A Report on Classical Cytogenetics of Hihgfin Barb Fish, *Cyclocheilichthys armatus* (Cypriniformes, Cyprinidae)

Patcharaporn Chaiyasan¹, Weerayuth Supiwong², Pasakorn Saenjundaeng²,
Kriengkrai Seetapan³, Sithisak Pinmongkhonkul⁴ and Alongklod Tanomtong^{1*}

¹Toxic Substance in Livestock and Aquatic Animals Research Group, Department of Biology, Faculty of Science, Khon Kaen University, Muang, Khon Kaen 40002, Thailand

²Faculty of Applied Science and Engineering, Khon Kaen University, Nong Khai Campus, Muang, Nong Khai 43000, Thailand

³School of Agriculture and Natural Resources, University of Phayao, Tumbol Maeka, Muang, Phayao 56000, Thailand

⁴Department of Biology, School of Science, University of Phayao, Tumbol Maeka, Muang, Phayao 56000, Thailand

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Summary Conventional staining and Ag-NORs banding techniques were applied on the chromosomes of hihgfin barb fish, *Cyclocheilichthys armatus*. Specimens were collected from the Chao Phraya River, Thailand. The mitotic chromosome preparations were directly obtained from kidney tissues of eight males and eight females. The results revealed that the diploid chromosome numbers of *C. armatus* was $2n=50$ and the fundamental number (NF) was 94 in both males and females. The karyotype consists of four large metacentric, six large submetacentric, two large acrocentric, eight medium metacentric, 12 medium submetacentric, 12 medium acrocentric and six medium telocentric chromosomes. No sex chromosome was observed. Ag-NORs indicating NORs were clearly exhibited at the region adjacent to telomere of the short arm of some chromosomes. The NORs located on chromosomes of the metacentric chromosome pair 3 and the submetacentric chromosome pair 7. The karyotype formula of *C. armatus* is $2n=50=L_4^m+L_6^{sm}+L_2^a+M_8^m+M_{12}^{sm}+M_{12}^a+M_6^t$. Cytogenetic data of cyprinid fishes can be used as the basic knowledge for enhancing the existing cytotaxonomic information and chromosome evolution of the Cyprinidae especially in the genus *Cyclocheilichthys*.

Key words Hihgfin barb fish, *Cyclocheilichthys armatus*, Karyotype, NOR, Fundamental number, Chromosome.

Family Cyprinidae is the most abundant and globally widespread family of freshwater fish, comprising 3000, living and extinct species in about 370 genera (Eschmeyer and Fong 2015). Hihgfin barb fish, *C. armatus* belongs to the family Cyprinidae. Hihgfin barb fish is widely distributed in freshwater of Laos, Cambodia, Vietnam, Peninsular Malaysia and the Greater Sunda Islands of Borneo, Sumatra and Java, with additional recorded from the Philippines. In Thailand, it is found in the Mekong, Mae Klong and Chao Phraya Rivers as well as a host of smaller river basins, lakes and reservoirs. They feed on zooplankton, small crustaceans, chironomids and other insect larvae (Rainboth 1996). *Cyclocheilichthys* has eight species (Rainboth 1996). Although chromosome numbers of most species in the *Cyclocheilichthys* have been described (Magtoon and Arai 1989, 1993, Donsakul and Magtoon 1995, Donsakul and Poopitayasathaporn 2002, Donsakul *et al.* 2005, 2006, Seetapan 2007), there is no report for *C. armatus*. Chromosome analyses of *Cyclocheilichthys* species have shown the diploid chromosome number of $2n=50$ (Table 1).

The study on fish chromosomes has received considerable attention in recent years because of their importance in classification, evolution, heredity, systematic (Gold *et al.* 1990, Ueda *et al.* 2001, Barat *et al.* 2002, Barat and Sahoo 2007), fish breeding, rapid production of inbred lines and cytotaxonomy (Kirpichnikov 1981). Moreover, cytogenetic studies of fishes have been used as biological indicator for determination of water pollution (Klinkhardt 1993) and cytogenetic techniques have been widely applied to improve farmed stocks in many aquaculture species in the World (Beardmore *et al.* 2001, Desprez *et al.* 2003). An important characteristic of NORs in fish is related to that it has inter- and intra-species polymorphism. NORs characterization can be a cytogenetic marker for cytotaxonomic studies and have been used for studying phylogenetic relationships between the Cyprinids (Amemiya and Gold 1988, Gatetti Jr. 1998, Almeida-Toledo *et al.* 2000).

