Karyotype and Idiogram of Indian Hog Deer (*Hyelaphus porcinus*) by Conventional Staining, GTG-, High-Resolution and Ag-NOR Banding Techniques

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Summary Standardized karyotype and idiogram of Indian hog deer (*Hyelaphus porcinus*) at Khon Kaen Zoo, Thailand was explored. Blood samples were taken from two male and two female deer. After standard whole blood T-lymphocytes were cultured at 37°C for 72h in presence of colchicine, metaphase spreads were performed on microscopic slides and air-dried. Conventional staining, GTG-, high-resolution and Ag-NOR banding techniques were applied to stain the chromosome. The results show that the diploid chromosome number of *H. porcinus* was $2n=68$, the fundamental number (NF) was 70 in both males and females. The types of autosomes observed were 6 large telocentric, 18 medium telocentric and 42 small telocentric chromosomes. The X chromosome was large metacentric chromosome and Y chromosome was small submetacentric chromosome. The GTG-banding and high-resolution techniques showed that the numbers of bands and locations in *H. porcinus* are 239 and 301, respectively, and each chromosome pair could be clearly differentiated. In addition, the subtelomeric q-arm of chromosome pairs 1 and 2 showed clearly observable nucleolar organizer regions (NORs). Our results are the first reports of GTG-, high-resolution and Ag-NOR banding techniques on this species. The karyotype formula of *H. porcinus* is as follows:

$$2n(68) = L_6^t + M_{18}^t + S_{42}^t + \text{Sex-chromosomes}$$

Key words *Hyelaphus porcinus*, Karyotype, GTG banding, Ag-NOR banding.

Deer are the ruminant mammals forming the family Cervidae. The deer family has at least 90 species; the list is based on several molecular and phylogenetic studies. The family Cervidae is organized as two subfamilies, Cervinae (old world deer) and Capreolinae (new world deer). The Cervidae is divided into two tribes, Muntiacini (muntjac) and Cervini (true deer). The Cervini consist of eight genera including *Dama*, *Axis*, *Rucervus*, *Cervus*, *Panolia*, *Elaphurus*, *Hyelaphus* and *Rusa*. The genus *Hyelaphus* includes four species, Indian hog deer (*H. porcinus*), Indochinese hog deer (*H. annamiticus*), Calamian deer (*H. calamianensis*) and Bawean deer (*H. kuhlii*) (Meijaard and Groves 2004, Pitra *et al.* 2004).

The extant deer species have diverse karyotypes; their diploid chromosome numbers range from $2n=6$ in the female Indian muntjac (*Muntiacus muntjak vaginalis*) to $2n=80$ in the Siberian roe deer (*Capreolus capreolus pygargus*) (Wurster and Benirschke 1970, Neitzel 1987, Tanomtong *et al.* 2005, Huang *et al.* 2005, Tanomtong *et al.* 2010, Khongcharoensuk *et al.* 2017).

The Indian hog deer is a small deer whose habitat ranges from Pakistan, through northern India, to mainland Southeast Asia, which inhabits much of Pakistan, Northern India, Nepal, Bangladesh, and Southwestern Yunnan (China); introduced populations also exist in Australia, the United States and Sri Lanka. The Indian hog deer's coat is quite thick and generally a uniform dark-brown in winter except for the underparts of the body and legs which are lighter in colour. During late

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spring, the change to a summer coat of rich reddish brown commences, although this may vary between individuals. Many hog deer show a dark dorsal stripe extending from the head down the back of the neck and

along the spine. In summer, there is usually a uniform row of light-coloured spots along either side of the dorsal stripe from the shoulders to the rump. The tail is fairly short and brown but tipped with white. The underside

Fig. 1. General characteristics of male (A) and female (B) Indian hog deer (*Hyelaphus porcinus*).

Table 1. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from ten metaphases of male and female Indian hog deer (*Hyelaphus porcinus*) in Thailand, $2n=68$.

