Dual control of host actin polymerization by a *Legionella* effector pair

Authors

M. Pillon[1], C. Michard[1#], N. Baïlo[1#], J. Bougnon[1,2], K. Picq[3], O. Dubois[1], C. Andrea[1], L. Attaiech[3], V. Daubin[2], S. Jarraud [1], E. Kay[1], P. Doublet[1]*

SUPPLEMENTAL DATA LEGENDS

Supplementary Data S1 – List of all cells, bacteria strains used in this study.

Supplementary Data S2 – List of plasmids used in this study.

Supplementary Data S3 – List of oligonucleotides used to realize mutant strains of *Legionella* as well as for cloning.

Supplementary Data S4 – Accession numbers of genomes used for evolutionary analyses.

Supplementary Data S5 – Fasta files of sequencing data of *ΔlegK2* and *ΔlegK2/ΔvipA Legionella pneumophila* strains

Supplementary Data S6 – LegK2 and VipA mutations do not alter axenic growth of *L. pneumophila* Paris strains. Axenic growth kinetics of *L. pneumophila* Paris WT, Δ*dotA,* Δ*legK2,* Δ*vipA,* Δ*legK2/*Δ*vipA,* mutant strains transformed with mCherry-expressing plasmids. AYE broth was seeded with 0.1 x10^9 bacteria/mL and then plated in a 96well-Greiner plate, with black wells and transparent flat bottom. OD_{600nm}absorbance was measured every 30min at 595 nm on an Infinite M200 microplate reader (Tecan).

Supplementary Data S7 – VipA and LegK2 are produced at the onset of the stationary phase in axenic medium at 30°C. Luminescence emission fromchromosomal translational fusions of *vipA::luc***(A)** and *legK2::luc***(B)**in *L. pneumophila* Paris strain grown in AYE medium at 30°C. Luminescence emission of VipA-Luc and LegK2-Luc fusion proteins is maximal at the onset of the stationary growth phase (growth at 30°C measured by $OD_{600,}$ dashed red line), with VipA-Luc slightly ahead of LegK2-Luc.The data shown are representative of 3 independent clones of each fusion.

Supplementary Data S8 – Mass Spectrometry data following protein expression in *Legionella pneumophila* Paris WT at OD_{600nm} 1, 2, 3, 4 and 5. VipA accumulates in *L. pneumophila* up to DO_{600}= 4. Three independent cultures of *L. pneumophila* Paris WT were grown in liquid medium AYE (at 30°C). At the desired OD_{600} (1, 2, 3, 4 and 5), samples were collected, and their protein content was analysed by mass spectrometry after sample-specific labelling (see Material and methods for details). The graphs show the quantity detected at each OD_{600} or as a ratio compared to the quantity at OD_{600}. The RocC protein is used as a control as it is detected at the same range of quantity as VipA and its production during growth was previously studied by Western-blot [23]. Of note the RocC pattern of production detected by mass spectrometry corresponds to the one previously obtained by Western-blot[23].

Supplementary Data S9 - LegK2/VipA effector pairs are restricted and strongly conserved in the *L. pneumophila* species. Phylogenetic tree representing the phylogenetic diversity of *Legionella* genomes displaying either LegK2 (blue green squares) or VipA genes (purple squares). The squares are filled when the genes have been found in corresponding genome and empty when genes have not been detected.

