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Leptin in Darkbarbel Catfish *Pseudobagrus vachellii*: Molecular Characterization, Synteny and Phylogeny, Tissue Distribution, and Expression in Response to Different Feeding Status

Zheng-Yong Wen[1, 2]*, Chuan-Jie Qin[1,2], Bin Li[1,2], Rui Li[1,2] and Xiao-Tao Shi[3]*

[1]*Key Laboratory of Sichuan Province for Fishes Conservation and Utilization in the Upper Reaches of the Yangtze River, Neijiang Normal University, Neijiang, 641100, China*

[2]*College of Life Science, Neijiang Normal University, Neijiang, 641100, China*

[3]*Engineering Research Center of Eco-environment in Three Gorges Reservoir Region, Ministry of Education, China Three Gorges University, Yichang 443002, China*

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Key words
Leptin, Phylogeny, Fasting, Refeeding, *Pseudobagrus vachellii*

ABSTRACT

Leptin is a small peptide secreted by adipocytes and it plays important roles in regulating appetite, energy homeostasis, bodyweight, reproduction and immunity. However, its roles are still limited in teleosts. In the present study, a *leptin* gene was characterized from the darkbarbel catfish (*Pseudobagrus vachellii*) and its expression patterns in response to different feeding status were investigated. The cDNA of the *pvleptin* was 1186 bp long, containing a 519 bp open reading frame (ORF) that predicted to encode a protein of 172 amino acids. Multiple Leptins alignment showed that four a-helix domains and two cysteine residues were conserved in vertebrates. Three-dimensional (3D) structure modeling revealed that pvLeptin was highly conserved with that of other tetrapods. Genetic synteny analysis revealed that *lepB* had specifically lost in siluriformes teleosts. Phylogenetic analysis showed that fish lineage contained two clades of *leptinA* and *leptinB*, and the *pvleptin* was grouped into *leptinA* clade and shared a close relationship with its counterpart in *P. fulvidraco*. Tissue distribution analysis showed that *pvleptin* was widely distributed with the highest mRNA expression level in liver. Two-week fasting significantly decreased the transcription level while refeeding elevated the mRNA expression level of *pvleptin* in liver, suggesting *leptin* may be involved in regulating food intake and energy metabolism in darkbarbel catfish. These findings may expand our understanding about the evolutionary history and functional roles of Leptin in teleost, as well as lay a solid foundation for commercial production of darkbarbel catfish.

INTRODUCTION

Leptin is a class-I helical cytokine peptide primarily secreted by adipocytes in mammals, which was first discovered from mouse in 1994 (Zhang *et al*., 1994). Subsequently, *leptin* was cloned in human (Cohen *et al*., 1996) and other mammals (Denver *et al*., 2011). In non-mammalian animals, *leptin* was first cloned from puffer fish (*Takifugu rubripes*) in 2005 (Kurokawa *et al*., 2005), the delay may be due to the low identity and similarity of Leptin in different lineages (Londraville *et al*., 2017). Thus far, it has been well demonstrated that Leptin plays vital roles in suppressing food intake, modulating cell morphology and cytokine release, stimulating the reproductive endocrine system, promoting bone formation, and maintaining energy homeostasis (Barash *et al*., 1996; Steppan *et al*., 2000; Friedman, 2002; Klok *et al*., 2006; Lafrance *et al*., 2010). However, the roles of *leptin* are variable due to its divergent evolution in vertebrates.

The evolutionary history of *leptin* seems to be complex among different vertebrates. Generally, a single copy of *LEPTIN* gene was identified in mammals (Londraville *et al*., 2017), while two copies of *leptin* were proved to be widely existed in teleosts (Xu *et al*., 2018). To date, two paralogs of *leptin* (*lepa* and *lepb*) were identified in medaka (*Oryzias latipes*) (Kurokawa and Murashita, 2009), zebrafish (*Danio rerio*) (Gorissen *et al*., 2009), orange-spotted grouper (*Epinephelus coioides*) (Zhang *et al*., 2013),

* Corresponding author: zhengyong_wen@126.com, fishlab@163.com
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mandarin fish (*Siniperca chuatsi*) (He *et al*., 2013; Yuan *et al*., 2016), Nile tilapia (*Oreochromis niloticus*) (Shpilman *et al*., 2014), tongue sole (*Cynoglossus semilaevis*) (Xu *et al*., 2018), and Northern snakehead (*Channa argus*) (Wen *et al*., 2020d). It is now clear that this phenomenon might be caused by a whole genome duplication (WGD) event in teleosts (Londraville *et al*., 2017; Xu *et al*., 2018; Wen *et al*., 2020d). Meanwhile, two copies of *lepa* genes (*lepa1* and *lepa2*) were identified in common carp (*Cyprinus carpio* L.) (Huising *et al*., 2006), Jian carp (*C. carpio* var. Jian) (Tang *et al*., 2013), goldfish (*Carassius auratus*) (Yan *et al*., 2016), Atlantic salmon (*Salmo salar* L.) (Angotzi *et al*., 2013), and rainbow trout (*Oncorhynchus mykiss*) (Murashita *et al*., 2008; Gong *et al*., 2013a). Moreover, two copies of *lepb* genes (*lepb1* and *lepb2*) were also identified in Atlantic salmon, rainbow trout, brown trout (*Salmo trutta*), and Arctic charr (*Salvelinus alpinus*) (Angotzi *et al*., 2013). These novel paralogs may originate from an additional WGD event in cypriniformes and salmoniformes teleosts (Lien *et al*., 2016; Xu *et al*., 2019). Interestingly, it seems that only a single copy of *leptin* is existed in siluriformes teleosts, such as channel catfish (*Ictalurus punctatus*) (Kobayashi *et al*., 2011) and yellow catfish (*Pelteobagrus fulvidraco*) (Gong *et al*., 2013b). However, the potential mechanisms of this phenomenon are still unclear and need for further investigation.

