Chapter

Ecological and Histological Notes on the Luminous Springtail, *Lobella* sp. (Collembola: Neanuridae), Discovered in Tokyo, Japan

Tadasu Sano, Yukimasa Kobayashi, Ikuko Sakai, Katsunori Ogoh and Hirobumi Suzuki

Abstract

Some species of springtail (Collembola) are luminous, but it is not known whether light emitted by springtail is due to self-luminescence, feeding on luminous fungi, or accidental infection by luminous bacteria. To address this question, we characterized the luminescence of a luminous springtail, *Lobella* sp. (family Neanuridae) discovered in Tokyo, Japan. The emitted light was yellowish-green (540 nm) and was found to originate from tubercles on the thorax (segments II and III) and abdomen (segments I–VI) using a low-light imaging system. The luminescence persisted for several seconds but showed occasional oscillations in a laboratory environment. We also observed fat bodies containing eosin-positive granules under the integument of the tubercles in the tergum by hematoxylin and eosin (HE) staining that were not present in a nonluminous springtail (*Vitronura* sp.). The fat bodies in *Lobella* sp. are presumably photocytes analogous to the firefly lantern, and the eosin-positive granules are the likely source of bioluminescence, which implies that springtails are self-luminescent.

Keywords: luminous springtail, *Lobella*, light organ, fat body, histology

1. Introduction

Some species of springtail (Collembola) are luminous. *Lipura noctiluca* [1], *Anurida* sp. [1], and *Anurida granaria* [2] of the family Neanuridae, *Anurophorus fimetareus* [1] of the family Isotomidae, and *Onychiurus armatus* [3] of the family Onychiuridae exhibit luminescence throughout the body and glow continuously, while *Neanura muscorum* [1] and *Neanura quadrioculata* [1] of the family Neanuridae emit flashes of light upon stimulation. The color of emitted light is diverse from bluish-green to greenish-yellow in each species. However, it is not known whether light emitted by springtail is due to self-luminescence, feeding on luminous fungi, or accidental infection by luminous bacteria. Springtails typically live in the soil of damp wooded areas that contains the mycelia of luminous

fungi. The midgut of the luminous springtail species *Achorutes muscorum* (family Neanuridae) was found to be full of luminous mycelia, but species from other localities were nonluminous [3], suggesting that the light originates from fungi consumed by springtails. However, this is unlikely for species that glow only when stimulated. On the other hand, *Onychiurus armatus* exhibited luminescence even after rearing for 6 months on sterilized agar, providing support for the self-luminescence hypothesis [3]. However, to date there are no detailed descriptions of the histology of light organ and chemistry of luminescence in luminous springtails [3–5].

One of authors (Sano) discovered a luminous springtail, *Lobella* sp., belonging to the family Neanuridae in Tokyo, Japan [6]. This species emits light not from the whole body but from spots on the abdominal segments, which has never been previously reported in springtail [3–5]. This makes *Lobella* sp. suitable for histological studies of a potential light organ. In this work we describe the habitat of *Lobella* sp., the nature and origin of the luminescence, and histological findings.

2. Materials and methods

2.1 Springtails

Lobella sp., which is closely related to *Lobella sauteri*, was collected from two sites: (1) Yokosawa (Akiruno-shi, Tokyo, Japan) and (2) Mitake (Oume-shi, Tokyo, Japan). A nonluminous species, *Vitronura* sp. (family Neanuridae) collected from Takiyama (Hachioji-shi, Tokyo, Japan), served as a control.

2.2 Luminescence imaging

The luminescence of the specimen was captured on Fujicolor 1600 film (Fujifilm, Tokyo, Japan) with a Nikon FM camera (Nikon, Tokyo, Japan) with Nicol 50-mm (F1.4) and 2× teleconverter lenses. Luminescence images were also acquired with a luminescence microscope using a short focal length lens system [7, 8] equipped with an ImagEM electron-multiplying charge-coupled device (CCD) camera (C9100-13; Hamamatsu Photonics, Hamamatsu, Japan). The total magnification was reduced from 4× (UPLSAPO4× objective lens; Olympus, Tokyo, Japan) to 0.8× using the short focal length imaging lens (f = 36 mm, NA = 0.2) in order to capture low intensity light.

