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Cloning, partial sequencing and expression analysis of the neural form of P450 aromatase (*cyp19a1b*) in the South America catfish *Rhamdia quelen*

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ABSTRACT

Brain aromatase is a key enzyme exclusively expressed in fish radial glial cells that convert androgens into estrogens, thus controlling neuroendocrine functions and neurogenesis. As an important step in characterizing the neuroendocrine systems of *Rhamdia quelen* (jundiá), a partial cDNA sequence (1045 bp) of brain aromatase (*cyp19a1b*) was cloned and sequenced. At the nucleotide level the cDNA sequence was found to be 88% identical to *cyp19a1b* of two species of catfish, *Ictalurus punctatus* and *Silurus meridionalis*. The predicted amino acid sequence was between 80 and 91% similar to other teleosts. Real-time RT-qPCR analysis revealed that *cyp19a1b* was detected in pituitary, hypothalamus, telencephalon, head and posterior kidneys, liver and gonads (testis and ovary) of both males and females. The effects of E2 on *cyp19a1b* expression are sexually dimorphic in *R. quelen*. The injection of 17β-estradiol (E2) decreased head kidney mRNA levels of *cyp19a1b* in males and increased *cyp19a1b* mRNA levels in the pituitary and head kidney of females. This study demonstrated that the *R. quelen* *cyp19a1b* gene is expressed in brain, pituitary and peripheral tissues in both males and females.

1. Introduction

Rhamdia quelen (jundiá, Silurifomes, Heptapteridae) is a neotropical catfish widely distributed in Central and South America (Silfvergrip, 1996). This species shows high potential for the aquaculture industry, due to an elevated growth rate, good carcass yield, and easy reproduction in the subtropical climate (de Amorim et al., 2009; Fracalossi et al., 2004). *R. quelen* is the most commercialized native species in the State of Rio Grande do Sul, Brazil (Baldisserotto, 2009), representing ~50% of fish markets supplied from aquaculture and 50% from artisanal fisheries. Some aspects of the biology of this species are known, for example, growth and activity patterns, gonadal development, and responses to several contaminants (Gomes et al., 2000; Mela et al., 2013; Pereira et al., 2016; Salhi et al., 2004; Schulz and Leuchtenberger, 2006). However, very little is known about the endocrinology of this species. Therefore, the endocrine physiology and molecular biology of *R. quelen* requires more attention because this is essential to enhance the reproductive and growth potential of the species in aquaculture.

As a first step to characterize critical neuroendocrine genes in this species, we focus here on aromatase (cyp19), the only enzyme able to convert androgens to estrogens, and a key player in vertebrate brain sexual differentiation (Behl, 2002; Brinton, 2009). In teleost fish, two aromatase genes arising from genome duplication have been identified. These are *cyp19a1a*, mainly expressed in gonads and *cyp19a1b*, mainly expressed in the brain (Piferrer and Blázquez, 2005; Tchoudakova and Callard, 1998). In contrast to mammals in which brain aromatase activity is maximal during embryonic development, aromatase expression in fish brains increases with age in parallel with the levels of sexual steroids (González and Piferrer, 2003). It is notable that in the teleost central nervous system *cyp19a1b* is exclusively expressed in radial glial cells, which serve as neuronal progenitor cells and, therefore, explains the high regenerative capacity of the fish brain (Diotel et al., 2013; Forlano et al., 2001; Xing et al., 2014).

It is well established in mammals and other vertebrates that adult brain can *de novo* synthesize steroids from cholesterol (Do Rego et al., 2009). Indeed, preliminary evidence indicated that goldfish radial glial cells have the complete enzymatic machinery to synthesize estrogens from cholesterol (Xing et al., 2014). There is also growing evidence indicating that 17β-estradiol (E2) modulates neurogenesis in teleost

