1   **A high-quality *de novo* genome assembly from a single parasitoid wasp**

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31   **Abstract**

32   Sequencing and assembling a genome with a single individual have several advantages, such as

33   lower heterozygosity and easier sample preparation. However, the amount of genomic DNA of

34   some small sized organisms might not meet the standard DNA input requirement for current

35   sequencing pipelines. Although few studies sequenced a single small insect with about 100 ng

36   DNA as input, it may still be challenging for many small organisms to obtain such amount of

37   DNA from a single individual. Here, we use 20 ng DNA as input, and present a high-quality

38   genome assembly for a single haploid male parasitoid wasp (*Habrobracon hebetor*) using

39   Nanopore and Illumina. Because of the low input DNA, a whole genome amplification (WGA)

40   method is used before sequencing. The assembled genome size is 131.6 Mb with a contig N50 of

41   1.63 Mb. A total of 99% Benchmarking Universal Single-Copy Orthologs are detected, suggesting

42   the high level of completeness of the genome assembly. Genome comparison between *H. hebetor*

43   and its relative *Bracon brevicornis* shows a high-level genome synteny, indicating the genome of

44   *H. hebetor* is highly accurate and contiguous. Our study provides an example for *de novo*

45   assembling a genome from ultra-low input DNA, and will be used for sequencing projects of

46   small sized species and rare samples, haploid genomics as well as population genetics of small

47   sized species.

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Introduction

A high-quality genome assembly is one of the most important resources for studying biological questions in organisms. However, genome sequencing and assembly can be complicated by the small body size of many organisms (i.e., very low genomic DNA from a single individual) and high heterozygosity [1, 2]. In particular, many arthropod (insect) genome projects face these problems, and obtain highly fragmented genomes with very low Contig N50 or/and Scaffold N50 value [1]. These fragmented genomes will have problems in genome annotation, as some genes are incomplete in genome assembly. In addition, gene synteny analysis, chromosome evolution, quantitative trait locus mapping also will fail in such fragmented genomes [1].

Mostly, the amount of genomic DNA is very low when obtained from single small-sized insect, which makes it hard to meet the standard DNA input requirement of long-reads sequencing (Pacbio or Nanopore) or even short-reads sequencing (Illumina) [1, 3-6]. Over the past two decades, many insects with small body size (e. g., parasitoid wasps, aphids, many *Drosophila*) were sequenced by using the DNA from pooled samples [2]. But pooling method raises heterozygosities in genomic regions, which will be assembled into more fragmented contigs. To reduce the heterozygosity level in pooled sample, inbreeding species were used for DNA extraction and sequencing in many cases [1, 7, 8]. Using the current hybrid genome sequencing and assembly approaches, many high-quality genome assemblies (some are chromosome-level genomes) were released [9]. However, most of insects are difficult to collect or cannot be well reared in the lab. Even if they can be reared in the lab, they might be difficult to inbreed [1, 3, 6]. Therefore, obtaining a high-quality genome assembly is still a problem for some small sized species and rare species in the wild.

Recently, to resolve these problems, some approaches were developed in sequencing from a single individual with low DNA input. Kingan et al. reported a genome assembly from a single mosquito (about 100 ng genomic DNA), *Anopheles coluzzii*, sequenced with three PacBio SMRT Cells [6]. Adams et al. developed a hybrid method (Illumina, Nanopore and Hi-C) to obtain a chromosome-level genome assembly from a single *Drosophila melanogaster* (totally ~200ng genomic DNA) [10]. These two studies provided good examples for sequencing a single small insect, but obtaining about 100 ng DNA from a single individual may still be challenging for many small insects such as parasitoid wasps.

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91   How to sequence and assemble a high-quality genome from a single insect with ultra-low

92   input DNA is still a problem, and no practical experience in this field up to now. Because of the

93   ultra-low input DNA, it is difficult to construct library for sequencing at this time. A whole

94   genome amplification (WGA) method has to be used to increase the total amount of DNA to meet

95   the lowest requirement of sequencing library construction [11]. The WGA method is widely used

96   to identify single-nucleotide polymorphisms, copy number variations in low DNA sample, e. g.

