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Morphological and Genetic Analyses of *Melanoides tuberculata* Populations in Egypt

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Abstract: *Melanoides tuberculata* snail (Thiaridae) gained recently extra medical and or veterinary importance since several new types of cercariae were procured from it in Egypt. Therefore the morphological and genetic analyses of its populations in this country were studied. Thousands of snails were collected from various localities and the chonchological data of them were gathered. Five morphs were distinguished depending on colour pattern, sculpture, form of sutures and tubercles. The most different two morphs were subjected to genetic analysis by differentiating DNA of snails using RAPD -PCR technique. The individual implication of the two morphs with eight primers ($UBC_{475,}$ $UBC_{476,}$ UBC_{477}, UBC_{478}, UBC_{479}, UBC_{483}, UBC_{486} and UBC_{487}) showed differences between the bands in number and location. However, calculating the genetic distance based on the proportion of shared alleles proved that there was no genetic difference between the morphs. This result confirms that morphologically different *Melanoides* snails in Egyptian habitats belong to one and the same species *Melanoides tuberculata*.

Key words: *Melanoides tuberculata* · Morphs · Genetic analysis

INTRODUCTION

The snail *Melanoides (Melanoides) tuberculata* Müller (1774), family Thiaridae, gained recently extra medical and or veterinary importance since it has been found by the present authors to act as intermediate host of several trematodes in Egypt. Thirteen types of cercariae belonging to xiphidio, furcocercous, gymnocephalous and pleurolophocercous groups of cercariae were procured from this snail [1] *Melanoides* snails is known to be polymorphic and variation in shell ornamentation allowed the definition of discrete entities referred to as morphs which were also distinguished from different localities in the world [2-4]. Meanwhile, random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) methodology represents an adequate approach for analysis of genetic polymorphism of several organisms. Based on this technology studies were previously carried out on the genetic variation among different populations of vector snails [5-7]. In the case of *M.tuberculata,* as far as can be ascertained, no previous PCR work has been carried out on its morphs in Egypt. Therefore, the objective of the present work was to carry out morphological and genetic analyses of *Melanoides* populations in Egyptian habitats to elucidate the potential of correlation between them.

MATERIAL AND METHODS

Morphological Studies of *Melanoides* Shells: Thousands of *Melanoides* snails were collected from several localities in Egypt. (Giza, Qalyoubyia, Behaira, Ismailiya and Sinai Governorates). Shells were treated with Clorox [8] so that details of sculpture and colour were clearly seen. For each shell, the number of whorls (Whrl), the length (L) and width (W) and those of the aperture were measured as by [2]. The ratio between width and length of shell (W/L), number of whorls and length (Whrl / L) and ratio between aperture length and shell length (A/L) were calculated and analysed.

Genetic Variability in *Melanoides* Morphs: Two morphs showing most morphological differences were chosen to study the genetic variability between them with the objective to elucidate whether *Melanoides* snail morphs in Egypt represent indeed separate species or all morphs

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belong to one species showing intraspecific variation. Thus the chosen morphs were utilized to differentiate between their DNA using RAPD.Therefore, snail samples of these morphs were carefully dissected and their soft parts carefully isolated and stored at -20°C until used for genetic analysis. Genomic DNA was extracted from snail tissues by CTAB precipitation method as described by [9] with some modifications [10]. Eight primers (UBC_{475}, UBC_{476}, UBC_{477}, UBC_{478}, UBC_{479}, UBC_{483}, UBC_{486} and UBC_{487}) were used in the RAPD-PCR reaction as follows:

475: 5` - CCA GCA TAT T-3`
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`
483: 5` - GCA CTA AGA C-3`
486 5` - CCA GCA TCA G-3`
487 5` - GTG GCT AGG T-3`

Amplifications were performed by the protocol reported by [11] with some modifications.

RESULTS

Melanoides has a dextral turreted operculated shell, somewhat thick, hard, with solid columella and closed umbilicus. The spire is long and the apex is more of less pointed. The shell aperture is almost oval in outline, broader below than above. When seen from the side, the lateral lip appears slightly convex and more or less parallel to the vertical axis of the shell. The inner lip is almost inconspicuous and closely applied to the collumella. The sculpture of the shell consists mainly of parallel rows of tubercles of various sizes which appear more of less continuous with each other thus forming transverse lines. It includes also successive fine transverse lines of growth. The shell varies in colour being either whitish, grayish to dark brownish. Spiral rows of brown elongated flames of different sizes lie mainly on the spiral lines described above. *M. tuberculata* is an operculated snail and the operculum in adult snails is thin flexible brownish corneous and oval. It lies transversally on the posterodorsal side of the foot. The left or columellar edge of the operculum presents growth

Table 1: Main morphological differences in various shell morphs of Melanoides tuberculata in Egypt