SUPPLEMENTARY DATA

Strains		
Names	**Genotypes**	**References**
Dictyostelium discoideum		
DBS0236184	Calnexin-GFP	Müller-Taubenberger et al. (2001)
DBS0235534	Ax2-214	Watts & Ashworth, (1970)
Acanthamoeba castellanii		
Environmental isolate		
Mammalian cells		
HeLa cells		Inserm U1111, Lyon, France
U937	Human monocytes ATCC CRL1593.2	Sundstrom et al. (1976)
Legionella pneumophila		
WT	Virulent *L. pneumophila* strain Paris CIP 107629	Cazalet et al. (2004)
ΔdotA	Paris *lpp2740*::Km	Cazalet et al. (2004)
ΔlegK2	Paris Δ*lpp2076* (scar-free deletion of lpp2076)	This study
ΔvipA	Paris Δ*lpp0457* (scar-free deletion of lpp0457)	This study
ΔlegK2ΔvipA	Paris Δ*lpp2076* Δlpp0457 (scar-free deletion of lpp2076 and lpp0457)	This study
legK2::luc		This study
vipA::luc		This study
legK2::gfp		This study
Escherichia coli		
DH5α	*endA1 hsdR17 supE4, thi-1 recA1 gyrArelA, Δlac*	Laboratory
BL21	B *dcmompThsdS(rB-mB-) gal*	Laboratory
XL1 Blue	*endA1 gyrA96(nalR) thi-1 recA1 relA1 lac glnV44* F'[//Tn10 *proAB+ lacIqΔ(lacZ)*M15] *hsdR17*(rK- mK+)	Stratagene

SUPPLEMENTARY DATA S1

Plasmids		
Names	**Characteristics**	**References**
Donor vectors for Gateway cloning		
pDONR^{TM}207	Donor Gateway vector	Invitrogen
pDONR^{TM}207-*vipA*	Donor Gateway vector with insertion of lpp0457 gene	This study
Mammalian cells expression vectors for Gateway cloning		
pDEST27	Expression Gateway vector for mammalian cells allowing overexpression of GST-tagged protein	Invitrogen
pDEST27-*legK2*	pDEST27 with insertion of lpp2076 gene for expression of GST-LegK2 fusion protein	This study
pDEST27-*legK2*$_{K112M}$	pDEST27 with insertion of lpp2076 gene for expression of GST-LegK2$_{K112M}$ dead kinase-mutant fusion protein	This study
pCI-Neo3Flag	Expression Gateway vector for mammalian cells allowing overexpression of Flag-tagged protein	Invitrogen
pCI-Neo3Flag-*ArpC1b*	pCI-Neo3Flag with insertion of ARPC1B human gene for expression of Flag-ARPC1B fusion protein	(Michard et al. 2015)
peGFP-Nterm	Expression Gateway vector for mammalian cells allowing overexpression of Nterminal GFP-tagged protein	INSERM U1111, Lyon, France
peGFP-Cterm	Expression Gateway vector for mammalian cells allowing overexpression of Nterminal GFP-tagged protein	INSERM U1111, Lyon, France
peGFP-N-*vipA*	peGFP-Nterm with insertion of lpp0457 gene for expression of VipA-GFP fusion protein	This study
peGFP-C-*vipA*	peGFP-Cterm with insertion of lpp0457 gene for expression of GFP-VipA fusion protein	This study
Bacteria expression vectors		
pXDC50	Expression plasmid for *L. pneumophila* allowing expression of mCherry fluorescent protein	(Hervet et al. 2011)
pXDC50-lpp2076	Expression plasmid for *L. pneumophila* allowinginductible expression of mCherry fluorescent protein and expression of LegK2 under control of its own promoter	This study
pXDC61	Expression plasmid for *L. pneumophila* allowing overexpression of β-Lactamase	Charpentier et al. (2009)
pXDC61-*legK2*	Expression plasmid for *L. pneumophila* allowing overexpression of β-Lactamase-LegK2 fusion protein	This study
pXDC61-*vipA*	Expression plasmid for *L. pneumophila* allowing overexpression of β-Lactamase-VipA fusion protein	This study
pGEX-*legK2*	Overproduction and *in vitro* purification of GST-LegK2	This study
pQE30-*vipA*	Overproduction and *in vitro* purification of GST-LegK2	This study

