



การขาดหายของแขนข้างสั้นบนโครโมโซมคู่ที่ 15 ในลิงเสน (*Macaca arctoides*) ที่มีภาวะเผือก จากสาธารณรัฐประชาธิปไตยประชาชนลาว  
The Short Arm of Chromosome Pair 15 Deletion (15p-) of Albino Stump-tailed Macaque (*Macaca arctoides*) in Laos PDR

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### บทคัดย่อ

รายงานการศึกษาพันธุศาสตร์เซลล์ครั้งแรกในลิงเสนที่มีภาวะเผือก (เพศผู้ 1 ตัว และเพศเมีย 1 ตัว) จากสวนสัตว์แห่งชาติ สาธารณรัฐประชาธิปไตยประชาชนลาว เตรียมโครโมโซมด้วยเทคนิคการเพาะเลี้ยงเซลล์เม็ดเลือดขาว ย้อมสีโครโมโซมแบบธรรมดา ผลการศึกษาพบว่าลิงเสนเผือกมีจำนวนโครโมโซมดิพลอยด์ (2n) เท่ากับ 42 แห่ง มีจำนวนโครโมโซมพื้นฐาน (NF) เท่ากับ 82 ทั้งในเพศผู้และเพศเมีย ออโตโซมประกอบด้วยโครโมโซมชนิดเมทาเซนทริก 18 แห่ง ซับเมทาเซนทริก 20 แห่ง และเทโลเซนทริก 2 แห่ง โครโมโซมเอ็กซ์และวายเป็นชนิดซับเมทาเซนทริก โครโมโซมคู่ที่ 13 เป็นโครโมโซมเครื่องหมายโดยมีรอยคอดที่สอง (secondary constriction) บนแขนข้างสั้น ตรวจพบความผิดปกติของโครโมโซมคู่ที่ 15 มีแขนข้างสั้นที่ขาดหายไปทั้งเพศผู้และเพศเมีย (del,XX,XY,42,15p-) ทำให้เปลี่ยนโครโมโซมจากชนิดซับเมทาเซนทริกไปเป็นเทโลเซนทริกในโครโมโซมคู่นี้ คาดว่าเป็นผลทำให้เกิดการขาดหายของจีนที่ควบคุมการสร้างเม็ดสีเมลานิน ส่งผลทำให้เกิดภาวะเผือกในลิงเสน ลิงเสนเผือกมีสูตรคาริโอไทป์ คือ

$$2n \text{ (diploid) } 42 = L^m_6 + L^{sm}_8 + M^m_4 + M^{sm}_{10} + M^t_2 + S^m_8 + S^{sm}_2 + \text{โครโมโซมเพศ (XX/XY)}$$

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## ABSTRACT

The first cytogenetic study of one male and one female of albino stump-tailed macaque, *Macaca arctoides* (Primates, Cercopithecidae) in, Laos PDR was carried out. Blood samples were collected from this species maintained in Laos Zoo. The standard whole blood lymphocytes were cultured in the presence of colchicine, metaphase cells were spread on slides and air-dried. Conventional staining was applied to stain the chromosome. The results showed that the number of diploid chromosomes of albino stump-tailed macaque was  $2n=42$ , the fundamental number (NF) were 82 in both sexes. The types of autosomes were 18 metacentric, 20 submetacentric and 2 telocentric chromosomes. The X and Y chromosome was the submetacentric chromosome and founded that a chromosome pair 13 is a clearly observable satellite chromosome that has secondary constriction on short arm chromosomes. However, detected abnormality of chromosome pairs 15 is deletion in the short arms (del,XX,XY,42,15p-), cause the transition of chromosome type from submetacentric to telocentric. This might involved in the loss of melanin pigment that causes fractures in albino stump-tailed macaque. The karyotype formula for the albino *M. arctoides* is as follows:

$$2n \text{ (diploid) } 42 = L^m_6 + L^{sm}_8 + M^m_4 + M^{sm}_{10} + M^t_2 + S^m_8 + S^{sm}_2 + \text{sex chromosomes (XX/XY)}$$

**คำสำคัญ:** พันธุศาสตร์เซลล์ การขาดหาย โครโมโซม ลิงเสนเผือก (*Macaca arctoides*)

**Keywords:** Cytogenetics, Deletion, Chromosome, Albino stump-tailed macaque (*Macaca arctoides*)

## Introduction

There are 13 families, 60 genera and 232 species of animals in the order Primates (Wilson and Cole, 2000), of these 17 species belong to the genus *Macaca*. This genus was found in South Asia; Cambodia, China, India, Lao People's Democratic Republic, Malaysia, Myanmar, Thailand and Vietnam (Choudhury, 1988). 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 (Makino and Takagi, 1965).

