KARYOTYPE ANALYSIS OF DECCAN CARP, *LABEO POTAIL* (SYKES, 1839) AND STRIPED CATFISH, *PANGASIANODON HYPOPHTHALMUS* (SAUVAGE, 1878)

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Abstract

The deccan carp *Labeo potail* and striped catfish *Pangasianodon hypophthalmus* were obtained from Arrthit Private Fish Farm, Patheingyi Township to investigate their chromosomal characteristics by blocking the metaphase stage of mitotic division during January to August 2022. The spreads of metaphase stages of chromosomes were observed in the colchicine concentration 0.50 % for duration 4 hrs 30 mins in *L. potail* and 5 hrs 30 mins in *P. hypophthalmus* with 0.56 % hypotonic solution for duration 1 hr. The various organs of fishes were used for chromosome preparation. Karyological analysis revealed that 2n = 56 having 1 m (metacentric) + 3 sm (submetacentric) + 4 a (acrocentric) + 20 t (telocentric) with fundamental arm number (64) in *L. potail*, and 2n = 60 having 1 m (metacentric) + 8 sm (submetacentric) + 4 a (acrocentric) + 17 t (telocentric) with fundamental arm number (78) in *P. hypophthalmus*. The best metaphase spreads of chromosomes were observed in kidney and gill filaments compared with the liver, blood cells and oral cells in both of the fish species.

Keywords *Labeo potail, Pangasianodon hypophthalmus,* karyotype, colchicine

Introduction

Labeo is a large genus having several species which are of considerable importance as an article of food. Some of the species of the *Labeo* genus are reared for ornamental purpose, some as food species, some for extracting oil and some are considered to be of medicinal value. Among them, *Labeo potail* has good market value and high consumer preference, importance to fisheries and in aquaculture activities (Sarma *et al*., 2017). *Pangasianodon hypophthalmus* is one of the major fish species in the Mekong River Fishery, one of the largest and most important inland fisheries in the world. Striped catfish is also riverine freshwater species that can be found in Ayeyarwady Basin of Myanmar. Myanmar and other Asian countries followed after Vietnam in export sector (Griffiths *et al*., 2020).

A karyotype is the complete diploid set of chromosomes grouped together in pairs, arranged in order of decreasing in size within a nucleus of every cell in eukaryotes and haploid set of chromosomes observed in prokaryotes. The global fish fauna consists of about 2, 8900 species of fishes which 2,200 species are cytogenetically studied (Biswal *et al*., 2010).

The chromosomal characteristics of an individual species vary under the same genus or species. These characters may be different depending on the researchers' assumptions to identify chromosomal patterns (Levan *et al*., 1964).

Cytogenetic research on *L. potail* and *P. hypophthalmus* has not been carried out yet in Myanmar. That is why, these fishes from Arrthit Private Fish Farm were selected to investigate their chromosomal characteristics of these fishes.

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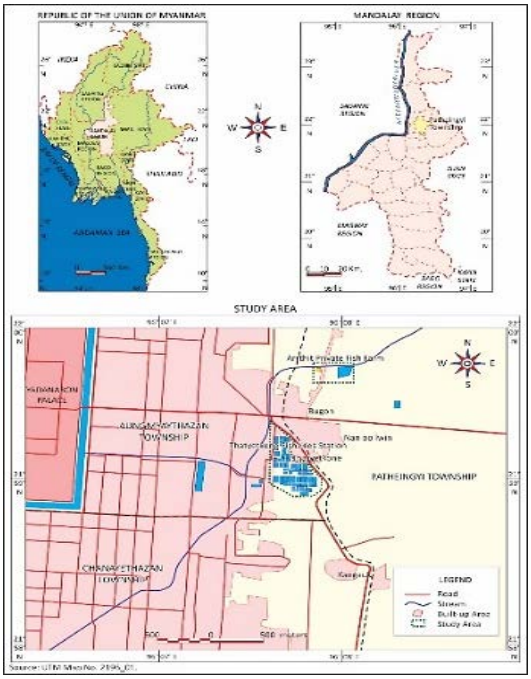


Figure 1 Map showing Arrthit Private Fish Farm, Patheingyi Township, Mandalay Region (Source from Universal Thermatic Map)

Materials and Methods

Study site

Arrthit Private Fish Farm is located around 21°59´50.90"N and 96° 07´50.23"E, Patheingyi Township, Mandalay Region (Fig. 1).

