Figure S1. Example of the lack of genomic synteny between *Bi*G and Orbales with Enterobacteriales and Pasteurellales displayed in the IMG/ER "Gene Cart: Gene Neighborhood" viewer. Shown is an alignment of genomic regions from bacteria that share the *sucD* gene, which is part of a conserved cluster of TCA cycle genes. The *sucABCD* operon region (α-ketogluterate dehydrogenase, succinyl-CoA synthetase) is boxed. The pink highlighted genes within the boxed area is a region of synteny unique to, and conserved among the Orbales, which includes *Gilliamella* ("Gamma" bin) and *Bi*G. The *sdh* operon (succinate dehydrogenase) is highlighted in blue, which is syntenic among Enterobacteriales and Vibrionales adjacent to *sucABCD*, but absent in Orbales. Highlighted in green are the cytochrome bd complex operon (*cydAB*) and the Tol-Pal operon (*ybgC*, *tolQRAB*, *pal*, *ybgF*), which have a similar genomic location and gene order across *Bi*G, the *A. mellifera* "Gamma" bin, and Enterobacteriales.

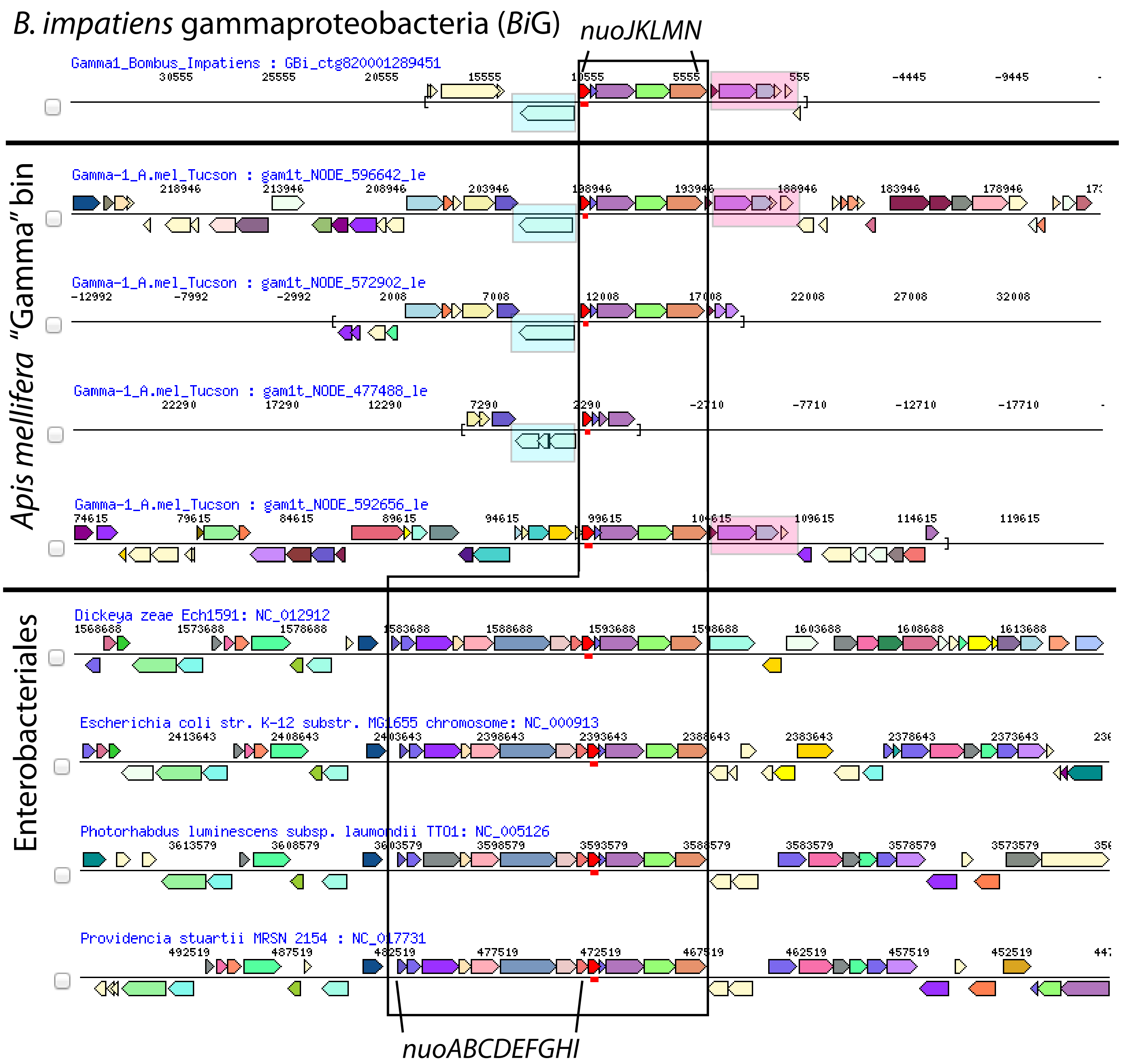

Figure S2. Alignment of the genomic regions from bacteria that harbor the *nuoJ* gene from the NADH dehydrogenase complex I operon (NDH-I, *nuoA-N*), the boxed region shows shared synteny of this operon. Unlike most Enterobacteriales, *Bi*G and contigs from the *A. mellifera* "Gamma" bin do not contain genes *nuoA-I*. Similar to the region around *sucCD*, *Bi*G and the "Gamma" bin have unique syntenic regions that are highlighted: in blue is a phosphoenolpyruvate carboxylase and in pink is *yajC*, *secD*, *secF*, and tRNA Ala (GGC).

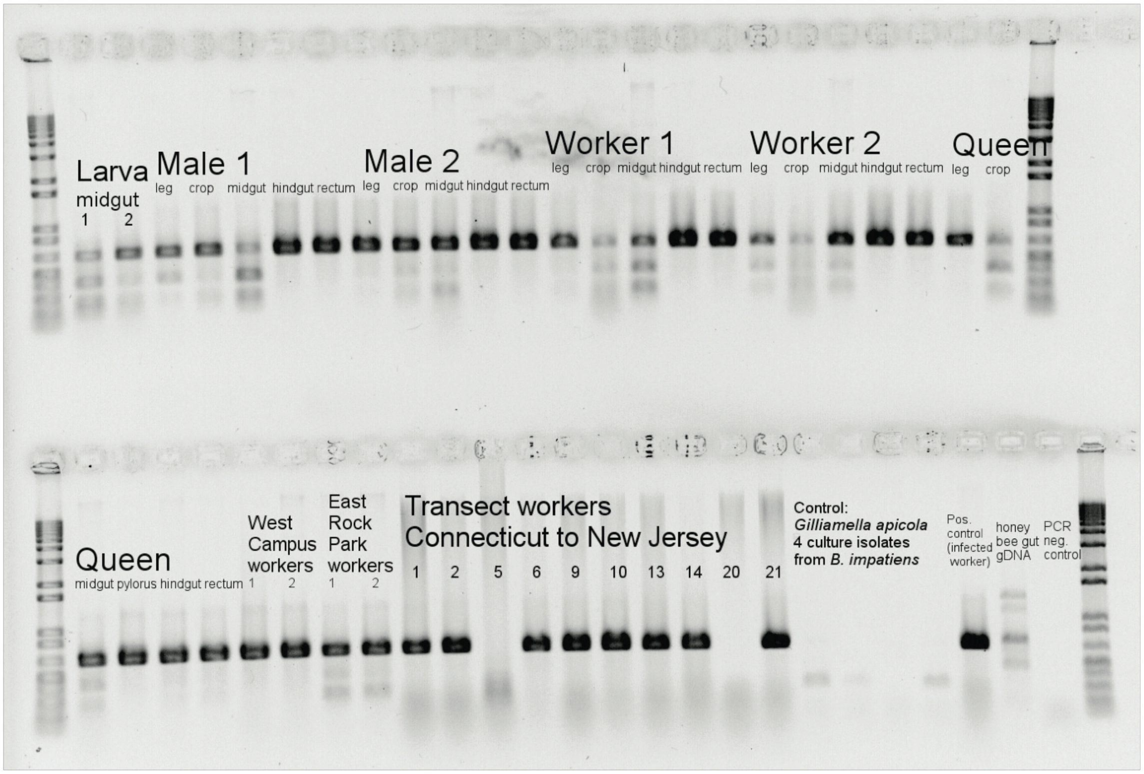

Figure S3. Image of gel showing the results of the survey for *Ca*. Schmidhempelia bombi with specific primers. Note that the primers did not amplify cultured isolates of *Gilliamella apicola*.

