Karyotype Analysis of *Channa punctata* Bloch and *Channa orientalis* Schneider with Giemsa, CMA and DAPI

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**Summary** Mitotic metaphase chromosomes of *Channa punctata* and *C. orientalis* were stained with Giemsa, CMA and DAPI. *Channa punctata* possessed 2n=32 chromosomes whereas *C. orientalis* had 2n=78 chromosomes. The range of chromosomal length of *C. punctata* and *C. orientalis* was 2.70–6.60 μm and 1.50–3.00 μm, respectively. It indicates the gradual decrease in chromosomal length in *C. punctata* than *C. orientalis*. The centromeric formulae were 24m+2sm+6t in *C. punctata* and 34m+2sm+42t in *C. orientalis*. More telocentric chromosomes are present in *C. orientalis*, thus it possesses more advanced characters than *C. punctata*. Seven CMA-positive bands were found in *C. punctata* and 12 in *C. orientalis*. The CMA-positive bands in *C. punctata* are thicker than that of *C. orientalis*. Heteromorphicity in respect of CMA-positive bands was found in some homologous pairs of both the species. It reveals the probable presence of facultative heterochromatin in the non-banded homologue members. Seven DAPI-negative bands were observed in *C. punctata* at the same position where CMA-positive bands appeared. This reversible banding indicates that those portions of chromosomes are composed of fully GC-rich repeats. In *C. orientalis*, 20 DAPI-positive bands were observed. Seven DAPI-negative bands were present at the same position where CMA-positive bands appeared. This reversible banding indicates that those portions of chromosomes are composed of fully GC-rich repeats. Three chromosomes of *C. orientalis* showed tandem presence of GC- and AT-rich repeats. These chromosomes could easily be identified and used as marker. The 2 species possessed a distinct karyotype and therefore, could be identified authentically with these methods.

**Key words** Fluorescent banding, Karyotype, *Channa*, Snakehead fish.

Species diversity of Bangladesh fish fauna is known to a greater extent but there are very few records of genetic diversity of these fishes. To conserve any species it is important to know their genetic diversity. It is well known that genetic diversity is the raw material for species diversity. According to Rajts and Akanda (2004) genetic diversity gives species the ability to adapt to changing environments, including new pests, diseases and new climatic conditions. The knowledge of genetic diversity is important for proper breeding and to conserve original gene pool for future application. The use of genetic diversity through field experimentation or in sophisticated gene transfer procedures remains arguably the best route to securing our food. Therefore, it has now become an essential task to record the genetic information of our native fishes.

There are several ways to determine the genetic make up of an individual. Karyotype analysis is the most classical 1. It includes the determination of 2n chromosome number, relative length of particular chromosome, centromeric formulae, centromeric index, total chromatin length, etc. Sometimes this conventional karyotype does not yield satisfactory result, especially during comparative study of closely related species. In that case an unambiguous identification of the individual chromosome is not possible because of their highly symmetrical morphology. Even the considera-
tion of chromosome length, arm ratio, position and number of secondary constrictions are not always sufficient to differentiate individual chromosome. The fluorescent chromosome banding technique may be helpful to solve this problem by providing additional markers to them. This technique has been in use since seventies of the last century in both plants and animals (Hilwig and Gropp 1972, Weisblum and Haenssler 1974, Schweizer 1976, Kondo and Hizume 1982, Hizume et al. 1989, Alam and Kondo 1995, Alam and Kondo 1996, Alam et al. 1995). Among the fluorochromes CMA and DAPI are used widely because they produce characteristic band on the chromosome. The chromosomes which produce these bands can be identified individually. Distribution of GC- and AT-repeats in the genome probably plays an important role in the karyotype diversification.

*Channa punctata* and *C. orientalis* are 2 native snakehead fish belonging to the family Channidae (Talwar and Jhingran 1991). These 2 species are morphologically very similar. However, they have been classified as 2 distinct species on the basis of their few morphological differences. But no genetic information was available in Bangladesh for their proper identification. Therefore, the present investigation was undertaken for the authentic identification of these 2 native fish species. The aim of the study was to i) determine the 2n chromosome number, ii) compare the karyotypes, and iii) distinguish DNA-base specific chromosome banding.

