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# PLASMA ESTERASE POLYMORPHISM: A FEASIBLE TOOL IN RESEARCH ON DISCUS FISH STOCKS

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ABSTRACT

Starch gel eletrophoresis was applied to investigate a plasma esterase polymorphism at the locus Est-4 in discus fish *Symphysodon aequifasciatus* and *Symphysodon discus* which are very popular and expensive ornamental cichlids found in the Amazon Basin. Five presumptive genotypes: Est-$4^{AA}$, Est-$4^{AB}$, Est-$4^{AC}$, Est-$4^{BB}$ and Est-$4^{BC}$ were observed in *S. aequifasciatus* and two: Est-$4^{AB}$ and Est-$4^{AC}$ in *S. discus* of six theoretically possible ones. The genotypes were supposedly encoded by three co-dominant alleles: Est-$4^{A}$, Est-$4^{B}$ and Est-$4^{C}$ with allelic segregation following a Mendelian model. This polymorphism may contribute to researches towards the identification and delimitation of fish discus stocks in the Amazon region.

**Keywords:** discus fish, eletrophoresis, esterase, polymorphism.

RESUMO

Eletroforese em gel de amido foi aplicada para investigar um polimorfismo no loco Est-4 da enzima esterase do plasma sanguíneo de peixes ciclídeos ornamentais de alto valor econômico, popularmente conhecidos na Amazônia como acarás-disco: *Symphysodon aequifasciatus* e *Symphysodon discus*. Cinco supostos genótipos foram observados em *S. aequifasciatus*: Est-$4^{AA}$, Est-$4^{AB}$, Est-$4^{AC}$, Est-$4^{BB}$ e Est-$4^{BC}$, e dois em *S. discus*: Est-$4^{AB}$ e Est-$4^{AC}$, dos seis teoricamente possíveis. Os genótipos foram supostamente codificados por três alelos codominantes: Est-$4^{A}$, Est-$4^{B}$ e Est-$4^{C}$ com segregação alélica seguindo o padrão Mendeliano. Este polimorfismo pode contribuir com as pesquisas relacionadas à identificação e delimitação dos estoques de acarás-disco na região amazônica.

**Palavras-chave**: acarás-disco, eletroforese, esterase, polimorfismo.

## INTRODUCTION

The ornamental fishing trade in Amazon commercializes annually about 20 million of fishes generating US$ 3 million (Chao, 2001; Silva *et al*., 2008). As part of this trade, the discus fishes, genus *Symphysodon* Heckel, 1840 are popular and expensive ornamental cichlids restricted to areas where seasonal flooding occurs near the mainstream of the Amazon River itself and in the lower reaches of tributaries on the Amazonian floodplain (Kullander, 1996; Bleher *et al*., 2007). They are one of the most intriguing and distinctive groups of fishes among the South American Cichlidae (Ready *et al*., 2006). The polemic on the taxonomic status of these fishes has persisted since the genus *Symphysodon* was first described by Heckel (1840) *apud* Koh et al. (1999). In fact, the validity of the species formally described in this genus has frequently been questioned (Mazeroll & Weiss, 1995; Koh *et al*., 1999) until now. Nevertheless, most recently Ready *et al*. (2006) based on color patterns, morphology and mitochondrial

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DNA (mtDNA) analyses of *Symphysodon* specimens sampled along the Solimões-Amazon River, provided evidence for the existence of a third species, *S. tarzoo*. According to Ready *et al*. (*op. cit*.), the diagnostic phenotypic character of red spots on the anal fin and on the sides of the body distinguishes *S. tarzoo* from the other species (*S. aequifasciatus* and *S. discus*) that have reticulations (Figure 1). Therefore, efforts should be done to find appropriate genetic markers in order to complement studies on the taxonomic complexity of these species.

Plasma proteins (albumin, transferrin, haemoglobin, and one or more esterases) are among the more rapidly evolving genetic markers (Sarich, 1977). Plasma esterases are relatively easy to detect and appear to be polymorphic in several vertebrate species, including fish (Diakov, 1998), reptiles (Flint *et al*., 2000; Bock *et al.,* 2001), birds (Corbin *et al.*, 1974; Kuznetsov *et al*., 1998; Slavénaité & Sruoga, 2002), mammals (Bell *et al.*, 1998; Díaz *et al*., 2002; Jiskrová *et al.,* 2002; Vinocur *et al*., 2003; Erdoğan & Özbeyaz, 2004; Nunes *et al*., 2005; Šveistienė & Jatkauskienė, 2006), among others, and thus suitable in population genetic studies.

The aim of this study is to consider the potential application of plasma esterase polymorphism in detecting and delimiting natural stocks of discus fish.

