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Original Article

Cytogenetic study on Thai brow-antlered deer, *Cervus eldi siamensis* and Thamin brow-antlered deer, *Cervus eldi thamin* (Artiodactyla, Cervidae) by conventional staining method

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Abstract

Cytogenetics of Thai brow-antlered deer (*Cervus eldi siamensis*) were studied in comparison with those of Thamin brow-antlered deer (*Cervus eldi thamin*). Blood samples were taken from the two subspecies kept in Khoa Kheow Open Zoo, Chonburi Province. After the standard whole blood lymphocyte were cultured in presence of colchicine, the metaphase spreads were performed on microscopic slides and air-dried. Conventional Giemsa's staining were applied to stain chromosome. Thai and Thamin brow-antlered deers exhibited the same karyotype with diploid number of 2n = 58 (NF = 70) for females and 2n = 58 (NF = 71) for males. The types of autosome are 6 large metacentric, 6 large submetacentric, 8 large telocentric, 20 medium telocentric and 16 small telocentric chromosomes. In addition, satellites are clearly observed in terminal position on the short arm of a pair of chromosome 7. The X chromosome is the largest telocentric and the Y chromosome is the smallest metacentric chromosome. The karyotype formula of Thai and Thamin brow-antlered deer is as follows:

$2n\ (58) = L^{m}_{6}+L^{sm}_{6}+L^{t}_{8}+M^{t}_{20}+S^{t}_{16}$+sex chromosome

Keywords: chromosome, cytogenetics, Thai brow-antlered deer (*Cervus eldi siamensis*), Thamin brow-antlered deer (*Cervus eldi thamin*)

1. Introduction

Thai brow-antlered deer (*Cervus eldi siamensis*) and Thamin brow-antlered deer (*Cervus eldi thamin*) were classified in kingdom Animalia, phylum Chrodata, class Mammalia, order Artiodactyla (even-toed ungulates), family Cervidae, genus *Cervus*. *C. eldi* comprises 4 subspecies which are; *C. e. siamensis* found in Thailand and Indo-China countries, *C. e. thamin* found in Thailand and Myanmar, *C. e. eldi* found in Assam State of India and *C. e. hainanus* found in Hainan Island (Lekagul and McNeely, 1977, 1988).

The different characteristics of Thai brow-antlered and Thamin brow-antlered deer are the top of anther and the body color. The antler of Thai brow-antlered is more branching out like fingers, than that of the Thamin brow-antlered deer. Moreover, the previous one has a darker red colored body than the first one. The height of the Thai brow-antlered and Thamin brow-antlered deer are 1.2-1.5 meters from ground to shoulder and both have 95-100 kilograms bodyweight. Females does not have antlers. Their body have red-brown hair but the ear rims, eye rims and chins have white

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hair. Their herd stays in clear forest, since they do not prefer to live in dense forest and highland (Lekagul and McNeely, 1977, 1988).

The chromosome number of wild animals in family Cervidae vary from 2n = 6 in Indian muntjac (*Muntiacus muntjak*) to 2*n* = 68 in hog deer (*Cervus porcinus*) or 2*n* = 70 in Roe deer (*Capreolus capreolus*) and Rein deer (*Rangifer tarandus*) (Wurster and Atkin, 1972; Chavananikul, 1995). In some species, males and females contain different chromosome numbers such as Sika deer (*Cervus nippon*), where males contain 2n = 68 while females contain 2*n* = 64, 65, 66, 67 and 68. Moreover, there are animals in genus *Muntiacus* such as *M. muntjak* containing 2*n* = 7 in males and 2*n* = 6 in females and *M. fae* which contains 2*n* = 14 in males and 2*n* = 13 in females (Wurster and Benirschke, 1967; Wurster and Atkin, 1972; Suwattana *et al.*, 1996; Tanomtong *et al.*, 2005). Previously, there are some studies on cytogenetics of Thai and Thamin brow-antlered deer (Chavananikul *et al.*, 1995; Bonnet *et al.,* 2002). In 2000, Thévenon *et al.* studied karyotypes of Thai and Thamin brow-antlered deers kept in zoological parks at the Paris Museum of Natural History, France. Their aim was to compare chromosome banding analysis of *C. e. siamensis* and *C. e. thamin*. They found that there is no difference among them. This study aimed to confirm and compare the result from the earlier studies on cytogenetics of Thai and Thamin brow-antlered deer. Moreover, this study exhibited the first report about chromosome measuring for determining the size and karyotype formula which were excluded in the previous studies.

