AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Mitochondrial Genome Sequences of *Diorhabda carinata* and *Diorhabda carinulata*, Two Beetle Species Introduced to North America for Biological Control

A. R. Stahlke,[a,b] A. Z. Ozsoy,[c] D. W. Bean,[d] P. A. Hohenlohe[a]

[a]Institute for Bioinformatics and Evolutionary Studies, University of Idaho, Moscow, Idaho, USA
[b]Bioinformatics and Computational Biology Graduate Program, University of Idaho, Moscow, Idaho, USA
[c]Department of Biological Sciences, Colorado Mesa University, Grand Junction, Colorado, USA
[d]Colorado Department of Agriculture, Palisade, Colorado, USA

ABSTRACT We announce the complete circularized mitochondrial genome assemblies of *Diorhabda carinata* and *Diorhabda carinulata*, beetle species introduced to North America for the biological control of invasive shrubs of the genus *Tamarix* L. (Tamaricaceae). The assemblies (16,232 and 16,298 bp, respectively) each comprise 13 protein-coding genes, 22 tRNAs, two rRNAs, and a noncoding region.

The tamarisk beetle, a cryptic species complex in the genus *Diorhabda* (Coleoptera: Chrysomelidae), originated from Eurasia and was introduced to North America for the biological control of invasive *Tamarix* spp. (1). To better understand evolution within this group of beetles, we assembled and annotated the mitochondrial genomes of *Diorhabda carinata* and *Diorhabda carinulata*, which are the only introduced *Diorhabda* species that are sympatric in their native ranges (1).

Bean and colleagues (2) examined evolutionary relationships among introduced *Diorhabda* spp. This study revealed polyphyly based on the cytochrome oxidase subunit I mitochondrial gene, while analysis of nuclear loci (amplified fragment length polymorphism analysis) grouped samples into four clades corresponding to their morphospecies designations. Additionally, *D. carinata* readily hybridizes with *Diorhabda sublineata* and *Diorhabda elongata* under laboratory conditions without a reduction in fecundity (2, 3) and appears to do so in the field (4). *D. carinulata* failed to produce stable hybrids with the other three clades (2). These results warrant further work to determine the possible influence of introgression, mitochondrial selection, or sex-biased dispersal patterns (5).

For this study, we used a single male from full-sibling inbred lines developed from continuous cultures of each species at the Palisade Insectary, Palisade, CO. We produced a 26-generation inbred line of *D. carinata* originating from Qarshi, Uzbekistan (38.86°N, 65.72°E). A five-generation inbred line of *D. carinulata* was produced from a laboratory culture established from field-collected beetles in Lovelock, NV (40.02°N, 118.52°W), where *D. carinulata* from Fukang, China (44.17°N, 87.98°E), was released in 2001. For *D. carinata,* we dissected the testes and extracted DNA with a MagAttract high-molecular-weight (HMW) DNA kit (Qiagen). For *D. carinulata*, we dissected the head, thorax, and testes using a DNeasy blood and tissue kit (Qiagen). We constructed whole-genome shotgun sequencing libraries using the NEBNext Ultra II DNA library prep kit for both species. The *D. carinata* library was sequenced on a HiSeq 4000 platform (Illumina) to produce paired 150-bp reads. The *D. carinulata* library was sequenced on a MiSeq platform (Illumina) using v3 reagents to produce paired 300-bp reads.

We trimmed adapters from raw reads using Sickle 1.33 (6). With 44,665,534 reads for

Citation Stahlke AR, Ozsoy AZ, Bean DW, Hohenlohe PA. 2019. Mitochondrial genome sequences of *Diorhabda carinata* and *Diorhabda carinulata*, two beetle species introduced to North America for biological control. Microbiol Resour Announc 8:e00690-19. https://doi.org/10.1128/MRA.00690-19.

Editor Antonis Rokas, Vanderbilt University

Address correspondence to A. R. Stahlke, astahlke@uidaho.edu.

Received 7 June 2019
Accepted 29 June 2019
Published 29 August 2019

D. carinata and 28,532,798 reads from *D. carinulata,* we used NOVOPlasty 2.7.2 (7) to assemble each mitochondrial genome. The *Diabrotica barberi* mitochondrion (GenBank accession number KF669870) was used to seed the *D. carinata* assembly. Then, we used the *D. carinata* assembly to seed the *D. carinulata* assembly. Annotations were performed with MITOS2 (last modified 16 June 2017; Git hash 6b33f95) (8) using RefSeq 63 Metazoa and the invertebrate genetic code.

From the *D. carinata* and *D. carinulata* reads, we assembled one circularized mitochondrion assembly per species of lengths 16,232 and 16,298 bp, average coverages of 3,323× and 3,846×, and G+C contents of 22.1% and 21.1%, respectively. Annotations of both genomes comprise 13 protein-coding genes, 22 tRNAs, two rRNAs, and a noncoding region (d-loop). We did not identify light origin (OL) in either assembly. The assembly sizes, G+C contents, and annotations are consistent with those of other chrysomelid mitochondrial genomes (9–13).

