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# Cytogenetic analysis of Black Shark Minnow, *Labeo chrysophekadion* (Bleeker, 1850) (Cypriniformes: Cyprinidae) in Thailand

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## Abstract

In the present study, we investigated the karyotype and chromosomal characteristic of nucleolar organizing regions (NORs) in *Labeo chrysophekadion* from Mae khong River, Nong Khai Province, Thailand. The metaphase chromosomes were prepared from kidney cells of five male and five female fish. Conventional and Ag–NOR staining techniques were applied to the chromosomes. Karyological analysis revealed that 2*n*= 50 and NF= 86. The karyotype of *L. chrysophekadion* comprising five pair of metacentric, three pairs of submetacentric, ten pairs of acrocentric and seven pairs of telocentric chromosomes. Thus, the chromosome formula could be suggested as 2*n*=50, 10m+6sm+20a+14t. There are two pairs of NOR–bearing chromosomes which are located at the telomeric position of the short arm of the acrocentric chromosome pair 12 and 13. The chromosome characters between male and female fish cannot be distinguished by conventional and silver straining. The number of active NORs in metaphase cell varies between two and four among the observed metaphase cells in the same sample.

**Keywords:** Black Shark Minnow, *Labeo chrysophekadion*, Karyotype, Chromosome, NOR

## Introduction

*Labeo chrysophekadion*, known as Black shark minnow, is the member of subfamily Labeoninae, tribe Labeonini, distributed mainly in Asian region; the Mekong and Chao Phraya

Basin, Malay Peninsula, Sumatra, Java and Borneo and the Tonle Sap in Cambodia (Romero, 2002). *L. chrysophekadion* is a predominantly herbivorous species occurring in flowing and standing water (Rainboth, 1996).

Cytogenetics has become popular in fish classification such as cyprinids fish group (Yang et al., 2015). Karyological studies of fishes provide the comprehensive knowledge to solve the problem in many areas (Alsabti, 1985), such as taxonomy, systematic, phylogenetic relationship (Campiranont, 2003), evolution (Tanomtong et al., 2014; Cioffi et al., 2015) and environmental toxicology (Ishidate et al., 1998).

In the genus *Labeo*, karyotype has been reported in 17 species. The 2*n* number varies between 48 and 54, while the NF varies between 72 and 90. Most of them, 13 species, have 2*n*=50, but their chromosome morphology and NF number are different. The lowest 2*n* number, 2*n*=48, is found in *L. caeruleus* and *L. dero*. The highest 2*n* number, 2*n*=54, is found in *L. gonius*. Intra-species variation of 2*n* can be found only in *L. dero*, 2*n*=48 and 50. The knowledge of the karyotype and chromosomal characteristic of NOR in *L. chrysophekadion* (Bleeker, 1850) would also improve our understanding of karyotype diversity and chromosomal evolution of the Cyprinids.

## Research Objectives

1. To investigate the karyotype of *L. chrysophekadion* (Bleeker, 1850) from Thailand.
2. To investigate the chromosomal characteristic of NOR in *L. chrysophekadion* (Bleeker, 1850) from Thailand.

## Materials and methods

### Chromosome preparation

The samples of *L. chrysophekadion* (five males and five females) were obtained from Mae khong River, Nong Khai Province, Thailand. The fish were alive when transported to the laboratory and were kept for 72 hrs. prior to processing. The metaphase chromosomes were prepared *in vivo*. The protocol is adapted from the method of Chen and Ebeling (1968) and Nanda et al. (1995). Colchicine solution (0.05% w/v) was injected into the intramuscular and/or abdominal cavity (1 ml/100 g body weight) and left for 1 hr. Kidney, especially the anterior portion of the kidney, was removed and cut into small pieces. Then, kidney tissue was mixed with 0.075 M KCl. After the large pieces of tissues were discarded, cell sediment was transferred to centrifuge tubes and 0.075 M KCl was added up to 7 ml. Thereafter, cell

suspension was incubated at room temperature for 30 minutes. When the incubation process is done, the KCl activities were stopped by adding 0.2 ml of fresh and cold fixative solution (3: 1 v/v methanol: glacial acetic acid) into cell suspension. Cell suspension was centrifuged at 1,200 rpm for eight minutes. After discarded supernatant is discarded, cell sediment was rinsed and mixed with fixative solution again. The fixative solution was gradually filled up to 7 ml before recentrifugation at 1,200 rpm for eight minutes. The fixation process was repeated until the supernatant is clear. The obtained cell sediments were used for a further step.

