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1 **Recovery of primary sporocysts in vivo in the *Schistosoma mansoni* / *Biomphalaria***
2 ***glabrata* model using a simple fixation method suitable for extraction of genomic DNA**
3 **and RNA.**

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22

23 **Abstract**

24 Detailed studies of host/parasite interactions are currently limited because *in-situ* gene
25 sequencing or monitoring of parasite gene expression is so far limited to genes presenting a
26 high loci copy number in the Schistosoma genome or a high level of expression. Indeed, how
27 to investigate the host parasite molecular interplay when parasites are not directly accessible
28 in vivo? Here we describe a method to circumvent this problem and to analyze DNA and
29 RNA of *Schistosoma mansoni* during the interaction with its intermediate snail host
30 *Biomphalaria glabrata*. We propose a technique for improved DNA and RNA extraction
31 from the intra-molluscan stage of the parasite recovered after fixation of infected snails in
32 Raillet-Henry solution. The extractions can be used for genetic analysis, transcription studies
33 and microsatellite genotyping.

34

35 **Keywords:** *Schistosoma mansoni*, *Biomphalaria glabrata*, intramolluscan stage, DNA/RNA
36 extraction.

37

38

39 1. Introduction

40 Schistosomiasis or bilharzia is a tropical parasitic disease affecting 200 million humans in
41 74 countries, causing 200,000 deaths annually (WHO, 2002). It is the second most important
42 tropical disease in terms of morbidity after malaria. Schistosomiasis is caused by flatworms of
43 the genus *Schistosoma* (Platyhelminth, Digenea) (Chitsulo, et al., 2004, Gryseels, et al.,
44 2006). The cycle of the parasite is complex. It requires humans as definitive hosts and
45 freshwater snail species as intermediate hosts. Infection occurs in water by the free-living
46 larval stages (cercaria for the definitive host and miracidia for the intermediate host).
47 Significant attention has been paid to freshwater snails in the past because of both their
48 medical and epidemiological importance as intermediate hosts for schistosome parasites.
49 Moreover, the interaction between *Schistosoma mansoni* and the snail *Biomphalaria glabrata*
50 provides a model of choice in evolutionary biology to investigate the host-parasite co-
51 evolutionary dynamics but also invertebrate immune response (Baeza Garcia, et al., 2010,
52 Lockyer, et al., 2008, Mone, et al., 2010, Roger, et al., 2008). While snail response to
53 infection can be readily studied particularly at the molecular level (Adema, et al., 2010, Baeza
54 Garcia, et al., 2010, Guillou, et al., 2007, Hanington, et al., 2010) it is not easy for the *S.*
55 *mansoni* intramolluscan stage parasite that is not directly accessible. To avoid this problem, *in*
56 *vitro* culture is often used in this model to obtain pure parasites and/or secretion products to
57 identify the molecules involved in host-parasite interplay (Bender, et al., 2002, Coppin, et al.,
58 2003, Guillou, et al., 2007, Roger, et al., 2008), to perform pharmacological investigations
59 (Mattos, et al., 2006), or to study parasite development (Azzi, et al., 2009). However,
60 miracidia to sporocyst *in vitro* transformation can be achieved only for a short period of time.
61 For a longer period an artificial snail environment must be used involving sporocysts co-
62 cultured with *Biomphalaria glabrata* embryonic cells (Bge cell line) (Coustau and Yoshino,
63 2000, Taft, et al., 2009, Vermeire, et al., 2004). These approaches has been used with great

64 success in the past, however, the *in-vivo* response of the parasite to the authentic host snail
65 environment was never investigated.

66 Here we describe a new efficient method for improved DNA and RNA extraction from the
67 *S. mansoni* intra molluscan stage. We show that the method can be used for PCR
68 amplification, analysis of gene expression and microsatellite genotyping approaches.

