

ORIGINAL ARTICLE

Transferability of heterologous primers in *Brycon falcatus*

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ABSTRACT

The genus *Brycon* comprises fish species of significant socioeconomic and biological importance in Brazil. Despite that, the genetic knowledge about these species is scarce, especially regarding *Brycon falcatus*. Thus, the objective of this study was to evaluate the transferability of heterologous microsatellite primers in *B. falcatus* for the first time. Heterologous primers obtained from *B. opalinus*, *B. hilarii*, *B. insignis*, *B. orbignyanus*, *B. amazonicus*, *Prochilodus argenteus*, *Prochilodus lineatus*, *Piaractus mesopotamicus*, and *Colossoma macropomum* were evaluated. The primers that showed the best amplification patterns were applied to a sample of 22 individuals and the genetic parameters were calculated. Nine primers displayed satisfactory cross-amplification with *B. falcatus*: BoM5 (*Brycon opalinus*); Bh8, Bh13 and Bh16 (*B. hilarii*); Borg59 (*B. orbignyanus*); Bag22 (*B. amazonicus*); Par12 and Par80 (*P. argenteus*), and Cm1A8 (*C. macropomum*). The genetic parameters (number of alleles, effective alleles, allele richness, and expected and observed heterozygosity) and the polymorphic information content (PIC) confirmed the viability of these primers for population genetics analyses. Our study demonstrates the potential of transferability of microsatellite markers from related species and even different genera to *B. falcatus*, providing usefull tools for future population genetic studies in this species.

KEYWORDS: Bryconidae, cross amplification, genetic conservation, genetic variability, SSR

Transferibilidade de *primers* heterólogos em *Brycon falcatus*

RESUMO

O gênero *Brycon* compreende um grupo de espécies de peixes de grande importância socioeconômica e biológica no Brasil. Entretanto, o conhecimento genético dessas espécies é escasso, principalmente no caso do *Brycon falcatus*. Diante disso, objetivou-se verificar a transferibilidade de *primers* heterólogos em *B. falcatus*. Foram avaliados *primers* heterólogos provenientes das espécies *B. opalinus*, *B. hilarii*, *B. insignis*, *B. orbignyanus*, *B. amazonicus*, *Prochilodus argenteus*, *Prochilodus lineatus*, *Piaractus mesopotamicus* e *Colossoma macropomum*. Os *primers* que demonstraram melhores padrões de amplificação foram aplicados a uma amostra de 22 indivíduos e os parâmetros genéticos foram calculados. Nove *primers* apresentaram resultados satisfatórios de amplificação cruzada com *B. falcatus*: BoM5 (*Brycon opalinus*), Bh8, Bh13 e Bh16 (*B. hilarii*); Borg59 (*Brycon orbignyanus*); Bag22 (*B. amazonicus*); Par12 and Par80 (*Prochilodus argenteus*) e Cm1A8 (*Colossoma macropomum*). Os parâmetros genéticos (número de alelos, alelos efetivos, riqueza de alelos e heterozigosidade esperada e observada) e o conteúdo de informação polimórfica (PIC) demonstraram a viabilidade da utilização dos *primers* para análises genéticas populacionais. Nosso estudo demonstra o potencial de transferibilidade de marcadores microssatélites de espécies relacionadas e até de gêneros diferentes para *B. falcatus*, fornecendo ferramentas úteis para futuros estudos genéticos populacionais nessa espécie.

PALAVRAS-CHAVE: Bryconidae, amplificação cruzada, conservação genética, variabilidade genética, SSR

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INTRODUCTION

The Neotropical region has the largest number of freshwater fish species in the world, with fish of the order Characiformes standing out for the vast diversity of species, many not yet described (Guisande *et al.* 2012; Ota *et al.* 2015). Within this order, the genus *Brycon* (Müller & Troschel, 1844) is considered one of the main neotropical freshwater fish (Antunes *et al.* 2010). It has a wide geographic distribution, occurring in the Amazonas, Paraná, Paraguai, Orinoco, Magdalena, and Tocantins-Araguaia river basins (Howes, 1982; Antunes *et al.* 2010). *Brycon* species are medium to large sized, live in small shoals, have omnivorous feeding habits (Lima and Castro 2000), and perform fairly extensive reproductive migrations (Goulding 1980). These fish are mostly used for sport fishing and human consumption (Lima 2017).

