A Chromosome Study on Two Cyprinid Fishes, Acrossocheilus labiatus and Pseudorasbora pumila pumila, with Notes on Eurasian Cyprinids and their Karyotypes

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Chromosomes of cyprinid fishes have been studied more intensively than those of any other family of fishes except the family Cyprinodontidae (VASILIEV, 1980). As regards Japanese cyprinids, chromosomes of 20 genera and 35 species-subspecies have been hitherto reported (OJIMA & HITOTSUMACHI, 1967; OJIMA *et al.*, 1970, 1972, 1973, 1976; ITOH & NIIYAMA, 1972; KOBAYASI *et al.*, 1970, 1973a, 1973b; ARAI & FUJIKI, 1977).

As I had the chance to observe chromosomes of two cyprinid fishes, *Acrossocheilus labiatus* from Taiwan and *Pseudorasbora pumila pumila* from Japan, their karyotypes are here described.

As regards Eurasian cyprinid fishes, karyotypes of 58 genera and 141 speciessubspecies have been reported, including those of two species in this paper. Their karyotypes are reviewed in this paper and phylogenetic systematics of Eurasian cyprinid fishes are discussed on the basis of karyotypes and several morphological characters.

Method of chromosome preparation is the same as that of ARAI (1973). Classification of chromosomes is adopted from Levan *et al.* (1964). Metacentrics and submetacentrics are described as two-arm chromosomes, and subtelocentrics and acrocentrics as one-arm chromosomes. The definition of the new arm number (NAN) is referred to ARAI and NAGAIWA (1976).

All the specimens used for the experiments are deposited in the fish collection of the Department of Zoology, National Science Museum, Tokyo.

Acrossocheilus labiatus (REGAN)

(Figs. 1 A, C)

Two specimens (Nos. $A \cdot 10 \cdot 1$ and $A \cdot 10 \cdot 2$), 84.0 and 86.6 mm in total length, were used for experiments. These specimens were offsprings of fish which had been caught at Taiwan by Mr. Eizo KIMURA. Characters of material fish are shown in Table 1.

As shown in Table 2, the diploid chromosome number of this species is 50. The karyotype comprises 8 pairs of metacentric, 6 pairs of submetacentric, 2 pairs of sub-

telocentric and 9 pairs of acrocentric chromosomes. The arm number is 78.

As regards Acrossocheilus, the karyotype of A. sumatranus was reported (SUZUKI & TAKI, 1981). Its diploid chromosome number is 98 which differs from 2n=50 of A. labiatus. Acrossocheilus labiatus also differs from A. sumatranus in the number of branched dorsal rays, i. e., 8 in A. labiatus versus 9 in A. sumatranus.

Species	No. of fish	S. L. (mm)	Dorsal	Anal	Vertebrae
Acrossocheilus labiatus	2	67.9-72.1	iv, 8	iii, 5	21 + 17 - 18
Pseudorasbora pumila pumila	2	54.2-55.6	iii, 7	iii, 6	20-21+15

Table 1. Characters of two species of material fishes.

Table 2. Frequency distributions of diploid chromosome counts in two species of material fishes.

Si	2n								
Species	44	45	46	47	48	49	50	51	Total
Acrossocheilus labiatus		1		2	1	3	16	2	25
Pseudorasbora pumila pumila		1	1		2	8	36	1	49

Pseudorasbora pumila pumila MIYADI "Shinai-motsugo"

(Figs. 1 B, D)

Two specimens (Nos. $A \cdot 3 \cdot 2$ and $A \cdot 3 \cdot 3$), 66.1 and 64.6 mm in total length, were collected from Akita Prefecture, Japan (Table 1).

The diploid chromosome number is 50 (Table 2). The karyotype comprises 13 pairs of metacentric, 10 pairs of submetacentric and 2 pairs of subtelocentric chromosomes. The arm number is 96.

The karyotype of this species agrees with that of *P. parva* in the diploid chromosome number, but differs from *P. parva* in the number of metacentrics, i. e., 26 in *P. p. pumila* versus 14 in *P. parva* (OJIMA et al., 1972). The lateral line of *P. parva* is complete, while that of *P. p. pumila* is incomplete. From the viewpoint of the lateral line, *P. p. pumila* is considered to be more specialized than *P. parva*. Such interpretation seems to be supported by karyological analysis.

Eurasian Cyprinid Fishes and their Karyotypes

The family Cyprinidae is the greatest in diversity in most areas within its distribution range. It contains about 275 genera and about 1600 species, the largest number of species in fish families (Nelson, 1976).

The Cyprinidae is so large that its phylogenetic study is very difficult. Howes (1978, p. 61) said "Various classifications of the Cyprinidae have been made by attaching significance to superficial resemblances and by utilizing only single character or a



Fig. 1. Photomicrographs of mitotic metaphase chromosomes and karyotypes from gill epithelial cells of two cyprinid fishes. — A, *Acrossocheilus labiatus* (No. A·10·2), 2n=50, ×1,350; B, *Pseudorasbora pumila pumila* (No. A·3·2), 2n=50, ×1,650; C, *Acrossocheilus labiatus*, from Fig. A, NF=78, ×1,710; D, *Pseudorasbora p. pumila*, from Fig. B, NF=96, ×1,750.

Table 3. Eurasian cyprinid fishes and their chromosomes.

