Published in final edited form as:

Dev Comp Immunol. 2020 March ; 104: 103557. doi:10.1016/j.dci.2019.103557.

Biomphalaria glabrata immunity: Post-genome advances

Maria G. Castillo[a,*], **Judith E. Humphries**[b], **Marina M. Mourão**[c], **Joshua Marquez**[a], **Adrian Gonzalez**[a], **Cesar E. Montelongo**[a]

[a]Department of Biology, New Mexico State University, Las Cruces, NM 88003, USA

[b]Department of Biology, Lawrence University, Appleton WI 54911, USA

[c]Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz, Fiocruz Minas, Brazil

Abstract

The freshwater snail, *Biomphalaria glabrata*, is an important intermediate host in the life cycle for the human parasite *Schistosoma mansoni*, the causative agent of schistosomiasis. Current treatment and prevention strategies have not led to a significant decrease in disease transmission. However, the genome of *B. glabrata* was recently sequenced to provide additional resources to further our understanding of snail biology. This review presents an overview of recently published, postgenome studies related to the topic of snail immunity. Many of these reports expand on findings originated from the genome characterization. These novel studies include a complementary gene linkage map, analysis of the genome of the *B. glabrata* embryonic (Bge) cell line, as well as transcriptomic and proteomic studies looking at snail-parasite interactions and innate immune memory responses towards schistosomes. Also included are biochemical investigations on snail pheromones, neuropeptides, and attractants, as well as studies investigating the frontiers of molluscan epigenetics and cell signaling were also included. Findings support the current hypotheses on snail-parasite strain compatibility, and that snail host resistance to schistosome infection is dependent not only on genetics and expression, but on the ability to form multimeric molecular complexes in a timely and tissue-specific manner. The relevance of cell immunity is reinforced, while the importance of humoral factors, especially for secondary infections, is supported. Overall, these studies reflect an improved understanding on the diversity, specificity, and complexity of molluscan immune systems.

1. Introduction

1.1. Snails and their importance

Snails (phylum Mollusca, class Gastropoda) are a diverse group of molluscs that remains understudied despite their important roles in community ecosystems (reviewed in Wallace and Webster, 1996; Dillon, 2000; Sturrock, 2001; Gutiérrez et al., 2003), and disease transmission (Bayne and Loker, 2018). Snails are of medical, veterinary, and economic

*Corresponding author.mcastill@nmsu.edu (M.G. Castillo).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dci.2019.103557.

importance as they transmit diseases that affect many animals for instance birds, reptiles, amphibians, and mammals including humans (Loker and Mkoji, 2005). Notably, around the turn of the 20th century, freshwater snails were recognized as key players in the transmission of schistosomiasis, an important chronic parasitic disease of humans caused by trematode infections in tropical and subtropical regions around the world (reviewed in Sturrock, 2001; Miyairi and Suzuki, 1914; Leiper and Atkinson, 1915; Cook, 2007). Since then, these gastropods have been included among other medically relevant invertebrates such as mosquitos and ticks, as essential factors for control of parasitic diseases that affect humans.

The World Health Organization (WHO) acknowledges that snails serve as intermediary hosts in the parasitic zoonoses: schistosomiasis, trematodiasis, and angiostrongyliasis, 3 of 18 neglected tropical diseases (NTDs) (WHO, 2019). Snails are considered a target and integral component in long-term measures for controlling transmission of these aforementioned diseases. Despite challenges and transient setbacks in the efforts to control NTDs, progress has been made (Touré et al., 2008; Savioli et al., 2015). In recent years these endeavors have been reenergized worldwide, in part catalyzed by the London Declaration of 2012 and the World Health Organization's 2020 Roadmap that aim at, beyond controlling morbidity, the elimination of NTDs including schistosomiasis (Wang et al., 2012a; WHO, 2012). Special interest is given to snail-related research, specifically, studies focused on the immune system of vector snails and the role invertebrate immunity plays in defense against schistosome parasites. One goal of continuing the characterization of the snail immune system (e.g. of *Biomphalaria glabrata*) is to find biological targets that may lead to the development of complementary strategies to block or prevent schistosome infection in snails to prevent parasite transmission to the human host. Such investigations are anticipated to further advance the discovery of determinants of host susceptibility (permissive to infection and transmission) or resistance (refractory to infection, no transmission) to the parasite that can later be selected (Mendelian genetics) or genetically modified to reduce or prevent snail-to-human transmission.Furthermore, a better understanding of snails' genetics and physiology can also reveal unique characteristics in these organisms toward the identification of new targets for biological snail control in the field, or the development of novel chemicals that may be used as targeted molluscicides.

Thanks to advances in molecular and analytical tools, we are at a point where abundant genomic, transcriptomic, proteomic, and metabolomic data are available that provide information related to the immunobiome of invertebrates and disease-vectors, including gastropod snails such as those that transmit schistosomiasis (Hanelt et al., 2008; Adema et al., 2010; Ittiprasert et al., 2010; Lockyer et al., 2012; Zahoor et al., 2014; Buddenborg et al., 2017). The recent publication of the genome of the snail *Biomphalaria glabrata* (Adema et al., 2017), added a much-needed level of overarching genetic resources that have, and will continue to improve our understanding of the mechanistic basis of gastropod immunity by building further on the knowledge generated by previous studies. This is reflected by several recent post-genome studies that expand and advance insights into the diversity and function of *B. glabrata*'s immunity, most with special emphasis on the snail response to larval schistosome parasites and their secreted products. Considering that many recent investigations focus on a specific snail tissue or the interaction with either miracidia or

sporocysts, specific schistosome developmental stages, this review is organized according to these hostparasite interfaces. First we describe an overview of the intramolluscan schistosome larval stages and their specific association with snail intermediate hosts. Subsequent sections discuss novel scientific contributions and insights to snail immunity and how these associate with specific host-parasite interfaces. The final section identifies several current challenges and areas of research that may advance our understanding of snail-schistosome immune interactions.

2. *Biomphalaria glabrata*: A molluscan model

The three most important freshwater snails transmitting schistosome parasites to humans are gastropods in the families Planorbidae and Pomatiopsidae, with subclasses Pulmonata and Prosobranchiata respectively. *Schistosoma mansoni* and *Schistosoma haematobium* are transmitted by multiple species of *Biomphalaria* and by *Bulinus* snails respectively; while *Schistosoma japonicum* utilizes *Oncomelania* as vector snails (reviewed in Adema and Loker, 2015). In this review, we will focus on the most studied snail vector model, *Biomphalaria glabrata* that transmits *S. mansoni* in South America, the Caribbean, and in some regions of North Africa.

Globally, nine species of *Biomphalaria* have been found naturally infected with *S. mansoni*: *Biomphalaria glabrata* (Say, 1818), *Biomphalaria pfeifferi* (Krauss, 1848), *Biomphalaria alexandrina* (Engels et al., 2002), *Biomphalaria sudanica* (Martens, 1870), *Biomphalaria tenagophila* (Orbigny, 1835), *Biomphalaria straminea* (Dunker, 1848), *Biomphalaria choanomphala* (Martens, 1879), *Biomphalaria prona* (Martens, 1873), and *Biomphalaria camerunensis* (Boettger, 1941) (Carvalho et al., 2008; Caldeira et al., 2016). However, many other *Biomphalaria* species are susceptible to infection if experimentally exposed in the laboratory. Of all these species, research emphasis has historically been placed on *B. glabrata* as a laboratory research model to study snailtrematode interactions, culminating in selection of a Brazilian strain of this species to study the genome of *B. glabrata* (BB02) as intermediate host of *S. mansoni* (Adema et al., 2017).

3. Schistosomiasis

3.1. Disease transmission, prevention, treatment, and control

Schistosomiasis is a chronic parasitic disease that has been reported in 78 countries around the world (WHO, 2019), with more than 220.8 million people requiring preventive treatment in 2017, and 770 million people at risk of infection (Steinmann et al., 2006; Hotez et al., 2014; WHO, 2017WHO, 2019). In 2016, the global burden of schistosomiasis was estimated at over 1.9 million disability adjusted life years (DALYs) (GBD, 2016 DALYs and HALE Colley et al., 2017). Until recently, the WHO's morbidity reduction and eradication strategies relied heavily on chemotherapy administration as a preventive and therapeutic measure (WHO, 2002; Wilson et al., 2008). For many years control has entailed the use of just a single drug, praziquantel, the only drug effective against the adult stage of all *Schistosoma* species, although resistance has been described in the laboratory (Fallon et al., 1996, 1997; William et al., 2001; Botros et al., 2005) and suspected in the field (Ismail et al., 1999; Melman et al., 2009; Wang et al., 2012b). The low efficacy of drug-

centered strategies stresses the need to incorporate complementary approaches for disease control, including implementation of sanitary and hygiene measures, and intermediate host suppression (Engels et al., 2002; Colley et al., 2017; Toor et al., 2018). In addition to the risk of spreading or shifting schistosomiasis to new areas through human migration (Boissier et al., 2015; Kincaid-Smith et al., 2017), there is the impending threat that climate change may alter the distribution and natural susceptibility of vector snails (Stensgaard et al., 2019). Vertebrate definite hosts are infected through contact with contaminated water that contains cercariae of *Schistosoma* parasites (e; Fig. 1). Several key aspects contribute to the reduced efficacy of human-based interventions to control schistosomiasis infections, including that a vaccine is not yet available, and that treatment with drugs does not prevent (rapid) reinfection, especially when life-sustaining activities of endemic populations challenge avoidance of contaminated waters. Thus, populations at risk are usually exposed to multiple infections throughout their lives causing long-term and permanent developmental and physiological problems, additional to chronic abdominal pain, diarrhea, anemia and malnutrition as prominent pathologies (King et al., 2015). With transmission driven not only by biological but also by ecological, sociocultural, economic, and political factors the complex nature of this disease require multifaceted strategies must be adopted to control, and eventually eradicate schistosomiasis (Krauth et al., 2019).

In the past, several methods to control snail populations in the field have been implemented with various degrees of success (reviewed in Sokolow et al., 2016). However, efficiency of such methods was highly dependent on the specific locality and environment, making their implementation on a global scale challenging (Clennon et al., 2006; Gurarie and Seto, 2009; Mari et al., 2017; Gurarie et al., 2018). These strategies included the use of chemical and biological molluscicides, snail competitors/predators, and environmental management (McCullough et al., 1980; McCullough, 1981; Pieri and Thomas, 1987; Lardans and Dissous, 1998; Mkoji et al., 1999; King et al., 2015). Similar to other vector organisms of human disease (Wang and JacobsLorena, 2013; Kyrou et al., 2018), snails are considered as target for genetic modification to enhance or improve natural traits by which they resist infection or prevent the intramolluscan development of schistosome parasites. In addition, it is expected that novel transgenic techniques such as CRISPR-Cas9 will be valuable in functional genomic studies of important immune candidate genes as the methods continue to be adapted to molluscan systems (Abe and Kuroda, 2019; McVeigh and Maule, 2019). An obvious group of potential targets for genetic modification are immune-related genes and the molecules associated with expression and modulation of such genes. Thus, it is critical to understand the snail response and immunity to the parasite.

3.2. Snails as obligate intermediate hosts

A characteristic of schistosome parasite life cycles is the involvement of two animal hosts; in addition to the vertebrate definitive host, freshwater snails are required as (obligate) intermediate host. The snail species suitable as host varies depending on the schistosome species, usually with a strict parasite-host compatibility at the level of species and strain. The development of *S. mansoni* in the intermediate host *B. glabrata* (described below) is characteristic for the complex intramolluscan biology and intimate, long term interactions with the snail host of various schistosome species.

With excreta, vertebrate hosts release fully embryonated **eggs** into aquatic habitats of snails (a; Fig. 1), where the changes in osmolarity, light, and temperature (15–30 °C) stimulate the hatching of ciliated **miracidia** (Upatham, 1972) (b; Fig. 1), the schistosome larval stage that is infective to the snail intermediate host. **Miracidia** are freeliving, highly motile swimmers due to multiple rows of ciliated plates that cover their body surface. Miracidia locate snail hosts by employing chemical signals that are as yet not well understood (see section 5.3, Wang et al., 2019). After attaching with the apical papilla by suction or adhesion to snail surface epithelia (usually on the exposed headfoot area; reviewed in Whittington and Cribb, 2001; LoVerde, 1975), miracidia penetrate into the snail soft tissue, aided by secretions from the penetration glands and possibly the primordial gut (Kinoti, 1971; Pan, 1980). One of these secretory components may be *S. mansoni* venom allergen-like 9 (SmVAL9), proposed to also aid in egg release from the mammalian host, infection of the snail host, and intra-molluscan migration and development (Yoshino et al., 2014). Upon penetration into snail tissues, a **miracidium** sheds its ciliated plates exposing a tegument with microvilli (Basch and DiConza, 1974; Bayne et al., 1980). At this stage, the parasite has transformed in to a **primary sporocyst** and will remain close to the infection site for at least the next 48 h (c; Fig. 1). This developmental stage of the parasite is considered the main target of the snail's immune response (see Snail immunity, section 4). The surface of primary sporocysts is covered by a continuous tegument or syncytium (Smith and Chernin, 1974) that is about 0.5 μm thick and contains multiple vesicles (Basch and DiConza, 1974). The schistosome syncytium is thought to be an important antigenic target and a challenge to the host's immune system, as it changes in composition throughout the life stages of the parasite (Simpson et al., 1984; Robijn et al., 2005; Braschi et al., 2006). If the **primary sporocyst** is in a compatible snail host that permits development, the larval parasite will survive and grow. In this regards, a recent study identified a sporocyst secreted metalloprotease, *S. mansoni* leishmanolysin (Hambrook et al., 2018), that interferes with snail hemocyte migration, and thus encapsulation of sporocysts. *In vivo* studies describe that primary sporocysts actively migrate inside host tissues, possibly following open sinuses towards the mantle, leading to concentrating parasites in the snail hepatopancreas (digestive gland) (Jourdane and Théron, 1987). At this time, primary sporocysts contain multiple **secondary** (also called **daughter**) **sporocysts** (d; Fig. 1). As described by Smith and Chernin (1974), 6–13 days after infecting the snail host, a primary sporocyst is "a sack filled with daughter sporocysts in various developmental stages". **Daughter sporocysts** originate from germinal cells and mature in about 8–10 days with observed densities of 20–25 per primary sporocyst (Hansen, 1975). Mature secondary sporocysts are released from the mother sporocyst and migrate towards the snail ovotestis, while germinal cells inside the daughter sporocysts produce additional generations of (secondary) sporocysts. Alternatively, numerous **cercariae** (the last intramolluscan larval stage) will develop inside a proportion of these secondary sporocysts (d, e; Fig. 1). Cercarial output per miracidium ranges from hundreds to thousands depending on the schistosome species, but is also influenced by the snail age, size, nutrition status, other parasite coinfections, and the level of snailschistosome compatibility (reviewed in Sturrock, 2001). **Cercariae** are the infective stage for the (vertebrate) definite host (e; Fig. 1). Ceracariae exit the secondary sporocyst and migrate towards the snail's surface epithelia and emerge from the snail host into their aquatic habitat and swim in search of a mammalian host to continue the transmission cycle. Cercariae are small (~150 μm in length), almost

invisible to the naked eye (Salter et al., 2000). Infection occurs by means of skin penetration, a rapid (few minutes) process that is not detected by the definitive host. Initial adhesion to and penetration of human skin is aided physically via the cercarial oral sucker, and chemically by adhesive secretions and proteolytic enzymes from the preacetabular and post-acetabular glands of the parasite (reviewed in Whittington and Cribb, 2001; Haas et al., 1997a,b). During skin penetration of the vertebrate host, a cercaria loses its tail and transforms to a schistosomulum, the last and only larval stage of the parasite in the definite host. After about 72–96 h the schistosomula leave the epidermis and migrate through the vascular and lymphatic systems to reach the liver where they mature (Wilson, 1987). In 4–5 weeks, male and female adult worms pair up (f; Fig. 1) and using their ventral suckers migrate via the portal system to their final residency, the mesenteric vessels. Once there, adult worms remain paired for the rest of their lives while producing eggs that will be released in the environment for the life cycle to continue.

4. Snail immunity

Starting at the moment a *Schistosoma* miracidium penetrates the surface epithelium of a compatible snail, signals released from damaged tissue and parasite-derived molecules (released or secreted) are detected by the host. If the snail is from a resistant strain, these signals will initiate an immune response to prevent the parasite from establishing and continuing development to sporocyst. This is not true for susceptible snails, where the response is somewhat inadequate and the parasite finds a favorable environment to grow and develop. Technically, it has been difficult to study snail host-parasite *in situ* interactions, due mainly to the small size of the parasites, their nonhomogenous distribution in snail tissues, and the variability among individual snails in terms of resistance and susceptibility to infection. Recognizing the pivotal role in the transmission of the disease to humans, many of the longstanding studies of schistosomiasis have focused on understanding the physiology and biology of the snail. Research focusing on the snail immune response has taken multiple approaches to elucidate what defines host suitability for schistosome development. Recent transcriptome studies are examples of investigations of systemic reactions in whole snail tissues (Lockyer et al., 2007, 2008; Hanington et al., 2010a; Dheilly et al., 2014; Buddenborg et al., 2017; Mansour et al., 2017; Portet et al., 2017). Following discovery of the importance of hemocytes (Cheng, 1975) (i; Fig. 1) in the snail antiparasitic response (Harris, 1975; Harris and Cheng, 1975; Bayne et al., 1980; Loker et al., 1982), these cells have been the focus of much research, as well as the soluble components found in the plasma (blood fluid) that hemocytes are bathed in, and the tissues associated with hemocyte proliferation such as the amebocyte producing organ (Joky et al., 1985; Sullivan and Spence, 1994; Sullivan et al., 1995). A different approach to study snail immunity has been to examine tissues relevant to specific stages of schistosome infection, such as the headfoot region, which is penetrated during early infection by primary sporocysts (c; Fig. 1), or the hepatopancreas region where secondary sporocysts develop (d; Fig. 1). In addition to *in vivo* studies, *in vitro* experiments using snail secretions, hemolymph, or the *B. glabrata* embryonic (Bge) cell line (h; Fig. 1) in co-culture with parasite larval stages have also provided important insights into the molecular aspects of host-parasite interactions. Finally, studies on the interactions of schistosomes with other snail developmental stages

(embryos, juveniles; g; Fig. 1) and snail species (*Bulinus spp.*, *Oncomelania spp.*) (j; Fig. 1) are also extremely valuable. Table 1 lists a summary of molecules for which direct activity has been assessed and linked to the *B. glabrata* immune response to *S. mansoni*. Many more molecules have been associated with the response or resistance to schistosomes, especially through transcriptome and proteomic studies, however, the specific functions of these molecules in defense require further characterization. There is still much to learn about the mechanisms and molecular pathways of *B. glabrata* anti-schistosome responses, and about the complexities of species and strain compatibilities that are observed in the field. Several recent reviews provide comprehensive cover of the major aspects of cellular and humoral immunity in snails (Bayne, 2009; Yoshino and Coustau, 2011; Knight et al., 2014; Adema and Loker, 2015; Coustau et al., 2015; Pila et al., 2017; Famakinde, 2018; Loker and Bayne, 2018; Schultz et al., 2018). In the present review we focus on the advances in snail immunology and related topics that have been made since the publication of the *B. glabrata* genome (Adema et al., 2017). In addition, two sections include new insights in relation to immune receptors, cytokines, and signaling pathways that were only made possible through the analysis of genomic data. Finally, and with a look toward the future, we discuss areas of scientific exploration that likely will advance our understanding of snail immune processes and that may be utilized in the pursuit of eradicating schistosomiasis. Whenever possible, information is organized in reference to the snail-schistosome interfaces defined by the parasite developmental stages (miracidia, sporocysts, and cercariae).

As invertebrates, the *B. glabrata*'s defense system consists only of innate immune components, with representatives of the major common groups of innate immune molecules. These include pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) and lipopolysaccharide-binding proteins and bactericidal/permeability-increasing proteins (LBPs/BPIs) that recognize and bind to pathogen-associated molecular patterns (PAMPs). Snails also possess soluble immune molecules such as antimicrobial peptides, lectins, and complement-related molecules as part of their humoral response capabilities (see Table 1). Some of these immune constituents are expressed in a generalized (nonspecific) manner in response to immune challenge (Coustau et al., 2015). Others are more pathogen specific, as reported in transcriptome analyses (Hanelt et al., 2008; Adema et al., 2010 and Deleury et al., 2012) and with the expression of selective molecules such as fibrinogen-related proteins (FREPs) (Zhang et al., 2008). A prominent aspect of the detectable response in snail tissues during a primary *S. mansoni* infection is contributed by the hemocytes (Bender et al., 2005; reviewed in Loker, 2010). These defense cells circulate in the hemolymph and are found throughout snail tissues. Hemocytes migrate to sites where schistosome sporocysts are located, bind to the sporocysts' tegument and form a multi-layered cellular capsule around these parasites (Loker et al., 1982). Hemocytes within these encapsulating layers secrete toxic compounds, mainly reactive oxygen species (ROS) that will eventually kill the invading parasite (Hahn et al., 2001a). Direct hemocyte-mediated cytotoxicity is not the only means to killing *S. mansoni* larvae; in resistant snails, some primary sporocysts are killed without any evidence of a cellular encapsulation or the presence of hemocytes in the surrounding areas. This suggests that humoral components can also contribute effective anti-schistosome reactions (Galinier et al., 2013). In addition, recent publications report

the protective characteristics of humoral components and their involvement in antiparasitic responses during secondary infections (see section 5.4.3).

5. Post-genome advances

5.1. B. glabrata genome

The *B. glabrata* genome (Adema et al., 2017) plus accompanying analyses and datasets represent valuable resources for a variety of research topics including host-parasite interactions, invertebrate biology, and innate immunity. Furthermore, it also represents a good source of information for those interested in other molluscan and invertebrate organisms for which transcriptome data exists but no genomic resources are currently available. In addition, for *Biomphalaria*, access to the genome data offers an opportunity to study gene families for which only transcriptomic and proteomic data was previously available, and the prospect to correlate previous experimental observations with genetic data. This report offered quantitative information regarding the specifics of *B. glabrata* genomics that can be used in comparative analyses with other molluscs. For example, comparative analyses revealed a relatively small number of genes coding for antimicrobial peptides in *B. glabrata* compared to other invertebrates, and *B. glabrata* has uncommon biosynthetic pathways lacking several enzymes that are commonly used in invertebrates to make steroids from cholesterol. The genome publication also highlights several areas that could be explored to aid in the efforts to control snail populations in the field. To illustrate, the presence of the *Capsaspora* snail symbiont was confirmed (Hertel et al., 2002), and a previously unknown mycoplasma endosymbiont was discovered; both symbionts could potentially be used as vehicles for introducing genetic modifications to the snail host. In addition, genes involved in biomineralization, detoxification mechanisms, and snail chemosensory attraction, were identified as potential targets to be used in developing snail control methods.

The innate immune system of *B. glabrata* was a key topic in the genome report. Important to note is that many of the stress-and immune-related molecules previously reported to be important for the snail, particularly in response to schistosome parasites, were not present as single coding genes, but instead were members of multigene families. This important information may in part explain the previous difficulties in determining differences in gene expression between resistant and susceptible strains of *B. glabrata*, especially if gene identification was based on short or shared sequences. The genomic data now offers the opportunity to investigate whether the differentially expressed gene variants are associated with resistance traits. This is not a new idea, as previous research has demonstrated the complexity of *B. glabrata's* immune response exemplified with the fibrinogen related proteins (FREPs) (Gordy et al., 2015). This diverse family of proteins is coded by about 20 genes that can be somatically mutated differently in each individual snail (Zhang and Loker, 2004). Another molecule that has raised interest is biomphalysin, a putative pore-forming protein associated with innate immune memory (Pinaud et al., 2016, 2019). Sequencing of the genome revealed that these proteins are actually encoded by a family of 21 different genes. It would be useful to determine if all of these gene products share similar functions, or if expression of unique genes or alleles associates with specific tissues

and immune responses to various pathogens. Interestingly, the *B. glabrata* genome also contains multigene families of pattern recognition receptors such as TLRs, PGRPs, and LBPs/BPIs; with 56 genes (27 coding complete proteins), eight, and five respectively. Furthermore, the genome analysis confirmed the presence of genes important for stress responses, such as heat shock proteins (HSPs), and proteins associated with production of reactive oxygen and nitrogen species (ROS/NOS). The snail genome also revealed a role for epigenetic regulation, harboring enzymes involved in chromatin modification, including methyltransferases, demethylases, deacetylases and acetyltransferases, as well as transcript regulation by microRNAs. Exploration of cytokines and signaling pathways involved in cellular communication and activation resulted in finding homologs of IL-17, TNF, and components of the NF-κB pathway. This is just the "tip of the iceberg", and an example of yet additional data that can be extracted from the genome; sections 5.5.4 and 5.7 below present new insights into the diversity and signaling associated with thioester-containing proteins and cytokines, and their potential transcription binding sites.

