Czech University of Life Sciences Prague

Faculty of Agrobiology, Food, and Natural Resources

Department of Zoology and Fisheries

Topic:
Exploring strategies and approaches to schistosomiasis control and elimination - the role of basic and applied research

Thesis Dissertation

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DECLARATION

I, Enoch Mensah Boateng, hereby declare that the work herein presented is the result of my own investigation under the supervision of RNDr. Jan Dvorak, Ph.D. and Mgr. Marta Chanova, Ph.D. as my consultant except for other people's work and collaboration which have been duly acknowledged. This thesis has not been presented to this university or elsewhere for any degree. I also declare I did not infringe the copyright of third parties in connection of creation this Ph.D. thesis.

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ACKNOWLEDGMENT

I thank God for bringing me this far in my academic pursuit and for making all things possible in His time. I am also most grateful to my supervisor, Jan Dvorak, for accepting me into this new area of studies despite my limited background and for all the support you provided during my study. I admit that it was tough for us both but ultimately, we made it, and that is all that matters. To Marta Chanova, I am indebted to you for all the support. You are kind and very supportive and without your counsel and timely advice, I wouldn't come this far. I can't thank you enough for the supervisor role you assumed when I need it most. Thank you also for the job opportunity you offered and the pieces of training you provided; I say God bless you beyond imagining. Hello Lenka, I do appreciate you a lot for the role you played in providing me with the basic skills I needed to succeed in this Ph.D. program. Thank you for your dedication, corrections, and mentorship. They mean a lot to me. I will forever remember that you are an integral part of my skills and knowledge development process. And to Lucie Jedlickova, although it was a short experience, it was very impactful and very educative. Thank you for your selfless support and guidance. My heartfelt appreciation goes to Prof. Irene Ayi of Noguchi Memorial Institute for Medical Research for opening her department to me for internship and the various research collaboration. I pray the good Lord will replenish in manifolds all the time and resources you expended towards my wellbeing. To Yvonne Ashong, I say thank you and God bless you for the collaborations. Finally, I want to say a big thank you to Stepanka Nedvedova, Lucie Panska, Kristyna Peterkova, and Vojtech Vacek for the various cooperation.

DEDICATION

This piece of work is dedicated to my beautiful, hardworking, and lovely mother, **Mary Akoto** who did not have formal education herself, but invested her **all** in ensuring that her children are educated. I thank her for her prayers, motivation, support, and most of all sacrifices till this day. To all families and friends who have supported my journey thus far, I dedicate this thesis to you as well.

List of abbreviation

Bi – *Biomphalaria* spp.

BSA – Bovine serum albumin

Bu – *Bulinus* spp.

CD – Clusters of differentiation

CKBP – Cytokines binding protein.

DALYs – Disability-adjusted life years.

DAPI – 4',6-diamidino-2-phenylindole

DDT – Dithiothreitol

DEPC: Diethyl pyrocarbonate

EDTA - Ethylenediaminetetraacetic acid

ICAM – Intercellular adhesion molecule.

IF – Interferons.

IL – Interleukins.

IPSE – Inducing principle of *S. mansoni* eggs.

LFA – Lymphocyte function-associated antigen.

MDA – Mass drug administration.

NSCH – Non-schistosomes.

NTS – Newly transformed schistosomula

PBS - Phosphate-buffered saline

PC – Preventive chemotherapy.

PeCAM – Platelet and endothelial cell adhesion molecule.

PZQ – Praziquantel.

SCH – Schistosome.

SDS-PAGE - Sodium dodecyl sulfate polyacrylamide gel electrophoresis

SmCKBP – *S. mansoni* cytokines binding protein.

SSA – sub-Saharan Africa.

VLA – Very late antigen.

WASH – Water, sanitation, and hygiene.

Abstract

Schistosomiasis continues to exert debilitating effects and public health threat to over 240 million people with about 700 million people at risk of infection. The health and economic implication of this neglected tropical disease is devastating hence there is a need for the adoption of effective control and elimination strategies by endemic countries. However, there is a significant gap between schistosomiasis research on one side and control in practice on the other side.

In this thesis, various aspects were investigated to identify obstacles and gains associated with schistosomiasis control. The study was conducted in three parts: a literature review of the schistosomiasis situation in Ghana, a field-based study of praziquantel mass drug administration efficacy using an alternative approach of xenomonitoring in snail intermediate hosts, and finally, an experimental study on SmM28B (*S. mansoni* ortholog of human glutamate carboxypeptidase II) and its role in the development and survival of the parasite.

The thesis comprises an introduction that provides a foundation and aims for the studies, a literature review of the existing corpus of knowledge as well as knowledge gaps in the control of schistosomiasis, three independent studies each addressing a particular aim, and an overall summary (discussion with concluding remarks).

The studies included in this thesis present insightful findings that are useful for schistosomiasis control. Numerous research and knowledge gaps have been identified in Ghana as a schistosomiasis endemic region, followed by suggestions for future actions to bridge these gaps to reach recent WHO targets and eliminate schistosomiasis as a public health problem. Xenomonitoring as one of the proposed approaches has been evaluated in Ghana for the first time and indicated a limited effect of praziquantel mass drug administration on schistosomiasis transmission in areas under study. Among other findings, both studies confirm the urgent need for alternative drugs and/or vaccine candidates. The result of the experimental study provides first-hand information about the expression and localization of SmM28B in the reproductive and digestive tissues/organs across all developmental stages of *Schistosoma mansoni* and identified its biological functions that make this molecule a potential target in future drug/vaccine development.

Table of content

1.0 INTRODUCTION

Schistosomiasis attracts a lot of research attention due to its debilitating nature and public health threat. The research spans from field epidemiological studies to basic and applied laboratory-based research where most effort is geared towards finding a lasting solution to the medical and veterinary menace it presents. Despite the enormous research, interventions, and elimination breakthroughs for some countries, 51 countries are still endemic to schistosomiasis (Chitsulo et al., 2000; WHO, 2023), hence there remains the need to explore all strategies and approaches to achieve effective control and elimination of the disease. On the issues of control, several aspects such as preventive chemotherapy (PC) by praziquantel (PZQ) mass drug administration (MDA), control of snail intermediate host, a strict culture of water, sanitation, and hygiene (WASH), and monitoring and evaluation of control programs are the main recommendations to be adopted and practiced by affected countries (WHO, 2023). However, drawbacks to these recommendations abound. The present thesis aimed at exploring different aspects of schistosomiasis as they pertain to the control and elimination from basic and applied laboratory as well as field-based research perspective. The thesis consists of a general literature review that captures relevant studies conducted on schistosomiasis in general and specifically on the control of schistosomiasis, three different studies each addressing a particular control strategy, and a general discussion and concluding remarks that brings all three studies together in respect of improving control with a holistic approach.

Aims of the Thesis

1. To review schistosomiasis in Ghana to bring out knowledge gaps and recommend steps to improve control.
2. To evaluate the use of xenomonitoring as a tool in endemic regions and analyze field data as they relate to the control and elimination of schistosomiasis in Ghana.
3. To investigate the biological role of the parasite's peptidase (*Schistosoma mansoni* M28B) as a potential drug/vaccine/prophylactic candidate.

2.0 LITERATURE REVIEW

2.1 Brief history and general characteristics of schistosomiasis

Schistosomiasis, also known by other names including but not limited to bilharziasis and schistosomosis, is reported to have existed in 1500 BC in ancient Egyptian medical records, while in the ancient northern part of Syria the knowledge of the disease dates back to over 6000 years ago (Di Bella et al., 2018). Meanwhile, the first discovery of one of the causative agents was not until the mid-19th century in 1851. During this period, a German scientist, medic, and surgeon Theodore Bilharz identified one of the etiological agents of schistosomiasis (*Schistosoma haematobium*) while working in Cairo (McManus et al., 2018). Since then, various aspects of the disease have been investigated by researchers, physicians, and scientists. Currently, it is one of the main neglected tropical diseases of major concern for WHO and public health experts due to its economic, health, and veterinary implications on affected communities and countries (Chitsulo et al., 2004).

Schistosomiasis is an infectious water-borne trematode disease found in many tropical and sub-tropical countries (Baker et al., 2010; Bakuza et al., 2017). The causative agents of schistosomiasis are species of the genus *Schistosoma*, which use various freshwater snails as intermediate hosts for asexual reproduction (Caffrey, 2015; Edwards & Mccullough, 1954). They infect humans, sheep, goats, pigs, cattle, buffalos, camels, and horses (Colley et al., 2014; Yihunie et al., 2019). Human schistosomiasis is further divided into intestinal and urogenital (Boissier et al., 2016; Gryseels et al., 2006).

While human schistosomiasis is a public health threat due to loss of productivity arising from DALYs, animal schistosomiasis is a topical issue under one health due to zoonotic potentials and economic losses incurred by animal farmers as a result of diseased animals (Catalano et al., 2018; Gordon et al., 2019; Leger et al., 2020). The severe and deadly nature of the disease calls for an urgent need to control and eliminate it from affected regions. The world health organization (WHO) has recommended various control and prevention strategies to ultimately achieve the elimination of the disease as a public health issue. Although some countries have attained elimination status, 51 countries are still endemic to schistosomiasis, the majority of which are sub-Saharan African (SSA) countries (Al-Shamiri et al., 2011; WHO, 2020a, 2023).

2.2 Taxonomy, morphology, and life cycle of *Schistosoma* spp.

Schistosomes are dioecious and quite distinct from other pathologically significant hermaphroditic trematodes (Mone & Boissier, 2004). They are a group of blood-feeding digenean fluke that live in the vascular system of their definitive hosts as obligate parasites. The order of classification is as follows.

Kingdom – Animalia

Phylum – Platyhelminthes

Class – Trematoda

Order – Diplostomida

Family – Schistosomatidae

There are 23 recognized *Schistosoma* species infecting humans and various animals (Colley et al., 2014; Lockyer et al., 2003; Webster et al., 2006), however, 6 of them are important agents of human schistosomiasis (Table 1). The adult worms are about 1 cm long, and the male has a deep ventral groove or schist (hence the term 'schistosome') called gynaecophoral canal (*canalis gynaecophorus*) in which the female worm resides. Worms of each sex have an oral sucker at the anterior end which they use for blood meal pumping and regurgitation since there is only one gut opening (Collins et al., 2011). The oral and ventral suckers serve as a means of attachment to the venous epithelium of the host and for the locomotion of the paired worms. These suckers are more pronounced in males than females due to the relatively larger size of the male in comparison to the female. The outer body is made of large tegument which helps the parasite to evade the host's immune response while at the same time assisting in nutrient uptake from the host (Collins et al., 2011; Montgomery, 2020; Pearce & MacDonald, 2002). The prominent external/internal morphology of adult males shows some organs that are unique such as genital pores, testes, and gynaecophoral canal. The female parasite has distinct parts such as uterus, Mehlis' glands, ootype, oviduct, ovary, vitelline duct, and vitellaria. Structures/organs that are common to both sexes are oral sucker, esophagus, ventral sucker, cecum, intestine, and tegument (**Figure 1**). (Cogswell et al., 2011; Collins et al., 2011; Greer et al., 1989)

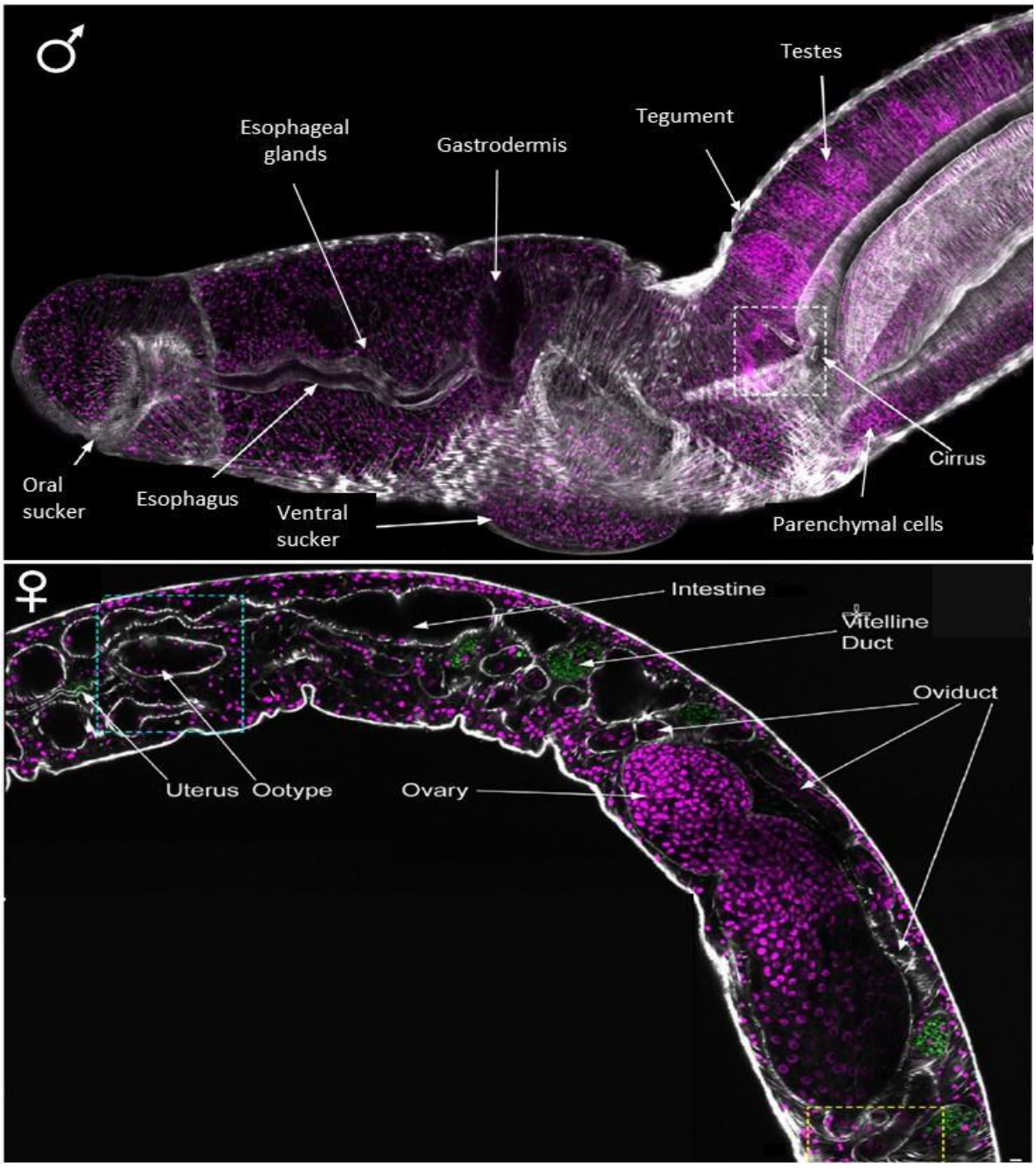

Figure 1. A labelled adult male and female *S. mansoni* image showing some important male and female-specific organs and structures based on gene atlas and confocal microscopy techniques. The female-specific organs shown in this image are uterus, ovary, ootype, oviduct, and vitelline whilst male-specific organs are the testes and cirrus duct. Image was adopted and slightly modified from (Collins et al., 2011).

The life cycle of *Schistosoma* spp. includes an intermediate mollusk host where larval development takes place and a definitive mammalian host where schistosomula, adults and egg production occur (**Figure 2**). Schistosomes use species-specific intermediate hosts, hence different species infect specific snails. Among human schistosomes, *Biomphalaria* spp. is a specific intermediate host for *S. mansoni*, *Oncomelania* spp. for *Schistosoma japonicum*, *Tricula* spp. (*Neotricula aperta*) for *Schistosoma mekongi* and *Bulinus* spp. for *S. haematobium* and *Schistosoma intercalatum* (Sturrock et al., 2001).

Cercariae are shed from the infected snail into the surrounding fresh water to begin a new cycle by infecting a definitive host (Chitsulo et al., 2004). Cercariae are transformed into schistosomula during skin penetration by shedding tails and outer glycocalyx layer while mounting double-lipid-bilayer tegument that is highly resistant to host immune responses (Greer et al., 1989; Nelwan, 2019).

Upon skin penetration, transformed schistosomula move around through the skin to dermal veins and in several days, to the pulmonary vasculature. The developing worms are subsequently transported to the portal veins, where they mature sexually into males and females (Ahmed et al., 2017; Pearce & MacDonald, 2002). Adult males and females pair within the portal vasculature (**Figure 2**) and together, they migrate along the endothelium against portal blood flow to the mesenteric veins or urinary bladder venous plexus where they begin eggs production (Ahmed et al., 2017).

The eggs from the oviposition site are carried toward the bladder or small intestine lumen with the help of various molecules at the host-parasite interface to be excreted via feces or urine (Pearce & MacDonald, 2002; Peterkova et al., 2023); for details see chapter 2.4.1. Eggs that are not excreted successfully through urine or feces may remain in the tissues or be swept back to the portal circulation (from the mesenteric vessels and be entrapped in the liver of the definite host) or to the pulmonary circulation (from the vesicular vessels via the inferior *vena cava*) (Corachan, 2002; Houston et al., 2004; Peterkova et al., 2023). These eggs entrapped in various tissues induce a granulomatous response; for details see chapter 2.4.1.

It takes approximately 10 days for released eggs to mature and form miracidia inside (Ashton et al., 2001; Wynn et al., 2004). Miracidia hatch from the excreted eggs in freshwater environment.

and specifically search for susceptible snail hosts to complete the life cycle (**Figure 2**). Two generations of asexual multiplication occur following the infection of snails: 1) sporocyst multiplication involving a division of germ cells in the mother sporocyst to produce daughter sporocysts that move to the snail's digestive gland called hepatopancreas, 2) Within the hepatopancreas, germ cells inside the daughter sporocysts divide again, producing thousands of infective larvae known as cercariae which are released by the snail into the freshwater environment in the presence of adequate light (Mone & Boissier, 2004). These cercariae must infect a viable host within a few hours to continue the life cycle in the definitive host.

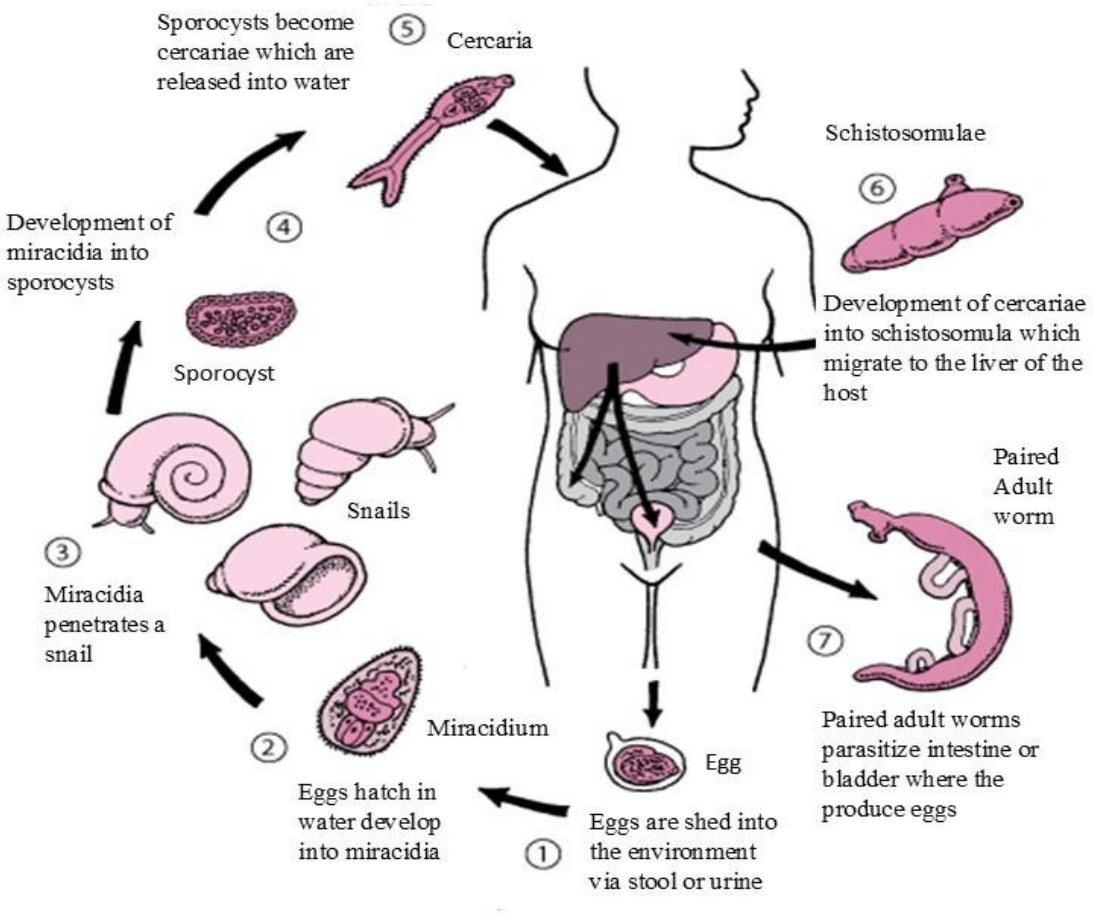

Figure 2. Description of the generalized life cycle of *Schistosoma* spp.: 1) The diagnostic stage where infected person releases eggs via stool or urine or both depending on particular species. 2) Miracidium in a freshwater environment hatch from matured viable egg. 3) Miracidium finds and penetrates a susceptible snail host. 4) Within the snail, miracidium first develops into mother sporocysts and later produces daughter sporocysts. 5) Inside the hepatopancreas, germ cells inside the daughter sporocysts later produce several cercariae which are then released into water. The cercaria finds a suitable definitive host (human) and penetrates the skin. 6) During penetration, the cercaria transforms into schistosomulum by molting off its tail and outer glycocalyx. 7) Finally, the schistosomulum migrates and develops into adult worm which lives in pairs within the host blood vessels. The figure was adopted and modified accordingly from https://www.msdmanuals.com/home/multimedia/figure/life-cycle-of-schistosoma. Accessed on 17.01.2023.

2.3 Prevalence, transmission, and distribution of schistosomiasis

It is estimated that over 240 million people are infected globally while around 700 million people are at risk of infection (Caffrey, 2015; Chitsulo et al., 2000). In recent documentation by the WHO, a little over 251 million people needed preventive chemotherapy out of which an estimated 73.5 million were reported treated in 2021 (WHO, 2023). An estimated 3.31 million disability-adjusted life years (DALYs) and 11,700 deaths per year are attributed to schistosomiasis (Molyneux et al., 2017). An estimated 90% of global schistosomiasis cases are in Africa (Chitsulo et al., 2000). According to (WHO, 2023), schistosomiasis has been eradicated from Japan and the Lesser Antilles islands, and transmission to the human population has been curtailed in Tunisia while diminished to a minimum in several countries such as Morocco, Saudi Arabia, Venezuela, and Puerto Rico. However, the dominant agents of human schistosomiasis are still highly pervasive in most tropical and sub-tropical countries (Chitsulo et al., 2000). In recent times, reports have shown that some temperate European countries such as France, Greece, Italy, Portugal and Spain have preadaptation necessary for the thriving of *S. haematobium* snail intermediate hosts and the establishment of urogenital schistosomiasis (Mulero et al., 2019; Poddighe et al., 2016) while local transmission of the disease has been recorded in Corsica and Spain (Bisoffi et al., 2016; Reguera-Gomez et al., 2023; Villasante Ferrer et al., 2018). Cases of hybrids and crossbreeds between animal-specific species (*S. bovis*) and anthropogenic-specific species (*S. haematobium*) have been recorded in different countries including Corsica in France (Boissier et al., 2016; Huyse et al., 2009; Leger & Webster, 2017; Webster et al., 2013). Five notable species (*S. haematobium*, *S. mansoni*, *Schistosoma japonicum*, *Schistosoma intercalatum* and *Schistosoma. mekongi*) are implicated in human schistosomiasis in tropics and sub-tropics (Weerakoon et al., 2015). The geographical distribution of these species is listed in **Table 1**

Table 1. Geographical distribution of *Schistosoma* spp. infecting humans (WHO, 2023)

	Species	Geographical distribution	Number of countries
Intestinal	*S. mansoni*	Africa, the Middle East, the Caribbean, Brazil, Venezuela, and Suriname	52
	S. japonicum	China, Indonesia, the Philippines	3
	S. mekongi	Cambodia and the Laos	2
	S. intercalatum S. guineensis	Rain forest areas of central Africa and part of West Africa	10
Urogenital	*S. haematobium*	Africa, the Middle East, Corsica (France)	54

2.4 Host-parasite interactions

2.4.1. Immune response induced by worms and eggs

The host parasite interaction of schistosomes is better explored from experimental animal models rather than in humans because of obstacles such as concurrent infection in humans, environmental factors, prior exposure to treatment, and medical history of humans (Schwartz & Fallon, 2018). On the contrary, animal models provide naïve environment to study the uncompromised interaction of schistosomes with their definitive hosts (Maezawa et al., 2018; Schwartz & Fallon, 2018). The use of experimental animals also allows for infection with the exact dose of cercariae and easy study of effects on organ through dissection.

Right from penetration of cercariae to excretion of eggs from the host, the parasite interacts with various cells and tissues of the definitive host. The onset of infection and migration of schistosomula is characterized by the production of type 1 immune response which lasts for approximately 35 days (Schwartz & Fallon, 2018). This response leads to appreciable production of interleukins 12 and gamma interferon whose main target are worm antigens (Vella et al., 1992). Upon eggs production, a type 2 dominant immune response is activated leading to the induction of $CD4^+$ cells while the type 1 immune response declines, especially gamma interferon production (Brunet et al., 1997; Fallon et al., 2000). The protective type 2 immune response also causes the production of basophils, eosinophils, elevated levels of IL-3, IL-4, IL-5, IgG1, IgE, and polarization of macrophages towards M2 phenotype. Eggs antigens are the main target of type 2 immune response (Vella et al., 1992). The interaction of the eggs with the type 2 immune response culminates into granuloma formation around the eggs which decreases over time at the chronic stage of the disease (Pearce & MacDonald, 2002; Schwartz et al., 2014).

Generally, the granuloma provides protection for the host against foreign agents including pathogens and their molecules as well as protecting the host's tissues against prolonged immune response such as the one elicited by schistosome eggs. During *S. mansoni* infection, mostly intestinal and hepatic granulomas are formed dues to the extravasation of eggs from the bloodstream into these organs. Granulomas in the intestines are like early-stage liver granulomas, however, in the gut, granulomas have fewer B-cells, T-cells, eosinophils, and more macrophages. On the other hand, hepatic granulomas are collagen-rich (Amaral et al., 2017; Weinstock & Boros, 1983).

The granulomatous induces Th-2 polarized immune reaction which benefits the parasite as it allows egg transport into the gut/bladder lumen for onward excretion (Schwartz & Fallon, 2018). Thus, through immunomodulatory process facilitated by bioactive molecules secreted by the eggs, the host's immune response is regulated to achieve successful excretion of eggs (Ashton et al., 2001; deWalick et al., 2012). The molecules used predominantly to aid eggs excretions are alpha-1 (IPSE or SmCKBP) and omega-1 (Everts et al., 2012; Fahel et al., 2010; Pennington et al., 2017; Wuhrer et al., 2006). Alpha-1 is a dimeric glycoprotein (Dunne et al., 1984) which induces the release of IL-4 from basophils and clones the recombinant protein called IL-4 inducing principle of *S. mansoni* eggs (IPSE) (Schramm et al., 2007; Wuhrer et al., 2006). At the C-terminus of IPSE,

there is a nuclear localization signal that promotes infiltrations through the cytoplasm and cell membrane into the nucleus but not their uptake (Kaur et al., 2011; Pennington et al., 2017). Additionally, IPSE causes enlargement of hepatic granulomas (Fahel et al., 2010) and regulates chemokines to change local cellular recruitment and activation known as chemokines binding proteins (CKBP) (Smith et al., 2005). *S. mansoni* eggs are rich in CKBP with chemokines binding activity thus blocking chemokines. In the gut and livers, SmCKBP from matured eggs are actively involved in granuloma formation around viable eggs (Smith et al., 2005). Alpha-1 has therefore shown to be essential to the perpetuity of *S. mansoni* life cycle and infection. Besides Alpha-1, omega-1, a hepatotoxic egg glycoprotein (Everts et al., 2009) has T2 RNase activity and can activate Th2-type immune response. It primes dendritic cells to induce Th2-type response expansion (Everts et al., 2012; Steinfelder et al., 2009). The combined effect of innate glycosylation and T2 RNase activities enables omega-1 to bind to mannose receptor on the dendritic cells and be internalized (Everts et al., 2012; Hussaarts et al., 2013). As glycan-depending molecules, alpha-1 and omega-1 interacts with selected C-type lectin receptors on the immune cells and modulate the host's immune responses to the advantage of the parasite and egg excretion (Meevissen et al., 2012; Thomas & Harn, 2004).

Four stages of eggs excretion may be recognized: 1) Release of egg into the bloodstream and its attachment to the endothelium, 2) Immune-dependent granuloma formation, 3) Transition between endothelium and epithelium, and 4) Released into the gut lumen/bladder. The rigidity of *S. mansoni* eggs prevents inherent active movement of the egg hence certain molecules are needed from both the parasite and host to ensure that eggs are excreted successfully (Schwartz & Fallon, 2018). Female worms produce enolase (phosphopyruvate hydratase) and glyceraldehyde-3-P-dehydrogenase (GAPDH) on the surface of eggshells which act as surface receptors to bind plasminogen thus increasing the fibrinolytic activity of the eggs (Samoil et al., 2018; Yang et al., 2010). It has been observed that freshly deposited eggs enhance the active migration of endothelial cells as well as bind to and activate platelets which helps to activate endothelial cells (File, 1995; Ritter & McKerrow, 1996). Furthermore, enolase can function as plasminogen receptor and induce plasminogen conversion to plasmin (Figueiredo et al., 2015). Plasmin anti-blood clot activities together with recruiting monocytes assist the intravasation of eggs. Schistosome eggs antigen is also responsible for the upregulation of ICAM-1 which has been demonstrated by the overexpression of liver granulomas (Lukacs et al., 1994; Ritter & McKerrow, 1996; Secor et al.,

1994). Other bioactive molecules used at the host-parasite interface are LFA-1, VLA-4, ICAM-2, and PeCAM (Jacobs et al., 1998; Ritter & McKerrow, 1996). Overall, the immune responses of the host are actively involved in stage two of excretion whereas the exit of eggs from the endothelium to the epithelium and later into the gut lumen/bladder are facilitated by numerous cells and proteins of the homeostatic system (Schwartz & Fallon, 2018). The recent study (Peterkova et al., 2023) also enumerates peptidases excreted by *S. mansoni* eggs and their inhibitors many of them involved in the host-parasite interface which might be useful for eggs excretion among other functions.

2.4.2. Pathophysiology and clinical symptoms in acute and chronic disease

Depending on the stage of infection, acute and chronic phases of the disease can be distinguished. Each phase of the disease is characterized by certain signs and symptoms. Symptoms of acute infection can be observed within a few hours of the first day of infection to several weeks post-infection. First-time, hosts of *Schistosoma* spp. may experience tingling or itchy sensation and rashes at the point of entry by the cercariae within 60 minutes after contact with contaminated water (Boros, 1989; Gryseels, 2012; Gryseels et al., 2006). In hosts with previous infection or exposure, the immune system produces eosinophils, protective antibody (IgE), and macrophages to combat schistosomula from subsequent infections. Cercariae and early schistosomula who are unsuccessful may die in the skin, get surrounded by edema and cell infiltrates which may trigger maculopapular rash (so-called swimmers itch or cercarial dermatitis) characterized by edema, erythema, papules, pruritus, and vesicles (Curwen & Wilson, 2003; He et al., 2005; Pearce & MacDonald, 2002; Whitfield et al., 2003). Katayama fever also known as snail fever may arise from the successful penetration and further migration and development of schistosomula. It is a systemic hypersensitivity reaction resulting from the exposure of the host to parasite's molecules during schistosomula migration and egg deposition by adult worms which is commonest with *S. japonicum* but less common in *S. haematobium* and *S. mansoni* infections (Corachan, 2002; Jaureguiberry et al., 2010; Ross et al., 2007). The manifestation of Katayama fever occurs within 14-84 days post-infection and is predominantly characterized by symptoms including cough, diarrhea, fatigue, headache, fever, and myalgia. The proposed duration of recovery from acute schistosomiasis is 2-10 weeks, however, in worse cases, patients may develop more persistent disease characterized by abdominal pain, diarrhea, dyspnea, hepatomegaly, rash and weight loss

(Bottieau et al., 2006; Camprubi-Ferrer et al., 2021; Clerinx & Van Gompel, 2011; de Jesus et al., 2002; Gryseels, 2012; Jaureguiberry et al., 2010; Ross et al., 2007).

Chronic infection persists for years and decades and severe morbidity results from host immune responses to the eggs entrapped in various host tissues and organs such as the bladder, brain, colon, genitals, intestine, kidney, liver, and lungs when the infection is not detected and treated early (Pearce & MacDonald, 2002; Sombetzki et al., 2019). At the preliminary stages, the inflammatory reaction is readily reversible, however, in the later stages of the disease, the pathology is associated with collagen deposition and fibrosis, resulting in organ damage that may be only partially reversible (Burke et al., 2009; Colley et al., 2014; Corachan, 2002; Hams et al., 2013; Houston et al., 2004). Prolong chronic schistosomiasis in children may also lead to cognitive malfunction, stunted/retarded growth and weakened memory (Carvalho, 2013; Inobaya et al., 2014). Besides the period post-infection, the severity of manifestation is a function of infection intensity (worm burden and egg production), host immune response, and the localization of adults worms and eggs of the parasites (Ashton et al., 2001; Rutitzky & Stadecker, 2011; Schwartz & Fallon, 2018).

For chronic intestinal schistosomiasis caused by *S. japonicum* and *S. mansoni*, notable signs and symptoms include abdominal pain, diarrhea with intermittent constipation, and blood in the stool. Severe infection may cause appendicitis, bowel obstruction, perforation of the intestinal tract, and rectal stenosis (Gryseels, 2012; Gryseels et al., 2006; King & Dangerfield-Chay, 2008). In chronic urogenital schistosomiasis, egg deposition in the urinary system has well-defined symptoms such as bladder calcification, micro and macro hematuria, obstruction of the ureter, painful urination, proteinuria, renal colic. In extreme cases, obstructive uropathy, renal failure, hydroureter, hydronephrosis, and bladder cancer may occur (Ashton et al., 2001; Rutitzky & Stadecker, 2011; Schwartz & Fallon, 2018).

Eggs deposition in the liver causes periportal fibrosis and presinusoidal inflammation arising from the granulomatous formation which may consequently converge to form Symmer's pipe stem fibrosis which further obstructs the portal veins, resulting in portal hypertension. The resultant effect of this portal hypertension is the occurrence of ascites, hepatosplenomegaly, hypersplenism, variceal bleeding, and varices (Goncalves-Macedo et al., 2017; Li et al., 2011) In some cases, the lung may be affected due to extravasation of eggs from the bloodstream into the lungs (Gryseels, 2012; Hams et al., 2013). Eggs can be entrapped also in the female genitals causing granuloma

formation in the uterus, fallopian tube, and ovaries causing female genital schistosomiasis (FGS). Similarly, the entrapment of eggs in male genitals results in male genital schistosomiasis (MGS) (Burke et al., 2009; Bustinduy et al., 2022).

The central nervous system becomes affected when the embolization of eggs from the portal mesenteric system to the brain and spinal cord through the paravertebral venous plexus occurs (Corachan, 2002; Houston et al., 2004). The main clinical presentation of neuronal schistosomiasis resulting from the presence of *S. haematobium* and *S. mansoni* in the central nervous system is traverse myelitis (Al-Abdulwahhab et al., 2018; Machiels et al., 2018; Ross et al., 2012). Major reported symptoms are loss of bladder and bowel sphincter control, loss of sensation pain in lower limbs and lumbar region, and paraplegia (Burke et al., 2009; Nelwan, 2019; Norseth et al., 2014). In case of *S. japonicum* induced neuroschistosomiasis, associated symptoms such as epilepsy, meningoencephalitis, and paralysis of the host are reported (Gryseels, 2012).

2.5 Prevention and transmission control of schistosomiasis

Factors that promote schistosomiasis are hygiene and sanitation issues coupled with human contact with infected water. General measures of schistosomiasis prevention and control are morbidity control, snail control, proper sanitation, provision of portable clean water, and sensitization program for affected communities. Integrated control strategies combine two or more control measures to enhance effective and efficient control. The main recommended integrated control and prevention is a water, sanitation, and hygiene (WASH) control program (Lo et al., 2022; WHO, 2020a).

Morbidity control is achieved by the MDA of PZQ to mainly school-aged children in endemic countries and communities. Several case studies of PZQ MDA have reported a significant reduction in worm burden and prevalence (Bajiro et al., 2016; Guidi et al., 2010; Inobaya et al., 2014; Lamberton et al., 2017). Through PZQ MDA, reduced susceptibility to reinfection by increasing the IgE levels of the studied populations have been recorded (Asuming-Brempong et al., 2022; Fukushige et al., 2019), however, it never completely stop reinfection (Fukushige et al., 2019; Montgomery, 2020). Several countries have achieved significant success with the control of schistosomiasis through consistent use of MDA since its introduction (Abou-El-Naga, 2018; Anto

et al., 2011; Bergquist et al., 2017; Chitsulo et al., 2000). However, in the year 2021, about 30% of people at risk of infection only had access to PC through MDA globally (WHO, 2023).

Many local studies have exploited various ways to control snail intermediate host of *Schistosoma* spp. (Abe et al., 2018; Abou-El-Naga, 2018; Allan et al., 2020; Anto et al., 2005). In Ghana, for instance, the use of environmentally friendly modifications and molluscicides have been tested for their effectiveness against schistosomes (Chu, 1978). The removal of aquatic weeds reduced snail population but did not interrupt schistosomiasis transmission, however, the application of 0.5 mg of niclosamide was effective in killing snails at focal human water contact points and thus reduced cercarial transmission significantly (Chu et al., 1981). The potential of predatory snails *Lanistes varicus* in controlling *B. truncatus* and *B. pfeifferi* has also been studied showing a favorable outcome in the reduction of schistosome intermediate snail host population and subsequently cercarial transmission (Anto & Bimi, 2017; Anto et al., 2005). Focal snail control is thus recommended to complement MDA to achieve sustainable control.

WASH is an official terminology used to describe an intervention to minimize or prevent water, sanitation, and hygiene related diseases or risks. According to the WHO, all NTDs require WASH to sustain control and elimination efforts as well as morbidity management (WHO, 2020a). Regarding schistosomiasis, this intervention involves providing access to quality non-infected water as well as sanitation and hygiene facilities (toilets, bathrooms, urinals, etc) in affected and endemic communities to limit their contact with contaminated water (Lo et al., 2022). WASH has been demonstrated to be effective at reducing schistosomiasis in endemic countries. There is evidence, that the establishment of recreational water facilities in an endemic community resulted in a significant reduction of schistosomiasis in the community (Kosinski et al., 2012). Improved access to water for drinking and adjustments of the household's everyday activities might be necessary to reduce the incidence of schistosomiasis in vulnerable communities (Kulinkina et al., 2017; Pitchford, 1966, 1970a, 1970b).

Public education and sensitization about schistosomiasis are necessary tools for preventing infection in endemic countries and communities. Sensitization creates awareness of the disease and promotes at least certain basic knowledge of the disease which may subsequently lead to beneficial behavioral changes of the population in the endemic areas, as recommended by the

WHO in complementary preventive plans (Aryeetey et al., 1999; Danso-Appiah et al., 2004; Martel et al., 2019; WHO, 2020a, 2023).

2.6 Treatment of schistosomiasis

The treatment of schistosomiasis goes back to the early part of the 20th century when antimony drugs were administered as chemotherapy (Christopherson, 1918). Afterwards Metrifonate (Davis & Bailey, 1969; Mgeni et al., 1990), Hycanthone (Farid et al., 1972; Oostburg, 1972), and Ambilhar (Shekhar, 1991), were used but were later recalled due to associated severe side effects.

Currently, praziquantel is the drug of choice for treating both urogenital and intestinal schistosomiasis. It is a derivate of pyrazinosoquinolone with a broad spectrum activity against helminths infecting both humans and animals such as cestodes and trematodes (e.g., cysticercosis, taeniasis, and schistosomiasis) (Gonnert & Andrews, 1977). Initially manufactured to be used in animals, the antischistosomal activity of PZQ was revealed in 1972 and was found to be effective against the adult form of all major schistosomes infecting humans (Chai, 2013; Cupit & Cunningham, 2015; Doenhoff et al., 2008; Gonnert & Andrews, 1977).

Investigations into the mechanism of action although inconclusive, have revealed that PZQ disrupts calcium-mediated processes in the adult worm in antagonism of the parasite's voltage-gated calcium channel and/or molecule which interact with and regulate these channels (Doenhoff et al., 2008; Greenberg, 2005a, 2005b; Salvador-Recatala & Greenberg, 2012). It can also influence the membrane permeability of adult schistosomes leading to rapid calcium influx, sustained muscle contractions and profuse damage of the tegument. The disruption of the adult worm tegument exposes the parasite to the host's immune response which reacts in the attack against worm surface antigens (Becker et al., 1980; Fetterer et al., 1980; Pax et al., 1978). The contraction of worm musculature mediated by PZQ administration causes paralysis of the worm, detachment from host tissues, and later expulsion from the host (Harnett & Kusel, 1986; Mehlhorn et al., 1981). Other schistosome molecules identified to interact with PZQ are glutathione S-transferase, myosin, G-protein-coupled receptor ligand (Chan et al., 2017), kinases (Chienwichai et al., 2020), nucleoside (adenosine and uridine) (Angelucci et al., 2007) and transient receptor of potential channels (Nogueira et al., 2022). Further studies have posited that PZQ administration promotes immunoregulatory activities by increasing T regulatory type 1 (Tr1) cell differentiation

while decreasing inflammation. In summary, PZQ mechanism of action is broad and multichannel. This mechanism can be studied in anticipation of drug and vaccine discovery.

A greater percentage (estimated 80%) of PZQ is absorbed rapidly into the intestinal tract and then metabolized in the liver and finally excreted via urine or stool (Vale et al., 2017). The drug is administered orally as a single dose (Doenhoff & Pica-Mattoccia, 2006; Ross et al., 2002). A recommended dosage 40 mg/kg per day orally in two divided doses has proven to be effective against *S. haematobium*, *S. intercalatum*, and *S. mansoni*, whereas 60 mg/kg per day orally in three divided doses for one day is recommended for *S. japonicum* and *S. mekongi (Caffrey, 2015; Chai, 2013)*. However, a study proposed three monthly doses of 60 mg/kg PZQ is efficacious against *S. haematobium* and safe for patients (Darko et al., 2020).

A cure rate within the range of 75%-100% has been reported when persons infected with *S. haematobium* and *S. mansoni* were treated with 40 mg/kg per day on one hand and persons infected with *S. japonicum* were treated with two doses of 30 mg/kg per day on another hand in a multi-site study (Olliaro et al., 2011). Several related studies have reported different ranges of cure rate among school children who were infected with *S. haematobium* and *S. mansoni* when treated with 40 mg/kg body weight. The cure rate ranges from as less than 40% to 88.9% (Coulibaly et al., 2012; Coulibaly et al., 2018; Erko et al., 2012; Obonyo et al., 2010; Reta & Erko, 2013). When similar treatment was meted out to preschool children, over 77% cure rate was observed among first-time PZQ users whereas the cure rate was less than 70% in children who have previously been exposed to PZQ (Borrmann et al., 2001; Keiser et al., 2010; Obonyo et al., 2010; Sissoko et al., 2009; Sousa-Figueiredo et al., 2012).

PZQ has some obvious demerits such as: 1) its ineffectiveness against the migratory larval stages and eggs (Cioli & Pica-Mattoccia, 2003; Cioli et al., 2014). 2) Possibility of the parasites developing resistance to the drug due to extensive use over a long period of time and also reduced efficacy arising from continuous use exists (for example, in Senegal PZQ treatment of *S. mansoni* gave a cure rate between 18%-36%)(Cioli et al., 2014; Doenhoff et al., 2008; Doenhoff & Pica-Mattoccia, 2006; Inobaya et al., 2014). However, PZQ is the most favored antischistosomal drug owing to the relatively mild to moderate side effects, high efficacy, easy to administer, and safe use across all age groups (Inobaya et al., 2014)

Other drugs used in the treatment or tested against schistosomiasis are enumerated below:

1. Oxamniquine: This drug is a derivative of aminoethyltetrahydroquinolone and was used for the treatment of *S. mansoni* in the early 1960s. It was found to be very effective against invasive, migratory, as well as adult stages with adult males being the most sensitive to this drug (Foster, 1987; Gentile & Oliveira, 2008). After the ingestion of oxamniquine, the drug is absorbed rapidly hence reaching its maximum concentration in the plasma within 1 to 4 hours approximately. The main mechanism of drug metabolism is via oxidation while the main route for excretion is through urine. The recommended dose to achieve high medicinal efficacy is 15-60 mg/kg for 2 to 3 days consecutively, however, this drug is not currently in use for treatment the disease owing various side effects and multiple administration (Inobaya et al., 2014).

2. Artemisinin and its derivatives: They are plant-based compounds first discovered in China for the treatment of malaria (Araujo et al., 1991; Inobaya et al., 2014; Woodrow et al., 2005). Their effectiveness against schistosomiasis has been experimented under various situations. Artesunate and artemether have been used against *S. mansoni* (Utzinger et al., 2000). Trials show that artemisinin is very potent against juvenile stages of the parasites but less effective against adult worms (Obonyo et al., 2010; Utzinger et al., 2000). Artemether showed significant damage on adult female parasites in infected mice (Araujo et al., 1991). When artesunate with sulfadoxine was combined with pyrimethamine to treat *S. mansoni* infection, a cure rate ranging between 14%-100% was recorded (Abay et al., 2013). In a similar manner, a cure rate within 44% to 100% was observed when artesunate with sulfadoxine/sulfalene was administered in tandem with amodiaquine or pyrimethamine to treat *S. haematobium* infection (Fortin & Jansen, 2011; Sissoko et al., 2009). Furthermore, the use of artemether and lumefantine in combination to treat simultaneous infection of *Plasmodium falciparum* and *S. mansoni* resulted in 100% cure rate (Abay et al., 2013) thus showing high antischistosmal potency against *S. mansoni* which is comparable to PZQ in some cases hence their potential as alternative medication for schistosomiasis must be investigated further.

3. Corticosteroids: They are steroid hormones produced and secreted by the adrenal glands in response to pituitary adrenocroticotropic hormone and regulated by hypothalamic croticotropin releasing hormone. Functions of these hormones include homeostasis control, stress management, and regulation of major endocrine system (Kaliner, 2006). They serve as adjuvants in the treatment

of schistosomiasis (Ross et al., 2012; Vale et al., 2012). When administered against neuroschistosomiasis and urogenital schistosomiasis, it regulates the immune responses thereby reducing granulomatous formation and subsequent damage of surrounding tissues (Fowler et al., 1999; Miller & Reid, 1967). Although several related case reports seem to show antischistosomal activities of corticosteroids (Badr et al., 2011; Lambertucci et al., 2008; Miller & Reid, 1967; Vale et al., 2012), more research is needed to reveal exact roles.

2.7 Advances in schistosomiasis vaccine development

Over the past two decades, experts have become more concerned about the development of vaccines for the prevention of schistosomiasis owing to the numerous lapses in the existing control strategies. Arrays of *Schistosoma* spp. molecules have been exploited as vaccine targets in both, basic and translational research (Bergquist & Colley, 1998; Capron et al., 2002; De Bont et al., 1997). The criteria set by the expert committee for the ideal schistosomiasis vaccine is that it should be able to reduce worm burden to at least 75% with a nearly 75% reduction in eggs production and excretion in vaccinated persons (Mo & Colley, 2016; Molehin et al., 2016). These criteria are because achieving sterile immunity is not the goal of schistosomiasis vaccine development due to the heteroecious nature of schistosomes which makes them unable to complete the life cycle in their mammalian hosts. As the core of vaccine candidates search is antigen, several arrays of antigens have been tested against *S. haematobium*, *S. japonicum*, and *S. mansoni* in mice and non-human primates, however, most of them have not made it to the first clinical trial stage (Hotez et al., 2019; Molehin, 2020; Molehin et al., 2022; Molehin et al., 2016).

Irrespective of the odds that have besotted the development of schistosomiasis vaccines in the past, four antigen molecules have been identified to be at the forefront of vaccine development for all major medically important human schistosomes, one from *S. haematobium*, 28-kD glutathione S-transferase (Sh28GST/Alhydrogel) (Riveau et al., 2018) and the other three are coming from *S. mansoni*, calpain (Sm-p80/GLA-SE) (Molehin et al., 2022), a 14-kDa fatty acid-binding protein (Sm14/GLA-SE) (Santini-Oliveira et al., 2016; Santini-Oliveira et al., 2022), and tetraspanins (Sm-TSP-2/Alhydrogel) (Keitel et al., 2019; Loukas et al., 2007).

Glutathione S-transferase enzyme plays a significant function in schistosomes by modulating the host's immune system at the onset of infection (De Bont et al., 1997; Mitchell, 1989; Riveau et al.,

2018). Calpain is a cysteine peptidase with catalytic and proteolytic subunits. The main site of expression is the tegument of schistosomes and facilitates host's immune evasion (Molehin et al., 2017; Suzuki et al., 2004). The 14-kDa fatty acid-binding protein is universally expressed at all developmental stages of schistosomes. This protein allows the parasite to take up essential fatty acids from the host at various stages of development (Fonseca et al., 2005; Garcia et al., 2008; Santini-Oliveira et al., 2016). Tetraspanins are proteins found on the surface membrane of schistosomes with regulatory functions for other proteins (Pinheiro et al., 2014). All these molecules are at various stages of clinical trials. *S. haematobium* recombinant glutathione-S-transferase (rSh28GST) has undergone phase 3 clinical randomized trials in Senegal (Riveau et al., 2018). *S. mansoni* Calpain (Sm-p80) has passed pre-clinical trial phase and phase 1a clinical trials have commenced in first-time infected adults in the USA (Molehin et al., 2022). *S. mansoni* 14-kDa fatty acid-binding protein (Sm14) has also completed phase 2b clinical trials in Senegal (Molehin et al., 2022). *S. mansoni* Tetraspanin (Sm-TSP-2) has successfully undergone phase 1a and 1b trials and is currently undergoing Phase 1 and 2b trials in Uganda with a focus on healthy adults (Molehin et al., 2022). Molehin et al. is giving more detailed characteristics of these notable vaccine candidates (Molehin et al., 2022).

Despite the seeming progress and advances made in schistosomiasis vaccine development, several obstacles impede the smooth and fast development of schistosomiasis vaccines. The complexity of the schistosome life cycle and their ability to evade the host immune responses make it difficult for vaccine advancement (Molehin, 2020; Molehin et al., 2022). Besides, several vaccine studies are done in animal models whose immune systems do not reflect exact human parameters hence success in animal models does not automatically translate into success with human subjects (Abdul-Ghani & Hassan, 2010; Wilson et al., 2016). From an economic and political perspective, vaccine development is very cost/demanding. Yet schistosomiasis affects the world's poorest populations and does not result in immediate death, therefore, governments have shown low to zero interest in vaccine development (Adenowo et al., 2015; Al-Naseri et al., 2021). Amid these advances and challenges, there is still the need for the search for vaccine candidates thus identification of the biological roles of schistosome molecules is paramount to the vaccine development discourse owing to their numerous roles at the host-parasite interplay. Special attention is paid to schistosome peptidases.

