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## **A comparative chromosome analysis of Thai wild boar (*Sus scrofa jubatus*) and relationship to domestic pig (*S. s. domestica*) by conventional staining, G-banding and high-resolution technique**

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### **Abstract**

**Tanomtong, A., Supanuam, P., Siripiyasing, P. and Bunjonrat, R.**

**A comparative chromosome analysis of Thai wild boar (*Sus scrofa jubatus*) and relationship to domestic pig (*S. s. domestica*) by conventional staining, G-banding and high-resolution technique**

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This research is the first comparative chromosome analysis report of Thai wild boar (*Sus scrofa jubatus*) and its relationship to domestic pig (*S. s. domestica*) by conventional staining, G-banding and high-resolution technique. Blood samples of the Thai wild boar were taken from two males and two females kept in Nakhon Ratchasima Zoo. After standard whole blood lymphocyte culture at 37 °C for 72 hr. in the presence of colchicine, the metaphase spreads were performed on microscopic slides and air-dried. Conventional staining, G-banding and high-resolution technique were applied to stain the chromosomes.

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The results showed that the number of diploid chromosomes of Thai wild boar was  $2n$  (diploid) = 38, and the fundamental numbers (NF) were 62 in the male and female. The type of autosomes were 12 metacentric, 14 submetacentric, 4 acrocentric and 6 telocentric chromosomes, with X and Y chromosomes being metacentric chromosomes. We found that chromosomes 1, 5, 7, 8, 10, 11, 12, 13, 14, 16, 17, 18, X and Y had the same G-banding and high-resolution technique patterns as those of domestic pig chromosomes. Chromosomes 2, 3, 4, 6, 9 and 15 are similar to those of domestic pig chromosomes. These results show the evolutionary relationship between the Thai wild boar and the domestic pig.

**Key words :** chromosome, conventional staining, G-banding, high-resolution technique, Thai wild boar (*Sus scrofa jubatus*), domestic pig (*Sus scrofa domestica*)

## บทคัดย่อ

องค์กร แทนออมทอง<sup>1</sup> ประวีรัล สุพรผลอ้วน<sup>2</sup> พรณรงค์ สิริปียะสิงห์<sup>3</sup> และ เรืองวิทย์ บรรจงรัตน์<sup>4</sup> การเปรียบเทียบความสัมพันธ์ของโครโนไซมของสุกรป่าไทย (*Sus scrofa jubatus*) และสุกรเลี้ยง (*S. s. domestica*) ด้วยเทคนิคการย้อมสีแบบธรรมด้า ย้อมแอบสีแบบบี และการย้อมแอบสีที่ให้รายละเอียดสูง

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เป็นรายงานครั้งแรกของการเปรียบเทียบความสัมพันธ์ของโครโนไซมของสุกรป่าไทยและสุกรเลี้ยง ด้วยเทคนิคการย้อมสีแบบธรรมด้า ย้อมแอบสีแบบบี และการย้อมแอบสีที่ให้รายละเอียดสูง ใช้ตัวอย่างเลือดสุกรป่าเพศผู้ 2 ตัว และเพศเมีย 2 ตัว จากส่วนสัตว์คราราชสีมา เตรียมโครโนไซมด้วยการเพาะเลี้ยงเซลล์เม็ดเลือดขาวที่อุณหภูมิ 37°C เป็นเวลา 72 ชม. เก็บเกี่ยวเซลล์ด้วยเทคนิคโคลอชิชิน-ไอโอล์ฟินิก-ฟิกเซชั่น-แอร์ดาร์ยอยิ่ง ทำการย้อมสีแบบธรรมด้า ย้อมแอบสีแบบบี และแอบสีที่ให้รายละเอียดสูง ผลการศึกษาพบว่าสุกรป่าไทยมีจำนวนโครโนไซม  $2n$ (diploid) เท่ากับ 38 แท่ง มีจำนวนโครโนไซมพื้นฐานเท่ากับ 62 แท่งในเพศผู้และเพศเมีย โครโนไซมร่างกาย ประกอบด้วยโครโนไซมชนิดเมทาเซนทริก 12 แท่ง ชั้นเมทาเซนทริก 14 แท่ง อะโครเซนทริก 4 แท่งและชนิดเทโลเซนทริก 6 แท่ง โครโนไซมเออกซ์และวายเป็นชนิดเมทาเซนทริก โครโนไซมของสุกรป่าไทยติดแอบสีบีและแอบสีที่ให้รายละเอียดสูง เมื่อเทียบกับโครโนไซมของสุกรเลี้ยง 13 คู่ คือ โครโนไซมคู่ที่ 1, 5, 7, 8, 10, 11, 12, 13, 14, 16, 17, 18, โครโนไซมเออกซ์และโครโนไซมวาย และแอบสีบีนั้นโครโนไซมสุกรป่าไทยมีความคล้ายคลึงกับแอบสีบีนั้น โครโนไซมสุกรเลี้ยงมี 6 คู่ คือ โครโนไซมคู่ที่ 2, 3, 4, 6, 9, และ 15 ผลการศึกษาแสดงให้เห็นว่าสุกรป่าไทยมีสายวัฒนาการร่วมกับสุกรเลี้ยง