Species	2n	NF_1	NF_2	Literature
Subfamily Cyprininae				
Carassius auratus	100	152		ZAN & SONG, 1980a
C. a. buergeri	100	160		Kobayasi et al., 1973a
C. a. cuvieri	100	148		Олма et al., 1966
C. a. gibelio	100	160		Kobayasi et al., 1973b
C. a. grandoculis	100	160		Kobayasi et al., 1973a
C. a. langsdorfi	100	160		Kobayasi et al., 1970
C. a. subsp. (Kin-buna)	100	160		Kobayasi et al., 1970
C. carassius	∫100	160		Kobayasi et al., 1970
	100		ca. 164	HAFEZ et al., 1978b
Catla catla	50	74	88	Manna & Khuda-Bukhsh, 197
Cirrhina reba	48	86	92	Manna & Khuda-Bukhsh, 197
Cyprinus carpio	(100		ca. 152	HAFEZ et al., 1978b
	(100	148		Оліма & Нітотѕимасні, 1967
C. c. chilia	100	152		ZAN & SONG, 1980b
C. c. rubrofuscus	100	152		ZAN & SONG, 1980b
C. longipectoralis	100	152		Zan & Song, 1980b
C. megalophthalmus	100	152		Zan & Song, 1980b
C. micristius fuxianensis	100	152		ZAN & SONG, 1980b
C. pellegrini barbatus	100	152		Zan & Song, 1980b
Labeo bata	50	74	90	Manna & Khuda-Bukhsh, 197
L. calbasu	50	64	86	Manna & Khuda-Bukhsh, 197
*Morulius chrysophekadion	50	64	82	Muramoto <i>et al.</i> , 1968
Subfamily Barbinae				
Acrossocheilus labiatus	50	78	82	This paper
A. sumatranus	98	142	158	Suzuki & Taki, 1981
Barbus barbus	100		ca. 160	HAFEZ et al., 1978b
B. b. plebejus	100	144	162	CATAUDELLA et al., 1977
B. meridionalis	100	142	156	CATAUDELLA et al., 1977
B. m. petenyi	100			Sofradžija & Berberović, 1973
Garra lamta	50	74	86	Khuda-Bukhsh et al., 1980
Puntius arulius	50		82	Taki & Suzuki, 1977
P. binotatus	50		92	Taki <i>et al.</i> , 1977
P. chola	50		56	Taki & Suzuki, 1977
P. conchonius	50		94	Taki & Suzuki, 1977
P. cumingi	50		94	Taki & Suzuki, 1977
P. everetti	50		86	Такі <i>et al.</i> , 1977
P. fasciatus	(50		82	Такі et al., 1977
	52	82	86	Онно et al., 1967
P. filamentosus	50		84	Taki & Suzuki, 1977
P. japonicus	50	70	78	Khuda-Bukhsh, 1975
P. lateristriga	50		88	Taki <i>et al.</i> , 1977
P. nigrofasciatus	50		100	Taki & Suzuki, 1977
P. oligolepis	50		88	Taki et al., 1977
P. orphoides	50		92	Taki et al., 1977

^{*} Morulius was reported as Labeo.

Table 3 (continued).

Species	2n	NF ₁	NF ₂	Literature
•		1411		
Puntius partipentazona	50 50		90 98	TAKI et al., 1977
P. pentazona	50		98 84	TAKI et al., 1977
P. schwanenfeldi	48	54	04	Такі <i>et al.</i> , 1977 Rishi <i>et al.</i> , 1977
P. sophore	50	84	90	Ohno <i>et al.</i> , 1967
P. tetrazona	50	04	84	Taki <i>et al.</i> , 1977
P. ticto	50		100	Taki & Suzuki, 1977
P. titteya	50		98	TAKI & SUZUKI, 1977 TAKI & SUZUKI, 1977
Tor putitora	100	134	148	Khuda-Bukhsh, 1980
Subfamily Gobioninae	100	134	140	KHODA-DOKHSH, 1700
•	100	148		Proprovide et al. 1072
Aulopyge huegeli	50	146	94	Berberović <i>et al.</i> , 1973 Ojima <i>et al.</i> , 1972
Gnathopogon	30		94	Олма ет ап., 1972
elongatus elongatus Gobio albipinnatus	50	98	98	Parculat al 1072
vladykovi	30	98	98	Raicu <i>et al.</i> , 1973
G. gobio	(50	96	98	Raicu <i>et al.</i> , 1973
J. 80010	50	88	,,,	Sofradžija & Berberović, 1975
	50		98	HAFEZ et al., 1978b
G. kessleri banaticus	50	94	98	RAICU <i>et al.</i> , 1973
G. uranoscopus	52	88	100	RAICU <i>et al.</i> , 1973
Hemibarbus barbus	50	84	94	Олма et al., 1970
H. labeo	50		90	Kang & Park, 1973
H. longirostris	50		92	KANG & PARK, 1973
Pungtungia herzi	50		100	Олма et al., 1972
Pseudogobio esocinus	50	84	94	Олма <i>et al.</i> , 1970
Pseudorasbora parva	50		100	Олма et al., 1972
P. pumila pumila	50	96	100	This paper
Sarcocheilichthys	50		92	Олим <i>et al.</i> , 1972
variegatus				
Subfamily Rasborinae				
Amblypharyngodon mola	50	82	90	Manna & Khuda-Bukhsh, 1977
Esomus danrica	50	78	88	Manna & Khuda-Bukhsh, 1977
Rasbora buchanani	50	98	100	Manna & Khuda-Bukhsh, 1977
R. daniconius	50	74	80	KHUDA-BUKHSH, 1979
Subfamily Gobiobotiinae				
Gobiobotia brevibarba	50		82	Ueno, 1981
Microphysogobio	50		100	Ueno, 1981
tafangensis				
longidorsalis				
M. yalnensis	50		100	Ueno, 1981
Subfamily Abramidinae				
Abramis ballerus	52	70		Nygren et al., 1975
A. brama	(52	82		Nygren <i>et al.</i> , 1975
	50		ca. 80	Hafez <i>et al.</i> , 1978b
Alburnus	50	87		Fontana <i>et al.</i> , 1970
albidus alborella		370.0		,,

Table 3 (continued).