2. Materials and Methods

The jugular vein blood samples were collected from one male and one female of each Thai and Thamin brow-antlered deers maintained in Khoa Kheow Open Zoo, Thailand, by using aseptic technique. The blood samples were kept in 10 ml vacuum tubes containing heparin to prevent blood clotting and cooled on ice until arriving at the laboratory.

2.1 Cell preparation

The lymphocytes were cultured using the whole blood microculture technique modified from Rooney (2001) and Kampiranont (2003). RPMI 1640 medium with 2% PHA (Phytohemagglutinin) was prepared as a mitogen in 5 ml culture bottles and 0.5 ml of blood sample was added and well mixed. The culture bottle was loosely capped and incubated at 37°C, 5% CO_2 with regularly shaken in the morning and evening. At the 72nd hour of incubation, colchicine was added and well mixed followed by further incubation for 30 minutes.

2.2 Cell harvesting

The blood sample mixture was centrifuged at 1,200 rpm for 10 minutes and the supernatant was discarded. Then, 10 ml of hypotonic solution (0.075 M KCl) was applied to the pellet and incubated for 30 minutes, 37°C. KCl was discarded by centrifugation. Cells were fixed with fresh cool fixative (3 methanol : 1 glacial acetic acid) by gradually adding up to 8 ml before centrifuging and discarding the supernatant. The fixation was repeated until the supernatant was clear. The pellet was then mixed with 1 ml fixative. The mixture was dropped onto a clean and cold slide using micropipette followed by the air-dry technique. The slide was conventionally stained with 20 % stock Giemsa's solution for 30 minutes.

2.3 Chromosomal checking, karyotyping and idiograming

Mitotic metaphase chromosomes were checked under light microscope. Twenty cells were clearly observable and well-cells spread chromosomes of each male and female were selected and photographed. The lengths of short arm chromosomes (Ls) and long arm chromosomes (Ll) were measured and calculated to the length of total arm chromosome (LT, LT = Ls + Ll). 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 idiograming.

3. Results and Discussion

After T-lymphocyte culturing, cell harvesting, chromosome checking, karyotyping and idiograming, the results show that there are no differences in chromosome number and type between Thai and Thamin brow-antlered deers (Figures 1, 2, 3 and 4). The results agree with Thévenon *et al.* (2000) which shown that the karyotypes of Thai and Thamin brow-antlered deers were similar by using RBG-banding and karyotypic perspective.

Diploid chromosomes (2n) of both Thai and Thamin brow-antlered deer are 58 that agree with Chavananikul *et al.* (1995) and Bonnet *et al.* (2002). The fundamental number (NF) of Thai and Thamin brow-antlered deer are 70 in female and 71 in male which corresponds to Chavananikul *et al.* (1995). Thai and Thamin brow-antlered deer autosomes composed of 6 large metacentric, 6 large submetacentric, 8 large telocentric, 20 medium telocentric and 16 small telocentric chromosomes (Table 1 and 2). This is not in agreement with Chavananikul *et al.* (1995) that reported the autosomes of Thamin brow-antlered deer maintained kept in Dusit Zoo contains 12 metacentric and submetacentric and 44 acrocentric chromosomes. The mean of the short arm length (Ls), long arm length (Ll) and chromosome length (Lt) are shown in centimeter. Relative length (RL), centromeric index (CI) and standard deviation (SD) of Thai and Thamin brow-antlered deer are shown in Tables 1 and 2, respectively. The idiogram of the Thai and Thamin brow-antlered deer shows gradually decreasing length of the auto-

