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Molecular Evidence of *Leptospira* spp. in Isolated Fijian Bats

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ABSTRACT: Bats host several zoonotic pathogens. Island biogeography and epidemiologic theory predict small remote islands have lower infection diversity. Molecular studies of urine and feces from three species at 10 sites from three islands suggest multiple pathogenic *Leptospira*, but not coronavirus, paramyxovirus, or *Histoplasma*, circulate in isolated Pacific Fijian bat populations.

Bats play important ecologic roles worldwide (Fenton and Simmons 2014) and are hosts of diverse zoonotic pathogens. In Australasia, Oceania, and Southeast Asia, multiple viral families with proven or probable zoonotic potential have been isolated from bat guano (e.g., coronaviruses) and urine (e.g., paramyxoviruses) historically (Mackenzie et al. 2003; Rahman et al. 2010; Drexler et al. 2011). Alpha- and β-coronaviruses share a common ancestry in bats and cause human diseases, including coronavirus disease 2019, severe acute respiratory syndrome, and Middle East respiratory syndrome. Additionally, zoonotic paramyxoviruses have caused severe and fatal disease in both intermediate hosts and humans, including Hendra virus with horses as an intermediate host and Nipah and Menangle viruses with swine as an intermediate host (Philbey et al. 1998; Clayton et al. 2013; Anderson and Marsh 2015). Other pathogens of zoonotic concern recovered from bats and their guano include *Leptospira* spp. from urine and *Histoplasma* spp. from guano (Monchy et al. 1998; Lagadec et al. 2012).

There are six bat species in Fiji: *Chaerephon bregullae* (Fijian free-tailed bat), *Notopteris macdonaldi* (Fijian blossom bat), *Pteropus samoensis* (Samoan flying fox), *Pteropus tonganus* (Pacific flying fox), *Mirimiri acrodont* (Fijian monkey-faced bat), and *Emballonura semicaudata* (Pacific sheath-tailed bat; Palmeirim et al. 2007). These species are declining because of anthropogenic activities, including guano mining, hunting and consumption of bats, cave tourism, and human-wildlife conflicts around farming (Palmeirim et al. 2007; Mickleburgh et al. 2009). Because of human encroachment into bat habitats, the risk of human-bat interactions increases. *Leptospira* is an important zoonotic disease in Fiji, but previous studies regarding risk factors of human leptospirosis in Fiji have not investigated bats (Lau et al. 2016). Paramyxoviruses, coronaviruses, and *Leptospira* have been isolated from bats in Australia and Southeast Asia. Fiji's geographic isolation and small bat population sizes may limit the ability of infections to be introduced and persist, but given Fiji's proximity to these regions and the known human-bat interactions, our objective was to investigate the presence of several pathogens in Fijian bats. We used molecular techniques to investigate the presence of coronaviruses, paramyxoviruses, *Histoplasma*, and *Leptospira* in the urine and feces of Fijian bats.

During August–October 2018, we collected 176 samples (78 pooled urine and 98 pooled fecal samples) by free-catch methods onto plastic drop sheets under 10 arboreal and cave roosting sites (Fig. 1a and Supplementary Material Appendix 1). Animal ethics were approved by the Fijian government (RA 35/18). Samples were added to 2 mL of RNAlater (Sigma, St. Louis, Missouri, USA) in 5-mL cryovials with sterile swabs and pipettes, stored and transported at 4 C where possible, and taken to the Hopkirk Research Institute (Palmerston North, New Zealand). Nucleic acids were extracted from samples to amplify pathogen markers (Supplementary Material Appendix 2). Briefly, nucleic acids

