Wuchereria bancrofti Lymphatic Filariasis, Barrancabermeja, Colombia, 2023

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We describe a recent case of lymphatic filariasis in Colombia caused by *Wuchereria bancrofti* nematodes. Our study combines clinical-epidemiologic findings with phylogenetic data. Resurgence of lymphatic filariasis may be linked to increasing urbanization trends and migration from previously endemic regions. Fieldwork can be a beneficial tool for screening and containing transmission.

"…could not avoid a spasm of horror at the sight of men with ruptures sitting in their doorways on hot afternoons, fanning their enormous testicle as if it were a child sleeping between their legs […] well-carried rupture was, more than anything else, a display of masculine honor."

"For a long time and with great pride, the scrotal hernia that many men in the city endured was attributed to the water from the cisterns, not only without shame but even with a certain patriotic insolence."

—Gabriel García Márquez,
Love in the Time of Cholera

Lymphatic filariasis (LF) is caused by an infection with the mosquitoborne filarial nematodes *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori* (*1*). Transmission has occurred in various regions, including Africa, Southeast Asia, and the Pacific basin (*1*). In addition, cases have been documented in specific areas across the Middle East, Caribbean, and South America (*1*,*2*). Historically, LF was endemic in 24 countries within the Americas (*1*,*3*); currently, 4 countries remain endemic for LF (Brazil, Haiti, Guyana, and the Dominican Republic), and 13.4 million persons are at risk for infection (*2*). Aside from Guyana, little is known about the occurrence and endemicity of LF caused by the *W. bancrofti* nematodes in northern South America, particularly in Colombia and Venezuela, where no recent cases were reported. We describe an LF case in Colombia caused by *W. bancrofti* infection.

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The Study

A 14-year-old boy residing in an urban area of Barrancabermeja, Santander, Colombia, located to the east bank of the Magdalena River, sought care for a history of progressive lymphedema lasting for 3 years. His symptoms began after a hunting trip to the San Rafael plateau, a forest area situated in the foothills of the eastern mountain range, ≈37 km west of Barrancabermeja. He was referred to Fundación Cardiovascular de Colombia because of progressive enlargement of both testicles, which had become painful over the previous few weeks, and recurrent episodes of fever, erythema, urticarial-like lesions, itching, and pain in the left lower limb. The patient had previously been under outpatient care with a vascular surgery team, who had considered a diagnosis of arteriovenous malformation. Of note, the patient did not experience symptom onset until age 11.

Physical examination of the patient showed extensive lymphedema of the left lower limb, extending from the foot to the genital region, along with areas of induration in the left thigh and painful bilateral inguinal lymphadenopathy (Figure 1, panel A). We also found severe scrotal edema (Figure 1, panel B). Complete blood count results showed leukocytosis with 87.4% neutrophils, indicating neutrophilia (reference

