**Supplementary materials for Journal of Wildlife Diseases DOI: 10.7589/JWD-D-22-00038: Jessica L. McCutchan,** **Matthew A. Knox,** **Alivereti Naikatini,** **David T. S. Hayman,** **and Brett D. Gartrell. Molecular Evidence of *Leptospira* spp. in Isolated Fijian Bats.**

**Supplementary Methods 1. RNA Extraction and PCR Protocols**

RNA was extracted with Roche High Pure Viral Nucleic Acid Kit ((Roche, Basel, Switzerland) using ~200 mg of feces or 200 μL urine added to 0.5 mL PBS and incubated for 1 hour at 4 ̊C. Following centrifugation (6,000 xg for 5 min) 450 μL supernatant was filtered through a 0.45uM syringe filter resulting in 200 μL sample for extraction following kit instructions. DNA from ~200 mg feces was extracted with a Zymo Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (Zymo Research, Freiburg, Germany). Approximately 1 mL urine was pelleted by centrifugation (16,000 xg for 3 min) and extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The isolation was. Extracted RNA and DNA was stored at-80 ̊C.RNA extractions were amplified by nested RT PCR in 25 μL volumes using Superscript™ III two-step RT-PCR system with platinum™ taq DNA polymerase kit (ThermoFisher, Massachusetts, USA). We used 11 μL of DNAse treated (Invitrogen DNA-free™ DNA Removal Kit) sample and outer primers. Inner primers used 4 μL 5x HOT-FIREPol PCR MasterMix (Solis BioDyne, Estonia) and 2μL product from the RT-PCR in a 20 μL reaction volume. PCR of DNA samples was carried out in 20 μL volumes using 5x HOT-FIREPol PCR MasterMix with 2 μL template (extracted DNA or PCR product from outer primers). For nested PCRs, the outer PCR product was diluted 1:20 before amplification with inner primer sets. All PCRs used 1 μL of 10 mM primer.

The NEB PCR Cloning Kit from New England BioLabs and TOPO TA Cloning Kit from Invitrogen was used to clone copies of the synthetically generated DNA, which was amplified using primers as shown in Supplementary Table 2.

**Supplementary Methods 2. Control material**

**a) Positive control material. A 1000 bp sequence with primer binding sites for all PCR tests**

TTCACTGTTATTTAATGATTTATTACTAAGTGCGACAAGTCGCCCTCCATGATACAAGGCACGCCCGACAACTCGTCCTCCAGTAAGCGTATCCTCGCATTTATGGTATCCTGATGATCTTAGGCTTCGTCTTATGCCCATAAGGCGTCTGAAGGITATTGTCAIAARNTNTGGACACATGTTTGACCCCTTCGTTAGGGTTGCTTCAATGGTTCARGGNGAYAACCAGATTCATATTAAACCCCTATGCGTTGGIACWAAYBTVCCWYTICARBTRGGAGAGCGAGTGTGTAGGCCGCGGCWCCWCCHGGNGARCAATTCCCGTGAACGGTCGACGGGATTCCATGTAGCGGGGTTGGCTCTGCTCTTACAAGAGTATACCGTGACTTTACGAGGTCACGACTGTATCCTGTTGTTCGATAATGTGCGCGGTGGTGATCGCTTCAAACTTACTCAGATAACCAGGGGGGATCTGCGAGTATGGTAGCAAACGTGGAGTTAGTCTGGTGCAGGTTATCTTATTAAGAAGTCCTCAGCATCGGATATGGCGGACAAGCGTGCTAATGTACATTACAAATTCTTGTGGTGTTCTTTCTTTCGCTAACAAACGCTAGGATATGTCCGAGATCTTGACCTCTGAAGCTATCGCAAGAATTACGCACGACATCTCACCCTTCCGGGGGTGAACAGCCTACTATGAGTACTCTTGGTGATTACCCAGAYRTWCARCARTGGGGWTWCCGTCGGTGCCGTCGCGCATGTNGTNGCWWSTKBTGAYGCTATMATGACCTGGGTACACTCAAGCCCGGGGTTTCCAACTTAGNAAYATHGGHGAHCCHGTAACTTCAGCTCCATATGACTGCGAAGCCCGTCAGCCCTCACAGGTTCTAGCGCTCCGGGCTCACAGCCCGGCGGTTCATGGGGCTTAAAGCAAGACGATTCTTCCGAGGTACCGGACTTAATTCAAGGCAGCCCGCTCAAGGTTGACAACCATCCTCAT

