Y chromosome is the smallest submetacentric chromosome. These features are similar to the report of Mensher et al. (1989); ISCNDA 1989 (1990); Di Meo et al. (1991, 2005); Hayes et al. (1991); Gallagher & Womack (1992); Iannuzzi & Di Meo (1995) that revealed goat have acrocentric X chromosome and submetacentric Y chromosome. In comparison with the other ruminant species in Thailand, the X chromosomes of buffalo (*B. bubalis*), gaur (*B. gaurus*), banteng (*B. javanicus*), cattle (*B. taurus*) and sheep (*O. aries*) are telocentric, submetacentric, submetacentric, submetacentric and acrocentric chromosome, respectively and the Y chromosome of all those species are telocentric, metacentric, submetacentric, submetacentric and submetacentric chromosome, respectively (Wurster & Benirschke, 1968; Kakampuy et al., 2007; Jantarat et al., 2009; Gomontean et al., 2009; Supanuam et al., 2010).

GTG-banded revealed that number on 1 set of haploid chromosomes, which includes autosomes, X and Y chromosomes, is 174 bands. (Figs. 4, 5, 10). Similar from the reported by Di Berardino et al. (2001) and Iannuzzi et al. (1994). G-banded provide a clearly chromosome band which represent in black (Dark band) and white (Light band) regions on chromosome. The level of GTG-banding techniques (Band numbers) is defined by a visible and in a haploid set which compose of autosomes, X and Y chromosome. Thus, the haploid set of Thai native goat consist of 29 autosome pairs include X and Y chromosomes.

CBG-banded demonstrated dark bands (C-positive) on all acrocentric autosomes (29 pairs) of Thai native goat, the representative of constitutive heterochromatin. However, there is no dark band (Light or C-negative) on the X and the Y chromosome (Figs. 6, 7, 11). The dark bands those appear by C-banding technique are obviously arises on centromeres, telomeres and some parts of its regions (Campiranont, 2003).

In this investigation, the nucleolar organizer regions (NORs), which represents the chromosome marker, locates on the long arms near centromere of 6 acrocentric autosomes. In contrast,

Mayr & Czaker (1981), and Andraszek et al. (2009) indicated active NORs in terminal parts of q arms of pair 2, 3, 4, 5 and 28 chromosomes. In addition, Di Meo et al. (1993) reported NOR location in sheep at the telomeres of chromosome pairs 1p, 2q, 3q, 6 and 25 on the basic of the standard R-banded karyotype. The NORs are the chromosomal sites of genes, which transcribe for 18s and 28s ribosomal RNA, that were presumably transcribed at preceding interphase and are important in view of their intimate relationship with protein synthesis (Howell & Black, 1980).

NORs, as ribosomal gene clusters, that were active in previous interphase form prominent cytogenetic features, namely secondary constrictions. The main, defining characteristic of these constrictions is under condensation in comparison with the rest of the chromosome. Genes encoding rRNA are associated with proteins UBF are characterized by silver-binding properties. In interphase the cell synthesizes nucleoli on the basis of rRNA genes (Weisenberg & Scheer, 1995; Miller et al., 2001). Information on the cytogenetic markers can also be used for molecular cytogenetic assignment of genes on chromosomes (Donate et al., 2003).

The length in centimeters of 20 cells of Thai native goat chromosomes in mitotic metaphase cells was measured. The mean length of the short arm chromosome (Ls), the length of long arm chromosome (Ll), total length of arm chromosome (LT), relative length (RL), centromeric index (CI), size and the type of presented in Table 1. The Thai native goat revealed that the chromosome marker is the X chromosome, which is the largest acrocentric chromosome. The important chromosome marker of Thai native goat is asymmetrical karyotype, which is all two types of chromosomes were found (submetacentric and acrocentric chromosomes). The largest and smallest chromosomes show different sizes (approximately 4 folds). Figures 9 and 11 shows idiograms of Thai native goat from conventional and CBG-banded techniques, while Figure 10 show idiogram from GTG-banded technique with landmarks, regions and bands. The karyotype formula of Thai native goat was as follows:2n (diploid) 60 = L^{a}_{22} + M^{a}_{14} + S^{a}_{22} + sex chromosomes

