



HAL
open science

Horizontal transfer of the rfb cluster in *Leptospira* is a genetic determinant of serovar identity

Cecilia Nieves, Antony Vincent, Leticia Zarantonelli, Mathieu Picardeau, Frédéric Veyrier, Alejandro Buschiazzo

► **To cite this version:**

Cecilia Nieves, Antony Vincent, Leticia Zarantonelli, Mathieu Picardeau, Frédéric Veyrier, et al.. Horizontal transfer of the rfb cluster in *Leptospira* is a genetic determinant of serovar identity. Life Science Alliance, 2022, 6 (2), pp.e202201480. 10.26508/lisa.202201480 . pasteur-04117799

HAL Id: pasteur-04117799

<https://pasteur.hal.science/pasteur-04117799v1>

Submitted on 5 Jun 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



Horizontal transfer of the *rfb* cluster in *Leptospira* is a genetic determinant of serovar identity

Cecilia Nieves¹ , Antony T Vincent^{1,2}, Leticia Zarantonelli³ , Mathieu Picardeau^{4,5}, Frédéric J Veyrier^{1,*} , Alejandro Buschiazco^{3,5,*}

***Leptospira* bacteria comprise numerous species, several of which cause serious disease to a broad range of hosts including humans. These spirochetes exhibit large intraspecific variation, resulting in complex tabulations of serogroups/serovars that crisscross the species classification. Serovar identity, linked to biological/clinical phenotypes, depends on the structure of surface-exposed LPS. Many LPS biosynthesis-encoding genes reside within the chromosomal *rfb* gene cluster. However, the genetic basis of intraspecific variability is not fully understood, constraining diagnostics/typing methods to cumbersome serologic procedures. We now show that the gene content of the *rfb* cluster strongly correlates with *Leptospira* serovar designation. Whole-genome sequencing of pathogenic *L. noguchii*, including strains of different serogroups, reveals that the *rfb* cluster undergoes extensive horizontal gene transfer. The *rfb* clusters from several *Leptospira* species disclose a univocal correspondence between gene composition and serovar identity. This work paves the way to genetic typing of *Leptospira* serovars, and to pinpointing specific genes within the distinct *rfb* clusters, encoding host-specific virulence traits. Further research shall unveil the molecular mechanism of *rfb* transfer among *Leptospira* strains and species.**

DOI [10.26508/lsa.202201480](https://doi.org/10.26508/lsa.202201480) | Received 12 April 2022 | Revised 22 November 2022 | Accepted 23 November 2022 | Published online 9 December 2022

Introduction

Leptospirosis is a bacterial disease that affects humans and animals. Despite being one of the most extended zoonoses worldwide, leptospirosis remains a neglected and underdiagnosed febrile illness. Pathogenic *Leptospira* species, the etiological agents of leptospirosis, infect a broad spectrum of hosts, with a global annual incidence of 1 million human cases and ~60,000 deaths (Costa et al,

2015; Torgerson et al, 2015). Leptospirosis constitutes an important case model within the “One Health” perspective (Jancloes et al, 2014), being a zoonotic disease that spreads among symptomatic and asymptomatic hosts, with transmission strongly influenced by environmental conditions (Mwachui et al, 2015).

Spirochetes belonging to the genus *Leptospira* have traditionally been divided into three groups: pathogens, intermediates, and saprophytes (Ko et al, 2009). This classification relied on bacterial virulence, isolation from infected hosts, and phylogeny. Recently, expanded and more elaborate phylogenetic analyses resulted in a comprehensive new classification scheme, beyond the species’ infectious capacity. The 68 species of *Leptospira* that have been identified so far (Vincent et al, 2019; Korba et al, 2021) are thus classified into two major clades: P (pathogenic) and S (saprophytic), each of which subdivided into two subclades (Vincent et al, 2019).

Leptospira noguchii belongs to the first subclade within the P group (P1), which comprises the most important species causing human and animal diseases such as *L. interrogans* and *L. borgpetersenii* (Vincent et al, 2019). Many human infections by *L. noguchii* have been recorded since 1940 (Gochenour et al, 1952; Fraser et al, 1973), but it was not until 1987 that it was recognized as a distinct species (Yasuda et al, 1987), baptized after the Japanese bacteriologist Hideyo Noguchi, himself responsible for choosing the genus name. Despite the clinical importance of *L. noguchii* and its extended geographical distribution, it has received far less attention compared with other pathogenic *Leptospira* species. Noticeably, no finished or closed whole-genome sequence (WGS) of *L. noguchii* is currently available, data that would otherwise boost the power of comparative genomics analyses.

Why could *L. noguchii* WGS contribute with novel insights into leptospirosis? Comprising the largest reported genome among *Leptospira* spp. (Fouts et al, 2016), *L. noguchii* also ranks among the species with more genes and predicted proteins of the entire genus, particularly compared with the closely related pathogens *L. interrogans* and *L. borgpetersenii*, both of which have been studied

¹Bacterial Symbionts Evolution, Centre Armand-Frappier Santé Biotechnologie, Institut National de la Recherche Scientifique, Université du Québec, Laval, Canada
²Département des Sciences Animales, Faculté des Sciences de l’agriculture et de l’alimentation, Université Laval, Québec City, Canada
³Laboratory of Molecular and Structural Microbiology, Institut Pasteur de Montevideo, Montevideo, Uruguay
⁴Institut Pasteur, Université Paris Cité, CNRS UMR 6047, Biology of Spirochetes Unit, Paris, France
⁵Integrative Microbiology of Zoonotic Agents, Pasteur International Joint Research Unit, Paris/Montevideo, France/Uruguay

Correspondence: frederic.veyrier@inrs.ca; alebus@pasteur.edu.uy

*Frédéric J Veyrier and Alejandro Buschiazco contributed equally to this work

Leticia Zarantonelli’s present address is Unidad Mixta Pasteur INIA, Institut Pasteur de Montevideo, Montevideo, Uruguay

with much more detail. Besides being isolated from humans, *L. noguchii* has been isolated from armadillos, cattle, sheep, dogs, frogs, and opossums, among others (Silva et al, 2007, 2009), demonstrating its remarkably high adaptability to infecting a very broad range of hosts. Human infections by *L. noguchii* have been reported in geographic areas where the same strains were previously detected in other hosts (Silva et al, 2009; Flores et al, 2017), confirming *L. noguchii*'s capacity for zoonotic transmission. Found predominantly in the Americas, and more rarely in Asia (Guglielmini et al, 2019), *L. noguchii* exhibits high genetic diversity among circulating strains (Martins et al, 2015; Hamond et al, 2016; Zarantonelli et al, 2018), with no apparent correlation between genotypes and hosts or geographic distribution (Loureiro et al, 2020). Particularly in South America, systematic field studies of infected animal hosts reveal a much larger diversity of *L. noguchii* serovars than that encountered for *L. interrogans* and *L. borgpetersenii* (Zarantonelli et al, 2018).

The presence of many serovars (serologic variants) is a common attribute within *Leptospira* species. With >300 serovars having been reported, their classification into serogroups has been instrumental, clustering together related serovars that express overlapping antigenic determinants (Bharti et al, 2003). Probably related to variable structures of the surface-exposed LPS antigen on the bacterial cell wall (Adler & de la Peña Moctezuma, 2010), different serovars trigger distinct antibody responses during infection. Interestingly, a number of known serovar–host associations have been pinpointed, leading to the concept of serovar adaptation and chronicity of infection for particular hosts, also correlating to more acute virulence when non-adapted serovars accidentally infect heterologous hosts (Ellis, 2015). Despite the relevance of this phenomenon in terms of epidemiology and clinical outcomes, the molecular mechanisms that underlie serovar determination are not fully understood (de la Peña-Moctezuma et al, 1999; Adler & de la Peña Moctezuma, 2010; Fouts et al, 2016). A connection between serovar determination and gene content has been proposed (Bulach et al, 2000, 2006; Santos et al, 2018), but not demonstrating a direct, biunivocal link among each of the many different serovars and a defined set of genes (genetic presence/absence profiles). Such unequivocal link has also been hampered by the scarcity of precise serovar identification for most reported isolates, and the lack of finished whole-genome sequencing data, particularly so for *L. noguchii* and other understudied species.

By sequencing the genomes of 12 *L. noguchii* strains (10 closed genomes and 2 drafts), we undertook an extensive comparative genomics approach, uncovering underlying reasons for *Leptospira* phenotypic complexity. We now reveal (i) the detailed genomic features and plasmid repertoire of *L. noguchii* and its phylogenetic structure; (ii) that the cluster comprising most of the LPS synthesis enzyme-encoding genes, known as *rfb* (Patra et al, 2015; Picardeau, 2017), exhibits clear signs of horizontal gene transfer (HGT) among different *Leptospira* species; and (iii) that serovar identity is univocally linked to the presence/absence of specific genes within this *rfb* cluster.

In sum, this work constitutes the first report of complete genomes of *L. noguchii*, which allowed a comprehensive analysis of its genetic variability. Remarkably, after comparing with known serovars of different *Leptospira* species, it was possible to reveal

serovar-specific genetic fingerprints encoded within a horizontally transferred gene cluster, paving the way toward genome-based serotyping and further molecular studies of the HGT mechanisms at play.

Results

L. noguchii WGSs: general features

Whole genomes from 12 *L. noguchii* strains (Table 1) were sequenced using a long-read sequencing approach (PacBio technology). These strains were isolated from different hosts at four distant geographic locations in Central and South America: Barbados (two isolates from amphibian hosts), Guadeloupe island/France (one, human), Uruguay (eight, cattle), and Venezuela (one, human). Exhibiting an average genome size of 4,863,036 ± 99,185 bp, all were larger than other well-studied species such as *L. interrogans* (~4.6 Mb) and *L. borgpetersenii* (~3.9 Mb). Most genomes reached a finished status, with three to eight contigs (Table 1) corresponding to chromosomes 1 (Chr1) and 2 (Chr2), plus a variable number of plasmids. The whole genomes from strains “bajan” and “201102933” were the only ones not closed, albeit rendering very high-quality draft sequences (five contigs in the case of strain bajan, and six for strain 201102933, with N50s of 2,827,750 and 3,551,682 bp, respectively).

The average nucleotide identity (ANI) among all sequenced strains in this study (using *L. noguchii* sv Panama strain CZ214 as reference) was >95% (Table S1 and Fig S1; see all Supplemental Material in Supplemental Data 1), consistent with previous determinations based on 16S rDNA sequence (Zarantonelli et al, 2018). Similar identity figures with all reported draft WGSs from *L. noguchii* strains further confirm the taxonomic determination, and ANIs ≤90% with respect to closely related species such as *L. interrogans* and *L. kirschneri*. However, the percentage of conserved proteins among the 12 sequenced strains did not exceed 98.1% (Fig S2), uncovering a significant intraspecies phenotypic complexity. Especially diverse in terms of protein repertoire is the cluster comprising the three Caribbean strains (bajan, barbudensis, and 2011029331), which consistently exhibit highest ANI figures among them. Regarding replicon content, *L. noguchii* displays the same genetic organization as reported for other *Leptospira* species (Picardeau et al, 2008), with two chromosomes and a variable number of plasmids (Table 1). Consistent with their larger genome size, *L. noguchii* also exhibits more CDSs (~4,000 on average) compared with other *Leptospira* species (Picardeau et al, 2008), with other features such as number of tRNA genes and GC content being similar. The number of predicted CRISPR sequences was more variable, as were those of transposases and IS transposase-like CDSs, ranging from 95 to 145 (Table 1), in any case much more numerous than in *L. interrogans* (26 in sv Copenhageni strain Fiocruz) or in the saprophyte *Leptospira biflexa* (8 in sv Patoc strain Ames) (Picardeau et al, 2008).

Analysis of the *L. noguchii* pangenome of the 12 strains sequenced in this study (Fig S3) showed an open profile (Heaps' law parameter $\alpha = 0.36$ [Tettelin et al, 2008]), confirming the high genetic variability among *L. noguchii* strains. Out of the 7,963 genes that constitute the pangenome, only 2,671 were found in almost all the

Table 1. *L. noguchii* whole-genome sequences.

