

# Supplementary materials

1  
2  
3 **Mitochondrial diversity.** The mtDNA sequences used to compare nucleotide diversity  
4 between Culicidae species were available on GenBank: *Ae. albopictus* (*cytb*: AJ970990-  
5 AJ9701002, AY072044; *COI*: AF253022, AY072044, AY100666-671, AY101848-  
6 AY101854, DQ181451, DQ181457, DQ181458, DQ397908-DQ397912), *Ae. aegypti* (*cytb*:  
7 AJ970943-AJ970958; *ND4*: AF203344-AF203366, AF334841-AF334859, AF334861-  
8 AF334865), *Ae. caspius* (*COI*: FJ210902-FJ210908; *COII*: DQ300479-DQ300499), *Ae.*  
9 *vexans* (*COI*: AY645241- AY645247; *COII*: AY645304- AY645309, GU229896); *C. pipiens*  
10 (*ND4*: AY793688-AY793693, EF028084, EF030092, EF033661; *COI*: AJ557889, AJ557891,  
11 AJ557892, AJ633083-AJ633086, AY33086, GQ255648-GQ255651, GQ255659- GQ25564,  
12 GQ255666; *COII*: EU014281, EU014282, L344351); *Culex* sp. (*ND4*: AY793694-  
13 AY7937003), *C. tarsalis* (*nad4*: EF125799-EF125862), *An. aconitus* (*COI*: AY423055,  
14 DQ000253-DQ000264; *COII*: AJ194448-AJ194451, AJ547367-AJ547369, AY626951-  
15 AY626978), *An. funestus* (*cytb*: AF062501-AF062511), *An. gambiae* (*COI*: AF020967,  
16 AF020968, AF020970, AF020971, AF020973, AF020980, AF020988, AF020989,  
17 AF020991-AF02093, AF020998, AF020999, AF021002, AF021003, AF021011-AF021023)  
18 and *An. maculipennis* (*COI*: AF342716-AF342722, AF491682-AF491736).

Gene	Putative product	Locus tag in wPip(Pel)	Primer (5'-3')	Size (bp)	Number of alleles found in this study (accession numbers)	References
<i>gatB</i>	Glutamyl-tRNA(Gln) amidotransferase, subunit B	WPa_0087	gatB_F1-GAKTTAAAYCGYGCAGGBGTT gatB_R1-TGGYAAAYTCRGGYAAAGATGA	369	1	(Baldo et al. 2006)
<i>coxA</i>	Cytochrome c oxidase, subunit I	WPa_0082	coxA_F1-TTGGRGCATYAACITTTATAG coxA_R1-CTAAAGACTTTKACRCCAGT	402	1	(Baldo et al. 2006)
<i>hcpA</i>	Conserved hypothetical protein	WPa_1214	hcpA_F1-GAAATARCAGTTGCTGCAAA hcpA_R1-GAAAGTYRAGCAAGYTCTG	444	1	(Baldo et al. 2006)
<i>ftsZ</i>	Cell division protein	WPa_0577	ftsZ_F1-ATYATGGARCATATAAARGATAG ftsZ_R1-TCRAGYAATGGATTRGATAT	435	1	(Baldo et al. 2006)
<i>fbpA</i>	Fructose-bisphosphate aldolase	WPa_1081	fbpA_F1-GCTGCTCCRCTTGGYWTGAT fbpA_R1-CCRCCAGARAAAAYACTATTC	429	1	(Baldo et al. 2006)
<i>wsp</i>	Surface protein	WPa_0937	81F-TGGTCCAATAAGTGATGAAGAAAC 691R-AAAAATTAACGCTACTCCA	602	1	(Braig et al. 1998)
<i>MutL</i>	DNA mismatch repair protein	WPa_0278	F- ACTTCATTGCCCTTCCAGCT R -GGCATCAAATTAAGGGACA	1000-1,063	6 (HQ709389-HQ709394)	This study
<i>ank2</i>	Ankyrin domain protein	WPa_0652	F-CTTCTTCTGTGAGTGTACGT R2-TCCATATCGATCTACTGCGT	313-511	5 (AM397068-AM397072)	(Duron et al. 2007)
<i>pk1</i>	Ankyrin domain protein	WPa_0256 (1) WPa_0313 (2) WPa_1306 (3)	F-CCACTACATTCGCTATAGA R-ACAGTAGAACTACTCCTCCA	1,334-1,349	5 (AM397075-AM397079)	(Sinkins et al. 2005) (Duron et al. 2007)
<i>pk2</i>	Ankyrin domain protein	WPa_0299 (1) WPa_0413 (2)	F-ATTATGATAAAGCTTGGTAAGAA R-TTAGCCCTTCATAAATAGCTT	453	4 (AM397073-AM397073; DQ000471-DQ000472)	(Sinkins et al. 2005) (Duron et al. 2007)
<i>GPI2</i>	Phage related DNA methylase-like protein	WPa_0258 (1) WPa_0317 (2) WPa_1310 (3) WPa_0429 (4)	F-ATGAATTTAGCAATCCACTACT R-TTACTAAATAACAGACATATTGCT	1,215-1,302	7 (GU827985-GU827987; HQ709395-HQ709398)	(Atyame et al. in press)
<i>GPI5 (=vrIC)</i>	Phage related probable secretory protein	WPa_1322	F1-ACCATTACAGAAGCTTGAGGA R1-TAGACGTTTCATAGGCAACCA F2-ACCTGACTCTGCAGTACTTGA R2-ACTGCTTCTCATAAATTCA	1,511-1,538	7 (GU827988-GU827991; HQ709399-HQ709401)	(Duron, Fort, and Weill 2006) (Atyame et al. in press)
<i>RepA</i>	Phage related replication protein	WPa_1312	Tr1e-F1-ACITTTAGAGGGTGTCTTCT Tr1e-R2- ACAAACAACGGCACAGATT	583-1,501	2 (AJ646884 ; AJ646887)	(Duron et al. 2005)