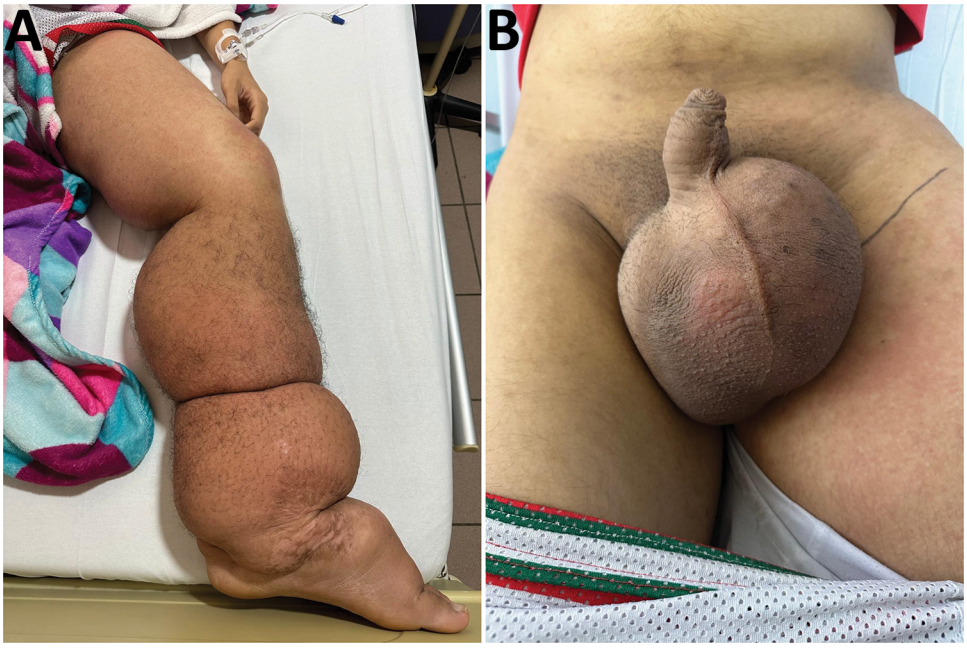

Figure 1. Images of a 14-year-old patient from Colombia experiencing severe progressive lymphedema caused by a *Wuchereria bancrofti* nematode infection. A) Patient's left lower limb, showing generalized lymphedema extending from the foot to the genital region along with erythematous areas involving the left thigh and areas of perimaleolar hypocromia and hypercromia reflective of post-inflammatory trophic skin changes. B) Severe scrotal edema (hydrocele).

range 45%–70%). All other laboratory test results were unremarkable, including no eosinophilia. Doppler ultrasound revealed bilateral giant scrotal hydrocele. A computed tomography scan of the abdomen and pelvis, performed 3 months earlier, had demonstrated bilateral hydrocele along with left para-aortic, right iliac, and bilateral inguinal lymphadenopathy.

Our suspicion of chronic filariasis prompted nocturnal direct examination by using the Knott concentration method, which yielded inconclusive results (Appendix, https://wwwnc.cdc.gov/EID/article/30/7/23-1363-App1.pdf). We then pursued molecular characterization. We extracted DNA from the patient's blood sample and conducted a PCR-based filarial detection by using specific primers for *W. bancrofti* nematodes. Those primers targeted the cytochrome c oxidase subunit I, along with short and long fragments of the 18S gene (Appendix).

We identified the species by using the MinION Sequencing System (Oxford Nanopore, https://nanoporetech.com) for the 3 gene fragments, as described previously (*4*,*5*). We evaluated phylogenetic relationships of the sequenced isolate by comparison against other filarial species, confirming its taxonomic assignment as *W. bancrofti* (Figure 2; Appendix Figure 1). We started the patient on treatment consisting of clindamycin (600 mg intravenously every 8 h for 7 d) and single doses of ivermectin (100 μg/kg) and albendazole (400 mg) while awaiting diethylcarbamazine (DEC), which is currently unavailable in Colombia.

LF was a prevalent clinical condition in Colombia from the 16th Century until the mid-20th Century, prominent in the Caribbean city of Cartagena de Indias, where the initial cases were documented (*6*). However, it was not until 1930 that its endemicity was identified in communities residing along the Magdalena River, specifically in the oil city of Barrancabermeja, where the last case was reported in the late 1940s (*7*). The estimated incidence in that area was ≈16 cases/1000 oil-workers annually, making it one of the country's major endemic regions (*7*). The incidence of the disease markedly declined during the 1960s and 1970s, resulting in only sporadic and subclinical cases, eventually leading to the apparent disappearance of the disease foci for reasons that remain undetermined. Since that time, no new cases were reported in Colombia until 2016, when a case of giant penile and scrotal lymphedema was documented in a 33-year-old patient in the city of Cali, Valle del Cauca (*8*). However, there was no confirmation of parasite presence or verification of a travel history or residency in endemic areas (*8*).