	Strain	Host	Replicon	Size (bp)	CDSs	rRNA	tRNA	CRISPRs	TPases	GC (%)	Coding ratio (%)	Accession number
Finished genomes												
	barbudensis (AUS/BRB)	Amphibian	Chr1	4,408,823	3,883	5	37	6	101	35.5	75.9	CP091967
			Chr2	359,178	337	—	—	—	3	35.3	76.8	CP091968
			p1	47,641	65	—	—	—	0	42.2	45.2	CP091969
			p2	44,440	63	—	—	—	0	42.5	46	CP091970
			Total	4,860,082								
	IP1512017 (ND/URY)	Cattle	Chr1	4,297,194	3,375	5	37	8	104	35.6	73.3	CP091957
			Chr2	370,404	327	—	—	—	14	36	77.1	CP091958
			p1	178,101	139	—	—	—	7	38.3	74.3	CP091959
			p2	67,330	48	—	—	—	3	37.2	76.6	CP091960
			p3	43,769	33	—	—	—	0	35.4	73	CP091961
	Total	4,956,798										
	IP1605021 (PYR/URY)	Cattle	Chr1	4,438,826	3,555	5	37	10	121	35.8	73.4	CP091953
			Chr2	395,164	368	—	—	—	10	36.2	79.5	CP091954
			p1	169,819	136	—	—	—	11	38.1	75	CP091955
			p2	47,268	29	—	—	—	3	36.4	67.3	CP091956
			Total	5,051,077								
	IP1611024 (AUS/URY)	Cattle	Chr1	4,248,034	3,329	5	37	10	83	35.6	74	CP091947
			Chr2	351,624	291	—	—	—	10	35.6	76.6	CP091948
			p1	89,758	93	—	—	—	0	32.4	83.1	CP091949
			p2	74,081	60	—	—	—	5	34.6	67	CP091950
			p3	57,447	50	—	—	—	3	34.3	61.2	CP091951
			p4	41,294	30	—	—	—	1	32.4	71.2	CP091952
	Total	4,862,238										
	IP1703027 (ND/URY)	Cattle	Chr1	4,329,282	3,408	5	37	12	94	35.7	73.7	CP091943
			Chr2	338,457	275	0	0	1	3	35.7	76.4	CP091944
			p1	83,272	52	—	—	—	1	35.9	71.2	CP091945
			p2	61,325	44	—	—	—	3	35.7	73.1	CP091946
			Total	4,812,336								
	IP1705032 (AUT/URY)	Cattle	Chr1	4,342,451	3,435	5	37	10	98	35.7	74	CP091940
			Chr2	343,634	286	—	—	—	4	35.6	77.3	CP091941
			p1	58,982	49	—	—	—	0	35.9	66.1	CP091942
			Total	4,745,067								
	IP1709037 (AUT/URY)	Cattle	Chr1	4,199,394	3,354	5	37	8	81	35.7	74.1	CP091936
			Chr2	376,569	326	—	1	—	3	36.1	76.8	CP091937
			p1	90,518	74	—	—	—	6	35.3	73.5	CP091938
			p2	74,070	61	—	—	—	5	34.5	65.7	CP091939
			Total	4,740,551								

(Continued on following page)

Table 1. Continued

	Strain	Host	Replicon	Size (bp)	CDSs	rRNA	tRNA	CRISPRs	TPases	GC (%)	Coding ratio (%)	Accession number
	IP1712055 (ND/URY)	Cattle	Chr1	4,329,115	3,455	5	37	12	93	35.7	73.6	CP091928
			Chr2	338,447	277	—	—	1	3	35.7	76.2	CP091929
			p1	83,272	52	—	—	—	1	35.9	71.2	CP091930
			p2	61,322	44	—	—	—	3	35.7	71.5	CP091931
			p3	35,952	34	—	—	—	0	41.3	53.1	CP091932
			p4	26,771	7	—	—	—	0	41.1	5.7	CP091933
			p5	24,253	38	—	—	—	0	42	71.5	CP091934
			p6	13,148	11	—	—	—	0	40.3	36.8	CP091935
			Total	4,912,280								
	IP1804061 (ND/URY)	Cattle	Chr1	4,322,667	3,447	5	37	9	93	35.7	73.4	CP092112
			Chr2	330,637	280	—	—	—	16	35.8	76	CP092113
			p1	127,018	100	—	—	—	5	37.3	70.8	CP092114
			p2	100,275	71	—	—	—	0	36.3	74.1	CP092115
			p3	51,374	37	—	—	—	2	35.2	72.1	CP092116
					Total	4,931,971						
	201601331 (ND/VEN)	Human	Chr1	4,334,850	3,447	5	37	8	134	35.7	74	CP091962
			Chr2	350,933	293	—	—	—	5	35.7	76.8	CP091963
			p1	65,245	46	—	—	—	1	35.9	65.2	CP091964
			p2	38,153	33	—	—	—	2	33.4	57	CP091965
			p3	28,484	26	—	—	—	1	35.8	34.1	CP091966
			Total	4,817,665								
Draft genomes												
	bajan (AUS/BRB)	Amphibian	Five (5) contigs	4,850,434	4,326	5	37	6	104	35.7	76.4	JAKNBP000000000
	201102933 (AUS/GLP)	Human	Six (6) contigs	4,697,964	3,869	5	38	6	103	35.5	76.9	JAKNBO000000000

The “Strain” column provides information about the serogroup of each strain (AUS, Australis; AUT, Autumnalis; PYR, Pyrogenes; ND, not determined) and its country of origin (BRB, Barbados; GLP, Guadeloupe; URY, Uruguay; VEN, Venezuela), in parentheses. The other columns provide information as described in the column headings. “TPases” stands for transposases.

strains thus comprising the core genome. Indeed, the cloud genome was defined by 2,183 genes (accessory genes), uncovering a rich array of unique attributes that distinguish strains.

To further explore the properties of such strain-specific genes, likely underlying phenotypic variability, profile hidden Markov models were calculated for each one of the genes present in only one of the strains and absent in all others. These hidden Markov model profiles were then mapped (Aramaki et al, 2020) onto the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to investigate whether these variant-specific genes are enriched in particular biochemical functions, or instead randomly distributed (Table S2). Among the four top-ranking pathways—(i) metabolic pathways, (ii) biosynthesis of nucleotide sugars, (iii) amino sugar and nucleotide sugar metabolism, and (iv) *O*-antigen nucleotide sugar biosynthesis—a clear enrichment is observed in functions related to carbohydrate metabolism, and glycoside modification

and synthesis. This KEGG mapping analysis was systematically extended to accessory genes present only in two strains, three, and further, confirming the importance of variations in carbohydrate-related metabolism as a main source of strain-specific genetic variability. Of note, a number of carbohydrate-related genes, including several that encode LPS biosynthesis enzymes, were present only in particular groups of strains. For example, UDP-glucuronate 4-epimerase (GalE [Bulach et al, 2000]) or 3-deoxy-D-manno-octulosonate 8-phosphate phosphatase (KdsC [Biswas et al, 2009; Valvano, 2015]), among others, clusters according to serogroup identity.

Plasmid repertoire in *L. noguchii*

Besides the two chromosomes, *L. noguchii* strains harbor a variable number of plasmids (Tables 1 and S3, first sheet), ranging from only

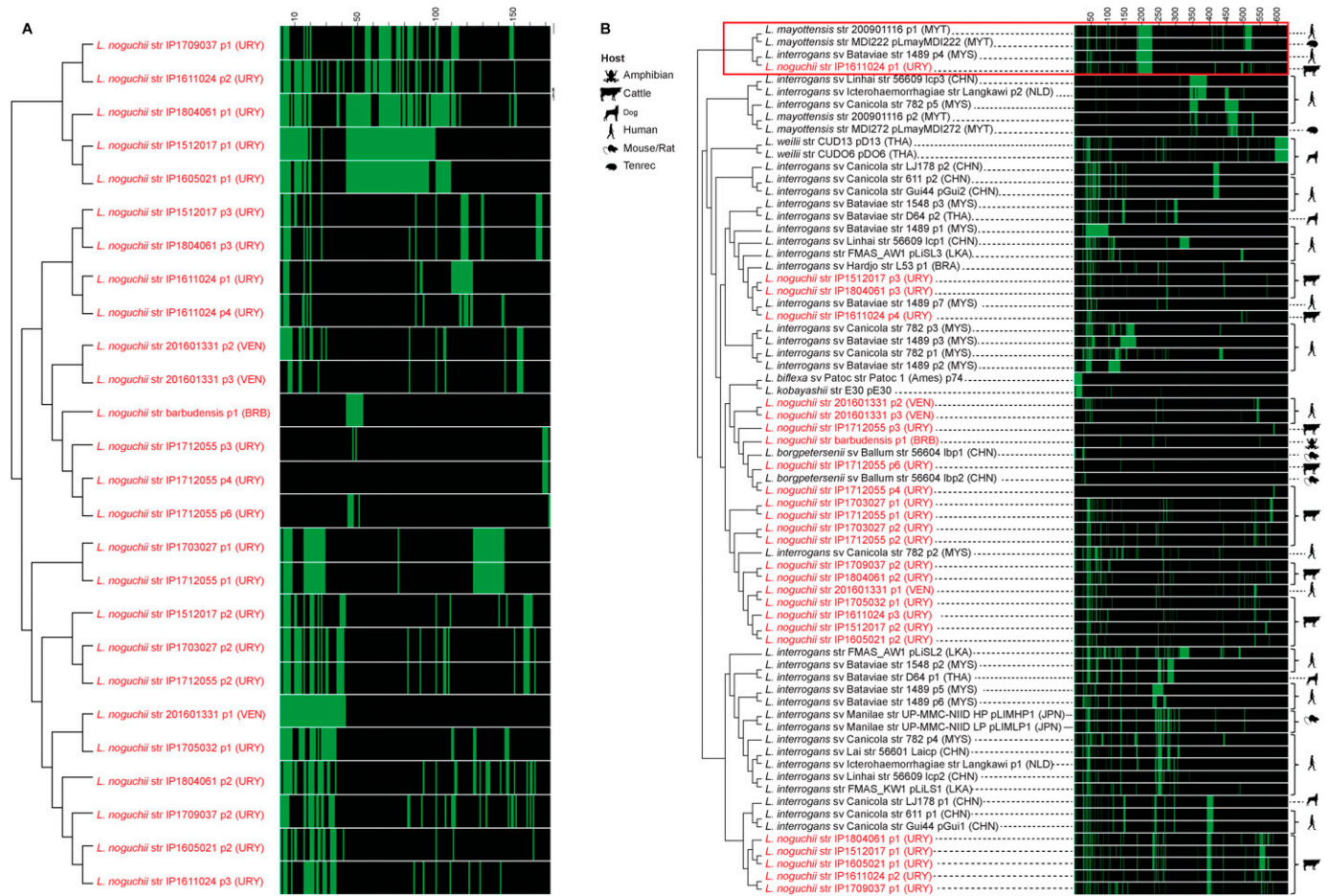


Figure 1. Conservation of protein-encoding genes across *Leptospira* plasmids.

Sequence network association analysis by hierarchical clustering, based on the presence/absence of plasmidic protein-encoding genes. Matrices on the right of each clustering depict individual genes with vertical lines, green meaning that the gene is present in that strain (60% similarity cutoff), and black meaning absence. Scales on the top of the matrices indicate the number of different genes being compared. **(A)** Plasmids from *L. noguchii* strains sequenced in this study. Plasmid names are indicated right after the strain designation, and country of origin in parentheses (BRB, Barbados; URY, Uruguay; VEN, Venezuela). **(B)** Plasmids from different *Leptospira* species. Plasmid names as in (A), and country of origin in parentheses (BRA, Brazil; BRB, Barbados; CHN, China; JPN, Japan; LKA, Sri Lanka; MYS, Malaysia; MYT, Mayotte; NLD, the Netherlands; THA, Thailand; URY, Uruguay; VEN, Venezuela). Strains sequenced in this study are highlighted in red. The hosts from which they were isolated are indicated with cartoons on the right side of the matrix. The red square encloses plasmids from different *Leptospira* species sharing a large number of protein-encoding genes.

one to as much as six replicons. The plasmid repertoire is unique to each strain. A network association analysis was performed to compare the plasmid-encoded proteins in different *L. noguchii* strains (Fig 1A). Some plasmids showed identical or nearly identical presence/absence patterns of protein-encoding genes, suggesting that plasmids may be transferred among strains. For example, the two plasmids of strain IP1703027 bear identical gene composition compared with two of the plasmids in IP1712055 (p1 and p2); plasmids p1 from strains IP1512017 and IP1605021, and plasmids p3 from IP1512017 and IP1804061 share many genes.

