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Incorrect Identification in the Marketing of Serrasalmid Fishes: A Threat to Native Species and Productivity in the Aquaculture Industry

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Abstract: Interspecific hybridization can offer advantages in fish aquaculture by enhancing traits like growth rate and disease resistance. However, improper management can result in heterogeneous breeding stocks, which pose risks if hybrids interbreed with native populations. This can lead to loss of genetic diversity and alterations in population structure. This study aimed to evaluate the prevalence of hybridization within the Brazilian aquaculture industry, focusing on the economically significant Serrasalmid species, such as *Piaractus mesopotamicus* (pacu), *Piaractus brachypomus* (pirapitinga), *Colossoma macropomum* (tambaqui), and their hybrids. Using molecular markers (TROP and APOC SNP markers), 312 individuals from the Companhia de Entrepostos e Armazéns Gerais de São Paulo (CEAGESP), the largest warehouse in Latin America, were assessed. The results revealed that 80% of the samples were misidentified, with a substantial proportion of hybrids (72.12%). Among these hybrids, 71.6% were classified as F1 hybrids, while 28.4% were post-F1 hybrids. These findings highlight the need for improved genetic monitoring and management practices in Brazil's fish production industry, emphasizing the importance of sustainable practices to ensure the long-term viability of aquaculture while preserving native genetic resources.

Keywords: biological conservation; fish farms; molecular markers; hybrids; food security

Key Contribution: The molecular markers used proved to be an effective tool for the certification of pure and hybrid strains of fish. Considering only the lots that were marketed as pure lineages, 89.85% of the samples were marketed erroneously. Adaptations are necessary in the production chain of Serrasalmid fish to ensure the sustainability of this productive modality.

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1. Introduction

Fish farming, the most prominent subcategory of global aquaculture, reached a production volume of 57.5 million tons in 2022 [1], highlighting its crucial role in global food security and economic growth. In Brazil, fish farming plays a significant part in the

aquaculture sector, with the latest IBGE report (2018) [2] indicating a total production of 519,000 tons. Of this, 143,000 tons consisted of Serrasalmidae roundfish, including pacu (*Piaractus mesopotamicus*), pirapitinga (*Piaractus brachypomus*), tambaqui (*Colossoma macropomum*), and their hybrids.

The production of interspecific hybrids is a widespread practice in Brazilian aquaculture, aiming to improve zootechnical traits through "hybrid vigor" or "positive heterosis", in which hybrids may exhibit enhanced growth or resilience compared to their parental species [3–5]. Despite these advantages, some studies suggest that pure species may still outperform hybrids in certain aspects, such as reproductive success or disease resistance [6,7]. The extensive production of hybrids, however, raises significant environmental concerns, especially when fertile hybrids interbreed with native populations, potentially altering genetic diversity and impacting ecosystem dynamics [8–11].

Monitoring the genetic composition of Serrasalmidae species has become challenging due to the frequent hybridization in fish farms [9,12–19]. While morphological characteristics have traditionally been used for identification, they often prove inadequate for distinguishing hybrids from pure species. In this context, molecular markers have emerged as a valuable and cost-effective tool for accurately identifying hybrid strains, thereby addressing the limitations of conventional methods [9,20]. Notably, instances of backcrossing with hybrids such as "patinga" (female *P. mesopotamicus* × male *P. brachypomus*) have already been reported, posing further challenges for stock management [9].

Building on previous findings of mislabeling and intergenus backcrossing at private fish farms, this study used molecular markers to assess the prevalence of misidentification and hybridization in Serrasalmidae fish sold at the Companhia de Entrepostos e Armazéns Gerais de São Paulo (CEAGESP), Latin America's second largest warehouse, influencing the commerce of fishes in at least four states of Brazil (São Paulo, Rio de Janeiro, Santa Catarina, and Rio Grande do Sul). Our findings provide insights that could enhance monitoring practices and support sustainable aquaculture.