This case shows the potential for reemergence of LF in Colombia and highlights the clinical characteristics associated with LF. The patient was a 14-year-old boy with chronic manifestations of the disease, including lymphedema and hydrocele, typically observed in the adult population (*9*). The case features dermatolymphangioadenitis attacks characteristic of the acute phase (*9*,*10*). Studies of LF in children suggest advanced stages of lymphedema and the presence of hydrocele, although rare, tend to be more prevalent in male children during puberty or older, which aligns with the observations in our case (*10*,*11*).

DEC is the drug of choice for *W. bancrofti*–caused LF, because of its dual action; primary microfilaricidal and partial macrofilaricidal effects typically result in a 50% reduction in adult filaria (*12*). Because DEC is unavailable in Colombia, we made the decision to initiate treatment with ivermectin in combination with albendazole, leveraging the microfilaricidal properties of ivermectin (*10*). Clindamycin therapy was started to mitigate the risk of secondary bacterial infection because of the patient's acute dermatolymphangioadenitis. While awaiting DEC availability, initiating doxycycline treatment is being considered, because of its potential for controlling the adult parasite forms (*13*).

Because the patient's medical history did not indicate travel, we believe the resurgence of LF may be associated with increasing urbanization trends, leading to the breakthrough of new ecologic niches and migration from previously known endemic regions such as Venezuela. From an epidemiologic perspective, it is important to highlight that *W. bancrofti* filaria exhibit a nocturnal periodicity of microfilaremia, which coincides with the peak activity hours of its main vector, *Culex quinquefasciatus* mosquitoes (*14*). *C. quinquefasciatus* mosquitoes demonstrate anthropodomestic habits, which are determining factors in the focal transmission of *W. bancrofti* infection in the vicinity of human dwellings (*14*). The patient's

