

# Dynamics of *Schistosoma Mansoni* Development in The Intermediate Host, *Biomphalaria Glabrata*, A Freshwater Snail

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## Research

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# Abstract

## Background

Schistosomiasis is one of the most significant and prevalent waterborne parasitic diseases. Even though many studies have been reported about schistosomiasis, the dynamics of schistosome in intermediate host snails is little known. In the present study, the dynamics of *Schistosoma* larvae in infected snails was histologically investigated.

## Methods

To examine the localization of *Schistosoma mansoni* (*S. mansoni*) parasites in the snails, *Biomphalaria glabrata* snails infected with miracidia were harvested and examined by stereoscopic observation. Then, frozen sections were prepared and stained with H&E. Furthermore, immunohistochemical detection of parasites was performed using anti-*S. mansoni* antibody, and their localization in the snails was analyzed.

## Results

Snails infected with *S. mansoni* miracidia were harvested at 10 and 56 days post-infection (DPI) and analyzed. In the stereoscopic observations, white spots were observed at 56 DPI, while no spots were observed at 10 DPI. However, histological investigations visualized the larvae specifically in the head-foot area of the snail at 10 DPI. Further, it was observed that the larvae relocated to the hepatopancreas and ovotestis areas at 56 DPI.

## Conclusions

The present study revealed the dynamics of *Schistosoma* larvae in intermediate snails, shown as the differential localization of *S. mansoni* larvae at early and late infection stages.

## Background

Schistosomiasis is one of the most significant and prevalent waterborne parasitic diseases, as it affects more than 240 million people worldwide (WHO 2020) [1, 2]. Six *Schistosoma* species (*Schistosoma haematobium*, *S. mansoni*, *S. japonicum*, *S. intercalatum*, *S. guineensis*, *S. mekongi*) are pathogenic in humans and freshwater snails are known to be their intermediate hosts [3, 4]. Following excretion of schistosome eggs from an infected human or animal, miracidia hatch from the eggs and infect intermediate host snails, with developing into cercariae in the snails [5, 6]. After cercariae are released from the snails, they percutaneously infect humans and animals, then become schistosomulae, migrate into the portal vein via the bloodstream, and mature into adult worms [5, 7]. Combinations of male and female adult worms lay eggs in intestinal or pelvic veins, and the schistosome lifecycle is repeated [8].

A large number of studies have been reported about schistosomiasis, including case reports and developments of new drugs and vaccination methods [9-14]. On the other hand, few studies that focused on intermediate hosts have been presented [15-17]. Notably, the dynamics of schistosome in intermediate host snails is little known, though it has been well known that the infection begins with miracidia invasion and ends with explosively increased production of cercariae. Since *Schistosoma* cercariae infect the final host, it is crucial to suppress outbreak production of cercariae to control the prevalence of schistosomiasis.

In the present study, the dynamics of *Schistosoma* larvae in infected snails was histologically visualized and we revealed that the larvae initially invade the surface of the head-foot area of the snail and localize there in the early period of their development, then relocate to the hepatopancreas and ovotestis areas in the late period of the infection. Thus, it is elucidated that the process of *Schistosoma* larvae growth and development is related to the selection of preferential locations inside intermediate host snails.

## Methods

### Ethical statement

All of the present experiments using *Schistosoma mansoni* (*S. mansoni*) parasites, *Biomphalaria glabrata* (*B. glabrata*) snails, and mice were approved by the Institutional Animal Research Committee of Nara Medical University (#12813, 12776), and performed according to Japanese statutes regarding Humane Treatment and Management of Animals.

### Animals and parasites

Female ICR mice (eight weeks old) were purchased from Japan SCL (Hamamatsu, Japan). A strain of *S. mansoni* (NIH-Sm-PR-1) was maintained at the animal facilities of Nara Medical University by passage through albino *B. glabrata* snails (Newton's NIH Puerto Rican/Brazilian M-line strain) and ICR mice [18]. Mice were subcutaneously infected with 250 *S. mansoni* cercariae, then the livers were harvested eight weeks after initiation of infection. Parasite eggs were separated from the livers and collected following treatments with actinase and collagenase. Miracidia were then hatched from the eggs in sterile water and used to infect *B. glabrata* snails (5-mm shell size). Each snail was infected with five miracidia of *S. mansoni*. They were placed in 24-well plates containing tap water for 24 hours at 28°C, then transferred into a 15-L aquarium. The release of cercariae was observed by illumination with an LED lamp from four weeks up to 12 weeks after infection.