Video recording of the luminous specimen was performed using a C2400 high-resolution SIT video camera system (Hamamatsu Photonics) equipped with a Zuiko auto-zoom 35–105 mm (F3.5–4.5) lens (Olympus). The video was converted to an avi format file, and time-lapse image analysis was performed using TiLIA software [9] in order to determine the time course of luminescence intensity in a region of interest.

2.3 Spectroscopy

The luminescence spectrum of the specimen was determined using a U-2900 spectrometer (Hitachi High-Technologies, Tokyo, Japan) under the following conditions: transmittance mode without incident light; scan range, 450–650 nm in 5-nm steps; scan speed, 240 nm/min; and response time, 2 s.

2.4 Histology

The whole body of each specimen was fixed in alcohol Bouin's solution and stored at room temperature. The sample was dehydrated in a graded series of ethanol, embedded in paraffin, and sectioned at a thickness of 5 μm with a microtome (Microm HM310; Leica Biosystems, Nussloch, Germany). The sections were stained with Delafield's hematoxylin and eosin (HE). A BX53 microscope (Olympus) with a DP74 color CCD camera (Olympus) was used for light microscopy observation.

3. Results

3.1 Habitat description

Lobella sp. was collected at Yokosawa, which is a hill region (200–300 m in height) surrounded by valleys that is a typical secondary forest used for agriculture (**Figure 1A**). In one area, shiitake mushrooms (*Lentinula edodes*) are cultured on logs on the ground surface of the forest. After the mushrooms are harvested, the logs proceed to decay (**Figure 1B**). *Lobella* sp. was found under or in the decaying logs (**Figure 2A**) from April to the end of October in 1997–2004. Luminous mushrooms of an unidentified species were also present in this area. *Lobella* sp. was also collected from a mountain pass beside Mount Mitake (900 m in height); the specimens were found under litter, particularly from *Magnolia obovata* (family Magnoliaceae) (**Figure 2B**). Although the luminous springtail, *Lobella* sp., inhabits in such different habitats, this study does not consider taxonomically whether they

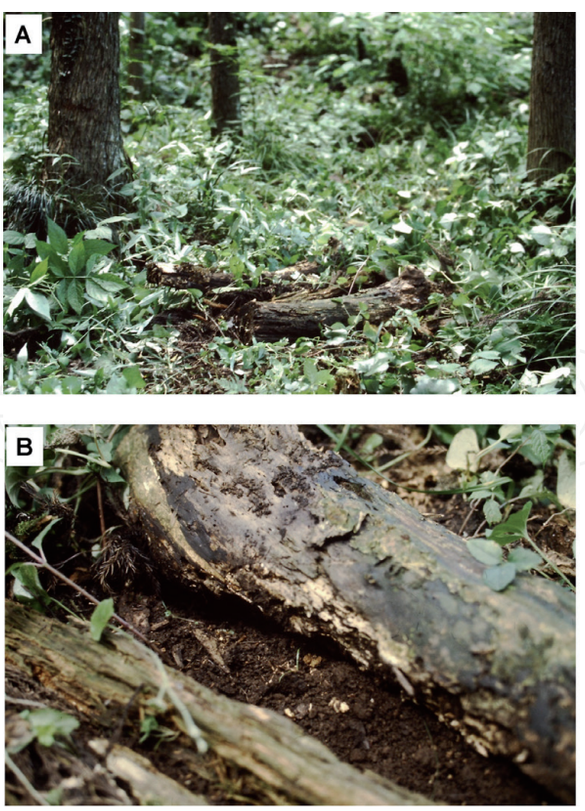

Figure 1.
Habitat of the luminous springtail (Lobella sp.). (A) Overview of the habitat at the Yokosawa site (Akiruno, Tokyo, Japan). (B) Shiitake mushroom (Lentinula edodes) is cultured on logs, which proceed to decay on the ground after the mushrooms are harvested. Springtails are found under or on decaying logs.

