คาริโอไทป์ของชะนีมือขาว (*Hylobates lar*) ด้วยวิธีการย้อมสีแบบธรรมดา

Karyological of the White-handed Gibbon, *Hylobates lar* (Primates, Hylobatidae) by Conventional Staining Technique

Alongkoad Tanomtong[1], *Praween Supanuam*[2],
Sumpars Khunsook[3] *and Roungvit Bunjonrat*[4]

บทคัดย่อ

การศึกษาคาริโอไปท์ของชะนีมือขาวจากสวนสัตว์นครราชสีมาทำขึ้นโดยการ เตรียมโครโมโซมจากการเพาะเลี้ยงเซลล์เม็ดเลือดขาวจากชะนีมือขาวเพศผู้ 2 ตัว และเพศเมีย 2 ตัว หลังจากนั้นเก็บเกี่ยวเซลล์ด้วยเทคนิคโคลชิซิน-ไฮโปโทนิค-ฟิกเซชั่น-แอร์ดรายอิง และย้อมสีโครโมโซมแบบธรรมดาด้วยสีจิมซ่า ผลการศึกษาพบว่าชะนีมือขาวมีจำนวนโครโมโซมดิพลอยด์เท่ากับ 44 แท่ง มีจำนวนโครโมโซมพื้นฐานเท่ากับ 88 ในเพศเมียและเพศผู้ โครโมโซมร่างกายประกอบด้วยโครโมโซมชนิดเมทาเซน ทริกขนาดใหญ่ 12 แท่ง เมทาเซนทริกขนาดกลาง 8 แท่ง ซับเมทาเซนทริกขนาดกลาง 4 แท่ง อะโครเซนทริกขนาดกลาง 2 แท่ง เมทาเซนทริกขนาดเล็ก 8 แท่ง และซับเมทาเซนทริกขนาดเล็ก 8 แท่ง แขนข้างยาวของโครโมโซมคู่ที่ 13 จัดเป็นแซทเทลไลท์โครโมโซม โครโมโซมเอ็กซ์เป็นชนิดซับเมทาเซนทริกขนาดกลาง และโครโมโซมวายเป็นชนิดอะโครเซนทริกขนาดเล็กมากที่สุด

Abstract

Karyotype of the white-handed gibbon (*Hylobates lar*) kept in Nakhonratchasima Zoo, was studied. Blood samples were taken from two female and two male gibbons. After lymphocyte culture, the mitotic chromosome preparation was done by air-drying method and conventional Giemsa's staining. The results showed that diploid chromosome number was 2*n*=44 and the fundamental number (NF) were 88 chromosomes in both female and male. The autosomes consisted of 12 large metacentric, 8 medium metacentric, 4 medium submetacentric, 2 medium acrocentric, 8 small metacentric and 8 small submetacentric chromosomes. In addition, a pair of the long arm of chromosome 13 showed clearly observable satellite chromosomes. The X-chromosome was the medium submetacentric and the Y-chromosome was the smallest acrocentric chromosome.

คำสำคัญ: คาริโอไทป์, การย้อมสีแบบธรรมดา, ชะนีมือขาว

Keywords: karyotype, conventional staining, white-handed gibbon (*Hylobates lar*)

[1]*Asst. Prof., Department of Biology, Faculty of Science, Khon Kaen University*

[2]*M.Sc. Student, Department of Biology, Faculty of Science, Khon Kaen University*

[3]*Lecturer, Department of Biology, Faculty of Science, Khon Kaen University*

[4]*Asst. Prof., Department of Botany, Faculty of Science, Chulalongkorn University*

Introduction

A white-handed gibbon belongs to kingdom Animalia, phylum Chordata, class Mammalia, order Primates, family Hylobatidae, genus *Hylobates* and species *Hylobates lar*. The common characteristics of the white-handed gibbons are dimorphic, with some individuals chocolate to nearly black and other individuals whitish to very light brown, the coloration is not related to age or sex and the same color is maintained throughout the life of the animal. In both color phase, the backs of the hands and feet are white and the necked black face is circled with a border of white hairs. (Lekagul and McNeely, 1977, 1988; Brokelman, 1981). (Figure 1)