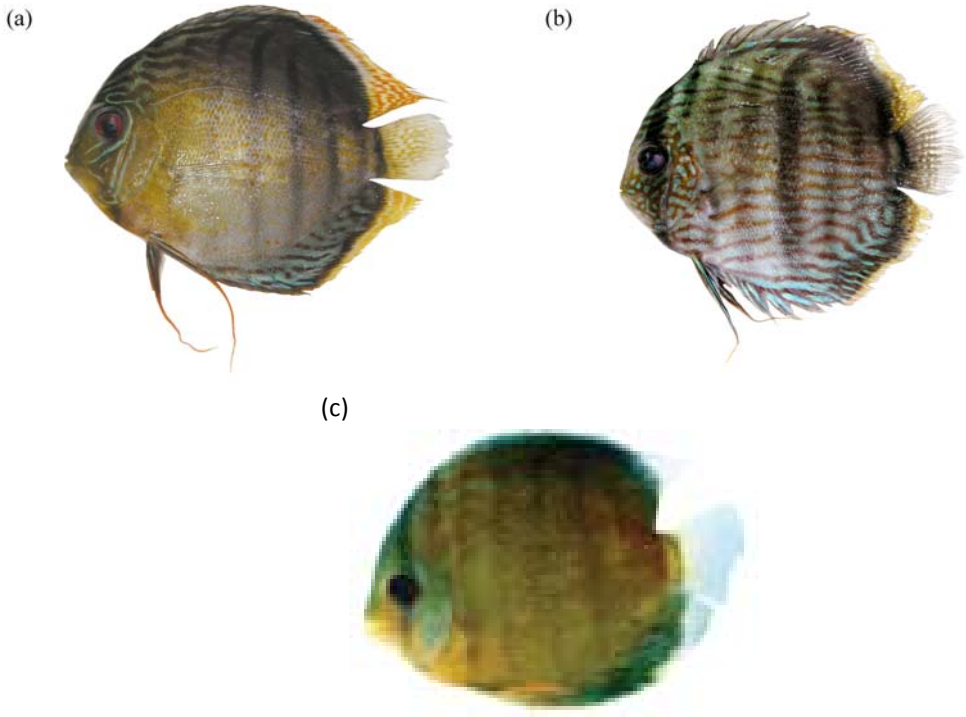


**Figure 1.** Discus fish species: (a) *Symphysodon aequifasciatus*; (b) *S. discus*; and (c) *S. tarzoo*. Picture of *S. tarzoo* from Ready et al. (2006).

## MATERIAL AND METHODS

Blood samples were drawn from twenty five live specimens of discus fish which were kindly donated by the ornamental fish exporters in Manaus, Amazon State, Brazil: Turkys Aquarium Ltda (10 *Symphysodon aequifasciatus* and 5 *S. discus*) and K2-Peixes Tropicais (10 *Symphysodon aequifasciatus*). Due to the minute amount of blood in discus fish, small needles of BD (0.55×20 - 24 G1/4) were used to collect about 1 ml of blood from each fish specimen. Blood was drawn from the dorsal veins and plasma specimens were separated by centrifugation, stored in a freezer, and later thawed moments before the electrophoresis experiments according to the method described by Teixeira *et al*. (2002).

Electrophoresis separation of discus fish plasma esterases was best resolved on Sigma starch gel at a concentration of 8.35%. All other electrophoretic procedures, related to the preparation of gel-electrode buffers and staining recipe to detect esterases by using á-naphthyl acetate as specific substract were those described by Ridgway *et al*. (1970). Plasma specimens were absorbed in 8×5 mm rectangular filter papers (Whatman 3 MM), and then inserted on the gels. A potential of 150 V for each gel slab was applied for a period of 4 h and 30 minutes with the electrophoretic migration taking place towards the anode. The putative co-dominant esterase alleles were classified alphabetically according to their decreasing electrophoretic mobilities (Figure 2).

## RESULTS AND DISCUSSION

The esterase enzyme extracted from the blood plasma of discus fish *Symphysodon aequifasciatus* and *Symphysodon discus*, revealed a polymorphism at the locus Est-4, detected in a zone of eletrophoretic activity located in the intermediate region of the gel. Individual homozygote fish was identified as a single esterase band, and individual heterozygote fish as double esterase bands (Figure 2). In *S. aequifasciatus* five genotypes: Est-$4^{AA}$, Est-$4^{AB}$, Est-$4^{AC}$, Est-$4^{BB}$ and Est-$4^{BC}$; in *S. discus* two: Est-$4^{AB}$ and Est-$4^{AC}$ of six theoretically possible ones, were detected presumably encoded by three co-dominant alleles: Est-$4^{A}$, Est-$4^{B}$ and Est-$4^{C}$ segregating at the Est-4 locus following a Mendelian pattern (Figure 2, Table 1). This polymorphism differs from the esterase monomorphism described by Silva *et al*. (2008) in white skeletal muscles of these two species, in which three fixed loci: Est-1 and Est-2 (in the anodic region of the gel) and Est-3 (in the cathode region) were identified. On the other hand, the present report confirms what was predicted by the above authors who did not rule out an eventual existence of discus fish isoenzyme polymorphism in other gene loci to be analyzed in the future.

