Genetic diversity of oriental river prawn (*Macrobrachium nipponense* De Haan) revealed by ISSR markers

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Abstract: Oriental river prawn *Macrobrachium nipponense* is a commercially important aquaculture species in China. In this study, genetic diversity among five populations of *M. nipponense* was determined using inter-simple sequence repeat (ISSR) markers. The five populations were collected from Tai Lake of Jiangsu Province (JS), Poyang Lake of Jiangxi Province (NC), Nanman River of Yunnan Province (YN), Yangtze River of Hubei Province (HB) and Bositeng Lake of Xinjiang Uygur Autonomous Region (XJ). A total of 142 bands were produced from 60 individuals by nine ISSR primers. At species level, the percentage of polymorphic loci (PPL), observed number of alles per locus (N_o), effective number of alleles per locus (N_e), Nei's gene diversity index (H) and Shannon's information index (I) were 97.18%, 1.972, 0.312, 0.120 and 0.323, respectively. At population level, the percentage of polymorphism loci (PPL) varied greatly among populations, ranging from 29.58% to 61.97%. The observed number of alleles per locus ranged from 1.296 to 1.620, the effective number of alleles varied from 1.165 to 1.281. Nei's gene diversity indices ranged from 0.098 to 0.172. Shannon's information indices averaged 0.135 at the population level (H_{pop}) and 0.323 at the species level (H_{sp}). The total genetic diversity (H_T) and within-population genetic diversity (H_s) were 0.201 and 0.135. The coefficient of genetic differentiation between populations (G_{ST}) was 0.327 as estimated by partitioning of the total gene diversity (0.201). NC population exhibits great level of variability (PPL: 61.97%, N_o: 1.620, N_e: 1.281, H: 0.172, I: 0.267), whereas the population HB exhibits the lowest level of variability (PPL: 29.58%, N_o: 1.296, N_e: 1.165, H: 0.098, I: 0.147). Compared with other populations, XJ population and JS population were the most similar genetically (F_{ST}: 0.192 3, D: 0.054 2), while XJ population and YN population were the most dissimilar (F_{ST}: 0.595 0, D: 0.155 9). The result of this study may be useful for conservation and utilization of wild genetic resources of oriental river prawn. [Journal of Fishery Sciences of China, 2010, 17(5): 913-921]

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Oriental river prawn, *Macrobrachium nipponense* is a commercially important aquaculture species in China, especially in southern China. Its estimated production was 120 000 t in China in 2001[1]. Currently, most fries used for stock pond stocking come from wild populations. Farms prefer wild fries to pond-reared ones because wild shrimps are larger, with higher fecundity and yield more viable nauplii[2]. A few allozyme-based and PCR-based studies have been taken[3-4], but genetic variations among *M. nipponense*

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populations are largely unknown. The numbers of wild *M. nipponense* populations declined over the last 20 years mainly because of overexploitation, pollution, and construction of hydropower projects[5]. To understand the genetic variation of a species is fundamentally important for genetic enhancement programs that maximize the benefits of selective breeding and avoide potential inbreeding and random genetic drift[6]. And information of genetic variation is vital for design and implementation of adequate management strategies for a species[7]. Furthermore, in order to relieve pressure on wild stock and improve industry products through selective breeding, developing a domestication program for *M. nipponnense* is important, which requires baseline information concerning genetic background and genetic variations.

Inter-Simple Sequence Repeats (ISSR) amplifies inter-microsatellite sequences at multiple loci throughout the genome[8-9]. ISSR molecular marker technique permits the detection of polymorphism in microsatellite and intermicrosatellite loci without knowledge of DNA sequences[10]. This technique has been widely used to investigate genetic diversity and population genetic structure[11-12], due to its advantages in overcoming limitations of allozyme and RAPD techniques[13-15]. ISSR has been widely used in plant, vertebrate[16-19] and terrestrial invertebrate researches[20-22]. This study is to determine the genetic variations among five populations of *M. nipponense* and provide preliminary information for potential selective breeding program of this species.

