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Original Article

A study on karyotype of the pileated gibbon, *Hylobates pileatus* (Primates, Hylobatidae), by conventional staining

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Abstract

Cytogenetics of the pileated gibbon (*Hylobates pileatus*) at Nakhon Ratchasima Zoo, Thailand, was studied. Blood samples were taken from two female and two male gibbons. After lymphocyte culture, the mitotic chromosome preparation was done by hypotonic-fixation-air-drying method and conventional Giemsa's staining. The results show that diploid chromosome number was 44 (2n=2x=44), and the fundamental number (NF) were 88 chromosomes in both female and male. The autosomes consist of 12 large metacentric, 6 medium metacentric, 2 medium submetacentric, 2 medium acrocentric, 12 small metacentric and 8 small submetacentric chromosomes. In addition, the chromosome 15 showed clearly observable satellite chromosomes. The X chromosome was a medium submetacentric chromosome and the Y chromosome was a tiny acrocentric chromosome. The karyotype formula for the pileated gibbon is as follows:

$$2n\ (44) = L^{m}_{12}+M^{m}_{6}+M^{sm}_{2}+M^{a}_{2}+S^{m}_{12}+S^{sm}_{8}+\text{sex-chromosomes}$$

Key words: karyotype, pileated gibbon (*Hylobates pileatus*), conventional staining

1. Introductions

There are three species of gibbon (order Primates, family Hylobatidae) in Thailand including white-handed gibbons (*Hylobates lar*), dark-handed gibbons (*H. agilis*), and pileated gibbons (*H. pileatus*). In the past, pileated gibbons were considered to be a subspecies of white-handed gibbon because they are similar to white-handed gibbons. After that taxonomists have raised the taxon of pileated gibbons to the one of gibbon species in Thailand. The pileated gibbons are sexually dimorphic in color; both sexes are grayish white at birth, but by the age of 4-6 months, two black patches appear, one on the chest, another on the crown, by the age of 3-4 years the male turns black all over except for the circle around the face, the genital area, and the crown just above the ears. Females turn black by the age of 2-3 years, then become buff again by the time they are adults, retaining a triangular black patch on the breast and a black crown. These color changes seem to be hormonally controlled (Figure 1). The pileated gibbons are found in the evergreen

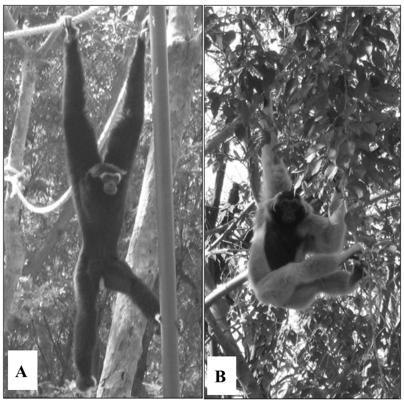

Figure 1. Pileated gibbon, *Hylobates pileatus* (Primate, Hylobatidae), male (A) and female (B).

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forests and in the tropical rainforests of the far southeast. Their diet comprises plants and animals, such as fruit, leaves, buds, insects, spiders, birds' egg and lizards (Brokelman, 1981; Lekagul and McNeely, 1988).

There are only two reports of cytogenetic studies of pileated gibbon, Dutrillaux *et al*. (1975), and Stanyon (1987), which reported karyotype by R-band and G-band, respectively. We found that the basis cytogenetics of the pileated gibbon by conventional staining had not been studied. Moreover, the standardizations of chromosome including chromosome measuring to determined shape and size, karyotype formulating, and idiograming have not been previously reported. Therefore, the standardizations of the pileated gibbon chromosome were studied in this insertization. In addition, we confirm and compare the results with previous reports. This study, therefore, provides important basic knowledge and can be applied as the basis for further research.

2. Materials and Methods

Blood samples from the jugular vein were collected from two males and two females pileated gibbons, kept in Nakhon Ratchasima Zoo, by 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.

2.1 Cell preparation

The lymphocytes were cultured using the whole blood microculture technique adapted from Rooney (2001) and Kampiranont (2003).