19

20 **Table S1.** List of primers and characteristics of genes used to examine the *Wolbachia* polymorphism.

Mitochondrial forward primers (5'-3')		Mitochondrial reverse primers (5'-3')	
1F	AATGAATTGCCTGATAAAAAGGA	417R	TGAAGAGGCCAAAAGCTTGAGT
161F <sup>a</sup>	GCTATTGGGTTCATACCCAC	773R <sup>a</sup>	GCTATTAATATTCAACCTAAG
286F	TGGCTTGGTGCTTGAATAGGGT	1442R	AATGGCTGAAGTTTAGGCGAT
1254F	ACTAATAGCCTTCAAAGCTGA	2123R	TGGATCTCCTCCTCCAATTGGA
2045F	AGCTGGTGCTATTACTATGT	3921R	AGTTAATCATCTAATAGGGGCT
2768F	TCCAGATAGTACTTAGCATGA	4798R	AGCTCCAATAGCTCCTGT
3738F	TTCATTAGATGACTGAAAGCA	5968R	TTAGGTCGAAACTAATTGCA
4781F	ACAGGAGCTATTGGAGCT	7002R	CTTTTTTAGCAGGGTTTTATTC
5949F	TGCAATTAGTTTCGACCTAA	7723R <sup>b</sup>	GGGTGGGATGGATTAGGATTGG
6290F	CATCTTCAGTGTCATGCTCT	8112R <sup>b</sup>	GATTTGTGGTGTCAATGATA
6981F <sup>b</sup>	GAATAAAACCTGCTAAAAAAG	8871R	TGATTACCTAAGGCTCATGT
7702F <sup>b</sup>	CCAATCCTAATCCATCCACCC	9259R	AGCAAGAGAAAAGAGTTGTACGA
7940F	TGAAACAATTTCCCATTCA	10099R	AATAAACTAATATTCCTCCT
8636F	TGAGCAACAGAAGAATAAGCA	11217R <sup>c</sup>	ACTAAAGGATTAGCAGGAATGA
8781F	GTAATAATCCATATCCTCCT	12178R	TACGAGCGGTTGCTCAAACA
9239F	CGTACAACCTTTTCTTTGCT	12409R	TACTAAGGAACAACTTATCCT
9851F	AGAAATCTCTTTGTCACTAAT	13182R	TGAATGAGATATATACTGTCT
10366F <sup>c</sup>	CTTTATTAGTAAGTGTAAAAATTAC	13587R	TATTTTAAGGATTAGCTTTAA
10912F	ACAATGGATTTGAGGAGGA	13706R	TAATTAGAAATGAAATGTTAATCG
11985F	AGGAGTACGATTAGTTTCAGCT	14067R	TTAAAGCTTAATTAGTAAAGTA
12387F	AGGATAAGTTTGTTCCCTTAGTAA	14998R	AGCAATGGGAAGGCTTACACT
12856F	TCCAACATCGAGGTCGCAATC		
13338F	GCCGAATTCCTTATTTAAACCTTTC		
13566F	TTAAAGCTAATCCCTTAAAATA		
13802F	ACCCTGATACACAAGGTACA		
14793F	AATTCACACAAAAATTTACATGT		

22

23 **Table S2.** List of primers used to examine the *Culex pipiens* mitochondrial polymorphism. The name of the primers indicates their position in the

24 mitochondrial genome. <sup>a,b,c</sup>, primers used to amplified fragments of the *ND2*, *ND5* and *cytb* genes, respectively. GenBank accession numbers:

25 *ND2* (HQ709410-HQ709413), *ND5* (HQ724607-HQ724613), *cytb* (HQ709402-HQ709409), complete mitochondrial genomes (HQ724614-

