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Karyotypes and Distribution of Nucleolus Organizer Regions in Cyprinid Fishes from Thailand

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Abstract The karyotypes of seven species of cyprinid fishes in Thailand are as follows: *Cirrhinus jullieni*, 2n=50, NF=90, NORs on 2 acrocentrics; *C. mrigala*, 2n=50, NF=72; *Osteochilus waandersi*, 2n=50, NF=92, NORs on 2 submetacentrics; *Cyclocheilichthys enoplos*, 2n=50, NF=90, NORs on 2 submetacentrics and 2 acrocentrics; *Labeo rohita*, 2n=50, NF=80, NORs on 2 submetacentrics; *Tor soro*, 2n=100, NF=144; *Puntioplites proctozysron*, 2n=50, NF=76. The karyotype of *Puntioplites proctozysron*, the first report for the genus, suggests that *P. proctozysron* is more closely related to *Puntius* than to *Procypris*, *Cyprinus*, *Carassioides*, or *Carassius*.

There are many genera and species of the family Cyprinidae in South-East Asia for which the systematic positions are vague. Because comparative karyology has become a useful tool in fish systematic studies (Arai, 1982; Buth et al., 1991), the intention of clarifying the systematics and zoogeography of cyprinid fishes in Thailand has resulted in the karyotypes of 7 genera and 13 species of the Cyprinidae being reported to date (Magtoon and Arai, 1989, 1990; Arai and Magtoon, 1991). Recently, the chromosomes of seven further cyprinid species from Thailand were examined, viz. *Cirrhinus jullieni* Sauvage, 1878, *C. mrigala* (Hamilton, 1822), *Osteochilus waandersi* (Bleeker, 1852), *Cyclocheilichthys enoplos* Bleeker, 1850, *Labeo rohita* (Hamilton, 1822), *Tor soro* (Valenciennes, 1842), and *Puntioplites proctozysron* (Bleeker, 1865). In addition, the nucleolus organizer regions (NORs) of four species were examined.

Materials and Methods

Collection localities and some morphological characters of the specimens used are shown in Table 1. Chromosome preparation followed Ojima and Kurishita (1980). For detection of NORs, slides were stained following Howell and Black (1980). Classification of chromosomes follows Levan et al. (1964). Metacentrics (M) and submetacentrics (SM) are described as two-arm chromosomes, and subtelocentrics (ST) and acrocentrics (A) as one-arm chromosomes.

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

Species	No. of fishes	SL (mm)	Dorsal rays	Anal rays	Localities
Cirrhinus jullieni	3	93.7–105.0	iv, 8	iv, 5	Uthai Thani Prov.
C. mrigala	1	62.2	iv, 12	iii, 5	Ayuthaya Prov.
Osteochilus waandersi	1	93.0	iv, 11	iii, 5	Kanchana Buri Prov.
Labeo rohita	2	65.0–87.7	iv, 12–13	iii, 5	Ayuthaya Prov.
Cyclocheilichthys enoplos	1	66.0	iv, 8	iii, 5	Uthai Thani Prov.
Puntioplites proctozysron	4	42.3–128.1	iv, 8	iii, 5	Uthai Thani Prov.
Tor soro	2	72.0–110.0	iv, 8–9	iii, 5	Kanchana Buri Prov.

