SUPPLEMENTARY MATERIALS

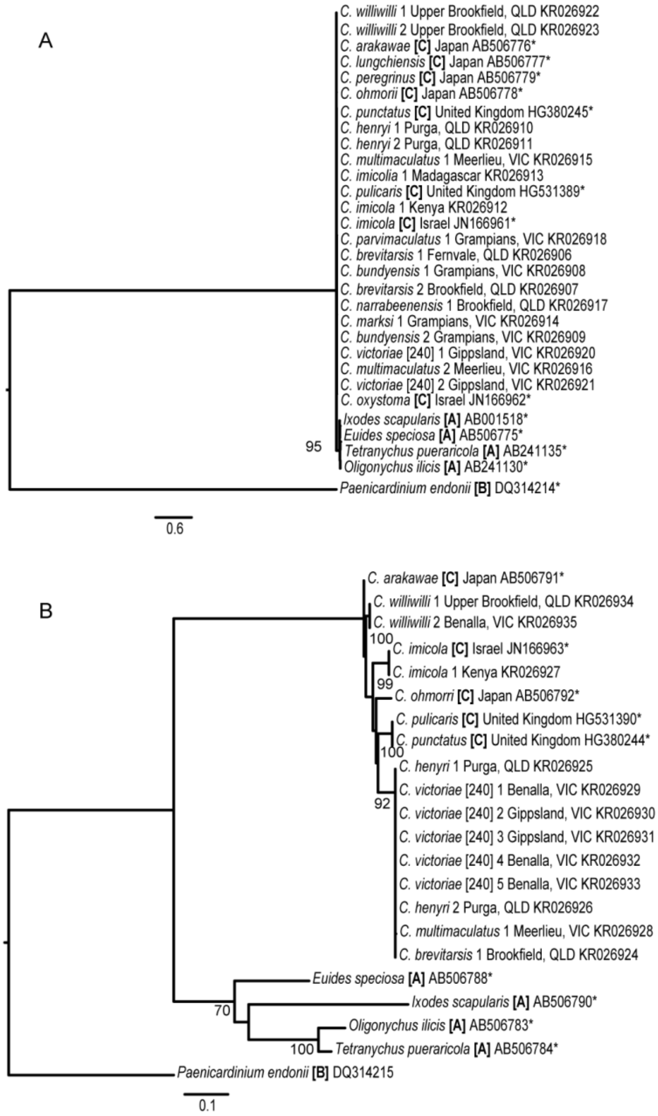

FIG S1. Maximum-likelihood phylogenetic trees of *Cardinium.* Based on (A) a 505 bp region of 16S rDNA and (B) a 1,088 bp region the *gyrB* gene. Nucleotide sequences were aligned using the Clustal W algorithm employing the Hasegawa-Kishino-Yano (HKY) substitution model for 16S rDNA and the General Time-Reversible (GTR) model for the *gyrB* gene, based on JModelTest2 analysis with 1,000 bootstrap replicates. Bootstrap proportions (BSP ≥ 70%) are indicated beside nodes. Three *Cardinium* groups included group **[A]** found in arthropods, group **[B]**

found in plant parasitic nematodes and group **[C]** found in *Culicoides*. Group **[B]** *Cardinium* was used to root the trees. Capture location is indicated beside species name. Asterisks (*) denote sequences obtained from GenBank; all other sequences were generated in this study.

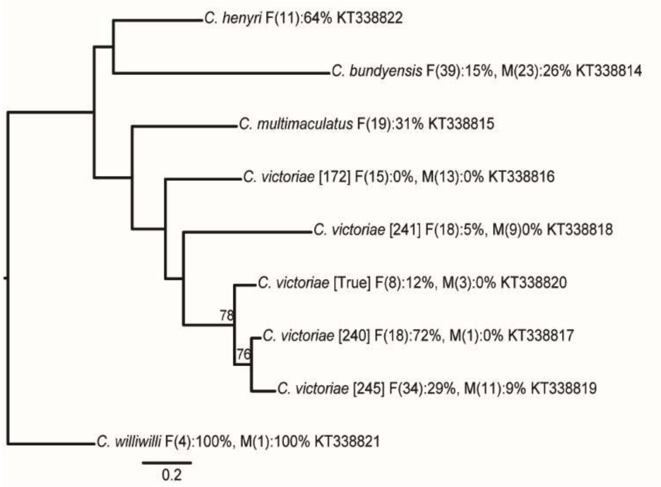

Fig S2. Maximum-likelihood phylogenetic tree of COI and *Cardinium* infection status. This was based on a 548 bp region of cytochrome oxidase subunit 1 (COI) of the *C. victoriae* gp. Nucleotide sequences were aligned using the Clustal W algorithm employing the General Time-Reversible (GTR) model substitution model, based on JModelTest2 analysis with 1,000 boostrap replicates. The number of females ‘F’ and males ‘M’ tested are indicated in parenthesis, with infection status in percentage. Bootstrap proportions (BSP ≥ 70%) are indicated beside nodes. *C. williwilli* is not part of *C. victoriae* but is included to root the tree. All sequences were generated in this study.

Supplementary Table 1. Quantification cycle (Cq) values for positive detections with quantitative *Wolbachia* and *Cardinium* assays.

Species	*Cardinium*					*Wolbachia*				
	sex	no.	min	mean	max	sex	no.	min	mean	max
C. austropalpalis	F	7	37.53	40.12	42.01	F	1		40.84	
	M	3	38.31	41.32	42.50					
C. narrabeenensis	F	1		40.86		F	3	35.73	36.83	38.67
C. marksi	F		36.35	39.86	42.18		1		40.28	
C. parvimaculatus	F	8	33.03	37.23	42.68	F	4	35.55	38.35	41.21
	M	2	41.94	42.31	42.68					
C. dycei	F	2	39.14	40.81	42.48					
C. williwilli	F	4	26.58	29.34	31.32					
	M	1		39.78						
C. henryi	F	7	33.49	36.37	40.40	F	1		40.12	
C. brevitarsis	F	6	36.60	38.83	42.10	F	5	34.50	38.58	41.30
	M	1		39.07						
C. imicola (Madagascar)	F	3	31.03	34.41	41.08		2	39.29	39.80	40.31
C. imicola (Kenya)	F	1		25.92						
C. wadai	F	1		40.62						
C. bundyensis	F	6	39.95	41.23	42.50	F	1		37.56	
	M	6	31.37	38.93	42.35	M	2	35.71	36.79	37.86
C. victoriae [245]	F		29.21	38.09	42.12	F	3	35.96	38.62	40.08
	M			27.79						

C. victoriae [241]	F	1		36.87		F	1		39.84	
C. victoriae [240]	F	13	27.00	32.39	40.32					
C. victoriae [True]	F	1		34.53						
C. antennalis	M	8	33.07	38.02	42.18	M	5	30.53	37.38	38.55
C. Molestus gp sp no2	F	1		37.42						
C. marmoratus	F	6	34.35	38.54	41.59	F	1		41.42	
C. multimaculatus	F	6	26.80	34.65	39.60					