

Karyological evidence for interspecific hybridization between *Cichla monoculus* and *C. temensis* (Perciformes, Cichlidae) in the Amazon

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Cichla monoculus, *Cichla temensis* (peacock bass or tucunaré), and its presumed hybrids, were cytogenetically analyzed. The fish were collected at three distinct sites in the central Amazon basin, namely in the Uatumã (*C. monoculus*, *C. temensis* and their natural hybrid), Jaú (*C. temensis*), and Solimões rivers (*C. monoculus*). The two species and the natural hybrid showed the same diploid number, $2n = 48$ acrocentric chromosomes. Single NORs were detected in the distal region of the long arm in all three species. However, in *C. monoculus*, the NOR was found on the second pair of the complement, in *C. temensis*, on the third pair and in the hybrid two NOR patterns were found, one on the second pair and the other on the third pair of chromosomes. The two species and the hybrid have their constitutive heterochromatin located in the pericentromeric region of all chromosomes and an interstitial C-band located on the largest chromosome pair. The great similarity in the chromosome number and morphology, chromosome size class differences, the NOR patterns and C-banding suggested chromosomal stasis during speciation and hybridization of *Cichla*.

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In the mid 1950s the peacock bass or tucunaré (*Cichla* spp.) became very popular and were introduced in several lakes and culture ponds throughout Brazil, as well as in other countries (NASCIMENTO et al. 2001). The genus *Cichla*, a widely distributed endemic Neotropical cichlid in the Amazon basin, consists of five species which have been described in the literature: *C. temensis*, restricted to clear and black water tributaries such as the Orinoco, Negro and Tapajós Rivers; *C. monoculus*, distributed throughout central Amazonia; *C. orinocensis*, in the Orinoco and Negro basins; *C. intermedia*, found in the upper Negro and middle Orinoco Rivers; and *C. ocellaris*, found in Suriname (MACHADO-ALLISON 1971; KULLANDER 1986; KULLANDER and NIJSSEN 1989). However, it has been indicated that there are at least seven more species to be described (S. O. Kullander, pers. com.).

Despite the importance of the *Cichla* species, both as a food resource for the general population, and sport fishing for tourists, since late 1970s (THOMPSON 1979), no cytogenetic study has been published on the genus, although such studies have been presented in meetings and two (unpublished) master's theses (ALVES 1998; NISHIYAMA 1999) have been generated. According to THOMPSON (1979) *Cichla temensis* have 48 acrocentric chromosomes.

Recently, a presumed new species of *Cichla* from the Uatumã River, that together with *C. monoculus* and

C. temensis occurs in Lake Balbina (a large manmade reservoir created in the middle of the Brazilian Amazon to supply hydroelectric power) was reported. Lake Balbina (formed in 1987) and its flooded water surface area of 2400 km², mostly of forest and inundated trunks of dead and leafless trees, recently provided an appropriate habitat for the explosive reproduction of peacock bass. In Lake Balbina, *C. monoculus* is the most common peacock bass and *Cichla* sp. the least common. *Cichla* sp. presents intermediate morphometric characteristics between *C. monoculus* and *C. temensis* (OLIVEIRA JÚNIOR 1998).

The radically disturbed habitat that must have prevailed during the formation of Lake Balbina as well as the morphometric similarities between the peacock bass species taken from Lake Balbina suggest that *Cichla* sp. (sensu OLIVEIRA JÚNIOR 1998) is a natural hybrid. Taking this into account, ANDRADE et al. (2001) analyzed the 16S mtDNA gene of *C. monoculus*, *C. temensis* and the presumed *Cichla* hybrid and confirmed that natural hybridization occurs between *C. monoculus* and *C. temensis* in Lake Balbina. They also showed that hybridization events are quite common in many localities in the Amazon, where the two species occur sympatrically.

The incidence of hybridization of fish species is not rare and there is evidence of hybridization being a

mechanism for speciation. Thus, hybridization may be viewed as another source of variation in evolution where hybrids represent novelties and the new allele combinations could promote adaptive radiation (ARNOLD 1997). Anthropogenic disturbances, related to aquacultural activities, species introductions and loss or alteration of habitats, are the major contributing factors in interspecific hybridization of fish species (SCRIBNER et al. 2001).

