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Intraspecific genetic variation in mitochondrial 16S rRNA and COI genes in domestic and wild populations of Huaguizhikong scallop *Chlamys nobilis* Reeve

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ABSTRACT

A 468 base-pair fragment of the mitochondrial 16S rRNA gene and a 481 base-pair fragment of the mitochondrial COI gene were used to study the geographic structure and genetic diversity within Huaguizhikong scallop *Chlamys nobilis* Reeve. The samples were collected from one Thailand population (TH) which sequences were obtained from the GenBank database, five domestic stocks [Fujian (FJ), Guangxi (GX), Hainan (HN), Daya Bay (DW), and Zhanjiang (ZJ)] and two wild populations [Guangxi (GY) and Daya Bay (DY)]. Sixty polymorphic sites were found in COI gene, and thirty-nine haplotypes were detected in all samples. In addition, twenty-four polymorphic sites were found, and twenty-seven haplotypes were detected in all samples of 16S rRNA gene. The analysis of haplotype frequency distribution and molecular variation indicated that genetic variation and geographic structure were significant. To COI gene and combined data set, the majority (COI: 78.7%, combined data set: 78.5%) of the genetic variation was distributed within populations, furthermore, less than 37.0% genetic variation of 16S rRNA could be distributed within populations. Variance of 16S rRNA gene (4.3%), COI gene (26.1%), and combined data set (23.4%) could be attributed among different geographic regions. The richest variation of 16S rRNA was observed in TH population among the eight populations supported the fact that TH has richer genetic polymorphism than Chinese population, which may sustain the idea of stock introduction. Otherwise, in the present study, we found that the gene diversity (Hd) level of wild population was higher than cultured populations of *C. nobilis* in three data sets. Our results indicated that genetic composition of cultured *C. nobilis* populations in southern China have been significantly affected by hatchery performance. Besides, the FJ stock which was the center seed stock of scallop *C. nobilis* cultured populations has been preliminarily confirmed in our research.

1. Introduction

Distributed mainly along the south coast of China, Japan, and Malaysia, the Huaguizhikong scallop *Chlamys nobilis* Reeve, which live free in rocky or sandy bottoms, has been one of the important economic species of the shellfish aquaculture industry along the south coast of China for several decades. And the culture regions principally distributed along four coastal provinces (Fujian, Guangdong, Guangxi, and Hainan) of South China Sea. Hatchery cultured stock has been used as brood stock to produce the next cultured generation every year. Such closed cycle operation could lead to reproductive isolation from the wild populations and reduction in genetic variability due to low effective population size and inbreeding (Gosling, 1982; Hedgecock and Sly, 1990). Genetic divergence among these isolated hatchery-propagated stocks would be expected to increase with time (Hedgecock and Sly, 1990). Wada (1986) found a reduction in the number of alleles per locus in four polymorphic allozyme loci of Japanese pearl oyster, *Pinctada fucata martensii* after six generations of selection. In China, the culture of scallop *C. nobilis* was originated in Fujian Province in 1970s, but in recent years, the situation of its aquaculture industry was in chaos. No one knew the origin of parents and the seed stocks. The mortality rate was higher than ever before. A proposed explanation for this problem was the loss of genetic diversity which came from inbreeding depression, few males used in the hatchery and culture environment (Jiang and Wei, 1985). Refreshing the scallop stock by introducing new stocks from wild populations outside the coast of southern China and increasing the numbers of male parent in the brood stock were suggested. Therefore investigation and evaluation of its stock structure throughout its geographic range were required.

It has been proposed that benthic marine species with pelagic larvae have population genetic variation reflecting the dispersal capacity of larvae. Most of them are thought to have little genetic structure. Considerable work has been conducted to test this hypothesis on many species, including scallops (Wilbur et al., 1997; Saavedra and Peña, 2003; Barucca et al., 2004), oysters (Reeb and Avise, 1990; Small and Chapman, 1997; Yu et al., 2003), mussels (Karakousis and Skibinski, 1992; Geller et al., 1993), gastropods (Kyle and Boulding, 2000), abalones (Gruenthal and Burton, 2008), pearl oysters (Yu and Chu, 2006), ophiuroids (Baric

