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1 Correspondence

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3 **No evidence for lateral gene transfer from salmonids to *Schistosomes* (or vice**
4 ***versa*)**

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14 A study in 2004 in number 8 of this journal concluded that in the past some sort of
15 lateral gene transfer occurred from the salmonids to the schistosomes ¹. Schistosomes
16 are parasitic helminths that cause a severe disorder called bilharziosis or
17 schistosomiasis in humans and several animals. The deduction of gene transfer was
18 based on the analysis of an EST library for *Schistosoma japonicum* (Adult SjC 7/94,
19 GenBank accession number BU712912) and on PCR on genomic and cDNA of *S.*
20 *mansoni* and *S. japonicum*. The study identified salmon transposon-like sequences in
21 schistosomes, and these sequences were deposited in GenBank as schistosomes-
22 specific, salmon-like repeats (GenBank accession numbers AY834394 to AY834403).
23 Naturally, the report stirred up a considerable interest in the scientific community
24 since the evolutionary conclusions were far-reaching ²⁻⁴. Nowadays, schistosomatidae
25 are parasitic helminths of mammals and birds, and a relation to fish that would be
26 close enough to allow for gene transfer made it necessary to reconsider the
27 evolutionary history of these blood-flukes. We show here that we find no evidence for
28 the proposed transfer of repetitive sequences from salmonids to *S. mansoni* and *S.*
29 *japonicum*.

30 We re-sequenced the genomes of female and male *S. mansoni* using the Genome
31 Analyzer II (Illumina) by single end sequencing according to the manufacturers
32 protocol. We obtained a total of 17,955,578 reads (~645 Mb). Sequences are available
33 at the NCBI sequence read archive (study accession number SRA012151.6). These

34 sequences were aligned to the 1278 repeats (total number of bp: 1,305,387) in the
35 repeat masker database of *S. mansoni*
36 (ftp://ftp.tigr.org/pub/data/Eukaryotic_Projects/s_mansoni/preliminary_annotation/homology_evidence/sma1.repeats.gz) that includes the above-mentioned Sm_salmonid
37 repeat (GenBank AY834402) but not the other putative salmon-like repeats. A total of
38 2,810,622 reads (15.66 %) aligned to the repeats database (2-fold coverage). The
39 Sm_salmonid sequence was the only one that did not match with any of the sequence
40 reads. In other words, among roughly 18 million sequence bits of 36 bp, not a single
41 read correspond to the 419 bp long previously in *S. mansoni* identified sequence of
42 putative salmonid origin. We then used Blast searches for GenBank accession
43 numbers AY834402, AY834401, AY834399, AY834397 and AY834395 against a
44 local genome database of *S. mansoni* (*S. mansoni* assembly version 3.1 from
45 ftp://ftp.sanger.ac.uk/pub/pathogens/Schistosoma/mansoni/genome/gene_predictions/GFF/S.mansoni_080508.fasta.gz ; updated march 20, 2007; 19,022 sequences;
46 381,096,674 base pairs; blastn default parameters; E \leq 0.05). In addition, *Schistosoma*
47 whole-genome shotgun reads and the nucleotide collection at the NCBI Blast server
48 (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>, blastn default parameters) was searched but
49 did not deliver significant hits (\geq 50% coverage, \geq 50% identity) other than the above-
50 mentioned GenBank entries. Finally, we attempted to PCR amplify the Sm_salmonid
51 sequences. First we used primers that were designed based on the GenBank entry
52 AY834402 and genomic DNA extracts of *S. mansoni* larvae (cercariae) or adult
53 worms of African and Middle American origin (Oman, Egypt, Guadeloupe and
54 Brazil) as template. Rainbow trout (*Oncorhynchus mykiss*) DNA served as control for
55 a touch-up PCR with 30 cycles starting at 42°C (18 degrees below the calculated T_m,
56 *i.e.* low stringency). In no case, amplification occurred for the schistosomes
57 (supplementary figure 1 A). We then tested the primer pairs that were originally used
58 by Melamed *et al.* ¹ (personal communication) with genomic DNA of *S. mansoni* and
59 *S. japonicum*. In none of the reactions, amplification occurred for the schistosome
60 samples (supplementary figure 1 B). Finally, we performed PCR with a temperature
61 gradient between 40 and 60°C for the annealing temperature, 35 amplification cycles
62 and 50 ng *S. mansoni* genomic DNA. At low temperatures, amplification occurred for
63 primer pairs Igf, PRL and LH (supplementary figure 1 C). The PCR products were
64 cloned and sequenced. For LH, a PCR product of 461 bp was amplified. This is larger
65 than the expected size of 424 bp based on GenBank entry BU711870.1 cited in ¹.

