Comparative Karyotype Analysis with Differential Staining in Two Forms of *Anabas testudineus* Bloch

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**Summary**  Differential staining with Giemsa, CMA and DAPI was compared in 2 forms of *Anabas testudineus* (non-spotted and spotted form). The somatic chromosome number of these forms was determined as 2n=46. The range of chromosomal length of non-spotted form and spotted form was 1.86–5.33 μm and 1.60–5.32 μm, respectively. It indicates the gradual decrease in chromosome length of both the forms. The centromeric formulae were 14 m/32 t in non-spotted form and 6 m/40 t in spotted form. More telocentric chromosomes are present in spotted form, thus it is relatively advanced. Facultative heterochromatins were found in the spotted form, it was absent in non-spotted 1. Only one CMA-band was found in non-spotted form, whereas 4 CMA-bands present in the spotted form. Absence of CMA-positive band in a member of pair VI indicates a small deletion of GC-rich repeats in the non-spotted form. The percentage of GC-rich repeats is much more in spotted form. Neither DAPI positive nor negative band could be detected in any form. Since 2 forms possessed distinct centromeric formulae and CMA-banded karyotypes, they could be characterized authentically in these methods. The karyotypic features suggest for placing the 2 forms in at least different taxonomic variety.

**Key words**  Karyotype, Fluorescent banding, *Anabas testudineus*.

Fishes are the important sources of animal protein in Bangladesh. About 80% animal proteins come from fishes (Karim and Ahsan 1989). Due to habitat loss and various anthropogenic activities such as unwise use of pesticides, herbicides and mismanagement of industrial, municipal and household waste discharges, several species of fresh water fishes are facing different categories of threats (IUCN 2000). These lead to the destruction of favourable native fish germplasms.

To conserve any species it is important to know their genetic diversity. The knowledge of genetic diversity is important for proper breeding and to conserve original gene pool for future application. 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. Sometimes this conventional karyotype does not yield satisfactory result, especially during comparative study of closely related species. An unambiguous identification of the individual chromosome is not possible because of their highly symmetrical morphology. Even the consideration of chromosome length, arm ratio, position and number of secondary constrictions are not always sufficient to differentiate individual chromosome. In such cases, the fluorescent chromosome banding technique may be helpful by providing additional markers to them. This technique has been using since seventies of the last century (Schweizer 1976, Kondo and Hizume 1982,

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Karyotype characterization at varietal level is not an easy task, since varieties usually possess the same chromosome number and even same karyotypes. Fluorescent banding may be able to characterize the varieties. Different fluorochromes have been used in fluorescent banding.

CMA is such a fluorochrome which has GC-repetitive base specificity. This fluorochrome may play an important role to bind GC-rich repetitive sequences in the genomes. On the other hand, DAPI is specific to AT-rich repeats of the chromosomes. Therefore, it would be very important to see whether fluorescent banding is able to characterize the varieties of a species. In this experiment, 2 forms of *A. testudineus* Bloch (non-spotted and spotted) were investigated to characterize the karyotypes. *A. testudineus*, commonly known as climbing perch belonging to the family Anabantiidae. These 2 forms differ morphologically on the basis of presence or absence of spot on the body.

The aim of this research was to—

i) compare the karyotypes of 2 forms after differential staining with Giemsa, CMA and DAPI.

ii) locate the GC- and AT-rich repeats in their genomes.

iii) mark the chromosome for easy identification.

Materials and methods

The materials were collected from Polashi bazar fish market in Dhaka city. These were then reared in a well-aerated aquarium in the Department of Fisheries, University of Dhaka. Chromosome preparations were made by “Flame drying” standard methods using kidney and gill cells. Giemsa staining was done by using the procedure of Pandey and Lakra (1997) with slight modifications. The air-dried slides were dipped in a coupling jar with 6% Giemsa solution at pH 6.8 for an hour. The slides were then rinsed in running tap water for 20–25 s and air-dried for a few hours. The air-dried slides were then mounted by DPX with cover slip and observed under microscope.

For CMA banding, the method proposed by Alam and Kondo (1995) was followed with some minor modifications. After 48 h of air-drying the slides were first pre-incubated in McIlvaine’s buffer (pH 7.0) for 30 min. A drop of 0.1 mg/ml Distamycin A was added to the materials of slide and a cover glass placed on it. The slide was rinsed mildly in McIlvaine’s buffer supplemented with 5 mM MgSO₄ for 15 min. One drop of Chromomycin A₃ (0.1 mg/ml) was added to the material of slide and a clean cover glass placed on it. The slide was kept in humid chamber for 15 min. Then the slide was treated again for 10 min in McIlvaine’s buffer with Mg²⁺ and McIlvaine’s buffer without Mg²⁺. The slide was mounted in 50% glycerol and kept at 4°C for overnight before observation. This was observed under a fluorescent microscope with blue violet (BV) filter cassette. For DAPI staining, the slide was treated in 0.25 mg/ml Actinomycin D for 10 min in a humid chamber. After antibiotic treatment the slide was washed with distilled water in such a way that the cover glass is also removed. The slide was immersed again in McIlvaine’s buffer (pH 7.0) for 10 min followed by treating in DAPI solution (0.1 mg/ml) for 15 min. After rinsing in McIlvaine’s buffer (pH 7.0) for 10 min, it was mounted with 50% glycerol and observed under a fluorescent microscope with ultraviolet (UV) filter cassette.