Species	2n	NF_1	NF_2	Literature
Alburnus alburnus	(50	76	92	Cataudella et al., 1977
	50		86	Hafez et al., 1978a
	50	82		Sofradžija et al., 1979a
Alburnoides bipunctatus	50	88		Sofradžija et al., 1979a
Aspius aspius	52	94		Nygren et al., 1975
Vimba vimba carinata	50	82		Rudek, 1974
V. v. vimba	₅₀	82		Rudek, 1974
	52	76–80		Shinderite & Vasiliev, 1976
Subfamily Cultrinae				
Anabarilius alburnops	48	82, 84	96	ZAN & SONG, 1980b
A. andersoni	48	84	96	ZAN & SONG, 1980b
A. grahami	48	82	96	Zan & Song, 1980b
A. macrolepis	48	84	96	Zan & Song, 1980b
Ischikauia steenackeri	48		88	Олма <i>et al.</i> , 1972
Megalobrama	48	92		ZAN & SONG, 1979
amblycephala				
Rohtee cotio	48	84	92	Manna & Khuda-Bukhsh, 1977
*Salmostoma bacaila	50	72	82	Manna & Khuda-Bukhsh, 1977
Subfamily Hypophthalmicht	hyinae			
Aristichthys nobilis	ſ 48	86	96	Воzнко et al., 1976
	{ 48	86		ZAN & SONG, 1980a
Hypophthalmichthys	(48	80	86	Manna & Khuda-Bukhsh, 1977
molitrix	1 48	86		ZAN & SONG, 1980a
Subfamily Danioninae				
Barilius barila	50	78	86	KHUDA-BUKHSH & NAYAK, 1981
B. bendelisis	(50	62	72	KHUDA-BUKHSH, 1979
	50	74	94	SHARMA & TRIPATHI, 1981
*Bola bola	50	70	80	KHUDA-BUKHSH & NAYAK, 1981
Danio albolineatus	50	99		FONTANA et al., 1970
D. rerio	50	99		Fontana et al., 1970
D. devario	50	60		Fontana et al., 1970
D. malabaricus	50	60		Fontana et al., 1970
Opsariichthys uncirostris	78		86	Олма et al., 1972
Zacco platypus	48		88	Олма <i>et al.</i> , 1972
Z. temmincki	48		88	Олма et al., 1972
Subfamily Acheilognathinae				
*Acanthorhodeus	44	72		Олма <i>et al.</i> , 1973
longipinnis				,
Acheilognathus	44	72		Олма <i>et al.</i> , 1973
cyanostigma		. –		,
A. lanceolata	48	76		Олма <i>et al.</i> , 1973
A. limbata	48	76		Олим <i>et al.</i> , 1973
A. moriokae	44	72		Олма <i>et al.</i> , 1973
A. tabira	44	72		Олим <i>et al.</i> , 1973

^{*} Each of Salmostoma, Bola and Acanthorhodeus was reported as Chela, Barilius and Acheilognathus, respectively.

Table 3 (continued).

Species	2n	NF_1	NF_2	Literature
*Paracheilognathus rhombea	44	72		Олма <i>et al.</i> , 1973
Pseudoperilampus typus	44	72		Олма <i>et al.</i> , 1973
Rhodeus atremius	46	50		Олма <i>et al.</i> , 1973
R. ocellatus ocellatus	48	76		Олма <i>et al.</i> , 1973
R. o. smithii	48	76		Олма et al., 1973
R. sericeus amarus	(48	82		Sofradžija <i>et al.</i> , 1975
	48	80	84	Воzнко et al., 1976
	(48		ca. 86	HAFEZ et al., 1978b
R. suigensis	46	50		Олма <i>et al.</i> , 1973
Tanakia tanago	48	76		Олма <i>et al.</i> , 1973
Subfamily Leuciscinae				
Ctenopharyngodon	(48		84	Олма et al., 1972
idellus	48	96	96	Воzнко et al., 1976
	₹ 48	82	90	Manna & Khuda-Bukhsh, 1977
	48	88		Zan & Song, 1979
	48	96	96	Liu, 1980
Leuciscus aula	50	88		Fontana et al., 1970
L. cephalus	[50	90		Fontana et al., 1970
•	50		ca. 88	HAFEZ et al., 1978b
L. c. cabeda	50	78	90	CATAUDELLA et al., 1977
*L. idus	52			Lueken & Foerster, 1969
L. leuciscus	50		ca. 88	Hafez et al., 1978b
L. souffia muticellus	[50	88		Fontana et al., 1970
	50	82	90	Cataudella et al., 1977
L. svallize	50	74		Berberović & Sofradžija, 1972
L. turskyi	50	68		Berberović & Sofradžija, 1972
Moroco jouyi	52		92	Олма <i>et al.</i> , 1970
M. lagowskii	50		90	KANG & PARK, 1973
M. oxycephalus	50		90	KANG & PARK, 1973
M. steindachneri	50	86	94	Олма et al., 1976
Pachychilon pictum	50			Berberović & Sofradžija, 1972
Paraphoxinus adspersus	50	88		Berberović & Sofradžija, 1972
P. alepidotus	50	76		Berberović et al., 1969
P. croaticus	50	86		Berberović & Sofradžija, 1972
P. metohiensis	50	82		Berberović & Sofradžija, 1972
P. pstrossi	50			Sofradžija et al., 1972
Phoxinus phoxinus	(50	82		Berberović & Sofradžija, 1974
•	50	76	92	CATAUDELLA et al., 1977
	50		ca. 90	Hafez et al., 1978b
Rutilus rubilio	(50	84		Berberović & Sofradžija, 1972
	50	78	94	CATAUDELLA et al., 1977
R. rutilus	(50	68	, ,	Berberović & Sofradžija, 1972
/ 10111110	50	76		Nygren et al., 1975
	50	70	82	Hafez <i>et al.</i> , 1978

^{*} Each of Paracheilognathus and Leucisus was reported as Acheilognathus and Idus, respectively.

Table 3 (continued).

Species	2n	NF_1	NF_2	Literature
Scardinius	(48	60		Fontana et al., 1970
erythrophthalmus	48	90		Nygren et al., 1975
	₹ 50	82	94	CATAUDELLA et al., 1977
	50		ca. 84	HAFEZ et al., 1978b
	50	82		Sofradžija, et al., 1979b
Tinca tinca	/ 48	82		FONTANA et al., 1970
	48	84		Nygren et al., 1975
	48	84	90	Воzнко et al., 1976
	48	68	78	Cataudella et al., 1977
	48	84		Berberović et al., 1978
	48		ca. 86	HAFEZ et al., 1978b
Tribolodon ezoe	₅ 50	80	92	ITOH & NIIYAMA, 1972
	₹ 50	84	92	Олма et al., 1976
T. hakonensis	50	80	92	Iтон & Nііуама, 1972
	(50	84	92	Олма <i>et al.</i> , 1976
Subfamily Chondrostomat	inae			
Chondrostoma kneri	50	80		Berberović et al., 1970
C. phoxinus	50	86		Berberović et al., 1970
C. soetta	50	80	94	CATAUDELLA et al., 1977
C. toxostoma	50	80	94	Cataudella et al., 1977
	(50		ca. 90	Hafez et al., 1978b

series of too few characters. In some cases the significance of these features has defied interpretation."