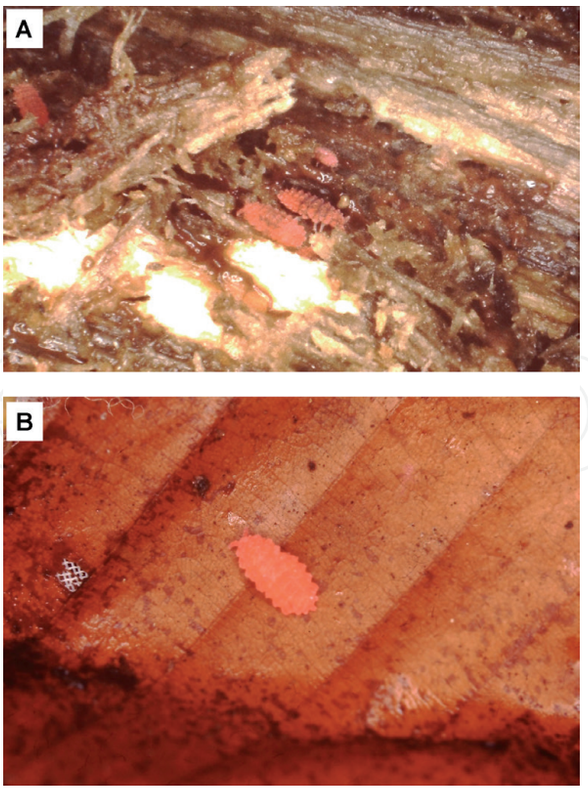

Figure 2.
(A and B) luminous springtail (Lobella sp.) found in decayed logs at the Yokosawa site (A) and under Magnolia obovata *(family Magnoliaceae) litter at the Mitake site (B).*

Figure 3.
Habitat of nonluminous springtail (Vitronura sp.) collected at the Takiyama site (Hachioji, Tokyo, Japan). (A) Cattle barn. (B) Vitronura sp. inhabits soil-containing cattle dung.

are the same or different species. The nonluminous springtail *Vitronura* sp. was collected at Takiyama (**Figure 3A**), a cattle barn, from soil-containing cattle dung (**Figure 3B**).

3.2 Luminescence

In a field observation at the Yokosawa site, we observed flashes of light on the ground when lifting the shiitake logs. However, we were unable to discern the color of the light by the naked eye since it was too weak. **Figure 4** shows *Lobella* sp. and its luminescence. Several tubercles were present on the surface of the thorax and abdomen (**Figure 4A**), and the luminescence was strongest at the posterior margin of the abdomen (**Figure 4B**). The luminescence was elicited for several seconds by mild mechanical stimulation (e.g., by shaking the container or blowing on the specimen), although there were long intervals between bursts of light. The luminescence spectrum showed a maximum peak wavelength of 540 nm (**Figure 5**), which is visible as a yellowish-green color. Mechanical stimulation also induced the secretion of mucus from the body surface; at such instances, no luminescence was observed either from the body or the mucus.

To identify the luminescent region of the body, luminescence image of the specimen was captured by microscopy (**Figure 6**). The luminous spots corresponded to tubercles on the thorax (segments II and II) and abdomen (segments I through VI). Time-lapse image analysis showed that the luminescence persisted for several seconds (less than half a minute) in the laboratory environment with occasional oscillations with a 3.0-s flash interval, 2.1-s pulse duration, 0.9-s inter-pulse duration, and 3.0-s oscillatory peak interval (determined from the average of four peaks in **Figure 7**).