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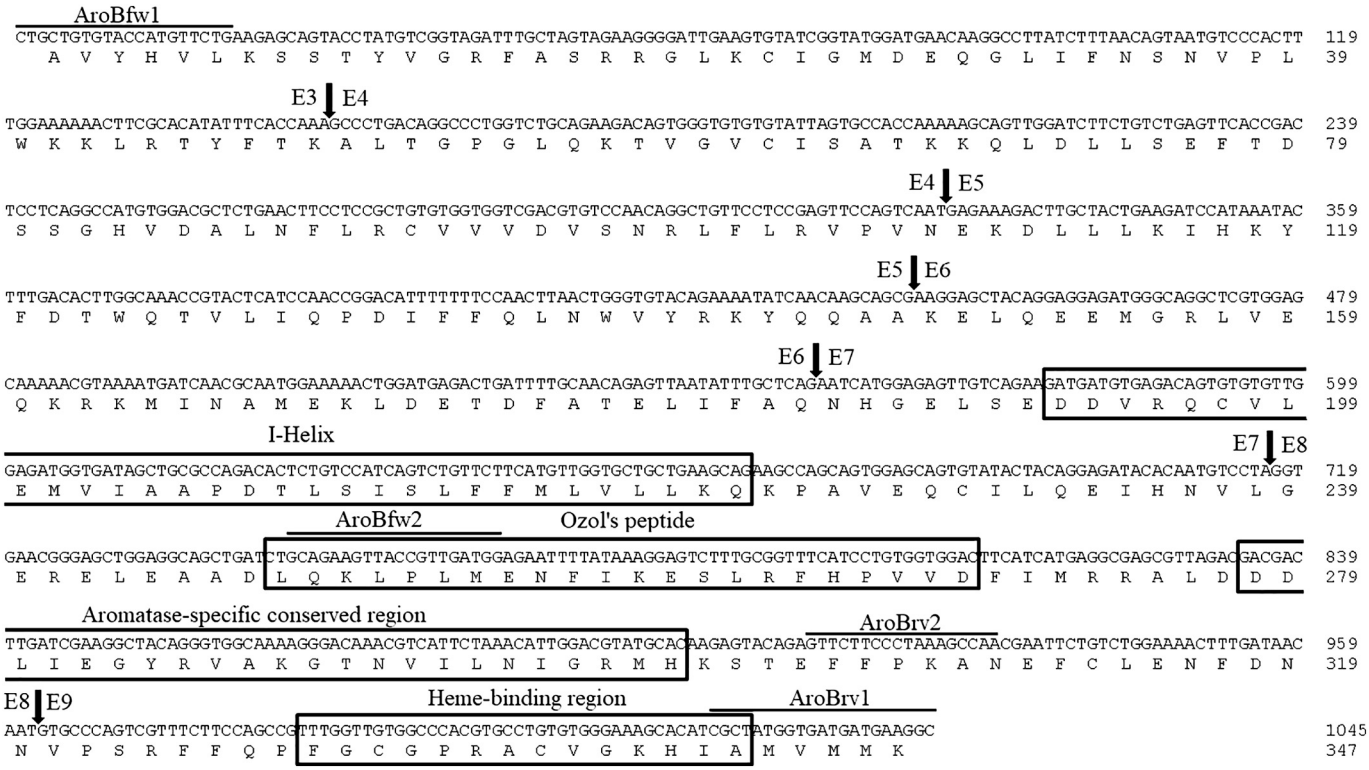

Fig. 1. Partial nucleotide and predicted amino acid sequences of *cyp19a1b* of *Rhamdia quelen*. Conserved domains are shown (boxes). Primers name and localization are indicated by thick bars. The arrows indicate the limit between exons. The numbers at the end of each line indicate the position of nucleotides and amino acids. E (Exons).

fish (Pellegrini et al., 2016).

A limited number of important genes or cDNAs of *R. quelen* has been sequenced, e.g., growth hormone, prolactin and somatolactin from the pituitary (Dolci et al., 2014; Pês et al., 2016; Vaz et al., 2010). However, neuroendocrine genes in the brain have been not explored. At this way there is an increasing concern about the detrimental effects of many natural and synthetic chemicals present in the environment on the endocrine system of organisms. The *cyp19a1b* and other genes as vitellogenin and *cyp19a1a* can be used as a tool for biomonitoring studies and water quality assessment. Therefore, the aim of this study was to identify and characterize brain aromatase gene expression (*cyp19a1b*) in *R. quelen*, and to evaluate the effects of E2 on *cyp19a1b* in different tissues.

2. Material and methods

2.1. Animals

R. quelen males (12.78 ± 0.53 cm; 18.79 ± 1.84 g) and females (12.72 ± 0.74 cm; 18.57 ± 2.64 g) were obtained from a local hatchery and maintained in glass aquaria (100 L capacity, with maximum loading of 1.5 g fish L^{-1}). Water was maintained saturated with oxygen through continuous aeration; temperature was at 25 ± 1 °C, under a photoperiod of 12 h light:12 h darkness. Fish were fed with balanced fish food suitable for this species (FRI-ACQUA 40, Fri-Ribe São Paulo, Brazil). In all cases, fish were anesthetized with 0.02% MS222 (ethyl 3-aminobenzoate methanesulfonate) and euthanized by spinal section. Tissues were dissected and stored in RNA later (Ambion) until further analysis. All procedures using animals were performed according to the NIH guidelines and to the Committee on Ethics in Animal Experimentation of Federal University of Paraná, Brazil.

