General and Comparative Endocrinology 176 (2012) 52–61

Contents lists available at SciVerse ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Cloning, phylogenetic analysis and expression of somatolactin and its receptor in *Cichlasoma dimerus*: Their role in long-term background color acclimation

Maximiliano M. Cánepa [a,b,1], Yong Zhu [c], Mariana Fossati [a], John W. Stiller [c], Paula G. Vissio [a,b,*]

[a] Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, C1428EHA Buenos Aires, Argentina
[b] CONICET, Argentina
[c] Department of Biology, East Carolina University, Greenville, NC 27858, USA

A R T I C L E   I N F O

Article history:
Received 26 September 2011
Revised 29 November 2011
Accepted 17 December 2011
Available online 29 December 2011

Keywords:
Somatolactin
Somatolactin receptor
Pituitary
Background color exposure
Chromatophore
Melanophore
Teleost
Cichlid

A B S T R A C T

Somatolactin (SL) and SL receptor (SLR) belong to the growth hormone and cytokine type I receptor superfamilies, respectively. However, further research is required to define the duplications and functions of SL and its receptors in basal vertebrates including environmental background color adaptation in fish. In the present study, we cloned and sequenced SL and its putative receptor (SLR), classified and compared the sequences phylogenetically, and determined SL and SLR mRNA expression levels during long-term background color exposure in *Cichlasoma dimerus*, a freshwater South American cichlid. Our results show that *C. dimerus* SL and SLR share high sequence similarity with homologous from other perciform fish. Phylogenetic analysis indicates that *C. dimerus* SL belongs to the SLα clade sub-group. *C. dimerus* SLR is clearly a member of the GHR1 receptor subgroup, which includes the experimentally validated SLR from salmonids. Higher transcript levels of SLα in the pituitary and SLR in the epidermis and dermis cells of fish scales were observed in fish following long-term black background color exposure compared to those exposed to a white background. A higher number of melanophores was also observed in fish exposed for 10 days to a black background compared to those exposed to a white background. These changes were concomitant to differences in SL or SLR transcript levels found in fish exposed to these two different background colors. Our results suggest, for the first time, that SLR is expressed in fish scales, and that there is an increase in SL in the pituitary and the putative SLR in likely target cells, i.e., melanophores, in long-term black background exposure in *C. dimerus*.

© 2011 Elsevier Inc. All rights reserved.

1. Introduction

Somatolactin (SL) is a fish specific hormone produced mainly in the pituitary gland [18,35]. Initial studies suggested possible involvements of SL in a variety of physiological processes, including reproduction, stress responses, Ca^{2+} homeostasis, acid–base balance, growth, metabolism, and immune responses [2,14–17,20,21,25,27,28,32–34]. More recent studies suggested that SL is involved in the generation of chromatophores and the regulation of pigment movements in them [3,8,38]. In red drum, SL mRNA increased in the pituitary of fish adapted to a dark background color concomitant with an increase in plasma SL protein concentrations [38]. A decrease in the number of melanophores and increase in the number of leucophores were found in a medaka mutant lacking a functional SL [8]. Rescuing abnormal chromatophore proliferation by expressing a functional form of SL in mutants strongly supports the role of SL in the regulation of chromatophores [10]. An increase in number and area of SL immunoreactive cells was observed in *Cichlasoma dimerus* exposed to a black background compared to fish exposed to a white background [3]. However, no study has localized SLR in target tissues (i.e., chromatophores in fish scales) involved in changes of body color that are essential for regulation of fish body color and background color adaptation. In addition, there is no consensus regarding the physiological functions of SL and its receptor in fish, including their roles in the control of body color [9].

Cichlasoma dimerus is a cichlid that easily can be maintained and bred in the laboratory. Body color varies, depending on the mood of the fish and its social status, between greenish with light and dark gray (subordinate fish), or golden-yellow with light blue reflections (dominant fish). This fish also has several dark-brown vertical stripes that are controlled by the neuronal system. *C. dimerus* has predetermined breeding activities which can be observed and recorded easily in the laboratory. A dominant pair aggressively defends a previously established territory and

* Corresponding author at: Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, C1428EHA Buenos Aires, Argentina. Fax: +54 1145763384.

E-mail addresses: maximiliano.canepa@flinders.edu.au (M.M. Cánepa), paulav@bg.fcen.uba.ar, pvissio@gmail.com (P.G. Vissio).

[1] Present address: School of Biological Sciences, Flinders University, G.P.O. Box 2100, Adelaide, SA 5001, Australia.

0016-6480/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygcen.2011.12.023

prospective spawning site. They interact aggressively, biting (eyes and fins) and chasing other fish. Three to four days prior to spawning, the ventral head regions of the dominant pair turn black in color. After spawning, the defended territory increases in size and members of the pair alternately defend the breeding site. The frequency of aggressive interactions increases as fries grow. All these characteristics make *C. dimerus* a good model for studying neuroendocrine, reproductive and behavioral physiology.

In this study, we first cloned and phylogenetically classified cDNAs of SL and SLR from *C. dimerus*. Then, we examined the effects of long-term background color exposure on expression of SL and SLR transcript levels measured by RT-PCR in pituitary and skin, respectively. Changes in chromatophore number and morphology in fish scales were also determined. For the first time, we have localized SLR mRNA in epidermis and dermis cells obtained from fish scales and have shown changes of SLR transcript level concomitant with changes of SL-likely target cells (melanophores) in a cichlid.

2. Materials and methods

2.1. Fish collection and maintenance

Adult *C. dimerus* were transferred to the laboratory immediately following collection near coordinates 27°12′50″S, 58°11′50″W, Esteros del Riachuelo, Corrientes, Argentina. These fish were acclimated to a constant temperature (25 ± 2 °C) and photoperiod (14L:10D) in clear glass tanks (400 L) supplied with fresh water for at least one month prior to the experiments. Fish were fed to satiation daily with commercial pellets (Tetra Pond Variety Blend, Tetra). Experiment protocols were approved in accordance with the Guiding Principles for the Care and Use of Research Animals of The Comisión Institucional para el Cuidado y Uso de Animales de Laboratorio, Facultad de Ciencias Exactas y Naturales, Buenos Aires, Argentina.

2.2. Cloning of SL and SLR cDNAs

Pituitaries and livers were collected from five male fish (50–70 g) and placed into ten individual collecting tubes, following an overdose with 0.1% benzocaine and humane decapitation. Total RNA was extracted from each tissue sample by TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. RNA samples were quantified and purity of the samples was confirmed by spectrophotometry. Samples were then treated with DNase I (Sigma, St. Louis, MO, USA) to eliminate potential contamination of genomic DNA. First strand cDNA was synthesized using a SuperScript II (Invitrogen, Carlsbad, CA, USA). Reverse transcription (RT) reactions were performed using 2 μg of total RNA template at 45 °C for 50 min and 70 °C for 10 min. Degenerate primers (Tables 1 and 2) were designed according to conserved cDNA sequences of teleost SLs or SLRs from Perciform fish found in the GenBank database. PCR amplifications were performed in 20 μl reaction volumes using GoTaq Flexi DNA polymerase (Promega, Madison, WI, USA). Following an initial 2 min denaturation at 94 °C, a PCR cycle was repeated 40 times with denaturation at 94 °C for 30 s, annealing at 48–55 °C for 30 s and elongation at 72 °C for 30 s, with a final extension step at 72 °C for 10 min. PCR products were separated on a 1% agarose gel by electrophoresis. Specific bands, which were assumed to be SL or SLR, were extracted and purified from the agarose using a AccuPrep gel purification kit (Bioneer, Burenos Aires, Argentina). The purified PCR products were sequenced using the Big-Dye Terminator kit and an ABI Prism 377 DNA sequencer (Perkin–Elmer) and confirmed to be partial SL or SLR sequences.

Table 1
PCR primers for SL cloning and sequencing.

Name	Sequence (5′ → 3′)
degSLf	TGCTCTGGCCCYATYTRMTWAC
degSLr	TAAAATAACAACTGAGCAGGG
cdSLf1	CCATGCCTTAGCGAATCAACCTTG
cdSLf2	GCCCGTTCCAGTATGATGTGC
cdSLr1	TGAGATGGAGGGGCAGCGAGT
cdSLr2	GGAGAGGGAGTGGGATGAACA

Table 2
PCR primers for SLR cloning and sequencing.

