รายงานพันธุศาสตร์ระดับเซลล์ครั้งแรกของปลานวลจันทร์เทศ (*Cirrhinus cirrhosus,* Cyprinidae) ด้วยการย้อมสีแบบธรรมดาและการย้อมแถบซิลเวอร์นอร์

First Cytogenetic Report of Mrigal Carp (*Cirrhinus cirrhosus*, Cyprinidae) by Conventional Staining and Ag-NOR banding

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บทคัดย่อ

การศึกษานี้เป็นการศึกษาพันธุศาสตร์ระดับเซลล์ครั้งแรกของปลานวลจันทร์เทศ (*Cirrhinus cirrhosus*) ด้วยการย้อมสีแบบธรรมดาและการย้อมแถบซิลเวอร์นอร์ โดยเตรียมโครโมโซมจากไตและเหงือกของตัวอย่างปลาเพศผู้ 10 ตัว และเพศเมีย 10 ตัว จากอำเภอเมือง จังหวัดอุบลราชธานี จากนั้นย้อมสีโครโมโซมด้วยสีจิมซ่าและซิลเวอร์ไนเตรต ผลการศึกษาพบว่าปลานวลจันทร์เทศมีโครโมโซมดิพลอยด์เท่ากับ 50 แท่ง มีจำนวนโครโมโซมพื้นฐานเท่ากับ 72 โครโมโซมประกอบด้วยซนิดซับเมทาเซนทริกขนาดใหญ่ 12 แท่ง เทโลเซนทริกขนาดใหญ่ 8 แท่ง เมทาเซนทริกขนาดกลาง 10 แท่ง เทโลเซนทริกขนาดกลาง 14 แท่ง และเทโลเซนทริกขนาดเล็ก 6 แท่ง ไม่พบความแตกต่างของโครโมโซมเพศ นอกจากนี้พบตำแหน่งนอร์บนแขนข้างยาวใกล้กับบริเวณเทโลเมียร์ของโครโมโซมคู่ที่ 6 และสูตรแคริโอไทป์ของปลานวลจันทร์เทศแสดงได้ดังนี้

$$2n\ (50) = L^{sm}_{12} + L^{t}_{8} + M^{m}_{10} + M^{t}_{14} + S^{t}_{6}$$

คำสำคัญ: ปลานวลจันทร์เทศ แคริโอไทป์ นอร์

Abstract

This study was the first cytogenetic study of mrigal carp (*Cirrhinus cirrhosus*) by conventional staining and Ag-NOR banding. Fish chromosome preparation was conducted by squash technique. Gill and kidney tissues were taken from ten male and ten female fish from Muang district, Ubon Ratchathani province. The metaphase spreads were performed on microscopic slide and air-dried. Giemsa staining and Ag-NOR banding techniques were applied to stain the chromosome. Results showed that the diploid chromosomes number of *C. cirrhosus* was 2n=50, the fundamental numbers (NF) was 72 in both male and female. The type of chromosomes can be defined as 12 large submetacentric, 8 large telocentric, 10 medium metacentric, 14 medium telocentric and 6 small telocentric chromosomes. There were no strange size chromosomes related to sex. The long arm

near telomere of chromosome pair 6 showed clearly observable nucleolar organizer regions (NORs). The karyotype formula of *C. cirrhosus* was as follows:

$$2n\ (50) = L^{sm}_{12} + L^{t}_{8} + M^{m}_{10} + M^{t}_{14} + S^{t}_{6}$$

Key words*: Cirrhinus cirrhosus*, Karyotype, NORs

Introduction

The genus *Cirrhinus* is native to freshwater in South Asia, Indochina and southern China. There are about 11 species including *C. caudimaculatus, C. cirrhosis*, *C. fulungee*, *C. inornatus*, *C. jullieni*, *C. macrops*, *C. microlepis*, *C. molitorella*, *C. mrigala*, *C. reba* and *C. rubirostris*. In Thailand, there are 5 species of this genus i.e. *C. caudimaculatus, C. cirrhosis*, *C. jullieni*, *C. microlepis* and *C. molitorella* (Roberts, 1997; Menon, 1999; Kottelat, 2001).

