Walailak J Sci & Tech 2005; 2(2): 231-240.

Cytogenetics of Stump-tailed Macaque, *Macaca arctoides* (Primate, Cercopithecidae) in Thailand by Conventional Staining

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ABSTRACT

The cytogenetics of the stump-tailed macaque (*Macaca arctoides*) in Thailand was studied. Blood samples were collected from this species maintained in Nakhon Ratchasima Zoo and Songkhla Zoo. After standard whole blood lymphocytes were cultured in the presence of colchicine, metaphase cells were spreaded on the slide and air-aired. Chromosomes were observed using Conventional Giemsa staining under a light microscope. The results show that the number of diploid chromosomes of the stump-tailed macaque is 2n=42, and the fundamental number (NF) are 84 chromosomes in the female and male. The types of autosomes are 6 large metacentric, 8 large submetacentric, 4 medium metacentric, 12 medium submetacentric, 8 small metacentric and 2 small submetacentric chromosomes. In addition, the pair of chromosome 13 is a clearly observable satellite chromosome. The X chromosome is medium submetacentric and the Y chromosome is the smallest submetacentric chromosome. The karyotype formula for the stump-tailed macaque is as follows :

$$2n\ (42) = L_6^{m}+L_8^{sm}+M_4^{m}+M_{12}^{sm}+S_8^{m}+S_2^{sm} + \text{sex chromosomes}$$

Key words: Cytogenetics - Conventional staining - Stump-tailed macaque (*Macaca arctoides*)

INTRODUCTION

There are 13 families, 60 genus and 232 species of animals in the order Primate, but only 3 families, 5 genus and 13 species are found in Thailand. There are 5 species belonging to the genus *Macaca*, which are stump-tailed macaque (*Macaca arctoides* Geoffroy, 1831), assam macaque (*Macaca assamensis* McClelland, 1839), long-tailed macaque (*Macaca fascicularis* Raffles, 1821), rhesus monkey (*Macaca mulatta* Zimmermann, 1780) and pig-tail macaque (*Macaca nemestrina* Linnaeus,

1766) (1,2,3). These species are listed as protected species according to the Wild Animal Reservation and Protection Act (1992). Furthermore, the International Union for the Conservation of Nature and National Resources (IUCN) classified them as vulnerable species. They are also in the Appendix II of the Convention on International Trade in Endangered Species (CITES) of Wild Fauna and Flora which means that trading of these species is under the control of the CITES (14).

A stump-tailed macaque belongs to Kingdom Animalia, Phylum Chrodata, Class Mammal, Order Primate, Family Cercopithecidae, Subfamily Cercopithecinae, genus *Macaca*, species *Macaca arctoides* (2,3). The distinct characteristics of the stump-tailed macaque are the same stout build as the pig-tailed macaque, though somewhat larger in size. The upper parts are variable from blackish to brownish to reddish, becoming darker and more grizzled with age; the hairs from the back sides of the head down the shoulders and the back are very long and shaggy, especially in older individuals. The face is sparsely haired and the forehead is bald behind the prominent brow ridges; the whiskers under the chin may form a rather well-developed beard in mature males. (1,2,3)

From a literature review about cytogenetic studies of genus *Macaca* and according to Chiarelli (5); Hsu and Benirschke (6); Napier and Napier (7); Small and Stanyon (8); Brown et al (9); Hirai et al (10) **(Table 1)**, the cytogenetics of the stump-tailed macaque in Thailand has not been studied. Thus, it is important to conduct this study, as it should be basic knowledge that can be applied to accommodate further research. In this study, lymphocyte culture and conventional staining of the mitotic chromosomes were carried out to compare with previous reports.

MATERIALS AND METHODS

Blood samples were collected from 2 male and 2 female stump-tailed macaque, which were kept in Nakhon Ratchasima Zoo and Songkhla Zoo, from the jugular vein using an aseptic technique. The samples were kept in vacuum tubes containing heparin to prevent blood clotting and were cooled on ice until arriving at the laboratory. The procedure was divided into 2 parts as follows.