26 HQ724617).

27

28

Gene	No. of alleles <sup>a</sup>	Fragment size	% of VI <sup>b</sup>	$\pi$ <sup>b</sup>	G+C content (%) <sup>b</sup>	$Ka/Ks$ <sup>b</sup>	Intragenic recombination (Sawyer's test) <sup>a</sup>
<i>MutL</i>	6	960-1,023	7.1	0.03	35.2	0.25	Yes ( $P < 10^{-4}$ )
<i>ank2</i>	5	273-471	3.3	0.01	38.6	0.00	No ( $P = 0.33$ )
<i>pk1</i>	5	1,292-1,307	16.2	0.07	33.7	0.17	Yes ( $P < 10^{-4}$ )
<i>pk2</i>	4	409	11.2	0.03	38.3	0.04	Yes ( $P < 10^{-4}$ )
<i>GP12</i>	7	1,193-1,278	7.9	0.03	37.5	0.05	Yes ( $P < 10^{-4}$ )
<i>GP15</i>	8 <sup>c</sup>	1,470-1,497	12.4	0.04	37.6	0.12	Yes ( $P < 10^{-4}$ )
<i>RepA</i>	2	544-1,462	0.0	0.00	32.6	0.00	not reliable

29

30 **Table S3.** Genetic characteristics of the seven polymorphic genes used for wPip characterization. VI; number of variable sites;  $\pi$  : pairwise  
31 nucleotide diversity based on the average of all pairwise comparisons; <sup>a</sup> Characteristics estimated considering indels in sequence alignments; <sup>b</sup>  
32 characteristics assessed excluding indels; <sup>c</sup> including the null *GP15* wPip(JHB) allele. Note that primer regions were not considered in these  
33 analyses.

34

35

Genes	<i>MutL</i>	<i>ank2</i>	<i>pk1</i>	<i>pk2</i>	<i>GP12</i>	<i>GP15</i>	<i>RepA</i>
<i>MutL</i>		0.000***	0.000***	0.087	0.000***	0.000***	0.060
<i>ank2</i>	0.966		0.000***	0.013	0.000***	0.000***	0.009
<i>pk1</i>	0.966	0.999		0.020	0.000***	0.000***	0.010
<i>pk2</i>	0.639	0.700	0.700		0.478	0.206	0.008
<i>GP12</i>	0.914	0.967	0.967	0.600		0.000***	0.501
<i>GP15</i>	0.911	0.999	0.999	0.700	0.999		0.058
<i>RepA</i>	0.688	0.750	0.750	0.750	0.667	0.750	

36

37 **Table S4.** Linkage disequilibrium (LD) measures and tests of association between the *wPip* genes. The upper half shows probabilities based on  
38 the null hypothesis of random association of allelic diversity between loci. The lower half shows LD measures ( $D'$  values). \*\*\*, the null  
39 hypothesis is rejected at  $\alpha = 0.001$  taking into account a Bonferonni's adjustment for 21 comparisons.

Gene	Position, Direction of transcription	tRNA anticodon/Position	Start codon	End codon
t-RNA <sup>Ile</sup>	2-69, CW	GAU/31-33	–	–
t-RNA <sup>Gln</sup>	70-138, CCW	UUG/108-106	–	–
t-RNA <sup>Met</sup>	142-210, CW	CAU/172-174	–	–
ND2	211-1233, CW	–	ATC (Ile)	TAA
tRNA <sup>Trp</sup>	1235-1303, CW	UCA/1265-1267	–	–
tRNA <sup>Cys</sup>	1303-1369, CCW	GCA/1340-1338	–	–
tRNA <sup>Tyr</sup>	1382-1447, CCW	GUA/1416-1414	–	–
COI	1446-2983, CW	–	TCG (Ser)	T
tRna <sup>Leu</sup>	2983-3049, CW	UAA/3012-3014	–	–
COII	3055-3739, CW	–	ATG (Met)	T
tRNA <sup>Lys</sup>	3740-3810, CW	CUU/3770-3772	–	–
tRNA <sup>Asp</sup>	3821-3888, CW	GUC/3852-3854	–	–
ATPase8	3888-4050, CW	–	ATT (Ile)	TAA
ATPase6	4044-4724, CW	–	ATG (Met)	TAA
COIII	4724-5512, CW	–	ATG (Met)	TAA
tRNA <sup>Gly</sup>	5512-5578, CW	UCC/5543-5545	–	–
ND3	5578-5932, CW	–	ATT (Ile)	TAA
tRNA <sup>Arg</sup>	5931-5994, CW	UCG/5960-5962	–	–
tRNA <sup>Ala</sup>	5995-6060, CW	UGC/6024-6026	–	–
tRNA <sup>Asn</sup>	6061-6127, CW	GUU/6091-6093	–	–
tRNA <sup>Ser</sup>	6131-6197, CW	GCA/6170-6172	–	–
tRNA <sup>Glu</sup>	6198-6263, CW	UUC/6228-6230	–	–
tRNA <sup>Phe</sup>	6262-6328, CCW	GAA/6296-6294	–	–
ND5	6329-8071, CCW	–	GTG (Val)	TAA
tRNA <sup>His</sup>	8072-8137, CCW	GUG/8107-8105	–	–
ND4	8137-9480, CCW	–	ATG (Met)	TAA
ND4L	9474-9770, CCW	–	ATG (Met)	TAA
tRNA <sup>Thr</sup>	9776-9840, CW	UGU/9806-9808	–	–
tRNA <sup>Pro</sup>	9841-9906, CCW	UGG/9876-9874	–	–
ND6	9907-10427, CW	–	ATA (Met)	TAA
CytB	10428-11567, CW	–	ATG (Met)	TAA
tRNA <sup>Ser</sup>	11562-11627, CW	UGA/11590-11592	–	–
ND1	11646-12596, CCW	–	TTG (Phe)	TAA
tRNA <sup>Leu</sup>	12597-12663, CCW	UAG/12634-12632	–	–
Large rRNA	12664-13999, CCW	–	–	–
tRNA <sup>Val</sup>	14000-14071, CCW	UAC/14038-14036	–	–
Small rRNA	14072-14865, CCW	–	–	–
A + T rich region	14867-15587	–	–	–