2.2. Cloning and sequencing of R. quelen cyp19a1b

Total RNA from one male brain was isolated using RNeasy mini kit (including RNase-free DNase step; Qiagen) following the manufacturer's instructions. The extracted RNA was suspended in RNase-free water and stored at −80 °C until further analysis. Two micrograms of total RNA were reverse transcribed into cDNA using Superscript II (Invitrogen) and 1 μL (200 ng μL^{-1}) of random primers according to manufacturer's protocol. The resulting cDNA was used to amplify and clone *R. quelen cyp19a1b* by the polymerase chain reaction (PCR) using the following primers (AroBfw1: 5′-CTGCTGTGTACCATGTTCTG-3′ and AroBrev1: 5′-GCCTTCATCATCACCATAGC-3′). Primers were designed based on homologous brain *cyp19a1b* sequences from *Ictalurus punctatus* (GenBank, AF417239.1) and *Silurus meridionalis* (GenBank, AY325907.1). PCR was carried out in the presence of native *Taq* DNA polymerase (Invitrogen) and under the following conditions: 95 °C for 2 min followed by 40 cycles of 95 °C for 45 s, 55 °C for 30 s, and 72 °C for 45 s, and a final extension step at 72 °C for 10 min.

The expected product size amplified by PCR was excised and purified using QIAquick Gel Extraction kit (Qiagen). The purified PCR product was inserted into pCR®2.1-TOPO vector and cloned in *Escherichia coli* Top10 chemically competent cells (Invitrogen) following the manufacturer's instructions. Plasmids were purified using QIAprep® spin miniprep kit (Qiagen). To obtain the consensus sequence, three plasmid clones were sequenced at Ontario Genomics Innovation Centre (Ottawa Health Research Institute, Ottawa, Ontario, Canada).

2.3. Prediction of the amino acid sequence, alignment and phylogenetic analysis

The predicted protein sequence was determined from the obtained nucleotide sequence using the Expert Protein Analysis System (Expasy) translate program (http://www.expasy.ch/tools/dna.html). Domain