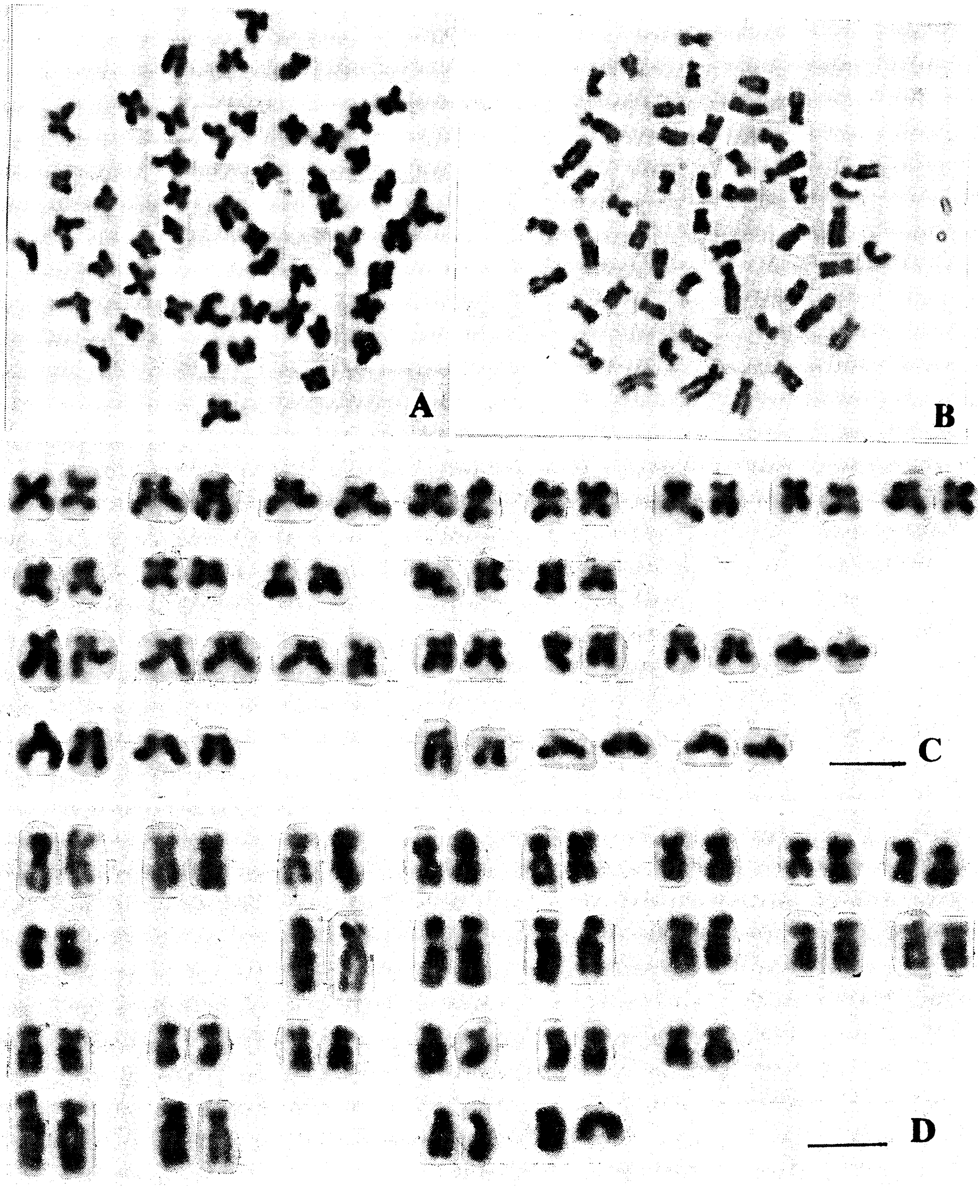

Fig. 1. Mitotic metaphase chromosomes and karyotypes of A, C) *Cirrhinus jullieni* and B, D) *Osteochilus waandersi*. Each scale indicates 5 μm.

Results

Cirrhinus jullieni (Fig. 1A, C)

As shown in Table 2, the diploid chromosome number was 50. The karyotype comprised 26 metacentric, 14 submetacentric, 4 subtelocentric, and 6 acrocentric chromosomes. The arm number was 90. NORs were found as small, dot-like blocks on the terminal regions of an acrocentric chromosome pair (Fig. 5A).