41

42 **Table S5.** Summary of the *Culex pipiens* mitochondrial genome. Position: expressed in

43 nucleotides based on the Pel sequence. Direction of transcription: CW, clockwise; CCW,

44 counterclockwise.

Mitotype	Gene, position																						Mosquito line
	ND2					ND5									cytb								
	256	470	543	591	660	7,061	7,106	7,280	7,341	7,345	7,571	7,824	7,826	7,927	10,502	10,554	10,758	10,887	10,918	10,943	10,952	11,118	
<i>pi1</i>	A	T	C	T	T	T	A	T	G	C	G	A	C	A	A	A	G	G	G	C	A	A	Pel
<i>pi2</i>	G	-	-	-	-	-	-	-	-	-	-	G	-	-	-	G	-	-	-	-	-	-	Cot-A, Cot-B, Ma-B
<i>pi3</i>	G	-	-	-	-	-	-	-	-	T	-	G	-	-	-	G	-	-	-	-	-	-	Ep-A, Ep-B
<i>pi4</i>	G	-	-	C	-	A	-	-	-	-	-	G	-	-	-	G	-	A	-	-	-	-	Ko, Tn
<i>pi5</i>	G	-	-	C	-	-	-	-	-	-	-	G	-	-	-	G	-	A	-	-	-	-	Bf-A
<i>pi6</i>	G	-	-	-	A	-	G	-	-	-	-	G	T	-	-	G	-	-	-	-	-	G	Au
<i>pi7</i>	G	-	-	-	A	-	-	-	-	-	-	G	T	-	-	G	-	-	-	-	-	G	Lv
<i>pi8</i>	G	C	-	-	A	-	-	C	-	-	-	G	T	G	-	G	-	-	-	-	-	G	Ke-A
<i>pi9</i>	G	C	-	-	A	-	-	-	-	-	-	G	T	-	-	G	-	-	-	-	-	G	Ke-B
<i>pi10</i>	G	-	-	-	A	-	-	-	-	-	-	G	T	-	G	G	-	-	-	-	-	G	Bf-B, Mc
<i>pi11</i>	G	-	-	-	A	-	-	-	-	-	-	G	T	-	G	G	-	-	-	-	G	G	SI
<i>pi12</i>	G	-	G	-	A	-	-	-	A	-	A	G	T	-	-	G	-	-	A	-	-	-	Is
<i>pi13</i>	G	-	-	-	A	-	-	-	-	-	-	G	T	-	-	G	A	-	-	T	-	-	Ka-C
<i>pi14</i>	G	-	-	-	A	-	-	-	-	-	-	G	T	-	-	G	-	-	-	T	-	-	Ma-A

46

47 **Table S6:** Nucleotide polymorphism in the *ND2*, *ND5* and *cytb* mitochondrial genes of *Culex pipiens*. Mosquito lines are listed according to  
48 mitotype (*pi1* to *pi14*). Only polymorphic site are indicated, and a dash indicates similarity with the top sequence. Position: expressed in  
49 nucleotides based on the complete mitochondrial sequence of the Pel *C. pipiens* line.



## 50 **Supplementary figures**

51 **Figure S1.** *Wolbachia* phylogeny constructed using Bayesian inferences on concatenated  
52 sequences of the five MLST genes *gatB*, *coxA*, *hcpA*, *ftsZ* and *hcpA*. *Wolbachia* of major  
53 supergroups (A, B, D, F and H) were included in the analysis to delineate the *wPip* group  
54 (highlighted). Host species of *Wolbachia* are reported, followed by the name of the  
55 *Wolbachia* strain. The scale bar is in units of substitutions/site.