Previous studies revealed that Leptin is also involved in food intake and energy homeostasis in teleosts, and its mRNA expression level can be modulated by various feeding status (Dar *et al*., 2018; Chen *et al*., 2020). Food deprivation or starvation significantly increased the *leptin* transcription while refeeding decreased the corresponding mRNA expression level in goldfish (Volkoff *et al*., 2003), Atlantic salmon (Ronnestad *et al*., 2010), rainbow trout (Jorgensen *et al*., 2016), minnow (*Tanichthys albonubes*) (Chen *et al*., 2016), and snakehead (Wen *et al*., 2020d). On the contrary, a totally reversed pattern was observed in Ya-fish (*Schizothorax prenanti*) (Yuan *et al*., 2014), seabream (*Sparus aurata*) (Babaei *et al*., 2017), Indian major carp (*Labeo rohita*) (Dar *et al*., 2018), and Yangtze sturgeon (*Acipenser dabryanus*) (Chen *et al*., 2020). Additionally, two recent studies demonstrated that *leptin* was not affected by fasting or food deprivation in pacu (*Piaractus mesopotamicus*) (Volkoff *et al*., 2017) and silver dollar (*Metynnis argenteus*) (Butt *et al*., 2018). The differences among studies might be due to different tissues examined, variable durations of starvation, different types of diets, or differentially evolutionary history of teleost *leptin* genes (Butt *et al*., 2018; Wen *et al*., 2020d). Therefore, more related studies are required to illustrate the potential roles of *leptin* in teleosts.

The darkbarbel catfish (*P. vachelli*) belongs to siluriformes, bagridae, and is an omnivorous freshwater fish native to Asia, which has become an economically important aquaculture species in China due to its fast growth and valuable taste traits (Qin *et al*., 2017, 2018a). In the present study, a *leptin* gene was cloned and characterized in darkbarbel catfish (*Pvleptin*), and its expression level in response to different feeding status was examined. These findings will help us to better understand the evolutionary history and functional roles of Leptin in teleosts, as well as provide potential feeding management measures to improve the production of this species.

MATERIALS AND METHODS

Fish sampling

Darkbarbel catfish (bodyweight 4.9 ± 0.3 g) used in this study were purchased from local aquatic market in Neijiang city of China and fishes were transported to the experimental aquarium in the Key Laboratory of Sichuan Province for Fishes Conservation and Utilization in the Upper Reaches of the Yangtze River (Neijiang Normal University). Fishes were cultured in 100 L tanks with a constant flow of filtered water under natural light-dark conditions (12 L/12 D). The aquicultural water was aerated using an air pump, and the water temperature was maintained at 20.0±0.5°C. For cloning and tissue distribution experiments, a total of five fishes were randomly selected and then anesthetized with 10 mg.L^{-1} MS-222. Subsequently, fishes were sacrificed by decapitation and tissues samples including adipose, brain, gill, heart, intestine, kidney, liver, muscle, spleen and stomach were collected and immersed in liquid nitrogen immediately, then, were kept at -80°C for further utilization.

For fasting and refeeding experiments, fishes were assigned to 3 groups (with triplicate tanks per group; 15 fishes per tank) and then conducted as described in several previous studies (Qin *et al*., 2018b; Yang *et al*., 2018; Wen *et al*., 2020d). Fishes in control tanks were fed once daily at 19:00, while fishes in fasting group were not fed for two weeks. For refeeding group, fishes were fasted for two weeks and then fed at 19:00. Fishes were allowed to feed for 30 minutes then five fishes from each tank (three replicates, a total of 15 fishes for each group) were randomly selected and their livers were collected. All samples were treated as described above and finally were kept at -80°C for further experiments.

The animal experiments were conducted following the approval of the Neijiang Normal University Animal Care and Use Committee and in full compliance with its ethics guidelines.

