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Full Length Research Paper

Comparative study of the karyotypes and electrophoretic patterns of *Biomphalaria alexandrina* and *Bulinus truncatus* and the ova of their corresponding trematode hosts

Fayez A. Bakry[1]* and Manal F. El Garhy[2]

[1]Medical Malacology Department, Theodor Bilharz Research Institute, Giza, Egypt.
[2]Zoology Department, Faculty of Science, Cairo University, Egypt.

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The karyotypes of *Biomphalaria alexandrina* and *Bulinus truncatus* were analyzed comparatively and classified on the basis of centromere position and the electrophoretic analysis of tissue protein has been used to determine the relationships between the ova of *Schistosoma mansoni* and *Schistosoma haematobium* and their respective snail hosts. The two species have the same diploid chromosome number, 2n = 36. The mitotic chromosomes of *B. alexandrina* are organized in three groups and consist of 8 metacentric pairs, 8 submetacentric pairs and 2 subtelocentric pairs of chromosomes. While, the karyotype of *B. truncatus* is organized in four groups and consists of 10 metacentric pairs, 4 submetacentric pairs, 2 telocentric pairs, and 2 subtelocentric pairs of chromosomes. Also, SDS-PAGE revealed 7 similar protein bands in the ova of *S. mansoni* and its snail host *B. alexandrina* and 5 similar protein bands in *S. haematobium* and its snail host *B. truncatus*. Additionally, the highest similarity coefficient was found between *B. alexandrina* and *B. truncatus* and their respective trematode hosts. It is hoped in the near future to identify targets in the snail host that interfere with parasite survival and develop alternate and/or novel methods to disrupt the transmission of schistosomiasis.

Key words: *Biomphalaria alexandrina, Bulinus truncatus, Schistosoma mansoni, Schistosoma haematobium*, chromosomes, electrophoretic analysis.

INTRODUCTION

The freshwater snails *Biomphalaria alexandrina* and *Bulinus truncatus* (class gastropoda, subclass pulmonata, order basommatophora and family planorbidae) are the intermediate snail hosts for *Schistosoma mansoni* and *Schistosoma haematobium*, respectively, which are responsible for the widespread transmission of schistosomiasis in humans. This chronic disease is believed to affect up to 10% of the world's population (Crompton, 1999) making it a significant international medical concern. Since these snails play a potential role in the transmission of schistosomiasis in developing countries and since the host/parasite association is known to be highly complex, studies have focused on the genetics of parasite/snail compatibility (Thompson, 1985). However, there are still some significant gaps in our knowledge concerning snail/parasite relationship, the susceptibility of snails to infection by their respective trematodes and their suitability for parasite development.

The aim of the present investigation is to study the karyotypes of two important snail hosts of schistosomiasis in Egypt; *B. alexandrina* and *B. truncatus* and also to reveal the relationship between the protein patterns of their soft tissue and the ova of their corresponding trematode hosts using SDS-polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

The snails *B. alexandrina* and *B. truncatus* were collected from the Nile and irrigation scheme in Giza, Egypt and brought to the laboratory where they were washed thoroughly and examined successively for natural trematode infection. Healthy snail's

*Corresponding author. E-mail: Fayezbakery@yahoo.com.

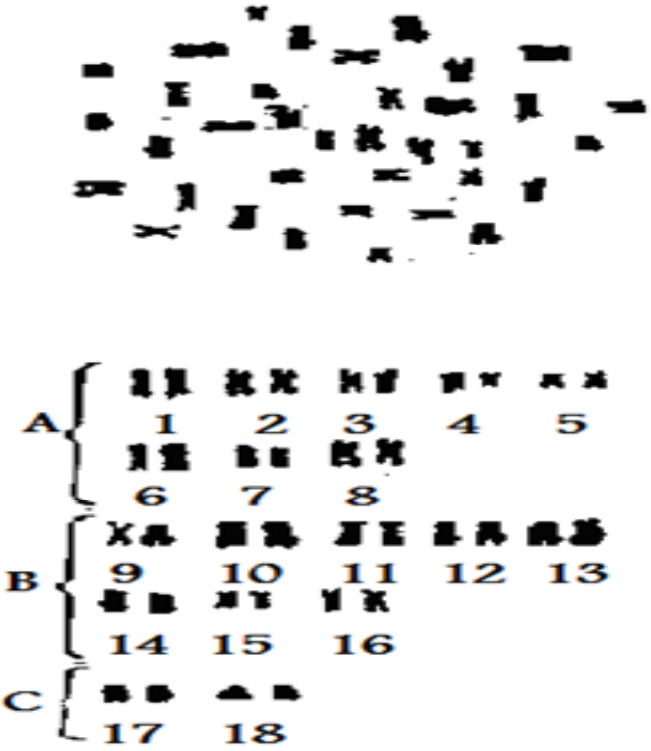

Figure 1. Photomicrographs showing the cell spread and karyotype of *B. alexandrina* snail (2n=36). A = metscentric, B = submetacentric, C = subtelocentric chromosomes.

