Electronic Supporting Information

Reagents

Riboflavin, FMN, FAD, palmitic acid, *cis*-11-hexadecenal, spectroscopy ethanol, ammonium formate (LC MS), methanol (MeOH) (LC MS) and acetonitrile (ACN) (LC MS) were purchased from Sigma Aldrich (Czech Republic).

Identification and description of the earthworm Eisenia lucens

Specimens of Eisenia lucens were collected in two areas within the Czech Republic: the Bohemian-Moravian Highlands and the Moravian-Silesian Beskids

Adults have 60–130 segments and reach 4.5–18 cm in length and 5–6.5 mm in width. The body colouration is conspicuous: each segment has a transverse band of red-brown to red-violet colour in its middle, whereas the parts towards the intersegmental groves are of lighter yellowish to greyish colour (this colour also prevails ventrally). The prostomium is epilobic, the clitellum saddle-shaped. Typical of montane forest habitats in the Carpathian arch but also found in other parts of Europe, west to northern Spain; missing in northern Europe. Mostly dwelling under the bark and in wet decomposing wood of downed tree trunks and stumps, but also reported from the organic soil horizon, in particular under moist to wet conditions.

Earthworms were stored in plastic containers filled with decaying wood and soil at a constant temperature of 10 °C. Earthworms were chemically stimulated by 50% ethanol. The expelled fluid was collected, centrifuged, and diluted 10 times with water.

Luminescence spectrometer

All spectra (photoluminescence and bioluminescence) were measured on the spectroluminometer Aminco-Bowman Series 2 (Thermospectronic, USA), equipped with a Xe lamp (for excitation between 200-900 nm).

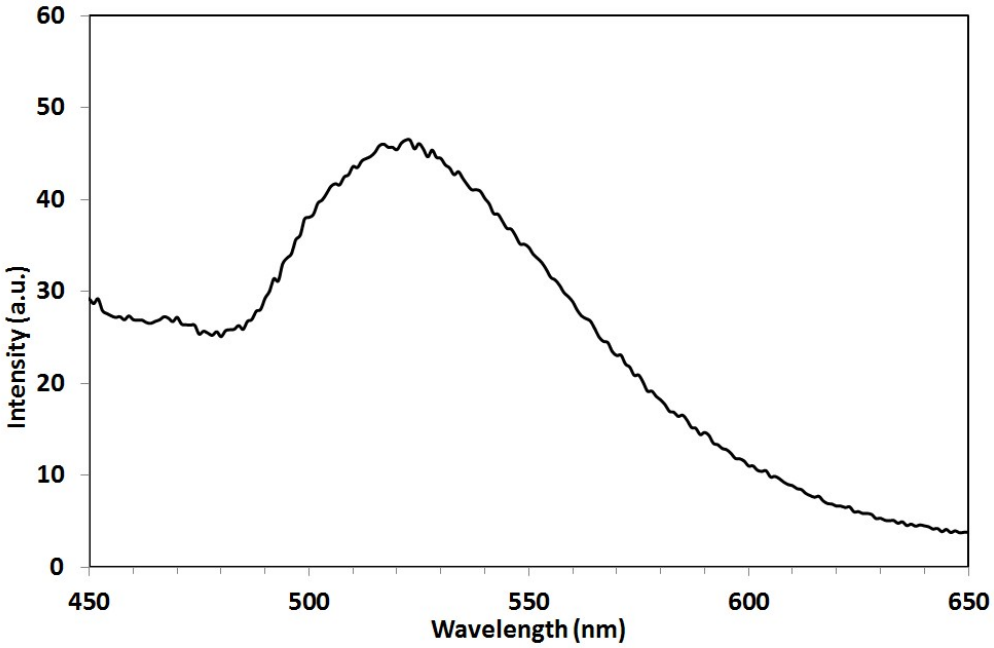

Fig E1 A photoluminescence emission spectrum of the expelled coelomic fluid. The spectrum is discussed in the article but not shown.