Molecular cloning of Pvleptin

Total RNA was isolated from liver with the Trizol

reagent (Invitrogen, USA) following the manufacturer's instruction, and 1 μg of the RNA from each sample was reversely transcribed to cDNA by using Super Script™ II RT reverse transcriptase (Takara, Japan). Two pairs of primers were designed basing on a transcriptome data established in our previous study (Qin *et al*., 2017), and then they were used to amplify the complete open reading frame (ORF) sequences of *Pvleptin* by using *leptin* sequences from yellow catfish and channel catfish as references (Kobayashi *et al*., 2011; Gong *et al*., 2013b), and the primer information is shown in Table I. The basic cycling conditions of the PCR were set as follows: a denaturing stage at 94°C for 30 s, an annealing stage at gene-specific temperature for 45 s and an elongation stage at 72°C for 60 s, a total of 34 cycles. The products were purified from agarose gel using the Universal DNA Purification Kit (Tiangen, China), and then cloned into the pMD-19T vector (TaKaRa, Dalian, China) and finally sequenced at BGI-Wuhan (Wuhan, China).

Table I. PCR primers used for cloning and gene expression studies.

Primers	Primer sequence (5′→3′)
leptin 01	F TCCTGAAGTGATTCACTG
	R CACTGGGAATACAAGGCT
leptin 02	F TACTACATCACCGTGCGTCA
	R GCTTAGAGAACTGTGCT
Pvleptin q	F ACTTCCAGCGAGTCCTTC
	R GGTTGAGCCTCTGTATGTATT
β-actin q	F GGTCCAGACGCAGAATAGC
	R AATCCCAAAGCCAACAGG

Multiple sequences alignment and three-dimension (3D) structure prediction

Two cDNA sequences obtained from the sequencing clones were assembled into one complete sequence. Subsequently, the ORF finder (https://www.ncbi.nlm.nih.gov/gorf/gorf.html) and Primer Premier 5.0 software were used to determine the ORF and predict the putative protein sequence of *Pvleptin*, respectively (Wen *et al*., 2020a). Meanwhile, signal peptide was predicted using the online tool Signal P 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/). Furthermore, ClustalX and BioEdit were conducted to perform the multiple alignments as described in our previous studies (Wen *et al*., 2019, 2020b). Finally, the SWISS-MODEL (https://swissmodel.expasy.org/) was utilized to predict the Leptin three-dimension (3D) structures of representative species (Li *et al*., 2020; Wen *et al*., 2020c).

Table II. Listing of Leptin amino acid sequences used in this study. Protein IDs are given to allow access to the protein sequence on GenBank or Ensembl website.

No.	Taxon name	Gene name	Protein ID
1	*Channa argus*	*leptin A*	MF504015.1
2	*Ctenopharyngodon idella*	*leptin A*	ACI32423.1
3	*Danio rerio*	*leptin A*	NP_001122048.1
4	*Epinephelus coioides*	*leptin A*	JX406147.1
5	*Oncorhynchus mykiss*	*leptin A*	BAG09232.1
6	*Oreochromis niloticus*	*leptin A*	ENSONIT00000018810.1
7	*Salmo salar*	*leptin A*	FJ830677.1
8	*Siniperca chuatsi*	*leptin A*	KC778775.1
9	*Channa argus*	*leptin B*	MK559418.1
10	*Ctenopharyngodon idella*	*leptin B*	AFU35432.1
11	*Danio rerio*	*leptin B*	NP_001025357.2
12	*Epinephelus coioides*	*leptin B*	JX406148.1
13	*Oncorhynchus mykiss*	*leptin B*	AGG81493.1
14	*Oreochromis niloticus*	*leptin B*	ENSONIT00000014459.1
15	*Salmo salar*	*leptin B*	AGG81488.1
16	*Siniperca chuatsi*	*leptin B*	KC778776.1
17	*Ictalurus punctatus*	*leptin*	ENSIPUP00000010365.1
18	*Lepisosteus oculatus*	*leptin*	ENSLOCT00000019160.1
19	*Pseudobagrus vachellii*	*leptin*	MW251477
20	*Tachysurus fulvidraco*	*leptin*	AFO67938.1
21	*Bubalus bubalis*	*LEPTIN*	NP_001277830.1
22	*Columba livia*	*LEPTIN*	CDL67225.1
23	*Gallus gallus*	*LEPTIN*	APC23099.1
24	*Homo sapiens*	*LEPTIN*	NP_000221.1
25	*Ovis aries*	*LEPTIN*	CCE35540.1
26	*Rattus norvegicus*	*LEPTIN*	NP_037208.1
27	*Xenopus laevis*	*LEPTIN*	NP_001089183.1

Synteny and phylogenetic analyses

Genetic synteny was conducted to better understand the evolutionary history of *leptin* genes in representative species. *In silico* protein similarity-based blast was executed to against the genome datasets of representative species using zebrafish Leptin and its flanking proteins as queries. Genome datasets were downloaded from

Ensemble (http://asia.ensembl.org/index.html) or NCBI (https://www.ncbi.nlm.nih.gov/) databases. Meanwhile, phylogenetic analysis was performed to declare the relationship of *leptin* genes in vertebrates. A series of Leptin protein sequences of representative species were also downloaded from NCBI or Ensemble databases, and their accession numbers were listed in Table II. The protein dataset was aligned by using ClustalX software, and then the best-fitting model was evaluated by Mrmodeltest 2.0 and ProtTest 2.4. Subsequently, phylogenetic tree was constructed with neighbor-joining method by using Mega 6.0 software (Wen *et al*., 2017). The robustness of the tree topology was assessed by nonparametric bootstrap analysis with 1,000 resampling replicates. The tree was beautified with FigTree software and spotted gar (*Lepisosteus oculatus*) was selected as the outgroup species.

