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Karyological analysis of ten freshwater fish species from Lumphachi river basin, Thailand

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Abstract - This research aims to study karyotype of ten species of freshwater fishes from Lumphachi river basin, Suan Phueng district, Ratchaburi province. Chromosomes were prepared directly from kidney tissue of each fish species (ten individual/species) and were then stained by conventional staining technique (Giemsa's stain). The karyotype results of all ten fish species were shown as followed: Clown featherback (*Chitala ornata*) (2n = 42; NF = 44; 2a+40t), Striped tiger leaffish (*Pristolepis fasciatus*) (2n = 48; NF = 48; 48t), Three spot gourami (*Trichopodus trichopterus*) (2n = 46; NF = 46; 46t), Silver barb (*Barbonymus gonionotus*) (2n = 50; NF = 78; 6m+16sm+6a+22t), Red tailed tinfoil (*Barbonymus altus*) (2n = 50; NF = 86; 12m+20sm+4a+14t), Tire track eel (*Mastacembelus favus*) (2n = 48; NF = 68; 10m+6sm+4a+28t), Black sharkminnow (*Labeo chrysophekadion*) (2n = 50; NF = 86; 10m+8sm+18a+14t), *Poropuntius melanogrammus* (2n = 50; NF = 86; 10m+12sm+14a+14t), Hampala barb (*Hampala macrolepidota*) (2n = 50; NF = 86; 6m+18sm+12a+14t), and Roho labeo (*Labeo rohita*) (2n = 50; NF = 80; 8m+14sm+8a+20t), respectively. No sex chromosome differences were found in all fish karyotypes. Based on the data, this is the first report of cytogenetic study of fish species in this area. The information obtained can be used to support ~~the~~ taxonomic classification of freshwater fishes, manipulated for conservation of genetic resources of fishes in nature, and can be useful to understanding of chromosomal evolution of freshwater fishes in the future. Moreover, this finding can be used as a basis for genetic resources conservation and chromosomal evolution among fishes in the future.

Keywords: Freshwater fishes, chromosome, karyotype

1. Introduction

Phachi river basin, Suan Phueng district, Ratchaburi province is a river in the west of Thailand. It originated from the Tanaosri mountains and consists of many streams flows through Suan Phueng district and Chom Bueng district in Ratchaburi province and then meets the Kwai Noi river in Mueang Kanchanaburi district, Kanchanaburi province. This river brings great benefits for agriculture and consumption to the people who live on both sides of the river but cannot be used as a transport route because of the winding and the water is full of rapids caused the Phachi river has a relatively high biodiversity of fish. In addition, its unique landscape, unlike other wetlands, is a contributing factor in the emergence and conserve of fish populations that bring it possible to find highly specific fish and different from the other areas since the fish have adapted to the environment.

Freshwater fish plays an important role in the national economy of Thailand. The export value of freshwater fish is several hundred million baht a year (Rainboth, 1996). The understanding of fish genetic especially the chromosome which is represents the structure of the whole genome can be applied in further studies on stain improvement of the fish for preferable characteristics. Moreover, it can be used in planning, management, selection to increase the high potential of the fish improvement in the future. The present study aimed to analyze the chromosomal structure in ten species of freshwater fishes from Lumphachi River Basin, Suan Phueng district, Ratchaburi province namely, *Chitala ornata*, *Pristolepis fasciatus*, *Trichopodus trichopterus*, *Barbonymus gonionotus*, *Barbonymus altus*, *Mastacembelus favus*, *Labeo chrysophekadion*, *Poropuntius melanogrammus*, *Hampala macrolepidota* and *Labeo rohita*, by using cytogenetic techniques. The knowledge will provide the useful information for fish breeding in strain improvement program, conservation, commercials, evolution, systematics, phylogenetics, fish fauna management and suitable conservation.

