Bioluminescent properties of *Mesochaetopterus japonicus* (Polychaeta: Chaetopteridae) with comparison to *Chaetopterus*

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Abstract: *Mesochaetopterus* is a bioluminescent polychaete that belongs to the family Chaetopteridae. It secretes a blue luminescent mucus as a response to mechanical stimulation similar to the species in *Chaetopterus* (Chaetopteridae). However, unlike *Chaetopterus*, the biochemical properties of *Mesochaetopterus* bioluminescence are largely unexplored. In this study, we examined the basic biochemical properties of the bioluminescence seen in *M. japonicus* and compared them to those seen in *Chaetopterus*. The comparison revealed that similar blue luminescence peaked at approximately 460 nm were induced by the addition of Fe$^{2+}$ and H$_2$O$_2$, suggesting that bioluminescence in *M. japonicus* and *Chaetopterus* has similar basic biochemical properties. On the other hand, the gel filtration analyses showed that the elution volumes of active proteins were different between *Mesochaetopterus* and *Chaetopterus*. The molecular weights of these proteins were estimated to be 150 kDa and 90 kDa (approximately) for *Mesochaetopterus* and *Chaetopterus*, respectively.

Key words: Bioluminescence, Chaetopteridae, Chaetopterus, Mesochaetopterus, Polychaeta

Introduction

The marine benthic polychaete *Chaetopterus* shows an internal brilliant blue luminescence and secretion of glowing mucus when disturbed, which are supposed to contribute their survival by startling or making the predators more visible to its predator’s enemies (Verdes & Gruber 2017). The light emission has been reported as a biochemical reaction and by the enzyme-substrate complex called *Chaetopterus* photoprotein (CP). Fe$^{2+}$ and H$_2$O$_2$ stimulate the light production of CP (Shimomura & Johnson 1966). CP of *Chaetopterus variopedatus* was isolated and crystallized (Shimomura & Johnson 1968), but neither the gene nor the chromophore chemistry has been elucidated (Rawat & Deheyn 2016, Purtov et al. 2019). On the other hand, studies on the bioluminescence of *Mesochaetopterus* are very few.

*Mesochaetopterus* is the sister genus of *Chaetopterus* in the family Chaetopteridae (Moore et al. 2017). Members of both these genera are benthic, tubicolous organisms which are commonly found in shallow water. Organisms belonging to the genus *Chaetopterus* construct a U-shaped tube, whereas those from the genus *Mesochaetopterus* construct an l-shaped tube (Fujiwara 1935). *M. japonicus* is abundantly present in the coasts of the Tokai region of Japan and can be collected by hand digging, but it is challenging to collect undamaged specimens because of their fragile tube and body (Fujiwara 1934). The structure of luminous glands has been studied (Fujiwara 1935), but the bioluminescence properties have not yet been reported.

Luminous organisms mostly have similar luminescent systems within the same taxa, such as family level. However, recent studies reported the presence of at least two distinctive bioluminescence systems within a same family, as seen in Lumbricidae and Enchytraeidae (Annelida), Mycetophilidae (Insecta), Linophrynidae and Apogonidae (ray-finned fish) (Rodionova & Petushkov 2019, Viviani et al. 2001, Hansen & Herring 1977, Thacker & Roje 2009). Interestingly, for every pair mentioned in the previous here, the light emitted by the both the members was similar in color. In this study, we examined the basic biochemi-
**Materials and Methods**

**Materials**

*M. japonicus* and *C. cautus* were collected from intertidal zones of Ago-Bay, Japan, in winter of 2018–2019. Worms were frozen using liquid nitrogen and stored at −80°C until use. Partial sequences of COI and 18S rDNA were sequenced and deposited in GenBank (accession numbers, LC533808 and LC533810). Species identification was confirmed by high similarity (>99.8%) to the sequences deposited in GenBank (DQ209218 for 18S rDNA of *M. japonicus* and KX896507 for cytochrome oxidase c subunit I of *C. cautus*, respectively).