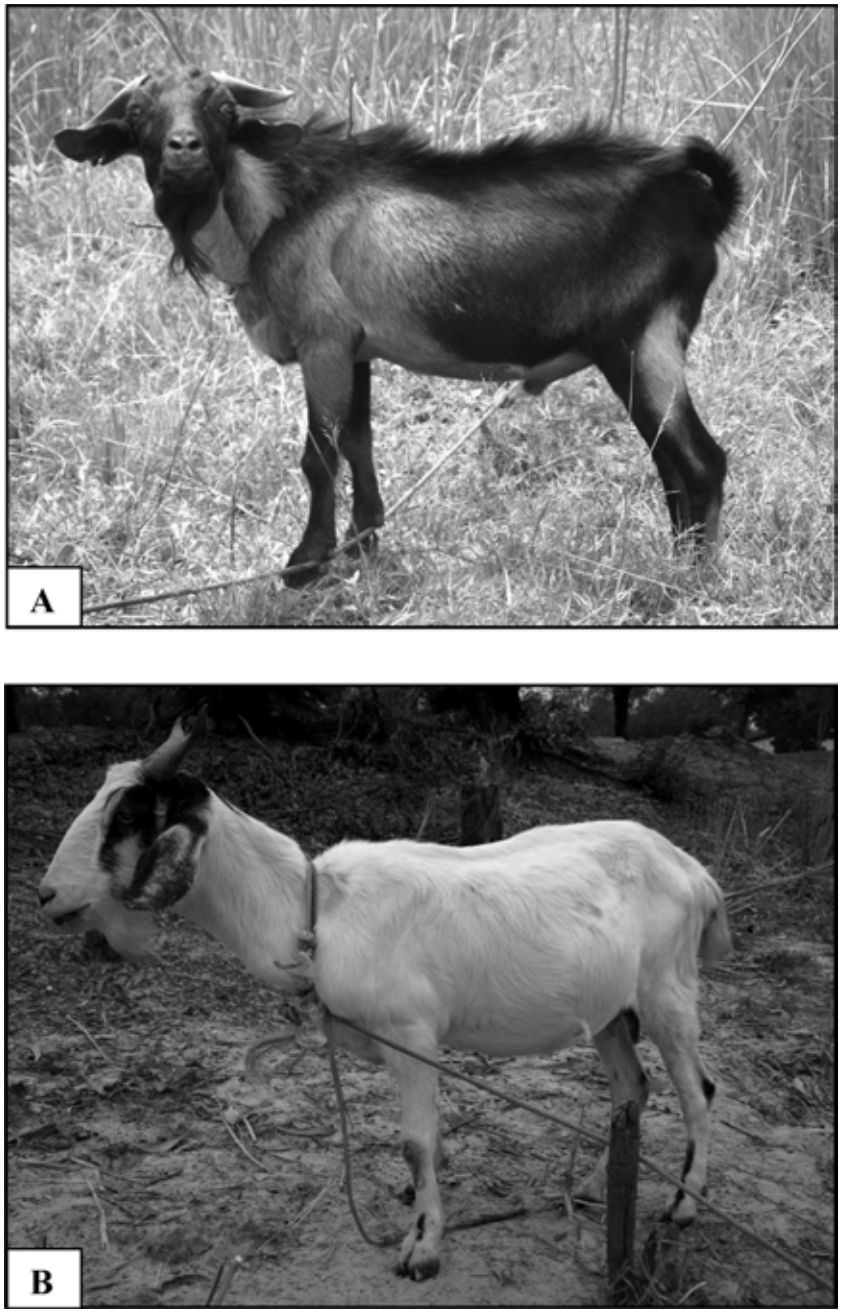

Figure 1. The male (A.) and female (B.) Thai native goat (*Capra hircus*).

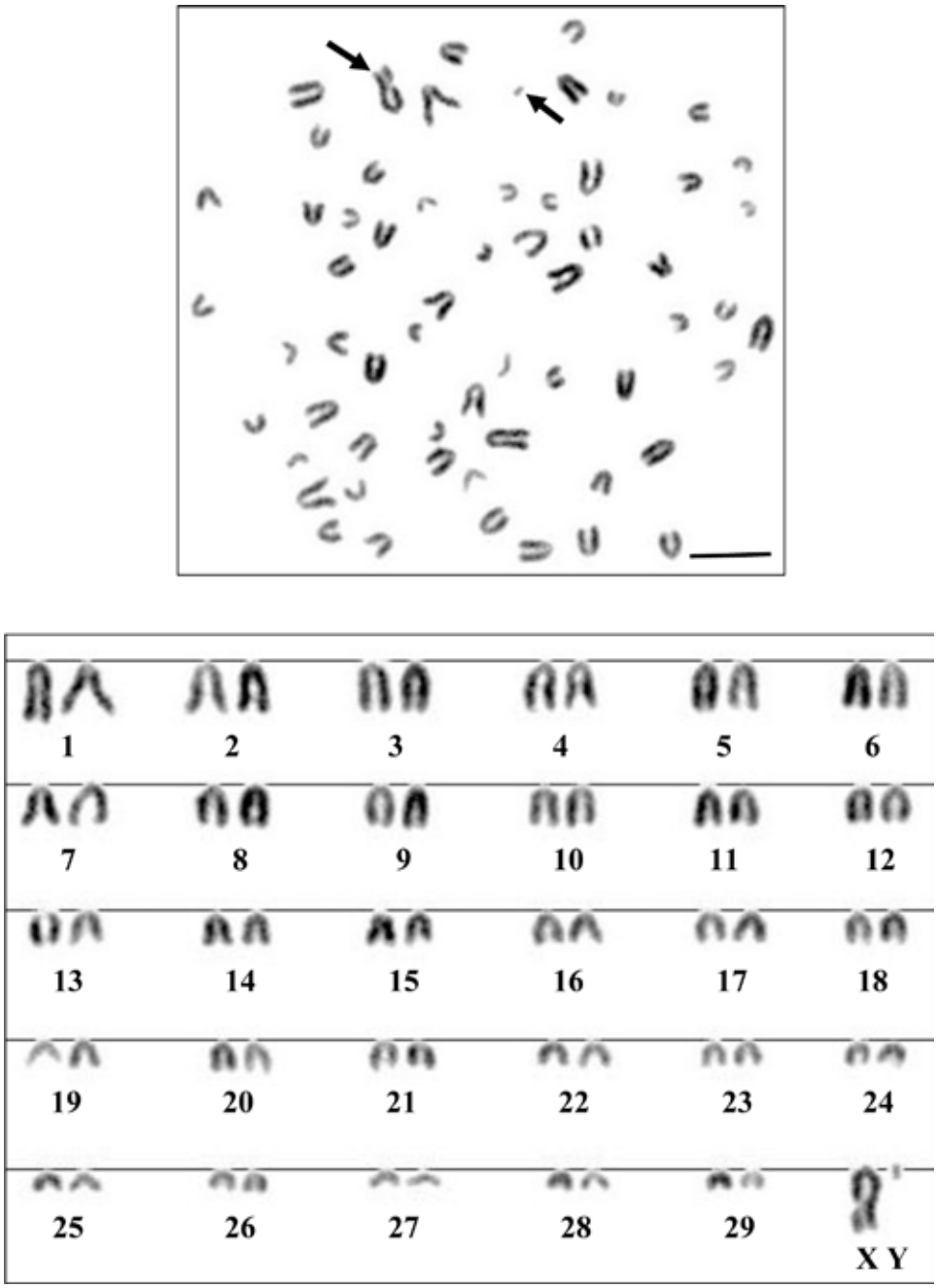

Figure 2. Metaphase chromosome plate and karyotype of male Thai native goat (*Capra hircus*) 2n (diploid) = 60 by conventional staining technique, showing sex chromosomes (arrows), scale bars = 10 µm.

