

Genomic Resources for *Goniozus legneri*, *Aleochara bilineata* and *Paykullia maculata*, Representing Three Independent Origins of the Parasitoid Lifestyle in Insects

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ABSTRACT Parasitoid insects are important model systems for a multitude of biological research topics and widely used as biological control agents against insect pests. While the parasitoid lifestyle has evolved numerous times in different insect groups, research has focused almost exclusively on Hymenoptera from the Parasitica clade. The genomes of several members of this group have been sequenced, but no genomic resources are available from any of the other, independent evolutionary origins of the parasitoid lifestyle. Our aim here was to develop genomic resources for three parasitoid insects outside the Parasitica. We present draft genome assemblies for *Goniozus legneri*, a parasitoid Hymenopteran more closely related to the non-parasitoid wasps and bees than to the Parasitica wasps, the Coleopteran parasitoid *Aleochara bilineata* and the Dipteran parasitoid *Paykullia maculata*. The genome assemblies are fragmented, but complete in terms of gene content. We also provide preliminary structural annotations. We anticipate that these genomic resources will be valuable for testing the generality of findings obtained from Parasitica wasps in future comparative studies.

KEYWORDS

parasitoid
insect
genome
whole genome
sequencing
genome report

Parasitoid insects have long been used as model systems for the study of a wide variety of topics in biology, including life history, chemical ecology and population dynamics (Godfray 1994; Hawkins and Sheehan 1994; Jervis *et al.* 2008; Wajnberg and Colazza 2013). Parasitoids are also widely employed as agents of biological control against insect pests (Heimpel and Mills 2017). In recent years, the study of parasitoid insects has received new impetus through the availability of a steadily growing number of available genome sequences. Genomes of 13 parasitoid insects have recently become available, all from within one

monophyletic clade of parasitoid wasps (Branstetter *et al.* 2018). These genomes are rapidly becoming a rich source of information on many aspects of parasitoid biology, *e.g.*, evolution of venom, Wolbachia, and transition to asexual reproduction (Werren *et al.* 2010; Kraaijeveld *et al.* 2016).

The vast literature on insect parasitoids deals almost exclusively with Hymenopteran parasitoids, which all share a single evolutionary origin (Eggleton and Belshaw 2013). The stinging wasps (Aculeata) diverged from this group and lost the parasitoid life style. However, the parasitoid Chrysoidea may represent an additional evolutionary origin, as recent phylogenetic studies place this group within the Aculeata, suggesting that they may have re-evolved the parasitoid life style secondarily (Carr *et al.* 2010; Peters *et al.* 2017). Outside the Hymenoptera, parasitoid lifestyles have evolved in multiple insect groups, including Diptera, Coleoptera, Lepidoptera, and Neuroptera (Eggleton and Belshaw 2013). Dozens, or even hundreds, of evolutionarily independent parasitoid lineages are found within the Diptera and Coleoptera (Feener and Brown 1997; Eggleton and Belshaw 2013). It has been estimated that 20% of all parasitoid insect species are Dipterans (Feener and Brown 1997). Some of these are economically important, either as biological control agent (Grenier 1988) or as pest (Prabhakar *et al.* 2017). The study of

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doi: <https://doi.org/10.1534/g3.119.300584>

Manuscript received April 10, 2018; accepted for publication January 25, 2019; published Early Online January 31, 2019.

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Supplemental material available at Figshare: <https://doi.org/10.25387/g3.7655840>.

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such non-hymenopteran parasitoid systems would benefit from genomic resources, as it is unknown to what extent insights from hymenopteran parasitoids can be extrapolated to non-hymenopteran parasitoids. Unfortunately, no sequenced genomes are available for any of these groups as yet.