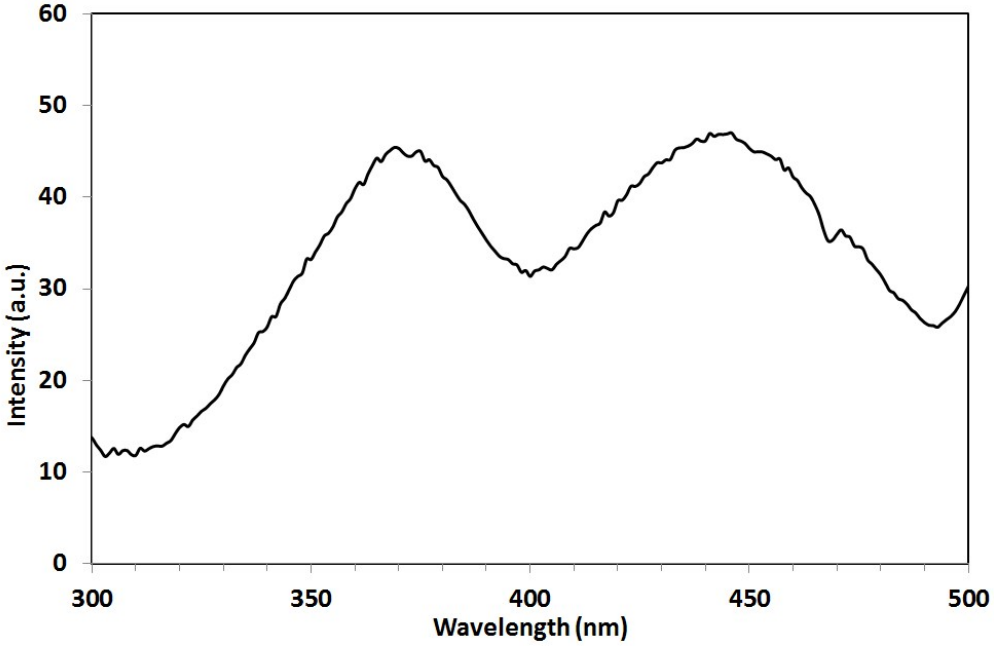

Fig E2 A photoluminescence excitation spectrum of the expelled coelomic fluid.

Fluorescence microscopy

An Olympus BX60 fluorescence microscope with digital image analysis system (Stream Motion 1.9.2) was employed. Blue light was used for excitation in study of native photoluminescence.