1 Materials and methods

1.1 Prawn samples

Totally 60 individuals of *M. nipponense* were collected from Tai Lake of Jiangsu Province (JS), Poyang Lake of Jiangxi Province (NC), Nanman River of Yunnan Province (YN), Yangtze River of Hubei Province (HB) and Bositeng Lake of Xinjiang Uygur Autonomous Region (XJ) (Tab.1). Samples were preserved in 75% ethanol until DNA was isolated for genetic analysis.

Tab. 1 Information of five *Macrobrachium nipponense* populations for ISSR analysis
表1 日本沼虾5个地理群体采样点位置

Population 群体	Code 代号	Sample size 数量	Locality 采样地	Latitude and longitude 经纬度
Jiangsu	JS	12	Tai Lake, Suzhou, Jiangsu Province	31° 10′ N 120° 38′ E
Nanchang	NC	12	Poyang Lake, Nanchang, Jiangxi Province	29° 22′ N 116° 20′ E
Yunnan	YN	11	Nanman River, Xishuangbanna, Yunnan Province	21° 10′ N 100° 40′ E
Hubei	HB	13	Yangtze River, Yidu, Hubei Province	30° 38′ N 111° 45′ E
Xinjiang	XJ	12	Bositeng Lake, Bohu, Xinjiang Uygur Autonomous Region	42° 10′ N 86° 50′ E

1.2 DNA extraction and ISSR PCR

Ventral muscle tissue was dissected and washed thoroughly with water to clean the ethanol, then incubated in standard buffer [0.06 mmol/L ethylenediaminetetraacetic acid (EDTA), 0.1 mmol/L Tris, 0.5% sodium dodecyl sulfate (SDS) pH 8.6] overnight at 37 ℃ in the presence of Proteinase K. After buffer incubation, DNA was extracted using standard phenol/chloroform extraction protocols and concentrated by column purification. The purified DNA was dried, dissolved in TE buffer and stored at −20 ℃.

Nine inter-simple sequence repeat (ISSR) primers from the University of British Columbia (ISSR Kit #9) were used for this study (Tab.2). Primers contained different di- and tri-nucleotide repeat motifs to screen different parts of the genome. The PCR reaction

mixture of 20 μL volume contained 0.8 units of *Taq* DNA polymerase, 1×reaction buffer, 2 mmol/L $MgCl_2$, 0.2 mol/L primer, 200 μmol/L of each dNTP, and up to 30 ng of genomic DNA. The PCR cycling included a pre-denaturing step for 7 min at 94 ℃ and 45 cycles of 30 s at 94 ℃, 45 s at 50 ℃, and 2 min at 72 ℃ and a final extension of 7 min at 72 ℃. For each primer, negative controls and three replicates were conducted in the amplifications to determine repeatability.

The PCR products were separated by electrophoresis using 1.5% agarose gel in 0.5×TBE buffer. After electrophoresis at 60 V for 2 h , gels were stained with 0.5 μg/mL ethidium bromide and photographed under ultraviolet light. One hundred base pair DNA ladders (100 bp ladder, Dongsheng Biotech Ltd.) were used as markers to estimate the size of amplified products. Only bands that appeared consistently in all three replicates were scored and used for genetic analysis, while weak bands were not considered for analysis.

Tab. 2　Primers used in ISSR analysis of *Macrobrachium nipponense* and number of reproducible bands
表2　本研究选用的9个引物和扩增条带数

Primer name 引物代号	Sequence (5′−3′) 序列(5′−3′)	Number of fragment 扩增条带数
ISSR 1	GTCGTCGTCGTCGTCGTC	8
ISSR 2	GTGCGTGCGTGCGTGC	15
ISSR 3	ACCACCACCACCACC	12
ISSR 4	ACACACACACACACACC	20
ISSR 5	GAGAGAGAGAGAGAGAYT	18
ISSR 6	AGAGAGAGAGAGAGAGYT	7
ISSR 7	TGTGTGTGTGTGTGTGRG	24
ISSR 8	GAGAGAGAGAGAGAGAC	23
ISSR 9	AGAGAGAGAGAGAGAGG	15

