

A Cytotaxonomical Study in Some Freshwater Cottoid Fishes (Cottidae, Pisces)

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The Cottidae, a closely related family of percoid fishes, has a world-wide distribution over the northern hemisphere as seawater and freshwater residents and contains a number of species which differentiate at various levels in form and size (Matsubara 1955). According to Watanabe (1958), only 6 species of cottoid fishes are freshwater residents among 74 species occurring in Japan. They are included in two genera, *Cottus* and *Trachidermus*. Since the first description of Günther (1873), the definition of *Cottus* species in Japan has been a subject of animated discussion in ichthyology. Recent advances in cytogenetic techniques allow the cyto-systematic analysis in fishes to a considerable extent (Ojima *et al.* 1973, Uyeno and Miller 1973).

Chromosome studies of the freshwater cottoid fishes were done by Hann (1927), Nogusa (1960), and Starmach (1967) with the classical testis-section method, or squash techniques. The present paper deals with the chromosomes of 6 cottoid species of Japan, with special regard to their karyological and phylogenic aspects.

Materials and methods

Six species studied here are listed in Tables 1 and 2. They were identified in reference to the description of Watanabe (1958) and Nakamura (1963), based on the morphology of adult specimens. The specimens for study were immediately received an intraperitoneal injection of colchicine (5 $\mu\text{g/g}$ body weight) for 5 to 6 hours prior to sacrificing. Pieces of kidneys and gonads (mostly testes) were dissected from the body, minced with scissors in a shalot and suspended in a 0.075 M KCl hypotonic solution for 20 to 30 minutes at room temperature. The suspended cells were fixed with 3:1 methanol-acetic acid. Slides were prepared according to the flame-drying or air-drying methods followed by Giemsa staining. Chromosome counts were made in at least 30 well spread metaphase plates. Karyograms were constructed on 10 metaphase plates from kidney cells in each species.

Results

In karyotype analysis, the chromosomes were categorized into two groups: biarmed chromosomes including meta- and submetacentrics, and unarmed chromosomes of acrocentrics with or without the minute arms. The arm-number was calculated as two for the biarmed chromosomes and as one for the unarmed ones.

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1. Genus *Trachidermus*: The genus *Trachidermus* in Japan is represented by only one species, *T. fasciatus* Heckel. The species is known to inhabit in a restricted area of Kyushu, Japan, while it has a wide distribution from China to Korea along the eastern coasts of the Asiatic Continent (Tsukahara 1952).

Table 1. Numbers and localities of four species of Cottidae

Species	Number of individuals		Localities
	(Sex)	M. T. L.*	
Genus <i>Trachidermus</i>			
<i>T. fasciatus</i>	4 (?)	7.7	Yabe river (Fukuoka Pref.)
Genus <i>Cottus</i>			
<i>C. kazika</i>	12 (11♀ and 1♂)	11.3	Kuzuryu river (Fukui Pref.)
<i>C. pollux</i>	41 (16♀ and 25♂)	10.1	Ado river (Shiga Pref.) Iwaki river (Aomori Pref.) Hida river (Gifu Pref.) Oguni river (Yamagata Pref.)
<i>C. reinii</i>	1 (1♀)	9.5	Kuji river (Iwate Pref.)

* M. T. L.; mean total length (cm).

Table 2. Results of karyotype analysis of six Japanese and two foreign species of Cottidae

Species	Biarmed chromosomes	Uniarmed chromosomes	2N	NF*
	M and SM**	A**		
<i>T. fasciatus</i>	24	16	40	64
<i>C. kazika</i>	18	22	40	58
<i>C. pollux</i>	10	38	48	58
<i>C. reinii</i>	12	36	48	60
<i>C. nozawae</i> ⁺	10	38	48	58
<i>C. hangiongensis</i> ⁺	6	42	48	54
<i>C. poecilopus</i> ⁺⁺	8	40	48	56
<i>C. gobio</i> ⁺⁺	6	46	52	58

* NF; total number of chromosome arms (nombre fondamental).

** M; metacentrics, SM; submetacentrics, A; acrocentrics.

⁺ Cited from the previous report (Abe 1972).

⁺⁺ Cited from Starmach (1972).

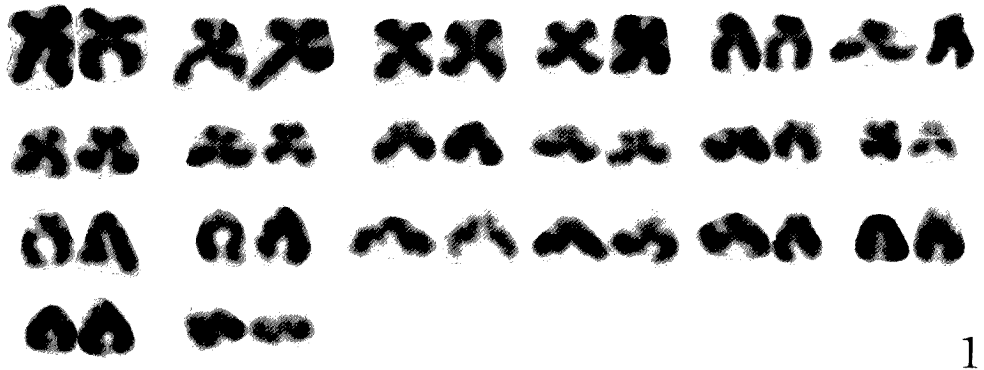
Cells from kidney tissue of 4 individuals provided 40 chromosomes (Fig. 1). The sexuality of these specimens was unknown due to immature gonads. The karyotype consisted of 24 biarmed chromosomes, 4 pairs of metacentrics and 8 pairs of submetacentrics. They were rather surely distinguished because of their characteristic shape and size. The remaining 16 uniarmed chromosomes appeared as acrocentrics, being mostly equal in their length. The arm-number calculated was 64.

