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A method for capturing images of Collembola bioluminescence using a smartphone camera

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Abstract

Although bioluminescence in Collembola has been known since 1851, photographic evidence remains sparse. Photographs serve as essential evidence to confirm bioluminescence and facilitate further investigations into this phenomenon. We present a method for capturing images of Collembola bioluminescence using a smartphone camera, which will facilitate the discovery and description of luminous Collembola taxa worldwide.

Keywords Enclosure | photographic evidence | still photograph | video

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1. Introduction

Collembola are terrestrial micro-arthropods approximately a few millimetres in body length and include light-emitting species. Since light emission in Collembola was first reported by Allman (1851), only about a dozen species, including Anurida granaria (Nicolet, 1847) (A. Fjellberg pers. com.), Lobella sauteri (Börner, 1906) (Ohira et al. 2023) and Neanura muscorum (Templeton, 1836) (Molisch 1904), have been documented to emit light, despite over 9,000 species of Collembola being described (Bellinger et al. 1996–2024).

Understanding the geographic distribution, phylogenetic relationships and ecological significance of luminous Collembola species could lead to broader insights into the evolutionary advantages of bioluminescence in terrestrial arthropods. Photographic evidence plays a crucial role in confirming bioluminescence and serves as a foundation for deeper investigations. However, capturing images of the low-intensity light emitted by small specimens is challenging.

A limited number of bioluminescence images are available for Collembola species (Kashiwabara 1997; Oba et al. 2011; Sano et al. 2019; Ohira et al. 2023), some of which were taken using high-sensitivity cameras with a macro lens (e.g., Ohira et al. 2023). The use of smartphones in biology is increasing due to their accessibility and portability (Kim et al. 2017; Schaefer et al. 2023). However, there are no reported applications for capturing images of small bioluminescent organisms, such as Collembola. In this study, we present the first protocol for capturing light emission in Collembola using a smartphone camera with an external macro lens. This accessible method facilitates screenings and preliminary studies on the distribution and diversity of luminous Collembola.

2. Materials and methods

2.1 Collembola specimens

We used a laboratory population of *Lobella sauteri* originating from 25 and 29 identified specimens collected at Bugenji, Yokohama, Japan, on November 15 and 25, 2022, respectively. The population was fed plasmodia of *Fuligo septica* (for collection records, see Ohira et al. 2023), as described by Kataoka & Nakamori (2020).

2.2 Preparation of live specimens

Live specimens were prepared on glass slides (26 mm × 76 mm × 1 mm; S1112; Matsunami Glass, Osaka, Japan), each covered with a sheet of moistened paper (26 mm × 76 mm, 50 g/m² graph paper; Kokuyo Co. Ltd., Osaka, Japan) and a vinyl sheet (1 mm thick, 26 mm × 76 mm) with a 6-mm-diameter hole in the centre, followed by another glass slide (1 mm thick, 26 mm × 76 mm; S1112; Matsunami Glass) (Fig. 1A). Live specimens were transferred into the hole in the vinyl sheet by using an aspirator before the top slide was put in place (Fig. 1B). The layers were secured using rubber bands or clips. Images were taken through the top glass slide.

2.3 Imaging

We used an iPhone 13 Pro (Apple Inc., Cupertino, CA, USA) as a representative example of smartphone technology and an Apexel 100X Microscope Lens for Smartphone (Apexel Technology Co. Ltd., Shenzhen, China). The method described is adaptable to other smartphone models (e.g., the iPhone 15 was able to capture images; the iPhone 6 could not) and other macro lenses (e.g., Smartphone Lens Set, Daiso Industries Co., Ltd., Japan and Phone Camera Lens Kit, Selvim Corporation were effective), provided they offer similar functionality, thereby ensuring broader accessibility and applicability. The microscope lens was attached to the wide lens of the iPhone. The camera app was opened in video mode, and the camera was moved close to the subject in the light. Automatic macro switching was turned off by tapping the Macro button. To lock the automatic exposure (AE) and automatic focus (AF), the focus area was touched and held until the AE/AF lock was seen. Performing this operation in complete darkness set the ISO sensitivity to a maximum of 12,500, based on information extracted from still photographs. The camera or subject was then moved to manually adjust the focus (Fig. 1D). The exposure was adjusted to the maximum level by dragging up the Adjust Exposure button, and then the Record button was tapped to start recording (Fig. 1E). The specimens were stimulated as described below. Finally, the Record button was tapped again to stop recording.

During recording, lights in the room were turned off to observe the bioluminescence more clearly.

