

Presented to the  
Scientific Working Group on Schistosomiasis  
World Health Organization  
Geneva, Switzerland, 14-16 November 2005

## Research on the Molluscan Intermediate Hosts for Schistosomiasis: What are the Priorities?

Eric S. Loker  
Department of Biology  
The University of New Mexico  
Albuquerque, New Mexico 87131-0001 U.S.A.  
esloker@unm.edu  
505-277-2496  
505-277-0304 fax

**Abstract:** Human-infecting schistosomes, and the snails that support their life cycles, remain all too common in today's world, and we must remain diligent in funding studies related to these organisms. Dramatic changes in the distribution and abundance of snails hosting schistosomes are underway or can be anticipated, so we must closely monitor such changes because where the appropriate snails go, the schistosomes typically follow. We also need to continue to develop modern tools to gain a deeper basic understanding of snail biology, and to better comprehend the interactions between schistosomes and snails, particularly in real-life field settings. We need to apply modern techniques to learn more about the unseen natural enemies of both snails and schistosome sporocysts, and to learn more about other relevant snail-transmitted pathogens, including the species of non-human schistosomes that cause cercarial dermatitis. We must improve the dissemination of relevant information to workers in endemic areas who often suffer from poor access to new knowledge. Finally, there is an important need to maintain a critical mass of malacologists, both to capitalize on the promise offered by exciting tools now or soon to be available, and to avoid losing our accumulated practical knowledge of medically important snails and their role in transmission of schistosomiasis.

**Introduction:** Without snails, there can be no schistosomiasis. Because schistosome-transmitting snails occur in very particular ecological circumstances that are subject to rapid change in an increasingly human-dominated world, one of the greatest challenges to understanding the future of this neglected yet persistent human disease is to try to understand how snails will be affected by global changes in climate, increased pollution of aquatic habitats, continued transport of exotic and invasive species, construction of dams and irrigation systems, mass movements of humans, and changes in human population density and standard of living. Some of these factors such as continued high levels of poverty and civil unrest will have more predictable effects on transmission, but others, like climate change, pollution of aquatic habitats or movement of exotic species, have impacts that are far harder to predict. For example, increased pollution of water bodies with human wastes might favor transmission, but pollution with agricultural or industrial wastes may have the beneficial impact of eliminating transmission, but for all the wrong reasons. Because schistosomes by necessity follow the snails, we must not ignore the snails as they will ultimately dictate where in the world schistosomiasis can occur, and likely at what level of prevalence it can occur.

Having said this, snails should not be viewed as the enemy—or the target. Indeed, they are, like us, unwitting participants in the life cycles of schistosomes and other helminths of medical or veterinary significance. Snails are an integral part of the biosphere and attempts to eradicate them are not only misguided from a conservation point of view (Kristensen and Brown, 1999), but unlikely to be successful (Lardans and Dissous, 1998). Given the pervasive problems of pollution and introduction of

exotics (Pointier et al., 2005a), the day will likely come when the presence of indigenous schistosome-transmitting snails in an aquatic habitat will be considered a sign of environmental health and something about which to be happy. Rather it is the *parasites* that depend on snails and humans for their survival that must be controlled. This is somewhat different from contemplating the control of a typical arthropod-borne pathogen like malaria where the vector, such as a mosquito, is also obnoxious and harmful to people in its own right. Snails are by contrast innocent bystanders.

So, if snails are not the enemy, is there any real imperative to learn more about their biology? The answer is unequivocally “yes” because the more we know about the intricacies of the parasite-snail relationship (Lockyer et al., 2004), the broader is our conceptual base from which to draw unique solutions regarding parasite control. Furthermore, as noted above, conditions in the developing tropics will favor massive changes in the distribution and abundance of snails of medical or veterinary significance. We need to be able to anticipate and respond to such changes.

**Monitoring and Preventing Changes in Snail Distribution and Abundance:** The aquarium and aquatic plant trade, the incessant movement of people and their goods, and even natural dispersal events all conspire to move snails from one location to another (Pointier et al., 2005a). The hermaphroditic or parthenogenetic reproductive habits of many snails (Jarne and Stadler, 1995) favor their successful introduction into new locations. Thus a *Biomphalaria glabrata*-like snail was dispersed naturally to Africa within the past five million years creating dramatic new opportunities for schistosome parasites there (Woodruff and Mulvey, 1997; Campbell et al., 2000; DeJong et al., 2001). Through the activities of humans, ironically likely those involved in schistosomiasis research, the “second coming” of *B. glabrata* to Africa, to the Nile Delta, was accomplished in the last 50 years (Lotfy et al., 2005). The Neotropical *Biomphalaria tenagophila* has appeared in central Africa (Pointier et al., 2005b), and *B. straminea*, a particularly invasive exotic, now thrives in Asia and many other parts of the world (Pointier et al., 2005a). The latter two species can both host *S. mansoni*. *Indoplanorbis exustus*, the host for three schistosome species of ruminants in southern Asia, has also appeared in Africa and the Caribbean region (Pointier et al., 2005a). Fortunately, other introductions, such as of *Bulinus* species to the Neotropics, have not to our knowledge yet been effected, and all measures should be used to prevent this from occurring.

At the same time that potential schistosome hosts are being redistributed, other snails such as *Melanoides tuberculata* or *Helisoma duryi* are also being widely introduced around the globe, including to the Neotropics, and although these introductions may have beneficial impacts on schistosome transmission, as observed particularly on the Caribbean islands, these introductions ultimately have many unfavorable aspects (Kinzelbach, 1995), such as displacement of native (often endangered) snails and the potential threat of introduction of associated trematodes (Scholz and Salgado-Maldonado, 2000) that may also threaten endangered native vertebrate species.