Feature Morphs	SINAI (Fig. a)	MYF (Fig. b)	ASHO (Fig. c)	RAWASH(Fig. d)	H0S (Fig. e)
Sites of collection	Tameer drain, Um Apttal village, North Sinai and Saqia Abu Sha'ara drain, Ashmoon, (Menoufiya Gov)	Matta'eid and Abu Adam drains,Manyef, (Ismailiya Gov.)	Saqia Abu Sha'ara drain, Ashmoon, (Menoufiya Gov.)	Mansouriya drain, Abu Rawash, (Giza Gov.)	Hadad drain, Hosh Issa, (Behaira Gov.)
Body whorl	Slightly rounded	Slightly rounded	Well rounded	Slightly rounded	Slightly rounded
Sutures	Shallow	Shallow	Deep	Deep	Deep
Tubercles	Form axial ribs in the spire connected between sutures.	Spiral rows of tubercles marked in the suture	Axial ribs extend along the spiral whorls only.	Shell smooth without tubercles	Shell smooth without tubercles
Colour	Pale to dark brown with reddish brown flames between axial ribs	Pale with dark brown flames and spots	Pale to light brown with reddish brown flames	Pale brown to dark with flames connected forming regular undulating lines in body whorl	Brown with numerous reddish brown flames
Conchological W/L	0.32±0.040*	0.37±0.030*	0.37±0.040*	0.40±0.05*	0.32±0.02*
measurements Whrl/L	0.40±0.07	0.4±0.01	0.43±0.06	0.47±0.04*	0.35±0.05*
A/L	0.30±0.03	0.31±0.02	0.33±0.04	0.36±0.04*	0.30±0.02*

*Number of shells measured (n) 10-18, significantly different from other morphs

Table 2: Genetic analysis between two morphs of Melanoides tuberculata using PARD- PCR technique

UBC 475	
Morph 1 (e)	Band 1 at ~ 180 bp
	Band 2 at ~ 220 bp
	Band 3 at ~ 350 bp
Morph 11(b)	Band 1 at ~ 300 bp
	Band 2 at ~ 220 bp
UBC 476	

Table 2: Continued

Morph 1	Band 1 at ~ 180
	Band 2 at ~ 300 bp
Morph 11	Band 1 at ~ 180 bp
	Band 2 at ~ 300 bp
UBC 477	
Morph 1	Band 1 at ~ 180 bp
	Band 2 at ~ 280 bp
Morph 11	Band 1 at ~ 180 bp
	Band 2 at ~ 280 bp
UBC 478	
Morph 1 (5)	Band 1 at ~ 180 bp
	Band 2 at ~ 280 bp
	Band 3 at ~ 320 bp
Morph 11 (2)	Band 1 at ~ 180 bp
	Band 2 at ~ 280 bp
	Band 3 at ~ 320 bp
	Band 4at ~ 390 bp
UBC 479	
Morph 1	Band 1 at ~ 120 bp
	Band 2 at ~ 150 bp
Morph 11	Band 1 at ~ 150 bp
	Band 2 at ~ 160 bp
	Band 3 at ~ 170 bp
	Band 4 at ~ 180 bp
	Band 5 at ~ 220 b
UBC 483	
Morph 1	Band 1 at ~ 180 bp
Morph 11	Band 1 at ~ 180 bp
	Band 2 at ~ 220 bp
	Band 3 at ~ 300 bp
	Band 4 at ~ 390 bp
UBC 486	
Morph 1	Band 1 at ~ 100 bp
	Band 2 at ~ 180 bp
Morph 11	Band 1 at ~ 100 bp
	Band 2 at ~ 180 bp
UBC 487	
Morph 1	Band 1 at ~ 120 bp
	Band 2 at ~ 150 bp
	Band 3 at ~ 200 bp
Morph 11	Band 1 at ~ 200 bp

lines which extend out of the excentric nucleus in spiral manner. The nucleus lies on the left columellar side of the operculum.

The shells showed wide variation in shape, thus five morphs were distinguished in the snails examined in this study. These morphs were given symbolic names depending on sites of their collection as well as their specific characteristics (Table1 and Fig 1).