1.3　Data analysis

Each ISSR band was treated as a character and was scored as present (1) or absent (0) for each DNA sample by Crosscheck software[23]. The data were transformed into 0/1 binary character matrix. The data matrix of ISSR was analyzed using POPGENE version 1.31[24] to estimate the percentage of polymorphic loci (PPL), observed number of alleles per locus (N_o), effective number of alleles per locus (N_e), Shannon's information index (I) and Nei's gene diversity index (H) (Tab. 3). At species level, total population gene diversity (H_T), coefficient of gene differentiation [G_{ST}: $G_{ST}=(H-H_s)/H_T$] and the level of gene flow [N_m: $N_m=0.5(1-G_{ST})/G_{ST}$] were measured using Nei's gene diversity statistics[25]. Genetic diversity was also estimated using Shannon's information measure[26]. The parameters include average diversity within populations (H_{pop}) and total diversity (H_{sp}). The analysis of molecular variance (AMOVA)[27] was performed to partition total phenotypic variance into variance within populations and among populations by using ARLEQUIN software[28].

To examine the genetic relationship among populations, a dendrogram was generated from the above matrix using unweighted pair group method with arithmetical averages (UPGMA)[29] with MEGA (version 3.1)[30].

2　Results and analysis

2.1　Genetic variability of five populations

Nine ISSR primers generated 142 bands across the five populations investigated. Length of the fragment

was 300–3 000 base pairs (bps). The average number of bands generated by individual primers varied from 7 to 24 (Tab. 2). At species level, the percentage of polymorphic loci (PPL), observed number of alleles per locus (N_o), effective number of alleles per locus (N_e), Nei's gene diversity index (H) and Shannon's information index (I) were 97.18%, 1.972, 0.312, 0.120 and 0.323, respectively. The percentage of polymorphic loci (PPL) for a single population varied from 29.58% for the Hubei population (HB) to 61.97% for the Nanchang (NC) population (Tab.3). The observed numbers of alleles per locus (N_o) ranged from 1.296±0.458 to 1.620±0.487. Similarly, the effective number of alleles varied from 1.165±0.301 to 1.281±0.340. Nei's gene diversity ranged from 0.098 to 0.172. The average Shannon's index was 0.135±0.013 at population level (H_{pop}) and 0.323 at species level (H_{sp}). Among the five populations, population NC exhibits the highest level of variability (PPL: 61.97%, N_o: 1.620, N_e: 1.281, H: 0.172, I: 0.267), whereas the population HB exhibits the lowest level of variability (PPL: 29.58%, N_o: 1.296, N_e: 1.165, H: 0.098, I: 0.147).

Tab. 3 Population genetic parameters of five *Macrobrachium nipponense* populations
表3 日本沼虾5群体的群体遗传参数

Population 群体	PPL 多态位点百分率/%	N_o 等位基因数	N_e 有效等位基因数	H Nei's基因多样性指数	I Shannon's信息指数
JS	45.77	1.458±0.500	1.254±0.356	0.149±0.192	0.225±0.275
XJ	45.77	1.458±0.500	1.209±0.316	0.128±0.173	0.200±0.252
NC	61.97	1.620±0.487	1.281±0.340	0.172±0.184	0.267±0.260
HB	29.58	1.296±0.458	1.165±0.301	0.098±0.170	0.147±0.248
YN	38.03	1.380±0.487	1.223±0.350	0.129±0.189	0.194±0.271

Note: PPL–percentage of polymorphic loci; N_o–observed number of alleles per locus; N_e–the effective number of alleles per locus; H–Nei's gene diversity; I–Shannon's information index.

2.2 Genetic divergence in five populations

The total genetic diversity (H_T) and within-population genetic diversity (H_s) were 0.201 and 0.135. The coefficient of genetic differentiation between populations (G_{ST}) was 0.327 which was estimated by partitioning total gene diversity (0.201). The level of gene flow (N_m) was estimated to be 1.029 among populations.

Molecular variance within and among *M. nipponense* populations was analyzed with AMOVA (Tab.4). Significance tests after 1 000 permutations. There were highly significant ($P<0.001$) genetic differences among the five *M. nipponense* populations. Of the total genetic diversity, 38.59% was from among-populations and the rest (61.41%) from within-population.