### Post-infection histological analysis of *B. glabrata* in snails

To examine the localization of the parasites in the snails, *B. glabrata* snails infected with miracidia were harvested at 10 and 56 days post-infection (DPI) (Fig. 1). After removing the shell, each snail was fixed with 4% PFA for two days at 4°C, followed by 20% sucrose in PBS for 12 hours. Next, the specimens were embedded in an OTC compound, then sections (10 µm thick) were prepared using a cryostat (Thermo)

and stained with hematoxylin-eosin (H&E) using a standard protocol. Immunohistochemical detection of *S. mansoni* parasites was performed in each of 10 sections and their localization in the snails was analyzed. For immunohistochemistry, the sections were permeabilized with 0.2% Triton X-100 in PBS (TPBS) containing 1% BSA, followed by blocking with 5% normal goat serum, then treated with a mouse anti-*S. mansoni* monoclonal antibody (BGN/1A7, dilution 1:100, Novus Biologicals). The following incubation at 4°C overnight and washing three times with TPBS, an AlexaFluor 546 conjugated goat anti-mouse secondary antibody (dilution 1:200, Molecular Probes, Invitrogen) was used to detect primary antibodies. All nuclei were stained with DAPI (Dojin), followed by washing three times with TPBS. Fluorescence was visualized using a fluorescent microscopy system (Zeiss Axiovert 200).

## Results

### Localization of *S. mansoni* larvae in infected-*B. glabrata* snails

Snails infected with *S. mansoni* miracidia were harvested at 10 and 56 DPI and analyzed. At 10 DPI (early infection), no gross abnormalities were observed in the infected snails as compared with uninfected snails (Fig. 2A). At 56 DPI (late infection), white spots were noted in the infected snails (Fig. 2B, 2F-2J), whereas those were not observed in uninfected snails at any time (Fig. 2A-2E), indicating that the spots were formed where *S. mansoni* sporocysts or cercariae existed. The snails were fixed and sliced, then the sections were subjected to H&E staining. In sections from the head-foot (HF) area at 10 DPI, deep purple colored structures were detected, in which the presence of *S. mansoni* larvae was suspected (Fig. 3A-3C, 3E, 3F), while it was not detected in the hepatopancreas (HP) area (Fig. 3D). At 56 DPI, *S. mansoni* larvae were detected in the deep purple colored structures in the sections of the HP area (Fig. 3G), while more precise observations revealed their distribution in other organs, including the ctenidium (Ct), and ovotestis (OT) (Fig. 3I-3K), but not in HF area (Fig. 3H).

After serial sections were stained by H&E (Fig. 4A, 4B, 4N, 4O), immunohistochemical analysis was conducted using the anti-*S. mansoni* antibody that reacts to both *S. mansoni* miracidia and cercariae (Supplementary Fig. S1). Figures A, B, N and O in Figure 4 are same respectively to Figures A, B, G and J in Figure 3. *S. mansoni* larvae were immunohistochemically confirmed to be present in the HF area in the early period of their development (10 DPI) (Fig. 4A-4M). However, they were predominantly present in the HP areas in the late period of their development (56 DPI) (Fig. 4N-4R).

### Mapping of *S. mansoni* larvae in *B. glabrata* snails

We attempted to produce a map showing where *S. mansoni* larvae existed in *B. glabrata* snails (Fig. 5A). Immunohistochemical analysis of serial sections of *S. mansoni*-infected *B. glabrata* snails at 10 DPI showed larvae in 30 out of 45 sections from the HF area (66%) (Fig. 5B), while those were observed in only 7 of 45 sections from other areas (15%), such as Hr and Ct. At 56 DPI, immunopositivity for *S. mansoni* larvae was observed in all 15 successive specimens from the HP and OT areas, which was in contrast to sparse distribution in the other areas, indicating accumulation of *S. mansoni* larvae in the HP + OT (HPOT) area (Fig. 5C). These findings indicate that *S. mansoni* larvae become located in the HF area

during the early infection period, then move to the HPOT area by the time of late infection, and suggest the presumable location-specific assistance.

