Schistosomes of small mammals from the Lake Victoria Basin, Kenya: new species, familiar species, and implications for schistosomiasis control

B. HANELT[1]*, I. N. MWANGI[2], J. M. KINUTHIA[2], G. M. MAINA[2], L. E. AGOLA[2], M. W. MUTUKU[2], M. L. STEINAUER[1], B. R. AGWANDA[3], L. KIGO[3], B. N. MUNGAI[2], E. S. LOKER[1] *and* G. M. MKOJI[2]

[1] *Department of Biology, University of New Mexico, MSC03 2020, Albuquerque, New Mexico, 87131-0001, USA*
[2] *Center for Biotechnology Research and Development, Kenya Medical Research Institute, Mbagathi Road, P.O. Box 54840-00200, City Square, Nairobi, Kenya*
[3] *National Museum of Kenya, Zoology Department, Mammal Section, Museum Hill Road, P.O. Box 40658, Westlands, Nairobi, Kenya*

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SUMMARY

Recent schistosomiasis control efforts in sub-Saharan Africa have focused nearly exclusively on treatment of humans with praziquantel. However, the extent to which wild mammals act as reservoirs for *Schistosoma mansoni* and therefore as sources of renewed transmission following control efforts is poorly understood. With the objective to study the role of small mammals as reservoir hosts, 480 animals belonging to 9 rodent and 1 insectivore species were examined for infection with schistosomes in Kisumu, in the Lake Victoria Basin, Kenya. Animals were collected from 2 sites: near the lakeshore and from Nyabera Marsh draining into the lake. A total of 6·0% of the animals captured, including 5 murid rodent species and 1 species of shrew (*Crocidura olivieri*) were infected with schistosomes. Four schistosome species were recovered and identified using *cox*1 DNA barcoding: *S. mansoni*, *S. bovis*, *S. rodhaini* and *S. kisumuensis*, the latter of which was recently described from Nyabera Marsh. *Schistosoma mansoni* and *S. rodhaini* were found infecting the same host individual (*Lophuromys flavopunctatus*), suggesting that this host species could be responsible for the production of hybrid schistosomes found in the area. Although the prevalence of *S. mansoni* infection in these reservoir populations was low (1·5%), given their potentially vast population size, their impact on transmission needs further study. Reservoir hosts could perpetuate snail infections and favour renewed transmission to humans once control programmes have ceased.

Key words: schistosomiasis control, rodents, insectivores, reservoir hosts, Africa, Kenya, *Schistosoma kisumuensis*, *Schistosoma mansoni*, *Schistosoma bovis*, *Schistosoma rodhaini*, control, barcoding.

INTRODUCTION

Schistosomiasis has been described as a ''three factor disease'' involving schistosomes, snails and humans (Kloos, 1985). However, a fourth factor, involving reservoir hosts, could maintain the infection in nature and serve as sources of infection for humans, thus may have potentially significant implications for transmission, parasite hybridization and control efforts. In East Africa, *Schistosoma mansoni* and *S. haematobium*, the two predominant species responsible for human schistosomiasis, have been found in many domestic mammals (Nelson, 1960; Nelson *et al*. 1962; Ouma and Fenwick, 1991). However, for these infected mammals to be considered true reservoir hosts, they must also pass viable and infective eggs and maintain the life cycle of the parasite (Duplantier and Sene, 2006).

Whereas *S. haematobium* has only been found in a few non-human primates and in a small number of rodents (Ouma and Fenwick, 1991), *S. mansoni* has been found in numerous host species, including rodents, in which the parasites mature and produce large numbers of viable eggs (McMahon and Baalawy, 1967; Kawashima *et al*. 1978; Rodrigues-Silva *et al*. 1992). Rodents are capable of sustaining natural populations of *S. mansoni* with little transmission input by humans (Théron *et al*. 1992), and have been implicated as important reservoir hosts in South America, the Caribbean and West Africa (D'Andrea *et al*. 2000; Duplantier and Sene, 2000; Gentile *et al*. 2006). However, the role of small mammals in East Africa, especially in the Lake Victoria Basin where rates of incidence are extremely high, requires additional investigation (Nelson, 1983).

