SCAN: Schistosomiasis Collections at the NHM

Aidan Emery

NATURAL HISTORY MUSEUM

Mission





- A global repository for schistosomiasis-related specimens
- A collection of schistosomes and snails as a genetic resource
- Support projects as they collect schistosomes and snails

  - Legacy collections
    - Spirit collection of African freshwater snails (1950s-2000s)
    - Adult schistosomes stored in liquid nitrogen (1980s-2010s)
  - New collections
    - Larval stages of schistosomes, mostly on FTA cards
    - Snails preserved in ethanol

wellcome

Numbers (to end of 2017)

- > 450,000 larval schistosomes on >8,000 FTA cards
- 64,000 snails
- 10,000 archived DNA extractions
- 26 countries

Archived within NHM's molecular collections facility (Jackie Mackenzie-Dodds)

More ancient archives exist…

NOTE ON THE PRESENCE OF "BILHARZIA HAEMATOBIA" IN EGYPTIAN MUMMIES OF THE TWENTIETH DYNASTY

[1250–1000 B.C.].

By MARC ARMAND RUFFER, M.D.,

PRESIDENT OF THE SANITARY, MARITIME, AND QUARANTINE COUNCIL OF EGYPT, ALEXANDRIA.

In a previous note published in this JOURNAL I described a process by which mummified tissues could be prepared for histological examination. I ventured to predict that it was highly probable that, by this method, one would be able to recognize pathological changes, such as cirrhosis, cancer, etc.

Thanks to the kindness of Professor Elliot Smith, Professor Flinders Petrie, and Professor Keatinge, I have obtained several organs from mummies of the eighteenth to the twentieth dynasty, and I may state at once that such diseases as atheroma, pneumonia, renal abscesses, and cirrhosis of the liver are plainly recognizable. In the renal abscesses and in other lesions I have stained micro-organisms with methylene blue, fuchsin, haematoxylin, and even by Gram's method.

At the present time there is perhaps no disease more important to Egypt than that caused by the *Bilharzia haematobia*. So far no evidence has been produced to show how long it has existed in this country, although medical papyri contain prescriptions against one of its most prominent symptoms—namely, haematuria. The lesions of this disease are best seen in the bladder and rectum, but unfortunately these are just the two mummified organs which I have not been able to obtain so far. Nevertheless, in the kidneys of two mummies of the twentieth dynasty I have demonstrated in microscopic sections a large number of calcified eggs of *Bilharzia haematobia*, situated, for the most part, among the straight tubules. Although calcified, these eggs are easily recognizable and cannot be mistaken for anything else. I may add that I showed some of my sections to Professors Looss and Ferguson, whose paramount authority on such a subject cannot be disputed, and both confirmed my diagnosis.

I have examined microscopically the kidneys of six mummies. The kidneys of two were apparently healthy; the left kidney of another was congenitally atrophied; those of the fourth contained multiple abscesses with well-staining bacteria and other lesions, which so far I have not diagnosed; those of the fifth and sixth showed bilharzia eggs, and the latter had other lesions as well, which, owing to the shrunken state of the organ, I am unable to define accurately as yet.

Renal disease, therefore, was not infrequent among Egyptians living over three thousand years ago.

1910

Yearbook of Mummy Studies, Vol. 2, pp. 39–47, 4 figs., 1 tab., March 2014

Molecular Confirmation of *Schistosoma* and Family Relationship in two Ancient Egyptian Mummies

