Firefly genomes illuminate the origin and evolution of bioluminescence in beetles

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**ABSTRACT.** Fireflies represent one of the most widely appreciated-examples of bioluminescence. Despite long-term interest in the biochemistry, neurobiology, evolution and biotechnological applications of firefly flash signals, only a limited number of genes related to this complex trait have been described. To investigate the genetic basis of firefly bioluminescence, we generated a high-quality reference genome for *Photinus pyralis*, the North American species from which laboratory luciferase is derived, using long-read (PacBio), short-read (Illumina), and Hi-C sequencing technologies. To facilitate comparative genomics, we also generated short-read genome assemblies for *Aquatica lateralis*, a Japanese firefly of conservation interest, and *Ignelator luminosus*, a bioluminescent click-beetle relative. Analyses of these datasets provide new insights into the evolution of beetle bioluminescence. In particular, we reveal a physical clustering of firefly lantern-associated luciferase (Luc1) with several tandemly duplicated long chain fatty acyl-CoA synthetases, supporting its origin at this locus via gene duplication followed by neofunctionalization. We report the presence of the luciferase paralog Luc2 in *P. pyralis*, which is located on a separate chromosome from the Luc1 cluster. We anticipate the genomes presented here will serve as a valuable resource for future investigations of the molecular mechanisms underlying the origin and evolution of firefly bioluminescence.

**Key words:** Photinus pyralis, Aquatica lateralis, Lampyridae, Pyrophorus, luciferase, de novo genomics, de novo transcriptomics