

I.S.M., R.O.L., J.C.S., and J.M.E. designed the study; J.S.M., L.S.D.P., S.R.R., and M.D.D.G. performed the experiments; I.S.M., L.S.D.P., C.H.G., and V.F.E. analyzed the data; I.S.M., L.S.D.P., R.O.L., J.C.S., and J.M.E. wrote the manuscript; R.O.L., J.C.S., and J.M.E. revised the manuscript; all authors edited and revised the final version of the manuscript.

About the Author

Dr. Sanz-Muñoz is a virologist, responsible for science and virological surveillance at the National Influenza Center of Valladolid, Spain. His main interests are virologic and epidemiologic surveillance of influenza viruses, as well as serological studies in diverse populations.

References

- Centers for Disease Control and Prevention. Interim clinical considerations for use of JYNNEOS and ACAM2000 vaccines during the 2022 U.S. mpox outbreak [cited 2022 Jun 21]. <https://www.cdc.gov/poxvirus/monkeypox/clinicians/smallpox-vaccine.html>
- Centers for Disease Control and Prevention. Smallpox [cited 2022 Jul 20]. <https://www.cdc.gov/smallpox/index.html>
- World Health Organization. Multi-country monkeypox outbreak in non-endemic countries. 2022 [cited 2022 Jul 20]. <https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON385>
- European Centre for Disease Prevention and Control. Monkeypox multi-country outbreak – first update [cited 2022 Jul 20]. <https://www.ecdc.europa.eu/sites/default/files/documents/Monkeypox-multi-country-outbreak-first-update-8-July-FINAL3.pdf>
- Carlos III Health Institute. Analysis of healthcare in Spain throughout the 20th century [in Spanish] [cited 2022 Jul 26]. <http://gesdoc.isciii.es/gesdoccontroller?action=download&id=19/10/2012-3c0cfd4ca3#page=228>
- Comité Asesor de Vacunas de la Asociación Española de Pediatría. Vaccination against smallpox in Spain in the years prior to its eradication [in Spanish] [cited 2022 Jul 26]. <https://vacunasep.org/profesionales/noticias/la-vacunacion-contrala-viruela-en-espana-en-los-anos-anteriores-su-erradicacion>
- Rieckmann A, Villumsen M, Hønge BL, Sørup S, Rodrigues A, da Silva ZJ, et al. Phase-out of smallpox vaccination and the female/male HIV-1 prevalence ratio: an ecological study from Guinea-Bissau. *BMJ Open*. 2019; 9:e031415. <https://doi.org/10.1136/bmjopen-2019-031415>
- Sørup S, Villumsen M, Ravn H, Benn CS, Sørensen TIA, Aaby P, et al. Smallpox vaccination and all-cause infectious disease hospitalization: a Danish register-based cohort study. *Int J Epidemiol*. 2011;40:955–63. <https://doi.org/10.1093/ije/dyr063>
- Belongia EA, Naleway AL. Smallpox vaccine: the good, the bad, and the ugly. *Clin Med Res*. 2003;1:87–92. <https://doi.org/10.3121/cm.1.2.87>
- Centers for Disease Control and Prevention. History of smallpox [cited 2022 Jul 26]. <https://www.cdc.gov/smallpox/history/history.html>
- Ministerio de Sanidad. Carlos III Health Institute. Epidemiological situation of monkeypox cases in Spain [in Spanish] [cited 2022 Oct 31]. <https://www.isciii.es/>

QueHacemos/Servicios/VigilanciaSaludPublicaRENAVE/EnfermedadesTransmisibles/Documents/archivos%20A-Z/MPOX/SITUACION%20EPIDEMIOLOGICA%20DE%20LOS%20CASOS%20DE%20VIRUELA%20DEL%20MONO-30082022.pdf

Address for correspondence: Iván Sanz-Muñoz, Centro Nacional de Gripe, Edificio Rondilla, Hospital Clínico Universitario de Valladolid, Calle Rondilla de Santa Teresa s/n, 47009, Valladolid, Spain; email: isanzm@saludcastillayleon.es