Brycon falcatus (Muller and Troschel, 1844), regionally known in Brazil as *matrinxã miúda* or *voadeira*, is highlighted within *Brycon* (Bini 2012) because it is a highly consumed species and has great commercial importance in sport and artisanal fishing (Matos *et al.* 2018). It is broadly distributed in the Amazon basin, also occurring in the Araguaia-Tocantins River basin (Antunes *et al.* 2010; Isaac *et al.* 2015; Matos *et al.* 2018). Its rich fish fauna in these basins makes *Brycon* an important exploitable resource, but information on the biological, biogeographical, ecological, and genetic characteristics of its species are generally scarce, especially for *B. falcatus*.

Microsatellites are promising molecular markers to generate genetic information for *B. falcatus*, since they allow to access important genetic information such as population genetic structure, demographic history, kinship and mating system. The most important characteristics of microsatellites are high polymorphism, marker co-dominance and relative abundance with uniform genome coverage (Gasques *et al.* 2013; Abdul-Muneer 2014). One of the main limitations of using microsatellite markers is the need for species-specific primers. However, pairs of primers designed for one species can be used for cross-amplification in phylogenetically related species, allowing genetic studies to use transferred markers or heterologous primers, which help to save time and money (Abdul-Muneer 2014; Castro *et al.* 2017; Lopera-Barrero *et al.* 2016a).

Accordingly, the objective of this study was to evaluate, for the first time, the transferability of heterologous primers from *Brycon opalinus* (Cuvier, 1819), *B. hilarii* (Valenciennes, 1850), *B. insignis* (Steindachner, 1877), *B. orbignyanus* (Valenciennes, 1850), *B. amazonicus* (Spix & Agassiz, 1829), *Prochilodus argenteus* (Agassiz, 1829), *P. lineatus* (Valenciennes, 1836), *Piaractus mesopotamicus* (Holmberg, 1887), and *Colossoma macropomum* (Cuvier, 1818) to *B. falcatus*.

MATERIAL AND METHODS

Caudal fin fragments (approximately 0.5 cm²) were collected from 22 wild *Brycon falcatus* individuals sampled in the Araguaia River (15°53'21.52"S, 52°14'47.79"W), near the municipality of Barra das Garças, state of Mato Grosso, Brazil. The samples were preserved in 70% alcohol for subsequent analysis in the laboratory. This study was approved by the ethics committee in the use of animals of Universidade Estadual de Londrina (CEUA-UEL # 21383.2017.93).

DNA extraction followed the methodology described by Lopera-Barrero *et al.* (2008). The DNA concentration was evaluated in a spectrophotometer SLIPQ 026 - L-Quant Quantifer (Loccus Biotecnologia, Ribeirão Preto, Brazil), and the samples were diluted to a concentration of 30 ng µL⁻¹. DNA integrity was assessed on 1% agarose gel stained with SYBR Safe DNA (Invitrogen). Electrophoresis was performed in 0.5X TBE buffer (250 mM Tris-HCl, 30 mM boric acid, and 41.5 mM EDTA) for one hour at 70 V. The gel was observed using a trans-illuminator and the image was captured by a photo-documentation system. Analyzes were performed at the Aquaculture and Genetics Research Center (NEPAG) of Universidade Estadual de Londrina (UEL).

