Supplemental Table 1. List of homologs (>50% identity) and unique genes in VPIα, VPaIβ and VPaIγ.

A: VPaIα (VPaI-7) gene content and corresponding homologs in VPaIβ and VPaIγ

RIMD2210633 VPIα (VPaI-7) NCBI annotated	**MAVP-Q VPaIγ**	**TH3996 VPaIβ**	**MAVP-R VPaIβ**
VPA1310_-_hypothetical_protein			
VPA1311_-_hypothetical_protein			
VPA1312_-_hypothetical_protein	QPI_00008	PI_00006	RPI_00014
VPA1313_-_hypothetical_protein			
VPA1314_-_thermostable_direct_hemolysin_A (***tdh2***)	***tdh3***:QPI_00012		
VPA1315_-_hypothetical_protein			
VPA1316_-_transposase	QPI_00086		
VPA1317_-_hypothetical_protein			
VPA1318_-_hypothetical_protein	QPI_00088	PI_00083	
VPA1320_-_hypothetical_protein	QPI_00089		
VPA1319_-_IS1655_transposase			
VPA1321_-_cytotoxic_necrotizing_factor (***vopC***[1])			
VPA1322_-_zinc_finger_protein	QPI_00079	PI_00076	RPI_00086
VPA1323_-_hypothetical_protein			
VPA1324_-_hypothetical_protein	QPI_00080	PI_00077	RPI_00087
VPA1325_-_hypothetical_protein			
VPA1326_-_hypothetical_protein			
VPA1327_-_exoenzyme_T (***vopT***[1])			
VPA1328_-_hypothetical_protein			
VPA1329_-_TraA_protein			
VPA1330_-_hypothetical_protein			
VPA1331_-_*ospC2*			
VPA1332_-_transcriptional_regulator_(***vtrA)***			
VPA1333_-_hypothetical_protein	QPI_00042	PI_00037	RPI_00048
VPA1334_-_hypothetical_protein[2]			
VPA1335_-_type_III_secretion_apparatus_protein[2] ***(vscS2)***	QPI_00043	PI_ 00038	RPI_00049
VPA1336_-_hypothetical_protein (***vopZ***[1]***)***			
VPA1337_-_hypothetical_protein	QPI_00045	PI_00040	RPI_00051
VPA1338_-_ATPase_YscN (***vscN2***[2]***)***	QPI_00046	PI_00041	RPI_00052
VPA1339_-_type_III_secretion_system_EscC_protein (***vscC2***[2]***)***	QPI_00047	PI_00042	RPI_00053
VPA1340_-_hypothetical_protein			
VPA1341_-_Spa29,_component_of_the_Mxi-Spa_secretion_machinery (***vscT2***)	QPI_00049	PI_00044	RPI_00055
VPA1342_-_type_III_secretion_system_protein (***vscR2***[2]***)***	QPI_00050	PI_00045	RPI_00056
VPA1343_-_hypothetical_protein	QPI_00051	PI_00046	RPI_00057
VPA1344_-_hypothetical_protein			
VPA1345_-_hypothetical_protein (***vopW***[2])			
VPA1346_-_targeted_effector_protein_YopP (***vopA/P***[1])	QPI_00075	PI_00072	RPI_00082
VPA1347_-_hypothetical_protein			
VPA1348_-_transcriptional_activator (***vtrB)***	QPI_00052	PI_00048	RPI_00058
VPA1349_-_Type_III_secretion_protein_Spa33 (***vscQ2***[2]***)***	QPI_00053	PI_00049	RPI_00059
VPA1350_-_hypothetical_protein T3SS2[3]	QPI_00054	PI_00050	RPI_00060
VPA1351_-_hypothetical_protein			

