Cytogenetic Analysis of Silver Carp, *Hypophathalmichthys molitrix* (Valenciennes in Cuvier and Valenciennes, 1844) and Thai Silver Barb, *Barbonymus gonionotus* (Bleeker, 1850)

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Abstract

Cytogenetic investigation on silver carp *Hypophthalmichthys molitrix* and silver barb *Barbonymus gonionotus* belonging to family Cyprinidae from Thatyetkone Fisheries Station, Mandalay Region, was carried out. The optimal effect of colchicine concentration was observed at 0.50 % for duration 5 hrs with 0.56 % hypotonic solution treatment of 1 hr 30 mins period in silver carp and colchicine concentration 0.50 % for 6 hrs with 1 hr duration of hypotonic treatment in silver barb. The diploid chromosome number of silver carp *H. molitrix* was 2n = 48 with karyotype formula 2 m (metacentric) + 4 sm (submetacentric) + 6 a (acrocentric) + 12 t (telocentric) and fundamental arm number 60. The chromosomal number of silver barb *B. gonionotus* was 2n = 50 with karyotype formula 1 m (metacentric) + 9 sm (submetacentric) + 2 a (acrocentric) + 12 t (telocentric) and fundamental arm number 68.

Keywords *Hypophthalmichthys molitrix, Barbonymus gonionotus,* karyotype, frequency

Introduction

Myanmar is rich in natural resources such as flora as well as fauna. Not only the indigenous freshwater fishes distributed throughout the rivers, ponds and lakes, but also marine fishes are populated in costal, beaches and bays of Myanmar.

Among the fish species, silver carp *Hypophthalmichthys molitrix* (Valenciennes in Cuvier and Valenciennes, 1844) is a freshwater fish native to East Asia and introduced around the world for aquaculture and control of algal blooms. Silver barb *Barbonymus gonionotus* (Bleeker, 1850) is recorded from Vietnam Mekong delta and the Dong Nai River, the Mekkong basin in Lao PDR, Cambodia and Thailand. These fishes are introduced from Malaysia, Peninsular Malaysia and distributed in Southeast Asia.

A karyotype is the arrangement of pairing a set of metaphase chromosomes according to their sizes and shapes. The precise number of complete sets of metaphase chromosomes would be observed within the nucleus of a cell of an individual species, genus or other grouping (Levan *et al.,* 1964). Cytogenetic studies are very useful for taxonomic, genetic, cyto-toxicology, race improvement and biotechnological investigations (Nandini *et al.,* 2014).

The detail information on karyological studies of these fishes from Thatyetkone Fisheries Station is necessary to fill the gap of research areas. In addition, the different methodological approaches on cytogenetic studies have been limited in various research areas. Therefore, these fishes are selected to investigate the effects of colchicine on the inhibition of metaphase checkpoint of cells in *H. molitrix* and *B. gonionotus,* and to evaluate the metaphase checkpoint and karyotypes of these fishes.

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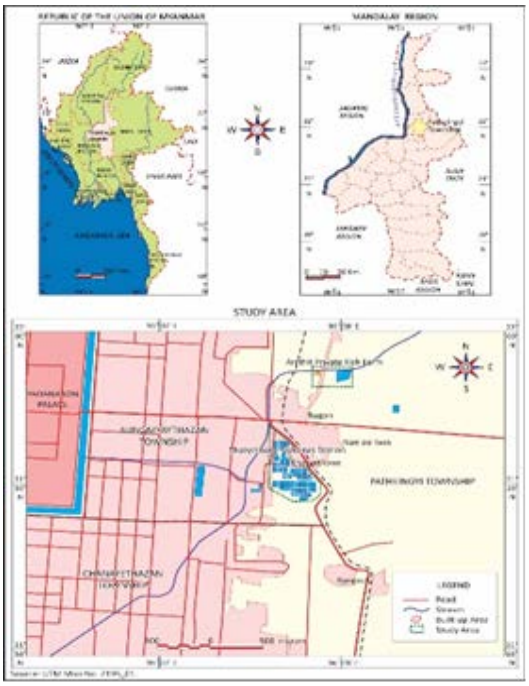


Figure 1 Study map of Thatyetkone Fisheries Station, Mandalay Region (Source: UTM)

Materials and Methods

Study site

Thatyetkone Fisheries Station is situated around 21° 59' 28.53" N, and 96° 7' 44.60" E, Patheingyi Township, Mandalay Region (Fig. 1).

