INTRODUCTION

Schistosomiasis is a major neglected tropical disease (NTD) and one of the most prevalent snail borne infections, especially in developing countries. It affects more than 249 million people all over the world, among which more than 90% of those requiring treatment for schistosomiasis live in Africa. Ranking second following malaria, schistosomiasis lies among human parasitic diseases in tropical and subtropical countries causing morbidity, mortality together with socioeconomic and public health impact in these areas. [1] It is believed to be the most important water-based disease from a global public-health perspective. [2,3] Additionally, the disease is one of the ten tropical diseases especially targeted for control by the Special Program for Research and Training in Tropical Diseases of the United Nations Development Program, the World Bank and the World Health Organization (WHO).[4] The human cost of schistosomiasis remains considerable despite consistent efforts to develop control strategies and therapeutics. [5]

Historical review of Schistosomiasis

Schistosomiasis is known since ancient times, with the oldest human cases dated to Pharaonic Egypt. Haematuria, was a very common complaint in Egypt during historic times. [6] Many Egyptologists considered the disease that was referred to as 'aaa' in many papyri to be hematuria. The antiquity of the disease was approved by both; recovery of *Schistosoma* ova from Egyptian mummies and also by finding shells of snail intermediate hosts for thousands of years. [7]

Both *Schistosoma haematobium* (*S. haematobium*) and *Schistosoma mansoni* (*S. mansoni*) infections were present during the Pharaonic period. *S. haematobium* was present in Upper and Lower Egypt after the arrival of infected slaves, while *S. mansoni* was present in Lower Egypt after the downward dispersal of imported infected slaves. It is likely that infection with *S. mansoni* has not been identified because the discovered mummies that were dissected were generally from Upper and not Lower Egypt, as Egyptian mummies from the former area were better preserved due to the dry air in this region. While in humid Lower Egypt, where *S. mansoni* was initially present, the majority of all mummies have perished. Another important factor was the lack of satisfactory mummified tissue from the lower social classes that were exposed to the parasite, as adequate mummification was performed mainly in the higher classes. [8] During the later dynasties, mummification was offered to all classes of society; however, it was rare in the earlier periods during which schistosomiasis had established itself in Egypt. Also, due to the fact that *S. mansoni*'s symptomatology is usually less dramatic than that for *S. haematobium*, thus, its presence have understandably been overlooked by the ancients. All these factors have made *S.mansoni* to be thought of to arrive Egypt after *S. haematobium*.[8]

Despite the great antiquity of the disease, related scientific studies started only about 150 years ago, [7,9] when the Japanese workers, Fuji *et al*, described acute katayama syndrome for the first time in 1847.[10] However *Schistosoma* was originally named Bilharzia in the honor of Theodor Bilharz, the young pathologist who described it in 1851 at the Kasr El-Aini hospital in Cairo, Egypt. Bilharz found the distome trematodes in the urogenital blood vessels during post mortem examination of the Egyptian corpses.[11] The recent history of intestinal schistosomiasis caused by *S. mansoni* dates back to 1902, in

London, when Sir Patrick Manson diagnosed a case of intestinal bilharziasis, that was originally contracted in the West Indies, Manson found the characteristic lateral-spined eggs in the patient's stool. [12] In 1907, Sambon named the new species as *S.mansoni* after Sir Patrick Manson. [13] The name was officially accepted in 1913. [14]

In 1914, just before the First World War, the prosobranch snail, *Oncomelania* was discovered as an intermediate host of *S. japonicum* by Miyairi and Suzuki. [15] In 1915, Leiper demonstrated the life cycle of schistosomes and showed that the aquatic pulmonate snails of the genera *Bulinus* and *Biomphalaria* are the transmitters of *S. haematobium* and *S. mansoni*, respectively. The term "bilharziasis" was commonly used for schistosomiasis.[16] In 1967, "schistosomiasis" became the official terminology following WHO expert report on the epidemiology and control of schistosomiasis. [17]

Types of human Schistosomes

The main three schistosome species that can infect humans are; *S. mansoni*, *S. haematobium* and *S. japonicum.* Additionally, humans can be infected by other species which are more localized geographically. Those are; *S. mekongi* and *S.malayi* (both of which are similar to *S.japonicum* clinically and geographically). *S. intercalatum* and *S.mattheei* are species that cause infections in sheep, cattle and horses, however, less commonly they can cause human intestinal schistosomiasis. Each of the previously mentioned species is with different epidemiology and geographical distribution. Less importantly, other schistosomes with avian or mammalian primary hosts can cause severe dermatitis in humans (as in swimmer's itch secondary to *Trichobilharzia*). In Egypt, the first two mentioned species are the endemic ones causing intestinal and urinary schistosomiasis, respectively. [2-5]

Epidemiology of Schistosomiasis

Worldwide, it is estimated that 779 million people live at risk of *Schistosoma* infection and around 1 of 30 people has schistosomiasis, this means that about 250 million people, mostly children are actually infected. Of them, about 20 million are suffering from severe disease. It continues to disrupt lives of millions of people in about 74 countries in Africa, Latin America, South West and South East Asia, inflicting misery and precluding the individuals' reasonable expectations of productive lives. Hence it represents a great risk to health in rural areas of developing countries.[1-4] At present, 85% of all these cases are concentrated in sub-Saharan Africa with estimated morbidity burden of 3.5 million disability-adjusted life years (DALYs). [5]

Egypt is one of the affected countries, where schistosomiasis is undoubtly one of most widespread public health problems. Around 70% of the adult chronic liver diseases and 35% of liver diseases in children are due to schistosomiasis. [18] In 2007, about 3% of rural population showed *S. mansoni* eggs in their stools. [19]

Owing to the special nature of *Schistosoma* life cycle, and its need for the fresh water snail intermediate host to complete this cycle, transmission relies upon natural water polluted with human excreta. This condition is often found in areas of poverty or low income, where lack of facilities forces people to use natural water bodies for domestic, occupational, or religious purposes as performing ablution. Daily life in rural Egypt resulted in frequent and intense contact with Nile and canal water in the domestic

purposes. [9] Conditions in the village communities have predisposed the population to infection, working and recreational activities have brought many people into direct contact with infective water near riverbanks and in the canals. Hence, schistosomiasis particularly affected agricultural population. Moreover, fishing population were also at risk. Brickmaking was another activity which involves close contact with water. Women performing daily domestic activities in infective water, such as washing clothes and utensils, are also at risk. Playing or bathing children in contaminated waters are also vulnerable to infection. [2, 9, 17, 20, 21]

Hence, in contrary to malaria and other vector-transmitted diseases, schistosomiasis transmission depends on the active role of the human host, through contaminative activities of snail habitats. This occurs either by direct excretion or by sewage disposal into water, and also through exposure activities by direct contact with infective water. [7, 21]

Both *S. mansoni* and *S. haematobium* have been and are still endemic in Egypt *S. haematobium* had been the predominant species of *Schistosoma* throughout Egypt since Bilharz first described the infection in Cairo, in 1851, while *S. mansoni* was confined to the north central Nile Delta.[22] Individuals found infected with *S. mansoni*, who resided in the southern areas of Nile Delta or in Upper Egypt, were always found to have acquired the infection during their visits to the northern endemic areas.[23]

Since few decades, the epidemiological distribution of schistosomiasis in Egypt has been changed, where *S. mansoni* has replaced *S. haematobium* in the Delta and has become well established in Middle and Upper Egypt. [24] By 1979, *Biomphalaria* snails were recovered from canals in Aswan and Lake Nasser, indicating that the snail had colonized the Nile from the Delta to Lake Nasser. This has resulted in a noticed decrease in the prevalence of *S. haematobium* infection with increase in *S. mansoni* infection rates. [24, 25]

This change was attributed to several factors. The change in the hydrology of the Nile basin, with controlled water flow, following construction of the Aswan High Dam was one of these factors. This has led to changes in Nile water velocity and to shorter winter closure period, resulting in more stable snail habitats, and so *Biomphalaria* snails, which were previously restricted to the Delta, have extended to Upper Egypt. [2, 7, 8, 26]

Another important factor was the refugees who moved from Suez Canal zone to the Delta during the war time and became heavily infected with *S. mansoni*.[27] When they returned back home, transmission became established because of the presence of *Biomphalaria* snails. Large scale perennial irrigation in Egypt began with the reign of Mohamed Ali (1805-1848), who promoted the cultivation of long staple cotton, a major Egyptian export crop.[7] This change from basin to perennial irrigation in Upper Egypt has led to an increase in the prevalence of *S. mansoni* infection due to the flourishing of the aquatic snails in absence of the annual drying period.[7]