## Discussion

Schistosome infects intermediate host snails with miracidia, then miracidia become cercariae by approximately four weeks [5, 19]. Although it is known that the intra-snail development process includes mother sporocysts, daughter sporocysts, and cercariae [4, 7], their distributions in snails have not been analyzed in detail. In the present study, localization of *S. mansoni* larvae during the early (10 DPI) and late (56 DPI) development periods in intermediate host *B. glabrata* snails was investigated, and the distribution of *S. mansoni* larvae was clearly demonstrated to be different between those early and late periods, as the predominant location at 10 DPI was the head-foot (HF) area, while the hepatopancreas (HP) and ovotestis (OT) areas were predominant at 56 DPI. However, the larval developmental stage was not determined due to the limitation that anti-*S. mansoni* monoclonal antibody used in the present study recognizes both *S. mansoni* miracidia and cercariae (Supplementary Fig. S1) but does not discriminate each stages in the snails. Bahia *et al.* reported that the *S. mansoni* protein kinase C1 (SmPKC1) is differentially expressed during the lifecycle of this parasite (adult worm, miracidium, sporocyst, cercaria, schistosomula) [20], and Fernandes *et al.* found that expression patterns of the *S. mansoni* venom allergen-like protein (SmVAL) family differed among the germ ball, cercaria, and schistosomula [21]. We consider that immunohistological analysis of SmPKC1 and SmVAL expression patterns would make it possible to identify the developmental stage of larvae found at 10 and 56 DPI.

Schistosome infects only certain species of intermediate host snails, while *B. glabrata* is known to be the host snail for *S. mansoni* [6, 7]. The albino *B. glabrata* snails (Newton's NIH Puerto Rican/Brazilian M-line strain) used in the present study have been reported to produce *S. mansoni* cercariae [18], which was confirmed by our findings. Some strains of *B. glabrata*, such as the NMRI and M line, are also susceptible to the infection by *S. mansoni* [22, 23], while resistant strains such as BS-90 also allow infection with *S. mansoni* miracidia, though it does not lead to production of cercariae [24, 25]. Snails have innate immune defense systems that can specifically or non-specifically target schistosomes for destruction [26, 27]. Even following successful infection, miracidia and sporocysts are exposed to humoral factors, and attacked by motile hemocytes in the hemolymph and other tissues of the snail [28], resulting in production failure of cercariae. Therefore, an immunohistological investigation of larval distribution using resistant snails such as BS-90 may provide temporal and spatial information regarding the arrest of larval development. Indeed, larval encapsulation and several factors including reactive oxygen species [29] and other resistance-associated factors [30] have been noted as possible mechanisms by which the development of larvae fails to proceed.

The present results demonstrated changes in larval locations inside snails between early and late infection, strongly suggesting that the environment favoring *Schistosoma* larvae differs depending on the developmental stage. Several methods for culturing *Schistosoma* larvae *in vitro* have been reported [31, 32]. The Bge cell line, established from 4- to 5-day-old embryos of an albino *B. glabrata* snail, has been

utilized to support the development and growth of *Schistosoma spp* [33]. larvae *in vitro*. According to the report by Yoshino *et al.*, Bge cells supported the induction of sporocysts but failed to produce cercariae [34]. Furthermore, Ivanchenko *et al.* observed the generation of daughter sporocysts, whereas cercariae production was scarcely observed in the cultures with Bge cells [35]. Considering previously reported information about culture systems, the use of Bge cells alone seems to be inadequate to achieve complete production of sporocysts and cercariae. Identification of environmental factors required for the development of those larvae would make it possible to grow them *in vitro*.

## Conclusion

The present study revealed differential localization of *S. mansoni* larvae at early and late infection stages in intermediate snails. Our findings may lead to the development of novel methods for *in vitro* larvae cultivation using larval stage-specific environmental factors.

## Abbreviations

***S. mansoni***: *Schistosoma mansoni*

***B. glabrata***: *Biomphalaria glabrata*

**DPI**: days post-infection

**H&E**: hematoxylin-eosin

**TPBS**: 0.2% Triton X-100 in PBS

**HF**: head-foot

**HP**: hepatopancreas

**Ct**: ctenidium

**OT**: ovotestis

**Hr**: heart

**HPOT**: hepatopancreas and ovotestis

**SmPKC1**: *S. mansoni* protein kinase C1

**SmVAL**: *S. mansoni* venom allergen-like protein

## Declarations

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## **Availability of data and materials**

All data generated or analyzed during this study are included in this article and its supplementary information files.