As the mention above, a chromosome study in fishes is very important and clearly exhibits the benefits. Moreover, the karyological and NORs characteristics in the *C. armatus* were not reported. This research is the description of the *C. armatus* chromosomes from Thailand using conventional staining and Ag-NOR banding

* Corresponding author, e-mail: tanomtong@hotmail.com
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techniques.

Materials and methods

Sample collection

The *C. armatus* specimens (eight males and females) (Fig. 1) were obtained from the Chao Phraya River, Sing Buri province in the central part of Thailand. Species was identified morphologically using the keys and description of Rainboth (1996).

Chromosome preparation

Chromosomes were directly prepared *in vivo* as follows by Supiwong *et al.* (2013, 2017). The 0.05% colchicine (1 mL to 100 g body weight) was injected into the intramuscular or abdominal cavity of fish and left for 1 h before sacrificing. The kidney tissues were cut into small pieces then squashed, mixed with hypotonic solution (0.075 M KCl) and incubated for 30 min. The samples were centrifuged at 1200 rpm for 8 min and cells were fixed in fresh and cold Carnoy's fixative (3 methanol:1 acetic acid) about 2–3 times. The fixation was repeated until the supernatant was clear and the pellet was mixed with 1 mL fixative. The mixture was dropped onto a clean and cold glass slide by micropipette followed by an air-drying technique.

Chromosome staining

Conventional staining was performed using 20% Giemsa for 30 min (Rooney 2001). Ag-NOR banding was

carried out by adding four drops of 50% silver nitrate and two drops of 2% gelatin on the glass slides (Howell and Black 1980). The glass slides were then sealed with cover slips and incubated at 60°C for 5 min. After that they were soaked in distilled water until the cover slips were separated. The glass slides were stained with 20% Giemsa for 1 min.

Chromosomal checks, karyotyping and idiogramming

Chromosome counting was performed on mitotic metaphase cells under a microscope. Twenty clearly observable and well-spread metaphase cells from each male and female were selected and photographed. The length of short arm (Ls) and long arm (Ll) of each chromosome was measured and calculated for total chromosome length (LT=Ls+Ll) to classify the sizes of chromosomes as large, medium and small. The relative length (RL) and the centromeric index (CI) were estimated. The CI was also computed to classify the types of chromosomes according to Turpin and Lejeune (1965). The centromeric index (CI, $q/p+q$) between 0.50–0.59, 0.60–0.69, 0.70–0.89 and 0.90–0.99 were described as metacentric, submetacentric, acrocentric and telocentric chromosomes, respectively. NF is obtained by assigning a value of two arms to metacentric, submetacentric and acrocentric chromosomes, and one arm to telocentric chromosome. All parameters were used in karyotyping and idiogramming.

Results and discussion

Diploid chromosome number, fundamental number and karyotype

The diploid chromosome number of *C. armatus* was found as 50 (Figs. 2A, 3A). This result is consistent with previous reports in the *Cyclocheilichthys* (Magtoon and Arai 1989, 1993, Donsakul and Magtoon 1995, Donsakul and Poopitayasathaporn 2002, Donsakul *et al.* 2005, 2006, Seetapan 2007). These species have the chromosome diploid number of $2n=50$, which is an apparent modal diploid number of the *Cyclocheilichthys*. Accordingly, it can be concluded that chromosome number in this genus is conserved as in other genera of the Barbinae subfamily (*e.g.*, *Acrossocheilus*, *Balantiocheilus*).

Fig. 1. General characteristics of hihgfin barb fish, *Cyclocheilichthys armatus* (Cypriniformes: Cyprinidae). Scale bar=2 cm.

Table 1. Cytogenetical data of the genus *Cyclocheilichthys*.