Cell Preparation

The lymphocytes were cultured using a technique adapted from Kampiranont (11) for 72 hours in the RPMI 1640 medium with Phytohemagglutinin (PHA) as the mitogen. The cultured cells were examined by the colchicine-hypotonic-fixation-air-drying technique followed by conventional staining with Giemsa's stain.

Chromosomal Checks

Chromosomal checks were performed on mitotic metaphase cells under a light microscope. Twenty cells of each male and female with clearly observable and well-spread chromosomes were selected and photographed. The length of the long arm chromosome (Ll) and the length of the short arm chromosome (Ls) were measured to calculate the total arm chromosome length (LT), the relative length (RL) and the centromeric index (CI). The means of Ll, Ls, LT, RL and CI and the standard deviation (SD) of RL and CI were computed while the type and size of chromosomes were

distinguished by CI and LT respectively. For the determination of the length of the chromosome, chromosome 1 was assigned to be the largest. The medium chromosome was selected from chromosomes that were shorter than the average half-length of the largest and smallest chromosome (12).

Table 1. Karyotypic studies of animals species in the subfamily Cercopithecinae (Primate, Cercopithecidae).

Species	2n	NF	M	sm	X	Y	Reference
Stump-tailed macaque (*Macaca arctoides*)	42	84 in male and female	18	22	sm(-)	sm(-)	Brown et al (9)
	42		12	28	sm(-)	sm(-)	Chiarelli (5)
Long-tailed macaque (*Macaca fascicularis*)	42	84 in male and female	18	22	m(-)	t(-)	Brown et al (9)
	42	83 in male and 84 in female	18	22	m(M)	t(S)	Hirai et al (10)
Rhesus monkey (*Macaca mulatta*)	42	84 in male and female	18	22	m(-)	m(-)	Brown et al (9)
	42	84 in male and female	18	22	m(M)	m(S)	Napier and Napier (7)
	42	84 in male and female	18	22	m(M)	m(S)	Small and Stanyon (8)
Pig-tailed macaque (*Macaca nemestrina*)	42	84 in male and female	18	22	sm	t	Brown et al (9)

Remark : 2n = diploid number NF = fundamental number m = metacentric
sm = submetacentric t = telocentric X = X-chromosome
Y = Y-chromosome M = medium chromosome S = small chromosome

RESULTS AND DISCUSSION

Cytogenetic study of the stump-tailed macaque using lymphocyte culture and the conventional staining procedures revealed that the chromosome number is 2n (diploid) = 42, which consists of 40 (20 pairs) autosomes and 2 (1 pair) sex chromosomes. This is the same chromosome number as that for the rhesus monkey as reported by Chiarelli (5) and Brown et al (9). Comparison with primates in the subfamily Cercopithecinae, the chromosome numbers as that were all the same according to Brown et al (9), who reported that the chromosome numbers of primates in genus *Macaca* (pig-tailed macaque, rhesus monkey, long-tailed macaque, celebes crested monkey, bonnet monkey) and genus *Cercocebus* (mangabey monkey) are 2n=42. Furthermore, Hirai et al (10) also reported that the chromosome number of the long-tailed macaque is 2n=42.

This examination also revealed that the fundamental number (NF) of the stump-tailed macaque is 84 chromosomes in male and female. This is the same fundamental number as that for the stump-tailed macaque as reported by Chiarelli (5) and Brown et al (9). This number is different from that of a long-tailed macaque, which is 83 chromosomes in male and 84 in female (10). The chromosomes in mitotic

metaphase and the karyotype of the stump-tailed macaque are shown in **Figures 1 and 2**.

The stump-tailed macaque has 2 types of autosomes, which are metacentric (18 chromosomes) and submetacentric (22 chromosomes) types. The 18 metacentric autosomes were classified by size into 6 large, 4 medium and 8 small chromosomes while the 22 submetacentric autosomes were distinguished to be 8 large, 12 medium and 2 small chromosomes. Similar chromosomal features were reported by Brown et al (9), which indicated that the stump-tailed macaque has 18 metacentric and 22 submetacentric autosomes. Compared to other species in the genus *Macaca*, Brown et al (9) reported that a pig-tailed macaque, long-tailed macaque and rhesus monkey has 18 metacentric and 22 submetacentric autosomes. Similar numbers were also reported by Hirai et al (10) indicating that the autosomes of a long-tailed macaque are 18 metacentric and 22 submetacentric chromosomes. The idiogram of the stump-tailed macaque shows the gradually decreasing length of the autosomes **(Figure 3)**.