Here, we present draft genome assemblies for three parasitoid insect species that each represent an evolutionary independent acquisition of the parasitoid lifestyle (Figure 1A). *Goniozus legneri* is a parasitoid wasp from the superfamily Chrysoidea (family Bethyloidea) that is not part of the species-rich and well-studied Parasitica clade. *G. legneri* can therefore function as an outgroup in comparative analyses of Parasitica wasps and may have re-evolved the parasitoid lifestyle after it was lost at the base of the Aculeata. *G. legneri* is a gregarious parasitoid of Lepidopteran larvae that stings and paralyzes its prey before ovipositing on it externally (Khidr *et al.* 2013). The female then guards the host against utilization by other females (Khidr *et al.* 2013). *Aleochara bilineata* is a Coleopteran parasitoid of Dipteran pupae that represents another evolutionary independent acquisition of the parasitoid lifestyle. Females lay their eggs in the proximity of hosts (Bili *et al.* 2016). After hatching, the first instar larvae search for, enter and parasitize host

pupae (Bili *et al.* 2016). *Paykullia maculata* is a parasitoid fly from the family of the Rhizophoridae, representing one of the many independent acquisitions of the parasitoid mode within the Diptera. Like all Rhizophoridae, *P. maculata* parasitizes isopods. Females lay their eggs in the vicinity of isopod aggregations. The larvae latch on to a passing isopod and enter it through an intersegmental membrane (Wijnhoven 2001). They feed on the isopod's hemolymph and later also on non-vital tissues, like the female ovaria (Wijnhoven 2001). The isopod continues to feed and molt normally, until the parasitoid kills it and pupates within the host exoskeleton (Wijnhoven 2001). Here, we present draft genome sequences for these three species.

MATERIALS AND METHODS

Samples

Specimens of *G. legneri* were obtained from a long-term culture at the University of Nottingham (UK), which is descended from founders originally collected in Uruguay (I. Hardy, pers. comm.). Specimens of *A. bilineata* used for genome sequencing were obtained from a culture maintained at the University of Rennes (France). Specimens of