1) Cell culture

The 5 ml of RPMI 1640 medium was prepared with 2% PHA (phytohemagglutinin) as a mitogen and kept in blood culture flasks. A blood sample of 0.5 ml was dropped into a medium bottle and well mixed. The culture bottles were 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 72nd hour of incubation, 0.01% colchicine was added and well mixed, followed by further incubation for 30 minutes.

2) Cell harvest

The blood sample mixture was centrifuged at 1,200 rpm (100 xg) for 10 minutes and the supernatant discarded. Ten milliliters of hypotonic solution (0.075 M KCl) was applied to the pellet and the mixture incubated for 30 minutes. KCl was discarded from the supernatant after centrifugation again at 1,200 rpm (100 xg) for 10 minutes. Cells were fixed in fresh cold 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 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 by micropipette followed by the air-drying technique. The slide was conventionally stained with 20% stock Giemsa's solution for 30 minutes.

2.2 Chromosome counting, karyotyping and idiograming

Chromosome counting was 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 of short arm (Ls) and the length of long arm of chromosome (Ll) were measured to calculate the length of 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 Turpin and Lejeune (1965). All parameters were used in karyotyping and idiograming according to Nash and O'Brien (1987), and Wada *et al*. (1991).

3. Results and Discussion

Cytogenetic study of the pileated gibbon using lymphocyte culture and the conventional staining procedures revealed that the chromosome number was 44 (2n=2x=44), comprising 42 (21 pairs) autosomes and 2 (1 pair) sex chromosomes. This is the same chromosome number for the pileated gibbon as reported before by Dutrillaux *et al*. (1975), Stanyon (1987), and Geissmann (2002). In the genus *Hylobates*, the chromosome numbers are 44 was comfirmed by Chiarelli (1972) who studied in *H. lar*, *H. agilis*, *H. moloch*, *H. hoolock* and *H. klossii*. Furthermore, Stanyon (1987) also reported that the chromosome numbers of *H. lar*, *H. agilis*, *H. moloch*, *H. muelleri* and *H. klossii* were 2n = 44. The gibbon species in family Hylobatidae were classified by chromosome number into 4 genera (11 species), namely *Hylobates*, *Hoolock*, *Nomascus* and *Symphalangus* with chromosome number 44, 38, 52 and 50 respectively (Geissmann, 2002).

This examination also revealed that the fundamental number (NF, number of chromosome arms) of the pileated gibbon was 88 in a male and a female. This is the same NF for the pileated gibbon as reported by Dutrillaux *et al*. (1975) and Liu *et al* (1996). The chromosomes of mitotic metaphase cells and the karyotypes of the pileated gibbons in male and female are shown in Figures 2 and 3.

The pileated gibbon has 3 types of autosomes, metacentric (30 chromosomes), submetacentric (10 chromosomes) and acrocentric (2 chromosomes). The 30 metacentric autosomes were classified by size into 12 large, 6 medium and 12 small chromosomes, the 10 submetacentric autosomes were classified by size into 2 medium and 8 small chromosomes while the 2 acrocentric autosomes were all 2 medium chromosomes. The idiogram of the pileated gibbon shows the