Chromosome pair	Ls	Ll	LT	RL $\pm$ SD	CI $\pm$ SD	Size	Type
1*	0.000	9.907	9.907	0.044 $\pm$ 0.005	1.000 $\pm$ 0.000	Large	Telocentric
2*	0.000	8.785	8.785	0.039 $\pm$ 0.002	1.000 $\pm$ 0.000	Large	Telocentric
3	0.000	8.527	8.527	0.038 $\pm$ 0.003	1.000 $\pm$ 0.000	Large	Telocentric
4	0.000	8.450	8.450	0.038 $\pm$ 0.002	1.000 $\pm$ 0.000	Medium	Telocentric
5	0.000	7.894	7.894	0.035 $\pm$ 0.004	1.000 $\pm$ 0.000	Medium	Telocentric
6	0.000	7.782	7.782	0.035 $\pm$ 0.001	1.000 $\pm$ 0.000	Medium	Telocentric
7	0.000	7.763	7.763	0.035 $\pm$ 0.001	1.000 $\pm$ 0.000	Medium	Telocentric
8	0.000	7.520	7.520	0.033 $\pm$ 0.005	1.000 $\pm$ 0.000	Medium	Telocentric
9	0.000	7.360	7.360	0.033 $\pm$ 0.001	1.000 $\pm$ 0.000	Medium	Telocentric
10	0.000	7.217	7.217	0.032 $\pm$ 0.001	1.000 $\pm$ 0.000	Medium	Telocentric
11	0.000	6.969	6.969	0.031 $\pm$ 0.001	1.000 $\pm$ 0.000	Medium	Telocentric
12	0.000	6.824	6.824	0.030 $\pm$ 0.001	1.000 $\pm$ 0.000	Medium	Telocentric
13	0.000	6.611	6.611	0.029 $\pm$ 0.001	1.000 $\pm$ 0.000	Small	Telocentric
14	0.000	6.475	6.475	0.029 $\pm$ 0.001	1.000 $\pm$ 0.000	Small	Telocentric
15	0.000	6.295	6.295	0.028 $\pm$ 0.001	1.000 $\pm$ 0.000	Small	Telocentric
16	0.000	6.199	6.199	0.028 $\pm$ 0.001	1.000 $\pm$ 0.000	Small	Telocentric
17	0.000	5.982	5.982	0.027 $\pm$ 0.001	1.000 $\pm$ 0.000	Small	Telocentric
18	0.000	5.870	5.870	0.026 $\pm$ 0.001	1.000 $\pm$ 0.000	Small	Telocentric
19	0.000	5.836	5.836	0.026 $\pm$ 0.001	1.000 $\pm$ 0.000	Small	Telocentric
20	0.000	5.685	5.685	0.025 $\pm$ 0.001	1.000 $\pm$ 0.000	Small	Telocentric
21	0.000	5.561	5.561	0.025 $\pm$ 0.001	1.000 $\pm$ 0.000	Small	Telocentric
22	0.000	5.527	5.527	0.025 $\pm$ 0.001	1.000 $\pm$ 0.000	Small	Telocentric
23	0.000	5.397	5.397	0.024 $\pm$ 0.000	1.000 $\pm$ 0.000	Small	Telocentric
24	0.000	5.386	5.386	0.024 $\pm$ 0.001	1.000 $\pm$ 0.000	Small	Telocentric
25	0.000	5.276	5.276	0.024 $\pm$ 0.001	1.000 $\pm$ 0.000	Small	Telocentric
26	0.000	5.214	5.214	0.023 $\pm$ 0.001	1.000 $\pm$ 0.000	Small	Telocentric
27	0.000	5.059	5.059	0.023 $\pm$ 0.001	1.000 $\pm$ 0.000	Small	Telocentric
28	0.000	4.962	4.962	0.022 $\pm$ 0.001	1.000 $\pm$ 0.000	Small	Telocentric
29	0.000	4.896	4.896	0.022 $\pm$ 0.000	1.000 $\pm$ 0.000	Small	Telocentric
30	0.000	4.797	4.797	0.021 $\pm$ 0.001	1.000 $\pm$ 0.000	Small	Telocentric
31	0.000	4.734	4.734	0.021 $\pm$ 0.001	1.000 $\pm$ 0.000	Small	Telocentric
32	0.000	4.728	4.728	0.021 $\pm$ 0.001	1.000 $\pm$ 0.000	Small	Telocentric
33	0.000	4.399	4.399	0.020 $\pm$ 0.001	1.000 $\pm$ 0.000	Small	Telocentric
X	4.584	5.268	9.852	0.044 $\pm$ 0.003	0.535 $\pm$ 0.038	Large	Metacentric
Y	1.842	2.903	4.745	0.021 $\pm$ 0.001	0.612 $\pm$ 0.028	Small	Submetacentric

Remark: *=Satellite chromosome (nucleolar organizer regions/NORs).

of the tail is white and the deer can fan the white hairs out in a distinctive alarm display (Fig. 1). This species is listed on CITES appendix I and IUCN is Endangered

(Grzimek 2004, Groves 2006).

There are few cytogenetics reports of the *H. porcinus* as follows, Wurster and Benirschke (1967), Hsu and Be-

Fig. 2. Metaphase chromosome plates and karyotypes of male (A) and female (B) Indian hog deer (*Hyelaphus porcinus*), $2n=68$ by conventional staining technique.

Fig. 3. Metaphase chromosome plates and karyotypes of male (A) and female (B) Indian hog deer (*Hyelaphus porcinus*), $2n=68$ by Ag-NOR banding technique. Arrows indicate nucleolar organizer regions/NORs.

nirschke (1977) and Chavananikul (1995) which report karyotypes of conventional staining of this animal. In our study, we confirm and compare our finding with previous reports. Moreover, this is the first report about standardized karyotype and idiogram measurements by GTG-, high-resolution and Ag-NOR banding techniques. This finding will represent basic knowledge and can be applicable to genetic diversity, taxonomy, conservation and evolution.

