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Research Article

Karyotypes of three species of cyprinid fishes *Garra cambodgiensis*, *G. fasciacauda* and *G. notata* (Cypriniformes, Cyprinidae) from Thailand

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Abstract - Karyotype analyses revealed that three species of cyprinid fishes from Thailand, *Garra cambodgiensis*, *G. fasciacauda* and *G. notata*, have the same diploid chromosome number of 2n = 50. However, they have different karyotype formulae. The karyotype formula of *G. cambodgiensis* is 10 metacentric (m) + 6 submetacentric (sm) + 2 subtelocentric (st) + 7 acrocentric (T) and the fundamental chromosome arm number (NF) is 82. *G. fasciacauda* is 9M + 7SM + 1ST + 8T and NF = 84, whilst *G. notata* is 10M + 5SM + 10T and NF = 80. This is the first report.

Keywords: Cypriniformes, *Garra cambodgiensis*, *Garra fasciacauda*, *Garra notata*, karyotypes

1. Introduction

Cyprinidae (Cypriniformes) is the largest family of fresh-water fishes. This family comprises 220 genera and approximately 2,420 species (Nelson, 2006). *Garra* is a genus belonging to the family Cyprinidae. The fish in genus *Garra* (Hamilton, 1822) are characterized by having a more or less well-developed sectorial disc on the undersurface just behind the mouth. This genus is distributed widely from Southern China, across Southeast Asia, India and the Middle East as far as to North and Central Africa (Menon, 1964). The majority of the more than 140 species that have been reported of the Graras are native in Asia, but only eight species, *Garra taeniata*, *G. fasciacauda, G. parvifilum, G. fuliginosa, G. cambodgiensis ,G. fisheri, G. nasuta* and *G. pingi,* were found in Thailand and Southeast Asia (Smith, 1945; Vidthayanon *et al*., 1977).

Cytogenetic studies provide information about the chromosome number and karyotypes in each species and can help to identify species and phylogeny relationships. The chromosome number and karyotypes of fishes in the family Cyprinidae in Thailand have been reported (Magtoon and Arai, 1989, Arai and Magtoon, 1991; Donsakul and Magtoon, 2010; Donsakul *et al*., 2011). However, genetic information of fish species in the genus *Garra* (*G. cambodgiensis*, *G. fasciacauda* and *G. notata)* in the Salween and Mekong river basins of Thailand has not been published. In the present study, the chromosome number and karyotypes of *G. cambodgiensis*, *G. fasciacauda* and *G. notata* from Thailand were described and karyotype evolution in this family is discussed.

2. Materials and methods

G. cambodgiensis (Tirant, 1884) and *G. notata* (Blyth, 1860) with total lengths (TL) of 50.0-60.0 mm and 100.0-120.0 mm, respectively, were obtained from the Salween River Basin, Mae Hong Son Province, Northern Thailand. *G. fasciacauda* (Fowler, 1937) with a total length of 42.0-60.0 mm were obtained from the Mekong River Basin, Nong Khai Province, Northeast Thailand (Table 1). Eight specimens each of *G. cambodgiensis* (Tirant, 1884) and *G. notata* (Blyth, 1860), and five specimens of *G. fasciacauda* (Fowler, 1937) were used for chromosome observation and preparation. They were identified as species reported by Smith (1945), Taki (1974), Vithayanon (2004), Rainboth (1996) and Kottelat (2001).

Mitotic chromosome preparations were obtained from kidney suspensions and gill epithelial tissue using the air drying technique of Ojima and Kurishita (1980). Fish were injected intraperitoneally with a 0.1% colchicine solution (1 ml/100 g body weight). The kidney and gill epithelial tissues were collected two hours later. The techniques for the preparation of the cell suspensions, hypotonic treatment and fixation of cells have been described previously (Donsakul and Magtoon, 2010). Preparations were stained with 4% giemsa. The best 5-10 metaphase figures of each specimen were used to make the karyotype arrangement following the method of Levan *et al*. (1964). The arm number of each chromosome is described by the NF, i.e., metacentric and submetacentric described as two-arm chromosomes, and subtelocentric and acrocentric as one-arm chromosomes (Aria, 1982; Nakamura, 1985).

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Table 1. Morphological characters and collection localities of specimens used in this study.