3.3 Histology

HE staining of a cross section of the second abdominal segment of *Lobella* sp. from the Yokosawa site revealed that the midgut cavity occupied most of the body and the tubercles had a pointy seta within a socket (**Figure 8**). The dorsal vessel (heart), ventral nerve cord, hemocytes, and muscle tissues were also identified. Under the integument of the tubercle in the tergum, fat bodies occupy a large space between the integument and midgut and comprise large trophocytes whose cellular boundary is obscure. Each trophocyte contains many eosin-positive granules (EPG) in its peripheral cytoplasm. Such granules were not present in fat bodies under the

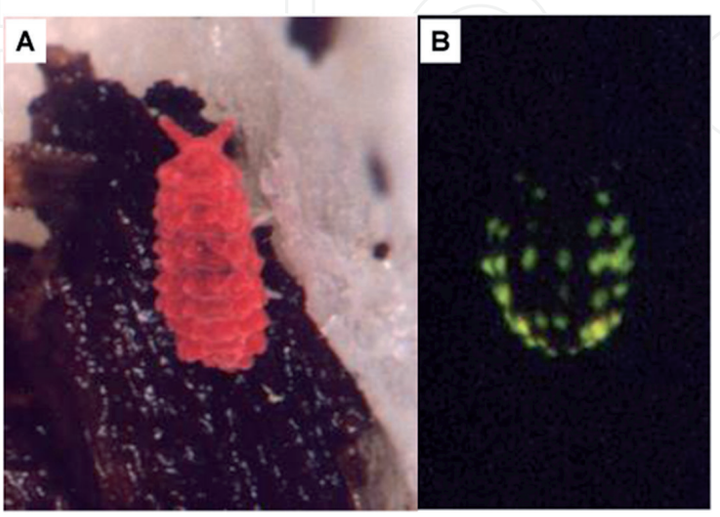

Figure 4.
Photographs of Lobella sp. (A) Bright-field image of Lobella sp. collected at the Yokosawa site. Tubercles are present on the body surface of the thorax and abdomen. (B) Bioluminescence images of Lobella sp. collected at the Mitake site. Luminescent spots are present on the body surface; a higher signal intensity is observed at posterior margin of the abdomen.

integument of the sternum or pleuron. The posterior fat bodies were larger than those in the anterior region and were surrounded with hemolymph (**Figure 9**). No eosin-positive granules were observed in fat bodies of *Vitronura* sp. (**Figure 10**).

