

Asian Fisheries Society, Manila, Philippines

# Chromosomal Karyotyping from Peripheral Blood Lymphocytes of the Mekong Giant Catfish (*Pangasianodon gigas*, Chevey)

J. MANOSROI<sup>1</sup>, K. MENG-UMPHAN<sup>2</sup>, U. MEEVATEE<sup>3</sup>, and A. MANOSROI<sup>1\*</sup>

<sup>1</sup>Faculty of Pharmacy/Pharmaceutical Cosmetic Raw Materials and Natural Products Research and Development Center (PCRNC)  
Institute for Science and Technology Research and Development (IST)  
Chiang Mai University, Chiang Mai 50200, Thailand

<sup>2</sup>Department of Fisheries Technology  
Faculty of Agricultural Production  
Maejo University, Sansai  
Chiang Mai 50290, Thailand

<sup>3</sup>Department of Anatomy  
Faculty of Medicine  
Chiang Mai University  
Chiang Mai 50200, Thailand

## Abstract

Chromosomal karyotyping of Mekong Giant Catfish (*Pangasianodon gigas*, Chevey) were determined from peripheral blood lymphocytes of caudal vasculature in 15 fish reared in earthen ponds and 4 fish caught from reservoirs in Chiang Mai Province, Thailand. The diploid chromosome number was 60. The karyotype comprised 5 pairs of metacentric, 13 pairs of sub-metacentric, 7 pairs of subtelocentric and 5 pairs of acrocentric chromosomes. No variation of chromosomal numbers and karyotype between fish in ponds and reservoirs was observed. The sex determination mechanism was more likely to have homogametic female (XX) and heterogametic male (XY). This study presented a modified technique for karyotypic examination from peripheral blood lymphocytes of the Mekong Giant Catfish and provided valuable information for cytogenetic and taxonomic studies on *P. gigas*.

## Introduction

The Mekong Giant Catfish (*Pangasianodon gigas*,; MGC; family: Pangasiidae) is the largest fresh water catfish in the world and is considered as an endangered species according to the Convention on International Trade in Endangered Species of Wild Fauna and Flora [CITES; Tongsanga

---

\*Corresponding author

and Pholprasit 1991]. It is endemic to the Mekong River, one of the longest international rivers in Indochina. This river runs through many countries including Thailand, Laos, Cambodia, Myanmar, Vietnam and China. For these countries, MGC is a highly valuable species of food fish, with potential for aquaculture. Every year during May to June, the mature MGC at the age of about 16-24 years, weighing 200-350 kilograms, migrate upstream for mating (Pholprasit and Thevarat 1998). Some of them are caught by local fishermen at Chiang Khong district in Chiang Rai Province, which is located at the border between Thailand and Laos. From the period of 1986 to 2001, a total of 429 captured MGC were recorded at Chiang Khong. The highest catch of individuals was 62 in 1990. In 1998, only one fish was caught and no captured fish was recorded in 2000 (Meng-Umphphan 2000).

As a measure to conserve this valuable international resource, since 1988, MGC have been stocked in reservoirs and earthen ponds in Thailand (Tonsanga and Pholprasit 1991). Further, the karyotype of *P. gigas*, with chromosome spreads derived from kidney cells has been previously reported (Donsakul and Magtoon 1998). However, this approach requires sacrifice of the animals for chromosome preparations. To circumvent this limitation, this study undertook and established a non-invasive technique of preparing chromosomal spreads derived from peripheral blood lymphocytes.

## Materials and Methods

### *Fish samples*

Nineteen *P. gigas* were used in this study. Fifteen fish with an average weight of 15 kg and an average age of 9 years old were collected from earthen ponds at Maejo University, Chiang Mai, Thailand. One, weighing 60 kg, and 6 years old, was from Mae-Kuang reservoir and the other three with an average weight of 145 kg and age of 13 years old were from Mae-Ngad reservoir in Chiang Mai, Thailand. These fish samples were from artificial breeding of the same MGC brood stock from the Mekong river.