Extending the analysis of plasmid repertoires to other strains and *Leptospira* species (Table S3, second sheet) revealed no core or even softcore genes, highlighting the extreme plasmid diversity in *Leptospira*. A network association analysis considering this extended set of plasmids revealed species-specific clustering of plasmid sequences, again uncovering cases of strong similarity/identity in the arrays of protein-encoding genes between different

strains (Fig 1B). For instance, plasmids p1/p2, along with p2/p3 from distinct *L. interrogans* strains D64 and 1548, exhibit nearly identical profiles. The same was observed for plasmids p1 and p2 from *L. interrogans* strains 611, Gui44, and LJ178; and for comparing plasmids pD13 and pDO6 from *L. weilii*. Overall, plasmids from *L. noguchii* clustered together and did not show similar patterns to those from other species, except just in one case. Plasmid p1 from strain IP1611024 shared a significant number of protein-encoding genes with plasmids p1 from *L. mayottensis* str 200901116, pLMayMDI222 from *L. mayottensis* str MDI222, and p4 from *L. interrogans* str 1489 (Fig 1B, red square).

A functional/biological analysis of *L. noguchii* plasmid-encoded genes is difficult, as most of the constituent proteins are hypothetical (Table S3, third sheet). A first general inquiry about the potential link between plasmid identity and environmental factors did not result in a clear association. Neither the geographical locations of strains, nor the infected host from which they were isolated, showed clear-cut connections with plasmids and their

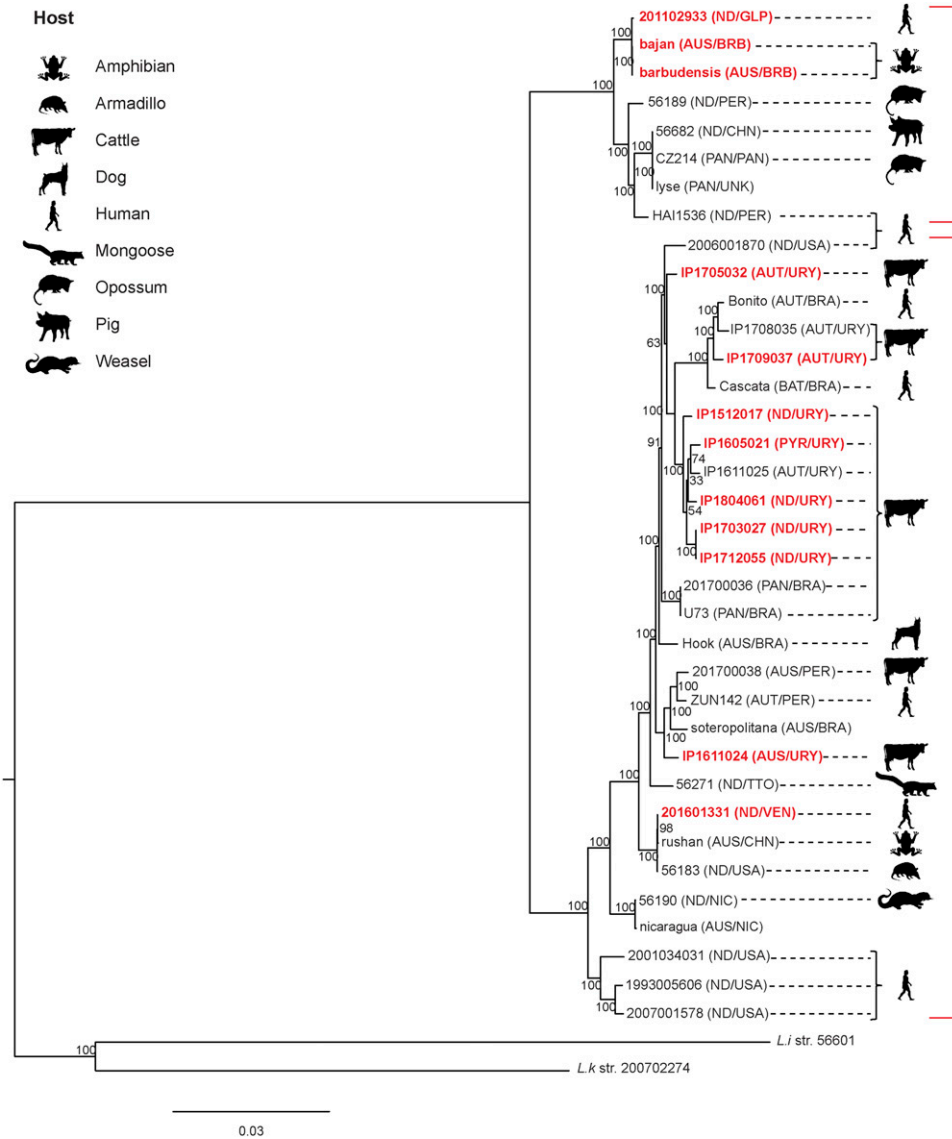


Figure 2. Phylogenetic tree of *Leptospira noguchii*. The maximum-likelihood phylogenetic tree is based on the softcore genes (present in more than 95% of the genomes). *L. interrogans* str 56601 and *L. kirschneri* str 200702274 were included as outgroups. The serogroup of each strain (AUS, Australis; AUT, Autumnalis; BAT, Bataviae; ND, not determined; PAN, Panama; PYR, Pyrogenes) and its country of origin (BRB, Barbados; BRA, Brazil; CHN, China; GLP, Guadeloupe; NIC, Nicaragua; PAN, Panama; PER, Peru; TTO, Trinidad & Tobago; USA, United States of America; URY, Uruguay; UNK, unknown; VEN, Venezuela) are indicated in parentheses. Strains sequenced in this study are outlined in bold red. The hosts from which they were isolated are indicated with cartoons (some hosts were not specified in the original reports). Two clades can be distinguished, highlighted with red brackets.

protein-encoding gene compositions. Plasmid-borne virulence factors and antibiotic resistance determinants were also explored, recognizing two genes encoding putative multidrug efflux proteins of the resistance-nodulation-division family (Nishino et al, 2007), MtdA and MtdB, in plasmids p1 (from strains IP1512017, IP1605021, IP1709037, and IP1804061), and p2 (from IP1611024). The limited identity with bona fide antibiotic resistance proteins did not allow for conclusive antibiotic specificity and/or functional prediction. These genes were always found as a cluster in *Leptospira* plasmids, with *mtdA* followed by *mtdB* and *cusA*, the latter encoding a cation efflux pump. The extended network association analysis including also other *Leptospira* species (Table S3, fourth sheet) revealed this same cluster in plasmid p1 from three *L. interrogans* strains (611, Gui44, and LJ178). Further work is needed to uncover the biological role of these proteins in *Leptospira*, especially considering that antibiotic resistance is not a usual feature in spirochetes. Functional analysis of clusters of orthologous genes (COG) showed a few

categories to be absent from plasmidic genes in *L. noguchii* and other *Leptospira* species: (i) RNA processing and modification; (ii) chromatin structure and dynamics; (iii) carbohydrate metabolism and transport; (iv) nuclear structure; (v) cytoskeleton; and (vi) general function prediction. On the contrary, among the most represented COG categories were those related to (i) unknown function, (ii) replication and repair, (iii) transcription, and (iv) signal transduction. Surprisingly, the functional category linked to amino acid metabolism and transport was completely absent in *L. noguchii* plasmids, in stark contrast to other *Leptospira* species.

***L. noguchii* phylogeny**

The complexity and high diversity of this species' pangenome could also be related to the adaptation of *L. noguchii* to different hosts and geographic locations. To uncover such potential genotype/phenotype associations, the phylogenetic structure of *L. noguchii*

was explored in greater detail by analyzing the genomes from 11 different geographic locations and nine types of hosts (Fig 2 and Table S4) applying a maximum-likelihood approach.

Two clades could be distinguished, which do not correlate to geographic distribution nor to host. Further studies to increase the number of strains shall enable a more conclusive statement. The Uruguayan strains isolated from cattle cluster within one of the clades, but they are not phylogenetically distant from other host species including humans, other mammals, and even amphibians. Focusing the analysis on *L. noguchii* strains isolated from human infections, the distribution is once again extremely broad throughout the phylogenetic tree, indicative of transmission among different reservoirs (Fig 2).

Genetic variability of the *rfb* cluster in *L. noguchii*

Considering that serologic variability is a particularly relevant phenotypic trait in *Leptospira* (Adler & de la Peña Moctezuma, 2010), that the *L. noguchii* phylogenetic structure did not reveal clear genotype/phenotype associations including host tropism, and that *L. noguchii* strain-specific accessory genes were found to be highly enriched in carbohydrate pathways and LPS biosynthesis, a more detailed analysis focusing on particular genomic regions was done. Genes coding for the cell wall LPS biosynthesis have been linked to serovariation, and in *Leptospira* tend to concentrate within a gene cluster known as *rfb* (de la Peña-Moctezuma et al, 1999; Fouts et al, 2016). Access to complete/finished WGSs is particularly relevant to analyze delimited loci, avoiding inaccurate gene composition descriptions that result from fuzzy boundaries and/or incompleteness (Denton et al, 2014). Exploiting the WGS of *L. noguchii* strains that we are now reporting, a reliable evaluation of gene diversity related to LPS biosynthesis is feasible.

The core moiety of LPS known as lipid A (Raetz & Whitfield, 2002; Que-Gewirth et al, 2004) is synthesized by several enzymes encoded in a cluster of 13 genes: *lpxA*, *lpxC*, *lpxD1*, *lpxD2*, *lpxB1*, *lpxB2*, *lpxK*, *kdtA*, *kdsB1*, *kdsB2*, *lnt*, *kdsA*, and *htrB*. The composition of this gene cluster was almost identical in all the strains analyzed, including genomes reported in this work and those from other *Leptospira* strains of known serovar identity (Table S5, first sheet). This result confirms previous reports analyzing several different pathogenic *Leptospira* species (Fouts et al, 2016).

A second component of LPS is the core oligosaccharide (Raetz & Whitfield, 2002), whose biosynthesis starts with the addition of 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo) molecules to the lipid A moiety, subsequently incorporating heptoses and further modifications (Bertani & Ruiz, 2018). Comparison of the genes coding for core synthesis enzymes (such as WaaA that adds Kdo molecules; RfaC, RfaD, RfaE, and RfaF that attach ADP-L-glycero-β-D-mannoheptose intermediates; and other glycosyltransferases such as RfaG, which append glucose units to the heptoses) among the different strains (Table S5, first sheet) revealed no variation in terms of differential presence of genes. The only difference concerned *L. interrogans* sv Weerasinghe, in which three genes coding for WaaA isoforms were found (~99% identical among them).

Finally, the outermost section of the LPS known as the O-antigen is the most variable part of the structure and most exposed to interact with the environment (Raetz & Whitfield, 2002). The

Table 2. Overall composition of *rfb* clusters in *L. noguchii*.

Strain	Size (bp)	CDSs	GC content (%)
IP1512017 (ND/URY)	84,097	74	33.7
IP1605021 (PYR/URY)	113,593	110	34.0
IP1611024 (AUS/URY)	106,137	97	32.8
IP1703027 (ND/URY)	82,932	74	33.7
IP1705032 (AUT/URY)	93,324	83	31.9
IP1709037 (AUT/URY)	96,628	90	32.4
IP1712055 (ND/URY)	82,927	77	33.7
IP1804061 (ND/URY)	82,821	74	33.7
201102933 (ND/GLP)	103,442	100	33.8
201601331 (ND/VEN)	99,122	89	32.2
bajan (AUS/BRB)	104,988	105	33.8
barbudensis (AUS/BRB)	103,476	103	33.8

The “Strain” column informs, in parentheses, about the serogroup of each strain (AUS, Australis; AUT, Autumnalis; PYR, Pyrogenes; ND, not determined) and its country of origin (BRB, Barbados; GLP, Guadeloupe; URY, Uruguay; VEN, Venezuela).

O-antigen is an oligosaccharide comprising a fairly large number of diverse monosaccharides, synthesized and oligomerized together by the action of several enzymes, most of which are encoded in the *rfb* cluster in *Leptospira* (Mitchison et al, 1997). The genetic composition of the *rfb* clusters of the 12 *L. noguchii* genomes revealed a striking variability (Table 2). High *rfb* variability had previously been described comparing different *Leptospira* species (Fouts et al, 2016), hereby confirmed within a single species.

