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COMPARATIVE VECTORIAL COMPETENCE OF *BIOMPHALARIA SUDANICA* AND *BIOMPHALARIA CHOANOMPHALA*, SNAIL HOSTS OF *SCHISTOSOMA MANSONI*, FROM TRANSMISSION HOTSPOTS IN LAKE VICTORIA, WESTERN KENYA

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ABSTRACT

Schistosoma mansoni, which causes human intestinal schistosomiasis, continues to be a major public health concern in the Lake Victoria basin in western Kenya, with *Biomphalaria sudanica* (a shoreline inhabiting snail) and *Biomphalaria choanomphala* (a deep-water snail) playing roles in transmission. A recent study showed that *B. sudanica* was abundantly present near all study villages on the lakeshore, but *B. choanomphala* was significantly more abundant near villages known to be persistent transmission hotspots. The present study investigated the relative compatibility of *B. sudanica* and *B. choanomphala* with *S. mansoni*. A reciprocal cross-infection experiment used young adult F1 generation *B. sudanica and B. choanomphala* that were exposed to either 1, 5, or 10 sympatric or allopatric human-derived *S. mansoni* miracidia. Three weeks post-exposure (PE) and weekly thereafter, the snails were counted and screened for schistosome cercariae, and at 7 wk PE, total cercariae shed during a 2 hr period by each infected snail was determined. Pre-patent periods for *S. mansoni* in both *B. sudanica* and *B. choanomphala* were similar, and most snails in all exposure combinations started shedding cercariae 5 wk PE. Prevalences were significantly higher in *B. choanomphala* (12.2–80.9%) than in *B. sudanica* (5.2–18.6%) at each dose, regardless of whether miracidia were of an allopatric or a sympatric source ($P < 0.0001$). Overall, the odds of a snail becoming infected with 5 or 10 miracidia were significantly higher than the odds of being infected with 1 miracidium, ($P < 0.0001$), and fewer cercariae were produced by snails exposed to single as compared to 5 or 10 miracidia. On average, *B. choanomphala* produced more cercariae ($\bar{X} = 458$, SD = 414) than *B. sudanica* ($\bar{X} = 238$, SD = 208) ($P < 0.0001$). These results suggest that *B. choanomphala* is more compatible with *S. mansoni* than *B. sudanica*. Though *B. choanomphala* can be found in shallow shoreline waters, it is, for the most part, a deeper-water taxon. Because dredging is a relatively inefficient means of sampling, *B. choanomphala* is likely underestimated with respect to its population size, the number of *S. mansoni*–positive snails, and its role in maintaining transmission.

Intestinal schistosomiasis caused by *Schistosoma mansoni* continues to be a major public health concern in the Lake Victoria basin in western Kenya despite various chemotherapy-based control efforts (Onkanga et al., 2016; Karanja et al., 2017; Wiegand et al., 2017; King et al., 2020; Secor et al., 2020). Within the region, the prevalence of *S. mansoni* is high on the islands in the lake and villages along the lakeshore, and it declines with increasing distance from the lakeshore (Odiere et al., 2012; Samuels et al., 2012). Transmission of *S. mansoni* within the Lake Victoria basin is perpetuated by 3 *Biomphalaria* snail taxa, each of which occupies distinct habitats: (1) *Biomphalaria pfeifferi*, an inhabitant of streams, canals, ponds, and other small water impoundments, (2) *Biomphalaria sudanica*, which lives in shallow waters on the shores of Lake Victoria and the surrounding swamps, and (3) *Biomphalaria choanomphala*, usually found on the lake bottom up to depths of 12 m and possibly deeper but sometimes swept to the shoreline by strong water currents (Loker et al., 1993; Brown, 1994; DeJong et al., 2001; Mutuku et al.,

2019). Molecular studies of mitochondrial and nuclear markers of *B. sudanica* and *B. choanomphala* suggest that the 2 taxa are not highly divergent genetically and should probably be considered as a single species (Standley et al., 2011; Zhang et al., 2018).

In a recent study that involved a 4-yr annual praziquantel treatment of school children in villages around the lakeshore, it was observed that in some villages, the prevalence of *S. mansoni* decreased markedly to less than 30% of pre-control levels (responding [RESP] villages), whereas in other villages, prevalence remained high and above 30% after the 4-yr treatment campaign, and these were designated as persistent hotspots (PHS villages) (Wiegand et al., 2017). A follow-up study indicated there were no significant differences between RESP and PHS villages in relative abundance of *B. sudanica* in shoreline habitats sampled or prevalence of *S. mansoni* infection in the snail populations (Mutuku et al., 2019). However, *B. choanomphala* was recovered from all PHS villages, which were all located along the west-facing shoreline of the lake, and only from one of the RESP villages located near the mouth of Winam Gulf. The three remaining RESP villages were all located farther east, along the shores of the Winam Gulf, Lake Victoria. *Biomphalaria choanomphala* was significantly more abundant in the PHS villages, and the prevalence of *S. mansoni* among villages both before and after control was positively correlated with *B. choanomphala* abundance (Mutuku et al., 2019). Differences in offshore ecology may explain the differential presence of *B. choanomphala* in the PHS and RESP villages. Of the total *Biomphalaria* snails retrieved from the PHS villages that were infected with *S. mansoni*, 3.5% were *B. choanompahala*. In a different study done in Mwanza, Tanzania, farther south on the shore of Lake Victoria, 12.2% of all *Biomphalaria* positive for *S. mansoni* were *B. choanomphala* (Gouvras et al., 2017). Both studies concluded that although most transmission occurs via the shore-inhabiting *B. sudanica*, *B. choanomphala* also plays an underestimated role in the transmission of schistosomiasis in the Lake Victoria basin. Also, in both these studies, there was no obvious indication that *B. pfeifferi* played a role in *S. mansoni* transmission in the lake.

A key determinant of schistosomiasis transmission success is the compatibility of the local snail population with local schistosomes. Compatibility, as defined here, has several components, including the likelihood that exposure of a snail to miracidia leads to a cercariae-producing infection. The greater the population level compatibility, the more snail infections are expected to result from a given level of schistosome egg input into the habitat (Anderson and May, 1979; French et al., 2010). Another key factor in the compatibility of snails as intermediate host is the length of time required to complete sporocyst development to enable the first release of cercariae following exposure to miracidia (the pre-patent period). The longer the pre-patent period, the more likely the infected snail might be to suffer mortality and never bring an infection to culmination. The remaining key factor in compatibility we consider is the daily and/or total output of cercariae produced by infected snails (Ibikounlé et al., 2012).

