CHROMOSOMAL INVESTIGATIONS ON *CYPRINUS CARPIO* LINNAEUS, 1758 AND *LABEO ROHITA* (HAMILTON, 1822) FROM MONDAING DAM, MEIKTILA TOWNSHIP, MANDALAY REGION

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Abstract

The present research was conducted to study chromosomal variations and karyogram of *Cyprinus carpio* and *Labeo rohita* from Mondaing Dam, Meiktila Township, Mandalay Region. The study period lasted from July 2022 to January 2023. For chromosome preparation, 0.5 ml of blood sample was extracted from the caudal vasculature of selected specimens and mixed with 0.5% colchicine 0.5 ml and 100% sodium chloride (hypotonic solution) 1 ml. Then, tested for different durations of 1 hour 30 minutes, 1 hour 45 minutes, 2 hours, 2 hours 15 minutes, 2 hours 30 minutes and 2 hours 45 minutes and stained with undiluted Giemsa for 15 minutes. Finally, the two slides of the treated cell suspensions were fixed in two or three drops of Carnoy's fixative solution. The optimum metaphase spreads were observed at 2 hours and 15 minutes in *C. carpio* and 2 hours and 30 minutes and 2 hours and 45 minutes in *L. rohita.* The karyotype results of two fish species indicated that the diploid number of *C. carpio* was 2n = 100 with 28 metacentric (m), 38 submetacentric (sm), 12 acrocentric (a) and 22 telocentric (te) chromosomes with the number of fundamental arms was 166. The diploid number of *L. rohita* was 2n = 50 with 16 metacentric (m), 14 submetacentric (sm), 12 acrocentric (a) and 8 telocentric (te) chromosomes with the number of fundamental arms was 82.

Keywords: chromosomes, colchicine, hypotonic solution, karyotype, fundamental arms

Introduction

Myanmar is commonly regarded as a carp country, with carps accounting for 85% of the nation's overall aquaculture production (Fishery Statistics of Myanmar, 2009-2010). The common carp (*Cyprinus carpio*, Linnaeus, 1758), locally known as (shwe war Nga gyin) is a member of the family Cyprinidae and the order Cypriniformes. It is classified into seven subfamilies, 220 genera, and around 20,000 recognized species (Howes, 1991).

A prominent member of the Cyprinidae family, *Labeo rohita (Hamiltion 1822),* locally known as (Nga myint chin) is prevalent in the natural river systems of Bangladesh, India, Pakistan, and Myanmar (Talwar and Jhingran 1991).

The study of chromosome shape, structure, disease, function, and behavior is known as cytogenetics. Chromosomes are examined during mitotic or meiotic metaphase, but some studies such as fluorescent in situ hybridization (FISH) techniques, may study interphase cells (Lawce *et al*., 2017). Chromosomal studies in fishes are restricted to about 10 % of the total fishes all over the world (Sahoo *et al.*, 2007)

Understanding of the fundamental information on cytogenetics can be used to the development of commercially economic species in the future. The investigations on the karyotypes help to study the genetic construction of aquatic animal species in their respective habitats, thus it can decide what species are relationship to each other in a correct manner. This may aid to easy the hybridization between them in the future for strain advancement (Sofy *et al*. 2008).

There are many species of fishes in Myanmar because of the abundance of rivers, lakes, ponds, streams, and dams. The study on the karyotype of fish is very restricted compared to other area of researches. So, the karyotypes of fish species still need to be studied. The study of karyotypes helps to understand the karyomorphological

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variations of fishes, to identify the nature of chromosomes and to identify species accurately. For these purposes, the present study was conducted for chromosomal investigations on *Cyprinus carpio* and *Labeo rohita* fishes from Mondaing Dam, Meiktila Township, Mandalay Region in detail with the following objectives such as to investigate the chromosomal variations in *Cyprinus carpio* and *Labeo rohita*, to examine the karyogram of these two species.

Materials and Methods

Study area for specimen collection

Fish specimen collection was conducted at Mondaing Dam situating approximately 16 km on the west of Meiktila, Meiktila Township, Meiktila District, Mandalay Region, Myanmar. It located between North Latitude 20º 48′ 0″ N and 20º 50′ 15″ N and between East Longitude 95º 41′ 15″ E and 95º 45′ 0″ E. (Fig-1).

