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SHORT COMMUNICATION

Diversity of *Leptospira* spp. in bats and rodents from Papua New Guinea

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Abstract

Leptospirosis is the most common bacterial zoonosis globally. The pathogen, *Leptospira* spp., is primarily associated with rodent reservoirs. However, a wide range of other species has been implicated as reservoirs or dead-end hosts. We conducted a survey for *Leptospira* spp. in bats and rodents from Papua New Guinea. Kidney samples were collected from 97 pteropodid bats (five species), 37 insectivorous bats from four different families (six species) and 188 rodents (two species). Leptospires were detected in a high proportion of pteropodid bats, including *Nyctimene* cf. *albiventer* (35%), *Macroglossus minimus* (34%) and *Rousettus amplexicaudatus* (36%). Partial sequencing of the *secY* gene from rodent and bat leptospires showed host species clustering, with *Leptospira interrogans* and *L. weilii* detected in rodents and *L. kirschneri* and a potential novel species of *Leptospira* detected in bats. Further research is needed in Papua New Guinea and other locales in the Pacific region to gain a better understanding of the circulation dynamics of leptospires in reservoir species and the risks to public and veterinary health.

KEYWORDS
bats, leptospira, Papua New Guinea, rats, rodents

Sarah Javati, Vanina Guernier-Cambert, Mohammad Yazid Abdad and Paul F. Horwood contributed equally to this article.

1 | INTRODUCTION

Leptospirosis is a zoonosis of global distribution with the highest incidence rates reported in tropical areas. A systematic review conducted in the Pacific Islands showed that human and animal leptospirosis is widespread in the region while displaying some epidemiological heterogeneity (Guernier et al., 2018). In Papua New Guinea, data are scarce, mostly dating from the 1960s when animal leptospirosis was investigated through microscopy or serology in selected animal species (livestock, dogs, bandicoots and rodents; Guernier et al., 2018). The most recently published survey, conducted in 2004, investigated renal carriage in cattle and pigs from three Papua New Guinea provinces, with the detection of *Leptospira borgpetersenii* in cattle (Wai'in, 2007). Human cases of leptospirosis in Papua New Guinea have not been investigated for at least 50 years (Emanuel, 1959; Forbes & Wannan, 1955; Kariks & Stallman, 1968; Willis & Wannan, 1966), but the high incidence of this disease in the Pacific region (Guernier et al., 2018), and the prevailing social and environmental conditions in the country (Horwood et al., 2019), make it likely that leptospirosis incidence is very high in this setting. Recent global studies have estimated that the rate of leptospirosis in Papua New Guinea is likely amongst the highest in the world, ranging from > 100 (Costa et al., 2015) to > 500 (Torgerson et al., 2015) cases per 100,000 population.

Bats have been shown to be an important reservoir of leptospires in various tropical islands in the Indian Ocean (Dietrich et al., 2015; Gomard et al., 2016; Lagadec et al., 2012), Malaysia (Thayaparan et al., 2015) and the West Indies (Bevans et al., 2020). However, to date, they have not been investigated in Papua New Guinea, even though the country contains a diverse bat fauna of at least 91 species that includes 21 pteropodids (flying foxes, fruit and blossom bats) and 70 insectivorous bats (Bonaccorsco, 1998; Helgen, 2007).

