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GENTIC DIVERSITY OF WHITE SHRIMP *METAPENAEUSAFFINIS* USING RAPD-PCR FROM THE BAHERALNAJAF DEPRESSION

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Abstract: *The aim of this study is to adopt a molecular diagnostic method using RAPD-PCR technology to differentiate between* M. *affinis shrimp samples collected from the Bahr al-Najaf Depression.* Metapenaeusaffinis a species of shrimp from the Metapenaeus genus, is found in the central and southern regions of Iraq. *was recorded for the first time in the Bahr al-Najaf depression, as its external appearance was a light gray color, and the vertical area was covered with a shield, which extends forward to form the snout, which reached an average length of 10 mm, and the snout contains teeth ranging in number from 8 -12 According to the size of the crustacean shrimp .Six M. affinis samples were tested using two random oligonucleotide primers in PCR under standard conditions. High repeatability and consistency with the RAPD profile were achieved by both primers. Two different random examined primers (AM1 and G32) reflected intraspecific polymorphisms within shrimp samples , AM1 primer yielded three patterens among the isolates ,the number of framgments varied from two to five bands ranging between approximately 240 to 585 bp in length, samples 2,3,4 and 5 have the same profiles. While sample 1 and 6 have different profiles whileG32 primer yielded six patterens among the isolates also the number of framgments varied from two to five bands ranging between approximately 206 to 808 bp in length, each sample has different profiles .*

Keywords: *Metapenauesaffinis, RAPD-PCR, genetic diversity, Baheralnajaf depression.*

INTRODUCTION

To the west and south-west of Holy Najaf City lies a marsh depression region known as Bahr Al-Najaf. It stretches from north to south-east over an area of 360 to 750 km2, with coordinates of longitude 43° 40′ to 44° 25′ and latitude 31° 40′ to 32° 10′ and an elevation of approximately 11 m asl. (Al-Atia, 2006, Benni and Al-Tawash, 2011) It consists of a lake- or marsh-like area with a few cultivated orchards beyond, and is bordered by a sizable amount of semi-desert or desert land. The region is identified as a component of the ecoregion of the Arabian Desert and East Sahero-Arabic Xeric Shrub Lands (PA1303). (Bachmann *et al*., 2011). It was once a portion of a very extensive water surface connected to the Arabian Gulf by water canals that functioned as a route for travel between the ancient Levant and Europe via Syria. (Al-Hakeem, 2004). Biodiversity is a feature of a region and refers in particular to the diversity within and among organisms, groups of organisms, biotic communities, and biological processes, whether naturally occurring or supervised by humans. Biodiversity can be measured in terms of genetic diversity and species identity. Different types and numbers, groups of species, biotic communities, biological processes, quantity (e.g. abundance, biomass, cover, rate and structure of each)(DeLong,1996).Aquatic organisms that can exist in both salty and fresh water environments are known as euryhaline organisms. Aquatic organisms can also live in fresh water environments. Many times, scientists classify aquatic species based on prospective classification because they believe that some of the phenotypic classifications are too restrictive. The current work used genetics to deal with this issue (Lima, *et al* .2010).

In several countries, the Metapenaeus genus was divided into numerous species. A number of countries depended on phenotypic classification, while others used genetic classification; yet others relied on both. One species of the *Metapenaeus* genus, *M. affinis,* has been recorded in Iraq; this species, Khour Abd Allah or Al-faw, exists in saline water. However, another unidentified species, Shatt al-Arab, or marsh, lives in fresh water and shares many morphological characteristics with Metapenaeusaffinis species (Abdul-Sahib and Sultan,2006).The diversity of organisms is determined by a variety of evolutionary processes, which can have an impact on a plant's or animal's morphology, ecology, and other biological activities. Most species clearly display

biological diversity, which results in individual variances in a wide range of traits(Ebiamadon,*et al*.2017). Environmental factors, genetic variation, or a both combination, indicate the distinctions in species that result from variety. There are several species of pawns in the genus Metapenaeus, including the marine water pawn Metapenaeusaffinis. (Kapoor , 2014). Although the marine pawn has been identified and given a name, freshwater bodies have also been habitat to another closely related critter. It is smaller than the marine pawn but shares many of the same appearance characteristics as its marine counterpart. (Thanh, 2015).