populations were maintained in the laboratory under 24±1°C in plastic aquaria with aerated filtered dechlorinated tap water and were fed either dry or fresh lettuce leaves. Laboratory-reared snails (6 to 8 mm) produced egg masses containing embryos used in this study. The Ova of *S. mansoni* and *S. haematobium* were obtained from *Schistosoma* Biological Supply Center (SBSC), Theodor Bilharz Research Institute (TBRI). Chromosome preparations were made according to the technique of Goldman et al. (1984) (Colchicine-air drying-Giemsa technique) with slight modification by Vitturi et al. (1988). For karyotyping, chromosomes were cut out of the photographs and paired on the basis of size and centromere position. Within each population, measurements of the chromosomes were made using digitzer table (Odoemelam et al., 2009). Relative chromosome length was expressed µm as a percentage of the total length of the haploid complement. The centromeric index was calculated by dividing 100 times the length of the short arm by the total chromosome length. The centromere position was also expressed in terms of the arm ratio (length of long arm divided by the length of short arm). The terminology of chromosome type and centromere position followed that of Levan et al. (1964).

For electrophoretic analysis, the soft parts of snails *B. alexandrina* and *B. truncatus* and the ova of *S. mansoni* and *S. haematobium* were weighed and homogenized individually in double volume of 10 mM potassium phosphate buffer (pH 6.8). Then, they were centrifuged for 20 min at 25000 g/min (Marchat et al., 1994) and the supernatant was frozen at -20°C for electrophoresis. SDS-PAGE electrophoresis was done according to the method of Laemmli (1970). The percentage of shared bands in a given lane was compared with those in other lanes of the same gel. A similarity matrix was constructed according to the similarity coefficient as described by Dice (1945).

RESULTS

Chromosome analysis of *B. alexandrina* snails showed that the karyotype consists of three groups. Group A has 8 metacentric pairs of chromosomes with relative length ranging from 5.57 to 13.92%, arm ratio from 1.05 to 1.17 and centromeric index from 43.12 to 50. Group B is made up of 8 submetacentric pairs with relative length ranging from 2.21 to 4.35%, arm ratio from 1.21 to 1.5 and centromeric index from 40 to 45.16. Group C contains 2 subtelocentric pairs with relative length ranging from 1.99 to 1.99%, arm ratio from 1.33 to 1.55 and centromeric index from 39.29 to 42.86. None of the chromosomes examined were telocentric (Table 1 and Figure 1). The karyotype of *B. truncatus* was identified and classified into four groups. Group A has 10 metacentric pairs of chromosomes with relative length ranging from 4.68 to 10.16%, arm ratio from 1.05 to 1.19 and centromeric index from 44.90 to 48.68. Group B is composed of 4 submetacentric pairs with relative length ranging from 3.07 to 4.01%, arm ratio from 1.08 to 1.3 and centromeric index from 43.48 to 48.15. Group C contains 2 telocentric pairs with relative length 2.81, arm ratio 1.33 and centromeric index 42.86. Group D has 2 subtelocentric pairs with relative length ranging from 2.34 to 2.54%, arm ratio from 1.33 to 1.38 and centromeric index from 42.11 to 42.86 (Table 2 and Figure 2). The pattern of protein

Table 1. Measurements and classification of the chromosomes of *B. alexandrina* snails.