Less dramatic range extensions, such as of *B. glabrata* into southern Brazil (Graeff-Teixeira et al., 2004) or of *B. alexandrina* down the course of the Nile (Lotfy et al., 2005) also bear scrutiny. As noted recently in *Nature* (Marshall, 2005), and a phenomenon seen all too commonly while collecting snails in both the Neotropics and Africa, many of the aquatic habitats in which schistosome-transmitting snails occur are now incredibly fouled by pollution. It is often necessary to kick away the refuse to get to the water to find snails which are nonetheless there and even thriving. However, the impact that such rampant pollution will have on schistosomiasis will be significant. Thus, for example, what is causing the change in the distribution of schistosomiasis along the course of the Nile (Abdel Wahab et al., 1993), with sharply falling prevalence of *S. haematobium* and a rise in the relative abundance of *S. mansoni*? Among the many possibilities that must be considered is the role of pollution, now extensive in the canals of the Delta. A reasonable expectation is that such habitats will increasingly be colonized by globe-trotting snails with high levels of tolerance for pollution, such as physids (Dillon et al., 2002). Fortunately, although physids do transmit dermatitis-causing avian

schistosomes, they do not play a role in transmission of other parasites of medical or veterinary significance, although the likelihood of them acquiring such a capability can not be discounted. The uncertainties regarding where and when new situations will arise with respect to snails is further compounded by the impact of global climate change (Martens et al., 1997; Sutherst, 2004) and the construction of massive water development projects such as dams that influence snail habitats across huge areas (Zheng et al., 2002; Sow et al., 2002).

WHO has a role to play with respect to encouraging regulations that make it less likely for snails to be accidentally distributed, for providing updates about where and when new introductions have occurred, and possibly assisting focal control programs where feasible to eradicate newly-introduced exotics of medical significance. It is also imperative to highlight the global degradation of freshwater habitats and the host of public health problems associated with this degradation. It is sobering and ironic to imagine that WHO could someday announce the eradication of schistosomiasis from the Nile Delta, but any excitement would potentially be muted by the realization that the eradication was because the aquatic habitats there were so fouled as to prevent any self-respecting macroinvertebrate such as a snail from surviving.

Because of the massive scale of anticipated environmental change, studies to understand the basic ecology and ecological preferences of schistosome-transmitting snails remain critical. In particular, studies that help to define the nature of competitive interactions with invasive snails such as *Melanoides* (Giovanelli et al., 2002b; 2005), or that help to define the tolerances of snails with respect to different categories of pollutants are particularly important. Also helpful for understanding the impact of changing habitats across broad geographic scales are remote sensing techniques (Seto et al., 2002).

The highest priority for the WHO with respect to molluscan aspects of schistosomiasis is to recognize that snail and parasite faunas are in a state of rapid flux and that this may create surprising new opportunities for transmission in some places, may prevent transmission in others, and may serve as a bell weather for detecting even more profound environmental and health changes associated with degradation of aquatic environments around the world.

### **Developing a Modern Toolkit to Study the Basic Biology of Snails and**

**Associated Parasites:** At the same time that we maintain a global overview of the biogeography of snails and associated parasites, it is also necessary to “turn inward” and gain a greater appreciation of the basic biology of snails, trematode larval stages, and their interactions. For this, a modern toolkit is required. Given the enormous impact that modern approaches have had on furthering our understanding of mosquitoes and their parasites (Heckel, 2003; Christophides et al., 2004), no less should be sought for the snails that transmit schistosomes and other parasites.

One of the most prominent model organisms among snails is *Biomphalaria glabrata*: it is relatively easy to rear in the laboratory, as is its associated parasite, *Schistosoma mansoni*. One disadvantage of the widespread study of *B. glabrata* is the possibility it will be accidentally introduced into new locations. Studies with *B. glabrata* have already shown potential to illuminate general principles of invertebrate immunobiology (Zhang et al., 2004) and host-parasite interactions (Lockyer et al., 2004), so the impact of these studies is important on a broader stage as well.

Development of modern tools for use with *B. glabrata* is well under way, with the support and encouragement of a consortium of biologists working with snails, and several funding organizations, including WHO. NHGRI has supported the construction of a high quality BAC library that is now available to the public (<http://www.genome.gov/page.cfm?pageID=10001852>). Furthermore, *B. glabrata* has been selected by the NHGRI to be the subject of a genome sequencing project (<http://www.genome.gov/12511858>), which will likely be completed before the decade is over. Both BAC library and genome sequencing projects feature the BB02 strain of *B. glabrata*, an *S. mansoni*-susceptible isolate recently collected from Minas Gerais in Brazil

(<http://biology.unm.edu/biomphalaria-genome/BB02STRAIN.html>). This will be among the first molluscan genome sequence to be completed.

Other important tools are already being developed. To go along with the genome sequence, it will be invaluable to have microarrays so that the transcriptional activity of snail genes, such as in response to schistosome infection or changing environmental circumstances, can be monitored. cDNA microarrays with over 2000 non-redundant features have already been developed in the U.K. (C. S. Jones, personal communication), and oligo-based arrays are under construction in the U.S. The microarrays can be updated and enlarged as we obtain information about more snail genes. Going hand-in-hand with the development of microarrays is the development of EST libraries, both by individual investigators (C. M. Adema, personal communication; Mitta et al., 2005; Davison and Blaxter, 2005) or through the assistance of sequencing centers. The Sanger Center for example is likely to provide 100 thousand ESTs to complement the *B. glabrata* genome sequencing effort.

