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

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

31 **Gastropods, parasites and vertebrates**

32 The Mollusca, one of the largest phyla of living creatures, includes gastropod species able to
33 colonise every humid corner of the planet [1]. Given their adaptability to a range of diverse
34 ecosystems, molluscs have been long known to serve as ideal hosts for a number of parasites,
35 including nematodes and trematodes [2]. Indeed, gastropods act as intermediate hosts for a range of
36 helminth parasites of medical and veterinary concern [2,3], including more than 18,000 digenean
37 trematodes and about 50 roundworm species ranked into the superfamily Metastrongyloidea [4,5].
38 Currently, diseases caused by gastropod-borne helminths (GBHs) are estimated to affect more than
39 300 million people worldwide (<http://www.who.int/mediacentre/factsheets/fs115/en>). Some of these
40 GBHs, such as the zoonotic liver flukes *Fasciola hepatica* and *Fasciola gigantica*, significantly
41 affect the livestock industry [6], while others (e.g., *Angiostrongylus vasorum* and *Aelurostrongylus*
42 *abstrusus*) have long been in the spotlight as causes of significant concern for companion animal
43 health [7,8]. In spite of major global efforts to control GBHs, many of these diseases are still
44 endemic in vast areas of the world (<http://www.who.int/mediacentre/factsheets/fs115/en>).
45 Therefore, there is a constant need to discover novel strategies to effectively reduce the burden of
46 disease caused by these parasites, in both humans and animals. The development of adequate
47 control strategies against any disease heavily relies on a thorough understanding of the pathogen
48 biology, ecology and epidemiology. In the case of parasites with indirect life cycles, this includes a
49 profound knowledge of the intermediate hosts. Accordingly, for GBHs, current and future efforts
50 aimed at controlling the diseases they cause must take into account measures to reduce the burden
51 of infections in snails [2,9].

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

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

70 **A snail for each schistosome**

71 Recent estimates provided by the World Health Organization (WHO) indicate that, in 2013, at least
72 260 million people required preventative treatment for schistosomiasis
73 (<http://www.who.int/mediacentre/factsheets/fs115/en>), which translated into losses estimated at
74 ~3.3 million disability-adjusted life years (DALYs) [19]. Schistosomiasis, also known as
75 bilharziasis (see Glossary), is endemic amongst poor communities of tropical and subtropical areas,
76 where sanitation conditions are below standards and snail intermediate hosts are endemic [20].
77 Most human infections are caused by *Schistosoma mansoni*, *S. haematobium*, or *S. japonicum*, with
78 the latter known to infect 46 species of animals, which therefore serve as reservoir hosts for human
79 infections [21]. The distribution of these species of *Schistosoma* overlaps that of the snail
80 intermediate hosts; *S. mansoni*, transmitted by aquatic snails of the genus *Biomphalaria*, is
81 estimated to infect >80 million people, mainly in the sub-Saharan Africa, isolated Middle East
82 areas, southern America and the Caribbean, whereas *S. haematobium*, transmitted by *Bulinus*
83 freshwater planorbids, is widespread throughout sub-Saharan Africa and the Eastern Mediterranean
84 countries, where it affects >110 million people [22]. Conversely, the distribution of *Oncomelania*
85 snails, the intermediate hosts of *S. japonicum*, is limited to Southeast Asia and China, where ~1.8
86 million people are infected by this flatworm [23,24].

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

98 Conversely, the control of schistosomiasis japonica in China, the Philippines and Sulawesi Island is
99 challenged by the resistance to silting and amphibious nature of *Oncomelania hupensis* snails,
100 which may inhabit ditches, wetlands and marshy ground in both hilly and mountains regions [24].
101 Similarly, *Biomphalaria* snails, responsible for transmission of *S. mansoni* in the Caribbean, South
102 America, Egypt and sub-Saharan Africa [29-31] are adapted to a large number of ecosystems (e.g.,
103 lakes, fish ponds and rice fields) and benefit from the presence of controlled water flows, dams and
104 irrigation networks [30]; in addition, planorbids of the genus *Bulinus* are known to successfully
105 breed in a range of environmental conditions and can be detected in several regions of Africa (i.e.,
106 the Nile River valley, Mahgreb, most of southern and sub-Saharan Africa, including Madagascar)
107 [32], and the Middle East (e.g., Iran, Malawi, Sudan) [33], where they are efficient intermediate
108 hosts of *S. haematobium*.

