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Studing Pontogammarus Maeoticus among Southern Coast of Caspian Sea

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Abstract: For the first time, the genetic variability of southern Caspian Sea population of the brackish water amphipod [Pontogammarus maeoticus] was analyzed using randomly amplified polymorphic DNA [PCR-RAPD]. We analyzed 4 populations along the Mazandaran coast with 10 arbitrary primers, which produce 21 Interpretable bands. There was no significant polymorphism and 95.2% of bands were monomorphic. These results [molecular identification] are consistent with those of morphological identification. This was the first time that the southern Caspian Sea amphipods [p. maeoticus] has been genetically surveyed; therefore to monitor further alteration, variation, mutation and modulation, a continues, precise and great project with the Guilan province coast results, is needed.

Key Words: Genetic variability · Pontogammarus maeoticus · RAPD marker · Southern Caspian sea coasts · Monomorphic bands

INTRODUCTION

A vast amount of scientific literature recognizes the importance of the amphipods of the genus Gammarus in aquatic ecosystem worldwide. Gammarus in a wide variety of freshwater, brackish and marine habitats, where they are often dominant members, playing a key role in the structure and function of aquatic communities. Also they act as a good carbon transfer organ in the food chain [1,2]. Despite their ecological importance and the research interest they have long received, there are still important taxonomic uncertainties and problematic species identifications with in Gammarus. Although many attempts have been made to employ morphological characters to resolve phylogenetic relationships within the amphipod, the results are tentative and based on only a few characters for most groups. Gammarus is a particularly specious genus with 204 species currently described the vast majority of which are freshwater species [3].

In Iran, dominant species of Caspian Sea's gammarus is pontogammarus maeoticus [4]. In Caspian Sea, 9 orders of crustaceans are recognized. Order of amphipods contains 5 families and the family of gammaridea has 16 recognized species in Caspian Sea [5].

These 16 species has a significant role in food chain of sturgeons, mullets, carps, trouts and many different birds Such as flamingos and snipes [6]. To resolve the uncertainties in identification the species, using molecular markers can be very useful. For resumption of process, due to lack of genetic identification, RAPD markers will be the best and reasonable choice. RAPD have been used to analyze intra-specific genetic differentiation of many similar species such as Gammarus locusta world wide [7].

In Iran this was the first time that molecular markers used to identify pontogammarus maeoticus. We intend to depart from this case study to explore the potential of RAPD fingerprinting to identify amphipod species, by determining tentative species-specific markers that may be applied universally to distinguish amphipods.

For these purpose 4 different zones have been selected along Caspian Sea and careful morphological identification was applied simultaneously as a validation system for the resultant molecular markers [6].

MATERIAL AND METHOD

Collecting the Samples and Primary Process: P. Maeoticus were collected among 4 different zones along Mazandaran province shore. These zones were: Sari (Farah abad beach), Neka (Azadi beach), Babolsar (Shahrdari beah) and Noor (tafrihbeach). These zones were selected cause of following reasons:

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Morphological identification of the mentioned species has been done along the mentioned coasts, therefore, the zones are which that in Seifabadi *et al*. reports, contains more than 98% Pontogammarus maeoticus. Gammarus are very sensitive to environmental pollutions, thus these zones contains high pollution rate so in case of mutation and the mutations were easier to find [4].

Sampling: Sampling was done from May 2009 to May 2010 periodically. Season differing do not affect the result but also, in calm and warm weather, the gammarus are bigger and easier to collect. Sampling was done with a canvass sampler through digging a part of beach [intertidal zone] and put the sampler inside the hole. 20-30 samples were collected along each zone.

Morphological Identification: All samples have been transferred to the lab and have been identified through stock *et al*. identification codes and binocular microscope.

DNA Extraction: 5 different methods have been used to determine the best way of extraction and finally the method which has been used by costa *et al*. shown the best result [7-9].