Further insights were obtained by aligning the *rfb* clusters of these 12 *L. noguchii* strains, highlighting the synteny among their constituent genes (Fig 3A).

Delimiting the boundary ends of the *rfb* cluster, genes coding for a transcriptional regulator (MarR) and a sodium/sulfate symporter (SdcS) were consistently found, as in other pathogenic *Leptospira* species (Fouts et al, 2016). The 3' end is more conserved; toward this end, a gene subcluster is located, composed of *rfbC*, *rfbD*, *rfbB*, and *rfbA*, which encodes enzymes involved in the dTDP-rhamnose biosynthesis, implicated in LPS assembly in pathogenic strains (Mitchison et al, 1997). The order of appearance of these four genes was conserved in all strains. Only strain IP1605021 (serogroup Pyrogenes) presented an extra copy of *rfbC* within the *rfb* cluster but separated from the dTDP-rhamnose biosynthesis subcluster. Systematically, the *rfb* cluster was found in preferential locations within Chr1, approximately at ~1.75 and ~2.50 Mb from the origin (Fig 3B), and run in opposite senses comparing locations 1 versus 2. The regions that flank the *rfb* clusters are conserved (Fig 3B), although this depends on the location site. An interesting example of this is illustrated in cases where the same *rfb* cluster is identified in one genomic site or the other in different strains (e.g., strains IP1705032 versus IP1709037, or IP1804061 versus IP1703027; see Fig 3B). A detailed examination of both *rfb* locations revealed an explanation to this feature: genomic inversions are coincident with the *rfb* clusters being located at one site or the other (Fig 3C). Although

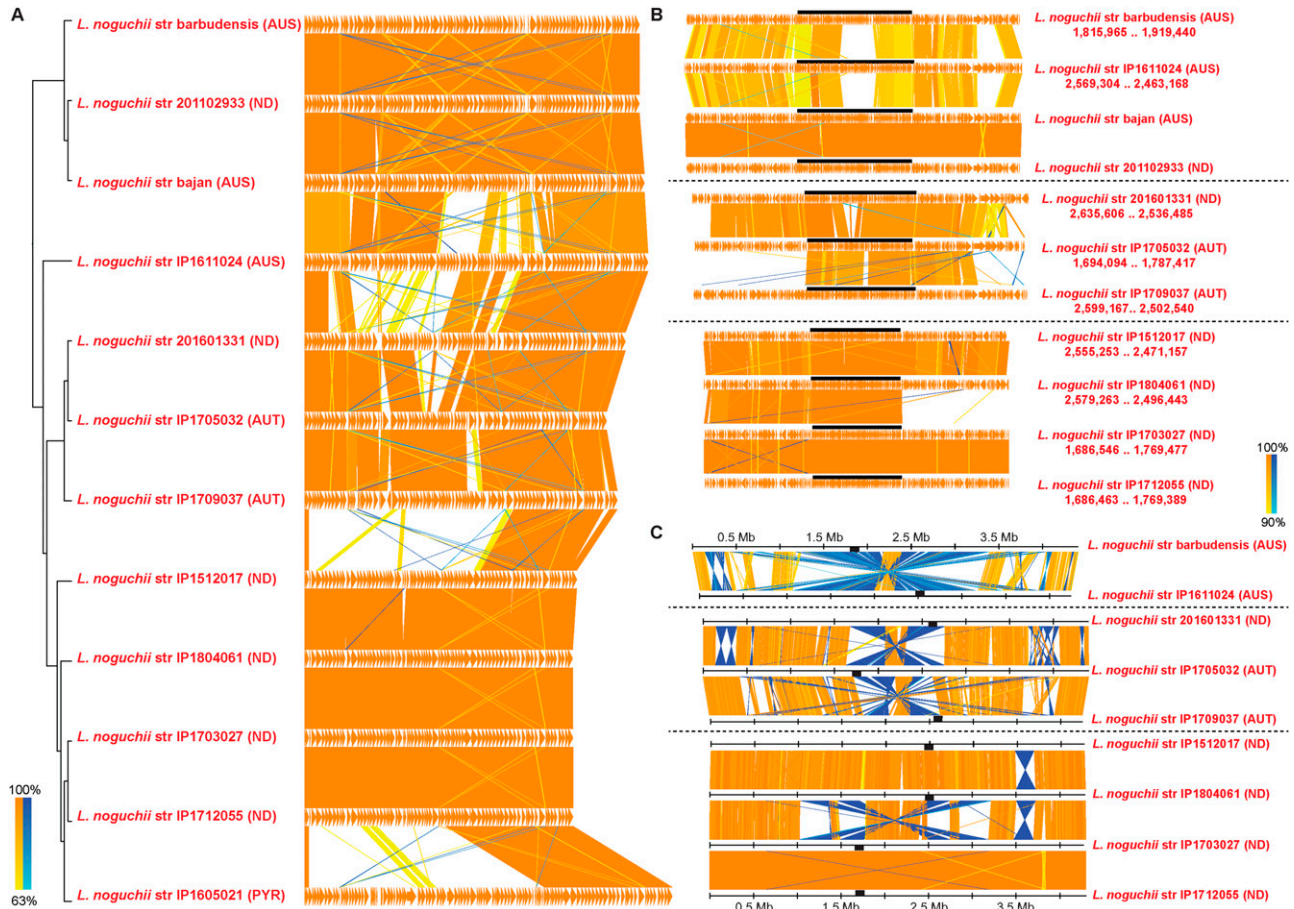


Figure 3. Comparison of *rfb* clusters in *L. noguchii* shows highly variable gene composition.

(A) softcore *rfb* genes from *L. noguchii* strains aligned and clustered considering a 60% identity cutoff and gene presence in at least 60% of the *rfb* clusters analyzed (left). Serogroup identity is indicated in parentheses (AUS, Australis; AUT, Autumnalis; PYR, Pyrogenes; ND, not determined). To the right, *rfb* cluster gene content and shared synteny are depicted. Homologous regions are linked with orange lines (same orientation) and blue lines (inverted regions), from lighter to darker colors according to identity level (as marked by the lower left scale index). (B) Close-up of *rfb* clusters (highlighted as black bars) from *L. noguchii* strains that belong to the same serogroup, or with highly similar *rfb* clusters, including 100,000 extra bp flanking at each side. The genomic coordinates of *rfb*-delimiting genes *marR* and *sdC5* (in base pairs, with *dnaA* at position 0) are indicated below the strains' names for those strains with closed/finished genomes, and their delimitations within the alignments are highlighted as black bars. Nucleotide alignment was performed considering a 90% identity cutoff. Homologous regions are linked with orange lines (same orientation) and blue lines (inverted regions), from lighter to darker colors according to identity level (as marked by the lower right scale index). (C) Nucleotide alignments (90% identity cutoff) of entire chromosome 1 from *L. noguchii* strains that possess highly similar *rfb* clusters (barbudensis versus IP1611024; 201601331 versus IP1705032/IP1709037; and IP1512017 versus IP1804061/IP1703027/IP1712055). Color references as in (B). The *rfb* clusters are highlighted as black bars.

larger in some genomes, such inversions do not implicate the entire genomic range between ~1.75 and ~2.50 Mb, wherein colinear regions are also observed. Interestingly, in some of the strains the *rfb* cluster is located precisely at the boundary where the inversion occurs, thereby explaining why on those cases there is only one conserved *rfb* flanking region (Fig 3B). Of note, insertion sequence (IS) transposase-like CDSs were found at or near the boundaries of the *rfb* cluster (Fig S4 and Table S5, fourth and fifth sheets). Genomic rearrangements involving inversions have been reported in other *Leptospira* species (Nascimento et al, 2004; Olo Ndela et al, 2021), some of which are indeed IS-mediated (Nascimento et al, 2004).

The *rfb* cluster shows hallmarks of HGT

Signs of HGT were readily identified when analyzing the *rfb* cluster of genes in *L. noguchii*, and in other *Leptospira* species. A significant

decrease in the GC content was systematically observed at the cluster position in all *L. noguchii* genomes reported in this study (Fig 4A). Moreover, the deviated GC content that identifies *rfb* clusters as islands within *L. noguchii* Chr1 was further confirmed by extending these analyses to 10 additional *Leptospira* species for which complete WGSs are available (Fig S5). A conspicuously low-GC-content region corresponds with the position of the *rfb* cluster in all cases, in several of the species being the only such deviated segment, whereas additional ones are present in other cases as well. *L. interrogans* is the species that displays the least pronounced decrease, although the deviation is still evident. In *L. biflexa*, a second nearby segment exhibits a noticeable GC content decrease other than the *rfb* cluster itself (Fig S5).

A second feature pointing to HGT of *rfb* is the decreased sequence conservation of flanking regions. This is more difficult to observe when only *L. noguchii* genomes are compared (Fig 3B and

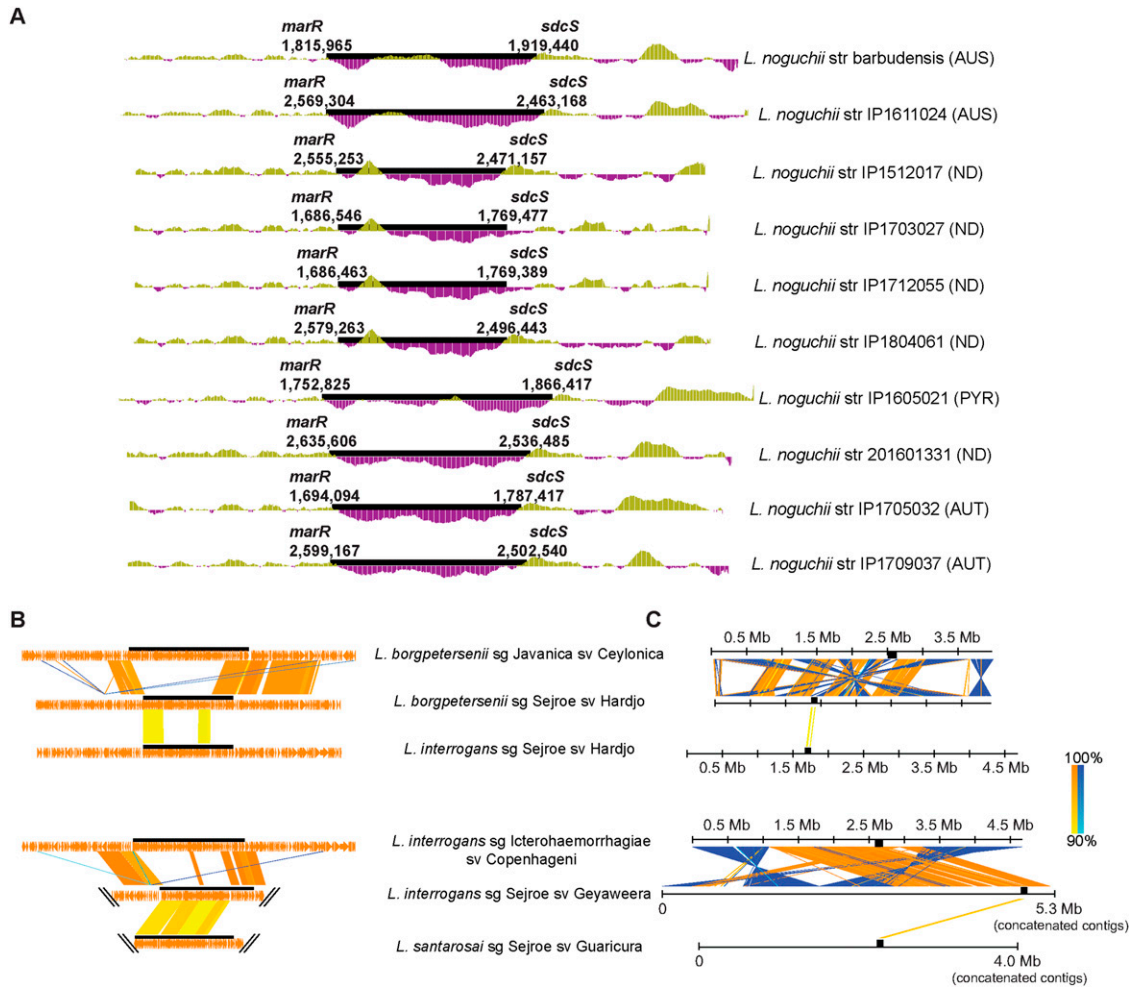


Figure 4. Genomic details of the *rfb* cluster in *Leptospira* reveal hallmarks of HGT.

