

Reviewer Report

Title: Chromosome-level reference genome of the European wasp spider *Argiope bruennichi*: a resource for studies on range expansion and evolutionary adaptation

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Reviewer Comments to Author:

I was excited to read about the first chromosome-scale spider genome, but this manuscript will still require a little bit of work before I can recommend it for publication.

Major concerns:

The manuscript revolves around the presentation of a high-quality chromosome-level assembly, but the evidence supporting the quality of the assembly is a bit sketchy: merely contiguity statistics, BUSCO scores and a contact map. To be convinced by the quality of the assembly, I would need to see a KAT plot (<https://kat.readthedocs.io/en/latest/walkthrough.html#genome-assembly-analysis-using-k-mer-spectra> - as the Illumina sequencing depth is only 30X, the authors will probably need to play a bit with the k-mer size parameter to generate a satisfying plot in which the peaks are well separated from one another) and a k-mer completeness estimate. Also, as the amount of repetitions seems fairly high it would be interesting to see coverage plots (obtained by remapping the PacBio reads on the one hand and the Illumina reads on the other hand on the genome assembly) in order to assess whether some repeated parts have been overcollapsed or (conversely) some haplotypes have not been properly merged, resulting in artefactual duplications.

On line 99 it is mentioned that the genome size was estimated at 1.7 Gb but there is no explanation regarding this estimation: was it obtained using flow cytometry, or by analyzing the k-mer distribution of Illumina reads?

Running KAT (with default parameters) on the data downloaded from the FTP server provided by the authors yielded a genome size estimate of 1.62 Gb. Also, KAT estimated a k-mer completeness of only 88.9% (for the homozygous peak, which should have a 100% k-mer completeness for a haploid assembly of a diploid genome): this may be due to the 21.5X PacBio coverage used for the assembly being too low for the consensus step to fully correct the sequencing errors, followed by a polishing step with Pilon using an Illumina coverage once again on the lower side (30X). The authors could possibly obtain a better polished assembly with a higher k-mer completeness by performing their polishing using HyPo, which utilizes both PacBio and Illumina data.

As the PacBio, Illumina and Hi-C data were generated from different individuals collected several years apart, the mapping rates of the Illumina data on the initial PacBio assembly as well as the mapping rate of the the Hi-C data on the polished assembly should be mentioned.

The part entitled "whole-genome duplication" does not really look into WGS per se but rather only analyzes the duplication of the Hox gene cluster, which could also result from a segmental duplication involving this cluster. *Argiope bruennichi* being the first chromosome-scale assembly made available for any arachnid, the authors should seize this opportunity to perform a synteny and/or microsynteny

analysis at chromosome level in order to check whether they find evidence supporting an actual whole-genome duplication.

Minor concerns:

- line 38: "Arachnids" should be spelled "arachnids";
- lines 38-39: "whole-genome duplication" is normally with a hyphen;
- lines 53 and 230: I checked reference 7 and it does not really seem to support the assertion that "chromosome-level genome assembly would greatly increase the potential for inference on evolutionary adaptation and modes of speciation" (there is no discussion about the need for a chromosome-level genome assembly in that paper);
- line 305: I could not find the "Wasp spider hub" on the UCSC genome browser, please provide a direct link;
- figure 3b could be removed as it does not bring much relevant information that is not already present in the text.

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