2. Materials and methods

2.1 Specimens collection

Ten species of freshwater fish were analyzed including, Clown featherback (*Chitala ornata*), Striped tiger leaffish (*Pristolepis fasciatus*), Three spot gourami (*Trichopodus trichopterus*), Silver barb (*Barbonymus gonionotus*), Red tailed tinfoil (*Barbonymus altus*), Tire track eel (*Mastacembelus favus*), Black sharkminnow (*Labeo chrysophekadion*), *Poropuntius melanogrammus*, Hampala barb (*Hampala macrolepidota*), and Roho labeo (*Labeo rohita*) from Lumphachi river basin, Suan Phueng district, Ratchaburi province. The specimens were caught using hand-net. After capture, animals were placed in sealed plastic bags containing oxygen with clean water and transported to the research station.

2.2 Chromosomal preparations and staining

The procedures followed ethical protocols; an aesthesia was conducted by kept in freeze before euthanasia. The process was approved by the Ethics Committee of Muban Chombueng Rajabhat University and by the Ethics of Animal Experimentation of the National Research Council of Thailand

under licence no. U1-04484-2559. Mitotic chromosomes were obtained from cell suspensions of the anterior kidney: a solution of 0.02% colchicine (1 ml per 100 g body weight) was injected into the abdominal cavity and left for one hour. Chromosomes were prepared from the kidney cells of the fish by the squash technique. Kidney tissues were cut into small pieces then mixed with hypotonic solution (0.075 M KCl). After discarding all large pieces of tissues, 7 ml of cell sediments were transferred to a 15 ml centrifuge tube and incubated for 45 min. Hypotonic solution was discarded from the supernatant after centrifugation at 2500 rpm for 8 min. Cells were fixed in a fresh cool fixative (3 absolute methanol :1 glacial acetic acid) to which up to 7 ml by gradually added before being centrifuged again at 2500 rpm for 8 min, at which time the supernatant was discarded. The fixation was repeated until the supernatant was clear and the pellet was then mixed with 1 ml fixative. The mixture was dropped onto a clean slide by a plastic pipette followed by air-dry technique (Getlekha *et al*., 2016a, 2016b). The slide was then conventionally stained with 20% Giemsa's solution for 20 min. The specimens were deposited in the fish collection of the Department of Biology, Faculty of Science and Technology, Muban Chombueng Rajabhat University.

2.3 Image processing

At least 20 metaphase spreads were analyzed to confirm the 2n, karyotype structure. Images were captured using a digital microscope Olympus CX31 with visualization device based on LCD screen and camera. Chromosomes were classified as metacentric (m), submetacentric (sm), acrocentric (a) or telocentric (t) according to their relative length (Chaiyasut, 1989).

3. Results and discussion

All 10 fish species that analysis. It consists of 4 orders, 5 families, arranged in order of taxonomy and systematics as follows:

1. Order Osteoglossiformes has 1 family; Notopteridae has 1 species.

2. Order Cypriniformes has 1 family; Cyprinidae has 6 species.

3. Order Synbranchiformes has 1 family; Mastacembelidae has 1 species.

4. Order Perciformes has 2 families; Anabantidae, 1 species and Pristolepidae, 1 species.

The ten analyzed freshwater fish species share the diploid number (2n) = 42-50; however, they can be divided into two major groups in terms of their chromosomal formulas: *C. ornata* (2n = 42; NF = 44; 2a+40t), *P. fasciatus* (2n = 48; NF = 48; 48t), and *T. trichopterus* (2n = 46; NF = 46; 46t) display karyotypes formed exclusively by telocentric chromosomes, while the karyotypes of *M. favus* (2n = 48; NF = 68; 10m+6sm+4a+28t), *B. gonionotus* (2n = 50; NF = 78; 6m+16sm+6a+22t), *B. altus* (2n = 50; NF = 86; 12m+20sm+4a+14t), *L. chrysophekadion* (2n = 50; NF = 86; 10m+8sm+18a+14t, *P. melanogrammus* (2n = 50; NF = 86; 10m+12sm+14a+14t), *H. macrolepidota* (2n = 50; NF = 86; 6m+18sm+12a+14t), and *L. rohita* (2n = 50; NF = 80; 8m+14sm+8a+20t) are composed of mono-armed and bi-armed chromosomes. Here, the metaphase chromosomes and karyotypes of ten species of freshwater fishes are shown in Figure 1 and Figure 2.

