

## DIFFERENTIATION OF *SCHISTOSOMA HAEMATOBIMUM* FROM RELATED SCHISTOSOMES BY PCR AMPLIFYING AN INTER-REPEAT SEQUENCE

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**Abstract.** *Schistosoma haematobium* infects nearly 150 million people, primarily in Africa, and is transmitted by select species of local bulinid snails. These snails can host other related trematode species as well, so that effective detection and monitoring of snails infected with *S. haematobium* requires a successful differentiation between *S. haematobium* and any closely related schistosome species. To enable differential detection of *S. haematobium* DNA by simple polymerase chain reaction (PCR), we designed and tested primer pairs from numerous newly identified *Schistosoma* DNA repeat sequences. However, all pairs tested were found unsuitable for this purpose. Differentiation of *S. haematobium* from *S. bovis*, *S. matthei*, *S. curassoni*, and *S. intercalatum* (but not from *S. margrebowiei*) was ultimately accomplished by PCR using one primer from a newly identified repeat, Sh110, and a second primer from a known schistosomal splice-leader sequence. For evaluation of residual *S. haematobium* transmission after control interventions, this differentiation tool will enable accurate monitoring of infected snails in areas where *S. haematobium* is sympatric with the most prevalent other schistosome species.

### INTRODUCTION

*Schistosoma haematobium*, which causes urinary schistosomiasis in humans, afflicts ~150 million people in 53 countries in Africa and the Middle East.<sup>1</sup> In many areas, *S. haematobium* is sympatric with related schistosome parasites (most of other mammals) (i.e., *S. bovis*, *S. matthei*, *S. curassoni*, *S. intercalatum*, and *S. margrebowiei*), and these related species are known to develop in the same bulinid intermediate snails that transmit *S. haematobium*.<sup>2,3</sup> Because *S. haematobium* and its related schistosome species often infect snails inhabiting the same bodies of water, the assessment of the transmission of *S. haematobium* by examining cercariae or infected snails is confounded by the need to differentially identify *S. haematobium*.<sup>4–6</sup> Of the schistosome species related to *S. haematobium*, *S. bovis* is the most widespread, with reported distribution in the Middle East, the Mediterranean basin, and most parts of Africa.<sup>7,8</sup> The other species of schistosomes examined in this study, *S. matthei*, *S. margrebowiei*, and *S. curassoni*, are also present in various parts of Africa, although less prevalent. In addition, *S. intercalatum*, which causes mixed intestinal and urinary schistosomiasis in humans, can also be found in transmission foci in Africa that are co-inhabited by *S. haematobium*.<sup>9</sup> Previously, the differential identification of snails infected by *S. haematobium* needed careful examination and testing of shed cercariae. Given that cercariae belonging to the *S. haematobium*-related species are not readily distinguishable morphologically from others within the group, a variety of approaches have been taken for differential identification,<sup>10</sup> with the standard method being infection of laboratory animals and subsequent parasite species identification based on the morphology of the adult worms. The importance of discriminating *S. haematobium* from other species later led to the development of molecular approaches, which have included isoenzyme profiling,<sup>11</sup>

Southern blot analysis employing rDNA gene probes,<sup>12</sup> randomly amplified DNA,<sup>13</sup> and polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis of the ITS2 region.<sup>10</sup> Amplification by simple PCR using defined primers, considered more suitable for large-scale screening, has not been accomplished for this purpose thus far.

We have recently accomplished identification of cercariae<sup>14</sup> and of snails infected with *S. haematobium*<sup>15</sup> by using primers designed from a repeated sequence, *DraI*, in a PCR assay. *DraI* was not found in *S. mansoni* or *S. japonicum* by dot hybridization.<sup>14</sup> However, because the *DraI* repeat is present in DNA of other schistosomes belonging to the *S. haematobium* group,<sup>14</sup> the identification of *S. haematobium*-infected snails by a simple PCR assay has been limited, thus far, to areas where these other *S. haematobium*-related schistosome species are absent or very rare.<sup>15</sup>

PCR, unlike other methods previously used for identifying snails with prepatent infection, has enabled detection of snail infection from its very earliest stages.<sup>15</sup> It thus identifies the entire population of infected snails, regardless of whether they eventually shed cercariae, representing in quantitative terms the direct outcome of human contamination of water bodies.<sup>15</sup> This approach has been applied to large-scale monitoring of infection in field snails at transmission sites in Coast Province, Kenya.<sup>15</sup> Because differential identification of snails infected with *S. haematobium* is important for further evaluation of integrated control approaches in other endemic regions, we have undertaken a search for DNA sequences from which primers can be designed for differential identification of *S. haematobium* by means of simple PCR techniques.