VPA1352_-_hypothetical_protein			
VPA1353_-_outer_membrane_protein (***ompA***) [3]	QPI_00057	PI_00053	RPI_00063
VPA1354_-_type_III_secretion_system_EscU_protein (***vscU2***[2]**)**	QPI_00058	PI_00054	RPI_00064
VPA1355_-_type_III_secretion_system_EscV_protein (***vcrD2***[2]**)**	QPI_00059	PI_00055	RPI_00065
VPA1356_-_hypothetical_protein[3]	QPI_00060	PI_00056	RPI_00066
VPA1357_-_hypothetical_protein (***vopV***[1]**)**			
VPA1358_-_dimethyladenosine_transferase			
VPA1359_-_hypothetical_protein	QPI_00064	PI_00060	RPI_00070
VPA1360_-_hypothetical_protein[3]	QPI_00065	PI_00061	RPI_00071
VPA1361_-_hypothetical_protein (***vopD2***[2])	QPI_00066	PI_00062	RPI_00072
VPA1362_-_secreted_protein_EspD (***vopB2***[2])	QPI_00067	PI_00063	RPI_00073
VPA1363_-_chaperone[3]	QPI_00068	PI_00064	RPI_00074
VPA1364_-_hypothetical_protein [3]			
VPA1365_-_two-component_response_regulator	QPI_00069	PI_00066	RPI_00076
VPA1366_-_hypothetical_protein[3]			
VPA1367_-_type_III_secretion_system_lipoprotein_*eprK* (***vscJ2***[2])	QPI_00071	PI_00068	RPI_00078
VPA1368_-_hypothetical_protein	QPI_00072	PI_00069	RPI_00079
VPA1369_-_hypothetical_protein			
VPA1371_-_hypothetical_protein			
VPA1370_-_hypothetical_protein (***vopL***[1])			
VPA1372_-_hypothetical_protein			
VPA1373_-_hypothetical_protein			
VPA1374_-_transposase,_partial			
VPA1375_-_hypothetical_protein			
VPA1376_-_hypothetical_protein			
VPA1377_-_hypothetical_protein			
VPA1378_-_thermostable_direct_hemolysin_S (***tdh1***)			
VPA1379_-_transposase			
VPA1380_-_OspB_protein			
VPA1381_-_hypothetical_protein			
VPA1382_-_integral_membrane_protein			
VPA1383_-_hypothetical_protein			
VPA1384_-_hypothetical_protein			
VPA1385_-_integral_membrane_protein			
VPA1386_-_hypothetical_protein			
VPA1387_-_hypothetical_protein	QPI_00095	PI_00085	
VPA1388_-_hypothetical_protein	***csy2***:QPI_00096	PI_00087	
VPA1389_-_hypothetical_protein	***csy3***:QPI_00097	PI_00088	
VPA1390_-_hypothetical_protein	***csy4***:QPI_00098	PI_00089	
VPA1391_-_hypothetical_protein	QPI_00099	PI_00090	
VPA1392_-_hypothetical_protein	***vspR***:QPI_00100	PI_00091	
VPA1393_-_hypothetical_protein	QPI_00101	PI_00092	
VPA1394_-_transposition_protein			
VPA1395_-_transposase			
VPA1396_-_hypothetical_protein			

[1]Denotes an identified T3SS2 effector, [2]denotes T3SS2 apparatus gene, and [3]denotes gene required for T3SS structure formation (2, 4)

B: Genes in VPaIβ with corresponding homologs in VPaIγ, absent or divergent in VPaIα