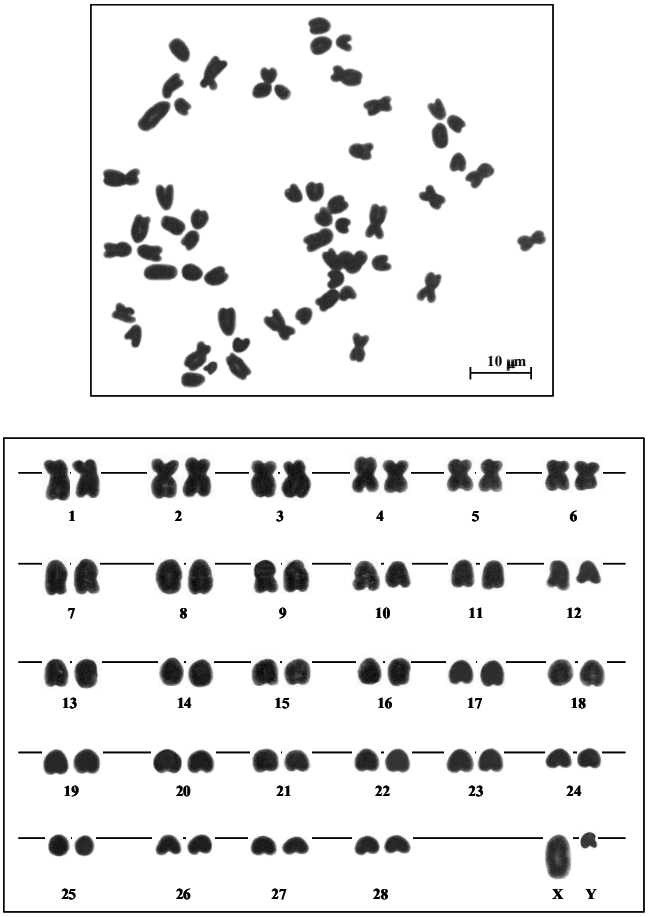


Figure 1. Metaphase chromosome plates (top) and karyotype (bottom) of male Thai brow-antlered deer (*Cervus eldi siamensis*) 2n (diploid) = 58, by conventional staining method.