**Materials and methods**

Two snakehead fish species *viz. C. punctata* and *C. orientalis* were used in this study. These were collected from Rayer Bazar fish market of Dhaka city and reared in a well-aerated aquarium of the Zoological Garden, Department of Zoology, University of Dhaka. After sacrificing, the materials were preserved in the museum of the department as voucher specimens. Chromosome preparations were made by “Flame drying” standard methods using kidney and gill cells in the Cytogenetics Laboratory, Department of Botany, University of Dhaka. A small quantity of cell suspension was drawn out by means of a dropper. A clean slide was placed at 45° angle. A drop of the suspension was allowed falling at the upper side of the slide. It ran down the slide quickly. Then the slide was shaken by hand in order to spread the liquid properly on the slide. The slide was placed on a flame for 5–6 seconds only to remove excess fixative. The slides were then air-dried for at least 24 h before staining. Then Giemsa staining was done by using the procedure of Pandey and Lakra (1997) with slight modifications. The air-dried slides were dipped in a coplin jar with 6% Giemsa solution at pH 6.8 for an hour. The slides were then rinsed in running tap water for 20–25 seconds and air-dried for few hours. The air-dried slides were then mounted by DPX with cover slip and observed under microscope. For CMA- and DAPI-staining, the methods proposed by Alam and Kondo (1995) were followed with slight modification. Briefly, the air-dried slides were first pre-incubated in McIlvaine’s buffer (pH 7.0) for 30 min followed by distamycin A (0.1 mg/ml) treatment for 10 min in a humid chamber. The slides were then treated with CMA (0.1 mg/ml) and kept in humid chamber for 15 min. The slides were then treated again for 10 min in both McIlvaine’s buffer with MgSO<sub>4</sub> (5 mM) and McIlvaine’s buffer without Mg<sup>2+</sup>, mounted in 50% glycerol and kept overnight at 4°C. These were examined under a Nikon-fluorescent microscope with BV (blue violet) filter cassette. After CMA-staining same slides were counter-stained with DAPI. These were rinsed with distilled water followed by de-staining in 45% acetic acid for 15 min. The slides were then washed in distilled water and air-dried over night. The de-stained preparations were immersed in McIlvaine’s buffer (pH 7.0) for 20 min. The slides were treated in actinomycin D (0.25 mg/ml) for 15 min in a humid chamber. The slides were then washed in distilled water and mounted with 50% glycerol. These were observed under a Nikon-fluorescent microscope with Ultra Violet (UV) filter cassette.
Results and discussion

Giemsa stained karyotypes

*Channa punctata* and *C. orientalis* were found to possess $2n=32$ and $2n=78$ chromosomes, respectively (Figs. 1, 4). Similar results for these species were also reported by early workers (Banerjee *et al.* 1988). Thus the present finding regarding $2n$ chromosome number of these 2 species confirms the earlier reports. However, Dhar and Chatterjee (1984) reported $2n=34$ chromosomes for *C. punctata*. The probable reason for this disagreement might be due to miscounting of chromosomes, as the chromatids of telocentric chromosomes sometimes seem to be different chromosomes.

The chromosome of *C. punctata* is larger than that of *C. orientalis*. The total length of $2n$ chromosome complement of *C. punctata* and *C. orientalis* was 137.00 μm and 162.00 μm, respectively (Table 1). The range of chromosomal length in *C. punctata* was 2.70–6.60 μm and it was 1.50–3.00 μm in *C. orientalis*. The smallest chromosome (2.70 μm) of *C. punctata* was about the same size of the largest chromosome (3.00 μm) of *C. orientalis*. It indicates that *C. punctata* possesses a gradual decrease in chromosome length whereas most of the chromosomes of *C. orientalis* are homomorphic in respect of chromosome length. Gradual decrease of chromosome length reveals asymmetric karyotype. According to Stebbins (1971), the asymmetric karyotype is an advanced character. Thus *C. punctata* may be considered as having relatively advanced characters. On the other hand, the centromeric formulae 24 $m+6t$ and 34 $m+42t$ were found in *C. punctata* and *C. orientalis*, respectively (Table 1). Here 42 chromosomes (out of 78) were telocentric in *C. orientalis*, which was more than 50% of the total chromosome number. In contrast, only 6 telocentric chromosomes were found in *C. punctata*. The result indicates that *C. orientalis* possesses more asymmetric karyotype in respect of centromeric type. Stebbins (1971) also reported that presence of acrocentric and telocentric chromosomes indicates an advanced character. Therefore, in respect of centromeric type, *Channa orientalis* is much more advanced than *C. punctata*.

CMA banding

Seven CMA-positive bands were observed in each of 7 different chromosomes in *C. punctata* (Fig. 2 arrows). On the other hand, 12 CMA-positive bands appeared in 12 different chromosomes of *C. orientalis* (Fig. 5 arrows). CMA-positive bands indicate the presence of GC-rich repeats on the respective chromosomes. CMA bands appeared at the terminal region in most of the chromosomes in both the species (Fig. 2, 5 arrows). This indicates that most of the GC-rich repeats of the complement in both the species are distributed in the terminal region rather than the other part of the chromosomes. *Channa punctata* possesses 6.22% of GC-rich repeat (in 7 chromosomes) whereas 8.95% GC-rich repeats (in 12 chromosomes) present in *C. orientalis* (Table 1). The CMA bands of *C. punctata* are bigger and thicker than those of *C. orientalis*. This reveals much accumulation of GC-rich repeats in the chromosome of former species. This result reflects the percentage of GC-rich areas of 2 species (Table 1).