Name	Sequence (5′ → 3′)
degSLRf1	CTTCTCATCCTTTCCTSC
degSLRf2	AAACTGGACCCTGCTGAA
degSLRf3	CTCAGACACCCAGARRCT
degSLRr1	GTCCAATGCTTCCAGTT
degSLRr2	TGACCTGAGCGTAGAAGT
degSLRr3	ATTGTGAGAGGTTCCCCA
cdSLRf1	CAGTGGTTCAGGAGTTAGACAG
cdSLRf2	CACAGCACCCACTCAAGGCA
cdSLRr1	TACCCACAGTCCCAAACACAAGAACC
cdSLRr2	CACGCATCCATCCCAACCCAACAT

Full-length SL or SLR sequences were obtained by RNA ligase-mediated rapid amplification of 5′ and 3′ cDNA ends (RLM-RACE) using the GeneRacer Kit (Invitrogen, Carlsbad, CA, USA). The 1st strand cDNA was synthesized according to the kit instructions using 2 μg total RNA and SuperScript III RT (Invitrogen, Carlsbad, CA, USA). The *C. dimerus* reverse and forward gene specific primers were designed based on partial sequences of SL or SLR obtained initially (Tables 1 and 2). Touchdown PCR was performed in a 20 μl reaction volume using GoTaq Flexi DNA polymerase (Promega). Following an initial 2 min denaturation at 94 °C, a PCR cycle was repeated five times with denaturation at 94 °C for 30 s, annealing/elongation at 72 °C for 2 min, then 5 cycles with denaturation at 94 °C for 30 s and annealing/elongation at 70 °C for 2 min, and 30 cycles with 94 °C for 30 s, annealing at 55–65 °C for 30 s and elongation at 72 °C for 2 min, with a final extension step at 72 °C for 10 min. PCR products were purified using a MinElute PCR Purification Kit (Qiagen, Valencia, CA, USA) and ligated into a pCR4-TOPO plasmid vector. The plasmids were transformed into TOP10 chemically-competent *Escherichia coli* cells (Invitrogen, Carlsbad, CA, USA). Multiple colonies were selected and plasmid vectors that contained PCR products were purified and sequenced as above. Confirmation of complete *C. dimerus* SL or SLR sequences was made using the BLASTN program (http://blast.ddbj.nig.ac.jp/).

2.3. Phylogenetic analysis

Phylogenetic analyses on protein sequences inferred from our putative *C. dimerus* SL and SLR genes were performed to verify their respective position in these hormone and receptor families. Protein sequences of putative homologs of both SLR and GHR were retrieved from NCBI across the diversity of teleost fish available, as well as GHR from lungfish and tetrapods to serve as phylogenetic outgroups. The same strategy was employed for teleost SL sequences using sturgeon and lungfish SL as outgroups. Sequences were aligned initially using Clustal X [13] to locate conserved domains and trim alignments to regions useful for broad scale phylogenetic reconstruction. These trimmed alignments were further refined in Muscle [6] on the EMBL-EBI web-server (http://www.ebi.ac.uk/Tools/msa/muscle/), and regions unique to single sequences were removed by hand.

Maximum-likelihood analyses were performed in PHYML [12] using the following parameters: an LG substitution matrix and gamma (4 categories) + invariable model for rate variation across

sites, with α and the fraction of invariable sites estimated from the data. One hundred non-parametric bootstrap replicates were performed to provide relative support values for membership of each *C. dimerus* sequence within a given protein subfamily.

2.4. Protein structure prediction

The SOSUI program [11] (http://www.bp.nuap.nagoya-u.ac.jp/sosui/sosuisignal/sosuisignal_submit.html) and NetNGlyc 1.0 server (http://www.cbs.dtu.dk/services/NetNGlyc/) were used for predicting N-terminus signal peptides, a transmembrane region, and potential N-glycosylation sites in *C. dimerus* SL and SLR sequences. To estimate SL and SLR molecular weights, the "Compute pI/Mw program" tool from the ExPASy server (http://www.expasy.ch/tools/pi_tool.html) was used. A ClustalW alignment (http://www.ebi.ac.uk/clustalw/) between the obtained sequences and published SL and GHR1 sequences was performed to locate consensus sequences.

2.5. Experiment on long-term background color exposure and SL expression in the pituitary gland

Fish (body length from 11.5 to 14.0 cm; body weight from 43.1 to 61.6 g; one fish per tank, 12 fish per treatment, six males and six females) were transferred to experimental glass tanks covered by a black background (BB) or white background (WB) polyethylene sheet, except for the top surface. Because *C. dimerus* is highly sociable, fish were maintained individually in the tanks for only 10 days in order to minimize stress. At the end of 10-day exposures, fish were sacrificed as described previously for the examination of the SL expression. Briefly, fish were anesthetized by immersion in a 0.1% benzocaine buffered solution. Pituitary glands were removed from overdosed fish and analyzed individually. Total RNA extraction, quantification and first-strand cDNA synthesis were performed as described above. Specific primers (Table 3) were designed to amplify a SL fragment of 500 bp. PCR was performed in a 20 μl reaction volume using a GoTaq Flexi DNA polymerase (Promega). Following an initial 2 min denaturation at 94 °C, a PCR cycle was repeated 35 times, consisting of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and elongation at 72 °C for 30 s, with a final extension step at 72 °C for 10 min. The optical densities of specific bands were photographed and quantified following electrophoresis of PCR products using image analysis software (Image Gauge version 3.12; Fuji Photo Software). Acidic ribosomal phosphoprotein (ARP) and β-actin RNA levels were used as housekeeping control genes (GenBank numbers GU244484 and EU158257, respectively). An optimal number of 25 amplification cycles was determined empirically for these control genes, at which the linear phase of PCR amplification was observed.

2.6. Experiment on long-term background color exposure and SLR expression in fish scales

The experimental design was similar to that described in the above section. However, fish (12 adult *C. dimerus*, average total body length: 10.1 ± 0.1 cm, average body weight: 27.1 ± 2.2 g, one fish per tank, six fish per treatment) were maintained in experimental tanks for 15 instead of 10 days. We chose a longer period for this experiment because a pilot study determined that a 15-day exposure to a black or white background was an appropriate duration to reach maximum differentiation or degeneration of melanophores (Cánepa, personal observations). For examining the expression and changes of SLR, ten scales were removed from the dorsal part of the trunk above the lateral line of each fish. Scales were used instead of direct evaluation of SLR expression on skin samples in order to avoid potential contamination from muscle cells. Epidermis and dermis cells were detached from scales

Table 3
PCR primers for RT-PCR.

Name	Sequence (5′ → 3′)
cdSLf	ATGCCACTAGACTGTAAAGA
cdSLr	TATGCACAGTTGTAGTTGTCAGC
cdSLR1f	GTGGTTCAGGAGTTAGAC
cdSLR1r	AACAGACACCCGTACACA
cdARPf	TTTGAAAATCATCCAACTTTTGGAT
cdARPr	GCAGGGACAGACGGATGGT
ACTf	GGATGATATGGAGAAGATCTG
ACTr	ATGGTGATGACCTGTCCGTC

by stirring the ten scales pooled together in plastic tubes for at least 30 min at room temperature in TRIzol reagent. Scales were discarded and the dissociated cells were homogenized using a micro pestle (Eppendorf) and processed for total RNA extraction. RNA was quantified by spectrophotometry and treated with DNase I (Sigma) to eliminate potential contamination of genomic DNA. First-strand cDNA was synthesized using an AMV enzyme (Promega) and 1 μg total RNA. Specific primers for SLR were designed to evaluate its presence in the scales (Table 3). PCR amplification was performed in a 20 μl reaction volume using GoTaq Flexi DNA polymerase (Promega). After 2 min of denaturation at 94 °C, the PCR cycle was repeated 40 times, consisting of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and elongation at 72 °C for 30 s. Liver and RNase-treated skin RNAs were used as positive and negative controls, respectively. Three additional scales also were collected from each fish. These scales were kept in physiological solution (NaCl, 16 mM; KCl, 8.6 mM; $CaCl_2$, 3 mM; $MgCl_2$, 2.5 mM; $NaHCO_3$, 1 mM; Na_2HPO_4, 1.3 mM; D-glucose 5 mM) and were photographed under a Nikon Microphot FX microscope with a Nikon Coolpix 4500 digital camera. Numbers of melanophores and xanthophores were counted manually in an area of approximately 3000 μm^2. Dopamine (10 mM) was added to the medium to induce pigment aggregation to facilitate the counting of chromatophores [5,23,29].

2.7. Statistical analysis

Experimental data were analyzed by Student's *t*-tests. Melanophore number was analyzed by a nested ANOVA design (scales nested within each experimental fish). Differences were considered significant at a *p*-value of 0.05 or less. Data were presented as mean ± SEM.

3. Results

3.1. Cichlasoma dimerus SL sequence (cdSLα)

A partial SL cDNA fragment of 879 bp was amplified by PCR from pituitary cDNAs using degenerate primers. The full-length SL cDNA was obtained by 5′ and 3′-RACE, and has 1549 nucleotides (GenBank accession number: EF192603, Fig. 1). The primary structure of *C. dimerus* SL is highly similar to SLs identified from other fish species (Supplementary Table 1). *C. dimerus* SL shares over 80% aa identity with SLα from medaka (*Oryzias latipes*) and other percomorpha species, and approximately 70% aa identity with SLα from other teleost species including Atlantic salmon, grass carp, goldfish and zebrafish. In contrast, it was far less (approximate 50% aa identity) similar to known SLβ sequences (Supplementary Table 1). Hereafter, this sequence is referred as cdSLα. The entire cdSLα cDNA has 31 bp in the 5′-untranslated region (UTR) followed by 69 bp encoding a signal peptide, 621 bp encoding the mature SL peptide, and finally 828 bp of 3′ UTR. A typical AATAAA polyadenylation motif was located 14 nucleotides upstream of the poly (A) tail. The open reading frame (ORF) of cdSLα cDNA encodes a pre-hormone of 230 aa, with a signal peptide of 23 aa and a mature protein of 207 aa.