Cytogenetic studies of white-handed gibbon was studied by Bender and Chu (1963); Hamerton et al. (1963); Chiarelli (1972); Warburton et al. (1975); Stayon et al. (1987, 1995); Jauch et al. (1992); Tuinen et al. (1999); Mueller et al. (2002, 2003). We found that the cytogenetics of the white-handed gibbon in Thailand has not been yet studied. In this study, we confirm and compare the result with previous report. In addition , this is the first report about chromosome measuring for determine size and formula karyotyping that have not been studied before. Thus, it is important to conduct this study, as it should be basic knowledge and can be applied to accommodate further research.

Materials and Methods

Blood samples from the jugular vein were collected from two males and two females white-handed gibbon, which were kept in Nakhonratchasima 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 the laboratory at department of Biology, faculty of Science,Khonkaen University.

1. Cell preparation

The lymphocytes were cultured using the whole blood microculture technique adapted from Rooney (2001) and 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^{o}C under 5% of carbondioxide environment and regularly shaken in the morning and evening. When reaching harvest time at the 72nd 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 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. The supernatant containing KCL was discarded after centrifugation again at 1,200 rpm for 10 minutes. Cells were fixed by fresh cold fixative (methanol : glacial acetic acid = 3 : 1) gradually added up to 8 ml before centrifuging again at 1,200 rpm for 10 minutes, and the supernatant was 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-drying technique. The slide was conventionally stained with 20% stock Giemsa's solution for 30 minutes.

A. The male white-handed gibbon B. The female white-handed gibbon

Figure 1. The male (A) and female (B) white-handed gibbon, *Hylobates lar* Linnaeus, 1771 (Primates, Hylobatidae) in Thailand.

Photograph by: *Alongkoad Tanomtong and Praween Supanuam from Nakhonratchasima Zoo*

2. Chromosomal checking, karyotyping and idiograming

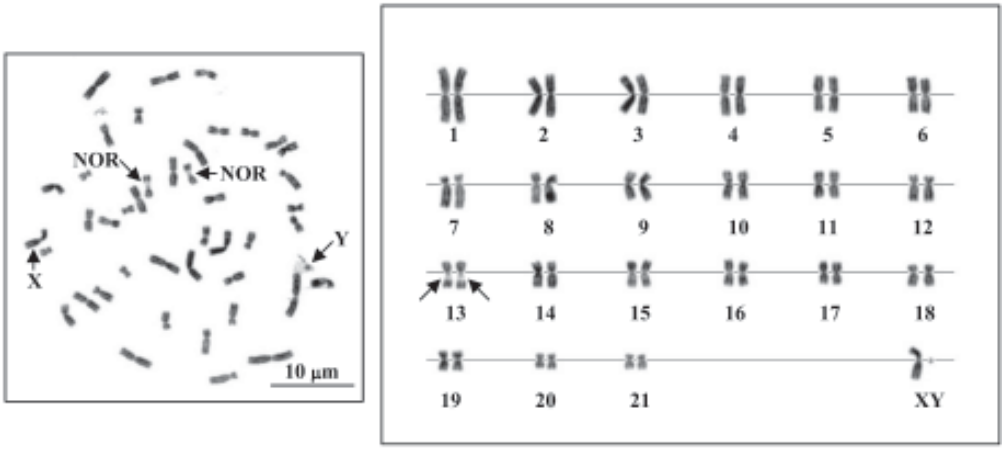
Chromosomal checking were performed on mitotic metaphase cells under light microscope. Twenty cells each of male and female with clearly observable and well-spread chromosomes were selected and photographed. The length short arm chromosome (Ls) and the length long arm chromosome (Ll) were measured to 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 and size of chromosomes according to Chaiyasut (1989). All parameters were used in karyotyping and idiograming according to Nash and O'Brien (1987); Wada et al. (1991).