Real-time quantitative PCR

Real-time quantitative PCR (qPCR) was used to detect the mRNA expression level of *Pvleptin*, which could be helpful for better understanding the tissue distribution pattern and nutritional regulation of *leptin* in the darkbarbel catfish. RNA isolation and first strand cDNA synthesis were conducted as described above. The qPCR reaction system contained 10 µL SYBR Green Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), 8 µL double distilled H_2O, 0.5 µL forward/reverse specific primer (10 µM), and 1 µL reverse transcribed product, with a final volume of 20 µL. Then qPCR was conducted on a Light Cycler Real-Time system and the running procedure was designed following the manufacturer's instruction. The end products of qPCR were verified with the melting curves that showing a single peak specific for the target gene. Relative *leptin* mRNA expression was calculated by using method described in previous studies (Pfaffl, 2001; Da *et al*., 2021; Wen *et al*., 2021), and *β-actin* was selected as reference gene after assessing the stability of several potential housekeeping genes. Primers were provided in Table I.

Statistical analysis

Statistical analysis was performed with SPSS 22.0 (IBM, Armonk, NY, USA) and GraphPad Prism (San Diego, CA, USA). All data were shown as mean normalized values ± standard error of the mean. Significant differences were evaluated by using one-way analysis of variance (ANOVA), followed by the post hoc test (least significant difference test and Duncan's multiple range test), after confirming for data normality and homogeneity of variances. Differences were considered to be significant if $P < 0.05$.

RESULTS

cDNA characterization of the Pvleptin

The characterized cDNA sequence of *Pvleptin* was 1186 bp long containing a 148 bp 5'-UTR, a 539 bp 3'-UTR, and a 519 bp ORF that predicted to encode a Leptin precursor of 172 amino acids (Fig. 1). A putative signal peptide with 23 amino acids was identified at the N-terminal of the Leptin precursor (Fig. 1). Similar to other teleosts, four conserved a-helix domains were discovered in the mature Leptin of darkbarbel catfish with length ranging from 16-23 amino acids (Fig. 1). The electronic point and molecular weight of the putative PvLeptin were calculated to be 7.96 and 19.89 kDa, respectively. The cDNA sequence of *Pvleptin* has been deposited into GenBank database with an accession number MW251477.

Multiple sequences alignment and 3D structure prediction

Multiple sequences alignment was performed based on the protein sequences to better understand the structural and functional properties of the vertebrate Leptins. We observed that Leptins commonly contained a signal peptide, four a-helix domains and two conserved cysteine residues were also identified in vertebrates (Fig. 2A). Meanwhile, sequence identity analysis was also conducted, and results showed that PvLeptin shared low identity with Leptin in tetrapods (human, 19.2%; rat, 19.7%; chicken, 20.1%; frog, 25.9%) and most teleosts (zebrafish LepA, 37.3%; zebrafish LepB, 22.0%; snakehead LepA, 22.1%; snakehead LepB, 15.3%; rainbow trout LepA, 28.4%; rainbow trout LepB, 17.4%), whereas shared high identity with that in siluriformes fish including yellow catfish (99.4%) and channel catfish (90.1%) (Supplemental Table I). Interestingly, despite PvLeptin shares low identity with that in most teleosts, it seems that PvLeptin is closer to teleost LepA than LepB. Additionally, 3D structures modeling revealed that the 3D structures of Leptin in four representative species (human, rat, frog and darkbarbel catfish) were highly conserved (Fig. 2B).

Genetic synteny and phylogenetic analyses

Genetic synteny and phylogenetic analysis were performed to better understand the evolutionary history and phylogenetic relationship of *leptin* genes in vertebrates. Synteny analysis showed that only a single copy of *LEPTIN* gene was found in mammals including human and rat, while two copies of *leptin* gene were extensively existed in teleosts, such as in zebrafish, pachon cavefish (*Astyanax mexicanus*) and snakehead fish (Fig. 3). Meanwhile, two conserved gene clusters of *PAX4-SND1-LRRC4-LEPTIN-RBM28-PRRT4-IMPDH1* and *cacna2d1-hgfb-lrrc4b-lepA-rbm28* were identified in mammalian