### *Blood samples and culture*

About 1 ml of blood was drawn from caudal vasculature of the fish and 10 drops of blood were cultured in 5 ml of RPMI 1640 media containing 0.25 % w/v ampicillin, 0.01 % w/v streptomycin, 20 % v/v fetal bovine serum, 100 ml of phytohaemagglutinin and 300 ml of concanavalin A. The mixture was incubated at 37°C for 96 hrs (Seabright 1971; Meevatee 1988).

### *Peripheral blood lymphocytes and chromosome preparation*

An amount of 100 ml of colcemid solution (0.2 mg/ml) was added to the above mixture, which was then further incubated at room temperature (30±1°C) for 30 mins. The mixture was centrifuged at 1200 rpm for 5 mins.

The supernatant was removed and then 5 ml of phosphate buffer saline (PBS) was added to the cells. The mixture was centrifuged at 1200 rpm for 5 mins. The supernatant was removed and then 5 ml of hypotonic solution (0.075 M KCl) was added to swell up and lyse the cells. The mixture was centrifuged at 1200 rpm for 5 mins. The cells were fixed with 5 ml of Carnoy fixative [methanol/acetic acid (3:1)] and centrifuged at 1200 rpm for 5 mins. The fixing step was repeated twice. The resulting cells were kept at  $4\pm 1^{\circ}\text{C}$  for chromosome banding and staining (Ida and Kyo 1980; Meevatee 1988).

### ***Preparation of chromosomal G-banding and staining***

About one milliliter of Carnoy fixative was added to the cells. The mixture was dropped on a clean slide and air dried. The slide was warmed at  $90^{\circ}\text{C}$  and then dipped in 0.25% w/v trypsin solution for 15 seconds, washed with PBS and air dried. The slide was immersed in 10 % w/v Giemsa in Sorenson's phosphate buffer solution (pH 7) for 40 mins and followed by rinsing with distilled water (Uwa and Ojima 1981).

### ***Chromosomal analysis***

The slides of the chromosomes were examined using a light microscope (Zeiss Axioskop, Germany) at a magnification of 1,000 X with the Matrox inspector version 2.1 Program. Metaphases of the chromosomes were classified, photographed and counted. Representative metaphases were printed on high contrast paper and the karyotypes were arranged according to chromosomal morphology, centromere, size and arm (Meevatee 1988). Chromosomes were grouped into four categories which were metacentrics (M), submetacentric (SM), subtelocentric (ST) and acrocentric (A). The chromosome number was determined. Sex chromosomes were also investigated.

## **Results**

From 148 metaphase spreads pooled from nineteen fish, the total diploid chromosome numbers of 60 ( $2n = 60$ ) were observed with the frequency 63.51 %, seventy five (69.44 %) of 108 metaphase spreads from fish reared in earthen ponds, six (50 %) of 12 metaphase spreads from Mae-Kuang reservoir and thirteen (46.23 %) of 28 metaphase spreads from Mae-Ngad reservoir (Table 1).

Karyotypes, arranged from metaphase chromosomes of fish from all locations, were composed of 5 M, 13 SM, 7 ST, and 5 A. Figures 1 and 2 represent karyotypes of a female and a male fish from ponds, respectively. The female and male *P. gigas* were more likely to have homogametic sex chromosome (XX) and heterogametic sex chromosome (XY), respectively. These sex determinations were observed from 18 metaphases of 2 female and 13 metaphases of 1 male fish from earthen ponds.

### Discussion

According to the analysis of metaphase spreads of nineteen *P. gigas* reared in earthen ponds and caught from two reservoirs (Mae-Kuang and Mae-Ngad) in Chiang Mai, Thailand, the diploid chromosome number was found to be 60. Karyotypes of the fish from the three locations were not different. This is most likely due to the fact that these three populations originated from the same brood stock population in Mekong River. Since the genetic bases of the fish samples were close, any chromosomal polymorphisms could be less likely. Other study on *Pangasianodon gigas* showed different karyotypes from our study. Donsakul and Magtoon (1998) reported that the chromosomal karyotype from kidney cells comprised of 16 M, 4 SM, 6 ST and 4 A with fundamental arm numbers of 100. This difference may be due to the different classification system. They used the arm ratio system (Levan et al 1964 ; Arai 1982), while we used the position of centromere, number and sizes of chromosome for classification (Meevatee 1988). For karyotype of

Table 1. Distribution of chromosome numbers of *P. gigas* from earthen ponds and reservoirs.