No cytologically distinguishable sex chromosomes were observed. It is possible that the change in the sex chromosomes of the fish depends on the onset of differentiation. (Supiwong *et al*., 2012). Therefore, chromosomes containing sex-determination gene cannot be found by cytogenetic analyses. The origin and development of sex-chromosome has been reported on Neotropical fish in Brazil (Bertollo *et al*., 2004).

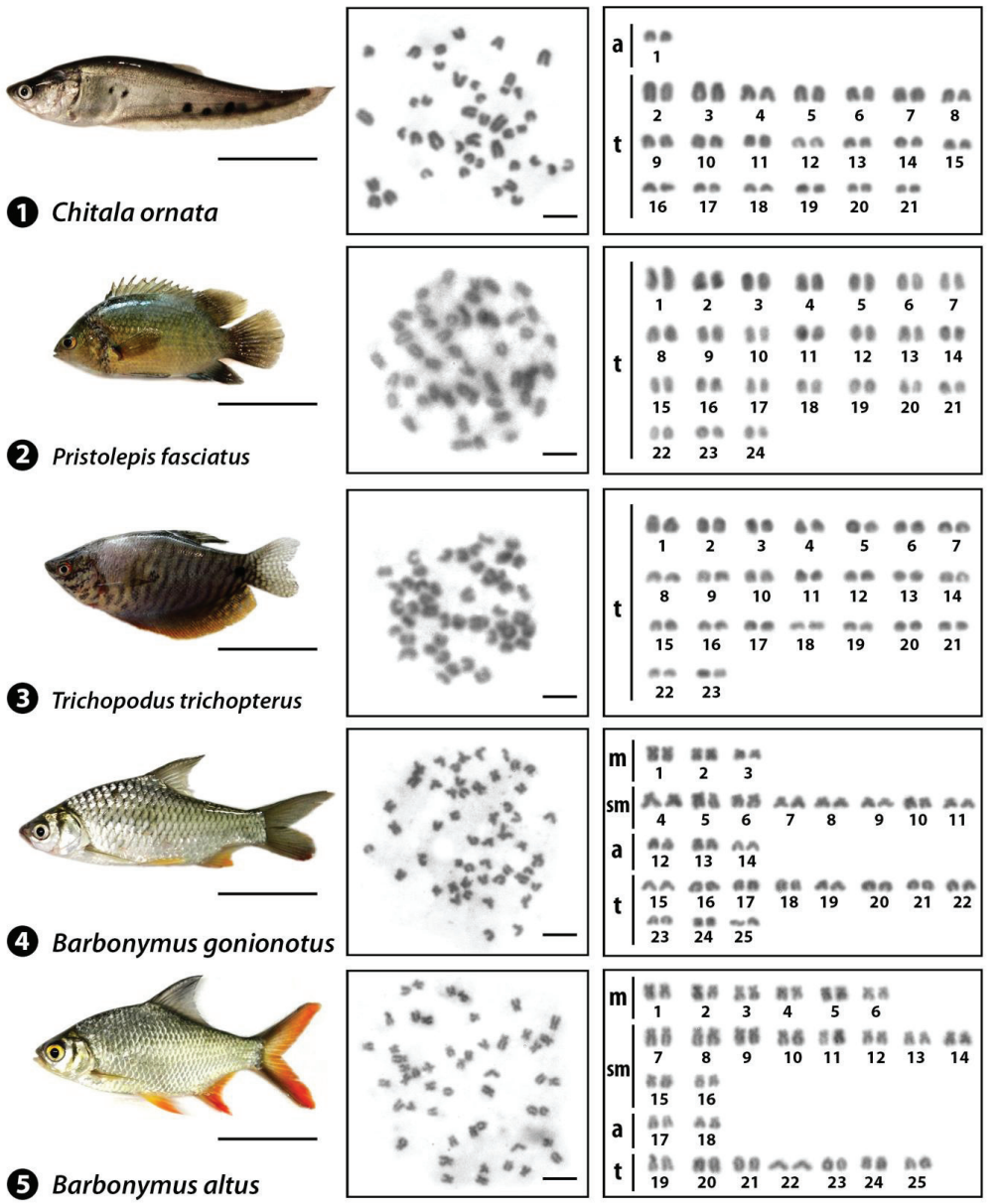

Figure 1. Metaphase and karyotypes of Clown featherback (*Chitala ornata*), Striped tiger leaffish (*Pristolepis fasciatus*), Three spot gourami (*Trichopodus trichopterus*), Silver barb (*Barbonymus gonionotus*), Red tailed tinfoil (*Barbonymus altus*) arranged from conventionally Giemsa-stained. Bar = 5 cm (fish) and 5 μm (chromosome).

