Gastropod-borne helminths: a look at the snail-parasite interplay

Alessio Giannelli[1]*, Cinzia Cantacessi[2]*, Vito Colella[1], Filipe Dantas-Torres[1,3], Domenico Otranto[1]**

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari, Valenzano 70010, Italy
[2]Department of Veterinary Medicine, University of Cambridge, Cambridge CB3 0ES, United Kingdom
[3]Departamento de Imunologia, Centro de Pesquisas Aggeu Magalhães (Fiocruz-PE), Recife, Pernambuco, Brazil

*Equal contributions

**Corresponding author: Otranto D. (domenico.otranto@uniba.it)

Keywords: gastropod-borne diseases, *Schistosoma*, *Angiostrongylus*, metastrongylids, snail-parasite interactions

More than 300 million people suffer from a range of diseases caused by snail-borne helminths, predominantly flatworms and roundworms, whose life cycles are characterised by a diversified ecology and epidemiology. Despite the plethora of data on these parasites, very little is known on the fundamental biology of their gastropod intermediate hosts, and of the interactions occurring at the snail-helminth interface. In this article, we focus on schistosomes and metastrongylids of human and animal significance and review current knowledge of snail-parasite interplay. Future efforts aimed at elucidating key elements of the biology and ecology of the snail intermediate hosts, along with an improved understanding of snail-parasite interactions, will aid to identify, plan and develop new strategies for disease control focused on gastropod intermediate hosts.

Gastropods, parasites and vertebrates

The Mollusca, one of the largest phyla of living creatures, includes gastropod species able to colonise every humid corner of the planet [1]. Given their adaptability to a range of diverse ecosystems, molluscs have been long known to serve as ideal hosts for a number of parasites, including nematodes and trematodes [2]. Indeed, gastropods act as intermediate hosts for a range of helminth parasites of medical and veterinary concern [2,3], including more than 18,000 digenean trematodes and about 50 roundworm species ranked into the superfamily Metastrongyloidea [4,5]. Currently, diseases caused by gastropod-borne helminths (GBHs) are estimated to affect more than 300 million people worldwide (http://www.who.int/mediacentre/factsheets/fs115/en). Some of these GBHs, such as the zoonotic liver flukes *Fasciola hepatica* and *Fasciola gigantica*, significantly affect the livestock industry [6], while others (e.g., *Angiostrongylus vasorum* and *Aelurostrongylus abstrusus*) have long been in the spotlight as causes of significant concern for companion animal health [7,8]. In spite of major global efforts to control GBHs, many of these diseases are still endemic in vast areas of the world (http://www.who.int/mediacentre/factsheets/fs115/en). Therefore, there is a constant need to discover novel strategies to effectively reduce the burden of disease caused by these parasites, in both humans and animals. The development of adequate control strategies against any disease heavily relies on a thorough understanding of the pathogen biology, ecology and epidemiology. In the case of parasites with indirect life cycles, this includes a profound knowledge of the intermediate hosts. Accordingly, for GBHs, current and future efforts aimed at controlling the diseases they cause must take into account measures to reduce the burden of infections in snails [2,9].

To date, the majority of studies on gastropod-borne parasitic diseases have involved trematodes belonging to family Schistosomatidae and Opisthorchiidae [9-11]. Nonetheless, some GBHs may potentially threaten a larger number of people in the near future, as a consequence of climate change and/or enhanced movement of people and goods [12]. The rat lungworms *Angiostrongylus cantonensis* and *Angiostrongylus costaricensis* represent two key examples of such GBHs [3,13]. The life cycles of these helminths are strictly associated with the distribution of their gastropod intermediate hosts [14], which makes improvement of current knowledge of snail-parasite interactions a priority. Over the last few years, a range of studies has explored the fundamental biology of snail intermediate hosts of GBHs, as well as key molecular and immunological interactions occurring at the snail-parasite interface [15-18]. This improved understanding provides a solid basis for the development of future strategies of disease intervention based on control of infected gastropods.

In this article, we provide an account of recent advances in knowledge of snail-parasite interactions, focussing in particular on schistosomes and zoonotic metastrongylids and, in line with the principles of the One Health Initiative (www.onehealthinitiative.com), we emphasize the need for enhanced communications amongst research groups investigating human and animal GBHs, in order to support the design of integrated strategies to combat these diseases.

A snail for each schistosome

Recent estimates provided by the World Health Organization (WHO) indicate that, in 2013, at least 260 million people required preventative treatment for schistosomiasis (http://www.who.int/mediacentre/factsheets/fs115/en), which translated into losses estimated at ~3.3 million disability-adjusted life years (DALYs) [19]. Schistosomiasis, also known as bilharziasis (see Glossary), is endemic amongst poor communities of tropical and subtropical areas, where sanitation conditions are below standards and snail intermediate hosts are endemic [20]. Most human infections are caused by *Schistosoma mansoni, S. haematobium*, or *S. japonicum*, with the latter known to infect 46 species of animals, which therefore serve as reservoir hosts for human infections [21]. The distribution of these species of *Schistosoma* overlaps that of the snail intermediate hosts; *S. mansoni*, transmitted by aquatic snails of the genus *Biomphalaria*, is estimated to infect >80 million people, mainly in the sub-Saharan Africa, isolated Middle East areas, southern America and the Caribbean, whereas *S. haematobium*, transmitted by *Bulinus* freshwater planorbids, is widespread throughout sub-Saharan Africa and the Eastern Mediterranean countries, where it affects >110 million people [22]. Conversely, the distribution of *Oncomelania* snails, the intermediate hosts of *S. japonicum*, is limited to Southeast Asia and China, where ~1.8 million people are infected by this flatworm [23,24].