The degree of compatibility can be influenced strongly by whether the parasite and host derive from the same environment (are sympatric), or if host snails are exposed to *S. mansoni* miracidia from distant environments (allopatric combinations) (Southgate et al., 2000; Ibikounlé et al., 2012; Adriko et al., 2013; Mutuku et al., 2014, 2017). Theory generally predicts that a parasite should be more adapted to sympatric than to allopatric hosts, and that the superior adaptation of a parasite to local hosts should be more pronounced when the hosts have discontinuous sporadic distribution rather than continuous distribution (Thompson, 1994; Morand et al., 1996). Adaptation of parasites to their local hosts is a common phenomenon but not universal, and sometimes the pattern is even reversed (Kaltz and Shykoff, 1998). A number of factors, including high rates of local extinction (such that co-evolutionary associations do not have a chance to develop), high rates of migration of host or parasite populations, or time lags in response may also break down or obscure patterns of local adaptation (Thompson, 1994; Lively and Dybdahl, 2000).

Several studies have determined the vectorial competence of snail species that transmit *S. mansoni* in the East African region. A study of Kenyan field-derived *B. sudanica* and *B. pfeifferi* using *S. mansoni* derived from allopatric or sympatric sources established that *S. mansoni* developed faster and consistently had higher prevalence in *B. pfeifferi* (39.6–80.7%) than in *B. sudanica* (2.4–21.5%), regardless of the source of *S. mansoni* (Mutuku et al., 2017). Mean daily cercariae production was greater for *B. pfeifferi* exposed to sympatric than for the snails exposed to allopatric *S. mansoni* (583–1,686 vs. 392–1,232 respectively). Furthermore, mean daily cercariae output among *B. sudanica* was consistently low (50–590), with no significant differences between sympatric or allopatric combinations. In another study using Ugandan *Biomphalaria* snail isolates, *B. sudanica* produced more cercariae than *B. pfeifferi*, even though the difference in cercariae output between the 2 species was not significant (Adriko et al., 2013). Although experimentation with additional isolates is needed, the low measures of compatibility retrieved with *B. sudanica* following experimental exposure to *S. mansoni* in our hands are suggestive of the presence in *B. sudanica* of resistance traits that may affect the force of transmission of the parasite to the definitive host.

The present study examines the relative compatibility of *S. mansoni* with *B. sudanica* and *B. choanomphala*, the 2 snail taxa responsible for the transmission of intestinal schistosomiasis to human populations living near the lake shore. The objective was to determine if the 2 snail hosts differ in their susceptibility to *S. mansoni* infection derived from a sympatric locality (one whose transmission depends on either *B. choanomphala or B. sudanica*) compared to allopatrically sourced *S. mansoni* (one collected from a different location where transmission depends on *B. pfeifferi*). The study also sought to determine daily cercariae output in the 2 snail taxa, another key parameter associated with vectorial competence.

MATERIALS AND METHODS

Experimental design

A reciprocal cross-infection experiment using first generation lab-reared snails was conducted in which young adult snails (2 mo old), *B. sudanica* (6–9 mm shell diameter) or *B. choanomphala* (5–7 mm shell diameter), were exposed to sympatric miracidia of *S. mansoni* from Kanyibok (Siaya County) or allopatric miracidia from Asao (Kisumu County) where transmission is usually perpetuated by *B. pfeifferi*. For each combination, 100 snails were exposed to either 1, 5, or 10 miracidia of *S. mansoni*. Another group of 100 snails for each of the 2 snail species were

not exposed to the parasite and served as negative controls. A total of 1,400 snails and 6,400 miracidia were used in this experiment. Observations were made once a week over a period of 10 wk. Snails were counted, screened for schistosome infections by the "shedding" method as described by Mutuku et al., (2014) starting from 3 wk post-exposure (PE), and the number of snails surviving and those shedding cercariae recorded. For snails that were found to be positive for schistosome infections, the total number of cercariae they produced within a 2 hr period was determined at 7 wk PE.

Parasite and snail sources

Schistosoma mansoni eggs were obtained from pooled fecal samples from 5 adults from Asao, Nyakach Sub-County, Kisumu County, western Kenya (00°19′01″S, 035°00′22″E) and from 5 adults from Kanyibok, Bondo Sub-County, Siaya County, western Kenya (00°05′22.49″S, 34°05′09.34″E). Schistosome eggs were concentrated and hatched, and miracidia used to infect snails as described previously (Mutuku et al., 2014). Initial snail populations of *B. sudanica* and *B. choanomphala* snails were collected by scooping and dredging, respectively, in Anyanga beach in Kanyibok (00°05′22.49″S, 34°05′09.34″E) as described previously (Mutuku et al., 2019). Kanyibok village is 3 km away from where the *B. choanomphala* were collected for the mitochondrial genome characterization (Zhang et al., 2018). The snails were then transported to the laboratory at the Center for Global Health Research (CGHR), Kenya Medical Research Institute (KEMRI), Kisian, Kisumu, where they were sorted by species and screened individually for trematode infections. Any snails found to be shedding any type of cercariae were discarded.

Raising F1 generations of *B. sudanica* and *B. choanomphala*

The field-derived *B. sudanica* or *B. choanomphala* snails were maintained for breeding in plastic aquaria measuring 60 cm long × 30 cm wide × 15 cm deep in outdoor, "semi-field" ambient conditions in a roofed, open-sided, screened structure at CGHR, KEMRI Kisian, as described previously (Mutuku et al., 2014). The goal was to provide conditions that were close to what the snails would experience in the field, which ensures natural survival of the snails and provides an environment conducive for snail breeding. After 1 mo in the aquaria, the field-collected snails were removed, and the upcoming F1 generation of juvenile snails were allowed to grow for another 1 mo (shell diameter 6–9 and 5–7 mm for *B. sudanica* and *B. choanomphala*, respectively) and then used in the experiment.

Determination of cercariae output from infected snails

Cercariae produced by each individual snail was determined as described previously (Mutuku et al., 2017). Briefly, each snail was placed in an individual well of a 24-well plastic culture plate, containing 1 ml of aged de-chlorinated tap water. The plate was placed in indirect sunlight for 2 hr between 1000 hr and 1200 hr. Individual wells were then examined under a dissecting microscope for the presence of cercariae. For the snails that had shed cercariae, the contents of the well were mixed gently using a micropipette, and an aliquot of 50 µl was then taken and placed in a gridded Petri dish. Two drops of Lugol's iodine were then added to stain and immobilize the cercariae, and these were then counted with the aid of a dissecting microscope using a tally counter. The number of cercariae counted was multiplied by 20 to obtain the total number of cercariae that were produced by the snail during the 2 hr shedding period. This procedure was used for all the shedding snails at 7 wk PE.

