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Karyotype Analysis of the New Catfish *Mystus ngasep* (Siluriformes: Bagridae) from Manipur, India

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Abstract

Karyotypic and cytogenetic characteristics of catfish *Mystus ngasep*, a new species of bagrid catfish described from the Northeast India, Manipur was studied for the first time by examining 200 metaphase spreads chromosome from the kidney cells of 25 healthy specimens. The diploid chromosome number of this species was 2n=56 and the total fundamental arm number was determined as NF=90. The karyotype consisted of 12 Metacentric (m), 22 Submetacentric (Sm), 8 Subtelocentric (St) and 14 Telocentric (t) chromosomes. The chromosome formula can be represented as 2n= 12m + 22Sm + 8St + 14t. No heteromorphic sex chromosomes were cytologically detected. Chromosome number, formula and karyotype of Mystus ngasep differentiate it from other closely related species. The largest chromosome in this species is a pair of metacentric chromosomes and the present study is the first report on the karyology of this particular species which will pave the way for the future studies on this line of karyology for this genus. Thus, this study is of importance in the analysis of karyotypic evolutionary trends, classification and taxonomy of the genus Mystus and better understanding of the karyotype diversity and chromosome evolution processes.

Keywords: Catfish, chromosome, metaphase.

Introduction

India has rich fish diversity of about 800 species (600 freshwater and the rest, brackish water) belonging to 272 genera and 71 families (Jayaram, 2010). Out of this Northeast India has as many as 300 species under 111 genera and 35 families. Above all, it has two of the world's biodiversity hotspots: the Himalaya having the highest mountain peaks of more than 8000 metres and the deepest river gorges of the world (McGinley, 2008) and the Indo-Burma constituted by the Ganga-Brahmaputra lowlands, a part of the Indo-Chinese subregion. Though Northeast India has rich diversity of ichthyofauna, knowledge of cytogenetic characterisation of fishes of this region is very scanty since, studies on the chromosome of fishes have not been successful or widespread as in other vertebrate groups. Fish karyotypes are generally characterized by a large number of small chromosomes, discouraging researchers from pursuing fish-karyotype analysis. Therefore, no karyological data on fishes of this region are available so far.

The family Bagridae is one of the most species rich and important taxon among the Siluriformes and its members are distributed throughout the world (Day, 1878). The phylogenetic relationships among the Bagridae are not very clear, especially in the genus *Mystus*. Fishes of the genus *Mystus* Scopoli are small to medium sized stripe catfishes which are economically important and distributed extensively in the two headwaters of Manipur, the Brahmaputra basin in the west and the Chindwin basin in the east. Ferraris (2007) listed 33 species of *Mystus* as valid. Now with the description of a new species *Mystus ngasep* from the headwaters of Chindwin drainage of Manipur, India by Darshan *et al*. (2011) 34 species of *Mystus* are valid. Out of this 34 species of *Mystus* only five (5) species were cytogenetically studied as shown in Table 1. It has been reported that the number of chromosomes varies between species in genus *Mystus* (Table 1). Among these, *Mystus bleekeri* is the nearest congener in terms of morphological characteristics (Darshan *et al*., 2011).

Detailed chromosome analyses of the genus *Mystus* are rare. The study on fish chromosome has received considerable attention in recent years because of their importance in classification,

evolution and heredity (Gold *et al*., 1990). Moreover cytogenetic studies of fish provide a complementary data source for more accurate and precise identification of fishes to morphology method. Considering the importance of chromosomal studies and lack of data on karyotype of fishes of Northeast, India led to the present investigation. Thus the present study gives the detailed karyotype of *Mystus ngasep* a bagrid catfish for the first time and enhances the understanding of cytotaxonomic information for the evolution of Bagridae family.

