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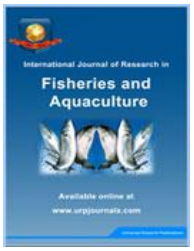

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Original Article

A cytogenetical study on *Barilius ngawa*, Vishwanath and Manojkumar, 2002 (Cypriniformes: Cyprinidae) from Northeast India, Manipur

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Abstract

Karyotypic characteristics of *Barilius ngawa* described from Manipur, northeast India reported here for the first time revealed diploid complements of 50 chromosomes. The karyotype consisted of 12 metacentric, 10 submetacentric, and 28 acrocentric chromosomes (Fundamental arm numbers, NF = 72). No heteromorphic sex chromosomes were observed in the species. The present study reports the karyotypic and cytogenetic data of bariline cyprinid fishes of northeast India thereby, enhances the existing cytotaxonomic information and chromosome evolution of Cyprinidae family in particular the genus *Barilius*.

Key words: Karyotype, *Barilius ngawa*, cytogenetic, cytotaxonomic, Manipur.

Introduction

Fishes of the genus *Barilius* Hamilton are freshwater fishes of the family Cyprinidae (Order Cypriniformes). They are generally characterized by their relatively elongate compressed body, blue-black bars or spots on the body and dorsal fin inserted behind the middle of the body [17]. Species of *Barilius* are inhabitants of small, clean, medium to fast flowing torrential mountain streams of China, western Asia, South and mainland South-east Asia. As of 2012 there are eleven species of *Barilius* in the northeast region of India out of the thirteen species known from the Eastern Himalaya region [7]. They are: *B. chatricensis* Selim and Vishwanath, *B. dogarsinghi* Hora, *B. lairokensis* Arunkumar and Tombi, *B. ngawa* Vishwanath and Manojkumar from the Chindwin drainage; *B. barila* (Hamilton), *B. barna* (Hamilton), *B. bendelisis* (Hamilton), *B. shacra* (Hamilton), *B. tileo* (Hamilton), *B. vagra* (Hamilton) from the Ganga-Brahmaputra drainage and *B. profundus* Dishma and Vishwanath from the Kolodyne drainage. Out of the eleven species four species of the genus are hitherto known their cytogenetic characteristics as shown in Table 1.

The study on fish chromosomes has received considerable attention in recent years because of their importance in classification, evolution, heredity [15], fish breeding, rapid production of inbred lines, and cytotaxonomy [23]. Basic information on the number, size, and morphology of chromosomes are needed to undertake genetic investigations such as hybridization and chromosomal manipulations in fish [19]. It also provides a complementary data source (beside the morphological methods) for more accurate and precise identification of fishes [10]. Considering the importance of chromosomal studies and lack of karyological information of fishes of northeast India led to the present investigation. The present study reports the first description of the chromosome number and karyotype of *B. ngawa*, as a contribution to the existing cytogenetic data to understand the chromosome evolution of bariline fishes which have immense ornamental potential for aquarium trade.

Materials and methods

Ten adult specimens (6 males and 4 females) of *Barilius ngawa* (Figure 1) were captured from Lokchao River (23°45´N–24°45´N latitude and 93°45´E–94°30´E longitude) of Chindwin drainage Manipur, by the local fishermen with cast nets and transported live in oxygen filled polythene bags to the laboratory. Then fishes were kept into well aerated tank of 20–25°C for acclimatization for 48 hours before experimentation. Species were identified following Viswanath *et al*., [31]. A voucher specimen was catalogued into the fish collection centre of Institute of Bioresources and Sustainable Development,

Table 1. Cytogenetic data the genus *Barilius* from northeast, India.

Species	Locality	2n	Karyotype	Sex	NF	Reference
B. bendelisis	Assam (India)	50	24m+4sm+22t		78	Khuda-Bukhsh *et al*. (1986)
B. bendelisis	Manipur (India)	50	16m+14sm+20t		80	Sanjabihari *et al*. (2013)
B. ngawa	Manipur (India)	50	12m+10sm+28t		72	Present paper
B. shacra	Assam (India)	52	22m+23sm+7t	Male XY	97	Chanda (1989)
B. tileo	Assam (India)	50	12m+32sm+6t		94	Chanda (1989)
B. vagra	Assam (India)	50	14m+26sm+10t		90	Chanda (1989)

2n: Diploid number; NF: Fundamental arm; m: Metacentric; sm: Submetacentric; t: acrocentric

Figure 1. *Barilius ngawa*

Manipur, India (IBSD FM C5).