Study period

The study lasted from July 2022 to January 2023.

Sample collection

Ten specimens each of *Cyprinus carpio* and *Labeo rohita* were collected from Mondaing Dam from local fishermen and transported to the laboratory of Zoology Department, Meiktila University and kept in a well-aerated aquarium before study.

Identification of species

The collected fish specimens were identified according to Talwar and Jhingram (1991) and Froese and Pauly (2022).

Chromosomes preparation and analyses

For chromosome preparation, 0.5 ml of blood sample was extracted from the caudal vasculature of selected specimens and put into a blood collecting tube. Blood collecting tube was shaken for about 5 minutes to prevent blood clotting. 0.5 ml of 0.5% colchicine was added to blood sample collecting tube. One ml of 100% hypotonic solution (sodium chloride) was added to colchicine treated blood sample and was shaken for 5 minutes. Blood sample treated with colchicine 0.5 ml and sodium chloride 1 ml were exposed to different durations of 1 hour 30 minutes, 1 hour 45 minutes, 2 hours, 2 hours 15 minutes, 2 hours 30 minutes and 2 hours 45 minutes respectively. Supernatant was not removed. Finally, the treated cell suspensions were fixed in two or three drops of Carnoy's fixative solution. One or two drops of treated blood sample dropped on to glass slides, made a thin blood smear and left 15 minutes to dry. Then, the slides were stained undiluted Giemsa for 15 minutes and then rinsed in tap water. When the slides dried, Canada balsam was added and covered with cover slip. Chromosomes preparation was conducted according to Arsham *et al.* 2017. Chromosome metaphase spreads were examined under a microscope (Primostar, equipped with Axiocam ERc 5 s digital camera) with an oil immersion lens at 1000 magnification. The number of chromosomes were determined. Karyotyping was conducted according to Levan *et al.,* (1964). The homologous pairs were arranged based on the centromere position.

