

Detection of four patients who were infected by *Schistosoma haematobium* in Vietnam

Nguyen Van De¹
Truong La²
Pham Ngoc Minh¹
Pham Thi Bich Dao¹
Le Van Duet³

¹Department of Parasitology, Hanoi Medical University, Hanoi, Vietnam;

²Department of Microbiology and Parasitology, The Western Highlands Agriculture and Forestry Science Institute (WASI), Hanoi, Vietnam;

³Department of Molecular Diagnosis, National Hospital for Tropical Diseases, Hanoi, Vietnam

Introduction: *Schistosoma* lives as a parasite in the portal vein causing intestinal lesions. It also lives in the liver, spleen, and the vein of the urinary bladder causing lesions in the urinary system. Angola is an endemic area of *Schistosoma haematobium*, which causes lesions in the urinary system, including the urinary bladder. In this study, we aimed to identify and classify the parasites that were collected from four patients from Angola, who currently live in Vietnam, by morphological and molecular methods.

Patients and methods: The main clinical symptoms of the patients were collected, and *Schistosoma* eggs were taken from urine by a centrifugal method from the four patients in 2016. Identification of the species by morphological method was taken using a microscope. The DNA of the *Schistosoma* was also isolated and was identified by cytochrome C oxidase subunit 1 (*Cox1*) sequence.

Results: The four Vietnamese patients infected with *S. haematobium* in Angola returned to Vietnam. All the patients felt strange and had cystalgia and hematuria (blood urine), and one of them was diagnosed with urinary bladder cancer, where surgery was necessary for that patient. *Schistosoma* eggs, which were collected from the urine of the four patients, were identified as *S. haematobium* by morphological and molecular methods. These patients were the first reports of *Schistosoma* in Vietnam.

Conclusion: Four Vietnamese schistosomiasis patients returned from Angola: three were diagnosed with schistosomiasis and one was diagnosed with urinary bladder cancer. They had similar symptoms including a strange feeling, cystalgia, hematuria, and eosinophilia and were detected with *Schistosoma* eggs in urine.

Keywords: hematuria, *Schistosoma haematobium*, Vietnam

Introduction

Schistosomiasis is an acute and chronic infection caused by the parasite of the genus *Schistosoma*. It was estimated in 2015 that 218 million people in the world needed to use prophylactic treatment for the disease. Prophylaxis of schistosomiasis is essential and must be repeated to prevent and reduce the prevalence of schistosomiasis. Schistosomiasis has been reported in 78 countries around the world. However, extensive community-based prophylaxis is required in only 52 countries with moderate to high rates of disease transmission.¹

The *Schistosoma* parasite in human beings seems to have gone through at least three evolutionary stages in both the Asian and African continents. Species of the *Schistosoma* genus include *Schistosoma haematobium*, known as “bladder fluke”,

Correspondence: Nguyen Van De
Department of Parasitology, Hanoi Medical University, No 1 Ton That Tung, DongDa, Hanoi 1000, Vietnam
Tel +84 91 237 7281
Email ngvdeyhn@gmail.com

originated in Africa, the Near East, and the Mediterranean Basin and were imported to India during World War II. The freshwater snail species of the *Bulinus* genus were identified as the intermediate vector of the *Schistosoma* parasite. *Schistosoma mansoni* is found in Africa, Brazil, Venezuela, Suriname, Antilles, Puerto Rico, and Dominican Republic. Freshwater snail species belonging to the *Biomphalaria* genus are important parasite mediators of this parasite; *Schistosoma japonicum*, known as “blood fluke”, is widely circulated in East Asia and the South-West Pacific area. Freshwater snail species, *Oncomelania*, is an important mediating host for *S. japonicum*; *Schistosoma mekongi*, and like the *S. japonicum*, affects superior and inferior mesenteric veins, but *S. mekongi* differs from that where they have smaller eggs, different intermediate hosts (*Neotricula aperta*), and longer incubation periods in mammals with the final hosts being human beings and dogs. Experimental results suggest that *N. aperta* snails may also be infected with *S. mekongi*; *Schistosoma intercalatum* is usually parasitized in human host and is mediated by *Bulinus* spp. Whereas *Schistosoma malayensis* rarely causes disease in human beings and is mediated by the host of *Robertsiella* spp., and *S. guineensis* is a recently described species, found in the western African region, and its intermediate host is *Bulinus forskalii*.²