Species	No. of fishes	TL (mm)	Lateral line scales	Localities
Garra cambodgiensis	8	50.0-60.0	32-35	Salween River Basin, Mae Hong Son Province
G. fasciacauda	5	42.0-60.0	29-31	Mekong River Basin, NongKhai Province
G. notata	8	100.0-150.0	33-34	Salween River Basin, Mae Hong Son Province

3. Results

Chromosome observation revealed that *G. cambodgiensis*, *G. fasciacauda* and *G. notate* possessed many relatively small chromosomes. Unfortunately, the sex chromosomes were not identified.

Observation of thirty-six cells from kidney and gill epithelial cells of eight *G. cambodgiensis* specimens revealed that the diploid chromosome number (2n) of thirty-four chromosome counts was 50. Only two from thirty-six chromosome counts showed different chromosome numbers, which were 48 and 52 (Table 2). The karyotypes had 10 pairs of metacentric, six pairs of submetacentric, two pairs of subtelocentric and seven pairs of acrocentric chromosomes. The fundamental chromosome arm number (NF) was 82 (Table 3 and Fig. 1).

Observation of thirty-eight cells from kidney and gill epithelial cells of five *G. fasciacauda* specimens revealed that the diploid chromosome number (2n) of thirty-six chromosome counts was 50. Only two from thirty-eight chromosome counts showed different chromosome numbers, which were 47 and 51 (Table 2). The karyotypes had 9 pairs of metacentric, seven pairs of submetacentric, one pair of subtelocentric and eight pairs of acrocentric chromosomes. The fundamental chromosome arm number (NF) was 84 (Table 3 and Fig. 2).

Observation of forty-two cells from kidney and gill epithelial cells of eight *G. notata* specimens revealed that the diploid chromosome number (2n) of forty chromosome counts was 50. Only two from forty-two chromosome counts showed different chromosome numbers, which were 48 and 51 (Table 2). The karyotypes had 10 pairs of metacentric, five pairs of submetacentric and ten pairs of acrocentric chromosomes pairs. The fundamental chromosome arm number (NF) was 80 (Table 3 and Fig. 3).

Table 2. Frequency distribution of diploid chromosome counts in *Garra cambodgiensis*, *G. fasciacauda* and *G. notata*.

Species	Chromosome counts						No. of cells observed
	47	48	49	50	51	52	
Garra cambodgiensis	0	1	0	34	0	1	36
G. fasciacauda	1	0	0	36	1	0	38
G. notata	0	1	0	40	1	0	42

Table 3. Summary of chromosomal features of *Cyprinid* fish genus *Garra* and related species in family Cyprinidae as well as fundamental numbers (NF) and metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (t) chromosomes.

Species	Localities	Karyotype						References
		2n	NF	m	sm	st	t	
1. *Cyprinuscarpio*	China	100	150	-	-	-	-	Wang *et al*., 1985
2. *Tor soro*	Thailand	100	144	24	20	6	50	Magtoon and Arai, 1993
3. *G. gotylagotyla*	India, Itanagar, A.P	50	70	12	8	8	22	Sahoo*et al*., 2007
4. *G. kempi*	India, Itanagar, A.P	50	78	14	14	10	12	Sahoo *et al*., 2007
5. *G. lissorhynchus*	India, Itanagar, A.P	50	82	16	16	6	12	Sahoo *et al*., 2007
6. *G. lamta*	India Simlipal Hills, Orissa	50	86	12	24	2	12	Barat, 1985
7. *G. mullya*	India Chalakkudy river, Kerala	50	82	18	14	10	8	Nagpure *et al*., 2006
8. *G. persica*	Iran	48	94	15	8	1	0	Esmaeili *et al*., 2008
9. *G. surendranathanii*	Unspecified	50	-	14	20	8	8	Nagpure *et al*., 2006
10. *G. cambodgiensis*	Unspecified	52	-	-	-	-	-	Vasil'ev, 1980
11. *G. dembeensis*	Unspecified	50	82	-	-	-	-	Krysanov and Golubtsov, 1993
12. *G. makiensis*	Unspecified	50	84	34	1	16	1	Barat, 1985

Table 3. Summary of chromosomal features of *Cyprinid* fish genus *Garra* and related species in family Cyprinidae as well as fundamental numbers (NF) and metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (t) chromosomes. (Cont.)

