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Occurrence of Intersexuality in "Lambaris", *Astyanax scabripinnis* (Jenyns, 1842), Small Characids from the Brazilian Streams

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ABSTRACT

During studies carried out with a small characid (Astyanax scabripinnis), from the Brazilian streams located in Cerrado biome, some gonads of fishes exhibited sparse oocytes in perinucleolar stage embedded in normally developing testicular tissues. Another pattern of gonad intersex with vitellogenic oocytes and mature lobules was observed in the gonads of fishes collected from a reservoir supplied by the Atlantic Forest stream. These gonads in different stages of maturation were examined histologically. The results revealed the patterns occurring in fishes of the same species, from four different populations.

Key Words: Intersexuality, Neotropical ichthyofauna, Characidae, Tetras, Stream fishes

INTRODUCTION

Among the Characiformes, the freshwater fishes of the family Characidae and its subfamily Tetragonopterinae, the so-called South American tetras (Nelson, 1994), have the largest richness of species. This is a paraphyletic group predominating in the streams of several Brazilian hydrographic basins (Buckup, 1998). Among the Tetragonopterinae, *Astyanax scabripinnis* (Jenyns, 1842), known as "lambari" is frequently found in the small streams (Castro and Casatti, 1997), particularly in the headwaters (Moreira Filho and Bertollo, 1991). Several studies on this species have been performed for geographically isolated populations from small tributaries located in mountain regions of two major Brazilian biomes: the "Cerrado" (savannah type formation) and the Atlantic Forest (Britski et al., 1988; Moreira Filho and Bertollo, 1991; Barbieri, 1992; Néo et al., 2000; Veregue and Orsi, 2003). Based on the morphological and kariotypic characteristics, Moreira-Filho and Bertollo (1991) proposed that these *A. scabripinnis* populations might constitute a species complex.

In studies carried out on *A. scabripinnis* populations by Fenerich-Verani et al. (1997); Ferro et al. (2001), Ferro et al. (2003), Fragoso (2000), Sá et al. (2000) and Sá (2000), intersex gonads have been repeatedly found. Chan and Yeung (1983) also reported this, whereby animals possessed both the male and female gonadal

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tissues, considering it an abnormal occurrence in gonochoristic species of the fishes. In fact, intersexuality is very infrequently observed under natural or normal breeding conditions and, as might be expected, according to Quillet et al. (2004), very little is known about the physiological mechanisms of gonad differentiation in spontaneous intersex individuals recorded for gonochoristic species.

Although not usual, except for undifferentiated species (Yamamoto, 1969), several studies have already reported the occurrence of intersexuality in a few individual from gonochoristic fishes: *Cyprinus carpio* (Gupta and Meske, 1976), *Rutilus rutilis* (Jafri and Ensor, 1979; Lukšienė et al., 2000; Nolan et al., 2001), *Thymallus thymallus* (Blachuta et al., 1991), *Salvelinus alpinus* (Fraser, 1997), *Acipenser oxyrinchus* (Van Eenennaam and Dorosov, 1998), *Esox lucius* (Lukšienė et al., 2000), and *Oncorhynchus mykiss* (Quillet et al., 2004). Except for *O. mykiss*, which presented an overall frequency of 5.4%, the frequency of occurrence was usually low, varying from 0.1 to 0.6%.

Nevertheless, some studies have reported intersex percentage values higher than 10 % in wild populations of *Esox lucius* (Vine et al., 2005), *Gasterosteus aculeatus* and *Perca fluviatilis* (Gerchen and Sordyl, 2002), and *Xiphias gladius* (DeMetrio, 2003). In the present paper, the occurrence of intersexuality is reported for some *Astyanax scabripinnis* individuals collected in wild populations from streams in Minas Gerais State and in a reservoir in São Paulo State, Southeastern region of Brazil.

