A note on karyotype structure in Barilius bendelisis (Hamilton, 1807) (Cypriniformes: Cyprinidae) from...

Article · July 2013

CITATION
1

READS
233

6 authors, including:

Singh C B
Institute of Bioresources and Sustainable De...
69 PUBLICATIONS **358** CITATIONS
SEE PROFILE

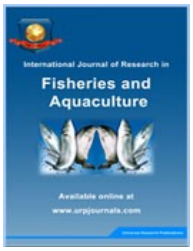
Labee Thoidingjam
Institute of Bioresources and Sustainable De...
6 PUBLICATIONS **2** CITATIONS
SEE PROFILE

Gusheinzed Waikhom
Institute of Bioresources and Sustainable De...
12 PUBLICATIONS **36** CITATIONS
SEE PROFILE

Basdeo Kushwaha
National Bureau of Fish Genetic Resources
84 PUBLICATIONS **728** CITATIONS
SEE PROFILE

Available online at http://www.urpjournals.com

International Journal of Research in Fisheries and Aquaculture



ISSN 2277-7729

Original Article

A note on karyotype structure in *Barilius bendelisis* (Hamilton, 1807) (Cypriniformes: Cyprinidae) from Northeast India, Manipur

Sanjabihari Sukham[1], Brajakishor Chingakham[1],* Labee Thoidingjam[1], Gusheinzed Waikhom[1], Ravindra Kumar[2], Basdeo Kushwaha[2]

[1]Institute of Bioresources and Sustainable Development, Imphal-795001, Manipur, India

[2]National Bureau of Fish Genetic Resources, Canal Ring Road, Lucknow-226002, U.P. India

***Corresponding author: Brajakishor Chingakham**

E-mail: kishore.ibsd@nic.in Tel.: +91-385-2446122, Fax: +91-385-2446120

Received 22 May 2013; accepted 08 June 2013

Abstract

Karyotypic characteristics of *Barilius bendelisis* from Manipur, northeast India reported here revealed diploid count of 50 chromosomes having a karyotype of 16 metacentric, 14 submetacentric and 20 acrocentric chromosomes with fundamental arm numbers, NF = 80. The results were correlated to the available cytogenetic data and analyses the karyotype variations and chromosomal evolution that occurs in the same species of different geographical locations. No sex chromosomes were observed in the species and the comparative study corroborates the hypothesis of chromosomal evolutionary process like centric inversions were responsible for variation in the karyotypic structure in the species. The present study shows that basic information of chromosome number and morphology analysis, are no longer obsolete and can be used to compare the degree of chromosomal diversity over their geographical range, providing important tools for phylogeographic, evolutionary and taxonomic status besides increasing the existing cytogenetic data of the region.

Key words: Karyotype, *Barilius bendelisis*, Cytogenetic, Taxonomic, Manipur.

Introduction

Northeast India, falling under two biodiversity hotspots namely the Eastern Himalayas and the Indo-Burma, has rich diversity of ichthyofauna with 300 species of fishes under 111 genera and 35 families [15]. However, cytogenetic characterization of fishes of this region is very scanty; only 68 species (22.6%) were characterized [26] and with the recent karyotype report of a new cat fish species, *Mystus ngasep* from this region [31] increased to 69 species (23%) so far. Nagpure *et al*., [26] also highlighted the need for intensive cytogenetic studies to bridge the gap between morphological and karyological information for many species of the region. However, studies on the chromosome of fishes have not been widespread as in other vertebrate groups. This is because fish chromosomes are characterized by a large number of small chromosomes [17] discouraging the researchers from pursuing fish karyotype analysis and difficulty in inventorying and precise identification of the species. Therefore, karyological data on fishes of this region are available for only a small percentage.

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 [14]. 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 [5]. 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 per literature.

