A reference genome for *Trichogramma kaykai*: A tiny desert-dwelling parasitoid wasp with competing sex-ratio distorters

Jack Culotta & Amelia RI Lindsey*

Department of Entomology, University of Minnesota, Saint Paul, MN, USA 51108

*corresponding: alindsey@umn.edu

JC ORCID: 0000-0002-2533-0802

ARIL ORCID: 0000-0003-3354-7419

ABSTRACT

The tiny parasitoid wasp *Trichogramma kaykai* inhabits the Mojave Desert of the southwest United States. Populations of this tiny insect variably host up to two different sex-distorting genetic elements: (1) the endosymbiotic bacterium *Wolbachia* which induces the parthenogenetic reproduction of females, and (2) a B-chromosome, “Paternal Sex Ratio” (PSR), which converts would-be female offspring to PSR-transmitting males. We report here the genome of a *Wolbachia*-infected *Trichogramma kaykai* isofemale colony KSX58. Using Oxford Nanopore sequencing we produced a final genome assembly of 203 Mbp with 45x coverage, consisting of 213 contigs with an N50 of 1.9 Mbp. The assembly is quite complete, with 91.41% complete BUSCOs recovered: a very high score for Trichogrammatids that have been previously characterized for having high levels of core gene losses. We also report a complete mitochondrial genome for *T. kaykai,* and an assembly of the associated *Wolbachia*, strain *w*Tkk. We identified copies of the parthenogenesis-inducing genes *pifA* and *pifB* in a remnant prophage region of the *w*Tkk genome. The *Trichogramma kaykai* assembly is the highest quality genome assembly for the genus to-date and will serve as a great resource for understanding the evolution of sex and selfish genetic elements.

Key words

Wolbachia, sex ratio, selfish genetic element, symbiosis, B chromosome, *Trichogramma kaykai*

INTRODUCTION

Trichogramma wasps (Hymenoptera: Trichogrammatidae) are some of the smallest animals on the planet (Polilov 2015). The genus contains more than 200 described species: all parasitoids that complete their development within the eggs of other insects (Burks et al. 2024; Pinto 2006). Trichogrammatid research has largely focused on (1) their application as biological control agents of insect pests (Knutson 1998; Cherif et al. 2021), (2) innovations associated with extreme miniaturization (Polilov 2012), and (3) sex allocation, especially due to relationships with sex-distorting elements (Stouthamer et al. 1990; Stouthamer and Kazmer 1994; Russell and Stouthamer 2010). The most common sex-ratio distorter is the intracellular, maternally transmitted bacterium *Wolbachia,* a common associate of many arthropods and nematodes (Kaur et al. 2021). In *Trichogramma*, most *Wolbachia* strains are "parthenogenesis-inducing" (PI), and enable the asexual reproduction of females (i.e., "thelytokous parthenogenesis") (Stouthamer et al. 1990; Stouthamer et al. 1993; Ma and Schwander 2017).

To date all instances of microbe-mediated PI are in animals with haplodiploid sex determination (Ma and Schwander 2017; Verhulst et al. 2023). Under haplodiploidy (and without PI-*Wolbachia*) males typically develop from unfertilized (i.e., haploid) eggs, and females are typically derived from fertilized, diploid, eggs (De La Filia et al. 2015). PI-*Wolbachia* diplodize the unfertilized eggs, resulting in a female (Stouthamer and Kazmer 1994). In one species with PI-*Wolbachia*, *Trichogramma kaykai* (Figure 1A-B), a second sex-distorter is sometimes present: a supernumerary B-chromosome, "Paternal Sex Ratio" (PSR) (van Vugt et al. 2003; Stouthamer et al. 2001). PSR achieves the opposite outcome of *Wolbachia*'s PI: haploid males with PSR mate, and any fertilized eggs develop into more PSR-transmitting males (Van Vugt et al. 2009). PSR facilitates destruction of the paternal genome (except for itself), resulting in a haploid embryo (the maternal copy) and the untouched PSR chromosome. In populations where *Wolbachia* and PSR are present, a curious pattern of reproduction is present: males are derived

57 from fertilized eggs (with PSR-containing sperm), and females are derived from unfertilized
58 eggs (with PI-*Wolbachia*) (Figure 1C). Unlike many other PI-*Wolbachia* systems where PI is
59 accompanied by a decay of sexual function (Stouthamer et al. 2010; Russell and Stouthamer
60 2011; Jeong and Stouthamer 2005; Stouthamer and Mak 2002; Gottlieb and Zchori-Fein 2001),
61 *Trichogramma kaykai* are easily cured of their *Wolbachia* in the lab, and readily return to a fully
62 functional sexual form (Hohmann and Luck 2000; Hohmann et al. 2001; Miura and Tagami
63 2004; Russell et al. 2016). The PSR chromosome ensures males and sexual reproduction are
64 maintained.
65
66 As host to PI-*Wolbachia* and PSR, *Trichogramma kaykai* is a valuable model for understanding
67 the evolution of sex ratios and interactions between selfish genetic elements. This species was
68 described in 1997 (Pinto et al.) and is native to the deserts of the Southwest United States
69 (Figure 1D). We report a reference genome for an isofemale colony of *Trichogramma kaykai*
70 from the Mojave Desert, plus the genome of its PI-*Wolbachia* strain, *w*Tkk. To our knowledge,
71 there are currently no *Trichogramma kaykai* PSR chromosomes in culture, but this reference
72 genome will aid in future efforts to understand how this selfish element alters chromosome
73 dynamics and sex ratios.