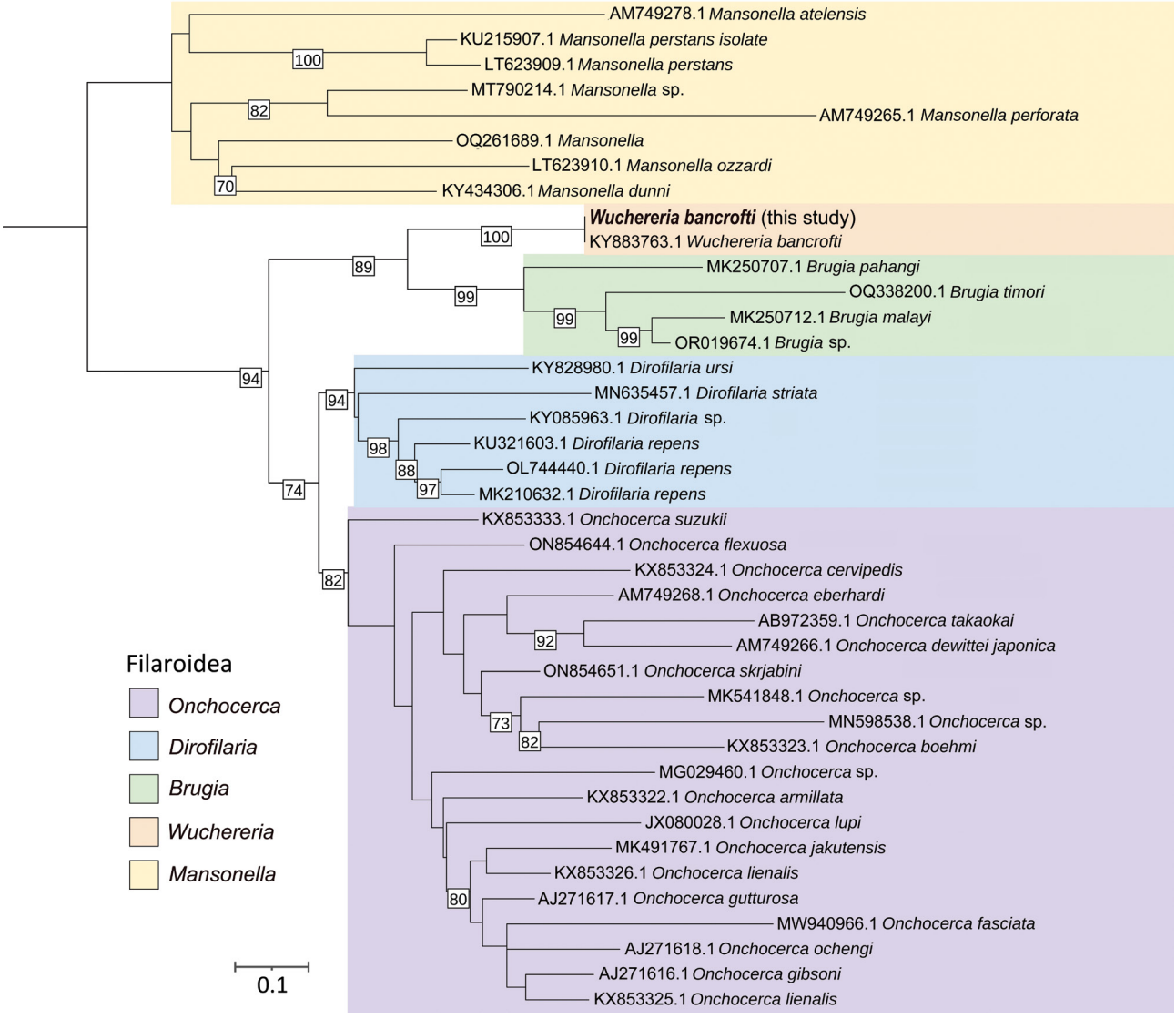

Figure 2. Phylogenetic reconstruction of consensus sequences of filaria, generated from a sample collected from a 14-year-old patient in Colombia (bold text). Cytochrome c oxidase I (COI) gene marker was used for this reconstruction. GenBank accession numbers are provided for reference sequences. Numbers along branch lengths indicate measures of support. Scale bar indicates the distance scale.

place of residence (Barrancabermeja) is an endemic area not only for *Culex* spp. mosquitoes but also for other vectors involved in the urban transmission of *W. bancrofti* filaria, such as *Aedes* spp. mosquitoes.

Conclusions

As previously reported, exposure to the LF infection within households appears to be a major contributor to childhood LF infection (*10*,*14*). Investigating familial clustering in this case is warranted. Despite renewed control efforts led by the Global Programme for the Elimination of LF, *W. bancrofti* infection foci persist in the region. Vigilance is crucial to prevent the reactivation of former endemic foci or a resurgence of cases in hypoendemic regions, which is potentially the scenario in this case. Parasite and entomologic surveillance should be promptly established to help develop and implement targeted interventions, including mass drug administration, vector control measures, and other strategic approaches. Such measures are essential for preventing the potential emergence of additional cases of LF within this geographic region of Colombia.

About the Author

Dr. Suárez is a clinician research scientist in the Panamanian Investigation System and CIDES, Panama City, Panama, and a professor at the International University SEK-Ecuador, Quito, Ecuador, and the University of Panama, Panama City, Panama. His primary research interests are tropical medicine, and fungal and parasitic diseases.

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Appendix.

Methods

Sample collection

In this study, we obtained a blood sample from a patient suspected of lymphatic filariasis, which was sent for processing from the Cardiovascular Foundation in Bucaramanga, Colombia.

Microscopy analysis

We conducted a thick blood smear as well as a peripheral blood smear to verify the presence of the parasite. Staining was performed using the GIEMSA dye, and the results were observed through an optical microscope.

DNA extraction

DNA extraction from the blood sample was performed using the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Penzberg, Germany), following the manufacturer's instructions. Subsequently, a PCR assay was conducted using primers targeting the small ribosomal subunit gene of 18S rRNA, an employed genetic marker in helminth identification. To enhance amplicon size and ensure better taxonomic characterization, we employed a combination of previously reported primers: Nem_18S_F (5′-CGCGAATRGCTCATTACAACAGC-3′) and 1289-R (5′- ACTAAGAACGGCCATGCACC-3′) with an amplicon size of 1300bp (*1*,*2*).