2.8 Overview of *S. mansoni* peptidases

Peptidases also referred to as proteases or proteolytic enzymes, hydrolyze proteins or peptides. These enzymes are found in all types of living organisms including schistosomes (Barrett, 2001; van der Velden & Hulsmann, 1999). In biomedical research in general and parasitology in specific, peptidases have become attractive molecules since their roles in both acute and chronic stages of many parasitic diseases including schistosomiasis cannot be undermined (McKerrow et al., 2006). Generally, peptidases are pivotal in host-parasite interactions such as invasion, migration through the tissues, hatching of eggs, reproduction, excystment, nutrients acquisition through hemoglobin digestion, immune evasion and activation, modulation of the host immune responses, inflammation, and excretion (Caffrey et al., 2004; Delcroix et al., 2006; deWalick et al., 2012; Dvorak et al., 2005; Horak et al., 2008; Kasny et al., 2011; McKerrow et al., 2006). Different classes and types of peptidases are found in parasites with specific functions as mentioned above and others as targets for disease diagnosis in recent years (Atkinson et al., 2009; Baig et al., 2002; Barrett, 2001; Bos et al., 2009; Caffrey et al., 2004; Dvorak et al., 2016).

Our laboratory is actively studying specific roles of *S. mansoni* peptidases at the host-parasite interface in mice models using varieties of platforms and tools such as transcriptomics, recombinant expression, immunohistochemistry, RT-qPCR, RNAi, *in situ* hybridization and a host of other techniques.

Among the schistosomes infecting humans, *S. mansoni* has become an attractive model for various genomic and proteolytic studies owing to relatively easy life cycle maintenance under laboratory conditions (Ulrychova et al., 2020). The life cycle is maintained via *Biomphalaria* spp. acting as the snail intermediate host and murine definitive hosts (Edwards & Mccullough, 1954; Jedlickova et al., 2022). Another reason why *S. mansoni* stands out among the trematodes whose peptidases have been well studied is due to available genomic information in various databases (Berriman et al., 2009; Howe et al., 2017; Wendt et al., 2020). A good number of aspartic, cysteine, metallopeptidases, and serine peptidases of *S. mansoni* have been identified and characterized biochemically or biologically in various stages of the parasite's life cycle. In this chapter, common groups of *S. mansoni* peptidases with an emphasis on biological characterization and identifiable functions are presented.

Metallopeptidases in parasites in general and helminths in particular, have demonstrated immunesuppression and modulation roles (Hambrook et al., 2018). The matrix metallopeptidase from M8 family, *S. mansoni* leishmanolysin (SmLeish) is a variant of *Leishmania* spp. metallopeptidase, leishmanolysin (Gp63) that disrupt the hemocytes of its snail intermediate host, *Biomphalaria glabrata* thus preventing the encapsulation of the sporocysts. When the gene encoding SmLeish was silenced in miracidia, the penetrability of the miracidia was weakened which resulted in further delay of infection in the snail intermediate host and subsequent lesser cercariae production (Hambrook et al., 2018). Another known *S. mansoni* peptidase which plays a significant role in the larval stage of the parasite is cercariae invadolysin (SmCI-1), a matrix metallopeptidase known for immunomodulatory function. They are one of the major genes families found in *Schistosoma* spp. With records of homologs in *S. haematobium* and *S. japonicum*.

A total of seven *S. mansoni* invadolysins are identified, five of which are found in germ cells of daughter sporocysts and the other two in cercariae (Parker-Manuel et al., 2011; Schistosoma japonicum Genome & Functional Analysis, 2009; Silva et al., 2011; Young et al., 2012). SmCI-1 is released from the acetabular glands of cercariae. Its involvement in the immunological environment and for the ultimate survival of parasites during host invasion and transformation into schistosomula have been unearthed when SmCI-1 cleaved collagen type V, gelatin, fibrinogen, and C3b. The peptidase has shown a protective function by shielding the parasite from complement-mediated lysis in the human plasma. It promotes an anti-inflammatory microenvironment and suppresses the production of inflammatory cytokine-like IL-1B and IL-12P70 (Hambrook & Hanington, 2023). Leucine aminopeptidases (LAPs) also belong to the M17 family metallopeptidase with affinity cleavage of leucine residue at the N-terminus of proteins and short peptides. Two of these LAPs, SmLAP1 and SmLAP2 are predominantly expressed in mammalian life stages of *S. mansoni*, eggs and adult worms. Both peptidases are localized to the epithelial cell of the intestine, gastrodermis, and vitellaria, of the adult worms. The expression of these peptidases in eggs is linked to the hatching of the eggs whereas the localization in the intestine and gut imposes digestive function whereby the ingested hemoglobin from the host is degraded by the enzymes. Further function of SmLAP1 in the modulation of host's immune response is associated with its localization in the sub-tegument of adult worms (Rinaldi et al., 2009).

Despite numerous studies on various classes of *S. mansoni* peptidases including some families of metallopeptidases, currently, there is no information about any *S. mansoni* M28B subfamily peptidase. Some mammalian orthologs/homologs of M28B peptidases have been well studied but with some complexities about biological functions in mammals (Bacich et al., 2002; Barinka et al., 2008; Barinka et al., 2012; Bzdega et al., 2004; Devlin et al., 2001; Hlouchova et al., 2007; Hlouchova et al., 2009) hence laying the foundation for investigation of the *S. mansoni* ortholog. This further presents a rare opportunity to add to the existing quantity of *S. mansoni* peptidases as well as fill the knowledge gap by pioneering investigation into SmM28B and its potential for drug or vaccine development in study number 3 in the present thesis.

Several other *S. mansoni* peptidases belonging to different classes or groups are known to be expressed and/or localized in certain tissues with functions in host-parasite interplay. Table 3 outlines non-metallopeptidase *S. mansoni* peptidases and their functions.

Table 2. Functions of *S. mansoni* peptidases

Peptidase	Class	Localization	Possible function(s)	Reference
Cathepsin D-like (SmCD)	Aspartic	Gut	Hemoglobin digestion	(Caffrey et al., 2004; Kasny et al., 2009)
S. mansoni Cathepsin B like (SmCB1)	Cysteine	Gut	Hemoglobin digestion	(El Ridi et al., 2014; Jilkova et al., 2020; Sajid et al., 2003)
S. mansoni cathepsin B2 (SmCB2)	Cysteine	Tegument surface	Modulation and immune evasion	(Caffrey et al., 2002)
S. mansoni Cathepsin L like (SmCL1)	Cysteine	Gut	Digestion of serum protein	(Caffrey et al., 2004)
S. mansoni asparaginyl endopeptidase (SmAE)	Cysteine	Gut	Activation of SmCB1, digestion of serum protein an hemoglobin	(Caffrey et al., 2000; Caffrey & Ruppel, 1997)
S. mansoni Cathepsin C (SmCC)	Dipepdyl peptidase I	Gut	Hemoglobin digestion	(Sojka et al., 2016)
S. mansoni cercarial elastase (SmCE)	Serine	Circumacetabular	Host invasion	(Dvorak & Horn, 2018; Dvorak et al., 2008)
S. mansoni serine peptidase 2 (SmSP2)	Serine	Esophageal glands, reproductive organs, teguments	Digestion and reproduction	(Horn, Fajtova, Rojo Arreola, et al., 2014; Ulrychova et al., 2021)
S. mansoni prolyl oligopeptide (SmPOP)	Serine	Parenchyma and tegument	Modulation of immune response, immune evasion	(Fajtova et al., 2015)

3.0 STUDY 1

Title: Situation of schistosomiasis in Ghana

OVERVIEW - **From this study, a peer-reviewed article was published in a 2nd quartile journal, Transactions of the Royal Society for Tropical Medicine and Hygiene (TRSTMH) with impact factor 2.6:**

Boateng EM, Dvorak J, Ayi I, Chanova M. A literature review of schistosomiasis in Ghana: a reference for bridging the research and control gap. Trans R Soc Trop Med Hyg. 2023 Jan 23:trac134. doi: 10.1093/trstmh/trac134.

The full article is attached as Appendix 1 to this thesis.

Ghana remains one of the endemic countries on the schistosomiasis map of Africa with an appreciable long history. To understand the research landscape as well as the control, relevant literature along with socio-economic and demographical characteristics were reviewed to evaluate the success of control programs thus far. This study focused on identification of knowledge gaps, providing reference for bridging the gap between research and control programs, and offering recommendations that will facilitate efficient control and subsequent elimination as a public health issue.

Although literature review is an untraditional assessment tool, it plays a significant role in the retrospective assessment of an intervention to identify progress, pitfalls, strengths, weaknesses, challenges, and the way forward. In view of this, a review of schistosomiasis situation in Ghana presents an opportunity to assess the pros and cons of schistosomiasis in general and the overall achievements regarding the control of the disease especially when there is no nationally defined assessment tool for assessing control. It is likely that, the schistosomiasis situation of Ghana mimics the situation of other endemic countries in the sub-region hence, the adoption of a situational literature review as an evaluation tool may help overcome some of the bottlenecks faced in the control of the disease in sub-Saharan Africa.

Methodology: Identification and validation of Ghana-related schistosomiasis literature

A comprehensive data search was performed using PubMed, Scopus, and Google Scholar search engines. The search was done using "schistosomiasis or bilharzia or schistosomosis or Schistosoma" and "Ghana" as the main keywords in combination with at least one of the following: animal, control, distribution, elimination, livestock, mass drug administration (MDA), neglected tropical diseases (NTDs), prevalence, snail intermediate host and transmission. Further selection was performed according to the following criteria: studies conducted in Ghana between 1954 to 2021 and published as an original research article; research must be in English and must have an abstract. Reviews, case reports, and short communications were excluded. Other relevant sources such as online documents by the Center for Disease Control (CDC), the Global Atlas of Helminth Infections (GAHI), World Bank (WB), Ghana Statistical Service (GSS), WHO, and the Ministry of Environment, Science and Technology (MoEST) were also used.

RESULTS AND DISCUSSIONS

Geography, demography, and socioeconomic characteristic of Ghana Relevant to schistosomiasis occurrence

Ghana is one of the anglophone countries in SSA on the west coast of Africa to be precise. The geographical area of Ghana is estimated to be 238,533 km^2. Land mass represents 95% of the territory, and the remaining 5% represents the hydrological network. The country is divided into six agroecological zones, namely; Sudan, Guinea, and coastal savannahs, forest/savannah transitional zone, deciduous forest zone, and the rain forest zone (MoEST, 2011). Three main hydrological basins are the Volta basin which covers 70% of the country, stretching from south to north of the country, (Mone et al., 2010). The other two are the southwestern rivers and the coastal basin draining 22% and 8% of the area, respectively.

Since the year in 2018, Ghana is administratively partitioned into 16 regions country from the previous 10 regions (Baguio, 2011). For the present study, Upper East, Upper West, and the Northern regions (Northern region now separated into Savannah, North-East, and the Northern regions), are reported as the northern sector of the country. As for the southern sector, Ashanti, Brong Ahafo (now Ahafo, Bono, and Bono East regions), Central, Eastern, Greater Accra, Volta

(now Oti and Volta regions), and Western region (now Western North and Western regions) are described.

According to the World Bank, Ghana belongs to a lower-middle-income economy (WB, 2021), with nearly a quarter (23.4%) poverty rate (WB, 2020). The housing and population census (GSS, 2021) estimated a total population of around 30.8 million and a population density of 129 persons per km^2. Young people (15-35 years) and (0-14 years) are the dominating year groups making up 38.2% and 35,3% of the population, respectively. Among the employable category (i.e., economically active population 15 years and older), Almost 11.5 million people (58.1%) are working in various private and public sectors, with a predominant 32.0% of the employed population engaged in agriculture, forestry, and fishery. In addition, 182 thousand (2.6%) children (aged 5-14 years) are engaged in agriculture-related economic activities. Of all people 6 years of age and older, 30.2% are illiterate. Rural folks made up 43.3% and only 7 out of 16 regions are urbanized. Households with access to a bathroom for exclusive use was 38.9% and those with a private toilet constituted 59.3%. The most prevalent method of disposing of household wastewater is by throwing it onto the ground/street/outside (70.6%). Over 17.7% of households did not have access to any toilet facility and open defecation is practiced in 6.2-68.5% of households in particular regions. Almost a tenth of households (8.0%) did not have access to an improved source of drinking water. Of those, 79.9% (i.e., nearly 2 million people) rely on surface water (river/stream/dugout/pond/dam/canal). Most poverty-related characteristics are seen in rural communities and in northern Ghana.

The geography of Ghana provided a suitable environment for schistosomiasis to thrive. The disease transmission is further promoted by socioeconomic characteristics and the economic activities of poor communities such as widespread open defecation and disposal of wastewater to the environment that enables uncontrolled contamination of natural water sources. Predominant agricultural and fishing activities, as well as deprived access to safe water for some households, all resulting in regular contact with contaminated water, predispose a significant proportion of the population to schistosomiasis. Moreover, increasing demands of the growing population have contributed to the rapid spread of schistosomiasis in general and *S. mansoni* in particular, thereby changing both prevalence and distribution throughout the country over the decades. Generally, human migration contributes significantly to the introduction or spread of infectious diseases

(Soto, 2009). In addition, hydrological changes arising from anthropogenic factors such as dams and irrigation constructions intensified the transmission of water-borne diseases, including schistosomiasis, e.g. by providing a more stable snail habitat or by changing human water-related activities (Hunter, 2003).

Schistosomiasis research in Ghana

The most topical studies were epidemiology of human schistosomiasis in Ghana (assessed in 33 articles), control of schistosomiasis transmission (21 articles), and schistosomiasis diagnostic methods (18 articles). Other topics on human and livestock schistosomiasis were also covered. 15% represented purely laboratory-based studies with most of the studies being field bases or a combination of the field and laboratory. Of all the studies, the Greater Accra region featured prominently (33 articles focused on the region alone). This was followed by the Eastern region (13 studies). Other regions are subject to zero to 10 studies.

The first published manuscript on schistosomiasis was in 1954 (Edwards & Mccullough, 1954). Until this date, data on the disease was only available in some hospitals in the then Gold Coast (McCullough, 1956). Several studies presented local prevalence (Adu-Gyasi et al., 2010; Afrifa et al., 2017; Amankwa et al., 1994; Anto, 2013; Anyan et al., 2019; Anyan et al., 2020) as well as estimated (derived) national prevalence for 6 specific time points as shown in Figure 3. Various aspects of schistosomiasis such as genital, livestock/animal, molecular identification, and characterization of schistosomes, malacological studies were giving little to no attention over the last seven decades of research. The utter absence of a recent nationwide epidemiological survey covering all regions and people can hinder effective control program since the absence of real data may lead to overestimation or underestimation of people in need of treatment. The only recent national surveillance was started in 2007 and completed in 2010 with a focus on school-aged children. From this survey, MDA was started progressively in 2008 and since then, the program has continued (GHS, 2016).

The quest to effectively control schistosomiasis and further eliminate it requires that all aspects of the disease are carefully researched in an integrated manner to determine a holistic control strategy and approach to combat the disease. Oftentimes, genital schistosomiasis is confused with sexually transmitted infections thereby precluding the right control or treatment regime (Bustinduy et al.,

2022). The lack of extensive research on genital schistosomiasis poses threat to the control the disease. Records of animals serving as a reservoir host abound in Senegal and elsewhere and can significantly influence the transmission of the disease, (Catalano et al., 2018; Gentile et al., 2006; Standley et al., 2012) yet very little attention has been given to animal schistosomiasis in Ghana. Besides, the interplay of animal-specific and human-specific *Schistosoma spp*. has given rise to hybrids that infect humans (Boissier et al., 2016; Boon et al., 2019; Huyse et al., 2009; Leger et al., 2020; Leger et al., 2016). The sensitivity of these hybrids to PZQ is not well studied and the possibility of zoonosis cannot be undermined (Catalano et al., 2018). Understanding the status of animal schistosomiasis and its relationship with human infections in Ghana will be very crucial to combat this disease in the following years and decades.

Molecular studies of diseases and parasites provide detailed yet precise information about diseases and parasites. The schistosomiasis scientific community has used various molecular tools and platforms to unearth the mystery surrounding species identification, diagnosis, and treatment (Barrett et al., 2001; Caffrey, 2015; Caffrey et al., 2004; Caffrey et al., 2002; Kasny et al., 2009). In Ghana, *S. haematobium* and *S. mansoni*, are the well-studied species however, other human-infecting species have been identified in Senegal and other countries in the SSA sub-region (Leger et al., 2020; Pennance et al., 2020; Webster et al., 2013). The low interest in molecular studies in Ghana means that some vital information that will be useful in controlling the disease is lacking. Increasing molecular studies will be very necessary if elimination of the disease as a public health issue is the goal.

Schistosomiasis transmission control hinges on several recommended strategies as stated earlier on. One of those strategies is the control of snail intermediate hosts (WHO, 2020a). Although molluscicide comes with certain demerits, a targeted approach using the right chemical formulation in focused areas has been recommended by the WHO and other control program experts. However, there is no recent nationwide attempt to control the snail intermediate host in Ghana indicating a huge gap in the control programs in Ghana. There is, therefore, an urgent need to improve schistosomiasis malacological studies to identify which strategy of snail intermediate hosts control works best for the country so that it can be recommended and adopted at the national level to complement the main MDA that's currently ongoing.

Precision mapping, surveillance, and monitoring of schistosomiasis are key components of the control and elimination program (Kabatereine et al., 2011; Kabore et al., 2013; Soares Magalhaes et al., 2011; Tchuem Tchuente et al., 2017; WHO, 2020a). The nationwide epidemiological survey provides precise and accurate data on the disease that are useful to ensure policy design and implementation. A sound epidemiological survey of schistosomiasis will provide information on group age, communities, and time of need of treatment among other things without which there will be overestimation/underestimation, both of which have a high potential of impeding control progress. The only known national epidemiological survey was conducted between 2007 and 2010 where 170 districts were surveyed and over 6.5 million school children were considered at risk (GHS, 2016), however, the survey did not include adults and out-of-school-aged children who might be at risk, and a number of infected people was not reported.

Prevalence and geographical distribution of schistosomiasis in Ghana

Few national prevalence and distribution studies have been conducted between 1954-2021, however, data on national prevalence is quite old. The study demonstrated that focal prevalence can be as high as 95%. National prevalence as estimated for the first time in 1963 was between 15-20% of *S. haematobium* (Doumenge, 1987). Schistosomiasis prevalence has since progressed profusely to over 70% based on the estimation of PZQ need in 1986 (Utroska JA, 1989), 2003, and 2010 (Rollinson et al., 2013) timepoints consecutively. The recent estimated national prevalence in 2015 is a little over 23% (Lai et al., 2015). Although the estimated national prevalence values shows that Ghana achieved a significant progress in terms of controlling the disease (**Figure 1**), data shown from the website of WHO suggests that a vast part of the country is moderate to high endemic status (WHO, 2020b) hence the need to intensify control campaign. While prevalence appreciates over the years, the distribution of schistosomiasis in general and *S. mansoni* specifically expanded rapidly from northern Ghana to other parts of the country, although the prevalence of *S. mansoni* remains relatively low in comparison to *S. haematobium* (GAHI, 2015; WHO, 1987). One of the key contributors to the exacerbation of schistosomiasis distribution is the construction of the Akosombo dam and its related irrigation dams in Northern Ghana to promote agricultural activities. These dams promoted the growth of water plants which further provided and conducive habitat for snail intermediate hosts and served as conduit for their dispersal nationwide (Hunter, 2003).

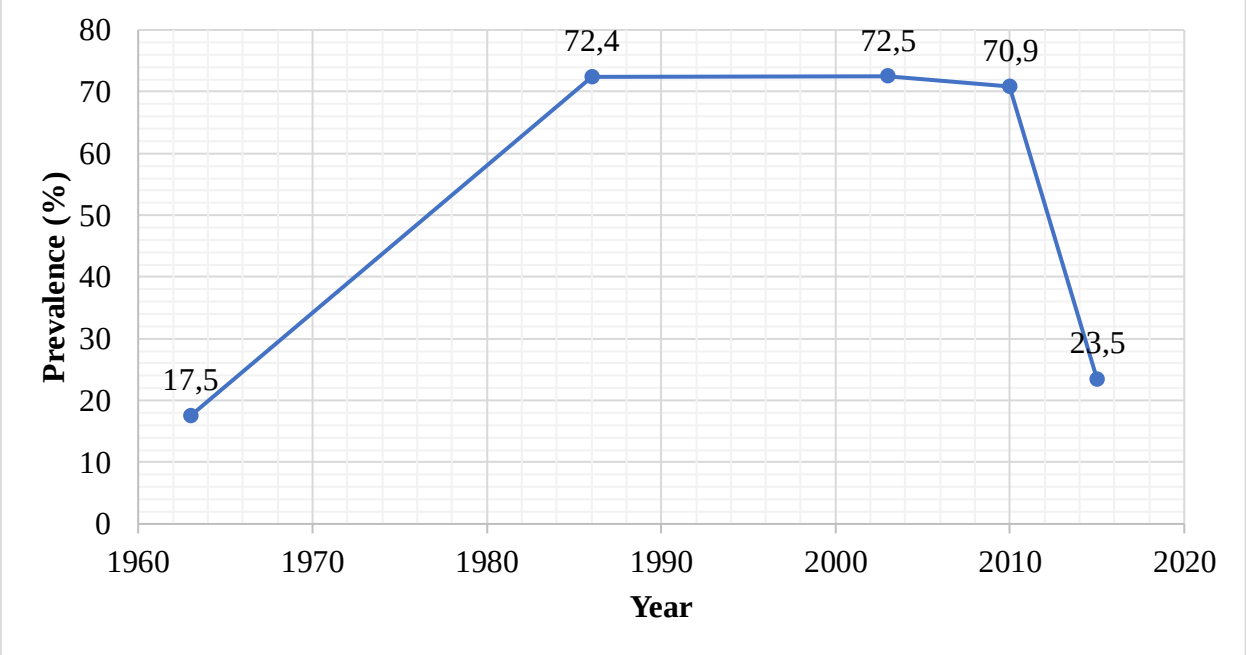

Figure 1. The estimated nationwide prevalence of human schistosomiasis in Ghana published from 1963 to 2015 (Doumenge, 1987; Lai et al., 2015; Rollinson et al., 2013; Utroska JA, 1989). Prevalence was low in 1963 and rose significantly to 72.4% in 1986 which plateaued for almost 3 decades. The prevalence then declines almost a quarter of the Ghanaian population in 2015.

Conclusion

The literature review of schistosomiasis in Ghana revealed several knowledge gaps which need to be filled to ensure effective control. Researchers must be actively involved in the nationwide control programs. Research on animals and its relationship with human schistosomiasis including molecular identification and characterization of schistosome species in Ghana should be promoted. Besides, it's high time for precision mapping and a nationwide epidemiological survey that will inform and guide appropriate control programs toward the right target group. Also, there is a scanty study on malacology which needs to be improved significantly since malacology plays a significant role in transmission control. The roles of basic and applied research in disease control have been presented in this study. The information herein is necessary for improving control of the disease at national level. Unfortunately, review of literature is not considered as a tool for the assessment of national control and prevention programs. For the first time, a singles document that encompasses the salient studies, progress, and pitfalls of control and elimination has been produce

that provides reference for bridging the research and control gap. Based on the findings of this study, situational literature review is highly recommended to be integral part of national evaluation of the disease prevention, control, and subsequent elimination as a public health threat.

STUDY - 2

Title: Evaluation and monitoring of praziquantel communitywide mass drug administration through xenomonitoring

OVERVIEW

One key aspect of the MDA campaigns is monitoring and evaluation. Effective monitoring and evaluation have been recommended to help assess the effectiveness or otherwise of the MDA campaigns in endemic areas (WHO, 2020a, 2023). In medical epidemiology, monitoring and evaluation are effective tools for the assessment of the success or otherwise of a medical or public health intervention or program. Toor et al., define monitoring and evaluation in the context of schistosomiasis as "programs used to collect data which are required to assess the impact of current interventions on their progress towards achieving the WHO goals of morbidity control and elimination of schistosomiasis as a public health problem" (Toor et al., 2018). In the history of schistosomiasis control and elimination, continuous assessment of control programs, especially PZQ MDA via monitoring and evaluation has played a significant role (WHO, 2020a).

Post-treatment monitoring and evaluation of schistosomiasis provided reliable, timely, and vital feedback for any further action and follow-ups where necessary. Countries that are known to achieve elimination status have kept a strict culture of continuous sound monitoring and evaluation. In Egypt, annual monitoring for 10 years showed significant progress made in disease control at the national level (Abou-El-Naga, 2018). Morocco, Japan, and parts of China have used monitoring and evaluation extensive to achieve significant strides in their fight against schistosomiasis, some of which have been declared "schistosomiasis-free". This sets a good precedence for endemic countries to integrate monitoring and evaluation of schistosomiasis control programs in their national control policies and programs (WHO, 2020b).

The traditional method for monitoring and evaluating schistosomiasis MDA campaign is by collecting pre-treatment and post-treatment samples from the treated human population and determining the prevalence of the disease at these time points. Where a larger population is involved, a representative sample is collected, and estimation is derived for the entire group. The frequent collection of samples from humans can be quite challenging on various levels as listed

previously in Chapter 1.2 (c). In high transmission areas, the diagnostic tool for monitoring and evaluation is still the detection of parasitic eggs in stool or urine via microscope, however, there is a call for a more sensitive diagnostic tool for disease surveillance in low transmission (Abdel-Fattah et al., 2011; Ajibola et al., 2018; Camprubi-Ferrer et al., 2021). At the national level, monitoring and evaluation of MDA preclude the impact of PZQ MDA on the changes in antibody responses at the population level for most countries (Abou-El-Naga, 2018; Albonico et al., 2015; Boateng et al., 2023; Exum et al., 2019).

Cercariae infection in susceptible snail intermediate hosts has been used to study transmission dynamics of schistosomiasis in several endemic countries, however, when it comes to monitoring and evaluation of mass drug administration programs, snail xenomonitoring remains untouched. In this study, the possibility of using xenomonitoring to monitor and evaluate the effectiveness or otherwise of communitywide MDA was exploited.

Xenomonitoring involves the monitoring of incidence in vector hosts, in this case, snail intermediate hosts. The study was conducted in collaboration with colleagues at the Noguchi Memorial Institute for Medical Research, University of Ghana. The field data collection and primary experiments had been performed between 2017 and 2019 before my involvement.

METHODOLOGY

Characteristics of the study location

The study was conducted in three townships [Tomefa (TOM), Manheam (MHM) and Toghakorpe-Adakope (TGP_ADP)] in the Ga South Municipal District, a peri-urban setting about 17 km west of Accra, all in the district capital Weija. All these townships share a border with the Weija Lake (dam), a manmade reservoir on the river Densu (Cunningham et al., 2020). There is no health facility in these three neighboring communities and the closest health center is located approximately 10 km away. There are also no government schools in these communities, private organizations provide basic education in these communities (Campbell et al., 2018). The majority of inhabitants of the three communities are of the Ewe and Ga-Adamgbes ethnic origin. The main economic activity for men in these communities is fishing and with that associated business involving women as well.

Study design

A series of surveys involving snail collections incorporating all human water contact points in these localities were carried out pre- and post-community wide MDA with PZQ. The MDA was executed by the National Neglected Tropical Disease (NTD) Control program in conjunction with the district health directorate. A total of six snail sampling time points were conducted for eighteen months. The baseline collection was done before the first MDA in October 2017. The subsequent samples were collected at 1 month, 2 months, 6 months, 12 months, and 18 months after baseline collection and the first MDA. The 12^{th} month collections were done immediately after the second MDA whiles the 18^{th} month collection was done six months after the second MDA. Finally, the sampling was completed in March 2019. A total of 31 human water contact points bordering each of the study areas along the lake were identified through interviews of participants and community leaders as well as observations by field research staff. Bathing, wadding to board canoes, fishing, fetching water, and washing were some activities observed at the various contact points.

Collection of snails and their screening for trematode infections

Hand-held scooping devices were used to scrape the bottom of the riverbank and surrounding vegetation. Several scoops of material were collected into bowls and all snails present were carefully picked out (Amoah et al., 2017). Snails were also handpicked around the shores of the water, parked canoes, water plants, or underneath other floating items (broken branches, plastics, or water plants). Collected snails were washed clean of any debris, placed in containers or beakers filled with some of the river water, and covered. Geographical coordinates for all the collection sites were recorded using a global positioning system (GPS) device. The snails were transported to the parasitology laboratory of Noguchi Memorial Institute for Medical Research (NMIMR), identified by shell morphology to the genera level (David, 1994), transferred into labeled aquaria, and counted.

After 24 hours in the aquaria, snails were singly placed in wells of a 24-well plate, each containing 1 ml of dechlorinated water. The plates were then placed under an artificial source of light for 2hrs to stimulate maximum cercarial shedding. Subsequently, snails with their water pools were observed by 4x and 10x objectives of a CK30-F200 inverted microscope to record and determine cercariae based on the morphological indicators, swimming behavior, and resting position

(Faltynkova et al., 2007; Frandsen & Christensen, 1984). Snails that shed no cercariae were returned to their respective aquaria containing dechlorinated water and placed in a dark environment. Light exposure of non-shedding snails was repeated once a week for 5 weeks period to allow enough time for snails with pre-patent infection at sample collection time to begin cercariae shedding. Snails that did not shed cercariae over the five weeks were excluded from further analysis. For further analyses, cercariae shedding snails with the pool of cercariae were each kept in 2 ml Eppendorf tubes and kept frozen at -20 ^{o}C.

Molecular detection of schistosome cercariae

Primer selection and design: To evaluate the presence of *Schistosoma* species, each pool of cercariae and snails was subjected to molecular analysis. Genus' primers targeting the second internal transcribed spacer *(*ITS2*)* region of the ribosomal *gene* complex (rDNA) for schistosomes were designed to amplify schistosome cercariae within the population at the genus level while species-specific primers targeting the cytochrome c oxidase subunit 1 (*cox1*) gene of the mitochondrial genome for *Schistosoma* species were designed to amplify specifically *Schistosoma mansoni*, *Schistosoma bovis* and *Schistosoma haematobium*.

For the genus primer (SCH_ITS) design, ITS2 gene sequences for *S haematobium, S. mansoni, S. bovis, Schistosoma guineensis, Schistosoma curassoni, Schistosoma rodhani* and *Schistosoma matheei* with NCBI GenBank accession numbers MG554667.1, MF776596.1, MF776589.1, OX103898.1, MT580947.1, AF531312.1 and MW046871.1, respectively, were aligned to derive a consensus sequence using MULTALIN online tool (Corpet, 1988). For the species-specific primers, COX 1 gene sequences for *S. haematobium* (GenBank accession KT354661.1), *S. mansoni* (KX011043.1), *S. bovis* (AY157212.1) and COX 1 primers sequences for *S. haematobium* and *S. bovis* described by (Sady et al., 2015; Tuffour et al., 2018) were aligned to form a consensus sequence for primer design. Afterward, Oligo Explorer 1.4 (Gene Link Inc., USA) program was used to design primers sets (**Table 1**) from the consensus sequences.

The primers were analyzed by BLAST using NCBI primer blast tool (Rawlings & Morton, 2008; Ye et al., 2012) to check their specificity to their target *Schistosoma* spp. SnapGene v 3.2.1 (GSL Biotech LLC, USA) was used to check the region of annealing for each primer designed. The fragment sizes were then confirmed from the Oligo Explorer 1.4 (Gene Link Inc., USA). All

primers were synthesized by (Inqaba Biotec, W/A). The primer sets after synthesis were tested through serial dilution of the various primer sets and the template DNAs until ideal dilution factors were determined.

DNA extraction: DNA of schistosomes was extracted from each cercariae sample using Quick DNA Plus kits (Zymo Research) according to the manufacturer's protocol with little modification during sample preparation (Grube, 2005). The suspension was centrifuged at 1600 RPM for 5 minutes to concentrate the cercariae. The supernatant was then pipetted off before adding 200µl of TE buffer to the cercarial pellet. Afterward, samples were vortexed and further lysed with MagNA Lyser instrument (Hoffman-La Roche Ltd.) to increase DNA yield. The mixture was lyzed by buffer (Zymo Research), DNA washing and elution following the manufacturer's protocol. The concentration of DNA was determined by nanodrop.

Amplification by polymerase chain reaction (PCR): Single PCR was performed for each primer set (**Table 1**) on each of the extracted DNA in a total reaction volume of 10µl comprising 1x OneTaq master mix [$MgCl_2$, OneTaq DNA polymerase, and dNTPs (New England Biolabs Inc., UK)], 200 nM each of forward primer and reverse primer and 3 µl of genomic DNA template. Each reaction was done with a known adult worm DNA of each *Schistosoma* species (BEI Resources, USA) as a positive control. Thermal cycling was performed as previously described by (Pennance et al., 2020) with some modification. The PCR conditions were 3 min denaturing at 94^{o}C, 45 cycles of 45 seconds at 94^{o}C, 45 seconds at 72^{o}C; followed by a final extension period of 5 min at 72^{o}C. Fragment sizes of each PCR product (amplicon) were gel verified and then visualized under UV light and images were captured with VILBER Smart Imaging system.

Data analysis

Data were entered into Microsoft Excel and analyzed using Statistical Package for Social Sciences version 18.0 (IBM, USA). The abundance of snail species was reported as frequencies and proportions. The prevalence of snail infection with schistosome cercariae and non-schistosome cercariae was reported as the ratio of infected snails to the total population of snails within the subgroup. Variations in infection prevalence were evaluated using One-Way ANOVA with multiple comparisons using Bonferroni Method. Statistical significance was set at $p < 0.05$.

Table 1. Primers sequence for molecular identification of schistosome cercariae

	Primer Sequence	**Size (bp)**	**Annealing Temperature**
Schistosoma spp.	Forward: TCT TGA CCG GGG TAC CTA Reverse: ATT AAG CCA CGA CTC GAG CAC	691	60.1°C
S. mansoni (Sm)	Forward: GAG GGG TCT GGT TTT GGT GT Reverse: GCA GAT AAA GCC ACC CCT GT	659	58.7°C
S. haematobium (Sh)	Forward: TTG AGC CTAT GGG TGG TGG T Reverse: ACC AGT AAC ACC ACC TAT CGT	410	58.7°C
S. bovis (Sb)	Forward: TGG GCA TCC TGA GGT GTA T Reverse: CAC AGG ATC AGA CAA ACG AGT ACC	301	55.6°C

RESULTS AND DISCUSSION

Presence and distribution of freshwater snails

A total of 4223 live snail specimens were collected from all contact points throughout the survey period. All snails were classified by shell morphology (Figure 4) into 4 genera comprising 1644 *Bulinus* spp., 557 *Biomphalaria* spp., 958 *Physa* spp., and 1064 *Melaniodes* spp. All four snail genera were present at all collection sites and across all survey time points. Stratified by timepoint, a large percentage (36.8%, n=1556) of snails were collected at baseline, followed by the collection 6 months after baseline collection (20.2%, n=853) (where n = number of snails) and the least number of snails have been collected 12 months post-baseline collection.

The transmission of the disease depends strongly on the snail intermediate host. Several studies report that transmission of schistosomiasis in Ghana is facilitated by *Biomphalaria pfeifferi* and *Bulinus globosus and Bulinus truncatus* aquatic snails (Anyan et al., 2019; Aryeetey et al., 2013; Mc, 1959; McCullough, 1956; Yirenya-Tawiah et al., 2011). The use of both, *Biomphalaria* and *Bulinus* snails but not snails of any other abundant genera present in Ghana have been confirmed also in the present study that aimed to evaluate the potential of the snail vector investigation for monitoring human infection. *Biomphalaria* spp., *Bulinus* spp., *Physa* spp., and *Melanoides* spp. snails were investigated for schistosome transmission but only *Bulinus* spp and *Biomphalaria* spp vectors were identified as intermediate hosts of schistosomes in the studied area. *Bulinus* spp. snails were found in large numbers across all human water contact points and time points whereas *Biomphalaria* spp. was sparsely distributed agreeing with studies by (Amoah et al., 2017). The high occurrence of the intermediate host snails coupled with the intense human activities that occur at the water contact sites present an ideal situation for the active transmission of schistosomes to human hosts as reported previously by (Anyan et al., 2019; Cunningham et al., 2020; Nyarko et al., 2018).

Figure 1. Morphological characterization of snails collected during the survey. Snails collected were differentiated into 4 main genera based on their shell morphological characteristics (A) *Bulinus* spp., (B) *Biomphalaria* spp., (C) *Melanoides* spp., *and* (D) *Physa* spp.

Shedding and characterization of cercariae from snail intermediate hosts

Seven cercarial morphotypes were identified in this study; *Monostoma* spp. (MON), *Megalurous* spp. (MEG), *Echinostoma* spp. (ECH), Lophocercous apharyngeate (LAP), Longifurcate pharyngeate distome (LPD) and Bifurcate-apharyngeate distome (Schistosomes "SCH") (BAD). An overall cercarial infection prevalence of 6.84% was recorded for all snails sampled. No single snail was found to shed more than one cercariae morphotype. Most prevalent among these cercarial morphotypes was MEG (32.9%) shed by all 4 genera of collected snails. Cercariae of MON and AXI morphotypes were recorded in *Bulinus* spp., *Biomphalaria* spp. and *Physa* spp., BAD (SCH) and LPD were found in *Bulinus* spp. and *Biomphalaria* spp. only, while ECH and LAP were exclusively found in *Bulinus* spp. (**Figure 2**). A total of 71 snails were identified as shedding schistosome cercariae (BAD morphotype) comprising 39 (54.93%) *Biomphalaria* spp. and 32 (45.07%) *Bulinus* spp. snails.

Molecular identification of schistosome cercariae

DNA analyses of cercariae from all 289 shedding snails confirmed that 77 (26.6%) snails (*Bulinus* spp. and *Biomphalaria* spp. only) were infected with *Schistosoma* spp. (identified at the genus level), with 61 of these identified as either *S. mansoni* (49.2%; n = 30), *S. haematobium* (18.0%; n = 11) or *S. bovis* (13.1%; n = 8) infection, together with 19.7% (n = 12) of snails had *S. bovis* and *S. haematobium* co-infection. (**Table 2**). The molecular analysis showed the infection in 16 snails did not belong to any of the species that were identified. Hence these schistosome cercariae may belong to other species not yet identified in Ghana. The observation sets the stage to undertake elaborate molecular characterization and identification of schistosomes infecting animals, humans, and snail intermediate hosts in Ghana apart from *S. mansoni*, *S. haematobium*, and *S. bovis*

The study confirms that *Bulinus* spp. and *Biomphalaria* spp. are hosts to different types of cercariae (Kaukas et al., 1994; Pennance et al., 2020) and the only snail intermediate hosts for Schistosoma species in this study locality. Despite the presence of mixed infection in both snail intermediate hosts of *Schistosoma* spp, *Bulinus* spp. was not found to be infected by *S. mansoni* neither did any of the *Biomphalaria* spp. snails harbor *S. bovis* nor *S. haematobium* infections, confirming the intermediate host specificity to *Schistosoma* spp. (Allan et al., 2017; Campbell et al., 2010; Hailegebriel et al., 2020; Pennance et al., 2020). This could be due to complex intra- molluscan mechanisms such as the antagonism, or induced immunoregulation and adaptive immunity of the snail during multiple schistosome infection challenges that may inhibit the occurrence of this co-infection (Pennance et al., 2020)

Snails of *Bulinus* spp. had the highest number of diverse cercarial infections (7 types), followed by *Biomphalaria* spp. (5 types), *Melaniodes* spp. (3 types) and *Physa* spp. (1 type) (Figure 5). The prevalence of any cercarial infection in *Biomphalaria* spp. was (22.48%) followed by 6.33% prevalence in *Bulinus* spp. Despite the abundance of *Bulinus* spp. at the various human water contact points This finding indirectly corroborates the outcome of a previous study where there was a high prevalence of intestinal schistosomiasis of *S. mansoni* origin compared to urogenital schistosomiasis in humans in the same communities (Cunningham et al., 2020). The axiom supposes that there is a somewhat direct relationship between the infectivity of *Schistosoma* cercariae in snail intermediate hosts and the prevalence of schistosomiasis in humans as propounded earlier (Macdonald, 1965)

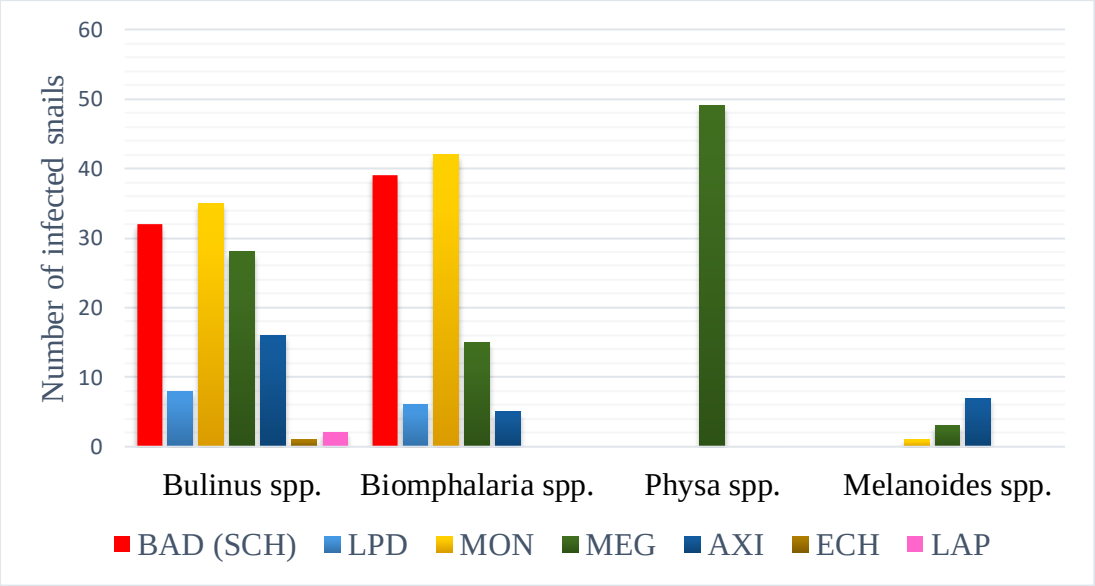

Figure 2. Distribution of cercariae based on morphology across the four genera of snails in the study area. *Bulinus* spp. harbored the largest diversity of schistosomes followed by *Biomphalaria* spp. *Melanoides* spp. was infected by only one type of cercariae. The highest number of schistosome cercariae was found in *Biomphalaria* spp.

Comparing the production of SCH by both *Bulinus* spp. and *Biomphalaria* spp. at particular survey time points based on molecular identification, prevalence decreased from 3.99% at baseline to 3.17% 1 month after treatment and to 0.91% 2 months post-baseline but suddenly increased to 5.46 % at collection performed 6 months post-baseline. In snails collected 12 months post-baseline, prevalence of SCH was 8.33% which declined significantly to 1.03% at 18 months post-baseline (6 months post 2nd MDA) (**Figure 3** and **Table 2**). This demonstrates that multiple MDA will result in a significant decline in infection of schistosomes in snails and help interrupt transmission in the long term compared to a one-time MDA.

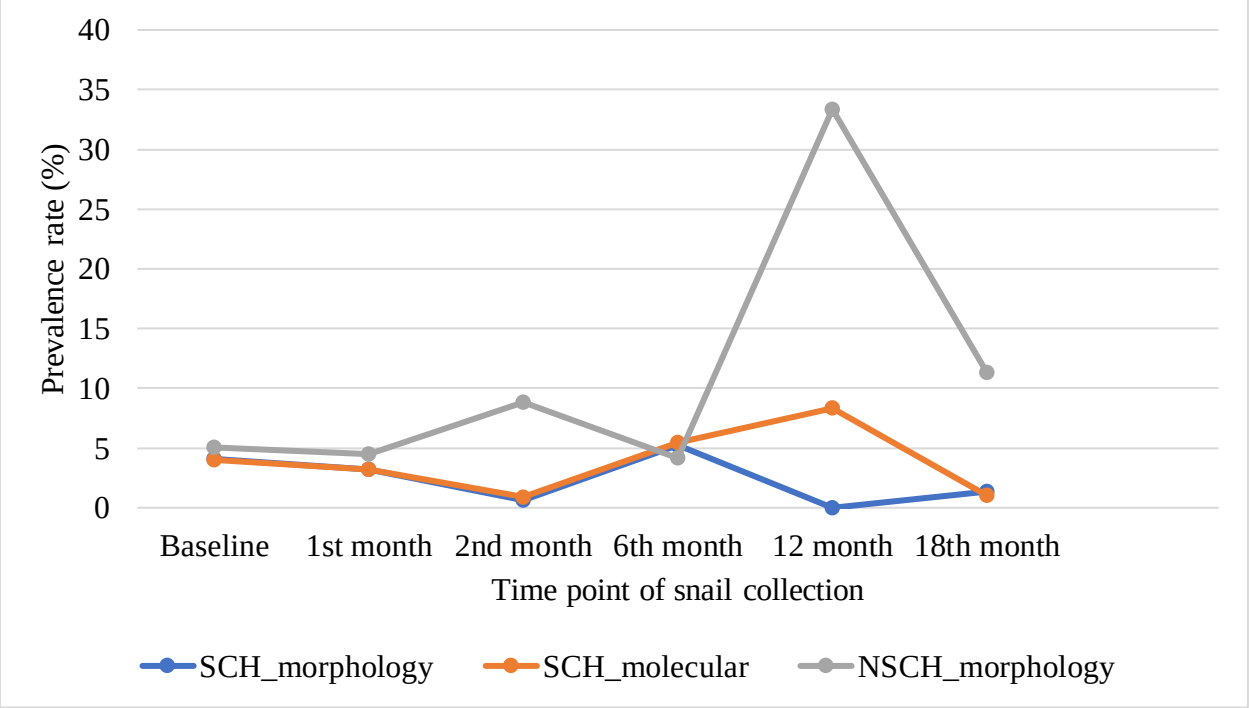

Figure 3: Trend of SCH prevalence by morphotype and molecular analysis and prevalence of NSCH by morphotype in *Bulinus* and *Boimphalaria* spp. snails across all study time points. The prevalence of SCH by morphological and molecular analysis and the prevalence of NSCH by the morphological analysis were almost the same at baseline prior to community MDA. The prevalence then declines insignificantly for all variables at one-month post-MDA. At 2 months post-MDA, SCH prevalence generally declines to a significant 0.91% for molecular analysis and 0.61% for morphological analysis while NSCH prevalence increases significantly to 8.79%. The prevalence of SCH at 6 months post MDA altogether saw an appreciable increase 5.46% compared to the baseline prevalence. While SCH prevalence appreciates, there is a steady decline in the prevalence of NSCH to a 1.97% at 6 months post-MDA. On the 12th month post-MDA, morphological analysis revealed that there was no infection of SCH in susceptible snails while molecular analysis showed a significant increase in the prevalence of SCH from a baseline value of 3.99% to 8.33%. NSCH at 12th month time point had the highest prevalence (33.37%) across all time points and all variables. Finally, at 18th month time point, SCH prevalence declines again to a significant 1.37% for morphotype and 1.03% for molecular identification.

Table 2. Distribution of schistosomes cercariae infection in snails based on morphology and molecular analysis.

	Number of snails and their infection rate at each timepoint (%)					
	Baseline	1 month	2 months	6 months	12 months	18 months
No of snails collected	1556	448	580	853	222	564
Number of snails shedding cercariae	86 (5.52)	18 (4.01)	32 (5.52)	43 (5.04)	25 (10.81)	85 (15.12)
Number of snails shedding SCH	34 (2.18)	7 (1.56)	2 (0.34)	24 (2.81)	0 (0.00)	4 (0.71)
Number of snails shedding NSCH	52	11	30	19	25	81
Number of Bi+Bu snails collected	827	221	330	458	74	291
Number of Bi+Bu snails shedding SCH (morphology)	34 (4.11)	7 (3.17)	2 (0.60)	24 (5.24)	0	4 (1.37)
Number of Bi+Bu snails shedding SCH (Molecular)	33 (3.99)	7 (3.17)	3 (0.91)	25 (5.46)	6 (8.33)	3 (1.03)
Number of Bi+Bu snails shedding NSCH	52 (6.29)	10 (4.52)	29 (8.79)	14 (3.06)	25 (33.78)	28 (11.68)

*Bu = *Bulinus* spp. and Bi = *Biomphalaria* spp.

The highest number of snails and their infections were observed at baseline collections for both schistosomes infected and infected snails by other trematode species. One-month post-MDA, there was a slight decline in the prevalence of both SCH and NSCH which is probably influenced by a reduced number of snails collected and disturbance of snail habitat during baseline collection. Since the decline in prevalence at one month is not unique to only SCH, we cannot attribute it to the effect of MDA alone. Besides, infection in snails requires at least 4 weeks to manifest in the intermediate host as snails with pre-patent infection at baseline but was not captured during sample collection will start shedding cercariae after 4 weeks (Mattos et al., 2007). Generally, the number of infected snails declined as the total number of snails collected declined with a continuous collection of snails at various time points, however, this decline is not proportional. This observation suggests that snail collection/control can significantly contribute to the control of transmission of schistosomiasis in endemic communities as suggested by WHO and confirmed by various studies (WHO, 2023).

To find out whether the decline in the prevalence of schistosome cercariae has been influenced by the community-wide MDA, we assumed a linear relationship between snail infection and human infectivity (Macdonald, 1965; Tuljapurkar, 1991). We compared the prevalence of SCH and NSCH (positive control since they are not targeted by the MDA) at various time points using a common denominator (combined number of *Bulinus* spp. and *Biomphalaria* spp. collected per time point and their infections by SCH and NSCH). Two months after the MDA, the prevalence of SCH declines significantly in contrast to NSCH, however, this decline was not sustained for 6 months and 12 months after the first treatment. observed decline in prevalence is likely influenced by the MDA campaign. The observed phenomenon can be attributed to PZQ being a target of schistosome species, killing a large portion of adult parasites (Lamberton et al., 2017; Rollinson et al., 2013; Vale et al., 2017), resulting in a reduction in egg production in human hosts and subsequently reduces miracidia infection in snail intermediate hosts. Consequently, the community-wide MDA had a short-term effect in reducing the prevalence of schistosomes in snail intermediate hosts. The observed fluctuations in the prevalence of SCH over the time after 1st and 2nd MDAs suggest that xenomonitoring of schistosomes snail intermediate host using molecular analysis has demonstrated the potential of being a tool for indirect assessment and evaluation of the efficacy of MDA campaigns in schistosomiasis endemic communities. However, a parallel study in both snails and humans from different study localities will be necessary to validate this method as a sole tool for monitoring and evaluating schistosomiasis community-wide MDA campaigns.

Conclusion

This pivotal study presents the potential of xenomononitoring of schistosomes snail intermediate as a tool for monitoring and evaluating schistosomiasis MDA programs however comes with several limitations such as a) inability to identify snails to species level, b) undefined sampling strategy which might account for the wide variability in the number of snails collected per timepoints thereby introducing biases in the prevalence of cercariae and c) lack of ecological data at the time of sampling which may influence availability and viability if snail population and help explain some observed trends. d) Lack of data on human water contact activities post-MDA which could be helpful to identify the determinants of cercariae prevalence. Despite the numerous drawbacks, the study still presents some insightful and interesting preliminary data such as the longevity of PZQ MDA on the prevalence of infection in the snail intermediate host, the interplay of schistosome and non-schistosome trematodes in the same ecological niche (competing for the same intermediate host), the diversity of snail intermediate hosts and their respective cercariae types which can serve as a basis for a comprehensive study to elicit the use of snail xenomonitoring to monitor, assess, and evaluate schistosomiasis MDA programs in endemic countries.

5.0 STUDY 3 – Appendix 2

Title: Biological characterization of *S. mansoni* ortholog of human glutamate carboxypeptidases 2

OVERVIEW: **This study forms part of the published article named "Characterization of trematodes ortholog of human GCP2" in a 1st quartile peer review journal, "Parasites and Vectors" of impact factor 4.052** Jedlickova, L, Peterkova, K, Boateng, E. M., Ulrychova, L., Vacek, V., Kutil, Z., Jiang, Z., Novakova, Z., Snajdr, I., Kim, J., O'Donoghue, A. J., Barinka C., & Dvorak J. This article has been attached as appendix 2 of this thesis.