2. Materials and Methods

2.1. Sampling

Specimen sampling was conducted at the CEAGESP, São Paulo, Brazil, to assess individuals from the fish species *Piaractus mesopotamicus* (pacu), *Piaractus brachypomus* (pirapitinga), and *Colossoma macropomum* (tambaqui). A total of 312 specimens were collected from 15 sales booths. To facilitate subsequent molecular analyses, fin clips were excised from each fish and immediately preserved in 95% ethanol for DNA extraction. Additionally, the nomenclature used by sellers during commercial trade was documented, allowing for later cross-referencing with molecular identification results. Pure specimens, as described [18], were used as control samples in the present study to ensure the accuracy of the molecular identification process. DNA was extracted from tissue samples using the "Wizard Genomic DNA Purification Kit-Promega".

2.2. Molecular Markers and PCR

For hybrid identification, the TROP (*α-tropomyosin*) SNP marker [18] was selected for analysis through multiplex PCR (Table 1). In addition, we developed a new PCR multiplex molecular marker in this study, named APOC (*Apoliprotein C-I like*). To achieve this, we analyzed transcriptome libraries from *Piaractus mesopotamicus* [21], *Colossoma macropomum* [22], and *Piaractus brachypomus* [23], with SRA accession numbers SRR2924956, SRR5122711, and SRR6303971, respectively. These libraries, obtained in previous studies, were sequenced using the Roche/454 GS FLX Titanium platform. Further details can

be found in the original publications, and additional information about these libraries is provided in Supplementary Table S1.

Table 1. Species-specific and universal primers used for amplification of markers. Pm: *Piaractus mesopotamicus*, Pb: *Piaractus brachypomus*, Cm: *Colossoma macropomum*, Serra: Serrasalmidae, F: Forward, R: Reverse, Trop: *α-tropomyosin*, Apoc: *Apoliprotein C-I like*.

Primer	Primer Sequence	References
Trop Pm R	5′-CTTCAGCTGGATCTCCTGA-3′	[18]
Trop Pb R	5′-TTGACTTTATGCCACACAAAT-3′	
Trop Cm R	5′-ATACAACAATGCCATCGCT-3′	
Trop Serra F	5′-GAGTTGGATCGGGCTCAG-3′	
Apoc Pm F	5′-AGAAGCTGGAAGAGAGCGAGC-3′	Present study
Apoc Pb R	5′-TTTAACTTACTCATCTGCTCATTGAT-3′	
Apoc Cm R	5′-TCGGACAGATCCTTTCCCAA-3′	
Apoc Serra F	5′-CGCTGATGCTCGTGCTTCTT-3′	
Apoc Serra R	5′-AATGAGGCGTAGCAATATCACA-3′	

Initially, sequence quality filtering was performed using Roche Newbler software (v. 2.6) [24] to ensure high-quality data. Following this, the transcriptomes of the three species were *de novo* assembled according to the method described by [25]. *De novo* transcriptome assemblies were performed using CLC Genomics Workbench (Aarhus, Denmark). Two different approaches were used: (i) RNA-seq sequences were assembled combining sequencing reads from two species in the following combinations: *Piaractus mesopotamicus* × *Piaractus brachypomus*, *Colossoma macropomum* × *Piaractus mesopotamicus*, and *Colossoma macropomum* × *Piaractus brachypomus*. For these mixed assemblies, all sequencing reads from each species pair were combined prior to assembly to maximize transcript representation. (ii) Species-specific assemblies: Separate, independent assemblies were performed for each species (*Piaractus mesopotamicus*, *Piaractus brachypomus*, and *Colossoma macropomum*) using their respective RNA-Seq sequences. Then, SNPs were identified using CLC Genomics Workbench with a minimum read coverage of 15, minor allele frequency of 20% (at least 4 reads), and base quality (Q) $\geq$ 20 [26]. Finally, the contigs were annotated through a similarity search using the BlastX tool.

PCR reactions were conducted using a Mastercycler Personal thermocycler (Eppendorf) with the following conditions: 150 µM of each dNTP, 1.5 mM of MgCl2, 1X Taq Buffer, 0.5 U of Taq polymerase (Invitrogen, Carlsbad, CA, USA), 0.1 to 0.4 µM of each primer, and 10 to 50 ng of genomic DNA in a final volume of 25 µL. Detailed cycling conditions are provided in Table 2.