The development of new irrigation networks and water resources which are essential for agricultural expansion, together with land reclamation projects have further exacerbated the problem. Invasion of the snail vector into El-Salam Project area in Northern Sinai, which is irrigated by a mixture of Nile and agricultural run-off, was inevitable with resulting emergence of schistosomiasis there. Toshka is another reclaimed project, which is located at the West Desert at a low altitude where the River Nile expands and branches to form a new irrigation system. Water velocity at this area is low and

suitable for snail reproduction. In these areas, there are inadequate infrastructure for water, sanitation and health services with increased chance for schistomiasis transmission. [2, 7, 8, 28]

Life cycle of *Schistosoma mansoni* [29-35]

In 1915, the life cycle of *Schistosoma* was first demonstrated by Leiper in Egypt.[16] It includes two generations that alternate between two hosts, the sexual generation which comprises the adult schistosomes of both sexes in the definitive host, including man and the asexual generation in the snail intermediate host. [29]

S. mansoni adult worms measure about 10 to 15 mm long with the female being longer and thinner than her male. They live permanently twined together in mated female-male pairs in the radicles of the inferior mesenteric venules, draining the terminal ileum and the large intestine. [29] The female lay hundreds of immature eggs daily, those are laid in the very fine capillaries of the inferior mesenteric venules. Using their lateral spine, eggs stretch the capillary wall finding their way from the blood vessels to the submucosa of the terminal ileum and the colon. Egg maturation takes about six days, which is the time taken during the egg journey from the submucosal blood vessels till reaching the colonic lumen. [29] Eggs break out of the venules in the intestinal submucosa, aided by the egg spine and miracidial antigen, they escape into the intestinal lumen to be excreted in patient's feces. [30] Egg excretion starts five to six weeks after skin penetration by cercariae.[31] They are fully embryonated, non operculated, oval, transparent with lateral spine, about 115 to 175µm × 45 to 68 µm in diameter. [30, 31]

Excreted eggs in warm fresh water hatch and release ciliated actively swimming miracidia. Each miracidium is approximately 0.2 mm long and has anterior penetration glands to assist in snail penetration, flickering flame cells which constitute its excretory system, and a non-functional primitive gut. Miracidium is positively phototropic by its photoreceptors , but it has no eye spots. [29,31] Miracidia can live for up to 24-48 hours, during this time they actively search for their intermediate host that they must find. Then the miracidium penetrates the correct suitable fresh water snail which acts as an intermediate host. It has been stated that, one miracidium is capable of producing up to 200,000 cercariae of the same sex. [31-33]

The suitable snail for *S. mansoni* is of the genus *Biomphalaria*, its species differ according to the geographical distribution. In Egypt, it is *Biomphalaria alexandrina* (*B. alexandrina*). [28,34] Whereupon the miracidia penetrate the tegument of the snail, preferentially targeting near the tentacles. During penetration of the tegument and once within the tissue of the snail, morphological and physiological changes occur in the miracidium. First, miracidia lose their cilia. They develop into primary sporocysts in the head-foot region of the snail about one week post infection. Two weeks later, the primary sporocysts give birth to secondary sporocysts or cercarial releasing sporocysts that migrate to the digestive glands (hepatopancreas) and ovitestes (reproductive organs). There is no redial stage in the life cycle of *Schistosoma*. [29-33]

Life cycle inside the snail host takes about four weeks, starting from snail exposure to miracidia, to the time of shedding of the furcocercus cercariae. [29,30] Cercaria of *Schistosoma* is about 500 µm in length, with a pear shaped body that is 200 µm long ×55-100 µm wide, a tail which is 250 µm long × 35-50 µm wide and a pair of furci of about 50-

100 µm length. In water, thousands of cercariae can be released over weeks from the snails if there is direct sun light, a period which coincides with the peak time of human water-contact activity. Cercaria of *Schistosoma* are usually found near the water surface, swimming vigorously, with their body upside down. They can live for one to three days in the fresh water searching for a suitable host, and can swim at about four meters per hour. (29, 33)

When people come in contact with cercariae infested water, the cercariae come into contact with the skin while the water evaporates. Aided by their lytic secretions, produced from the cercarial penetration glands present in their body, they penetrate the skin of their definitive host. Skin penetration is completed within half an hour. One cercaria gives rise only to one fluke, this means that no multiplication takes place in the human host. (29-33) Following skin penetration, the cercaria loses its tail and becomes a worm-like schistosomule, which stays in the skin for a few hours. (29)

Schistosomulae enter the venules of the peripheral circulation to be carried to the lungs and distributed through the aorta to the whole body.(29,30,33) Those which reach the portal blood vessels, in the liver sinusoids, undergo maturation. (33) Sexual dimorphism into male and female adults occurs about one month following skin penetration, the male carries the female in its gynecophoric canal and migrates in the portal tributaries against the blood stream to settle in the inferior mesenteric plexus, and the female starts egg laying. (29, 33) Life cycle of *S. mansoni* is shown in figure (1).

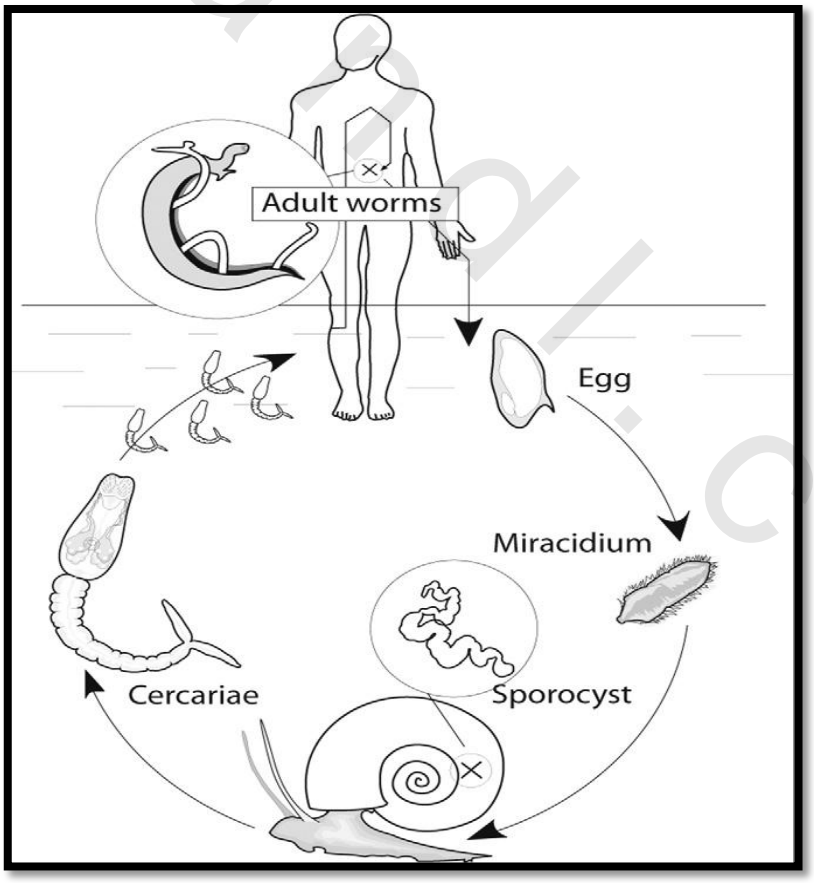

Figure 1: *Schistosoma mansoni* life cycle. (35)

Control of Schistosomaisis *mansoni*

Owing to the complex life cycle of *Schistosoma* that runs in two hosts, different preventive and control measures can be used to combat the disease. Those are either targeting the human definitive host, or are directed towards the control of the snail intermediate host.