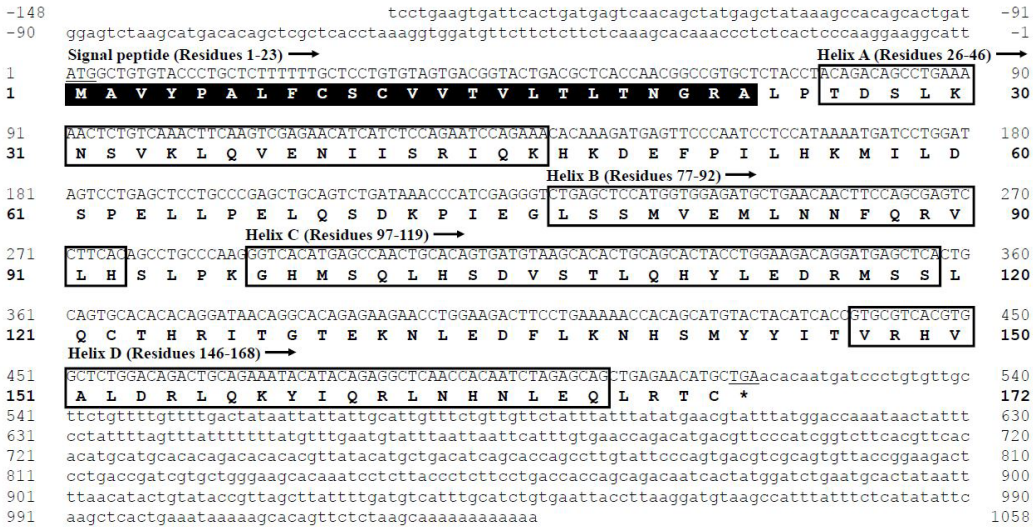

Fig. 1. Nucleotide and putative amino acid sequences of leptin in darkbarbel catfish. Numbers on both sides represent the positions of the nucleotide and amino acid sequences. Signal peptide is marked with white type in black box. Four putative α-helix domains are boxed and numbered with helix A-D. Small letters in the top and bottom positions represent the 5′- and 3′-untranslated regions (UTRs). Underlines represent initiation codon and termination codon respectively.

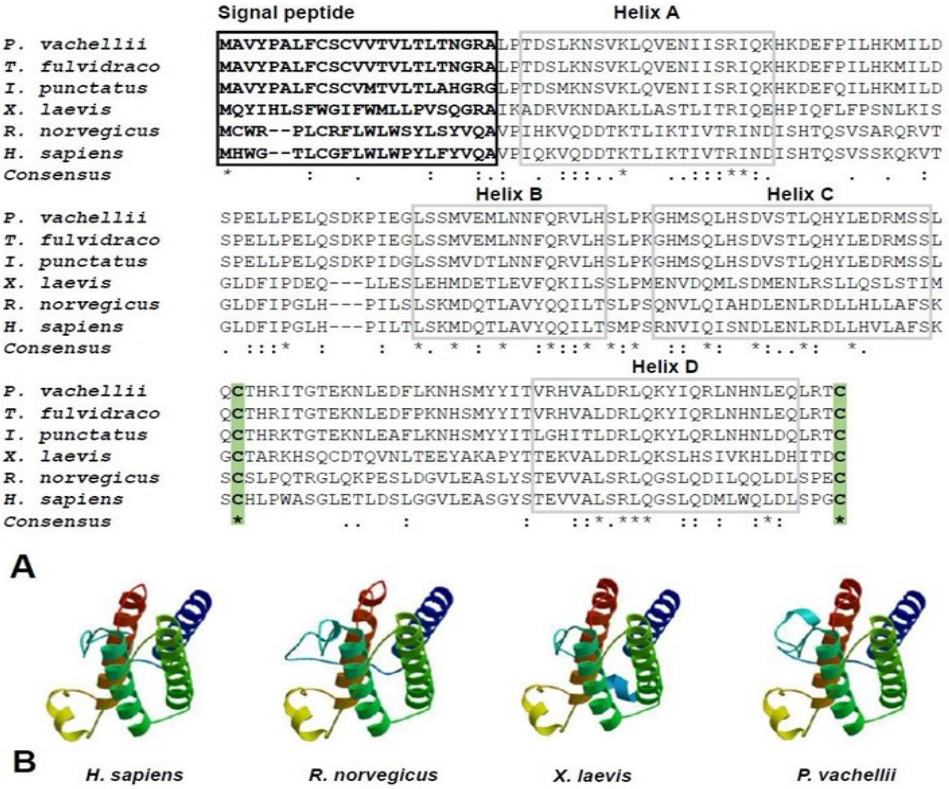

Fig. 2. Multiple Leptin sequences alignment (A) and three-dimensional structure modeling of Leptins in four representative species (B). Signal peptide sequences are marked by black box and highlighted with bold font. Four α-helix domains were labeled by gray boxes and numbered A-D. Two conservative cysteine residues were marked by green background with bold font. The representative species including human (*H. sapiens*), rat (*R. norvegicus*), frog (*X. laevis*) and darkbarbel catfish (*P. vachellii*).