As part of an ongoing effort to karyotype the Amazonian cichlids, the aim of this article is to analyze the chromosomes of *C. monoculus* and *C. temensis* from the central Amazon, and addressing a particular problem involving natural hybridization between them in Lake Balbina.

MATERIAL AND METHODS

Specimens of the peacock bass or tucunaré were collected from their natural habitat in the Amazon

basin: *C. monoculus* (16 males, 13 females) from the Uatumã River (59° 20'W, 1° 00'S) up and downstream from the Balbina hydroelectric power plant, and two females from the Solimões River (60° 20'W, 3° 40'S); *C. temensis* (three females) from the Uatumã River and (two males, one female) from the Jaú River (61° 29'42''W, 1° 58' 42''S); and *Cichla* hybrids (four males, eight females, one undetermined sex) from the juncture of Lake Balbina with the Uatumã River (Fig. 1).

We followed the meristic and morphometrics outlined by OLIVEIRA JÚNIOR (1998) for the *Cichla* species from the Uatumã River and the summary of the diagnostic features are: *Cichla monoculus*-yellowish green color pattern; three dark vertical bands extending partially down the sides of the body, not reaching the pectoral fin; one dark horizontal band at the pectoral fin; no yellow spots; short snout; less than 86 and 48 scales on the lateral line and caudal peduncle, respectively; and less than 18 scale rows below the lateral line. *Cichla temensis*-4-6 longitudinal