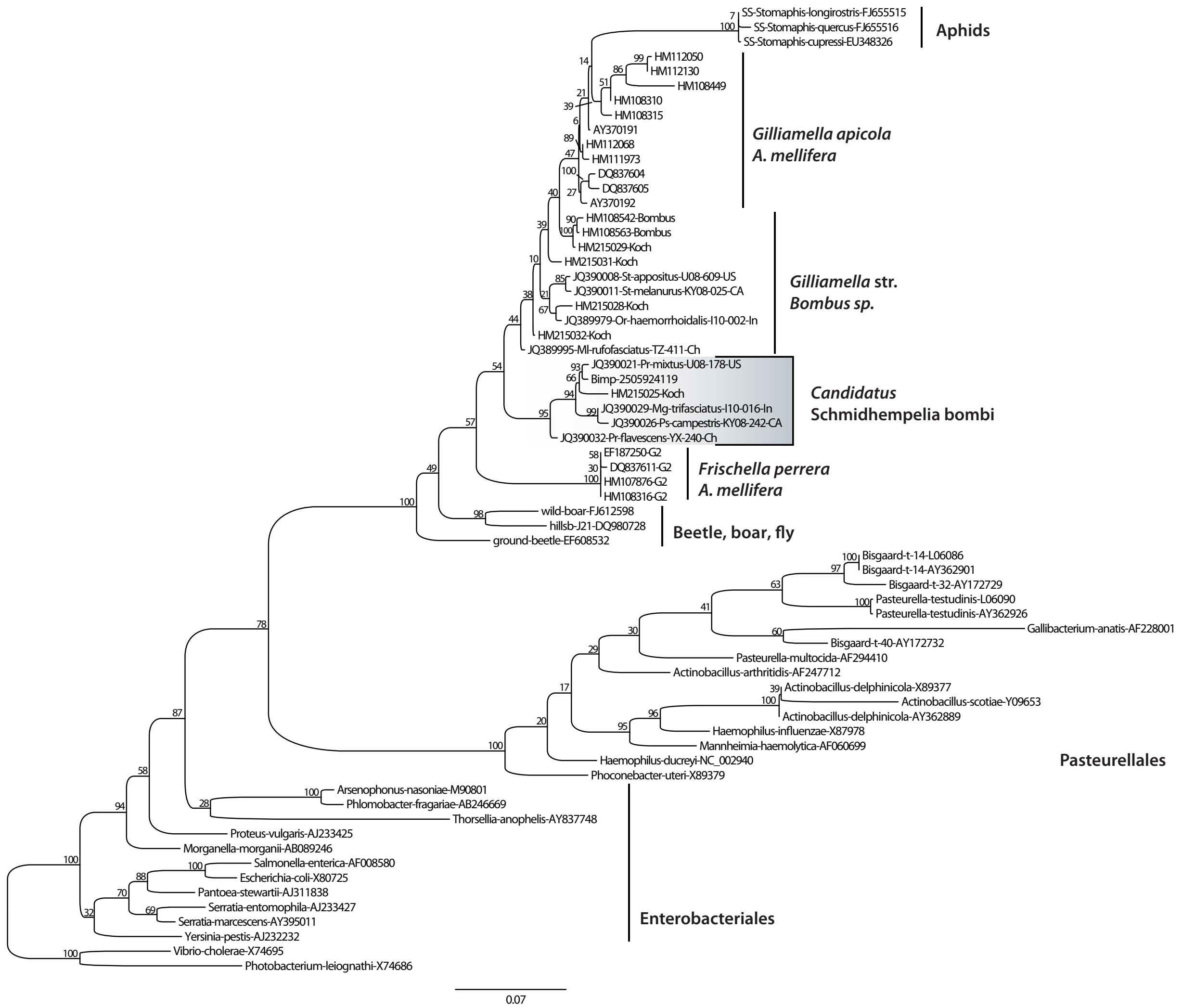

Figure S4. Full 16S rRNA gene maximum likelihood tree with sequences from Pasteurellales, Enterobacteriales, and the Orbales that includes *Bi*G (RAxML, GTRGAMMA, 100 bootstrap replicates).

Table S1: COGs represented in the *Bi*G genome and absent from the "Gamma" bin of the *A. mellifera* metagenome

