Karyotypes of Three *Tanakia* Bitterlings (Pisces, Cyprinidae) from East Asia

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**Summary** Silver stained-NOR patterns as well as routine Giemsa stained karyotypes of 3 *Tanakia* species/subspecies from East Asia, *T. himantegus himantegus*, *T. h. chii*, and *T. somjinensis*, were described. This is the first description of karyotypes for *T. h. himantegus* and *T. somjinensis*. *Tanakia h. himantegus* (2n/H11005 = 48: 8M/H11001 = 20SM/H11001 = 18ST/H11001 = 2A) and *T. h. chii* (2n/H11005 = 48: 8M+20SM+18ST+2A) had distinct acrocentric chromosomes. In *T. somjinensis* (2n/H11005 = 48: 8M+20SM+20ST), acrocentric chromosomes were not found and Ag-NORs on the terminal region of the long arm were more prevalent than those on the terminal region of the short arm, being a characteristic that is unusual in bitterlings.

**Key words** Bitterlings, *Tanakia*, Cyprinidae, Karyotype, Ag-NORs.

The bitterling group, subfamily Acheilognathinae (Pisces, Cyprinidae) includes approximately 50 species/subspecies that are widely distributed in Eurasia, particularly in East Asia (Nakamura 1969, Holcik and Jedlicka 1994, Lin 1998, Kim and Park 2002, Chen and Chang 2005). An unusual characteristic of reproduction in the Acheilognathinae is the deposition of eggs with an ovipositor into the gills of freshwater bivalves where the larvae develop (Nakamura 1969).

Three genera were classified by Arai and Akai (1988), *i.e.*, *Acheilognathus*, *Rhodeus*, and *Tanakia*. This generic classification was supported by a molecular phylogeny using mitochondrial 12S ribosomal DNA sequences of 27 species/subspecies of Acheilognathinae (Okazaki et al. 2001). The genus *Tanakia* includes 8 species/subspecies. *Tanakia lanceolata*, *T. limbata*, and *T. tanago* are known from Japan (Nakamura 1969), *T. lanceolata*, *T. koreensis*, *T. somjinensis*, and *T. signifer* from Korea (Kim and Park 2002), *T. lanceolata* and *T. himantegus chii* from China (Arai et al. 1995, Lin 1998), and *T. himantegus himantegus* from Taiwan (Chen and Chang 2005).

As for bitterling karyotypes, 6 species/subspecies of the genus *Tanakia* analyzed to date have 2n=48 and a FN (fundamental number of arms)=76 (Ojima et al. 1973, Takai and Ojima 1986, Ueda et al. 1997, Ueda et al. 2001). *Rhodeus* species have 2n=48, FN=76 and 2n=46, FN=50, and *Acheilognathus* species have 2n=42 or 44, FN=68 to 74. The karyotype comprised of 48 chromosomes and FN=76 was assumed to be the ancestral condition in bitterlings (Ueda et al. 2001).

The number of the nucleolar organizer regions (NORs), the morphology of NOR-bearing chromosomes, and the position of the NORs on the chromosomes have frequently been observed to differ, even in closely related species with very similar karyotypes (Takai and Ojima 1986, Sola et al. 2003). Therefore, in the present report, silver stained-NOR (Ag-NOR) patterns as well as routine Giemsa-stained karyotypes of 3 *Tanakia* bitterlings from East Asia are described.

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Materials and methods

Specimens of *Tanakia himantegus himantegus*, *T. h. chii* and *T. somjinensis* were collected from Taipei-hsien in Taiwan, Shanghai in China, and Jeollabuk-do in South Korea, respectively. Air-dried chromosome slides of gastrula cells and the kidney cells of adult fishes were prepared by the direct (Ueda et al. 1991, 1997) and short-term (for 7 d at 20°C) culture (Yamamoto and Ojima 1973) methods. The cells were stained with Giemsa. The silver banding technique of Howell and Black (1980) was used for staining NORs. Chromosome classification followed that of Levan et al. (1964). As for fundamental number (FN), metacentric (M) and submetacentric (SM) chromosomes were counted as 2, and subtelocentric (ST) and acrocentric (A) chromosomes were counted as 1. Ag-NORs were classified into 3 types: Ag-NORs on the terminal region of the short arm (type A), on the terminal region of the long arm (type B), and on both terminal regions (type C).