The density of snail populations in lentic and lotic ecosystems (see Glossary) fluctuates along with the availability of several abiotic and biotic environmental factors (e.g., temperature of the water, conductivity, pH and presence of suitable vegetation) [25,26]. In addition, the adaptability of snail species serving as intermediate hosts of GBHs to changing environments, as a consequence of climatic variations and/or human-driven modifications of ecosystems, is bound to play key roles in the epidemiology of these diseases, as well as on the robustness of intervention strategies based on the control of snail populations. For example, *Neotricula aperta*, implicated in the transmission of *Schistosoma mekongi* in Cambodia, Laos and Thailand, where ~140,000 people are at risk of infection, occupies exclusively shallow areas characterised by hard water and stony river beds, close to karst springs [27]. Therefore, given the specific ecological requirements of this snail species, eradication of disease based on control of *N. aperta* is a feasible option [28].

Conversely, the control of schistosomiasis japonica in China, the Philippines and Sulawesi Island is challenged by the resistance to silting and amphibious nature of *Oncomelania hupensis* snails, which may inhabit ditches, wetlands and marshy ground in both hilly and mountains regions [24]. Similarly, *Biomphalaria* snails, responsible for transmission of *S. mansoni* in the Caribbean, South America, Egypt and sub-Saharan Africa [29-31] are adapted to a large number of ecosystems (e.g., lakes, fish ponds and rice fields) and benefit from the presence of controlled water flows, dams and irrigation networks [30]; in addition, planorbids of the genus *Bulinus* are known to successfully breed in a range of environmental conditions and can be detected in several regions of Africa (i.e., the Nile River valley, Mahgreb, most of southern and sub-Saharan Africa, including Madagascar) [32], and the Middle East (e.g., Iran, Malawi, Sudan) [33], where they are efficient intermediate hosts of *S. haematobium*.

Therefore, given the ability of these gastropods to colonise a range of different habitats, their control and that of the GBHs they transmit presents inevitable challenges. For this reason, gaining a profound knowledge of snail-parasite interactions will represent a key arrow in our quiver of potential weapons against GBHs, as it will allow researchers to identify parasite 'Achille's heels' on which to address future efforts aimed at developing disease intervention strategies based on parasite control.

Gastropods and trematodes

The majority of studies performed to explore snail-parasite relationships have been focused on schistosomes and their intermediate hosts, primarily because of the availability of experimental systems that allow maintenance of these parasites in several species of molluscs in the laboratory [9]. As a consequence, a plethora of information has been collected over the last few years on the biological and molecular interactions occurring between schistosome parasites and their gastropod

intermediate hosts (**Box 1**) [34-37], including the intramolluscan life cycle of those flatworms. Miracidia of *S. mansoni* infect *Biomphalaria* gastropods through the exposed mantle epithelium, and frequently through the antennae or the head-foot. In the fibromuscular tissue of the cephalopodal region, the parasite undergoes morphological and physiological changes, developing into a primary or mother sporocyst; this stage generates several secondary or daughter sporocyts, which migrate to the digestive glands or the hepatopancreas of the mollusc. Finally germinative cells of the daughter sporocyst produce water-living furcocercous cercariae (**Figure 1**) [38]. Infections by trematodes inevitably impact on snail longevity and fitness, with variable outcomes [39]. For instance, accelerated shell growth or gigantism has been observed in *Lymnaea* snails infected by plagiorchids, whereas retarded development or stunting has been recorded in *Biomphalaria* planorbids exposed to *S. mansoni* [39]. The occurrence of these changes is often associated with impairment of the snail fecundity, an event also referred to as parasitic castration [40], during which the trematode gradually redirects the host metabolism towards its own needs [41]. Although the exact mechanisms that lead to the snail castration are currently unknown, it has been suggested that the parasite may act as a 'competitor' for nutrients required for reproduction (e.g., vitelline glands), or may directly interfere with selected physiological processes of the gastropod [41]. For instance, preliminary studies in the *Lymnea stagnalis-Trichobilharzia ocellata* system had pointed towards a role of schistosomin, a host-derived host factor, in the occurrence of parasitic castration [42]; however, recent data indicate that changes in expression levels of this neuropeptide in *B. glabrata* snails are not directly linked to active development of schistosomes [43].

Clearly, the availability of basic parasitological information on snails-schistosome interactions has assisted scientists in the acquisition of a better understanding of GBHs epidemiology. However, substantial gaps still exist in our knowledge of the cascade of molecular events that regulate the development of nematodes within their mollusc intermediate hosts. Such gaps are particularly pronounced for snail hosts of metastrongylid parasites of humans and animals, as illustrated in the following section.

Zoonotic *Angiostrongylus* infection: the state of the art

The superfamily Metastrongyloidea includes several GBHs of veterinary concern and two zoonotic species of public health interest, namely *A. cantonensis* and *A. costaricensis*. The former is the causative agent of eosinophilic meningitis, which affects ~3000 people throughout Southeast Asia, Australia, Pacific Islands and the Caribbean [3,44,45], whereas *A. costaricensis* is emerging in the New World, causing life-threatening human abdominal angiostrongyliasis [13,46]. Although gastropods serve as intermediate hosts for both parasites, a range of paratenic hosts (e.g., shrimps, prawns, crabs, toads, planarians) act as vehicles of the infection to humans [47]. In particular, the completion of the life cycle of *Angiostrongylus* relies on rats as definitive host, which shed first-stage larvae (L1s) in the environment with their faeces. As for most metastrongylids (**Figure 2**), L1s infect gastropods (for *A. cantonensis*, more than 160 snail or slug species under natural or experimental conditions; [3]), in which they moult twice before developing to infective third stage larvae (L3s). Rats and other dead-end hosts become infected when they ingest gastropod molluscs or paratenic hosts [47]. While studies on *Angiostrongylus*-gastropod interactions are currently limited (**Table 1**), a number of surveys have investigated the main factors involved in the distribution and possible expansion of eosinophilic meningitis from endemic regions to geographical areas previously considered 'parasite-free' [48-50]. For instance, the spreading of *A. cantonensis* via newly-introduced gastropod species [3] has been investigated in the Hawaiian Islands, where the parasite was detected in 16 snail species (2 native, 14 non-native), four of which were acknowledged as intermediate hosts for the first time [3]. Similarly, this parasite has also been newly-introduced *via* terrestrial snails (i.e., *Achatina fulica*, *Zachrysia provisoria*, *Bradybaena similaris* and *Alcadia striata*) imported in the Gulf Coast region of the United States [51].