Study period

The study period was from January to August 2022.

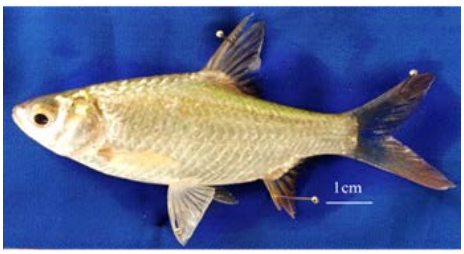
Specimen collection

Twenty fishes for each species were collected from Thatyetkone Fisheries Station and cultured in the Laboratory, Department of Zoology, University of Mandalay (Plate 1 A and B; 2 A). The fishes were fed twice a day with formulated commercial feeds. The water was changed twice a week and kept in well-aerated aquarium.

Identification of species

The species identification followed after Talwar and Jhingran (1991) and Integrated Taxonomic Information System (ITIS, 2022).

A. Lateral view of *Hypophthalmichthys molitrix*



B. Lateral view of *Barbonymus gonionotus*

Plate 1 Collected fishes

Injection technique

The total length and standard length of fish were measured by plastic ruler to the nearest 0.1 cm. The body weight of fish was recorded by digital balance to the nearest 0.01 g. Depending on the weight of fish (1ml / 100 g), the different concentrations of colchicine (Avi Chem, India) solutions such as 0.10 % for duration 1 hr 30 mins, 2 hrs, 2 hrs 30 mins; 0.30 % for 2 hrs and 0.50 % for 3 hrs, 4 hrs, 4 hrs 45 mins, 5 hrs and 6 hrs were applied to block the metaphase stage of cells. Fishes were injected at the base of pelvic fin (Plate 2 B and C).

Collection of blood and tissues

The blood was collected directly from colchicine treated fish by using syringe (0.5 cm) and diluted into the culture tube with 0.56 % hypotonic solution (24 Well Cell Culture Cluster, USA)**.** Then, each fish was sacrificed with one or two drops of 10 % formaldehyde solution (Laba Chemie Put. Ltd, India). The sample tissues such as liver, heart, kidney, oral cells and gill filaments were taken out for further study (Plate 2 D).

Extraction of cells

Each sample was kept in block-cup filled with 0.56 % hypotonic solution and incubated for various duration 10 mins, 20 mins, 30 mins, 45 mins, 1 hr, 1 hr 30 mins and 2 hrs 45 mins. After that, these samples were mixed homogeneously by Advanced Vortex mixer (2 × 3, Nanova**)** and centrifuged (Firlabo.sa, CBA 1015**)** at 1000 rpm for 10 mins (or) 2000 rpm for 5 mins. The supernatant was discarded and the pellets were treated with Carnoyl's fixative (3 methanol: 1 acetic acid) and kept for 15 mins. Then, the samples were centrifuged again at 1000 rpm for 10 mins (or) 2000 rpm for 5 mins. The supernatant was excluded from the test tube by pipette. After that, the pellets were treated with fixative solution again for stabilization of the cells (Plate 2 E, F, G and H).

Preparation of slide and Giemsa stain

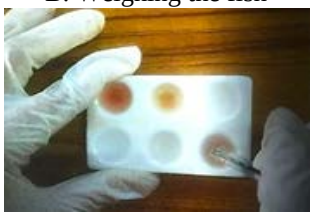
One or two drops of samples were placed onto the pre-warmed slides with a far distance and dehydrated at room temperature. Then, these slides were stained with undiluted Giemsa stain (Avi Chem, India) for 10 mins, washed under running tap water and dried at room temperature. The permanent slides were prepared by dropping one or two drops of immersion oil, covered with cover slip and coated with Canada balsam (Kanto Chemical Co. Inc, Tokyo, Japan) (Plate 2 I, J, K and L).

