



Draft Genome Assembly of the False Spider Mite *Brevipalpus yothersi*

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ABSTRACT The false spider mite *Brevipalpus yothersi* infests a broad host plant range and has become one of the most economically important species within the genus *Brevipalpus*. This phytophagous mite inflicts damage by both feeding on plants and transmitting plant viruses. Here, we report the first draft genome sequence of the false spider mite, which is also the first plant virus mite vector to be sequenced. The ~72 Mb genome (sequenced at 42× coverage) encodes ~16,000 predicted protein-coding genes.

Brevipalpus yothersi Baker (Tenuipalpidae), previously misidentified as *Brevipalpus phoenicis* (Geijskes), was recently resurrected, redescribed, and placed in the *B. phoenicis sensu stricto* group (1). The false spider mite *B. yothersi* is a vector of several plant viruses, some of which cause diseases in economically important crops, such as citrus and passion fruit (2). More than 40 plant species have been reported as natural hosts of *Brevipalpus*-transmitted viruses (BTVs) (3). Despite the intense use of pesticides and acaricides (4), even low population densities of the false spider mites are sufficient to infest citrus orchards and spread diseases such as citrus leprosis (CL) in Brazil (5) and potentially in the United States and the European Union (6). Most tropical and subtropical regions in the world have resident *Brevipalpus* mites (1), and these pose a major threat to crops affected by the transmitted viruses.

Although information on the economic impact of false spider mites in agriculture is limited, it was estimated that almost 10% of the total world acaricide market value is spent on the control of *Brevipalpus* spp. (7). *Brevipalpus phoenicis sensu lato* species, which include *B. yothersi* (1), reproduce by thelytoky parthenogenesis, controlled by a symbiotic relationship with *Cardinium* bacteria. As a result of the reproductive manipulation, *B. yothersi* populations almost exclusively consist of haploid females ($n = 2$ chromosomes) (8).

For sequencing, a *B. yothersi* population was identified by molecular and morphological traits, as described in Navia et al. (9) and Beard et al. (1), respectively. An isofemale line was reared on sweet orange [*Citrus sinensis* (L.) Osbeck] fruits. For DNA extraction, 8,000 male mites, lacking *Cardinium*, were collected, flash frozen, and

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TABLE 1 Details on the Roche 454 GS FLX+ and Illumina sequencing and libraries used

| Accession no. | Technology used | No. of reads | No. of nucleotides | Coverage (×) | Library type ^a | Molecule | Read length | Insert size |
|----------------------------|---------------------|---------------|--------------------|--------------|---------------------------|-------------------|---------------------|-------------|
| SRX4717572 | Roche (454 GS FLX+) | 600,711 | 421.8 million | 5.9 | SE | gDNA ^b | 1 kb | — |
| SRX4676376 | Roche (454 GS FLX+) | 631,875 | 432 million | 6.1 | SE | gDNA | 1 kb | — |
| SRX4676374 | Illumina (MiSeq) | 11.4 million | 5.3 billion | 74.5 | PE | gDNA | 250 nt ^c | 250 nt |
| SRX4676377 | Illumina (MiSeq) | 15.7 million | 4.6 billion | 64.6 | PE | gDNA | 250 nt | 250 nt |
| SRX4676378 | Illumina (MiSeq) | 2.1 million | 611.3 million | 8.6 | MP | gDNA | 250 nt | 6 kb |
| SRX4676375 | Illumina (MiSeq) | 5.1 million | 1.6 billion | 22.5 | MP | gDNA | 250 nt | 6 kb |
| SRX4676380 | Illumina (MiSeq) | 5.6 million | 1.4 billion | 19.7 | MP | gDNA | 250 nt | 3 kb |
| SRX4676381 | Illumina (MiSeq) | 2.3 million | 697.7 million | 9.8 | MP | gDNA | 250 nt | 3 kb |
| SRX4676379 | Illumina (MiSeq) | 253.5 million | 50.9 billion | — | PE | RNA-seq | 100 nt | 200 nt |

^a SE, single pair; PE, paired ends; MP, mate pairs.

^b gDNA, genomic DNA.

^c nt, nucleotides.

ground with tungsten beads in batches of 2,000 mites. Batches were homogenized, and total DNA was extracted with a DNeasy blood and tissue kit (Qiagen). The DNA samples were pooled and sequenced on a Roche 454 GS FLX+ system with one kit for unidirectional sequencing and one for the mate-pair library preparation protocol (spacing, 3 to 8 kb; reads, 700 to 1,000 bp). Additional sequencing of paired-end libraries was prepared with the Gel-Free protocol (Nextera) and performed with an Illumina MiSeq next-generation sequencer (MiSeq run, Nextera kit, 2 × 250 bp reads, 10 to 15 Gb data; see Table 1).