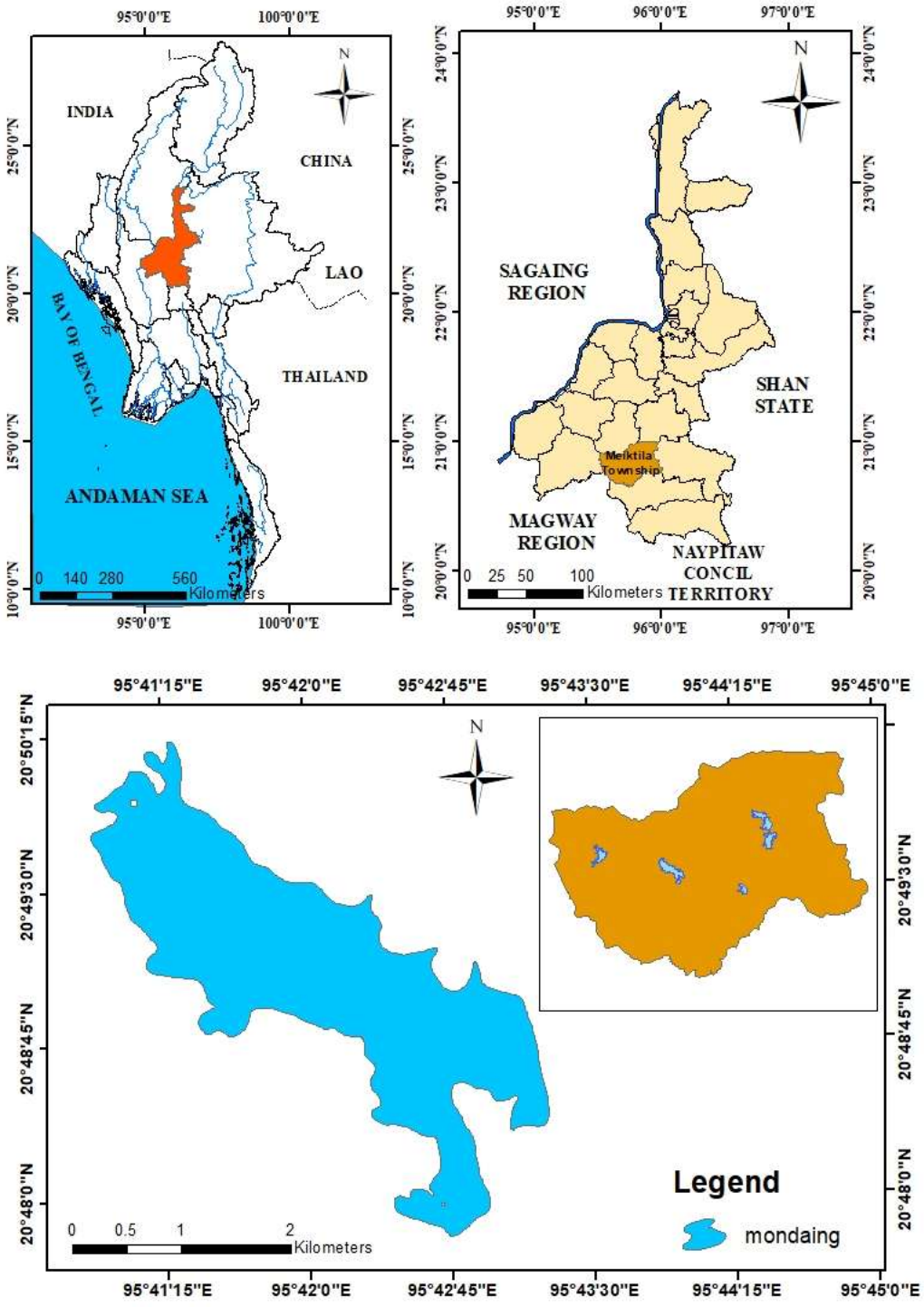


Figure. 1. Location map showing Mondaing Dam, Meiktila Township, Mandalay Region

Results

Morphological characteristics of studied fish species

Cyprinus carpio

Common name - Common carp

Vernacular name - Shwe War Nga Gyin

Body is stout, slightly compressed. Head is moderate, triangular; snout obtusely rounded. Mouth is small, oblique and protrusible; lips are thick and fleshy. Barbels are two pairs. Colour is usually olivaceous, with silvery or golden sides. Fins are yellowish, reddish, or golden. *Cyprinus carpio* is omnivorous and mostly bottom feeder (Plate 1A).

Labeo rohita

Common name - Rohu

Vernacular name - Nga myit chin or Nga gyi myet san ni

D = III 14, C = 22-24, A =I 7, Pc =17, Pv =9, LLS =42, LLS/A =7, LLS/B =6

Body is moderately elongate and devoid lateral lobe. Eyes are large. Mouth is small and inferior; lips are thick and fringed. Barbels are a pair of small maxillary barbels concealed in lateral groove. Color is bluish along back, with a reddish mark on each scale during breeding season; eyes are reddish. Fins are greyish or dark; pectoral fins are dusky. Rohu is a bottom feeder (Plate 1B).

(A) *Cyprinus carpio* (B) *Labeo rohita*

Plate 1. Studied fish species collected from from Mondaing Dam

Chromosome counts and karyotype

In the chromosome counts of *Cyprinus carpio*, the diploid number of 50 chromosomes with 24.3% (f = 17), 56 chromosomes with 12.9% (f = 9), 78 chromosomes with 1.4 % (f = 1), 88 chromosomes with 4.3 % (f = 3), 98 chromosomes with 18.6 % (f = 13) and 100 chromosomes with 38.6 % (f = 27) were observed. The karyotype formula was m = 28, sm = 38, a = 12, te = 22 with number of fundamental arms (NF) 166. As a result, the optimum metaphase spreads were observed at 2 hours and 15 minutes (Table 1 and 2; Figure 2 & Plate 2).

In the chromosome counts of *Labeo rohita*, the diploid number of 20 chromosomes with 8.6% (f = 6), 24 chromosomes with 4.3% (f =3), 25 chromosomes with 21.4% (f = 15), 26 chromosomes with 5.7% (f = 4), 28 chromosomes with 2.9% (f = 2), 38 chromosomes with 2.2% (f =2), 48 chromosomes with 8.6% (f =6), 50 chromosomes with 44.3% (f = 31) and 54 chromosomes with 1.4% (f =1) were found. The karyotype formula was m = 16, sm = 14, a = 12, te = 8 with number of fundamental arms (NF) 82. In consequence, the optimum metaphase spreads were observed at 2 hours and 30 minutes and 2 hours and 45 minutes (Table 3 and 4; Figure 3 & Plate 3).

