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Chronology of emergence of the genus *Leptospira* and over-representation of gene families enriched by vitamin B2, B12 biosynthesis, cell adhesion and external encapsulating structure in *L. interrogans* isolates from asymptomatic dogs

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ABSTRACT

The spirochete species *Leptospira interrogans* is the most common cause of leptospirosis, producing acute to chronic infections in most mammalian species. This pathogenic bacterium has an ability to evolve in many ways to occupy various environments and hosts. In this study, we performed chronology analysis to look for insight into the emergence of *Leptospira* species, focusing on *L. interrogans*, and investigated gene gain and loss related to their adaptation in strains isolated from asymptomatic dogs. Chronology analysis revealed that the emergence of *L. interrogans* was around 53.7 million years ago (MYA), corresponding to the Paleogene period that coincided with an optimal climatic temperature and the evolution of suitable mammalian hosts. Gene families encoding for vitamin B2, B12 biosynthesis, cell adhesion and external encapsulating structure were found to be enriched in *L. interrogans* isolated from the urine of asymptomatic dogs. The activity of these gene families may have favored adaptations resulting in chronic infections.

1. Introduction

The genus *Leptospira* includes 34 species of Gram-negative spirochete bacteria (Picardeau, 2017; Thibeaux et al., 2018a; Thibeaux et al., 2018b). Some species can cause the important zoonotic disease known as leptospirosis, which has a worldwide distribution (Adler and de la Pena Moctezuma, 2010). *Leptospira* species can infect a wide range of mammalian hosts causing different clinical outcomes varying from acute life-threatening disease to chronic infections (Evangelista and Coburn, 2010). *L. interrogans* is the most common of the pathogenic species, and strains have been shown to contain numerous virulence factors (Picardeau, 2017). This pathogenic bacterium has an ability to adapt in many ways to persist in environments and hosts (Fouts et al., 2016). Although infections with *L. interrogans* cause high mortality and morbidity rates worldwide, and the molecular pathogenesis has been widely studied (Costa et al., 2015), to date there has been limited information available about their evolutionary emergence and genomic adaptations, especially in relation to strains causing chronic infections.

Previously, the genomes of *L. interrogans* serovar Paidjan strain CUDO5 and serovar Dadas strain CUDO8 recovered from the urine of asymptomatic dogs in Thailand were sequenced (Kurilung et al., 2019; Kurilung et al., 2018), and in this study these were used as representative examples of strains associated with chronic carriage of leptospirosis. The strains may provide useful information regarding genetic adaptation in chronic infection and extend our knowledge about the molecular pathogenesis of this pathogen. In the present study, we analyzed the chronology of emergence of *L. interrogans* and the other *Leptospira* species. Furthermore, gene gain and loss analyses were performed focusing on *L. interrogans* serovar Paidjan strain CUDO5 and serovar Dadas strain CUDO8 in comparison with other *L. interrogans* strains of distinct worldwide origin to investigate gene families possibly enriched in chronic infections.

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Table 1
Summary of genomic features of *Leptospira* species and other bacterial species used in the study.

