Karyotype Analysis on *Oreochromis* spp. from Thatyetkhone Fisheries Station, Mandalay

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Abstract

Chromosomal characteristics of *Oreochromis* spp. was investigated using mitotic cell division of liver tissues to designate the genome composition of tilapia species. Karyological data was automated by SmartType 3 indicated the modal diploid number of *O. niloticus* was 4 m (metacentric), 10 sm (submetacentric), 2 st (subtelocentric) and 6 t (telocentric) with arm number 72; *O. mossambicus* was 2 m, 8 sm, 4 st and 8 t with arm number 64 and *Oreochromis* sp. was 2 m, 5 sm, 6 st and 9 t with 58 arm number indicating the significant difference in chromosomal characteristics. This study may provide the strain classification of chromosome analysis on tilapia species.

Key words: tilapia, karyotype, liver tissue, diploid, mitotic

Introduction

Tilapias, genus *Tilapia* and *Oreochromis* of family Cichilidae, are native fish of Africa (Trewavas, 1983) and the first recorded scientifically was conducted in Kenya in 1924 (Gupta and Acosta, 2004). They have been introduced either accidently or deliberately in too many countries around the world for last five decades (Pillary, 1990). Thus, the importance of tilapia culture is confirmed by continuous reviews and manual published over the year (Lim and Webster, 2006).

Tilapias have become globally aquatic species in many tropical and sub-tropical countries worldwide. In Myanmar, *Oreochromis mossambicus* was introduced in 1953 from Thailand, the *O. niloticus* and *O. aureus* were imported by People's Pearl and Fisheries Corporation from Israel in 1976 (Soe Tun, 2016). The red tilapia *O. hybrid* have been cultured intensively and distributed in rural farms since 2001. Later, genetics improvement programs were also initiated by administration of methyl testosterone hormone to produce monosex tilapia in Fisheries Station, and distributed through the local fish farms since 2002.

The cultured of pure stains population are important for successful development of fishery resource management by characterizing the morphometric characters vs cytological assessment of species. Karyological studies on fishes can contribute significantly to the solution of many problems in areas of research ranging from taxonomy, systematic on genetic to evolutionary phylogenetic or environmental toxicology in aquaculture (Al-Sabti, 1991; Sofy, Layla and Iman, 2008).

In Myanmar, the cytological data concerning tilapia have never been carried out in aquaculture research. The detail information on their genetic inheritance of pure species as well as hybrid strains is required for researchers, observers and scientific research, especially in fishery management.

The objectives of the present study were

- to gain information in technique of complete chromosome complement of *Oreochromis* spp.
- to know the karyotypic arrangement of *Oreochromis* spp.
- to determine the basic karyological structures of *Oreochromis* spp.

Materials and methods

Study site

The Thayetkhone Fisheries Station is situated in Patheingyi Twonship, Mandalay District. It is located at 21º 58' 58.72" - 21º 59' 28.53" N, 96º 7' 23.91" - 96º 7' 44.60" E (Plate.1).

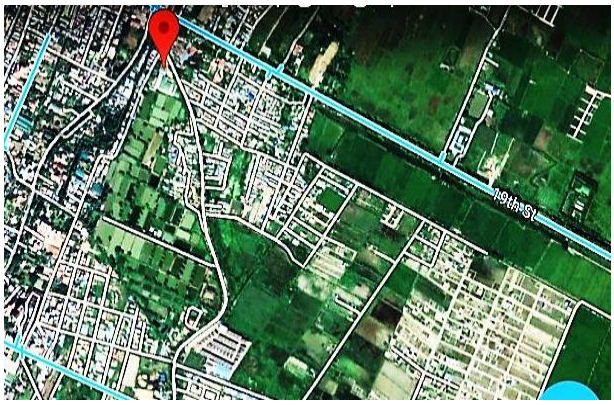

Plate 1. Map of the No.1. Thatyetkhone Fisheries Station, Patheingyi Twonship, Mandalay. Google Source: 2019

Study period

This study was conducted from June, 2017 to February, 2018.

Collection of specimens

Fifteen *Oreochromis niloticus*, 10 red tilapia *Oreochromis* sp. and eight *O. mossambicus* were used in this present study. All fishes were provided by Thayetkhone Fisheries Station, Mandalay. After capturing, the fish were transferred to the oxygenated plastic bags and cultured with well aerated in plastic tank in the Laboratory at Zoology Department in Meiktila University.