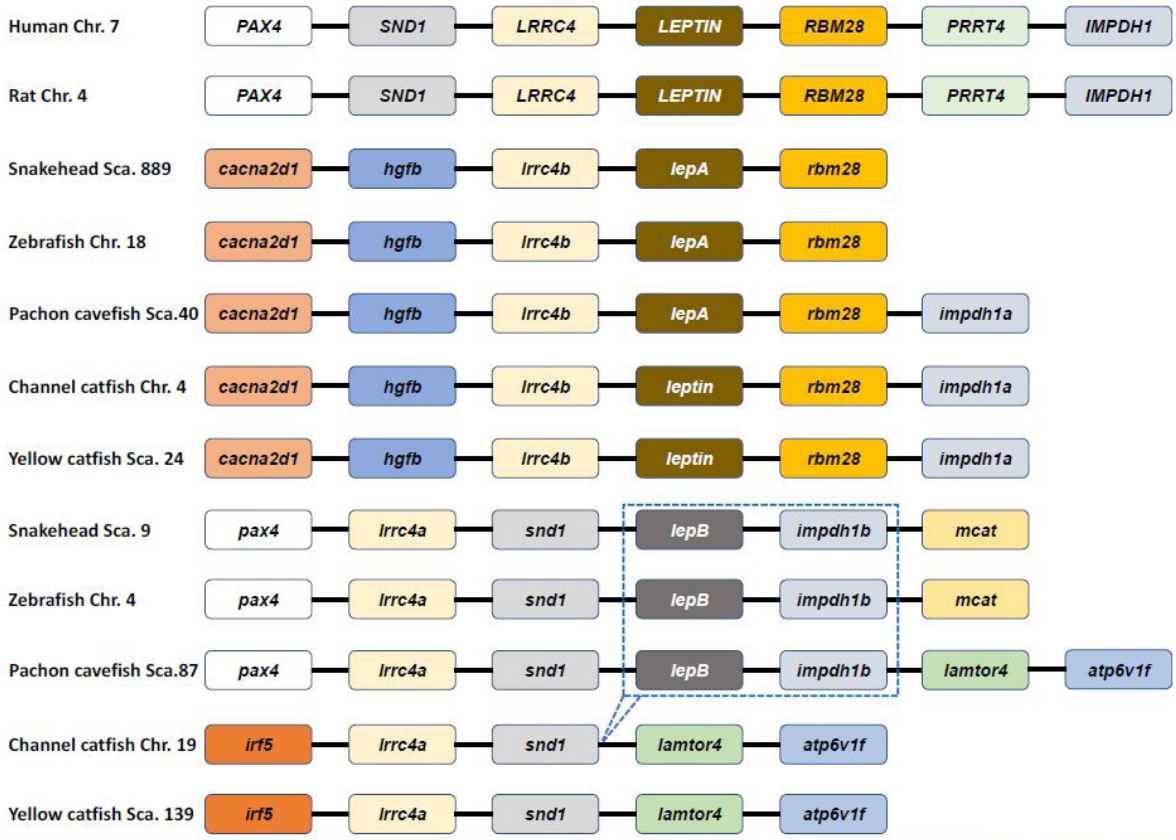

Fig. 3. Genetic synteny comparison of Pvleptin and its homologs in vertebrates. The colorful blocks and solid lines represent genes and intergenic regions, respectively. The specific cluster (*lepB-impdh1b*) that has lost in siluriformes are marked by dotted box.

genomes and teleost genomes respectively, sharing same core cluster of *lrrc4-leptin-rbm28* (Fig. 3). Differently, a specific cluster of *pax4-lrrc4a-snd1-lepB-impdh1b* was found in teleost genomes (Fig. 3). Interestingly, it seems that *lepB* but not *lepA* has lost in Siluriformes fishes including yellow catfish and channel catfish (Fig. 3). Phylogenetic analysis showed that the neighbor joining tree was divided into two groups of teleost *leptin* and tetrapod *leptin*, and the former was further clustered into two subgroups including teleost *lepA* and teleost *lepB* (Fig. 4). The *Pvleptin* was clustered into *lepA* clade and shared a close relationship with *leptin* in yellow catfish and channel catfish (Fig. 4), consistent with the protein identity described above. All clades were supported with high scores, and the spotted gar (*Lepisosteus oculatus*) was selected as outgroup species due to its special evolution potion in teleost.

Tissue distribution of Pvleptin

Quantitative real-time PCRs were conducted to detect the tissue distribution pattern of *Pvleptin*. Results showed that the *Pvleptin* was widely distributed in examined tissues including adipose, brain, gill, heart, intestine, kidney, liver, muscle, spleen, and stomach (Fig. 5). The highest mRNA expression level of *Pvleptin* was detected in liver, while relative high expression was tested in adipose, heart, intestine, muscle, spleen and stomach (Fig. 5). While, *Pvleptin* was hardly detectable in brain, gill and kidney (Fig. 5).

Effect of fasting and refeeding on Pvleptin *mRNA expression*

To investigate the expression patterns of *Leptin* associated with starvation and feeding schemes, the mRNA expression level of *Pvleptin* in the liver was detected after food deprivation and refeeding. The mRNA expression level of hepatic *Pvleptin* was significantly decreased in fishes after a two-week fasting in comparison with that of those in feeding group, while refeeding increased the transcription of hepatic *Pvleptin* of the fasted fish (Fig. 6). Groups with significant differences were indicated by different letters above the bars. Data were shown as mean ± SEM.