68 Alignment of the sequence of BU711870.1 spanning the primer binding sites with the
69 sequence of the PCR product delivered 47.6% sequence identity (Needle,
70 <http://www.ebi.ac.uk/Tools/emboss/align/>). Comparison with the genomic sequence
71 of *S.mansoni* identified the PCR product as part of a unique sequence on scaffold
72 000213 (position 409215-409650, 99% identity). For the Hpa primer pairs ¹, the PCR
73 product has a size of 175 bp instead of 159 bp predicted size, and alignment with
74 AY834401.1 gives only 45% similarity. Blast finds a unique 100% match on *S.*
75 *mansoni* genomic scaffold 000001 (position 1882577-1882721). For the Igf primer
76 pair, the predicted size is 266 bp, the observed size is 1041 bp and the similarity to
77 AY834397.1 is very low (18.7%). Primer sequences are given in supplementary table
78 1. In short, we did not observe PCR amplification with the previously used primers
79 for the putative salmonid-like repeat sequences in *S. mansoni* or *S. japonicum*. Taken
80 together, our experimental results, our *in-silico* analysis, and the schistosome and
81 salmon eco-ethology and the literature (see supplementary note) do not support the
82 view that gene transfer occurred from salmonids to schistosomes or between their
83 ancestors.

84 One might wonder how such a relation could have been established in the first place.
85 The sample history of the EST library “Adult SjC 7/94” is well documented in the
86 “note” section of GenBank accession number BU712912. The authors of the database
87 entry mention there that 2-3% of the clones contain inserts with homology to salmon
88 DNA and apparently they considered this as contamination. Salmon sperm DNA has
89 traditionally been used as a carrier material in many laboratories. We hypothesize that
90 such a contamination had misguided the authors of the original article. Cross-species
91 and vector contamination would not be surprising and are found in many databases ⁵.
92 We hope to initiate with the present work a re-examination of evolutionary theories
93 concerning Schistosomatidae and Salmonoidae that were based on the initial - and as
94 we think erroneous - report of horizontal gene transfer between these clades.

95

96 **Author contributions**

97 C.G. and J.B. designed the experiment and wrote the manuscript. C.G. performed the
98 experimental work and data analysis

99

100 **Competing financial interests**

101 The authors declare no competing financial interests.

102

103 **References:**

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Supplementary note

Lateral gene transfer could only be imagined if in one point of the evolutionary history, salmonid fishes were in close physical contact to schistosomes (e.g. parasited by the latter). Consequently, both groups of organisms must have lived at the same time in the same place and schistosomes must have got inside (and out) the salmonids somehow. We refer to this as encounter and compatibility filters ¹. From what is known about the biology of both taxonomic groups, both filters are probably closed in a hypothetical Salmonid-Schistosome host-parasite system. First, salmonids are typically cold-water fishes living higher the northern tropic, while schistosomes and their intermediate hosts occur in the intertropical zones. Moreover, both species, salmon and snail, are highly sensible to temperature variations: schistosomes do not infest under 20°C, while upper lethal limits of salmonids is around 25°C. For instance, in the *Biomphalaria glabrata* / *S. mansoni* host parasite system, the mortality rate of infected molluscs is 96% at 16°C, and after 2 weeks at 15°C the cercarial production cease completely ². In the *Oncomelania hupensis* / *S. japonicum* host parasite system, Zhou *et al.* ³ and Yang *et al.* ⁴ described that at 21°C the duration of development of the parasite inside its mollusc host is two times longer compared to 30°C. For salmonids, the negative effects of high temperatures (>15°) are also extensively documented in the literature (see ⁵ for review). Warm temperature reduces fecundity, decrease egg survival, retard growth of fry and smolts, and increase mortality in all developmental stages. For instance, the maximum temperature for spawning and for egg or alevin to survive is only 12°C. In other words, when schistosomes start to infect, their hypothetical salmon hosts start to die (and *vice versa*). Second, schistosomes parasitize only endothermic animals (birds and mammals) ⁶. Even if schistosome cercariae could be found in the same area as a salmon species (*i.e.* the encounter filter would be open) it remains highly improbable for the parasite cercariae to infect the fish because host temperature is a key component of the host finding and host penetration for both *S. mansoni* and *S. japonicum* ⁷⁻⁹. *S. mansoni* cercariae are attracted by a thermal gradient ⁷ and do not attach to a cold support (< 25°C) ⁹. Also *S. japonicum* cercariae migrate in the direction of high temperature and penetration starts only at 30°C surface temperature ⁸. The infection of an ectothermic host is not impossible but highly improbable. One might argue that even if today a Salmonid-Schistosome host-parasite