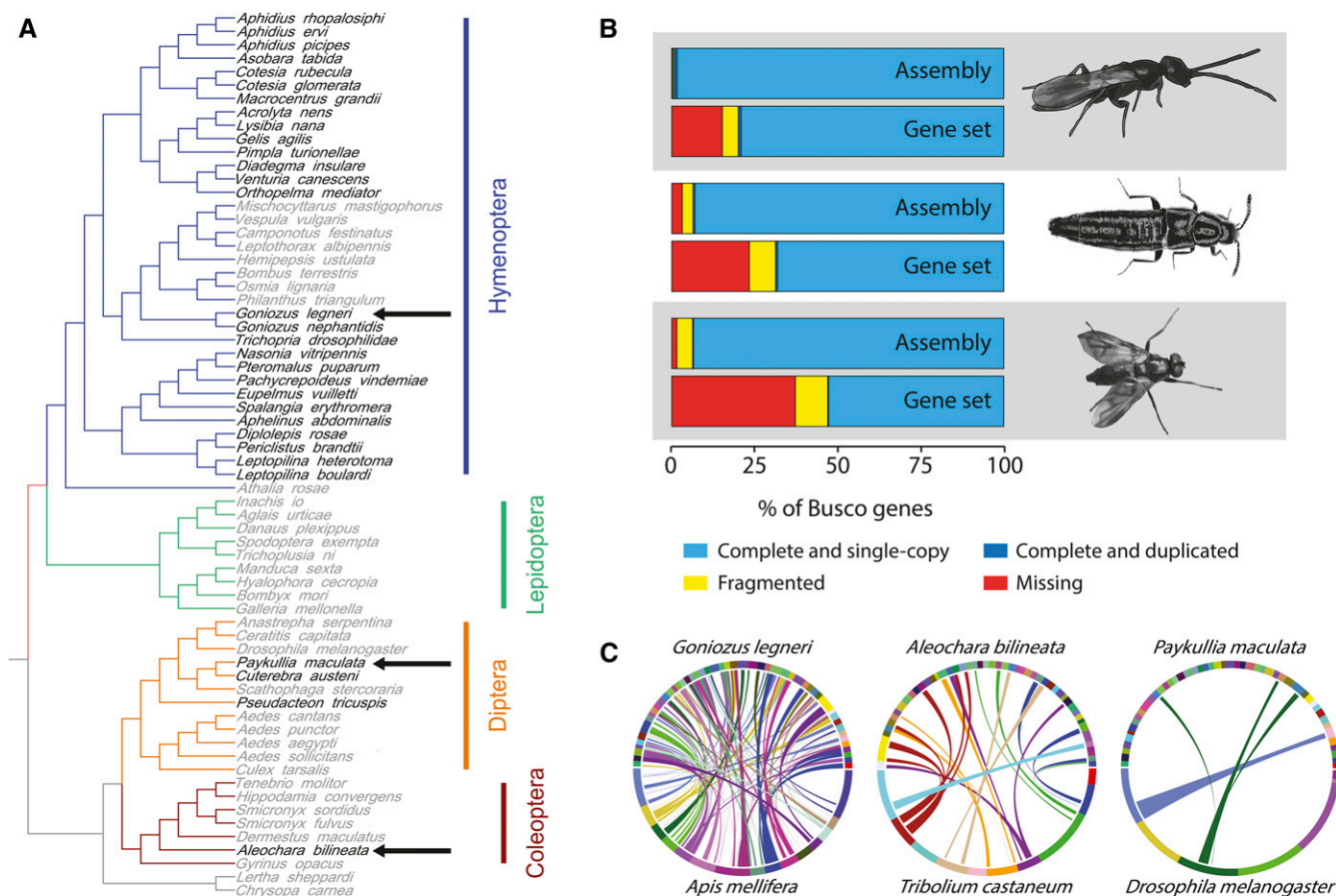


Figure 1 Phylogenetic context and genome features. (A) Phylogeny of selected insect species from (Visser *et al.* 2010). Parasitoid species are indicated in black font and non-parasitoid species in gray font. The three species for which we provide draft genome assemblies are indicated by arrows. (B) Completeness of the genome assemblies and annotation. Orthologs of 1658 genes that are present as single copies in at least 90% of insects were retrieved using Busco v3. From top to bottom: *Goniozus legneri* genome, *G. legneri* gene set, *Aleochara bilineata* genome, *A. bilineata* gene set, *Paykullia maculata* genome and *P. maculata* gene set. (C) Circle plots illustrating the synteny between the 50 largest scaffolds of the genome assemblies of *G. legneri*, *A. bilineata* and *P. maculata* (top half of each circle) and the genomes of the closest available relative with chromosome- or linkage group- level assemblies (*A. mellifera*, *T. castaneum* and *D. melanogaster*, respectively; bottom half of each circle). Links are colored to match the chromosome or linkage group of the model species.

A. bilineata used for flow cytometry were obtained commercially from De Groene Vlieg (www.degroenevlieg.nl). Specimens of *P. maculata* were cultured from *Porcellio scaber*, collected in the field in The Netherlands (Wageningen and Amsterdam) during 2015. For each species, DNA was extracted from a single adult female using different spin column protocols. For *G. legneri* and *A. bilineata*, DNA was obtained by crushing the insect in 100–200 μ l PBS and adding 100 μ l Nuclei Lysis Solution, 5 μ l RNase (4 mg/ml)(both Promega), 4 μ l Proteinase K (20 mg/ml, Roche) and incubating at 60° for 15 min. A further 340 μ l Wizard SV Lysis Buffer (Promega) was then added and the sample was centrifuged at full speed for 5 min. The supernatant was then transferred to a spin column (Promega), rinsed with 500 μ l Wizard SV Wash Solution (Promega) three times and eluted in 100 μ l H₂O. *P. maculata* was crushed in liquid nitrogen and DNA was extracted using QIAamp DNA Mini Kit (Qiagen) following the manufacturer's protocol. The quantity and quality of the DNA samples was assessed using Nanodrop (Thermo Fisher Scientific) and Qubit 2.0 Fluorometer (Invitrogen).

