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การศึกษาโครโมโซมของชะนีแก้มขาว (*Nomascus leucogenys*) ด้วยวิธีการย้อมสีโครโมโซมแบบธรรมดา

A Study on the Chromosomes of White-cheeked Gibbon, *Nomascus leucogenys* (Primate, Hylobatidae) by Conventional Staining Technique

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บทคัดย่อ

คาริโอไทป์และอิดิโอแกรมมาตรฐานของชะนีแก้มขาว (*Nomascus leucogenys*) เพศเมีย 1 ตัว และเพศผู้ 2 ตัว จากสวนสัตว์นครราชสีมา เก็บตัวอย่างเลือดมาเพาะเลี้ยงเซลล์เม็ดเลือดขาวที่อุณหภูมิ 37 องศาเซลเซียส เป็นระยะเวลา 72 ชั่วโมง ย้อมสีโครโมโซมด้วยวิธีย้อมแบบธรรมดา ผลการศึกษาพบว่าชะนีแก้มขาวมีจำนวนโครโมโซมดิพลอยด์เท่ากับ 52 แท่ง มีจำนวนโครโมโซมพื้นฐานเท่ากับ 104 ทั้งในเพศเมียและเพศผู้ โครโมโซมร่างกายประกอบด้วยโครโมโซมชนิดเมทาเซนทริกขนาดใหญ่ 22 แท่ง ซับเมทาเซนทริกขนาดใหญ่ 8 แท่ง เมทาเซนทริกขนาดกลาง 10 แท่ง ซับเมทาเซนทริกขนาดกลาง 4 แท่ง และอะโครเซนทริกขนาดเล็ก 6 แท่ง โครโมโซมเอ็กซ์เป็นชนิดอะโครเซนทริกขนาดใหญ่ และโครโมโซมวายเป็นชนิดซับเมทาเซนทริกที่มีขนาดเล็กมากที่สุด สูตรคาริโอไทป์มาตราฐานของชะนีแก้มขาว ดังนี้

ดิพลอยด์ (52) = $L^{m}_{22}\ L^{sm}_{8}+M^{m}_{10}+M^{sm}_{4}+S^{a}_{6}$ + โครโมโซมเพศ

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ABSTRACT

Standardized karyotype and idiogram of white-cheeked gibbon (*Nomascus leucogenys*) at Nakhonratchasima Zoo, was study. Blood sample were taken from 1 female and 2 males gibbons. 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 technique was applied to stain the chromosomes. The results show that diploid chromosome number is 2*n* = 52, and the fundamental number (NF) is 104 in both female and male. The autosomes consist of 22 large metacentric, 8 large submetacentric, 10 medium metacentric, 4 medium submetacentric and 6 small acrocentric chromosomes. The X-chromosome is the large acrocentric and the Y-chromosome is the smallest submetacentric chromosome. The karyotype formula for the white-cheeked gibbon is as follows:

$2n\ (52) = L^{m}_{22}\ L^{sm}_{8} + M^{m}_{10} + M^{sm}_{4} + S^{a}_{6}$ + sex-chromosomes

คำสำคัญ: คาริโอไทป์ โครโมโซม การย้อมสีแบบธรรมดา ชะนีแก้มขาว

Keywords: Karyotype, Chromosome, Conventional staining, White-cheeked gibbon

Introduction

Habitat loss has occurred violently throughout Southeast Asia over the past 20 years and these gibbon species are vulnerable to population pressures and habitat fragmentation. Forest destruction has negatively affected on wild animals such as family Hylobatidae. It reduces habitat for wild animals and causes population fragmentation due to the loss of genetic heterogeneity and thus they become vulnerable to environmental change and risk extinction.

