

## Phylogenetic analyses of individual loci

Phylogenetic analyses were implemented in BEAST v2.6.0 (1) using the most appropriate substitution models as determined Bayesian Information Criterion (BIC) by model test in MEGA7 (2) and linked clocks (strict) and trees. Single-locus analyses were run using a chain length of  $1 \times 10^7$  and sampled every  $1 \times 10^3$  runs with a burn-in of 10%. TRACER v1.7.1 (3) was used to verify that the effective sample size (ESS) was greater than 200 and TREEANNOTATOR v2.6.0 used to generate a maximum clade credibility tree using mean node heights annotated by posterior probabilities greater than 0.9.

Electropherograms of sequences generated in the course of this study were examined by eye and trimmed for quality. *Leptospira* reference sequences were obtained by querying sequences against the NCBI refseq\_genome database using the BLASTn algorithm limited to *Leptospira* (taxid 171) belonging to the two species (*L. interrogans* and *L. borpetersenii*) identified in this study. Aligned BLAST hits for each locus were linked by biosample and representative sequences for each *Leptospira* species and serovar combination selected as reference sequences.

## 1. *L. borgpetersenii*

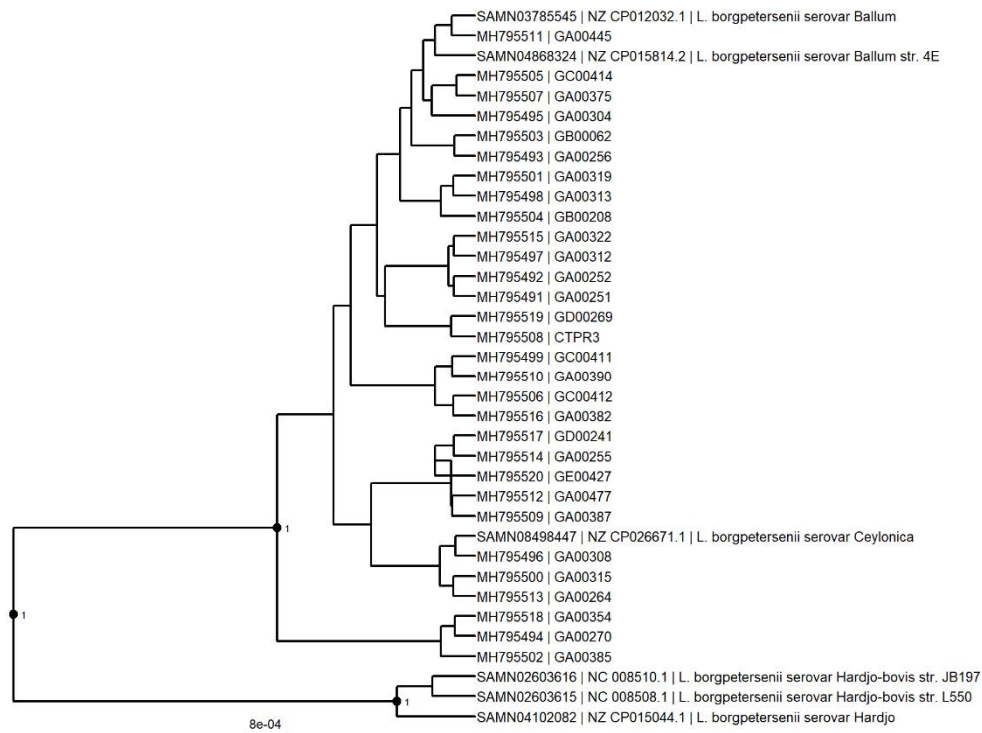


Figure S3: Maximum clade credibility tree based on *L. borgpetersenii lfb1* sequences (167bp) implemented using the Jukes-Cantor evolutionary model. Nodes with posterior support greater than 0.9 are shown. Reference sequences are labelled by biosample, sequence accession, and *Leptospira* strain and sequences from this study labelled by accession and sample code. Sample codes beginning with “G” are from the Johannesburg study site and codes beginning with “C” from Cape Town. Although 37 *lfb1* products were sequenced to confirm the *Leptospira* species identification inferred from melt curve analysis, eight sequences were of insufficient quality for phylogenetic analysis and were excluded

