Karyotypic Diversity Among Three Species of *Garra* (Family: Cyprinidae) from River Dikrong, Arunachal Pradesh, India

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Summary Karyotype analyses of 3 *Garra* species, *G. gotyla gotyla* (Gray), *G. kempfi* Hora and *G. lissorhynchus* (McClelland) from Arunachal Pradesh, India, are presented. All the 3 species have a diploid chromosome number of $2n=50$, but different karyotype formulae. The species-specific karyotypes were observed in the 3 species. It was also tried to draw a possible phylogenetic relationship among the 3 species.

Key words *Garra*, Cyprinidae, Karyotype, Phylogenetic relationship.

Garra, a genus belonged to family cyprinidae, consists of nearly about 34 species, among which 18 are available in India (Talwar and Jhingran 1992). They are widely distributed in Asia and Africa and adopted to swift flowing mountain streams using small sucking disc on the lower lip that enables the fish to hold fast in a strong current. Morphologically all species are very similar with minor variations which causes a great difficulties in proper identification, as for example, *G. grave-lyi*, *G. kempfi* and *G. orientalis* from the upper Irrawaddy river basin in Yunnan, South China (Zhang 2006). Hence, information on the cytogenetics related to chromosome number and karyotype of each species would help in species identification as also focus on their phylogenetic relationship. Cytogenetic studies in recent years gained a considerable importance, concerning species characterization, evolution and systematic (Barat *et al.* 2002, Gold *et al.* 1990). In the present studies, it was tried to characterize and to draw a relationship among the three species of *Garra* on the basis of their respective karyotype. This finding would help in solving the species identification.

Materials and methods

Ten live samples, (4 each for *Garra gotyla gotyla*, *G. kempfi* and 2 for *G. lissorhynchus*, respectively) were collected from River Dikrong, near Itanagar, Arunachal Pradesh. After several trials we failed to get some more samples, hence we restricted our studies on the above nos. of sample. The initial species identification was made on the basis of morphology (Talwar and Jhingran 1992, Nath and Dey 2000).

Chromosome and karyotype Analysis

All the samples were injected intramuscularly with 1 ml of 0.05% colchicine (Sigma,

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US)/100 g of body weight and kept the fishes alive for 2–3 h. with proper aeration. Gill and kidney tissues were processed for chromosome preparation following conventional KCl-acetomethanol-flame drying protocols (Khuda-Bukhsh and Barat 1987). The slides were stained with 4% Giemsa stain in phosphate buffer (pH 6.8). 50–60 well spread metaphase complements were obtained for each individual. The chromosomes of three well spread metaphase complements for each species were individually measured and their centromeric indices and arm ratios were determined in order to ascribe the morphology as suggested by Levan *et al.* (1964).

Results

The overwhelming majority of metaphase complements in the kidney and gill tissues of each species contained 50 chromosomes, though a few plates had 46 and 48 chromosomes. Therefore, the diploid number of all species under study was ascertained to be 50. The details of each chromosome pair of each species are presented in Table 1.

Garra gotyla gotyla: The diploid metaphase complements consisted of 50 chromosomes measuring between 8.02 to 3.0 μm . *G. gotyla gotyla* showed a karyotype (Fig. 1A) formula of 12 metacentric (m)+8 submetacentric (sm)+8 subtelocentric (st)+22 telocentric (T) with fundamental arm number (FN) as 70.

Garra kempfi: Revealed $2n=50$ chromosomes, with a karyotype (Fig. 1B) formula of 14M+14SM+10ST+12T and a fundamental arm number (FN) of 78. The size of the chromosomes varies between 10.37 to 5.0 μm .

Garra lissorhynchus had also diploid chromosome number as 50, but a different karyotype (Fig. 1C) of 16M+16SM+6ST+12T and a fundamental arm number (FN) of 82. The size of the chromosomes varies between 7.62 to 3.0 μm .

In each karyotype the largest chromosome pair was a submetacentric (Fig. 1). That chromosome pair may be regarded as the marker chromosomes for Genus *Garra*.

Discussion

Out of 34 species of *Garra*, *G. gotyla gotyla*, *G. lamta*, *G. mullya* from Indian water and *G. rufa* from Turkey were cytogenetically studied (Table 2). So far the authors are aware cytological work on *G. kempfi* and *G. lissorhynchus*, had not been carried out earlier.

Though the cytogenetical data on *G. gotyla gotyla* was reported earlier (Khuda-Bukhsh and Barat 1987), but the samples were collected from different geographical localities. The karyotypes were also differing significantly though diploid chromosome number was in conformity. The variations in the chromosome formula may be an inherent in nature due to geographical isolation and possibilities of population subdivision of this species may not be ruled out. This can be confirmed by using some molecular tools taking a larger sample number.

It is also observed that except the species, *G. rufa*, from Turkey all other species had a consistent diploid number of 50. All the species so far studied had also a species-specific pattern of karyotype with a unique largest submetacentric chromosome pair in all karyotypes. This chromosome pair can be defined as marker pair of this genus *Garra* and this seems to be very stable in this genus. The same pair of chromosome was also observed in the earlier reports (Khuda-Bukhsh and Barat 1987, Khuda-Bukhsh 1984, Nagpure *et al.* 2006, Gozukara and Cavas 2004).

In *G. gotyla gotyla*, more number of telocentric chromosomes were observed in comparison to other 2 species, *G. kempfi* and *G. lissorhynchus*, but diploid chromosome number is same and chromosome formula is variable. This indicates that Robertsonian rearrangements as well as pericentric inversions were the main rearrangements related with the karyotype diversification of this genus *Garra*. It was also observed from the fundamental arm number (FN), that the 2 species, *G. kempfi*

Fig. 1. A. Karyotype of *G. gotyla gotyla*, B. Karyotype of *G. kempi*, C. Karyotype of *G. lissorhynchus*.