69

70 **2. Material and Methods**

71 *2.1. Biological material, Raillet-Henry fixation and parasite recovery*

72 *Schistosoma mansoni* (Guadeloupean strain) was maintained in its sympatric *Biomphalaria*
73 *glabrata* strain and in hamsters (*Mesocricetus auratus*), as described previously (Théron, et
74 al., 1997). Miracidia were hatched from eggs recovered from 60-day-infected hamster livers.
75 The livers were homogenized and the eggs were filtered out, washed, and transferred to spring
76 water. The miracidia were allowed to hatch under exposure to artificial light. For each
77 experiment, snails (7-9 mm in diameter) were exposed individually to 20 miracidia. For
78 primary sporocysts (SpI) detection and recovery, the snails were fixed 15 days post-exposure,
79 according to a modified method, previously described (Mone, et al., 2010, Theron and
80 Gerard, 1994). In brief, each infected snail was relaxed in pond water containing an excess of
81 crystalline menthol for 6 h (menthol relaxation help in shell removing and snail anatomic
82 observation), the snail body was recovered and fixed in modified Raillet-Henry's solution
83 (0.6% NaCl; 2% acetic acid; 2% formalin). After 24 hours in fixative, a dissection of the
84 head-foot zone was performed, and SpIs (Figure 1) were recovered, washed two times in
85 Phosphate Buffer Saline (PBS) for 15 min on ice and kept at -80°C until use.

86 An alternative procedure can be used with a rapid fixation procedure when studying gene
87 transcription. Infected snails were snap-frozen in liquid nitrogen and fixed directly with the

88 shell in modified Raillet-Henry's solution (0.6% NaCl; 2% acetic acid; 2% formalin). After
89 24 hours in fixative, the shell is removed and SpI recovery was performed as above.

90

91 *2.2. DNA and RNA extraction and reverse transcription*

92 Genomic DNA (gDNA) was extracted from single Raillet-Henry fixed *S. mansoni* sporocysts
93 according to the following protocol. Sixty μ l of TE (Tris 10mM; EDTA 1mM; ph 8)
94 containing 1.67mg/ml of Proteinase K (Merck) was added to the SpI. Samples were put 3
95 hours at 55°C and vortexed each 15 min. Then samples were heated 10 min at 100°C for
96 proteinase K inactivation. gDNA were kept at -20°C until use.

97 Total RNA was isolated from single or pool of 5 SpIs using the High Pure FFPE RNA
98 microkit (Roche) according to manufacturer recommendations. Reverse transcription was
99 performed using random hexamer primer and the RevertAid H minus First Strand cDNA
100 Synthesis kit (Fermentas) following the manufacturer's protocol. cDNA were kept at -80°C
101 until use. It as been largely documented that these new extraction methods have been now
102 optimized for RNA extraction from formalin-fixed tissue to achieve high quality microarray
103 and qRT-PCR downstream applications (April, et al., 2009, Ribeiro-Silva, et al., 2007,
104 Serinsoz, et al., 2005).

105

106 *2.3. PCR amplifications of gDNA and cDNA*

107 gDNA PCR amplifications of fixed *S. mansoni* sporocyst were performed with the Advantage
108 2 PCR Enzyme System (Clontech). To test PCR on gDNA as template, the SmPoMuc loci
109 (Roger, et al., 2008, Roger, et al., 2008, Roger, et al., 2008) were amplified using specific
110 primers designed to amplify specific fragment lengths (see Table 1 for primer sequences and
111 PCR cycling conditions). PCR conditions were: 0.5 μ M primers; 1X final Advantage 2 PCR

112 buffer (2mM MgCl₂); 0.4mM dNTPs; 1X final Advantage 2 polymerase and 5μl of gDNA in
113 a total volume of 20μl of RNase, DNase free water.