SUPPLEMENTARY DATA S2

Primers		
Names	**Sequences**	**Description**
1_vipA fwd	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAatgcctatcagtaatgcc ttt	*vipA* insertion in pDONR207 vector for peGFP-C-VipA
2_vipA rev	GGGGACCACTTTGTACAAGAAAGCTGGGTTgagatttttttttttcgacggt agtg	
3_vipA fwd	GGGGACCACTTTGTACAAGAAAGCTGGGTTgagatttttttttttcgacggt agtg	*vipA* insertion in pDONR207 vector for peGFP-N-VipA
4_vipA rev	GGGGACCACTTTGTACAAGAAAGCTGGGTTgagatttttttttttcgacggt agtg	
5_legK2 fwd	attggggaagcggtacccgggtttattacataaatttgaagga	*legK2* insertion in pXDC61 vector in XmaI site
6_legK2 rev	tatctagaggatccccgggttttagcttgggcctcgcat	
7_vipA fwd	cattggggaagcggtacccggcctatcagtaatgcctttcttaa	*vipA* insertion in pXDC61 vector in XmaI site
8_vipA rev	tatctagaggatccccggctagagatttttttttttcgacggta	
9_P1 vipA	TTCAACTCATCCTAGCATTG	*vipA* inactivation in *Legionella* by KanMazF technique
10_P2 vipA	CAGCAATATGATATTCTGGC	
11_P3 vipA	GGCCCAATTCGCCCTATAGTGAGTCGATGGATGGTCAAGCATTAT C	
12_P4 vipA	GGGTTTGCTCGGGTCGGTGGCATATGGTGCTCTATGATACTGACA TC	
13_P5 vipA	GATGTCAGTACATAGAGCACATGGATGGTCAAGCATTATC	
14_P6 vipA	GATAATGCTTGACCATCCATGTGCTCTATGATACTGACATC	
19_P1 legK2	TTACCAATGTAATGAGACATCG	*legK2* inactivation in *Legionella* by KanMazF technique
20_P2 legK2	GGCCCAATTCGCCCTATAGTGAGTCGtcttgaggtagaggttgttcc	
21_P3 legK2	GGGTTTGCTCGGGTCGGTGGCATATGCTGCAAATCAAGATAAGCA ACC	
22_P4 legK2	ATAGCATGCACAACTTTGATATCAAGCATCC	
23_P5 legK2	GGTTGCTTATCTTGATTTGCAGtcttgaggtagaggttgttcc	
24_P6 legK2	GGAACAACCTCTACCTCAAGACTGCAAATCAAGATAAGCAACC	
vipA linker REV	GCTGCCCCCACCTCCGAGATTTTTTTTTTCGACGGTAGTG	Insertion of gene *luc* for translational fusion by kanMazF technique
luc REV	CAATTTGGGCTTTCCGCCCTTC	
pLLA01_TRAD FW	GGAGGTGGGGGCAGCAGATCCGCCAAAAAC ATAAAGAAAGG	
lpp0457 trad after luc	GAAGGGCGGAAAGCCCAAATTGTAGTTCAGCCAATTTCTGTTCC	
legK2linker REV	GCTGCCCCCACCTCCCCTTGGGCCTCGCATCAATGAAG	
legK2 fusion luc after	GAAGGGCGGAAAGCCCAAATTGTAAAAAACGTCACTGAAGTGTT AG	
SD ATG lpp2076	CATAATGCTCTCCTATAAGCC	Insertion of gene *sfGFP*for translational fusion by kanMazF technique
SD ATG lpp2076 SfGFP	GGCTTATAGGAGAGCATTATGTCTAAAGGTGAAGAACTGTTC	
Comp start lpp2076-end sfGFP	CTTCAAATTTATGTAATAAACTTTGTAGAGCTCATCCATGCC	
start lpp2076	GTTTATTACATAAATTTGAAG	
25_Fwd pGEX	GGGCCCCTGGAACAGAAC	Construction of pGEX-l*egK2* plasmid by PCR SLIC
26_Rev pGEX	CTGGGATCCCCGAATTCCC	
27_Fwd legK2 pGEX	GTTCTGTTCCAGGGGCCCgtttattacataaatttgaaggaac	
28_Rev legK2 pGEX	GGGAATTCGGGGATCCCAGttagcttgggcctcgcatc	
31_Fwd vipA pQE30	GCTCGCATGCGGATCctagagatttttttttttcgacggtag	Construction of pQE30-*vipA* plasmid by BamHI digestion SLIC
32_Rev vipA pQE30	TCACCATCACGGATCccctatcagtaatgcctttcttaagtt	