Table 1. Comparative chromosome data of three species

SN	<i>G. gotyla gotyla</i>					<i>G. kempi</i>					<i>G. lissorhynchus</i>				
CN	SA	LA	TL	AR	CM	SA	LA	TL	AR	CM	SA	LA	TL	AR	CM
1	3.0	3.1	6.1	1.03	m	3.8	4.2	8.0	1.1	m	2.65	2.95	5.60	1.11	m
2	2.7	2.75	5.45	1.01	m	2.95	3.5	6.45	1.18	m	2.5	2.5	5.0	1.0	m
3	2.42	2.8	5.22	1.15	m	2.75	3.5	6.25	1.27	m	2.25	2.37	4.62	1.25	m
4	2.5	2.5	5.0	1.0	m	2.45	3.65	6.10	1.48	m	2.0	2.5	4.50	1.05	m
5	2.3	2.5	4.8	1.08	m	2.62	3.12	5.74	1.19	m	2.0	2.5	4.50	1.05	m
6	2.0	2.0	4.0	1.0	m	2.45	3.0	5.45	1.22	m	2.0	2.12	4.12	1.06	m
7	2.42	5.6	8.02	2.31	sm	1.95	2.2	4.15	1.12	m	2.0	2.0	4.0	1.0	m
8	1.95	4.7	6.65	2.41	sm	3.12	7.25	10.37	2.32	sm	1.87	2.0	3.87	1.06	m
9	1.57	3.75	5.32	2.38	sm	2.25	6.25	8.5	2.77	sm	2.37	5.25	7.62	2.21	sm
10	1.15	2.65	3.8	2.3	sm	2.0	4.95	6.95	2.47	sm	1.95	5.0	6.95	2.56	sm
11	0.9	3.05	3.95	3.38	st	2.0	4.12	6.12	2.06	sm	2.0	4.0	6.0	2.0	sm
12	0.75	2.95	3.7	3.93	st	2.0	4.0	6.0	2.0	sm	1.0	3.12	4.12	3.12	sm
13	0.51	2.7	3.21	5.29	st	1.95	3.87	5.82	1.98	sm	1.0	3.0	4.0	3.0	sm
14	0.45	2.2	2.65	4.88	st	1.9	3.62	5.52	1.90	sm	1.0	2.83	3.83	2.83	sm
15	0.0	4.0	4.0	∞	T	1.45	5.87	7.32	4.04	st	1.27	2.35	3.62	1.85	sm
16	0.0	4.0	4.0	∞	T	1.0	6.25	7.25	6.25	st	1.0	2.0	3.0	2.0	sm
17	0.0	3.85	3.85	∞	T	1.65	5.0	6.65	3.03	st	0.93	4.0	4.93	4.32	st
18	0.0	3.8	3.8	∞	T	0.5	5.25	5.75	10.5	st	0.98	3.88	4.86	3.97	st
19	0.0	3.8	3.8	∞	T	0.62	4.25	4.87	6.85	st	1.13	3.75	4.88	3.33	st
20	0.0	3.8	3.8	∞	T	0.0	6.25	6.25	∞	T	0.0	3.75	3.75	∞	T
21	0.0	3.72	3.72	∞	T	0.0	6.25	6.25	∞	T	0.0	3.63	3.63	∞	T
22	0.0	3.5	3.5	∞	T	0.0	5.78	5.78	∞	T	0.0	3.50	3.50	∞	T
23	0.0	3.5	3.5	∞	T	0.0	5.75	5.75	∞	T	0.0	3.25	3.25	∞	T
24	0.0	3.25	3.25	∞	T	0.0	5.0	5.0	∞	T	0.0	3.0	3.0	∞	T
25	0.0	3.0	3.0	∞	T	0.0	5.0	5.0	∞	T	0.0	3.0	3.0	∞	T

SN, Species name; CN, Chromosome no.; SA, Short arm; LA, Long arm; TL, Total arm; AR, arm ratio; CM, Chromosome morphology; m, metacentric; sm, submetacentric; st, subtelocentric; T, telocentric.

Table 2. Cytogenetical data of fishes under *Garra*

Sl. No	Species name	Locality	2n	Chromosome formula	FN	Authors
1	<i>G. gotyla gotyla</i>	Itanagar, A. P	50	12m+8sm+8st+22T	70	Present studies
2	<i>G. gotyla gotyla</i>	Ooty, TN.	50	14m+26sm+10T	90	Khuda-Bukhsh and Barat 1987
3	<i>G. gotyla gotyla</i>	Jammu, J & K	50	14m+10sm+10st+16T		Khuda-Bukhsh, 1984
4	<i>G. kempi</i>	Itanagar, A. P	50	14m+14sm+10st+12T	78	Present studies
5	<i>G. lamta</i>		50	12m+24sm+2st+12T	86	KhudaBukhsh <i>et al.</i> 1986
6	<i>G. lissorhynchus</i>	Itanagar, A. P	50	16m+16sm+6st+12T	82	Present studies
7	<i>G. mullya</i>	Chalakkudy river, Kerala	50	18m+14sm+10st+8T	82	Nagpure <i>et al.</i> 2006
8	<i>G. rufa</i>	Turkey	44	22m+20sm+2A	85	Gozukara and Cavas, 2004

and *G. lissorhynchus* are genetically closer than *G. gotyla gotyla* forming two distinct clusters, one consisted of *G. gotyla gotyla* and the other consisted of *G. kempi* and *G. lissorhynchus*.

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