In the neighboring countries of Vietnam, including China, there is an endemic area of schistosomiasis, *S. japonicum*, with 900,000 infected human beings with a prevalence of *S. mekongi* in Laos, 14% in Khong Island¹⁰ and in Cambodia 11.2% in Kratie.^{3–6} But in Vietnam, only the intermediate hosts including *Tricula aperta*, *Oncomelania*, and *Manillgila* spp. were detected (Nguyen Van De, 2000).⁷

The clinical symptoms of schistosomiasis are the consequence of the body's response to the parasite's eggs. Intestinal schistosomiasis can cause abdominal pain, diarrhea, and bloody stool. In addition, hepatomegaly is a common symptom in the early stages of the disease and is often accompanied by a fluid accumulation in the peritoneal cavity and an increase of pressure in the intestinal vessels. Patients with these symptoms can also lead to splenomegaly.²

The common sign of urogenital schistosomiasis is bloody urine. Bladder and ureter fibrosis and kidney damage are sometimes used to diagnose the early stages of the disease. The possible complication of urogenital schistosomiasis in the later stage can also be bladder cancer. In women, urogenital schistosomiasis can occur with symptoms such as genital trauma, vaginal bleeding, and pain during sexual intercourse

and vulvar nodules. In men, urogenital schistosomiasis can cause disease in the seminal vesicles, prostate gland, and other organs. This disease can also cause long-term, unrecoverable consequences including infertility. This paper reports about the three cases of schistosomiasis in Vietnam, which were imported from Angola.

Patients and methods

- Description of cases: description for the main clinical symptoms of four patients was done.
- Parasite samples: samples of *Schistosoma* eggs were taken from the urine of four patients by a centrifugal method in 2016 (using 50 mL urine per patient), using a microscope for the detection of eggs, and the camera was used to take photos.
- Identification of species: the species was identified by morphological method in Parasitology Department at Hanoi Medical University and molecular methods with cytochrome C oxidase subunit 1 (*Cox1*) and in Molecular Department of National Hospital of Tropical Diseases.

DNA isolation

To isolate the genetic materials from *Schistosoma* eggs, DNeasy Blood and Tissue kit from Qiagen NV (Venlo, the Netherlands) was used to extract the total DNA following the manufacturer's instructions. Worm eggs were resuspended in 100 µL of lysis buffer (ATL solution) with 20 µL of proteinase K, which was incubated at 56°C for 30 minutes. Then, 4 µL of RNase and 200 µL of AL buffer were added and continued to follow the manufacturer's protocol.

PCR amplification of the *Cox1* of the mitochondrial genome

To amplify the 267 bp fragment of the *Cox1* gene of *Schistosoma*, PCR was performed in a total volume of 50 µL with the following components: 25 µL of 2X Taq PCR Master Mix Kit (Qiagen NV), 40 pmol of forward primer (ShmF: 5'-GGATTGATTTGTGCTATGGC-3') and 40 pmol of reverse primer (ShmR: 5'-CACCGCCWAYCGTAAATAAA-3'),⁸ 5 µL DNA template, 4 mM MgCl₂, fill ddH₂O to 50 µL. PCR amplification was performed on a Proflex Cyclor (Thermo Fisher Scientific, Waltham, MA, USA) with the following thermal parameters: initial denaturation and activation at 94°C for 3 minutes, followed by 35 cycles of 94°C for 30 seconds (template denaturation), 58°C for 30 seconds (primer annealing) and 72°C for 1 minute (product extension), PCR was terminated by a thermostatic extension at 72°C for 5 minutes.

Sanger sequencing

The fragment 267 bp of *Schistosoma*'s *Cox1* gene was sequenced using the Bigdye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific), which was followed by the manufacturer's protocol. In PCR sequencing, the forward primer (ShmF) and primer (ShmR) are used to multiply the PCR products. The nucleotide sequence of the *Cox1* gene was read on the 3130 sequencers (Thermo Fisher Scientific), and the archived nucleotides were analyzed by software v5.4 (Thermo Fisher Scientific).