Species	Strain	Serovar	Host	Genome status	Size (Mbp)	Number of CDS	GC%	Accession number
L. interrogans	56601	Lai	Human	Complete	4.62	3780	35	NC_004342.2
L. kirschneri	M110/06	Pomona	Fox	Draft	4.61	3799	35.9	NZ_MVIT00000000.1
L. noguchii	56682	Undetermined	Wild boar	Draft	4.66	3830	35.4	NZ_JQRF00000000.1
L. borgpetersenii	203	Hardjo-bovis	Cattle	Complete	3.90	3244	40.2	NZ_CP021412
L. weilii	56622	Undetermined	Human	Draft	4.28	3885	40.7	NZ_JQRQ00000000.1
L. alexanderi	56650	Undetermined	Human	Draft	4.04	3588	40.3	NZ_JQGS00000000.1
L. alstonii	GWTS #1	Room22	Shrew	Complete	4.59	4274	42.4	NZ_CP015217
L. santarosai	U233	Undetermined	Cattle	Complete	3.94	3567	41.9	NZ_CP028377.1
L. kmetyi	Bejo-Iso9	Malaysia	Soil	Draft	4.41	4062	44.8	NZ_AHMP00000000.2
L. ellisii	ATI7-C-A5	Undetermined	Soil	Draft	4.31	4212	47.8	NPDV01000001.1
L. barantonii	FH4-C-A1	Undetermined	Soil	Draft	4.39	4033	43.9	NPDV01000001.1
L. adleri	FH2-B-C1	Undetermined	Soil	Draft	4.82	4626	43.5	NPDV01000001.1
L. mayottensis	VS2413	Undetermined	Undetermined	Complete	4.13	3554	39.5	NZ_CP030142.1
L. licerasiae	VAR 010	Varillal	Human	Draft	4.20	3864	41.1	NZ_AHOO00000000.2
L. wolffii	Khorat-H2	Khorat	Human	Draft	4.31	3951	45.7	NZ_AKWX00000000.2
L. inadai	M34/99	Lyme	Rodent	Draft	4.55	4126	44.5	NZ_MCRM00000000
L. broomii	5399	Hurstbridge	Human	Draft	4.39	3991	43	NZ_AHMO00000000.2
L. fainei	BUT 6	Hurstbridge	Wild boar	Draft	4.28	3873	43.5	NZ_AKWZ00000000
L. venezuelensis	CLM-U50	Undetermined	Human	Draft	4.30	3995	39.2	NZ_NETS00000000.1
L. perolatii	FH1-B-B1	Undetermined	Soil	Draft	3.98	3631	42.3	NPDZ00000000.1
L. neocaledonica	ES4-C-A1	Undetermined	Soil	Draft	4.21	3889	40.1	NPEA00000000.1
L. saintgironsiae	FH4-C-A2	Undetermined	Soil	Draft	4.08	3736	45.8	NPDR00000000.1
L. haakeii	ATI7-C-A4	Undetermined	Soil	Draft	4.19	3821	39.8	NPEG00000000.1
L. hartskeelii	MCA2-B-A3	Undetermined	Soil	Draft	4.05	3708	37.8	NPDL00000000.1
L. biflexa	Patoc	Patoc I (Paris)	Water	Complete	3.95	3687	38.9	NC_010602.1
L. terpatrae	LT 11–33	Hualin	Undetermined	Draft	4.09	3794	38.2	NZ_AOGW00000000.2
L. yanagawae	Sao Paulo	Saopaulo	Water	Draft	4.05	3771	38.2	NZ_AOGX00000000.2
L. wolbachii	CDC	Codice	Undetermined	Draft	4.08	3767	39.2	NZ_AOGZ00000000.2
L. meryeri	Hardjo	Went 5	Undetermined	Draft	4.12	3808	38.1	NZ_AKXE00000000.1
L. vanthielii	Waz Holland	Holland	Water	Draft	4.23	3885	38.9	NZ_AOGY00000000.2
L. harrisiae	FH2-B-A1	Undetermined	Soil	Draft	3.94	3651	37.8	NPDX00000000.1
L. macculloughii	ATI2-C-A1	Undetermined	Soil	Draft	6.92	6615	37.9	NPEK00000000.1
L. brenneri	JW2-C-A2	Undetermined	Soil	Draft	4.11	3835	38.3	NPDQ00000000.1
L. levettii	MCA2-B-A1	Undetermined	Soil	Draft	3.87	3579	37.61	NPDM00000000.1
Leptonema illini	56270	Undetermined	NA	Draft	4.42	4098	54.3	NZ_JQDG00000000
Salmonella enterica	CT18	Typhi	NA	Complete	4.78	4614	52.1	NC_003197
Escherichia coli	K-12	NA	NA	Complete	5.14	4962	50.6	NC_000913
Rhizoium spp.	WSM1689	NA	Clover	Complete	7.54	7023	60.8	NZ_CP007045
Agrobacterium spp.	Ach5	NA	Sneezewort	Complete	5.66	5148	58.4	NZ_CP011246
Nostoc spp.	PCC 7120	NA	NA	Complete	7.21	5842	41.2	NC_003272
Gloeobacter violaceus	PCC 7421	NA	NA	Complete	4.65	4430	62	NC_005125

NA: Not applicable.

