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Application of RAPD-PCR in Taxonomy of Certain Freshwater Bivalves of Genus *Caelatura*

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Abstract: Adult specimens of the two common freshwater bivalves, *Caelatura* (*Caelatura*) *companyoi* and *Caelatura* (*Caelatura*) *prasidens* belonging to family Unionidae were collected from the River Nile at Giza governorate in Egypt. These two species were described by some authors as one species and others separated them as two different species. In the present study, random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) markers were used to determine the genetic distance (D) between these two unionid species. 5 out of 13 random primers generated good DNA patterns that ranged from 1 to 3 bands (200-1000 bp). By application of the genetic distance equation, the result was above 0.5. Based on this result, the high value of D (0.64) proved that the present two specimens must be actually separated as 2 different species.

Key words: RAPD-PCR · Genetic variability · Freshwater mussels · *Caelatura* spp · River nile

INTRODUCTION

Freshwater bivalves occurring in Egypt represent a neglected animal group and little is known about them or their diversity; perhaps due to the fact that they have no apparent economic or medical importance, although they are eaten in Asia as a supplemental protein source. According to Ibrahim *et al.* [1], most bivalves, especially unionids, possess a great ecological adaptability, which often finds expression in the shape of the shell. Such ecotypes are well known in European and African unionds, that most of them have so variable shell morphology leading to confused nomenclature and classification. This variation, coupled with the relatively few constant characters, has rendered the Bivalvia a systematically difficult group for almost a life time. Therefore, a taxonomical revision for some of the species is necessary, using more advanced technique like RAPD-PCR. Most of the previous taxonomical studies on bivalves were based on the morphology only (mainly on the shape of shell valves, teeth and colour of the nacre). The main aspects of the general taxonomy of the Unionida were recently reviewed by Bogan [2]. This author summarized in table the freshwater bivalve fauna of Africa and Egypt, based on the five most recent publications [1, 3, 4, 5, 6]. Different authors have treated this variation as just a variation within a species, while Ibrahim *et al.* [1] have treated many of the named forms as separated species. Mandahl-Barth [5] reduced all the species of *Caelatura* into only one species, namely *Caelatura aegyptiaca*. Recently a preliminary review of the freshwater Unionida of Northern Africa with an emphasis on the Nile was carried out by Graf and Cummings [7].

Nothing is yet known about the basic biology and reproduction of these unionid species found in Egypt beyond the information provided on the anatomy of one species by Soliman [8]. Moreover, nothing is known about the genetic variation of any freshwater bivalve species, but several studies were concerned with the genetic information of some marine bivalve species [9-14].

As far as we know, no previous RAPD-PCR analysis has been so for carried out on the freshwater bivalves or unionids found in Egypt. Therefore, the present work was designed to study the genetic variation between two morphologically almost similar freshwater unionid bivalves common in the Egyptian Nile at Giza; namely: *Caelatura(Caelatura) companyoi* and *Caelatura* (*Caelatura*) *prasidens*, which were described by Mandahl-Barth [5] as one species and by Ibrahim *et al.* [1] as 2 different species.

MATERIALS AND METHODS

Biological Material: Adult mussels of the two unionid forms were collected from rocky muddy bottom of

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Mansouria Canal, Giza Governorate. Specimens were placed in a bucket of Nile water to be transported to the laboratory at Zoology Department, Faculty of Science, Ain Shams University for the present study. Samples were dissected and their soft parts were carefully isolated and preserved in 100% ethyl alcohol at -20°C until use.

DNA Extraction: The preserved mantle samples were cut into small pieces of about 0.5 cm in length and kept in ddH2O in the refrigerator (5°C) overnight for rehydration. These samples were centrifuged for 1 min. at 1400 rpm. Qiagen Dneasy tissue kits were used to isolate and purify the DNA (these kits contain proprietary buffers used to purify the DNA). 180 µl of lysis buffer (ATL) and 20 µl of proteinase K were added to each 1.5ml tube and vortexed for 1min. Another 20 µl of proteinase K were added and the samples were centrifuged for 20 seconds. Then they were incubated at 55°C for about 3 hours. Following incubation, 200 µl of (AL) lysis buffer were added and the samples were vortexed for 10 seconds.200 µl of ethyl alcohol, were then added and vortexed for 10 seconds. Samples were spun for 1 min, then the DNA was washed with 500 µl (AW1) buffer and 500 µl (AW2) washing buffer. Finally 200 µl of (AE) elution buffer were added.