system is impossible, gene transfer could have occurred in an earlier system. Indeed, a sister group of the schistosomes, the sanguinicolidae, are parasites of fishes and we could imagine a common ancestor that was a parasite of ancient salmonid fishes. The genetic distance of schistosomes to sanguinicolidae was estimated using the 18S and 28S RNA genes, and the mitochondrial cytochrome c oxidase CO1 gene. Sequence divergence is 8%, 11.5% and 30%, respectively⁶. Sequence divergence of the putative *S. mansoni* salmonid-style repeats to the closest salmonid sequences is 3-5% with the exception of GenBank acc. number AY834399, a 123 bp long SINE fragment (divergence 9.6%) (supplementary table 2). These sequence divergence values correspond to the genetic distance to the closest salmonid species (supplementary table 2). That is to say the genetic distance of schistosomes to a sister group is larger than the genetic distance to the putative donor species for the gene transfer. Consequently, either there is stronger selective pressure on the repeats than on essential genes such as rRNA, or the transfer has occurred after the separation of the sister groups. Neither the first nor the latter (as outlined above) is likely in our opinion.

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Locus	Abbreviation used in the text	Forward primer	Reverse primer
<i>S.mansoni</i> 28S rDNA	28S	28SF1 GCTGTAGTGGATCTGTGC	28SR1 CTACGTCATGGGACGG
<i>S. mansoni</i> and <i>S. japonicum</i> 28S rDNA	28S	28SF1b GGTGTAGTGGTAGACGA	
<i>S.mansoni</i> specific repeat W7	W7	W7F ATTTACAGTTCAATCAATTGG	W7R AGATTTGCGCACAATTCC
RSg-1-like salmonid retrotransposon	Rsg	Sm_salmonid_F192 GGCCATGGTGGAAAAGTAAA	Sm_salmonid_R410 AAGCACCAACTGTCAGAGCA
	Rsg*	RsgF CTAGCTTTAAGCACCAACTGTCAG AGC	RsgR GTAGGCAAGTTGAGAACAAGTTCTC
IGF-I precursor-like gene, intron 2	Igf*	IgfF GAGGGAGGTGTTTAGTCCCAGG	IgfR TAGCCATGAAGTGCTTTGAAAGGCT
prolactin II-like flanking sequence	PRL*	PRLF GCAAACCTTCTTGCCACAGCTCGC	PRLR CAGACCACTTCTCAGTTCCTATGCTTCC
SINE SmaI	SmaI*	SmaF GACGCTTTTATCCAAAGCGACTTA CAG	SmaR CTGTAGCTCAGTTGGTAGAGCATGGCG
LH-like sequence	LH*	LHF TGCTGGTTAAATGTGCCTTGA ACTC	LHR GTGCATTAGAGTTAACTGCATCTCAG
SINE HpaI	HpaI*	HpaF CGGCAGCGTAGCCTAGTGGTTAG	HpaR TAACCAGGCAAGTCAGTTAAGAACATA

Supplementary Table 1: Primers used in this work. * indicate primers used in experiments described in ¹ (personal communication P. Melamed)

