



Abyssal glowing sea spider, first insight into pycnogonid luminescence

Jérôme Mallefet^{a,*}, David A. Staples^b, Timothy D. O'Hara^b, Laurent Duchatelet^{a,1}

^a Université catholique de Louvain – UCLouvain, Earth and Life Institute, Marine Biology Laboratory, 3 Croix du Sud, Louvain-La-Neuve, 1348, Belgium

^b Museums Victoria Research Institute, PO Box 666, Vic, Melbourne, 3001, Australia

ARTICLE INFO

Keywords:

Bioluminescence
Photoprotein
Abyss
Sea spiders
Colossendeis

ABSTRACT

We report the first observations of bioluminescence in two giant deep-sea pycnogonid species from the family Colossendeidae, *Colossendeis tasmanica* Staples, 2007 and *Colossendeis minor* Schimkewitsch, 1893, both collected from the Australian abyss. Notably, *C. tasmanica* was abundant enough to allow for detailed study. This species emits a bright deep-blue light ($\lambda_{\max} = 449 \text{ nm}$) from its legs, displaying a distal-to-proximal luminescence gradient. Luminescence is triggered by H_2O_2 and KCl, while adrenaline does not induce light emission. The lack of response to the hot and cold assay suggests that the luminescence system of *C. tasmanica* is not luciferin-luciferase-based, but instead resembles a photoprotein-based system. Catalase and Fenton assays also elicited light responses, with Fenton's reagent response increasing with calcium concentration. These results suggest that calcium ions, catalase, and H_2O_2 may be involved in the luminescence mechanism. Histological analysis indicates that light is produced in gut-associated cells within the legs, implying that trophic acquisition of a factor may be necessary. The ecological function of *C. tasmanica* bioluminescence, whether for anti-predation, predation, or reproduction, remains speculative and warrants further investigation of this abyssal species.

1. Introduction

The Pycnogonida, or sea spiders, have a fossil record extending back to the Silurian period, approximately 425 million years ago (Waloszek and Dunlop, 2002; Arango and Wheeler, 2007; Bamber, 2007; Sabroux et al., 2023) but an even earlier specimen from the Upper Ordovician, possibly also an adult pycnogonid, dates the group to around 450 million years ago (Siveter et al., 2004). Pycnogonids are classified within Chelicerata, which includes animals such as scorpions, horseshoe crabs and spiders among others (Giribet and Edgecombe, 2019; Ballesteros et al., 2020; Frankowski et al., 2022). It is difficult to discuss any aspect of their biology without encountering an inevitable exception. The number of legs varies from the typical eight to ten or twelve in some deep-sea and Polar species. Leg spans in the range of 3–60 mm are most common in coastal waters but much larger specimens, frequently with a size range of 20–50 cm, are common in deep-sea collections. Records of still larger specimens are not unusual. One specimen in the US National Museum of Natural History, at the Smithsonian Institution, is reported to have a leg span of 30 inches (c. 76 cm, Child, 1986). Eyes (ocelli) are located on a tubercle of various shapes and sizes. There are one, two or four pairs of eyes but true to their being an exception, blind

species also exist amongst the deep-sea fauna.

Their evolutionary success can be attributed *inter alia* to their morphological diversity and unorthodox physiology (Frankowski et al., 2022). Their diversity is most evident in the development of the anteriorly placed proboscis with terminal jaws of various structures (Staples, 2020) and the presence, absence, and development of three pairs of anterior appendages attached to the cephalosoma or cephalon; the chelifores, palps and ovigers. When combined with the varying shapes of the proboscis and mouth parts, they represent important taxonomic characters at family and genus level. This diverse morphology has allowed pycnogonids to adapt to a wide range of host substrates (Arnaud and Bamber, 1988; Staples, 1997; Soler-Membrives et al., 2013; Dietz et al., 2018). The body terminates in an anal tubercle (referred to as the abdomen in traditional terminology). The ovigers, when present, are unique to pycnogonids and are carried beneath the body, attached either to the ventral surface of the cephalon or to the neck, anterior to the first pair of trunk lateral processes. They are multi-segmented prehensile appendages, and when sufficiently developed, they are used by both sexes for grooming but exclusively by the male to carry eggs following their release by the female. In many cases, eggs are deposited elsewhere and enter into a parasitic relationship (Staples and Watson, 1987;

* Corresponding author.

E-mail address: jerome.mallefet@uclouvain.be (J. Mallefet).

¹ These authors have contributed equally to the work.

Staples, 2005). In the absence of ovigers, or where they are so reduced that they no longer serve to carry eggs, eggs are adhered directly to the ventral surface of the male.

The sluggish and measured movement of specimens suggests limited dispersal capabilities, but observations of specimens treading water (swimming) to maintain suspension in the water column whilst being carried by the tides indicate that this is an effective means of distribution far beyond their initial site (Arnaud and Bamber, 1988; Staples, 1997, 2007). Their anatomical simplicity is reflected in unconventional systems for respiration and digestion. Respiration occurs through cuticular diffusion of oxygen from the surrounding water (Lane et al., 2017). Gut diverticula extend from each trunk segment into the corresponding legs and chelifores (when present), and peristaltic contractions of the gut primarily drive the circulation of gut content and haemolymph. Digestion is intracellular and mediated by pinocytosis within the midgut epithelial cells (Richards and Fry, 1978; Soler-Membrives et al., 2013; Dietz et al., 2018; Fahrenbach and Arango, 2007; Frankowski et al., 2022). Circulation of the haemolymph is driven by a tube-like heart (when present) supported or maintained by the peristaltic contractions of the midgut and diverticula (Lane et al., 2017; Woods et al., 2017; Frankowski et al., 2022). A heart is absent in at least two species of *Pycnogonum*, and circulation is driven entirely by strong peristalsis of the gut (Firstman, 1973; Frankowski et al., 2022). The organisation of the nervous system is varied and linked to the external morphology of the specimen. Broadly speaking, the brain is located ventral to the eye tubercle from which optic nerves extend into the eye tubercle and other nerves extend to innervate the proboscis, chelifores, palps and ovigers (Lehmann et al., 2012). A chain of segmental ganglia extends posteriorly to innervate the pairs of legs. For an understanding of the nervous system and its various deviations, reference should be made to the excellent analysis by Frankowski et al., 2022).

Pycnogonids have a worldwide distribution ranging from the shoreline to abyssal depths. Approximately 1400 extant species have been described, and this number continues to grow as benthic sampling continues to expand (Bamber et al., 2025).

Bioluminescence is a relatively important phenomenon for deep-sea benthic organisms, where it is estimated that 50–60% of species glow in the dark (Martini et al., 2019). Defined as the emission of cold visible light by a living organism, bioluminescence results from a natural exergonic biochemical reaction involving either a luciferin-luciferase system or a photoprotein system. In the former, a free luciferin substrate is oxidized by an enzyme called luciferase, providing the energy required for photon emission. The increase of luciferase will modify the kinetics but not the intensity of light emitted. In the latter, a stable luciferin-luciferase complex within a single protein emits light when activated by a cofactor, such as ions (Shimomura, 2012; Delroisse et al., 2021). In this case, total emission correlates to the amount of photoprotein.

While bioluminescence is well documented for several arthropod groups (e.g., crustaceans), there are very few records of luminous pycnogonids. The genus *Colossendeis* is the only one for which bioluminescence has been reported, with scattered references appearing in recent inventories of luminous marine species (Harvey, 1952; Claes et al., 2024). Given the limited data, the extent to which bioluminescence occurs among pycnogonids remains unknown. The first recorded observation was made by Wood-Mason and Alcock (1891) who described a *Colossendeis colossea* Wilson (1881) (as *C. gigas*) specimen collected in the Indian Ocean as exhibiting a “brilliant blue-green metallic luster” on its ventral body and leg surface. Alcock later reported that “the creature, as it lay on its back, shone like a star, all its legs being lit along their ventral surface with a strange blue radiance” (Alcock, 1902). These early observers were quite specific in noting that the bioluminescence was limited to the ventral surfaces of the body and legs, which could be consistent with the luminescent material being transferred from another source. For instance, many deep-sea cnidarians exhibit bioluminescence (DeLeo et al., 2024; Duchatelet et al. 2025)

and, based on observations by one of us (DAS via ROV cameras) of a pycnogonid belonging to the genus *Bathypallenis*, splayed on a black coral, the transfer of luminescent material in the form of slime to the ventral surfaces might be probable.

The apparent lack of sophisticated integumentary structures such as photophores found in squid and fish suggests that pycnogonid luminescence is not localized in specific organs but rather related to a diffuse reaction within internal tissues. A personal communication, cited by King (1973), from Jarvis & Isaac described “phosphorescence” in the terminal segments of the first pair of legs of *Nymphon gracile* Leach (1814), during laboratory experiments. However, this report lacks validation and remains anecdotal. Whilst the purpose of pycnogonid light emissions is not known, King speculated that it might serve functions such as mate attraction, predator deterrence, or transmitting other messages to individuals of its own or other species. Herring (1983) studied the spectral characteristics of luminous marine organisms in the laboratory and found the legs of *Colossendeis* sp. (mentioned as “*C. gigantea*?”) emitted a blue-green light in response to H₂O₂ stimulation (Herring, 1983). It appears that *C. gigantea* is not a validated name and is likely a synonym of *C. proboscidea* (see <https://www.biodiversitylibrary.org/page/2112094#page/586/mode/1up>). Herring's record of a spectrum maximum emission of 485 nm with a half bandwidth of 84 nm at 18–25°C, corresponds to a light to which the visual pigments of most marine animals are very sensitive (Munz, 1958; Frank and Case, 1988; Warrant and Locket, 2007; Tsutsui et al., 2015).

The scarcity of data on deep-sea spider bioluminescence stems from difficulties in obtaining living specimens in optimal conditions. The RV Investigator cruise expedition, ‘Sampling the Abyss’, supported by the CSIRO Marine National Facility and the NESP Marine Biodiversity Hub and led by T. O'H. (Museum Victoria), provided a unique chance to study deep-sea spiders from Australia's eastern coast. We documented bioluminescence through on board stimulation of two species of giant sea spider belonging to the family *Colossendeidae* Hoek 1881; *Colossendeis tasmanica* Staples (2007) and *C. minor* Schimkewitsch (1893) capturing the first photographs of luminous sea spiders. With an abundance of *C. tasmanica*, we conducted detailed studies on the pattern, biochemistry, and physiology of light, offering the first insights into the light-emission process of deep-sea spider species.