In the present study, a survey of schistosomes was conducted in small mammals collected from sites in

* Corresponding author: Department of Biology, University of New Mexico, MSC03 2020, Albuquerque, New Mexico, 87131-0001, USA. Tel: +505 2773174. Fax: +505 2770304. E-mail: bhanelt@unm.edu

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Table 1. Collection localities, rodent/insectivore numbers, and prevalence of schistosomes

Site name	GPS coordinates	Rodents/ insectivores	Number infected
Kisumu Lake Sites			
ADC Farm	−0·0884, 34·7538	43	0
Car wash	−0·0959, 34·7492	2	0
Tilapia beach	−0·0937, 34·7522	28	0
Power house	−0·0926, 34·7529	80	1
Nawa	−0·0941, 34·7076	2	0
Other Lake Sites			
Rota	−0·0964, 34·6803	1	0
Usare	−0·1057, 34·6743	2	0
Nyabera Swamp Sites			
Nyabera West	−0·1086, 34·7731	38	1
Nyabera East	−0·1088, 34·7748	280	24
Kachok	−0·1957, 34·7724	4	0

Kisumu city in the Lake Victoria Basin, western Kenya. The aims of this study were to determine if schistosome species infect local small mammals, to identify species able to host schistosomes, and to determine parasite prevalence and intensity. In addition, we explored whether these hosts can become coinfected with multiple schistosome species. Finally, we consider and discuss the implications of our findings with respect to schistosomiasis control.

MATERIALS AND METHODS

Small mammal collection and examination

Small mammals were collected in western Kenya from habitats near the shore of Lake Victoria, and from Nyabera Marsh within the Kisumu city boundaries (Table 1), using Sherman's rodent traps (Tallahassee, FL, USA). Traps were baited with a mixture of peanut butter and cupcakes (locally known as queen cakes). Traps were checked at dusk and dawn, and captured animals were returned live to the laboratory in Kisian, Kenya, 12 km west of Kisumu. Collections were done about once monthly between January 2007 and December 2008, with each collection session lasting about 5 days. For all captured individuals, sex, weight and several size measurements were taken to aid in taxonomic identification.

Small mammals were anaesthetized with ether, and injected with 0·5 ml of a mixture of 26% pentobarbital containing 100 units of heparin. The abdominal cavity of each animal was opened and worms were recovered from the hepatic portal system by perfusion (Lewis, 1998). A liver press was made from each animal and examined using a compound microscope to determine if schistosome eggs were present. After dissection, carcasses were preserved in 95% ethanol and submitted to the National Museum of Kenya (NMK), Mammal Section, for identification. Rodents and insectivores were identified and placed in the NMK permanent collection (NMK Accession numbers: 16527–16800, 167802–167823, 168269–168356, 169349–169422).

Processing of worms

The worms recovered from the dissected animals were placed in 100% ethanol, and stored at 4 °C for 2–10 weeks. The worms were then photographed, before the posterior 10–15% of each worm was removed, retained in 100% ethanol, and saved for DNA analysis. Genomic DNA from whole or parts of adult worms, was extracted using a modified HotShot method (Truett *et al*. 2000; Steinauer *et al*. 2008*b*), and was stored at 4 °C until polymerase chain reaction (PCR) amplification.

Partial sequences of the *cox*1 mDNA were amplified using TaKaRa Ex Taq (Takara Bio Inc., Otsu, Japan) following the manufacturer's instructions and using the following primers: '*cox*1F4', ATT TGG WAC TGC TTT TTT TGA GCC, and '*cox*1 Schist 3' TAA TGC ATA CGG AAA AAA ACA (Lockyer *et al*. 2003). PCR reactions were analysed by agarose gel electrophoresis: 1·0% agarose gels were stained with 0·5% GelRed™ Nucleic acid gel stain (Biotium, Hayward, CA, USA), and visualized on a UV transilluminator. Amplicons were purified by ethanol precipitation and products were sequenced with BigDye version 3.1 kit (Applied Biosystems, Foster City, CA, USA) in an ABI 3130x sequence analyzer (Applied Biosystems). The resulting DNA sequences were verified by aligning reads from both 5′- and 3′ directions, using Sequencher software version 4.9 (Gene Codes, Ann Arbor, Michigan), and manually corrected for ambiguous base calls.

