Copeia, 2001(3), pp. 866–869

Karyological Evidence for a Cryptic Species of Piranha within *Serrasalmus rhombeus* (Characidae, Serrasalminae) in the Amazon

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Cytogenetic data obtained for *Serrasalmus rhombeus* revealed two cytotypes (cytotype 1 and cytotype 2) at four sampling sites. Cytotype 1 consists of 2n = 60, 20M + 24SM + 6ST + 10A and was detected in specimens from Negro River (Anavilhanas Islands), Solimões River (Camaleão Lake), confluence of Negro and Solimões Rivers (Catalão Lake), and Uatumã River. Cytotype 2 consists of 2n = 58, 22M + 24SM + 2ST+10A and was detected in specimens from Catalão and Camaleão Lakes. In Cytotype 2, the first chromosome pair was almost twice the size of second pair, and the constitutive heterochromatin pattern of each cytotype is distinct. The two cytotypes occurred sympatrically at Catalão and Camaleão Lakes, and no intermediates between the cytotypes were detected, suggesting each cytotype represents a different fish species.

MANY examples of cytogenetic variation have been reported in Neotropical fishes. For example, intraspecific cytogenetic variation in the diploid number, karyotype structures or C-banding patterns occur in nominal forms of *Hoplias malabaricus, Astyanax scabripinnis, Eigenmannia virescens,* and *Callichthys callichthys*. Debates remain concerning the status of the cytotypes within these nominal species (Oliveira et al., 1988; Porto et al., 1992; Almeida-Toledo, 1998). In the Amazon, intraspecific cytotypic variation has been reported in *Callichthys callichthys* (Porto and Feldberg, 1993), *Plagioscion* sp. (Feldberg et al., 1999) and *Serrasalmus spilopleura* (Centofante, 2000; Nakayama et al., 2000). However, only a few studies have associated both morphological and karyotypic difference (Moreira Filho and Bertollo, 1991).

The genus *Serrasalmus* is distributed throughout the tropical regions of South America eastward from the Andean Cordillera. The taxonomy and systematics of several species of this genus are not well known because most analyses were based on small sample sizes usually consisting of immature specimens (Machado-Allison and Fink, 1996). Thus, the number of recognized species continues to fluctuate (Merckx et al., 2000). *Serrasalmus rhombeus* is considered to be one of the most common piranhas in the Amazon. It was described from Surinam but is widespread throughout the Amazon Basin, and Orinoco and Guiana coastal basins. Its pigmentation changes with age, being silvery with small dark spots when young (known as white piranha) and becoming progressively darker when adult (when they are known as black piranha or red-eyed piranha). According to Géry (1976) *S. rhombeus* is a species complex (*rhombeus* group) comprising six to nine species (*S. rhombeus, S. albus, S. spilopleura, S. marginatus, S. brandtii, S. sanchezi* plus probably *S. gibbus, S. humeralis* and *S. pingke* = *S. elongatus*) that are recognized by a deep and compressed body. The aim of this paper is to document the cytogenetic variation found in nominal forms of *S. rhombeus* collected in Central Amazon near Manaus, Amazonas, Brazil, and to focus on a particular taxonomic problem that may be assisted by chromosomal analyses.

Materials and Methods

A total of 71 specimens of the nominal *S. rhombeus* were collected in the Central Amazon Basin between 25 March 1984 and 30 September 1996 at the following sites: (1) 45 specimens (17 males and 28 females) at the confluence of Negro and Solimões Rivers (Catalão Lake); (2) 16 specimens (nine males and seven females) on Solimões River (Camaleão Lake-Marchantaria Island); (3) two specimens (two females) in the Uatumã River; (4) six specimens (two males and four females) in the Jatapu River; (5) one female specimen at Capucapu River; (6) one female specimen at Anavilhanas in the Negro River (Fig. 1).

Mitotic chromosomes, nucleolar organizing regions (NORs), and C-band were obtained through the methods of Bertollo et al. (1978), Howell and Black (1980), and Sumner (1972), respectively. The chromosomes were analyzed by measuring the short arm length, long arm length, and total length, by means of a dry-tip compass and a calliper. Mean values were calculated for each chromosome pair. The relative length (RL%) of each chromosome pair in relation to the total length of the haploid chromosome length was obtained from these values. Chromosomes were identified by the arm ratio criteria proposed by Levan et al. (1964). Metacentric (M),

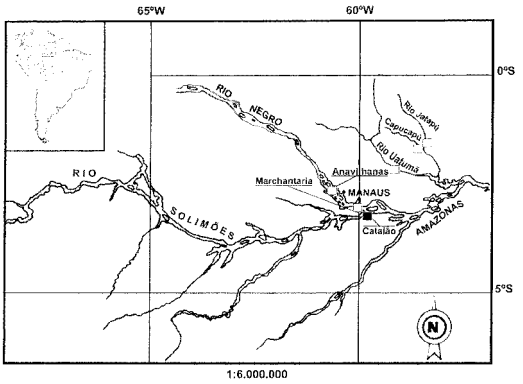

Fig. 1. Geographical distribution of distinct cytotypes found in nominal forms of *Serrasalmus rhombeus*. White squares represent collection localities for cytotype 1 (2n = 60), and black squares are localities for cytotype 2 (2n = 58).

submetacentric (SM), and subtelocentric (ST) chromosomes were considered to be biarmed, and acrocentric (A) chromosomes were considered to be monoarmed.