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There are 5 genera and 16 species of animals in the family Suidae, but only 1 species is found in Thailand, which is *Sus scrofa* including wild boar, domestic pig and native pig (Wilson and Reeder, 1992). The Thai wild boar belongs to the kingdom Animalia, phylum Chordata, class Mammalia, order Artiodactyla, family Suidae, genus *Sus*, species *Sus*

*scrofa* and subspecies *Sus scrofa jubatus*. Its common name is wild boar (Parr et al., 2003).

The common characteristics of the Thai wild boar are : body weight about 75-200 kg, the body shape shows a large head and a smaller hind part with small legs and hooves, the face is small with tiny ears, big black eyes, and strong snout used for

digging with 2 fangs pointing up out of the mouth. A piglet has light brown longitudinal stripes and the bristly hair and the color will change to black or blown in the adult. The Thai wild boar is omnivorous and can be found all over parts of Thailand (Lekakul and McNeely, 1977, 1988) (Figure 1).

The wild boar, domestic pig and native pig are descended from the same ancestor and belong to the same species with the same chromosome number, ( $2n = 38$ ) However, the European wild boar has the chromosome number of  $2n = 36$ , which

resulted from the Robertsonian translocation of 2 pairs of acrocentric chromosomes to a pair of submetacentric chromosomes. Recently, breeding for commercial purposes has yielded many hybrids of pigs and natural crossbreeding among different subspecies leads to more polymorphism, resulting in those pigs have different chromosome numbers of  $2n = 36, 37$  or  $38$  (Sysa et al., 1984; Popescu et al., 1989; Gustavsson, 1990; Macchi et al., 1995; Miranda and Lui, 2003; Rejduch et al., 2003).

According to Mayr et al. (1984); Sysa et al.



**A. Male Thai wild boar**



**B. Female Thai wild boar**

**Figure 1. Thai wild boar, *Sus scrofa jubatus* (Artiodactyla, Suidae)**

(1984); Popescu *et al.* (1989); Gustavsson (1990); Bosma *et al.* (1991); Chen *et al.* (1991); Yerle *et al.* (1991); Mellink *et al.* (1992); Macchi *et al.* (1995); Ronne (1995); Miranda and Lui (2003); Rejduck *et al.* (2003); Bosma *et al.* (2004), a comparative cytogenetic study of the Thai wild boar and the domestic pig in Thailand has not been accomplished. Using G-banding and high resolution techniques increases the validity of the classification of Thai wild boars, which have high numbers of subspecies as the reported by Oliver (1995) was showed that there are 17 subspecies of *Sus scrofa*. Furthermore, this study provides information for the evolutionary study of chromosomes of pigs and their relatives because of the varieties of subspecies. Also, the information can be used to conserve pure lines of the species, since the number of hybrids is increasing due to commercial activities and natural breeding.

### Materials and Methods

Blood samples from the jugular vein were collected from two males and two females Thai wild boars, which were kept in Nakhon Ratchasima Zoo, using aseptic technique. The samples were kept in 10 ml vacuum tubes containing heparin to prevent blood clotting and they were cooled on ice until arriving at the laboratory.

#### 1. Cell preparation

The lymphocytes were cultured using the whole blood microculture technique adapted from Kampiranont (2003).

#### Cell culture

The RPMI 1640 medium was prepared with 2% PHA (phytohemagglutinin) as a mitogen and kept in blood culture bottles of 5 ml each. A blood sample of 0.5 ml was dropped into a medium bottle and well mixed. The culture bottle was loosely capped, incubated at 37 °C under 5% of carbon dioxide environment and regularly shaken in the morning and evening. When reaching harvest time at the 72<sup>nd</sup> hour of incubation, colchicine was introduced and well mixed followed by further incubation for

30 minutes.