Species	2n	NF	Karyotype formula	NOR	Reference
<i>C. amatus</i>	50	94	12m+18sm+14a+6t	S(TR)4	Present study
<i>C. apogon</i>	50	70	12m+8sm+6a+24t	—	Magtoon and Arai (1989)
	50	76	18m+8sm+4a+20t	—	Donsakul and Poopitayasathaporn (2002)
<i>C. enoplos</i>	50	90	10m+30sm+4a+6t	4	Magtoon and Arai (1993)
	50	72	14m+8sm+10a+18t	—	Donsakul and Magtoon (1995)
<i>C. lagleri</i>	50	86	24m+12sm+2a+12t	—	Donsakul <i>et al.</i> (2006)
<i>C. repasson</i>	50	—	8m+10sm+12a+20t	—	Seetapan (2007)
	50	78	12m+16sm+6a+16t	—	Donsakul <i>et al.</i> (2005)

TR: telomeric region. —: no data.

Fig. 2. Metaphase chromosome plates and karyotype of *C. armatus* (female) $2n=50$ by conventional staining (A) and Ag-NOR banding techniques (B). (C) Karyotype arranged by chromosome shape, and chromosome pairs 3 and 7 having Ag-NOR (arrows). Scale bars= $5\mu\text{m}$.

Fig. 3. Metaphase chromosome plates and karyotype of *C. armatus* (male) $2n=50$ by conventional staining (A) and Ag-NOR banding techniques (B). (C) Karyotype arranged by chromosome shape and length and two chromosome pairs 3 and 7 with Ag-NOR (arrows). Scale bars= $5\mu\text{m}$.

los, Barbonymus, Catla, Poropuntius, and Puntius) (Arai 2011). The diploid chromosome number is rather a conservative characteristic which is used as an indicator of the closeness of species inter-relationships within families (Moyle and Cech 2004). The diploid number, $2n=50$ would probably be the ancestral diploid number of the family because the characteristic that frequently occurs in a group can be considered as ancestral (Estabrook 1977).

The NF of *C. armatus* was 94 in both male and female. Although the NF in the genus *Cyclocheilichthys* varies from 70 to 90 (Magtoon and Arai 1989, 1993), *C. armatus* showed higher NF when compared with *C. apogon*, *C. enoplos*, *C. lagleri* and *C. repasson* from Thailand. It is considered that species with high NF is advanced state or apomorphic while one with low NF is a primitive state or plesiomorphic (Nirchio *et al.* 2002). The *C. armatus* karyotype consists of four large metacentric, six large submetacentric, two large acrocentric, eight medium metacentric, 12 medium submetacentric, 12 medium acrocentric and six medium telocentric chromosomes. The karyotype formula for this species is $2n=50=L_4^m+L_6^{sm}+L_2^a+M_8^m+M_{12}^{sm}+M_{12}^a+M_6^t$. It is different from other *Cyclocheilichthys* species (Magtoon and Arai 1989, 1993). In view of this fact, the *Cyclocheilichthys* is similar in diploid chromosome number but there are different in NF and karyotypes. The NF and karyotypes might be the result from pericentric inversions of the telocentric chromosome which can be transformed to a new bi-arm chromosome. In addition, the NF values may be due to the variability of fish in this group (Galetti *et al.* 2000, Sukham *et al.* 2014). Moreover, the difference of the karyotypes probably dues to interspecific

variations and/or the use of different criteria for classification of chromosome types. The chromosomes characteristics of other *Cyclocheilichthys* species (*C. apogon*, *C. enoplos*, *C. lagleri* and *C. repasson*) have been only reported in Thailand. Thus, the chromosome evolution of this group is still incomplete savvy.

There is no evidence of sexual dimorphism of the chromosomes in this species which accord to the reports on *C. apogon*, *C. enoplos*, *C. lagleri* and *C. repasson* (Magtoon and Arai 1989, 1993, Donsakul *et al.* 2005, 2006, Seetapan 2007). Similar to several cyprinid fishes, no cytologically distinguishable sex chromosome was found (Ueda *et al.* 2006, Mani *et al.* 2009, Monteiro *et al.* 2009, Kaewmad *et al.* 2014, Sukham *et al.* 2014). It may be possible that sex chromosomes are at the initiation step of differentiation which cannot be detected by cytogenetic analyses (Na-Nakron 2000). However, there are the reports found sex chromosomes of the XY system (Chanda 1989, Karahan and Ergene 2009, 2010) and the ZW system (Barat 1985) in some species of Cyprinids.