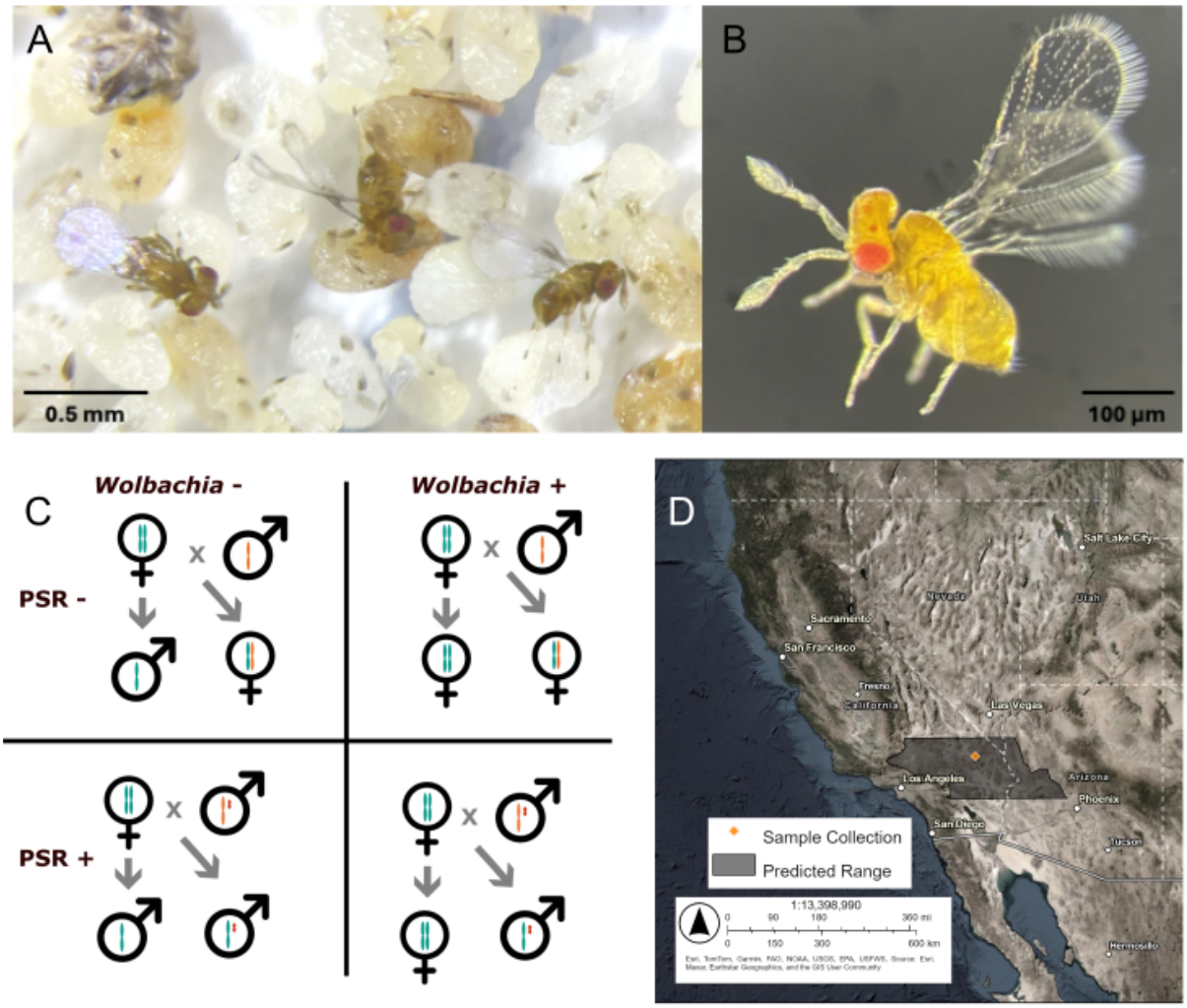


Figure 1. *Trichogramma kaykai* biology. (A) Three *Trichogramma kaykai* females ovipositing into host moth eggs (*Ephestia kuehniella*). **(B)** An exemplary specimen of *T. kaykai* (female). **(C)** Sex in *T. kaykai* is determined based on haplodiploidy, mediated by the presence or absence of *Wolbachia* (maternally transmitted) and the PSR chromosome (paternally transmitted). **(D)** The sample collection site for KSX58 and predicted geographic range of *Trichogramma kaykai*.

MATERIALS & METHODS

Species Origin and Sampling Strategy

Genome sequencing and assembly was performed for *Trichogramma kaykai* line “KSX58”, an isofemale laboratory culture. A single unmated *Wolbachia*-infected, thelytokous female was

85 reared out of a parasitized *Apodemia mormo* egg collected off an *Eriogonum inflatum* stem and
86 used to initiate an isofemale line. The founding female was collected in May 2010 in Kelso, CA,
87 USA, by R. Stouthamer and J. Russell (Figure 1D). The colony has since been maintained in 5
88 ml glass culture tubes stopped with cotton, and kept at 25°C with a 12:12 light:dark cycle.
89 Wasps are hosted every 12 days on sterilized *Ephestia kuehniella* eggs adhered to cardstock
90 alongside a streak of honey. *Wolbachia* infection status was confirmed by PCR with *Wolbachia*
91 specific “Wspec" primers (Werren and Windsor 2000), and *Trichogramma* species was
92 confirmed by molecular identification (Stouthamer et al. 1999), both as detailed previously
93 (Lindsey and Stouthamer 2017). To collect wasps for DNA extraction, freshly emerged females
94 were allowed to crawl up into a sterile tube attached to the colony culture vial. The pool of
95 wasps was flash frozen in liquid nitrogen and stored at -80°C for further processing.
96
97 **Geographic Range Map**
98 Locations of *Trichogramma kaykai* are centered around the Southern Mojave Desert (Pinto et
99 al. 1997; Russell et al. 2018; Tulgetske and Stouthamer 2012; Russell et al. 2016; Van Vugt et
100 al. 2009; van Vugt et al. 2003). The predicted northern and southern boundaries of this species’
101 range were estimated from these observations. As it is assumed *Trichogramma kaykai* is
102 restricted to desert habitat, the eastern and western borders of range are indicated by the
103 Southern Mojave Desert and Northern Sonoran Desert. The map was generated in ArcGIS
104 Online (www.arcgis.com).
105
106 **Sequencing Methods and Sample Preparation**
107 DNA was extracted from 25 mg of whole insect tissues using the MagAttract High Molecular
108 Weight kit (Qiagen), following manufacturer’s instructions. The DNA was concentrated to 25 uL
109 using Sergi Lab Supplies magnetic beads and went through the PacBio SRE kit to deplete
110 fragments shorter than 10kb. The sample was barcoded and library prepped with the ONT SQK-

