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Identification and analysis of a novel microsatellite marker within the growth hormone gene promoter of *Colossoma macropomum* (Characiformes: Characidae) detected by TAIL-PCR

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Summary

While in all vertebrates, growth hormone (GH) promotes post-natal growth, in fishes it also affects such metabolic functions as foraging rate, digestion, osmoregulation, and reproduction. The promoter region of the GH gene is an important target for studies of mechanisms regulating its expression, and polymorphisms within the promoter have been associated with performance traits in fishes. We used Thermal Asymmetric Interlaced PCR (TAIL-PCR) to amplify and sequence the 5′-flanking regions of the *Colossoma macropomum* GH (cmGH) gene. Based on a sequence of 1,038 bp, we designed three specific nested primers (R-290, R-186 and R-26) which were used with shorter arbitrary degenerate primers to amplify the 5′ proximal region of the cmGH gene. We identified a tetranucleotide $(ATCC)_4$ microsatellite motif in this region, exhibiting four alleles (118, 122, 126 and 130 bp) within the population study. Genotypes at this locus deviated significantly from Hardy–Weinberg expectations ($p \leq .05$) and showed a low level of polymorphism (polymorphic information content = 0.163). High homozygosity ($F_{IS} = 0.147$) was observed in the overall population. The polymorphism at the microsatellite makes it an important candidate for association studies between the respective genotypes, growth rate and other traits in farmed populations. Such studies may contribute to future breeding programs using marker-assisted selection upon this aquaculturally important species.

KEYWORDS
breeding program, cachama negra, short tandem repeat, polymorphism, tambaqui

1 | INTRODUCTION

Growth of vertebrates is controlled by different physiological pathways. The growth hormone (GH) axis is one of the most important pathways *regulating somatic growth of body tissues* (Sellier, 2000). In fish, GH is also involved in foraging rate, digestion, osmoregulation and reproduction (Almuly et al., 2005; Sakamoto et al., 1997; Zou et al., 1997). An increase of circulating GH levels in fish results in significant gains in growth rate (Devlin et al., 2004). Injection of GH increases growth rate in a number of farmed fish species. Cavari et al. (1993) showed that gilthead seabream (*Sparus aurata*) exhibited a 20% increase in growth rate after a treatment with native GH injection for only two weeks. Silverstein et al. (2000) determined that two different channel catfish (*Ictalurus punctatus*) strains had higher feed efficiency and growth rate when treated with bovine GH. Furthermore, transgenic fishes with an exogenous GH gene copy introduced into their genomes exhibited a higher growth rate than their non-transgenic siblings (reviewed by

Hallerman et al., 2007), as observed in coho salmon (*Oncorhynchus kisutch*; Devlin et al., 2004), Atlantic salmon (*Salmo salar*; Du et al., 1992), channel catfish (*I. punctatus*; Dunham et al., 1992), common carp (*Cyprinus carpio*; Chen et al., 1993), northern pike (*Esox lucius*; Gross et al., 1992) and Nile tilapia (*Oreochromis niloticus*; Martinez et al., 1996). Considering its importance to growth of farmed animals, molecular characterization of the entire GH gene or its cDNA has been carried out for chicken (Kuhnlein et al., 1997), cattle (Curi et al., 2006; Pereira et al., 2005), goat (Zhang et al., 2011) and several fishes (e.g., *Tinca tinca*; Kocour & Kohlmann, 2011; pacu, *Piaractus mesopotamicus*; Pinheiro et al., 2008).

Physiological mechanisms underlying tissue maintenance and somatic growth have been assessed in studies investigating the activation and regulation of the GH gene in vivo in GH-deficient rats and cultured cells (Woelfle et al., 2003). Thus, the expression of this gene is known to be controlled by pituitary-specific transcription factors, which bind to *cis* elements in the promoter, rich in AT sequences (Yowe & Epping, 1995). Ma et al. (2012) identified binding sites for four transcription factors—Pit1/GHF-1, CRE, HNF-3β and TER—in the GH gene promoter of tongue sole (*Cynoglossus semilaevis*). Moriyama et al. (2006) identified binding sites for three transcription factors—Pit-1/IGF-1, CRE and TER—in the GH gene promoter of sea lamprey (*Petromyzon marinus*), one of the most ancient vertebrates. These results demonstrate that the GH gene of teleosts is regulated by synergistic interactions among a number of transcription factors within the promoter region, wherein Pit-1/IGF-1, HNF-3β and TER are specifically responsible for promoter activation (Yamada et al., 1993). Thus, the promoter is an important target for studies of regulatory mechanisms of the GH gene. Polymorphisms within the GH promoter have been associated with performance characters of fishes (reviewed by De-Santis & Jerris, 2007), including growth in Atlantic salmon (*S. salar*; Gross & Nilsson, 1999) and gilthead sea bream (Sánchez-Ramos et al., 2006), morphometric traits in Nile tilapia (Blanck et al., 2009).