**Extraction of luminescent proteins**

The crude extracts of *Mesochaetopterus* and *Chaetopterus* were prepared separately. The segments around aliform parapodia were dissected from three frozen specimens (approximately 1.5 g) and were ground with 15 mL of the ice-cold buffer (50 mM Tris–HCl at pH 7.5, containing 150 mM NaCl) using a mortar. Homogenates were centrifuged at 7 745 g for 30 min at 0°C, and the supernatants were collected as the crude extracts. The effects of Fe²⁺ and H₂O₂ on the light emission was measured by adding 10 µL of 5 µM FeCl₂ and 10 µL of 1 µM H₂O₂ to 80 µL of the extract, using a luminometer (Centro LB960, Berthold). The luminescent spectra were measured using 800 µL of the extract, 100 µL of 5 µM FeCl₂, and 100 µL of 1 µM H₂O₂ by a spectrophotometer (FP-777, Jasco). As these crude extracts produced light even when added water only, the measurements were taken after most of the luminescence had subsided.

**Partial purification of luminescent proteins**

Ammonium sulfate precipitation and gel filtration analysis were performed to examine the biochemical properties of the proteins responsible for bioluminescence. Ammonium sulfate (3.13 g) was added to 10 mL of crude extracts up to 50% saturation, and this solution was centrifuged at 7 745 g for 30 min. Both the pellets and the floating materials were dissolved in 1 mL of the elution buffer (50 mM Tris–HCl at pH 7.5, containing 150 mM NaCl) containing 0.05% Triton X-100. The solutions were applied onto a gel filtration column (HiLoad 16/60 Superdex 200 pg, GE) that was equilibrated with the elution buffer and chromatographed using an AKTA Prime Plus (GE). The molecular weights were calculated using Gel Filtration Calibration Kit LMW and HMW (GE). The luminescent activity of these fractions was measured in a manner same to that used in the crude extracts.

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**Results and Discussion**

To characterize the basic biochemical properties of *M. japonicus* bioluminescence, we performed three independent experiments reported in previously published studies about *Chaetopterus*: i) measurement of luminescent spectrum, ii) evaluation of the effect of Fe²⁺ and H₂O₂, and iii) estimation of molecular weight of the protein responsible for bioluminescence. We also studied the properties of *C. cautus*, which are very similar to those seen in the well-studied species, *C. variopedatus*; using the same equipment for a precise comparison.

In vivo bioluminescence of *M. japonicus* and *C. cautus* was similar to previously reported observations in multiple aspects (Fujiwara 1935, Nicol 1952). In both the organisms, they occurred in various body parts of the worm and in secreted mucus (Fig. 1). The potassium chloride solution (400 mM) and freshwater efficiently triggered the luminescent response in living specimens. Light emission of both species appeared blue to our naked eyes, and the spectra had a peak around 460 nm (457 nm in *M. japonicus*, 459 nm in *C. cautus*) (Fig. 2a), which is identical to the value observed for *C. variopedatus* (Nicol 1957, Shimomura & Johnson 1966). The light production by buffer extracts from both species was stimulated similarly by the addition of Fe²⁺ and H₂O₂ (Fig. 2b). These results suggest that the luminescent system of *Mesochaetopterus* is essentially the same as that of *Chaetopterus*. Thus, the bioluminescent characteristics seem to be acquired in a common ancestor of the two genera in Chaetopteridae.

In the gel-filtration analysis, the luminescent activity of both extracts was eluted in three different volume areas; void volume (~50 mL), elution volume (50–100 mL), and near column volume (100– mL). We performed the gel-filtration analyses thrice and twice for *Mesochaetopterus* and *Chaetopterus*, respectively, and found that the elution times and light intensity of the first and the third active fractions varied about a few milliliters, but remained constant for the second active fraction for both species.

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**Fig. 1.** Bioluminescence of *Mesochaetopterus japonicus* (a, b) and *Chaetopterus cautus* (c, d) under white light field (a, c) and dark field after KCl stimulation (b, d). Scale bars = 10 mm.
The first active fraction probably corresponds to the aggregated protein, and the light production observed in the third active fraction could be caused by the chemiluminescence of the small organic compounds, such as flavins occurring in the presence of Fe$^{2+}$ (Zeng & Jewsbury 1995). The association of flavins with light production by *Chaetopterus* has been reported in the literature (Branchini et al. 2014). The molecular weight of the second active fraction for an elution volume of 73 mL in *C. cautus* was estimated to 90 000 (Fig. 3a, b), which is in agreement with the latest reports in *C. variopedatus* (80 kDa) (Purtov et al. 2019). On the other hand, the molecular weight of the *M. japonicus* luminescent protein, appeared at an elution volume of 67 mL, was calculated to be approximately 145 000 (Fig. 3a, b). It is still unclear if the actual molecular sizes of the active proteins show any significant difference between *Mesochaetopterus* and *Chaetopterus*, but it may be a contributing factor leading to the physiological and ecological differences between them. *Mesochaetopterus* luminous glands are not located on the aliform parapodia, which is one of the most intensely luminescent parts in *Chaetopterus*, and are instead situated in the segments...
Bioluminescence of Mesochaetopterus

Bioluminescence of Mesochaetopterus present immediately before and after the aliform parapodia (Fuj iwara 1935).