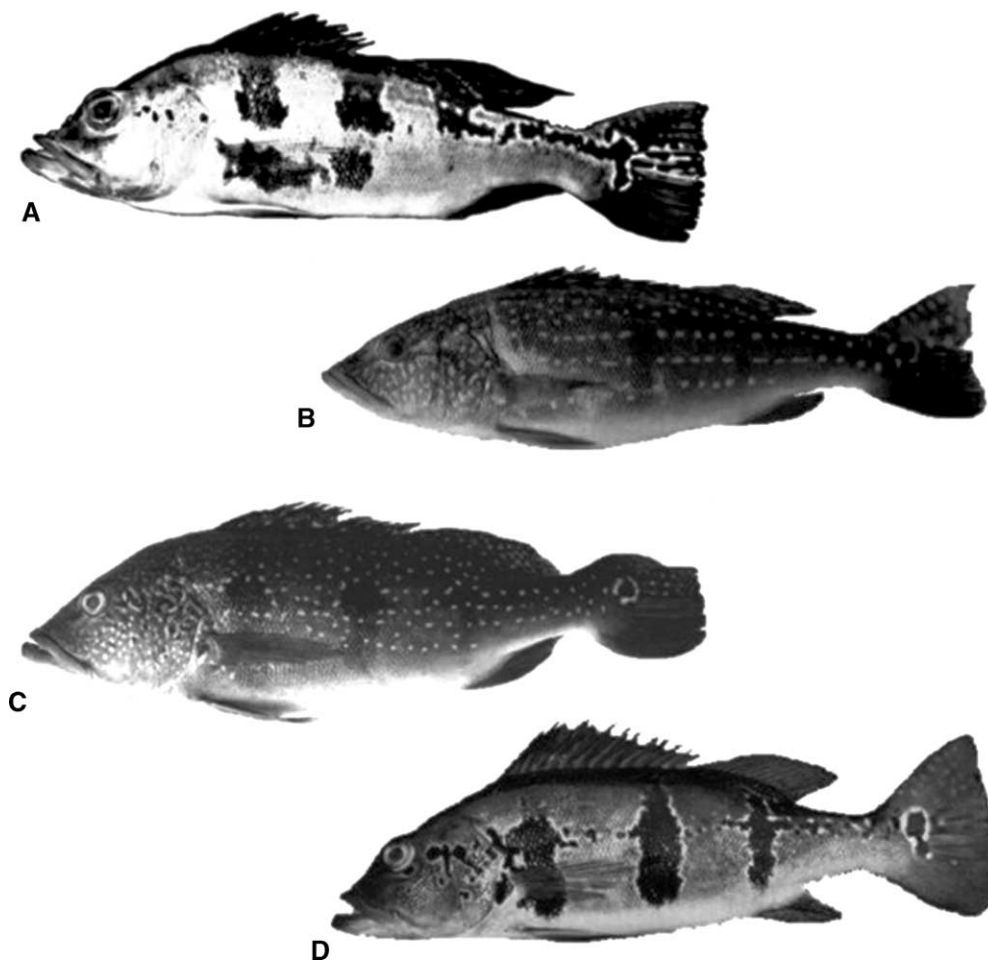


Fig. 1A–D. *Cichla* species: (A) *Cichla monoculus* (260 mm standard length); (B) *C. temensis* (270 mm standard length); (C) *Cichla* hybrid, similar to *C. temensis* (350 mm standard length); (D) *Cichla* hybrid, similar to *C. monoculus* (340 mm standard length). (Photos: Arnaldo Oliveira Júnior, 1998).

series of uniform yellow spots on a brownish body; three faint dark vertical bands reaching or extending to the pectoral fin; no horizontal band at the pectoral fin; long peduncle; more than 100 and 54 scales on the lateral line and caudal peduncle, respectively; and more than 19 scales rows below the lateral line. *Cichla* hybrids-spotted body, but non uniform, brownish color pattern; when unspotted presented a yellowish-green color pattern; no horizontal band at the pectoral fin; 76–99 scales on the lateral line; 50–62 scales on the caudal peduncle; three dark vertical bands reaching or extending past the pectoral fin. During taxonomic identification of the species we recognized two different groups in *Cichla* hybrids, one of them being more similar to *C. monoculus* and the other to *C. temensis*.

Mitotic induction with biological yeast (LOZANO et al. 1988) was performed in order to induce a cell response. To obtain somatic metaphases after colchicine treatment, kidney cells were prepared according to the air-drying technique of BERTOLLO et al. (1978) but with a lower colchicine concentration (0.025%). Nucleolar organizer regions (NORs) were identified by silver nitrate staining (Ag-NORs) and the C bands by the barium hydroxide according to the techniques described by HOWELL and BLACK (1980) and SUMNER (1972), respectively. When possible slides were stained sequentially with Giemsa, C-banding and silver nitrate solution (Ag-NOR) according to the procedures described in CENTOFANTE et al. (2002).

Length features of chromosomes (the total length of chromosome, the length of long arms and the relative length in relation to the total haploid length) were taken from three (*C. temensis*) to eight (*C. monoculus* and *Cichla* hybrids) specimens, from the Uatumã River where the species supposedly hybridize. In order to visualize the chromosome size gradient, the total length of chromosomes was grouped into relative size classes.

RESULTS

A total of 700, 173 and 427 metaphases were analyzed for *C. monoculus*, *C. temensis* and *Cichla* hybrids, respectively. Nineteen karyotypes were measured. $2n=48$ acrocentric chromosomes (FN=48) were found for the two species and the hybrids (Fig. 2). There were no differences in modal diploid chromosome number or in chromosome morphology between males and females.

A single pair of nucleolar organizer regions (NOR) was observed. These NOR were located at the distal position on the long arms and assigned to the second pair in *C. monoculus*, and to the third pair in

C. temensis. However, in *Cichla* hybrids five individuals presented the NOR in the second pair and three presented it in the third chromosome pair (Fig. 2). Morphometric analyses and sequential Giemsa-NOR staining assigned these positions.

The constitutive heterochromatin pattern obtained for the two species plus hybrids was very similar. The heterochromatin blocks were located preferentially in the pericentromeric region of all chromosomes, with some of them being more evident than others. One interstitial band on the long arm of the largest pair was seen in all samples. A faint distal C-band was also found in the NOR sites of *C. monoculus*, *C. temensis* and *Cichla* hybrids (Fig. 3).