2 | MATERIALS AND METHODS

Bats were trapped using mist nets and harp traps as part of biodiversity surveys conducted in October 2014 in Manus Province (Manus Island) and New Ireland Province (Mussau Island) of Papua New Guinea as previously described (Aplin et al., 2015). Kidney samples were collected from fruit-eating bats (Pteropodidae): *Nyctimene* cf. *albiventer* (*n* = 29), *Macroglossus minimus* (*n* = 35), *Rousettus amplexicaudatus* (*n* = 11), *Pteropus* cf. *admiralitatum* (*n* = 17) and *Dobsonia anderseni* (n = 5); and insectivorous bats: *Mosia nigrescens* (n = 2) (Emballonuridae), *Aselliscus tricuspidatus* (n = 5; Hipposideridae), *Hipposideros calcaratus* and *H. cervinus* (*n* = 21; Hipposideridae), *Pipistrellus angulatus* (*n* = 1; Vespertilionidae) and *Miniopterus* cf. *propitristis* (*n* = 8; Miniopteridae; Table 1). Animal collections were conducted following approval from the Papua New Guinea Department of Environment and Conservation, the Provincial Governments of Manus and New Ireland. Bat specimens were euthanized with isoflurane inhalation in accordance with an approved animal care and use proposal from the Smithsonian Institution (NHB-ACUC #2009-4), exported under permit #014273 (dated 18 November 2014) from the Papua New Guinea Department of Environment and Conservation and deposited and available for study as voucher specimens at the National Museum of Natural History, Smithsonian Institution, Washington. DC, USA.

Rodent specimens (*Rattus rattus*, *n* = 39; *R. exulans*, *n* = 149) were collected during 2014–2015 as previously described (Robby et al., 2017) in the Eastern Highlands Province of Papua New Guinea. Kidney samples were collected in the field (5–10 mm pieces) and stored in RNAlater (Merck) before transport to the laboratory where they were stored at -80°C until testing.

Total nucleic acids were extracted from kidneys using the DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer's instructions. Extracts were screened for pathogenic *Leptospira* spp. with a TaqMan real-time polymerase chain reaction (PCR) targeting the *rrs* (16S) gene (Smythe et al., 2002). *Leptospira*-positive samples were subjected to conventional PCR amplification, targeting the *secY* gene (549-bp fragment), which has been shown to be suitable for species identification and phylogenetic studies (Ahmed et al., 2006; Guernier et al., 2017; Medeiros et al., 2020; Perez & Goarant, 2010; Victoria et al., 2008). All PCRs were run with negative and positive controls. The resulting PCR products were sent to Macrogen for Sanger sequencing.

Consensus sequences and alignments were generated with Geneious Prime 2019.1.1 (Biomatters Ltd). Phylogenetic trees were constructed using PhyML with Smart Model Selection (Guindon et al., 2010; Lefort et al., 2017) with the following settings: the substitution model selection used Akaike Information Criterion; the tree searching used default settings; the phylogeny was based on the maximum-likelihood method with 1000 bootstraps. Trees were visualized in FigTree v1.3.1 (http://tree.bio.ecd.ac.uk/).

3 | RESULTS

In total, kidney samples were collected from 97 pteropodid bats (five species), 37 insectivorous bats from four different families (six species) and 188 rodents (two species). Real-time PCR screening of these samples resulted in the detection of *Leptospira* spp. in a high proportion of fruit-eating bats (Pteropodidae) from *N.* cf. *albiventer* (34.5%), *M. minimus* (34.3%) and *R. amplexicaudatus* (36.4%; Table 1). Leptospires were also detected from a high proportion of rodents from *R. rattus* (20.5%) and *R. exulans* (24.2%) as previously reported (Robby et al., 2017). Molecular testing did not result in the detection of *Leptospira* sp. in any of the insectivorous bats or the two other species of pteropodid bats. However, we cannot make any definite conclusions about leptospiral carriage in these species as sample sizes were small (one to eight individuals per species, except for *Hipposideros* spp. for which we collected 21 individuals).

Leptospira-positive samples (26 bats, 44 rodents) were targeted for conventional PCR (*secY* gene), and subsequent Sanger sequencing successfully generated *Leptospira* data from nine bat samples and 30 rodent samples. Micromammals collected from Papua New Guinea were infected with at least four *Leptospira* species, one of which was

TABLE 1 Detection of *Leptospira* spp. in kidney samples collected from bats and rodents from Papua New Guinea