### MATERIALS AND METHODS

**Parasites.** Adult worms, preserved in liquid nitrogen as part of the schistosome collection at the Natural History Museum, London, UK, were preserved in ethanol and shipped, and kept in ethanol until subjected to DNA extraction in Jerusalem. These included *S. haematobium* from Cameroon, Mauritius, and Zanzibar; *S. bovis* from Tanzania and Senegal; *S. margrebowiei* from Zambia; *S. curassoni* from Senegal; *S.*

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*mattheei* from Zambia and Malawi; and *S. intercalatum* from Cameroon and Zaire.

**Normal and infected snails.** *Bulinus nasutus* field snails were collected during 2001 from transmission sites in the Msambweni area of Kwale District, Coast Province, Kenya. Details of the study area have been described elsewhere.<sup>16</sup> *B. nasutus* collected from transmission sites in this area exhibited high rates of prepatent *S. haematobium* infection as determined by PCR, ranging from 29% to 50% and patent infection ranging between 0.14% and 3.4%. Schistosomes of ruminants in these sites were found to be rare.<sup>15</sup>

Uninfected *B. nasutus* used for these studies were laboratory bred, taken from first- to fourth-generation stocks derived from the above-mentioned field collected snails (C. Kariuki and others, unpublished data). Snails were preserved in ethanol until subjected to DNA extraction.

**DNA extraction.** DNA was prepared from adult schistosome worms of all species as previously described.<sup>14</sup> Briefly, the worms were kept in lysis buffer containing (0.1 mol/L Ethylenediaminetetraacetate, pH 8.0, 0.1 mol/L Tris-HCl, pH 7.5, 0.2 mol/L NaCl, 1% SDS, 0.2% 2-mercaptoethanol, and 100 µg Proteinase K) at 65°C for 1–2 hours. This was followed by phenol and chloroform extraction.

DNA from snails was extracted by the hexadecyltrimethylammonium bromide (CTAB) extraction method as previously described.<sup>15</sup> Briefly, snail bodies were removed from their shell and incubated in 0.6 mL of lysis buffer (100 mmol/L Tris base, 20 mmol/L EDTA, 1.4 mol/L NaCl, 0.2% β-mercaptoethanol, 2% CTAB) and Proteinase K. After incubation for 3 hours at 60°C, nucleic acids were extracted twice by chloroform and precipitated with ethanol.

**Construction of genomic DNA libraries.** Genomic libraries were prepared from DNA of all the schistosomes included in this study. Materials for recombinant DNA technology, including enzymes used for restriction, ligation, and dephosphorylation, were from MBI Fermentas (Vilnius, Lithuania). Genomic DNA was partially digested by Sau3A restriction endonuclease, followed by phenol/chloroform extraction and ethanol precipitation. Digested DNA was ligated into the *Bam*HI site of the Bluescript plasmid (Stratagene, La Jolla, CA), by standard methods.<sup>17</sup> For additional genomic libraries of *S. haematobium*, DNA was digested by *Alu*I or *Rsa*I, and digestion products ligated into the *Sma*I site of the Bluescript plasmid. Ligation products were transformed into *Escherichia coli*, strain XL1-Blue, and were plated for color selection of recombinants by standard methods.<sup>17</sup>

**Selection of repetitive DNA clones.** The selected white recombinant colonies were transferred onto nylon filters and probed with <sup>32</sup>P-labeled total *S. haematobium* genomic DNA. Colony lysis, DNA denaturation, and fixation onto the nylon filter were carried out in a microwave oven as previously described.<sup>18</sup> Clones initially selected were those that exhibited strong hybridization signals with the homologous total DNA probe, indicating the presence of repeated sequences. These were transferred to a grid on a nylon filter, and a second level of selection was applied by hybridization with the *Dra*I repeat of *S. haematobium*, shared by all of the schistosome species studied in this paper.<sup>15</sup> Colonies not hybridizing with the *Dra*I probe underwent a third level of selection involving cross-hybridization with total DNA from the other species and selection of those giving hybridization signals with *S. haematobium* DNA only. A fourth level of selection of

candidate clones followed sequence analysis, with selection of those that exhibited the following features: 1) they were not similar to the *Dra*I repeat; 2) they were not similar to known sequences from the other schistosomes included in this study nor to schistosomal sequences present in GenBank; and 3) when aligned with other sequences of the same species, sequences thereof were found to be represented more than once in the same species, suggesting a relatively high copy number.