As regards subfamilies, classification of cyprinid subfamily has not been fixed. Hensel (1970) reviewed the history of cyprinid classification. For example, Günther (1868) classified 11 subfamilies excepting Catostomina, Homalopterina and Cobitidina. Thereafter, his Cyprinina was divided into four subfamilies, Cyprininae, Barbinae, Schizothoracinae and Gobioninae by Nikolskii (1954). Similarly, his Leuciscina was separated into Leuciscinae and Chondrostomatinae, and his Abramidina into Leuciscinae and Cultrinae (Nikolskii, 1954). On the contrary, Günther's three subfamilies, Leuciscina, Rasborina and Danionina were united into a single subfamily Leuciscinae (Chen, 1935). Berg (1912) separated the Russian Cyprinidae into 10 subfamilies, but later he (1940) lumped them into 4 subfamilies. Chu (1935) classified Chinese cyprinid fishes into 8 subfamilies, and Wu et al. (1964) separated Chu's Cyprininae into Cyprininae and Barbinae. Kryzanovsky (1947) divided all the Cyprinidae mainly on the basis of the embryonic and the zoogeographical data into 4 subfamilies. These facts show how to difficult to study phylogenetic systematics of the Cyprinidae.

Several investigators concluded that the family cannot be divided readily into subfamilies or other well-defined groupings. However, it will be more convenient to divide the Cyprinidae into subfamilies when the whole scheme of the Cyprinidae is viewed. Therefore, cyprinid fishes are separated into subfamilies in Tables 3 and 4.

On the other hand, karyological approach to fish phylogeny has been considered

to be very important. Cyprinid karyotypes have been studied rather well. However, the Cyprinidae is so large that their karyological data are not enough to analyze interrelationships of cyprinids. Nevertheless, relationships between karyotypes and Eurasian cyprinids will be discussed in the following lines. Table 3 lists Eurasian cyprinid chromosomes. Classification of subfamilies is adopted generally from GÜNTHER (1868), although GÜNTHER'S Cyprinina, Leuciscina and Abramidina are divided into Cyprininae, Barbinae, Gobioninae, Leuciscinae, Chondrostomatinae, Abramidinae and Cultrinae. In this paper, Cyprininae is represented by GÜNTHER'S 5th to 12th genera, Barbinae by his 13th to 23rd genera, Gobioninae by his 30th to 43rd genera, Leuciscinae by his 56th to 62nd genera, Abramidinae by his 79th to 82nd genera and Cultrinae by his 83rd to 95th genera.

Diploid chromosome number. Species with 2n=50 are distributed widely in Cyprininae, Barbinae, Gobioninae, Rasborinae, Leuciscinae, Chondrostomatinae, Danioninae, Abramidinae and Gobiobotiinae. Species with 2n=48 are found in Cyprininae, Cultrinae, Hypophthalmichthyinae, Danioninae, Acheilognathinae and Leuciscinae. Species with 2n=44-46 are limited in Acheilognathinae. Species with 2n=98-100 are distributed in Cyprininae, Barbinae and Gobioninae. Species with 2n=52 are found in Barbinae, Gobioninae, Abramidinae and Leuciscinae. Only a single species with 2n=78 is found in Danioninae.

Arm number. The arm number as well as 2n has been considered to be valuable in karyotype analysis. Polarity of the arm number is from small to large. There have been two types of definition on the arm number. In one definition (NF_1) , metacentrics and submetacentrics are counted as two, in the other (NF_2) , metacentrics, submetacentrics and subtelocentrics as two.

As regards cyprinid karyotypes, these two types of arm numbers have been reported. As these two types of NF have been confused frequently, both NF₁ and NF₂ are separately presented in Table 3.

When the karyotype of the same species is studied by plural investigators, it is very frequent that NF does not agree with one another (see *Puntius fasciatus*, *P. tetrazona*, *Gobio gobio*, *Alburnus alburnus*, *Hypophthalmichthys molitrix*, *Barilius bendelisis*, *Rhodeus sericeus amarus*, *Ctenopharyngodon idellus*, *Leuciscus cephalus*, *L. souffia muticellus*, *Phoxinus phoxinus*, *Rutilus rutilus*, *Scardinius erythrophthalmus*, *Tinca tinca*, *Tribolodon ezoe*, *T. hakonensis* and *Chondrostoma toxostoma* in Table 3). Such disagreement in NF may be caused by intraspecific variation or by experimental artifacts. Actually, the disagreements by artifacts are more possible than those by intraspecific variation, because gradual changes of centromeric position make difficult to separate submetacentrics from subtelocentrics or subtelocentrics from acrocentrics. Therefore, it should be noted that NF in the Cyprinidae is not always useful for karyotype analysis. This is the reason why NF is excluded from Table 4.