Data analysis

In addition to 47 newly collected sequences, 3 GenBank sequences from 3 species were included as

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Table 2. Numbers of rodents and insectivore species collected at ten sites in the Lake Victoria Basin

	Kisumu Lake Sites					Other Lake Sites		Nyabera Swamp Sites		
	ADC Farm	Car wash	Tilapia beach	Power house	Nawa	Rota	Usare	West	East	Kachok
Order Rodentia										
Aethomys kaiseri			1	1				3	36	
Aethomys sp.	2								3	
Arvicanthis niloticus			1			1			1	
Dasymys incomptus							1		12	
Dasymys sp.									2	
Lemniscomys striatus	5		9	6						
Lophuromys flavopunctatus	12		8	26			1	7	6	
Lophuromys sp.	1			8						
Mastomys natalensis	18		4	12				7	31	2
Mastomys sp.	5			6					2	
Mus minutoides		2		2				1	3	
Mus sp.				3						
Otomys sp.				1						
Pelomys isseli								10	126	1
Pelomys sp.									3	
Rattus rattus									1	
Unknown								3	1	
Order Insectivora										
Crocidura sp.			5	15				1	9	
Crocidura olivieri					2			6	44	1

reference samples (*S. mansoni* AJ519524, *S. bovis* AJ519521 and *S. rodhaini* AY157202). A reference sample for *S. kisumuensis* could not be included, since the species was described from the same material considered in the present study (Hanelt *et al*. 2009). New sequences are available in the 'Schistosomes of Kenyan Rodents and Insectivores' project on the BOLD database (www.barcodinglife.org) and on GenBank (Accession numbers: GU294793–GU294839). Alignment was performed by eye and was trivial, as there were no insertions or deletions present in the fragments sequenced. The Kimura 2-parameter (K2P) model of base substitution (Kimura, 1980) was used as a measure of pairwise sequence distances. To visualize these distances, a neighbour-joining (NJ) tree was produced in MEGA4 (Tamura *et al*. 2007). A bootstrap test was done using 500 replicates. The goal of this analysis was to cluster individuals into similar groups for identification purposes, and the resulting tree should not be interpreted as a phylogenetic hypothesis. Worm species identifications were made according to clustering with the reference sequences.

RESULTS

Small mammals

Rodents of 10 genera and insectivores of 1 genus were collected from 3 different collection sites (Table 1) located within the city of Kisumu. The rodents comprised 9 identified species and 8 unidentified groups (Table 2). Overall, the most abundant species in the study area were *Pelomys isseli* (Issel's groove-toothed swamp rat), *Mastomys natalensis* (Natal multimammate mouse), *Lophuromys flavopunctatus* (Yellow-spotted brush-furred rat), and *Crocidura olivieri* (African giant shrew). *Mastomys natalensis* was common across all collection sites, whereas *P. isseli* was only collected from Nyabera Marsh, and *L. flavopunctatus* was predominantly collected from the lake sites.

Schistosome genetic data

A *cox*1 amplicon for schistosomes was obtained from all 47 individuals collected. Sequence alignments comprised 607 bp. No insertions, deletions or stop codons were observed in any sequence. The lack of stop codons, the consistent length of all sequences and the lack of variance between the 2 sequencing primers suggests that the obtained sequences were coding *cox*1 and not NUMTs (nuclear mitochondrial DNA) (Buhay, 2009).

Parasites

The results of the overall NJ analysis of distances among the 47 individuals are summarized in Fig. 1. Sequences clustered into 4 groups, representing 4 schistosome species, *S. mansoni* Sambon, 1907, *S. rodhaini* Brumpt, 1931, *S. bovis* (Sonsino, 1876) Blanchard, 1895 and *S. kisumuensis* Hanelt *et al*. 2009.