At the end of the First World War, the discovery of antischistosomal properties of Tartar Emetic, raised hope of therapeutic interventions against *Schistosoma*. However, long courses of painful injections and serious side effects, were major disadvantages of the drug.[36] Schistosomiasis control began in Egypt in 1922, when the country's government set up specialized clinics for treating people that were infected by the parasite. [37]

Between the two World Wars, control was based mainly on the combined treatment with antimonial drugs and snail control with copper sulfate.[38] Since then and until the mid-'80s, control of schistosomiasis was mainly directed against the snail vector. Yet trials to find more efficient chemotherapeutic drugs kept going on. Among which, oxamniquine was both effective and less toxic. After a half-century searching for an efficient chemotherapy, the real breakthrough was the discovery of praziquantel (PZQ). The drug that was invented in 1970s and was proven to be safe and effective when given in a single oral dose, with negligible side effects and broad spectrum activity against all schistosome species. Experimental evidence showed that praziquantel increases the permeability of the membranes of schistosome cells towards calcium ions. The drug thereby induces contraction of the parasite, resulting in its paralysis in the contracted state. The dying parasites are then dislodged from their site in the host and may enter systemic circulation or may be destroyed by host immune reaction by phagocytosis. In 1988, under sponsorship of the Ministry of Health and the United States Agency for International Development (USAID), the Schistosomiasis Research Project (SRP) was started. This ten-year campaign supported the investigation of the prevalence and magnitude of morbidity caused by schistosomiasis and the changing pattern of distribution of *S.mansoni* and *S. haematobium*. Additionally, this program included treating patients with PZQ, with developing a suspension formulation of praziquantel suitable for young children. [39]

By 1989, the distribution of Praziquantel doses, free of charge, to all diagnosed schistosomiasis cases was implemented through different health facilities including the network of rural health units. Although chemotherapy yielded favorable results regarding reduction of morbidity, drugs did not prevent reinfections, so reinfection persisted to occur due to re-exposure. Furthermore, schistosome strains that are less susceptible to praziquantel started to appear in some Egyptian villages within the Nile Delta region indicating the need for additional antischistosomal drugs. [40-42]

Later on, a newer antischistosomal drug, mirazid (Myrrh) was introduced to the Egyptian market. It causes contraction of adult *Schistosoma* worms' musculature, with loss of their anchorage to blood vessel walls, then tegumental destruction takes place with separation of male and female couples. Then, their shift to the liver, where host immune system destroys and phagocytoses the shifted worms. However, there are serious doubts about its therapeutic effect. [43]

Another measure that targets the human host is, the use of protective vaccination that represents an important strategy for long-term control of this disease. However, due to the

complex life cycle of schistosomes and their different immune evasion strategies, the tried approaches for vaccine development were unfortunately not successful. [44]

As a result, the role of other measures of intervention has been amplified to aid in reduction of disease transmission, such as; improvement of sanitation, safe water supply to population, health education and snail control. [45]

Snail control was achieved by several methods such as; chemical antagonists, environmental modifications and biological approaches.

Based on a postulate that the snail intermediate host is the weakest link in the parasite's life cycle, the struggle began against the mollusk transmitters of schistosomiasis in Egypt in the mid of the previous century, using chemical control as rapid and effective means for reduction of the transmission of schistosomiasis. This control method entailed treatment of water bodies with molluscicides to reduce the number of snail intermediate hosts, thereby breaking the disease transmission cycle. This included the use of synthetic molluscicides such as copper sulfate, which was the only safe chemical readily available in the 1950s. It affected both *Biomphalaria* and *Bulinus*, however it acted more efficiently on *Biomphalaria* species. [46]

Niclosamide, is the only remaining commercially available chemical molluscicide now. It acts by depleting snail's glycogen stores, and has so far proven effective. The high cost of synthetic molluscicides, along with the possible building up of snail resistance to these compounds, the environmental hazards and their toxicity to non-target organisms, all these factors , have increased the need for plant molluscicides as alternatives for synthetic ones.[47,48]

In conclusion, control through molluscicides is costly and also, it leads to accumulation of the applied chemical agents in the treated environment. Moreover, treating patients using chemotherapeutic agents is not enough for combating the disease, because of the common occurrence of reinfection after patients' treatment by keeping their same habits that lead to reinfection. [49,50] This is why other methods should always be thought of to be added to the control measures. Those methods include; environmental measures that are used to get rid of these snails including; regular cleaning of canals from vegetation, use of concrete or cement lining of canals and ditches with provision of a clean source for drinking water. All of which are effective in many countries, however, these strategies are often expensive when compared with the health budgets of developing countries. [45]

The previously mentioned factors have encouraged the development of new biological control measures against the snail vector. Those have been reviewed by the WHO in 1985. Biological control methods that were tried, included; the use of living organisms such as competitor snails, or predators as snail host antagonist to interrupt the cycle of human schistosome transmission. [51] For biological control methods to be achieved, accurate information about *Biomphalaria* taxonomy, physiology and bionomics should be well known to establish and implement these control measures.

Taxonomic Classification of *Biomphalaria* [52]

The snail belongs to;
phylum *Mollusca* ,
class *Gastropoda*,
subclass *Pulmonata*,
family *Planorbida*,
subfamily *Planorbinae* ,
genus *Biomphalaria*.

Identification of *Biomphalaria* snail

This is performed based on; geographical distribution, morphological and anatomical characters and molecular tools. [52-56]

A) Geographical distribution:

There are more than 34 identified species of *Biomphalaria* in the Neotropic regions and in Africa. It has been hypothesized that *Biomphalaria* snails originated in South America after the continental divide, which occurred approximately 95–106 million years ago. The Neotropic *Biomphalaria* snails in Central and South America include, *B. glabrata* which was discovered by Say in 1818, *B. tenagophila* and *B. peregrina* that were discovered by Orbigny in 1835 and *B. straminea* that was discovered by Dunker in 1848. [8, 53, 57, 58]

In Africa, there are approximately 12 species of *Biomphalaria* present. The African species of *Biomphalaria* appeared as a result of the relatively recent west-to-east trans-Atlantic dispersal of the *B. glabrata*-like taxon. These snails may have been transported via the feathers of aquatic birds or on vegetation rafted across the ocean. Then, successful colonization occurred because of the hermaphroditic nature of *Biomphalaria* and its capablility of self-fertilization. [8] The most important species in Central Africa, the Ethiopian plateau and in the Nile Basin are *B. alexandrina* , which was discovered by Ehrenberg in 1831, *B. pfeifferi*, which was discovered by Krauss in 1848 and *B. sudanica* which was discovered by Martens in 1870. [53, 57, 58]

Of the several species of *Biomphalaria* snails worldwide that serve as the intermediate host for *S. mansoni*, *B. alexandrina* is the species that is indigenous to Egypt. [28,34] *B. alexandrina* is thought to be originated in the area between Alexandria and Rosetta and has historically been confined to the Nile Delta. However, the snail has expanded its range upstream as far as Lake Naser. [8, 27, 28] So, it is only recently that *B. alexandrina* colonized the Egyptian Nile from the Delta to Lake Nasser. This change was likely due to factors mentioned above such as; changes in the hydrology of the Nile basin following construction of the Aswan High Dam. Additionally, construction of huge water projects, the development of new irrigation networks and new water resources essential for land reclamation projects such as Tushka and El-Salam Project have further exacerbated the problem. [8]

Long-distance dispersal of snails occurred probably through attachment to suitable vehicles such as boats and also by being carried in water inside tires that can be used as boat fenders. Spread of *B.alexandrina* snails in Middle & Upper Egypt has led to

S.mansoni transmission to these areas after establishment of the snail in this sluggish freshwater. [18, 28] This was helped by the absence of local adaptation between *S. mansoni* and *B. alexandrina* throughout the water bodies located in Egypt, as found in a recent meta-analysis. This finding also suggests the possibility of future invasion of the new reclaimed areas by schistosomiasis. [59]

Using computer-based geographical information system (GIS) that is performed in Egypt because of its dry atmosphere and clear sunny sky, criteria of *Biomphalaria* inhabited waters and their locations were identified. *Biomphalaria* snails were found to be common in low lands in the northern parts of Egyptian Delta. In general, *Biomphalaria* species are abundant in the lower portions of major tributary streams and are rare in the fast-flowing headwaters. Moreover, slowly flowing canals that are heavily vegetated, with their substrate mostly formed of clay or organic material, are also heavily inhabited with these snails. They are more abundant in drains than in irrigation canals and more plentiful towards the distal ends of irrigation canals, because they thrive more in slower currents. Snails are absent in wells due to their location on higher ground away from the flooding streams and also due to different water chemistry and lack of vegetation and pollution. *Biomphalaria* are also not found in fish ponds stocked with adult *Tilapia*, which acts as a predator for the snail. [60,61]

B) Morphology:

Biomphalaria is a sinistral or left handed fresh water snail, with two well defined body parts. **A bony part**, which is formed of discoid button like shell, with a color varying from light to dark grayish brown. This bony shell is formed of a spirally coiled disc that gradually increases in diameter around a central axis or columella , from the blind end or the snail apex towards the snail aperture through which the body of the snail can protrude or retract. The shell is concave on either side with a wide open funnel-shaped deep umbilicus. The shell possesses separate coils that are called whorls. The line occurring between every two whorls is called the suture. The diameter of the snail shell varies according to the species of *Biomphalaria* and also according to the age of the snail. When fully grown, *B. alexandrina* can reach up to about 15 mm in diameter, with five round whorls, with the last whorl being the oldest and named as the body whorl. [52, 53] The shell is secreted by the mantle of the snail, it is made up of calcium carbonate, therefore, its formation is influenced by the presence of calcium carbonate in the water. So, environmental factors together with food conditions affect shell formation. [62]

The second part of the snail body is **the soft part** which is formed of the head-foot region and the visceral mass. The front part contains the head with a pair of tentacles, a pair of eyes and a mouth. The muscular foot is covered with a flat creeping sole. Internal organs including; circulatory, digestive, renal and reproductive systems are located in the visceral mass in the body whorl and are surrounded by a large fold of skin called the mantle. The mantle cavity has contact with the outside world through the pseudostome (aperture). Usually the head foot region is present outside the shell, with the rest of soft part being protected inside the bony shell. When endangered by a predator, or even when handled in the laboratory, the head-foot region is completely withdrawn inside the shell by a strong retractor muscle that is attached to the columella of the shell. The head foot region is withdrawn inside the shell also during periods of snail rest. [52,53]

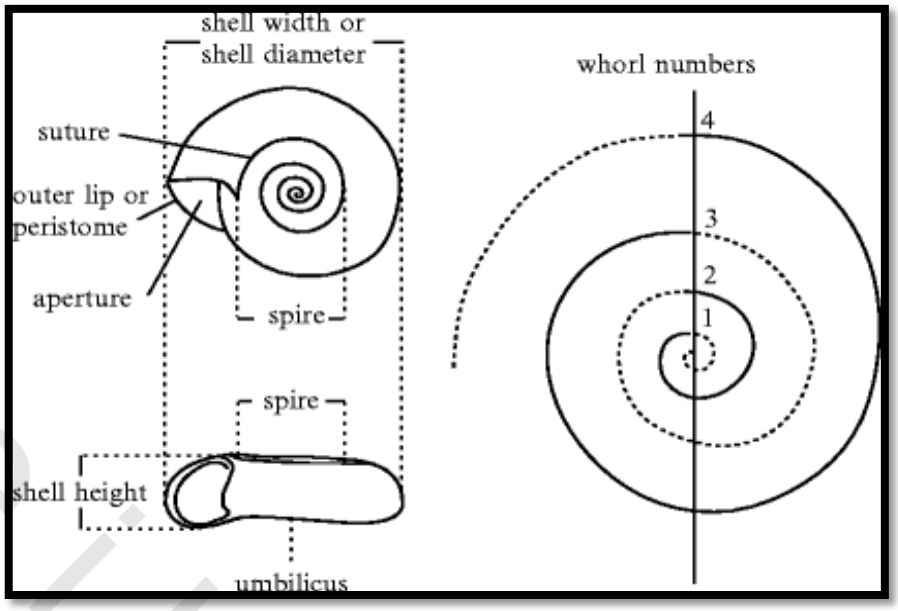

Figure 2: **Schematic representation of *Biomphalaria* shell. The figure on the right indicates how whorl numbers can be counted. The dorsal side of the living animal is oriented upward (bottom left figure) or toward the reader (top left figure).** [63]

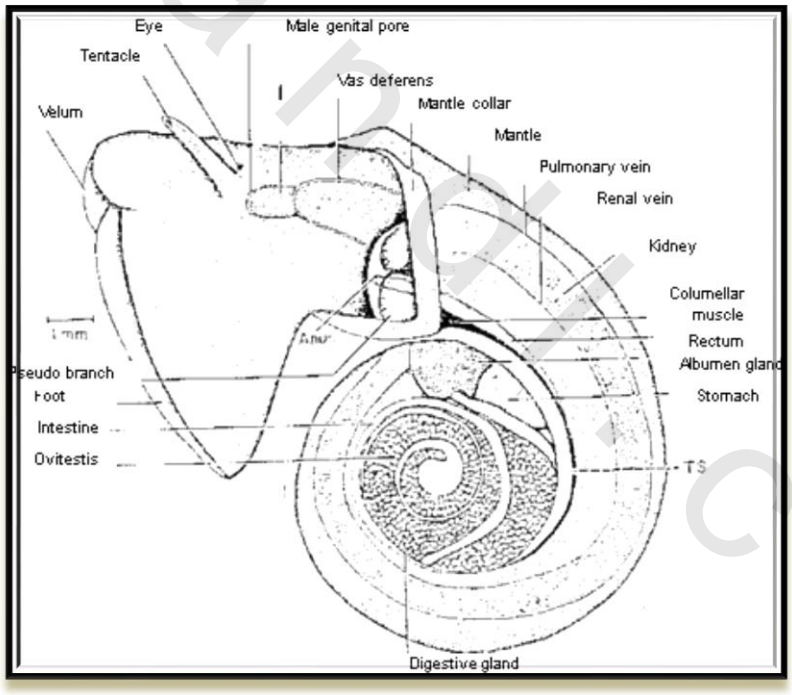

Figure 3: **Morphological illustration of *Biomphalaria alexandrina* soft tissue (right side view).** [53]

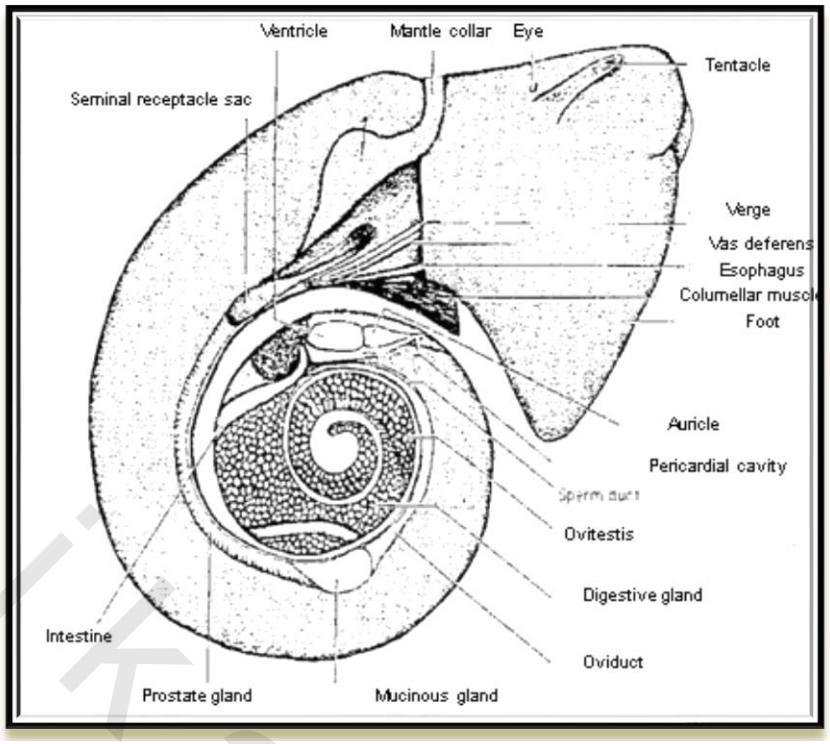

Figure 4: Morphological illustration of *Biomphalaria alexandrina* soft tissue (left side view). [53]

C) Molecular tools:

Many molecular methods were used for *Biomphalaria* species' identification. Of these tools, polymerase chain reaction-based analysis of restriction fragment length polymorphism (PCR-RFLP) has been used. [54,55]

Bionomics of *Biomphalaria* snails

A) Life cycle, fecundity and reproduction:

Biomphalaria snails are hermaphrodites, each individual possesses both complete and functional male and female reproductive systems, so they are capable of self-fertilization, as well as of cross-fertilization or out-crossing, with the latter being the preferred method in most species. However, self-fertilization has the merit to enhance the dispersal abilities of these hermaphrodite snails, as only a single founder can establish a new colony.[64, 65]

They are oviparous, laying eggs in masses or in clutches. The egg masses when first deposited are soft, with translucent outer envelope and numerous suspended minute granules. They are small, yellowish, rounded to oval, flattened gelatinous masses. They become harder and darker as they stay in water, attaching themselves to flat surfaces, such as water plant leaves and stones. In case of laboratory bred *Biomphalaria* snails, the gelatinous egg masses are found on the wall of the laboratory aquaria, filtration devices or the glass-covered heaters. Each egg mass is about 0.5 cm in diameter and it contains up to