A. Fish rearing in aquarium

B. Weighing the fish

C. Injection of fish

D. Extraction of blood



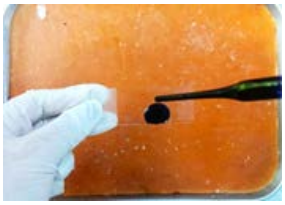
E. Mechanical dissociation of tissue

F. Homogenization of tissues

Plate 2 Preparation of cytological process from fish tissues

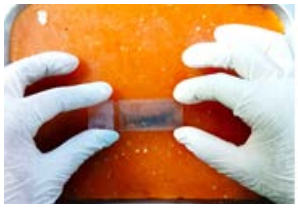
G. Centrifugation of pellets

H. Discarding the supernatant from the test tube



I. Staining on the slide

J. Wash under tap water



K. Covering with coverslip

L. Examination under microscope

Plate 2 Preparation of cytological process of fish tissues

Chromosomal analysis and preparation of karyotype

The microphotographs were recorded by biological microscope with attached camera (G303p, Taiwan), (x1000). The metaphase stages were counted and the chromosomal numbers were recorded by ImageJ. (1.52a, USA). Acrocentric and telocentric chromosomes were scored as uniarmed, metacentric and submetacentric chromosomes which were suggested as biarmed. Chromosome pairs were arranged in decreasing order of largest to smallest ones according to their sizes from each cell point.

Statistical analysis

The frequency and the range of metaphase chromosomes spreads from silver carp and silver barb were analyzed using Microsoft Excel (2010).

Results

Different tissues of fish such as liver, oral cells, kidney, heart, gill filaments and blood were taken out from the fish. Among these tissues, the kidney tissue was the best for karyological studies in these fishes. The optimal concentration of colchicine was 0.50 % for 5 hrs and hypotonic treatment was 0.56 % hypotonic solution for 1hr 30 mins in silver carp. But the best duration of colchicine solution was 6 hrs and duration of 0.56 % hypotonic solution treatment was 1 hr in silver barb.

Systematic position of *Hypophthalmichthys molitrix*

Kingdom	-	Animalia
Phylum	-	Chordata
Class	-	Actinopterygii
Order	-	Cypriniformes
Family	-	Cyprinidae
Subfamily	-	Xenocyprididae
Genus	-	*Hypophthalmichthys* (Bleeker, 1860)
Species	-	*H.molitrix*
Common name	-	Silver Carp
Vernacular name	-	Ngwe-Yaung-Nga-Gyin

Hypophthalmichthys molitrix with mean total length 14.3 ± 0.80 cm, standard length 11.45 ± 0.70 cm and the body weight 23.3 ± 6.06 g (n = 4) was used. The mean meristic

characters of silver carp were dorsal fin (D) – I 7, pectoral fin (P) – i 14, ventral fin (V) – i 7, anal fin (A) – ii 10 and caudal fin (C) – 26.

Systematic position of *Barbonymus gonionotus*

Kingdom	-	Animalia
Phylum	-	Chordata
Class	-	Actinopterygii
Order	-	Cypriniformes
Family	-	Cyprinidae
Genus	-	*Barbonymus*
Species	-	*B.goinonotus* (Bleeker, 1850)
Common name	-	Silver barb or Java barb
Vernacular name	-	Nga-khone-ma-kyee

Barbonymus gonionotus with mean total length 10.00 ± 1.20 cm, standard length 7.98 ± 1.10 cm and the body weight 9.25 ± 3.40 g (n = 4) was used. The mean meristic characters of silver carp were dorsal fin (D) – II 9, pectoral fin (P) – i 13, ventral fin (V) – i 7, anal fin (A) – I 7 and caudal fin (C) – 20.