Of the 397 rodents and 83 insectivores examined, 24 rodents (6·8%) and 2 shrews (2·4%) harboured

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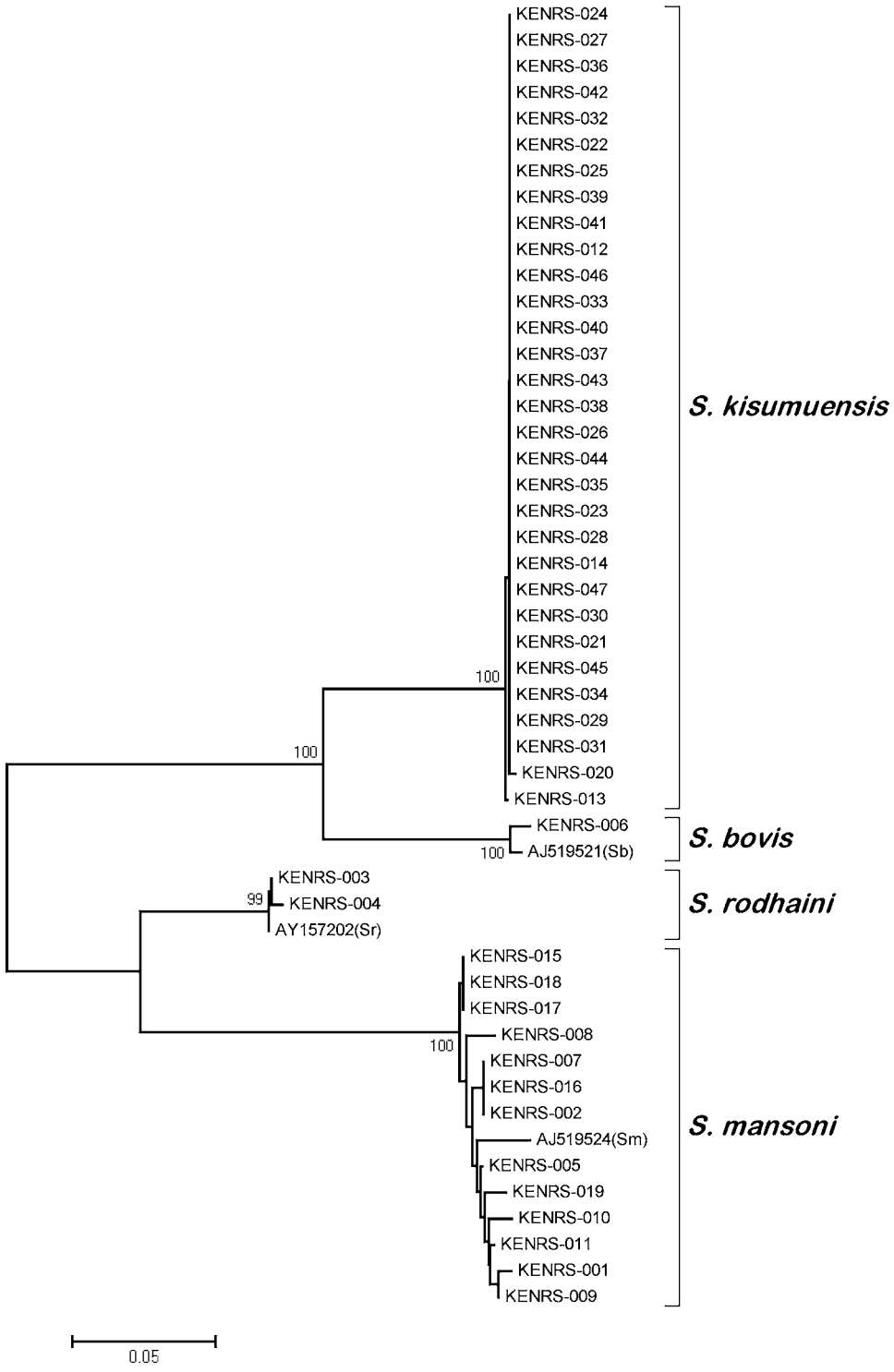

Fig. 1. Neighbour-joining tree based on *cox*1 barcode nucleotide distances (K2P) for schistosomes recovered from rodents. Bootstrap values ⩾95% given, together with a K2P distance scale bar.

schistosomes (Table 3). The large majority of infected rodents (96·6%) and all of the infected shrews were collected from Nyabera Marsh; 67% of all animals were collected at Nyabera Marsh. Only a single infected rodent was found outside of Nyabera, at the Power house site (Table 3, host #3). Worms were not recovered from 3 hosts containing eggs in their liver. This indicates either error in the worm collection protocol or worm mortality.