Above all, global travel, climate change and globalization act as major drivers for the emergence of

Angiostrongylus infection worldwide. For instance, molluscs consumed as food or kept as domestic pets (e.g., *Achatina fulica*), along with the expansion of the distribution range of some invasive species (e.g., *Pomacea canaliculata*) are considered key determinants of the increasing prevalence of infections by *A. cantonensis* in Mainland China and South America [52]. Nonetheless, the detection of this roundworm in European native gastropods (e.g., *Cornu aspersum* or *Theba pisana*) [50], together with the availability of predictive models based on climatic factors suggesting an increase in suitable habitats for this pathogen in Europe [12], is worrisome. For this reason, preventative measures should be developed to face a potential introduction of rat definitive hosts to non-endemic areas. To achieve these goals and accurately identify risk factors for disease transmission in Europe, comprehensive investigations of the complex interactions occurring at the gastropod-angiostrongylid interface are needed.

Delving into the great unknown: zoonotic metastrongylid-snail interactions

Thus far, knowledge of snail-*Angiostrongylus* interactions is limited to reports dating back to the '70s and '80s. For instance, the detection of *A. cantonensis* larvae in the kidney and rectum of *B. glabrata*, suggested that these might represent the main routes of larval migration to the snail mantle collar and head-foot, i.e. the 'exit doors' to the outer environment [53]. In the same study, angiostrongylid larvae were encapsulated by the snail 24-48 hours post-infection following a two-phase process, consisting of an initial infiltration and aggregation of basophilic haemolymph cells around the parasite, followed by encasement in fibrous-appearing nodules [53]. As a likely consequence of the increasing interest towards GBHs [2], recent studies have focussed on the metabolic responses of gastropods infected by *A. cantonensis*. For instance, the energetic balance and oxidative metabolism of *B. glabrata* infected by *A. cantonensis* under experimental conditions, displayed a sharp decline in glucose content immediately following infection, likely as a consequence of the competition for nutrients between the nematode and the snail which, in turn, is forced to activate its anaerobic metabolism (i.e., increased activity of enzymes involved in the glycolytic pathway mediated by lactate dehydrogenase), in order to survive [54]. The angiostrongylid also promote the protein metabolism of snails, as demonstrated by the increased production of nitrogen catabolites such as urea, and particularly the conversion of uricotelic into ureotelic acid, probably as a detoxification strategy, thus favouring the vital functions of the snail and, indirectly, parasite development [55]. Similarly, co-infections by *Echinostoma paraensei*, a trematode of wild rodents, and *A. cantonensis* [56] trigger a progressive depletion of the carbohydrates reserves in *B. glabrata* snails, which, in turn, increase the rate of deamination of amino acids. Moreover, the enhanced demand for nutrients by the parasites modifies the kinetic behaviour of *A. cantonensis* and *E. paraensi* in the gastropod tissues, inducing the former to pursue new migration routes [56]. Indeed, the intense cellular disorganization induced by *E. paraensei* in the digestive gland-gonad complex of the snail (i.e., the site for *A. cantonensis* moulting) forces the nematode to continue its development into the kidneys [56]. Further immune-molecular studies on the fundamental *Angiostrongylus*-gastropod interplay are crucial to implement strategies for the control of the infection. Even though a suitable experimental snail model is currently unavailable, such a system would provide a ready-to-use infrastructure for in-depth studies of biological pathways specifically involved in snail-parasite interactions, as recently proposed for the *Bithynia-Opisthorchis* complex [57]. Until that, research on phylogenetically-related animal parasites (e.g., *A. vasorum* or *A. abstrusus*) [46] may represent a useful way to overcome these gaps, thus opening new opportunities for a thorough investigation on GBHs of medical and veterinary concern.

Opening new fields in GBHs research

Over the past couple of years, a few fundamental studies have opened new and exciting avenues for research on gastropod hosts of parasites, that may pave the way towards much needed comparative studies between trematode- and nematode-bearing snail intermediate hosts. For instance, new scientific evidence now points towards the occurrence of an alternative mode of transmission of the

cat lungworm *A. abstrusus* among gastropods. Indeed, after being shed in the mucus trails of the land snails or in the water where gastropods had died [58], L3s of this metastrongylid are able to infect new intermediate hosts, in a mechanism referred to as intermediesis. This phenomenon may represent a dynamic survival-and-transmission strategy for nematodes, allowing spread of parasites to other susceptible intermediate hosts [59]. While it is tempting to speculate on the potential advantages that intermediesis may present for spreading and survival of snail-borne nematodes, a clear understanding of this phenomenon can only be achieved via in depth studies of snail-nematode interactions. Indeed, while a plethora of information is available on the molecular and immunological interactions occurring at the snail-schistosome interface (mainly as a consequence of the availability of suitable experimental systems, including a draft genome sequence for *B. glabrata* http://www.vectorbase.org), its embryonic cell lines (Bge) [60], and schistosome genomes [61-63], studies of the immune-molecular mechanisms that govern the snail-metastrongylid interplay are minimal (**Table 1**). Beside a single attempt to cultivate *A. cantonensis* from L3s to fourth-stage larvae in a defined culture medium [64], the development of metastrongyloids *in vitro* is still un unexplored field; progress in this area is required to provide essential information on the physiology of helminths, as well as for the advancement of parasitological and biomedical research on GBHs. Therefore, an improved knowledge of snail-parasite interactions will not only result in a better understanding of the ecology, epidemiology and basic biology of GBHs, but will also represent the necessary infrastructure for hypothesis-driven studies aimed at interrupting the spreading of the diseases they cause.