**Dot-blot and Southern blot analysis.** Comparative dot-blot hybridization was performed to estimate the abundance of the sequence amplified by applying PCR using the selected primers (see below). Various concentrations of *S. haematobium* genomic DNA (ranging from 0.01 to 10 ng) and DNA of the said amplification product, ranging between 0.1 and 10 µg, were dotted onto a nitrocellulose membrane using a dot-blot apparatus (BioRad, Hercules, CA). These underwent denaturation and neutralization by standard procedures<sup>17</sup> and were finally hybridized with radiolabeled total DNA probe and with the cloned amplification product probe.

Radiolabeling with <sup>32</sup>P of genomic DNA, repetitive DNA clones, or PCR products used as probes was done by the Random Primer DNA Labeling Kit (Biologic Industries Co., Beit Haemek, Israel). Analysis of the dot hybridization signals was performed using the public domain NIH Image program (developed at the Research Services Branch at the US National Institute of Mental Health and available on the Internet at <http://rsb.info.nih.gov/nih-image/about.html>).

Southern blot analysis was performed, according to standard protocols<sup>17</sup> for determining the distribution of the amplified DNA sequence (assumed to represent the DNA segment stretching between the primer sequences used). *S. haematobium* genomic DNA (1 µg) was partially digested by *Rsa*I or *Alu*I restriction enzymes followed by Southern transfer to nitrocellulose filter and probing by the corresponding amplification product.

**Filter hybridization.** This was done according to standard procedures.<sup>17</sup> The filters were incubated for 2 hours at 65°C in a prehybridization solution (6× sodium chloride, sodium citrate [SSC], 10× Denhardt solution, 0.1% SDS, and 100 µg/mL denatured salmon sperm DNA). The radiolabeled probe was directly added to the filters in the prehybridization solution at 60°C for overnight. The filters were washed once in 2× SSC at room temperature for 30 minutes, followed by two washes with 2× SSC, 0.1% SDS at 60°C for 15 minutes each time. Filters were air-dried and exposed to x-ray film.

**Primer design and PCR assay.** Primers were designed by using the PRIME software version 9.0, of the Wisconsin Package (Genetic Computer Group [GCG], Madison, WI) based on the sequence information of the newly described repeated sequences of *S. haematobium* selected for these experiments.

The primer combination ultimately enabling specific amplification of *S. haematobium* DNA included one primer (Sh110: 5' TTCCTCCAACCTACCATCTTATCTC) based on a newly identified *S. haematobium* repetitive sequence termed Sh110 (Figure 1A), and a second primer (Sm-SL: 5' AACCGTCACGGTTTTACTCTTGTTG) based on the highly abundant *S. mansoni* splice leader sequence.<sup>19</sup> The degree of similarity of the *S. mansoni* splice leader sequence to that of *S. haematobium* and of other schistosomes is likely to be high, given that the same splice leader primer sequence is

TABLE 1

Summary: The search for repeated sequences and corresponding primers relevant for differentiating *Schistosoma haematobium* (Sh-Diff.) from related species by PCR

Species included	Library constructed by:	NOC* selected <sup>a</sup>	NOC* sequenced (%) <sup>b</sup>	NOC* selected for <i>Sh</i> -diff.	NOC* tested for <i>Sh</i> -diff.	NOP** tested in intra-repeat PCR <sup>c</sup>	NOP** used with the Sm-SI primer
<i>S. haematobium</i>	<i>Sau3AI</i>	68	7 (10.3)	5	5	15 <sup>d</sup>	10
<i>S. haematobium</i>	<i>AluI</i>	147	55 (37.4)	15	9	18	18
<i>S. haematobium</i>	<i>RsaI</i>	236	95 (40.2)	4	4	8	8
<i>S. mattheei</i>	<i>Sau3AI</i>	103	21 (20.4)	4	2	6 <sup>d</sup>	4
<i>S. bovis</i>	<i>Sau3AI</i>	43	20 (46.5)	6	4	8	4
<i>S. intercalatum</i>	<i>Sau3AI</i>	88	18 (20.4)	4	2	4	4
<i>S. margrebowiei</i>	<i>Sau3AI</i>	64	17 (25.6)	4	2	4	4
<i>S. curassoni</i>	<i>Sau3AI</i>	64	17 (25.6)	0	0	0	0
Total		813	250	42	28	63	52

<sup>a</sup> Hybridization with homologous schistosomal DNA yielded strong hybridization signals, characteristic of highly repetitive sequences.