Chromosome markers

The NOR is the location of 18S and 28S ribosomal RNA genes (rDNA) functioning to ribosome synthesis. The silver staining procedure is commonly used to visualize the activity of rDNA, since only functionally active genes at NORs of metaphase chromosomes are stained by silver (Galetti Jr. *et al.* 1984, Schmid *et al.* 2006). NORs are used as makers to indicate intra- and inter-specific chromosome polymorphism in many groups of fishes (Ráb *et al.* 2008). This study was accomplished by

Table 2. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL) and centromeric index (CI) from 20 metaphases of male and female, hihi fish barb fish (*C. armatus*) $2n=50$.

Chromosome pair	Ls (μm)	Ll (μm)	LT (μm)	RL $\pm$ S.D.	CI $\pm$ S.D.	Chromosome size	Chromosome type
1	1.068	1.154	2.222	0.048 $\pm$ 0.002	0.519 $\pm$ 0.009	Large	Metacentric
2	0.997	1.080	2.076	0.045 $\pm$ 0.002	0.520 $\pm$ 0.011	Large	Metacentric
3*	0.924	1.013	1.936	0.042 $\pm$ 0.002	0.523 $\pm$ 0.011	Medium	Metacentric
4	0.870	0.978	1.848	0.040 $\pm$ 0.002	0.529 $\pm$ 0.017	Medium	Metacentric
5	0.832	0.938	1.770	0.038 $\pm$ 0.002	0.530 $\pm$ 0.016	Medium	Metacentric
6	0.773	0.872	1.644	0.035 $\pm$ 0.002	0.530 $\pm$ 0.016	Medium	Metacentric
7*	0.902	1.664	2.566	0.055 $\pm$ 0.003	0.648 $\pm$ 0.023	Large	Submetacentric
8	0.844	1.487	2.330	0.050 $\pm$ 0.002	0.638 $\pm$ 0.022	Large	Submetacentric
9	0.803	1.325	2.128	0.046 $\pm$ 0.002	0.622 $\pm$ 0.018	Large	Submetacentric
10	0.738	1.241	1.979	0.043 $\pm$ 0.002	0.627 $\pm$ 0.014	Medium	Submetacentric
11	0.710	1.167	1.877	0.041 $\pm$ 0.002	0.621 $\pm$ 0.022	Medium	Submetacentric
12	0.698	1.119	1.817	0.039 $\pm$ 0.001	0.616 $\pm$ 0.021	Medium	Submetacentric
13	0.661	1.099	1.760	0.038 $\pm$ 0.002	0.625 $\pm$ 0.019	Medium	Submetacentric
14	0.640	1.041	1.681	0.036 $\pm$ 0.002	0.619 $\pm$ 0.016	Medium	Submetacentric
15	0.616	0.980	1.596	0.034 $\pm$ 0.001	0.614 $\pm$ 0.022	Medium	Submetacentric
16	0.468	1.651	2.119	0.046 $\pm$ 0.003	0.778 $\pm$ 0.025	Large	Acrocentric
17	0.461	1.470	1.930	0.042 $\pm$ 0.003	0.762 $\pm$ 0.030	Medium	Acrocentric
18	0.469	1.308	1.776	0.038 $\pm$ 0.002	0.737 $\pm$ 0.025	Medium	Acrocentric
19	0.469	1.233	1.702	0.037 $\pm$ 0.002	0.724 $\pm$ 0.023	Medium	Acrocentric
20	0.445	1.190	1.635	0.035 $\pm$ 0.001	0.728 $\pm$ 0.023	Medium	Acrocentric
21	0.413	1.129	1.542	0.033 $\pm$ 0.002	0.733 $\pm$ 0.027	Medium	Acrocentric
22	0.396	1.060	1.456	0.031 $\pm$ 0.001	0.728 $\pm$ 0.020	Medium	Acrocentric
23	0.000	1.985	1.985	0.043 $\pm$ 0.002	1.000 $\pm$ 0.000	Medium	Telocentric
24	0.000	1.749	1.749	0.038 $\pm$ 0.003	1.000 $\pm$ 0.000	Medium	Telocentric
25	0.000	1.461	1.461	0.032 $\pm$ 0.003	1.000 $\pm$ 0.000	Medium	Telocentric

* Chromosome having NOR.