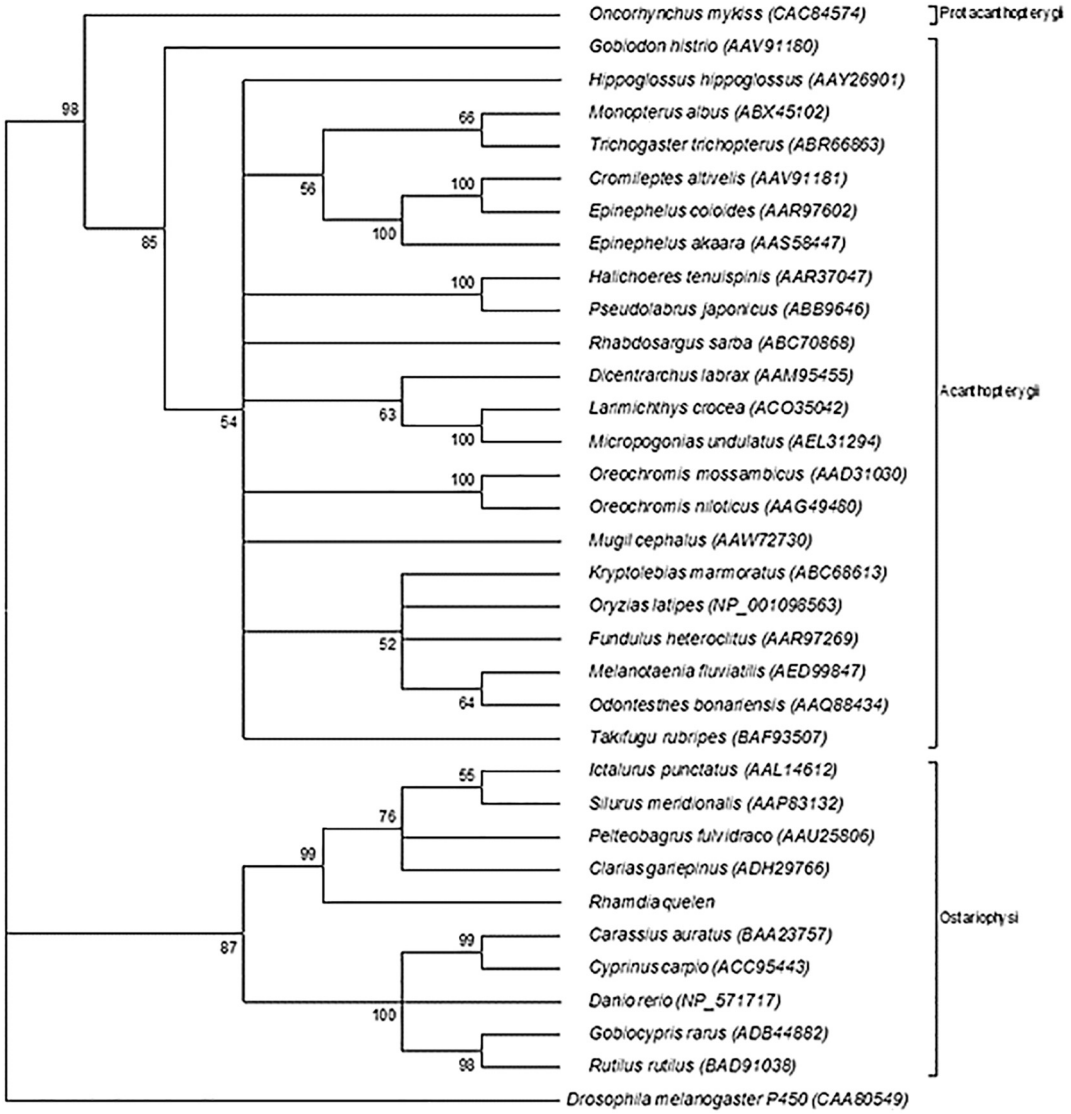

Fig. 2. Phylogenetic tree for vertebrate *cyp19a1b* proteins. Sequences are presented by species name followed by GenBank accession number in parenthesis. Taxonomic superorder is indicated (brackets). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Branches corresponding to partitions reproduced in < 50% bootstrap replicates are collapsed. Bootstrap values (percent) are shown next to branches.

sequences and intron/exon boundaries were determined by comparing the cloned sequence with other available and characterized fish *cyp19a1b* sequences using BLAST (http://www.ncbi.nlm.nih.gov/BLAST) and Clustal W multiple alignment programs (http://www.ch.embnet.org/software/ClustalW.html).

A phylogenetic tree was compiled using MEGA5 (Tamura et al., 2011). The analysis involved full-length amino acid sequences from 33 species and the *R. quelen* predicted protein sequence cloned in this study. GenBank accession numbers for those sequences were as follows: *Oncorhynchus mykiss* (CAC84574), *Gobiodon histrio* (AAV91180), *Hippoglossus hippoglossus* (AAY26901), *Monopterus albus* (ABX45102), *Trichogaster trichopterus* (ABR66863), *Cromileptes altivelis* (AAV91181), *Epinephelus coioides* (AAR97602), *Epinephelus akaara* (AAS58447), *Halichoeres tenuispinis* (AAR37047), *Pseudo labrus japonicus* (ABB9646), *Rhabdosargus sarba* (ABC70868), *Dicentrarchus labrax* (AAM95455), *Larimichthys crocea* (ACO35042), *Micropogonias undulatus* (AEL31294), *Oreochromis mossambicus* (AAD31030), *Oreochromis niloticus* (AAG49480), *Mugil cephalus* (AAW72730), *Kryptolebias marmoratus* (ABC68613), *Oryzias latipes* (NP_001098563), *Fundulus heteroclitus* (AAR97269), *Melanotaenia fluviatilis* (AED99847), *Odontesthes bonariensis* (AAQ88434), *Takifugu rubripes* (BAF93507), *Ictalurus punctatus* (AAL14612), *Silurus meridionalis* (AAP83132), *Pelteobagrus fulvidraco* (AAU25806), *Clarias gariepinus* (ADH29766), *Carassius auratus* (BAA23757), *Cyprinus carpio* (ACC95443), *Danio rerio* (NP_571717), *Gobio cyprisrarus* (ADB44882) and *Rutilus rutilus* (BAD91038). The tree was rooted with *Drosophila melanogaster* P450 (CAA80549). The amino acid sequences were aligned using Clustal W. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was processed according to Felsenstein (1985). Branches corresponding to partitions reproduced in < 50% bootstrap replicates are collapsed. Bootstrap values (percent) are shown next to branches. The evolutionary distances were computed using the Poisson correction method (Zuckerkandl and Pauling, 1965) and they are in the units of the number of amino acid substitutions per site. All ambiguous positions were eliminated only in pairwise sequence comparisons. There were a total of 552 amino acids positions in the final dataset.