Study period

The study period was from January to August 2022.

Collection of the fishes

Forty fishes were collected from Arrthit Private Fish Farm and reared in the laboratory, Department of Zoology (Plate 1 and 2). The fishes were fed twice a day with formulated commercial feeds. The water was changed twice a week and kept in well-aerated aquarium (Plate 3 A).

Identification of species

The identification of fish species was conducted following after Talwar and Jhingran (1991) and Khamees *et al*. (2013).

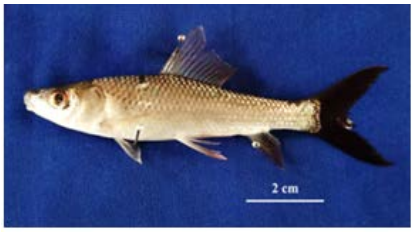


Plate 1 Lateral view of *Labeo potail*

Plate 2 Lateral view of *Pangasianodon hybpphthalmus*

Injection technique

Each fish was weighed with balance (kitchen scale) to the nearest 0.01 g and measured their standard length and total length to the nearest 0.1cm by using scaled ruler. The two different concentrations of colchicine (AVI CHEM, AC01318, India) solutions, 0.30 % and 0.50 %, were prepared and treated intramuscularly to fishes depending upon their weight (1ml /100 g) (Plate 3 B and C).

Collection of tissues

After injection with colchicine, fishes were placed into the aerated bowl to attain the designated time points. Blood was extracted from caudal peduncle by a syringe. Then, fishes were anesthetized with one or two drops of 10 % formaldehyde solution onto the head. The tissues samples such as oral cells, gill, heart, liver and kidney were harvested immediately for chromosomal preparation (Plate 3 D and E).

Extraction of cells

The sample tissues were incubated in hypotonic solution (0.56 % KCL) (MERCK Limited, Worll, Munbal) for 15 mins, 20 mins, 1hr and 1hr 30 mins and then minced these tissues with glass rod to get very fine particles. The incubated samples were mixed thoroughly with vortex mixer (NANOVA) by adding 3 methanol (Pure Chemical Industries., Ltd): 1 acetic acid (BDH Chemicals Ltd Poole England) and centrifuged (Firlabo) for 5 mins at 2000 rpm to get the pellets. The supernatant was discarded by using pipette. This procedure was repeated again (Plate 3 F, G and H).

Preparation of slides

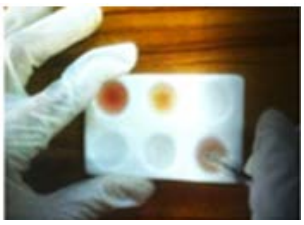
The slides were heated in the oven (Gallenhamp). One or two drops of pellets were placed onto the pre-warmed slides in a far distance. These slides were stained with undiluted Giemsa stain (AVI CHEM, India) for 10 mins, 15 mins and with diluted Giemsa stain for 20 mins. The stained slides were washed under running tap water. To prepare the permanent slides, the stained slides were covered with pre-cleaned coverslips and finally coated with Canada balsam (Kanto Chemical Co., Inc, Tokyo, Japan) (Plate 3 I, J and K).

