Karyotype Relationships among Species of Subfamily Tetragonopterinae (Pisces, Characidae): Cytotaxonomy and Evolution Aspects

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Summary Chromosome analyses were carried out on 3 species of fish, Moenkhausia sanctaefilomenae, Moenkhausia intermedia and Hemigrammus marginatus (Tetragonopterinae) from the upper Paraná River floodplain (PR, Brazil). Species showed diploid number 2n=50 chromosomes, with different chromosome formulae. Karyotype of M. sanctaefilomenae was composed of 12M+36SM+2ST, while M. intermedia had 16M+34SM, with FN=100 for both species. Diploid numbers and chromosome formulae presented a predominance of 2-arm chromosomes, similar to that of other species of the genus. Since the chromosome formulae of H. marginatus is 10M+34SM+6A (FN=94), it differed from the previous data on this species. NOR studies for the 3 species revealed a simple system, obtained by silver nitrate staining and CMA3, whereas M. sanctaefilomenae presented strong heterochromatic blocks in the interstitial and centromeric regions of the chromosomes, heterochromatin of the other 2 species was mainly located in the NOR bearing chromosomes. Evolutionary and cytotaxonomic aspects of these genera are discussed.

Key words Chromosome evolution, Cytotaxonomy, Hemigrammus, Moenkhausia, Tetragonopterinae.

The subfamily Tetragonopterinae is one of the most numerous of the Characidae family with approximately 400–500 species distributed throughout South and Central America (Britski 1972). The group is incorrectly delimited, certainly non-monophyletic, and no extensive evaluation about its phylogenetic relationships among many genera has been undertaken (Menezes 1992). Many characid groups with numerous species, which include genera of the subfamily Tetragonopterinae, such as Astyanax, Hemigrammus, Hynessobrycon, are at present being phylogenetically restructured and reorganized within the family. In some cases they are being relocated to other Characidiform groups (Weitzmann and Malabarba 1998). Recent data have attributed some 45 species for Hemigrammus and 55 for Moenkhausia, distributed throughout several regions of South America (Froese and Pauly 2000).

Most cytogenetic studies on Tetragonopterinae focus on species of the genus Astyanax (Morelli et al. 1983, Moreira-Filho and Bertollo 1991, Salvador and Moreira-Filho 1992, Vicente et al. 1996, Mizoguchi and Martins-Santos 1998a, Moreira-Filho et al. 2001). In their revision of karyotype data on neotropical fish Oliveira et al. (1988) presented the diploid and haploid values for 91 species of Tetragonopterinae. They ranged from 2n=36 (Astyanax schubartii) to 2n=52 (Bryconamericus stramineus, Tetragonopterus chalceus, T. argenteus, Piabina argentea, Hemigrammus hynauary, Hynessobrycon flaminaeus, H. herbertaxelrodi, among others). Most chromosome data in this subfamily merely refer to haploid numbers, as may be observed in the genera Hemigrammus and Hyphessobrycon. Cytogenetic studies with descriptions of diploid number, kary-

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Since cyrogenetic data in the group are lacking, current research aims at an analysis of karyotypes of *Moenkhausia sanctaefilomenae*, *M. intermedia* and *Hemigrammus marginatus* by means of Giemsa coloration, C-banding, NOR and GC-specific fluorochrome (CMA₃). Further information on these genera and the establishment of certain karyotype relationships useful in future taxonomic reevaluations are enhanced.

Materials and methods

Eighteen specimens of *Moenkhausia sanctaefilomenae* (8 males, 10 females), 10 specimens of *M. intermedia* (2 males, 8 females) and 12 specimens of *Hemigrammus marginatus* (2 males, 10 females) were collected from the River Paraná, in the city of Porto Rico (Paraná state Brazil).

Mitotic chromosomes were obtained from kidney cells by air-drying (Bertollo et al. 1978). Nucleolus organizer regions (NOR) were identified by staining with silver nitrate, according to methodology by Howell and Black (1980). C-banding and distamycin/chromomycin A₃ staining followed the basic procedures of Sumner (1972) and Schmid (1980), respectively.

