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Chromosomal Studies on a Threatened Fish *Cyprinion semiplotus* (Teleostei: Cyprinidae) from Arunachal Pradesh

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Abstract

Cyprinion semiplotus (McClelland) (Fam: Cyprinidae), also known as Assamese Kingfish, is a minor carp that occurs naturally in the Hill Rivers of North East India. This species is recognized as threatened as its occurrence has declined considerably in the rivers of Arunachal Pradesh in recent times. Hence, the present study is undertaken to establish base chromosomal data for application in future conservation measures. Five live specimens were collected from a tributary of River Dikrong near Itanagar, Arunachal Pradesh. Samples were processed for chromosome preparation using a colchicine- KCl flame drying method (Khuda Bukhsh & Manna 1976). The diploid chromosome number (2n) for *C. semiplotus* was ascertained to be 50. The karyotype consisted of 12 metacentric, 8 submetacentric, 8 subtelocentric, and 22 telocentric chromosomes with a fundamental arm number (FN) 70. No sex chromosomes could be identified. This is the first report of a karyotype for *Cyprinion semiplotus*. The possible evolutionary significance of chromosome number is discussed.

Introduction

Cytogenetic studies of fish in recent years have grown in importance with regard to species characterization, evolution, and systematics. These studies are limited to approximately 10% of the total fish species known taxonomically across the world. The fish fauna in the northeastern states of India, particularly in Arunachal Pradesh, are well diversified in the Dikrong River and its tributaries. In this river system, 87 species of fish have been listed (Nath & Dey 2000). Among them, *Cyprinion semiplotus*, Cyprinidae, also known as the Assamese Kingfish, occurs in almost all rivers of the northeastern states of India. In recent years, its population has declined drastically due

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to various natural and anthropogenic stresses. A cytogenetical study on this endangered species was undertaken to determine the presence of natural genetic variation to aid future conservation programmes.

Materials and Methods

Five live specimens of *C. semiplotus* (Fig.1) were collected from a tributary of Dikrong River near Itanagar, Arunachal Pradesh, using a cast net and the fish were acclimatized in an aquarium for a few hours

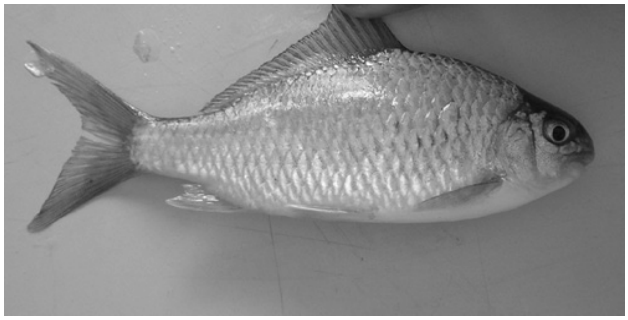

Figure 1. *Cyprinion semiplotus* (McClelland, 1839)

The sex of the specimens could not be identified, as specimens were not sexually matured. All the specimens were injected intraperitoneally with 0.05% Colchicine (1mL/ 100gm of body weight). After three hours the fish were sacrificed and the gill and kidney tissues were processed for chromosome preparation following KCl-Aceto methanol-flame drying method (Khuda Bukhsh & Manna 1976). Chromosome preparations were stained with 2% Giemsa in phosphate buffer (pH 6.8) and microscopically examined. The best metaphase spreads were identified and photographed consequently. For each fish 50 to 60 metaphase spreads were studied. Chromosome complements of three well spread metaphase spreads were measured individually and their centromeric indices and arm ratios were determined to ascribe the morphology, as suggested by Levan et al. (1964).

Results

Somatic metaphase complements contained 50 chromosomes in 52 cells out of the 60 cells studied. Thus, the diploid chromosome number in this species was ascertained to be 2n=50 (Fig.2). Size of the individual chromosomes ranged from 4.27 to 2.0 µm. The karyotype consisted of 12 metacentric, 8 submetacentric, 8 subtelocentric, and 22 telocentric chromosomes with a fundamental arm number (FN) 70. No karyotype

variation was observed within sampled specimens. No sex chromosomes were identified in the karyotype (Fig.3) because of the absence of any heteromorphic pair ((either in the form of differential staining or differential size) in unidentified sex of the specimens).

Figure 2. Metaphase complement

Figure 3. The karyotype of *Cyprinion semiplotus* consists of 2n=50

Discussion

This is the first report of karyotypic data for C*yprinion semiplotus*. Cytogenetic studies of a conspecific *Cyprinion macrostomus*, Cyprinidae, with Nucleolus Organizer Regions (NOR) were reported from Turkey (Yuksel & Gaffaroglu 2008). Diploid chromosome number for *C. macrostomus* was also 2n=50. However, the karyotype differed with 6 metacentric, 24 submetacentric, 12 subtelocentric, and 8 acrocentric chromosomes. In *C. semiplotus* more telocentric chromosomes were observed in comparison with *C. macrostomus* although the diploid chromosome number was the same. This suggests that Robertsonian rearrangements, and possibly pericentric inversions have influenced the karyotype evolution in the genus *Cyprinion.* Hence, both species possess a specific unique karyotype. Manna (1983; 1984) has studied the diploid chromosome number in most of the cyprinid species (70%) and suggested that the modal chromosome number in family Cyprinidae was 2n=50. The sex chromosomes have been detected only in a few species of fishes (Manna 1984). Khuda Bukhsh et al. (1986) observed female heterogamety (females (ZW) and male (WW)) in *Garra lamta* (Fam. Cyprinidae). Recent reviews on sex chromosome and sex determination in fish (Baroiller & D'Cotta 2001; Devlin & Nagahama 2002; Volf & Schartl 2002; Volff 2005) show various forms of genetic sex determination, which include both male heterogamety (males are XY and females are XX) and female heterogamety (males are ZZ and females are ZW). Sex determination mechanism in fish may have autosomal and polygenic influences. Recent studies on the medaka, *Oryzias latipes*, Adrianichthyidae, show a sex-determining gene, *DMY* (DM- domain gene on the Y chromosome). This gene on the Y chromosome is the master gene for male sex determination in the medaka (Matsuda 2005). Therefore, the medaka is expected to become a model species for studies on the

mechanism of sex determination in fishes. In the present study, the sex of the individuals could not be identified and in the karyotype no heteromorphic pair (either in the form of differential staining or differential size) were detected that can be defined as sex chromosomes. No explanation has been given in regards to the sex chromosome in *C. macrostomus* (Yuksel & Gaffaroglu 2008).

Conclusion

The basic karyotype for *C. semiplotus* was 2n=50, similar to that reported for *C. macrostomus* and 70% of other cyprinids. Karyotype evolution in this group of fish appears to have occurred largely through chromosome rearrangements and inversion events.

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