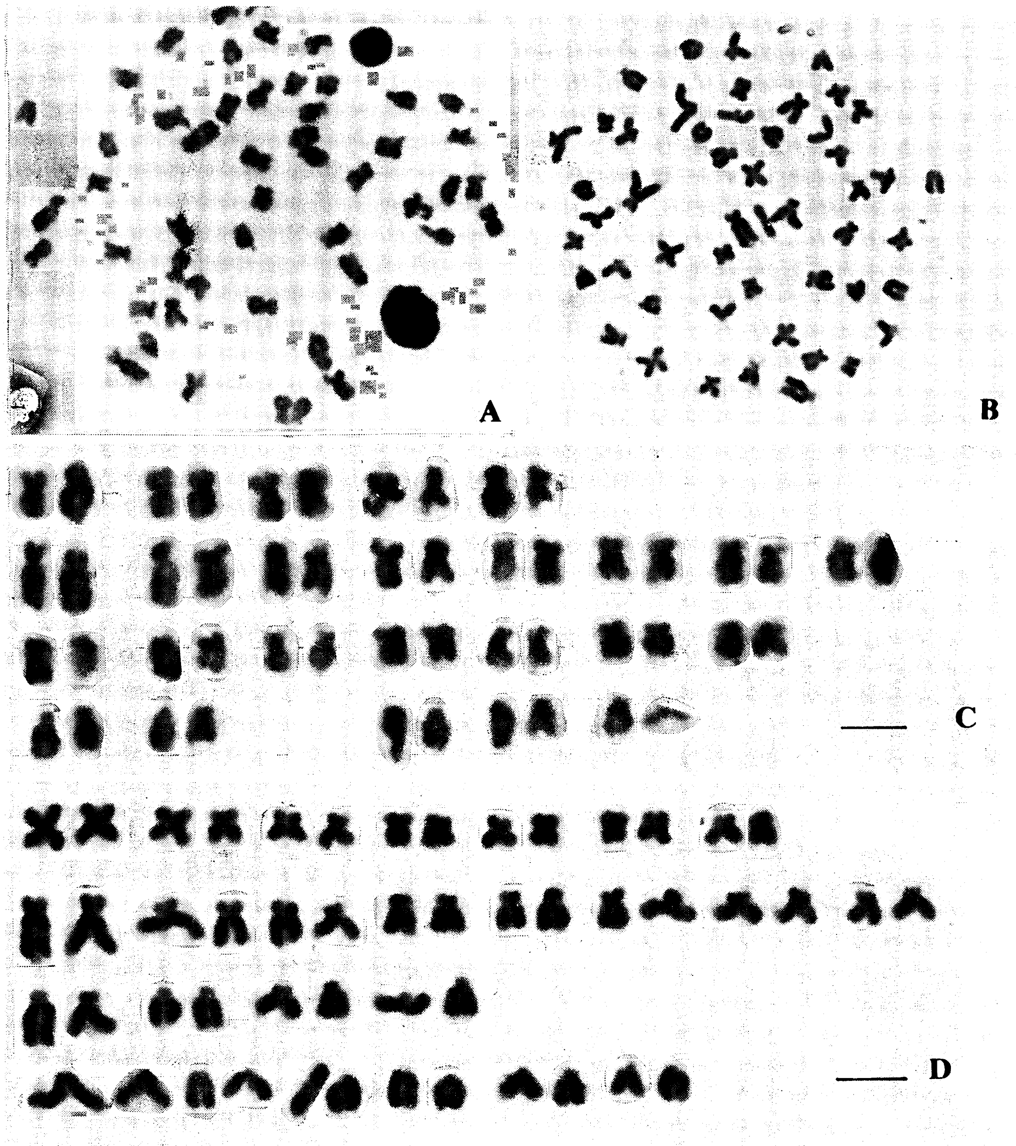

Fig. 2. Mitotic metaphase chromosomes and karyotypes of A, C) *Cyclocheilichthys enoplos* and B, D) *Labeo rohita.* Each scale indicates 5 μm.

Table 2. Frequency distributions of diploid chromosome counts

Species	2n														Total
	46	47	48	49	50	51	......	75	79	83	87	90	97	100	
Cirrhinus jullieni	4		8		25										37
Cirrhinus mrigala					9										9
Osteochilus waandersi	1		1	1	8										11
Labeo rohita	3		12	2	28	1									46
Cyclocheilichthys enoplos			2		16										18
Puntioplites proctozysron	3	1	8		21										33
Tor soro								1	1	1	2	1	1	7	14