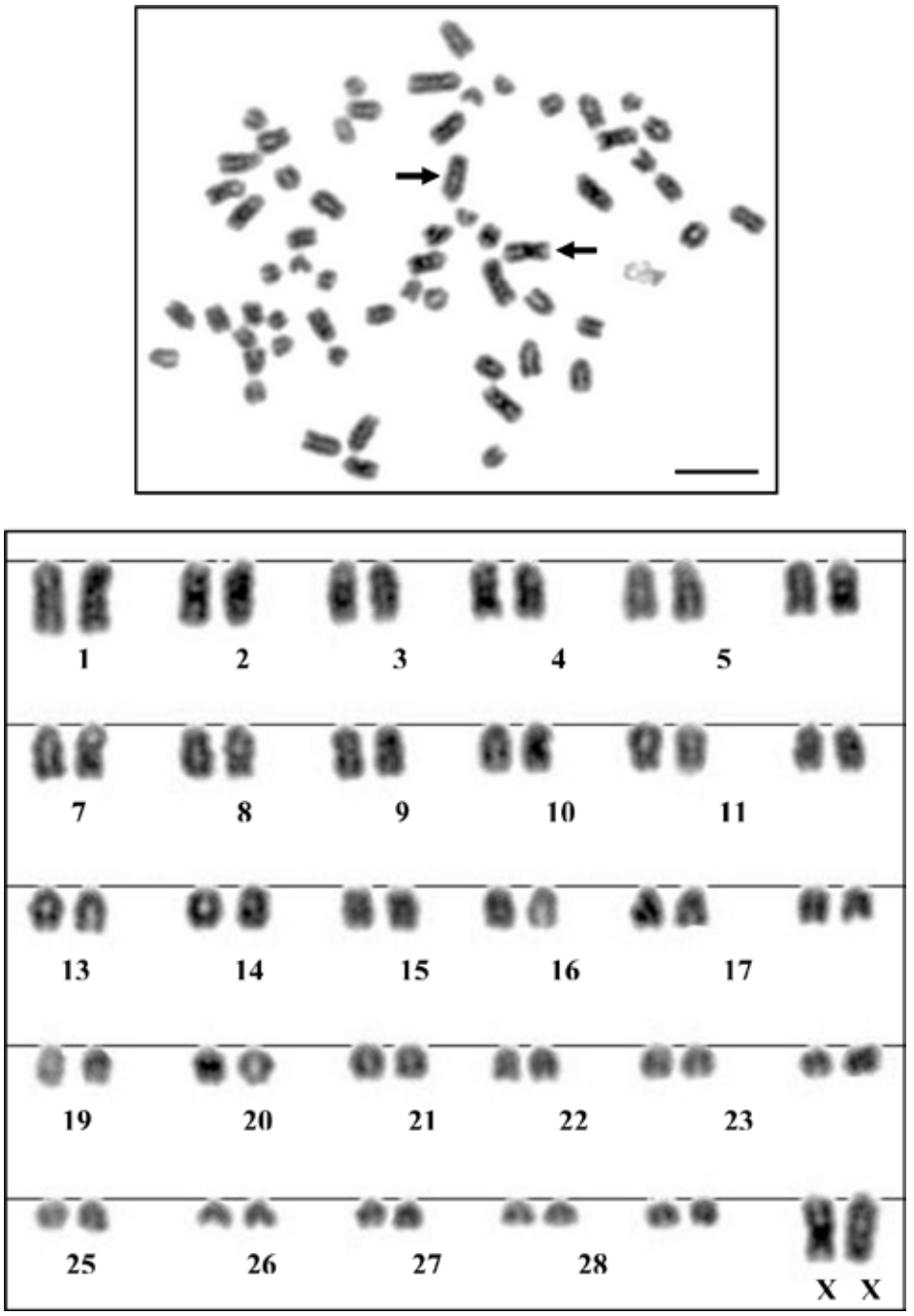

Figure 3. Metaphase chromosome plate and karyotype of female Thai native goat (*Capra hircus*) 2n (diploid) = 60 by conventional staining technique, showing sex chromosomes (arrows), scale bars=10 μm.