Our model system, tambaqui, (*Colossoma macropomum*), known as cachama in Spanish-speaking countries, is distributed throughout the Amazon and Orinoco river basins (Araujo-Lima & Goulding, 1998). It is the second-largest species in the Amazon basin, and can reach one meter in total length and 30 kg in weight (Isaac & Ruffino, 1996). It is the object of an important freshwater fishery in the Amazon River and tributaries and is the second-largest Brazilian aquaculture species in production volume, which amounted to 101,079,464 tons in 2019 (Corrêa et al., 2014; IBGE, 2019).

Considering the lack of information for members of Order Ostariophysi and for Neotropical fishes generally, to gain insight into mechanisms regulating expression of the GH gene and any polymorphism in the GH promoter, we: (i) characterized the GH gene promoter of *C. macropomum* using TAIL PCR methodology, (ii) determined the presence of short tandem repeats (STR) polymorphism in the GH promoter sequence, and (iii) assessed the level of STR polymorphism in different *C. macropomum* populations.

2 | MATERIALS AND METHODS

2.1 | Isolation of the 5′ flanking region of the *C. macropomum* GH gene

2.1.1 | Sequencing of the GH gene intron-I

Genomic DNA was isolated using the phenol-chloroform method (Sambrook & Russell, 2006) from a wild tambaqui caudal fin-clip sample stored in 95% ethanol. To characterize the 5′ flanking region of the *C. macropomum* GH gene (cmGH) gene, we applied the Thermal Asymmetric Interlaced PCR (TAIL-PCR) methodology (Liu & Whittier, 1995), which permits amplification of an unknown genomic region for which only partial sequence information is available (Session et al., 2002). The coding region of the GH gene being highly conserved among vertebrates (Ma et al., 2012), we inferred the positions of introns within the cmGH gene by aligning complete GH sequences from other fish species (Almuly et al., 2000; Ber & Daniel, 1992; Ma et al., 2011; Poen & Pornbanlualap, 2013; Sekkali et al., 1999; Yowe & Epping, 1995). As the exon-I sequence is not transcribed into the GH mature hormone because it is part of the signal peptide sequence (Miller & Eberhardt, 1983), we characterized GH intron-I based on the cDNA sequence of the GH gene for *C. macropomum* (Souza et al., 2016; GenBank: KU312263.1) to design the primers (TambF-1: ATGGCTAAAGGATTGGTGCTGCTC; TambR-224: GAGTCAGAATTGCAGAAGGACAG) using the Primer3 tool (Untergasser et al., 2012).

The conditions for PCR amplification of intron-I were carried out in a final volume of 50 μl containing *Taq* Buffer 1X; 3.7 mM $MgCl_2$; 0.2 mM dNTPs; 0.2 μM of each primer; 0.2 U/μl of *Taq* DNA polymerase and 2 μl of DNA template, performed in a MJ Research thermocycler, with initial denaturation at 94°C for 5:00 min; followed by 34 cycles with denaturation at 94°C for 1:00 min, annealing at 60°C for 1:00 min and extension at 72°C for 1:30 min; and finishing with an extension at 72°C for 7:00 min. The product was analyzed in 1.5% agarose gels and purified with the Illustra™ GFX™ PCR DNA and Gel Band Purification kit (GE Healthcare). The DNA sequencing was carried out on an ABI Prism™ 3,730 DNA Analyzer (Applied Biosystems) using the BigDye® Terminator v 3.1. The consensus sequences were generate using CodonCode Aligner software, version 3.7.1 (http://www.codoncode.com/aligner/). All consensus sequences were aligned using the ClustalW tool (Higgins, 1994) implemented in MEGA5 software (Tamura et al., 2011) and optimized by eye using Bioedit version 7.0.9.0 (Hall, 1999).

