# Standardized Karyotype and Idiogram of the Agile Gibbon, *Hyloba Tesagilis* (Primate, Hylobatidae) by Conventional Staining Technique

Krit Pinthong[1*] and Sarawut Kaewsri[2]

## Abstract

Standardized karyotype and idiogram of the agile gibbon (*Hylobates agilis*) at Songkla Zoo, was studied. Blood sample were taken from 1 female and 2 males agile gibbon. After lymphocyte culture, the mitotic chromosome preparation was done by air-drying method and conventional Giemsa's staining technique. The results show that diploid chromosome number is 2*n*=44, and the fundamental number (NF) are 88 in both female and male. The autosomes consist 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 near centromere of chromosome 12 showed clearly observable satellite chromosomes. The X-chromosome is the medium submetacentric and the Y-chromosome is the smallest acrocentric chromosome. The karyotype formula for the agile gibbon is as follows:

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

**Keywords:** karyotype, idiogram, chromosome, agile gibbon, *Hylobates agilis*

[1]Biology Program, Department of Fundamental Science, Faculty of Science, SurinRajabhat University

[2]Biology Program, Faculty of Science, BurirumRajabhat University

[*]Corresponding author, e-mail: k_pinthong@yahoo.com

## 1. Introduction

There are 12 species of gibbon in the world and subdivided into 4 genera, namely *Hylobates* (2*n*=44), *Hoolock* (2*n*=38), *Nomascus* (2*n*=52) and *Symphalangus* (2*n*=50) (Wilson & Cole, 2002; Geissmann, 2002; Mootnick & Grove, 2005). Three species have been found in Thailand including white-handed gibbon (*H. lar*), pileated gibbon (*H. pileatus*) and agile gibbon or dark-handed gibbon (*H. agilis*) that are only in the lar group (Lekagul & McNeely, 1977, 1988; Brokelman, 1981).

The common characteristics of the agile gibbon are closely resemble *H. lar*, but without white hands and with much less white around the face. The species is asexually dimorphic, with one phase buff to light gray, the other black or dark brown, almost maroon. The paler forms often have the under parts and inner sides of the limbs darker, while in the dark phase, the rump is palest (Lekagul and McNeely, 1977, 1988).

According to the cytogenetic studies of agile gibbon by Chiarelli (1972); Stanyon *et al*. (1987); Van Tuinen *et al*. (1999); Hirai *et al*. (2003, 2005); Tanomtong *et al*. (2008) and Supanuam *et al*. (2012) found that the cytogenetics of the agile gibbon in Thailand has not been studied. In this study, we confirm and compare the results with previous report. In addition to, this is the first report about chromosome measuring for determining 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.

## 2. Materials and methods

Blood samples from the jugular vein were collected from 1 female and 2 males agile gibbon, which were kept from Songkla Zoo, Songkla Province using aseptic technique. The samples were kept in 10 ml vacuum tubes containing heparin to prevent blood clotting and 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) & Campiranont (2003).

### *2.2 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°C under 5% of carbon dioxide environment and regularly shaken in the morning and evening. When reaching harvest time at the 72$^{nd}$ hour

of incubation, colchicine was added and well mixed, followed by further incubation for 30 minutes.

### *2.3 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. KCl was discarded with the supernatant 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.

### *2.4 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) and Wada *et al.* (1991).

## 3. Results and discussion

Cytogenetic study of the agile gibbon using lymphocyte culture and the conventional staining procedures revealed that the chromosome number is 2*n* (diploid) = 44, which consists of 42 autosomes and 2 sex chromosomes (X and Y-chromosome). This is the same chromosome number for the agile gibbon as reported by Chiarelli (1972); Stanyon *et al.* (1987); Van Tuinen *et al.* (1999; Hirai *et al.*(2003, 2005; Tanomtong *et al.* (2008) and Supanuam *et al.* (2012). 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*) are 2*n*=44. Furthermore, Stanyon (1987) also reported that the chromosome numbers of *H. lar*, *H. Agilis*, *H. moloch*, *H. muelleri*

and *H. klossii* are 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 44, 38, 52 and 50, respectively (Geissmann, 2002).

This examination also revealed that the fundamental number, NF (number of chromosome arms) of the agile gibbon is 88 in male and female. This is the same NF for the agile gibbon as reported by Chiarelli (1972). The chromosomes in mitotic metaphase plate and the karyotype of the agile gibbon are shown in Figure 2.