Frequency distribution of metaphase chromosomes

In silver carp *Hypophthalmichthys molitrix*, the highest percentage of chromosome counts was found in the range of 46 – 50 (32.61 %, n = 15) with 39.13 % cumulative frequency (CF), followed by 51 – 55 (23.91 %, n = 11) and 56 – 60 (21.74 %, n = 10). The ranges of chromosomes with 40 – 45 and 61 – 65 (6.52 %, n = 3 each), 66 – 70 and 71 – 75 (4.35 %, n = 2 each) were observed (Table 1 and Fig 2).

In silver barb *Barbonymus gonionotus*, the highest range of chromosome count n = 17 was found in 46 – 50 (38.64 %) with 52.28 % cumulative frequency (CF), followed by 51 – 55 (22.73 %, n = 10), 40 – 45 (13.64 %, n = 26), 61 – 65 (11.36 %, n = 5) and the lowest percentage was found in 66 – 70 and 71 – 75 (2.27 %, n = 1 each) (Table 2 and Fig 3).

Table 1 Percent and frequency distribution of diploid number of chromosomes counts in silver carp *Hypophthalmichthys molitrix*

Treatment	Range	Frequency	Percentage (%)	Cumulated frequency (CF)
0.50 %	40-45	3	6.52	6.52
Colchicine	46-50	15	32.61	39.13
(5 hr)	51-55	11	23.91	63.04
0.57 %	56-60	10	21.74	84.78
KCL	61-65	3	6.52	91.30
(1hr 30	66-70	2	4.35	95.65
min)	71-75	2	4.35	100.00

Table 2 Percent and frequency distribution of diploid number of chromosomes counts in silver barb *Barbonymus gonionotus*

Treatment	Range	Frequency	Percentage (%)	Cumulated frequency (CF)
0.50 %	40-45	6	13.64	13.64
Colchicine	46-50	17	38.64	52.28
(5 hr)	51-55	10	22.73	75.01
0.57 %	56-60	4	9.09	84.10
KCL	61-65	5	11.36	95.46
(1hr 30	66-70	1	2.27	97.73
min)	71-75	1	2.27	100.00

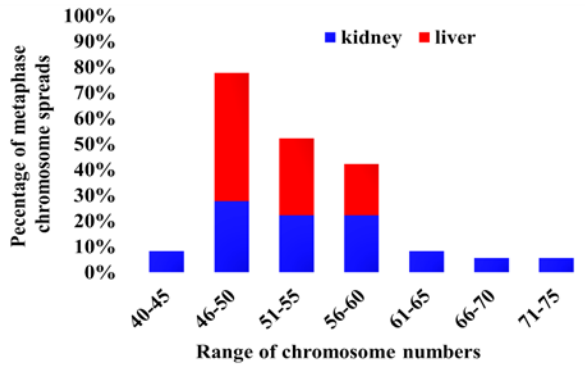


Figure 2 Frequency distribution of metaphase chromosomes spreads from liver and kidney cells of silver carp *Hypophthalmichthys molitrix*

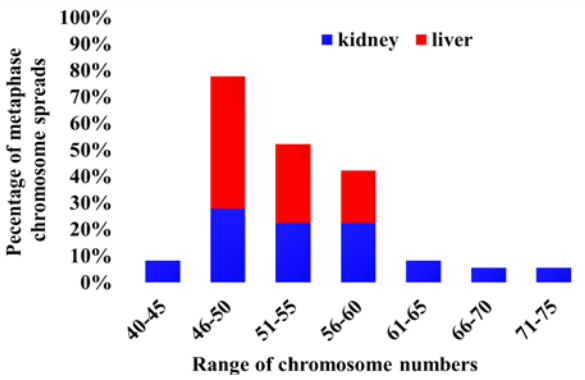
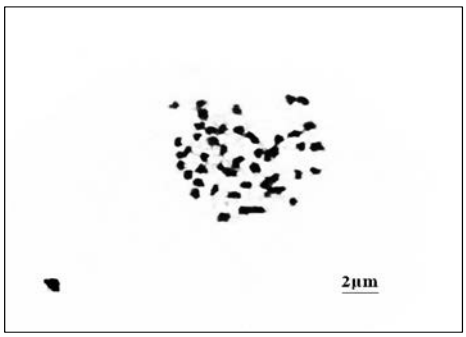