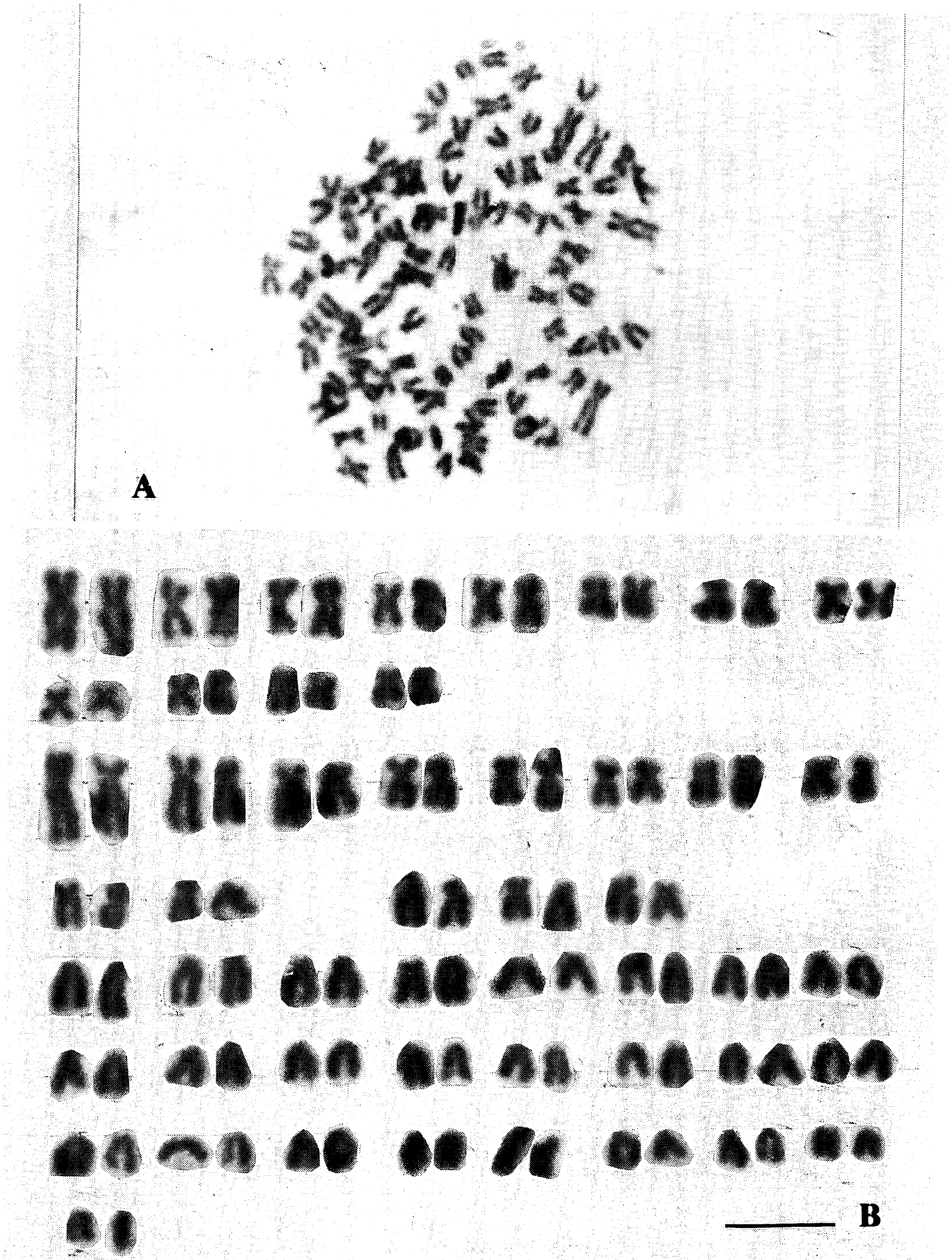

Fig. 3. Mitotic metaphase chromosomes and a karyotype of *Tor soro*. Scale indicates 5μm.

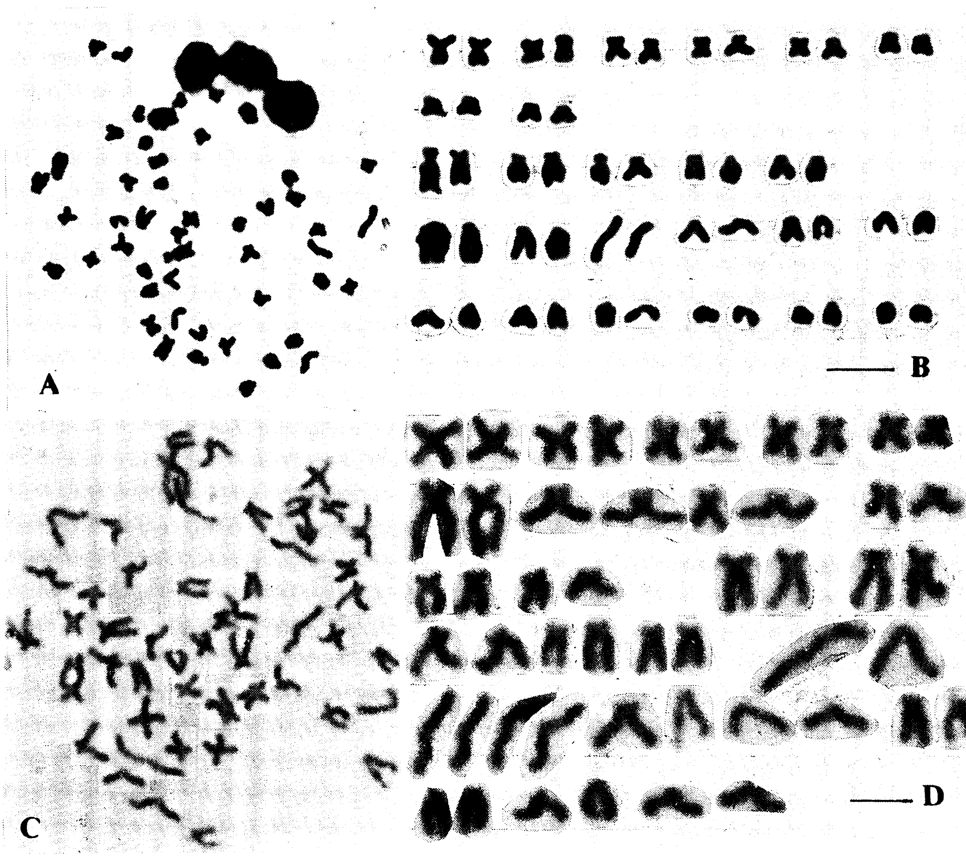

Fig. 4. Mitotic metaphase chromosomes and karyotypes of A, B) *Puntioplites proctozysron* and C, D) *Cirrhinus mrigala.* Each scale indicates 5 μm.