#### Cell harvest

The blood sample mixture was centrifuged at 1,200 rpm (100 xg) for 10 minutes and the supernatant was discarded. Ten ml of hypotonic solution (0.075 M KCl) was applied to the pellet and the mixture was incubated for 30 minutes. KCl was discarded with the supernatant after centrifugation again at 1,200 rpm (100 xg) for 10 minutes. Cells were fixed by fresh cool fixative (methanol : glacial acetic acid=3:1) gradually added up to 8 ml before centrifuging again at 1,200 rpm (100 xg) for 10 minutes and the supernatant discarded. The fixation was repeated until the supernatant was clear and the pellet was 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. G-banding method

G-banding technique was adapted from Kampiranont (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 10% fetal calf serum (FCS) or phosphate buffer. FCS was washed out by 50% methanol and the slide was stained with 10% Giemsa's solution for 30 minutes.

#### 3. High-resolution staining method

After the lymphocytes were cultured for 72 hours, 0.05 ml of 10<sup>-5</sup> M methotrexate was applied into the cultured lymphocytes to induce synchronization. The mixture was incubated again for 17 hours before the methotrexate was discarded with the supernatant by centrifugation at 2,800 rpm. (450 xg) The pellet was mixed with 5 ml of the RPMI 1640 medium and centrifuged at 2,800 rpm (450 xg). The supernatant was discarded before the cultured cells were released by adding 0.2 ml thymidine and incubating for 5 hours and 15 minutes. The cells were harvested at the exact time and stained by using the G-banding procedure.

#### 4. Chromosomal checks, karyotyping and idiograming

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

### Results and Discussion

Cytogenetic study of the Thai wild boar using lymphocyte culture revealed that the chromosome number is  $2n$  (diploid) = 38. This is the same chromosome number for the wild boar, and the domestic pig as reported in previous studies. However this number differs from the chromosome number of the European wild boar, which is  $2n = 36$  (Sysa *et al.*, 1984; Popescu *et al.*, 1989; Gustavsson, 1990; Macchi *et al.*, 1995; Miranda and Lui, 2003; Rejduck *et al.*, 2003). The previous studies in Thailand by Chuanchai (1982), Pintong *et al.* (1994) and Kuntongeg (1994) reported that the domestic pig and the subspecies hybrid have the same chromosome number, which is  $2n = 38$ .

The autosomes of the Thai wild boar are 8 submetacentric, 12 metacentric, 4 acrocentric and 12 telocentric chromosomes (Figure 2 and 3). This result agrees with the previous studies by Kuntongeg (1994), Chuanchai (1982), Sysa *et al.* (1984), Popescu *et al.* (1989), Gustavsson (1990), Bosma *et al.* (1991), Chang *et al.* (1991), Macchi *et al.* (1995), Ronne (1995) and Miranda and Lui (2003) indicating that a member of the genus *Sus*, such as a wildboar, a domestic pig and a native pig (except for a European wild boar), has 20 submetacentric and metacentric autosomes and 16 acrocentric and telocentric autosomes.

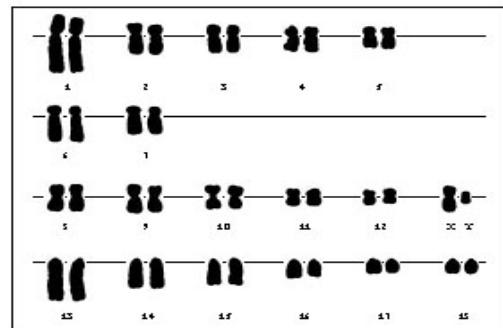
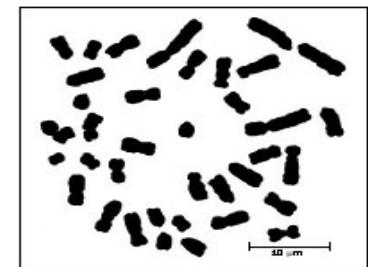


Figure 2. Metaphase chromosome and karyotype of the male Thai wild boar (*Sus scrofa jubatus*)  $2n$  (diploid) = 38 by conventional staining technique.

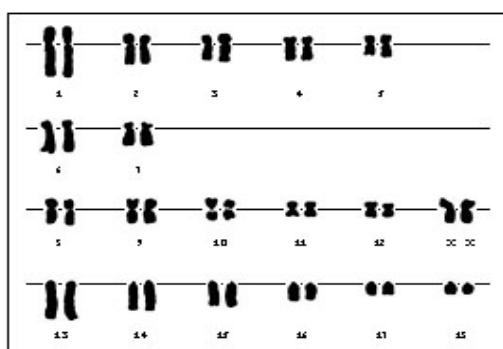


Figure 3. Metaphase chromosome and karyotype of the female Thai wild boar (*Sus scrofa jubatus*)  $2n$  (diploid) = 38 by conventional staining technique.