Mrigal carp (*Cirrhinus cirrhosus*) is a species of ray-finned fish in the family Cyprinidae. This fish distribution is a native to streams and rivers in India but it has been introduced to many countries in Southeast Asia such as Myanmar, Thailand, Laos and Cambodia. The IUCN (International Union foe Conservation of Nature) evaluated this fish as vulnerable (Roberts, 1997; Kottelat, 2001).

The cytogenetic study of the genus *Cirrhinus* has been reported from 6 species including *C. caudimaculatus, C. jullieni*, *C. microlepis*, *C. mrigala*, *C. reba* and *C. molitorella* (Manna and Khuda-Bukhsh, 1977; Wu *et al*., 1989; Zhang and Reddy, 1991; Magtoon and Arai, 1993; Ren and Yu, 1993; Donsakul and Magtoon, 1997; Nangpure *et al.*, 2001; Donsakul *et al.*, 2007; Biswal *et al.*, 2008; Saravanan *et al.*, 2012; Yadav *et al.*, 2013; Bhatnagar *et al.*, 2014; Nadini *et al.*, 2014; Neeru *et al.*, 2018). However, the cytogenetics study of this *C. cirrhosus* is inadequate.

The present study aimed for a cytogenetic analysis of *C. cirrhosus*. From the present study, we exhibited the standardization of karyotype and idiogram. This report described the first chromosome staining by conventional staining and Ag-NOR banding techniques in this species. The Ag-NOR banding technique is to reach out the NORs which is the representative location of genes (loci) that function in ribosome synthesis (18S and 28S ribosomal RNA) (Sobit *et al*., 2002). The NORs could be used to distinguish species with similar karyotypes. The obtained results can provide more cytogenetic information for future studies on taxonomy and evolutionary relationships of this genus and family. Moreover, it provides useful basic information for the conservation and breeding practices. Cytogenetics can provide invaluable insight for the management of threatened species. Matings between individuals characterized by different cytotypes can result in perinatal mortality or,

at a later stage, in reduced fertility of offspring heterozygous for chromosomal rearrangements (Potter and Daekin, 2018).

Materials and Methods

The study of standardized karyotype and idiogram by conventional staining and Ag-NOR banding of mrigal carp (*C. cirrhosus*) was divided into 4 steps including the fish chromosome preparation, chromosome counting, chromosome measuring and chromosome analysis.

1. Fish chromosome preparation: The 10 males and 10 females of *C. cirrhosus* were collected from natural area from Muang district, Ubon Ratchathani, Thailand. The samples were checked by identification key, Roberts (1997) and Kottelat (2001). Chromosome preparation was conducted by the colchicine-hypotonic-fixation-air drying technique, from gill filaments and kidney tissues. The chromosomes were stained with 20% Giemsa staining solution for 30 min and identified for NORs by Ag-NOR staining according to Howell and Black (1980) and Verma and Babu (1995).

2. Chromosome counting: Many pictures of the chromosomes in metaphase cells were taken for counting the number of chromosomes. Twenty metaphase cells of each male and female were measured for length of short arm (Ls) and long arm (Ll) chromosomes.

3. Chromosome measuring: The Ls and Ll were measured and calculated for the length of total arm chromosomes (LT, LT=Ls+Ll). Relative length (RL), centromeric index (CI) and standard deviation (SD) were also calculated. The CI was also computed to classify the shape of chromosomes, respectively according to Chaiyasut (1989) and Tanomtong (2011). All parameters were used in standardized karyotyping and idiograming.

4. Chromosome analysis: In karyotype formula, the size and type of chromosome were classified, which the symbol "L, M and S" represented large, medium and small chromosomes, respectively. The symbol "m, sm, a and t" represented metacentric, submetacentric, acrocentric and telocentric type of chromosomes, respectively. The fundamental number (NF) was obtained by assigning a value of two to metacentric, submetacentric and acrocentric chromosomes and one to telocentric chromosome.

Results

The general character of mrigal carp (*C. cirrhosis*) is shown on Figure 1. The body is elongated and streamlined or laterally compressed. Dorsal profile is more convex than that of abdomen. Ventral profile is slightly convex. There are grayish or greenish color on the back and silvery at the sides and below. Fins are slightly orange colored in larger specimen. Lateral line is present and complete with about 39-46 scales.