The agile gibbon has 3 types of autosomes, which are 28 metacentric, 12 submetacentric and 2 acrocentric chromosomes. The 28 metacentric autosomes are classified by size into 12 large, 8 medium and 8 small chromosomes, the 10 submetacentric autosomes are classified by size into 4 medium and 8 small chromosomes while the 2 acrocentric autosomes are distinguished to be 2 medium chromosomes. Difference chromosomal features were reported by Chiarelli (1972), which indicated that the agile gibbon had 24 metacentric and 18 submetacentric autosomes. The idiogram of the agile gibbon shows the gradually decreasing length of the autosomes (Figure 3).

The important chromosome marker of the agile gibbon is the asymmetrical karyotype, which is all 3 types of chromosomes are found (metacentric, submetacentric and acrocentric chromosomes). The largest and smallest chromosomes show high size difference (approximately 9 folds). The largest and second chromosomes are metacentric chromosomes, and the Y-chromosome is the smallest acrocentric chromosome.

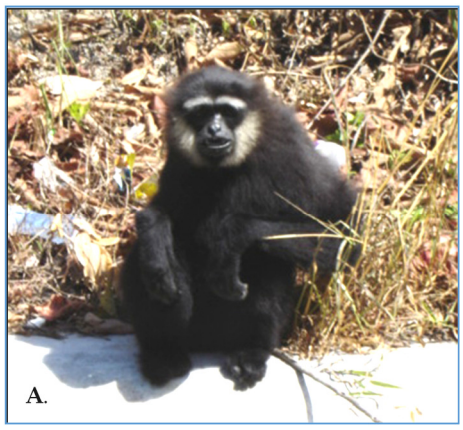
The X-chromosome of the agile gibbon is a medium submetacentric chromosome and the Y-chromosome is the smallest acrocentric chromosome. These features are difference to that reported by Chiarelli (1972) indicating that an agile gibbon had a submetacentric X-chromosome and a submetacentric Y- chromosome. In comparison with gibbon species in the genus *Hylobates* in Thailand, that the X-chromosomes of the *H. lar* and *H. pilaetus* are metacentric or submetacentric chromosomes. The Y-chromosomes of those species are submetacentric or acrocentric chromosomes (Bender & Chu, 1963; Hamerton *et al*., 1963; Chiarelli, 1972; Warburton *et al*., 1975 & Supanuam *et al*., 2007). Dutrillaux *et al*. (1975) indicated that the Y chromosomes of the members of the genus *Hylobates* are tiny (dot like) 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 & 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 near centromere of the pair metacentric autosomes 12. In contrast, Stanyon (1987) indicated that the NORs of lar group gibbons present of the pair autosome 21. This difference may due to the different methods of karyotyping and measuring. Charelli (1972) reported that the NORs of genus *Hylobates* in Thailand, *H. lar* and *H.agilis*, located on the short arms of the pair autosomes 21, while Warburton *et al*. (1975) indicated that the NORs located on the long arms of the pair autosomes 21. The study by Jones *et al*. (1994) indicated that only 1 pair of NORs is found in the autosomes of gibbons and baboons. Supanuam *et al*. (2012) reported that the *H. Agilis* had NORs on the short arms of chromosome pair 10 (submetacentric chromosomes).

After measuring the length in centimeters of the chromosomes in mitotic metaphase plate 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 agile gibbon show in tables 1 and 2. The idiogram of the agile gibbon shows gradually decreasing length of the autosomes and sex chromosomes. The karyotype formula for the agile gibbon was as follows:

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




A. The male agile gibbon


B. The female agile gibbon

**Figure 1** The male (A) and female (B) agile gibbon, *Hylobates agilis* (Cuvier, 1821) (Primate, Hylobatidae) from Songkla Zoo, Thailand.

