demarcated boundary of erythema with a tiny scab (Figure, panel D).

A new species of *Rickettsia* was detected from leeches in Japan (*5*,*6*). Furthermore, certain leech species, parasitizing frogs or fish, can complete the vertical transmission of *Rickettsia* spp. with possible horizontal transmission (*6*). The leech is reported to be a potential vector for human rickettsial infections (*7*,*8*). Slesak et al. described the case of a 39-year-old woman with *R. felis* infection confirmed by eschar PCR after a leech bite in northern Laos (*7*). Balcells et al. reported the case of a 54-year-old man with scrub typhus–like illness after a leech bite in southern Chile (*8*). In our previous study (*4*), 13% (4/31) of patients with Japanese spotted fever and 2% (4/188) of patients with scrub typhus diagnosed by serologic tests had a history of land leech bite before the symptom onset.

Our report is limited because we did not have the land leech for testing by PCR. The patient might have had rickettsia on his skin and then been inoculated by the leech bite or by scratching after the bite (*7*). Further investigations, including an experimental model, are needed to support the potential role of leeches in the transmission of *R. japonica* and other *Rickettsia* spp.

Acknowledgments

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Schistosome Interactions within the *Schistosoma haematobium* Group, Malawi

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Molecular analysis of atypical schistosome eggs retrieved from children in Malawi revealed genetic interactions occurring between human (*Schistosoma haematobium*) and livestock (*S. mattheei* and *S. bovis*) schistosome species. Detection of hybrid schistosomes adds a notable new perspective to the epidemiology and control of urogenital schistosomiasis in central Africa.

Urogenital schistosomiasis is a waterborne disease transmitted by certain freshwater snails that occurs throughout much of sub-Saharan Africa. Until recently, this disease was attributed solely to *Schistosoma haematobium*, which was considered to have limited zoonotic potential (*1*). However, genetic analysis of natural infections with noninvasive larval sampling (*2*) has provided new evidence. In West Africa, for example, species interactions with hybrid combinations of *S. haematobium* and the bovine or ovine species of *S. bovis* and *S. curassoni* are commonly encountered in humans and snails (*3*). Although key biologic features of hybrids may not always be apparent, the risk for zoonotic transmission along with enhanced definitive and intermediate host compatibilities needs investigation (*2,3*). The recent emergence and persistent transmission of *S. haematobium–bovis* hybrids on the Mediterranean island of Corsica (*4*) demonstrates the public health impact of such genetic introgression.

Genetic analysis of *S. haematobium* group species in central and southern Africa is a high priority. Atypical egg morphologies suggest a capacity for natural hybridization of *S. haematobium* with the bovine species *S. mattheei*, later confirmed with biochemical markers and experimental infections demonstrating viable progeny (*3*). During ongoing surveillance of urogenital schistosomiasis in Chikhwawa District, Malawi, we encountered atypical *S. haematobium* eggs in urine samples from several infected children (*5*). We report the further genetic characterization of atypical eggs collected from epidemiologic surveys of

children within Chikhawa, Nsanje, and Mangochi Districts (Figure, panel A).

Ethics approvals for the epidemiological surveys were granted by Liverpool School of Tropical Medicine, College of Medicine, Malawi, and Ministry of Health and Population, Malawi. All children found infected were treatment with praziquantel.

We filtered schistosome eggs from the urine of infected children, then photographed and measured them before storing them on Whatman FTA cards for molecular analysis (*2*). We alkaline-eluted and genotyped DNA from individual eggs using both the mitochondrial cytochrome oxidase subunit 1 (*cox*1) and the nuclear ribosomal internal transcribed spacer (rITS) DNA regions (*2*) (Appendix Table, https://wwwnc.cdc.gov/EID/article/25/6/19-0020-App1. pdf). In addition, for the samples from Mangochi District, we analyzed a partial region (300-bp) of the nuclear ribosomal 18S DNA to confirm the presence of *S. mattheei* nuclear DNA (*2,6*) (Appendix).

Of 6 atypical eggs from Chikhwawa, all had a pure *S. haematobium* genetic profile (Figure, panels B, C). Of 19 eggs from Nsanje, 18 had a pure *S. haematobium* genetic profile; 4 eggs had atypical morphology, but only 1 atypical egg had a discordant genetic profile (i.e., *cox*1 *S. bovis* and rITS *S. haematobium*). Of 20 eggs from Mangochi, 16 typical *S. haematobium* eggs had a pure *S. haematobium* genetic profile, whereas the 4 atypical eggs had the same discordant genetic profiles (*cox*1 *S. mattheei* and rITS *S. haematobium-mattheei*). Inspection of the partial 18S gene

