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Chromosomes and DNA Values of Two Cyprinid Fishes of the Subfamily Barbinae

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The family Cyprinidae includes a number of tetraploid-origin species. These tetraploid species fall into only three of the many subfamilies of the Cyprinidae, i.e. Cyprininae, Barbinae and Schizothoracinae, and are predominantly temperate Eurasian in geographic distribution. From Southeast Asia, where barbine fishes abound, tetraploidy has been known in two species, *Tor putitora* (Khuda-Bukhsh, 1980) and *Acrossocheilus sumatranus* (Suzuki and Taki, 1981). In the course of our karyological study of Southeast Asian cyprinids, we found a tetraploid-originated karyotype in *Probarbus jullieni* Sauvage. The karyotype and relative DNA values of this species are reported here together with those of *Leptobarbus hoevenii* (Bleeker).

Materials and methods

Two specimens of *Probarbus jullieni,* 82.2 mm and 89.4 mm SL, and two specimens of *Leptobarbus hoevenii,* 95.4 mm and 149.0 mm SL, were used for chromosome observations. The smaller specimen of each species was also used for DNA value determination.

Methods of chromosome preparation were the same as described in Suzuki and Taki (1981) except for the concentration of KCl solution (0.055 M instead of 0.075 M). Classification of chromosomes followed Levan *et al.* (1964).

Erythrocytes were used for DNA value determination. A drop of blood of each species was smeared separately on the same slide and was dried for 1 hour in the air. Then the smears were fixed in 3: 1 methanol-acetic acid for 2 hours, and were hydrolized in 5 N HCl solution for 15 minutes at room temperature. The slide was washed in several changes of SO_2 water for 30 minutes after staining in Feulgen solution for 2 hours. Dehydration was done with ethanol, changing its concentration gradually from 30% to 100%, and the slide was cleared in several changes of xylene. Then it was mounted by a piece of coverglass with Bioliet. Relative DNA values were determined from measurements of the Feulgen stain density of erythrocyte nuclei with an Olympus microspectrophotometer (MMSP) by the two-wavelength method (Mendelsohn, 1958).

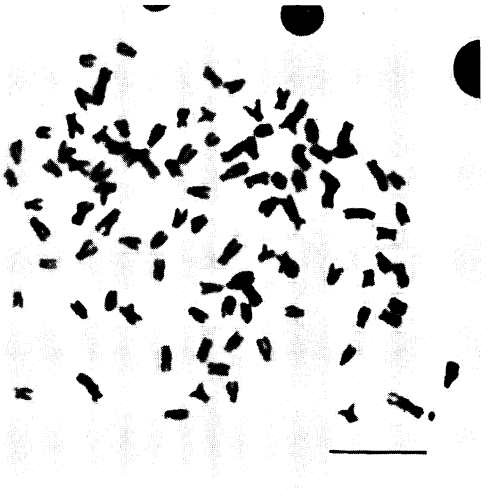

Fig. 1. Metaphase chromosomes from a kidney cell of *Probarbus jullieni*. Scale indicates 10 μm.

Results

Ten excellent metaphase plates obtained from the two specimens of *Probarbus jullieni* clearly showed 98 chromosomes (Fig. 1). The diploid complement was made up of 18 metacentric, 28 submeta- and subtelocentric, and 52 acrocentric chromosomes (Fig. 2A).

Table 1. DNA values of *Probarbus jullieni* and *Leptobarbus hoevenii.*

Species	2n	No. of cells measured	Amount of DNA (Arbitrary unit)	Standard error	Relative DNA values (Control: *L. hoevenii*)
Probarbus jullieni	98	62	0.0231	0.0029	1.686
Leptobarbus hoevenii	50	54	0.0137	0.0008	1.000