111 NBD114.24 kit. The libraries were sequenced on a P2 Solo instrument using PromethION
112 10.4.1 flow cells. Every 24 hours the libraries were recovered and flowcells were flushed with
113 nuclease (EXP-WSH004 kit) and reloaded.
114
115 **Nuclear Genome Assembly, Curation, and Quality Control**
116 Samples were originally basecalled within Minknow using 'super accuracy' mode with
117 5mC_5hmC modified base calling. Reads were then re-basecalled with dorado v.0.7.2 using
118 basecall model dna_r10.4.1_e8.2_400bps_sup\@v5.0.0. Reads at least 5kb in length were
119 maintained, processed with 'dorado correct', and used for generating an assembly with Hifiasm
120 v.0.19.9 and default parameters. The genome was manually curated, and cytoplasmic genomes
121 were identified through tblastn results implemented in Blobtools v.1.1.1 (Challis et al. 2020).
122 Assemblies were assessed with Compleasm v.0.2.6 (Huang and Li 2023) with the hymenoptera
123 lineage flag ('-l hymenoptera').
124
125 **Genomic Methylation**
126 Methylation and hydroxymethylation of genomic DNA at 5' cytosines (5mC and 5hmC) in a
127 cytosine-guanine dinucleotide (CpG) context was determined from the basecalling information
128 stored in the unmapped modBAM files (Flack et al. 2024). These were aligned to the final
129 assembly using Minimap v.2.17 (Li 2016), converted to bedMethyl format with Modkit v.0.4.1
130 (https://github.com/nanoporetech/modkit), and the 5mC and 5hmC percentages were calculated
131 with an AWK script.
132
133 ***Trichogramma* Phylogeny**
134 A whole-genome phylogeny was reconstructed with SANS v.2.4_10, which uses a pangenomic
135 approach to calculate splits in a phylogenetic tree (Rempel and Wittler 2021). SANS parameters
136 included '--filter strict' with an output Newick tree file and 100 bootstrap replicates. Taxa

137 included the available *Trichogramma* genomes (for *Trichogramma brassicae*, which is
138 represented by two assemblies, only GCA_902806795.1 was used; Table 1), and an outgroup
139 species from a closely related family (Cruaud et al. 2024), *Phymastichus coffea* (Hymenoptera:
140 Eulophidae) GCF_024137745.1. Tree topology was configured in FigTree v.1.4.4
141 (https://github.com/rambaut/figtree/) and annotated in Inkscape (https://www.inkscape.org).
142
143 **Table 1. Available *Trichogramma* genome assemblies.**

Species	Accession	Size (bp)	Contig/ Scaffold Count*	N50 (bp)*	*Wolbachia* Genome	Citation
Trichogramma brassicae	GCA_902806795.1	235,386,796	1,570 (C)	556,663	No	Ferguson et al. (2020)
Trichogramma brassicae	GCA_030522885.1	203,810,232	87,792 (S)	18,131	No	Guinet et al. (2023)
Trichogramma dendrolimi	GCA_034770305.1	215,209,100	316 (S)	1,412,680	No	Zhang et al. (2023)
Trichogramma evanescens	GCA_902732785.1	213,671,129	146,286 (S)	38,173	No	N/A
Trichogramma pretiosum	GCA_000599845.3	187,641,947	925 (S)	1,825,723	*w*Tpre (Lindsey et al. 2016)	Lindsey et al. (2018a)
Trichogramma kaykai	Processing	203,423,343	213 (C)	1,898,390	*w*Tkk	This study

144 *If assembly is scaffolded, metrics reported are for scaffolds and an (S) is indicated in the count column. If
145 there are only contigs, those metrics are reported and (C) is indicated in the count column.
146
147 **Repeat Assembly Techniques**
148 We identified and masked repetitive sequences in each genome. First a custom *de novo* repeat
149 library was crated with RepeatModeler v.2.0.5 (Flynn et al. 2020) with the -LTRStruct parameter
150 included. Then this library was used to mask the genome with RepeatMasker v.4.1.1 (Tarailo-
151 Graovac and Chen 2009) with the -s (sensitive mode) parameter included.
152
153

154 **Gene Finding Methods**

155 To annotate the *T. kaykai* genome, a soft masked genome was used for gene model prediction
156 with Galba v.1.0.11 (Brůna et al. 2023), using the RefSeq annotations for *Trichogramma*
157 *brassicae* (GCA_902806795.1), *Trichogramma pretiosum* (GCA_000599845.3), *Nasonia*
158 *vitripennis* (GCA_009193385.2), *Copidosoma floridanum* (GCF_000648655.2), *Phymastichus*
159 *coffea* (GCF_024137745.1) and *Ceratosolen solmsi marchali* (GCF_000503995.2) as
160 references. The Galba pipeline was executed using Singularity with parameters to output a gff3
161 file. Summary statistics for the resulting gff3 file were computed with GAG v.2.0.1 (Geib et al.
162 2018). Split genes (those encoded across the ends of two contigs) were manually re-assigned
163 gene identifiers as per NCBI best practices.

164

165 **Synteny Analysis**

166 We identified conserved regions and mapped synteny between the *T. kaykai* and *T. pretiosum*
167 genomes (Table 1) using D-GENIES webtool (https://dgenies.toulouse.inra.fr/run) (Cabanettes
168 and Klopp 2018) employing Minimap v.2.28 (Li 2016), the “many repeats” flag, and the “hide
169 noise” option.