Figure. Investigation of atypical schistosome eggs retrieved from children in Malawi. A) Locations where urine samples containing *Schistosoma haematobium* eggs were collected from children in Mangochi (Samama village, 14°41′74.65″S, 35°21′75.80″E), Chikhwawa (Mpangani village, 16°03′62.99″S, 34°84′10.63″E), and Nsanje (Kastiano village, 16°90′63.98″S, 35°26′65.78″E) districts. Of the children sampled, ≈10% had atypical eggs in their urine, in an approximate atypical:typical ratio of 1:25. Note that the Shire River flows southward from Lake Malawi, linking the 3 sampled locations within the same drainage basin. B) Photomicrographs of a representative atypical egg from each location. Corresponding genotypes assigned for the mitochondrial *cox*1 and nuclear rITS loci: Mangochi, *cox*1 *S. mattheei* and rITS *S. haematobium-mattheei*; Chikhwawa, *cox*1 and rITS *S. haematobium*; Nsanje, *cox*1 *S. bovis* and rITS *S. haematobium*. A typical *S. haematobium* egg is shown for comparison. Sizes are not to scale. C) Histogram of length measurements for 83 typical *S. haematobium* eggs collected from Nsanje. Solid line indicates the associated density distribution. The mean length of this sample of typical eggs was 135 ± 28 μm (1 SD), with minimum 86 μm and maximum 180 μm. Arrows with dashed lines at right indicate the length of the 3 atypical eggs, which fall well outside the range of length variation of the 83 typical eggs as measured.
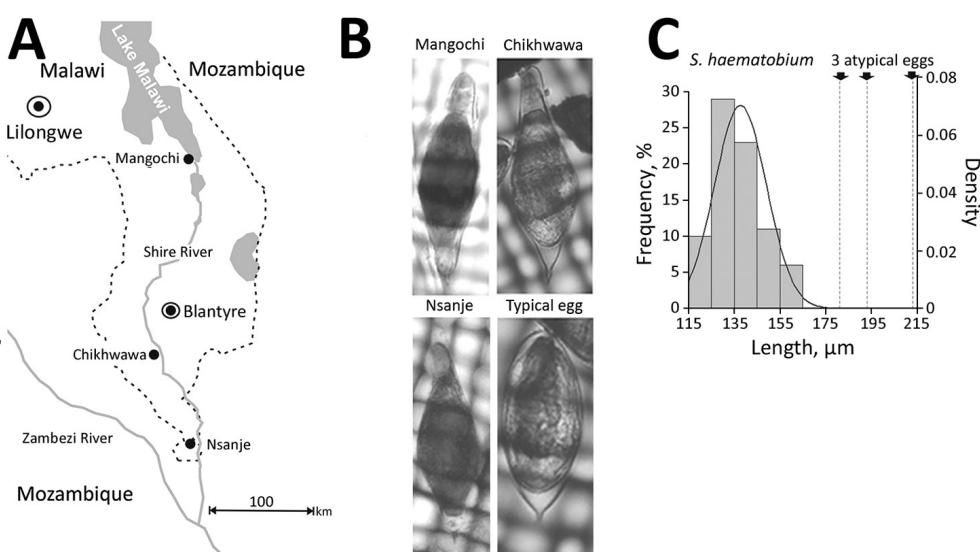
sequence confirmed *S. haematobium–mattheei* hybrids (Appendix). We deposited all sequence data into GenBank (accession nos. MK358841–MK358858).

Our genetic analysis demonstrated the presence of *S. haematobium* group hybrids in Malawi as introgressed forms of *S. haematobium–mattheei* and *S. haematobium–bovis*. Of note, an unusual egg morphology may not always correspond with the ability to detect introgression with the current combination of genetic markers used (*6*; Appendix). As described by Boon et al., successive backcrossings of hybrid progeny may obscure our ability to detect ancestral introgression, and the development of a wider panel of nuclear genetic markers is needed (*6*). Nonetheless, detection of these 2 hybrid schistosomes strongly suggests interactions of *S. haematobium* with the ungulate schistosomes *S. mattheei* and *S. bovis*. That *S. bovis* has not been reported in Malawi implies a changing species dynamic with possible zoonotic transmission along the drainage basin of Lake Malawi, adding a new dimension to the epidemiology and control of urogenital schistosomiasis in Malawi (*7*).

Because we did not attempt miracidial hatching during this study, we cannot confirm that these hybrids or introgressed forms are fully viable in autochthonous natural transmission. However, the process of ancestral introgression with subsequent natural selection may help explain unexpected shifts in local snail–schistosome relationships (e.g., the changing compatibility of *Bulinus nyassanus* snails in Lake Malawi with *S. haematobium* schistosomes) (*8*). Further studies are needed to better characterize schistosomes involved in human infection, investigate more thoroughly any zoonotic potential, and assess all possible combinations of interspecies introgressions.