This is the first report on *C. cirrhosus* cytogenetics study. The results revealed the diploid number was 50 and the fundamental number was 72 in both male and female. The type of chromosomes can be defined as 12 large submetacentric, 8 large telocentric, 10 medium metacentric, 14 medium telocentric and 6 small telocentric chromosomes (Table1). No heteromorphic chromosomes indication sex chromosomes were detected (Figure 2 and 3). Twenty metaphase cells of each male and female were used for Ls, LI, LT, CI, RL, SD, chromosome sizes and types analysis as shown on Table 1. This species has NOR on telomeric region of chromosome pair 6 (Figure 3). The standardized idiogram of *C. cirrhosus* was shown on Figure 5. The karyotype formula can be deduced as: 2n (50) = $L^{sm}_{12} + L^{t}_{8} + M^{m}_{10} + M^{t}_{14} + S^{t}_{6}$

Discussion

The cytogenetic studies have been firstly performed on *C. cirrhosus* from Thailand. The results showed that 2n=50, which is the same as other species on this genus (Table 2). The chromosomal data on *C. reba* was reported earlier by Manna and Khuda-Bukhsh (1977) with 2n=48 but all later publications on this genus which reported the diploid number is 2n=50. This may cause by different fish sample or technical error. The NF of *Cirrhinus* genus varied from 70 to 90 (Wu *et al*., 1989; Zhang and Reddy, 1991; Ren and Yu, 1993; Donsakul and Magtoon, 1997; Nangpure *et al*., 2001; Donsakul *et al*., 2007; Biswal *et al*., 2008; Saravanan *et al*., 2012; Yadav *et al*., 2013; Bhatnagar *et al*., 2014; Nadini *et al*., 2014; Neeru *et al*., 2018). The NF of *C. cirrhosus* was 72 which similar to *C. mrigala* and *C. caudimaculatus* (Magtoon and Arai, 1993; Donsakul *et al*., 2007).

The autosome type of *C. cirrhosus* were 12 large submetacentric, 8 large telocentric, 10 medium metacentric, 14 medium telocentric and 6 small telocentric chromosomes. The types of autosome were not related to previous reports (Manna and Khuda-Bukhsh, 1977; Wu *et al*., 1989; Zhang and Reddy, 1991; Magtoon and Arai, 1993; Ren and Yu, 1993; Donsakul and Magtoon, 1997; Nangpure *et al*., 2001; Donsakul *et al*., 2007; Biswal *et al*., 2008; Saravanan *et al*., 2012; Yadav *et al*., 2013; Bhatnagar *et al*., 2014; Nadini *et al*., 2014; Neeru *et al*., 2018). The cause of different type of chromosome may be genetic variation between species and populations (Tanomtong, 2011).

No sex chromosomes could be found in the form of heteromorphic pair in the *C. cirrhosus*. Also, the sex chromosome dimorphism was not found in this genus. However, the sex chromosome determination of both XY/XX system and ZZ/ZW system were found in some species of cyprinid fishes. The cyprinid fishes such as, *Acheilognathus cyanostigma*, *A. rhombeus, A. tabira, Danio rerio*, *Leuciscus aula, L. cephalus, L. carolitertii*, *L. pyrenaicus*, *L. souffia, Rhodeus uyekii, Scardinius erythrophthalm*, *Tanakia lanceolate* and *T. limbate* has ZZ/ZW sex determination system (Koehler *et*

al., 1995; Collares-Pereira *et al*., 1998; Sharma *et al*., 1998; Kawamura and Hosoya, 2000). However, the XY/XX system was found in *Onychostoma alticorpus* and *O. barbatulum* (Han *et al*., 2017: online).

From the result, *C. cirrhosus* had the positive Ag-NOR marks on a single pair of homologous chromosomes. The telomeric NOR on the long arm of a pair of the large submetacentric chromosome pair (the sixth pair) was observed. The result showed similarity with *C. mrigala* (Magtoon and Arai, 1993; Nangpure *et al*., 2001; Neeru, 2014; Neeru *et al*., 2018).

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Figure 1 General characteristics of mrigal carp, *Cirrhinus cirrhosus* (Cyprinidae) from Muang district, Ubon Ratchathani (Scale bars = 2 cm).