114 cDNA PCR amplification of fixed *S. mansoni* sporocyst was performed with the GoTaq
115 hotstart Enzyme (Promega). Five genes were tested (see Table 2 for gene name, primer
116 sequences and PCR cycling conditions). PCR mix was: 0.4μM primers; 1.5mM MgCl₂;
117 0.2mM dNTPs, 1 unit GoTaq hot-start and 1μl of cDNA in a total volume of 20μl of RNase,
118 DNase free water. All PCR products were separated by electrophoresis through 1% agarose
119 gels, and visualized by staining with ethidium bromide.

120

121 *2.4. PCR amplification of microsatellite loci*

122 Fifteen microsatellite markers, SmC1, SmDO11, SmDA28 (Curtis, et al., 2001), R95529,
123 SmD57, SmD28, SmD25, SCMSMOXII, L46951 (Durand, et al., 2000), SmBR16, SmBR10,
124 SmBR13 (Rodrigues, et al., 2007), SmS7-1 (Blair, et al., 2001), SmBR1, SmBR6 (Rodrigues,
125 et al., 2002) were used in this study (Table 3). The relevant DNA fragments were amplified
126 using PCR. Details on microsatellite sequences, primers and PCR conditions are available in
127 Table 3. To maximise efficiency and minimize costs, these PCRs were performed in three
128 multiplex reactions using the QIAGEN multiplex kit. The PCR amplifications of loci:
129 R95529, SmC1, SmDO11, SmBR16 and SmD57 were grouped in the multiplex 1 (M1); loci:
130 SmDA28, SmBR1, SmS7-1, SmD28, SCMSMOXII were grouped in the multiplex 2 (M2);
131 and loci: SmD25, L46951, SmBR6, SmBR10 and SmBR13 were grouped in the multiplex 3
132 (M3). These multiplex reactions were carried out according to the manufacturer's standard
133 microsatellite amplification protocol in a final volume of 10μL and with 57°C as annealing
134 temperature. PCR products were diluted in Sample Loading Solution (Beckman Coulter) with
135 red-labelled size standard (CEQ™ DNA size standard kit, 400, Beckman Coulter) and
136 electrophoresis was done on an automatic sequencer (CEQ™ 8000, Beckman Coulter).

137 Microsatellite sizes were determined using the fragment analyzer package of Beckman
138 Coulter. To confirm that the fixation procedure did not modify the microsatellite length and
139 thus did not affect negatively microsatellite analyses, we used a clonal strain of *S. mansoni*
140 (Brazilian strain selected for locus homozygosity) to compare the microsatellite profile
141 between fixed and non-fixed material. These samples were processed as described above.

142

143 *2.5. Ethical Statements*

144 Our laboratory has received the permit # A66040 for experiments on animals from both the
145 French Ministry of Agriculture and Fishing and the French Ministry of National Education,
146 Research and Technology. Housing, breeding and animal care of the hamster followed the
147 ethical requirements of the French government. The experimenter possesses the official
148 certificate for animal experimentation delivered by both ministries (Décret # 87-848 du 19
149 octobre 1987).

150

151

152 **3. Results**

153 *3.1. Sporocysts detection and dissection.*

154 After 24 hours in Raillet-Henry fixation, SpIs are readily observable as translucent white
155 bodies within an opaque grey tissue background (Figure 1A). At higher magnifications we
156 could see the spherical aspect of SpI (Figure 1B and C). Figure 1 shows a snail at 15 DPI.
157 SpIs were at their optimal development (filled with secondary sporocysts (SpIIs)), the biggest
158 reached 1mm in diameter and could be recovered by careful dissection of the snail head-foot
159 zone under a binocular microscope. Dissecting SpIs before 6 DPI remains very difficult. It is
160 important to recover the SpI without taking snail tissue in order to enrich the parasite signal
161 for genomic DNA or transcript RNA extractions.