Cirrhinus mrigala (Fig. 4C, D)

The diploid chromosome number was 50 (Table 2). The karyotype comprised 10 metacentric, 12 submetacentric, 10 subtelocentric, and 18 acrocentric chromosomes. The arm number was 72.

Osteochilus waandersi (Fig. 1B, D)

The diploid chromosome number was 50 (Table 2). The karyotype comprised 18 metacentric, 24 submetacentric, 4 subtelocentric, and 4 acrocentric chromosomes. The arm number was 92. NORs were found on the short arms of a submetacentric chromosome pair (Fig. 5B).

Cyclocheilichthys enoplos (Fig. 2A, C)

The diploid chromosome number was 50 (Table 2). The karyotype comprised 10 metacentric, 30 submetacentric, 4 subtelocentric, and 6 acrocentric chromosomes. The arm number was 90. NORs were found on a submetacentric and an acrocentric chromosome pair (Fig. 5C).

Labeo rohita (Fig. 2B, D)

The diploid chromosome number was 50 (Table 2). The karyotype comprised 14 metacentric, 16 submetacentric, 8 subtelocentric, and 12 acrocentric chromosomes. The arm number was 80. NORs were found on a submetacentric chromosome pair (Fig. 5D).

Tor soro (Fig. 3A, B)

The diploid chromosome number was 100 (Table 2). The karyotype comprised 24 metacentric, 20

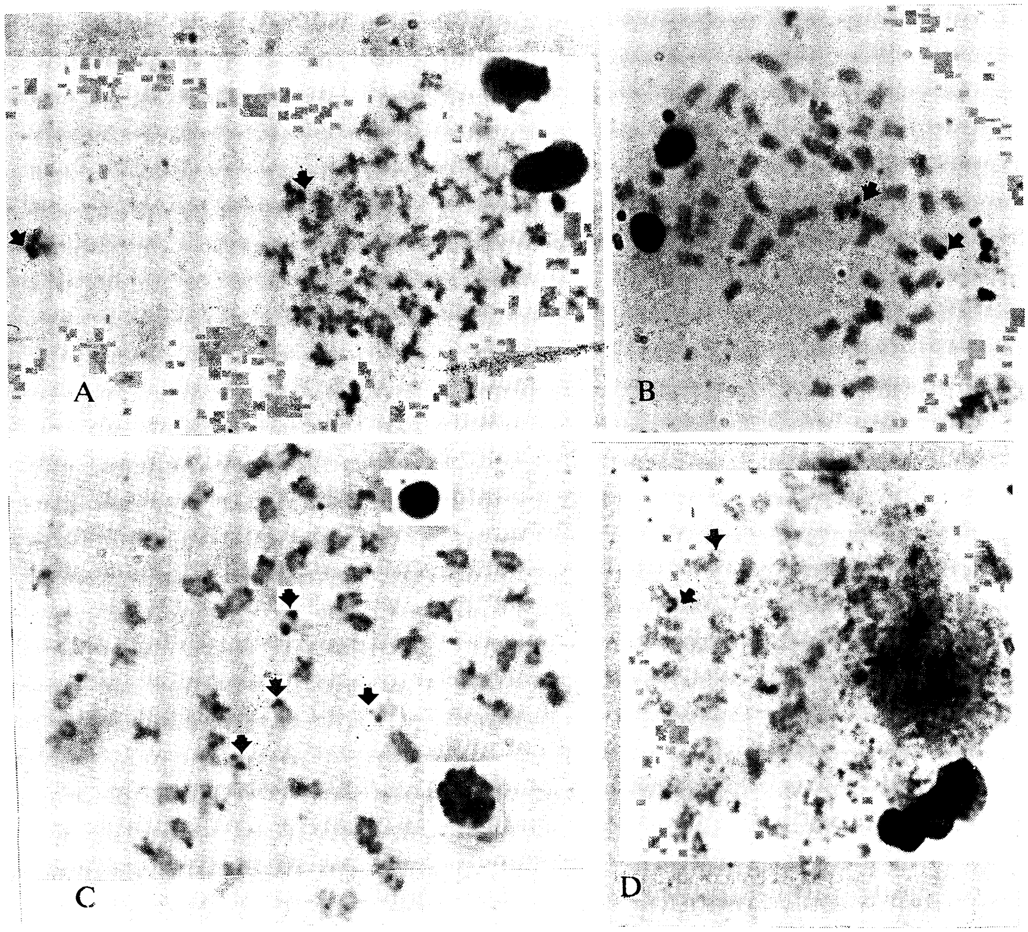

Fig. 5. Metaphase figures and NORs in A) *Cirrhinus jullieni*; B) *Osteochilus waandersi*; C) *Cyclocheilichthys enoplos*; D) *Labeo rohita*. Arrows indicate NORs.

submetacentric, 6 subtelocentric, and 50 acrocentric chromosomes. The arm number was 144.