Results and discussion

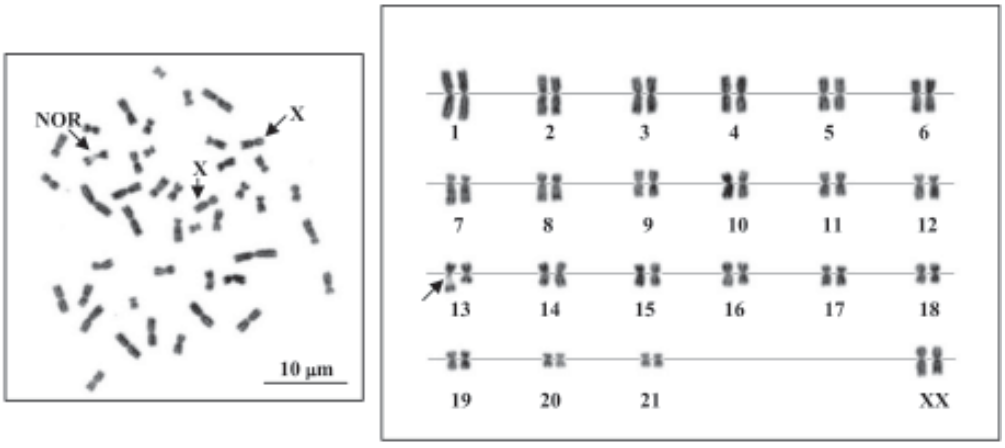
Karyological studies of the white-handed gibbon using lymphocyte culture and the conventional staining procedures revealed that the chromosome number was 2*n* (diploid) = 44, which consists of 42 autosomes and 2 sex chromosomes (The X and Y-chromosome). This is the same chromosome number for the white-handed gibbon as reported by Bender and Chu (1963); Hamerton et al. (1963); Chiarelli (1972); Warburton et al. (1975); Stayon et al. (1987, 1995); Jauch et al. (1992); Tuinen et al. (1999); Mueller et al. (2002, 2003). Comparing with gibbon species in the genus *Hylobates*, the chromosome numbers were all the same according to Chiarelli (1972), who reported the chromosome numbers of gibbon species in the

genus *Hylobates* (*H. lar*, *H. agilis*, *H. moloch*, *H. hoolock* and *H. klossii*) were 2*n*=44. Furthermore, Stanyon (1987) also reported that the chromosome numbers of *H. lar*, *H. agilis*, *H. moloch*, *H. muelleri* and *H. klossii* were 2*n*=44. The gibbon species were classified by chromosome number into 4 genera (12 species) namely *Hylobates*, *Hoolock*, *Nomascus* and *Symphalangus* with chromosome number (2*n*) 44, 38, 52 and 50, respectively (Geissmann, 2002).

This examination also revealed that the fundamental number (NF) of the white-handed gibbon was 88 in male and female. This is the same NF for the white-handed gibbon as reported by Bender and Chu (1963); Hamerton et al. (1963); Chiarelli (1972); Warburton et al. (1975). The chromosomes in mitotic metaphase plates and the karyotype of the white-handed gibbon are shown in figure 2.



A. The male white-handed gibbon



B. The female white-handed gibbon

Figure 2. Metaphase chromosome plate and karyotype of the male (A) and female (B) white-handed gibbon (*Hylobates lar* Linnaeus, 1771) 2*n* (diploid) = 44 by conventional staining technique, satellite chromosomes (nucleolar organizer regions, NORs), arrows.