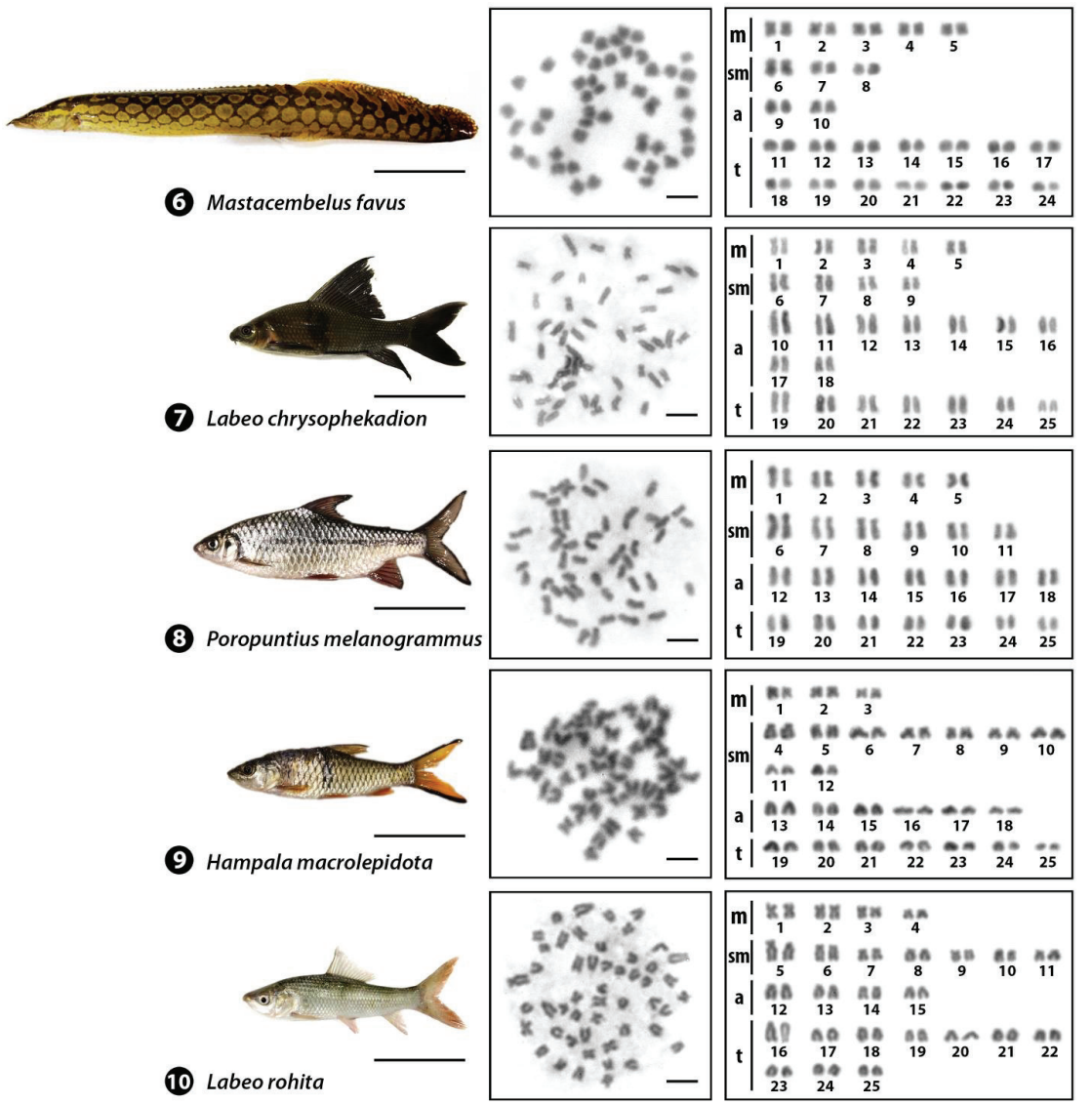

Figure 2. Metaphase and karyotypes of Tire track eel (*Mastacembelus favus*), Black sharkminnow (*Labeo chrysophekadion*), *Poropuntius melanogrammus*, Hampala barb (*Hampala macrolepidota*), and Roho labeo (*Labeo rohita*) arranged from conventionally Giemsa-stained. Bar = 5 cm (fish) and 5 μm (chromosome).

Table 1. Comparative karyotype and reviews of cytogenetic reports in the ten species of freshwater fishes

No.	Subfamily/Species	2*n*	NF	Formula	References
1	Chitala ornata	42	42	2st+40a	Donsakul and Magtoon (1990)
		42	44	2a+40t	Supiwong *et al.*, (2012)
		42	44	2a+40t	Present study
2	Pristolepis fasciatus	48	-	48t	Swaminathan *et al.*, (2013)
		48	48	48t	Present study
3	Trichopodus trichopterus	46	46	46t	Supiwong *et al.*, (2010)
		46	46	46t	Present study
4	Barbonymus gonionotus	50	72	2m+20sm+4st+24a	Magtoon and Arai (1989)
		50	74	16m+8sm+26a	Donsakul and Magtoon (1997)
		50	72	6m+16sm+6st+22a	Piyapong (1999)