TH3996 PI VPaIβ	**Annotation**	**MAVP-Q VPaIγ**	**MAVP-RPI VPaIβ**
PI_00001	Manganese ABC transporter substrate-binding lipoprotein precursor		
PI_00002	hypothetical protein		
PI_00003	ChrR Cupin-like domain protein		
PI_00004	Hemolysin secretion protein D	***hlyD*_1**:QPI_00003	PI_00008
PI_00005	hypothetical protein		
	Transposase DDE domain protein	QPI_00005	RPI_00017
	Integrase core domain protein	QPI_00007	RPI_00013
	hypothetical protein	QPI_00008	RPI_00014
	hypothetical protein	QPI_00009	RPI_00015
	Hypothetical protein	QPI_00010	RPI_00016
PI_00007	Bicyclomycin resistance protein		
PI_00008	Transposase	QPI_00013	PI_00020
PI_00009	hypothetical protein		
PI_00010	*ureG*	QPI_00016	RPI_00024
PI_00011	*ureF*	QPI_00017	RPI_00025
PI_00012	*ureE*	QPI_00018	RPI_00026
PI_00013	*ureC*	QPI_00019	RPI_00027
PI_00014	*ureB*	QPI_00020	RPI_00028
PI_00015	*ureA*	QPI_00021	RPI_00029
PI_00016	*ureD*	QPI_00022	RPI_00030
PI_00017	*appA*	QPI_00023	RPI_00031
PI_00018	*oppB*	QPI_00024	RPI_00032
PI_00019	*ddpC*	QPI_00025	RPI_00026
PI_00020	*oppD*	QPI_00026	RPI_00033
PI_00021	*ddpF*	QPI_00027	RPI_00034
PI_00022	*ureR*	QPI_00029	RPI_00035
PI_00023	hypothetical protein		
PI_00024	*trh*	QPI_00030	RPI_00037
PI_00025	hypothetical protein		
PI_00026	transposase	QPI_00031	RPI_00038
PI_00027	putative *ospB*	QPI_00032	RPI_00040
PI_00028	H-NS like protein	*stpA*:QPI_00033	RPI_00041
PI_00029	accessory colonization factor *acfD*	QPI_00034	RPI_00042
PI_00030	hypothetic protein	QPI_00035	RPI_00043
PI_00031	hypothetic protein		
PI_00032	hypothetic protein	QPI_00036	RPI_00044
PI_00033	***yedA***	QPI_00037	RPI_00045
PI_00034	hypothetic protein	QPI_00039	RPI_00075
PI_00035	putative transcriptional regulator (***vtrA*)**	QPI_00040	RPI_00046
PI_00036	hypothetic protein	QPI_00041	RPI_00047
PI_00039	hypothetical protein (*vopZ* location)	QPI_00044	RPI_00050
PI_00043	hypothetical protein	QPI_00048	RPI_00054
PI_00047	hypothetical protein		
PI_00051	hypothetical protein	QPI_00055	RPI_00061
PI_00052	hypothetical protein	QPI_00056	RPI_00062
	repetitive sequence (*vopV* location)	QPI_00061	RPI_00067
PI_00057	hypothetical protein		

PI_00058	hypothetical protein	QPI_00062	RPI_00068
PI_00059	hypothetical protein	QPI_00063	RPI_00070
PI_00065	hypothetical protein		
PI_00067	hypothetical protein	QPI_00070	RPI_00077
PI_00070	hypothetical protein	QPI_00073	RPI_00080
PI_00071	TH3996 VopL (***vopL***[4])	QPI_00074	RPI_00081
PI_00073	putative TraA	QPI_00076	RPI_00083
PI_00074	effector protein VopC (***vopC***[4])	QPI_00077	RPI_00084
PI_00075	hypothetical protein	QPI_00078	RPI_00085
PI_00076	hypothetical protein		
PI_00077	hypothetical protein		
PI_00079	hypothetical protein	QPI_00082	
PI_00080	Metallo-beta-lactamase superfamily protein		
PI_00081	hypothetical protein		
PI_00082	hypothetical protein	QPI_00085	
PI_00083	hypothetical protein		
PI_00084	putative integrate membrane protein	*kcsA*: QPI_00092	

[4]Denotes annotated [see (3)] effector genes that were highly divergent from their presumed homolog in VPaIα