170

171 **Mitogenome**

172 A single circular contig was identified as the mitochondrial genome based on GC content, size,
173 and coverage. Mitogenome annotation was completed with MITOS2 v.2.1.9 (Bernt et al. 2013;
174 Donath et al. 2019) and the circular mitogenome was started at Cox1 per convention with
175 rearrangement in SnapGene v.7.2. MITOS2 parameters were the RefSeq63 Metazoa reference
176 and the invertebrate mitochondrial translation code. Manual curation of the control region and
177 inferences of gene structure were made based on comparisons to other *Trichogramma*
178 mitochondrial genomes (Chen et al. 2018).

179

180 ***Wolbachia* Strain *w*Tkk Genome**

181 Four contigs were identified as a *Wolbachia* genome based on cumulative size and Blobtools
182 results. Genome completeness was analyzed against the rickettsiales_odb10 database with
183 Compleasm v.0.2.6 (Huang and Li 2023). Prophage regions and mobile elements were
184 identified with VirSorter2 v.2.2.4 (Guo et al. 2021) and mobileOG-db v.1.0.1 (Brown et al. 2022),
185 implemented in proksee (Grant et al. 2023)(https://proksee.ca/) with default parameters. To
186 identify putative parthenogenesis-inducing genes (*pifs*) (Fricke and Lindsey 2024), we leveraged
187 annotation and orthology data generated by Prokka v.1.14.6 (Seemann 2014) and OrthoFinder
188 v.2.5.4 (Emms and Kelly 2019), implemented in the *Wolbachia* Phylogeny Pipeline (WHOP;
189 https://github.com/gerthmicha/WHOP). Phylogenetic analysis was performed based on the
190 clustering results from WHOP/OrthoFinder results. Single-copy orthologs were aligned with
191 MAFFT L-INS-i v.7.487 (Katoh and Standley 2013), recombining genes were eliminated with
192 PhiPack v.1.1 (Bruen and Bruen 2005), and alignments were concatenated for phylogenetic
193 reconstruction in IQtree v.2.2.3 (Nguyen et al. 2015), run with model optimization and 1000
194 ultrafast bootstrap replicates.

195

196 **RESULTS & DISCUSSION**

197 **Sequencing and Assembly**

198 We generated 10.5 billion base pairs of nanopore sequencing data: a total of 1,543,039 reads
199 with a read N50 of 13,472 (Supplementary Table S1). A draft assembly from reads longer than
200 5,000 base pairs was generated with HiFiasm which produced a 204.6 Mbp assembly contained
201 in 226 contigs. A combination of coverage, GC%, and blast hits from BlobTools results were
202 used to identify non-nuclear contigs and curate the assembly. After removing spurious and
203 contaminant contigs (Supplemental Table S2), and extracting the *Wolbachia w*Tkk and
204 mitochondrial genomes, the final assembly was 203.4 Mbp in 213 contigs, with an average of
205 45x coverage (Table 2). The *Trichogramma kaykai* assembly falls in the middle of the size

206 range for the genus, (187.6 Mbp in *T. pretiosum* to 235.4 Mbp in one of the *T. brassicae* (Table
207 1). Additionally, this size closely aligns with a flow cytometry-based estimate of 216 Mbp for a
208 different colony of *T. kaykai*, "LC19-1" (van Vugt et al. 2005). The GC% of *Trichogramma*
209 genomes appears to be highly conserved, with all at 40%.
210
211 Quality assessments indicate that the genome assembly is quite complete, with 91.41% of
212 Hymenopteran BUSCO loci present as complete coding sequences (Table 2). These metrics
213 are on par with other well-assembled *Trichogramma* genomes (e.g., *T. pretiosum,* Hymenoptera
214 BUSCO: C:92.7%[S:90.9%,D:1.8%],F:0.8%,M:6.5%,n:5991)(Lindsey et al. 2018a). Comparative
215 genomics of *T. pretiosum* relative to other hymenopterans indicated that these wasps have
216 undergone a large number of core gene losses and have highly accelerated rates of protein
217 evolution (Lindsey et al. 2018a) so we do not expect BUSCO scores close to 100% even for a
218 "perfect" assembly.
219
220 **Table 2. *Trichogramma kaykai* genome assembly statistics.**

Metric	**Draft**	**Final**
Contigs	226	213
Total Length (bp)	204,642,443	203,423,343
Min contig length	5,287	6,150
Average contig length	905,498	955,039
Max contig length	6,625,044	6,625,044
N50	1,898,390	1,898,390
L50	34	34
%GC	39.60	39.63
Compleasm*	91.44% [S:90.32%, D: 1.12%] F: 0.78%, M:7.78%, n=5991	91.41% [S:90.29%, D: 1.12%] F: 0.82%, M:7.78%, n=5991

221 *Standard BUSCO annotation: Complete BUSCOs (C) [Complete and single-copy BUSCOs (S),
222 Complete and duplicated BUSCOs (D)], Fragmented BUSCOs (F), Missing BUSCOs (M), Total BUSCO
223 groups searched (n). Hymenoptera dataset used for determining completeness.
224

225 **Genome Methylation**

226 We determined 5' methylation at cytosines in a CpG context based on the direct sequencing
227 basecalls. Less than 1% of CpGs were methylated: 0.67% of CpGs had 5mC (methyl)
228 modifications and 0.18% had 5hmC (hydroxymethyl) modifications. While this is a low level of
229 methylation as compared to vertebrates, this is not atypical for insects (Hunt et al. 2013).
230 Importantly, this level of methylation closely mirrors the number of methylated CpG sites
231 identified in *T. pretiosum* using bisulfite sequencing (Lindsey et al. 2018a; Wu et al. 2020).
232