Materials and Methods

Twenty five (18 males, 7 females) mature healthy fish specimens weighing 9-20 gms of *Mystus ngasep* were collected from Iril River, Imphal East as shown in Figure 1 which lies between the latitude 24°40′N and 25°25′N and longitude 93°55′E and 94°20′E. It is one of the headwaters of the Chindwin River drainage in Manipur. Fishes were caught hiring the local fishermen with gill 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 before experimentation. Species identification was done following the diagnostic characters described by Darshan *et al*. (2011). Voucher specimens are available at the fish collection museum of Institute of Bioresources and Sustainable Development (IBSD), Manipur, India (IBSD FM C1) and in the Manipur University Museum of Fishes (MUMF 9500) as holotype.

For chromosome preparations, the specimens were treated intramuscularly with 0.05% colchicine at a dose of 1ml 100 g m^{-1} body weight using an insulin syringe to arrest the chromosomes in metaphase stage and kept alive in a well aerated plastic bucket. After 2 hours, the specimens were sacrificed, the kidney tissues were dissected out and further processed for chromosome preparations using hypotonic treatment in 0.56% KCl solution – fixation using fresh chilled Carnoy's fixative (Methanol : Acetic acid in 3:1 ratio) - flame drying technique. The chromosome slides were stained with 6% Giemsa in phosphate buffer of pH 6.8 for 15 minutes and wash with distilled water, air dried. The slides were observed under the microscope and screened for good metaphase spreads. From 200 chromosome spreads of cells exhibiting the complete somatic chromosome number ten best spreads were selected, to establish the modal chromosome number of this species. The selected spreads were photographed under 100x oil immersion lens by Leica ATC 2000 light microscope mounted with a camera.

Karyotype was prepared by arranging homologous pairs of chromosomes in order of decreasing size. For each chromosome, the average lengths of the short (p) and long arms (q) and arm ratio (the ratio of the long arm length to the short arm length of chromosomes) of each chromosome were calculated to classify the chromosomes as metacentric (m), submetacentric (sm), subtelocentric (st) and telocentric (t), as suggested by Levan *et al*. (1964). Fundamental arm number (NF) was established by

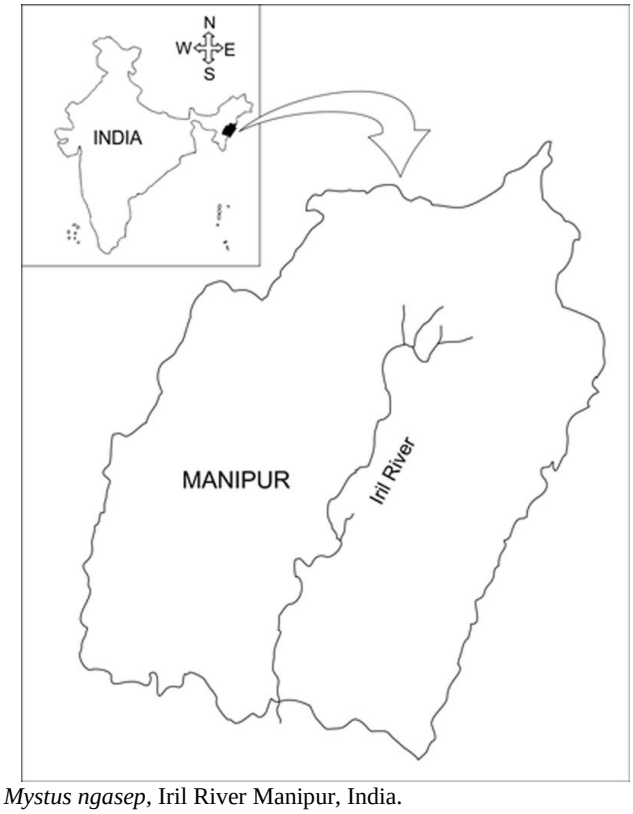

Figure 1. Collection sites of *Mystus ngasep*, Iril River Manipur, India.

assigning st and t as uniarmed while m and sm as biarmed chromosomes. The karyogram was prepared in Excel 2010 software (Microsoft).