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## **Contributions**

YO, SH, MY designed the experiments. YO, MH, MM performed the experiments. YO, MH, SH, MY collected and analyzed the data. MM, MH, TK contributed reagents/materials/analysis. YO, SH, MY wrote the paper. All authors read and approved the final version of the manuscript.

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## Ethics declarations

### Ethics approval and consent to participate

All of the present experiments were approved by the Institutional Animal Research Committee of Nara Medical University (#12813, 12776), and performed according to Japanese statutes regarding Humane Treatment and Management of Animals.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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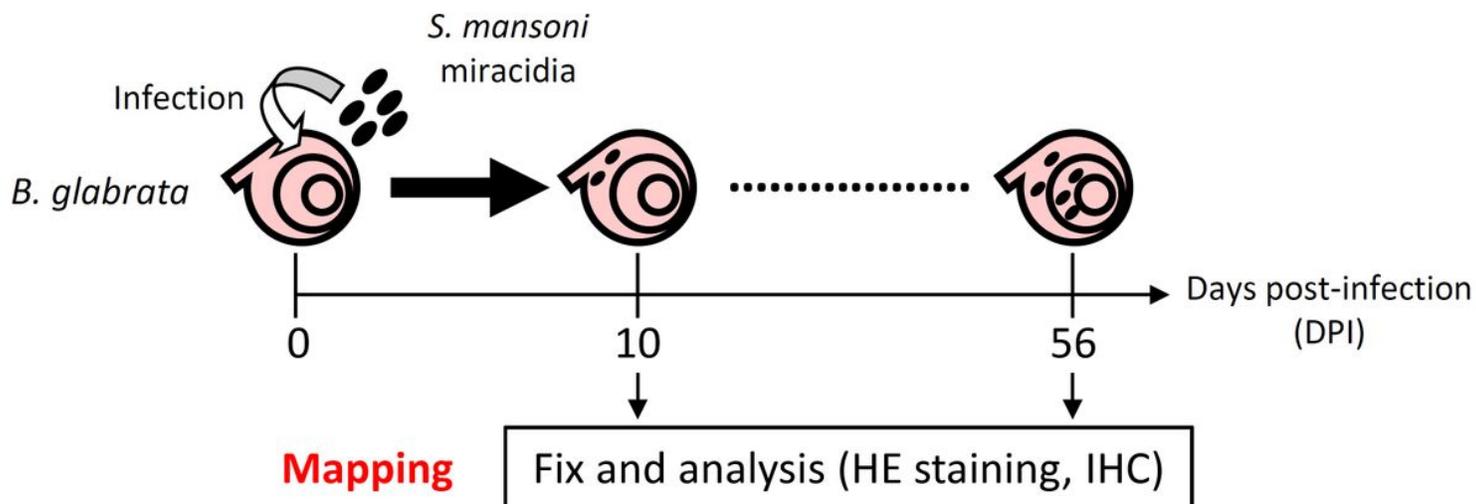
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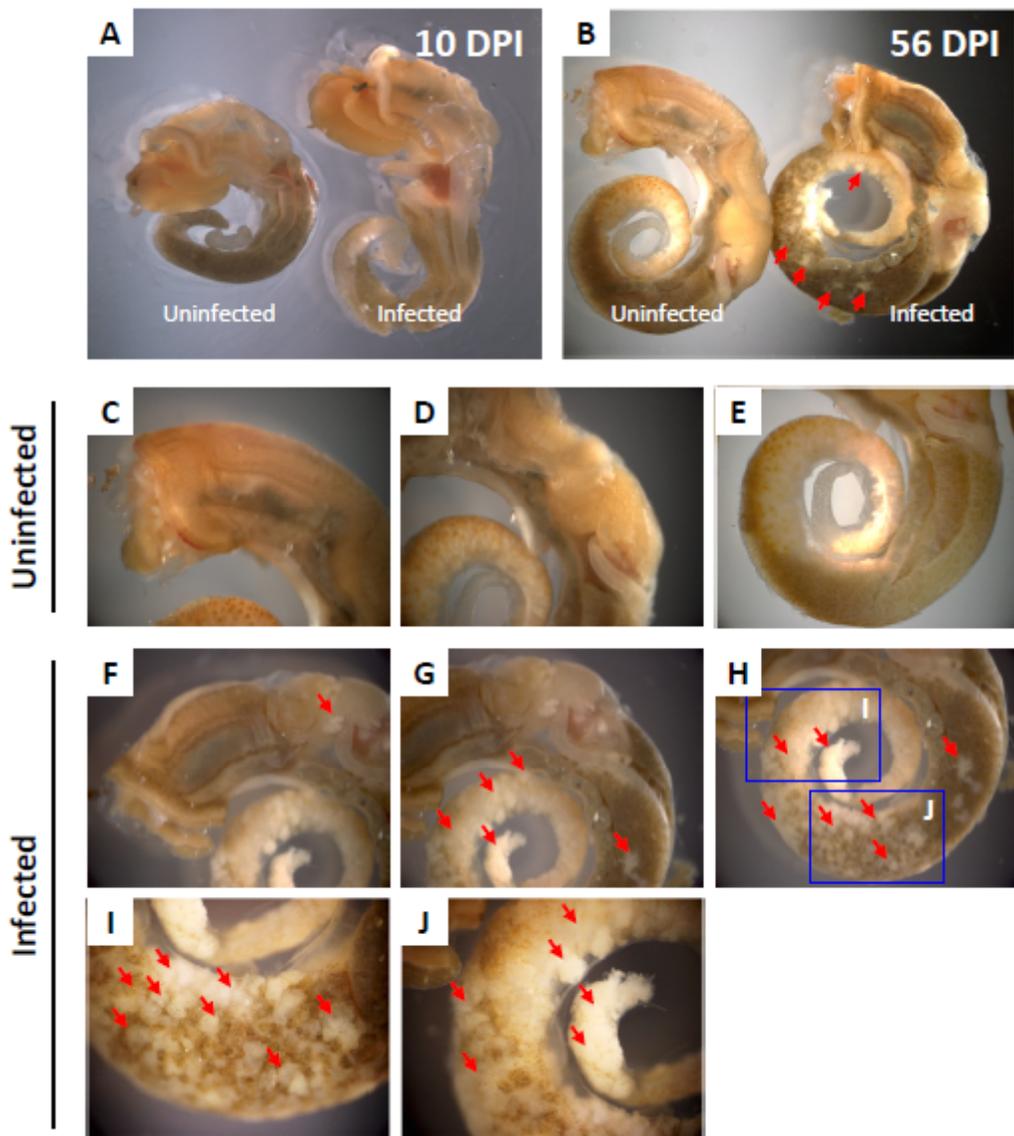
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## Figures



