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Presence of *Spirometra mansoni*, Causative Agent of Sparganosis, in South America

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We report molecular identification of an adult *Spirometra mansoni* tapeworm retrieved from a crab-eating fox (*Cerdocyon thous*) in Colombia, confirming presence of this parasite in South America. This tapeworm is the causative agent of human sparganosis, commonly reported from Southeast Asia, and represents the second congeneric species with known zoonotic potential in the Americas.

Sparganosis is a neglected human zoonosis caused by migrating larval stages of the broad tapeworm genus *Spirometra* (Diphyllobothriidea), whose natural definitive hosts include wild and domestic canids and felids. The life cycle of this tapeworm involves 2 intermediate hosts: a freshwater copepod crustacean as the first and various vertebrates, mostly amphibians, as the second. Human infections are commonly reported from Southeast Asia and propagate most often in the form of subcutaneous sparganosis; however, the larvae can enter other organs or parts of central nervous system and cause damage.

Taxonomy of *Spirometra* remains highly complicated. Numerous species of *Spirometra* have been described, often poorly (*1*), and representatives of just 6 species-level lineages have been characterized molecularly so far, a key prerequisite to achieve a convincing tapeworm identification when only strobila fragments or larval stages are available. Limitations of morphologic characters of *Spirometra* are numerous and include characters' great intraspecific and even intra-individual variability (overview of problematic traits in *2*). Molecular sequence data thus represent the only unequivocal method of species identification.

Previous phylogenetic analysis of *Spirometra* has shown that the geographic distribution of the 6 lineages respects continental borders (*2*). North

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and South America were shown to share 2 lineages found exclusively on those continents (*3*), provisionally termed *Spirometra decipiens* complex 1 and 2 because of the lack of essential morphologic data precluding conclusive species determination (*2*). *S. decipiens* complex 1 was shown to house, among parasites of canids and felids, causative agents of cutaneous and proliferative sparganosis. Representatives of *S. decipiens* complex 2, on the other hand, have not yet been shown to cause the zoonosis. The frequently reported human cases of sparganosis from Southeast Asia, as well as numerous specimens from wildlife from the region, corresponded to *S. mansoni* (*2*).

We report molecular identification of a tapeworm specimen retrieved from a dead crab-eating fox (*Cerdocyon thous*) from the vicinity of Ciudad Bolívar, Antioquia, Colombia. We characterized the specimen through Sanger-sequencing of 3 genetic loci (Appendix, https://wwwnc.cdc.gov/EID/article/28/11/22-0529-App1.pdf), including the complete mitochondrial cytochrome c oxidase subunit I gene (*cox1*) as the most densely sampled and phylogenetically informative gene of broad tapeworms.