The post-genomic publications reviewed here present a genome for the Bge cells (Wheeler et al., 2018), proteomic studies on parasite-host interactions using *in vitro* assays (Dinguirard et al., 2018; Wu et al., 2017), innate memory and antiparasitic responses (Gourbal et al., 2018; Portet et al., 2018; Pinaud et al., 2019), the genetic basis of resistance (Tennessen et al., 2017; Allan et al., 2017; Allan et al., 2018a, b), snail pheromones and neuropeptides (Pila et al., 2017; Wang et al., 2019), attractants for miracidia (Wang et al., 2019), and epigenetics (Geyer et al., 2017; Queiroz et al., 2017).

5.2. Bge cell line genome

The availability of an immortal cell line from *B. glabrata*, established by Hansen (1976), has always been considered a useful tool to investigate the molecular and genomic aspects of snail biology (Coustau and Yoshino, 2000; Yoshino et al., 2013b). The Bge cell line was instrumental for the development of an *in vitro* culture system for schistosome and echinostome larval stages (DiConza and Basch, 1974; Basch and DiConza, 1974; Yoshino and Laursen, 1995; Yoshino and Laursen, 1995; Coustau et al., 1997; Ataev et al., 1998; Bixler et al., 2001), and for the study of cell-to-cell interactions between snails and parasites (Yoshino et al., 1999; Vermeire and Yoshino, 2007; Humphries and Yoshino, 2006; Castillo et al., 2007; Geyer et al., 2017; Wright et al., 2017). The Bge cell line originated from a trypsinized 4–5-day-old embryo of an albino strain *B. glabrata* that was susceptible to *S. mansoni* infection (Hansen, 1976). Although the precise cell type that gave rise to Bge cells is not known, the morphological and behavioral characteristics are very similar to those of snail hemocytes, and as a result Bge cells have been extensively used to study host-parasite interactions and encapsulation reactions. In recent years, interest in Bge cells has grown because of potential uses as a tool in the development of genemodifying protocols such as gene editing via CRISPR, or transcript control modification with miRNA (see Epigenetics section 5.6).

As a resource for the use of the Bge cell line, Wheeler et al. (2018) sequenced the genome of one of the three Bge cell line subcultures available (Bge3). This effort addressed several key questions to answer were: how similar/dissimilar are the genomes of these Bge cells

compared to the whole organism (B. glabrata)? And, can research findings from Bge cells be easily translated with confidence to the *in vivo* system? Such concerns deserve significant consideration, especially in light of previously report of extensive mutations and aneuploidy in two other Bge subcultures (Odoemelam et al., 2009). Wheeler et al. (2018) reported mapping 98.6% of the Bge3 cell genome reads to the *B. glabrata* reference genome and confirmed aneuploidy in Bge cells by determining that the read coverage varied among the 18 linkage groups previously published by Tennessen et al. (2017). The genomic differences between Bge3 and the reference genome were further evident from analysis that identified 10,031,395 single nucleotide variants (SNPs) that after filtering, were determined to affect 3,277 transcripts. These gene products contained “high impact” variants due to mutations with a high theoretical possibility of affecting protein structure and/or function, that is, mutations predicted to occur in exon/intron splice regions, or that would result in the gain or loss of a start/stop codon. These Gene ontology (GO) analysis showed that the majority of the affected transcripts were in categories involved in binding activity (cell-substrate or cell-matrix), and many were cytoskeletal components. It was concluded that some of these mutations are characteristic of immortalized cell lines, likely resulting from selection pressures that favor the capacity to adhere to a substrate during culture conditions. Verification by PCR and Sanger sequencing confirmed mutations in 88% of 151 *in-silico* predicted transcript variants. Data from the variant analysis are accessible through VectorBase (https://www.vectorbase.org) in support of research employing the Bge cell line.

The above findings suggest that many years of active culture and passaging of Bge cells in laboratories have resulted in mixed, non-clonal cell populations showing considerable aneuploidy from one another, giving rise to the need to verify sequences of interest before engaging in experimental procedures. The original cell line had a diploid chromosome number of 36, as snails do (Hansen, 1976; Bayne et al., 1978), while the population of Bge3 cells studied had an average of 62 chromosomes, similar to a previous study that showed two other Bge subcultures to be a mixture of cells with 63 and 67 chromosomes each (Odoemelam et al., 2009).

Regardless, the Bge cell line is still the only molluscan immortal cell line; its ease of use and maintenance makes it a valuable resource and tool that is considered in the development and design of experimental procedures difficult to perform on whole snails, especially gene editing and transgenics.

5.3. Attractants and pheromones: Miracidia interface

It remains a basic question as to how *S. mansoni* miracidia locate snail hosts in the water. The parasite is thought to employ chemosensory receptors to follow bioactive molecules released or secreted by their invertebrate hosts. Previous reports have shown that miracidia swim following a chemical gradient from snail secretions (Kalbe et al., 1997), including those of *S. mansoni* (Chernin, 1970; Roberts et al., 1979, 1980). If the chemicals responsible for attracting newly hatched miracidia were identified, then miracidial migration could be possibly manipulated to prevent infection of new snail hosts. Recently Wang et al. (2019) followed this notion, with the development of an assay to observe and measure the movements of *S. mansoni* miracidia in an aqueous environment to test and record

the effects of whole and fractionated snail secretions, or synthetic peptides on miracidial swimming behavior (velocity, angularity, and tortuosity). Mass spectrometry allowed the identification of a single fraction from snail secretions containing multiple peptides that attracted miracidia. Within that fraction, one peptide designated P12, was responsible for the activity. P12 was identified as a 13 amino acid long peptide containing alpha-helix secondary structure and derived from a larger, water soluble precursor. The precursor was found in various tissues including the central nervous system. This study is the first to specifically identify a short compound of snail origin that attracts *S. mansoni*. Previous studies have only identified small chemical molecules (Macinnis, 1965) or a mixture of snail mucus glycoconjugates (Haberl and Haas, 1992; Kalbe et al., 1996; Haberl and Haas, 1992). Wang et al. (2019) suggest that P12 peptides may be used to lure miracidia away from snails in natural environments or to identify the receptor in the parasite in order to block it and reduce snail infections.

An alternative approach to control transmission involves removing snails from areas where people and other animals risk exposure to parasites. This may be effected by using an attractant for the snail host. An example of such candidate attractant is a recently reported temptinlike protein produced by *B. glabrata* snails (Pila et al., 2017). Temptin was originally identified as a pheromone released by *Aplysia californica* (Sea Hare, Euopistobranchiae, Cummins et al., 2004, 2005) along with other proteins during mating aggregations (Cummins and Bowie, 2012). Based on a partial EST with homology to temptin (Adema et al., 2006), Pila et al. (2017) a complete coding sequence from the *B. glabrata* genome was obtained and subsequently used as a recombinantlyexpressed protein in maze experiments to quantitatively test attractiveness of temptin. Results showed that *B. glabrata* snails move towards temptin in a specific and concentration-dependent manner. The authors indicated that it remains to be tested whether snails infected with *S. mansoni* respond in a similar manner to this pheromone, or if infection changes the behavior of the snail.

5.4. Sporocyst interface

The following section summarize new findings from either the use of sporocyst stages or that focused on interaction between snail host and the sporocyst stage.

5.4.1. Guadaloupe resistance complex—Tennessen et al. (2015a) selected laboratory resistant and susceptible snails out of a field population from the island of Guadaloupe. After 10 generations of in-breeding, they identified a region of the *B. glabrata* genome associated with snail resistance to *S. mansoni* infection containing 15 genes. This genomic region was named the Guadeloupe Resistance Complex (GRC), with three phenotype forms: one resistant and two susceptible. Additionally, the resistant haplotype was dominant. The 15 genes in the GRC had no known homologs, but seven genes contained domains characteristic of transmembrane proteins, and therefore could serve as cell surface receptors. Interestingly, there was no significant difference in expression of GCR-genes between resistant and susceptible individuals when exposed to the parasite. This led the authors to note that the correlation between the GRC genes and snail phenotype (resistant or susceptible) was not due to different gene expression levels, but instead most likely due to the inherent amino acid divergence of the products from the various alleles.

Tennessen et al. (2015a) described the natural *B. glabrata* population in Guadaloupe to be naturally resistant at a level of 50–60%; after selection, this resistance increased to about 80–90% in homozygous RR snails, while homozygous susceptible snails were infected at a rate of about 80% (Tennessen et al., 2015b; Allan et al., 2018). Most of the protein divergence among phenotypes was located on the extracellular domains of the putative transmembrane proteins. The authors hypothesized that these proteins could recognize PAMPs such as *S. mansoni* surface glycoproteins (SmPoMucs) (Roger et al., 2008a, b) in a similar fashion to FREPs (Adema et al., 1997; Moné et al., 2010; Mitta et al., 2012), and that resistant snails have more efficient receptors than susceptible individuals.

Subsequent studies further characterized two of the GRC transmembrane-coding genes (*grctm5* and *grctm6*). These two genes deserved focus because comparison of resistant and susceptible alleles showed non-synonymous amino acid substitutions (Allan et al., 2017). *grctm5* encoded for a fibrinogen type-III protein similar to chitinase, while *grctm6* encoded a single-pass transmembrane protein with structural similarity to TLRs and Fc receptors. The Grctm6 transcript was found in all tissues tested but expression was more abundant in the headfoot area. However, the GRC story is complicated; one of the susceptible GRC-genotypes was found to have about two-times higher constitutive expression levels of *grctm6* than the resistant phenotype. This further support the notion that it is more plausible that resistance correlates with the actual amino acid differences among GRC alleles and the function of the proteins *in vivo*. At protein level, Grctm6 could only be detected in whole hemolymph, but not in hemocytes or cell-free plasma alone. Furthermore, the immune role of Grctm6 was confirmed when RNA interference knocking down led to an increased the cercarial release, by 3–4 times in resistant snails challenged with *S. mansoni* miracidia when Grctm6 protein was low (day three post RNAi-treatment). This led to the conclusion that Grctm6 may control the number of miracidia infecting a snail, or alternatively, is part of the mechanism that regulates parasite development into cercariae and/or their release. Once genetic manipulation is available, genes in the GRC such as *grctm6*, could be manipulated in *B. glabrata* field populations to reduce host suitability and susceptibility to schistosome infection. Finally, Allan et al. (2017) noted that not only is the study of the gene products associated with the GRC valuable, but that identification of the antigenic targets of these snail receptors could be used to make the parasites easier to recognize and more susceptible to attack by snail defense responses.

Two additional studies furthered the analyses of the GRC phenotypes (Allan et al., 2018a, b). To test the effect of the genes in the GRC during the early stages of infection, snails with resistant or susceptible haplotypes were exposed to *S. mansoni*. Histological studies disclosed no difference in the composition or structure of the headfoot's surface epithelia among snails, nor in the number of invading miracidia, or the number of developing sporocysts within the first 24–48 h. However, snails carrying the susceptible alleles had more unencapsulated sporocysts after 24 h exposure than snails with the resistant haplotypes. In addition, resistance could be transferred to snails with susceptible GRC genotypes by injecting whole hemolymph from resistant snails. Furthermore, this resistance was not transferable when injecting cell-free plasma, suggesting the importance of hemocytes in the early stages of antiparasitic response. These results differ from other studies that report the transfer of resistance to susceptible snails through cell free

hemolymph (Pereira et al., 2008; Pinaud et al., 2016). An explanation for such different results could be that genes not coded in the GRC can confer resistance to snails, also Pereira et al. (2008) used different species and strains of snails (*B. tenagophila*) and parasites, and the Pinaud group (2016) was testing the immune properties of plasma on snails with secondary *S. mansoni* infections. To investigate the possible differences in cytotoxic capabilities among snails from various GRC haplotypes, Allan and Blouin (2018b) tested the release of hydrogen peroxide (H_2O_2) by snail hemocytes after exposure to various PAMPs. Hemocytes from the three GRC haplotypes demonstrated similar responses to 6 of the 8 PAMPs used. There was no difference in hemocyte capacity to produce H_2O_2, this indicates that the cellular machinery to produce ROS in a defensive manner is not affected by the genetic variance in GRC alleles. Only one of the susceptible GRC haplotypes had ~60% reduction in H_2O_2 production compared to the other two genotypes when exposed to BSA-galactose. Based on these results, it was concluded that one or more of the GRC products act as lectin-like receptors with a binding specificity for galactose-containing glycoconjugates. Because none of the GRC genes was directly linked to the re-cognition of galactose, an alternative explanation could be that a different gene (not in the GRC) is responsible for the lectin activity, or that one of the genes in the GRC may be working in conjunction with another gene not accounted for in the genotype testing.

In a separate but related publication (Tennessen et al., 2017), a new linkage map for the *B. glabrata* genome was generated using targeted capture methods. This work was performed in order to reduce the number of more than 300,000 scaffolds in the original genome assembly (Adema et al., 2017). The updated approach mapped ~75% of the genes in the *B. glabrata* genome to 18,613 scaffolds. Eighteen of these scaffolds were large enough (> 10 Mb) to theoretically represent the snail haploid chromosomes. As an example of the accuracy of the new linkage map, three genes (*actin*, *ferritin*, and *hsp70*) that were previously localized in different chromosomes (Adema et al., 2017), were assigned to separate linkage groups. This linkage map was used for associating gene loci of interest with nearby genes and control regions. With this strategy, it was confirmed that genes known to be important for the response to schistosome parasites are organized into what could be considered immunity loci or clusters. For instance, the newly discovered GRC was localized in cluster IX along with the previously identified genes for *sod1*, *prx4*, *cat*, and biomphalysin (Blouin et al., 2013; Tennessen et al., 2015b), as well as with genes for a TLR and spondin-1 (Mitta et al., 2005; Pila et al., 2016a).

5.4.2. Proteomics—Soluble snail proteins are thought to be important in the recognition and killing of sporocysts. Nonetheless, the molecules that are specifically involved in binding to the parasite surface, and how this binding contributes to effective defense, remain to be fully characterized. Facilitated by the genome sequence data, proteomics approaches were utilized to help answer these queries by investigating the interaction between *S. mansoni* sporocysts and plasma components of *B. glabrata*; Wu et al. (2017) used BS90 and NMRI snail strains, while Tetreau et al. (2017) used BRE *B. glabrata*. Both studies employed *in vitro*-transformed *S. mansoni* sporocysts for mass spectrometry analysis after incubation with *B. glabrata* plasma (cell-free hemolymph) from unexposed snails. Proteins identified in plasma from unexposed snails comprised the constitutively

expressed *B. glabrata* plasma-proteome. Any snail proteins found bound to sporocysts represented components that could be associating with the parasite during the first hours of infection *in vivo*. These two studies corroborated each other in revealing similar cohorts of snail proteins that bind to sporocysts, including: biomphalysin, dermatopontin, collagen, hemoglobin, acetylcholine binding protein, adisintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), and alpha amylase (Tetreau et al., 2017; Wu et al., 2017). However, the results of the two studies also contrasted; for instance, Tetreau et al. (2017) did not detect the binding to sporocysts of plasma lectins, such as FREPs. In contrast, Wu et al. (2017) identified the presence of FREPs, CREPS, and GREPs proteins associated with sporocysts. Perhaps the lack of lectin binding in Tetreau et al.'s study is due to methodological differences. Furthermore, Wu et al. (2017) utilized two different snail strains, allowing the comparison of the plasma of binding properties of resistant (BS90) and susceptible (NMRI) snails. Interestingly, the latter study showed that multiple immune factors are constitutively expressed in both strains prior to infection: LPS-BPI, TEP, CD109, HSP 70 and 60, biomphalysin, dermatopontin, FREPs, CREPs and GREPs. Between the two strains, however, protein expression patterns clearly change following parasite exposure, in agreement with previous reports of the differential expression of some of these proteins (Hertel et al., 2005; Hanington et al., 2010b; Portet et al., 2018). Although the plasma of NMRI and BS90 snails shared the presence of several sporocyst-binding proteins, strain specific differences were also apparent. ADAMTS binding to sporocysts was detected from the NMRI plasma only. In addition, a new GREP was identified with different isoforms in BS90 (GREP1.1) and NMRI (GREP1.2). Interestingly, GREP1.1 expression was detected in all 10 BS90 snails whereas GREP1.2 transcripts were detected in just 4 of 10 NMRI snails. It is possible that these strain-specific differences contribute to the resistant and susceptible phenotypes of BS90 and NMRI snails, respectively.

Many of the plasma proteins that bind to *S. mansoni* sporocysts as recorded by proteomics analyses have previously been linked with the *B. glabrata* immune system (Cu/Zn SOD1: Goodall et al., 2004, 2006; Bender et al., 2007; FREPs: Hanington et al., 2010b; HSP70, 90: Zahoor et al., 2010; MIF: Baeza-Garcia et al., 2010; Biomphalysin: Galinier et al., 2013; TLR: Pila et al., 2016a; Granulin: Pila et al., 2016b; TEP: Portet et al., 2018). However proteomics also identified sporocystbinding proteins that are not typically associated with immunity and defense, such as actin, collagen, hemoglobin, GAPDH, apolipophorin, and histone 4. The independent corroboration suggests that these unexpected plasma protein-sporocyst interactions are specific, and the functional significance of such interactions warrants further examination. Future research into the binding of such "non-immune" proteins may indeed indicate a yet undescribed role in immunity. To illustrate, recent research suggests that apolipophorin in the greater wax moth, *Galleria mellonella* (Whitten et al., 2004) and histone 4 can function in immune responses in various organisms (reviewed in Hoeksema et al., 2016).

The interaction between plasma and sporocysts is followed by hemocyte-sporocyst interactions, including hemocyte defense effector functions. A proteomic profiling of hemocytes of BS90 and NMRI snails, when encapsulating *S. mansoni* sporocysts *in vitro* (Dinguirard et al., 2018) provides insight into such hemocytes-sporocysts interactions within the first 24 h of infection and/or encapsulation. In accordance with previous

gene expression studies, different proteomic profiles were evident from NMRI and BS90 hemocytes. Compared to the BS90 hemocyte encapsulation, there was a greater number of down-regulated proteins in the NMRI hemocyte encapsulation, and many of these proteins were associated with protein synthesis or trafficking, metabolism, cell signaling, and redox reactions. In addition, some downregulated proteins may play a role in defense such as those reported for LPS-BPI, superoxide dismutase, and apolipophorin. Interestingly, just a few proteins in encapsulating NMRI hemocytes were substantially increased in abundance, such as IRAK, argonaute 18, arginase 1 and bacterial permeability protein (BPI). Of these, IRAK, argonaute 18 and BPI may function in defense. However, arginase can compete for arginine, the substrate required for NO synthesis, so increased arginase may lead to a decrease in NO production and therefore weaken the immune response. NO was previously found to be directly involved in the cytotoxic killing of sporocysts in resistant (13–16-R1) snails (Hahn et al., 2001b). In contrast to NMRI (susceptible) hemocytes, BS90 hemocytes encapsulating sporocysts demonstrated increased expression of protein synthesis machinery constituents and anti-apoptotic factors (compared to control BS90 hemocytes). Likewise, factors associated with immune defense, such as those involved in ROS production (dual oxidase), autophagy and the proteasome, were increased in encapsulating hemocytes from BS90 (Dinguirard et al., 2018).

Cumulatively, these studies highlight the down regulation and the up regulation of immune responses in susceptible and resistant *B. glabrata* strains, respectively. However, despite numerous comparative studies, the factor(s) determining the contrasting gene expression changes in resistant and susceptible strains remain unclear, as do their origin (host or parasite) and the mechanism(s) that cause such modulations. One possibility is that differences in the plasma lectin profile between snail strains is responsible. One can imagine a scenario where parasite products with the ability to interfere with snail immune responses are mopped up in a complex comprising lectins, and other associating molecules (TEPs, etc.) in resistant snails, and therefore down regulation does not occur. Whereas in susceptible snails, the specific lectins required to neutralize these parasite molecules are absent, are expressed at a low level, or different alleles of the lectins are expressed that are not as efficient as the resistant forms. Furthermore, the signaling pathways and transcription factors that facilitate the strainspecific changes have yet to be identified as well as the possible influence of these entities on resistance to infection.

5.4.3. Immune memory—Immune memory was previously considered an exclusive characteristic of the adaptive immune system. That paradigm, however, is shifting as evidence suggests that in both invertebrates and vertebrates, the innate immune system may produce an enhanced secondary response to a pathogen (Gourbal et al., 2018). The first indication of *B. glabrata* induced resistance to *S mansoni* was reported over 30 years ago by exposing snails to irradiated miracidia (Lie et al., 1983); and the first report of immune memory to *S. mansoni* infection was reported 20 years ago, where schistosome-susceptible *B. glabrata* infected with a single miracidium, demonstrated resistance to reinfection two weeks following the initial infection (Sire et al., 1998). Furthermore, the second infection elicited a humoral response and there was no evidence of cellular encapsulation (Sire et al., 1998). A more recent study has likewise reported immune memory in *B. glabrata*; 10 days

following a primary infection, snails were resistant to additional (secondary) infection by the same strain of *S. mansoni* (Portela et al., 2013). It was demonstrated that the initial immune response is primarily cellular (hemocyte-associated), whereas the second or memory immune response is humoral (Pinaud et al., 2016). This last molecular-level study implicated FREPs 2, 3 and 4 in the generation of immune memory. In addition, the pore-forming factor biomphalysin was identified as a component of the humoral, second response (Pinaud et al., 2016). More recently, Pinaud et al. (2019) addressed invertebrate adaptive immune responses by using a variety of tools and techniques (transcriptome, proteomics, snail "vaccination", and *in vitro* sporocyst-toxicity assays) to identify the molecules and possible mechanism responsible for the observed immunological memory, with special emphasis on the differences associated diverse parasite genotypes. Major conclusions from this study are: (i) *B. glabrata*'s immune memory to the parasite is strainspecific and long lasting (for the life span of a snail); (ii) Of the molecules that were associated with immune memory, some were modulated irrespective of the parasite strain used in the immune challenge, and included several transcript isoforms; (iii) The response to immune challenge was stronger (greater abundance) and more efficient (protective) when the parasite strain used as immune challenge was more genetically related (homologous) to that of the initial infection; (iv) The three main types of immune molecules represented in the immune memory response were: immune receptors, immune effectors, and immune modulators/activators. The most abundant protein found in the components of the hemolymph responsible for immune memory was biomphalysin, but also present were C-type lectins, FREPs, galectins, LBP/BPI, as well as proteins characteristic of the extracellular matrix; (v) Vaccination experiments showed that the primary sporocyst stage is the most immunogenic to snails, but is not equivalent to a natural infection, and that vaccination efficacy also depends on the genetic background of the parasite used in the challenge; (vi) Cell-free plasma from infected snails also provided some protection (up to ~50%), but only if taken from the donor at one day after challenge and used to protect against homologous parasite infections. In addition to providing protection against challenge infection, this plasma was also effective in killing homologous strains of parasites in *in vitro* assays.

Considered together, these studies support early observations that suggested a molecular mechanism for innate immune memory in invertebrates like snails. In addition, the protective effect of plasma is observed when the challenge parasite is genetically identical or similar to that of the initial infection. As expected, the humoral response included a complex mixture of proteins with a variety of isoforms (or alleles) being expressed, and that these variations depended on the parasite strain used as challenge. The authors suggest that rather then one or a few molecules being responsible for the observed immune protection, a variety of proteins is required and these may need to be used in a specific manner sequentially, or simultaneously to form complex multimers to enable efficient recognition of the pathogen challenge and respond appropriately. Still, there are many more aspects yet to be investigated in relation to snails immune memory; for example, does immune memory offer any advantage to snails that already are carrying an infection? does innate memory provide any fitness to the snail host or their offspring? what is the mechanism involved in immune memory production? are hemocytes major player in this process as has been found in other invertebrates (Tassetto et al., 2017)? As a final note, the parasite

does not necessarily have a passive role, rather there is the likely possibility that some of the differences and lack of complete protection in vaccination experiments and plasma injections are due to molecules secreted by the schistosomes (Lodes and Yoshino, 1990; Guillou et al., 2007; Yoshino et al., 2014; Nowacki et al., 2015) that prevent or block recognition, similar to those operating in antigenic or molecular mimicry (Yoshino and Bayne, 1983; Damian, 1997; Yoshino et al., 2013a).