Results

In 200 metaphases from the cells of kidney tissues of twentyfive (25) mature species of *Mystus ngasep*, (males and females) the frequency of diploid chromosome number ranged from 53 to 58 per metaphases and modal chromosome number of this species was found to be 2n=56 which is valid over 86% of metaphase cells as highlighted in Figure 2. Cells different than 2n=56 were probably caused by losses during preparation or additions from nearby cells. The investigation of metaphases showed notable difference in size and type of chromosomes in *Mystus ngasep*. The representative karyotype on the basis of centromere position is shown in Figure 3 and the karyotype formula is 2n=12m+22sm+8st+14t. The number of fundamental chromosome arms was determined NF=90. No morphologically different chromosomes related to sex were seen. The morphological and numerical data were summarized

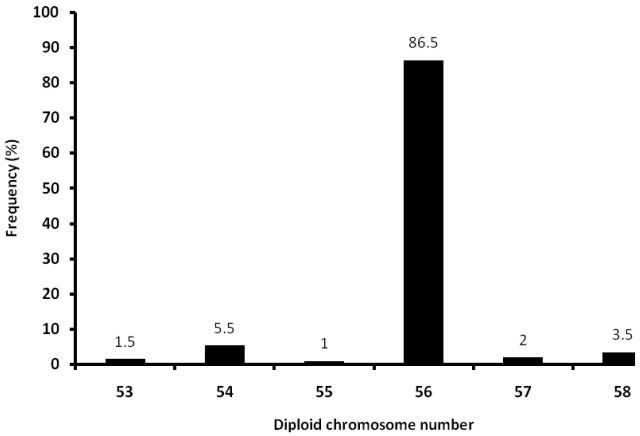

Figure 2. Frequency of diploid chromosome number recorded in 200 metaphases of *Mystus ngasep*.

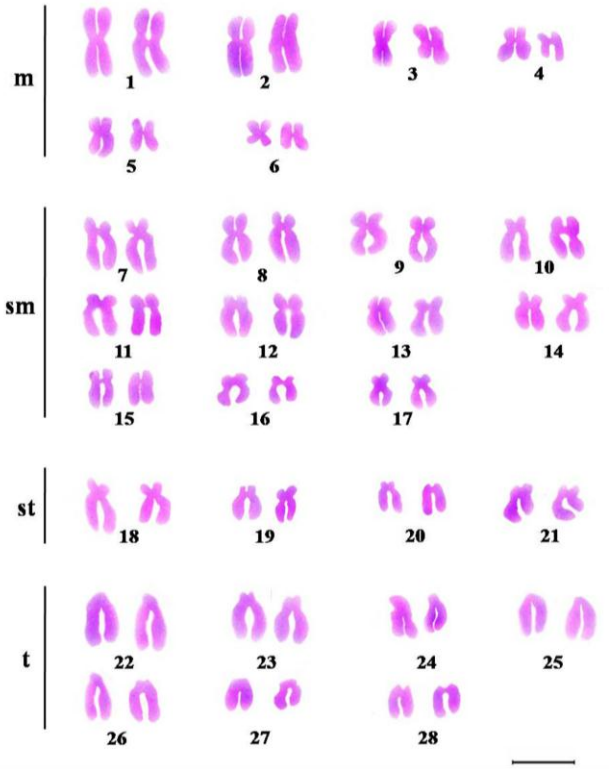

Figure 3. Karyotype of *Mystus ngasep*. Bar = 10µm.
m: Metacentric; sm: Submetacentric; st: Subtelocentric; t: Telocentric

in Table 1. Total arm length and arm ratio of the chromosomes were between 6.16-18.36 and 1.11-∞ respectively. The largest chromosome was a pair of metacentric chromosomes. The morphometry karyogram is represented in Figure 4 on the basis of short arm to long arm measurements.