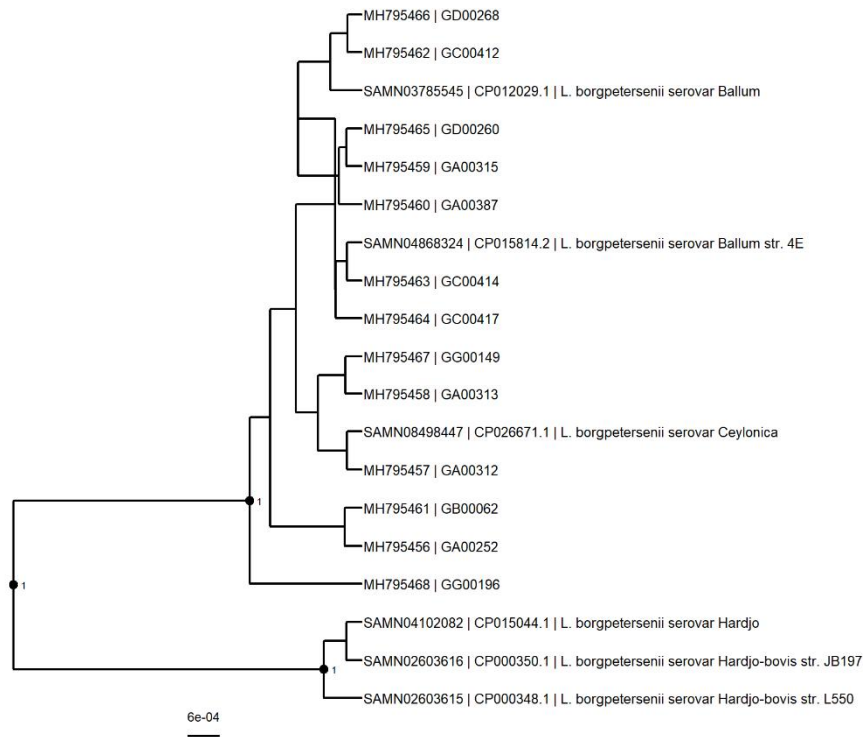


Figure S4: Maximum clade credibility tree based on *L. borgpetersenii* *secY* sequences (433bp) implemented using the Jukes-Cantor evolutionary model. Nodes with posterior support greater than 0.9 are shown. Reference sequences are labelled by biosample, sequence accession, and *Leptospira* strain and sequences from this study labelled by accession and sample code