Biological control of snails: a feasible option?

Nowadays, the control of GBH infections is based on a combination of preventative measures, which, in the case of schistosomiasis, include early diagnosis and treatment of infected people, improvement of life quality and implementation of health education [65]. Nonetheless, the monitoring of susceptible snail populations, through wide-scale malacological surveys, will provide basic essential data towards planning adequate strategies to reduce the transmission risk of GBHs. For instance, in areas where schistosomiasis is endemic, campaigns involving gastropod control measures are mandatory to achieve a long-term effect on disease transmission [66]. In line with the agenda of the World Health Assembly, which endorsed an integration of non drug-based interventions to prevent parasite transmission [67], the scientific community is now seeking alternative means for interrupting the life cycles of snail-borne parasites [66]. For example, the introduction of gastropod intermediate hosts resistant to the infection, as opposed to the spreading of molluscicides (e.g., niclosamide) in the water, has been proposed as an effective and environmentally friendly strategy to reduce the burden of disease [68,69]. The impact of biological control of snails on the epidemiology of GBHs has long been debated (reviewed by [70]), with a number of reports documenting how competitors and snail predators might be exploited to reduce populations of molluscs in the environment [70]. For instance, fishes of the family Cichlidae or Cyprinidae are natural predators of gastropods, and their introduction may result in a significant reduction of schistosome intermediate hosts [70]. However, due to the broad-spectrum diet of these fishes and their low population density, this method is considered unfeasible [70]. Conversely, rhabditid nematodes of the genus *Daubaylia* (e.g., *D. potomaca*) [71, 72] and bacteria such as *Candidatus* Paenibacillus glabratella [73] could provide a means to the control of schistosome snail hosts, although the potential efficacy of this approach remains to be clearly demonstrated [72]. Competing and predatory snails have been considered a valid, promising and relatively inexpensive option for the control of gastropods. For example, the ampullarids *Marisa cornuarietis* and *Pomacea australis* have been successfully employed to reduce the populations of *Biomphalaria* and *Bulinus* under natural condition [70]; however, these species are considered economically-important agricultural pests [52]. Similarly, the re-introduction of the prawn *Macrobrachium vollenhoveni*, a predator of snails, was effective for the control of *Bulinus* planorbids, and ultimately resulted in a detectable reduction of the number of cases of urinary schistosomiasis in a village of Senegal [74].

However, caution is required when hypothesising the use of snail predators (e.g., other snails or prawns) for the control of certain GBHs, as they may serve as hosts for lung flukes (e.g., *Paragonimus* spp.), lungworms (*Angiostrongylus* spp.) and other foodborne helminths (*Clonorchis* and *Opisthorchis* spp.)[70]. Fly larvae of the genus *Sciomyzidae* are strictly malacophagous and harmless to humans and vertebrates; however, their use is made impractical by the different degrees of vulnerability displayed by snails [70]. Finally, trematode predators, including annellids of the genus *Chaetogaster*, mosquito larvae and Hydrozoa, may provide effective means to reduce the burden of GBHs Also, guppies (*Lebistes reticulatus*) are highly cercariophagous, with up to 1000 cercariae ingested per hour, but only in cases of densely established fish populations [70]. These key examples suggest that, today, the control of GBHs cannot solely rely on the use of biological agents, mainly because of the unfeasibility of such approaches on a global scale. Therefore regular administrations of praziquantel remain the most effective strategy to reduce the burden of schistosomiasis in exposed populations [65].

Concluding remarks

Given the well-known issues with widespread anthelmintic resistance involving a number of parasites of livestock, and the realistic possibility that such mechanisms may eventually emerge in human helminths, we advocate for the development of an integrated approach to combat diseases caused by GBHs. This could include i) information campaigns on GBHs and on their prevention in endemic areas, ii) reduction of the molluscicide dispersal, which often negatively affects organisms that may interfere with the helminth transmission; iii) implementation of research programs on alternative/novel ways to reduce the gastropod burden in the environment; iv) enhanced circulation of information among physicians, veterinarians, parasitologists and malacologists.

In conclusion, further data on snail-parasite biology is needed to enhance our knowledge of host-parasite interactions, and ultimately to provide new potential tools for GBH control. In particular, the application of so called -omic technologies (e.g. genomics, transcriptomics, proteomics, metabolomics) to large-scale explorations of snail-nematode interactions is bound to accelerate this progress, ultimately leading to the development of integrated strategies of GBHs control, based on extensive knowledge of snail biology and immunology. Altogether, these efforts will be determinant in the near future to identify the parasite ‘Achille’s heel’, thus translating these fundamental discoveries into potential control measures.