Puntioplites proctozysron (Fig. 4A, B)

The diploid chromosome number was 50 (Table 2). The karyotype comprised 16 metacentric, 10 submetacentric, and 24 acrocentric chromosomes. The arm number was 76.

Discussion

Excepting for *Cirrhinus mrigala* (see Rishi, 1981) and *Labeo rohita* (see Gui et al., 1986; Rishi and Mandhan, 1990), the karyotypes of the above species are reported here for the first time. Localization of the NORs of 4 of the species are also apparently reported for the first time. NORs of fishes were included in Takai and Ojima (1986) and Aref'yev (1990), but because those of cyprinid fishes from South-East Asia have not been reported, a phylogenetic analysis of such cannot be made here.

The diploid chromosome numbers of *Cirrhinus jullieni* and *C. mrigala* were the same, 2n=50, but the species differed in the arm number, i.e., NF=90 in *C. jullieni* and NF=72 in *C. mrigala*. The karyotype of *C. mrigala* from India, reported by Rishi (1981), differed from that found in the present study in the arm number, i.e., NF=72 from Thailand vs. NF=64 from India. Such a difference may be geographic or may reflect a need to review the taxonomy of this species. Within the genus *Osteochilus*, *O. waandersi*, *O. hasselti* and *O. vittatus* have the same diploid chromosome number, but differ in the arm number, that of the former being smaller than in the other species (Magtoon and Arai, 1990). *Cyclocheilichthys apogon* has 2n=50 and NF=70 (Magtoon and Arai, 1989), but *C. enoplos* differs considerably from the former in the arm number. All *Labeo* species that have been karyotyped have 2n=50, but the arm number of *L. rohita* in Thailand is the largest of the 4 species known (Magtoon and Arai, 1990). The different arm numbers of *Labeo rohita* from China (NF=76), Thailand (NF=80), and India (NF=70) reflect local differentiation in the karyotype or artifacts (Gui et al., 1986).

As shown in Table 3, the karyotype of *Tor soro* (2n=100) is similar to those of six other *Tor* species, confirming the generic placement of the former.

The systematic position of *Puntioplites* has been problematical. Wu et al. (1977) and Cheng and Zheng (1987) included *Puntioplites* in the subfamily Cyprininae which thereby comprised 5 genera, *Puntioplites*, *Procypris*, *Cyprinus*, *Carassioides*, and *Carassius*. However, Taki and Katsuyama (1979) referred *Puntioplites* to the subfamily Barbinae, considering it to be closely related to *Puntius*. Regarding the karyotypes of the Cyprininae as defined by Wu et al. (1977), all genera except *Puntioplites*, which was unknown to the present time, have 2n=100, c. 150 or c. 200 (Arai, 1982; Gui et al., 1985; Zan et al., 1986; Yu et al., 1989). In this study, the karyotype of *Puntioplites proctozysron* was found to have 2n=50, a markedly different value to those above. Morphologically, *Puntioplites* is very similar to *Puntius* except for the anal simple ray being serrated (Taki and Katsuyama, 1979). In addition, the karyotype of *Puntius* varies from 2n=48 to 2n=50, and NF=54 to NF=90, variation within which the karyotype of *Puntioplites proctozysron* is included (Taki et al., 1977; Magtoon and Arai, 1989; Arai and Magtoon, 1991). These facts suggest that *Puntioplites* is more closely related to *Puntius* than to *Procypris*, *Cyprinus*, *Carassioides*, or *Carassius*, and that the former should be included in the subfamily Barbinae.

Acknowledgments

We wish to thank Mr. Thawat Donsakul, Srinakharinwirot University at Bangkhen, Bangkok, for his

Table 3. Karyotypes of *Puntioplites*, *Tor*, *Procypris*, *Cyprinus*, *Carassioides* and *Carassius* species