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

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116 **Gastropods and trematodes**

117 The majority of studies performed to explore snail-parasite relationships have been focused on
118 schistosomes and their intermediate hosts, primarily because of the availability of experimental
119 systems that allow maintenance of these parasites in several species of molluscs in the laboratory
120 [9]. As a consequence, a plethora of information has been collected over the last few years on the
121 biological and molecular interactions occurring between schistosome parasites and their gastropod

122 intermediate hosts (**Box 1**) [34-37], including the intramolluscan life cycle of those flatworms.
123 Miracidia of *S. mansoni* infect *Biomphalaria* gastropods through the exposed mantle epithelium,
124 and frequently through the antennae or the head-foot. In the fibromuscular tissue of the
125 cephalopodal region, the parasite undergoes morphological and physiological changes, developing
126 into a primary or mother sporocyst; this stage generates several secondary or daughter sporocysts,
127 which migrate to the digestive glands or the hepatopancreas of the mollusc. Finally germinative
128 cells of the daughter sporocyst produce water-living furcocercous cercariae (**Figure 1**) [38].
129 Infections by trematodes inevitably impact on snail longevity and fitness, with variable outcomes
130 [39]. For instance, accelerated shell growth or gigantism has been observed in *Lymnaea* snails
131 infected by plagiorchids, whereas retarded development or stunting has been recorded in
132 *Biomphalaria* planorbids exposed to *S. mansoni* [39]. The occurrence of these changes is often
133 associated with impairment of the snail fecundity, an event also referred to as parasitic castration
134 [40], during which the trematode gradually redirects the host metabolism towards its own needs
135 [41]. Although the exact mechanisms that lead to the snail castration are currently unknown, it has
136 been suggested that the parasite may act as a ‘competitor’ for nutrients required for reproduction
137 (e.g., vitelline glands), or may directly interfere with selected physiological processes of the
138 gastropod [41]. For instance, preliminary studies in the *Lymnea stagnalis-Trichobilharzia ocellata*
139 system had pointed towards a role of schistosomin, a host-derived host factor, in the occurrence of
140 parasitic castration [42]; however, recent data indicate that changes in expression levels of this
141 neuropeptide in *B. glabrata* snails are not directly linked to active development of schistosomes
142 [43].
143 Clearly, the availability of basic parasitological information on snails-schistosome interactions has
144 assisted scientists in the acquisition of a better understanding of GBHs epidemiology. However,
145 substantial gaps still exist in our knowledge of the cascade of molecular events that regulate the
146 development of nematodes within their mollusc intermediate hosts. Such gaps are particularly
147 pronounced for snail hosts of metastrongylid parasites of humans and animals, as illustrated in the
148 following section.

149

150 **Zoonotic *Angiostrongylus* infection: the state of the art**

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

173 *Angiostrongylus* infection worldwide. For instance, molluscs consumed as food or kept as domestic
174 pets (e.g., *Achatina fulica*), along with the expansion of the distribution range of some invasive
175 species (e.g., *Pomacea canaliculata*) are considered key determinants of the increasing prevalence
176 of infections by *A. cantonensis* in Mainland China and South America [52]. Nonetheless, the
177 detection of this roundworm in European native gastropods (e.g., *Cornu aspersum* or *Theba pisana*)
178 [50], together with the availability of predictive models based on climatic factors suggesting an
179 increase in suitable habitats for this pathogen in Europe [12], is worrisome. For this reason,
180 preventative measures should be developed to face a potential introduction of rat definitive hosts to
181 non-endemic areas. To achieve these goals and accurately identify risk factors for disease
182 transmission in Europe, comprehensive investigations of the complex interactions occurring at the
183 gastropod-angiostrongylid interface are needed.

184 185 **Delving into the great unknown: zoonotic metastrongylid-snail interactions**