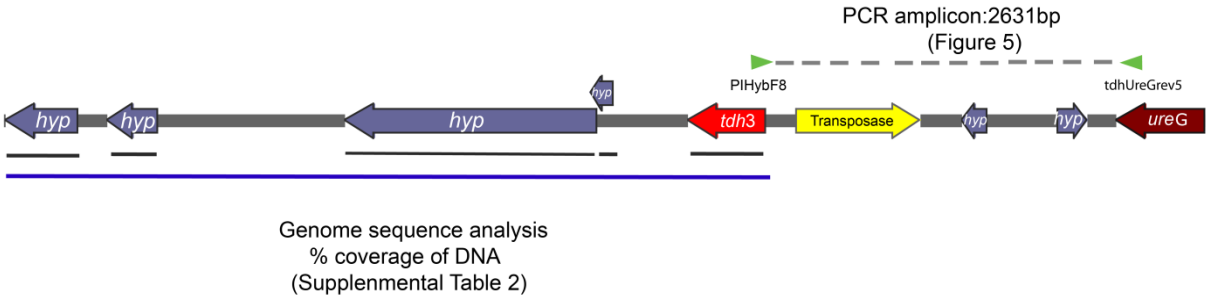
C: Genes unique to VPaIγ absent or divergent in both VPaIα and VPaIβ

MAVP-Q VPaIγ	Annotation
QPI_00001	putative hydrolase
QPI_00002	hypothetical protein
QPI_00004	*hlyD*_2:Hemolysin secretion protein D
QPI_00006	hypothetical protein
QPI_00011	hypothetical protein
QPI_00014	hypothetical protein
QPI_00015	hypothetical protein
QPI_00028	hypothetical protein
QPI_00038	Insertion element 4 transposase
QPI_00083	hypothetical protein
QPI_00084	hypothetical protein
QPI_00085	hypothetical protein
QPI_00087	Integrase core domain protein
QPI_00090	hypothetical protein
QPI_00091	hypothetical protein
QPI_00093	hypothetical protein
QPI_00094	hypothetical protein
QPI_00102	Integrase core domain protein
QPI_00103	putative transposition protein
QPI_00104	*tnsB*: Transposon Tn7 transposition protein
QPI_00105	*tnsA*: endonuclease N terminal

Supplemental Table 2. Analysis of presence and co-occurrence of DNA encoding each of four hypothetical-proteins and *tdh*, as well as the contiguous five-gene module in representative draft genomes, by sequence type depicted in Supplemental Fig. 1[a]

