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Short Communication

Sequence analysis of the growth hormone gene of the South American catfish *Rhamdia quelen*

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ABSTRACT. *Rhamdia quelen* is an important Neotropical catfish species for fisheries and aquaculture in southern Brazil, where it is called Jandia. Like other native Brazilian species of economic importance, *R. quelen* genetics needs more attention for animal breeding programs. The growth hormone gene is known to be linked to a number of molecular markers and quantitative trait loci. We sequenced the coding region of the growth hormone gene with the primer walking technique. As in other Siluriformes, the *R. quelen* growth hormone gene has four introns and five exons, in a 1465-bp coding region. The tertiary structure of the encoded protein was predicted by bioinformatics; it has four α-helix structures connected by loops, which form a compressed complex maintained by two disulfide bridges.

Key words: *Rhamdia quelen*; Siluriformes; Growth hormone; Bioinformatics; Primer walking

Jundia (*Rhamdia quelen*) is a Neotropical catfish that shows a high potential for aquaculture in Rio Grande do Sul State, Southern Brazil (Baldisserotto, 2009), due to an elevated growth rate, good carcass yield, and easy reproductive handling under the subtropical climate (Gomes et al., 2000; Francalossi et al., 2004; de Amorin et al., 2009). Jundia is the most commercialized native species in Rio Grande do Sul State (750 tons/year) (Boscardin, 2008; Baldisserotto, 2009), and 50% is supplied from aquaculture and 50% from artisanal fishery, corresponding to an annual amount of U$882,800. Rio Grande do Sul and Santa Catarina States are the only ones that significantly explore the Jundia fishery stocks, as revealed by the annual fishery statistics from the Brazilian government (IBAMA, 2008). Despite vast knowledge about this species, molecular genetics of Jundia requires more attention since it may provide useful information to support animal breeding programs, genetic conservation and understanding of phylogeny (Gomes et al., 2000).

Growth hormone (GH) is synthesized in the somatotrophic axis and is phenotypically associated with characteristics of interest to animal breeding, such as growth, reproduction (Duan, 1998; Gomez et al., 1998) and osmoregulation (Mccormik, 2001). In addition, growth hormone may be the most promising growth-promoting agent in aquaculture (Zohar, 1989), since it is essential for somatic growth and reproduction in bony fishes and osmoregulation in euryhaline fishes (Sciara et al., 2006). Among vertebrates, GH is essential for normal growth and is involved in the regulation of several anabolic processes (Xu et al., 2001).

The teleost *gh* genes can be grouped into two types: on the one hand are genes of the Siluriforms and Cypriniforms, which consist of five exons and four introns (5-exon type), and on the other hand are those of the Salmoniforms, Perciforms, and Tetradontiforms, which consist of six exons and five introns (6-exon type). Structurally, the latter differs from the former by the presence of an intron inserted in the 5th exon (Moriyama et al., 2006). The *gh* gene has been shown to serve as a natural marker for studies of evolutionary genetics of various fishes because of its sequence conservation, sufficient length and minimal amount of homoplasy (Marins et al., 2003; Chen et al., 2004; Pinheiro et al., 2008). The aim of this study was to annotate the coding sequence of the *R. quelen gh* gene and perform a *gh*-based phylogenetic analysis within the Siluriformes family.

Primers were designed by the use of the Vector NTi 10.0 software (Invitrogen), and they were based on the alignment of the several *gh* gene sequences from the diverse Siluriformes species available in GenBank. The sequences were aligned in the Align-X software (Invitrogen). The degenerated primers used for polymerase chain reaction (PCR) amplification correspond to GH_forward: 5'-GCARAAATGGCTMGAGGTAAGG-3' and GH_reverse: 5'-CARRGTGCAGTTGGAATCC-3', while those employed in the primer walking method correspond to GH_forward2: 5'-TTACCAGAAGAACGCAAACAGC-3', GH_forward3: 5'-GCACAATTTCAATCCTCACCAG-3', GH_reverse2: 5'-CACTGTCAAAGAAGAAAA GAGGTAG-3', and GH_reverse3: 5'-ATCAGGCGGTAGGAAGTGTGC-3'. The *gh* gene was amplified by PCR as follows: 94°C for 3 min, 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 2 min, and a final extension step of 72°C for 7 min. The products were amplified by the use of Taq DNA Polymerase (Invitrogen), and amplification was confirmed by agarose gel electrophoresis. The PCR product was purified by adding one volume of 20% polyethylene glycol and 2.5 M NaCl. The solution was incubated at 37°C for 1 h and centrifuged at 16,000 *g* for 1 h at room temperature. The supernatant was removed and 125 µL cold 80% ethanol was added to the microtube. The material was centrifuged for 10 min at room temperature