5.4.4. Thioester proteins—Among the newly recognized snail immune molecules that interact with *S. mansoni* sporocysts is the thioester-containing protein (BgTEP), for which continued attention provided an update (Portet et al., 2018) on a previously reported study (Moné et al., 2010). This molecule was originally identified in 2008 by Yoshino and coworkers (GenBank: FJ480411.1) and later characterized by Moné et al. (2010). BgTEP is a plasma protein from snails that forms a heterocomplex with FREPs and interacts with *S. mansoni* sporocysts polymorphic surface mucins (SmPoMucs). Moné et al. (2010) used antibodies against sporocyst mucins to co-immunoprecipitate several snail proteins for identification with mass spectrophotometry. Among these proteins were several FREPs (2, 12, and 13), a galectin, cell adhesion proteins (peroxinctinlike protein and Dec-1/Matrilin-like protein), a protease inhibitor cystatin-like molecule, a homolog to the pore-forming toxin aerolysin, and AIF (allograph inflammatory factor, a pro-inflammatory protein). The detection of FREPs was in line with previous reports that these are important players in immune recognition against schistosomes (Adema et al., 1997; Zhang and Loker, 2004; Zhang et al., 2004a; 2008; Hanington et al., 2010b). FREPs are highly diversified molecules that show expression variations of single genes even among individuals of the same snail strain (Zhang and Loker, 2004), and the authors speculated that FREPs are a logical host immune component to recognize and bind to the polymorphic components found on parasite mucins (Roger et al., 2008a, b), resembling the capacity of antibodies in vertebrate immune systems to recognize highly diverse antigenic epitopes. It is of note however, that Moné et al. (2010) found that sporocyst mucins were bound by a complex that included two snail plasma proteins, FREP2 and a fragment of BgTEP, suggesting a possible link between immune recognition and immune activation.

Thioester-containing proteins (TEPs) are well recognized as part of the battery of pattern recognition molecules that are employed by invertebrates to differentiate and defend against potential pathogens. TEPs are involved in innate immunity, and originally were defined as containing a characteristic thioester domain (TED) (Dodds and Law, 1998) that contains a β-cysteinyl-γ-glutamyl bond that is highly reactive and allows TEPs to form covalent bonds with target molecules (Law et al., 1980; Tack et al., 1980); The binding to the surfaces of microorganisms by invertebrates TEPs has been associated with immune functions, (Levashina et al., 2001; Blandin et al., 2004, 2008; Kopacek et al., 2012; Li et al., 2012; Urbanová et al., 2015). TEPs are organized into three major groups based on their structural domains: the alpha-2-macroglobulin (A2M) group, the complement C3-related (C3) group, and the classical thioester-containing proteins (TEPs) (Blandin and Levashina, 2004; Fujito et al., 2010; Sekiguchi et al., 2012). Each group is further divided into subgroups depending on structural or functional characteristics. For example, the A2M group includes proteins such as CPAMD8 (C3 and PZP-like alpha-2-macroglobulin domain-containing protein 8)

and PZP (pregnancy-zone protein); the C3 group is further divided into the complement C3, C4, and C5 subgroups; and the TEP group is subdivided into classical TEPs or insect TEPs (iTEPs), and CD109 (Blandin and Levashina, 2004; Urbanová et al., 2015). The application of next generation sequencing methods has identified multiple members of the TEP family in all major groups of metazoan (deuterostomes and protostomes). Although the mechanism of action is not well understood, and functional data is limited for many of them, most invertebrate TEPs have been associated with immune-related functions. For example, complement factors and activity have been identified in invertebrates (reviewed in Smith et al., 1996; Blandin and Levashina, 2004; Pinto et al., 2007; Armstrong, 2010; Nonaka, 2014), including early ancestral species such as cnidarians, molluscs, arthropods, and echinoderms (Spycher et al., 1987; Iwaki et al., 1996; Iwanaga et al., 1998; Blandin et al., 2004; Nonaka and Kimura, 2006; Zhang et al., 2007; Obbard et al., 2008; Castillo et al., 2009; Fujito et al., 2010; Nonaka and Satake., 2010; Bou Aoun et al., 2011; Buresová et al., 2011; Sekiguchi et al., 2012; Urbanová et al., 2015; Yazzie et al., 2015; Gorbushin, 2018; and many others). In the snail *B. glabrata*, the only known representative of the TEP superfamily (referred to as TEP1 in this review), recorded by Moné et al. (2010), was studied in greater detail by Portet et al. (2018). Computational characterizations showed that TEP1 had all the major structural domains and motifs characteristic of TEPs proteins, and phylogenetic analysis placed this molecule among the insect TEPs and invertebrate TEP/CD109 subgroup. TEP1 was differentially expressed among snail tissues, with the highest abundance in ovotestis, headfoot, and circulating non-phagocytic hemocytes. TEP1 expression was differentially modulated in a time-dependent manner when snails were exposed to microbes (Gram+ and Gram–bacteria, and yeast). Immuoblotting with anti-TEP1 antibody identified three different-sized protein bands in snail plasma, correlating with proteolytic cleavage that commonly leads to activation of many TEPs. *In vitro* binding and western blot analysis also revealed that TEP1 from snail plasma bound to the surface of all microorganisms tested, including *S. mansoni* miracidia and sporocysts, with a greater affinity to sporocysts. Treatment with the inhibitor methylamine resulted in loss of activity indicating that this binding was mediated via the TED motif. To investigate opsonic properties following previous reports of similar activity in mosquitos (Blandin et al., 2008), the role of TEP1 in encapsulation of *S. mansoni* was tested. Immunocytochemical labeling of snail tissue, sectioned 24 h post-exposure to miracidia, revealed a diffuse positive signal surrounding the parasite and into the hemocytic capsule, as well as in the hemocytes in close proximity to encapsulations, suggesting these cells were responsible for TEP1 secretion. Still, the mechanics of TEP1 binding remains to be characterized. Although it was proposed that this TEP1 is the same molecule that was previously characterized biochemically and termed alpha-2-macroglobulin by Bender and Bayne (1996) and Fryer et al. (1996), Portet et al. (2018) conclusion was not confirmed by phylogenetic analysis, neither proteinase activity tested for this protein. Aa an alternative explanation, the molecule responsible for the proteinase activity in *B. glabrata* could be another yet unidentified member of the TEP family in the snail.

In continuation of investigating this topic, we are presenting new data that was obtained by searching the *B. glabrata* genome for additional members of the TEP superfamily. It was hypothesized that the *B. glabrata* genome contains a variety of TEP factors, and

that further characterization of these molecules will improve our understanding of snail immunity. Furthermore, localization of such genes provided upstream genome sequences to help predict potential transcription factorbinding sites, and thus, associate the expression of TEPs with particular stimuli and identify the specific signaling pathway involved. For detailed methods please refer to supplemental file 1.

Screening the genome assembly and associated RNAseq databases yielded no less than 11 novel (partial) TEP-like sequences in *B. glabrata*, that did not correspond to the sequence or genome locus for the previously identified *B. glabrata* TEP1 (NCBI accession No. MK583203, Moné et al., 2010). Sequences were improved by mapping RNA-Seq reads to the *B. glabrata* genome (Table 2). Accordingly, *B. glabrata* has at least 12 members of the TEP family. Based on sequence domain composition and BLAST results, lacking functional data and full-length data for all sequences, these TEPs are tentatively classified as three members of the A2M group (A2M-1, A2M-2, CPAMD8–1); three complement C3-like molecules (C3–1, C3–2, C3–3), and six classical TEPrelated proteins (TEP-1, TEP-2, TEP-3, TEP-4, TEP-5, and CD109–1) (Fig. 2) (Table 2). Since the publication of the genome (Adema et al., 2017), ongoing automated annotation has resulted in ID assignments to several of these TEPs by VectorBase, and most can also be found as predicted proteins in the NCBI database, see Table 2 for accession numbers of verified TEPs.

To further this new TEP diversity contributions, we examined whether the expression of *B. glabrata* TEP genes may be under the regulation of NF-κB transcription factors, the genomic sequences upstream of coding regions were analyzed following methods described in Humphries and Deneckere (2018) and supplemental file 1. Putative binding sites for NF-κB (κB) were predicted upstream of all genes examined except for TEPs 1 and 2 (Table 3). TEP-4 was excluded from the analyses, as the upstream genomic sequence was not found. It is not yet known whether *B. glabrata* TEPs function downstream of binding to a target and if they interact with a signaling pathway(s). Moita et al. (2005) implicated two transmembrane receptors in TEP signaling pathways in *Anopheles* mosquitoes: a low density lipoprotein (LDL)receptor related protein, LRP1, and an integrin β subunit, BINT. Interestingly, an integrin β subunit was previously identified in *B. glabrata* (Davids et al., 1999), and an LRP1 homolog was predicted from the *B. glabrata* genome assembly (XP_013085247.1). In addition, Moita et al. (2005) implicated intracellular proteins, CED-2, CED-5 and CED-6 in TEP signaling, and a *ced*-6 like gene was detected in the *B. glabrata* genome (XP_013075601.1). As components of these pathways appear to be present in *B. glabrata*, perhaps they function in TEP signaling pathways in snails as well. It is also possible that there is an interaction between TEPs and TLR signaling in *B. glabrata*, similar as has been reported in insects (Shokal and Eleftherianos, 2017; Dostálová et al., 2017). For example, in *Drosophila*, inactivation of TEP4 led to increased activity in Toll and IMD signaling pathways but a reduction in Jak/STAT and Jnk pathway activity (Shokal and Eleftherianos, 2017). In contrast, Dostálová et al. (2017) demonstrated that *Drosophila* TEPs contribute to activating a Toll signaling pathway in response to Gram + bacterial and fungal infections. Perhaps the TLR signaling pathway can induce TEP expression through NF-κB in *B. glabrata*, as putative κB binding sites were predicted upstream of several TEP genes (Table 3). The feasibility of hypothetical regulation of *B. glabrata* TEPs by a TLR–NF–κB signaling pathway is supported by recent demonstration that in the crustacean, *Littopeneaus*

vannamei, TEP expression is dependent on NF-κB and activator protein 1 (AP1) (Li et al., 2012).

5.5. Epigenetics-methylation and non-coding small RNA

Epigenetics is a topic that has generated considerable interest lately. This is mainly, because understanding the underlying mechanisms could facilitate the development of methods to genetically manipulate snails in ways that may modulate immune function and general gene expression in snails to possibly interfere with the transmission of schistosomiasis.

DNA methylation processes have not been well studied in invertebrates. However studies with the pacific oyster *Crassostrea gigas* (Gavery and Roberts, 2010) and the scallop *Chlamys farreri* (Sun et al., 2014) have identified common targets of the methylation machinery in molluscs. In *B. glabrata*, searches of the genome assembly identified core components of methylation pathways including DNMT1 (a maintenance DNA methyltransferase), DNMT2 (a DNA/tRNA methyltransferase), and MDB2/3 (a methyl-CpG-binding domain protein) (Geyer et al., 2017). Additionally, the finding that BgDNMT1 and BgMBD2/3 transcripts were upregulated in snail gonads and in Bge cells after exposure to *S. mansoni* larval transformation products (LTPs are molecules released by parasites during the *in vitro* transformation of miracidia to sporocyst stages; Wu et al., 2009) suggest that epigenetics play an important role in snail reproduction and defense against parasites. This concords with previous findings, including methylation of cytidine residues of the *hsp70* gene in heat-stressed or *S. mansoni*exposed snails, possibly leading to increased gene expression, and the observation of a “mosaic DNA methylation” pattern in the *B. glabrata* genome (Fneich et al., 2013; Adema et al., 2017), described as consisting of regions with high methylation in “house-keeping” genes, interspaced by low- or non-methylated (encoding inducible genes) DNA segments. Moreover like in other molluscan species studied, DNA methylation in *B. glabrata* appears to be restricted to CpG sites (Geyer et al., 2017).

Gene regulation may also be influenced by small non-coding RNAs (ncRNAs), including microRNAs (miRNAs) and PIWI-interacting RNAs (piRNAs) (Hirose et al., 2014; Morris and Mattick, 2014). Analyses of the *B. glabrata* genome led to the identification of 95 conserved premiRNAs, and the prediction of a further 107 novel pre-miRNAS. In addition, homologs of 9 genes associated with the processing of small ncRNA processing genes, were also identified in the genome (Adema et al., 2017). Queiroz et al. (2017) have since investigated the potential role of miRNAs and piRNAs in *B. glabrata* development and in *S. mansoni* infection, by determining the expression profiles of the ncRNA processing genes Argonaute, Piwi, Drosha, Exportin-5, and Tudor. Although the expression profiles of these genes differed, overall the data suggest that all genes except Tudor, play a role in the first 40 days of development. Furthermore, the potential role of small ncRNAs in the interaction between *B. glabrata* and *S. mansoni* was probed at several time points post-infection. Though the expression profiles of the 5 genes were not uniform, all were downregulated at 4 h and 30 days postinfection, and most were also downregulated at various time points in between. The results suggest that downregulation of these genes in *B. glabrata* facilitates infection by the parasite. However, this study was performed on a single,

schistosome-compatible strain of *B. glabrata*, so it will be important to examine whether small ncRNAs play a role in defense against infection in a non-compatible snail strain. In addition, the possible manipulation of *B. glabrata* gene expression by schistosome-produced small ncRNAs should be considered and deserves examination.

5.6. Signaling

5.6.1. Cytokine signaling—Whereas pregenomics studies suggested the presence of an interleukin 1(IL-1)-like factor and a tumor necrosis factor (TNF) functioning in regulation of the immune system of *B. glabrata* (Granath et al., 1994; Ouwe-Missi-Oukem-Boyer et al., 1994), specific analyses on the evolution of cytokines so far suggest that homologs of IL-17, TNF, transforming growth factor β (TGFβ) and AIF (apoptosis inducing factor) are the only vertebrate-like cytokines in molluscs (DeFilippo and Beck, 2018). This notion is supported by *B. glabrata*, as IL-17 and TNF homologs were predicted from the genome, but IL-1-like sequences were not (Adema et al., 2017). An additional survey of the genome provided evidence of a TGF signaling pathway, although we were unable to detect an AIF-like sequence.

Specifically, eleven TNF-like genes were predicted from the *B. glabrata* genome (supplementary data 17, Adema et al., 2017). In a new analysis, we further investigated the predicted genes, and considered sequences to be TNF homologs if they were supported by the RNA-Seq data and contained a TNF domain as indicated by SMART (http://smart.embl.de; Letunic and Bork, 2018). The annotations of eight TNF homologs were confirmed, though two of the transcripts were incomplete (lacking 5′ termini and 3’ stop codons); in addition, BgTNF3 appears to have two variants (Table 4). Transmembrane domains, considered a feature of mammalian TNFs (MacEwan, 2002; Aggarwal et al., 2011), were predicted in all BgTNFs using TMHHM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM).

Subsequent survey of the genome assembly and RNA-Seq data identified three potential TNF receptors based on the presence of a TNFR superfamily domain, however the transcript for BgTNFR3 lacked a 5’ start codon (Table 5). All BgTNFRs were classified as TNFR2 subtype because of a lack of a cytoplasmic death domain (DD) in the cytoplasmic tails, according to the nomenclature of mammalian BgTNFRs (MacEwan, 2002; Aggarwal et al., 2011).

The RNA-Seq data collected as part of the characterization of the *B. glabrata* genome not only allows for the confirmation and/or correction of predicted gene models but also provides information on specific tissue localization of transcripts, as mRNA sequences were obtained from 12 different snail tissues/organs. This information indicated that the various TNFs were not detected in all tissues, and in those in which they were, they were differentially expressed, such that: (i) No TNF transcripts were detected in salivary glands; (ii) TNF4 transcripts were detected in only 2 tissues; (iii) TNF2 and 3 transcripts were found in four to five tissues; (iv) TNF1 transcripts were detected in seven tissues; (v) Transcripts representing TNF7 were found in eight tissues; (vi) TNFs 6 and 8 were found in ten tissues; and (vii) TNF5 transcripts were found in eleven of the twelve tissues (Table 6). Similar expression patterns were recorded for TNF receptors: (i) No TNFR transcripts were detected

in either the albumen gland or terminal genitalia; (ii) TNFR1 and 2 were found in seven and nine different tissues respectively; and (iii) TNFR3 was only detected in transcripts from the stomach; interestingly the stomach was the only tissue in which all BgTNFRs were found (Table 7). Apart from TNF4 and TNFR3, the *B. glabrata* TNFs and TNFRs demonstrated constitutive and ubiquitous tissue expression, similar to what has been reported for other molluscs (De Zoysa et al., 2008; Xiang et al., 2016; Xing et al., 2016). In addition, the number of TNF and TNFR transcripts, as well as tissue distribution patterns, suggest that each TNFR can bind more than one TNF.

In mammalians, the cytoplasmic DD of TNFR1 allows the receptor to interact with apoptosis-associated signaling proteins such as TRADD (TNF receptor-associated DD) and RIP (receptor-interacting protein), through DD to DD binding (MacEwan, 2002). It is notable that all three putative BgTNFRs lack a cytoplasmic DD and in addition, homologs to TRADD and RIP were not found in the *B. glabrata* genome. Likewise, transcriptomic study of embryonic *B. glabrata*, identified transcripts for TNF and TNFR whereas those for TRADD and RIP were absent (Kenny et al., 2016). These findings suggest that the BgTNFRs signal via direct interactions with a TRAF2 protein, similar to mammalian TNFR2 receptors (MacEwan, 2002). This is further supported by the recent identification of a TNF receptor associated factor 2 (TRAF2) homolog in *B. glabrata* (Adema et al., 2017; Humphries and Deneckere, 2018). Based on mammalian studies, ligand binding by BgTNFRs may lead to the activation of mitogen-activated protein kinase (MAPK), NF-κB and AP1 signaling pathways (Aggarwal et al., 2011, Fig. 3); components of all these pathways have been identified in *B. glabrata* (Humphries and Yoshino, 2006; Zhang and Coultas, 2011; Humphries and Harter, 2015; Adema et al., 2017).

The identification of a transmembrane domain in each of the BgTNFs, in conjunction with the presence of a putative TNF-α-converting enzyme (TACE) in the *B. glabrata* genome (XP_013083719.1), support the notion that *B. glabrata* TNFs are first expressed as membrane proteins that may be subsequently cleaved to a soluble form, similar to TNF processing in mammalians (MacEwan, 2002). Furthermore, both the membrane and soluble forms may be capable of inducing signaling in receptor-bearing cells through TNFR binding. Interestingly, the binding of membrane TNFs by a TNFR can cause signaling in the TNF-bearing cell; this is known as outside-inside or reverse signaling (Harashima et al., 2001; MacEwan, 2002; Aggarwal et al., 2011).

The identification of TNF and TNFR homologs in *B. glabrata* complements the discovery of similar homologs in other molluscs (De Zoysa et al., 2008; Zhang et al., 2015; Xiang et al., 2016; Xing et al., 2016). Pending functional confirmation, it is hypothesized that TNF signaling functions in *B. glabrata* immunity just as TNF signaling participates in mammalian immune regulation. Furthermore, studies of other molluscan species have demonstrated increased expression levels of TNF and TNFR genes in response to pathogen exposures, thus supporting a role for TNF signaling in the mollusca immune defenses (De Zoysa et al., 2008; Xiang et al., 2016; Xing et al., 2016).

As stated earlier, studies on the evolution of cytokines suggest that, like TNFs, homologs of IL-17 are among the categories of vertebrate-like cytokines to be found in molluscs

(DeFilippo and Beck, 2018). The *B. glabrata* genome project (supplementary data 17, Adema et al., 2017) yielded twelve predicted IL-17-like genes. Investigation using the tissue-derived RNA-Seq data led to recovery of four of the twelve predicted *B. glabrata* IL-17 homologs, as characterized by containing IL-17 domains, as expressed transcripts (http://smart.embl.de; Table 8). In contrast to the TNF expression patterns described above, IL-17-like transcripts were not ubiquitously expressed across tissues. Each of the four BgIL-17 transcript variants was found in only one or two tissues; two of the BgIL-17 homologs were represented in the terminal genitalia sequences and two were found in the stomach. It should be noted, however, that the RNA-Seq data represent constitutive expression, as the tissues were isolated from uninfected snails. Perhaps most IL-17s in *B. glabrata* are expressed in response to stimuli, whereas TNFs are expressed constitutively to be upregulated further during an immune response. With confirmation of BgIL-17 homologs, a survey of the genomic and RNA-Seq data identified a single putative *B. glabrata* IL-17 receptor sequence that shares similarity with both invertebrate and vertebrate IL-17 receptors entries in NCBI.

The identification of IL-17 and IL-17 receptor homologs in *B. glabrata* is corroborated by the identification of similar homologs in other molluscs, such as *Octopus vulgaris* and *Crassostrea gigas* (Castellanos-Martinez et al., 2014; Zhang et al., 2015). Changes in the expression of other molluscan IL-17 homologs following pathogen exposure (Zhang et al., 2015), support the concept that BgIL-17s may have an immune role. Mammalian IL-17 homologs play a pro-inflammatory role in the adaptive immune system and regulate innate immunity. In the latter,IL-17 signaling pathways increase the expression of chemokines, antimicrobial molecules, and acute phase proteins for example (Gaffen, 2008). Based on this, BgIL-17 homologs are assumed to function in the snail immune system, perhaps by regulating the expression and release of humoral factors that play a role in immunity.

5.6.2. Toll-like receptor (TLR) signaling—Toll-like receptors (TLRs) are an ancient family of pattern recognition receptors (PRRs) that has been identified in numerous animal phyla ranging from sponges and cnidarians (Prebilateria) to protostomes and deuterostomes including the chordates (Gilmore and Wolenski, 2012; Brennan and Gilmore, 2018). Vertebrate TLRs have been studied the most extensively, and are known to function as dimers that collectively bind a diversity of PAMPs, including double stranded RNA, CpG DNA, and lipopolysaccharide (Takeda et al., 2003). Targeted searches of the *B. glabrata* genome assembly led to the identification of twenty-seven complete TLRs (Adema et al., 2017). Of these, only one TLR (designated TLR1, most similar to TLR5 as listed in Adema et al., 2017) has been studied at the functional level (Pila et al., 2016a). It should be noted that the numbering of *B. glabrata* TLRs does not imply functional homology with mammalian TLRs. In comparing the responses of two *B. glabrata* strains following exposure to *S. mansoni*, BgTLR1 expression levels increased in BS90 snails (resistant) at 12 and 24 h post infection, whereas no significant changes were reported for M-line snails (susceptible). Furthermore, RNAi knockdown of BgTLR1 led to a reduction in hemocyte phagocytosis of beads coated with *S. mansoni* excreted-secreted products (ESP, Johnston and Yoshino, 2001), and 43% of siRNA-treated BS90 snails (normally resistant) became susceptible to infection (Pila et al., 2016a). These findings indicate that BgTLR1 functions in defense

against *S. mansoni* in resistant *B. glabrata* of the BS90 strains. The specific ligand for BgTLR1 has not yet been identified, but Aksoy et al. (2005) previously demonstrated that a mammalian TLR3 bound *S. mansoni* dsRNA. It is possible therefore, that BgTLR1 directly binds a schistosome molecule. It is of interest to determine whether other TLRs contribute to *B. glabrata* anti-schistosome defense and to identify the ligands for each of these receptors. In addition, it is yet to be resolved whether *B. glabrata* TLRs function as monomers or dimers, given that mammalian TLRs typically work as dimers. A comprehensive TLR signaling pathway was identified through the *B. glabrata* genome project (Adema et al., 2017; Humphries and Deneckere, 2018), and we hypothesize functions downstream of TLR-ligand binding. Possibly, activation of TLRs in *B. glabrata* leads to amplification of TLR signaling as indicated by the occurrence of kappabinding DNA sites, and demonstrated by BgNF-κB being able to bind upstream of the genes for several members of the TLR signaling pathway (Humphries and Deneckere, 2018). As mentioned above (section 5.5.4), perhaps TEP- and TLR-mediated signaling intersect in *B. glabrata*, such that some TEPs can activate the TLR signaling pathway, which leads to increased expression of certain TEPs, while in contrast, other TEPs function to down regulate TLR signaling and the immune response.