The study on fish chromosomes has received considerable attention in recent years because of their importance in classification, evolution, heredity [12], 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 [18]. It also provides a complementary data source (beside the morphological methods) for more accurate and precise identification of fishes [7]. Considering the importance of classical cytogenetic and small number of karyological information on species of *Barilius bendelisis* of different geographical locations, led to the present study. The study aims to contribute new information about karyotypic relations, by comparing the cytogenetic data available and discuss the aspects of karyotypic evolution over their wide geographical range, providing important tools for phylogeographic, evolutionary and taxonomic studies of this species, besides increasing the existing cytogenetic data to understand the chromosome evolution of bariline fishes which have immense ornamental potential for aquarium trade.

Materials and methods

Twenty adult specimens (12 males and 8 females) of *Barilius bendelisis* (Fig. 1) were captured from Tupul River (24°28′N–25°32′N latitude and 93°10′E–93°45′E longitude) of Brahmaputra basin 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*., [34]. A voucher specimen was catalogued into the fish collection centre of Institute of Bioresources and Sustainable Development, Manipur, India (IBSD FM C8).

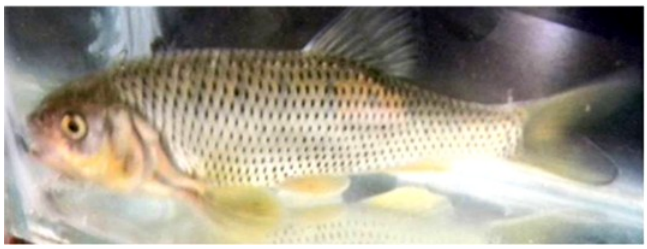


Figure 1. *Barilius bendelisis*

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 (a), following Levan *et al*., [24]. Fundamental number of chromosome arms (NF) was established by assigning a value of one to all acrocentric chromosomes and a value of two to all metacentric and submetacentric chromosomes.

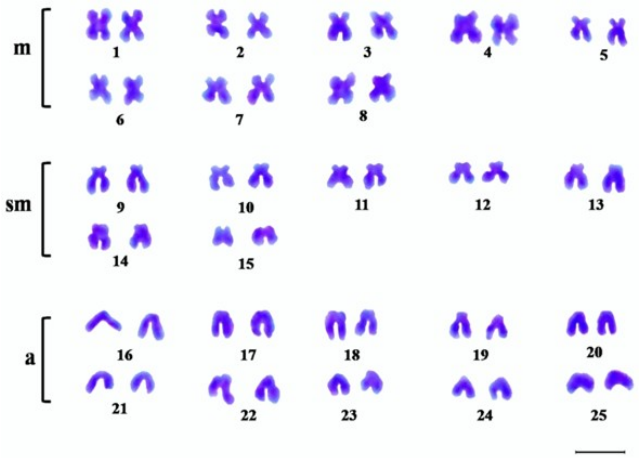


Figure 2. Giemsa stained karyotype of *Barilius ngawa* (Bar = 5µm).
m: Metacentric; sm: Submetacentric; a: acrocentric chromosomes.

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 82% (Table 1). The representative karyotype obtained on the basis of chromosome size and centromere position (based on the long arm to short arm ratio), consisted of 16 metacentric, 14 submetacentric, and 20 acrocentric chromosomes. The fundamental number of chromosome arms (NF) was 80. 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 2 shows the giemsa stained standard karyotype of *B. bendelisis*.

Discussion

As per the karyotypic data available for *B. bendelisis* from different geographical locations (Table 2), the diploid count 2n = 50 is an apparent model diploid number of the species. 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 species is conserved despite of different geographical locations as in other bariline fishes (*Barilius gatensis*, *Barilius vagra*, *Barilius naseeri*, *Barilius pakistanicus* and *B. tileo*) [3]. 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 [1, 4, 13, 28] 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 family.

Table 1. Chromosome complements of *Barilius bendelisis*

Species	No. of metaphase plates	No. of chromosomes	Percentage
B. bendelisis	100	45	2%
		47	9%
		49	4%
		50	82%
		51	3%

Table 2. Cytogenetic data of *Barilius bendelisis* from different locations.