Table 1. Frequency and percentage of different chromosome counts in *Cyprinus carpio*

Number of Chromosome counts	Frequency (f)	Percent	Cumulative percent
50	17	24.3	24.3
56	9	12.9	37.1
78	1	1.4	38.6
88	3	4.3	42.9
98	13	18.6	61.4
100	27	38.6	100.0
Total	70	100.0	

Table 2. Karyotype of *Cyprinus carpio*

Diploid chromosome number (2n)	Karyotype formula				Number of fundamental arms (NF)
	m	sm	a	te	
100	28	38	12	22	166

m = metacentric, sm = submetacentric, a = acrocentric, te = telocentric

Table 3. Frequency and percentage of different chromosome counts in *Labeo rohita*

Number of Chromosome counts	Frequency (f)	Percent	Cumulative Percent
20	6	8.6	8.6
24	3	4.3	12.9
25	15	21.4	34.3
26	4	5.7	40.0
28	2	2.9	42.9
38	2	2.9	45.7
48	6	8.6	54.3
50	31	44.3	98.6
54	1	1.4	100.0
Total	70	100.0	

Table 4. Karyotype of *Labeo rohita*

Diploid chromosome number (2n)	Karyotype formula				Number of arms (NF)
	m	sm	a	te	
50	16	14	12	8	82