2. Materials and methods

2.1. Specimens sampling

Specimens studied here resulted from a one-month deep-water survey along Australia's east coast, in May–June 2017 at depths between 1006 and 4977 m using beam trawl and Brenke epibenthic sledge (see Supplementary Figure 1 and Table 1Table 1; O'Hara, 2019). Seven specimens of *C. tasmanica* and two specimens of *C. minor* were provided from the larger collection of pycnogonids for bioluminescence study. The pycnogonids were transferred into ice-cold sea water (prepared using a dedicated seawater ice system to avoid osmotic stress) in the sorting laboratory, then maintained in a 30L internally-blackened box and placed in a cold darkroom before being observed with dim red light. Taxonomic identification of *C. tasmanica* is based on the description by Staples (2007) independently supported by subsequent unpublished molecular data. Bamber (2010) provides a guide to the identification of *C. minor*. Specimens were tested for luminescence by application of 400 mM KCl and H₂O₂ 0.2% stock solutions on fresh specimens (Gouvenaux and Mallefet, 2013; Mallefet, 2009; Mallefet et al., 2020; Duchatelet et al., 2023) and photographed (i.e., the animals and their bioluminescence) with a Sony Alpha 7SII camera. The light emission spectrum was recorded on three *C. tasmanica* specimens using a Hamamatsu microspectrophotometer (C10083) equipped with an optical fibre (A976201). Pieces of legs (2 to 3.5 cm) were dissected and either (i) immediately tested for luminescence, (ii) stored at –80°C, or (iii) fixed in 4% paraformaldehyde phosphate buffer saline (PBS:

123 mM NaCl, 2.6 mM KCl, 12.6 mM Na₂HPO₄, 1.7 mM KH₂PO₄, pH 7.4) for 24 h and then, rinsed and stored in PBS until use.

2.2. Onboard assay

Fresh leg pieces of four specimens of *C. tasmanica* were placed in a luminometer tube with 2 mL of artificial seawater (ASW; 400 mM NaCl; 9.6 mM KCl; 52.3 mM MgCl₂; 9.9 mM CaCl₂; 27.7 mM Na₂SO₄; 20 mM Tris; pH 8.2). After 10 s, 2 mL of solution of either (i) KCl stock concentration of 400 mM, (ii) H₂O₂ stock concentration of 0.2%, (iii) ASW as control for mechanical stimulation, (iv) adrenaline stock concentrations of 1 mM and 0.5 mM (Adrenaline tartrate, Sterop, Bruxelles, Belgium), was added, and luminous response recorded. Bioluminescence emission was recorded during 3 min thanks to an FB12 Berthold luminometer (Titertek-Berthold, Pforzheim, Germany) coupled with FB12-Sirius software (Titertek-Berthold, Pforzheim, Germany) in a dark room. The recorded light emission parameters were the maximum light intensity (L_{max}) in megaquanta per second (Mq s⁻¹) and the total amount of light emitted during the experiment (L_{tot}), expressed in megaquanta (Mq mg⁻¹). All measurements were standardized per milligram of leg tissue. In addition, one leg was cut into five pieces (every 3.5 cm) to test the distal-proximal luminescent capabilities through the application of 200 mM of KCl. Light emission was recorded as described above. The distribution of replicates performed on board the RV Investigator is shown in [Supplementary Table 2](#).

Finally, a leg piece of the one of the two collected specimen of *C. minor* was stimulated with a KCl 400 mM stock solution, and light emission was recorded as described above.

2.3. Biochemical assay

Frozen leg pieces of two *C. tasmanica* specimen, maintained at -80°C onboard of the RV Investigator then brought back on dry ice to Belgium, were lyophilized under freezing conditions ([Tran et al., 2007](#); [Puzyr et al., 2017](#)), weighed and crushed with a mortar and pestle, and mixed with 500 µL PBS (0.15 M NaCl, pH 7.4) on ice in a dark room. These extracts were subjected to three different biochemical assays: (i) catalase activity assay, (ii) Fenton reaction, and (iii) hot and cold extract test. For all these assays, luminescence was recorded for 5 min following the same protocol as described above. The distribution of replicates performed on the frozen legs of the two *C. tasmanica* is summarized in [Supplementary Table 3](#).

2.3.1. Catalase assay

To infer the involvement of catalase activity in the light emission reaction ([Chou et al., 2014](#)), 850 µL of Tris buffer (0.1 M Tris-HCl; pH 8.5) and 50 µL of catalase (Catalase from Bovine Liver, Merck, Darmstadt, Germany; 2 mg/mL) were added to either 50, 100 or 200 µL leg extracts. After the initial 10 s luminometer recording, 50 µL of H₂O₂ 5% was added to trigger the reaction, and L_{tot} was recorded on the luminometer for 3 min. A negative control was performed by omitting the leg extract in the assay (0 µL).

2.3.2. Fenton reaction assay

Fenton reaction assay was performed to evaluate the reactive oxygen species involvement in the light reaction ([Nicolas et al., 1982](#)). Briefly, 800 µL of PBS pH 6.6 with either EGTA 10 mM (Sigma Chemical Co., St. Louis, MO, USA), or a graded series of CaCl₂ (0, 0.01, 0.02, 0.1, 0.5, 1 M; Sigma), mixed with 10 µL of H₂O₂ 1% were added to 50 µL of the leg extract. Again, after 10 s luminometer recording, 100 µL of FeSO₄ 0.1 mM (Sigma) was added to trigger a putative catalytic reaction in dark conditions, and L_{tot} was recorded on the luminometer.

2.3.3. Hot and cold assay

To test if the bioluminescence of *C. tasmanica* is the result of a luciferin/luciferase reaction, the classical hot and cold method was

performed ([Dubois, 1886](#); [Oliveira et al., 2012](#)). Briefly, 75 µL of the extract was placed in boiling water (i.e., hot extract), and 50 µL of the extract was kept on ice (i.e., cold extract). The warm extract putatively contains only luciferin since the luciferase (a protein) is inactivated by heat, whereas the cold extract should contain only active luciferase that has exhausted any luciferin present. By mixing hot and cold extracts, the luminous reaction should be detected ([Viviani et al., 2007](#)). In our protocol, 800 µL of PBS was added to the 50 µL cold extract in a tube and placed in the luminometer. The light recording was started, and after 10 s, 50 µL of the hot extract was injected into the tube. The total amount of light emitted during the 3 min experiment (L_{tot}) was recorded.

2.4. Morphological localization of peroxidase

Fixed leg pieces were bathed in a decalcifying solution (OsteoRAL R, Fast Decalcifier for Large Anatomical Specimens, Ral Diagnostics, France) overnight to soften the tissues and facilitate slicing. Leg pieces were successively placed in PBS with an increasing sucrose concentration (1h at 10%; 1h at 20%; and overnight at 30%), then embedded in optimal cutting medium compound (OCT) and quickly frozen. 10 µm transversal and longitudinal sections were performed using a cryostat microtome (CM3050S, Leica, Germany) and spread out on pre-cleaned ground edges coated slides (Superfrost ultra plus, Thermo Scientific). Slides were left to rest overnight to dry, then subjected to a peroxidase revelation assay. Briefly, slides were rinsed and rehydrated in distilled water (5 min), then covered with DAB substrate (Sigma), which highlighted peroxidase activity for 10 min. The reaction was stopped by washing the slides with tap water. Finally, slides were dehydrated and mounted (Mowiol® 4-88, Sigma) for observation. Sections were examined with a Leitz Diaplan microscope (Leica, Germany) equipped with a Nikon DS-U1 digital camera coupled with NIS-elements AR software.

3. Results

3.1. Onboard assay

3.1.1. Luminescence pattern

Fresh specimens of *C. tasmanica* in good condition ([Fig. 1A](#)) and with all legs intact, displayed little activity when observed in the 30L box filled with cold water. When stimulated by gently pinching the legs, a weak localized glow lasting from a few seconds to more than 2 min was induced but with no sign of spreading along the legs. Immersion in a potassium chloride (KCl 200 mM) bath triggered a long-lasting blue light from the legs. The intensity of the light increased with the addition of 0.1% peroxide. All of the legs lit up similarly and mostly at the same time, some slight delay could be observed (JM personal observation). Bioluminescence lasted for 5 to 10 min and became increasingly evident as the eyes adapted to the dark. Light was first observed distally, moving and decreasing in intensity from the propodus to the tibiae, femur, then to finally disappearing at the level of the coxae ([Fig. 1B](#)). No luminescence was observed elsewhere. A light emission gradient was measured along the leg upon KCl application ([Fig. 1C](#)). A highest L_{tot} value of 106.76 10³ Mq mg⁻¹ was recorded for the distal part (i.e., the propodus), while the remaining parts presented successively decreasing values ([Fig. 1C](#)). Normalized *C. tasmanica* bioluminescence spectra peaked at 449 nm with a half-bandwidth of 23 nm ([Fig. 1D](#)).

Two specimens of *C. minor* were collected and used to test their potential bioluminescence. The first specimen was used to document the organism under dim light ([Fig. 2A](#)) and its bioluminescence after immersion in a KCl solution ([Fig. 2B](#)). The second specimen was used for luminometric measurements, which revealed light production, as illustrated by the original recording of a glow lasting more than 3 min following KCl application ([Fig. 2C](#)).