RAPD-PCR: 13 primers were used in the present work but only 5 of them worked as follows:

476: 5`-TTG AGG CCC T-3`
477: 5`-TGT TGT GCC C-3`
478: 5`-CGA GCT GGT C-3`
479: 5`-CTC ATA CGC G-3`
487: 5`-GTG GCT AGG T-3`

Amplifications were performed by modifying the protocol reported by Williams *et al.* [15]. The 25 µl mixture contained 25 ng of template DNA, 1.5 unit of Taq. Polymerase, 10 mM dNTPs, 10 pM primer, and 2.5 µl of 10x PCR buffer. Amplifications were performed in T-personal thermal cycler (Biometra), programmed for 45 cycles of 94°C for 1 min., 35°C for 1 min., and 72°C for 1 min. An initial denaturation step (3 min, 94°C) and a final extension holding (10 min, 72°C) were included in the first and last cycles, respectively. Reaction products (10 µl) were resolved by 2% agarose gel electrophoresis at 85 volt in 1x TAE buffer. The gel was stained with ethedium bromide and photographed by Polaroid camera under UV transilluminator. From the comparison of the amplified products, population-specific fragments were detected. Two replicas per individual and primers were carried out. Only primers producing similar patterns in the two replicas were considered.

RESULTS AND DISCUSSION

RAPD analysis was performed using DNA extracted from two forms of *Caelatura* bivalves, the first was *Caelatura* (*Caelatura*) *companyoi* (Fig. 1 A and B) and the second was *Caelatura* (*Caelatura*) *prasidens* (Fig. 2 C and D).

Individual amplifications of both forms with the five primers: UBC 476, UBC 477, UBC 478, UBC 479 and UBC 487 which are commonly used in the bivalve RAPD PCR as shown in Fig. 3.

One of the simplest estimations of genetic distance is based on the proportion of shared alleles [16]. For individual pairwise comparisons, the proportion of shared alleles was estimated by:

$PSA_1 = \Sigma uS/2u$, where the number of shared alleles S summed the overall loci u. Distance between individuals (*DSA*1) is estimated by,

*DSA*1 = 1-PSA1

UBC 476	
Form 1	Band at ~ 550 bp
	Band at ~ 1000 bp
Form 2	Band at ~ 650 bp
UBC 477	
Form 1	Band at ~ 250 bp
	Band at ~ 400 bp
Form 2	Band at ~ 420 bp
	Band at ~ 550 bp
UBC 478	
Form 1	Band at ~ 260 bp
	Band at ~ 320 bp
	Band at ~ 400 bp
Form 2	Bands at ~ 350 bp
	Band at ~ 420 bp
UBC 479	
Form 1	Band at ~ 320 bp
	Band at ~ 450 bp
Form 2	Band at ~ 360 bp
	Band at ~ 520 bp
UBC 487	
Form 1	Band at ~ 360 bp
	Band at ~ 520 bp
Form 2	Band at ~ 270 bp
	Band at ~ 360 bp
	Band at ~ 450 bp

Fig. 1: Shell valves of *Caelatura* (*Caelatura*) *companyoi* A&B interior and exterior of the shell respectively

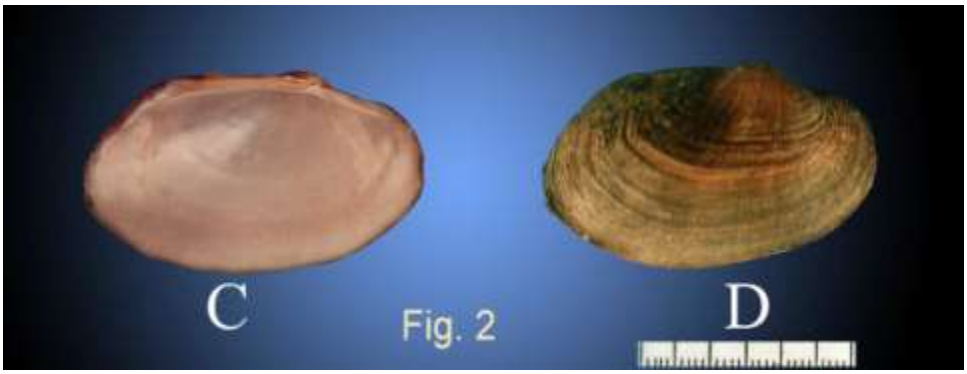

Fig. 2: Shell valves of *Caelatura* (*Caelatura*) *prasidens* C&D interior and exterior of the shell respectively