The use of peptidases by parasites to interact with hosts makes peptidases one of the main foci in basic research as therapeutic/vaccine targets. In the case of schistosomiasis, discussion on alternative drugs and vaccines to complement the existing chemotherapy is far advanced as such investigation of GCP2 which is universally expressed across all phyla to elucidate its biological function and as a potential drug or vaccine candidate is not farfetched. GCP2 is the most well-studied member of the subfamily M28B metallopeptidase with a well-defined three-dimensional structure and partially identified functions. GCP2 was first discovered in 1987 under different names concurrently by different groups of researchers in two completely unrelated research areas. A group of researchers in neurological study found N-acetylated alpha-linked acidic dipeptidase (NAALADase), responsible for hydrolysis of neurotransmitter N-acetyl-L-aspartyl-L-glutamate (NAAG) in the brain of rat (Robinson et al., 1987). In an oncological study, (Horoszewicz et al., 1987), produced monoclonal antibody (7E11-C5.3) against a new marker of epithelial prostatic cancer cell line LNCaP (lymph node carcinoma of the prostate) called prostate-specific membrane antigen (PSMA). Four years after, a folate hydrolase (FOLH) was also found in the jejunum of a pig. This enzyme was found to play a role in the hydrolysis and absorption of folate from the diet (Halsted, 1991).

For five years, this enzyme existed independently under the above-mentioned names owing to the unrelated fields of study without the realization that it was a single protein entity. The discrepancies surrounding the nomenclature began to wane when Pinto *et al*, showed that PSMA possesses folate hydrolase activity (Pinto et al., 1996) and that it also embodies the characteristics of neuropeptides (Carter et al., 1996). An analysis of the protein in 1998 initiated the need for a unified name for the single protein that has existed under 3 different names for over a decade (Luthi-Carter et al., 1998). Owing to their similar biochemical properties, the name "glutamate carboxypeptidase 2" was suggested by (Halsted et al., 1998; Luthi-Carter et

al., 1998) which was later adopted by the International Union of Biochemistry and Molecular Biology (IUBMB). Nonetheless, the names PSMA and NAALADase still appear in various studies till now.

GCP2 consists of 750 amino acids with a short intracellular N-terminal part (AAs 1-18), one transmembrane helix (AAs 19-43) with an extracellular C-terminal part composed of 707 amino acids (AAs 44-750) (Mesters et al., 2006). GCP2 contains 10 N-glycosylation motifs (Asn-X-Ser/Thr) within its primary sequence and these post-translational modifications were shown to be necessary for GCP2 enzymatic activity (Barinka, Mlcochova, et al., 2004; Ghosh & Heston, 2003). A properly modified GCP2 molecule embodies a molecular weight of approximately 100 kDa while the protein without posttranslational modifications has a molecular weight of 84 kDa (Israeli et al., 1994).

GCP2 cleaves off C-terminal glutamate from its substrate. In mammals, the enzymatic activity of GCP2 is manifested in 1) the cleavage of N-acetyl-L-aspartyl-L-glutamate (NAAG), the most abundant peptide neurotransmitter in the human brain (Neale et al., 2000), yielding N-acetyl-L-aspartate and L-glutamate (Robinson et al., 1987) and 2) the hydrolysis of polyglutamate found in diet to a form that can be absorbed and used by mammals. The consecutive cleaving of polyglutamates and the absorption of folate in the small intestine by the epithelial cells is facilitated by the presence of GCP2 located at the jejunal brush border of mammals (Halsted, 1991; Horoszewicz et al., 1987). Besides these two endogenous substrates, GCP2 can process other peptide substrates. Barinka *et al*., showed that GCPII efficiently processes N-acetylated dipeptides which contain acidic residues (Glu/Asp) in P1 position and or methionine in P1’ position (e.g., N-acetyl-L-glutamyl-L-glutamate or N-acetyl-L-aspartyl-L-methionine) (Barinka et al., 2002).

Regarding the MEROP peptidase database, other peptidases and homologs of M28B subfamily are altered meristem program 1-like peptidase (M28.007), NAALADASE L peptidase (M28.011), glutamate carboxypeptidase 3 (GCP3) (M28.012), transferrin receptor protein (M28.972), transferrin receptor 2 protein (M28.973), At5g19740 (Arabidopsis thaliana)-type peptidase (M28.A02), C35C5.2g.p *Caenorhabditis elegans* (M28.A18), and R57.1g.p of *C. elegans* (M28.A19) (Rawlings et al., 2018). On the other hand, the gene encoding SmM28B protein, (naaladasel) is found in MEROP database, but nothing is known about the protein expression, localization, and biological functions of the protein in *S. mansoni*. Thus, unveiling the biological role of SmM28B will give a firsthand idea for any further translational research.

Expression and localization of mammalian/human GCP2

Since its discovery, several studies have been conducted to identify biological and physiological functions of GCP2 mostly in higher organisms (Bzdega et al., 2004; Chandler et al., 1991; Knedlik et al., 2017; Rovenska et al., 2008). Methods such as protein expression, localization of mRNA transcript, gene knockout, and immunohistochemistry have been used to reveal the function partially and to identify expression sites of GCP2 (Cao et al., 2007; Cunha et al., 2006; Gala et al., 2000; Haffner et al., 2012; Halsted et al., 1998; Kinoshita et al., 2006; Knedlik et al., 2017). Prostate tissues emerged to have a significantly higher level of GCPII in comparison to other studied tissues and organs (Cunha et al., 2006; Pangalos et al., 1999a; Renneberg et al., 1999). (Chang et al., 1999) posited that GCP2 mRNA expression is induced in malignant non-prostatic tissues such as the bladder, lung, or pancreas. However, a subsequent study by (Pangalos et al., 1999a), detected mRNA expression in a variety of human tissues including brain, colon, heart, kidneys, lungs, ovary, pancreas, spleen, small intestine, and testis. These data suggest that the GCP2 mRNA expression is widespread in humans, but it may be further induced by the malignant processes within the cell.

In humans, GCP2 is mainly expressed in organs and systems such as the nervous system (astrocytes and Schwann cells), jejunal brush membranes, and kidney. Lacrimal glands, heart, pancreas, bladder, skin, breast, liver, lung, colon, and testis have recorded low levels of GCP2 expression (Rovenska et al., 2008; Silver et al., 1997). The absence of GCP2 activities in knockout mice is compensated for by GCP2, thus, hindering the study of GCP2-specific functions in the brain and other tissues (Bacich et al., 2002; Hlouchova et al., 2007).

Despite the extensive studies of GCP2 expression in human tissues, except for a high protein expression in both benign and malignant prostate tissue, the data on GCP expression in other human tissues are somewhat inconsistent (Kinoshita et al., 2006; Troyer et al., 1995). The explanation for the discrepancies may lie in a variety of detection methods used in these studies. Different methods have variable detection limits, and their results also often rely on the experience of the researcher. Moreover, different mAbs recognize distinct epitopes (e.g., within intracellular or extracellular portion of GCP2) and may bind to its antigen in different forms (e.g., native, or denatured form of GCP2) (Gala et al., 2000; Lopes et al., 1990; Silver et al., 1997).

This study explored the putative biological functions of *S. mansoni* ortholog of human GCP2 (SmM28B) using gene expression, immunolocalization, carboxypeptidase/aminopeptidase activities using synthetic library, and gene silencing techniques. It was revealed that SmM28B was expressed across all developmental stages of the parasite with highest expression percentage in schistosomula. The peptidase was localized to mainly the digestive and reproductive organs of both female and male adult worms. In vitro RNAi-mediated gene silencing on schistosomula revealed about 92% success, however, when knockdown worms were introduced into mice hosts, there were no significant phenotypic manifestations. No substrate activity was identified for SmM28B.

The localization of SmM28B in adult worms is similar to that of human GCP2 in functionally similar organs and might play similar digestion and reproduction functions in *S. mansoni* as in humans or mammals. The study thus sets the stage for further investigation into the likely pharmacological importance of SmM28B and its theoretical potential in treating and controlling schistosomiasis.

METHODOLOGY

Identification of *S. mansoni* ortholog (SmM28B) of GCP2

This aspect of my thesis forms part of a bigger project by my supervisor to characterize helminths orthologs of human GCP2, particularly in trematodes (*Fasciola hepatica* and *S. mansoni*) and roundworm model (*Caenorhabditis elegans*). Before the study, the single gene coding M28B metallopeptidases was identified in *S. mansoni* based on the sequence similarities from originally annotated sequence submission (SmM28B) as *S. mansoni* NAALADase L peptidase (GenBank XP_018651911). The open reading frame was PCR amplified, cloned in *E. coli* bacteria, and verified by Sanger sequencing.

Acquisition of parasite materials

The life cycle of a Puerto Rican strain of *S. mansoni* was maintained in the intermediate host snail (*B. glabrata*) and the final host ICR (CD -1) mice as previously described (Dvorak et al., 2016; Leontovyc et al., 2018). After being infected with miracidia for 4 weeks, the snails were exposed to light for approximately 1 h to release the cercariae in a beaker of pure water. The mice were exposed to 50 ml of water containing approximately 200 cercariae. Six weeks after infection, the mice were sacrificed by perfusion and the adult worms were collected. Worms

were separated into male and female and stored at -80 °C for later processing. Schistosomula were prepared *in vitro* from cercariae and cultured in RPMI 1640 according to previously published standard protocols (Basch, 1981; Horn, Fajtova, Arreola, et al., 2014; Stefanic et al., 2010). Briefly, cercariae were washed three times in RPMI 1640 and mechanically transformed using a 22-G needle and syringe by aspirating and releasing the cercariae several times in RMPI 1640 containing 5% fetal bovine albumin (FBS). Newly transformed schistosomula (NTS) were collected into Eppendorf tubes using a light microscope. The NTS were washed several times in Basch medium and then incubated for 1 hour at 37 °C and 5% CO_2 in Basch medium containing antibiotics (Penicillin and streptomycin) in a 1:100 dilution factor and fungizone. Incubation was followed by several washes to remove fungizone prior to 5 days of incubation of schistosomula. Approximately, 300 NTS were dispensed into each well and 200 µl of complete medium (Basch + 1% penicillin +1% streptomycin + 5% FBS) was added to each well to bring the final volume to approximately 200 ml. The culture was kept in an incubator at a constant temperature of 37 °C and 5% CO_2 for 5 days and regularly checked for any contamination. When necessary, the additional culture medium was added to respective wells.

Sample fixation, embedding, and sectioning.

Part of the freshly collected male and female *S. mansoni* adults and mechanically transformed schistosomula were fixed as published previously (Ulrychova et al., 2020). All samples were fixed in Bouin solution by incubating for 90 and 30 minutes for adult worms and schistosomula respectively.

Fixed samples were further dehydrated in ethanol series of increasing ethanol concentrations 25%, 50%, 70%, 90%, 96%, 100% v/v ethanol for 5 min each (*S. mansoni*). A drop of Chromotrope 2R dye (Sigma-Aldrich, Germany) was added to 90% ethanol step for *S. mansoni* samples. Prior to embedment in paraffin *S. mansoni* adults, males were incubated in methyl benzoate (Sigma-Aldrich, Germany), for 45, and 20 min of incubation time for both *S. mansoni* adult female and schistosomula. Following incubation in methyl benzoate, the worms were washed twice for 5 min in benzene (Sigma-Aldrich). All tissues were finally embedded into hot paraffin- Paraplast (Sigma-Aldrich) at 60 °C and incubated at the same temperature for 1 h. A second and third incubations in hot paraffin were done at 12 and 1 hour respectively (Ulrychova et al., 2020). Semi-thin sections (6 µm) were cut using Shandon Finesse® ME+ (Thermo Fisher) and placed onto X-tra adhesive slides (Leica). Good-quality tissue sections were then selected under a light microscope and were later used for immunohistochemistry (IHC).

RNA isolation and cDNA synthesis.

RNA was isolated from 6 different life stages of *S. mansoni* comprising daughter sporocysts, cercariae, 5-day *in vitro* cultivated transformed schistosomula, adults, eggs, and miracidia. The aforementioned stages of *S. mansoni* were washed 3 times in PBS and then re-suspended in Trizol reagent (Thermo Fisher) and incubated overnight at 4 ^{o}C. Samples were briefly chilled on dry ice and stored at -80 ^{o}C until use or processed immediately. Samples were homogenized by a pestle tissue homogenizer while on ice. Where necessary, sample volumes were modified by addition of Trizol reagent and processed according to the manufacturer's instructions. Finally, precipitated RNA was air-dried and resuspended in DEPC-treated H_2O. Single-stranded cDNA was synthesized from total RNA using SuperScript IV reverse transcriptase (Thermo Fisher) and an oligo d(T)18 reverse primer according to the manufacturer's protocol. The resulting cDNA was purified using a QIAquick PCR purification kit (Qiagen) and stored in DEPC-H2O at -20 ^{o}C.

RT-qPCR analysis of *S. mansoni* gene expression

Analysis of *SmM28b* gene expression for 6 life stages was done through RT-qPCR to analyze the relative expression of *SmM28b* at various life stages. Specific primers were designed and selected for studied gene targets coding SmM28B, *S. mansoni* cathepsin B1.1 (SmCB1.1, GenBank AJ506157) and *S. mansoni* cytochrome C oxidase I (SmCOX I, GenBank AF216698) as the sample reference gene transcript (Table 2). Primer3 software (http://frodo.wi.mit.edu/ was used to design specific new primers for SmM28B target. Primers were evaluated by serial dilutions of both the primers and the cDNA template as described (Horn, Fajtova, Arreola, et al., 2014; Stefanic et al., 2010) while primers for SmCB1.1. and SmCOX I were adopted from previous studies (Horn, Fajtova, Arreola, et al., 2014; Stefanic et al., 2010) (Table 5). Reactions containing LightCycler 480 SYBR Green I Master (Roche) were prepared in final volumes of 25 mL in 96-well plates and carried out as described previously (Stefanic et al., 2010). PCR reactions were performed in triplicate with at least one biological replicate. Analysis using SmCOX I as the sample reference gene transcript was calculated as previously published (Horn, Fajtova, Arreola, et al., 2014; Stefanic et al., 2010) by the 2-CT method to measure transcript levels (Livak & Schmittgen, 2001). The whole experiment was repeated when CT values of technical replicates fluctuated by 0.5 or more. Finally, the resulting transcript levels were calculated as a percentage of given unspecific control (mCherry dsRNA for RNAi) or the highest transcript level that was set as 100%.

Table 1. Primers sets used for qPCR analysis.

Primer	Sequence	Use
M28B_SM_qPCRf	TCTTGGTTCTTGGGATGGAG	Forward primer
M28B_SM_qPCRr	TCGGTTCGAGCTGAAAAGTT	Reverse primer
CB1.1_SM_qPCRf	ACTTGGTGGGCACGGTATAC	Forward primer
CB1.1_SM_qPCRr	TAATTCGACCGGCTGTTACC	Reverse primer
COX1_SM_qPCRf	TACGGTTGGTGGTGTCACAG	Forward primer
COX1_SM_qPCRr	ACGGCCATCACCATACTAGC	Reverse primer

Cloning and expression of recombinant SmM28B

To express recombinant SmM28B (rSMM28B), Gateway cloning technology was used to clone plasmid made in the laboratory of Dr. Cyril Barinka (IBT, CAS, Biocev). Codon optimized sequence encoding full length of SmM28B (see primers in Table 6) was cloned to approximately 10 ng of pEC527 destination vector (Addgene plasmid 268 #11518) in frame with the TEV-cleavable hexahistidine tag. The destination vector was added to a thawed vial of BL21 (DE3) *E. coli* competent cells (Genscript) and incubated on ice for 30 minutes at 37 ºC. The mixture was heat-shocked for 30 seconds at 42 ºC and then transferred immediately onto ice. 250 µl of SOC at RT was added to the mixture which was incubated for 30 minutes with shaking at 37 ºC. The transformed cells were added to 10ml Lysogeny broth (LB) with 50 µg/ml of ampicillin and cultured overnight at 37 ºC.

The expression of SmM28B was induced by 1mM IPTG in a culture medium composed of [6L LB + ampicillin (50 µg/ml) + glucose (2 g/L) and incubated at 37 ºC until OD600 = 0.7 was obtained. The isolation was done by using lysis buffer of composition; [200 ml of 1x PBS, 200mg of lysozyme, 2 tablets of protease inhibitor cocktail, 60 µl of 1x benzonase and 80 µl of 1mM PMSF inhibitor (phenylmethyl sulfonyl fluoride)] for 30 minutes and sonicated on ice for 10 minutes. Several centrifugations and re-suspensions were done to obtain pure pellet. Re-suspension of pellet in buffer made up of (180 ml of ice-cold 1x PBS + 1.8 ml of 5mM EDTA + 900 µl of 5 mM DTT + 16.4 ml of 1M urea) was done followed by sonication on ice for 3 minutes. Content was again centrifuged at 5000 g for 10 minutes and supernatant discarded. Inclusion bodies was further dissolved in guanidine-based buffer composed of (30 ml of ice-cold 50mM phosphate, pH 8.0 + 6 M guanidine + 1mM DTT) then sonication. Centrifugation

of the mixture at 30000 g for 10 minutes was done successively. The supernatant was then collected for further purification.

Table 2. Primer sets for Gateway expression.

Primer	Sequence
SmM28B_GTWf	GAGAACCTGTACTTCCAGTCTAACATGTGGCAAGA AATATATCACAAAATTTG
SmM28B_GTWr	GGGGACCACTTTGTACAAGAAAGCTGGGTTATTAT AATGCTAATGAAAAATCAGTTAAAC

Purification of SmM28B

Purification of recombinant SmM28B started with disruption of the cell pellet by sonication in phosphate-buffered saline (PBS) and inclusion bodies (IBs) isolated by centrifugation at 5000 g for 10 min. Following the cell disruption, a further purification step involving the solubilization of IBs was ensured. The solubilization process of IBs involved serial sonication and sequential centrifugation after each sonication step in solubilization buffer (PBS + 1 M urea, PBS + 1 M NaCl, and PBS + 1% Triton X-100). Each centrifugation lasted 10 min at 5000 g. After the last centrifugation of the solubilization process, IBs were further dissolved in an equilibration buffer composed of (10M urea + 100 mM Tris-HCl at pH 8.0) and final centrifugation was done at 30000 g for 30 min. A Ni^{2+} charged chelating Sepharose column was equilibrated with equilibrated buffer (10 M urea + 100 mM Tris–HCl, pH 8.0) before loading supernatant obtained from final centrifugation. The protein (SmM28B) was finally eluted with equilibration buffer containing 200 mM imidazole The resultant protein was authenticated by SDS-PAGE. The pure protein fraction was later used to produce recombinant polyclonal antibody.

Antibody production and immunohistochemistry

The purified recombinant SmM28B was used to produce polyclonal antibodies in rabbit (New Zealand white) raised by the animal facility of the Institute of Microbiology at the Czech Academy of Sciences

Embedded worm samples were deparaffinized in Xylen (Sigma Aldrich, Czech Republic) and absolute ethanol and subsequently rehydrated in ethanol series of decreasing concentration (90%, 75%, 50%, and 30%) and finally in H_2O as previously (Ulrychova et al., 2020). Antigen retrieval was performed by incubating slides in sodium citrate of pH 6.0 for 10 minutes in a water bath at 100 ^oC (Collins et al., 2011; Ulrychova et al., 2020). Sections were washed 3 times in 1x PBS followed by permeabilization in freshly prepared PBS-Tx (1x PBS + 0.25% Triton X-100 + pH 7.5) for 20 min and subsequent overnight incubation in the blocking buffer (1x PBS + 2% BSA + 0.25% Triton X-100). Sections were washed once in antibody diluent (AbD) (1x PBS + 1%BSA + 0.25% Triton X-100) and subsequently probed with anti-SmM28B and pre-serum (control) at the dilution1:50 in antibody diluent. Slides were incubated overnight at 4 ^oC in the wet chamber. A control slide with no serum treatment to exclude non-specific reactions of secondary antibodies was also included. Following the overnight incubation, 3 washes in (PBS-Tx) for 10 min each, 45 minutes incubation with anti-mouse Alexa Fluor 647 secondary antibodies (Invitrogen) with dilution ratio 1:500 at 37 ^oC were done successively on the slides. Final steps involved 3 washes in PBS-Tx for 10 min each a 10 min wash in PBS, mounting in ProLong Diamond Antifade Mountant with DAPI (Invitrogen), and visualization of signals by Nikon Ni-E fluorescence microscope. Images were processed with imageJ software.

RNA interference mediated gene knockdown in *S. mansoni*

In vitro gene knockdown involved the synthesis of double stranded RNA (dsRNA), mechanical transformation of cercariae into schistosomula, treatment of NTS with respective dsRNA and cultivation of treated schistosomula. Briefly, dsRNA for SmM28B and *S. mansoni* cathepsin B1.1 (SmCB1.1, GenBank AJ506157) were prepared by PCR amplification of cDNA isolated from six-day old schistosomula by following previously established protocol (Stefanic et al., 2010). To check for unspecific reaction of dsRNA, DNA coding mCherry protein was used. Sizes of amplicons we around 500bp (Table 3). Following amplification, PCR template were gel-purified was used for synthesizing dsRNA using the T7 RiboMAX Express RNAi System (Promega) as outlined by manufacturer's protocol and (Stefanic et al., 2010). Here, the schistosomula used were mechanically transformed as described previous in chapter 3.2. Schistosomula were cultured in 96 wells plate with 7 wells replications for each treatment and control (i.e DNA coding mCherry, SmM28B dsRNA, and SmCB1.1 dsRNA). Each well was filled with 200 ml of Basch medium supplemented with 5% FBS with approximately 350 NTS per well. 30 ug/ml of respective dsRNA were added to each well and incubated for 5 days at a

temperature of 37 ºC and 5% CO_2 (Stefanic et al., 2010). Immunolocalization on harvested schistosomula and RT-qPCR were used to evaluate the effectivity of gene silencing on. For schistosomula samples used for immunolocalization, they were collected, washed thrice in PBS, and further processed as described in chapters 3.2 and 3.8. In the case of samples used for RT-qPCR analysis, after three washes in PBS, schistosomula were immersed in Trizol reagent (50 ml) for RNA isolation. The analysis of gene expression levels was done in accordance with previously established protocols (Horn, Fajtova, Arreola, et al., 2014; Stefanic et al., 2010).

Table 3. Primer sets used for dsRNA synthesis.

Target gene	Amplicon size	Primers	Sequence
mCherry	521	Cherry-T7-s	**TAATACGACTCACTATAGGG**AGTGGTGAGCAAAGGGCGAGGAG
		Cherry-as	TTACTTGTACAGCTCGTCC
		Cherry-s	ATGGTGAGCAAGGGCGAGGAG
		Cherry-T7-as	**TAATACGACTCACTATAGGG**TTACTTGTACAGCTCGTCC
SmCB1.1	526	SmCB1.1-T7-s	**AAGTAATACGACTCACTATATAGGG**ATGCTCACATCTATTTTGTGTATTAC
		SmCB1.1-as	CAATACCTTCCTTCACCCAGTAATC
		SmCB1.1-s	ATGCTCACATCTATTTTGTGTATTGC
		SmCB1.1-T7-as	**AAGTAATACGACTCACTATATAGGG**AATACCTTCCTTCACCCAGTAATC
SmM28B	483	SmM28B-T7-s	**TAATACGACTCACTATAGGG**TATCGTTTGGGGATTCCATCAACAT
		SmM28B-as	ATGATTTTTCACATTGACCAGCAGC
		SmM28B-s	TATCGTTTGGGGATTCCATCAACAT
		SmM28B-T7-as	**TAATACGACTCACTATAGGG**ATGATTTTTCACATTGACCAGCAGC

RESULTS AND DISCUSSIONS

Identification and expression of gene coding SmM28b in various stages of life cycle of *S. mansoni*

Gene expression level was determined via RT-qPCR in eggs, miracidia, sporocysts, cercariae, NTS, and adults using *smcoxi* as the reference gene and measured constitutively. The relative gene level was highest in NTS, followed by cercariae and adults. The least expression was observed in eggs. The difference in expression between NTS and adults was statistically significant (**Figure 1**)

Based on the sequence alignment in **Figure 2**, SmM28B shares 30% sequence similarity with hGCP2. Three domain organizations namely, protease domain, apical domain, and C-terminal domain was observed to be identical to all three proteins. The sequence alignment shows the absence of the intracellular and transmembrane SmM28B compared to hGCP2. This difference might indicate a different secretion route of the trematode orthologs. Finally, we see highly conserved catalytic glutamate residue and residues useful for coordinating zinc ions at the active site in SmM28B, an indicator of the potential enzymatic activity of the protein.

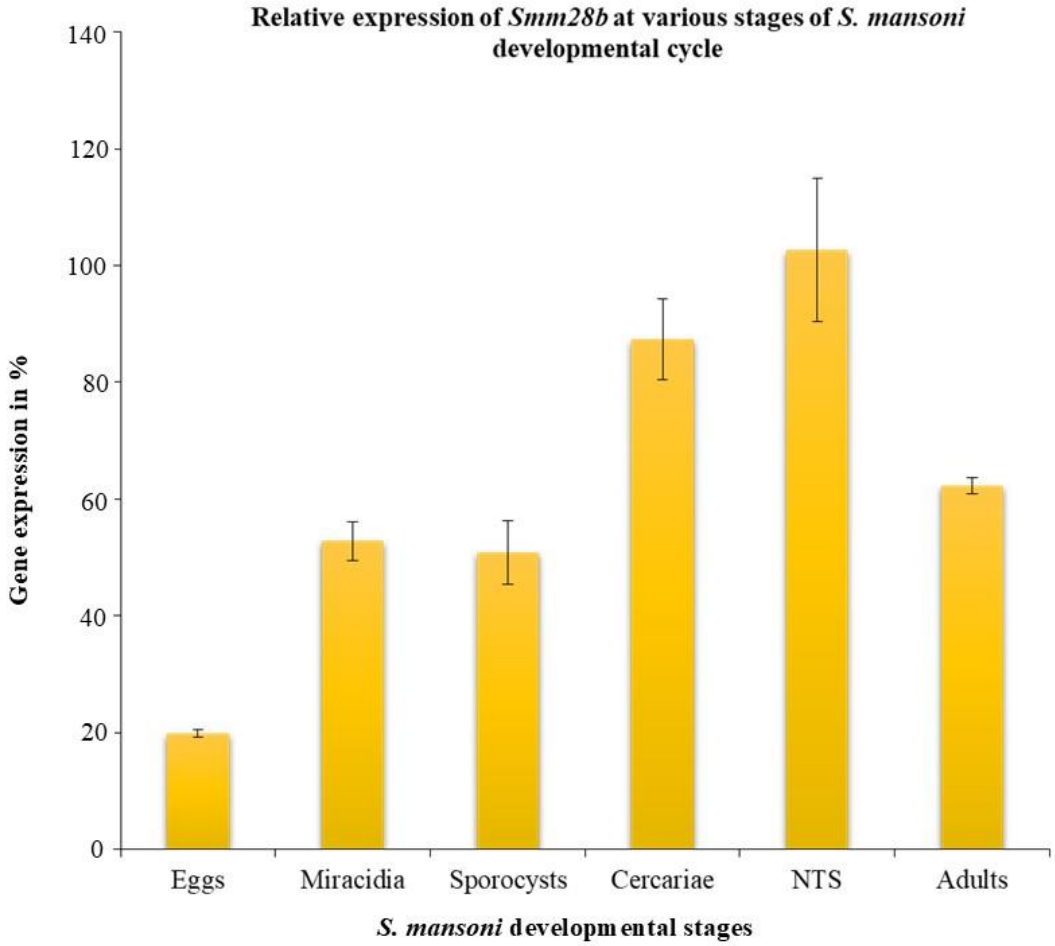

Figure 1 shows relative gene expression of *smM28b* in various life stages of *S. mansoni*. Newly transformed schistosomula (NTS) had the highest gene expression followed by cercariae. The least gene expressed was found in the eggs.

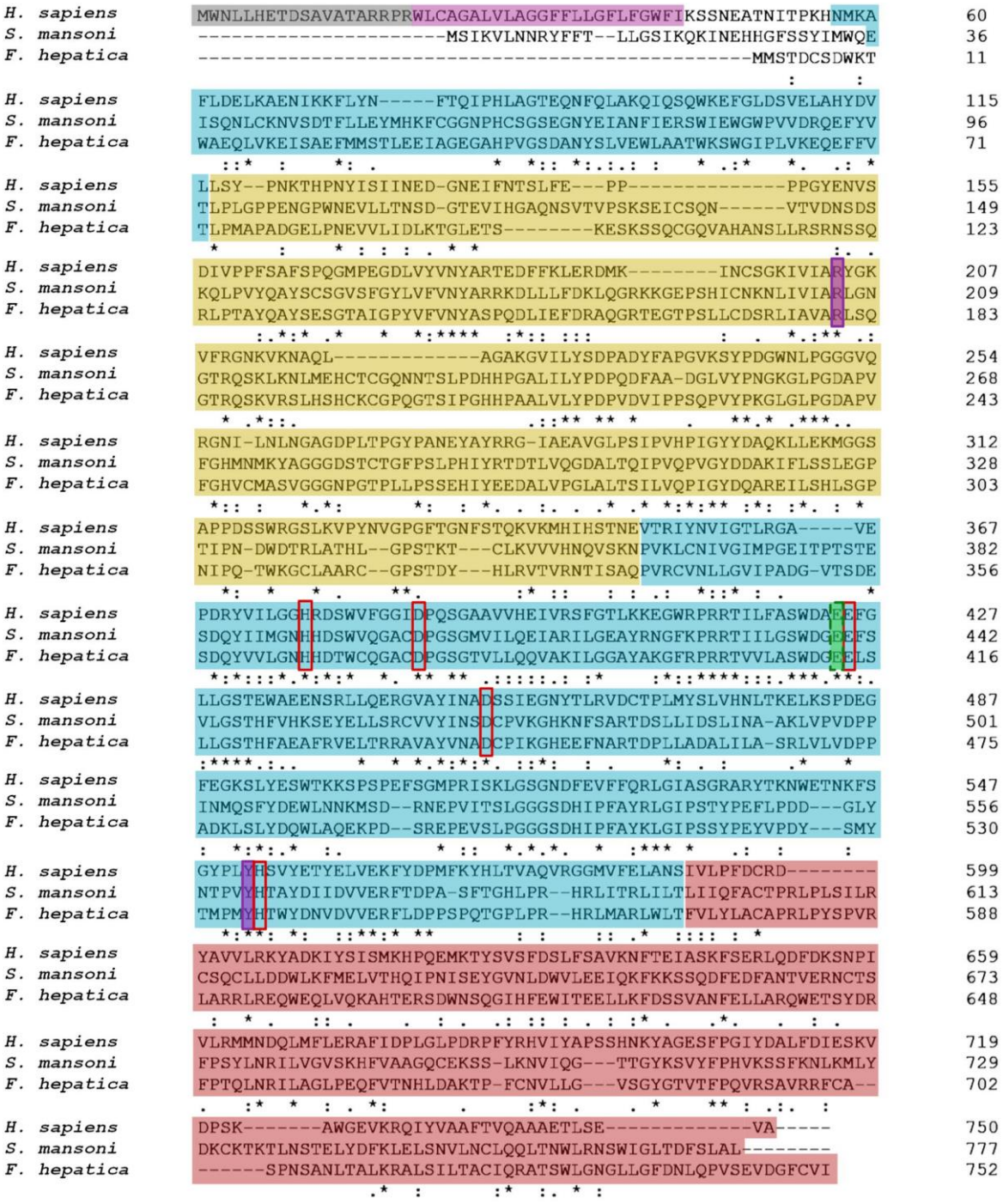

Figure 2. A consensus sequence alignment of human GCP2, *Fasciola hepatica* M28B (FhM28B), and SmM28B. The intracellular part and the transmembrane domain of human GCP2 are shaded *gray* and *magenta*, respectively. Protease-like (*blue*), apical (*yellow*), and C-terminal dimerization (*red*) domains are present. The catalytic acid/base glutamate is highlighted by the green background in the rectangular box. Residues coordinating zinc ions are marked by red rectangular boxes, and residues coordinating α-carboxylate binding are shaded in the violet background in rectangular boxes adopted from our recently published work (Jedlickova et al., 2022)

Gel Analysis of recombinant SmM28B protein.

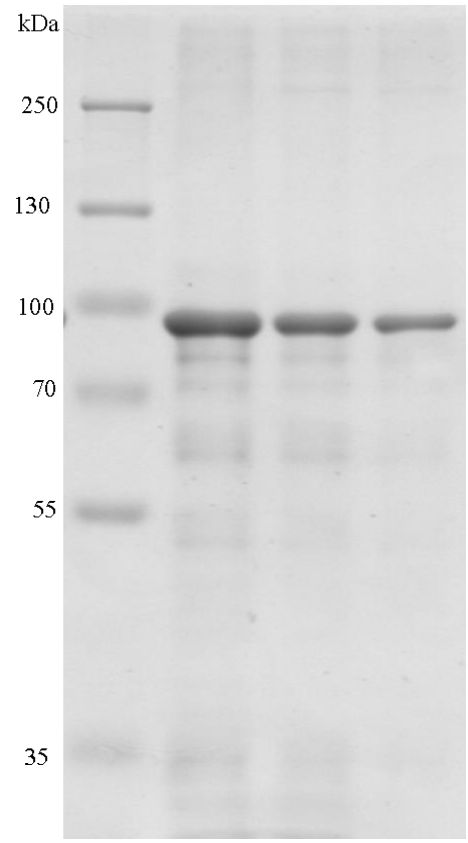

Figure 3. SDS page analysis of purified recombinant SmM28B. Lane M represents molecular marker. Lane 1-3 are three loads of purified Histidine tagged SmM28B recombinant protein expressed in *E. coli* vector. The estimated weight of the rSmM28B is 85 kDa.

Immunolocalization of SmM28B in adult *S. mansoni*

Immunohistochemistry was used to localize SmM28B antibodies in the adult worm. The result shows that the enzyme is overexpressed mainly in the reproductive organs parenchyma and gastrodermis, except for *S. mansoni* females, where a signal in the gastrodermis was missing (Figure 12). The pattern of expression is similar to a concurrent on F. hepatica in our lab , and also shared some similarities with the expression of GCP2 in humans, mice, and rats (Devlin et al., 2001; Knedlik et al., 2017; Rovenska et al., 2008; Sacha et al., 2007)

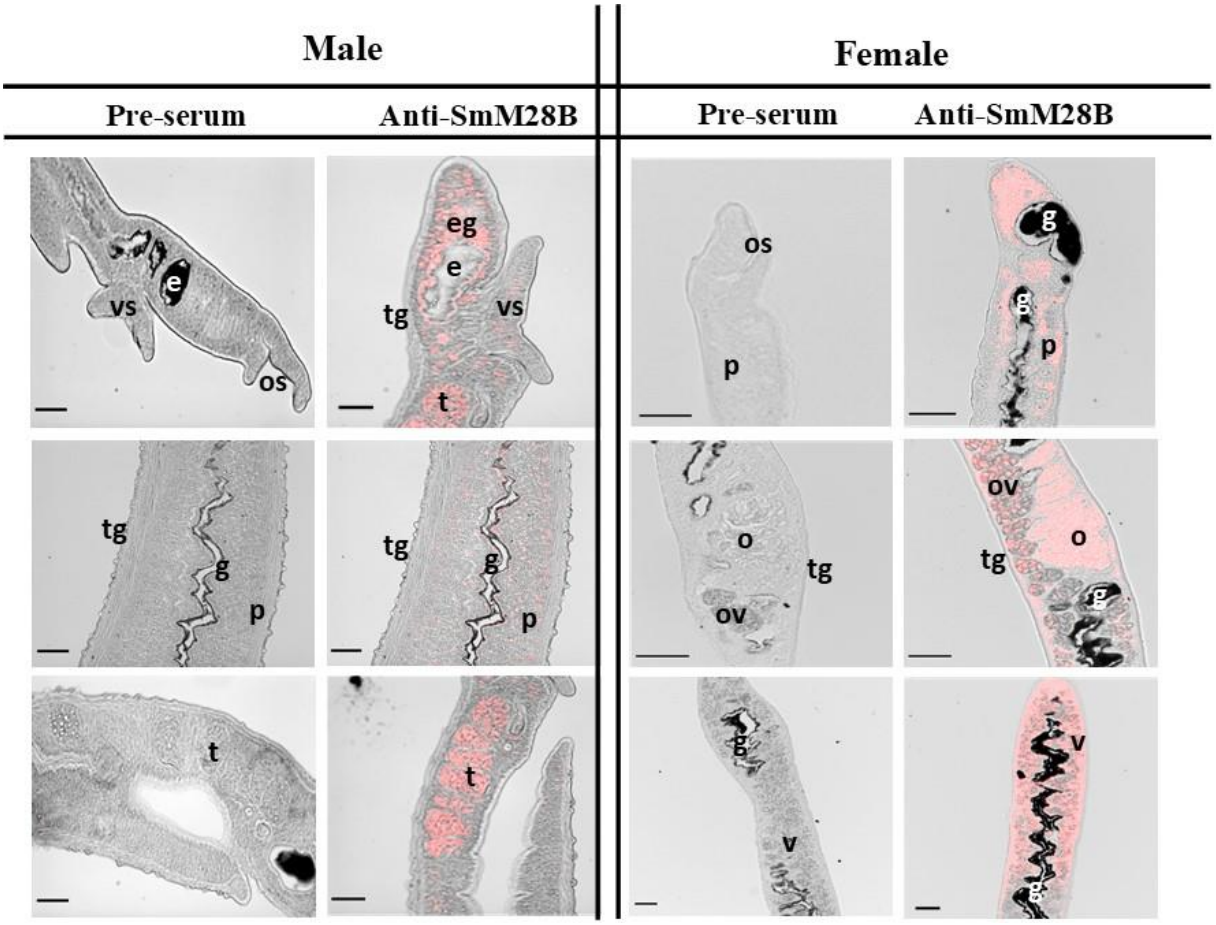

Figure 4. represents immunolocalization (red) of anti-SmM28B in adult male and female *S. mansoni* worms. Semi-thin sections of *S. mansoni* adult male and female were probed with Anti-SmM28B and pre-serum (control). Anti-SmM28B was localized in the gastrodermis of male (g), ventral sucker (vs), testis (t), parenchyma cells (p), ovary (o), oviduct (ov), and vitellaria (v). There was no localization in the gut of female as well as tegument of both male and female.

RNAi-mediated knockdown of SmM28B expression

NTS of *S. mansoni* were subjected to gene silencing *in vitro*. Schistosomula were treated with specific dsRNA for 5 days; the gene expression levels post-treated were later assessed by RT-qPCR. RNAi reduced the expression of SmM28B by around 92% in schistosomula while the expression level of the positive control was nearly 100%. Successful gene knockdown was also confirmed by immunolocalization on targeted dsRNA treated schistosomula (Figure 13) Despite significant knockdown of *Smm28b* gene, no observable phenotypic changes was detected when 2-days old RNAi schistosomula were injected in mice and sacrificed after 7 weeks of infection (result not shown).

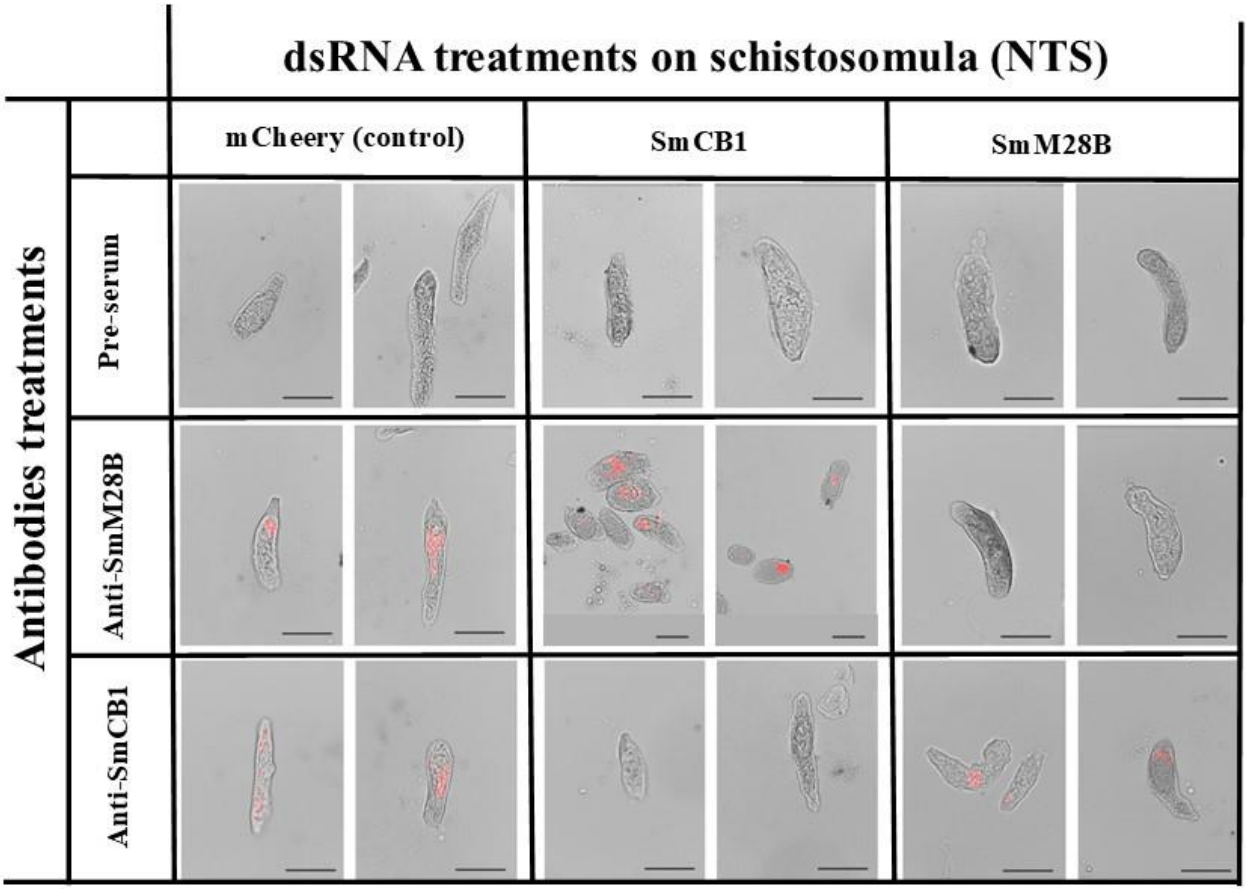

Figure 5. presents results of immunolocalization of SmM28B on schistosomula following *in vitro* RNAi. Thin sections of schistosomula were probed with anti-SmM28B, anti-SmCB1 (+ control) polyclonal antibodies, and pre-serum (control). Anti-SmM28B was localized in mCherry and SmCB1 dsRNA treated NTS. SmCB1 was localized in mCherry and SmM28B dsRNA treated NTS. Localization is shown in tissues with red coloration. The scale bar represents 50 μm.

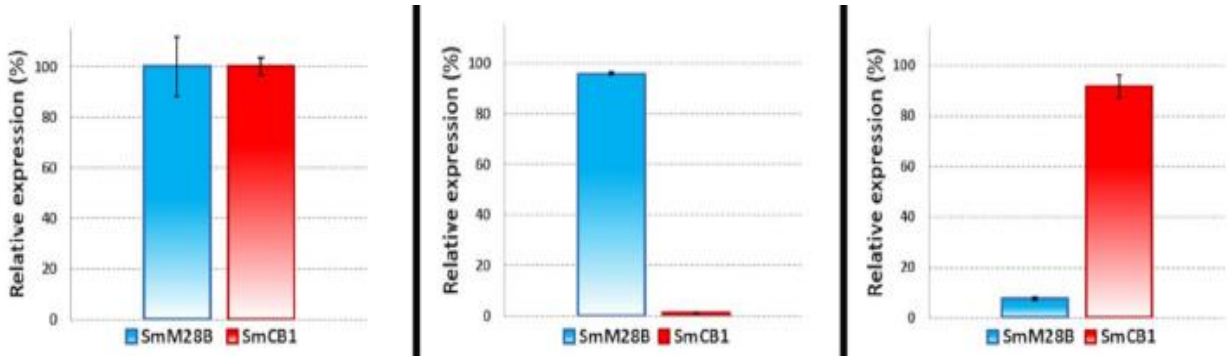

Figure 6 shows relative expression of SmM28B and SmCB1 genes following in vitro gene knockdown. The figure shows approximately 99% and 92% successful gene silencing for SmCB1 and SmM28B respectively. Adopted from (Jedlickova et al., 2022).

The study was informed by the integral role that peptidases play in the overall survival of parasites at the host-parasite interface, their use in disease diagnosis, treatment, and others (Atkinson et al., 2009; Barrett, 2001; Dvorak et al., 2008). Besides, several *S. mansoni* cysteine and serine peptidases are well known (Baig et al., 2002; Caffrey et al., 2004; Caffrey & Ruppel, 1997; Caffrey et al., 2002; Delcroix et al., 2006; Dvorak et al., 2016; Dvorak et al., 2008; El Ridi et al., 2014; Fishelson et al., 1992; Horn, Fajtova, Arreola, et al., 2014) but there is no information about metallopeptidases of this organism. Furthermore, human ortholog (hGCP2) of SmM28B has been studied for its therapeutic potential among others (Bacich et al., 2002; Barinka et al., 2008; Barinka et al., 2002; Barinka et al., 2012; Barinka, Sacha, et al., 2004; Bzdega et al., 2004; Davis et al., 2005; Hlouchova et al., 2009). All these together set the stage to study SmM28B, an ortholog of mammalian GCP2 in parasites to identify the biological role of the peptidase in *S. mansoni*.

The proportionate expression of *SmM28b* gene across all developmental stages of *S. mansoni* is an indication that the gene might be useful for certain biological activities at every stage of the parasite's development. The sequence alignment confirms relatively high similarities in the amino acid sequence of the three orthologs. It also revealed that there are highly conserved amino acid residue and protein domains among hGCP2, FhM28B (concurrent study with SmM28B), and SmM28B, irrespective of diversifications from the ancestral protein, indicating possible enzymatic activity of SmM28B (Jedlickova et al., 2022).

Under normal circumstances, hGCP2 is expressed in tissues and organs such as the brain, kidney, jejunal brush-border of the small intestine (Bzdega et al., 2004; Devlin et al., 2001; Knedlik et al., 2017; Rovenska et al., 2008; Sacha et al., 2007). Many authors have

demonstrated that hGCP2 is expressed and localized in other tissues, organs, or systems such as bladder, breast, colon, heart, lacrimal glands, liver, lungs, pancreas, skin, and testis in relatively lower amount (Pangalos et al., 1999b; Rovenska et al., 2008; Tykvart et al., 2015; Tykvart et al., 2014). Other human paralogs have also been expressed in mainly nervous tissues and limited expression in the intestine showing the proteins likely involvement in nutrients digestion as well as absorption (Bacich et al., 2002; Chandler et al., 1991; Halsted, 1991; Halsted et al., 1998; Hlouchova et al., 2007; Hlouchova et al., 2009; Knedlik et al., 2017; Lambert & Mitchell, 2007). The enigma surrounding the total function of hGCP2 has not been fully unraveled, thanks to the several paralogs and spliced variants that makes it difficult to study in detail, the function of the protein. Unlike hGCP2, *S. mansoni* has single gene encoding SmM28B, making it a simplified model to study the ancestral biological role of the protein before later diversification. The localization of anti-SmM28B partially mimics the expression of GCP2 pattern in functionally similar organs or tissues in human. Expression of *S. mansoni* metallopetidases SmLAPs in eggs is associated with egg hatching, in our study, SmM28B was profusely localized in ova, and vitellaria and minimally expressed in eggs suggesting a role in eggs development, nourishment and hatching.

This study has demonstrated that, SmM28B is mainly localized in reproductive organs and tissues, partially in digestive organs and muscles/parenchyma tissue. These localization pattern support my M.Sc. thesis research where SmM28B mRNA transcript was localized in similar organs through *in situ* hybridization. Also, immunolocalization pattern of FhM28B in adult *F. hepatica*, another trematode model for studying M28B peptidase in trematodes shows similar localization pattern (Jedlickova et al., 2022). The localization of SmM28B in the above-mentioned organ systems and tissues suggest that the protein might me involved in biological activities related to reproduction, digestion or nutrients update and probably movement and coordination of the parasite in the host's environment. The overexpression of *SmM28b* in NTS, the migratory larval and other motile stages of the parasite lend credence to a putative role of the protein in the migration of schistosomula as well as movement of cercariae and adults. This assumption led gene-silencing in NTS to further study the specific role.

The evaluation of *in vitro* RNAi on schistosomula showed approximately 92% gene knockdown success, however, there was no phenotypic manifestation in a further *in vivo* study. The shape, sizes, and number of adult worms recovered from mice infected with RNAi-treated NTS was similar to the negative and positive controls (result not shown). Thus, the near absence of *SmM28b* gene didn't affect the migration of the parasite in the host. Another assumption could

be that the little (8%) available gene was enough to promote migration, or the effect of RNAi wane off with time hence the worms recovered and initiated the migration to their destination. Further studies will be necessary to ascertain the reason behind this observation.

Finally, this purely basic study provides first inside on the localization and expression of M28B family homologue *S. mansoni* and hence paves the way for further translated research on this peptidase to elicit its therapeutic potential if any, and how that potential can be harnessed to help the control of intestinal schistosomiasis (*S. mansoni*) in particular and schistosomiasis in general.

6.0 GENERAL DISCUSSION AND CONCLUDING REMARKS

The debilitating nature and public health threats associated with schistosomiasis make it imperative to adopt effect control and elimination strategies to ensure the achievement of the WHO roadmap 2020-2030 the main strategy is PZQ MDA as preventive chemotherapy to endemic communities and school-aged children who have been shown to be the most vulnerable age group to *Schistosoma* spp. infections. My thesis focused on investigating a multifaceted approach to schistosomiasis control and elimination as a public health threat - the role of basic and applied research. Specifically, I investigated how a situational literature review can contribute to the promotion of effective control of the disease. Also, I went ahead to explore the role of alternative non-traditional monitoring and evaluation tools that can contribute to enhanced control. Finally, my laboratory study is about how host-parasite interaction can be a turning point in vaccine or drug development toward disease control.

Although policies recommendations and implementations by the WHO have emerged out of strict expert reviews of research and control around the world, my study revealed that literature review is never a reference point for implementation of control programs in Ghana. Again, I observed a divergent relationship between researchers and control program implementers thus creating a gap that hinders successful control. The literature review brought out fundamental knowledge gaps that are crucial for effective disease control including the absence of precision mapping and real-time nationwide survey which are necessary for targeting the right group in need of preventive chemotherapy. Instead, most MDA and praziquantel needs were based on estimations of archaic data thus the likelihood of underestimating or overestimating PZQ needs and missing out of the actual target groups and communities. The study established that literature review can be a rich source for the assessment of control programs in endemic

countries and can be the reference point for control policy revision. It is highly recommended that researchers would be an integral part of the disease control in Ghana and by extension, in endemic countries to provide research base evidence for policy formulation and implementation. Endemic countries should consider consistent literature review for at least every 5-10 years as a tool to reflect on and react to the disease control.

Inadequate monitoring and evaluation of control programs especially MDA led to the preliminary exploration of xenomonitoring of snail intermediate hosts as an alternative tool for monitoring and evaluation of community control programs. The study revealed that xenomonitoring can be an effective, less expensive, and non-invasive tool for the evaluation of the effectiveness of control programs as well at the efficacy of PZQ towards transmission interruption. Through this study, it was demonstrated that the effect of PZQ towards transmission is short-lived and persistent annual or semi-annual MDA campaigns in endemic communities might be necessary to achieve significant progress in control.

Despite several years of use of PZQ, less than 30% of endemic countries have reached elimination status by the WHO (WHO, 2023). This, along with the findings of the literature review and monitoring and evaluation provide solid evidence for an investigation into molecules that can be potential vaccine or drug candidates for schistosomiasis to complement the already existing chemotherapy. Peptidases are one of the major molecules targeted for vaccine and drug development. Our investigation of the biological functions or roles of *S. mansoni* ortholog of human GCP2, a universally expressed peptidase across all phyla of living organisms showed that the functions of SmM28B in *S. mansoni* might mimic that of GCP2 in functionally similar organs in human or mammals. These findings lay a solid foundation for elaborate future studies towards the pharmacological, therapeutic, or clinical benefits of SmM28B as it pertains to *S. mansoni* control specifically and schistosomiasis control in general.