RNA interference (RNAi), to facilitate functional studies of snail gene products is also currently under development, and the first paper documenting use of this technique with *B. glabrata* is soon to be published (Zhang et al., in final revision). The *FREP2* gene, normally expressed at increased levels following exposure to digenetic trematode parasites such as *S. mansoni* or *Echinostoma paraensei*, was targeted for knockdown. Double-stranded RNA (dsRNA) corresponding to specific regions of the *FREP2* gene was introduced into snails by direct injection into hemolymph. Knockdown efficiency was examined at the transcript level using quantitative-PCR (qPCR) and Northern blot analysis, and expression levels were shown to be significantly reduced (~70-80% knockdown). The establishment of RNAi techniques in *B. glabrata* will enable us to elucidate the function of genes which we believe play a role in defense against pathogens such as *S. mansoni*.

Proteomics is yet another important approach to be developed with respect to snail-schistosome studies. Automated mass spectrometry approaches offer the promise of providing enormous quantities of information regarding the protein and peptide composition of individual snail organs or tissues. Having information about the snail proteome will prove to be useful in providing annotation to the huge amount of snail genome sequence anticipated to be forthcoming. Another valuable tool already available for use is the ability to culture trematode larvae in the presence of cells of the *Biomphalaria glabrata* embryonic cell line (Bge cells) (Coustau and Yoshino, 2000; Bixler et al., 2001; Coppin et al., 2003), raising the potential for eventual routine culture of schistosome sporocysts without the need to maintain snail colonies.

Although the first priority will be to develop the toolkit to support studies of *B. glabrata* as a model, thus enabling us to gain deeper insights into snail-schistosome interactions, we should most emphatically not lose sight of the fact that most human cases of schistosomiasis still occur in Africa, and probably always will. Thus it will be relevant to learn more about species such as *Biomphalaria pfeifferi*, *Bulinus truncatus*, and *Bulinus globosus* which play important roles in transmission in tropical Africa. Transferal of much of the technology to these species should then not only be possible but should be encouraged because it should not be assumed that the fundamental nature of all schistosome-snail relationships are the same. This might be particularly true for the Asian schistosomes transmitted by pomatiopsid snails which are in general understudied.

**Some Additional Tools to Facilitate our Understanding of Schistosome-Snail Interactions in the Field:** Additional tools and approaches are also needed to better understand schistosomes and snails in the field. For example, one useful approach to serve as an alternative to classical methods for determining prevalence of schistosome infection among snail hosts has been the development of PCR-based methods to detect the presence of even minute amounts of schistosome DNA in snails, including cryptic prepatent infections (Hanelt et al., 1997; Jannotti-Passos et al., 1997; Hamburger et al., 1998, 2004).

One important phenomenon of direct relevance to transmission is the degree of compatibility between local snails and schistosomes. Although much of the recent work on schistosome-snail compatibility has focused on the differences between isolates or inbred snail lines that are either fully susceptible or strongly resistant to schistosome infection (Coelho et al., 2004; Lockyer et al., 2004; Carton et al., 2005), the evidence from the field suggests that absolute resistance is rare, and that that the “success or failure of an infection does not depend on the snail susceptibility/resistance status, but on the 'matched' or 'mismatched' status of the host and parasite phenotypes” (Théron and Coustau, 2005). The basic idea here is that if a particular schistosome miracidium has a genetic constitution that appropriately matches a snail of a particular genetic constitution, a successful encounter will occur. If such a match does not occur, then the parasite will be recognized and destroyed. The low prevalence of infection noted following exposure to low doses of miracidia in natural snail–schistosome combinations suggests that such mismatches regularly occur in the field, and can play an important role in diminishing the number of patent infections achieved. Having better tools to assess the early fate of sporocysts in snails would help us to understand the role of this phenomenon in nature. For example, using a PCR-based assay to monitor the presence of *S. haematobium* larvae in bulinid snails, Hamburger et al. (2004) showed that many more snails had been exposed to parasites than actually developed cercariae-producing infections. An explanation consistent with the data is that miracidium-snail encounters are more common than realized, but that a failure of some sporocysts to develop in some snails keeps patent infection rates low. This has fundamental implications with respect to schistosome transmission.

Other practical tools for the study of schistosomes in snails have already been devised, such as the use of hybridization of Southern blots to a polymorphic repetitive DNA element (Minchella et al., 1995) to provide insights into how many different schistosome genotypes are present within a given snail. Additional techniques, such as an ability to quantify the amount of schistosome biomass in a particular snail would also be useful for understanding the epidemiology of snail infections in the field.

With respect to the snails themselves, one of the long-standing needs for schistosomiasis workers in the field has been to have reliable means to determine which species of snails with which they were working. The application of molecular techniques to both amplify and provide sequence data for key reference genes like ITS 1 and 2, 18S, 28S, 16S, ND1 and CO I is proving to be extremely helpful, not only with respect to providing a more reliable yardstick for species determinations (Vidigal et al., 2002, 2004; Lotfy et al., 2005), but also for development of robust phylogenies that have allowed us to gain a much greater appreciation for the evolutionary history of schistosome-transmitting snails (DeJong et al., 2001; Jones et al., 2001; Morgan et al., 2002; Atwood et al., 2004). By application of molecular methods, two species of bulinid snails differing in susceptibility to *S. haematobium* have been identified on Zanzibar, thus considerably clarifying where transmission is possible and directing control efforts (Stothard et al., 2002). This provides a good example of the importance of accurate snail identification and the value of medical malacology for eventual control efforts.

