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Geographic Distributions of Karyological Races of *Cobitis biwae* (Cobitididae)

Yoshiteru Kimizuka and Hiromu Kobayasi

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Chromosomal polymorphism has been known in spinous loaches of the genus *Cobitis*, *C. biwae* Jordan et Snyder, *C. taenia taenia* Linnaeus and *C. taenia striata* Ikeda (Kobayasi, 1976; Takahasi and Oka, 1976; Ueno and Ojima, 1976; Sofradžija and Berberović, 1978; Ueno et al., 1980; Vasiliev and Vasilieva, 1982). In *Cobitis biwae*, Kobayasi (1976) reported the existence of two karyological races, i.e., a diploid race with 48 chromosomes and a polyploid race with 96 chromosomes. Aneuploid specimens with 46 chromosomes have also been recorded (Ueno, 1981). The purpose of this study is to elucidate the geographic distributions of these races or populations with different karyotypes on the basis of materials from numerous localities covering almost the whole of the range of the species, and to examine their distributional patterns from a zoogeographical viewpoint.

Materials and methods

The specimens examined for the present study were collected from May 1977 to July 1981, in a total of 146 localities in 114 river systems all over Honshu and Shikoku, Japan. Samples were captured mostly by scoop nets.

In the present study, *Cobitis biwae* is identified from the following two characters: 1) lamina circularis (Canestrini's organ) in the pectoral fins of mature males consisting of a beak-shaped tip and a round to oval base, in contrast to the simply roundish plate in *C. taenia*; 2) a row of blackish blotches running along the middle of the sides of the body (Ikeda, 1936, 1937).

In the western part of Honshu, a few specimens with a continuous blackish band along the sides of the body, as in *C. taenia striata*, were collected together with normally dotted specimens. In these cases, males were identified by the shape of the lamina circularis, and females could be identified by their markings on the dorsal and caudal fins, i.e., with irregular striations, not with two to three horizontal or V-shaped dotted lines as in *C. taenia*.

For karyotype analysis each fish was given an intraperitoneal injection of colchicine (0.5 or 1.0%), two to three hours prior to sacrifice. Gill and kidney tissues were removed, minced with scissors, treated with hypotonic solution, and fixed in Carnoy's solution. Slide preparation was made by means of the routine flame-drying and Giemsa-staining methods. The classification of chromosomes followed Levan et al. (1964). Specimens used in the present study are deposited in the Yokosuka City Museum, Kanagawa Prefecture, Japan.

Results and discussion

Longitudinal distribution. *Cobitis biwae* occurs mostly in the middle and a part of the lower courses of the streams. Its habitats are generally restricted to slow-flowing waters with sandy bottoms. In drainages where *Cobitis taenia striata* occurs together with *C. biwae*, the latter inhabits slightly upper portions. Segregation in accordance with bottom features is recognized: *C. biwae* inhabits gravel to sandy bottoms, while *C. taenia striata* is seen on muddy-sand bottoms. In drainages where *C. taenia striata* does not occur, *C. biwae* extends its habitats to lower reaches.

Geographic distribution. *Cobitis biwae* was collected from 135 localities in Honshu, north to the Noheji River (st. 1 in Fig. 1) and the Shinjo River (st. 2), southwest to the Takatsu River (st. 22) and Nishiki River (st. 26), and from 11 localities (sts. 28～31) in Shikoku. Reliable records of *C. biwae* outside the range of our collections have been made from the Abu and Shimata rivers (sts. 23 and 27; Fujioka, 1977). Our field observations indicate the absence of this species in the southeastern slope of the Kii Peninsula and the southwestern tip of Shikoku.

C. biwae has been recorded from northwestern Kyushu (Ikeda, 1937; Okada and Ikeda, 1939), but we have collected only *C. taenia taenia* throughout this area. The specimens from the upper reaches of the Sendai River in Miyazaki Prefecture reported by Yamane (1969) were reexamined by us and identified as *C. taenia taenia*. Our samples from the Sendai River system were also identified as *C. taenia taenia* both morphologically and karyologically.