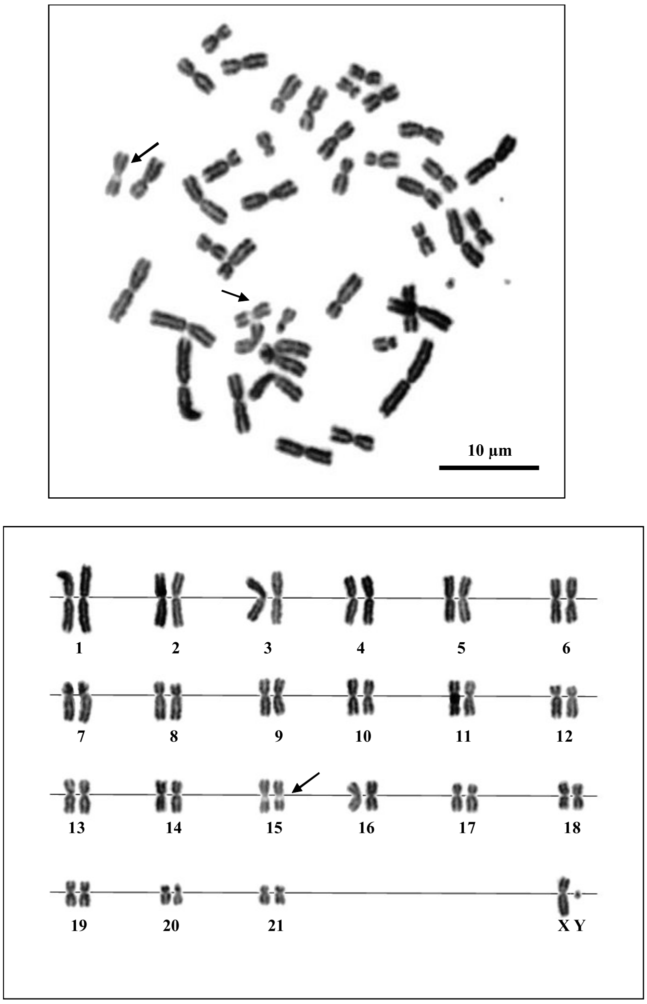

Figure 2. Metaphase chromosomes (A) and karyotype of male pileated gibbon (*Hylobates pileatus*) 2n=44, by conventional staining (B), arrows indicate satellite chromosomes.

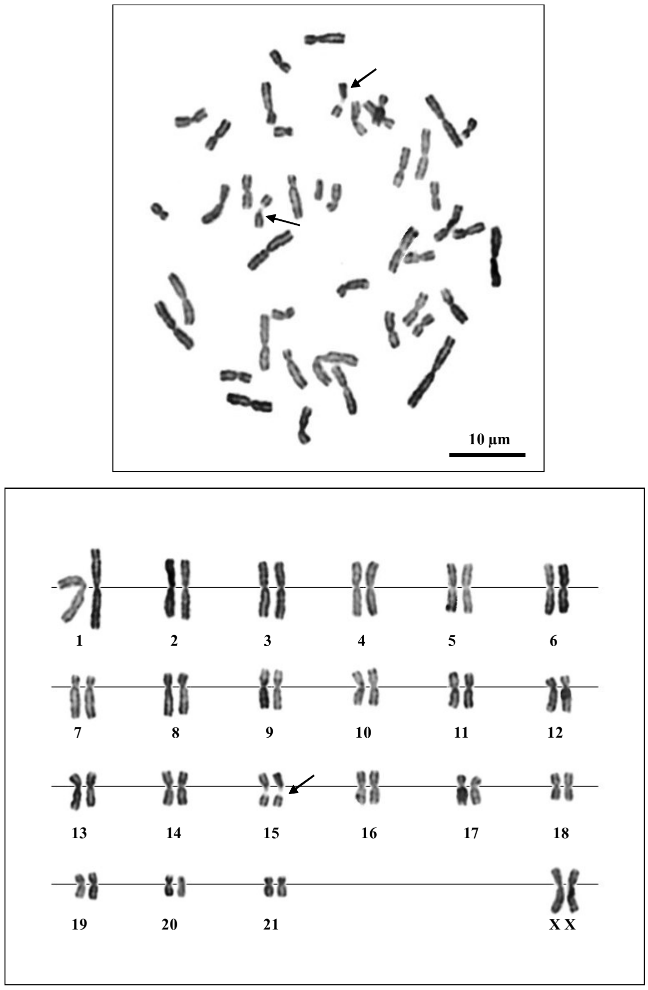

Figure 3. Metaphase chromosomes (A) and karyotype of female pileated gibbon (*Hylobates pileatus*) 2n=44, by conventional staining (B), arrows indicate satellite chromosomes.

gradually decreasing length of the autosomes (Figure 4). Different chromosomal features were reported by Liu *et al* (1996), that the pileated gibbon has 42 metacentric and submetacentric autosomes. Furthermore, Dutrillaux *et al.* (1975) also reported that the autosomes of the pileated gibbon has 24 metacentric and 18 submetacentric autosomes.