186 Thus far, knowledge of snail-*Angiostrongylus* interactions is limited to reports dating back to the
187 '70s and '80s. For instance, the detection of *A. cantonensis* larvae in the kidney and rectum of *B.*
188 *glabrata*, suggested that these might represent the main routes of larval migration to the snail
189 mantle collar and head-foot, i.e. the 'exit doors' to the outer environment [53]. In the same study,
190 angiostrongylid larvae were encapsulated by the snail 24-48 hours post-infection following a two-
191 phase process, consisting of an initial infiltration and aggregation of basophilic haemolymph cells
192 around the parasite, followed by encasement in fibrous-appearing nodules [53]. As a likely
193 consequence of the increasing interest towards GBHs [2], recent studies have focussed on the
194 metabolic responses of gastropods infected by *A. cantonensis*. For instance, the energetic balance
195 and oxidative metabolism of *B. glabrata* infected by *A. cantonensis* under experimental conditions,
196 displayed a sharp decline in glucose content immediately following infection, likely as a
197 consequence of the competition for nutrients between the nematode and the snail which, in turn, is
198 forced to activate its anaerobic metabolism (i.e., increased activity of enzymes involved in the
199 glycolytic pathway mediated by lactate dehydrogenase), in order to survive [54]. The
200 angiostrongylid also promote the protein metabolism of snails, as demonstrated by the increased
201 production of nitrogen catabolites such as urea, and particularly the conversion of uricotelic into
202 ureotelic acid, probably as a detoxification strategy, thus favouring the vital functions of the snail
203 and, indirectly, parasite development [55]. Similarly, co-infections by *Echinostoma paraensei*, a
204 trematode of wild rodents, and *A. cantonensis* [56] trigger a progressive depletion of the
205 carbohydrates reserves in *B. glabrata* snails, which, in turn, increase the rate of deamination of
206 amino acids. Moreover, the enhanced demand for nutrients by the parasites modifies the kinetic
207 behaviour of *A. cantonensis* and *E. paraensei* in the gastropod tissues, inducing the former to pursue
208 new migration routes [56]. Indeed, the intense cellular disorganization induced by *E. paraensei* in
209 the digestive gland-gonad complex of the snail (i.e., the site for *A. cantonensis* moulting) forces the
210 nematode to continue its development into the kidneys [56]. Further immune-molecular studies on
211 the fundamental *Angiostrongylus*-gastropod interplay are crucial to implement strategies for the
212 control of the infection. Even though a suitable experimental snail model is currently unavailable,
213 such a system would provide a ready-to-use infrastructure for in-depth studies of biological
214 pathways specifically involved in snail-parasite interactions, as recently proposed for the *Bithynia-*
215 *Opisthorchis* complex [57]. Until that, research on phylogenetically-related animal parasites (e.g.,
216 *A. vasorum* or *A. abstrusus*) [46] may represent a useful way to overcome these gaps, thus opening
217 new opportunities for a thorough investigation on GBHs of medical and veterinary concern.

218 219 **Opening new fields in GBHs research**

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

224 cat lungworm *A. abstrusus* among gastropods. Indeed, after being shed in the mucus trails of the
225 land snails or in the water where gastropods had died [58], L3s of this metastrongylid are able to
226 infect new intermediate hosts, in a mechanism referred to as intermediation. This phenomenon may
227 represent a dynamic survival-and-transmission strategy for nematodes, allowing spread of parasites
228 to other susceptible intermediate hosts [59]. While it is tempting to speculate on the potential
229 advantages that intermediation may present for spreading and survival of snail-borne nematodes, a
230 clear understanding of this phenomenon can only be achieved via in depth studies of snail-
231 nematode interactions. Indeed, while a plethora of information is available on the molecular and
232 immunological interactions occurring at the snail-schistosome interface (mainly as a consequence
233 of the availability of suitable experimental systems, including a draft genome sequence for *B.*
234 *glabrata* <http://www.vectorbase.org>), its embryonic cell lines (Bge) [60], and schistosome genomes
235 [61-63], studies of the immune-molecular mechanisms that govern the snail-metastrongylid
236 interplay are minimal (**Table 1**). Beside a single attempt to cultivate *A. cantonensis* from L3s to
237 fourth-stage larvae in a defined culture medium [64], the development of metastrongylids *in vitro*
238 is still an unexplored field; progress in this area is required to provide essential information on the
239 physiology of helminths, as well as for the advancement of parasitological and biomedical research
240 on GBHs. Therefore, an improved knowledge of snail-parasite interactions will not only result in a
241 better understanding of the ecology, epidemiology and basic biology of GBHs, but will also
242 represent the necessary infrastructure for hypothesis-driven studies aimed at interrupting the
243 spreading of the diseases they cause.

244

245 **Biological control of snails: a feasible option?**

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

275 However, caution is required when hypothesising the use of snail predators (e.g., other snails or
276 prawns) for the control of certain GBHs, as they may serve as hosts for lung flukes (e.g.,
277 *Paragonimus* spp.), lungworms (*Angiostrongylus* spp.) and other foodborne helminths (*Clonorchis*
278 and *Opisthorchis* spp.) [70]. Fly larvae of the genus *Sciomyzidae* are strictly malacophagous and
279 harmless to humans and vertebrates; however, their use is made impractical by the different degrees
280 of vulnerability displayed by snails [70]. Finally, trematode predators, including annellids of the
281 genus *Chaetogaster*, mosquito larvae and Hydrozoa, may provide effective means to reduce the
282 burden of GBHs. Also, guppies (*Lebistes reticulatus*) are highly cercariophagous, with up to 1000
283 cercariae ingested per hour, but only in cases of densely established fish populations [70]. These
284 key examples suggest that, today, the control of GBHs cannot solely rely on the use of biological
285 agents, mainly because of the unfeasibility of such approaches on a global scale. Therefore regular
286 administrations of praziquantel remain the most effective strategy to reduce the burden of
287 schistosomiasis in exposed populations [65].
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289