Species	2n	NF*	NF**	NORs	Source
Puntioplites proctozysron	50	76	76	—	Present study
Tor douronensis	100	152	—	—	Zan et al., 1986
T. khudree	100	—	—	—	Khuda-Bukhsh, 1982
T. mosal mahanadicus	100+2	—	—	—	Khuda-Bukhsh et al., 1986
T. putitora	100	—	—	—	Khuda-Bukhsh, 1980
T. sinensis	100	148	—	—	Zan et al., 1986
T. soro	100	144	150	—	Present study
T. tor	100	—	—	—	Khuda-Bukhsh, 1982
Procypris rabaudi	100	148	170	—	Yu et al., 1989
Cyprinus carpio	100	—	—	2SM	Takai and Ojima, 1986
	100	150	—	2SM	Wang et al., 1985
Cy. carpio chila	100	152	—	2SM	Wang et al., 1985
Cy. longipectoralis	100	152	—	2SM	Wang et al., 1985
Carassioides cantonensis	100	150	168	—	Gui et al., 1985
Carassius auratus auratus	100	—	—	2SM	Takai and Ojima, 1986
Ca. a. buergeri	100	148	—	2SM	Takai and Ojima, 1986
Ca. a. cuvieri	100	148	—	2SM	Takai and Ojima, 1986
Ca. a. gibelio	162	246	—	4SM	Wang et al., 1988
Ca. a. grandoculis	100	148	—	2SM	Takai and Ojima, 1986
Ca. a. langsdorfii	154	238	—	2SM	Takai and Ojima, 1986
	100	148	—	—	Takai and Ojima, 1990
Ca. carassius	100	—	164	2–3SM	Hafez et al., 1978; Mayr et al., 1986

*Metacentrics and submetacentrics are counted as two-arm chromosomes; **metacentrics, submetacentrics, and subtelocentrics are counted as two-arm chromosomes.

help in collecting specimens of *Cirrhinus jullieni*. We also wish to express our gratitudes to Prof. Yasuhiko Taki, Tokyo University of Fisheries, Tokyo, for valuable advice on cyprinid fishes in Thailand, and Prof. Hiroshi Uwa, Shinshu University, Matsumoto, for his useful suggestion regarding chromosome preparation.

Literature Cited

Arai, R. 1982. A chromosome study on two cyprinid fishes, *Acrossocheilus labiatus* and *Pseudorasbora pumila pumila*, with notes on Eurasian cyprinids and their karyotypes. Bull. Natn. Sci. Mus., Tokyo, (A), 8: 131–152.

Arai, R. and W. Magtoon. 1991. Karyotypes of four cyprinid fishes from Thailand. Bull. Natn. Sci. Mus., Tokyo, (A), 17: 183–188.

Aref'yev, V. A. 1990. Cytogenetic analysis and nucleolar organization of the sea bass *Dicentrachus labrax*. J. Ichthyol., 29: 1–12.

Buth, D. G., T. E. Dowling and J. R. Gold. 1991. Molecular and cytological investigations. Pages 83–126 *in* I. J. Winfield and J. S. Nelson, eds. Cyprinid fishes: systematics, biology and exploitation. Chapman & Hall, New York.

Cheng, Q. and B. Zheng. (eds.). 1987. Systematic synopsis of Chinese fishes. (Vol. 1). Science Press, Beijing, China. xxiii+643+iii pp. (In Chinese.)

Gui, J., Y. Li, K. Li, Y. Hong and T. Zhou. 1985. Studies on the karyotypes of Chinese cyprinid fishes. VI. Karyotypes of three tetraploid species in Barbinae and one tetraploid species in Cyprininae. Acta Gen. Sin., 12: 302–308, pls. 1–2. (In Chinese with English abstract.)

Gui, J., Y. Li, Y. Zhou and T. Zhou. 1986. Studies on the karyotypes of Chinese cyprinid fishes. VIII. Karyotypic analyses of fifteen species of Barbinae with a consideration for their phyletic evolution. Trans. Chinese Ichthyol. Soc., 5: 119–127, 2 pls. (In Chinese with English abstract.)

Hafez, R., R. Labat and R. Quillier. 1978. Etude cytogénétique chez quelques espèces de Cyprinidés de la région Midi-Pyrénées. Bull. Soc. Hist. Nat. Toulouse, 114: 122–159.

Howell, W. M. and D. A. Black. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia, 36: 1014–1015.

Khuda-Bukhsh, A. R. 1980. A high number of chromosomes in the hill stream cyprinid, *Tor putitora* (Pisces). Experientia, 36: 173–174.

Khuda-Bukhsh, A. R. 1982. Karyomorphology of two species of *Tor* (Pisces; Cyprinidae) with a high number of chromosomes. Experientia, 38: 82–83.

Khuda-Bukhsh, A. R., T. Chanda and A. Barat. 1986. Karyomorphology and evolution in some Indian hill-stream fishes with particular reference to polyploidy in some species. Pages 886–898 *in* T. Uyeno, R. Arai, T. Taniuchi and K. Matsuura, eds. Indo-Pacific fish biology. Ichthyol. Soc. of Japan, Tokyo.

Levan, A., K. Fredga and A. A. Sandberg. 1964. Nomenclature of centromeric position on chromosomes. Hereditas, 52: 201–220.

Magtoon, W. and R. Arai. 1989. Karyotypes of five *Puntius* species and one *Cyclocheilichthys* species (Pisces, Cyprinidae) from Thailand. Bull. Natn. Sci. Mus., Tokyo, (A), 15: 167–175.