SARS-CoV-2 Infection in a Hippopotamus, Hanoi, Vietnam

Vuong Nghia Bui,¹ Tung Duy Dao,¹ Long Hoang Tran, Thanh Thi Vu, Trang Huyen Nguyen, Giang Hoang Nguyen, Kien Viet Dung Tran, Huyen Xuan Nguyen, Anh Ngoc Bui, Fred Unger, Hung Nguyen-Viet, Hu Suk Lee

Author affiliations: National Institute of Veterinary Research, Hanoi, Vietnam (V.N. Bui, T.D. Dao, L.H. Tran, T.T. Vu, T.H. Nguyen, G.H. Nguyen, K.V.D. Tran, A.N. Bui); International Livestock Research Institute, Hanoi (F. Unger, H.S. Lee); International Livestock Research Institute, Nairobi, Kenya (H. Nguyen-Viet); Chungnam National University College of Veterinary Medicine, Daejeon, South Korea (H.S. Lee)

DOI: <https://doi.org/10.3201/eid2903.220915>

While investigating the death of a hippopotamus at a zoo in Hanoi, Vietnam, we isolated SARS-CoV-2 and sequenced the RNA-dependent RNA polymerase gene from different organs. Phylogenetic analysis showed that the SARS-CoV-2 strain was closely related to 3 human SARS-CoV-2 strains in Vietnam.

On December 4, 2021, a 20-year-old female hippopotamus (*Hippopotamus amphibius*) at a zoo in Hanoi, Vietnam, was treated for lethargy, depression, and reduced appetite. Veterinary staff initiated antimicrobial drug treatment on the basis of the clinical signs.

¹These first authors contributed equally to this article.

Table. Identification and isolation of SARS-CoV-2 from tissue samples of a hippopotamus, Vietnam

Tissue	Real-time RT-PCR result		Virus isolation	
	All betacoronaviruses	SARS-CoV-2	Vero cells	Real-time RT-PCR result
Lung	27.09	26.67	Positive	26.30
Spleen	33.96	33.53	Positive	33.91
Liver	32.29	31.8	Positive	38.34
Intestine	37.84	36.96	Negative	NA
Blood	Negative	Negative	NA	NA

*NA, not applicable; RT-PCR, reverse transcription PCR.

Six days after onset of clinical signs, the hippopotamus was anorexic; she died 17 days after onset. Zoo staff conducted necropsy; the main finding was severe pneumonia. Tissue samples from the liver, spleen, lung, intestine, and blood were collected and sent to the National Institute of Veterinary Research in Hanoi for further diagnosis of viral and bacterial diseases.

We screened the samples by real-time PCR to detect SARS-CoV-2, in accordance with World Health Organization (WHO) PCR protocol (1). The lung, spleen, liver, and intestine samples tested positive; cycle threshold (Ct) values for tissue types were 26.67 for lung, 33.53 for spleen, 31.8 for liver, and 36.96 for intestine. No other viral testing was pursued, and tissues were not examined histologically (data not shown).

To obtain the viral isolate, we inoculated the samples into Vero cells according to a method described previously (2). After 3 days, we successfully recovered the virus from the lung, spleen, and liver samples (Table). We confirmed that the recovered viruses from Vero cells were SARS-CoV-2 by real-time PCR. We gave the virus the temporary designation SARS-CoV-2/hippo/zoo/Vietnam/2021.