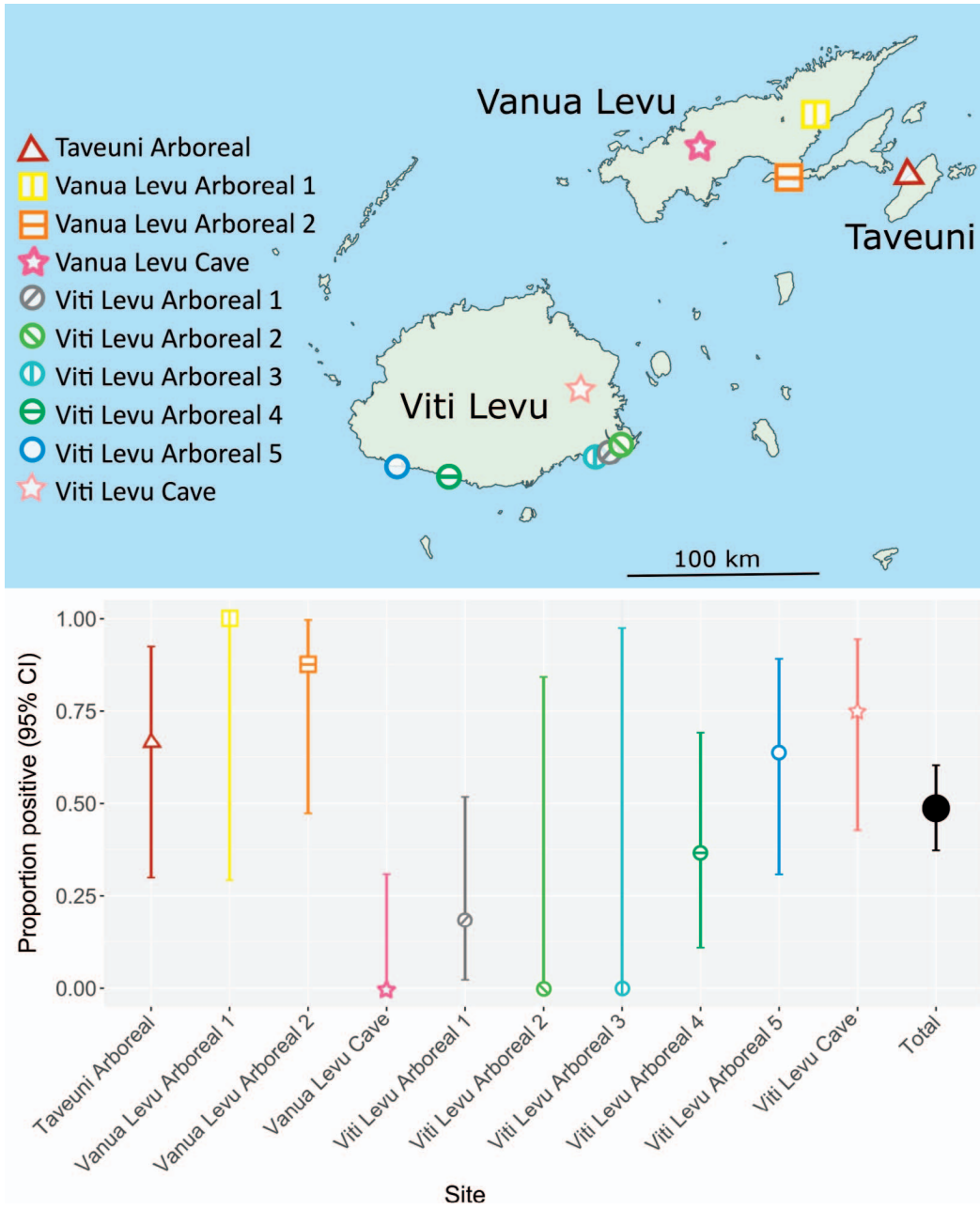

FIGURE 1. (a) Study site locations across three Fijian islands (Viti Levu, Vanua Levu, and Taveuni) for collection of bat urine and feces from below roost sites and (b) the proportion of *Leptospira*-positive urine samples at each study site.

were extracted from approximately 200 mg of feces or 200 µL of urine and amplified by reverse transcription PCR for RNA or PCR for DNA (for full details, see Supplementary Material Methods 1).

To confirm the host species (because samples were collected by free-catch methods), DNA from feces was tested by PCR using LCO1490 and HCO2198 primers designed for amplifying the cytochrome *c*

oxidase subunit I gene (Folmer et al. 1994). All PCR products were checked by gel electrophoresis on 1% agarose gels run at 70 volts for 60 min. Positive control material was synthesized with primer binding sites for the primers used in coronavirus, paramyxovirus, and *Histoplasma* sp. amplifications (see Supplementary Material Methods 2). Cloning kits (see Supplementary Material Methods 1) were used to clone copies of the synthetically generated DNA, which was amplified with previously published primers (see Supplementary Material Table S2). The *Leptospira*-positive controls were available from our laboratory *Leptospira* cultures.