The X chromosome of the pileated gibbon is a medium submetacentric chromosome and the Y chromosome is the tiny acrocentric chromosome. These features are similar to the report by Dutrillaux *et al.* (1975) and Liu *et al.* (1996) that the pileated gibbon has a submetacentric X chromosome and a dot Y chromosome. In comparison with the other gibbon species in the genus *Hylobates* in Thailand, the X chromosomes of the *H. lar* and *H. agilis* are metacentric and submetacentric chromosomes, respectively. The Y chromosomes of those species are acrocentric and submetacentric chromosomes, respectively (Figure 5) (Hamerton *et al.*, 1963; Chiarelli, 1972; Warburton *et al.*, 1975).

Furthermore, the researches cited above elucidated that the shape and size variation on the sex chromosomes of gibbons in the genus *Hylobates*. According to those reports and this investigation, the X chromosome of *H. pileatus* 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 shapes. Occasionally, the centromere is not obvious and the type of the chromosome is difficult to classify. In contrast, this report clearly identified the Y chromosome of pileated gibbon.

In this investigation, the nucleolar organizer regions (NORs), which represents the chromosome marker, in located only on the long arms of the pair metacentric autosome 15. In contrast, Stanyon (1987) indicated that the NORs of pileated gibbon is present only on the short arms of the pair autosome 21. This difference may be due to the different methods of karyotyping and measuring. Chiarelli (1972) reported that the NORs of genus *Hylobates* in Thailand such as *H. lar* and *H. agilis*, were located on the short arms of the

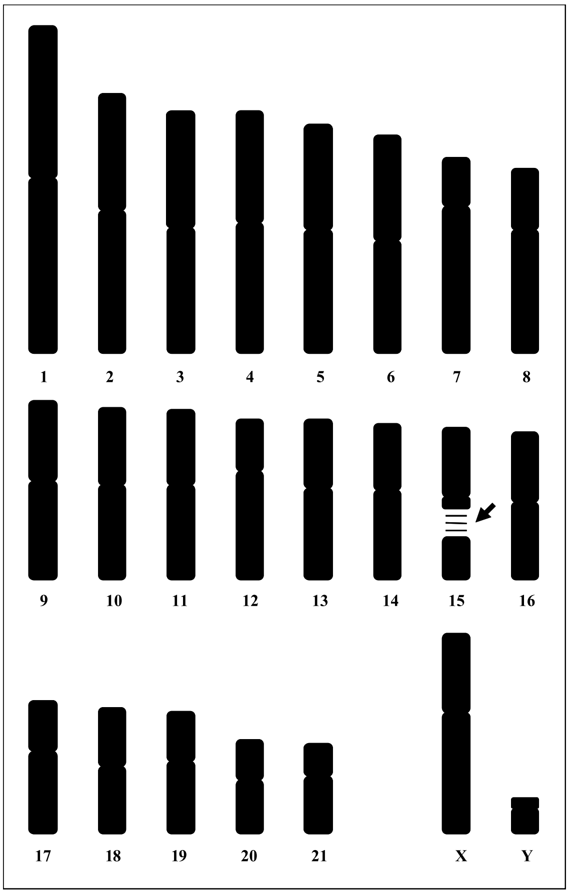

Figure 4. Idiogram of the pileated gibbon (*Hylobates pileatus*) by conventional staining, arrow indicate satellite chromosome.