MATERIALS AND METHODS

Specimens of three populations of *A. scabripinnis*, were sampled from three streams situated in the savannah region of the adjoining states of Minas Gerais and São Paulo, as well as in a reservoir receiving headwaters from some highlands in the Atlantic Forest mountain region of São Paulo State. Fishes were collected using a trawl net (4.20m x 0.95m) with 1mm mesh in the "Curral das Éguas" stream (18°07'11''S and 42°25'09''W), located in Minas Gerais State in the Central region of Brazil. The stream watershed included areas used for cattle farming and agriculture. Using the same type of net, fishes were also sampled in "Fazzari" (21°58'08''S and 47°53'09''W) and "Lagoa" (21°56'12''S and 47°54'15''W) streams located on the "Cerrado" of the São Paulo State (Southeastern Brazil). The streams cross cattle areas, where chemical substances are used. "Lagoa" stream presents a stretch that consists in a cattle drinking place, with detritus accumulation. The fish from "Capivari" (22°43'00"S and 45°34'00"W) - a stream inside Atlantic Forest - were captured in a reservoir located in the central urban area of Campos de Jordão town– a famous touristic center. The reservoir receives inputs from agricultural areas drainage and is also used by local population for recreational purposes.

After biometry and ventral incision, the gonadal maturation stage and sex were identified macroscopically. Gonads were removed by dissection and weighted before fixation in Bouin's fluid or glutaraldehyde (GTA). Small fish (*LT*<3.5 cm) were fixed whole in order to avoid damaging the gonads. Due to their reduced size, even in the larger fish (*LT* = 9.4 cm), whole gonads were embedded in either paraffin wax or glycol methacrylate plastic resin. After 5 µm sectioning - transversally or longitudinally, staining was carried out with hematoxylin-eosin (HE) or methylene blue plus fucsine. The sections were examined by light microscopy. For each fish, the gonadal development stage and cell type were recorded.

RESULTS

The maximum total length (*LT*) recorded in the studied populations of *Astyanax. scabripinnis* was 9.4 cm. Microscopic analysis involved 219 fish gonads from the Curral das Éguas Stream; as well as 247 fish from the Fazzari, 228 from the Lagoa, and 41 from the Capivari streams**.** The gonads of this species were paired structures fixed to the body cavity**,** as observed in the majority of teleost fishes. While the testes were of the lobular type described by Grier and Taylor (1999), the ovaries were cystovarian (like those described by Takashima and Hibiya, 1995). The normal developmental pattern of the ovaries of *A. scabripinnis* regarding all the populations studied was asynchronic (*sensu* Nagahama, 1983), with oocytes in different developmental stages during the year, which were typical of species spawning several times in an annual cycle. An asynchronic

developmental pattern of the testis was also observed.

In this study, some gonads showed intersex characters under microscopic examination. Four intersex fish were found in the Curral das Éguas Stream (1.9%), six in the Fazzari (2.4%), five in the Lagoa (2.2%) and four in the Capivari streams (9.8%). Intersex fishes from all the streams were microscopically identified as males with testes in different maturation stages (resting, maturation, and mature). The predominance of testicular tissue, with oocytes in perinucleolar stage inserted in the lobular wall (Figs. 1a, b, c and 2a, b), was observed in these fish. The fish from the Capivari stream presented yet another pattern of intersexuality, when both gonads of a single fish had distinct areas with the male and female germinative cells in the mature stage (Fig. 3).