The gibbons, genus *Nomascus*, represent one the most successful populations within the order Primate. They consist of 12 gibbon species of the world and are subdivided into 4 morphologically and karyologically distinct genera, namely *Hylobates* (diploid chromosome number, 2*n* = 44), *Hoolock* (2*n* = 38), *Nomascus* (2*n* = 52) and *Symphalangus* (2*n* = 50) (Wilson and Cole, 2000; Geissmann, 2002; Mootnick and Grove, 2005). A white-cheeked gibbon belongs to kingdom Animalia, phylum Chordata, class Mammalia, order Primates, family Hylobatidae, genus *Nomascus* and species *Nomascus leucogenys* (Brokelman, 1981) (Figure 1).

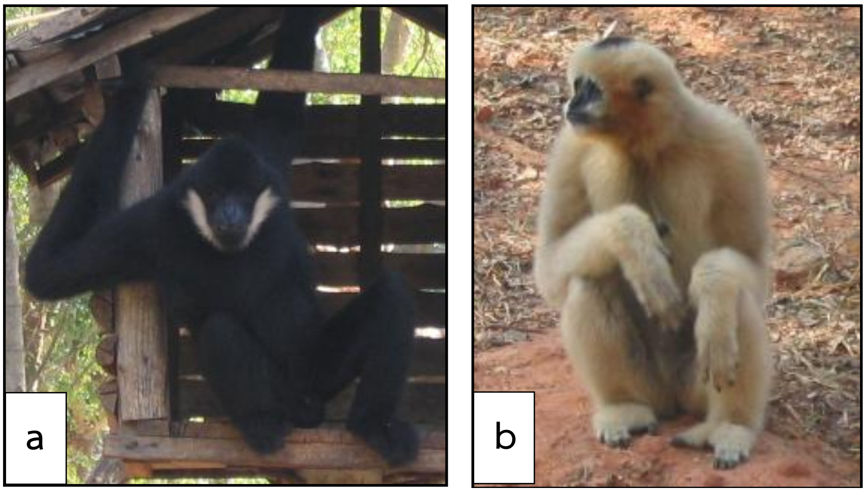


Figure 1. The male (A) and female (B) white-cheeked gibbon, *Nomascus leucogenys* Cuvier, 1821 (Primate, Hylobatidae) in Thailand from Nakhonratchasima Zoo.

Conventional and karyotype analysis is an important taxonomic tool for assessing the diversification between species, subspecies, and populations. According to the cytogenetic studies of white-cheeked gibbon by Hsu and Benirschke (1969); Chiarelli (1972); Dutrillaux et al. (1975) and Liu et al. (1996), they found that the cytogenetics of the white-cheeked gibbon in Thailand has not been studied. In this study, we confirm and compare the result with previous report. In addition to, this is the first report about chromosome measuring for determined size and formula karyotyping that has 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 1 female and 2 males white-cheeked gibbon, which were kept in Nakhonratchasima Zoo, Nakhonratchasima Province 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 Rooney (2001) and Campiranont (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 $^{\circ}$C under 5% of carbondioxide environment and regularly shaken in the morning and evening. At the harvesting time, the 72th hour of incubation, colchicine was added and well mixed, following by the 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. The 10 milliliters of hypotonic solution (0.075 M KCl) was applied to the pellet and the mixture was incubated for 30 minutes. KCl was discarded from the supernatant after centrifuged again at 1,200 rpm for 10 minutes. Cells were fixed by fresh cool fixative (3 methanol: 1 glacial acetic acid) and gradually added up to 8 milliliters before centrifuged again at 1,200 rpm for 10 minutes. After that the supernatant was discarded, the fixation was repeated until the supernatant was clear and then the pellet was mixed with 1 milliliter fixative. The mixture was dropped onto a clean and cold slide by micropipette following by the air-drying technique. The slide was conventionally stained with 20% stock Giemsa's solution for 30 minutes.