Table 2. Conditions of reactions used to obtain amplicons.

Gene		Denaturation (95 °C)	Cycles	Extension (72 °C)	Reference
Nuclear	TROP	5 min	35× 95 °C/30 s, 60 °C/30 s, 72 °C/10 s	7 min	[18]
	APOC	5 min	35× 95 °C/30 s, 61 °C/30 s, 72 °C/30 s	7 min	Present study

Min: minutes; s: seconds; TROP: *α-tropomyosin*; APOC: *Apoliprotein C-I like*.

The expected and observed banding patterns for each of the pure species using the different molecular markers are exemplified in Figure 1.

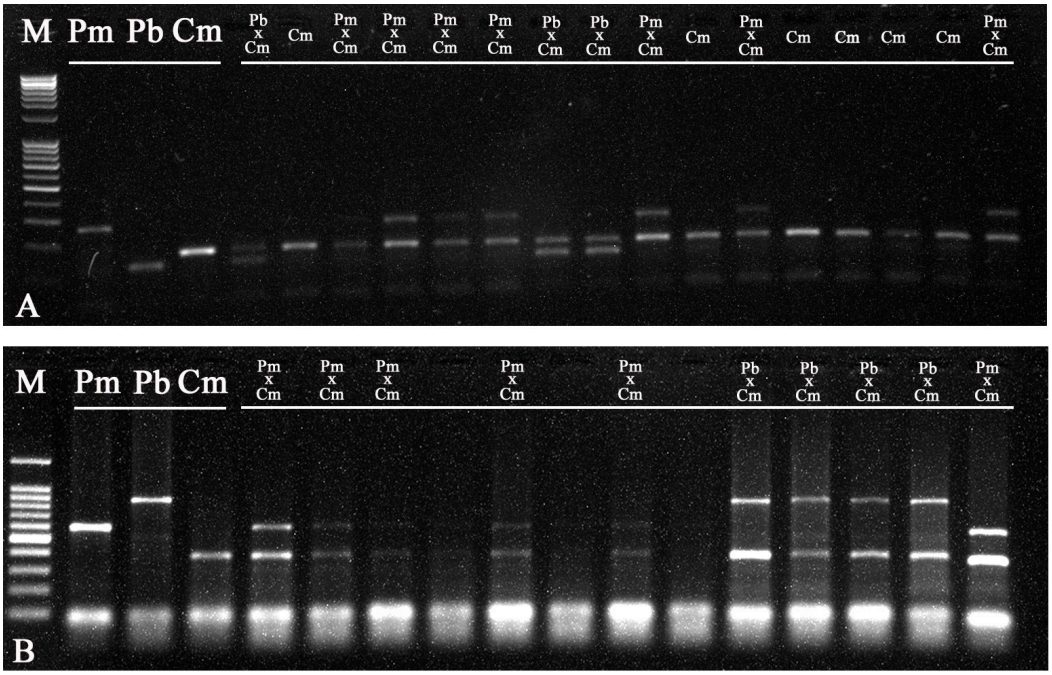

Figure 1. Agarose gel containing three control individuals per species for each marker. (**A**) TROP control (*Piaractus mesopotamicus*–269 bp; *Piaractus brachypomus*–131 bp; *Colossoma macropomum*–172) and the respective hybrids; (**B**) APOC control (*Piaractus mesopotamicus*–400 bp; *Piaractus brachypomus*–640 bp; *Colossoma macropomum*–280) and the respective hybrids; Pm (*Piaractus mesopotamicus*); Pb (*Piaractus brachypomus*); Cm (*Colossoma macropomum*); Pb × Cm (*Piaractus brachypomus* × *Colossoma macropomum*); Pm × Cm (*Piaractus mesopotamicus* × *Colossoma macropomum*); (M) 1kb Ladder.