Fig. 4. Idiograms showing shape and length of chromosome of *C. armatus* represented the haploid set ($n=25$) by conventional staining (A) and Ag-NOR staining (B). Arrows indicate NORs on the short arm of metacentric chromosome pair 3 and submetacentric chromosome pair 7.

using Ag-NOR staining. The Ag-NORs were observed on near the telomeric regions of the short arms of the metacentric chromosome pair 3 and the submetacentric chromosome pair 7 in both male and female fishes (Figs. 2B, C and 3B, C). The result is consistent with previous reports of the *C. enoplos* which have two pair of Ag-NORs bearing chromosomes (Magtoon and Arai 1993). Most of the other species belonging to the family Cyprinidae have one pair chromosomes carrying of the NOR (Arai 2011). The occurrence of multiple NORs

in fishes was considered to be apomorphic or derived condition whereas single pair of NORs was considered to be plesiomorphic or a primitive condition (Gold and Amemiya 1986).

The important chromosome marker of the *C. armatus* is the asymmetrical karyotype that was found for four types of chromosomes regarding the centromere position. The idiogram shows a continuous length gradation of chromosomes. The largest and smallest chromosomes show approximately two-fold size differences. The marker chromosomes were the chromosome pair 7, which is the largest submetacentric and the chromosome pair 22, which is the smallest acrocentric. The data of the chromosome measurement on mitotic metaphase cells are shown in Table 2. Idiograms by conventional staining and Ag-NOR staining are shown in Fig. 4.

Furthermore, the information of chromosome morphology and the position as well as number of NORs in *C. armatus* suggested that these cytotaxonomic markers could be utilized in contribute toward clarifying the karyotypic evolution and phylogenetic relationships in this group. Further analysis, the additional species of *Cyclocheilichthys* of different regions and different staining techniques such as fluorescent staining with the CG specific chromomycin A_3 and fluorescence *in situ* hybridization technique will be used to study for better understanding of the chromosome evolution in the group and confirming the apparent conservative nature of the diploid number in this cyprinid fish.