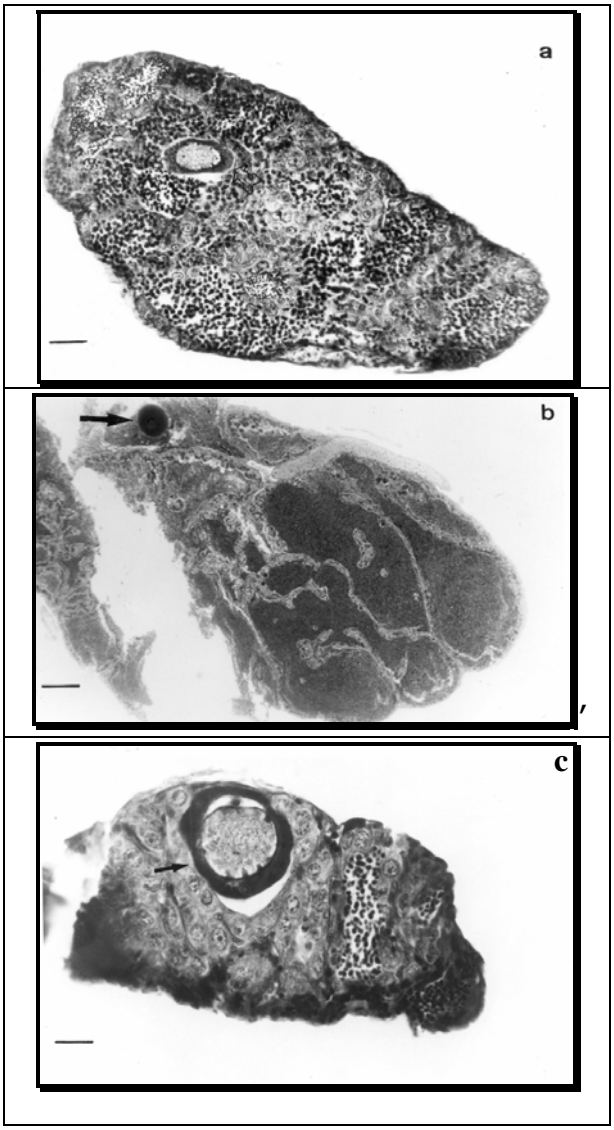

Figure 1 - Testes of *Astyanax scabripinnis* from the Curral das Éguas Stream, identified as males, showing ovarian follicles with perinucleolar stage oocytes (a) advanced maturation stage [HE; scale bar: 40 μm]; (b) mature stage [methilene blue plus fucsine; Scale bar: 200 μm]; (c) initial maturation stage [HE; scale bar: 20 μm]

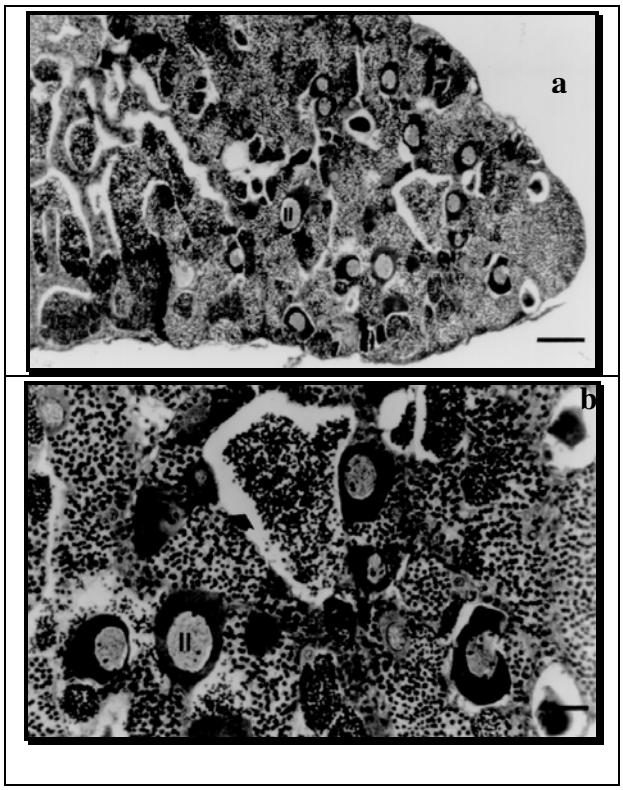

Figure 2 - Testes of *Astyanax scabripinnis* from Lagoa Stream, identified as males, (a) in advanced maturation stage, showing perinucleolar stage oocytes (II) [HE; scale bar: 100 µm]; (b) a detail showing oocytes (II) in the walls of the lobules [HE; Scale bar: 40 µm]

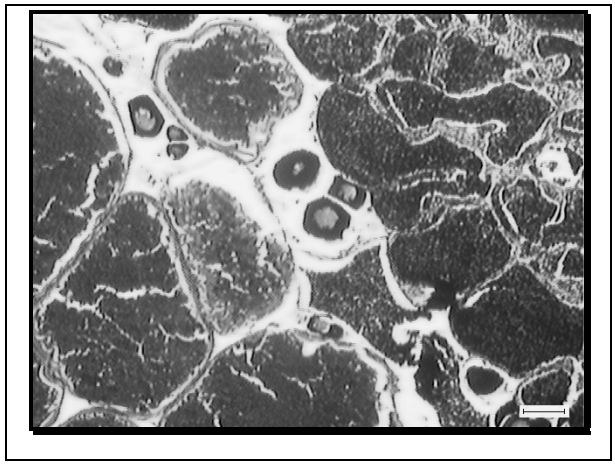

Figure 3 - Testes of *Astyanax scabripinnis* from the Capivari Stream, identified as males, showing vitellogenic oocytes (left) and spermatozoa (right) in same gonad [HE; scale bar: 100 µm]