Along with the currently employed standard genetic analysis capillary sequencer, additional molecular biology techniques can be applied to compare the genetic variety (Chen , 2017).Gene dependent methods that concentrate on variations in DNA code at various loci are used to characterize various species molecularly. Possibly from the same organism, the genus Metapenaeus sp. evolved, but because of environmental limitations and resultant mutations, the unidentified species may have evolved smaller and capable to thrive in bodies of fresh-water. Gene-mutation and chromosom alterations, which are connected to biological processes like meiosis and fertilization, are frequent sources of genetic variety (Baldwin and Bass 2012). The variations in the amino acid or DNA nucleotide sequence of distinct proteins can be evaluated to comprehend the molecular biodiversity of the two animals. DNA sequencing is currently the most widely utilized technology, there are alternative less expensive and labor-intensive options as well. One tool that can be used to characterize the diversity of organisms is the Rapid Amplified Polymorphic DNA (RAPD) technique (Ibrahim, *et al*. 2010).

This technique amplified genomic DNA with randum primers to find potential polymorphism markers (Nehemiah and Marc , 2017).Numerous procedures were used to use RAPD to identify the genetic differences between the two shrimp species. In the beginning, DNA was taken from the two species in accordance with conventional practice. (Williams ,*et al*.1990). The procedure was designed to prevent DNA breakdown, which could interfere with the amplification and detection of polymorphism regions. (ltukhov , 2006). Without a specified target of loci, primers are designed at random. The creation of primers uses knowledge of the genetic makeup of the *Metapenaeus* species. (Chareontawee,*et al*.2007). Furthermore, the primers specified to target specific conserved sequences to define homology and demonstrate an area of evolutionary diversion (Song , *et al* . 2016).Once primers introduced, the next step was the amplification through polymerase chain reaction (PCR). The amplification process duplicates the genetic loci that bind the primers, further increasing the number of copies. Once the fragments were amplified, separation for the different samples was undertaken using gel electrophoresis, a process that exploits the charge and size of the fragments (Eliboland Behiye, 2017). A kilo base lambda DNA was used in the gel to help in the determination of the fragment sizes (Chareontawee,*et al*. 2007). The fragments were seen under UV light after they were separated on 1% agarose gel with ethidium bromide. When comparing the two species, the band with the highest movement has the smallest size. (Thanh, 2015; Thanh,*et al.*2015). When used RAPD-PCRthey identified polymorphic bands that can differentiate the *Metapenaeus* species and demonstrate the difference in ecosystems. Because it was quick, easy, and effective, the approach was suitable for this categorization. Only the agarose gel electrophoresis apparatus and the thermocycling machine were required for the procedure.(Rumisha, *et al.*2017). However, prior knowledge on the size of conserved sequences may be needed to compare and identify points of polymorphic diversion (Franco and Julian . 2012) .

MATERIALS AND METHODS

Collection of samples

A total of sixsamples of *M. affinis* were collected from Baheralnajaf depression which situated in Alnajaf Which is located in the middle of Iraq.

Genomic DNA isolation

Total genomic DNA was isolated from a piece of pleopod of each samples of shrimp using a phenol-chloroform-proteinase K method (Thaewnon-ngiw, *et al.*2004), DNAase Tissue Kit (Germany). DNA concentrations were spectrophotometrically determined at using a NanoDrop; 1000 Spectrophotometer at the absorbance of 260 (A260) and 280 nm (A280). The purity of extracted DNA was determined by using