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References

- Almeida-Toledo, L. F., Foresti, F. and Toledo-Filho, S. A. 2000. Karyotypic evolution in neotropical freshwater fish. *Chromosome Today* **13**: 169–181.
- Amemiya, C. T. and Gold, J. R. 1988. Chromosomal NORs as taxonomic and systematic characters in North American cyprinid fishes. *Genetica* **76**: 81–90.
- Arai, R. 2011. *Fish Karyotypes: A Check List*. Springer, Tokyo.
- Barat, A. 1985. A Study of Chromosomes in Some Indian Teleost (Pisces). Ph.D. Thesis. Kalyani University, West Bengal.
- Barat, A. and Sahoo, P. K. 2007. Karyotype analysis of *Channa punctatus* (Pisces) using restriction endonucleases. *Cytologia* **72**: 471–473.
- Barat, A., Sahoo, P. K. and Ponniah, A. G. 2002. Karyotype and Nucleolar Organizer Regions (NORs) in a Few Hill Stream Fishes. In: Ayyappan, S., Jena, J. K. and Joseph, M. M. (eds.). *The Fifth Indian Fisheries Forum Proceedings*, AFSIB, Mangalore and AoA, Bhubaneswar. pp. 111–114.
- Beardmore, J. A., Mair, G. C. and Lewis, R. I. 2001. Monosex male production in finfish as exemplified by tilapia: Applications, problems, and prospects. *Aquaculture* **197**: 283–301.
- Chanda, T. 1989. A study of Chromosome in Some Hill Stream Fishes of Assam India. Ph.D. thesis. Kalyani University, West Bengal.
- Desprez, D., Mélard, C., Hoareau, M. C., Bellemène, Y., Bosc, P. and Baroiller, J. F. 2003. Inheritance of sex in two ZZ pseudofemale lines of tilapia, *Oreochromis aureus*. *Aquaculture* **218**: 131–140.
- Donsakul, T. and Magtoon, W. 1995. Karyological studies of *Puntius brevis* (Bleeker, 1850) and *Cyclocheilichthys enoplos* in Thailand. In: *Proceeding of 21st Congress on Science and Technology of Thailand*, BO 14. pp. 292–293. (in Thai)
- Donsakul, T., Magtoon, W. and Rangsiruji, A. 2005. Karyotypes of four cyprinid fishes: *Cyclocheilichthys repasson*, *Cosmochilus harmandi*, *Poropuntius deauratus* and *Sikukia gudgeri* from Thailand. In: *Proceedings of 43rd Kasetsart University Annual Conference*, Kasetsart University, Bangkok. pp. 344–351. (in Thai)
- Donsakul, T., Magtoon, W. and Rangsiruji, A. 2006. Karyological studies of four Cyprinid Fishes: *Barbichthys nitidus*, *Mystacoleucus argenteus*, *Cychocheilichthys lagleri* and *Systomus* sp. from Thailand. In: *Proceedings of the 44th Kasetsart University Annual Conference*, Kasetsart University, Bangkok. pp. 469–476. (in Thai)
- Donsakul, T. and Poopitayasathaporn, A. 2002. Karyotype of Fifteen Species of Cyprinid Fishes (Family Cyprinidae) from Thailand. Srinakharinwirot University, Bangkok. (in Thai)
- Estabrook, G. F. 1977. Does common equal primitive? *Syst. Bot.* **2**: 36–42.
- Eschmeyer, W. N. and Fong, J. D. 2015. *The Catalog of Fishes*. Institute for Biodiversity Science and Sustainability. Academy of Sciences, California.
- Galetti, P. M. Jr. 1998. Chromosome diversity in neotropical fish: NOR studies. *Ital. J. Zool.* **65** suppl: 53–56.
- Galetti, P. M., Aguilar, C. T. and Molina, W. F. 2000. An overview of marine fish cytogenetics. *Mar. Genet.* **420**: 55–62.
- Galetti, P. M. Jr., Foresti, F., Bertollo, L. A. C. and Moreira, O. 1984. Characterization of eight species of Anostomidae (Cypriniformes) fish on the basis of the nucleolar organizing region. *Caryologia* **37**: 401–406.
- Gold, J. R. and Amemiya, C. T. 1986. Cytogenetic studies in North American minnows (Cyprinidae) XII. Pattern of chromosomal nucleolus organizer region variation among 14 species. *Can. J. Zool.* **65**: 1869–1877.
- Gold, J. R., Li, Y. C., Shipley, N. S. and Powers, P. K. 1990. Improved methods for working with fish chromosomes with a review of metaphase chromosome banding. *J. Fish Biol.* **37**: 563–575.
- Howell, W. M. and Black, D. A. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia* **36**: 1014–1015.
- Kaewmad, P., Monthatong, M., Supiwong, W., Saowakoon, S. and Tanomtong, A. 2014. Natural autotetraploid and chromosomal characteristics in the subfamily Botiinae (Cypriniformes, Cobitinae) from northeast Thailand. *Cytologia* **79**: 299–313.
- Karahan, A. and Ergene, S. 2009. Cytogenetic variation of geographically isolated four populations of *Garra rufa* [(Heckel, 1843) (Pisces, Cyprinidae)] in Turkey. *Caryologia* **62**: 276–287.
- Karahan, A. and Ergene, S. 2010. Cytogenetic analysis of *Garra variabilis* (Heckel, 1843) (Pisces, Cyprinidae) from Savur Stream (Mardin), Turkey. *Turk. J. Fish Aquat. Sci.* **10**: 483–489.
- Kirpichnikov, V. S. 1981. *Genetic Bases of Fish Selection*. Springer Verlag, New York.
- Klinkhardt, M. 1993. Fish Chromosomes as Sensitive Toxicity Indicators-Possibilities and Limits. In: Braunbeck, T. H., Hanke, W. and Segner, S. (eds.). *Fish in Ecotoxicology and Ecophysiology. Proceedings of an International Symposium*, Heidelberg. pp. 45–54.
- Magtoon, W. and Arai, R. 1989. Karyotypes of five *Puntius* species and one *Cyclocheilichthys* species (Pisces, Cyprinidae) from Thailand. *Bull. Natl. Mus. Nat. Sci. Ser. A* **15**: 167–175.
- Magtoon, W. and Arai, R. 1993. Karyotypes and distribution of nucleolus organizer regions in cyprinid fishes from Thailand. *Jpn. J. Ichthyol.* **40**: 77–85.
- Mani, I., Kumar, R., Singh, M., Kushwaha, B., Nagpure, N. S., Srivastava, P. K., Murmu, K., Rao, D. S. K. and Lakra, W. S. 2009. Karyotypic diversity and evolution of seven mahseer species (Cyprinidae) from India. *J. Fish Biol.* **75**: 1079–1091.
- Monteiro, R., Carvalho, C. and Collares-Pereira, M. J. 2009. Karyotype and genome size of *Iberochondrostoma almcai* (Teleostei, Cyprinidae) and comparison with the sister-species *I. lusitanicum*. *Genet. Mol. Biol.* **32**: 268–275.
- Moyle, P. B. and Cech, J. J. 2004. *Fishes. An Introduction to Ichthyology*. Prentice Hall, Upper Saddle River.
- Na-Nakron, U. 2000. *Fish Genetics*. Kasetsart University Press, Bangkok.
- Nirchio, M., Turner, B. J., Perez, J. E., Gaviria, J. I. and Cequea, H. 2002. Karyotypes of three species of toadfish (Batrachoididae: Teleostei) from Venezuela. *Sci. Mar.* **66**: 1–4.
- Ráb, P., Rábová, M., Pereira, C. S., Collares-Pereira, M. J. and Pelikánová, Š. 2008. Chromosome studies of European cyprinid fishes: Interspecific homology of leuciscine cytotoxic marker-the largest subtelocentric chromosome pair as revealed by crossspecies painting. *Chromosome Res.* **16**: 863–873.
- Rainboth, W. J. 1996. *FAO Species Identification Field Guide for Fisheries Purposes. Fishes of the Cambodian Mekong*. FAO, Rome.
- Rooney, D. E. 2001. *Human Cytogenetics: Constitutional Analysis, a Practical Approach*. Oxford University Press, London.
- Schmid, M., Ziegler, C. G., Steinlein, C., Nanda, I. and Scharf, M. 2006. Cytogenetics of the bleak (*Alburnus alburnus*), with special emphasis on the B chromosomes. *Chromosome Res.* **14**: 231–242.
- Seetapan, K. 2007. Karyotypes of sex fish species of the family Cyprinidae. In: *Proceedings of the 45th Kasetsart University Annual Conference*. Kasetsart University, Bangkok. pp. 749–758. (in Thai)