DISCUSSION

Common among the fishes, the hermaphroditism is a normal mode of reproduction, related to 34 families of teleosts (Kuwamura and Nakashima, 1998) that present, especially in reef fishes, a diversity of patterns that can be either sequentially (protandrous or protogynous fishes) or simultaneously hermaphroditic. There are several theories about the adaptative significance of sex change in those teleosts that normally signals hermaphroditism (Robertson and Warner, 1978; Fischer and Hardison, 1987; Lejeune, 1987; Shapiro, 1987; Siau, 1994; Sadovy and Donaldson, 1995).

The occurrences observed in the present study were not common cases of hermaphroditism, but they could be more accurately described as intersexuality. According to Yamamoto (1969), intersex is the term used to denote either sporadically appearing or experimentally produced hermaphroditic individuals of a species in which all or nearly all individuals are gonochoristic. Characids species are considered to be gonochoristic.

There are several records of intersexuality among gonochoristic species. In an experimental study of reproduction in *Rutilus rutilus* (L.), Jafri and Ensor (1979) registered a condition of apparently functional intersexuality during the microscopical examination of a testis exhibiting normal stages of spermatogenesis.

In *Astyanax scabripinnis,* there were cysts of small oogonia in the chromatin-nucleolus stage, while a few larger oocytes in the perinucleolar stage were situated beside the cyst or randomly scattered throughout the testis itself, as observed experimentally in *R. rutilus.* The occurrence of testicular and ovary tissues in the same gonad, characterized by the presence of vitelogenic oocytes and mature male germinative cells, was observed in the present study. In *Cyprinus carpio,* Gupta and Meske (1976) described a similar structure, which they named the ovotestis.

The evolution of sex change as an alternative style on the life history of gonochorist fishes has been explained by the size-advantage model (Ghiselin, related by Chan and Yeung, 1983). Tobin et al. (1997) reported that the simple presence of intersex or transitional tissues in the gonads of histologically examined fishes did not provide direct evidence of a sex change process. Bruslé and Bruslé (1983) emphasized that the presence of intratesticular oocytes did not constitute a sufficient argument for differentiation of the indirect type. Nolan et al. (2001) presented a study based on 150 intersex individuals of a gonochoristic species, *Rutilus rutilus,* collected from some British rivers. This impressive and well-known record has been attributed to the exposition of those fishes to endocrine-disrupting substances present in the effluents of the British rivers, lakes, and streams. However, according to the authors, the functional significance and the reproductive consequences of intersex in *R. rutilus* remain to be determined.

In a large-scale sampling study of rainbow trout *Oncorhynchus mykiss* spontaneous intersexes, Quillet et al. (2004) found that this was usually considered teratological. Although, according to these authors, environmental factors could be the possible causes, intersex condition in rainbow trout was interpreted due to sex-modifying genetic factors. Fraser (1997) mentioned studies that showed increasing evidence that alkylphenol polyethoxylates, a group of compounds widely used in industrial and agricultural processes, when introduced into the aquatic environments through sewage effluents, were responsible for promoting vitellogenesis in the male fish resulting in feminization of the reproductive organs. Lukšienė et al. (2000) observed that high temperatures in the thermal effluent area at the nuclear power plant in Swedish and Lithuanian negatively influenced the gametogenesis in *Perca fluviatilis, Rutilus rutilus* and *Esox lucius* females, thus provoking hermaphroditism among other alterations observed. Jobling et al. (2002) demonstrated that male gamete production and male gamete quality were reduced in intersex roach (*Rutilus rutilus*) living in rivers with a history of contamination from treated sewage effluents known to contain estrogenic contaminants. Intersex was not observed in hatchery-reared white perch or in white perch collected by Kavanagh et al. (2004) from an uncontaminated reference site in the United States.