Of the 26 hosts from which adult worms were recovered, *S. kisumuensis* was the most common schistosome encountered (65·9%), followed by *S. mansoni* (27·7%), *S. rodhaini* (4·3%), and *S. bovis* (2·1%). Intensities ranged from 1 to 5 (mean = 1·81).

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Table 3. Hosts infected with schistosome adults and/or eggs

(N East: Nyabera East; N West: Nyabera West, PH: Power house; Sm: *Schistosoma mansoni*; Sr: *S. rodhaini*; Sb: *S. bovis*, Sk: *S. kisumuensis*; E: immature eggs; L: lateral-spined egg; T: terminal-spined egg; NE: no eggs seen; NA: no adults recovered.)

Host						Worm		
Number	NMK Cat. number	Species	Liver Exam	Site	Date	ID	Sex	Species
1	15962	*Crocidura olivieri*	NE	N East	21 Feb 2007	KENRS-001	M	Sm
2	15968	*Pelomys isseli*	NE	N East	21 Feb 2007	KENRS-005	F	Sm
3	15970	*Lophuromys flavopunctatus*	NE	PH	22 Feb 2007	KENRS-002	F	Sm
						KENRS-003	M	Sr
						KENRS-004	F	Sr
4	169428	*Pelomys isseli*	NE	N East	20 June 2007	KENRS-013	M	Sk
5	167902	*Mastomys natalensis*	E	N East	22 June 2007		NA	
6	16514	*Mastomys natalensis*	NE	N East	10 July 2007	KENRS-006	F	Sb
7	16517	*Aethomys kaiseri*	NE	N East	10 July 2007	KENRS-007	F	Sm
8	16530	*Mastomys natalensis*	NE	N East	12 July 2007	KENRS-008	M	Sm
9	16548	*Mastomys natalensis*	L	N East	23 August 2007	KENRS-009	F	Sm
						KENRS-010	M	Sm
						KENRS-011	M	Sm
10	16522	*Pelomys isseli*	T	N East	24 August 2007		NA	
11	16593	*Pelomys isseli*	NE	N East	21 September 2007	KENRS-012	M	Sk
12	16791	*Pelomys isseli*	NE	N East	13 December 2007	KENRS-014	M	Sk
13	16695	*Mastomys natalensis*	L	N West	19 October 2007	KENRS-015	M	Sm
						KENRS-016	M	Sm
						KENRS-017	M	Sm
						KENRS-018	F	Sm
						KENRS-019	F	Sm
14	167811	*Pelomys isseli*	T	N East	14 December 2007	KENRS-020	M	Sk
15	167821	*Pelomys isseli*	NE	N East	14 December 2007	KENRS-021	M	Sk
16	167807	*Pelomys isseli*	NE	N East	15 December 2007	KENRS-022	F	Sk
						KENRS-023	F	Sk
17	167806	*Dasymys incomptus*	NE	N East	15 December 2007	KENRS-024	M	Sk
18	16593	*Pelomys isseli*	T	N East	21 September 2007		NA	
19	168316	*Pelomys isseli*	T	N East	12 September 2008	KENRS-025	M	Sk
						KENRS-026	F	Sk
						KENRS-027	M	Sk
20	168295	*Pelomys isseli*	T	N East	16 September 2008	KENRS-028	M	Sk
						KENRS-029	F	Sk
21	168313	*Pelomys isseli*	NE	N East	16 September 2008	KENRS-030	M	Sk
22	169358	*Pelomys isseli*	T	N East	4 December 2008	KENRS-031	M	Sk
						KENRS-032	F	Sk
						KENRS-033	F	Sk
23	169362	*Pelomys isseli*	NE	N East	5 December 2008	KENRS-034	F	Sk
						KENRS-035	F	Sk
24	169389	*Pelomys isseli*	NE	N East	5 December 2008	KENRS-036	M	Sk
25	169364	*Pelomys isseli*	NE	N East	6 December 2008	KENRS-037	M	Sk
						KENRS-038	M	Sk
26	169350	*Pelomys isseli*	T	N East	8 December 2008	KENRS-039	M	Sk
						KENRS-040	M	Sk
						KENRS-041	M	Sk
27	169418	*Crocidura* sp.	NE	N East	11 December 2008	KENRS-042	M	Sk
						KENRS-043	M	Sk
						KENRS-044	M	Sk
28	169370	*Pelomys isseli*	T	N East	11 December 2008	KENRS-045	M	Sk
						KENRS-046	F	Sk
29	169390	*Pelomys isseli*	NE	N East	20 December 2008	KENRS-047	M	Sk

