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Genetic diversity and the relationship of *Nibea japonica* and *Nibea miichthioides* using mitochondrial DNA gene

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1. Introduction

Species of *Nibea* belong to a family of warm water carnivorous fish and consist of *Nibea coibor*, *Nibea albiflora*, *Nibea semifascitata*, *Nibea diacanthus*, *Nibea acuta*. *Nibea miichthioides* and *Nibea japonica* (Zhu et al., 1963; Cheng and Zheng, 1987). Since *Nibea* species can tolerate and live under widely varying salinities and temperatures, the *Nibea* genus is distributed widely along the coastal waters of China, Japan, Korea and Southeast Asia and India. In the early 1990s, *Nibea* fish were mainly obtained from wild populations. However, overfishing and ocean pollution in the past decade have led to a dramatic decrease of fish in the wild fisheries of China. At present, three kinds of *Nibea*, including *N. japonica, N. miichthioides* and *Nibea alibiflora* have been propagated artificially in China. Thus, enhancement and release has become the main route for replenishing the stock population of *Nibea* to promote ecological restoration. More importantly, *Nibea japonca* as the endemic species in the East China Sea, also possesses features such as fast growth and strong adaptability to the environment and, thus, is an ideal release species.

As an economically important marine fishes, *N. japonica* was mainly distributed along East China Sea and southern Sea of Japan, while *N. miichthioides* appeared in the seacoast of Guangdong and Fujian in late March every year. From April to May, some fry of *N.miichthioides* could be captured in the Yuhuan, Dongtou of Zhejiang Province (Xu, 2005) (Fig. 1). Nowadays, research on *N. japonica* and *N. miichthioides* have recently been initiated on the artificial breeding and fry

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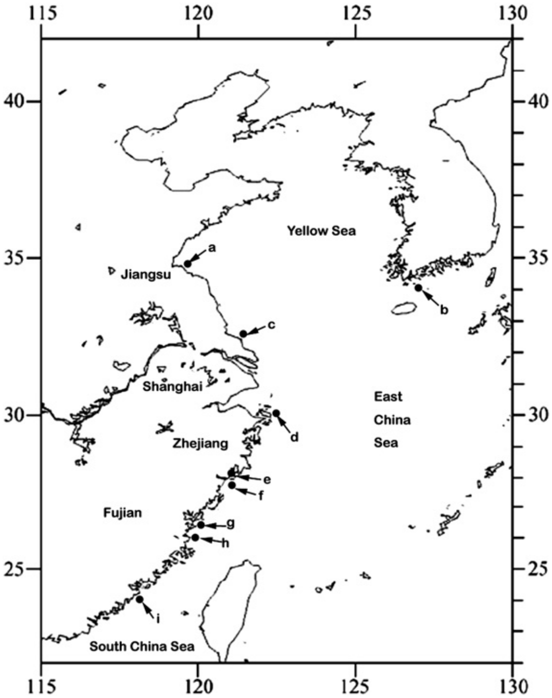

Fig. 1. The geographical locations of *Nibea japonica* and *N. miichthioides* around the seacoast of China (arrows: a. Lianyungang; b. Southern Sea of Japan; c. Lvsi; d. Zhoushan; e. Yuhuan; f. Dongtou; g. Fuding; h. Xiapu; i: Dongshan).

production (Lou et al., 2002; Chai et al., 2007a; Hu et al., 2007). Liang et al. (2010) indicated that the enhancement release of *Sparus macrocephalus* and *N. japonica* in artificial reef habitat waters increased fisheries production, restored populations to a certain extent and maintained the diversity of natural waters. However, there is little known about the genetic diversity and relationship of two *Nibea* species, besides the molecular identification using RAPD markers and the mitochondrial (mt) Cyt *b* gene (Chai et al., 2007b, 2009). From 2009 to 2012, a total of 4.45 million *N. japonica* fries have been released in the Zhoushan Sea area due to the depletion of *N. japonica* resource (Notification of Enhancement and Releasing for *Nibea japonica*, 2009; Enhancement and Releasing of *Nibea japonica*, 2010; Enhancement and Releasing of *Nibea japonica*, 2011; Enhancement and Releasing Briefing for *Nibea japonica*, 2012). It is important to assess the genetic diversity of *N. japonica in* order to ensure the released fry quality. Compared to isozymes, RAPD and other markers, DNA sequence analysis has proven to be rapid, accurate and reliable for assessing the genetic diversity of populations (Buonnacorsi et al., 2001). Because the mt DNA genome possesses features including a fast evolution rate, maternal inheritance, and the easy detection of genetic divergence among populations, it is regarded as an effective genetic marker for assessing the genetic variation and distinguishing different species. Up to now, genes such as mtDNA COI (Lakra et al., 2009; Ma et al., 2011; Yuan et al., 2009), COII (Wang and Li, 2004, 2008), Cyt *b* (Cheng et al., 2008), 16S rRNA (Lakra et al., 2009; Yuan et al., 2009) and the D-loop gene (Liu et al., 2004; Peng et al., 2010) were successfully utilised to assess the genetic diversity and genetic structure of some aquatic animal populations. However, there were few reports on the monitoring of genetic diversity of *Nibea* using mtDNA. Furthermore, despite the fact that Talwar (1995) were in favour of their classification of *Argyrosomus*, the related experimental results were deficient and require more relevant data for authentication via molecular markers. Hence, this study intended to investigate the mt 16S rRNA and COI genes to better identify genetic diversity between *N. japonica* and *N. miichthioides*, and their relationship with other related Sciaenidae species.

2. Material and methods

2.1. Sample collection and DNA extraction

N. japonica was collected in June 2011 from an experimental farm of Marine Fisheries Research Institute of Zhejiang province. *N. miichthioides* fish were sampled from the Aquaculture Association of Fodu deep sea net-cage. Each population had 20 individuals, from which fin rays from live fish were collected and fixed in 75% ethanol for subsequent analysis.

The total genomic DNA of fin rays was isolated following the specification of the animal genome extraction Kit (Sangon, China). The DNA was quantified by UV spectrophotometer and stored at −20 °C until use.