Putative <i>Schistosoma mansoni</i> sequences from reference 1	Closest salmonid sequences	% difference between <i>S.mansoni</i> sequence (column 1) and closest salmonid sequence (column 2)	% difference between salmonid sequence (column 2) and closest sequence of another salmonid species
AY834402.1: <i>Schistosoma mansoni</i> salmonid retrotransposon RSg-1-like element	M37215.1: SMORSRSG12 <i>Oncorhynchus mykiss</i> RSg-1 repeat DNA	5	4
AY834401.1: <i>Schistosoma mansoni</i> SINE HpaI genomic sequence	D49900.1: ONHHOM49 <i>Oncorhynchus mykiss</i> DNA, repeat sequence	3.5	4
AY834399.1: <i>Schistosoma mansoni</i> SINE SmaI	AB001880.1: <i>Oncorhynchus keta</i> SINE SmaI	9.6	2
AY834397.1: <i>Schistosoma mansoni</i> IGF-I precursor-like gene	EU621899.1: <i>Salmo salar</i> , growth hormone 2 gene,	5	5
AY834395.1: <i>Schistosoma mansoni</i> salmonid prolactin II-like flanking sequence	FJ969490.1: <i>Salmo salar</i> MHC class I antigen	3	1

Supplementary Table 2: Relation of putative salmonid sequences in *Schistosoma mansoni* to sequences of contemporary salmonid species.

figure 1 A)

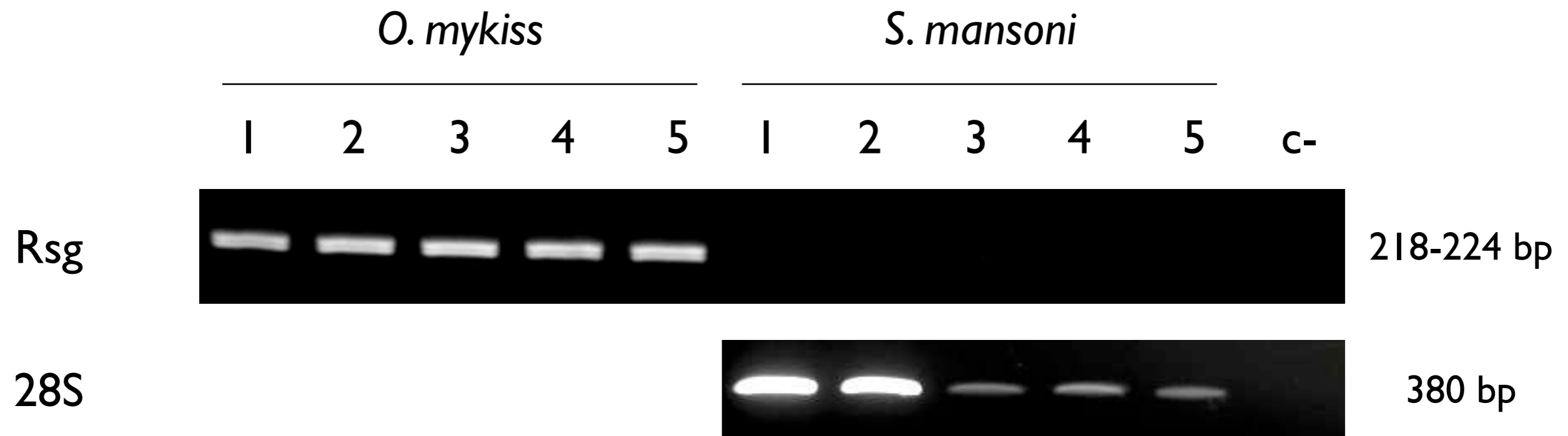


figure 1 B)

