Figure S1 Locations of *Drosophila* universal primers, *RpL40.*

```
D. pseudoobscura   AACTGCCGCAAGAAGAAGTGCGGACACACCAACAACTTGCGCCCCAAGAAGAAGTTGAAGTAG
D. persimilis      AACTGCCGCAAGAAGAAGTGCGGACACACCAACAACTTGCGCCCCAAGAAGAAGTTGAAGTAG
D. grimshawi       AACTGCCGCAAGAAGAAGTGTGGACACACCAACAATCTGCGCCCCAAGAAGAAGTTGAAGTAG
D. sechellia       AACTGCCGCAAGAAGAAGTGCGGACACACCAACACCCTGCGCCCCAAGAAGAAGTTGAAGTAG
D. simulans        AACTGCCGCAAGAAGAAGTGCGGACACACCAACAACCTGCGCCCCAAGAAGAAGTTGAAGTAG
D. virilis         AACTGCCGCAAGAAGAAGTGCGGACACACCAACAACTTGCGCCCCAAGAAGAAGTTGAAGTAG
D. erecta          AACTGCCGCAAGAAGAAGTGCGGACACACCAACAACCTGCGCCCCAAGAAGAAGTTGAAGTAG
D. yakuba          AACTGCCGCAAGAAGAAGTGCGGACACACCAACAACCTGCGCCCCAAGAAGAAGTTGAAGTAG
D. ananassae       AACTGCCGCAAGAAGAAGTGTGGACACACCAACAACCTGCGTCCCAAGAAGAAGTTGAAGTAG
D. mojavensis      AACTGCCGCAAGAAGAAGTGTGGACACACCAACAACTTGCGTCCCAAGAAGAAGTTGAAGTAG
D. melanogaster    AACTGCCGCAAGAAGAAGTGCGGACACACCAACAACCTGCGCCCCAAGAAGAAGTTGAAGTAG
D. willistoni      AACTGCCGCAAGAAGAAGTGCGGACACACTAACAACTTGCGCCCCAAGAAGAAGTTGAAGTAG
Dros_RpL40_F       AACTGCCGCAAGAAGAAGTG
Dros_RpL40_R                                                  CCCAAGAAGAAGTTGAAGTAG


                     |330     |340     |350     |360     |370     |380
                   AACTGCCGCAAGAAGAAGTGYGGACACACYAACAMYYTGCGYCCCAAGAAGAAGTTGAAGTAG
                   ...................................................................
                                           •         •     •••     •
```
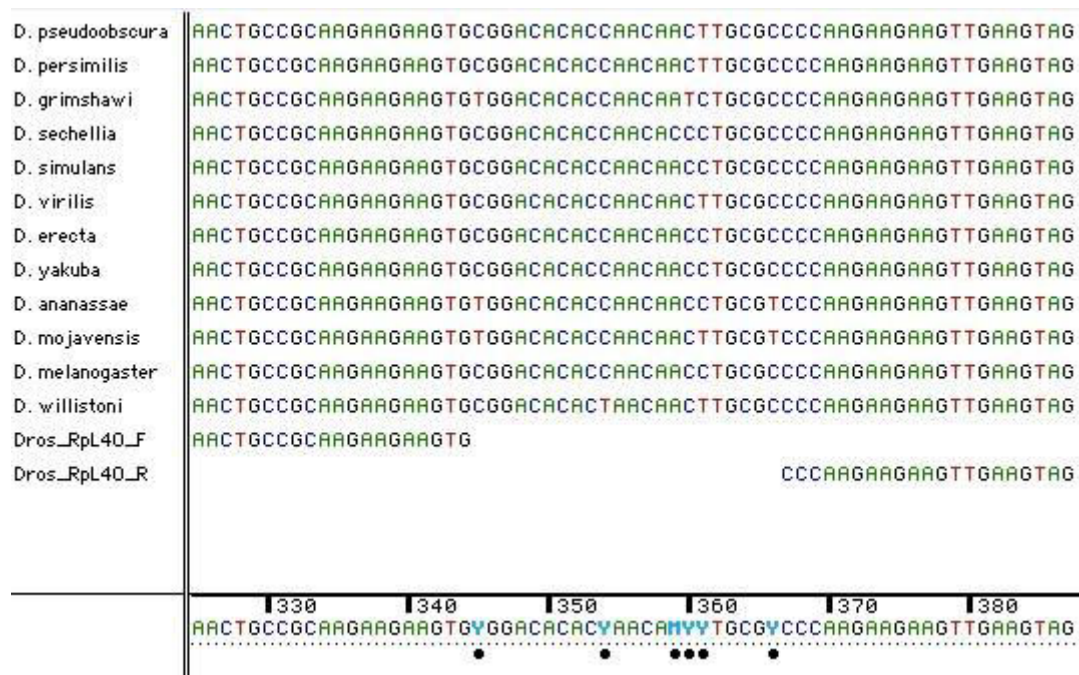
Table S1 Summary of sequences included in *Wolbachia* species tree analysis (Fig 1). 'Reference' refers to the publication describing the sequences used, '*Wolbachia* strain name' is listed only if referred to in the reference publication and 'isolate number' refers to the identity of the sequence in the *Wolbachia* MLST database at http://pubmlst.org/wolbachia/ (Jolley and Maiden, 2010). Where sequences were obtained from whole genome sequences, the region used has been listed next to the 'Genbank accession'.