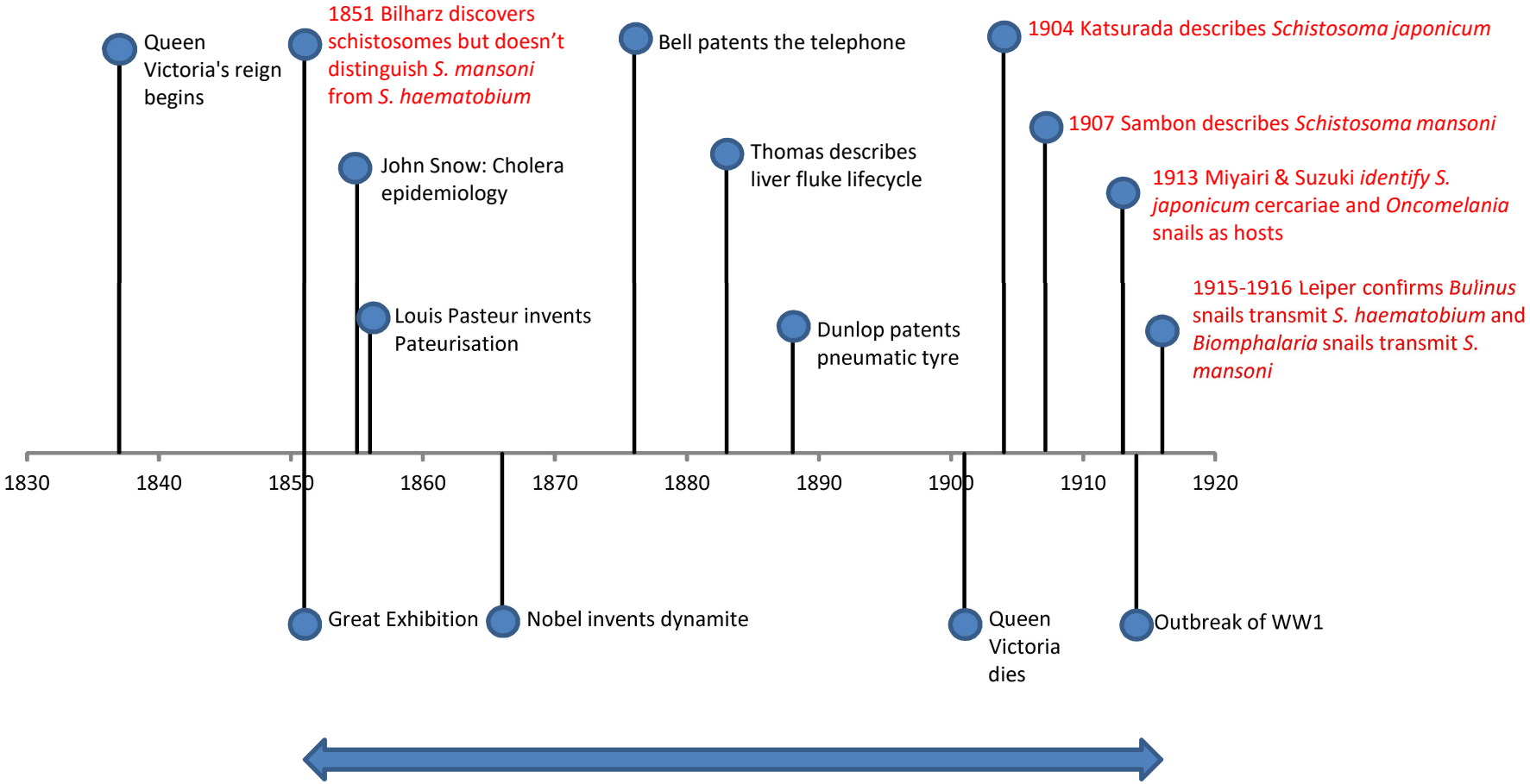
Carney D. Matheson[1], Rosalie David[2], Mark Spigelman[3,4] and Helen D. Donoghue[4,5]

Abstract

Egg morphology and immunocytochemistry have identified schistosomiasis in ancient Egypt. Our study aimed to detect and characterize schistosomal DNA in mummified human tissue. Liver samples from the mummy Nekht-Ankh (c. 3900 BP) and intestinal samples from Khnum-Nakht, possibly his brother, were analyzed using PCR primers suitable for fragmented ancient DNA, specific for either *Schistosoma mansoni* or *Schistosoma haematobium*. Mitochondrial primers examined any relationship between the supposed brothers. Two independent laboratories confirmed *S. mansoni* DNA from the Nekht-Ankh liver. One laboratory detected *S. haematobium* DNA in both the Nekht-Ankh liver and intestinal samples from Khnum-Nakht in repeat experiments. We believe this is the first verified report of *S. mansoni* in ancient Egypt. Although no *S. haematobium* DNA sequence was obtained, the results support earlier histological findings of *S. haematobium* in ancient Egyptian mummies. These findings demonstrate that both *S. mansoni* and *S. haematobium* were present in Central Egypt during the Middle Kingdom, around 3900 years ago. From the mitochondrial DNA analysis it appears that these two individuals were not maternally related, which is consistent with the morphology of the skulls. The lack of genetic relatedness between these supposed brothers throws light upon the habit of adoption in this society.