<sup>b</sup> These clones exhibited cross hybridization with DNA of *S. haematobium* but not with DNA of the other *Schistosoma* species, nor with the *Dral* repeated<sup>14</sup> sequence.

<sup>c</sup> Most of the primers were used in both intra and inter repeat amplifications.

<sup>d</sup> For some clones more than one pair of primers were designed.

NOC\* = number of clones; NOP\*\* = number of primers.

found (with complete identity) in protein mRNA sequences of *S. japonicum*, as shown by a Blast search in the GenBank.

PCR assays for sensitivity and specificity studies were carried out in a total volume of 100  $\mu$ L containing 200  $\mu$ mol/L of each dNTPs, 25 pmol of the two amplified primers, 2.5 units of *Taq* polymerase (MBI Fermentas, Vilnius, Lithuania), and the target DNA. *Taq* polymerases from various other sources were also used for establishing reproducibility. These included Red Hot *Taq* Polymerase (Advanced Biotechnologies, Epsom, UK), A Sigma *Taq* Polymerase (Sigma-Aldrich, St. Louis, MO) and a *Taq* Polymerase from an unspecified source. The reaction buffer consisted of 10 mmol/L tris-HCl, pH 8.3, 25 mmol/L KCl, and 3.5 mmol/L MgCl<sub>2</sub>. A thermal cycler (MJ Research, Watertown, MA) was used with a thermal profile involving 5 minutes at 95°C, followed by 35 cycles each of 1 minute at 95°C, 1 minute at 50°C, followed by 1 minute at 72°C, and a final elongation step at 72°C for 10 minutes.

For detecting *S. haematobium*-infected snails by PCR, preliminary experiments were carried out for establishing DNA extraction conditions and PCR conditions by testing snail bodies spiked with schistosomal DNA. Further examinations were carried out with infected and uninfected field-collected snails. Conditions for snail PCR have been described elsewhere.<sup>15</sup>

**Nucleotide sequencing.** Plasmid Isolation Kit (Quiagen, Chatsworth, CA) was used according to the instructions of the manufacturer for obtaining DNA from recombinant plasmids selected for sequence analysis. Nucleotide sequence analysis was carried out using Dye Terminator Cycle Sequencing kit (Perkin Elmer, Wallington, UK) according to the instructions of the manufacturer. An ABI PRISM 377 DNA Sequencer and ABI Sequencing Analysis software (Perkin Elmer, Foster City, CA) were used. The thermal profile was 10 seconds at 96°C, 5 seconds at 50°C, and 4 minutes at 60°C for 25 cycles. Consensus sequence was analyzed by employing the Wisconsin Package (Genetic Computer Group, Madison, WI).

## RESULTS

**Search for repeated sequences and primers specific for *S. haematobium*.** An extensive preliminary search (Table 1) failed to identify repeated DNA sequences that were suitable, by themselves, for differentiation of *S. haematobium* by direct

PCR (unpublished data).† We next examined an alternative possibility to accomplish this goal. This involved attempts at inter-repeat amplification using mixed primer pairs—on one end, a primer designed from newly identified *S. haematobium* repeat sequences, and on the other end, a primer based on known *Schistosoma* splice-leader sequence (taken from *S. mansoni* sequence<sup>19</sup> and common to *S. japonicum*). A total of 52 such mixed primer pairs were used in this part of the search (Table 1). The primer designed from the newly identified repeated sequence clone Sh110 (Figure 1A) in combination with the splice leader primer, Sm-S1, (Figure 1B), were selected for further testing for differential PCR identification of *S. haematobium* based on an initial demonstration of differentiation from *S. bovis*.