Host group (family)	Host genera/species	Collection province[a]	Leptospira positive (%)
Bats (Pteropodidae)	*Nyctimene* cf. *albiventer*	Manus and New Ireland	10/29 (34.5)
	Macroglossus minimus	Manus and New Ireland	12/35 (34.3)
	Rousettus amplexicaudatus	Manus and New Ireland	4/11 (36.4)
	Dobsonia anderseni	New Ireland	0/5 (0)
	Pteropus cf. *admiralitatum*	New Ireland	0/3 (0)
Bats (Emballonuridae)	*Mosia nigrescens*	Manus	0/2 (0)
Bats (Hipposideridae)	*Aselliscus tricuspidatus*	New Ireland	0/5 (0)
	Hipposideros cervinus and *H. calcaratus*	New Ireland	0/21 (0)
Bats (Vespertilionidae)	*Pipistrellus angulatus*	Manus	0/1 (0)
Bats (Miniopteridae)	*Miniopterus* cf. *propitristis*	New Ireland	0/8 (0)
Rats (Muridae)	*Rattus rattus*	Eastern Highlands	8/39 (20.5)[b]
	R. exulans	Eastern Highlands	36/149 (24.2)[b]

[a] Bats were collected in October 2014 on Manus Island (Manus Province) and Mussau Island (New Ireland Province) of Papua New Guinea (Aplin et al., 2015).
[b] Results were reported in Robby et al. (2017).

possibly an undescribed species (Figure 1). Three leptospires from *R. exulans* rodents clustered with *L. weilii*, while the rest of the rodent samples (six *R. rattus* and 21 *R. exulans*) clustered with *L. interrogans*, with a low diversity observed in this cluster. Seven bat leptospires were clustered with *L. kirschneri*, with a high diversity observed and host species-associated clustering. The remaining three bat (all *R. amplexicaudatus*) leptospires clustered together and were closely related to *L. alexanderi* and *L. mayottensis* but did not belong to these species (maximum of 91% similarity when basic local alignment seach tool searched against the National Center for Biotechnology Information database). Interestingly, one *R. amplexicaudatus* was co-infected with two different *Leptospira* (OM811606 and OM811607; Figure 1).

To see how Papua New Guinean bat leptospires compared to *Leptospira* collected from other bats worldwide, we built a phylogeny including 42 *secY Leptospira* sequences from bats only (Figure 2). The first *Leptospira* genetic cluster from Papua New Guinean bats was most closely related to a *Leptospira kirschneri* strain infecting *M. natalensis* collected in South Africa. The second *Leptospira* cluster from Papua New Guinean bats was detected in four *R. amplexicaudatus* individuals and was putatively an undescribed species closely related to *L. alexanderi* and *L. mayottensis*. The most closely related sequences to this potential undescribed species were from genetically related *Leptospira* infecting other *Rousettus* species collected in South Africa and Madagascar.

4 | DISCUSSION

Although human leptospirosis has not been recently described in Papua New Guinea, it was confirmed in the 1950s and 1960s (; Emanuel, 1959; Forbes & Wannan, 1955; Kariks & Stallman, 1968; Willis & Wannan, 1966) mostly through seroprevalence studies in the general population. The detection of several species of pathogenic *Leptospira* in various micromammals in this study, especially *L. interrogans* in rodents (a *Leptospira* species commonly related to human clinical cases worldwide) suggests that human leptospirosis is underdiagnosed in Papua New Guinea. The genetic clustering of the rodent leptospires detected in this study with regional human cases further supports the importance of rodents as the primary reservoir for human cases of leptospirosis.

The findings from this study suggest that fruit-eating bats (Pteropodidae) may be an underappreciated reservoir of *Leptospira* spp. in Papua New Guinea and potentially other locales in the Pacific region. Previous studies have found a similar prevalence of leptospires in bats from Australia (11%; Cox et al., 2005), Madagascar (35%; Lagadec et al., 2012), China (50%; Han et al., 2018) and Grenada (27%; Bevans et al., 2020). *Leptospira kirschneri* was the most commonly detected leptospire in Papua New Guinean bats, with the strains clustering in a distinct clade despite the samples being from three different genera of bats and the two collection sites on different island provinces separated by > 400 km. A potential novel *Leptospira* sp. was also detected in three *R. amplexicaudatus* bats, adding to previous reports of the detection of diverse *Leptospira* species in bats (Dietrich et al., 2015). This finding shows the potential circulation of an undescribed *Leptospira* species in Papua New Guinea but also highlights the rich biodiversity of this phylum yet to be discovered, in bats especially. The *Leptospira*-positive samples from 17 bats and 14 rodents that we were unable to sequence could hide further diversity, and uncultured samples might prove insufficient to sequence and uncover undescribed *Leptospira* species. Comprehensive one-health studies that consider multiple potential reservoirs and the risk factors for zoonotic transmission are needed to gain a greater understanding of the transmission