6. Discussion

The sequencing of *B. glabrata*'s genome has transformed the field of snail biology, and will continue to do so. This is exemplified by recent post-genomic studies that utilize the genome resources to advance the understanding of immunity and general biology of this snail. This review examined several such reports that focused on snail-schistosome interactions and further develop previous findings aimed to better understating of snail-parasite (immune) interactions, potentially benefitting the control of human schistosomiasis. These studies included the sequencing of the Bge cell genome, the identification of a family of thioester proteins, analyses of TNF and IL-17 cytokines in *B. glabrata*, discovery of the potential roles for small nc-RNAs in snail development and during *S. mansoni* infection, and explorations of the immune memory in *B. glabrata*. These topics provide a preview of what promises to be a component-diverse molluscan immune system, and indicate a high complexity of snail-parasite interactions as well as yet to be discovered mechanisms underlying immune function in *B. glabrata*. With the now available *B. glabrata* genome and its associated data resources, newly gained insights can be more easily interpreted in connection to previously gained knowledge and observations to begin and facilitate development of more holistic hypotheses regarding the mechanisms of immunity in snails and invertebrates in general. What at this moment seems to constitute independently working, disconnected, pieces of a puzzle, may instead be parts of a larger, interconnected polygenic network. Are these pieces part of generalized immune reactions or unique to anti-helminthic responses in *B. glabrata*? Whereas it has become evident that snail- and parasite-derived molecules interact to form multimeric complexes, it remains to be clarified whether and how these complexes are part of the parasite pathogenic strategy, or perhaps play a role in effective snail immune defense against parasite infection. The *B. glabrata* genome and its associated data has allowed for the discovery of extended families of immune and immune related molecules as described here (e.g. TEPs). It is anticipated that more specific analyses of these resources could

reveal novel immune targets to be characterized in *B. glabrata* as transcriptome explorations performed in the common periwinkle snail *Littorina littorea* transcriptome improving our understanding of molluscan lectin (Gorbushin and Borisova, 2015; Gorbushin, 2019) and complement-like (Gorbushin, 2018) families. Postgenomic investigations of B. glabrata can now begin to address diverse novel questions. What are the biological bases of the diverse levels of susceptibility of *B. glabrata* to schistosome infection seen in the wild? Are studies in the laboratory representative of what is happening in snail-parasite interactions in the field? Are parasite products being "mopped up" in multimeric complexes in resistant snails to prevent downregulation of snail's immunity or do these complexes facilitate immune recognition and activation to initiate downstream signaling (e.g., the TLR–NF–κB pathway) that leads to the synthesis and release of immune factors such as cytokines and immune effectors? Can snail immunity be artificially manipulated and improved? Fortunately, and despite that all these and many more questions remain to be answered, efforts are being made in related fields of study to further our knowledge and understanding of snail immunity and genomics; some of these include research on schistosome biology (Mickum et al., 2014; Yan et al., 2018), transgenics (Hagen et al., 2014; Ittiprasert et al., 2019), post-transcriptional regulation (Fiscon et al., 2018), vaccine and diagnostic tools (Sousa-Figueiredo et al., 2013; Prasanphanich et al., 2014; Sato et al., 2018), snail ecology (Perez-Saez et al., 2016; Civitello et al., 2018), computational modeling (Gurarie et al., 2018), and gene networks (Ruprecht et al., 2017) among others. Multidisciplinary and multifaceted approaches (reviewed in Colley et al., 2014; and McManus et al., 2018) will accelerate discovery and development of methods to decrease the burden of schistosomiasis in the future while at the same time expanding our understanding of immune function in *B. glabrata*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors would like to acknowledge Ricardo Castillo for the art in this review and to Nathalie Dinguirard for helpful discussion of the manuscript. Portions of this research was supported by the National Institute of General Medical Sciences of the National Institutes of Health under award number 5SC2AI133645.

References

May 14 Abe M, Kuroda R, 2019. The development of CRISPR for a mollusc establishes the formin Lsdia1 as the long-sought gene for snail dextral/sinistral coiling. pii: dev175976. Development (9), 146. 10.1242/dev.175976. PubMed PMID: 31088796.

Adema CM, Loker ES, 2015. Digenean-gastropod host associations inform on aspects of specific immunity in snails. Feb. Dev. Comp. Immunol. 48 (2), 275–283. 10.1016/j.dci.2014.06.014. Epub 2014 Jul 14. Review. PubMed PMID: 25034871; PubMed Central PMCID: PMC4258543. [PubMed: 25034871]

Adema CM, Hertel LA, Miller RD, Loker ES, 1997. A family of fibrinogen-related proteins that precipitates parasite-derived molecules is produced by an invertebrate after infection. Aug 5. Proc. Natl. Acad. Sci. U. S. A. 94 (16), 8691–8696 PubMed PMID: 9238039; PubMed Central PMCID: PMC23082. [PubMed: 9238039]

Adema CM, Luo MZ, Hanelt B, Hertel LA, Marshall JJ, Zhang SM, DeJong RJ, Kim HR, Kudrna D, Wing RA, Soderlund C, Knight M, Lewis FA, Caldeira RL, Jannotti-Passos LK, Carvalho Odos S, Loker ES, 2006. A bacterial artificial chromosome library for Biomphalaria glabrata, intermediate snail host of Schistosoma mansoni. Sep. Mem. Inst. Oswaldo Cruz 101 (Suppl. 1), 167–177 PubMed PMID: 17308766. [PubMed: 17308766]

Adema CM, Hanington PC, Lun CM, Rosenberg GH, Aragon AD, Stout BA, Lennard Richard ML, Gross PS, Loker ES, 2010. Differential transcriptomic responses of Biomphalaria glabrata (Gastropoda, Mollusca) to bacteria and metazoan parasites, Schistosoma mansoni and Echinostoma paraensei (Digenea, Platyhelminthes). Jan. Mol. Immunol. 47 (4), 849–860. 10.1016/j.molimm.2009.10.019. Epub 2009 Dec 3. PubMed PMID: 19962194; PubMed Central PMCID: PMC2814977. [PubMed: 19962194]

Adema CM, Hillier LW, Jones CS, Loker ES, Knight M, Minx P, Oliveira G, Raghavan N, Shedlock A, do Amaral LR, Arican-Goktas HD, Assis JG, Baba EH, Baron OL, Bayne CJ, Bickham-Wright U, Biggar KK, Blouin M, Bonning BC, Botka C, Bridger JM, Buckley KM, Buddenborg SK, Lima Caldeira R, Carleton J, Carvalho OS, Castillo MG, Chalmers IW, Christensens M, Clifton S, Cosseau C, Coustau C, Cripps RM, Cuesta-Astroz Y, Cummins SF, di Stephano L, Dinguirard N, Duval D, Emrich S, Feschotte C, Feyereisen R, FitzGerald P, Fronick C, Fulton L, Galinier R, Gava SG, Geusz M, Geyer KK, Giraldo-Calderón GI, de Souza Gomes M, Gordy MA, Gourbal B, Grunau C, Hanington PC, Hoffmann KF, Hughes D, Humphries J, Jackson DJ, JannottiPassos LK, de Jesus Jeremias W, Jobling S, Kamel B, Kapusta A, Kaur S, Koene JM, Kohn AB, Lawson D, Lawton SP, Liang D, Limpanont Y, Liu S, Lockyer AE, Lovato TL, Ludolf F, Magrini V, McManus DP, Medina M, Misra M, Mitta G, Mkoji GM, Montague MJ, Montelongo C, Moroz LL, Munoz-Torres MC, Niazi U, Noble LR, Oliveira FS, Pais FS, Papenfuss AT, Peace R, Pena JJ, Pila EA, Quelais T, Raney BJ, Rast JP, Rollinson D, Rosse IC, Rotgans B, Routledge EJ, Ryan KM, Scholte LLS, Storey KB, Swain M, Tennessen JA, Tomlinson C, Trujillo DL, Volpi EV, Walker AJ, Wang T, Wannaporn I, Warren WC, Wu XJ, Yoshino TP, Yusuf M, Zhang SM, Zhao M, Wilson RK, 2017. Whole genome analysis of a schistosomiasistransmitting freshwater snail. 2017 May 16. Nat. Commun. 8, 15451. 10.1038/ncomms15451. Erratum in: Nat Commun. Aug 23; 8:16153. PubMed PMID: 28508897; PubMed Central PMCID: PMC5440852. [PubMed: 28508897]

Aggarwal BB, Gupta SC, Kim JH, 2011. Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. 2012 Jan 19. Blood 119 (3), 651–665. 10.1182/blood-2011-04-325225. Epub Nov 3. Review. PubMed PMID: 22053109; PubMed Central PMCID: PMC3265196. [PubMed: 22053109]

Aksoy E, Zouain CS, Vanhoutte F, Fontaine J, Pavelka N, Thieblemont N, Willems F, Ricciardi-Castagnoli P, Goldman M, Capron M, Ryffel B, Trottein F, 2005. Double-stranded RNAs from the helminth parasite Schistosoma activate TLR3 in dendritic cells. Jan 7. J. Biol. Chem. 280 (1) 277–83. Epub 2004 Nov 1. PubMed PMID: 15519998. [PubMed: 15519998]

Allan ERO, Blouin MS, 2018. Allelic variation partially regulates galactose-dependent hydrogen peroxide release from circulating hemocytes of the snail Biomphalaria glabrata. Jan. Fish Shellfish Immunol. 72, 111–116. 10.1016/j.fsi.2017.10.037. Epub 2017 Oct 28. PMID: 29107743 Free PMC Article. [PubMed: 29107743]

Allan ER, Tennessen JA, Bollmann SR, Hanington PC, Bayne CJ, Blouin MS, 2017. Schistosome infectivity in the snail, Biomphalaria glabrata, is partially dependent on the expression of Grctm6, a Guadeloupe Resistance Complex protein. Feb 3. PLoS Neglected Trop. Dis. 11 (2), e0005362. 10.1371/journal.pntd.0005362. eCollection 2017 Feb. PubMed PMID: 28158185; PubMed Central PMCID: PMC5310918.

Allan ERO, Gourbal B, Dores CB, Portet A, Bayne CJ, Blouin MS, 2018. Clearance of schistosome parasites by resistant genotypes at a single genomic region in Biomphalaria glabrata snails involves cellular components of the hemolymph. Apr. Int. J. Parasitol. 48 (5), 387–393. 10.1016/j.ijpara.2017.08.008. Epub 2017 Nov 12. PMID: 29137971 Free PMC Article. [PubMed: 29137971]

Armstrong PB, 2010. Role of α2-macroglobulin in the immune responses of invertebrates. Invertebr. Surviv. J. 7 (2), 165–180.

Ataev GL, Fournier A, Coustau C, 1998. Comparison of Echinostoma caproni mother sporocyst development in vivo and in vitro using Biomphalaria glabrata snails and a B. glabrata embryonic cell line. Apr. J. Parasitol. 84 (2), 227–235 PubMed PMID: 9576492. [PubMed: 9576492]

Baeza Garcia A, Pierce RJ, Gourbal B, Werkmeister E, Colinet D, Reichhart JM, Dissous C, Coustau C, 2010. Involvement of the cytokine MIF in the snail host immune response to the parasite Schistosoma mansoni.Sep. 23. PLoS Pathog. 6 (9), e1001115. 10.1371/journal.ppat.1001115. PubMed PMID: 20886098; PubMed Central PMCID: PMC2944803.

Basch PF, DiConza JJ, 1974. The miracidium-sporocyst transition in Schistosoma mansoni: surface changes in vitro with ultrastructural correlation.Dec. J. Parasitol. 60 (6), 935–941 PubMed PMID: 4436765. [PubMed: 4436765]

Bayne CJ, 2009. Successful parasitism of vector snail Biomphalaria glabrata by the human blood fluke (trematode) Schistosoma mansoni: a 2009 assessment. May. Mol. Biochem. Parasitol. 165 (1), 8–18. 10.1016/j.molbiopara.2009.01.005. Epub 2009 Jan 22. [PubMed: 19393158]

Bayne CJ, Loker ES, 2018. Molluscan immunobiology: challenges in the anthropocene epoch. In: Advances in Comparative Immunology. Springer, Cham, pp. 343–407.

Bayne CJ, Owczarzak A, Allen JR, 1978. Molluscan (Biomphalaria) cell line: serology, karyotype, behavioral, and enzyme electrophoretic characterization. J. Invertebr. Pathol. 32 (1), 35–39.

Bayne CJ, Buckley PM, DeWan PC, 1980. Macrophagelike hemocytes of resistant Biomphalaria glabrata are cytotoxic for sporocysts of Schistosoma mansoni in vitro. Jun. J. Parasitol. 66 (3), 413–419 PubMed PMID: 7391885. [PubMed: 7391885]

Bender RC, Bayne CJ, 1996. Purification and characterization of a tetrameric alphamacroglobulin proteinase inhibitor from the gastropod mollusc Biomphalaria glabrata. Jun 15. Biochem. J. 316 (Pt 3), 893–900 PubMed PMID: 8670168; PubMed Central PMCID: PMC1217434. [PubMed: 8670168]

Bender RC, Broderick EJ, Goodall CP, Bayne CJ, 2005. Respiratory burst of Biomphalaria glabrata hemocytes: Schistosoma mansoni-resistant snails produce more extracellular H2O2 than susceptible snails.Apr. J. Parasitol. 91 (2), 275–279 PubMed PMID: 15986600. [PubMed: 15986600]

Bender RC, Goodall CP, Blouin MS, Bayne CJ, 2007. Variation in expression of Biomphalaria glabrata SOD1: a potential controlling factor in susceptibility/resistance to Schistosoma mansoni. Dev. Comp. Immunol. 31, 874–878 [PubMed: 17292470]. [PubMed: 17292470]

Bixler LM, Lerner JP, Ivanchenko M, McCormick RS, Barnes DW, Bayne CJ, 2001. Axenic culture of Schistosoma mansoni sporocysts in low O2 environments. Oct. J. Parasitol. 87 (5), 1167–1168 PubMed PMID: 11695386. [PubMed: 11695386]

Blandin S, Levashina EA, 2004. Thioester-containing proteins and insect immunity. Feb. Mol. Immunol. 40 (12), 903–908 Review. PubMed PMID: 14698229. [PubMed: 14698229]

Blandin S, Shiao SH, Moita LF, Janse CJ, Waters AP, Kafatos FC, Levashina EA, 2004. Complement-like protein TEP1 is a determinant of vectorial capacity in the malaria vector Anopheles gambiae. Mar 5. Cell 116 (5), 661–670 PubMed PMID: 15006349. [PubMed: 15006349]

Blandin SA, Marois E, Levashina EA, 2008. Antimalarial responses in Anopheles gambiae: from a complement-like protein to a complement-like pathway. Jun 12. Cell Host Microbe 3 (6), 364–374. 10.1016/j.chom.2008.05.007. Review. PubMed PMID: 18541213. [PubMed: 18541213]

Blouin MS, Bonner KM, Cooper B, Amarasinghe V, O'Donnell RP, Bayne CJ, 2013. Three genes involved in the oxidative burst are closely linked in the genome of the snail, Biomphalaria glabrata. Jan. Int. J. Parasitol. 43 (1), 51–55. 10.1016/j.ijpara.2012.10.020. Epub 2012 Dec 1. PubMed PMID: 23207063; PubMed Central PMCID: PMC3733350. [PubMed: 23207063]

Boissier J, Moné H, Mitta G, Bargues MD, Molyneux D, Mas-Coma S, 2015. Schistosomiasis reaches europe. Jul. Lancet Infect. Dis. 15 (7), 757–758. 10.1016/S1473-3099(15)00084-5. [PubMed: 26122434]

Botros S, Sayed H, Amer N, El-Ghannam M, Bennett JL, Day TA, 2005. Current status of sensitivity to praziquantel in a focus of potential drug resistance in Egypt. Jun. Int. J. Parasitol. 35 (7), 787–791 Epub 2005 Mar 18. PubMed PMID: 15925597. [PubMed: 15925597]

Bou Aoun R, Hetru C, Troxler L, Doucet D, Ferrandon D, Matt N, 2011. Analysis of thioester-containing proteins during the innate immune response of Drosophila melanogaster. J Innate Immun 3 (1), 52–64. 10.1159/000321554. Epub 2010 Nov 9. PubMed PMID: 21063077; PubMed Central PMCID: PMC3031515. [PubMed: 21063077]

Braschi S, Borges WC, Wilson RA, 2006. Proteomic analysis of the shistosome tegument and its surface membranes. Mem. Inst. Oswaldo Cruz 101, 205–212. 10.1590/S0074-02762006000900032. [PubMed: 17308771]

Brennan JJ, Gilmore TD, 2018. Evolutionary origins of Toll-like receptor signaling. Mol. Biol. Evol. 35 (7), 1576–1587. 10.1093/molbev/msy050. [PubMed: 29590394]

Buddenborg SK, Bu L, Zhang SM, Schilkey FD, Mkoji GM, Loker ES, 2017. Transcriptomic responses of Biomphalaria pfeifferi to Schistosoma mansoni: investigation of a neglected African snail that supports more S. mansoni transmission than any other snail species. 2017 Oct 18. PLoS Neglected Trop. Dis. 11 (10), e0005984. 10.1371/journal.pntd.0005984. eCollection Oct. PubMed PMID: 29045404; PubMed Central PMCID: PMC5685644.

Buresová V, Hajdusek O, Franta Z, Loosova G, Grunclova L, Levashina EA, Kopacek P, 2011. Functional genomics of tick thioester-containing proteins reveal the ancient origin of the complement system. J Innate Immun 3 (6), 623–630. 10.1159/000328851. Epub 2011 Jul 30. PubMed PMID: 21811049. [PubMed: 21811049]

Caldeira RL, Teodoro TM, Jannotti-Passos LK, Lira-Moreira PM, Goveia CO, Carvalho OS, 2016. Characterization of South American snails of the genus Biomphalaria (basommatophora: Planorbidae) and Schistosoma mansoni (Platyhelminthes: trematoda) in molluscs by PCR-RFLP. BioMed Res. Int. 2016 10.1155/2016/1045391. Article ID 1045391.

Carvalho OS, Jannotti-Passos LK, Caldeira RL, 2008. Importância Epidemiologica e Biologia Molecular Aplicada ao estudo dos Moluscos do Genero Biomphalariain. In: Carvalho OS, Coelho PMC, Lenzi HL (Eds.), Schistosoma mansoni e Esquistossomose: Uma visão multidisciplinar Cap9, pp. 311–346.

Castellanos-Martinez S, Arteta D, Catarino S, Gestal C, 2014. De novo transcriptome sequencing of the Octopus vulgaris hemocytes using Illumina RNA-Seq Technology: response to the infection by the gastrointestinal parasite Aggregata octopiana. PLoS One 9 (10), 10783.

Castillo MG, Wu XJ, Dinguirard N, Nyame AK, Cummings RD, Yoshino TP, 2007. Surface membrane proteins of Biomphalaria glabrata embryonic cells bind fucosyl determinants on the tegumental surface of Schistosoma mansoni primary sporocysts. Aug. J. Parasitol. 93 (4), 832–840 PubMed PMID: 17918362. [PubMed: 17918362]

Castillo MG, Goodson MS, McFall-Ngai M, 2009. Identification and molecular characterization of a complement C3 molecule in a lophotrochozoan, the Hawaiian bobtail squid Euprymna scolopes. Dev. Comp. Immunol. 33 (1), 69–76. 10.1016/j.dci.2008.07.013. PubMed PMID: 18765250; PubMed Central PMCID: PMC2642888. [PubMed: 18765250]

Cheng TC, 1975. Functional morphology and biochemistry of molluscan phagocytes. Ann. N. Y. Acad. Sci. 266, 343–379 Review. PubMed PMID: 829473. [PubMed: 829473]

Chernin E, 1970. Behavioral responses of miracidia of Schistosoma mansoni and other trematodes to substances emitted by snails. Apr. J. Parasitol. 56 (2), 287–296 PubMed PMID: 5445826. [PubMed: 5445826]

Civitello DJ, Fatima H, Johnson LR, Nisbet RM, Rohr JR, 2018. Bioenergetic theory predicts infection dynamics of human schistosomes in intermediate host snails across ecological gradients. May. Ecol. Lett. 21 (5), 692–701. 10.1111/ele.12937. Epub 2018 Mar 12. PubMed PMID: 29527787. [PubMed: 29527787]

Clennon JA, Mungai PL, Muchiri EM, King CH, Kitron U, 2006. Spatial and temporal variations in local transmission of Schistosoma haematobium in Msambweni, Kenya. Am. J. Trop. Med. Hyg. 75 (6), 1034–1041. [PubMed: 17172362]

Colley DG, Bustinduy AL, Secor WE, King CH, 2014. Human schistosomiasis. Jun 28. Lancet 383 (9936), 2253–2264. 10.1016/S0140-6736(13)619492. Epub 2014 Apr 1. Review. PubMed PMID: 24698483; PubMed Central PMCID: PMC4672382. [PubMed: 24698483]

Colley DG, Andros TS, Campbell CH Jr., 2017. Schistosomiasis is more prevalent than previously thought: what does it mean for public health goals, policies, strategies, guidelines and intervention programs? Mar 22. Infect Dis Poverty 6 (1), 63. 10.1186/s40249-017-0275-5. Review. PubMed PMID: 28327187; PubMed Central PMCID: PMC5361841. [PubMed: 28327187]

Cook GC, 2007. Tropical Medicine: an Illustrated History of the Pioneers. Academic Press, London, UK, pp. 157–165.

Coustau C, Yoshino TP, 2000. Flukes without snails: advances in the in vitro cultivation of intramolluscan stages of trematodes. Jan. Exp. Parasitol. 94 (1), 62–66 Review. PubMed PMID: 10631085. [PubMed: 10631085]

Coustau C, Ataev G, Jourdane J, Yoshino TP, 1997. Schistosoma japonicum: in vitro cultivation of miracidium to daughter sporocyst using a Biomphalaria glabrata embryonic cell line. Oct. Exp. Parasitol. 87 (2), 77–87. [PubMed: 9326883]

Coustau C, Gourbal B, Duval D, Yoshino TP, Adema CM, Mitta G, 2015. Advances in gastropod immunity from the study of the interaction between the snail Biomphalaria glabrata and its parasites: a review of research progress over the last decade. Sep. Fish Shellfish Immunol. 46 (1), 5–16. 10.1016/j.fsi.2015.01.036. Epub 2015 Feb 7. [PubMed: 25662712]

Cummins SF, Bowie JH, 2012. Pheromones, attractants and other chemical cues of aquatic organisms and amphibians.Jun. Nat. Prod. Rep. 29 (6), 642–658. 10.1039/c2np00102k. Epub 2012 Apr 12. Review. PubMed PMID: 22495567. [PubMed: 22495567]

Cummins SF, Nichols AE, Amare A, Hummon AB, Sweedler JV, Nagle GT, 2004. Characterization of Aplysia enticin and temptin, two novel water-borne protein pheromones that act in concert with attractin to stimulate mate attraction. Jun 11. J. Biol. Chem. 279 (24), 25614–25622 Epub 2004 Mar 30. PubMed PMID: 15054104. [PubMed: 15054104]

Cummins SF, Nichols AE, Warso CJ, Nagle GT, 2005. Aplysia seductin is a waterborne protein pheromone that acts in concert with attractin to stimulate mate attraction. Peptides. Mar 26 (3), 351–359 PubMed PMID: 15652640.

Damian RT, 1997. Parasite immune evasion and exploitation: reflections and projections. Parasitology 115 (Suppl. l), S169–S175 Review. PubMed PMID: 9571701. [PubMed: 9571701]

Davids BJ, Wu XJ, Yoshino TP, 1999. Cloning of a beta integrin subunit cDNA from an embryonic cell line derived from the freshwater mollusc, Biomphalaria glabrata. Mar 4. Gene 228 (1–2) 213–23. PubMed PMID: 10072774. [PubMed: 10072774]

De Zoysa M, Jung S, Lee J, 2008. First molluscan TNF-alpha homologue of the TNF superfamily in disk abalone: molecular characterization and expression analysis. Fish Shellfish Immunol. 26 (4), 625–631. 10.1016/j.fsi.2008.10.004. Epub 2008 Oct 18. [PubMed: 18984056]

DeFilippo J, Beck G, 2018. Cytokines of Invertebrate Immunity, Reference Module in Life Sciences. Elsevier 10.1016/B978-0-12-809633-8.90751-9.

Deleury E, Dubreuil G, Elangovan N, Wajnberg E, Reichhart JM, Gourbal B, Duval D, Baron OL, Gouzy J, Coustau C, 2012. Specific versus non-specific immune responses in an invertebrate species evidenced by a comparative de novo sequencing study. 2012. PLoS One 7 (3), e32512. 10.1371/journal.pone.0032512. EpubMar 12. PubMed PMID: 22427848; PubMed Central PMCID: PMC3299671.

