

Supplementary materials

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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-TGGYAAATCRGGYAAAGATGA	369	1	(Baldo et al. 2006)
<i>cox4</i>	Cytochrome c oxidase, subunit I	WPa_0082	cox4_F1-TTGGRCRATYAACTTTATAG cox4_R1-CTAAAGACTTKACRCCAGT	402	1	(Baldo et al. 2006)
<i>hcpA</i>	Conserved hypothetical protein	WPa_1214	hcpA_F1-GAAATARCAGTTGCTGCAAA hcpA_R1-GAAAGTYRAGCAAGYTCGT	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-GCTGCTCRCTGGYWTGAT fbpA_R1-CCRCAGARAAAAYACTATTC	429	1	(Baldo et al. 2006)
<i>wsp</i>	Surface protein	WPa_0937	81F-TGGTCCAATAAGTGATGAAGAAAC 691R-AAAAAATTAACGCTACTCCA	602	1	(Braig et al. 1998)
<i>MutL</i>	DNA mismatch repair protein	WPa_0278	F- ACTTCATTTGCCCTTCCAGCT R- GGCAATCAAAATTAAGGGACA	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-CCACTACATTCGGCTATAGA R-ACAGTAGAACTACACTCTCCA	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-ATTAATGATAAAGCTTGGTAAGAA 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) WPa_1322	F-ATGAATTTAGCAAICCACTACT R-TTACTAAATAACAGACATATTGCT	1,215-1,302	7 (GU827985-GU827987; HQ709395-HQ709398)	(Abyame et al. in press)
<i>GPI5 (=v/C)</i>	Phage related probable secretory protein	WPa_1322	F1-ACCATTACAGAACTTGAGGA R1-TAGACGTTTCATAGGCAACCA F2-ACCTGACTCTGCAGTACTTGA R2-ACTGGTTCCTCTATAAAATCA	1,511-1,538	7 (GU827988-GU827991; HQ709399-HQ709401)	(Duron, Fort, and Weill 2006) (Abyame et al. in press)
<i>RepA</i>	Phage related replication protein	WPa_1312	TriE-F1-ACTTTAGAGGGGTGCTTTCT TriE-R2-ACAAACAACGGCACAGATT	583-1,501	2 (AJ646884 ; AJ646887)	(Duron et al. 2005)

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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	AATGAATTGCCTGATAAAAAAGGA	417R	TGAAGAGGCAAAAAGCTTGAGT
161F ^a	GCTAATGGGTTCATACCCAC	773R ^a	GCTAATTAATATCAACCTAAG
286F	TGGCTTGGTGTGAATAGGGT	1442R	AATGGCTGAAAGTTTAGGCGAT
1254F	ACTAATAGCCTTCAAAAGCTGA	2123R	TGGATCTCTCCTCCAATTGGA
2045F	AGCTGGTACTACTATGT	3921R	AGTTAATCATCTAATAGGGGCT
2768F	TCCAGATAGTACTTAGCATGA	4798R	AGTCCAATAGCTCCTGT
3738F	TTCATTAAGTACTGAAAGCA	5968R	TTAGTTCGAAACTAATTGCA
4781F	ACAGGACTATTGGAGCT	7002R	CTTTTTAGCAGGGTTTTATTTC
5949F	TGCAATTAGTTTCGACCTAA	7723R ^b	GGGTGGGATGGATTAGGATTGG
6290F	CATCTTCAAGTGTATGCTCT	8112R ^b	GATTTGTGGTGTCAATGATA
6981F ^b	GAATAAAAACCCTGCTAAAAAAG	8871R	TGATTACCTAAGGCTCATGT
7702F ^b	CCAAATCCTAATCCATCCACCC	9259R	AGCAAGAGAAAAGAGTTGTACGA
7940F	TGAAACAATITCCCATTCA	10099R	AATAAAAATAATATTCCTCCT
8636F	TGAGCAACGAGAATAAAGCA	11217R ^c	ACTAAAGGATTAGCAGGAAATGA
8781F	GTAATAATCCATATCCTCCT	12178R	TACGAGCGGTGCTCAAAACA
9239F	CGTACAACCTTTCTCTTGCT	12409R	TACTAAGGAACA AACTTATCCT
9851F	AGAAATCTCTTGTCACTAACT	13182R	TGAATGAGATATATACTGTCT
10366F ^c	CTTTATTAGTAACTGTAAAAATTAC	13587R	TATTTAAGGGATTAGCTTTAA
10912F	ACAATGGATTTGAGGAGGA	13706R	TAATTAGAAAATGAAAATGTTAAATCG
11985F	AGGATACGATTAGTTTCAGCT	14067R	TTAAAAGCTTAATTAGTAAAAGTA
12387F	AGGATAAGTTTGTTCCTTAGTAA	14998R	AGCAATGGGAAGGCTTACACT
12856F	TCCAACATCGAGGTCGCAATC		
13338F	GCCGAAATTCCTTATTTAAAACCTTTC		
13566F	TTAAAAGCTAATCCCTTAAAATA		
13802F	ACCCTGATACACAAAGGTACA		
14793F	AATTCACACAAAAATTTACATGT		