Discussion

Karyotypes are descriptions of the number and morphology of chromosomes. As per the karyotypic data already available on 5 species of the genus *Mystus* the diploid chromosome number ranges from 54 to 58 (Table 2). A perusal of literature as shown in Table 2 indicates a diploid count of 2n=56±2 to be the modal number of the genus *Mystus* and it is in conformation with the reports of Sharma and Tripathi (1986) of the Bagridae family. The diploid count (2n=56) of the present species shows similarity to that of *Mystus bleekeri*, the nearest congener, from Jammu province and Cachar region, India (Sharma and Tripathi, 1986; Chanda, 1989) as shown in Table 2. The apparent modal diploid number of 2n=56±2 in the genus *Mystus* is also similar to some other fishes of Bagridae family such as, *Hemibagrus wyckii* (Ferraris, 2007) previously described as *Mystus wyckii*

Table 1. Chromosome measurements and classification of *Mystus ngasep* chromosomes

Chromosome Pair No.	Long arm (µm) Mean ± SD	Short arm (µm) Mean ± SD	Arm ratio	Total arm length (µm)	Chromosome Type
1	9.63 ± 0.48	8.63 ± 0.63	1.12	18.36	m
2	7.63 ± 0.75	6.88 ± 0.25	1.11	14.51	m
3	6.00 ± 0.00	5.25 ± 0.75	1.14	11.00	m
4	5.88 ± 0.25	4.00 ± 0.82	1.53	9.88	m
5	6.13 ± 0.14	3.75 ± 0.29	1.63	9.55	m
6	4.88 ± 0.25	4.25 ± 0.29	1.15	9.13	m
7	10.25 ± 0.29	4.13 ± 0.48	2.51	14.38	Sm
8	9.13 ± 0.85	4.63 ± 0.75	2.03	13.76	Sm
9	9.00 ± 0.41	4.38 ± 0.63	2.10	13.38	Sm
10	9.00 ± 0.41	4.00 ± 0.00	2.25	13.00	Sm
11	9.25 ± 0.65	3.63 ± 0.75	2.66	12.88	Sm
12	8.50 ± 0.41	3.88 ± 0.63	2.25	12.38	Sm
13	7.50 ± 0.41	4.00 ± 0.00	1.88	11.50	Sm
14	7.75 ± 0.96	3.63 ± 0.48	2.19	11.38	Sm
15	6.88 ± 0.63	3.13 ± 0.48	2.25	10.01	Sm
16	6.88 ± 0.85	3.00 ± 0.41	2.35	9.88	Sm
17	8.13 ± 0.85	4.13 ± 0.25	1.97	6.16	Sm
18	10.38 ± 0.95	3.50 ± 0.58	3.04	13.88	St
19	8.86 ± 0.48	3.00 ± 0.41	3.01	11.86	St
20	8.63 ± 0.25	2.88 ± 0.25	3.02	11.51	St
21	7.50 ± 0.91	2.50 ± 0.58	3.19	10.00	St
22	14.50 ± 0.41	0.00	∞	14.50	t
23	12.38 ± 0.48	0.00	∞	12.38	t
24	12.13 ± 0.63	0.00	∞	12.13	t
25	11.38 ± 0.48	0.00	∞	11.38	t
26	11.00 ± 0.41	0.00	∞	11.00	t
27	8.13 ± 0.48	0.00	∞	8.13	t
28	7.75 ± 0.65	0.00	∞	7.75	t

m: Metacentric; Sm: Submetacentric; St: Subtelocentric; t: Telocentric

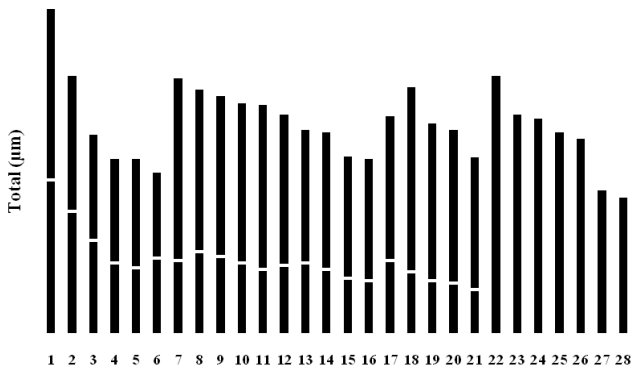

Figure 4. Haploid Karyogram of *Mystus ngasep.*

Table 1. Chromosome measurements and classification of *Mystus ngasep* chromosomes