2014

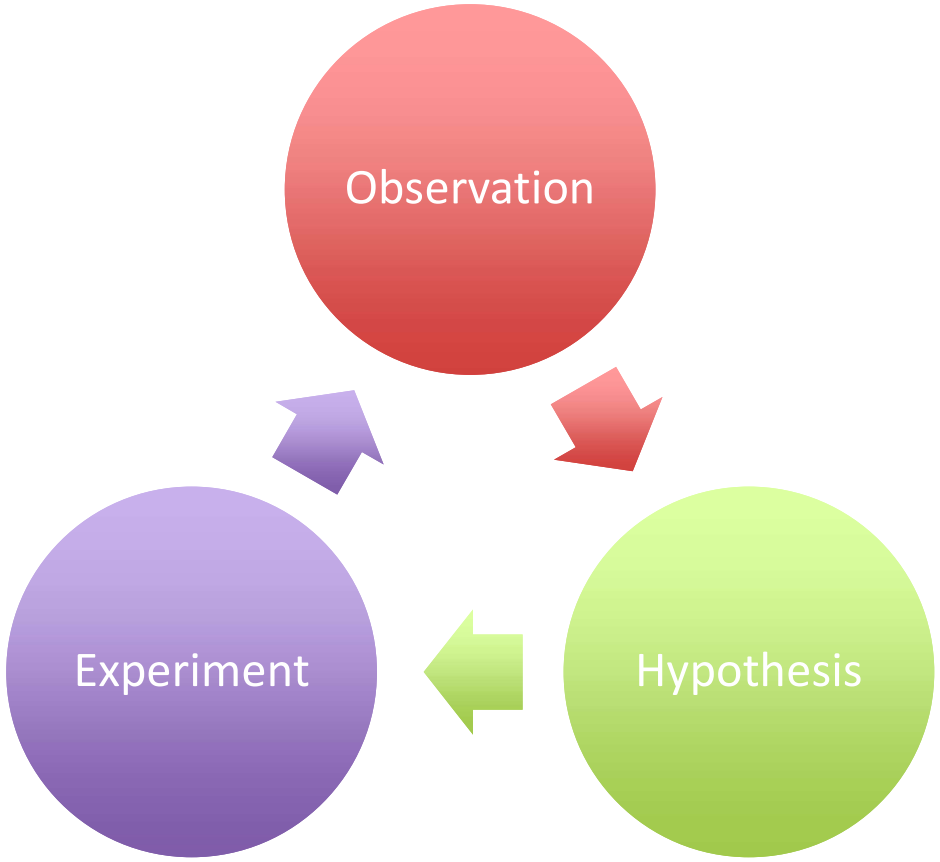
Schistosomiasis: 1851-1916



65 years!

It is a capital mistake to theorize before one has data.

Doyle, A.C. (1887) A Study in Scarlet. *Beeton's Christmas Annual* 28: 1-95



"If Dr. Sambon's view were correct, all of us who have devoted attention to the subject, would have indeed been wandering in the dark since the time of Bilharz himself, fifty-seven years ago."

LOOSS A. (1908) What is "*Schistosomum mansoni*" Sambon 1907? *Annals of Tropical Medicine and Parasitology* 2: 153-191

The legacy

- Geographic spread
  - Out of scope for a single project

OPEN ACCESS Freely available online

PLOS | NEGLECTED TROPICAL DISEASES

Genetic Diversity within *Schistosoma haematobium*: DNA Barcoding Reveals Two Distinct Groups

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Abstract

Background: Schistosomiasis in one of the most prevalent parasitic diseases, affecting millions of people and animals in developing countries. Amongst the human-infective species *S. haematobium* is one of the most widespread causing urogenital schistosomiasis, a major human health problem across Africa, however in terms of research this human pathogen has been severely neglected.