56

57 **Figure S2.** Examples of recombination breakpoints along the *pk1* (A, B), *pk2* (C, D, E) and  
58 *GP12* (F) sequences. For each alignment, only polymorphic sites around the breakpoints are  
59 shown. Polymorphisms shared with the underlined sequence are highlighted in grey. Arrows  
60 indicated the significant breakpoints and the nucleotide position detected by Sawyer's  
61 procedure.

62

63 **Figure S3.** Mapping of the 13 genes examined in this study on the *wPip*(Pel) genome and on  
64 the five major contigs of the *wPip*(JHB) genome. Black boxes designate prophage genes, or  
65 genes inserted in phage regions. Lines connect orthologous genes. The *wPip*(JHB) genome  
66 description corresponds to the current situation and could change when the assembling is  
67 achieved.

68

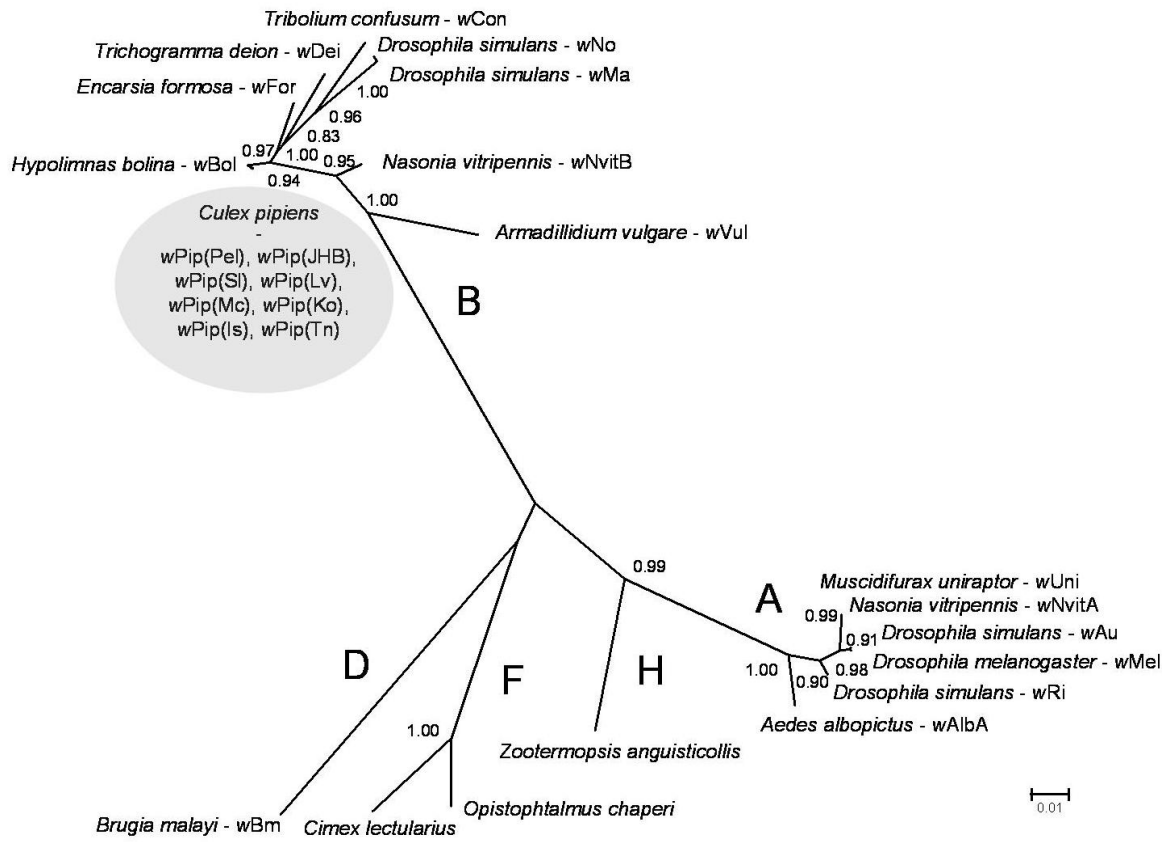
69 **Figure S4.** *Wolbachia* phylogenies constructed with six *wPip* polymorphic genes. **A:** *MutL*;  
70 **B:** *ank2*; **C:** *pk1*; **D:** *pk2*; **E:** *GP12*; **F:** *GP15*. The phylogeny of the *RepA* gene was not  
71 performed because the polymorphism with this gene is only based on the presence or the  
72 absence of the transposon *Tr1*. The scale bar is in units of substitutions/site.