Cytological study

Liver tissues were excised from anesthetized adult fish for chromosomal studies. These excised tissues were put into blocked cup and crushed with glass rod to dissociate fine cells. These cells were mixed 0.06 % colchicine solution with vortex mixer (VM-1000) and were treated for 15 min, 30 min and 45 min to block the metaphase stages.

To obtain fine cell suspension, the fixed cells were centrifuged twice at 2000 rpm for 10 min (DSC - 200A - 2). The supernatant was removed by a Pasteur pipette. The tissues were transferred to hypotonic solution of 0.56 % KCl for 1 hr, 2 hr and 3 hr for cells swelling. The supernatant was discarded by centrifugation for 5 min at 2000 rpm. This procedure was made twice.

After treatment, the tissues were transferred to freshly prepared Carnoy's fixative solution (3 methanol: 1 acetic acid). The Carnoy's fluid was changed twice at an interval of 20 min and the later fixed solution was stored overnight. Then, the supernatant was discarded after centrifugation for 10 min at 2000 rpm.

The preparations were processed under air-drying technique and slides were stained with undiluted Giemsa stain for 2 min, 4 min and 6 min. The stained slides were dipped in xylene for 2 min according to Pradeep *et al.,* 2011, washed under running tap water and dry at room temperature. The slides were checked with immersion oil under Olympus CX-21FS1 microscope (x 1000). The microphotographs were taken in oil immersion using blue sub-stage filter with VCL camera software with Olympus CX - 40.

Chromosome pairing

The microphotographs recorded were generated by SmartType Demo SDB - 459 for chromosomal arrangement and all these microphotographs were converted pixel to micrometer by Micro ImageJ.

The length of each arm was measured with micrometer and the representative metaphases for the karyotype were arranged and classified into metacentric (M), submetacentric (SM), subtelocentric (ST), telocentric (T), with centromeric index (i) ranges of 40 - 47.5, 27.5 - 37.5, 15 - 25 and less than 12.5 according to chromosomal classification of Levan *et al.*,(1964). The length of each arm was measured the location of the centromere r = l / s, centromeric index i = 100 s/c, s is the short arm, l is the long arm, r is the ratio of chromosome arm, and c is the total length of the chromosome.

The fundamental arm number (FN) expressed as metacentric and submetacentric chromosomes were considered bi-armed; the subtelocentric and telocentric chromosomes were considered mono-armed (Levan *et al*., 1964). Chromosomal characteristics were resolved by Smart Type 3.

Statistical analysis

From microphotograph, all representative data of metaphases for the Karyotype were analyzed by Microsoft Excel 2010.

Results

A total of fifteen *Oreochromis niloticus*, eight *O.mossambicus* and five pair of red tilapia *Oreochromis* sp. were collected from Thayetkhone Fisheries Station, Mandalay. Their liver tissues were used for karyological studies to confirm their species status.

The different morphological characteristics of three types of *Oreochromis* spp. were shown in Table 1.

Table 1. Meristic characters of *Oreochromis niloticus, O.mossambicus* and *Oreochromis* sp. from Thatyetkhone Fisheries Station

Species	DF	PF	PLF	AF	CF	GR	LS	Remarks
Oreochromis niloticus	XVII 12-14	i, 13	I/5	III 9 – 11	18	19 - 22	Interrupted (23 + 20)	Caudal fin with 7-12 narrow vertical stripes
Oreochromis mossambicus	XVII 12-14	i, 13	I/5	III 9 – 11	20	18 - 23	Complete (34)	Caudal fin with a broad red margin
Oreochromis sp.	XV 11	i, 13	I/5	III 10	18	16 - 22	Interrupted (24 + 21)	Caudal fin vague or variable

DF = dorsal fin, PF = pectoral fin, PlF = pelvic fin, AF = anal fin, CF = caudal fin, GR = gill raker, LS = lateral line scale

Classification and systematic position of *Oreochromis* spp.

Kingdom - Animalia
Phylum - Chordata
Class - Actinopterygi
Order - Perciformes
Family - Cichilidae
Genus - *Oreochromis* Guntter, 1889
Species - *Oreochromis niloticus* (Linnaeus, 1758)
O. mossambicus (Peters, 1852)
Oreochromis sp.
(Behrends and Smitherman, 1984)

Mitotic phases in the fish species collected

Of the diploid 2n = 44 chromosomes the long arm found on chromosome one with arm ratio 1.852 by centromeric index 35.05 and short arm on chromosome 21. *Oreochromis niloticus* consisted of 4 metacentric (6, 9, 10, 11), 10 sub-metacentric (1, 4, 5, 7, 8, 12, 13, 14, 15, 22), 2 sub-telocentric (2, 3), 6 telocentric (16, 17, 18, 19, 20, 21) and the fundamental arm number NF = 72 observed (Table 2 and Plate 2).