A. Fish reared in glass tank

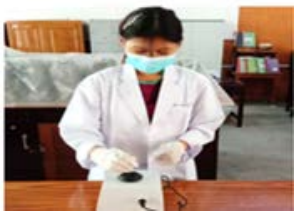
B. Weighing the fish

C. Injection of fish

D. Extraction of blood



E. Mechanical dissociation of tissues

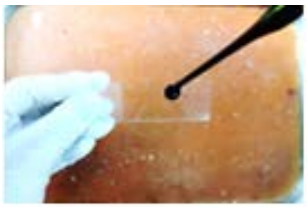


F. Homogenization of tissues

Plate 3 Preparation of cytological process from fish tissues

G. Centrifugation of pellets

H. Sucking the pellet from test tube to drop on slide



I. Dropping the Giemsa stain onto the slide

J. Washing the slide under tap water

K. Covering with cover slip

L. Examining the tissue

Plate 3 Preparation of cytological process from fish tissues

Chromosomal analysis and preparation of karyotype

The microphotographs were recorded with biological microscope with attach camera (G-303P, Taiwan) (x1000). Good quality metaphase spreads were recorded to analyse various stages of metaphase spreads, counting chromosomal numbers and microphotographed for karyotyping in mitotic division. The chromosomal numbers were counted by using ImageJ (1.52a, USA). All chromosome pairs were arranged linearly from biggest to smallest ones according to their size. The chromosome patterns were classified into metacentric (m), submetacentric (sm), acrocentric (c), and telocentric (t) with visual observation according to their designated chromosomal patterns (Levan *et al*., 1964) (Plate 3 L).

Statistical analysis

All recorded data was performed by Microsoft Excel 2010 to check the effects of mitotic inhibitors on mitotic division of cells from different tissues of designated fishes.

Results

The same concentration of 0.50 % colchicine solution was exposed to different organs of deccan carp *Labeo potail* and striped catfish *Pangasianodon hypophthalmus* such as gill filaments, oral cells, kidney, blood and liver. The effect of colchicine solution varied depending upon the type of somatic tissues. The optimum colchicine concentration and duration of *Labeo potail* was 0.50 % for 4 hrs 30 mins in gill filaments and 0.50 % for duration 5 hrs 30 mins in *Pangasianodon hypophthalmus* especially in the kidney tissues rather than other organs.

The best treatment of hypotonic solution was 1 hr on all extracted organs in *L. potail* and 1 hr 30 mins especially in kidney tissues of *P. hypophthalmus*. When staining with undiluted Giemsa stain on prepared slides, chromosomal patterns were clearly distinguished to designate the stage of mitotic division.

Systematic position of fishes

Kingdom	-	Animalia
Phylum	-	Chordata
Class	-	Actinopterygii
Order	-	Cypriniformes
Family	-	Cyprinidae
Genus	-	Labeo
Species	-	*L.potail* (Sykes, 1839)
Common Name	-	Deccan Carp

In *Labeo potail*, the mean total length of deccan carp (n = 10) used was 16.80 ± 1.09 cm, standard length 13.90 ± 3.70 cm and the body weight 10.32 ± 3.40 g. The meristic characters of *Labeo potail* were dorsal fin (D) ii 13, pectoral fin (P) i 13-14, ventral fin (V) - i 8, anal fin (A) ii 5-6, caudal fin (C) 22-26, lateral line scale (Lts) 37-38 and branchiostegal rays 3 (Plate 1).

Kingdom	-	Animalia
Phylum	-	Chordata
Class	-	Actinopterygii
Order	-	Siluriformes
Family	-	Pangasiidae
Genus	-	Pangasianodon
Species	-	*P.hypophthalmus* (Sauvage, 1878)
Common name	-	Striped catfish
Vernacular name	-	Nga Khu Zin Nga Dan

In *Pangasianodon hypophthalmus*, the mean total length of striped catfish (n =4) used was 22.80 ± 1.37 cm, standard length 21.00 ± 1.19 cm and the body weight 37.90 ± 0.97 g**.** The meristic characters of *P. hypothalamus* were dorsal fin (D) I 7, pectoral fin (P) I 10, ventral fin (V) i 7, anal fin (A) 29, caudal fin (C) 31 and lateral line scale (Lts) 50-51 (Plate 2).