Methodology/Principal Findings: To elucidate the genetic diversity of *Schistosoma haematobium*, a DNA 'barcoding' study was performed on parasite material collected from 41 localities representing 18 countries across Africa and the Indian Ocean Islands. Surprisingly low sequence variation was found within the mitochondrial cytochrome oxidase subunit I (*cox*1) and the NADH-dehydrogenase subunit 1 *snad*1). The 61 haplotypes found within 1978 individual samples split into two distinct groups; one (Group 1) that is predominately made up of parasites from the African mainland and the other (Group 2) that is made up of samples exclusively from the Indian Ocean Islands and the neighbouring African coastal regions. Within Group 1 there was a dominance of one particular haplotype (H1) representing 1574 (80%) of the samples analyzed. Population genetic diversity increased in samples collected from the East African coastal regions and the data suggest that there has been movement of parasites between these areas and the Indian Ocean Islands.

Conclusions/Significance: The high occurrence of the haplotype (H1) suggests that at some point in the recent evolutionary history of *S. haematobium* in Africa the population may have passed through a genetic 'bottleneck' followed by a population expansion. This study provides novel and extremely interesting insights into the population genetics of *S. haematobium* on a large geographic scale, which may have consequence for control and monitoring of urogenital schistosomiasis.

Citation: Webster BL, Emery AM, Webster JP, Gouvras A, Garba A, et al. (2012) Genetic Diversity within *Schistosoma haematobium*: DNA Barcoding Reveals Two Distinct Groups. PLoS Negl Trop Dis 6(10): e1882. doi:10.1371/journal.pntd.0001882

Editor: Aaron R. Jex, University of Melbourne, Australia

Received May 11, 2012; **Accepted** September 12, 2012; **Published** October 25, 2012

Funding: This research was funded by an FP6 (European Community Framework Programme for Research, Technological Development and Demonstration) grant from the European Union. The programme was called CONTRAST (FP6 STREP contract no: 032203), http://www.eu-contrast.eu. This research was funded by the EU grant CONTRAST (FP6 STREP contract no: 032203), http://www.eu-contrast.eu. The authors were supported by The Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) during the writing of the manuscript and from SCAN (http://www.nhm.ac.uk/research-curation/collections/curation-groups/scan/) for covering the publication costs, which is funded by the Wellcome Trust. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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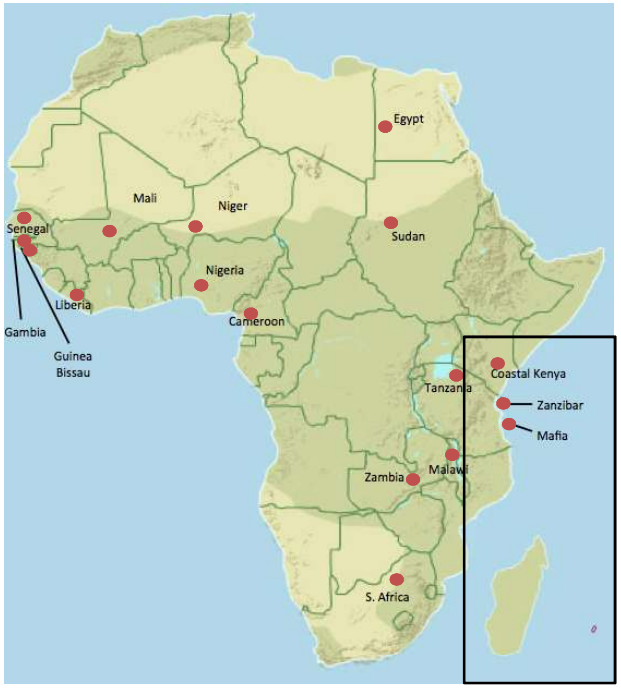
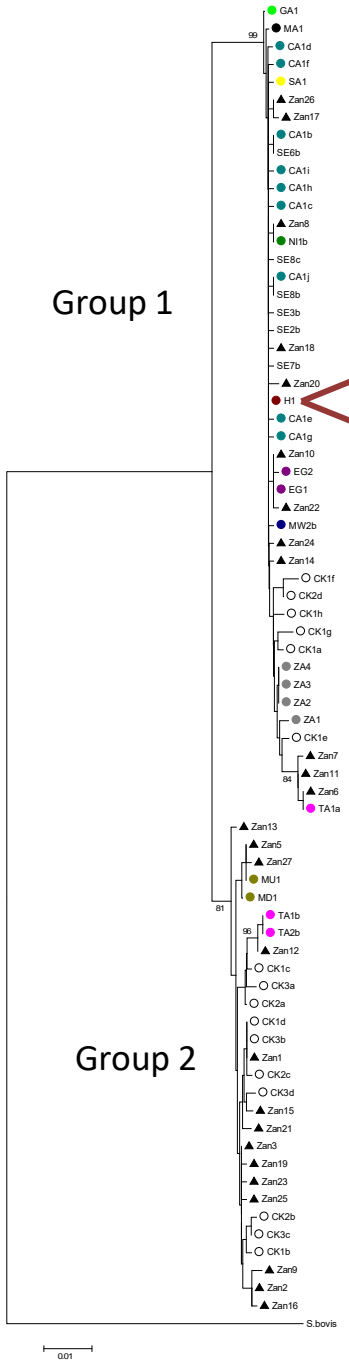
S. haematobium