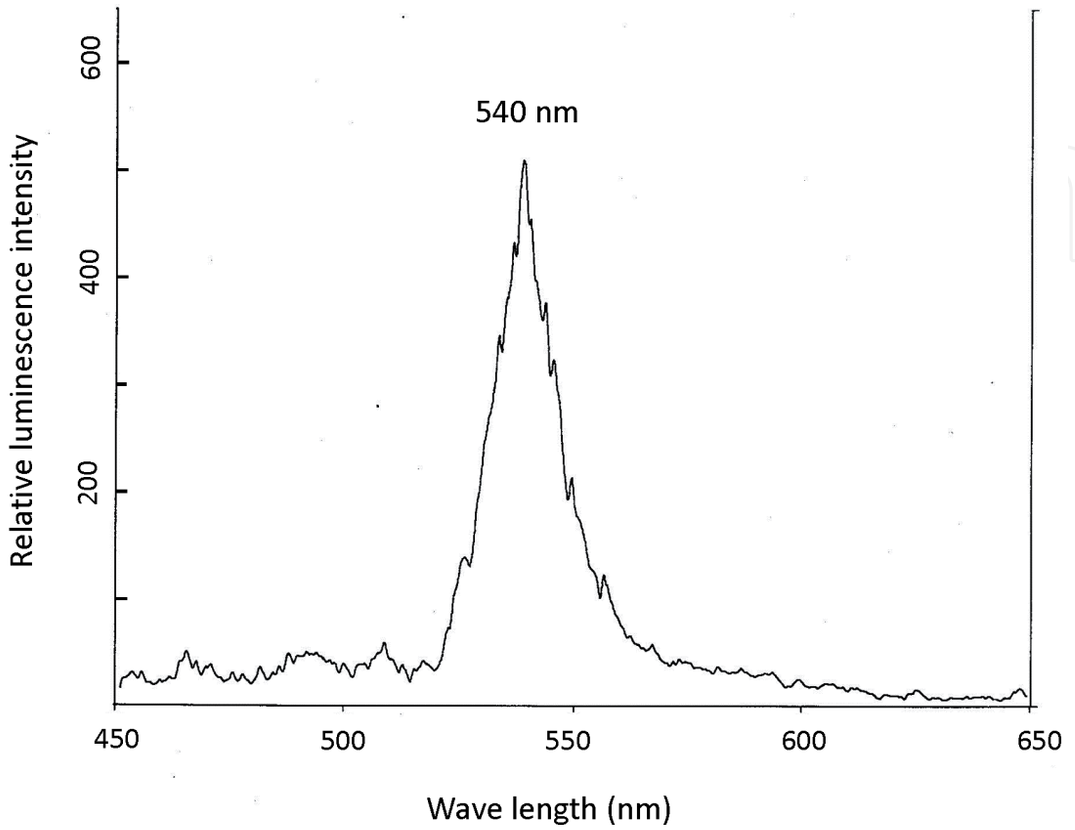

Figure 5.
Bioluminescence spectra of Lobella sp.; the maximum peak wavelength is 540 nm.

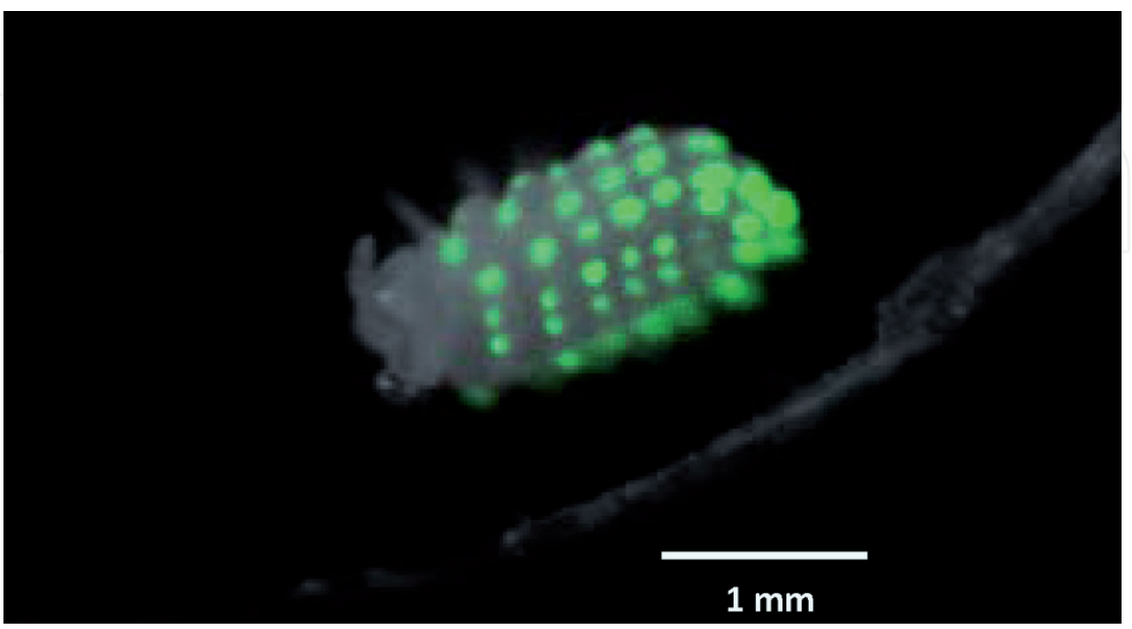

Figure 6.
Merged bioluminescence and bright-field images (pseudocolored green) captured with the luminescence microscope. The luminous spots correspond to tubercles on the thorax (segments II and III) and abdomen (segments I–VI). Scale bar: 1 mm.