Thirty-five primers were tested, of which seventeen belonged to *Brycon*: two were described by Barroso *et al.* (2005) for *B. opalinus* (BoM5 and BoM13), five were described by Sanches and Galetti (2006) for *B. hilarii* (Bh5, Bh6, Bh8, Bh13, Bh16), one was described by Matsumoto and Hilsdorf (2009) for *B. insignis* (Bc48-10), three were described by Souza *et al.* (2018a) for *B. orbignyanus* (Borg13, Borg25, and Borg59), and six were described by Araújo (2012) for *B. amazonicus* (Bag22, Bag25, Bag27, Bag31, Bam6, and Bam11). Additionally, we tested primers of related species: five described by Barbosa *et al.* (2006) (Par12, Par14, Par15, Par21, and Par43) and two described by Barbosa *et al.* (2008) (Par80, and Par82) for *Prochilodus argenteus*, one described by Yazbeck and Kalapothakis (2007) for *Prochilodus lineatus* (Pli30), five described by Calcagnotto *et al.* (2001) for *Piaractus mesopotamicus* (Pme2, Pme4, Pme5, Pme20, and Pme28) and five described by Santos *et al.* (2009) for *Colossoma macropomum* (Cm1A8, Cm1A11, Cm1C8, Cm1D1, and Cm1E3).

The DNA was amplified in a 15-µL reaction volume with 1X Tris-KCl buffer, 2.0 mmol L⁻¹ of MgCl₂, 0.4 µmol L⁻¹ of each primer (Forward and Reverse), 0.2 mmol L⁻¹ dNTP, 1U Platinum Taq DNA Polymerase, and 30 ng of the target DNA. A PCR was performed under the following conditions: initial denaturation at 94 °C during four minutes, followed by 35 cycles of denaturation at 94 °C during 30 seconds, annealing (primer-dependent temperature) during 30 seconds, and extension at 72 °C during one minute, and a final extension at 72 °C during 10 minutes. Three different annealing temperatures were tested for each primer: the specific

temperature for each primer (described by the authors), 2 °C below and 2 °C above the specific temperatures. The temperature that generated the best banding pattern was selected for use in the population analysis (Table 1). The amplified samples were run using 10% polyacrylamide gel electrophoresis (acrylamide:bisacrylamide ratio of 29:1), 6 M urea, in 0.5X TBE buffer at 180 V and 250 mA for eight hours. The methodology proposed by Bassam *et al.* (1991) was followed in gel staining, with modifications. The gel was immersed in a fixation solution (10% ethanol and 0.5% acetic acid) for 20 minutes, and then in 6 mM silver nitrate solution for 30 minutes, and revealed in solution with 0.75 M NaOH and 0.22% of 40% formaldehyde, then was photographed for later analysis.

Initially, six *B. falcatus* DNA samples were amplified for each primer for an initial quality assessment and selection of

the best annealing temperature. Primers that presented an adequate band pattern, i.e., a clear pattern and no nonspecific bands, were selected for the next step. The primers with good transferability in the initial test were used in 22 specimens of *B. falcatus* following the same methodology described previously, and genetic parameters were calculated. The number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), and the Hardy-Weinberg equilibrium (Hw) were calculated for each locus using the GenAlex software version 6.5, according to Peakall and Smouse (2012). The coefficient of inbreeding (F_{IS}), the allelic richness (Ra) and allelic frequencies were calculated for each locus using FSTAT software (Goudet 2005). The polymorphic information content (PIC) was analyzed using the software Cervus 3.0.7 (Kalinowski *et al.* 2007). The PIC classification was performed according to Botstein *et al.* (1980) considering

Table 1. Characterization of each heterologous locus of microsatellite primers tested for *Brycon falcatus*. TA°C = annealing temperature; PIC = polymorphic information content; Di-: dinucleotide; Tri-: trinucleotide; bp = base pairs.