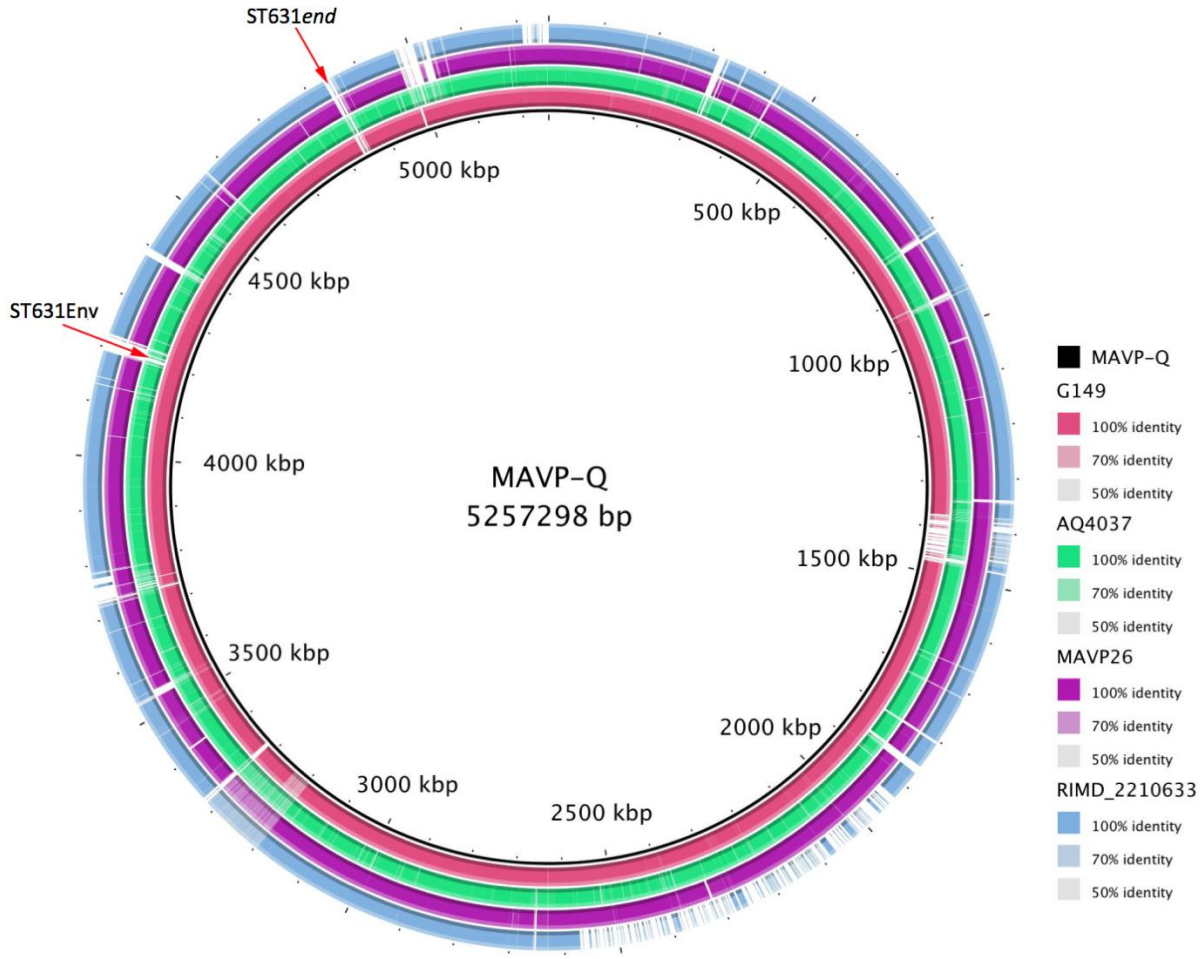
Sequence type	**Percent coverage of VPaI gene or contiguous five-gene module**						**VPaI type**
	hyp AB831_22090 526 bp	*hyp* AB831_22095 369 bp	*hyp* AB831_22100 1827 bp	*hyp* AB831_22105 108 of 177 bp	*tdh* 570 bp	All five genes 6118 bp	
3	0	0	0	13.89	100	24.21	α
36	100	100	100	100	100	100	γ
631	100 100 0	100 100 0	100 100 0	100 85.90 0	100 0 0	100 80.94 4.04	γ β absent
43	100	100	100	100	100	100	γ
636	100	100	100	100	100	99.48	γ
1127	100	100	100	85.19	0	80.94	β
110	100	100	100	100	100	99.98	γ
34/324	100	100	100	100	100	100	γ
674	100 0	100 0	100 0	100 0	100 0	100 5.26	γ absent
308	100	100	100	100	100	100	γ
23	100	100	100	100	100	100	γ
749	100	100	100	100	100	100	γ

[a]Analyses were performed on 300 quality draft genomes compared to the corresponding sequences in MAVP-Q as a reference, to determine the percent coverage of each gene (see methods). Data reported only for sequence types identified among regional clinical isolates as part of this study, where results from the comprehensive analyses are available upon request. ST308 isolate MAVP-67 was sequenced subsequent to this analysis, and presence of all five genes and the contiguous five-gene module was confirmed through manual analysis of its draft genome.