3.1.2. Physiological assay

The number of specimens allowed us to gain some insight into the

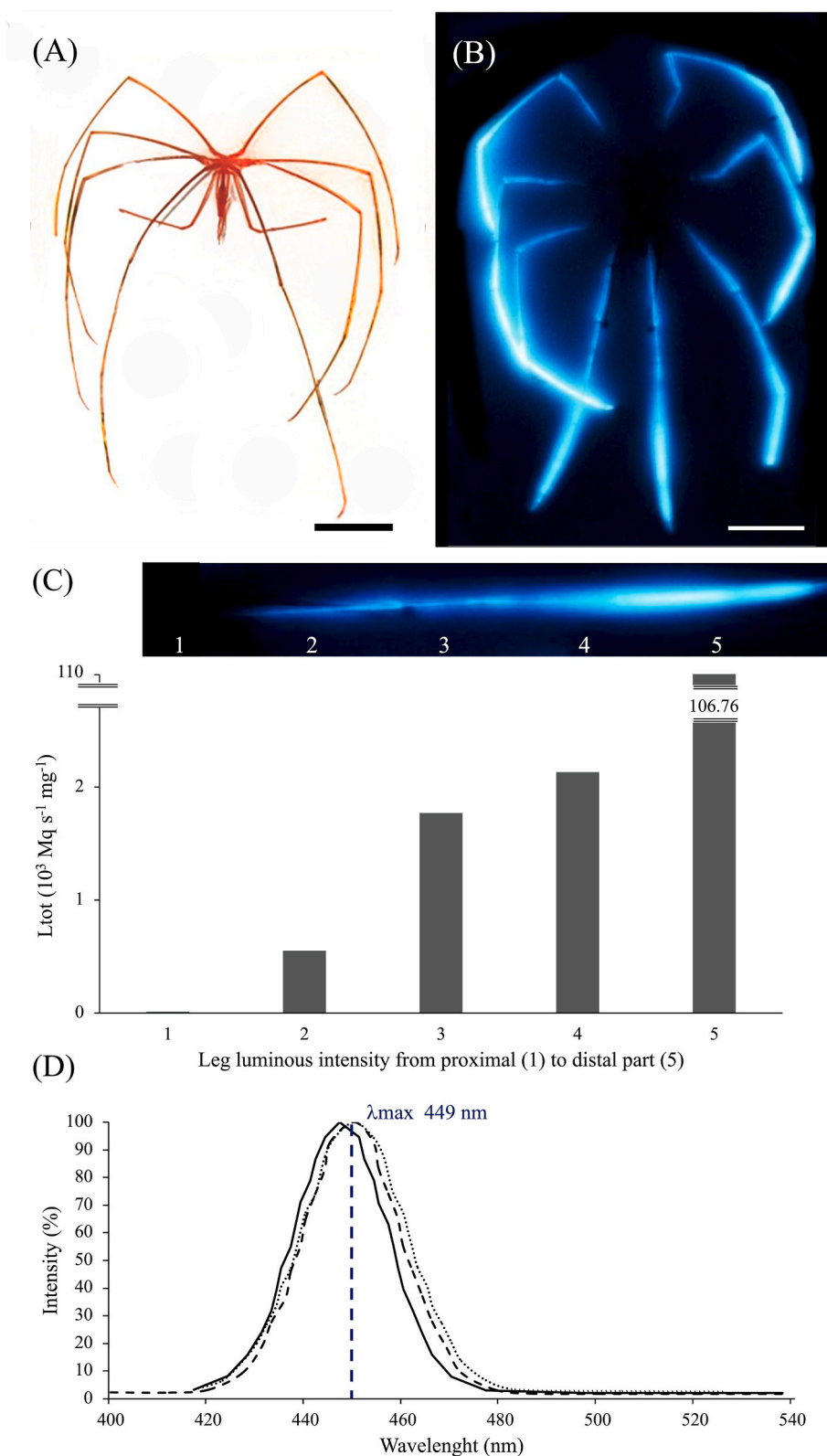


Fig. 1. Bioluminescence of *Colossendeis tasmanica*. (A) In vivo picture of an individual under dim light (Sony a7SII, 1000 ISO, f 1.4, 1/8s). (B) The deep-blue bioluminescence of the legs in the dark (Sony a7SII, 40000 ISO, f 1.4, 2.5s). (C) Glowing leg showing light gradient from proximal to distal with corresponding values in the graph below, illustrating the total light produced (Mq mg^{-1}) under KCl application. Broken scaling due to extreme light intensity is represented for the distal part (5). (D) Normalized spectrum of three specimens (full, dotted, and dashed lines) with a peak of intensity at 449 nm. Scale bars = 5 cm.

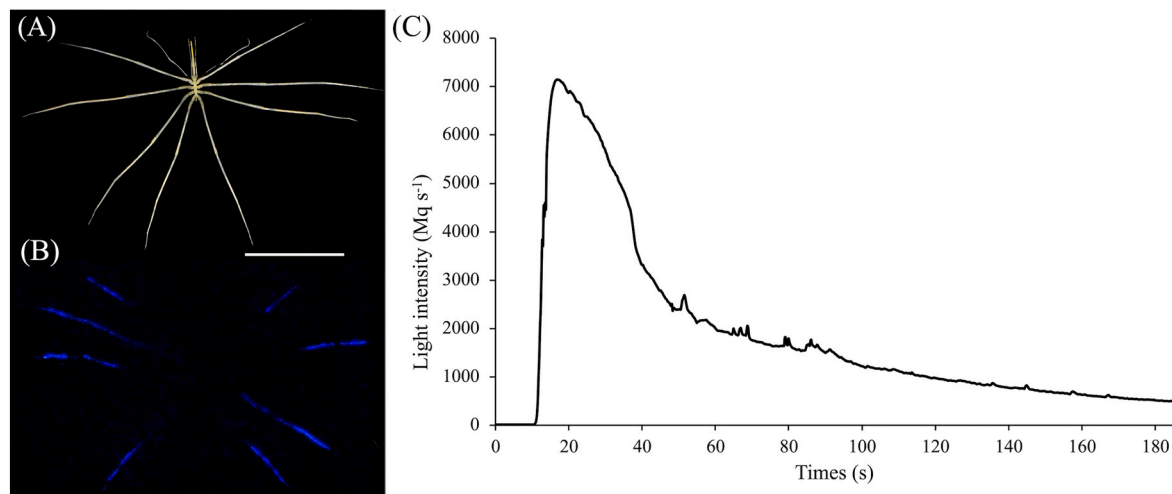


Fig. 2. Bioluminescence of *Colossendeis minor*. (A) In vivo pictures of the general morphology under dim light (Sony a7SII, 1250 ISO, f 2.0, 1/25s) (B) luminescence (Sony a7SII, 102400 ISO, f 1.4, 6s). (C) Typical curve obtained under KCl 200mM application. Scale bar = 5 cm.

bioluminescence response to various stimuli of *C. tasmanica*. In pharmacological experiments, depolarization of an isolated leg segment with the application of 200 mM KCl resulted in the production of a long glow, steadily increasing during 3 min of recording (broken line Fig. 3A); yielding a mean L_{max} value of $36.18 \pm 19.28 \text{ Mq s}^{-1} \text{ mg}^{-1}$ ($n = 7$). On the other hand, the application of peroxide induces a rapid increase of luminescence after injection, reaching an average peak of $365.37 \pm 130.58 \text{ Mq s}^{-1} \text{ mg}^{-1}$ ($n = 4$) within the first minute, followed by a rapid decrease (Fig. 3A). The mean total amount of light recorded for 3 min on fresh tissue under various stimuli is summarized in Fig. 3B. Regardless of the location of the piece of leg, the mean L_{tot} value recorded for the KCl application ($n = 7$; $5290 \pm 3349 \text{ Mq mg}^{-1}$) was lower than the ones obtained for the H_2O_2 applications ($n = 4$; $14299 \pm 4752 \text{ Mq mg}^{-1}$), both being 10 to 100-fold higher than the ASW control ($n = 2$; $438 \pm 324 \text{ Mq mg}^{-1}$). Adrenaline injection at 0.25 and 0.5 mM on *C. tasmanica* induced a very weak light production ($L_{tot} = 2.63$ and 4.03 Mq mg^{-1} , respectively).

3.2. Biochemical assay

Different biochemical assays were performed in the laboratory on defrosted lyophilized tissue extracts. Fig. 4A illustrates one original recording of light emission obtained during the catalase assay using 100 μL of leg tissue crude extract. The application of H_2O_2 5% to 50, 100 and 200 μL leg extracts induced a rapid peak of light emission, followed

by a drastic decrease. Omission of crude extract in the luminometer tube, i.e. 0 μL , represented the mean control value for the peak of light L_{max} of $0.10 \pm 0.02 \text{ Mq}$; with 50 μL extract, a mean L_{max} value of $4.22 \pm 1.46 \text{ Mq mg}^{-1}$ was recorded, and for 100 μL , the L_{max} value averaged $4.98 \pm 2.55 \text{ Mq mg}^{-1}$ ($n = 3$ for each test). One 200 μL extract test provided a value of 6.63 Mq mg^{-1} . Mean total light values are represented in Fig. 4B; the following values were measured: $0.10 \pm 0.02 \text{ Mq mg}^{-1}$; $1.84 \pm 0.09 \text{ Mq mg}^{-1}$; $4.11 \pm 0.26 \text{ Mq mg}^{-1}$; and 5.78 Mq mg^{-1} for 0, 50, 100 and 200 μL , respectively (Supplementary Table 4). The total emission was dependent and proportional to the extract volume.

Typical Fenton assay curve presented a rapid increase of the light emission for a few seconds before dropping down to the basal background value (Fig. 4C). The Fenton reaction assay performed on *C. tasmanica* without calcium resulted in a weak light emission response (Fig. 4D–Supplementary Table 4). Raising the calcium concentration in the medium (from 10 mM to 1 M) resulted in a gradual increase in the responses (Fig. 4D).