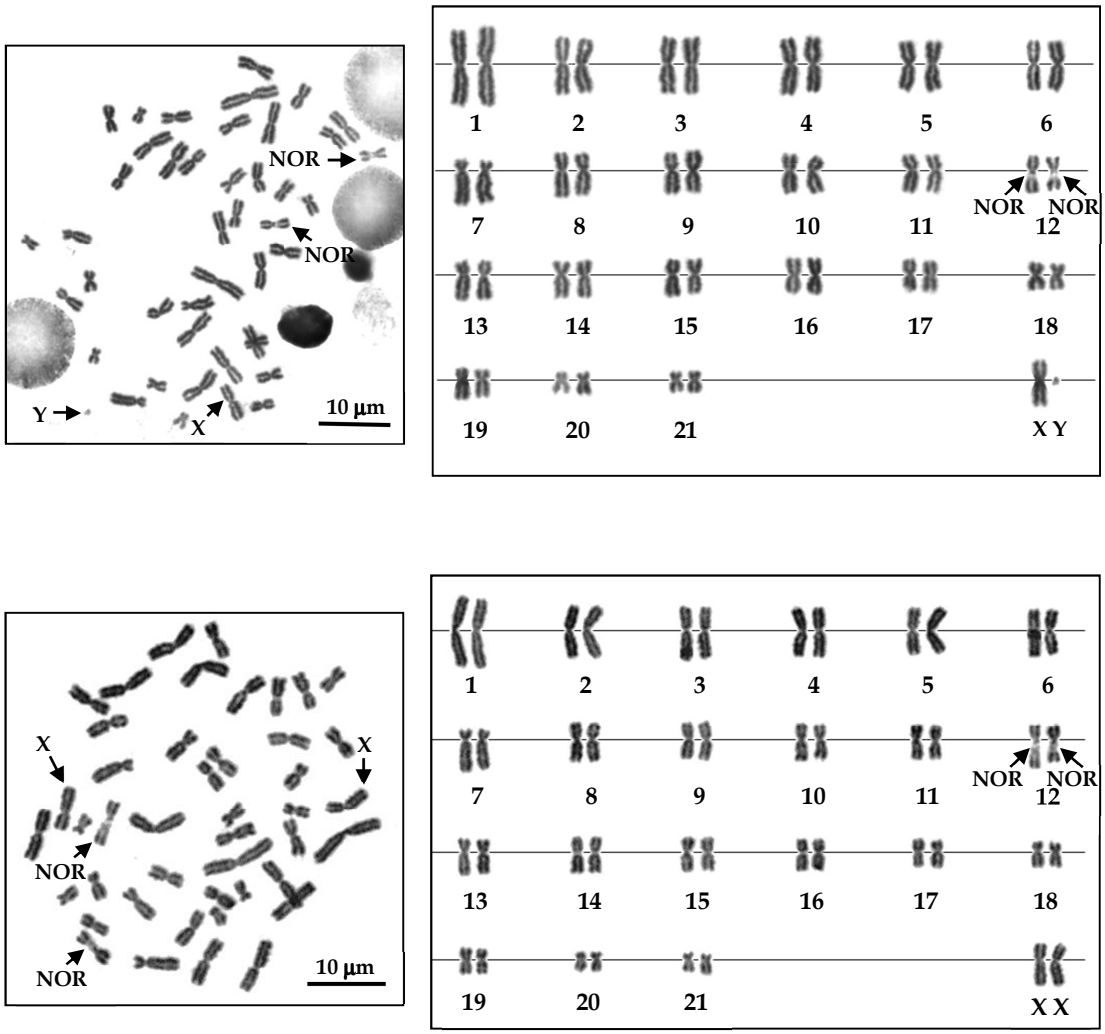



**Figure 2** Metaphase chromosome plates and karyotypes of the male (A) and female (B) Agile gibbon (*Hylobates agilis*) 2*n* (diploid) = 44 by conventional staining technique, arrows indicate satellite chromosomes (nucleolar organizer regions) and sex- chromosomes