25 eggs. A mature snail can lay up to 10,000 or more eggs/year. The hatchlings measure about half to one mm in diameter. [52, 53, 66]

B) Survival and longevity:

In nature the life span of non-infected *Biomphalaria* can reach twelve to eighteen months. However their survival in the laboratory under closely monitored conditions rarely exceeds twelve months. Since the generation time is approximately about five weeks, this snail can undergo several generations over the twelve months period. [67] *Schistosome* infection has been shown to negatively affect the snail's lifespan and its reproductive capacity, especially the egg production ability. Snails which are exposed to smaller dosage of miracidia, survive longer than those receiving larger numbers. [52, 53, 68-70]

C) Diet and feeding habits:

Biomphalaria is mainly herbivorous, feeding on vegetation, aquatic weeds and other decaying leaves that are present in their aquatic natural environment. Young snails prefer feeding on unicellular organisms and the small soft decaying organic particles. Older snails subsist in part on vegetable matter and in part upon the microflora of their environment. They have strong chemoreceptors that can locate food. [53]

In case of laboratory bred *Biomphalaria,* fresh green lettuce leaves are used as the main source for their nutrition. Tetramin fish food is known to add more nutritive value to the breeding medium. Calcium carbonates in the form of chalk pieces also promote snail growth and shell hardening. [71]

Environmental factors that affect *Biomphalaria* snails and their susceptibility to *Schistoma mansoni* infection

These are **biological, physical** and **chemical** factors

(i) Biological factors ;

1- Starvation:

Diet enhances life span, growth rate (shell diameter and height), egg production, hatchability of snails' eggs, also it affects cercarial output by snails. [72,73]

2-Vegetation:

Water plants and aquatic weeds form an important element in the snail habitat. They function as nutritive source. Also, they provide suitable surfaces for the deposition of eggs and for the growth of unicellular green algae which form a favorite kind of food. Water plants also provide the snails with shelter and adhering surfaces. Moreover, they are used for protection from intense sunlight and fast water currents. The underside of leaves offer higher oxygen tension, lower temperature and calm water currents which attract snails into the zone where miracidia are concentrated. [74]

Some water plants have molluscicidal properities. One of the best plant molluscicide studied in Egypt for several years is the herb *Ambrosia maritima* (*Damsissa*), which causes mortality of snails, their egg masses, as well as miracidia and cercariae of *Schistosoma*. It

has the merit of being of low toxicity against fish and mammals at concentrations that are lethal to the target snail. [75, 76]

3- Crowding:

The density of snails in an environment is a critical ecological factor that can affect their growth, survivorship and fecundity, with their growth being depressed by crowding. [77, 78] *Biomphalaria* snails are able to limit their population size at high densities, either by self -inhibition or due to nutritional deficiencies and competition for food items which lead to reduction in both snail growth and fecundity.[79, 80]

Moreover, the inhibition of respiratory capacity at high densities, with lower capacity to consume iron has adverse effects on snails'growth and fecundity.[81] Crowding also leads to anaerobiosis with release of organic acids, as lactic and succinic acids and so, it leads to decrease in pH, which is not favorable for snail growth. Additionally, crowded snail population produce an inhibitory factor in their feces. [82] In conlusion, competition for food resources, depletion of oxygen and/or calcium and the production of growth inhibitory or toxic factors, all play roles in crowding effect.

Crowding effect is more obvious in case of infected snails due to their more fragile condition, where infected snails should be kept at a population density between 15 and 20 snails per liter. However, uninfected *Biomphalaria* grow and reproduce well over a wide population density up to 50 snails per unit volume of water when adequate nutrition and properly conditioned water are provided. [81]

4- Natural enemies:

Different non-target competitor mollusks as; *Helisoma duryi*, *Melanoides tuberculatus* and *Physa acuta* exist in the habitat of vector snails, interfering with their nutrition. This reducing effect is much more pronounced when the density of these snails increases. So, one of the successful methods tried for biological control of *Biomphalaria* was the competitive displacement through introducing non-vector snails, which have similar ecological requirements. [83-85]

***Tilapia* fish** is an effective **predator** that feeds on *Biomphalaria*, so, it can control *S.mansoni* infection in fishponds by destroying the environment of its vector. [61,86]

(ii) Physical Factors;

1-Water Related Factors;

Water Body Type: *Biomphalaria* needs perennial streams irrigation channels, this is why the snail dissemination has flourished after building the High Dam, *Biomphalaria* invaded the whole Nile valley and the Delta, this has led to increased incidence of *S.mansoni* in Upper Egypt. [18]

Water Depth: *Biomphalaria* inhabit shallow waters, especially along shore line, that acts as a muddy substratum for crawling and ovi-position. Also, this medium is a favorable shelter, rich in decaying organic matter, with a lot of vegetation and algae that are necessary for snail nutrition, so, they are surface feeders.[87]

Water Velocity: *Biomphalaria* snails prefer stagnant or gently flowing waters. They can withstand only a narrow range of water velocity, the maximum tolerated flow of water velocity is 0.3 m/sec, so, heavy rainfall and fast flowing rivers prevent establishment of snail population. Fluctuation in water level plays a role in dissemination of *Biomphalaria* , this is why it can be used as a means of snail control. [88,89]

Water Salinity: *Biomphalaria* snails inhabit fresh water, with less tolerance to high salt content in water than *Bulinus*. High salinity prevents hatching of *S. mansoni* ova and affects infectivity of miracidia. [90-92]

2-Effect of temperature and seasonal changes:

Suitable temperature for *Biomphalaria* is between 10 ºC and 30ºC. Optimum temperature ranges between 22-26 ºC.[93] Snails' egg hatching, growth and development are affected by water temperature. Egg laying increases proportionally with temperature up to 30°C, however more than this, both snail and egg mortality rises dramatically. On the other hand, *Biomphalaria* do not lay eggs below 18°C. [94]

Besides, temperature is one of the important environmental factors affecting *S. mansoni* life cycle inside its intermediate host.There is a direct relationship between the temperature and the snail infection rate, with lower infection level of *Biomphalaria* with *S. mansoni* in the cold months of the year. The ideal temperature at the time of contact between the host and the parasite ranges from 25°C to 26°C. Also, miracidium-cercaria transformation takes about 30 days at this temperature. [94,95]

It was found that, induction of stress genes after heat-shock by incubation of *B. glabrata* about 4 hours at 32°C prior to infection by *S. mansoni* has turned resistant snails to become susceptible to infection. This was attributed to the effect of temperature modulation in these resistant snails. This suggests that susceptibility to infection may be temperature-sensitive. [96]

3- Light:

It was found that *Biomphalaria*'s mating behavior, crossbreeding activities and their egg laying capacities vary under different illumination regimes, being the best under the usual daily illumination rhythm, followed by the constant daily illumination regime and being the least under constant darkness. [97]

Regarding the effect of light on *Schistosoma* infection, miracidia are positively phototropic. Darkness seems to inhibit the activity of miracidia. [90, 98] Light stimulates cercarial shedding. So, cercariae are usually concentrated near the water surface.[29,31,33]

(iii) Chemical Factors;

1- Minerals and Ions

Essential Minerals

A) Calcium

Calcium (Ca++) is the most important mineral required for *Biomphalaria*. The major part of calcium requirement of the snail is acquired by direct absorption from the surrounding water and from their food.[99] Calcium is essential for the lives of mollusks, because snail shell is mainly made up of calcium carbonate and so its formation is influenced by its presence in the inhabited water. Calcium content is significantly higher in the shells than in the soft parts of the snails, [100,101] also, calcium is needed for snail reproduction.[102] Moreover, the presence of calcium ions in water affects miracidia-mollusk contact. It is necessary for the penetration, maturation and development of the penetrating cercariae. [103,104] The phagocytic activity of hemocytes and lectins cooperating in snails' defense reactions, depends on the presence of calcium ions in their hemolymph.[105]

Snail fauna with <2 mg/L Ca++ give small and extremely fragile shells. Snail mortality is significantly greater when calcium levels are very low (1.5 mg/L), with 30 mg/L being the optimal calcium concentration for the greatest snail fecundity. In case of laboratory bred *Biomphalaria*, calcium is supplied to the breeding waters through putting chalk pieces in these waters, also lettuce itself contains calcium. [106]

B) Iron and copper

Small amounts of iron are beneficial for growth of algae,that are important for snail feeding. Additionally, iron and copper play important role by acting as catalysts in the oxidant system of *Biomphalaria*, where the enzyme superoxide dismutase (SOD) facilitates dismutation of oxygen (O_2) into hydrogen peroxide (H_2O_2), which can then, upon reaction with reduced iron or copper salts, produce -OH that is in turn toxic to *S. mansoni* sporocysts, and so protective to the snail itself against infection by *S. mansoni*.[107]