Location	No. of fish	Chromosome number											No. of Metaphase cells	Maximum frequency at 2N=60 (%)
		<50	52	54	55	56	57	58	59	60	61	62		
Earthen Ponds	15	7	2	2		2	1	10	6	75	3		108	69.44
Mae-Kuang Reservoir	1	2	1		1	1					6		12	50
Mae-Ngad Reservoir	3	1	2			1	1	2	6	13	2		28	46.23
<b>Total</b>	<b>19</b>	<b>10</b>	<b>5</b>	<b>2</b>	<b>1</b>	<b>4</b>	<b>2</b>	<b>13</b>	<b>12</b>	<b>94</b>	<b>2</b>	<b>3</b>	<b>148</b>	<b>63.51</b>

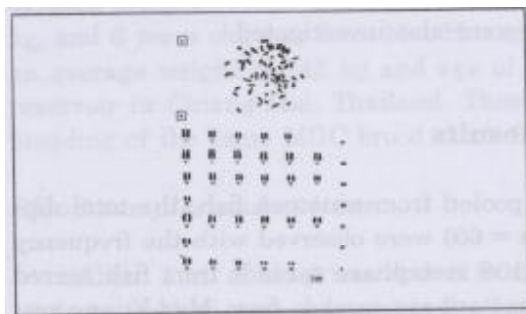


Fig. 1. Photomicrographs of a mitotic metaphase chromosome spread (a) and a karyotype (b) of female *P. gigas* from earthen ponds reared at Maejo University, Chiang Mai Thailand; diploid chromosome 2N=60

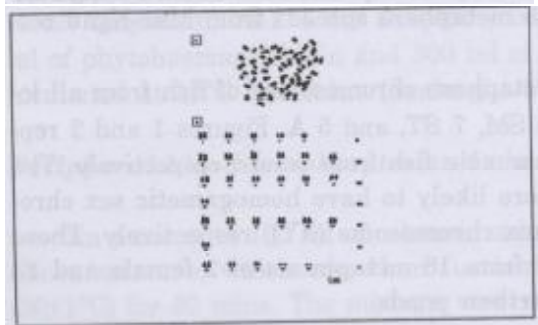


Fig. 2. Photomicrographs of a mitotic metaphase chromosome spread (a) and a karyotype (b) of male *P. gigas* from earthen ponds reared at Maejo University, Chiang Mai Thailand; diploid chromosome 2N=60

*Pangasius sanitwongsei*, it comprised of 10 M, 3 SM, 9 ST and 8 A with fundamental arm numbers of 86 (Magtoon and Donsakul 1987). The karyotype of *P. hypophthalmus* consisted of 10 M, 6 SM, 2 ST and 12 A with fundamental arm numbers of 92. The karyotype of *P. lamaudii* comprised 12 M, 10 SM, 2 ST and 6 A with fundamental arm numbers of 104. The diploid number of these four species was 60. The chromosomal karyotype of *P. pangasius* was 14 M, 6 SM, 18 ST and 24 A with fundamental arm numbers of 82 and comprised a chromosome diploid number of 62 (Manna and Prasad 1971).

The sex determination mechanism of *P. gigas* from peripheral blood lymphocytes by G-banding technique in this study was more likely to have homogametic (XX) in females and heterogametic (XY) in males that reared in earthen ponds. Similar to the gonochoristic fish, such as the Rainbow Trout, the homogametic sex (XX) was found in females and heterogametic sex (XY) was found in males (Thorgaard 1979). These were also reported in *Ctenopharyngodon idella* (Stanley 1976); *Clarias macrocephalus* (Na-Nakorn 1995), *Oreochromis niloticus* (Jalabert et al. 1974) and *Oreochromis mossambicus* (Chen 1969).