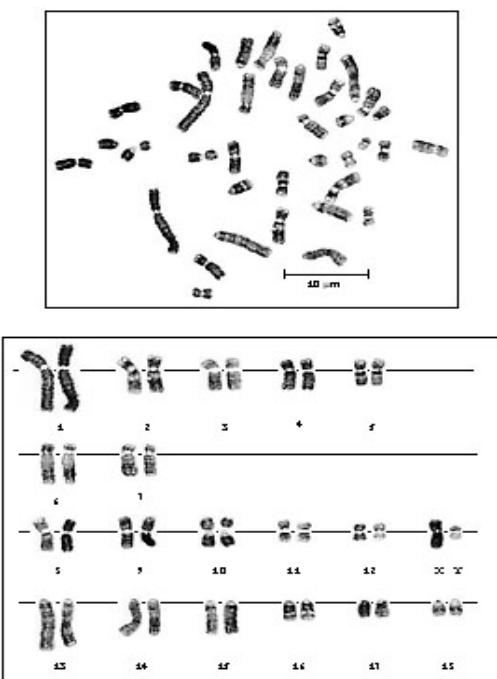
The fundamental number (NF) of the Thai wildboar is 64 in male and female. The sex chromosomes, both X and Y are metacentric type (Figure 2 and 3). This agrees with the previous studies on the domestic pigs by Sysa *et al.* (1984), Popescu *et al.* (1989), Gustavsson (1990), Bosma *et al.* (1991), Chen *et al.* (1991), Macchi *et al.* (1995), Ronne (1995) and Miranda and Lui (2003).

However, a recent study showed that the Thai wildboar hasn't satellite chromosome, which is a chromosome with secondary constriction or nucleolar organizer regions (NORs). In contrast, Miranda and Lui (2003) reported that the chromosome pairs 7 and 10 of the hybrid of European wild boar and native pig are satellite chromosomes. Furthermore, Mayr *et al.* (1984) and Popescu *et al.* (1989) also reported that the chromosome pairs 8 and 10 of the European wild boar and the subspecies hybrid are satellite chromosomes. This difference feature may be due to the methods of karyotyping. The

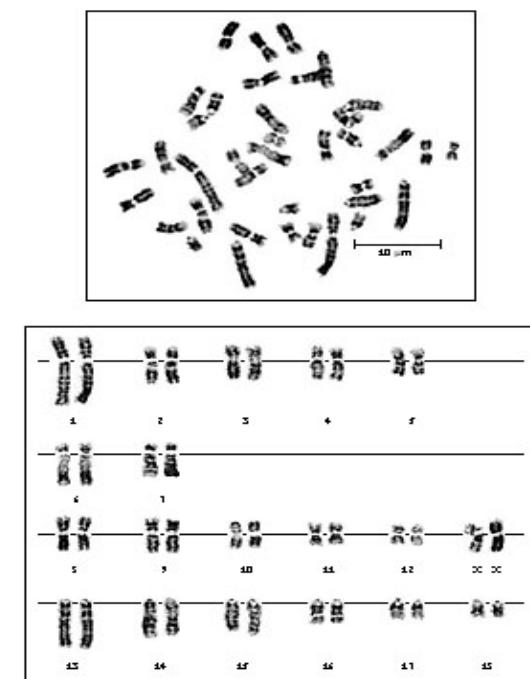
methods of staining used in the recent study are conventional, G-band and high-resolution staining, which may not be sufficient to identify the satellite chromosome. The NOR-banding such as silver staining is needed in order to confirm the results.

The important chromosome marker of the Thai wild boar is the asymmetrical karyotype, which is all four types of chromosomes are found (metacentric, submetacentric, telocentric and acrocentric). The largest and smallest chromosomes show high size difference (approximately 3.6 folds). The largest chromosome is submetacentric, while the second large chromosome is telocentric and the Ychromosome is the smallest metacentric chromosome (Figures 2 and 3). Comparing with the studies by Sysa *et al.* (1984) and Gustavsson (1999), this feature is the same in the domestic pig.

The G-banding revealed that the number of G-bands on 1 set of haploid chromosomes, which includes autosomes, X and Y chromosomes, is 239



**Figure 4.** metaphase chromosome and karyotype of the male Thai wild boar (*Sus scrofa jubatus*)  $2n$  (diploid) = 38 by G-banding technique.



**Figure 5.** metaphase chromosome and karyotype of the female Thai wild boar (*Sus scrofa jubatus*)  $2n$  (diploid) = 38 by G-banding technique.

bands for the Thai wild boar (Figures 4 and 5). The number of bands in 1 set of prometaphase haploid chromosomes from the high-resolution method is 275 bands (Figures 6 and 7). Comparing with the study in a native pig by Ronne (1995), there are 300 bands on 1 set of the metaphase haploid chromosomes. The numbers of bands for the prometaphase chromosomes of the native pig studied by Chen *et al.* (1991), Yerle *et al.* (1991) and Ronne (1995) using high-resolution technique are 444, 539 and 600 bands, respectively. This study showed the lower number of bands compared to previous studies because only clearly observable bands of the chromosomes were counted.