Figure 3 Frequency distribution of metaphase chromosomes spreads from liver and kidney cells of silver carp *Hypophthalmichthys molitrix*

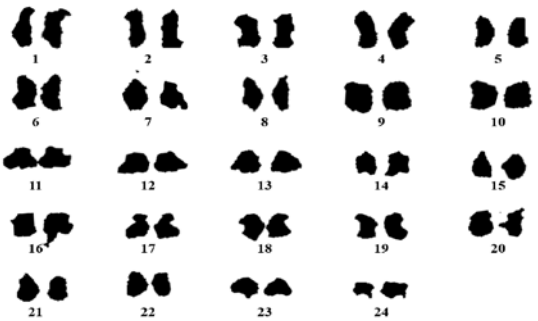
Karyotype

Forty-six well spread metaphase complements of chromosomes in silver carp *Hypophthalmichthys molitrix* and forty-four in silver barb *Barbonymus gonionotus* were selected and counted to construct the karyotypes of these fishes. The number of diploid chromosomes ranged from 40 - 74 in silver carp and 40 - 70 in silver barb (Plate 3A and 4A).

In silver carp, the modal diploid chromosome numbers was 2n = 48 consisting with 2 metacentric, 4 submetacentric, 6 acrocentric and 12 telocentric with 60 fundamental arm numbers (Plate 3 B). In silver barb, the chromosomal formula was 2n = 50 with 1 metacentric, 9 submetacentric, 2 acrocentric and 12 telocentric with 68 fundamental arm numbers (Plate 4 B).

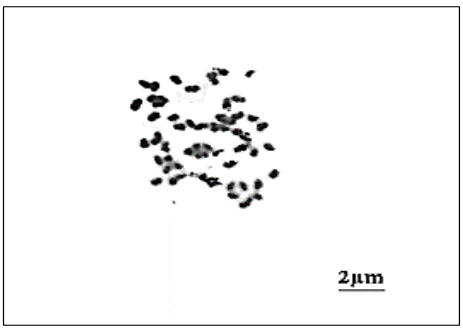


(A) Mitotic metaphase chromosome spread (x1000)

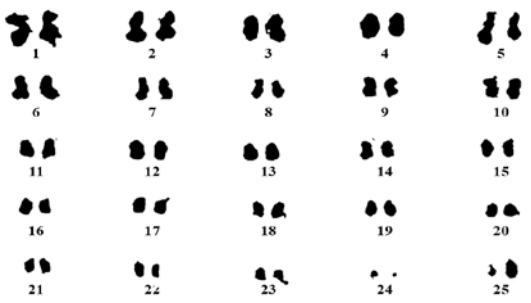


(B) Karyotype

Plate 3 Silver carp *Hypophthalmichthys molitrix*



(A)Mitotic metaphase chroosome spread (x1000)



(B) Karyotype

Plate 4 Silver barb *Barbonymus gonionotus*

Discussion

In silver carp *Hypophthalmichthys molitrix*, late prophase stages of cells were more observed in colchicine (0.50 %) for 3 hrs treatment with 0.56 % hypotonic solution for 45 mins. However, the injection of colchicine treatment 4 hrs with 0.56 % hypotonic solution for 1 hr generated the early metaphase stages of chromosomes through the breakdown of nuclear envelope.