2000 samples





80% (1574) identical

- Low diversity

Region	Diversity (π)
Mainland Africa	0.00068
Indian Ocean Islands	0.01177
Zanzibar	0.01137

- Higher diversity
- Unique haplotypes to Indian Ocean Islands

Webster e*t al*., 2012. Plos NTD

The legacy

- Geographic spread
  - Out of scope for a single project
- Making samples available for new projects
  - Specimens available without new collections

SCIENTIFIC REPORTS

OPEN

Received: 08 October 2015
Accepted: 13 January 2016
Published: 16 February 2016

Whole genome resequencing of the human parasite *Schistosoma mansoni* reveals population history and effects of selection

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Schistosoma mansoni **is a parasitic fluke that infects millions of people in the developing world. This study presents the first application of population genomics to *S. mansoni* based on high-coverage resequencing data from 10 global isolates and an isolate of the closely-related *Schistosoma rodhaini*, which infects rodents. Using population genetic tests, we document genes under directional and balancing selection in *S. mansoni* that may facilitate adaptation to the human host. Coalescence modeling reveals the speciation of *S. mansoni* and *S. rodhaini* as 107.5–147.6KYA, a period which overlaps with the earliest archaeological evidence for fishing in Africa. Our results indicate that *S. mansoni* originated in East Africa and experienced a decline in effective population size 20–90KYA, before dispersing across the continent during the Holocene. In addition, we find strong evidence that *S. mansoni* migrated to the New World with the 16–19th Century Atlantic Slave Trade.**

Schistosoma mansoni is a dioecious trematode (fluke) and an aetiological agent of the neglected tropical disease schistosomiasis, which infects over 250 million people and causes over 11 thousand deaths annually[1,2]. The trematode has a wide geographic range; the majority of *S. mansoni* infections are found in sub-Saharan Africa and Madagascar, though transmission foci also exist in the Arabian Peninsula, South America and the Caribbean. Chronic pathology is caused when eggs laid by the adult worms, residing in the mesenteric veins, are swept by the bloodstream into internal organs causing an inflammatory response that may result in fibrosis and calcification of the liver and spleen[3]. *Schistosoma mansoni* has a complex lifecycle with an intermediate freshwater-snail host (*Biomphalaria spp.*), consequently its epidemiology is closely tied with water contact and prevalence of the disease is greatest in communities that live close to endemic freshwater lakes and rivers[4].

Since the sequencing of the 380 megabase (Mb) *S. mansoni* reference genome[5], genomic resources have contributed to comparative[6] and functional studies[7], including the discovery of the molecular basis for oxamniquine resistance[8], but little is known about genome-wide variation between parasites in the field. While large populations of *S. mansoni* have been investigated[9–11], all previous studies in this field have been restricted to analysing a small number of markers, typically using the mitochondrial *cox*1 gene, nuclear ITS region or <10 microsatellite loci. In this study we present the first population genomic study of *S. mansoni* based on whole genome re-sequencing data for nine isolates collected from field sites across Africa and the New World. African individuals were selected from distinct clades based on previous phylogenetic data[10] to represent the diversity of extant *S. mansoni*. We also include the NMRI (Puerto Rican) laboratory strain that was used to build the reference genome[5]. In addition we have sequenced *Schistosoma rodhaini*, a rodent schistosome species that is firmly established as the closest outgroup to *S. mansoni*[12,13].