73

74 **Figure S5.** Map of the *Culex pipiens* mitochondrial genome. The map has been linearized and  
75 nucleotide 1 is arbitrary allocated to tRNA<sup>Ile</sup> transcription start. All genes are indicated as  
76 boxes above (transcription from left to right) or below (transcription from right to left) the  
77 baseline. tRNAs are represented by the single-letter code for the cognate amino acid. Sites  
78 found polymorphic between the five *C. pipiens* mtDNA genomes (without the A+T rich  
79 region) are indicated by stars.

80

81



82

83

84

85

Figure S1

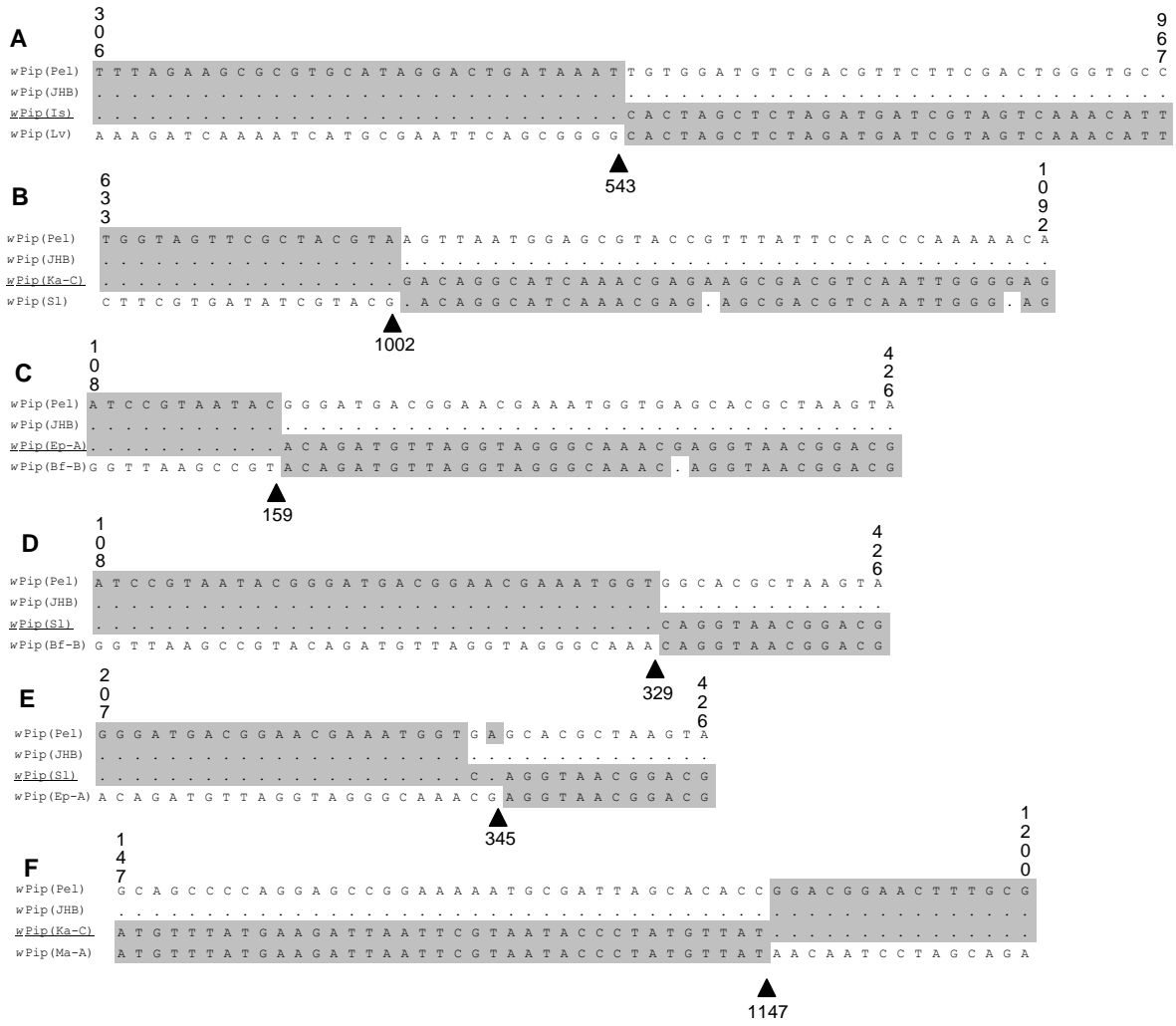


Figure S2

90

wPip(JHB) contig 1299  
(478,325bp)