Raw sequencing reads were quality trimmed, and all ends were removed with a quality Phred score below 20. The MiSeq read pairs were joined into pseudoreads and assembled with the 454 reads with an overlap-layout approach (Newbler 2.9.1). The resulting contigs were further scaffolded with the mate-pair reads (SSpace 2.0) (10), and resulting gaps were locally filled through an iterative process (GapFiller 1.10) (11). The obtained genome sequence was assembled into 3,467 contigs scaffolded into 849 larger genomic segments (N_{50} , 632 kb; 71.18 Mb; GC, 36.8%) and was annotated with both EuGene (12) and AUGUSTUS (13). We predicted 15,929 protein-coding genes, with an average coding DNA sequence (CDS) length of 1,266 bp. The core eukaryotic protein-coding gene presence was assessed with BUSCO (14) (v3.0, 303 reference genes), with 86.5% complete orthologs present (83.2% single copy, 3.3% duplicates, 2.3% fragments, and 34 genes missing). BLASTP hits against the reference genome of the spider mite *Tetranychus urticae* identified 11,721 homologous genes. A search with the InterProScan tool could assign known motifs and gene ontology (GO) terms, for, respectively, 10,171 and 7,831 genes.

Data availability. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank as BioProject number [PRJNA490612](#) under the accession number [QZCP00000000](#). The version described in this paper is the first version.

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REFERENCES

1. Beard JJ, Ochoa R, Braswell WE, Bauchan GR. 2015. *Brevipalpus phoenicis* (Geijskes) species complex (Acari: Tenuipalpidae)—a closer look. *Zootaxa* 3944:1–67. <https://doi.org/10.11646/zootaxa.3944.1.1>.
2. Freitas-Astúa J, Ramos-González PL, Arena GD, Tassi AD, Kitajima EW. 2018. *Brevipalpus*-transmitted viruses: parallelism beyond a common vector or convergent evolution of distantly-related pathogens? *Curr Opin Virol* 33:66–73. <https://doi.org/10.1016/j.coviro.2018.07.010>.
3. Kitajima EW, Rodrigues JCV, Freitas-Astua J. 2010. An annotated list of ornamentals naturally found infected by *Brevipalpus* mite-transmitted viruses. *Sci Agric* 67:348–371. <https://doi.org/10.1590/S0103-90162010000300014>.
4. Della Vecchia JF, Ferreira MC, Andrade DJ. 2018. Interaction of spirodiclofen with insecticides for the control of *Brevipalpus yothersi* in citrus. *Pest Manag Sci* 74:2438–2443. <https://doi.org/10.1002/ps.4918>.

5. Andrade DJ, Lorençon JR, Siqueira DS, Novelli VM, Bassanezi RB. 2018. Space—time variability of *Citrus leprosis* as strategic planning for crop management. *Pest Manag Sci* 74:1798–1803. <https://doi.org/10.1002/ps.4877>.
6. EFSA Panel on Plant Health (PLH), Jeger M, Bragard C, Caffier D, Dehnen-Schmutz K, Gilioli G, Gregoire J-C, Miret JAJ, MacLeod A, Navajas Navarro M, Niere B, Parnell S, Potting R, Rafoss T, Rossi V, Urek G, Van Bruggen A, Van der Werf W, West J, Chatzivassiliou E, Winter S, Catara A, Duran-Vila N, Hollo G, Candresse T. 2017. Pest categorisation of *Citrus leprosis* viruses. *EFSA J* 15:5110. <https://doi.org/10.2903/j.efsa.2017.5110>.
7. Van Leeuwen T, Tirry L, Yamamoto A, Nauen R, Dermauw W. 2015. The economic importance of acaricides in the control of phytophagous mites and an update on recent acaricide mode of action research. *Pestic Biochem Phys* 121:12–21. <https://doi.org/10.1016/j.pestbp.2014.12.009>.
8. Weeks A, Marec F, Breeuwer JAJ. 2001. A mite species that consists entirely of haploid females. *Science* 292:2479–2482. <https://doi.org/10.1126/science.1060411>.
9. Navia D, Mendonça RS, Ferragut F, Miranda LC, Trincado RC, Michaux J, Navajas M. 2013. Cryptic diversity in *Brevipalpus* mites (Tenuipalpidae). *Zool Scr* 42:406–426. <https://doi.org/10.1111/zsc.12013>.
10. Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W. 2011. Scaffolding pre-assembled contigs using SSPACE. *Bioinformatics* 27: 578–579. <https://doi.org/10.1093/bioinformatics/btq683>.
11. Nadalin F, Vezzi F, Policriti A. 2012. GapFiller: a de novo assembly approach to fill the gap within paired reads. *BMC Bioinformatics* 2012(Suppl 14):S8. <https://doi.org/10.1186/1471-2105-13-S14-S8>.
12. Foissac S, Gouzy J, Rombauts S, Mathé C, Amselem J, Sterck L, Van de Peer Y, Rouze P, Schiex T. 2008. Genome annotation in plants and fungi: EuGene as a model platform. *Curr Bioinformatics* 3:87–97. <https://doi.org/10.2174/157489308784340702>.
13. Stanke M, Schöffmann O, Morgenstern B, Waack S. 2006. Gene prediction in eukaryotes with a generalized hidden Markov model that uses hints from external sources. *BMC Bioinformatics* 7:62. <https://doi.org/10.1186/1471-2105-7-62>.
14. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31:3210–3212. <https://doi.org/10.1093/bioinformatics/btv351>.