Species	Locality	2n	Karyotype	NF	Reference
B. bendelisis	Assam (India)	50	24m+4sm+22a	78	Khuda-Bukhsh *et al*., (1986)
B. bendelisis	Bihar (India)	50	6m+6sm+10st+20a	72	Khuda-Bukhsh, (1979)
B. bendelisis	Jammu (India)	50	6m+18sm+20st+6a	94	Sharma & Tripathi , (1981)
B. bendelisis	Tamil (India)	50	-------	---	Khuda-Bukhsh *et al*., (1986)
B. bendelisis	Manipur (India)	50	16m+14sm+20a	80	Present paper

2n = Diploid number; NF = Fundamental arm; m = Metacentric; sm = Submetacentric; st = Subtelocentric; a = Acrocentric;

Though chromosome numbers of *B. bendelisis* species are conserved despite of different geographical locations, the fundamental arm numbers (NF) are different. The differences in the fundamental number of chromosome arms within the same species of *B. bendelisis* of different geographical locations suggest the structural rearrangement in chromosome complements, as a consequence changes in chromosome morphology without change in chromosome number [29]. This divergence may be attributed to differences in the karyotype macrostructure, reflecting a real geographical variation common to widespread species [33] or may be the result of differences in the scoring of submetacentric or metacentric chromosomes as different degrees of chromosome condensation, leads to differences in chromosome classification among authors [3]. This intra-individual similarity in diploid chromosome number but dissimilarity in fundamental number of chromosome arms, within the same species of *B. bendelisis* irrespective of different locations indicate that, centric inversions are the main chromosomal rearrangements which played a substantial role in the karyotypic differentiation in this species, which corroborates to *Puntius* species as suggested by Sahoo et al., [30], and it is considered to be the main mechanism of karyotypic evolution resulting in the variations of NF within the group [11]. Another characteristic observed in this species is the frequent variation of chromosome formulae from different populations as shown in Table 2 and appears that pericentric inversions represent the preponderant path of chromosome evolution in this bariline fish.

Considering the species with low NF value as plesiomorphic or a primitive condition and high NF as apomorphic or derived condition [27], *B. bendelisis* of Jammu region could be considered comparatively to be the recent appearance as the karyotype shows large number of biarmed chromosomes which might have resulted from a series of pericentric inversions, resulting in high NF value in the evolutionary history of the bariline lineage. The present species and the species from Assam and Bihar regions show the cytological closeness in having rich number of acrocentric chromosomes resulting to low FN value as highlighted (Table 2). 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 from different locations so far reported. Similarly, sex chromosomes were indistinguishable in several cyprinid fishes reported so far [6, 7, 8, 9, 10, 16, 21, 22]. Occurrence of cytologically differentiated sex chromosomes in large number of living marine fish species appears to be rare [11] although it has been described in some catfishes [2].

The data of the present study on the chromosome composition and classic cytogenetic studies involving the karyotypic characterization of other *Barilius* species, which have not been investigated so far, would contribute toward clarifying the karyotypic divergences and also testing the

validity of the hypothesis that maintaining the diploid number 2n=50, with inversion chromosomal re--arrangements is responsible for the variation in *bendelisis* species. Further analysis, including additional species of *bendelisis* of different regions and different staining techniques will provide a better understanding of the degree of chromosomal diversity in the group over their wide geographical range, providing important tools for phylogeographic, evolutionary and taxonomic status and confirm the apparent conservative nature of the diploid number in this cyprinid fish. Our results show the need for broader cytogenetic studies on *Barilius* so as to analyze and correlate chromosomal data with different approaches to clarify the existence of karyotypic diversity and chromosomal evolution.

Acknowledgements

The first author is thankful to the Director, IBSD for the facilities provided and to Department of Biotechnology, Government of India for financial assistance under DBT-Research Associateship Program in Biotechnology and Life Sciences.