97   single cell [12, 13]. However, this method is rarely used in *de novo* genome assembly. It is

98   important to note that WGA has some disadvantages, such as potential amplification biases and

99   contaminant problems [12, 14-16], which might influence the quality of genome assembly. There

100   are only a few cases on *de novo* genome assembly based on WGA data, most of them are in

101   bacteria [17, 18]. Recently, WGA was used to build a *de novo* genome assembly for a fungus [19].

102   But there is no report about WGA application in *de novo* genome assembly in insects or other

103   more complex eukaryotic species.

104   Here, starting with 20 ng DNA, we present a high-quality *de novo* genome assembly of a

105   single male parasitoid wasp (*Habrobracon hebetor*) using WGA and Nanopore, Illumina

106   sequencing. This approach provides an example for genome sequencing and assembly using

107   ultra-low DNA input, and is applicable for small size organisms and rare samples.

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109   **Results and discussion**

110   **Parasitoid wasp for sequencing**

111   Parasitoid wasps are interesting and important organisms for studying fundamental biological

112   questions such as evolution and sex determination, and some of them are important natural

113   enemies for insect pest management [20, 21]. In addition, parasitoid wasps are often in very small

114   size, which makes the genome projects of them complicated. Although many studies have

115   assembled genomes from pooled inbred lab strains [8, 22-27], most of parasitoid wasps are

116   difficult to be reared in the lab to establish such lab strains for sequencing. There are also some

117   problems and uncertain factors in sequencing field collected samples, such as high heterozygosity

118   and insufficient sample. These issues are major hindrances to the development of parasitoid wasp

119   genomics. To test the feasibility of sequencing and assembling genome from a single parasitoid

120   wasp, we sequenced a single male adult wasp of *H. hebetor* (Braconidae) in the study. *H. hebetor*

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121   wasp is an important biological control agent for managing multiple lepidopteran pests [28], and

122   an ideal model for Hymenoptera sex determination researches [29, 30].

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124   **Genome sequencing**

125   In total, 102 ng high molecular weight DNA was extracted from a single male adult wasp of *H.*

126   *hebetor*. Two studies already had provided good cases to obtain a high-quality genome assembly

127   with approximately 100 ng of DNA [6, 10]. However, it still might be difficult to obtain ~100 ng

128   DNA from a single individual in many small sized species. To challenge the lower input DNA for

129   sequencing and make our method useful for more small size species, only 20 ng DNA was used

130   for subsequent study.

131   DNA was subjected to whole genome amplification, yielding ~4.37 μg amplified DNA. 2.02

132   μg amplified DNA was used for Nanopore sequencing. In total, we obtained 51 Gb high-quality

133   reads (~372X, genome size is about 137 Mb) from a single Oxford Nanopore Technology (ONT)

134   PromethION flow cell (Table. S1). According to the handbook of this WGA method, the average

135   amplified product length is in a range between 2 kb and 100 kb. The distribution of the Nanopore

136   reads showed the similar pattern (Table. S1 and Figure. S1). The average length of total reads is

137   4,484 bp, and the N50 of total reads is 7,311 bp. We also generated 13.8 Gb Illumina clean data

138   reads using the rest of amplified DNA (Table. S2).

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140   **Genome assembly**

141   We assembled a genome assembly using Flye [31, 32] with ~250X >5K Nanopore reads. This

142   assembly was then corrected and polished by both Nanopore reads and Illumina reads. The final

143   assembly size is 131.6 Mb, consisting of 765 contigs with a Contig N50 of 1.63 Mb (Table 1). The

144   GC content of the genome assembly is 35.49%. Assembly statistic of *H. hebetor* are compared to

145   four additional braconid genomes, and the result shows a higher N50 value of *H. hebetor* than

146   *Fopius arisanus* (0.98 Mb) [33], *Diachasma alloeum* (0.65 Mb) [23], *Macrocentrus cingulum*

147   (0.19 Mb) [26] and *Microplitis demolitor* (1.1 Mb) [34]. Our analysis indicates that this assembly

148   of a single wasp is more continuous than most wasp genomes which generated by pooled samples.