Box 1. Snail immunobiology and resistance to schistosome infection

Gastropods can activate mechanisms of innate immunity to cope with *Schistosoma* development [17,75], modulated by the activity of the mollusc internal defence system (IDS). The snail IDS includes two populations of haemocyte effector cells (granulocytes and hyalinocytes) [75] and a range of soluble factors in the hemolymph, which are involved in pathogen recognition and inflammatory responses [17]. Among these molecules, fibrinogen-related proteins (FREPs) are of major interest to the scientific community, due to their conserved structure throughout animal evolution; in addition, gastropod FREPs are the only known proteins, which combine immunoglobulin superfamily domains (IgSF) with their fibrinogen domain [18,76]. Fourteen subfamilies of FREPs have been described thus far, and eight more have been detected using RNA-seq (reviewed by [18]). Some of these molecules (i.e., FREP3) may act as opsonins [77] for a number of monosaccharide and mucin antigens expressed on the surface of schistosome larval stages [18]. In addition to FREPs, several cytokines, such as the macrophage migration inhibitory factor [78], and other proteins (e.g., biomphalysin) [79] have been hypothesized to be involved in the immune defence of *B. glabrata* against schistosomes. Once stimulated by the presence of miracidia, the haemocytes undergo enhanced mitosis within 24-72 hours, and produce reactive oxygen species (ROS), including hydrogen peroxide and hypochlorous acid, or phagocytise portions of *Schistosoma* tegument, leading to mechanical damage and destruction of the invader [17]. The expression of immunological factors that regulate snail resistance to trematode infection (e.g., production of FREPs, cathepsins, actins, heat-shock proteins) varies according to snail species, strain and age [80]. Studies on snail immunology [16-18], along with new insights on snail gene regulation and expression [81], are essential for elucidating the interactions between GBHs and their intermediate hosts, including those that result in unsuccessful infections. For instance, the *Biomphalaria tenagophila* Taim/RS strain is resistant to *S. mansoni* challenge, due to the clustering of haemocytes in a thick layer which surrounds, encapsulates and destroys the *S. mansoni* miracidium, soon after its penetration [82,83]. Considering that the resistance factor is transmitted as a dominant character with Mendelian inheritance [69,84,85], the identification of other genes linked to schistosome resistance (e.g., the guadeloupe resistance complex [GRC] in *B. glabrata*) and the genetic manipulation of snails might pave the way towards the insertion of such genes in susceptible gastropod populations [81]. Altogether, this approach could potentially help reducing the burden of infection in endemic areas of disease.

References

1. Bouchet, P. et al (2005) Classification and nomenclator of gastropod families. Malacologia 47, 1–397
2. Adema, C.M. et al (2012) Will all scientists working on snails and the diseases they transmit please stand up? PLoS Negl Trop Dis 6, e1835
3. Kim, J.R. et al (2014) Diverse gastropod hosts of *Angiostrongylus cantonensis*, the rat lungworm, globally and with a focus on the Hawaiian Islands. PLoS One 9, e94969
4. Kostadinova, A., Pérez-del-Olmo, A. (2014) Systematics of the Trematoda in Digenetic Trematodes, R. Toledo and B. Fried, eds., vol. 766 of Advances in Experimental Medicine and Biology, Springer-Verlag, New York, 2014, 21–44
5. Grewal, P.S. et al (2003) Parasitism of molluscs by nematodes: types of associations and evolutionary trends. J Nematol 35, 146–156
6. Knubben-Schweizer, G., Torgerson, P.R. (2015) Bovine fasciolosis: control strategies based on the location of *Galba truncatula* habitats on farms. Vet Parasitol 208, 77-83
7. Morgan, E.R. et al (2012) Parasite epidemiology in a changing world: can molecular phylogeography help us tell the wood from the trees? Parasitology 139, 1924-1938
8. Beugnet, F. et al (2014) Parasites of domestic owned cats in Europe: co-infestations and risk factors. Parasit Vectors 7, 291
9. Knight, M. et al (2014) Schistosomes and snails: a molecular encounter. Front Genet 5, 230
10. Huang, S.Y. et al (2012) Genomics and molecular genetics of *Clonorchis sinensis*: current status and perspectives. Parasitol Int 61, 71-76
11. Chen, Y. et al (2013) Development and evaluation of loop-mediated isothermal amplification (LAMP) for rapid detection of *Clonorchis sinensis* from its first intermediate hosts, freshwater snails. Parasitology 140, 1377-1383
12. York, E.M., Butler, C.J., Lord, W.D. (2014) Global decline in suitable habitat for *Angiostrongylus* (=*Parastrongylus*) *cantonensis*: the role of climate change. PLoS One 9, e103831
13. Romero-Alegría, A. et al (2014) *Angiostrongylus costaricensis*: systematic review of case reports. Adv Infect Dis 4, 36–41
14. Littlewood, D.T.J., Bray, R.A. (2001) Interrelationships of the Platyhelminthes, eds Taylor & Francis
15. Morley, N.J. (2010) Aquatic molluscs as auxiliary hosts for terrestrial nematode parasites: implications for pathogen transmission in a changing climate. Parasitology 137, 1041–1056
16. Negrão-Corrêa, D. et al (2007) Molluscan response to parasite, *Biomphalaria* and *Schistosoma mansoni* interaction. Invert Surviv J 4, 101–111
17. Coustau, C. et al (2015) Advances in gastropod immunity from the study of the interaction between the snail *Biomphalaria glabrata* and its parasites: A review of research progress over the last decade. Fish Shellfish Immunol 46, 5–16
18. Gordy, M.A., Pila, E.A., Hanington, P.C. (2015) The role of fibrinogen-related proteins in the gastropod immune response. Fish Shellfish Immunol 46, 39–49
19. Hotez, P.J. et al (2014) The global burden of disease study 2010: interpretation and implications for the neglected tropical diseases. PLoS Negl Trop Dis 8, e2865
20. Elmorshedy, H. et al (2015) Can human schistosomiasis mansoni control be sustained in high-risk transmission foci in Egypt? Parasit Vectors 8, 372
21. He, Y.X., Salafsky, B., Ramaswamy, K. (2001) Host--parasite relationships of *Schistosoma japonicum* in mammalian hosts. Trends Parasitol 17, 320–324
22. Hotez, P.J. et al (2006) Helminth Infections: Soil-transmitted Helminth Infections and Schistosomiasis In: Disease Control Priorities in Developing Countries, 2nd edition Disease Control Priorities Project, Jamison D.T et al. eds World Bank, Washington (DC) ISBN-10: 0-8213-6179-1