2. Chromosomal checks, karyotyping and idiograming

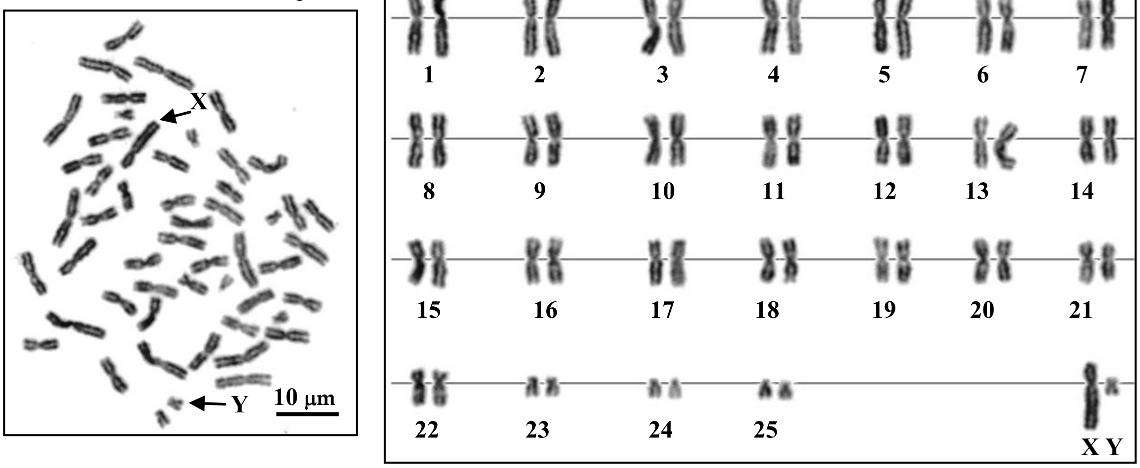
Chromosomal checks were performed on mitotic metaphase cells under light microscope. Twenty cells each of male and female with clearly observable and well-spread chromosomes were selected and photographed. The length short arm chromosome (Ls) and the length long arm chromosome (Ll) were measured to calculate 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

Cytogenetics study of white-cheeked gibbon using lymphocyte culture and the conventional staining procedures revealed that the chromosome number is 2*n* (diploid) = 52, which consists of 50 (25 pairs) autosomes and 2 (1 pair) sex chromosomes. This is the same chromosome number for the white-cheeked gibbon as reported by Hsu and Benirschke (1969); Chiarelli (1972); Dutrillaux et al. (1975) and Liu et al. (1996). The gibbon species of the world 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 (number of chromosome arms) of the white-cheeked gibbon is 104 in male and female. This is the same NF for the white-cheeked gibbon as reported by Chiarelli (1972) and Dutrillaux et al. (1975). The chromosomes in mitotic metaphase plates and the karyotype of the white-cheeked gibbon are shown in figure 2.