When fish were treated with colchicine solution 5 hrs injection with 1 hr fixation of hypotonic solution, the early and middle metaphase stages were observed. The complete set of metaphase chromosomal configurations was observed in 5 hrs colchicine treatment with hypotonic treatment for 1 hr 30 mins. This is the optimum treatment for desired condensation degree of metaphase chromosomes in silver carp. To sum up these consecutive tests on blocking the metaphase stage of cells in these two fishes (silver carp and silver barb), the effects of optimal concentration on colchicine concentration and hypotonic solution did not synchronize on the mitotic checkpoints of cells.

The condensed chromosomal configuration was observed in 0.50 % colchicine concentration for 4 hrs 45 mins duration with 0.56 % hypotonic solution for 1 hr indicating the optimal stage of metaphase check point of cells in silver barb. The middle stage of metaphase stage was observed in injection duration for 5 hrs and hypotonic treatment duration for 1 hr. The complete metaphase stages were observed in colchicine injection duration for 6 hrs and hypotonic solution treatment for 1 hr. However, the desired metaphase chromosomes were not observed in 6 hrs colchicine solution with hypotonic treatment duration for 1 hr 30 mins.

In this study, the duration of Carnoyl's fixative for 15 mins was the optimal condition for the preservation and suspension of the cells in silver carp and silver barb. The good shapes of miotic chromosome spreads were observed by using pre-warmed slides and stained with undiluted Giemsa stain for 10 mins. Undiluted Giemsa stain was very effective for getting the good shape of chromosomes in prepared slides.

The model chromosome number of silver carp was optimized as 2n = 48. The chromosomal formula of silver carp *Hypophthalmichthys molitrix* has 2 metacentric (m), 4 submetacentric(sm), 6 acrocentric (a) and 12 telocentric (t) with fundamental arm number (60) in this study. The similar result was not found in other researchers. Nandini, *et al.* (2014) stated that the chromosomal formula of *H. molitrix* has 11 pairs (metacentric), 11 pairs (submetacentric), and 2 pairs (acrocentric), 11 m, 7 sm and 6 t (Márián, and Krasznai, 1979); and 4 m , 12 sm and 8 st-a (Sember *et al.*, 2020). The diploid chromosomal number of silver barb *Barbonimus gonionotus* was 2n = 50 with a chromosomal formula 1 metacentric (m), 9 submetacentric (s.m), 2 acrocentric (a) and 12 telocentric (t) with fundamental arm number (68). This study does not support to Magtoon and Arai (1989) who described that the karyotype composition of silver barb was 2 (m), 20 (s.m), 4 (st) and 24 (a). The fundamental arm number of this fish was 72. The differential chromosomal configurations on these fishes may be due to the researcher's assumption on karyological study even though the same methodological approach.

The present result on the range of diploid chromosome number on each cell varied from 40 - 74 (n = 46) in silver carp and 40 – 70 (n = 44) in silver barb with various chromosomal configurations in each well spread cell. This was due to the result of losses or additions of chromosomes during karyotype preparation including splashing due to their downfall from various heights from nearby cells (Ganai and Yousuf, 2011). Therefore, Biswal, *et al.* (2008) reported the broad spectrum of karyotype of some fishes is needed to assay with an aid of DNA maker.

Conclusion

Silver carp, *Hypophathalmichthys molitrix* had the optimal chromosomal number 2n = 48 while that of silver barb, *Barbonymus gonionotus* had the modal diploid number 2n = 50. And the basic information on karyotypes of these fishes was indication of further genetic researches in diverse areas. Concerning with the chromosomal configurations in selected fishes, the chromosomal preparation is vital process to designate the karyomorphological studies on haploid or diploid species.

Acknowledgements

We are grateful to Dr Nwe Nwe Khaing, Professor and Head of Zoology Department of Mandalay University, Dr Thant Zin, Professor, Curriculum Central Unit, Department of Education Research, Planning and Training, Yangon (Former Professor and Head, Department of Zoology, University of Mandalay), Dr Kay Thi Mya and Dr Ni Ni Win, Professors, Department of Zoology, Mandalay University for their suggestions and comments on this paper.

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