Successful PCR products were cleaned by gel excision and sequenced in both orientations at the Massey Genome Services (Palmerston North, New Zealand) with the ABI 3730 capillary electrophoresis DNA analyzer (Applied Biosystems, Foster City, California, USA). Sequences were assembled in Geneious 10.2.6 (Kearse et al. 2012) by de novo assembly to align reads and generate consensus sequences used for phylogenetic analyses. Representative *Leptospira* glmU sequences were selected (Wilkinson et al. 2021), downloaded from the National Center for Biotechnology Information (NCBI 2019) nucleotide database, and aligned with our study sequences by MAFFT 7 software (Katoh and Standley 2013). A phylogenetic tree was generated by PhyML 3.0 (Guindon et al. 2010), with parameters determined by smart model selection (Lefort et al. 2017) and branch support by a Shimodaira-Hasegawa–like approximate likelihood ratio test (Anisimova and Gascuel 2006). Tree annotations and edits were produced in iTOL v5.3 (Letunic and Bork 2006).

No samples were positive for coronaviruses (n=98, feces), paramyxoviruses (n=78, urine), or *Histoplasma* sp. (n=98, feces). We discovered a high prevalence (n=38, 49%, 95% confidence interval 37–60) of 78 urine samples *Leptospira* sp.–positive, with high genetic diversity (Fig. 1b). All were new sequences with little similarity (88.8–96.8%) with known alleles. All *Leptospira* sequences have been deposited in GenBank (accessions OL694103–OL694136; Supplementary Material Appendix 5). Phylogenies showed clustering with *Leptospira kirschneri* and *Leptospira interrogans* (Fig. 2).

The Pacific flying fox was detected at five sites (Taveuni arboreal, Viti Levu arboreal 1 and 4, and Vanua Levu arboreal 1 and 2; see Supplementary Material Appendix 1). A different bat species was detected at Vanua Levu Cave. The closest basic local alignment search tool (BLAST) match for the sequence was to *Chaerephon plicatus*, at 95.6% similarity, and may represent the Fijian mastiff bat *Chaerephon bregullae*, which is not represented in the NCBI sequence database. No *Leptospira*-positive samples were found at Vanua Levu Cave, and no host DNA could be isolated at the remaining sites. All bat cytochrome oxidase c subunit 1 sequences have been deposited in GenBank (accessions OL693647–OL693655; Supplementary Material Appendix 5).

We found no molecular evidence of coronaviruses, paramyxoviruses, or *Histoplasma* in the samples tested. Study limitations include relatively small sample sizes, intermittent shedding of the organisms leading to seasonal excretion (Drexler et al. 2011), nucleic acid degradation in tropical conditions, and false negative tests. Zoonotic pathogens have been isolated from Chiroptera species worldwide, including regions neighboring the Pacific, so additional studies, including serology for viral infections, are warranted before it can be concluded that these pathogens are absent from these bat populations. However, geographic isolation and small population sizes may limit the ability for these infections to be introduced and perpetuate if they require critical community sizes larger than these populations.

Leptospira may be able to persist in small populations by chronic infection and shedding (Buhnerkempe et al. 2017). Bacterial isolation with fresh samples will be needed to characterize fully the bacteria we detected by molecular methods. Surveillance for and characterization of *Leptospira* among Fijian bats, other wildlife, domestic animals, and humans will be useful; genetic characteriza-