Species	Localities	Karyotype						References
		2n	NF	m	sm	st	t	
13. *G. quadrimaculata*	Ethiopia	50	88	38	1	12	1	Krysanov and Golubtsov, 1993
14. *G. variabilis*	Turkey	102	186	42	18	24	18	Karahan and Ergene, 2010
15. *G. rufa*	Turkey	44	85	22	20	-	2	Ergene, Gozukara and Cavas, 2004
16. *G. cambodgiensis*	Thailand	50	82	10	6	2	7	Present study
17. *G. fasciacauda*	Thailand	50	84	9	7	1	8	Present study
18. *G. notata*	Thailand	50	80	10	5	0	10	Present study

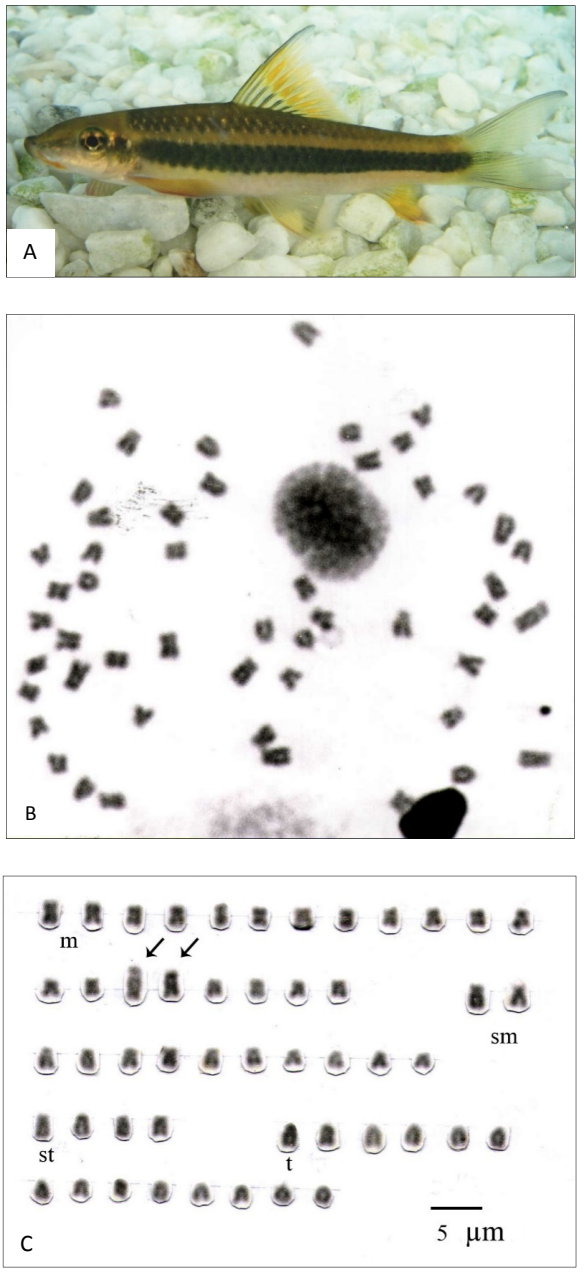

Figure 1. A) Photograph of lateral view of *Garra cambodgiensis* in an aquarium; B) photomicrographs of mitotic metaphase chromosome; C) karyotype of *G. cambodgiensis* (m = metacentric, sm = submetacentric, st = subtelocentric and t = acrocentric chromosomes). Arrows show satellite site on terminal short metacentric chromosome.