Results

*T. h. himantegus* had 48 chromosomes (2n), consisting of 8M, 20SM, 18ST, and 2A chromosomes (Fig. 1). The FN was 76. The number and positions of Ag-NORs were variable (Table 1). The number of Ag-NOR bearing chromosomes per diploid genome ranged from 0 to 4 in the kidney cells and from 2 to 4 in the embryos; the highest number was observed in the kidney cells and the embryo cells. Ag-NORs of Types A and/or B were observed in both the kidney cells and the embryo cells. The numbers of chromosomes with Type A were 0–4 in the kidney cells and 2–4 in the embryo cells. The number of chromosomes with Type B was 0–1 in both the kidney cells and the embryo cells. Although frequencies of cells having diploid genome with Type A or Type B were not shown, the prevalent Ag-NORs on chromosome were type A.

*T. h. chii* had 48 chromosomes (2n), consisting of 8M, 20SM, 18ST, and 2A chromosomes (Fig. 2). The FN was 76. Chromosomes with Ag-NORs per diploid genome were 0–3 (2 in mode)
in the kidney cells and 1–4 in the embryo cells (Table 1). Types A and/or B were observed in both the kidney cells and the embryo cells. Chromosomes with Type A were 0–3 in the kidney cells and 0–4 in the embryo cells. Type B was 0–1 in both the kidney cells and the embryo cells. Although frequencies of cells having diploid genome with Type A or Type B were not shown, the prevalent Ag-NORs on chromosome were type A.

Table 1. Frequencies of cells bearing karyotypes with various number of Ag-NORs (0 to 6) and the numbers of chromosomes with various Ag-NOR type (A to C) per diploid genome

<table>
<thead>
<tr>
<th>Specimen no.</th>
<th>Species/subspecies</th>
<th>Tissue</th>
<th>Number of Ag-NORs</th>
<th>Type of Ag-NORs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 1 2 3 4 5 6 A</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><em>T. h. himantegus</em></td>
<td>kidney</td>
<td>2 0 18 4 1 0 0</td>
<td>0–4 0–1 0</td>
</tr>
<tr>
<td>2</td>
<td>kidney</td>
<td>0 10 0 0 0 0 0</td>
<td>2 0 0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>embryo</td>
<td>0 2 3 1 0 0 0</td>
<td>2–4 0–1 0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>embryo</td>
<td>0 0 3 0 0 0 0</td>
<td>2–3 0–1 0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><em>T. h. chii</em></td>
<td>kidney</td>
<td>1 1 12 0 0 0 0</td>
<td>1–2 0 0</td>
</tr>
<tr>
<td>6</td>
<td>kidney</td>
<td>2 1 6 4 0 0 0</td>
<td>0–3 0–1 0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>embryo</td>
<td>0 7 6 3 2 0 0</td>
<td>0–3 0–1 0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>embryo</td>
<td>0 0 2 3 1 0 0</td>
<td>2–4 0–1 0</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td><em>T. somjinensis</em></td>
<td>kidney</td>
<td>0 19 10 5 1 0 0</td>
<td>1–4 0–2 0</td>
</tr>
<tr>
<td>10</td>
<td>kidney</td>
<td>0 2 7 12 2 0 0</td>
<td>0–2 0–2 0–1</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>kidney</td>
<td>0 0 16 13 9 0 1</td>
<td>0–2 1–4 0</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>kidney</td>
<td>0 0 2 21 7 3 1</td>
<td>0–3 0–4 0</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. Karyotype of *Tanakia himantegus chii*. 2n=48: 8M, 20SM, 18ST, and 2A chromosomes. Routine Giemsa stain (upper row) and Ag stain (lower row). Arrows indicate Ag-NORs.
**Discussion**

*T. somjinensis* had 48 chromosomes (2n), consisting of 8M, 20SM, and 20ST chromosomes (Fig. 3). The FN was 76. Chromosomes with Ag-NORs per diploid genome were 1–6 (mainly 1–3) in the kidney cells (Table 1). Chromosomes with Type A, B and/or C were observed in the kidney cells. Up to 4 chromosomes were Type A, 0–4 in Type B, and 0–1 in Type C. Many cells with only Type B were recognized. Therefore, it was considered that Ag-NORs on the terminal region of the long arm (type B) were more prevalent than those on the terminal region of the short arm (type A).

**As for Ag-NORs,** *Tanakia somjinensis* differs from 2 subspecies of *Tanakia himantegus* by more numerous Ag-NORs per diploid genome in the kidney cells than *T. himantegus* (Table 1). Furthermore, in *T. somjinensis*, the Ag-NORs on the terminal region of the long arm (type B) occurred with greater frequency than those on the terminal region of the short arm (type A), which is unusual in bitterlings. A similar observation has been made in *T. koreensis* (Ueda et al. 2001), but not in other bitterlings. Though *T. somjinensis* and *T. koreensis* have been considered as different species (Kim and Kim 1991), the marked similarity between the karyotypes of 2 species was shown.
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References