To further characterize and compare the virus isolated from the hippopotamus and the recent human SARS-CoV-2, we used a seminested reverse transcription PCR assay (3) to amplify 599–602 bp of the conserved RNA-dependent RNA polymerase (RdRp) genome sequence of 3 human SARS-CoV-2 strains from COVID-19 patients in Vietnam (selected at the same time as the hippopotamus isolate and afterwards) and the isolates from the dead hippopotamus. We sent the purified PCR products to 1st BASE Company (<http://www.base-asia.com>), Singapore, to sequence the 599–602-bp nucleotide of the RdRp genome. We submitted the sequences to GenBank (hippopotamus, accession no. ON365747; human, ON365835–7). We conducted multiple alignments of the obtained sequences of the dead hippopotamus and 3 human COVID-19 patients, together with representative nucleotide sequences of SARS-CoV-2 and other betacoronaviruses available in GenBank, using ClustalW in BioEdit version 7.2.5 as previously described (4). We performed phylogenetic

analysis in MEGA-X software using the maximum-likelihood method with the best-fit model general time reversible plus gamma 4 plus invariate sites and 1,000 bootstrap replicates (5). We constructed a Bayesian maximum-clade credibility host discrete traits tree by using BEAST version 1.10.4 (<http://tree.bio.ed.ac.uk/software/blast>).

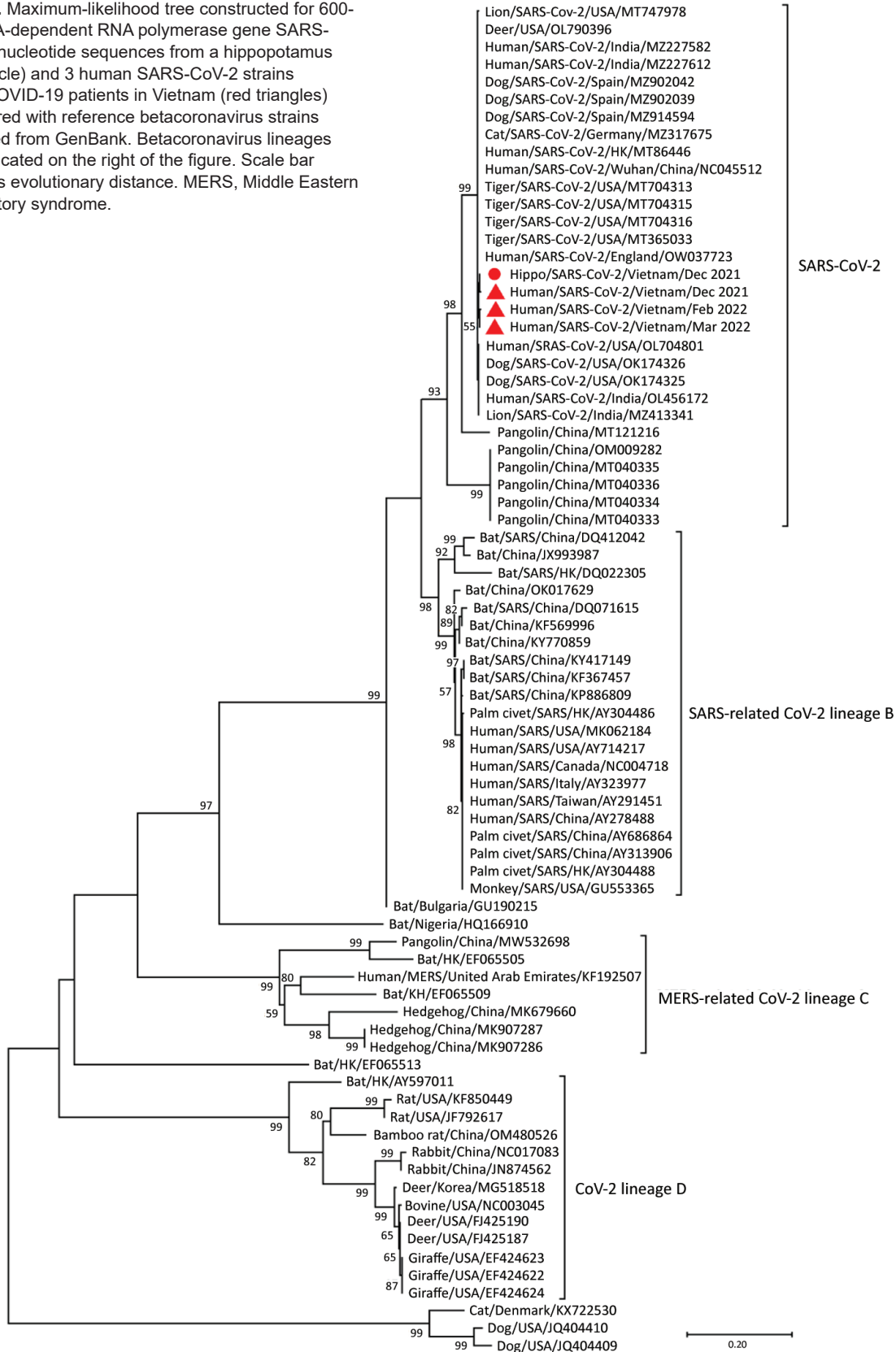
Phylogenetic analysis indicated that the sequences obtained from the dead hippopotamus and 3 human COVID-19 patients were SARS-CoV-2 (Figure; Appendix, <https://wwwnc.cdc.gov/EID/article/29/3/22-0915-App1.pdf>). The source of the hippopotamus' infection was difficult to pinpoint because the zoo had been open to the public; a visitor or staff member could have been transmitted the virus. As a precaution, all zoo staff were required to wear uniforms, facemasks, and gloves and to disinfect their boots when servicing the animal areas. However, those biosecurity measures were not sufficient to prevent the airborne transmission of the virus from humans to animals. To prevent anthroponotic disease, zoos must closely monitor the health status of zoo staff to eliminate virus transmission from humans to animals. Active surveillance using nasal or oral swab specimens, or fecal samples from animals, is needed for early detection of viral infection. In addition, stricter biosecurity measures are required in zoo exhibit areas to reduce the potential transmission of viruses by visitors to animals. For example, zoos should install glass barriers to separate exhibit areas from pathways for visitors.