162

163 3.2. *S. mansoni* sporocysts fixation and genomic DNA amplification

164 We tested the possibility to amplify gDNA of single SpI after Raillet-Henry fixation. PCR
165 products of up to 2 kb in length could be amplified from gDNA (columns 1, 2, 3; Figure 2).
166 At 2.5 and 3 kb amplification did not work anymore or only occasionally for some individuals
167 (see individual of column 3 at 2.5kb, Figure 2). The results were compared to a positive
168 control (classical gDNA extraction) for which PCR amplifications worked for up to 3kb
169 (column 4; Figure 2). To achieve this fragment length specific amplification, we design
170 primers for SmPoMuc genes for *Schistosoma mansoni* polymorphic mucins. These mucins
171 were specific of *S. mansoni* and no related genes were present in the snail host *B. glabrata*,
172 thus no cross-amplifications did occur (data not showed).

173

174 3.3. *S. mansoni* sporocysts fixation and microsatellite amplification

175 Microsatellite PCR on DNA extracted from Raillet-Henry fixed SpIs shown good results
176 (Figure 3). All microsatellite loci were amplified and fragment lengths were in agreement
177 with the expected sizes (see Figure 3 and Table 3) based on previous studies done on DNA
178 extracted from adults of the same parasite strain (Bech, et al., 2010, Durand, et al., 2000). In
179 addition using a clonal strain of *S. mansoni* without microsatellite diversity we showed that
180 microsatellite profiles were the same for fixed and non-fixed material (Table 4). The fixation
181 procedure did not modify the microsatellite length and thus did not affect negatively
182 microsatellite analyses.

183

184 3.4. *S. mansoni* sporocysts fixation and reverse-transcription PCR.

185 Five genes were selected to study the sensitivity of transcript amplification, (i) alpha tubulin
186 is a highly expressed gene involved in microtubules (one of the main components of the

187 cytoskeleton) bio-synthesis, (ii) two genes involved in different metabolic pathways, the
188 superoxide dismutase (SOD) that is part of the antioxidant defence against reactive oxygen
189 species and the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) involved mainly in
190 glycolysis pathway ; (iii) calreticulin is a multifunctional calcium binding protein encoded by
191 a single copy gene in *S. mansoni* genome (Berriman, et al., 2009). Finally (iv) the *S. mansoni*
192 antigen 10-3, a developmentally regulated surface antigen known to be expressed specifically
193 in cercaria, male and female worms, was tested. Transcript amplifications were tested for
194 these five genes after menthol anaesthesia and Raillet-Henry fixation or after snail were snap-
195 frozen in liquid nitrogen and Raillet-Henry fixation and the same results were obtained for
196 both techniques. For the four first tested genes, PCR products could be obtained when 5 SpI
197 were used for cDNA preparation (Column 1, Figure 4). cDNA extracted from a single fixed
198 SpI gave only an amplification for alpha-tubulin (Column 2, Figure 4) probably due to the
199 greater amount of transcript for this highly expressed gene. PCR amplifications were highly
200 specific as no cross amplifications with the intermediate host *B. glabrata* cDNA could be
201 observed (Column 4, Figure 4). All those four genes were known to be expressed in miracidia
202 and sporocysts of *S. mansoni*. Antigen 10-3 known not to be expressed was also tested as an
203 internal control and as expected gave no amplifications (Figure 4).

204

205 **4. Discussion**

206 Identification of *S. mansoni* sporocyst intramolluscan stage using Raillet-Henry fixation is a
207 classical technique used mainly to investigate prevalence and intensity or intramolluscan
208 development of the larval stages in the *S. mansoni* / *B. glabrata* model (Sire, et al., 1998,
209 Théron, et al., 1997, Theron, et al., 1998). However, recovery of these intramolluscan fixed
210 parasite stages for extraction of DNA or RNA was never described. Indeed, study of genetic
211 information in formalin-fixed tissues is often hampered by the impossibility to amplify the

212 desired DNA or RNA as a consequence of nucleic acid damage (Zimmermann, et al., 2008).
213 It is thus important to dissect snails and to recover the parasites after a short period of fixation
214 (less than 24 hours). Studying genomic or transcriptomic information of these fixed
215 intramolluscan stages will be of interest in many fields of research including the molecular
216 dialogue between parasite and the host's immune system, distribution of infrapopulation
217 genotypes or gene expression during development and maturation from SpIs to cercariae. The
218 present work describes an improved technique for DNA and RNA extraction from such
219 samples and delivers proof for their use for the study of genomic DNA, transcript expression
220 or microsatellite genotyping.