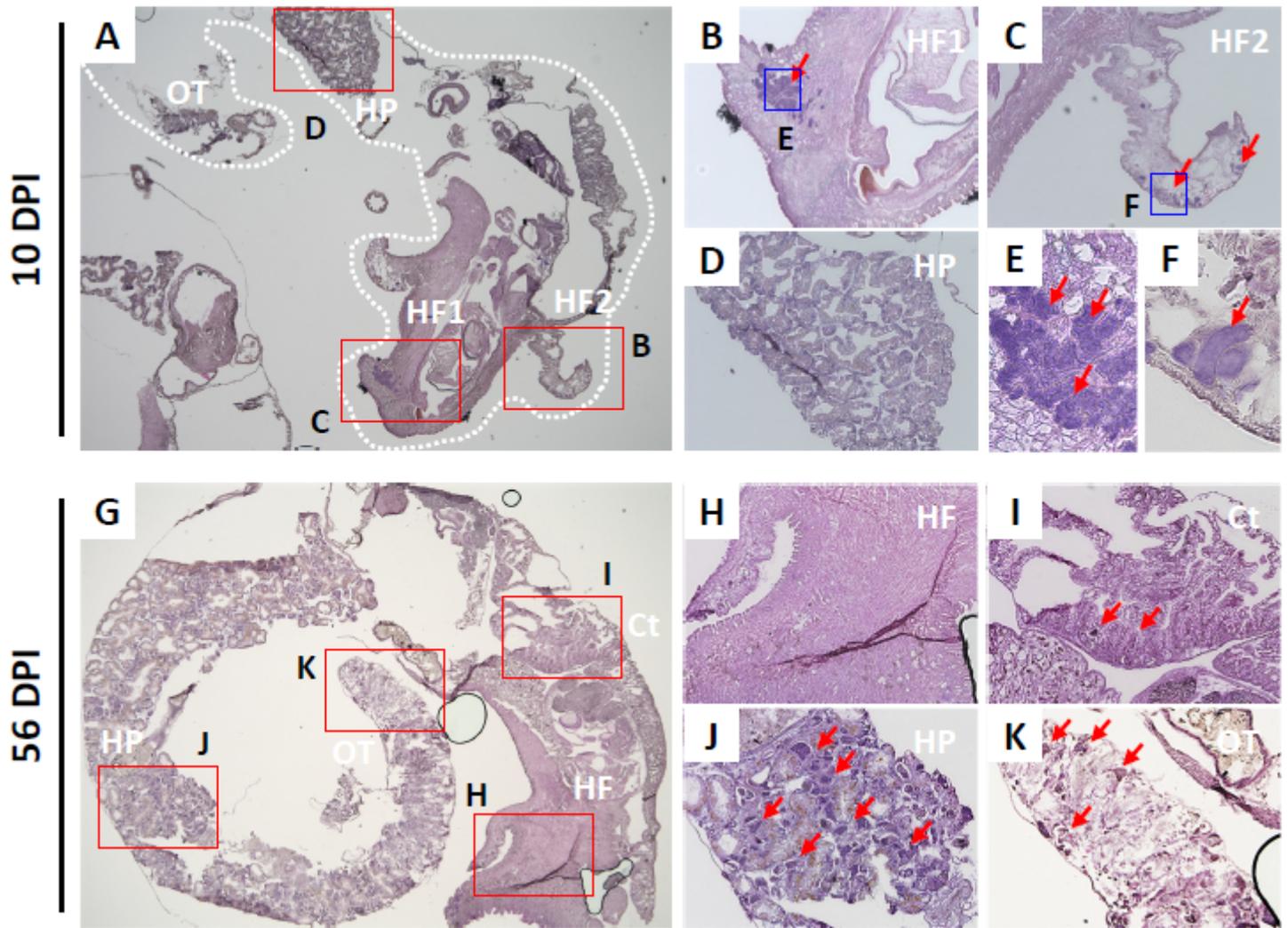
**Figure 1**

Study schema Snails infected by *S. mansoni* miracidia were harvested at 10 and 56 DPI. Sections of fixed snails were analyzed by H&E staining and immunohistochemistry using anti-*S. mansoni* antibodies.



