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Research Article
Identification of Invasive *Biomphalaria glabrata* Prevalence Using Internal Transcribed Spacer in Egyptian Governorates

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Abstract
Background and Objective: *Biomphalaria* species widely distributed all over the Egyptian Governorates considered as an the intermediate host of *Schistosoma mansoni*. This study aimed to investigate the interspecific variations between two *Biomphalaria* species that collected from five different governorates namely; Gharbia, Beni Suef, Giza, Menoufia and Qalyubia by the amplification of the internal transcribed spacer (ITS) region using polymerase chain reaction (PCR) then comparing using restriction fragment length polymorphism (RFLP). **Materials and Methods:** All wild *Biomphalaria* snails were collected from the terminal irrigation canals. Laboratory well identified *B. alexandrina* and *B. glabrata* were obtained from Theodor Bilharz Research Institute (TBRI) to be used as a reference snails. The DNA of wild and laboratory snails were extracted and ITS region was amplified using PCR, then comparing using the restriction enzyme Dde I. **Results:** The present study showed that the amplification of ITS region revealed the appearance of single band with 1100 bp in both wild and laboratory *Biomphalaria* species. After RFLP profile, three bands of 480, 260 and 100 bp were detected in snails that collected from Giza and Beni Suef governorates. These bands were similar to bands obtained in laboratory *B. glabrata*. While Gharbia, Menoufia and Qalyubia snails' expressed two bands (480 and 100 bp) only which were similar to laboratory *B. alexandrina*. **Conclusion:** *Biomphalaria* species collected from Giza and Beni Suef governorates were identical to *B. glabrata* and species that collected from the other governorates were identical to *B. alexandrina*.

Key words: *Biomphalaria alexandrina*, *B. glabrata*, genetic variation, ITS region, PCR, RFLP, schistosomiasis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Biomphalaria spp., are freshwater aquatic pulmonated gastropod belonging to the family Planorbidae which play a great role in schistosomiasis transmission[1]. In Egypt, *B. alexandrina* is the most common species, but another species, *B. glabrata* was detected in different irrigation canals and had successfully established in the Nile Delta. This species was susceptible to *S. mansoni* infection, effective to transmit schistosomiasis, tolerant to high temperature and has a long life span[2]. Worsley, the appearance of a hybrid species of *B. alexandrina* and *B. glabrata* increased the opportunity of schistosome transmission[3].

Identification of the *Biomphalaria* species using traditional morphological and anatomical methods were difficult for non-specialist because of their high similarity between them[4].

The interspecific variation of this genus at the morphological and genetic levels complete the correct identification of *Biomphalaria* species[5,6]. Identification of *Biomphalaria* spp., by molecular tools is considered the most accurate method as RAPD analysis, which was proved to be useful in population structure analysis but ineffective in species identification due to the interspecific genetic variations between species[7]. The allozyme variations between the different *Biomphalaria* species collected from different habitats showed that the allelic diversity pattern was reduced in species collected from Upper Egypt, Alexandria and Ismailia than those collected from Qalyubia[8]. Determination of the snail species using molecular techniques that amplify internal transcribed spacer (ITS) regions, has been useful in confirming the presence of *B. alexandrina*, *B. glabrata* and their hybrid[9]. The ITS region amplification technique is an easy method that used in the molecular identification of closely related species even with minimal DNA quantity[10]. A combination of PCR amplification and the RFLP technique was used by Abdulmawjood and Bülte[11] to show interspecific variations between two species of Helix.

Having the correct identification of these snails, increase the chance to control their spreading which in turn influence the spread of schistosomiasis. The most effective approach is an application of the molecular technique in parasite detection[12]. Using of qPCR technique to detect infection of *B. glabrata* with *S. mansoni* is an effective method[13].

The purpose of the study is to investigate the genetic interspecific variations between *Biomphalaria* species which collected from terminal irrigation canals in five different Egyptian governorates, Gharbia, Beni Suef, Giza, Menoufia and Qalyubia.

MATERIALS AND METHODS

Study area: The study was carried out at central laboratory, Department of Zoology, Faculty of Science, Tanta University, Egypt from June to October, 2019.