Whole genome sequencing

Two Illumina Truseq DNA libraries with slightly different insert sizes (400 and 500 bp) were constructed from each DNA sample. The two libraries for each species were barcoded, pooled and sequenced using 2x100 bp paired-end sequencing on Illumina HiSeq 2000 (*G. legneri* and *A. bilineata*), or 2x125 bp paired-end on Illumina HiSeq 2500 (*P. maculata*).

De novo genome assembly

Prior to *de novo* assembly, we characterized the raw read data using SGA (Simpson 2014) and estimated genome size using KmerGenie (Chikhi and Medvedev 2014). Furthermore, 21-mer counts were obtained using Jellyfish (Marçais and Kingsford 2011) and plotted as histograms using GenomeScope (Vurture *et al.* 2017). GenomeScope also provided an additional genome size estimate. Ploidy structure was estimated from the 21-mer counts using Smudgeplot (<https://github.com/tbenavi1/smudgeplot>). Furthermore, we removed co-sequenced genomes to reduce complexity of the read set. To this end, reads were mapped to the mitochondrial DNA of well-sequenced related species (*Apis mellifera* L06178.1, *Tribolium castaneum* NC_003081.2 and *Drosophila melanogaster* U37541.1 for *G. legneri*, *A. bilineata* and *P. maculata*, respectively) and to a panel of 12 *Wolbachia* strains (wAu GCA_000953315.1; wBm GCA_000008385.1; wCle GCA_000829315.1; wPip_Pel GCA_000073005.1; wHa GCA_000376605.1; wNo GCA_000376585.1; wRi GCA_000022285.1; wMel GCA_000008025.1; wFol CP015510.1; wOo GCA_000306885.1; wOv GFA_000530755.1; wPip GCA_000208785.1) using Bowtie2 (Langmead and Salzberg 2012) with default settings. Unmapped reads were extracted from the Sam file using samtools view with -f 4 (Li *et al.* 2009) and reverted back to fastq using bedtools bamToFastq (Quinlan and Hall 2010). Reads were trimmed using platanus_trim (Kajitani *et al.* 2014). Given that the results from the SGA analysis indicated high levels of heterozygosity for *A. bilineata* and *P. maculata*, we chose to perform the *de novo* assembly for all three species in Platanus, which is specifically geared to deal with short-read data from heterozygous genomes (Kajitani *et al.* 2014). Assembly was followed by scaffold and gap_close steps as implemented in the Platanus pipeline. To assess coverage, reads were mapped back to the assembly using Bowtie2 with default settings and per-base genome coverage was calculated using samtools depth.

■ **Table 1 Raw reads generated for assembly**

Species	Read pairs	Base pairs aligned	Coverage
<i>Goniozus legneri</i>	140.0 M	22.52 Gb	160.8x
<i>Aleochara bilineata</i>	170.3 M	18.55 Gb	217.8x
<i>Paykullia maculata</i>	211.9 M	43.56 Gb	103.4x

Annotation

Structural annotation was performed using Maker2 using default settings (Holt and Yandell 2011). For each species, we included a protein training set from the closest relative with a well-annotated reference genome (*A. mellifera* Amel_4.5, *T. castaneum* Tcas5.2 and *D. melanogaster* release 6 for *G. legneri*, *A. bilineata* and *P. maculata*, respectively). Augustus was provided with gene models for the same combinations of species. Annotation statistics were obtained using GAG (Hall *et al.* 2014). The completeness of the genomes and gene sets was assessed by identifying the number of insect Benchmark Universal Single-Copy Orthologs (BUSCOs) (Simão *et al.* 2015). BUSCO v3.0.2 was run on both the genome assembly (using -m geno) and the Maker gene set at the peptide level (using -m prot) with the insecta_odb9 lineage dataset as reference. We compared the draft genome of each species to that of its closest available relative with a chromosome- or linkage group-level genome sequence available (*A. mellifera* Amel_4.5, *T. castaneum* Tcas5.2 and *D. melanogaster* release 6 for *G. legneri*, *A. bilineata* and *P. maculata*, respectively) using SyMap v4.2 (Soderlund *et al.* 2011).