Oreochromis mossambicus consists of the diploid number of 22 pairs with the longest chromosome one was 2.613 µm, the arm ratio 1.685 and centromeric index 37.23 belonged to sub-metacentric; the smallest chromosome 21 with 0.632 µm belonged to telocentric type. Its chromosomal formula was 2n = 44 (2 m +8 sm + 4 st + 8 t) and fundamental chromosome number 64 (Table 3 and Plate 3).

The modal diploid number 44 also occurred in red tilapia *Orechromis* sp. possessed the long arm chromosome one was sub-metacentric 2.39 µm with arm ratio 2.219 and centromeric index 31.07. The short arm was chromosome 20 with 0.611 µm. Its chromosomal formula was 2n = 44 (2 m + 5 sm + 6 st + 9 t) and fundamental chromosome number 58 (Table 4 and Plate 4).

Representative mitotic interphase chromosomes of the microphotographs were shown in Plate 5. Several types of chromosomal patterns such as leptotene (initiation of chromosome shortly), zygotene (initiation of synapsis), pachytene (completion of chromosome synapsis), diplotene (chiasma and crossing over), and diakinesis (terminization) were observed in this study.

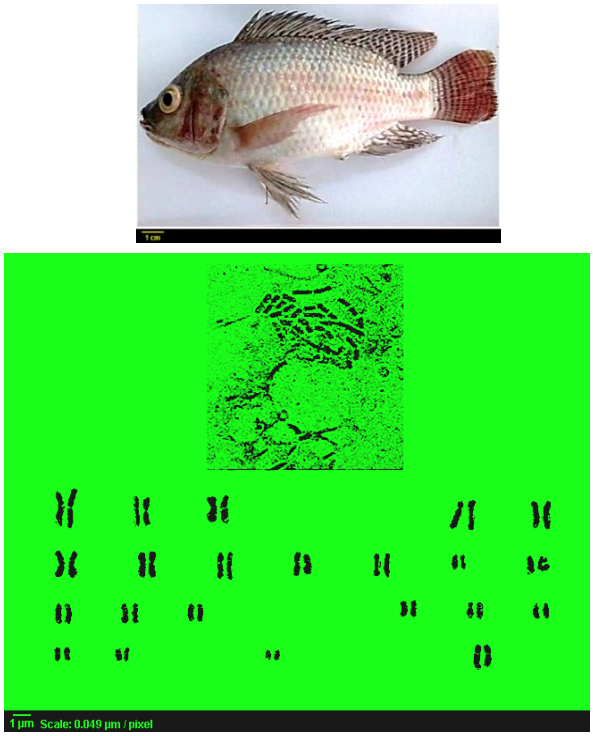

Plate 2. Karyotype in *Oreochromis niloticus* (2n = 44, NF = 72), and a metaphase spread of chromosomes.

Table 2. Measurements on the chromosome complement and classification of *Oreochromis niloticus*

Oreochromis niloticus (n=30)						
No	LSA (µm)	LLA (µm)	TL (µm)	AR (l / s)	CMI	C
1	0.909	1.684	2.593	1.852	35.05	sm
2	0.496	1.558	2.054	3.141	24.14	st
3	0.480	1.656	2.136	3.450	22.47	st
4	0.681	1.164	1.845	1.709	36.91	sm
5	0.578	1.108	1.686	1.916	34.28	sm
6	0.672	0.972	1.644	1.446	40.87	m
7	0.439	1.150	1.589	2.620	27.61	sm
8	0.496	1.053	1.549	2.122	32.02	sm
9	0.616	0.844	1.461	1.369	42.20	m
10	0.620	0.918	1.538	1.480	40.31	m
11	0.679	0.831	1.510	1.223	44.96	m
12	0.404	1.078	1.482	2.668	27.26	sm
13	0.436	1.019	1.455	2.338	29.95	sm
14	0.412	0.963	1.375	2.337	29.96	sm
15	0.438	0.929	1.367	2.121	32.04	sm
16	0	0.638	0.638	∞	∞	t
17	0	0.624	0.624	∞	∞	t
18	0	0.669	0.669	∞	∞	t
19	0	0.576	0.576	∞	∞	t
20	0	0.779	0.779	∞	∞	t
21	0	0.563	0.563	∞	∞	t
22	0.681	1.164	1.845	1.709	36.91	sm