Results

Two cytotypes were found in samples of *S. rhombeus* (cytotype 1 and cytotype 2). Cytotype 1 had 2n = 60 chromosomes (20M + 24SM + 6ST + 10A) and a difference of less than 0.5% in relative length (RL%) between the two first chromosome pairs. This karyotype was detected in specimens collected from Uatumã, Jatapu, Capucapu, Negro and Solimões (Marchantaria Island) Rivers and at Catalão Lake in the confluence of the latter two rivers (Fig. 2). Cytotype 2 had 2n = 58 chromosomes (22M + 24SM + 2ST + 10A) and 2.1% of difference in relative length (RL%) between the first and second chromosome pairs; thus, the largest pair is about twice the size of chromosome pair 2 (Fig. 3). This karyotype was detected in specimens from Catalão and Camaleão (Marchantaria Island) Lakes. Multiple NORs in short arms of 5–12 ST-A chromosomes were detected in both cytotypes (Table 1). Number, size, and intensity of NORs were variable intraindividually (Figs. 2B, 3B).

Constitutive heterochromatin pattern of cytotypes 1 and 2 are different (Figs. 2C, 3C). Heterochromatic blocks in cytotype 1 were mostly pericentromeric, some being more conspicuous than others, although faint telomeric blocks were also detected. Heterochromatic blocks were more evident in cytotype 2 than in cytotype 1, especially in the first and second metacentric pairs, and 12th, 15th, and 21th submetacentric pairs. The main heterochromatic simi-

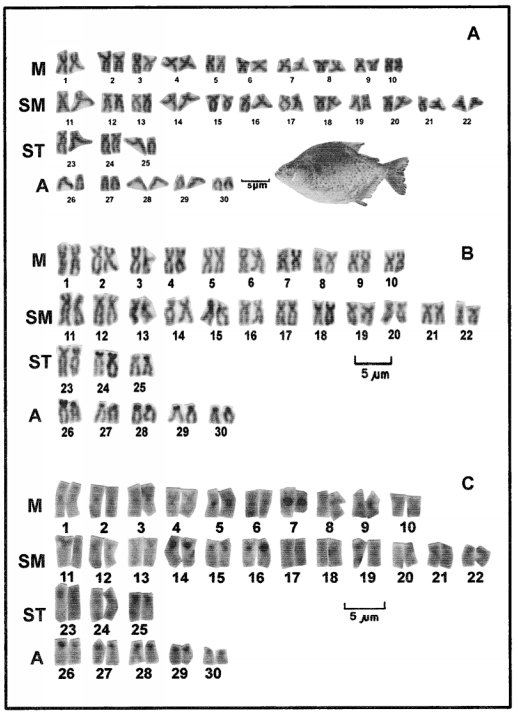

Fig. 2. Giemsa (A), NOR (B) and C-Band (C) karyotypes of *Serrasalmus rhombeus* cytotype1.

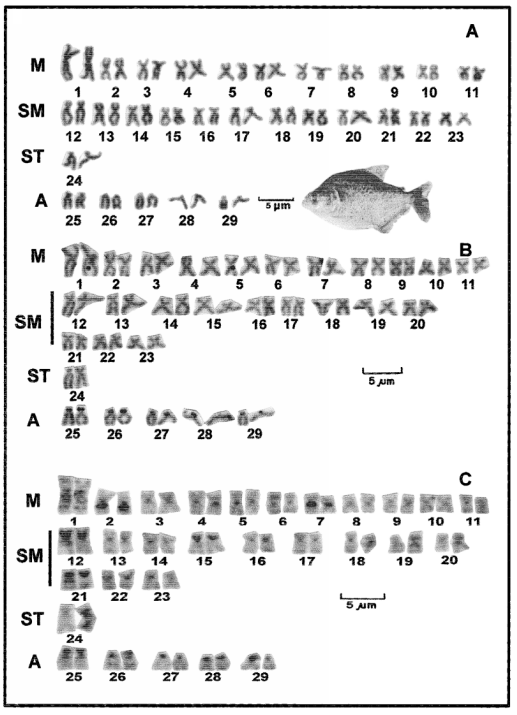

Fig. 3. Giemsa (A), NOR (B) and C-Band (C) karyotypes of *Serrasalmus rhombeus* cytotype 2.