2.1.2 | TAIL-PCR for isolation of the cmGH gene 5′ flanking region

To perform TAIL-PCR (Liu & Whittier, 1995), we designed three nested reverse primers (R-290, R-186 e R-26; Table 1) based on the GH intron-I and cDNA sequences. Each specific primer was tested with six shorter arbitrary degenerate primers (AD2 to AD7; Amadeo et al., 2000; Parinov et al., 2004; Session et al., 2002; see Table 1)

TABLE 1 Primers tested in TAIL-PCR reactions for amplification of the initial region of tambaqui (*Colossoma macropomum*) GH gene

Primer	Sequence (5′–3′)	Reference
R-290	CACTGCAGAGTTAAATCGGTGA	Designed in this work
R-186	GATGACTGCATTGTTGAAGAGC	Designed in this work
R-26	GAGAGCAGCACCAATCCTTTAG	Designed in this work
AD2-S	TGWGNAGSANCASAGA	Session et al. (2002)
AD3-S	AGWGNAGWANCAWAGG	Session et al. (2002)
AD5-P	WCAGNTGWTNGTNCTG	Parinov et al. (2004)
AD11-P	NCASGAWAGNCSWCAA	Parinov et al. (2004)
AD6-A	WGCNAGTNAGWANAAG	Amadeo et al. (2000)
AD7-A	AWGCANGNCWGANATA	Amadeo et al. (2000)

Abbreviation: GH, growth hormone; TAIL-PCR, Thermal Asymmetric Interlaced PCR.

to amplify the target region. The TAIL-PCR process consisted of three successive reactions. In the primary PCR amplification, specific primer (R-290), annealing farthest from the target region, was used with the genomic DNA template. In the secondary reaction, the primer (R-186), with the intermediate binding site, was used with the product of the first reaction. Finally, in the tertiary reaction, the specific primer (R-26), binding closest to the target region, was used with the product of the second reaction. In each reaction step, each one of the specific primers was tested using all of the six arbitrary primers to boost the chances of successfully obtaining amplicons. The concentrations of all TAIL-PCR components are given in File S1, and the thermal cycling conditions are shown in Table 2.

The tertiary reactions PCR products were subjected to electrophoresis through a 1.5% agarose gel. The single amplicon bands with great concentration were excised from the gel, purified with the Illustra™ GFXTM PCR DNA Purification kit and cloned into *Escherichia coli* DH5α cells using the pGEM®T—Easy Vector System (Promega). Plasmids were isolated from white colonies with the Illustra PlasmidPrep Mini Spin kit (GE Healthcare Life Sciences) and sequenced. Sequences were analyzed as described above, and the 3′ end of each sequence was evaluated to verify that it could align with the 5′ end of the cDNA sequence.

2.2 | Characterization of the cmGH gene 5′ flanking region and identification of microsatellite

By aligning and comparing the sequence of the 5′ flanking region of the cmGH gene with those of other teleost *GH* gene sequences and using the PROMO tool (http://www.lsi.upc.es/~alggen; Messeguer et al., 2002), we characterized potential exon-I, intron-I and putative regulatory regions. The 5′ flanking region also was assessed in silico using Microsatellites Repeats Finder (http://insilico.ehu.es/mini_tools/microsatellites/; Bikandi et al., 2004). Then, using the Primer3 package, we designed primers to amplify the short tandem repeat (STR) locus found in this region.

TABLE 2 Cycle settings used for TAIL-PCR. Modified from Liu and Whittier (1995)

Reaction	Cycles number	Thermal settings
Primary	1	94° for 2 min; 95° for 1 min
	5	94° for 30 s; 66° for 1 min; 72° for 2:30 min
	1	94° for 30 s; 30° for 3 min; 72° for 3min; 72° for 2 min
	10	94° for 5 s; 44° for 1 min; 72° for 2:30 min
	15	94° for 5 s; 66° for 1 min; 72° for 2:30 min
	15	94° for 5 s; 66° for 1 min; 72° for 2:30 min
	15	94° for 5 s; 44° for 1 min; 72° for 2:30 min
	1	72° for 7 min
Secondary	15	94° for 10 s; 66° for 1 min; 72° for 2 min
	15	94° for 10 s; 66° for 1 min; 72° for 2 min
	15	94° for 10 s; 44° for 1 min; 72° for 2 min
	1	72° for 5 min
Tertiary	1	94° for 10 s
	30	94° for 15 s; 42° for 15 s; 72° for 2 min
	1	72° for 7 min

Abbreviation: TAIL-PCR, Thermal Asymmetric Interlaced PCR.