Materials and methods

Blood samples of the *H. porcinus* (two males and two females) were collected from Khon Kaen Zoo, Thailand and then applied to cytogenetic studies by lymphocyte culture of whole blood samples. The culture cells were treated with a colchicine-hypotonic-fixation-air-drying technique followed by conventional staining, GTG-, high-resolution and Ag-NOR banding techniques (Rooney 2001, Campiranon 2003, Sangpakdee *et al.* 2017). For 20 cells of each individual chromosome, checks, length measurements, karyotype and idiogram analysis were accomplished by using a light microscope as previously described (Choo-seangjaew *et al.* 2017).

Results and discussion

The cytogenetic study of *H. porcinus* using lymphocyte culture demonstrated that the chromosome number

is $2n$ (diploid)=68 and the fundamental number (NF, number of chromosome arms) for both sexes is 70 (Table 1). These results agree with Wurster and Benirschke (1967), Hsu and Benirschke (1977) and Chavananikul (1995). The diploid number of Cervini species ranges from 56 to 68 with a constant fundamental number of 70. The decrease in chromosome number is due to an increase in the number of bi-armed chromosomes. Robertsonian fusions or translocations are described as the main rearrangement involved in this variation (Bonnet-Garnier *et al.* 2003, O'Brien *et al.* 2006).

The autosomes of *H. porcinus* consisted of 6 large telocentric, 18 medium telocentric and 42 small telocentric chromosomes. The X chromosome was large metacentric chromosome and Y chromosome was small submetacentric chromosome. Conventional, GTG-, high-resolution and Ag-NOR banding karyotypes of *H. porcinus* demonstrate on Figs. 2–5, respectively. Moreover, the idiogram of those are illustrated on Figs. 6–9, respectively. These results disagree with Wurster and Benirschke (1967) and Hsu and Benirschke (1977) which reported that the autosomes of *H. porcinus* were two metacentric and 64 acrocentric (mono-armed), X was acrocentric and Y was acrocentric or submetacentric chromosomes. The sex chromosomes of *H. porcinus* in this study are like the sex chromosomes of some deer, *Alces alces* and *Ca. capreolus* (Gustavsson and Sundt 1968). The banding pattern of *H. porcinus* X chromosome is similar to human X chromosome. The

Fig. 4. Metaphase chromosome plates and karyotypes of male (A) and female (B) Indian hog deer (*Hyelaphus porcinus*), $2n=68$ by GTG-banding technique.

Fig. 5. Prometaphase chromosome plates and karyotypes of male (A) and female (B) Indian hog deer (*Hyelaphus porcinus*), $2n=68$ by high-resolution GTG-banding technique.

Fig. 6. Standardized idiogram of Indian hog deer (*Hyelaphus porcinus*), $2n=68$ by conventional staining technique.

morphology of chromosome is different because of the classification of chromosome type in each system; previous studies used Levan *et al.* (1964) but this study used Turpin and Lejeune (1965) and Chaiyasut (1989). The diploid chromosome number in deer varies greatly, but the causes of this variation are obscure. The NF in the tribe Cervini appears to be 70; the NF in New World deer is 74. The actual $2n$ is a function of chromo-

Fig. 7. Standardized idiogram of Indian hog deer (*Hyelaphus porcinus*), $2n=68$ by Ag-NOR banding technique, arrow indicates nucleolar organizer regions/NORs on the subtelomeric long arms of chromosome pairs 1 and 2.

some fusions. New World deer differ from Old World deer by having metacentric or submetacentric X chromosomes (Geist 1998). The first reported standardized idiogram of GTG-banding technique on *H. porcinus* show a pattern of transverse light and heavy bands; the amount of banding

Fig. 8. Standardized idiogram of Indian hog deer (*Hyelaphus porcinus*), $2n=68$ by GTG-banding technique.

Fig. 9. Standardized idiogram of Indian hog deer (*Hyelaphus porcinus*) $2n=68$ by high-resolution GTG-banding technique.

pattern on a set of haploid number (n) including autosomes and sex-chromosomes is 240 and 300 bands on metaphase and prometaphase chromosomes, respectively. This result can be compared with Herzog (1987) and Bonnet *et al.* (2001) which reported the number of red deer (*Ce. elaphus*) and Vietnamese sika deer (*Ce. nippon psuedaxis*) banding pattern on metaphase chromosomes as 353 and 409 bands, respectively. This study showed a lower number of bands compared to previous studies because only clearly observable bands of the chromosomes were counted.

Ag-NOR banding showed clearly nucleolar organizer regions of *H. porcinus* located on the subtelomeric q-arm of chromosome pairs 1 and 2. There are no heteromorphic NOR detection. This is a first report of NORs of this species. Numerous repetitive of 28S and 18S ribosomal gene are found in eukaryotic NORs. Multiplication of ribosomal DNA is able to increase or decrease

size of NORs (Campiranon 2003). In conclusion, an asymmetrical karyotype is an important appearance of *H. porcinus*, in which three types of chromosomes consisting of metacentric, submetacentric and telocentric chromosomes were found. The karyotype formula for *H. porcinus* is as follows:

$$2n (68) = L_6^t + M_{18}^t + S_{42}^t + \text{Sex-chromosomes.}$$

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