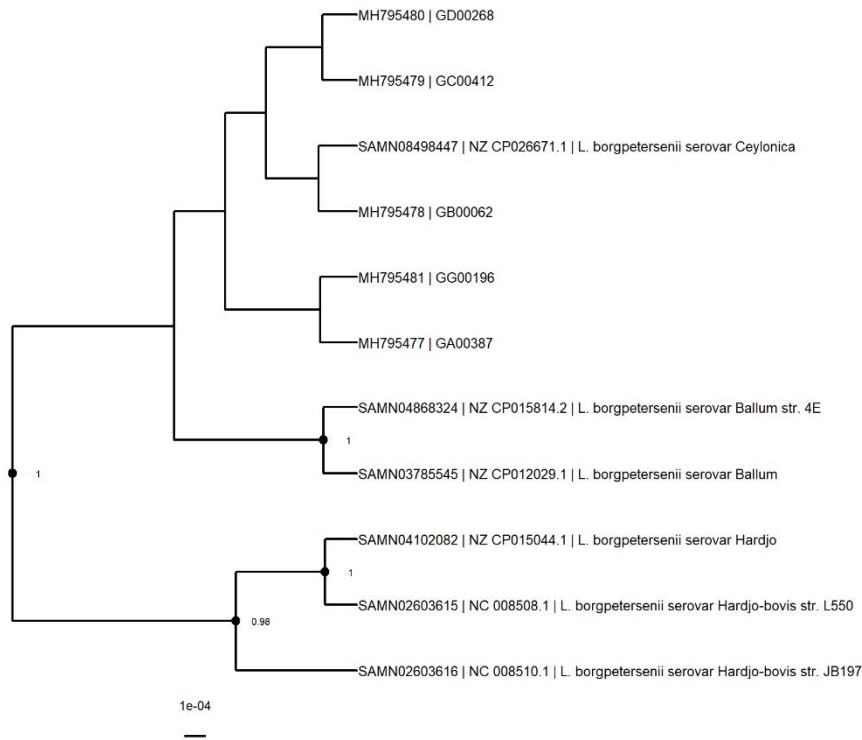


Figure S5: Maximum clade credibility tree based on *L. borgpetersenii* *lipL41* sequences (594bp) implemented using the Jukes-Cantor evolutionary model. Nodes with posterior support greater than 0.9 are shown. Reference sequences are labelled by biosample, sequence accession, and *Leptospira* strain and sequences from this study labelled by accession and sample code

## 2. *L. interrogans*

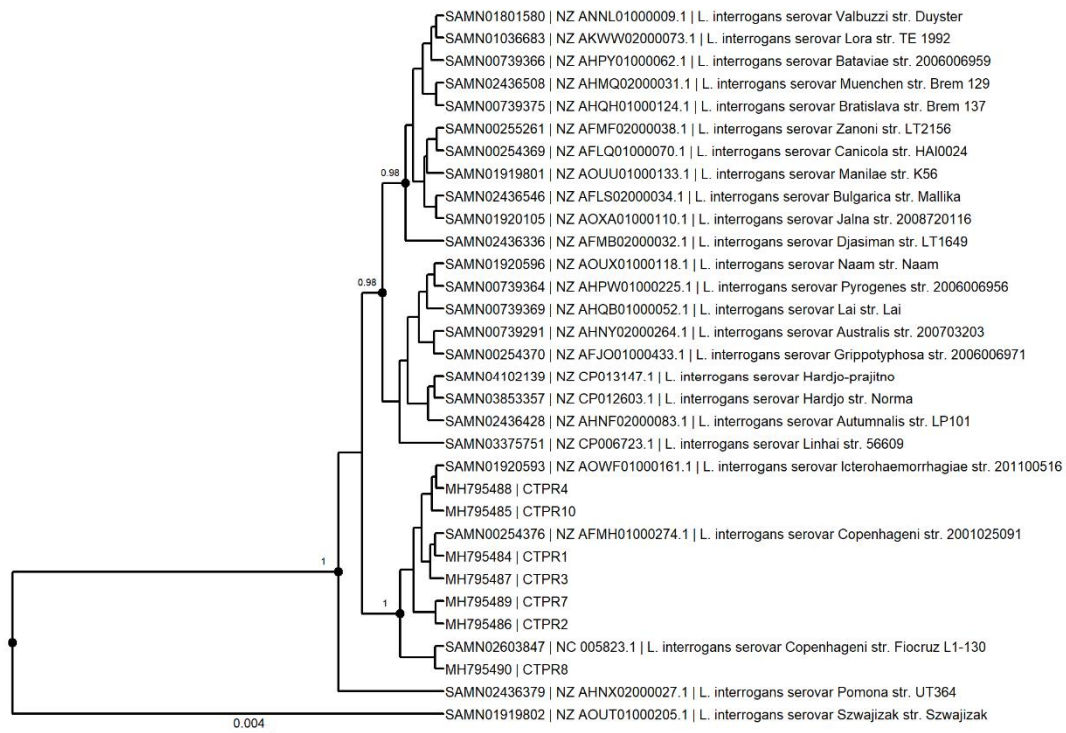


Figure S6: Maximum clade credibility tree based on *L. interrogans lfb1* sequences (261bp) implemented using the Jukes-Cantor substitution model. Nodes with posterior support greater than 0.9 are shown. Reference sequences are labelled by biosample, sequence accession, and *Leptospira* strain and sequences from this study labelled by accession and sample code