221 Raillet-Henry fixation permits to recover SpIs that are more than 6 days old. To isolate SpIs
222 before 6 DPI was quite impossible due to the minute size of these parasites at these steps. The
223 use of this technique to study post-miracidial stage or SpI within the first days of infection
224 remains therefore difficult. This constitutes the main limitation of this approach. However
225 recovery of SpIIs or cercariae is feasible (data not shown). Our extraction protocols are
226 efficient on SpIs but also on all other intramolluscan parasite stages (data not shown).

227 However for gDNA a limited size of PCR amplification exists. Apparently, Raillet-Henry
228 fixation results in gDNA breaks at around 2kb because amplification of fragment of 2.5 kb
229 was difficult and of 3kb was impossible. Fragmentations in DNA extracted from formalin
230 preserved samples has been described before and is based on nicks and double-strand breaks
231 (Zimmermann, et al., 2008). Amplifications of microsatellite fragments of less than 500
232 nucleotides worked very well using our protocols. There is no difference in microsatellite
233 amplification between fixed and non-fixed material. Raillet-Henry fixation did not affect
234 negatively the microsatellite analyses. The technique will allow for genotyping of single
235 parasite intramolluscan stages and thus could permit reconstruction of the population structure
236 of sporocyst infra-populations inside the snail. Finally, RNA extraction and RT-PCR showed

237 good results, cDNA transcript amplification from Raillet-Henry fixed material with or without
238 nitrogen freezing works well. In our model this will facilitate the study of gene expression for
239 all intra-molluscan parasite developmental stages at 6 DPI and older.

240 We hope that the technique developed herein paves the way to a better understanding of the
241 host/parasite molecular dialogue by taking into account more easily an often neglected partner
242 in this interaction, the parasite.

243

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Table 1: Primers used for genomic DNA PCR

Fragment length	Forward primer name	Forward primer sequence	Reverse primer name	Reverse primer sequence	Cycling conditions
1010 bp	Exon6F	TGAAGCTCAACTCAGTAAGCTGAAC	Exon5R	CTTGTATCGCCTTCGATTCCAATTC	Ta: 58°C - elong: 68°C, 3min 30 - 40 cycles
1422 bp	10483ex13.F2	ACGAGGATTAATGATTACAAATATGC	Exon11R	TAGATAATGTACTGCCCACTTTGTG	Ta: 58°C - elong: 68°C, 3min 30 - 40 cycles
1999 bp	InTron14/15.F	CACTTGTTTCATAAACACGTGTCTTC	Exon11R	TAGATAATGTACTGCCCACTTTGTG	Ta: 58°C - elong: 68°C, 3min 30 - 40 cycles
2517 bp	Exon11F	ATTTCTTCTAGAATGTCTGAG	InTron6/7.R	TAAAGGTGGAATATGCCAAACTCAC	Ta: 58°C - elong: 68°C, 3min 30 - 40 cycles
3052 bp	InT3/4-3.3.F	CTATGGACACTATGAACAATATTCG	InTron6/7.R	TAAAGGTGGAATATGCCAAACTCAC	Ta: 58°C - elong: 68°C, 3min 30 - 40 cycles

bp: base pairs

Ta: Primers annealing temperature in centigrade; elong: temperature in centigrade and duration of elongation in minutes.

