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Parallel evolution evidenced by molecular data in the banded-tetra (*Astyanax fasciatus*)

Rubens Pazza*, Letícia Aparecida Cruvinel, Karine Frehner Kavalco

Universidade Federal de Viçosa, Campus Rio Paranaíba, Laboratório de Genética Ecológica e Evolutiva, Rodovia BR 354, Km 310 (1300m), 38810-000, PO Box 22, Rio Paranaíba, Minas Gerais, Brazil

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ABSTRACT

Astyanax is well known as a model for developmental biology studies, particularly with regard to Mexico's cave populations. More than 130 species of *Astyanax* are already known, most of which live in South America. The occurrence of cryptic species and species complexes elucidated by chromosomal and genetic studies demonstrates that the relationship between morphology and molecular evolution is quite complex within this group. In this work, we demonstrate that morphology does not follow the path of vicariant processes observed in *Astyanax fasciatus* populations, which separated about three million years ago, although molecular data suggests its separation in two species.

1. Introduction

Brazil is a huge country with some major hydrographic basins. The largest and best known of these is the Amazon, but the basins of the São Francisco and Upper Paraná should also be highlighted for their wide area and endemism. Nevertheless, these basins are home to various common species, remnant of the geological processes of its genesis.

The Upper Paranaíba arc is the watershed between the São Francisco and Paraná river basins and is located in a North-South arrangement in the Southern Brazil (Campos and Dardenne, 1997). This region seems to be responsible for the shared ichthyofauna between these two hydrographic basins (Hubert and Renno, 2006). The region, which in some places is elevated above 1100 m, began its geological elevation about 117 million years ago (Ma). This was then followed by draining during neotectonic events at the end of the Tertiary, around 3 Ma (Saadi et al., 1991).

From this time of divergence, for the initial vicariant processes in the region, we tested if morphological evolution followed the genetic structure and phylogeography within one species of the genus *Astyanax*, *Astyanax fasciatus*, which was widely distributed among these watersheds, in order to determine whether this time of divergence was enough for differentiation as separate species.

Although widely known, this species presents taxonomic problems since its description in the nineteenth century to more recent chromosomal characterizations. Its type location is referred to as “rivers from Brazil,” although Eigenmann suggests that the type location is the São Francisco river (Eigenmann, 1921). On the other hand, cytogenetic studies suggest that *Astyanax fasciatus* is more than one species but that it has a common denomination due to interesting chromosomal variation in Upper Paraná river basin with divergent cytotypes found in sympatry and alopatry (Pazza et al., 2006, 2008).

* Corresponding author.
E-mail address: rpazza@ufv.br (R. Pazza).

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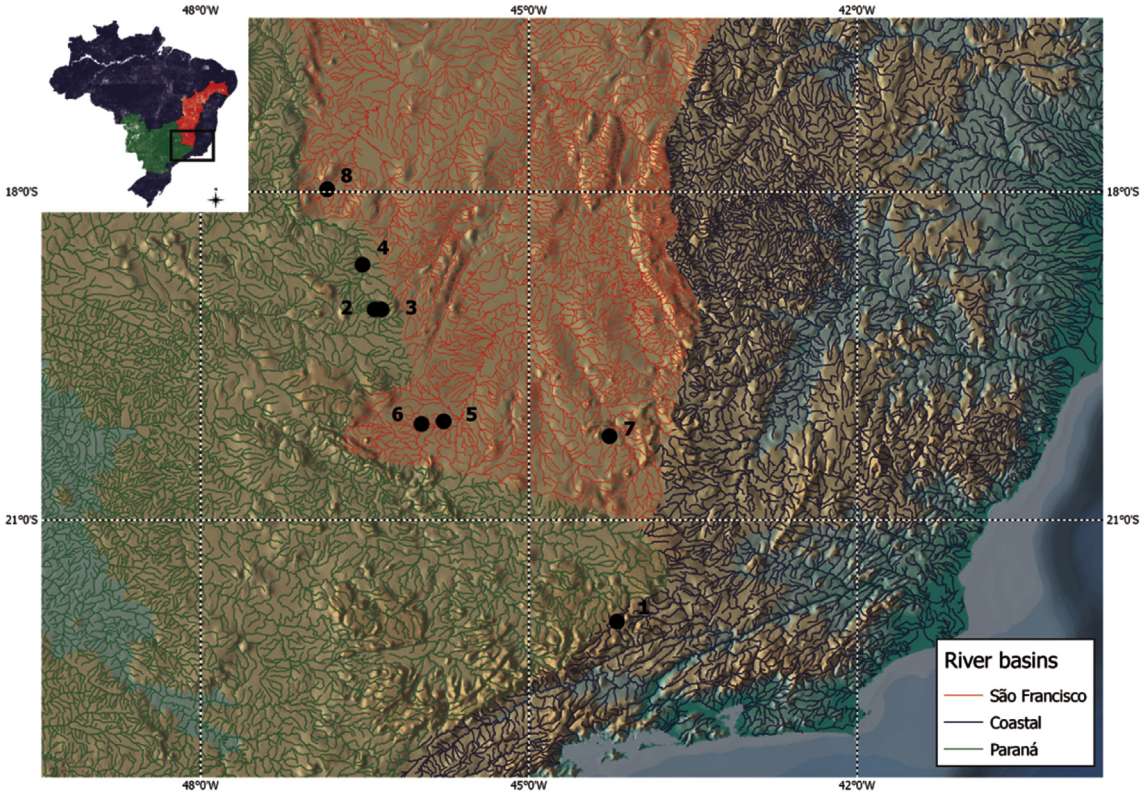