References

1. Al-Sabti, K. 1991. Handbook of genotoxic effects and fish chromosome. Joseph Stephan Institute Press Ljubljana, Yugoslavia, 221 pp.
2. Alves, A.L., Oliveira, C., Nirchio, M., Granado, A. and Foresti, F. 2006. Karyotypic relationships among the tribes of Hypostominae (Siluriformes: Loricariidae) with description of XO sex chromosome system in a Neotropical fish species. Genetica. 128: 1-9.
3. Arai, R. 2011. Fish Karyotypes: A checklist. Springer Tokyo Berlin Heidelberg, New York, 340 pp.
4. Collares-Pereira, M.J. 1989. Hybridization in European Cyprinids: Evolutionary potential of Unisexual populations. In: R.M. Dawley and J.B. Bogart (Eds.), Evolution and Ecology of Unisexual Vertebrates, New York State Museum, Albany, USA: 281-288.
5. Dishma, M. and Vishwanath, W. 2012. *Barilius profundus*, a new cyprinid fish (Teleostei: Cyprinidae) from the Koladyne basin, India. Journal of Threatened Taxa. 4: 2363-2369.
6. Ergene, S. Karahan, A. and Kuru, M. 2010. Cytogenetic analysis of *Pseudophoxinus antalyae*, Bogustkaya, 1992 (Pisces: Cyprinidae) from the Eastern Mediterranean River Basin, Turkey. Turkish Journal of Zoology. 34: 111-117.
7. Esmaeili, H.R., Ebrahimi, M. and Saifali, M. 2008. Karyological analysis of five tooth- carps (Actinopterygii: Cyprinodontidae) from Iran. Micron. 39: 95-100.
8. Esmaeili, H.R., Ebrahimi, M. Ansari, T.H., Teimory, A. and Gholamhosseini, G. 2009. Karyotype analysis of Persian stone lapper, *Garra persica* Berg, 1913 (Actinopterygii: Cyprinidae) from Iran. Current Science India. 96: 959-962.
9. Esmaeili, H.R., Piravar, Z. and Shiva, A.H. 2007 Karyological Analysis of Two Endemic Tooth-Carps, *Aphanius persicus* and *Aphanius sophiae* (Pisces: Cyprinodontidae), from Southwest Iran. Turkish Journal of Zoology. 31: 69-74.
10. Esmaeili, H.R., Zareian, H., Gholamhosseini, A., Ebrahimi, M., Gholami, Z., Teimori, A. and Ansari, T.H. 2010. Karyotype Analysis of the King Nase Fish, *Chondrostoma regium* (Heckel, 1843) (Actinopterygii: Cyprinidae) from Iran. Turkish Journal of Fisheries and Aquatic Sciences. 10: 477-481.
11. Galleti, P.M., Aguilar, C.T. and Molina, W.F. 2000. An overview of marine fish cytogenetics. Hydrobiologia. 420: 55-62.
12. Gold, J.R., Li, Y.C., Shipley, N.S., and Powers, P.K. 1990. Improved methods for working with fish chromosomes with a review of metaphase chromosome banding. Journal of Fish Biology. 37: 563-575.
13. Gul, S., Colak, A., Sezgin, I. and Kaloglu, B. 2004. Karyotype analysis of *Alnurnus heckeli* (Battalgil, 1943) from Lake Hazer. Turkish Journal of Veterinary and Animal Sciences. 28: 309-314.
14. Hamilton, F. 1822. An account of the fishes found in the river Ganges and its branches. Archibald Constable and Company Edinburgh, London, 405 pp.
15. Jayaram, K.C. 2010. The Freshwater Fishes of the Indian Region. Narendra Publication Company, Delhi.
16. Kalbassi, M.R., Hosseini, S.V., and Tahergorabi, R. 2008. Karyotype Analysis in *Schizothorax zarudnyi* from Hamoon Lake, Iran. Turkish Journal of Fisheries and Aquatic Sciences. 8: 335-340.
17. Karahan, A. and Ergene, S. 2010. Cytogenetic analysis of *Garra variabilis* (Heckel, 1843)(Pisces, Cyprinidae) from Savur stream (Mardin), Turkey. Turkish Journal of Fisheries and Aquatic Sciences. 10: 483-489.
18. Khan, T.A., Bhise, M.P. and Lakra, W.S. 2000. Chromosome manipulation in fish: a review. Indian Journal of Animal Sciences. 70: 213-321.
19. Khuda-Bukhsh, A.R. (1979) Karyology of two species of hillstream fishes, *Barilius bendelesis* and *Rasbora daniconius* (fam.: Cyprinidae). Current Science. 48: 793-794.
20. Khuda-Bukhsh, A.R., Chanda, T. and Barat, A. 1986. Karyomorphology and evolution in some Indian hillstream fishes with particular reference to polyploidy in some species. In: T. Uyeno, R. Arai, T. Taniuchi and K. Matsuura (Eds.), Proceedings of the 2nd International Conference on Indo-Pacific Fishes, Ichthyological Society of Japan, Tokyo: 886-898.
21. Kilic-Demirok, N. and Ünlü, E. 2001. Karyotypes of Cyprinid Fish *Capoeta trutta* and *Capoeta capoeta umbla* (Cyprinidae) fromn the Tigris River. Turkish Journal of Zoology. 25: 389-393.
22. Kilic-Demirok, N. and Ünlü, E. 2004. Karyotype of the Cyprinid Fish *Alburnoides bipunctatus* (Cyprinidae) from the Tigris River. Folia Biologica-Krakow. 52: 57-59.
23. Kirpichnikov, V.S. 1981. Genetic Bases of Fish Selection. Springer Verlag, Berlin, Heidelberg, New York, 410 pp.