Molecular evidence for ancestral hybridization between *S. haematobium* and *S. mansoni* schistosomes was presented recently (*9*); given autochthonous transmission of intestinal schistosomiasis in Lake Malawi (*10*), there may be sufficient epidemiologic opportunity for other introgression events to occur with the hybrids we report. We therefore advise heightened concurrent surveillance of urogenital and intestinal schistosomiasis, entailing a One-Health approach with molecular vigilance for interspecies interactions along with phenotypic assessments for any altered host pathogenicity or susceptibility to praziquantel treatment. Detection of the hybrid schistosomes we report adds a new perspective to the epidemiology and control of urogenital schistosomiasis in central Africa.

Acknowledgments

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Schistosome Interactions within the *Schistosoma haematobium* Group, Malawi

Appendix

DNA Extraction

DNA preparation from FTA stored schistosome eggs and larvae:

1. Add 14 µL of Solution 1 (0.1M NaOH, 0.3mM EDTA, pH13.0) to the punchout 2 mm FTA disc.

2. Incubate at room temperature for 5 min.

3. Add 26 µL of Solution 2 (0.1M Tris-HCl, pH7.0).

4. Pulse vortex 3 times.

5. Incubate at room temperature for 10 min.

6. Pulse vortex 10 times.

7. Use 3 µL of the DNA elution in a 25 µL PCR.

PCR Analysis and Sequencing

We eluted DNA as described above from the schistosome eggs stored on the FTA cards. In separate PCRs, run on a Perkin Elmer 9600 Thermal Cycler, we amplified the *cox*1, ITS and 18S DNA regions (Appendix Table). We performed a 25 µL PCR reaction for each using illustraTM puReTaq Ready-To-Go PCR Beads (GE Healthcare, Hertfordshire, UK, https://www.gehealthcare.com) and 10 pmol of each primer (Appendix Table) and 3µL of the DNA elution.

We checked all PCR reactions for positive amplification of the correct band size by gel electrophoresis using 0.8% Gelred agarose gels (Biotium, https://biotium.com). We purified PCR amplicons and Sanger sequenced them in both directions using a dilution of original PCR primer.

We used Sequencher version 5.1 (Gene Codes Corp., http://www.genecodes.com) to visualize and manually edit all sequence data.

We confirmed mitochondrial *cox*1 sequence identity using the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi). We analyzed the ITS and 18S sequence identity by visual comparison to personal reference sequences for each species (*S. haematobium*, *S. bovis,* and *S. mattheei*). We visually checked known interspecies SNP regions (Appendix Figure) to identify homogenous or heterogenous ITS and 18S DNA.

We inspected mitochondrial and nuclear genetic profiles to identify hybrids (and any discordance of mitochondrial and nuclear DNA data).

The mean egg length of this sample of 83 typical eggs was 135 ± 28µm (1 SD), which was very similar to the 137 ± 15µm (1 SD) reported by Boon et al (*1*). We referred to additional sources on unusual egg morphology (*2–4*).

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Appendix Table. PCR primers used to amplify each DNA region and their associated PCR thermal cycle in study of *Schistosoma haematobium* hybridization, Malawi

DNA region	Forward primer (name)	Reverse primer (name)	PCR thermal cycle
ITS1+2 rDNA	TGCTTAAGTTCAGCGGGT (ITS1)	AACAAGGTTTCCGTAGGTGAA (ITS2)	5 min at 95°C: 40 cycles of 30 s at 95°C, 30 s at 58°C, 1.30 min at 72°C: 10 min at 72°C.
Partial 18S rDNA	GCGAATGGCTCATTAAATCAG (WA)	TCCGGAGAGGGAGCCTGA (300R)	5 min at 95°C: 40 cycles of 30 s at 95°C, 30 s at 60°C, 1 min at 72°C: 10 min at 72°C.
Partial *cox*1 mt DNA	TAATGCATMGGAAAAAAACA (cox1Schisto5′)	TCTTTRGATCATAAGCG (cox1Schisto3′)	5 min at 95°C: 40 cycles of 30 s at 95°C, 30 s at 40°C and 1.30 min at 72°C 10 min at 72°C.

Marker	18S (300bp)			ITS (906bp)										
SNP	138	163	210	18	26	50	51	91	92	120	170	225	490	877
S. haematobium	T	C	T	C	T	G	T	C	G	C	G	C	T	T
S. bovis	C	T	C	C	T	A	T	C	G	T	A	T	T	C
S. mattheei	C	T	T	T	A	A	C	T	A	T	A	T	A	T

Appendix Figure. Comparison of DNA sequences from eggs of 3 *Schistosoma* species, Malawi.