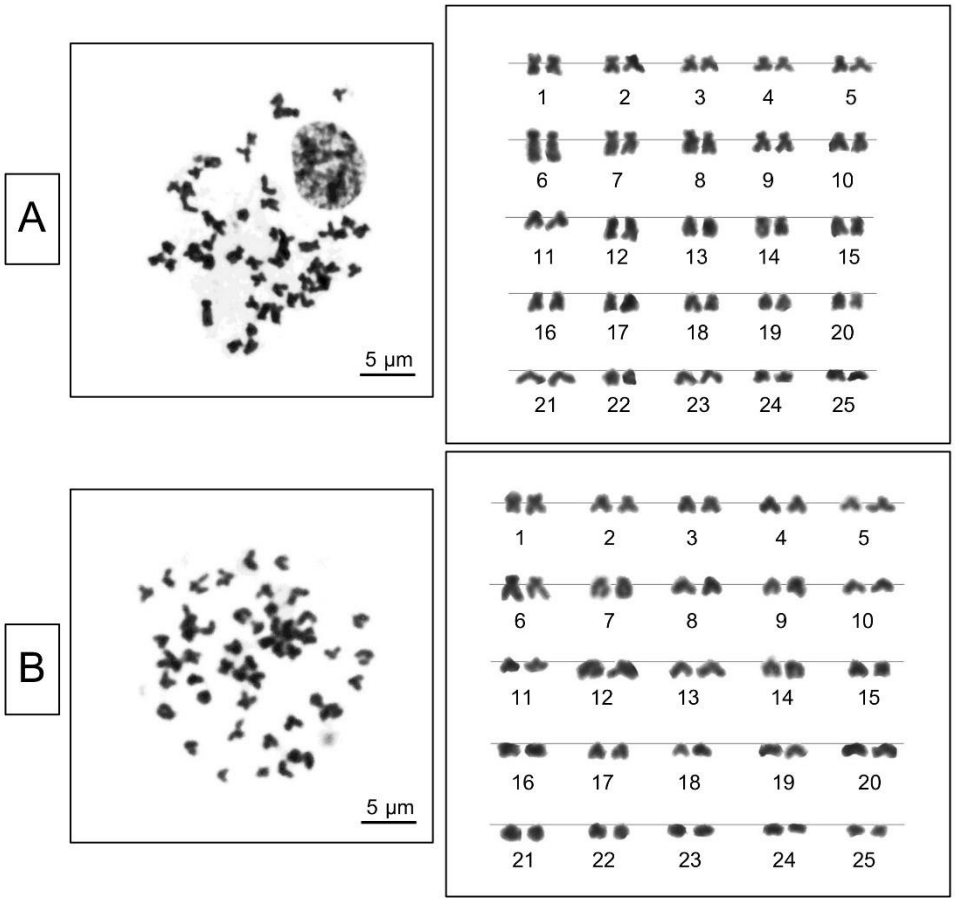

Figure 2 Metaphase plates and standardized karyotypes of male (A) and female (B) mrigal carp, (*C. cirrhosus*) 2n=50 by conventional staining (Scale bars = 5 µm).