In summary, basic and applied research go hand in hand when it comes to the disease control and elimination. While applied research (field studies) help to implement recommended interventions, assess policies, and provide a realistic assessment of control interventions, basic research dwells on these assessments to identify lapses and provide knowledge and interventions that better the existing ones. These studies reveal yet the need for alternative treatment whether in the form of an alternative drug or vaccine, however, a vaccine seems to be the obvious option. Basic research is the fulcrum around which vaccine/prophylaxis or alternative drug for the disease revolves since PZQ has not completed significant elimination

of schistosomiasis in most endemic country. Therefore, to ensure that the disease is effectively controlled and eliminated, an integrated and collaborative research approach encompassing basic and translational research, applied and field research as well as clinical research must be harnessed together rather than independent disjointed studies.

Reference

Abay, S. M., Tilahun, M., Fikrie, N., & Habtewold, A. (2013). Plasmodium falciparum and Schistosoma mansoni coinfection and the side benefit of artemether-lumefantrine in malaria patients. *J Infect Dev Ctries*, *7*(6), 468-474. https://doi.org/10.3855/jidc.2658

Abdel-Fattah, M., Al-Sherbiny, M., Osman, A., Charmy, R., & Tsang, V. (2011). Improving the detection limit of quantitative diagnosis of anti-S. haematobium antibodies using Falcon Assay Screening Test (FAST) ELISA by developing a new standard curve. *Parasitol Res*, *108*(6), 1457-1463. https://doi.org/10.1007/s00436-010-2198-y

Abdul-Ghani, R. A., & Hassan, A. A. (2010). Murine schistosomiasis as a model for human schistosomiasis mansoni: similarities and discrepancies. *Parasitol Res*, *107*(1), 1-8. https://doi.org/10.1007/s00436-010-1855-5

Abe, E. M., Guan, W., Guo, Y. H., Kassegne, K., Qin, Z. Q., Xu, J., Chen, J. H., Ekpo, U. F., Li, S. Z., & Zhou, X. N. (2018). Differentiating snail intermediate hosts of Schistosoma spp. using molecular approaches: fundamental to successful integrated control mechanism in Africa. *Infect Dis Poverty*, *7*(1), 29. https://doi.org/10.1186/s40249-018-0401-z

Abou-El-Naga, I. F. (2018). Towards elimination of schistosomiasis after 5000 years of endemicity in Egypt. *Acta Trop*, *181*, 112-121. https://doi.org/10.1016/j.actatropica.2018.02.005

Adenowo, A. F., Oyinloye, B. E., Ogunyinka, B. I., & Kappo, A. P. (2015). Impact of human schistosomiasis in sub-Saharan Africa. *Braz J Infect Dis*, *19*(2), 196-205. https://doi.org/10.1016/j.bjid.2014.11.004

Adu-Gyasi, D., Kye-Amoah, K., & Sorvor, E. (2010). Prevalence of urinary schistosomiasis in Offinso-South Municipality in Ashanti Region-Ghana. *Int J Infect Dis*, *14*, E270-E270. https://doi.org/10.1016/j.ijid.2010.02.2087

Afrifa, J., Gyedu, D., Gyamerah, E. O., Essien-Baidoo, S., & Mensah-Essilfie, I. (2017). Haematological Profile and Intensity of Urogenital Schistosomiasis in Ghanaian Children. *J Env and Pub Health*, *2017*. https://doi.org/Artn 424832510.1155/2017/4248325

Ahmed, N. S., Mahmoud, S. F., El-Samman, M. K., & Khalifa, R. M. A. (2017). Histopathological Analysis of Schistosomiasis in the Gastrointestinal Tract with First Record of Schistosomal Appendicitis from Sohag, Upper Egypt. *J Egypt Soc Parasitol*, *47*(1), 13-18. https://www.ncbi.nlm.nih.gov/pubmed/30157328

Ajibola, O., Gulumbe, B. H., Eze, A. A., & Obishakin, E. (2018). Tools for Detection of Schistosomiasis in Resource Limited Settings. *Med Sci (Basel)*, *6*(2). https://doi.org/10.3390/medsci6020039

Al-Abdulwahhab, A. H., Al-Sharydah, A. M., Al-Suhibani, S. S., Al-Jubran, S. A., Al-Haidey, A. K., Al-Hifzi, A. I., & Al-Issawi, W. (2018). Neuroschistosomiasis mimicking lower back pain: case report of a rare differential diagnosis in a pediatric patient. *Patient Saf Surg*, *12*, 28. https://doi.org/10.1186/s13037-018-0175-z

Al-Naseri, A., Al-Absi, S., El Ridi, R., & Mahana, N. (2021). A comprehensive and critical overview of schistosomiasis vaccine candidates. *J Parasit Dis*, *45*(2), 557-580. https://doi.org/10.1007/s12639-021-01387-w

Al-Shamiri, A. H., Al-Taj, M. A., & Ahmed, A. S. (2011). Prevalence and co-infections of schistosomiasis/hepatitis B and C viruses among school children in an endemic areas in Taiz, Yemen. *Asian Pac J Trop Med*, *4*(5), 404-408. https://doi.org/10.1016/S1995-7645(11)60113-2

Albonico, M., Levecke, B., LoVerde, P. T., Montresor, A., Prichard, R., Vercruysse, J., & Webster, J. P. (2015). Monitoring the efficacy of drugs for neglected tropical diseases controlled by

preventive chemotherapy. *J Glob Antimicrob Resist*, *3*(4), 229-236. https://doi.org/10.1016/j.jgar.2015.08.004

Allan, F., Ame, S. M., Tian-Bi, Y. T., Hofkin, B. V., Webster, B. L., Diakite, N. R., N'Goran, E. K., Kabole, F., Khamis, I. S., Gouvras, A. N., Emery, A. M., Pennance, T., Rabone, M., Kinung'hi, S., Hamidou, A. A., Mkoji, G. M., McLaughlin, J. P., Kuris, A. M., Loker, E. S., Rollinson, D. (2020). Snail-Related Contributions from the Schistosomiasis Consortium for Operational Research and Evaluation Program Including Xenomonitoring, Focal Mollusciciding, Biological Control, and Modeling. *Am J Trop Med Hyg*, *103*(1_Suppl), 66-79. https://doi.org/10.4269/ajtmh.19-0831

Allan, F., Sousa-Figueiredo, J. C., Emery, A. M., Paulo, R., Mirante, C., Sebastiao, A., Brito, M., & Rollinson, D. (2017). Mapping freshwater snails in north-western Angola: distribution, identity and molecular diversity of medically important taxa. *Parasit Vectors*, *10*(1), 460. https://doi.org/10.1186/s13071-017-2395-y

Amankwa, J. A., Bloch, P., Meyerlassen, J., Olsen, A., & Christensen, N. O. (1994). Urinary and Intestinal Schistosomiasis in the Tono Irrigation Scheme, Kassena-Nankana District, Upper East Region, Ghana. *Trop Med Parasitol*, *45*(4), 319-323. <Go to ISI>://WOS:A1994PY81700006

Amaral, K. B., Silva, T. P., Dias, F. F., Malta, K. K., Rosa, F. M., Costa-Neto, S. F., Gentile, R., & Melo, R. C. N. (2017). Histological assessment of granulomas in natural and experimental Schistosoma mansoni infections using whole slide imaging. *PLoS One*, *12*(9), e0184696. https://doi.org/10.1371/journal.pone.0184696

Amoah, L. A. O., Anyan, W. K., Aboagye-Antwi, F., Abonie, S., Tettey, M. D., & Bosompem, K. M. (2017). Environmental Factors and their Influence on Seasonal Variations of Schistosomiasis Intermediate Snail Hosts Abundance in Weija Lake, Ghana. *J Advoc, Res Edu*, *4*(2), 68-80.

Angelucci, F., Basso, A., Bellelli, A., Brunori, M., Pica Mattoccia, L., & Valle, C. (2007). The anti-schistosomal drug praziquantel is an adenosine antagonist. *Parasitol*, *134*(Pt 9), 1215-1221. https://doi.org/10.1017/S0031182007002600

Anto, F. (2013). Water Contact Activities and Prevalence of Schistosomiasis Infection among School-age Children in Communities along an Irrigation Scheme in Rural Northern Ghana. *Journal of Bacteriol & Parasitol*, *04*(04). https://doi.org/10.4172/2155-9597.1000177

Anto, F., Asoala, V., Anyorigiya, T., Oduro, A., Adjuik, M., Akweongo, P., Aborigo, R., Bimi, L., Amankwa, J., & Hodgson, A. (2011). Simultaneous administration of praziquantel, ivermectin and albendazole, in a community in rural northern Ghana endemic for schistosomiasis, onchocerciasis and lymphatic filariasis. *Trop Med Int Health*, *16*(9), 1112-1119. https://doi.org/10.1111/j.1365-3156.2011.02814.x

Anto, F., & Bimi, L. (2017). Potential of Lanistes varicus in limiting the population of Bulinus truncatus. *BMC Res Notes*, *10*(1), 509. https://doi.org/10.1186/s13104-017-2837-9

Anto, F., Bosompem, K., Kpikpi, J., Adjuik, M., & Edoh, D. (2005). Experimental control of Biomphalaria pfeifferi, the intermediate host of Schistosoma mansoni, by the ampullariid snail Lanistes varicus. *Ann Trop Med Parasitol*, *99*(2), 203-209. https://doi.org/10.1179/136485905x17425

Anyan, W. K., Abonie, S. D., Aboagye-Antwi, F., Tettey, M. D., Nartey, L. K., Hanington, P. C., Anang, A. K., & Muench, S. B. (2019). Concurrent Schistosoma mansoni and Schistosoma haematobium infections in a peri-urban community along the Weija dam in Ghana: A wake up call for effective National Control Programme. *Acta Trop*, *199*, 105116. https://doi.org/10.1016/j.actatropica.2019.105116

Anyan, W. K., Pulkkila, B. R., Dyra, C. E., Price, M., Naples, J. M., Quartey, J. K., Anang, A. K., & Lodh, N. (2020). Assessment of dual schistosome infection prevalence from urine in an endemic

community of Ghana by molecular diagnostic approach. *Parasite Epidemiol Con*, *9*, e00130. https://doi.org/10.1016/j.parepi.2019.e00130

Araujo, N., Kohn, A., & Katz, N. (1991). Activity of the artemether in experimental schistosomiasis mansoni. *Mem Inst Oswaldo Cruz*, *86 Suppl 2*, 185-188. https://doi.org/10.1590/s0074-02761991000600042

Aryeetey, M. E., Aholu, C., Wagatsuma, Y., Bentil, G., Nkrumah, F. K., & Kojima, S. (1999). Health education and community participation in the control of urinary schistosomiasis in Ghana. *East African Medical Journal*, *76*(6), 324-329. <Go to ISI>://WOS:000083525300006

Aryeetey, Y. A., Essien-Baidoo, S., Larbi, I. A., Ahmed, K., Amoah, A. S., Obeng, B. B., van Lieshout, L., Yazdanbakhsh, M., Boakye, D. A., & Verweij, J. J. (2013). Molecular Diagnosis of Schistosoma Infections in Urine Samples of School Children in Ghana. *Am J of Trop Med Hyg*, *88*(6), 1028-1031. https://doi.org/10.4269/ajtmh.12-0571

Ashton, P. D., Harrop, R., Shah, B., & Wilson, R. A. (2001). The schistosome egg: development and secretions. *Parasitol*, *122*(Pt 3), 329-338. https://doi.org/10.1017/s0031182001007351

Asuming-Brempong, E. K., Ayi, I., van der Puije, W., Gyan, B. A., Larbi, I. A., Ashong, Y., Frempong, N. A., Quartey, J. K., Otchere, J., Jones, F. M., Wilson, S., Dunne, D. W., & Boakye, D. A. (2022). Increased ShTAL1 IgE responses post-Praziquantel treatment may be associated with a reduced risk to re-infection in a Ghanaian S. haematobium-endemic community. *PLoS Neg Trop Dis*, *16*(3). https://doi.org/ARTN e001011510.1371/journal.pntd.0010115

Atkinson, H. J., Babbitt, P. C., & Sajid, M. (2009). The global cysteine peptidase landscape in parasites. *Trends Parasitol*, *25*(12), 573-581. https://doi.org/10.1016/j.pt.2009.09.006

Bacich, D. J., Ramadan, E., O'Keefe, D. S., Bukhari, N., Wegorzewska, I., Ojeifo, O., Olszewski, R., Wrenn, C. C., Bzdega, T., Wroblewska, B., Heston, W. D. W., & Neale, J. H. (2002). Deletion of the glutamate carboxypeptidase II gene in mice reveals a second enzyme activity that hydrolyzes N-acetylaspartylglutamate. *J Neurochem*, *83*(1), 20-29. https://doi.org/DOI 10.1046/j.1471-4159.2002.01117.x

Badr, H. I., Shaker, A. A., Mansour, M. A., Kasem, M. A., Zaher, A. A., Salama, H. H., & Safwat, M. I. (2011). Schistosomal myeloradiculopathy due to Schistosoma mansoni: Report on 17 cases from an endemic area. *Ann Indian Acad Neurol*, *14*(2), 107-110. https://doi.org/10.4103/0972-2327.82796

Baguio, B. R. (2011). *Ghana: administrative areas and boundaries 1874-2009*. Ghana University Press.

Baig, S., Damian, R. T., & Peterson, D. S. (2002). A novel cathepsin B active site motif is shared by helminth bloodfeeders. *Exp Parasitol*, *101*(2-3), 83-89. https://doi.org/10.1016/s0014-4894(02)00105-4

Bajiro, M., Dana, D., Ayana, M., Emana, D., Mekonnen, Z., Zawdie, B., Garbi, A., Kure, A., & Zeynudin, A. (2016). Prevalence of Schistosoma mansoni infection and the therapeutic efficacy of praziquantel among school children in Manna District, Jimma Zone, southwest Ethiopia. *Parasit Vectors*, *9*. https://doi.org/ARTN 56010.1186/s13071-016-1833-6

Baker, M. C., Mathieu, E., Fleming, F. M., Deming, M., King, J. D., Garba, A., Koroma, J. B., Bockarie, M., Kabore, A., Sankara, D. P., & Molyneux, D. H. (2010). Neglected Tropical Diseases 3 Mapping, monitoring, and surveillance of neglected tropical diseases: towards a policy framework. *Lancet*, *375*(9710), 231-238. https://doi.org/Doi 10.1016/S0140-6736(09)61458-6

Bakuza, J. S., Denwood', M. J., Nkwengulila, G., & Mablel, B. K. (2017). Estimating the prevalence and intensity of Schistosoma mansoni infection among rural communities in Western Tanzania: The influence of sampling strategy and statistical approach. *PLoS Negl Trop Dis*, *11*(9). https://doi.org/ARTN e000593710.1371/journal.pntd.0005937

Barinka, C., Byun, Y., Dusich, C. L., Banerjee, S. R., Chen, Y., Castanares, M., Kozikowski, A. P., Mease, R. C., Pomper, M. G., & Lubkowski, J. (2008). Interactions between human glutamate carboxypeptidase II and urea-based inhibitors: structural characterization. *J Med Chem*, *51*(24), 7737-7743. https://doi.org/10.1021/jm800765e

Barinka, C., Mlcochova, P., Sacha, P., Hilgert, I., Majer, P., Slusher, B. S., Horejsi, V., & Konvalinka, J. (2004). Amino acids at the N- and C-termini of human glutamate carboxypeptidase II are required for enzymatic activity and proper folding. *Eur J Biochem*, *271*(13), 2782-2790. https://doi.org/10.1111/j.1432-1033.2004.04209.x

Barinka, C., Rinnova, M., Sacha, P., Rojas, C., Majer, P., Slusher, B. S., & Konvalinka, J. (2002). Substrate specificity, inhibition and enzymological analysis of recombinant human glutamate carboxypeptidase II. *J Neurochem*, *80*(3), 477-487. https://doi.org/DOI 10.1046/j.0022-3042.2001.00715.x

Barinka, C., Rojas, C., Slusher, B., & Pomper, M. (2012). Glutamate Carboxypeptidase II in Diagnosis and Treatment of Neurologic Disorders and Prostate Cancer. *Curr Med Chem*, *19*(6), 856-870. https://doi.org/Doi 10.2174/092986712799034888

Barinka, C., Sacha, P., Sklenar, J., Man, P., Bezouska, K., Slusher, B. S., & Konvalinka, J. (2004). Identification of the N-glycosylation sites on glutamate carboxypeptidase II necessary for proteolytic activity. *Protein Sci*, *13*(6), 1627-1635. https://doi.org/DOI 10.1110/ps.04622104

Barrett, A. J. (2001). Proteases. *Curr Protoc Protein Sci*, *Chapter 21*, Unit 21 21. https://doi.org/10.1002/0471140864.ps2101s21

Barrett, A. J., Rawlings, N. D., & O'Brien, E. A. (2001). The MEROPS database as a protease information system. *J Struc Bio*, *134*(2-3), 95-102. https://doi.org/DOI 10.1006/jsbi.2000.4332

Basch, P. F. (1981). Cultivation of Schistosoma mansoni in vitro. II. production of infertile eggs by worm pairs cultured from cercariae. *J Parasitol*, *67*(2), 186-190. https://www.ncbi.nlm.nih.gov/pubmed/7241278

Becker, B., Mehlhorn, H., Andrews, P., Thomas, H., & Eckert, J. (1980). Light and Electron-Microscopic Studies on the Effect of Praziquantel on Schistosoma-Mansoni, Dicrocoelium-Dendriticum, and Fasciola-Hepatica (Trematoda) Invitro. *Zeitschrift Fur Parasitenkunde-Parasitol Res*, *63*(2), 113-128. https://doi.org/Doi 10.1007/Bf00927527

Bergquist, N. R., & Colley, D. G. (1998). Schistosomiasis vaccine:research to development. *Parasitol Today*, *14*(3), 99-104. https://doi.org/10.1016/s0169-4758(97)01207-6

Bergquist, R., Utzinger, J., & Keiser, J. (2017). Controlling schistosomiasis with praziquantel: How much longer without a viable alternative? *Infect Dis Poverty*, *6*. https://doi.org/ARTN 7410.1186/s40249-017-0286-2

Berriman, M., Haas, B. J., LoVerde, P. T., Wilson, R. A., Dillon, G. P., Cerqueira, G. C., Mashiyama, S. T., Al-Lazikani, B., Andrade, L. F., Ashton, P. D., Aslett, M. A., Bartholomeu, D. C., Blandin, G., Caffrey, C. R., Coghlan, A., Coulson, R., Day, T. A., Delcher, A., DeMarco, R., El-Sayed, N. M. (2009). The genome of the blood fluke Schistosoma mansoni. *Nature*, *460*(7253), 352-358. https://doi.org/10.1038/nature08160

Bisoffi, Z., Buonfrate, D., & Beltrame, A. (2016). Schistosomiasis transmission in Europe. *Lancet Infect Dis*, *16*(8), 878-880. https://doi.org/10.1016/S1473-3099(16)30061-5

Boateng, E. M., Dvorak, J., Ayi, I., & Chanova, M. (2023). A literature review of schistosomiasis in Ghana: a reference for bridging the research and control gap. *Trans R Soc Trop Med Hyg*. https://doi.org/10.1093/trstmh/trac134

Boissier, J., Grech-Angelini, S., Webster, B. L., Allienne, J. F., Huyse, T., Mas-Coma, S., Toulza, E., Barre-Cardi, H., Rollinson, D., Kincaid-Smith, J., Oleaga, A., Galinier, R., Foata, J., Rognon, A., Berry, A., Mouahid, G., Henneron, R., Mone, H., Noel, H., & Mitta, G. (2016). Outbreak of

urogenital schistosomiasis in Corsica (France): an epidemiological case study. *Lancet Infect Dis*, *16*(8), 971-979. https://doi.org/10.1016/S1473-3099(16)00175-4

Boon, N. A. M., Mbow, M., Paredis, L., Moris, P., Sy, I., Maes, T., Webster, B. L., Sacko, M., Volckaert, F. A. M., Polman, K., & Huyse, T. (2019). No barrier breakdown between human and cattle schistosome species in the Senegal River Basin in the face of hybridisation. *Int J Parasitol*, *49*(13-14), 1039-1048. https://doi.org/10.1016/j.ijpara.2019.08.004

Boros, D. L. (1989). Immunopathology of Schistosoma mansoni infection. *Clin Microbiol Rev*, *2*(3), 250-269. https://doi.org/10.1128/CMR.2.3.250

Borrmann, S., Szlezak, N., Faucher, J. F., Matsiegui, P. B., Neubauer, R., Binder, R. K., Lell, B., & Kremsner, P. G. (2001). Artesunate and praziquantel for the treatment of Schistosoma haematobium infections: a double-blind, randomized, placebo-controlled study. *J Infect Dis*, *184*(10), 1363-1366. https://doi.org/10.1086/324004

Bos, D. H., Mayfield, C., & Minchella, D. J. (2009). Analysis of regulatory protease sequences identified through bioinformatic data mining of the Schistosoma mansoni genome. *BMC Genomics*, *10*, 488. https://doi.org/10.1186/1471-2164-10-488

Bottieau, E., Clerinx, J., de Vega, M. R., Van den Enden, E., Colebunders, R., Van Esbroeck, M., Vervoort, T., Van Gompel, A., & Van den Ende, J. (2006). Imported Katayama fever: Clinical and biological features at presentation and during treatment. *J Infection*, *52*(5), 339-345. https://doi.org/10.1016/j.jinf.2005.07.022

Brunet, L. R., Finkelman, F. D., Cheever, A. W., Kopf, M. A., & Pearce, E. J. (1997). IL-4 protects against TNF-alpha-mediated cachexia and death during acute schistosomiasis. *J Immunol*, *159*(2), 777-785. https://www.ncbi.nlm.nih.gov/pubmed/9218595

Burke, M. L., Jones, M. K., Gobert, G. N., Li, Y. S., Ellis, M. K., & McManus, D. P. (2009). Immunopathogenesis of human schistosomiasis. *Parasite Immunol*, *31*(4), 163-176. https://doi.org/10.1111/j.1365-3024.2009.01098.x

Bustinduy, A. L., Randriansolo, B., Sturt, A. S., Kayuni, S. A., Leustcher, P. D. C., Webster, B. L., Van Lieshout, L., Stothard, J. R., Feldmeier, H., & Gyapong, M. (2022). An update on female and male genital schistosomiasis and a call to integrate efforts to escalate diagnosis, treatment and awareness in endemic and non-endemic settings: The time is now. *Adv Parasitol*, *115*, 1-44. https://doi.org/10.1016/bs.apar.2021.12.003

Bzdega, T., Crowe, S. L., Ramadan, E. R., Sciarretta, K. H., Olszewski, R. T., Ojeifo, O. A., Rafalski, V. A., Wroblewska, B., & Neale, J. H. (2004). The cloning and characterization of a second brain enzyme with NAAG peptidase activity. *J Neurochem*, *89*(3), 627-635. https://doi.org/10.1111/j.1471-4159.2004.02361.x

Caffrey, C. R. (2015). Schistosomiasis and its treatment. *Future Med Chem*, *7*(6), 675-676. https://doi.org/10.4155/fmc.15.27

Caffrey, C. R., Mathieu, M. A., Gaffney, A. M., Salter, J. P., Sajid, M., Lucas, K. D., Franklin, C., Bogyo, M., & McKerrow, J. H. (2000). Identification of a cDNA encoding an active asparaginyl endopeptidase of Schistosoma mansoni and its expression in Pichia pastoris. *Febs Letters*, *466*(2-3), 244-248. https://doi.org/10.1016/s0014-5793(99)01798-6

Caffrey, C. R., McKerrow, J. H., Salter, J. P., & Sajid, M. (2004). Blood 'n' guts: an update on schistosome digestive peptidases. *Trends Parasitol*, *20*(5), 241-248. https://doi.org/10.1016/j.pt.2004.03.004

Caffrey, C. R., & Ruppel, A. (1997). Cathepsin B-like activity predominates over cathepsin L-like activity in adult Schistosoma mansoni and S. japonicum. *Parasitol Res*, *83*(6), 632-635. https://doi.org/10.1007/s004360050310

Caffrey, C. R., Salter, J. P., Lucas, K. D., Khiem, D., Hsieh, I., Lim, K. C., Ruppel, A., McKerrow, J. H., & Sajid, M. (2002). SmCB2, a novel tegumental cathepsin B from adult Schistosoma

mansoni. *Mol Biochem Parasitol*, *121*(1), 49-61. https://doi.org/10.1016/s0166-6851(02)00022-1

Campbell, G., Noble, L. R., Rollinson, D., Southgate, V. R., Webster, J. P., & Jones, C. S. (2010). Low genetic diversity in a snail intermediate host (Biomphalaria pfeifferi Krass, 1848) and schistosomiasis transmission in the Senegal River Basin. *Mol Ecol*, *19*(2), 241-256. https://doi.org/10.1111/j.1365-294X.2009.04463.x

Campbell, S. J., Osei-Atweneboana, M. Y., Stothard, R., Koukounari, A., Cunningham, L., Armoo, S. K., Biritwum, N. K., Gyapong, M., MacPherson, E., Theobald, S., Woode, M. E., Khan, J., Niessen, L., & Adams, E. R. (2018). The COUNTDOWN Study Protocol for Expansion of Mass Drug Administration Strategies against Schistosomiasis and Soil-Transmitted Helminthiasis in Ghana. *Trop Med Infect Dis*, *3*(1). https://doi.org/10.3390/tropicalmed3010010

Camprubi-Ferrer, D., Romero, L., Van Esbroeck, M., Wammes, L. J., Almuedo-Riera, A., Rodriguez-Valero, N., Balerdi-Sarasola, L., Hoekstra, P. T., Subira, C., Valls, M. E., Micalessi, I., Corstjens, P., Cortes-Serra, N., Huyse, T., Benegas, M., Alvarez-Martinez, M. J., Munoz, J., & van Lieshout, L. (2021). Improving the diagnosis and management of acute schistosomiasis with antibody, antigen and molecular techniques: lessons from a cluster of six travellers. *J Travel Med*, *28*(6). https://doi.org/ARTN taab10110.1093/jtm/taab101

Cao, K. Y., Mao, X. P., Wang, D. H., Xu, L., Yuan, G. Q., Dai, S. Q., Zheng, B. J., & Qiu, S. P. (2007). High expression of PSM-E correlated with tumor grade in prostate cancer: a new alternatively spliced variant of prostate-specific membrane antigen. *Prostate*, *67*(16), 1791-1800. https://doi.org/10.1002/pros.20664

Capron, A., Capron, M., & Riveau, G. (2002). Vaccine development against schistosomiasis from concepts to clinical trials. *Br Med Bull*, *62*, 139-148. https://doi.org/10.1093/bmb/62.1.139

Carter, R. E., Feldman, A. R., & Coyle, J. T. (1996). Prostate-specific membrane antigen is a hydrolase with substrate and pharmacologic characteristics of a neuropeptidase. *Proc Natl Acad Sci U S A*, *93*(2), 749-753. https://www.ncbi.nlm.nih.gov/pubmed/8570628

Carvalho, O. A. (2013). Mansonic neuroschistosomiasis. *Arq Neuropsiquiatr*, *71*(9B), 714-716. https://doi.org/10.1590/0004-282X20130157

Catalano, S., Sene, M., Diouf, N. D., Fall, C. B., Borlase, A., Leger, E., Ba, K., & Webster, J. P. (2018). Rodents as Natural Hosts of Zoonotic Schistosoma Species and Hybrids: An Epidemiological and Evolutionary Perspective From West Africa. *J Infect Dis*, *218*(3), 429-433. https://doi.org/10.1093/infdis/jiy029

Chai, J. Y. (2013). Praziquantel treatment in trematode and cestode infections: an update. *Infect Chemother*, *45*(1), 32-43. https://doi.org/10.3947/ic.2013.45.1.32

Chan, J. D., Cupit, P. M., Gunaratne, G. S., McCorvy, J. D., Yang, Y., Stoltz, K., Webb, T. R., Dosa, P. I., Roth, B. L., Abagyan, R., Cunningham, C., & Marchant, J. S. (2017). The anthelmintic praziquantel is a human serotoninergic G-protein-coupled receptor ligand. *Nat Commun*, *8*(1), 1910. https://doi.org/10.1038/s41467-017-02084-0

Chandler, C. J., Harrison, D. A., Buffington, C. A., Santiago, N. A., & Halsted, C. H. (1991). Functional Specificity of Jejunal Brush-Border Pteroylpolyglutamate Hydrolase in Pig. *Am J Physiol*, *260*(6), G865-G872. <Go to ISI>://WOS:A1991FU84100055

Chang, S. S., O'Keefe, D. S., Bacich, D. J., Reuter, V. E., Heston, W. D., & Gaudin, P. B. (1999). Prostate-specific membrane antigen is produced in tumor-associated neovasculature. *Clin Cancer Res*, *5*(10), 2674-2681. https://www.ncbi.nlm.nih.gov/pubmed/10537328

Chienwichai, P., Ampawong, S., Adisakwattana, P., Thiangtrongjit, T., Limpanont, Y., Chusongsang, P., Chusongsang, Y., & Reamtong, O. (2020). Effect of Praziquantel on Schistosoma mekongi Proteome and Phosphoproteome. *Pathogens*, *9*(6). https://doi.org/10.3390/pathogens9060417

Chitsulo, L., Engels, D., Montresor, A., & Savioli, L. (2000). The global status of schistosomiasis and its control. *Acta Trop*, *77*(1), 41-51. https://doi.org/Doi 10.1016/S0001-706x(00)00122-4

Chitsulo, L., Loverde, P., & Engels, D. (2004). Schistosomiasis. *Nat Rev Microbiol*, *2*(1), 12-13. https://doi.org/10.1038/nrmicro801

Christopherson, J. B. (1918). Intravenous Injections of Antimonium Tartaratum in Bilharziosis. *Br Med J*, *2*(3024), 652-653. https://doi.org/10.1136/bmj.2.3024.652

Chu, K. Y. (1978). Trials of ecological and chemical measures for the control of Schistosoma haematobium transmission in a Volta Lake village. *Bull World Health Organ*, *56*(2), 313-322.

Chu, K. Y., Klumpp, R. K., & Kofi, D. Y. (1981). Results of three years of cercarial transmission control in the Volta Lake. *Bull World Health Organ*, *59*(4), 549-554. https://www.ncbi.nlm.nih.gov/pubmed/6976224

Cioli, D., & Pica-Mattoccia, L. (2003). Praziquantel. *Parasitol Res*, *90 Supp 1*, S3-9. https://doi.org/10.1007/s00436-002-0751-z

Cioli, D., Pica-Mattoccia, L., Basso, A., & Guidi, A. (2014). Schistosomiasis control: praziquantel forever? *Mol Biochem Parasitol*, *195*(1), 23-29. https://doi.org/10.1016/j.molbiopara.2014.06.002

Clerinx, J., & Van Gompel, A. (2011). Schistosomiasis in travellers and migrants. *Travel Med Infect Dis*, *9*(1), 6-24. https://doi.org/10.1016/j.tmaid.2010.11.002

Cogswell, A. A., Collins, J. J., 3rd, Newmark, P. A., & Williams, D. L. (2011). Whole mount in situ hybridization methodology for Schistosoma mansoni. *Mol Biochem Parasitol*, *178*(1-2), 46-50. https://doi.org/10.1016/j.molbiopara.2011.03.001

Colley, D. G., Bustinduy, A. L., Secor, E., & King, C. H. (2014). Human schistosomiasis. *Lancet*, *383*(9936), 2253-2264. https://doi.org/10.1016/S0140-6736(13)61949-2

Collins, J. J., 3rd, King, R. S., Cogswell, A., Williams, D. L., & Newmark, P. A. (2011). An atlas for Schistosoma mansoni organs and life-cycle stages using cell type-specific markers and confocal microscopy. *PLoS Negl Trop Dis*, *5*(3), e1009. https://doi.org/10.1371/journal.pntd.0001009

Corachan, M. (2002). Schistosomiasis and international travel. *Clinical Infectious Diseases*, *35*(4), 446-450. https://doi.org/Doi 10.1086/341895

Corpet, F. (1988). Multiple sequence alignment with hierarchical clustering. *Nucleic Acids Res*, *16*(22), 10881-10890. https://doi.org/10.1093/nar/16.22.10881

Coulibaly, J. T., N'Gbesso Y, K., Knopp, S., Keiser, J., N'Goran, E. K., & Utzinger, J. (2012). Efficacy and safety of praziquantel in preschool-aged children in an area co-endemic for Schistosoma mansoni and S. haematobium. *PLoS Negl Trop Dis*, *6*(12), e1917. https://doi.org/10.1371/journal.pntd.0001917

Coulibaly, J. T., Panic, G., Yapi, R. B., Kovac, J., Barda, B., N'Gbesso, Y. K., Hattendorf, J., & Keiser, J. (2018). Efficacy and safety of ascending doses of praziquantel against Schistosoma haematobium infection in preschool-aged and school-aged children: a single-blind randomised controlled trial. *BMC Med*, *16*(1), 81. https://doi.org/10.1186/s12916-018-1066-y

Cunha, A. C., Weigle, B., Kiessling, A., Bachmann, M., & Rieber, E. P. (2006). Tissue-specificity of prostate specific antigens: Comparative analysis of transcript levels in prostate and non-prostatic tissues. *Cancer Lett*, *236*(2), 229-238. https://doi.org/10.1016/j.canlet.2005.05.021

Cunningham, L. J., Campbell, S. J., Armoo, S., Koukounari, A., Watson, V., Selormey, P., Stothard, J. R., Idun, B., Asiedu, M., Ashong, Y., Adams, E. R., & Osei-Atweneboana, M. Y. (2020). Assessing expanded community wide treatment for schistosomiasis: Baseline infection status and self-reported risk factors in three communities from the Greater Accra region, Ghana. *PLoS Negl Trop Dis*, *14*(4), e0007973. https://doi.org/10.1371/journal.pntd.0007973

Cupit, P. M., & Cunningham, C. (2015). What is the mechanism of action of praziquantel and how might resistance strike? *Future Med Chem*, *7*(6), 701-705. https://doi.org/10.4155/Fmc.15.11

Curwen, R. S., & Wilson, R. A. (2003). Invasion of skin by schistosome cercariae: some neglected facts. *Trends Parasitol*, *19*(2), 63-66; discussion 66-68. https://doi.org/10.1016/s1471-4922(02)00019-3

Danso-Appiah, A., De Vlas, S. J., Bosompem, K. M., & Habbema, J. D. (2004). Determinants of health-seeking behaviour for schistosomiasis-related symptoms in the context of integrating schistosomiasis control within the regular health services in Ghana. *Trop Med Int Health*, *9*(7), 784-794. https://doi.org/10.1111/j.1365-3156.2004.01267.x

Darko, S. N., Hanson, H., Twumasi-Ankrah, S., Baffour-Awuah, S., Adjei-Kusi, P., Yar, D., & Owusu-Dabo, E. (2020). Three monthly doses of 60 mg/kg praziquantel for Schistosoma haematobium infection is a safe and effective treatment regimen. *BMC Infect Dis*, *20*(1), 323. https://doi.org/10.1186/s12879-020-05053-z

David, B. (1994). *Freshwater Snails of Africa and their Medical Importance* (Second ed.). Taylor & Francis.

Davis, A., & Bailey, D. R. (1969). Metrifonate in urinary schistosomiasis. *Bull World Health Organ*, *41*(2), 209-224. https://www.ncbi.nlm.nih.gov/pubmed/5308698

Davis, M. I., Bennett, M. J., Thomas, L. M., & Bjorkman, P. J. (2005). Crystal structure of prostate-specific membrane antigen, a tumor marker and peptidase. *Proc Natl Acad Sci U S A*, *102*(17), 5981-5986. https://doi.org/10.1073/pnas.0502101102

De Bont, J., Vercruysse, J., Grzych, J. M., Meeus, P. F., & Capron, A. (1997). Potential of a recombinant Schistosoma bovis-derived glutathione S-transferase to protect cattle against experimental and natural S. mattheei infection. *Parasitol, 115 (Pt 3)*, 249-255. https://doi.org/10.1017/s0031182097001352

de Jesus, A. R., Silva, A., Santana, L. B., Magalhaes, A., de Jesus, A. A., de Almeida, R. P., Rego, M. A. V., Burattini, M. N., Pearce, E. J., & Carvalho, E. M. (2002). Clinical and immunologic evaluation of 31 patients with acute schistosomiasis mansoni. *J Infect Dis*, *185*(1), 98-105. https://doi.org/Doi 10.1086/324668

Delcroix, M., Sajid, M., Caffrey, C. R., Lim, K. C., Dvorak, J., Hsieh, I., Bahgat, M., Dissous, C., & McKerrow, J. H. (2006). A multienzyme network functions in intestinal protein digestion by a platyhelminth parasite. *J Biol Chem*, *281*(51), 39316-39329. https://doi.org/10.1074/jbc.M607128200

Devlin, A. M., Swenson, J. V., Villanueva, J. A., & Halsted, C. H. (2001). Expression of glutamate carboxypeptidase II (GCPII) in human small intestine. *Faseb J*, *15*(5), A955-A955. <Go to ISI>://WOS:000167454201391

deWalick, S., Tielens, A. G., & van Hellemond, J. J. (2012). Schistosoma mansoni: the egg, biosynthesis of the shell and interaction with the host. *Exp Parasitol*, *132*(1), 7-13. https://doi.org/10.1016/j.exppara.2011.07.018

Di Bella, S., Riccardi, N., Giacobbe, D. R., & Luzzati, R. (2018). History of schistosomiasis (bilharziasis) in humans: from Egyptian medical papyri to molecular biology on mummies. *Pathog Glob Health*, *112*(5), 268-273. https://doi.org/10.1080/20477724.2018.1495357

Doenhoff, M. J., Cioli, D., & Utzinger, J. (2008). Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. *Curr Opin Infect Dis*, *21*(6), 659-667. https://doi.org/10.1097/QCO.0b013e328318978f

Doenhoff, M. J., & Pica-Mattoccia, L. (2006). Praziquantel for the treatment of schistosomiasis: its use for control in areas with endemic disease and prospects for drug resistance. *Expert Rev Anti Infect Ther*, *4*(2), 199-210. https://doi.org/10.1586/14787210.4.2.199

Doumenge, J., Mott, KE, Cheung, C, Villenave, D, Chapuis, O, Perrin, MF, Reaud-Thomas, G. (1987). *Atlas of the global distribution of schistosomiasis = Atlas de la repartition mondiale des*

schistosomiases. Presses Universitaires de Bordeaux. https://fr.ircwash.org/resources/atlas-global-distribution-schistosomiasis-atlas-de-la-repartition-mondiale-des

Dunne, D. W., Bain, J., Lillywhite, J., & Doenhoff, M. J. (1984). The stage-, strain- and species-specificity of a Schistosoma mansoni egg antigen fraction (CEF6) with serodiagnostic potential. *Trans R Soc Trop Med Hyg*, *78*(4), 460-470. https://doi.org/10.1016/0035-9203(84)90061-0

Dvorak, J., Delcroix, M., Rossi, A., Vopalensky, V., Pospisek, M., Sedinova, M., Mikes, L., Sajid, M., Sali, A., McKerrow, J. H., Horak, P., & Caffrey, C. R. (2005). Multiple cathepsin B isoforms in schistosomula of Trichobilharzia regenti: identification, characterisation and putative role in migration and nutrition. *Int J Parasitol*, *35*(8), 895-910. https://doi.org/10.1016/j.ijpara.2005.02.018

Dvorak, J., Fajtova, P., Ulrychova, L., Leontovyc, A., Rojo-Arreola, L., Suzuki, B. M., Horn, M., Mares, M., Craik, C. S., Caffrey, C. R., & O'Donoghue, A. J. (2016). Excretion/secretion products from Schistosoma mansoni adults, eggs and schistosomula have unique peptidase specificity profiles. *Biochimie*, *122*, 99-109. https://doi.org/10.1016/j.biochi.2015.09.025

Dvorak, J., & Horn, M. (2018). Serine proteases in schistosomes and other trematodes. *Int J Parasitol*, *48*(5), 333-344. https://doi.org/10.1016/j.ijpara.2018.01.001

Dvorak, J., Mashiyama, S. T., Braschi, S., Sajid, M., Knudsen, G. M., Hansell, E., Lim, K. C., Hsieh, I., Bahgat, M., Mackenzie, B., Medzihradszky, K. F., Babbitt, P. C., Caffrey, C. R., & McKerrow, J. H. (2008). Differential use of protease families for invasion by schistosome cercariae. *Biochimie*, *90*(2), 345-358. https://doi.org/10.1016/j.biochi.2007.08.013

Edwards, E. E., & Mccullough, F. S. (1954). Studies on the Life-Cycles of Schistosoma-Haematobium and S-Mansoni in the Gold-Coast. *Ann Trop Med Parasitol*, *48*(2), 164-177. https://doi.org/Doi 10.1080/00034983.1954.11685612

El Ridi, R., Tallima, H., Selim, S., Donnelly, S., Cotton, S., Gonzales Santana, B., & Dalton, J. P. (2014). Cysteine peptidases as schistosomiasis vaccines with inbuilt adjuvanticity. *PLoS One*, *9*(1), e85401. https://doi.org/10.1371/journal.pone.0085401

Erko, B., Degarege, A., Tadesse, K., Mathiwos, A., & Legesse, M. (2012). Efficacy and side effects of praziquantel in the treatment of Schistosomiasis mansoni in schoolchildren in Shesha Kekele Elementary School, Wondo Genet, Southern Ethiopia. *Asian Pac J Trop Biomed*, *2*(3), 235-239. https://doi.org/10.1016/S2221-1691(12)60049-5

Everts, B., Hussaarts, L., Driessen, N. N., Meevissen, M. H., Schramm, G., van der Ham, A. J., van der Hoeven, B., Scholzen, T., Burgdorf, S., Mohrs, M., Pearce, E. J., Hokke, C. H., Haas, H., Smits, H. H., & Yazdanbakhsh, M. (2012). Schistosome-derived omega-1 drives Th2 polarization by suppressing protein synthesis following internalization by the mannose receptor. *J Exp Med*, *209*(10), 1753-1767, S1751. https://doi.org/10.1084/jem.20111381

Everts, B., Perona-Wright, G., Smits, H. H., Hokke, C. H., van der Ham, A. J., Fitzsimmons, C. M., Doenhoff, M. J., van der Bosch, J., Mohrs, K., Haas, H., Mohrs, M., Yazdanbakhsh, M., & Schramm, G. (2009). Omega-1, a glycoprotein secreted by Schistosoma mansoni eggs, drives Th2 responses. *J Exp Med*, *206*(8), 1673-1680. https://doi.org/10.1084/jem.20082460

Exum, N. G., Kibira, S. P. S., Ssenyonga, R., Nobili, J., Shannon, A. K., Ssempebwa, J. C., Tukahebwa, E. M., Radloff, S., Schwab, K. J., & Makumbi, F. E. (2019). The prevalence of schistosomiasis in Uganda: A nationally representative population estimate to inform control programs and water and sanitation interventions. *PLoS Negl Trop Dis*, *13*(8). https://doi.org/ARTN e000761710.1371/journal.pntd.0007617

Fahel, J. S., Macedo, G. C., Pinheiro, C. S., Caliari, M. V., & Oliveira, S. C. (2010). IPSE/alpha-1 of Schistosoma mansoni egg induces enlargement of granuloma but does not alter Th2 balance

after infection. *Parasite Immunol*, *32*(5), 345-353. https://doi.org/10.1111/j.1365-3024.2009.01192.x

Fajtova, P., Stefanic, S., Hradilek, M., Dvorak, J., Vondrasek, J., Jilkova, A., Ulrychova, L., McKerrow, J. H., Caffrey, C. R., Mares, M., & Horn, M. (2015). Prolyl Oligopeptidase from the Blood Fluke Schistosoma mansoni: From Functional Analysis to Anti-schistosomal Inhibitors. *PLoS Negl Trop Dis*, *9*(6), e0003827. https://doi.org/10.1371/journal.pntd.0003827

Fallon, P. G., Richardson, E. J., McKenzie, G. J., & McKenzie, A. N. (2000). Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 and IL-13: IL-13 is a profibrotic agent. *J Immunol*, *164*(5), 2585-2591. https://doi.org/10.4049/jimmunol.164.5.2585

Faltynkova, A., Nasincova, V., & Kablaskova, L. (2007). Larval trematodes (Digenea) of the great pond snail, Lymnaea stagnalis (L.), (Gastropoda, Pulmonata) in Central Europe: a survey of species and key to their identification. *Parasite*, *14*(1), 39-51. https://doi.org/10.1051/parasite/2007141039

Farid, Z., Smith, J. H., Bassily, S., & Sparks, H. A. (1972). Hepatotoxicity after treatment of schistosomiasis with hycanthone. *Br Med J*, *2*(5805), 88-89. https://doi.org/10.1136/bmj.2.5805.88

Fetterer, R. H., Pax, R. A., & Bennett, J. L. (1980). Praziquantel, potassium and 2,4-dinitrophenol: analysis of their action on the musculature of Schistosoma mansoni. *Eur J Pharmacol*, *64*(1), 31-38. https://doi.org/10.1016/0014-2999(80)90366-0

Figueiredo, B. C., Da'dara, A. A., Oliveira, S. C., & Skelly, P. J. (2015). Schistosomes Enhance Plasminogen Activation: The Role of Tegumental Enolase. *PLoS Pathogens*, *11*(12), e1005335. https://doi.org/10.1371/journal.ppat.1005335

File, S. (1995). Interaction of schistosome eggs with vascular endothelium. *J Parasitol*, *81*(2), 234-238. https://www.ncbi.nlm.nih.gov/pubmed/7707199

Fishelson, Z., Amiri, P., Friend, D. S., Marikovsky, M., Petitt, M., Newport, G., & McKerrow, J. H. (1992). Schistosoma mansoni: cell-specific expression and secretion of a serine protease during development of cercariae. *Exp Parasitol*, *75*(1), 87-98. https://doi.org/10.1016/0014-4894(92)90124-s

Fonseca, C. T., Cunha-Neto, E., Goldberg, A. C., Kalil, J., de Jesus, A. R., Carvalho, E. M., Correa-Oliveira, R., & Oliveira, S. C. (2005). Human T cell epitope mapping of the Schistosoma mansoni 14-kDa fatty acid-binding protein using cells from patients living in areas endemic for schistosomiasis. *Microbes Infect*, *7*(2), 204-212. https://doi.org/10.1016/j.micinf.2004.10.012

Fortin, A., & Jansen, F. H. (2011). ACTs for the treatment of schistosomiasis. *Lancet Infect Dis*, *11*(7), 498. https://doi.org/10.1016/S1473-3099(11)70157-8

Foster, R. (1987). A review of clinical experience with oxamniquine. *Trans R Soc Trop Med Hyg*, *81*(1), 55-59. https://doi.org/10.1016/0035-9203(87)90282-3

Fowler, R., Lee, C., & Keystone, J. S. (1999). The role of corticosteroids in the treatment of cerebral schistosomiasis caused by Schistosoma mansoni: case report and discussion. *Am J Trop Med Hyg*, *61*(1), 47-50. https://doi.org/10.4269/ajtmh.1999.61.47

Frandsen, F., & Christensen, N. O. (1984). An introductory guide to the identification of cercariae from African freshwater snails with special reference to cercariae of trematode species of medical and veterinary importance. *Acta Trop*, *41*(2), 181-202. https://www.ncbi.nlm.nih.gov/pubmed/6206702

Fukushige, M., Mutapi, F., & Woolhouse, M. E. J. (2019). Population level changes in schistosome-specific antibody levels following chemotherapy. *Parasite Immunol*, *41*(1), e12604. https://doi.org/10.1111/pim.12604

GAHI. (2015). *Distribution of schistosomiasis survey data in Ghana*. Retrieved 21.02.2021 from https://www.thiswormyworld.org/maps/distribution-of-schistosomiasis-survey-data-in-ghana

Gala, J. L., Loric, S., Guiot, Y., Denmeade, S. R., Gady, A., Brasseur, F., Heusterspreute, M., Eschwege, P., De Nayer, P., Van Cangh, P., & Tombal, B. (2000). Expression of prostate-specific membrane antigen in transitional cell carcinoma of the bladder: prognostic value? *Clin Cancer Res*, *6*(10), 4049-4054. https://www.ncbi.nlm.nih.gov/pubmed/11051255

Garcia, T. C., Fonseca, C. T., Pacifico, L. G., Duraes Fdo, V., Marinho, F. A., Penido, M. L., Caliari, M. V., de Melo, A. L., Pinto, H. A., Barsante, M. M., Cunha-Neto, E., & Oliveira, S. C. (2008). Peptides containing T cell epitopes, derived from Sm14, but not from paramyosin, induce a Th1 type of immune response, reduction in liver pathology and partial protection against Schistosoma mansoni infection in mice. *Acta Trop*, *106*(3), 162-167. https://doi.org/10.1016/j.actatropica.2008.03.003

Gentile, R., Costa-Neto, S. F., Goncalves, M. M., Bonecker, S. T., Fernandes, F. A., Garcia, J. S., Barreto, M. G., Soares, M. S., D'Andrea, P. S., Peralta, J. M., & Rey, L. (2006). An ecological field study of the water-rat Nectomys squamipes as a wild reservoir indicator of Schistosoma mansoni transmission in an endemic area. *Mem Inst Oswaldo Cruz*, *101 Suppl 1*, 111-117. https://doi.org/10.1590/s0074-02762006000900018

Gentile, R., & Oliveira, G. (2008). Brazilian studies on the genetics of Schistosoma mansoni. *Acta Trop*, *108*(2-3), 175-178. https://doi.org/10.1016/j.actatropica.2008.09.003

Ghosh, A., & Heston, W. D. (2003). Effect of carbohydrate moieties on the folate hydrolysis activity of the prostate specific membrane antigen. *Prostate*, *57*(2), 140-151. https://doi.org/10.1002/pros.10289

GHS. (2016). Master plan for neglected tropical diseases programme, Ghana *(2016 – 2020).* Retrieved from https://espen.afro.who.int/system/files/content/resources/GHANA_NTD_Master_Plan_2016_2020.pdf

Goncalves-Macedo, L., Domingues, A. L., Lopes, E. P., Luna, C. F., Mota, V. G., Becker, M. M., & Markman-Filho, B. (2017). Pulmonary shunts in severe hepatosplenic schistosomiasis: Diagnosis by contrast echocardiography and their relationship with abdominal ultrasound findings. *PLoS Negl Trop Dis*, *11*(4), e0005417. https://doi.org/10.1371/journal.pntd.0005417

Gonnert, R., & Andrews, P. (1977). Praziquantel, a new board-spectrum antischistosomal agent. *Zeitschrift Fur Parasitenkunde-Parasitol Res*, *52*(2), 129-150. https://doi.org/10.1007/BF00389899

Gordon, C. A., Kurscheid, J., Williams, G. M., Clements, A. C. A., Li, Y., Zhou, X. N., Utzinger, J., McManus, D. P., & Gray, D. J. (2019). Asian Schistosomiasis: Current Status and Prospects for Control Leading to Elimination. *Trop Med Infect Dis*, *4*(1). https://doi.org/10.3390/tropicalmed4010040

Greenberg, R. M. (2005a). Are Ca2+ channels targets of praziquantel action? *Int J Parasitol*, *35*(1), 1-9. https://doi.org/10.1016/j.ijpara.2004.09.004

Greenberg, R. M. (2005b). Ca2+ signalling, voltage-gated Ca2+ channels and praziquantel in flatworm neuromusculature. *Parasitology*, *131 Suppl*, S97-108. https://doi.org/10.1017/S0031182005008346

Greer, G. J., Kitikoon, V., & Lohachit, C. (1989). Morphology and life cycle of Schistosoma sinensium Pao, 1959, from northwest Thailand. *J Parasitol*, *75*(1), 98-101. https://www.ncbi.nlm.nih.gov/pubmed/2918446

Grube, M. (2005). Nucleic acid isolation from ecological samples--fungal associations, lichens. *Methods Enzymol*, *395*, 48-57. https://doi.org/10.1016/S0076-6879(05)95004-0

Gryseels, B. (2012). Schistosomiasis. *Infect Dis Clin North Am*, *26*(2), 383-397. https://doi.org/10.1016/j.idc.2012.03.004

Gryseels, B., Polman, K., Clerinx, J., & Kestens, L. (2006). Human schistosomiasis. *Lancet*, *368*(9541), 1106-1118. https://doi.org/Doi 10.1016/S0140-6736(06)69440-3

GSS, G. S. S. (2021). *Ghana 2021 population and housing census*. Retrieved from https://census2021.statsghana.gov.gh/

Guidi, A., Andolina, C., Makame Ame, S., Albonico, M., Cioli, D., & Juma Haji, H. (2010). Praziquantel efficacy and long-term appraisal of schistosomiasis control in Pemba Island. *Trop Med Int Health*, *15*(5), 614-618. https://doi.org/10.1111/j.1365-3156.2010.02488.x

Haffner, M. C., Laimer, J., Chaux, A., Schafer, G., Obrist, P., Brunner, A., Kronberger, I. E., Laimer, K., Gurel, B., Koller, J. B., Seifarth, C., Zelger, B., Klocker, H., Rasse, M., Doppler, W., & Bander, N. H. (2012). High expression of prostate-specific membrane antigen in the tumor-associated neo-vasculature is associated with worse prognosis in squamous cell carcinoma of the oral cavity. *Modern Pathol*, *25*(8), 1079-1085. https://doi.org/10.1038/modpathol.2012.66

Hailegebriel, T., Nibret, E., & Munshea, A. (2020). Prevalence of Schistosoma mansoni and S. haematobium in Snail Intermediate Hosts in Africa: A Systematic Review and Meta-analysis. *J Trop Med*, *2020*. https://doi.org/Artn 885084010.1155/2020/8850840

Halsted, C. H. (1991). Jejunal Brush-Border Folate Hydrolase - a Novel Enzyme. *Western J Med*, *155*(6), 605-609. <Go to ISI>://WOS:A1991GV45500001

Halsted, C. H., Ling, E. H., Luthi-Carter, R., Villanueva, J. A., Gardner, J. M., & Coyle, J. T. (1998). Folylpoly-gamma-glutamate carboxypeptidase from pig jejunum. Molecular characterization and relation to glutamate carboxypeptidase II. *J Biol Chem*, *273*(32), 20417-20424. https://www.ncbi.nlm.nih.gov/pubmed/9685395

Hambrook, J. R., & Hanington, P. C. (2023). A cercarial invadolysin interferes with the host immune response and facilitates infection establishment of Schistosoma mansoni. *PLoS Pathogens*, *19*(2), e1010884. https://doi.org/10.1371/journal.ppat.1010884

Hambrook, J. R., Kabore, A. L., Pila, E. A., & Hanington, P. C. (2018). A metalloprotease produced by larval Schistosoma mansoni facilitates infection establishment and maintenance in the snail host by interfering with immune cell function. *PLoS Pathogens*, *14*(10), e1007393. https://doi.org/10.1371/journal.ppat.1007393

Hams, E., Aviello, G., & Fallon, P. G. (2013). The schistosoma granuloma: friend or foe? *Front Immunol*, *4*, 89. https://doi.org/10.3389/fimmu.2013.00089

Harnett, W., & Kusel, J. R. (1986). Increased exposure of parasite antigens at the surface of adult male Schistosoma mansoni exposed to praziquantel in vitro. *Parasitol*, *93 (Pt 2)*, 401-405. https://doi.org/10.1017/s0031182000051568

He, Y. X., Salafsky, B., & Ramaswamy, K. (2005). Comparison of skin invasion among three major species of Schistosoma. *Trends Parasitol*, *21*(5), 201-203. https://doi.org/10.1016/j.pt.2005.03.003

Hlouchova, K., Barinka, C., Klusak, V., Sacha, P., Mlcochova, P., Majer, P., Rulisek, L., & Konvalinka, J. (2007). Biochemical characterization of human glutamate carboxypeptidase III. *J Neurochem*, *101*(3), 682-696. https://doi.org/10.1111/j.1471-4159.2006.04341.x

Hlouchova, K., Barinka, C., Konvalinka, J., & Lubkowski, J. (2009). Structural insight into the evolutionary and pharmacologic homology of glutamate carboxypeptidases II and III. *Febs J*, *276*(16), 4448-4462. https://doi.org/10.1111/j.1742-4658.2009.07152.x

Horak, P., Mikes, L., Rudolfova, J., & Kolarova, L. (2008). Penetration of Trichobilharzia cercariae into mammals: dangerous or negligible event? *Parasite*, *15*(3), 299-303. https://doi.org/10.1051/parasite/2008153299

Horn, M., Fajtova, P., Arreola, L. R., Ulrychova, L., Bartosova-Sojkova, P., Franta, Z., Protasio, A. V., Opavsky, D., Vondrasek, J., McKerrow, J. H., Mares, M., Caffrey, C. R., & Dvorak, J. (2014).