m = metacentric, sm = submetacentric, a = acrocentric, te = telocentric

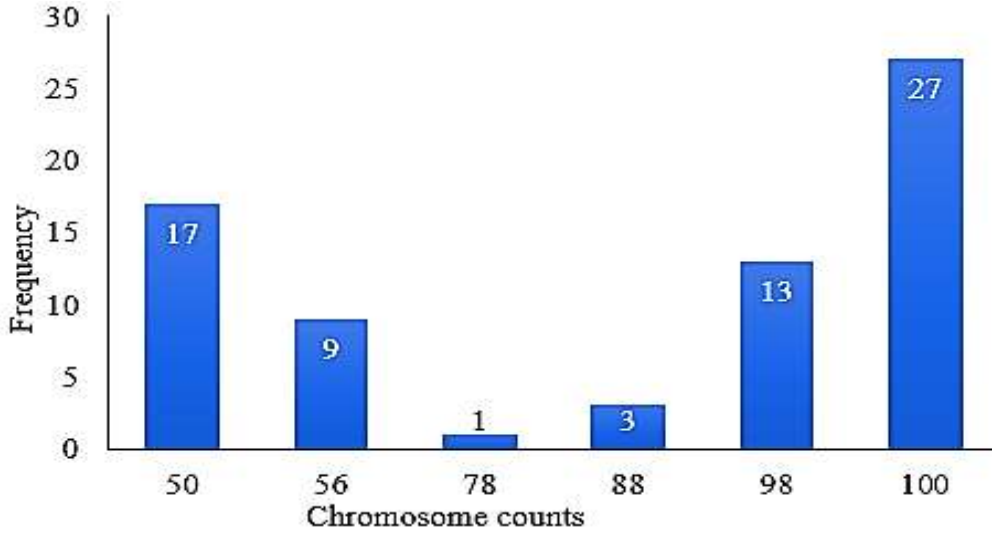


Figure. 2. Frequency of different chromosome counts in *Cyprinus carpio*

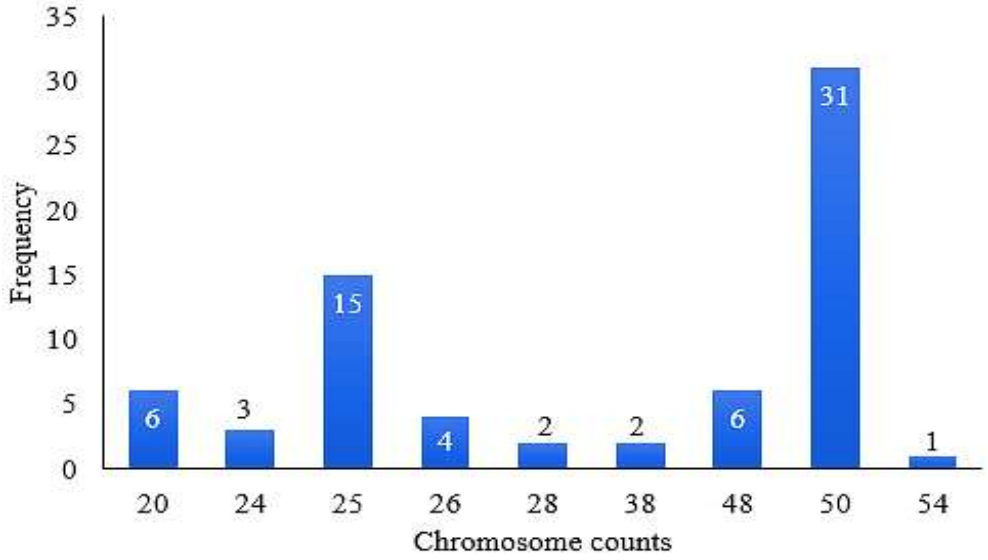


Figure. 3. Frequency of different chromosome counts in *Labeo rohita*

(A)

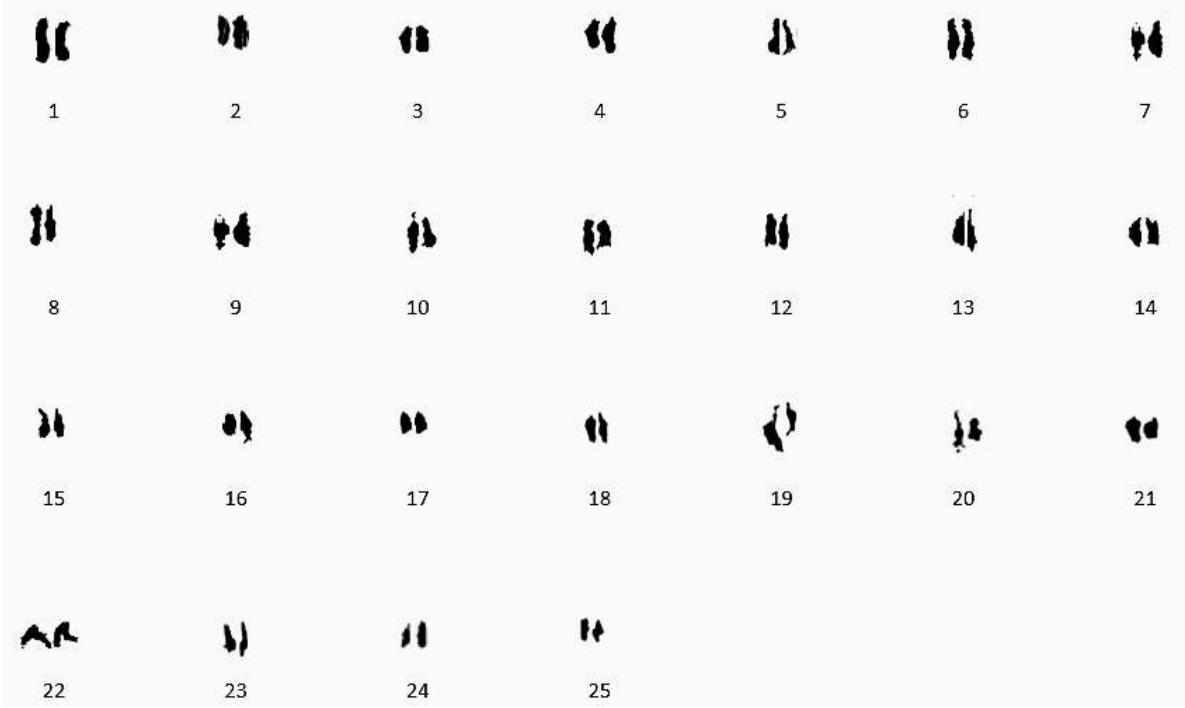
(B)

Plate 2. (A) Chromosomal spread of *Cyprinus carpio*

(B) Karyotype (28 m, 38 sm, 12 a, 22 te)

m=1-14, sm=15-33, a=34-39, te=40-50

(A)



(B)

Plate 3. (A) Chromosomal spread of *Labeo rohita*,
(B) Karyotype (16m, 14sm, 12a, 8t)
m=1-8, sm=9-15, a=16-21, te=22-25

Discussion

The species *Cyprinus carpio* and *Labeo rohita* are major carps in the aquaculture of Myanmar. In the present study, specimens of these two species were collected from Mondaing Dam during July 2022 to January 2023.