Hazardous ions and minerals

Ammonia has a molluscicidal activity against *B.alexandrina*, it decreases its growth rate, increases its mortality rate and it reduces its susceptibility to *S. mansoni* miracidia. Urea was found to be less harmful to the snail than ammonia. [108,109]

2-Dissolved gases and water pH

Oxygen

In *Biomphalaria* species, respiration occurs by both pulmonary chambers (lung) and gills which are elaborate to ensure enough oxygen uptake.[110] Low oxygen level reduces snail movement, impairs their feeding and reproduction. The production of reactive free oxygen radicals by hemocytes in *Biomphalaria* snails is linked to their ability to kill *S. mansoni* miracidia. Hemocytes from resistant snails generate significantly more H_2O_2, this

suggests that the capacity to produce H_2O_2 is critical in determining susceptibility or resistance of *Biomphalaria* to *S. mansoni*. [107]

Hydrogen ions and pH

Biomphalaria species are tolerant to wide range of pH in their habitat water. However, infection of the snail by *S. mansoni* miracidia is highest at pH of 7-9. [111]

Compatibility between *Biomphalaria* snail - *Schistosoma mansoni*

The concept of compatibility between *Biomphalaria* snail and *S. mansoni* worms, is defined as the interaction of physiological properties of both organisms. The outcome of this complex interaction enables the parasite to penetrate, develop and propagate inside the invertebrate host, or not. [112, 113] It depends on both; the **ability of the mollusk** internal defense system (both cellular and humoral factors) to recognize and destroy the invading parasite and also on the **ability of the parasite** to evade the defensive response of the snail host. [114] Therefore, the success or failure of snail host attack by *S. mansoni* represents this compatibility.

The dynamic interaction between molluscs and their trematode parasites leads either to a state of co-existence, in which the trematode thrives and produces subsequent stages of its life cycle, or to incompatibility, where the trematode is either destroyed and eliminated by the host snail defensive responses or fails to develop because the host is physiologically unsuitable. [115] In other words, compatibility between the parasite and the snail host involves; the invasion of miracidia, their development into sporocyst within the host and the escape of the cercariae. These processes if occurred with suitability as hosts for continued parasite development, the snail is said to be susceptible. Whereas, in non-susceptible snails, invasion occurs but the sporocyst undergoes encapsulation by the vigorous host tissue reaction with failure of cercaria to develop. [116]

Within the *Biomphalaria* genus, there is a large diversity in the susceptibility of strains to infection by *Schistosoma* parasites. Physiological and genetic aspects of the snail hosts, as well as the genetic factors of the *S. mansoni* parasite, contribute to this diversity. [117] Susceptible snails are those which allow successful schistosome development and emergence of cercariae, with the ability of becoming infected after being challenged by a parasite, while resistant (refractory or incompatible) ones are unable to function as hosts.[118]

Schistosoma cause substantial deleterious effects to *Biomphalaria*, therefore, it acts as a major driving force in the snail evolution. At the same time, it has to cope with host-defense mechanisms to avoid being eliminated. This represents reciprocal antagonistic co-evolution between both partners. At a particular time of their evolution, parasite virulence and host defense can be in equilibrium in natural populations. Compatibility is a characteristic of a host–parasite system where the parasite species is capable of establishing infection and achieving transmission using this host species. To achieve compatibility, the parasite has to evade host defense systems in order to complete its life cycle. In certain host–parasite systems, compatibility is incomplete: sometimes the host wins and the parasite is eliminated and sometimes the parasite wins and succeeds in infecting the host. This can lead to a phenomenon called **compatibility polymorphism**.[35,114]

Even among susceptible snails there are degrees of susceptibility. In 1979, Frandsen assessed the degrees of compatibility between *S. mansoni* and *Biomphalaria*, he classified the compatibility according to the total cercarial production/ 100 exposed snails (**TCP**) parameter, into seven classes; [119]

Class 0: 0 cercariae / 100 exposed snails; Resistant, Incompatible.

Class 1: 1-10.000 cercariae / 100 exposed snails; Not very compatible.

Class 2: 10.001-50.000 cercariae / 100 exposed snails; Poor compatibility.

Class 3 : 50.001-150.000 cercariae / 100 exposed snails; Compatible.

Class 4: 150.001-250.000 cercariae / 100 exposed snails; Well compatible.

Class 5: 250.001-500.000 cercariae / 100 exposed snails; Very compatible.

Class 6: ≥ 500.001- cercariae / 100 exposed snails ; Extemely compatible.

Factors influencing *Biomphalaria - Schistosoma mansoni* compatibility

When *S. mansoni* miracidium penetrates its potential snail host, its fate depends on both parasite infectivity and snail host susceptibility. Continued development of the parasite is possible only in the absence of harmful immune response against it in physiologically compatible snail. Therefore, factors influencing this relationship are either snail host related, or parasite related. [120]

A) Snail factors

Mollusks must possess some mechanisms that would allow them to defend themselves against foreign parasites. If not, they would not have survived through evolutionary time. [113]

1- Snails' Internal Defense System (cellular and humoral immune factors)

Unlike the vertebrate immune system, snails as invertebrates have no acquired adaptive immunity with only innate, non-specific immune response. Also, they do not have lymphocytes, immunoglobulins, nor complement system. Nevertheless, they do have the capacity to recognize self from non-self structures. [114, 121] Instead of the usual vertebrate blood, the invertebrate *Biomphalaria* has what is known as hemolymph that circulates in the snail circulatory system that comprises a heart together with a network of vessels and capillaries. [122]

The snail's internal defense system (IDS) is the most important defensive mechanism against *S. mansoni* infection. It comprises cells that are named as **hemocytes** and **soluble factors** contained in this hemolymph. These components act together to determine self from non-self molecular patterns to eliminate any threats. [114,123]

Hemocytes or hemolymph cells originate mainly from an organ that was named as, haemocyte producing organ (HPO) which is a special organ that is present in a tiny area of the snail reno-pericardial zone, this organ is supposed to be the counter part of the vertebrate bone marrow. [124, 125] More recently, it is termed as the **amoebocyte-producing**

organ (APO). This organ was approved to be the origin of the hemocytes based on its hyperplasia and mitoses during parasitic infection. Moreover, hemocytes can be produced by the vascular and intestinal systems of the snail. [126]

The hemocytes are the principle line of cellular defense in *Biomphalaria*. They are mobile phagocytic cells that are found in the circulating hemolymph, also they are present within the snail interstitial tissues. The circulating hemocytes play a central role in innate immunity. They are involved in recognition of foreign bodies, encapsulation responses, phagocytosis and cytotoxic reactions.They contribute to phagocytic and scavenging functions, hence they are compared to the vertebrate macrophages. [127] The number of hemocytes in the hemolymph is influenced by age and physiological conditions of the snail. [128]

Biomphalaria circulating hemocytes are classified into **3 main subpopulations** with variable morphology, size, ultrastructure, enzymatic activity, different abilities for adhesion, phagocytosis and encapsulation for the invading parasite. These are blast-like cells, granulocytes and hyalinocytes. **Blast-like cells** are spherical with a large centrally located nucleus that almost filled the whole cell. Cytoplasm only occupied a narrow area around the nucleus. This kind of cell may be regarded as young cells or precursor cells for other hemocytes. **Granulocytes** are cells with both phagocytic and encapsulating activities.They are polymorphic and filled with a variable number of granules distributed in the cytoplasm. These granules could differ in number and could be distributed either on the periphery or at the center of the cell. **Hyalinocytes** are engaged in snail tissue repair. Subsets of hyalinocytes differ among different species of *Biomphalaria*. [123,124, 129-132]

Encapsulation of the parasite occurs through granulocytes. They are nucleated amoeboid granular hemocytes resembling cells of the vertebrate monocyte macrophage series, both morphologically and functionally. They circulate freely in the hemolymph, connective tissue and in several organs and accumulate in specific sites around sporocyst forming focal, granulomatous structures. Ultrastructural examination indicates that they are actively involved in parasite endocytosis, with extensive Golgi apparatus, lysosomes like structures, granular endoplasmic reticulum and elongate mitochondria in their cytoplasm.[133,134]