		50	66	2m+4sm+10st+34a	Seetapan (2007)
		50	74	6m+18sm+16st+10a	Khuda-Bukhsh and Das (2007)
		50	78	6m+16sm+6a+22t	Present study
5	Barbonymus altus	50	80	20m+10sm+4st+16t	Donsakul and Poopityastaporn (2002)
		50	86	12m+20sm+4a+14t	Present study
6	Mastacembelus favus	48	68	10m+4sm+4st+30a	Donsakul and Magtoon (1989)
		48	68	10m+6sm+4a+28t	Present study
7	Labeo chrysophekadion	50	74	10m+14sm+26a/t	Donsakul and Magtoon (2002)
		50	86	10m+6sm+20a+14t	Sarasan *et al.*, (2019)
		50	86	10m+8sm+18a+14t	Present study
8	Poropuntius melanogrammus	50	86	10m+12sm+14a+14t	Present study
9	Hampala macrolepidota	50	72	10m+12sm+8st+20a	Donsakul and Poopitayasathaporn (2002)
		50	86	6m+18sm+12a+14t	Present study
10	Labeo rohita	50	70	14m+6sm+4a+26t	Magtoon and Donsakul (1993)
		50	80	8m+14sm+8a+20t	Present study

Note: 2n = diploid chromosome number, NF = fundamental number (number of chromosome arm), m = metacentric, sm = submetacentric, a = acrocentric, st = subtelocentric and t = telocentric chromosome.