In conclusion, our results show that the major biochemical properties of Mesochaetopterus bioluminescence are similar to those of Chaetopterus in the context of the luminescence spectrum, iron sensitivity, and involvement of the proteinaceous component. This suggests that the bioluminescence in Mesochaetopterus and Chaetopterus could have possibly originated from a common ancestor.

References

Branchini BR, Behney CE, Southworth TL, Rawat R, Deheyn DD (2014) Chemical analysis of the luminous slime secreted by the marine worm Chaetopterus (Annelida, Polychaeta). Photochem Photobiol 90: 247–251.

Fujiwara T (1934) On a new chaetopterid, Mesochaetopterus japonicus sp. nov. J Sci Hiroshima Univ Ser B Div I Zool 3: 1–14.

Fuj iwara T (1935) On the light production and the luminous organs in a Japanese chaetopterid, Mesochaetopterus japonicus Fuj iwara. J Sci Hiroshima Univ Ser B Div I Zool 3: 185–192.

Hansen K, Herring PJ (1977) Dual bioluminescent systems in the anglerfish genus Linophryne (Pisces: Ceratioidea). J Zool Lond 182: 103–124.

Moore JM, Nishi E, Rouse GW (2017) Phylogenetic analyses of Chaetopteridae (Annelida). Zool Scr 46: 596–610.

Nicol JAC (1952) Studies on Chaetopterus variopedatus (Reni er). I. The light-production glands. J Mar Biol Assoc UK 30: 417–431.

Nicol JAC (1957) Spectral composition of the light of Chaetopterus. J Mar Biol Assoc UK 36: 629–649.

Osborn KJ, Rouse GW, Goffredi SK, Robison BH (2007) Description and relationships of Chaetopterus pugapor cinus, an unusual pelagic polychaete (Annelida, Chaetopteridae). Biol Bull 212: 40–54.

Purtov KV, Petushkov VN, Rodionova NS, Pakhomova VG, Myasnyanko IN, Myshkina NM, Tsarkova AS, Gitelson JI (2019) Luciferin-luciferase system of marine polychaete Chaetopterus variopedatus. Dokl Biochem Biophys 486: 209–212.

Rawat R, Deheyn DD (2016) Evidence that ferritin is associated with light production in the mucus of the marine worm Chaetopterus. Sci Rep 6: 36854.

Rodionova NS, Petushkov VN (2019) Comparison of earthworm bioluminescence systems. Dokl Biochem Biophys 485: 157–161.

Shimomura O, Johnson FH (1966) Partial purification and properties of the Chaetopterus luminescence system. In: Bioluminescence in Progress (eds Johnson FH, Haneda Y). Princeton University Press, Princeton, New Jersey, pp. 495–521.

Shimomura O, Johnson FH (1968) Chaetopterus photoprotein: Crystallization and cofactor requirements for bioluminescence. Science 159: 1239–1240.

Shimomura O, Yampolsky I (2019) Bioluminescence: Chemical Principles and Methods—third edition. World Scientific, Singapore.

Thacker CE, Roje DM (2009) Phylogeny of cardinalfishes (Tele ostei: Gobiiformes: Apogonidae) and the evolution of visceral bioluminescence. Mol Phylogenet Evol 52, 735–745.

Verdes A, Gruber DF (2017) Glowing Worms: Biological, Chemical, and Functional Diversity of Bioluminescence Annelids. Integr Comp Biol 57: 18–32.

Viviani VR, Hastings JW, Wilson T (2001) Two bioluminescent Diptera: The North America Orfelia fultoni and Australian Arachnocampa flava. Similar niche, different bioluminescent systems. Photochem Photobiol 75: 22–27.

Zeng J, Jewsbury RA (1995) Chemiluminescence of flavin in the presence of Fe(II). J Photochem Photobiol 91: 117–120.