Oculocutaneous albinism (OCA) is a syndrome that encompasses a group of individual inborn errors, each inherited as a Mendelian autosomal recessive trait and

characterized by the absence or near absence of melanin pigmentation in the skin, hair, and eyes (King and Summers, 1988). Tyrosinase (monophenol monooxygenase; monophenol, L-dopa: oxygen oxidoreductase; EC 1.14.18.1) is a copper containing enzyme that catalyzes the first two reactions in the melanin biosynthetic pathway: the hydroxylation of tyrosine to dihydroxyphenylalanine (dopa) and the subsequent oxidation of dopa to dopaquinone (Leaner and Fitzpatrick, 1950). It is a 58-kDa glycoprotein composed of 529 amino acid (Kwon et al., 1987; Shibahara et al., 1988). The human tyrosinase (TYR) gene consists of five exons spanning ~50 kb of DNA (Giebel and Strunk, 1991) in chromosome segment 11q14–q21 (Barton et al., 1988).

There are three previous reports based on conventionally Giemsa-stained chromosomes of *M. arctoides* (normal stump-tailed) by Brown et al. (1986) and Tanomtong et al. (2005; 2006) showing  $2n = 42$ ,  $NF = 84$  in the female and male. Chromosome with the karyotype composed of  $18m + 24sm$ . In addition, the four species in the genus *Macaca* namely, *M. assamensis*, *M. fascicularis*, *M. mulatta* and *M. nemestrina* were karyological studied. The same diploid chromosome number ( $2n = 42$ ) and karyotype formula consist of 18 metacentric and 22 submetacentric were founded. However, difference NF

(Fundamental number), type and shape of sex chromosomes were showed among these species (Napier and Napier, 1976; Small and Stanyon, 1985; Brown et al., 1986; Hirai et al., 1991 and Tanomtong et al., 2006) (Table 1).

This cytogenetic study using conventional staining technique provides the first report on chromosomal characteristic of the *M. arctoides* which being with the deleted region of 15p-.

### Research Methodology

Blood samples were collected from one male and one female albino stump-tailed macaque (figure 1) which were kept in Laos Zoo, Laos PDR, 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 two parts as follows.

### Cell Preparation

The lymphocytes were cultured using a technique adapted from Kampiranont (1997) for 72 hours in the RPMI 1640 medium with Phytohemagglutinin (PHA) as the mitogen. The cultured cells were examined by the colchicines-hypotonic-fixation-air drying technique followed by conventional staining with Giemsa's solution.



**Figure 1.** General characteristic of the albino stump-tailed macaque, *Macaca arctoides* (Primate, Cercopithecidae) from Laos PDR.