(A) Close-up of each of the *rfb* clusters (shown as black bars), including 100,000 extra bp flanking at each side. GC % (calculated every 1,000 bp) is plotted, with purple or green indicating, respectively, a reduced or increased percentage compared with the average found in the whole chromosome 1. Relative position of genes *marR* and *sdcS* delimiting the *rfb* cluster is indicated in base pairs (*dnaA* at the origin). **(B)** Nucleotide alignment (90% identity cutoff) of the *rfb* clusters (black bars) and 100,000 extra base pairs flanking at each side, comparing more distant species all belonging to different serovars within serogroup Sejroe (*L. borgpetersenii* sv Hardjo str L550, *L. interrogans* sv Hardjo str Hardjoprajitno, *L. interrogans* sv Geyaweera str 1L-int, and *L. santarosai* sv Guaricura str M4/98). Unrelated serovars (*L. borgpetersenii* sv Ceylonica str Piyasena and *L. interrogans* sv Copenhageni str Fiocruz L1-130) were also added to compare species-specific conservation, outside of the *rfb* cluster. Homologous genes are linked with orange lines (if they share the same orientation) and blue lines (for inverted orientations), from lighter to darker colors according to identity percentage as marked by the right scale index. Of note, the genomes from *L. interrogans* sv Geyaweera and *L. santarosai* sv Guaricura are draft genomes. In these cases, contig boundaries are indicated with parallel black slashes, as the analysis could not be reliably extended beyond those limits. **(C)** Nucleotide alignment (90% identity cutoff) over the entire chromosome 1 (depicted as straight black lines), comparing the same set of species as in (B). The position of *rfb* clusters is indicated with black square blocks. Reference colors as in (B).

(C), because of the overall higher conservation within a species. However, the *rfb* flanking regions' variability becomes evident when comparing different species of the same serovar and serogroup. In these cases, high percentage of identity (>90%) is only observed within the *rfb* cluster, and not in the immediate surrounding segments (Fig 4B), nor in other parts of the whole genome (Fig 4C), a clear sign that a large extension of the *rfb* cluster is being horizontally transferred.

Certain genomic rearrangements, including inversions, can also be related to HGT (Oliveira et al, 2017), further highlighting the relevance of the above-mentioned inversions observed in *L. noguchii* strains (Fig 3C). And lastly, even though the positions of the *rfb* clusters have a slight variation among Chr1 from different

L. noguchii strains and *Leptospira* species (Figs 3C and 4C), they do locate at restricted positions, both when they are on the sense strand at position 1, or on the antisense at position 2. Considering all the evidence together, our data strongly suggest that genes within the *rfb* cluster are horizontally transferred among different strains and species of *Leptospira*.

***Leptospira* strains with identical/similar serologic identity display identical/similar *rfb* cluster gene composition**

Considering that the *rfb* cluster uncovers clear signs of horizontal transfer, and comprises a great genetic variability, we then wished to explore whether the specific *rfb* cluster present in a given strain

serves as a genetic signature underlying serovar identity. A number of observations support such hypothesis. Four of the Uruguayan strains for which the serogroup identity could not be assigned—because of undetectable agglutination by standard serogroup-specific antisera panels (Zarantonelli et al, 2018)—presented remarkably similar *rfb* clusters, inviting to posit that they may belong to the same serogroup/serovar (Fig 3). On the contrary, strains IP1705032 and IP1709037, both belonging to serogroup Autumnalis, were indeed grouped together according to their *rfb* gene composition (Fig 3A). It must be stressed, however, that the Uruguayan strains have not been assigned to specific serovars yet (Zarantonelli et al, 2018). We thus extended this analysis using genomic data from a wide diversity of strains of different *Leptospira* species with known serovar identities, indeed confirming that the serovar/*rfb* identity link holds (Fig 5A). By constructing matrices where presence/absence of *rfb* genes are crossed with different strains, serovar-specific patterns or signatures were unambiguously uncovered (Fig 5).

Very few genes were conserved in all the *rfb* clusters from different serovars, consistent with the previous observations (Table S6). A detailed analysis by serogroup unveiled several important observations: (i) a few serovars belonging to the same serogroup showed indistinguishable patterns (e.g., Bratislava versus Lora, Ceylonica versus Javanica, Copenhageni versus Icterohaemorrhagiae, and Lai versus Naam); extreme relatedness had been previously reported for Copenhageni/Icterohaemorrhagiae (Santos et al, 2018), where only one indel frameshift in a single LPS biosynthesis gene explains their differentiation; (ii) differential profiles were evident within most serogroups, featuring genes that may discriminate serovars; (iii) *L. noguchii* strains did not show similar patterns to other species with well-typed serovars, not even to those belonging to shared serogroups, suggesting that these *L. noguchii* strains may represent novel serovars; and (iv) the four non-agglutinating *L. noguchii* strains showing an identical *rfb* composition (except for slight differences in IP1512017) did not share many genes with other serogroups, suggesting they perhaps belong to a new serogroup altogether.

Worth highlighting is the fact that serovars belonging to the same serogroup shared all or the vast majority of genes among their *rfb* clusters. In this regard, Australis was the most variable serogroup (Fig 5A), with strains barbudensis, bajan, and 201102933 almost clonal, and IP1611024 more closely related to serovars Bratislava and Lora. Of note, the serogroup of strain 201102933 is not known, but the proximity to barbudensis and bajan, and the clustering with other strains of serogroup Australis, strongly suggests that strain 201102933 belongs to Australis, a hypothesis-driven prediction amenable for future testing.

In further support of serovar-specific *rfb* genetic signatures is the presence of 26 *rfb* genes only present in, and shared among, those Uruguayan strains, which were not amenable to serogroup assignment (genes framed within a yellow square in Fig 5A). Almost identical gene arrays were recognized among the shared signature gene sets. After a search of orthologous protein sequences (Blast best hits' accession numbers are indicated in Table S6), among the shared signature genes several were found to encode carbohydrate-active enzymes such as UDP-glucuronate 4-epimerase [EC:5.1.3.6], GDP/UDP-N,N'-diacetylbacillosamine

2-epimerase [EC:3.2.1.184], N,N'-diacetyllegionamine synthase [EC:2.5.1.101], and CMP-N,N'-diacetyllegionamic acid synthase [EC:2.7.7.82] (using PFAM and KEGG Mapper). These enzymes likely play key roles in generating the—yet to be determined—serovar-distinctive structures of LPS O-antigens.

The comparative analyses described above (Fig 5A) have limitations, because not all serovars are represented. Either because of a lack of information in the characterization of isolates, or because not enough finished and good-quality draft genomes are available, information loss is inevitable, eventually hampering reliable reconstructions of the relevant *rfb* genetic clusters. To further address these issues, and as a means of testing the decisive role of gene composition in serovar determination, representative genomes corresponding to identical serovar but belonging to different *Leptospira* species were analyzed in detail. Seven reliably determined serovars were found to belong to different species. In four of them, the *rfb* clusters were split into more than one contig so that for the sake of maximum reliability, only strains corresponding to three serovars were used: Hurstbridge (from *L. broomii* and *L. fainei* strains), Hardjo (from *L. borgpetersenii* and *L. interrogans*), and Valbuzzi (from *L. interrogans* and *L. kirschneri*) (Table S5, second sheet). The gene presence/absence matrix calculated for the *rfb* clusters from this subgroup (Fig 5B) readily confirms that specific groups of *rfb* genes are associated with each serovar (Table S6, second sheet), irrespective of the species. Despite the previously stated constraint in terms of genomic fragmentation, a “pan-*rfb*” was created considering the genes resulting from the analysis in Fig 5A. The comparison of this artificial *rfb* against WGSs from different serovars, including more than one strain in each case, showed the same genetic pattern for several examples (Fig 5C and Table S6, third sheet). These findings constitute a solid starting point to define a comprehensive set of serovar-specific genetic signatures, eventually revising the current protocols for serogroup and serovar assignments, which are extremely useful in clinical and epidemiologic work.

Discussion

L. noguchii strains are pathogenic members of the genus *Leptospira*, of worldwide distribution, and, together with *L. interrogans*, one of the species most involved in human leptospirosis (Vincent et al, 2019). Beyond human infections, *L. noguchii* has been detected or isolated from different hosts (Silva et al, 2007; Martins et al, 2015; Barragan et al, 2016; Zarantonelli et al, 2018), exhibiting a singular host adaptation capacity, and being one of the few *Leptospira* species isolated not only from different mammals but also from amphibians (Everard et al, 1988; Gravekamp et al, 1991). However, *L. noguchii* is still a poorly characterized species, with scarce information about the circulating serovars. A few draft genomes of *L. noguchii* have been published (Moreno et al, 2015; Nieves et al, 2019), but no complete genomes had been reported so far.

The 12 *L. noguchii* strains that we have now sequenced were selected such that a broad range of serogroup variants were included, and different hosts including cattle, human, and amphibians. These genomes were analyzed in terms of their core

genome and strain-specific genes and put in a broader context by studying their phylogenetic relationship with other *L. noguchii* strains. Special attention was given to analyzing the genetic composition of the *rfb* cluster, which is notoriously linked to serovar determination (Patra et al, 2015; Picardeau, 2017). Moreover, this analysis was extended to other serovars from different *Leptospira* species.

The genome architecture in terms of replicon content and its organization is overall similar to that found in other *Leptospira* species (Picardeau et al, 2008), exhibiting two main chromosomes and a diverse repertoire of plasmids. On average, *L. noguchii* has a larger genome (4.8 Mb) compared with other relevant pathogens such as *L. interrogans* (4.6 Mb) and *L. borgpetersenii* (3.9 Mb), with a larger array of accessory genes that might be involved in *L. noguchii*'s remarkable adaptability. Indeed, ~30% of the genes from the strains sequenced in this study correspond to accessory genome, with a clear enrichment in carbohydrate biochemistry pathways. Concerning the plasmid repertoire, some strains showed identical or nearly identical protein-encoding plasmidic genes (Fig 1), suggesting that these replicons may be transferred between strains. Such exchange among different species may be evolutionarily ancient, considering the large number of shared proteins that can be identified in several cases (e.g., *L. mayottensis* str 200901116 p1, *L. mayottensis* str MDI222 pLmayMDI222, *L. interrogans* sv Bataviae str 1489 p4, and *L. noguchii* str IP1611024 p1). On the contrary, plasmid-borne gene variability seems larger in some *Leptospira* species (Table S3, first sheet), perhaps reflecting a varying contribution of horizontal plasmid acquisition as a source of adaptation. COG analyses revealed no variation in functional representation between plasmids from *L. noguchii* versus those from other species, except for the absence of genes related to amino acid metabolism and transport in *L. noguchii* plasmids.

Phylogenetic analyses using *L. noguchii* WGS data (Fig 2) did not reveal a correlation of genotype with geographical distribution nor with host specificity traits. This is consistent with previous reports (Loureiro et al, 2020), now further including the set of 10 finished genomes and two high-quality drafts that were not previously available. This led us to focus on the study of genes associated with LPS synthesis, to explore genotype–phenotype relationships that could underlie the rich phenotypic complexity exhibited among and within *Leptospira* species, which is expressed as disparate serovars/serogroups. It is known that the expression of surface epitopes in the LPS is a major determinant for serovar identity, particularly concerning the LPS's composition and spatial arrangement of sugars (Adler & de la Peña Moctezuma, 2010). The *rfb* cluster, which harbors most of the genes encoding enzymes for the LPS O-antigen synthesis, has been studied in *Leptospira* species of different serovars (de la Peña-Moctezuma et al, 1999; Kalambaheti et al, 1999; Bulach et al, 2000; Casas et al, 2005; Fouts et al, 2016), showing great variability in terms of genetic content and synteny. LPS O-antigens are frequently synthesized through a so-

called Wzx/Wzy-dependent pathway, implicating an O-flippase (Wzx) and an O-polymerase (Wzy). This biosynthesis system is complex and includes several proteins highly conserved in Gram-negative and Gram-positive bacteria that possess glycan polymers on their cell surfaces, such as LPS O-antigen, spore coats, enterobacterial common antigen, and outer capsules (Islam & Lam, 2014). *Leptospira* spp. appear to conserve the Wzx/Wzy-dependent O-antigen biosynthesis pathway, exhibiting orthologues of the genes encoding these two enzymes within the *rfb* cluster (Nascimento et al, 2004), even though variations of the typical pathway are anticipated based upon the sporadic presence of *wzy* and *wzz* genes (Fouts et al, 2016). However, a definite association between the presence of genes and serovar determination has not been shown. Some studies in *Leptospira* have been insightful, albeit focused on identifying pathogenicity determinants via characterization of LPS products (Patra et al, 2015; Vanithamani et al, 2021), but limited to the examination of subsets and not all the genes present within *rfb* clusters.