The results revealed that the diploid chromosome number of common carp was to be 2n = 100 frequency 27 with 38.6 percent. The karyotype formula of *C. carpio* are comprised of 28 m, 38 sm,12 a, 22 te (NF = 166). From a tetraploid origin, *C. carpio* is a teleostean species. Carp contains a great number of very small chromosomes; it has been moderately well examined by the cytogenetic researchers. Anjum (2005) described karyotype of *C. carpio* 2n = 100 (16 m, 34 sm, 50 a). And also, Salish and Majeed (2012) reported the karyotype of *C. carpio* as 2n = 100 (22 m, 32 sm, 12 te, 34 a). Similarly, the present studied carp species had the same number of 2n = 100 diploid chromosomes. Nevertheless, the morphologies of the investigated karyotypes varied somewhat from earlier researches. Cucchi and Baruffaldi, (1990) stated that bony fish possess relatively small size and many chromosomes leading to technological challenges for karyological research that are not found in other vertebrates. Chromosomes are organized according to their size and shape. In the present study, chromosomes in *C. carpio* were tiny and numerous, making it difficult to separate and arrange in homologous pairs.

In *L. rohita*, the diploid number was found as 2n = 50 chromosomes with the chromosome counts 50 frequency 31 with 44.3 percent. The karyotype consisted of 16 m, 14 sm, 12 a, 8 te (NF = 82). According to the previous literatures such as Win Mar *et al*. (2011) have been studied the karyotype formula 2n = 50 (10 m, 16 sm, 24 a) (NF = 76), 2n = 50 (36 m, 12 sm, 2 st) (Mahfuj *et al*., 2013), 2n = 50 (32 a, 4 st, 6 sm, 8 m) (Bhatanagar *et al*., 2014), 2n = 50; NF = 80; 8 m,14 sm, 8 a, 20 te) (Getlekha, *et al*., 2022). So, the present result of diploid number in *L. rohita* 2n = 50 was a conformity with above reports.

Buth *et al*., (1991) stated that the total chromosome number in cyprinids is wide-ranging between 42 and over 200. In the present study, the number of metacentric, submetacentric, acrocentric and telocentric chromosomes varied a little compared by previous literatures. The number of chromosomes ranged was from 50 to 100 in *Cyprinus carpio* whereas *L. rohita*, the number of chromosomes ranged from 20 to 54. These variances might be the result of chromosomal overlaps and loss during preparation, and staining and the various factors such as centromere inversions, amount of hypotonic solution, colchicine, and duration of time for preparation of chromosomes.

According to Sarasan *et al*., 2019, acrocentric chromosome is readily be mistaken as telocentric chromosome if they are highly constricted which is caused by excessive exposure to colchicine. Colchicine and hypotonic solution (NaCL) were crucial to produce the optimum chromosomal spreads in preparation of chromosomes. Colchicine 0.5 ml, 100% hypotonic solution (sodium chloride) 1 ml and undiluted Giemsa stain for 15 minutes gave the best result for present study.

The karyotype analysis is a key step towards improving the stock by polyploidy manipulation, hybridization and related generic engineering (Tan *et al*., 2004). Thus, like other animals, karyotype of aquatic species will be needed to study thoroughly.

The present research of cytogenetic investigation on *C. carpio* and *L. rohita* revealed variations in karyomorphology between the two species and there was no previous study in this field of research from fishes of Mondaing Dam. Thus, these are the first data of cytogenetic study of two fish species in this area and hope to provide basic information for future cytogenetic analyses of other Myanmar freshwater fishes.

Conclusion

The karyotype of two fish species described the diploid number of *Cyprinus carpio* 2n = 100 with 28 metacentric (m), 38 submetacentric (sm), 12 acrocentric (a) and 22 telocentric (te) chromosomes and the number of fundamental arms was 166. The diploid number of *Labeo rohita* 2n = 50 with 16 metacentric (m), 14 submetacentric (sm), 12 acrocentric (a) and 8 telocentric (te) chromosomes and the number of fundamental arms was 82. In *Cyprinus carpio*, optimum chromosome metaphase spreads were observed at 2 hours 15 minutes and in *Labeo rohita*, optimum chromosome metaphase spreads were observed at 2 hours 30 minutes and 2 hours 45 minutes.

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