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# Assessment of genetic diversity and population structure of swamp eel *Monopterus albus* in China

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ABSTRACT

Swamp eel has become one of the most economically important fish in China. However, the wild swamp eel is facing the serious challenge of declining population and germplasm degeneration because most of farming swamp eel fingerlings was collected by fishing wild individuals. In this study, the genetic variation of *Monopterus albus* in six dominant farming regions was investigated based on the mitochondrial DNA D-Loop of 1008 bp in length. 180 individuals from 6 populations were examined and 74 haplotypes were observed. The overall genetic diversity was abundant and which its SD population was highest but CQ population was lowest. There was obvious genetic differentiation among investigated populations. Phylogenetic analysis revealed that these individuals were divided into four distinct genetic clades, clade A, B, C, and D. Clade A should be the most common ancestor clade. AH and CQ populations might originate from one single ancestor in maternal clade A. Clade C should be a native important clade in China. Though the genetic diversity did not suffered obvious decreasing, it is still imperative to take effective conservation measurements and establish an efficient selective breeding program.



## 1. Introduction

The swamp eel (*Monopterus albus*) is a family of freshwater eel-like teleost fishes, which belongs to the family Synbranchidae, order Synbrachiformes and class Actinopterygii. It originates widely in tropical, subtropical and temperate freshwater regions from Southeast Asia to East Asia (Zhou et al., 2002) and commonly found in rice fields, swamps, ponds, and muddy areas (Siang et al., 2007; Li et al., 2013). Because of its delicious taste, high nutrition and medicinal value, swamp eel has become one of the most economically important economical fishes, especially in China (Qu et al., 2014; Hu et al., 2015a). The worldwide production of *Monopterus albus* has reached 321,006 tons in 2012 (FAO Yearbook Fishery and Aquaculture Statistics, 2014), while most of the production was provided Chinese fisheries (320,966 tons, China Fisheries Yearbook, 2013).

Swamp eel is distributed throughout most regions, except the Qinhai-Tibet Plateau in China. In 2013, the total annual production of swamp eel was 346,077 tons (China Fisheries Yearbook, 2014). Six dominant farming regions, which are Hubei, Jiangxi, Anhui, Hunan, Sichuan, and Shandong, have the first six production with 161,837 tons, 79,471 tons, 43,643 tons, 29,999 tons, 11,409 tons, and 2083 tons, respectively (China Fisheries Yearbook, 2014). Nowadays most of the cultured swamp

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eel fingerlings are collected from fishing wild larvae. Thus, the wild swamp eel resources are facing serious challenges with the populations declining due to overfishing and large-scale application of pesticides (Yang et al., 2011a; Lei et al., 2012). What's more, the germplasm quality has declined sharply in terms of resistance and growth (He et al., 2010; Shao et al., 2015). In order to conserve and utilize this species well, it is vital to carry out fundamental breeding research. In the past ten years, some researchers have started utilizing artificial reproduction (Bing, 2005), culture technology (Yang et al., 2011b; Ma et al., 2014), sex differentiation of the swamp eel (Zhou et al., 2002; Hu et al., 2014).

Genetic diversity provides the fundamental material for biological diversity and selective breeding (Frankham et al., 2002; Hao et al., 2006). The genetic structure investigation is an important step to gain background knowledge about all kinds of species. Some studies analyzed the genetic variation of swamp eel by using different molecular markers, such as RAPD (Yin et al., 2005), microsatellites (Li et al., 2013; Lei et al., 2012), ISSR (Li et al., 2013), and mitochondrial DNA (Cai et al., 2008). All of these studies involved a small sample or a limited distribution, such as the Sichuan Basin (Cai and Zhang, 2011; Cai et al., 2013), south China (Sun et al., 2015), and the Anhui province (Hu et al., 2015b). However, not all populations from the dominant farming districts of swamp eel were used to analyze and evaluate the genetic diversity. It will be very useful to compare the dominant cultured populations to establish basic breeding populations. To determine the genetic variations and investigate the genetic background of dominant farming districts, six populations are analyzed in this study.

## 2. Materials and methods

### 2.1. Sample collection and DNA extraction

The experimental procedures for swamp eel were performed according the standards of the Animal Care Policy of YFI (Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences). The alcohol-preserved muscle tissues obtained from 180 individuals of wild swamp eel were collected to six cultured dominant regions, including Hubei (HB), Jiangxi (JX), Anhui (AH), Hunan (HN), ChongQing (CQ, representing Sichuan Basin) and Shandong (SD). The detailed information of sample is listed in Table 1 and Fig. S1. Total genomic DNA was extracted by the described method (Taggart et al., 1992).

### 2.2. Mitochondrial DNA D-loop amplification and sequencing

The complete mitochondrial DNA D-Loop sequences of 180 samples were amplified using the primers 5′ TCAAATCCCTCTCATTACTCA 3′ and 5′ GATAAAGCCAGGACCAAAC 3′, which designed according the swamp eel mtDNA sequence deposited in GenBank (accession number KP779624.1). Each 30 μL PCR reaction system contained 2 μL of 10 mM each primer, 2U *Taq* DNA polymerase (TaKaRa, Japan), 3 μL 10 × PCR buffer (100 mM Tris-HCl, 500 mM KCl, 15 mM $MgCl_2$; TaKaRa), 2 μL 10 mM dNTP (TaKaRa, Japan) and about 100 ng DNA template. PCR amplification was carried on S1000™ Thermal Cycler (BIO-RAD, USA) using the following procedure: pre-denaturing at 95 °C for 3 min; 35 cycles of denaturing at 94 °C for 30 s, annealing at 58 °C for 30 s, and extending at 72 °C for 45 s; and a final extension at 72 °C for 5 min. The PCR products were detected by 2.0% agarose gel in 1 × TBE buffer at 80 V for 1 h and then purified using a DNA Agarose Gel Extraction Kit (Axygen, USA). The purified PCR products were cloned into pMD 18-T vector and sequenced by ABI 3730 automated sequencer (Applied Biosystems, USA).

### 2.3. Data analysis

All the sequenced D-loop fragments were edited and aligned using Clustal W program (Larkin et al., 2007). The genetic diversity parameters were analyzed by DNAsp5.0 program (Librado and Rozas, 2009), which included the number of polymorphic sites (S), number of haplotypes (h), haplotype diversity (Hd), nucleotide diversity ($\pi$), and average number of nucleotide differences (K). Genetic distance, fixation index, molecular variance, and neutrality tests were calculated by using Arlequin version 3.1 (Excoffier et al., 2005).

Based on the haplotypes of complete mitochondrial D-Loop sequences, the molecular phylogenetic tree of neighbor-joining (NJ) was constructed by MEGA version 5.0 (Tamura et al., 2011) and set the *Synbranchus marmoratus* (GenBank

**Table 1**
Samples collection information.

| Population | Abbreviation | Location | Longtitude (E)/latitude(N) | Sample size |
|---|---|---|---|---|
| Hubei | HB | Qianjiang, Hubei Province | 112°45′/30°06′ | 30 |
| Jiangxi | JX | Xinyu, Jiangxi Province | 114°56′/27°49′ | 30 |
| Anhui | AH | Hefei, Anhui Province | 117°16′/31°51′ | 30 |
| Hunan | HN | Yueyang, Hunan Province | 113°09′/29°37′ | 30 |
| Shandong | SD | Weishanhu, Shandong Province | 117°58′/34°53′ | 30 |
| Chongqing | CQ | Chongqing | 106°31′/29°32′ | 30 |