In the present work, it was observed that the intersexuality occurrences in the populations were inside the range observed for other species, as found in the literature: from 0.1% (Jafri and Ensor, 1979; Fraser, 1997; van Eenennaam and Dorosov,

1998; Lukšienė et al., 2000; Barnhoorn et al., 2004) to 25% (De Metrio et al., 2003) or more (Gercken and Sordyl, 2002; Kavanagh et al., 2004). However, it was noteworthy that the highest frequency of intersex (9.8%) was recorded for the fish population collected in Capivari stream, the water outflow from an urban reservoir receiving both, the agricultural runoff and the domestic sewage from a touristic town.

Barnhoorn et al. (2004) found intersexuality in 20% of catfish (*Clarias gariepinus*) collected ramdomly from the two dams, which received drained waters from a stream that received effluent from the industrial sites, agricultural activities, informal settlements, and municipal treatment plants. Chemical analyses showed high values of p-nonylphenol (p-NP) in the water and sediment of these dams, indicating estrogenic water pollution. According to Barnhoorn et al. (2004), commonly found in the effluent from sewage treatment plants, p-NP might affect wildlife and human dependent on these sources.

To assess the estrogenicity of a municipal sewage treatment plant, Diniz et al. (2005) exposed sexually mature crucian carp (*Carassius carassius*) for 28 days to domestic treated sewage effluent (25, 50 and 100%). Morphological changes were detected by histological evaluation, revealing severe effects on the testes, with spermatogenesis progressively reduced to total inhibition in fish exposed to 100% effluent. The presence of oocytes was detected in 20% of the males exposed to 100% sewage effluent.

In order to explain the evidence for sexual disruption, van Aerle et al. (2001) collected specimens of *Gobio gobio* from the rivers in the United Kingdom, in several locations that received high-volume discharges of sewage treatments work. Intersex gonads were found at all the sites, but the highest incidences (12 to 15%) occurred at one of places with still water; the elevated concentrations of plasma vitellogenin indicated that fish had been exposed to estrogens.

De Metrio et al. (2003) reported evidence of a high percentage of intersex (25%) in a wild population of Mediterranean swordfish (*Xiphias gladius*) and immunohistochemically demonstrated the presence of vitellogenin in the liver of both, intersex and normal males. According to the authors, these findings could be due to the exposure to endocrine disrupting substances that promoted estrogenic effects. Man-made chemicals are among the substances causing environmental pollution. These endocrine-disrupting substances (EDSs), according to Kavanagh et al. (2004), are from industrial origin, such as alkylphenol surfactants, bisphenol A, and phthalate plasticizers, beyond include natural estrogens, such as 17β (E_2) and estrone, and the syntetic estrogen 17 α-ethinyllestradiol (EE_2). These authors opened the question on the risk to human health of consumption of fishes containing bio-accumulated endocrine disrupters. These alterations were not restricted to Mediterranean region and to fish population, but there is a vast literature about this problem concerning to different regions of the world and different groups of animals. Colborn et al.. (1997) provided information of emerging scientific research on animal and human studies carried out in the world that linked the wide range of manmade chemicals and the disruptions of hormone systems.

In the present study, the intersex occurrences observed in the wild populations of *A.scabripinnis* from four different localities were not conclusive regarding the causes. In a general way, the possible action of toxic agents present in the environments could not be ruled out. Nevertheless, experimental studies are necessary to provide a basis for constructing an explanatory hypothesis to the occurrence of intersexuality in the populations of *A. scabripinnis* here analyzed.

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RESUMO

Durante estudos efetuados com pequenos caracídeos (*Astyanax scabripinnis*) de riachos do Cerrado brasileiro, localizados nos Estados de Minas Gerais e de São Paulo, foram encontrados alguns peixes com gônadas exibindo oócitos em estágio perinucleolar dispersos, embebidos em tecidos testiculares em desenvolvimento. Em exemplares da mesma espécie, provenientes de um riacho situado na Mata Atlântica do Estado de São Paulo, outro padrão de gônadas intersexuadas foi registrado, mostrando oócitos vitelogênicos e lóbulos maduros presentes na mesma gônada. As gônadas em diferentes estágios de maturação foram submetidas a análises histológicas. Os resultados obtidos mostram os padrões morfológicos e as freqüências de ocorrência dos intersexos encontrados em peixes do "complexo" *A. scabripinnis* provenientes de quatro diferentes populações. São discutidas as prováveis causas ambientais responsáveis por estas alterações.

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