The X chromosome of the stump-tailed macaque is a medium submetacentric chromosome and the Y chromosome is the smallest submetacentric chromosome. These features are similar to that reported by Chiarelli (5) indicating that a stump-tailed macaque has a submetacentric X chromosome and a submetacentric Y chromosome. In comparison with other species in the genus *Macaca*, Brown et al (9) reported that the X chromosomes of the rhesus monkey, pig-tailed macaque and long-tailed macaque are metacentric, submetacentric and metacentric chromosomes respectively. The Y chromosomes of those species are metacentric, telocentric and telocentric chromosomes respectively. Hirai et al (10) also reported that a long-tailed macaque has a medium metacentric X chromosome and a small telocentric Y chromosome.

Furthermore, the research cited above elucidated that the variation of both type and size occurred in the sex chromosomes of monkeys in the genus *Macaca*. According to those reports and this investigation, the X chromosome is metacentric or submetacentric type while the Y chromosome is metacentric, submetacentric, or telocentric type. Brown et al (9) showed that there were variations in X and Y chromosomes among long-tail macaque, stump-tail macaque, rhesus monkey, pig-tail macaque, celebes crested monkey and bonnet monkey. Napier and Napier (7) indicated that the Y chromosomes of the members of the genus *Macaca* are tiny with varying shape. Occasionally, the centromere is not obvious and the type of the chromosome is difficult to classify. Hirai et al (10) showed that variation in size and shape of X and Y chromosomes was also found in the rhesus monkey, pig-tail macaque, Japanese monkey and Taiwanese monkey (*M. cyclopis*). Stanyon et al (13) found that the Y chromosome of the Japanese monkey (genus *Macaca*) and *Cercocebus aterrimus* showed different bands stained in C-banding. Moreover, the report of variation in human Y chromosome by Makino and Takagi (14) revealed that the length of the acrocentric Y chromosome varies among individuals.

In this investigation, the nucleolar organizer region (NORs), which represents the chromosome marker, locates only on the short arms of the pair metacentric autosomes 13. In contrast, Brown et al (9) indicated that the NORs of the rhesus monkey, long-tailed macaque, stump-tailed macaque, celebes crested monkey, bonnet monkey and mangabey monkey are present only on the short arms of the pair autosome 9. This difference may due to the different methods of karyotyping and measuring. The study by Jones et al (15) indicated that only 1 pair of NORs is found in the autosomes of gibbons and baboons. Stanyon (13) also found that a pair of NORs

located at the pair autosome 13 in the Japanese monkey (genus *Macaca*) and *Cercocebus aterrimus*, which are in the same family, but the size of the NORs is slightly different. The chromosomal checks of the mitotic metaphase cells of species in the genus *Macaca* in Thailand revealed that the chromosome marker is the pair autosome 1, which is the biggest metacentric chromosome. Furthermore, the chromosome marker can be used to classify monkeys in the genus *Macaca* into species, which agrees with the reports by Napier and Napier (7), Small and Stanyon (8), Brown et al (9) and Hirai et al (10).

After measuring the length in centimeters of the chromosomes in mitotic metaphase cells for 20 cells in males and females, the results and the calculations are shown in **Table 2**. The karyotype formula for the stump-tailed macaque is as follows:

$$2n\ (42) = L_6^{m}+L_8^{sm}+M_4^{m}+M_{12}^{sm}+S_8^{m}+S_2^{sm} + \text{sex chromosomes}$$

Table 2. Means of length short arm chromosome (Ls), length long arm chromosome (Ll), length total arm chromosome (LT), relative length (RL) with its standard deviation (SD) and centromeric index (CI) with its SD from chromosomes in mitotic metaphase for 20 cells of each male and female stump-tailed macaque, 2n (diploid) = 42.