Co-sequenced genomes

To remove any co-sequenced genomes (in addition to *Wolbachia* and mitochondria, which were removed prior to assembly), we employed the Blobology pipeline (Kumar *et al.* 2013).

Flow cytometry

Since the genome size predicted for *A. bilineata* was small (see Results), we performed flow cytometry to provide an independent estimate of genome size for this species. Flow cytometry was performed at Plant Cytometry Services (www.plantcytometry.nl) using the following protocol. Three replicate measurements were obtained. For each measurement, a single head of *A. bilineata* together with a whole specimen of *D. melanogaster* (obtained from long-term culture held at the Vrije Universiteit Amsterdam) was fragmented with a sharp razor blade in 500 μ l extraction buffer (Sysmex), in a plastic petri disc. After 30 – 60 sec of incubation, 2 ml staining buffer (Sysmex: propidium iodide, RNase, 0.1% dithiothreitol and 1% polyvinylpyrrolidone). The sample was then passed through a nylon filter of 50 μ m mesh size. After 30 min incubation at room temperature, the filtered solution with stained nuclei was sent through the flow cytometer (Partec Cube). The fluorescence of the stained nuclei, passing through the focus of the light beam of a 50 mW, 532 nm green laser, was measured by a photomultiplier and converted into voltage pulses. These voltage pulses are electronically processed to yield integral and peak signals.

Data availability

The Whole Genome Shotgun projects have been deposited at DDBJ/ENA/GenBank under the accessions NCVS00000000 (*G. legneri*), NBZA00000000 (*A. bilineata*) and NDXZ00000000 (*P. maculata*). The versions described in this paper are versions NCVS01000000, NBZA01000000 and NDXZ01000000, respectively. Mapped reads and genome annotations are available through <http://parasitoids.labs.vu.nl/parasitoids/>. This website also includes genome browsers and viroblast

■ **Table 2 Assembly summary statistics**

Metric	<i>G. legneri</i>	<i>A. bilineata</i>	<i>P. maculata</i>
GC (%)	40.6	39.9	28.4
Scaffold count	7,863	33,003	147,656
Contig count	13,705	40,228	169,825
Total length (Mb)	140.1	85.9	422.4
Predicted length KmerGenie (Mb)	142	112	536
Predicted length GenomeScope (Mb)	167	123	429
Gap (%)	0.18	1.1	0.29
Scaffold N50 (kb)	167.3	54.1	7.7
Contig N50 (kb)	37.8	12.1	5.9
Max. scaffold length (Mb)	1.5	0,876	0.17
Max. contig length (kb)	371.7	316.8	86.6
Number of scaffolds > 50 kb	684	445	108
% of genome in scaffolds > 50 kb	82.4	52.1	1.8

instances for each genome. Supplemental material available at Figshare: <https://doi.org/10.25387/g3.7655840>.

RESULTS AND DISCUSSION

We generated 18.6–43.6 Gb data per species, covering each genome >100x (Table 1). These data were assembled into draft genomes that were reasonably close to the predicted size from k-mer analysis for each species (Table 2). The genome size of *G. legneri* appears small compared to other sequenced genomes of Hymenoptera, but is within the range for parasitoid wasps (e.g., *Macrocentrus cingulum*: 128 Mb, *Fopius arisanus*: 153 Mb). The estimated genome size of *A. bilineata* is smaller than that of other sequenced Coleoptera (smallest to date is *Hypothenemus hampei*: 151 Mb; see below). The genome size of *P. maculata* is within the range observed for Diptera (e.g., *Zaprionus indianus* 124 Mb; *Rhagoletis zephyria*: 795 Mb). Further work is required to establish the accuracy of our genome size estimates.