2.3 | Polymorphism assessment of the STR in the 5′ flanking region of the cmGH gene

The polymorphism analysis was performed using 170 *C. macropomum* individuals from six different farmed stocks and one wild population (Figure 1). The farmed stocks were obtained from: Biofish fish farm, Porto Velho, RO (BF; 31 samples); Projeto Prosperidade, Santarém,PA (PP; 17 samples), Tajá fish farm, Manaus, AM; (TJ; 30 samples), National Department of Works Against Drought, Pentecoste, CE (DNOCS; 30 samples), Brumado fish farm, Mogi Mirim, SP (BM; 30 samples) and National Center for Research and Conservation of Continental Fish, Pirassununga, SP (CEPTA; six samples). The wild population came from Balbina Reservoir, Manaus, AM (BW; 26 samples).

The genomic DNA were isolated using a high salt concentration method (Aljanabi & Martinez, 1997). DNA integrity and concentration

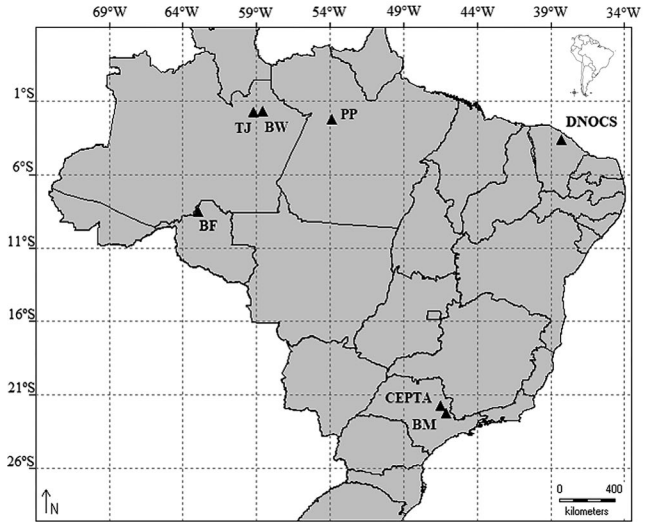

FIGURE 1 Map of sampling locations for tambaqui (*Colossoma macropomum*): BF, Biofish fish farm, Porto Velho-RO; BM, Brumado fish farm, Mogi Mirim-SP. DIVA-GIS (2017); BW, wild population from Balbina Reservoir, Manaus-AM; CEPTA, National Center for Research and Conservation of Continental Fishes, Pirassununga-SP; DNOCS, National Department of Works Against Drought, Pentecoste-CE; PP, Projeto Prosperidade, Santarém-PA; TJ, Tajá fish farm, Manaus-AM

were assessed by electrophoresis through a 1% agarose gel with the lambda/*Hind*III molecular weight ladder (Fermentas). The amplification conditions were carried out in a final volume of 25 μl, containing: 1X *Taq* Buffer, 1.5 mM $MgCl_2$, 0.2 mM of dNTPs; 0.2 mM of each primer (Cm_MiF2 and Mi_CmR2), 2 U *Taq* DNA polymerase (Sinapse Biotecnologia) and 1.5 μl of template genomic DNA, using a Veriti® thermal cycler (Applied Biosystems) with an initial denaturation at 95°C for 10 min; followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 1 min, and extension at 72°C for 30 s; and a final extension step at 72°C for 10 min.

Electrophoresis and genotyping of the microsatellite locus were carried out in a Matrix Plus denaturing 6.5% polyacrylamide gel inside a Li-Cor 4300 DNA Analyzer (LI-COR Biosciences, Lincoln, NE, USA) using the IRDye®700 marker (LI-COR Biosciences) and universal M13 tail primer as described by Schuelke (2000). Allele sizes were estimated by interpolating their position relative to the molecular weight markers (50–350 bp DNA Sizing Standard IRDye® 700) using the SagaGT Client program (LI-COR Biosciences). Putative genotyping errors (Pompanon et al., 2005) were minimized by semi-automated scoring followed by visual inspection by two independent people.

The levels of polymorphism and the allelic diversity were quantified as allelic richness (*Ar*), allele frequencies, and genotypic frequencies using the Fstat software (Goudet, 1994). The observed (*Ho*) and expected (*He*) heterozygosities and departures of genotype frequencies from those expected under Hardy–Weinberg equilibrium (HWE) were estimated using Arlequin 3.0 software (Excoffier et al., 2005), and the polymorphic information content (PIC) was calculated using CERVUS (Marshall et al., 1998).