This study highlights an urgent need to establish comprehensive monitoring systems for SARS-CoV-2 in animals. Our findings underscore hippopotamuses' susceptibility to SARS-CoV-2 and further contribute to the knowledge of the epidemiology of SARS-CoV-2, especially regarding the virus's host range. Whole-genome sequencing will provide information about SARS-CoV-2 lineage to help track transmission pathways.

Acknowledgments

We thank Ngo Thi Minh Quyen and other members of our laboratory in the Department of Virology at the National Institute of Veterinary Research for technical support. We thank Tezira Lore for language editing work.

Figure. Maximum-likelihood tree constructed for 600-bp RNA-dependent RNA polymerase gene SARS-CoV-2 nucleotide sequences from a hippopotamus (red circle) and 3 human SARS-CoV-2 strains from COVID-19 patients in Vietnam (red triangles) compared with reference betacoronavirus strains obtained from GenBank. Betacoronavirus lineages are indicated on the right of the figure. Scale bar denotes evolutionary distance. MERS, Middle Eastern respiratory syndrome.



This study was funded by the Consultative Group for International Agricultural Research (CGIAR) COVID-19 Hub and the CGIAR Initiative “Protecting human health through a One Health approach.”

About the Author

Dr. Bui is a research scientist and leader in the Department of Virology, National Institute of Veterinary Research, Hanoi, Vietnam. His research interests are molecular epidemiology, pathogenesis of viruses, and viral diseases. Dr. Dao is a research scientist in the Department of Virology, National Institute of Veterinary Research. His research interests include molecular epidemiology, biology, and bioinformatics analysis of influenza virus, coronavirus, foot-and-mouth disease virus, classical swine fever, African swine fever, porcine reproductive and respiratory syndrome, porcine circovirus type 2, hepatitis E virus, dengue virus, and other viruses.