**b) Positive control material showing locations of primer binding sites (see Supplementary Table 2 for primer details)**



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| **Supplementary Table 1. Fijian sample site locations, bat species (visualized and DNA isolation), number of samples collected and tested by type, and number of *Leptospira* positive (+ve) samples**  |
| **Location** | **Bat Sp.** | **No. urinesamples** | **No. fecal****samples** | ***Leptospira* +ve samples** |
| Viti Levu Arboreal 1 | PFF (DNA) | 11 | 11 | 2 |
| Viti Levu Arboreal 2 | PFF | 2 | 9 | 0 |
| Viti Levu Arboreal 3 | PFF | 1 | 9 | 0 |
| Viti Levu Arboreal 4 | PFF (DNA) | 11 | 11 | 4 |
| Viti Levu Arboreal 5 | PFF | 11 | 11 | 7 |
| Viti Levu Cave | FBB | 12 | 12 | 9 |
| Vanua Levu Arboreal 1 | PFF (DNA) | 3 | 8 | 3 |
| Vanua Levu Arboreal 2 | PFF (DNA) | 8 | 9 | 7 |
| Vanua Levu Cave | FFTB (DNA) | 10 | 10 | 0 |
| Taveuni Arboreal | PFF (DNA) | 9 | 8 | 6 |
| **10 sites** | **3 sp. (pos. 4)** | **78** | **98** |  |
| Note: PFF (Pacific flying fox, *Pteropus tonganus*), FBB (Fijian blossom bat, *Notopteris macdonaldi*), FFTB (Fijian free-tailed bat. *Chaerephon bregullae*), pos. (possibly), DNA (deoxyribonucleic acid), No. (number) |  |

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| **Supplementary Table 2 . PCR Primers and thermocycling conditions**  |  |
| **Pathogen** | **Locus** | **Amplicon size** | **Primer sequences** | **PCR conditions** | **Citation** |
| Coronavirus | RNA-dependent RNA polymerase (RdRp) | 400 | COR-F1: CGT TGG IAC WAA YBT VCC WYT ICA RBT RGG | RT 45°C for 30 min 94°C for 2 mins followed by PCR 95°C for 15 min; 15 cycles at 95°C for 30 s 65°C for 30 s (−1°C/cycle) and 72°C for 45 s; 35 cycles at 94°C for 30 s 50°C for 30 s and 72°C for 45 s; and 1 cycle at 72°C for 5 min. | 1 |
| COR-R1: GGT CAT KAT AGC RTC AVM ASW WGC NAC NAC ATG |
| COR-F2: GGC WCC WCC HGG NGA RCA ATT |
| COR-R2: GGW AWC CCC AYT GYT GWA YRT C |
| Paramyxovirus | RNA polymerase L gene | 650 | PAR-F1: GAA GGI TAT TGT CAI AAR NTN TGG AC | RT 45°C for 30 min 94°C for 2 mins followed by PCR 94°C for 15 min and then 40 cycles at 94°C for 15 s 48°C for 30 s 72°C for 30 s and a final extension at 72°C for 7 min. | 2 |
| PAR-R: GCT GAA GTT ACI GGI TCI CCD ATR TTN C |
| PAR-F2: GTT GCT TCA ATG GTT CAR GGN GAY AA |
| PAR-R: GCT GAA GTT ACI GGI TCI CCD ATR TTN C |
| Histoplasma (1) | H antigen gene | 439 | HC2: GCGGGGTTGGCTCTGCTCT | 95°C for 15 min; 35 cycles of 94°C for 1 min 59°C for 1 min and 72°C for 1 min; and a final extension at 72°C for 10 min. | 3 |
| HC3: TTGGAAACCCCGGGCTTG |
| 330 | HC2: GCGGGGTTGGCTCTGCTCT |
| HC1: TCATAGTAGGCTGTTCACCCCCG |
| Histoplasma (2) | Internal transcribed spacer region | 600 | ITS-1: TCCGTAGTAACCTGCGG | 95°C for 15 min; 40 cycles of 95°C for 1 min 55°C for 1 min and 72°C for 1 min; and a final extension at 72°C for 10 min. | 4 |
| ITS-4: TCCTCCGCTTATTGATATGC |
| 400 | HC-1: GGAGCCTCTGACCGGGAC |
| HC-2: GCACGTCCCACCGGTCAG |
| *Leptospira* | glmU | 600 | glmU\_DW\_F: CCCGTATGAAAACGGATCAGCC | 94°C for 5 min; 5 cycles of 94°C for 30s 50°C for 40s and 72°C for 1 min; 50 cycles of 94°C for 30s 55°C for 40s and 72°C for 1 min; and a final extension at 72°C for 10 min. | 5 |
| glmU\_DW\_R: ATTCTCCCTGAGCGTTTTGATTTC |
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| Host (bat) | Cytochrome c oxidase subunit 1 (COI) | 658 | LCO-1490F: GGTCAACAAATCATAAAGATATTGG | 95°C for 15 min; 35 cycles of 95°C for 1 min 43°C for 1 min and 72°C for 1 min; and a final extension at 72°C for 10 min | 6 |
| HCO-2198R: TAAACTTCAGGGTGACCAAAAAATCA |