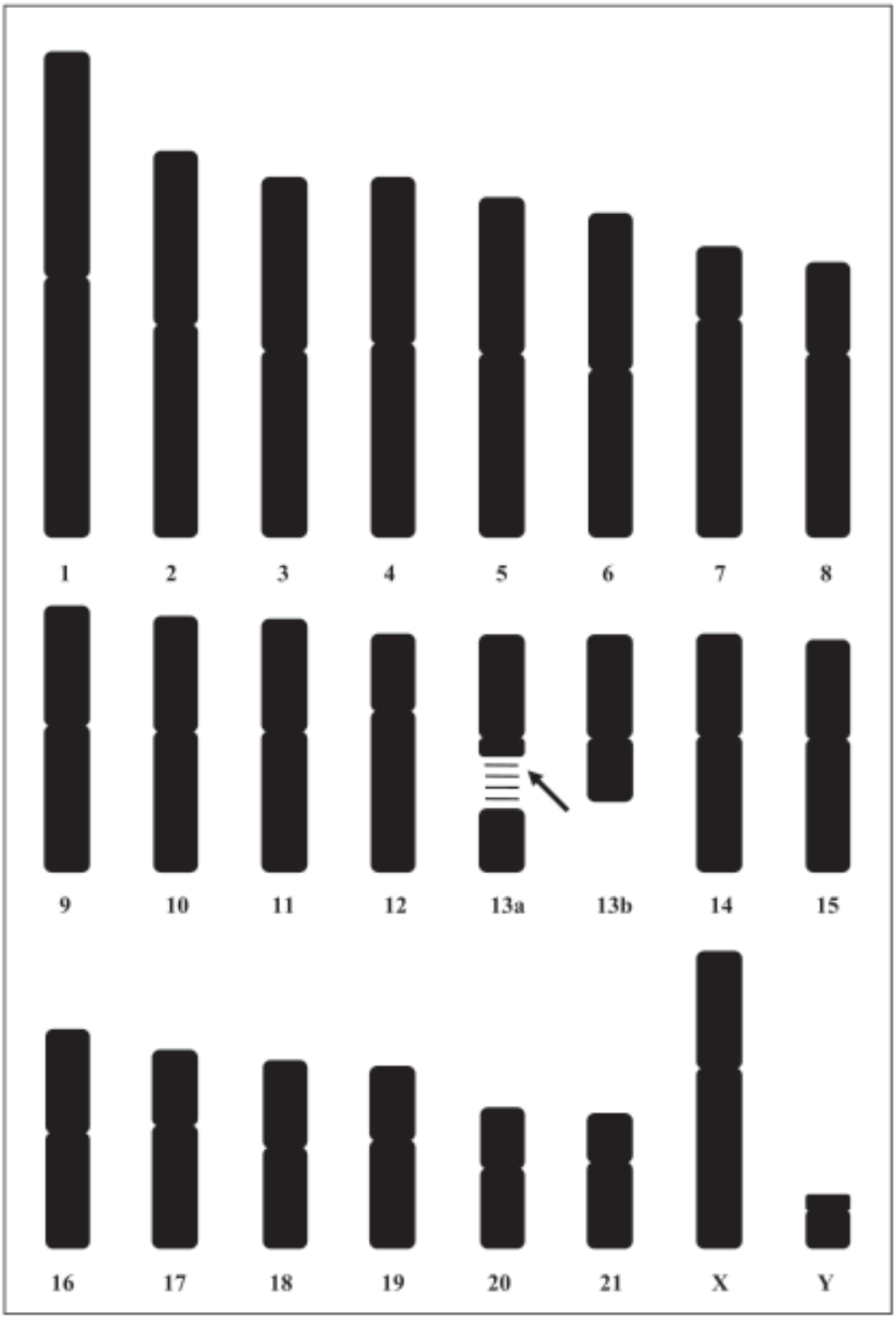


Figure 3. Idiogram of white-handed gibbon (*Hylobates lar* Linnaeus, 1771) 2*n* (diploid) = 44 by conventional staining technique, satellite chromosome (nucleolar organizer region, NOR), arrow.

The white-handed gibbon has 3 types of autosomes, which are metacentric (28 chromosomes), submetacentric (12 chromosomes) and acrocentric (2 chromosomes). The 28 metacentric autosomes were classified by size into 12 large, 8 medium and 8 small chromosomes, the 12 submetacentric autosomes were classified by size into 4 medium and 8 small chromosomes while the 2 acrocentric autosomes were distinguished to be 2 medium chromosomes. Difference chromosomal features were reported by Bender and Chu (1963), which indicated that the white-handed gibbon had 38 metacentric and 6 submetacentric autosomes, Hamerton et al. (1963), indicated that the white-handed gibbon had 42 metacentric autosomes, Chiarelli (1972), indicated that the white-handed gibbon had 24 metacentric and 18 submetacentric autosomes. Furthermore, Warburton et al. (1975) also reported that the autosomes of the white-handed gibbon had 32 metacentric and 10 submetacentric autosomes. The idiogram of the white-handed gibbon shows the gradually decreasing length of the autosomes (Figure 3).