2. Genus *Cottus*: The genus *Cottus* includes 5 species in Japan, *C. kazika* Jordan et Starks, *C. pollux* Günther, *C. reinii* (Hilgendorf), *C. nozawae* Snyder, and

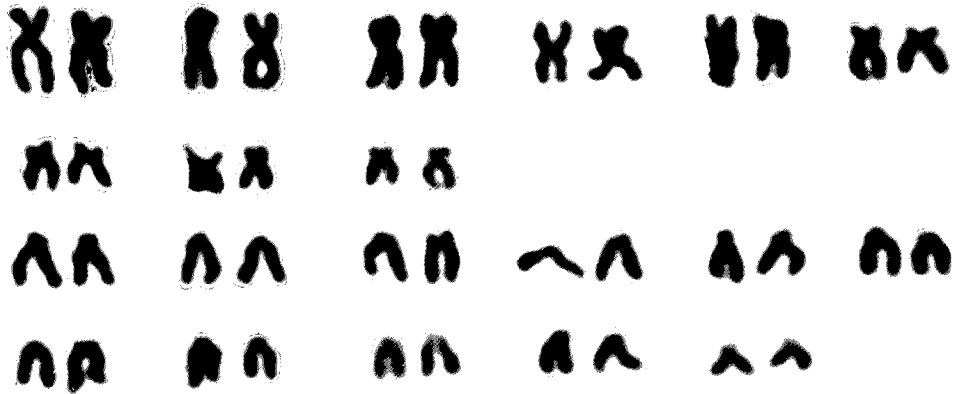
C. hangiongensis Mori. The chromosomes of the latter 2 species were already reported by the present author (Abe 1972).

C. kazika: The diploid number of this species was 40 in accordance to that of *T. fasciatus*. The karyotype consisted of 9 pairs of biarmed chromosomes and 11 pairs of acrocentrics, with the arm-number of 58. Five pairs of biarmed chromosomes were relatively larger than any of acrocentric elements (Fig. 2).

C. pollux: This is a common species in Japan and has been the object of taxono-



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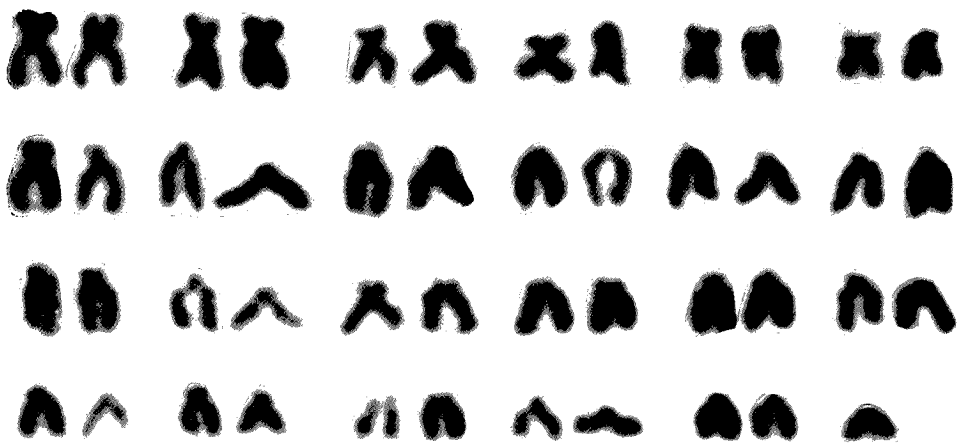
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Figs. 1-2. 1, karyotype of *T. fasciatus* from kidney cell (sex unknown). 2, karyotype of *C. kazika* from female.

mical and ecological studies (Tamura 1936, Koyama 1950, Watanabe 1958, Mizuno and Niwa 1961, Mizuno 1963). According to Mizuno and Niwa (1961), this species includes ecological dimorphic groups consisting of a large egg-type and a small egg-type. There was no meristic difference between the two groups except the number of pectoral fin rays. All specimens studied were of the large egg-type.