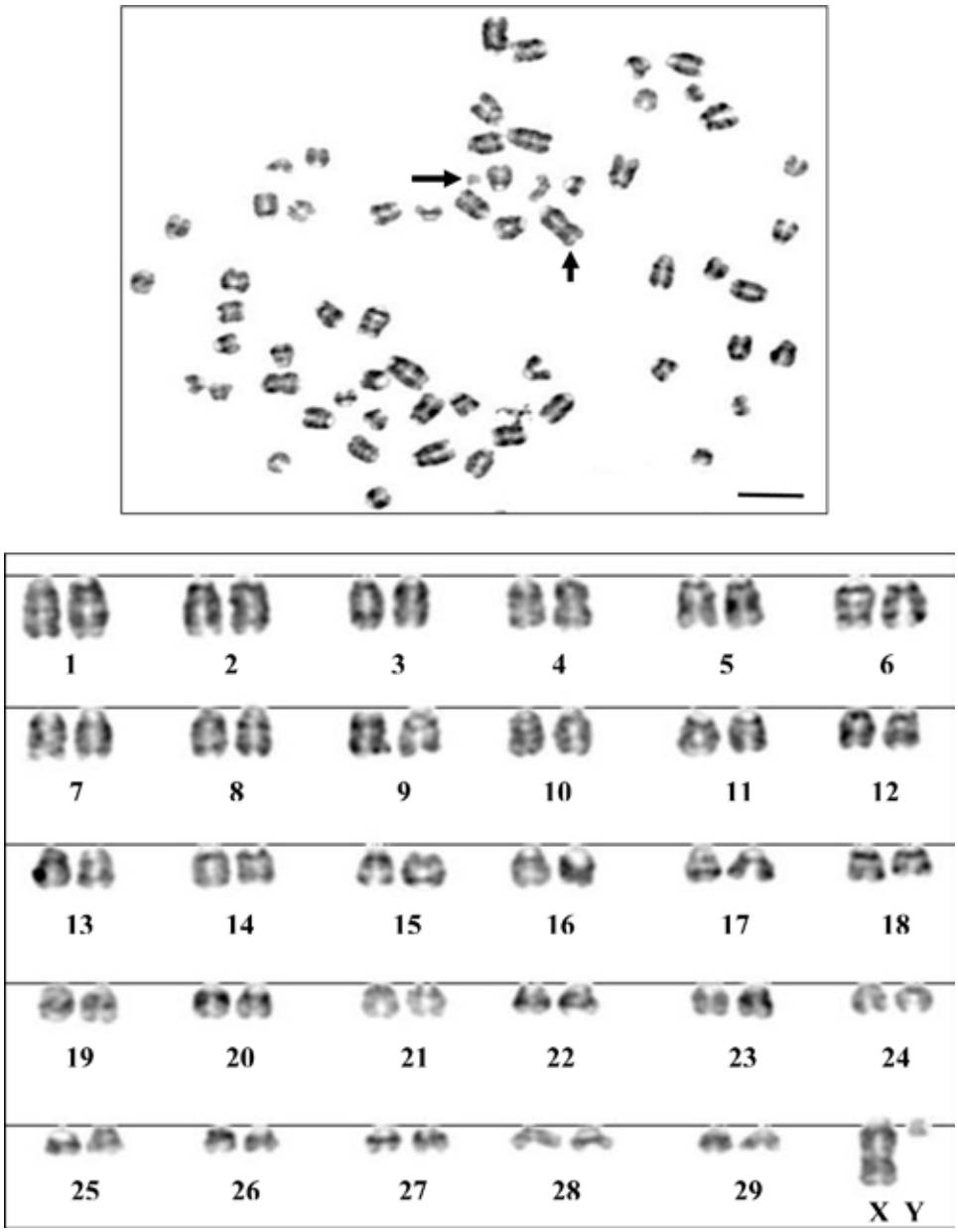

Figure 4. Metaphase chromosome plate and karyotype of male Thai native goat (*Capra hircus*) 2n (diploid) = 60 by GTG-banding technique, showing sex chromosomes (arrows), scale bars = 10 μm.

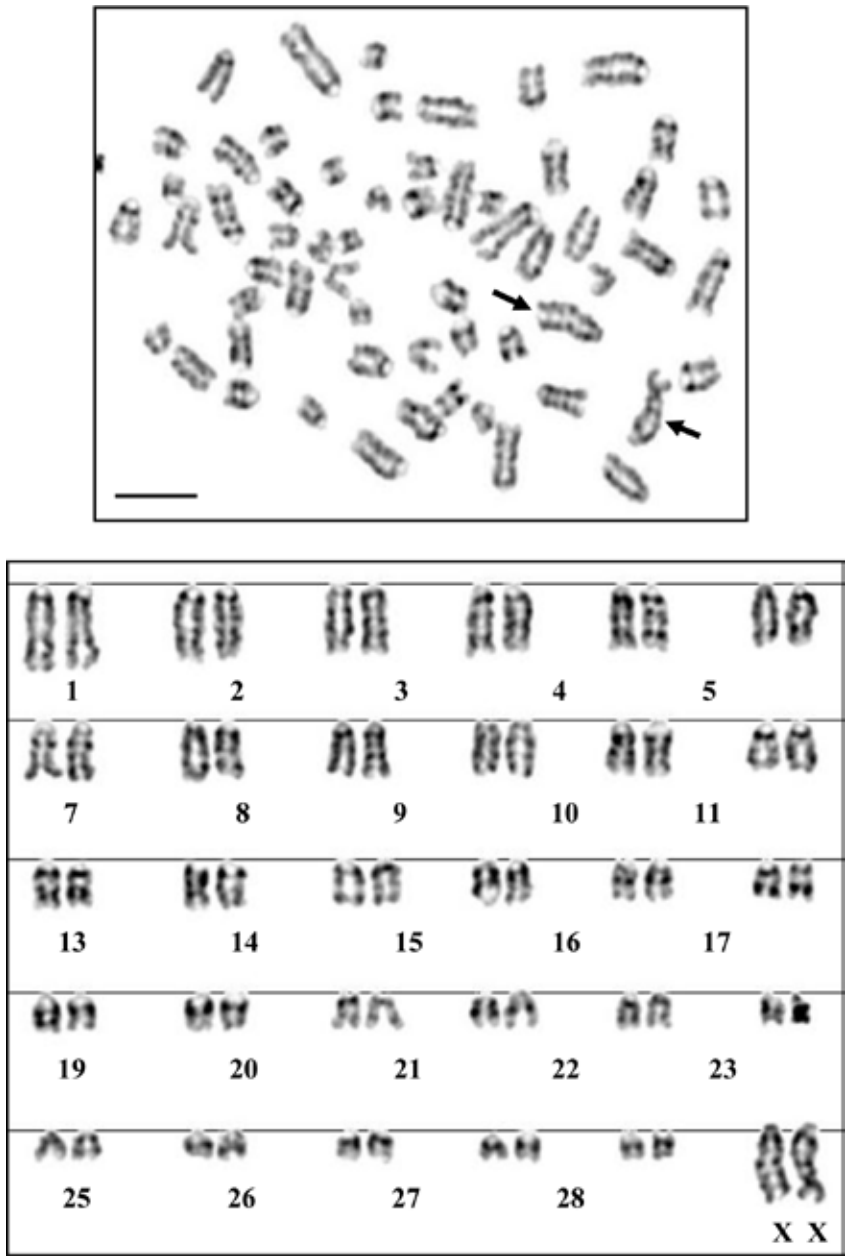

Figure 5. Metaphase chromosome plate and karyotype of female Thai native goat (*Capra hircus*) 2n (diploid) = 60 by GTG-banding technique, showing sex chromosomes (arrows), scale bars = 10 μm.