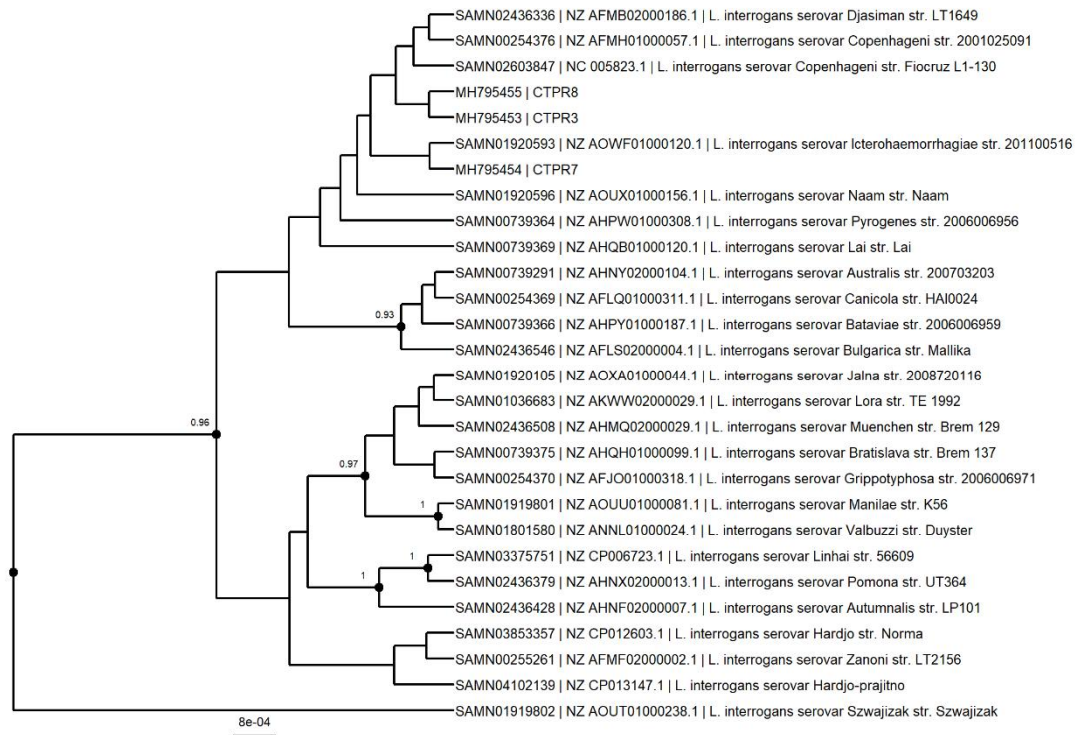


Figure S7: Maximum clade credibility tree based on *L. interrogans secY* sequences (433bp) implemented using the Hasegawa-Kishino-Yano evolutionary model (4) substitution model. Nodes with posterior support greater than 0.9 are shown. Reference sequences are labelled by biosample, sequence accession, and *Leptospira* strain and sequences from this study labelled by accession and sample code