A comparative analysis of the chromosomal measurements among the species was performed (Fig. 4). A greater similarity between *C. temensis* and *Cichla* hybrids, with NOR in the third chromosome pair was observed. However only a weak similarity between *C. monoculus* and *Cichla* hybrids, with NOR in the second chromosome pair was observed. In addition, no correlation between the two morphs of *Cichla* hybrids and the two chromosomal groups (NORs phenotypes) was observed. In *C. monoculus* the size of the first chromosomal pair was responsible for the presence of a unique size class (larger size class), which was not found for *C. temensis* and *Cichla* hybrids.

DISCUSSION

It is well known that cichlids are an evolutionary complex puzzle for those studying speciation, mainly due to adaptive evolution and explosive speciation (FELDBERG et al. 2003). The fact that many cichlid species are easily hybridized in the laboratory as well as evidence for viable progeny from crosses indicate that the differences between the karyotypes do not prevent the generation of inter-specific hybrids and support the idea that cichlids are well suited for studying questions in evolutionary biology. Hybridization between two species of *Cichla* has already been observed by SAWAYA and MARANHÃO (1946), who reported the reproductive behavior in captivity between *C. temensis* and *C. ocellaris*. However, because *C. ocellaris* does not occur in Brazilian Amazonia (KULLANDER and NIJSSEN 1989) most probably the original report of *C. ocellaris* was really *C. monoculus*.

Viable hybrids have also been produced by inter and intrageneric crossings in the Cichlidae family, such as in *Tilapia* × *Oreochromis*, *O. niloticus* × *O. karongae*, *Cichlasoma nigrofasciatum* × *C. cyanoguttatum*, *Symphysodon aequifasciatus* × *S. discus* and *Labeotropheus fuelleborni* × *Metriaclima zebra* (MCELROY and KORNFIELD 1993; MAZEROLL and WEISS 1995;