Host species name	*Wolbachia* strain name	Reference	Isolate number	Genbank accession
Drosophila bifasciata	N/A	(Baldo *et al*, 2006)	isolate 5	*coxA* - DQ842279.1
	"	"	"	*gatB* - DQ842427.1
	"	"	"	*hcpA* - DQ842390.1
	"	"	"	*fpbA* - DQ842353.1
	"	"	"	*ftsZ* - DQ842315.1
	"	"	"	*wsp* - DQ842463.1
D. innubila	N/A	(Baldo *et al*, 2006)	isolate 6	*coxA* - DQ842280.1
	"	"	"	*gatB* - DQ842428.1
	"	"	"	*hcpA* - DQ842391.1

"	"	"	*fpbA* - DQ842354.1
"	"	"	*ftsZ* - DQ842316.1
"	"	"	*wsp* - DQ842464.1
D. melanogaster	*w*Mel	(Wu *et al*, 2004)	N/A	*coxA* - AE017196.1; 280987-281432
	"	"	"	*gatB* - AE017196.1; 131993-132423
	"	"	"	*hcpA* - AE017196.1; 466250-465885
				fpbA - AE017196.1; 1185651-
	"	"	"	1186117
	"	"	"	*ftsZ* - AE017196.1; 699096-699564
				wsp - AE017196.1; 1023310 -
	"	"	"	1023601
D. pandora	MK	(Richardson *et al*, 2016)	N/A	*coxA* – KU686370.1
	"	"	"	*gatB* – KU686373.1
	"	"	"	*hcpA* – KU686374.1

	"	"	"	*fpbA* – KU686371.1
	"	"	"	*ftsZ* – KU686372.1
	"	"	"	*wsp* – KU686375.1
D. pandora	*w*Ri	(Richardson *et al*, 2016)	N/A	*coxA* – KU686364.1
	"	"	"	*gatB* – KU686367.1
	"	"	"	*hcpA* – KU686368.1
	"	"	"	*fpbA* – KU686365.1
	"	"	"	*ftsZ* – KU686366.1
	"	"	"	*wsp* – KU686369.1
D. simulans	*w*Ri	(Baldo *et al*, 2006)	isolate 11	*coxA* - DQ842285.1
	"	"	"	*gatB* - DQ842433.1
	"	"	"	*hcpA* - DQ842396.1
	"	"	"	*fpbA* - DQ842359.1
	"	"	"	*ftsZ* - DQ842323.1