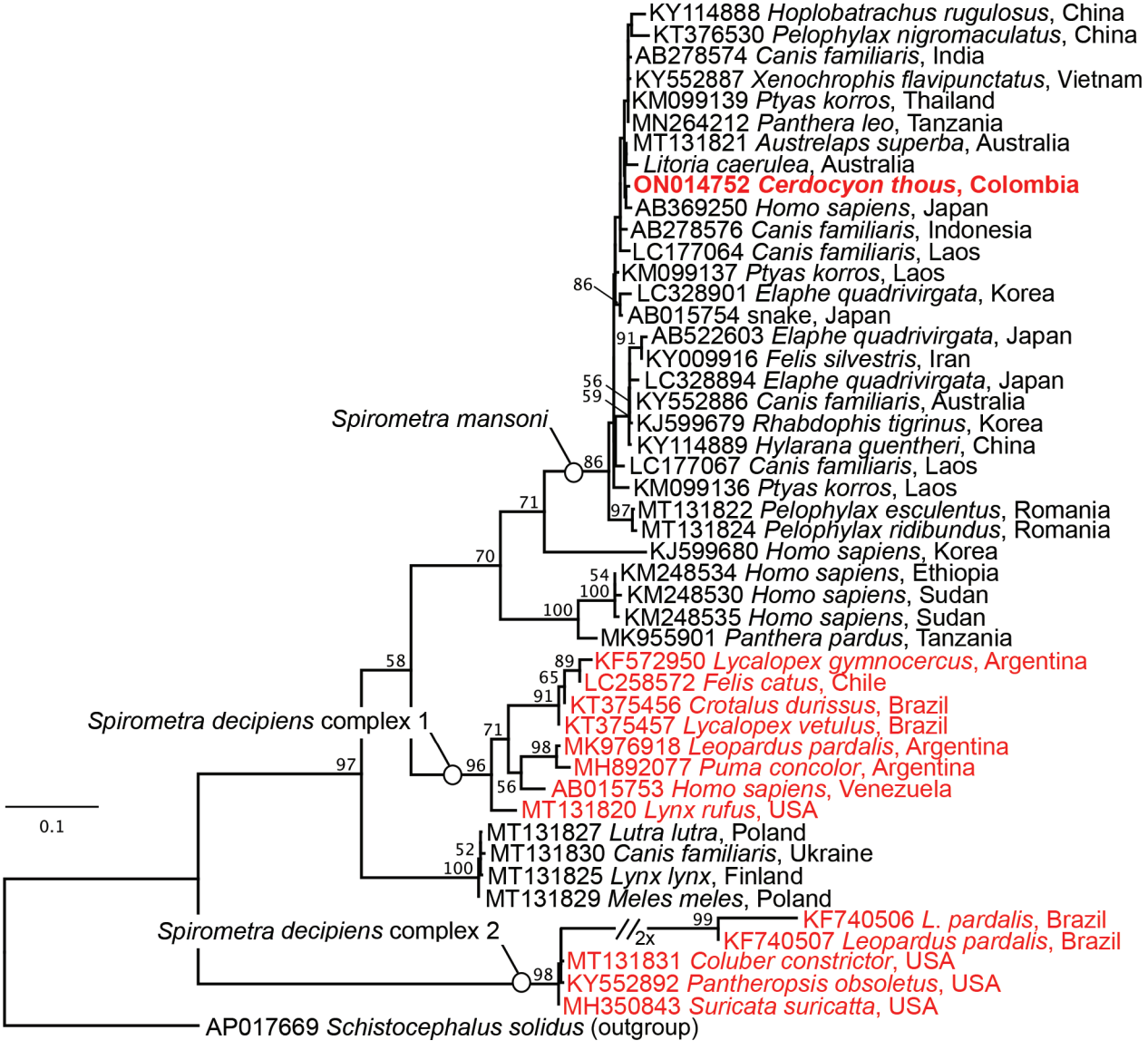

Figure. Maximum-likelihood estimate of the phylogenetic position of a *Spirometra mansoni* tapeworm collected from a crab-eating fox (*Cerdocyon thous*) in Colombia. Red indicates specimens from South America; bold indicates newly characterized *S. mansoni* from this report. Names of the 3 species-level lineages of *Spirometra* in South America are indicated; GenBank numbers are provided. Nodal support values show standard bootstrap supports >50. Scale bar indicates number of substitutions per site.

Phylogenetic analysis under maximum-likelihood criterion resolved the position of the tapeworm nested deep within the clade of *S. mansoni* (Figure), proving the presence of this causative agent of human sparganosis on the American continents.

S. mansoni represents by far the most frequently reported causative agent of sparganosis, previously misidentified as *S. erinaceieuropaei* (2). This species is responsible for virtually all human cases in Asia but has been also shown to infect wildlife in Africa, Australia, and Eastern Europe (2). Our finding of *S. mansoni* in Colombia in a crab-eating fox, a definitive host endemic and widely distributed across South America, from Panama to the Entre Ríos province of Argentina (4), expands the known distribution of *S. mansoni* into broader range than previously thought. This finding contrasts with the distribution of the remaining 5 lineages of *Spirometra*, which seem limited to continental regions (2). *S. mansoni* has been sporadically reported from the Americas in the past; however, morphology-altering fixation techniques and lack of critical molecular evidence did not support species identification. Reported hosts mostly included domestic cats (Appendix) and a single report from a crab-eating fox in Brazil (5).