M = metacentric, sm = sub-metrcentric, st = sub-telocentric, t = telocentric, C = classification, LSA = length of short arm, LLA = length of long arm, TL = total length, AR = arm ratio, CMI = centromeric index, l = long arm and s = short arm

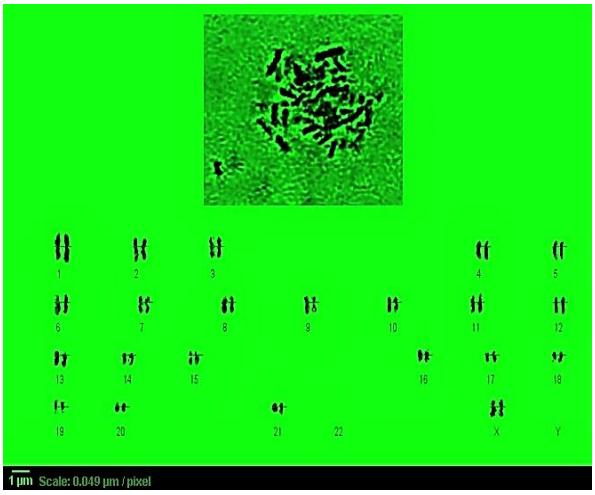

Plate 3. Karyotype in *Oreochromis mossambicus* (2n = 44, NF = 64), and a metaphase spread of chromosomes.

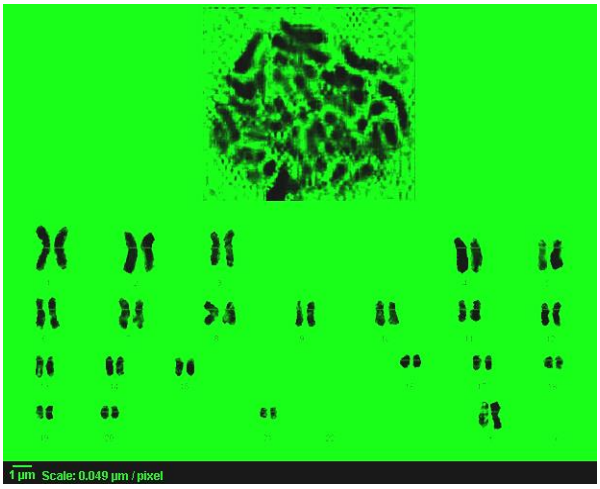

Plate 4. Karyotype in *Oreochromis* sp. (2n = 44, NF = 58), and a metaphase spread of chromosomes.

Table 3. Measurements on the chromosome complement and classification of *Oreochromis mossambicus*

Oreochromis mossambicus (n=30)						
No	LSA (µm)	LLA (µm)	TL (µm)	AR (l / s)	CMI	C
1	0.973	1.64	2.613	1.685	37.23	sm
2	0.625	1.492	2.117	2.385	29.53	sm
3	0.457	1.851	2.309	4.046	19.81	st
4	0.584	1.746	2.330	2.989	25.06	st
5	0.600	1.863	2.463	3.102	24.37	st
6	0.587	1.400	1.987	2.382	29.55	sm
7	0.610	1.293	1.903	2.119	32.05	sm
8	0.397	1.314	1.712	3.306	23.21	st
9	0.476	1.236	1.712	2.593	27.82	sm
10	0.586	1.371	1.957	2.339	29.94	sm
11	0.415	1.096	1.511	2.640	27.46	sm
12	0.719	0.767	1.486	1.067	48.36	m
13	0.760	0.900	1.66	1.184	45.78	m
14	0	1.148	1.148	∞	∞	t
15	0	0.888	0.888	∞	∞	t
16	0	1.000	1.000	∞	∞	t
17	0	0.879	0.879	∞	∞	t
18	0	0.992	0.992	∞	∞	t
19	0	0.806	0.806	∞	∞	t
20	0	0.856	0.856	∞	∞	t
21	0	0.632	0.632	∞	∞	t
22	0.555	1.362	1.917	2.454	28.951	sm

M = metacentric, sm = sub-metrcentric, st = sub-telocentric, t = telocentric, C = classification, LSA = length of short arm, LLA = length of long arm, TL = total length, AR = arm ratio, CMI = centromeric index, l = long arm and s = short arm

Table 4. Measurements on the chromosome complement and classification of *Oreochromis* sp.