The diploid number was 48. Five pairs of biarmed chromosomes and 19 pairs of uniarmed ones were defined among them (Fig. 3). The arm-number was

58. Thus, the karyological feature of this species accords with that of *C. nozawae* (Abe 1972), except the largest pair of metacentrics as marker chromosomes of this species. The length of these metacentrics was estimated 1.5–2 times that of the submetacentrics of next rank which decreased gradually in length. According to



Figs. 3–4. 3, karyotype of *C. pollux* from male. The largest metacentric pair as marker chromosomes of this species. 4, karyotype of *C. reinii* from female.

Nogusa (1960), the present species showed the same diploid number, while all the elements were acrocentrics. Probably, the different results may be caused artificially by technical procedures. The karyotype showed no local variations, so far as our specimens collected in various localities from Aomori to Shiga Prefecture are concerned.

C. reinii: The diploid number was 48, in agreement with that of *C. pollux* (Fig. 4). The biarmed chromosomes were represented by 6 pairs of meta- and submetacentrics. The remaining 18 uniarmed pairs were acrocentrics. The arm-number was 60.

In all the 6 species considered here, there was no karyological evidence for the occurrence of the heteromorphic elements in both sexes, though Nogusa (1960) reported heteromorphic chromosomes in the male of *C. pollux*.

Discussion

The present study has revealed that 6 cottoid species under consideration contain 2 groups which are different in diploid numbers, 40 and 48. The former group includes *T. fasciatus* and *C. kazika*, while the remaining 4 species of *Cottus* belong to the latter (Table 2). According to Starmach (1967), the diploid number of *C. poecilopus* was 48 consisting of 8 metacentrics and 40 acrocentrics, while *C. gobio* had 52 chromosomes comprising 6 metacentrics and 46 acrocentrics. Therefore, the former species is to be categorized into the 48-chromosome group, while the latter is outstanding by the highest diploid number (52) among the cottoid species so far studied. According to Matsubara (1955), cottoid fishes are originally derived from a percoid ancestor, bearing a close similarity to perciform fishes in their morphological characters. In perciform fishes, the basic chromosome number has been considered as 48, since 2n, 48 has occurred in a number of species studied (Post 1965, Nayyar 1966, Ohno *et al.* 1968, Chen and Ebeling 1971). Amongst the 8 species of cottoid fishes so far concerned, the diploid number was 48 in 5 species, 40 in 2 species, and 52 in 1 species.

The 4 Japanese species having 2n, 48 are of freshwater-adapted or land-locked type, while *T. fasciatus* and *C. kazika* are of catadromous type, or a less freshwater-adapted species (Tsukahara 1952, Watanabe 1958, Mizuno and Niwa 1961, Nakamura 1963). Watanabe (1958) has mentioned that there is some close relation between freshwater sculpins occurring in the Asiatic Continent and those in Japan, and that the latter are derived from a migratory ancestor occurring in the Northeast coast of the Continent. Further, Mizuno (1963) has suggested that the less freshwater-adapted sculpins are more conservative than the freshwater sculpins. With the above view in mind, the following statements may be made that the karyotype of the freshwater species might have been derived from that of the seawater or less freshwater-adapted forms. However, the cytogenetic features in the species having 2n, 48 and 2n, 40 are not in favor of the above view.

According to Watanabe (1958), *C. pollux* is a common ancestor of *C. reinii* and *C. nozawae*. The chromosome constitutions of these 3 species strongly suggest the occurrence of a close kinship among them. *C. pollux* here concerned is of the

large egg-type, which is considered as a derivative from the amphidromous small egg-type (Mizuno and Niwa 1961). Though no karyological evidence was provided, it seems probable that the differentiation of *C. pollux* from the small egg-type to the large egg-type might have occurred rather recently than 3 species, *C. pollux*, *versus*, *C. reinii* and *C. nozawae* (Mizuno 1963). Karyologically, *C. hangiongensis* differs from the above 3 species, in the number of biarmed chromosomes and arm-number (Abe 1972). Very probably, this species might have been differentiated through a different pathway from the 2n, 48 species, since this species is of the amphidromous type, and closely related to the species occurring in Sakhalin and Korea (Berg 1965).

The above karyological data in the cottoid fishes seem to correlate to their morphological characters such as the number of preopercular spines and with or without the palatine teeth. Two species having, 2n, 40 have the preopercular spines in the same number (4) having palatine teeth, while each of 2n, 48 species shows preopercular spines varying from 1 to 3, without having the palatine teeth. The meristic characters of *C. kazika* seem to be rather close to that of *T. fasciatus*. Karyological and morphological characters of this species occurring in Japan are of special interest in relation to its taxonomical situation.

Summary

The chromosomes of 6 species of cottoid fishes from Japan are studied. *Trachidermus fasciatus* and *Cottus kazika* have the diploid number of 40, the arm-number being 64 in the former, while 58 in the latter. The remaining 4 species belonging to the genus *Cottus* have the diploid number of 48. The arm-number is 58 in *C. pollux* and *C. nozawae*, 54 in *C. hangiongensis*, and 60 in *C. reinii*. The taxonomical relationship between the 48-chromosome group and the 40-chromosome-group are discussed on the basis of the chromosome morphology.

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