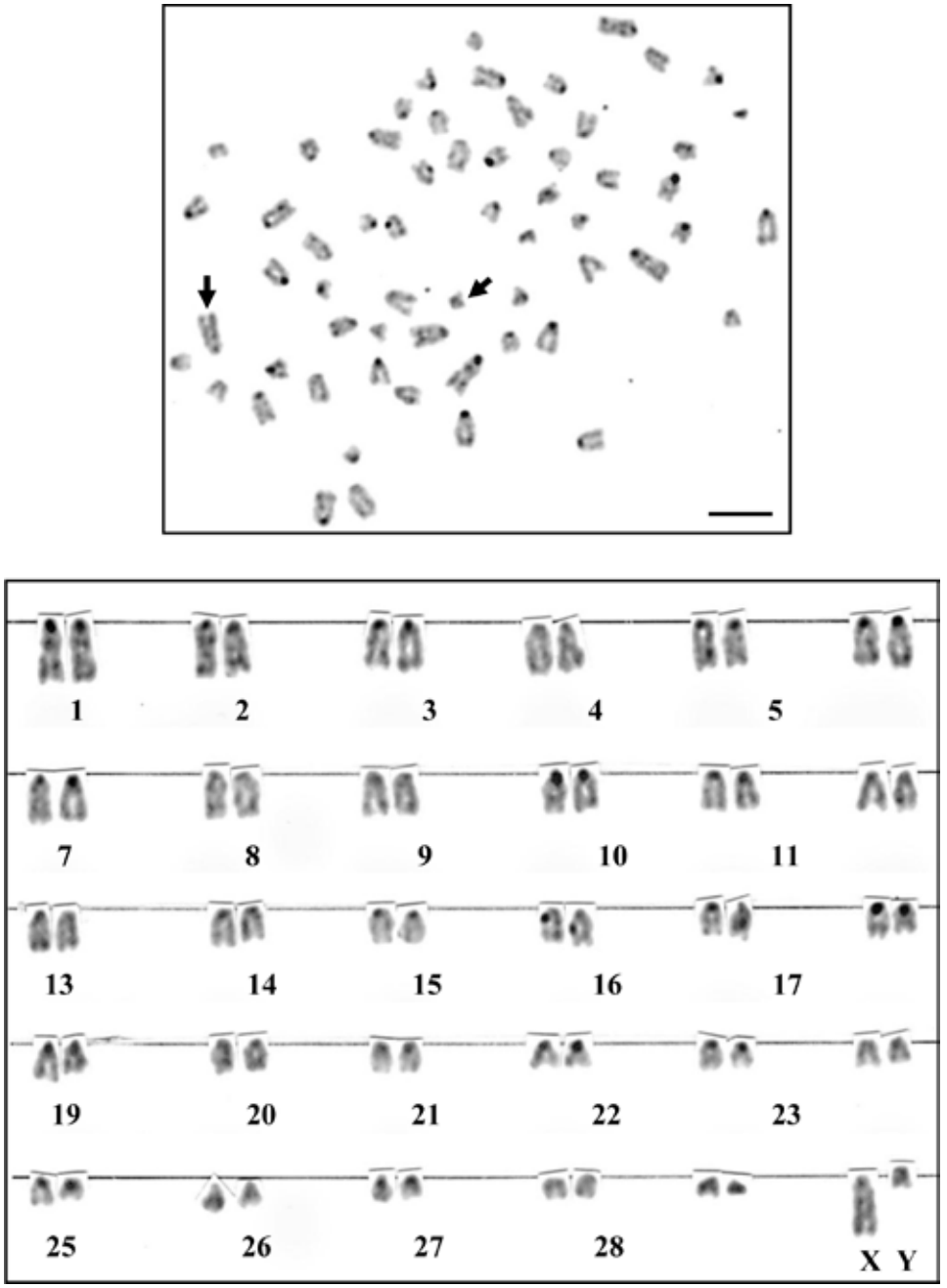

Figure 6. Metaphase chromosome plate and karyotype of male Thai native goat (*Capra hircus*) 2n (diploid) = 60 by CBG-banding technique, showing sex chromosomes (arrows), scale bars = 10 μm.

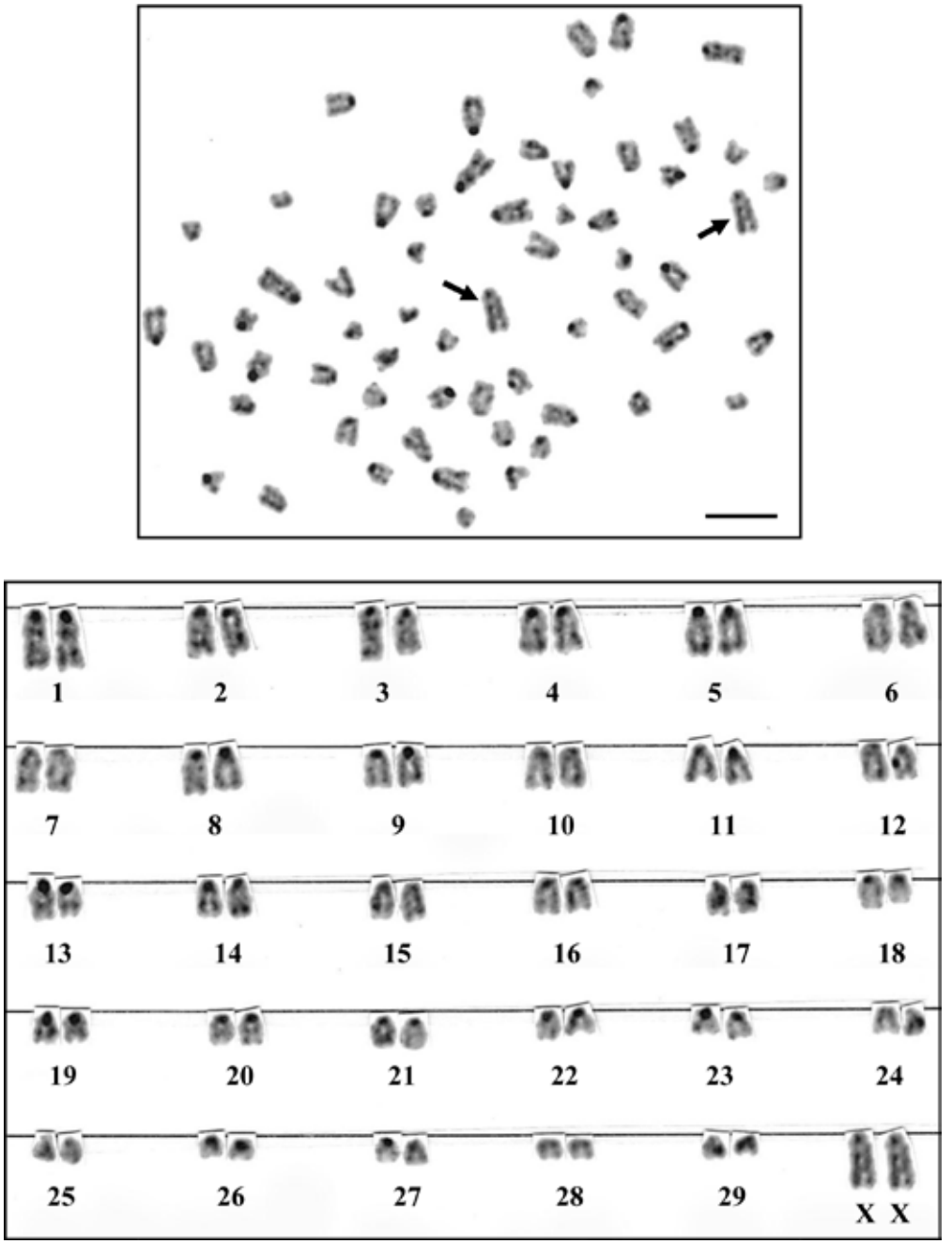

Figure 7. Metaphase chromosome plate and karyotype of female Thai native goat (*Capra hircus*) 2n (diploid) = 60 by CBG-banding technique, showing sex chromosomes (arrows), scale bars = 10 μm.