The crab-eating fox inhabits savannah and woodland areas of various Neotropical habitats from coastal plains to montane forests and is considered omnivorous, opportunistically feeding on fruits, insects, and small vertebrates including amphibians and reptiles, with seasonal shifts to its diet (6,7). A broad range of Neotropical amphibians and reptiles has been found to serve as intermediate hosts of *Spirometra*; however, the record remains skewed toward herpetofauna of the more intensively surveyed coastal regions (8), and species identification of the parasite larvae has been, thanks to the lack of accompanying molecular data, either absent or ungrounded. As a result, the real range and the relevance of different intermediate hosts for the transmission of the sympatric South America species of *Spirometra* remain unknown. The situation in North America is even more obscure because of the virtually missing intermediate host record (1,9). Given the wide spectrum of suitable intermediate hosts of *S. mansoni*, which includes omnivores such as wild boar in Europe (10), the natural pools and the importance of different host species in the etiology of the zoonosis remain dubious. The concurrent presence of the second congeneric species with zoonotic potential urges deeper investigations into the parasite's life cycles and the epizootiology of a disease that could affect public health in the Americas.

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TIGIT Monoallelic Nonsense Variant in Patient with Severe COVID-19 Infection, Thailand

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A heterozygous nonsense variant in the *TIGIT* gene was identified in a patient in Thailand who had severe COVID-19, resulting in lower *TIGIT* expression in T cells. The patient's T cells produced higher levels of cytokines upon stimulation. This mutation causes less-controlled immune responses, which might contribute to COVID-19 severity.

To investigate SARS-CoV-2 genomic variants, we recruited 46 COVID-19 patients from King Chulalongkorn Memorial Hospital in Bangkok, Thailand, in January 2020. Recruited patients were 16–79 years of age and had moderate to severe COVID-19 symptoms according to World Health Organization interim guidelines (https://apps.who.int/iris/bitstream/handle/10665/331446/WHO-2019-nCoV-clinical-2020.4-eng.pdf). We performed whole-exome sequencing on peripheral blood samples as described (*1*). The institutional review board of the Faculty of Medicine, Chulalongkorn University, Bangkok, approved this study (COA no. 738/2020).

We filtered variants by using the following criteria. Variants had to pass the quality standards, have read depth >10, and be from the coding regions or canonical splice sites of 1,810 immune-related genes, including immune checkpoint genes (*2*). Variants also had to have <1% allele frequency in the Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org), Exome Variant Server (University of Washington, https://evs.gs.washington.edu/EVS), 1000 Genomes Project Consortium (https://www.genome.gov), dbSNPs (https://www.ncbi.nlm.nih.gov/projects/SNP), and Thai Reference Exome (T-Rex) database (*3*). We called candidate variants novel pathogenic variants when they were not previously identified in patients in the literature.

In our patient cohort, exome sequencing identified no variants in type I interferon genes, which previously have been commonly observed in patients with severe COVID-19 (*4*). Of note, we identified a heterozygous nonsense variant (rs1386709957) in the T-cell immunoglobulin and ITIM domain (*TIGIT*) gene in 1 patient (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/29/11/22-0914-App1.pdf). We did not identify this nonsense variant among 3,742 persons in the T-Rex database but did observe it in 1 of 31,390 alleles in the gnomAD database, in an allele from a female patient from East Asia. This variant truncates the 245-amino acid residue proteins at residue 56 and is classified as a pathogenic variant American College of Medical Genetics guidelines (https://www.acmg.net).

We investigated *TIGIT* gene expression in T cells of the patient from our study (Co45), a 43-year-old man, and compared it with 2 other sex- and age-matched patients who had severe COVID-19 (Co6 and Co84) (Appendix). We collected peripheral blood mononuclear cells (PBMCs) from each of the patients 1 month after they recovered. We used RNA extracted from PBMCs for real-time reverse transcription PCR and found patient Co45 had the lowest *TIGIT* mRNA level (Figure, panel A). Because *TIGIT* is mainly expressed in T cells, we used flow cytometry to measure the mean fluorescence intensity of TIGIT expressed in the cytoplasmic domain (CD) T cells. Patient Co45 had lower TIGIT gene expression in all CD3+, CD4+, and CD8+ T cells than the other 2 patients, most remarkably in the CD8+ T cells (Figure, panels B–D). The percentages of CD3+, CD4+, and CD8+ T cells in patient Co45 were comparable those in the other 2 patients (Appendix Fig-

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Appendix

Additional historical reports of *Spirometra mansoni* from domestic cats mentioned in the main text include Puerto Rico, Chile and Costa Rica (*1*,*5*,*7*).

