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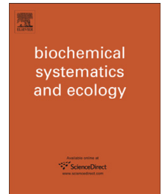

An analysis of genetic diversity in *Marphysa sanguinea* from different geographic populations using ISSR polymorphisms

Huan Zhao, Yixiao Wang, Dazuo Yang, Xinda Zhao, Na Li, Yibing Zhou*

Key Laboratory of Marine Bio-resource Restoration and Habitat Reparation in Liaoning Province, Dalian Ocean University, Dalian 116023, PR China

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The genetic diversity of five wild populations of marine polychaete *Marphysa sanguinea* found in China was investigated using Inter-Simple Sequence Repeat-PCR (ISSR-PCR) polymorphisms. The results of the ISSR-PCR showed that 108 (90.8%) of the 119 ISSR loci tested were polymorphic. The Shannon's information index value was 0.4981, Nei's gene diversity was 0.3418, and the coefficient of gene differentiation (Gst) was 0.3671, which indicated that the among-population component accounted for 36.7% of the total variation, while the within-population component accounted for 63.3%. A UPGMA tree showed that the five populations clustered into two branches. Populations from Dalian, Xingcheng, and Rushan clustered together, while the two Guangxi populations, A and B, clustered into a unique group. The results indicated that the genetic diversity among the five populations of *M. sanguinea* is high, which will provide useful information for the protection of biodiversity among marine polychaetes.

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1. Introduction

Marphysa sanguinea, which is globally distributed, belongs to the phylum Annelida, class Polychaete, order Eunicidae, and family *Marphysa*. This polychaete is a keystone species inhabiting the shallow marine and estuarine waters of China, where it represents a major component of the benthic biomass and an important food resource for crustaceans, fishes, and birds. In addition, because they are nutritionally rich, they are commonly used as bait in recreational fishing and aquaculture (Yu et al., 2005). Considering their short-distance migration and deposit-feeding characteristics, they usually accumulate organic matter from the sediment, and their steady-state body burdens are a function of the biotransformation and elimination processes. Thus, this polychaete is considered as a good candidate for toxicity detection. With the growing ecological and economic value of *M. sanguinea*, the demand of this marine polychaete continues to increase, which may lead to its depletion from natural sources in China (Yang et al., 2011).

Previous reports on *M. sanguinea* mostly concentrated on morphology, reproduction, development, physiology, and artificial breeding, as well as other aspects (Yu et al., 2005; Prevedelli et al., 2007; Parandavar and Kim, 2015); however, there is little information on the genetic diversity of this species. To protect the important genetic resources of this species, the genetic diversity and structure of five different natural populations of *M. sanguinea* from the Yellow and Bohai Seas, as well as the South China Sea region, were analysed in this study using ISSR polymorphisms. The results of this study will provide useful information on understanding the genetic relationships and carrying out artificial breeding in *M. sanguinea.*

* Corresponding author. Key Laboratory of Marine Bio-resources Restoration and Habitat Reparation in Liaoning Province, Dalian Ocean University. No 52, HeiShiJiao Street, Dalian, Liaoning, 116023, PR China.

E-mail addresses: ybzhou@dlou.edu.cn, ybzhou2011@hotmail.com (Y. Zhou).

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2. Materials and methods

2.1. Sample collection and DNA extraction

A total of 150 *M. sanguinea* were collected from five different wild populations. In total, 150 samples were collected, with 30 samples each came from Dalian (Liaoning Province, DL), Xingcheng (Liaoning Province, XC), and Rushan (Shandong Province, RS) and 30 samples came from each of two areas of Beihai (Guangxi Province, GXA and GXB) (Fig. 1). Total DNA was extracted using a DNA extraction kit (MiniBEST Universal Genomic DNA Extraction Kit, TAKARA) according to the manufacturer's instructions. The quality of DNA was examined by 1% agarose gel electrophoresis, and the DNA concentration was measured using a UV/Visible spectrophotometer at 260 nm absorption.