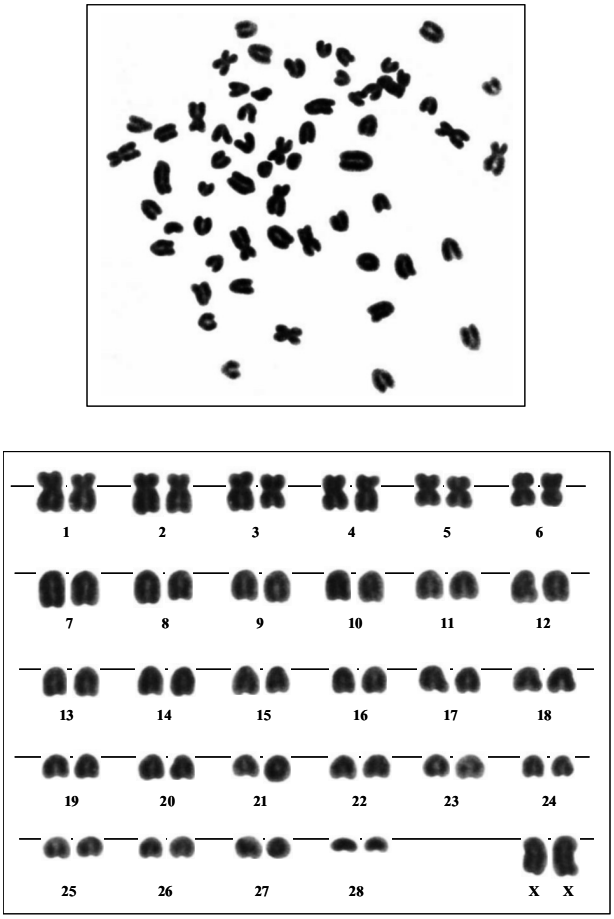


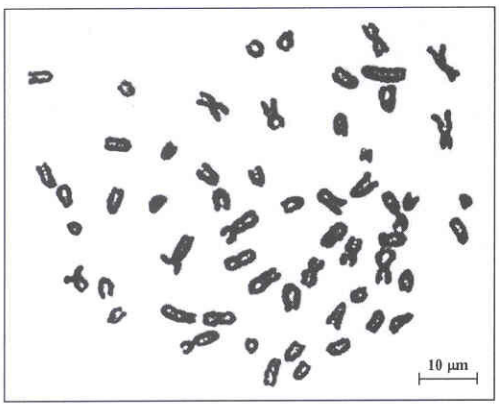
Figure 2. Metaphase chromosome plates (top) and karyotype (bottom) of female Thai brow-antlered deer (*Cervus eldi siamensis*) 2n (diploid) = 58, by conventional staining method.

somes and sex chromosomes (Figure 5).

We also found that sex chromosome of Thai and Thamin brow-antlered deer are chromosome markers. The X chromosome is the largest telocentric while the Y chromosome is the smallest matecentric (Figures 1, 2, 3, 4 and 5). This is not in agreement with Chavananikul *et al.* (1995) which reported that Thamin brow-antlered deer has a large acrocentric X chromosome and a submetacentric Y chromosome. The telocentric Chromosome 7 of both deers contains nucleolar organizer region (NOR) so called satellite chromosome. This is not in agreement with Chavananikul *et al.* (1995) that reported satellite chromosome of the Thamin brow-antlered deer is two large acrocentric chromosomes.

When the karyotype of Thai and Thamin brow-antlered deers were compared with those from other animals in the same family such as sambar deer (*Cervus unicolor*), the numbers and types of autosomes are similar but the sex chromosomes are different. Sambar deer has a large acrocentric X chromosome and a small acrocentric Y chromosome (Chandra *et al.*, 1967). When comparing the chromosomes of brow-antlered deer with the chromosomes of the other deer, the difference are in the numbers and types of autosomes and sex chromosomes for example the 2*n* of rusa deer (*C. timoremsis*), white-lipped deer (*C. albriostris*), nilgai (*Boselaphus tragocamelus*), barasingha deer (*C. duvauceli*), Himalayan thar (*Hemitragus jemlahicus*) and dik-dik antelope (*Rhynchotragus kirki*) are 60, 66, 46, 56, 48 and 46, respectively (Chandra *et al.*, 1967; Bonnet *et al.,* 2001)

Fontana and Rubini (1990) studied chromosome evolution in the family Cervidae and reported that primitive karyotype is composed of with 70 acrocentric chromosomes (2*n* = 70). In the evolutional history, animals in family Cervidae can be divided into 3 groups using chromosome arrangement. The first is the subfamily Cervinae which by chromosome fusion causing the autosomes decreasing to 68 and NF=70. An example of an animal in this subfamily is the red deer (*C. elaphus*). The second group is the subfamily Odocoileinae evolving by pericentric inversion and Robertsonian translocation that lead to the submetacentric X chromosome and one submetacentric autosome, so that the final



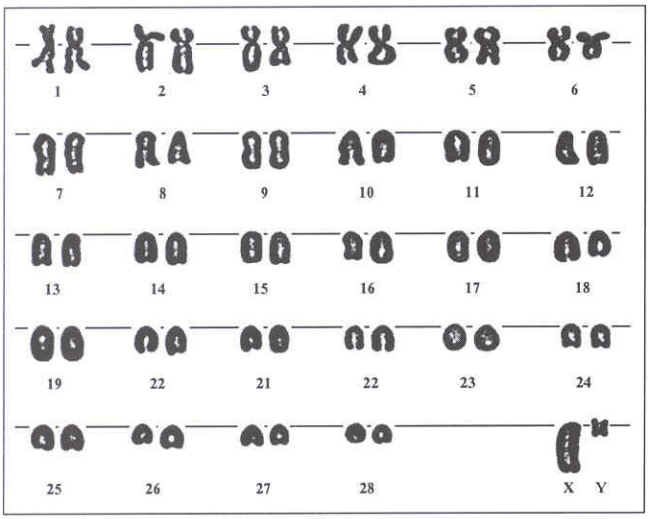
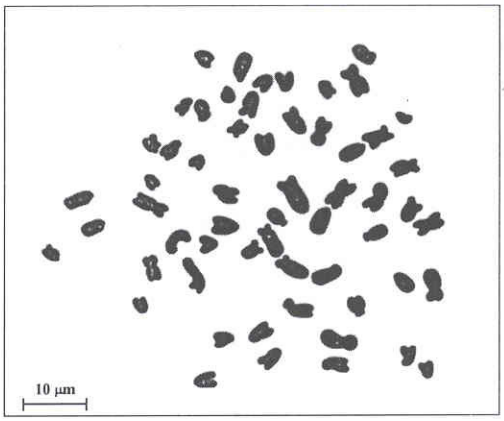