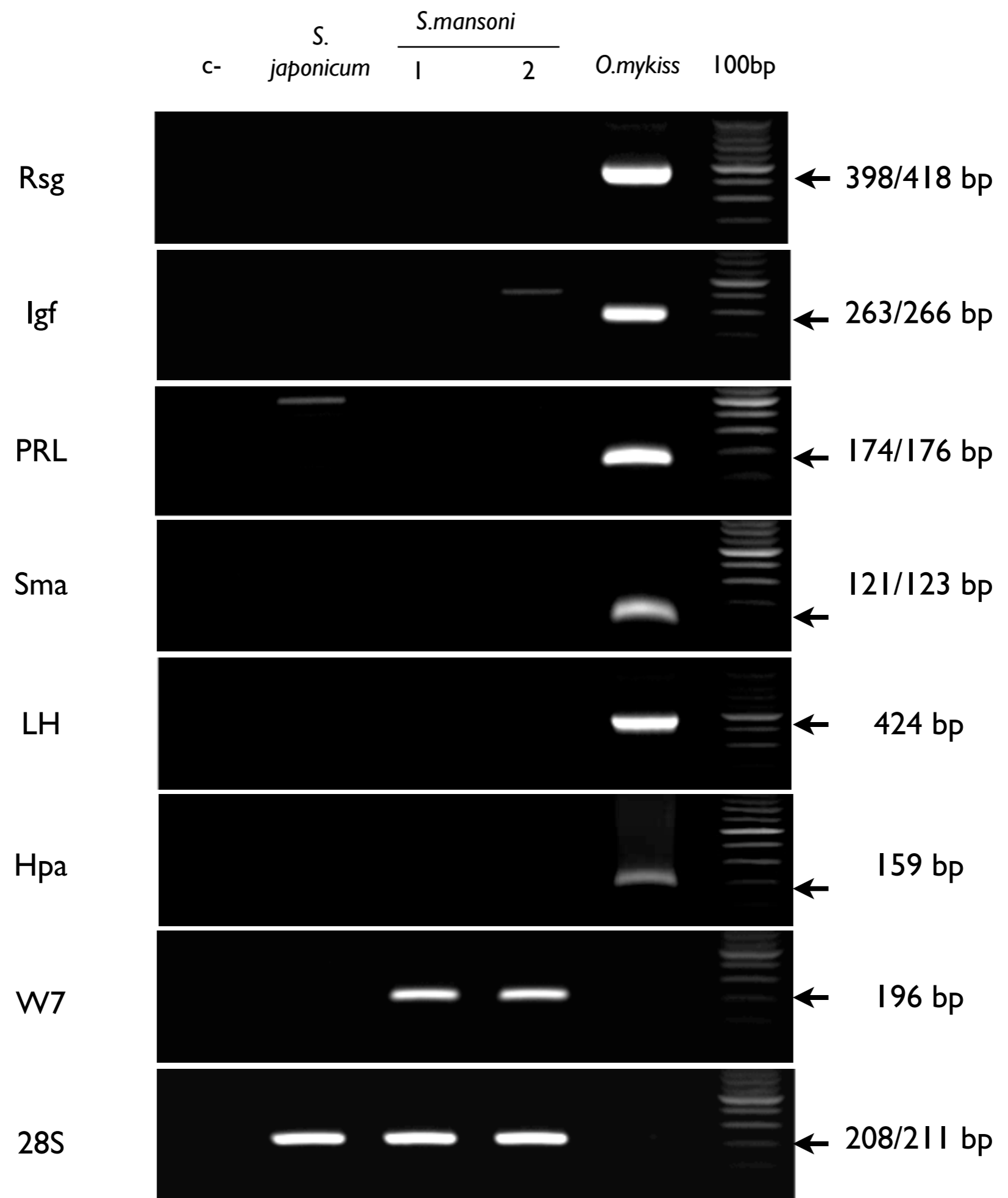


figure 1 C)