Table 2: Primers used for cDNA PCR

Gene name	Accession No.	Forward primer	Reverse primer	Cycling conditions
Alpha tubulin	SCMSAT1A	AGCAGTTAAGCGTTGCAGAAATCA	TGACGAGGGTCACATTCACCAT	Ta: 53°C - elong: 72°C, 30sec - 40 cycles
Superoxide dismutase Cu/Zn (SOD)	XM_002580438	AGTGGACTCAAGGCTG	CCACGGCCTAAATCAT	Ta: 54°C - elong: 72°C, 30sec - 40 cycles
Glyceraldehydes-3-phosphate dehydrogenase (GAPDH)	XM_002576947	GCGAGGTTTCGACTGA	AACAACGAACATGGGTG	Ta: 55°C - elong: 72°C, 30sec - 40 cycles
Calreticulin	XM_002574439	ATACGCTCTGGGACAT	CCTTGCTTCTCGGCATTA	Ta: 54°C - elong: 72°C, 30sec - 40 cycles
<i>S.mansoni</i> antigen 10-3 (Ag10-3)	M22346.1	CACAAGGGTCTACTGCTAACGGA	CCTTTAACATGGAATTTATCAGTCTGG	Ta: 54°C - elong: 72°C, 30sec - 40 cycles

Ta: Primer annealing temperature in centigrade; elong: temperature in centigrade and duration of elongation in seconds.
GenBank Accession numbers (No.) are indicated.

Table 3: Primers used for Microsatellite PCR

Multiplex	Microsatellite name	Accession No.	Range size (nt)	Repeat motif	Forward sequence	Reverse sequence	Cycling conditions
M1	R95529	R95529	228/275	(CAT)10	GTGATTGGGGTGATAAAG	CATGTTTCTTCAGTGTCC	Ta: 57°C - elong: 72°C, 1min - 35 cycles
	SmC1	AF325694	287-296	(AAT)6-16	TGACGAGGTGACCATAATTCTAC	AACACAGATAAGAGCGTCATGG	Ta: 57°C - elong: 72°C, 1min - 35 cycles
	SmD57	AF202967	276-300	(TA)22(GA)9	TCCTTGATTCCACTGTTG	GCAGTAATCCGAAAGATTAG	Ta: 57°C - elong: 72°C, 1min - 35 cycles
	SmBR16	L04480	337-341	(TA)10	TGTGACTTTGATGCCACTGA	GGCCTGATACAATTCTCCGA	Ta: 57°C - elong: 72°C, 1min - 35 cycles
	SmDO11	AF325698	303-367	(GATA)20-37	TGTTTAAGTCGTCGGTGCTG	ACCCTGCCAGTTTAGCGTAG	Ta: 57°C - elong: 72°C, 1min - 35 cycles
M2	SmDA28	AF325695	91-115	(GATA)7-14	CATGATCTTAGCTCAGAGAGCC	AGCCAGTATAGCGTTGATCATC	Ta: 57°C - elong: 72°C, 1min - 35 cycles
	SmBR1	L81235	154	(AC)9	GAGTATACGGCTTCTTGGA	CGGAACGACAAGAAAATCAT	Ta: 57°C - elong: 72°C, 1min - 35 cycles
	SmS7-1	AF330105	184	(AC)17	TCCTCCTCTCTATTTTCTCTTTG	ATTACGATTGCACAGATACTTTTG	Ta: 57°C - elong: 72°C, 1min - 35 cycles
	SmD28	AF202966	240-244	(CAA)5	CATCACCATCAATCACTC	TATTCACAGTAGTAGGCG	Ta: 57°C - elong: 72°C, 1min - 35 cycles
	SCMSMOXII	M85305	283-295	(CAT)9CGT(CAT)6	TTCTACAATAATACCATCAAC	TTTTTTCTCACTCATATACAC	Ta: 57°C - elong: 72°C, 1min - 35 cycles
M3	SmBR10	DQ448293	109-133	(GATA)10	CATGATCTTAGCTCAGAGAGC	GTACATTTTATGTCAGTTAGCC	Ta: 57°C - elong: 72°C, 1min - 35 cycles
	L46951	L46951	168-174	(GAA)7	CAAACATATACATTGAATACAG	TGAATTGATGAATGATTGAAG	Ta: 57°C - elong: 72°C, 1min - 35 cycles
	SmBR13	DQ137790	205-225	(CTAT)16	GTCACAGATACCTGACGAGCTG	ACTCCCCAGCAATTTGTC	Ta: 57°C - elong: 72°C, 1min - 35 cycles
	SmBR6	AF009659	272-278	(CTT)10	CTTAACAGACATACACGC	GAATACAGGCTATAATCTACA	Ta: 57°C - elong: 72°C, 1min - 35 cycles
	SmD25	AF202965	268-274	(CA)10	GATTCCAAGATTAATGCC	GCCATTAGATAATGTACGTG	Ta: 57°C - elong: 72°C, 1min - 35 cycles