Effects of solutions on blocking stages of cells

The metaphase stages of cells were observed at 4 hrs 30 mins with 0.56 % hypotonic solution in kidney cells and gill filaments, 5hrs in liver, oral cells and blood cells in *L. potail.* The range of chromosome count 56 – 60 (41.66 %) in gill filaments, 20 % in kidney cells and 36.36 % in liver cells. Among three tissues, gill filaments were the best for karyotype analysis, followed by liver cells (36.36 %) and kidney cells (20 %) (Fig. 2)

In *P. hypophthalmus*, the effect of colchicine solution on blood cells and kidney tissues with duration 5 hrs 30 mins and other tissues with 5 hrs were operated at the metaphase stages of chromosomes. Especially, the kidney cells generated the metaphase stage of cells with the optimal range of 56 – 60 (32.25 %), followed by the range 45 – 50 (25.80 %), 40 – 45 (19.35 %), 71 – 75 (12.90 %) and 61– 65 (3.22 %) (Fig. 3)

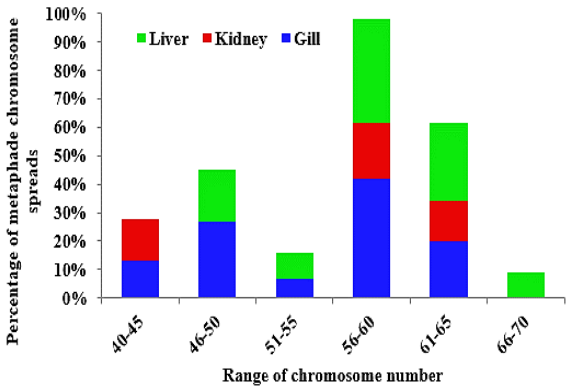


Figure 2 Frequency distribution of metaphase chromosome spread from different tissues of deccan carp *Labeo potail*

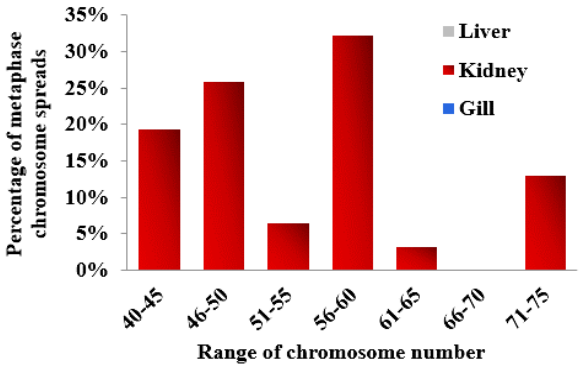


Figure 3 Frequency distribution of metaphase chromosome spread from different tissues of striped catfish *Pangasianodon hypophthalmus*

Percent and frequency distribution

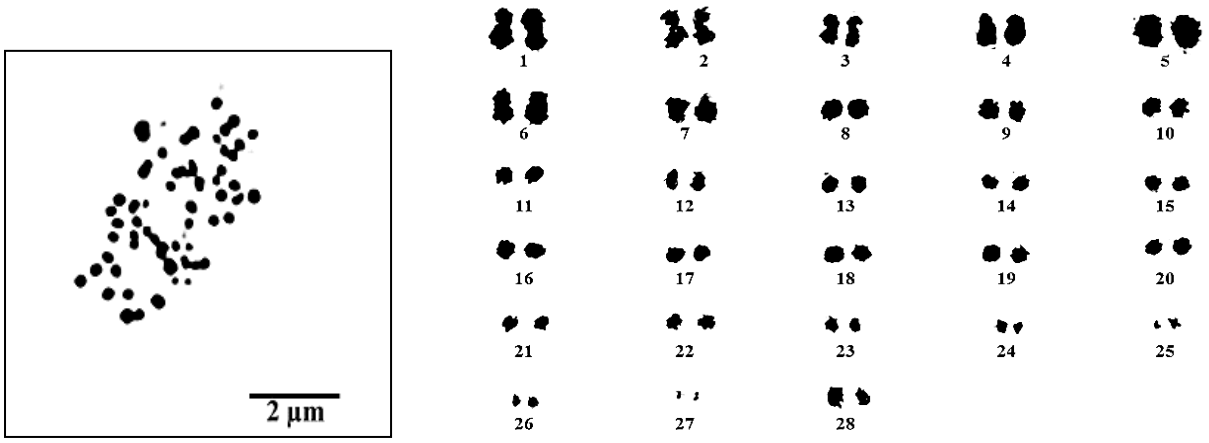
Sixty-three from *Labeo potail* and sixty-one from *P. hypophthalmus* well-spread metaphase complements were counted. In *L. potail*, the highest range of metaphase chromosome count was 56 – 60 with 43 % (n =14) having the cumulative frequency 76, followed by 61 – 65 with 21 % (n = 7), 46 – 50 with 18 % (n = 6), 40 – 45 with 9 % (n = 3) and 66 – 70 with 3 % (n = 1) except the range of 71 – 75. In *P. hypophthalmus*, the range of metaphase count 56 – 60 was the highest percentage 32 % (n = 10) with cumulative frequency 84 and the lowest range of 61 – 65 had 3 % (n = 1) with cumulative frequency 87. The second largest range of chromosome count was 46 - 50 with 26 % (n = 8). Unfortunately, the metaphase chromosome was not observed in the range of 66 – 70. However, the range 71 – 75 had 13 % (n = 4) of metaphase stage of cells (Table 1).