The 2 infected shrews harboured 1 worm species each, *S. mansoni* (host #1) and *S. kisumuensis* (host #27). One rodent host contained a mixed schistosome species infection of *S. mansoni* and *S. rodhaini* (host #3). Male to female sex ratios were 1·16 for *S. mansoni*, and 2·44 for *S. kisumuensis*.

Genetic differences between *S. kisumuensis* individuals (all collected at Nyabera, but some collected more than a year apart) were extremely low, containing only 3 polymorphic sites (*cox*1 genetic distance $<0{\cdot}01\%$). On the other hand, genetic differences within the *S. mansoni* population collected

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at Nyabera were much higher, containing 28 polymorphic sites (*cox*1 genetic distance = 1·07%).

DISCUSSION

The role of rodents as reservoir hosts for *S. mansoni* has been well established in South America, and the Caribbean Islands. For example, a comprehensive study of *S. mansoni* on the island of Guadeloupe suggested complicated transmission dynamics between the intermediate host, humans and the Black rat *Rattus rattus* (for a review, see Théron and Pointier, 1995). Sylvatic cycles in which rats serve as the primary (or perhaps only) definitive host exist in this region (Théron, 1984; Sire *et al*. 1999; Prugnolle *et al*. 2002). In Brazil, longitudinal studies of *S. mansoni* in the Scaly-footed water rat, *Nectomys squamipes*, suggested that the rodent host can increase parasite transmission and spread infection to new areas (D'Andrea *et al*. 2000; Gentile *et al*. 2006). However, the role of rodents in the transmission of schistosomes in East Africa has received little attention.

In Africa, *S. mansoni* is frequently recovered from naturally infected small mammals (for reviews see Nelson, 1960; Nelson *et al*. 1962; Pitchford, 1977; Kawashima *et al*. 1978). However, compared with the relatively high prevalences found among rodents in the New World, ranging from 30% to 100% (Théron *et al*. 1992; Rey, 1993; D'Andrea *et al*. 2000), prevalences reported from Africa are usually much lower, less than 5% (for example, see Schwetz, 1954, 1956; Nelson, 1960; Pitchford and Visser, 1962; McMahon and Baalawy, 1967; Karoum and Amin, 1985). Only a single report, from eastern Kenya (Kawashima *et al*. 1978), describes higher prevalences, up to 44% in *Pelomys* sp.

In Kisumu, Kenya, we recorded a relatively low overall schistosome prevalence (6·0%). However, 27% of the schistosome-infected rodents and shrews harboured *S. mansoni*, and nearly a third of these were infections consisting of both male and female worms. Surprisingly, we found 2 schistosome species in shrews, *S. kisumuensis* and *S. mansoni*; the host record of *C. olivieri* for *S. kisumuensis* is new. Shrews have previously been reported to be infected with *S. mansoni*, in the Democratic Republic of the Congo (Stijns, 1952) and Egypt (Kuntz, 1958). Although the current study and Kuntz (1958) found only immature worms, Stijns (1952) observed fully developed eggs in the host's intestine. Thus, shrews cannot be ruled out as possible reservoir hosts.