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. ^{a,b,c}, 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).

Gene	No. of alleles ^a	Fragment size	% of VI ^b	π^b	G+C content (%) ^b	Ka/Ks ^b	Intragenic recombination (Sawyer's test) ^a
<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>GPI2</i>	7	1,193-1,278	7.9	0.03	37.5	0.05	Yes ($P < 10^{-4}$)
<i>GPI5</i>	8 ^c	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 **Table S3.** Genetic characteristics of the seven polymorphic genes used for wPip characterization. VI; number of variable sites; π : pairwise
- 30 nucleotide diversity based on the average of all pairwise comparisons; ^a Characteristics estimated considering indels in sequence alignments; ^b
- 31 characteristics assessed excluding indels; ^c including the null *GPI5* wPip(JHB) allele. Note that primer regions were not considered in these
- 32 analyses.

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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	

34

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

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

39

40 **Table S5.** Summary of the *Culex pipiens* mitochondrial genome. Position: expressed in
41 nucleotides based on the Pel sequence. Direction of transcription: CW, clockwise; CCW,
42 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	10,302	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	G	C	A	Pel
<i>pi2</i>	G	-	-	-	-	-	-	-	-	-	-	G	-	-	-	G	-	-	-	-	-	-	Cot-A, Cot-B, Ma-B
<i>pi3</i>	G	-	-	-	-	-	-	-	-	T	-	-	-	-	-	G	-	-	-	-	-	-	Ep-A, Ep-B
<i>pi4</i>	G	-	-	C	-	A	-	-	-	-	-	G	-	-	-	G	-	A	-	-	-	-	Ko, Th
<i>pi5</i>	G	-	-	C	-	-	-	-	-	-	-	G	-	-	-	G	-	A	-	-	-	-	Bf-A
<i>pi6</i>	G	-	-	-	A	-	G	-	-	-	-	G	T	-	-	G	-	-	-	-	-	-	Au
<i>pi7</i>	G	-	-	-	A	-	-	-	-	-	-	G	T	-	-	G	-	-	-	-	-	-	Lv
<i>pi8</i>	G	C	-	-	A	-	-	C	-	-	-	G	T	G	-	G	-	-	-	-	-	-	Ke-A
<i>pi9</i>	G	C	-	-	A	-	-	-	-	-	-	G	T	-	-	G	-	-	-	-	-	-	Ke-B
<i>pi10</i>	G	-	-	-	A	-	-	-	-	-	-	G	T	-	G	G	-	-	-	-	-	-	Bf-B, Mc
<i>pi11</i>	G	-	-	-	A	-	-	-	-	-	-	G	T	-	G	G	-	-	-	-	G	-	Sl
<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

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

48 **Supplementary figures**

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

54

55 **Figure S2.** Examples of recombination breakpoints along the *pk1* (A, B), *pk2* (C, D, E) and
56 *GPI2* (F) sequences. For each alignment, only polymorphic sites around the breakpoints are
57 shown. Polymorphisms shared with the underlined sequence are highlighted in grey. Arrows
58 indicated the significant breakpoints and the nucleotide position detected by Sawyer's
59 procedure.

