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COMMENTS & REPLIES

Standards of evidence for bioluminescence in cockroaches

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A group of cockroaches from Brazil is purported to release bioluminescence through a pair of eye-like cuticular structures on the pronotum (Vršanský et al. 2012). Because living specimens taken from their natural habitat are rare, the authors examined dead material. It is my opinion that the methods employed provide no direct evidence that the insects are indeed bioluminescent; rather, the authors base their conclusions about bioluminescence in the genus on two factors, (1) a single pre-1999 report of a bio-luminescent specimen, and (2) the use of cuticle autofluorescence of the putative light organ as a proxy for bioluminescence capability. I propose that a single report is not sufficient for assuming all individuals of a species are bioluminescent and I contend that cuticle autofluorescence is not a reliable indicator of the presence of light-emitting cells beneath the cuticle.

In 1999, the first record of bioluminescence in an orthopteroid insect was described in the journal *Amazoniana* (Zompro and Fritzsche 1999). The paper related the observation by a field collector that a specimen was bioluminescent. A second line of evidence for bioluminescence—the focus of the *Naturwissenschaften* paper—is the fluorescence characteristics of the external cuticle of the purported light organs. It is worth clarifying the difference between bioluminescence and fluorescence. Fluorescence is the emission of light of one wavelength upon excitation by a different wavelength. Most insect cuticles show endogenous fluorescence—often called auto-fluorescence—upon excitation by ultraviolet and visible light, related to the protein composition of the cuticle (Zill et al. 2000). Flexible cuticle between segments tends to have different autofluorescence to the harder, more sclerotized plates (Hepburn and Joffe 1976). To date, no systematic studies have elucidated a "standard" fluorescence spectrum for cuticle or indeed explained precisely why different cuticles show different autofluorescence spectra (Michels and Gorb 2012).

Bioluminescence is the product of enzymatically-mediated light production within living cells; it is a chemical reaction. In insects and crustaceans, light is produced internally in the cells of a specialised light organ, covered by a transparent cuticle. Cells within the light organ produce luciferin (the substrate) and luciferase (the enzyme) that react to produce light. Dead insects do not bioluminesce. The one exception might be dead or dying insects infected with parasitic nematodes that release a bioluminescent bacteria inside their hosts (Forst et al. 1997).

The approach was to characterise the autofluorescence characteristics of the cuticle of two dead specimens of the cockroach, *Lucihormetica*, and two bioluminescent beetles and display the spectra after normalisation to the intensity of each at 530 nm. The similarities of the spectra were taken as indication of a spectral curve "signature" for bioluminescent organs. My immediate reaction was to ask what is the spectrum of adjacent cuticle that does not cover a putative light organ? The argument would unravel if adjacent cuticles showed similar spectra. These crucial control experiments were not done. The authors state that other cuticles such as the legs or wings of *L. luckae* "were much dimmer and different in shape from the lanterns". Brightness is irrelevant because the authors normalised the curves derived from the test species' light organs, eliminating any value of brightness as a component of the signature. If the shape of the spectrum curve is different, it is crucial that it be shown.

In addition, the normalised autofluorescence spectrum of an aqueous solution of firefly luciferin was shown to approximately match the cuticle spectra, and was used to confirm that "the flashing colour of cockroaches is identical with the light of luminescent click beetles". In fact, the bioluminescence emission spectra in beetles show no similarity to the autofluorescence curves of their light organs. Bioluminescence tends to be of narrow bandwidth and symmetrical with peaks ranging between 550 to 575 nm (Hastings 1996). One would not expect the putative bioluminescence of *Lucihormetica* to match its cuticle autofluorescence either.

The observation that cuticles of dried light organs from diverse bioluminescent species share a common autofluorescence fingerprint is unremarkable, but to use this to conclude that insects showing the pattern are bioluminescent and to infer the colour of the bioluminescence goes too far, especially when no experiments were conducted that attempted to disprove the hypotheses. To my mind, the conclusions about the evolution of bioluminescence in insects are unsupported because of the distinct possibility that *Lucihormetica* is not bioluminescent at all: a single report—even from a reliable source—is not a sufficient basis for such arguments. In fact, this report should be balanced against the observation that captive-reared *Lucihormetica* do not bioluminesce (Vršanský et al 2012) and that a captive-reared

This is a comment to Vršanský, P., Chorvát, D., Fritzsche, I., Hain, M. and Ševčík, R. (2012) Light-mimicking cockroaches indicate Tertiary origin of recent terrestrial luminescence. Naturwissenschaften 99:739-749.

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adult male *Lucihormetica fenestrata* collected as a nymph in a rotting log in its native Brazilian habitat did not bioluminesce (George Beccaloni, personal communication).

Conflict of Interest The author declares no conflict of interest.

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