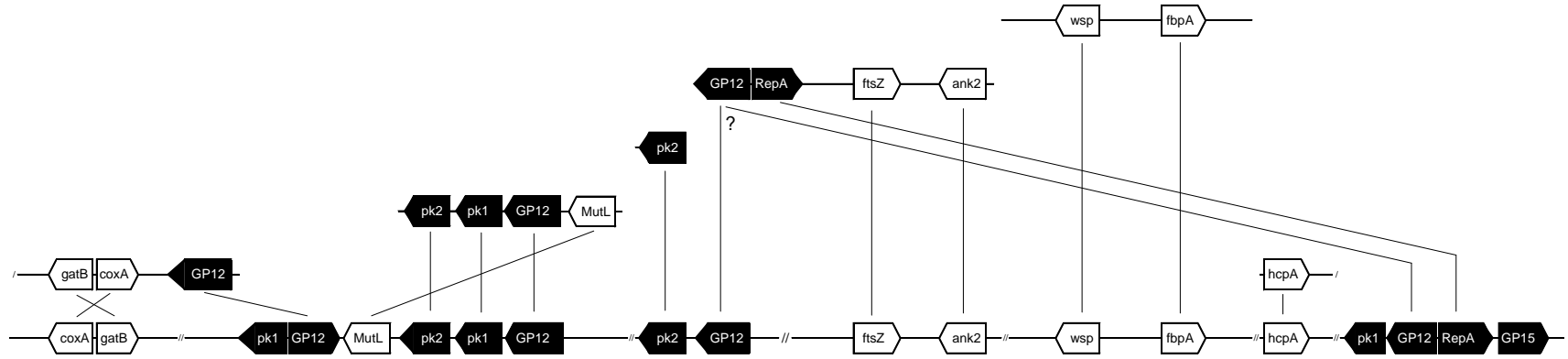
wPip(JHB) contig 1298  
(316,943bp)

wPip(JHB) contig 1302  
(42,565bp)

wPip(JHB) contig 1301  
(126,623bp)

wPip(JHB) contig 1300  
(466,173bp)

wPip(Pel) genome  
(1,482,355bp)



91

92

93

Figure S3

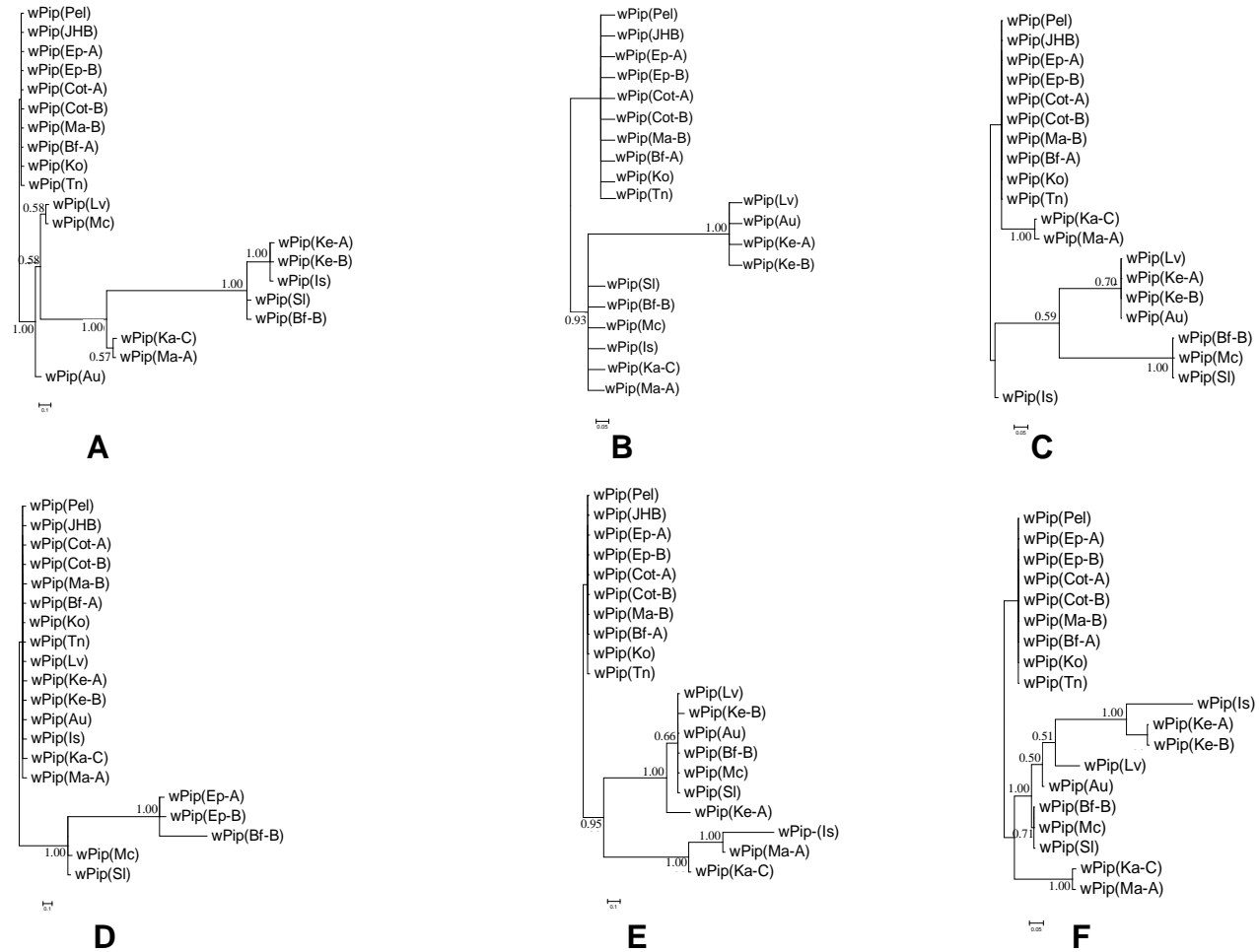
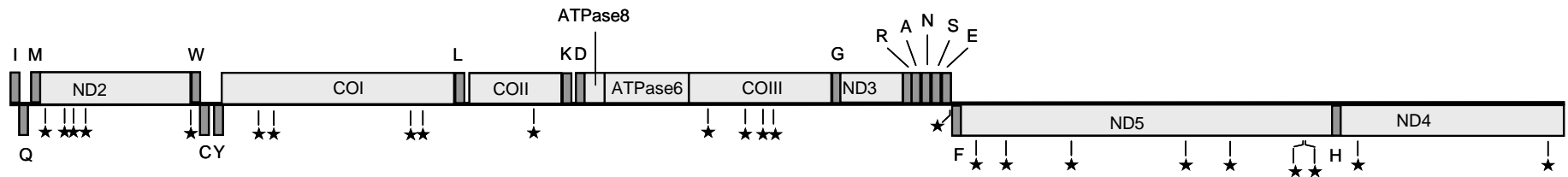