Polarity of the diploid chromosome number and some morphological characters. When phylogenetic systematics are studied by cladistic analysis, polarity of each character needs to be determined. In this study, several characters known in all sub-

Table 4. Comparison of selected characters. Data of morphological characters are limited to species whose karyotypes have been reported.

			reported.			
Genus	2n	Rows of pharyngeal teeth	Barbels	Branched dorsal rays (br. D)	Branched anal rays (br. A)	br. D minus br. A
Cyprininae						
Carassius	100	. 1	0	12-18	5, 6	+ 7-13
Catla	50	3	0	14-16	5	+ 9-11
Cirrhina	48	3	2	8- 9	5	+ 3- 4
Cyprinus	100	3	4, 2	10-22	5	+ 5-17
Labeo	50	3	4, 2	9-15	5	+ 4-10
Morulius	50	3	4	15-18	5	+10-13
Barbinae						
Acrossocheilus	(50	3	4	8	5	+ 3
	198	3	4	9	5	+ 4
Barbus	100	3	4	8	5	+ 3
Garra	50	3	4	8- 9	5	+ 3- 4
Puntius	50	3	4	8	5	+ 3
Tor	100	3	4	9	5	+ 4
Gobioninae						
Aulopyge	100	1	4	7	5	+ 2
Gnathopogon	50	2	2	7	6	+ 1
Gobio	50	2	2	7	6	+ 1
Hemibarbus	50	3	2	7	6	+ 1
Pseudogobio	50	2	2, 0	7	6	+ 1
Pseudorasbora	50	1	0	7	6	+ 1
Pungtungia	50	1	2	7	6	+ 1
Sarcocheilichthys	50	2	2, 0	7	6	+ 1
Rasborinae			,			
Amblypharyngodon	50	3	0	7	5	+ 2
Esomus	50	1	2	6	5	+ 1
Rasbora	50	3	0	7	5	+ 2
Gobiobotiinae						. –
Gobiobotia	50	2	8	7	6	+ 1
Microphysogobio	50	1	2	7	6	+ 1
Abramidinae						
Abramis	50, 52	1	0	8- 9	24-44	-15-36
Alburnoides	50	2	0	7- 8	12–17	- 5- 9
Alburnus	50	2	0	7- 8	15–20	- 8-12
Aspius	52	2	0	8- 9	12–14	- 5- 6
Vimba	50, 52	1	0	8	15–21	- 7-13
Cultrinae	,	•	v	O	15-21	7-13
Anabarilius	48	3	0	7	9–12	- 2- 5
Ischikauia	48	3	0	7	9–12 13–14	- 2- 3 - 6- 7
Megalobrama	48	3	0	7	27–32	-6-7 $-20-25$
Rohtee	48	3	0, 2	8	27–32	-20-25 $-19-25$
Salmostoma	50	3	0, 2	8 7	27–33 11–13	- 19-25 - 4- 6

Table 4 (continued).

Genus	2n	Rows of pharyngeal teeth	Barbels	Branched dorsal rays (br. D)	Branched anal rays (br. A)	br. D minus br. A
Hypophthalmichthyinae	2					
Aristichthys	48	1	0	7	12-13	- 5- 6
Hypophthalmichthys	48	1	0	7	12-13	- 5- 6
Danioninae						
Barilius	50	3	0, 4	7	7–10	- 0-
Bola	50	2	0	7- 8	10	- 2-
Danio	50	3	0, 2, 4	7-16	12-16	- 0-
Opsariichthys	78	3	0	7	9	- 2
Zacco	48	3	0	7	9-10	- 2-
Acheilognathinae						
Acanthorhodeus	44	1	0	14-16	13-15	+ 0-
Acheilognathus	(48	1	2	8- 9	9-11	- 1-
	(44	1	2	8-10	8- 9	+ 0-
Paracheilognathus	44	1	2	12-13	9-10	+ 2-
Pseudoperilampus	44	1	0	10	10	0
Rhodeus	(48	1	0	8-12	8-12	0, 1, -
	(46	1	0	10-11	10-11	- 0-
Tanakia	48	1	2	8	8	0
Leuciscinae						
Ctenopharyngodon	48	2	0	7	8	- 1
Leuciscus	50	2	0	7- 8	8- 9	- 1
Moroco	50, 52	2	0	7	7	0
Pachychilon	50	1	0 -	7- 8	8- 9	- 0-
Paraphoxinus	50	1	0	7	7	0
Phoxinus	50	2	0	7	7	0
Rutilus	50	1	0	9-11	9-11	0
Scardinius	50	2	0	8- 9	9-11	— 1–
Tinca	48	1	2	8	6-8	+ 0-
Tribolodon	50	2	0	7	7- 8	- 0-
Chondrostomatinae						
Chondrostoma	50	1	0	8- 9	8-11	- 0-

families are selected. Polarity is determined mainly by Maslin (1952). Table 4 shows 2n, number of rows of pharyngeal teeth, number of barbels, number of branched dorsal rays, number of branched anal rays, and the value of number of branched dorsal rays minus number of branched anal rays in Eurasian cyprinid fishes whose karyotypes have been reported. Data of these five characters except 2n are adopted from Günther (1868), Day (1875), Berg (1949), Wu et al. (1964, 1977), Sterba (1967), Nakamura (1969) and others. Although it is desirable that osteological characters are included in Table 4, they have been studied in very limited groups in the Cyprinidae to be included therein.

Interrelations in the diploid chromosome number, number of rows of pharyngeal

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teeth, the value of number of branched dorsal rays *minus* number of branched anal rays, and number of barbles are shown in Table 5.

1) Diploid chromosome number. In Cyprinidae, 2n ranges from 44 to 100 (2n=153-206 in gynogenetic Carassius is excluded). Ninety eight to one hundred in 2n may be resulted by polyploidization of 2n=50 or 48. Seventy eight in 2n is extraordinarily large number observed in only Opsariichthys uncirostris. According to Howes (1980), O. uncirostris is not so primitive as Barilius species whose 2n is 50. Therefore, 2n=78 is not considered to represent plesiomorphic character state. On the other hand, O. uncirostris has the largest number of one-arm chromosomes among the Cyprinidae except for species with 2n=98 or 100. This fact seems to demonstrate that 2n=78is formed by centric fission of 2n=50 or 48. The diploid chromosome number of Abramis, Aspius and Vimba, all of which belong to the subfamily Abramidinae, is 50 or 52. These diploid chromosome numbers may reflect interspecific variation. Pharyngeal teeth of Abramidinae are arranged in one or two rows. Fifty two in 2n has not been found in species with 3 rows of pharyngeal teeth. Although Ohno et al. (1967) reported 2n=52 in Barbus fasciatus (=Puntius fasciatus), TAKI et al. (1977) reported 2n=50 in the same species. From these facts, it is estimated that 2n=52 is resulted by aneuploidy or centric fission. Fifty in 2n is distributed most widely in Cyprinidae and has been found in all the subfamilies except for Hypophthalmichthyinae and Acheilognathinae. On the other hand, distribution of 2n=48 is more limited than that of 2n=50. A pair of large chromosomes in Anabarilius (2n=48, NAN=50) and Acheilognathus lanceolata and A. limbata (2n=48, NAN=50) suggest that 2n=48 in them is resulted from Robertsonian fusion. These facts seem to show that 2n=50 is more primitive than 2n=48.