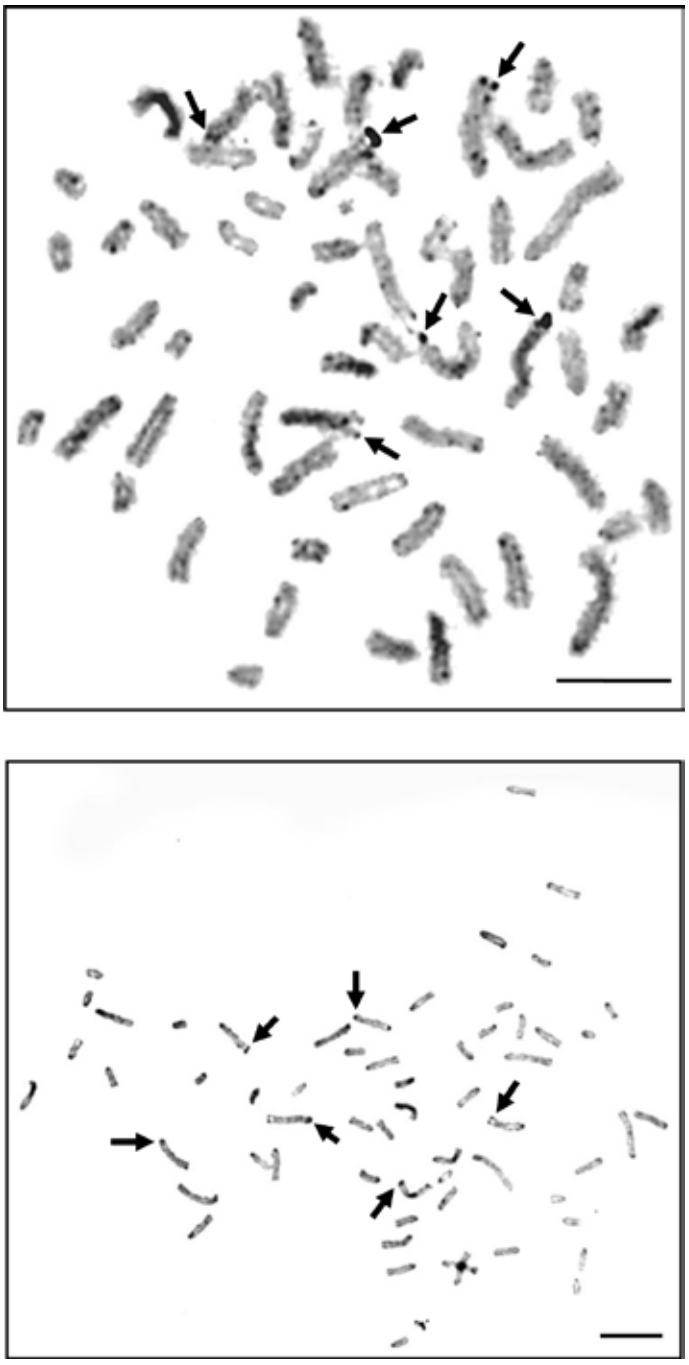

Figure 8. Metaphase chromosome plate of male and female Thai native goat (*Capra hircus*) 2n (diploid) = 60 by Ag-NOR banding technique, showing satellite chromosomes (arrows), scale bars = 10 μm.