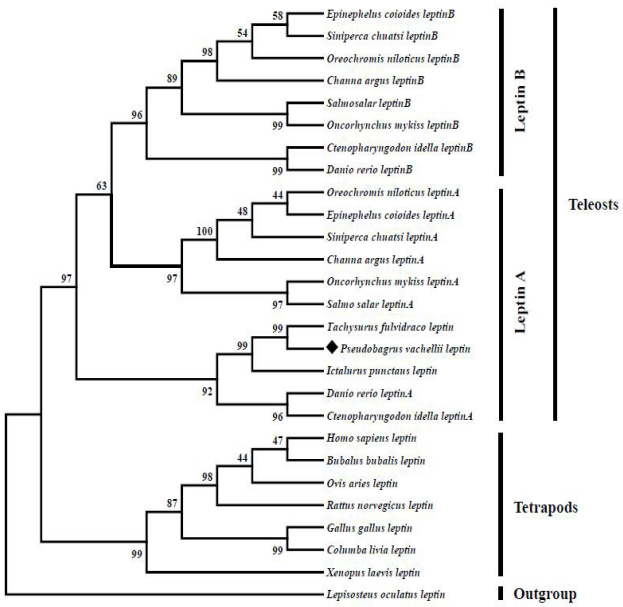

Fig. 4. Neighbor joining tree reveals the phylogenetic relationship among vertebrate leptin genes. The tree was constructed by using MEGA 6.0 program based on a protein dataset. Values on the nodes represent bootstrap percentages from 1000 replicates. The aimed species was highlighted by a rhombic diamond and spotted gar (*Lepisosteus oculatus*) was used as the outgroup species.

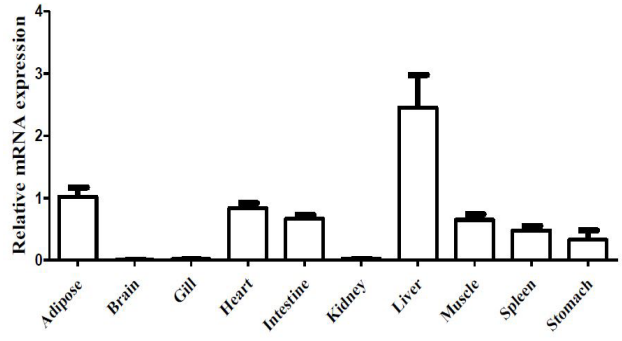

Fig. 5. Tissue distribution pattern of *leptin* in darkbarbel catfish. Results were expressed as relative expression levels and normalized by the *β-actin* gene. Each error bar represents a standard error of the mean (n = 5).

DISCUSSION

In the present study, we characterized a *leptin* gene from the darkbarbel catfish (*P. vachelli*) for the first time. The cDNA of *Pvleptin* contained a 519 bp long ORF that predicted to encode a precursor protein of 172 amino acids, which was in line with the findings in yellow catfish (Gong *et al*., 2013b) and channel catfish (Kobayashi *et al*., 2011). *PvLeptin* was predicted to contain four conserved a-helix domains and two cysteine residues, which was similar to previous studies in pufferfish (Kurokawa *et al*., 2005), Jian carp (Tang *et al*., 2013), mandarin fish (He *et al*., 2013; Yuan *et al*., 2016), and snakehead fish (Wen *et al*., 2020d), suggesting these conservative domains or amino acid residues are especially important for maintaining the 3D structures and functions of the vertebrate Leptin. Multiple sequences alignment revealed that Leptins were variable and shared low identity with each other in vertebrates, which was consistent with most studies related to this hormone (Londraville *et al*., 2017). It is noticed that PvLeptin shared higher identity with teleost LepA in comparison of that with teleost LepB, suggesting the *Pvleptin* identified in present study may be the ortholog of *lepA* in teleosts (Kobayashi *et al*., 2011). Although low sequence identities of Leptins were observed among different animals, 3D structure modeling showed that the 3D structures of Leptins were highly conserved, implying they may experience an independent evolution process while still potentially restrain similar functional roles in vertebrates (Munzberg and Morrison, 2015; Londraville *et al*., 2017).

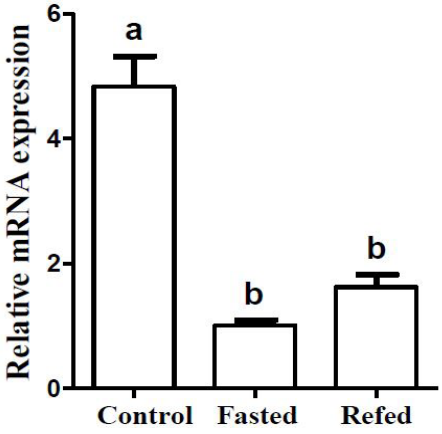

Fig. 6. Effects of two-week fasting and refeeding on the mRNA expression level of *Pvleptin* in liver. Results were expressed as relative expression levels and normalized by the *β-actin* gene. Each error bar represents a standard error of the mean (n = 5).