290 **Concluding remarks**

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

299 In conclusion, further data on snail-parasite biology is needed to enhance our knowledge of host-
300 parasite interactions, and ultimately to provide new potential tools for GBH control. In particular,
301 the application of so called -omic technologies (e.g. genomics, transcriptomics, proteomics,
302 metabolomics) to large-scale explorations of snail-nematode interactions is bound to accelerate this
303 progress, ultimately leading to the development of integrated strategies of GBHs control, based on
304 extensive knowledge of snail biology and immunology. Altogether, these efforts will be
305 determinant in the near future to identify the parasite 'Achille's heel', thus translating these
306 fundamental discoveries into potential control measures.
307

308 **Box 1. Snail immunobiology and resistance to schistosome infection**

309 Gastropods can activate mechanisms of innate immunity to cope with *Schistosoma* development
310 [17,75], modulated by the activity of the mollusc internal defence system (IDS). The snail IDS
311 includes two populations of haemocyte effector cells (granulocytes and hyalinocytes) [75] and a
312 range of soluble factors in the hemolymph, which are involved in pathogen recognition and
313 inflammatory responses [17]. Among these molecules, fibrinogen-related proteins (FREPs) are of
314 major interest to the scientific community, due to their conserved structure throughout animal
315 evolution; in addition, gastropod FREPs are the only known proteins, which combine
316 immunoglobulin superfamily domains (IgSF) with their fibrinogen domain [18,76]. Fourteen
317 subfamilies of FREPs have been described thus far, and eight more have been detected using RNA-
318 seq (reviewed by [18]). Some of these molecules (i.e., FREP3) may act as opsonins [77] for a
319 number of monosaccharide and mucin antigens expressed on the surface of schistosome larval
320 stages [18]. In addition to FREPs, several cytokines, such as the macrophage migration inhibitory
321 factor [78], and other proteins (e.g., biomphalysin) [79] have been hypothesized to be involved in
322 the immune defence of *B. glabrata* against schistosomes. Once stimulated by the presence of
323 miracidia, the haemocytes undergo enhanced mitosis within 24-72 hours, and produce reactive
324 oxygen species (ROS), including hydrogen peroxide and hypochlorous acid, or phagocytise
325 portions of *Schistosoma* tegument, leading to mechanical damage and destruction of the invader
326 [17]. The expression of immunological factors that regulate snail resistance to trematode infection
327 (e.g., production of FREPs, cathepsins, actins, heat-shock proteins) varies according to snail
328 species, strain and age [80]. Studies on snail immunology [16-18], along with new insights on snail
329 gene regulation and expression [81], are essential for elucidating the interactions between GBHs
330 and their intermediate hosts, including those that result in unsuccessful infections. For instance, the
331 *Biomphalaria tenagophila* Taim/RS strain is resistant to *S. mansoni* challenge, due to the clustering
332 of haemocytes in a thick layer which surrounds, encapsulates and destroys the *S. mansoni*
333 miracidium, soon after its penetration [82,83]. Considering that the resistance factor is transmitted
334 as a dominant character with Mendelian inheritance [69,84,85], the identification of other genes
335 linked to schistosome resistance (e.g., the guadeloupe resistance complex [GRC] in *B. glabrata*)
336 and the genetic manipulation of snails might pave the way towards the insertion of such genes in
337 susceptible gastropod populations [81]. Altogether, this approach could potentially help reducing
338 the burden of infection in endemic areas of disease.

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549 **Glossary**

550

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

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

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

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

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

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

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

571

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 intermediates, 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 <i>A. cantonensis</i> .	Gastropod species to be used as reference model for <i>A. cantonensis</i> studies, especially land snails.
Epidemiology of <i>A. cantonensis</i> .	Epidemiology of metastrongylids of pets.
Life cycle of <i>A. cantonensis</i> in the intermediate host.	Snail-parasite relationships.
Biochemical alterations induced by <i>A. cantonensis</i> 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., <i>Angiostrongylus vasorum</i>)
Availability of a draft genome sequence for <i>B. glabrata</i>	Large scale sequence datasets for lungworm-transmitting snails.

589