By including 12 genome sequences from *L. noguchii*, and systematically extending the analysis to all *Leptospira* species with assigned serovar identity, we have now substantiated a definite and biunivocal link between *rfb* gene composition and serogroup/serovar identity (Fig 5). The requirement of closed genomes in studies that depend on the analysis of gene presence/absence cannot be overstated, as even minor assembly mistakes, often introduced in multiple contig draft genomes, can lead to major misinterpretations. Many available draft genomes suffer from incompleteness artifacts because of contig closure errors (Kosugi et al, 2015; Lu et al, 2020). Progress in the elucidation of the structure of different LPS variants from *Leptospira* can be anticipated using NMR, as done with other bacterial genera (Fontana et al, 2014). Such an approach critically depends on accurate WGS information, to single out ambiguous alternatives from the carbohydrates' NMR spectra according to the specific set of glycosyltransferases present in the genome. The LPS structures shall thus be conclusive about their dependence on distinct sets of genes present in the genomes, rather than on regulation of gene expression. A serovar-specific genetic fingerprint such as the one we are now reporting shall be instrumental to shifting from serologic techniques to simpler and more accurate PCR-based serovar determination protocols. Considering the clinical impact that different *Leptospira* serovars exhibit, associated with distinct host adaptation and virulence phenotypes, such molecular genetic approaches have been attempted, but only at the level of serogroup (Cai et al, 2010) or for some serovars (Bezerra da Silva et al, 2011; Medeiros et al, 2022) suffering from considerable cross-detection among strains. As further and reliably complete *rfb* gene clusters from finished/closed genomes become available, more precise gene composition assessments among *Leptospira* serovars are expected to be made.

one corresponding to two different *Leptospira* species as marked, is shown side by side with the same matrix representation as in panel (A). (C) Comparison of the *rfb* cluster in whole genomes from different species (Lsan, *L. santarosai*; Lint, *L. interrogans*; Lkir, *L. kirschneri*; Lbor, *L. borgpetersenii*; Lbro, *L. bromii*; Lfai, *L. fainei*; Lina, *L. inadai*; Lliger, *L. licerasiae*; Lbif, *L. biflexa*; Lsp, *Leptospira* sp.), presented side by side by grouping species with the same serovar (labeled in bold red) and serogroup (enclosed in dotted brackets and bold lettering). The numbers at the top of each column correspond to the number of contigs for draft genomes, whereas complete/finished genomes are marked as *Com*.

Added to this serovar-specific signature found in the *rfb* cluster, strong indications that the *rfb* functions as a large genomic island, dispersed via events of HGT, were uncovered. Hallmarks of HGT include (i) the characteristic low GC content within the *rfb* clusters, ostensibly distinct from its flanking DNA segments, (ii) signs of genomic rearrangements (inversions) in the cluster's surroundings, and (iii) the fact that identical/nearly identical *rfb* clusters were found in different *Leptospira* species. A similar HGT scenario of genomic islands has been described in different classes of Proteobacteria, concerning LPS(*O*-antigen)- and capsule polysaccharide(*K*-antigen)-encoding loci (Thrane et al, 2015; Bian et al, 2020; Huszczyński et al, 2020; Buzzanca et al, 2021). Such loci have been observed to locate at highly plastic regions of the genomes from enterobacteria such as *Escherichia* and *Salmonella*, *Pseudomonas*, *Vibrio*, and *Aliarcobacter*, among other genera, exhibiting clear evidence of HGT underlying locus exchange.

Taking all the evidence together, our results strongly suggest that serovar identity can change in *Leptospira* by HGT of a part or even the entire *rfb* cluster, acting as an LPS *O*-antigen-encoding genomic island. This HGT phenomenon seems to occur within and among species from the *Leptospira* genus, contributing to population diversity and adaptability. This observation is consistent with the large variation of gene composition among different *rfb* clusters, with more or less genes being transferred in different cases, together with the ill-defined downstream limit of the island that had already been reported (Fouts et al, 2016). The molecular HGT mechanism explaining *rfb* exchange in *Leptospira* remains to be determined, potentially by homologous recombination, IS elements—which are indeed observed surrounding and within the locus—or phages (Wang & Quinn, 2010).

Materials and Methods

DNA extraction, sequencing, and assembly

Genomic DNA was extracted from 100 ml of a 10⁸ bacteria/ml culture of each isolate using QIAGEN Genomic-tip 100/G and Genomic DNA Buffer Set (QIAGEN). PacBio SMRT sequencing was performed with RSII technology (McGill University/Genome Quebec; Eurofins). De novo assembly was performed with HGAP v.4 (Chin et al, 2013) available on SMRT Link v.7 (default parameters, except, min. subread length: 500; estimated genome size: 4.8 Mb), Canu (<https://github.com/marbl/canu>) (Koren et al, 2017), Unicycler (<https://github.com/rrwick/Unicycler>) (Wick et al, 2017), or Tricycler (<https://github.com/rrwick/Tricycler>) (Wick et al, 2021). The polishing step was run on SMRT Link v.7 using the Resequencing application (default parameters).

Genome data sets

Genomes included in the phylogenetic analyses were downloaded from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) or from the Institut Pasteur Bacterial Isolate Genome Sequence Database (<https://bigsd.bpasteur.fr/leptospira/>). The metadata for all isolates, including for those sequenced in this study, are summarized

in Table S4. Genomes from strains of known serovar used for the *rfb* cluster comparisons were downloaded from Patric (<https://www.patricbrc.org>), and their metadata are summarized in Table S5. Plasmid sequences from other *Leptospira* species were also obtained from GenBank, and their associated metadata are summarized in Table S3, along with the general features of plasmids from the strains sequenced in this study. Of note, plasmids p2 from strain “barbudensis” and p5 from “IP1712055” were not included in the network analysis (Fig 1 and Table S3, third and fourth sheets) to simplify the comparison, as they show high similarities with plasmids p1 and p3 from those strains, respectively. Nevertheless, all GenBank plasmid sequence files are included as Supplemental Data 2.

Phylogenetic analyses

All 38 genome sequences (including *L. interrogans* str. 56601 and *L. kirschneri* str. 200702274 as outgroups) were annotated using Prokka version 1.13.7 (Seemann, 2014). Orthology between the coding sequences was inferred using the combination of the two algorithms COG and OMCL through GET_HOMOLOGUES version 20190411 (Conterras-Moreira & Vinuesa, 2013). The sequences of orthologous genes that are single copy and corresponding to the softcore (sequences present in more than 95% of the genomes) were aligned using MAFFT version 7.407 (Kato & Standley, 2013). The resulting alignments were filtered using BMGE version 1.12 (Criscuolo & Gribaldo, 2010) and concatenated in a partitioned supermatrix using AMAS (Borowiec, 2016). The best-fit model of each partition and the maximum-likelihood phylogeny was performed using IQ-TREE version 1.6.11 (Nguyen et al, 2015) and 10,000 ultrafast bootstraps (Hoang et al, 2018). The same protocol was followed for the construction of the phylogenetic tree, but sequences were codon-aligned using TranslatorX version 1.1 (Abascal et al, 2010).

The ANI index was calculated using the OrthoANIu algorithm available at EzBioCloud (<https://www.ezbiocloud.net/tools/ani>) (Yoon et al, 2017).

Genomic analyses

Comparative pangenome analysis was performed using Roary version 3.11.2 (Page et al, 2015). By combining the use of Blast+ (Camacho et al, 2009) and KEGG (Aramaki et al, 2020; Kanehisa & Sato, 2020), it was possible to assign functions of selected core and accessory genes (Table S2). *Leptospira* genomes with assigned serovars and less than 500 contigs (Table S5) were downloaded, and their *rfb* clusters, lipid A, and core oligosaccharide biosynthesis-encoding clusters were analyzed. Location of sites and gene cluster sequence extraction were done with the bioinformatics tools included in Emboss 6.6.0 (Rice et al, 2000) and then annotated using Prokka version 1.13.7. Softcore *rfb* alignments were performed with MAFFT through Roary version 3.11.2, considering 60% identity and gene presence in at least 60% of strains included in the analysis. The resulting alignment was then used to calculate the phylogenetic tree using IQ-TREE version 1.6.11. The synteny of *rfb* clusters, and pairwise genome comparisons to determine conservation, was inferred and represented using Easyfig

2.2.2 (Sullivan et al, 2011). Gene presence/absence analyses among *rfb* clusters from different serovars (Supplemental Data 3) were performed by protein-level cross-matching and subsequent network associations. Briefly, a pairwise comparison of each *rfb* cluster with one another was conducted using Blastp. The network connection was thereafter established using the previously generated Blast files and NetworkX version 2.6.2 (Hagberg et al, 2008) with 60% similarity threshold; thus, proteins having $\geq 60\%$ similarity were grouped together generating the gene presence/absence matrices. Close-up plots of GC content along linearized sequence fragments were performed with DNAPlotter (Carver et al, 2009). Network association analysis of plasmidic protein-encoding gene repertoire was carried out as described for the *rfb* cluster, considering a 60% similarity cutoff. Hierarchical clustering according to the shared protein-encoding genes (options used: Euclidean distance, ward linkage) was performed using available tools at <https://mev.tm4.org>. Functional annotation was done using eggno-mapper v2 (Huerta-Cepas et al, 2017). Transposase positions across Chr1 of *L. noguchii* strains were obtained from the Prokka annotation and then represented using the online version of shinyCircos (<https://venyao.xyz/shinycircos/>) (Yu et al, 2018).

Data Availability

The genome sequences have been deposited in DDBJ/ENA/GenBank under the BioProject PRJNA803166, specifically with the following accession numbers: *L. noguchii* strain barbudensis, CP091967–CP091970; *L. noguchii* strain 201601331, CP091962–CP091966; *L. noguchii* strain IP1512017, CP091957–CP091961; *L. noguchii* strain IP1605021, CP091953–CP091956; *L. noguchii* strain IP1611024, CP091947–CP091952; *L. noguchii* strain IP1703027, CP091943–CP091946; *L. noguchii* strain IP1705032, CP091940–CP091942; *L. noguchii* strain IP1709037, CP091936–CP091939; *L. noguchii* strain IP1712055, CP091928–CP091935; *L. noguchii* strain IP1804061, CP092112–CP092116; *L. noguchii* strain bajan, JAKNBP000000000; and *L. noguchii* strain 201102933, JAKNBO000000000.

Supplementary Information

Supplementary Information is available at <https://doi.org/10.26508/lsa.202201480>

Acknowledgements

This research was supported by Institut Pasteur through grant PTR 30-2017 (M Picardeau, A Buschiazio, and FJ Veyrier); by Institut Pasteur & Institut Pasteur de Montevideo through their Pasteur International Joint Research Units program “Integrative Microbiology of Zoonotic Agents” grant IMiZA-2017 (M Picardeau and A Buschiazio); and by the Natural Sciences and Engineering Research Council of Canada discovery grant (RGPIN-2016-04940) (FJ Veyrier). C Nieves received a Ph.D. studentship Calmette & Yersin from the Institut Pasteur International Network. FJ Veyrier received a Junior 1 and Junior 2 research scholar salary award from the Fonds de Recherche du Québec—Santé. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the article. We thank Howard

Takiff and Lizeth Caraballo (Venezuelan Institute of Scientific Investigation) for providing a *L. noguchii* isolate; and Gregorio Iraola and Ignacio Ferrés for helpful discussions.

Author Contributions

C Nieves: conceptualization, data curation, formal analysis, validation, investigation, visualization, methodology, and writing—original draft, review, and editing.

AT Vincent: data curation, formal analysis, investigation, methodology, and writing—review and editing.

L Zaranonelli: resources and writing—review and editing.

M Picardeau: resources, funding acquisition, and writing—review and editing.