Heteromorphicity in respect of CMA-positive band was observed in *C. punctata* and *C. orientalis* i.e., in some cases CMA-band did not appear in both the members of a homologue (Figs. 7, 9). The reason for this heteromorphicity of CMA-positive bands was unknown. Alam and Kondo (1995) reported this kind of preferential banding in plants. They suggested that it might be due to presence of facultative heterochromatins. In this investigation, the non-banded chromosomes of the respective homologue pair may have some facultative heterochromatins and thus did not bind with CMA stains.

DAPI banding

In *C. punctata*, 7 DAPI-negative bands were observed at exactly the same position where
Figs. 1–6. Differential staining of *Channa punctata* and *C. orientalis*. 1) Giemsa stained metaphase chromosomes of *C. punctata*, 2) CMA-stained metaphase chromosomes of *C. punctata*, 3) DAPI-stained metaphase chromosomes of *C. punctata*, 4) Giemsa stained metaphase chromosomes of *C. orientalis*, 5) CMA-stained metaphase chromosomes of *C. orientalis*, 6) DAPI-stained metaphase chromosomes of *C. orientalis*, Bar=10 µm.

Table 1. Comparative Giemsa, CMA- and DAPI-karyotype analysis in *C. punctata* and *C. orientalis*

<table>
<thead>
<tr>
<th>Species</th>
<th>2n chromosomes</th>
<th>Total chromatin length (µm)</th>
<th>Giemsa karyotype</th>
<th>No. of CMA-positive bands</th>
<th>No. of CMA-negative bands</th>
<th>GC-rich portion (%)</th>
<th>No. of DAPI-positive bands</th>
<th>No. of DAPI-negative bands</th>
<th>AT-rich portion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. punctata</em></td>
<td>32</td>
<td>137.00</td>
<td>24m+2sm</td>
<td>7</td>
<td>—</td>
<td>6.22</td>
<td>—</td>
<td>7</td>
<td>—</td>
</tr>
<tr>
<td><em>C. orientalis</em></td>
<td>78</td>
<td>162.00</td>
<td>34m+2sm+42t</td>
<td>12</td>
<td>—</td>
<td>8.95</td>
<td>20</td>
<td>7</td>
<td>13.26</td>
</tr>
</tbody>
</table>

m=metacentric chromosome, sm=submetacentric chromosome, t=telocentric chromosome.
CMA-positive bands appeared (Fig. 3, 8 arrows). This reversible fluorescent banding indicates that those portions were composed of completely GC-rich repeats. No DAPI-positive bands were observed in this species. *Channa orientalis* possessed 20 DAPI-positive bands indicated the presence of AT-rich repeats on the respective chromosomes (Fig. 6 arrows). This species possessed 13.26% of AT-rich repeat (in 20 chromosomes) whereas no AT-rich repeats were found in *Channa punctata* (Table 1). In *C. orientalis*, 7 DAPI-negative bands were present at the same position where CMA-positive bands (out of 12 CMA-positive bands) were appeared (Fig. 6 arrowheads). Thus, these portions were composed of completely GC-rich repeats. Both the number of pair V and a member of pair X of *C. orientalis* showed remarkable features. These three chromosomes had terminal CMA-positive band. After counter staining, these chromosomes were looked smaller when the length compared with CMA figure (Fig. 6 big arrowheads). It is possible if DAPI-negative band appeared at the terminal region of these chromosomes, and thus they looked smaller. The reversible banding (CMA-positive and DAPI-negative) indicates that those portions were full of GC-rich repeats. Moreover, a thick and prominent DAPI-positive band appeared just beneath the DAPI-negative (CMA-positive) band of these three chromosomes (Fig. 10). It indicates the tandem presence of GC- and AT-rich repeats of these portions. These chromosomes could easily be identified and used as marker.

The 2 species possess a distinct 2n chromosome number, range of chromosomal length, centromeric formulae, CMA- and DAPI-banding pattern, percentage of GC- and AT-rich areas. Therefore, with the help of these features the karyotypes of these 2 species could easily be distinguished.
References

—, Kondo, K. and Hoshi, Y. 1995. Study on diffused centromeric nature of *Drosera* chromosomes. Cytologia 60: 43–47.