Locus	Micro-satellite class	Motif and Repetition	Species	5'-3' Sequence	TA °C	Fragment size - bp (Frequency)	PIC
Bh8	Tri-	(GAT) ₅	<i>Brycon hilarii</i>	F: CCATGGCTCAACACAGATAT R: TGTACGAATCCTGAAATGCT	54	190 (0.182); 200 (0.318); 215 (0.500)	0.542
Bh13	Di-	(AT) ₇	<i>Brycon hilarii</i>	F: AGCAATTTAAGCAAGTGAAG R: GCGTCGGAGCAGTAGTTATA	54	150 (0.250); 165 (0.750)	0.305
Bh16	Tri-	(TAA) ₈	<i>Brycon hilarii</i>	F: CCTCCAATGAAAACAGTGCG R: ACGACTTAGCCACCCACCCT	58	145 (0.182); 160 (0.818)	0.253
BoM5	Complex	(AC) ₄ T(AC) ₁₀ AT(AC) ₅	<i>Brycon opalinus</i>	F: CGACCACAATAGGATTAGGG R: CTGGAGTTTGTGTGTGGA	52	106 (0.159); 110 (0.114); 115 (0.682); 150 (0.045)	0.457
Borg59	Complex	(CT) ₄ CC(CT) ₅ TT (CT) ₅ (CA) ₉ (CT) ₃ N(CT) ₇	<i>Brycon orbignyanus</i>	F: TCCCTCTCTGTCCAATGTCT R: GAAGTCAAGGTTAGAGCGGC	55	225 (0.050); 230 (0.950)	0.090
Bag22	Di-	(GA) ₁₄	<i>Brycon amazonicus</i>	F: TGTAGTAGTTCTGTCTGCTG R: TGGAGTTGTTGTTGTAATC	61	319 (0.563); 376 (0.438)	0.371
Par12	Tetra-	(AAAC) ₇	<i>Prochilodus argenteus</i>	F: CGAGCTGGTACCGTCACATA R: AGCATGATGCAAAGGATCTG	56	234 (0.531); 244 (0.125); 251 (0.344)	0.505
Par80	Di-	(CT) ₃₇	<i>Prochilodus argenteus</i>	F: CTAACCTACAAACCTCATTC R: CTGTAAGGCTCCACTTATC	50	190 (0.154); 195 (0.461); 200 (0.385)	0.535
Cm1A8	Complex	(CT) ₁₈ (CA) ₁₂	<i>Colossoma macropomum</i>	F: TGCTCTCCTGCAGTCTCTCA R: TCATGGTTGCCACTCATCTC	65	170(0.045); 180 (0.955)	0.083

PIC values above 0.5 as very informative, between 0.25 and 0.50 as moderately informative, and below 0.25 as poorly informative. The presence of null alleles was determined using the software Micro-Checker 2.2.3 (Van Oosterhout *et al.* 2004).

RESULTS

Nine of the 35 primers tested showed a well-defined pattern of bands and polymorphism. The name of each cross-amplified primer and of the species for which it was originally developed are: BoM5 (*Brycon opalinus*), Bh8, Bh13, and Bh16 (*B. hilarii*), Borg59 (*B. orbignyanus*), Bag22 (*B. amazonicus*), Par80, and Par12 (*Prochilodus argenteus*), and Cm1A8 (*Colossoma macropomum*). The remaining primers were discarded, as they did not provide cross-amplification or were monomorphic (Supplementary Material, Table S1). *Brycon* species provided the most successful transferability, with five loci with satisfactory and reproducible results.

The size of the alleles ranged between 106 bp (Bom5) and 376 bp (Bag22) (Table 1). The primers that showed the highest number of alleles were BoM5 (four alleles); followed by Bh8, Par12 and Par80 (three alleles each); and Bh13, Bh16, Borg59, Bag22 and Cm1A8 (two alleles each) (Table 2).

The mean effective number of alleles was 1.934, ranging from 1.095 (Cm1A8) to 3.000 (Par12), which was smaller than the average number of alleles (2.556) (Table 2). This difference suggests the presence of rare or low-frequency alleles. Accordingly, three alleles had a frequency of less than 0.100 (BoM5: 150; Borg59: 225; Cm1A8: 170) and six alleles had a frequency between 0.100 and 0.200 (Bh8: 190; Bh16: 145; BoM5: 106 and 110; Par12: 244, and Par80: 190). Nevertheless, values of allelic richness were high and ranged from 1.838 (Cm1A8) to 3.830 (BoM5) (Table 2). The PIC values ranged between 0.083 (Cm1A8) and 0.542 (Bh8) (Table 1).