3 | RESULTS

3.1 | Sequencing of the 5′ flanking region of the cmGH gene

3.1.1 | Sequencing of cmGH gene intron-I

A fragment of about 900 bp was obtained by the amplification of intron-I (GenBank: MN433469) of the cmGH gene using the primer pair TambF-1/TamR-224 and we confirmed that is was intron-I (887 bp) by its alignment in proper position relative to the cDNA (File S2).

3.1.2 | TAIL-PCR for isolation and confirmation of the cmGH gene 5′ flanking region

With the cmGH sequence of 1,038 bp (151 bp of exon-II and 887 bp of intron-I), we designed three specific nested primers (R-290, R-186 and R-26; File S2) required to perform TAIL-PCR. By testing six different arbitrary degenerate primers, we ascertained two specific and more intense amplicons (“A” and “B”; Figure 2), which were sequenced. These bands were the PCR products of primer pairs R-26/AD6-A and R-26/AD7-A, respectively. Assessing the alignment of sequences using ClustalW tool, the 3′ end of the amplicon “B” (Figure 2)

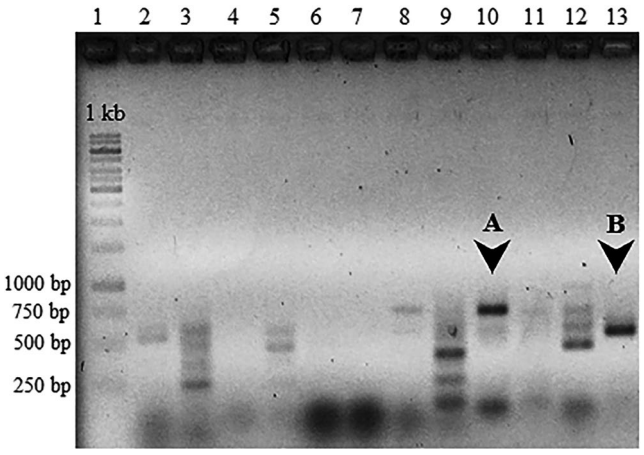

FIGURE 2 Electrophoresis gel (1.5%) showing products of TAIL-PCR tertiary reactions for amplification of 5′ flanking region of *Colossoma macropomum* GH gene. (a) product of TAIL-PCR tertiary reaction with primers R-26/AD6-A; (b) product of TAIL-PCR tertiary reaction with primers R-26/AD7-A. (1) molecular weight reference 1 kb DNA ladder (Thermo Scientific™); (2) secondary reaction with primers R-186/AD2-S; (3) tertiary reaction with primers R-26/AD2-S; (4) secondary reaction with primers R-186/AD3-S; (5) tertiary reaction with primers R-26/AD3-S; (6) secondary reaction with primers R-186/AD5-P; (7) tertiary reaction with primers R-26/AD5-P; (8) secondary reaction with primers R-186/AD11-P; (9) tertiary reaction with primers R-26/AD11-P; (10) secondary reaction with primers R-186/AD6-A; (11) tertiary reaction with primers R-26/AD6-A; (12) secondary reaction with primers R-186/AD-7A; (13) tertiary reaction with primers R-26/AD7-A. GH, growth hormone; TAIL-PCR, Thermal Asymmetric Interlaced PCR

sequence was aligned with the 5′ end of the cDNA sequence. As a result, amplicon 'B' was the TAIL-PCR product associated with the 5′ flanking region of the cmGH gene 5′-flanking region. Therefore, using a homemade primer design, Pm_16F (5′gcccttataacttcaccccttga3′) that anneals to the beginning of the 5′ flanking sequence and Pm_713 (5′cgaacagaggtaaggttgaaa3′) that anneals in the intron-I (File S3), we confirmed that the 873-bp sequence obtained by TAIL-PCR was the cmGH gene 5′ flanking region (GenBank: MN433469). Further, the 3′ end of this fragment aligned with the 5′ end of *C. macropomum* cDNA sequence, which ascertains the identity of the target sequence.