SUPPLEMENTARY DATA S3

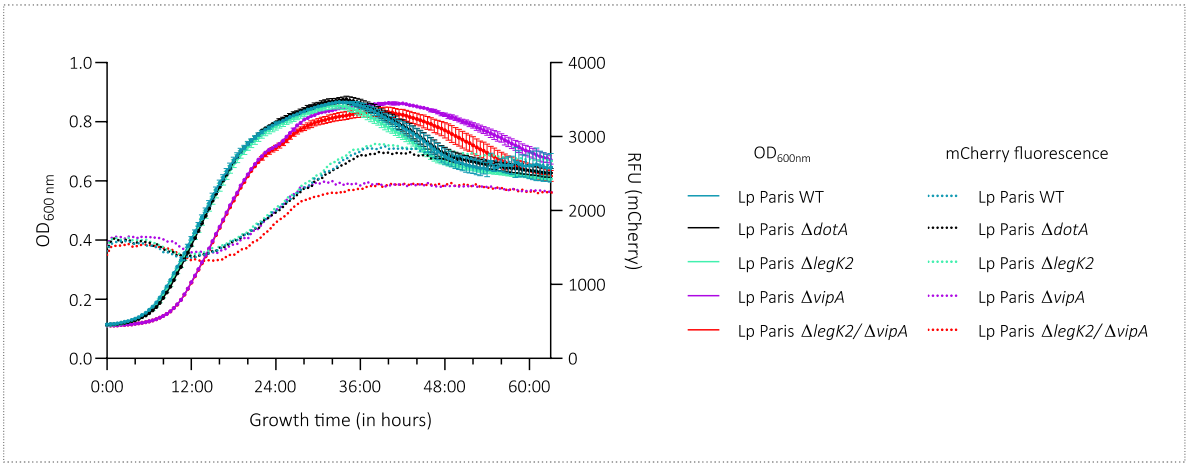
Accession #	Strain names	Accession #	Strain names
GCF000621365.1	*L. geestiana* DSM 21217	**GCF001468085.1**	*L. waltersii*ATCC51914
GCF001467645.1	*L. geestiana* ATCC49504	**GCF900187095.1**	*L. waltersii* NCTC13017
GCF001468165.1	*L. spiritensis* Mt.St.Helens-9	**GCF000770585.1**	*L. norrlandica* LEGN
GCF900186965.1	*L. spiritensis* NCTC11990	**GCF001582625.1**	L. *pneumophila* FS_4_1103abu
GCF001467615.1	*L. erythra* SE-32A-C8	**GCF000586195.1**	L. *pneumophila* subsp. fraseri ATCC35251
GCF001468125.1	*L. rubrilucens* WA-270A-C2	**GCF000586315.1**	L. *pneumophila* subsp. fraseri ATCC33156
GCF001467505.1	*L. birminghamensis* CDC#1407-AL-14	**GCF001639045.1**	*L. pneumophila*PtVFX/2014
GCF001467975.1	*L. quinlivanii* CDC#1442-AUS-E	**GCF001582535.1**	*L. pneumophila* SZ069
GCF001467025.1	*L. brunensis* ATCC43878	**GCF001886835.1**	*L. pneumophila* subsp. fraseri Dallas 1E
GCF000953655.1	*L. hackeliae* ATCC35250	**GCF001582295.1**	*L. pneumophila* SZ099
GCF001467705.1	*L. hackeliae* 798-PA-H	**GCF001886795.1**	*L. pneumophila*subsp. fraseri Detroit-1
GCF002240035.1	*L. clemsonensis* CDC-D5610	**GCF001583645.1**	*L. pneumophila* SZ026
GCF001691475.1	*L. jamestowniensis* 974010_12	**GCF001600905.1**	*L. pneumophila* NY24
GCF001467745.1	*L. jamestowniensis* JA-26-G1-E2	**GCF000586255.1**	L. pneumophil*a subsp. pascullei*ATCC33737
GCF900187355.1	*L. lansingensis* NCTC12830	**GCF001582645.1**	*L. pneumophila* WD_4_1102b-36
GCF001467795.1	*L. lansingensis* ATCC49751	**GCF000953935.1**	*L. pneumophila* Ymt294
GCF001467765.1	*L. jordanis* BL-540	**GCF001582405.1**	*L. pneumophila* FS_10_1101a-3
GCF001648675.1	*L. jordanis* ATCC33623	**GCF000092625.1**	*L. pneumophila* 2300/99 Alcoy
GCF000308315.1	*L. tunisiensis*LegM	**GCF000092545.1**	*L. pneumophila* str. Corby
GCF001467625.1	*L. feeleii* WO-44C	**GCF000823425.1**	*L. pneumophila* 12_4117
GCF001648615.1	*L. feeleii*ATCC35072	**GCF000586095.1**	*L. pneumophila subsp. pneumophila* ATCC43703
GCF000621525.1	*L. fairfieldensis* ATCC49588	**GCF001582385.1**	*L. pneumophila* SH135
GCF000756695.1	*L. massiliensis*	**GCF900063795.1**	*L. pneumophila* 2531STDY5467313
GCF000756815.1	*L. massiliensis*LegA	**GCF000586075.1**	*L. pneumophila subsp. pneumophila* ATCC43736
GCF001467585.1	*L. drozanskii* ATCC700990	**GCF001592705.1**	*L. pneumophila subsp. pneumophila* Toronto-2005
GCF001467895.1	*L. nautarum* ATCC49506	**GCF900119765.1**	*L. pneumophila* ST62
GCF900167045.1	*L. maceachernii* ATCC35300	**GCF900053665.1**	*L. pneumophila* 2531STDY5467288
GCF002085735.1	*T. micdadei* NZ2016	**GCF900119755.1**	*L. pneumophila* ST23
GCF002085715.1	*T. micdadei* NZ2015	**GCF000048645.1**	*L. pneumophila* str. Paris
GCF001467055.1	*L. adelaidensis* 1762-AUS-E	**GCF001583655.1**	*L. pneumophila* SH003
GCF001467825.1	*L. londiniensis* ATCC 49505	**GCF900060375.1**	*L. pneumophila* 2531STDY5467394
GCF000512715.1	*L. oakridgensis* RV-2-2007	**GCF000695015.1**	*L. pneumophila* TUM 13948
GCF001648605.1	*L. oakridgensis* ATCC 33761	**GCF001582555.1**	*L. pneumophila* TL-12
GCF001467785.1	*L. israelensis*Bercovier 4	**GCF000586235.1**	*L. pneumophila subsp. pneumophila* ATCC33823
GCF000162755.2	*L. drancourtii* LLAP12	**GCF000950745.1**	*L. pneumophila* Twr292
GCF001465875.1	*L. saoudiensis* LH-SWC	**GCF001582225.1**	*L. pneumophila* ATCC35096
GCF001467695.1	*L. gratiana* Lyon 8420412	**GCF000306865.1**	*L. pneumophila subsp. pneumophila* Lorraine
GCF001467545.1	*L. cincinnatiensis* CDC#72-OH-14	**GCF900050185.1**	*L. pneumophila* 2531STDY5467306
GCF001468135.1	*L. santicrucis* SC-63-C7	**GCF001582215.1**	*L. pneumophila* ATCC43130