A260/A280 ratio. 1% Agarose Gel Electrophoresis was performed to detect the genomic DNA using Gel documentation. The DNA was diluted using TE buffer to a final concentration of µg/ml for RAPD analysis. Two random primers were used for RAPD-PCR analysis to amplified gene (AM1 and G32) .Polymerase Chain Reaction of primers were achieved in 25 µl volumewhich were comprising 1x of PCR-buffer (100 mMTris-HCl (pH 8.3), 1.5 mMof MgCl2 and 50 mM of KCl, 0.2 µM of primer,100 µm from dATP, dCTP, dGTP and dTTP 0.75 Uint of *Taq* DNA polymerase 20 ng of template- DNA (Williams ,*et al* .1990).
The condition of PCR were, 94ºC for 5 min for initial denaturation , followed by 94ºC for 1 min with 45 cycles, 37ºC for 1 min and 72ºC for 1.30 min, one cycle at 72ºC for 7 min, and finally 4ºC. The amplification samples were stored at-20ºC for further test. PCR products were visualized on agarose gel 1%, and identified by its size in base pairs and its associated primer. A pair of primers at the conserved regions of *16S*rDNA was designed and tested against 6 shrimp individuals sample (AM1= GAGAGAGAGAGACC and G32=GTGTGTGTGTGTCC)

RESULTS AND DISCUSSION

Metapenaeusaffinis (Figure 1) was recorded for the first time in the Bahr al-Najaf depression, as its external appearance was a light gray color, and the vertical area was covered with a shield, which extends forward to form the snout, which reached an average length of 10 mm, and the snout contains teeth ranging in number from 8 -12 According to the size of the crustacean shrimp , at the base of the snout, the eyes are located on a short stalk and are clearly prominent. The thorax also bears a number of appendages, represented by the maxillary, canine, and walking feet. The average length of the cephalothoracic region was (22 mm), while the ventral region was characterized by several body rings. At the back of the body, the animal has a caudal fan, which was separated into two parts with an average length of up to 5 mm.
The largest size of Metapenaeusaffinis obtained during this study was (80-90 mm), and it also appeared in a light gray color. The reason may be due to the effect of the environment on the external appearance of marine shrimp. This is consistent with the study of (Salman,*et al* ,1997).

Figure (1): Samples of *Metapenaeus affinis*shrimp exist in Bahr Al-Najaf is a wetland depression (salty water)

Six samples of M.affinis shrimp were subjected to PCR under standard conditions using two random primers. Both primers generated good repeatability results that were compatible with the RAPD profile and produced monomorphic and polymorphic fragments.Two different random examined primers (AM1 and G32) reflected intraspecific polymorphisms within *Metapenaeus* affinis shrimp samples , AM1 primer yielded three patterens among the isolates ,the number of framgments varied from two to five bands ranging between approximately 240 to 585 bp in length, samples 2,3,4 and 5 have the same profiles. While sample 1 and 6 have different profiles (Figure 2) . In the other hand G32 primer yielded six patterens among the isolates also the number of framgments varied from two to five bands ranging between approximately 206 to 808 bp in length, each sample has different profiles (Figure 3).Species diagnostic primers from DNA segments should exhibit minimal genetic polymorphic within a specific-species but considerable genetic divergence between different samples. The number and size of the amplified products varied depending on the genetic characterisation of the samples(Thaewnon-ngiw , *et al.* 2004).

The challenge of separating samples from species that are morphologically similar was made easier by RAPD-PCR. It was also very helpful in figuring out where the various species samples were distributed.The RAPD method of DNA fingerprinting has become quite popular for many stydies and has been applied successfully .RAPD technology is simples rapid and the absence of specific nucleotied sequence information for many shrimp species able to generate intraspecific DNA polymorphisms on the basis of characteristic band patterns.