Chromosome Pairs	Ls	Ll	LT	RL±SD	CI±SD	Chromosome Size	Chromosome Type
1	0.901	1.265	2.166	0.072± 0.006	0.584± 0.038	L	m
2	0.695	1.185	1.880	0.062± 0.004	0.630± 0.028	L	sm
3	0.669	1.095	1.764	0.059± 0.006	0.621± 0.024	L	sm
4	0.778	0.960	1.738	0.058± 0.004	0.552± 0.026	L	m
5	0.578	1.119	1.697	0.056± 0.005	0.659± 0.035	L	sm
6	0.585	1.043	1.629	0.054± 0.007	0.641± 0.033	L	sm
7	0.644	0.965	1.610	0.053± 0.004	0.600± 0.037	L	m
8	0.559	0.878	1.437	0.048± 0.005	0.611± 0.026	M	sm
9	0.530	0.890	1.420	0.047± 0.005	0.626± 0.034	M	sm
10	0.478	0.938	1.415	0.047± 0.007	0.663± 0.022	M	sm
11	0.480	0.908	1.387	0.046± 0.007	0.654± 0.024	M	sm
12	0.610	0.668	1.278	0.042± 0.007	0.522± 0.020	M	m
13	0.614	0.658	1.272	0.042± 0.003	0.517± 0.023	M	m
14	0.403	0.863	1.266	0.042± 0.005	0.681± 0.039	M	sm
15	0.382	0.853	1.235	0.041± 0.004	0.69±0.0171	M	sm
16	0.507	0.554	1.061	0.035± 0.004	0.522± 0.025	S	m
17	0.377	0.650	1.028	0.034± 0.006	0.633± 0.037	S	sm
18	0.429	0.462	0.891	0.030± 0.005	0.518± 0.016	S	m
19	0.415	0.441	0.856	0.028± 0.007	0.515± 0.023	S	m
20	0.365	0.489	0.853	0.028± 0.008	0.573± 0.019	S	m
X	0.558	0.857	1.414	0.047± 0.005	0.606± 0.031	M	sm
Y	0.331	0.502	0.833	0.028± 0.009	0.60± 0.0382	S	sm

Remark: L = large chromosome, M = medium chromosome, S = small chromosome, m = metacentric chromosome and sm = submetacentric chromosome