Dheilly NM, Jouaux A, Boudry P, Favrel P, Lelong C, 2014. Transcriptomic profiling of gametogenesis in triploid Pacific Oysters Crassostrea gigas: towards an understanding of partial sterility associated with triploidy. Nov 6. PLoS One 9 (11), e112094. 10.1371/journal.pone.0112094. eCollection 2014. PubMed PMID: 25375782; PubMed Central PMCID: PMC4222980.

DiConza JJ, Basch PF, 1974. Axenic cultivation of Schistosoma mansoni daughter sporocysts. Oct. J. Parasitol. 60 (5), 757–763 PubMed PMID: 4430941. [PubMed: 4430941]

Dillon RT, 2000. The Ecology of Freshwater Molluscs. Cambridge University Press.

Dinguirard N, Cavalcanti MGS, Wu X-J, Bickham-Wright U, Sabat G, Yoshino TP, 2018. Proteomic analysis of Biomphalaria glabrata hemocytes during in vitro encapsulation of Schistosoma mansoni sporocysts. Front. Immunol. 9, 2773. 10.3389/fimmu.2018.02773. [PubMed: 30555466]

Dodds AW, Law SK, 1998. The phylogeny and evolution of the thioester bond-containing proteins C3, C4 and alpha 2-macroglobulin. Dec. Immunol. Rev. 166, 15–26 Review. PubMed PMID: 9914899. [PubMed: 9914899]

Dostálová A, Rommelaere S, Poidevin M, Lemaitre B, 2017. Thioester-containing proteins regulate the Toll pathway and play a role in Drosophila defence against microbial pathogens and parasitoid wasps. Sep. 5. BMC Biol. 15 (1), 79. 10.1186/s12915-017-0408-0. PubMed PMID: 28874153; PubMed Central PMCID: PMC5584532. [PubMed: 28874153]

Engels D, Chitsulo L, Montresor A, Savioli L, 2002. The global epidemiological situation of schistosomiasis and new approaches to control and research. May. Acta Trop. 82 (2), 139–146 PubMed PMID: 12020886; PubMed Central PMCID: PMC5633073. [PubMed: 12020886]

Fallon PG, Tao LF, Ismail MM, Bennett JL, 1996. Schistosome resistance to praziquantel: fact or artifact? Aug. Parasitol. Today 12 (8), 316–320. [PubMed: 15275183]

Fallon PG, Mubarak JS, Fookes RE, Niang M, Butterworth AE, Sturrock RF, Doenhoff MJ, 1997. Schistosoma mansoni: maturation rate and drug susceptibility of different geographical isolates. Exp. Parasitol. 86, 29–36. 10.1006/expr.1997.4149. [PubMed: 9149238]

Famakinde DO, 2018. Treading the path towards genetic control of snail resistance to schistosome infection. Aug 15. Trav. Med. Infect. Dis. 3 (3), E86. 10.3390/tropicalmed3030086. Review. PubMed PMID: 30274482; PubMed Central PMCID: PMC6160955.

Fiscon G, Conte F, Farina L, Paci P. Network-based approaches to explore complex biological systems towards network medicine. Genes 2018 Aug 31;9(9). pii: E437. doi: 10.3390/genes9090437. Review. PubMed PMID: 30200360; PubMed Central PMCID: PMC6162385. [PubMed: 30200360]

Fneich S, Dheilly N, Adema C, Rognon A, Reichelt M, Bulla J, Grunau C, Cosseau C, 2013. 5-methyl-cytosine and 5-hydroxy-methyl-cytosine in the genome of Biomphalaria glabrata, a snail intermediate host of Schistosoma mansoni. Jun 6. Parasites Vectors 6, 167. 10.1186/1756-3305-6-167. PubMed PMID: 23742053; PubMed Central PMCID: PMC3681652. [PubMed: 23742053]

Fryer SE, Bender RC, Bayne CJ, 1996. Inhibition of cysteine proteinase from Schistosoma mansoni larvae by alpha-macroglobulin from the plasma of Biomphalaria glabrata. Apr. J. Parasitol. 82 (2), 343–347 PubMed PMID: 8604113. [PubMed: 8604113]

Fujito NT, Sugimoto S, Nonaka M, 2010. Evolution of thioester-containing proteins revealed by cloning and characterization of their genes from a cnidarian sea anemone, Haliplanella lineate.Jul. Dev. Comp. Immunol. 34 (7), 775–784. 10.1016/j.dci.2010.02.011. Epub 2010 Mar 5. PubMed PMID: 20188753. [PubMed: 20188753]

Gaffen SL, 2008. An overview of IL-17 function and signaling. Sep. Cytokine 43 (3), 402–407. 10.1016/j.cyto.2008.07.017. Epub 2008 Aug 12. Review. PubMed PMID: 18701318; PubMed Central PMCID: PMC2582446. [PubMed: 18701318]

Galinier R, Portela J, Moné Y, Allienne JF, Henri H, Delbecq S, Mitta G, Gourbal B, Duval D, 2013. Biomphalysin, a new β pore-forming toxin involved in Biomphalaria glabrata immune defense against Schistosoma mansoni. Mar. PLoS Pathog. 9 (3), e1003216. 10.1371/journal.ppat.1003216. Epub 2013 Mar 21. PubMed PMID: 23555242; PubMed Central PMCID: PMC3605176.

Gavery MR, Roberts SB, 2010. DNA methylation patterns provide insight into epigenetic regulation in the Pacific oyster (Crassostrea gigas). Aug 27;11:483. BMC Genomics. 10.1186/1471-2164-11-483. PubMed PMID: 20799955; PubMed Central PMCID: PMC2996979.

GBD, 2016. DALYs and HALE Collaborators. 2017. Global, regional, and national disability-adjusted life-years (DALYs) for 333 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Sep. 16. Lancet 390 (10100), 1260–1344. 10.1016/S0140-6736(17)32130-X. Erratum in: Lancet. 2017 Oct 28;390(10106):e38. PubMed PMID: 28919118; PubMed Central PMCID: PMC5605707.

Geyer KK, Niazi UH, Duval D, Cosseau C, Tomlinson C, Chalmers IW, Swain MT, Cutress DJ, Bickham-Wright U, Munshi SE, Grunau C, Yoshino TP, Hoffmann KF, 2017. The Biomphalaria glabrata DNA methylation machinery displays spatial tissue expression, is differentially active in distinct snail populations and is modulated by interactions with Schistosoma mansoni. May 16. PLoS Neglected Trop. Dis. 11 (5), e0005246. 10.1371/journal.pntd.0005246. eCollection 2017 May.PubMed PMID: 28510608; PubMed Central PMCID: PMC5433704.

Gilmore TD, Wolenski FS, 2012. NF-κB: where did it come from and why?Mar. Immunol. Rev. 246 (1), 14–35. 10.1111/j.1600-065X.2012.01096.x. Review. PubMed PMID: 22435545. [PubMed: 22435545]

Goodall CP, Bender RC, Broderick EJ, Bayne CJ, 2004. Constitutive differences in Cu/Zn superoxide dismutase mRNA levels and activity in hemocytes of Biomphalaria glabrata (Mollusca) that are

either susceptible or resistant to Schistosoma mansoni (Trematoda). Oct. Mol. Biochem. Parasitol. 137 (2), 321–328 PubMed PMID: 15383302. [PubMed: 15383302]

Goodall CP, Bender RC, Brooks JK, Bayne CJ, 2006. Biomphalaria glabrata cytosolic copper/zinc superoxide dismutase (SOD1) gene: association of SOD1 alleles with resistance/susceptibility to Schistosoma mansoni.Jun. Mol. Biochem. Parasitol. 147 (2), 207–210 Epub 2006 Mar 9. PubMed PMID: 16564582. [PubMed: 16564582]

Gorbushin AM, 2018. Immune repertoire in the transcriptome of Littorina littorea reveals new trends in lophotrochozoan proto-complement evolution. Jul. Dev. Comp. Immunol. 84, 250–263. 10.1016/j.dci.2018.02.018. Epub 2018 Feb 28. PubMed PMID: 29501422. [PubMed: 29501422]

Gorbushin AM, 2019. Derivatives of the lectin complement pathway in Lophotrochozoa. May. Dev. Comp. Immunol. 94, 35–58. 10.1016/j.dci.2019.01.010. Epub 2019 Jan 22. PubMed PMID: 30682446. [PubMed: 30682446]

Gorbushin AM, Borisova EA, 2015. Lectin-like molecules in transcriptome of Littorina littorea hemocytes. Jan. Dev. Comp. Immunol. 48 (1), 210–220. 10.1016/j.dci.2014.10.007. Epub 2014 Oct 22. PubMed PMID: 25451301. [PubMed: 25451301]

Gordy MA, Pila EA, Hanington PC, 2015. The role of fibrinogen-related proteins in the gastropod immune response. Sep. Fish Shellfish Immunol. 46 (1), 39–49. 10.1016/j.fsi.2015.03.005. Epub 2015 Mar 10. Review. PubMed PMID: 25765166. [PubMed: 25765166]

Gourbal B, Pinaud S, Beckers GJM, Van Der Meer JWM, Conrath U, Netea MG, 2018. Innate immune memory: an evolutionary perspective. May. Immunol. Rev. 283 (1), 21–40. 10.1111/imr.12647. Review. PubMed PMID: 29664574. [PubMed: 29664574]

Granath WO Jr., Connors VA, Tarleton RL, 1994. Interleukin 1 activity in haemolymph from strains of the snail Biomphalaria glabrata varying in susceptibility to the human blood fluke, Schistosoma mansoni: presence, differential expression, and biological function. Jan. Cytokine 6 (1), 21–27 PubMed PMID: 8003629. [PubMed: 8003629]

Guillou F, Roger E, Moné Y, Rognon A, Grunau C, Théron A, Mitta G, Coustau C, Gourbal BE, 2007. Excretory-secretory proteome of larval Schistosoma mansoni and Echinostoma caproni, two parasites of Biomphalaria glabrata. Sep. Mol. Biochem. Parasitol. 155 (1), 45–56 Epub 2007 May 29. PubMed PMID: 17606306. [PubMed: 17606306]

Gurarie D, Seto EY, 2009. Connectivity sustains disease transmission in environments with low potential for endemicity: modelling schistosomiasis with hydrologic and social connectivities. Jun 6. J. R. Soc. Interface 6 (35), 495–508. 10.1098/rsif.2008.0265. Epub 2008 Sep 9. PubMed PMID: 18782722; PubMed Central PMCID: PMC2575370. [PubMed: 18782722]

Gurarie D, Lo NC, Ndeffo-Mbah ML, Durham DP, King CH, 2018. The humansnail transmission environment shapes long term schistosomiasis control outcomes: implications for improving the accuracy of predictive modeling. May 21. PLoS Neglected Trop. Dis. 12 (5), e0006514. 10.1371/journal.pntd.0006514. eCollection 2018 May. PubMed PMID: 29782500; PubMed Central PMCID: PMC5983867.

Gutiérrez JL, Jones CG, Strayer DL, Iribarne OO, 2003. Mollusks as ecosystem engineers: the role of shell production in aquatic habitats. Oikos 101 (1), 79–90.

Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J, 2006. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. Jul. Lancet Infect. Dis. 6 (7), 411–425 Review. PubMed PMID: 16790382. [PubMed: 16790382]

Haas W, Diekhoff D, Koch K, Schmalfuss G, Loy C, 1997a. Schistosoma mansoni cercariae: stimulation of acetabular gland secretion is adapted to the chemical composition of mammalian skin. Dec. J. Parasitol. 83 (6), 1079–1085 PubMed PMID: 9406783. [PubMed: 9406783]

Haas W, Diekhoff D, Koch K, Schmalfuss G, Loy C, 1997b. Schistosoma mansoni cercariae: stimulation of acetabular gland secretion is adapted to the chemical composition of mammalian skin. Dec. J. Parasitol. 83 (6), 1079–1085 PubMed PMID: 9406783. [PubMed: 9406783]

Haberl B, Haas W, 1992. Miracidium of Schistosoma mansoni: a macromolecular glycoconjugate as signal for the behaviour after contact with the snail host. Feb. Comp. Biochem. Physiol. A Comp. Physiol. 101 (2), 329–333 PubMed PMID: 1348464.

Hagen J, Young ND, Every AL, Pagel CN, Schnoeller C, Scheerlinck JP, Gasser RB, Kalinna BH, 2014. Omega-1 knockdown in Schistosoma mansoni eggs by lentivirus transduction reduces

granuloma size in vivo. Nov 17;5:5375. Nat. Commun. 10.1038/ncomms6375. PubMed PMID: 25400038; PubMed Central PMCID: PMC4243216.

Hahn UK, Bender RC, Bayne CJ, 2000. Production of reactive oxygen species by hemocytes of Biomphalaria glabrata: carbohydrate-specific stimulation. Dev. Comp. Immunol. 24 (6–7), 531–541. [PubMed: 10831788]

Hahn UK, Bender RC, Bayne CJ, 2001a. Killing of Schistosoma mansoni sporocysts by hemocytes from resistant Biomphalaria glabrata: role of reactive oxygen species. Apr. J. Parasitol. 87 (2), 292–299 PubMed PMID: 11318558. [PubMed: 11318558]

Hahn UK, Bender RC, Bayne CJ, 2001b. Involvement of nitric oxide in killing of Schistosoma mansoni sporocysts by hemocytes from resistant Biomphalaria glabrata. Aug. J. Parasitol. 87 (4), 778–785 PubMed PMID: 11534641. [PubMed: 11534641]

Hambrook JR, Kaboré AL, Pila EA, Hanington PC, 2018. A metalloprotease produced by larval Schistosoma mansoni facilitates infection establishment and maintenance in the snail host by interfering with immune cell function. Oct 29. PLoS Pathog. 14 (10), e1007393. 10.1371/journal.ppat.1007393. eCollection 2018 Oct. PubMed PMID: ; PubMed Central PMCID: PMC6224180. [PubMed: 30372490]

Hanelt B, Lun CM, Adema CM, 2008. Comparative ORESTES-sampling of transcriptomes of immune-challenged Biomphalaria glabrata snails. Oct. J. Invertebr. Pathol. 99 (2), 192–203. 10.1016/j.jip.2008.06.002. Epub 2008 Jun 12. PubMed PMID: 18590737; PubMed Central PMCID: PMC2724063. [PubMed: 18590737]

Hanington PC, Lun CM, Adema CM, Loker ES, 2010a. Time series analysis of the transcriptional responses of Biomphalaria glabrata throughout the course of intramolluscan development of Schistosoma mansoni and Echinostoma paraensei. Jun. Int. J. Parasitol. 40 (7), 819–831. 10.1016/j.ijpara.2009.12.005. Epub 2010 Jan 18. PubMed PMID: 20083115; PubMed Central PMCID: PMC2866805. [PubMed: 20083115]

Hanington PC, Forys MA, Dragoo JW, Zhang SM, Adema CM, Loker ES, 2010b. Role for a somatically diversified lectin in resistance of an invertebrate to parasite infection. Dec 7. Proc. Natl. Acad. Sci. U. S. A. 107 (49), 21087–21092. 10.1073/pnas.1011242107. Epub 2010 Nov 17. PubMed PMID: 21084634; PubMed Central PMCID: PMC3000291. [PubMed: 21084634]

Hansen EL, 1975. Secondary daughter sporocysts of Schistosoma mansoni: their occurrence and cultivation. Ann. N. Y. Acad. Sci. 266, 426–436. [PubMed: 1072603]

Hansen EL, 1976. A cell line from embryos of Biomphalaria glabrata (Pulmonata): establishment and characteristics. In: Maramorosch K. (Ed.), Invertebrate Tissue Culture: Research Applications. Academic Press, Inc., Cambridge, pp. 75–98.

Harashima S, Horiuchi T, Hatta N, Morita C, Higuchi M, Sawabe T, Tsukamoto H, Tahira T, Hayashi K, Fujita S, Niho Y, 2001. Outside-to-inside signal through the membrane TNF-alpha induces E-selectin (CD62E) expression on activated human CD4+ T cells. Jan 1. J. Immunol. 166 (1), 130–136 PubMed PMID: 11123285. [PubMed: 11123285]

Harris KR, 1975. The fine structure of encapsulation in Biomphalaria glabrata. Ann. N. Y. Acad. Sci. 266, 446–464 PubMed PMID: 1072605. [PubMed: 1072605]

Harris KR, Cheng TC, 1975. The encapsulation process in Biomphalaria glabrata experimentally infected with the metastrongylid Angiostrongylus cantonensis: light microscopy. Oct. Int. J. Parasitol. 5 (5), 521–528 PubMed PMID: 1158554. [PubMed: 1158554]

Hertel LA, Bayne CJ, Loker ES, 2002. The symbiont Capsaspora owczarzaki, nov. gen. nov. sp., isolated from three strains of the pulmonate snail Biomphalaria glabrata is related to members of the Mesomycetozoea. Aug. Int. J. Parasitol. 32 (9), 1183–1191 PubMed PMID: 12117501. [PubMed: 12117501]

Hertel LA, Adema CM, Loker ES, 2005. Differential expression of FREP genes in two strains of Biomphalaria glabrata following exposure to the digenetic trematodes Schistosoma mansoni and Echinostoma paraensei. Dev. Comp. Immunol. 29 (4), 295–303 PubMed PMID: 15859234. [PubMed: 15859234]

Hirose T, Mishima Y, Tomari Y, 2014. Elements and machinery of non-coding RNAs: toward their taxonomy. May. EMBO Rep. 15 (5), 489–507. 10.1002/embr.201338390. Epub 2014 Apr 14. Review. PubMed PMID: 24731943; PubMed Central PMCID: PMC4210095. [PubMed: 24731943]

Hoeksema M, van Eijk M, Haagsman HP, Hartshorn KL, 2016. Histones as mediators of host defense, inflammation and thrombosis. Future Microbiol. 11 (3), 441–453. 10.2217/fmb.15.151. Epub 2016 Mar 4. Review. PubMed PMID: 26939619; PubMed Central PMCID: PMC5549641. [PubMed: 26939619]

Hotez PJ, Alvarado M, Basáñez MG, Bolliger I, Bourne R, Boussinesq M, Brooker SJ, Brown AS, Buckle G, Budke CM, Carabin H, Coffeng LE, Fèvre EM, Fürst T, Halasa YA, Jasrasaria R, Johns NE, Keiser J, King CH, Lozano R, Murdoch ME, O'Hanlon S, Pion SD, Pullan RL, Ramaiah KD, Roberts T, Shepard DS, Smith JL, Stolk WA, Undurraga EA, Utzinger J, Wang M, Murray CJ, Naghavi M, 2014. The global burden of disease study 2010: interpretation and implications for the neglected tropical diseases. Jul 24. PLoS Neglected Trop. Dis. 8 (7), e2865. 10.1371/journal.pntd.0002865. eCollection 2014 Jul. PubMed PMID: 25058013; PubMed Central PMCID: PMC4109880.

Humphries JE, Deneckere LE, 2018. Characterization of a Toll-like receptor (TLR) signaling pathway in Biomphalaria glabrata and its potential regulation by NF-kappaB. Sep. Dev. Comp. Immunol. 86, 118–129. 10.1016/j.dci.2018.05.003. Epub 2018 May 7. PubMed PMID: 29746981. [PubMed: 29746981]

Humphries J, Harter B, 2015. Identification of nuclear factor kappaB (NF-κB) binding motifs in Biomphalaria glabrata. Dec. Dev. Comp. Immunol. 53 (2), 366–370. 10.1016/j.dci.2015.08.004. Epub 2015 Aug 12. PubMed PMID: 26277107. [PubMed: 26277107]

Humphries JE, Yoshino TP, 2006. Schistosoma mansoni excretory-secretory products stimulate a p38 signalling pathway in Biomphalaria glabrata embryonic cells. Jan. Int. J. Parasitol. 36 (1), 37–46 Epub 2005 Sep 15. PubMed PMID: 16194541. [PubMed: 16194541]

Ismail M, Botros S, Metwally A, William S, Farghally A, Tao LF, Day TA, Bennett JL, 1999. Resistance to praziquantel: direct evidence from Schistosoma mansoni isolated from Egyptian villagers. Am. J. Trop. Med. Hyg. 60, 932–935. [PubMed: 10403323]

Ittiprasert W, Miller A, Myers J, Nene V, El-Sayed NM, Knight M, 2010. Identification of immediate response genes dominantly expressed in juvenile resistant and susceptible Biomphalaria glabrata snails upon exposure to Schistosoma mansoni. Jan. Mol. Biochem. Parasitol. 169 (1), 27–39. 10.1016/j.molbiopara.2009.09.009. Epub 2009 Oct 6. PubMed PMID: 19815034; PubMed Central PMCID: PMC2785114. [PubMed: 19815034]

Ittiprasert W, Mann VH, Karinshak SE, Coghlan A, Rinaldi G, Sankaranarayanan G, Chaidee A, Tanno T, Kumkhaek C, Prangtaworn P, Mentink-Kane MM, Cochran CJ, Driguez P, Holroyd N, Tracey A, Rodpai R, Everts B, Hokke CH, Hoffmann KF, Berriman M, Brindley PJ, 2019. Programmed genome editing of the omega-1 ribonuclease of the blood fluke, Schistosoma mansoni. Jan 15;8. Elife, e41337. 10.7554/eLife.41337. PubMed PMID: 30644357; PubMed Central PMCID: PMC6355194.

Iwaki D, Kawabata S, Miura Y, Kato A, Armstrong PB, Quigley JP, Nielsen KL, Dolmer K, Sottrup-Jensen L, Iwanaga S, 1996. Molecular cloning of Limulus alpha 2-macroglobulin. Dec 15. Eur. J. Biochem. 242 (3) 822–31. PubMed PMID: 9022715. [PubMed: 9022715]

Iwanaga S, Kawabata S, Muta T, 1998. New types of clotting factors and defense molecules found in horseshoe crab hemolymph: their structures and functions. Jan. J. Biochem. 123 (1), 1–15 Review. PubMed PMID: 9504402. [PubMed: 9504402]

Johnston LA, Yoshino TP, 2001. Larval Schistosoma mansoni excretory-secretory glycoproteins (ESPs) bind to hemocytes of Biomphalaria glabrata (Gastropoda) via surface carbohydrate binding receptors. Aug. J. Parasitol. 87 (4), 786–793 PubMed PMID: 11534642. [PubMed: 11534642]

Joky A, Matricon-Gondran M, Benex J, 1985. Response to the amoebocyte-producing organ of sensitized Biomphalaria glabrata after exposure to Echinostoma caproni miracidia. Jan. J. Invertebr. Pathol. 45 (1), 28–33 PubMed PMID: 3968444. [PubMed: 3968444]

Jourdane J, Théron A, 1987. Larval development: eggs to cercariae. In: Erasmus DA (Ed.), The Biology of Schistosomes. Academic Press Ltd., London, New York, pp. 83–113.

Kalbe M, Haberl B, Haas W, 1996. Schistosoma mansoni miracidial host-finding: species specificity of an Egyptian strain. Parasitol. Res. 82 (1), 8–13 PubMed PMID: 8825437. [PubMed: 8825437]

Kalbe M, Haberl B, Haas W, 1997. Miracidial host-finding in Fasciola hepatica and Trichobilharzia ocellata is stimulated by species-specific glycoconjugates released from the host snails. Parasitol. Res. 83 (8), 806–812 PubMed PMID: 9342748. [PubMed: 9342748]

Kenny NJ, Truchado-García M, Grande C, 2016. Deep, multi-stage transcriptome of the schistosomiasis vector Biomphalaria glabrata provides platform for understanding molluscan disease-related pathways. Oct 28. BMC Infect. Dis. 16 (1) 618. PubMed PMID: 27793108; PubMed Central PMCID: PMC5084317. [PubMed: 27793108]

Kincaid-Smith J, Rey O, Toulza E, Berry A, Boissier J, 2017. Emerging schistosomiasis in europe: a need to quantify the risks. Aug. Trends Parasitol. 33 (8), 600–609. 10.1016/j.pt.2017.04.009. Epub 2017 May 21. Review. PubMed PMID: 28539255. [PubMed: 28539255]

King CH, Sutherland LJ, Bertsch D, 2015. Systematic review and meta-analysis of the impact of chemical-based molluscicide for control of Schistosoma mansoni and S. haematobium transmission. Dec 28. PLoS Neglected Trop. Dis. 9 (12), e0004290. 10.1371/journal.pntd.0004290. eCollection 2015 Dec. Review. PubMed PMID: 26709922; PubMed Central PMCID: PMC4692485.