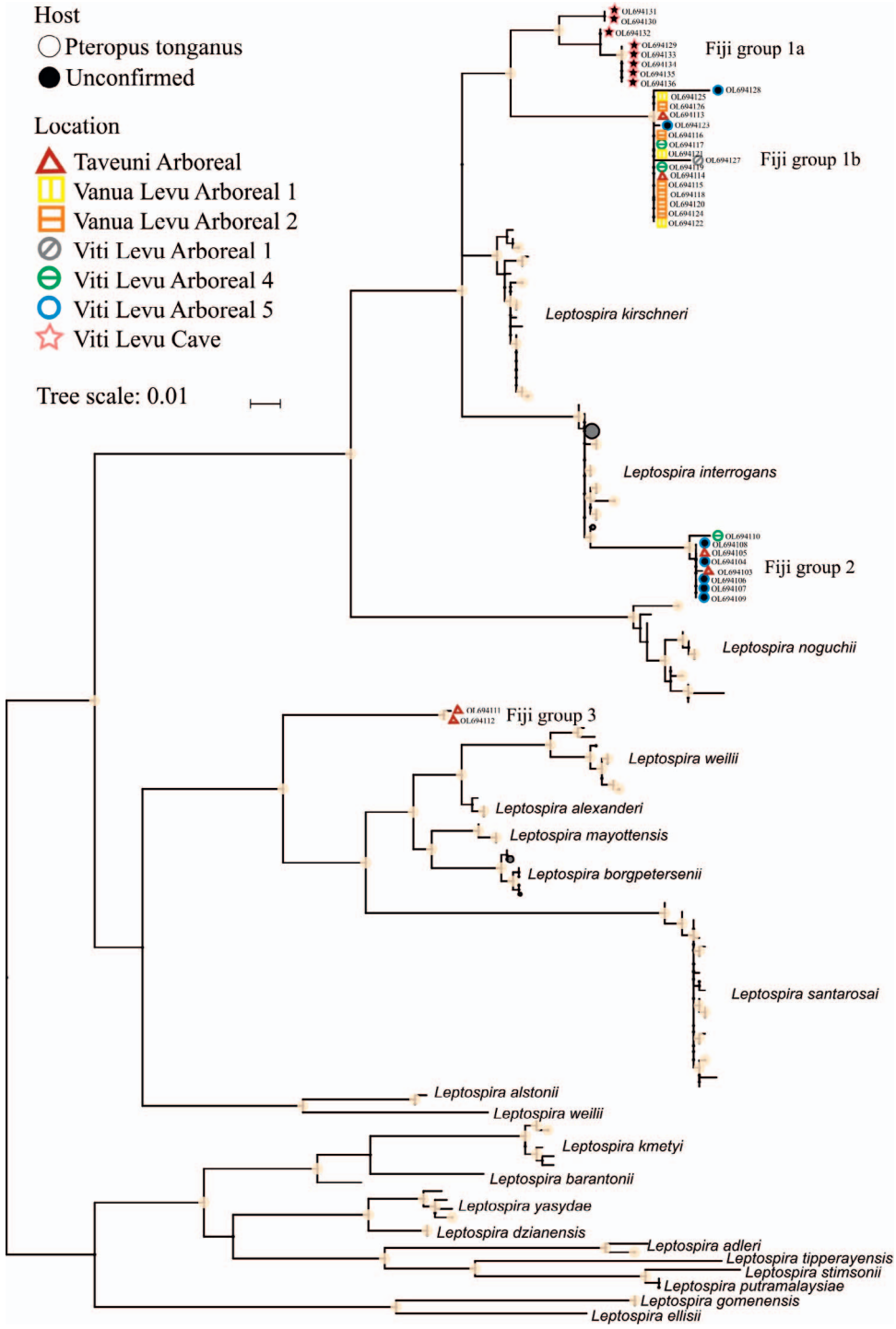

FIGURE 2. *Leptospira* phylogenetic tree based on the glmU gene with representatives from the NCBI (2019) nucleotide database and Fijian bat urine samples from three Fijian islands (Viti Levu, Vanua Levu, and Taveuni) presented with bat host species and study site information. Branch support >80% by approximate likelihood ratio test (aLRT) is indicated at pale pink nodes. Collapsed nodes within *Leptospira interrogans* and *Leptospira borgpetersenii* groups are indicated with gray circles.

tion of *Leptospira* human cases should be able to identify whether bats are the source, given the distinct clusters of the Fiji bat *Leptospira*.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-22-00038.