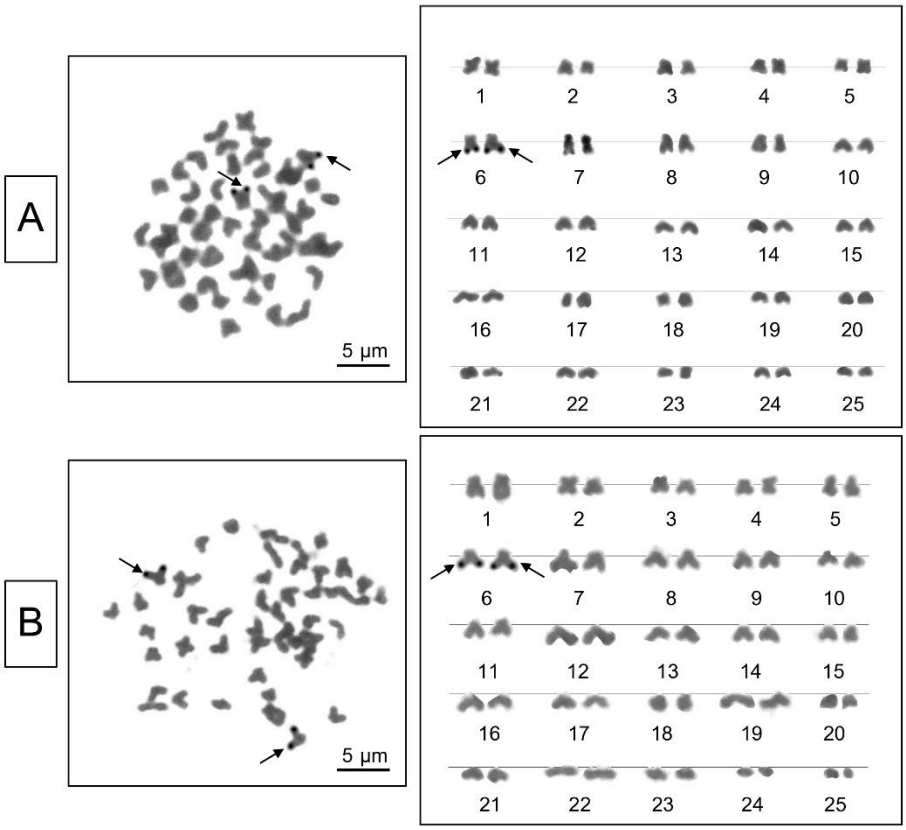

Figure 3 Metaphase plates and standardized karyotypes of male (A) and female (B) mrigal carp, (*C. cirrhosus*) 2n=50 by Ag-NOR banding, arrows indicate nucleolar organizer regions (Scale bars = 5 μm).

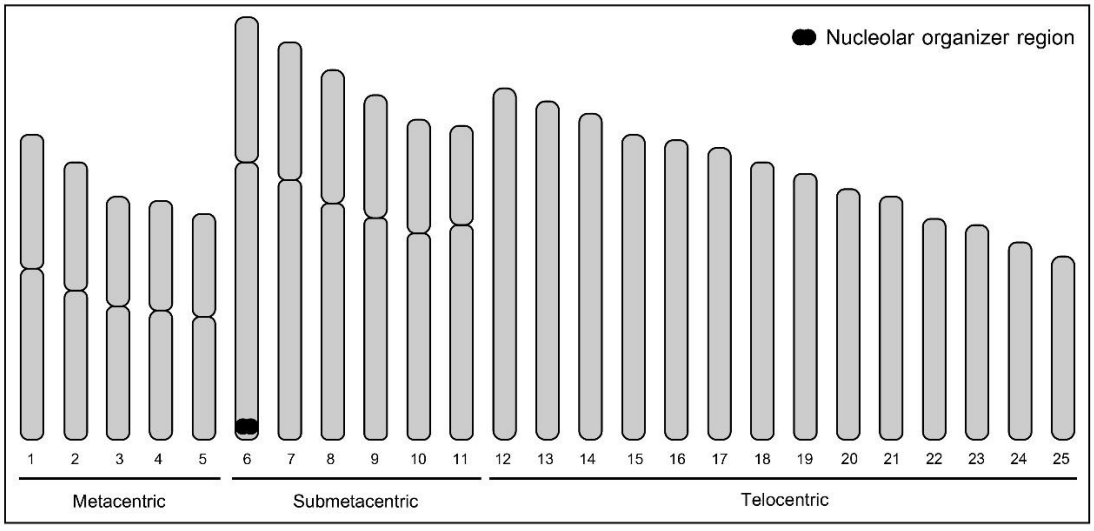

Figure 4 Standardized idiogram of mrigal carp, (*C. cirrhosus*) 2n=50 by Ag-NOR banding.

Table 1 Mean length of short arm chromosome (Ls) , long arm chromosome (LI) , total arm chromosome (LT), centromeric index (CI), relative length (RL) and standard deviation (SD) of CI, RL from 40 karyotypes of male and female mrigal carp, (*C. cirrhosus*), 2n=50.