A. The male white-cheeked gibbon



B. The female white-cheeked gibbon

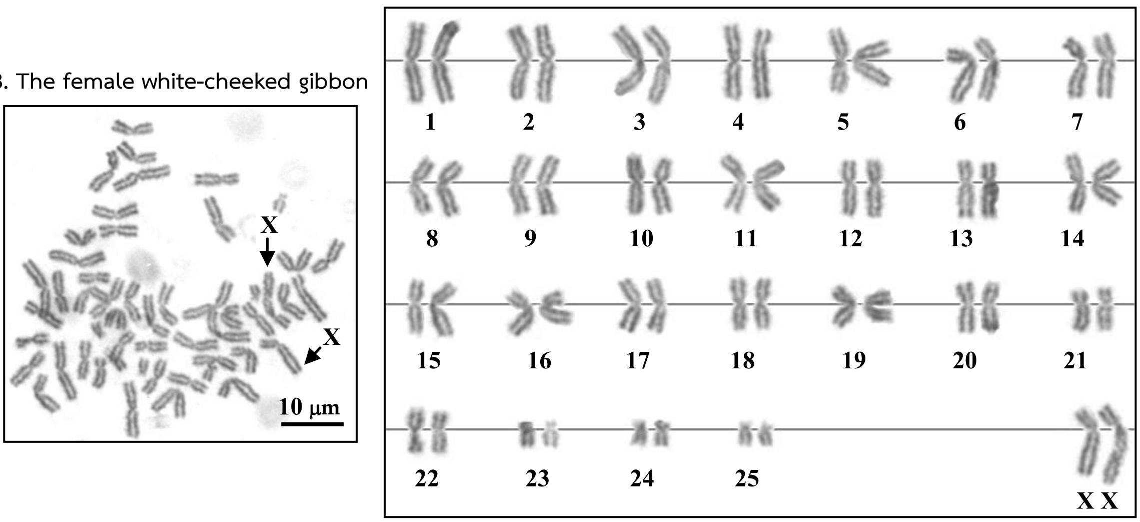


Figure 2. Metaphase chromosome plates and karyotypes of the male (A) and female (B) white-cheeked gibbon (*Nomascus leucogenys* Cuvier, 1821) 2*n* (diploid) = 52 by conventional staining technique, arrows indicate sex-chromosomes.

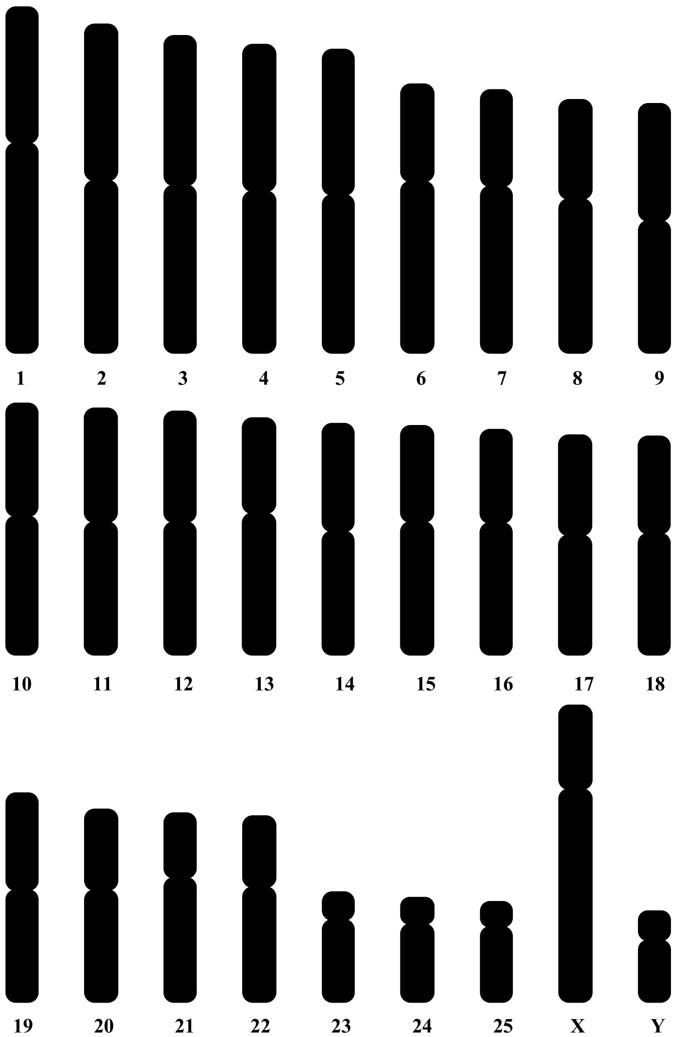


Figure 3. Idiogram of white-cheeked gibbon (*Nomascus leucogenys* Cuvier, 1821) 2*n* (diploid) = 52 by conventional staining technique.