Phylogenetic analysis

Alignments of the nucleotide sequences of the *Cox1* gene obtained from strains of *Schistosoma* isolated in Vietnam and other strains of *Schistosoma* was performed using ATGC software version 7.0.2 (Japanese) and Clustal W software to identify the nucleotide and amino acid similarities. Phylogenetic tree reflects the genetic linkage between the *Schistosoma* strains of Vietnam and other countries analyzed by MEGA 6.1 software using a Neighbor Joining cluster algorithm in which the evolutionary distance was calculated based on the reference of Kimura 2 parameter model. The bootstrap method was used to calculate the pseudo-replicates.

Ethics in research

The authors confirm that written informed consent has been provided by the patients to have the case details published. This study was approved by the ethics committee of Ha Noi Medical University.

Case report

Description of four patients

The first patient was a 30-year-old male and the second was a 49-year-old male residing in Dien Hoa commune, Dien Chau district, Nghe An province. The third was a 43-year-old male residing in Quang Minh commune, Gia Loc district, Hai Duong province. The fourth patient was a 27-year-old male residing in Co Thanh commune, Chi Linh district, Hai Duong province. Although they were working in Angola from January 2015 to December 2015, they used to catch fish in the local lake on the weekends and were infected with *Schistosoma*. They had similar symptoms including feeling uncomfortable in lower part of the abdomen, cystalgia, and hematuria but no fever from the end of 2015 to early 2016. In early 2016, they returned to Vietnam. The first, second, and fourth patient went to a medical doctor (parasitologist) in Hanoi for examination, and they were diagnosed with schistosomiasis. The collected urine of the three patients was examined by a centrifugal method to detect parasite eggs.

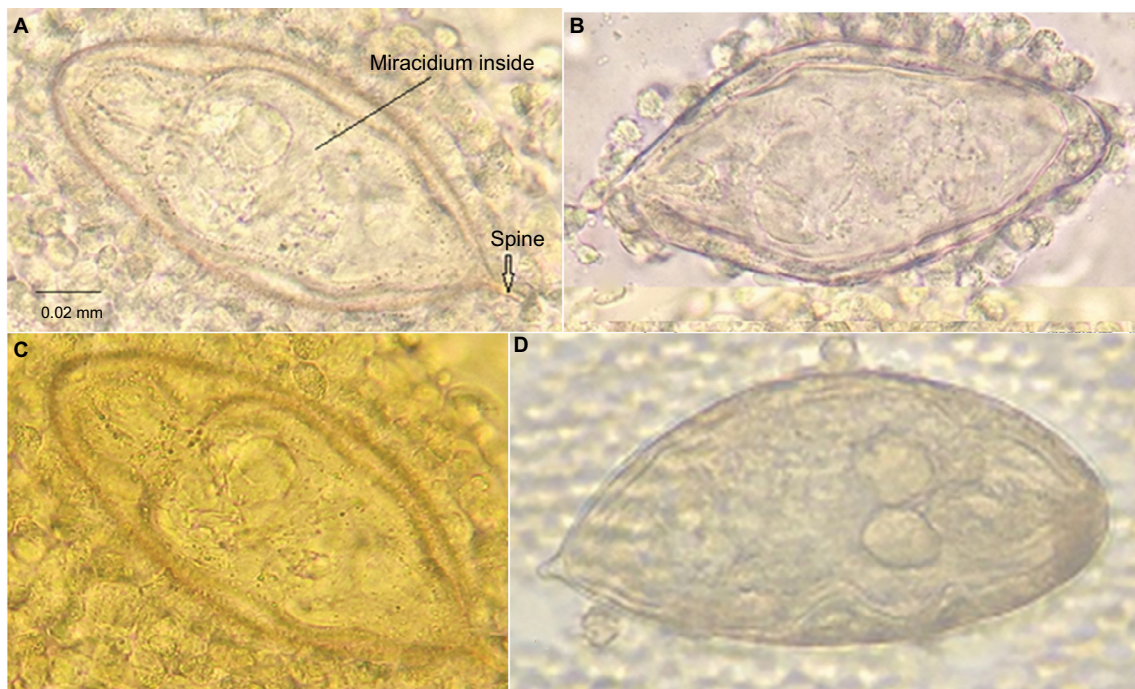


Figure 1 *S. haematobium* eggs collected from the patients.

Notes: (A) From the first patient. (B) From the second patient. (C) From the third patient. (D) From the fourth patient.

Abbreviation: *S. haematobium*, *Schistosoma haematobium*.