**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 agile gibbon (*Hylobates agilis*) 2*n* (diploid) = 44.

| Chromosome pair | Ls | LL | LT | RL±SD | CI±SD | Chromosome Size | Chromosome Type |
|---|---|---|---|---|---|---|---|
| 1 | 1.79 | 1.97 | 3.76 | 0.080±0.001 | 0.524±0.013 | Large | Metacentric |
| 2 | 1.29 | 1.59 | 2.88 | 0.061±0.003 | 0.552±0.011 | Large | Metacentric |
| 3 | 1.37 | 1.47 | 2.84 | 0.061±0.002 | 0.518±0.021 | Large | Metacentric |
| 4 | 1.26 | 1.51 | 2.77 | 0.059±0.002 | 0.545±0.017 | Large | Metacentric |
| 5 | 1.22 | 1.37 | 2.59 | 0.055±0.003 | 0.529±0.023 | Large | Metacentric |
| 6 | 1.12 | 1.41 | 2.53 | 0.054±0.002 | 0.557±0.009 | Large | Metacentric |
| 7 | 0.57 | 1.71 | 2.28 | 0.049±0.001 | 0.750±0.036 | Medium | Acrocentric |
| 8 | 0.96 | 1.26 | 2.22 | 0.047±0.001 | 0.568±0.011 | Medium | Metacentric |
| 9 | 1.06 | 1.14 | 2.20 | 0.047±0.001 | 0.518±0.008 | Medium | Metacentric |
| 10 | 0.90 | 1.14 | 2.04 | 0.043±0.003 | 0.559±0.021 | Medium | Metacentric |
| 11 | 0.86 | 1.15 | 2.01 | 0.043±0.002 | 0.572±0.018 | Medium | Metacentric |
| 12* | 0.77 | 1.23 | 2.00 | 0.043±0.002 | 0.615±0.031 | Medium | Submetacentric |
| 13 | 0.75 | 1.25 | 2.00 | 0.043±0.001 | 0.625±0.032 | Medium | Submetacentric |
| 14 | 0.80 | 1.08 | 1.88 | 0.040±0.002 | 0.574±0.015 | Small | Metacentric |
| 15 | 0.77 | 1.05 | 1.82 | 0.039±0.003 | 0.577±0.016 | Small | Metacentric |
| 16 | 0.85 | 0.95 | 1.80 | 0.038±0.001 | 0.528±0.010 | Small | Metacentric |
| 17 | 0.55 | 0.98 | 1.53 | 0.033±0.001 | 0.641±0.024 | Small | Submetacentric |
| 18 | 0.69 | 0.81 | 1.50 | 0.032±0.002 | 0.540±0.018 | Small | Metacentric |
| 19 | 0.57 | 0.86 | 1.43 | 0.030±0.001 | 0.601±0.034 | Small | Submetacentric |
| 20 | 0.45 | 0.69 | 1.14 | 0.024±0.001 | 0.605±0.020 | Small | Submetacentric |
| 21 | 0.38 | 0.71 | 1.09 | 0.023±0.002 | 0.651±0.044 | Small | Submetacentric |
| X | 0.88 | 1.36 | 2.24 | 0.048±0.001 | 0.607±0.032 | Medium | Submetacentric |
| Y | 0.10 | 0.34 | 0.44 | 0.009±0.001 | 0.773±0.015 | Small | Acrocentric |

Remark: *satellite chromosome (nucleolar organizer region, NOR)

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**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 agile gibbon (*Hylobates agilis*) 2*n* (diploid) = 44.

| Chromosome pair | Ls | LL | LT | RL±SD | CI±SD | Chromosome Size | Chromosome Type |
|---|---|---|---|---|---|---|---|
| 1 | 1.86 | 2.07 | 3.93 | 0.081±0.003 | 0.527±0.011 | Large | Metacentric |
| 2 | 1.33 | 1.67 | 3.00 | 0.062±0.001 | 0.557±0.007 | Large | Metacentric |
| 3 | 1.48 | 1.51 | 2.99 | 0.061±0.002 | 0.505±0.012 | Large | Metacentric |
| 4 | 1.33 | 1.58 | 2.91 | 0.060±0.002 | 0.543±0.008 | Large | Metacentric |
| 5 | 1.26 | 1.42 | 2.68 | 0.055±0.002 | 0.530±0.015 | Large | Metacentric |
| 6 | 1.13 | 1.47 | 2.60 | 0.053±0.001 | 0.565±0.012 | Large | Metacentric |
| 7 | 0.57 | 1.78 | 2.35 | 0.048±0.002 | 0.757±0.017 | Medium | Acrocentric |
| 8 | 1.03 | 1.32 | 2.35 | 0.048±0.001 | 0.562±0.018 | Medium | Metacentric |
| 9 | 1.10 | 1.23 | 2.33 | 0.048±0.001 | 0.528±0.019 | Medium | Metacentric |
| 10 | 0.92 | 1.24 | 2.16 | 0.044±0.002 | 0.574±0.010 | Medium | Metacentric |
| 11 | 0.88 | 1.27 | 2.15 | 0.044±0.001 | 0.591±0.013 | Medium | Metacentric |
| 12* | 0.94 | 1.15 | 2.09 | 0.043±0.002 | 0.608±0.028 | Medium | Submetacentric |
| 13 | 0.81 | 1.28 | 2.09 | 0.043±0.001 | 0.612±0.033 | Medium | Submetacentric |
| 14 | 0.81 | 1.15 | 1.96 | 0.040±0.001 | 0.587±0.015 | Small | Metacentric |
| 15 | 0.79 | 1.11 | 1.90 | 0.039±0.002 | 0.584±0.016 | Small | Metacentric |
| 16 | 0.88 | 1.02 | 1.90 | 0.039±0.002 | 0.537±0.027 | Small | Metacentric |
| 17 | 0.61 | 1.01 | 1.62 | 0.033±0.003 | 0.623±0.027 | Small | Submetacentric |
| 18 | 0.69 | 0.83 | 1.52 | 0.031±0.001 | 0.546±0.012 | Small | Metacentric |
| 19 | 0.57 | 0.88 | 1.45 | 0.030±0.002 | 0.607±0.025 | Small | Submetacentric |
| 20 | 0.48 | 0.73 | 1.21 | 0.025±0.001 | 0.603±0.035 | Small | Submetacentric |
| 21 | 0.38 | 0.74 | 1.12 | 0.023±0.002 | 0.661±0.024 | Small | Submetacentric |
| X | 0.91 | 1.43 | 2.34 | 0.048±0.002 | 0.611±0.010 | Medium | Submetacentric |