Trypsin- and Chymotrypsin-Like Serine Proteases in Schistosoma mansoni - 'The Undiscovered Country'. *PLoS Negl Trop Dis*, *8*(3). https://doi.org/ARTN e276610.1371/journal.pntd.0002766

Horn, M., Fajtova, P., Rojo Arreola, L., Ulrychova, L., Bartosova-Sojkova, P., Franta, Z., Protasio, A. V., Opavsky, D., Vondrasek, J., McKerrow, J. H., Mares, M., Caffrey, C. R., & Dvorak, J. (2014). Trypsin- and Chymotrypsin-like serine proteases in schistosoma mansoni-- 'the undiscovered country'. *PLoS Negl Trop Dis*, *8*(3), e2766. https://doi.org/10.1371/journal.pntd.0002766

Horoszewicz, J. S., Kawinski, E., & Murphy, G. P. (1987). Monoclonal-Antibodies to a New Antigenic Marker in Epithelial Prostatic Cells and Serum of Prostatic-Cancer Patients. *Antican Res*, *7*(5), 927-936. <Go to ISI>://WOS:A1987L903900001

Hotez, P. J., Bottazzi, M. E., Bethony, J., & Diemert, D. D. (2019). Advancing the Development of a Human Schistosomiasis Vaccine. *Trends Parasitol*, *35*(2), 104-108. https://doi.org/10.1016/j.pt.2018.10.005

Houston, S., Kowalewska-Grochowska, K., Naik, S., McKean, J., Johnson, E. S., & Warren, K. (2004). First report of Schistosoma mekongi infection with brain involvement. *Clin Infect Dis*, *38*(1), e1-6. https://doi.org/10.1086/379826

Howe, K. L., Bolt, B. J., Shafie, M., Kersey, P., & Berriman, M. (2017). WormBase ParaSite - a comprehensive resource for helminth genomics. *Mol Biochem Parasitol*, *215*, 2-10. https://doi.org/10.1016/j.molbiopara.2016.11.005

Hunter, J. M. (2003). Inherited burden of disease: agricultural dams and the persistence of bloody urine (Schistosomiasis hematobium) in the Upper East Region of Ghana, 1959-1997. *Soc Sci Med*, *56*(2), 219-234. https://doi.org/10.1016/s0277-9536(02)00021-7

Hussaarts, L., Smits, H. H., Schramm, G., van der Ham, A. J., van der Zon, G. C., Haas, H., Guigas, B., & Yazdanbakhsh, M. (2013). Rapamycin and omega-1: mTOR-dependent and -independent Th2 skewing by human dendritic cells. *Immunol Cell Biol*, *91*(7), 486-489. https://doi.org/10.1038/icb.2013.31

Huyse, T., Webster, B. L., Geldof, S., Stothard, J. R., Diaw, O. T., Polman, K., & Rollinson, D. (2009). Bidirectional Introgressive Hybridization between a Cattle and Human Schistosome Species. *PLoS Pathogens*, *5*(9). https://doi.org/ARTN e100057110.1371/journal.ppat.1000571

Inobaya, M. T., Olveda, R. M., Chau, T. N., Olveda, D. U., & Ross, A. G. (2014). Prevention and control of schistosomiasis: a current perspective. *Res Rep Trop Med*, *2014*(5), 65-75. https://doi.org/10.2147/RRTM.S44274

Israeli, R. S., Powell, C. T., Corr, J. G., Fair, W. R., & Heston, W. D. (1994). Expression of the prostate-specific membrane antigen. *Cancer Res*, *54*(7), 1807-1811. https://www.ncbi.nlm.nih.gov/pubmed/7511053

Jacobs, W., Bogers, J. J., Timmermans, J. P., Deelder, A. M., & Van Marck, E. A. (1998). Adhesion molecules in intestinal Schistosoma mansoni infection. *Parasitol Res*, *84*(4), 276-280. https://doi.org/10.1007/s004360050395

Jaureguiberry, S., Paris, L., & Caumes, E. (2010). Acute schistosomiasis, a diagnostic and therapeutic challenge. *Clin Microbiol Infection*, *16*(3), 225-231. https://doi.org/10.1111/j.1469-0691.2009.03131.x

Jedlickova, L., Peterkova, K., Boateng, E. M., Ulrychova, L., Vacek, V., Kutil, Z., Jiang, Z., Novakova, Z., Snajdr, I., Kim, J., O'Donoghue, A. J., Barinka, C., & Dvorak, J. (2022). Characterization of glutamate carboxypeptidase 2 orthologs in trematodes. *Parasit Vectors*, *15*(1), 480. https://doi.org/10.1186/s13071-022-05556-5

Jilkova, A., Horn, M., & Mares, M. (2020). Structural and Functional Characterization of Schistosoma mansoni Cathepsin B1. *Methods Mol Biol*, *2151*, 145-158. https://doi.org/10.1007/978-1-0716-0635-3_12

Kabatereine, N. B., Standley, C. J., Sousa-Figueiredo, J. C., Fleming, F. M., Stothard, J. R., Talisuna, A., & Fenwick, A. (2011). Integrated prevalence mapping of schistosomiasis, soil-transmitted helminthiasis and malaria in lakeside and island communities in Lake Victoria, Uganda. *Parasit Vectors*, *4*, 232. https://doi.org/10.1186/1756-3305-4-232

Kabore, A., Biritwum, N. K., Downs, P. W., Magalhaes, R. J. S., Zhang, Y. B., & Ottesen, E. A. (2013). Predictive vs. Empiric Assessment of Schistosomiasis: Implications for Treatment Projections in Ghana. *PLoS Negl Trop Dis*, *7*(3). https://doi.org/ARTN e205110.1371/journal.pntd.0002051

Kaliner, M. A. (2006). Pharmacologic characteristics and adrenal suppression with newer inhaled corticosteroids: A comparison of ciclesonide and fluticasone propionate. *Clin Ther*, *28*(3), 319-331. https://doi.org/10.1016/j.clinthera.2006.03.003

Kasny, M., Mikes, L., Doleckova, K., Hampl, V., Dvorak, J., Novotny, M., & Horak, P. (2011). Cathepsins B1 and B2 of Trichobilharzia SPP., bird schistosomes causing cercarial dermatitis. *Adv Exp Med Biol*, *712*, 136-154. https://doi.org/10.1007/978-1-4419-8414-2_9

Kasny, M., Mikes, L., Hampl, V., Dvorak, J., Caffrey, C. R., Dalton, J. P., & Horak, P. (2009). Chapter 4. Peptidases of trematodes. *Adv Parasitol*, *69*, 205-297. https://doi.org/10.1016/S0065-308X(09)69004-7

Kaukas, A., Dias Neto, E., Simpson, A. J., Southgate, V. R., & Rollinson, D. (1994). A phylogenetic analysis of Schistosoma haematobium group species based on randomly amplified polymorphic DNA. *Int J Parasitol*, *24*(2), 285-290. https://doi.org/10.1016/0020-7519(94)90040-x

Kaur, I., Schramm, G., Everts, B., Scholzen, T., Kindle, K. B., Beetz, C., Montiel-Duarte, C., Blindow, S., Jones, A. T., Haas, H., Stolnik, S., Heery, D. M., & Falcone, F. H. (2011). Interleukin-4-inducing principle from Schistosoma mansoni eggs contains a functional C-terminal nuclear localization signal necessary for nuclear translocation in mammalian cells but not for its uptake. *Infect Immun*, *79*(4), 1779-1788. https://doi.org/10.1128/IAI.01048-10

Keiser, J., N'Guessan, N. A., Adoubryn, K. D., Silue, K. D., Vounatsou, P., Hatz, C., Utzinger, J., & N'Goran, E. K. (2010). Efficacy and safety of mefloquine, artesunate, mefloquine-artesunate, and praziquantel against Schistosoma haematobium: randomized, exploratory open-label trial. *Clin Infect Dis*, *50*(9), 1205-1213. https://doi.org/10.1086/651682

Keitel, W. A., Potter, G. E., Diemert, D., Bethony, J., El Sahly, H. M., Kennedy, J. K., Patel, S. M., Plieskatt, J. L., Jones, W., Deye, G., Bottazzi, M. E., Hotez, P. J., & Atmar, R. L. (2019). A phase 1 study of the safety, reactogenicity, and immunogenicity of a Schistosoma mansoni vaccine with or without glucopyranosyl lipid A aqueous formulation (GLA-AF) in healthy adults from a non-endemic area. *Vaccine*, *37*(43), 6500-6509. https://doi.org/10.1016/j.vaccine.2019.08.075

King, C. H., & Dangerfield-Chay, M. (2008). The unacknowledged impact of chronic schistosomiasis. *Chron Illness*, *4*(1), 65-79. https://doi.org/10.1177/1742395307084407

Kinoshita, Y., Kuratsukuri, K., Landas, S., Imaida, K., Rovito, P. M., Wang, C. Y., & Haas, G. P. (2006). Expression of prostate-specific membrane antigen in normal and malignant human tissues. *Wor J Surg*, *30*(4), 628-636. https://doi.org/10.1007/s00268-005-0544-5

Knedlik, T., Vorlova, B., Navratil, V., Tykvart, J., Sedlak, F., Vaculin, S., Franek, M., Sacha, P., & Konvalinka, J. (2017). Mouse glutamate carboxypeptidase II (GCPII) has a similar enzyme activity and inhibition profile but a different tissue distribution to human GCPII. *FEBS Open Bio*, *7*(9), 1362-1378. https://doi.org/10.1002/2211-5463.12276

Kosinski, K. C., Adjei, M. N., Bosompem, K. M., Crocker, J. J., Durant, J. L., Osabutey, D., Plummer, J. D., Stadecker, M. J., Wagner, A. D., Woodin, M., & Gute, D. M. (2012). Effective control of Schistosoma haematobium infection in a Ghanaian community following installation of a water recreation area. *PLoS Negl Trop Dis*, *6*(7), e1709. https://doi.org/10.1371/journal.pntd.0001709

Kulinkina, A. V., Kosinski, K. C., Plummer, J. D., Durant, J. L., Bosompem, K. M., Adjei, M. N., Griffiths, J. K., Gute, D. M., & Naumova, E. N. (2017). Indicators of improved water access in the context of schistosomiasis transmission in rural Eastern Region, Ghana. *Sci Total Environ*, *579*, 1745-1755. https://doi.org/10.1016/j.scitotenv.2016.11.140

Lai, Y. S., Biedermann, P., Ekpo, U. F., Garba, A., Mathieu, E., Midzi, N., Mwinzi, P., N'Goran, E. K., Raso, G., Assare, R. N. K., Sacko, M., Schur, N., Talla, I., Tchuente, L. A. T., Toure, S., Winkler, M. S., Utzinger, J., & Vounatsou, P. (2015). Spatial distribution of schistosomiasis and treatment needs in sub-Saharan Africa: a systematic review and geostatistical analysis. *Lancet Infect Dis*, *15*(8), 927-940. https://doi.org/10.1016/S1473-3099(15)00066-3

Lambert, L. A., & Mitchell, S. L. (2007). Molecular evolution of the transferrin receptor/glutamate carboxypeptidase II family. *J Mol Evol*, *64*(1), 113-128. https://doi.org/10.1007/s00239-006-0137-4

Lamberton, P. H. L., Faust, C. L., & Webster, J. P. (2017). Praziquantel decreases fecundity in Schistosoma mansoni adult worms that survive treatment: evidence from a laboratory life-history trade-offs selection study. *Infect Dis Poverty*, *6*(1), 110. https://doi.org/10.1186/s40249-017-0324-0

Lambertucci, J. R., Voieta, I., & Silveira Idos, S. (2008). Cerebral schistosomiasis mansoni. *Rev Soc Bras Med Trop*, *41*(6), 693-694. https://doi.org/10.1590/s0037-86822008000600029

Leger, E., Borlase, A., Fall, C. B., Diouf, N. D., Diop, S. D., Yasenev, L., Catalano, S., Thiam, C. T., Ndiaye, A., Emery, A., Morrell, A., Rabone, M., Ndao, M., Faye, B., Rollinson, D., Rudge, J. W., Sene, M., & Webster, J. P. (2020). Prevalence and distribution of schistosomiasis in human, livestock, and snail populations in northern Senegal: a One Health epidemiological study of a multi-host system. *Lancet Plan Hlth*, *4*(8), E330-E342. <Go to ISI>://WOS:000573750600010

Leger, E., Garba, A., Hamidou, A. A., Webster, B. L., Pennance, T., Rollinson, D., & Webster, J. P. (2016). Introgressed Animal Schistosomes Schistosoma curassoni and S. bovis Naturally Infecting Humans. *Emerg Infect Dis*, *22*(12), 2212-2214. https://doi.org/10.3201/eid2212.160644

Leger, E., & Webster, J. P. (2017). Hybridizations within the Genus Schistosoma: implications for evolution, epidemiology and control. *Parasitol*, *144*(1), 65-80. https://doi.org/10.1017/S0031182016001190

Li, Y., Chen, D., Ross, A. G., Burke, M. L., Yu, X., Li, R. S., Zhou, J., & McManus, D. P. (2011). Severe hepatosplenic schistosomiasis: clinicopathologic study of 102 cases undergoing splenectomy. *Hum Pathol*, *42*(1), 111-119. https://doi.org/10.1016/j.humpath.2010.05.020

Lo, N. C., Bezerra, F. S. M., Colley, D. G., Fleming, F. M., Homeida, M., Kabatereine, N., Kabole, F. M., King, C. H., Mafe, M. A., Midzi, N., Mutapi, F., Mwanga, J. R., Ramzy, R. M. R., Satrija, F., Stothard, J. R., Traore, M. S., Webster, J. P., Utzinger, J., Zhou, X. N., . . . Garba, A. (2022). Review of 2022 WHO guidelines on the control and elimination of schistosomiasis. *Lancet Infect Dis*. https://doi.org/10.1016/S1473-3099(22)00221-3

Lockyer, A. E., Olson, P. D., Ostergaard, P., Rollinson, D., Johnston, D. A., Attwood, S. W., Southgate, V. R., Horak, P., Snyder, S. D., Le, T. H., Agatsuma, T., McManus, D. P., Carmichael, A. C., Naem, S., & Littlewood, D. T. (2003). The phylogeny of the Schistosomatidae based on three genes with emphasis on the interrelationships of Schistosoma Weinland, 1858. *Parasitol*, *126*(Pt 3), 203-224. https://doi.org/10.1017/s0031182002002792

Lopes, A. D., Davis, W. L., Rosenstraus, M. J., Uveges, A. J., & Gilman, S. C. (1990). Immunohistochemical and pharmacokinetic characterization of the site-specific immunoconjugate CYT-356 derived from antiprostate monoclonal antibody 7E11-C5. *Cancer Res*, *50*(19), 6423-6429. https://www.ncbi.nlm.nih.gov/pubmed/1698122

Loukas, A., Tran, M., & Pearson, M. S. (2007). Schistosome membrane proteins as vaccines. *Int J Parasitol*, *37*(3-4), 257-263. https://doi.org/10.1016/j.ijpara.2006.12.001

Lukacs, N. W., Chensue, S. W., Strieter, R. M., Warmington, K., & Kunkel, S. L. (1994). Inflammatory granuloma formation is mediated by TNF-alpha-inducible intercellular adhesion molecule-1. *J Immunol*, *152*(12), 5883-5889. https://www.ncbi.nlm.nih.gov/pubmed/7911491

Luthi-Carter, R., Barczak, A. K., Speno, H., & Coyle, J. T. (1998). Molecular characterization of human brain N-acetylated alpha-linked acidic dipeptidase (NAALADase). *J Pharmacol Exp Ther*, *286*(2), 1020-1025. https://www.ncbi.nlm.nih.gov/pubmed/9694964

Macdonald, G. (1965). The dynamics of helminth infections, with special reference to schistosomes. *Trans R Soc Trop Med Hyg*, *59*(5), 489-506. https://doi.org/10.1016/0035-9203(65)90152-5

Machiels, J. D., Cobussen, M., Bosboom, R. W., van Os, N. J. H., Hageman, A. T. M., & Hassing, R. (2018). [Myelitis transversa caused by neuroschistosomiasis]. *Ned Tijdschr Geneeskd*, *162*. https://www.ncbi.nlm.nih.gov/pubmed/30040277 (Myelitis transversa door schistosomiasis.)

Maezawa, K., Furushima-Shimogawara, R., Yasukawa, A., Ohta, N., & Iwanaga, S. (2018). Real-time observation of pathophysiological processes during murine experimental Schistosoma japonicum infection using high-resolution ultrasound imaging. *Trop Med Health*, *46*. https://doi.org/ARTN 110.1186/s41182-017-0082-5

Martel, R. A., Osei, B. G., Kulinkina, A. V., Naumova, E. N., Abdulai, A. A., Tybor, D., & Kosinski, K. C. (2019). Assessment of urogenital schistosomiasis knowledge among primary and junior high school students in the Eastern Region of Ghana: A cross-sectional study. *PLoS One*, *14*(6). https://doi.org/ARTN e021808010.1371/journal.pone.0218080

Mattos, A. C. A., Pereira, G. C., Jannotti-Passos, L. K., Kusel, J. R., & Coelho, P. M. Z. (2007). Evaluation of the effect of oxamniquine, praziquantel and a combination of both drugs on the intramolluscan phase of Schistosoma mansoni. *Acta Trop*, *102*(2), 84-91. https://doi.org/10.1016/j.actatropica.2007.04.002

Mc, C. F. (1959). The susceptibility and resistance of Bulinus (Physopsis) globosus and Bulinus (Bulinus) truncatus rohlfsi to two strains of Schistosoma haematobium in Ghana. *Bull World Health Organ*, *20*(1), 75-85.

McCullough, F. S. (1956). Transmission of Schistosoma haematobium by Bulinus sp. in the Ke district of the Gold Coast. *Trans R Soc Trop Med Hyg*, *50*(5), 449-457. https://doi.org/10.1016/0035-9203(56)90091-8

McKerrow, J. H., Caffrey, C., Kelly, B., Loke, P., & Sajid, M. (2006). Proteases in parasitic diseases. *Annu Rev Pathol*, *1*, 497-536. https://doi.org/10.1146/annurev.pathol.1.110304.100151

McManus, D. P., Dunne, D. W., Sacko, M., Utzinger, J., Vennervald, B. J., & Zhou, X. N. (2018). Schistosomiasis. *Nat Rev Dis Primers*, *4*(1), 13. https://doi.org/10.1038/s41572-018-0013-8

Meevissen, M. H., Yazdanbakhsh, M., & Hokke, C. H. (2012). Schistosoma mansoni egg glycoproteins and C-type lectins of host immune cells: molecular partners that shape immune responses. *Exp Parasitol*, *132*(1), 14-21. https://doi.org/10.1016/j.exppara.2011.05.005

Mehlhorn, H., Becker, B., Andrews, P., Thomas, H., & Frenkel, J. K. (1981). In vivo and in vitro experiments on the effects of praziquantel on Schistosoma mansoni. A light and electron microscopic study. *Arzneimittelforschung*, *31*(3a), 544-554. https://www.ncbi.nlm.nih.gov/pubmed/7195245

Mesters, J. R., Barinka, C., Li, W., Tsukamoto, T., Majer, P., Slusher, B. S., Konvalinka, J., & Hilgenfeld, R. (2006). Structure of glutamate carboxypeptidase II, a drug target in neuronal damage and prostate cancer. *Embo J*, *25*(6), 1375-1384. https://doi.org/10.1038/sj.emboj.7600969

Mgeni, A. F., Kisumku, U. M., McCullough, F. S., Dixon, H., Yoon, S. S., & Mott, K. E. (1990). Metrifonate in the control of urinary schistosomiasis in Zanzibar. *Bull World Health Organ*, *68*(6), 721-730. https://www.ncbi.nlm.nih.gov/pubmed/2127381

Miller, M. J., & Reid, E. C. (1967). Steroid therapy in chronic urinary schistosomiasis (bilharziasis). *Cancer Med Assoc J*, *97*(11), 594-599. https://www.ncbi.nlm.nih.gov/pubmed/6050892

Mitchell, G. F. (1989). Glutathione S-transferases - potential components of anti-schistosome vaccines? *Parasitol Today*, *5*(2), 34-37. https://doi.org/10.1016/0169-4758(89)90185-3

Mo, A. X., & Colley, D. G. (2016). Workshop report: Schistosomiasis vaccine clinical development and product characteristics. *Vaccine*, *34*(8), 995-1001. https://doi.org/10.1016/j.vaccine.2015.12.032

MoEST. (2011). The national portfolio formulation document *(NPFD)*. The Ministry of Environment, Science and Technology Retrieved from https://www.thegef.org/sites/default/files/documents/Ghana_-_NPFD_0.pdf

Molehin, A. J. (2020). Current Understanding of Immunity Against Schistosomiasis: Impact on Vaccine and Drug Development. *Res Rep Trop Med*, *11*, 119-128. https://doi.org/10.2147/RRTM.S274518

Molehin, A. J., McManus, D. P., & You, H. (2022). Vaccines for Human Schistosomiasis: Recent Progress, New Developments and Future Prospects. *Int J Mol Sci*, *23*(4). https://doi.org/10.3390/ijms23042255

Molehin, A. J., Rojo, J. U., Siddiqui, S. Z., Gray, S. A., Carter, D., & Siddiqui, A. A. (2016). Development of a schistosomiasis vaccine. *Expert Rev Vaccines*, *15*(5), 619-627. https://doi.org/10.1586/14760584.2016.1131127

Molehin, A. J., Sennoune, S. R., Zhang, W., Rojo, J. U., Siddiqui, A. J., Herrera, K. A., Johnson, L., Sudduth, J., May, J., & Siddiqui, A. A. (2017). Cross-species prophylactic efficacy of Sm-p80-based vaccine and intracellular localization of Sm-p80/Sm-p80 ortholog proteins during development in Schistosoma mansoni, Schistosoma japonicum, and Schistosoma haematobium. *Parasitol Res*, *116*(11), 3175-3188. https://doi.org/10.1007/s00436-017-5634-4

Molyneux, D. H., Savioli, L., & Engels, D. (2017). Neglected tropical diseases: progress towards addressing the chronic pandemic. *Lancet*, *389*(10066), 312-325. https://doi.org/10.1016/S0140-6736(16)30171-4

Mone, H., & Boissier, J. (2004). Sexual biology of schistosomes. *Adv Parasitol*, *57*, 89-189. https://doi.org/10.1016/S0065-308X(04)57002-1

Mone, H., Ibikounle, M., Massougbodji, A., & Mouahid, G. (2010). Human Schistosomiasis in the Economic Community of West African States: Epidemiology and Control. *Adv Parasitol, Vol 71*, *71*, 33-91. https://doi.org/10.1016/S0065-308x(10)71001-0

Montgomery, S. (2020). *Chapter 4: Travel-Related Infectious Diseases*. Retrieved from https://wwwnc.cdc.gov/travel/yellowbook/2020/travel-related-infectious-diseases/schistosomiasis#map411

Mulero, S., Rey, O., Arancibia, N., Mas-Coma, S., & Boissier, J. (2019). Persistent establishment of a tropical disease in Europe: the preadaptation of schistosomes to overwinter. *Parasit Vectors*, *12*(1), 379. https://doi.org/10.1186/s13071-019-3635-0

Neale, J. H., Bzdega, T., & Wroblewska, B. (2000). N-Acetylaspartylglutamate: the most abundant peptide neurotransmitter in the mammalian central nervous system. *J Neurochem*, *75*(2), 443-452. https://doi.org/10.1046/j.1471-4159.2000.0750443.x

Nelwan, M. L. (2019). Schistosomiasis: Life Cycle, Diagnosis, and Control. *Curr Ther Res Clin Exp*, *91*, 5-9. https://doi.org/10.1016/j.curtheres.2019.06.001

Nogueira, R. A., Lira, M. G. S., Lica, I. C. L., Frazao, G., Dos Santos, V. A. F., Filho, A., Rodrigues, J. G. M., Miranda, G. S., Carvalho, R. C., & Nascimento, F. R. F. (2022). Praziquantel: An update on the mechanism of its action against schistosomiasis and new therapeutic perspectives. *Mol Biochem Parasitol*, *252*, 111531. https://doi.org/10.1016/j.molbiopara.2022.111531

Norseth, H. M., Ndhlovu, P. D., Kleppa, E., Randrianasolo, B. S., Jourdan, P. M., Roald, B., Holmen, S. D., Gundersen, S. G., Bagratee, J., Onsrud, M., & Kjetland, E. F. (2014). The Colposcopic Atlas of Schistosomiasis in the Lower Female Genital Tract Based on Studies in Malawi, Zimbabwe, Madagascar and South Africa. *PLoS Negl Trop Dis*, *8*(11). https://doi.org/ARTN e322910.1371/journal.pntd.0003229

Nyarko, R., Torpey, K., & Ankomah, A. (2018). Schistosoma haematobium, Plasmodium falciparum infection and anaemia in children in Accra, Ghana. *Trop Dis Travel Med Vaccines*, *4*, 3. https://doi.org/10.1186/s40794-018-0063-7

Obonyo, C. O., Muok, E. M., & Mwinzi, P. N. (2010). Efficacy of artesunate with sulfalene plus pyrimethamine versus praziquantel for treatment of Schistosoma mansoni in Kenyan children: an open-label randomised controlled trial. *Lancet Infect Dis*, *10*(9), 603-611. https://doi.org/10.1016/S1473-3099(10)70161-4

Olliaro, P. L., Vaillant, M. T., Belizario, V. J., Lwambo, N. J., Ouldabdallahi, M., Pieri, O. S., Amarillo, M. L., Kaatano, G. M., Diaw, M., Domingues, A. C., Favre, T. C., Lapujade, O., Alves, F., & Chitsulo, L. (2011). A multicentre randomized controlled trial of the efficacy and safety of single-dose praziquantel at 40 mg/kg vs. 60 mg/kg for treating intestinal schistosomiasis in the Philippines, Mauritania, Tanzania and Brazil. *PLoS Negl Trop Dis*, *5*(6), e1165. https://doi.org/10.1371/journal.pntd.0001165

Oostburg, B. F. (1972). Clinical trial with hycanthone in Schistosomiasis mansoni in Surinam. *Trop Geogr Med*, *24*(2), 148-151. https://www.ncbi.nlm.nih.gov/pubmed/4402580

Pangalos, M. N., Neefs, J. M., Somers, M., Verhasselt, P., Bekkers, M., van der Helm, L., Fraiponts, E., Ashton, D., & Gordon, R. D. (1999a). Isolation and expression of novel human glutamate carboxypeptidases with N-acetylated alpha-linked acidic dipeptidase and dipeptidyl peptidase IV activity. *J Biol Chem*, *274*(13), 8470-8483. https://doi.org/DOI 10.1074/jbc.274.13.8470

Pangalos, M. N., Neefs, J. M., Somers, M., Verhasselt, P., Bekkers, M., van der Helm, L., Fraiponts, E., Ashton, D., & Gordon, R. D. (1999b). Isolation and expression of novel human glutamate carboxypeptidases with N-acetylated alpha-linked acidic dipeptidase and dipeptidyl peptidase IV activity. *J Biol Chem*, *274*(13), 8470-8483. https://doi.org/10.1074/jbc.274.13.8470

Parker-Manuel, S. J., Ivens, A. C., Dillon, G. P., & Wilson, R. A. (2011). Gene expression patterns in larval Schistosoma mansoni associated with infection of the mammalian host. *PLoS Negl Trop Dis*, *5*(8), e1274. https://doi.org/10.1371/journal.pntd.0001274

Pax, R., Bennett, J. L., & Fetterer, R. (1978). A benzodiazepine derivative and praziquantel: effects on musculature of Schistosoma mansoni and Schistosoma japonicum. *Naunyn Schmiedebergs Arch Pharmacol*, *304*(3), 309-315. https://doi.org/10.1007/BF00507974

Pearce, E. J., & MacDonald, A. S. (2002). The immunobiology of schistosomiasis. *Nat Rev Immunol*, *2*(7), 499-511. https://doi.org/10.1038/nri843

Pennance, T., Allan, F., Emery, A., Rabone, M., Cable, J., Garba, A. D., Hamidou, A. A., Webster, J. P., Rollinson, D., & Webster, B. L. (2020). Interactions between Schistosoma haematobium group species and their Bulinus spp. intermediate hosts along the Niger River Valley. *Parasit Vectors*, *13*(1). https://doi.org/ARTN 26810.1186/s13071-020-04136-9

Pennington, L. F., Alouffi, A., Mbanefo, E. C., Ray, D., Heery, D. M., Jardetzky, T. S., Hsieh, M. H., & Falcone, F. H. (2017). H-IPSE Is a Pathogen-Secreted Host Nucleus-Infiltrating Protein (Infiltrin) Expressed Exclusively by the Schistosoma haematobium Egg Stage. *Infect Immun*, *85*(12). https://doi.org/10.1128/IAI.00301-17

Peterkova, K., Vorel, J., Ilgova, J., Ostasov, P., Fajtova, P., Konecny, L., Chanova, M., Kasny, M., Horn, M., & Dvorak, J. (2023). Proteases and their inhibitors involved in Schistosoma mansoni egg-host interaction revealed by comparative transcriptomics with Fasciola hepatica eggs. *Int J Parasitol*. https://doi.org/10.1016/j.ijpara.2022.12.007

Pinheiro, C. S., Ribeiro, A. P., Cardoso, F. C., Martins, V. P., Figueiredo, B. C., Assis, N. R., Morais, S. B., Caliari, M. V., Loukas, A., & Oliveira, S. C. (2014). A multivalent chimeric vaccine composed of Schistosoma mansoni SmTSP-2 and Sm29 was able to induce protection against infection in mice. *Parasite Immunol*, *36*(7), 303-312. https://doi.org/10.1111/pim.12118

Pinto, J. T., Suffoletto, B. P., Berzin, T. M., Qiao, C. H., Lin, S., Tong, W. P., May, F., Mukherjee, B., & Heston, W. D. (1996). Prostate-specific membrane antigen: a novel folate hydrolase in human prostatic carcinoma cells. *Clin Cancer Res*, *2*(9), 1445-1451. https://www.ncbi.nlm.nih.gov/pubmed/9816319

Pitchford, R. J. (1966). Findings in relation to schistosome transmission in the field following the introduction of various control measures. *S Afr Med J*, *40*(36), Suppl 40:41-16. https://www.ncbi.nlm.nih.gov/pubmed/5920426

Pitchford, R. J. (1970a). Control of bilharziasis by rural management. *Cent Afr J Med*, Suppl:31-33. https://www.ncbi.nlm.nih.gov/pubmed/5471345

Pitchford, R. J. (1970b). Further observations on bilharzia control in the Eastern Transvaal. *S Afr Med J*, *44*(16), 475-477. https://www.ncbi.nlm.nih.gov/pubmed/5443302

Poddighe, D., Castelli, L., Pulcrano, G., Grosini, A., Balzaretti, M., Spadaro, S., & Bruni, P. (2016). Urinary Schistosomiasis in an Adolescent Refugee from Africa: An Uncommon Cause of Hematuria and an Emerging Infectious Disease in Europe. *J Immigr Minor Health*, *18*(5), 1237-1240. https://doi.org/10.1007/s10903-015-0272-3

Rawlings, N. D., Barrett, A. J., Thomas, P. D., Huang, X., Bateman, A., & Finn, R. D. (2018). The MEROPS database of proteolytic enzymes, their substrates and inhibitors in 2017 and a comparison with peptidases in the PANTHER database. *Nucleic Acids Res*, *46*(D1), D624-D632. https://doi.org/10.1093/nar/gkx1134

Rawlings, N. D., & Morton, F. R. (2008). The MEROPS batch BLAST: a tool to detect peptidases and their non-peptidase homologues in a genome. *Biochimie*, *90*(2), 243-259. https://doi.org/10.1016/j.biochi.2007.09.014

Reguera-Gomez, M., Valero, M. A., Artigas, P., De Elias-Escribano, A., Fantozzi, M. C., Luzon-Garcia, M. P., Salas-Coronas, J., Boissier, J., Mas-Coma, S., & Bargues, M. D. (2023). Geographical Influence on Morphometric Variability of Genetically "Pure" Schistosoma haematobium Eggs from Sub-Saharan Migrants in Spain. *Trop Med Infect Dis*, *8*(3). https://doi.org/10.3390/tropicalmed8030144

Renneberg, H., Friedetzky, A., Konrad, L., Kurek, R., Weingartner, K., Wennemuth, G., Tunn, U. W., & Aumuller, G. (1999). Prostate specific membrane antigen (PSM) is expressed in various human tissues: implication for the use of PSM reverse transcription polymerase chain reaction to detect hematogenous prostate cancer spread. *Urol Res*, *27*(1), 23-27. https://doi.org/DOI 10.1007/s002400050085

Reta, B., & Erko, B. (2013). Efficacy and side effects of praziquantel in the treatment for Schistosoma mansoni infection in school children in Senbete Town, northeastern Ethiopia. *Trop Med Int Health*, *18*(11), 1338-1343. https://doi.org/10.1111/tmi.12187

Rinaldi, G., Morales, M. E., Alrefaei, Y. N., Cancela, M., Castillo, E., Dalton, J. P., Tort, J. F., & Brindley, P. J. (2009). RNA interference targeting leucine aminopeptidase blocks hatching of Schistosoma mansoni eggs. *Mol Biochem Parasitol*, *167*(2), 118-126. https://doi.org/10.1016/j.molbiopara.2009.05.002

Ritter, D. M., & McKerrow, J. H. (1996). Intercellular adhesion molecule 1 is the major adhesion molecule expressed during schistosome granuloma formation. *Infect Immun*, *64*(11), 4706-4713. https://doi.org/10.1128/iai.64.11.4706-4713.1996

Riveau, G., Schacht, A. M., Dompnier, J. P., Deplanque, D., Seck, M., Waucquier, N., Senghor, S., Delcroix-Genete, D., Hermann, E., Idris-Khodja, N., Levy-Marchal, C., Capron, M., & Capron, A. (2018). Safety and efficacy of the rSh28GST urinary schistosomiasis vaccine: A phase 3 randomized, controlled trial in Senegalese children. *PLoS Negl Trop Dis*, *12*(12), e0006968. https://doi.org/10.1371/journal.pntd.0006968

Robinson, M. B., Blakely, R. D., Couto, R., & Coyle, J. T. (1987). Hydrolysis of the Brain Dipeptide N-Acetyl-L-Aspartyl-L-Glutamate - Identification and Characterization of a Novel N-Acetylated Alpha-Linked Acidic Dipeptidase Activity from Rat-Brain. *J Biol Chem*, *262*(30), 14498-14506. <Go to ISI>://WOS:A1987K522100022

Rollinson, D., Knopp, S., Levitz, S., Stothard, J. R., Tchuente, L. A. T., Garba, A., Mohammed, K. A., Schur, N., Person, B., Colley, D. G., & Utzinger, J. (2013). Time to set the agenda for schistosomiasis elimination. *Acta Trop*, *128*(2), 423-440. https://doi.org/10.1016/j.actatropica.2012.04.013

Ross, A. G., Bartley, P. B., Sleigh, A. C., Olds, G. R., Li, Y., Williams, G. M., & McManus, D. P. (2002). Schistosomiasis. *N Engl J Med*, *346*(16), 1212-1220. https://doi.org/10.1056/NEJMra012396

Ross, A. G., McManus, D. P., Farrar, J., Hunstman, R. J., Gray, D. J., & Li, Y. S. (2012). Neuroschistosomiasis. *J Neurol*, *259*(1), 22-32. https://doi.org/10.1007/s00415-011-6133-7

Ross, A. G., Vickers, D., Olds, G. R., Shah, S. M., & McManus, D. P. (2007). Katayama syndrome. *Lancet Infect Dis*, *7*(3), 218-224. https://doi.org/10.1016/S1473-3099(07)70053-1

Rovenska, M., Hlouchova, K., Sacha, P., Mlcochova, P., Horak, V., Zamecnik, J., Barinka, C., & Konvalinka, J. (2008). Tissue expression and enzymologic characterization of human prostate specific membrane antigen and its rat and pig orthologs. *Prostate*, *68*(2), 171-182. https://doi.org/10.1002/pros.20676

Rutitzky, L. I., & Stadecker, M. J. (2011). Exacerbated egg-induced immunopathology in murine Schistosoma mansoni infection is primarily mediated by IL-17 and restrained by IFN-gamma. *Eur J Immunol*, *41*(9), 2677-2687. https://doi.org/10.1002/eji.201041327

Sacha, P., Zamecnik, J., Barinka, C., Hlouchova, K., Vicha, A., Mlcochova, P., Hilgert, I., Eckschlager, T., & Konvalinka, J. (2007). Expression of glutamate carboxypeptidase II in human brain. *Neuroscience*, *144*(4), 1361-1372. https://doi.org/10.1016/j.neuroscience.2006.10.022

Sady, H., Al-Mekhlafi, H. M., Webster, B. L., Ngui, R., Atroosh, W. M., Al-Delaimy, A. K., Nasr, N. A., Chua, K. H., Lim, Y. A., & Surin, J. (2015). New insights into the genetic diversity of Schistosoma mansoni and S. haematobiumin Yemen. *Parasit Vectors*, *8*, 544. https://doi.org/10.1186/s13071-015-1168-8

Sajid, M., McKerrow, J. H., Hansell, E., Mathieu, M. A., Lucas, K. D., Hsieh, I., Greenbaum, D., Bogyo, M., Salter, J. P., Lim, K. C., Franklin, C., Kim, J. H., & Caffrey, C. R. (2003). Functional expression and characterization of Schistosoma mansoni cathepsin B and its trans-activation by an endogenous asparaginyl endopeptidase. *Mol Biochem Parasitol*, *131*(1), 65-75. https://doi.org/10.1016/s0166-6851(03)00194-4

Salvador-Recatala, V., & Greenberg, R. M. (2012). Calcium channels of schistosomes: unresolved questions and unexpected answers. *Wiley Interdiscip Rev Membr Transp Signal*, *1*(1), 85-93. https://doi.org/10.1002/wmts.19

Samoil, V., Dagenais, M., Ganapathy, V., Aldridge, J., Glebov, A., Jardim, A., & Ribeiro, P. (2018). Vesicle-based secretion in schistosomes: Analysis of protein and microRNA (miRNA) content of exosome-like vesicles derived from Schistosoma mansoni. *Sci Rep*, *8*(1), 3286. https://doi.org/10.1038/s41598-018-21587-4

Santini-Oliveira, M., Coler, R. N., Parra, J., Veloso, V., Jayashankar, L., Pinto, P. M., Ciol, M. A., Bergquist, R., Reed, S. G., & Tendler, M. (2016). Schistosomiasis vaccine candidate Sm14/GLA-SE: Phase 1 safety and immunogenicity clinical trial in healthy, male adults. *Vaccine*, *34*(4), 586-594. https://doi.org/10.1016/j.vaccine.2015.10.027

Santini-Oliveira, M., Machado Pinto, P., Santos, T. D., Vilar, M. M., Grinsztejn, B., Veloso, V., Paes-de-Almeida, E. C., Amaral, M. A. Z., Ramos, C. R., Marroquin-Quelopana, M., Coler, R., Reed, S., Ciol, M. A., Savino, W., Parra, J. C., Almeida, M., & Tendler, M. (2022). Development of the Sm14/GLA-SE Schistosomiasis Vaccine Candidate: An Open, Non-Placebo-Controlled, Standardized-Dose Immunization Phase Ib Clinical Trial Targeting Healthy Young Women. *Vaccines (Basel)*, *10*(10). https://doi.org/10.3390/vaccines10101724

Schistosoma japonicum Genome, S., & Functional Analysis, C. (2009). The Schistosoma japonicum genome reveals features of host-parasite interplay. *Nature*, *460*(7253), 345-351. https://doi.org/10.1038/nature08140

Schramm, G., Mohrs, K., Wodrich, M., Doenhoff, M. J., Pearce, E. J., Haas, H., & Mohrs, M. (2007). Cutting edge: IPSE/alpha-1, a glycoprotein from Schistosoma mansoni eggs, induces IgE-dependent, antigen-independent IL-4 production by murine basophils in vivo. *J Immunol*, *178*(10), 6023-6027. https://doi.org/10.4049/jimmunol.178.10.6023

Schwartz, C., & Fallon, P. G. (2018). Schistosoma "Eggs-Iting" the Host: Granuloma Formation and Egg Excretion. *Front Immunol*, *9*, 2492. https://doi.org/10.3389/fimmu.2018.02492

Schwartz, C., Oeser, K., Prazeres da Costa, C., Layland, L. E., & Voehringer, D. (2014). T cell-derived IL-4/IL-13 protects mice against fatal Schistosoma mansoni infection independently of basophils. *J Immunol*, *193*(7), 3590-3599. https://doi.org/10.4049/jimmunol.1401155

Secor, W. E., dos Reis, M. G., Ramos, E. A., Matos, E. P., Reis, E. A., do Carmo, T. M., & Harn, D. A., Jr. (1994). Soluble intercellular adhesion molecules in human schistosomiasis: correlations with disease severity and decreased responsiveness to egg antigens. *Infect Immun*, *62*(7), 2695-2701. https://doi.org/10.1128/iai.62.7.2695-2701.1994

Shekhar, K. C. (1991). Schistosomiasis drug therapy and treatment considerations. *Drugs*, *42*(3), 379-405. https://doi.org/10.2165/00003495-199142030-00004

Silva, L. L., Marcet-Houben, M., Zerlotini, A., Gabaldon, T., Oliveira, G., & Nahum, L. A. (2011). Evolutionary histories of expanded peptidase families in Schistosoma mansoni. *Mem Inst Oswaldo Cruz*, *106*(7), 864-877. https://doi.org/10.1590/s0074-02762011000700013

Silver, D. A., Pellicer, I., Fair, W. R., Heston, W. D., & Cordon-Cardo, C. (1997). Prostate-specific membrane antigen expression in normal and malignant human tissues. *Clin Cancer Res*, *3*(1), 81-85. https://www.ncbi.nlm.nih.gov/pubmed/9815541

Sissoko, M. S., Dabo, A., Traore, H., Diallo, M., Traore, B., Konate, D., Niare, B., Diakite, M., Kamate, B., Traore, A., Bathily, A., Tapily, A., Toure, O. B., Cauwenbergh, S., Jansen, H. F., & Doumbo, O. K. (2009). Efficacy of artesunate + sulfamethoxypyrazine/pyrimethamine versus praziquantel in the treatment of Schistosoma haematobium in children. *PLoS One*, *4*(10), e6732. https://doi.org/10.1371/journal.pone.0006732

Smith, P., Fallon, R. E., Mangan, N. E., Walsh, C. M., Saraiva, M., Sayers, J. R., McKenzie, A. N., Alcami, A., & Fallon, P. G. (2005). Schistosoma mansoni secretes a chemokine binding protein

with antiinflammatory activity. *J Exp Med*, *202*(10), 1319-1325. https://doi.org/10.1084/jem.20050955

Soares Magalhaes, R. J., Biritwum, N. K., Gyapong, J. O., Brooker, S., Zhang, Y., Blair, L., Fenwick, A., & Clements, A. C. (2011). Mapping helminth co-infection and co-intensity: geostatistical prediction in ghana. *PLoS Negl Trop Dis*, *5*(6), e1200. https://doi.org/10.1371/journal.pntd.0001200

Sojka, D., Pytelkova, J., Perner, J., Horn, M., Konvickova, J., Schrenkova, J., Mares, M., & Kopacek, P. (2016). Multienzyme degradation of host serum albumin in ticks. *Ticks Borne Dis*, *7*(4), 604-613. https://doi.org/10.1016/j.ttbdis.2015.12.014

Sombetzki, M., Rabes, A., Bischofsberger, M., Winkelmann, F., Koslowski, N., Schulz, C., & Reisinger, E. C. (2019). Preventive CTLA-4-Ig Treatment Reduces Hepatic Egg Load and Hepatic Fibrosis in Schistosoma mansoni-Infected Mice. *Biomed Res Int*, *2019*, 1704238. https://doi.org/10.1155/2019/1704238

Soto, S. M. (2009). Human migration and infectious diseases. *Clin Microbiol Infect*, *15 Suppl 1*, 26-28. https://doi.org/10.1111/j.1469-0691.2008.02694.x

Sousa-Figueiredo, J. C., Betson, M., Atuhaire, A., Arinaitwe, M., Navaratnam, A. M., Kabatereine, N. B., Bickle, Q., & Stothard, J. R. (2012). Performance and safety of praziquantel for treatment of intestinal schistosomiasis in infants and preschool children. *PLoS Negl Trop Dis*, *6*(10), e1864. https://doi.org/10.1371/journal.pntd.0001864

Standley, C. J., Mugisha, L., Dobson, A. P., & Stothard, J. R. (2012). Zoonotic schistosomiasis in non-human primates: past, present and future activities at the human-wildlife interface in Africa. *J of Helminthol*, *86*(2), 131-140. <Go to ISI>://WOS:000303891800001

Stefanic, S., Dvorak, J., Horn, M., Braschi, S., Sojka, D., Ruelas, D. S., Suzuki, B., Lim, K. C., Hopkins, S. D., McKerrow, J. H., & Caffrey, C. R. (2010). RNA Interference in Schistosoma mansoni Schistosomula: Selectivity, Sensitivity and Operation for Larger-Scale Screening. *PLoS Negl Trop Dis*, *4*(10). https://doi.org/ARTN e85010.1371/journal.pntd.0000850

Steinfelder, S., Andersen, J. F., Cannons, J. L., Feng, C. G., Joshi, M., Dwyer, D., Caspar, P., Schwartzberg, P. L., Sher, A., & Jankovic, D. (2009). The major component in schistosome eggs responsible for conditioning dendritic cells for Th2 polarization is a T2 ribonuclease (omega-1). *J Exp Med*, *206*(8), 1681-1690. https://doi.org/10.1084/jem.20082462

Sturrock, R. F., Diaw, O. T., Talla, I., Niang, M., Piau, J. P., & Capron, A. (2001). Seasonality in the transmission of schistosomiasis and in populations of its snail intermediate hosts in and around a sugar irrigation scheme at Richard Toll, Senegal. *Parasitol*, *123 Suppl*, S77-89. https://doi.org/10.1017/s0031182001008125

Suzuki, K., Hata, S., Kawabata, Y., & Sorimachi, H. (2004). Structure, activation, and biology of calpain. *Diabetes*, *53 Suppl 1*, S12-18. https://doi.org/10.2337/diabetes.53.2007.s12

Tchuem Tchuente, L. A., Rollinson, D., Stothard, J. R., & Molyneux, D. (2017). Moving from control to elimination of schistosomiasis in sub-Saharan Africa: time to change and adapt strategies. *Infect Dis Poverty*, *6*(1), 42. https://doi.org/10.1186/s40249-017-0256-8

Thomas, P. G., & Harn, D. A., Jr. (2004). Immune biasing by helminth glycans. *Cell Microbiol*, *6*(1), 13-22. https://doi.org/10.1046/j.1462-5822.2003.00337.x

Toor, J., Turner, H. C., Truscott, J. E., Werkman, M., Phillips, A. E., Alsallaq, R., Medley, G. F., King, C. H., & Anderson, R. M. (2018). The design of schistosomiasis monitoring and evaluation programmes: The importance of collecting adult data to inform treatment strategies for Schistosoma mansoni. *PLoS Negl Trop Dis*, *12*(10), e0006717. https://doi.org/10.1371/journal.pntd.0006717

Troyer, J. K., Beckett, M. L., & Wright, G. L., Jr. (1995). Detection and characterization of the prostate-specific membrane antigen (PSMA) in tissue extracts and body fluids. *Int J Cancer*, *62*(5), 552-558. https://doi.org/10.1002/ijc.2910620511

Tuffour, I., Ayi, I., Gwira, T. M., Dumashie, E., Ashong, Y., & Appiah-Opong, R. (2018). Schistosoma Egg Antigen Induces Oncogenic Alterations in Human Prostate Cells. *Anal Cell Pathol (Amst)*, *2018*, 4675380. https://doi.org/10.1155/2018/4675380

Tuljapurkar, S. (1991). Infectious-Diseases of Humans - Dynamics and Control - Anderson,Rm, May,Rm. *Science*, *254*(5031), 591-592. https://doi.org/DOI 10.1126/science.254.5031.591

Tykvart, J., Barinka, C., Svoboda, M., Navratil, V., Soucek, R., Hubalek, M., Hradilek, M., Sacha, P., Lubkowski, J., & Konvalinka, J. (2015). Structural and Biochemical Characterization of a Novel Aminopeptidase from Human Intestine. *J Biol Chem*, *290*(18), 11321-11336. https://doi.org/10.1074/jbc.M114.628149

Tykvart, J., Navratil, V., Sedlak, F., Corey, E., Colombatti, M., Fracasso, G., Koukolik, F., Barinka, C., Sacha, P., & Konvalinka, J. (2014). Comparative analysis of monoclonal antibodies against prostate-specific membrane antigen (PSMA). *Prostate*, *74*(16), 1674-1690. https://doi.org/10.1002/pros.22887

Ulrychova, L., Horn, M., & Dvorak, J. (2020). Sensitive Fluorescence In Situ Hybridization on Semithin Sections of Adult Schistosoma mansoni Using DIG-Labeled RNA Probes. *Methods Mol Biol*, *2151*, 43-53. https://doi.org/10.1007/978-1-0716-0635-3_4

Ulrychova, L., Ostasov, P., Chanova, M., Mares, M., Horn, M., & Dvorak, J. (2021). Spatial expression pattern of serine proteases in the blood fluke Schistosoma mansoni determined by fluorescence RNA in situ hybridization. *Parasit Vectors*, *14*(1), 274. https://doi.org/10.1186/s13071-021-04773-8

Utroska JA, C. M., Dixon H, Yoon S, Helling-Borda M, Hogerzeil HV, Mott KE. (1989). An Estimate of Global Needs for Praziquantel within Schistosomiasis Control Programmes. whqlibdoc.who.int at<http://whqlibdoc.who.int/HQ/1989/WHO_SCHISTO_89.102_Rev1.pdf>

Utzinger, J., N'Goran, E. K., N'Dri, A., Lengeler, C., Xiao, S., & Tanner, M. (2000). Oral artemether for prevention of Schistosoma mansoni infection: randomised controlled trial. *Lancet*, *355*(9212), 1320-1325. https://doi.org/10.1016/s0140-6736(00)02114-0

Vale, N., Gouveia, M. J., Rinaldi, G., Brindley, P. J., Gartner, F., & Correia da Costa, J. M. (2017). Praziquantel for Schistosomiasis: Single-Drug Metabolism Revisited, Mode of Action, and Resistance. *Antimicrob Agents Chemother*, *61*(5). https://doi.org/10.1128/AAC.02582-16

Vale, T. C., de Sousa-Pereira, S. R., Ribas, J. G., & Lambertucci, J. R. (2012). Neuroschistosomiasis mansoni: literature review and guidelines. *Neurologist*, *18*(6), 333-342. https://doi.org/10.1097/NRL.0b013e3182704d1e

van der Velden, V. H., & Hulsmann, A. R. (1999). Peptidases: structure, function and modulation of peptide-mediated effects in the human lung. *Clin Exp Allergy*, *29*(4), 445-456. https://doi.org/10.1046/j.1365-2222.1999.00462.x

Vella, A. T., Hulsebosch, M. D., & Pearce, E. J. (1992). Schistosoma mansoni eggs induce antigen-responsive CD44-hi T helper 2 cells and IL-4-secreting CD44-lo cells. Potential for T helper 2 subset differentiation is evident at the precursor level. *J Immunol*, *149*(5), 1714-1722. https://www.ncbi.nlm.nih.gov/pubmed/1387150

Villasante Ferrer, A., Iranzo Tatay, A., Aznar Oroval, E., & Mollar Maseres, J. (2018). [Study of the current status of Schistosoma haematobium infection in the European Union. An approach to the possible risk in Spain]. *Rev Esp Salud Publica*, *92*. https://www.ncbi.nlm.nih.gov/pubmed/29637924 (Estudio de la situacion actual de la infeccion

por Schistosoma haematobium en la Union Europea. Una aproximacion al posible riesgo en Espana.)