at 16,000 *g* and the supernatant was removed. Again, 125 µL cold 80% ethanol was added and immediately removed. The microtube was incubated at 37°C until complete evaporation of the ethanol. Sequencing was performed in a MegaBACE 500 DNA sequencer (GE-Healthcare) by the use of the Dyenamic ET-terminator technology. Contigs were assembled using the ContigExpress software (Invitrogen). The assembled sequence was submitted to BLAST alignment analysis (www.ncbi.nlm.nih.gov/BLAST) for confirmation. The *R. quelen gh* gene sequence was deposited in GenBank under accession No. EF101341. The Genescan software was used for the annotation of the exon/intron regions of the *gh* gene sequence. *In silico* translation and protein analysis were performed by the use of the Vector NTi 10.0 software (Invitrogen). The phylogenetic analysis was performed with the use of the Mega 4.1 software (Grisoft). The neighbor-joining method using the p-distance model was applied, and a bootstrap value of 1000 replications was used for the inference of the tree structure. The tertiary structure of GH was predicted on Poseidon Linux platform (Ferreira et al., 2009) using Modeller (Eswar et al., 2003), employing a homology prediction analysis (Lambert et al., 2002), and the protein structure was visualized with the use of PyMOL (DeLano, 2002).

Initially, six animals, which were maintained in the same breeding tank, were randomly selected for chromosomal DNA extraction. Since there are no known *R. quelen gh* sequences, several other *gh* entries in GenBank, from different bony fish species, were aligned for identification of conserved sites for primer design. Primer degeneracy was employed to ensure hybridization. The coding region of the *R. quelen gh* gene was amplified by PCR from the six different animals, but only one was selected for further sequencing. As expected, all individuals showed the same amplification pattern, with bands of approximately 1500 bp in length. The PCR product was directly sequenced without any cloning steps, in order to avoid the occurrence of point mutations that could provide incorrect information. Primer walking was necessary to close gaps in the *R. quelen gh* gene sequence. By *in silico* analysis, the partial *R. quelen gh* gene sequence was determined to have 1565 bp, thus distributed as six introns and five exons (exon nucleotide positions range from 1 to 10, 209 to 348, 438 to 554, 1038 to 1169, and 1254 to 1457, based on GenBank accession No. EF101341). The predicted mRNA sequence contains 603 nucleotides and encodes a protein of 22 kDa.

The extent of *gh* identity was evaluated among all bony fishes. For this purpose, the few sequences available in GenBank were used for global alignment, including that from *R. quelen gh* (Table 1). A clear evidence of sequence heterogeneity was found at the nucleotide level. Point mutations were evenly distributed, but, interestingly, gaps were found occupying mostly the 3'end portion of the *gh* genes. Amino acid substitutions showed a higher number of non-synonymous mutations within the carboxy-terminal portion of the GH protein. This suggests that selection pressure possibly contributed to environmental adaptation rather than species evolution. The coding sequences of the *gh* gene were used for phylogenetic analysis. As expected, within the assembled tree, three major clusters can be observed containing the Cypriniformes, Salmoniformes and Siluriformes species. *Rhamdia quelen* is positioned within the Siluriformes group, and based on the *gh* gene sequences analysis, it appears to be the most ancient in relation to the other species (Figure 1).