Results

All the species showed a diploid number equal to 50 chromosomes, with distinct chromosome formulae. *Moenkhausia sanctaefilomenae* had 12M+36SM+2ST, while *M. intermedia* presented 16M+34SM, with FN=100 for both species (Fig. 1). *Hemigrammus marginatus* showed 10M+34SM+6A and FN=94 (Fig. 3a).

Chromosome data of *Moenkhausia sanctaefilomenae* had been previously given, which male specimens had 1 to 2 supernumerary chromosomes in 82.82% of somatic cells (Portela-Castro et al. 2001).

Two Ag-NOR sites were detected in the chromosome complement of the 3 species. The NOR is located in the subterminal position of the short arm of a pair of submetacentric chromosomes, where a secondary constriction was frequently observed. In *M. sanctaefilomenae* and *Moenkhausia intermedia*, Ag-NOR bearing chromosomes were evidenced in pairs 13 and 12, respectively (Figs. 1, 4a, b). Fluorescent CMA₃ bands were observed coincident with these Ag-NOR sites (Fig. 4d). In *M. sanctaefilomenae* some specimens presented differences in NOR size between the 2 homologous (pair 13) seen by secondary constriction (Fig. 1a) and silver nitrate/CMA₃ staining. In *Hemigrammus marginatus*, Ag-NOR⁺/CMA₃⁺ sites are located on pair 19 (Figs. 3a, 4c, d).

With regard to C-band pattern, discernible heterochromatic blocks in *M. sanctaefilomenae* were detected in interstitial regions in the majority of the chromosomes and in centromere regions of pairs 1, 3, 4, 5, 6 (Fig. 2a). Heterochromatic blocks in *M. intermedia* were detected in the centromere regions of certain chromosome pairs (2, 3, 5, 8). However, markings in the Ag-NOR pair are salient (Fig. 2b). Constitutive heterochromatin in *H. marginatus* consists of small blocks in the centromere regions of most chromosomes (M-SM) after C-banding. Nonetheless, it is noticeable through a stronger coloration in the pericentromere regions of the acrocentric pairs 23, 24 and 25 and in the short arm of pair 19, Ag-NOR⁺, (Fig. 3b).
Fig. 1. Giemsa stained karyotypes of (a) *Moenkhausia sanctaefilomenae* and (b) *M. intermedia*. In detail, pair 13 showing a heteromorphism of secondary constrictions.

Fig. 2. Karyotype after C-banding: (a) *Moenkhausia sanctaefilomenae* and (b) *M. intermedia.*
Discussion

Diploid number $2n=50$ chromosomes in *M. intermedia*, *M. sanctaeilomenae* and *Hemigrammus marginatus* is frequently reported in many Tetragonopterinae species, chiefly in the genus *Astyanax*. Table 1 demonstrates current position with regard to karyotypic data of these genera. However, in this analysis information with merely haploid numbers has not been taken into account. Diploid number $2n=50$ chromosomes is most frequent in *Moenkhausia*, with a variable FN (88 to 100). Although several species have symmetric karyotypes, some divergences are worth mentioning, or rather, cytotypes with $2n=49$ chromosomes in *M. pittieri* and $2n=48$ in cytotype B of *M. gracilima*. These have the smallest FN values of the group (Table 1). According to Arefjev (1990) data from *M. pittieri* show heterogeneity in the genus and are evidence against a suggested conservatism in the *Moenkhausia*.

A characteristic common to the 3 species consists of the large metacentric pair. It is a characteristic shared by many characids, initially noted by Scheel (1973) and confirmed by studies on the Characidae family. The R-banding was applied to several species of this family revealed similarity of banding pattern in the marker chromosomes (Almeida-Toledo 2000). In the case of the subfamily Tetragonopterinae this pair is noticeable in species of the genus *Astyanax*, *Moenkhausia* and *Hemigrammus* and indicates an extremely conservative trait that should be taken into account in comparative analyses within a group. According to Santos (1999) cytotype B of *Moenkhausia gracilima* is different from that of other species of the genus both in the diploid number and in the absence of big metacentric chromosomes. A revision of its taxonomic position is consequently envisaged.