233 **Analysis of Repetitive DNA**

234 *Trichogramma kaykai* is sister to all other *Trichogramma* species with published genomes
235 (Figure 2A). Across the genus, repetitive content appears to be relatively conserved. Repetitive
236 sequences account for between 17.9% - 29.39% of the total genome lengths (Table 1, Figure
237 2B, Supplemental Table S3). This is in contrast to the outgroup species, *Phymastichus coffea*
238 (Hymenoptera: Eulophidae), that has a 421 Mbp genome with more than half (57.21%)
239 attributed to repetitive sequences (Figure 2B, Supplemental Table S3). Across *Trichogramma*,
240 the majority of repetitive sequences are unclassified. In *T. kaykai*, 4% of the genome is derived
241 from retroelements, <1% from DNA transposons, and around 3% of the genome is simple and
242 low complexity repeats (Table 3). We then assessed the level of synteny between *T. kaykai* and
243 *T. pretiosum* by cross-mapping similar genomic sequences with D-GENIES (Figure 3). A large
244 proportion (60.34%) of the *T. kayaki* genome shares 50-75% identity with *T. pretiosum*, and
245 there are high levels of synteny across the two assemblies (Figure 3).
246

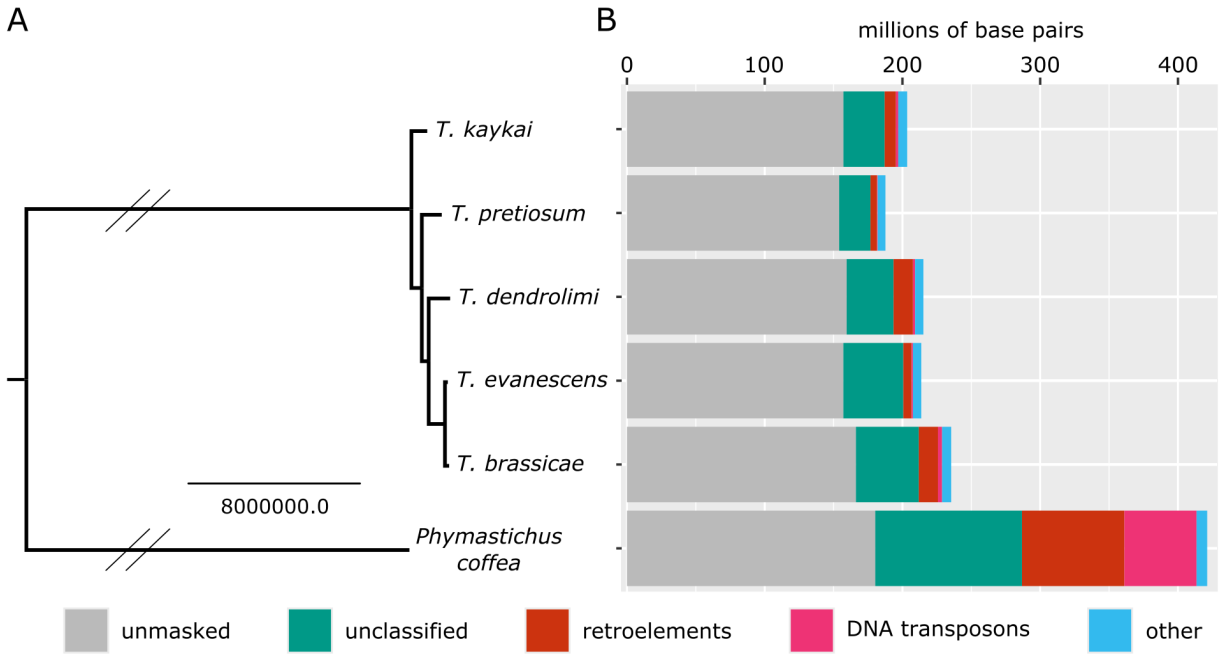


Figure 2. Comparative genomics of *Trichogramma.* (A) Whole genome phylogeny of five *Trichogramma* species and outgroup *Phymastichus coffea* (Hymenoptera: Eulophidae). Double slashes indicate branches that were shortened to half their length for ease of visualization. **(B)** Repetitive content of each genome, corresponding to the taxa in (A). “Other” includes rolling circles, simple repeats, and low complexity repeats.

255 **Table 3. Interspersed repeats in *Trichogramma kaykai.***

Name	Number	Length (bp)	Percent (%)
Retroelements	8,591	8,132,788	4.00%
Penelope class	203	76,919	0.04 %
LINE class	5,232	5,084,665	2.50 %
L2/CR1/Rex	674	423,279	0.21 %
R1/LOA/Jockey	1,929	1,977,765	0.97 %
R2/R4/NeSL	2,056	2,301,966	1.13 %
LTR class	3,359	3,048,123	1.50 %
BEL/Pao	484	436,375	0.21 %
Ty1/Copia	497	368,036	0.18 %
Gypsy/DIRS1	2,378	2,243,712	1.10 %
DNA transposons	2,812	1,863,150	0.92 %
hobo-Activator	497	176,867	0.09 %
Tc1-IS630-Pogo	370	118,645	0.06 %
Rolling-circles	797	350,441	0.17 %
Unclassified	110,502	29,820,645	14.66 %
Total interspersed repeats		39,816,583	19.57 %
Simple repeats	147,687	5,403,933	2.66 %
Low complexity	15,687	704,136	0.35 %
Bases masked		46,275,093	22.75 %