Comparison of the chromosome banding pattern between the Thai wild boar and the domestic pig (Gustavsson, 1990) revealed that 13 chromosome pairs show the same pattern (pairs 1, 5, 7, 8,

10, 11, 12, 13, 14, 16, 17, 18, X and Y) and 6 pairs share the similarity (pairs 2, 3, 4, 6, 9 and 15) (Figure 8). This indicates that there is an evolutionary relationship between the Thai wild boar and the domestic pig. For further studies, more information about genetic differences is needed which may be accomplished by using molecular biology or molecular genetics.

The data of the chromosomal checks on mitotic metaphase cells of the Thai wild boar are shown in Tables 1 and 2. Figure 9 shows the idiogram for the Thai wild boar from the G-band staining, while figure 10 shows the idiograms from the high-resolution banding, with landmarks, bands and sub-bands. The karyotype formula for the Thai wild boars are as follows:

$$2n = 38 = L^{\text{sm}}_2 + L^{\text{a}}_2 + L^{\text{t}}_2 + M^{\text{m}}_4 + M^{\text{sm}}_6 + M^{\text{a}}_2 + M^{\text{t}}_4 + S^{\text{m}}_6 + S^{\text{t}}_6 + \text{Sex-chromosome}$$

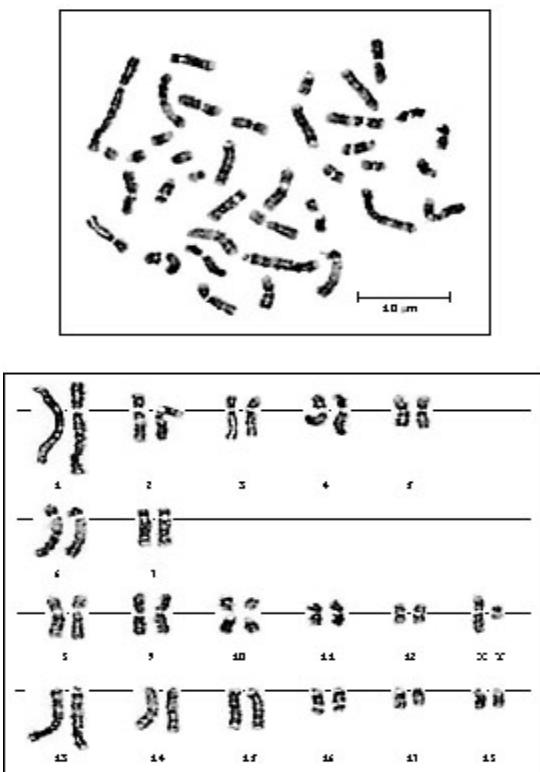


Figure 6. Prometaphase chromosome and karyotype of the male Thai wild boar (*Sus scrofa jubatus*)  $2n$  (diploid) = 38 by high-resolution technique.