Ethical considerations

This study was approved by KEMRI's Scientific and Ethics Review Unit (SERU), reference SERU No. 3540, and by the Institutional Review Board of the University of New Mexico (UNM), reference 18115. Villagers living around Asao stream and fishermen in Kanyibok were included in this study because they are likely to be positive due to their water-associated activities, and they were easily accessible to our team from the beach sites and the stream. Prior to recruitment, screening, selection, or treatment of any participant, the purpose of the study was explained to them in a language they easily understood. Participation was voluntary, and participants were allowed to withdraw at any time, without penalty. Written and signed consent were sought from the participants. Any participant found positive for *S. mansoni* was offered standard treatment with praziquantel (40 mg/kg body weight). To ensure confidentiality, each participant was given a personal identification number as an identifier.

Statistical analyses

Descriptive statistics such as proportions or percentages were used to summarize categorical variables, while measures of central tendency such as mean and range were used to summarize continuous variables. To determine proportionate mortality at 7 wk PE, the number of dead snails for each category (parasite source or miracidia dose) was divided by the number of dead snails from the negative control. A Wilcoxon rank sum test with a continuity correction was used to determine the effect of species and sympatry on the proportionate mortality, whereas a Kruskal-Wallis rank sum test was used to determine the effect of dose on the proportionate mortality as the dose had 3 levels.

To determine the effects of snail taxon and parasite source on infection status (yes/no) at the 3 different doses, a generalized linear model was used with a binomial distribution and logit link function due to the binary response variable. Thereafter, models were split by snail taxon in order to detect parasite source differences at 3 different doses within a taxon. For the latter individual taxon models, parasite source was nested within dose, and dispersion was tested with a chi-squared test. Estimates and confidence intervals (CI) were back transformed to represent odds ratios.

To determine whether snail taxon, parasite source, and dose have an effect on the number of cercariae produced, a generalized linear model with a negative binomial distribution and a log link function were fitted to account for the response variable (number of cercariae) being count data and overdispersion (Kleiber and Zeileis, 2008). The "dispersion test" function from the AER package in R (European Environment Agency, 2020) were used to detect overdispersion. Thereafter, models were split by taxon to determine whether there are any differences between parasite source within each taxon at 3 different doses. For the latter individual taxon models, parasite source was nested within dose.

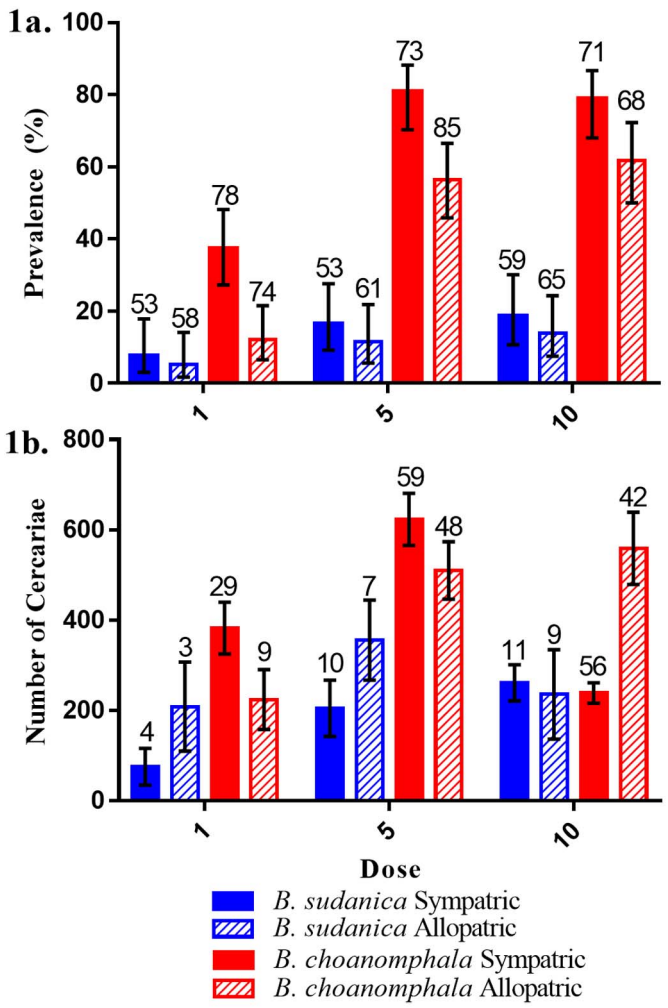

Figure 1. (**a**) *Schistosoma mansoni* infection prevalence in *Biomphalaria choanomphala* or *Biomphalaria sudanica* 6 wk post-exposure to a dose of either of 1, 5, or 10 miracidia from allopatric or sympatric combinations. Error bars represent 95% confidence interval. (**b**) Average number of cercariae produced by snail taxon *B. choanomphala* or *B. sudanica* upon exposure to allopatric or sympatric *Schistosoma mansoni*, following 3 different miracidia doses 1, 5, and 10. Error bars represent standard error of the mean. Number on top of each bar represents number of snails in each group. Color version available online.

RESULTS

Biomphalaria pre-patency infection period and mortality

Overall pre-patent periods for *S. mansoni* in *B. sudanica* and *B. choanomphala* snails were not different, with the majority of snails in all exposure combinations starting to shed cercariae by 5 wk PE. By 6 wk PE all the combinations attained their peak prevalence except for *B. choanomphala* exposed to 1 miracidium from Asao and *B. sudanica* exposed to 1 or 5 miracidia from Asao, and 1 miracidium from Kanyibok. For these groups, all had another single snail start shedding cercariae at 7 wk PE.

On average, at 7 wk PE, *B. sudanica* snails had a proportionate higher mortality of 0.43 compared to *B. choanomphala* snails; however, this difference was only marginal (Wilcoxon rank sum test, $W = 29$, $P = 0.0921$). There were no significant differences between parasite dose and source on the proportionate mortality (Kruskal-Wallis rank sum test, $\chi^2_{df=2} = 0.81$, $P = 0.6668$ and Wilcoxon rank sum test, $W = 15.5$, $P = 0.7483$, respectively).

Biomphalaria infection prevalence post-exposure to *S. mansoni* miracidia

As expected, none of the snails in the unexposed negative control groups became infected, and both *B. choanomphala* and *B. sudanica* had a high survival rate of 88% and 84%, respectively. *Biomphalaria choanomphala* had a higher infection prevalence (number of shedding/numbers of surviving) than *B. sudanica* for each miracidia dose, regardless of the source of miracidia (Fig. 1a). For *B. choanomphala*, the sympatric combination (Kanyibok snails-Kanyibok parasite) generally yielded higher prevalences (in the range 37.2–80.9%) than for the allopatric combination (Kanyibok snails-Asao parasite), which produced prevalences in the 12.2–61.8% range (Fig. 1a). For *B. sudanica*, prevalences were consistently low, regardless of miracidial dose or source, with the sympatric combination producing marginally a higher prevalence of 7.5–18.6%, relative to the allopatric combination, which produced a prevalence between 5.2–13.8% (Fig. 1a).