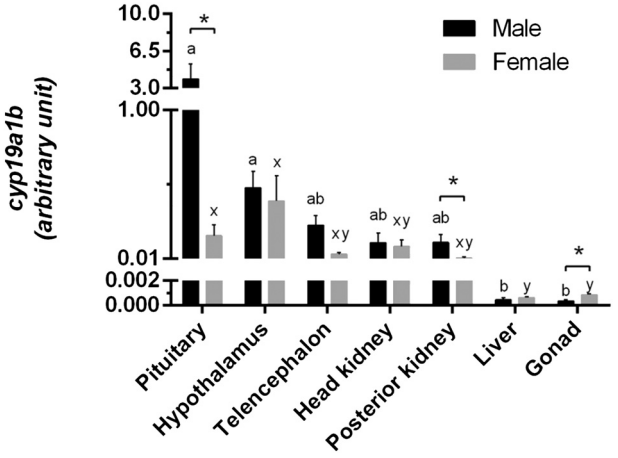

Fig. 3. *Cyp19a1b* mRNA levels in different tissues of 6 males and 6 females of *Rhamdia quelen*. abc indicate significant differences among tissues in males, while xyz indicate significant differences among tissues in females (Kruskal-Wallis, Dunn's test, $p < 0.05$). * indicates significant difference between male and female for each tissue (Student's *t* or Mann Whitney tests, $p < 0.05$). Data are expressed as mean ± S.E.M.

2.4. Effect of E2 on cyp19a1b in vivo

Six animals of each sex were injected intraperitoneally with 10 mg kg^{-1} E2 dissolved in canola oil and another six animals for each sex were injected with canola oil alone (vehicle control). This dose was used based on previous study that tested three doses of E2 (Moura Costa et al., 2010) and observed physiological effects in *R. quelen* with 10 mg kg^{-1} after 17 days of administration (Moura Costa et al., 2010, 2016). The fish were anesthetized with benzocaine 1%, and euthanized by spinal section. Telencephalon and hypothalamus brain regions, pituitary, head and posterior kidneys, liver and gonads were dissected and stored at −80 °C.

2.4.1. RNA isolation, cDNA synthesis and Real-time RT-PCR analysis

Total RNA from tissues was isolated using ReliaPrep™ RNA Tissue Miniprep System (including DNase step; Promega) following the manufacturer's instructions. The extracted RNA was suspended in RNase-free water and stored at −80 °C until further analysis. A 260/280 nm OD ratio ≥1.8 was obtained to ensure high RNA purity, and RNA integrity was confirmed via electrophoresis on 2% agarose gels. One microgram of total RNA was reverse transcribed into cDNA using AMV reverse transcriptase (Promega) and 1 μL (500 ng μL^{-1}) of Oligo(dT)$_{15}$ primer. The reaction was performed for 10 min at 20 °C, 45 min at 45 °C and 5 min at 95 °C.

Specific *cyp19a1b* cDNA from each tissue was amplified by quantitative polymerase chain reaction (qPCR) using specific primers (AroBfw2: 5′-GCAGAAGTTACCGTTGATGGA-3′ and AroBrev2: 5′-TTG GCTTTAGGGAAGAAC-3′) and SYBR Green PCR Master Mix (Applied Biosystems). The same procedure was carried for expression of references genes, β-actin (βacFw: 5′-CACTGGTATTGTGATGGACTC-3′ and βacRv: 5′- TCATGAGGTAGTCAGTCAGGTC-3′) and elongation factor-1α (ElFFw: 5′- GTTGGAGTCAACAAGATGG-3′ and ElFRv: 5′-GGGTTG TAGCCGATCTTC-3′).