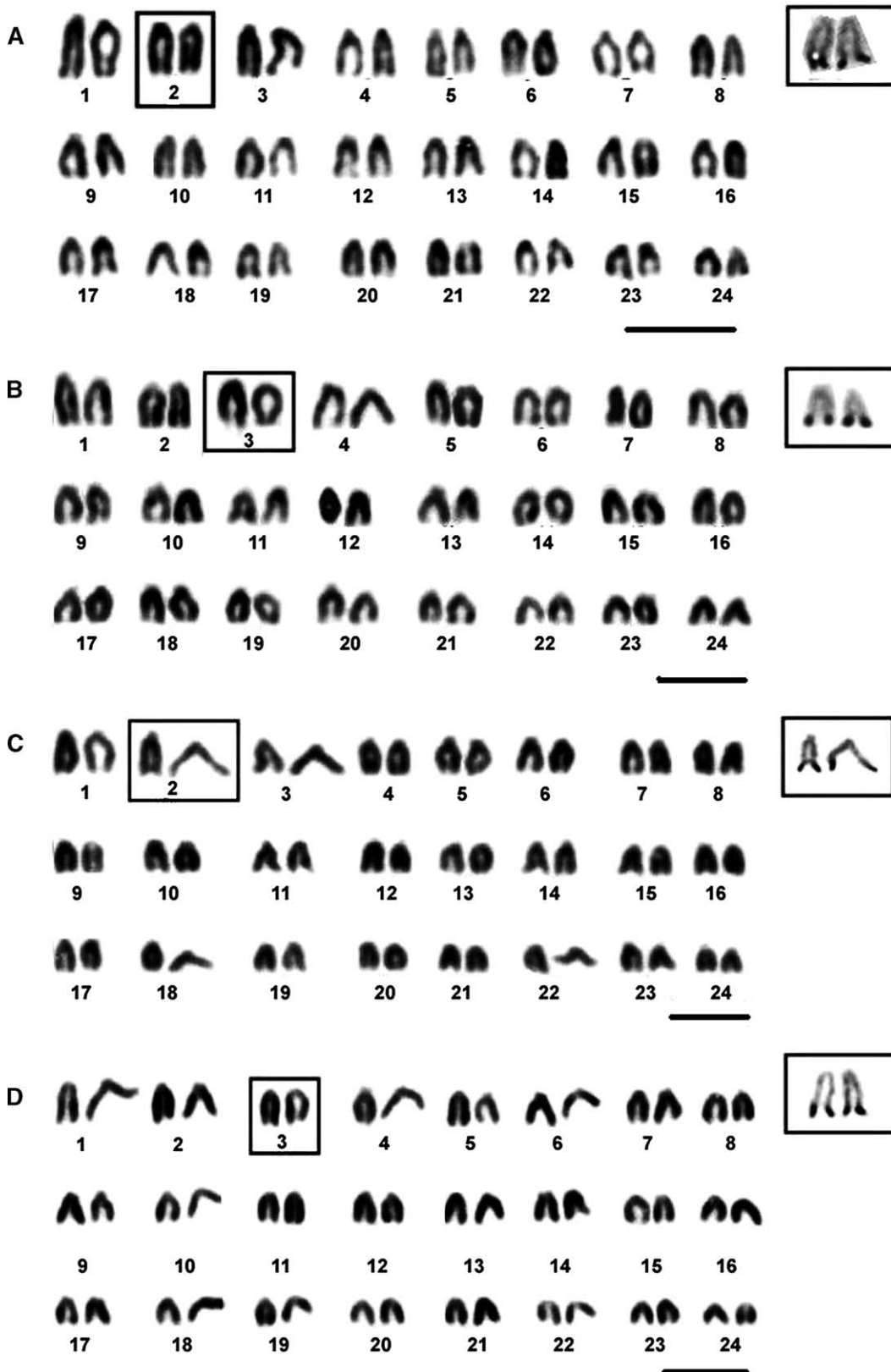


Fig. 2A–D. Karyotype and nucleolar chromosomes (NOR) of: (A) *Cichla monoculus*, NOR on the second pair; (B) *C. temensis*, NOR on the third pair; (C) *Cichla* hybrid, NOR on the second pair; (D) *Cichla* hybrid, NOR on the third pair (bar = 5 µm). The NOR bearing chromosomes appear in the square inset.

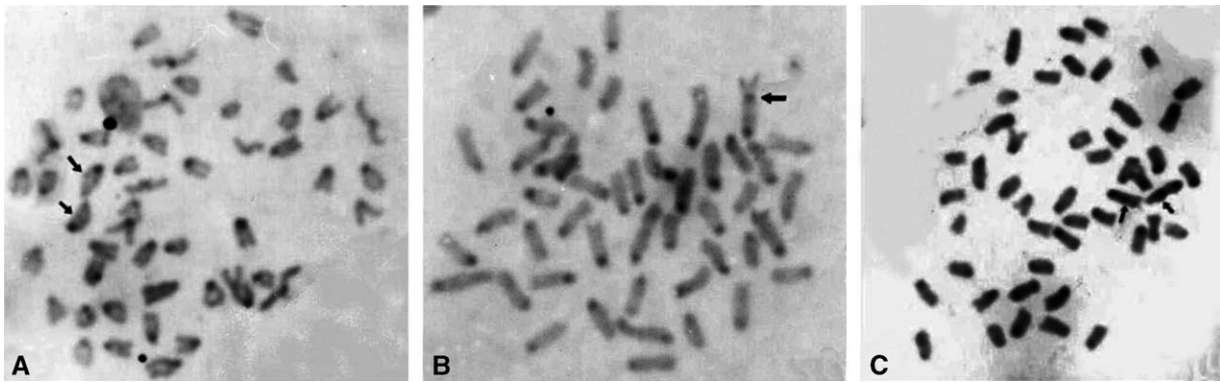


Fig. 3A–C. Somatic metaphases showing the C-banding pattern: (A) *Cichla monoculus*; (B) *C. temensis*; (C) *Cichla* hybrid. The arrows indicate the interstitial band in the long arm of the marker chromosome pair.

HARVEY et al. 2002; ALBERTSON et al. 2003). Hybrids from some crossings resemble more closely one of the parents, whereas others are intermediate (KORNFIELD 1984). Further, a case of introgressive hybridization between two ancient and genetically distinct species of Lake Tanganyika cichlids may have led to the formation of *Neolamprologus marunguensis* (SALZBURGER et al. 2002).