## Conclusion

The karyotypes of the *P. gigas* from the three locations were identical comprising of 5 M, 13 SM, 7 ST and 5 A with a diploid chromosome number of 60. There are very few reports on chromosome karyotype of fish obtained from peripheral blood lymphocytes. Most chromosomal karyotypes of fish are usually determined from kidney cells and gills. This study has demonstrated the modified G-banding technique for chromosomal preparation from peripheral blood lymphocytes of *P. gigas*. It appeared to be successful identifying karyotype and sex chromosomes of *P. gigas* by this modified method. This results will not only be beneficial to our fish breeding study, but also in cytogenetic and taxonomic studies as well.

## Acknowledgements

The authors are grateful to the Thailand Research Fund (TRF), the National Science and Technology Development Agency (NSTDA) and Graduate School of Chiang Mai University for the financial support of this study. We also thank to Assoc. Prof. Dr. P. Jarayabhand, Dr. A. Termvidchakorn and Dr. S. Ngamwongchon for their valuable suggestions.

## References

- Arai, R. 1982. A chromosome study on two cyprinid fishes *Acrossocheilus labiatus* and *Pseudorasbora pumila* with note on Eurasian Cyprinids and their karyotypes Bull. Natn. Sci. Mus. Ser. A. 8(3): 131-181.

- Chen, F.Y. 1969. Preliminary studies on the sex-determining mechanism of *Tilapia mossambica*. Proceeding Theory Apply Limnology 17:719-724.
- Donsakul, T. and W. Magtoon. 1998. A chromosome study of three fishes, *Pangasius sp.*, *P. saniwongsei* and *Oxyeleotris mamoratus*, from Thailand. Faculty of Science, Srinakarinwirot University Prasanmitra, Bangkok. 18 pp.
- Ida, H. and Y. Kyo. 1980. Karyotypic variation found among five species of the family platycephalidae. Japan Journal Ichthyology 27:122-128.
- Jalabert, B., J. Moreau, P. Planquette and R. Billard. 1974. Sex determination in *Tilapia macrochir* and *Tilapia nilotica* : Effect of methyltestosterone administered in fry feed on sex-reversed males. Animal Biology, Biochemistry and Biophysics 14:729-739.
- Levan, A.K. Fredga and A.A. Sandberg. 1964. Nomenclature for centromeric position of chromosome. Hereditas. 52:201-202.
- Magtoon, W. and T. Donsakul. 1987. Karyotypes of Pangasii catfishes, *Pangasius sutchi* and *P. lamaudii*, from Thailand. Japanese Journal of Ichthyology 34:396-398.
- Manna, G.K. and R. Prasad. 1971. A new perspective in the mechanism of evolution of chromosomes in fishes. Proceedings India Congress 1st Cytologia Genetics. 237-240.
- Meevatee, U. 1988. Human cytogenetics. Department of Anatomy, Chiangmai University, Chiangmai. 150 pp.
- Meng-Umphun, K. 2000. Plabuk (Thai). Thailand Research Fund (TRF). O.S. Printing House Company, Bangkok. 125 pp.
- Pholprasit, S. and P. Thevarat. 1998. The Mekong giant catfish biology and breeding. Thai Fisheries Gazette 51:107-115.
- Na-Nakorn, U. 1995. Comparison of cold and heat shocks to induce diploid gynogenesis in Thai walking catfish (*Clarias macrocephalus* ) and performance of gynogens. Aquatic Living Resource 8:333-341.
- Seabright, M. 1971. A rapid banding technique for human chromosomes. Lancet 2(7731) 971-972.
- Stanley, J.G. 1976. Female homogamety in grass carp (*Ctenopharyngodon idella*) determined by gynogenesis. Fish Research 33:1373-1374.
- Thorgaard, G.H. 1979. Heteromorphic sex chromosomes in male rainbow trout. Science. 196:900 - 902.
- Tongsanga, S. and S. Pholprasit. 1991. Some aspect on the biology of the Mekong giant catfish (*P. gigas*, Chevey). Proceeding Kasetsart University Annual Conference (29):499-511.
- Uwa, H and Y. Ojima. 1981. Detailed and banding karyotype analysis of the medaka *Oryzias latipes* in culture cell. Proceeding Japan Academic 57B:39-43.