Genetic Analysis Between HOS and MYF Morphs of *Melanoides* Using RAPD- PCR Technique: Individual amplification of the two morphs with the 8 primers used showed several differences between the bands in number and location (Table 2, Figs.2 and3).For individual pair wise comparisons the proportion of shared alleles is estimated by calculating the genetic distance based on the proportion of shared alleles, as by [12] confirmed that

Fig. 1: Photos of *Melanoides tuberculata* snails from Egypt
a. SINAI morph b. MYF morph
c. ASHO morph d. RAWASH morph e.HOS morph

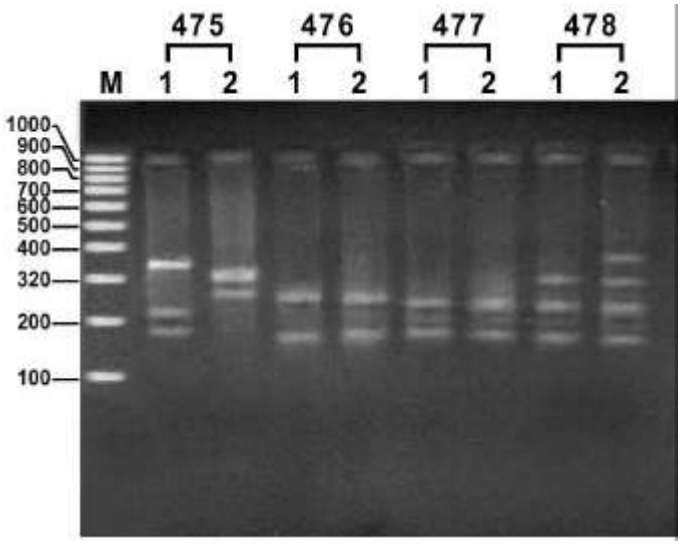

Fig. 2: RAPD-PCR profiles from two morphs of *Melanoides tuberculata* snails (1 and2) using primers UBC $_{475,477,478}$. M: DNA maker

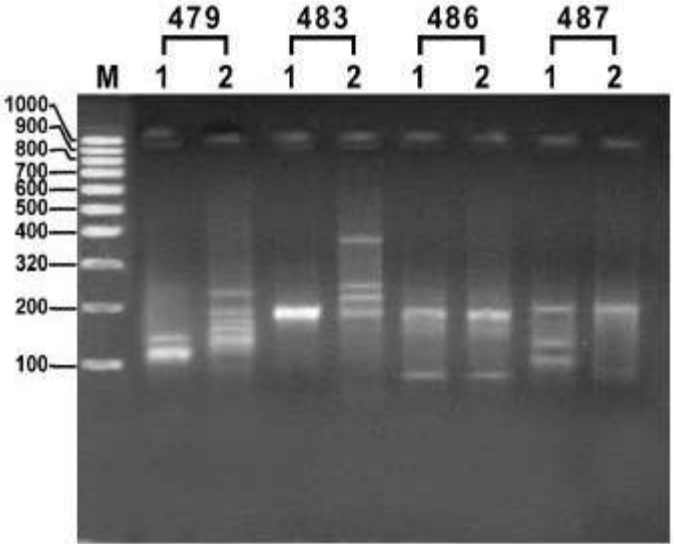

Fig. 3: RAPD-PCR profiles from two morphs of *Melanoides tuberculata* snails (1 and2) using primers UBC $_{479,483,487}$. M: DNA maker

there is no genetic difference between the morphs. Therefore, in the 2 morphs under investigation no genetic difference could be indicated and consequently they belong to one and same species *tuberculata*

$$PSAI = \Sigma \underline{S} \; 2U$$

Where the number of shared alleles *S* is summed over all loci U. Distance between individuals (DsA1) is estimated by

$$DSA1 = 1 - PSAI$$
$$PSA1 = 37/2x40 = 0.4625$$

DISCUSSION

The present collection of *Melanoides* snails from several localities in the Nile Delta and Sinai in Egypt showed wide range of variation in shape of adult snail shells. Thus, five morphs were distinguished depending on certain characteristics namely shell colour, shape of body whorl and certain conchological measurements, etc. Studies of the morphology of this snail species from Egypt by previous authors just reported on the diversity of the shell [2]. Later on, the life history was studied of 3 morphs of *M. tuberculata* from Guadeloupe and Martinique [3].Other morphs of the same snail species were also described by other authors [4].

RAPD primers are were reported to be useful for distinguishing between morphs of the same species [7, 13-15] and therefore this technique was used in this study for genetic analysis of two of the present *Melanoides* morphs. The obtained results prove that there is no genetic difference between the morphs investigated. Consequently this provides a genetic confirmation that variation in shell morphology, sculpture and coloration of *M. tuberculata* may be mainly due to environmental effects during shell secretion and maturation. This meant that *Melanoides* snails collected from several sites in Egypt belong to only one species showing intraspecific variation. Moreover, observations from this study provide more evidence that RAPD can be highly useful for the phylogenetic analysis among closely related individuals. This agrees with [16] who studied two populations of *Pseudosuccinea collumella*, the intermediate host of *Fasciola hepatica*. In Bivalvia the problem of polymorphism among the shells of the oyster has been also solved by molecular techniques [17-20]. Despite that shell characters have been used for species identification, the present results give more confirmation that molecular methods, enzyme electrophoresis and DNA amplification provide considerable help in the taxonomy of snail vectors as previously claimed by [21].

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