Results and discussion

The 2 forms of *A. testudineus* were found to possess 2n = 46 chromosomes (Figs. 1, 2). The same chromosome number for this species was reported earlier (Manna and Prasad 1974). Therefore, the present findings regarding 2n chromosome number of this species confirms the earlier reports. However, Kaur and Srivastava (1965) reported 2n = 48 chromosomes for this species. The probable reason for this disagreement might be due to miscounting of chromosomes, as the chro-
matids of telomeric chromosomes sometimes seem to be different chromosomes.

The total length of $2n$ chromosome complements were 141.63 $\mu$m in non-spotted and 146.44 $\mu$m in spotted form (Table 1). It indicates that both the forms have almost similar chromatin length. The range of chromosomal length in non-spotted form was 1.86–5.33 $\mu$m and it was 1.60–5.32 $\mu$m in spotted form (Table 1). It indicates that both the forms of *A. testudineus* possess a gradual decrease in chromosome length. Gradual decrease in chromosome length reveals asymmetric karyotype.

The centromeric formula of non-spotted form was $14m/H_{11001}^{32}t$ whereas it was $6m/H_{11001}^{40}t$ in spotted form (Table 1). This result indicates a distinct centromeric formula for each form. Since both the forms possess combination of metacentric and telocentric chromosomes, indicates an asymmetric karyotype. According to Stebbins (1971) the asymmetric karyotype is an advanced character. Thus *A. testudineus* may have advanced characters. Further, Stebbins (1971) reported that presence of acrocentric or telocentric chromosomes indicates an advanced character. In this experiment, it was found that the spotted form of *A. testudineus* has more telocentric chromosomes than that of non-spotted form. Therefore, spotted form is relatively advanced in this respect.

In case of the spotted form, it was found that few metaphase chromosomes in some cells did not stain homogenously with Giemsa. Lightly and darkly stained regions were found on the respective chromosomes (Fig. 3, arrow). This kind of staining property indicates asynchronous coiling of chromatids. It reveals the presence of facultative heterochromatins. Therefore, this character might be considered as a distinguishing feature.

In the non-spotted form of *A. testudineus*, 1 CMA-positive band was observed at the terminal region in only a member of pair VI (Figs. 4, 6, arrow). The band was thick, bright, and prominent. The length of the band was 1.52 $\mu$m, which was about 1.07% of the total chromatin length. The other member of pair VI is supposed to have this kind of band. However, the other homologue did not show any such band. On the other hand, in the spotted form both the members of pair VI possessed a CMA-positive band at the terminal region (Figs. 5, 7, arrow). The length of the band was 1.52 $\mu$m which is similar to that of non-spotted form of *A. testudineus*. It indicates that a small deletion of GC-rich repeats from the terminal portion in a member of pair VI of the spotted form may

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**Table 1.** Comparative karyotype analysis of non-spotted and spotted forms of *Anabas testudineus*

<table>
<thead>
<tr>
<th>Forms</th>
<th>$2n$</th>
<th>Range of chromosomal length ($\mu$m)</th>
<th>Total length of $2n$ chromosome complements ($\mu$m)</th>
<th>Centromeric formulae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-spotted</td>
<td>46</td>
<td>1.86–5.33</td>
<td>141.63</td>
<td>$14m+32t$</td>
</tr>
<tr>
<td>Spotted</td>
<td>46</td>
<td>1.60–5.32</td>
<td>146.44</td>
<td>$6m+40t$</td>
</tr>
</tbody>
</table>

$m=$metacentric chromosome, $t=$telocentric chromosome.

**Table 2.** Comparative CMA-banding analysis of non-spotted and spotted forms of *Anabas testudineus*

<table>
<thead>
<tr>
<th>Forms</th>
<th>No. of CMA-bands</th>
<th>CMA-positive banded region</th>
<th>CMA-banded karyotype formulae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Total banded length ($\mu$m)</td>
</tr>
<tr>
<td>Non-spotted</td>
<td>1</td>
<td>—</td>
<td>1.52</td>
</tr>
<tr>
<td>Spotted</td>
<td>4</td>
<td>—</td>
<td>5.04</td>
</tr>
</tbody>
</table>

$\beta=$band in terminal region, $\varphi=$no band.
evolve the karyotype of the non-spotted form.

In addition to the above mentioned bands, the spotted form possessed another pair of band in chromosome pair IX (Figs. 5, 7, arrowhead). The percentage of GC-rich repeats (3.44%) in spotted form is much more than that of non-spotted form (1.07%) (Table 2). This reveals much accumulation of GC-rich repeats in the chromosome of former forms.

Neither DAPI positive nor negative band was observed in any form of *A. testudineus*. However, the chromosomes were fluoresced throughout the entire length (Figs. 8, 9). Absence of DAPI positive band may indicate the lack of AT-rich repeats in these forms.

In spite of certain similarities, the karyotypes of the 2 forms differed from each other. The 2 forms showed distinct centromeric formulae. Only the spotted form possessed facultative heterochromatins. Different CMA banding pattern and percentage of GC-rich repeats were found in the 2 forms. Therefore, the foregoing discussion suggests for placing the 2 forms in at least different taxonomic varieties.

References

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