C. dimerus SLα sequence

```
GAAAAGGAAAAACTTGAAGAAGACAGATAGAATGAGCATGACTGGCATCCAGCGAGGTGT   60
                                   -23 M  S  M  T  G  I  Q  R  G  V   -14
GTGGGGTCTACTGCTCTGGCCTTATATTCTTACTGTAAGCATGCCACTAGACTGTAAAGA  120
  W  G  L  L  L  W  P  Y  I  L  T  V  S  M  P  L  D  C  K  E    7
AGAGCCGGGCAGCTTTACTCGCTGCCCCTCCATCTCACAAGAGAAACTTCTCGACAGAGT  180
  E  P  G  S  F  T  R  C  P  S  I  S  Q  E  K  L  L  D  R  V   27
CATCCATCATGCTGAGCTCATCTACCGTGTCTCAGAAGAAGCGTGTTCCTTATTTGAGGA  240
  I  H  H  A  E  L  I  Y  R  V  S  E  E  A  C  S  L  F  E  E   47
GATGTTCATCCCACTCCCTCTCCGACTTCAGAGTAACCAGGCTGGCTATGCGTGTATCAC  300
  M  F  I  P  L  P  L  R  L  Q  S  N  Q  A  G  Y  A  C  I  T   67
CAATGCATTACCGATCCCCAGCTCCAAAAGTGAAATTCAACAGATATCCGATAGATGGTT  360
  N  A  L  P  I  P  S  S  K  S  E  I  Q  Q  I  S  D  R  W  L   87
GCTCCACTCTGTGCTGATGCTGGTTCAGTCATGGATTGAGCCCTTGGTCTACCTGCAAAC  420
  L  H  S  V  L  M  L  V  Q  S  W  I  E  P  L  V  Y  L  Q  T  107
AACACTGGATCGCTACGATCATGCTCCTGAAATGCTGCTCAACAAGACAAAATGGGTTTC  480
  T  L  D  R  Y  D  H  A  P  E  M  L  L  N  K  T  K  W  V  S  127
TGAGAAGCTGGTCAGTCTGGAGCAAGGGGTGGTTGCCCTCATTAAAAAGATGCTGGATGA  540
  E  K  L  V  S  L  E  Q  G  V  V  A  L  I  K  K  M  L  D  E  147
AGGGACGTTTACCACAACCTACAGTGAACAAGGCCCGTTCCAGTATGATGTGCTGCCAGA  600
  G  T  F  T  T  T  Y  S  E  Q  G  P  F  Q  Y  D  V  L  P  D  167
TATGCTGGAATCTGTTATGAGAGACTATACCTTGCTCAGCTGCTTCAAAAAGGATGCTCA  660
  M  L  E  S  V  M  R  D  Y  T  L  L  S  C  F  K  K  D  A  H  187
TAAGATGGAGACTTTCCTCAAGCTTCTTAAGTGTCGTCAGGCTGACAACTACAACTGTGC  720
  K  M  E  T  F  L  K  L  L  K  C  R  Q  A  D  N  Y  N  C  A  207
ATAAAATATAAACTGCAGCTAATAAATAATACAGTGCTTAGCTTTAAATGAATTCCTAAA  780
  *
GTTGGTAGTGTGCACTTAAAGATATGACCATGCCTTAGCGAATCAACCTTGCTTGTAATG  840
CAGTGCATTCCTTATTGATTGTTTTGGAACACCTTCACACAAAACTAAGTAGATGTAATG  900
CTTTGCCCTTTCTTCAACATACTGCATTTTATATTTTTTTTTCCCTGCTTAGTTGCTATT  960
TTAACCTTGCAAAGGAAGCAGAGAGCGAACTCTCAAAAGATTATTTGTGTGTTGAGTTGT 1020
CAAAAAAAATCTGCATATGGTGCCATTGATTTCCATTTCCTTTGTTCCTGACTGGTGTTT 1080
CTATTCCTTGCTGGGTCTTGCAGTGTTGTGATTATTCTCACAGCCCCTAGAGTACTGAGC 1140
GAGACGCTCATTTTTAATGGAGCTGGTTTCACCTCTGCATTAGTGAAATGAAACACTTTC 1200
ACCAAAGATCGCAGACACACAGAGCGCAATCACTATTAAAAATTCGATATATTTTGATTG 1260
GTGAAAGAGAGTGTGGATGAGACAGAGACAAAATAAATATCAGTCAAATTGAGAGCCTAA 1320
ATGTGTATAATTTATCATGAATATAACATATATAAAGAATGTGCCTTATTTACTTAAATT 1380
GTTCAGGGAATAAACAAGTTAAATGTATATTGTTAGATGTTATAAATACATGATGCAGAG 1440
CATAAAAAATACCTTTATCTTGTCAACAAAAGAACCTTATGGTGTACTCTCGATGCATTT 1500
CCATGCAACTGCTGTTCATTTAAGAGGCTAATAAAGCAAGACACAAAGCAAAAAAAAAAA 1560
AAAAAAAAAA
```

Fig. 1. Nucleotide and deduced amino acid sequences of *C. dimerus* somatolactin α (cdSLα). The putative signal peptide, polyadenylation signal, glycosylation site and conserved cysteine residues are underlined. Numbers on the right indicate the number of nucleotides and amino acids (italics) from the transcription start site or beginning of the mature peptide. An asterisk (*) represents the stop codon.

The estimated molecular weight of cdSLα is 23.81 kDa. The cdSLα sequence has seven cysteine residues at positions 5, 15, 42, 65, 181, 198, and 206 and a potential glycosylation site at position 121 (Asn-Lys-Thr, N-K-T) (Fig. 1).

Phylogenetic analysis showed cdSLα to nest deeply within the SLα clade, specifically related to SLα sequences from other cichlid fish. The analysis also provided strong support for a distinct and distant clade of the SLβ forms and cdSLα does not belong to this alternative sub-family of fish SL hormones (Fig. 2).

3.2. Cichlasoma dimerus SLR sequence (cdSLR)

The cdSLR cDNA consists of 2679 nucleotides, including 229 bp of 5′ UTR and 557 bp of 3′ UTR (GenBank accession number: FJ208943, Fig. 3). The ORF of cdSLR cDNA encodes 630 aa, which includes a 26 aa signal peptide, a 231 aa extracellular domain, a 22 aa single transmembrane domain, and a 351 aa intracellular domain. The estimated molecular weight of cdSLR is 67.75 kDa. The cdSLR peptide shares typical domains and characteristics with GH receptors: a FGEGS motif in the extracellular region, and Box 1 (PPVPAPKIKGI) and Box 2 (EPWVELIEVDVE) domains in the intracellular portion. The cdSLR has seven cysteine residues in the extracellular domain, in contrast to the five usually present in GH and PRL receptors of teleosts. Five cysteine residues in the cdSLR sequence share characteristic positions with those found in teleost GH and PRL receptors. The two additional cysteine residues are located in the medial part of the extracellular domain, at aa positions 115 and 132. Five potential N-glycosylation sites are also present in the extracellular domain, as well as seven tyrosine residues in the intracellular domain. The cdSLR has a strong similarity with GHR1 homologs of cichlid fishes from the genus of *Oreochromis* (85%) and other related teleosts (>60%) (Supplementary Table 2).

Phylogenetic analysis (Fig. 4) grouped cdSLR in a well-defined clade composed of GHR1 sequences from Acanthopterygii fish, which in turn are related to GHR1 variants from salmonids, zebrafish and eel. This global clade of fish GHR1 sequences also contains the SLR sequence from Masu salmon that has been defined experimentally as a somatolactin receptor [7]. GHR type 2 sequences from *Oreochromis* species and other cichlids formed a separate, well-defined clade with a parallel branching pattern of the common taxa available for analysis. These parallel topologies indicate that GHR and SLR originated by a gene duplication relatively early in fish evolution.