Chromosome pair	Ls (μm)	LI (μm)	LT (μm)	RL±SD	CI±SD	Chromosome size	Chromosome type
1	0.87	1.10	1.97	0.041±0.009	0.559±0.037	Medium	Metacentric
2	0.85	1.01	1.86	0.038±0.009	0.541±0.031	Medium	Metacentric
3	0.71	0.86	1.57	0.032±0.007	0.549±0.036	Medium	Metacentric
4	0.68	0.85	1.53	0.032±0.007	0.557±0.025	Medium	Metacentric
5	0.65	0.79	1.45	0.040±0.007	0.548±0.035	Medium	Metacentric
6	0.99	1.88	2.87	0.059±0.012	0.655±0.028	Large	Submetacentric
7	0.94	1.77	2.71	0.056±0.009	0.653±0.026	Large	Submetacentric
8	0.91	1.60	2.51	0.052±0.008	0.637±0.030	Large	Submetacentric
9	0.79	1.53	2.32	0.048±0.006	0.659±0.026	Large	Submetacentric
10	0.74	1.40	2.14	0.044±0.005	0.654±0.029	Large	Submetacentric
11	0.71	1.43	2.14	0.044±0.005	0.668±0.029	Large	Submetacentric
12	0.00	2.39	2.39	0.049±0.005	1.000±0.000	Large	Telocentric
13	0.00	2.27	2.27	0.047±0.002	1.000±0.000	Large	Telocentric
14	0.00	2.19	2.19	0.045±0.003	1.000±0.000	Large	Telocentric
15	0.00	2.05	2.05	0.042±0.005	1.000±0.000	Large	Telocentric
16	0.00	2.03	2.03	0.042±0.003	1.000±0.000	Medium	Telocentric
17	0.00	1.97	1.97	0.041±0.004	1.000±0.000	Medium	Telocentric
18	0.00	1.85	1.85	0.038±0.005	1.000±0.000	Medium	Telocentric
19	0.00	1.80	1.80	0.037±0.006	1.000±0.000	Medium	Telocentric
20	0.00	1.68	1.68	0.035±0.004	1.000±0.000	Medium	Telocentric
21	0.00	1.62	1.62	0.033±0.003	1.000±0.000	Medium	Telocentric
22	0.00	1.48	1.48	0.031±0.003	1.000±0.000	Medium	Telocentric
23	0.00	1.41	1.41	0.029±0.003	1.000±0.000	Small	Telocentric
24	0.00	1.38	1.38	0.029±0.005	1.000±0.000	Small	Telocentric
25	0.00	1.21	1.21	0.025±0.004	1.000±0.000	Small	Telocentric

Table 2 Cytogenetic publications of the genus *Cirrhinus*

Species	2n	NF	Chromosome type				NOR position	Reference
			m	sm	a	t		
C. reba	48	86	36	2	6	4	-	Manna and Khuda-Bukhsh (1977)
	50	90	18	22	-	10	-	Wu *et al*. (1989)
	50	-	8	10	6	26	-	Biswal *et al*. (2008)
C. mrigala	50	76	10	16	16	12	-	Wu *et al*. (1989)
	50	80	12	18	10	10	-	Zhang and Reddy (1991)
	50	72	10	12	10	18	6q	Magtoon and Arai (1993)
	50	86	12	18	6	14	1 pair (q)	Nangpure *et al*. (2001)
	50	-	18	12	6	14	-	Saravanan *et al*. (2012)
	50	80	30	-	-	20	-	Yadav *et al*. (2013)
	50	70	6	6	8	30	-	Bhatnagar *et al*. (2014)
	50	-	8	26	-	16	-	Nadini *et al*. (2014)
	50	-	6	8	22	14	8q	Neeru (2014); Neeru *et al*. (2018)
C. jullieni	50	90	26	14	4	6	23p	Magtoon and Arai (1993)
C. molitorella	50	-	16	24	10	-	21, 23	Ren and Yu (1993)
C. microlepis	50	80	22	8	8	12	-	Donsakul and Magtoon (1997)
C. caudimaculatus	50	72	12	10	2	26	-	Donsakul *et al*. (2007)
C. cirrhosus	50	72	10	12	-	28	6q	This study

Remarks: 2n = diploid chromosome, NF = fundamental number, m = metacentric chromosome, sm = submetacentric chromosome, a = acrocentric chromosome, t = telocentric chromosome, p =short arm, q = long arm and - = not available.