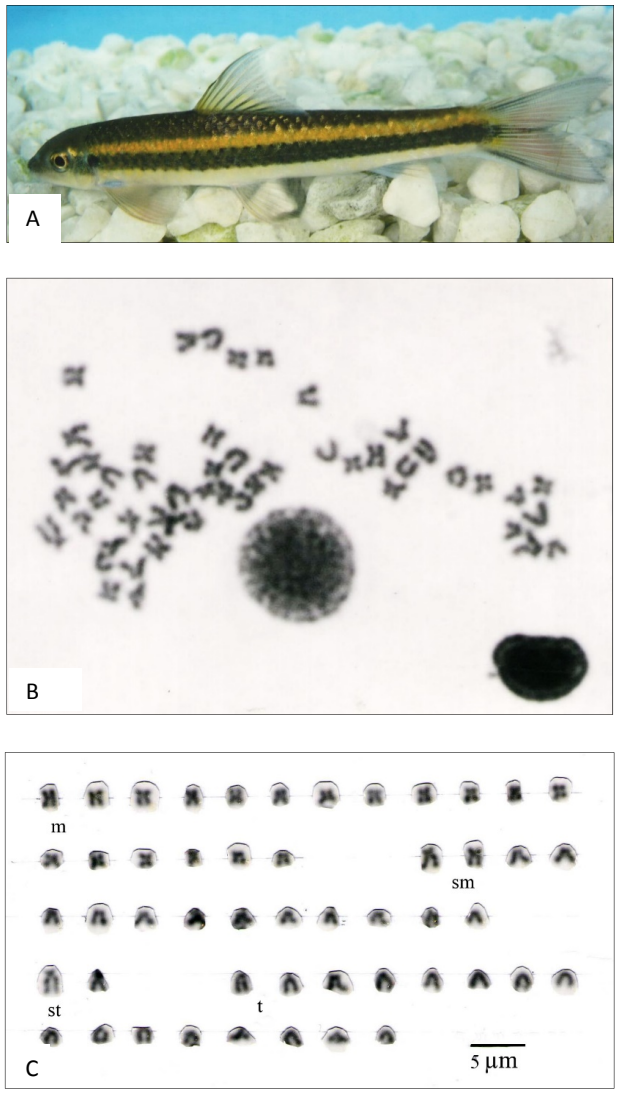

Figure 2. A) Photograph of lateral view of *Garra fasciacuada* in an aquarium; B) photomicrographs of mitotic metaphase chromosome; C) karyotype of *G. fasciacuada* (m = metacentric, sm = submetacentric, st = subtelocentric and t = acrocentric chromosomes).

4. Discussion and conclusion

As shown in (Table 3), sixteen species of *Garra* had diploid chromosome numbers (2n) ranging from 44 to 102. The diploid chromosome numbers of *G. gotyla gotyla*, *G. kempi*, *G. lissorhynchus*, *G. lamta*, *G. mullya* and *G. quadrimaculat* from India and Ethiopia were 2n = 50. Whereas, the diploid chromosome numbers of *Garra* from Iran and Turkey were reported to vary from 44 to 102. In the present study, the diploid chromosome numbers of *G. cambodgiensis, G. fasciacauda* and, *G. notata* from Thailand were 2n = 50 as well. Interestingly, diploid chromosome variation was found in the genus *Garra*. However, the majority of species of *Garra* had 2n = 50, which could be suggested as the model number for this genus. The chromosome numbers in *Carra* are reportedly different. The differences are possibly due to the intraspecific variability.

In other cyprinid fishes, *Cyprinus carpio* and *Tor soro,* the 2n value is 100 (Wang *et al*., 1985; Magtoon and Arai, 1993). The occurrence of 102 chromosomes in *G. variabilis* could arise due to polyploidization (tetraploidization) of the chromosome number of 50 in this genus. Some species of the subfamily Cyprininae have reportedly evolved polyploidy. Cytogenetic pattern indications of polyploidy have been shown in three genera: *Garra, Cyprinus* and *Tor* (Table 3). The role played by polyploidy in the evolution of the fish karyotype seems to be significant.

In the present study, the *Carra* fishes from Thailand, *G. cambodgiensis, G. fasciacauda* and *G. notata,* have the same diploid chromosome numbers with 2n = 50. However, the different species possessed different fundamental chromosome arm numbers, i.e., NF = 82 in *G. cambodgiensis*, NF = 84 in *G. fasciacauda* and NF = 80 in *G. notata*.

Some studies on the morphology of these three species also revealed the differences in the lateral line scales (LS), i.e., LS = 29 - 31 in *G. fasciacauda*, LS = 32 – 35 in *G. cambodgiensis* and LS = 33 - 34 in *G. notata* (Rainboth, 1996; Kottelat, 2001 and 2013). This information suggests that *G. cambodgiensis* is more closely related to *G. notata* than to *G. fasciacauda*.

In addition, the difference in the fundamental chromosome arm number within *G. cambodgiensis, G. fasciacauda* and *G. notata* individuals from different geographical localities has been observed. It is likely that the structural rearrangement in the chromosome complement was a consequence of changes in the chromosome morphology without changes in the chromosome number.

In the genus *Garra*, therefore, percentric inversions are the main chromosomal rearrangement, which plays an important role in the karyotypic differentiation and this could be the main mechanism of karyotypic evolution resulting in the arm number differences within the three species.