A gammarus was homogenized in a 1.5 ml micro-tube containing 500 µl TEN (50 mM Tris, 2.5 mM EDTA and 100 mM NaCl) and mixing with 50 µl sarcosyl. To omit RNA and proteins, ribonuclease A and proteinase K have been added for 1 and 2 hour at 55°C water bath, respectively. The solution was extracted twice with PCI (phenol: Choloroform: isoamyl alcohol, 25:24:1 V/V/V). DNA was precipitated from the aqueous phase with ethanol and re-suspended in TE (10mM Tris and 1 mM EDTA, pH 8.0). Randomely primed ampilifications were performed in a final volume of 12.5 µl, containing 1 mM mixed dNTPs, 1 U of Taq DNA polymerase, 1X PCR buffer (20 mM Tris- HCl----pH 8.4, 50 mM KCl, 3.5 mM $MgCl_2$), 1 µM of primer, 10 ng of extracted DNA. The primers used and their nucleotide sequences were as follows: OPA2 (5'-TGCCGAGCTG-3'), OPA10 (5'-GTGATCGCAG-3'), OPA16 (5'-AGCCAGCGAA-3') AND 4GACA (5'-GACAGACAGACA-3'). All primers were purchased from Cinnagene Company. Amplifications were conducted in a thermocycler eppendorf, consisting of an initial denaturation at 94°C for 3 minutes followed by 40 cycles of amplification (denaturation 93°C-1 minute, annealing 55°C- 1minute, extension 70°C- 2 minutes) and a final extension of 6 minutes at 72°C. The products were maintained at 4°C until loaded onto the gels [7-9].

Electrophoresis was conducted in 1.5% agarose gels in TAE buffer (40mM Tris--- pH7.6, 20 mM acetic acid and 1mM EDTA) run at 90 volts for 1.5 hours, followed by staining with gelred. Molecular size standards consisting of 100 base pair ladders were run in lanes flanking groups of about 8-10 samples.

RESULTS

Samples' Identification: The samples had short first and second antennas, Antenna 1 with robust peduncle segments, 10-segmented flagellum and 4-segmented accessory flagellum. Flagella segments with long setae in ventral section. Peduncle segments 3 to 5 and flagellum with numerous setae along the ventral side. The length of the samples were approximately 12 millimeters, their color were light grey with white patches. Mandibles in females are small and fragile with same length, but in males, are disparate and the second mandibles are longer [5].

The smallest sample was collected along Neka zone with 5 millimeters length and the largest one were collected along Babolsar zone with 14 millimeters length.

Results of RAPD-PCR: Primer 4GACA, shown 7 specific bands which were monomorphic [Figure 1]. Primer OPA 10, shown 3 specific bands which were monomorphic too [Figure 2]. Primer OPA2, shown 4 specific bands which were monomorphic either [Figure 3]; but primer OPA16, shown 7 specific bands which 6 of it were monomorphic [Figure 4]. In other word, form 21 produced bands, 20 bands (95.2%) were monomorphic, so we can say that there isn't any visible difference or polymorphism among pontogamarus. The results have been shown in the Table1.

Each RAPD test has been repeated twice to examine and prove the repeatability of the bands. This repeatability happens cause of high quality extracted DNA.

Table 1: Results of RAPD-PCR

Primer's name	Number of specific bands	Number of monomorphic bands	Number of polymorphic bands
4GACA	7	7	0
OPA2	4	4	0
OPA10	3	3	0
OPA16	7	6	1
Total	21	20	1

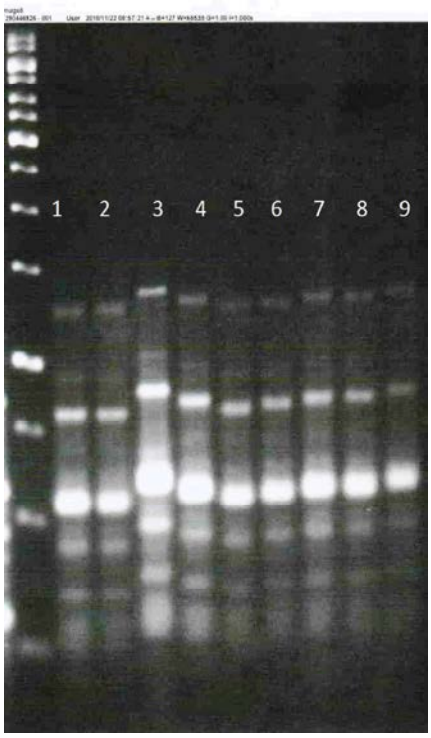

Fig. 1: 4GACA RAPD-PCR profile
First and second lane are Noor's samples, third and fourth are Babolsar's samples, fifth to seventh are Sari's samples and eighth and ninth are Neka's samples.