WB. (2020). *Ghana Poverty Assessment*. World Bank. Retrieved 16.08.2022 from https://openknowledge.worldbank.org/handle/10986/34804

WB. (2021). *GDP per capita (current US$) - Ghana, Lower middle income. World Development Indicators.* The World Bank Group. Retrieved 24. 08. 2022 from https://data.worldbank.org/indicator/NY.GDP.PCAP.CD?locations=GH-XN

Webster, B. L., Diaw, O. T., Seye, M. M., Webster, J. P., & Rollinson, D. (2013). Introgressive Hybridization of Schistosoma haematobium Group Species in Senegal: Species Barrier Break Down between Ruminant and Human Schistosomes. *PLoS Negl Trop Dis*, *7*(4). https://doi.org/ARTN e211010.1371/journal.pntd.0002110

Webster, B. L., Southgate, V. R., & Littlewood, D. T. (2006). A revision of the interrelationships of Schistosoma including the recently described Schistosoma guineensis. *Int J Parasitol*, *36*(8), 947-955. https://doi.org/10.1016/j.ijpara.2006.03.005

Weerakoon, K. G., Gobert, G. N., Cai, P., & McManus, D. P. (2015). Advances in the Diagnosis of Human Schistosomiasis. *Clin Microbiol Rev*, *28*(4), 939-967. https://doi.org/10.1128/CMR.00137-14

Weinstock, J. V., & Boros, D. L. (1983). Organ-dependent differences in composition and function observed in hepatic and intestinal granulomas isolated from mice with Schistosomiasis mansoni. *J Immunol*, *130*(1), 418-422. https://www.ncbi.nlm.nih.gov/pubmed/6600190

Wendt, G., Zhao, L., Chen, R., Liu, C., O'Donoghue, A. J., Caffrey, C. R., Reese, M. L., & Collins, J. J., 3rd. (2020). A single-cell RNA-seq atlas of Schistosoma mansoni identifies a key regulator of blood feeding. *Science*, *369*(6511), 1644-1649. https://doi.org/10.1126/science.abb7709

Whitfield, P. J., Bartlett, A., Khammo, N., Brain, A. P., Brown, M. B., Marriott, C., & Clothier, R. (2003). Delayed tail loss during the invasion of human skin by schistosome cercariae. *Parasitol*, *126*(Pt 2), 135-140. https://doi.org/10.1017/s0031182002002676

WHO. (1987). *Atlas of the global distribution of schistosomiasis- Ghana*. World Health Organization (WHO). Retrieved 23/01/2021 from https://www.who.int/schistosomiasis/epidemiology/en/ghana.pdf

WHO. (2020a). *Ending the neglect to attain the sustainable development goals: a road map for neglected tropical diseases 2021–2030: overview*. World Health organization Retrieved from https://apps.who.int/iris/handle/10665/332094

WHO. (2020b). *Schistosomiasis: status of schistosomiasis endemic countries 2020*. https://apps.who.int/neglected_diseases/ntddata/sch/sch.html

WHO. (2023). *Schistosomiasis*. Retrieved 15th January 2023 from https://www.who.int/news-room/fact-sheets/detail/schistosomiasis

Wilson, R. A., Li, X. H., & Castro-Borges, W. (2016). Do schistosome vaccine trials in mice have an intrinsic flaw that generates spurious protection data? *Parasit Vectors*, *9*, 89. https://doi.org/10.1186/s13071-016-1369-9

Woodrow, C. J., Haynes, R. K., & Krishna, S. (2005). Artemisinins. *Postgrad Med J*, *81*(952), 71-78. https://doi.org/10.1136/pgmj.2004.028399

Wuhrer, M., Balog, C. I., Catalina, M. I., Jones, F. M., Schramm, G., Haas, H., Doenhoff, M. J., Dunne, D. W., Deelder, A. M., & Hokke, C. H. (2006). IPSE/alpha-1, a major secretory glycoprotein antigen from schistosome eggs, expresses the Lewis X motif on core-difucosylated N-glycans. *Febs J*, *273*(10), 2276-2292. https://doi.org/10.1111/j.1742-4658.2006.05242.x

Wynn, T. A., Thompson, R. W., Cheever, A. W., & Mentink-Kane, M. M. (2004). Immunopathogenesis of schistosomiasis. *Immunol Rev, 201*, 156-167. https://doi.org/10.1111/j.0105-2896.2004.00176.x

Yang, J., Qiu, C., Xia, Y., Yao, L., Fu, Z., Yuan, C., Feng, X., & Lin, J. (2010). Molecular cloning and functional characterization of Schistosoma japonicum enolase which is highly expressed at the schistosomulum stage. *Parasitol Res, 107*(3), 667-677. https://doi.org/10.1007/s00436-010-1913-z

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., & Madden, T. L. (2012). Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics, 13*, 134. https://doi.org/10.1186/1471-2105-13-134

Yihunie, A., Urga, B., & Alebie, G. (2019). Prevalence and risk factors of bovine schistosomiasis in Northwestern Ethiopia. *BMC Vet Res, 15*(1), 12. https://doi.org/10.1186/s12917-018-1757-9

Yirenya-Tawiah, D., Amoah, C., Apea-Kubi, K. A., Dade, M., Ackumey, M., Annang, T., Mensah, D. Y., & Bosompem, K. M. (2011). A survey of female genital schistosomiasis of the lower reproductive tract in the volta basin of Ghana. *Ghana Med J, 45*(1), 16-21. https://doi.org/10.4314/gmj.v45i1.68917

Young, N. D., Jex, A. R., Li, B., Liu, S., Yang, L., Xiong, Z., Li, Y., Cantacessi, C., Hall, R. S., Xu, X., Chen, F., Wu, X., Zerlotini, A., Oliveira, G., Hofmann, A., Zhang, G., Fang, X., Kang, Y., Campbell, B. E., . . . Gasser, R. B. (2012). Whole-genome sequence of Schistosoma haematobium. *Nat Genet, 44*(2), 221-225. https://doi.org/10.1038/ng.1065

Trans R Soc Trop Med Hyg 2023; **0**: 1–11
https://doi.org/10.1093/trstmh/trac134 Advance Access publication 0 2023

REVIEW ARTICLE

A literature review of schistosomiasis in Ghana: a reference for bridging the research and control gap

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Received 1 April 2022; revised 13 September 2022; editorial decision 25 December 2022; accepted 30 December 2022

Schistosomiasis is endemic in most sub-Saharan African countries, including Ghana, where the need for effective control including preventive chemotherapy was indicated by the WHO. Mass drug administration commenced in 2008 and has continued since then in Ghana, but the country remains highly endemic. Here, we review the literature on schistosomiasis to identify research and knowledge gaps potentially affecting disease control. A total of 100 Ghana-related schistosomiasis literature sources were reviewed, showing that most studies were conducted on epidemiology, control of transmission and diagnosis. By contrast, many aspects of this disease remain neglected, including livestock schistosomiasis and its zoonotic potential, recent distribution of disease vectors or widely overlooked genital schistosomiasis. Stratified by region, the highest number of studies focus on Greater Accra, while studies are limited or absent for several other regions. Although this review shows apparent progress in terms of schistosomiasis research and control, a considerable amount of work remains to achieve at least a reduction in the prevalence of the disease, which affects a significant proportion of the population. National epidemiological data based on a nationwide survey, integrated control and improved monitoring and evaluation must be ensured.

Keywords: disease control strategies, Ghana, mass drug administration, neglected tropical disease, schistosoma, zoonosis

Introduction

Schistosomiasis is a waterborne helminthic disease caused by blood flukes of the genus *Schistosoma*.[1] The disease is prevalent in tropical and subtropical areas, mostly affecting poor rural communities without access to safe drinking water and adequate sanitation. Activities that expose people to infested natural freshwater bodies such as fishing, agriculture, washing clothes or swimming lead to infection.[2] An estimated 240 million people are infected, several million experience severe morbidity and 700 million live at risk of infection.[3] Approximately 85% of cases occur in Africa,[4] where almost all countries report schistosomiasis.

There are three major agents of human schistosomiasis (*Schistosoma haematobium*, *Schistosoma mansoni*, *Schistosoma japonicum*) and several other less common (e.g. *Schistosoma intercalatum*, *Schistosoma guineensis* and *Schistosoma mekongi*).[5] *Schistosoma haematobium* is predominantly present in Africa, *S. mansoni* in both Africa and Latin America and *S. japonicum* in Asia. Other species occur only focally in Asia (*S. mekongi*) and Africa (*S. intercalatum*, *S. guineensis*).[6] Concerning host specificity, *S. haematobium* and *S. mansoni* are considered primarily human parasites, while others are reported to be zoonotic with a broad spectrum of mammalian hosts (e.g. bovines, dogs, pigs and sheep).[7,8] Intermediate hosts include several snail species of the genera Biomphalaria (*S. mansoni*), Oncomelania (*S. japonicum*), Bulinus (*S. haematobium*, *S. intercalatum*, *S. guineensis*) and the single species Neotricula aperta (*S. mekongi*).[4]

Humans become infected when infective larvae (cercariae) are released into water from snail intermediate hosts and penetrate the skin. They subsequently transform into migrating larvae (schistosomula) that move via the circulatory system where they feed on red blood cells, mature into adult worms and lay eggs in species-specific locations.[9] The eggs are transported either to the lumen of the urinary bladder (urogenital form caused by *S. haematobium*) or intestine (intestinal form by *S. mansoni*,

S. japonicum, *S. guineensis*, *S. intalactum* and *S. mekongi*) and are passed with excreta. However, a significant proportion of the eggs become entrapped in tissues both at the oviposition site and in distant organs such as the liver due to hematogenous spread. The eggs generally provoke tissue granuloma formation and fibrosis, resulting in associated symptoms.[10]

Apart from human schistosomiasis, livestock/animal schistosomiasis exists. The species causing animal schistosomiasis include *Schistosoma bovis*, *Schistosoma curassoni*, *Schistosoma hippopotami*, *Schistosoma indicum*, *S. intercalatum*, *Schistosoma mattheei*, *Schistosoma nasalis*, *Schistosoma rohhaini* and *Schistosoma spindale*. They infect a wide range of domestic and wild animals such as goats, sheep, pigs, cattle, horses, camels and buffaloes.[11] In addition, hybrids between *S. haematobium* and *S. bovis* and/or *S. curassoni* exist, demonstrating hybridization within the *Schistosoma* genus. These hybrids can infect both humans and animals, indicating a potential spillover either from animals to humans or vice versa.[12]

Human schistosomiasis is ranked among the most devastating tropical diseases and is a major cause of morbidity and mortality in endemic countries.[13] It is of public health concern due to years of productivity loss resulting from disease conditions. An estimated 3.31 million disability-adjusted life years and 11 700 deaths per year are attributed to schistosomiasis alone.[14,15] Moreover, livestock or animal schistosomiasis is a One Health concern due to animal morbidity and mortality, associated economic losses as well as the zoonotic threats that those infections pose, especially when there is no program targeting control of animal schistosomiasis.[16,17]

The devastating nature of schistosomiasis calls for the implementation of proper control and elimination strategies. According to the WHO, preventive chemotherapy through mass drug administration (MDA) of at-risk groups is needed in endemic countries with moderate to severe transmission. This covers about 242 million people living in 51 countries mostly in Africa, including an estimated 10.6 million in Ghana.[18] In 2019, only about 45% of these people had been treated by preventive chemotherapy.[2] Within the recently revised WHO 2021–2030 roadmap for Neglected Tropical Diseases (NTDs), a target to eliminate schistosomiasis as a public health problem in all endemic countries, and elimination in several of them, was established. Core strategic interventions have also been identified to achieve these targets, including improvements in MDA, water, sanitation and health (WASH), vector control, veterinary public health, case management and others, such as behavioral changes.[19]

The objectives of this review are to sum up the basic geographic, demographic and socioeconomic characteristics of Ghana with a focus on those that are relevant for transmission of schistosomiasis, and to provide an up-to-date literature review. Research and knowledge gaps concerning schistosomiasis in Ghana are presented, followed by suggestions for future research and control bridging necessary to reach WHO targets over the next decade.

Methods

Guided by the objectives, a comprehensive data search was performed using PubMed, Scopus and Google Scholar search engines. The search was carried out using 'schistosomiasis' and 'Ghana' as the main keywords in combination with at least one of the following: animal, control, distribution, elimination, livestock, MDA, NTDs, prevalence, snail intermediate host and transmission. Further selection was performed according to the following criteria: studies conducted in Ghana from 1954 to 2021 and published as original research articles; research must be in English and must have an abstract. Reviews, case reports and short communications were excluded. Other relevant sources such as online documents by the Centre for Disease Control, the Global Atlas of Helminth Infections (GAHI), World Bank, Ghana Statistical Service, the WHO and Ministry of Environment, Science and Technology were also used. The sources have been comprehensively reviewed and research gaps have been identified, which formed the basis of the recommendations (see Knowledge gaps, future perspectives on schistosomiasis research and control).

Results and Discussion

Geography, demography and socioeconomics of Ghana

Ghana occupies a total area of 238 533 km^2 in West Africa, of which 95% is landmass, while the remaining 5% represents waterbodies. The territory comprises six agroecological zones, including Sudan, Guinea and coastal savannahs, forest/savannah transitional zone, deciduous forest zone and the rainforest zone.[20] The main river system draining 70% of the country is formed by the Volta basin, stretching from north to south, including Lake Volta with the significantly large Akosombo Dam, as well as other minor dams constructed for agricultural purposes.[21] Two other major basin systems, the south-western rivers and the coastal basin, drain 22% and 8% of the area, respectively (Supplementary Figure S1a).

The country is divided into 16 (formerly 10) administrative regions, as shown in the map in Supplementary Figure S1b.[22] For the present study, the Upper East, Upper West and the Northern regions (the Northern region now separated into Savannah, North-East and the Northern regions) are reported as the northern sector of the country. As for the southern sector, Ashanti, Brong Ahafo (now the Ahafo, Bono and Bono East regions), Central, Eastern, Greater Accra, Volta (now the Oti and Volta regions) and the Western region (now the Western North and Western regions), are reported.

Ghana is a lower-middle income economy with a gross domestic product per capita of 2445.3 US$[23] and a poverty rate of 23.4%.[24] According to the 2021 population and housing census,[25] Ghana's total population is >30.8 million with a density of 129 persons per km^2. Its society is dominated by young people (aged 15–35 y), followed by children (aged 0–14 y), making up 38.2% and 35.3% of the total population, respectively. About 11.5 million people (58.1%) represent the labor force (i.e. the economically active population, aged ≥15 y), with a predominant 32.0% of the employed population engaged in agriculture, forestry and fishery. In addition, 182 000 (2.6%) children (aged 5–14 y) are engaged in agriculture-related economic activities. Of all people aged ≥6 y, 30.2% are illiterate. The rural population forms 43.3% of all inhabitants and only 7 out of 16 regions are urbanized. Concerning water, hygiene and

sanitation, just 38.9% of households have access to a bathroom for exclusive use and 59.3% of households possess a private toilet. The most prevalent method of disposing of household wastewater is by throwing it onto the ground/street/outside (70.6%). More than 17.7% of households do not have access to any toilet facility and open defecation is practiced in 6.2%–68.5% of households in particular regions. Almost one-tenth of households (8.0%) do not have access to an improved source of drinking water. Of those, 79.9% (i.e. nearly 2 million people) rely on surface water (river/stream/dugout/pond/dam/canal). Most poverty-associated characteristics are more prominent in rural compared with urban areas, and in northern compared with southern sectors of the country.

Overall, geographical factors make Ghana suitable for schistosomiasis. Its transmission is further enhanced by widespread open defecation and disposal of wastewater to the environment that enables uncontrolled contamination of natural water sources. Predominant agricultural and fishing activities, as well as deprived access to safe water for some households, all resulting in regular contact with contaminated water, predispose a significant proportion of the population to schistosomiasis.

Moreover, increasing demands of the growing population have contributed to the rapid spread of schistosomiasis in general and *S. mansoni* in particular, thereby changing both prevalence and distribution throughout the country over the decades (Figure 1A,B). Generally, human migration contributes significantly to the introduction or spread of infectious diseases.[26] In addition, hydrological changes due to the development and management of water resources such as dams and irrigation constructions intensified the transmission of waterborne diseases, including schistosomiasis (e.g. by providing a more stable snail habitat or by changing human water-related activities).[27]

Schistosomiasis research in Ghana

Overall, a literature search using defined keywords returned 270 items, out of which 100 were included in this review (all the included papers are listed in Table S1). The epidemiology of human schistosomiasis in Ghana is a dominating topic (assessed in 33 articles). It is followed by studies on control of transmission (21 articles) and studies focused on diagnostic methods (18 articles). Other topics on human and livestock schistosomiasis were also covered. Out of all included articles, 15 represented purely laboratory-based studies. Concerning both laboratory and field studies, the Greater Accra region featured prominently (33 articles focused on the region alone). This was followed by the Eastern region (13 studies). Other regions are a subject of 0–10 studies.

Epidemiology of schistosomiasis

Prevalence Since the early 1950s, numerous data on local prevalence of human schistosomiasis have been reported, showing values of up to 95% (Table 1). However, the data have arisen from studies focused on various groups, usually with a limited number of subjects, and performed for different purposes, such as evaluation of novel therapeutic or diagnostic approaches. National prevalence data were comprehensively summarized into prevalence map in 1987 for the first time.[28] This document was later used for several prevalence estimations,[29,30] although limited comparability and representativity of original data make any further conclusions uncertain.

Generally, almost all widely referred national prevalence data have arisen from estimations using various methods. The first national prevalence estimation was reported within 15% to 20% in 1963, based on *S. haematobium* data only.[28] Later, national prevalence estimation of 72.4% based on mathematical calculation using various parameters was reported in 1986,[29] and 70.9% based on extrapolations from the Ministry of Health and United States Agency for International Development control programs in 2010.[30] In 2015, a national prevalence estimation of 23.3% based on geostatistical analysis of some selected local data was reported in a systematic review paper.[31]

The only known national epidemiological survey was conducted during 2007–2010, when 170 districts were surveyed and >6.5 million school children were considered at risk[32]; however, the survey did not include adults and out of school-aged children who might be at risk, and the number of infected people was not reported. Common use of estimations and a lack of national epidemiologic surveys may result in overestimated or underestimated status of schistosomiasis in Ghana and significantly influence the control efforts.

Geographical distribution and transmission of schistosomiasis in Ghana The first schistosomiasis distribution map of Ghana was published by the WHO in 1987 (Figure 1A). The map depicts the co-dominance of *S. mansoni* and *S. haematobium* in the northern sector of the country, while the southern sector was mostly dominated by *S. haematobium*.[28] Later, a distribution map (Figure 1B) produced by the GAHI in 2015 depicted the widespread presence of both *S. haematobium* and *S. mansoni* throughout all regions of Ghana.[33] Maps generated by the WHO during 2016–2019 (Figure 1C) confirm that schistosomiasis is endemic in all regions of Ghana. Significant parts of southern Ghana show moderate to high endemicity, while for Brong Ahafo, Northern, Upper East and Upper West regions, low prevalence is reported. The status of endemicity changed mildly from 2016 to 2019 as some communities (Figure 1C, highlighted in the rectangle) in the Brong Ahafo and Northern regions shifted from low to high endemicity status, which is a cause for concern.

Disease diagnosis

Generally, studies on diagnosis evaluated various diagnostic tools suitable for different settings in comparison with the egg detection method by microscope, which is the 'gold standard' for schistosomiasis and still seems to represent the ideal method for disease diagnosis, surveillance and monitoring of schistosomiasis in highly endemic countries like Ghana. More complex laboratory-based diagnostic tools such as antibody/antigen detection, real-time PCR and other modified molecular methods have been evaluated as the most sensitive and specific for diagnosing schistosomiasis under various conditions[34,35]; however, their application in the field is very limited (Supplementary Table S1). Several papers evaluated field applicable methods, for example, self-reporting metrics through questionnaires and urine reagent strips/dipsticks as low-cost, rapid and easy diagnostic approaches on-site in

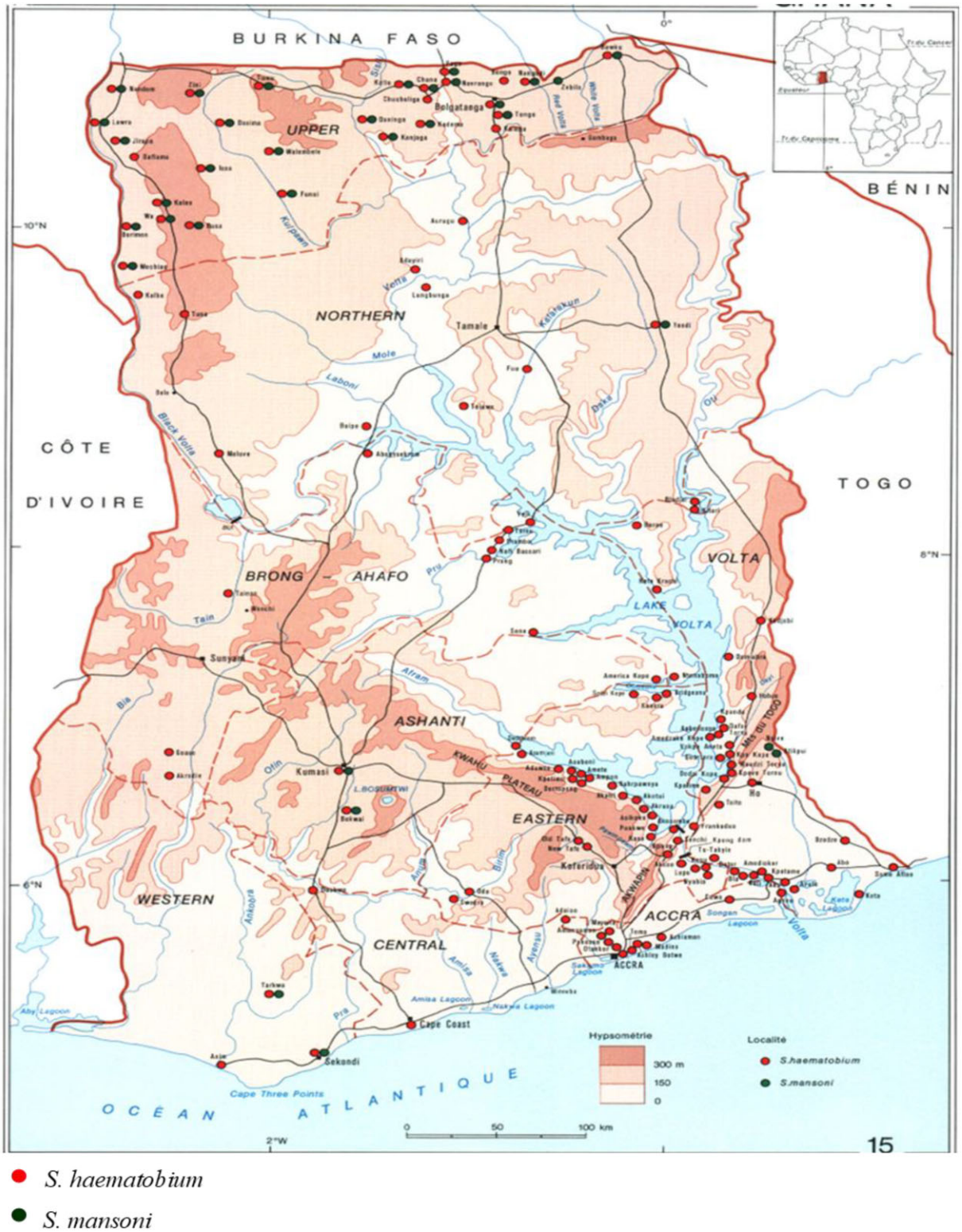
Table 1. Overview of regional/local prevalence studies of human schistosomiasis in Ghana published from 2000 to 2021 (14 studies)

SECTOR	Region	Community	Target group	Study participants	% Prevalence		Year of study	Reference
					S. mansoni	*S. haematobium*		
Northern	Northern	Kuli	Mixed	208	-	6.8	2008	36
	Upper East	Kassena-Nankana District	SAC*	1001	-	30	Not stated	37
	Upper East	Kassena-Nankana District	SAC*	1764	14.2	38	Not stated	38
Southern	Ashanti	Bunuso	Children	100	-	95	2009	39
	Central	Okyereko	Infants	97	-	33.0	2004	40
	Eastern	Adasawase	SAC*	255	-	43.6	2008	41
	Eastern and Greater Accra	Akuapem and Ga South districts	Children	354	-	83.9	Not stated	42
	Eastern and Greater Accra	Akuapem and Ga South districts	Mixed	2562	-	57.4	1992/1993	43
	Greater Accra	Accra	Mixed	91/163	49.5	12.9	Not stated	44
	Greater Accra	Accra	SAC*	730	-	7.8	Not stated	45
	Greater Accra	Doblo, Chento, Ntoaso	Adults	220	-	15.5	Not stated	45
	Greater Accra	Kasseh-sub- district	SAC*	417	-	20.9	Not stated	46
	Greater Accra	Manheam	Mixed	696	30.0	3.3	2016	47
	Greater Accra	Tomefa	Mixed	635	78.3	14.3	2016	47
	Greater Accra	Torgahkope-Adakope	Mixed	598	31	19	2016	47
	Greater Accra	Zenu	SAC*	274	-	30.7	Not stated	48
	Greater Accra	Zenu and Weija	SAC*	420	-	18.3	2016/2017	34

Only the studies that adopted the standardized microscopy diagnostic method with the stated sample size are included.

*SAC, school-age children.

Mixed: both adults and children.



● *S. haematobium*

● *S. mansoni*

Figure 1A. *Schistosoma* species distribution map of Ghana, 1987.[1] The map was adopted from the WHO with some modifications. It depicts the widespread of *S. haematobium* countrywide, while *S. mansoni* is limited to the upper part of the northern sector of the country. Most of the cases occurred along Lake Volta. https://www.who.int/schistosomiasis/epidemiology/en/ghana.pdf (accessed on 26 October 2021)

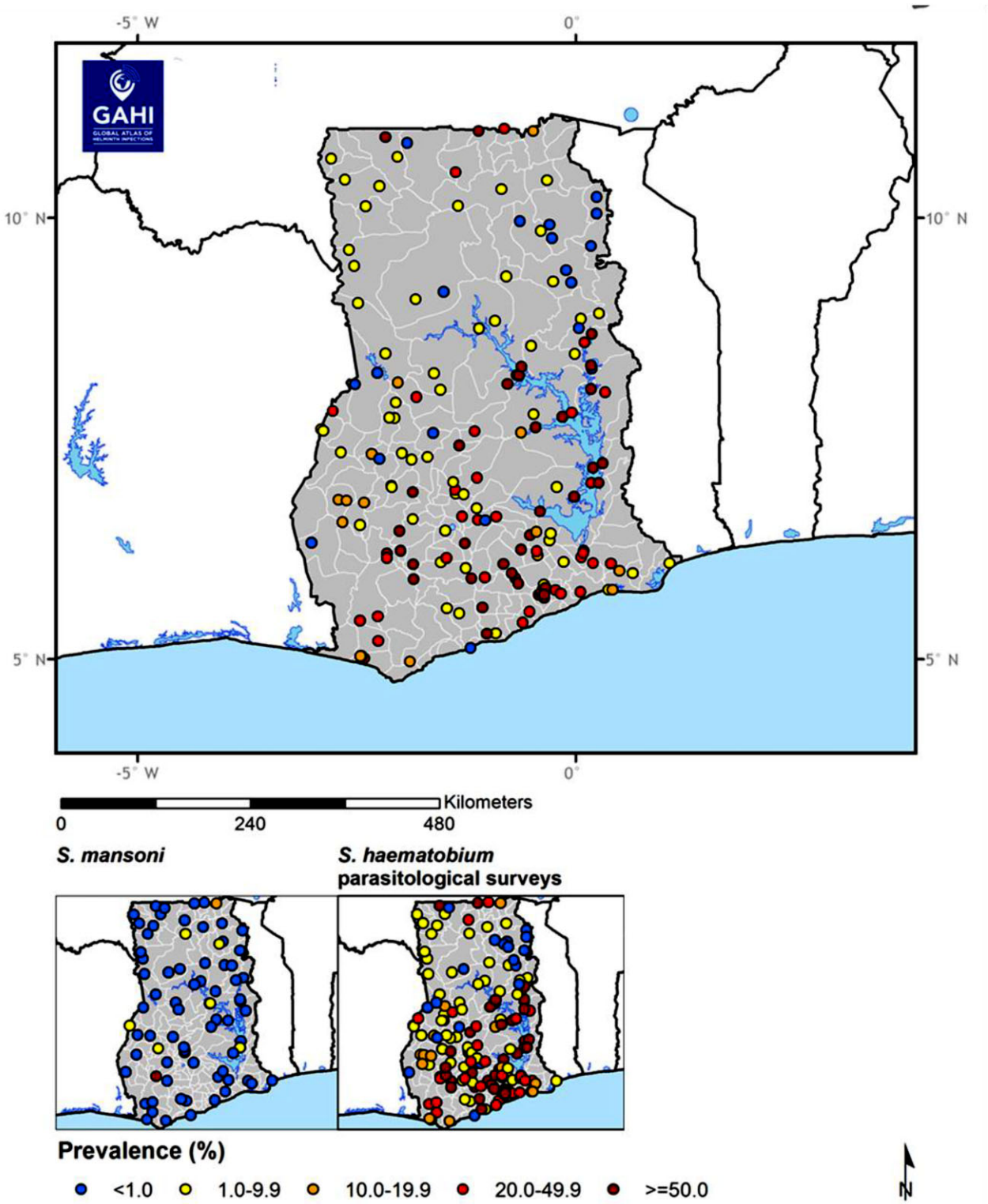


Figure 1B. *Schistosoma* species distribution and prevalence map of Ghana, 2015.[2] Adopted from Global Atlas of Helminths Infection (GAHI) with modification. The map depicts widespread of both *S. haematobium* and *S. mansoni* throughout Ghana; however, the prevalence of *S. mansoni* was very low compared with *S. haematobium*. https://www.thiswormyworld.org/maps/distribution-of-schistosomiasis-survey-data-in-ghana (accessed on 5 June 2021).

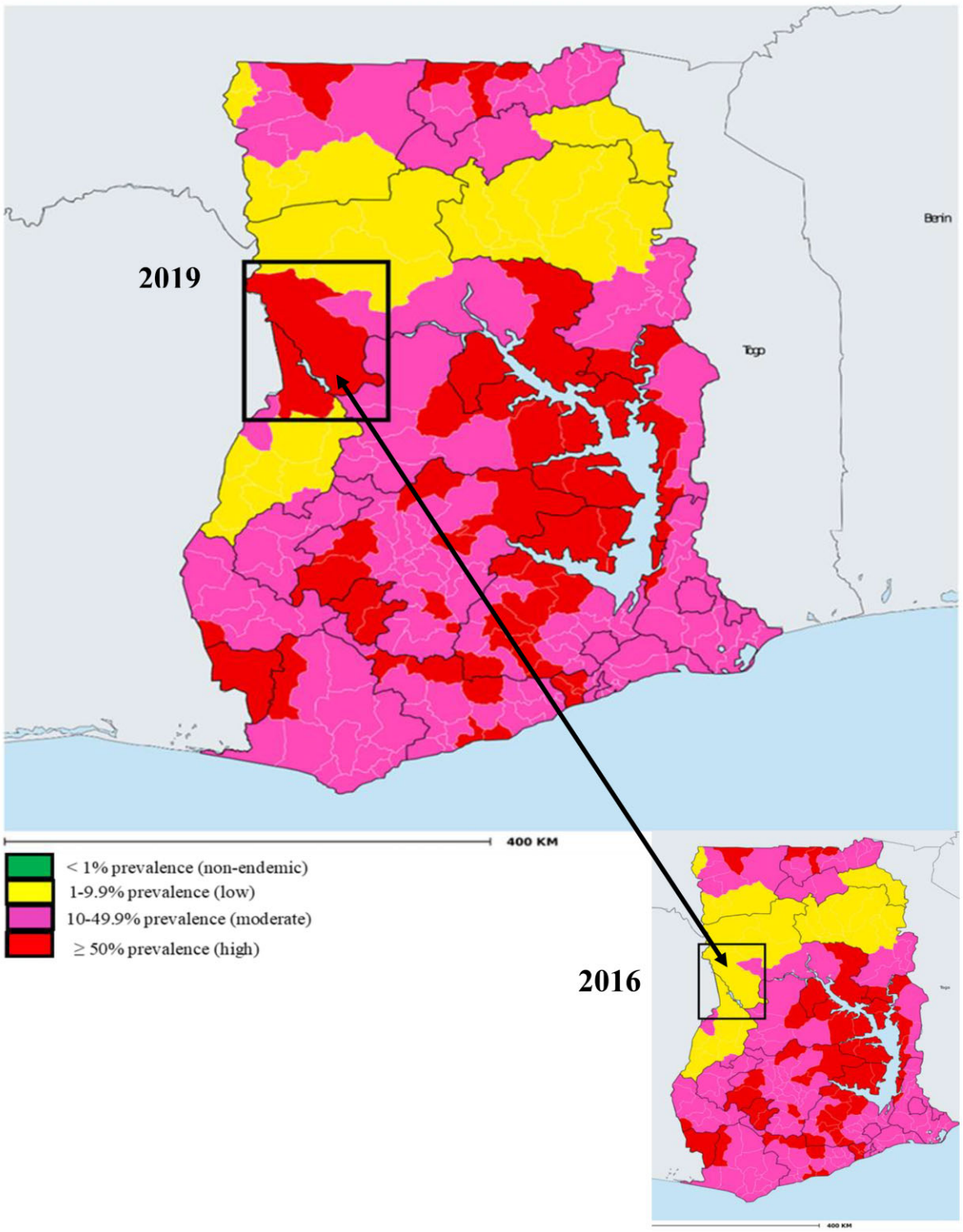


Figure 1C. Schistosomiasis distribution and prevalence map of Ghana, 2016 and 2019.[3] The maps were adopted from the WHO and modified accordingly. The maps depict that the whole country is endemic to schistosomiasis, with the vast part of Ghana having moderate to high prevalence. There is evidence of change in the endemicity status of some localities from low endemic status in 2016 to high endemic status in 2019 marked by the squares. https://espen.afro.who.int/countries/ghana (accessed on 6 June 2022).

both light and heavy infection settings.[41] Also, the potential of portable mobile phone-based devices for quality diagnostics on-site, where standard laboratory facilities are unavailable, has been documented.[49] However, none of these field-applicable methods, which would be of extremely high importance for monitoring schistosomiasis in very remote communities without access to electricity, have been implemented into routine use.

Control of schistosomiasis transmission in Ghana

Articles on transmission control are focused on snail intermediate host control, water and sanitation quality control, evaluation of human water contact activities, knowledge, awareness of schistosomiasis and finally on evaluation of MDA campaigns (Supplementary Table S1). Among efficient environmental interventions, snail control due to the removal of weeds and the application of molluscicides in water contact sites is reported.[50] On the contrary, increased levels of schistosomiasis transmission have been attributed to dam constructions, irrigation channels and proliferation of aquatic weeds.[27,51] Metrics such as water contact activities, disease symptoms awareness, knowledge of disease transmission, health-seeking behavior, knowledge of disease treatment and prevention/control were assessed. The overall knowledge of schistosomiasis in terms of symptoms among studied groups, including females and health workers, was very poor.[52] Respondents tended to have more knowledge about urogenital compared with intestinal schistosomiasis; however, for example, symptom-aware children were highly infected with *S. haematobium*.[42] The health-seeking behavior on schistosomiasis, compared with other diseases (e.g. malaria/fever), was very low and practices such as the fetching of surface water, swimming and wading were predominant among school-aged children[53] (Supplementary Table S1).

Preventive chemotherapy as a measure to control the disease was almost nonexistent in the early years[54] until 2008, a year after a nationwide survey had commenced, and the target group of this nationwide MDA was school-aged children. The most updated data on schistosomiasis MDA in Ghana and endemicity status were published in 2019 on the WHO website.[18]

Pathogenesis of human schistosomiasis

Articles on the pathogenesis of human schistosomiasis cover studies focused on female genital schistosomiasis (FGS), oncogenic alterations and immune response (Supplementary Table S1). Greater than 10% prevalence of FGS has been reported in a schistosomiasis endemic community. Also, poor knowledge has been reported elsewhere: interviewees thought that schistosomiasis was a 'boys' disease'.[52,55] However, these studies are too sparse to paint a clear picture of FGS specifically and genital schistosomiasis in general in Ghana.

Snail intermediate host

Snail surveys conducted in Ghana during 1954–1980 indicated the widespread presence of *Bulinus* spp. and *Biomphalaria pfeifferi* as vectors for *S. haematobium* and *S. mansoni*, respectively. These studies showed the presence of snail intermediate hosts in an environment dominated by water dams, drainage and irrigation channels for agricultural activities.[51,56] In addition, other studies have explored the susceptibility of snails to different strains of schistosomes in different localities, while others examined snail intermediate host control using chemical compounds together with ecological modifications (Supplementary Table S1). Data on snail distribution and prevalence for the past 3 decades are absent, although the importance of snail intermediate hosts in the transmission of schistosomiasis is clear.

Animal schistosomiasis

Two studies conducted on intestinal parasites of livestock in southern Ghana are the only available records of prevalence, reporting distant values of 0.2% and 21.7%, particularly for cattle schistosomiasis (Supplementary Table S1).[57] In a related study, the prevalence of schistosomiasis in pigs was 0.4% in northern Ghana.[58] Such a negligible focus on animal schistosomiasis in Ghana neither reflects close relationships of animal and human schistosomiasis nor the economic impact of livestock schistosomiasis.

Knowledge gaps, future perspectives on schistosomiasis research and control

According to the documents reviewed, several issues regarding schistosomiasis arise:

1. To ensure effective control, sound national epidemiological data are required for proper planning and implementation of control programs, especially for MDA campaigns. Real data, rather than estimations and inferences on at-risk group, infected people, their age groups, respective communities and parasite species, will ensure the right amount of medication is procured and the right people in need of treatment are reached. Besides, epidemiological data will also enhance the monitoring and evaluation of control programs effectively. In the past, some countries that achieved effective control and elimination (e.g. Morocco) have employed effective screening and treatment of infected persons.[59] A significant lack of reliable data in Ghana, especially on prevalence, but also, for example, on general morbidity from a large part of the country, might negatively influence any control-related efforts at local level. Thus, the focus of schistosomiasis research in neglected regions needs to be improved drastically.
2. It is also necessary to fully implement international strategies and protocols aimed at controlling and eliminating NTDs in general and schistosomiasis in particular (e.g. adhering strictly to the current WHO 2021–2030 roadmap on NTDs would help to improve schistosomiasis control). MDA campaigns need to be expanded, regularized and consistent. Provision of recreational water centers in at-risk communities, along with control of snail intermediate hosts, are needed to achieve low endemic status in the long term. Proper monitoring and impact evaluation of control programs are required to identify cases of resurgence and reinfection borne out of reduced efficacy of praziquantel (PZQ). This will help to

improve control of schistosomiasis in Ghana significantly. We recommend that improving control of schistosomiasis in Ghana requires: (a) integration of political will for the formulation of effective and efficient health-supportive policies; (b) cooperation of control program managers and implementers with local communities; and (c) active involvement of researchers in planning and implementing schistosomiasis control programs, without which little or no success would be achieved towards the goal of disease elimination.

3. To improve disease diagnosis, training of clinical laboratory technicians must be ensured by the National NTD program and partners. Also, practices, including routine checks for the presence of schistosome eggs during every routine stool and urine examination, should be introduced.
4. Genital schistosomiasis is an emerging topic and evidence points to incidence of FGS in some communities in Ghana; however, no study on male genital schistosomiasis (MGS) has been conducted, although records of MGS do exist.[60] Hence, critical attention must be paid to genital schistosomiasis, starting from awareness creation and sensitization of health workers in endemic communities with updates on the complex symptoms of schistosomiasis.
5. A few limited malacological studies have been conducted in Ghana, but these are archaic. New nationwide studies on snail distribution, susceptibility to schistosomes and prevalence are necessary to indirectly monitor the transmission trend of schistosomiasis.
6. There is an urgent need to establish research focused on livestock schistosomiasis, population structure and genetics, the interrelatedness of animal and human schistosomiasis, zoonotic potentials, their transmission routes, susceptibility to PZQ and, subsequently, to implement this knowledge in control programs. This will equally enhance molecular research on schistosomiasis that is currently lacking in Ghana.

Conclusion

This pivotal comprehensive literature review of schistosomiasis in Ghana covers almost 7 decades of published research. Most of the studies included in the present review are focused on the epidemiology, diagnosis and control of schistosomiasis. As per the estimated prevalence values, Ghana has achieved significant progress in disease control over recent years by reducing prevalence from >70% in 2010 to <25% in 2015. However, the estimation is already 7 y old and more recent data are needed. Moreover, the control effort that yielded such results was heavily dependent on morbidity control in humans through PZQ MDA, with very little on snail intermediate hosts control and other relevant strategies. Hence, there remains the need for animal schistosomiasis studies and improvement in other control strategies such as monitoring and evaluation of MDAs, control of snail intermediate hosts, WASH and community sensitization.

Supplementary data

Supplementary data are available at *Transactions* online.

Authors' contributions: All authors contributed immensely to this review article. EMB conceived the idea, reviewed the literature and drafted the review. IA expanded the scope of coverage and restructured the conclusion; JD performed the structuring and organization of topics and subtopics and introduced technicalities to the review. He was also instrumental in the internal review of the article. MC supervised and carried out the internal revision of the revised version and participated in the literature search. All the authors read and approved the final version of the manuscript.

Acknowledgements: We acknowledge the contributions of Prof. John Brooker of JBK Consulting, for providing outstanding language and stylistic editing. Our appreciation is also given to Mr. Frank Boahen for his inspiration during the conception stage of this review.

Funding: 1. Czech University of Life Sciences, Prague, Czech Republic—Operational Programme Research, Development and Education (OP RDE) [grant number CZ.02.2.69/0.0/0.0/19_073/0016944]. 2. ARES Trading S.A., an affiliate of Merck KGaA, Darmstadt, Germany [grant number FR4003935]. 3. Ministry of Health of the Czech Republic, Prague, Czech Republic [grant number NV18-05-00345]. 4. Charles University, Prague, Czech Republic [COOPERATIO Program—Immunity and Infection]. 5. Czech University of Life Sciences, Prague, Czech Republic—NutRisk Centre [grant number CZ.02.01/0.0/0.0/16_019/0000845].

Competing interests: We declare that there are no competing interests.

Ethical approval: Not required.

Data availability: The data supporting the findings of this study are available within the article and/or its supplementary materials. Secondary data are also available at duly cited primary sources in the manuscript.

Disclaimer: The authors listed below originally compiled this review article and apart from the materials duly cited and referenced, no part of the review has been presented anywhere prior to this submission.

References

1 Lockyer AE, Olson PD, Ostergaard P, et al. The phylogeny of the Schistosomatidae based on three genes with emphasis on the interrelationships of Schistosoma Weinland, 1858. Parasitology. 2003;126(3):203–24.

2 WHO. Schistososiasis. Available at: https://www.who.int/news-room/fact-sheets/detail/schistosomiasis [accessed 21 June 2021].

3 Caffrey CR. Schistosomiasis and its treatment. Future Med Chem. 2015;7(6):675–6.

4 Montgomery S. Travel-related infectious diseases. Schistosomiasis. CDC. 2020;78. https://wwwnc.cdc.gov/travel/yellowbook/2020/travel-related-infectious-diseases/schistosomiasis [accessed 14 April 2021].

5 Weerakoon KG, Gobert GN, Cai P, et al. Advances in the diagnosis of human schistosomiasis. Clin Microbiol Rev. 2015;28(4):939–67.

6 Chitsulo L, Engels D, Montresor A, et al. The global status of schistosomiasis and its control. Acta Trop. 2000;77(1):41.

7 Voge M, Bruckner D, Bruce JI. Schistosoma mekongi sp. n. from man and animals, compared with four geographic strains of Schistosoma japonicum. J Parasitol. 1978;64(4):577–84.

8 Gordon CA, Kurscheid J, Williams GM, et al. Asian schistosomiasis: current status and prospects for control leading to elimination. Trop Med Infect Dis. 2019;4(1).

9 Pearce EJ, MacDonald AS. The immunobiology of schistosomiasis. Nat Rev Immunol. 2002;2(7):499–511.

10 Schwartz C, Fallon PG. Schistosoma "Eggs-Iting" the host: granuloma formation and egg excretion. Front Immunol. 2018;9:2492.

11 Yihunie A, Urga B, Alebie G. Prevalence and risk factors of bovine schistosomiasis in Northwestern Ethiopia. BMC Vet Res. 2019;15(1):12.

12 Leger E, Webster JP. Hybridizations within the genus schistosoma: implications for evolution, epidemiology and control. Parasitology. 2017;144(1):65–80.

13 Lo NC, Bezerra FSM, Colley DG, et al. Review of 2022 WHO guidelines on the control and elimination of schistosomiasis. Lancet Infect Dis. 2022;22(11):e327–35.

14 Molyneux DH, Savioli L, Engels D. Neglected tropical diseases: progress towards addressing the chronic pandemic. Lancet. 2017;389(10066):312–25.

15 Tchuem Tchuente LA, Rollinson D, Stothard JR, et al. Moving from control to elimination of schistosomiasis in sub-Saharan Africa: time to change and adapt strategies. Infect Dis Poverty. 2017;6(1):42.

16 Gower CM, Vince L, Webster JP. Should we be treating animal schistosomiasis in Africa? The need for a One Health economic evaluation of schistosomiasis control in people and their livestock. Trans R Soc Trop Med Hyg. 2017;111(6):244–7.

17 Leger E, Borlase A, Fall CB, et al. Prevalence and distribution of schistosomiasis in human, livestock, and snail populations in northern Senegal: a One Health epidemiological study of a multi- host system. Lancet Planetary Health. 2020;4(8):E330–42.

18 WHO. Schistosomiasis: status of schistosomiasis endemic countries 2020. Available at: https://apps.who.int/neglected_diseases/ntddata/sch/sch.html [accessed 21 June 2021].

19 WHO. Ending the neglect to attain the sustainable development goals: a road map for neglected tropical diseases 2021–2030: overview. Geneva, Switzerland: World Health Organization; 2020;4(8):E330–42.

20 MoEST (The Ministry of Environment SaT; THE NATIONAL PORTFOLIO FORMULATION DOCUMENT (NPFD). The Ministry of Environment, Science and Technology. Accra, Ghana: Ministry of Enovironment, Science and Technology. 2011.

21 Mone H, Ibikounle M, Massougbodji A, et al. Human schistosomiasis in the economic community of West African states: epidemiology and control. Adv Parasitol. 2010;71:33–91.

22 Baguio BR. Ghana: administrative areas and boundaries 1874-2009: Ghana University Press; 2011.

23 WB. GDP per capita (current US$) - Ghana, Lower middle income. World Development Indicators. Available at: https://data.worldbank.org/indicator/NY.GDP.PCAP.CD?locations=GH-XN [accessed 15 August 2022].