Table 1 Percent and frequency distribution of diploid number of chromosomes counts in deccan carp *Labeo potail* and striped catfish *Pangasianodon hypophthalmus*

Deccan carp *Labeo potail*					Striped catfish *Pangasianodon hypophthalmus*				
Treatment	Range	Frequency	Percentage (%)	Cumulative Frequency	Treatment	Range	Frequency	Percentage (%)	Cumulative Frequency
0.50 % Colchicine (4 hrs 30 mins) 0.56 % KCL (1 hr)	40-45	3	9	9	0.50 % Colchicine (5hrs 30 min) 0.56 % KCL (1 hr 30 mins)	40-45	6	19	19
	46-50	6	18	27		46-50	8	26	45
	51-55	2	6	33		51-55	2	7	52
	56-60	14	43	76		56-60	10	32	84
	61-65	7	21	97		61-65	1	3	87
	66-70	1	3	100		66-70	0	0	87
	71-75	0	0	0		71-75	4	13	100

Karyotype

The numbers of diploid chromosomes ranging from 40 - 65 in *Labeo potail* and 40 - 75 in *Pangasianodon hypophthalmus* were observed. The modal diploid chromosome number was 2n = 56 as it constituted 38 % and 2n = 64, constituted 14 % of counted plates in *Labeo potail* consisting of 1 metacentric, 3 submetacentric, 4 acrocentric and 20 telocentric with fundamental arm number (64) (Table 1, Plate 4 and 5).

