Supplementary materials: Methods

Isolate collection. A. nasoniae was isolated from *Nasonia vitripennis* collected from fly pupae from spent birds' nests provided by Dr. Lehikoinen (Turku, Finland). This was achieved using previously described methods, with the modification that adult emerging wasps were used rather than pupae (1). A clone was isolated on BHI plates, and then preserved to glycerol stock. For the experiments described in this manuscript, pure *Arsenophonus nasoniae* cultures stored at -80ºC were then streaked on a BHI (Brain Heart Infusion) agar plate and incubated at 30ºC for 6 days till visible colonies appeared on the agar surface. This slow growth rate is one of the typical characteristics from this species. A second verification of the bacterium's identity was conducted by confirming its characteristic colony morphology under a dissecting scope (Supplementary figure 1. Nadal-Jimenez et al. (2019). Env. Microbiol.). A single colony was then used to inoculate 5 ml of BHI broth, which were then incubated at 30ºC and 250 r.p.m. for 48h. A third verification method is related to the OD600 obtained with this bacterium: *A. nasoniae* grows to a maximum OD600 = 0.6-0.8 which is easily observed as a weakly cloudy, translucent culture in BHI broth in comparison to the milky cultures that most bacteria produce (as a result of their usual OD600 = 3-6) in this same medium. In addition, nanopore and Illumina genome-sequencing libraries did not reveal any contaminant DNA in the cultures.

Extraction methods. High molecular weight (HMW) genomic DNA was extracted from a 50 ml culture of *A. nasoniae* using a phenol-chloroform extraction method or a modified CTAB protocol (2-3). DNA purity was assessed on the basis of OD 260/280 and OD 260/230 using Nanodrop while the quality of the HMW DNA was assessed by agarose gel electrophoresis.

Nanopore sequencing and base calling. A nanopore sequencing library was prepared using the Rapid Sequencing Kit (SQK-RAD004) (Oxford Nanopore) with slight modifications of the manufacture's protocol. In total 3.7 μg of HMW DNA was used as input instead of the 400 ng originally suggested. The volume of the DNA sample was adjusted to 15 µl with nuclease-free water and 1.5 µl of the FRA fragmentation mix was added following an incubation at 30°C for 1 min and then 80°C for 1 min. The fragmented DNA was then used for adapter ligation according to manufacturer's recommendations. The DNA library was finally prepared for loading into a FLO-MIN106 R9.4 MinION flow cell by omitting the loading beads. Nanopore sequencing was performed for 24 hr and the raw nanopore signals were live basecalled using the processing pipeline implemented in MinKNOW software v18.01.6 (Oxford Nanopore). Low quality reads (quality score < 7) were discarded from downstream analyses.

Illumina sequencing. This was performed by MicrobesNG (Birmingham) using the Nextera XT library prep protocol on a MiSeq platform (Illumina, San Diego, USA) and reads were adapter trimmed using Trimmomatic 0.30 with a sliding window quality cutoff of Q15 (4).

PacBio sequencing. This was performed by the Centre for Genomic Research (University of Liverpool), size selected 15-20kb libraries were produced and run on the PacBio RSII system (Pacific Biosciences). Contamination with a *Staphylococcus* species was identified, so reads were BLAST filtered to remove the contaminating sequence. The resulting *A. nasoniae* coverage was adequate for assembly and these PACBIO reads were assembled with CANU.

Genome assembly and annotation. Both Illumina and long Nanopore reads were used to produce a hybrid assembly using the Unicycler pipeline version 0.4.5 under the normal mode (5). Additionally, *de novo* assembly was performed for each individual dataset as

follows. Illumina reads were assembled using SPAdes version 3.7.0 (6) under the default parameters while the Nanopore and PacBio reads were de novo assembled using canu version 1.7. The assemblies from the three individual datasets were then mapped on the hybrid assembly and manually inspected for mis-assemblies and errors. Genome annotation was carried out using Prokka version 1.12 (7), while the identification and annotation of phage sequences was performed using the PHAge Search Tool Enhanced Release (PHASTER) web server (8). Finally, functional annotation of the predicted protein sequences was performed by searching for Pfam domains using InterProScan 5 (9). Synteny blocks larger than 5kb between and within the *A. nasoniae* main chromosome and extrachromosomal elements were identified using Sibelia software version 3.0.7 (10). Circular plots were produced using Circos software version 0.69 (11). Taxonomic classification of *A. nasoniae* ORFs was performed using Kaiju web server (12) and results were visualized with Krona charts (13).

References

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