24 WB. Ghana Poverty Assessment. Available at: https://openknowledge.worldbank.org/handle/10986/34804 [accessed 15 August 2022].

25 (GSS) GSS; Ghana 2021 Population and Housing Census. In: Service GS (ed); Accra, Ghana: Ghana statistical service, 2021:1–148.

26 Soto SM. Human migration and infectious diseases. Clin Microbiol Infect. 2009;15(Suppl 1):26–8.

27 Hunter JM. Inherited burden of disease: agricultural dams and the persistence of bloody urine (Schistosomiasis hematobium) in the Upper East Region of Ghana, 1959-1997. Soc Sci Med. 2003;56(2):219–34.

28 Doumenge J, Mott KE, Cheung C, et al. Atlas of the global distribution of schistosomiasis = Atlas de la repartition mondiale des schistosomiases. Talence, France: Presses Universitaires de Bordeaux; 1987.

29 Utroska JACM, Dixon H, Yoon S, et al. An Estimate of Global Needs for Praziquantel within Schistosomiasis Control Programmes; 1989. https://apps.who.int/iris/handle/10665/61433 [accessed 15 July 2021].

30 Rollinson D, Knopp S, Levitz S, et al. Time to set the agenda for schistosomiasis elimination. Acta Trop. 2013;128:423–40.

31 Lai YS, Biedermann P, Ekpo UF, et al. Spatial distribution of schistosomiasis and treatment needs in sub-Saharan Africa: a systematic review and geostatistical analysis. Lancet Infect Dis. 2015;15(8):927–40.

32 GHS. Master plan for neglected tropical diseases programme, Ghana (2016–2020). In: Division GHSPHs (ed); Accra, Ghana: Ghana Health Service, 2016.

33 GAHI. Distribution of schistosomiasis survey data in Ghana. Available at: https://www.thiswormyworld.org/maps/distribution-of-schistosomiasis-survey-data-in-ghana [accessed 22 February 2021].

34 Tetteh-Quarcoo PB, Akuetteh BK, Owusu IA, et al. Cytological and wet mount microscopic observations made in urine of Schistosoma haematobium-infected children: hint of the implication in bladder cancer. Can J Infect Dis Med Microbiol. 2019;7912186.

35 Lodh N, Naples JM, Bosompem KM, et al. Detection of parasite-specific DNA in urine sediment obtained by filtration differentiates between single and mixed infections of Schistosoma mansoni and S. haematobium from endemic areas in Ghana. PLoS One. 2014;9(3):e91144.

36 Bogoch II, Andrews JR, Ephraim RK, et al. Simple questionnaire and urine reagent strips compared to microscopy for the diagnosis of Schistosoma haematobium in a community in northern Ghana. Trop Med Int Health. 2012;17(10):121.

37 Anto F, Asoala V, Anyorigiya T, et al. Simultaneous administration of praziquantel, ivermectin and albendazole, in a community in rural northern Ghana endemic for schistosomiasis, onchocerciasis and lymphatic filariasis. Trop Med Int Health. 2011;16(9):11.

38 Anto F, Asoala V, Adjuik M, et al. Childhood activities and schistosomiasis infection in the Kassena- Nankana District of Northern Ghana. J Infect Dis Therapy. 2014;2(4):152.

39 Ayeh-Kumi PF, Obeng-Nkrumah N, Baidoo D, et al. High levels of urinary schistosomiasis among children in Bunuso, a rural community in Ghana: an urgent call for increased surveillance and control programs. J Parasit Dis. 2015;39(4):613–23.

40 Bosompem KM, Bentum IA, Otchere J, et al. Infant schistosomiasis in Ghana: a survey in an irrigation community. Trop Med Int Health. 2004;9(8):91.

41 Kosinski KC, Bosompem KM, Stadecker MJ, et al. Diagnostic accuracy of urine filtration and dipstick tests for Schistosoma haematobium infection in a lightly infected population of Ghanaian schoolchildren. Acta Trop. 2011;118(2):123–7.

42 Wagatsuma Y, Aryeetey ME, Nkrumah FK, et al. Highly symptom-aware children were heavily infected with urinary schistosomiasis in southern Ghana. Cent Afr J Med. 2003;49(1-2):16–9.

43 Aryeetey ME, Wagatsuma Y, Yeboah G, et al. Urinary schistosomiasis in southern Ghana: 1. Prevalence and morbidity assessment in three (defined) rural areas drained by the Densu river. Parasitol Int. 2000;49(2):155–63.

44 Anyan WK, Pulkkila BR, Dyra CE, et al. Assessment of dual schistosome infection prevalence from urine in an endemic community of Ghana by molecular diagnostic approach. Parasite Epidemiol Control. 2020;9:e00130.

45 Koukounari A, Webster JP, Donnelly CA, et al. Sensitivities and specificities of diagnostic tests and infection prevalence of Schistosoma haematobium estimated from data on adults in villages Northwest of Accra, Ghana. Am J Trop Med Hyg. 2009;80(3):435–41.

46 Tetteh AK, Otchere J, Bimi L, et al. Evaluation of field recognizable morbidity indicators of Schistosoma haematobium infection among primary school children in Ghana. Russian Open Med J. 2016;5(3): e303.

47 Anyan WK, Abonie SD, Aboagye-Antwi F, et al. Concurrent Schistosoma mansoni and Schistosoma haematobium infections in a peri-urban community along the Weija dam in Ghana: a wake up call for effective National Control Programme. Acta Trop. 2019;199:105116.

48 Tetteh-Quarcoo PB, Attah SK, Donkor ES, et al. Urinary schistosomiasis in children—still a concern in part of the Ghanaian capital city. Open J Med Microbiol. 2013;3:151–8.

49 Bogoch II, Koydemir HC, Tseng D, et al. Evaluation of a mobile phone-based microscope for screening of Schistosoma haematobium infection in rural Ghana. Am J Trop Med Hyg. 2017;96(6):1468–71.

50 Chu KY. Trials of ecological and chemical measures for the control of Schistosoma haematobium transmission in a Volta Lake village. Bull World Health Organ. 1978;56(2):313–22.

51 Klumpp RK, Chu KY. Importance of the aquatic weed ceratophyllum to transmission of Schistosoma-Haematobium in the Volta Lake, Ghana. Bull World Health Organ. 1980;58(5):791–8.

52 Kukula VA, MacPherson EE, Tsey IH, et al. A major hurdle in the elimination of urogenital schistosomiasis revealed: identifying key gaps in knowledge and understanding of female genital schistosomiasis within communities and local health workers. PLoS Negl Trop Dis. 2019;13(3):e0007207.

53 Anto F, Asoala V, Adjuik M, Anyorigiya T, Oduro A, et al. Childhood activities and schistosomiasis infection in the Kassena-Nankana District of Northern Ghana. J Infect Dis Ther. 2014;2:152.

54 van der Werf MJ, Bosompem KM, de Vlas SJ. Schistosomiasis control in Ghana: case management and means for diagnosis and treatment within the health system. Trans R Soc Trop Med Hyg. 2003;97(2):146–52.

55 Yirenya-Tawiah D, Amoah C, Apea-Kubi KA, et al. A survey of female genital schistosomiasis of the lower reproductive tract in the volta basin of Ghana. Ghana Med J. 2011;45(1):16–21.

56 Klumpp RK, Chu KY. Ecological-studies of Bulinus-Rohlfsi, intermediate host of Schistosoma- Haematobium in Volta Lake. Bull World Health Organ. 1977;55(6):715–30.

57 Squire SA, Robertson ID, Yang R, et al. Prevalence and risk factors associated with gastrointestinal parasites in ruminant livestock in the Coastal Savannah zone of Ghana. Acta Trop. 2019;199:105126.

58 Permin A, Yelifari L, Bloch P, et al. Parasites in cross-bred pigs in the Upper East region of Ghana. Vet Parasitol. 1999;87(1):63–71.

59 Laamrani H, Mahjour J, Madsen H, et al. Schistosoma haematobium in Morocco: moving from control to elimination. Parasitol Today. 2000;16(6):257–60.

60 Bustinduy AL, Randriansolo B, Sturt AS, et al. An update on female and male genital schistosomiasis and a call to integrate efforts to escalate diagnosis, treatment and awareness in endemic and non-endemic settings: the time is now. Adv Parasitol. 2022;115:1–44.

Parasites & Vectors

RESEARCH

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Characterization of glutamate carboxypeptidase 2 orthologs in trematodes

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Abstract

Background: Glutamate carboxypeptidase 2 (GCP2) belongs to the M28B metalloprotease subfamily encompassing a variety of zinc-dependent exopeptidases that can be found in many eukaryotes, including unicellular organisms. Limited information exists on the physiological functions of GCP2 orthologs in mammalian tissues outside of the brain and intestine, and such data are completely absent for non-mammalian species. Here, we investigate GCP2 orthologs found in trematodes, not only as putative instrumental molecules for defining their basal function(s) but also as drug targets.

Methods: Identified genes encoding M28B proteases *Schistosoma mansoni* and *Fasciola hepatica* genomes were analyzed and annotated. Homology modeling was used to create three-dimensional models of SmM28B and FhM28B proteins using published X-ray structures as the template. For *S. mansoni*, RT-qPCR was used to evaluate gene expression profiles, and, by RNAi, we exploited the possible impact of knockdown on the viability of worms. Enzymes from both parasite species were cloned for recombinant expression. Polyclonal antibodies raised against purified recombinant enzymes and RNA probes were used for localization studies in both parasite species.

Results: Single genes encoding M28B metalloproteases were identified in the genomes of *S. mansoni* and *F. hepatica*. Homology models revealed the conserved three-dimensional fold as well as the organization of the di-zinc active site. Putative peptidase activities of purified recombinant proteins were assayed using peptidic libraries, yet no specific substrate was identified, pointing towards the likely stringent substrate specificity of the enzymes. The orthologs were found to be localized in reproductive, digestive, nervous, and sensory organs as well as parenchymal cells. Knockdown of gene expression by RNAi silencing revealed that the genes studied were non-essential for trematode survival under laboratory conditions, reflecting similar findings for GCP2 KO mice.

Conclusions: Our study offers the first insight to our knowledge into M28B protease orthologs found in trematodes. Conservation of their three-dimensional structure, as well as tissue expression pattern, suggests that trematode GCP2 orthologs may have functions similar to their mammalian counterparts and can thus serve as valuable models for future studies aimed at clarifying the physiological role(s) of GCP2 and related subfamily proteases.

Keywords: Platyhelminth, M28B metalloproteases, Prostate specific-membrane antigen, *Schistosoma mansoni*, *Fasciola hepatica*, RNA in situ hybridization, Immunohistochemistry, Folate hydrolase, NAALADase

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Introduction

Parasitic organisms affect millions of people and animals worldwide, causing serious disease. Flukes are important parasites from the class Trematoda (Platyhelminthes),

and we selected two medically and veterinary important species, *Schistosoma mansoni* and *Fasciola hepatica*, as models to demonstrate putative roles of their metalloproteases from the subfamily M28B.

Schistosoma mansoni is a blood fluke and together with several other related species causes schistosomiasis (schistosomosis, bilharziasis) [1]. This is a chronic and potentially deadly disease with more than 200 million people infected globally [2, 3]. Their free-swimming larvae (cercariae), released into water from snail intermediate hosts, penetrate human skin and consequently migrate into the circulatory system where they mature into adult worms. Adult worms reside in the portal and mesenteric or bladder veins as male/female pairs producing hundreds of fertilized eggs per day. Chronic infection persists for years or even decades, and severe morbidity results from host immune responses to eggs in host tissues [4, 5]. Existing treatments rely heavily on one drug, praziquantel (PZQ), and no vaccine has yet been developed [2–4]. While schistosomes survive for decades in the veins of their mammalian hosts, their ability to survive and modulate host physiological processes, using a variety of secreted molecules, is crucial for survival [6]; thus, they are natural targets for specific drug/vaccine research development.

The liver fluke *F. hepatica* is among several related species and is the causative agent of fascioliasis (fasciolosis). Contrary to schistosomes, *F. hepatica* is a hermaphrodite, so it possesses both male and female reproductive organs. The disease affects livestock primarily as well as many other mammals worldwide, including humans. Cercariae are released from the intermediate snail host and encyst on the surface of herbage. Disease is transmitted by the ingestion of these cysts. After ingestion, the parasite migrates via the intestine into the peritoneal cavity and later enters the liver parenchyma, where it causes extensive tissue damage. The post-feeding larvae move to the bile duct, where they mature and produce eggs. Liver flukes generally cause serious economic losses in livestock production worldwide [7, 8], and the drug of choice is triclabendazole (TCBZ), although resistance is spreading [9]. Disease symptoms include anemia at the early stage, bile duct inflammation and fibrosis, a decrease in bile production, liver atrophy, cirrhosis, and weight loss; for some animals, disease is lethal [10]. Human infection can occur in areas where farm management is poor; thus, fascioliasis is recognized as a food-borne disease with millions of people infected or at risk [7].

In our study, we investigated two fluke species with already well-characterized genomes and body organization [11, 12] as research models to uncover and compare the physiological roles of metalloenzymes from the subfamily M28B (human GCP2 orthologs). The M28B protease family belongs to a group of membrane-bound di-zinc metalloproteases with either aminopeptidase or carboxypeptidase activity [13]. This subfamily contains proteases that are ubiquitously expressed across all phyla, from yeasts through plants to animals [14]. Additionally, homologous receptors with distinctive non-enzymatic functions, such as the transferrin receptor, also belong to the same family [14]. Human glutamate carboxypeptidase 2 (HsGCP2), also known as prostate-specific membrane antigen (PSMA), N-acetylated-alpha-linked acidic dipeptidase (NAALADase), and folate hydrolase (FOLH), is the most studied member of M28B subfamily proteases [14, 15]. In addition to GCP2, the human genome contains four other genes encoding M28B paralogs with only partially known or unknown functions [13, 16, 17]. Under physiological conditions, the primary sites of human GCP2 expression in humans include the nervous system (astrocytes and Schwann cells), kidney, prostate, salivary glands, and jejunal brush membranes [18, 19]. Despite GCP2 expression in several major human organs, there is a marked absence of data on GCP2 physiology in virtually all tissues, with the exception of the nervous system and small intestine. In the nervous system, GCP2 is involved in communication between neurons and glial cells by hydrolyzing N-acetyl-aspartyl-glutamate (NAAG), the most abundant neuropeptide in the mammalian brain. In the digestive system, GCP2 plays an important role in the absorption of dietary folates by removing their C-terminal polyglutamylated tails, thus enabling transcytosis of the resulting monoglutamylated folate [20, 21]. In addition to functioning as hydrolase/proteases, several reports allude to a non-proteolytic role(s) of GCP2 [22]. The closest human M28B paralogs to GCP2 are poorly characterized, PSMA-L expressed intracellularly [23] and GCP3 [24, 25], with 98% and 67% sequence identities, respectively. Human NAALADase L and NAALADase L2, also GCP2 orthologs, are much less characterized enzymes with likely intestine-restricted expression and aminopeptidase activity involved in protein/peptide degradation and absorption in the digestive system [15].

HsGCP2 is also a valid target for the in vivo imaging of prostate cancer because dysplastic and neoplastic transformation of prostate tissue is accompanied by a substantial increase in GCP2 expression [26]. Furthermore, inhibition of the enzyme was shown to be neuroprotective in preclinical models of numerous neurological conditions, including traumatic brain injury, neuropathic and inflammatory pain, cancer, amyotrophic lateral sclerosis [27, 28], stroke [29], and schizophrenia [30]. However, studying specific functions of GCP2 in higher organisms such as mammals is complicated because of several factors, the most notable being the presence of several

paralogs; more basal models might thus be beneficial to clarify these issues.

In contrast to higher organisms, platyhelminths possess only a single gene encoding the human GCP2 ortholog [11], allowing us to study ancestral proteases before later diversification. In this study, we precisely annotated *S. mansoni* and *F. hepatica* M28B metalloproteases at the gene level. Homology modeling of both enzymes confirmed a conserved three-dimensional fold with the di-zinc active site typical for this enzyme group. Open reading frames (ORFs) for both genes were verified by sequencing, and codon-optimized versions were used for heterologous recombinant protein expressions. Despite recombinant proteins being tested for proteolytic activities in a wide range of combinatorial peptidic libraries, we did not identify any specific substrates, suggesting most likely stringent substrate specificity of the enzymes. Polyclonal antibodies and mRNA probes of both targets were produced and used for RNA in situ hybridization and immunohistochemistry to identify respective localization within parasites. For both parasites, we localized their GCP2 orthologs to reproductive, digestive, nervous, and sensory organs as well as parenchymal cells. Those localizations correlated with the expression pattern of their mammalian orthologs; thus, functional commonalities within the entire group cannot be ruled out. A comparable finding known for GCP2 KO mice [24, 25] was recorded in the case of the expression of *S. mansoni* ortholog as RNAi silencing did not lead to phenotypic changes under experimental conditions in vitro. RNAi silencing thus revealed that the genes were non-essential for immediate trematode survival under laboratory conditions. Based on our results, the real functions of trematode GCP2 orthologs remain elusive, but there are strong indications that they share common biological roles due to localizations in similar organ structures.

Methods

Parasite material

Schistosoma mansoni (a Puerto Rican strain) is routinely maintained in the laboratory in the intermediate snail host (*Biomphalaria glabrata*) and definitive host NMRI (Naval Medical Research Institute) mouse as described previously [31, 32]. Laboratory mice are maintained by a certified person (certificate number CZ 02627) in the laboratories accredited according to the animal welfare laws of the Czech Republic and EU. Free-swimming larvae (cercariae) were shed by light stimulation from infected snails into the water. Adult female mice were infected by immersing their feet and tails into 50 ml water containing approximately 200 cercariae for 45 min. Seven weeks post-infection, mice were killed by intraperitoneal injection of ketamine (Narkamon 5%—1.2 ml/kg body weight) and xylazine (Rometar 2%—0.6 ml/kg body weight) and with heparin to prevent blood clotting. Adult worms were recovered from the mouse hepatic portal system by transcardial perfusion with RPMI 160 medium. Worms in pairs were then transferred into a petri dish containing medium and chilled on ice for male and female separation by gentle prodding with a small brush. Migratory larvae (schistosomula) were prepared from cercariae in vitro and cultivated in Complete Medium 169 according to standard protocols published previously [33–35].

Living *F. hepatica* worms were collected from the liver of infected cattle (Cesky Krumlov district, Czechia) and carefully washed in phosphate-buffered saline (PBS). Worm samples were immediately frozen on dry ice and stored at − 80 °C.

RNA isolation

Total RNA from *S. mansoni* samples (excised snail hepatopancreases with daughter sporocysts, cercariae, 5-day cultivated in vitro transformed schistosomula, adults, eggs, and miracidia) and from *F. hepatica* adults were isolated according to the TRIzol reagent (Thermo Fisher Scientific) protocol. The final precipitated RNA was air-dried and resuspended in DEPC-treated H_2O. Single-stranded cDNA was synthesized from total RNA using SuperScript IV reverse transcriptase (Thermo Fisher Scientific) and an oligo d(T)18 reverse primer according to the manufacturer's protocol. The resulting cDNA was purified using a QIAquick PCR purification kit (Qiagen) and stored in DEPC-H_2O at − 20 °C.

Sample preparation for microscopy

For RNA in situ hybridization and immunohistochemistry, the adults and schistosomula of *S. mansoni* were freshly collected and fixed in hot 4% formaldehyde heated to approximately 60 °C, but the solution did not boil (90 min for adults/30 min for schistosomula) and was cooled to 25 °C. All fixed samples were dehydrated through progressively concentrated ethanol (25%, 50%, 70%, 90%, 96%, 100% v/v ethanol) for 5 min for each step. A drop of Chromotrope 2R dye (Sigma-Aldrich) was added to the 90% ethanol step. Before embedding in paraffin, *S. mansoni* adults and schistosomula were incubated in methyl benzoate (Sigma-Aldrich) for 45 min in the case of adults and 20 min for schistosomula. Subsequently, the worms were washed twice for 5 min in benzene (Sigma-Aldrich). *Fasciola hepatica* adults were fixed in Bouin's solution (Sigma-Aldrich) for 24 h at RT and then rinsed in PBS containing 0.02% sodium azide until the picric acid was washed off. Fixed samples were dehydrated with increasing concentrations of ethanol as was described for *S. mansoni* for 30 min for each step.

All tissues were finally embedded in paraffin-Paraplast (Sigma-Aldrich). Sections (*S. mansoni* 5 µm, *F. hepatica* 7 µm) were cut using a Shandon Finesse® ME + microtome (Thermo Fisher Scientific) and placed onto X-tra adhesive slides (Leica).

Annotation of M28B metalloproteases

Single genes coding M28B metalloproteases were identified in *S. mansoni* and *F. hepatica* based on the sequence similarities from previous sequence submissions: SmM28B as *S. mansoni* NAALADase L (GenBank XP_018651911) and FhM28B as *F. hepatica* NAALADase 2 (GenBank THD24162.1). Respective ORFs were amplified, cloned, and verified by sequencing. Fully annotated sequences were deposited into the GenBank as MZ456528 and MZ456529 for SmM28B and FhM28B, respectively.

Homology modeling

The amino acid sequences of SmM28B and FhM28B were used for homology modeling. The 3BXM HsGCP2 structure was selected as a modeling template [36], and Modeler 9.23 software was used to construct the target-template sequence alignment and to generate a set of 3D homology models; the best model for each enzyme was selected based on discrete optimized protein energy (DOPE) scores [37]. DOPE scores for SmM28B and FhM28B homology models were − 82,422 and − 81,303, respectively. The GA341 score of both models was 1. Finally, the SmM28B and FhM28B homology models and HsGCP2 structures were superimposed in PyMol and analyzed by visual inspection.

Cloning and mutagenesis

Codon-optimized genes encoding the SmM28B and FhM28B proteins were custom-made by the Thermo Fisher gene-string synthesis protocol. Coding sequences were PCR amplified with corresponding sets of gene-specific primers (Additional file 1: Table S1), and pD221 donor vectors were constructed using the BP Gateway cloning protocol (Invitrogen). Expression plasmids featuring N-terminal purification His-Strep-HALO tags were prepared by recombining the donor vectors and the in-house expression pDEST320 destination vector (Additional file 2: Fig. S1) using the LR Gateway reaction mix. SmM28B(E439M) and FhM28B(E413M) mutants, harboring inactivating mutations of the putative proton shuttle glutamate, were constructed by Quick-change site-directed mutagenesis using corresponding expression plasmids as templates. To this end, target glutamate residues were mutated to methionine via PCR with mutagenic primers (Additional file 1: Table S1) followed by elimination of the template by DpnI treatment, as described for the HsGCP2 ortholog [38]. *Escherichia coli* clones transformed by a plasmid containing the modified sequence were verified using Sanger sequencing.

Expression and purification of trematode M28B metalloproteases in HEK-293T cells

For eukaryotic expression, metalloproteases were cloned into the pDEST320 destination vector in frame with the TEV-cleavable His-Strep-HALO tag (Additional file 2: Fig. S1) and expressed and purified essentially as described previously [39]. Briefly, all variants were expressed using HEK-293T/17 cells following transient transfection, mediated by linear polyethyleneimine (PEI) (Polysciences). The suspension culture was grown in Erlenmeyer flasks in FreeStyle F17 medium (Thermo Fisher Scientific) at 110 rpm under a humidified 5% CO_2 atmosphere at 37 °C. For large-scale expression, an expression plasmid was diluted in PBS (1 mg in 25 ml of PBS for 1 l of the cell culture). Then 3 ml of the PEI stock solution (1 mg/ml) was added; the mixture was vortexed briefly, incubated for 10 min at room temperature, and then added to the cell suspension (4×10^6 cell/ml). Four hours post-transfection, the cell suspension was diluted with an equal volume of ExCell serum-free medium. Cells were harvested 72 h post-transfection by centrifugation at 500 g for 5 min, and then the cell pellet was frozen in liquid nitrogen and stored at − 80 °C until further use.

The cell pellet was lysed by sonication in ice-cold lysis buffer (100 mM Tris–HCl, 10 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 10% glycerol, 0.2% Nonidet P-40, pH 8.0) supplemented with the protease inhibitor cocktail cOmplete EDTA-free (Roche). Following incubation on ice for 30 min, the cell lysate was cleared by centrifugation at 40,000 *g* for 30 min, and the supernatant was loaded on a Strep-Tactin column (IBA-Lifesciences). The fusion was eluted with the lysis buffer supplemented with 2 mM desthiobiotin. Alternatively, when deemed beneficial, the N-terminal fusion tag was cleaved-off by TEV protease (at a 1:10 TEV to target ratio) at 8 °C overnight. The final purification step comprised size exclusion chromatography on a Superose 6 column (GE Life Sciences) with 20 mM MOPS, 150 mM NaCl, pH 7.4, as a mobile phase. Purified proteins were concentrated at 1 mg/ml and flash-frozen in liquid nitrogen until further use.

Recombinant production of SmM28B in *E. coli*

For polyclonal antibody production, the SmM28B antigen was heterologously expressed in a prokaryotic expression system. The codon-optimized sequence encoding SmM28B was cloned into the pEC527 destination vector (a gift from Dominic Esposito; Addgene plasmid #11518) in frame with the TEV-cleavable hexahistidine tag. The

recombinant protein was expressed in *E. coli* BL21(DE3) cells in LB medium at 37 °C upon 1 mM IPTG induction and further purified from inclusion bodies using Ni^{2+} chelating chromatography under denaturing conditions. Briefly, the cell pellet was disrupted by sonication in PBS, and inclusion bodies (IBs) were isolated by centrifugation at 5000 *g* for 10 min. IBs were further purified by consecutive sonication-assisted solubilization in PBS + 1 M urea, PBS + 1 M NaCl, and PBS + 1% Triton X-100, where each solubilization step was followed by centrifugation at 5000 *g* for 10 min. Following the final centrifugation, the IBs were dissolved in an equilibration buffer (10 M urea, 100 mM Tris–HCl, pH 8.0) and centrifuged at 30,000 *g* for 30 min, and the supernatant was loaded onto a 5-ml Ni–NTA column. His-tagged SmM28B was eluted with the equilibration buffer supplemented with 200 mM imidazole. Fractions containing the purified protein were pooled and used to prepare polyclonal rabbit antiserum.

Expression and purification of human GCP2

Expression and purification of the extracellular part of HsGCP2 (residues 44-750) were carried out as described previously [40].

Aminopeptidase library screening

The aminopeptidase activities of GCP2-like enzymes were evaluated using the library of proteinogenic amino acids, except cysteine, labeled with a fluorophore, 7-amino-4-methylcoumarin (AMC). The enzymatic assays were carried out in 384-well black plates in a final volume of 50 µl. The reaction mixtures consisted of 5 µM of individual fluorophore-labeled amino acids and 500 nM of enzyme in an assay buffer comprising 50 mM Tris–HCl, 150 mM NaCl, 0.001% $C_{12}E_8$ (dodecyloctaglycol), pH 7.4. The reactions were incubated at room temperature for 60 min, and the release of free AMC was monitored using a CLARIOstar fluorimeter (BMG Labtech GmbH) with excitation/emission wavelengths set at 365 and 440 nm, respectively. The two-fold dilution series of AMC with a starting concentration of 5 µM was used as a positive control and for quantification of the reaction products. The substrate without enzyme was used as a negative control and as a signal background.

Carboxypeptidase library screening

The carboxypeptidase activities of GCP2 enzymes were evaluated using the library of dipeptides. The library was synthesized as 19 mixtures, each containing a defined N-terminal residue and a mixture of 19 proteinogenic amino acids at the C-terminus. Cysteine was not present in the library. Enzymatic assays were carried out in 96-well plates with a final volume of 50 µl. Reaction mixtures consisted of 500 µM peptide mixture and 500 nM enzyme in the assay buffer. Reaction mixtures were incubated at room temperature for 60 min, quenched by the addition of 0.1% trifluoroacetic acid in 5% acetonitrile, and released amino acids were quantified by Waters Corporation AccQ-Tag Ultra chemistry package on RP-HPLC (Shimadzu, HPLC Prominence system) according to the manufacturer's instructions. The complementary peptide library comprising a defined C-terminal residue and a mixture of 19 proteinogenic amino acids at the N-terminus was used for the control experiments.

Multiplex substrate profiling by mass spectrometry (MSP-MS)

The MSP-MS assays were performed as previously described [31]. Briefly, protease samples were incubated in triplicate reaction tubes with a mixture of 228 synthetic tetradecapeptides (0.5 µM each) in the reaction buffer. Then, 20 µl of the reaction mixture was removed from each replicate after a set incubation time (15, 60, 120, 240, and 1200 min of incubation), and enzyme activity was quenched by adding 40 µl of 6.4 M guanidinium chloride (GuHCl). Samples were flash frozen immediately at − 80 °C. A control reaction consisted of enzyme pretreated with GuHCl prior to peptide library exposure. All samples were subsequently thawed, acidified to pH < 3.0 with 1% formic acid, desalted with C18 LTS tips (Rainin), injected into a Q Exactive Mass Spectrometer (Thermo) equipped with an Ultimate 3000 HPLC (Thermo), and analyzed as previously described [32].

RT-qPCR analysis of *S. mansoni* gene expression

RT-qPCR involved specific primers designed and selected for gene targets encoding SmM28B, *S. mansoni* cathepsin B1.1 (SmCB1.1, GenBank AJ506157), and *S. mansoni* cytochrome C oxidase I (SmCOX I, GenBank AF216698) as the sample reference gene transcript (Additional file 3: Table S2). Primer3 software (http://frodo.wi.mit.edu/ [41]) was used to design specific novel primers for the gene encoding SmM28B. Primers were evaluated by serial dilutions of both the primers and the cDNA template as described [33, 42, 43], while SmCB1.1 primers were adopted from our previous studies [33, 43]. The cDNA from various life stages was generated using the mRNA isolation protocol described above and previously [33, 43]. Reactions containing LightCycler 480 SYBR Green I Master (Roche) were prepared in final volumes of 25 µl in 96-well plates and carried out as described previously [33]. PCR reactions were performed in triplicate with at least one biological replicate. Analysis using the reference gene transcript and the 2^{-CT} method was as previously published [33, 43] to measure transcript levels [44]. The whole experiment was repeated when CT values of technical replicates fluctuated by 0.5 or more. The

resulting transcript levels were calculated as a percentage of the nonspecific control (mCherry dsRNA for RNAi) or the highest transcript level that was set at 100%.

Probes design for RNA in situ hybridization

The specific PCR products were amplified by polymerase chain reaction (PCR) from the first-strand cDNA of adults *S. mansoni* and *F. hepatica* using gene-specific primers (Additional file 4: Table S3). The PCR fragments of approximately 450 bp were ligated into the pGEM-T Easy Vector (Promega), and cloned sequences were verified by DNA sequencing. Verified constructs were linearized and used as a template for the production of sense/antisense RNA probes according to the manufacturer's instructions (DIG RNA labeling kit SP6/T7, Roche).

RNA in situ hybridization (ISH)

Sections (7 µm *F. hepatica*/5 µm *S. mansoni*) of adult worms were de-paraffinized in xylene, rehydrated, and washed in diethyl-pyrocarbonate (DEPC, Sigma-Aldrich)-treated water. The *S. mansoni* sections were incubated in 0.01 M sodium citrate buffer, pH 6.0, in a boiling water bath for 15 min and were then cooled for 30 min. In the case of *F. hepatica*, the sections were treated with proteinase K (final concentration 2 µg/ml, Roche) for 5 min at 37 °C. Hybridization was performed for 16 h at 42 °C (*S. mansoni*) and 55 °C (*F. hepatica*) with RNA probes diluted to 1:200 in a hybridization mixture (5 × SSC, 1 × PBS, 0.1% Torula yeast RNA (Sigma-Aldrich), 50% formamide, 10% dextran sulfate molecular weight 4000 (Sigma-Aldrich), 1% Tween 20) according to the modified protocols [45, 46]. After hybridization, slides were washed and incubated in blocking solution as described [45, 46]. Subsequently, final detection in the tissue of *F. hepatica* was carried out using Anti-DIG-AP antibodies (Roche) at a 1:500 dilution and SIGMAFAST™ Fast Red TR/Naphthol AS-MX tablets (Sigma-Aldrich); results were visualized with a Nikon ECLIPSE Ni-E microscope. In the case of the significantly smaller *S. mansoni*, hybridized probes were labeled with Anti-DIG-HRP antibodies (Roche) at a 1:500 dilution, and signal amplification was performed using the Tyramide Signal Amplification (TSA) system with the Cyanine Plus 5 Tyramide Reagent fluorescence system (Perkin Elmer) according to the manufacturer's protocol. Sections were embedded in ProLong™ Diamond Antifade Mountant (Thermo Fisher Scientific) and visualized with an Olympus IX83 microscope equipped with PCO edge 5.5. camera and CoolLED pE-4000 LED illumination system. As negative controls, sections were incubated under the same conditions with sense probes and without any probe. Images from both *S. mansoni* and *F. hepatica* slides were processed using ImageJ software version 1.52 u [47].

Polyclonal antibody production and immunohistochemistry (IHC)

Purified recombinant SmM28B (BL21 *E. coli*) and FhM28B (HEK-293FT source, with the N-terminal HALO tag removed) were used to produce rabbit polyclonal antibodies at the service facility of the Institute of Microbiology, Czech Academy of Sciences. Rabbit polyclonal antibodies against major *S. mansoni* gut protease SmCB1 were raised against recombinantly produced protein [48] and kindly provided by Dr. Daniel Sojka, from the Biology Centre, Czech Academy of Sciences. Sample fixation, deparaffinization, rehydration, and antigen retrieval followed the same protocol as was described in the RNA in situ hybridization section below [49, 50]. After antigen retrieval with sodium citrate buffer, pH 6.0, sections were washed three times in 1 × PBS. Then, sections were permeabilized in fresh PBS, pH 7.5, supplemented with 0.25% (v/v) Triton X-100 (PBS-Tx) for 20 min followed by overnight incubation in the blocking buffer composed of PBS-Tx + 2% BSA. Finally, the sections were washed once in antibody diluent (AbD; PBS-Tx + 1% BSA) and subsequently probed with respective immunized sera at a dilution of 1:50. Slides were incubated in the wet chamber at 4 °C overnight. Respective controls included primary sera and no sera to exclude non-specific reactions of sera or secondary antibodies, respectively. After three washes in (PBS-Tx) for 10 min each, slides were incubated with goat anti-rabbit IgG H&L Alexa Fluor Plus 647 secondary antibodies (ThermoScientific) at a dilution of 1:500, followed by 45 min of incubation at 37 °C in the wet chamber. Slides were again washed 3 × in PBS-Tx for 10 min each followed by a final 10 min wash in PBS. Slides were mounted in ProLong Diamond Antifade Mountant with DAPI (Thermo Fisher Scientific) and visualized by Nikon ECLIPSE Ni-E fluorescence microscope. Images were processed with ImageJ software version 1.52u.

RNA interference in *S. mansoni*

As templates for dsRNA synthesis, PCR products of targeted SmM28B and positive control *S. mansoni* cathepsin B1.1 (SmCB1.1, GenBank AJ506157) were PCR amplified from cDNA prepared from 5-day-old in vitro-cultivated *S. mansoni* schistosomula as described previously [33]. DNA coding mCherry protein was used as a nonspecific control dsRNA. PCR products were approximately 500 bp in size (Additional file 5: Table S4). Synthesis of dsRNA from gel-purified PCR templates was accomplished using the T7 RiboMAX Express RNAi System (Promega) as described previously [33]. Schistosomula

transformed from cercariae or adults perfused from the infected mice [33–35] were incubated in 24-well plates containing 1 ml of Complete Medium 169 with 5% FBS [34], and dsRNA 30 µg/ml at 37 °C and 5% CO_2 [33]. (i) For monitoring of gene silencing levels, parasites were collected after 5 days of cultivation, washed three times in PBS, and resuspended in 50 ml of Trizol reagent (Thermo Fisher Scientific). RNA isolations and evaluations of gene expression levels by RT-qPCR followed previous protocols [33, 43] as described for the reaction design above. (ii) For observing phenotypic changes, dsRNA-treated parasites were monitored regularly for 14 days.

Results

Identification of M28B metalloproteases in *S. mansoni* and *F. hepatica*

The sequence alignment of *S. mansoni* and *F. hepatica* M28B proteases with their human GCP2 ortholog is shown in Fig. 1. The SmM28B and FhM28B protein sequences share 48% and 30% similarity with HsGCP2, respectively. All three proteins share an identical domain organization (also verified by homology modeling below) comprising the protease, apical, and C-terminal dimerization domain, which is responsible for protease dimer formation [51]. At the same time, both SmM28B and FhM28B lack the intracellular and transmembrane domains present in HsGCP2, indicating their intracellular functions or a different secretory mechanism. Importantly, the catalytic glutamate residue, as well as residues responsible for coordinating active-site zinc ions, are conserved in FhM28B and SmM28B, pointing toward their putative proteolytic functions (Fig. 1).

Homology modeling

To provide structural insight into SmM28B and FhM28B, we constructed homology models of SmM28B and FhM28B and compared them with the X-ray crystal structure of HsGCP2 (PDB code 3BXM). As shown in Fig. 2, the overall fold of all three proteins was virtually identical. The active site of HsGCP2 comprises two zinc ions that are critical for GCP2 folding and enzymatic activity [52]. Our homology models revealed that the three-dimensional arrangement of the active sites, including residues coordinating Zn^{2+} ions of GCP2, were conserved in *S. mansoni* and *F. hepatica* orthologs. Similarly, Glu424, the proton shuttle residue in HsGCP2, which is essential for its catalytic activity, was also conserved in studied orthologs. Consequently: (i) it can be assumed that both orthologs might retain protease activity; (ii) based on these predictions we designed E413M and E439M inactive mutants for FhM28B and SmM28B, respectively, which served as negative controls for activity profiling (see below; [38]).

Analysis of the homology models further highlighted marked differences in the characteristics of residues delineating substrate specificity pockets in the internal cavity of the enzyme (Fig. 2, Refs. [53, 54]). The positively charged arginine patch, comprising residues Arg463, Arg534, and Arg536, is the hallmark of the non-prime pocket of HsGCP2 and imparts specificity for negatively charged residues at the P1 position of HsGCP2 substrates (Asp, Glu) [54]. Corresponding residues in SmM28B are Arg480, Tyr546, and Glu548, while, in FhM28B, these positions are occupied by Asn452, Tyr520, and Glu522. Similarly, Arg210 and Lys699, interacting with α- and γ-carboxylates of the C-terminal glutamate residues in HsGCP2 substrates, respectively, are critical determinants of HsGCP2 substrate specificity in the S1' pocket [53, 55, 56]. However, while residues corresponding to Arg210 are conserved in both orthologs, Lys699 is replaced by small uncharged Ser712 and Gly684 in SmM28B and FhM28B, respectively. Overall, these findings point towards different substrate specificities of the two orthologs when compared to the HsGCP2 preference for negatively-charged glutamate-containing substrates [55].

Heterologous expression and purification of SmM28B and FhM28B

Both orthologs were successfully cloned into vectors for heterologous expression in HEK-293T/17 that are more suitable for the production of complex, multidomain proteins. All wild-type enzymes, together with their corresponding putative inactive mutants, were purified from HEK-293T/17 lysates using Strep Tactin affinity chromatography. For the FhM28B construct, the purification protocol further followed comprised by a size-exclusion chromatography step. Finally, for antibody preparation, the HALO-FhM28B fusion was cleaved by TEV protease so only the FhM28B ortholog without the tag was used for rabbit immunization (Additional file 6: Fig. S2). The overall purity, as estimated by Coomassie-stained SDS-PAGE, was 60% and 90% for wild-type SmM28B and wild-type FhM28B, respectively (Fig. 3). Importantly, the identities of all constructs were confirmed by mass spectrometry, and elution profiles from size-exclusion chromatography revealed a predominant monodisperse peak corresponding to the expected dimeric molecular mass, indicating the production of correctly folded protein species that were further used for profiling of peptidase activities. Additionally, recombinant SmM28B was also expressed in *E. coli* and purified by Ni–NTA chromatography (Fig. 3), and this recombinant protein, together with TEV-cleaved FhM28B described above, was used for the production of polyclonal rabbit antibodies for immunohistochemistry experiments.

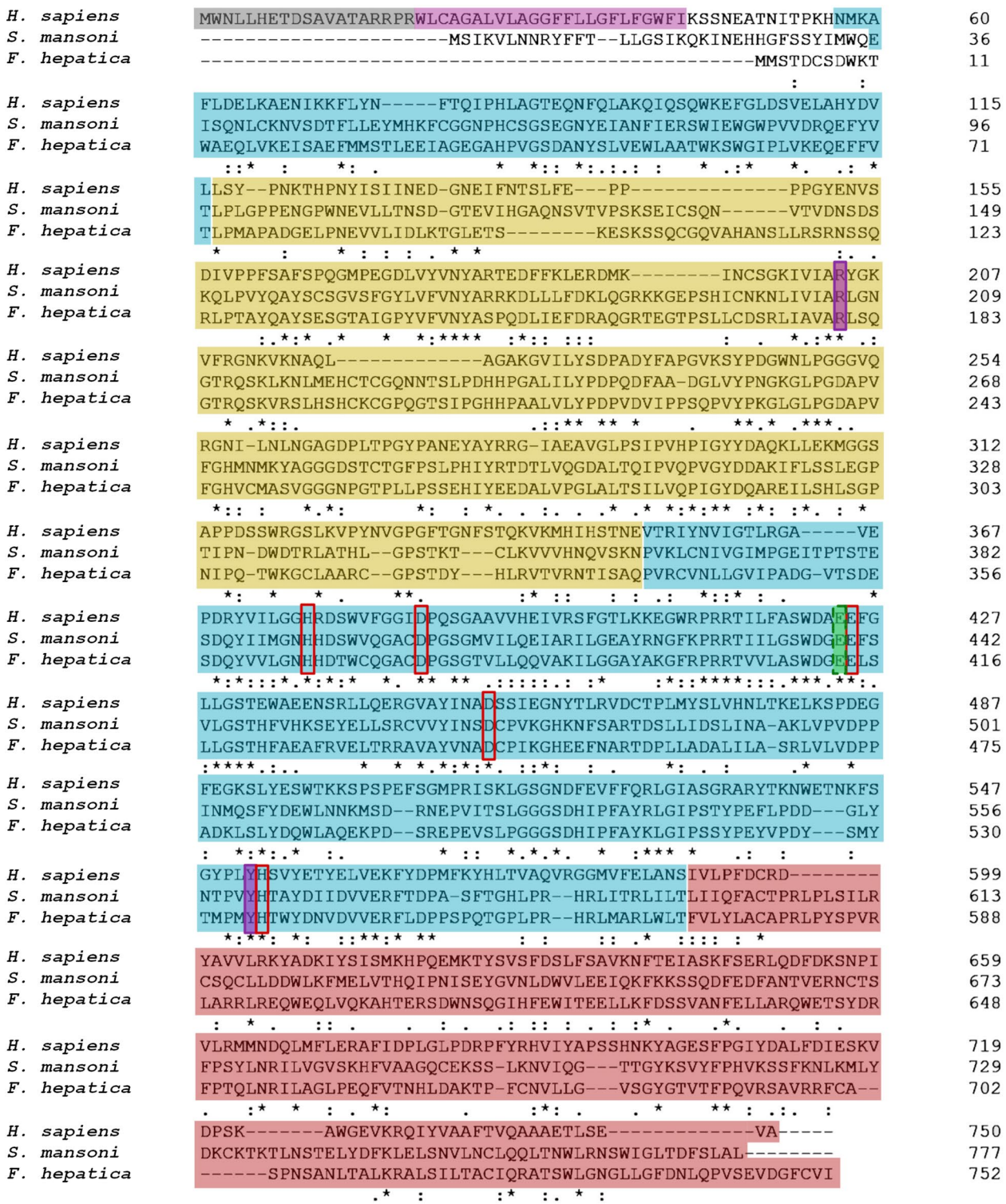

Fig. 1 Sequence alignment of SmM28B, FhM28B, and HsGCP2. The intracellular part and the transmembrane domain of human GCP2 are shaded *gray* and *magenta*, respectively. Protease-like (*blue*), apical (*yellow*), and C-terminal dimerization (*red*) domains are present. The catalytic acid/base glutamate is highlighted by the green background in the rectangular box. Residues coordinating zinc ions are marked by red rectangular boxes, and residues coordinating α-carboxylate binding are shaded in the violet background in rectangular boxes

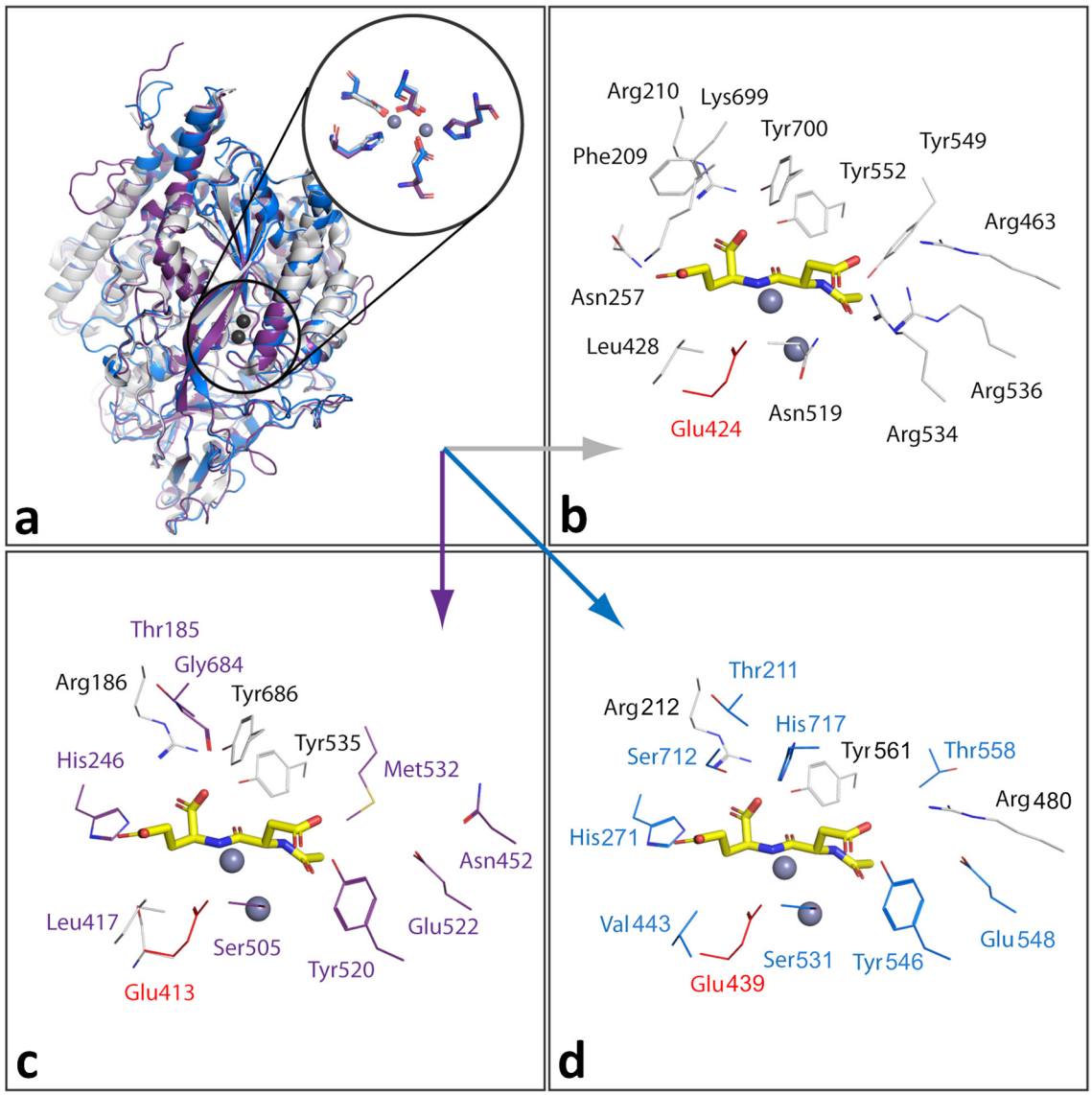

Fig. 2 Homology models of SmM28B and FhM28B. (**a**) The superposition of the HsGCP2 structure (pdb: 3BXM, gray cartoon representation), SmM28B (blue), and FhM28B (violet) homology models reveals the conservation of their overall fold and the coordination sphere of active-site zinc ions (gray spheres). The major differences are observed in residues forming the substrate-binding pocket. (**b**) Residues delineating the substrate-binding pocket of HsGCP2 are shown as gray lines. The active site-bound NAAG is shown in yellow stick representation. The FhM28B (**c**) and SmM28B (**d**) residues that differ from the human ortholog are highlighted in violet and blue, respectively. The critical proton shuttle glutamate residue is highlighted in red and was mutated to methionine to prepare inactive variants of the enzymes. The figure was generated using PyMol 2.4.1

Profiling peptidase activities

Given the structural conservation of the active sites of HsGCP2 orthologs, we hypothesized that trematode enzymes should have intrinsic peptidase activity, although with specificity differing from human counterparts because of marked amino acid variations in the S1 and S1′ pockets. To evaluate the enzymatic activity of HALO-FhM28B, we used three complementary approaches for profiling aminopeptidase, carboxypeptidase, and endopeptidase enzymatic activities against specific combinatorial peptidic libraries (Fig. 4). HsGCP2 and the HALO-FhM28B (E413M) were used as positive and negative controls, respectively. We also included HALO-SmM28B (and HALO-SmM28B (E439M) as a negative control) in preliminary activity screens, but, upon data evaluation, we decided not to pursue these

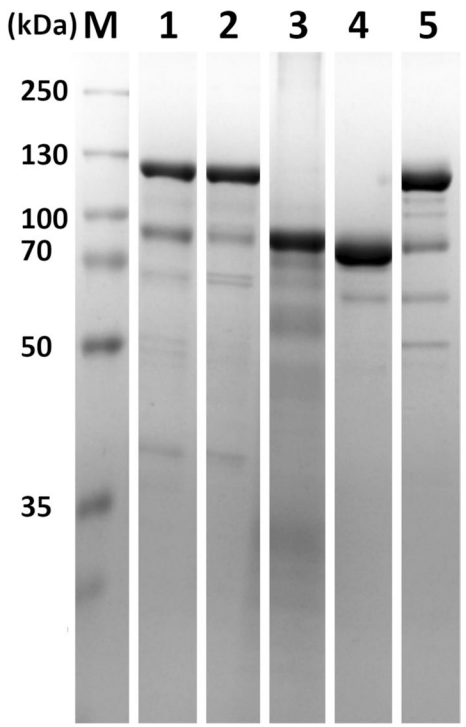

Fig. 3 SDS-PAGE analysis of purified recombinant SmM28B and FhM28B. Lanes **1** through **5** show HALO-SmM28B (123 kDa), HALO-SmM28B (E439M) (123 kDa), His-SmM28B (85 kDa), FhM28B (83 kDa), and HALO-FhM28B (E413M) (126 kDa), respectively. Calculated molecular masses of individual constructs are shown in brackets. His-SmM28B was expressed in *E. coli*, while the remaining constructs were expressed in HEK-293FT cells as fusion proteins with the N-terminal TEV-cleavable His-Strep-HALO tag. For FhM28B, the N-terminal tag was cleaved off by TEV protease. Proteins were separated by reducing SDS-PAGE and stained by Coomassie Brilliant Blue R-250

constructs further because of insufficient purity and the high likelihood of false positives stemming from the presence of contaminating cellular peptidases. The results from carboxypeptidase activity screening are shown in Fig. 4. While substrate preferences of HsGCP2 were consistent with previously published data and thus provided confirmation of the usability of our assay conditions, carboxypeptidase activity was not detected for either wild-type HALO-FhM28B or the HALO-FhM28B (E413M) mutant. Similarly, no aminopeptidase or endopeptidase activities were identified when using either traditional aminopeptidase substrates, represented by amino acids labeled by AMC at their N-terminus, or by the 228-peptide library of tetradecapeptides, respectively (data not shown).