23. Gordon, C.A. et al (2012) High prevalence of *Schistosoma japonicum* infection in Carabao from Samar Province, the Philippines: implications for transmission and control. PLoS Negl Trop Dis 6, e1778
24. Attwood, S.W. et al (2015) Comparative Phylogenetic Studies on *Schistosoma japonicum* and Its Snail Intermediate Host *Oncomelania hupensis*: Origins, Dispersal and Coevolution. PLoS Negl Trop Dis 9, e0003935
25. McCreesh, N., Booth, M. (2013) Challenges in predicting the effects of climate change on *Schistosoma mansoni* and *Schistosoma haematobium* transmission potential. Trends Parasitol 29, 548–555
26. Rollinson, D., Stothard, J.R., Southgate, V.R. (2001) Interactions between intermediate snail hosts of the genus *Bulinus* and schistosomes of the *Schistosoma haematobium* group. Parasitology 123, S245–260
27. Attwood, S.W., Upatham, E.S. (2013) A population growth trend analysis for *Neotricula aperta*, the snail intermediate host of *Schistosoma mekongi*, after construction of the Pak-Mun dam. PLoS Negl Trop Dis 7, e2539.
28. Fukuhara, K. et al (2011) Analysis of the effectiveness of control measures against *Schistosoma mekongi* using an intra- and inter-village model in Champasak Province, Lao PDR. Parasitol Int 60, 452–459
29. Scholte, R.G. et al (2012) Spatial distribution of Biomphalaria spp., the intermediate host snails of *Schistosoma mansoni*, in Brazil. Geospat Health 6, S95–101
30. Abou-El-Naga, I.F. et al (2015) Impact of the age of *Biomphalaria alexandrina* snails on *Schistosoma mansoni* transmission: modulation of the genetic outcome and the internal defence system of the snail. Mem Inst Oswaldo Cruz 110, 585–595
31. McCreesh, N., Nikulin, G., Booth, M. (2015) Predicting the effects of climate change on *Schistosoma mansoni* transmission in eastern Africa. Parasit Vectors 8, 4
32. Zein-Eddine, R. et al (2014) Phylogeny of seven *Bulinus* species originating from endemic areas in three African countries, in relation to the human blood fluke *Schistosoma haematobium*. BMC Evol Biol 14, 271
33. Jørgensen, A. et al (2007) Molecular phylogenetic investigations of *Bulinus* (Gastropoda: Planorbidae) in Lake Malawi with comments on the topological incongruence between DNA loci. Zoologica Scripta 36, 577
34. Faro, M.J. et al (2013) Biological, biochemical and histopathological features related to parasitic castration of *Biomphalaria glabrata* infected by *Schistosoma mansoni*. Exp Parasitol 134, 228–234
35. Alberto-Silva, A.C. et al (2015) Changes in the locomotory and reproductive behavior of *Biomphalaria glabrata* infected with *Schistosoma mansoni*. Exp Parasitol 153, 68-74
36. Brant, S.V., Loker, E.S. (2013) Discovery-based studies of schistosome diversity stimulate new hypotheses about parasite biology. Trends Parasitol 29, 449–459
37. Adema, C.M., Loker, E.S. (2015). Digenean-gastropod host associations inform on aspects of specific immunity in snails. Dev Comp Immunol 48, 275–283
38. Negrão-Corrêa, D. et al (2012) Interaction of *Schistosoma mansoni* sporocysts and hemocytes of *Biomphalaria*. J Parasitol Res 2012, 743920
39. Sorensen, R.E., Minchella, D.J. (2001) Snail-trematode life history interactions: past trends and future directions. Parasitology 123, S3–18
40. Lafferty, K.D., Kuris, A.M. (2009) Parasitic castration: the evolution and ecology of body snatchers. Trends Parasitol 25, 564–572
41. Théron, A., Moné, H., Gérard, C. (1992) Spatial and energy compromise between host and parasite: the *Biomphalaria glabrata-Schistosoma mansoni* system. Int J Parasitol. 1992 Feb;22(1):91-4.
42. de Jong-Brink, M. et al (1992) The anti-gonadotropic neuropeptide schistosomin interferes with peripheral and central neuroendocrine mechanisms involved in the regulation of