3.3. Effect of long-term background color exposure on the expression of SLα mRNA in the pituitary

Black background color exposure increased SLα transcript contents significantly in the pituitary compared to a WB exposure ($p = 0.032$; Fig. 6a and b). Visually, fish exposed to BB had darker body color than those fish maintained with a WB (Fig 5). Actin

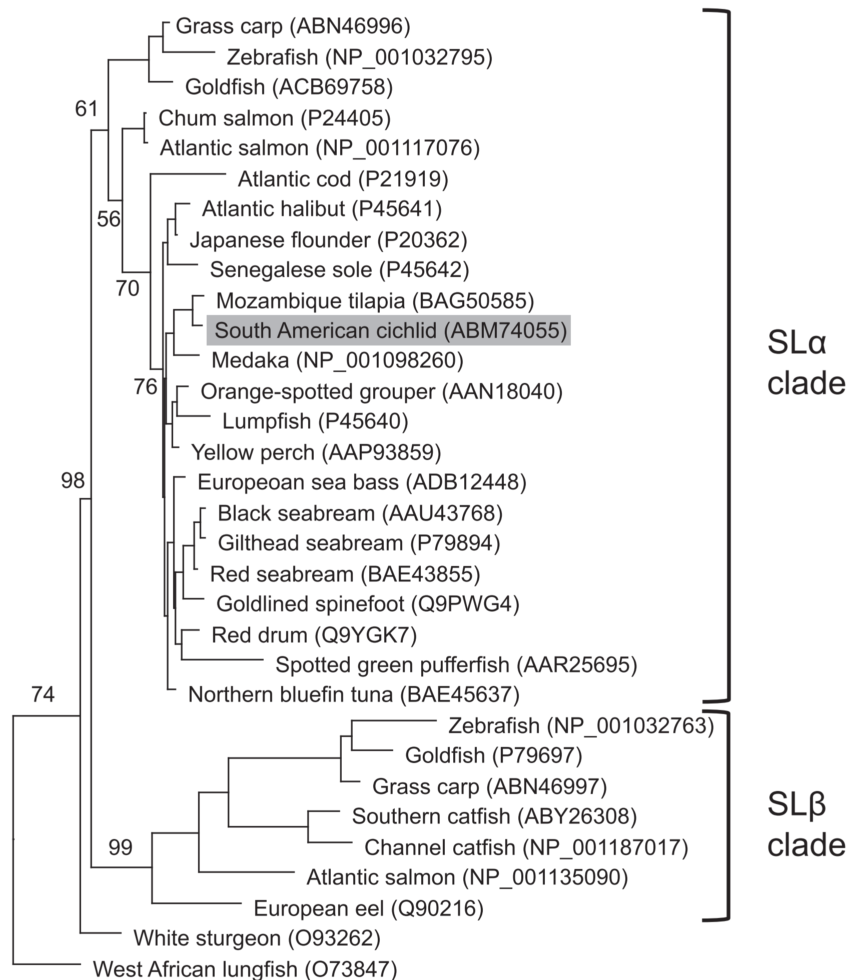

Fig. 2. Maximum-likelihood phylogeny of somatolactin (SL) sequences across the diversity of fish species. The tree resolves the SLα and SLβ sub-families recovered in previous investigations (e.g. [39]), and shows that the *C. dimerus* sequence (shaded in gray) nests deeply within the SLα clade, specifically related to SLα sequences from other cichlids. Support values from 100 nonparametric bootstrap replicates are shown for nodes important for defining the two SL subfamilies and the branching position of the *C. dimerus* sequence. NCBI accession numbers for each sequence are shown in parentheses. Lungfish and sturgeon SL sequences were used to root the tree of teleost SLs.

and ARP (controls) mRNA levels determined by RT-PCR did not differ among experimental conditions (data not shown).

3.4. Effect of long-term background color exposure on the expression of SLR mRNA in fish scales

A SLR specific product was found in epidermis and dermis cells samples obtained from the dorsal trunk region scales and in liver samples of adult fish by RT-PCR and confirmed by sequencing. No SLR product was detected in the absence of reverse transcriptase in the RT-PCR mixture, indicating no contamination from genomic DNA was present in the samples (Fig. 7).

An increase in expression of SLR transcripts in epidermis and dermis cells was observed in fish exposed to a BB compared to those exposed to a WB (Fig. 8, $p = 0.014$) along with an increase in the number of melanophores (Fig. 9, $p < 0.0001$). In contrast, no significant change in the number of xanthophores was observed under this background color exposure.

4. Discussion

SL was first identified in the early 1990s as a novel pituitary hormone in Atlantic cod [26], and now is known to be present in all actinopterygian fishes but absent from tetrapods [19]. Currently, there is no consensus on the function(s) of SL in fish [9]. Our study, for the first time, localizes the SL receptor (SLR) in the epidermis and dermis cells fish scales, and shows changes in the expression of SLR concomitant with phenotypic changes of likely SL target cells (melanophores) during background-color adaptation. Our present results are consistent with the previous findings in red drum, zebrafish and medaka, which all suggest a likely role of SL in the regulation of body color in fish [8,10,22,35,36].

Tissue localization of GHR1/SLR has only been carried out in a few fish species. In tilapia, mRNA for GHR1/SLR was detected in brain, pituitary, gills, heart, stomach, gall bladder, gut, fat, kidney, spleen, ovary, testis, muscle and skin [24]. In the present study, great care was employed to process skin to avoid muscle contamination, since the presence of GHR1/SLR has been reported in muscle cells [2,24]. Following scale removal and RNA extraction, RT-PCR showed the presence of SLR mRNA in epidermis and dermis cells, likely in chromatophores. Fukamachi and co-workers suggested a possible involvement of SL in chromatophore differentiation and proliferation, based on results obtained from a mutant medaka with a truncated SL gene [8]. One of the observed changes in the mutant medaka was variation in numbers of chromatophores. Indeed, changes in chromatophores were the phenotypes that could be rescued by over-expression of a normal form of SL [10]. When SL was over-expressed in mutant medaka, melanophore numbers in-