GCF000621685.1	*L. sainthelensi*ATCC35248	**GCF001601075.1**	*L. pneumophila* NY27
GCF001468105.1	*L. sainthelensi*Mt.St.Helens-4	**GCF900062515.1**	*L. pneumophila* 2531STDY5467417
GCF000091785.1	*L. longbeachae* NSW150	**GCF000048665.1**	*L. pneumophila* str. Lens
GCF000176095.1	*L. longbeachae* D-4968	**GCF000586355.1**	*L. pneumophila subsp. pneumophila* ATCC33154
GCF000701265.1	*L. wadsworthii*ATCC33877	**GCF001582565.1**	*L. pneumophila* ATCC33154
GCF001467945.1	*L. parisiensis* PF-209-C-C2	**GCF900057235.1**	*L. pneumophila* 2531STDY5467316
GCF001736145.1	*L. parisiensis*DSM 19216	**GCF900092465.1**	*L. pneumophila* Lpm7613
GCF001468035.1	*L. tucsonensis* ATCC49180	**GCF001753125.1**	*L. pneumophila* C11_O
GCF000333755.1	*L. anisa*Linanisette	**GCF001752705.1**	*L. pneumophila* E10_P
GCF002082905.1	*L. anisa*FDAARGOS_200	**GCF001753265.1**	*L. pneumophila* E2_N
GCF001468065.1	*L. steigerwaltii* SC-18-C9	**GCF000008485.1**	*L. pneumophila subsp. pneumophila* str. Philadelphia 1
GCF000621385.1	*L. cherrii* DSM 19213	**GCF001601055.1**	*L. pneumophila* NY17
GCF001467035.1	*L. cherrii*ORW	**GCF900058585.1**	*L. pneumophila* 2532STDY5467522
GCF001468005.1	*L. steelei* IMVS3376	**GCF900062435.1**	*L. pneumophila* 2532STDY5467504
GCF000953135.1	*L. fallonii* LLAP-10	**GCF900062395.1**	*L. pneumophila* 2532STDY5467530
GCF000373765.1	*L. shakespearei* DSM 23087	**GCF000823645.1**	*L. pneumophila* 12_4904
GCF001468025.1	*L. shakespearei* ATCC49655	**GCF900055205.1**	*L. pneumophila* 2532STDY5467502
GCF001467535.1	*L. worsleiensis* ATCC49508	**GCF900062385.1**	*L. pneumophila* 2532STDY5467524
GCF000423305.1	*L. moravica* DSM 19234	**GCF900053395.1**	*L. pneumophila* 2532STDY5467482
GCF001467865.1	*L. moravica* ATCC43877	**GCF000826165.1**	*C. burnetii* Cb171_QLYMPHOMA
GCF001467955.1	*L. quateirensis* ATCC49507	**GCF000019865.1**	*C. burnetii* CbuG_Q212