reproduction and growth in the schistosome-infected snail *Lymnaea stagnalis*. Prog Brain Res 92, 385-396
43. Zhang, S.M. et al (2009) Schistosomin from the snail *Biomphalaria glabrata*: expression studies suggest no involvement in trematode-mediated castration. Mol Biochem Parasitol 165, 79–86
44. Lv, S. et al (2009) Invasive snails and an emerging infectious disease: results from the first national survey on *Angiostrongylus cantonensis* in China. PLoS Negl Trop Dis 3, e368
45. Wang, Q.P. et al (2012) Human *Angiostrongylus cantonensis*: an update. Eur J Clin Microbiol Infect Dis 31, 389–395
46. Yong, H.S. et al (2015) Molecular phylogeography of *Angiostrongylus cantonensis* (Nematoda: Angiostrongylidae) and genetic relationships with congeners using cytochrome b gene marker. Acta Trop 148, 66-71
47. Graeff-Teixeira, C., Silva, A.C.A., Yoshimura, K. (2009) Update on eosinophilic meningoencephalitis and its clinical relevance. Clin Microbiol Rev 22, 322–348
48. Chan, D. et al (2015) The Prevalence of *Angiostrongylus cantonensis*/*mackerrasae* Complex in Molluscs from the Sydney Region. PLoS One 10, e0128128
49. Iwanowicz, D.D. et al (2015) Spread of the Rat Lungworm (*Angiostrongylus cantonensis*) in Giant African Land Snails (*Lissachatina fulica*) in Florida, USA. J Wildl Dis 51, 749-753
50. Martin-Alonso, A. et al (2015) Intermediate hosts of *Angiostrongylus cantonensis* in Tenerife, Spain. PLoS One 10, e0120686
51. Stockdale-Walden, H.D. et al (2015) *Angiostrongylus cantonensis* in Introduced Gastropods in Southern Florida. J Parasitol. 2015 Apr;101(2):156-9. doi: 10.1645/14-553.1. Epub 2015 Jan 7.
52. Wang, Q.P., Chen, X.G., Lun, Z.R. (2007) Invasive freshwater snail, China. Emerg Infect Dis 13, 1119–1120
53. Harris, K.R., Cheng, T.C. (1975). The encapsulation process in *Biomphalaria glabrata* experimentally infected with the metastrongylid *Angiostrongylus cantonensis*: light microscopy. Int J Parasitol 5, 521–528
54. Tunholi-Alves, V.M. et al (2014) Activation of anaerobic metabolism in *Biomphalaria glabrata* (Mollusca: Gastropoda) experimentally infected by *Angiostrongylus cantonensis* (Nematoda, Metastrongylidae) by high-performance liquid chromatography. Parasitol Int 63, 64-68
55. Tunholi-Alves, V.M. et al (2012) Effects of infection by larvae of *Angiostrongylus cantonensis* (Nematoda, Metastrongylidae) on the metabolism of the experimental intermediate host *Biomphalaria glabrata*. Exp Parasitol 131, 143–147
56. dos Santos Bonfim, T.C. et al (2014) Biochemical and histopathological alterations in *Biomphalaria glabrata* due to co-infection by *Angiostrongylus cantonensis* and *Echinostoma paraensei*. J Invertebr Pathol 115, 80–85
57. Cantacessi, C. et al (2013) Coming out of the shell: building the molecular infrastructure for research on parasite-harbouring snails. PLoS Negl Trop Dis 7, e2284
58. Giannelli, A. et al (2015) Release of lungworm larvae from snails in the environment: potential for alternative transmission pathways. PloS Negl Trop Dis 9, e0003722
59. Colella, V. et al (2015) Feline lungworms unlock a novel mode of parasite transmission. Sci Rep 5, 13105
60. Yoshino, T.P., Bickham, U., Bayne, C.J. (2013) Molluscan cells in culture: primary cell cultures and cell lines. Can J Zool 91, 6
61. Berriman, M. et al (2009) The genome of the blood fluke *Schistosoma mansoni*. Nature 460, 352–358
62. Young, N.D. et al (2012) Whole-genome sequence of *Schistosoma haematobium*. Nat Genet 44, 221–225

63. *Schistosoma japonicum* Genome Sequencing and Functional Analysis Consortium (2009) The *Schistosoma japonicum* genome reveals features of host-parasite interplay. Nature 460, 345-351
64. Lin, R.J. et al (2013) *Angiostrongylus cantonensis* (Nematode: Metastrongiloidea): in vitro cultivation of infective third-stage larvae to fourth-stage larvae. PLoS One 8, e72084
65. Rollinson, D. et al (2013) Time to set the agenda for schistosomiasis elimination. Acta Trop 128, 423–440
66. King, C.H., Bertsch, D. (2015) Historical perspective: snail control to prevent schistosomiasis. PLoS Negl Trop Dis 9, e0003657
67. World Health Assembly (2012) Elimination of schistosomiasis. Sixty-fifth World Health Assembly. Agenda item 13.11. WHA 65.21. May 26, 2012.
68. Coelho, P.M. et al (2008) Transmission control of schistosomiasis mansoni by introduction of a resistant strain of *Biomphalaria tenagophila* in areas where transmission is maintained by this species. Acta Trop 108, 245–248
69. Richards, C.S. (1970) Genetics of a molluscan vector of Schistosomiasis. Nature 227, 806-810
70. Berg, C.O. (1973) Biological control of snail-borne diseases: a review. Exp Parasitol 33, 318-330
71. Chernin, E., Michelson, E.H., Augustine, D.L. (1960) *Daubaylia potomaca*, a nematode parasite of *Helisoma trivolvis*, transmissible to *Australorbis glabratus*. J Parasitol 46, 599-607
72. Zimmermann, M.R., Luth, K.E., Esch, G.W. (2011) The unusual life cycle of *Daubaylia potomaca*, a nematode parasite of *Helisoma anceps*. J Parasitol 97, 430-434
73. Duval, D. et al (2015) A novel bacterial pathogen of *Biomphalaria glabrata*: a potential weapon for schistosomiasis control? PLoS Negl Trop Dis 9, e0003489
74. Sokolow, S.H. et al (2015) Reduced transmission of human schistosomiasis after restoration of a native river prawn that preys on the snail intermediate host. Proc Natl Acad Sci USA 112, 9650–9655
75. Donaghy, L. et al (2010) First characterisation of the populations and immune-related activities of hemocytes from two edible gastropod species, the disk abalone, *Haliotis discus discus* and the spiny top shell, *Turbo cornutus*. Fish Shellfish Immunol 28, 87e97.
76. Hanington, P.C., Zhang, S.M. (2010) The primary role of fibrinogen-related proteins in invertebrates is defense, not coagulation. J Innate Immun 3, 17–27
77. Hanington, P.C., Forys, M.A., Loker, E.S. (2012) A somatically diversified defense factor, FREP3, is a determinant of snail resistance to schistosome infection. PLoS Negl Trop Dis 6, e1591
78. Baeza Garcia, A. et al (2010) Involvement of the cytokine MIF in the snail host immune response to the parasite *Schistosoma mansoni*. PLoS Pathog 6, e1001115
79. Galinier, R. et al (2013) Biomphalysin, a new β pore-forming toxin involved in *Biomphalaria glabrata* immune defense against *Schistosoma mansoni*. PLoS Pathog 9, e1003216
80. Knight, M. et al (2015) Susceptibility of Snails to Infection with Schistosomes is influenced by Temperature and Expression of Heat Shock Proteins. Epidemiology (Sunnyvale), 5 pii: 189. Epub 2015 Jun 21.
81. Tennessen, J.A. et al (2015) Hyperdiverse gene cluster in snail host conveys resistance to human schistosome parasites. PLoS Genet 11, e1005067
82. Théron, A., Coustau, C. (2005) Are *Biomphalaria* snails resistant to *Schistosoma mansoni*? J Helminthol 79, 187-191