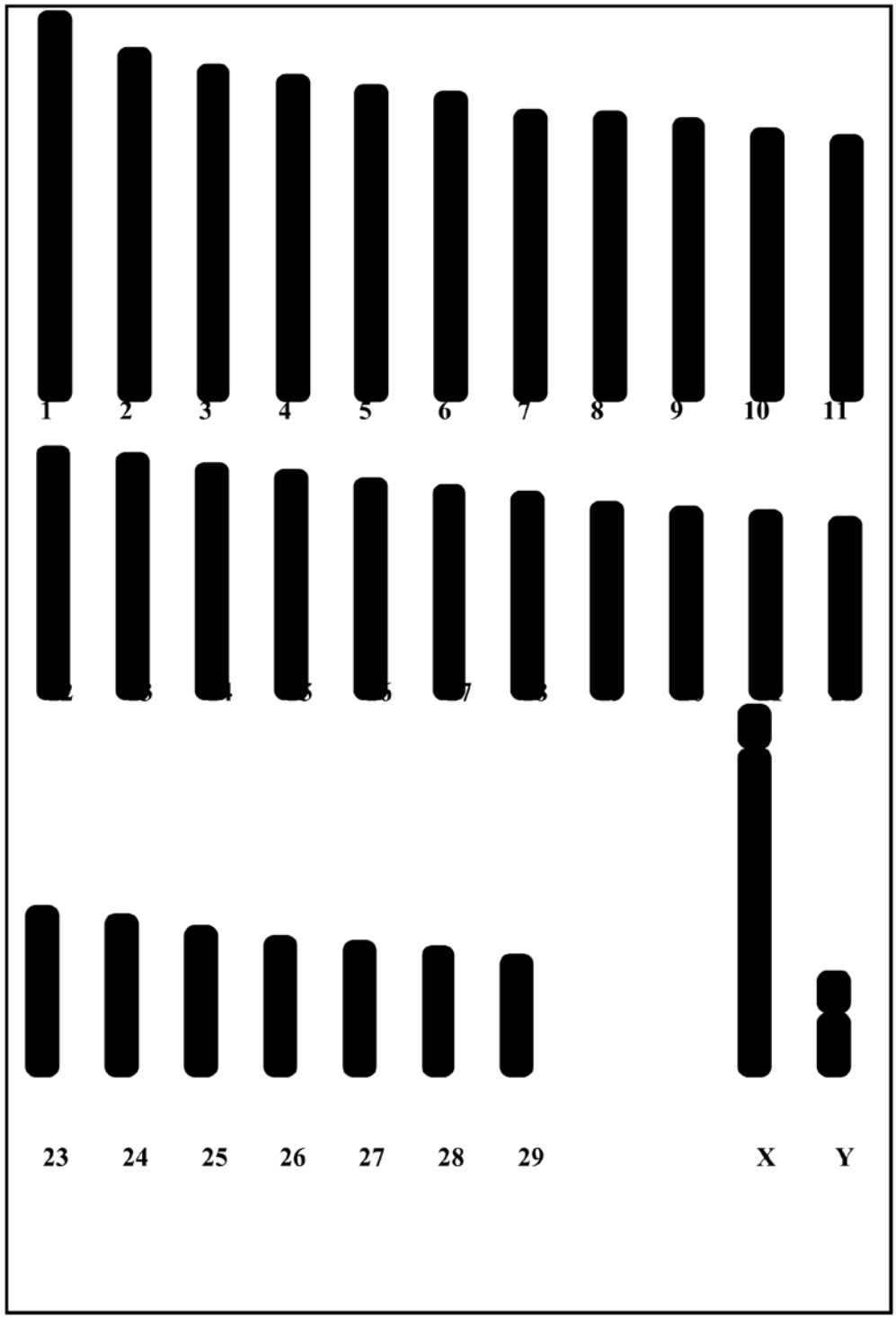

Figure 9. Idiogram of Thai native goat (*Capra hircus*) 2n=60 by conventional staining