Methods

Under the scope of a survey of parasites of Colombian canids, we carried out a necropsy of an adult male crab-eating fox (*Cerdocyon thous*) found at 1,084 meters above sea level in the Andean municipality of Ciudad Bolívar, Antioquia, Colombia (5°52′02″N, 75°57′15.42″W). During detailed examination of the carcass and cavitary organs we recovered an entire cestode specimen and a strobila fragment from the duodenum. The two strobila fragments were rinsed in saline and preserved in 90% ethanol, unwittingly of the fixative's detrimental effects on conserving the soft tissue quality and associated morphological characteristics necessary for morphological identification and morphometrics.

Total genomic DNA was extracted from snippets of the two ethanol-preserved strobila pieces using the Monarch Genomic DNA Purification kit (New England Biolabs, Inc.) following the manufacturer's protocol. The complete sequence of the *cox1* gene (1,566 bp) was PCR amplified with primers Cox1Forward and Cox1Reverse of Wicht et al. (*7*) using the Phusion High-Fidelity DNA Polymerase (New England Biolabs, Inc.) and the following cycling conditions: 35 cycles of 10 s at 98 °C, 15 s at 53 °C, 45 s at 72 °C. Nearly complete sequence of the nuclear small subunit ribosomal RNA gene (ssrDNA) was amplified over 30 cycles of 10 s at 98 °C, 15 s at 58 °C, 60 s at 72 °C using the primers WormA and WormB. Partial nuclear large subunit ribosomal RNA gene (lsrDNA) was amplified over 30 cycles of 10 s at 98 °C, 15 s at 63 °C, 45 s at 72 °C using the primers LSU5 and 1500R.

PCR products were gel-checked and enzymatically purified with Exonuclease I and FastAP alkaline phosphatase (Thermo Fisher Scientific). Sanger sequencing was carried out by SeqMe (Czech Republic) using the PCR as well as the following internal primers:

Cox1Forward (TATCAAATTAAGTTAAGTAGACTA) **cox1**

Cox1Reverse (CCAAATAGCATGATGCAAAAG) **cox1**

JB3 (TTTTTTGGGCATCCTGAGGTTTAT) **cox1**

JB4.5 (TAAAGAAAGAACATAATGAAAATG) **cox1**

WormA (GCGAATGGCTCATTAAATCAG) ssrDNA

WormB (CTTGTTACGACTTTTACTTCC) ssrDNA

600R (AACCGCGGCKGCTGGCACC) ssrDNA

1270F (ACTTAAAGGAATTGACGG) ssrDNA

1270R (CCGTCAATTCCTTTAAGT) ssrDNA

LSU5 (TAGGTCGACCCGCTGAAYTTAAGCA) lsrDNA

1500R (GCTATCCTGAGGGAAACTTCG) lsrDNA

ECD2 (CTTGGTCCGTGTTTCAAGACGGG) lsrDNA

900F (CCGTCTTGAAACACGGACCAAG) lsrDNA

400R (GGCAGCTTGACTACACCCG) lsrDNA

Contiguous gene sequences were assembled and inspected for errors in Geneious Prime 202.0.5 (http://www.geneious.com). The complete *cox1* sequences of the two strobila fragments were compared and found identical, suggesting their common origin from a single tapeworm individual. Newly characterized gene sequences were deposited in GenBank under accession nos. ON014752, ON016172 and ON032355.

The complete c*ox1* sequence was aligned with 47 representatives of the global genetic diversity of *Spirometra*, using the G-INS-i algorithm of the program MAFFT (*2*) implemented in Geneious. Uncharacterized parts of the partial sequences downloaded from GenBank were encoded as missing data. The maximum likelihood tree was estimated under the following best-scoring model and partitioning scheme TIM3+F+I+G4: part1, TN+F+I: part2, TIM2+F+R3:

part3 selected by ModelFinder (*3*) according to the corrected Akaike information criterion in the program IQ-TREE (*4*). Nodal support values were estimated through running 100 standard nonparametric bootstrap resamples in IQ-TREE. Pairwise sequence similarity of the newly characterized *cox1* to the remaining representatives of *S. mansoni* ranged between 99.49 and 96.55 %.

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