2. Materials and methods

The genome sequences of 45 strains of *L. interrogans* from different geographical locations and hosts of origin (Supplementary data 1), and the genomes of single strains of 33 other *Leptospira* species, and *Leptonema illini*, *Salmonella enterica*, *Escherichia coli*, *Rhizobium* spp., *Agrobacterium* spp., *Nostoc* spp. and *Gloeobacter violaceus* were downloaded from NBCI and included in this study (Table 1). Molecular dating analysis was performed to estimate the time of emergence of *L. interrogans* using a phylogenetic tree and fossil calibration time points. To investigate how the *L. interrogans* serovar Paidjan strains CUDO5 and serovar Dadas CUDO8 may have adapted to facilitate a carrier state in dogs, the AnGST DTL reconciliation method was used to investigate birth-and-death for gene gain, gene loss, gene duplication and horizontal gene transfer (David and Alm, 2011). Moreover, gene orthologous (GO) enrichment analysis was performed using GOstats (Beissbarth and Speed, 2004) to identify gene families that were over-represented in the gene families gained and lost groups with a *q*-value < 0.05. Detailed information about the materials and methods are presented in Supplementary data 1.

3. Results and discussion

The molecular dating results are summarized in Fig. 1. The most recent common ancestor (MRCA) of *Leptospira* was estimated to have appeared around 2.02 billion years ago (BYA) (95% HPD, 4.87–0.73 BYA) when it diverged from *Leptonema illini*. This period was consistence with the divergence time of *Borrelia* and *Treponema* (~1.9 BYA) (Battistuzzi et al., 2004), and suggests that member of the *Spirochaetes* diverged from each other around this time. The saprophytic *Leptospira* diverged from the other *Leptospira* species around 923 MYA (95% HPD, 2202–332 MYA), and the intermediate species diverged from the progenitor of the pathogenic species at around 631 MYA (95% HPD, 1503–218 MYA). Adaptation of *Leptospira* species to mammalian hosts has been associated with the loss of some sensory transduction signal receptors, with the gain of several virulence genes (Xu et al., 2016), and these events presumably occurred around this time. From around 258 MYA (95% HPD, 620–93 MYA) the various pathogenic species started to diverge from each other. *L. interrogans* diverged from the closely related *L. kirschneri* around 53.7 MYA (95% HPD, 130–16 MYA) (Supplementary data 2), corresponding to the Paleogene period (~66–23.03 MYA). The climate of the earth in this period was warm and moist with an average temperature of around 23–29 °C (± 4.7 °C) (Naafs et al., 2018), and coincided with the evolution of mammals (e.g. rodents, dogs and pigs) during this time (Luterbacher, 2004). Therefore, these findings show that the emergence of *L. interrogans* coincided with optimal climatic conditions and the evolution of suitable mammalian hosts.

To estimate time divergence and investigate genetic diversity among *L. interrogans* strains, the concatenated sequence of seven housekeeping genes from the MLST scheme (Boonsilp et al., 2013) was used