The cytogenetic data available so for *Hemigrammus* are few and not representative of the
Fig. 4. NOR-bearing chromosomes stained by silver nitrate (a, b, c) and by DA/CMA, (d), (a, d, pair I) *M. sanctaeofilomenae*; (b, d, pair II) *M. intermedia*; (c, d, pair III) *Hemigrammus marginatus*.

![Image of chromosomes](image)

Table 1. Chromosome characteristics of genera *Hemigrammus* and *Moenkhausia*

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>2n</th>
<th>NF</th>
<th>Chromosome Types</th>
<th>Ref.</th>
</tr>
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<tbody>
<tr>
<td><em>Hemigrammus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>H. caudovittatus</em></td>
<td>—</td>
<td>50</td>
<td>84</td>
<td>2M+32SM, ST+16A</td>
<td>1</td>
</tr>
<tr>
<td><em>H. cythrozonus</em></td>
<td>—</td>
<td>48</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td><em>H. hyamnary</em></td>
<td>—</td>
<td>52</td>
<td></td>
<td>22M−SM+30ST−A</td>
<td>3</td>
</tr>
<tr>
<td><em>H. marginatus</em></td>
<td>R. São Francisco (MG)</td>
<td>50</td>
<td>98</td>
<td>12M+36SM+2A</td>
<td>4</td>
</tr>
<tr>
<td><em>H. marginatus</em></td>
<td>R. Paraná (PR)</td>
<td>50</td>
<td>94</td>
<td>10M+34SM+6A</td>
<td>**</td>
</tr>
<tr>
<td><em>H. ocellifer</em></td>
<td>—</td>
<td>48</td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td><em>H. aff. schmardae</em></td>
<td>—</td>
<td>52</td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td><em>Moenkhausia</em></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. costae</em></td>
<td>R. São Francisco (MG)</td>
<td>50</td>
<td>100</td>
<td>50M−SM</td>
<td>7</td>
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<tr>
<td><em>M. dichroma</em></td>
<td>R. Cuiabá (MT)</td>
<td>50</td>
<td>100</td>
<td>32M+14SM+4ST</td>
<td>8</td>
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<tr>
<td><em>M. gracilina</em> (cit.A)*</td>
<td>R. Amazonas (AM)</td>
<td>50</td>
<td>96</td>
<td>14M+26SM+6ST+4A</td>
<td>9</td>
</tr>
<tr>
<td><em>M. gracilina</em> (cit.B)*</td>
<td>R. Amazonas (AM)</td>
<td>48</td>
<td>88</td>
<td>4M+24SM+12ST+8A</td>
<td>9</td>
</tr>
<tr>
<td><em>M. intermedia</em></td>
<td>R. Mogi-Guaçu (SP)</td>
<td>50</td>
<td>100</td>
<td>50M−SM (0−1B)</td>
<td>7</td>
</tr>
<tr>
<td><em>M. intermedia</em></td>
<td>R. Paraná (PR)</td>
<td>50</td>
<td>100</td>
<td>16M+34SM</td>
<td>**</td>
</tr>
<tr>
<td><em>M. pittieri</em></td>
<td>—</td>
<td>50</td>
<td>94</td>
<td>4M+40SM−ST+6A</td>
<td>3</td>
</tr>
<tr>
<td><em>M. pittieri</em></td>
<td>—</td>
<td>49</td>
<td>92</td>
<td>4M+39SM−ST+6A</td>
<td>3</td>
</tr>
<tr>
<td><em>M. sanctaeofilomenae</em></td>
<td>R. Tietê (SP)</td>
<td>50</td>
<td>98</td>
<td>48M−SM+2A</td>
<td>10</td>
</tr>
<tr>
<td><em>M. sanctaeofilomenae</em></td>
<td>R. Aquatey (Argentina)</td>
<td>50</td>
<td>98</td>
<td>48M−SM+2ST−A (1−3B)</td>
<td>11</td>
</tr>
<tr>
<td><em>M. sanctaeofilomenae</em></td>
<td>R. Paraná (PR)</td>
<td>50</td>
<td>100</td>
<td>12M+36SM+2ST (0−2B)</td>
<td>12**</td>
</tr>
</tbody>
</table>