Characteristics of *Schistosoma mansoni* strain Guadeloupe (GUA) microsatellite loci, including locus name, GenBank Accession number (No.), size of PCR products in nucleotides (nt), nature of repeated motifs, forward and reverse primer sequences, annealing temperature in centigrade (Ta); elong: temperature in centigrade and duration of elongation in minutes.

Table 4: Comparison of microsatellite sizes for fixed and non-fixed materials.

Multiplex	Microsatellite name	Size (bp) for fixed material	Size (bp) for non-fixed material
M1	R95529	275	275
	SmC1	290	290
	SmD57	296	296
	SmBR16	337	337
	SmDO11	332	332
M2	SmDA28	115	115
	SmBR1	154	154
	SmS7-1	184	184
	SmD28	240	240
	SCMSMOXII	295	295
M3	SmBR10	133	133
	L46951	168	168
	SmBR13	221	221
	SmBR6	272	272
	SmD25	292	292

Microsatellite locus sizes of *Schistosoma mansoni* Brazilian clonal strain.

Legends to figures

Figure 1. Visualisation of implanted SpI in snail tissue at 15DPI after Raillet-Henry fixation. The SpI were readily observable as translucent white bodies within an opaque tissue background (A). Higher magnification showing the spherical aspect of SpI (B, C). *Biomphalaria glabrata* was exposed to 20 miracidia of *Schistosoma mansoni*.

Figure 2. PCR amplification of genomic DNA recovered from Raillet-Henry fixed sporocysts. Columns 1, 2 and 3 correspond to individual sporocysts fixed in Raillet-Henry. Column 4 is a PCR positive control using gDNA extracted from non fixed miracidia and column 5 correspond to the negative PCR control. Specific fragment lengths were amplified using specific primer couples designed on SmPoMuc gene (Roger, et al., 2008, Roger, et al., 2008, Roger, et al., 2008). See Table 1 for primer sequences and PCR conditions.

MW: molecular weight in kilo bases.

Figure 3. Multiplex microsatellite fragments profiles visualised after PCR amplifications and sequencing using the CEQ 8000 fragment analyzer package (Beckman Coulter). For each multiplex (M1, M2, M3) the name and size position of microsatellites were indicated (see Table 3 for primer sequences and PCR conditions). For SCMSMOXII, as the dye signal is faint compared to the others, we showed a higher magnification of this microsatellite in the upper right corner of the M2 picture.

nt: nucleotide; RFU: relative fluorescent units.

Figure 4. PCR amplification of cDNA transcripts recovered from Raillet-Henry fixed sporocysts. Column 1 corresponds to the pool of cDNA originating from 5 sporocysts fixed in

Raillet-Henry. Column 2 corresponds to the cDNA of 1 sporocyst fixed in Raillet-Henry. Column 3 corresponds to a positive control using cDNA originating from non fixed miracidia. Column 4 corresponds to *Biomphalaria glabrata* cDNA. Column 5 corresponds to retro-transcription negative control. Column 6 corresponds to PCR negative control. See Table 2 for primer sequences and PCR conditions.

MW: molecular weight in base pairs.