Of the 4 rodent species infected with *S. mansoni*, the most likely to contribute to natural transmission is *M. natalensis*, because it was the most common host for *S. mansoni*, it contained the majority of the *S. mansoni* worms recovered, and 2 of these hosts contained mature male and female worms, and mature *S. mansoni* eggs in the liver. *Mastomys natalensis* is semi-domestic, found in close association with humans (Isaacson, 1975), is tolerant of habitat modification, it readily follows roads and is often transported around Africa accidentally by vehicles (Granjon *et al*. 2008). *Mastomys natalensis* has been noted to be a very clean animal, pushing refuse out of the nests; droppings have rarely been found in its burrows (Isaacson, 1975). In most parts of its range, it is considered a major pest (Sluydtsa *et al*. 2009). It is widespread throughout sub-Saharan Africa, except for areas in the south-western portion of the continent. Previous work suggests that *M. natalensis* can host *S. mansoni* throughout its range, including Kenya (Nelson *et al*. 1962), northern South Africa (Pitchford, 1959; Pitchford and Visser, 1962) and the Democratic Republic of the Congo (Schwetz, 1954, 1956). Furthermore, experimental infections of field-derived *M. natalensis* exposed to *S. mansoni* in the field (Pitchford and Visser, 1962) and laboratory (for example, see Lämmler and Petranyi, 1971), produced mature worms that passed large numbers of eggs, a finding which has led some to use this mouse as a model schistosome host. In nature, *M. natalensis* could be infected by direct contact with water, as they are known to be good swimmers (Isaacson, 1975), or by the ingestion of infected snails, which has been shown possible with *Mastomys coucha* (Luttermoser, 1963).

With an infection prevalence of 7·5% and high population densities, *M. natalensis* could contribute significantly to schistosome eggs in the environment. Studies of rodent population densities conducted in mosaic-structured agricultural environments have measured 50–150 *M. natalensis* per ha (Makundi *et al*. 2007; Sluydtsa *et al*. 2009). Assuming that Nyabera Marsh and the connected, downstream Dunga Marsh (2500 ha) contain an average of 100 mice/ha, with an infection rate similar to that found in our study, there may be as many as 18 750 *M. natalensis* mice infected with *S. mansoni* in and around the marshes surrounding Kisumu. Again, compared to the large number of people infected with *S. mansoni* in Kisumu, rodents probably play but a minor role in transmission. However, it is nearly impossible to envision that the many, and largely uncharacterized numbers of eggs shed by rodents each day do not lead to the infection of snails and subsequent transfer to humans. Although small, murine-snail transmission could potentially be of considerable significance in areas with active schistosomiasis control programmes, since the transmission from rodents to snails would not be interrupted. This could serve to maintain transmission and a source of new infections or reinfections to humans after successful treatment.

Another schistosome we encountered was *S. rodhaini*, which is endemic within the Lake Victoria Basin (Saoud, 1966; Handzel *et al*. 2003; Morgan

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et al. 2003) and was first discovered in western Kenya in the early 1960s (Nelson *et al*. 1962). Its rediscovery in the area in snails during earlier surveys (Mungai *et al*. 2003; Steinauer *et al*. 2008*c*), and its occurrence in a rodent examined during the present survey suggest that the parasite remains present in western Kenya. Prevalence of *S. rodhaini* in the mammals surveyed was much lower than that of *S. mansoni*, which is surprising because *S. rodhaini* is considered a rodent parasite. However, the prevalence of *S. rodhaini* in snails was also low and temporally very sporadic (Steinauer *et al*. 2008*c*). The possibility that other mammals transmit *S. rodhaini* in the Kisumu area or that *S. rodhaini* is transient in the Kisumu area cannot be ruled out, and deserves further investigation.