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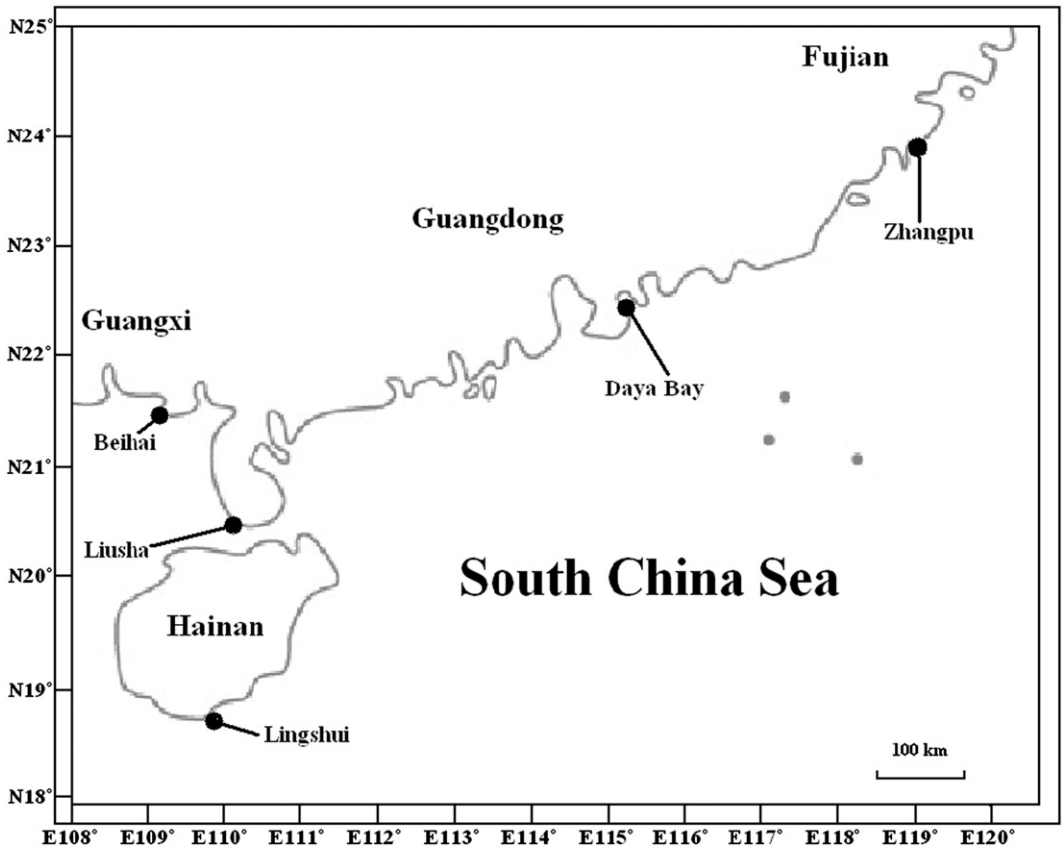

Fig. 1. Sampling locations of wild and cultured *Chlamys nobilis* populations in Zhangpu, Daya Bay, Liusha, Beihai and Lingshui in South China.

and Sturmbauer, 1997), anostracans (Elpidio and Paul, 2000), and many other invertebrates with planktonic larvae (Palumbi and Wilson, 1990; Arndt and Smith, 1998; Schizas et al., 1999). Mitochondrial DNA sequences (including 16S rDNA and COI) were used for many of these studies. In the scallop *C. nobilis*, this hypothesis has not been tested. The intraspecific genetic variation of *C. farreri*, which has been one of another major species of the shellfish aquaculture industry on northern China, has been studied by using allozyme frequency data among five populations along the northern coast of China (Zhang and Zhang, 1997) and 16S rRNA gene fragment data among six populations of China, Japan, and South Korea (Kong et al., 2003), but the question of whether significant geographic structure existed was not directly answered. Moreover, as the results of other researchers have shown, when different genetic systems were used in the same species, the resulting population genetic structures might differ (Karl and Avise, 1992). Thus, this also led to our interest in examining the genetic structure of the population using mitochondrial gene sequence data among samples from the geographic range of the species.

2. Materials and methods

2.1. Population sampling and DNA extraction

Scallop *C. nobilis* samples were collected from five cultured populations of Fujian (FJ) Zhangpu, Guangxi (GX) Beihai, Zhanjiang (ZJ) Liusha, Hainan (HN) Lingshui, and Shenzhen (DW) Daya Bay, and two wild populations from the coast of Guangxi (GY) Beihai, Shenzhen (DY) Daya Bay, respectively (Fig. 1). Two hundred and twenty-one scallop specimens (FJ: 28, GX: 29, GY: 28, ZJ: 28, HN: 30, DY: 30, DW: 30) were sequenced to examine 16S rRNA gene. Sequences of 16 S rRNA in Thailand population (TH) were obtained from GenBank database (Accession Nos.DQ640848.1–DQ640865.1, Na-Nakorn et al., 2006). And two hundred and one scallop specimens (FJ: 30, GX: 26, GY: 28, ZJ: 30, HN: 29, DY: 30, DW: 28) were sequenced to examine COI gene.

Total DNAs were extracted from adductor muscles using CATB extraction procedure (Adamkewicz and Harasewych, 1996). Tissues (1 g) were homogenized and incubated in pre-warmed (60 °C) CTAB

Table 1
The haplotype frequencies of 16S rRNA gene in eight *Chlamys nobilis* populations

Haplotype	Haplotype frequency								
	GY	HN	TH	ZJ	FJ	GX	DY	DW	Total
H1	–	–	1	–	14	12	12	12	51
H2	11	30	1	24	13	14	17	18	128
H3	–	–	1	3	1	–	–	–	5
H4	–	–	–	–	–	3	–	–	3
H5	1	–	–	–	–	–	–	–	1
H6	2	–	–	–	–	–	–	–	2
H7	10	–	–	–	–	–	–	–	10
H8	1	–	–	–	–	–	–	–	1
H9	1	–	–	–	–	–	–	–	1
H10	1	–	–	–	–	–	–	–	1
H11	1	–	–	–	–	–	–	–	1
H12	–	–	–	1	–	–	–	–	1
H13	–	–	1	–	–	–	–	–	1
H14	–	–	1	–	–	–	–	–	1
H15	–	–	1	–	–	–	–	–	1
H16	–	–	1	–	–	–	–	–	1
H17	–	–	1	–	–	–	–	–	1
H18	–	–	1	–	–	–	–	–	1
H19	–	–	1	–	–	–	–	–	1
H20	–	–	1	–	–	–	–	–	1
H21	–	–	1	–	–	–	–	–	1
H22	–	–	1	–	–	–	–	–	1
H23	–	–	1	–	–	–	–	–	1
H24	–	–	1	–	–	–	–	–	1
H25	–	–	1	–	–	–	–	–	1
H26	–	–	1	–	–	–	–	–	1
H27	–	–	–	–	–	–	1	–	1