Magtoon, W. and R. Arai. 1990. Karyotypes of three cyprinid fishes, *Osteochilus hasselti*, *O. vittatus* and *Labiobarbus lineatus*, from Thailand. Japan. J. Ichthyol., 36: 483–487.

Mayr, B., P. Rab and M. Kalat. 1986. NORs and counter-stain-enhanced fluorescence studies in Cyprinidae of different ploidy level. Genetica, 69: 111–118.

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

Rishi, K. K. 1981. Chromosomal studies on four cyprinid fishes. Intl. J. Acad. Ichthyol., 2: 1–4.

Rishi, K. K. and R. P. Mandhan. 1990. Analysis of C-banded heterochromatin in the chromosomes of *Labeo rohita* (Ham.) (Cyprinidae). Chromosome Information Service, (48): 3–4.

Takai, A. and Y. Ojima. 1986. Some features on nucleolus organizer regions in fish chromosomes. Pages 899–909 *in* T. Uyeno, R. Arai, T. Taniuchi and K. Matsuura, eds. Indo-Pacific fish biology. Ichthyol. Soc. of Japan, Tokyo.

Takai, A. and Y. Ojima. 1990. Characteristics of chromosomes and electrophoretic patterns of muscle protein and lactate dehydrogenase in fish of genus *Carassius* (Cypriniformes). Cytologia, 55: 255–263.

Taki, Y. and A. Katsuyama. 1979. Differentiation and zoogeography of two species of the cyprinid genus *Puntioplites*. Japan. J. Ichthyol., 26: 253–265.

Taki, Y., T. Urushido, A. Suzuki and C. Serizawa. 1977. A comparative chromosome study of *Puntius* (Cyprinidae: Pisces). I. Southeast Asian species. Proc. Japan Acad., 53B: 231–235.

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

Wang, R., L. Shi and W. He. 1988. A comparative study of the Ag-NORs of *Carassius auratus* from different geographic districts. Zool. Res. (Kunming), 9: 165–169, pls. 1–3. (In Chinese with English abstract.)

Wu, H. W. and 9 others. 1977. Cyprinid fishes of China, 2. Shanghai People's Press, Shanghai. 1–2+229–598 pp., pls. 1–109. (In Chinese.)

Yu, X., T. Zhou, Y. Li, K. Li and M. Zhou. 1989. Chromosomes of Chinese fresh-water fishes. Science Press, Beijing. vi + 179 pp. (In Chinese.)

Zan, R., Z. Song and W. Liu. 1986. Studies on karyotypes and nuclear DNA contents of some cyprinoid fishes, with notes on fish polyploids in China. Pages 877–885 *in* T. Uyeno, R. Arai, T. Taniuchi and K. Matsuura, eds. Indo-Pacific fish biology. Ichthyol. Soc. of Japan, Tokyo.

タイ国産コイ科魚類の核型と仁形成部位

Wichian Magtoon・新井良一

タイ国産コイ科魚類7種の染色体と，そのうち4種の仁形成部位を観察した．*Cirrhinus jullieni* では 2n = 50, 核型は 26 本の中部着糸染色体 (M), 14 本の次中部着糸染色体 (SM), 4 本の次端部着糸染色体 (ST), 6 本の端部着糸染色体 (A) からなり，染色体腕数 (NF) は 90, 仁形成部位 (NORs) は 2 本の A 染色体上にあった．以下同様に他の 6 種では，*Cirrhinus mrigala*: 2n = 50, 核型，10M, 12SM, 10ST, 18A, NF = 72; *Osteochilus waandersi*: 2n = 50, 核型，18M, 24SM, 4ST, 4A, NF = 92, NORs = 2SM; *Cyclocheilichthys enoplos*: 2n = 50, 核型，10M, 30 SM, 4ST, 6A, NF = 90, NORs = 2SM + 2A; *Labeo rohita*: 2n = 50, 核型，14M, 16SM, 8ST, 12A, NF = 80, NORs = 2SM; *Puntioplites proctozysron*: 2n = 50, 核型，16M, 10SM, 24A, NF = 76; *Tor soro*: 2n = 100, 核型，24M, 20SM, 6ST, 50A, NF = 144 であった．これらのうち，*Cirrhinus mrigala* および *Labeo rohita* を除く 5 種の核型，および 4 種の NORs は初めての報告である．特に *Puntioplites proctozysron* の核型は *Puntioplites* 属では最初の報告である．*Puntioplites* 属の亜科の所属について，従来，コイ亜科説，バルブス亜科説が提唱されてきたが，比較核型学の立場から本属はバルブス亜科に含めるべきであることが示唆された．

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訂　　正・Erratum

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魚類学雑誌第40巻1号に下記の訂正があります.

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