From above discussion, it may be concluded that 2n=50 is the most primitive character state in the Cyprinidae and that 2n=50 is specialized into several different directions, i. e., (1) increase by aneuploidy or centric fission, $2n=50\rightarrow52$, (2) increase by centric fission, 2n=50 or $48\rightarrow78$, (3) increase by polyploidization, $2n=50\rightarrow100$ or 98, (4) decrease by Robertsonian fusion, $2n=50\rightarrow48$ (Anabarilius, Acheilognathus), $2n=48\rightarrow44$ (Acanthorhodeus, Acheilognathus, Paracheilognathus, Pseudoperilampus), and (5) decrease by deficiency, $2n=48\rightarrow46$ (Rhodeus).

- 2) Pharyngeal teeth. Polarity of pharyngeal teeth is from three rows to one row (Chu, 1935). Such interpretation seems to be supported by many ichthyologists, e. g., Vasnecov (1939), Kryzanovsky (1947), and Hensel (1970).
- 3) Barbels. The number of barbels is rather conservative in cyprinid genus, although there are genera whose species have 0-4 barbels, i. e., *Cyprinus*, *Barbus*, *Puntius* and others.

Species with barbels belong to Cyprininae, Barbinae, Gobioninae, Rasborinae, Gobiobotiinae, Cultrinae, Danioninae and Acheilognathinae, but are not found in Abramidinae, Hypophthalmichthyinae, Leuciscinae except for *Tinca*, and Chondrostomatinae. Eight barbels are limited in only subfamily Gobiobotiinae. As this subfamily is considered as one of specialized subfamilies, eight barbels are not plesio-

Table 5. Interrelation between 2n and some morphological characters in Eurasian cyprinids.

Genus	Aulopyge, Carassius Acrossocheilus, Barbus, Cyprinus, Tor	Opsariichthys	Abramis, Vimba Aspius	Esonnus, Microphysogobio, Pseudorasbora, Pungtungia Chondrostoma, Pachychilon, Paraphoxinus, Rutilus	Gnathopogon, Gobio, Gobiobotia, Fseudogobio, Sarcocheulchthys (Alburnoides, Alburnus, Bola, Leuciscus, Moroco, (Phoxinus, Scardinius, Tribolodon	Acrossocheilus, Amblypharyngodon, Catla, Garra, Hemibarbus, Labeo, Morulius, Puntius, Rasbora Barilius, Danio, Salmostoma	Rhodeus, Tinca Acheilognathus, Aristichthys, Hypophthalmichthys, Tanakia	Ctenopharyngodon Cirrhina	Anabarilius, Ischikauia, Megalobrama, Rohtee, Zacco	Rhodeus	Acheilognathus, Acanthorhodeus, Paracheilognathus Pseudoperilampus
Barbels	4, 0	0	0	2, 0	2, 8 0	4,0	2,0	0	2,0	0	2, 0
br. D minus br. A	+2, 7-13 +3-17	-2	-7-36 -5- 6	$\left\{\begin{matrix} +1\\ -0-2\end{matrix}\right.$	$\left\{egin{array}{c} +1 \ -0-12 \end{array} ight.$	$\begin{pmatrix} +1-13 \\ -0-6 \end{pmatrix}$	$\begin{cases} +0-2 \\ -0-6 \end{cases}$	-1 + 3 + 4	$\{-2-25\}$	-0- 1	+0-3
Rows of pharyngeal teeth	3	3	1 2	-	2	33	1	2	93	1	1
2n	100, 98	78	52		50			48		46	44

morphic. As six barbels on the lower jaw of Gobiobotia are not found in all other cyprinid genera, these barbels may have been formed secondarily. Four barbels are found in species with 3 rows of pharyngeal teeth in Cyprininae, Barbinae and Danioninae, but not in species with two rows of pharyngeal teeth. As regards species with one row of pharyngeal teeth, four barbels have been recognized exceptionally in one species, Aulopyge huegeli. Two barbels are shared by species with 1-3 rows of pharyngeal teeth in Cyprininae, Gobioninae, Rasborinae, Cultrinae, Danioninae and Acheilognathinae. Species without barbels are found in all subfamilies except for Gobiobotiinae. All species have no barbels in both of Hypophthalmichthyinae and Leuciscinae (except Tinca), which consist of species exclusively with one or two rows of pharyngeal teeth. It was suggested that species with barbels are more primitive than those without barbels in Japanese bitterlings (ARAI, 1978). Howes (1978, p. 60) suggested that barbel-less state was plesiomorphic, but later he (1980, p. 177) changed his opinion, i. e., having barbels was plesiomorphic character state. These facts seem to show that the posession of 4 barbels is the most primitive character state. The number of barbels is specialized into two different directions, i. e., (1) decrease from 4 to 2 and to 0, and (2) increase from 2 to 8 (Gobiobotia).

4) Branched dorsal and anal rays. The number of branched dorsal rays ranges from 6 to 22 in species whose karyotypes have been studied. Seven or eight branched dorsal rays are found in species of all subfamilies. Branched dorsal rays of *Carassius*, *Cyprinus*, *Catla* and *Morulius* in Cyprininae and 3 genera in Acheilognathinae are more than 9 in number. Among these 7 genera, pharyngeal teeth are arranged in one row in *Carassius* and in three genera in Acheilognathinae. *Cyprinus* is very characteristic in having serrated 3rd anal spine which is considered to be more specialized character state than non-serrated one. From these facts, it may be evaluated that 7 or 8 branched dorsal rays are plesiomorphic.