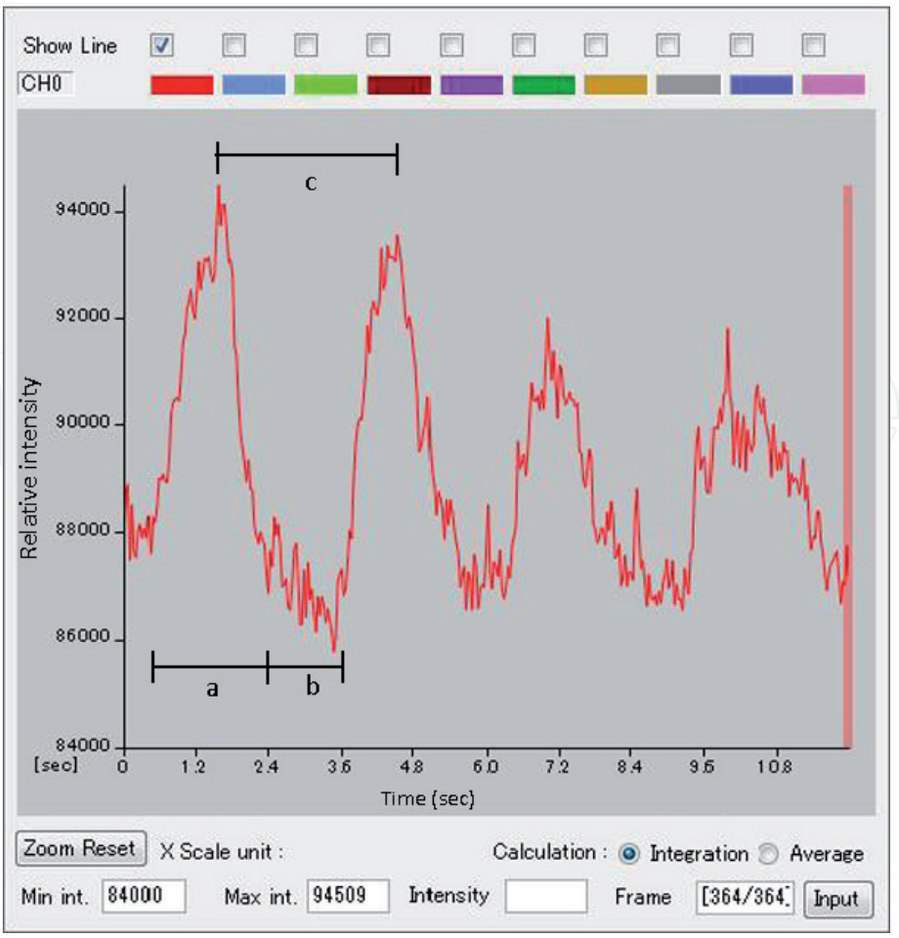

Figure 7.
Time course of luminescence captured by time-lapse imaging. The X and Y axes show time(s) and relative luminescence intensity, respectively. Occasional oscillations were observed, and the flash interval (a + b) is 3.0 s with 2.1 pulse duration (a) and 0.9 s inter-pulse duration (b), and oscillatory peak interval (c) is 3.0 s.