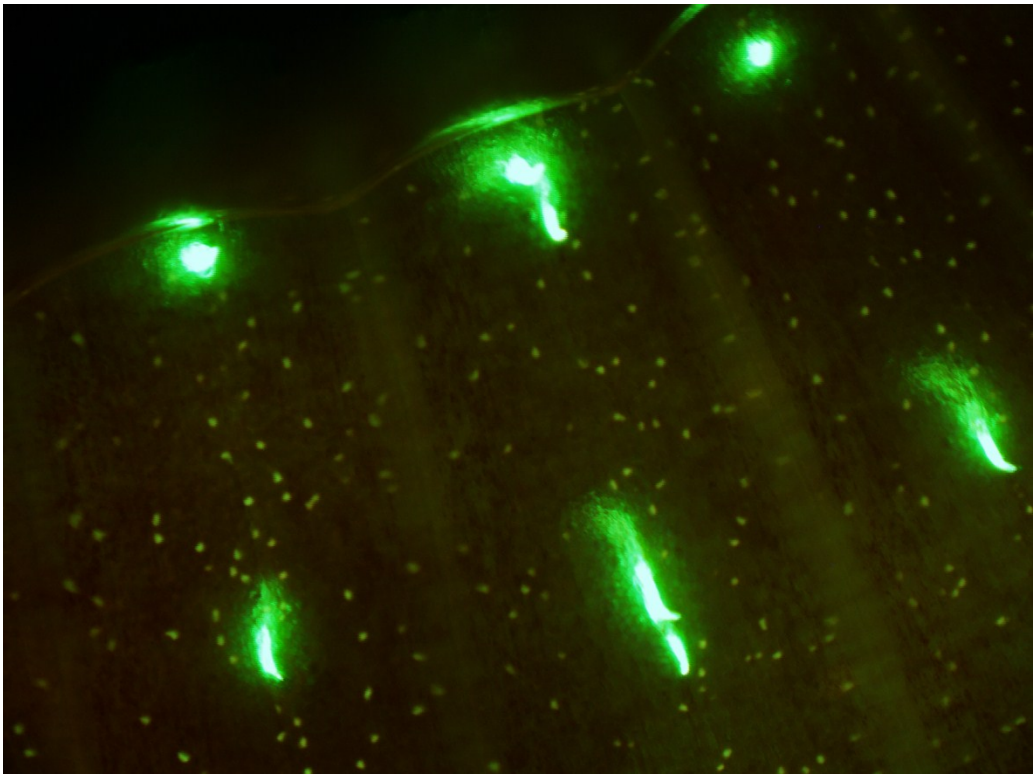

Fig E3 A fluorescence microscopy image. Large bright-green spots correspond to photoluminescence of bristles; smaller ones represent cells floating in the coelomic fluid (coelomocytes).

FLIM

FLIM (Fluorescence Lifie-Time Imaging) measurements were done using the confocal microscope Leica TCS SP8 X, equipped with tuneable pulse white laser (WLL). Pulse laser 405 nm was used as an excitation source.

LC MS

The LC MS method was developed using a Dionex Ultimate 3000RS (Thermo Scientific, CA) module. Compound separation was achieved with a 3.0×100 mm, 2.6 μm Kinetex C18 (Phenomenex, CA) column equipped with a guard column at 23 °C, and a flow rate of 0.3 mL/min. The binary mobile phase system consisted of 0.1% ammonium formate (pH = 6.2) and MeOH or ACN. The injection volume was 10 μl. For separation of isoalloxazines (method A), MeOH was linearly increased from 15% to 75% over 20 min, kept constant at 75% over the next 2 min and followed by equilibration at the initial conditions for 3.0 min. For separation of fatty compounds (method B), ACN was held at initial 50% for 2 min and then linearly increased to 95% over 10 min, kept constant at 95% over the next 10 min and followed by equilibration at the initial conditions for 3.0 min. A complete LC run in both cases took 25 min. The isoalloxazine ring was detected by monitoring absorbance at 448

nm. The HPLC system was connected to a MicrOTOF-QII (Bruker, Germany) mass spectrometer operated in positive electrospray (ESI+) mode with the method A, negative electrospray (ESI–) mode and positive atmospheric pressure chemical ionization (APCI+) mode with the method B. The ionization conditions were set by the software according to the mode used; nebulizing and desolvation gas was nitrogen.

ESI+: capillary voltage +4500 V, end plate offset –500 V, source temperature 250 °C, desolvation gas flow 5 L/min, nebulizer pressure 200 kPa, and collision cell voltage 35 eV.

ESI–: capillary voltage –4500 V, end plate offset +500 V, source temperature 250 °C, desolvation gas flow 5 L/min, nebulizer pressure 200 kPa, and collision cell voltage 35 eV.

APCI+: capillary voltage +4500 V, end plate offset –500 V, corona needle 4000 nA, source temperature 210 °C, vaporizer temperature 250 °C, desolvation gas flow 2 L/min, nebulizer pressure 100 kPa, and collision cell voltage 35 eV.

A base-peak chromatogram (BPC) was acquired in MS mode by monitoring the range of 50 to 2000 m/z with a spectra sample time of 1 s. Identification of target compounds relied on a combination of accurate m/z, isotopic pattern, MS/MS and retention behaviour. High-resolution MS and MS/MS spectra were first investigated to obtain the elemental formula of each compound. A compound was unambiguously identified if the fragmentation patterns of the unknown and the standard compound were identical. Due to the lack of fragmentation spectra of non-polar compounds obtained by the method B, compounds were identified by comparing accurate m/z and retention time with the standard compound.