Species	Locality	2n	NF	Karyotype formula	References
M.bleekeri	India (Jammu Province)	56	90	20m+14sm+10st+12t	Sharma and Tripathi (1986)
M.bleekeri	India (Cachar, Assam)	56	102	32m+14sm+0st+10t	Chanda (1989)
M.ngasep	India (Manipur)	56	90	12m+22sm+8st+14t	Present paper
M.cavasius	India (Jammu province)	58	92	18m+16sm+10st+14t	Sharma and Tripathi (1986)
M.cavasius	India (Bihar)	58	98	14m+26sm+4st+14t	Khuda-Bukhsh *et al*. (1980)
M.cavasius	India (Orissa)	58	98	18m+22sm+0st+18t	Tripathy and Das (1980)
M.gulio	India (West Bengal)	58	100	30m+12sm+2st+14t	Manna and Khuda-Bukhsh (1978)
M.gulio	India (Maharashtra)	58	100	30m+12sm+2st+14t	Das and khuda-Bukhsh (2007)
M.tengara	India	54	64	10m+0sm+0st+44t	Nayyar (1966)
M.tengara (Male)	India (Harayana)	54	102	10m+38sm+0st+6t	Rishi (1973)
M.tengara (Female)	India (Harayana)	54	101	9m+38sm+0st+7t	Rishi (1973)
M.vittatus	India (Jammu Province)	54	96	22m+20sm+12st+0t	Sharma and Tripathi (1986)
M.vittatus	India	54	102	22m+26sm+6st+0t	Tripathy and Das (1980)
M.vittatus (Type A)	India (West Bengal)	54	98	20m+24sm+10st+0t	Manna and Prasad (1974)
M.vittatus (Type B)	India (West Bengal)	58	84	16m+10sm+20st+12t	Manna and Prasad (1974)
M.vittatus	India	50	--	--	Srivastava and Das (1969)
M.vittatus	India	50	64	14m+0sm+0st+36t	Das and Srivastava (1973)
M.vittatus	India (Tamilnadu)	54	78	6m+18sm+0st+30t	Ramasamy *et al*. (2010)

with 2n=54 from Thailand, Nakhon Phanom Province (Wichian and Ryoichi, 1988), *Hemibagrus menoda* (Ferraris, 2007) previously described as *Mystus menoda* with 2n=56 from Cachar, India (Chanda, 1989) and *Mystus corsula* with 2n=58 from West Bengal, India (Barat and Khuda-Bukhsh, 1986). This finding suggests the close relationship between the two genera, *Mystus* and *Hemibagrus* of Bagridae family and also support the conservative nature of the karyotype macrostructure within the group, especially regarding the diploid chromosome number 2n=56, which the ancestors of all Siluriformes probably had (Oliveira and Gosztonyi, 2000).

However, *Mystus bleekeri* of Cachar region has different fundamental number, NF=102 from the one found described here, (NF=90) and also that of Jammu province. This divergence of fundamental arms may be attributed to differences in the karyotype macrostructure, reflecting a real geographical variation common to widespread species (Thaís *et al*., 2010) or may be the result of differences in the scoring of subtelocentric or telocentric chromosomes in different species of *Mystus* from India (Das and Khuda-Bukhsh, 2003). *Mystus bleekeri* of Jammu province shows the real cytological closeness with the present species having the same chromosome number and fundamental arm numbers of these two species of different geographical locations comparing with other species reported from different regions of India. The only difference observed is in karyotype formula caused by the presence of telocentric chromosomes indicating that pericentric inversions might have played a substantial role during the evolutionary pathway of these fishes. The differences in the fundamental arm numbers (NF) within the same species of *Mystus bleekeri, Mystus cavasius, Mystus gulio, Mystus tengara* and *Mystus vittatus* of different geographical locations, inspite of conserved diploid number (2n) as shown in Table 2 suggested the structural rearrangement in chromosome complements, as a consequence changes in chromosome morphology without change in chromosome number (Rishi *et al*., 1998).This intra-individual similarity in chromosome number but dissimilarity in fundamental arm numbers and karyotype formula in *Mystus* species cannot be fully explained by pericentric inversion alone. Though, pericentric inversions might have played significant role during the speciation and evolution of catfishes (Esmaeili *et al*., 2009).