C. dimerus SLR sequence

```
GAAAAGCAAATAAGGCAAAAACCTCGCCAGGAAAGCACGCACCCGATTCTGAGATTGAGTGATATGTAAAAAATCGTCCCGGGGAGATCT 90
GACYCGGGCCGAACAGTCTGCAGTGAGGAAACAGGACTTGTACCCGTTAGAGCTTGCGCTTGCCATCAGATGAGCAACTTCTGAAAAGTA 180
AATCTGCGCTGTGCTTGCAGTTTTCAGCTCAACACTTCGGAAGAACATCATGGCTCTCTCGCCCTCCGCTAATCTCCTGATTCTTCTCAT 270
                                                      -26 M  A  L  S  P  S  A  N  L  L  I  L  L  I -13
TCTTTCCTCCCTGGATTGGCTGCCCTCTCCAGGATCGACATTTCTCACCGACTGGGACCACATGACATCACCCGCTCCCATTGAGCCTCA 360
 L  S  S  L  D  W  L  P  S  P  G  S  T  F  L  T  D  W  D  H  M  T  S  P  A  P  I  E  P  H 18
TTTTTCTGAGTGCATATCAAGGGACCAGGCGACGTTCCGCTGTTGGTGGAGTCCGGGCAGCTTCCAAAACCTGTCCTCCCCTGGAGCGCT 450
 F  S  E  C  I  S  R  D  Q  A  T  F  R  C  W  W  S  P  G  S  F  Q  N  L  S  S  P  G  A  L 48
CAGAGtCTtCTACCTGAAGAAAGACTCTCATGCCAGCCAATGGAAGGAGTGTCCTGtGTATATCCATTCAAGCAGGGAATGTTTCTTTGA 540
 R  V  F  Y  L  K  K  D  S  H  A  S  Q  W  K  E  C  P  V  Y  I  H  S  S  R  E  C  F  F  D 78
TGAAAACCACACATCTATTTGGATCACTTACTGCATGCAGCTTCGCACTCAAAACAACGTCACCTATTTCAATGAAGATGACTGTTTCAC 630
 E  N  H  T  S  I  W  I  T  Y  C  M  Q  L  R  T  Q  N  N  V  T  Y  F  N  E  D  D  C  F  T 108
TGTGGAGAATATTGTACGTCCTGACCCACCAGTGTCTCTAAACTGGACCCTGCTGAATATAAGTCCTTCTGGGCTAAATTATGATGTCAA 720
 V  E  N  I  V  R  P  D  P  P  V  S  L  N  W  T  L  L  N  I  S  P  S  G  L  N  Y  D  V  K 138
AGTTAACTGGGAGCCCCCGCCCACTGCTGATGTTGGGTTGGGATGGATGCGTGTTGAGTATGAGCTGCAGTACAGAGGGAGAAATACCAC 810
 V  N  W  E  P  P  P  T  A  D  V  G  L  G  W  M  R  V  E  Y  E  L  Q  Y  R  G  R  N  T  T 168
AAACTGGGAAGCAATGGAGATTCAGCGAAACACTCATCAGACAATCTACGGTCTGCACTTGGGAAAAGAATATGAAGTACACATCCGCTG 900
 N  W  E  A  M  E  I  Q  R  N  T  H  Q  T  I  Y  G  L  H  L  G  K  E  Y  E  V  H  I  R  C 198
CAGGATGCAGGCCTTCATTAAGTTTGGGGAGTTCAGCGACTCCATCTTCATTCAAGTGACTGAGATTCCTAGCGCAGAGTCTGCTGTCCA 990
 R  M  Q  A  F  I  K  F  G  E  F  S  D  S  I  F  I  Q  V  T  E  I  P  S  A  E  S  A  V  H 228
TCTCACAGTGGTTCTTGTGTTTGGGACTGTGGGTATCCTCATACTCTTCATGCTCATAGTCATCTCTCAGCAGAACCGATTAATGATATT 1080
 L  T  V  V  L  V  F  G  T  V  G  I  L  I  L  F  M  L  I  V  I  S  Q  Q  N  R  L  M  I  F 258
TCTGCTGCCGCCTGTTCCTGCACCCAAAATCAAAGGCATCGATTCAGAGCTATTAAAGAAGGGGAAACTGGATGAGCTGAATTTTATGCT 1170
 L  L  P  P  V  P  A  P  K  I  K  G  I  D  S  E  L  L  K  K  G  K  L  D  E  L  N  F  M  L 288
                 Box 1
GAGTGGTGGAGGAATCGACTGCCTACCCACTTACGCACCAGATTTCTACCAAGATGAGCCATGGGTGGAGCTAATCGAGGTGGATGTGGA 1260
 S  G  G  G  I  D  C  L  P  T  Y  A  P  D  F  Y  Q  D  E  P  W  V  E  L  I  E  V  D  V  E 318
                                                                     Box 2
GGATGAAGATAGTGGAGGGAAGGAGGATAACCGAGGCTCAGACACCCAGAAGCTCCTGGGTCAGCCCCAGCACATCAACATAGGCTGCTC 1350
 D  E  D  S  G  G  K  E  D  N  R  G  S  D  T  Q  K  L  L  G  Q  P  Q  H  I  N  I  G  C  S 348
CAATGCAGTCAGTGGCCCTGATGCTGACTCAGGGCGGGCCGGCTGTTACGACACAGATCTGCCTGAACAAGAAACCCTAATGCTGATGGC 1440
 N  A  V  S  G  P  D  A  D  S  G  R  A  G  C  Y  D  T  D  L  P  E  Q  E  T  L  M  L  M  A 378
CACCCTTCTGCCAGGACAACCTGATGACAAAGAAGCCTCCCTTGATGTTGTAGAAAGATCCTCAGCCTCTGAGACAGGTGATAGGCCTCT 1530
 T  L  L  P  G  Q  P  D  D  K  E  A  S  L  D  V  V  E  R  S  S  A  S  E  T  G  D  R  P  L 408
CATCCAAACCCAAACTAGAGGGCCCCAGACCTGGGTCAACACAGACTTCTATGCTCAGGTCAGCAATGTTATGCCCTCTGGTGGTGTGGT 1620
 I  Q  T  Q  T  R  G  P  Q  T  W  V  N  T  D  F  Y  A  Q  V  S  N  V  M  P  S  G  G  V  V 438
GTTGTCTCCTGGACAGCAACTCAGAATCCAGGAGAGCATCTCAGCCACCGAGGAGGAAACAAAAAAGAAGCGAAAGGGAAATGGAGACAG 1710
 L  S  P  G  Q  Q  L  R  I  Q  E  S  I  S  A  T  E  E  E  T  K  K  K  R  K  G  N  G  D  S 468
TGAGGAGTCTGAGGAGCGGAAGCAAAAAGAGCTGCAGTTTCGGCTGATAGTAGTGGATCCAGAGGGGAATGGCTACACTACAGAGAGCAA 1800
 E  E  S  E  E  R  K  Q  K  E  L  Q  F  R  L  I  V  V  D  P  E  G  N  G  Y  T  T  E  S  N 498
TGTCCAGCAGATCAGCACTCCTCCCCCCAGCTCTCTCATGCCTGGTGAGGGGTACCATATTATTCACCCTCAGCCAGTGGAGCCTAGACC 1890
 V  Q  Q  I  S  T  P  P  P  S  S  L  M  P  G  E  G  Y  H  I  I  H  P  Q  P  V  E  P  R  P 528
TACAGTTACAACAGAGGTTAATCTGTCACCTTACATTCTTCCTGACTCTTCCCAGTCTTTTGCTCCTGTTGCAGACTACACAGTGGTTCA 1980
 T  V  T  T  E  V  N  L  S  P  Y  I  L  P  D  S  S  Q  S  F  A  P  V  A  D  Y  T  V  V  Q 558
GGAGTTAGACAGTCATCACAGTCTGCTCCTTAACCCATCTACCCACCACACACCCCCTCCCTGCCTGCCACAGCACCCATTCAAGGCACC 2070
 E  L  D  S  H  H  S  L  L  L  N  P  S  T  H  H  T  P  P  P  C  L  P  Q  H  P  F  K  A  P 588
TATGCCTGTGGGGTACATTACCCCAGACTTGCTGGGGAACCTCTCACAATGAAATGACAATGGCATCAGATATAAGGCTGATTTACCAGG 2160
 M  P  V  G  Y  I  T  P  D  L  L  G  N  L  S  Q  *                                          604
AAGTTCCCCACAGTCGTCCTACCTGATTCTTGCTGGAAACCAAGTAACCGGGTGGATGAGATGTGTACGGGTGTCTGTTTTCAGAGGATG 2250
CTGAAAGGCAGATATAAAAAGCTGCGAGCTATTCTCTTAACTTCTTTGCATCTGCTCGAACAGTTTTTTTTTGAGCCAGGCAAATAACAT 2340
CACGTACCAAATCCACAGTTTGCTCTTGTCAATATATTCATGCATCCTTGCATGATTGTAATGAGTCAAACAAGCATAATGTCAGCGCTT 2430
AACTGCCTTGTGTCACATTGCAGCTCTGGCTTATTGTGTTCAGAGCAATGACACCATTACAGTCTTAATTTTACAGAGCACTGTCAGCGT 2520
ATGATTGATGGTGTATGATTTCATCAAACATCAAACAGAAGCCRCTGATGTTTACACCACCGAGGTACTTTGTCAGTTTTATTTTCTTAT 2610
AGGAGATCGTTCTTATCCTTCTTATCYCATCATGTCATGTTAGTACTTCACTAGAGATATATTTTTTATAAAAAAAAAAAAAAAAAAAAA 2700
AAA
```

Fig. 3. Nucleotide and deduced amino acid sequence of the putative *C. dimerus* somatolactin receptor (cdSLR). The putative signal peptide (underlined), polyadenylation signal (underlined with double line), potential extracellular N-glycosylation sites (open boxes), conserved cysteine residues, potential intracellular tyrosine phosphorylation sites (Y), and tyrosine residues are underlined. FGEFS (bold italics underlined), transmembrane (underlined with double line) and consensus sequences BOX1 and BOX2 (shaded boxes) also are highlighted. Numbers on the right indicate the number of nucleotides and amino acids (italics) from transcription start site or beginning of the mature peptide.

creased along with changes of other chromatophores, suggesting that SL plays a role in pigment cell proliferation and/or differentiation in fish.

In vitro assays performed in red drum and zebrafish suggest a high likelihood of finding SLR in melanophores, since SL stimulates pigment movement in a dose dependent, specific and reversible way [22,35]. Particularly in red drum, pre-incubation with SL affects melanophore dispersion induced by MSH in scale cultures [35]. In addition, the PI3K inhibitor LY-294002 inhibits melanophore aggregation provoked by NE in *Labrus bimaculatus*, linking the PI3K pathway with pigment dynamics [1]. Given that GHR1 belongs to the cytokine family of receptors, it is possibly involved in the signaling pathway of insulin receptor substrate 1 (IRS-1) and 2, and interacts with PI3K through JAK proteins pathways that include regulation of MAPK activity (Mitogen-activated protein kinases) and the cell cycle progression [4]. It is reasonable to suggest that SL and SLR can signal via the PI3K to affect pigment dynamics.

Variation in background color occurs frequently in nature due to diverse and unpredictable changes in the habitats occupied by this species (personal observations). Our study indicates that the pattern of body color shows a parallel change with SL transcript levels in the pituitary. Similar results were reported in other perciform fish including red drum, *Sciaenops ocellatus*, Atlantic croaker, *Micropogonias undulatus*, and medaka, *O. latipes* [8,37,38]. No significant difference in pituitary SL mRNA was found between male and female *C. dimerus*, indicating that the hormone's effects on background color are not sex-linked in this species. Current results showing elevated SL transcript in the pituitary are consistent with previous observations that an increased number and area of SL producing cells in fish pituitary occur in fish exposed to BB compared to those exposed to WB [3]. Moreover, SL plasma