2.2. ISSR primer and PCR amplification

The primers for ISSR-PCR amplification were designed based on the University of British Columbia Nucleic Acid-Protein Service Unit, UBC Primers set #9. The primers were synthesized by Takara Inc. (Dalian, China). Ten ISSR primers that detected polymorphisms with clear bands were selected for this experiment (Table 1). The ISSR-PCR reaction was carried out in a 25 μl volume containing 15 ng DNA, 0.2 mM dNTPs, 2.0 μM each primer, 1.5 mM Mg^{2+}, 2.0 U Taq polymerase, and 2.5 μl 10 × Buffer (Mg^{2+} Free). The reaction program was as follows: 94°C denaturation for 5 min, followed by 35 cycles of 94 °C for 30 s, 50 °C for 1 min and 72 °C for 1 min, and a final extension at 72 °C for 10 min. PCR products were detected using 1% agarose gel electrophoresis.

2.3. Data analysis

The ISSR bands were scored as present (1) or absent (0) for each sample using Crosscheck freeware (Buntjer, 1999), and transformed into a 0/1 binary character matrix. The number of alleles (Na), effective number of alleles (Ne), Shannon's information index, Nei's genetic diversity index (H), gene flow (Nm), and genetic differentiation coefficient (Gst) were

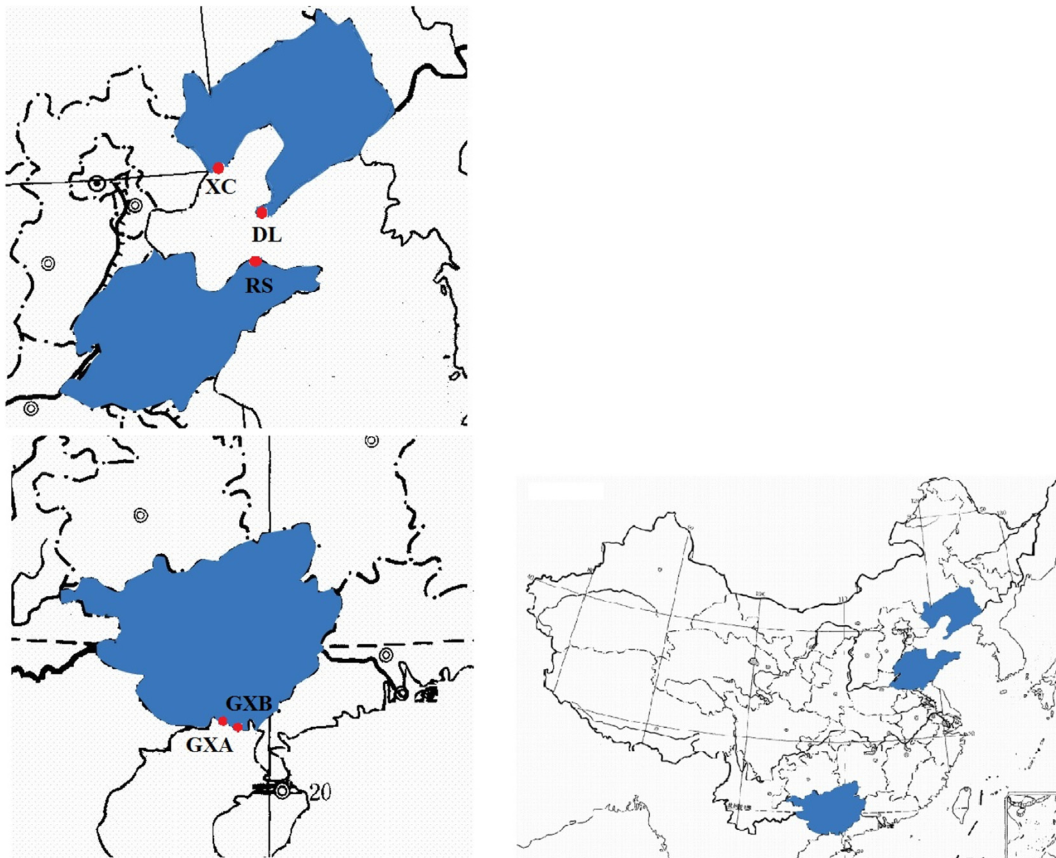

Fig. 1. A map showing the sampled populations of *M. Sanguinea* (XC: Xicheng; DL: Dalian; RS: Rushan; GX: Beihai in Guangxi Province).

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