83. Marques, D.P. et al (2014) Reduced susceptibility of a *Biomphalaria tenagophila* population to *Schistosoma mansoni* after introducing the resistant Taim/RS strain of *B. tenagophila* into Herivelton Martins stream. PLoS One 9, e99573
84. Richards, C.S., Knight, M., Lewis, F.A. (1992) Genetics of *Biomphalaria glabrata* and its effect on the outcome of *Schistosoma mansoni* infection. Parasitol Today 8, 171-174.
85. Lie, K.J., Heyneman, D., Richards, C.S. (1979) Specificity of natural resistance to trematode infections in *Biomphalaria glabrata*. Int J Parasitol 9, 529-531

Glossary

Bilharziasis: synonymous of schistosomiasis, named after Theodor Bilharz (1825–1962), German parasitologist who described in 1851 the adult worms of *Schistosoma haematobium* during the autopsy of an Egyptian patient with a clinical history of haematuria.

Dead-end host: a host from which the parasite is not transmitted to other susceptible hosts, thus blocking the parasite life cycle.

DALY: the disability-adjusted life year measures the overall disease burden, expressed as the number of healthy years lost due to ill-health, disability or early death.

Intermediate host: a fundamental host for the parasite life cycle that supports the immature or asexual developmental stage of a parasite.

Lentic and lotic habitat: lentic refers to an aquatic ecosystem featured by stationary or still water, including lakes, wetlands or ponds, whereas lotic involves flowing terrestrial waters, such as rivers, streams, or springs, featured by unidirectional flow and continuous physical change.

Metastrongyloidea: superfamily ranked into the order Strongylida, includes the so-called lungworms of vertebrates. Metastrongylids show a wide range of definitive anatomical localization, ranging from the pulmonary arteries and right ventricle to the mesenteric veins and the bronchioles of the lung. All first-stage larvae pass through the gastrointestinal tract, before being shed in the faeces. Most of species display an indirect life cycle, which requires the presence of gastropods as intermediate hosts, and some species may also use paratenic hosts.

Paratenic host: a host that may be important for the maintenance of a parasite life cycle and in which no dramatic development of the parasite occurs.

572 **Figure 1. Intramolluscan cycle of schistosomes.** Miracidia of infect gastropods (1), developing
573 into a primary or mother sporocyst (2), which generates secondary or daughter sporocysts after 2-3
574 weeks (3). The latter stage migrates to the digestive glands or the hepatopancreas of the mollusc,
575 where its germinative cells give birth to furcocercous cercariae (4). Drawing by Viviana Domenica
576 Tarallo.
577
578 **Figure 2. Life cycle of the cat lungworm *Aelurostrongylus abstrusus* with new insights on its**
579 **biology in the gastropod intermediate host.** Infected cats shed L1 larvae through their faeces (1)
580 that may be ingested by susceptible gastropod intermediate hosts or may penetrate through the snail
581 tegument. Within the host tissues, L1 moult to L3 (2). Infected gastropods may be ingested by a
582 new felid host (3) or by a range of paratenic hosts (e.g., rodents, birds, lizards) (4-5), thus closing
583 the biological life cycle when predated by cats (6). Alternatively, metastrongylid L3 can be released
584 with the snail mucous trails (7), potentially contaminating the cat food (8) or infecting other
585 intermediate hosts (also referred as to intermediesis, 9), thus broadening the number of gastropod
586 hosts available to the paratenic and definitive hosts. Drawing by Viviana Domenica Tarallo.
587

588 **Table 1. Summary of our current understanding on gastropod-borne nematodes.**

What we know	**What we do not know**
Gastropod species involved in the transmission of *A. cantonensis.*	Gastropod species to be used as reference model for *A. cantonensis* studies, especially land snails.
Epidemiology of *A. cantonensis* .	Epidemiology of metastrongylids of pets.
Life cycle of *A. cantonensis* in the intermediate host.	Snail-parasite relationships.
Biochemical alterations induced by *A. cantonensis* in gastropods.	Immunological reactions of gastropod following metastrongylid infection.
Possibility of co-infection with more than one nematode species.	Effect of co-infections on helminth transmission and snail survival.
Zoonotic role of rat lungworms.	Zoonotic role of pet lungworms (e.g., *Angiostrongylus vasorum*)
Availability of a draft genome sequence for *B. glabrata*	Large scale sequence datasets for lungworm-transmitting snails.

589