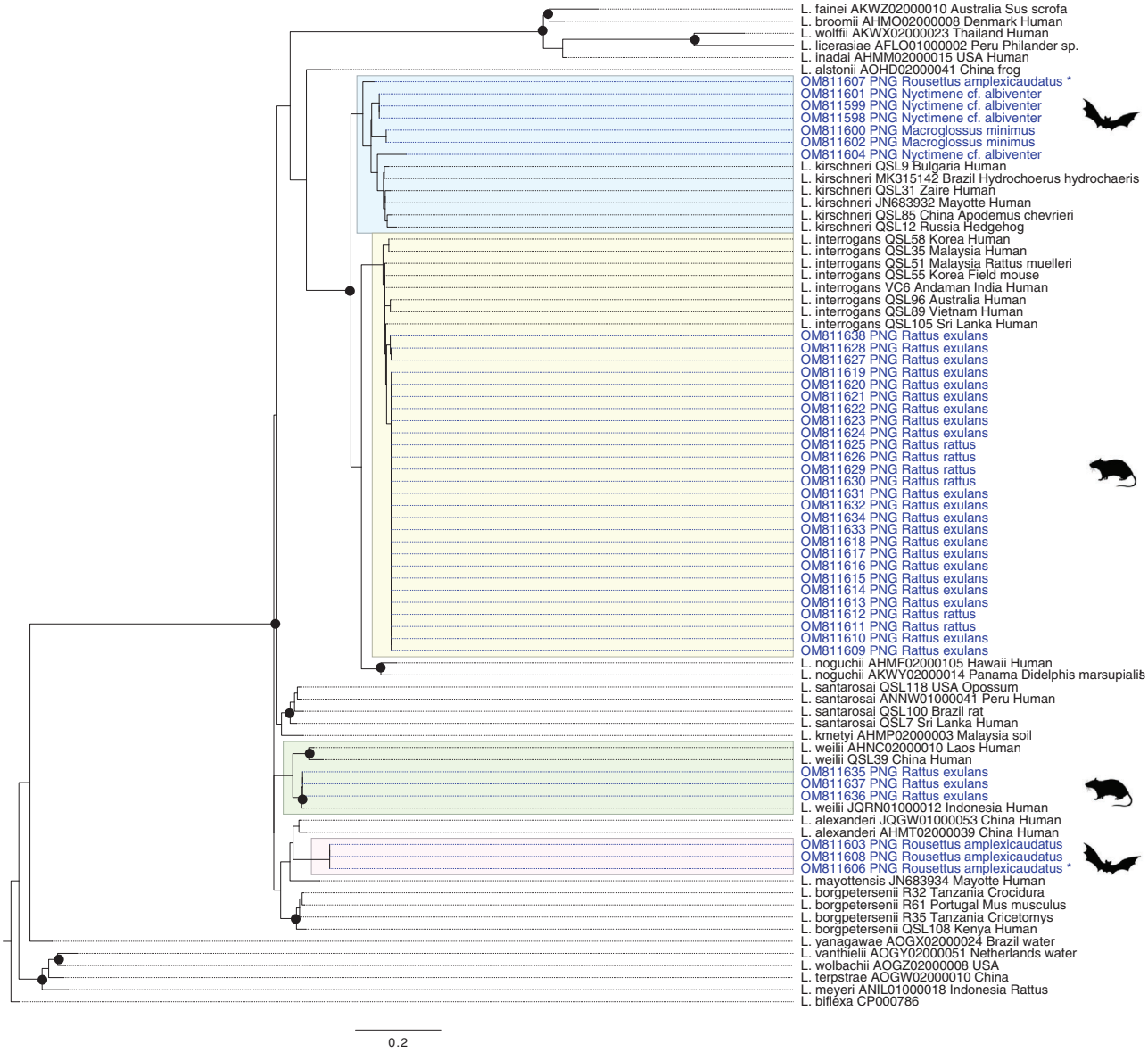

FIGURE 1 Maximum-likelihood (ML) phylogenetic tree (model GTR+G+I; 1000 replicates) inferred from the *Leptospira secY* gene (515-bp sequence). Phylogenetic trees were constructed using PhyML based on the ML method with 1000 bootstraps. Trees were visualized in FigTree v1.3.1 (http://tree.bio.ecd.ac.uk/). *Leptospira secY* sequences from Papua New Guinea are shown in blue (GenBank accession numbers OM811598 to OM811638), sequences from other countries are in black. Names include *Leptospira* species (when provided in GenBank), identifier or GenBank accession number, country of collection and host species. Sequences not from GenBank are from a global collection previously published (Nalam et al., 2010). The four genetic groups including Papua New Guinea samples are highlighted in colour. Bootstrap values higher than 70% are indicated by a dark circle. Black silhouettes represent Papua New Guinea host groups (i.e., rodents or bats). Of note, sample OM811605 (*Leptospira* from a *Rousettus amplexicaudatus*) was not included in this phylogeny as the *secY* sequence was incomplete (476 bp). * *Leptospira* OM811606 and OM811607 were obtained from the same individual (co-infection).

dynamics between leptospiral reservoirs such as rodents and bats and the potential role that bat-borne leptospires may play in the public and veterinary health threat of leptospirosis. To date, the role of bats in the transmission of leptospirosis to humans is unclear as direct transmission has not been conclusively established. However, the increasing encroachment of humans into bat habitats coupled with certain cultural practices may increase the risk for zoonotic cross-over in some settings as established for the transmission of Nipah virus from bats to humans in Bangladesh (McKee et al., 2021).

This study is the first investigation of the molecular diversity of leptospires in Papua New Guinean micromammals and the first to report the detection of leptospires in Papua New Guinean bats. The results show that Papua New Guinean rodents and bats carry diverse *Leptospira* sp. that are phylogenetically related to strains with known public and veterinary health impacts. Although leptospirosis has not been recently reported in Papua New Guinea, this is likely due to the lack of diagnostic capacity in most hospitals in the country (Greenhill et al., 2012). Further studies are needed to determine the public health