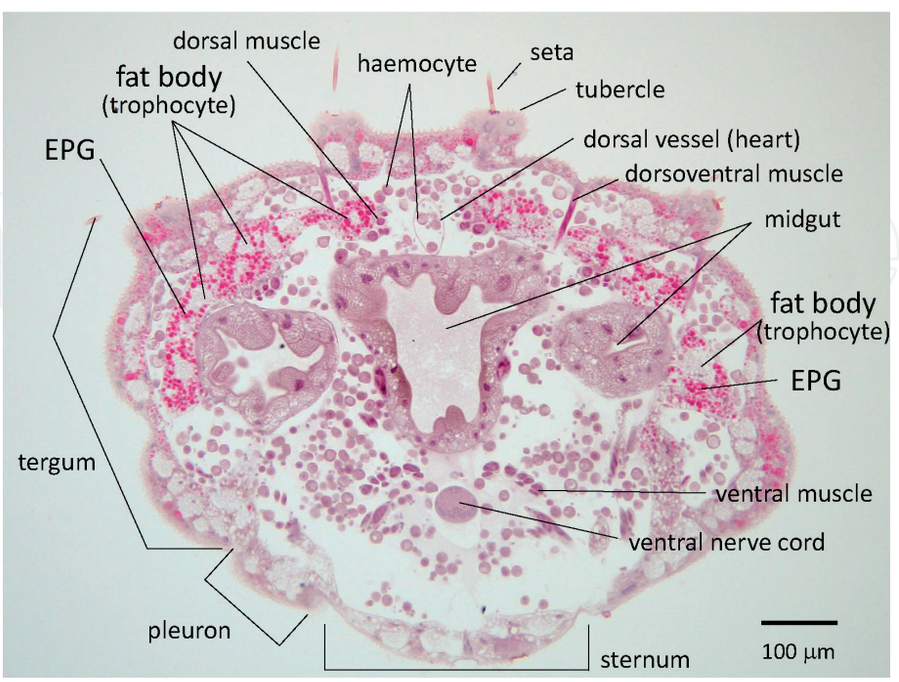

Figure 8.
Cross section of the second abdominal segment visualized by HE staining of Lobella sp. from the Yokosawa site. Fat bodies containing eosin-positive granules (EPG) are present under the integument of the tubercles in the tergum. Scale bar, 100 μm.

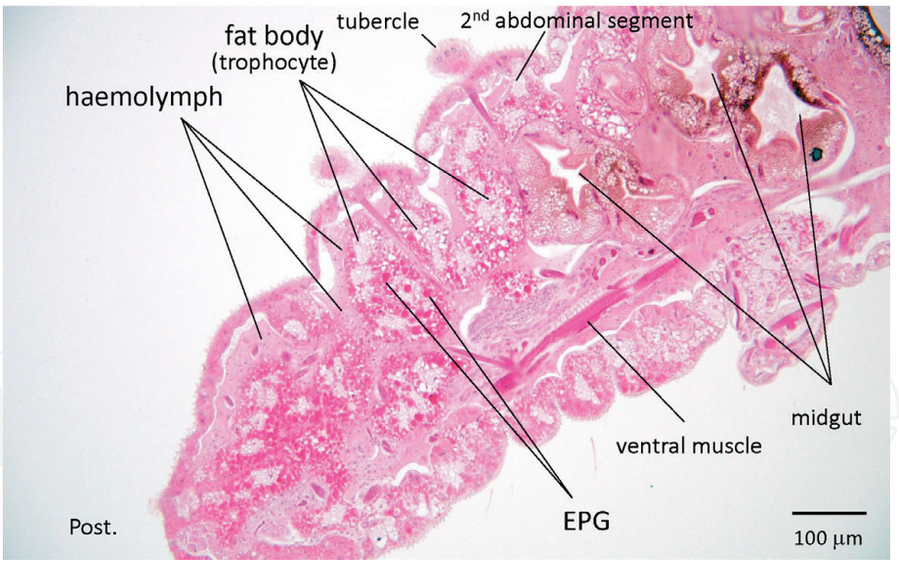

Figure 9.
Longitudinal section of Lobella sp. from the Yokosawa site visualized by HE staining. The fat bodies in the posterior region are larger than those in the anterior region and contain eosin-positive granules (EPG) surrounded by hemolymph. Scale bar, 100 μm.