**Structure, abundance, and arrangement of the Sh110 repeated sequence.** This 120-bp sequence, derived from a *Sau3AI* library of *S. haematobium* (Figure 1A), is flanked by a GAT-rich region with GAT repeated 50 times (results not shown). Although our study detected the presence of this repeated sequence in *S. haematobium*, its relation to any particular repeated gene or non-coding sequence of *S. haematobium* is yet unknown.

The abundance of the Sh110 sequence has not been directly determined but can be inferred, with some approximation, from the abundance of the amplified sequence (see below).

The arrangement of the Sh110 repeated sequence was determined by Southern blot analysis using a Sh110 clone as probe. Several bands of different sizes were obtained (results not shown), as was also found with the Sm-SI/Sh110 PCR product (see below), but there was no ladder banding pattern typical of tandem repeated sequences.<sup>14,20,21</sup> These results suggested that the Sh110 repeat sequence is dispersed in the genome.

**Structure, abundance, and arrangement of the Sh110/Sm-SI PCR product.** The DNA amplification by PCR using the Sh110 and Sm-S1 primers yielded a 525-bp product (Gen-

†The structure and other characteristics of the various repeated sequences found in the course of our search are beyond the scope of the present report, which centers on differential identification of *S. haematobium*, and will be detailed in a future publication. Those 27 clones that provided primers for inter-repeat amplification with the Sm-SI primer were deposited in the GenBank (accession nos. DQ831697–DQ831706).

**A. Sh110 *S. haematobium* repetitive sequence**

5' GATGATGATC AAGTTGTAAT ACGGTTACCA GTTTAGGTGA TTCAACATTG  
 AGTGTACTAT GTT**GAGATAA** **GATGGTAGTT** **GGAGGAA**CTG ATATACATGA

←  
 Sh110R<sub>2</sub>

CACTGATTAC CAACCGATGA TC 3'

**B. *Schistosoma mansoni* splice leader sequence**

5' AACCGTCACGGTTTTACTCTTGATTGTTGCATG 3'

→  
 Sm-S1

FIGURE 1. **A.** The newly identified *S. haematobium* repeated sequence clone Sh110. **B.** The *S. mansoni* splice leader sequence from *S. mansoni*—Sm-S1. Primers designed from both (in bold) were used in PCR assays for species-specific discrimination between *S. haematobium* DNA and the DNA of other related schistosome species.

Bank accession no. DQ831696). The position of the 5' end was inferred from the known position of the *S. mansoni* splice leader sequence.<sup>19</sup> The PCR product's sequence and the corresponding primers are presented in Figure 2. A Blast search in the GenBank identified a high degree of sequence homology between parts of this PCR product (93- to 122-bp-long segments) and a number of schistosomal sequences, including *S. haematobium* acetylcholinesterase gene (accession no. AY167025). However, the amplified sequence, as presented in its entirety (Figure 2), is not represented in the GenBank.

The abundance of the PCR product in the schistosomal genome was determined by comparative dot blot hybridization, as previously described for repeated sequences of *S. mansoni*,<sup>20</sup> *S. haematobium*,<sup>14</sup> and *Echinococcus granulosus*.<sup>21</sup> Signal intensity obtained with 50 ng of total *S. haematobium* DNA was similar to that obtained with 0.1 ng of Sm-S1/Sh110 PCR product. Thus, the Sm-S1/Sh110 PCR product constitutes ~0.002% of the genome. Given the estimated genome size of *S. haematobium*,  $2.7 \times 10^8$  bp,<sup>22</sup> the approximate copy number of Sm-S1/Sh110 repeat PCR product (525 bases) is 1,026 copies. This figure, however, is much higher than the copy number of the *S. mansoni* splice-leader sequence and may represent a higher number of splice-leader sequences in *S. haematobium* and/or closely related sequences, which could initiate amplification.