These defense mechanisms have been described in resistant snails, whereas in susceptible ones, the hemocytes bind to the parasites in a transitory and inefficient way allowing successful evolution of the parasite. [135] This means that, resistance in major part is due to the ability of circulating hemocytes to recognize and bind the parasite surface and then undergo a cytotoxic activation with release of reactive ions resulting in killing , phagocytosis and encapsulation of the parasite. [129] In the resistant snail, there is diffuse hemocyte proliferation, with formation of encapsulating reactions around sporocysts and developing cercariae. A strong host reaction occurs which is an expression of an innate cellular internal defense mechanism, in which *S. mansoni* sporocysts are quickly attacked and encapsulated by circulating phagocytic cells in the snails' hemolymph. Moreover, hemocytes release a variety of cytotoxic agents. Parasite recognition and hemocyte activation are mainly mediated by lectins. Also, some proteins, which are similar to mammal cytokines, such as TNF-α, that are associated with the activation and cellular proliferation in *S. mansoni* infections. [129,136-138]

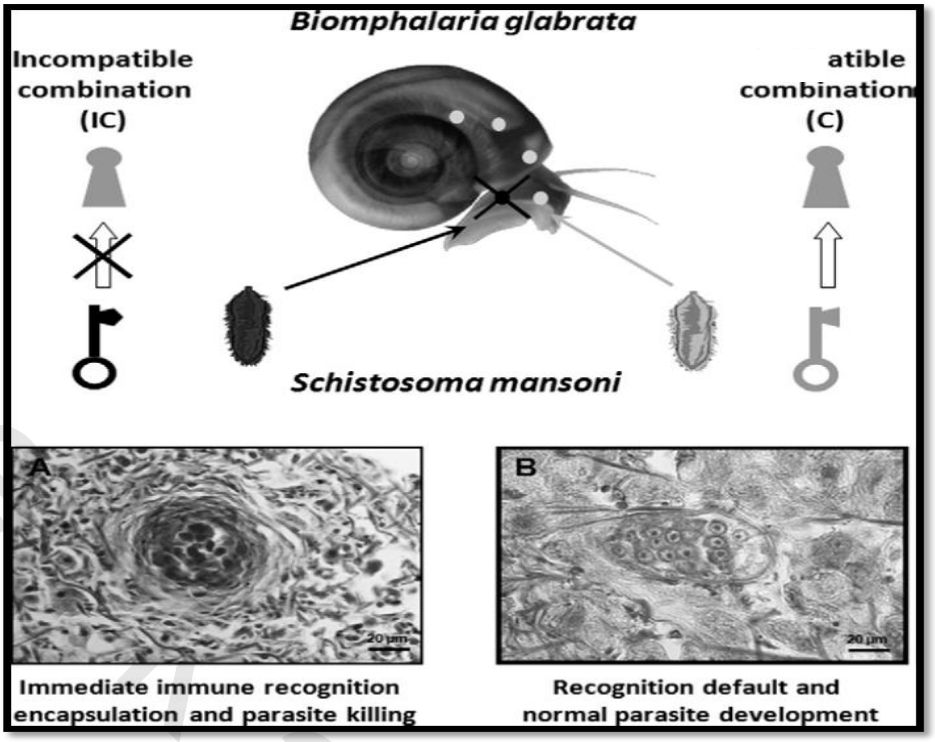

Figure 5: *Schistosoma mansoni/Biomphalaria* interactions. [35]

When activated to a cytotoxic state, hemocytes undergo a respiratory burst, which generates reactive oxygen and nitrogen species (ROS and RNS), to eliminate the invading parasite. Specifically, hydrogen peroxide and nitric oxide appear to be directly involved in hemocyte-mediated killing of schistosomes. *Biomphalaria* production of ROS, particularly H_2O_2 is known to play a crucial role in anti-schistosome defense. Moreover, hemocytes from *S. mansoni*-resistant snails have been shown to generate significantly more ROS than susceptible snails, and a reciprocal coevolution has been demonstrated between ROS and ROS scavengers produced by sympatric populations of *B. glabrata* and *S. mansoni*. [139-141]

Superoxide Dismutase enzyme (SOD) is a ubiquitous protein that is involved in several cellular functions inside *Biomphalaria* in the context of the snail immune response against *S. mansoni* infection. [142,143] According to the metal present at their active center, two distinct classes of SOD exist in metazoans: a mitochondrial form which contains manganese (Mn) and a cytosolic form which contains copper and zinc (Cu/Zn), the latter is named as (SOD_1). [144] Among the various functions of SOD_1, it catalyzes the reduction of highly reactive superoxide (O_2^-), into molecular oxygen (O_2) and H_2O_2. Hydrogen peroxide is in turn a known cytotoxic component in the process of the oxidative burst, which is the primary defense mechanism for parasite clearance in molluscs.When a schistosome invades a snail, hemocytes surround the invading parasite and then they generate H_2O_2 as a part of the killing mechanism. So, SOD plays an important role in snail's IDS. [107, 139,145.146]

Reactive superoxide (O_2^-) $\xrightarrow{\textbf{SOD}}$ **Hydrogen peroxide (H_2O_2)**

Another group of proteins was discovered and proven to aid in the immune response of *Biomphalaria*. Those are lectins named as, **Fibrinogen-related proteins (FREP)**, they are related to fibrinogen in vertebrate animals. They are present in the snail hemolymph, have been found to play a role in extracellular protein-protein interactions such as adhesion, coagulation, tissue repair, shell formation and receptor-ligand interactions forming a matrix that immobilizes the pathogen in a network of hemocytes. FREPs are encoded by a multigene family of at least fourteen members. The diverse genes encoding the FREP molecules undergo somatic modification involving gene conversion and point mutations, leading to a remarkable degree of diversification. These molecules are hemolymph lectins that precipitate soluble antigens derived from trematodes. They bind to sporocysts and have opsonic properties. It was found that FREP2 is markedly up-regulated following exposure of *B. glabrata* to *S. mansoni*. Additionally, FREP3 molecules differ among subsets of hemocytes, carrying the implication that all hemocytes of *Biomphalaria* are not functionally equivalent. [35,147]

Another interesting snail factor that was found to be co-immunoprecipitated with the molecular complex that comprised FREPs and parasite extracts, was an opsonin, named as ThioEster-containing Protein from *B. glabrata* **(BgTEP)**. Precursor and phylogenetic analyses suggest that BgTEP shares features of invertebrate TEPs. It was found to be involved in antiparasitic defence and microbe phagocytosis. [35,148]

Additional mollusk immune effector protein that is found to be involved in killing *S. mansoni*, is a cytolytic protein with hemolytic activity. It is related to ß pore forming toxin family (ß -PFT protein). This protein is named **Biomphalysin,** that was found to form channels in targeted parasite membranes leading to its cytolysis. Moreover, it is cytotoxic to the primary sporocysts, by binding to them leading to their vacuolization, focal lysis of the tegumental matrix and underlying muscle fibers and hence leads to their mortality. [149]

2- Snail Genetics

The interest in studying snail genetics was attributed to that fresh water snails in general, including *Biomphalaria* species, have been recognized as interesting biological models for studying population genetics, as they are hermaphrodites, so they can reproduce by self-fertilization beside cross-fertilization. Self-fertilization in turn, reduces genetic diversity within a population and increases genetic differentiation among populations. [150]

Although IDS is the major defending system in *Biomphalaria* against *S. mansoni* infection, [114,123] nevertheless, this molluscan innate immunity is primarily a genetically controlled one. Hence, snail genes represent part of the defense response. Over the past decade, a wealth of modern molecular tools have been made available to study snail genetics. The expression patterns of the known immune-related genes were found to differ between more resistant and less resistant strains when each is challenged with the same strain of parasite. [151,152] In this context, a single locus has been identified at which allelic variation clearly associates with resistance of *B. glabrata* to the parasite: copper-zinc superoxide dismutase (SOD_1). [153,154]

In the natural population, there is a varying degrees of susceptible snails, with certain individuals are more resistant than others, resistance being the dominant character.[155] Resistance to *S. mansoni* infection in *Biomphalaria* is a heritable character

that can occur in laboratory and field populations, and is almost certainly controlled by multiple loci. [156]

One of the earliest studies in the snail genetics field was done by Newton in 1952, who was the first to consider that susceptibility or resistance of *B. glabrata* to *S. mansoni* infection is a hereditary character. [157] Later on, Richards and Merritt confirmed these findings in 1972. [158]