Effect of snail species and parasite source on infection status of snails

After accounting for snail taxon and parasite source, in comparison to snails exposed to a single miracidium, the odds of a snail becoming infected was higher when exposed to a dose of 5 miracidia (494%, GLM, $\beta_0 = 0.55$, $\beta = 5.95$, $z = 7.65$, $P \leq 0.0001$, Suppl. Table S1) or 10 miracidia (569%, GLM, $\beta_0 = 0.55$, $\beta = 6.69$, $z = 7.95$, $P \leq 0.0001$; Table S1). Although a dose of 10 miracidia had higher odds of infection than a dose of 5 miracidia, this difference was not statistically significant (GLM, $\beta_0 = 3.70$, $\beta = 0.89$, $z = -0.58$, $P = 0.5620$; Table S1). *Biomphalaria sudanica* have 92% lower odds of being infected compared to *B. choanomphala*, after accounting for miracidia dose and source (GLM, $\beta_0 = 0.55$, $\beta = 0.08$, $z = -12.05$, $P \leq 0.0001$; Table S1). The odds of infection with miracidia from the allopatric source is 59% lower than infection with miracidia from the sympatric source, after accounting for species and dose (GLM, $\beta_0 = 0.55$, $\beta = 0.41$, $z = -4.93$, $P \leq 0.0001$; Table S1).

Effect of parasite source and miracidia dose on infection prevalences for *B. choanomphala*

For *B. choanomphala*, after accounting for a parasite source, as compared to snails exposed to 1 miracidium, the odds of becoming infected with a dose of 5 miracidia (612%, GLM, $\beta_0 = 0.59$, $\beta = 7.12$, $z = 5.19$, $P \leq 0.0001$) or 10 miracidia (531%, GLM, $\beta_0 = 0.59$, $\beta = 6.31$, $z = 4.93$, $P \leq 0.0001$) was higher in both cases (Table S2). Snails exposed to a dose of 5 miracidia had higher odds of infection than those exposed to a dose of 10 miracidia, but this difference was not statistically significant (GLM, $\beta_0 = 3.73$, $\beta = 1.13$, $z = 0.29$, $P = 0.7707$; Table S2), as was the case with *B. sudanica* (GLM, $\beta_0 = 0.23$, $\beta = 0.86$, $z = -0.32$, $P = 0.7460$; Table S3). Thus, with either snail species, increasing the dose from 5 to 10 miracidia did not have a statistically significant impact on the prevalence of infection. Exposure to miracidia from an allopatric as opposed to sympatric source of *S. mansoni* significantly reduced the odds of infection by 77% (GLM, $\beta_0 = 0.59$, $\beta = 0.23$, $z = -3.45$, $P = 0.0006$), 69% (GLM, $\beta_0 = 0.59$, $\beta =$

0.31, $z = -3.19$, $P = 0.0014$), or 57% (GLM, $\beta_0 = 0.59$, $\beta = 0.43$, $z = -2.19$, $P = 0.0288$) when exposed with 1, 5, or 10 miracidia, respectively (Table S2).

Effect of parasite source and miracidia dose on infection prevalences for *B. sudanica*

After accounting for parasite sources, there were no significant differences in the odds of infection among *B. sudanica* snails given doses of *S. mansoni* (GLM, Dose 5 vs. Dose 1: $\beta_0 = 0.08$, $\beta = 2.40$, $z = 1.40$, $P = 0.1610$; Dose 10 vs. Dose 1: $\beta_0 = 0.08$, $\beta = 2.81$, $z = 1.67$, $P = 0.0950$; Dose 5 vs. Dose 10: $\beta_0 = 0.23$, $\beta = 0.86$, $z = -0.32$, $P = 0.7460$; Table S3). Numerically, miracidia from an allopatric source have lower odds of infection than those from sympatric sources by 33%, 35%, and 30% when exposed to 1, 5, and 10 miracidia, respectively. However, these differences were not statistically significant (GLM, Dose 1: $\beta_0 = 0.08$, $\beta = 0.67$, $z = -0.51$, $P = 0.6090$; Dose 5: $\beta_0 = 0.08$, $\beta = 0.65$, $z = -0.82$, $P = 0.4150$; Dose 10: $\beta_0 = 0.08$, $\beta = 0.70$, $z = -0.72$, $P = 0.4700$; Table S3).

Effect of snail taxon, parasite source and miracidia dose on number of cercariae produced by snails

On average, individual infected *B. choanomphala* produced more cercariae than *B. sudanica* (458 ± 414 and 238 ± 208 respectively; $\bar{X}$ ± SD; Fig. 1b). The expected number of cercariae shed by *B. sudanica* was 46% lower than for *B. choanomphala*, after accounting for parasite dose and source (GLM, $\beta_0 = 314.60$, $\beta = 0.54$, $z = -4.34$, $P \leq 0.0001$; Table S4). Cercarial production was also dose dependent; however, the most cercariae were produced with a dose of 5. While accounting for snail taxon and parasite source, the expected number of cercariae recovered from snails given a dose of 5 miracidia were 65% and 50% higher than for doses of 1 and 10 miracidia, respectively (GLM, Dose 1: $\beta_0 = 314.60$, $\beta = 1.65$, $z = 3.24$, $P = 0.0012$; Dose 5: $\beta_0 = 346.82$, $\beta = 1.50$, $z = 3.57$, $P = 0.0004$; Table S4), and there was no statistically significant difference between the cercariae produced from infections with 1 or 10 miracidia (GLM, $\beta_0 = 314.60$, $\beta = 1.10$, $z = 0.63$, $P = 0.5298$; Table S4). After accounting for snail taxon and dose, the expected number of cercariae shed following exposure to allopatric parasites was 23% higher compared to sympatric parasites (GLM, $\beta_0 = 314.60$, $\beta = 1.23$, $z = 1.98$, $P = 0.0475$; Table S4). This pattern was more evident in the dose 10 group from *B. choanomphala* and dose 1 and 5 groups from *B. sudanica*, but appeared to be reversed in the dose 1 and 5 groups from *B. choanomphala* and dose 10 group from *B. sudanica*. For example, *B. choanomphala* exposed to 10 miracidia from an allopatric source shed 134% more cercariae than snails exposed to 10 miracidia from a sympatric source (GLM, $\beta_0 = 382.32$, $\beta = 2.34$, $z = 5.05$, $P \leq 0.0001$; Table S5), whereas exposure to an allopatric source at dose 1 and 5 were lower (GLM, Dose 1: $\beta_0 = 382.32$, $\beta = 0.59$, $z = -1.69$, $P = 0.0914$; Dose 5: $\beta_0 = 382.32$, $\beta = 0.82$, $z = -1.24$, $P = 0.2142$; Table S5). Although there were no significant differences in the case of *B. sudanica* snails, both dose 1 and 5 shed more cercariae under allopatric vs. sympatric exposure, whereas at dose 10 it was reverse (GLM, Dose 1: $\beta_0 = 75$, $\beta = 2.78$, $z = 1.51$, $P = 0.1304$; Dose 5: $\beta_0 = 75$, $\beta = 1.74$, $z = 1.27$, $P = 0.2026$; Dose 10: $\beta_0 = 75$, $\beta = 0.90$, $z = -0.26$, $P = 0.7965$; Table S6).