The arrangement in the *S. haematobium* genome of the DNA segment amplified by Sm-S1/Sh110 PCR seems to be dispersed given that several bands of different sizes were exhibited (Figure 3). Bands with corresponding sizes of about 260 bp (Figure 3, lane 1-RsaI cut DNA) and both 260 and 520 bp (Figure 3, lane 2-AluI cut DNA) were exhibited, the latter

←  
 Sm-SL primer  
 →

5' -**AACCGTCACG** **GTTTTACTCT** **TGTGATTTGT** GTTAATGTCC AATCATAATT  
 AATTCGCGAT ATFGCATAAT CTTTATCCAT TATTATTATT ATTATTATCA  
 AAAATATTAA AACGTTTTAT AGGCGTTCTT ATAACCTGAA CGAGAACACA  
 GACAAGAACA TTCAAATCTA CTTTATTACA ACATTACAGA ACTCCGCCTG  
 GAGTCCCGTC TGGGGCTACT GCCGGTCCCA AGCCCGGATA AAGAAGGAGG  
 GTCGGGCATG GGGTTAGCTA CCCCATCCCG TAGAAACTAA CTCGCTAAAA  
 AGCGCTAACC AGAATATCTG AATAACATAC AGTAAAAATA AATACATCAT  
 TCTGTAATAAT ATCAATCGAT TGTCCTCATAT TTCATTGTTC TTTCGTTTCC  
 ATATCAATCA TTCTTTGTTT TCATTCTGTT CTCTTCAATC TTCTTTATAT  
 TCTACCATCT ACTTTCTATT AGTGAATAAT ACATACTACT TATATATGTT  
 GAGATAAGAT GGTCAATTGG AGGAA-3'

←  
 Sh110 primer

FIGURE 2. The 525-bp DNA sequence of the amplification product of PCR using the Sm-S1 primer and the reverse Sh110 primer. The primers and their positions are marked in bold.

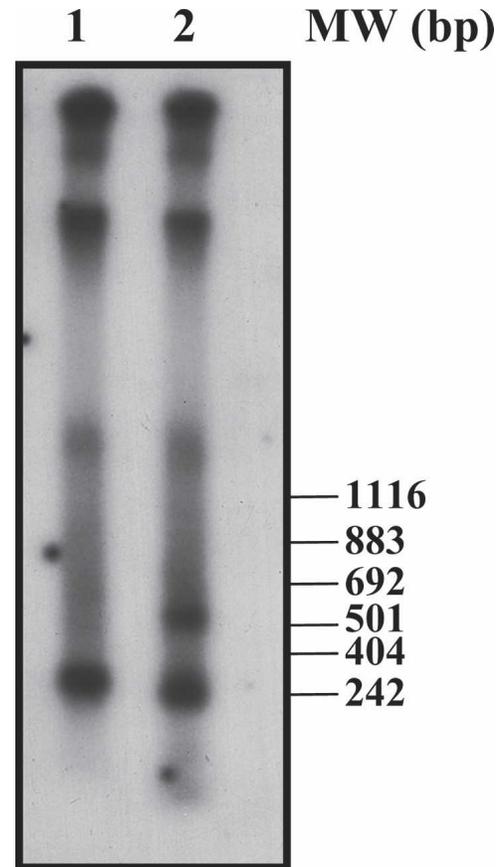


FIGURE 3. The distribution of the DNA segment amplified by the Sh110 and Sm-S1 primers. Total *S. haematobium* DNA digested by *Rsa*I (1) or *Alu*I (2) underwent Southern blot and probing with labeled Sm-S1/Sh110 PCR product.

identical to the size of the amplification product. Given that discrete bands were obtained by Southern hybridization with the corresponding probe (Figure 3), it is unlikely that hybridization occurred with small segments having only partial sequence homology (e.g., parts of the splice leader sequence) but rather with the full sequence of the amplification product or with large parts of it.

**Differential identification of *S. haematobium* by PCR: detection sensitivity and specificity.** Sensitivity of detection by PCR was determined using different concentrations of *S. haematobium* genomic DNA. As shown in Figure 4, 1 pg of *S. haematobium* DNA could be detected in the standard Sm-S1/Sh110 PCR reaction.

For specificity of detection, varying concentrations of genomic DNA from *S. haematobium* and related schistosome species were subjected to PCR, and their products examined by gel electrophoresis (Figure 5). Results indicate that 1 pg of *S. haematobium* DNA was clearly detectable by Sm-S1/Sh110 PCR, whereas no amplification was exhibited with 1 and 10 ng DNA (1000–10,000 times more DNA) of *S. bovis* or *S. matthei*. The other species examined exhibited PCR positivity with 10 ng DNA, four orders of magnitude more than was needed to obtain a positive amplification signal with *S. haematobium* DNA. This quantitative difference can enable differentiation of *S. haematobium* but not differential identification of the other schistosome species. Such