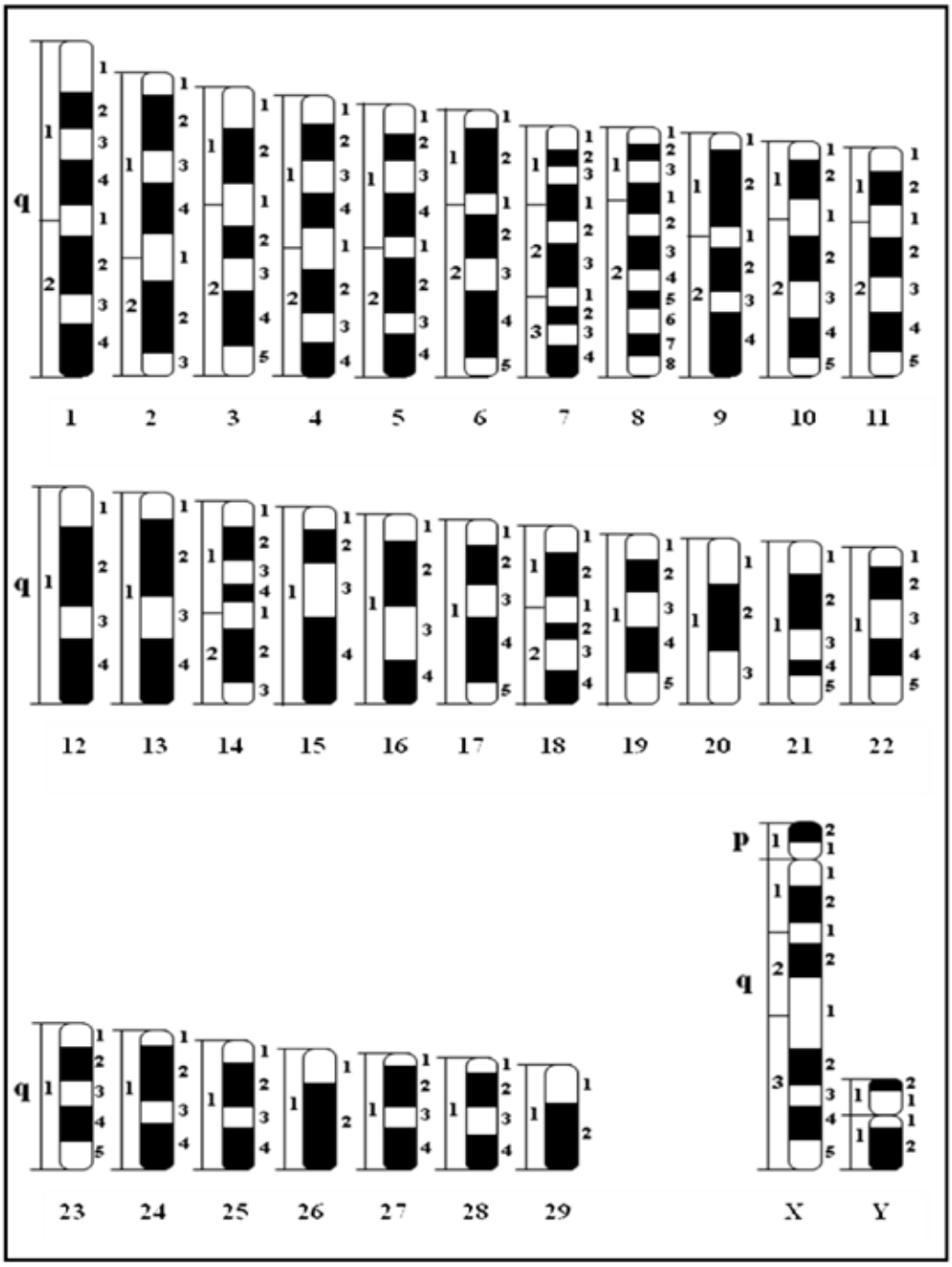

Figure 10. Idiogram of Thai native goat (*Capra hircus*) 2n=60 by GTG-banding

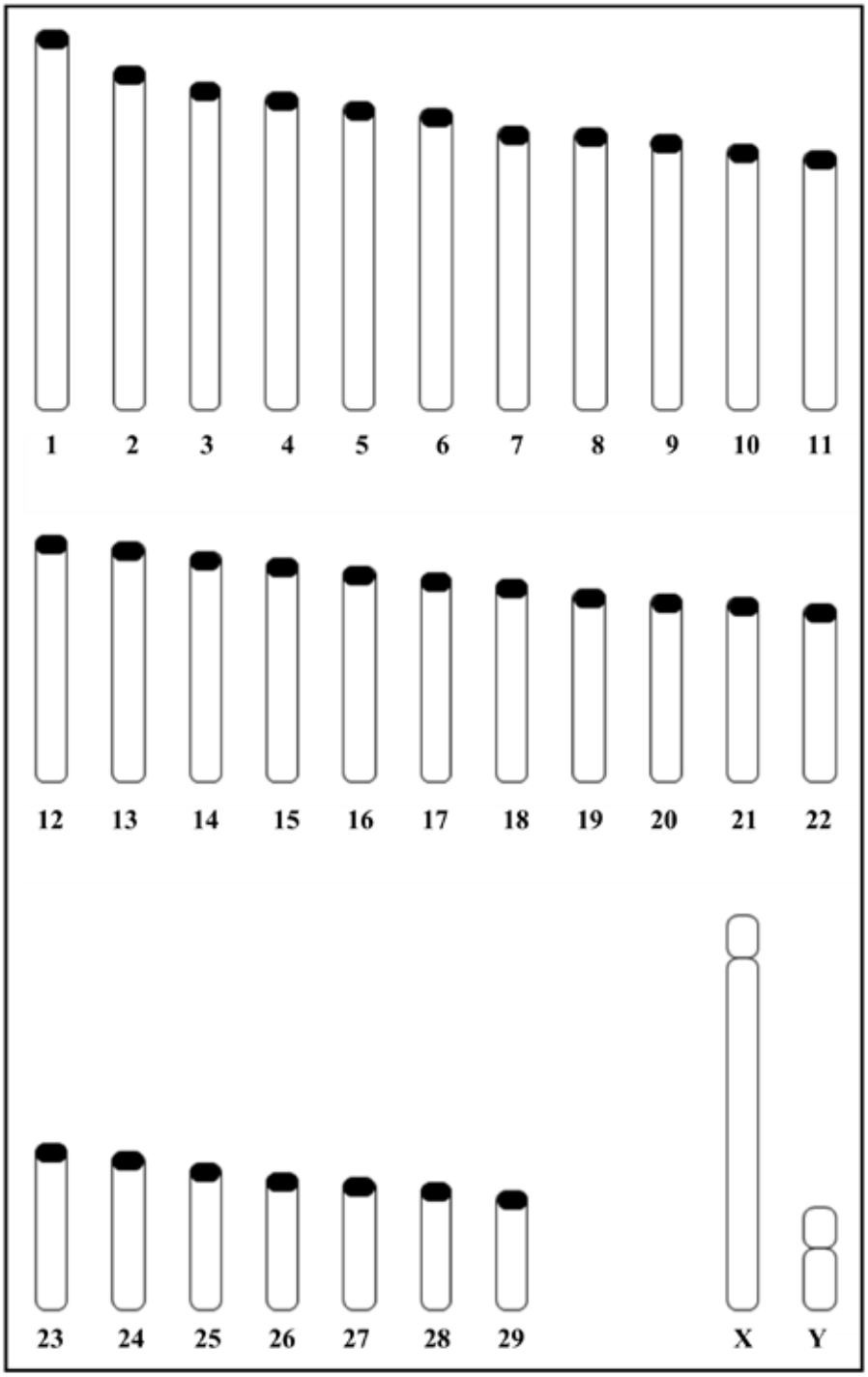

Figure 11. Idiogram of Thai native goat (*Capra hircus*) 2n=60 by CBG-banding

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), chromosome size and chromosome type from metaphase chromosomes of 20 cells in male and female the Thai native goat (*Capra hircus*), 2n (diploid) = 60.

Chromosome Pair	Ls (cm)	Ll (cm)	LT (cm)	RL	CI	Size	Type
1	0.000	1.446	1.446	0.055	1.000	L	a
2	0.000	1.308	1.308	0.050	1.000	L	a
3	0.000	1.248	1.248	0.048	1.000	L	a
4	0.000	1.212	1.212	0.046	1.000	L	a
5	0.000	1.174	1.174	0.045	1.000	L	a
6	0.000	1.145	1.145	0.044	1.000	L	a
7	0.000	1.082	1.082	0.041	1.000	L	a
8	0.000	1.070	1.070	0.041	1.000	L	a
9	0.000	1.045	1.045	0.040	1.000	L	a
10	0.000	1.011	1.011	0.039	1.000	L	a
11	0.000	0.986	0.986	0.038	1.000	L	a
12	0.000	0.937	0.937	0.036	1.000	M	a
13	0.000	0.909	0.909	0.035	1.000	M	a
14	0.000	0.875	0.875	0.033	1.000	M	a
15	0.000	0.847	0.847	0.032	1.000	M	a
16	0.000	0.817	0.817	0.031	1.000	M	a
17	0.000	0.796	0.796	0.030	1.000	M	a
18	0.000	0.767	0.767	0.029	1.000	M	a
19	0.000	0.734	0.734	0.028	1.000	S	a
20	0.000	0.713	0.713	0.027	1.000	S	a
21	0.000	0.701	0.701	0.027	1.000	S	a
22	0.000	0.675	0.675	0.026	1.000	S	a
23	0.000	0.631	0.631	0.024	1.000	S	a
24	0.000	0.604	0.604	0.023	1.000	S	a
25	0.000	0.559	0.559	0.021	1.000	S	a
26	0.000	0.519	0.519	0.020	1.000	S	a
27	0.000	0.504	0.504	0.019	1.000	S	a
28	0.000	0.481	0.481	0.018	1.000	S	a
29	0.000	0.451	0.451	0.017	1.000	S	a
X	0.157	1.341	1.498	0.056	0.895	L	a
Y	0.155	0.237	0.392	0.015	0.604	S	sm

Remarks: L = large chromosome, M = medium chromosome, S = small chromosome, a = acrocentric chromosome and sm = submetacentric chromosome.

Acknowledgements

The authors gratefully acknowledge the financial support received from The Zoological Park Organization under the Royal Patronage of H.M. The King. We also thank Dr. Sopon Dumnui, Director of the organization, Dr. Sumat Kamolnaranath, Chief of the Educational Division and the staffs of Surindra Rajabhat University, for their valuable help.

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