The expected heterozygosity (He) surpassed the observed heterozygosity (Ho) in six (Bh8, Bh13, Bh16, BoM5, Par12 and Par80) of nine primers. The p value of F_{IS} was compared with alpha 0.05 after the adjusted nominal level of 0.0055, and the result was positive and significant ($p < 0.05$) in three loci (Bh8, BoM5, and Par80). The lowest and highest He value was found with primers Cm1A8 (0.087) and Bh8 (0.616), respectively (Table 2). A significant deviation in the Hardy-Weinberg equilibrium (Hw) ($P < 0.05$) was observed with four primers (Bh8, BoM5, Bag22 and Par80), possibly influenced by the presence of null alleles in three of these primers (Bh8, BoM5 and Par80), which was confirmed through analysis by the Micro-Checker software 2.2.3.

Table 2. Number of alleles (Na), number of effective alleles (Ne), allele richness (Ra), observed heterozygosity (Ho), expected heterozygosity (He), Hardy-Weinberg equilibrium (p values) and inbreeding coefficient (F_{IS}) per microsatellite loci tested for *Brycon falcatus*.

Locus	Na	Ne	Ra	Ho	He	Hw (p value)	F_{IS}
Bh8	3.0	2.602	3.000	0.182	0.616	0.000*	0.716*
Bh13	2.0	1.600	2.000	0.227	0.375	0.065 ^{ns}	0.413
Bh16	2.0	1.424	2.000	0.273	0.298	0.696 ^{ns}	0.106
BoM5	4.0	1.980	3.830	0.182	0.495	0.000*	0.646*
Borg59	2.0	1.105	1.883	0.100	0.095	0.814 ^{ns}	-0.027
Bag22	2.0	2.0	3.0	0.875	0.492	0.002*	-0.765
Par12	3.0	3.0	3.0	0.563	0.584	0.868 ^{ns}	0.069
Par80	3.0	2.600	3.000	0.154	0.615	0.002*	0.767*
Cm1A8	2.0	1.095	1.838	0.091	0.087	0.823 ^{ns}	-0.024
Mean	2.556	1.934	2.616	0.294	0.406	-	0.303*

ns: non-significant; *: significant at $p < 0.05$

DISCUSSION

No previous studies have evaluated in detail the transferability of heterologous primers in *B. falcatus*. Here we demonstrate that the cross-amplification among species of *Brycon* is possible and applicable. Previous studies have shown that primers of *B. opalinus* and *B. hilarii* can be transferred to *B. orbignyanus* (Carmo *et al.* 2015; Castro *et al.* 2017). The possibility of cross-amplification of primers from two species of *Salminus* (*S. brasiliensis* and *S. franciscanus*) in *B. orbignyanus* was also reported (Carmo *et al.* 2015). The locus of *P. argenteus* (Par80) can also be used in *B. orbignyanus* (Castro *et al.* 2017). These previous studies confirm the plasticity of cross-amplification of microsatellites, even if they involve different genera. Our study corroborates these findings, as two loci of *P. argenteus* (Par12 and Par80) and one of *C. macropomum* (Cm1A8) displayed satisfactory amplification in *B. falcatus*. However, it is noteworthy that most validated loci belong to *Brycon*, which is expected due to the greater genetic proximity among species of the same genus.

The number of alleles per locus in most primers was similar to that found by Castro *et al.* (2017) in cross-amplification of microsatellite markers of *B. hilarii* and *B. opalinus* in *B. orbignyanus*; and similar to that found by Souza *et al.* (2018b) in cross-amplification of microsatellite of *B. hilarii*, *B. opalinus* and *B. orbignyanus* in *B. goudingi*. In a study with natural populations of *Prochilodus lineatus*, Lopera-Barrero *et al.* (2016b) found a variation of three to six alleles produced by the Par12 locus and three to five alleles produced by the Par80 locus. The number of alleles produced in our study was lower than that reported by Bignardi *et al.* (2016) for *Brycon hilarii* using the loci Bh8, Bh13, and Bh16. The same applies to the primers Par80 and Cm1A8, which showed a lower