LITERATURE CITED

Anderson DE, Marsh GA. 2015. Bat paramyxoviruses. In: *Bats and viruses: A new frontier of emerging infectious diseases*. Wang LF, Cowled C, editors. John Wiley & Sons, Hoboken, New Jersey, pp. 99–126.

Anisimova M, Gascuel O. 2006. Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. *Syst Biol* 55:539–552.

Buhnerkempe MG, Prager KC, Strelioff CC, Greig DJ, Laake JL, Melin SR, DeLong RL, Gulland FMD, Lloyd-Smith JO. 2017. Detecting signals of chronic shedding to explain pathogen persistence: *Leptospira interrogans* in California sea lions. *J Anim Ecol* 86: 460–472.

Clayton BA, Wang LF, Marsh GA. 2013. Henipaviruses: An updated review focusing on the pteropid reservoid and features of transmission. *Zoonoses Public Health* 60:69–83.

Drexler JF, Corman VM, Wegner T, Tateno AF, Zerbinati RM, Gloza-Rausch F, Seebens A, Müller MA, Drosten C. 2011. Amplification of emerging viruses in a bat colony. *Emerg Infect Dis* 17:449–456.

Fenton MB, Simmons NB. 2014. *Bats: A world of science and mystery*. University of Chicago Press, Chicago, 303 pp.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol* 3:294–299.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Syst Biol* 59:307–321.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol Biol Evol* 30:772–780.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, et al. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649.

Lagadec E, Gomard Y, Guernier V, Dietrich M, Pascalis H, Temmam S, Ramasindrazana B, Goodman SM, Tortosa P, Dellagi K. 2012. Pathogenic *Leptospira spp*. in bats, Madagascar and Union of the Comoros. *Emerg Infect Dis* 18:1696–1698.

Lau CL, Watson CH, Lowry JH, David MC, Craig SB, Wynwood SJ, Kama M, Nilles EJ. 2016. Human leptospirosis infection in Fiji: An eco-epidemiological approach to identifying risk factors and environmental drivers for transmission. *PLoS Negl Trop Dis* 10: e0004405.

Lefort V, Longueville JE, Gascuel O. 2017. SMS: Smart model selection in PhyML. *Mol Biol Evol* 34:2422–2424.

Letunic I, Bork P. 2006. Interactive Tree Of Life (iTOL): An online tool for phylogenetic tree display and annotation. *Bioinformatics* 23:127–128.

Mackenzie JS, Field HE, Guyatt KJ. 2003. Managing emerging diseases borne by fruit bats (flying foxes) with particular reference to henipaviruses and Australian bat lyssavirus. *J Appl Microbiol* 94:59S–69S.

Mickleburgh S, Waylen K, Racey P. 2009. Bats as bushmeat: A global review. *Oryx* 43:217–234.

Monchy D, Huerre MR, de Bievre C. 1998. Deep-seated fungal diseases in the South Pacific, especially in New Caledonia. *Mycoses* 41:31–34.

NCBI (National Center for Biotechnology Information). 2019. *Nucleotide*. https://www.ncbi.nlm.nih.gov/nucleotide/. Accessed August 2019.

Palmeirim JM, Champion A, Naikatini A, Niukula J, Tuiwawa M, Fisher M, Yabaki-Gounder M, Thorsteinsdottir S, Qalovaki S, Dunn T. 2007. Distribution status and conservation of the bats of the Fiji Islands. *Oryx* 41:509–519.

Philbey AW, Kirkland PD, Ross AD, Davis RJ, Gleeson AB, Love RJ, Daniels PW, Gould AR, Hyatt AD. 1998. An apparently new virus (family paramyxoviridae) infectious for pigs humans and fruit bats. *Emerg Infect Dis* 4:269–271.

Rahman SA, Hassan SS, Olival KJ, Mohamed M, Chang LY, Hassan L, Saad NM, Shohaimi SA, Mamat ZC, et al. 2010. Characterization of Nipah virus from naturally infected *Pteropus vampyrus* bats Malaysia. *Emerg Infect Dis* 16:1990–1993.

Wilkinson DA, Edwards M, Benschop J, Nisa S. 2021. Identification of pathogenic *Leptospira* species and serovars in New Zealand using metabarcoding. *PLoS One* 16:e0257971.

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