To confirm the presence of the parasite, an additional set of primers designed for the amplification of the 18S ribosomal RNA gene of *W. bancrofti* microfilariae specifically was used. Conventional PCR was performed for amplification of an ≈1700 bp fragment using primers

G18S4 and 18P (available at http://nema.cap.ed.ac.uk/biodiversidad/sourhope/nemoprimers.html) (*3*). The amplified fragments were visualized by 1.5% agarose gel electrophoresis, and the DNA concentration was quantified using Qubit.

We also carried out amplification of the mitochondrial cytochrome c oxidase I (COI) gene using forward (5-ATRGTTTATCAGTCTTTTTTTTTTTTTTATTATTGG-3) and reverse (5-GCAATYCAAATAGAAGAAGCAAAAGT-3) primers (*4*). These primers were designed for amplification of a fragment of ≈500bp in filarial positive samples. The amplified fragments were visualized by 1.5% agarose gel electrophoresis, and the DNA concentration was quantified using Qubit.

Sequencing and taxonomic assignment

Sequencing libraries were prepared from the obtained amplicons for rRNA18S and COI. Long-read sequencing on the Oxford Nanopore MinION platform was performed using the MinKNOW 23.04.5 application. Raw Fast5 files were base-called and demultiplexed using Guppy. Subsequently, sequences were quality and length filtered, removing potential chimeric and low-quality sequences.

For bioinformatic analyses, we created a custom database with sequences reported in NCBI for the Filaroidea family for the rRNA18S and COI genes. Taxonomic assignment was subsequently executed through Centrifuge, employing the custom databases, considering reads with a minimum length of 1500bp and 500bp for 18S and COI, respectively. The results of taxonomic assignment were visualized using Pavian (https://fbreitwieser.shinyapps.io/pavian/), and the taxonomic assignments were further validated through BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Consensus sequences generation from Oxford Nanopore reads

The reads assigned to *Wuchereria* by centrifuge were filtered using Seqtk (https://github.com/lh3/seqtk). Subsequently, a mapping was performed with Minimap2 (version 2.24) using the selected reference sequences of the small ribosomal subunit gene of 18S rRNA (GenBank: OR662183.2), large ribosomal subunit gene of 18S rRNA (GenBank: PP343069) and the cytochrome c oxidase subunit I (COI) gene (GenBank: PP342541) for *Wuchereria bancrofti*.

From the mapped sequences, a Variant Call Format (VCF) file was generated using Samtools (v 1.17). This VCF file was used to identify differences compared to the reference sequences of *Wuchereria bancrofti*. Finally, Bedtools (version 2.25.0) was employed to generate the consensus sequence, which served as the basis for subsequent analyses.

Phylogeny

We conducted a phylogenetic reconstruction to analyze the evolutionary relationships between *Wuchereria* sequences obtained by 18S (long and short fragments) and COI genes and reference sequences from other Filaroidea groups, including *Onchocerca, Dirofilaria, Brugia* and *Mansonella* species. These sequences were obtained from the 18S rRNA small ribosomal subunit gene and the cytochrome c oxidase subunit I (COI) gene, downloaded from NCBI. For 18S rRNA, sequences shorter than 1000 bp were excluded to ensure that the analysis included significant genetic information of the gene. For COI, sequences shorter than 300 bp were excluded considering that an amplified fragment of ≈500 bp was expected to be obtained. To improve computational capacity and to better elucidate phylogenetic relationships, we carried out a removal of duplicate sequences and clustering of sequences with a percentage of identity greater than 97% using Vsearch (https://github.com/torognes/vsearch). Subsequently, we perform multiple sequence alignment using MAFFT v7.407 and used this alignment to construct a maximum likelihood (ML) tree in IQ-TREE multicore v1.6.12, using the best substitution model and other parameters with default values. The phylogenetic tree was graphically represented in Interactive Tree Of Life (iTOL) v5.