**Studies of the natural enemies and symbionts of snails:** Except in a focal or insular context, it is difficult to imagine how populations of snails would ever be controlled at a level sufficient to interrupt transmission, in a sustainable, cost-effective, environmentally acceptable way. Snails transmitting schistosomiasis often have considerable abilities to aestivate, to be passively dispersed, or to quickly re-populate areas because of their rapid rates of reproduction. Attempts to control snails through the application of molluscicidal chemicals derived from either the chemical industry or from local indigenous plants, would seem to face a severe uphill battle, given the increasingly strong public resistance to the widespread application of chemicals to the environment. The evidence that such chemicals have specific effects on target snails is often equivocal (Monkiedje et al., 1991; Oliveira and Paumgarten, 2000; Giovanelli et al., 2002a).

Also likely of limited potential for future control efforts on continental scales are most methods of biological control that have been proposed, including the use of exotic generalist predators/competitors which carry unacceptable ecological risks (Cowie, 2001). It seems unlikely that indigenous predatory/competitor molluscs can be counted on to achieve sustainable control, otherwise we would already see ample evidence of their effectiveness. Even the introduction of indigenous species into environments where they do not already occur may carry hazards, especially with respect to rice-growing or creating new opportunities for disease-transmission (Yousif and Lämmler, 1975; Teo, 2001).

Here it is argued that even though sustainable, environmentally-acceptable snail control may be a near-impossible goal, it is nonetheless important to continue to learn more about the natural enemies or symbionts of snails, particularly the ones that are at present either poorly characterized or unknown. When snail symbionts have been examined with modern molecular-based methods, some surprising results have emerged. Thus snails and other molluscs have been shown to harbor members of a poorly known clade of eukaryotic symbionts lying close to the divergence between fungi and animals, the Mesomycetozoa (Hertel et al., 2002).

As an example of our ignorance with respect to snail symbionts, not a single virus from any snail of medical significance, or to my knowledge from any gastropod, has ever been isolated. Such viruses almost certainly exist as it would be unusual for any group of organisms to be devoid of viruses. They may routinely kill snails in the field for all we know. Furthermore, many of the tools needed to characterize snail viruses already in hand, most notably the Bge snail cell line. Thus extracts from snails from natural populations could be readily prepared and plated onto Bge monolayers. Then, any plaques revealed could be subjected to further study in search of viral particles. Snail viruses could be useful as control agents, may adversely affect schistosome development, or may be convenient agents for generating transgenic snails. Virtually nothing is known about how snails defend themselves from viruses. It is conceivable that RNAi-related mechanisms are used to discourage viral infections, and if so, then the study of molluscan viruses may offer valuable clues for how to better exploit RNAi to knockdown snail genes, potentially including those required for snails to nurture larval trematodes.

Also poorly known are the bacterial associates of schistosome-transmitting snails. Although some studies of culturable bacteria have been undertaken using *Biomphalaria* including snails from natural habitats (Ducklow et al., 1979, 1981), no studies using PCR-based methods to survey the bacterial diversity associated with snails have been undertaken. Thus it is not at all clear if snails have a bacterial flora that simply mimics the aquatic environment in which they live, is typical of other aquatic invertebrates, or if they harbor specialized and unculturable species. Knowing more about the bacterial flora of snails is important because such bacteria may offer specific opportunities for control, or for introducing exotic genes into snails. Also, bacterial associates with snails may influence the susceptibility of snails to schistosomes or other trematodes.

Two final examples of poorly known symbionts of freshwater snails deserving of study are nematodes and chaetogasters. Some nematodes from terrestrial snails, like their entomopathogenic relatives from insects, carry potentially lethal bacteria into snails (Glenn and Wilson, 1997; Grewal et al., 2003). It is not known if freshwater snails have similar counterparts, or if molluscopathogenic nematodes of terrestrial snails could be adapted to freshwater snails. Also unknown is the impact such nematodes and their bacterial associates might have on developing larval trematodes. Chaetogasters are ectosymbiotic oligochaetes of snails and are of interest because they can consume both schistosome miracidia and cercariae, and may play a role in protecting snails from infection (Rodgers et al., 2005). There is much to learn about snail symbionts, and it seems certain that some of these associates will prove to be biologically unique and may have useful properties with respect to controlling snails or their larval trematodes.

**Sporocysts as the true targets of control efforts – the need to develop new approaches:** As noted above, with respect to controlling schistosomiasis at the level of the molluscan host, the true enemy is not the snail that is hosting the parasite, but the schistosome sporocysts that colonize the snail. It is these sporocysts that will eventually produce the infective stages—cercariae—that infect humans. In general, especially with the advent of a host of molecular techniques, particularly PCR, there are now new opportunities to look for, identify, and to potentially manipulate natural enemies or symbionts of sporocysts. Since the molecular era began, there has been virtually no attempt to follow up on promising studies of the microsporidan hyperparasites of trematode larvae (Canning, 1981). How common are these in nature? How many different taxa are capable of infecting trematode, including schistosome larvae? Can these be grown in vitro (for example in cultures of Bge cells) and then introduced into natural populations of schistosome-transmitting snails?

Some trematodes are well-known to serve as hosts for rickettsial parasites, most notably *Neorickettsia helminthoeca* transmitted by *Nanophyetus salmincola* in the Pacific Northwest. There is now evidence, based on the use of PCR amplifications, to indicate that rickettsiae may be more commonly associated with larval trematodes than previously thought (Park et al., 2003; Chae et al., 2003; Pusterla et al., 2003; Gibson et al., 2005). Do rickettsia infect the intramolluscan stages of human- or animal-infecting schistosomes? We don't know.

Other groups of symbionts of larval trematodes very well might come to light, including viruses or potentially members of the Mesomycetozoa. In general, it would be helpful for funding agencies to encourage the exploration, using modern approaches, for novel associates of larval trematodes that may have considerable control potential, including with something typically lacking, the possibility of specificity with respect to controlling sporocysts.