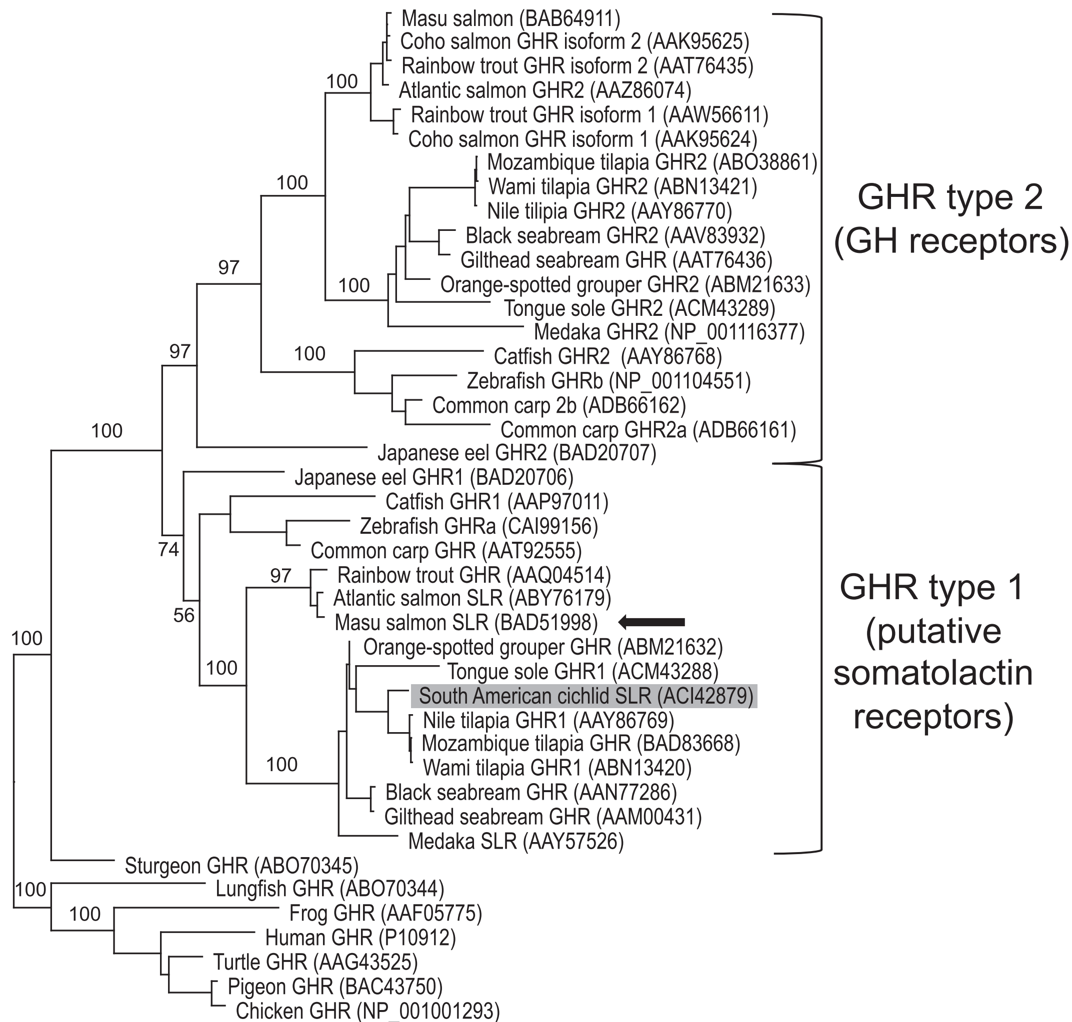

Fig. 4. Maximum-likelihood phylogeny of growth hormone receptor (GHR) and putative somatolactin receptor (SLR) sequences across the diversity of fish species. The tree resolves two major and well-supported receptor clades with parallel topologies across the diversity of teleost fish taxa. The *C. dimerus* sequence is shaded in gray and characterization of SLR and GHR subfamilies is based on our results combined with previous experimental evidence supporting members of the GHR1 clade as SL receptors; for example, SLR identified experimentally from Masu salmon [7] is indicated by the arrow. Support values from 100 nonparametric bootstrap replicates are shown for nodes important for defining the GHR and SLR subfamilies and the position of the *C. dimerus* sequence. Receptor names are based on their designations in NCBI accessions (shown in parentheses), which do not always conform to SLR/GHR1 and GHR2 subfamily designations defined by our phylogenetic analysis. Tetrapod and lungfish GHR sequences were used to root the tree of fish receptors.

levels were also affected by a black background in red drum and Atlantic croaker [37]. Taken together, these data indeed point to physiological roles of SL in background exposure, regulation of pigment movement, and generation of chromatophores, especially melanphores, in fishes.

As described previously, *C. dimerus* changes body color dramatically in response to changes in the background color (black or white). These changes can be achieved by aggregation/dispersion of pigments within chromatophores, and/or by an increase or decrease the number of chromatophores per area. Our study indicates that fish in BB have twice number of melanophores compared to those in WB. In contrast, background color has no effects on the number of xanthophores in our experimental setting, suggesting that this type of pigment cell is not involved in black/white background acclimation. Similar results were observed in tilapia and medaka, in which background conditions (white and black backgrounds) caused differences in melanophore morphology [30]. In particular, melanophores from *C. dimerus* exposed to a BB had an increased number of cells and great pigment dispersion. Several studies have shown that melanophores recover their shape, and occasionally their size, when animals are moved from a WB to a BB [30]. It was suggested that SL stimulates dispersion of pigments in melanophores [31], and, together with noradrenaline, MCH, and αMSH, affects dynamics of melanophore formation, dispersion and aggregation.

Two distinct SL variants encoded by paralogous genes and synthesized by different pituitary cells have been isolated in fish [39]. SLα is present in all teleost fish studied to date. In contrast, SLβ is retained only in earlier derived fish groups including zebrafish, goldfish, European eel, channel catfish and the salmonidae, but has been lost in late derived fish [2,39]. As expected, cdSLα shares high sequence identity with SL from another cichlid fish, Mozambique tilapia. It has the characteristic N-glycosylation sequence found in all SLα published to date and the seven cysteine residues present in α variants. Two cysteine residues were identified near the N-terminal end and four close to the C-terminal end; these allow the formation of three intra-disulfide bonds. The third cysteine closest to the N-terminus is not thought to be involved in the formation of intra-peptide disulfide bonds [26]; rather, it is proposed to participate in a regulatory mechanism involving rapid protein dimerization [39]. Characterization of the SL sequence from *C. dimerus* opens new areas of research for this species

Fig. 5. Effect of long-term background color acclimation on skin coloration of *C. dimerus*. Fish exposed to a black background (B) show a darker body color compared with those exposed to a white background (A). Scale bar = 1 cm. BB: black background, WB: white background.

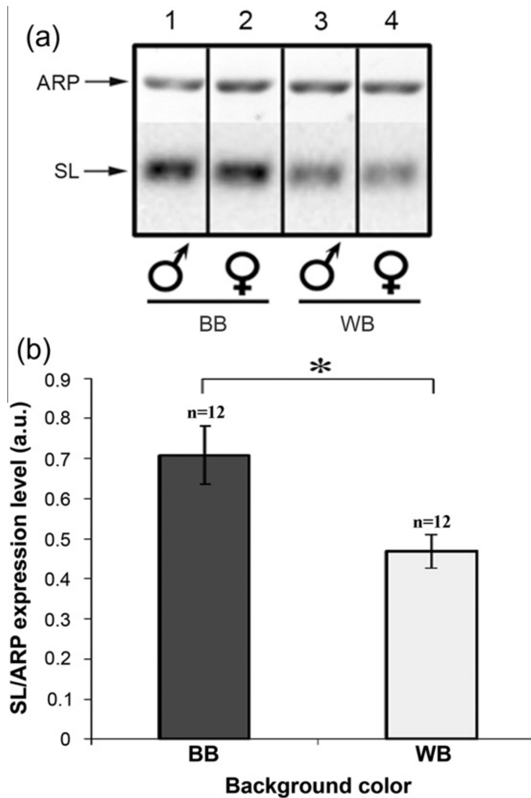

Fig. 6. Effects of black or white background on the expression of SL transcripts in fish pituitaries during a long-term background color acclimation. (a) Expression of SL transcript shown in a representative gel image. (b) SL transcripts levels were normalized using ARP. Each bar represents the mean ± SEM (n = 12). Significance is designated by an asterisk (student t-test, p = 0.0318). BB: black background, WB: white background.

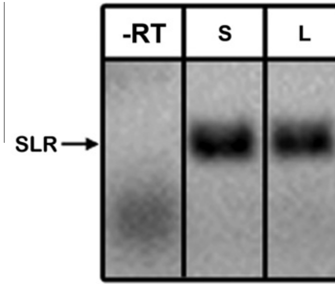

Fig. 7. Presence of SLR RNA transcript in epidermis and dermis cells detached from fish scales (S). cDNA from liver (L) was used as a positive control. (−RT) indicates a negative control PCR reaction carried out under the same conditions except with no reverse transcriptase enzyme (see Section 2 for detail methods).

