Karyotypic Analysis of *Neolissocheilus hexagonolepis* (McClelland), *Puntius ticto* (Ham.) and *P. chola* (Ham.) (Family: Cyprinidae, Pisces)

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**Summary** Karyotypic study of 3 species of *Neolissocheilus hexagonolepis*, *Puntius ticto* and *P. chola* belonging to family Cyprinidae, from Arunanchal Pradesh, India, were carried out. The diploid chromosome number in *Neolissocheilus hexagonolepis* was 100 with a chromosomal formula of 32m+16sm+6st+46T and fundamental arm number (FN) as 148. The other 2 species, *Puntius ticto* and *P. chola* have 50 chromosome with chromosomal formula of 28m+16sm+6st (FN=94) and 2m+2sm+46T (FN=54), respectively. The evolutionary significance of these 3 karyotypes was discussed.

**Key words** *Neolissocheilus hexagonolepis*, *Puntius ticto*, *P. chola*, Karyotype, Evolutionary significance.

Cytogenetic studies in recent years gained a considerable importance, concerning species characterization, evolution and systematic (Gold *et al.* 1990, Barat *et al.* 2002). The cytogenetical studies in fishes are limited to just about 10% of the total fishes known taxonomically all over the world (Barat *et al.* 1996). The fish fauna in the North-Eastern States of India, particularly in Arunachal Pradesh are well diversified in the river Dikrong and its tributaries. In this river system 87 species of fishes were listed (Nath and Dey, 2000). Among them *Neolissocheilus hexagonolepis*, commonly known as chocolate mahseer, was available in almost all the rivers in North-Eastern India. In recent years as its population size has been drastically declined due to various natural and other anthropogenic stresses, it is considered as one of the endangered species (Menon 1999). Therefore, the cytogenetical study of this species is an urgent prerequisite for determining its natural genetic variation for future conservation programme.

The present paper deals with the karyomorphological analysis, of *Neolissocheilus hexagonolepis*, which has not been done so far and 2 other cyprinids (*Puntius ticto* and *P. chola*), which are reinvestigated to confirm the cytogenetical status in the North-East area of this Sub-continent.

**Materials and methods**

Fifteen live samples, (five each for *Neolissocheilus hexagonolepis*, *Puntius ticto* and *P. chola*, respectively) were collected from River Dikrong, near Itanagar, Arunachal Pradesh. The initial species identification was made on the basis of morphology (Talwar and Jhingran 1992, Nath and Dey 2000).

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Chromosome and karyotype Analysis

All the samples were injected intramuscularly with 0.05% colchicine (Sigma, US) 1 ml/100 gm. 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-aceto methanol-flame drying protocols (Khuda Bukhsh and Barat 1987). The sex of any individuals could not be detected due to their small size. The slides were stained with 4% Giemsa stain in phosphate buffer (pH 6.8). Fifty to sixty well spread metaphase complements were obtained for each individual. The chromosomes of 3 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

*Neolissocheilus hexagonolepis*: The overwhelming majority (82%) of metaphase complements in the kidney and gill tissues of *Neolissocheilus hexagonolepis* contained 100 chromosomes, though a few plates had a range within 98 to 102. The diploid metaphase complements consisted of 100 chromosomes measuring between 7.0 to 2.0 μm. *N. hexagonolepis* showed a karyotype (Fig. 1A) formula of 32 metacentric (m)+16 submetacentric (sm)+6 subtelocentric (st)+46 telocentric (T) with fundamental arm number (FN) as 148 (Table 1). The karyotype yielded no indication of sex element(s) by way of any heteromorphic chromosome pair.

*Puntius ticto*: The somatic metaphase complements contained 50 chromosomes in 52 out of 60 cells studied. Therefore, the diploid chromosome number in this species was ascertained to be 50 and the karyotype (Fig. 1B) consisted of 2n=28m+16m+6st with a fundamental arm number (FN) of 94. The size of the chromosomes varies between 5.27 to 2.4 μm. However, no sex chromosomes could be identified in this species.

*Puntius chola*: The diploid metaphase complements of unidentified sex of this species contained 50 chromosomes in 42 out of 48 cells studied, while rest had aneuploid numbers ranging within 45 to 49. The karyotype (Fig. 1C) consisted of 2n=2m+2sm+46T and a fundamental arm number (FN) of 54. The size of the chromosomes varies between 6.37 to 1.8 μm. The largest submetacentric chromosome pair (6.37 μm) could be designated as ‘Marker pair’.

Discussion

The present cytogenetical studies on this chocolate mahseer *N. hexagonolepis* may be the first report as far as the authors are aware. The cytogenetical studies on some of closely related mahseer species such as *T. khudree, T. mosal mahanadicus, Tor putitora* and *T. tor* were worked out earlier (Khuda-Bukhsh, 1980, 1982, Khuda-Bukhsh et al. 1986, Barat and Ponniah 1998). The karyotypes and the fundamental arm numbers of different mahseer species studied (Table 2) so far, revealed a species-specific pattern with same diploid chromosome number but variation in FN. This indicates that pericentric inversions at different lengths of chromosomes played a major role in the karyotypic evolution/phylogenetic relationships in this group of fishes. It is also assumed that the modal chromosomal number in the members of mahseers of upland water areas as 100. Manna (1983, 1984) studied the diploid chromosome number as 50 in most of the cyprinid species (70%) and suggested as the modal chromosome number 50 in this family. Therefore, the occurrence of 100 chromosomes in some species could arise due to polyploidization (tetraploidization) of the modal number of 50 in this family as suggested by Khuda-Bukhsh et al. (1986).