"	"		"	*wsp* - DQ842468.1
D. simulans	*w*Au	(Kriesner *et al*, 2013)	isolate 10	*coxA* - KF278668.1
"	"		"	*gatB* - KF278671.1
"	"		"	*hcpA* - KF278672.1
"	"		"	*fpbA* - KF278669.1
"	"		"	*ftsZ* - KF278670.1
"	Paraskevopoulos et al., unpublished			*wsp* - DQ235409.1
Nasonia longicornus	2.1	(Baldo *et al*, 2006)	isolate 16	*coxA* - DQ842295.1
"	"		"	*gatB* - DQ842443.1
"	"		"	*hcpA* - DQ842406.1
"	"		"	*fpbA* - DQ842369.1
"	"		"	*ftsZ* - DQ842331.1
"	"		"	*wsp* - DQ842478.1

Culex quinquefasciatus				
Pel	*w*Pip	(Klasson *et al*, 2008)	N/A	*coxA* - AM999887.1; 79211-78766
	"	"	"	*gatB* - AM999887.1; 84844-85266
	"	"	"	*hcpA* - AM999887.1; 1287994 - 1288353
	"	"	"	*fpbA* - AM999887.1; 1152376- 1152835
	"	"	"	*ftsZ* - AM999887.1; 624131-624599
	"	"	"	*wsp* - AM999887.1; 1006350- 1006062
Rhagoletis cerasi	*w*Cer5	(Arthofer *et al*, 2011)	isolate 70	*coxA* - KJ546843.1
	"	"	"	*gatB* - KJ546847.1
	"	"	"	*hcpA* - KJ546855.1
	"	"	"	*fpbA* - KJ546851.1
	"	"	"	*ftsZ* - KJ546858.1

	"	"		*wsp* - EU344971.1
Acraea encedon	N/A	(Baldo *et al*, 2006)	isolate 22	*coxA* - DQ842269.1
	"	"	"	*gatB* - DQ842417.1
	"	"	"	*hcpA* - DQ842380.1
	"	"	"	*fpbA* - DQ842343.1
	"	"	"	*ftsZ* - DQ842306.1
	"	"	"	*wsp* - DQ842454.1
Ostrinia scapulalis	N/A	(Baldo *et al*, 2006)	isolate 32	*coxA* - DQ842298.1
	"	"	"	*gatB* - DQ842446.1
	"	"	"	*hcpA* - DQ842409.1
	"	"	"	*fpbA* - DQ842372.1
	"	"	"	*ftsZ* - DQ842334.1
	"	"	"	*wsp* - DQ842481.1
Brugia malayi	N/A	(Baldo *et al*, 2006)	isolate 37	*coxA* - DQ842273.1

"	"	"	*gatB* - DQ842421.1
"	"	"	*hcpA* - DQ842384.1
"	"	"	*fpbA* - DQ842347.1
"	"	"	*ftsZ* - DQ842341.1
"	Garlipp and Isseroff, unpublished		*wsp* - AY527201.1
Onchocerca ochengi	*w*Oo	(Darby *et al*, 2012)	N/A	*coxA* - HE660029.1; 505781-506225
	"	"	"	*gatB* - HE660029.1; 814921-814507
	"	"	"	*hcpA* - HE660029.1; 605568-605203
	"	"	"	*fpbA* - HE660029.1; 437142-436676
	"	"	"	*ftsZ* - HE660029.1; 473656-474124
	"	"	"	*wsp* - HE660029.1; 675278 - 674989
Cimex lectularis	N/A	(Baldo *et al*, 2006)	isolate 36	*coxA* - DQ842275.1
	"	"	"	*gatB* - DQ842423.1
	"	"	"	*hcpA* - DQ842386.1
	"	"	"	*fpbA* - DQ842349.1

	"	"	"	*ftsZ* - DQ842311.1
	"	"	"	*wsp* - DQ842459.1
Folsomia candida	N/A	(Bordenstein *et al*, 2009)	N/A	*coxA* - FJ390247.1
	"	Baldo and Werren, unpublished	"	*gatB* - N/A
	"	Baldo and Werren, unpublished	"	*hcpA* - N/A
	"	Baldo and Werren, unpublished	"	*fpbA* - N/A
	"	Baldo and Werren, unpublished	"	*ftsZ* - N/A
	"	Li, unpublished	"	*wsp* - KP746935.1

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Table S2 Prior settings used in the *BEAST species tree analysis.