The hot and cold assay was performed on *C. tasmanica*. Fig. 4E illustrates the mean curve of light recorded for hot and cold mixing extracts of 5 replicates from a leg segment. The mean maximal intensity, L_{max} , was calculated to peak at $0.00011 \pm 0.00003 \text{ Mq s}^{-1} \text{ mg}^{-1}$ for a mean L_{tot} of $0.0065 \pm 0.003 \text{ Mq mg}^{-1}$. These values are extremely low compared to all other assays performed. Comparison can be found in the Supplementary Table 4 ratio between hot/cold, either L_{max} or L_{tot}

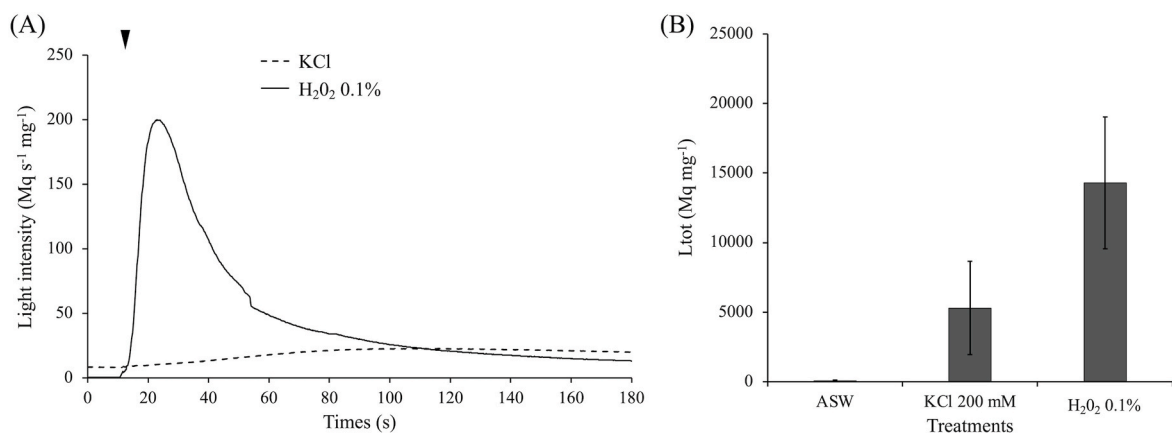


Fig. 3. Bioluminescence induction on *Colossendeis tasmanica*. (A) Original recording obtained under H_2O_2 0.1% and KCl 200 mM applications over three minutes. Arrowhead indicates the time of application. (B) Total light emission recorded for ASW, KCl, and H_2O_2 0.1% applications on fresh tissues.

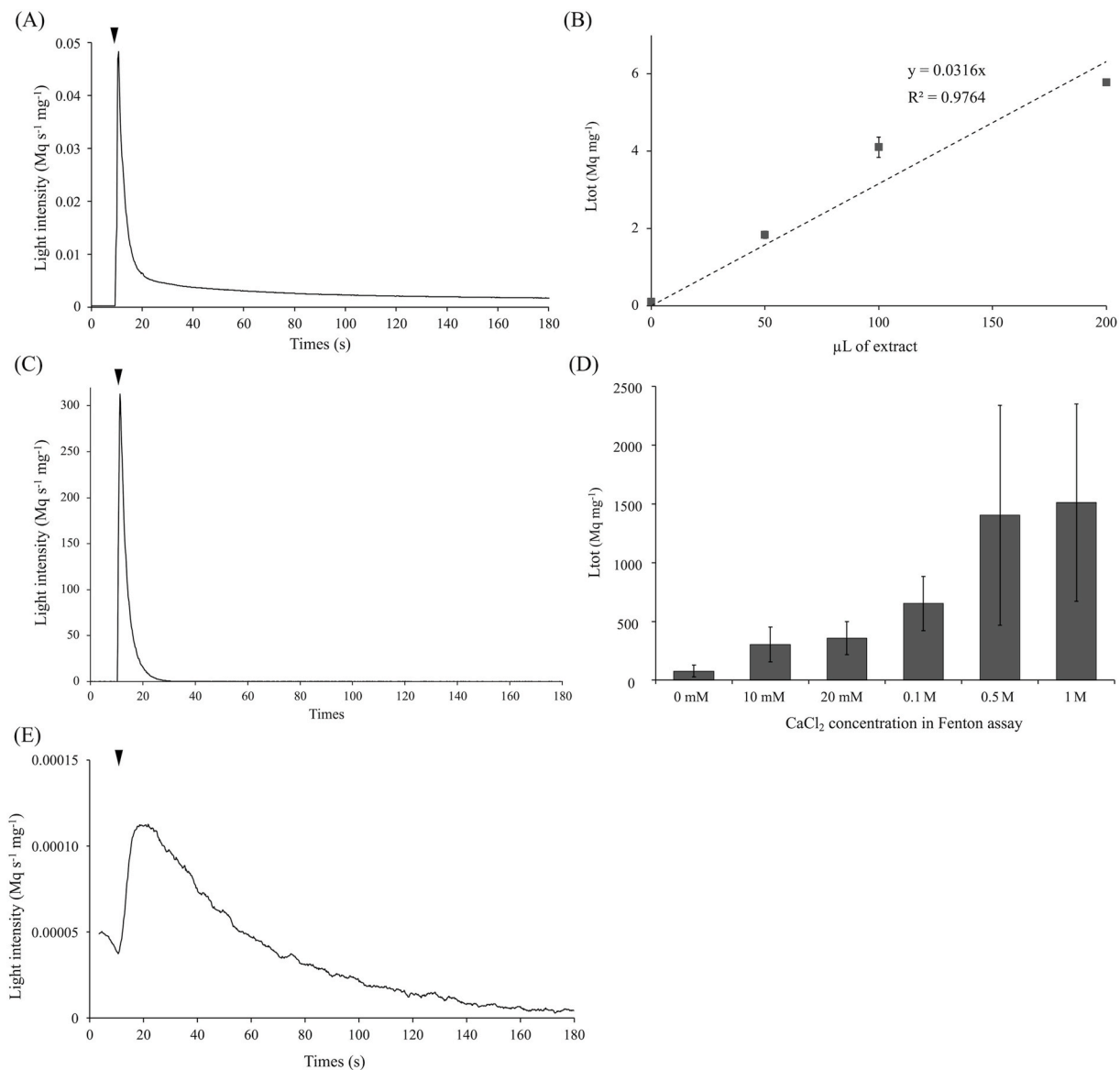


Fig. 4. Biochemical assays performed on *Colossendeis tasmanica* (A) Original recording obtained during catalase assays with 100 µL extract from frozen lyophilized tissues over three minutes. (B) Total light emission for catalase assay obtained with different sample extract quantities (0, 50, 100 and 200 µL). (C) Original recording obtained during the Fenton assay with 1 M of CaCl₂. (D) Effect of calcium concentration on total light emission induced by Fenton assays: from 0 mM Ca²⁺ (medium devoid of calcium without and with the addition of EGTA) to 1 M Ca²⁺. (E) Mean curve obtained during the hot and cold assays over three minutes. Arrowheads indicate the time of application (A, C, E). Error bars represent S.E.M. (B, D).

values to the KCL, peroxide assay, reveals that, at most, the hot/cold is 10⁵ to 10⁷-fold lower than the responses on fresh tissues. This hot and cold value remains 10³ to 10⁵-fold lower than peroxide (catalase assay; 50 µL, $n = 3$, $L_{max} = 4.22 \pm 1.46 \text{ Mq s}^{-1} \text{ mg}^{-1}$) and Fenton responses (at 10 mM calcium), respectively, all done on lyophilized samples. The hot/cold values were even lower than the control value (see 0 µL extract with catalase).

Data used during the analysis are compiled as [Supplementary Table 6](#).

3.3. Morphological localization of peroxidase within the *C. tasmanica* legs

Cross sections of *C. tasmanica* legs show a thin layer at the edges corresponding to the cuticle, enclosing the first multiple layers of basal cells from which bud cells extend toward the lumen of the gastrointestinal projection within the legs (Fig. 5A). Peroxidase stainings on unstimulated individual parts were specifically observed within vesicles,

including the cells present in the inner part of the gastrointestinal projections of the legs (Fig. 5B–E). Conversely, peroxidase stainings were barely present in the labelling assay performed on legs previously treated with KCl (Fig. 5F and G).

4. Discussion

New deep-sea invertebrates are described each year from abyssal research expeditions. Among these, an increasing number of species have been shown to exhibit bioluminescence (e.g. [Bessho-Uehara et al., 2020a, 2024](#); [Mallefet et al., 2023](#); [Kise et al., 2025](#)). In this study, we document the ability of two abyssal pycnogonids, *C. tasmanica* and *C. minor*, to produce light, raising the total number of known bioluminescent pycnogonid species to four. The two others, not examined here, are *Colossendeis gigantea* (possibly = *C. proboscidea*) and *Nymphon gracile* ([Wood-Mason and Alcock, 1891](#); [Arnaud and Bamber, 1988](#)). Two additional pycnogonid specimens tested in this study, *Colossendeis* sp.4

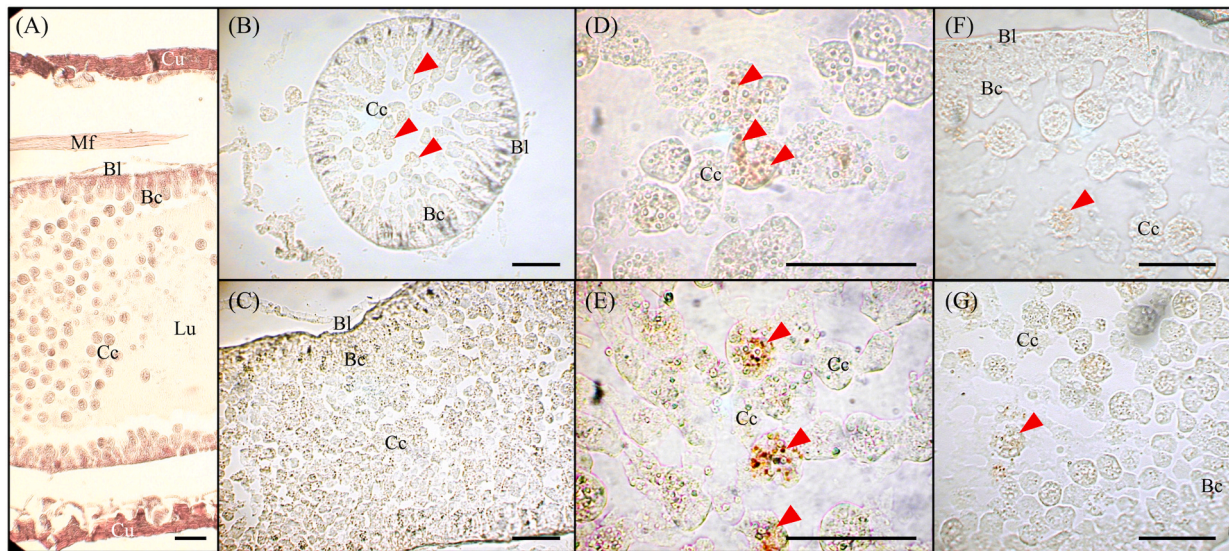


Fig. 5. Peroxidase staining on *C. tasmanica* leg sections. (A) longitudinal section of a leg showing the different histological structures. Peroxidase immunostaining (red arrowhead) on transversal sections (B, D, F) and longitudinal section (C, E, G), before (B-E) and after (F-G) KCl application. Bc, budding cells; Bl, basal lamina; Cc, central cells; Cu, cuticle; Lu, lumen of the gastrointestinal tract; Mf, muscular fibers. Scale bars = 100 μ m.

and *Nymphon* sp.1 (see [Supplementary Table 1](#)) failed to incite luminescence, and further investigation is needed. *In vivo* images and/or luminometric recordings of leg bioluminescence are provided for *C. tasmanica* and *C. minor*.