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The legacy

- Geographic spread
  - Out of scope for a single project
- Making samples available for new projects
  - Specimens available without new collections
  - Incidental “unusual” specimens

e.g. *S. intercalatum* & *S. guineensis*

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PLOS | NEGLECTED TROPICAL DISEASES

A *Schistosoma haematobium*-Specific Real-Time PCR for Diagnosis of Urogenital Schistosomiasis in Serum Samples of International Travelers and Migrants

Lieselotte Cnops*, Patrick Soentjens, Jan Clerinx, Marjan Van Esbroeck

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Abstract

Background: Diagnosis of urogenital schistosomiasis by microscopy and serological tests may be elusive in travelers due to low egg load and the absence of seroconversion upon arrival. There is need for a more sensitive diagnostic test. Therefore, we developed a real-time PCR targeting the *Schistosoma haematobium*-specific *Dra1* sequence.

Methodology/Principal Findings: The PCR was evaluated on urine (n = 111), stool (n = 84) and serum samples (n = 135), and one biopsy from travelers and migrants with confirmed or suspected schistosomiasis. PCR revealed a positive result in 7/7 urine samples, 11/11 stool samples and 1/1 biopsy containing *S. haematobium* eggs as demonstrated by microscopy and in 22/23 serum samples from patients with a parasitological confirmed *S. haematobium* infection. *S. haematobium* DNA was additionally detected by PCR in 7 urine, 3 stool and 5 serum samples of patients suspected of having schistosomiasis without egg excretion in urine and feces. None of these suspected patients demonstrated other parasitic infections except one with *Blastocystis hominis* and *Entamoeba* cyst in a fecal sample. The PCR was negative in all stool samples containing *S. mansoni* eggs (n = 21) and in all serum samples of patients with a microscopically confirmed *S. mansoni* (n = 22), *Ascaris lumbricoides* (n = 1), *Ancylostomidae* (n = 1), *Strongyloides stercoralis* (n = 1) or *Trichuris trichuria* infection (n = 1). The PCR demonstrated a high specificity, reproducibility and analytical sensitivity (0.5 eggs per gram of feces).

Conclusion/Significance: The real-time PCR targeting the *Dra1* sequence for *S. haematobium*-specific detection in urine, feces, and particularly serum, is a promising tool to confirm the diagnosis, also during the acute phase of urogenital schistosomiasis.

Citation: Cnops L, Soentjens P, Clerinx J, Van Esbroeck M (2013) A *Schistosoma haematobium*-Specific Real-Time PCR for Diagnosis of Urogenital Schistosomiasis in Serum Samples of International Travelers and Migrants. PLoS Negl Trop Dis 7(8): e2413. doi:10.1371/journal.pntd.0002413

Editor: Patrick J. Lammie, Centers for Disease Control and Prevention, United States of America

Received December 21, 2012; **Accepted** July 27, 2013; **Published** August 29, 2013

Funding: The ITM receives funding from the Ministry of Health for its tasks as reference laboratory for the diagnosis and treatment of infectious and tropical diseases. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Urogenital schistosomiasis due to *Schistosoma haematobium* is a serious underestimated public health problem. It is endemic in 53 countries of the African continent and of the Middle East [1,2]. Adult worms live in the capillary plexus of the bladder and other parts of the urino-genital system and eggs are excreted in the urine and occasionally found in feces.

Diagnosis of *S. haematobium* infections is traditionally done by microscopy but is often unreliable due to the circadian and day-to-day variations in egg excretion, and to low parasite load, especially in the traveler. Antibody-based assays are useful to confirm infection, but do not distinguish active infection from past exposure, and false-negative results occur, mainly in *S. haematobium* infections. Antibody tests are usually negative during acute symptomatic schistosomiasis. Assays that detect circulating antigens seem very promising in the early phase of infection but still lack sensitivity in the diagnosis of light infections [3,4,5,6].

Recently, we developed a genus-specific real-time PCR (further called ‘genusPCR’) that sensitively detect all human infectious *Schistosoma* species in feces and urine [7]. The genusPCR was not able to detect schistosome DNA in serum although molecular analysis of serum is of interest in acute schistosomiasis before detectable levels of eggs are excreted [8–12]. In 2009, Wichmann and colleagues [10] described a real-time PCR, targeting a highly repeated 121-bp sequence of *S. mansoni* (named Sm1-7) to detect cell-free schistosome DNA in serum. This was proven successful in acute and chronic *S. mansoni* infection, but not so much in *S. haematobium* infection. To fill that gap, we developed a real-time PCR specific for the diagnosis of *S. haematobium* in serum samples. The real-time PCR targets *Dra1*, a *S. haematobium*-specific 121-bp repeat sequence originally described by Hamburger *et al.* and present in hundreds to thousands of copies and representing at least 15% of its genome [13]. We first tested this PCR (further called ‘draPCR’) on urine and feces samples to evaluate its species-specificity and its performance in comparison with microscopy, and then on serum samples to determine its potential as diagnostic tool for acute phase schistosomiasis.