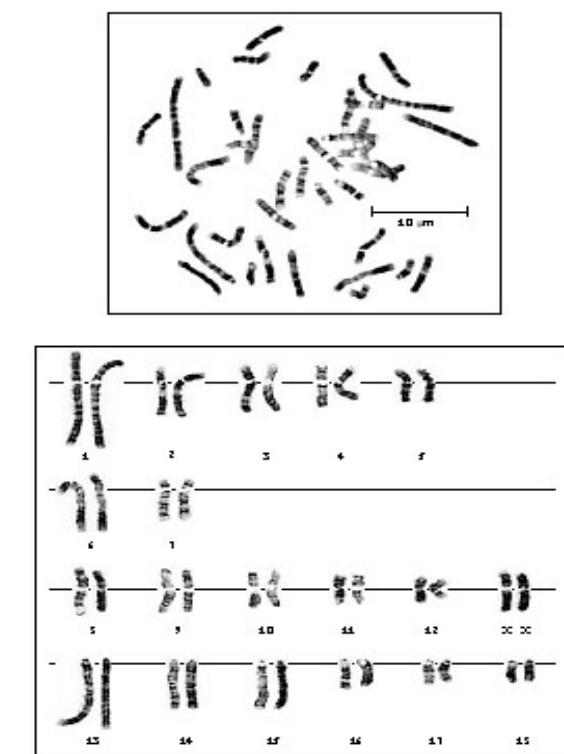
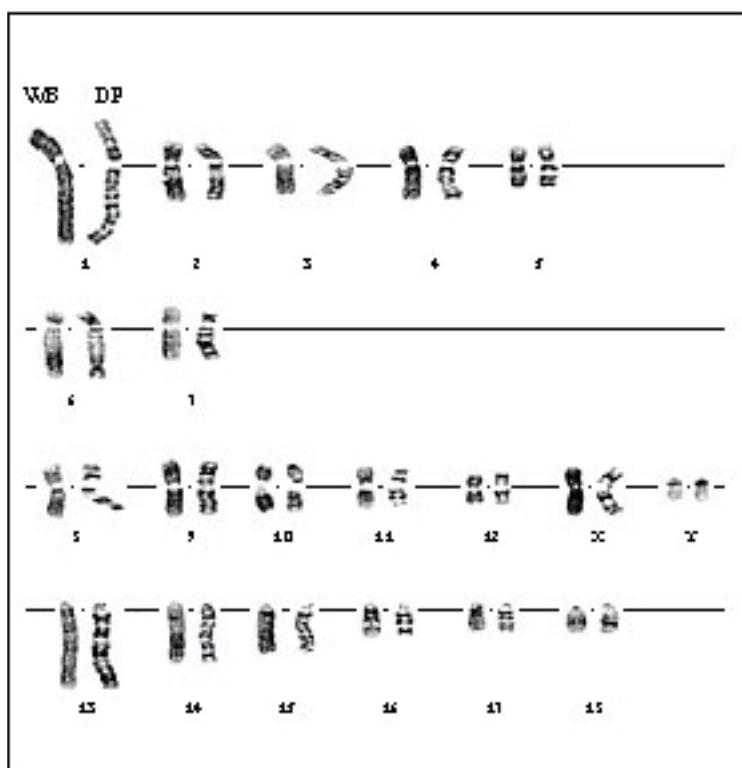


Figure 7. Prometaphase chromosome and karyotype of the female Thai wild boar (*Sus scrofa jubatus*)  $2n$  (diploid) = 38 by high-resolution technique



**Figure 8.** A comparison of the chromosome pair between Thai wild boar (WB), *Sus scrofa jubatus* and Domestic Pig (DP), *S.s.domestica* have the same G-banding patterns as those of Thai wild boar Chromosomes (Chromosome 1,5,7,8,10,11,12,13,14,16,17,18, X and Y), similar to those of Thai wild boar chromosomes (Chromosome 2,3,4,6,9 and 15).

### Conclusions

This cytogenetic study of the Thai wild boar revealed that the chromosome number of the Thai wild boar is  $2n = 38$  and the fundamental number is 62 in male and female. The types of autosomes are 12 metacentric, 14 submetacentric, 4 acrocentric and 6 telocentric chromosomes. The banding

patterns of 13 chromosome pairs, which are 1, 5, 7, 8, 10, 11, 12, 13, 14, 16, 17, 18, X and Y, of the Thai wild boar are the same as that of the domestic pig. Moreover, the other 6 pairs, which are 2, 3, 4, 6, 9 and 15, share similarities. The results show the evolutionary relationship between the Thai wild boar and domestic pig.

**Table 1. Mean of length short arm chromosome (Ls), length long arm chromosome (Ll), length total arm chromosome (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from metaphase chromosome 20 cells in male Thai wild boar (*Sus scrofa jubatus*) 2n (diploid) = 38.**

Chromosome Pairs	Ls	Ll	LT	RL+SD	CI+SD	Size of chromosome	Type of chromosome
1	0.657	1.266	1.922	0.099±0.006	0.658±0.024	L	sm
2	0.442	0.758	1.200	0.062±0.002	0.632±0.018	M	sm
3	0.405	0.662	1.067	0.055±0.003	0.619±0.015	M	sm
4	0.372	0.624	1.006	0.051±0.002	0.625±0.02	M	sm
5	0.349	0.465	0.814	0.042±0.002	0.570±0.024	S	m
6	0.329	0.909	1.238	0.064±0.004	0.734±0.017	L	a
7	0.283	0.718	1.001	0.052±0.003	0.717±0.019	M	a
8	0.451	0.574	1.025	0.053±0.001	0.559±0.029	M	m
9	0.468	0.540	1.007	0.052±0.003	0.537±0.019	M	m
10	0.416	0.484	0.900	0.046±0.004	0.538±0.023	S	m
11	0.316	0.368	0.683	0.035±0.002	0.539±0.017	S	m
12	0.294	0.321	0.615	0.032±0.002	0.522±0.008	S	m
13	0.000	1.475	1.475	0.076±0.004	1.000±0.000	L	t
14	0.000	1.143	1.143	0.059±0.002	1.000±0.000	M	t
15	0.000	1.009	1.009	0.052±0.007	1.000±0.000	M	t
16	0.000	0.712	0.712	0.037±0.001	1.000±0.000	S	t
17	0.000	0.565	0.565	0.029±0.001	1.000±0.000	S	t
18	0.000	0.531	0.531	0.027±0.002	1.000±0.000	S	t
X	0.395	0.561	0.956	0.050±0.004	0.585±0.032	S	m
Y	0.216	0.258	0.474	0.025±0.003	0.547±0.029	S	m