3.2 | Characterization of the cmGH gene 5′ flanking region and polymorphism evaluation

The detailed sequence of the 5′ flanking region is depicted in Figure 3. Analysis of this sequence using the PROMO package and alignment with the same region of four other teleost species (Figure 4) revealed a TATA box (transcription-factor binding site) located at −24 bp, the position of nucleotide + 1, the possible position of the start codon (ATG), and putative transcription factor binding sites for Pit-1, HNF-3, AP-1, GRE, TRE and ERE (Figure 3).

The assessment of the 5′ flanking sequence also showed the presence of three STRs, two tetranucleotide repeats, $(ATCC)_4$ and $(CAGA)_3$, at positions −512 and −387, respectively and one $(AC)_5$ dinucleotide repeat at position −374. No STR polymorphism was found in either exon-I or in intron-I. The tetranucleotide $(ATCC)_4$ was selected because its molecular position to design a pair of primers (Cm_MiF2: GGGGCTTTTCTGTGATGTGG and Mi_CmR2: CAATGACAGCAGGGATAGG; File S4) to which amplify this STR region so that any polymorphisms in the proximal promoter region could be evaluated.

A total of 170 individuals from six cultured and one wild collections of *C. macropomum* were genotyped at the STR locus. The STR in the proximal 5′ flanking region proved to be polymorphic, with four alleles (118, 122, 126 and 130 bp) among all populations. The most frequent allele across populations was the 130-bp allele (File S5). We observed six of the 10 possible genotypes, and the most frequent in all populations was 130/130. Genotype 130/180 was found in two individuals only in population BW (File S5). Overall, this locus deviated significantly from HWE ($p < .05$) and showed a low level of polymorphism (PIC = 0.163). High homozygosity was observed in the overall population ($F_{IS} = 0.147$); the observed (*Ho*) and expected (*He*) heterozygosites were 0.183 and 0.215, respectively.

4 | DISCUSSION

Promoter elements are DNA sequences upstream of a gene's protein-encoding sequence that regulate in gene expression (Atkinson & Halfon, 2014; Roy & Singer, 2015). The structural elements of the promoter provide the transcriptional control domains for response elements and binding sites for transcription factors to recruit RNA polymerase to initiate transcription. In the 814 bp upstream of the cmGH gene, we identified putative transcription factor binding sites as well as three STR tracts. Comparing the sequence of the cmGH 5′-flanking region with those of other teleosts, we observed that the TATA box has a relatively conserved position, varying from the −21 to −24 position among five species (Almuly et al., 2005; Chiou et al., 1990; Tang et al., 1993; Yowe & Epping, 1995). Up to position −200, all species show putative N-Oct-3 and two Pit-1 binding sites. This region is conserved among perciform fishes (Almuly et al., 2005), and includes the presence of a $(CA)_n$ STR tract between the Pit-1 and HNF-3 binding sites. The putative NHF-3 binding site was detected in all species except for channel catfish (*I. punctatus*). However, the absence of a $(CA)_n$ STR tract in the three ostariophysian species (*C. macropomum*, *I. punctatus* and *C. carpio*) shows the higher degree of similarity among promoter sequences of species of the same phylogenetic order. Moriyama et al. (2006) demonstrated the presence of

FIGURE 3 Nucleotide sequence (802 bp) of 5′ flanking region of *Colossoma macropomum* growth hormone gene. TATA box and putative transcription factor binding sites are boldface and underlined; short tandem repeat sequences are highlighted in grey and +1 is used to represent transcription start point. AP-1, activating protein 1 binding element; ERE, estrogen response element; GRE, glucocorticoid response element; HNF-3, hepatocyte nuclear factor 3; N-Oct-3, neural POU-domain protein; Pit-1, binding site for pituitary-specific transcription factor; TRE, thyroid hormone response element

FIGURE 4 Multiple alignment of the GH gene promoter region of five fish growth hormone genes (200 pb). *Colossoma macropomum* (CM); *Cyprinus carpio* (CC; Chiou et al., 1990), Ictalurus punctatus (IP; Tang et al., 1993), *Sparus aurata* (SA; Almuly et al., 2005) and *Lates calcarifer* (LC; Yowe & Epping, 1995). The TATA box and putative binding sites for transcription factor are boldfaced and underlined. Microsatellites are highlighted in grey. Dashes represent gaps added to facilitate alignment. GH, growth hormone

putative Pit-1, CRE and TRE binding sites upstream of the GH gene of sea lamprey *P. marinus* and suggested that Pit-1 is primarily responsible for GH gene expression in this species, as well as in teleosts and mammals. In *C. macropomum*, in addition to the putative Pit-1 binding site, we detected putative binding sites for HNF-3, AP-1, GRE, ERE, TRE and N-Oct-3, although no complementary site for CRE was found. All of these binding sites having been detected in other fish species, the presence of multiple types of binding site in all species studied suggests that GH gene regulation in fishes depends upon the interaction of multiple regulatory factors (Almuly et al., 2005; Argenton et al., 1996; Moriyama et al., 2006; Von Schalburg et al., 2008).