All three patients were infected with the eggs of *Schistosoma* (Figure 1). These patients were cured with praziquantel 75 mg/kg body per day (three times for 1 day only).

However, during that time, the third patient went to a cancer hospital and was diagnosed with urinary bladder cancer and underwent surgery to cut the urinary bladder, but no cancer cells were found in the lesions. A week after the surgery, this patient contacted the first patient and then he went to a parasitologist (also medical doctor) for a urine examination. The result showed that he was also infected with *Schistosoma*, and the doctor used a centrifugal method for urine to detect the *Schistosoma* eggs (Table 1 and Figure 1). These *Schistosoma* eggs were collected from the four patients, which were used for the identification of species.

Identification of parasite

Morphology identification of the eggs of parasite: the eggs were lozenge, 165 $\mu\text{m} \times 65 \mu\text{m}$ diameter, with a spine (arrow) at the lower edge of the egg and miracidium inside. This form is *S. haematobium* (Figure 1).

Molecular identification of the eggs of parasite

The eggs collected from the four patients were identified by morphology as *S. haematobium* (Figure 1). The use of

diagnostic morphology for *Schistosoma* was performed as an initial step to preliminary evaluation, in order to confirm precisely the parasite, the 267 bp DNA fragment of *Schistosoma's Cox1* gene was amplified by PCR and sequenced. The *Schistosoma*-derived nucleotide sequences collected from patients were compared to other strains (GenBank; Table 2). The results showed that the nucleotide sequence homology between *Schistosoma* isolates in Vietnam and other strains of *Schistosoma* in the world is 99% (Table 3 and Figure 2). As shown in phylogenetic tree, *S. haematobium* in our study was in one group with the *S. haematobium* that reported in the GenBank (Figure 3). Therefore, the worm eggs collected from four patients were confirmed by morphological and molecular biology methods, namely *S. haematobium*.

Discussion

The four schistosomiasis patients reported in this paper were infected with *S. haematobium* in Angola and returned to Vietnam. Schistosomiasis due to haematobia is the most prevalent parasitic disease in Angola. The clinical symptoms of this disease are the presence of serious and irreversible lesions in the urogenital tract caused by chronic parasitic infections, which can eventually lead to squamous cell carcinoma of the bladder. Data from a study by Botelho et al of 300 randomized people aged 15–75 years in the period of 2017–2018 indicate that the prevalence of *S. haematobium* infection is 71.7% (215/300).¹² Clinical symptoms were mainly dyspnea (91.2%), hypogastralgia (88.7%), and hematuria (87.1%). These symptoms are closely related to *S. haematobium* infection. *S. haematobium*, a parasitic worm, has infected more than 100 million people, mostly in developing countries and is a major cause of urogenital schistosomiasis associated with high incidence of squamous cell carcinoma.⁹ In our study, the four patients had the clinical symptoms of a strange feeling, cystalgia, and hematuria with one of them being diagnosed with urinary bladder cancer.

Table 1 Symptoms of four patients (case)

Symptoms	First case	Second case	Third case	Fourth case
Fever	–	–	–	–
Strangury	–	–	–	–
Uncomfortable in the lower part of the abdomen	+	+	+	+
Cystalgia	+	+	+	+
Hematuria	+	+	+	+
Eosinophilia	+	+	+	+
Bladder cancer	–	–	+	–
<i>S. haematobium</i> eggs in urine	+	+	+	+

Abbreviation: *S. haematobium*, *Schistosoma haematobium*.

Table 2 Sequencing of the portion *Cox1* of different *Schistosoma* species from GenBank compared with *S. haematobium*^a in Vietnam

Notation	Origin	Host	Length (bp)	Species	GenBank	Study
SchiVN	Vietnam	Human	180	<i>S. haematobium</i> ^a	–	This study
Schi1	Gabon	–	180	<i>S. haematobium</i>	KT354659	Mone et al, 2015 ¹³
Schi2	Gabon	–	180	<i>S. haematobium</i>	KT354660	Mone et al, 2015 ¹³
Schi3	Benin	–	180	<i>S. haematobium</i>	KT354661	Mone et al, 2015 ¹³
Schi4	Kenya	–	180	<i>S. haematobium</i>	JQ397386	Webster et al, 2012 ¹⁴
Schi5	South Africa	–	180	<i>S. haematobium</i>	JQ397397	Webster et al, 2012 ¹⁴
Schi6	Mauritius	–	180	<i>S. haematobium</i>	JQ397398	Webster et al, 2012 ¹⁴
Schi7	Tanzania	–	180	<i>S. haematobium</i>	GU257354	Webster et al, 2013 ¹⁵
Schi8	Tanzania	–	180	<i>S. haematobium</i>	GU257355	Webster et al, 2013 ¹⁵

Note: ^aResult after study.