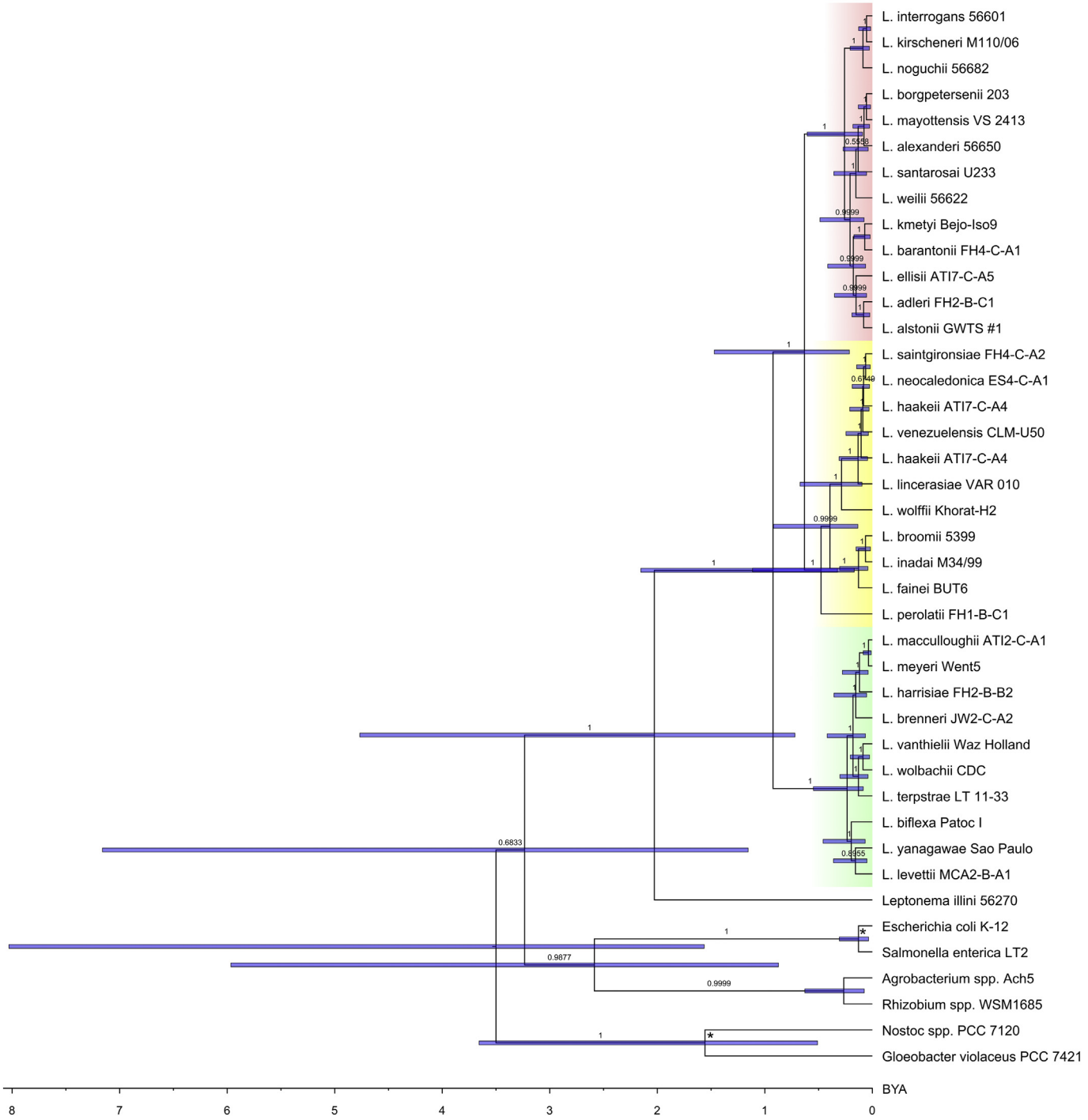

Fig. 1. Chronogram constructed using the Bayesian Markov chain Monte Carlo (MCMC) method to show the divergence time of *Leptospira interrogans*. The chronogram is fixed with two calibration time points using the emergence time of *Nostoc* spp. and divergence time of *Escherichia coli* and *Salmonella enterica* (marked with an asterisk). The emergence time of *Gloeobacter violaceus* was rooted at around 3.5 BYA (~3.5–2.7 BYA) (David and Alm, 2011; Falcon et al., 2010).The divergence time of *Leptospira* species occurred around 923 MYA with separation into three groups; pathogen (red), intermediate (yellow) and non-pathogen (green). *L. interrogans* was estimated to appear around 53.7 MYA. The chronology tree was supported by the previous proposal of divergence time for *Agrobacterium* spp. and *Rhizobium* spp. at around 266 MYA (~415–110 MYA) (Ochman and Wilson, 1987). Branch labels indicate the posterior probability values and blue bars represent node age with a 95% highest posterior density (HPD) interval. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to construct a phylogenetic tree with a combination of time of isolation from 1929 to 2014. All *L. interrogans* strains in this analysis ($n = 45$) shared a common ancestor (CA) that was present around the year 1350 CE. At this time the branch was separated into two lineages; Lineage 1 and Lineage 2. The Thai dog strains, where "Thai" refers to the Thai *Leptospira* isolates CUDO5 and CUDO8, were descended from Lineage 2. The CA of strain CUDO5 was estimated to have emerged around year 1840 CE, whereas strain CUDO8 shared a CA with strain UI 12758 isolated from human in Laos, and arose approximately in the year 1920 CE. Strain CUDO8 tended to have genome expansion (net gene gain > net gene loss) while strain CUDO5 possibly had a balanced genome associated with an equal net gain and loss of gene families