A final consideration with respect to larval schistosome population dynamics is the ultimate effect of human-mediated ecological simplification. This will also have the effect of eliminating other vertebrates and invertebrates that serve as hosts for metacercariae or adults of trematode species that also cycle through snails like *Biomphalaria*, *Bulinus* or *Oncomelania*. The significance of this is that these other trematodes, by virtue of competing with, or preying upon, the larvae of schistosomes, may exert a larger measure of natural control than we would otherwise suspect (Esch et al., 2001). Thus ecological simplification could potentially have the effect of intensifying schistosome transmission in some areas.

**Not parasites of humans but still in need of study – the schistosomes that cause cercarial dermatitis:** Throughout the world, the cercariae of schistosomes of animals, especially those from birds, cause dermatitis when they penetrate the skin of people in contact with waters of natural habitats (Verbrugge et al., 2004). Although it has generally been considered that such cercariae die in the skin, recent work suggests that this is not always the case, and that some parasites from such infections may persist and cause neurological problems (Hradkova and Horak, 2002). There is ample evidence that outbreaks of dermatitis occur continually around the world, both in marine and freshwater habitats (Larsen et al., 2004), in some cases involving exotic species of gastropods as hosts (Cohen, personal communication). We currently have only a hazy picture of the diversity of schistosome species involved in dermatitis outbreaks, a picture rendered all the more hazy by the complications of interpretation of the classical literature. Again, with the advent of molecular methods of identification of dermatitis-causing cercariae and corresponding adults (Brant et al., in press), and of the associated snails, we have significant new opportunities to learn more about this entire phenomenon. Studies of the basic biology of dermatitis, including the potential for prolonged human infection with non-human schistosome cercariae, should be undertaken.

**The need for reliable information transfer to developing countries and for maintaining a critical mass in medical malacology:** One of the ongoing ironies of schisto-

somiasis research is that much of the necessary background information and literature, and many of the new tools and techniques, are far more readily available in developed countries that are far from endemic areas. Some programs like the education of budding African malacologists by the Danish Bilharziasis Lab, have done much to improve not only the level of training of scientists from developing countries, but also the flow of relevant information to them. Primarily through the use of the worldwide web, potentially through the central organization of the WHO, there should be a renewed attempt to provide schistosomiasis field workers in developing countries as many opportunities as possible to get accurate and practical updates regarding various aspects of medical malacology. Provision of techniques to collect and identify snails using both classical and modern techniques, along with copies of the supporting references, would be very appropriate. Attention to regional differences in the snail fauna would also be very helpful. Additional topics worth of inclusion would be outlining the techniques for separating and isolating snails, identification of the types of cercariae that are produced by snails, again with the supporting original references, and protocols for how to apply some of the newer molecular methods for determining if snails are infected with schistosomes.

As a final comment, if we are to continue to capitalize on the new tools and discoveries available from recent studies of schistosomes and their associated snails, we need to be mindful of the need to train the next generation of malacologists so we do not lose critical mass and the accumulated knowledge and experience base we now have. It is also imperative that centers of malacological training be supported, not only for their own original research, but to permit training of students from developing countries where schistosomiasis or other snail-transmitted parasites are problematic.

**Summary of Needs:** A list of needs for the study of the molluscan aspects of schistosomiasis is provided, as follows:

To monitor changes in the distribution and abundance of schistosome-transmitting snails, preferably with the involvement of an active network of medical malacologists, incorporating new tools such as remote sensing and geographic information systems.

To continue to develop the modern tool kit to facilitate the in-depth study of snails, larval trematodes and their interactions.

To develop more specific modern tools to facilitate the study of snails and associated larval schistosomes in field settings.

To gain a more complete understanding of the natural symbionts and associates of schistosome-transmitting snails.

To investigate more thoroughly the natural enemies of schistosome sporocysts.

To learn more about the etiological agents and epidemiology of cercarial dermatitis.

To do a better job in facilitating relevant information transfer to scientists in developing countries.

To continue to support a critical mass of medical malacologists, including centers to support training of scientists from developing countries and support for scientists from developed countries willing to work in the difficult conditions in which schistosomiasis transmission often occurs in the field.

**Acknowledgments:** The author thanks the members of the “Parasites and Hosts” reading group at the University of New Mexico for their helpful feedback. This work was supported by NIH Grant Number RR-1P20RR18754 from the International Development Award (IDeA) program of the National Center for Research Resources, and by NIH grants AI24340 and AI44913.

**Literature Cited:**

Abdel-Wahab, M.F., Yosery, A., Narooz, S., Esmat, G., Elhak, S., Nasif, S. and Strickland, G.T. 1993. Is *Schistosoma mansoni* replacing *Schistosoma haematobium* in the Fayoum. American Journal of Tropical Medicine and Hygiene 49: 697-700.

Attwood, S.W., Upatham, E.S., Zhang, Y.P., Yang, Z.Q. and Southgate, V.R. 2004. A DNA-sequence based phylogeny for triculine snails (Gastropoda: Pomatiopsidae: Triculinae), intermediate hosts for *Schistosoma* (Trematoda : Digenea): phylogeography and the origin of *Neotricula*. Journal of Zoology 262: 47-56.

Bixler, L.M., Lerner, J.P., Ivanchenko, M., McCormick, R.S., Barnes, D.W., and Bayne, C.J. 2001. Axenic culture of *Schistosoma mansoni* sporocysts in low O<sub>2</sub> environments. Journal of Parasitology 87: 1167-1168.