Figure 3. Metaphase chromosome plates (top) and karyotype (bottom) of male Thamin brow-antlered deer (*Cervus eldi thamin*) 2n (diploid) = 58, by conventional staining method.



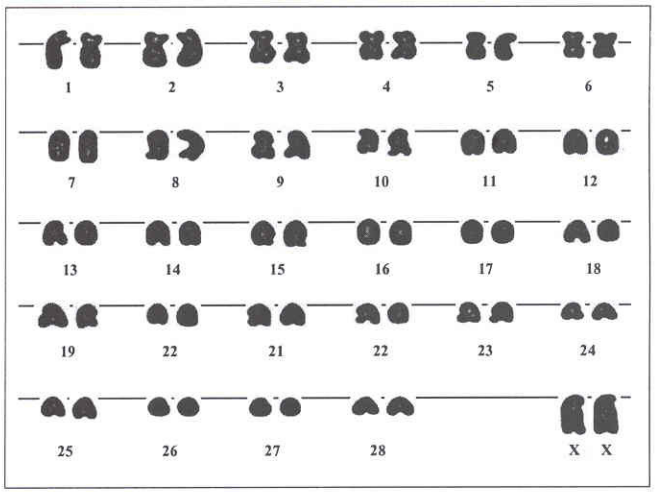


Figure 4. Metaphase chromosome plates (top) and karyotype (bottom) of female Thamin brow-antlered deer (*Cervus eldi thamin*) 2n (diploid) = 58, by conventional staining method.

chromosome number is $2n$ = 70 and NF = 74 such as the mule deer (*Odocoileus hemionus*). The last group is the subfamily Muntiacinae occurring by centric fusion and tandem fusion chromosome rearrangement resulting in $2n$ = 46, NF = 46, for example, the Reeve's muntjac (*M. reevesi*). It has the different chromosome number from the female Indian muntjac (*M. muntjak vaginalis*) and the Roosevelt's muntjac (*M. rooseveltorum*) that have $2n$ = 46. The Thai and Thamin brow-antlered deer karyotypes can be formula as follow:

$$2n\ (58) = L^{m}_{6}+L^{sm}_{6}+L^{t}_{8}+M^{t}_{20}+S^{t}_{16}+\text{sex-chromosome}$$

4. Conclusions

Cytogenetics of Thai brow-antlered deer (*Cervus eldi siamensis*) were studied in comparison with those of Thamin brow-antlered deer (*C. e. thamin*). Karyotypes of Thai and Thamin brow-antlered deers contain equal number of $2n$ = 58 with the same types of chromosomes. As a result of this, the NF are also equal to 70 chromosomes in females and 71 chromosomes in males. The types of autosomes are 6 large metacentric, 6 large submetacentric, 8 large telocentric, 20 medium telocentric and 16 small telocentric chromosomes. In addition, a pair of the short arm of chromosome 7 clearly shows an observable on satellite chromosome. The X chromosome is the largest telocentric chromosome and the Y chromosome is the smallest metacentric chromosome.