Kinoti GK, 1971. The attachment and penetration apparatus of the miracidium of Schistosoma. J. Helminthol. 45 (2), 229–235 PubMed PMID: 5123700. [PubMed: 5123700]

Knight M, Arican-Goktas HD, Ittiprasert W, Odoemelam EC, Miller AN, Bridger JM, 2014. Schistosomes and snails: a molecular encounter. 2014 Jul 21. Front. Genet. 5, 230. 10.3389/fgene.2014.00230. eCollection Review. PubMed PMID: 25101114; PubMed Central PMCID: PMC4104801. [PubMed: 25101114]

Kopacek P, Hajdusek O, Buresova V, 2012. Tick as a model for the study of a primitive complement system. Adv. Exp. Med. Biol. 710, 83–93. 10.1007/9781-4419-5638-5_9. PubMed PMID: 22127888. [PubMed: 22127888]

Krauth SJ, Balen J, Gobert GN, Lamberton PHL, 2019. A call for systems epidemiology to tackle the complexity of schistosomiasis, its control, and its elimination. Jan 29. Trav. Med. Infect. Dis. 4 (1), E21. 10.3390/tropicalmed4010021. PubMed PMID: 30699922; PubMed Central PMCID: PMC6473336.

Kyrou K, Hammond AM, Galizi R, Kranjc N, Burt A, Beaghton AK, Nolan T, Crisanti A, 2018. A CRISPR-Cas9 gene drive targeting doublesex causes complete population suppression in caged Anopheles gambiae mosquitoes. Dec. Nat. Biotechnol. 36 (11), 1062–1066. 10.1038/nbt.4245. Epub 2018 Sep 24. PubMed PMID: 30247490. [PubMed: 30247490]

Lardans V, Dissous C, 1998. Snail control strategies for reduction of schistosomiasis transmission. Oct. Parasitol. Today 14 (10), 413–417 PubMedPMID: 17040832. [PubMed: 17040832]

Law SK, Lichtenberg NA, Levine RP, 1980. Covalent binding and hemolytic activity of complement proteins. Dec. Proc. Natl. Acad. Sci. U. S. A. 77 (12), 7194–7198 PubMed PMID: 6938964; PubMed Central PMCID: PMC350468. [PubMed: 6938964]

Leiper RT, Atkinson EL, 1915. Oosevations on the spread of asicatic schistosomiasis. Jan 30. Br. Med. J. 1 (2822) 201–192.4. PubMed PMID: 20767468; PubMed Central PMCID: PMC2301688. [PubMed: 20767468]

Letunic I, Bork P, 2018. 20 years of the SMART protein domain annotation resource. Jan 4. Nucleic Acids Res. 46 (D1), D493–D496. 10.1093/nar/gkx922. PubMed PMID: ; PubMed Central PMCID: PMC5753352. [PubMed: 29040681]

Levashina EA, Moita LF, Blandin S, Vriend G, Lagueux M, Kafatos FC, 2001. Conserved role of a complement-like protein in phagocytosis revealed by dsRNA knockout in cultured cells of the mosquito, Anopheles gambiae. Mar 9. Cell 104 (5) 709–18. PubMed PMID: 11257225. [PubMed: 11257225]

Li BV, Williams M, Logarajah S, Baxter RH, 2012. Molecular basis for genetic resistance of Anopheles gambiae to Plasmodium: structural analysis of TEP1 susceptible and resistant alleles. PLoS Pathog. 8 (10), e1002958. 10.1371/journal.ppat.1002958. Epub 2012 Oct 4. PubMed PMID: 23055931; PubMed Central PMCID: PMC3464232.

Lie KJ, Jeong KH, Heyneman D, 1983. Acquired resistance in snails. Induction of resistance to Schistosoma mansoni in Biomphalaria glabrata. Jun. Int. J. Parasitol. 13 (3), 301–304 PubMed PMID: 6874229. [PubMed: 6874229]

Lockyer AE, Spinks JN, Walker AJ, Kane RA, Noble LR, Rollinson D, Dias-Neto E, Jones CS, 2007. Biomphalaria glabrata transcriptome: identification of cell-signalling, transcriptional control and immune-related genes from open reading frame expressed sequence tags (ORESTES). Dev. Comp. Immunol. 31 (8), 763–782 Epub 2006 Dec 14. PubMed PMID: 17208299; PubMed Central PMCID: PMC1871615. [PubMed: 17208299]

Lockyer AE, Spinks J, Kane RA, Hoffmann KF, Fitzpatrick JM, Rollinson D, Noble LR, Jones CS, 2008. Biomphalaria glabrata transcriptome: cDNA microarray profiling identifies resistant- and susceptible-specific gene expression in haemocytes from snail strains exposed to Schistosoma mansoni. Dec 29. BMC Genomics 9, 634. 10.1186/1471-2164-9-634. PubMed PMID: 19114004; PubMed Central PMCID: PMC2631019. [PubMed: 19114004]

Lockyer AE, Emery AM, Kane RA, Walker AJ, Mayer CD, Mitta G, Coustau C, Adema CM, Hanelt B, Rollinson D, Noble LR, Jones CS, 2012. Early differential gene expression in haemocytes from resistant and susceptible Biomphalaria glabrata strains in response to Schistosoma mansoni. PLoS One 7 (12), e51102. 10.1371/journal.pone.0051102. Epub 2012 Dec 26. PubMed PMID: 23300533; PubMed Central PMCID: PMC3530592.

Lodes MJ, Yoshino TP, 1990. The effect of schistosome excretory-secretory products on Biomphalaria glabrata hemocyte motility. Jul. J. Invertebr. Pathol. 56 (1), 75–85 PubMed PMID: 2376664. [PubMed: 2376664]

Loker ES, 2010. Gastropod immunobiology. Adv. Exp. Med. Biol. 708, 17–43 Review.PubMed PMID: 21528691. [PubMed: 21528691]

Loker ES, Bayne CJ, 2018. Molluscan immunobiology: challenges in the anthropocene epoch. In: Cooper Edwin L. (Ed.), Advances in Comparative Immunology. Springer International Publishing, pp. 343–501. 10.1007/978-3-319-76768-0.

Loker ES, Mkoji GM, 2005. Schistosomes and their snail hosts: the present and future of reconstructing their past. In: World Class Parasites: Vol 10. Schistosomes. Springer, N.Y, pp. 1–11 WE Secor and DG Colley.

Loker ES, Bayne CJ, Buckley PM, Kruse KT, 1982. Ultrastructure of encapsulation of Schistosoma mansoni mother sporocysts by hemocytes of juveniles of the 10-R2 strain of Biomphalaria glabrata. Feb. J. Parasitol. 68 (1), 84–94 PubMed PMID: 7077450. [PubMed: 7077450]

London Declaration on Neglected Tropical Diseases, 2012. Uniting to combat NTDs. Available from: http://unitingtocombatntds.org/london-declaration-neglectedtropical-diseases/.

LoVerde PT, 1975. Scanning electron microscope observations on the miracidium of Schistosoma.Feb. Int. J. Parasitol. 5 (1), 95–97 PubMed PMID: 1112635. [PubMed: 1112635]

MacEwan DJ, 2002. TNF ligands and receptors–a matter of life and death.Feb. Br. J. Pharmacol. 135 (4), 855–875 Review. PubMed PMID: 11861313; PubMed Central PMCID: PMC1573213. [PubMed: 11861313]

Macinnis AJ, 1965. Responses of Schistosoma mansoni miracidia to chemical attractants. Oct. J. Parasitol. 51 (5), 731–746 PubMed PMID: 5857269. [PubMed: 5857269]

Mansour TA, Habib MR, Rodríguez LCV, Vázquez AH, Alers JM, Ghezzi A, Croll RP, Brown CT, Miller MW. Central nervous system transcriptome of Biomphalaria alexandrina, an intermediate host for schistosomiasis. BMC Res. Notes. 2017 Dec 11;10(1):729. doi: 10.1186/s13104-017-3018-6. PubMed PMID: ; PubMed Central PMCID: PMC5725652. [PubMed: 29228974]

Mari L, Gatto M, Ciddio M, Dia ED, Sokolow SH, De Leo GA, Casagrandi R, 2017. Big-data-driven modeling unveils country-wide drivers of endemic schistosomiasis. Mar 28. Sci. Rep. 7 (1), 489. 10.1038/s41598-017-00493-1. PubMed PMID: 28352101; PubMed Central PMCID: PMC5428445. [PubMed: 28352101]

McCullough FS, 1981. Biological control of the snail intermediate hosts of human Schistosoma spp.: a review of its present status and future prospects. Mar. Acta Trop. 38 (1), 5–13 PubMed PMID: 6111917. [PubMed: 6111917]

McCullough FS, Gayral P, Duncan J, Chrissie JD, 1980. Molluscicides in schistosomiasis control. Bull. World Health Organ. 58, 681–689. [PubMed: 6975179]

McManus DP, Dunne DW, Sacko M, Utzinger J, Vennervald BJ, Zhou XN, 2018. Aug 9. Schistosomiasis. Nat Rev Dis Primers. 4 (1), 13. 10.1038/s41572-018-0013-8. Review. PubMed PMID: 30093684. [PubMed: 30093684]

McVeigh P, Maule AG, 2019. Can CRISPR help in the fight against parasitic worms? Jan 31;8. Elife, e44382. 10.7554/eLife.44382. PubMed PMID: 30702425; PubMed Central PMCID: PMC6355191.

Melman SD, Steinauer ML, Cunningham C, Kubatko LS, Mwangi IN, Wynn NB, Mutuku MW, Karanja DM, Colley DG, Black CL, Secor WE, Mkoji GM, Loker ES, 2009. Reduced susceptibility to praziquantel among naturally occurring Kenyan isolates of Schistosoma mansoni. Aug 18. PLoS Neglected Trop. Dis. 3 (8), e504. 10.1371/journal.pntd.0000504. PubMed PMID: 19688043; PubMed Central PMCID: PMC2721635.

Mickum ML, Prasanphanich NS, Heimburg-Molinaro J, Leon KE, Cummings RD. Deciphering the glycogenome of schistosomes. Front. Genet.. 2014 Aug 5;5:262. doi: 10.3389/fgene.2014.00262. eCollection 2014. Review. PubMed PMID: 25147556; PubMed Central PMCID: PMC4122909. [PubMed: 25147556]

Mitta G, Galinier R, Tisseyre P, Allienne JF, Girerd-Chambaz Y, Guillou F, Bouchut A, Coustau C, 2005. Gene discovery and expression analysis of immunerelevant genes from Biomphalaria glabrata hemocytes. Dev. Comp. Immunol. 29 (5), 393–407 Epub 2004 Dec 8. PubMed PMID: 15707661. [PubMed: 15707661]

Mitta G, Adema CM, Gourbal B, Loker ES, Theron A, 2012. Compatibility polymorphism in snail/schistosome interactions: from field to theory to molecular mechanisms. May. Dev. Comp. Immunol. 37 (1), 1–8. 10.1016/j.dci.2011.09.002. Epub 2011 Sep 16. Review. PubMed PMID: 21945832; PubMed Central PMCID: PMC3645982. [PubMed: 21945832]

Miyairi K, Suzuki M, 1914. Der Zwischenwirt des Schistosomum japonicum Katsurada. Der Universität.

Mkoji GM, Hofkin BV, Kuris AM, Stewart-Oaten A, Mungai BN, Kihara JH, Mungai F, Yundu J, Mbui J, Rashid JR, Kariuki CH, Ouma JH, Koech DK, Loker ES, 1999. Impact of the crayfish Procambarus clarkii on Schistosoma haematobium transmission in Kenya. Nov. Am. J. Trop. Med. Hyg. 61 (5), 751–759 PubMed PMID: 10586907. [PubMed: 10586907]

Moita LF, Wang-Sattler R, Michel K, Zimmermann T, Blandin S, Levashina EA, Kafatos FC, 2005. In vivo identification of novel regulators and conserved pathways of phagocytosis in A. gambiae. Jul. Immunity 23 (1), 65–73 PubMed PMID: 16039580. [PubMed: 16039580]

Moné Y, Gourbal B, Duval D, Du Pasquier L, Kieffer-Jaquinod S, Mitta G, 2010. A large repertoire of parasite epitopes matched by a large repertoire of host immune receptors in an invertebrate host/parasite model. Sep. 7. PLoS Neglected Trop. Dis. 4 (9), e813. 10.1371/journal.pntd.0000813. PubMed PMID: 20838648; PubMed Central PMCID: PMC2935394.

Morris KV, Mattick JS, 2014. The rise of regulatory RNA.Jun. Nat. Rev. Genet. 15 (6), 423–437. 10.1038/nrg3722. Epub 2014 Apr 29. Review. PubMed PMID:24776770; PubMed Central PMCID: PMC4314111. [PubMed: 24776770]

Nonaka M, 2014. Evolution of the complement system. Subcell. Biochem. 80, 31–43. 10.1007/978-94-017-8881-6_3. Review. PubMed PMID: 24798006. [PubMed: 24798006]

Nonaka M, Kimura A, 2006. Genomic view of the evolution of the complement system. Sep. Immunogenetics 58 (9), 701–713 Epub 2006 Aug 9. Review. PubMed PMID: 16896831; PubMed Central PMCID: PMC2480602. [PubMed: 16896831]

Nonaka M, Satake H, 2010. Urochordate immunity. Adv. Exp. Med. Biol. 708, 302–310 Review. PubMed PMID: 21528704. [PubMed: 21528704]

Nowacki FC, Swain MT, Klychnikov OI, Niazi U, Ivens A, Quintana JF, Hensbergen PJ, Hokke CH, Buck AH, Hoffmann KF, 2015. Protein and small non-coding RNA-enriched extracellular vesicles are released by the pathogenic blood fluke Schistosoma mansoni. Oct 5. J. Extracell. Vesicles 4, 28665. 10.3402/jev.v4.28665. eCollection 2015. PubMed PMID: 26443722; PubMed Central PMCID: PMC4595467.

Obbard DJ, Callister DM, Jiggins FM, Soares DC, Yan G, Little TJ, 2008. The evolution of TEP1, an exceptionally polymorphic immunity gene in Anopheles gambiae. Oct 7;8:274. BMC Evol. Biol. 10.1186/1471-2148-8-274. PubMed PMID: 18840262; PubMed Central PMCID: PMC2576239. [PubMed: 18840262]

Odoemelam E, Raghavan N, Miller A, Bridger JM, Knight M, 2009. Revised karyotyping and gene mapping of the Biomphalaria glabrata embryonic (Bge) cell line. May. Int. J. Parasitol. 39 (6), 675–681. 10.1016/j.ijpara.2008.11.011. Epub 2008 Dec 24. PubMed PMID: 19133265; PubMed Central PMCID: PMC2656398. [PubMed: 19133265]

Ouwe-Missi-Oukem-Boyer O, Porchet E, Capron A, Dissous C, 1994. Characterization of immunoreactive TNF alpha molecules in the gastropod Biomphalaria glabrata. May-Jun. Dev. Comp. Immunol. 18 (3), 211–218 PubMed PMID: 8001700. [PubMed: 8001700]

Pan SC, 1980. The fine structure of the miracidium of Schistosoma mansoni.Nov. J. Invertebr. Pathol. 36 (3), 307–372 PubMed PMID: 7452064. [PubMed: 7452064]

Pereira CA, Martins-Souza RL, Corrêa A Jr., Coelho PM, Negrão-Corrêa D, 2008. Participation of cell-free haemolymph of Biomphalaria tenagophila in the defence mechanism against Schistosoma mansoni sporocysts.Nov-Dec. Parasite Immunol. 30 (11–12), 610–619. 10.1111/j.1365-3024.2008.01062.x. PubMed PMID: 19067842. [PubMed: 19067842]

Perez-Saez J, Mande T, Ceperley N, Bertuzzo E, Mari L, Gatto M, Rinaldo A, 2016. Hydrology and density feedbacks control the ecology of intermediate hosts of schistosomiasis across habitats in seasonal climates. Jun 7. Proc. Natl. Acad. Sci. U. S. A. 113 (23), 6427–6432. 10.1073/pnas.1602251113. Epub 2016 May 9. PubMed PMID: 27162339; PubMed Central PMCID: PMC4988578. [PubMed: 27162339]

Pieri OS, Thomas JD, 1987. Snail host control in the eastern coastal areas of north-east (NE) Brazil. Mem. Inst. Oswaldo Cruz 82 (Suppl. 4), 197–201 PubMed PMID: 3151093.

Pila EA, Tarrabain M, Kabore AL, Hanington PC, 2016a. A novel toll-like receptor (TLR) influences compatibility between the gastropod Biomphalaria glabrata, and the digenean trematode Schistosoma mansoni. 2016 Mar 25. PLoS Pathog. 12 (3), e1005513. 10.1371/journal.ppat.1005513. eCollection. Mar. PubMed PMID: 27015424; PubMed Central PMCID: PMC4807771.

Pila EA, Gordy MA, Phillips VK, Kabore AL, Rudko SP, Hanington PC, 2016b. Endogenous growth factor stimulation of hemocyte proliferation induces resistance to Schistosoma mansoni challenge in the snail host. May 10. Proc. Natl. Acad. Sci. U. S. A. 113 (19), 5305–5310. 10.1073/pnas.1521239113. Epub 2016 Apr 25. PubMed PMID: 27114544; PubMed Central PMCID: PMC4868488. [PubMed: 27114544]

Pila EA, Li H, Hambrook JR, Wu X, Hanington PC, 2017. Schistosomiasis from a snail's perspective: advances in snail immunity. Nov. Trends Parasitol. 33 (11), 845–857. 10.1016/j.pt.2017.07.006. Epub 2017 Aug 11. Review. PubMed PMID: 28803793. [PubMed: 28803793]

Pinaud S, Portela J, Duval D, Nowacki FC, Olive MA, Allienne JF, Galinier R, Dheilly NM, Kieffer-Jaquinod S, Mitta G, Théron A, Gourbal B, 2016. A shift from cellular to humoral responses contributes to innate immune memory in the vector snail Biomphalaria glabrata. Jan 6. PLoS Pathog. 12 (1), e1005361. 10.1371/journal.ppat.1005361. eCollection 2016 Jan. PubMed PMID: 26735307; PubMed Central PMCID: PMC4703209.

Pinaud S, Portet A, Allienne JF, Belmudes L, Saint-Beat C, Arancibia N, Galinier R, Du Pasquier L, Duval D, Gourbal B, 2019. Molecular characterisation of immunological memory following homologous or heterologous challenges in the schistosomiasis vector snail, Biomphalaria glabrata. Mar. Dev. Comp. Immunol. 92, 238–252. 10.1016/j.dci.2018.12.001. Epub 2018 Dec 4. PubMed PMID: 30529491. [PubMed: 30529491]

Pinto MR, Melillo D, Giacomelli S, Sfyroera G, Lambris JD, 2007. Ancient origin of the complement system: emerging invertebrate models. Adv. Exp. Med. Biol. 598, 372–388 Review. PubMed PMID: 17892225. [PubMed: 17892225]

Portela J, Duval D, Rognon A, Galinier R, Boissier J, Coustau C, Mitta G, Théron A, Gourbal B, 2013. Evidence for specific genotype-dependent immune priming in the lophotrochozoan Biomphalaria glabrata snail. J Innate Immun 5 (3), 261–276. 10.1159/000345909. Epub 2013 Jan 22. PubMed PMID: 23343530. [PubMed: 23343530]

Portet A, Pinaud S, Tetreau G, Galinier R, Cosseau C, Duval D, Grunau C, Mitta G, Gourbal B, 2017. Integrated multi-omic analyses in Biomphalaria-Schistosoma dialogue reveal the immunobiological significance of FREP-SmPoMuc interaction. Oct. Dev. Comp. Immunol. 75, 16–27. 10.1016/j.dci.2017.02.025. Epub 2017 Feb 28. Review. PubMed PMID: 28257854. [PubMed: 28257854]

Portet A, Galinier R, Pinaud S, Portela J, Nowacki F, Gourbal B, Duval D, 2018. BgTEP: an antiprotease involved in innate immune sensing in Biomphalaria glabrata. May 29. Front. Immunol. 9, 1206. 10.3389/fimmu.2018.01206. eCollection 2018. PubMed PMID: 29899746; PubMed Central PMCID: PMC5989330. [PubMed: 29899746]

Prasanphanich NS, Luyai AE, Song X, Heimburg-Molinaro J, Mandalasi M, Mickum M, Smith DF, Nyame AK, Cummings RD, 2014. Immunization with recombinantly expressed glycan antigens from Schistosoma mansoni induces glycanspecific antibodies against the parasite. Jul. Glycobiology 24 (7), 619–637. 10.1093/glycob/cwu027. Epub 2014 Apr 11. PubMed PMID: 24727440; PubMed Central PMCID: PMC4038251. [PubMed: 24727440]

Queiroz FR, Silva LM, Jeremias WJ, Babá ÉH, Caldeira RL, Coelho PMZ, et al. , 2017. Differential expression of small RNA pathway genes associated with the Biomphalaria glabrata/Schistosoma mansoni interaction. PLoS One 12 (7), e0181483. 10.1371/journal.pone.0181483.

Roberts TM, Ward S, Chernin E, 1979. Behavioral responses of Schistosoma mansoni miracidia in concentration gradients of snail-conditioned water. Feb. J. Parasitol. 65 (1), 41–49 PubMed PMID: 448598. [PubMed: 448598]

Roberts TM, Linck RW, Chernin E, 1980. Effector mechanism in the response of Schistosoma mansoni miracidia to snail-conditioned water. Feb. J. Exp. Zool. 211 (2), 137–142 PubMed PMID: 7373270. [PubMed: 7373270]

Robijn ML, Wuhrer M, Kornelis D, Deelder AM, Geyer R, Hokke CH, 2005. Mapping fucosylated epitopes on glycoproteins and glycolipids of Schistosoma mansoni cercariae, adult worms and eggs. Jan. Parasitology 130 (Pt 1), 67–77 PubMed PMID: 15700758. [PubMed: 15700758]

Roger E, Mitta G, Moné Y, Bouchut A, Rognon A, Grunau C, Boissier J, Théron A, Gourbal BE, 2008a. Molecular determinants of compatibility polymorphism in the Biomphalaria glabrata/Schistosoma mansoni model: new candidates identified by a global comparative proteomics approach. Feb. Mol. Biochem. Parasitol. 157 (2), 205–216 Epub 2007 Nov 17. PubMed PMID: 18083248. [PubMed: 18083248]

Roger E, Grunau C, Pierce RJ, Hirai H, Gourbal B, Galinier R, Emans R, Cesari IM, Cosseau C, Mitta G, 2008b. Controlled chaos of polymorphic mucins in a metazoan parasite (Schistosoma mansoni) interacting with its invertebrate host (Biomphalaria glabrata). PLoS Neglected Trop. Dis. 2 (11), e330. 10.1371/journal.pntd.0000330. Epub 2008 Nov 11. PubMed PMID: 19002242; PubMed Central PMCID: PMC2576457.

Ruprecht C, Vaid N, Proost S, Persson S, Mutwil M, 2017. Beyond genomics: studying evolution with gene coexpression networks. Apr. Trends Plant Sci. 22 (4), 298–307. 10.1016/j.tplants.2016.12.011. Epub 2017 Jan 23. Review. PubMed PMID: 28126286. [PubMed: 28126286]

Salter JP, Lim KC, Hansell E, Hsieh I, McKerrow JH, 2000. Schistosome invasion of human skin and degradation of dermal elastin are mediated by a single serine protease. Dec 8. J. Biol. Chem. 275 (49) 38667–73. PubMed PMID: 10993899. [PubMed: 10993899]

Sato MO, Rafalimanantsoa A, Ramarokoto C, Rahetilahy AM, Ravoniarimbinina P, Kawai S, Minamoto T, Sato M, Kirinoki M, Rasolofo V, De Calan M, Chigusa Y, 2018. Usefulness of environmental DNA for detecting Schistosoma mansoni occurrence sites in Madagascar. Nov. Int. J. Infect. Dis. 76, 130–136. 10.1016/j.ijid.2018.08.018. Epub 2018 Sep 7. PubMed PMID: 30201503. [PubMed: 30201503]

Savioli L, Fenwick A, Rollinson D, Albonico M, Ame SM, 2015. An achievable goal: control and elimination of schistosomiasis. Aug 22. Lancet 386 (9995), 739. 10.1016/S0140-6736(15)61536-7. PubMed PMID: 26333971.