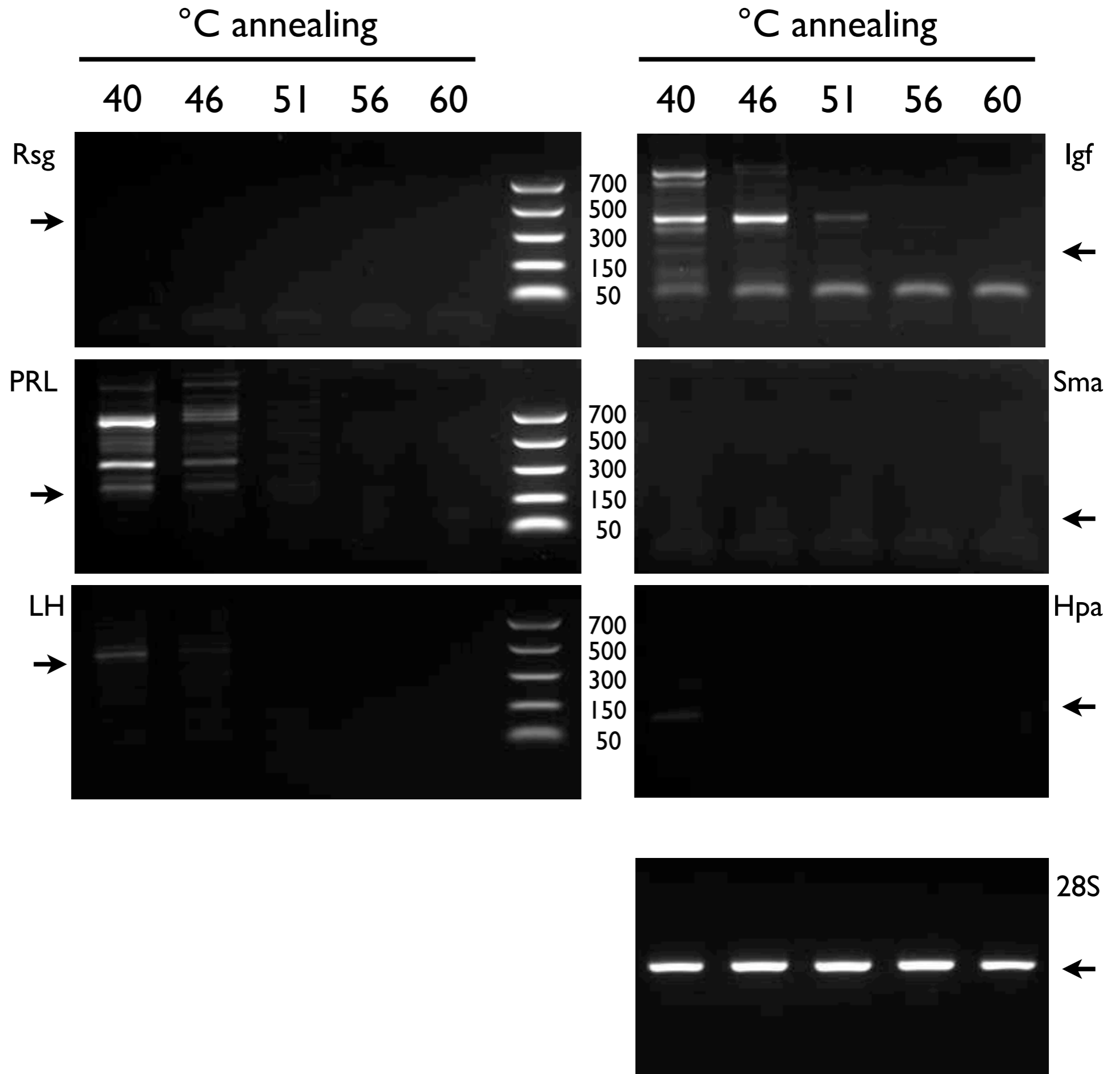


Figure legend for supplementary figure 1:

(A) Ethidiumbromide stained 1.5% agarose gels with bands corresponding to PCR products of PCR amplification of Sm_salmonid repeat Rsg and *Schistosoma mansoni* 28S rDNA as control for DNA presence. On the left control, reactions using *Oncorhynchus mykiss* genomic DNA extracts. Predicted size is 218-224 bp and corresponds to the observed one. On the right, 5 pools of *S. mansoni* genomic DNA served as templates (1: origin Guadeloupe (strain GH2), 2: origin Brazil (strain BRE), 3: origin Guadeloupe (strain DFO), 4 and 5: African origin, strains OmanM and Egypt). Negative control (c-) without template. (B) Ethidiumbromide stained 1.2% agarose gels with products of PCR amplification of Sm_salmonid repeat Rsg, IGF, PRL, SmaI, LH, HpaI, *S.mansoni* specific repeat W7, and *S. mansoni* and *S. japonicum* specific 28S rDNA (primer sequences in supplementary table 1). Fifty nanograms of template genomic DNA were used (touch down PCR annealing at 42-47°C for 5 cycles, constant 52°C for 30 cycles). (C) Ethidiumbromide stained 2% agarose gels with products of PCR amplification on 50 ng *S.mansoni* genomic DNA. A gradient of amplification temperature was used and is indicated on the top. Primer pairs as in (B). Arrowheads indicate expected size.