References

1. World Health Organization. Protocol: real-time RT-PCR assays for the detection of SARS-CoV-2. 2020 [cited 2023 Jan 20]. https://www.who.int/docs/default-source/coronaviruse/real-time-rt-pcr-assays-for-the-detection-of-sars-cov-2-institut-pasteur-paris.pdf?sfvrsn=3662fcb6_2
2. Killington RA, Stokes A, Hierholzer JC. Virus purification. In: Mahy BWJ, Kangro HO, editors. Virology method manual. New York: Academic Press; 1996. p. 71–89 [cited 2023 Jan 20]. <https://www.sciencedirect.com/book/9780124653306/virology-methods-manual>
3. Xiu L, Binder RA, Alarja NA, Koček K, Coleman KK, Than ST, et al. A RT-PCR assay for the detection of coronaviruses from four genera. *J Clinical Virol.* 2020; 128:104391.
4. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 1994;22:4673–80. <https://doi.org/10.1093/nar/22.22.4673>
5. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol.* 2018;35:1547–9. <https://doi.org/10.1093/molbev/msy096>

Address for correspondence: Hu Suk Lee, International Livestock Research Institute, Regional Office for East and Southeast Asia, Room 301-302, B1 Building, Van Phuc Diplomatic Compound, 298 Kim Ma St, Ba Dinh District, Hanoi, Vietnam; email: H.S.Lee@cgiar.org; or Hu Suk Lee, College of Veterinary Medicine, Chungnam National University, Daejeon, South Korea; email: hs.lee@cnu.ac.kr

Emergence of *Mycobacterium orygis*-Associated Tuberculosis in Wild Ruminants, India

Megha Sharma, Karikalan Mathesh, Premanshu Dandapat, Asok Kumar Mariappan, Ravi Kumar, Soni Kumari, Vivek Kapur, Sushila Maan, Naresh Jindal, Nitish Bansal, Riyaz Kadiwar, Abhishek Kumar, Nitin Gupta, A.M. Pawde, A.K. Sharma

Author affiliations: Indian Council of Agricultural Research—Indian Veterinary Research Institute, Izatnagar, India (M. Sharma, K. Mathesh, A.K. Mariappan, R. Kumar, S. Kumari, A.M. Pawde, A.K. Sharma); Indian Council of Agricultural Research—Indian Veterinary Research Institute, Eastern Regional Station, Kolkata, India (P. Dandapat); Pennsylvania State University, University Park, Pennsylvania, USA (V. Kapur); Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, India (S. Maan, N. Jindal, N. Bansal); Sakkarbaug Zoological Garden, Junagarh, India (R. Kadiwar, A. Kumar); Bandhavgarh National Park, Madhya Pradesh, India (N. Gupta)