**Chromosome staining**

**Conventional staining technique (Rooney, 2001)**

The mixture was dropped onto a clean and cold slide by micropipette, following by the air-dry technique. The slide was conventionally stained with 20% Giemsa's solution for 30 minutes.

**Ag-NOR banding technique (Howell and Black, 1980)**

The two drops of a colloidal developer solution (1% of pure formic acid in 2% of gelatin solution) and four drops of an aqueous silver nitrate solution (50% w/v $AgNO_3$ in deionized water) was dropped onto the microscope slide containing chromosome preparation. The slide was covered with a coverslip and incubated for five minutes at 60 °C. The staining solution turns to yellow-brown. The slide was rinsed off under running deionized water and air-dried.

**Chromosomal checks**

The slide was analysed under the light microscope. Twenty clearly observable and well-spread chromosomes of each male and female were selected and photographed. The types of chromosomes were classified according to Turpin and Lejeune (1965) and Chaiyasut (1989).

**Karyotyping**

The chromosome pictures were used for the homologous chromosome pairing. The determination of chromosome centromere was determined for chromosome pairing. Then, each length of long arm and short arm chromosome was measured and computed for the length of each chromosome. Karyotyping arrangement was ranged from long to short, except for sex chromosomes which always are the latest pair and show at the right bottom side of the range. The number of each chromosome is at the bottom of each picture. For the arrangement, short arm is on top where long arm is at the bottom of each chromosome picture.

## Results and discussion

### Karyotype

The conventional stained metaphase chromosomes and karyotypes of males and females *L. chrysophekadion* are shown in Figure 1. The results revealed 2*n*=50 and NF=86 in all specimens. The chromosomes of metaphase complement of *L. chrysophekadion* could also be aligned into 25 homomorphic pair of decreasing lengths comprising five pair of metacentric, three pairs of submetacentric, ten pairs of acrocentric and seven pairs of telocentric chromosomes. Thus, the chromosome formula could be suggested as 2*n*= 50, 10m+ 6sm+ 20a+ 14t. Karyotype of male and female fish cannot be distinguished by conventional and silver straining. So, no sex chromosome could be identified in *L. chrysophekadion*. The present finding of 2*n*=50 in *L. chrysophekadion* is in agreement with earlier report (Muramoto et al., 1968). However, there are slightly differences in the number of metacentric, acrocentric and telocentric chromosome. These differences may be due to several factor such as the chromosomal evolution, the intra-species chromosome variation and chromosomal misidentification. For example, acrocentric chromosome is easily be misidentified as telocentric chromosome if they are constricted at high degree which is caused by over exposure to colchicine (Zhang and Reddy, 1991). Study of karyotype of different population should be performed to confirm the variation of chromosomal characteristic of *L. chrysophekadion*. As a result, asymmetric karyotype, four pairs of metacentric and four pair of submetacentric chromosomes could easily be recognized and could be used as chromosome marker of *L. chrysophekadion*.

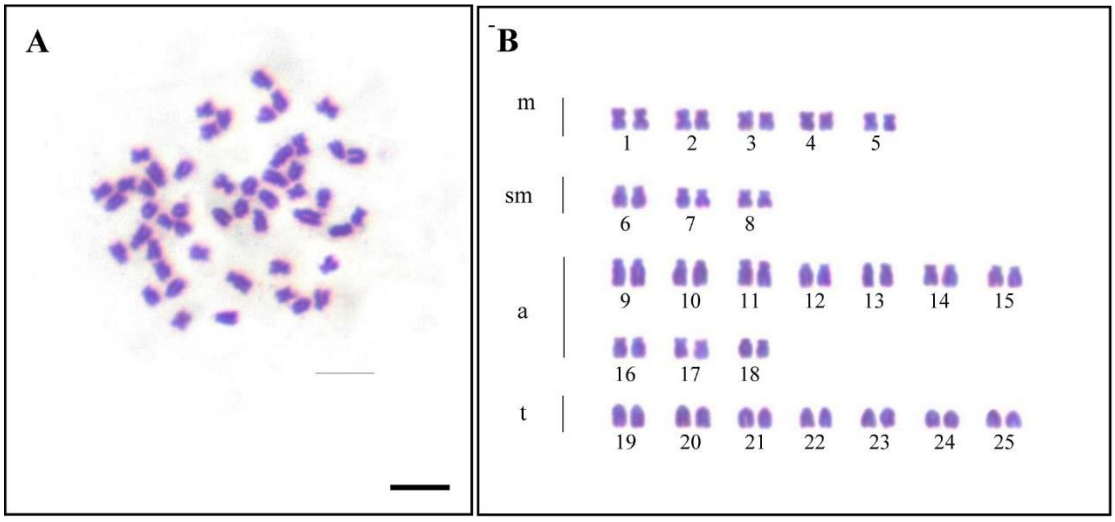


**Figure 1** Metaphase chromosome plate (A) and karyotype (B) by conventional staining of male *L. chrysophekadion* 2*n*=50. Scale bars=5 $\mu$m.