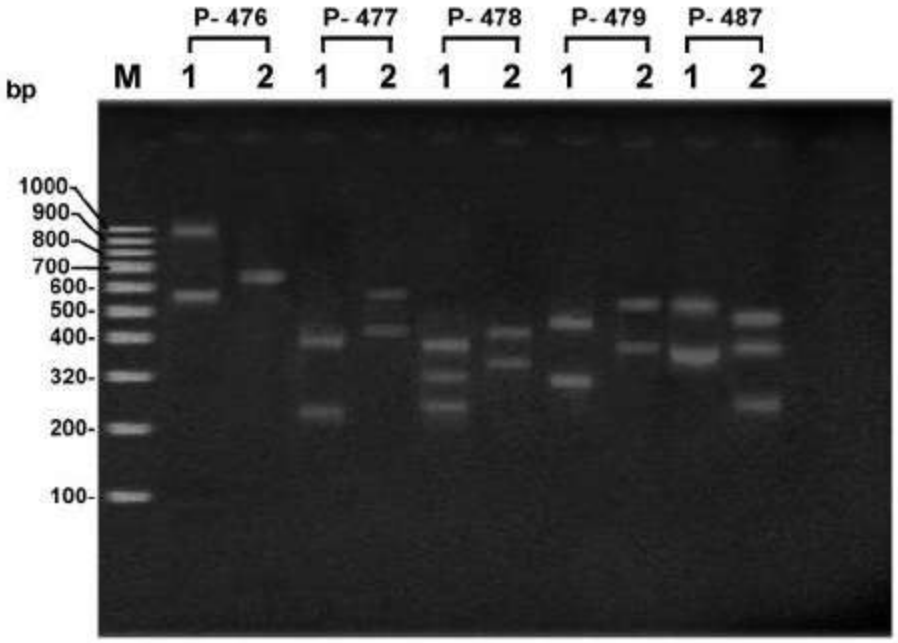

Fig. 3: RAPD-PCR profiles produced from the two species *Caelatura* (*Caelatura*) *companyoi* and *Caelatura* (*Caelatura*) *prasidens* using primers P-476,P-477,P-478,P-479 and P-487. M:DNA marker

$$PSA_1 = \Sigma uS/2u$$
$$= 15/2*21 = 0.357$$
$$DSA1 = 1-0.357 = 0.64$$

The RAPD-PCR analysis used in the present study has proven to be helpful in taxonomic studies of *Caelatura* spp. to evaluate the classical identification and to estimate genetic variations among their species. The molecular data have been confirmed as an effective tool for studying species with variable phenotypic plasticity [17,18].

Morphologically, *Caelatura* (*Caelatura*) *companyoi* and *Caelatura (Caelatura) prasidens* are two different species [1]. In the present study, by application of the genetic distance equation (D=0.64), it is confirmed that the 2 forms under investigation are two distinct species and this supports the opinion of Ibrahim *et al*. [1]. It was clear that no common bands could be detected in each primer. This means that the pattern detected with each primer could be specific for each species. The present results showed that RAPD-PCR is a simple and rapid technique, representing an important progress in studies on Egyptian unionids, which can be used to support classical morphological identification.

Also, RAPDs is a highly useful technique for phylogenetic analysis among closely related individuals. This agrees with Futoshi and Takane [9], Klinbunja *et al.* [11], Marin *et al.* [13], Ibrahim *et al*.[14], Kenchington *et al.* [19], Andre *et al.* [20] and Gutierrez *et al.* [21].

Many authors used RAPD-PCR to distinguish between different populations of gastropods [22-29]. They claimed that this advanced technique is highly sensitive and valuable tool for identification of species.

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