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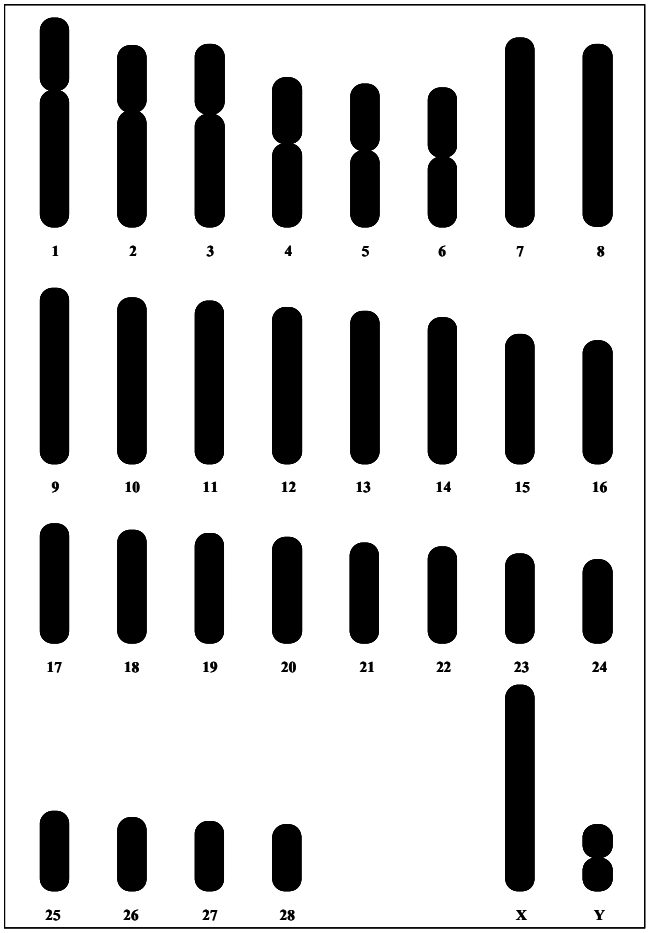

Figure 5. Idiogram of Thai brow-antlered deer (*Cervus eldi siamensis*) and Thamin brow-antlered deer (*Cervus eldi thamin*), 2n (diploid) = 58 by conventional staining.

deer blood samples. Thanks to the authorities and officers of the zoo for their kind cooperation.

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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) and standard deviation (SD) of RL, CI from metaphase chromosomes of 20 cells in Thai brow-antlered male and female deers (*Cervus eldi siamensis*), 2n (diploid) = 58.

Chromosome pairs	Ls	Ll	LT	RL±SD	CI±SD	Size of chromosome	Type of chromosome
1	0.242	0.427	0.670	0.049±0.002	0.637±0.040	L	sm
2	0.240	0.405	0.646	0.048±0.002	0.627±0.040	L	sm
3	0.247	0.400	0.648	0.048±0.002	0.617±0.040	L	sm
4	0.254	0.346	0.600	0.044±0.002	0.576±0.050	L	m
5	0.239	0.310	0.549	0.040±0.001	0.564±0.040	L	m
6	0.236	0.288	0.525	0.039±0.001	0.550±0.070	L	m
7	0.000	0.620	0.620	0.046±0.002	1.000±0.000	L	t
8	0.000	0.556	0.556	0.041±0.001	1.000±0.000	L	t
9	0.000	0.516	0.516	0.038±0.001	1.000±0.000	L	t
10	0.000	0.499	0.499	0.037+0.001	1.000+0.000	L	t
11	0.000	0.479	0.479	0.035±0.001	1.000±0.000	L	t
12	0.000	0.466	0.466	0.034±0.001	1.000±0.000	L	t
13	0.000	0.455	0.455	0.033±0.001	1.000±0.000	L	t
14	0.000	0.443	0.443	0.032±0.001	1.000±0.000	L	t
15	0.000	0.430	0.430	0.031±0.001	1.000±0.000	M	t
16	0.000	0.418	0.418	0.031±0.001	1.000±0.000	M	t
17	0.000	0.404	0.404	0.030±0.001	1.000±0.000	M	t
18	0.000	0.391	0.391	0.029±0.001	1.000±0.000	M	t
19	0.000	0.379	0.379	0.028±0.001	1.000±0.000	M	t
20	0.000	0.368	0.368	0.027±0.001	1.000±0.000	M	t
21	0.000	0.353	0.353	0.026±0.001	1.000±0.000	S	t
22	0.000	0.345	0.345	0.025±0.001	1.000±0.000	S	t
23	0.000	0.335	0.335	0.024±0.001	1.000±0.000	S	t
24	0.000	0.323	0.323	0.024±0.001	1.000±0.000	S	t
25	0.000	0.313	0.313	0.023±0.001	1.000±0.000	S	t
26	0.000	0.300	0.300	0.022±0.001	1.000±0.000	S	t
27	0.000	0.282	0.282	0.021±0.001	1.000±0.000	S	t
28	0.000	0.262	0.262	0.019±0.001	1.000±0.000	S	t
X	0.000	0.692	0.692	0.051±0.001	1.000±0.000	L	t
Y	0.100	0.096	0.192	0.014±0.001	0.5000±0.00	S	m