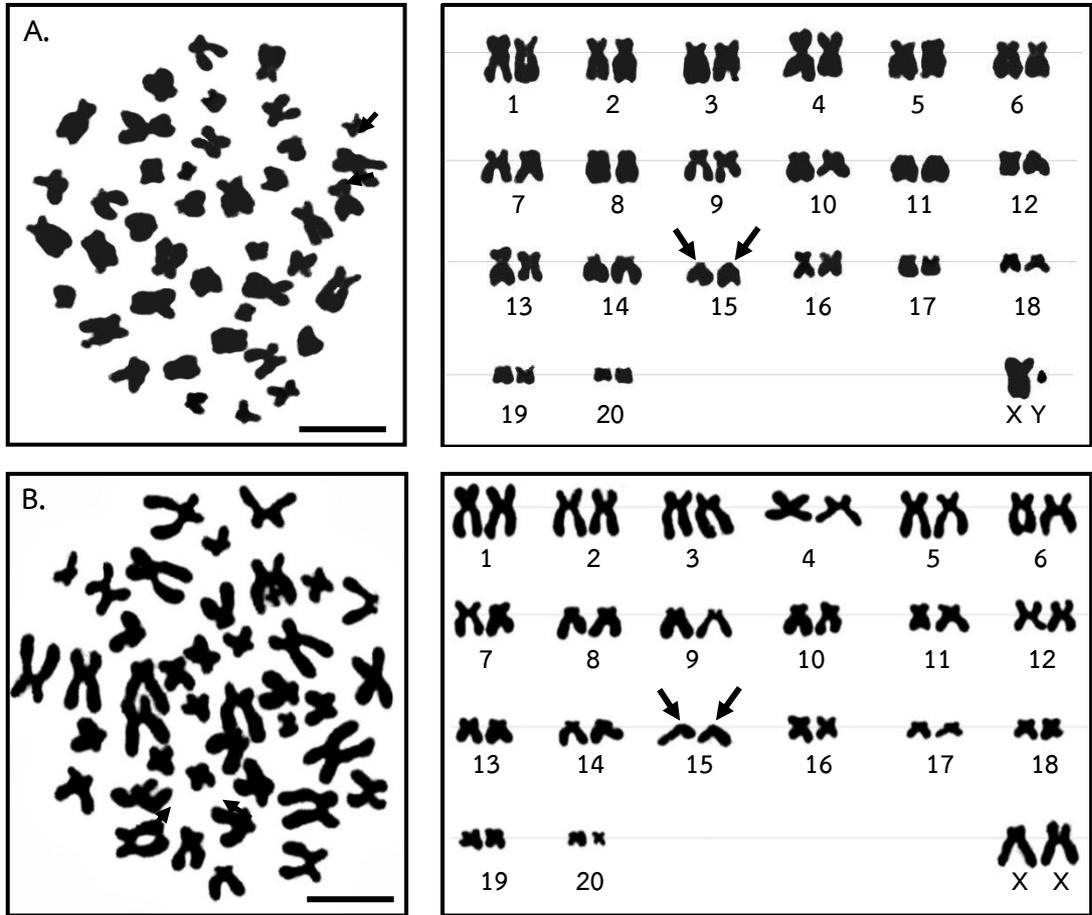
### **Chromosomal checks**

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,  $LT=Ls+Ll$ ). The relative length (RL) and the centromeric index (CI) and standard deviation (SD) of RL and CI were calculated (Chaiyasut 1989). The CI ( $q/p+q$ ) between 0.50–0.59, 0.60–0.69, 0.70–0.89 and 0.90–0.99 were described as metacentric, submetacentric, acrocentric and telocentric chromosomes, respectively. The fundamental number (number of chromosome arm, NF) was obtained by assigning a value of two to metacentric, submetacentric and acrocentric

chromosomes and one to telocentric chromosomes. All parameters were used in karyotyping and idiogramming.

### **Results**

Cytogenetic study of the albino stump-tailed macaque using lymphocyte culture and the conventional staining procedures revealed that the chromosome number is  $2n = 42$ . The autosomes composed of 18 metacentric, 20 submetacentric and two telocentric chromosomes. The X and Y chromosome were the submetacentric chromosomes and found that a chromosome pair 13 is a clearly observable satellite chromosome that has secondary constriction on short arm chromosomes (Figure 2, 3 and 4). However, detected abnormality of chromosome pairs 15 is deletion in the short arms ( $del,XX,XY,42,15p-$ ) (Figure 2, 3, 5 and Table 1).



**Figure 2.** Metaphase chromosome plates and karyotype of male (A.) and female (B.) albino stump-tailed macaque (*Macaca arctoides*),  $2n = 42$  by conventional straining technique. Arrows indicate deletion of short arm chromosome pair 15 (del,15p-), scale bars = 10 micrometers.

**Table 1.** Publications on karyological studies of the genus *Macaca* in Thailand.

Species	2n	NF	Karyotype	Sex-chromosome	Reference
<i>M. arctoides</i>	42	84(♂,♀)	18m+22sm	X(sm) Y(sm)	Brown et al. (1986)
	42	84(♂,♀)	18m+22sm	X(sm) Y(sm)	Tanomtong et al. (2005)
	42	84(♂,♀)	18m+22sm	X(sm) Y(sm)	Tanomtong et al. (2006)
	<b>42</b>	<b>82(♂,♀)</b>	<b>18m+20sm+2t</b>	<b>X(sm) Y(sm)</b>	<b>Present study</b>
<i>M. assamensis</i>	42	83(♂)/84(♀)	18m+22sm	X(sm) Y(t)	Tanomtong et al. (2006)
<i>M. fascicularis</i>	42	83(♂)/84(♀)	18m+22sm	X(m) Y(t)	Brown et al. (1986)
	42	83(♂)/84(♀)	18m+22sm	X(m) Y(t)	Hirai et al. (1991)
	42	83(♂)/84(♀)	18m+22sm	X(m) Y(t)	Tanomtong et al. (2006)