Unlike earlier report by [Wood-Mason and Alcock \(1891\)](#) who described light emission from the ventral body surfaces and legs of *C. gigas* [Hoek \(1881\)](#) (= *C. colossea* [Wilson, 1881](#)), the species examined here exhibited light emission exclusively from the legs, and this was not restricted to the ventral surface. Moreover, a gradient of light intensity was observed along the legs of *C. tasmanica*, from the coxae to the propodus. The recorded emission peak for *C. tasmanica* lies in the deep blue spectrum range (i.e., 449 nm), which differs from the 485 nm blue spectrum reported by [Herring \(1983\)](#) for the legs of an unconfirmed species of *Colossendeis*. One cannot exclude that this difference might be species-specific but also related to the temperature of recording, since Herring indicated he did his measurement in the laboratory at 18° - 25°C, while the present data were taken at 6°C. Violet, blue and blue-green bioluminescence is widespread and predominant among pelagic and deep-sea organisms, accounting for roughly 79% of recorded emission spectra ([Herring, 1983](#); [Widder et al., 1983](#); [Latz et al., 1988](#); [Widder, 2010](#); [Duchatelet and Dupont, 2025](#)).

The classical hot and cold assay ([Dubois, 1886](#)) has been successfully used multiple times as a preliminary step to reveal the presence of luciferin-luciferase systems in luminous organisms ([Harvey, 1922](#); [Viviani et al., 2007](#); [Oliveira et al., 2012](#)). However, the failure of the hot and cold assay to detect light, which was similar to the luminometer's background noise, suggests that *C. tasmanica* does not rely on a luciferin-luciferase system. Although these results argue against the presence of a classical luciferin-luciferase system under the tested conditions, the potential effects of sample preservation (including lyophilization) represent a methodological limitation that cannot be fully excluded. However, it should be noted that Fenton assays were performed with the same batch of lyophilized tissue and produced significant luminous levels, indicating the presence of active luminous compounds.

In our study, light emission from *C. tasmanica* was elicited using H_2O_2 , catalase, and Fenton's reagent, a dependence on calcium concentration was shown, all of which are consistent with known photoprotein systems. These results suggest that reactive oxygen species are involved in the light-emission system. Catalase-induced luminescence showed a dependency on the amount of tissue extract present in the

tube. This proportionality is in favour of the presence of a photoprotein system. First discovered in the jellyfish *Aequorea vitrina*, the photoprotein of *Aequorea*, named aequorin, was shown to produce light by the addition of calcium into the water, oxygen not being necessary, and the light emission being proportional to the amount of extracted photoprotein ([Shimomura, 2006](#)). Later, the failure of the hot/cold extraction in luminous scale worms led to the discovery of a membrane-bound photoprotein in the polynoid *Malmgrenia lunulata*, the protein was named polynoidin ([Lecuyer and Arrio, 1975](#); [Nicolas et al., 1982](#); [Plyuscheva and Martin, 2009](#)). The worm luminescence was elicited by the addition of Fenton's reagent (i.e., H_2O_2 and ferrous ions) in scale extract ([Lecuyer and Arrio, 1975](#); [Nicolas et al., 1982](#); [Plyuscheva and Martin, 2009](#)). Other photoprotein-based systems have been described in the ophiuroid *Ophiopsila californica* (ophiopsilin), the bivalve *Pholas dactylus* (pholasin), and the cephalopod *Eucleoteuthis luminosa* (symplectin), all triggered by peroxide or other oxidants ([Henry et al., 1975](#); [Michelson, 1978](#); [Shimomura, 1986, 2012](#); [Mallefet, 2009](#); [Kuse, 2020](#)).

Previous studies on symplectin and pholasin have also pointed to the role of catalase- H_2O_2 and peroxidase- H_2O_2 systems, respectively, in photoprotein-mediated light emission ([Shimomura, 1986](#); [Kuse, 2020](#); [Inouye et al., 2020](#)). In *C. tasmanica*, Fenton-induced light production appeared calcium dependent, with a 23-fold light increase between the EGTA chelator (0 $CaCl_2$) to 1 M $CaCl_2$ concentrations. It is known that high chloride levels (2 M Cl^-), originating from $CaCl_2$ dissociation, are known to exert a strong inhibitory effect on Fenton-base oxidative systems via hydroxyl radical scavenging and iron complexation ([Lipczynska-Kochany et al., 1995](#); [Maciel et al., 2004](#)) so higher $CaCl_2$ should induce a decrease of the Fenton response, but the contrary effect was observed. This result clearly supports the implication of Ca^{2+} in light generation. Calcium-activated photoproteins are well described in numerous cnidarians and ctenophores ([Ward and Seliger, 1974](#); [Shimomura and Shimomura, 1984](#); [Inouye and Tsuji, 1993](#); [Illarionov et al., 2000](#); [Markova et al., 2002](#); [Shimomura, 2006](#); [Burakova and Vysotski, 2019](#)).

However, our findings do not yet identify the chromophore (emitter inside the photoprotein) involved in the bioluminescent system of *C. tasmanica*. Most photoproteins use coelenterazine or a derivative substrate ([Shimomura, 2006](#)). Aequorin and mnemiopsin, for example, use coelenterazine ([Shimomura and Johnson, 1975](#); [Anttil and Shimomura, 1984](#)), while symplectin employs dehydrocoelenterazine ([Takahashi and Isobe, 1993](#)). The biochemical evidence presented here

suggests the involvement of a photoprotein in *C. tasmanica*, which is sensitive to reactive oxygen species and calcium. Further research is needed to validate and characterise at the molecular level this putative photoprotein.

Peroxidase staining revealed labeled cells in the inner part of the legs' gastrointestinal projections, consistent with the observed light emission pattern. Although peroxidase activities play various intracellular roles (Johansson, 1999; O'Brien, 2000; Schaffer and Bronnikova, 2012; Xu et al., 2013), the fading of peroxidase staining after KCl-stimulated luminescence remains puzzling. This result may be consistent with the exhaustion of a single-turnover photoprotein system. This plausible hypothesis remains speculative and requires further experimental validation to answer this open question.

Complementary biochemical studies will be necessary to understand the various steps of photogenesis in *C. tasmanica*. Nevertheless, considering the luminous reaction sensitivity to reactive oxygen species, one can suggest these peroxidase-positive cells likely represent a key component of *C. tasmanica* luminescence biochemical reaction. Their location within the gastrointestinal tract could suggest dietary acquisition of a luciferin substrate, as seen in the cnidarian *Aequorea victoria* and the brittle star *Amphiura filiformis* (Haddock et al., 2001; Coubris et al., 2024). Although the diet of *C. tasmanica* is unknown, other *Colossendeis* species (e.g., *C. megalonyx*, *C. colossea*, *C. japonica*, *C. scotti*) feed on various benthic and pelagic invertebrates such as cnidarians, molluscs, and ctenophores (Brady et al., 2009; Moran et al., 2018). Several of these prey taxa are known to be bioluminescent, such as ctenophores belonging to the genus *Beroë*, which also use a photoprotein system (Shimomura, 2006; Markova et al., 2012). Dietary acquisition of a luciferase enzyme is also known in the fish *Parapriacanthus ransonneti*, which consumes bioluminescent ostracod prey (Bessho-Uehara et al., 2020b).

The potassium chloride depolarization treatment triggers luminescence production, which suggests the presence of excitable cells that can be depolarised. This result raises the possibility that light production is under nervous control (Vanderlinden et al., 2003; Duchatelet et al., 2023; Mallefet et al., 2023). However, tests performed with adrenaline failed to elicit any significant glows or flashes. The recorded levels only represent 1% of the control value induced by ASW injection (mechanical stimulation in fresh tissue) and should be considered as the result of a weak mechanical stimulus to the leg caused by adrenaline injection. Given its arthropod affiliation, future studies on pycnogonids should test other neuroeffectors known to regulate bioluminescence in euphausiids and decapods, such as acetylcholine or serotonin (Doyle and Kay, 1967; Herring, 1976; Herring and Locket, 1978; Latz and Case, 1992; Herring et al., 1993; Fregin and Wiese, 2002; Krönström et al., 2007). The limited replication, particularly in pharmacological assays, constrains the strength of statistical inference and generalization. New opportunities to participate in an abyssal cruise or an abyssal remotely operated vehicle (ROV) collection should be focused on these captivating sea spiders in order to sample more specimens, hence being able to address the numerous open questions raised by this first study.

Finally, the light gradient observed along the legs of *C. tasmanica* raises questions regarding ecological function. The individuals examined in this study were recovered via deep-sea trawling, and as such, their behavioral responses may have been influenced by decompression, increase of surface water temperature, light in the sorting lab and handling, to cite some stress parameters. This limits the ability to confidently interpret the observed light emission patterns in an ecological context. The light emission pattern could suggest at least two uses: (i) it signals their presence to a potential mate in otherwise total darkness; (ii) as a stunning effect for predation, lighting up its legs all around a benthic prey item to stun it. As highlighted in recent works, bioluminescence represents a key ecological trait in deep benthic ecosystems, mediating interspecific interactions (Martini and Haddock, 2017; Martini et al., 2019). Whether for defence, attack, or intraspecific communication such as mating (Herring, 2007; Haddock et al.,

2010), the precise ecological roles of pycnogonid bioluminescence remain elusive and warrant further study. Again, using *in situ* observations, such as deep-sea video recordings obtained with ROVs equipped with highly sensitive cameras and minimal-light or red-light illumination, would be essential to document more accurately the natural behavior and better assess the ecological function of bioluminescence in this species.