FJ Veyrier: conceptualization, data curation, formal analysis, supervision, funding acquisition, methodology, and writing—review and editing.

A Buschiazio: conceptualization, formal analysis, funding acquisition, validation, investigation, and writing—original draft, review, and editing.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

References

- Abascal F, Zardoya R, Telford MJ (2010) TranslatorX: Multiple alignment of nucleotide sequences guided by amino acid translations. *Nucleic Acids Res* 38: W7–W13. doi:10.1093/nar/gkq291
- Adler B, de la Peña Moctezuma A (2010) Leptospira and leptospirosis. *Vet Microbiol* 140: 287–296. doi:10.1016/j.vetmic.2009.03.012
- Aramaki T, Blanc-Mathieu R, Endo H, Ohkubo K, Kanehisa M, Goto S, Ogata H (2020) KofamKOALA: KEGG ortholog assignment based on profile HMM and adaptive score threshold. *Bioinformatics* 36: 2251–2252. doi:10.1093/bioinformatics/bt2859
- Barragan V, Chiriboga J, Miller E, Olivás S, Birdsell D, Hepp C, Hornstra H, Schupp JM, Morales M, Gonzalez M, et al (2016) High leptospira diversity in animals and humans complicates the search for common reservoirs of human disease in rural Ecuador. *PLoS Negl Trop Dis* 10: e0004990. doi:10.1371/journal.pntd.0004990
- Bertani B, Ruiz N (2018) Function and biogenesis of lipopolysaccharides. *EcoSal Plus* 8: 10.1128/ecosalplus.ESP-0001-2018. doi:10.1128/ecosalplus.esp-0001-2018
- Bezerra da Silva J, Carvalho E, Hartskeerl RA, Ho PL (2011) Evaluation of the use of selective PCR amplification of LPS biosynthesis genes for molecular typing of leptospira at the serovar level. *Curr Microbiol* 62: 518–524. doi:10.1007/s00284-010-9738-7
- Bharti AR, Nally JE, Ricaldi JN, Matthias MA, Diaz MM, Lovett MA, Levett PN, Gilman RH, Willig MR, Gotuzzo E, et al (2003) Leptospirosis: A zoonotic disease of global importance. *Lancet Infect Dis* 3: 757–771. doi:10.1016/S1473-3099(03)00830-2
- Bian S, Zeng W, Li Q, Li Y, Wong NK, Jiang M, Zuo L, Hu Q, Li L (2020) Genetic structure, function, and evolution of capsule biosynthesis loci in *Vibrio parahaemolyticus*. *Front Microbiol* 11: 546150. doi:10.3389/fmicb.2020.546150
- Biswas T, Yi L, Aggarwal P, Wu J, Rubin JR, Stuckey JA, Woodard RW, Tsodikov OV (2009) The tail of KdsC: Conformational changes control the

- activity of a haloacid dehalogenase superfamily phosphatase. *J Biol Chem* 284: 30594–30603. doi:10.1074/jbc.m109.012278
- Borowiec ML (2016) AMAS: A fast tool for alignment manipulation and computing of summary statistics. *PeerJ* 4: e1660. doi:10.7717/peerj.1660
- Bulach DM, Kalambaheti T, de la Pena-Moctezuma A, Adler B (2000) Functional analysis of genes in the rfb locus of *Leptospira borgpetersenii* serovar Hardjo subtype Hardjobovis. *Infect Immun* 68: 3793–3798. doi:10.1128/iai.68.7.3793-3798.2000
- Bulach DM, Seemann T, Zuerner RL, Adler B (2006) The organization of *Leptospira* at a genomic level. In *Bacterial Genomes and Infectious Diseases*. Chan VL, Sherman PM, Bourke B (eds), pp 109–123. Totowa, NJ: Humana Press.
- Buzzanca D, Botta C, Ferrocino I, Alessandria V, Houf K, Rantsiou K (2021) Functional pangenome analysis reveals high virulence plasticity of *Aliarcobacter butzleri* and affinity to human mucus. *Genomics* 113: 2065–2076. doi:10.1016/j.ygeno.2021.05.001
- Cai CS, Zhu YZ, Zhong Y, Xin XF, Jiang XG, Lou XL, He P, Qin JH, Zhao GP, Wang SY, et al (2010) Development of O-antigen gene cluster-specific PCRs for rapid typing six epidemic serogroups of *Leptospira* in China. *BMC Microbiol* 10: 67. doi:10.1186/1471-2180-10-67
- Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL (2009) BLAST+: Architecture and applications. *BMC Bioinformatics* 10: 421. doi:10.1186/1471-2105-10-421
- Carver T, Thomson N, Bleasby A, Berriman M, Parkhill J (2009) DNAPlotter: Circular and linear interactive genome visualization. *Bioinformatics* 25: 119–120. doi:10.1093/bioinformatics/btn578
- Casas EMC, Rios RR, Bulach D, Adler B, de la Pena-Moctezuma A (2005) [Evaluation of the transcription of locus rfb for the biosynthesis of LPS in *Leptospira interrogans* subtype hardjoprajitno]. *Rev Cubana Med Trop* 57: 43–44.
- Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, et al (2013) Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10: 563–569. doi:10.1038/nmeth.2474
- Contreras-Moreira B, Vinuesa P (2013) GET_HOMOLOGUES, a versatile software package for scalable and robust microbial pangenome analysis. *Appl Environ Microbiol* 79: 7696–7701. doi:10.1128/aem.02411-13
- Costa F, Hagan JE, Calcagno J, Kane M, Torgerson P, Martinez-Silveira MS, Stein C, Abela-Ridder B, Ko AI (2015) Global morbidity and mortality of leptospirosis: A systematic review. *PLoS Negl Trop Dis* 9: e0003898. doi:10.1371/journal.pntd.0003898
- Crisuolo A, Gribaldo S (2010) BMGE (block mapping and gathering with entropy): A new software for selection of phylogenetic informative regions from multiple sequence alignments. *BMC Evol Biol* 10: 210. doi:10.1186/1471-2148-10-210
- de la Peña-Moctezuma A, Bulach DM, Kalambaheti T, Adler B (1999) Comparative analysis of the LPS biosynthetic loci of the genetic subtypes of serovar Hardjo: *Leptospira interrogans* subtype Hardjoprajitno and *Leptospira borgpetersenii* subtype Hardjobovis. *FEMS Microbiol Lett* 177: 319–326. doi:10.1016/S0378-1097(99)00333-x
- Denton JF, Lugo-Martinez J, Tucker AE, Schrider DR, Warren WC, Hahn MW (2014) Extensive error in the number of genes inferred from draft genome assemblies. *PLoS Comput Biol* 10: e1003998. doi:10.1371/journal.pcbi.1003998
- Ellis WA (2015) Animal leptospirosis. *Curr Top Microbiol Immunol* 387: 99–137. doi:10.1007/978-3-662-45059-8_6
- Everard COR, Carrington D, Korver H, Everard JD (1988) Leptospire in the marine toad (*Bufo marinus*) on Barbados. *J Wildl Dis* 24: 334–338. doi:10.7589/0090-3558-24.2.334
- Flores BJ, Perez-Sanchez T, Fuertes H, Sheleby-Elias J, Muzquiz JL, Jiron W, Duttmann C, Halaihel N (2017) A cross-sectional epidemiological study of domestic animals related to human leptospirosis cases in Nicaragua. *Acta Trop* 170: 79–84. doi:10.1016/j.actatropica.2017.02.031
- Fontana C, Lundborg M, Weintraub A, Widmalm G (2014) Rapid structural elucidation of polysaccharides employing predicted functions of glycosyltransferases and NMR data: Application to the O-antigen of *Escherichia coli* O59. *Glycobiology* 24: 450–457. doi:10.1093/glycob/cwu011
- Fouts DE, Matthias MA, Adhikarla H, Adler B, Amorim-Santos L, Berg DE, Bulach D, Buschiazzi A, Chang YF, Galloway RL, et al (2016) What makes a bacterial species pathogenic?: comparative genomic analysis of the genus *Leptospira*. *PLoS Negl Trop Dis* 10: e0004403. doi:10.1371/journal.pntd.0004403
- Fraser DW, Glosser JW, Francis DP, Phillips CJ, Feeley JC, Sulzer CR (1973) Leptospirosis caused by serotype Fort-Bragg. A suburban outbreak. *Ann Intern Med* 79: 786–789. doi:10.7326/0003-4819-79-6-786
- Gochenour WS, Jr., Smadel JE, Jackson EB, Evans LB, Yager RH (1952) Leptospirotic etiology of Fort Bragg fever. *Public Health Rep* 67: 811–813. doi:10.2307/4588208
- Gravekamp C, Korver H, Montgomery J, Everard CO, Carrington D, Ellis WA, Terpstra WJ (1991) Leptospire isolated from toads and frogs on the Island of Barbados. *Zentralbl Bakteriol* 275: 403–411. doi:10.1016/S0934-8840(11)80308-7
- Guglielmini J, Bourhy P, Schiettekatte O, Zinini F, Brisse S, Picardeau M (2019) Genus-wide *Leptospira* core genome multilocus sequence typing for strain taxonomy and global surveillance. *PLoS Negl Trop Dis* 13: e0007374. doi:10.1371/journal.pntd.0007374
- Hagberg AA, Schult DA, Swart PJ (2008) Exploring network structure, dynamics, and function using NetworkX. In *Proceedings of the 7th Python in Science Conference (SciPy 2008)*, Varoquaux G, Vaught T, Millman J (eds), pp 11–15. Available at: http://conference.scipy.org/proceedings/SciPy2008/paper_2/.
- Hamond C, Pestana CP, Medeiros MA, Lilenbaum W (2016) Genotyping of *Leptospira* directly in urine samples of cattle demonstrates a diversity of species and strains in Brazil. *Epidemiol Infect* 144: 72–75. doi:10.1017/S0950268815001363
- Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS (2018) UFBoot2: Improving the ultrafast bootstrap approximation. *Mol Biol Evol* 35: 518–522. doi:10.1093/molbev/msx281
- Huerta-Cepas J, Forslund K, Coelho LP, Szklarczyk D, Jensen LJ, von Mering C, Bork P (2017) Fast genome-wide functional annotation through orthology assignment by eggNOG-mapper. *Mol Biol Evol* 34: 2115–2122. doi:10.1093/molbev/msx148
- Huszczynski SM, Hao Y, Lam JS, Khursigara CM (2020) Identification of the *Pseudomonas aeruginosa* O17 and O15 O-specific antigen biosynthesis loci reveals an ABC transporter-dependent synthesis pathway and mechanisms of genetic diversity. *J Bacteriol* 202: e00347-20. doi:10.1128/jb.00347-20
- Islam ST, Lam JS (2014) Synthesis of bacterial polysaccharides via the Wzx/Wzy-dependent pathway. *Can J Microbiol* 60: 697–716. doi:10.1139/cjm-2014-0595
- Jancloes M, Bertherat E, Schneider C, Belmain S, Munoz-Zanzi C, Hartskeerl R, Costa F, Denis J, Benschop J (2014) Towards a “one health” strategy against Leptospirosis. *Planet@Risk* 2: 204–206.
- Kalambaheti T, Bulach DM, Rajakumar K, Adler B (1999) Genetic organization of the lipopolysaccharide O-antigen biosynthetic locus of *Leptospira borgpetersenii* serovar Hardjobovis. *Microb Pathog* 27: 105–117. doi:10.1006/mpat.1999.0285
- Kanehisa M, Sato Y (2020) KEGG Mapper for inferring cellular functions from protein sequences. *Protein Sci* 29: 28–35. doi:10.1002/pro.3711
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol Biol Evol* 30: 772–780. doi:10.1093/molbev/mst010
- Ko AI, Goarant C, Picardeau M (2009) Leptospira: The dawn of the molecular genetics era for an emerging zoonotic pathogen. *Nat Rev Microbiol* 7: 736–747. doi:10.1038/nrmicro2208