New collections

- Collecting larval stages on FTA cards
  - Refinements to Gower et al 2006
- Examples
  - SCORE population genetics
  - ZELS Epidemiology and evolution of zoonotic schistosomiasis in a changing world

Development and application of an ethically and epidemiologically advantageous assay for the multi-locus microsatellite analysis of *Schistosoma mansoni*

C. M. GOWER[1,2*], J. SHRIVASTAVA[1], P. H. L. LAMBERTON[1], D. ROLLINSON[2], B. L. WEBSTER[2], A. EMERY[2], N. B. KABATEREINE[3] *and* J. P. WEBSTER[1]

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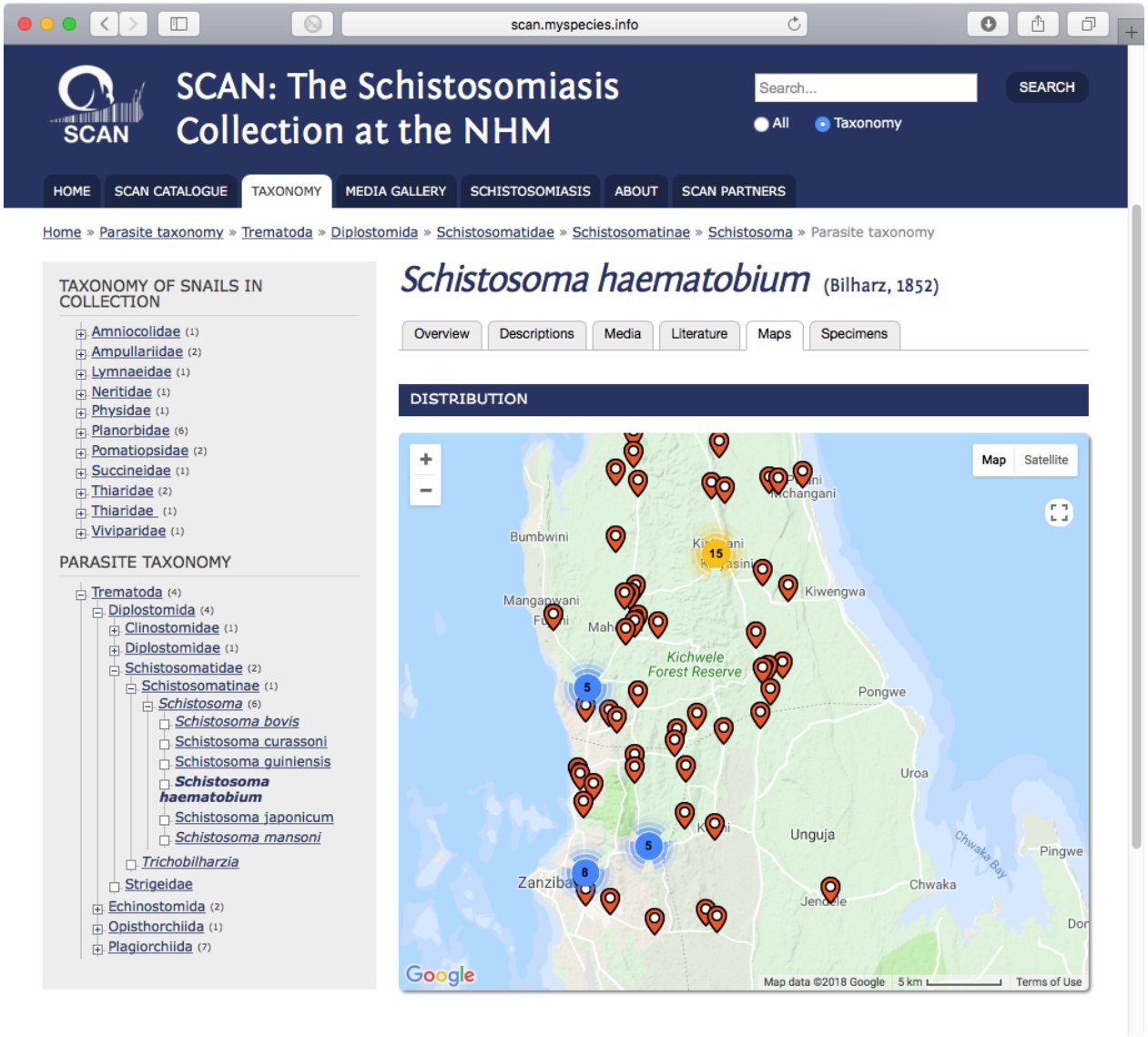
(*Received 28 September 2005; revised 27 April, 18 September and 21 September 2006; accepted 22 September 2006; first published online 13 November 2006*)