DISCUSSION

Throughout this study, including in the following discussion, we use the names *B. sudanica* and *B. choanomphala* in part for convenience and in part in deference to the classical literature that recognizes them as distinct species, e.g., Brown (1994). We hasten to add there is good evidence that snails of the 2 taxa are very closely related and probably should more correctly be considered as morphologically distinguishable forms of the same species (DeJong et al., 2001; Standley et al., 2011; Zhang et al., 2018). Which name, *sudanica* or *choanomphala*, deserves taxonomic precedence is a debatable point (Zhang et al., 2018). To avoid confusion in comparison of our study with others, we note that (Standley et al., 2011) used "*B. choanomphala*" to refer to both forms from Lake Victoria. For our purposes here, we wish to note that the form considered as "*choanomphala*" is the one usually dredged from deeper water but is also occasionally recovered from shoreline populations, including at Kanyibok. It has a smaller shell diameter and distinct conchological features such as "strongly angular whorls beneath" and a small umbilicus, whereas the form referred to as "*sudanica*" found in shoreline populations and in adjacent papyrus swamps has a larger diameter, flat shell with a wide umbilicus (Brown, 1994). We have established laboratory populations of both forms, both of which readily adapt to lab culture and at least over a span of months to a few years retain their characteristic shell features.

In the present study, we have again noted *B. sudanica* to have a relatively low prevalence of infection following experimental exposure to fresh, human-derived Kenyan isolates of *S. mansoni*, just as in our previous study (Mutuku et al., 2017) comparing *B. sudanica* and *B. pfeifferi* (Fig. 2). For instance, in those studies, we noted infection prevalence between 2.4 and 21.5% for *B. sudanica*, whereas *B. pfeifferi* had a much higher experimental prevalence (39.6–100%). The prevalence achieved for *B. choanomphala* were closer to but generally lower than those achieved with *B. pfeifferi* (Southgate et al., 2000; Ibikounlé et al., 2012; Mutuku et al., 2014, 2017). In comparison to the prevalence achieved by (Adriko et al., 2013) of 9.8% in Uganda, the susceptibility of *B. choanomphala* from Kenya was higher in our study (12.2–78.9%). There was a significant difference in prevalence between the 2 snail taxa, with the odds of infection being 92% lower for *B. sudanica* compared to *B. choanomphala* after accounting for parasite source and dose ($P \leq 0.0001$). In contrast to *B. pfeifferi*, which showed relatively high prevalences of infection with both sympatric and allopatric isolates of *S. mansoni*, both *B. choanomphala* and *B. sudanica* tended to have higher prevalence following exposure to sympatric *S. mansoni* isolates.

For both *B. choanomphala* and *B. sudanica*, increasing the dose of miracidia from 1 to either 5 or 10 increased the prevalence, but the response for *B. sudanica* was relatively modest (see Fig. 2), and no combination of *S. mansoni* with either snail taxon achieved 100% prevalence. From a dose of 1 miracidium, which is probably the most common exposure dose in nature, for *B. sudanica*, despite increasing by as much as 10-fold the number of parasite genotypes to which each snail was exposed, prevalences increased only about 2-fold and were lower than or comparable to the prevalences achieved by exposure of *B. choanomphala* to a single miracidium. For *B. choanomphala*, although the prevalence increased substantially by 2–4-fold with an increase in dose to 5 miracidia, a further increase to 10 miracidia did not have a large