Results

Microscopy analysis for the detection of microfilariae in a blood sample

We conducted a microscopic analysis to confirm the presence of the parasite in the patient's sample. Employing both peripheral blood smear and thick blood smear techniques, coupled with Giemsa staining, we examined the blood sample. The microscopic evaluation revealed a sheathed filariform-like structure for which microanatomical landmarks could not be fully resolved rendering diagnosis inconclusive. Therefore, the sample was analyzed by means of molecular detection by PCR.

Sequencing and taxonomic assignment

The reads obtained through long-read sequencing with Oxford Nanopore technology were taxonomically assigned using Centrifuge against a custom database created from 18S rRNA and COI gene sequences of the filaroidea family. The results of the taxonomic assignment corroborate *Wuchereria bancrofti* as the species involved in the case of filariasis reported here.

Phylogeny

We carried out a phylogenetic analysis of the sequences found for the rRNA18S and COI genes of *Wuchereria bancrofti*. The results obtained from the phylogenetic reconstruction of both molecular markers support the phylogenetic closeness of the sequence obtained here with previously reported *Wuchereria* species in a well-supported monophyletic cluster (Boostrap value >80). This is also supported by the fact that this sequence shows divergence from other filarial species responsible for cases of lymphatic filariasis such as *Brugia malayi* and *Brugia pahangi*. These findings support the hypothesis that the identified parasite in this patient corresponds to *Wuchereria bancrofti*.

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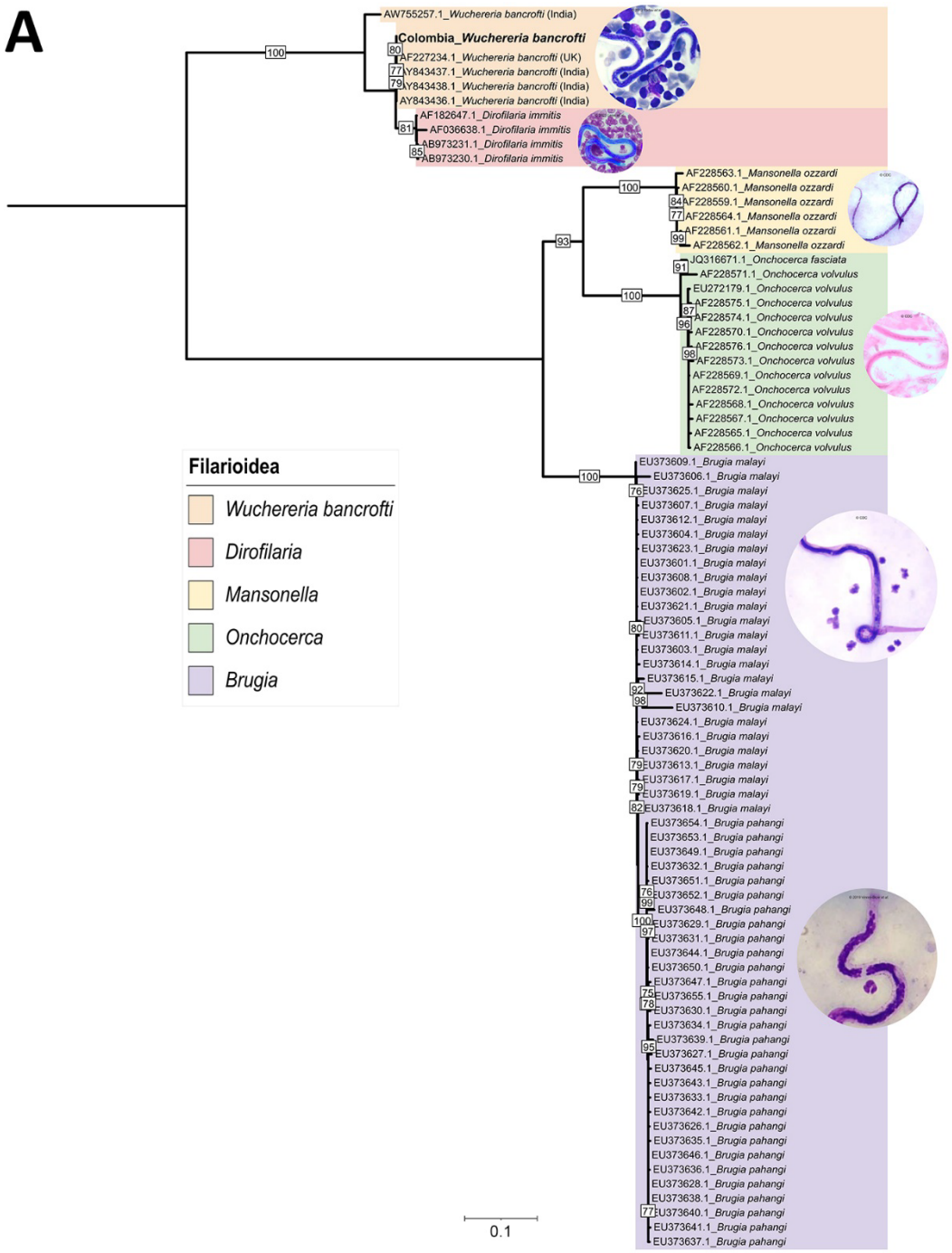