Sample collection: *Biomphalaria* species were collected from the terminal irrigation canals from different five governorates (Gharbia, Beni Suef, Giza, Menoufia and Qalyubia). From each region, at least 1000 snails were collected as shown in Table 1. The collected snails were transferred to the central laboratory, Department of Zoology, Faculty of Science, Tanta University, Egypt then each snail individually exposed to the light every hour for three consecutive days to let cercarial shedding if present. Later, these snails from each region were classified according to their collection sites and kept in dechlorinated tap water for further study. As a reference snails, identified laboratory *B. alexandrina* and *B. glabrata* snails (n = 50) were purchased from Theodor Bilharz Research Institute (TBRI), Egypt.

DNA extraction: All infected snails were excluded and uninfected clean snail groups were washed with distilled water and dissected for DNA extraction using a Gene Jet Genomic purification kit (Thermo Scientific, USA). Briefly, grinding of samples in liquid nitrogen using mortar was occurred, then adding digestion solution and proteinase k to break down the attached protein at 57EC for 1-3 hrs. After that add 20 µL RNAse, 200 µL lysis solutions and 400 µL 50% ethanol. The prepared lysis solution after RNA removal by RNAse was transferred to a purification column (Thermo Scientific, USA) inserted in a collection tube and centrifuged at 6000×g for 1 min (BOECO centrifuge SC-8, Germany). Finally, wash off the remaining solution twice with washing buffer. Genomic DNA concentration was obtained by using UV-spectrophotometer (V-730 double beam, Jasco, Japan) and reading the absorbance on OD 260 and 280. The DNA purity was based on OD 260/280 ratio.

DNA amplification using PCR: Amplification of 18s rRNA and 5.8 s rRNA gene was achieved using ETTS 1 and 2 with sequences of 5' CCATGAACGAGGAATTCCCAG 3' and 5' TTAG CAAACCGACCCTCAGAC 3' respectively. Genomic DNA was amplified using a PCR machine (Verti Well Thermal Cycler Applied Biosystem, USA). Each reaction was carried out in a final volume of 25 µL containing 2.5 µL of 10X DreamTaq Buffer, 1 µL dNTP Mix, 2 mM of each primer, 0.25 DreamTaq DNA Polymerase, 2 µL template DNA and deionized water. The amplification conditions were as follows: 1 cycle at 92°C for 5 min and 35 cycles of 92EC for 30 sec, 52°C for 30 sec, 72°C for 1 min. Finally, I cycle at 72°C for 10 min[14].

Agarose gel electrophoresis and staining: A total of 5 μL of the amplified product was resolved on ethidium bromide (1.5 μL mL^{-1}) stained Agarose gel (1.5%) using gel electrophoresis (electrophoresis power supplies E455, Consort, Belgium). Amplified bands were visualized using an Alpha Innotech® Ultraviolet Transilluminator (Alpha Innotech® USA) and photographed by using a high-resolution camera.

RFLP profiles: To evaluate interspecific variations between collected *Biomphalaria* snails, fast digest using endonucleases restriction Dde I enzyme (Promega Co., USA) was used in a total volume of 50 μL, 1 μL of the enzyme (10 units) is used to digest 1 μL of DNA. Adding 5 μL of 10x enzyme buffer and left for digestion for 5 hrs at 37EC. The products were evaluated using ethidium bromide-stained agarose gel (1.5%).

Analysis of polymorphism on agarose gel: The genetic variability between different Egyptian snail groups was determined by analysis of electrophoresis profile of the bands visualized on the gel by presence or absence of the gel and similarity coefficient was calculated according to Dice[15], by the following Eq.:

$$\frac{2a}{2}(a+b+c)\times 100$$

Hence a, no of shared bands. b, no of bands in the first sample only and c, no of bands in the second sample only.

RESULTS

Snails' collection and the rate of infection: The size of the snails that collected from different five Egyptian Governorates (Gharbia, Beni Suef, Giza, Menoufia and Qalyubia) ranged between 5-12 mm (Table 1). The sizes of laboratory *B. alexandrina* and *B. glabrata* from TBRI ranged between 6-10 and 8-13 mm, respectively. Post light exposure to let cercarial shedding from infected snails, the rate of infection with *S. mansoni* was recorded. The results showed that the percentage of the snail infection was more or less similar and ranged between 5-9%.

PCR amplification of ITS region of *Biomphalaria* snails: The PCR amplification of ITS region of both laboratory and wild *Biomphalaria* species, resulted in a product of approximately 1100 bp for all specimen (Fig. 1).