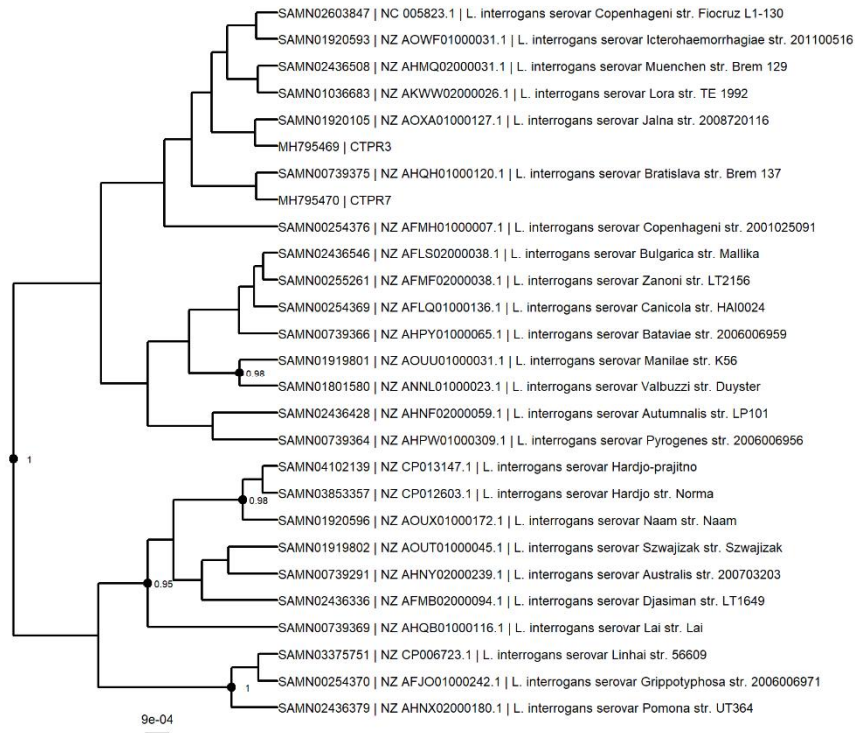


Figure S8: Maximum clade credibility tree based on *L. interrogans* MST1 sequences (174bp) implemented using the Hasegawa-Kishino-Yano evolutionary model (4) substitution model. Nodes with posterior support greater than 0.9 are shown. Reference sequences are labelled by biosample, sequence accession, and *Leptospira* strain and sequences from this study labelled by accession and sample code