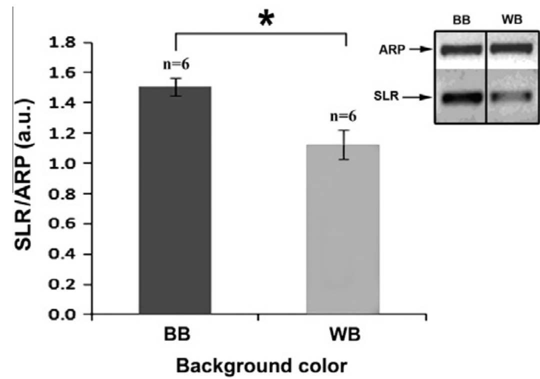

Fig. 8. Effect of background color exposure on the expression of cdSLR transcripts in skin. SLR levels were normalized using the housekeeping gene ARP. Insert picture shows a representative SLR gel image. Each bar represents mean ± SEM (n = 6). Significance is designated by an asterisk (student t-test, p = 0.014). BB: black background, WB: white background.

pertaining to the regulation of SL expression, and constitutes the first step in obtaining a recombinant protein that could be used in both *in vivo* and *in vitro* experiments to clarify further the role of this hormone.

Only masu salmon GHR1 has been characterized as a SLR using a functional receptor assay [7]. GHR1 in other fish species have been assumed to be putative SLR based on phylogenetic relationships [9,24]. In the present study, the nomenclature proposed by Fukamachi and Meyer [9] is used, which assumes that GHR1 is SLR. Based on SLR tissue distribution and transcript levels associated with different physiological conditions, Pierce et al. [24] also proposed, that GHR1 from Tilapia is SLR. The SLR sequence found in *C. dimerus* is very similar (identity 86%) to GHR1 obtained from three species of the tilapia genus *Oreochromis*, with similar structure and domains to GH receptors. However, it shares only 36% aa identity with GHR2 from *Oreochomis niloticus* and only 35% identity with GHR from *Protopterus dolloi*, a species that possesses only one copy of this gene [9]. SLR of *C. dimerus* has seven cysteine residues in extracellular domains capable of forming three disulfide bonds. The cdSLR intracellular domain is longer than the extracellular domain and possesses nine tyrosine residues susceptible to phosphorylation. These tyrosine residues would be involved in intracellular signaling, together with BOX1 and BOX2 domains, which are known to differ slightly between the types of GH receptors (type 1 and 2). Janus kinase (JAK) proteins bind to receptors of the cytokine family through consensus BOX1 and BOX2 sequences. These JAK proteins are capable of phosphorylating numerous different proteins involved in many signaling pathways, including STAT proteins that regulate gene transcription, and the IRS protein (insulin receptor substrate) that regulates the phosphatidiliositol 3́kinase (PI3K) pathway; these, in turn, regulate cellular metabolism. JAKs can also phosphorylate MAPK (Mitogen-activated protein kinases), which is involved in several cell processes [4].

From the phylogenetic analysis, *C. dimerus* SLR nests deeply within the putative SL receptor sub-group previously proposed by Fukamachi and Meyer [9]. Both sturgeon and lungfish have only one type of GH receptor, which is considered to be the ancestral

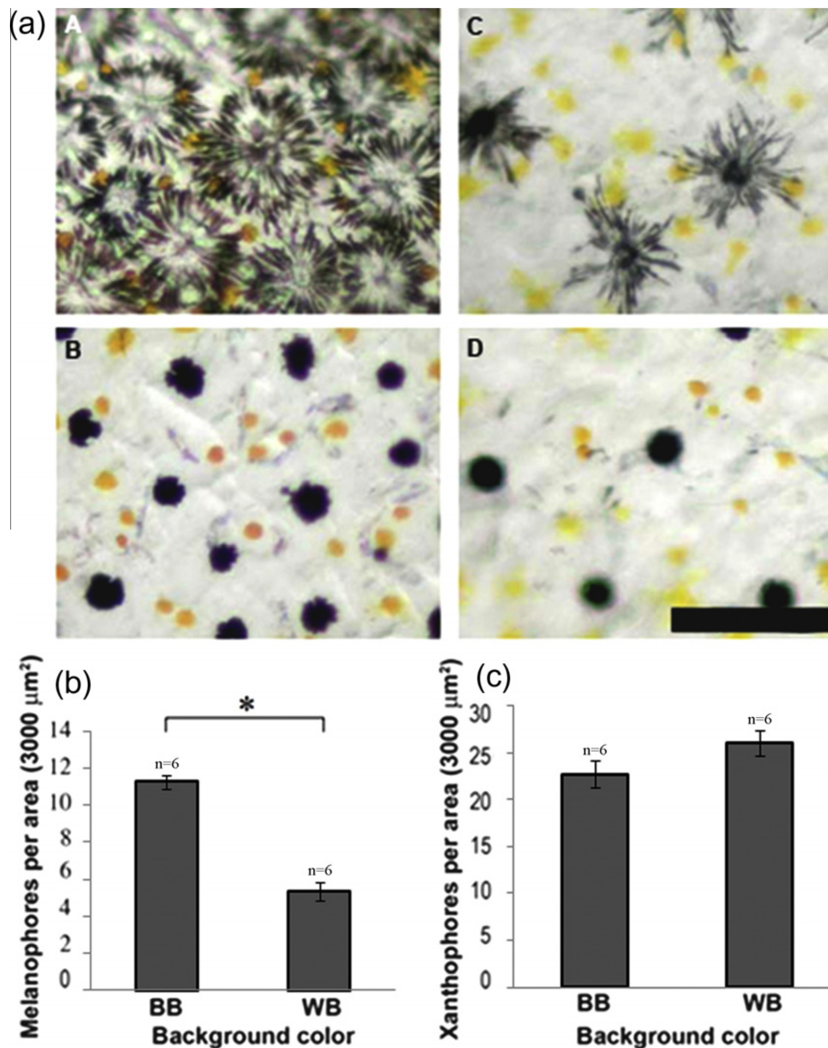

Fig. 9. Effects of a long-term background exposure on the chromatophores. (a) Representative scales from fish exposed to long-term black (picture A and B) or white (picture C and D) background color before (A and C) and after (B vs D) adding dopamine (10 mM) to the medium to induce pigment aggregation to facilitate the counting of chromatophores. Scale bar = 100 μm. (b) Comparing numbers of melanophores in fish exposed to a black background (BB) with those exposed to a white background (WB) in scales from the dorsal region of fish. Significant differences are indicated by an asterisk (nested ANOVA, $p < 0.0001$, $n = 6$). (c) Comparing the numbers of xanthophores in fish acclimated to BB to those acclimated to WB in scales from the dorsal region of fish. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

condition. As was the case for SL, GHR1 of the Percomorpha and Atherinomorpha are located in a well-defined clade with an overall topology that clearly reflects the phylogeny of fish species. Phylogenetic relationships are useful for comparing the distribution of SLR/GHR1 as well as the functions reported for SL among different fish species. New studies concerning possible SL functions are published every year, but the identity of the putative SL receptor remains under discussion. Therefore, it is of great importance to identify variations in SL levels with the presence of different receptors in target organs and, more precisely, in specific target cells.

In summary, *C. dimerus* exposed to a BB shows increased contents of SL transcript in the pituitary, which is consistent with previous findings that BB exposure induces an increase in the immunoreactive area of SL producing cells in the pituitary [3]. The increased number and dispersion of melanophores, likely SL target cells, and the elevation of SLR transcripts in scales of fish exposed to BB provides plausible evidence for SL and SLR functions in the regulation of chromatophores. Taken together, these results suggest that SL and SLR regulate body color by changing the numbers of melanophores and the movement of melanin within melanophores in the cichlid *C. dimerus*.

Acknowledgments

This work is supported in part by CONICET (grant number: PIP: 0276. P.V.), Agencia Nacional de Promoción Científica y Tecnológica (grant number: PICT 2005. P.V.), Universidad de Buenos Aires (grant number: 20020090200673. P.V.), the National Science Foundation Grant IBN-0315349 (Y.Z.) and East Carolina University Division of Research & Graduate Studies Research Development Award (Y.Z.). We thanks Tomás Delgadín and Daniela Perez Sirkin for their assistance. We would like to acknowledge Acaquatic Eco-Systems for a geneous gift, which supported the stay and visiting of Dr. Maximiliano M. Cánepa at Dr. Zhu's lab in Greenville, North Carolina.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ygcen.2011.12.023.