3. Results

The *de novo* transcriptome assembly, combining sequencing reads from different species, generated numerous contigs and identified interspecific SNPs, as summarized in Table 3. One gene, annotated as *Apolipoprotein C-I like* (herein called APOC), was selected, and species-specific primers were designed to be used in PCR multiplex reactions (Supplementary Figure S1).

Table 3. Summary of *de novo* transcriptome assemblies using mixed reads.

Assembly	Pacu × Pirapitinga	Tambaqui × Pacu	Tambaqui × Pirapitinga
Number of contigs	8273	12,431	11,714
Average contig length (bp)	775	767	766
Average reads per contig	8	7	6
N50	803	794	793
N90	501	502	499
Interspecific SNPs	811	2430	2080

Among the 312 specimens sampled from 15 sales booths, 80% were misidentified. This percentage was even more concerning when considering only commercial lots of pure species, where the misidentification rate reached 89.85%. Additionally, F1 hybrids and post-F1 hybrid strains accounted for 51.6% and 20.51% of the specimens (Figure 1), respectively (Table 4), while only 27.88% of the individuals were identified as pure specimens.

Table 4. Genetic identification of Serrasalmid fish species trade in stalls and frequency of erroneously sold products. (B): trade stalls (N): number of samples, Pm: *Piaractus mesopotamicus*, Pb: *Piaractus brachypomus*, Cm: *Colossoma macropomum*, HF_1: first hybrid generation, HA: post-F_1 hybrid generation), -: absence of fragment.

(B)/(N)	Merchant Identification	Genetic Identification					Error (%)
		Pm	Pb	Cm	HF_1	HA	
01/(34)	Hybrid	-	-	-	21	13	0
02/(35)	*C. macropomum*	-	-	-	16	19	100
03/(22)	Hybrid	-	-	-	16	6	0
04/(28)	*C. macropomum*	-	-	-	16	12	100
05/(21)	*P. mesopotamicus*	-	-	-	15	6	100
06/(8)	*P. mesopotamicus*	-	-	-	-	8	100
07/(29)	*P. mesopotamicus*	-	-	1	28	-	100
08/(24)	*P. mesopotamicus*	-	-	-	24	-	100
09/(5)	*P. mesopotamicus*	-	-	-	5	-	100
10/(26)	*C. macropomum*	-	-	26	-	-	0
11/(19)	*P. mesopotamicus*	-	-	15	4	-	100
12/(14)	*P. mesopotamicus*	-	-	13	1	-	100
13/(23)	*P. mesopotamicus*	-	-	19	4	-	100
14/(10)	*P. mesopotamicus*	-	-	1	9	-	100
15/(14)	*P. mesopotamicus*	-	-	12	2	-	100
Total (15/312)				87	161	64	
Identity frequency				27.88	51.60	20.51	
Error frequency							80

At only one booth (bench 10), the products being sold consisted exclusively of pure parental specimens, all identified as C. macropomum (Table 4). A significant proportion of specimens, particularly in booths 1 to 6 (Table 5, Supplementary Tables S2–S15), showed genomes from the three parental species or a higher proportion of one reference genotype, likely due to advanced hybridization processes leading to genetic introgression of the pure species (Figure 2).