The X-chromosome of the white-handed gibbon is a medium submetacentric chromosome and the Y-chromosome is the smallest acrocentric chromosome (dot like). These features are differences to that reported by Hamerton et al. (1963) indicating that a white-handed gibbon had a metacentric X-chromosome and a acrocentric Y-chromosome. Furthermore, Chiarelli (1972); Warburton et al. (1975) also reported that the sex-chromosomes of the white-handed gibbon had a metacentric X-chromosome and a submetacentric Y-chromosome. In comparison with gibbon species in the genus *Hylobates* in Thailand, that the X-chromosome of the agile gibbon (*H. agilis*) and pileated gibbon (*H. pileatus*) are submetacentric chromosomes. The Y-chromosome of those species are acrocentric chromosome (Hamerton et al., 1963; Dutrillaux et al., 1975; Supanuam et al., 2007).

Furthermore, the research cited above elucidated that the variation of both type occurred in the sex chromosomes of gibbons in the genus *Hylobates*. According to those reports and this investigation, the X-chromosome is metacentric or submetacentric type while the Y-chromosome is submetacentric or acrocentric type. Dutrillaux et al. (1975) indicated that the Y chromosomes of the members of the genus *Hylobates* are tiny with varying shape. Occasionally, the centromere is not obvious and the type of the chromosome is difficult to classify. Moreover, the report of variation in human Y-chromosome by Makino and Takagi (1965) revealed that the length of the acrocentric Y-chromosome varies among individuals.

In this investigation, the nucleolar organizer regions (NORs), which represents the chromosome marker, locates only on the long arms of the pair metacentric autosomes 13. In contrast, Chiarelli (1972); Stanyon et al. (1987) indicated that the NORs of white-handed gibbon present only on the short arms of the pair autosome 21, Warburton et al. (1975) indicated that the NORs of white-handed gibbon present only on the long arms of the pair autosome 15, Jauch et al. (1992) indicated that the NORs of white-handed gibbon present only of the pair autosome 12. This difference may due to the different methods of karyotyping and measuring. The study by Jones et al. (1994) indicated that only 1 pair of NORs is found in the autosomes of gibbons and baboons. Stanyon (1995) also found that a pair

of NORs located at the pair autosome 13 in 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 white-handed gibbon in Thailand revealed that the chromosome marker is the pair autosomes 1, which is the biggest metacentric chromosomes.

After measuring the length in centimeters of the chromosomes in mitotic metaphase cells for 20 cells in males and females. The mean of length short arm chromosome (Ls), length long arm chromosome (Ll), length total arm chromosome (LT), relative length (RL), centromeric index (CI), standard deviation (SD) of RL, CI, size and type of chromosome in male and female of the white-handed gibbon show in tables 1 and 2. The idiogram of the white-handed gibbon shows gradually decreasing length of the autosomes and sex chromosomes (Figure 3). The karyotype formula for the white-handed gibbon was as follows:

$$2n\ (44) = L^{m}_{12} + M^{m}_{8} + M^{sm}_{4} + M^{a}_{2} + S^{m}_{8} + S^{sm}_{8} + \text{sex- chromosomes}$$

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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 chromosomes of 20 cells in male white-handed gibbon (*Hylobates lar* Linnaeus, 1771) 2*n* (diploid) = 44.