Oreochromis sp. (n = 30)						
No	LSA (µm)	LLA (µm)	TL (µm)	AR (l / s)	CMI	C
1	0.745	1.653	2.398	2.219	31.07	sm
2	0.523	1.523	2.046	2.912	25.56	st
3	0.371	1.497	1.868	4.035	19.86	st
4	0.440	1.315	1.755	2.989	25.07	st
5	0.468	1.274	1.742	2.722	26.87	st
6	0.468	1.345	1.813	2.874	25.81	st
7	0.529	1.104	1.633	2.087	32.39	sm
8	0.435	1.1095	1.544	2.551	28.16	st
9	0.556	1.245	1.801	2.237	30.89	sm
10	0.618	1.037	1.655	1.677	37.36	sm
11	1.057	1.012	2.069	0.957	51.09	m
12	0.666	0.849	1.515	1.275	43.96	m
13	0	0.900	0.900	∞	∞	t
14	0	1.025	1.025	∞	∞	t
15	0	0.841	0.841	∞	∞	t
16	0	0.816	0.816	∞	∞	t
17	0	0.728	0.728	∞	∞	t
18	0	0.682	0.682	∞	∞	t
19	0	0.711	0.711	∞	∞	t
20	0	0.611	0.611	∞	∞	t
21	0	0.732	0.732	∞	∞	t
22	0.632	1.251	1.883	1.979	33.56	sm

M = metacentric, sm = sub-metrcentric, st = sub - telocentric, t = telocentric, C = classification, LSA = length of short arm, LLA = length of long arm, TL = total length, AR = arm ratio, CMI = centromeric index, l = long arm and s = short arm

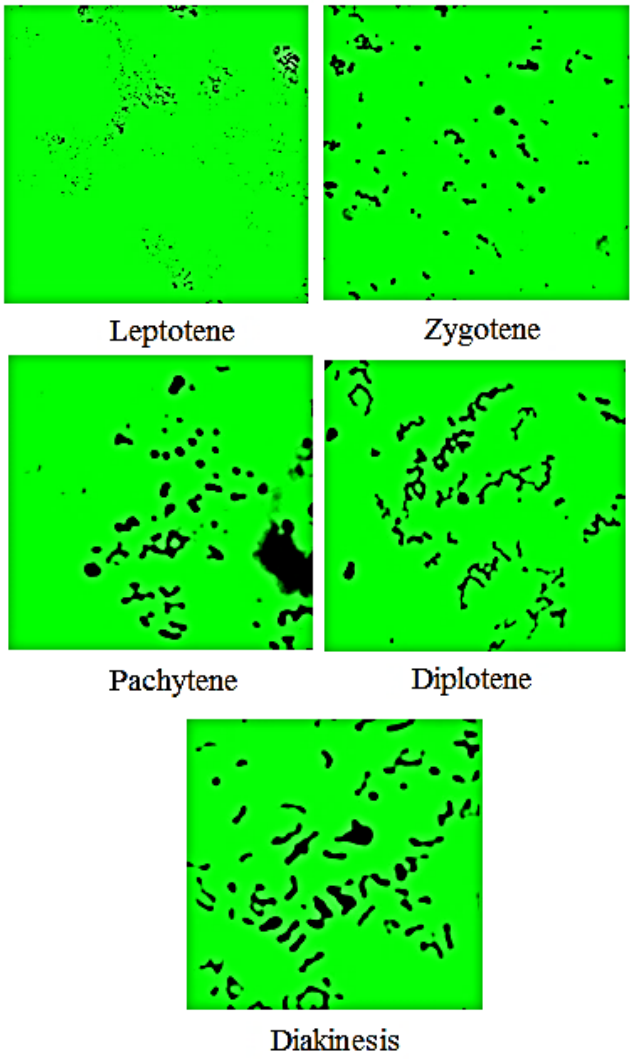

Plate 5. Mitotic cell division in interphase of *Oreochromis niloticus*

Discussion

Cytological investigation on three types of *Oreochromis* spp. revealed that the treatment duration of 0.06% colcichine to the anterior part of liver tissue cells for 45 min appeared as optimum to obtain the metaphase stage of cells. If the chromosomes are indiscreet to examine, the duration of treatment is required to rise up the designated time point. It must be emphasized that at most care, it should be taken to obtain the actual size of chromosomes because the longer treatment could lead to longer and the doubling the chromosomes numbers.