A dissociation curve was used to verify primer specificity. Standard curves for gene expression assays were obtained from serial dilutions of a randomly pooled subset of female hypothalamus cDNA samples. Each sample was run in duplicate in optically clear 96-well plates in a final volume of 25 μL. The reaction contained 40 ng of cDNA, 0.8 μM of each primer, and 12.5 μL of SYBR green PCR master mix (2×). No template and no RT controls were run to ensure the absence of DNA contamination. Cycling parameters were as follows: 95 °C for 15 min followed by 40 cycles of 95 °C for 15 s, 59 °C for 15 s, 72 °C for 40 s. The qPCR efficiency for all the genes was above 95%. The relative standard curve method (Applied Biosystems, 1997) was used to determine relative abundance of each gene on the different samples.

The ratio between the *cyp19a1b* abundance and the arithmetic mean of the abundance of reference gene was calculated for each sample. Prior to comparison the relative expression values, data were assessed for normality by Kolmogorov-Smirnov test. The non-parametric Mann-Whitney and Kruskal-Wallis tests were used for the non-normal data and the parametric Student's *t*-test for normal data. The differences of *cyp19a1b* expression levels of each tissue between male and female and the E2 effects in different tissues of each sex were compared by Mann-Whitney or Student's *t*-tests. The cyp*19a1b* expression levels among the tissues of females and males fish, separately, were tested by Kruskal–Wallis test followed by Dunn's post hoc test. Data were considered significantly different if $p < 0.05$.

3. Results

We successfully cloned, sequenced a fragment of *cyp19a1b* from *R. quelen*. The amplified product was 1045 bp long and corresponds to approximately 60–70% of the coding sequence of the expected size of the *cyp19a1b* based on a range of related teleost species. The conserved domains and the exons and introns boundaries were predicted (Fig. 1) based on the European sea bass *cyp19a1b* sequence (Blázquez and Piferrer, 2004). The *R. quelen cyp19a1b* partial sequence was deposited

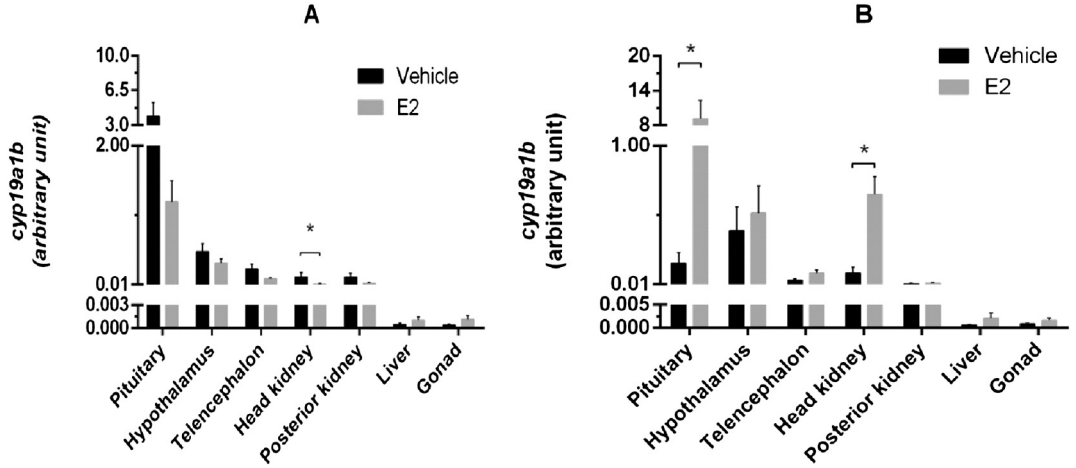

Fig. 4. Regulatory effects of 17β-estradiol (E2) in *cyp19a1b* mRNA levels in different tissues of 6 males (A) and 6 females (B) of *Rhamdia quelen*. * indicates significant difference between the treatments (vehicle control and E2) for each tissue (Student's *t*, or Mann Whitney tests $p < 0.05$). Data are expressed as mean ± S.E.M.

in GenBank under accession number KC525922.1. At the nucleotide level the sequence was found to be 88% identical to *Ictalurus punctatus* and *Silurus meridionalis*, both closely related species. Multiple amino acid (aa) alignments showed that the deduced amino acid sequence, with 347 aa of length, was between 80 and 91% similar to other teleosts.

Additionally, we used the predicted *Cyp19a1b* amino acid sequence 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. A phylogenetic tree was constructed and it included both full-length and partial fish *Cyp19a1b* protein sequences available in GenBank. The highest sequence similarity of *R. quelen Cyp19a1b* was with the Siluriformes, *Ictalurus punctatus* (88%), *Silurus meridionalis* (87%), and *Pelteobagrus fulvidraco* (86%), consistent with the known phylogenetic relationships of the catfish (Fig. 2).