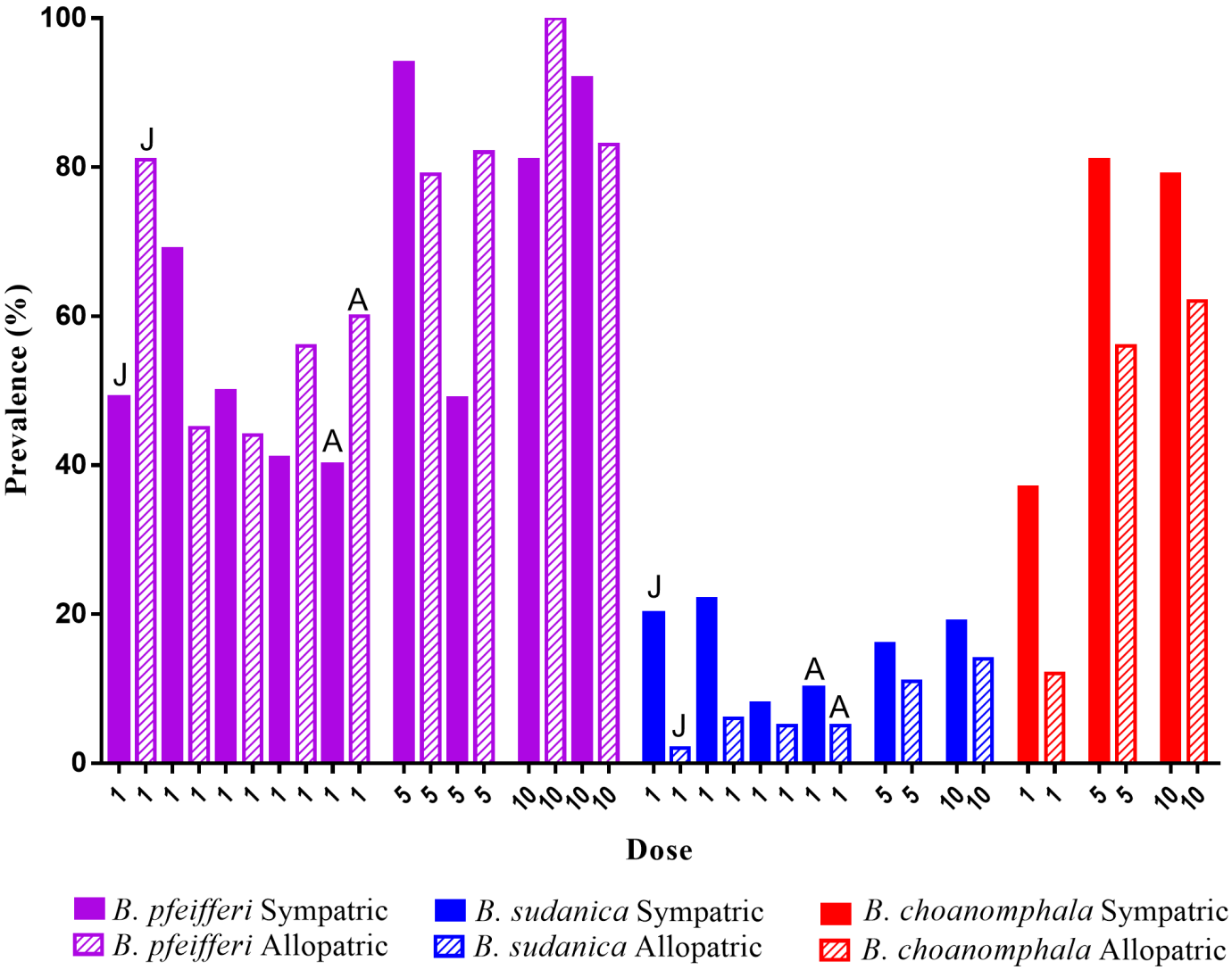

Figure 2. Summary of experimental exposures for young adult snails from Kenya representatives of *Biomphalaria pfeifferi*, *Biomphalaria choanomphala*, and *Biomphalaria sudanica* for different doses of *Schistosoma mansoni* miracidia, of either sympatric or allopatric origin. Abbreviations on top of a bar: adult snails, A; juvenile snails, J; while bars without any letter on top indicate young adult snails. Note that *B. sudanica* typically had lower compatibility than the other 2 snail taxa. Color version available online.

impact on further increasing the prevalence. The results suggest that up to 80% of *B. sudanica* are, for reasons yet to be determined, refractory to *S. mansoni* infection. Likewise, there may be about 20% of *B. choanomphala* that are refractory even when exposed to 10 miracidia. In contrast, prevalences of over 90% and even in 1 case, 100%, was achieved with *B. pfeifferi*, suggesting this species has a lower potential to be refractory. The basis for the refractory nature of *B. sudanica* deserves further study and may represent the presence of resistance genes that potentially could be exploited for control purposes.

With respect to other important components of compatibility, we noted *B. sudanica* and *B. choanomphala* did not differ significantly concerning the length of the pre-patent period (5–6 wk for most snails exposed). In contrast, *B. pfeifferi* supports more rapid development of *S. mansoni*, in the range of 3–6 wk (Southgate et al., 2000; Adriko et al., 2013; Mutuku et al., 2014, 2017). With respect to cercariae production, the expected number of cercariae shed by *B. sudanica* was 46% lower than for *B. choanomphala*, carrying the implication that the number of cercariae produced per penetrating miracidium by *B. choanomphala* is higher than for *B. sudanica*. Using similar methods, cercariae production was found to be substantially higher for *B. pfeifferi*, regardless of whether sympatric or allopatric combinations were studied (Mutuku et al., 2014, 2017). With respect to the relationship between miracidial dose and cercariae production, we observed a dose of one miracidium resulted in fewer cercariae produced than for doses of 5 or 10 miracidia, but the cercarial output for snails exposed to 5 miracidia was somewhat higher than for snails exposed to 10 miracidia each. This suggests a leveling off of cercariae production with miracidial dose, a result similar to that noted by Théron (1985). However, Southgate et al. (2000) noted that the relationship between miracidial dose and cercariae production is variable among experimental systems.

Considering the 3 components of compatibility together, for Kenyan combinations of snails and *S. mansoni*, we rank *B. pfeifferi* as the most compatible, followed by *B. choanomphala*, then *B. sudanica*. Care is required in extending our conclusions regarding compatibility to all parts of Lake Victoria or to other parts of the known ranges of the 3 snail taxa involved. For instance, Adriko et al. (2013), working with F1 generation snails collected from the Ugandan shore of Lake Victoria, following exposure of *B. choanomphala* to 20 miracidia/snail, reported prevalences less than 15% for both allopatric and sympatric combinations. They also reported relatively low compatibility levels for *B. pfeifferi*. Multiple factors, including real biological differences among snails and parasite isolates used, could account for the differences noted with the Ugandan studies.

At least with respect to lake-related *S. mansoni* transmission, even though generally found to have low compatibility, *B. sudanica* is nonetheless considered more important than *B. choanomphala* since the former dominates the lake shoreline by maintaining a larger and more visible population, and as evidenced by 2 recent studies (Gouvras et al., 2017; Mutuku et al., 2019), most snails collected from the lake with *S. mansoni* infections are of this taxon. However, given the higher experimental prevalence and cercariae production noted here for *B. choanomphala*, it raises the possibility that this species is a better overall producer of cercariae per penetrating miracidium than *B. sudanica*. In our view, *B. choanomphala* potentially plays a significant but an under-appreciated role in the transmission of *S. mansoni* in Lake Victoria, a role also noted in the earliest studies of this taxon (Magendantz, 1972). Its role may not be fully appreciated because it is easy to underestimate its population size because dredging provides an inefficient and crude means of sampling its potential habitat areas. Alternative means of sampling like direct inspection of the bottom facilitated by scuba diving would be preferable but are rendered difficult because of the dangers posed by schistosomiasis transmission and other aquatic animals like hippopotamuses and crocodiles. An alternative sampling procedure that might provide a needed new perspective could be a catch, mark, release, and re-catch sampling program.

The deep-water habitat of *B. choanomphala* can be viewed as a refugium in which the chances are lower for encountering miracidia of *S. mansoni* or of any other trematode whose eggs are deposited by definitive hosts living in or around the edges of the lake. At least in the case of *S. mansoni*, this effect might be partially offset by fishermen with the habit of defecating directly into deep water from their boats (Mutuku et al., 2019). Also, miracidia of trematodes infecting fish or turtles might be more likely to infect *B. choanomphala*. In addition to finding relatively few *B. choanomphala* infected with *S. mansoni* (Mutuku et al., 2019), we have found infections of only 4 other trematode species in *B. choanomphala*, each of these in small numbers. By contrast, *B. sudanica* inhabiting the shoreline is colonized by at least 26 species of trematodes (Laidemitt et al., 2019; Mutuku et al., 2019), including at higher overall prevalence; however, more offshore sampling needs to be done in different localities in the lake to ascertain the extent to which *B. choanomphala* is colonized by a variety of trematodes. Persistent exposure to high burdens of a variety of trematodes could lead to selection for higher levels of innate resistance, consistent with what we have observed for *B. sudanica* in its interactions with *S. mansoni*. Last, we note that although *B. sudanica* and *B. choanomphala* are very similar with respect to typical genetic markers like 28S nuclear rRNA and even mitochondrial genomes (Zhang et al., 2018), they do retain largely but not completely distinct habitat preferences, and they can be differentiated by conchological features that are retained in culture for several generations spanning over a period of 3 yr. Furthermore, this study suggests they have measurable differences in compatibility with *S. mansoni*, a feature that may not be independent of the differences in their preferred habitats.