A reference to the karyotypes of six *Mystus* species so far analysed and summarized in Table 2 indicates that species with large number of chromosomes *Mystus ngasep, Mystus bleekeri, Mystus cavasius, Mystus gulio* and *Mystus vittatus* (B) (2n=56 and 58) have higher number of subtelocentric and telocentric chromosomes than species of lower chromosome number (2n=54) *Mystus vittatus* and *Mystus tengara* (Kurukshetra, India) which is similar with the reports of Artoni and Bertollo (2001) in *Hypostomus* species. This observation supports the hypothesis of origin of biarmed chromosomes from acrocentric counterparts in *Mystus* species suggesting, centric fissions have occurred in the chromosome evolution of this genus (Sharma and Tripathi, 1986). Subsequently, the species *Mystus vittatus* of Jammu and West Bengal region of lowest chromosome number shows relative increase in metacentric and submetacentric chromosomes than that of higher chromosome number species *Mystus cavasius, Mystus ngasep* and *Mystus bleekeri*. The decrease in chromosome number with concurrent increase in metacentric and submetacentric number among the *Mystus* species suggested that interspecific centric fusions or Robertsonian translocations which is a well known phenomena that acrocentric chromosomes stick to each other by their centromeres to form metacentric chromosomes have occurred during chromosomal evolution in this genus, thus resulted in decrease chromosome number. According to the

published literature to date, it seems that fusion happens more frequently than fission and is widely observed in fishes (Yu and Zhou, 1996). Similar karyotypic variation in the species of other catfish genera *Clarias* and *Glyptothorax* attributed to centric fusions also have been reported (Ojo *et al*., 2011; Esmaeili *et al*., 2009). In the present study, no cytological evidence was found for sex chromosome dimorphism in the *Mystus ngasep* which agrees with the reports on other species of this genus (Khuda-Bukhsh, 1980; Ramasamy *et al*., 2010; Sharma and Tripathi, 1986) except the presence of heterogametic sex X and Y elements in *Mystus tengara* of Kurukshetra region, India by Rishi (1973) and in some catfishes (Alves *et al*., 2006).

Considering the difficulties in identifying several of the *Mystus* 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 (Artoni *et al*., 2009). Despite the apparently conserved diploid number of 2n=56±2 chromosomes composing different karyotype formulae within the genus *Mystus*, very little is known regarding the microstructural variability of these karyotypes. 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 *Mystus* and different staining techniques should provide a better understanding of the chromosome evolution in the group and confirm the apparent conservative nature of the diploid number in this fish family.

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References

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.

Artoni, R.F. and Bertollo, L.A.C. 2001. Trends in the karyotype evolution of Loricariidae fish (Siluriformes). Heriditas, 134: 201-210.

Artoni, R.F., Vicari, M.R., Almeida, M.C., Moreira-Filho, O. and Bertollo, L.A.C. 2009. Karyotype diversity and fish conservation of southern field from South Brazil. Reviews in Fish Biology and Fisheries, 19: 393-401. doi: 10.1007/s11160-009-9109-2

Barat, A. and Khuda-Bukhsh, A.R. 1986. Karyomorphological studies in two species of fishes, *Lepidocephalichthys guntea* (fam:Cobitidae) and *Mystus corsula* (fam: Bagridae). Perspectives in Cytology and Genetics, 5: 115-118.

Chanda, T. 1989. A study of chromosomes in some hill stream fishes of Assam, India. PhD thesis, Kalyani: Kalyani University.

Darshan, A., Vishwanath, W., Mahanta, P.C. and Barat, A. 2011. *Mystus ngasep*, a new catfish species (Teleostei: Bagridae) from the headwaters of Chindwin drainage in Manipur, India. Journal of Threatened Taxa, 3: 2177-2183.

Das, B. and Srivastava, M.D.L. 1973. The meiotic chromosomes of certain Indian teleosts. Proceedings of National Academy of Sciences, India 43: 29-40.

Das, J.K. and Khuda-Bukhsh, A.R. 2003. Karyotype, Ag-NOR, CMA_3 and SEM studies in a fish (*Mystus tengara*, Bagridae) with indication of female heterogamety. Indian Journal of Experimental Biology, 41: 609-613.