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

**Table 2. Mean of length short arm chromosome (Ls), length long arm chromosome (Ll), length total arm chromosome (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from metaphase chromosome 20 cells in female Thai wild boar (*Sus scrofa jubatus*) 2n (diploid) = 38.**

Chromosome Pairs	Ls	Ll	LT	RL+SD	CI+SD	Size of chromosome	Type of chromosome
1	0.707	1.383	2.090	0.103 $\pm$ 0.003	0.660 $\pm$ 0.016	L	sm
2	0.463	0.827	1.290	0.064 $\pm$ 0.003	0.641 $\pm$ 0.017	M	sm
3	0.436	0.701	1.139	0.056 $\pm$ 0.001	0.616 $\pm$ 0.013	M	sm
4	0.404	0.667	1.071	0.053 $\pm$ 0.003	0.620 $\pm$ 0.034	M	sm
5	0.379	0.510	0.888	0.044 $\pm$ 0.001	0.572 $\pm$ 0.028	S	m
6	0.357	0.984	1.340	0.066 $\pm$ 0.002	0.732 $\pm$ 0.023	L	a
7	0.289	0.756	1.045	0.051 $\pm$ 0.002	0.725 $\pm$ 0.028	M	a
8	0.491	0.647	1.138	0.056 $\pm$ 0.002	0.569 $\pm$ 0.023	M	m
9	0.503	0.572	1.074	0.053 $\pm$ 0.003	0.533 $\pm$ 0.020	M	m
10	0.399	0.476	0.890	0.043 $\pm$ 0.002	0.544 $\pm$ 0.026	S	m
11	0.333	0.379	0.712	0.035 $\pm$ 0.002	0.532 $\pm$ 0.017	S	m
12	0.308	0.364	0.671	0.033 $\pm$ 0.002	0.541 $\pm$ 0.030	S	m
13	0.000	1.594	1.594	0.079 $\pm$ 0.004	1.000 $\pm$ 0.000	L	t
14	0.000	1.175	1.175	0.058 $\pm$ 0.001	1.000 $\pm$ 0.000	M	t
15	0.000	1.124	1.124	0.056 $\pm$ 0.002	1.000 $\pm$ 0.000	M	t
16	0.000	0.764	0.764	0.038 $\pm$ 0.002	1.000 $\pm$ 0.000	S	t
17	0.000	0.639	0.639	0.032 $\pm$ 0.002	1.000 $\pm$ 0.000	S	t
18	0.000	0.562	0.562	0.028 $\pm$ 0.001	1.000 $\pm$ 0.000	S	t
X	0.440	0.588	1.028	0.051 $\pm$ 0.002	0.570 $\pm$ 0.023	S	m