Funding

JM was supported by an F.R.S.-FNRS travel grant. LD is supported by a postdoctoral researcher fellowship of the F.R.S.-FNRS awarded to the Université de Louvain – UCLouvain Marine Biology Laboratory and the Université de Mons Biology of Marine Organisms and Biomimetics Laboratory (T.0192.24) and by a Wallonia-Brussels International Excellence grant.

CRediT authorship contribution statement

Jérôme Mallefet: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **David A. Staples:** Writing – original draft, Writing – review & editing. **Timothy D. O'Hara:** Resources, Writing – review & editing. **Laurent Duchatelet:** Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We want to express our sincere thanks to the crew of RV Investigator on the; Sampling the Abyss voyage. This research was supported by a grant of sea time on RV *Investigator* from the CSIRO Marine National Facility (<https://ror.org/01mae9353>). Dr. T. O'Hara (T.O., Senior Curator at Museums Victoria) was the voyage chief scientist. J.M. is Research Associate to FRS-FNRS, D.S. is Honorary Associate of Museums Victoria, T.O. is Senior Curator at Museums Victoria, and L.D. is a post-doctoral researcher at Université catholique de Louvain - UCLouvain. This study is the contribution BRC #425 of the Biodiversity Research Center (BRC - UCLouvain) from the Earth and Life Institute Biodiversity (ELIV) and the "Centre Interuniversitaire de Biologie Marine" (CIBIM). We would like to thank the reviewers for their thorough and careful analysis of the manuscript. Their thoughtful feedback and suggestions for improvement were deeply appreciated.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.dsr.2026.104735>.

Data availability

The datasets generated during and/or analysed during the current study are available as supplementary materials.

References

- Alcock, A., 1902. *A Naturalist in Indian Seas: or, Four Years with the Royal Indian Marine Survey Ship "Investigator"* 238. London.

- Antil, M., Shimomura, O., 1984. Mechanism of photoinactivation and re-activation in the bioluminescence system of the ctenophore *Mnemiopsis*. *Biochem. J.* 221 (1), 269–272. <https://doi.org/10.1042/bj2210269>.
- Arango, C.P., Wheeler, W.C., 2007. Phylogeny of the sea spiders (Arthropoda: pycnogonida) based on direct optimization of six loci and morphology. *Cladistics* 23, 255–293. <https://doi.org/10.1111/j.1096-0031.2007.00143.x>.
- Arnaud, F., Bamber, R.N., 1988. The biology of Pycnogonida. *Adv. Mar. Biol.* 24, 1–96. [https://doi.org/10.1016/s0065-2881\(08\)60073-5](https://doi.org/10.1016/s0065-2881(08)60073-5).
- Ballesteros, J.A., Setton, E.V.W., Santibáñez-López, C.E., Arango, C.P., Brenneis, G., Brix, S., Corbett, K.F., Cano-Sánchez, E., Dandouch, M., Dilly, G.F., Eléaume, M.P., Gainett, G., Gallut, C., McAtee, S., McIntyre, L., Moran, A.L., Moran, R., López-González, P.J., Scholtz, G., Williamson, C., Woods, H.A., Zehms, J.T., Wheeler, W.C., Sharma, P.P., 2020. Phylogenomic resolution of sea spider diversification through integration of multiple data classes. *Mol. Biol. Evol.* 38 (2), 686–701. <https://doi.org/10.1093/molbev/msaa228>.
- Bamber, R.N., 2007. A holistic re-interpretation of the phylogeny of the Pycnogonida Latreille, 1810 (Arthropoda). *Zootaxa* 1668, 295–312. <https://doi.org/10.11646/zootaxa.1668.1.15>.
- Bamber, R.N., 2010. Sea Spiders (Pycnogonida) of the north-east Atlantic: keys and notes for the identification of the species. *Synopsis of the British Fauna, New Series* (5), 250.
- Bamber, R.N., El Nagar, A., Arango, C.P., 2025. Pycnobase: World Pycnogonida Database. <https://www.marinespecies.org/pycnobase>. (Accessed 25 September 2024).
- Bessho-Uehara, M., Francis, W.R., Haddock, S.H.D., 2020a. Biochemical characterization of diverse deep-sea anthozoan bioluminescence systems. *Mar. Biol.* 167, 114. <https://doi.org/10.1007/s00227-020-03706-w>.
- Bessho-Uehara, M., Mallefet, J., Haddock, S.H.D., 2024. In: Mercier, A., Hamel, J.-F., Suhrbier, A.D., Pearce, C.M. (Eds.), *Glowing Sea Cucumbers: Bioluminescence in the Holothuroidea in the World of Sea Cucumbers*. Academic Press, pp. 361–375. <https://doi.org/10.1016/B978-0-323-95377-1.00003-5>.
- Bessho-Uehara, M., Yamamoto, N., Shigenobu, S., Mori, H., Kuwata, K., Oba, Y., 2020b. Kleptoprotein bioluminescence: *parapriacanthus* fish obtain luciferase from ostracod prey. *Sci. Adv.* 6 (2), eaax4942. <https://doi.org/10.1126/sciadv.aax4942>.
- Brady, C.E., Pearce, V.B., Bain, B.A., Vrijenhoek, R.C., 2009. Pycnogonid-cnidarian interactions in the deep Monterey Submarine Canyon. *Invertebr. Biol.* 128 (4), 359–363. <https://doi.org/10.1111/j.1744-7410.2009.00176.x>.
- Burakova, L.P., Vysotski, E.S., 2019. Recombinant Ca²⁺-regulated photoproteins of ctenophores: current knowledge and application prospects. *Appl. Microbiol. Biotechnol.* 103 (15), 5929–5946. <https://doi.org/10.1007/s00253-019-09939-0>.
- Child, C.A., 1986. Spotlight on a sea-going 'spider'. *Animal Kingdom. New York Zoological Society* 89 (1), 36–38.
- Chou, C.-M., Tung, Y.-W., Isobe, M., 2014. Molecular mechanism of *Symplectoteuthis* bioluminescence – part 4: chromophore exchange and oxidation of the cysteine residue. *Bioorg. Med. Chem.* 22, 4177–4188. <https://doi.org/10.1016/j.bmc.2014.05.044>.
- Claes, J., Coubris, C., Haddock, S., Mallefet, J., 2024. Systematic distribution of bioluminescence in marine animals: a species-level inventory. *Life* 14 (4), 432. <https://doi.org/10.3390/life14040432>.
- Coubris, C., Duchatelet, L., Delroisse, J., Bayaert, W.S., Parise, L., Eloy, M.C., Pels, C., Mallefet, J., 2024. Maintain the light, long-term seasonal monitoring of luminous capabilities in the brittle star *Amphipura filiformis*. *Sci. Rep.* 14, 13238. <https://doi.org/10.1038/s41598-024-64010-x>.
- DeLeo, D., Bessho-Uehara, M., Haddock, S., McFadden, C., Quattrini, A., 2024. Evolution of bioluminescence in Anthozoa with emphasis on Octocorallia. *Proc. R. Soc. A B* 291, 20232626. <https://doi.org/10.1098/rspb.2023.2626>.
- Delroisse, J., Duchatelet, L., Flammang, P., Mallefet, J., 2021. Leaving the dark side? Insights into the evolution of luciferases. *Front. Mar. Sci.* 8, 673620. <https://doi.org/10.3389/fmars.2021.673620>.
- Dietz, L., Dömel, J.S., Leese, F., Lehmann, T., Melzer, R.R., 2018. Feeding ecology in sea spiders (Arthropoda: Pycnogonida): what do we know? *Front. Zool.* 15, 7. <https://doi.org/10.1186/s12983-018-0250-4>.
- Doyle, J.D., Kay, R.H., 1967. Some studies on the bioluminescence of the euphausiids, *Meganyctiphanes norvegica* and *Thysanoessa raschii*. *J. Mar. Biol. Assoc. U. K.* 47, 555–563. <https://doi.org/10.1017/S0025315400035189>.
- Dubois, R., 1886. Les Élatérides lumineux: contribution à l'étude de la production de la lumière par les êtres vivants. *Bull. Soc. Zool. Fr.* 11, 1–275.
- Duchatelet, L., Coubris, C., Pels, C., Dupont, S.T., Mallefet, J., 2023. Catecholamine involvement in the bioluminescence control of two species of Anthozoans. *Life* 13 (9), 1798. <https://doi.org/10.3390/life13091798>.
- Duchatelet, L., Dupont, S., 2025. Marine eukaryote bioluminescence: a review of species and their functional biology. *Mar. Life Sci. Technol.* 7 (2), 366–381. <https://doi.org/10.1007/s42995-024-00250-0>.