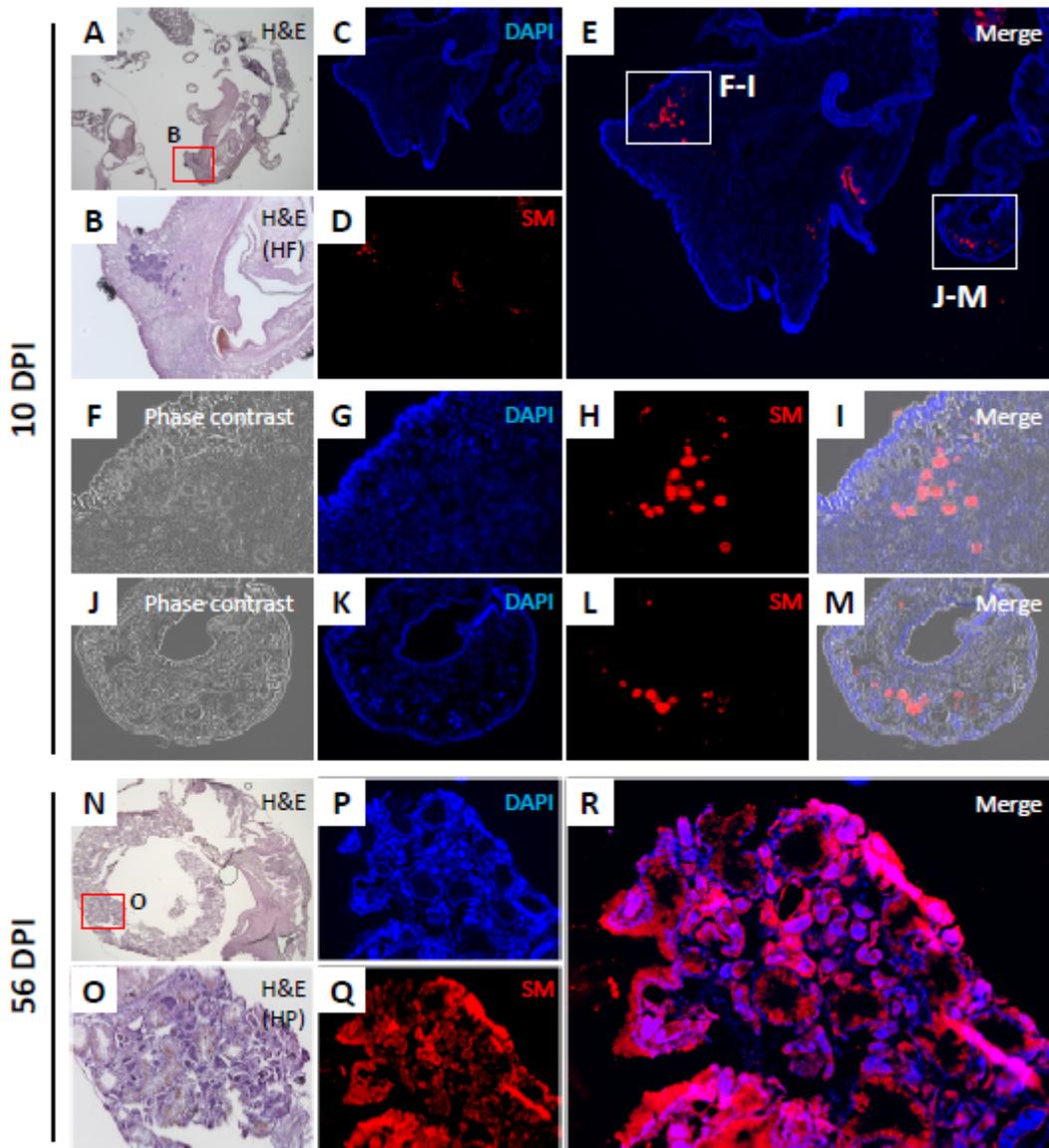
**Figure 2**

Stereoscopic observations of *B. glabrata* uninfected or infected with *S. mansoni* Photographs of *B. glabrata* snails without and with infection by *S. mansoni* miracidia at 10 DPI (A) and 56 DPI (B). (C-J) Photographs show *B. glabrata* snails uninfected (C-E) or infected (F-J) with *S. mansoni* at 56 DPI. (I, J) Magnification of blue squares shown in H. White mass spots were noted (red arrows).