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150   **Genome quality assessment**

151   The completeness of the assembly was assessed by using Benchmarking Universal Single-Copy

152   Orthologs (BUSCO) [35], 1,643 out of 1,658 (99%) conserved arthropod genes were found in the

153   genome, 97.3% occurred as single copies (Table 2). The complete and duplicated BUSCO

154   component of the genome was 1.7%. Only three BUSCOs (0.2%) were found fragmented in the

155   genome. There are still twelve BUSCOs (0.8%) cannot be deleted in this genome. We also

156   mapped the Illumina paired-end genomic sequencing reads to the assembled genome, 99.27% of

157   reads could be mapped to the genome. These results indicate that the genome assembly is both

158   highly accurate and near completion.

159   We next mapped *H. hebetor* genome to a genome of *Bracon brevicornis* which is a close

160   relative of *H. hebetor.* The mapping result shows a high-level genome synteny between these two

161   wasps, suggesting the genome assembly of *H. hebetor* obtained from a single wasp is accurate and

162   contiguous (Figure 1). From these results, we didn't find the evidence to support that

163   amplification biases of WGA could largely influence the quality of genome assembly. We

164   reasoned that might due to the relatively small size genome of parasitoid wasps.

165   In summary, we report a high-quality genome assembly of a single parasitoid wasp *H.*

166   *hebetor* (~20 ng starting DNA) using WGA, Nanopore and Illumina sequencing technologies.

167   This study presents an example for *de novo* assembling a genome from ultra-low input DNA,

168   which could be used for many small sized species sequencing projects, haploid genomics and

169   population genetics of small sized species.

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171   **Methods**

172   **DNA extraction and whole genome amplification**

173   High molecular weight DNA was extracted from a single male adult *H. hebetor* using TIANamp

174   Micro DNA Kit (DP316) following manufacturer's recommendations. Two DNA quantification

175   methods Qubit and Nanodrop were used to measure DNA concentration. Then, ~20 ng genomic

176   DNA was amplified using a whole-genome amplification (WGA) kit according to the

177   manufacturer's instructions (Qiagen REPLI-g Mini Kit, Qiagen, Valencia, CA). The REPLI-g Kit

178   is developed based on multiple displacement amplification (MDA), a WGA method with high

179   processivity and low error rate [36, 37]. Purified genomic DNA was firstly mixed with a

180   denaturation buffer by vortexing and centrifuge briefly. This reaction was quenched by 3 min

181   incubation (at room temperature) with neutralization buffer. The master mix components with

182   REPLI-g Mini DNA Polymerase were added to denatured DNA. The amplification step performed

183   by incubation at 30°C for 16 hours. Next, REPLI-g Mini DNA Polymerase was inactivated by

184   heating the sample for 3 min at 65°C. Following WGA, DNA concentration was determined by

185   using Qubit.

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187   **Nanopore sequencing**

188   A total amount of 2.02 μg DNA was used as input for ONT 1D library construction and

189   sequencing. In brief, the gDNA was sheared using the Megaruptor. Then, the large fragments were

190   selected and purified using AMPure beads. A ONT 1D sequencing library was prepared using the

191   Nanopore Ligation Sequencing Kit (SQK-LSK109; Oxford Nanopore, Oxford, UK) and was

192   sequenced on ONT PromethION 24 platform with one nanopore flow cell (FLO-PRO002).

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194   **Illumina sequencing**

195   Sequencing library was generated using Truseq Nano DNA HT Sample preparation Kit (Illumina

196   USA) following manufacturer's recommendations. Briefly, the DNA firstly sheared by Covaris S2

197   system (Covaris, Inc. Woburn, MA, USA), then DNA fragments were end polished, A-tailed, and

198   ligated with the full-length adapter for Illumina sequencing with further PCR amplification. At last,

199   PCR products were purified (AMPure XP system) and libraries were analyzed for size distribution

200   by Agilent2100 Bioanalyzer and quantified using real-time PCR. The final library was sequenced

201   by Illumina NovaSeq platform.

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203   **Genome assembly**

204   Nanopore long reads flagged as "passing" were corrected by NECAT

205   (https://github.com/xiaochuanle/NECAT). Flye (version: 2.7.1-b1590) [31, 32] was used to

206   assemble the genome with default parameters using >5K Nanopore reads (~250X). Then, Racon

207   (https://github.com/isovic/racon) was used for correcting the assembly. In addition, iterative

208   polishing was conducted using Pilon (version: 1.22) [38] with adapter-trimmed paired-end

209   Illumina reads. The Pilon program was run with default parameters to fix bases, fill gaps, and

210   correct local misassemblies.

