
The bioluminescent system of Keroplatidae (Diptera): characterization of *Orfelia fultonii* luciferase and occurrence of *Orfelia*-type luciferin in non-luminescent keroplatids

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Abstract

Bioluminescence in Diptera (flies), has been described only in Keroplatidae, in species of the genera *Arachnocampa*, *Orfelia* and *Keroplatus* living in temperate regions. Two distinct bioluminescent systems have been described in this family: (I) that of *Arachnocampa* spp in which bioluminescence is produced by lanterns derived from Malpighian tubules, and involves a luciferase, luciferin (substrate), and ATP and that (II) of the North-American *Orfelia fultonii*, in which the bioluminescence is associated to black bodies spread along the body, and requires a 140 kDa luciferase, a luciferin, and a > 300kDa substrate binding fraction (SBF) that is activated by DTT or ascorbic acid (Viviani et al., 2002). However, the identity of the luciferase, luciferin binding protein, and the chemical structure of the luciferin of *Orfelia fultonii* have remained unknown. Although the system of *Keroplatus* still lacks detailed studies, morphological and biochemical evidence suggests that it is similar to that of *Orfelia*. We now have purified and characterized the luciferase from *Orfelia fultonii*. The luciferase is active in dimeric and possibly in trimeric forms, displaying an optimum pH of 7.8, being stable between pH 7.0 and 8.5. MS analysis of purified samples associated with transcriptional analysis has provided tantalizing clues about the identity of the luciferase. We have recently found larvae of *Neoditomiya* sp living in caves in the Atlantic rain-forest in Brazil which despite being non-luminescent, display *Orfelia*-type luciferin as well as its associated binding protein. Molecular phylogenetic studies using mitochondrial markers show that *Neoditomiya* is closer to *Orfelia* and possibly to *Keroplatus* than to *Arachnocampa*. These results indicate that luciferin has other important biological functions in Keroplatids and that bioluminescence could be a recently evolved character. (FAPESP 2010/05426-8; FAPESP/NSF 2014/50583-5).

Keywords: *Orfelia fultonii*, Keroplatidae, luciferase, luciferin

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