Understanding the genetic mechanisms governing resistance and how resistance is maintained in vector populations is essential for the development of resistant vectors as a means of eradicating vector-borne diseases. This can lead to the development of a promising biological control method by driving resistance genes into *Biomphalaria* vector populations. In order to change the susceptibility of natural snail population from being predominantly highly susceptible to a non-susceptible state, the release of refractory snails into the snail natural habitats, has been tried as a new approach for biological control of schistosomiasis in endemic areas so that, snails that are resistant to parasitic infection could be used as biological competitors to replace existing susceptible snails. [159-162]

3- Snail Age

It was found that susceptibility to *S. mansoni* infection in *B. glabrata* is age dependant. Where, susceptibility of juvenile snails is controlled by at least four genes, each with multiple alleles. Once the snail reaches adulthood, most evidences indicate that it is a single gene trait with resistance dominance. [155]

Biomphalaria snails at all ages are liable to be infected with *S.mansoni*. However, age dependent variability in *B. glabrata* susceptibility to *S. mansoni* has been well documented with results showing that juvenile snails (even within the same stock) are, in general, more vulnerable than their adult counterparts to infection. [155,163,164]

B) Parasite Factors

There are two main mechanisms by which *S. mansoni* miracida locate their intermediate snail host. The **first** is their responses to the main physical factors present in the environment. *S.mansoni* miracidia possess positive phototaxis and negative geotaxis that match with *Biomphalaria* snails which naturally inhabit the superficial layers of water. The **second** is their response to chemical stimuli originating from snail hosts themselves.[165] Parsite related factors that can influence the compatibility between *S. mansoni* and *Biomphalaria* snail are;

1- Strain of *Schistosoma mansoni* Miracidia

Colonies of *Biomphalaria* were reported to be more susceptible to the local strain of *S.mansoni* than to strains from other localities. In this context, workers were unable to infect the Egyptian *B.alexandrina* with miracidia of a Puerto Rican strain of *S.mansoni*. Likewise, attempts to infect this Egyptian snail with West Indian, Venezuelan, Brazilian *S.mansoni* were unsuccessful. [166]

Furthermore, when snails from Giza Governorate were exposed to miracidia from Dakahlia Governorate, they showed lower susceptibility to the parasite than when they were exposed to miracidia from Giza Governorate, and vice versa. [116]

It was found that when *Biomphalaria* population and *S. mansoni* miracidia strain, are from the same locality, the number of cercarial output is higher than if they are from different localities. Accordingly, the genetic constitution of the miracidium is an important factor in determining its invasiveness to its host snail.[166,167]

2- Miracidial chemoreceptors and specificity of miracidial attraction

Characteristic pre-penetration behavioral changes in miracidia of *S.mansoni* were reported. Two chemicals might be involved in the process of penetration; one for attraction and another for stimulation of penetration. These chemical attractants may form component of snail mucus, and are composed of a mixture of aminoacids. Two types of chemo-receptors are present in the miracidium, one acting at a distance, possibly at low concentration and functions to attract the miracidium toward the snail, while the other one is a short range receptor that acts at higher concentrations, stimulating attachment and penetration.[168-170]

3- Number of Infecting Miracidia

The probability of infection of the snail host increases when the snail receives a higher number of infecting miracidia. [68] Cercarial output was found to rise with increasing number of the infecting miracidia from two to eight per individual snail. However, exposure to smaller dosages of miracidia renders snails survive longer than those received larger numbers of miracidia. [171]

4- Age of Infecting Miracidia

The hatchability of excreted *S. mansoni* eggs and also, the infectivity of the hatched *S. mansoni* miracidia decrease by time. The infectivity is maximum when the miracidium is freshly hatched within one to two hours of egg exposure to the environment outside the definitive host. [172] Miracidia of *S. mansoni* lose their ability to infect *Biomphalaria* snails gradually and they die about 24 to 48 hours after hatching. [33]

5- *Schistosoma mansoni* polymorphic mucins (SmPoMucs)

They are a family of schistosome antigens that share characteristics with molecules of mucin family. They are encoded by a multigene family of around 10 members that occupy four loci in the genome of *S. mansoni*. SmPoMuc precursor structure and expression analysis showed that these proteins display a mucin-like structure with an N-terminal domain containing a variable number of tandem repeats. They are highly glycosylated, only expressed by larval schistosome stages that interact with the snail intermediate host. Moreover, they are located in the apical gland of miracidia and sporocysts, secreted and released in excretory–secretory products and are highly polymorphic. Some *S. mansoni* individuals express only one gene, whereas others express several genes in combinations that differ among individuals. A comparative molecular approach was first developed using two *S. mansoni* laboratory strains: one of them is totally compatible (C strain) and the other is totally incompatible (IC strain) towards the same (Brazilian strain) *B. glabrata*. Newly penetrated parasites from the IC strain are contacted by host hemocytes within 1 hour post-infection and entirely encapsulated by 4–8 hours post-infection. In contrast, newly-penetrated miracidia of the C strain were not encapsulated and primary sporocyst developed normally. SmPoMucs are known to interact

with FREP immune receptors of *Biomphalaria*. This interaction may determine the compatible/ incompatible status the snail/schistosome combination. [35,173]

After summarizing factors affecting *Biomphalaria – S. mansoni* compatibility and after knowing that considerable variety of control strategies have been proposed within the current interest in eliminating schistosomiasis, with total elimination to have not been achieved yet, novel intervention tools to control schistosomiasis are needed. Additionally, given the limited options for treating *S. mansoni* infections in human host and knowing that breaking the chain of this parasite life cycle if achieved by targeting its intermediate host snail can for sure decrease its transmission, necessitate more research in this field. To make this goal a reality, better understanding of the immunobiological interactions between *Biomphalaria* and *S. mansoni* should be focused on. [2,174]

Recent advances in understanding the genetics of host-parasite interactions have increased the interest in driving resistance genes into susceptible vector populations to render them resistant or even with low susceptibility when challenged by the parasite. Making vector populations in the field resistant or even less susceptible to infection is of course a better ecologically safer long-term solution for breaking transmission cycles. [161,162,174,175] Thorough studying of the physiological and biochemical criteria that modulate *Biomphalaria* susceptibility to *S. mansoni* is pivotal to provide new insights in the control of the targeted mollusk. Studies concerning genetic variability of *Biomphalaria* with different degrees of susceptibility to *S. mansoni* infection can add to the development of control strategies invented for schistosomiasis. [176] In this context, the current work studied the impact of *B. alexandrina* snails' age, genetic background and internal defense on their compatibility pattern. This was achieved by using different parasitological parameters, SOD_1 enzyme assay and Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Enzyme assays are biochemical methods that are used to measure enzymatic activity where the quantity of active studied enzyme is measured. They measure either consumption of substrate or production of product over time. Many enzyme assay methods are available, they differ according to the sampling method.

In the current study, continuous spectrophotometric enzyme assay was used, where the assay gives a continuous reading of the enzyme activity through the course of reaction by measuring a change in how much light the assay solution absorbs, then color change can be seen. [177,178]

SDS-PAGE is an electrophoretic fractionation technique that is used for characterization of all proteins types and for estimation of protein subunit molecular weights (MW) with determination of the subunit compositions of purified proteins. It is used for separation of proteins from gels with estimation of protein concentration. This form of separation depends on the MW of polypeptides which in turn depends on their amino acid composition. Charged particles are caused to migrate towards the electrode of the opposite sign under the influence of an externally applied electric field. The mobility of the polypeptide particles are retarded by interaction with the surrounding gel matrix, which acts as a molecular sieving that result in different migration rates for the constituent proteins of a sample. During sample preparation for SDS-PAGE, sample proteins are treated with hot SDS which binds tightly to most proteins imparting a negative charge to the resultant complexes. Interaction with SDS disrupts all non-covalent protein bonds,

causing the macromolecules to unfold. Concomitant treatment with a disulfide-reducing agent, such as 2-mercaptoethanol, further denatures proteins, breaking them down to their constituent subunits. The electrophoretic mobility of the resultant detergent-polypeptide complexes assumes the same functional relationship to their MW. Migration occurs toward the anode at rates inversely proportional to their MW, thus polypeptides move through gels with low-molecular-weight complexes migrating faster than larger ones. [179,180]

The interplay of susceptibility of *Biomphalaria* snail to *S. mansoni* infection together with snail IDS represented in SOD_1 enzyme and with snail age is our concern in this work. This is a trial to clarify the factors affecting change of *B.alexandrina* susceptibility with snails' age and whether SOD_1 enzyme activity and protein composition of the snail have effect on snail susceptibility. This will be studied using parasitological parameters, spectrophotometric enzyme assay and SDS-PAGE at different snail ages.