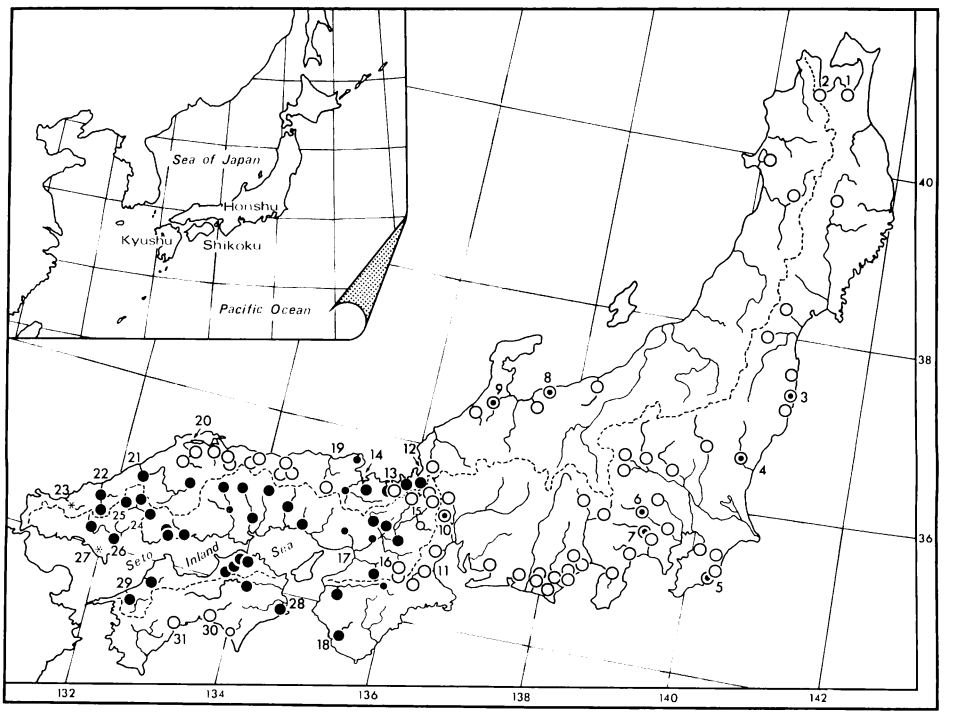

Fig. 1. Geographic distributions of karyological races of *Cobitis biwae*. ○, record of diploid specimens with 48 chromosomes (large circles indicate the present observations; small circles records by Ojima and Hitotsumachi, (1969) and Ueno et al., (1980)); ⊙, aneuploid specimens with 46, 49 and 50 chromosomes; ●, polyploid specimens with 96 chromosomes (large circles indicate the present observations; small circles records by Ueno et al., (1980)); ∗, collecting records by Fujioka (1977) (karyotype not examined). Station numbers are restricted only to those mentioned in the text, and specimens of non-numbering large circles also examined karyologically. Broken lines indicate principal watershed. 1, Noheji River; 2, Shinjo R.; 3, Tomioka R.; 4, Kuji R.; 5, Konta R.; 6, Hirai R.; 7, Sagami R.; 8, Funa-kawa R.; 9, Oyabe R.; 10, Fujiko R.; 11, Kumozu R.; 12, Shouno-kawa R.; 13. Okutanou R.; 14, Yura R.; 15, Lake Biwa; 16, Nabari R.; 17, Yodo R.; 18, Tonda R.; 19, Maruyama R.; 20, Hii-kawa R.; 21, Gouno R.; 22, Takatsu R.; 23. Abu R.; 24, Ohta R.; 25, Oze R.; 26, Nishiki R.; 27 Shimata R.; 28, Kuwano R.; 29, Hiji-kawa R.; 30, Monobe R.; 31, Shinjo R.

Karyotypes. Karyological examination was carried out for 329 specimens from 130 localities, and was successful in 283 specimens from 115 localities. Most of the specimens were either diploid with 48 chromosomes or polyploid with 96 chromosomes. Aneuploid specimens with various karyotypes were also found from eight localities. Their chromosomal constitutions are given in Table 1. Incorporating the present observations and published records (Ojima and Hitotsumachi, 1969; Kobayasi, 1976; Takahasi and Oka, 1976; Ueno and Ojima, 1976), *Cobitis biwae* can be considered to consist of two karyological races, i.e., diploid and polyploid races. Aneuploid specimens with 49 and 50 chromosomes were collected in most cases together with diploid specimens (Table 1), and the aneuploidy was represented by only one or two individuals among a sample of two to seven individuals at each locality. No diploid specimens were collected together with aneuploid specimens with 46 chromosomes. Sexual dimorphism was not seen.