Fig. 1. Map of the region of Upper Paranaíba showing collection sites. 1. Grande River; 2. Paranaíba River A; 3. Paraíso stream; 4. Paranaíba River B; 5. Mombaça River; 6. Das Araras weir; 7. Pará River; 8. Santa Catarina River. Brazil map with major hydrographic basins in the detail.

2. Material and methods

All the specimens and DNA samples used in this study were deposited in the DNA and Collection of Vertebrates and DNA of the Laboratory of Ecological and Evolutionary Genetics of the Federal University of Viçosa (Rio Paranaíba campus) (Suppl. 1) and were collected on the basis of field studies conducted during 2008–2010. Collecting permit SISBIO 15571-1 was issued to Prof. Rubens Pazza. The samples of *Astyanax fasciatus* analyzed in the current study originated from nine different locations, distributed throughout the hydrographic basins of the Rio Grande Paranaíba and São Francisco rivers (Fig. 1).

The following point-to-point measurements were taken from specimens using a digital caliper: standard length; head length; pre-dorsal distance; pre-pelvic distance; pre-pectoral distance; pre-anal distance; height of dorsal origin; height of tail peduncle; length of anal bases; length of dorsal bases; length of pelvic bases; length of pectoral bases; head height; snout length; eye diameter; interorbital diameter; and jaw length.

The multivariate analysis was performed using the PAST software package (Hammer et al., 2001) after correction in order to avoid the effects of size deviations among different populations (size-free).

Five representative individuals from each site sampled were used whenever possible. We examined mitochondrial segments of subunits 6 and 8 of the ATPase gene and Cytochrome Oxidase I (COI). For the polymerase chain reactions (PCRs) we used the pairs of primers ATP8.2-L8331 (5′-AAAGCRTTRGCCTTTTAAAGC-3′) and CO3.2-H9236 (5′-GTTAGTGGTCAGGGCTTGGRTC-3′) (Sivasundar et al., 2001); for ATPase and FishF1 (5′-TCAACCAACCACAAAGACATTGGCAC-3′); and FishR1 (5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′) (Ward et al., 2005) for COI, which amplified a segment of approximately 900 and 650 bp, respectively.

The amplification reactions were conducted in a thermal cycler with a total volume of 25 μL, containing 15 ng of DNA template, Tris-KCl (20 mM Tris-HCl pH 8.4 and 50 mM KCl), 1.5 mM $MgCl_2$, 2.5 μM of each primer, 0.1 mM of each dNTP, and 2.5 U Taq-polymerase. The reaction conditions were as follows: initial denaturation of 94 °C for 4 min, hydridization at 56 °C for 30 s, and extension at 72 °C for 2 min, followed by 40 cycles of 15 s at 94 °C, 30 s at 56 °C, 2 min at 72 °C, and a final extension step for 10 min at 72 °C (Prioli et al., 2002). After checking amplification via 1% agarose gel, the samples were sent to a purification and sequencing outsource service.

The sequences obtained were visualized and edited using the program Chromas Lite v2.01, and further verified at GenBank (http://www.ncbi.nlm.nih.gov) using the Blastn program. Subsequently, the sequences were aligned using the algorithm ClustalW v1.6 (Thompson et al., 1994) by the software MEGA v5 (Tamura et al., 2011), applying the penalties for the