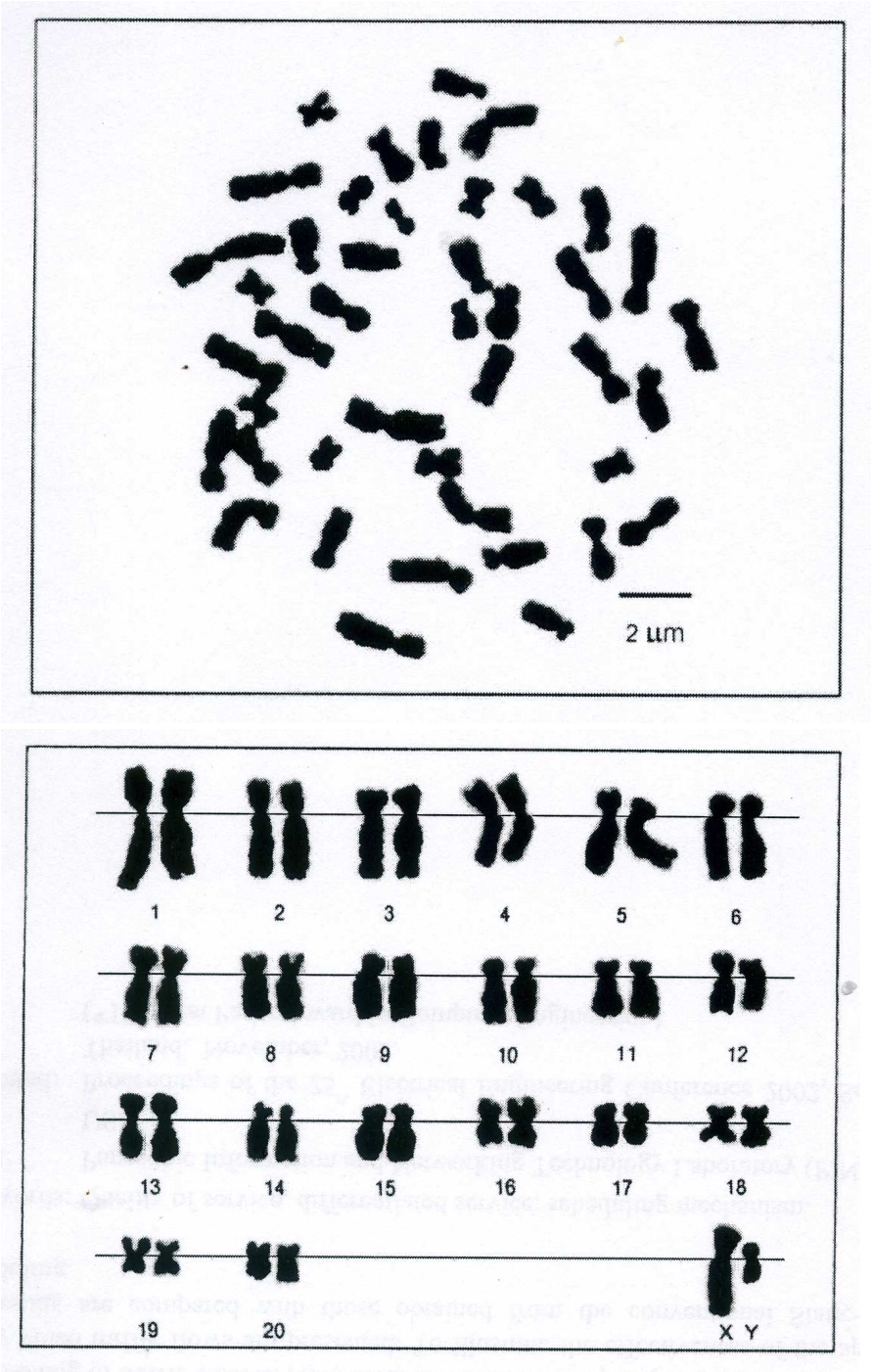

Figure 1. Conventional stained chromosomes in mitotic metaphase and karyotype of male stump-tailed macaque (*Macaca arctoides*), 2n (diploid) = 42.