Chromosome preparations were made from kidney as described by Manna and Prasad [25] with modification of colchicine concentration and duration of hypotonic treatment: each specimen was injected intramuscularly with 0.05% colchicine at a dose of 1 ml per 100 g of fish weight using an insulin syringe to arrest the mitotic division at the metaphase stage and kept alive in a well aerated plastic bucket. After 2 hours the specimens were sacrificed by an overdose of ethylene glycol. The kidneys were removed and placed in a hypotonic solution of 0.56% KCl. Each kidney was homogenized with a glass tissue homogenizer and treated in hypotonic solution for 45 min followed by fixation using fresh chilled fixative of methanol-acetic acid mixture (3:1 V:V). After thorough fixation, the cellular suspension was centrifuged at 1,500 rpm for 10 min. The supernatant was discarded and the cellular pellet was suspended again in the fresh fixative and washed 3 times or until a clear transparent cell suspension was obtained. One droplet of the cellular suspension was dropped on grease free, pre-cleaned glass slide from a height of 60-70 cm using pasture pipette. Immediately, the slide was swiftly passed over a flame 2-3 times and allowed to air-dry. The slides were then kept for aging in dust free place for 2-3 days before staining with 6% Giemsa solution (Sigma) in phosphate buffer of pH 6.8 for 15 minutes, wash with double distilled water and air dried. Then the slides were observed under Leica DM3000 microscope and screened for good metaphase plates. From a total of 100 mitotic spreads (50 per sex; atleast 10 per individual) exhibiting the complete chromosome number and characteristic morphology were scanned to determine the modal chromosome number. The selected metaphase spreads were photographed by Leica digital camera (DFC 310FX) coupled to the microscope under 100× oil immersion lens and images were captured using Leica Application Suite software (LAS) Version 4.0.0.

Homologous pairs of chromosomes were arranged in order of decreasing size within each morphological group and finally, karyotype was constructed on the basis of centromere position of ten best metaphases. Mean length of the short arm (p) and the long arm (q), and arm ratio (the ratio of the long arm to the short arm length) of each chromosome were calculated to classify the chromosomes as metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (t), following Levan *et al*., [24]. Fundamental number of chromosome arms (NF) was established by assigning a value of one to all t chromosomes and a value of two to all m and sm chromosomes.

Results

Analysis of 100 metaphase plates showed the frequency of diploid chromosome number ranging from 45 to 51with a modal diploid number 2n=50 which is valid over 83% (Figure 2). The representative karyotype obtained on the basis of chromosome size and centromere position (based on the long arm to short arm ratio), consisted of 12 metacentric, 10 submetacentric, and 28 acrocentric chromosomes. The fundamental number of chromosome arms (NF) was 72. The distribution of the number of chromosomes was asymmetrical with most 2n values appearing below the modal value. No morphologically different chromosomes related to sex were detected in the distribution of the number of chromosomes between male and female specimens examined. Figure 3 shows the giemsa stained standard karyotype of *Barilius ngawa*.

Discussion

Table 1 presents cytogenetic data of the bariline cyprinid fishes already studied from the northeast India. Except for *Barilius shacra* (2n = 52), the rest of the species have a chromosome diploid number of 2n = 50, which is an apparent modal diploid number of the genus *Barilius*. Cells lacking normal chromosome number (2n = 45, 47, 49, 51) were probably caused by losses during preparation or additions from nearby cells. Therefore, it can be concluded that chromosome number in this genus is conserved as in

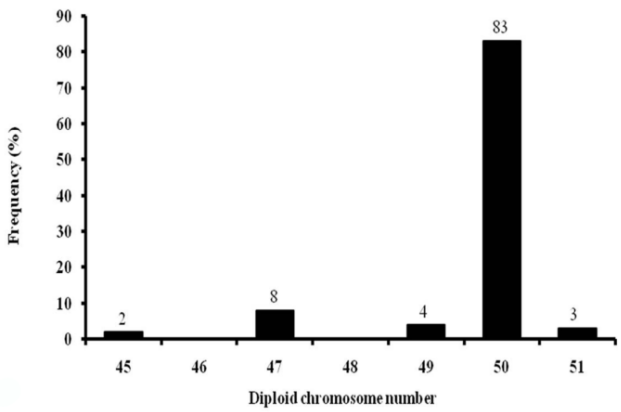

Figure 2. Frequency of diploid chromosome number recorded in 100 metaphases of *Barilius ngawa*.