**Ag-NOR**

Two pairs of NORs were detected in *L. chrysophekadion*. The NORs are located at the short arm of the acrocentric chromosome pair 12 and 13 (Figure 2). Two pairs of NOR have been reported in *L. dussumieri* (Nagpure et al., 2003), while most of their congeners such as *L. bata* (John et al., 1993), *L. calbasu* (Rishi and Rishi, 1981) and *L. gonius* (Lakra and Rishi, 1991) have only one pair. Whereas the number of NOR-bearing chromosomes is as a rule species-specific and constant, the variation of NOR at the intraspecific level has been reported in many species including fish (Garrido-Ramos et al., 1995). In present study, fifty well spread silver-stained metaphase chromosome were observed to evaluate the Ag-NOR polymorphism. The result revealed that Ag-NOR in each cell varies between two and four. Two pairs of NORs-bearing chromosomes in *L. chrysophekadion* are different from the majority of fishes in genus *Labeo*, which has only one pair of NOR. The increasing of NOR-bearing chromosome in genus *Labeo* may be caused by the translocation between some parts of chromosomes having NOR and another chromosome (Sharma et al., 2002). Further study including additional species of *Labeo* of different geographical locations, employing different staining techniques such as fluorescence *in situ* hybridization to investigate the distribution of repetitive DNA sequences in genome (Feldman and Levy 2005), the knowledge obtained should provide a better understanding of the chromosomal evolution in the genus *Labeo*

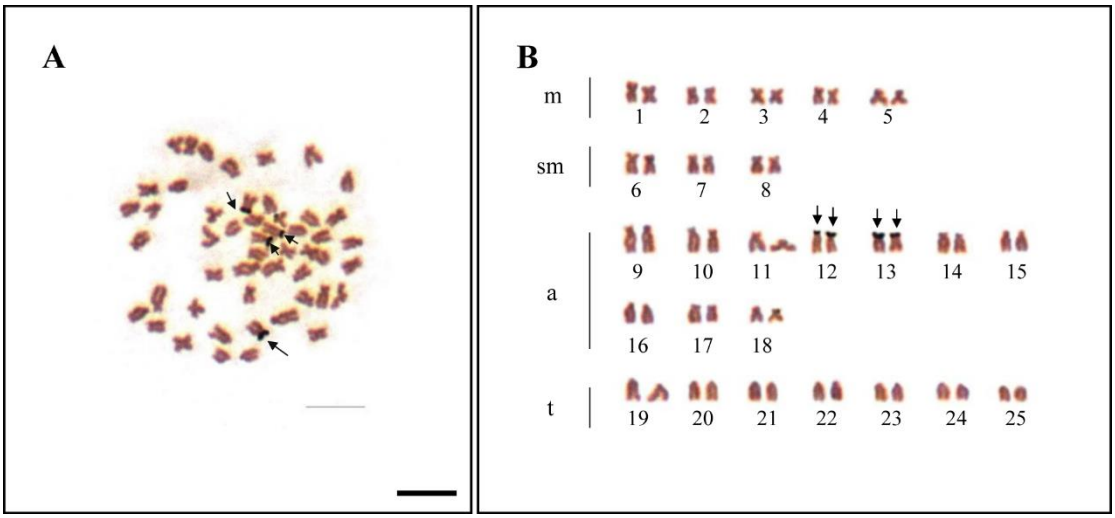


**Figure 2** Metaphase chromosome plate (A) and karyotype (B) of male *L. chrysophekadion* by Ag-NOR straining technique, arrows indicate Ag–NORs on the chromosome pairs 12 and 13. Scale bars = 5 $\mu$m**.**

## Conclusion

The 2*n* of *L. chrysophekadion* was 50, NF was 86, the karyotype formula was 2*n* = 50, 10m+6sm+20a+14t. The NORs are located at the telomeric position of the short arm of the acrocentric chromosome pair 12 and 13. The conventional and silver straining karyotype cannot be separate male and female.

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