Figure S4

98

99



100

101

102

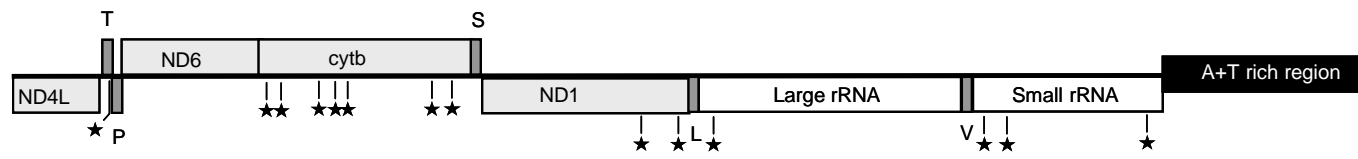


Figure S5

103 **Literature Cited**

- 104 Atyame, C., O. Duron, P. Tortosa, N. Pasteur, P. Fort, and M. Weill. 2011. Multiple  
105 *Wolbachia* determinants control the evolution of cytoplasmic incompatibilities in *Culex*  
106 *pipiens* mosquito populations. *Mol Ecol* (in press).
- 107 Baldo, L., J. C. Dunning Hotopp, K. A. Jolley, S. R. Bordenstein, S. A. Biber, R. R.  
108 Choudhury, C. Hayashi, M. C. Maiden, H. Tettelin, and J. H. Werren. 2006. Multilocus  
109 sequence typing system for the endosymbiont *Wolbachia pipientis*. *Appl Environ*  
110 *Microbiol* **72** :7098-7110.
- 111 Braig, H. R., W. Zhou, S. L. Dobson, and S. L. O'Neill. 1998. Cloning and characterization of  
112 a gene encoding the major surface protein of the bacterial endosymbiont *Wolbachia*  
113 *pipientis*. *J Bacteriol* **180** :2373-2378.
- 114 Duron, O., A. Boureux, P. Echaubard, A. Berthomieu, C. Berticat, P. Fort, and M. Weill.  
115 2007. Variability and expression of ankyrin domain genes in *Wolbachia* variants  
116 infecting the mosquito *Culex pipiens*. *J Bacteriol* **189** :4442-4448.
- 117 Duron, O., P. Fort, and M. Weill. 2006. Hypervariable prophage WO sequences describe an  
118 unexpected high number of *Wolbachia* variants in the mosquito *Culex pipiens*. *Proc Biol*  
119 *Sci* **273** :495-502.
- 120 Duron, O., J. Lagnel, M. Raymond, K. Bourtzis, P. Fort, and M. Weill. 2005. Transposable  
121 element polymorphism of *Wolbachia* in the mosquito *Culex pipiens*: evidence of genetic  
122 diversity, superinfection and recombination. *Mol Ecol* **14** :1561-1573.
- 123 Sinkins, S. P., T. Walker, A. R. Lynd, A. R. Steven, B. L. Makepeace, H. C. Godfray, and J.  
124 Parkhill. 2005. *Wolbachia* variability and host effects on crossing type in *Culex*  
125 *mosquitoes*. *Nature* **436** :257-260.