Day, F. 1878. Fishes of India, being a natural history of fishes known to inhabit the seas and freshwaters of India, Burma and Ceylon, William Dawson and Sons Ltd., London, 552 pp.

Esmaeili, H.R., Gholami, Z., Nazari, N., Gholamifard, A., Shahryari, F., Baghbani, S. and Ebrahimi, M. 2009. Karyotype analysis of an endemic sucker catfish, *Glyptothorax silviae* Coad, 1981 (Actinopterygii: Sisoridae), from Iran. Turkish Journal of Zoology, 33: 409-412.

Ferraris, C.J. 2007. Checklist of catfishes, recent and fossil (Osteichthyes: Siluriformes) and catalogue of siluriform primary types. Zootaxa, 1418: 1-628.

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.

Jayaram, K.C. 2010. The Freshwater Fishes of the Indian Region. Narendra Publication Company, New Delhi, 616 pp.

Khuda-Bukhsh, A.R., Gupta, S.K. and Goswami, S. 1980. Karyotypic studies in *Garra lamta* and *Mystus cavasius* (Pisces). Proceedings of Indian Academy of Sciences (Animal science), 89(6): 557-562.

Levan, A., Fredga, K. and Sandberg, A.A. 1964. Nomenclature of centromeric positions on chromosomes. Heriditas, 52: 201-220.

Manna, G.K. and Khuda-Bukhsh, A.R. 1978. Karyomorphological studies in three species of teleostean fishes. Cytologia, 43: 69-73.

Manna, G.K. and Prasad, R. 1974. Cytological evidence for two forms of *Mystus vittatus* (Bloch) as two species. Nucleus, 17: 4-8.

McGinley, M. 2008. Rwenzori Mountains National Park Uganda, In: J. Cleveland (Ed.), Encyclopedia of Earth, http://www.eoearth.org/article. (accessed May 9, 2012).

Nayyar, R.P. 1966. Karyotypic studies in thirteen species of fishes. Genetica, 37: 78-92.

Ojo, T.I., Adedapo, O.A., Adekunle, A.B., Akindayo, A.S., Adiaha, A.A.U. and Obin, A.U. 2011. Karyotypic description of six species of *Clarias* (Siluriformes: Clariidae) from South West Nigeria. International

Journal of Animal and Veterinary Advances, 3: 264-273.

Oliveira, C. and Gosztony, A.E. 2000. A cytogenetic study of *Diplomystes mesembrinus* (Teleostei, Siluriformes, Diplomystidae) with a discussion of chromosome evolution in Siluriforms. Caryologia, 53: 31-37.

Ramasamy, K., Bela, Z. and Kamatchi, B. 2010. Karyotype of a Bagrid catfish, *Mystus vittatus*, from the freshwater system of Chidambaram, Tamilnadu, India. Science Asia, 36: 157-160.

Rishi, K.K. 1973. Somatic karyotypes of three teleosts. Genen Phaenen, 16: 101-107.

Rishi, K.K., Shashikala and Rishi, S. 1998. Karyotype study on six Indian hill-stream fishes. Chromosome Science, 2: 9-13.

Sharma, O.P. and Tripathi, N.K. 1986. Karyotypic diversity in genus *Mystus* (Bagridae: Pisces). Cytologia, 51: 1-9.

Srivastava, M.D.L. and Das, B. 1969. Somatic chromosomes of teleostean fish. Journal of Heredity, 60: 57-58.

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.

Tripathy, N.K. and Das, C.C. 1980. Chromosomes in three species of Asian catfish. Copeia, 1980: 916-918.

Wichian, M. and Ryoichi, A. 1988. Karyotypes of Bagrid Catfishes, *Mystus wyckii* and *Bagroides macracanthus*, from Thailand. Bulletin of National Science Museum Tokyo, Series A, 14: 113-117.

Yu, Q. and Zhou, R. 1996. Analyses of Genetic Diversities in Chinese Fresh-water Fishes. Chinese Biodiversity, 4: 1-5.