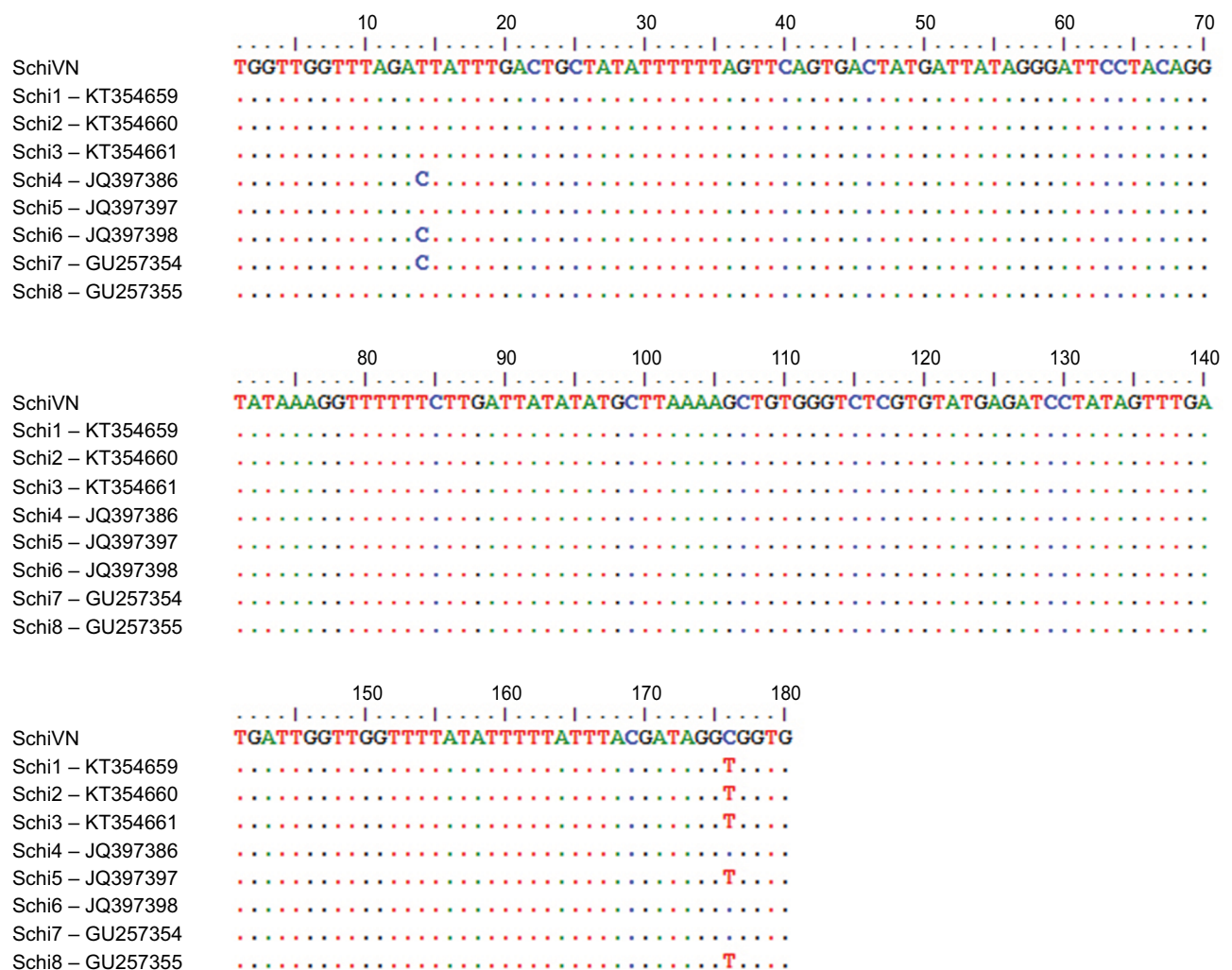
Abbreviations: *Cox1*, cytochrome C oxidase subunit I; *S. haematobium*, *Schistosoma haematobium*; SchiVN, Vietnamese *Schistosoma*.