Tab. 4 Analysis of molecular variance (AMOVA) within and among *Macrobrachium nipponense* populations
表4 5个日本沼虾群体分子变异分析结果

Source of variation 遗传差异来源	df 自由度	Variance component 遗传变异组分	Total variance 总变异	P
Among populations 群体间	4	6.87080	38.59	<0.001
Within population 群体内	43	10.93605	61.41	<0.001

Note: df–Degree of freedom. Significance tests after 1 000 permutations.
注: df– 自由度; 经1 000次随机置换来检测变异组分的显著性.

2.3 Phylogenetic analysis result

Fixation index (F_{ST}) and genetic distance (D, Nei's measure) between pairs of populations is presented in Tab. 5. These data suggested that among the five populations, Xinjiang population (XJ) and Jiangsu population (JS) were most similar (F_{ST}: 0.192 3 ; D: 0.054 2), while Xinjiang population (XJ) and Yunnan population (YN) were most dissimilar (F_{ST}: 0.595 0 ; D: 0.155 9).

The dendrogram produced by UPGMA based on genetic distance matrices for all the populations is presented in Fig. 1. Two main clusters were identified. The first cluster consists of two populations from Jiangsu and Xinjiang, and the second is composed of three populations from Nanchang, Hubei and Yunnan .

Tab. 5　Fixation index (F_{ST}) (blow diagonal) and genetic distance (above diagonal) in five populations of *Macrobrachium nipponense*
表5　日本沼虾群体间的遗传分化指数(对角线以下)和遗传距离(对角线以上)

Population 群体	JS	XJ	NC	HB	YN
JS		0.0542	0.0751	0.1387	0.1557
XJ	0.1923		0.0768	0.1350	0.1559
NC	0.1996	0.1904		0.0571	0.0594
HB	0.5035	0.5087	0.2169		0.0912
YN	0.4757	0.5950	0.2522	0.4361	

Note: Population codes are given in tab.1.
注: 群体代号同表 1 .

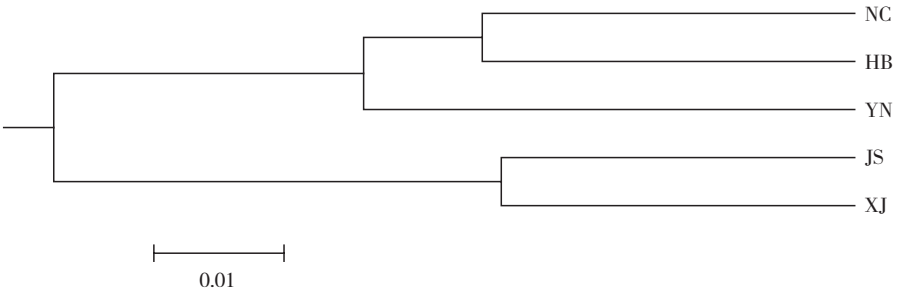

Fig. 1　UPGMA dendrogram of *Macrobrachium nipponense* based on Nei's (1978) genetic distances between populations
Population codes are gieven in tab.1.
图1　日本沼虾的UPGMA聚类图
群体代号同表1.

3 Discussion

Oriental river prawn is one of the few *Macrobrachium* species that has temperate distribution and is capable to reproduce naturally in most Chinese regions. And for this, over the past 20 years it has been a commercially important aquaculture species. However, the farming production has substantially declined due to diseases and small sizes[31]. It is urgent to know the genetic background of the species.

Compared with other PCR-based genetic markers, ISSR has many advantages such as quick, easy to handle, highly reproducible and polymorphic, and requiring no prior genome sequence information. The present study provided the first ISSR data for *M. nipponense* populations in China. Our results provided clear evidence for the reliability and usefulness of ISSR markers in defining population genetic structure. The nine primers generated total of 142 bands for the