- Sukham, S., Chingakham, B., Thoidingjam, L., Waikhom, G., Kumar, R. and Kushwaha, B. 2014. A cytogenetical study on *Barilius ngawa*, Vishwanath and Manojkumar, 2002 (Cypriniformes: Cyprinidae) from Northeast India, Manipur. *Int. J. Fish. Aquac.* **4**: 58–62.
- Supiwong, W., Boonsuk, J., Jantarat, S., Suvarnaksha, A., Pengseng, P. and Tanomtong, A. 2017. The first chromosomal characteristics of nucleolar organizer regions and karyological analysis of two chaetodontid fishes (Perciformes, Chaetodontidae). *Cytologia* **82**: 33–39.
- Supiwong, W., Tanomtong, A., Chaveerach, A., Tanee, T., Khakhong, S. and Sanoamuang, L. 2013. Interpopulational variation of NOR positions and karyotypic analysis of Siamese catfish (*Pseudomystus siamensis*) in Thailand. *Cytologia* **78**: 25–34.
- Turpin, R. and Lejeune, J. 1965. *Les Chromosomes Humains*. Gauthier-Pillars, Paris.
- Ueda, T., Iijima, K., Naoi, H., Arai, R., Ishinabe, T. and Jeon, S. R. 2006. Karyotypes of three *Tanakia* bitterlings (Pisces, Cyprinidae) from East Asia. *Cytologia* **71**: 251–255.
- Ueda, T., Naoi, H. and Arai, R. 2001. Flexibility on the karyotype evolution in bitterlings (Pisces, Cyprinidae). *Genetica* **111**: 423–432.
-