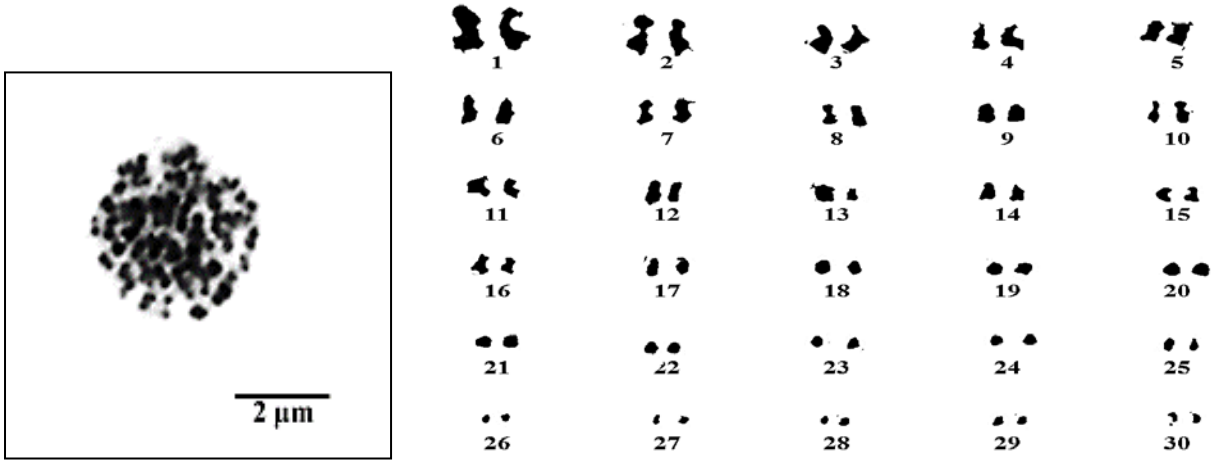


A. Mitotic metaphase chromosome Spread (x1000)

B. Karyotype

Plate 4 Chromosomes of deccan carp *Labeo potail*

The optimal diploid chromosome number was considered as 2n = 60, constituted 49 % and 2n = 74 with 13 % of metaphase plates in striped catfish *Pangasianodon hypophthalmus*. The modal diploid chromosome number of this fish was 1 metacentric (m), 8 submetacentric (sm), 4 acrocentric (a) and 17 telocentric (t) with fundamental arm number (NF = 78).



A. Mitotic metaphase chromosome Spread (x1000)

B. Karyotype

Plate 5 Chromosomes of striped catfish *Pangasianodon hypophthalmus*

Discussion

Fishes were widely cultivated in Arrthit Private Fish Farm, Patheingyi Township, Mandalay and distributed to local fishermen through Upper Myanmar. *Labeo potail* and *Pangasianodon hypophthalmus* were collected from Arrthit Private Fish Farm and investigated their genetics configuration on each species in this study.

Mahfuj *et al.* (2014) described that the variations of chromosomal characteristics are largely dependent on methods of chromosome preparation, staining procedure, tissue source of the body where the dividing cells. In the present study, the methods of chromosome preparation were based on testing with different concentrations of colchicine solutions using on various organs of fishes. The optimum colchicine concentration for *L. potail* and *P. hypophthalmus* was 0.50 % to block the metaphase checkpoint.

The successful outcome of check point was observed in liver tissues of *L. potail*. This result is an agreement with Mahfuj *et al.* (2014) who described that the liver tissues from two- days old larvae of *L. rohita* generated rather than using other somatic tissues.

The kidney tissues generated the best degree of chromosomal condensation rather than other tissues by using 0.50 % colchicine concentration for treatment 5 hrs 30 mins than 4 hrs 30 mins and 5 hrs. Therefore, this research is approved to John *et al.* (2017) who reported that the diploid number of chromosomes for *Labeo coubie* occurred more in the kidney plates than the liver plates.

In *Pangasianodon hypophthalmus*, the optimum concentration of colchicine solution 0.50 % for duration of 5 hrs 30 mins was good for kidney tissues and 5 hrs for other tissues. The present study is inconsistent with John *et al*. (2017) who stated that the best metaphase stages in *L. coubie* was 0.02 % colchicine solution for 4 hrs. The kidney tissues were the best tissues for metaphase spreads of mitotic division.

The hypotonic treatment of 0.56 % KCL for 1 hr was good for explosion of nuclear membrane (Win Win Mar and Thant Zin, 2020). According to the results of this study, longer treatment of 0.56 % KCL solution for 1 hr generates enough explosion of nuclear envelope and cytoplasmic membrane observed in all tissues and blood cells in *L. potail*. However, the same technique did not support the explosion of cells from all tissues in *P. hypophthalmus* expect the blood and oral cells. When the exposure time was raised up to 1 hr 30 mins, the check point of cells was observed.

Another important factor for cytogenetic analysis is Carnoyl's fixative (3 acetic acid: 1 methanol). In this study, Carnoyl's fixative was used to treat the extracted cells for 10 mins duration. The good shape of chromosomal configuration of *Labeo potail* and *Pangasianodon hypophthalmus* were obtained clearly leading to count and differentiated the chromosomal structure in detail.