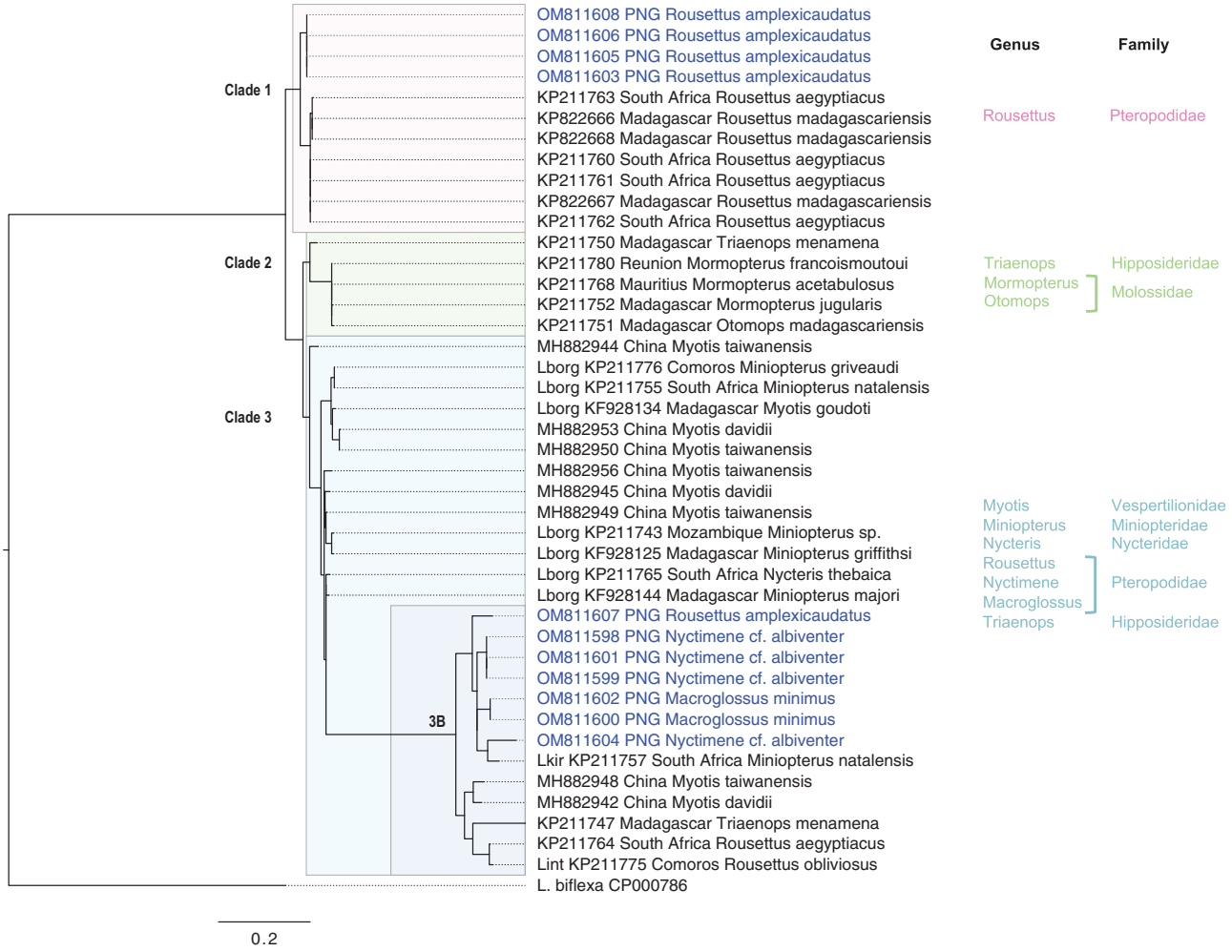

FIGURE 2 ML phylogenetic tree (model HKY85+G+I; 1000 replicates) inferred from the *Leptospira secY* gene (471-bp sequence) from bats only. Phylogenetic trees were constructed using PhyML based on the ML method with 1000 bootstraps. Trees were visualized in FigTree v1.3.1 (http://tree.bio.ecd.ac.uk/). *Leptospira secY* sequences from Papua New Guinea are shown in blue, sequences from other countries are in black. Names include *Leptospira* species (when provided in GenBank), identifier or GenBank accession number, country of collection and host species. GenBank accession numbers for our study are OM811598 to OM811638.

burden of leptospirosis in Papua New Guinea and the transmission links with rodents, bats and other animals.

AUTHOR CONTRIBUTIONS

P. F. Horwood and M. Y. Abdad sourced funding for the initial phases of the project and designed the experiments and supervized field and laboratory activities for the study. S Javati, M. Jonduo, S. Robby, J. Kimopa and T. Maure conducted the laboratory testing and molecular analysis of samples. V. Guernier-Cambert conducted the sequence and phylogenetic analysis of *Leptospira* spp. K. Aplin and K. M. Helgen conducted identification of bat species. E. S. McBryde and W. Pomat provided high-level support for the project and sourced funding for sequence analysis of *Leptospira* spp. P. F. Horwood wrote the first draft of the manuscript with assistance from V. Guernier-Cambert and M. Y. Abdad. All authors contributed to revisions of manuscript drafts and approved the final document for submission.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Information on the samples and sequence information generated are presented in Supplementary Table 1. All *Leptospira* sp. sequences were deposited in GenBank (accession numbers OM811598 to OM811638).

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. All relevant guidelines for the use of animals in scientific studies were followed.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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