Though both *P. ticto* and *P. chola* contained 50 chromosomes in their diploid metaphase complements, the karyotype differed considerably. They had shown their species-specific karyotype pattern. The present findings are also more or less in agreement in respect of total number of biarmed
Fig. 1. A. Karyotype of *Neolissocheilus hexagonolepis*, B. Karyotype of *Puntius ticto*, C. Karyotype of *Puntius chola*.
(m and sm) and mono-armed (st-T) chromosomes, described by Taki and Suzuki (1977). Few variations were observed in both the karyotypes against Taki and Suzuki (1977), which resulted in difference in fundamental arm number. These variations may be inherent in nature indicating polymorphism, which can be verified using certain molecular tools.

Altogether 24 species of *Puntius* had been cytogenetically studied so far (Ojima 1985) of which all had $2n/H_11005$ except in *P. sophore* and *P. stigma* in which have 50 (Rishi et al. 1977, Taki and Suzuki 1977, Khuda-Bukhsh et al. 1986). Hence, the modal diploid number of this genus is therefore, likely to be 50.

The wide karyological variation among genus *Puntius*, indicates that Robertsonian rearrangements as well as pericentric inversions were the main chromosomal rearrangements, which played a key role in the karyotypic diversification. Taki and Suzuki (1977) also suggested the speciation within this group seems to have dealt with chromosomal rearrangement in some of the chromosomes, resulting in the decrease of the arm number, though the diploid number retained to 50.

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### Table 1. Karyotype data of *Neolissocheilus hexagonolepis*, *Puntius ticto* and *P. chola*

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Species name</th>
<th>Locality</th>
<th>$2n$</th>
<th>Chromosome formula</th>
<th>FN</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Neolissocheilus</em></td>
<td>Itanagar, India</td>
<td>100</td>
<td>$32m+16sm+6st+46T$</td>
<td>148</td>
<td>Present studies</td>
</tr>
<tr>
<td></td>
<td><em>hexagonolepis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>P. ticto</em></td>
<td>Itanagar, India</td>
<td>50</td>
<td>$28m+16sm+6st$</td>
<td>94</td>
<td>Present studies</td>
</tr>
<tr>
<td>3</td>
<td><em>P. ticto</em></td>
<td>Kalyani, India</td>
<td>50</td>
<td>$14m+22sm+6st+8t$</td>
<td>86</td>
<td>Manna and Prasad, 1973</td>
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<tr>
<td>4</td>
<td><em>P. ticto</em></td>
<td><em>Tokyo, Japan</em></td>
<td>50</td>
<td>$28m+22sm$</td>
<td>100</td>
<td>Taki and Suzuki 1977</td>
</tr>
<tr>
<td>5</td>
<td><em>P. chola</em></td>
<td>Itanagar, India</td>
<td>50</td>
<td>$2m+2sm+46T$</td>
<td>54</td>
<td>Present studies</td>
</tr>
<tr>
<td>6</td>
<td><em>P. chola</em></td>
<td><em>Tokyo, Japan</em></td>
<td>50</td>
<td>$2m+4sm$</td>
<td>56</td>
<td>Taki and Suzuki 1977</td>
</tr>
</tbody>
</table>

* Obtained from some aquarium fish suppliers, Tokyo, but authors explained its origin from Southeast Asia.

### Table 2. Comparative karyotypes of some Mahseers

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Species name</th>
<th>Locality</th>
<th>$2n$</th>
<th>Chromosome formula</th>
<th>FN</th>
<th>Authors</th>
</tr>
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<td>1</td>
<td><em>Neolissocheilus</em></td>
<td>Itanagar, India</td>
<td>100</td>
<td>$32m+16sm+6st+46T$</td>
<td>148</td>
<td>Present studies</td>
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<td></td>
<td><em>hexagonolepis</em></td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>2</td>
<td><em>Tor khudree</em></td>
<td><em>Tawi river, J&amp;K</em></td>
<td>100</td>
<td>$16m+28sm+6st+50A$</td>
<td>144</td>
<td>Khuda-Bukhsh, 1982</td>
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<tr>
<td>3</td>
<td><em>T. mohanadicus</em></td>
<td>Simlipal, Orissa</td>
<td>100</td>
<td>$44m+14sm−40+44A$</td>
<td>158</td>
<td>Barat, 1986</td>
</tr>
<tr>
<td>4</td>
<td><em>T. putitora</em></td>
<td>Yamuna River, U.P</td>
<td>100</td>
<td>$10m+24sm+14st+52A$</td>
<td>100</td>
<td>Khuda-Bukhsh, 1980</td>
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<tr>
<td>5</td>
<td><em>T. putitora</em></td>
<td>Kosi (Uttaranchal) Beas &amp; Sutlej (H.P)</td>
<td>100</td>
<td>$20m+24sm+18st+38T$</td>
<td>144</td>
<td>Barat and Ponniah, 1998</td>
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<tr>
<td>6</td>
<td><em>T. tor</em></td>
<td>Bhimtal Lake, Uttarakhand</td>
<td>100</td>
<td>$24m+24sm+6st+46A$</td>
<td>148</td>
<td>Khuda-Bukhsh, 1982</td>
</tr>
</tbody>
</table>
References


Ojima, Y. 1985. Fish Chromosome Data Retrieval List. Ojima Lab., Kwansei Gakuin University, Nishinomiya, Japan.