In silico analysis of the three-dimensional structure of *R. quelen* GH demonstrated the presence of four α-helices connected by loops, which form a compressed complex maintained by covalent bonds of two disulfide bridges between amino acids Cys61 and

Table 1. *gh* gene sequences used in this study.

Species	Length (bp)	Accession Nos.
Carassius aurata	633+	DQ350437
Carassius cuvieri	633+	AF389237
Catla catla	894+	AY053361
Cirrhinus mrigala	1150+	AF140281
Clarias batrachus	663+	AF416485
Clarias gariepinus	1456*	AF416488
Coregonus autumnalis	742+	X77245
Ctenopharyngodon idella	1171+	X60474
Cyprinus carpio	1164+	M27000
Danio rerio	1270*	NM_001020492
Gymnocypris przewalskii	771+	AY707317
Heteropneustes fossilis	1132+	AF147792
Hypophthalmichthys molitrix	1170+	X60475
Hypophthalmichthys nobilis	1158+	S60473
Ictalurus punctatus	3379*	AF267989
Ictiobus bubalus	1279+	AY375301
Labeo bata	896+	AY691180
Labeo fimbriatus	893+	AY691182
Labeo gonius	894+	AY691183
Labeo kontius	895+	AY691184
Labeo rohita	885+	AF416490
Megalobrama amblycephala	1170+	AY170124
Misgurnus anguillicaudatus	1154+	AY334554
Misgurnus mizolepis	5099*	AF133815
Morulius calbasu	894+	AY691181
Mylopharyngodon piceus	633+	AF389238
Myxocyprinus asiaticus	633+	DQ350434
Oncorhynchus keta	1120+	X17593
Oncorhynchus masou	1176+	X59762
Oncorhynchus tshawytscha	1138+	S50867
Pangasianodon gigas	1176+	L27835
Pangasius pangasius	603+	M63713
Paramisgurnus dabryanus	633+	DQ350432
Pelteobagrus fulvidraco	603+	DQ112163
Pimephales promelas	1122+	AY643399
Rhamdia quelen	1465*	EF101341
Salmo salar	4397*	M21573
Silurus asotus	1082+	AY157496
Silurus meridionalis	602+	AF530481

+Corresponds to the mRNA. *Corresponds to the full-length gene without the regulatory region.

Cys163, and Cys188 and Cys180. Despite their close genetic relationship, Siluriformes, Salmoniformes and Cypriniformes show structural peculiarities with regard to GH. For example, Siluriformes GH is 10 amino acids shorter (200 amino acids) than that of Cypriniformes and Salmoniformes, although all three groups bear the same number of introns and exons in their respective *gh* genes (Figure 2).

In this study, we sequenced and characterized the *gh* gene from *R. quelen*, and its encoded GH protein. Additionally, we used the *gh* sequences from a number of bony fish species to infer their genetic relatedness and found that *R. quelen* occupies an ancient position within the Siluriformes group. Our results provide useful information for further studies on the *R. quelen* breeding and add to the previous knowledge of bony fish phylogeny. Further studies employing the *R. quelen gh* sequence are encouraged in the several fields of fish studies.