Gene	Partition prior settings	*coxA*	*fpbA*	*ftsZ*	*gatB*	*hcpA*	*wsp*
Best partitioning scheme		1+2+3	12+3	1+2+3	12+3	12+3	1+2+3
Codon position 1 partition	**Best model**	K80+I		TrN+I			HKY+I
	Proportion invariant (I): starting value [lower limit, upper limit]	0.75 [0.55, 0.85]		0.5 [0.15,0.85]			0.44 [0.34, 0.54]
	Nucleotide evolution model	HKY		TN93			HKY
	Base frequency setting	equal		estimated			estimated
	Kappa setting: starting value [lower limit, upper limit]	23 [5,40]		kappa1: 2.1 [0.5, 4]; kappa2: 11.5 [0.5, 23]			4 [2, 6]
Codon position 2 partition	**Best model**	HKY+I		K80+I			SYM+G
	Proportion invariant (I): starting value [lower limit, upper limit]	0.8 [0.6, 0.9]		0.71 [0.3, 0.9]			0

Gamma (G) category shape: starting value [lower limit, upper limit]				0.25 [0.15-0.4]
Nucleotide evolution model	HKY	HKY		GTR
Base frequency setting	estimated	equal		equal
Kappa setting: starting value [lower limit, upper limit]	10 [2, 20]	6 [1, 13]		
Mutation rate settings: starting value [lower limit, upper limit]				AC: 1 [0.5,2]; AG 1 [0.4,1.8]; AT 0.03 [0.001, 0.15]; CG 0.05 [0.001-0.2]; GT 0.03 [0.001, 0.1]

Codon position 1+2 partition

Best model	K80+I	HKY+I	TrN+G
Proportion invariant (I): starting value [lower limit, upper limit]	0.76 [0.66, 0.86]	0.76 [0.66, 0.86]	0
Gamma (G) category shape: starting value [lower limit, upper limit]			0.34 [0.14, 0.64]
Nucleotide evolution model	HKY	HKY	TN93
Base frequency setting	equal	estimated	estimated

	Kappa setting: starting value [lower limit, upper limit]		10 [5, 15]		20 [8, 36]	kappa1: 11.5 [5.5, 19.5]; kappa2: 23 [10, 36]	
Codon position 3 partition	Best model	GTR+G	GTR	HKY+I	HKY+I	HKY	TrN
	Proportion invariant (I): starting value [lower limit, upper limit]	0	0	0.17 [0.07, 0.27]	0.18 [0.08, 0.28]	0	0
	Gamma (G) category shape: starting value [lower limit, upper limit]	1.5 [0.5, 2.5]					
	Nucleotide evolution model	GTR	GTR	HKY	HKY	HKY	TN93
	Base frequency setting	estimated	estimated	estimated	estimated	estimated	estimated
	Kappa setting: starting value [lower limit, upper limit]			17 [11, 23]	28 [18, 44]	23 [13, 36]	kappa1: 17 [7, 27]; kappa2: 6.5 [2.5, 10.5]
	Mutation rate settings: starting value [lower limit, upper limit]	AC: 0.4 [0.24, 0.56]; AG 0.78 [0.48, 1.08]; AT: 0.02 [0.001, 0.04]; CG: 0.026 [0.001, 0.08]; GT 0.033 [0.001, 0.066]	AC 0.17 [0.07, 0.27]; AG: 1 [0.6, 1.4]; AT: 0.01 [0.001, 0.03]; CG: 0.27 [0.1, 0.45]; GT: 0.04 [0.001, 0.075]				