Despite the small population sample size of *Symphysodon aequifasciatus* examined (N = 20) and even conscious that in this case the application of Hardy-Weinberg equilibrium test can not lead to any conclusive result, the population sample of this species, when preliminarily submitted to this test, showed a good genetic balance for the Est-4 locus (Table 1). Due to the very small population sample size of *S. discus* (N = 5), the above test was not applied to this sample. Additional population samples of these two species should be analyzed in the future to test for genetic equilibrium at this locus.

The polymorphic locus Est-4 detected in the blood plasma of the *Symphysodon aequifasciatus* and *S. discus* may be included in the list of isozyme markers to be used in future surveys on population genetics of discus fish.

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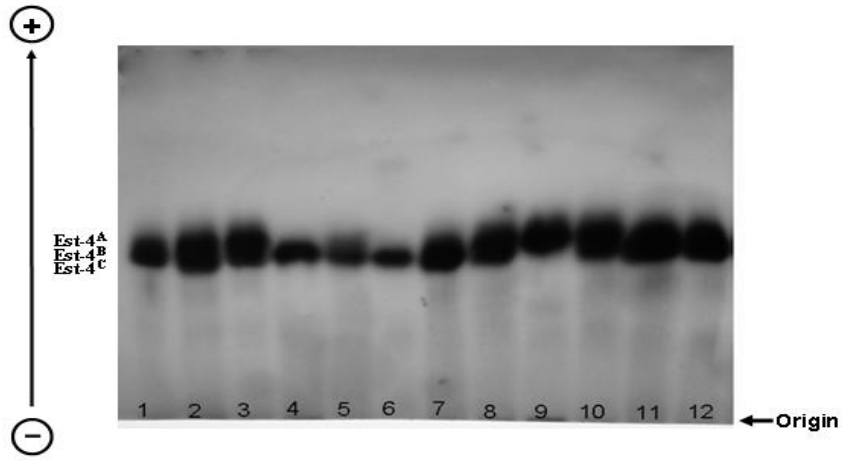


**Figure 2.** Zymogram of plasma esterase genotypes in discus fish showing the polymorphism at the locus Est-4. The sample genotypes are: in *Symphysodon aequifasciatus*, lane 1 (Est-$4^{AB}$), lanes 4 to 6 (Est-$4^{BB}$), lane 7 (Est-$4^{BC}$), lanes 8 and 10 (Est-$4^{AC}$), and lane 9 (Est-$4^{AA}$); in *Symphysodon discus*, lanes 2 and 3 (Est-$4^{AC}$), lanes 11 and 12 (Est-$4^{AB}$).

**Table 1.** Genotype and allele frequency distributions at the esterase locus Est-4 detected in the blood plasma of discus fish *Symphysodon aequifasciatus* and *S. discus.* Chi-square ($\chi^2$) test assuming Hardy-Weimberg equilibrium was applied to test for genetic balance in *Symphysodon aequifasciatus* population sample. Expected numbers of genotypes are shown in parentheses.

| | *Symphysodon aequifasciatus* N = 20* | *Symphysodon discus* N = 5 |
|---|---|---|
| **Plasma esterase genotypes** | | |
| Est- $4^{AA}$ | 2 (1.25) | 0 |
| Est- $4^{AB}$ | 2 (5.25) | 3 |
| Est- $4^{AC}$ | 4 (2.25) | 2 |
| Est- $4^{BB}$ | 7 (5.51) | 0 |
| Est- $4^{BC}$ | 5 (4.72) | 0 |
| Est- $4^{CC}$ | 0 (1.01) | 0 |
| **Plasma esterase allele frequencies** | | |
| Est- $4^{A}$ | 0.250 | 0.50 |
| Est- $4^{B}$ | 0.525 | 0.30 |
| Est- $4^{C}$ | 0.225 | 0.20 |
| **Hardy- Weimberg test** | | |
| df | 3 | - |
| $\chi^2$ | 5.252 | - |
| p | 0.20 - 0.10 | - |

*Pooled samples of *Symphysodon aequifasciatus* donated by the ornamental fish exporters Turkys Aquarium Ltda and K-2 Peixes Tropicais.