RFLP profile: The RFLP profile obtained by the restriction enzyme DdeI digestion showed the presence of three fragments of approximately 480, 260 and 100 bp (Fig. 2). It was clear that a band of 260 bp was the marker band for laboratory *B. glabrata* snails which was identical to specimens collected from irrigation canals in Beni Suef and Giza Governorates. Snails collected from irrigation canals in Gharbia, Menoufia and Qalyubia were identical to laboratory *B. alexandrina* snails with two bands of 480 and 100 bp.

Similarity coefficient: Table 2 shows the similarity coefficient was calculated between snails that collected from different Egyptian governorates and the laboratory snails. The present results revealed 80% similarity between *B. glabrata* and *B. alexandrina* from TBRI. It was clear that snails collected from Beni Suef and Giza were identical to laboratory *B. glabrata* with 100% similarity. While all samples collected from the other governorates, Gharbia, Menofia and Qalyubia were 100% similarity to *B. alexandrina*.

Table 1: Different component of collected snails from different sites

Snails	Collection sites	Irrigation canals	Number of snails	Size of snails (mm)	Rate of infection (%)
Biomphalaria species	Gharbia	Terat El Kased	1000	7-11	7
	Giza	Al-Mansoria Berkash	1000	6.2-9.3	8
	Beni suef	Ahnasia	1000	5-10.8	9
	Qaliubia	Manshia-El-Ka nater	1000	6-10	5
	Menoufia	El Bagor	1000	8-12	6
B. alexandrina	TBRI	---	50	6-10	---
B. glabrata	TBRI	---	50	8-13	---

Table 2: Similarity coefficient between *Biomphalaria* species

Samples	*B. glabrata* (lane 1) (%)	*B. alexandrina* (lane 2) (%)	Gharbia (lane 3) (%)	Beni suef (lane 4) (%)	Giza (lane 5) (%)	Menofia (lane 6) (%)	Qalyubia (lane 7) (%)
B. glabrata	100	80	80	100	100	80	80
B. alexandrina	80	100	100	80	80	100	100

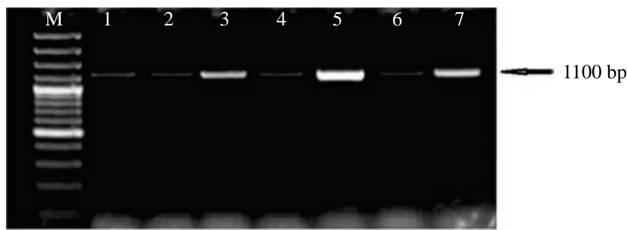

Fig. 1: Genetic variation between *Biomphalaria* snails collected from different Egyptian Governorates evaluated by using ITS primers

M: DNA ladder (ranged between 100-3000 bp), Lane 1: *B. glabrata* from SBCS, Lane 2: *B. alexandrina* from SBCS, Lane 3: *Biomphalaria* snails from Gharbia, Lane 4: *Biomphalaria* snails from Beni Suef, Lane 5: *Biomphalaria* snails from Giza, Lane 6: *Biomphalaria* snails from Menofia and Lane 7: *Biomphalaria* snails from Qalyubia

Fig. 2: RFLP profile using Dde I enzyme

M: DNA ladder (ranged between 100-3000 bp), Lane 1: *B. glabrata* from SBCS, Lane 2: *B. alexandrina* from SBCS, Lane 3: *Biomphalaria* snails from Gharbia, Lane 4: *Biomphalaria* snails from Beni Suef, Lane 5: *Biomphalaria* snails from Giza, Lane 6: *Biomphalaria* snails from Menofia and Lane 7: *Biomphalaria* snails from Qalyubia

DISCUSSION

Interspecific variations between *Biomphalaria* collected from five Egyptian governorates were detected in the present study. This study ensured the presence of species identical to *B. glabrata* in two governorates, Giza and Beni Suef. Developing countries, especially in Africa and Asia suffered tropical and epidemic disease as schistosomiasis[16]. *Biomphalaria* spp. and *Bulinus truncatus* known as intermediate hosts to transmit *S. mansoni* and *S. haematobium*, respectively. The presence of these snails in the terminal irrigation canals led to increase the prevalence and dissemination of schistosomiasis in Egypt[17]. Despite the programs of schistosomiasis control, it was an endemic disease in Egypt up to 1997. The infection rates of schistosomiasis was declined year by year[18]. In 2004, *S. mansoni* prevalence was 1.9% in the Nile delta governorates and from 2009-2012 the schistosomiasis prevalence reached 0.5 %. According to the Ministry of Health and Population (MOHP), the prevalence percentage was declined to 0.2% from 2013-2016[19].