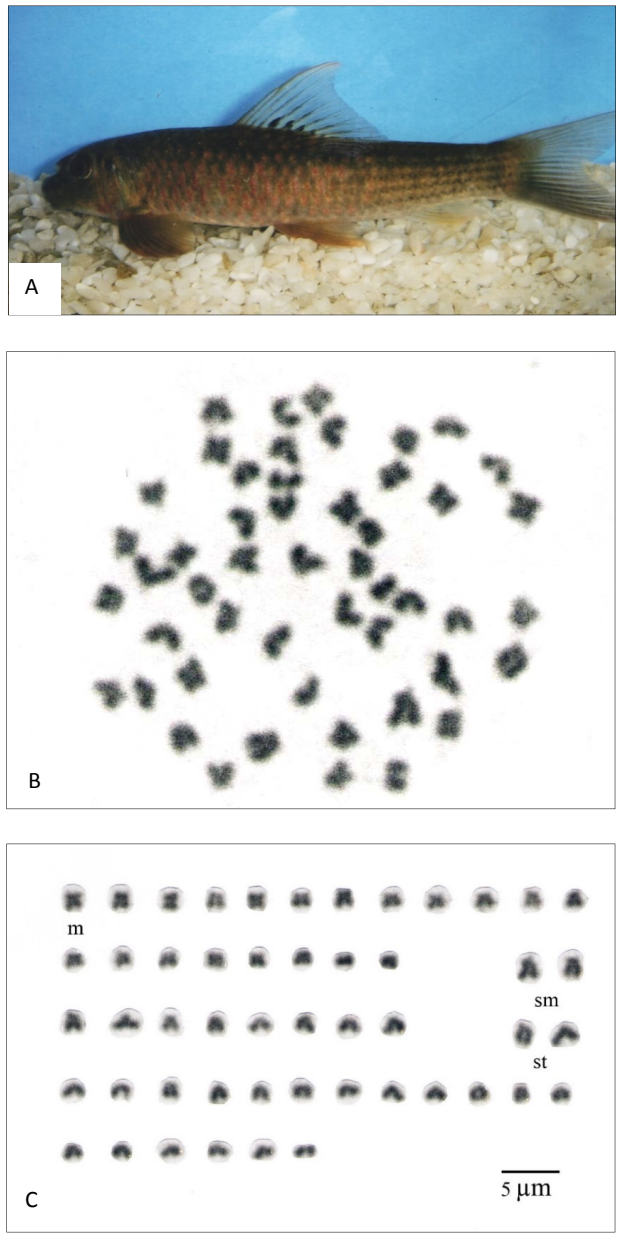

Figure 3. A) Photograph of lateral view of *Garra notata* in an aquarium; B) photomicrographs of mitotic metaphase chromosome; C) karyotype of *G. notata* (m = metacentric, sm = submetacentric, st = subtelocentric and t = acrocentric chromosomes).

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References

Arai, R. 1982. A chromosome study on two cyprinid fishes *Acrossocheilus abiatus* and *Pseudorasbora pumila* with note on Eurasian cyprinid and their karyotype. Bulletin of the National Science Museum A 8 (3), 131-182.

Arai, R. and Magtoon, W. 1991. Karyotypes of four cyprinid fish from Thailand. Bulletin of the National

Science Museum Tokyo A 17, 183-188.

Barat, A. 1985. A study of chromosomes in some Indian Teleost (Pisces). PhD thesis dissertation. West Bengal: Kalyani University.

Blyth, E. 1860. Report on some fishes received chiefly from the Sitang River and its tributary streams, Tenasserim Provinces. Journal of the Asiatic Society of Bengal 29 (2), 138-174.

Donsakul, T. and Magtoon, W. 2010. Karyotypes of four cyprinid fishes (Family Cyprinidae): *Hypophthalmichthys molitrix*, *H. nobilis*, *Henicorhynchus lineatus* and *H. lobatus* in Thailand. The 36th Congress on Science and Technology of Thailand (STT 36). Venue: Bangkok International Trade and Exhibition Centre (BITEC), Bangkok, Thailand, 26-28 October, 1-4.

Donsakul, T., Rangsiruji, A. and Magtoon, W. 2011. Karyotypes of five cyprinid fishes (Family Cyprinidae): *Bangana devdevi*, *Osteobrama alfredianus*,*S ystomusstoliczh anus*, *Oxygaster anomalura* and *Aspidoparia morar* in Thailand. The Proceeding of 49th Kasetsart University Annual Conference, Fisheries Section, 169-177.

Ergene, G. S. and Cavas, T. 2004. A karyological analysis of *Garra rufa* (Heckel, 1843) (Pisces, Cyprinidae) from the Eastern Mediterranean River Basin in Turkey. Turkish Journal of Veterinary and Animal Sciences 28, 497-500.