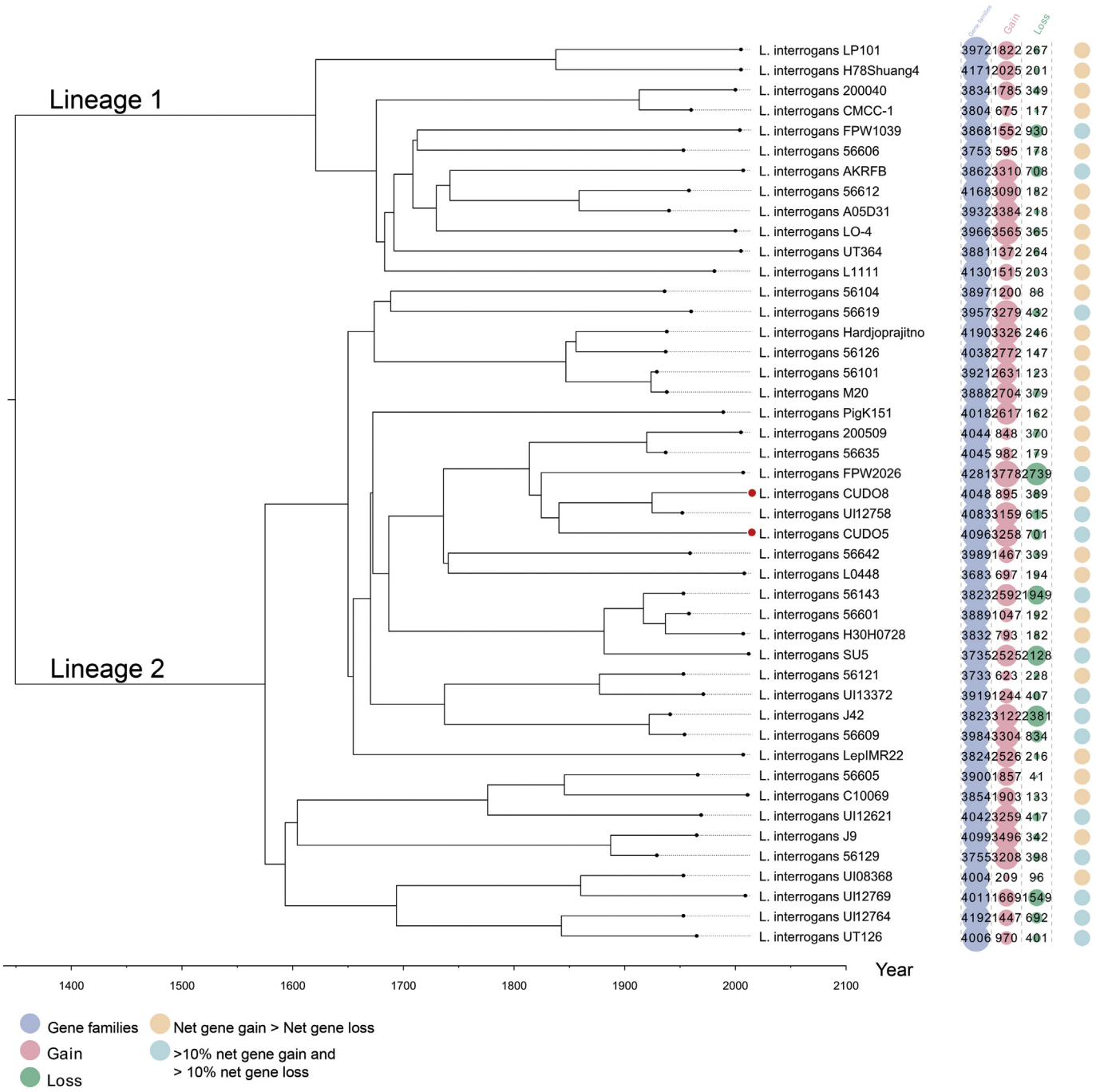

Fig. 2. Bayesian phylogenetic tree of *L. interrogans* strains with estimate divergence time mapping with gene gains and losses in each taxon. The tree was constructed using seven concatenated house-keeping genes from the MLST scheme. The common ancestor (CA) of all *L. interrogans* diverged into two lineages (Linage 1 and Linage 2) around the year 1350 CE. Lineage 2 was the CA of *L. interrogans* serovar Paidjan strain CUDO5 and serovar Dadas strain CUDO8 (red dot). The blue, pink, aquamarine, peach and light blue colored-circles indicate number of gene families, gene families gained, gene families lost, net gene gain > net gene loss and > 10% of net gene gain and > 10% of net gene loss in each *L. interrogans* strain, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(> 10% gene gain and > 10% gene loss) (Fig. 2).

After divergence, the CA of *L. interrogans* genome Lineage 2 had an over-representation of gene family encoding for signal transduction and ribosome (Table 2). Signal transduction might allow *Leptospira* to sense, respond and adapt in different ecological niches and hosts (Xu et al., 2016), whereas over-representation of ribosome was possibly associated with increased cell activity and rate of growth, with enhanced protein synthesis and replication (Levin et al., 2017). Notably, Thai dog strains that were isolated from the urine of asymptomatic dogs had gained gene families (over-represented) in the vitamin B biosynthetic process (cobalamin for strain CUDO5 and riboflavin for strain CUDO8), cell adhesion and external encapsulating structure (Table 2). Cobalamin (vitamin B12) and riboflavin (vitamin B2) are essential factors for leptospiral growth and survival (Herman et al., 2016). Moreover, *L. interrogans* possessed