Table 3 Percentage of the identification of nucleotide of *Cox I* sequences of Vietnamese *S. haematobium* and other strains of *Schistosoma* in GenBank

	SchiVN	Schi1	Schi2	Schi3	Schi4	Schi5	Schi6	Schi7	Schi8
SchiVN		99	99	99	99	99	99	99	99
Schi1	99		99	99	99	99	99	99	99
Schi2	99	99		99	99	99	99	99	99
Schi3	99	99	99		99	99	99	99	99
Schi4	99	99	99	99		99	99	99	99
Schi5	99	99	99	98	99		99	99	99
Schi6	99	99	99	99	99	99		99	99
Schi7	99	99	99	99	99	99	99		99
Schi8	99	99	99	99	99	99	99	99	

Notes: Schi1 and Schi2 are from Gabon *S. haematobium* (GenBank number: KT354659 and KT354660). Schi3 is from Benin (GenBank number: KT354661). Schi4 is from Kenya (GenBank number: JQ397386). Schi5 is from South Africa (GenBank number: JQ397397). Schi6 is from Mauritius (GenBank number JQ397398). Schi7 and Schi8 are from Tanzania (GenBank number: GU257354 and GU257355, respectively).

Abbreviations: *S. haematobium*, *Schistosoma haematobium*; SchiVN, Vietnamese *Schistosoma*.

**Figure 2** Comparison of 180 nucleotides of portion *Cox I* gene between Vietnamese *S. haematobium* and other species of *Schistosoma* including Gabon (*S. haematobium* [Schi1 and Schi2]), Benin (Schi3), Kenya (Schi4), South Africa, Mauritius (Schi6), and Tanzania (Schi7 and Schi8).

Notes: Difference between SchiVN and other species shown by their sign nucleotide. The mark (.) is similar to each other in nucleotide.

Abbreviations: *Cox I*, cytochrome C oxidase subunit I; SchiVN, Vietnamese *Schistosoma*.