Genetic synteny analysis showed that yellow catfish and channel catfish possessed a single *leptin* gene while the other teleosts contained two copies of *leptin* genes (namely *lepA* and *lepB*) in their genomes, and the two single *leptin* genes shared a consistent genetic synteny with that of other teleost *lepA* showing a same gene order (*cacna2d1-hgfb-lrrc4b-lepA-rbm28*) (Fig. 3). These findings were identical to several previous studies (Kobayashi *et al*., 2011; Gong

et al., 2013b; Wen *et al*., 2020d), suggesting that the ortholog of *lepA* was retained whereas *lepB* had lost in siluriformes genomes. A recent work has well illustrated the phylogenetic relationship of ray-finned fishes based on big genome and transcriptome datasets (Hughes *et al*., 2018), which revealed that Cypriniformes, Characiformes, Gymnotiformes and Siluriformes were belonged to Otophysa and the Cypriniformes was located at the root of this lineage. In present study, *lepB* was identified both in zebrafish and pachon cavefish, a representative species of Cypriniformes and Characiformes, respectively whereas *lepB-impdh1b* cluster has lost in Siluriformes including yellow catfish and channel catfish, suggesting the lost event of *lepB* should be specific in Siluriformes and therefore the *Pvleptin* should be the ortholog of teleost *lepA*.

Phylogenetic analysis showed that the teleost group consisted of two clades of teleost *lepA* and teleost *lepB*, which was in line with several previous phylogenetic studies (Yan *et al*., 2016; Yuan *et al*., 2016; Wen *et al*., 2019), suggesting two *leptin* genes were widely existed in teleosts and this phenomenon might be caused by a specific whole genome duplication (WGD) event in teleosts (Londraville *et al*., 2017; Xu *et al*., 2018; Wen *et al*., 2020d). Moreover, more copies of *leptin* were identified in common carp (Huising *et al*., 2006), Jian carp (Tang *et al*., 2013), goldfish (Yan *et al*., 2016), Atlantic salmon (Angotzi *et al*., 2013), and rainbow trout (Murashita *et al*., 2008; Gong *et al*., 2013a), which may be due to an additional WGD event was occurred in their genomes (Lien *et al*., 2016; Xu *et al*., 2019). In addition, *Pvleptin* was clustered into the clade of teleost *lepA* and shared a close relationship with yellow catfish and channel catfish *leptin* (Fig. 4), which further confirmed our assumption that mentioned above.

Tissue distribution pattern of *Pvleptin* was detected by using real-time quantitative PCR. Results showed that *Pvleptin* was widely distributed in various tissues with the highest expression level in liver, which was similar to the pattern of *leptin* in pufferfish (Kurokawa *et al*., 2005), channel catfish (Kobayashi *et al*., 2011), yellow catfish (Gong *et al*., 2013b), and *lepA* in mandarin fish (He *et al*., 2013), Ya-fish (Yuan *et al*., 2014) and snakehead (Wen *et al*., 2020d), indicating the *Pvleptin* may play similar roles in these teleosts and it also may be involved in regulating food intake and energy balance. Differently, *lepA* was observed to be highly expressed in brain of Atlantic salmon (Ronnestad *et al*., 2010), cerebellum of orange-spotted grouper (Zhang *et al*., 2013), and ovary of tongue sole (Xu *et al*., 2018), implying the distribution characteristic of teleost *lepA* or its ortholog is species-specific, and its roles may be variable among different teleosts. In addition, *lepB* was usually found to be highly distributed in central tissues, such as in orange-spotted grouper (Zhang *et al*., 2013), mandarin fish (Yuan *et al*., 2016) and snakehead fish (Wen *et al*., 2020d), suggesting the divergent evolution and function between the two paralogs of *leptin* in teleosts. However, the extract roles of teleost *leptin* genes are still not well understood and more studies are required to further clarify.

Previous studies have reported that feeding status can affect the mRNA expression level of *leptin* in various teleosts. In the present study, we observed that two-week fasting reduced the mRNA expression level of *Pvleptin* while refeeding improved the corresponding expression level in liver, which was consistent with related researches in Ya-fish (Yuan *et al*., 2014), seabream (Babaei *et al*., 2017), Indian major carp (Dar *et al*., 2018), and Yangtze sturgeon (Chen *et al*., 2020), implying that the *Pvleptin* is also involved in the regulation of energy balance in the liver, an important energy metabolic center in teleost. As an anorexigenic factor, Leptin has been reported to suppress the appetite in gold fish (De Pedro *et al*., 2006) and rainbow trout (Murashita *et al*., 2008). Therefore, the expression level of *Pvleptin* was decreased after a two-week food deprivation, suggesting this hormone protein may regulate energy homeostasis by reducing the metabolic energy demand among fasting period in darkbarbel catfish.

In summary, we identified a single *leptin* gene in darkbarbel catfish for the first time. Multiple sequences alignment and 3D structure modeling revealed low identity but similar 3D structure of Leptins in vertebrates. Genetic synteny and phylogenetic analysis suggested that *Pvleptin* was the ortholog of teleost *lepA* and the homolog of *lepB* had lost in siluriformes teleosts. Similar to other catfishes, *Pvleptin* was highly expressed in liver of darkbarbel catfish. Finally, fasting and refeeding experiments suggested that PvLeptin was also involved in regulation of food intake and energy homeostasis.

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Supplementary material

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Statement of conflict of interest

The authors have declared no conflict of interest.

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