a nearly complete set of genes involved in the cobalamin biosynthetic pathway, suggesting that these pathogens had adaptations involving *de novo* cobalamin production to facilitate survival in nutrient-limiting sources such as in convoluted tubules of the

Table 2
Representative of gene orthologous (GO) enrichment analysis in this study.

Emergence[a]	GO term[b]	GO:ID	Description	Count	Size	*q*-value	Presentation[c]
CA (L2)	BP	GO:0007165	Signal transduction	53	56	0.0321008	Over (Gain)
		GO:0023052	Signaling	53	56	0.0321008	Over (Gain)
		GO:0042221	Response to chemical	33	34	0.0363542	Over (Gain)
	CC	GO:0030529	Ribonucleoprotein complex	50	50	0.0017218	Over (Gain)
		GO:0005840	Ribosome	50	50	0.0017218	Over (Gain)
Paidjan CUDO5	BP	GO:0006766	Vitamin metabolic process	13	44	0.0441205	Over (Gain)
		GO:0009110	Vitamin biosynthetic process	13	44	0.0441205	Over (Gain)
		GO:0009236	Cobalamin biosynthetic process	6	16	0.0445599	Over (Gain)
		GO:0009235	Cobalamin metabolic process	6	16	0.0445599	Over (Gain)
	CC	GO:0044462	External encapsulating structure	13	39	0.0390022	Over (Gain)
		GO:0031975	Envelope	14	55	0.0441205	Over (Gain)
		GO:0030313	Cell envelope	13	52	0.0445599	Over (Gain)
Dadas CUDO8	BP	GO:0022610	Biological adhesion	4	5	0.0314982	Over (Gain)
		GO:0007155	Cell adhesion	3	4	0.0486509	Over (Gain)
		GO:0006766	Vitamin metabolic process	14	43	0.0486509	Over (Gain)
		GO:0009110	Vitamin biosynthetic process	14	43	0.0486509	Over (Gain)
		GO:0009231	Riboflavin biosynthetic process	4	8	0.0486509	Over (Gain)
		GO:0006771	Riboflavin metabolic process	4	8	0.0486509	Over (Gain)
	CC	GO:0044462	External encapsulating structure	14	39	0.0314982	Over (Gain)
		GO:0031975	Envelope	15	55	0.0486509	Over (Gain)
		GO:0030313	Cell envelope	14	52	0.0503742	Over (Gain)