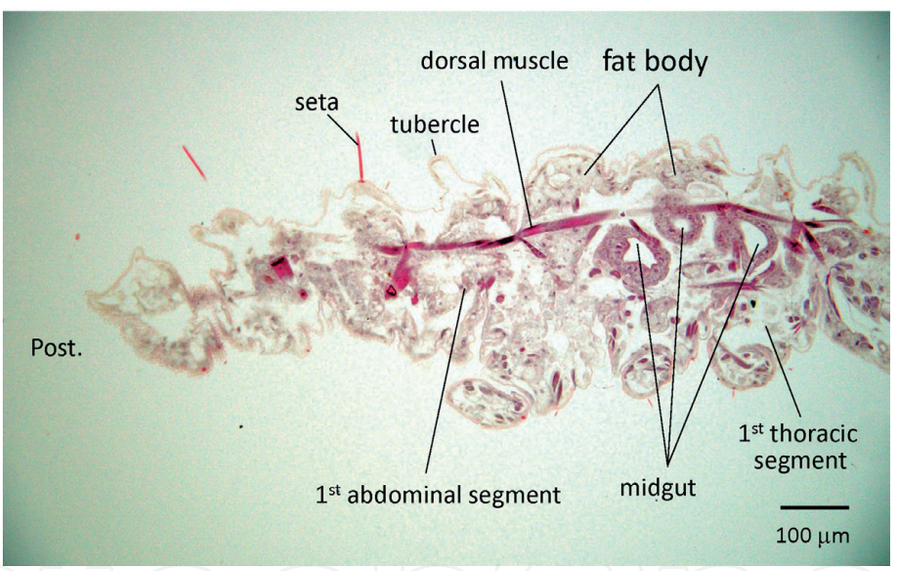

Figure 10.
Longitudinal section of Vitronura sp. from the Takiyama site visualized by HE staining. No eosin-positive granules were observed in the fat body. Scale bar, 100 μm.

4. Discussion

Lobella sp. emits flashes of light lasting several seconds upon stimulation. This is similar to *Neanura quadrioculata* and *N. muscorum* [1]. Furthermore, the luminescence of *Lobella* sp. was occasional oscillatory (with an interval of 3 s) (**Figure 7**), suggesting that it occurs in response to an external factor [1]. These results provide evidence for the self-luminescence of *Lobella* sp., which may have an intracellular origin since this species secretes mucus that is nonluminescent.

The light emitted by *Lobella* sp. was associated with tubercles on the abdomen (**Figure 6**), which may be light organs analogous to those of the firefly. A histological examination revealed fat bodies containing eosin-positive granules under the integument of the tubercles in the tergum (**Figures 8** and **9**), but we did not recognize any specific structures such as a photogenic or reflector layer of firefly

that could function as a light organ. Fat bodies were also present in the nonluminous springtail *Vitronura* sp., but these did not contain eosin-positive granules (**Figure 10**). Photocytes of the light organ in larval and adult fireflies are derived from fat bodies [10–13], and luciferase is located in the cytoplasm of photocytes in the photogenic layer [14]. Furthermore in a larval firefly, fat bodies are a precursor of photocytes that were observed to have some luminosity by ex vivo low-light microscopy [15]. We speculate that the eosin-positive granules in the fat bodies of *Lobella* sp. are related to the bioluminescence of this species in the same manner as peroxisomes of firefly photocytes [13]. Ex vivo low-light imaging of fat bodies could confirm this possibility. On the other hand, an anti-firefly luciferase antibody did not react with *Lobella* sp. tissue (data not shown), indicating that the bioluminescence of springtail is distinct from the luciferin-luciferase reaction in firefly.

There have been few studies on luminous springtails, and most of these have been review articles [2–5]. Some reasons for the lack of research are the difficulty of species identification by entomologists who do not study Collembola and the small size of specimens, which makes biochemical analyses challenging. The present findings provide a basis for future studies on the mechanisms as well as the evolution of bioluminescence in insects using a variety of experimental approaches—e.g., molecular modeling, genetic engineering, and omics technology with artificial intelligence processing—that are applicable to small-sized organisms and wild specimens [16–19]. Additionally, springtail displays a unique mating behavior where the male deposits spermatophores on the ground or close to a female and keeps other males away through jostling [20, 21]. It is possible that the bioluminescence of springtail plays an important role in mating, as is the case for firefly and other luminous organisms [22].

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Conflict of interest

The authors declare no conflict of interest.

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