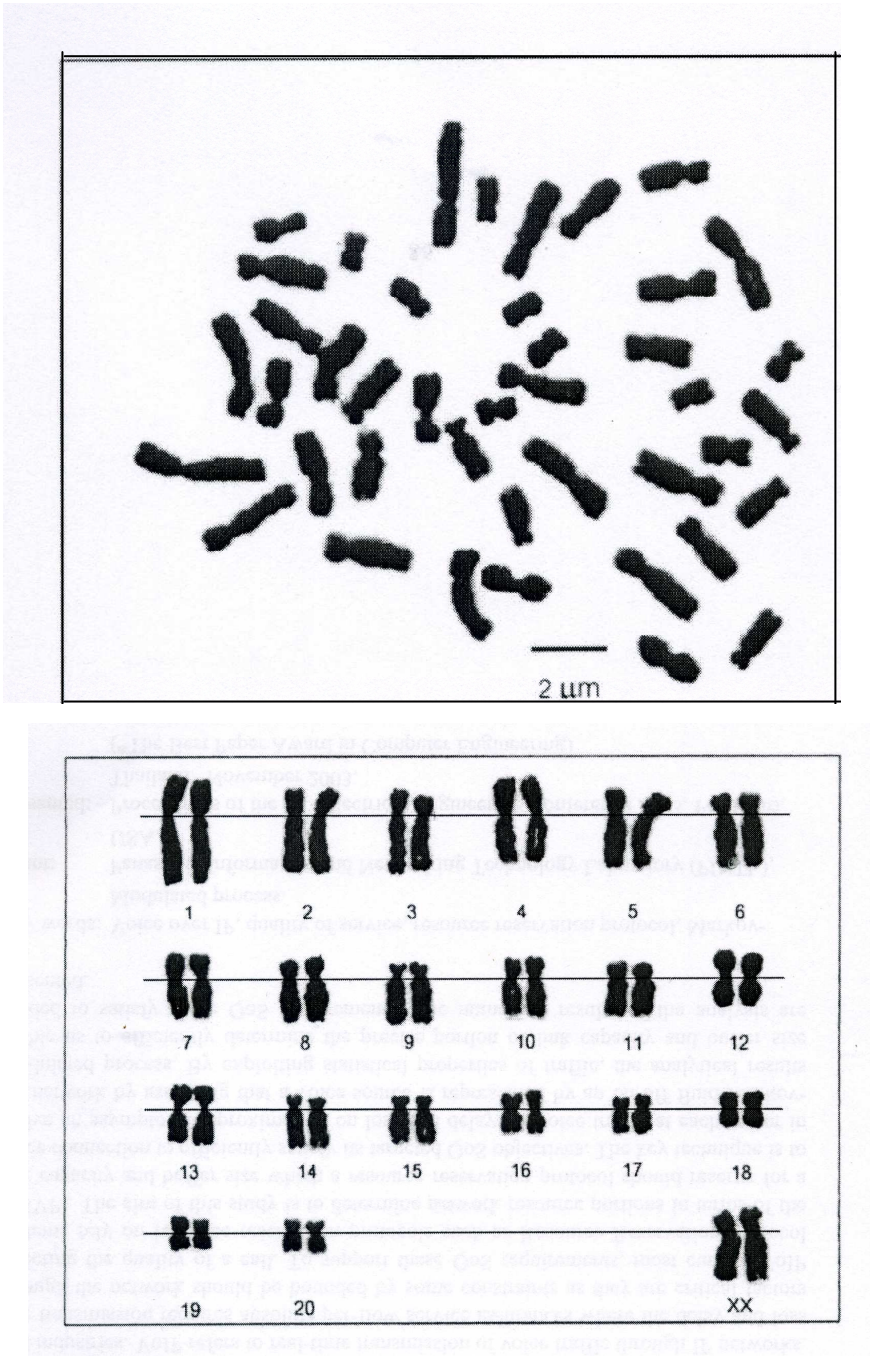

Figure 2. Conventional stained chromosomes in mitotic metaphase and karyotype of female stump-tailed macaque (*Macaca arctoides*), 2n (diploid) = 42.