DOI: <https://doi.org/10.3201/eid2903.221228>

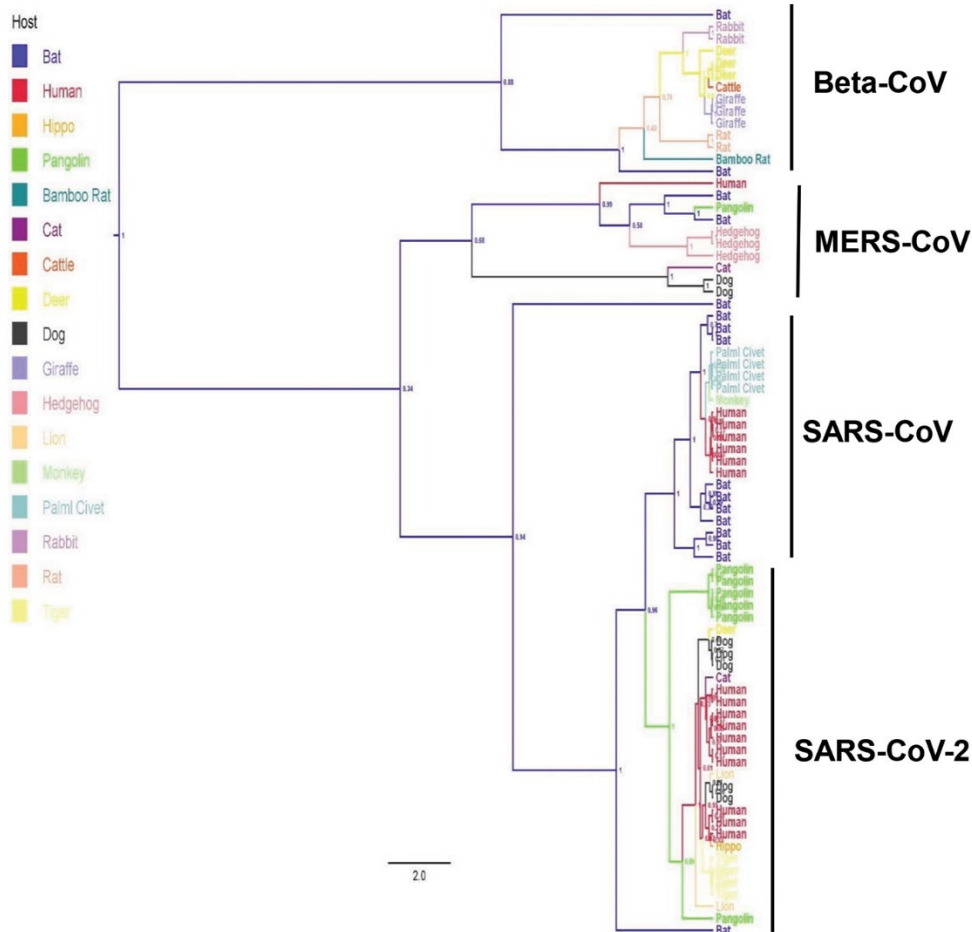
Tuberculosis caused by *Mycobacterium orygis* was detected in 2 spotted deer from a wildlife sanctuary in western India and an Indian bison from a national park in central India. Nationwide surveillance is urgently required to clarify the epidemiology of the *Mycobacterium tuberculosis* complex at the human–livestock–wildlife interface.

Tuberculosis (TB) caused by *Mycobacterium orygis* has been reported in humans, cattle, and, rarely, wild animals in India (1–3). We report 3 cases of *M. orygis*-associated TB in wild animals from among 85 unexplained deaths screened as part of disease investigations during February 2016–March 2020, which also revealed cases of suppurative bronchopneumonia (n = 32), TB caused by *M. tuberculosis* or *M. bovis* (n = 29), verminous pneumonia (n = 9), fungal granulomas (n = 6), and neoplasms (n = 6).

In February 2016, two adult free-range spotted deer (a male [case 1] and a female [case 2]) were found dead in Girnar Wildlife Sanctuary, Gujarat, western India. Postmortem examination revealed nonuniform, multifocal, coalescing pale-yellow nodules embedded in the parenchyma of the lungs with caseated yellowish-white material and enlarged liver and mesenteric lymph nodes with surface nodules. In January 2017, an emaciated adult male bison (case 3) was found dead at Bandhavgarh National Park, Madhya Pradesh, central

SARS-CoV-2 Infection in a Hippopotamus, Hanoi, Vietnam

Appendix



Appendix Figure. The Bayesian maximum clade credibility (MCC) host discrete traits tree for 600 bp RNA-dependent RNA polymerase gene nucleotide sequences of a hippopotamus, 3 human SARS-CoV-2 strains, and other betacoronavirus strains obtained from the GenBank database. The phylogenetic host tree indicated the transmission between betacoronavirus hosts. The number at the node indicates the posterior probability. The bar at the bottom of the figure denotes evolutionary distance. We used the uncorrelated relaxed clock with gamma distribution and the best fit general time reversible plus invariable plus gamma 4 sites (nucleotide substitution model with a constant population size coalescent tree. We ran the Bayesian Markov chain Monte Carlo at 50,000,000 generations and sampled at every 5,000

generations. The effective sample size (ESS) of the analysis was checked by Tracer version 1.6 software (<http://tree.bio.ed.ac.uk/software/tracer>). The MCC host discrete traits output tree was generated by TreeAnnotator version 1.10.4 (<http://tree.bio.ed.ac.uk/software/beast>) after burning 10% of the first trees. We reconstructed the host phylogenetic tree using FigTree version 1.4.3 software (<http://tree.bio.ed.ac.uk>).