256

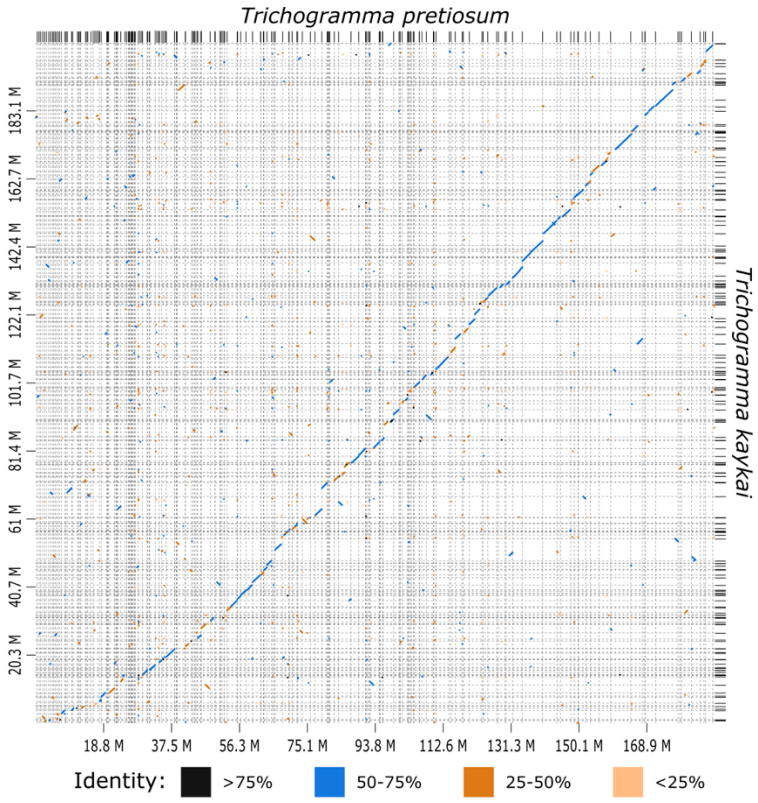


Figure 3. Synteny is highly conserved between *T. kaykai* and *T. pretiosum*. Dot plot indicating syntenic regions between *Trichogramma* genomes. Dots are colored according to percent identity.

Genome Annotation

We annotated the *T. kaykai* genome using a set of protein sequences from other wasps in the superfamily Chalcidoidea as a reference and identified 20,798 genes (Table 4). These genes corresponded to 24,714 transcripts, with a mean of four exons per mRNA (Table 4). Compared to other *Trichogramma* species, this is a larger number of annotated genes (e.g., 13,395 in *T. pretiosum*, 16,905 in *T. brassicae*). However, this could be due to differences in annotation pipelines, and or, lineage-specific differences in the patterns of gene gain and loss.

270 **Table 4. Annotation metrics for the *Trichogramma kaykai* genome.**

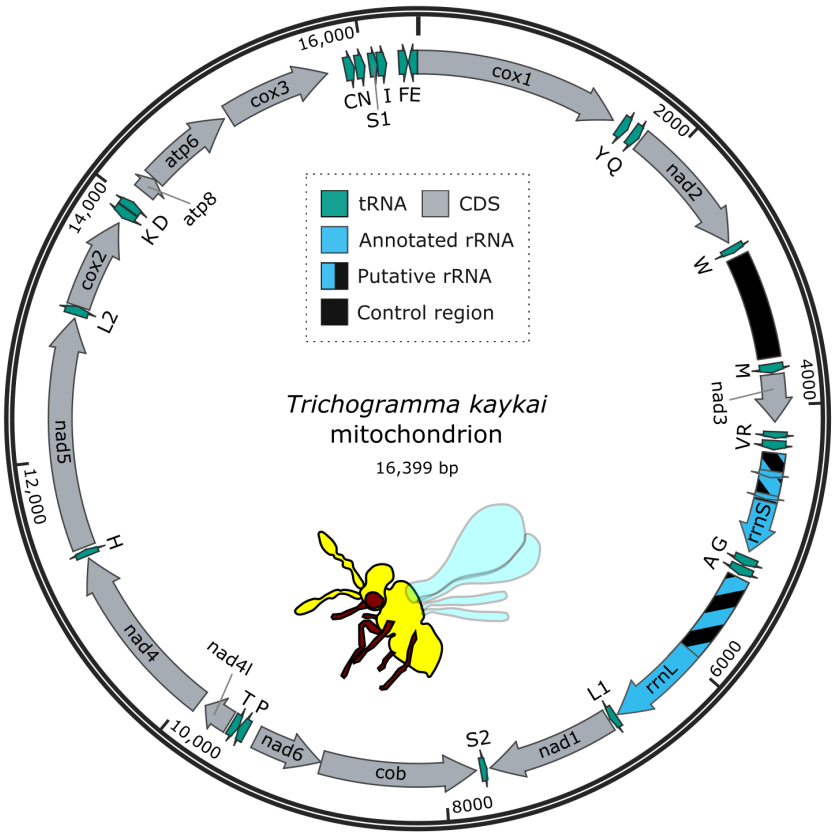
Metric	Value*
Number of genes	20,798
Number of mRNAs	24,714
Number of exons	116,714
Number of introns	92,000
Mean exons per mRNA	4
Total gene length	102,108,152 bp
Longest gene	160,105 bp
Mean gene length	4,910 bp
Longest CDS	54,789 bp
Mean CDS length	1,462 bp
Longest exon	14,469 bp
Mean exon length	310 bp

271 *Base pairs = bp

272

273 **Mitogenome**

274 We identified the mitogenome based on GC content (14.81%), size (16,399 bp), and coverage
275 (3708x). Annotation revealed all expected mitochondrial tRNAs and coding genes (Figure 4).
276 MITOS2 annotated a single large rRNA of only 712 bp and three regions (387, 49, and 38 bp)
277 as small rRNAs. Comparison to other Trichogrammatid mitochondrial genomes indicated that
278 the large rRNA annotation had been truncated on the 5' end, and the small rRNA annotation
279 had been fragmented (Figure 4), which is likely due to the extreme divergence of these
280 mitochondrial sequences. A 878 bp region between the tRNAs for tryptophan (W) and
281 methionine (M) corresponds to the putative control region identified in other Trichogrammatid
282 mitochondrial genomes (Chen et al. 2018).