Campbell G, Jones CS, Lockyer AE, *et al.* 2000. Molecular evidence supports an African affinity of the Neotropical freshwater gastropod, *Biomphalaria glabrata*, Say 1818, an intermediate host for *Schistosoma mansoni*. Proceedings of the Royal Society of London Series B. 267: 2351-2358.

Canning, E. U. 1981. Microsporidia for trematode control. Parasitology 82: 120-121.

Carton Y., Nappi, A.J. and Poirie M. 2005. Genetics of anti-parasite resistance in invertebrate. Developmental and Comparative Immunology 29: 9-32.

Chae, J.S., Kim, E.H., Kim, M.S., Kim, M., Cho, Y.H. and Park, B.K. 2003. Prevalence and sequence analyses of *Neorickettsia risticii*. Rickettsiology: Present and Future Directions 990: 248-256.

Christophides, G.K., Vlachou, D. and Kafatos, F.C. 2004. Comparative and functional genomics of the innate immune system in the malaria vector *Anopheles gambiae*. Immunological Reviews 198:127-148.

Coelho, P.M.Z., Carvalho, O.S., Andrade, Z.A., Martins-Sousa, R.L., Rosa, F.M., Oliveira, G.C., Franco, G.R., Teles, H.M.S. and Negrao-Correa, D. 2004. *Biomphalaria tenagophila/Schistosoma mansoni* interaction: Premises for a new approach to biological control of schistosomiasis. Memorias do Instituto Oswaldo Cruz 99:S109-111.

Coppin, J.F., Lefebvre, C., Caby, S., Coquerelle, C., Vicogne, J., Coustau, C., and Dissous, C. 2003. Gene expression changes in *Schistosoma mansoni* sporocysts induced by *Biomphalaria glabrata* embryonic cells. Parasitology Research 89: 113-119.

Coustau, C., and Yoshino, T.P. 2000. Flukes without snails: Advances in the in vitro cultivation of intramolluscan stages of trematodes. Experimental Parasitology 94: 62-66.

Cowie, R.H. 2001. Can snails ever be effective and safe biocontrol agents? International Journal of Pest Management 47: 23-40.

Davison, A. and Blaxter, M.L. 2005. An expressed sequence tag survey of gene expression in the pond snail *Lymnaea stagnalis*, an intermediate vector of *Fasciola hepatica*. Parasitology 130: 539-552.

DeJong, R.J., Morgan, J.A.T., Paraense, W.L., Pointier, J-P., Amarista, M., Ayeh-Kumi. P.F.K., Babiker, A., Barbosa, C.S., Bremond, P., Canese, A.P., de Souza, C.P., Dominguez, C., File, S., Gutierrez, A., Incani, R.N., Kawano, T., Kazibwe, F., Kpikpi, J., Lwambo, N.J.S., Mimpfoundi, R., Poda, J-N., Sene, M., Velasquez, L.E., Yong, M., Adema, C.M., Hofkin, B.V., Mkoji, G.M. and Loker, E.S. 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.

Dillon, R.T., Wethington, A.R., Rhett, J.M. and Smith, T.P. 2002. Populations of the European freshwater pulmonate *Physa acuta* are not reproductively isolated from American *Physa heterostropha* or *Physa integra*. Invertebrate Biology 121: 226-234.

## Molluscan Aspects of Schistosomiasis - Loker

Ducklow, H.W., Boyle, P.J., Mangel, P.W. Strong, C. And Mitchell, R. 1979.

Bacterial flora of the schistosome vector snail *Biomphalaria glabrata*. Applied and Environmental Microbiology 38: 667-672.

Ducklow, H.W., Clausen, K. And Mitchell, R. 1981. Ecology of bacterial communities in the schistosomiasis vector snail *Biomphalaria glabrata*. Microbial Ecology 7: 253-274.

Esch, G.W., Curtis, L.A. and Barger, M.A. 2001. A perspective on the ecology of trematode communities in snails. Parasitology 123: S57-S75

Gibson, K.E., Rikihisa, Y., Zhang, C.B. and Martin, C. 2005. *Neorickettsia risticii* is vertically transmitted in the trematode *Acanthatrium oregonense* and horizontally transmitted to bats. Environmental Microbiology 7: 203-212.

Giovanelli, A., da Silva, C.L.P.A.C., Medeiros, L. and de Vasconcellos, M.C. 2002a.

The molluscicidal activity of niclosamide (Bayluscide WP70 (R)) on *Melanooides tuberculata* (Thiaridae), a snail associated with habitats of *Biomphalaria glabrata* (Planorbidae). Memorias do Instituto Oswaldo Cruz 97: 743-745.

Giovanelli, A., Vieira, M.V. and da Silva, C.L.P.A.C. 2002b. Interaction between the intermediate host of schistosomiasis in Brazil *Biomphalaria glabrata* (Planorbidae) and a possible competitor *Melanooides tuberculata* (Thiaridae): I. Laboratory experiments. Memorias do Instituto Oswaldo Cruz 97: 363-369.

Giovanelli, A., Vieira, M.V. and da Silva, C.L.P.A.C. 2005. Interaction between the intermediate host of schistosomiasis in Brazil, *Biomphalaria glabrata* (Say, 1818) and a possible competitor, *Melanooides tuberculata* (Muller, 1774): A field study. Journal of Molluscan Studies 71: 7-13.

Glen, D.M. and Wilson, M.J. 1997. Slug-parasitic nematodes as biocontrol agents for slugs. Agro Food Industry Hi-Tech 8: 23-27.

Grewal, P.S., Grewal, S.K., Tan, L. and Adams, B.J. 2003. Parasitism of molluscs by nematodes: Types of associations and evolutionary trends. Journal of Nematology 35: 146-156.