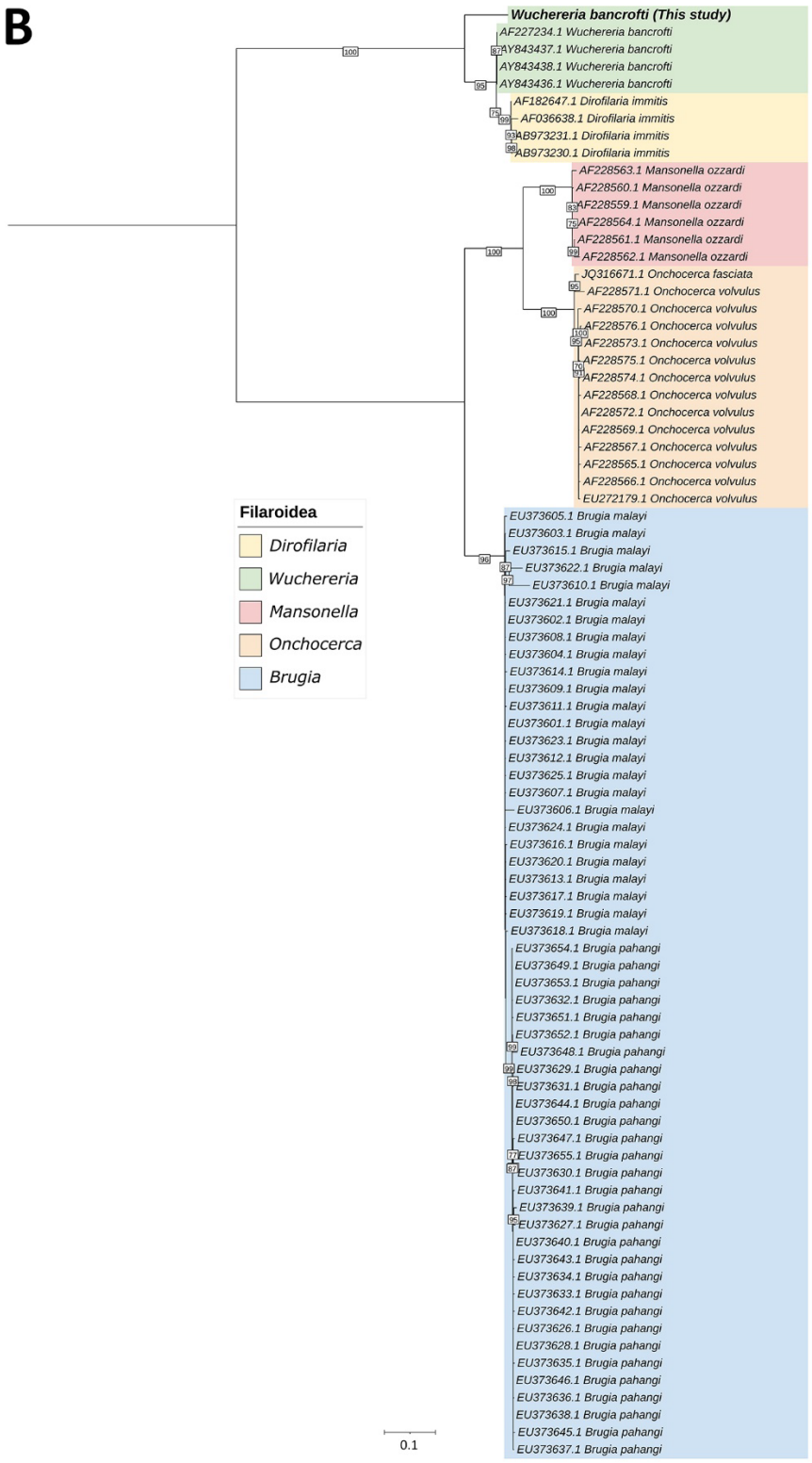

Figure 1. Phylogenetic reconstruction of consensus sequences, generated from a sample collected from a 14-year-old patient in Columbia. 