The genome assemblies presented here were obtained from outbred, heterozygous individuals. The Platanus assembler is specifically designed to handle such data and creates a mosaic of the two haplotypes (Kajitani *et al.* 2014). We tested this by mapping the sequence reads back to the genome assembly. Bowtie2 in default settings reports only the best alignment for each read and haplotypes that had assembled as separate contigs should have half the coverage as collapsed haplotypes, as reads would only map to one of the haplotypes. In our case, the coverage histograms were unimodal (Figure S1), indicating that haplotypes were successfully collapsed.

In the search for co-sequenced genomes, the assembly for *G. legneri* yielded hits to Rhabditida nematodes, some of which are known parasites of insects. A Blast search of the entomopathogenic Rhabditid *Oscheius sp.* (Lephoto *et al.* 2016) against the *G. legneri* genome assembly revealed two hits, upon which we removed one contig and one partial contig from the assembly. *A. bilineata* contained *Wolbachia*, but no other co-sequenced genomes. *P. maculata* contained no co-sequenced genomes.

The genome assemblies are very complete, with 97–99% of BUSCOs present and only 0.4–4.7% fragmented (Figure 1B). Lacking transcriptome data and other genomic resources for these or closely related species, the structural annotation is less complete. Maker2 annotated 5588–7463 genes per genome (Table 3), which is below the expected value for eukaryotes. BUSCO analysis indicated the gene sets to be 63–85% complete (Figure 1B). Genes missing from the annotation, but present in the genome assembly were more often fragmented, had relatively low bitscores and shorter alignment length to the BUSCO profile (Table S1). It is thus imperative for future studies to interrogate the genome assembly for genes missing from the gene set.

The level of synteny to well-characterized genome sequences of related model species varied (Figure 1C). The draft genome of *G. legneri* shows many collinear regions with the genome of *A. mellifera*, while the similarity between *P. maculata* and *D. melanogaster* is limited, with intermediate collinearity between *A. bilineata* and *T. castaneum* (Figure 1C). These differences are probably caused by a combination of factors, including quality of the draft genome assemblies, levels of relatedness to the selected model organism and rates of genome evolution.

As the genome size estimates and the assembled genome for *A. bilineata* were smaller than for other Coleoptera, we obtained an independent genome size estimate using flow cytometry. The three replicate measurements indicated a genome that was 1.32x larger than that of *D. melanogaster* (range 1.27 - 1.38, Figure S2). Given *D. melanogaster* has a genome of 180 Mb, our flow cytometry estimated the genome of *A. bilineata* to be 238 MB. Remarkably, this is almost exactly twice the size estimated from our genome sequencing analysis. BUSCO analysis indicated that our genome assembly was very complete, so the discrepancy cannot be explained by large amounts of coding sequence missing from the sequencing data. Alternatively, a high proportion of repeat sequences in this genome could result in an underestimation of genome size from the sequencing data. However, the k-mer profile (Figure S2B) provided no indication that this was the case. The most likely explanation seems endoreduplication in head

■ **Table 3 Gene annotation summary statistics**

Metric	<i>G. legneri</i>	<i>A. bilineata</i>	<i>P. maculata</i>
Number of genes	7463	7220	5588
Number of exons	46322	28720	20856
Total gene length (Mb)	25.5	17.2	18.1
Mean gene length	3418	2380	3232
% of genome covered by genes	18.2	20.0	4.3
mean exons per mRNA	6	4	4

tissue of this species. This was supported by the Smudgeplot analysis (Figure S3B), that indicated the presence of triploid and tetraploid cells.

In summary, we present fragmented, but relatively complete genome assemblies of three parasitoid insects, representing three independent evolutionary origins of the parasitoid lifestyle. These genomes will be valuable for comparisons to the widely studied parasitoid Hymenoptera, for which numerous genomes are available (Branstetter *et al.* 2018). Our study highlights that useful genomic resources can now be obtained from highly heterozygous individual insects collected from outbred lab cultures or even from the field, relieving the need for labor-intensive inbreeding procedures.

ACKNOWLEDGMENTS

We thank Ian Hardy, Denis Poinso, Mikael Bili, Mark Lammers and Rik Zoomer for providing insect samples.

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Communicating editor: S. Celniker