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Divergent adaptation to Qinghai-Tibetan Plateau implicated from transciptome study of *Gymnocypris dobula and Schizothorax nukiangensis*

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ABSTRACT

The Schizothoracine fishes are widely distributed in the Qinghai-Tibetan Plateau (QTP) area and its peripheral regions, which provide a prime example of adaptation in highland aquatic environments. Recent progresses have revealed various genetic adaptations of these fishes by comparing to distantly related lowerland species, however, comparative studies on closely-related species of different altitudes are still lacking. In this study, we sequenced and annotated a primitive Schizothoracine fish *Schizothorax nukiangensis Tsao* and a highly specialized one *Gymnocypris dobula*. We performed evolutionary analyses to investigate the candidate genes and signaling pathways involved QTP highland adaptation in both Schizothoracine fishes. Analysis of the 11,007 one-copy orthologs to the primitive cyprinid species, *Danio rerio*, revealed that both *G. dobula* and *S. nukiangensis* showed elevated evolutionary rates. A large number of genes related to hypoxia, including genes involved metabolic processes and cardiovascular system development, exhibited signatures of positive selection in both Schizothoracine fishes, but very few positively selected genes were found overlapping among these Schizothoracines. Our results indicated divergent genetic adaptation to highland environment for aquatic species living in QTP.

1. Introduction

The Qinghai-Tibet Plateau (QTP) is the highest and one of the biggest plateaus on earth, covering 2.5×10^6 square kilometers with an elevation of 3000–5000 m for most parts of the area. The QTP has been uplifting since approximately 45

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million years ago, resulted from collision of the India plate and the Eurasia plate (Li and Fang, 1999; Favre et al., 2014). The uplifting dramatically changed the environment conditions from an originally humid and warm climate to currently a dry and cold one (Wu et al., 2008). Characterized by factors including hypoxia, cold and strong ultraviolet radiation, the QTP environment posed harsh challenges to the endemic animals (Scheinfeldt and Tishkoff, 2010). Using comparative genomics and transcriptomics analysis, recent studies have identified various genes and signaling pathways that may be responsible for highland adaptation in both terrestrial and aquatic vertebrates. For example, genetic modifications to metabolism and cardiovascular system development are frequently found in the highland adapted animals (Qiu et al., 2012; Qu et al., 2013; Gou et al., 2014).

The family Cyprinidae is the most diverse clade in freshwater fishes (Chen and Mayden, 2009). The Schizothoracine fishes (Teleostei: Cyprinidae) dominate the lakes and rivers throughout the Tibetan Plateau and its peripheral regions. More than 70 species in twelve genera in the subfamily Schizothoracinae are endemic to the Tibetan Plateau (He and Chen, 2006). According to the degree of specialization of their morphological traits, the Schizothoracine fishes are divided into three grades: Primitive, specialized, and highly specialized, with Primitive ones live in the peripheral regions at around 1000 m above sea level (a.s.l) and the other two groups are intermingled with each other in the central part of QTP at above 3000 m a.s.l (He and Chen, 2006; Qi et al., 2012).

Recent transcriptome studies on Schizothoracines and other endemic Tibetan fishes have identified multiple biological processes and genes involved in highland adaptation, including genes that participate in metabolic processes and responses to hypoxia (Yang et al., 2014; Ma et al., 2015). Despite great progresses made in these investigations, most of current studies are limited to single highland species with comparisons made to distantly related teleosts. Such comparisons are limited in power to decipher the detailed molecular adaptations associated with Schizothoracine expansion in QTP. Therefore, genome-wide investigations of adaptive signals among Schizothoracine species of differently graded specialization are highly demanded to further reveal the genetic mechanisms of adaptation in these fishes.

The objective of this study was to carry out a comparative genome-wide screen for genes that might be involved in highland adaptation from species living at different altitudes. To achieve these aims, we generated transcriptomes of a primitive Schizothoracine fish *Schizothorax nukiangensis Tsao*, which lives at about 1,000 m a.s.l, and a highly specialized one *Gymnocypris dobula* which lives at 3000–4000 m a.s.l, and we performed evolutionary analyses on these data.

2. Materials and methods

2.1. Ethics statement

This study was approved by the Ethics Committee for the Use of Animal Subjects of Shanghai Ocean University.

2.2. Sample collection, cDNA library construction and illumina sequencing

G. dobula was collected from Lake Duoqing, situated at eastern QTP (28°03.37′, 89°17.83′) with an altitude of 4509 m a.s.l, and a temperature at 11.0 °C, the concentration of dissolved oxygen in the water was 1.9 mg/L; while *S. nukiangensis* was collected from Nujiang, Yunnan, situated on the eastern margin of QTP (25°41.24', 98°53.22), with an altitude of 1201 m a.s.l and at a temperature of 16.0 °C, the concentration of dissolved oxygen in the water was 7.8 mg/L. The collection loci were labelled in Fig. 1. For both species, five individuals were captured from the same sampling locations, and fishes with similar size were selected for tissue dissection. The fish were live trapped, anesthetized. Tissues including brain, gill, head kidney, kidney, liver, muscle, skin, spleen were quickly biopsied and placed in RNAlater (QIAGEN), and stored at −80 °C on arrival at the laboratory till RNA extraction.

Total RNAs from each tissue sample were extracted using TRIzol reagent (Invitrogen Corp., Carlsbad, CA). Library constructions from *G. dobula* and *S. nukiangensis* were made using Illumina Hiseq1500 RNA sample preparation kits (Illumina, San Diego, CA) following manufacturer's instructions. RNAseq libraries were quantified using an Agilent 2100 Bioanalyzer (Agilent Technologies). The libraries were then sequenced on an Illumina HiSeq 1500 platform (Illumina, Inc.) with 100 bps paired-end reads.

2.3. De novo assembly and annotation of transcripts

Raw reads were cleaned by removing adapter sequences using sequence pre-processing tool Trimmomatic. Reads with Phred score $\geq$25 were kept for sequence assembly. The transcriptomes of the tissues were pooled to generate an improved *de novo* assembly using the Trinity package, and open reading frames (ORFs) were predicted using the transdecoder with a minimum length of 50 amino acids (Haas et al., 2013). The assembled unigenes were first annotated by searching for homologous sequences against National Center for Biotechnology Information (NCBI) nonredundant protein (Nr) database and then were searched against the KOG database using BLASTP (evalue<1e-5). We also used the KOBAS software to annotate the unigenes with Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (Xie et al., 2011).

As zebrafish is currently the most closely related species to the Schizothoracines which has a completed and well annotated genome, we further annotated the unigenes of the two Schizothoracine fishes by mapping them to zebrafish proteins using BLASTP bidirectional best hit (BBH) method. The protein dataset of zebrafish (*Danio rerio*) was downloaded from