Germplasm Authentication of Mantis Shrimps (*Oratosquilla oratoria*) in China Sea by SNP and AS-PCR Method

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In the study, germplasm authentication of mantis shrimp in China sea was analyzed by SNP and AS-PCR method. Twenty-six stably single nucleotide polymorphisms (SNPs) between Bohai sea and South China sea were revealed, and population-specific primer pairs (BH065/BH326, SC065/SC326) were established based on SNPs. And topological structure analysis separated the mantis shrimps into two distinct lineages with 100% statistical support, which reveals significant divergence has happened in China sea though weak differentiation in morphology. These results showed SNPs and AS-PCR might be the useful tools for germplasm authentication of mantis shrimp.

**Keywords:** Mantis shrimp, *Oratosquilla oratoria*, SNP, AS-PCR, Authentication

Introduction

Mantis shrimps can be easily differentiated from other “shrimps” by the specific raptorial limbs that can be moved in astonishingly fast and furious strikes. As the impressive malacostracan crustacean, *Oratosquilla oratoria* has been regarded as the commercial valuable aquatic species for its good taste and rich nutrition in fishery in indo-pacific. The species occurs in a wide range of habitats from the shore down to coral reefs and level substrates along whole chinese coast, in which the Bohai sea and the South China sea are the important native distribution. Recently, natural resources of *O. oratoria* have been seriously damaged due to excessive exploitation and environmental pollution, e.g. oil spill event of Bohai bay in 2011. Significant differences have been found in growth rate, taste and nutrition in different sea areas. But germplasm authentication is difficult by traditional morphologic methods for their weak differentiation in morphology. Previously, it is usually thought that little genetic differentiation of mantis shrimps exists among different populations for their planktonic larva dispersion by ocean currents. However significant genetic differentiation has been revealed in some species similar to mantis shrimps. So we think to establish a reliable population-specific identification method based on the significant genetic differentiation.

Molecular markers are stable and less affected by external environment, which have been widely used for the species discrimination. And mt COI was considered to be the barcoding for high variability among different species. Our aim is to estimate a population-specific method by SNP and AS-PCR in germplasm authentication.

Materials and Methods

A total of seventy-three samples were collected in the study including forty-eight from Bohai sea and twenty-five sequences from South China sea. All samples were identified by morphological features. Genomic DNA was extracted from maxilliped muscle using standard phenol-chloroform method. A partial fragment of mitochondrial cytochrome coxidase I (COI) was amplified on a GeneAmp® PCR System 9700 (ABI) or Mastercycler® gradient (Eppendorf) thermocycler with the primers LCO1490 and HCO2198. PCR products were purified using PCR purification kit, then cycle sequenced following standard protocols (3μl purified PCR product, 4μl ABI PRISM Fluorescent dye terminators, 3μl of a 1μM of primer solution), followed by analysis on ABI 3730 automated DNA sequencer.

All sequences were aligned with Clustal 1.83.
using the multiple alignment default parameters, and corrected by hand. Single nucleotide variable sites and phylogenetic analyses were executed in Mega 3.015. Genetic diversity, nucleotide diversity (π), haplotype diversity (h) were calculated using the program DnaSP version 4.016. And genetic divergence within population and among populations were computed with Mega. Specific diagnostic primers were designed on the basis of the stably variable sites between populations.

**Result and Discussion**

In the study, seventy-three COI sequences with the length of 580bp were analyzed. And thirty-three haplotypes (15 of Bohai sea; 18 of South China sea) were defined. However none haplotype was shared between two areas. There were sixty-two polymorphic sites, in which twenty-six stably variable sites were found between Bohai and South China sea. These variable sites between populations were single nucleotide polymorphisms (SNPs). The whole average nucleotide diversity (π) was 0.03159. But a comparison of nucleotide diversity of the Bohai sea regions and South China sea was 0.00409 and 0.00669 respectively. So whole average nucleotide diversity (π) was about 10 times of within population. It revealed that significant divergence has happened between the two populations.

Topological structure analysis consistently separated all samples into two distinct lineages with 100% statistical support. One lineage was all samples from Bohai sea, and the other lineage was from South China sea. So population genetic structure was correspondence with the significant divergence between Bohai sea and the South China sea. Average genetic diversity intra-population of Bohai and South China sea was 0.0052 and 0.0075 respectively. But the mean value of inter-populations was 0.0677, which was also about 10 times of intra-population. Present results of none shared haplotype, high value of average π and genetic diversity proved significant genetic differentiation of mantis shrimps had existed among different populations though its planktonic larvae was dispersed by ocean currents.

Based on twenty-six SNPs, two primer pairs of AS-PCR (BH065/BH326, SC065/SC326) were established. And each primer’s nucleotide of 3-end was on the stable SNPs between populations. And 3-end nucleotide acid of primer pair BH065/BH326 was T and G, and 3-end of SC065/SC326 was C and A. Repeated experiments were conducted in every diagnostic primers, and primers BH065/BH326 yielded 300 bp positive fragment in samples from Bohai, but negative of South China sea. And primer pair SC065/SC326 showed the positive results in South China sea, but negative in Bohai. So combination of the two primer pairs of AS-PCR could identify mantis shrimps of Bohai from the South China sea. Therefore, primer pairs (BH065/BH326, SC065/SC326) were certified as the quick and effective markers in the two populations authentication. Compared to the authentication method by DNA sequence, AS-PCR was simple, timesaving and effective and it was particularly necessary for food safety in current oil spill situation of Bohai sea.

**Fig. 1**—AS-PCR identification of twenty mantis shrimps (S1-20) selected from fish markers by diagnostic primers BH065/BH326 (A) and SC065/SC326 (B); M: DL2000 DNA marker

In the study, twenty mantis shrimps were selected from markers of Qingdao and Shanwei and authenticated by diagnostic primer pairs (BH065/BH326, SC065/SC326). The results showed that nine individuals yielded positive fragment with BH065/BH326 (Fig1-A). And eight yielded the positive results by primer pair SC065/SC326 (Fig1-B). We concluded that nine samples were from Bohai sea and eight were from South China sea by the diagnostic results.

**Conclusion**

In the study, AS-PCR (BH065/BH326 and SC065/SC326) was proved effective in population authentication of Bohai and South China sea and the twenty-six SNPs was the population-specific barcoding sites. Compared to sequencing methods, AS-PCR method has demonstrated as a useful and
rapid tool for germplasm authentication. It will play an important role in germplasm authentication of mantis shrimps in future.

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