The infection rates in *B. pfeifferi* was 12%[20]. While in the current research the rate of infection ranged between 5-9% in *Biomphalaria* snails. Although the prevalence of schistosomiasis has been declined gradually in Egypt.

Morphological and anatomical differentiation between *B. alexandrina* and *B. glabrata* snails is so difficult for non-experienced persons due to their morphological identity[6]. So, using modern techniques as the RFLP-PCR technique is an excellent method for differentiation between closely related species[5]. It was noted that morphometric analysis, morphology, anatomical renal ridges with pallial cavity and buccal teeth were the key to differentiate between *Biomphalaria* spp.[3]. So, a lot of researches investigated modern tools for easy identification of species[21,22]. Interestingly, in 1996, *B. glabrata* and its hybrids with *B. alexandrina* were present in the Nile Delta[3]. Contradictory, researchers revealed the absence of *B. glabrata* in Egypt and the presence of only *B. alexandrina*[21,23].

On the molecular level, a lot of researches dealt with the identification of very closely related species using PCR as a fast, modern and sensitive method. It differentiates between susceptible and resistant *Biomphalaria* strains to reveal the snail/parasite relationship by using four primers in the RAPD-PCR profile[16]. In the past, the differentiation between many molluscan species was achieved using morphological characters of shell, renal and some structure of the reproductive system[4]. Molecular methods based on PCR, RFLP, microsatellite and simple sequence repeats (SSR) were investigated to overcome the difficulty of morphological, inter-and intra-specific similarity[24,25]. Also, molecular techniques are effective in *S. mansoni* detection in snail when the conventional methods as exposure to direct light for shedding and snail squeezes between two glass slides were insufficient. Amplifying of 18srDNA of *S. mansoni* in *B. glabrata* that infected with two types of trematodes was effective in the identification of the parasite[10]. Banaja *et al.*[26] compared the resistant and susceptible strains of *B. alexandrina* in Saudi Arabia using RAPD-PCR.

Many attempts were done by a lot of researchers to investigate interspecific variations between species using the RFLP technique and Low Stringency Polymerase Chain Reaction (LS-PCR)[7,27,28]. Furthermore, genetic variations between *Bulinus* sp. and *Biomphalaria* sp., snails using Arbitrarily Primed Polymerase Chain Reaction (AP-PCR) were studied[29].

In the present study, the PCR product showed single band with 1100 bp, which is closely similar to the 1300 bp band that investigated by Vidigal *et al.*[30] using the same primers to differentiate between different *Biomphalaria* spp., ITS 1 and ITS 2 sequencing used in genetic differentiation between snails.

Interestingly, a band of 361 bp has appeared in *B. glabrata* snail[23]. In the present study, the RFLP profile using the restriction enzyme DdeI revealed the appearance of 3 fragments of 480, 260 and 100 bp. While in Brazilian species, fragments ranged between 607 and 72 bp in RFLP profile using DdeI were revealed[30]. Interestingly, in the present study, a band of 260 bp was the marker for *B. glabrata* snails. So, it was obvious that *B. glabrata* was found in irrigation canals of Beni Suef and Giza governorates. This agrees with many authors who confirmed the presence of *B. glabrata* and its hybrid across the irrigation canals along the Nile in Egypt[3]. Contradictory, the study of Lotfy *et al.*[21] found no evidence of the presence of *B. glabrata* or its hybrids in Egypt. It worth noting that the spread of *B. glabrata* which is more tolerant to high temperature and has a long life span could increase the infection rates with Schistosoma due to its susceptibility to infection besides the high productivity rate of cercaria[2]. Although the low incidence of schistosomiasis in Egypt recently, the effort should be on snail control in irrigation canals to overcome disease spreading constantly.

CONCLUSION

To end the argument about the existence of *B. galbrata* in Egypt, RFLP-PCR was tested on different *Biomphalaria* snails collected from the different Egyptian government. It was concluded that *Biomphalaria* species collected from Giza and Beni Suef governorates were identical to *B. glabrata*, however species collected from the other governorates were identical to *B. alexandrina*.

SIGNIFICANCE STATEMENT

This study discovered the presence of *B. glabrata* with *B. alexandrina* in terminal irrigation canals of Egyptian governorates that could be beneficial to end the argument in the last few decades about the disappearance of *B. glabrata* along the Nile River. This study could help the researchers to take into consideration the critical role of *B. glabrata* in schistosomiasis prevalence. Thus, a new theory in enabling *Biomphalaria* identification using RFLP-PCR of internal transcribed spacer region may be arrived at.