SUMMARY

Non-availability of adult worms from living hosts remains a key problem in population genetic studies of schistosomes. Indirect sampling involving passage through laboratory animals presents significant ethical and practical drawbacks, and may result in sampling biases such as bottlenecking processes and/or host-induced selection pressures. The novel techniques reported here for sampling, storage and multi-locus microsatellite analysis of larval *Schistosoma mansoni*, allowing genotyping of up to 7 microsatellite loci from a single larva, circumvent these problems. The utility of these assays and the potential problems of laboratory passage, were evaluated using 7 *S. mansoni* population isolates collected from school-children in the Hoima district of Uganda, by comparing the associated field-collected miracidia with adult worms and miracidia obtained from a single generation in laboratory mice. Analyses of laboratory-passaged material erroneously indicated the presence of geographical structuring in the population, emphasizing the dangers of indirect sampling for population genetic studies. Bottlenecking and/or other sampling effects were demonstrated by reduced variability of adult worms compared to their parent field-collected larval samples. Patterns of heterozygote deficiency were apparent in the field-collected samples, which were not evident in laboratory-derived samples, potentially indicative of heterozygote advantage in establishment within laboratory hosts. Genetic distance between life-cycle stages in the majority of isolates revealed that adult worms and laboratory-passaged miracidia clustered together whilst segregating from field miracidia, thereby further highlighting the utility of this assay.

Key words: schistosome, sampling, laboratory passage, 3 Rs, bottlenecking, miracidia, cercariae, population genetics, multiplex.

Data (see scan.myspecies.info)



New uses for new collections!

Parasitology

cambridge.org/par

Special Issue Research Article

Cite this article: Le Clec'h W et al (2018). Whole genome amplification and exome sequencing of archived schistosome miracidia. Parasitology 1–9. https://doi.org/10.1017/S0031182018000811

Received: 31 January 2018
Revised: 27 March 2018
Accepted: 5 April 2018

Key words:
Exome sequencing; FTA cards; miracidia; quantitative PCR; Schistosoma; whole genome amplification

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Whole genome amplification and exome sequencing of archived schistosome miracidia

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Abstract

Adult schistosomes live in the blood vessels and cannot easily be sampled from humans, so archived miracidia larvae hatched from eggs expelled in feces or urine are commonly used for population genetic studies. Large collections of archived miracidia on FTA cards are now available through the Schistosomiasis Collection at the Natural History Museum (SCAN). Here we describe protocols for whole genome amplification of Schistosoma mansoni and Schistosome haematobium miracidia from these cards, as well as real time PCR quantification of amplified schistosome DNA. We used microgram quantities of DNA obtained for exome capture and sequencing of single miracidia, generating dense polymorphism data across the exome. These methods will facilitate the transition from population genetics, using limited numbers of markers to population genomics using genome-wide marker information, maximising the value of collections such as SCAN.

Matters arising

- Ownership and intellectual property
  - Agreements, MoUs, MTAs, etc

- Access and benefit sharing: The Nagoya Protocol to the Convention on Biological Diversity
  - Sovereign rights over genetic resources
  - https://www.cbd.int/abs/about/default.shtml/

Next steps

- Linked post with SCI. Can we incorporate genetic sampling into M&E and demonstrate its value?
- Consider new collection methods and build sample and data sets that the research community wants
  - Expand pre- and post- treatment sampling?
- How should SCAN evolve?
  - More than a repository: Expertise, facilities and support

Thanks

Thanks to our many collaborators!


NATURAL
HISTORY
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