Graeff-Teixeira, C., Valar, C., de Moraes, C.K., Salvany, A.M., Brum, C.D., Maurer, R.L., Ben, R., Mardini, L.B.F.L., Jobim, M.B. and do Amaral, R.S. 2004. The initial epidemiological studies in the low endemicity schistosomiasis area in Esteio, Rio Grande do Sul, the southernmost Brazilian state, 1997 to 2000. Memorias do Instituto Oswaldo Cruz 99: S73-78.

Hamburger, J., He-Na, Xin, X.Y., Ramzy, R.M., Jourdane, J. and Ruppel, A. 1998. A polymerase chain reaction assay for detecting snails infected with bilharzia parasites (*Schistosoma mansoni*) from very early prepatency. American Journal of Tropical Medicine and Hygiene 59: 872-876.

Hamburger, J., Hoffman, O., Kariuki, H.C., Muchiri, E.M., Ouma, J.H., Koech, D.K., Sturrock, R.F. and King, C.H. 2004. Large-scale, polymerase chain reaction-based surveillance of *Schistosoma haematobium* DNA in snails from transmission sites in coastal Kenya: A new tool for studying the dynamics of snail infection. American Journal of Tropical Medicine and Hygiene 71: 765-773.

Hanelt, B., Adema, C.M., Mansour, M.H., and Loker, E.S. 1997. Detection of *Schistosoma mansoni* in *Biomphalaria glabrata* using nested PCR. Journal of Parasitology. 83: 387-394.

Heckel, D.G. 2003. Genomics in pure and applied entomology. Annual Review of Entomology 48: 235-260.

## Molluscan Aspects of Schistosomiasis - Loker

- Hertel, L.A., Bayne, C.J. and Loker, E.S. 2002. The symbiont *Capsaspora owczarzaki*, nov. gen. nov. sp., isolated from three strains of the pulmonate snail *Biomphalaria glabrata* is related to members of the Mesomycetozoa. *International Journal for Parasitology*, 32: 1183-1191.
- Hradkova, K. and Horak, P. 2002. Neurotropic behaviour of *Trichobilharzia regenti* in ducks and mice. *Journal of Helminthology* 76: 137-141.
- Jannotti-Passos, L.K., Vidigal, T.H.D.A., Dias Neto, E., Pena, S.D.J., Simpson, A.J.G., Dutra, W.O., Souza, C.P. and Carvalho Parra, J.F. 1997. PCR amplification of the mitochondrial DNA minisatellite region to detect *Schistosoma mansoni* infection in *Biomphalaria glabrata* snails. *Journal of Parasitology* 83: 395-399.
- Jarne, P. and Stadler, T. 1995. Population genetic structure and mating system evolution in freshwater pulmonates. *Experientia* 51: 482-497.
- Jones, C.S., Rollinson, D., Mimpfoundi, R., Ouma, J., Kariuki, H.C. and Noble, L.R. 2001. Molecular evolution of freshwater snail intermediate hosts within the *Bulinus forskalii* group. *Parasitology* 123: S277-S292.
- Kinzelbach, R. 1995. Neozoans in European waters - exemplifying the worldwide process of invasion and species mixing. *Experientia* 51: 526-538.
- Kristensen, T.K., and Brown, D.S. 1999. Control of intermediate host snails for parasitic diseases - A threat to biodiversity in African freshwaters? *Malacologia* 41: 379-391.
- Lardans, V., and Dissous, C. 1998. Snail control strategies for reduction of schistosomiasis transmission. *Parasitology Today* 14: 413-417.
- Larsen, A.H., Bresciani, J. and Buchmann, K. 2004. Increasing frequency of cercarial dermatitis at higher latitudes. *Acta Parasitologica*. 49: 217-221.
- Lockyer, A.E., Jones, C.S., Noble, L.R. and Rollinson, D. 2004. Trematodes and snails: an intimate association. *Canadian Journal of Zoology – Revue Canadienne de Zoologie* 82: 251-269.
- Lotfy, W.M., DeJong, R.J., Kader, A.A. and Loker, E.S. 2005. A molecular survey of *Biomphalaria* in Egypt: is *B. glabrata* present? *American Journal of Hygiene and Tropical Medicine*. 73: 131-139.
- Marshall, J. 2005. Megacity, mega mess ... *Nature* 437: 312-314.
- Martens, W.J.M., Jetten, T.H. and Focks, D.A. 1997. Sensitivity of malaria, schistosomiasis and dengue to global warming. *Climatic Change* 35: 145-156.
- Minchella, D.J., Sollenberger, K.M., and Desouza, C.P. 1995. Distribution of schistosome genetic diversity within molluscan intermediate hosts. *Parasitology* 111: 217-220.
- Mitta, G., Galinier, R., Tisseyre, P., Allienne, J.F., Girerd-Chambaz, Y., Guillou, F., Bouchut, A. and Coustau, C. 2005. Gene discovery and expression analysis of immune-relevant genes from *Biomphalaria glabrata* hemocytes. *Developmental and Comparative Immunology* 29: 393-407.
- Monkiedje, A., Anderson, A.C., and Englande, A.J. 1991. Acute toxicity of *Phytolacca dodecandra* (Endod S) and niclosamide to snails, *Schistosoma mansoni* cercaria, tilapia fish, and soil microorganisms. *Environmental Toxicology and Water Quality* 6: 405-413.

## Molluscan Aspects of Schistosomiasis - Loker

Morgan, J.A.T., DeJong, R.J., Jung, Y., Khallaayoune, K., Kock, S., Mkoji, G.M., Loker, E.S., 2002. A phylogeny of planorbid snails, with implications for the evolution of *Schistosoma* parasites. *Molecular Phylogenetics and Evolution* 25: 477-488.