Karyotypes of the present species are distinguishable from those of *C. taenia* and *C. takatsuensis* Mizuno (Ueno and Ojima, 1976; Kimizuka et al., 1982) in having fewer acrocentric chromosomes.

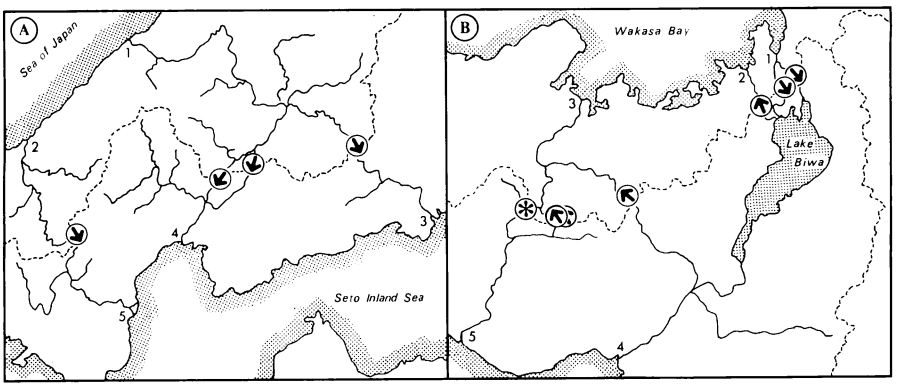

Fig. 2. Records of watershed migration in western part of Honshu District. Arrows indicate stream piracy with its direction of migration. Asterisk shows divide in valley. Broken lines denote principal watershed. A: 1, Gouno River; 2, Takatsu R.; 3, Ashida R.; 4, Ohta R.; 5, Nishiki R.; B: 1, Shouno-kawa R.; 2, Mimi-kawa R.; 3, Yura R.; 4, Yodo R.; 5, Kako R.

Table 1. List of materials for karyotype analysis of *Cobitis biwae*. For localities, see Fig. 1.

Ploidy	2n	Locality (St. no.)	No. of specimens (sex)
Diploid	48	81 stations	188 (♂, ♀)
Aneuploid	46	Oyabe River (9)	1 (unknown)
	46	Funa-kawa R.(8)	2 (♂, ♀)
	49	*Hirai R. (6)	2 (♂, ♀)
	49	*Fujiko R. (10)	1 (♂)
	49	*Tomioka R. (3)	1 (♀)
	49	Sagami R. (7)	1 (♀)
	49	*Kuji R. (4)	1 (♀)
	50	*Konta R. (5)	1 (♀)
Polyploid	96	37 stations	95 (♂, ♀)

* Diploid specimens with 48 chromosomes were also obtained.

Relationship between karyotypes and morphological characters. Morphological differences between two local races, i.e., small-body-sized from central Honshu and large-body-sized from western Honshu, was reported by Minamori (1951a, b), which differ in the sizes of the body, eggs, spermatozoal and of erythrocytic cells. These differences agree with our observations of diploid and polyploid specimens (Kobayasi, 1976). No other remarkable differences were recognized in morphological characters between the two races. Although the body size of the diploid race is fairly variable, it is smaller in the Kanto District than in other areas. Aneuploid specimens were not distinguishable morphologically from diploid individuals.

In the periphery of the geographic range of the polyploid race, i.e., the Shouno-kawa River (st. 12 in Fig. 1), the Tonda River (st. 18), the Kanoashi-kawachi River of the Takatsu system (st. 22), the Oze River (st. 25) and the Usa River of the Nishiki system (st. 26), a few specimens with an irregular continuous blackish band on the middle of the body were collected. Except for specimens from the Oze River (karyotype not examined), all of them were polyploid, and *C. taenia striata* was not seen in any of these drainages.

Geographic distributions of karyological races. Based on the present study and literature (Ueno et al., 1980; Ueno, 1981), the geographic distributions of the diploid and polyploid races of *Cobitis biwae* are shown in Fig. 1.