18S Ribosomal RNA Gene used for reconstruction (bootstrap >70) for both panels A) and B).

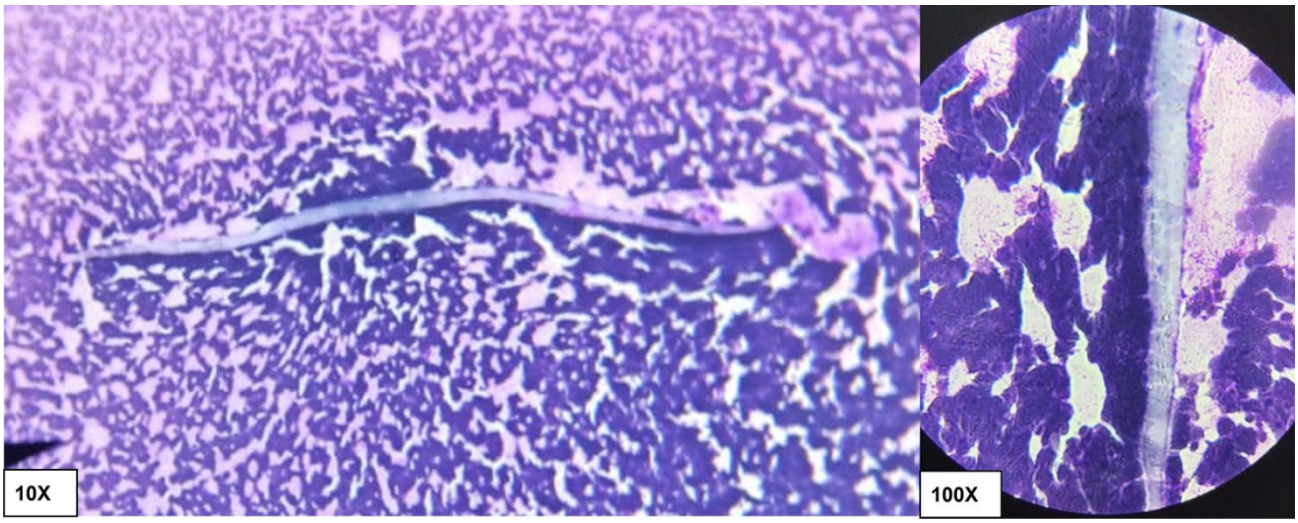

Figure 2. Identification of filariform-like structure in peripheral blood smear. Giemsa 10x and 100x