Chromosome number	Chromosome position	Chromosome length (µm)			Relative length (%)	Arm ratio	Centromeric index
		Long Arm±S.D	Short Arm±S.D	Total±S.D			
1	M	2.1±0.04	1.8±0.06	3.9±0.08	13.92	1.17±0.17	46.15±3.1
2	M	1.8±0.04	1.6±0.03	3.2±0.07	11.42	1.13±0.12	50±2.1
3	M	1.6 ±0.03	1.4±0.03	3±0.09	10.71	1.14±0.15	46.7±2.6
4	M	1.3 ±0.05	1.1±0.02	2.4±0.05	8.57	1.18±0.15	45.83±2,7
5	M	1.2±0.03	0.98 ±0.04	2.18±0.07	7.78	1.22±0.18	44.95±1.8
6	M	1.3±0.03	0.94 ±	2.24±0.06	7.99	1.39±0.11	43.12±2.1
7	M	1.1±0.04	0.84±0.02	1.94±0.04	6.92	1.31±0.15	43.30±2.4
8	M	0.80±0.02	0.76±0.02	1.56±0.06	5.57	1.05±0.12	48.72±2.4
9	SM	0.7 ±0.03	0.52 ±0.01	1.22±0.04	4.35	1.35±0.16	42.62±2.1
10	SM	0.6±0.05	0.45±0.02	1.04±0.03	3.71	1.33±0.12	43.27±1.8
11	SM	0.48±0.06	0.32±0.02	0.8±0.05	2.86	1.5±0.17	40±1.4
12	SM	0.46±0.02	0.32±0.03	0.78±0.04	2.78	1.4±0.14	41.03±1.2
13	SM	0.42±0.02	0.3±0.01	0.72±0.03	2.57	1.4±0.11	41.7±1.8
14	SM	0.4 ±0.03	0.28±0.02	0.68±0.06	2.43	1.43±0.15	41.18±1.1
15	SM	0.36±0.03	0.26±0.04	0.62±0.07	2.21	1.38±0.13	41.94±1.6
16	SM	0.34±0.04	0.28±0.03	0.62±0.08	2.21	1.21±0.11	45.16±0.98
17	ST	0.32±0.02	0.24±0.01	0.56±0.04	1.99	1.33±0.16	42.86±1.2
18	ST	0.34±0.03	0.22±0.02	0.56±0.03	1.99	1.55±0.12	39.29±0.84
				28.02			

M= metacentic, SM= submetacentric, ST= subtelocentric

Table 2. Measurements and classification of the chromosomes of *B. truncatus* snails.

Chromosome number	Chromosome position	Chromosome length (µm)			Relative length	Arm ratio	Centromeric index
		Long Arm±S.D	Short Arm±S.D	Total±S.D			
1	M	0.78 ±0.01	0.74±0.08	1.52±0.81	10.16	1.05±0.08	48.68
2	M	0.76±0.01	0.68±0.06	1.44±0.6	9.63	1.12	47.22
3	M	0.68±0.03	0.62±0.04	1.3± 0.54	8.69	1.1	47.68
4	M	0.64±0.04	0.58±0.06	1.22±0.32	8.16	1.1	47.54
5	M	0.62±0.06	0.52±0.04	1.14±0.81	7.62	1.19	45.61
6	M	0.56±0.02	0.52±0.04	1.08±0.42	7.22	1.08	48.15
7	M	0.58.±0.05	0.5±0.03	1.08±0.21	7.22	1.16	46.30
8	M	0.52±0.03	0.44±0.02	0.98±0.4	6.55	1.18	44.90
9	M	0.42 ±0.03	0.38 ±0.02	0.8±0.80	5.35	1.11	47.5
10	M	0.38 ±0.01	0.32±0.02	0.7±0.24	4.68	1.19	45.71
11	SM	0.32 ±0.01	0.28±0.03	0.6±0.53	4.01	1.14	46.66
12	SM	0.28 ±0.03	0.26±0.01	0.54±0.51	3.61	1.08	48.15
13	SM	0.28 ±0.03	0.25±0.01	0.53±0.45	3.54	1.12	47.17
14	SM	0.26 ±0.04	0.20±0.03	0.46±0.43	3.07	1.3	43.48
15	T	0.24 ±0.06	0.18±0.04	0.42±0.42	2.81	1.33	42.86
16	T	0.24 ±0.01	0.18±0.03	0.42±0.62	2.81	1.33	42.86
17	ST	0.22±0.01	0.16±0.02	0.38±0.61	2.54	1.38	42.11
18	ST	0.20 ±0.02	0.15±0.04	0.35±0.13	2.34	1.33	42.86
				14.96			

M= metacentic, SM= submetacentric, ST= subtelocentric.