REFERENCES

1. Bouchet, P. and J. Rocroi, 2005. Classification and nomenclature of gastropod families. Malacologia, 47: 1-397.
2. Abou-El-Naga, I.F., S.M.F. El-Nassery, S.R. Allam, E.A. Shaat and R.F.M. Mady, 2011. *Biomphalaria* species in Alexandria water channels. Parasitol. Int., 60: 247-254.
3. Yousif, F., N. Haroun, A. Ibrahium and S. El-Bardicy, 1996. *Biomphalaria glabrata*: A new threat for schistosomiasis transmission in Egypt. J. Egy. Soc. Parasitol., 26: 191-205.
4. Habib, M.R., Lv, S., D. Rollinson and X.N. Zhou, 2021. Invasion and dispersal of *Biomphalaria* species: Increased vigilance needed to prevent the introduction and spread of schistosomiasis. Front. Med., Vol. 8. 10.3389/fmed.2021.614797.
5. de Souza, M.A.A., 2017. Freshwater gastropods in the Northern littoral mesoregion of Espírito Santo, Brazil. Acta Sci. Biol. Sci., 39: 293-300.
6. Vidigal, T.H., E.D. Neto, O.S. Carvalho and A.J.G. Simpson, 1994. *Biomphalaria glabrata*: Extensive genetic variation in Brazilian isolates revealed by random amplified polymorphic DNA analysis. Exp. Parasitol., 79: 187-194.
7. Vidigal, T.H.D.A., E.D. Neto, A.J.G. Simpson and O.S. Carvalho, 1996. A low stringency polymerase chain reaction approach to the identification of *Biomphalaria glabrata* and *B. tenagophila*, intermediate snail hosts of *Schistosoma mansoni* in Brazil. Mem. Inst. Oswaldo Cruz., 91: 739-744.
8. Abou-El-Naga, I.F., 2013. *Biomphalaria alexandrina* in Egypt: Past, present and future. J. Biosci., 38: 665-672.
9. Hamburger, J., M. Weil, R. Klumpp, J.H. Ouma and D.K. Koech *et al.*, 1989. Identification of snails infected with Schistosomes by Elisa employing monoclonal antibodies: *Schistosoma mansoni* in laboratory snails (*Biomphalaria glabrata*) and in field snails (*Biomphalaria pfeifferi*) from Kenya. Am. J. Trop. Med. Hyg., 40: 613-619.
10. Rogers, S.O., 2012. Integrated molecular evolution. Yale J. Biol. Med., Vol. 85.
11. Abdulmawjood, A. and M. Bülte, 2001. Snail species identification by RFLP-PCR and designing of species-specific oligonucleotide primers. J. Food Sci., 66: 1287-1293.
12. Hanelt, B., C.M. Adema, M.H. Mansour and E.S. Loker, 1997. Detection of *Schistosoma mansoni* in *Biomphalaria* using nested PCR. J. Parasitol., 83: 387-394.
13. Zanardi, V.S., L.M. Barbosa, F.M. Simões, S.C. Thiengo and R.E. Blanton *et al.*, 2019. Prevalence of infection of *Biomphalaria glabrata* by *Schistosoma mansoni* and the risk of urban *Schistosomiasis mansoni* in Salvador, Bahia, Brazil. Rev. Soc. Bras. Med. Trop., Vol. 52. 10.1590/0037-8682-0171-2019.
14. Simpson, A.J.G., F.C. Reinach, P. Arruda, F.A. Abreu and M. Acencio *et al.*, 2000. The genome sequence of the plant pathogen *Xylella fastidiosa*. Nature, 406: 151-157.
15. Dice, L.R., 1945. Measures of the amount of ecological association between species. Ecology, 26: 297-302.