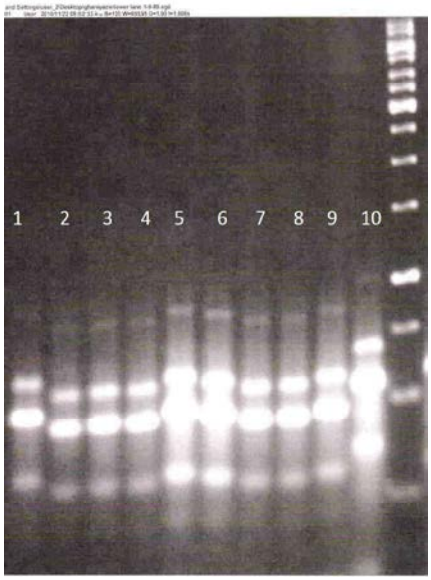

Fig. 2: OPA2 RAPD-PCR profile
First and second lane are Noor's samples, third and fourth are Babolsar's samples, fifth to seventh are Sari's samples and eighth to tenth are Neka's samples.

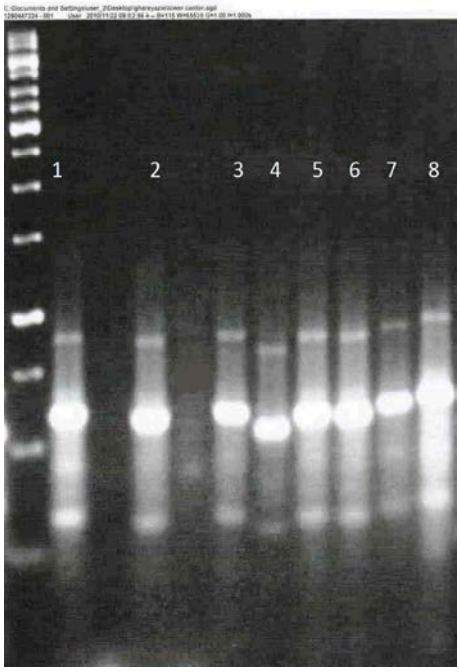

Fig. 3: OPA 10 RAPD-PCR profile
First and second lane are Noor's samples, third and fourth are Babolsar's samples, fifth and sixth are Sari's samples and seventh and eighth Neka's samples.

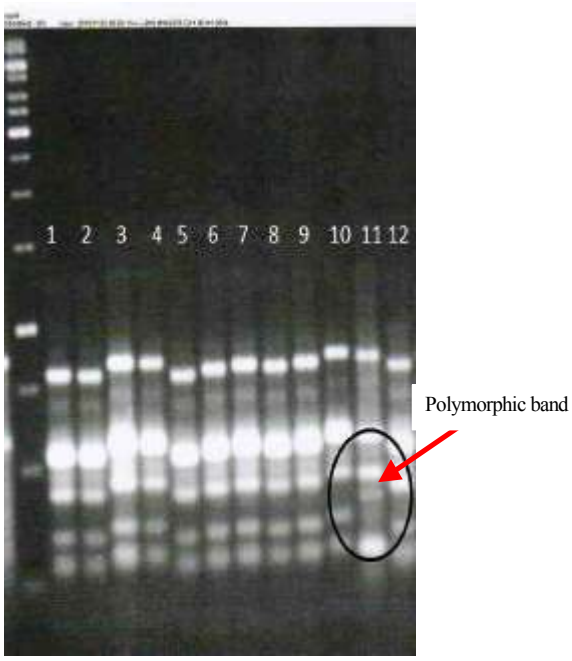

Fig. 4: OPA16 RAPD-PCR profile
First to third lane are Noor's samples, fourth to sixth are Babolsar's samples, seventh to ninth are Sari's samples and tenth to twelfth are Neka's samples.

DISCUSSION

Till today, identification of the gamaridea has been done through morphological methods, but due to lots of similarities, the probability of presence of a new sub-species or even a new species was high. Also to track any mutation, molecular methods are much more precise than morphological ones [4].

As we know the survival secret's of the creatures is summarize in biodiversity and these differences are not visible always phenotipically. We have many creatures that have same phenotype but they have lots of difference at their genome level. These differences will be visible through molecular markers methods.

These results [molecular identification] are consistent with those of morphological identification. Reasons for mutation are variable sometimes caused of bio-stresses such as environmental pollution and sometimes caused of wrong polymerase enzyme's action during replication.

This was the first time that the southern Caspian sea's amphipods [p. maeoticus] has been genetically surveyed; therefore to monitor further alteration, variation, mutation and modulation, a continus, precise and great project with the Guilin province coast results, is needed.

Right now, through this study an applicable and secure DNA extraction method and RAPD profile have been developed. It's suggested, to study the impact of environmental pollution at genomic level via microsatellite, SNP and AFLP markers.

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