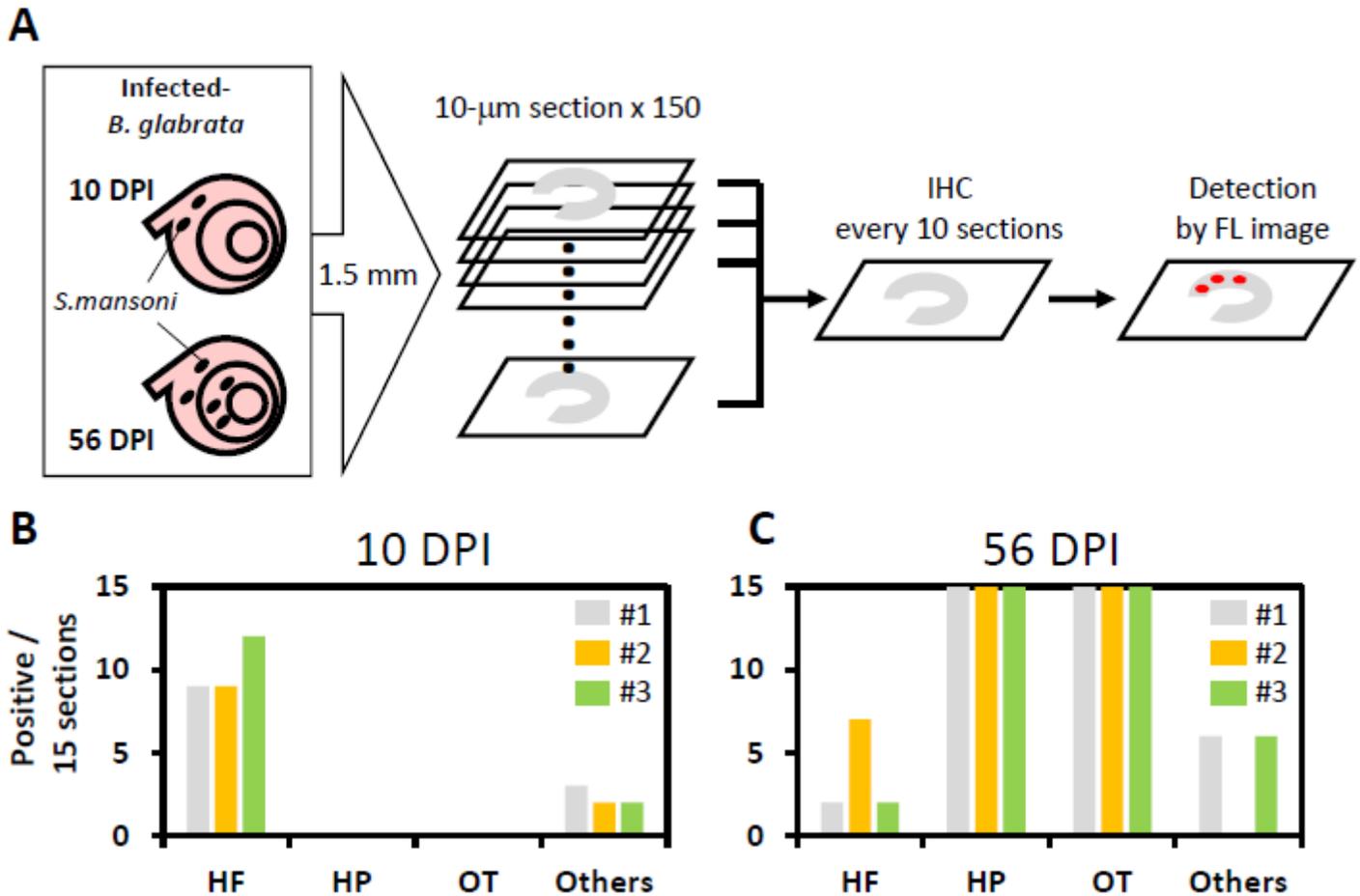
**Figure 3**

Histological analysis of *S. mansoni* larvae in infected-*B. glabrata* snails (A-F) Photographs show H&E stained section of *B. glabrata* snails infected with *S. mansoni* at 10 DPI. HF1, HF2: head-foot; HP: hepatopancreas; OT: ovotestis. (B-D) Magnification of red squares in A. Arrows indicate *S. mansoni* larvae infection. (E, F) Magnification of blue squares in B and C, respectively. (G-K) Photographs show H&E stained section of *B. glabrata* snails infected with *S. mansoni* at 56 DPI. Ct: ctenidium; HF: head-foot; HP: hepatopancreas; OT: ovotestis. (H-K) Magnification of red squares in G. Arrows indicate *S. mansoni* larvae infection.



**Figure 4**

Immunohistochemical analysis of *S. mansoni* infected with *B. glabrata*. Sections of infected snails were stained with H&E (A, B: 10 DPI; N, O: 56 DPI). Figures A, B, N and O in Figure 4 are same respectively to Figures A, B, G and J in Figure 3. Areas in red squares in A and N are shown magnified in B and O, respectively. Sections of infected snails were analyzed by immunohistochemistry using anti-*S. mansoni* antibodies (SM) (C-M: 10 DPI, P-R: 56 DPI). (F-I, J-M) Magnification of squares in E. F, J: phase contrast. C, G, K, P: staining with DAPI. D, H, L, Q: *S. mansoni* detected immunohistochemically using SM and AlexaFluor 546 conjugated secondary antibodies. E, I, M, R: Merged images with DAPI and SM staining.



**Figure 5**

Mapping of *S. mansoni* larvae in *B. glabrata* snails and extract preparation. (A) Immunohistochemical analyses were performed using serial sections of snails infected with *S. mansoni*. (B) At 10 DPI, *S. mansoni* larvae were predominantly detected in the head-foot (HF) area, though also observed in others such as the heart (Hr) and ctenidium (Ct). (C) At 56 DPI, immunopositivity for *S. mansoni* larvae was locally concentrated in the hepatopancreas (HP) and ovotestis (OT) areas, which was noted in all of the 15 successive specimens examined. A few *S. mansoni* larvae were also observed in other organs (HF, Ct).

## Supplementary Files

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