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Evaluation

213 Benchmarking Universal Single-Copy Orthologs method (BUSCO version 4.0) [35] was used to

214 search the 1,658 bench-marking universal single-copy orthologous genes in insecta_odb9.

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Genome comparison

217 An online tool D-GENIES [39] was used to compare the *H. hebetor* and *B. brevicornis* genome

218 [40].

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Data availability

221 All sequence data are available at the NCBI, Bioproject number PRJNA644201.

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Author contributions

232 GYY conceived and designed the works, and supervised the project. GYY, FL and QF coordinated

233 the project. KLY and SJX prepared the samples for sequencing. XHY, ZYT, HC sequenced and

234 assembled the genome. XHY and YY performed the bioinformatics analysis. XHY wrote the draft

235 manuscript. YY, LX, SX, CLY, HC, FL, QF and GYY improved and revised the manuscript. All

236 authors read and approved the final manuscript.

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Conflict of interest statement

239 The authors declare no competing interests.

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241   Table 1. Information of the genome assembly.

Statistic	*Habrobracon hebetor*
Total length (bp)	131,644,965
Total length without N (bp)	131,644,965
Contig number	765
GC content (%)	35.49
Contig N50 (bp)	1,625,734
Contig N90 (bp)	140,615
Average (bp)	172,084.92
Median (bp)	9,847.00
Min (bp)	177
Max (bp)	7,063,052

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243   Table 2. BUSCO assessment of the final assembly.

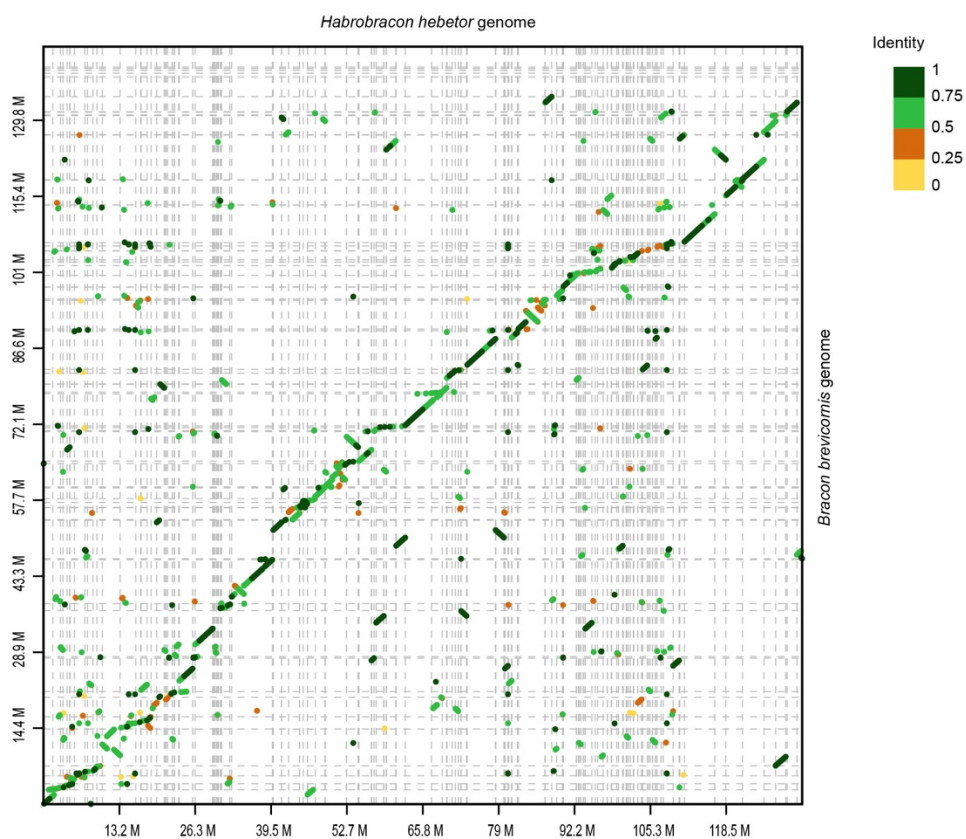| Category	Number of BUSCOs
Complete BUSCOs (C)	1,643 (99.0%)
Complete and single-copy BUSCOs (S)	1,614 (97.3%)
Complete and duplicated BUSCOs (D)	29 (1.7%)
Fragmented BUSCOs (F)	3 (0.2%)
Missing BUSCOs (M)	12 (0.8%)
Total BUSCO genes	1,658 (100%)

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253   Figure 1. Genome comparison between *H. hebetor* and *B. brevicornis* genome.