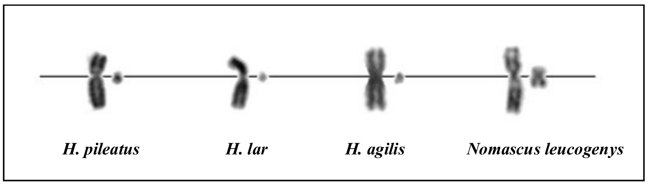

Figure 5. A comparison of the sex-chromosome of gibbon species in the family Hylobatidae in Thailand, pileated gibbon (*H. pileatus*), white-handed gibbon (*H. lar*), dark-handed gibbon (*H. agilis*) and Laos gibbon, white-cheeked gibbon (*Nomascus leucogenys*)

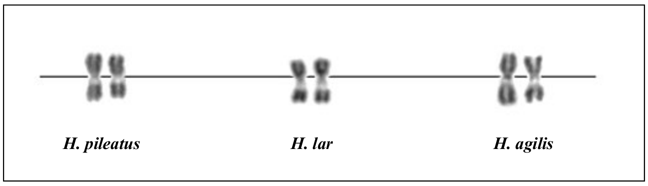

Figure 6. A comparison of the satellite chromosome (nucleolar organizer region, NOR) of gibbon species in the family Hylobatidae in Thailand, pileated gibbon (*H. pileatus*), white-handed gibbon (*H. lar*) and dark-handed gibbon (*H. agilis*).

pair autosomes 21, while Warburton *et al*. (1975) located NORs on the long arms of the pair autosomes 21 on the white-handed gibbon (Figure 6). The study by Jones *et al*. (1994) indicated that only 1 pair of NORs is found in the autosomes of gibbon and baboon. Stanyon (1983) also found that a pair of NORs were located at the pair autosome 13 in Japanese monkey (*Macaca fuscata*) and black mangabey (*Cercacebus aterrimus*), which are in the same family, but the size of the NORs is slightly different. In this study, the chromosome checks of the mitotic metaphase cells of the pileated gibbon in Thailand revealed that the chromosome marker is the chromosome pair 1, which are the largest metacentric chromosomes.

The length in centimeters of the chromosomes in mitotic metaphase cells for 20 cells in males and females 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 (SD) of RL, CI, size and type of chromosome in male and female of the pileated gibbon are presented in Tables 1 and 2. The idiogram of the pileated gibbon shows gradually decreasing length of the autosomes and sex chromosomes (Figure 4). The karyotype formula for the pileated gibbon was as follows:

$$2n\ (44) = L^{m}_{12}+M^{m}_{6}+M^{sm}_{2}+M^{a}_{2}+S^{m}_{12}+S^{sm}_{8}+\text{sex-chromosomes}$$

4. Conclusion

Cytogenetics of the pileated gibbon (*Hylobates pileatus*), the diploid chromosome number was 2n = 44, and the fundamental number (NF) were 88 chromosomes in both female and male. The autosomes consist of twelve large metacentric, six medium metacentric, two medium submetacentric, two medium acrocentric, twelve small metacentric and eight small submetacentric chromosomes. The X chromosome was a medium submetacentric chromosome and the Y chromosome was a tiny acrocentric chromosome. In addition, the chromosome pair 15 showed clearly observable satellite chromosomes.

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Table 1. The mean value of the short arm chromosome length (Ls), of the long arm chromosome length (Ll), of the total arm length of the chromosome (LT), of the relative length (RL) and of the centromeric index (CI). The standard deviation (SD) of RL and CI, the size and type of the chromosomes of 20 cells in male pileated gibbon (*Hylobates pileatus*), 2*n* = 44.