Remark: *satellite chromosome (nucleolar organizer region, NOR)

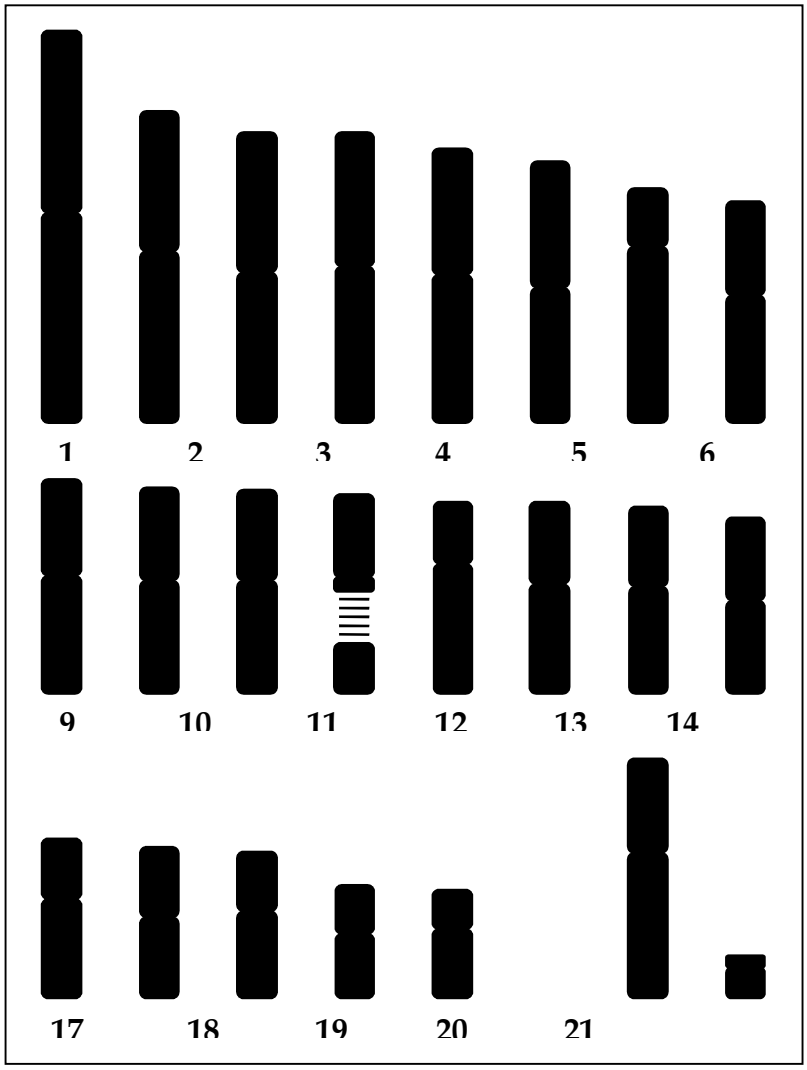


**Figure 3** Idiogram of agile gibbon (*Hylobates agilis*) 2*n* (diploid) = 44 by conventional staining technique, arrow indicate satellite chromosome (Nucleolar organizer region, NOR).

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