[a] The abbreviations for emergence terms are defined as: CA (L2), Common ancestor of L. *interrogans* lineage 2; Paidjan CUDO5, *L. interrogans* serovar Paidjan strain CUDO5; Dadas CUDO8, *L. interrogans* serovar Dadas strain CUDO8.
[b] GO term: BP, Biological process; CC, Cellular component.
[c] Over (Gain), Over-presentation in gene gained group.

kidneys (Fouts et al., 2016). Cell adhesion and external encapsulating structure (i.e. biofilm) play an important role for renal colonization and immune evasion of pathogenic *Leptospira* (Monahan et al., 2009). Over-representation of this gene family might enhance adhesive and protective niches in renal tubule (Yamaguchi et al., 2018), and be involved in adaption in chronic infections. The details of the adaptation need more detailed analysis.

4. Conclusion

The chronology tree suggested that *L. interrogans* emerged around 53.7 MYA, coinciding with optimal climatic conditions and the evolution of suitable mammalian hosts during the Paleogene period. Gene families encoding for vitamin B2, B12, cell adhesion and external encapsulating structure were enriched in *L. interrogans* isolates from asymptomatic dogs and might facilitate survival and colonization in the kidneys, leading to chronic infection. Our study helps to understanding the history of emergence of *L. interrogans* and genetic adaptations in strains causing chronic infections.

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Appendix A. Supplementary data