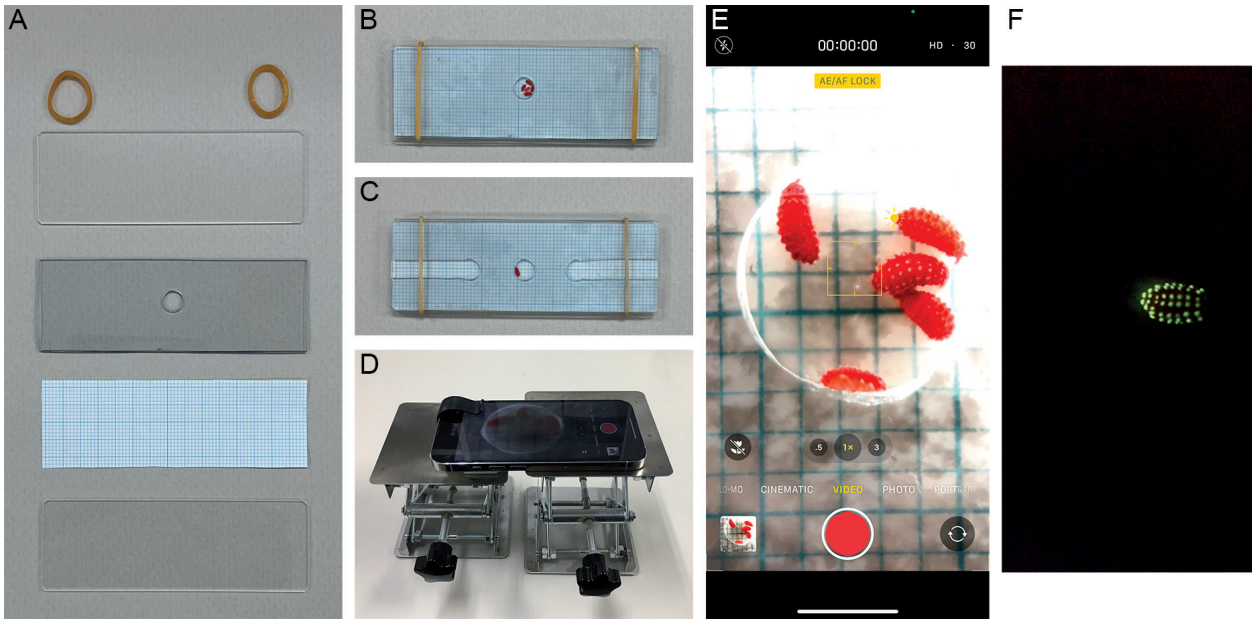

Figure 1. (A) Materials used to enclose live Lobella sauteri (top to bottom: rubber bands, slide glass, vinyl sheet, paper sheet, and glass slide). (B) Prepared glass slide. (C) Prepared glass slide with slits in the vinyl layer. (D) iPhone camera setup above the preparation. (E) Screenshot of the settings used to record a video. (F) Still photograph of Collembola bioluminescence captured by a smartphone camera with an external macro lens.

Using the proposed method, a still photograph can be taken during recording by pressing the Shutter button. We also captured still photographs from the video using Adobe Photoshop (Adobe Inc., San Jose, CA, USA).

2.4 Preparation of live specimens

Physical or chemical stimulation was applied to the specimens because the species used in this study emits light in response to stimuli. For physical stimulation, the specimen to be photographed was transferred into a preparation containing other locomoting individuals for body contact. For chemical stimulation, 50 µL lactic acid or ethanol was applied onto a moistened paper sheet via a slit (5–6 mm in width, 10 mm from the sample hole) cut into the vinyl sheet (Fig. 1C).

3. Results

Using the proposed method, we captured luminescent images of specimens, including greenish light emissions from individual tubercles, stimulated by other locomoting individuals (Fig. 1F), lactic acid, and ethanol. Stimulation with lactic acid and ethanol was fatal to the specimens.

4. Discussion

This study demonstrated that images of Collembola bioluminescence can be obtained using a smartphone camera by enclosing the specimens between glass slides sandwiching paper and vinyl layers. The use of an external macro lens facilitated the capture of these images. While smartphones may not match the performance of dedicated high-sensitivity cameras, they provide an accessible (i.e., widespread availability and relatively low cost compared to high-sensitivity cameras) means to document and present evidence regarding which parts of the body emit light and in what colours.

This method is particularly valuable for researchers without expertise in bioluminescence or without access to high-sensitivity cameras or spectrometers, enabling the discovery of luminous Collembola. To date, only about a dozen species of Collembola have been reported to emit light (e.g., Allman 1851; Molisch 1904; Ohira et al. 2023), despite the descrip-

tion of approximately 9,000 species (Bellinger et al. 1996–2024). Bioluminescence in Collembola may have been understudied due to the challenges associated with detecting and documenting such phenomena in small organisms. Additionally, an estimated 80% of Collembola species remain undescribed (Potapov et al. 2020). These figures highlight the significant potential for uncovering additional luminous species among both known and unknown taxa. The discovery of such species would provide essential groundwork for advancing the understanding of the evolution, ecological significance, and mechanisms of bioluminescence in this group.

While this study employed *L. sauteri* as a model species, the method is expected to be adaptable for a range of species with certain sensitivity. Its success depends on the interplay between the size and intensity of the species' light emissions and the sensitivity of the smartphone camera. Bioluminescence is not always a discrete phenomenon (either present or absent); emissions may range from weak to strong (Terashima et al. 2017; Park et al. 2021). The iPhone 13 achieves an ISO level of 12,500 using this method, implying its ability to detect weaker bioluminescent signals in smaller or less-luminous species.

Understanding what stimuli trigger bioluminescence is important for assessing the presence of this ability in species (Ramesh & Meyer-Rochow, 2021). Stimulation of *Lobella sauteri* specimens allowed us to take bioluminescence images for this species. Body contact with other locomoting individuals was found to be a suitable stimulus, and did not cause lethal damage to light-emitting individuals, although the locomoting individuals sometimes obscured the light emitted by the target specimen. Chemical stimulation with ethanol and lactic acid allowed direct application to single individuals, but this was fatal to the target specimen.

On the other hand, our method can also be used to investigate which stimuli elicit a bioluminescent response in Collembola. This is crucial for a better understanding of the ecological contexts in which bioluminescence is triggered (Ramesh & Meyer-Rochow, 2021), offering insights into the conditions that provoke bioluminescent behaviour in these organisms.

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