Schultz JH, Bu L, Adema CM, 2018. Comparative immunological study of the snail Physella acuta (Hygrophila, Pulmonata) reveals shared and unique aspects of gastropod immunobiology.Sep. Mol. Immunol. 101, 108–119. 10.1016/j.molimm.2018.05.029. Epub 2018 Jun 17. PubMed PMID: 29920433. [PubMed: 29920433]

Sekiguchi R, Fujito NT, Nonaka M, 2012. Evolution of the thioester-containing proteins (TEPs) of the arthropoda, revealed by molecular cloning of TEP genes from a spider, Hasarius adansoni. Feb. Dev. Comp. Immunol. 36 (2), 483–489. 10.1016/j.dci.2011.05.003. Epub 2011 May 31. PubMed PMID: 21663759. [PubMed: 21663759]

Shokal U, Eleftherianos I, 2017. Thioester-containing protein-4 regulates the Drosophila immune signaling and function against the pathogen photorhabdus. J Innate Immun 9 (1), 83–93. 10.1159/000450610. Epub 2016 Oct 22. PubMed PMID: 27771727. [PubMed: 27771727]

Simpson AJ, Payares G, Walker T, Knight M, Smithers SR, 1984. The modulation of expression of polypeptide surface antigens on developing schistosomula of Schistosoma mansoni. Nov. J. Immunol. 133 (5), 2725–2730 PubMed PMID: 6481169. [PubMed: 6481169]

Sire C, Rognon A, Theron A, 1998. Failure of Schistosoma mansoni to reinfect Biomphalaria glabrata snails: acquired humoral resistance or intra-specific larval antagonism? Aug. Parasitology 117 (Pt 2), 117–122 PubMed PMID: 9778633. [PubMed: 9778633]

Smith JH, Chernin E, 1974. Ultrastructure of young mother and daughter sporocysts of Schistosoma mansoni. Feb. J. Parasitol. 60 (1), 85–89 PubMed PMID: 4814802. [PubMed: 4814802]

Smith LC, Chang L, Britten RJ, Davidson EH, 1996. Sea urchin genes expressed in activated coelomocytes are identified by expressed sequence tags. Complement homologues and other putative immune response genes suggest immune system homology within the deuterostomes. Jan 15. J. Immunol. 156 (2), 593–602 PubMed PMID: 8543810. [PubMed: 8543810]

Sokolow SH, Wood CL, Jones IJ, Swartz SJ, Lopez M, Hsieh MH, Lafferty KD, Kuris AM, Rickards C, De Leo GA, 2016. Global assessment of schistosomiasis control over the past century shows targeting the snail intermediate host works best. Jul 21. PLoS Neglected Trop. Dis. 10 (7), e0004794. 10.1371/journal.pntd.0004794. eCollection 2016 Jul. PubMed PMID: ; PubMed Central PMCID: PMC4956325. [PubMed: 27441556]

Sousa-Figueiredo JC, Betson M, Kabatereine NB, Stothard JR, 2013. The urine circulating cathodic antigen (CCA) dipstick: a valid substitute for microscopy for mapping and point-of-care diagnosis of intestinal schistosomiasis. PLoS Neglected Trop. Dis. 7 (1), e2008. 10.1371/journal.pntd.0002008. Epub 2013 Jan 24. PubMed PMID: 23359826; PubMed Central PMCID: PMC3554525.

Spycher SE, Arya S, Isenman DE, Painter RH, 1987. A functional, thioester-containing alpha 2-macroglobulin homologue isolated from the hemolymph of the American lobster (Homarus americanus). Oct 25. J. Biol. Chem. 262 (30) 14606–11. PubMed PMID: 2444589. [PubMed: 2444589]

Stensgaard AS, Vounatsou P, Sengupta ME, Utzinger J, 2019. Schistosomes, snails and climate change: current trends and future expectations. Feb. Acta Trop. 190, 257–268. 10.1016/j.actatropica.2018.09.013. Epub 2018 Sep 24. Review. PubMed PMID: . [PubMed: 30261186]

Sturrock RF, 2001. The schistosomes and their intermediate hosts in Schistosomiasis. In: Mahmoud AAF (Ed.), Tropical Medicine: Science and Practice. Imperial College Press, London, pp. 7–83.

Sullivan JT, Spence JV, 1994. Transfer of resistance to Schistosoma mansoni in Biomphalaria glabrata by allografts of amoebocyte-producing organ. Jun. J. Parasitol. 80 (3), 449–453 PubMed PMID: 8195947. [PubMed: 8195947]

Sullivan JT, Spence JV, Nuñez JK, 1995. Killing of Schistosoma mansoni sporocysts in Biomphalaria glabrata implanted with amoebocyte-producing organ allografts from resistant snails. Oct. J. Parasitol. 81 (5), 829–833 PubMed PMID: 7472893. [PubMed: 7472893]

Sun Z, Jiang Q, Wang L, Zhou Z, Wang M, Yi Q, Song L, 2014. The comparative proteomics analysis revealed the modulation of inducible nitric oxide on the immune response of scallop Chlamys farreri. Oct. Fish Shellfish Immunol. 40 (2), 584–594. 10.1016/j.fsi.2014.08.015. Epub 2014 Aug 19. PubMed PMID: 25149594. [PubMed: 25149594]

Tack BF, Harrison RA, Janatova J, Thomas ML, Prahl JW, 1980. Evidence for presence of an internal thiolester bond in third component of human complement. Oct. Proc. Natl. Acad. Sci. U.S.A. 77 (10), 5764–5768 PubMed PMID: 6934510; PubMed Central PMCID: PMC350151. [PubMed: 6934510]

Takeda K, Kaisho T, Akira S, 2003. Toll-like receptors. Annu. Rev. Immunol. 21, 335–376 Epub 2001 Dec 19. Review. PubMed PMID: 12524386. [PubMed: 12524386]

Apr 6Tassetto M, Kunitomi M, Andino R, 2017. Circulating immune cells mediate a systemic RNAi-based adaptive antiviral response in Drosophila. e13. Cell 169 (2), 314–325. 10.1016/j.cell.2017.03.033. PubMed PMID: 28388413; PubMed Central PMCID: PMC5730277. [PubMed: 28388413]

Tennessen JA, Théron A, Marine M, Yeh JY, Rognon A, Blouin MS, 2015a. Hyperdiverse gene cluster in snail host conveys resistance to human schistosome parasites. Mar 16. PLoS Genet. 11 (3), e1005067. 10.1371/journal.pgen.1005067. eCollection 2015 Mar. PubMed PMID: 25775214; PubMed Central PMCID: PMC4361660.

Tennessen JA, Bonner KM, Bollmann SR, Johnstun JA, Yeh JY, Marine M, Tavalire HF, Bayne CJ, Blouin MS, 2015b. Genome-Wide scan and test of candidate genes in the snail Biomphalaria glabrata reveal new locus influencing resistance to Schistosoma mansoni. Sep. 15. PLoS Neglected Trop. Dis. 9 (9), e0004077. 10.1371/journal.pntd.0004077. eCollection 2015. PubMed PMID: ; PubMed Central PMCID: PMC4570800. [PubMed: 26372103]

Tennessen JA, Bollmann SR, Blouin MS, 2017. A targeted capture linkage map anchors the genome of the schistosomiasis vector snail, Biomphalaria glabrata. Jul 5. G3 (Bethesda) 7 (7), 2353–2361. 10.1534/g3.117.041319. PubMed PMID: 28526730; PubMed Central PMCID: PMC5499142. [PubMed: 28526730]

Tetreau G, Pinaud S, Portet A, Galinier R, Gourbal B, Duval D, 2017. Specific pathogen recognition by multiple innate immune sensors in an invertebrate. Oct 5. Front. Immunol. 8, 1249. 10.3389/fimmu.2017.01249. eCollection 2017. PubMed PMID: 29051762; PubMed Central PMCID: PMC5633686. [PubMed: 29051762]

Toor J, Alsallaq R, Truscott JE, Turner HC, Werkman M, Gurarie D, King CH, Anderson RM, 2018. Are we on our way to achieving the 2020 goals for schistosomiasis morbidity control using current world health organization guidelines? Jun 1. Clin. Infect. Dis. 66 (Suppl. l_4), S245–S252. 10.1093/cid/ciy001. PubMed PMID: 29860290; PubMed Central PMCID: PMC5982704. [PubMed: 29860290]

Touré S, Zhang Y, Bosqué-Oliva E, Ky C, Ouedraogo A, Koukounari A, Gabrielli AF, Bertrand S, Webster JP, Fenwick A, 2008. Two-year impact of single praziquantel treatment on infection in the national control programme on schistosomiasis in Burkina Faso. Oct. Bull. World Health Organ. 86 (10), 780–787 A. PubMed PMID: 18949215; PubMed Central PMCID: PMC2649514. [PubMed: 18949215]

Upatham ES, 1972. Rapidity and duration of hatching of St. Lucian Schistosoma mansoni eggs in outdoor habitats. J. Helminthol. 46 (3), 271–276 PubMed PMID: 4673291. [PubMed: 4673291]

Urbanová V, Šíma R, Šauman I, Hajdušek O, Kopáček P, 2015. Thioester-containing proteins of the tick Ixodes ricinus: gene expression, response to microbial challenge and their role in phagocytosis of the yeast Candida albicans. Jan. Dev. Comp. Immunol. 48 (1), 55–64. 10.1016/j.dci.2014.09.004. Epub 2014 Sep 16. PubMed PMID: 25224405. [PubMed: 25224405]

Vermeire JJ, Yoshino TP, 2007. Antioxidant gene expression and function in in vitrodeveloping Schistosoma mansoni mother sporocysts: possible role in self-protection. Sep. Parasitology 134 (Pt 10), 1369–1378 Epub 2007 Apr 20. PubMed PMID: 17445325. [PubMed: 17445325]

Wallace JB, Webster JR, 1996. The role of macroinvertebrates in stream ecosystem function. Annu. Rev. Entomol. 41, 115–139 PubMed PMID: 15012327. [PubMed: 15012327]

Wang S, Jacobs-Lorena M, 2013. Genetic approaches to interfere with malaria transmission by vector mosquitoes. Mar. Trends Biotechnol. 31 (3), 185–193. 10.1016/j.tibtech.2013.01.001. [PubMed: 23395485]

Wang X, Gurarie D, Mungai PL, Muchiri EM, Kitron U, King CH, 2012a. Projecting the long-term impact of school- or community-based mass-treatment interventions for control of Schistosoma infection. PLoS Neglected Trop. Dis. 6 (11), e1903. 10.1371/journal.pntd.0001903. Epub 2012 Nov 15. PubMed PMID: 23166850; PubMed Central PMCID: PMC3499404.

Wang W, Wang L, Liang YS, 2012b. Susceptibility or resistance of praziquantel in human schistosomiasis: a review. Nov. Parasitol. Res. 111 (5), 1871–1877. 10.1007/s00436-012-3151-z. Epub 2012 Oct 7. Review. PubMed PMID: 23052781. [PubMed: 23052781]

Wang T, Zhao M, Rotgans BA, Strong A, Liang D, Ni G, Limpanont Y, Ramasoota P, McManus DP, Cummins SF, 2016. Proteomic analysis of the Schistosoma mansoni miracidium. Jan 22. PLoS One 11 (1), e0147247. 10.1371/journal.pone.0147247. eCollection 2016. PubMed PMID: 26799066; PubMed Central PMCID: PMC4723143.

Wang T, Wyeth RC, Liang D, Bose U, Ni G, McManus DP, Cummins SF, 2019. A Biomphalaria glabrata peptide that stimulates significant behaviour modifications in aquatic free-living Schistosoma mansoni miracidia. Jan 22. PLoS Neglected Trop. Dis. 13 (1), e0006948. 10.1371/

journal.pntd.0006948. eCollection 2019 Jan. PubMed PMID: 30668561; PubMed Central PMCID: PMC6358113. Epub 2013 Feb 6. Review. PubMed PMID: 23395485; PubMed Central PMCID: PMC3593784.

Wheeler NJ, Dinguirard N, Marquez J, Gonzalez A, Zamanian M, Yoshino TP, Castillo MG, 2018. Correction to: sequence and structural variation in the genome of the Biomphalaria glabrata embryonic (Bge) cell line. Parasit Vectors. Oct 29 11 (1), 566. 10.1186/s13071-018-3135-7. PubMed PMID: 30373629; PubMed Central PMCID: PMC6206734. [PubMed: 30373629]

Whitten MM, Tew IF, Lee BL, Ratcliffe NA, 2004. A novel role for an insect apolipoprotein (apolipophorin III) in beta-1,3-glucan pattern recognition and cellular encapsulation reactions. Feb 15. J. Immunol. 172 (4) 2177–85. PubMed PMID: 14764684. [PubMed: 14764684]

Whittington ID, Cribb BW, 2001. Adhesive secretions in the Platyhelminthes. Adv. Parasitol. 48, 101–224 Review. PubMed PMID: 11013756. [PubMed: 11013756]

WHO, 2002. Prevention and control of schistosomiasis and soil-transmitted helminthiasis: report of a WHO expert committee.WHO Technical Report Series 912:1–57.

WHO, 2012. Accelerating Work to Overcome the Global Impact of Neglected Tropical Diseases: a Roadmap for Implementation Geneva. World Health Organization Available from: http://www.who.int/neglected_diseases/NTD_RoadMap_2012.

WHO, 2017. Schistosomiasis and soil-transmitted helminthiases: number of people treated in 2016. Wkly. Epidemiol. Rec. 92, 749–760. [PubMed: 29218962]

WHO, 2019. Neglected Tropical Diseases. Summary. https://www.who.int/neglected_diseases/diseases/summary/en/

William S, Sabra A, Ramzy F, Mousa M, Demerdash Z, Bennett JL, Day TA, Botros S, 2001. Stability and reproductive fitness of Schistosoma mansoni isolates with decreased sensitivity to praziquantel. Int. J. Parasitol. 31, 1093–1100. 10.1016/S0020-7519(01)00215-6. [PubMed: 11429173]

Wilson RA, 1987. Cercariae to liver worms: development and migration in the mammalian host. In: Rollinson D, Simpson AJG (Eds.), The Biology of Schistosomes from Genes to Latrines. Academic Press, London, UK, pp. 116–146.

Wilson RA, Langermans JA, van Dam GJ, Vervenne RA, Hall SL, Borges WC, Dillon GP, Thomas AW, Coulson PS, 2008. Elimination of Schistosoma mansoni adult worms by rhesus macaques: basis for a therapeutic vaccine? Sep. 17. PLoS Neglected Trop. Dis. 2 (9), e290. 10.1371/journal.pntd.0000290. PubMed PMID: 18820739; PubMed Central PMCID: PMC2553480.

Wright BJ, Bickham-Wright U, Yoshino TP, Jackson MB, 2017. H+ channels in embryonic Biomphalaria glabrata cell membranes: putative roles in snail host-schistosome interactions. Mar 20. PLoS Neglected Trop. Dis. 11 (3), e0005467. 10.1371/journal.pntd.0005467. eCollection 2017 Mar. PubMed PMID: 28319196; PubMed Central PMCID: PMC5373640.

Wu XJ, Sabat G, Brown JF, Zhang M, Taft A, Peterson N, Harms A, Yoshino TP, 2009. Proteomic analysis of Schistosoma mansoni proteins released during in vitro miracidium-to-sporocyst transformation. Mar. Mol. Biochem. Parasitol. 164 (1), 32–44. 10.1016/j.molbiopara.2008.11.005. Epub 2008 Nov 27. PubMed PMID: 19095013; PubMed Central PMCID: PMC2665799. [PubMed: 19095013]

Wu XJ, Dinguirard N, Sabat G, Lui HD, Gonzalez L, Gehring M, Bickham-Wright U, Yoshino TP, 2017. Proteomic analysis of Biomphalaria glabrata plasma proteins with binding affinity to those expressed by early developing larval Schistosoma mansoni. May 16. PLoS Pathog. 13 (5), e1006081. 10.1371/journal.ppat.1006081. eCollection 2017 May. PubMed PMID: 28520808; PubMed Central PMCID: PMC5433772.

Xiang Z, Xiao S, Wang F, Qin Y, Wu J, Ma H, Li J, Yu Z, 2016. Cloning, characterization and comparative analysis of four death receptorTNFRs from the oyster Crassostrea hongkongensis. Dec. Fish Shellfish Immunol. 59, 288–297. 10.1016/j.fsi.2016.09.041. Epub 2016 Sep 23. PubMed PMID: 27666188. [PubMed: 27666188]

Xing Q, Yu Q, Dou H, Wang J, Li R, Ning X, Wang R, Wang S, Zhang L, Hu X, Bao Z, 2016. Genome-wide identification, characterization and expression analyses of two TNFRs in Yesso scallop (Patinopecten yessoensis) provide insight into the disparity of responses to bacterial infections and heat stress in bivalves. May. Fish Shellfish Immunol. 52, 44–56. 10.1016/j.fsi.2016.03.010. Epub 2016 Mar 14. PubMed PMID: 26988286. [PubMed: 26988286]

Jul 27Yan HB, Smout MJ, Ju C, Folley AE, Skinner DE, Mann VH, Loukas A, Hu W, Brindley PJ, Rinaldi G, 2018. Developmental sensitivity in schistosoma mansoni to puromycin to establish drug selection of transgenic schistosomes. pii: e02568–17. Antimicrob. Agents Chemother. (8), 62. 10.1128/AAC.02568-17. Print 2018 Aug. PubMed PMID: 29760143; PubMed Central PMCID: PMC6105839.

Yazzie N, Salazar KA, Castillo MG, 2015. Identification, molecular characterization, and gene expression analysis of a CD109 molecule in the Hawaiian bobtail squid Euprymna scolopes. May. Fish Shellfish Immunol. 44 (1), 342–355. 10.1016/j.fsi.2015.02.036. Epub 2015 Mar 3. PubMed PMID: 25742727. [PubMed: 25742727]

Yoshino TP, Bayne CJ, 1983. Mimicry of snail host antigens by miracidia and primary sporocysts of Schistosoma mansoni. May. Parasite Immunol. 5 (3), 317–328 PubMed PMID: 6191268. [PubMed: 6191268]

Yoshino TP, Coustau C, 2011. Immunobiology of Biomphalaria–trematode. Interactions. In: Toledo R, Fried B. (Eds.), Biomphalaria Snails and Larval Trematodes. Springer, New York, pp. P159–P189.

Yoshino TP, Laursen JR, 1995. Production of Schistosoma mansoni daughter sporocysts from mother sporocysts maintained in synxenic culture with Biomphalaria glabrata embryonic (Bge) cells. Oct. J. Parasitol. 81 (5), 714–722 PubMed PMID: 7472861. [PubMed: 7472861]

Yoshino TP, Coustau C, Modat S, Castillo MG, 1999. The Biomphalaria glabrata embryonic (BGE) molluscan cellline: establishment of an in vitro cellular model for the study of snail host-parasite interactions. Malacologia 41, 331–343.

Yoshino TP, Dinguirard N, Kunert J, Hokke CH, 2008. Molecular and functional characterization of a tandem-repeat galectin from the freshwater snail Biomphalaria glabrata, intermediate host of the human blood fluke Schistosoma mansoni. Mar 31. Gene 411 (1–2), 46–58. 10.1016/j.gene.2008.01.003. Epub 2008 Jan 17. PubMed PMID: 18280060; PubMed Central PMCID: PMC2423817. [PubMed: 18280060]

Yoshino TP, Wu XJ, Gonzalez LA, Hokke CH, 2013a. Circulating Biomphalaria glabrata hemocyte subpopulations possess shared schistosome glycans and receptors capable of binding larval glycoconjugates. Exp Parasitol. Jan 133 (1), 28–36. 10.1016/j.exppara.2012.10.002. Epub 2012 Oct 22. PubMed PMID: 23085445; PubMed Central PMCID: PMC3647354.

Yoshino TP, Bickham U, Bayne CJ, 2013b. Molluscan cells in culture: primary cell cultures and cell lines. Jun 1. Can. J. Zool. 91 (6). 10.1139/cjz-20120258. PubMed PMID: 24198436; PubMed Central PMCID: PMC3816639.

Yoshino TP, Brown M, Wu XJ, Jackson CJ, Ocadiz-Ruiz R, Chalmers IW, Kolb M, Hokke CH, Hoffmann KF, 2014. Excreted/secreted Schistosoma mansoni venom allergen-like 9 (SmVAL9) modulates host extracellular matrix remodelling gene expression. Jul. Int. J. Parasitol. 44 (8), 551–563. 10.1016/j.ijpara.2014.04.002. Epub 2014 May 21. PubMed PMID: 24859313; PubMed Central PMCID: PMC4079936. [PubMed: 24859313]

Zahoor Z, Davies AJ, Kirk RS, Rollinson D, Walker AJ, 2010. Larval excretory-secretory products from the parasite Schistosoma mansoni modulate HSP70 protein expression in defence cells of its snail host, Biomphalaria glabrata. Sep. Cell Stress Chaperones 15 (5), 639–650. 10.1007/s12192-010-0176-z. Epub 2010 Feb 25. PubMed PMID: 20182834; PubMed Central PMCID: PMC3006636. [PubMed: 20182834]

Zahoor Z, Lockyer AE, Davies AJ, Kirk RS, Emery AM, Rollinson D, Jones CS, Noble LR, Walker AJ, 2014. Differences in the gene expression profiles of haemocytes from schistosome-susceptible and -resistant Biomphalaria glabrata exposed to Schistosoma mansoni excretory-secretory products. Mar 24. PLoS One 9 (3), e93215. 10.1371/journal.pone.0093215. eCollection 2014. PubMed PMID: 24663063; PubMed Central PMCID: PMC3963999.

Zhang SM, Coultas KA, 2011. Identification and characterization of five transcription factors that are associated with evolutionarily conserved immune signaling pathways in the schistosome-transmitting snail Biomphalaria glabrata. Sep. Mol. Immunol. 48 (15–16), 1868–1881. 10.1016/j.molimm.2011.05.017. Epub 2011 Jun 21. PubMed PMID: 21696828; PubMed Central PMCID: PMC3163751. [PubMed: 21696828]

Zhang SM, Loker ES, 2004. Representation of an immune responsive gene family encoding fibrinogen-related proteins in the freshwater mollusc Biomphalaria glabrata, an intermediate

host for Schistosoma mansoni. Oct. Gene 27 (341) 255–66. PubMed PMID: 15474308; PubMed Central PMCID: PMC3638878.

Zhang SM, Adema CM, Kepler TB, Loker ES, 2004. Diversification of Ig superfamily genes in an invertebrate. Jul 9. Science (5681), 305 251–4. PubMed PMID: 15247481.

Zhang H, Song L, Li C, Zhao J, Wang H, Gao Q, Xu W, 2007. Molecular cloning and characterization of a thioester-containing protein from Zhikong scallop Chlamys farreri. Jul. Mol. Immunol. 44 (14), 3492–3500 Epub 2007 May 10. PubMed PMID: 17498803. [PubMed: 17498803]

Zhang SM, Zeng Y, Loker ES, 2008. Expression profiling and binding properties of fibrinogen-related proteins (FREPs), plasma proteins from the schistosome snail host Biomphalaria glabrata. Jun. Innate Immun. 14 (3), 175–189. 10.1177/1753425908093800 . PubMed PMID: 18562576; PubMed Central PMCID:PMC3638879. [PubMed: 18562576]

Zhang L, Li L, Guo X, Litman GW, Dishaw LJ, Zhang G, 2015. Massive expansion and functional divergence of innate immune genes in a protostome. Sci Rep. Mar 3 (5), 8693. 10.1038/srep08693. PubMed PMID: 25732911; PubMed Central PMCID: PMC4346834.