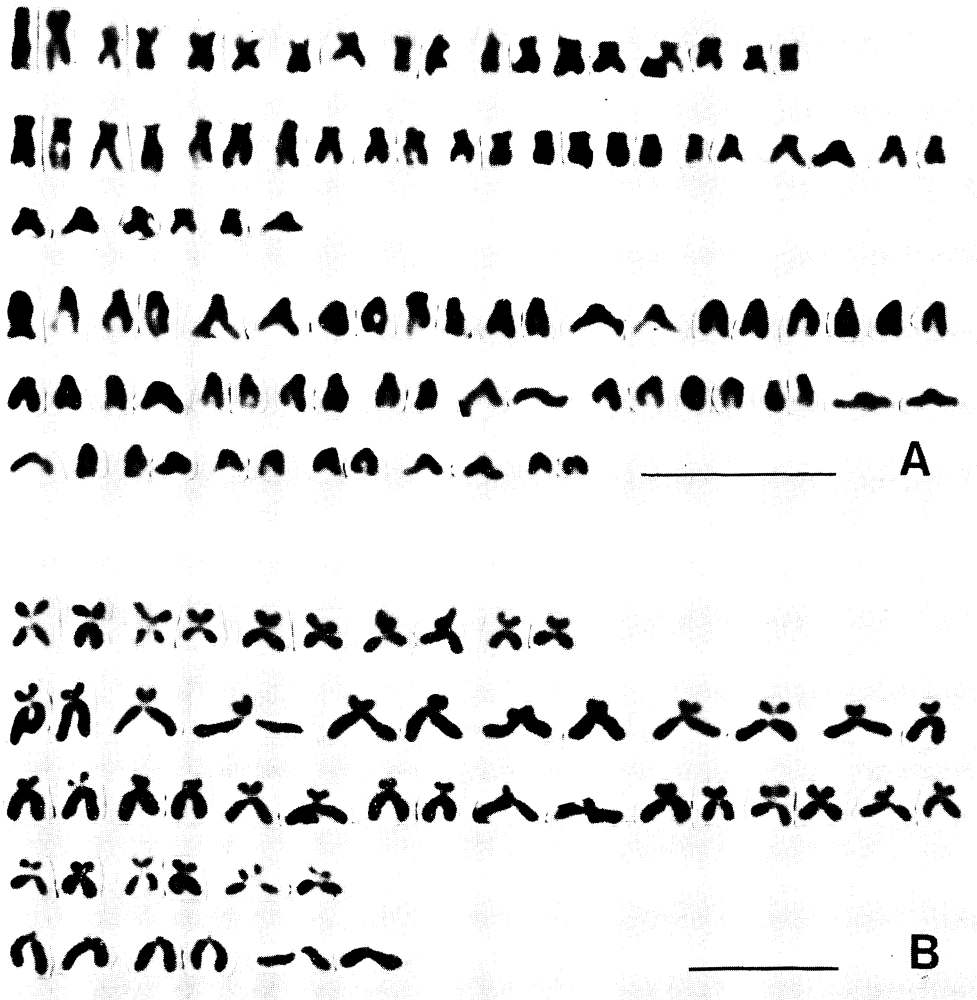

Fig. 2. Karyotype of two species of Barbinae. A, *Probarbus jullieni*, 2n=98; top row, metacentrics; second and third rows, submeta- and subtelocentrics; forth to sixth rows, acrocentrics. B, *Leptobarbus hoevenii*, 2n=50; top row, metacentrics; second to forth rows, submeta- and subtelocentrics, fifth row, acrocentrics. Scale bars indicate 10 μm.

Chromosome number of *Leptobarbus hoevenii* was determined as 2n=50, based on the observation of 10 well-spread metaphase plates from the two specimens. The karyotype consisted of 10 metacentric, 34 submeta- and subtelocentric, and 6 acrocentric chromosomes (Fig. 2B). No sex-associated heteromorphic elements were detected in both species.

The DNA value of *Probarbus jullieni* was 1.686 times as much as that of *Leptobarbus hoevenii* (Table 1).

Discussion

The karyotype of *Probarbus jullieni* comprises 98 chromosomes, in contrast to 50 chromosomes as in *Leptobarbus hoevenii* and in the majority of cyprinids, and the DNA value of the former species 1.686 times as much as that of the latter indicates a tetraploid origin of *P. jullieni*.

Including *P. jullieni* presently reported, tetraploid origins of karyotype are known in 24 cyprinid species which are classified in the sub-

Table 2. List of tetraploid species of Barbinae.

Species	2n	Distribution	References
Acrossocheilus sumatranus	98	Tropical Asia	Suzuki and Taki, 1981
A. hexagonolepis	100	Nepal	Masuda, 1985
Probarbus jullieni	98	Tropical Asia	This paper
Barbus barbus	100	Temperate Eurasia	Hafez *et al.*, 1978
B. meridionalis	100	Temperate Eurasia	Cataudella *et al.*, 1977
B. plebejis	100	Temperate Eurasia	Cataudella *et al.*, 1977
Percocypris pingi pingi	98	Temperate Eurasia	Zan *et al.*, 1984
Synocylocheilus grahami grahami	96	Temperate Eurasia	Li *et al.*, 1983
S. maculatus	96	Temperate Eurasia	Zan *et al.*, 1984
Tor khudree	100	Tropical Asia	Khuda-Bukhsh, 1982
T. putitora	100	Tropical Asia	Khuda-Bukhsh, 1980
T. tor	100	Tropical Asia	Khuda-Bukhsh, 1982
Barbodes (Spinibarbus) cardwelli	100	Temperate Eurasia	Zhou, 1984
B. (S.) denticulatus denticulatus	100	Temperate Eurasia	Zhou, 1984
Aulopyge huegeli	100	Temperate Eurasia	Berberovic *et al.*, 1973

families Cyprininae, Barbinae and Schizothoracinae. In no other cyprinid subfamilies are there known tetraploid species. Tetraploid species of the Barbinae are listed in Table 2.

As suggested by Suzuki and Taki (1981), the occurrence of tetraploidy in the Cyprininae and Barbinae may reflect close phylogenetic relationships of these two subfamilies. Further karyological investigations in the Schizothoracinae, Succeeding Khuda-Bukhsh and Nayak (1982) and Rishi *et al.* (1983), may throw light upon the elucidation of the evolutionary history of this highly specialized group of cyprinids.

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熱帯アジア産コイ科バルブス亜科 2 種の染色体と DNA 量

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東南アジア産コイ科バルブス亜科の *Probarbus jullieni* と *Leptobarbus hoevenii* の染色体と DNA 量を調査したところ，前者は 2n＝98，後者は 2n＝50 であること，DNA 量は後者を 1 とした場合，前者は 1.686 であることが判明した．これらから，*Probarbus jullieni* は 4 倍体起源の種であることが推定された．

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