The number of branched anal rays ranges from 5 to 44 for species whose karyotypes have been studied. In all genera of Cyprininae, Barbinae and Rasborinae, they are five in number, although those of *Carassius carassius* are exceptionally six. Six branched anal rays are found in all genera except for *Aulopyge* in Gobioninae and Gobiobotiinae. In Abramidinae, Cultrinae, Hypophthalmichthyinae, Danioninae and Chondrostomatinae, branched anal rays are generally more than 9. Branched anal rays in Leuciscinae and Acheilognathinae range from 7 to 15. From these facts, it seems to be difficult to determine polarity of the number of branched anal rays.

On the other hand, it has been known in many groups that the value of number of dorsal rays *minus* number of anal rays is conservative and not so variable as counts of dorsal and anal rays. Values of number of branched dorsal rays *minus* number of branched anal rays are examined. As the results, they are classified into three types of dorsal-anal ray patterns. In Type A, branched dorsal rays are more numerous than branched anal rays. In Type B, branched dorsal rays are not more numerous than branched anal rays. Type C is the mixture of Types A and B.

Type A is found in Cyprininae, Barbinae, Gobioninae, Rasborinae and Gobio-

botiinae. Type B occurs in Abramidinae, Cultrinae, Hypophthalmichthyinae, Danioninae, Leuciscinae (except for *Tinca*) and Chondrostomatinae. Type C is limited in Acheilognathinae. Species belonging to Type A have, in general, characters such as small number of branched anal rays and having barbels. Species with 2n=98-100 are limited to Type A. Large number of branched anal rays and no barbels are shared by fishes belonging to Type B except for the Danioninae. Difference of Type A from Type B seems to reflect that between habitats of fishes belonging to each Type, for instance, fishes of Type B are basically midwater groups (GOSLINE, 1975, p. 7) but fishes of Type A (except Rasborinae) are more benthic than those of Type B.

Systematics. From analysis of four characters discussed above, following problems are pointed out.

- 1. As reported by Berg (1949), phylogenetic position of *Tinca* is doubtful. Kryzanovsky (1947) separated *Tinca* from his Leuciscina, and erected Tincina on the basis of his experimental results that *Tinca* crossbred with representatives of the group Cyprinina, but not with some of Leuciscina. *Tinca* differs from all other genera in Leuciscinae by 2n=48, 2 barbels and Type A on dorsal-anal ray pattern. Therefore, it may be more reasonable that *Tinca* is transferred from Leuciscinae into one of subfamilies belonging to Type A on dorsal-anal ray pattern, e. g., Cyprininae.
- 2. Both species with 2n=50 and that with 2n=98 are included in *Acrossocheilus*. As regards fish karyotypes, it is very rare that fishes of the same genus have diploid (2n=50) and tetraploid chromosomes (2n=98). Morphologically *A. sumatranus* differs from *A. labiatus* in such characters as 2n=98 and 9 branched dorsal rays, which are shared by *Tor putitora*.

Acrossocheilus sumatranus was described originally as Lissochilus sumatranus (Weber & de Beaufort, 1916). As the name Lissochilus had been preoccupied by a genus of fossil mollusks, Smith (1945, pp. 196–197) classified this species into Acrossocheilus with some hesitation, although as originally defined Acrossocheilus did not apply to any of Lissochilus species. On the other hand, Weber and de Beaufort (1916) described two species of Lissochilus, L. sumatranus and L. dukai. The latter is closely related to Tor putitora (see Day, 1875, p. 557). From these facts, the generic name of A. sumatranus should be changed from Acrossocheilus to Tor.

- 3. Phylogenetic position of *Hemibarbus* is noteworthy. *Hemibarbus* shares 3 rows of pharyngeal teeth with genera of Barbinae, but differs from them in having 2 barbels, 7 branched dorsal and 6 branched anal rays which are in common with Gobioninae. These facts may mean that *Hemibarbus* is a link between Barbinae and Gobioninae.
- 4. Aulopyge is characteristic in combination of characters such as 2n=100 (apomorphic), one row of pharyngeal teeth (apomorphic), 4 barbels (plesiomorphic) and five branched anal rays, by which this genus is differentiated from all other genera of Gobioninae. Such mixture of plesiomorphic and apomorphic characters peculiar to Aulopyge may be related with its polyploidy (2n=100) whose morphological characters may be more hard to be influenced by mutation than those of the diploid. Aulopyge

shows a mosaic of barbine and gobionine characters.

5. Some ichthyologists (CHU, 1935; WU et al., 1964) have not differentiated Abramidinae from Cultrinae. However, karyologically these two subfamilies are specialized into different direction, i. e., decrease of 2n in Cultrinae (2n=50, 48) versus increase of 2n in Abramidinae (2n=50, 52). These facts may support NIKOLSKII's classification, in which he separated GÜNTHER's Abramidina into two groups. One group contained Abramis, Alburnoides, Alburnus, Aspius and Vimba, and the other belonged to Cultrinae. NIKOLSKII (1954) classified the former group into Leuciscinae, but I did them into Abramidinae.

From the viewpoint of comparative karyology, Abramidinae may be derived from Leuciscinae because almost genera of Leuciscinae is 50 in 2n, while 2n of Abramidinae is 50 or 52.

6. Gosline (1975) pointed out many characters which are shared by his Rasborinae (=Danioninae and Rasborinae by Günther) and cultrine *Salmostoma*. To conclude concerning the resemblances between his Rasborinae and Cultrinae in the *Chela-Salmostoma* area, he was of the opinion that they were the result of convergence.

As regards cultrine karyotypes, 2n of *Salmostoma* is 50, while 2n of all other genera examined is 48. As polarity of 2n is from 50 to 48, *Salmostoma* is considered to be the most primitive in cultrine genera as treated here. In all genera of Rasborinae and the Danioninae except for both *Opsariichthys* and *Zacco*, 2n is 50. From these facts, it may be estimated that Cultrinae is derived from Danioninae and that *Salmostoma* is a link between Danioninae and Cultrinae. My interpretation on interrelationships between Cultrinae and his Rasborinae differs from Gosline's one.