Data availability. Raw reads and mitochondrial DNA (mtDNA) genome sequences for *D. carinata* and *D. carinulata* have been deposited in GenBank under accession numbers PRJNA513507, MK359256, and MK359257.

ACKNOWLEDGMENTS

This research was supported by Agriculture and Food Research Initiative grant COLO-2016-01935 to Ruth Hufbauer, Ellyn Bitume, D.W.B., and P.A.H.; a Colorado Mesa University Faculty Professional Development Fund to A.Z.O.; and an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P30 GM103324.

We acknowledge Benji Oswald at the IBEST Computational Core, Dan New and Matt Fagnan at the IBEST Genomics Core, and the University of Oregon Genomics and Cell Characterization Core Facility for sequencing; the Colorado Department of Agriculture, especially Sonya Daly, Nina Louden, and Karen Rosen for aid in rearing cultures; Massimo Cristofaro for overseas expertise and collections; and Mason Linscott for suggesting this endeavor.

REFERENCES

1. Tracy JL, Robbins TO. 2009. Taxonomic revision and biogeography of the Tamarix-feeding *Diorhabda elongata* (Brullé, 1832) species group (Coleoptera: Chrysomelidae: Galerucinae: Galerucini) and analysis of their potential in biological control of Tamarisk. Zootaxa 2101:1–152. https://doi.org/10.11646/zootaxa.2101.1.1.
2. Bean DW, Kazmer DJ, Gardner K, Thompson DC, Reynolds B, Keller JC, Gaskin JF. 2013. Molecular genetic and hybridization studies of *Diorhabda* spp. released for biological control of *Tamarix*. Invasive Plant Sci Manag 6:1–15. https://doi.org/10.1614/IPSM-D-11-00093.1.
3. Bitume EV, Bean D, Stahlke AR, Hufbauer RA. 2017. Hybridization affects life-history traits and host specificity in *Diorhabda* spp. Biol Control 111:45–52. https://doi.org/10.1016/j.biocontrol.2017.05.009.
4. Michels GJ, Royer TA, Jones EN, Lange RA, Bynum ED, Ruthven DC, Tracy JL, Bible JB. 2013. New establishment and county records for *Diorhabda* spp. (Coleoptera: Chrysomelidae) and *Coniatus splendidulus* (Coleoptera: Curculionidae) in the Texas Panhandle and Western Oklahoma. Southwest Entomol 38:173–182. https://doi.org/10.3958/059.038.0203.
5. Toews DP, Brelsford A. 2012. The biogeography of mitochondrial and nuclear discordance in animals. Mol Ecol 21:3907–3930. https://doi.org/10.1111/j.1365-294X.2012.05664.x.
6. Joshi NA, Fass JN. 2011. Sickle: a sliding-window, adaptive, quality-based trimming tool for FastQ files. https://github.com/najoshi/sickle.
7. Dierckxsens N, Mardulyn P, Smits G. 2017. NOVOPlasty: de novo assembly of organelle genomes from whole genome data. Nucleic Acids Res 45:e18. https://doi.org/10.1093/nar/gkw955.
8. Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol 69:313–319. https://doi.org/10.1016/j.ympev.2012.08.023.
9. Song N, Yin X, Zhao X, Chen J, Yin J. 2018. Reconstruction of mitogenomes by NGS and phylogenetic implications for leaf beetles. Mitochondrial DNA A DNA Mapp Seq Anal 29:1041–1050. https://doi.org/10.1080/24701394.2017.1404044.
10. Wang Q, Tang G. 2018. The mitochondrial genomes of two walnut pests, *Gastrolina depressa depressa* and *G. depressa thoracica* (Coleoptera: Chrysomelidae), and phylogenetic analyses. PeerJ 6:e4919. https://doi.org/10.7717/peerj.4919.
11. Wang Q, Tang G. 2017. Genomic and phylogenetic analysis of the complete mitochondrial DNA sequence of walnut leaf pest *Paleosepharia posticata* (Coleoptera: Chrysomeloidea). J Asia Pac Entomol 20:840–853. https://doi.org/10.1016/j.aspen.2017.05.010.
12. Zhou X, Han H, Pang B, Zhang P. 2016. The complete mitochondrial genome of *Galeruca daurica* (Joannis) (Coleoptera: Chrysomelidae). Mitochondrial DNA A DNA Mapp Seq Anal 27:2891–2892. https://doi.org/10.3109/19401736.2015.1060424.
13. Li N, Wei J-N, Jia D, Li S, Ma R-Y. 2016. The complete mitochondrial genome of the flea beetle *Agasicles hygrophila*. Mitochondrial DNA A DNA Mapp Seq Anal 27:3670–3671. https://doi.org/10.3109/19401736.2015.1079848.