Gene Object ID	Locus Tag	Length (aa)	COG Name	COG Number	*Bi*G	"Gamma" Bin	AZ metagenome
2505924125	GBi_0010.00000060	190	Predicted esterase	COG3150	1	0	0
2505925004	GBi_0051.00000020	173	Predicted membrane protein	COG4905	1	0	0
2505925071	GBi_0053.00000040	269	Predicted membrane protein	COG4025	1	0	0
2505925088	GBi_0054.00000120	264	Predicted divalent heavy-metal cations transporter	COG0428	1	0	0
2505925174	GBi_0059.00000130	476	Na+/alanine symporter	COG1115	1	0	0
2505925725	GBi_0076.00000070	278	Outer membrane phospholipase A	COG2829	1	0	0
2505925792	GBi_0078.00000360	133	Predicted secreted protein	COG5513	1	0	0
2505924217	GBi_0012.00000210	251	Uridine phosphorylase	COG2820	1	0	2
2505924310	GBi_0016.00000470	145	Predicted acyltransferase	COG2153	1	0	12
2505924424	GBi_0019.00000010	1082	Nitrate reductase alpha subunit	COG5013	1	0	25
2505924425	GBi_0019.00000020	511	Nitrate reductase beta subunit	COG1140	1	0	16
2505924426	GBi_0019.00000030	236	Nitrate reductase delta subunit	COG2180	1	0	9
2505924427	GBi_0019.00000040	224	Nitrate reductase gamma subunit	COG2181	1	0	10
2505924446	GBi_0019.00000230	207	N-acyl-L-homoserine lactone synthetase	COG3916	1	0	6
2505924498	GBi_0022.00000010	248	Lipoate-protein ligase A	COG0095	1	0	9
2505924613	GBi_0029.00000140	90	Septum formation topological specificity factor	COG0851	1	0	3
2505924615	GBi_0029.00000160	230	Septum formation inhibitor	COG0850	1	0	4
2505924622	GBi_0029.00000230	170	Tfp pilus assembly protein PilE	COG4968	1	0	25
2505924725	GBi_0034.00000020	91	Antitoxin of toxin-antitoxin stability system	COG2161	1	0	5
2505924726	GBi_0034.00000030	109	Plasmid stabilization system protein	COG3668	1	0	6
2505924800	GBi_0038.00000360	241	Tfp pilus assembly protein, ATPase PilM	COG4972	1	0	12
2505924828	GBi_0041.00000110	100	Predicted methylated DNA-protein cysteine methyltransferase	COG3695	1	0	1
2505925044	GBi_0052.00000060	291	Putative glucose uptake permease	COG4975	1	0	2
2505925213	GBi_0060.00000210	184	Bacterioferritin (cytochrome b1)	COG2193	1	0	13
2505925494	GBi_0069.00000340	182	Guanyl-specific ribonuclease Sa	COG4290	1	0	1
2505925499	GBi_0069.00000390	62	Cobalamin-5-phosphate synthase	COG0368	1	0	7
2505925563	GBi_0070.00000550	376	Predicted Fe-S oxidoreductases	COG0535	1	0	13
2505925740	GBi_0076.00000220	540	Sigma54-dependent transcription regulator	COG4650	1	0	11
2505925743	GBi_0076.00000250	251	Predicted nucleotidyltransferase	COG3541	2	0	6
2505925744	GBi_0076.00000260	352	Predicted nucleotidyltransferase	COG3541	"	"	"
2505925787	GBi_0078.00000310	273	D-aminopeptidase	COG2362	1	0	13
2505925795	GBi_0078.00000390	337	Protein involved in chromosome segregation, interacts with SMC proteins	COG5185	1	0	2
2505925826	GBi_0079.00000270	128	Cytidylyltransferase	COG0615	1	0	5
2505925828	GBi_0079.00000290	336	Putative glycosyl/glycerophosphate transferases (TagF/TagB/EpsJ/RodC)	COG1887	1	0	9
2505925832	GBi_0079.00000330	476	Mannose-1-phosphate guanylyltransferase	COG0836	1	0	17

Table S2: Location of VgrG genes in the *Bi*G genome

Gene Object ID	Length	Locus Tag	VgrG	Adjacent gene lengths (aa)	Adjacent region function
2505925163	931	GBi_0059.00000030	intact	858	annexin repeat protein/insecticidal toxin
2505924397	786	GBi_0018.00000020	intact	322, 1413, 219, 219	unknown
2505924904	857	GBi_0047.00000020	intact	contig break	
2505924238	249	GBi_0014.00000010	partial	301, 1342, 274	unknown
2505924237	330	GBi_0013.00000150	partial	contig break	
2505925888	228	GBi_0084.00000080	partial	168, 111, 254, 239, 240, 241	unknown
2505925852	241	GBi_0081.00000010	partial	536, 246	unknown
2505925851	126	GBi_0080.00000090	partial	contig break	
2505925719	163	GBi_0076.00000010	partial	contig break	
2505925640	182	GBi_0073.00000010	partial	contig break	
2505925639	330	GBi_0072.00000220	partial	contig break	
2505925753	447	GBi_0077.00000010	partial	contig break	
2505925752	183	GBi_0076.00000340	partial	contig break	
2505925001	185	GBi_0049.00000270	partial	none	
2505924423	249	GBi_0018.00000280	partial	none	
2505925862	269	GBi_0081.00000110	partial	229, 280, 212, 1222	unknown