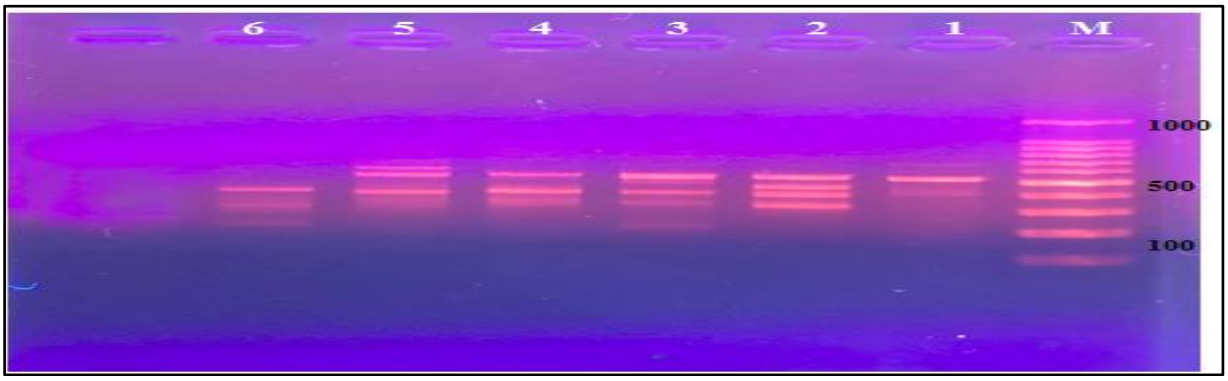

Figure (2): Agarose gel electrophoresis of PCR product in *16S* rDNAusing (AM1 primer) of different *Metapenaeusaffinis* samples. Lane (M): Size marker (100bp); Lane 1-6 *Metapenaeusaffinis* samples (1 % Agarose gel, 80 volts for 2 hours).

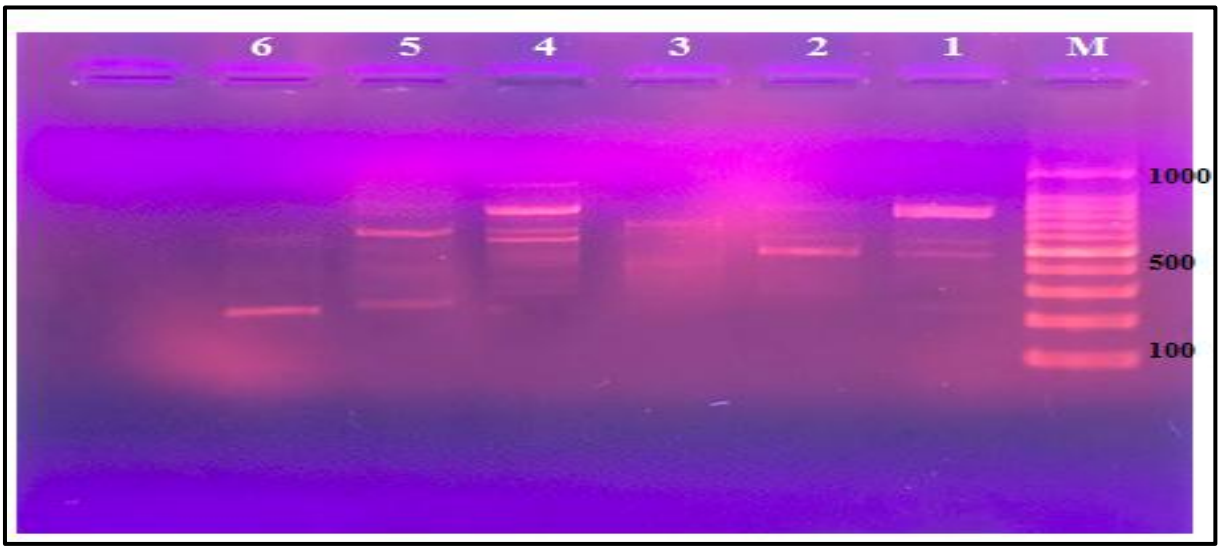

Figure (3): Agarose gel electrophoresis of PCR product in *16S* rDNA using (G32 primer) of different *Metapenaeusaffinis* samples. Lane (M): Size marker (100bp); Lane 1-6 *Metapenaeusaffinis* samples (1 % Agarose gel, 80 volts for 2 hours).

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