The white-cheeked gibbon has 3 types of autosomes, which are 32 metacentric, 12 submetacentric and 6 acrocentric chromosomes. The 32 metacentric autosomes are classified by size into 22 large and 10 medium chromosomes, the 12 submetacentric autosomes are classified by size into 8 large and 4 medium chromosomes while the 6 acrocentric autosomes are distinguished to be 6 small chromosomes. The results are difference to that reported by Hsu and Benirschke (1969) and Liu et al. (1996) indicating that white-cheeked gibbon had 44 metacentric and submetacentric, 6 acrocentric autosomes and Chiarelli (1972) reported that white-cheeked gibbon had 20 metacentric, 24 submetacentric and 6 acrocentric autosomes.

The X-chromosome of the white-cheeked gibbon is a large acrocentric chromosome and the Y-chromosome is the smallest submetacentric chromosome. These features are difference to that reported by Chiarelli (1972) and Liu et al. (1996) indicating that a white-cheeked gibbon had a metacentric X-chromosome and a acrocentric Y chromosome, Hsu and Benirschke (1969) indicating that a white-cheeked gibbon had a submetacentric X-chromosome and a acrocentric Y-chromosome and Dutrillaux et al. (1975) indicating that a white-cheeked gibbon had a acrocentric X-chromosome and a telocentric Y-chromosome.

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-cheeked gibbon (*Nomascus leucogenys*) 2*n* (diploid) = 52.

Chromosome pairs	Ls	LL	LT	RL±SD	CI±SD	Chromosome Size	Chromosome Type
1	1.04	1.58	2.61	0.056±0.002	0.602±0.020	Large	Submetacentric
2	1.19	1.28	2.47	0.053±0.001	0.518±0.016	Large	Metacentric
3	1.12	1.27	2.40	0.051±0.001	0.532±0.019	Large	Metacentric
4	1.11	1.21	2.33	0.050±0.001	0.522±0.013	Large	Metacentric
5	1.10	1.20	2.29	0.049±0.001	0.522±0.013	Large	Metacentric
6	0.73	1.30	2.02	0.043±0.002	0.642±0.021	Large	Submetacentric
7	0.74	1.26	2.00	0.043±0.001	0.629±0.029	Large	Submetacentric
8	0.75	1.19	1.94	0.042±0.002	0.612±0.011	Large	Submetacentric
9	0.93	1.00	1.92	0.041±0.001	0.527±0.013	Large	Metacentric
10	0.85	1.03	1.88	0.040±0.001	0.549±0.027	Large	Metacentric
11	0.85	1.00	1.85	0.039±0.001	0.542±0.031	Large	Metacentric
12	0.82	1.00	1.82	0.039±0.001	0.551±0.031	Large	Metacentric
13	0.73	1.04	1.78	0.038±0.001	0.584±0.045	Large	Metacentric
14	0.78	0.95	1.73	0.037±0.001	0.551±0.028	Large	Metacentric
15	0.74	0.97	1.71	0.036±0.001	0.566±0.031	Large	Metacentric
16	0.71	0.97	1.68	0.036±0.001	0.574±0.044	Medium	Metacentric
17	0.75	0.90	1.64	0.035±0.001	0.546±0.027	Medium	Metacentric
18	0.73	0.90	1.62	0.034±0.001	0.552±0.027	Medium	Metacentric
19	0.72	0.84	1.56	0.033±0.001	0.542±0.018	Medium	Metacentric
20	0.62	0.83	1.45	0.031±0.001	0.573±0.044	Medium	Metacentric
21	0.49	0.93	1.42	0.030±0.001	0.654±0.024	Medium	Submetacentric
22	0.53	0.85	1.38	0.030±0.002	0.605±0.035	Medium	Submetacentric
23	0.22	0.63	0.85	0.018±0.002	0.742±0.018	Small	Acrocentric
24	0.21	0.59	0.80	0.017±0.001	0.738±0.020	Small	Acrocentric
25	0.19	0.57	0.76	0.016±0.001	0.751±0.018	Small	Acrocentric
X	0.64	1.60	2.23	0.048±0.004	0.714±0.015	Large	Acrocentric
Y	0.22	0.48	0.71	0.015±0.002	0.684±0.030	Small	Submetacentric

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-cheeked gibbon (*Nomascus leucogenys*) 2*n* (diploid) = 52.