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268   Table S1. Nanopore sequencing data.

Type	TotalBase	TotalReads	MaxLen	AvgLen	N50	N90	meanQ
>0	51,597,252,675	11,507,945	124,237	4,484	7,311	2,127	11
>2000	46,957,240,951	7,372,598	124,237	6,369	8,039	3,113	11
>5000	34,318,006,006	3,510,066	124,237	9,777	10,379	5,877	11
>10000	18,071,218,227	1,193,518	124,237	15,141	14,941	10,769	11
>20000	4,376,715,930	170,092	124,237	25,731	24,876	20,737	11
>100000	534,472	5	124,237	106,894	102,023	100,525	10

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270   Table S2. Illumina sequencing data.

Raw Reads	Clean Reads	Clean Base (Gb)	Q20(%)	Q30(%)	GC Content (%)
46,619,030	46,061,685	13.82	95.51	91.23	35.27

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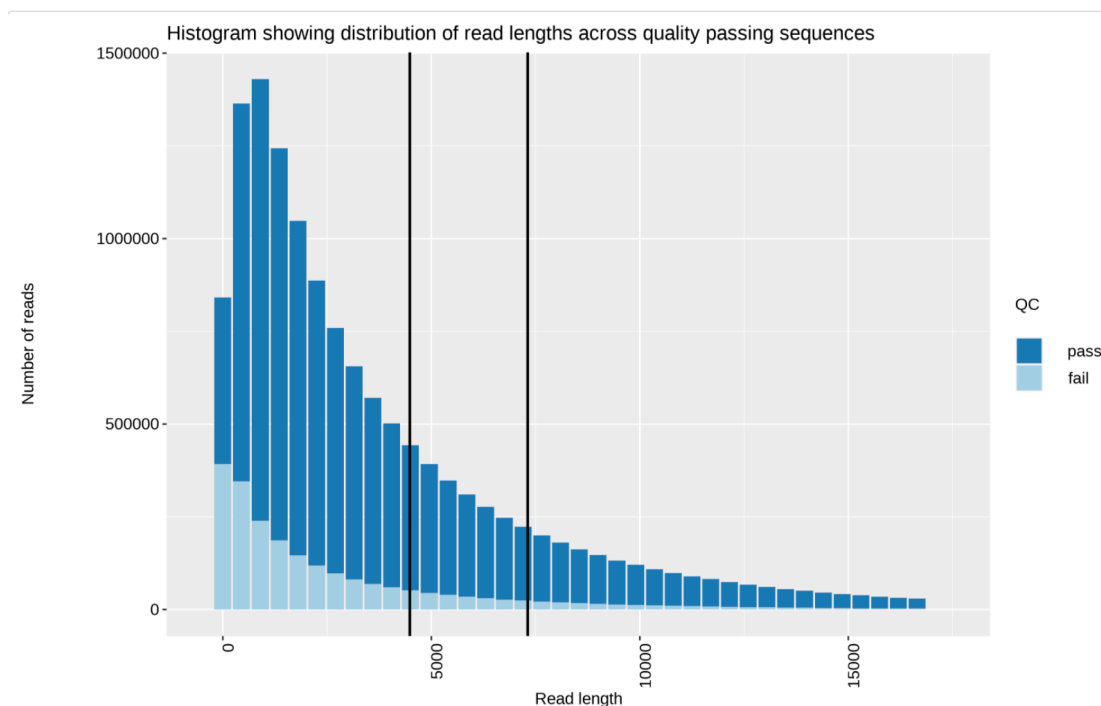274

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287 Figure S1. Distribution of the Nanopore reads.

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