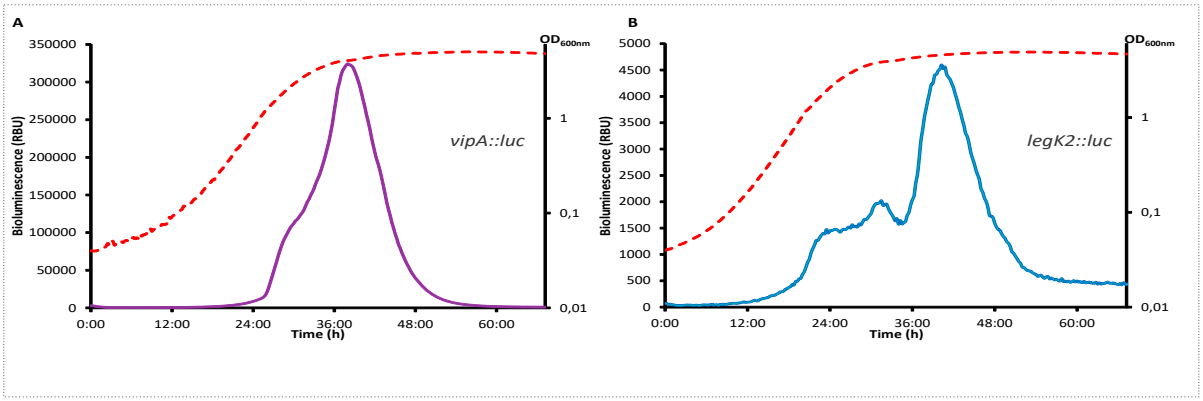
SUPPLEMENTARY DATA S4

Fasta files of sequencing data of *ΔlegK2* and *ΔlegK2/ΔvipA Legionella pneumophila* strains **-** RNA-seq and sequencing data have been deposited in European Nucleotide Archive database at EMBL-EBI (https://www.ebi.ac.uk/ena) under accession number PRJEB62121.