- Korba AA, Lounici H, Kainiu M, Vincent AT, Mariet JF, Veyrier FJ, Goarant C, Picardeau M (2021) *Leptospira ainlahdjerensis* sp. nov., *Leptospira ainzelensis* sp. nov., *Leptospira abararensis* sp. nov. and *Leptospira chreensis* sp. nov., four new species isolated from water sources in Algeria. *Int J Syst Evol Microbiol* 71. doi:10.1099/ijsem.0.005148
- Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM (2017) Canu: Scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. *Genome Res* 27: 722–736. doi:10.1101/gr.215087.116
- Kosugi S, Hirakawa H, Tabata S (2015) GMcloser: Closing gaps in assemblies accurately with a likelihood-based selection of contig or long-read alignments. *Bioinformatics* 31: 3733–3741. doi:10.1093/bioinformatics/btv465
- Loureiro AP, Jaeger LH, Di Azevedo MIN, Miraglia F, Moreno LZ, Moreno AM, Pestana CP, Carvalho-Costa FA, Medeiros MA, Lilenbaum W (2020) Molecular epidemiology of *Leptospira noguchii* reveals important insights into a One Health context. *Transbound Emerg Dis* 67: 276–283. doi:10.1111/tbed.13349
- Lu P, Jin J, Li Z, Xu Y, Hu D, Liu J, Cao P (2020) PGcloser: Fast parallel gap-closing tool using long-reads or contigs to fill gaps in genomes. *Evol Bioinform Online* 16: 1176934320913859. doi:10.1177/1176934320913859
- Martins G, Loureiro AP, Hamond C, Pinna MH, Bremont S, Bourhy P, Lilenbaum W (2015) First isolation of *Leptospira noguchii* serogroups Panama and Autumnalis from cattle. *Epidemiol Infect* 143: 1538–1541. doi:10.1017/s0950268814002416
- Medeiros EJS, Ferreira LCA, Ortega JM, Cosate MRV, Sakamoto T (2022) Genetic basis underlying the serological affinity of leptospiral serovars from serogroups Sejroe, Mini and Hebdomadis. *Infect Genet Evol* 103: 105345. doi:10.1016/j.meegid.2022.105345
- Mitchison M, Bulach DM, Vinh T, Rajakumar K, Faine S, Adler B (1997) Identification and characterization of the dTDP-rhamnose biosynthesis and transfer genes of the lipopolysaccharide-related rfb locus in *Leptospira interrogans* serovar Copenhageni. *J Bacteriol* 179: 1262–1267. doi:10.1128/jb.179.4.1262-1267.1997
- Moreno LZ, Loureiro AP, Miraglia F, Matajira CEC, Kremer FS, Esalbao MR, Dellagostin OA, Lilenbaum W, Moreno AM (2015) Draft genome sequence of Brazilian *Leptospira noguchii* serogroup Panama strain U73, isolated from cattle. *Genome Announc* 3: e01179–15. doi:10.1128/genomea.01179-15
- Mwachui MA, Crump L, Hartskeerl R, Zinsstag J, Hattendorf J (2015) Environmental and behavioural determinants of leptospirosis transmission: A systematic review. *PLoS Negl Trop Dis* 9: e0003843. doi:10.1371/journal.pntd.0003843
- Nascimento ALTO, Ko AI, Martins EAL, Monteiro-Vitorello CB, Ho PL, Haake DA, Verjovski-Almeida S, Hartskeerl RA, Marques MV, Oliveira MC, et al (2004) Comparative genomics of two *Leptospira interrogans* serovars reveals novel insights into physiology and pathogenesis. *J Bacteriol* 186: 2164–2172. doi:10.1128/jb.186.7.2164-2172.2004
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* 32: 268–274. doi:10.1093/molbev/msu300
- Nieves C, Ferrer I, Diaz-Viraque F, Buschiazio A, Zaranonelli L, Iraola G (2019) Draft genome sequences of 40 pathogenic leptospira strains isolated from cattle in Uruguay. *Microbiol Resour Announc* 8: e00893–19. doi:10.1128/mra.00893-19
- Nishino K, Nikaido E, Yamaguchi A (2007) Regulation of multidrug efflux systems involved in multidrug and metal resistance of *Salmonella enterica* serovar Typhimurium. *J Bacteriol* 189: 9066–9075. doi:10.1128/jb.01045-07
- Oliveira PH, Touchon M, Cury J, Rocha EPC (2017) The chromosomal organization of horizontal gene transfer in bacteria. *Nat Commun* 8: 841. doi:10.1038/s41467-017-00808-w
- Olo Ndela E, Enault F, Toussaint A (2021) Transposable prophages in leptospira: An ancient, now diverse, group predominant in causative agents of weill's disease. *Int J Mol Sci* 22: 13434. doi:10.3390/ijms222413434
- Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MT, Fookes M, Falush D, Keane JA, Parkhill J (2015) Roary: Rapid large-scale prokaryote pan genome analysis. *Bioinformatics* 31: 3691–3693. doi:10.1093/bioinformatics/btv421
- Patra KP, Choudhury B, Matthias MM, Baga S, Bandyopadhyay K, Vinetz JM (2015) Comparative analysis of lipopolysaccharides of pathogenic and intermediately pathogenic *Leptospira* species. *BMC Microbiol* 15: 244. doi:10.1186/s12866-015-0581-7
- Picardeau M, Bulach DM, Bouchier C, Zuerner RL, Zidane N, Wilson PJ, Creno S, Kuczek ES, Bommazzadri S, Davis JC, et al (2008) Genome sequence of the saprophyte *Leptospira biflexa* provides insights into the evolution of *Leptospira* and the pathogenesis of leptospirosis. *PLoS One* 3: e1607. doi:10.1371/journal.pone.0001607
- Picardeau M (2017) Virulence of the zoonotic agent of leptospirosis: Still terra incognita? *Nat Rev Microbiol* 15: 297–307. doi:10.1038/nrmicro.2017.5
- Que-Gewirth NLS, Ribeiro AA, Kalb SR, Cotter RJ, Bulach DM, Adler B, Girons IS, Werts C, Raetz CRH (2004) A methylated phosphate group and four amide-linked acyl chains in leptospira interrogans lipid A. The membrane anchor of an unusual lipopolysaccharide that activates TLR2. *J Biol Chem* 279: 25420–25429. doi:10.1074/jbc.M400598200
- Raetz CRH, Whitfield C (2002) Lipopolysaccharide endotoxins. *Annu Rev Biochem* 71: 635–700. doi:10.1146/annurev.biochem.71.110601.135414
- Rice P, Longden I, Bleasby A (2000) EMBOS: The European molecular biology open software suite. *Trends Genet* 16: 276–277. doi:10.1016/s0168-9525(00)02024-2
- Santos LA, Adhikarla H, Yan X, Wang Z, Fouts DE, Vinetz JM, Alcántara LCJ, Hartskeerl RA, Goris MGA, Picardeau M, et al (2018) Genomic comparison among global isolates of *L. interrogans* serovars Copenhageni and Icterohaemorrhagiae identified natural genetic variation caused by an indel. *Front Cell Infect Microbiol* 8: 193. doi:10.3389/fcimb.2018.00193
- Seemann T (2014) Prokka: Rapid prokaryotic genome annotation. *Bioinformatics* 30: 2068–2069. doi:10.1093/bioinformatics/btu153
- Silva EF, Brod CS, Cerqueira GM, Bourscheidt D, Seyffert N, Queiroz A, Santos CS, Ko AI, Dellagostin OA (2007) Isolation of *Leptospira noguchii* from sheep. *Vet Microbiol* 121: 144–149. doi:10.1016/j.vetmic.2006.11.010
- Silva EF, Cerqueira GM, Seyffert N, Seixas FK, Hartwig DD, Athanazio DA, Pinto LS, Queiroz A, Ko AI, Brod CS, et al (2009) *Leptospira noguchii* and human and animal leptospirosis, Southern Brazil. *Emerg Infect Dis* 15: 621–623. doi:10.3201/eid1504.071669
- Sullivan MJ, Petty NK, Beatson SA (2011) Easyfig: A genome comparison visualizer. *Bioinformatics* 27: 1009–1010. doi:10.1093/bioinformatics/btr039
- Tettelin H, Riley D, Cattuto C, Medini D (2008) Comparative genomics: The bacterial pan-genome. *Curr Opin Microbiol* 11: 472–477. doi:10.1016/j.mib.2008.09.006
- Thrane SW, Taylor VL, Freschi L, Kukavica-Ibrulj I, Boyle B, Laroche J, Pirnay JP, Levesque RC, Lam JS, Jelsbak L (2015) The widespread multidrug-resistant serotype O12 *Pseudomonas aeruginosa* clone emerged through concomitant horizontal transfer of serotype antigen and antibiotic resistance gene clusters. *mBio* 6: e01396–15. doi:10.1128/mbio.01396-15
- Torgerson PR, Hagan JE, Costa F, Calcagno J, Kane M, Martinez-Silveira MS, Goris MGA, Stein C, Ko AI, Abela-Ridder B (2015) Global burden of leptospirosis: Estimated in terms of disability adjusted life years. *PLoS Negl Trop Dis* 9: e0004122. doi:10.1371/journal.pntd.0004122
- Valvano M (2015) Genetics and biosynthesis of lipopolysaccharide. In *Molecular Medical Microbiology*, Tang Y-W, Sussman M, Liu D, Poxton I, Schwartzman J (eds), pp 55–89. Amsterdam: Academic Press.
- Vanithamani S, Akino Mercy CS, Kanagavel M, Sumaiya K, Bothammal P, Saranya P, Prasad M, Ponnurugan K, Muralitharan G, Al-Dhabi NA, et al

- (2021) Biochemical analysis of leptospiral LPS explained the difference between pathogenic and non-pathogenic serogroups. *Microb Pathog* 152: 104738. doi:[10.1016/j.micpath.2021.104738](https://doi.org/10.1016/j.micpath.2021.104738)
- Vincent AT, Schiettekatte O, Goarant C, Neela VK, Bernet E, Thibeaux R, Ismail N, Mohd Khalid MKN, Amran F, Masuzawa T, et al (2019) Revisiting the taxonomy and evolution of pathogenicity of the genus *Leptospira* through the prism of genomics. *PLoS Negl Trop Dis* 13: e0007270. doi:[10.1371/journal.pntd.0007270](https://doi.org/10.1371/journal.pntd.0007270)
- Wang X, Quinn PJ (2010) Endotoxins: Lipopolysaccharides of gram-negative bacteria. *Subcell Biochem* 53: 3–25. doi:[10.1007/978-90-481-9078-2_1](https://doi.org/10.1007/978-90-481-9078-2_1)
- Wick RR, Judd LM, Gorrie CL, Holt KE (2017) Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 13: e1005595. doi:[10.1371/journal.pcbi.1005595](https://doi.org/10.1371/journal.pcbi.1005595)
- Wick RR, Judd LM, Cerdeira LT, Hawkey J, Meric G, Vezina B, Wyres KL, Holt KE (2021) Trycycler: Consensus long-read assemblies for bacterial genomes. *Genome Biol* 22: 266. doi:[10.1186/s13059-021-02483-z](https://doi.org/10.1186/s13059-021-02483-z)
- Yasuda PH, Steigerwalt AG, Sulzer KR, Kaufmann AF, Rogers F, Brenner DJ (1987) Deoxyribonucleic acid relatedness between serogroups and serovars in the family leptospiraceae with proposals for seven new *Leptospira* species. *Int J Syst Bacteriol* 37: 407–415. doi:[10.1099/00207713-37-4-407](https://doi.org/10.1099/00207713-37-4-407)
- Yoon SH, Ha SM, Lim J, Kwon S, Chun J (2017) A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie Van Leeuwenhoek* 110: 1281–1286. doi:[10.1007/s10482-017-0844-4](https://doi.org/10.1007/s10482-017-0844-4)
- Yu Y, Ouyang Y, Yao W (2018) shinyCircos: an R/Shiny application for interactive creation of Circos plot. *Bioinformatics* 34: 1229–1231. doi:[10.1093/bioinformatics/btx763](https://doi.org/10.1093/bioinformatics/btx763)
- Zarantonelli L, Suanes A, Meny P, Buroni F, Nieves C, Salaberry X, Briano C, Ashfield N, Da Silva Silveira C, Dutra F, et al (2018) Isolation of pathogenic *Leptospira* strains from naturally infected cattle in Uruguay reveals high serovar diversity, and uncovers a relevant risk for human leptospirosis. *PLoS Negl Trop Dis* 12: e0006694. doi:[10.1371/journal.pntd.0006694](https://doi.org/10.1371/journal.pntd.0006694)



License: This article is available under a Creative Commons License (Attribution 4.0 International, as described at <https://creativecommons.org/licenses/by/4.0/>).