283

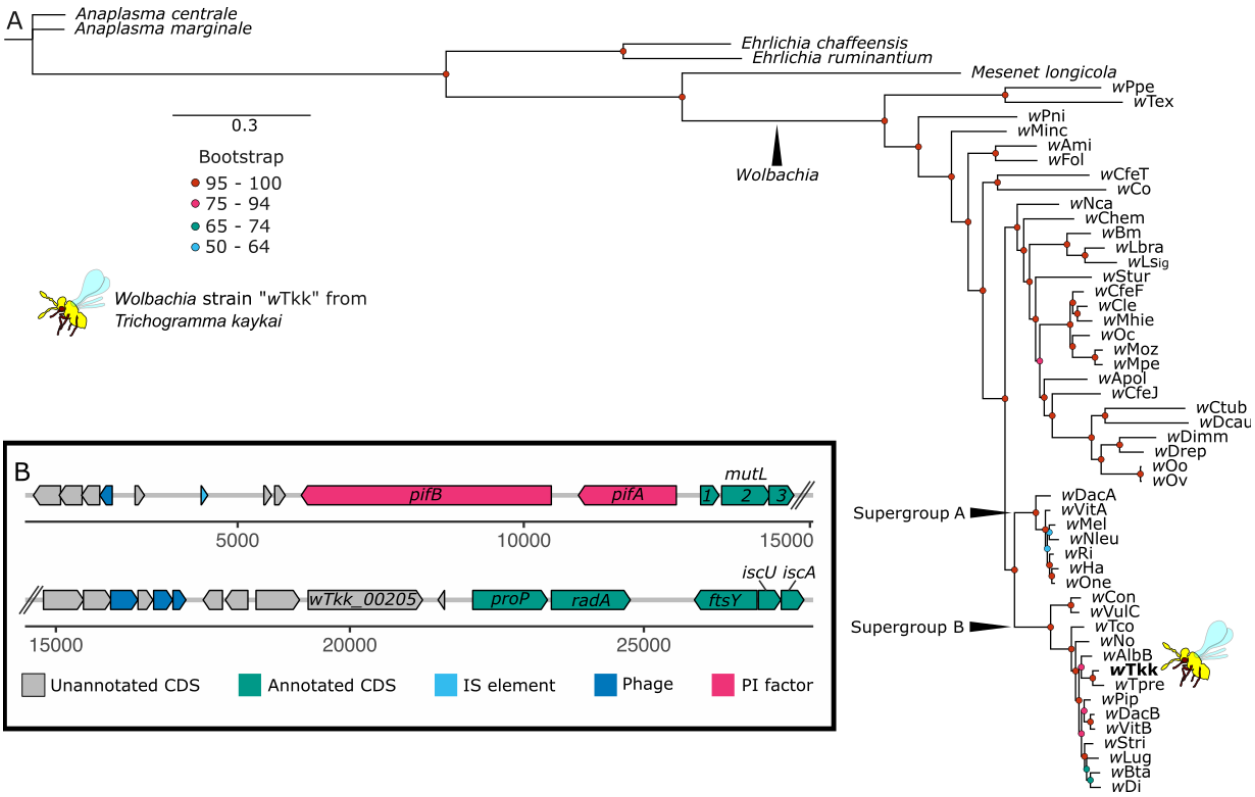
284 **Figure 4. Mitochondrial genome of *Trichogramma kaykai*.** Genes were annotated with MITOS2 (Bernt
285 et al. 2013). Putative regions of rRNAs that were not correctly annotated by MITOS2 are indicated with
286 stripes. The control region and the putative full length rRNAs were identified based on homology and
287 gene order of other *Trichogramma* mitochondria (Chen et al. 2018). Transfer RNAs (tRNAs) are denoted
288 by IPUC-IUB amino acid codes.

289

290 **Parthenogenesis-Inducing *Wolbachia* Strain *w*Tkk**

291 We assembled a near-complete *Wolbachia* genome of the *w*Tkk strain: ~1.12Mbp contained in
292 four contigs, sequenced at 55X coverage (Table 5). Phylogenetic reconstruction revealed that
293 *w*Tkk is in the "Supergroup B" clade of *Wolbachia*, and is sister to *w*Tpre, which infects
294 *Trichogramma pretiosum* (Lindsey et al. 2016)(Figure 5A). The *w*Tkk and *w*Tpre genomes are
295 similar in size: the *w*Tpre assembly (a single scaffold) is just slightly larger at 1,133,709 bp
296 (Lindsey et al. 2016). We queried the *w*Tkk proteins to identify the recently identified

297 parthenogenesis inducing factors, *pifA* and *pifB* (Fricke and Lindsey 2024). We identified a
298 single copy of each gene in the *w*Tkk genome, encoded next to each other within a remnant
299 prophage region (Figure 5B), as is typical of many other *Wolbachia* loci that induce host
300 reproductive manipulations (Fricke and Lindsey 2024; LePage et al. 2017; Lindsey et al. 2018b;
301 Shropshire et al. 2018; Perlmutter et al. 2019; Bordenstein and Bordenstein 2016). The *w*Tkk
302 PifA protein was 67% identical to the PifA from *w*Tpre, and 30% identical to the PifA from *w*Lcla
303 (another PI-*Wolbachia* infecting the parasitoid wasp *Leptopilina clavipes* [Hymenoptera:
304 Figitidae])(Pannebakker et al. 2004). In contrast, PifB proteins were more conserved: *w*Tkk and
305 *w*Tpre PifB were 93% identical, and *w*Tkk and *w*Lcla PifB shared 56% amino acid identity.
306



307

308 **Figure 5. Parthenogenesis-inducing *Wolbachia* strain *w*Tkk. (A)** Maximum likelihood-based
309 phylogeny of *Wolbachia* strains and Rickettsiales outgroups based on 78 core, single-copy, protein
310 coding genes (a total of 30,477 aligned amino acid sites). **(B)** Gene models for a predicted remnant
311 prophage region that contains the parthenogenesis factors *pifA* and *pifB.* Three tandem CDS were

312 annotated as *mutL*, which is likely a pseudogenization of *mutL* due to nonsense mutations and
313 fragmentation of the coding region into multiple open reading frames. Abbreviations: insertion element
314 (IS), parthenogenesis inducing factor (*pif*), coding sequence (CDS).
315
316 **Table 5. *Wolbachia* strain *w*Tkk genome assembly and annotation.**

Metric	***w*Tkk**
Contigs	4
Length (bp)	1,119,794
%GC	33%
Compleasm*	93.96% [S:93.96%, D: 0%] F: 0.55%, M:5.49%, n=364
CDS	1,265
rRNAs	3
tRNAs	34