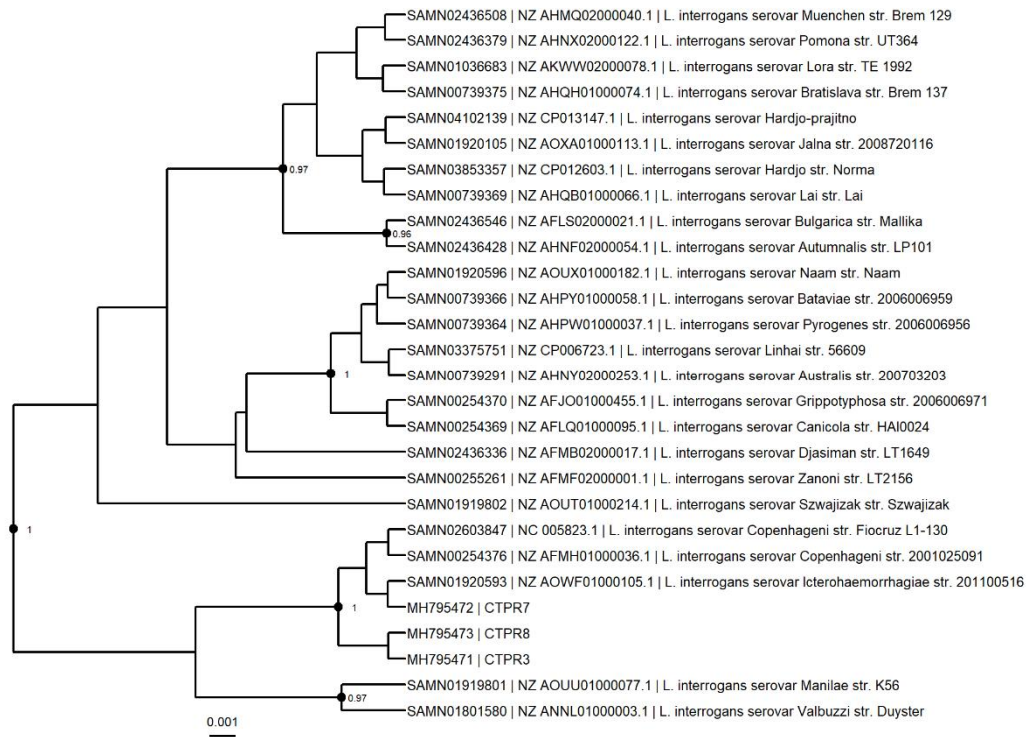


Figure S9: Maximum clade credibility tree based on *L. interrogans* MST3 sequences (220bp) implemented using the Hasegawa-Kishino-Yano evolutionary model (4) with a gamma distribution (4 categories). Nodes with posterior support greater than 0.9 are shown. Reference sequences are labelled by biosample, sequence accession, and *Leptospira* strain and sequences from this study labelled by accession and sample code

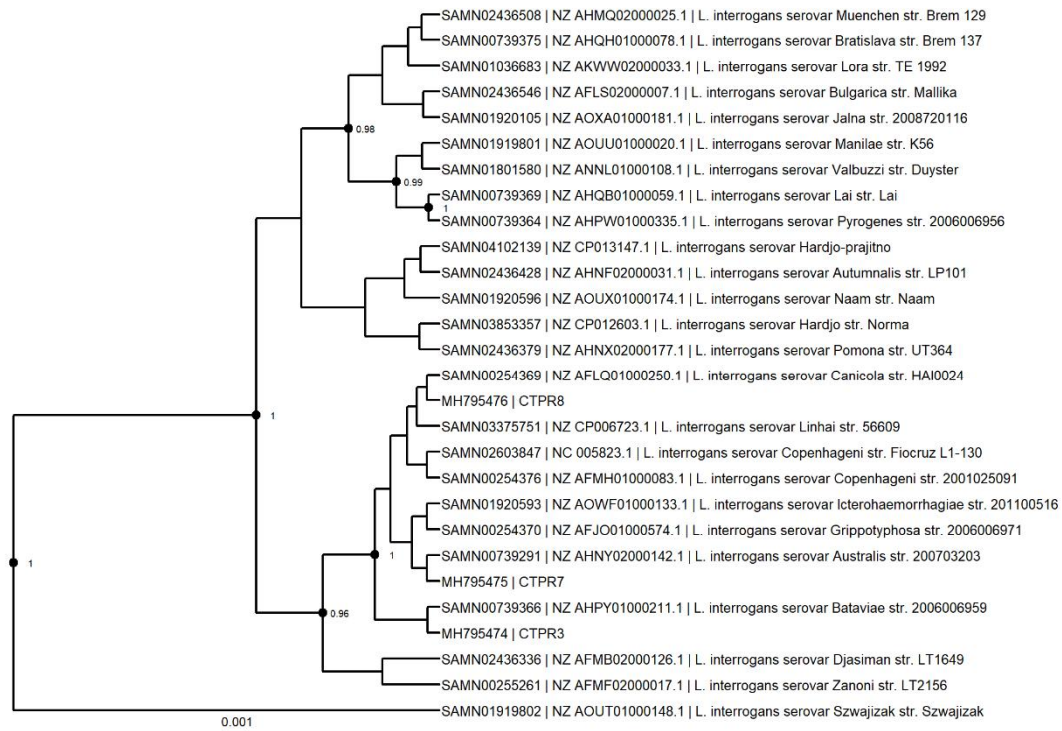


Figure S10: Maximum clade credibility tree based on *L. interrogans* MST9 sequences (204bp) implemented using the Jukes-Cantor evolutionary model. Nodes with posterior support greater than 0.9 are shown. Reference sequences are labelled by biosample, sequence accession, and *Leptospira* strain and sequences from this study labelled by accession and sample code

## References:

1. Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D, et al. BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Comput Biol [Internet]. 2014 Apr [cited 2014 Jul 11];10(4):e1003537. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3985171&tool=pmcentrez&rendertype=abstract>

2. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol.* 2016;33(7):1870–4.
3. Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Syst Biol.* 2018;67(5):901–4.
4. Hasegawa M, Kishino H, Yano T aki. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J Mol Evol.* 1985;22(2):160–74.