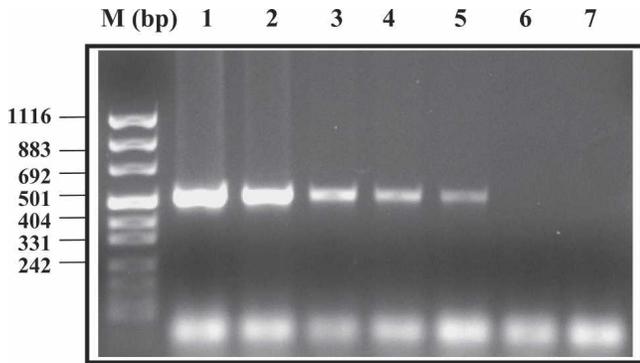


FIGURE 4. Sensitivity of detection by SmS1/Sh110 PCR. Different concentrations of *S. haematobium* genomic DNA were used: lane 1, 10 ng; lane 2, 1 ng; lane 3, 0.1 ng; lane 4, 10 pg; lane 5, 1 pg; lane 6, 0.1 pg; lane 7, no DNA. M, DNA size marker.

species identification may be possible in some cases by banding pattern. Thus, a distinctive banding pattern was obtained after Sm-SI/Sh110 PCR with 10 ng of *S. intercalatum* (two bands, the smaller of which, ~450 bp, is distinguishable from the *S. haematobium* 525-bp band). Amplification using three of the four *Taq* polymerases used (except for the one by Sigma) enabled *S. intercalatum* differentiation. *S. curassoni* uniformly yielded a single amplification band, ~450 bp, clearly distinguishable from that of *S. haematobium*, as well as from the other species examined. On the other hand, *S. magrebowiei*, regularly exhibited a single band indistinguishable from that of *S. haematobium* and therefore the Sm-SI/Sh110 PCR assay cannot be used to distinguish it from *S. haematobium*.

Although *S. haematobium* DNA amplification and amplification of DNA from *S. haematobium*-infected snails regularly yield a single amplification band (Figures 3–5), a second band of ~300 bp sometimes appears when  $\geq 10$  ng *S. haematobium* DNA is examined (results not shown). Differentiation between *S. haematobium* and *S. intercalatum*, the other species that exhibits a second amplification band (of ~450 bp in this case), remains possible by the different size of the second band, as well as by the higher amount of DNA required for obtaining amplification products with *S. intercalatum* DNA.

**Detection of *S. haematobium*-infected snails by PCR.** *Bulinus nasutus* snails, known to be either infected or uninfected with schistosomes based on *DraI*-PCR testing, were subsequently examined by Sm-SI/Sh110 PCR. Infected and unin-

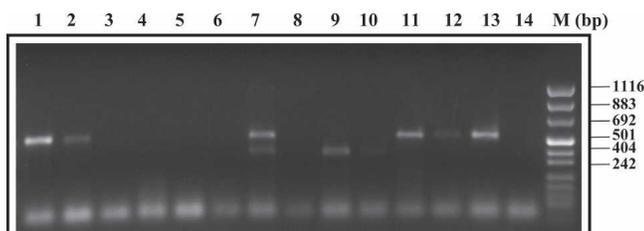


FIGURE 5. Specificity of detection of *S. haematobium* by SmS1/Sh110 PCR. Varying concentrations of genomic DNA from different species were tested. Lanes 1 and 2: 0.1 ng and 10 pg of *S. haematobium* DNA; lanes 3 and 4: 10 and 1 ng of *S. bovis* DNA; lanes 5 and 6: 10 and 1 ng of *S. mattheei* DNA; lanes 7 and 8: 10 and 1 ng of *S. intercalatum* DNA; lanes 9 and 10: 10 and 1 ng of *S. curassoni* DNA; lanes 11 and 12: 10 and 1 ng of *S. margrebowiei* DNA; lane 13: *S. haematobium* DNA 0.1 ng (positive control); lane 14: no DNA. M, DNA molecular size markers.

fecting snails were clearly distinguished (Figure 6), and sensitivity for *S. haematobium* DNA detection in snail extracts was further established by addition of DNA to extracts of uninfected snails (results not shown are similar to Figure 4).