Chromosome pairs	Ls	LL	LT	RL±SD	CI±SD	Chromosome Size	Chromosome Type
1	0.86	1.29	2.15	0.057±0.002	0.602±0.030	Large	Submetacentric
2	0.96	1.05	2.01	0.054±0.002	0.523±0.020	Large	Metacentric
3	0.89	1.08	1.97	0.052±0.001	0.549±0.015	Large	Metacentric
4	0.91	0.99	1.90	0.051±0.002	0.522±0.017	Large	Metacentric
5	0.88	0.97	1.85	0.049±0.001	0.523±0.011	Large	Metacentric
6	0.58	1.07	1.65	0.044±0.002	0.648±0.024	Large	Submetacentric
7	0.58	1.03	1.61	0.043±0.002	0.641±0.031	Large	Submetacentric
8	0.59	0.98	1.57	0.042±0.003	0.624±0.013	Large	Submetacentric
9	0.72	0.84	1.56	0.041±0.003	0.539±0.017	Large	Metacentric
10	0.68	0.81	1.49	0.040±0.002	0.543±0.025	Large	Metacentric
11	0.66	0.83	1.49	0.039±0.001	0.559±0.018	Large	Metacentric
12	0.64	0.82	1.46	0.039±0.001	0.560±0.018	Large	Metacentric
13	0.65	0.79	1.44	0.038±0.001	0.550±0.038	Large	Metacentric
14	0.61	0.79	1.40	0.037±0.002	0.565±0.025	Large	Metacentric
15	0.65	0.74	1.39	0.036±0.001	0.541±0.022	Large	Metacentric
16	0.60	0.74	1.34	0.036±0.001	0.553±0.015	Medium	Metacentric
17	0.60	0.72	1.32	0.035±0.001	0.548±0.030	Medium	Metacentric
18	0.56	0.74	1.30	0.034±0.000	0.572±0.024	Medium	Metacentric
19	0.56	0.68	1.24	0.033±0.002	0.548±0.011	Medium	Metacentric
20	0.52	0.68	1.20	0.032±0.001	0.568±0.033	Medium	Metacentric
21	0.41	0.77	1.18	0.031±0.001	0.654±0.026	Medium	Submetacentric
22	0.45	0.71	1.16	0.031±0.002	0.614±0.033	Medium	Submetacentric
23	0.21	0.58	0.79	0.021±0.002	0.731±0.015	Small	Acrocentric
24	0.20	0.52	0.72	0.019±0.001	0.727±0.021	Small	Acrocentric
25	0.15	0.47	0.62	0.016±0.001	0.755±0.014	Small	Acrocentric
X	0.53	1.34	1.87	0.050±0.002	0.716±0.020	Large	Acrocentric

Furthermore, the research cited above elucidated that the variation of both type occurred in the sex chromosomes of gibbons in the genus *Nomascus* (*N. concolor, N. leucogenys, N. gabriellae, N. sp. cf. nasutus*). According to those reports and this investigation, the X chromosome is metacentric or submetacentric or acrocentric type while the Y chromosome is submetacentric or acrocentric or telocentric type (Hsu and Benirschke, 1969; Chiarelli, 1972; Dutrillaux et al., 1975; Liu et al., 1996).

From the chromosomal checks of twenty cells each of male and female chromosomes in mitotic metaphase cells, the Ls, Ll, LT, RL, CI, SD of RL and CI, size and type of white-cheeked gibbon's chromosomes are show in tables 1 and 2. The idiogram of white-cheeked gibbon shows gradually decreasing length of the autosomes and sex chromosomes (Figure 3). The karyotype formula for the white-cheeked gibbon is as follows:

$2n$ (44) = $L^{m}_{22}\ L^{sm}_{8}+M^{m}_{10}+M^{sm}_{4}+S^{a}_{6}$ + sex-chromosomes

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