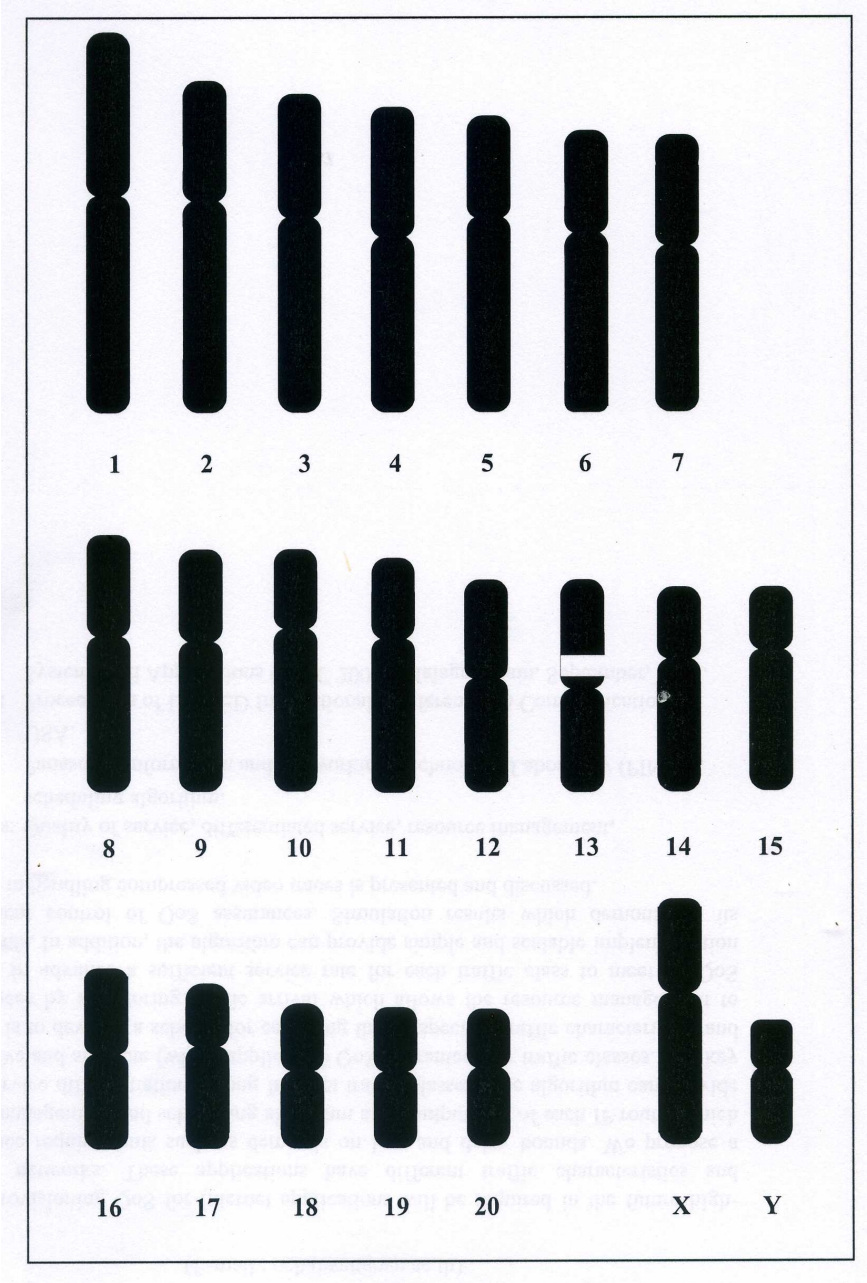

Figure 3. Idiogram of stump-tailed macaque (*Macaca arctoides*), 2n (diploid) = 42, from conventional stained chromosomes in the mitotic metaphase.

CONCLUSION

This cytogenetic study of stump-tailed macaque in Thailand revealed that the chromosome number of the stump-tailed macaque is 2n = 42 and the fundamental number is 84 chromosomes. These features apply to both male and female. The types of autosomes are 6 large metacentric, 8 large submetacentric, 4 medium metacentric, 12 medium submetacentric, 8 small metacentric and 2 small submetacentric chromosomes. The pair chromosome 13 is the satellite chromosome. The X chromosome is a medium submetacentric chromosome and the Y chromosome is the smallest submetacentric chromosome.

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บทคัดย่อ

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ศึกษาพันธุศาสตร์เซลล์ของลิงเสน (*Macaca arctoides*) ในประเทศไทยด้วยวิธีการย้อมแถบสีแบบธรรมดา

การศึกษาพันธุศาสตร์เซลล์ของลิงเสนในประเทศไทย ใช้ตัวอย่างสัตว์จากสวนสัตว์นครราชสีมา และสวนสัตว์สงขลา เตรียมโครโมโซมด้วยการเพาะเลี้ยงเซลล์เม็ดเลือดขาว เก็บเกี่ยวเซลล์ด้วยเทคนิคโคลชิซิน-ไฮโปโทนิค-ฟิกเซชั่น-แอร์ดาร์อิง ทำการย้อมสีแบบธรรมดา ผลการศึกษาพบว่าลิงเสนมีจำนวนโครโมโซม 2n (diploid) เท่ากับ 42 แท่ง มีจำนวนโครโมโซมพื้นฐาน (NF) เท่ากับ 84 ทั้งในเพศเมียและเพศผู้ โครโมโซมร่างกายประกอบด้วยโครโมโซมชนิดเมทาเซนทริกขนาดใหญ่ 6 แท่ง เมทาเซนทริกขนาดเล็ก 8 แท่ง และซับเมทาเซนทริกขนาดเล็ก 2 แท่ง โครโมโซมคู่ที่ 13 จัดเป็น satellite chromosome โครโมโซมเอ็กซ์เป็นชนิดซับเมทาเซนทริกขนาดกลาง และโครโมโซมวายเป็นชนิดซับเมทาเซนทริกขนาดเล็กมากที่สุด มีสูตรคาริโอไทป์ดังนี้

ลิงเสน 2n (42) = $L_6^{m}+L_8^{sm}+M_4^{m}+M_{12}^{sm}+S_8^{m}+S_2^{sm}+$ โครโมโซมเพศ

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