In conclusion, using the prevalence of infection following experimental exposure to 1, 5, or 10 miracidia of either sympatric or allopatric isolates of *S. mansoni*, length of pre-patent period, and daily numbers of cercariae produced as measures of compatibility, Kenyan *B. choanomphala* were more compatible with *S. mansoni* than *B. sudanica*. In comparison with earlier comparable studies, *B. pfeifferi* is more compatible with *S. mansoni* than either *B. choanomphala* or *B. sudanica*. The actual vectorial capacity of *B. choanomphala* is likely diminished by its preference for deeper water with ensuing lower probabilities for contact with *S. mansoni* miracidia or for any *S. mansoni* cercariae it might produce to reach human hosts. Nonetheless, the role *B. choanomphala* plays in *S. mansoni* transmission in the lake should not be ignored, and currents or winds might actually lead to greater shoreline contamination with cercariae emanating from this taxon than generally imagined. Additionally, *B. choanomphala* may be swept to the shoreline where they can persist on submerged vegetation for up to 5 mo, as demonstrated in a previous study (Mutuku et al., 2019). Conversely, even though *B. sudanica* generally has low compatibility with *S. mansoni*, the fact that it occurs in vast numbers and is a shoreline inhabitant increases the risk some snails will be successfully infected. Furthermore, its shoreline location increases the risk for cercarial-human contact. Consequently, despite its low compatibility, *B. sudanica* still plays a major role in perpetuating transmission. We further note that although *B. choanomphala* and *B. sudanica* are genetically very similar with respect to marker genes like cytochrome oxidase or even mitochondrial genome sequences, the differences we note in compatibility for these 2 species are noteworthy and suggest that individuals of *B. sudanica* are often refractory to infection, highlighting the need to reveal the underlying factors responsible for this lack of compatibility.

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LITERATURE CITED

Adriko, M., C. J. Standley, B. Tinkitina, G. Mwesigwa, T. K. Kristensen, J. R. Stothard, and N. B. Kabatereine. 2013. Compatibility of Ugandan *Schistosoma mansoni* isolates with *Biomphalaria* snail species from Albert Lake and Lake Victoria. Acta Tropica 128:303–308. doi:10.1016/j.actatropica.2013.02.014.

Anderson, R. M., and R. M. May. 1979. Prevalence of schistosome infections within molluscan populations: Observed patterns and theoretical predictions. Parasitology 79: 63–94. doi:10.1017/S0031182000051982.

Brown, D. S. 1994. Freshwater Snails of Africa and Their Medical Importance, 2nd edition. CRC Press, Boca Raton, Florida, 608 p. doi:10.1201/9781482295184.

DeJong, R. J., J. A. T. Morgan, W. L. Paraense, J. P. Pointier, M. Amarista, P. F. K. Ayeh-Kumi, A. Babiker, C. S. Barbosa, P. Brémond, A. Pedro Canese, et al. 2001. Evolutionary relationships and biogeography of *Biomphalaria* Gastropoda: Planorbidae with implications regarding its role as host of the human bloodfluke, *Schistosoma mansoni*. Molecular Biology and Evolution 18: 2225–2239. doi:10.1093/oxfordjournals.molbev.a003769.

European Environment Agency. 2020. R Core Team (2019). Methodgy reference. Available from: https://www.eea.europa.eu/data-and-maps/indicators/oxygen-consuming-substances-in-rivers/r-development-core-team-2006. Accessed 23 July 2020.

French, M. D., T. S. Churcher, M. Gambhir, A. Fenwick, J. P. Webster, N. B. Kabatereine, and M. G. Basáñez. 2010. Observed reductions in *Schistosoma mansoni* transmission from large-scale administration of praziquantel in Uganda: A mathematical modelling study. PLoS Neglected Tropical Diseases 4: e897. doi:10.1371/journal.pntd.0000897.

Gouvras, A. N., F. Allan, S. Kinung'hi, M. Rabone, A. Emery, T. Angelo, T. Pennance, B. Webster, H. Nagai, and D. Rollinson. 2017. Longitudinal survey on the distribution of *Biomphalaria sudanica* and *B. choanomophala* in Mwanza region, on the shores of Lake Victoria, Tanzania: Implications for schistosomiasis transmission and control. Parasites & Vectors 10: 316. doi:10.1186/s13071-017-2252-z.

Ibikounlé, M., G. Mouahid, R. Mintsa Nguéma, N. G. Sakiti, D. Kindé-Gasard, A. Massougbodji, and H. Moné. 2012. Life-history traits indicate local adaptation of the schistosome parasite, *Schistosoma mansoni*, to its snail host, *Biomphalaria pfeifferi*. Experimental Parasitology 132: 501–507. doi:10.1016/j.exppara.2012.09.020.

Kaltz, O., and J. A. Shykoff. 1998. Local adaptation in host–parasite systems. Heredity 81: 361–370. doi:10.1046/j.1365-2540.1998.00435.x.

Karanja, D. M. S., E. K. Awino, R. E. Wiegand, E. Okoth, B. O. Abudho, P. N. M. Mwinzi, S. P. Montgomery, and W. E. Secor. 2017. Cluster randomized trial comparing school-based mass drug administration schedules in areas of western Kenya with moderate initial prevalence of *Schistosoma mansoni* infections. PLOS Neglected Tropical Diseases 11: e0006033. doi:10.1371/journal.pntd.0006033.

King, C. H., S. Binder, Y. Shen, C. C. Whalen, C. H. Campbell, R. E. Wiegand, A. Olsen, W. E. Secor, S. P. Montgomery, R. Musuva, et al. 2020. SCORE studies on the impact of drug treatment on morbidity due to *Schistosoma mansoni* and *Schistosoma haematobium* infection. American Journal of Tropical Medicine and Hygiene 103: 30–35. doi:10.4269/ajtmh.19-0830.

Kleiber, C., and A. Zeileis. 2008. Applied econometrics with R. *In* Applied Econometrics with R. Springer, New York, New York, p. 1–6. doi:10.1007/978-0-387-77318-6.