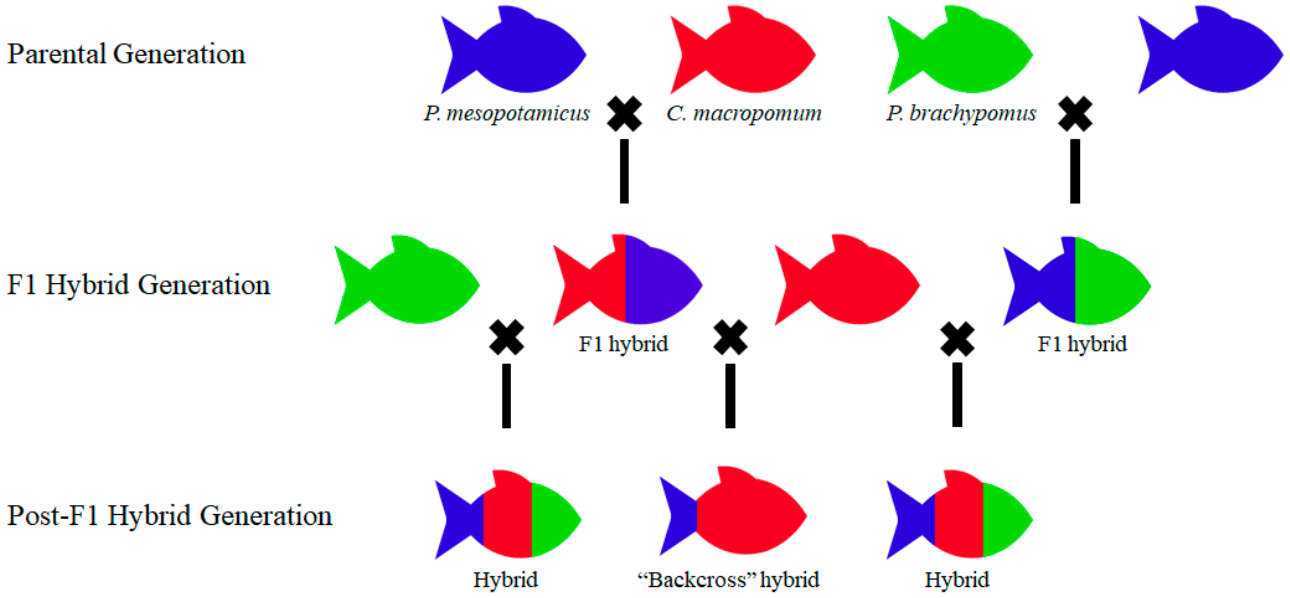

Figure 2. Illustrative scheme representing the possible crosses responsible for obtaining the hybrid strains identified by the analysis.

Table 5. Genetic identification of Serrasalmid fish species trade in sale booth 1.

Commercial Identification	Sample	TROP			APOC			Genetic Identification
		Pm	Pb	Cm	Pm	Pb	Cm	
Sale booth 1 Hybrids	1–1	-	-	X	X	-	X	HA
	1–2	X	-	X	X	-	X	HF_1
	1–3	X	-	X	X	-	X	HF_1
	1–4	-	-	X	X	-	X	HA
	1–5	-	-	X	X	-	X	HA
	1–6	X	-	X	X	-	X	HF_1
	1–7	X	-	X	-	-	X	HA
	1–8	X	-	X	X	-	X	HF_1
	1–9	X	-	X	X	-	X	HF_1
	1–10	X	-	X	X	-	X	HF_1
	1–11	-	-	X	X	-	X	HA
	1–12	X	-	X	X	-	X	HF_1
	1–13	-	-	X	X	-	X	HA
	1–14	-	-	X	X	-	X	HA
	1–15	-	X	X	X	-	X	HA
	1–16	-	-	X	X	-	X	HA
	1–17	-	-	X	X	-	X	HA
	1–18	X	-	X	X	-	X	HF_1
	1–19	X	-	X	X	-	X	HF_1
	1–20	X	-	X	X	-	X	HF_1
	1–21	X	-	X	-	-	-	HF_1
	1–22	X	-	X	X	-	X	HF_1
	1–23	X	-	X	X	-	X	HF_1
	1–24	X	-	X	X	-	X	HF_1
	1–25	X	-	X	-	-	-	HF_1
	1–26	X	-	X	X	-	X	HF_1
	1–27	-	X	X	-	-	-	HF_1
	1–28	X	-	X	X	-	X	HF_1
	1–29	-	X	X	X	-	X	HA
	1–30	X	-	X	X	-	X	HF_1
	1–31	-	X	X	-	-	-	HF_1
	1–32	-	-	X	X	-	X	HA
	1–33	X	-	X	-	-	-	HF_1
	1–34	-	X	X	X	-	X	HA

Pm: *Piaractus mesopotamicus*, Pb: *Piaractus brachypomus*, Cm: *Colossoma macropomum*, HF_1: first hybrid generation, HA: post-F_1 hybrid generation (highlighted), TROP: *α-tropomyosin*, APOC: *Apoliprotein C-I like*, -: absence of fragment, X: presence of fragment.