- Duchatelet, L., Galeazzo, G.A., Coubris, C., Bridoux, L., Rezhohazy, R., Melo, M.R.S., Marek, M., Amaral, D.T., Dupont, S., Oliveira, A.G., Delroisse, J., 2025. Insights into the bioluminescence systems of three sea pens (Cnidaria: anthozoa): from *de novo* transcriptome analyses to biobiochemical assays. *Open Biol.* 15 (4), 240262. <https://doi.org/10.1098/rsob.240262>.
- Fahrenbach, W.H., Arango, C.P., 2007. Microscopic anatomy of Pycnogonida. *Digestive System. J. Morphol.* 268 (11), 917–935.
- Firstman, B., 1973. The relationship of the chelicerate arterial system to the evolution of the endosternite. *J. Arachnol.* 1–54.
- Frank, T.M., Case, J.F., 1988. Visual spectral sensitivities of bioluminescent deep-sea Crustaceans. *Biol. Bull.* 175, 261–273. <https://doi.org/10.2307/1541567>.
- Frankowski, K., Miyazaki, K., Brenneis, G., 2022. A micro-CT-based atlas of the central nervous system and midgut in sea spiders (Pycnogonida) sheds first light on evolutionary trends at the family level. *Front. Zool.* 19, 14. <https://doi.org/10.1186/s12983-022-00459-8>.
- Fregin, T., Wiese, K., 2002. The photophores of *Meganyctiphanes norvegica* (M. Sars) (Euphausiacea): mode of operation. *Helgol. Mar. Res.* 56, 112–124. <https://doi.org/10.1007/s10152-002-0104-4>.
- Giribet, G., Edgecombe, G.D., 2019. The phylogeny and evolutionary history of arthropods. *Curr. Biol.* 29 (12), R592–R602. <https://doi.org/10.1016/j.cub.2019.04.057>.
- Gouvenaux, A., Mallefet, J., 2013. Physiological control of bioluminescence in a deep-sea planktonic worm, *Tomopteris helgolandica*. *J. Exp. Biol.* 216, 4285–4289. <https://doi.org/10.1242/jeb.090852>.
- Haddock, S.H.D., Moline, M.A., Case, J.F., 2010. Bioluminescence in the sea. *Ann. Rev. Mar. Sci.* 2, 443–493. <https://doi.org/10.1146/annurev-marine-120308-081028>.
- Haddock, S.H.D., Rivers, T.J., Robison, B.H., 2001. Can coelenterates make coelenterazine? Dietary requirement for luciferin in cnidarian bioluminescence. *Proc. Natl. Acad. Sci.* 98 (20), 11148–11151. <https://doi.org/10.1073/pnas.201329798>.
- Harvey, E.N., 1922. Studies on bioluminescence. XIV. The specificity of luciferin and luciferase. *J. Gen. Physiol.* 4, 285–295.
- Harvey, E.N., 1952. *Bioluminescence*. Academic Press.
- Henry, J.P., Monny, C., Michelson, A.M., 1975. Characterization and properties of *Pholas* luciferase as a metalloglycoprotein. *Biochemistry* 14 (15), 3458–3466. <https://doi.org/10.1021/bi00686a026>.
- Herring, P.J., 1976. Bioluminescence in decapod crustacea. *J. Mar. Biol. Assoc. U. K.* 56, 1029–1047. <https://doi.org/10.1017/S0025315400021056>.
- Herring, P.J., 1983. The spectral characteristics of luminous marine organisms. *Proc. R. Soc. A B* 220, 183–217. <https://doi.org/10.1098/rspb.1983.0095>.
- Herring, P.J., 2007. Sex with the lights on? A review of bioluminescent sexual dimorphism in the sea. *J. Mar. Biol. Assoc. U. K.* 87 (4), 829–842. <https://doi.org/10.1017/S0025315407056433>.
- Herring, P.J., Latz, M.I., Bannister, N.J., Widder, E.A., 1993. Bioluminescence of the poecilostomatoid copepod *Oncaea conifera*. *Mar. Ecol. Prog. Ser.* 94 (3), 297–309.
- Herring, P.J., Locket, N.A., 1978. The luminescence and photophores of euphausiid crustaceans. *J. Zool. Lond.* 186, 431–462. <https://doi.org/10.1111/j.1469-7798.1978.tb03932.x>.
- Hoek, P.P.C., 1881. Report on the Pycnogonida, dredged by HMS Challenger 1873–76. Reports of the Scientific Results of the Exploring Voyage of HMS Challenger 3 (10), 1–167. <https://doi.org/10.5962/bhl.title.13132>.
- Illarionov, B.A., Frank, L.A., Illarionova, V.A., Bondar, V.S., Vysotski, E.S., Blinks, J.R., 2000. Recombinant obelin: cloning and expression of cDNA, purification, and characterization as a calcium indicator. *Methods Enzymol.* 305, 223–249. [https://doi.org/10.1016/S0076-6879\(00\)05491-4](https://doi.org/10.1016/S0076-6879(00)05491-4).
- Inouye, S., Miura-Sahara, Y., Iimori, R., Sakata, Y., Hazama, Y., Yoshida, S., Nakamura, M., Hosoya, T., 2020. A novel yellow fluorescent protein of recombinant apoPholasin with dehydrocoelenterazine. *Biocem. Biophys. Res. Commun.* 526 (2), 404–409. <https://doi.org/10.1016/j.bbrc.2020.03.085>.
- Inouye, S., Tsuji, F.I., 1993. Cloning and sequence analysis of cDNA for the Ca²⁺-activated photoprotein, clytin. *FEBS Lett.* 315 (3), 343–346. [https://doi.org/10.1016/0014-5793\(93\)81191-2](https://doi.org/10.1016/0014-5793(93)81191-2).
- Johansson, M.W., 1999. Cell adhesion molecules in invertebrate immunity. *Dev. Comp. Immunol.* 23 (4–5), 303–315. [https://doi.org/10.1016/S0145-305X\(99\)00013-0](https://doi.org/10.1016/S0145-305X(99)00013-0).
- King, P.E., 1973. *Pycnogonids*, first ed. Hutchinson, London, p. 114.
- Kise, H., Bessho-Uehara, M., Kondo, K.C., Shimoji, K., Ito, S., Tsuchida, S., Fujiwara, Y., Reimer, J.D., 2025. Glow in the D-ARK: a new bioluminescent species of *Corallizanthus* (Anthozoa: zoantharia: parazoanthidae) from southern Japan. *R. Soc. Open Sci.* 12 (11), 250890. <https://doi.org/10.1098/rsos.250890>.
- Krönström, J., Dupont, S., Mallefet, J., Thorndyke, M., Holmgren, S., 2007. Serotonin and nitric oxide interaction in the control of bioluminescence in northern krill, *Meganyctiphanes norvegica* (M. Sars). *J. Exp. Biol.* 210 (18), 3179–3187. <https://doi.org/10.1242/jeb.002394>.
- Kuse, M., 2020. Marine bioluminescence with dehydrocoelenterazine, an imidazopyrazinone compound in *Marine Natural Products. Topics in Heterocyclic Chemistry*. In: Kiyota, H. (Ed.), 58. Springer, pp. 85–103. https://doi.org/10.1007/7081_2020_41.
- Lane, S.J., Shishido, C.M., Moran, A.L., Arango, C.P., Woods, H.A., 2017. Upper limits to body size imposed by respiratory–structural trade-offs in Antarctic pycnogonids. *Proc. R. Soc. A B* 284, 20171779. <https://doi.org/10.1098/rspb.2017.1779>.
- Latz, M.I., Case, J.F., 1992. Slow photic and chemical induction of bioluminescence in the midwater shrimp, *Sergestes similis* Hansen. *Biol. Bull.* 182, 391–400. <https://doi.org/10.2307/1542258>.
- Latz, M.I., Frank, T.M., Case, J.F., 1988. Spectral composition of bioluminescence of epipelagic organisms from the Sargasso Sea. *Mar. Biol.* 98, 441–446. <https://doi.org/10.1007/BF00391120>.
- Leach, W.E., 1814. *The zoology Miscellany* 1, 33–34, 43–45; X111, X1X. London.
- Lecuyer, B., Arrio, B., 1975. Some spectral characteristics of the light emitting system of the polynoid worms. *Photochem. Photobiol.* 22 (5), 213–215. <https://doi.org/10.1111/j.1751-1097.1975.tb06738.x>.
- Lehmann, T., Heß, M., Melzer, R.R., 2012. Wiring a periscope–ocelli, retinula axons, visual neuropils and the ancestry of sea spiders. *PLoS One* 7 (1), e30474.
- Lipczynska-Kochany, E., Sprah, G., Harms, S., 1995. Influence of some groundwater and surface waters constituents on the degradation of 4-chlorophenol by the Fenton reaction. *Chemosphere* 30 (1), 9–20.
- Maciel, R., Sant'Anna, G.L. Jr, Dezotti, M., 2004. Phenol removal from high salinity effluents using Fenton's reagent and photo-Fenton reactions. *Chemosphere* 57 (7), 711–719. <https://doi.org/10.1016/j.chemosphere.2004.07.032>.

