



# Genetic diversity and population structure analysis of Qinghai-Tibetan plateau schizothoracine fish (*Gymnocypris dobula*) based on mtDNA D-loop sequences



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## ABSTRACT

*Gymnocypris dobula* is a commercially important fishery species and mostly distributed in the freshwater of Tibetan plateau. In this study, genetic diversity and intraspecific population differentiation were examined by using mitochondrial DNA D-loop sequences in 97 individuals sampled from three localities (Pali, Lasa and Yanghu) in the Tibetan plateau. Two hundred and fourteen polymorphic sites and 50 haplotypes were defined among the three localities. Genetic diversity analysis showed that the highest genetic diversity level was found in Pali population. Phylogenetic relationships analysis results indicated that closer phylogenetic relationships were found between the Yanghu and Lasa populations. Genetic population differentiation analysis indicated that the majority of variation (84.91%) was attributed to variations among populations and the largest differentiation was found between Pali and Lasa localities ( $F_{ST} = 0.874$ ,  $N_m = 0.036$ ). Moreover, the historical demographic events were assessed by implementing the mismatch distribution analysis, Fajima's  $D$  test and Fu's  $F_s$  test. The results indicated that the Pali population had undergone a demographic expansion, possibly within the last 0.163 MYA (Million Years Ago). Our study firstly identified the population genetic structure of the *G. dobula*, which could be helpful for artificial breeding, fishery stock identification and resource conservation for this species.

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## 1. Introduction

Qinghai-Tibetan Plateau, the largest and highest plateau in the world, locates in the southwest of China. The uplift of the Qinghai-Tibetan plateau was created by the collisions of the Indian Plate and Asian Plate, which probably started from 50 million years ago (MYA) (Tapponnier et al., 2001; Royden et al., 2008). The high absolute elevation, extreme aridity, low temperature, and extreme hypoxia are the characteristics of the Tibetan plateau. Nonetheless, abundant freshwater and fishery resources were still contained in this region. As the most diverse clade of freshwater fishes on earth, order Cypriniformes are natively distributed on Qinghai-Tibetan Plateau and adapt to extreme environment. The major populations are

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schizothoracine fishes (Teleostei: Cyprinidae; also called “mountain carps”), which dominate river and lake drainages in Tibetan Plateau and its peripheral regions (Cao et al., 1981; Li et al., 2013).

The subfamily Schizothoracinae consists of 15 genera containing approximately 100 species (Mirza, 1991). The schizothoracines are among the most diverse subfamilies of cyprinids and are divided into three grades of specialization (primitive, specialized, and highly specialized) according to their habitat elevations and differences in their scales, pharyngeal teeth, and barbels (Cao et al., 1981). *Gymnocypris dobula*, which has hardly any scales and barbels, belongs to the highly specialized schizothoracines. It is an endemic species of cyprinidae fish, occupying the shallow lakes and tributaries at high altitudes (>4500 m) in the Himalayan mountains, and has a high economic value to marginal fishermen (Xu et al., 2016).

Given its economic importance, there is considerable interest in the genetic structure of *G. dobula*. Information on the population genetic structure of this species is of significance in developing effective strategies for management of this important fish species. In addition, it might also provide a genetic tool for fish breeding and offer a scientific support for resources conservation. As we know, the structure of hydrographic systems was influenced by climatic changes and crustal movements (Bermingham and Avise, 1986; Durand et al., 1999; Dominguez et al., 2008). Therefore, the genetic structure of the fish species in the hydrographic systems would be affected by the climatic and crustal changes. It was also indicated that the historical changes of rivers had been a driving force for the diversification and speciation of fish species (He and Chen, 2007). Therefore, the development processes of the historical biogeography for fish species could provide evidences to understand their population dynamics (Waters et al., 2007; Bernatchez and Wilson, 1998). The genetic structuring of the *G. dobula* populations might in return help to understand the hydrographic systems formation process in Qinghai-Tibetan Plateau. However, genetic analysis of the population structure of *G. dobula* has not been conducted so far.

Molecular techniques were widely used to study genetic variability and phylogenetic relationships among of variety of species. Because of its maternal inheritance and absence from intermolecular genetic recombination, Mitochondrial DNA (mtDNA) has been extensively used for studies in population structure, phylogeography, and phylogenetic relationships (Avise et al., 1987; Harrison, 1989). The Mitochondrial D-loop region, also called as mtDNA control region, is proved to be more variable sequences compared with other regions of mtDNA (Cann et al., 1984). It has been a useful molecular marker for revealing intraspecific genetic structure among populations in many previous researches (Rosel et al., 1995; Burrige, 1999). In addition, the mtDNA D-loop sequence has been widely used in the genetic diversity and population structure studies on a variety of plateau Schizothoracinae fish species, such as *Schizopygopsis chengi baoxingensis* (Liu et al., 2009), *Gymnodiptychus pachycheilus* (Su et al., 2012), *Schizopygopsis younghusbandi* (Guo et al., 2014) and *Diptychus maculatus* (Meng et al., 2015) and so on.

The present study concerned with the genetic variation background and population genetic structure of *G. dobula* populations distributed in Qinghai-Tibetan plateau. The aim of the present study is to provide genetic structure information of this commercially important species for fishery stock identification, management and conservation.

## 2. Materials and methods

### 2.1. Sample collection

A total of 97 samples were collected by using dip netting from three locations in Qinghai-Tibetan plateau, Pali, Lasa and Yanghu. The relevant information concerning the collections and habitat characteristics of the three localities were summarized in Fig. 1 and Table 1. Forty individuals from Pali, thirty-five individuals from Lasa and twenty-two samples from Yanghu were used for our study. For each individual, muscle tissues were taken from the tail and immediately preserved in  $-80^{\circ}\text{C}$  refrigerator until being used for further processing.

### 2.2. Genomic DNA isolation, PCR amplification and sequencing

Genomic DNA was extracted by using the method described by Sambrook. (Sambrook and Russell, 2001). The quality of DNA was tested on 1.0% agarose gels prepared in Tris-acetate EDTA buffer. The primers used for PCR amplification were shown as following: PRO: 5'-TCCCAAAGCTAGGATTCTAAACTAAAC-3' and PRE: 5'-TTCATCTTAACATCTTCAGTGTTATGC-3'. The primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. The mtDNA control region was amplified in a 25  $\mu\text{l}$  reaction volume with a final concentration of 1 U *Taq* polymerase, 1  $\times$  PCR buffer (50 mM KCl, 10 mM Tris-HCl, 1.5 mM MgCl<sub>2</sub>, PH 8.3), 200  $\mu\text{M}$  dNTPs (TAKARA), 10 pM of each primer and 100 ng of genomic DNA. Amplification was performed in a thermal cycler using a 5 min denaturation step at 95  $^{\circ}\text{C}$  followed by 34 cycles of 45 s at 94  $^{\circ}\text{C}$ , 45 s at 56  $^{\circ}\text{C}$ , 1 min at 72  $^{\circ}\text{C}$  and a final extension at 72  $^{\circ}\text{C}$  for 10 min. Products were cleaned by ethanol precipitation and sequences were resolved in an ABI Prism 3730 automated DNA sequencer by Sangon Biotech (Shanghai) Co., Ltd.

### 2.3. Data analysis

A total of 97 mtDNA D-loop sequences were aligned with CLUSTAL X (Thompson et al., 1997) and manually examined. The aligned DNA sequences were imported into MEGA version 6.0 (Tamura et al., 2013) for sequence comparisons and variation

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