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**Supplementary Table 3: Sequence accessions**

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| **Leptospira GlmU** |  |  |  |  |  |
| **Sequence** | **Accession** | **Location** | **Phylogenetic group (Fig. 2)** | **Putative bat host** | **Lat Long** |
| BankIt2525586 L009 | OL694103 | Taveuni Arboreal | 2 | *Pteropus tonganus*  | 16.79042 S 179.9958 W |
| BankIt2525586 L150 | OL694104 | Viti Levu Arboreal 5 | 2 | *Pteropus tonganus*  | 18.16528 S 177.4817 E |
| BankIt2525586 L017 | OL694105 | Taveuni Arboreal | 2 | *Pteropus tonganus*  | 16.79042 S 179.9958 W |
| BankIt2525586 L145 | OL694106 | Viti Levu Arboreal 5 | 2 | *Pteropus tonganus*  | 18.16528 S 177.4817 E |
| BankIt2525586 L151 | OL694107 | Viti Levu Arboreal 5 | 2 | *Pteropus tonganus*  | 18.16528 S 177.4817 E |
| BankIt2525586 L153 | OL694108 | Viti Levu Arboreal 5 | 2 | *Pteropus tonganus*  | 18.16528 S 177.4817 E |
| BankIt2525586 L152 | OL694109 | Viti Levu Arboreal 5 | 2 | *Pteropus tonganus*  | 18.16528 S 177.4817 E |
| BankIt2525586 L168 | OL694110 | Viti Levu Arboreal 4 | 2 | *Pteropus tonganus*  | 18.2115 S 177.7154 E |
| BankIt2525586 L010 | OL694111 | Taveuni Arboreal | 3 | *Pteropus tonganus*  | 16.79042 S 179.9958 W |
| BankIt2525586 L013 | OL694112 | Taveuni Arboreal | 3 | *Pteropus tonganus*  | 16.79042 S 179.9958 W |
| BankIt2525586 L015 | OL694113 | Taveuni Arboreal | 1b | *Pteropus tonganus*  | 16.79042 S 179.9958 W |
| BankIt2525586 L016 | OL694114 | Taveuni Arboreal | 1b | *Pteropus tonganus*  | 16.79042 S 179.9958 W |
| BankIt2525586 L062 | OL694115 | Vanua Levu Arboreal 2 | 1b | *Pteropus tonganus*  | 16.80389 S 179.3838 E |
| BankIt2525586 L064 | OL694116 | Vanua Levu Arboreal 2 | 1b | *Pteropus tonganus*  | 16.80389 S 179.3838 E |
| BankIt2525586 L175 | OL694117 | Viti Levu Arboreal 4 | 1b | *Pteropus tonganus*  | 18.2115 S 177.7154 E |
| BankIt2525586 L063 | OL694118 | Vanua Levu Arboreal 2 | 1b | *Pteropus tonganus*  | 16.80389 S 179.3838 E |
| BankIt2525586 L178 | OL694119 | Viti Levu Arboreal 4 | 1b | *Pteropus tonganus*  | 18.2115 S 177.7154 E |
| BankIt2525586 L050 | OL694120 | Vanua Levu Arboreal 2 | 1b | *Pteropus tonganus*  | 16.80389 S 179.3838 E |
| BankIt2525586 L048 | OL694121 | Vanua Levu Arboreal 1 | 1b | *Pteropus tonganus*  | 16.56419 S 179.4435 E |
| BankIt2525586 L046 | OL694122 | Vanua Levu Arboreal 1 | 1b | *Pteropus tonganus*  | 16.56419 S 179.4435 E |
| BankIt2525586 L136 | OL694123 | Viti Levu Arboreal 5 | 1b | *Pteropus tonganus*  | 18.16528 S 177.4817 E |