The mRNA of *cyp19a1b* was detected in all studied tissues including head and posterior kidney, liver and gonads, in addition to the expected expression in pituitary and brain regions in both male and female (Fig. 3). Females and males presented similar levels of mRNA *cyp19a1b* in pituitary, hypothalamus, telencephalon, anterior and posterior kidney tissues, while levels in liver and gonad were significantly lower than pituitary and hypothalamus. Males presented higher levels of *cyp19a1b* than females in pituitary and posterior kidney.

We observed sexually-dimorphic effects of E2 injection. In E2-treated males the expression of *cyp19a1b* was down-regulated in the head kidney (Fig. 4A), while in E2-treated females the expression in the pituitary and head kidney were up-regulated (Fig. 4B).

4. Discussion

To the best of our knowledge, the present study is the first to report the nucleotide sequences of *R. quelen* brain aromatase *cyp19a1b*. *R. quelen cyp19a1b* has high identity (~88%) to three other catfish species, *Ictalurus punctatus*, *Silurus meridionalis* and *Pelteobagrus fulvidraco*. The *R. quelen* deduced amino acid sequence, with 347 aa of length, corresponds to 60–70% of the total *Cyp19a1b* protein for several teleosts (Blázquez and Piferrer, 2004; Chaube et al., 2015; Gelinas et al., 1998; Strobl-Mazzulla et al., 2005).

Our predictions suggest that *R. quelen cyp19a1b* presents I-helix, Ozol's peptide, aromatase-specific conserved and heme-binding regions, which are the supposed functional regions of aromatase, confirming that *cyp19a1b* is a highly-conserved gene. The presence of these conserved regions may indicate the conservation of the enzymatic activity. We also examined potential sex differences in *cyp19a1b* mRNA levels in pituitary, telencephalon, hypothalamus, liver, gonads and head and posterior kidneys.

The presence of *cyp19a1b* in brain regions and pituitary is well documented for other fish species, for example, *Carassius auratus* (Gelinas et al., 1998), *Oncorhynchus mykiss* (Menuet et al., 2003), *Danio rerio* (Menuet et al., 2005; Pellegrini et al., 2007), *Hippoglossus hippoglossus* (Matsuoka et al., 2006), *Salaria pavo* (Gonçalves et al., 2008), *Heteropneutes fossilis* (Chaube et al., 2015). In the teleost brain aromatase is only expressed in radial glial cells (Coumailleau et al., 2015; Diotel et al., 2010). Sexually dimorphic expression in telencephalon and hypothalamus has been observed in *Danio rerio* (Goto-Kazeto et al., 2004) and *Odontesthes bonariensis* (Strobl-Mazzulla et al., 2005) with males showing higher expression than females. It is known that teleost have high sexually dimorphic neurogenic capacity, in part due to the high aromatase activity in the different brain regions (Ampatzis et al., 2012; Coumailleau et al., 2015).

The expression of *cyp19a1b* in several peripheral tissues (e.g., liver, head kidney, testis, ovary, gill, etc.) was reported for several species of fish (Chang et al., 2005; Chaube et al., 2015; Kwon et al., 2001; Shanthanagouda et al., 2012; Strobl-Mazzulla et al., 2005; Tang et al., 2010; Van Nes et al., 2005), although this normally occurs in lower levels than in brain or pituitary. However, there is no consensus among the species, sex or tissues where brain aromatase is expressed, which leads to an unclear role to *cyp19a1b* expression in these tissues. It is suggested that the estrogen produced in peripheral organs can act in local targets modulating mechanisms not related directly to reproduction, as growth and differentiation of tissues (Blázquez and Piferrer, 2004; Piferrer and Blázquez, 2005). Likewise, is suggested that the presence of estrogen receptors in different organs is associated with different functions or tissue-specific mechanisms of regulation (Marlatt et al., 2012; Strobl-Mazzulla et al., 2008). In this way, the presence of *cyp19a1b* in peripheral tissues can modulate, in a tissue-specific way, the transcription of several genes by the action of estrogen receptors.