TABLE 1. THE MAIN CYTOGENETIC DATA OF *Serrasalmus rhombeus*. (NORs = nucleolar organizer regions, *n* = number of specimens, NCel = number of cells analyzed, 2n = diploid number, FN = Fundamental number, M = metacentric, SM = submetacentric, ST = subtelocentric, pSTA = short arm of chromosomes subtelo-acrocentrics, Loc = Location).

					Formula				NORs	
Collecting sites	*n*	NCel	2n	NF	M	SM	ST	A	Range	Loc
CYTOTYPE 1										
Catalão Lake	10	519	60	110	20	24	06	10	5–12	pST-A
Camaleão Lake	09	313	60	110	20	24	06	10	5–12	pST-A
Uatumã River	02	64	60	110	20	24	06	10	5–12	pST-A
Jatapu River	06	268	60	110	20	24	06	10	5–12	pST-A
Capucapu River	01	26	60	110	20	24	06	10	5–12	pST-A
Anavilhanas Archip.	01	44	60	110	20	24	06	10	5–12	pST-A
CYTOTYPE 2										
Catalão Lake	35	1439	58	106	30	16	02	10	5–12	pST-A
Camaleão Lake	07	322	58	106	30	16	02	10	5–12	pST-A

larities observed among cytotypes encompass the proximal blocks on the long arms of one metacentric pair (seventh in both cytotype 1 and cytotype 2), the short arms totaly heterochromatic of one submetacentric pair (14th in the cytotype 1 and 15th in the cytotype 2) and the centromeric blocks of acrocentric chromosomes. Because NORs were detected in acrocentric chromosomes, the NORs sites were considered to be positive for C-banding.

All specimens are morphologically very similar, however, specimens with cytotype 2 are smaller than those with cytotype 1. Individuals of cytotype 2 never exceded 150 mm in standard length (SL), and females had ripe ovaries with 100 mm SL. Individuals with cytotype 1 exceded 250 mm in SL, and females have ripe ovaries with 160 mm SL.

DISCUSSION

This is the first report of karyotypes on specimens tentatively identified as *S. rhombeus*, and the chromosomal data presented here are part of a multidisciplinary study that encompasses parasitological, morphological, and molecular analyses on piranhas from the Amazon. In the Amazon floodplain near Manaus, *S. rhombeus* has two cytotypes that differ in diploid numbers, karyotypic formulae, and in C-banding patterns. It is noteworthy that cytotype 1 is the most widespread form being found in Uatumã, Negro, and Solimões River drainages, whereas cytotype 2 occurs only in the lower Negro River at the confluence with Solimões River. Because there is no cytological evidence of hybridization between the cytotypes (no intermediate cytotypes) where they coexist (Catalão and Camaleão Lakes), the cytotypes appear to represent distinct species.

Besides chromosomal characters, each cytotype (species) is uniquely defined by a set of morphological and parasitological character states. Preliminary morphometric and meristic data (including number of supraneural bones) indicate subtle differences between the two species (A. Merckx and M. Jégu, unpubl.). We also observed that adults from cytotype 1 are considerably larger than those from cytotype 2. Monogenoidea parasite data also support to the hypothesis that they might be distinct species because individuals representing the two cytotypes have different gill parasite species within the monogenoid genera *Anachantorus* (Kritsky et al., 1992), *Mymarothecium* and *Notozothecium* (Kritsky et al., 1996), *Amphitecium* (Kritsky et al., 1997), *Notothecium* and *Enallothecium* (Kritsky et al., 1998).

Examples of sympatric piranha species that possess a deep or compressed body typical of *S. rhombeus*-species complex seem not to be a rare event in Amazon River drainages: (1) *S. rhombeus* and *S. geryi* from Tocantins River (Jégu and Santos, 1988); (2) *S. altispinis* and *S. rhombeus* from Uatumã River (Merckx et al., 2000); and (3) cytotype 1 and cytotype 2 from lower Negro River (this work). Particularly in the latter example, external morphological characters do not help to differentiate the species, and a reasonable explanation might be a recent speciation. Additional studies are necessary to understand why individuals with cytotype 2 present such a limited distribution as well as to test the hypothesis whether all species included in the *S. rhombeus*-species complex are a monophyletic unit.

MATERIALS EXAMINED

Of 71 specimens examined in this study, eight voucher specimens of *S. rhombeus* (2n = 60) and

22 of *S rhombeus* (2n = 58) were selected and deposited at the ichthyology collection of the Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil, with the following accession numbers: INPA 7352–7356 (2n = 60) and INPA 7345–7351 (2n = 58).

Acknowledgments

We thank L. A. C. Bertollo (Universidade Federal de São Carlos), V. Thatcher, and A. T. Saturnino (Instituto Nacional de Pesquisas da Amazônia), and three anonymous reviewers for their contribution on preliminary versions of this paper. This work was supported by the Brazilian (CNPq and INPA) and the French (IRD) research institutions.

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