| BankIt2525586 L065 | OL694124 | Vanua Levu Arboreal 2 | 1b | *Pteropus tonganus*  | 16.80389 S 179.3838 E |
| BankIt2525586 L041 | OL694125 | Vanua Levu Arboreal 1 | 1b | *Pteropus tonganus*  | 16.56419 S 179.4435 E |
| BankIt2525586 L061 | OL694126 | Vanua Levu Arboreal 2 | 1b | *Pteropus tonganus*  | 16.80389 S 179.3838 E |
| BankIt2525586 L117 | OL694127 | Viti Levu Arboreal 1 | 1b | *Pteropus tonganus*  | 18.06631 S 178.4645 E |
| BankIt2525586 L146 | OL694128 | Viti Levu Arboreal 5 | 1b | *Pteropus tonganus*  | 18.16528 S 177.4817 E |
| BankIt2525586 L081 | OL694129 | Viti Levu Cave | 1a | *Notopteris macdonaldi*  | 17.76194 S 178.4069 E |
| BankIt2525586 L085 | OL694130 | Viti Levu Cave | 1a | *Notopteris macdonaldi*  | 17.76194 S 178.4069 E |
| BankIt2525586 L092 | OL694131 | Viti Levu Cave | 1a | *Notopteris macdonaldi*  | 17.76194 S 178.4069 E |
| BankIt2525586 L089 | OL694132 | Viti Levu Cave | 1a | *Notopteris macdonaldi*  | 17.76194 S 178.4069 E |
| BankIt2525586 L093 | OL694133 | Viti Levu Cave | 1a | *Notopteris macdonaldi*  | 17.76194 S 178.4069 E |
| BankIt2525586 L094 | OL694134 | Viti Levu Cave | 1a | *Notopteris macdonaldi*  | 17.76194 S 178.4069 E |
| BankIt2525586 L095 | OL694135 | Viti Levu Cave | 1a | *Notopteris macdonaldi*  | 17.76194 S 178.4069 E |
| BankIt2525586 L102 | OL694136 | Viti Levu Arboreal 1 | 1a | *Pteropus tonganus*  | 18.06631 S 178.4645 E |
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| **COI host DNA** |  |  |  |  |  |
| **Sequence** | **Accession** | **Location** | **Species** | **Lat. Long.** |  |
| SUB10746063 049 | OL693647 | Vanua Levu Arboreal 1 | *Pteropus tonganus*  | 16.56419 S 179.4435 E |  |
| SUB10746063 105 | OL693648 | Viti Levu Arboreal 1 | *Pteropus tonganus*  | 18.06631 S 178.4645 E |  |
| SUB10746063 008 | OL693649 | Taveuni Arboreal | *Pteropus tonganus*  | 16.79042 S 179.9958 W |  |
| SUB10746063 106 | OL693650 | Viti Levu Arboreal 1 | *Pteropus tonganus*  | 18.06631 S 178.4645 E |  |
| SUB10746063 158 | OL693651 | Viti Levu Arboreal 4 | *Pteropus tonganus*  | 18.2115 S 177.7154 E |  |
| SUB10746063 051 | OL693652 | Vanua Levu Arboreal 2 | *Pteropus tonganus*  | 16.80389 S 179.3838 E |  |
| SUB10746063 038 | OL693653 | Vanua Levu Cave | *Pteropus tonganus*  | 16.77792 S 178.9725 E |  |
| SUB10746063 003 | OL693654 | Taveuni Arboreal | *Pteropus tonganus*  | 16.79042 S 179.9958 W |  |
| SUB10746063 022 | OL693655 | Vanua Levu Cave | *Chaerephon bregullae*  | 16.77792 S 178.9725 E |  |