60

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

66

67 **Figure S4.** *Wolbachia* phylogenies constructed with six *wPip* polymorphic genes. **A:** *MutL*;
68 **B:** *ank2*; **C:** *pk1*; **D:** *pk2*; **E:** *GPI2*; **F:** *GPI5*. The phylogeny of the *RepA* gene was not
69 performed because the polymorphism with this gene is only based on the presence or the
70 absence of the transposon *Tr1*. The scale bar is in units of substitutions/site.

71

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

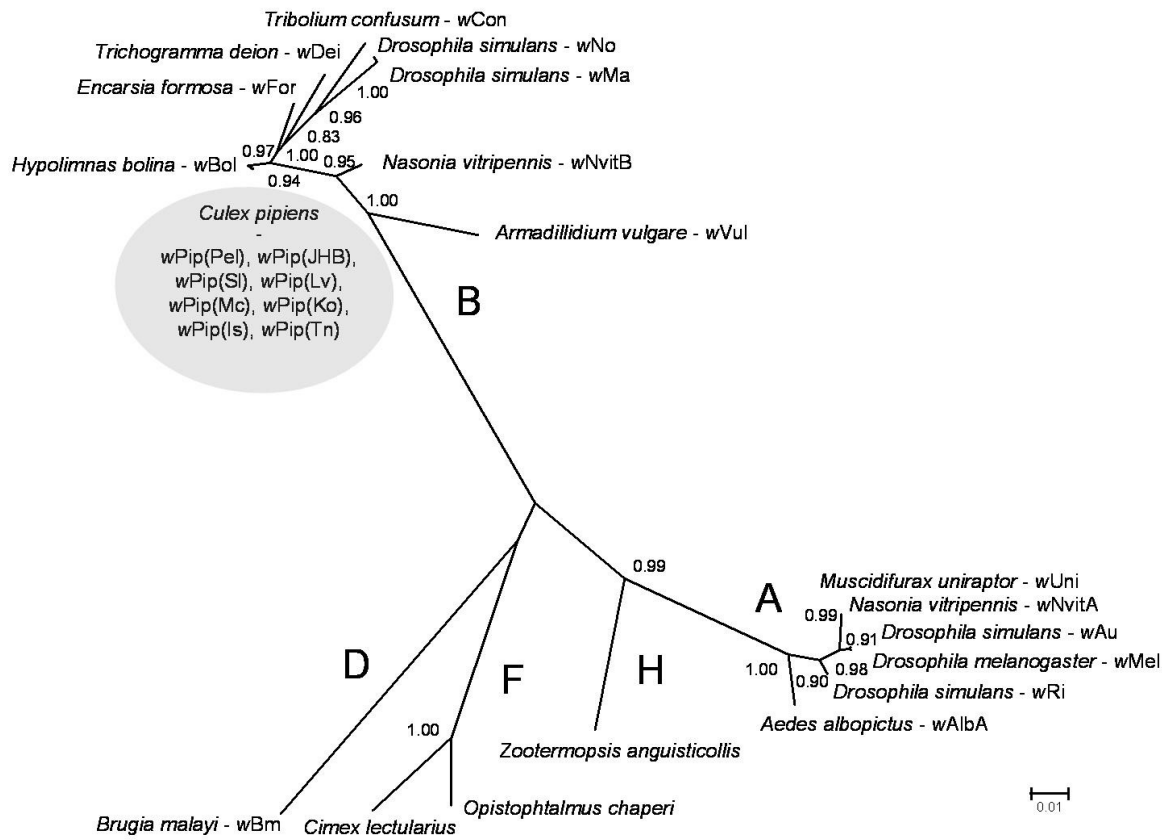


Figure S1

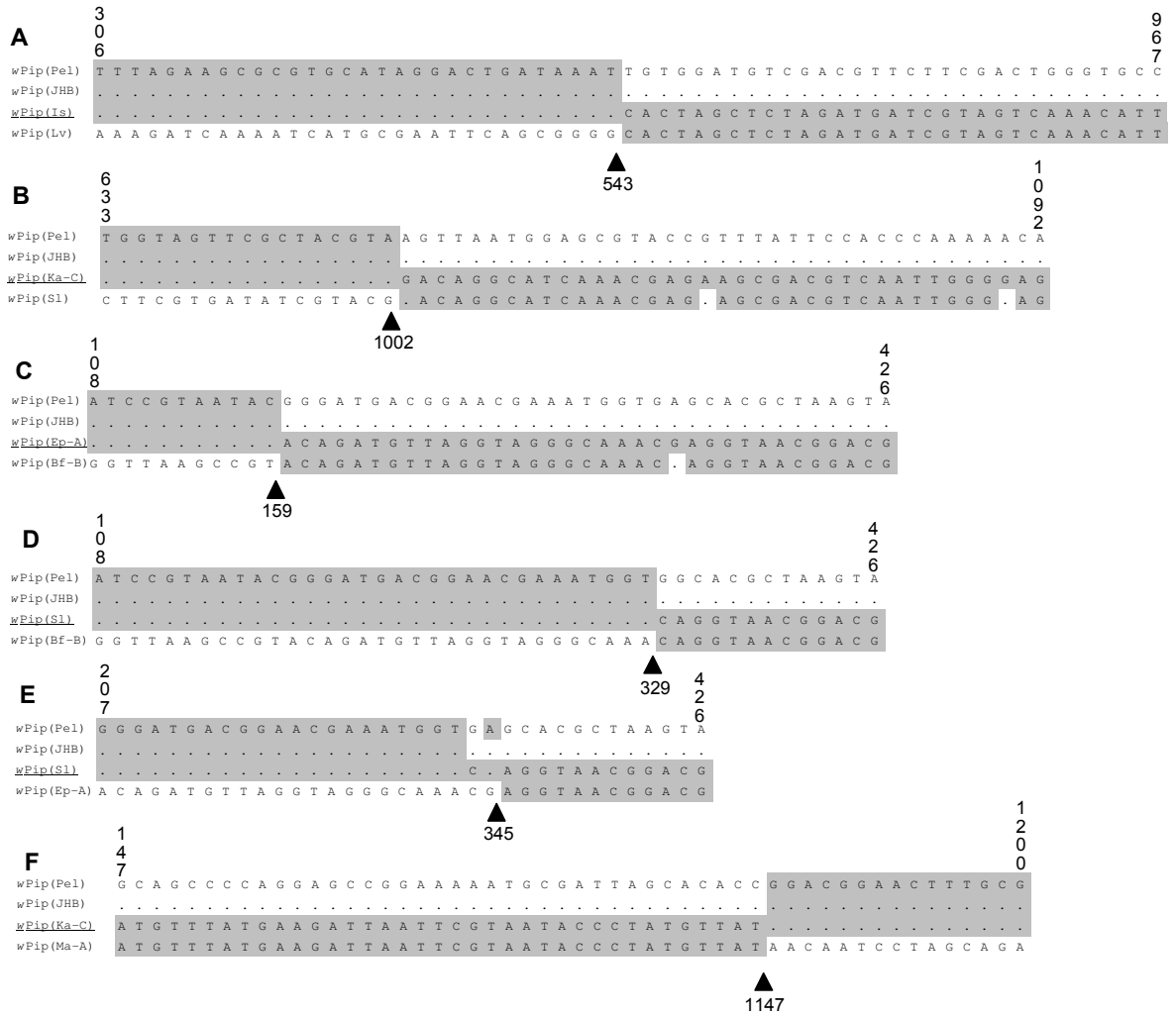
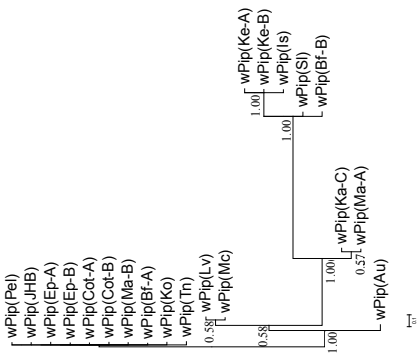
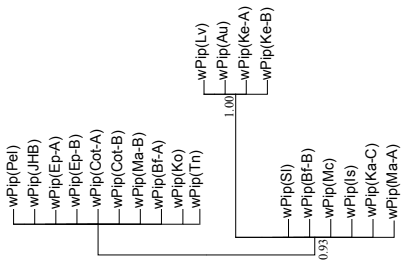