The results obtained here enabled the correct identification of pure lineages and clarified the potential crosses responsible for the observed hybrid categories. The F1 hybrids "patinga" (female *P. mesopotamicus* × male *P. brachypomus*) and "tambacu" (female *C. macropomum* × male *P. mesopotamicus*) were identified as the likely ancestors of the post-F_1 hybrid lineages (Figure 2).

4. Discussion

This study confirms the persistent disorganization within the Serrasalmid fish aquaculture industry. Previous studies have already identified challenges related to species misidentification and inaccurate descriptions during both the production and commercialization stages, with parental species frequently replaced by hybrids in juvenile stocks, which is a frequent issue in the production chain [9,18]. According to [27], only a limited number of producers manage both breeding and fattening stages, fragmenting the production process into two groups: (i) juvenile producers supplying stock to other facilities and (ii) fish farmers focused on cultivation and product preparation. This scenario poses significant risks, particularly when hybrid specimens are misidentified and traded, leading to biological and commercial threats to the integrity of Serrasalmid aquaculture.

The genetic introgression observed in this study poses a potential threat to aquaculture productivity. Hybrid backcrossing and interbreeding can lead to the dilution or loss of desirable zootechnical traits, increased mortality, and reduced offspring viability [9,28].

Additionally, many Brazilian aquaculture facilities operate under inconsistent licensing and regulatory frameworks, with fish production laws varying between states.

In the State of São Paulo, Decree No. 62.243 governs the production of hybrids involving exotic species, with the Fisheries Institute issuing authorizations for specific species. The decree regulates the production of hybrids such as "patinga" (*P. mesopotamicus* × *P. brachypomus*), "tambacu" (*C. macropomum* × *P. mesopotamicus*), and "tambatinga" (*C. macropomum* × *P. brachypomus*) within the Paraná, Southeast Atlantic, and South Atlantic basins. This raises conservation concerns, particularly since *P. mesopotamicus* (pacu) is native to the Paraná River basin. The cultivation of hybrids in reservoirs and pond nurseries also increases the risk of escape into natural environments, posing ecological risks.

In response to these challenges, researchers have called for urgent regulatory reforms, including the adoption of molecular tools for accurate species identification throughout the production chain [3,19]. Molecular markers are particularly useful as they surpass the limitations of morphological identification, which often underestimates hybrid frequencies [29]. As suggested by [3], the application of molecular markers at all stages of production could mitigate genetic introgression risks and prevent the mislabeling and sale of uncertified products [9].