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Table 2. 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) and standard deviation (SD) of RL, CI from metaphase chromosomes of 20 cells in Thamin brow-antlered male and female deers (*Cervus eldi thamin*), 2n (diploid) = 58.

Chromosome pairs	Ls	Ll	LT	RL±SD	CI±SD	Size of chromosome	Type of chromosome
1	0.220	0.410	0.630	0.027±0.000	0.652±0.015	L	sm
2	0.200	0.350	0.560	0.024±0.000	0.634±0.010	L	sm
3	0.210	0.340	0.540	0.023±0.000	0.619±0.009	L	sm
4	0.200	0.250	0.450	0.019±0.000	0.552±0.006	L	m
5	0.200	0.230	0.440	0.018±0.001	0.541±0.092	L	m
6	0.210	0.210	0.420	0.018±0.002	0.502±0.004	L	m
7	0.000	0.570	0.570	0.024±0.000	1.000±0.000	L	t
8	0.000	0.550	0.550	0.023±0.000	1.000±0.000	L	t
9	0.000	0.530	0.530	0.022±0.000	1.000±0.000	L	t
10	0.000	0.500	0.500	0.021±0.000	1.000±0.000	L	t
11	0.000	0.490	0.490	0.021±0.000	1.000±0.000	L	t
12	0.000	0.470	0.470	0.020±0.000	1.000±0.000	L	t
13	0.000	0.460	0.460	0.019±0.000	1.000±0.000	L	t
14	0.000	0.440	0.440	0.019±0.000	1.000±0.000	L	t
15	0.000	0.390	0.390	0.016±0.000	1.000±0.000	M	t
16	0.000	0.370	0.370	0.016±0.000	1.000±0.000	M	t
17	0.000	0.360	0.360	0.015±0.000	1.000±0.000	M	t
18	0.000	0.340	0.340	0.014±0.000	1.000±0.000	M	t
19	0.000	0.330	0.330	0.014±0.000	1.000±0.000	M	t
20	0.000	0.320	0.320	0.013±0.000	1.000±0.000	M	t
21	0.000	0.300	0.300	0.013±0.000	1.000±0.000	S	t
22	0.000	0.290	0.290	0.012±0.000	1.000±0.000	S	t
23	0.000	0.270	0.270	0.011±0.000	1.000±0.000	S	t
24	0.000	0.250	0.250	0.010±0.000	1.000±0.000	S	t
25	0.000	0.240	0.240	0.010±0.000	1.000±0.000	S	t
26	0.000	0.220	0.220	0.009±0.000	1.000±0.000	S	t
27	0.000	0.210	0.210	0.009±0.000	1.000±0.000	S	t
28	0.000	0.200	0.200	0.008±0.000	1.000±0.000	S	t
X	0.000	0.620	0.620	0.002±0.009	1.000±0.000	L	t
Y	0.100	0.100	0.200	0.008±0.000	0.500±0.000	S	m

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