317 *Standard BUSCO annotation: Complete BUSCOs (C) [Complete and single-copy BUSCOs (S),
318 Complete and duplicated BUSCOs (D)], Fragmented BUSCOs (F), Missing BUSCOs (M), Total BUSCO
319 groups searched (n). Rickettsiales dataset used for determining completeness.
320
321 **SUMMARY**
322 We report here a high-quality assembly for the parasitoid wasp *Trichogramma kaykai* along with
323 genomes for its mitochondrion and associated *Wolbachia* strain, *w*Tkk. There are five other
324 *Trichogramma* genomes currently available on NBCI: one each from the *Trichogramma* species
325 *pretiosum, dendrolimi,* and *evanescens*, and two assemblies for *Trichogramma brassicae*.
326 These species are some of the more commonly available *Trichogramma* sold as biological
327 control agents of lepidopteran pests (Knutson 1998; Cherif et al. 2021). The *Trichogramma*
328 *kaykai* assembly reported here is arguably the highest quality assembly available for the genus,
329 as it has the lowest number of contigs and the highest N50 (Table 1). To date, all *Trichogramma*
330 species assayed for karyotype have a haploid genome of five chromosomes (2n=10) (Gokhman

331 and Quicke 1995; Van Vugt et al. 2009; Gokhman 2020; Gokhman et al. 2017; Farsi et al.
332 2020). While chromosome number and approximate genome size are conserved, there do
333 appear to be species-specific differences in chromosome morphometrics (e.g., centromere
334 location, arm lengths, chromosome sizes) (Farsi et al. 2020; Gokhman et al. 2017; Gokhman
335 2020).
336
337 Of the *Trichogramma* genome sequencing efforts, one other reports a *Wolbachia* genome:
338 strain *w*Tpre, from *T. pretiosum* (Lindsey et al. 2016). The two *Trichogramma*-infecting strains,
339 *w*Tpre and *w*Tkk, are closely related members of the "Supergroup B" clade which contains a
340 suite of other arthropod-infecting strains, including other parthenogenesis-inducers from a range
341 of host insects (Scholz et al. 2020; Lindsey et al. 2016). While *Wolbachia* are maternally
342 transmitted, across longer evolutionary time scales there is a significant amount of horizontal
343 transfer, and often sister strains infect distantly related hosts (Bailly-Bechet et al. 2017; Scholz
344 et al. 2020). However, the PI-*Wolbachia* infecting *Trichogramma* appear to have a single origin
345 (Poorjavad et al. 2012; Schilthuizen and Stouthamer 1997; Almeida and Stouthamer 2017).
346 These PI-*Wolbachia* still undergo host switching (i.e., there is no co-cladogenesis) (Huigens et
347 al. 2000; Huigens et al. 2004; Almeida and Stouthamer 2017), but that this clade of *Wolbachia*
348 seem restricted to a single host genus makes them an interesting case study for host adaptation
349 and the evolution of their PI effector proteins.
350
351 In addition to the PI-*Wolbachia* present in *T. kaykai*, the PSR chromosome found in some males
352 offers another opportunity to understand the evolution of sex ratio distortion (Zhang and Ferree
353 2024). One other such PSR chromosome has been described: in the parasitoid wasp *Nasonia*
354 *vitripennis* (Hymenoptera: Pteromalidae)(Werren 1991; Nur et al. 1988). The PSR
355 chromosomes from these two wasp species appear to have independent origins, albeit a very
356 similar paternal genome elimination phenotype (van Vugt et al. 2003; Zhang and Ferree 2024).

357 Curiously, both PSR chromosomes seem to have originated from hybridization events in which
358 chromosomal regions with abundant repetitive elements were transferred in via a close relative
359 (McAllister and Werren 1997; van Vugt et al. 2005; Van Vugt et al. 2009). In contrast to *T.*
360 *kaykai*, *Nasonia* are not known to host any PI symbionts (Beukeboom and Van De Zande 2010).
361 However, some *Nasonia vitripennis* do host male-killing bacteria: *Arsenophonus nasoniae*
362 (Gherna et al. 1991; Ferree et al. 2008). The PSR chromosomes are likely playing a key role in
363 male-rescue which balances the male-eliminating cytoplasmic factors in both systems (either
364 elimination by conversion to female via PI-*Wolbachia* in *Trichogramma*, or, elimination via death
365 via *Arsenophonus* in *Nasonia*). The *Wolbachia*-infected line of *T. kaykai* reported here will
366 enable the long-term maintenance of PSR chromosomes in the lab, and in the future, we hope
367 to re-collect PSR-containing males from the native range to better understand the evolution of
368 these selfish genetic elements.
369
370 **Data Availability**
371 This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the
372 BioProject accession PRJNA1150630. BioSample accessions for *Trichogramma kaykai* and
373 *Wolbachia* strain *w*Tkk are SAMN43292057 and SAMN43292058, respectively. Sequencing
374 reads are deposited under SRR30339640. Genome assemblies and annotations for the
375 *Trichogramma kaykai* nuclear genome, *Trichogramma kaykai* mitochondrial genome, and
376 *Wolbachia* strain *w*Tkk are currently processing. Supplemental materials are available on the
377 GSA Figshare portal and include: (A) Table S1. Nanopore sequencing statistics, (B) Table S2.
378 Details on draft assembly curation, (C) Table S3. Comparison of interspersed repeats between
379 *Trichogramma kaykai* and related species, (D) File S1. *Trichogramma kaykai g*enome
380 annotations in GFF3 format, and (E) File S2. Analysis notebook with bioinformatics workflows
381 and scripts. A voucher of the *Trichogramma kaykai* KSX58 colony is available at the University
382 of California Riverside Insect Collection: UCRC_ENT00496298.

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Conflicts of Interest Statement

The authors declare no conflict of interest.

Author Contributions

ARIL provided samples, funding, analytical guidance, and performed some analyses. JC performed molecular work and bioinformatic analyses. JC and ARIL co-wrote the manuscript and created figures. Both authors read and approved the manuscript prior to submission.

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