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

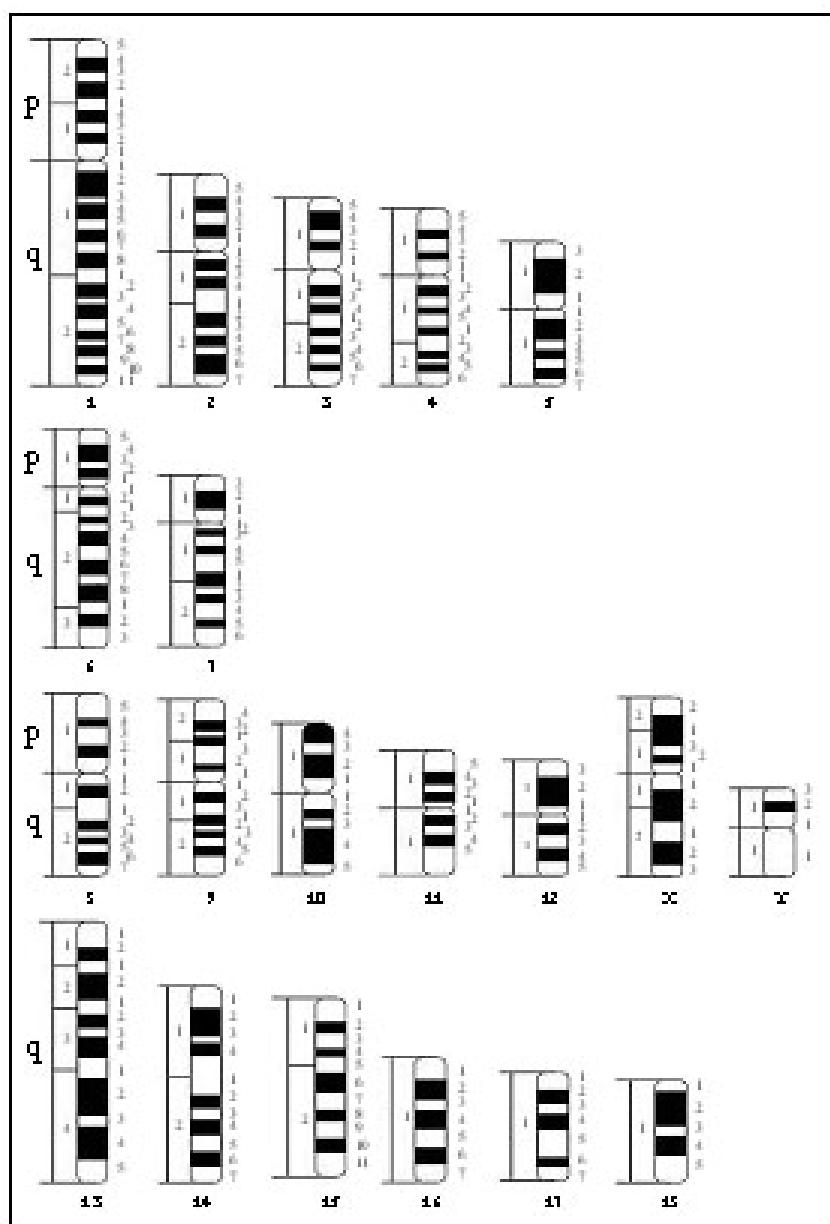


Figure 9. Idiogram of the Thai wild boar (*Sus scrofa jubatus*)  $2n$  (diploid) = 38 by G-banding technique

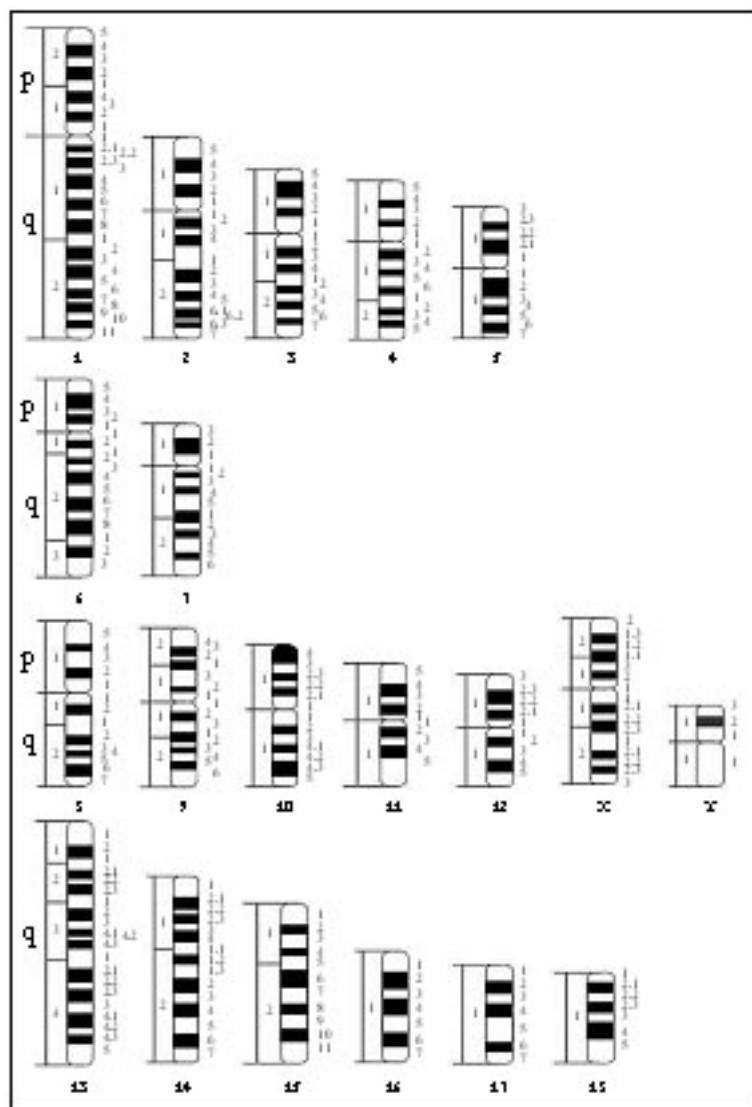


Figure 10. Idiogram of the Thai wild boar (*Sus scrofa jubatus*)  $2n$  (diploid) = 38 by high-resolution technique

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