ANDRADE et al. (2001), have recently analyzed the mitochondrial ribosomal 16S (rRNA) gene of the nominal species *C. monoculus* and *C. temensis* from six Amazonian sites and a clear indication of mitochondrial lineage introgression was found between *C. monoculus* and *C. temensis*, probably due to hybridization in all sites where the two species occur in sympatry. In addition, an ongoing project in *Cichla* species has demonstrated that hybridization is occurring in the wild as revealed by esterase enzymes (Teixeira and Oliveira, unpubl.).

Thus, the hypothesis that *Cichla* sp. (sensu OLIVEIRA JÚNIOR 1998) had been the product of a *C. monoculus* and *C. temensis* interspecific crossing, in the Uatumã River drainage basin is supported and cannot be discarded.

Some authors have cytogenetically studied the Cichlidae family and all of them indicate conservative chromosomal evolution, in contrast to high species diversity (KORNFIELD 1978; THOMPSON 1979; FELDBERG et al. 2003). The *Cichla* species presented very similar karyotypes, $2n=48$ acrocentric chromosomes, yet interspecific differences as to the nucleolar organizer region (NOR) position and chromosome size classes, were observed. *C. monoculus* presented the NOR in the second chromosome pair, *C. temensis* in the third pair. Yet in *Cichla* hybrids, two positions for the NOR were observed, i.e. in the second and third pair. However, no correlation was seen between the NOR location to the two morphotypes of hybrids, i.e.

the unspotted one resembling *C. monoculus* and the spotted one resembling *C. temensis* (Fig. 1).

Despite a general similarity between the karyotypes of *Cichla* species, the chromosomal comparisons by size classes provided a additional way of looking similarities and differences between *C. monoculus*, *C. temensis* and the hybrids. A greater similarity between *C. temensis* and *Cichla* hybrids, with the NOR in the third pair is evident. The most pronounced differences are found in classes 4 and 5, which correspond to the first chromosomal pairs. *C. temensis* has only one pair of chromosomes in the size 5 class and four chromosomal pairs in the size 4 class. *Cichla* hybrids with the NOR in the 3rd pair presents some uniformity in the first three pairs, as observed in class 5, and a reduction in the size 4 class.

It is evident that cytogenetically *Cichla* hybrids are split into two groups whether by the NOR phenotypes (position on the 2nd or 3rd pair) or by the chromosomal size class. Even though it would seem that chromosomal measurements may lead to mistakes, a blind analyses of the two cited characteristics suggest that their parentals are karyotypically distinct.

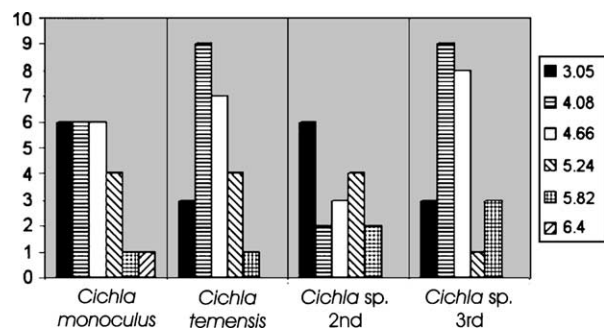


Fig. 4. Chromosome number frequency in the *Cichla* species analyzed distributed by relative size (% of the haploid lot) (Class 1 = 2.9–3.05; Class 2 = 3.06–4.08; Class 3 = 4.09–4.66; Class 4 = 4.67–5.24; Class 5 = 5.25–5.82; Class 6 = 5.83–6.4).

Hence, the genetics (chromosomes and mtDNA) and morphometrics plus the fact that *C. monoculus* and *C. temensis* occur in sympatry, led to the suggestion that *Cichla* sp. (sensu OLIVEIRA JÚNIOR 1998) and its morphs from Lake Balbina could have come from a possible natural hybridization and backcrosses in different generations along those years.

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