Moreover, the lack of standardization in commercial fish nomenclature exacerbates species substitution in trade [30]. Standardizing commercial names for economically important native species, as recommended by [30], would be critical for biodiversity conservation and consumer safety. One potential framework is the adoption of the NCM (Mercosur Common Nomenclature), an eight-digit code used to classify goods and species in international trade within Mercosur. Unifying trade nomenclatures for native Brazilian species would significantly improve market regulation, as inconsistent vernacular names across states contribute to disorganization in the production chain and undermine the sustainability of the aquaculture industry by enabling species mislabeling under a single commercial name.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes10020083/s1, Table S1. Summary statistics of three fish transcriptome single-read 454 Roche libraries obtained using seqkit stats; Table S2. Genetic identification of Serrasalmid fish species trade in sale booth 2. Pm: *Piaractus mesopotamicus*, Pb: *Piaractus brachypomus*, Cm: *Colossoma macropomum*, HF1: first hybrid generation, HA: post-F_1 hybrid generation, TROP: *α-tropomyosin*, APOC: *Apoliprotein C-I like*, -: Absence of fragment, X: Presence of frangment; Table S3. Genetic identification of Serrasalmid fish species trade in sale booth 3. Pm: *Piaractus mesopotamicus*, Pb: *Piaractus brachypomus*, Cm: *Colossoma macropomum*, HF1: first hybrid generation, HA: post-F_1 hybrid generation, TROP: *α-tropomyosin*, APOC: *Apoliprotein C-I like*, -: Absence of fragment, X: Presence of frangment; Table S4. Genetic identification of Serrasalmid fish species trade in sale booth 4. Pm: *Piaractus mesopotamicus*, Pb: *Piaractus brachypomus*, Cm: *Colossoma macropomum*, HF1: first hybrid generation, HA: post-F_1 hybrid generation, TROP: *α-tropomyosin*, APOC: *Apoliprotein C-I like*, -: Absence of fragment, X: Presence of frangment; Table S5. Genetic identification of Serrasalmid fish species trade in sale booth 5. Pm: *Piaractus mesopotamicus*, Pb: Piaractus brachypomus, Cm: Colossoma macropomum, HF1: first hybrid generation, HA: post-F_1 hybrid generation, TROP: α-tropomyosin, APOC: Apoliprotein C-I like, -: Absence of fragment, X: Presence of frangment; Table S6. Genetic identification of Serrasalmid fish species trade in sale booth 6. Pm: *Piaractus mesopotamicus*, Pb: *Piaractus brachypomus*, Cm: *Colossoma macropomum*, HF1: first hybrid generation, HA: post-F_1 hybrid generation, TROP: *α-tropomyosin*, APOC: *Apoliprotein C-I like*, -: Absence of fragment, X: Presence of frangment; Table S7. Genetic identification of Serrasalmid fish species trade in sale booth 7. Pm: *Piaractus mesopotamicus*, Pb: *Piaractus brachypomus*, Cm: *Colossoma macropomum*, HF1: first hybrid generation, HA: post-F_1 hybrid generation, TROP: *α-tropomyosin*, APOC: *Apoliprotein C-I like*, -: Absence of fragment, X: Presence of frangment; Table S8. Genetic identification of Serrasalmid fish

species trade in sale booth 8. Pm: *Piaractus mesopotamicus*, Pb: *Piaractus brachypomus*, Cm: *Colossoma macropomum*, HF1: first hybrid generation, HA: post-F_1 hybrid generation, TROP: *α-tropomyosin*, APOC: *Apoliprotein C-I like*, -: Absence of fragment, X: Presence of frangment; Table S9. Genetic identification of Serrasalmid fish species trade in sale booth 9. Pm: *Piaractus mesopotamicus*, Pb: *Piaractus brachypomus*, Cm: *Colossoma macropomum*, HF1: first hybrid generation, HA: post-F_1 hybrid generation, TROP: *α-tropomyosin*, APOC: *Apoliprotein C-I like*, -: Absence of fragment, X: Presence of frangment; Table S10. Genetic identification of Serrasalmid fish species trade in sale booth 10. Pm: *Piaractus mesopotamicus*, Pb: *Piaractus brachypomus*, Cm: *Colossoma macropomum*, HF1: first hybrid generation, HA: post-F_1 hybrid generation, TROP: *α-tropomyosin*, APOC: *Apoliprotein C-I like*, -: Absence of fragment, X: Presence of frangment; Table S11. Genetic identification of Serrasalmid fish species trade in sale booth 11. Pm: *Piaractus mesopotamicus*, Pb: *Piaractus brachypomus*, Cm: *Colossoma macropomum*, HF1: first hybrid generation, HA: post-F_1 hybrid generation, TROP: *α-tropomyosin*, APOC: *Apoliprotein C-I like*, -: Absence of fragment, X: Presence of frangment; Table S12. Genetic identification of Serrasalmid fish species trade in sale booth 12. Pm: *Piaractus mesopotamicus*, Pb: *Piaractus brachypomus*, Cm: *Colossoma macropomum*, HF1: first hybrid generation, HA: post-F_1 hybrid generation, TROP: *α-tropomyosin*, APOC: *Apoliprotein C-I like*, -: Absence of fragment, X: Presence of frangment.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclucions of this article will be made available by the authors on request.

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Conflicts of Interest: The authors declare no conflicts of interest.

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