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References

Adler, B., de la Pena Moctezuma, A., 2010. *Leptospira* and leptospirosis. Vet. Microbiol. 140, 287–296.
Battistuzzi, F.U., Feijao, A., Hedges, S.B., 2004. A genomic timescale of prokaryote evolution: insights into the origin of methanogenesis, phototrophy, and the colonization of land. BMC Evol. Biol. 4, 44.
Beissbarth, T., Speed, T.P., 2004. GOstat: find statistically overrepresented gene ontologies within a group of genes. Bioinformatics 20, 1464–1465.
Boonsilp, S., Thaipadungpanit, J., Amornchai, P., Wuthiekanun, V., Bailey, M.S., Holden, M.T., Zhang, C., Jiang, X., Koizumi, N., Taylor, K., Galloway, R., Hoffmaster, A.R., Craig, S., Smythe, L.D., Hartskeerl, R.A., Day, N.P., Chantratita, N., Feil, E.J., Aanensen, D.M., Spratt, B.G., Peacock, S.J., 2013. A single multilocus sequence typing (MLST) scheme for seven pathogenic *Leptospira* species. PLoS Negl. Trop. Dis. 7, e1954.
Costa, F., Hagan, J.E., Calcagno, J., Kane, M., Torgerson, P., Martinez-Silveira, M.S., Stein, C., Abela-Ridder, B., Ko, A.I., 2015. Global morbidity and mortality of leptospirosis: A systematic review. PLoS Negl. Trop. Dis. 9, e0003898.
David, L.A., Alm, E.J., 2011. Rapid evolutionary innovation during an Archaean genetic expansion. Nature 469, 93–96.
Evangelista, K., Coburn, J., 2010. *Leptospira* as an emerging pathogen: a review of its biology, pathogenesis and host immune responses. Future Microbiol 5, 1413–1425.
Falcon, L.I., Magallon, S., Castillo, A., 2010. Dating the cyanobacterial ancestor of the chloroplast. ISME J 4, 777–783.
Fouts, D.E., Matthias, M.A., Adhikarla, H., Adler, B., Amorim-Santos, L., Berg, D.E., Bulach, D., Buschiazzo, A., Chang, Y.F., Galloway, R.L., Haake, D.A., Haft, D.H., Hartskeerl, R., Ko, A.I., Levett, P.N., Matsunaga, J., Mechaly, A.E., Monk, J.M., Nascimento, A.L., Nelson, K.E., Palsson, B., Peacock, S.J., Picardeau, M., Ricaldi, J.N., Thaipandungpanit, J., Wunder Jr., E.A., Yang, X.F., Zhang, J.J., Vinetz, J.M., 2016. What makes a bacterial species pathogenic?:comparative genomic analysis of the genus *Leptospira*. PLoS Negl. Trop. Dis. 10, e0004403.
Herman, H.S., Mehta, S., Cardenas, W.B., Stewart-Ibarra, A.M., Finkelstein, J.L., 2016. Micronutrients and leptospirosis: a review of the current evidence. PLoS Negl. Trop. Dis. 10, e0004652.
Kurilung, A., Keeratipusana, C., Suriyaphol, P., Prapasarakul, N., 2018. Draft genome sequence of a *Leptospira interrogans* strain isolated from the urine of an asymptomatic dog in Thailand. Genome Announc 6.
Kurilung, A., Keeratipusana, C., Suriyaphol, P., Hampson, D.J., Prapasarakul, N., 2019. Genomic analysis of *Leptospira interrogans* serovar Paidjan and Dadas isolates from carrier dogs and comparative genomic analysis to detect genes under positive selection. BMC Genomics 20, 168.
Levin, B.R., McCall, I.C., Perrot, V., Weiss, H., Ovesepian, A., Baquero, F., 2017. A numbers game: ribosome densities, bacterial growth, and antibiotic-mediated stasis and death. MBio 8.
Luterbacher, H.P., 2004. The Paleogene period. A geologic time scale 384–408.
Monahan, A.M., Callanan, J.J., Nally, J.E., 2009. Review paper: host-pathogen interactions in the kidney during chronic leptospirosis. Vet. Pathol. 46, 792–799.
Naafs, B.D.A., Rohrssen, M., Inglis, G.N., Lähteenoja, O., Feakins, S.J., Collinson, M.E., Kennedy, E.M., Singh, P.K., Singh, M.P., Lunt, D.J., Pancost, R.D., 2018. High temperatures in the terrestrial mid-latitudes during the early Palaeogene. Nat. Geosci. 11, 766–771.
Ochman, H., Wilson, A.C., 1987. Evolution in bacteria: evidence for a universal substitution rate in cellular genomes. J. Mol. Evol. 26, 74–86.
Picardeau, M., 2017. Virulence of the zoonotic agent of leptospirosis: still terra incognita? Nat. Rev. Microbiol. 15, 297–307.

Thibeaux, R., Girault, D., Bierque, E., Soupe-Gilbert, M.E., Rettinger, A., Douyere, A., Meyer, M., Iraola, G., Picardeau, M., Goarant, C., 2018a. Biodiversity of environmental *Leptospira*: improving identification and revisiting the diagnosis. Front. Microbiol. 9, 816.

Thibeaux, R., Iraola, G., Ferres, I., Bierque, E., Girault, D., Soupe-Gilbert, M.E., Picardeau, M., Goarant, C., 2018b. Deciphering the unexplored *Leptospira* diversity from soils uncovers genomic evolution to virulence. Microb. Genom. 4.

Xu, Y., Zhu, Y., Wang, Y., Chang, Y.F., Zhang, Y., Jiang, X., Zhuang, X., Zhu, Y., Zhang, J., Zeng, L., Yang, M., Li, S., Wang, S., Ye, Q., Xin, X., Zhao, G., Zheng, H., Guo, X., Wang, J., 2016. Whole genome sequencing revealed host adaptation-focused genomic plasticity of pathogenic *Leptospira*. Sci. Rep. 6, 20020.

Yamaguchi, T., Higa, N., Okura, N., Matsumoto, A., Hermawan, I., Yamashiro, T., Suzuki, T., Toma, C., 2018. Characterizing interactions of *Leptospira interrogans* with proximal renal tubule epithelial cells. BMC Microbiol. 18, 64.