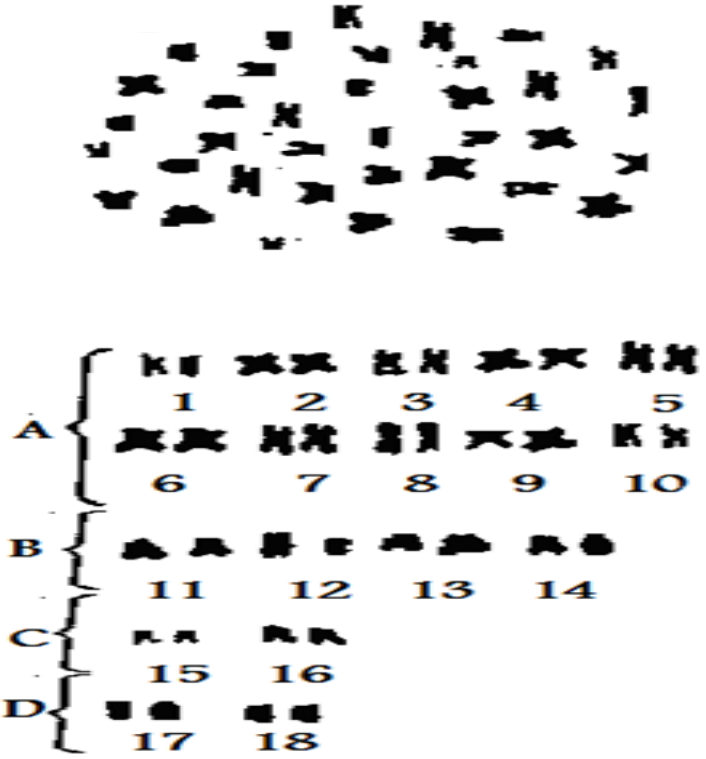

Figure 2. Photomicrographs showing the cell spread and karyotype of *B. truncatus* snail (2n = 36). A = metacentric, B = submetacentric, C = subtelocentric,D = telocentric chromosomes.

profile identified by SDS-PAGE electrophoresis for the parasites and their snail hosts is shown in Table 3 and Figure 3. The results indicate that the tissue protein exhibited a complex pattern ofpolypeptides with a total number of 11 and 9 bands for *B. alexandrina* and ova of *S. mansoni* respectively and their molecular weight ranged from 14 to 185.5 KDa. The results also pointed out that the number of bands in the snail *B. truncatus* and ova of *S. haematobium* is 12 and 8 bands respectively and their molecular weight ranged from 14 to 202.4 KDa. It was noticed that there are seven shared bands that appeared in the protein profile of the ova of *S. mansoni* and its snail host *B. alexandrina* (14, 20, 30, 71.2, 85.6, 132.2 and 185.5 KDa), in contrast to five shared bands (14, 19.1, 46.5, 63.5 and 202.4 KDa) in the ova of *S. haematobium* and its snail host *B. truncatus*. The protein profile also showed three shared bands (14, 46.5 and 55.2 KDa) in *B. alexandrina* and *B. truncatus* and two shared bands (14 and 63.5 KDa) in the ova of *S. mansoni* and *S. haematobium.* The present results (Table 4) indicate that the highest similarity index value (0.94) was between *B. alexandrina* snail and its corresponding trematode host *S. mansoni* and between *B. truncatus* and its trematode host *S. haematobium* (0.50).

On the other hand, the smallest "s" value (0.19) was observed between *B. alexandrina* and *S. haematobium* and between *B. truncatus* and *S. mansoni*.

DISSCUSSION

In this study, the karyotypes of the freshwater *B. alexandrina* and *B. truncatus* snails showed that the diploid chromosome number is 2n = 36. This result is in agreement with that reported by Kawano et al. (1987) who concluded that all chromosome preparations from the genus *Biomphalaria* (*B. glabrata, B. tenagophila* and *B. straminea*) had a model chromsosome number of 2n = 36. In addition, the results of these two species are in agreement with Giaco-mozzi et al. (1979), Raghunathan (1976), Goldman et al. (1983) and Odoemelam et al. (2009) who reported that the haploid chromosome number of *B. glabrata* is n = 18. Kawano et al. (1985) showed that the normal karyotype for *Helisoma duryi* (family planorbidae) is 2n = 36 chromosomes. The diploid chromosome numbers of freshwater *Cleopatra bulimoides* and *Bithynia* spp. (class Gastropoda, subclass Prosobranchia) are 28 and 32, respectively (Tohamy and Mohamed, 2006). In the current study, the comparison of the karyotypes of the two snail species under investigation revealed different morphological chromosome classification. They both have 2 subtelocentric pairs. *B. alexandrina* differs by showing 8 metacentic and 8 submetacentric pairs. On the other hand, *B. truncatus* has 10 metacentric and 4 submetacentric pairs of chromosomes. *B. truncatus* also

Table 3. Electrophoretic pattern of protein extracted ova of the parasites *S. mansoni* and *S. haematobium* and their snail hosts *B. alexandrina* and *B. truncatus*. Molecular weight (KD).