16. El-Nassery, S.M.F., I.F. Abou-El-Naga, S.R. Allam, E.A. Shaat and R.F.M. Mady, 2013. Genetic variation between *Biomphalaria alexandrina* snails susceptible and resistant to *Schistosoma mansoni* infection. Bio. Med. Res. Int., 2013: 1-6.
17. Yameny, A., 2017. The evolving schistosomiasis agenda 2017-2020 in Egypt: Moving from control toward final elimination. J. Biosci. Appl. Res., 3: 48-54.
18. World Health Organization, 2001. Report of the WHO informal consultation on schistosomiasis in low transmission areas: Control strategies and criteria for elimination. London, 10-13 April 2000 (No. WHO/CDS/CPE/SIP/2001.1). World Health Organization. https://apps.who.int/iris/bitstream/handle/10665/66904/WHO_CDS_CPE_SIP_2001.1.pdf
19. Ministry of health and population, 2017. Endemic disease control department. Annual report on schistosomiasis control. https://applications.emro.who.int/docs/EMROPUB_2018_EN_20396.pdf?ua=1
20. Bakuza, J.S., R. Gillespie, G. Nkwengulila, A. Adam, E. Kilbride and B.K. Mable, 2017. Assessing *S. mansoni* prevalence in *Biomphalaria* snails in the gombe ecosystem of western Tanzania: The importance of DNA sequence data for clarifying species identification. Parasites Vectors, Vol. 10. 10.1186/s13071-017-2525-6.
21. Lotfy, W.M., R.J. DeJong, B.S. Black and E.S. Loker, 2005. Specific identification of Egyptian *Biomphalaria* species and possible hybrids using the polymerase chain reaction based on nuclear and mitochondrial loci. Mol. Cell Prob., 19: 21-25.
22. Mbagnia, M.C.T., T.T.M. Tanekou, A.C.K. Fokam, D.N. Nguete, C.S. Wondji and F. Njiokou, 2020. PCR-based molecular identification of two intermediate snail hosts of *Schistosoma mansoni* in cameroon. Parasites Vectors, Vol. 13. 10.1186/s13071-020-04033-1.
23. Mohamed, A.M., A.T.S. El-Din, A.H. Mohamed and M.R. Habib, 2011. Identification of various *Biomphalaria alexandrina* strains collected from five Egyptian Governorates using RAPD and species-specific PCR techniques. Am. J. Mol. Biol., 1: 17-25.
24. Caldeira, R.L., T.H.D.A. Vidigal, L. Matinella, A.J.G. Simpson and O.S. Carvalho, 2000. Identification of planorbids from Venezuela by polymerase chain reaction amplification and restriction fragment length polymorphism of internal transcriber spacer of the RNA ribosomal gene. Mem. Inst. Oswaldo Cruz, 95: 171-177.
25. Campos, Y.R., O.S. Carvalho, C.O. Goveia and A.J. Romanha, 2002. Genetic variability of the main intermediate host of the *Schistosoma mansoni* in Brazil, *Biomphalaria glabrata* (Gastropoda: Planorbidae) assessed by SSR–PCR. Acta Trop., 83: 19-27.
26. Banaja, A., M. Jamjoom, I. Shalaby and Y. Gherbawy, 2008. Discrimination between susceptible and non-susceptible *Biomphalaria alexandrina* snails-intermediate hosts of *Schistosoma mansoni* in Western Saudi Arabia using random amplified polymorphic DNA analysis. Belg. J. Zool., 138: 191-195.
27. Knight, M., P.J. Brindley, C.S. Richards and F.A. Lewis, 1991. *Schistosoma mansoni*: Use of a cloned ribosomal rna gene probe to detect restriction fragment length polymorphisms in the intermediate host *Biomphalaria glabrata*. Exp. Parasitol., 73: 285-294.
28. Pires, E.R., T.H.D.A. Vidigal, H.M.S. Teles, A.J.G. Simpson and O.S. Carvalho, 1997. Specific identification of *Biomphalaria tenagophila* and *Biomphalaria occidentalis* populations by the low stringency polymerase chain reaction. Mem. Inst. Oswaldo Cruz, 92: 101-106.
29. Caldeira, R.L., O.S. Carvalho, R.C.G. Lage, P.C.M. Cardoso and G.C. Oliveira, 2002. Sequencing of simple sequence repeat anchored polymerase chain reaction amplification products of *Biomphalaria glabrata*. Mem. Inst. Oswaldo Cruz, 97: 23-26.
30. Vidigal, T.H.D.A., L. Spatz, D.N. Nunes, A.J.G. Simpson, O.S. Carvalho and E.D. Neto, 1998. *Biomphalaria* spp: Identification of the intermediate snail hosts of *Schistosoma mansoni* by polymerase chain reaction amplification and restriction enzyme digestion of the ribosomal RNA gene intergenic spacer. Exp. Parasitol., 89: 180-187.