24. Levan, A., Fredga, K. and Sandberg, A.A. 1974. Nomenclature of centromeric positions on chromoso--mes. Heriditas. 52: 201-220.
25. Manna, G.K. and Prasad, R. 1968. A study of somatic chromosomes of a common Indian live-fish *Channa punctatus* by a kidney technique. Proceedings of 55th Indian Science Congress Varanasi, India, 468 pp.
26. Nagpure, N.S., Srivastava, S.K., Kushwaha, B. and Ponniah, A.G. 2000. Current status on cytogenetics of Northeast Indian fishes. In: A.G. Ponniah and U.K. Sarkar (Eds.), Fish Biodiversity of North East India NBFGR-NATP, Publication 2: 161-167.
27. Nirchio, M., Turner, B.J., Perez, J.E., Gaviria, J.I., and Cequea, H. 2002. Karyotypes of three species of toadfish (Batrachoididae: Teleostei) from Venezuela. Scientia Marina. 66: 1-4.
28. Rishi, K.K. 1989. Current status of fish cytogenetics. In: P. Das and A.G. Jhingran (Eds), Fish Genetics in India, Today and Tomorrow Printers and Publishers, New Delhi: 1-20.
29. Rishi, K.K., Shashikala, and Rishi, S. 1998. Karyotype study on six Indian hill-stream fishes. Chromosome Science. 2: 9-13.
30. Sahoo, P.K., Nanda, P. and Barat, A. 2007. Karyotypic Analysis of *Neolissocheilus hexagonolepis* (McClelland), *Puntius ticto* (Ham.) and *P. chola* (Ham.) (Family:Cyprinidae,Pisces). Cytologia. 72: 409-413.
31. Sanjabihari, S.S., Chingakham, B.S. and Gusheinzed, W. 2013. Karyotype Analysis of the New Catfish *Mystus ngasep* (Siluriformes: Bagridae) from Manipur, India. Turkish Journal of Fisheries and Aquatic Sciences.13: 179-185.
32. Sharma, O.P. and Tripathi, N.K. 1981. Somatic and meiotic chromosomes of a fresh water teleost *Barilius bendelisis* (Pisces: Cyprinidae). Chromosome Information Service. 31: 20-22.
33. Thaís, S.S., Rafael, B.N., Marta, M.C. and Roberto, F.A. 2010. A comparative study of two marine catfish (Siluriformes, Ariidae): Cytogenetic tools for determining cytotaxonomy and karyotype evolution. Micron. 41: 193-197.
34. Vishwanath, W., Lakra, W.S. and Sarkar, U.K. 2007. Fishes of North East India. National Bureau of Fish Genetic Resources, Lucknow, ICAR, 264 pp.

Source of support: Nil; Conflict of interest: None declared