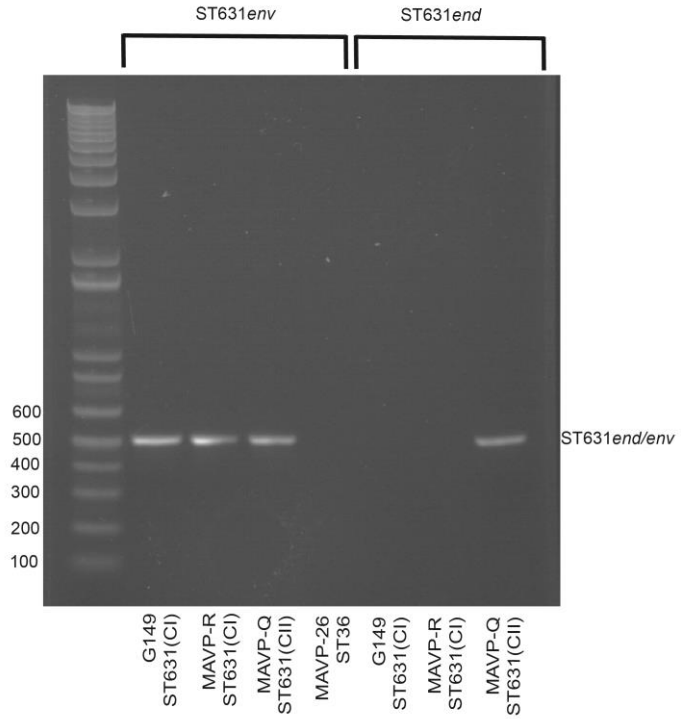


Supplemental Figure 1: Schematic depicting the complementary approaches for evaluating the potential conservation of *tdh* insertion location in various sequenced genomes and cultured isolates. The presence and percent coverage of each of four hypothetical protein encoding genes and *tdh* (black lines) or the entire five gene module (blue line) was determined from sequenced genomes (see methods). Specific primers (green) were subsequently designed and used to generate a PCR amplicon (grey dashed line) from *tdh* to the adjacent *ureG* gene, the results of which are presented in Fig. 5. Image generated with the aid of Snap Gene Viewer V3.3.4.

Supplemental Figure 2. Locus matching the insertion site of VPaI-β in MAVP-R, showing the absence of the island in MAVP-Q and G149.



Supplemental Figure 3. Illustration of genetic markers unique to ST631 utilized for the development of a lineage-specific amplicon assay. The shared and unique genome contents of ST631 clade II (MAVP-Q, innermost circle) and clade I (G149 second from interior) isolates when compared to non-ST631 reference genomes (AQ4037 third from interior, MAVP-26 fourth from interior, and RIMD_2210633 outermost circle) is depicted with unique genome content identified in ST631 (ST631env) and in clinically prevalent clade II ST631 (ST631*end*) using BRIG (1).



Supplemental Figure 4. PCR assays for the identification of ST631. The presence of ST631env and ST631*end* were determined by PCR amplification using gene-specific primers and visualized on a 1.2% agarose gel. The order from left to right is 1Kb+ ladder, clade I ST631 (G149, MAVP-R), clade II ST631 (MAVP-Q), ST36 (MAVP-26), clade I ST631 (G149, MAVP-R), and clade II ST631 (MAVP-Q). The corresponding sizes of the ladder fragments are as labeled to the left and the identity of the amplicons listed to the right of the gel image.

References:

1. Alikhan N-F, Petty NK, Zakour NLB, Beatson SA. 2011. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics **12:**402

2. Hubbard TP, Chao, MC, Abel, S, Blondel, CJ, zur Wiesch, PA, Zhou X, Davis, BM, Waldor M.K. 2016. Genetic analysis of *Vibrio parahaemolyticus* intestinal colonization. Proc Natl Acad Sci USA. **113**:6283-6288.

3. **Okada N, Iida T, Park K-S, Goto N, Yasunaga T, Hiyoshi H, Matsuda S, Kodama T, Honda T.** 2009. Identification and characterization of a novel type III secretion system in trh-positive *Vibrio parahaemolyticus* strain TH3996 reveal genetic lineage and diversity of pathogenic machinery beyond the species level. Infect Immun **77:**904-913.

4. Letchumanan V, Chan K-G, Lee L-H. 2014. *Vibrio parahaemolyticus*: a review on pathogenesis, prevalence and advance molecular identification techniques. Front Microbiol 5:705