In the pituitary of *R. quelen* mRNA levels of *cyp19a1b* were found to be higher in males than females, suggesting a sex-specific function, and perhaps a local action to regulate gonadotropins or other pituitary hormone secretion (Olivereau and Callard, 1985; Zhang et al., 2014). Interestingly, the highest mRNA levels were observed in male pituitary and this result is not similar to other fishes. In *Danio rerio* (Goto-Kazeto et al., 2004), in *Cyprinus carpio* (Tang et al., 2010) and in *Odontesthes bonariensis* (Strobl-Mazzulla et al., 2005) no sex differences were observed in this tissue.

In males, higher *cyp19a1b* was detected in posterior kidney, but this difference was not observed in *Hippoglossus hippoglossus* (Matsuoka et al., 2006), while in *Cyprinus carpio* (Tang et al., 2010) and in *Cichlasoma dimerus* (Ramallo et al., 2017) the expression of *cyp19a1b* in kidney was not detected. On the other hand, females presented higher expression of *cyp19a1b* in gonads than males, similar to that observed by Caulier et al. (2015) in *Danio rerio*. These authors suggested that the *cyp19a1b* present in oocytes could be necessary for preliminary synthesis of estrogens in the embryos. However, the recent development of *cyp19a1a* and *cyp19a1b* knockout lines in zebrafish point to a dominant role for gonadal aromatase *cyp19a1a* (Yin et al., 2017).

We are interested in *R. quelen cyp19a1b* because it may be a useful tool for further investigations of the effects endocrine disrupting chemicals (EDCs) in an ecologically relevant South American species. It is already known that *cyp19a1b* is responsive to sexual steroids, especially to estrogens, since there is an estrogen-responsive element located in the promoter region of the gene and for its up-regulation is also necessary the presence of a functional estrogen receptor (Coumailleau et al., 2015). In this study, we observed that both *R. quelen* males and females were sensitive to 17β-estradiol (E2) exposure. In females of *R. quelen* an up-regulation in *cyp19a1b* occurred in pituitary and head kidney following E2 injection. In males only a down-regulation in *cyp19a1b* in the head kidney after E2 treatment was observed. Little is known about the regulation of *cyp19a1b* in head kidney in other fish species. Therefore, this down-regulation was also observed in the brain of *Gobiocypris rarus* males exposed to 17α-ethinylestradiol (EE2) (Qin et al., 2014), and in brain of *Pimephales promelas* males exposed to E2 (Halm et al., 2002), but in peripheral tissues this effect was not observed. However, decreased expression induced by estrogenic compounds is most frequent observed in *cyp19a1a*, the gonadal isoform of aromatase gene (Cheshenko et al., 2007; Hinfray et al., 2006; Kazeto et al., 2004; Mills et al., 2014).

It is generally accepted that E2 and other estrogens time-and dose-dependently upregulate *cyp19a1b* in the brain of different fish species (Brion et al., 2012; Gupta et al., 2017; Le Page et al., 2010; Menuet et al., 2005; Pérez et al., 2012; Roggio et al., 2014). The lack of effect of E2 in the *R. quelen* hypothalamus and telencephalon was therefore a surprise. As this is one of the first South American species to be examined, so perhaps there is an important species difference in brain responsiveness to exogenous estrogens. The Chinese rare minnow serves as an interesting example that may not follow this pattern. It has been reported that *cyp19a1b* tends to be upregulated in the brains of female *Gobiocypris rarus* exposed to waterborne EE2 at 1–125 ng L^{-1} for 3–6 days (Qin et al., 2014). In the same study, EE2 increased *cyp19a1b* in male brain at 3 days, but the treatments either reduced *cyp19a1b* (1 ng L^{-1}) or had no effects on brain *cyp19a1b* at the higher doses

(5–125 ng L^{-1}). In contrast, *cyp19a1a* was inhibited by these doses of EE2 in ovaries of female and testes of male *Gobiocypris rarus* (Qin et al., 2014).

From these previously reported results, in comparison to those obtained following injection of E2 in jundia, it is evident that the response of the various forms of aromatase to estrogens depends on species, sex, time, dose and tissue considered. This regulation is likely indicative of combined direct effects via estrogen receptor-estrogen response elements in gene promoters, and indirect effects of estrogenic chemicals on other factors controlling expression of *cyp19a1b*. Here we show that the head kidney of *R. quelen* is also a site of *cyp19a1b* expression and target of estrogen action.

In conclusion, the partial sequencing and characterization of *R. quelen cyp19a1b* provides essential information about this gene and its presence in peripheral tissues outside of the central nervous system in a teleost. Furthermore, we covered sex differences in the effects of E2 on the *cyp19a1b* gene expression in *R. quelen*.

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