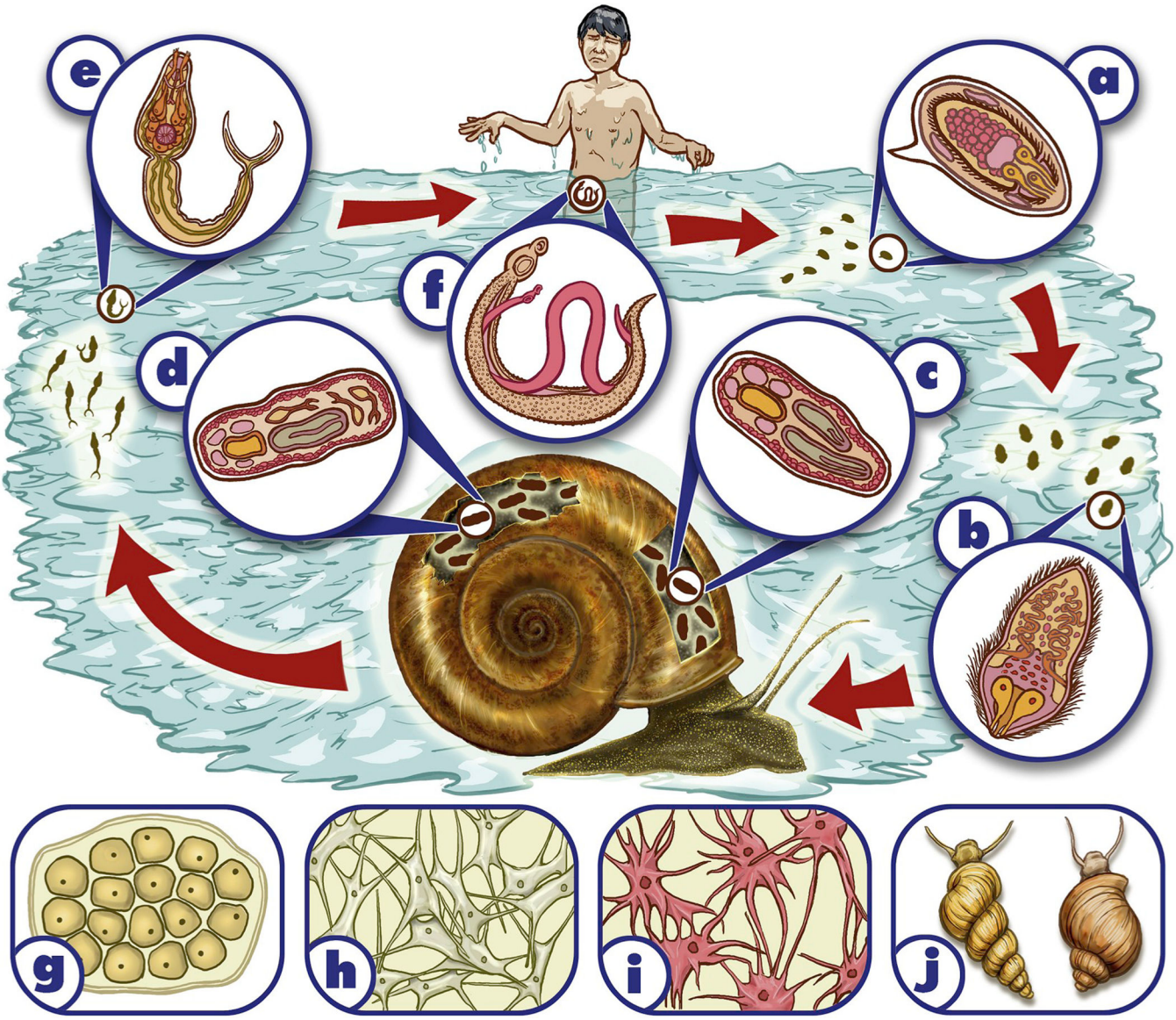

Fig. 1. *Schistosoma mansoni* intramolluscan larval stages in *Biomphalaria glabrata* and biological tools used in host-parasite studies.

The life cycle of the trematode *Schistosoma mansoni* uses the planorbid snail *Biomphalaria glabrata* as an obligatory intermediate host. Infection of snails is initiated when **eggs (a)** produced by adult *S. mansoni* worms found in the human host are released into the environment. Upon contact with fresh water, the eggs hatch into **miracidia (b)**. The miracidium is a free-living and motile larval stage of the parasite that after locating a snail host, it will penetrate its exposed epithelia. Inside snail tissues, miracidia transform into the **primary (mother) sporocyst (c)**, the first intramolluscan larval stage. After 24–48 h post-infection, primary sporocysts start migrating to other snail tissues, preferentially the digestive gland. In these internal tissues, germinal cells within primary sporocysts develop into **secondary (daughter) sporocysts (d),** which on their own will produce more sporocysts. Alternatively, secondary sporocysts will also produce **cercariae (e)**, the last

intramolluscan larval stage, that once matured will break out of snail tissues and swim in the aquatic environment in search of a new vertebrate host. Cercariae can penetrate the skin of humans and start a new cycle of infection when **adult worms (f)** develop, pair and start producing eggs. Studies in snail immunity utilize a variety of biological tools including various developmental stages of snails including **embryos and juveniles (g),** the ***B. glabrata*** **embryonic (Bge) cell line (h)**, cellular components such as **hemocytes (i)**, and other snail species for example ***Bulinus*** **spp. and** ***Oncomelania spp.*** **(j)**

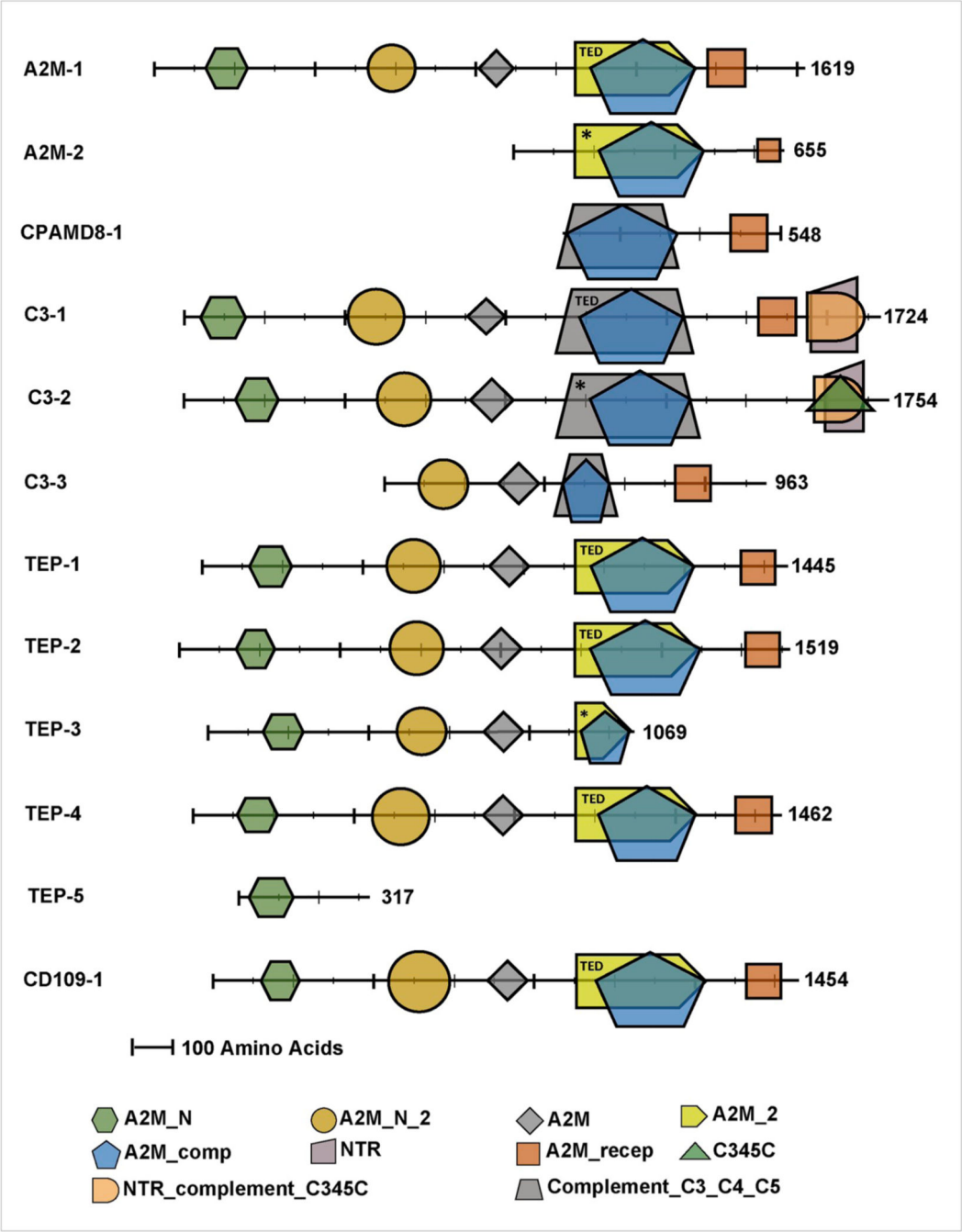

Fig. 2. Protein domains in *B. glabrata* TEP family.

Graphical representation of the putative conserved domains identified in the *B. glabrata* BB02 TEP sequences. The conserved domains found in all *B. glabrata* TEP-sequences have similar content and organization as those reported in other invertebrate and vertebrate TEPs. The National Center for Biotechnology Information (NCBI) Conserved Domain Database (CDD) (http://www.ncbi.nlm.nih.gov/cdd) was utilized for TEP domain nomenclature and abbreviations. A2M_N = MG2 (macroglobulin) domain of alpha-2-macroglobulin; A2M_N_2 = Alpha-2macroglobulin family N-terminal region; A2M =

Alpha-2-macroglobulin family, includes the C-terminal region of the alpha-2-macroglobulin family; Complement_C3_C4_C5 = Proteins similar to C3, C4 and C5 of vertebrate complement, thioester bond located within the structure of C3 and C4; A2M_2 = Proteins similar to alpha2-macroglobulin (alpha (2)-M). This group also contains the pregnancy zone protein (PZP); A2M_comp = Complement component region of the alpha-2-macroglobulin family; A2M_recep = Receptor domain region of the alpha-2-macroglobulin family; NTR_complement_C345C = NTR/C345C domain, NTR domains found in the C-termini of complement C3, C4 and C5; NTR = UNC-6/NTR/C345C module, sequence similarity between netrin UNC-6 and C345C complement protein family members; C345C = Netrin C-terminal Domain. The characteristic GCGEQ thioester domain is labeled with “TED” and regions labeled with an asterisk do not completely align wi4th the representative GCGEQ residues, having one or more dissimilar amino acids for the thioester domain sequence. No label signifies that no thioester domain was identified. The intervals of each domain and the length of each sequence are illustrated to scale with the most up-to-date amino acid length labeled at the 3′-end.

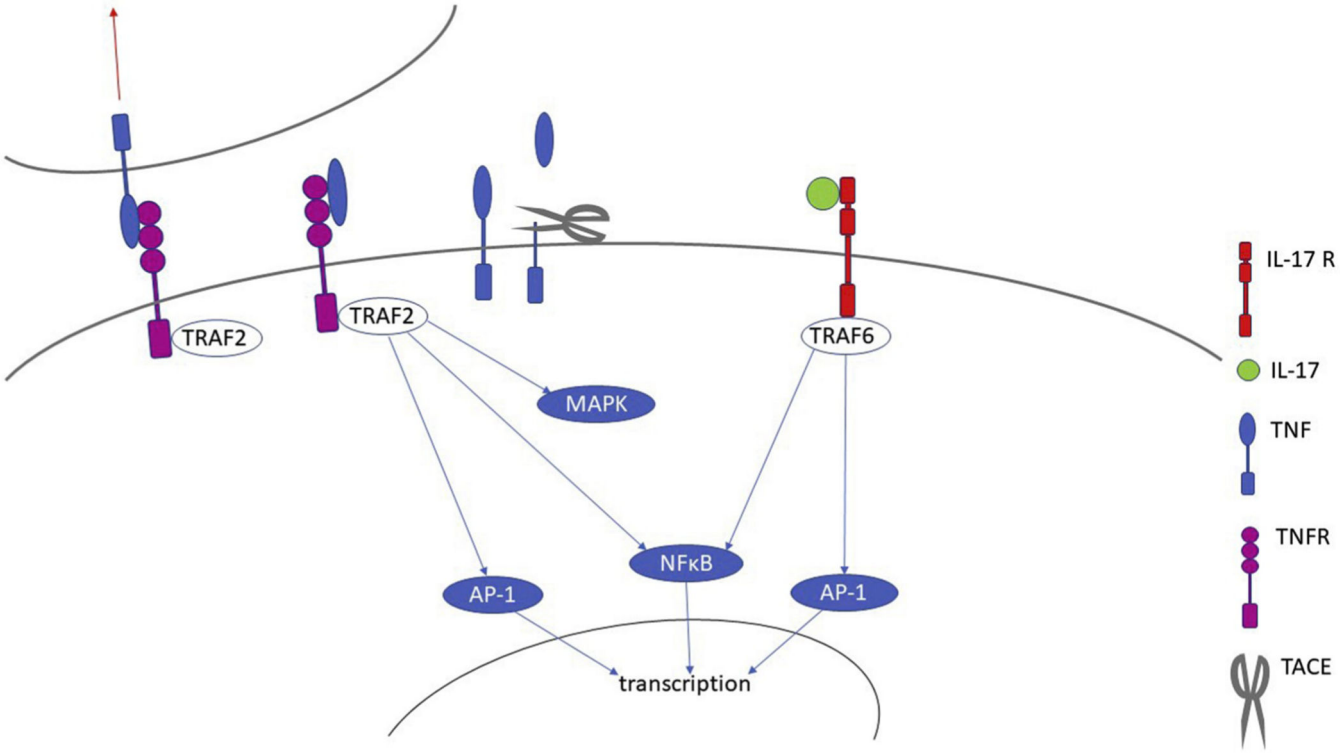

Fig. 3. Putative IL-17- and TNF-signaling pathways in *B. glabrata*.

TRAF2 = TNF receptor associated factor 2; AP1 = activator protein 1; NF-κB = nuclear factor κB; TNF = tumor necrosis factor; TNFR = TNF receptor; TACE = TNF-α-converting enzyme; IL-17 = interleukin 17; IL-17R = IL-17 receptor; red arrow indicates outside-in signaling via a TNF receptor; blue arrows indicate outside-in signaling via IL-17 and TNF receptors. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1

Functionally characterized immune molecules in *Biomphalaria glabrata*.

Molecules	Tissue Location	Type/Function	Target	Reference
Biomphalysin	Hemocytes, Bge cells, secretions	Opsonin/Porin?	Sporocysts	Galinier et al. (2013)
Cu/Zn SOD	Hemocytes cytoplasm	ROS Enzyme	Sporocyst	Goodall et al. (2004), 2006; Bender et al. (2007)
FREP2	Hemocytes, Bge cells, plasma	PRR, Opsonization	Miracidia, LTPs	Adema et al. (1997); Pinaud et al. (2016)
FREP3	Hemocytes, plasma	PRR, Opsonization	Sporocysts, LTPs, bacteria, fungi	Hanington et al. (2010b); Pinaud et al. (2016)
FREP4	Hemocytes, plasma	PRR, Opsonization	Sporocysts	Adema et al. (1997); Zhang et al. (2008); Moné et al. (2010); Pinaud et al. (2016)
Galectin	Hemocytes	Opsonization	Sporocysts	Yoshino et al. (2008)
Granulin	Unknown	Growth Factor	Hemocytes	Pila et al. (2016b)
Grctm6	Hemocytes	PRR	Sporocysts	Allan et al. (2017)
Hydrogen peroxide	Hemocytes secretions	ROS/Oxidative damage	Sporocysts	Adema et al., 1997; Hahn et al., 2000
LAPD2	CNS	Regulator/Hormone?	Miracidia	Wang et al. (2016)
MIF	Hemocytes, Bge & other cells, secretions	Cytokine/Proliferation, Encapsulation	LTPs, Sporocysts	Baeza Garcia et al., 2010
Nitric Oxide	Hemocytes secretions	NOS/Oxidative damage	Sporocysts	Hahn et al., 2001b
TEP1	Secretions, hemolymph	Opsonin/PRR	LTPs, SmPoMucs	Moné et al. (2010)
TLR	Hemocytes	PRR	Sporocyst	Pila et al. (2016a)

Note: To be classified as “characterized”, listed molecules have been reported to: (1) have complete CDS available (predicted genes are excluded), and (2) a direct role in defense or resistance to parasite has been demonstrated for example, through gene knockdown, or direct activity/binding to *S. mansoni* parasites or secreted/released products (ESPs/LTPs).

Table 2

Biomphalaria glabrata BB02 TEPs and closest homologs.

Name	Accession	Complete	BGLB ID	NCBI predicted	Closest Homolog
A2M-1	MK573558	Yes	016521-RB	XP_013081252.1 (96% identity)	A2M *L. littorea* AIC31934.1 (45%)
A2M-2	MK576002	No	3′ end- 022655-RA	XP_013084477.1 (100%)	CD109 *L. littorea* AVP12647.1 (55%)
C3-1	MK583200	Yes	018444-RA	XP_013068508.1 (99% identity)	C3 *L. littorea* AVP12644.1 (54%)
C3-2	MK583201	Yes	5′ end- 030610-RA	XP_013086914.1 (97% identity)	C3 *L. littorea* AVP12645.1 (33%)
			3′ end - 020436	XP_013087010.1	
				(100% identity)	
C3-3	MK583202	No	3′ end- 025256-RA	XP_013064315.1 (98% identity)	C3 *L. littorea* AVP12645.1 (32%)
CD109-1	MK576003	Yes	5′ end- 021085-RA	XP_013094127.1 (98% identity)	TEP *Epanerchodus* BAR45598.1 (29%)
			3′ end- 031746-RA	XP_013094132.1	
				(100% identity)	
				XP_013076313.1	
				(100% identity)	
CPAMD8-1	MK576004	No	3′ end- 035268-RA	XP_013061675.1 (100% identity)	CD109 *L. littorea* AVP12647.1 (62%)
TEP-1	MK583203	Yes	5′ end- 021162-RA	ADE45332.1 (98% identity)	CD109 *L. littorea* AVP12646.1 (38%)
			3′ end- 035158-RA		
TEP-2	MK583204	Yes	5′ end- 000155-RA	XP_013065920.1 (93% identity)	CD109 *M. yessoensis* OWF38485.1 (32%)
			3′ end- 032760-RA		
TEP-3	MK583205	No	5′ end- 000023-RB	XP_013091771.1 (100% identity)	TEP *E. tau* BAE44110.1 (53%)
TEP-4	MK583206	Yes	021854-RA	XP_013071291.1 (98% identity)	TEP *E. tau* BAE44110.1 (69%)
TEP-5	MK583207	No	5′ end- 021062-RA	XP_013075528.1 (100% identity)	C3 *L. littorea* AVP12645.1 (41%)

Table 3

Prediction of NF-κB binding sites upstream of complement-related genes.

Genomic sequences upstream of the coding region for complement-related genes were surveyed for putative NF-κB binding sites (κB) using LASAGNA 2.0. Based on a previously generated consensus *B. glabrata* κB, only sequences containing a G in position 2 and a C at position 10 in the predicted κB were included (Humphries and Deneckere, 2018). Positions represent the approximate locations of the predicted binding sites in nucleotides upstream of ATG start codon. The 2000bp upstream of TEP-4 were not available in the genome.

Gene	Scaffold region	Predicted binding site	Position
C3–1	LG48i_random_Scaffold305: 282201:284201	GGAATTTCTC	−48
		GGGGACGTTC	−1479
C3–2	LG7_random_Scaffold444: 159247:161247	GGGAAATCCC	−920
		AGGATTTCCC	−131
C3–3	LGUN_random_Scaffold16274: 7177:9177	GGTAATCTAC	−1794
		TGGGGACTTC	−1954
CD109–1	LGUN_random_Scaffold569: 102601:104601	GGGGTGTTCC	−1405
TEP-1	LGUN_random_Scaffold4524: 25148:27148	–	–
TEP-2	LG21_random_Scaffold104: 146283:148283	–	–
TEP-3	LG9_random_Scaffold563: 19966:21979	GGAAATTCAC	−493
TEP-5	LGUN_random_Scaffold15662: 110:2110	TGGAATTTTC	−385
		GGGAAGACCC	−757
CPAMD8–1	LGUN_random_Scaffold2480: 5066:7066	GGAAATCTCC	−1349
		GGAAATCTCC	−1399

Table 4

TNF homologs in *B. glabrata*.

TNF homologs predicted in the *B. glabrata* genome are identified by their NCBI accession number. Predicted genes that were confirmed using RNA-Seq data are listed in the table. Transcripts for which both 5′ and 3’ stop codons were present are classified as complete, whereas incomplete transcripts lacked one or both stop codons. Signal peptides, transmembrane domains and the TNF domains were identified using SignalP 3.0 server (http://www.cbs.dtu.dk/services/SignalP-3.0), TMHMM server, v2.0 (http://www.cbs.dtu.dk/services/TMHMM), and SMART (http://smart.embl.de).

	NCBI Predicted	Complete	Size	Predicted kD	Signal peptide	Transmembrane domain	TNF domain
BgTNF1	XP_013067535	No	309	35		36–58	163–302
BgTNF2	XP_013067539	Yes	322	36.19		41–63	152–308
BgTNF3v1	XP_013094494	No	240	27.3	1–36	15–37	106–232
BgTNF3v2	XP_013094495	No	210	24	1–36	15–37	106–232
BgTNF4	XP_013076957	Yes	271	30	1–30	12–34	106–252
BgTNF5	XP_013082590	Yes	366	41		95–117	206–358
BgTNF6	XP_013086591	Yes	618	69		382–404	480–612
BgTNF7	XP_013087321	Yes	384	43		85–107	235–384
BgTNF8	XP_013061250	Yes	369	41		116–138	235–369

Table 5

TNF-receptor (TNFR) homologs in *B. glabrata*.

The genomic and RNA-Seq data from *B. glabrata* were surveyed for the presence of BgTNFRs. Signal peptides, transmembrane domains, and TNF receptor domains were identified using SignalP 3.0 server (http://www.cbs.dtu.dk/services/SignalP-3.0), TMHMM server, v2.0 (http://www.cbs.dtu.dk/services/TMHMM), and SMART (http://smart.embl.de). The total amino acid length of each BgTNFR is given in column 1. The positions of signal peptides, regions outside the membrane, the transmembrane domains, and intracellular regions are given in columns 2–5 respectively.

	Length	Signal peptide	Outside	Transmembrane domain	Inside
BgTNFR1	362	1–19	1–271	272–94	295–362
BgTNFR2	225	1–42	1–181	182–204	205–225
BgTNFR3	186	1–20	1–159	160–182	183–186

Table 6

Tissue distribution of BgTNFs.

Tissue-specific RNA-Seq data was surveyed for the presence of BgTNF transcripts. Represented tissues include: albumen gland (AG); buccal mass (BUC); central nervous system (CNS); digestive gland/ hepatopancreas (DG/HP); muscular part of the headfoot (FOOT); heart including amebocyte producing organ (HAPO); kidney (KID); mantle edge (MAN); ovotestis (OVO); salivary gland (SAL); stomach (STO); terminal genitalia (TRG).

	BgTNF1	BgTNF2	BgTNF3v1	BgTNF3v2	BgTNF4	BgTNF5	BgTNF6	BgTNF7	BgTNF8
AG						✓	✓		
BUC	✓					✓	✓	✓	✓
CNS	✓					✓	✓	✓	✓
DG		✓	✓		✓	✓	✓	✓	✓
FOOT	✓	✓		✓		✓	✓	✓	✓
HAPLO	✓		✓	✓	✓	✓	✓	✓	✓
KID	✓	✓				✓	✓	✓	✓
MAN	✓		✓	✓		✓	✓	✓	✓
OVO						✓		✓	✓
SAL									
STO	✓	✓	✓	✓		✓	✓	✓	✓
TRG			✓	✓		✓	✓		✓

A ✓ indicates the presence of a BgTNF transcript.2

Table 7

Tissue distribution of BgTNF receptors.

Tissue-specific RNASeq data was surveyed for the presence of BgTNFR transcripts. Represented tissues include: albumen gland (AG); buccal mass (BUC); central nervous system (CNS); digestive gland/ hepatopancreas (DG/HP); muscular part of the headfoot (FOOT); heart including amebocyte producing organ (HAPO); kidney (KID); mantle edge (MAN); ovotestis (OVO); salivary gland (SAL); stomach (STO); terminal genitalia (TRG).

	BgTNFR1	BgTNFR2	BgTNFR3
AG			
BUC	✓	✓	
CNS		✓	
DG		✓	
FOOT	✓	✓	
HAPLO	✓	✓	
KID	✓	✓	
MAN	✓	✓	
OVO		✓	
SAL	✓		
STO	✓	✓	✓
TRG			

A ✓ indicates the presence of a BgTNFR transcript.

Table 8

IL-17 homologs in *B. glabrata*.

IL-17 homologs predicted in the *B. glabrata* genome are identified by their NCBI accession number. Predicted genes that were confirmed using RNA-Seq data are listed in the table. Transcripts for which both 5′ and 3’ stop codons were present are classified as complete. The presence of IL-17 domains was confirmed through SMART (http://smart.embl.de).

	NCBI predicted	IL-17 domain	Complete
BgIL-17-1	XP_013065647	Yes	Yes
BgIL-17-2	XP_013090467	Yes	Yes
BgIL-17-3	XP_013080675	Yes	Yes
BgIL-17-4	XP_013085850	Yes	Yes
BgIL-17-5	XP_013085675	Yes	Yes