Chromosome pairs	Ls	LL	LT	RL SD	CI SD	Chromosome size	Chromosome type
1	1.44	1.65	3.09	0.080 0.002	0.534 0.025	L	m
2	1.05	1.35	2.40	0.062 0.004	0.563 0.024	L	m
3	1.13	1.17	2.30	0.059 0.003	0.509 0.028	L	m
4	1.06	1.20	2.26	0.058 0.003	0.531 0.015	L	m
5	1.04	1.08	2.12	0.055 0.001	0.509 0.018	L	m
6	0.89	1.17	2.06	0.053 0.003	0.568 0.017	L	m
7	0.45	1.40	1.85	0.050 0.003	0.757 0.016	M	a
8	0.57	1.19	1.76	0.048 0.002	0.676 0.037	M	sm
9	0.78	0.97	1.75	0.045 0.002	0.554 0.017	M	m
10	0.76	0.92	1.68	0.045 0.001	0.548 0.019	M	m
11	0.78	0.87	1.65	0.043 0.001	0.527 0.021	M	m
12	0.63	1.00	1.63	0.042 0.001	0.613 0.031	M	sm
13*	0.66	0.96	1.62	0.042 0.002	0.593 0.012	M	m
14	0.64	0.90	1.54	0.040 0.001	0.584 0.007	S	m
15	0.61	0.90	1.51	0.039 0.003	0.596 0.012	S	m
16	0.69	0.76	1.45	0.037 0.003	0.524 0.033	S	m
17	0.45	0.88	1.33	0.034 0.003	0.662 0.032	S	sm
18	0.57	0.70	1.27	0.033 0.002	0.551 0.039	S	m
19	0.52	0.72	1.24	0.032 0.002	0.661 0.020	S	sm
20	0.39	0.64	1.03	0.027 0.002	0.621 0.023	S	sm
21	0.29	0.65	0.94	0.024 0.001	0.634 0.055	S	sm
X	0.76	1.12	1.88	0.048 0.003	0.621 0.028	M	sm
Y	0.10	0.28	0.38	0.010 0.002	0.751 0.048	S	a

Remarks: L = large chromosome, M = medium chromosome, S = small chromosome, m = metacentric chromosome, sm = submetacentric chromosome, a = acrocentric chromosome and * = satellite chromosomes (nucleolar organizer regions, NORs)

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 chromosomes of 20 cells in female white-handed gibbon (*Hylobates lar* Linnaeus, 1771) 2*n* (diploid) = 44.

Chromosome pairs	Ls	LL	LT	RL SD	CI SD	Chromosome size	Chromosome type
1	1.43	1.65	3.08	0.080 0.005	0.536 0.012	L	m
2	1.08	1.35	2.43	0.063 0.001	0.556 0.011	L	m
3	1.16	1.24	2.40	0.062 0.004	0.517 0.021	L	m
4	1.03	1.25	2.28	0.059 0.003	0.548 0.022	L	m
5	1.05	1.11	2.16	0.056 0.001	0.514 0.037	L	m
6	0.95	1.19	2.14	0.056 0.001	0.556 0.008	L	m
7	0.43	1.44	1.87	0.049 0.002	0.770 0.023	M	a
8	0.56	1.24	1.80	0.047 0.002	0.689 0.020	M	sm
9	0.74	1.00	1.74	0.045 0.002	0.575 0.020	M	m
10	0.72	0.95	1.67	0.043 0.002	0.569 0.011	M	m
11	0.74	0.89	1.63	0.042 0.002	0.546 0.020	M	m
12	0.57	1.02	1.59	0.041 0.001	0.642 0.014	M	sm
13a	0.66	1.16	1.82	0.040 0.001	0.551 0.024	M	m
13b	0.70	0.60	1.30				
14	0.64	0.90	1.54	0.040 0.002	0.582 0.020	S	m
15	0.62	0.91	1.53	0.040 0.002	0.584 0.016	S	m
16	0.69	0.75	1.44	0.037 0.003	0.521 0.034	S	m
17	0.46	0.85	1.31	0.034 0.002	0.649 0.032	S	sm
18	0.56	0.71	1.27	0.033 0.001	0.559 0.014	S	m
19	0.45	0.74	1.19	0.031 0.001	0.622 0.009	S	sm
20	0.37	0.64	1.01	0.026 0.002	0.634 0.022	S	sm
21	0.30	0.63	0.93	0.024 0.001	0.677 0.038	S	sm
X	0.74	1.21	1.95	0.051 0.002	0.621 0.007	M	sm

Remark: L = large chromosome, M = medium chromosome, S = small chromosome, m = metacentric chromosome, sm = submetacentric chromosome, a = acrocentric chromosome and * = satellite chromosomes (nucleolar organizer regions, NORs)