five populations, corresponding to an average of 15.8 bands per primer. All those bands were reproducible. Within each population, 29.58%–61.97% of the fragments were polymorphic. Jiang et al[4] investigated the genetic diversity of *M. nipponense* from Anhui and Jiangsu Province. Sixteen random primers generated 173 reproducible RAPD bands for 120 individuals, corresponding to 10.8 bands per primers. The percentage of polymorphic loci (PPL) was 43.4%. Li et al[32] studied the genetic variation of *M. rosenbergii* between a cultured population of Zhejiang Province and a natural population collected from Burma using RAPD method. 139 RAPD bands were detected by 22 random primers, corresponding to 6.3 bands per primer. The PPL of the cultured and the natural population were 30.22% and 33.81%, respectively. Compared with their results, the present research obained more reproducible bands per primer and higher percentage of polymorphic loci. Therefore, ISSR markers exhibit higher levels of polymorphism compared with RAPD markers, which suggests ISSR is a powerful technique in clarifying genetic differentiation among *M. nipponense* populations.

The coefficient of genetic differentiation between population (G_{ST}) was 0.327 as estimated by partitioning of the total gene diversity (0.201), which indicated that the populations are in the verge of genetic differentiation as they have accumulated about 32.7% genetic variability among themselves. Govindajaru[33] distinguished three levels of gene flow: high $N_m > 1$, intermediate $0.25 < N_m < 0.99$ and low $N_m < 0.25$. The value found here ($N_m = 1.029$) was therefore high, which was due to the high dispersal ability of this prawn and sampling locality of this study.

Among the five populations, population NC exhibits the highest level of variability (PPB: 61.97%, N_o: 1.620, N_e: 1.281, H: 0.172, I: 0.267). Samples of NC population were collected from Poyang Lake of Jiangxi Province. It receives water from five rivers of Gan River, Fu River, Xing River, Rao River and Xiu River, and is also connected to the Yangtze River[34]. The maintenance of genetic polymorphism in nature populations can reflect the process of adaptation to environmental heterogeneity[35]. The high polymorphism may suggest sufficient migration among populations of different river systems, and have important implications in conservation as well as utilization of wild genetic diversity.

Jiangsu (JS) and Xinjiang (XJ) populations have lower polymorphism than Nanchang (NC) population. The percentages of polymorphic loci (PPL) of Jiangsu (JS) and Xinjiang (XJ) population were both 45.77%. And the observed numbers of alleles per locus (N_o) were both 1.458. The above genetic parameters showed the genetic structures of these two populations are very similar. Among the five populations, the F_{ST} and genetic distance between Xinjiang and Jiangsu population (F_{ST}: 0.192 3, D: 0.054 2) indicated that they are genetically most similar. *M. nipponense* were introduced and cultured in Bohu County of Xinjiang Uygur Autonomous Region from Shanghai in 1991, but the reliable information about *M. nipponense* of Xinjiang (XJ) population is not clear. Based on the results, it can be speculated that Jiangsu (JS) and Xinjiang (XJ) population may have similar polymorphism. Xinjiang (XJ) and Jiangsu (JS) populations formed a clade, which indicated Xinjiang (XJ) population may come from Tai Lake. If this speculation is correct, they would have the same genetic origin.

This study represented the first application of ISSR to freshwater prawns *M. nipponense*. The results revealed ISSR is a suitable, quick, easy to apply, highly reproducible and polymorphous tool for screening genetic variation in population. The pattern of genetic diversity observed in the oriental river prawn in this study can be used as baseline information for protection and management of population and selective breeding programs.

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日本沼虾遗传多样性的ISSR分析

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摘要: 日本沼虾(*Macrobrachium nipponense*)是中国分布范围广、经济价值较大的一种重要淡水虾类。随着养殖规模的不断扩大,如何保持养殖群体的遗传品质已引起了人们的重视。但迄今为止,养殖的日本沼虾均来自未经系统遗传选育的野生群体,而养殖病害的日趋严重和养成规格、品质的下降已严重影响到日本沼虾养殖产业的健康发展。研究野生群体的遗传结构和遗传分化,揭示其遗传多样性是制定合理有效的保护和管理策略的前提和基础。ISSR(Inter-simple sequence repeats,简单重复序列中间区域)标记技术具有实验重复性好、信息量大、多态性高等优点,是一种理想的检测群体遗传变异的分子标记。因此,本研究应用ISSR标记技术对日本沼虾5个地理群体进行了初步的遗传分析,以期为合理开发和利用日本沼虾天然资源,以及建立和保护日本沼虾种质资源库及基因库提供理论依据。