References

[1] T.P.M. Andersson, H.N. Sköld, S.P.S. Svensson, Phosphoinositide 3-kinase is involved in *Xenopus* and *Labrus* melanophore aggregation, Cell Signal. 15 (2003) 1119–1127.
[2] S. Benedet, B.T. Björnsson, G.L. Taranger, E. Andersson, Cloning of somatolactin alpha, beta forms and the somatolactin receptor in Atlantic salmon: seasonal expression profile in pituitary and ovary of maturing female broodstock, Reprod. Biol. Endocrinol. 6 (2008) 42–58.
[3] M.M. Cánepa, M. Pandolfi, M.C. Maggese, P.G. Vissio, Involvement of somatolactin in background adaptation of the cichlid fish *Cichlasoma dimerus*, J. Exp. Zool. 305 (2006) 410–419.
[4] C. Carter-Su, L. Rui, J. Herrington, Role of the tyrosine kinase JAK2 in signal transduction by growth hormone, Pediatr. Nephrol. 14 (2000) 550–557.
[5] C.A. Cornil, C.B. Castelino, G.F. Ball, Dopamine binds to α2-adrenergic receptors in the song control system of zebra finches (*Taeniopygia guttata*), J. Chem. Neuroanat. 35 (2008) 202–215.
[6] R.C. Edgar, MUSCLE: multiple sequence alignment with high accuracy and high throughput, Nucleic Acids Res. 32 (2004) 1792–1797.
[7] H. Fukada, Y. Ozaki, A.L. Pierce, S. Adachi, K. Yamauchi, A. Hara, P. Swanson, W.W. Dickhoff, Identification of the salmon somatolactin receptor, a new member of the cytokine receptor family, Endocrinology 146 (2005) 2354–2361.
[8] S. Fukamachi, M. Sugimoto, H. Mitani, A. Shima, Somatolactin selectively regulates proliferation and morphogenesis of neural crest derived pigment cells in medaka, Proc. Natl. Acad. Sci. USA 29 (2004) 10661–10666.
[9] S. Fukamachi, A. Meyer, Evolution of receptors for growth hormone and somatolactin in fish and land vertebrates: lessons from the lungfish and sturgeon orthologues, J. Mol. Evol. 65 (2007) 359–372.
[10] S. Fukamachi, T. Yada, A. Meyer, M. Kinoshita, Effects of constitutive expression of somatolactin alpha on skin pigmentation in medaka, Gene 442 (2009) 81–87.
[11] M. Gomi, M. Sonoyama, S. Mitaku, High performance system for signal peptide prediction: SOSUI signal, Chem. Biol. Inf. J. 4 (2004) 142–147.
[12] S. Guindon, O. Gascuel, A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood, Syst. Biol. 52 (2003) 696–704.
[13] F. Jeanmougin, J.D. Thompson, M. Gouy, D.G. Higgins, T.J. Gibson, Multiple sequence alignment with Clustal X, Trends Biochem. Sci. 23 (1998) 403–405.
[14] L.L. Johnson, B. Norberg, M.L. Willis, H. Zebroski, P. Swanson, Isolation, characterization, and radioimmunoassay of Atlantic halibut somatolactin and plasma levels during stress and reproduction in flatfish, Gen. Comp. Endocrinol. 105 (1997) 194–209.
[15] S. Kakizawa, T. Kaneko, S. Hasegawa, T. Hirano, Effects of feeding, fasting, background adaptation, acute stress, and exhaustive exercise on the plasma somatolactin concentrations in rainbow trout, Gen. Comp. Endocrinol. 98 (1995) 137–146.
[16] S. Kakizawa, A. Ishimatsu, T. Takeda, T. Kaneko, T. Hirano, Possible involvement of somatolactin in the regulation of plasma bicarbonate for the compensation of acidosis in rainbow trout, J. Exp. Biol. 200 (1997) 2675–2683.
[17] T. Kaneko, T. Hirano, Role of prolactin and somatolactin in calcium regulation in fish, J. Exp. Biol. 184 (1993) 31–45.
[18] T. Kaneko, Cell biology of somatolactin, Int. Rev. Cytol. 169 (1996) 1–24.
[19] H. Kawauchi, S.A. Sower, The dawn and evolution of hormones in the adenohypophysis, Gen. Comp. Endocrinol. 148 (2006) 3–14.
[20] R. Laiz-Carrión, J. Fuentes, B. Redruello, J.M. Guzmán, M.P. Martín del Río, D. Power, J.M. Mancera, Expression of pituitary prolactin, growth hormone and somatolactin is modified in response to different stressors (salinity, crowding and food-deprivation) in gilthead sea bream *Sparus auratus*, Gen. Comp. Endocrinol. 162 (2009) 293–300.
[21] M.A. Mousa, S.A. Mousa, Implication of somatolactin in the regulation of sexual maturation and spawning of *Mugil cephalus*, J. Exp. Zool. 287 (2000) 62–73.
[22] N. Nguyen, M. Sugimoto, Y. Zhu, Production and purification of recombinant somatolactin beta and its effects on melanosome aggregation in zebrafish, Gen. Comp. Endocrinol. 145 (2006) 182–187.
[23] T. Nyrönen, M. Pihlavisto, J.M. Peltonen, A. Hoffrén, M. Varis, T. Salminen, S. Wurster, A. Marjamäki, L. Kanerva, E. Katainen, L. Laaksonen, J. Savola, M. Scheinin, M.S. Johnson, Molecular mechanism for agonist-promoted α2A-adrenoceptor activation by norepinephrine and epinephrine, Mol. Pharmacol. 59 (2001) 1343–1354.
[24] A.L. Pierce, B.K. Fox, L.K. Davis, N. Visitacion, T. Kitashashi, T. Hirano, E.G. Grau, Prolactin receptor, growth hormone receptor, and putative somatolactin receptor in Mozambique tilapia: tissue specific expression and differential regulation by salinity and fasting, Gen. Comp. Endocrinol. 154 (2007) 31–40.
[25] J.V. Planas, P. Swanson, M. Rand-Weaver, W.W. Dickho, Somatolactin stimulates in vitro gonadal steroidogenesis in coho salmon, *Oncorhynchus kisutch*, Gen. Comp. Endocrinol. 87 (1992) 1–5.
[26] M. Rand-Weaver, T. Noso, K. Muramoto, H. Kawauchi, Isolation and characterization of somatolactin, a new protein related to growth hormone and prolactin from Atlantic cod (*Gadus morhua*) pituitary glands, Biochemistry 30 (1991) 1509–1515.
[27] M. Rand-Weaver, P. Swanson, Plasma somatolactin levels in coho salmon (*Oncorhyncus kitsutch*) during smoltification and sexual maturation, Fish Physiol. Biochem. 11 (1993) 175–182.
[28] M. Rand-Weaver, T.G. Pottinger, J.P. Sumpter, Plasma somatolactin concentrations in salmonid fish are elevated by stress, J. Endocrinol. 138 (1993) 509–515.
[29] J. Ruuskanen, J. Laurila, H. Xhaard, V. Rantanen, K. Vuoriluoto, S. Wurster, A. Marjamäki, M. Vainio, M.S. Johnson, M. Scheinin, Conserved structural, pharmacological and functional properties among the three human and five zebrafish α2-adrenoceptors, Br. J. Pharmacol. 144 (2005) 165–177.
[30] M. Sugimoto, Morphological color changes in fish: regulation of pigment cell density and morphology, Microsc. Res. Tech. 58 (2002) 496–503.
[31] M. Sugimoto, N. Uchida, M. Hatayama, Apoptosis in skin pigment cells of the medaka, *Oryzias latipes* (Teleostei), during long-term chromatic adaptation: the role of sympathetic innervation, Cell Tissue Res. 301 (2000) 205–216.
[32] K. Uchida, S. Moriyama, J. Breves, B. Fox, A. Pierce, R. Borski, T. Hirano, E. Grau, DNA cloning and isolation of somatolactin in Mozambique tilapia and effects of seawater acclimation, confinement stress, and fasting on its pituitary expression, Gen. Comp. Endocrinol. 161 (2009) 162–170.
[33] L. Vargas-Chacoff, L. Astola, F. Arjona, M. Martín del Río, F. García-Cózar, J. Mancera, G. Martínez-Rodríguez, Gene and protein expression for prolactin, growth hormone and somatolactin in *Sparus aurata*: Seasonal variations, Comp. Biochem. Phys. Part B Biochem. Mol. Biol. 153 (2009) 130–135.
[34] P. Vissio, L. Andreone, D. Paz, M. Maggese, G. Somoza, C. Strussmann, Relation between the reproductive status and somatolactin cell activity in the pituitary of pejerrey, *Odontesthes bonariensis* (Atheriniformes), J. Exp. Zool. 293 (2002) 492–499.
[35] Y. Zhu, P. Thomas, Elevations of somatolactin in plasma and pituitaries and increased alpha-MSH cell activity in red drum exposed to black background and decreased illumination, Gen. Comp. Endocrinol. 101 (1996) 21–31.
[36] Y. Zhu, P. Thomas, Effects of somatolactin on melanosome aggregation in the melanophores of red drum (*Sciaenops ocellatus*) scales, Gen. Comp. Endocrinol. 105 (1997) 127–133.
[37] Y. Zhu, P. Thomas, Effects of light on plasma somatolactin levels in red drum (*Sciaenops ocellatus*), Gen. Comp. Endocrinol. 111 (1998) 76–82.
[38] Y. Zhu, Y. Yoshiura, K. Kikuchi, K. Aida, P. Thomas, Cloning and phylogenetic relationship of red drum somatolactin cDNA and effects of light on pituitary somatolactin mRNA expression, Gen. Comp. Endocrinol. 113 (1999) 69–79.
[39] Y. Zhu, J.W. Stiller, M.P. Shaner, A. Baldini, J. Scemama, A.A. Capehart, Cloning of somatolactin α and β cDNAs in zebrafish and phylogenetic analysis of two distinct somatolactin subtypes in fish, J. Endocrinol. 182 (2004) 509–518.