**Table 1.** Publications on karyological studies of the genus *Macaca* in Thailand. (continue)

Species	2n	NF	Karyotype	Sex-chromosome	Reference
<i>M. mulatta</i>	42	84(♂,♀)	18m+22sm	X(m) Y(m)	Napier and Napier (1976)
	42	84(♂,♀)	18m+22sm	X(m) Y(m)	Small and Stanyon (1985)
	42	84(♂,♀)	18m+22sm	X(m) Y(m)	Brown et al. (1986)
	42	84(♂,♀)	18m+22sm	X(m) Y(m)	Tanomtong et al. (2006)
<i>M. nemestrina</i>	42	83(♂)/84(♀)	18m+22sm	X(sm) Y(t)	Brown et al. (1986)
	42	83(♂)/84(♀)	18m+22sm	X(sm) Y(t)	Tanomtong et al. (2006)

**Remarks:** 2n = diploid chromosome, NF = fundamental number, m = metacentric, sm = submetacentric and t = telocentric chromosome.

After measuring the length 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 data of average chromosomal length, chromosome type and the position of

centromere were used for idiogram construction (fig. 3).

The karyotype formula for the albino stump-tailed macaque is as follows:

$$2n (42) = L^m_6 + L^{sm}_8 + M^m_4 + M^{sm}_{10} + M^t_2 + S^m_8 + S^{sm}_2 + \text{sex chromosomes (XX/XY)}$$

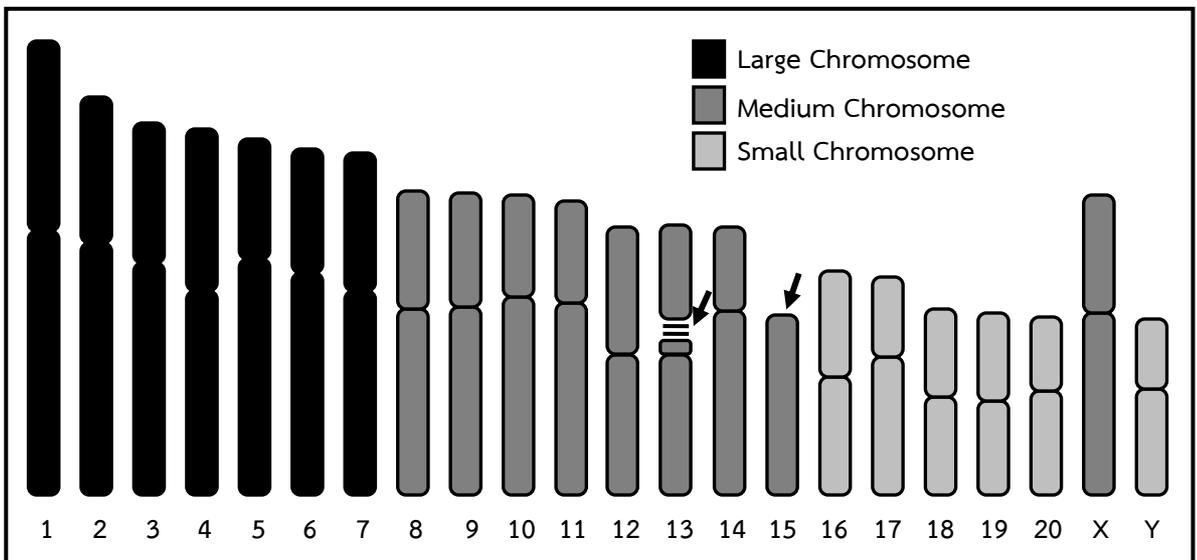
**Table 2.** Mean length of short arm chromosome (Ls), long arm chromosome (LL), total arm chromosome (LT), relative length (RL), centromeric index (CI), and standard deviation of RL, CI from 20 metaphases of male and female albino stump-tailed macaque (*Macaca arctoides*), 2n=42.