Oliveira, E.C. and Paumgarten, F.J.R. 2000. Toxicity of *Euphorbia milli* latex and niclosamide to snails and nontarget aquatic species. *Ecotoxicology and Environmental Safety* 46: 342-350.

Park, B.K., Kim, M.J., Kim, E.H., Kim, M.S., Na, D.G., and Chae, J.S. 2003. Identification of trematode cercariae carrying *Neorickettsia risticii* in freshwater stream snails. *Rickettsiology: Present and Future Directions* 990: 239-247.

Pointier, J.P., David, P. and Jarne, P. 2005a. Biological invasions: the case of planorbid snails. *Journal of Helminthology* 79:249-256.

Pointier, J.-P., DeJong, R.J., Tchuem Tchuente, L.A., Kristensen, T.K., and Loker, E.S. 2005b. A neotropical snail host of *Schistosoma mansoni* introduced into Africa and consequences for the schistosomiasis transmission: *Biomphalaria tenagophila* in Kinshasa (Democratic Republic of Congo). *Acta Tropica* 93: 191-199.

Pusterla, N., Johnson, E.M., Chae, J.S. and Madigan, J.E. 2003. Digenetic trematodes, *Acanthatrium* sp and *Lecithodendrium* sp., as vectors of *Neorickettsia risticii*, the agent of Potomac horse fever. *Journal of Helminthology* 77: 335-339.

Rodgers, J.K., Sandland, G.J., Joyce, S.R. and Minchella, D.J. 2005. Multi-species interactions among a commensal (*Chaetogaster limnaei limnaei*), a parasite (*Schistosoma mansoni*), and an aquatic snail host (*Biomphalaria glabrata*). *Journal of Parasitology* 91: 709-712.

Scholz, T. and Salgado-Maldonado, G. 2000. The introduction and dispersal of *Centrocestus formosanus* (Nishigori, 1924) (Digenea : Heterophyidae) in Mexico: A review. *American Midland Naturalist* 143: 185-200.

Seto, E., Xu, B., Liang, S., Gong, P., Wu, W.P., Davis, G., Qiu, D.C., Gu, X.G. and Spear, R. 2002. The use of remote sensing for predictive modeling of schistosomiasis in China. *Photogrammetric Engineering and Remote Sensing* 68: 167-174.

Sow, S., de Vlas, S.J., Engels, D. and Gryseels, B. 2002. Water-related disease patterns before and after the construction of the Diama dam in northern Senegal. *Annals of Tropical Medicine and Parasitology* 96: 575-586.

Stothard, J.R., Mgeni, A.F., Khamis, S., Seto, E., Ramsan, M., Hubbard, S.J. and Kristensen, T.K. 2002. Royal Society of Tropical Medicine and Hygiene Meeting at Manson House, London, 21 February 2002 - Fresh from the field - New insights into the transmission biology of urinary schistosomiasis in Zanzibar. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 96: 470-475.

Sutherst, R.W. 2004. Global change and human vulnerability to vector-borne diseases. *Clinical Microbiology Reviews* 17: 136-+.

Teo, S.S. 2001. Evaluation of different duck varieties for the control of the golden apple snail (*Pomacea canaliculata*) in transplanted and direct seeded rice. *Crop Protection* 20: 599-604.

Théron, A. and Coustau, C. 2005. Are *Biomphalaria* snails resistant to *Schistosoma mansoni*? *Journal of Helminthology* 79: 187-191.

Verbrugge, L.M., Rainey, J.J., Reimink, R.L. and Blankespoor, H.D. 2004. Swimmer's itch: Incidence and risk factors. *American Journal of Public Health* 94: 738-741.

## Molluscan Aspects of Schistosomiasis - Loker

Vidigal, T.H.D.A., Magalhaes, K.G., Kissinger, J.C., Caldeira, R.L., Simpson, A.J.G. and Carvalho, O.S. 2002. A Multiplex-PCR approach to identification of the Brazilian intermediate hosts of *Schistosoma mansoni*. *Memorias do Instituto Oswaldo Cruz* 97: S95-97.

Vidigal, T.H.D.A., Spatz, L., Kissinger, J.C., Redondo, R.A.F., Pires, E.C.R., Simpson, A.J.G. and Carvalho, O.S. 2004. Analysis of the first and second internal transcribed spacer sequences of the ribosomal DNA in *Biomphalaria tenagophila* complex (Mollusca : Planorbidae). *Memorias do Instituto Oswaldo Cruz* 99: 153-158.

Woodruff, D.S. and Mulvey, M. 1997. Neotropical schistosomiasis: African affinities of the host snail *Biomphalaria glabrata* (Gastropoda: Planorbidae). *Biological Journal of the Linnean Society* 60: 505-516.

Yousif, F. and Lämmler, G. 1975. The suitability of several aquatic snails as intermediate hosts for *Angiostrongylus cantonensis*. *Zeitschrift fur Parasitenkunde* 47: 203-210.

Zhang, S.-M., Adema, C.M., Kepler, T.B. and Loker, E.S. 2004. Diversification of Ig genes in an invertebrate. *Science* 305: 251-254.

Zheng, J., Gu, X.G., Xu, Y.L., Ge, J.H., Yang, X.X., He, C.H., Tang, C. Cai, K.P., Jiang, Q.W., Liang, Y.S.et. al. 2002. Relationship between the transmission of *Schistosomiasis japonica* and the construction of the Three Gorge Reservoir. *Acta Tropica* 82: S147-156.