From the results, some species exhibited karyotypes with broadly similar structural patterns, with all of their displaying 2n = 42-48 and karyotypes with a predominant of telocentric chromosomes. These characteristics, present in some freshwater fish species analyzed so far (Table 1), are considered a basal condition for fish karyotype (Brum & Galetti, 1997). Moreover, the 2n is slightly different among the three species (*C. ornata*, *P. fasciatus*, and *T. trichopterus*), may be caused by chromosome lose during evolution, especially the *C. ornata* that happened together with centric fussion of some chromosomal pairs. However, the remaining analyzed fish species share the same diploid number (2n=50) constant among the six species (*B. gonionotus*, *B. altus, L. chrysophekadion*, *P. melanogrammus*, *H. macrolepidota*, and *L. rohita*) except *M. favus* have 2n=48, slight variations in their NF indicate that pericentric inversions are the primary mechanism for karyotype diversification in these fishes (Arai and Inoue 1976). Indeed, the extremely high NF values in other freshwater fish, suggest the role of the pericentric inversions in their chromosomal differentiation (Getlekha *et al*., 2016a).

The present findings of 2n= 42-50 in the ten freshwater fish species analyzed are consistent with some of the previous reports (Table 1). However, there are slightly differences in the number of metacentric, submetacentric, acrocentric and telocentric chromosomes. These differences may be due to several factor such as the chromosomal evolution, the intra-species chromosome variation and chromosomal misidentification. For example, acrocentric chromosome is easily be misidentified as telocentric chromosome if they are constricted at high degree which is caused by over exposure to colchicine (Sarasan et al,. 2019). However, study of karyotype of different population should be performed to confirm the variation of chromosomal characteristic of these fishes as well as additional information and molecular techniques for chromosome analyses are expected to clarify and explain the reasons to support the karyotype polymorphism and chromosome evolution in these fishes.

4. Conclusion

Cytogenetic data, together with geographical and phylogenetic inferences, enable a better understanding of the differentiation trends in fish karyotypes. The chromosomal study of ten species of freshwater fish from Lumphachi river basin, Suan Phueng district, Ratchaburi province indicated : *C. ornata* (2n = 42; NF = 44; 2a+40t), *P. fasciatus* (2n = 48; NF = 48; 48t), *T. trichopterus* (2n = 46; NF = 46; 46t), *B. gonionotus* (2n = 50; NF = 78; 6m+16sm+6a+22t), *B. altus* (2n = 50; NF = 86; 12m+20sm+4a+14t), *M. favus* (2n = 44; NF = 68; 10m+6sm+4a+28t), *L. chrysophekadion* (2n = 50; NF = 86; 10m+8sm+18a+14t), *P. melanogrammus* (2n = 50; NF = 86; 10m+12sm+14a+14t), *H. macrolepidota* (2n = 50; NF = 86; 6m+18sm+12a+14t), and *L. rohita* (2n = 50; NF = 80; 8m+14sm+8a+20t), respectively. From the comparison of fish in the same family and the same order that we are studying, the data represent as follows:

1. Order Osteoglossiformes, most karyotypes are in the range 2n=40-56, but in the family Notopteridae there are 2n=34-42, The primitive karyotype has a lot of uni-arm chromosomes. The family

Notopteridae has different karyotypes from the other families in this order.

2. Order Cypriniformes, family Cyprinidae are all freshwater fish. The ancestors have a 2n=50 karyotype. The decedents have highly rearrangements. All chromosome types are found. Many species are found in polyploids.

3. Order Synbranchiformes, family Mastacembelidae, there are mainly 2n=48 karyotypes of all species. The primitive karyotype has a lot of uni-arm chromosomes, while the other family is Synbranchidae. There is a highly karyotype variation, 2n=18-46.

4. Order Perciformes has the most ancient karyotypes because their ancestors are marine fish, karyotype 2n=48 uni-arm chromosomes. The decedents have low rearrangement except for the betta group.

This is the first report of a fish cytogenetic study in this area. The information obtained can be used to support the taxonomic classification of fishes. In addition, the data obtained can be used as a basis for the conservation of natural fish genetic resources and the study of future chromosomal evolution.

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