Chromosome pair	Ls	LL	LT	RL±SD	CI±SD	Chromosome size	Chromosome type
1	0.901	1.245	2.146	0.070± 0.006	0.580± 0.038	Large	metacentric
2	0.690	1.184	1.874	0.061± 0.004	0.632± 0.028	Large	submetacentric
3	0.658	1.094	1.752	0.058± 0.006	0.624± 0.024	Large	submetacentric
4	0.766	0.959	1.725	0.057± 0.004	0.556± 0.026	Large	metacentric
5	0.573	1.110	1.683	0.056± 0.005	0.660± 0.035	Large	submetacentric
6	0.585	1.043	1.628	0.055± 0.007	0.641± 0.033	Large	submetacentric
7	0.645	0.965	1.610	0.053± 0.004	0.599± 0.037	Large	metacentric
8	0.558	0.878	1.436	0.048± 0.005	0.611± 0.026	Medium	submetacentric
9	0.531	0.891	1.422	0.047± 0.005	0.627± 0.034	Medium	submetacentric
10	0.478	0.938	1.416	0.047± 0.007	0.662± 0.022	Medium	submetacentric
11	0.480	0.905	1.385	0.046± 0.007	0.653± 0.024	Medium	submetacentric
12	0.613	0.658	1.271	0.042± 0.007	0.518± 0.020	Medium	metacentric

**Table 2.** Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), centromeric index (CI), and standard deviation of RL, CI from 20 metaphases of male and female albino stump-tailed macaque (*Macaca arctoides*), 2n=42. (continue)

Chromosome pair	Ls	Ll	LT	RL±SD	CI±SD	Chromosome size	Chromosome type
13*	0.610	0.656	1.266	0.042± 0.003	0.518± 0.023	Medium	metacentric
14	0.401	0.864	1.265	0.041± 0.005	0.683± 0.039	Medium	submetacentric
15**	0.000	0.853	0.853	0.041± 0.004	1.000± 0.017	Medium	telocentric
16	0.504	0.554	1.058	0.035± 0.004	0.524± 0.025	Small	metacentric
17	0.377	0.652	1.029	0.034± 0.006	0.634± 0.037	Small	submetacentric
18	0.420	0.462	0.882	0.030± 0.005	0.524± 0.016	Small	metacentric
19	0.414	0.442	0.856	0.028± 0.007	0.516± 0.023	Small	metacentric
20	0.355	0.488	0.843	0.028± 0.008	0.579± 0.019	Small	metacentric
X	0.557	0.855	1.412	0.047± 0.005	0.606± 0.031	Medium	submetacentric
Y	0.330	0.500	0.830	0.028± 0.009	0.602± 0.038	Small	submetacentric

Remark: \* = NOR-bearing chromosomes (satellite chromosomes), \*\* = deletion of short arm chromosome pair 15 (15p-).



**Figure 3.** Standardized idiogram showing lengths and shapes of chromosomes of albino stump-tailed macaque (*Macaca arctoides*), 2n=42. Arrows indicate nucleolar organizer region/NOR pair 13 and deletion of short arm chromosome pair 15 (del,15p-).

**Discussion**

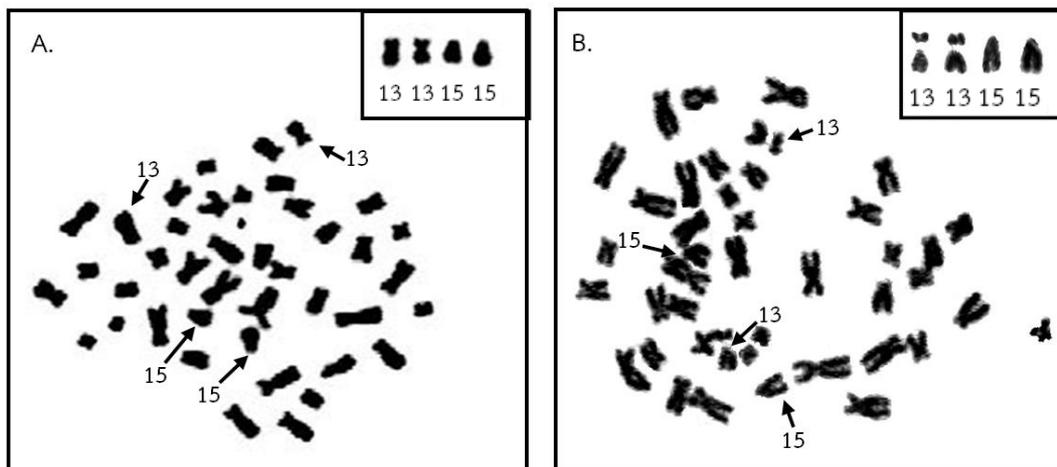
The chromosome of mitotic metaphase cells and the karyotypes of *M.*