Moreover, inadequate or over concentration of colchicine treatments could lead to clumsiness of cells and inhibit the breaking of cell membrane (Pradeep *et al*., 2011). The duration of colchicine treatments varies according to use of tissues samples: injection of larger fish for 2 - 4 hr, but larger fish more than 20 cm for at least 6 hr (Ferdaus *et al*., 2013) and red hybrid tilapia fish use 0.01 % for 4 - 6 hr (Hussain *et al*., 1944a).

Hypotonic treatment is an important and crucial factor in improving the chromosome spread, helping in removal of lipid and denaturing proteins, and swelling the cell which facilities cell dissociation and dispassion of chromosomes (Pradeep *et al*., 2011). They reported that the hypotonic treatment for red tilapia was standardized for 40 min, however, this study showed one hour treatment gave the best results than 2 hr and 3 hr, respectively. The hypotonic treatment of 0.56 % KCl for 1 hr was good for explosion of nuclear membrane. It was noticed that, fixation for longer duration led to random distribution of chromosomes outside the cells before preparation of slides.

Furthermore, the duration of Carnoy's fixative for one night was enough throughout the preparation process. To classify and designate the chromosomal pattern, chromosomes were stained with haemotoxylene treatment for 5 min, 6 min, and 8 min (Ferdaus *et al*., 2013); 10 % Giemsa stain for 30 min (Howell and Black, 1980), 20 min (Pradeep *et* al., 2011) and 5% for 15 - 20 min (Manosroi *et al*., 2013). Depending upon the classification of chromosomal banding patterns, different chemicals could be used by different intensity.

In this study, the staining in undiluted Giemsa stain for longer period led to blurring of the chromosome pattern and it was difficult to differentiate the chromosomal classification. Therefore, it is very critical to get good pattern of chromosomes for classification of cytotaxonomy. Staining solution for 2 min was found to be adequate to analyze chromosomal size and shape. Furthermore, the level of dropping sample solution on the slide must also be adjusted in order to prevent over flowering and overlapping on the slide while warming the slide with burning funnel. Finally, clearing in Xylene for 2 min was found to give the best shape of chromosomes pattern.

Chromosomal classification revealed that these three species have different diploid chromosomal characteristics with different fundamental arm number (NF): *Oreochromis niloticus* has 4 m, 10 sm, 2 st and 6 t with arm number 72; *O.mossambicus* has 2 m, 8 sm, 3 st and 9 t with arm number 64; and tilapia *Oreochromis* sp. has 2 m, 5 sm, 6 st and 9 t with arm number 58, respectively. These results are not consistent with other researchers. The karyotype of *O.niloticus* has 4 sm, 16 ac and 24 t with arm number NF = 64 reported by Arai and Koike (1980); 18 st, 26 ac with NF = 62 in *O. niloticus*, 2 sm, 24 st, 18 t with NF = 70 (Softy, Layla and Iman, 2008); 2 sm, 12 a, 30 t with NF = 58 (Supiwong *et al*, 2013); and 4 m, 3 sm,1 st, 36 t with NF = 52 (Ferdaus *et al*., 2013) ; 6 sm,10 ac, 28 a with NF = 60 in *O. mossambicus* and 2 s, 6 st, 36 a with NF = 50 in Thai Red Tilapia (Manosroi *et al*., 2013).

In fact, the chromosomal characteristics such as uni-armed and bi-armed are mainly depends on the majority of authors and the different scoring of sub-telocentric chromosomes (Philips and Rab, 2007). Fortunately, different stages of chromosomal types:

leptotene, zygotene, pachytene, diplotene and diakinesis were observed in this study. These could be the treatment time to cells did not coincide with the check point of metaphase cell division cycle. Nanda *et al*., (1995) reported that several incomplete metaphases were encountered as the results from hypotonic overtreatment. In this study, all cytological data of three *Oreochromis* spp. were conducted to be the first study to fulfill the requirement of cytological information and to understand the aspects of cytological variation in Tilapia from culture ponds in aquaculture.

Conclusion

The modal number of *Oreochromis* spp. revealed diploid number 2n = 44, however their chromosomal characteristics are quite different among them indicating their specific species status. This is the first report of cyto-taxonomis study of tilapia species. Further researches are needed to compromise the characteristics of tilapia species in other regions.

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