number of alleles than that found in the literature (Barbosa *et al.* 2008; Lopera-Barrero *et al.* 2016b; Santos *et al.* 2009). Based on the latter studies, we infer that the number of alleles per locus may vary, possibly due to factors, such as sample size, target species, effective size, etc. Although the number of alleles in our study was lower than in most referred studies, the mean observed (0.294) and expected (0.406) heterozygosity was similar to that found for some migratory fish like *B. orbignyanus* and *Brycon gouldingi* (Lima, 2004) (Ashikaga *et al.* 2015; Souza *et al.* 2018). In a study with wild populations of *B. orbignyanus*, Ashikaga *et al.* (2015) found $H_o = 0.233$ and $H_e = 0.497$ in a population in the Verde River, and $H_o = 0.324$ and $H_e = 0.578$ in the Sucuriu River. In other rivers such as the Paranapanema and Uruguay, the populations had $H_o < 0.2$ (Ashikaga *et al.* 2015). According to the authors, the Rio Verde population presented satisfactory genetic variability. A *B. gouldingi* population of the Araguaia River (Souza *et al.* 2018b) had lower H_o (0.157) and H_e (0.357) than those found for *B. falcatus* in our study. Our results demonstrate a moderate genetic variability in the *B. falcatus* population. However, the heterozygote deficit was notable through positive and significant values in the FIS index at the Bh8, BoM5, and Par80 loci. This heterozygous deficiency resulted in the Hardy-Weinberg equilibrium deviation in the same loci, possibly influenced by the presence of null alleles, which will be discussed below. Genetic analyzes of populations on a wider geographic scale will be necessary to estimate variability and the level of genetic structure in this species, and to evaluate the possibility of inbreeding in shoals, which would raise F_{IS} values.

The size of the alleles produced are close to the values reported by Bignardi *et al.* (2016) for *B. hilarii*. The primer Bag22 also produced a similar size to that found by Araújo (2012) for *B. amazonicus*. The size of the alleles reflects the number of repeating units in the microsatellite sequence. Therefore, because they share similar sizes, the *Brycon* species cited have similar sizes of short tandem repeats at the evaluated loci. However, care should be taken when establishing phylogenetic relationships, as the presence of homoplasia in regions with microsatellites may falsely infer kinship relationships, since in this condition alleles are identical by state, but not by ancestry (Turchetto-Zolet *et al.* 2017).

The allelic richness demonstrated that the heterologous primers were informative, which was corroborated by the PIC analysis, which yielded a high and moderately informative classification for seven of the nine heterologous primers tested (Bh8, Bh13, Bh16, BoM5, Bag22, Par12, and Par80). The higher the value, the greater is the ability of the marker to detect the variability between individuals (Preczenhak 2013).

The presence of null alleles in microsatellite markers is apparently recurrent in population studies in fish (Ashikaga *et al.*, 2015; Henriques *et al.*, 2017; Souza *et al.*, 2018b).

Cross amplification in related species increases the frequency of these alleles as the phylogenetic distance between species increases (Chapuis and Estoup 2007). This has already been demonstrated in the transferability of *B. orbignyanus*, *B. opalinus* and *P. argenteus* microsatellite primers in *B. gouldingi* (Souza *et al.* 2018b), and *B. opalinus* primers in *B. orbignyanus* (Ashikaga *et al.* 2015). In the latter study, the authors attributed the deviation in the Hardy-Weinberg equilibrium in some loci of natural populations of *B. orbignyanus* to the high frequency of these alleles. Likewise, the presence of null alleles in our study is presumably the reason for the heterozygote deficiency and the deviation in the Hardy-Weinberg equilibrium for some loci (Bh8, BoM5 and Par80). This occurs because the presence of these alleles can cause heterozygous individuals to be mistakenly identified as homozygous for these loci, underestimating the levels of genetic diversity (Turchetto-Zolet *et al.* 2017). Our results and other studies cited, point to an important limitation in the use of these markers in related species, which strengthens the importance of developing specific microsatellite primers for *B. falcatus*. However, other studies should be conducted with more populations and larger sample size per population, to ascertain if the inbreeding, genetic bottlenecks, Wahlund effect, null alleles, or a combination of these factors may be affecting the allelic frequencies of this population.