The majority of polymorphisms associated with fish GH genes occurs within introns and the proximal promoter (De-Santis & Jerry, 2007). Putative associations of GH sequence polymorphism and growth have been reported for some fishes, such as Atlantic salmon (*S. salar*; Gross & Nilsson, 1999), gilthead sea bream (*S. aurata*; Almuly et al., 2000; Almuly et al., 2005), Asian seabass (*Lates calcarifer*; Gopal et al., 2015; Yue et al., 2001), olive flounder (*Paralichthys olivaceus*; Kang et al., 2002), yellow catfish (*Pelteobagrus fulvidraco*; Li et al., 2017), common carp (*C. carpio*; Liu et al., 2017), and Nile tilapia (*O. niloticus*; Dias et al., 2019). STR polymprphism at the GH gene was related to culture history and differentiation among oceanic populaiuotns in European sea bass (*Dicentrarchus labrax*; Quéré et al., 2010).

STR repeats in the promoter region may affect the gene regulation, resulting in variation of levels of the protein encoded (Kashi & King, 2006; Kashi et al., 1997; Li et al., 2004), as has been shown in yeast (Russell et al., 1983), gilthead sea bream (Almuly et al., 2008), Nile tilapia (Streelman & Kocher, 2002), cattle (Hale et al., 2000) and human (Horan et al., 2003). In particular, STR polymorphisms in the promoter region may affect GH expression in fish, in turn affecting growth performance. Jaser et al. (2017) identified SNPs in the promoter region of the GH gene of a hatchery population of Nile tilapia and associated the markers with individual growth rate. Li et al. (2017) showed significant associations of variants at three STR loci with growth and several morphometric traits in yellow catfish. After introgressive crossbreeding between the Red-Stirling and Chitralada strains of Nile tilapia, Dias et al. (2019) showed that STR allelic variation at the promoter locus had a significant association with weight gain. Against this background, we note that the polymorphic STR identified in the present study is located very close to several putative complementary sequences for transcription factors, between an NHF-3 and a GRE site. Hence, we hypothesize that the contraction and expansion of such an STR array might affect the interaction of a transcription factor with the promoter, resulting in changes in the timing, location or level of cmGH gene expression.

The STR polymorphism in the proximal promoter of *C. macropomum* here reported is the first characterized in a Neotropical species of fisheries and aquaculture importance. The low genetic diversity (i.e., the low number of alleles) at the STR in the GH promoter of this species might be due to limited sampling, mostly from aquaculture stocks, to the tetranucleotide motif of this STR locus, which is characteristically less polymorphic than for di- or trinucleotide motifs, or to effects of variants upon GH expression and fitness.

Our findings open an avenue of population genetics investigations into whether wild populations from different locations in the Amazon

and Orinoco basins may reveal other alleles and genotypic combinations than those that we found. The characterization of the 5′ flanking region of the cmGH gene and the primers designed for this region in the present work can be used in future studies of aquaculture stocks to observe STR polymorphisms and to test their association with growth rate or other production traits. Should such associations be found, the knowledge can be applied as a performance-related molecular marker useful for marker-assisted selection and genetic improvement of aquaculture stocks (Fuji et al., 2007; Moen, 2010; Poompuang & Hallerman, 1997; Sonesson, 2007).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

S.K.K.J. performed the experimental design, laboratory work, and drafted the paper. L.C.L.F. performed the TAIL-PCR experimental procedures. C.A.P. performed the statistical analysis. E.H. contributed to manuscript drafting and preparation. A.W.S.H. conceived the study and prepared the final version of the manuscript. All the authors have read and approved the final version to submission.

DATA AVAILABILITY STATEMENT

The online version of this article contains Supporting Information, which is available to authorized users. Growth hormone gene sequence of *C. macropomum* and its partial 5′ flanking region have been submitted to GenBank with accession number MN433469.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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