The presence of *S. rodhaini* and *S. mansoni* together in at least one *L. flavopunctatus* individual further confirms that the two parasite species can co-occur in the same host, and indicates a potential host in which hybridization can occur. Hybrids have been found in snails in the Lake Victoria Basin, including at the same site where this individual rodent host was collected (Morgan *et al*. 2003; Steinauer *et al*. 2008*a*). Although *S. rodhaini* rarely infects humans (D'Haenens and Santele, 1955) and is not considered to be of public health significance, its interactions with *S. mansoni* in rodents might be significant with respect to the epidemiology of human schistosomiasis. Hybridization of pathogenic organisms could potentially result in the creation of new lineages or adaptive gene introgression (movement of advantageous alleles between species). Through this process, *S. mansoni* could obtain novel alleles from *S. rodhaini* that may alter characteristics such as virulence patterns, and ability to invade new host species or habitats (Arnold, 2004; Steinauer *et al*. 2008*c*). Introgression of neutrally evolving genes from *S. rodhaini* to *S. mansoni* has been reported in western Kenya, and thus indicates the potential for adaptive introgression (Allison and Seeley, 2004; Steinauer *et al*. 2008*c*). *S. rodhaini* and its hybrids with *S. mansoni* might also be of public health significance owing to the HIV pandemic affecting especially the Lake Victoria Basin (Allison and Seeley, 2004), since they (and possibly *S. kisumuensis* and *S. bovis*) could potentially break species barriers to become an opportunistic infection in immuno-compromised individuals. These findings highlight the need for additional studies of potential hybrids from rodent populations in endemic areas where *S. mansoni* and *S. rodhaini* are sympatric.

Based on the relatively low prevalence rates of *S. mansoni* in rodents in sub-Saharan Africa (including the current study), compared to the much higher rates in the New World, the importance of rodents in the transmission and maintenance of *S. mansoni* has been gauged from negligible (Nelson, 1960; Pitchford and Visser, 1962) to important (Ouma and Fenwick, 1991; Duplantier and Sene, 2000). Although the participation of rodents compared with humans in Africa appears to be considerably lower, several facts suggest that these reservoir hosts should be taken into consideration in future schistosomiasis control programmes. First, as noted above, several rodent species, including *M. natalensis*, are good hosts for *S. mansoni*. Second, studies have noted that *S. mansoni* has few deleterious effects on rodent hosts (D'Andrea *et al*. 2000), and in some cases, rodents (*Arvicanthis niloticus*, Nile rat) collected in the field and exposed and maintained in the laboratory, continued passing large numbers of eggs for up to 2·5 years (Kuntz and Malakatis, 1955), a time-period which could easily outlast some human treatment programmes. Third, although the infection rate with *S. mansoni* is low, the number of rodents per ha and the cohabitation of rodents such as *M. natalensis* with humans makes parasite transmission to humans a greater possibility. Finally, since this and other studies have found that rodents are capable of simultaneously hosting *S. rodhaini* and *S. mansoni*, they appear to be providing 'theatres' allowing interspecific interactions, which, as mentioned above, can lead to hybridization or genetic introgression (Arnold, 2004; Arnold *et al*. 2008).

Nyabera Marsh, at just over 5 ha, is a habitat with a remarkably complex pattern of schistosome species and transmission. Our rodent survey revealed the presence of 3 schistosome species: *S. mansoni*, *S. bovis* and *S. kisumuensis*. In addition, snail surveys at the same site, have identified the presence of *S. rodhaini* (Steinauer *et al*. 2008*c*), *S. haematobium* (Hanelt, personal observations), and avian schistosomes (Brant, personal observation). The presence in Nyabera of both *Biomphalaria* and *Bulinus* snails and the intense use of this peri-urban habitat by humans, domestic and wild animals contribute to the remarkable ability to support 5 *Schistosoma* life cycles. Similar habitats adjacent to African towns are by no means an uncommon occurrence and, if afforded the same degree of sampling intensity as undertaken in Nyabera, may yield similarly diverse transmission patterns.

Modern quantitative studies emphasizing the role of small mammals in serving as a source for snail infections are needed now that large-scale schistosomiasis control programmes are underway (Fenwick *et al*. 2009). Reservoir hosts may prove able to maintain cycles of infection until after successful chemotherapeutic intervention, thus providing a means for parasites like *S. mansoni* to re-infect humans. Alternatively, their role may prove to be neutral if they are unproductive relative to egg production or if they have separate cycles of *S. mansoni* transmission independent of those in humans. These alternatives need to be examined in a variety of African transmission settings. Thus, we believe that more focused studies on the role of reservoir hosts in

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Africa are needed, especially in areas with active schistosomiasis control programmes.

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