Closely related species have higher chances of amplification, and consequently more polymorphisms than phylogenetically distant species (Primmer *et al.* 1996), thus the knowledge of the phylogenetic relationships among species is important for the success of heterologous amplifications. Our results support this idea, as six of the nine primers that displayed satisfactory amplifications were derived from *Brycon*. Regarding the other three primers cross-amplified from *Prochilodus* and *Colossoma*, it is likely that flanking microsatellite regions were preserved during species differentiation, allowing the correct annealing of the primers in the corresponding regions. This hypothesis is reinforced by the fact that the primer Par80 had already been cross-amplified in another *Brycon* species, *B. orbignyanus* (Castro *et al.* 2017).

The transferability of heterologous primers is an important tool to reduce costs and time in developing species-specific primers. Genetic diversity indices and the polymorphic information content of the set of heterologous primers tested, demonstrate applicability in studies in natural or captive populations. Conservation of genetic variability in fish populations is critical for maintaining environmental adaptability (Frankham *et al.* 2008), and microsatellite markers are important tools for this assessment. In wild populations, microsatellite markers can be used to solve problems of taxonomic ambiguity, genetic structure and differentiation of isolated populations (Abdul-Muneer 2014). In addition, they can be used for restocking programs to control breeder mating and produce fingerlings with high

genetic diversity (Bignardi *et al.* 2016). The breeding and genetic control of this species in captivity could reduce fishing pressure and the demand for wild individuals (Oliveira *et al.* 2018), contributing to the conservation of the species. In this case, these markers could assist in strategies that reduce genetic erosion and minimize the risks of inbreeding depression (Aguiar *et al.* 2013). We demonstrated, for the first time, that the cross-amplification of microsatellite primers of different species and genera in *B. falcatus* is possible and can provide valuable genetic information that may assist conservation and production of this species. Yet, in view of the occurrence of null alleles in some *loci*, we emphasize the concomitant need to develop specific microsatellite markers for *B. falcatus*.

CONCLUSIONS

The transferability of microsatellite markers of *Brycon opalinus* (BoM5), *B. hilarii* (Bh8, Bh13 and Bh16), *B. orbignyanus* (Borg59), *B. amazonicus* (Bag22), *Prochilodus argenteus* (Par12 and Par 80), and *Colossoma macropomum* (Cm1A8) were validated for *B. falcatus*. These results can enable future genetic evaluations with this species, which has scarcely been studied in Brazilian river basins.

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SUPPLEMENTARY MATERIAL (only available in the electronic version)

PENHA *et al.* Transferability of heterologous primers to *Brycon falcatus*

Table S1. Species, locus and polymorphism evaluation in the amplification of the 35 microsatellite primers tested in *Brycon falcatus*. M: monomorphic; P: polymorphic; P*: polymorphic with null allele ($P < 0.05$); -: absence of amplification or nonspecificity.

Species	Locus	Amplification	Species	Locus	Amplification
<i>Brycon opalinus</i>	BoM5	P*	<i>Prochilodus argenteus</i>	Par12	P
<i>Brycon hilarii</i>	BoM13	M		Par14	-
	Bh5	-		Par15	-
	Bh6	-		Par21	-
	Bh8	P*		Par43	-
	Bh13	P		Par80	P*
	Bh16	P		Par82	-
<i>Brycon insignis</i>	Bc48-10	-	<i>Prochilodus lineatus</i>	Pli30	-
<i>Brycon orbignyanus</i>	Borg13	M	<i>Piaractus mesopotamicus</i>	Pme2	-
	Borg25	M		Pme4	-
	Borg59	P		Pme5	-
<i>Brycon amazonicus</i>	Bag22	P*		Pme20	-
	Bag25	-		Pme28	-
	Bag27	M	<i>Colossoma macropomum</i>	Cm1A8	P
	Bag31	-		Cm1A11	-
	Bam6	-		Cm1C8	-
	Bam11	-		Cm1D1	-
				Cm1E3	-