Marker KDa		*B. alexandrina*		*S. mansoni*		*B. truncatus*		*S. haematobium*	
KD	**%**	**KD**	**%**	**KD**	**%**	**KD**	**%**	**KD**	**%**
220	14	–		–		–			
						202.4		202.4	9.83
		185.5		185.5					
		132.2		132.2					
97						91.6			
								89.3	2.05
		85.6		85.6					
						76.4			
		71.2		71.2	9.11				
66									
				63.5		63.5		63.5	
		55.2				55.2			
		46.5				46.5		46.5	
45				45	3.50				
						41.4	10.1		
30		30	1.98	30					
								29.2	
						27.5			
								24.2	2.4
		22.4							
						21.5			
20		20		20					
						19.1		19.1	
		17							
						16			
14		14		14		14		14	
Sum in lane			100		100		100		100

differs from *B. alexandrina* by the presence of 2 telocentric chromosomes. Consequently, the chromosomes of *B. alexandrina* have been organized into three groups while those of *B. truncatus* into four groups.

Freshwater snails of the genus *Biomphalaria* are the most common host snail prevailing in developing countries and play a potential role in transmission of S*. mansoni*. *B. glabrata* is the most thoroughly studied species of all schistosome snail hosts and is responsible for the widespread of schistosomiasis in the western hemisphere. A comparison of the karyotypes of *B. alexandrina* in the current study with those of *B. glabrata* and *B. tenagophila* showed that all species have the same morphological classification. However, *B. glabrata* and *B. tenagophila* contained 12 metacentric, 4 submetacentric and 2 subtelocentric pairs of chromosomes (Kawano et al., 1987) but in the present result *B. alexandrina* has 8 metacentric, 8 submetacentric and 2 subtelocentric pairs of chromosomes. Despite the recent technical improvements in cytogenetics, there are still discrepancies in the results of many authors where chromosomes classification is concerned.

Raqhunathanm (1976) found that the karyotype of *B. glabrata* contains 10 metacentric, 4 submetacentric, 2 acrocentric and 2 telocentric pairs of chromosomes. However, Galdman et al. (1984) found that the karyotype of *B. glabrata* contains 15 metacentric, 1 submetacentric and 2 telocentric pairs of chromosomes. Additionally, Narang (1976) found that the karyotype of *B. glabrata* consists of 11 metacentric, 6 submetacentric, 1acrocentric and 2 telocentric pairs of chromosomes and *B. tenagophila* contains 13 metacentric, 4 submetacentric and 1 acrocentric pair of chromosomes. While Giaco-mozzi et al. (1979) found that *B. tenagophila* contains 8 metacentric, 8 submetacentric and 2 acrocentric pairs of chromosomes. Whether these differences in the obtained results stated above really exist between different populations or they are due to technical problems in chromosome preparation remain to be clarified. Both *B. alexandrina* and *B. truncatus* are snail species linked to a chronic and debilitating disease with a significant