## DISCUSSION

PCR primers were designed for differential identification of *S. haematobium* DNA content based on a newly identified repeated sequence of *S. haematobium* (Sh110; Figure 1A) and a second primer derived from the known splice leader sequence of *S. mansoni*.<sup>19</sup> Although the splice leader sequence analog in *S. haematobium* was not characterized in the present study, it is assumed that it is closely similar to that of *S. mansoni* and other schistosomes, given that sequences identical to the splice leader primer were identified in the GenBank among numerous protein mRNA sequences of *S. japonicum*. There is a high likelihood that the same splice leader primer sequence is present in the genomes of the other species studied. We assume that the presence or absence of the Sh110 sequence and differences in its relative location constitute the basis for the differential identification we have observed in our studies.

Of the *S. haematobium*-related schistosome species examined in this study, *S. bovis* is the most widespread and prevalent,<sup>7,8</sup> and thus has the widest potential geographical overlap with *S. haematobium*. The other species examined are less widely distributed. These are the animal schistosomes, *S. mattheei*, found in southern Africa,<sup>7</sup> *S. margrebowiei*, found in a relatively small area in west and southern Central Africa,<sup>7</sup> and *S. curassoni*, found primarily in western Africa.<sup>7,23</sup> *S. intercalatum*, belonging to the same group and causing sporadic schistosomiasis in humans, is found in foci in central Africa within Equatorial Guinea, Sao Tome, Nigeria, and Mali.<sup>9</sup> *S. leiperi*, another animal schistosome, the distribution of which overlaps that of *S. margrebowiei* in southern Central Africa,<sup>7</sup> was not examined in this study.

This report describes the differential identification of *S. haematobium* from the related schistosomes *S. bovis*, *S. mattheei*, *S. curassoni*, and *S. intercalatum* by simple PCR. In contrast, the much less common *S. margrebowiei* was not successfully differentiated from *S. haematobium*, as it yielded an amplification product of the same size (Figure 5). In practical terms, *S. haematobium*-infected snails can now be clearly differentiated from snails carrying the most prevalent, related

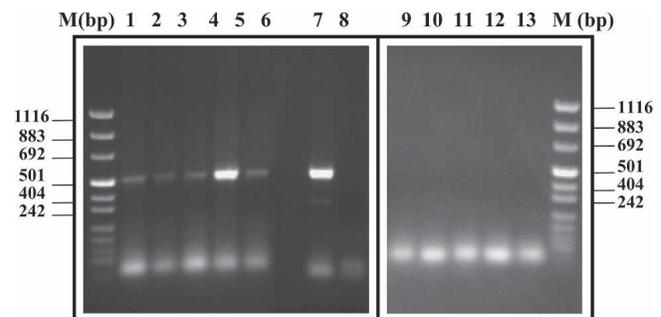


FIGURE 6. Detection of *S. haematobium*-infected snails by SmS1/Sh110 PCR. Lanes 1–6: PCR reaction run with infected snails; lanes 7–13: reaction run with uninfected snails; lane 7 only: 1 ng of *S. haematobium* DNA added to uninfected snail extract; lane 8: no DNA added; lanes 9–13: additional reactions run with uninfected snails. M, DNA size marker.

animal schistosomes in Africa (i.e., *S. bovis* and *S. mattheei*),<sup>7</sup> as well as from *S. curassoni* in areas where it is found alongside *S. haematobium*. These results will allow extension of large-scale PCR monitoring approaches for snails infected with *S. haematobium*,<sup>15</sup> which had been previously limited to areas where cross-reacting (*DraI* containing) animal schistosome species were absent or very rare.

In addition to differentiation between *S. haematobium* and its related species, specific identification of *S. intercalatum* (Figure 5) could facilitate better study of the distribution of this species and may also help to better define the effects of competition between *S. intercalatum* and *S. haematobium* in sympatric endemic areas.<sup>9</sup> The fact that *S. curassoni* can be differentiated from *S. haematobium*, as well as from the other species tested, may help to resolve doubts about the identity of *S. curassoni* as a distinct species.<sup>7</sup>

Further validation of the assay is needed for field studies because only a few field-collected snails were examined in this study. Such validation should include a whole range of infected and uninfected snails with different signal intensities (as detected by *DraI* PCR)<sup>15</sup> to assess the range of sensitivity among field-collected snails. Although PCR has been shown to be practical for large-scale monitoring of *S. haematobium* transmission in affected communities,<sup>15</sup> a more inexpensive and robust detection method will greatly facilitate implementation of molecular monitoring of transmission potential in large-scale control projects.

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