The diploid and polyploid races are clearly isolated from each other in their geographic ranges, and the two races are totally allopatric except in the Okutanou River of the Minami system (st. 13 in Fig. 1).

The diploid race occurs throughout northern and central Honshu and in a part of western Honshu westward to the Hii-kawa River (st. 20) on the Japan Sea slope, to Lake Biwa and the Nabari River (sts. 15 and 16) on the Seto Inland Sea slope, and to the Kumozu River (st. 11) on the Pacific slope. In Shikoku, it is found only on the Pacific slope (sts. 30 and 31).

The polyploid race occurs in western Honshu

and Shikoku, and its range is almost completely confined to the Seto Inland Sea slope (Fig. 1), except for the inflowing rivers of Lake Biwa, the rivers flowing into Wakasa Bay (sts. 12～14), and the rivers on the Japan Sea slope near the western tip of Honshu (sts. 21 and 22).

Minamori (1951a, b, 1957) recognized existence of two local races which differ mainly in body size. The distributional range of karyological races given in this study shows that his small-sized race is diploid and large-sized race polyploid. The sterility in the progeny in the cross between the two races observed by Minamori (1951a, b) seems to have resulted from difference in ploidy.

Consideration on the distribution of karyological races. The polyploid race of *Cobitis biwae*, restricted almost completely to the Seto Inland Sea slope of Honshu and Shikoku, is also found in the vicinities of Wakasa Bay and westernmost Honshu on the Japan Sea slope. In these two areas, wind gaps, divides in valley, underfit streams and other landforms indicating the migration of watersheds are found in many places (Inami, 1951; Nishimura, 1962; Okada and Takahashi, 1969; Togo and Nakagawa, 1973). For example, the riverheads of the Yodo and Kako rivers are considered to have been captured by the Yura River, the Gouno River by the Ashida and Ohta rivers, and the Takatsu River by the Nishiki River (Fig. 2).

When these geomorphological observations are taken into consideration, the occurrence of the polyploid race of *Cobitis biwae* in a few river systems outside the Seto Inland Sea slope can be interpreted as a result of its dispersal through the piracy or spill-over of the rivers. The polyploid race are most probably derived from a diploid ancestor in the slopes toward the present-day Seto Inland Sea, presumably when a large body of freshwater such as a paleo-river or lake was in existence.

The present-day geographic isolation of the two karyological races is remarkable. Considering the fact that the range of the polyploid race coincides almost completely with that of *Cobitis taenia striata*, the absence of the diploid race in the Seto Inland Sea slope may be related to the existence of *C. taenia striata* in the region, as well as to presumable competition between the two races of *C. biwae*. Further extensive studies are required to clarify the mechanisms of such isolation.

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(YK: 1-1-1, Kojima-cho, Chofu-shi, Tokyo 182, Japan; HK: Department of Biology, Japan Women's University, Mejirodai, Bunkyo-ku, Tokyo 112, Japan)

シマドジョウの核学的種族の地理的分布

君塚芳輝・小林　弘

ドジョウ科シマドジョウ亜科 (Cobitidinae) のシマドジョウ *Cobitis biwae* について，分布域のほぼ全域から多数の材料を集めて核型分析を行なった．本種は染色体数から主として 48 を数える 2 倍性種族（異数性の 2n=46, 49, 50 の少数個体を含む）と，96 を数える倍数性種族とに大別された．倍数性種族の地理的分布は本州・四国の瀬戸内海斜面地域に局限され，これらを取り囲むように他の地域を 2 倍性種族が占めていた．上野ほか (1980) が述べているように，両種族間には地理的隔離がほぼ完全に成立していた．これら核学的種族の分布を概観すると，(1) シマドジョウの倍数化は現在の瀬戸内海の位置にかつて存在した大淡水域（河川または湖沼）で起こり，(2) 日本海側の若狭湾流入河川や江川・高津川における倍数性種族の局所的な分布は，分水界を接する河川の流路変更（争奪あるいは溢流）に伴う瀬戸内海側からの浸潤によるものと推定された．

(君塚: 182 調布市小島町 1-1-1 RC-201; 小林: 112 東京都文京区日白台 2-8-1 日本女子大学生物学教室)