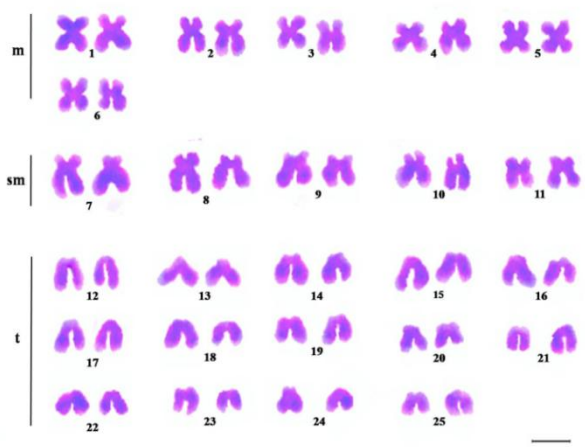

Figure 3. Giemsa stained karyotype of *Barilius ngawa* (Bar = 5µm).
m: Metacentric; sm: Submetacentric; t: acrocentric chromosomes.

other cyprinid fishes of Danioninae subfamily (e.g. *Danio rerio*, *Devario aequipinnatus*, *Rasbora rasbora*, *R. aurotaenia*, *R. daniconius*, *R. sumatrana*, *R. trilineata*, *Esomus danricus*) [3].The diploid chromosome number is rather a conservative characteristic used as an indicator of the closeness of species inter-relationships within families [26]. Thus, the conservative nature of diploid chromosome number in bariline fishes of Danioninae subfamily also suggests the monophyly of this group. Also, Danioninae subfamily shows similarity to many of the fish species of Cyprininae subfamily [6, 28, 1, 16] of different genera, such as *Chagunius*, *Cirrhinus*, *Labeo*, *Puntius*, and *Osteobrama* whose diploid numbers are 50. This finding suggests the close relationship between the two subfamily of Cyprinidae and supports the conservative nature of the chromosome number within the group. The diploid count 2n = 50, would probably be the ancestral diploid number for the family since the characteristic that occurs most frequently in a group or taxon can be considered as ancestral [8].

Though chromosome numbers of *Barilius* species are conserved despite of different geographical locations, the fundamental arm numbers (NF) are different. This divergence may be attributed to differences in the karyotype macrostructure, reflecting a real geographical variation common to widespread species [30] or may be the result of differences in the scoring of submetacentric or metacentric chromosomes in different species of *Barilius*. The difference in the fundamental arms of *Barilius* species of different geographical locations suggested the structural rearrangement in chromosome complements, as a consequence changes in chromosome morphology without change in chromosome number [29]. This inter-individual similarity in diploid chromosome number but dissimilarity in fundamental arm numbers and karyotype formula cannot be fully explained by pericentric inversion alone, though it is considered to be the main mechanism of karyotypic evolution resulting in the variations of NF within the group [14]. It is considered that species with low NF as plesiomorphic or a primitive condition and high NF as apomorphic or derived condition [27]. In view of this fact, *B. ngawa* could be considered comparatively primitive one when compared with *B. bendelisis*, *B. vagra*, *B. tileo* and *B. shacra* being the low NF value of 72 and *B. shacra* high NF value of 97 and occurrence of sex chromosome (table 1) as the more recent appearance in the evolutionary history of the bariline lineage. Karyotypes of other native *Barilius* species (*B. barila*, *B. barna*, *B. chatricensis*, *B. dogarsinghi*, *B. lairokensis* and *B. profundus* from northeast India) have not been investigated so far. As a result, chromosomal evolution of this group is not fully understood.

There is no evidence of sexual dimorphism of the chromosomes in the present species, which agrees with the reports on *B. bendelisis*, *B. tileo* and *B. vagra* except *B. shacra* (Table 1) Similarly, sex chromosomes were indistinguishable in several cyprinid fishes reported so far [21, 22, 18, 12, 10, 11, 13, 9]. Occurrence of cytologically differentiated sex chromosomes in large number of living marine fish species appears to be rare [14] although it has been described in some catfishes [2].

Considering the difficulties in identifying several of the *Barilius* species and its unclear phylogeny, cytogenetics may prove itself as an important tool in understanding the systematics of the genus. Thus, karyotype characteristics may contribute towards a better systematic interpretation, especially in the case of cryptic species, which are difficult to define [4]. The data of the present study on the chromosome composition would contribute toward clarifying the karyotypic evolution and phylogenetic relationships in this group. Further analysis, including additional species of *Barilius* of different regions and different staining techniques will provide a better understanding of the chromosome evolution in the group and confirm the apparent conservative nature of the diploid number in this bariline cyprinid fishes.

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