SmM28B expression is present in all life stages of *S. mansoni*

Expression levels of the gene encoding SmM28B were evaluated by RT-qPCR in the life stages of *S. mansoni.* The life stages for analysis were: stages residing in the mammalian host (newly transformed schistosomula, adults, and eggs); miracidia (infecting snail host); daughter sporocysts (producing cercariae within the snail); cercariae (leaving snail host and penetrating the skin of the mammalian host). Expression was normalized to the expression of the *smcoxi* gene and was observed, constitutively, with a slight increase in more motile stages (Fig. 5).

Localizations of M28B metalloproteases in *S. mansoni* and *F. hepatica* adults

To identify spatial gene expression of M28B proteases within the tissues of *S. mansoni* and *F. hepatica* adults, RNA in situ hybridization and indirect immunofluorescence microscopy demonstrated that enzymes occur with the same pattern in both species and follow the distribution of mammalian orthologs (Figs. 6, 7). Results showing localization of M28B on transcript and protein levels in both flukes are summarized in Table 1. Localization patterns in *S. mansoni* and *F. hepatica* (Figs. 6, 7) were identically detected in the reproductive organs and eggs of both flukes. Additionally, localization of the studied enzymes was confirmed in parenchyma and gastrodermis, except for *S. mansoni* females, where a signal in the gastrodermis was missing. In agreement with previous findings of such GCP2 orthologs, we were able to detect enzymes in the cerebral tissue of both species (Additional file 7: Fig. S3), despite not being confirmed by IHC in *F. hepatica*. Interestingly, the control sense probe revealed anti-sense transcripts (i.e. non-coding RNAs) in *S. mansoni* vitellaria and oviducts (Additional file 8: Fig. S4).

RNAi-mediated knockdown of SmM28B expression

Knockdown was tested on newly transformed schistosomula and adults of *S. mansoni* in vitro. Parasites were exposed by soaking to specific dsRNA for 5 days; subsequently, levels of remaining mRNAs coding particular proteins were evaluated by RT-qPCR. RNAi reduced expression of SmM28B by around 95% in schistosomula (Fig. 8), while in the adult worm knockdown did not exceed 30% (not shown). Our positive control targeting the major gut protease SmCB1 was around 98% in schistosomula (Fig. 8), which is consistent with our previous observations [33]. Despite significant knockdown of SmM28B expression, any measurable phenotypic changes were not observed in the parasites. Successful silencing of the major gut protease SmCB1 (a positive control) and SmM28B expressions in schistosomula were

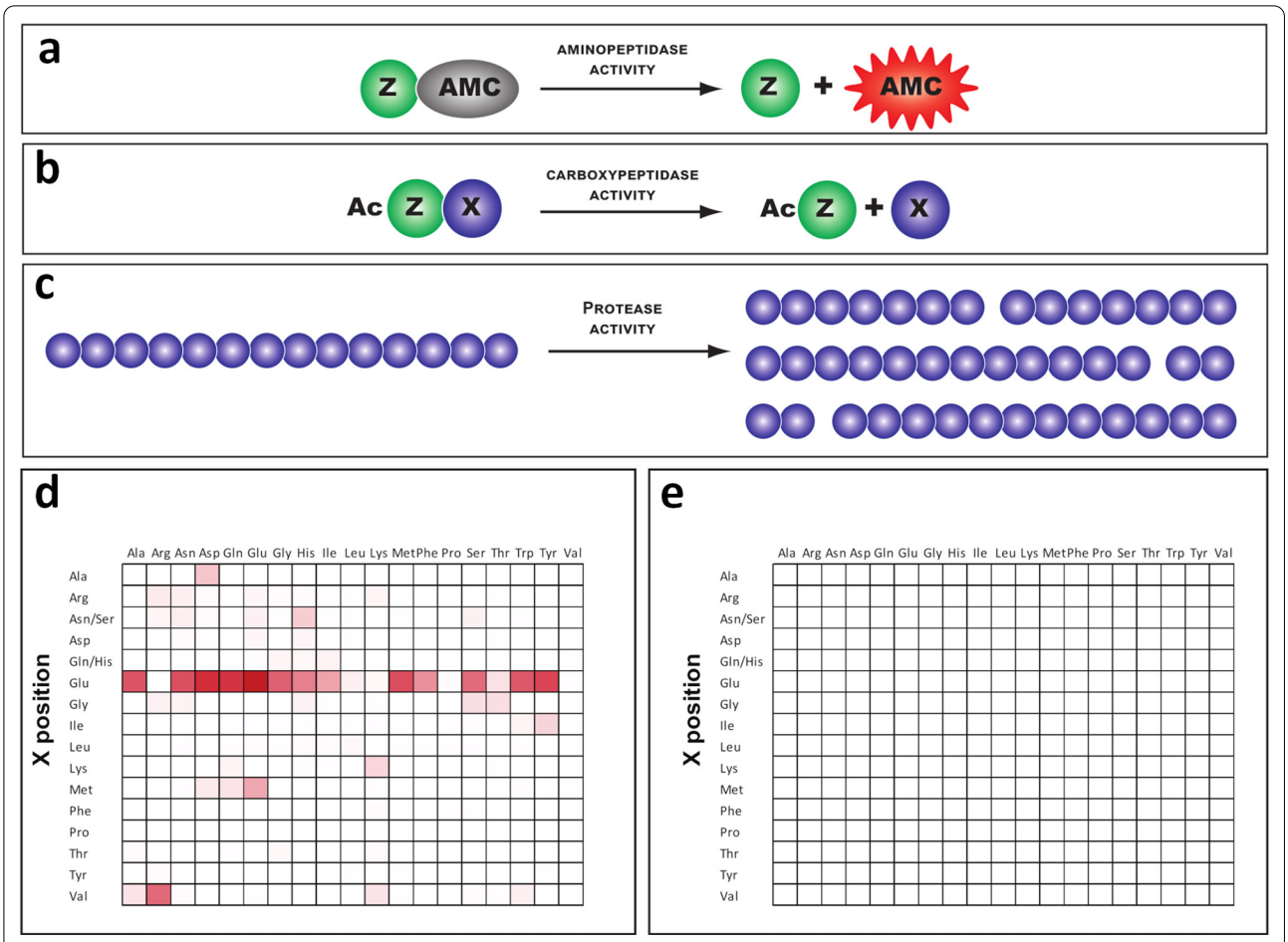

Fig. 4 The scheme of aminopeptidase library (**a**), carboxypeptidase library (**b**), and MSP-MS library screen (**c**). The results of HsGCP2 (**d**) and FhM28B (**e**) screening against the libraries. The data are presented in the form of heat maps with 0% and maximum 60% substrate conversion colored white and red, respectively

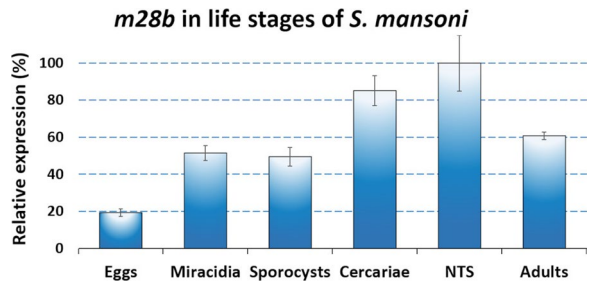

Fig. 5 Evaluation of the expression levels of the gene encoding SmM28B by the RT-qPCR in life stages of *S. mansoni*. *Smcoxi* was used as a suitable housekeeping gene for normalization of gene expression in samples of different life stages of *S. mansoni*

also confirmed visually by immunolocalization. The fluorescent localization signals of SmM28B detected in the head area, gut, and esophageal region of schistosomula and SmCB1 in the gut were not present in dsRNA-treated schistosomula (Fig. 8), thus confirming the efficiency of knockdown.

Discussion

Our focus on the subfamily M28B metalloproteases was guided by the fact that there was a complete lack of knowledge about these types of enzymes in parasitic platyhelminths. In mammals, this protease group contains several paralogs and splice variants with diverse and/or unknown functions. Their unique physiological roles remain unknown, as studies are complicated by their possible redundant roles or poor characterization. The primary expression sites of HsGCP2 under physiological conditions include the nervous system (astrocytes and Schwann cells), kidney (proximal tubules), and small intestine (jejunal brush border membranes). Several other studies have also pointed towards a broader GCP2 expression profile in humans, including lacrimal glands, heart, pancreas, bladder, skin, breast, liver, lung, colon,

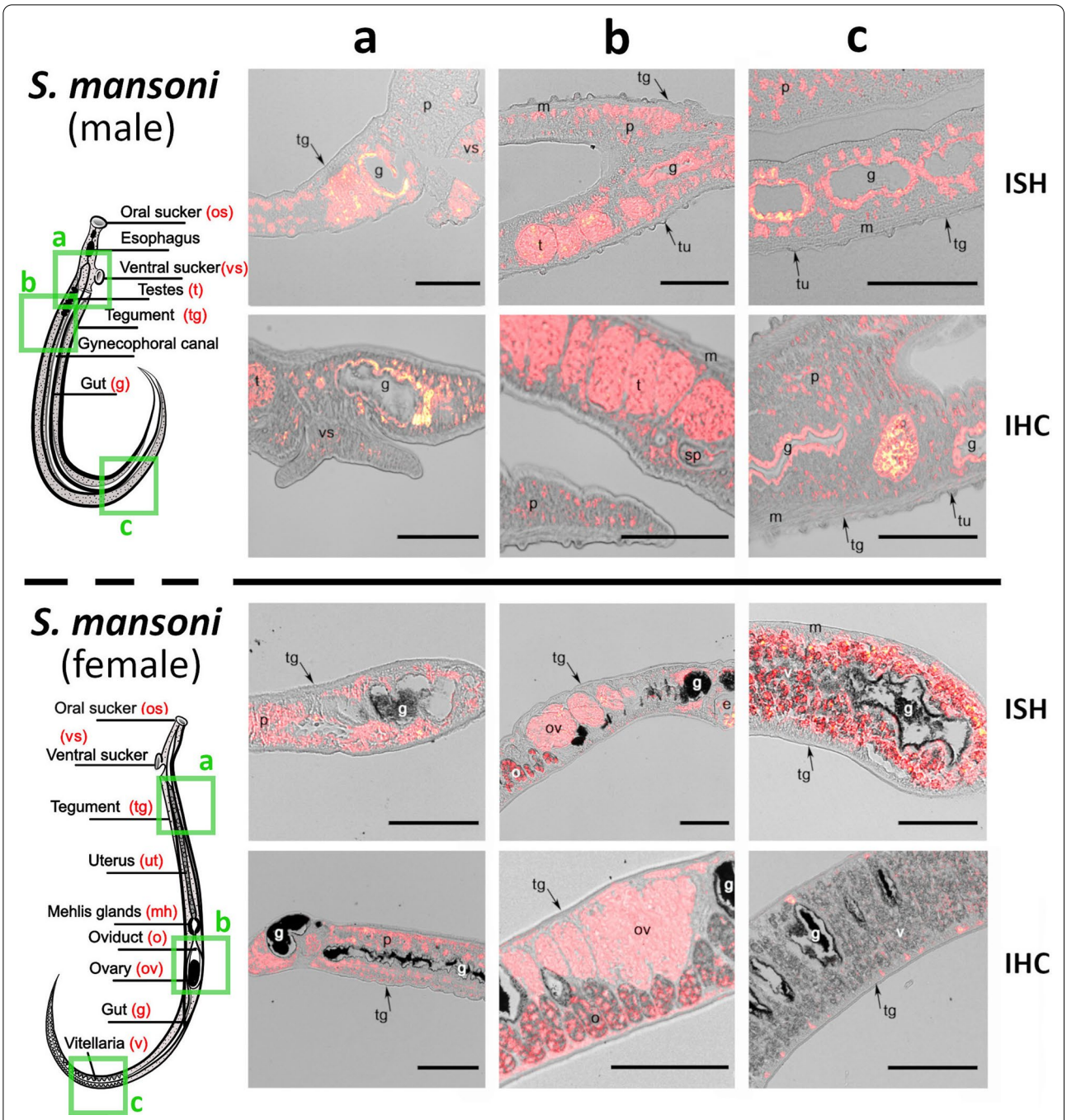

Fig. 6 Localization of SmM28B and M28B in adult male and female of *S. mansoni*. ISH-reaction of antisense DIG-labeled RNA probes designed to label SmM28B sense mRNA (protein-coding mRNA) with histological sections (5 µm) of *S. mansoni* adult (red). IHC—reaction of polyclonal antibodies against SmM28B with sections of *S. mansoni* adult (red). Adults were monitored in three parts: (**a**) head part; (**b**) middle part; and (**c**) posterior part of the worm with the focus on parenchyma, gut, and tegument. The green squares on the left diagram indicate where the cross-sections were taken. SmM28B peptidase was detected in ovary (ov), oviduct (o), vitellaria (v), testes (t), and parenchyma (p) of both sexes. Interestingly, SmM28B was localized exclusively in the gastrodermis of the male but not in the female. SmM28B was not detected in muscles (m), tegument (tg), and tubercules (tu) of both sexes. The scale bars represent 100 µm

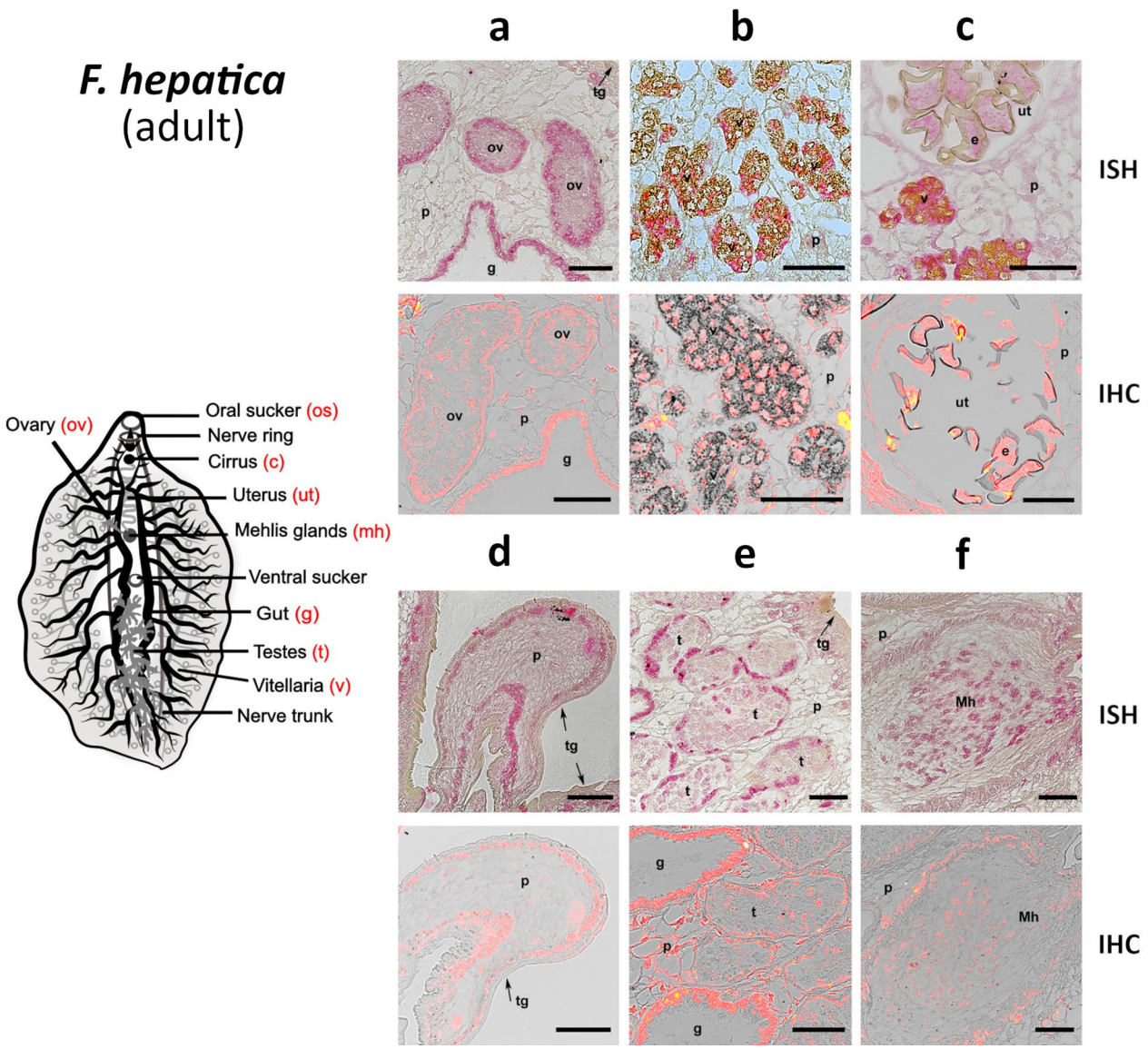

Fig. 7 Localization of mRNA encoding FhM28B and M28B protein in *F. hepatica* adults. ISH reaction of antisense DIG-labeled RNA probes designed to label FhM28B sense mRNA (protein-coding mRNA) with histological sections (7 µm) of *F. hepatica* adult (red). IHC—a signal of polyclonal antibodies against SmM28B with sections of *F. hepatica* adult (red). Adults were monitored in six parts: (**a**) ovary; (**b**) vitellaria; (**c**) uterus; (**d**) parenchyma and tegument; (**e**) testes; (**f**) Mehlis glands. FhM28B transcripts/proteins were detected in ovary (ov), eggs (e), vitellaria (v), Mehlis glands (Mh), gut (g), and parenchyma (p). FhM28B was not detected in uterus (ut), muscles (mu) and tegument (tg). The scale bars represent 100 µm

testis, etc., although expression levels in the latter tissues are thought to be significantly lower [18, 19]. Other human paralogs are localized in nervous tissue [24, 25] or with intestine-restricted expression and activity involved in protein/peptide degradation and absorption in the digestive system [15]. Contrary to mammals and many higher organisms, trematodes possess only a single gene encoding M28B subfamily orthologs. Their sequence alignment revealed the presence of all crucial conserved amino acid residues and domains that are essential for enzymatic function [15, 17, 57–59]. Nevertheless, the transmembrane domain and signal intracellular domain present in human GCP2 are absent from SmM28B and FhM28B orthologs, indicating that their intracellular function, or an alternative route of secretion [60, 61], are missing.

Our primary intention in investigating this group of enzymes in trematodes was to use these basal organisms

Table 1 Summary of M28B localization sites in model organisms *S. mansoni* and *F. hepatica*

Organ structure	*S. mansoni*				*F. hepatica*	
	Male		Female		Adult	
	ISH	IHC	ISH	IHC	ISH	IHC
Cirrus	−	−			+	+
Egg			−	−	−	+
Gastrodermis	+	+	−	−	+	+
Muscles	−	−	−	−	−	−
Nerve ring/cerebral ganglia	+	+	+	+	+	−
Ovary			+	+	+	+
Oviduct			+	+	−	−
Parenchyma	+	+	−	+	−	+
Seminal vesicles	−	+			−	−
Tegument	−	−	−	−	−	−
Testes	+	+			+	+
Uterus			−	−	−	−
Vitellaria			+	+	+	+

+, positive signal; −, negative signal; ISH (RNA in situ hybridization), IHC (immunolocalization)

as represented by *S. mansoni* and *F. hepatica*. We also hypothesized that these enzymes could lead to a druggable target as their in silico predicted substrate preferences are unique. Our principal effort was focused on basic topics such as localization, physiological functions, and essentiality for parasite survival. The localization of SmM28B and FhM28B approximately mirrors the expression profile of the partially characterized mammalian counterparts such as human GCP2, GCP3, and NAALADase L. Both proteases, SmM28B and FhM28B, were detected in the parasite organ structures that functionally correspond to those in mammals. Expression observed in the salivary glands and gut corresponded with localization in the digestive system of *S. mansoni* male and adult *F. hepatica*, particularly in the esophageal glands and the gastrodermis. Interestingly, we did not detect much of a signal in the gut of *S. mansoni* females, in agreement with the data presented in the single-cell RNA-seq atlas database [12]. However, the reason for this disproportional expression remains unclear until its physiological role becomes known. Other apparent expression localization sites are the reproductory organs of both flukes: gonads (ovaries and testes), oviducts, vitelline cells, and consequently in the eggs. In mammals, expression occurs in the reproductory organs; however, particular roles have not been clearly elucidated [18, 19]. Based on our research, both flukes express M28B proteases most likely in the cerebral ganglia, which resembles the roles of GCP2 and GCP3 in mammalian brain tissue. Our study revealed that SmM28B is also expressed as anti-sense RNA in *S. mansoni* vitellaria and oviducts. Anti-sense transcripts (i.e. non-coding RNAs) often play a regulatory role in post-transcriptional processes in various organisms [62] and are present in schistosomes [46, 63, 64].

The effectiveness of in vitro RNAi gene knockdown between schistosomula and adult *S. mansoni* was significantly different; nevertheless, no measurable phenotypic manifestations were observed. The low success with gene knockdown (approximately 30%) in adult worms, even in long-term experiments when fresh media containing dsRNA were replenished every second day, compared to approximately 95% in schistosomula, may be attributed to the significant differences in body sizes and morphologies of these two stages and tissue accessibility [33]. Unlike our observations in *Caenorhabditis elegans* (Dvorak lab, unpublished data), we could not achieve any significant phenotypic impact in vitro through targeting these proteases. Based on previous data [33] and a relatively low level of expression, in vitro conditions may not be ideal to reveal distinct functions in digestive, reproductive, and neuromotor functions. Thus, we set up a pilot experiment using in vitro exposed newly transformed schistosomula, which, after 2.5 days of incubation, were subsequently injected into laboratory mice (C57BL/6) as published previously [65]. Comparable numbers of worms, eggs, and granuloma formations were recorded, and the size and shape of recovered worms did not differ statistically. No obvious deformations or alterations were observed in treated worms 7 weeks p.i. (not shown). To conclude, our data based on RNAi do not provide adequate information. We can only speculate since we could not rule out, for example, that the loss of

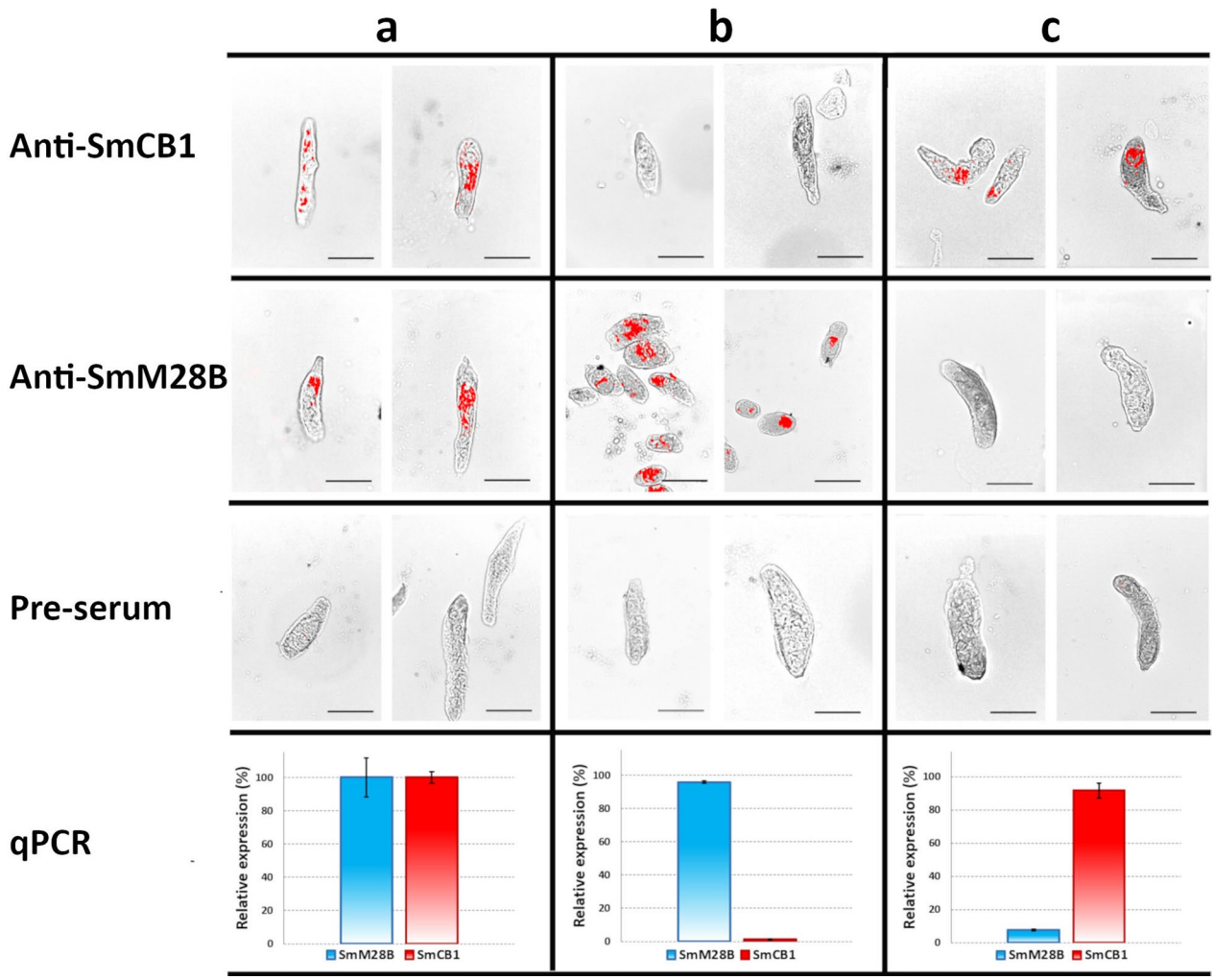

Fig. 8 Immunolocalization on schistosomula after 5 days of exposure to RNAi treatment. The panel presents the result of immunolocalization of SmM28B on *S. mansoni* schistosomula exposed to RNAi treatment. Sections with schistosomula were probed with anti-SmM28B, anti-SmCB1, and pre-serum respectively. Column (**a**) represents the result of immunohistochemistry on schistosomula treated with mCherry dsRNA serving as a control for RNAi. Column (**b**) presents the results of schistosomula treated with SmCB1 dsRNA. Column (**c**) shows the result of schistosomula treated with M28B dsRNA. All samples were probed with antibodies against SmCB1, SM28B, and control pre-serum. The qPCR charts show levels of SmM28B and SmCB1 expression in percentages compared to the controls. The scale bar represents 50 µm

an RNAi effect a few days after mice infections may have been sufficient to permit recovery from the impact of knockdown. When compared with the phenotypization of GCP2 KO laboratory mice, this would confirm that the presence of the enzyme in schistosomula was not crucial for survival, at least under laboratory conditions [25].

The unusual feature of the M28B protease subfamily is that it encompasses both aminopeptidases and carboxypeptidases. However, so far, little is known about their natural substrates. For example, HsGCP2 is highly restrictive as the enzyme specifically removes the C-terminal glutamate and with a low preference for methionine only from dipeptides but not peptides with three or more residues [55, 66]. As for longer peptides as substrates for HsGCP2, the C-terminal glutamate would only be cleaved if it is linked to the penultimate residue via its γ-carboxylate group [20, 67]. It should be noted that γ-linked peptide bonds are not abundant in proteins but exist, for example, in dietary folates, which are natural substrates for human GCP2. Not surprisingly then, only screenings against the dipeptidic library revealed positive hits for HsGCP2, while no substrates were identified using the aminopeptidase or tetradecapeptide libraries. Identification of the substrates of M28B metalloproteases from platyhelminth parasites *S. mansoni* and *F. hepatica* would therefore contribute to general knowledge of their

physiological roles [14]. Furthermore, different substrate specificities, compared to human orthologs, would offer an advantage for the design of selective inhibitors. Unfortunately, our screens could not identify any substrates of the protease from *F. hepatica*, yet the substrate specificity for HsGCP2 was consistent with known substrate preferences (Fig. 4) [60]. Several hypotheses can be offered to address this issue. First, although trematode orthologs possess all critical residues required for their enzymatic activity, they might lack the intrinsic hydrolase activity, similar to the transferrin receptor, which also belongs to the M28B family. Alternatively, there is a small chance that our HEK293 expression system was not suitable for the production of fully active proteins. For example, despite the absence of predicted signal/transmembrane sequences, proteins need to go through the secretory pathway, need to be N-glycosylated to possess peptidase activity (similarly to HsGCP2; [68]), or require an unknown co-factor for full activity. However, we believe that the most plausible explanation is a very narrow specificity of the proteases and that a preferred substrate recognized by the enzymes may be missing from our libraries. Further studies will be required to address these issues in more detail.

Conclusions

This work represents the first deep exploration and partial characterization of the M28B subfamily of trematode metalloproteases orthologous to human glutamate carboxypeptidase 2. The blood fluke *S. mansoni* and the liver fluke *F. hepatica* were both chosen as relatively well-established laboratory models because of their pathogenic importance, well-known genome, and body organizations. The study of the specific functions of these groups of proteases in higher organisms is complicated because of the presence of several paralogs in their genome. Platyhelminths have only a single gene encoding M28B metalloprotease, however, the lack of experimental data in non-mammalian species precludes any comparison. Sequences of both trematode proteases were fully annotated and their structures were predicted based on homology modeling. These metalloproteases were recombinantly expressed, purified, and partially characterized. RT-qPCR revealed a gene expression profile for all life stages of *S. mansoni*. RNAi silencing did not lead to any apparent phenotypic manifestations and revealed a non-essential role for schistosomula surviving under laboratory conditions. In situ hybridization and immunohistochemistry confirmed the almost identical distribution of metalloproteases within tissues of both adult trematodes. Their distribution in trematodes coincides with their localization in functionally similar organ structures in mammals. We therefore hypothesize that M28B might play similar basic and universal physiological roles as their mammalian orthologs. Therefore, these enzymes may serve as one of several suitable models for future studies that could reveal the real physiological roles of these metalloenzymes.

Abbreviations

AbD: Antibody diluent; AMC: 7-Amino-4-methylcoumarin; BSA: Bovine serum albumin; DEPC: Diethyl-pyrocarbonate; ESP: Excretory secretory products; FhM28B: *Fasciola hepatica* M28B; FOLH: Folate hydrolase; GCP2: Glutamate carboxypeptidase 2; GuHCl: Guanidine hydrochloride; HsGCP2: Human glutamate carboxypeptidase 2; HPLC: High-performence protein liquid chromatography; IBs: Inclusion bodies; IHC: Immunohistochemistry; ISH: RNA in situ hybridization; MSP-MS: Multiplex substrate profiling by mass spectrometry; NAAG: N-acetyl-aspartyl-glutamate; NAALADase: N-acetylated-alpha-linked acidic dipeptidase; Ni–NTA: Nickel-nitrilotriacetic acid; NMRI: Naval Medical Research Institute; NTS: New transformed schistosomula; ORFs: Open reading frames; PBS: Phosphate-buffered saline; PCR: Polymerase chain reaction; PSMA: Prostate-specific membrane antigen; PZQ: Praziquantel; RT: Room temperature; RT-PCR: Reverse transcription-PCR; SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis; SmCB1.1: *Schistosoma mansoni* Cathepsin B1.1; SmCOX I: *Schistosoma mansoni* cytochrome C oxidase I; SmM28B: *Schistosoma mansoni* M28B; SSC: Saline-sodium citrate buffer; TCBZ: Triclabendazole; TSA: Tyramide signal amplification.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13071-022-05556-5.

Additional file 1: Table S1. List of primers used for: **a** PCR amplification of the coding sequence of trematode M28B, used later for cloning into pD221 donor vectors by the BP Gateway cloning protocol (Invitrogen). All expression plasmids featuring N-terminal purification tags were prepared by recombining the donor vectors and in-house expression destination vectors using the LR Gateway reaction mix. **b** PCR amplification to obtain enzymatically inactive SmM28B (E407M) and FhM28B (E413M) mutants by the Quick-change site-directed mutagenesis using corresponding expression plasmids as templates.

Additional file 2: Figure S1. Map and detailed linker views of the pDEST320 destination vector.

Additional file 3: Table S2. List of primers used for RT-qPCR analyses of SmM28B, SmCB1, and SmCOXI.

Additional file 4: Table S3. Gene-specific primers for PCR amplification of SmM28B (**a, b**) and FhM28B fragments in sizes of 459 (**a**), 495 (**b**), and 451 bp, respectively. As templates, they served first-strand cDNA synthesis of adults *S. mansoni* and *F. hepatica*. The PCR fragments were ligated into the pGEM-T Easy Vector (Promega) and cloned sequences were verified by DNA sequencing.

Additional file 5: Table S4. List of primer sequences employed for dsRNA synthesis. Double-stranded RNA (dsRNA) was synthesized by the use of sense and antisense primers of each target gene. mCherry and SmCB1.1 were used as controls for our gene of interest, SmM28B. The T7 DNA polymerase-binding motif is shown in bold characters.

Additional file 6: Figure S2. SDS-PAGE analysis of FhM28B purification. The purified HALO-FhM28B fusion was cleaved by TEV protease and FhM28B was sequentially purified using Streptactin (columns **1–5**) and Ni-NTA (columns **6–8**) affinity chromatography. Proteins were separated by reducing SDS-PAGE and stained by Coomassie Brilliant Blue R-250. Lanes: **1**, purified HALO-FhM28B; **2**, reaction after TEV cleavage; **3** and **4**, flow-through from Streptactin column; **5**, elution from Streptactin column; **6** to **8**, flow-through from Ni-NTA column. Fractions **6** through **8** were pooled, concentrated, and used for rabbit immunization.

Additional file 7: Figure S3. Localization of mRNA encoding M28B protein in the cerebral tissues of *S. mansoni* and *F. hepatica* adults. ISH-reaction of antisense DIG-labeled RNA probes designed to label M28B sense mRNA (protein-coding mRNA) with histological sections (5 µm) of *S. mansoni* adults and (7 µm) of *F. hepatica* adults (red). Columns represent the head part of **a** female *S. mansoni*, **b** male *S. mansoni*, and **c** adult *F. hepatica* with the focus on cerebral tissues. M28B peptidase was detected in cerebral ganglia (cg) of both sexes of *S. mansoni* and the neural ring (nr) of adult *F. hepatica*. SmM28B was not detected in the ventral sucker (vs) or the oral sucker (os). The scale bars represent 50 µm.

Additional file 8: Figure S4. Localization of anti-sense RNA encoding SmM28B in *S. mansoni* adult female. Sections (7 µm) of *S. mansoni* female (**a-c**) were probed with DIG-labeled RNA probes designed to label SmM28B anti-sense RNA (non-protein coding RNA). The probe hybridized with transcripts was visualized by tyramide amplification assay (red). The adult female was monitored in three parts: **a** an anterior part of the worm, **b** oviduct and ovary, and **c** vitellaria. All columns show a fluorescent red signal merged with differential interference contrast. Gene expression of anti-sense SmM28B was detected in a few cells of oviduct (o) and vitellaria (v). Anti-sense RNA of SmM28B was not detected in muscles (mu) and tegument (tg), gut (g), parenchyma (p), ovaria (ov). The scale bars represent 100 µm.

Acknowledgements
The authors thank Dr. Marta Chanova, Institute of Immunology and Microbiology, First Faculty of Medicine, Charles University, for her help with immunohistochemistry. The authors thank Dr. Dominic Esposito, who kindly provided us with the pEC527 vector (Addgene plasmid #11518). The authors express our gratitude to Prof. J.D. Brooker for critically proofreading the manuscript.

Author contributions
L.J.—immunohistochemistry and RNAi of S. mansoni, data interpretation, and manuscript writing. .K.P—in situ hybridization and immunohistochemistry of F. hepatica, data interpretation, and manuscript writing. E.M.B.—in situ hybridization, immunohistochemistry, and RNAi of S. mansoni. L.U.—RNA probe design and RNA in situ hybridization. V.V.—purification of recombinant proteins. Z.K. and Z.N.—recombinant productions, performed homology models, participated in antibody preparation/characterization, and data interpretation. Z.J.—MSP-MS and data analysis. I.S. performed library screens and data analysis. J.K.—RNAi of S. mansoni. A.J.D.—analysis of MSP-MS data and in the study design, C.B.—study design, prepared bioinformatic annotation, and data interpretation, manuscript writing. J.D.—RT-qPCR, data interpretation, manuscript writing. All authors read and approved the final manuscript.

Funding
This project was supported by the Grant Agency of the Czech Republic (grants 18-14167S to J.D. and C.B. and 19-22269Y to Z.K.), and the Czech Academy of Sciences (RVO: 86652036).

Availability of data and materials
All materials and data are contained within the manuscript and supplementary figures. Sequences have been deposited in the NCBI GenBank database as *S. mansoni* SmM28B (GenBank MZ456528) and as *F. hepatica* FhM28B (GenBank MZ456529).

Declarations

Ethics approval and consent to participate
Laboratory mice were maintained by a certified person (certificate number CZ 02627) in the laboratories accredited by the Ministry of Agriculture of the Czech Republic under the animal welfare laws of the Czech Republic and EU.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 29 July 2022 Accepted: 19 October 2022
Published online: 20 December 2022

References

1. Lockyer AE, Olson PD, Ostergaard P, Rollinson D, Johnston DA, Attwood SW, et al. The phylogeny of the Schistosomatidae based on three genes with emphasis on the interrelationships of Schistosoma Weinland, 1858. Parasitology. 2003;126:203–24.
2. Caffrey CR. Schistosomiasis and its treatment. Future Med Chem. 2015;7:675–6.
3. Wilson RA. Schistosomiasis then and now: what has changed in the last 100 years? Parasitology. 2020;147:507–15.
4. Pearce EJ, MacDonald AS. The immunobiology of schistosomiasis. Nat Rev Immunol. 2002;2:499–511.
5. Sombetzki M, Rabes A, Bischofsberger M, Winkelmann F, Koslowski N, Schulz C, et al. Preventive CTLA-4-Ig treatment reduces hepatic egg load and hepatic fibrosis in *Schistosoma mansoni*-infected mice. Biomed Res Int. 2019;2019:1704238.
6. Da'dara A, Skelly PJ. Manipulation of vascular function by blood flukes? Blood Rev. 2011;25:175–9.
7. Mas-Coma S, Bargues MD, Valero MA. Fascioliasis and other plant-borne trematode zoonoses. Int J Parasitol. 2005;35:1255–78.
8. Piedrafita D, Spithill TW, Smith RE, Raadsma HW. Improving animal and human health through understanding liver fluke immunology. Parasite Immunol. 2010;32:572–81.
9. Fairweather I. Triclabendazole: new skills to unravel an old(ish) enigma. J Helminthol. 2005;79:227–34.
10. Mas-Coma S, Valero MA, Bargues MD. Climate change effects on trematodiases, with emphasis on zoonotic fascioliasis and schistosomiasis. Vet Parasitol. 2009;163:264–80.
11. Howe KL, Bolt BJ, Shafie M, Kersey P, Berriman M. WormBase ParaSite—a comprehensive resource for helminth genomics. Mol Biochem Parasitol. 2017;215:2–10.
12. Wendt G, Zhao L, Chen R, Liu C, O'Donoghue AJ, Caffrey CR, et al. A single-cell RNA-seq atlas of *Schistosoma mansoni* identifies a key regulator of blood feeding. Science. 2020;369:1644–9.
13. Rawlings ND, Barrett AJ, Thomas PD, Huang X, Bateman A, Finn RD. The MEROPS database of proteolytic enzymes, their substrates and inhibitors in 2017 and a comparison with peptidases in the PANTHER database. Nucleic Acids Res. 2018;46:D624–32.
14. Lambert LA, Mitchell SL. Molecular evolution of the transferrin receptor/glutamate carboxypeptidase II family. J Mol Evol. 2007;64:113–28.
15. Tykvart J, Barinka C, Svoboda M, Navratil V, Soucek R, Hubalek M, et al. Structural and biochemical characterization of a novel aminopeptidase from human intestine. J Biol Chem. 2015;290:11321–36.
16. Tykvart J, Schimer J, Barinkova J, Pachl P, Postova-Slavetinska L, Majer P, et al. Rational design of urea-based glutamate carboxypeptidase II (GCPII) inhibitors as versatile tools for specific drug targeting and delivery. Bioorg Med Chem. 2014;22:4099–108.
17. Hlouchova K, Navratil V, Tykvart J, Sacha P, Konvalinka J. GCPII variants, paralogs and orthologs. Curr Med Chem. 2012;19:1316–22.
18. Rovenska M, Hlouchova K, Sacha P, Mlcochova P, Horak V, Zamecnik J, et al. Tissue expression and enzymologic characterization of human prostate specific membrane antigen and its rat and pig orthologs. Prostate. 2008;68:171–82.

19. Silver DA, Pellicer I, Fair WR, Heston WD, Cordon-Cardo C. Prostate-specific membrane antigen expression in normal and malignant human tissues. Clin Cancer Res. 1997;3:81–5.
20. Navratil M, Ptacek J, Sacha P, Starkova J, Lubkowski J, Barinka C, et al. Structural and biochemical characterization of the folyl-poly-gamma-l-glutamate hydrolyzing activity of human glutamate carboxypeptidase II. FEBS J. 2014;281:3228–42.
21. Slusher BS, Vornov JJ, Thomas AG, Hurn PD, Harukuni I, Bhardwaj A, et al. Selective inhibition of NAALADase, which converts NAAG to glutamate, reduces ischemic brain injury. Nat Med. 1999;5:1396–402.
22. Rajasekaran AK, Anilkumar G, Christiansen JJ. Is prostate-specific membrane antigen a multifunctional protein? Am J Physiol Cell Physiol. 2005;288:C975–81.
23. Barinka C, Rojas C, Slusher B, Pomper M. Glutamate carboxypeptidase II in diagnosis and treatment of neurologic disorders and prostate cancer. Curr Med Chem. 2012;19:856–70.
24. Bacich DJ, Ramadan E, O'Keefe DS, Bukhari N, Wegorzewska I, Ojeifo O, et al. Deletion of the glutamate carboxypeptidase II gene in mice reveals a second enzyme activity that hydrolyzes N-acetylaspartylglutamate. J Neurochem. 2002;83:20–9.
25. Hlouchova K, Barinka C, Konvalinka J, Lubkowski J. Structural insight into the evolutionary and pharmacologic homology of glutamate carboxypeptidases II and III. FEBS J. 2009;276:4448–62.
26. Jeitner TM, Babich JW, Kelly JM. Advances in PSMA theranostics. Transl Oncol. 2022;22:101450.
27. Tsai G, Stauch-Slusher B, Sim L, Hedreen JC, Rothstein JD, Kuncl R, et al. Reductions in acidic amino acids and N-acetylaspartylglutamate in amyotrophic lateral sclerosis CNS. Brain Res. 1991;556:151–6.
28. Plaitakis A. Glutamate dysfunction and selective motor neuron degeneration in amyotrophc lateral sclerosis A hypothesis. Ann Neurol. 1990;28:3–8.
29. Sacha P, Zamecnik J, Barinka C, Hlouchova K, Vicha A, Mlcochova P, et al. Expression of glutamate carboxypeptidase II in human brain. Neuroscience. 2007;144:1361–72.
30. Fricker AC, Mok MH, de la Flor R, Shah AJ, Woolley M, Dawson LA, et al. Effects of N-acetylaspartylglutamate (NAAG) at group II mGluRs and NMDAR. Neuropharmacology. 2009;56:1060–7.
31. Leontovyc A, Ulrychova L, O'Donoghue AJ, Vondrasek J, Maresova L, Hubalek M, et al. SmSP2: a serine protease secreted by the blood fluke pathogen *Schistosoma mansoni* with anti-hemostatic properties. PLoS Negl Trop Dis. 2018;12:e0006446.
32. Dvorak J, Fajtova P, Ulrychova L, Leontovyc A, Rojo-Arreola L, Suzuki BM, et al. Excretion/secretion products from *Schistosoma mansoni* adults, eggs and schistosomula have unique peptidase specificity profiles. Biochimie. 2016;122:99–109.
33. Stefanic S, Dvorak J, Horn M, Braschi S, Sojka D, Ruelas DS, et al. RNA interference in *Schistosoma mansoni* schistosomula: selectivity, sensitivity and operation for larger-scale screening. PLoS Negl Trop Dis. 2010;4:e850.
34. Basch PF. Cultivation of *Schistosoma mansoni* in vitro. I. Establishment of cultures from cercariae and development until pairing. J Parasitol. 1981;67(2):179–85.
35. Leontovyc A, Ulrychova L, Horn M, Dvorak J. Collection of excretory/secretory products from individual developmental stages of the blood fluke *Schistosoma mansoni*. Methods Mol Biol. 2020;2151:55–63.
36. Klusák V, Barinka C, Plechanovová A, Mlcochová P, Konvalinka J, Rulísek L, et al. Reaction mechanism of glutamate carboxypeptidase II revealed by mutagenesis, X-ray crystallography, and computational methods. Biochemistry. 2009;48:4126–38.
37. Webb B, Sali A. Comparative protein structure modeling using MODELLER. Curr Protoc Bioinform. 2016;54:5.6.1–5.6.37.
38. Bim D, Navratil M, Gutten O, Konvalinka J, Kutil Z, Culka M, et al. Predicting effects of site-directed mutagenesis on enzyme kinetics by QM/MM and QM calculations: a case of glutamate carboxypeptidase II. J Phys Chem B. 2022;126:132–43.
39. Ustinova K, Novakova Z, Saito M, Meleshin M, Mikesova J, Kutil Z, et al. The disordered N-terminus of HDAC6 is a microtubule-binding domain critical for efficient tubulin deacetylation. J Biol Chem. 2020;295:2614–28.
40. Barinka C, Ptacek J, Richter A, Novakova Z, Morath V, Skerra A. Selection and characterization of Anticalins targeting human prostate-specific membrane antigen (PSMA). Protein Eng Des Sel. 2016;29:105–15.
41. Rozen S, Skaletsky H. Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol. 2000;132:365–86.
42. Nolan T, Hands RE, Bustin SA. Quantification of mRNA using real-time RT-PCR. Nat Protoc. 2006;1:1559–82.
43. Horn M, Fajtova P, Arreola LR, Ulrychova L, Bartosova-Sojkova P, Franta Z, et al. Trypsin- and chymotrypsin-like serine proteases in *Schistosoma mansoni*—'The Undiscovered Country'. PLoS Negl Trop Dis. 2014;8:e2766.
44. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) Method. Methods. 2001;25:402–8.
45. Ulrychova L, Horn M, Dvorak J. Sensitive fluorescence in situ hybridization on semithin sections of adult *Schistosoma mansoni* using DIG-labeled RNA probes. Methods Mol Biol. 2020;2151:43–53.
46. Ulrychova L, Ostasov P, Chanova M, Mares M, Horn M, Dvorak J. Spatial expression pattern of serine proteases in the blood fluke *Schistosoma mansoni* determined by fluorescence RNA in situ hybridization. Parasite Vector. 2021;14(1).
47. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 2012;9:671–5.
48. Jilkova A, Horn M, Rezacova P, Maresova L, Fajtova P, Brynda J, et al. Activation route of the *Schistosoma mansoni* cathepsin B1 drug target: structural map with a glycosaminoglycan switch. Structure. 2014;22:1786–98.
49. Collins JJ 3rd, King RS, Cogswell A, Williams DL, Newmark PA. An atlas for *Schistosoma mansoni* organs and life-cycle stages using cell type-specific markers and confocal microscopy. PLoS Negl Trop Dis. 2011;5:e1009.
50. Dillon GP, Illes JC, Isaacs HV, Wilson RA. Patterns of gene expression in schistosomes: localization by whole mount in situ hybridization. Parasitology. 2007;134:1589–97.
51. Barinka C, Hlouchova K, Rovenska M, Majer P, Dauter M, Hin N, et al. Structural basis of interactions between human glutamate carboxypeptidase II and its substrate analogs. J Mol Biol. 2008;376:1438–50.
52. Mesters JR, Barinka C, Li W, Tsukamoto T, Majer P, Slusher BS, et al. Structure of glutamate carboxypeptidase II, a drug target in neuronal damage and prostate cancer. EMBO J. 2006;25:1375–84.
53. Barinka C, Rovenska M, Mlcochova P, Hlouchova K, Plechanovova A, Majer P, et al. Structural insight into the pharmacophore pocket of human glutamate carboxypeptidase II. J Med Chem. 2007;50:3267–73.
54. Barinka C, Hlouchova K, Rovenska M, Majer P, Dauter M, Hin N, et al. Structural basis of interactions between human glutamate carboxypeptidase II and its substrate analogs. J Mol Biol. 2008;376:1438–50.
55. Barinka C, Rinnova M, Sacha P, Rojas C, Majer P, Slusher BS, et al. Substrate specificity, inhibition and enzymological analysis of recombinant human glutamate carboxypeptidase II. J Neurochem. 2002;80:477–87.
56. Mlcochova P, Plechanovova A, Barinka C, Mahadevan D, Saldanha JW, Rulisek L, et al. Mapping of the active site of glutamate carboxypeptidase II by site-directed mutagenesis. FEBS J. 2007;274:4731–41.
57. Hlouchova K, Barinka C, Konvalinka J, Lubkowski J. Structural insight into the evolutionary and pharmacologic homology of glutamate carboxypeptidases II and III. FEBS J. 2009;276:4448–62.
58. Barinka C, Rojas C, Slusher B, Pomper M. Glutamate carboxypeptidase II in diagnosis and treatment of neurologic disorders and prostate cancer. Curr Med Chem. 2012;19:856–70.
59. Pavlicek J, Ptacek J, Barinka C. Glutamate carboxypeptidase II: an overview of structural studies and their importance for structure-based drug design and deciphering the reaction mechanism of the enzyme. Curr Med Chem. 2012;19:1300–9.
60. Barinka C, Rinnova M, Sacha P, Rojas C, Majer P, Slusher BS, et al. Substrate specificity, inhibition and enzymological analysis of recombinant human glutamate carboxypeptidase II. J Neurochem. 2002;80:477–87.
61. Mesters JR, Barinka C, Li WX, Tsukamoto T, Majer P, Slusher BS, et al. Structure of glutamate carboxypeptidase II, a drug target in neuronal damage and prostate cancer. Embo J. 2006;25:1375–84.
62. Wahlestedt C. Natural antisense and noncoding RNA transcripts as potential drug targets. Drug Discov Today. 2006;11:503–8.
63. Vasconcelos EJR, daSilva LF, Pires DS, Lavezzo GM, Pereira ASA, Amaral MS, et al. The *Schistosoma mansoni* genome encodes thousands of long non-coding RNAs predicted to be functional at different parasite life-cycle stages. Sci Rep. 2017;7:10508.
64. Maciel LF, Morales-Vicente DA, Silveira GO, Ribeiro RO, Olberg GGO, Pires DS, et al. Weighted gene co-expression analyses point to long

non-coding RNA hub genes at different *Schistosoma mansoni* life-cycle stages. Front Genet. 2019;10:823.
65. Guidi A, Mansour NR, Paveley RA, Carruthers IM, Besnard J, Hopkins AL, et al. Application of RNAi to genomic drug target validation in Schistosomes. PLoS Negl Trop Dis. 2015;9:e0003801.
66. Robinson MB, Blakely RD, Couto R, Coyle JT. Hydrolysis of the brain dipeptide *N*-acetyl-L-aspartyl-L-glutamate. Identification and characterization of a novel N-acetylated alpha-linked acidic dipeptidase activity from rat brain. J Biol Chem. 1987;262(30):14498–506.
67. Pinto JT, Suffoletto BP, Berzin TM, Qiao CH, Lin S, Tong WP, et al. Prostate-specific membrane antigen: a novel folate hydrolase in human prostatic carcinoma cells. Clin Cancer Res. 1996;2:1445–51.
68. Barinka C, Sacha P, Sklenar J, Man P, Bezouska K, Slusher BS, et al. Identification of the N-glycosylation sites on glutamate carboxypeptidase II necessary for proteolytic activity. Protein Sci. 2004;13:1627–35.

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