Laidemitt, M. R., L. C. Anderson, H. J. Wearing, M. W. Mutuku, G. M. Mkoji, and E. S. Loker. 2019. Antagonism between parasites within snail hosts impacts the transmission of human schistosomiasis. eLife 8: e50095. doi:10.7554/eLife.50095.

Lively, C. M., and M. F. Dybdahl. 2000. Parasite adaptation to locally common host genotypes. Nature 405: 679–681. doi:10.1038/35015069.

Loker, E. S., B. Hofkin, G. M. Mkoji, B. Mungai, J. Kihara, and D. K. Koech. 1993. Distributions of freshwater snails in southern Kenya with implications for the biological control of schistosomiasis and other snail-mediated parasites. Journal of Medical and Applied Malacology 5: 1–20.

Magendantz, M. 1972. The biology of *Biomphalaria choanomphala* and *B. sudanica* in relation to their role in the transmission of *Schistosoma mansoni* in Lake Victoria at Mwanza, Tanzania. Bulletin of the World Health Organization 47: 331–341.

Morand, S., S. D. Manning, and M. E. J. Woolhouse. 1996. Parasite–host coevolution and geographic patterns of parasite infectivity and host susceptibility. Proceedings of the Royal Society of London. Series B: Biological Sciences 263: 119–128. doi:10.1098/rspb.1996.0019.

Mutuku, M. W., C. K. Dweni, M. Mwangi, J. M. Kinuthia, I. N. Mwangi, G. M. Maina, L. E. Agola, S. M. Zhang, R. Maranga, E. S. Loker, et al. 2014. Field-derived *Schistosoma mansoni* and *Biomphalaria pfeifferi* in Kenya: A compatible association characterized by lack of strong local adaptation, and presence of some snails able to persistently produce cercariae for over a year. Parasites & Vectors 7: 533. doi:10.1186/s13071-014-0533-3.

Mutuku, M. W., M. R. Laidemitt, B. R. Beechler, I. N. Mwangi, F. O. Otiato, E. L. Agola, H. Ochanda, B. Kamel, G. M. Mkoji, M. L. Steinauer, et al. 2019. A search for snail-related answers to explain differences in response of *Schistosoma mansoni* to praziquantel treatment among responding and persistent hotspot villages along the Kenyan shore of Lake Victoria. American Journal of Tropical Medicine and Hygiene 101: 65–77. doi:10.4269/ajtmh.19-0089.

Mutuku, M. W., L. Lu, F. O. Otiato, I. N. Mwangi, J. M. Kinuthia, G. M. Maina, M. R. Laidemitt, E. A. Lelo, H. Ochanda, E. S. Loker, et al. 2017. A comparison of Kenyan *Biomphalaria pfeifferi* and *B. sudanica* as vectors for *Schistosoma mansoni*, including a discussion of the need to better understand the effects of snail breeding systems on transmission. Journal of Parasitology 103: 669–676. doi:10.1645/17-72.

Odiere, M. R., F. O. Rawago, M. Ombok, W. E. Secor, D. M. S. Karanja, P. N. M. Mwinzi, P. J. Lammie, and K. Won. 2012. High prevalence of schistosomiasis in Mbita and its adjacent islands of Lake Victoria, western Kenya. Parasites & Vectors 5: 278. doi:10.1186/1756-3305-5-278.

Onkanga, I. O., P. N. M. Mwinzi, G. Muchiri, K. Andiego, M. Omedo, D. M. S. Karanja, R. E. Wiegand, W. E. Secor, and S. P. Montgomery. 2016. Impact of two rounds of praziquantel mass drug administration on *Schistosoma mansoni* infection prevalence and intensity: A comparison between community wide treatment and school based treatment in western Kenya. International Journal for Parasitology 46: 439–445. doi:10.1016/j.ijpara.2016.01.006.

Samuels, A. M., E. Matey, P. N. M. Mwinzi, R. E. Wiegand, G. Muchiri, E. Ireri, M. Hyde, S. P. Montgomery, D. M. S. Karanja, and W. E. Secor. 2012. *Schistosoma mansoni* morbidity among school-aged children: A SCORE project in Kenya. American Journal of Tropical Medicine and Hygiene 87: 874–882. doi:10.4269/ajtmh.2012.12-0397.

Secor, W. E., R. E. Wiegand, S. P. Montgomery, D. M. S. Karanja, and M. R. Odiere. 2020. Comparison of school-based and community-wide mass drug administration for schistosomiasis control in an area of western Kenya with high initial *schistosoma mansoni* infection prevalence: A cluster randomized trial. American Journal of Tropical Medicine and Hygiene 102: 318–327. doi:10.4269/ajtmh.19-0626.

Southgate, V. R., L. A. Tchuem Tchuenté, A. Théron, J. Jourdane, A. Ly, C. B. Moncrieff, and B. Gryseels. 2000. Compatibility of *Schistosoma mansoni* Cameroon and *Biomphalaria pfeifferi* Senegal. Parasitology 121: 501–505. doi:10.1017/S0031182099006708.

Standley, C. J., C. Wade, and J. R. Stothard. 2011. A fresh insight into transmission of schistosomiasis: A misleading tale of *Biomphalaria* in Lake Victoria. PLoS ONE 6: e26563. doi:10.1371/journal.pone.0026563.

Théron, A. 1985. Dynamiques de production des cercaires de *Schistosoma mansoni* en relation avec les variations de la dose miracidiale proposée au mollusque vecteur *Biomphalaria glabrata*. Annales de Parasitologie Humaine et Comparée 60: 665–674. doi.org/10.1051/parasite/1985606665.

Thompson, J. N. 1994. The Coevolutionary Process. University of Chicago Press, Chicago, Illinois, 376 p.

Wiegand, R. E., P. N. M. Mwinzi, S. P. Montgomery, Y. L. Chan, K. Andiego, M. Omedo, G. Muchiri, M. O. Ogutu, F. Rawago, M. R. Odiere, et al. 2017. A persistent hotspot of *Schistosoma mansoni* infection in a five-year randomized trial of praziquantel preventative chemotherapy strategies. Journal of Infectious Diseases 21: 1425–1433. doi:10.1093/infdis/jix496.

Zhang, S. M., L. Bu, M. R. Laidemitt, L. Lu, M. W. Mutuku, G. M. Mkoji, and E. S., Loker. 2018. Complete mitochondrial and rDNA complex sequences of important vector species of *Biomphalaria*, obligatory hosts of the human-infecting blood fluke, *Schistosoma mansoni*. Scientific Reports 8: 1–10. doi:10.1038/s41598-018-25463-z.