multi-species genus. However, divergent diploid numbers (2n=48 to 2n=52) occur in this group. Karyotypes of _H. marginatus_ of the Rivers Paraná and São Francisco basin mainly differ in the number of acrocentric chromosomes (Table 1). On the other hand, C-band patterns of this species and a simple NOR system (Ag-NOR/CMA3), corroborate data by Pfister (1997). From the taxonomic point of view, the _Hemigrammus_ genus is very complex. Thus, the hypothesis that _H. marginatus_ specimens of the Rivers Paraná and São Francisco may have different identities should not be discarded.

NOR detection by silver and GC-specific fluorochrome CMA3 impregnation denotes simple NOR system in the 2 species of _Moenkhausia_. Previous studies indicate 2 NOR-bearing chromosomes in the karyotype of _M. intermedia_ (Portela et al. 1988) and _M. costae_ (Portela et al. 1988, Pfister 1997). On the other hand, the occurrence of a single NOR pair in _M. sanctaefilomenae_ of the River Paraná contrasts with multiple NORs recorded in the population studied by Foresti et al. (1989). These authors have detected 2 NORs in a SM pair with a size difference between homologues and more than 1 to 6 silver-marked chromosomes. Divergence in NORs number in populations of _M. sanctaefilomenae_ (River Paraná and Tietê basin) may be related to a differential activity in the genomic expression of ribosomal cistrons, although the occurrence of distinct Ag-NOR standards among the same populations should not be discarded. NORs polymorphisms frequently occur in neotropical fish species (Foresti et al. 1981, Galetti Jr. 1998) with a well-documented example in _Astyanax scabripinnis_. They show extensive variations of intra- and inter-populational NORs ranging from a nuleolus pair (Souza et al. 1996) up to 15 nucleolus chromosomes (Rocon-Stange and Almeida-Toledo 1993, Mizoguchi and Martins-Santos 1998b, Marco-Ferro et al. 2001).

The 2 _Moenkhausia_ species were distinct in C-banding. Small heterochromatic blocks in _M. intermedia_ were restricted to centromere regions in most chromosomes, even though a heterochromatic block on the Ag-NOR correspondent pair is noticeable. This fact has been shown by Pfister (1997) to be the case in _M. costae_ too, with a karyotype similar to that of _M. intermedia_.

Heterochromatic blocks in _M. sanctaefilomenae_, present in the interstitial regions of most chromosomes, corroborate the standard reported in the population studied by Foresti et al. (1989). Although _M. intermedia, M. costae_ and _M. sanctaefilomenae_ are similar in their karyotypic macrostructures, heterochromatin organization sharply distinguishes _M. sanctaefilomenae_. This might mean that an important evolutionary diversification within the genus has occurred.

From the morphological point of view _Moenkhausia_ is greatly diversified and does not seem to be a taxonomic unit. According to classification criteria for the species of this genus, 3 groups, based on height/length relationship and number of scales, over and below the lateral line (Eigenmann 1917, Géry 1977), may be forwarded. Groups are labeled “lepidura”, “grandisquamis” and “chrysargyreus”. The species _M. intermedia, M. gracilima_ and _M. dichrous_ were included in the lepidura group; _M. sanctaefilomenae_ and _M. costae_ belong to the grandisquamis group, whereas _M. pittiari_ pertains to the chrysargyreus one. From the cytogenetic point of view data have shown great similarities in number and in chromosome formulae among _M. intermedia, M. costae, M. sanctaefilomenae_ and _M. dichrous_ whose karyotypes are more symmetric. However, _M. pittiari_ and _M. gracilima_ diverged from the others, mainly in acrocentric chromosomes. Data show that cytogenetic studies in _Moenkhausia_ indicate different trends in the karyotype diversification during species processing. It should be emphasized, however, that a greater number of species should be analyzed according to their karyotypes and taxonomic revisions must be undertaken so that the interrelationships in this group could be established.

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References