Mahfuj *et al*. (2014) who described that their proposed standard karyotype for *Labeo rohita* was 18 m , 6 sm and 1 st chromosome; *L. coubie* was 2n= 10 m + 11 sm + 5 st + 24 t (John *et al.*, 2017) and *Labeo rohita* 8 m+ 6 sm + 4 st + 30 a (Bhatnagar *et al.*, 2014). The modal diploid chromosome number of *Labeo potail* was 2n = 56 with the range of 40 - 65. Standard karyotype of this fish consisted of 1 m+ 3 sm + 4 a + 20 t with fundamental arm number NF 64. And the karyological structure of *Pangasianodon hypophthalmus* was the diploid number 2n = 60 having 1 m + 8 sm + 4 a + 17 t with fundamental arm number (78).

Therefore, the karyological data of studied fishes are standardized for their population. Hence, these data highly recommended for further phylogenetic studies and should research on the strains with various karyotype formulae within the same species.

Conclusion

The effect of colchicine concentrations 0.50 % was better than 0.30 % with various durations of 4 hrs 30 mins, 5 hrs and 5 hrs 30 mins in *Labeo potail* and *Pangasianodon hypophthalmus.* The cells extracted from kidney tissues and gills filament induced the metaphase spreads in *L. potail*, however, it was by blood cells in *P. hypophthalmus.* The modal diploid number of *L. potail* was 2n = 56 with fundamental arm number (64), and 2n = 60 with fundamental arm number (78) in *P. hypophthalmus* was observed.

Acknowledgements

We would like to extend our grateful thanks 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 various supports, valuable suggestions and comments.

References

Biswal, J.R., Sahoo, P.K. and Mohanty, P.K, (2008) "Chromosomal studies on four cyprinid fishes. *Journal of Aquaculture*, vol.6, pp.39-44.

Griffiths, D., Van Khanh, P. and Trong, T.O, (2020) "Cultured Aquatic Species Information Programme, *Pangasianodon hypophthalmus* (Sauvage, 1878)" In: FAO Fisheries and Aquaculture Department (online), Rome. Accessed date February, 2020.

John, A.O., Obafunke, O.O., Fatima O.T. and Choima, N.G, (2017) "Karyotypic analysis of *Labeo coubie* (Ruppell,1832) (African carp) from University of Ilorin Dam, Ilorin, Nigeria." *Animal Research International journal*, vol.14(2), pp.2710-2714.

Khamees, N.R., Ali, A.H., Abed, J.M. and Adday T.K, (2013) "First record of Striped Catfish Pangasianodon hypophthalmus (Sauvage, 1878) (Pisces: Pangasiidae) from Inland Water of Iraq." *Basrah Journal of Agriculture Science*, vol. A (26), pp.184-194.

Levan, A., Fredge, K. and Sandberg, A.A, (1964) "Nomenclature for centromeric position and chromosome. *Heredities*. vol.52, pp.201-202.

Mahfuj, M.S.E., Hossain, M.B., Rahman, M.M. and Hoshan, M.I, (2014) "Chromosomal studies and quantitative karyotypic analysis of Rohu, *Labeo rohita*" *Pakistan Journal of Biological Sciences*, vol.17(4), pp.490-496.

Sarma, K.J., Prajapati, M. and Mankodi, P.C, (2017) "Morphological description and taxonomic account of *Labeo* species (Cypriniformes, Family: Cyprinidae) from Gujarat, India." *Journal of Entomology and Zoology Studies*, vol.5(4), pp.1122.

Talwar, P.K. and Jhingran, A.G, (1991) *Inland Fishes of India and adjacent countries*. Oxford and IBH Publishing Co. Pvt. Ltd, New Delhi, Bombay and Calcutta, vol.1, pp.152-331.

Win Win Mar and Thant Zin, (2020) "Karyotype analysis on *Oreochromis* spp. from Thatyetkhone Fisheries Station, Mandalay District." *Meiktila University Research Journal*, vol.1, pp. 310-315.