- 7. Rasborinae is separated from Barbinae by lateral line running along the lower half of the tail being shared by Danioninae (GÜNTHER, 1868). Since Weber and DE Beaufort (1916) combined Danioninae with Rasborinae into a single subfamily, many ichthyologists followed their classification (Gosline, 1975). However, Rasborinae is similar to Barbinae in the number of branched dorsal and branched anal rays. As regards rasborine genera, *Esomus* differs from both *Amblypharyngodon* and *Rasbora* in having one row of pharyngeal teeth, 2 barbels and 6 branched dorsal rays. These facts suggest that Rasborinae is not monophyletic and that interrelationships in Barbinae, Rasborinae and Danioninae are in chaos, although Rasborinae may be more closely related to Danioninae than to Barbinae (Gosline, 1975).
- 8. Acheilognathinae is very characteristic among cyprinid fishes for the Type C in dorsal-anal ray pattern. In *Acheilognathus lanceolata* and *A. limbata*, anal branched rays are greater in number than branched dorsal rays (Type B). On the contrary, anal branched rays are not more numerous than branched dorsal rays in *A. cyanostigma*, *A. moriokae* and *A. tabira* (Type A). *Acheilognathus* contains both species with 2n=48 and those with 2n=44. Each dorsal-anal ray pattern of species with 2n=48 and those with 2n=44 is Type B and Type A, respectively.

Considering the fact that the difference between species with 2n=48 and those with 2n=44 in Acheilognathus is larger than the difference between Acheilognathus

species with 2n=44 and *Paracheilognathus* species or *Acanthorhodeus* species in karyotypes, the dorsal-anal ray pattern and embryological data, it seems to be more reasonable that *Acheilognathus* species should be divided into two different fish groups. Phylogenetically, *Acheilognathus* species with 2n=44 may occupy the position between *Acheilognathus* species with 2n=48 and *Paracheilognathus* or *Acanthorhodeus* (ARAI, 1978).

9. Both Aristichthys and Hypophthalmichthys of Hypophthalmichthyinae resemble the cultrine Ischikauia in the intestine convolution, possessing a keel along the ventral side, 13 branched anal rays, numerous lateral line scales and dorsally directed mouth, but differ from it in the shape of pharyngeal teeth (MIYADI et al., 1976). On the other hand, these two genera of Hypophthalmichthyinae are similar to the leuciscine Mylopharyngodon in the shape of pharyngeal teeth and the intestinal convolution, but not in its keel-less ventral side and 8 branched anal rays (CHU, 1935). MIYADI et al. (1976) combined the hypophthalmichthyine two genera with the leuciscine two genera, Ctenopharyngodon and Mylopharyngodon, and the cultrine Ischikauia into the subfamily Hypophthalmichthyinae.

As regards chromosomes, both Hypophthalmichthys and Aristichthys share 2n=48 with not only the leuciscine Ctenopharyngodon but also the cultrine Ischikauia. Chromosomes with secondary constriction are found in karyotypes of Hypophthalmichthys, Aristichthys and the cultrine Anabarilius and Megalobrama, but not in that of Ctenopharyngodon (ZAN & SONG, 1979, 1980a, 1980b). Presence or absence of secondarily constricted chromosomes in Ischikauia is unknown. Morphologically, each of the cultrine Ischikauia, the leuciscine two genera and the hypophthalmichthyine two genera cannot belong to the same monophyletic group because Ctenopharyngodon and Mylopharyngodon do not occupy the phylogenetic position between the cultrine and the hypophthalmichthyine genera. These facts suggest that the similarity in the shape of specialized pharyngeal teeth among them is the result of convergence. I currently adopt the hypothesis that Hypophthalmichthyinae may be derived from Cultrinae.

10. Gobiobotiinae was erected by Mori (1933) on the basis of the ossified gasbladder capsule, but not separated from Gobioninae by Nikolskii (1954) and Banarescu and Nalbant (1973). Wu et al. (1977) removed *Microphysogobio* (=Abbotina) and Saurogobio from Gobiobotiinae into Gobioninae, and only Gobiobotia was included in Gobiobotiinae.

The diploid chromosome number of both *Gobiobotia* and *Microphysogobio* is 50 which agrees with that of Gobioninae. *Gobiobotia* is very characteristic in having 8 barbels, 6 of which are placed on the lower jaw. As barbels on the lower jaw are not found in all other cyprinid genera, these six barbels may have been produced secondarily. These facts suggest that Gobiobotiinae is derived from Gobioninae.

11. Each of Barbinae, Cyprininae and Gobioninae is closely related one another in characteristics such as 5 or 6 branched anal rays, 2 or 4 barbels and presence of species with 2n=98-100. If polyploidization, just as seen in *Barbus* and *Tor* (Barbinae), *Aulopyge* (Gobioninae), *Carassius* and *Cyprinus* (Cyprininae), relates with

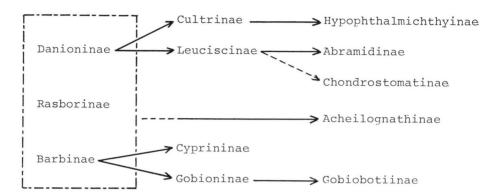


Fig. 2. Provisional interrelationships in Eurasian cyprinid subfamilies. Arrows show direction of specialization.

glaciation, some of these three subfamilies may have been distributed in common areas to them in the glacial period.

12. It is very difficult to construct phylogenetic tree of 12 subfamilies discussed in this paper, as the number of characters are too small to discuss on their interrelationships. Nevertheless, the provisional scheme of their interrelationships is presented here in Fig. 2 as the first step to the further investigation.

As far as I know, chromosomes of Schizothoracinae and Xenocyprininae have not been reported. Therefore, these subfamilies are excluded from Fig. 2. No data on chromosomes of Xenocyprininae which is considered to be closely related to Chondrostomatinae make it difficult to discuss on phylogenetic position of Chondrostomatinae in the family Cyprinidae.

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