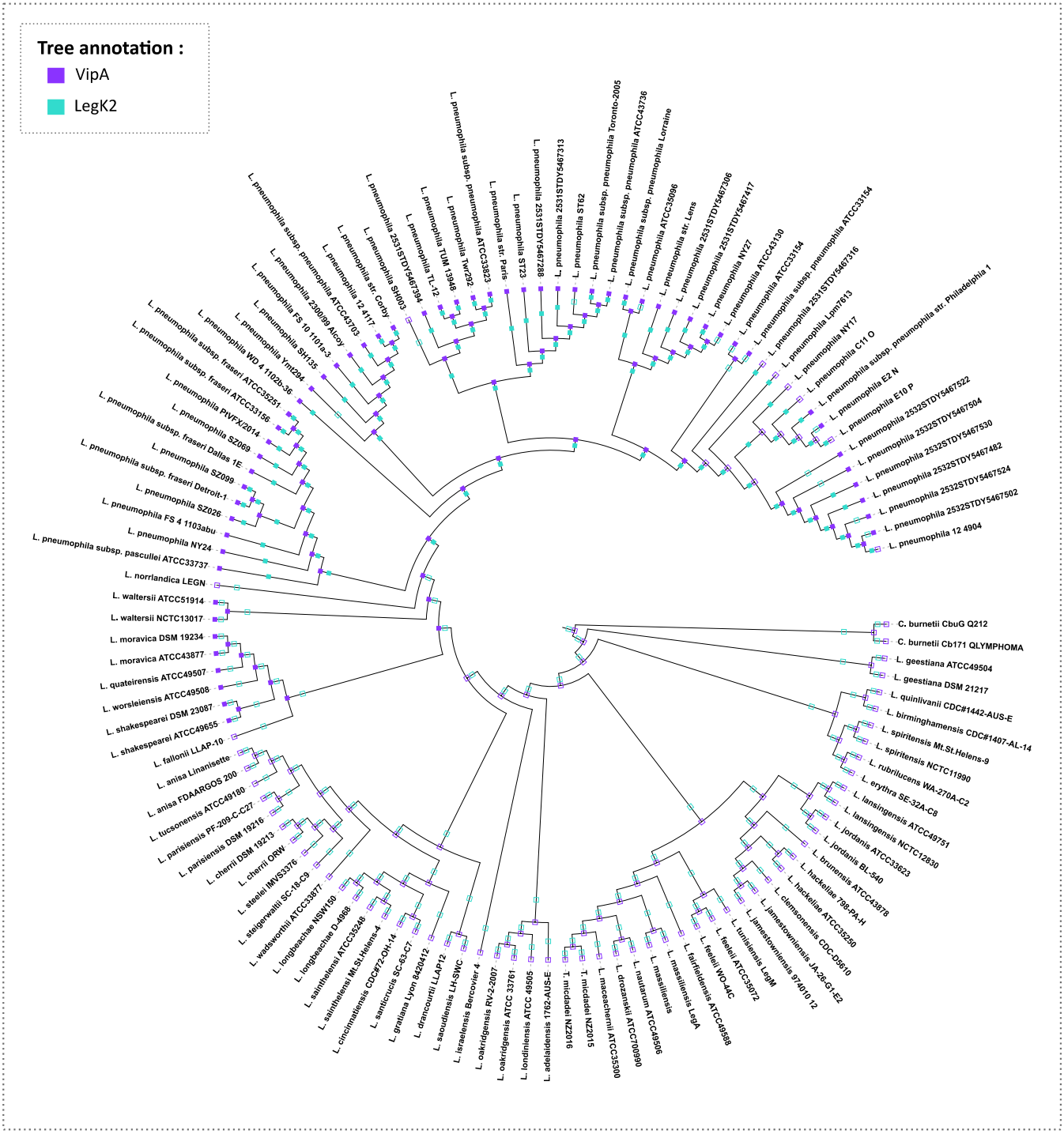
Supplementary Data S5



SUPPLEMENTARY DATA S6



SUPPLEMENTARY DATA S7



SUPPLEMENTARY DATA S9