本研究对采自江苏苏州、江西南昌、云南西双版纳、湖北宜都和新疆博湖的5个地理群体进行了初步研究。从50个ISSR引物中筛选出9个条带清晰、稳定性和重复性好,且产生相对较多条带的引物用于全部DNA样品的PCR扩增。对日本沼虾5个地理群体的群体遗传分析表明,在所有检测到的清晰且可重复的142个有效位点中,多态位点有138个。物种水平上,多态位点百分率(PPL)、等位基因数(N_o)、有效等位基因数(N_e)、Nei's 基因多样性指数(H)和Shannon信息指数(I)分别为97.18%、1.972、0.312、0.120和0.323。而在群体水平上,5个地理群体的多态位点百分率为29.58% ~ 61.97%; 等位基因数(N_o)为1.296 ~ 1.620; 有效等位基因数(N_e)为1.165 ~ 1.281; Nei's 基因多样性指数(H)为0.098 ~ 0.172; Shannon信息指数(I)为0.147 ~ 0.267。从各个地理群体看,江西南昌群体的遗传多样性最高(PPB: 61.97%, N_o: 1.620, N_e: 1.281, H: 0.172, I: 0.267),而湖北宜都群体最低(PPB: 29.58%, N_o: 1.296, N_e: 1.165, H: 0.098, I: 0.147)。群体内遗传多样度(H_s)和总基因多样度(H_T)分别为0.135和0.201,根据遗传多样性水平在群体内(H_s)和群体间(H_T–H_s)的分化,各个群体之间的Nei's基因分化系数[G_{ST}=(H_T−H_s)/H_T]是0.327。AMOVA分析表明,群体间的遗传变异占总遗传变异的38.59%,而61.41%的遗传变异源于群体内,群体之间表现出较高水平的遗传分化。与其他群体相比,新疆博湖群体和江苏苏州群体差异最小(F_{ST}: 0.1923,遗传距离D: 0.0542),而新疆博湖群体与云南西双版纳群体差异最大(F_{ST}: 0.5950, D: 0.1559)。采用UPGMA法构建的分子系统树显示,5个地理群体明显地聚为2个族群,来自新疆博湖和江苏苏州的日本沼虾群体聚为一支,而江西南昌、湖北宜都和云南西双版纳的群体聚在一起。

本研究使用9条引物对5个地理群体进行了扩增,共检测到138个多态位点,各群体的多态位点百分率(PPL)为29.58% ~ 61.97%。与其他相关研究结果进行比较,发现对日本沼虾而言,ISSR技术是一个理想的检测群体遗传变异的分子标记。与其他群体相比,新疆博湖群体和江苏苏州群体各遗传参数均相近,且UPGMA系统树亦显示新疆博湖群体和江苏苏州群体的亲缘关系较近,推测它们可能来自同一个祖先群体。与其他野生群体相比,江西南昌群体的遗传多样性最高,该群体采自江西省鄱阳湖,鄱阳湖是中国最大的淡水湖泊,它接纳赣江、抚河、信江、饶河、修河5大河及博阳河、漳田河、潼津河的来水经调蓄后由湖口注入长江,是一个过水性、吞吐型、季节型的湖泊。上游河流汇入鄱阳湖引起群体迁移使不同生态型的基因交流,增加了迁入地的遗传多样性。从遗传角度来讲,一个物种保持足够的遗传变异性是适应不同生境、生存和进化的首要保证。因此,较高水平的遗传多样性对于保护和利用野生群体具有重要意义。本研究表明,在日本沼虾的多样性研究方面,ISSR标记技术是一个非常有效的检测群体遗传变异的遗传标记。研究结果可以为合理开发和利用日本沼虾自然野生资源,以及建立和保护日本沼虾种质资源库及基因库提供基础资料。[中国水产科学,2010,17(5): 913–921]

关键词: 日本沼虾; 遗传多样性; ISSR

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