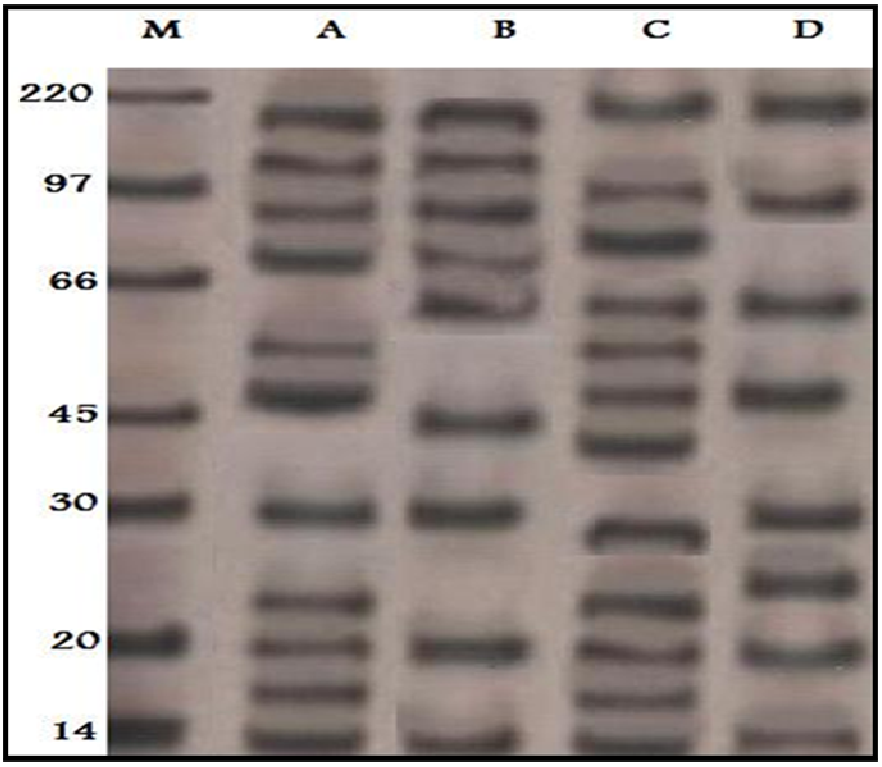

Figure 3. Protein fraction of ova of the parasites *S. mansoni* and *S. haematobium* and their snail hosts *B. alexandrina* and *B. truncatus*. Molecular weight (KD) A = *B. alexandrina*, B = Ova of *S. mansoni*, C = *B. trruncatus*, D = va of *S. haematobium* and M = Marker KDa.

Table 4. Dice's similarity coefficient (*S) of the protein profile bands between ova of the parasites *S. mansoni* and *S. haematobium* and their snail hosts *B. alexandrina* and *B. truncatus.*

	B. alexandrina	***S. mansoni***	***B. truncatus***	***S. haematobium***
B. alexandrina	1	0.94	0.26	0.19
S. mansoni	0.94	1	0.19	0.23
B. truncatus	0.26	0.19	1	0.5
S. haematobium	0.19	0.23	0.5	1

S = 2 a / 2 a + b + c, where: a = the number of shared bands between two individuals; b = the bands present in the 1st and not in the 2nd, and c = the bands present in the 2nd and not in the 1st.

economic and public health consequences in many developing countries. In a try to learn about the genetic basis of the snail/parasite relationship, the current research investigated the snails' soft parts and ova of their corresponded trematode using SDS-Page electrophoresis.

The host/parasite association was evaluated byanalyzing the electrophoretic band patterns and determining the similarity coefficient (Dice, 1945). The present results revealed similar bands between the parasites and their intermediate snail hosts. Seven similar bands appeared between *S. mansoni* ova and its intermediate host *B. alexandrina* and five similar bands appeared between *S. haematobium* and its intermediate host *B. truncatus*. Additionally, the results in the current study pointed out that the highest similarity index was between each snail species and its corresponding trematode and the lowest was among the non-compatible host/parasite association. Several investigators have focused largely on *B. glabrata* and its association with *S. mansoni* (Knight et al., 2000; Lewis et al., 2003). El-Dafrawy (2007) concluded that *S. mansoni* and *E. liei* and their snail's vector *B. alexandrina* and *B. glabrata* have three similar bands. These results could be explained by

the fact that there are shared antigens between *B. glabrata* snail and the parasite *S. mansoni* (Rasmussen et al., 1985). Also, Caustau et al. (2003) recorded that protein transcription in *B. glabrata* snails infected with *S. mansoni* is regulated by excretory-secretory products of the intermolluscan larval stages.

Additionally, El-Dafrawy et al. (2006) found that the electrophoresis of plasma protein of *B. alexandrina* infected with *S. mansoni* and *B. truncatus* infected with *S. haematobium* exhibited a wide variety of protein bands that is the infected snails led to the appearance and disappearance of several protein bands when compared to the controls. Since molecular information on the genes involved in the snail host/schistosome relationship is still rudimentary, further studies dealing with the genetics of parasite/snail compatibility are needed. It is hoped in the near future to identify targets in the snail host that interfere with parasite survival and develop alternate and/or novel methods to disrupt the transmission of schistosomiasis.

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