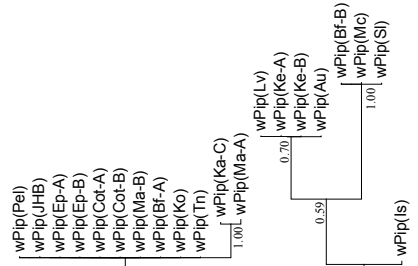
Figure S2



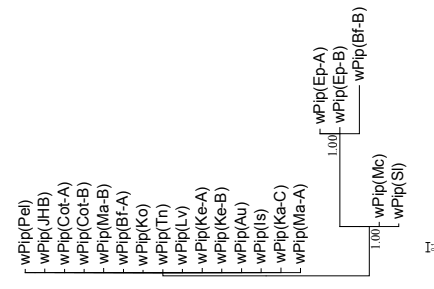
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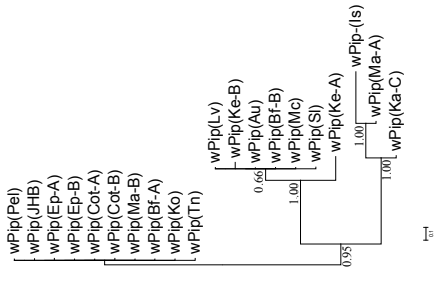
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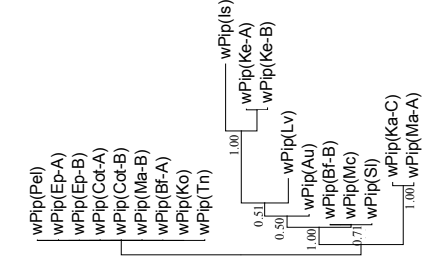
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E

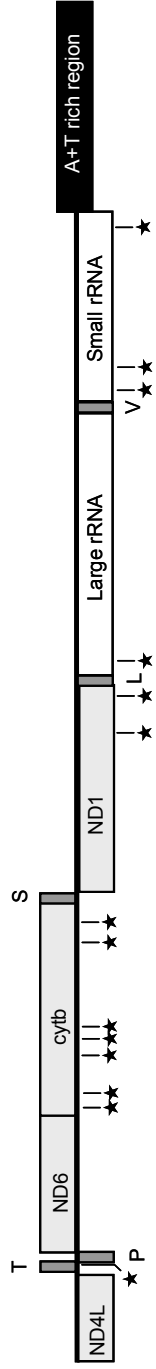
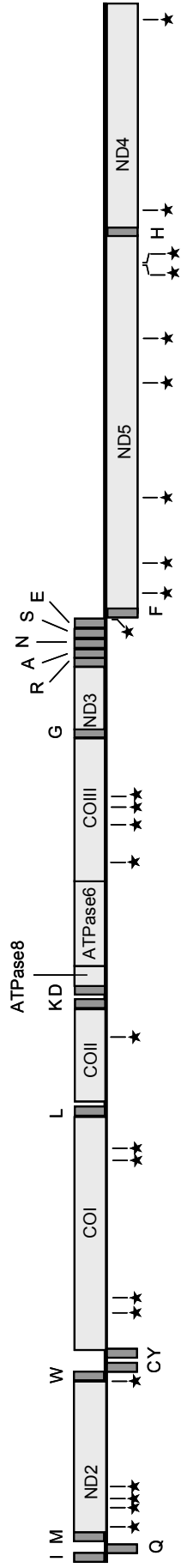


F

Figure S4

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95

96

Figure S5

97

98 **Literature Cited**

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