Esmaeili, H. R., Ebrahimi, M. and Saifali, M. 2008. Karyological analysis of fivetooth-carps (Actinopterygi i:Cyprinodontidae) from Iran. Micron 39, 95-100. doi:10.1016/j.micron.2006.12.007.

Fowler, H. W. 1937. Zoological results of the third De Schauensee Siamese Expedition. Part VIII – Fishes obtained in 1936. Proceedings of the Academy of Natural Sciences of Philadelphia 89, 125-164.

Hamilton, F. 1822. An Account of the Fishes of River Ganges and Its Branches. Edinburgh and London, pp 450.

Karahan, A. and Ergene, S. 2010. Cytogenetic analysis of *Garravariabilis* (Heckel, 1843) (Pisces, Cyprinidae) from Savur stream (Mardin), Turkey. Turkish Journal of Fisheries and Aquatic Sciences 10, 483-489. doi: 10.4194/trjfas.2010.0407.

Kottelat, M. 2001. Fishes of Laos. 4th Edition, Gunaratne Offset Ltd. Colombo, 195.

Kottelat, M. 2013. The fishes of the inland waters of Southeast Asia: a catalogue and core bibliography of the fishes known to occur in freshwaters, mangroves and estuaries. Raffles Bulletin of Zoology Supplement 27, 1-663.

Krysanov, E. Y. and Golubtsov, A. S. 1993. Karyotypes of the three *Garra* species from Ethiopia. Journal of Fish Biology 42 (3), 465-467.

Levan, A., Fredga, K. and Sandberg, A. A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52, 201-220.

Magtoon, W. and Arai, R. 1989. Karyotype of five Puntius species and one Cyclocheilichthys species (Pisces, Cyprinidae) from Thailand. Bulletin of the National Science Museum Tokyo A 15, 167-175.

Magtoon, W. and Arai, R. 1993. Karyotypes and distribution of nucleolus organizer regions in cyprinid fishes from Thailand. Japanese Journal of Ichthyology 40 (1), 77-85.

Menon, A. G. K. 1964. Monograph of the cyprinid fishes of the genus *Garra* Hamilton. Memoirs of the Indian Museum 14, 173-260.

Nagpure, N. S., Kumar, R., Srivastava, S. K., Gopalakrishnan, A., Basheer V. S. and Kapoor, K. 2006. Cytogenetic studies of two cyprinids *Garramullya* and *Bariliusbakeri* from the Western Ghats of India. Journal of Cytology and Genetics 7, 69-78.

Nakamura, H. K. 1985. A review of molluscan cytogenetic information based on the CISMOCH computerized index system for molluscan chromosomes. Bivalvia, Polyplacophora and Cephalopoda. Venus 44 (3), 193-225.

Nelson, J. S. 2006. Fishes of the world, John Wiley and Sons Inc., 4th edition. New York, 601.

Ojima, Y. and Kurishita, A. 1980. A new method to increase the number of mitotic cells in the kidney tissue for fish chromosome studies. Proceedings of the Japan Academy 56 B, 610-615.

Rainboth, W. J. 1996. Fishes of the Cambodian Mekong. Food and Agriculture Organization of the United Nation, Rome, pp. 233.

Sahoo, P. K., Nanda, P. and Barat, A. 2007.Karyotypic Diversity among Three Species of *Garra* (Family: Cyprinidae) from River Dikrong, Arunachal Pradesh, India, Cytologia 72 (3), 259-263.

Smith, H. M. 1945. The fresh-water fishes of Siam, or Thailand. Bulletin of the United States National Museum 188, 1-622.

Taki, Y. 1974. Fishes of the Lao Mekong Basin. United States Agency for International Development Mission to Lao. United Consultants, 106-108.

Tirant, G. 1884. Note surquelquese spèces de poisons des montagnes de Samrong Tong (Camcodge). Bulletin de la Société des Etudes Indochinoises 1, 167-173.

Vasil'ev, V. P. 1980. Chromosome numbers in fishlike vertebrates and fish. Journal of Ichthyology 20 (3), 1-38.

Vidthayanon, C. 2004. Manual of Freshwater Fishes. Sarakadee Press, Thailand, pp 230.

Vidthayanon, C., Karnasuta, J. and Nabhitabhata, J. 1997. Diversity of Freshwater Fishes in Thailand. Integrated Promotion Technology 103.

Wang, R., Shi, L. and He, W. 1985. Studies on nucleolus organizer regions in several species of carp (*Cyprinus*) by silver-staining. Zoological Research 6, 391-398. (In Chinese with English abstract).