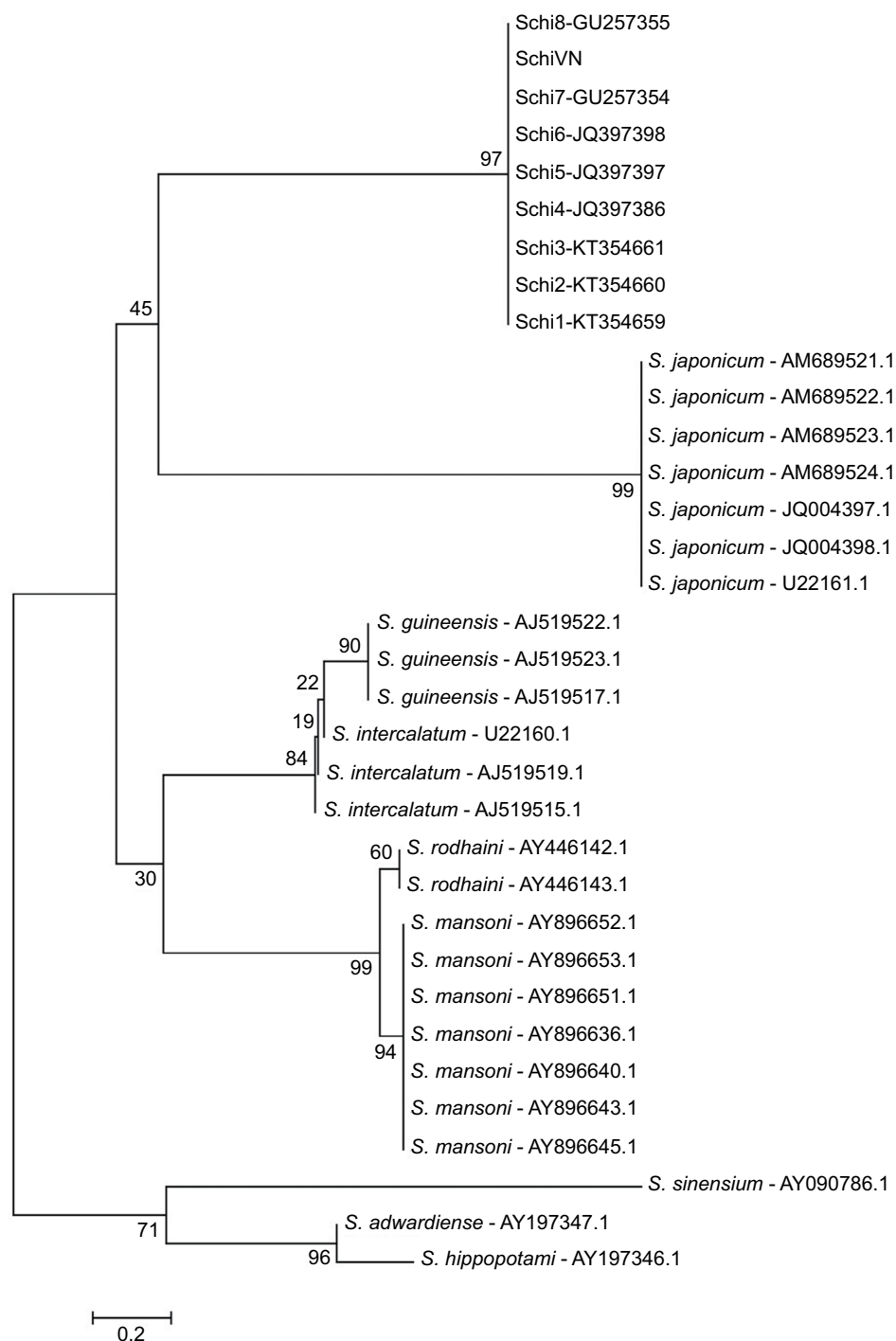


Figure 3 Phylogenetic tree of *S. haematobium* in Vietnam and other strains from *Cox I* gene nucleotide sequence estimated by NJ using MEGA5.4 software.¹⁶

Notes: Other *S. haematobium* (GenBank number: KT354659, KT354660, KT354661, JQ397386, JQ397397, JQ397398, GU257354, and GU257355). *S. japonicum* is from China (GenBank number: AM689521.1, AM689522.1, AM689523.1, AM689524.1, JQ004397.1, JQ004398.1, and U22161.1). *S. mansoni* is from Ghana (GenBank number: AY896636.1, AY896640.1, AY896643.1, AY896645.1, AY896651.1, AY896652.1, and AY896653.1). *S. rodhaini* is from Kenya (GenBank number: AY446142.1 and AY446143.1). *S. sinensium* is from China (GenBank number: AY090786.1). *S. intercalatum* (GenBank number: AJ519515.1, AJ519519.1, and U22160.1). *S. guineensis* is from Cameroon (GenBank number: AJ519517.1, AJ519522.1, and AJ519523.1). *Schistosoma adwardiense* is from Uganda (GenBank number: AY197347.1). *S. hippopotami* is from Uganda (GenBank number: AY197346.1).

Abbreviations: NJ, Neighbor Joining; *S. adwardiense*, *Schistosoma adwardiense*; *S. guineensis*, *Schistosoma guineensis*; *S. haematobium*, *Schistosoma haematobium*; *S. hippopotami*, *Schistosoma hippopotami*; *S. intercalatum*, *Schistosoma intercalatum*; *S. japonicum*, *Schistosoma japonicum*; *S. mansoni*, *Schistosoma mansoni*; *S. rodhaini*, *Schistosoma rodhaini*; *S. sinensium*, *Schistosoma sinensium*; SchiVN, Vietnamese *Schistosoma*.

Each of the four patients had eosinophilia and was detected with *S. haematobium* eggs in urine.

Conclusion

Among the four Vietnamese schistosomiasis patients from Angola who came to Vietnam, three were diagnosed as having schistosomiasis and one was diagnosed with urinary bladder cancer. They had similar symptoms including a strange feeling, cystalgia, hematuria, and eosinophilia and were detected with *Schistosoma* eggs in urine. These eggs from the four patients were identified as *S. haematobium* using morphological and molecular methods. This is the first report of schistosomiasis in Vietnam.

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Disclosure

The authors report no conflicts of interest in this work.

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