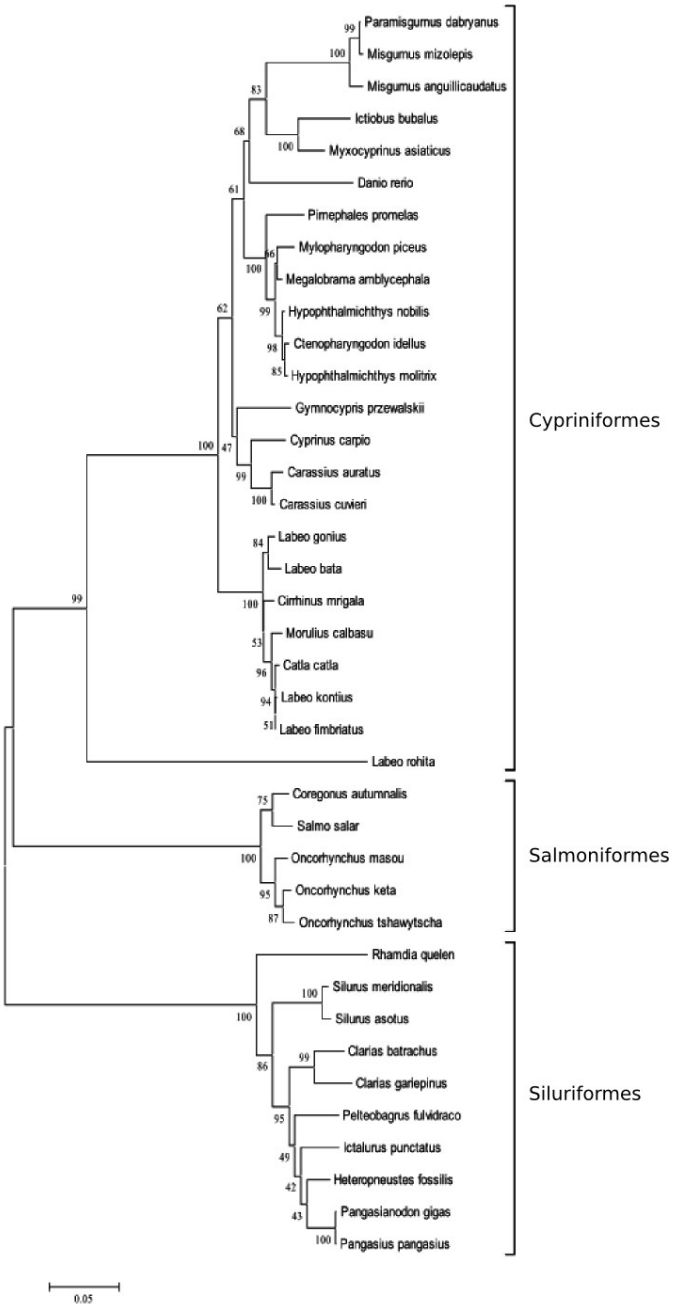

Figure 1. Unrooted phylogenetic tree was constructed based on the *gh* gene sequences (>600 bp), and it demonstrates the genetic relationship among the bony fish species. The tree was constructed by the neighbor-joining method employing the p-distance model. Bootstrap consensus values are indicated in the nodes. The use of one thousand replications was chosen for tree construction.

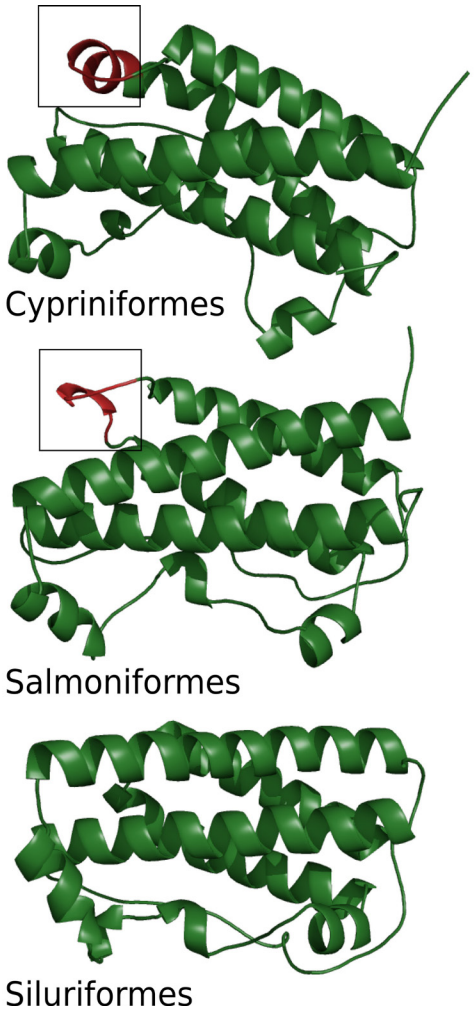

Figure 2. Three-dimensional model of the growth hormone from Cypriniformes, Salmoniformes and Siluriformes. Modeling was performed by homology search analysis. In detail is the additional 10-amino acid structure.

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