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High sequence variation and low population differentiation of mitochondrial control regions of wild Large yellow croaker in South China Sea

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ABSTRACT

As a highly successful mariculture candidate endemic to China, the wild genetic resource of Large yellow croaker (*Larimichthys crocea*) have collapsed in the northern and central East China Seas, which has impeded the wild sampling and assessment. Meanwhile, the wild population in South China Sea away from restocking and massive aquaculture is seldom reported. In this study, the complete mitochondrial control regions (CR) of 98 individuals of Naozhou stock were determined and the data indicated that the haplotype diversity of Naozhou stock ($H_d > 98\%$) is higher than all the reported values. Inconsistent with previous conclusion by traditional methods, the non-significant S_{nn} and F_{ST} owing to the strong gene flows (N_m), as well as the cluster tree without substructure reflected the lack of population differentiation in Large yellow croaker of Naozhou stock. Moreover, the demographic history was examined by using neutrality tests and mismatch distribution analysis, which revealed that the species obey the sudden expansion model. This result should be basic file for fisheries management and assessment of croaker.

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1. Introduction

Larimichthys crocea (Richardson, 1846), called the Croceine croaker, Large yellow croaker or just the Yellow croaker, is a species of croaker native to the western Pacific, generally in temperate waters such as the Taiwan Strait (http://en.wikipedia.org/wiki/Larimichthys_crocea). However, the wild Large yellow croaker declined in the 1970s due to overfishing, and stocks were almost exhausted by the mid-1980s to 1990s. Research into hatchery methods began in 1985 and artificial propagation was successful by 1987. By 2005, the annual production of large yellow croaker reached 69,600 tones, with two billion fingerlings produced (Wang et al., 2007). To date, all sources indicated that wild stocks of the croaker in the East China Sea never recovered despite specific management measures, including long-term restocking programs and successful mariculture (Liu and De Mitcheson, 2008). The South China Sea, on the other hand, considerably less important for croaker catches historically with a few thousand tons annually before the late-1980s, was reported to have improbably high catches of between 22,000 and 45,000 tons in the 1990s (Guangdong Province Survey Report, 1995), despite no significant management efforts for the croaker in the area.

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Three putative geographic stocks of the croaker were initially identified in coastal waters of China, i.e. the Daiquyang (Taichuyang) Min-Yuedong (Min-Uehtung) and Naozhou (Naochow) stocks. According to samples taken between 1957 and 1961, longevity, age of sexual maturation and growth rate differed between the three putative stocks. In the Daiquyang stock fish typically had long life, late sexual maturation and slow growth rate. In the Min-Yuedong stock, it had intermediate longevity and sexual maturation, and fast growth rate, with a relatively short life, early sexual maturation and slow growth rate in the Naozhou stock. Maximum age ranged from 8 to 29 years and age of 50% sexual maturation was between 2 and 4 years (Tian et al., 1962; Xu et al., 1984).

The most recent study using molecular techniques to analyze microsatellite loci and mitochondrial control region (CR) sequences mainly focused on the Daiquyang and Min-Yuedong stock, and suggested that the traditionally proposed two putative geographic stocks may represent only one genetic population (Mao et al., 2010; Wang et al., 2012a,b). Many researchers pointed out that the genetic homogeneity could be caused by the long-term restocking programs, rather than the approach inconsistence between traditional and molecular population genetics (Liu and De Mitcheson, 2008).

To date, there is still not any genetic analysis about Naozhou stock except that Cao divided this stock into Naozhou and Xuwen populations basing on the differences of five measurable factors (body length, body weight, age, feeding intensity, maturity of fish gonad, etc.) (Cao, 1998). Given no restocking records or massive aquaculture, the analysis of population structure of Naozhou stock could provide a good reference for the further discussion of the population relationship of croaker in China Seas.

In this study, we collected the wild populations of the large yellow croaker from Naozhou to Xuwen waters on the May and September. Furthermore, we determined the CR to achieve the following aims: (1) To estimate the levels of genetic variation and the putative temporal-spatial differentiation of Naozhou stock; (2) To compare the molecular genetic diversity among different stocks; (3) To stimulate the demographic history.

2. Materials and methods

2.1. Sampling and DNA extraction

Ninety eight wild large yellow croakers were randomly captured by the fishing boats from the west side of the South China Sea waters ($110-112^{\circ}$ E) in May 2012 and in September 2012 (Fig. 1 and Table 1). The specimen were taken to laboratory and stored under -20° C. Approximately 30 mg of muscles from each sample stored in a freezer was cut for DNA isolation using the method of proteinase K digestion (Lu, 1999).

2.2. Primers, PCR and sequencing of D-loop

The reference mitogenome of Large yellow croaker, *L. crocea* (Accession no. NC_011710.1) (Cui et al., 2009) were used to design the primers for amplification of the complete CR sequence (DLF: 5'-GCC TCA TAC CAC CCA TTT-3', DLR: 5'-GAT CCG AAG



Fig. 1. The three sampling sites (sNZ, fLZ and sXW) of Naozhou stock and distribution map of three stocks of Large yellow croaker in China (modified from Liu and De Mitcheson, 2008).

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