- Mallefet, J., 2009. Echinoderm bioluminescence: where, how and why do so many ophiuroids glow? In: Meyer-Rochow, V.B. (Ed.), *Bioluminescence in Focus – a Collection of Illuminating Essays*. Research Signpost, pp. 67–83.
- Mallefet, J., Duchatelet, L., Coubris, C., 2020. Bioluminescence induction in the ophiuroid *Amphiura filiformis* (Echinodermata). *J. Exp. Biol.* 223, jeb218719. <https://doi.org/10.1242/jeb.218719>.
- Mallefet, J., Martinez-Soares, P., Eléaume, M., O'Hara, T., Duchatelet, L., 2023. New insights on crinoid (Echinodermata: Crinoidea) bioluminescence. *Front. Mar. Sci.* 10, 1136138. <https://doi.org/10.3389/fmars.2023.1136138>.
- Markova, S.V., Burakova, L.P., Golz, S., Malikova, N.P., Frank, L.A., Vysotski, E.S., 2012. The light-sensitive photoprotein berovin from the bioluminescent ctenophore *Beroë abyssicola*: a novel type of Ca^{2+} -regulated photoprotein. *FEBS J.* 279 (5), 856–870. <https://doi.org/10.1111/j.1742-4658.2012.08476.x>.
- Markova, S.V., Vysotski, E.S., Blinks, J.R., Burakova, L.P., Wang, B.-C., Lee, J., 2002. Obelin from the bioluminescent marine hydroid *Obelia geniculata*: cloning, expression, and comparison of some properties with those of other Ca^{2+} -regulated photoproteins. *Biochemistry* 41 (7), 2227–2236. <https://doi.org/10.1021/bi0117910>.
- Martini, S., Haddock, S.H.D., 2017. Quantification of bioluminescence from the surface to the deep sea demonstrates its predominance as an ecological trait. *Sci. Rep.* 7 (1), 45750. <https://doi.org/10.1038/srep45750>.
- Martini, S., Kuhn, L., Mallefet, J., Haddock, S.H.D., 2019. Distribution and quantification of bioluminescence as an ecological trait in the deep sea benthos. *Sci. Rep.* 9, 14654. <https://doi.org/10.1038/s41598-019-50961-z>.
- Michelson, A.M., 1978. Purification and properties of *Pholas dactylus* luciferin and luciferase. *Methods Enzymol.* 57, 385–406. [https://doi.org/10.1016/0076-6879\(78\)57037-7](https://doi.org/10.1016/0076-6879(78)57037-7).
- Moran, A.L., Woods, H.A., Shishido, C.M., Lane, S.J., Tobalske, B.W., 2018. Predatory behavior of giant Antarctic sea spiders (*Colossendeis*) in nearshore environments. *Invertebr. Biol.* 137 (2), 116–123. <https://doi.org/10.1111/ivb.12210>.
- Munz, F.W., 1958. Photosensitive pigments from the retinae of certain deep-sea fishes. *J. Physiol.* 140, 220–235. <https://doi.org/10.1113/jphysiol.1958.sp005929>.
- Nicolas, M.-T., Bassot, J.-M., Shimomura, O., 1982. Polynoidin: a membrane photoprotein isolated from the bioluminescent system of scale-worms. *Photochem. Photobiol.* 35, 201–207. <https://doi.org/10.1111/j.1751-1097.1982.tb03832.x>.
- O'Brien, P.J., 2000. Peroxidases. *Chem. Biol. Interact.* 129 (1–2), 113–139. [https://doi.org/10.1016/S0009-2797\(00\)00201-5](https://doi.org/10.1016/S0009-2797(00)00201-5).
- O'Hara, Tim, 2019. The Eastern Australian Marine Parks: Biodiversity, Assemblage Structure, Diversity and Origin. *Report to Parks Australia from the National Environmental Science Program Marine Biodiversity Hub*. Museums Victoria, pp. 1–144.
- Oliveira, A.G., Desjardin, D.E., Perry, B.A., Stevani, C.V., 2012. Evidence that a single bioluminescent system is shared by all known bioluminescent fungal lineages. *Photochem. Photobiol.* 11, 848. <https://doi.org/10.1039/c2pp25032b>.
- Plyuscheva, M., Martin, D., 2009. On the morphology of elytra as luminescent organs in scale-worms (Polychaeta, Polynoidae). *Zoosymposia* 2, 379–389. <https://doi.org/10.11646/zoosymposia.2.1.26>.
- Puzyr, A.P., Medvedeva, S.E., Artemenko, K.S., Bondar, V.S., 2017. Luminescence of cold extracts from mycelium of luminous basidiomycetes during long-term storage. *Curr. Res. in Environm. Appl. Mycol.* 7, 227–235. <https://doi.org/10.5943/cream/7/3/9>.
- Richards, P.R., Fry, W.G., 1978. Digestion in pycnogonids: a study of some polar forms. *Zool. J. Linn. Soc.* 63 (1–2), 75–97. <https://doi.org/10.1111/j.1096-3642.1978.tb02091.x>.
- Sabroux, R., Edgcombe, G.D., Pisani, D., Garwood, R.J., 2023. New insights into sea spider fauna (Arthropoda, Pycnogonida) of La Voulte-sur-Rhône, France (Jurassic, Callovian). *Paleontology* 9 (4), 1515. <https://doi.org/10.1002/spp2.1515>.
- Schaffer, W.M., Bronnikova, T.V., 2012. Peroxidase-ROS interactions. *Nonlinear Dyn.* 68, 413–430. <https://doi.org/10.1007/s11071-011-0314-x>.
- Schminkewitsch, W., 1893. Reports on the dredging operations off the west coast of Central America to the Galapagos, to the west coast of Mexico, and in the Gulf of California, in charge of Alexander Agassiz, carried on by the U.S. Fish Commission Steamer "Albatross", during 1891, Lieut. Commander Z. L. Tanner, U.S.N., commanding. *Compte-rendu sur les Pantopodes recueillis pendant les Explorations de l'Albatross en 1891*. Bulletin of the Museum of comparative Zoölogy, Harvard 25 (2), 27–43.
- Shimomura, O., 1986. Bioluminescence of the brittle star *Ophiopsila californica*. *Photochem. Photobiol.* 44, 671–674.
- Shimomura, O., 2006. In: Daunert, S., Deo, S.K. (Eds.), *The Photoproteins in Bioanalysis*. Wiley-Vch Verlag GmbH & Co. KGaA, pp. 1–23.
- Shimomura, O., 2012. Bioluminescence: Chemical Principles and Methods. World Scientific.
- Shimomura, O., Johnson, F.H., 1975. Regeneration of the photoprotein aequorin. *Nature* 256 (5514), 236–238. <https://doi.org/10.1038/256236a0>.
- Shimomura, O., Shimomura, A., 1984. Effect of calcium chelators on the Ca^{2+} -dependent luminescence of aequorin. *Biochem. J.* 221 (3), 907–910. <https://doi.org/10.1042/bj2210907>.
- Siveter, D.J., Sutton, M.D., Briggs, D.E.G., 2004. A Silurian sea spider. *Nature* 431, 978–980. <https://doi.org/10.1030/nature02928>.
- Soler-Membrives, A., Arango, C.P., Cuadrado, M., Munilla, T., 2013. Feeding biology of carnivore and detritivore Mediterranean pycnogonids. *J. Mar. Biol. Assoc. U. K.* 93, 635–643. <https://doi.org/10.1017/S0025315411001287>.
- Staples, D.A., 1997. Sea spiders or pycnogonids. (Phylum Arthropoda). In: Shepherd, S. A., Davies, M. (Eds.), *Marine Invertebrates of Southern Australia*, pp. 1040–1072. Part 111.
- Staples, D.A., 2005. Pycnogonida (pycnogonids). In: Rhode, K. (Ed.), *Marine Parasitology*. CABI Publishing, Oxford University Press: Clarendon, pp. 215–225.
- Staples, D.A., 2007. A new species of *Colossendeis* (Pycnogonida: Colossendeidae) together with records from Australian and New Zealand waters. *Mem. Mus. Vic.* 64, 79–94.
- Staples, D.A., 2020. *Labrumoides vibrissa* (Arthropoda: pycnogonida), new Callipallenid genus and new species from North-west Australia. *Zootaxa* 4751 (3), 575–581. <https://doi.org/10.11646/zootaxa.4751.3.9>.
- Staples, D.A., Watson, J.E., 1987. Associations between pycnogonids and hydroids. In: Bouillion, J. (Ed.), *Modern Trends in the Systematics, Ecology and Evolution of Hydroids and Hydromedusae*. Oxford University Press: Clarendon, pp. 215–225.
- Takahashi, H., Isobe, M., 1993. *Symplectoteuthis* bioluminescence (1) structure and binding form of chromophore in photoprotein of a luminous squid. *Bioorg. Med. Chem. Lett* 3 (12), 2647–2652. [https://doi.org/10.1016/S0960-894X\(01\)80734-4](https://doi.org/10.1016/S0960-894X(01)80734-4).
- Tran, T.-H., Chang, W.-J., Kim, Y.-B., Yoon, J.-Y., Koo, Y.-M., Kim, E.-K., Kim, J.-H., 2007. Long-term preservation of high initial bioluminescence of lyophilized *Photobacterium phosphoreum*: effect of skim milk and saccharose at various temperatures. *Kor. J. Chem. Eng.* 24, 1053. <https://doi.org/10.1007/s11814-007-0120-9>.
- Tsutsui, K., Shimada, E., Tsuruoka, Y., 2015. Deep-sea anemone (Cnidaria: actiniaria) exhibits a positive behavioural response to blue light. *Mar. Biol. Res.* 11, 998–1003. <https://doi.org/10.1080/17451000.2015.1047382>.
- Vanderlinden, C., Dewael, Y., Mallefet, J., 2003. Screening of second messengers involved in photocycle bioluminescence control in three ophiuroid species (Ophiuroidea: echinodermata). *J. Exp. Biol.* 206, 3007–3014. <https://doi.org/10.1242/jeb.00520>.
- Viviani, V.R., Hastings, J.W., Wilson, T., 2007. Two bioluminescent Diptera: the North American *Orfelia fultoni* and the Australian *Arachnocampa flava*. Similar niche, different bioluminescent systems. *Photochem. Photobiol.* 75, 22–27. [https://doi.org/10.1562/0031-8655\(2002\)0750022TBDTNA2.0.CO2](https://doi.org/10.1562/0031-8655(2002)0750022TBDTNA2.0.CO2).
- Waloszek, D., Dunlop, J.A., 2002. A larval sea spider (Arthropoda: pycnogonida) from the upper Cambrian 'orsten' of Sweden, and the phylogenetic position of Pycnogonids. *Paleontology* 45, 421–446. <https://doi.org/10.1111/1475-4983.00244>.
- Ward, W.W., Seliger, H.H., 1974. Properties of mnemiopsin and berovin, calcium-activated photoproteins from the Ctenophores *Mnemiopsis* species and *Beroë ovata*. *Biochemistry* 13 (7), 1500–1510. <https://doi.org/10.1021/bi00704a028>.
- Warrant, E.J., Locket, N.A., 2007. Vision in the deep sea. *Biol. Rev.* 79, 671–712. <https://doi.org/10.1017/S1464793103006420>.
- Widder, E.A., 2010. Bioluminescence in the ocean: origins of biological, chemical, and ecological diversity. *Science* 328 (5979), 704–708. <https://doi.org/10.1126/science.1174269>.
- Widder, E.A., Latz, M.I., Case, J.F., 1983. Marine bioluminescence spectra measured with an optical multichannel detection system. *Biol. Bull.* 165 (3), 791–810. <https://doi.org/10.2307/1541479>.
- Wilson, E.B., 1881. Report on the Pycnogonida. Reports on the results of dredging, under the supervision of Alexander Agassiz, along the east coast of the United States, during the summer of 1880, by the U.S. Coast Survey Steamer *Blake*, COMMANDER J.R. BARTLETT, U.S.N., commanding. *Bull. Mus. Comp. Zool.* 8 (12), 239–256. Harvard.
- Wood-Mason, J., Alcock, A., 1891. On the results of the deep-sea dredging during the seasons 1890–91. Ser. 11, no. 1 of natural history Notes from H.M. Indian Marine Survey Steamer Investigator. *Ann. Mag. Nat. Hist.* 6 (8), 16–17, 269–286, 353–362.
- Woods, H.A., Lane, S.J., Shishido, C., Tobalske, B.W., Arango, C.P., Moran, A.L., 2017. Respiratory gut peristalsis by sea spiders. *Curr. Biol.* 27 (13), R638–R639. <https://doi.org/10.1016/j.cub.2017.05.062>.
- Xu, J., Strange, J.P., Welker, D.L., James, R.R., 2013. Detoxification and stress response genes expressed in a western North American Bumble bee, *Bombus huntii* (Hymenoptera: apidae). *BMC Genom.* 14, 874. <https://doi.org/10.1186/1471-2164-14-874>.