*arctoides* by conventional staining techniques are shown in Figs. 2. The length of chromosomes of 20 cells (males and females)

in mitotic metaphase was measured. The mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), total length of arm chromosome (LT), relative length (RL), centromeric index (CI), standard deviation of RL, CI, size and type of chromosomes are shown in Table 2. The idiogram of *M. arctoides* shows in Figs 3. This same diploid chromosome number as that for the stump-tailed macaque reported by Tanomtong et al. (2005) and Brown et al. (1986), and other *Macaca* (Napier and Napier, 1976; Small and Stanyon, 1985; Brown et al., 1986; Hirai et al., 1991). Although all species of the genus *Macaca* studied presented the  $2n=42$ . This research also revealed that the

fundamental number of the albino stump-tailed macaque is 82 in male and female. This is differ fundamental number as that for the stump-tailed macaque as reported by Brown et al. (1986) and Tanomtong et al. (2005). As a result of the loss of the short arm of chromosome pair 15, the number decreased by two arms in the albino stump-tailed macaque (Figure 4 and 5).

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, which is in accordance with Tanomtong et al. (2005).



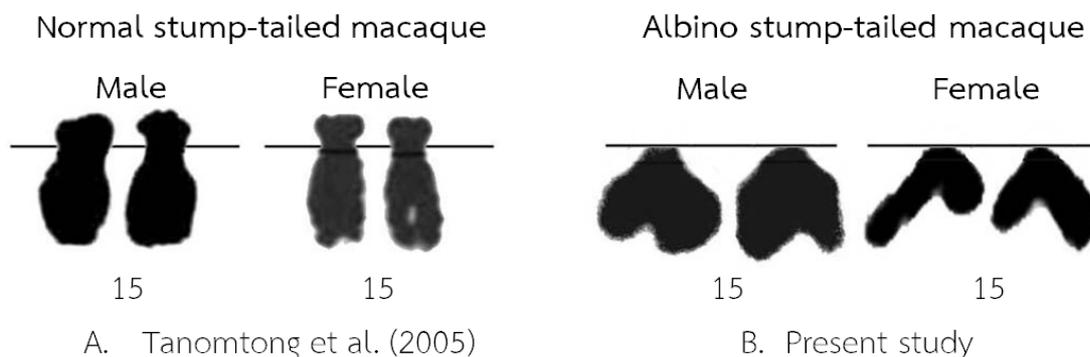
**Figure 4.** Metaphase chromosome plates of male (A.) and female (B.) albino stump-tailed macaque (*Macaca arctoides*)  $2n=42$  by conventional straining technique. Arrows indicate deletion of short arms chromosome pair 15 and satellite chromosome pair 13 (scale bars = 10 micrometers).

However, the present studies found that chromosome pairs 15 is deletion in the short arms (15p-), cause the transition of

chromosome type in from submetacentric to telocentric (Figure 4 and 5). The karyotype comparisons of the albino monkeys in both

male and female show that they have abnormalities of chromosome pair 15. This alteration is similar to the cause of the occurrence of albino in stump-tailed macaque. It would be much advantage to get

more explanation of the association among the chromosome pair 15 aberration and the occurrence of albino in stump-tailed macaque.



**Figure 5.** Comparison of normal chromosome (A.) and deletion of short arm chromosome of pair 15, del,15p- (B.).

## Conclusions

This cytogenetic study of albino stump-tailed macaque (*M. arctoides*) in Laos PDR revealed that the chromosome number  $2n$  (diploid) = 42 and the fundamental number is 82. These features apply to both male and female. The types of autosomes are six large metacentric, eight large submetacentric, four medium metacentric, ten medium submetacentric, two medium telocentric, eight small metacentric and two 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. We found that a chromosome pair 13 is a clearly observable satellite

chromosome that has secondary constriction on short arm chromosomes. This research discovery a new chromosome pairs 15 is deletion in the short arms (del,XX,XY,42,15p-), make the transition chromosome type in this pair from submetacentric to telocentric, is expected to the loss of melanin pigment that go along with albino in stump-tailed macaque.

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