chromosome pair	Ls	Ll	LT	RL±SD	CI±SD	size	shape
1	1.56	1.86	3.42	0.080±0.001	0.544±0.020	L	m
2	1.28	1.56	2.84	0.066±0.007	0.549±0.020	L	m
3	1.22	1.36	2.58	0.060±0.003	0.527±0.029	L	m
4	1.17	1.36	2.53	0.059±0.003	0.538±0.015	L	m
5	1.12	1.33	2.45	0.057±0.002	0.543±0.020	L	m
6	1.12	1.23	2.35	0.055±0.003	0.523±0.015	L	m
7	0.53	1.56	2.09	0.049±0.003	0.746±0.027	M	a
8	0.67	1.31	1.98	0.046±0.002	0.662±0.027	M	sm
9	0.86	1.02	1.88	0.044±0.002	0.543±0.013	M	m
10	0.82	1.00	1.82	0.043±0.002	0.549±0.020	M	m
11	0.81	1.01	1.82	0.043±0.001	0.555±0.019	M	m
12	0.57	1.15	1.72	0.040±0.002	0.669±0.031	M	sm
13	0.74	0.96	1.70	0.040±0.003	0.565±0.024	S	m
14	0.69	0.97	1.66	0.039±0.002	0.584±0.011	S	m
15	0.75	0.88	1.63	0.038±0.002	0.540±0.015	S	m
16	0.73	0.83	1.56	0.037±0.003	0.532±0.025	S	m
17	0.52	0.91	1.43	0.033±0.002	0.636±0.027	S	sm
18	0.61	0.74	1.35	0.032±0.002	0.548±0.030	S	m
19	0.50	0.78	1.28	0.030±0.002	0.609±0.033	S	sm
20	0.43	0.65	1.08	0.025±0.002	0.602±0.023	S	sm
21	0.35	0.63	0.98	0.023±0.001	0.643±0.048	S	sm
X	0.85	1.31	2.16	0.051±0.003	0.606±0.034	M	sm
Y	0.11	0.30	0.41	0.010±0.002	0.732±0.089	S	a

Notes: large (L), medium (M), and small (S) chromosomes, metacentric (m), submetacentric (sm), and acrocentric (a) chromosomes

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Table 2. The mean value of the short arm chromosome length (Ls), of the long arm chromosome length (Ll), of the total arm length of the chromosome (LT), of the relative length (RL) and of the centromeric index (CI). The standard deviation (SD) of RL and CI, the size and type of the chromosomes of 20 cells in female pileated gibbon (*Hylobates pileatus*), $2n = 44$.

chromosome pair	Ls	Ll	LT	RL±SD	CI±SD	size	shape
1	1.77	1.93	3.70	0.081±0.004	0.522±0.011	L	m
2	1.32	1.64	2.96	0.064±0.001	0.554±0.009	L	m
3	1.29	1.52	2.81	0.061±0.003	0.541±0.016	L	m
4	1.35	1.43	2.78	0.061±0.003	0.514±0.017	L	m
5	1.21	1.43	2.64	0.057±0.002	0.542±0.027	L	m
6	1.21	1.30	2.51	0.055±0.002	0.518±0.010	L	m
7	0.57	1.72	2.29	0.050±0.002	0.751±0.019	M	a
8	0.70	1.43	2.13	0.046±0.001	0.671±0.019	M	sm
9	0.94	1.16	2.10	0.046±0.002	0.552±0.019	M	m
10	0.91	1.09	2.00	0.044±0.002	0.545±0.012	M	m
11	0.88	1.10	1.98	0.043±0.002	0.556±0.016	M	m
12	0.61	1.25	1.86	0.040±0.002	0.672±0.027	M	sm
13	0.77	1.05	1.82	0.040±0.001	0.577±0.029	S	m
14	0.77	1.02	1.79	0.039±0.002	0.570±0.017	S	m
15	0.79	0.98	1.77	0.039±0.002	0.554±0.017	S	m
16	0.81	0.91	1.72	0.037±0.002	0.529±0.031	S	m
17	0.61	0.93	1.54	0.034±0.002	0.604±0.029	S	sm
18	0.70	0.78	1.48	0.032±0.001	0.529±0.014	S	m
19	0.55	0.90	1.45	0.032±0.002	0.621±0.018	S	sm
20	0.48	0.74	1.22	0.027±0.001	0.607±0.028	S	sm
21	0.41	0.66	1.07	0.023±0.001	0.617±0.032	S	sm
X	0.91	1.41	2.32	0.051±0.002	0.608±0.009	M	sm

Notes: large (L), medium (M), and small (S) chromosomes, metacentric (m), submetacentric (sm), and acrocentric (a) chromosomes

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