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Invited Review

Chaetopterus variopedatus Bioluminescence: A Review of Light Emission within a Species Complex

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ABSTRACT

***Chaetopterus variopedatus* has been studied for over a century in terms of its physiology, ecology and life history. One focus of research is on its intrinsic bioluminescent emissions, which can be observed as a blue light emitted from the extremities of individual body segments, or as a secreted mucus. Even though research shows that *C. variopedatus* is a species complex miscategorized as a single species, all of the variants of this polychaete produce light, which has been investigated in terms of both physiology and biochemistry. Despite decades of study, there are still many questions about the luminescence reaction, and, as of yet, no clear function for light emission exists. This review summarizes the current knowledge on *C. variopedatus* luminescence in addition to briefly describing its morphology, life cycle and ecology. Possible functions for luminescence were discussed using observations of specimens found in Brazil, along with a comparison of previous studies of other luminescent organisms. Further study will provide a better understanding of how and why *C. variopedatus* produces luminescence, and purifying the protein and luciferin involved could lead to new bioanalytical applications, as this reaction is unique among all known luminescent systems.**

INTRODUCTION

Bristle worms or polychaetes are a class of annelids that are highly abundant in marine ecosystems (1). Many have become specialized in their morphology allowing them to create their own micro-environments (2). One of the most differentiated groups is the family Chaetopteridae (3,4), which are mostly benthic when adults, except for one planktonic species described, *Chaetopterus pugaporcinus* which exhibits a mixture of larval and adult features and is found in deep pelagic waters (4). All other chaetopterids have a body divided into three distinct sections, are suspension or deposit feeders and inhabit tubes. The abundant and ubiquitous species *Chaetopterus variopedatus* (4,5) lives in a U-shaped parchment-like tube in soft sediments, normally around coastal, intertidal zones. *Chaetopterus variopedatus* has been described in a number of publications as a species complex, comprising of several distinct populations that at the moment are classified as separate subspecies (6-9). The animal is cosmopolitan in distribution, with different populations being found in coastal regions around the UK, mainland Europe, Japan, New Zealand, Brazil and the United States (4,10-14). Although in the past decade the taxonomy and systematics of the Chaetopteridae have been revisited (4,15), there is still no clear consensus regarding the phylogenetic relationships within the species complex *C. variopedatus*. While there is a lack of information and clarity on the phylogenetic relationships of this species, a great deal has been written on aspects of its physiology, life history and ecology.

One of the first detailed documentations of *C. variopedatus* was done by Joyeux-Laffuie (11), and following on from this there were studies that delved into its development, regenerative capabilities and feeding habits (3,10,16). A vast array of publications have added to these initial studies and much is now known about the polychaetes physiology and behavior. One aspect that is still poorly understood is its capacity to produce bioluminescence. Such ability is present in almost 100 annelid species that are distributed in thirteen families and show a high variation in bioluminescence colors, (17,18). *Chaetopterus variopedatus* can emit a blue luminescent light from the extremities of each body segment, or in the form of a glowing cloud of mucus. Documentations of luminescence have been made for more than a century (19), and chemical studies of this process have been conducted for several decades by different researchers (20-25). Despite this, much is still unknown as to why or how the animal can achieve this. In fact, Harvey wrote in 1952 “It is hard to imagine what the purpose of light can be to an animal which remains hidden in a tube on the sea bottom in mud or sand well below the surface, and which never wanders about” (26). The main aim of this review is to summarize what is currently known about the

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species in terms of its bioluminescence. To achieve this, the general morphology, life history and ecology of *C. variopedatus* will be briefly described. Finally, using new observations of *C. variopedatus,* collected in several locations of the São Sebastião Channel, on the Southeastern coast of Brazil, in addition to descriptions from previous studies, the possible ecological roles for luminescence will be discussed.

GENERAL MORPHOLOGY

Chaetopterus variopedatus is globally distributed in intertidal zones of coastal environments (14,27,28). The adults are 15–20 cm long when mature and spend their entire lives inside a parchment-like U-shaped tube made from a secreted mucus. The worm can be divided into anterior, middle and posterior body sections, and like all annelids, its body is segmented, with the number of segments increasing with age (1,10).

The anterior section is dorso-ventrally flattened and consists of eleven segments with the front two forming a trowel-like mouth with two antennae affixed to the dorsal lip, and the following nine displaying protruding bristle-like structures (chaetae) (3,10).

The middle section is divided into multiple regions adapted to facilitate its feeding behavior. Segment 12 bears a pair of highly modified, wing-like parapodia that secrete and support a mucous net that the animal utilizes in suspension feeding. The 13th segment contains the long, dark digestive tract within a transparent body wall, where the dark-green coloration is caused by a chlorophyll-like tetrapyrrole pigment (chaetopterin) within the gut epithelial cells (29). The main body of the worm, including an accessory feeding organ—a ball-like appendage attached to the lower ventral surface of the segment 13, is yellowish in color (30). Segments 14 to 16 bear enlarged circular fan-like structures that beat rhythmically, pumping water through the U-shaped tube and maintaining a current. The worm uses this current as a means of filter feeding, to trap detritus, diatoms, copepods and bacteria entering the tube using the produced mucous net (3,10,16) (Fig. 1).

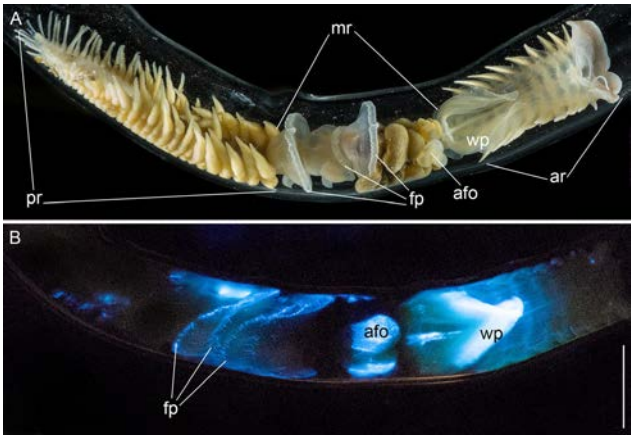

Figure 1. *Chaetopterus variopedatus* collected at São Sebastião Channel, Brazil, transferred from its burrow to a glass tube and photographed with a digital camera (Sony α7s with a Nikon AF Micro-NIKKOR 60 mm lens). (A) Under natural light, showing the anterior (*ar*), middle (*mr*) and posterior (*pr*) body sections; (B) in the dark, using a high ISO (64 000), a wide aperture (f/2.8), and a slow shutter speed (0.5 s), to show bioluminescent sections, following stimulation by addition of 1 M KCl, *afo*, accessory feeding organ, *fp*, fan-like parapodia, *wp*, wing-like parapodium. Scale bar: 1.0 cm.

Unlike the anterior and middle sections that remain the same, the posterior section increases in terms of the number of its body segments depending on the age of the individual. This section that comprises of the tail of *C. variopedatus* can consist of as many as fifty segments once the animal is mature (10,31). The worm can also regenerate tissue posteriorly from any body segment and anteriorly from segment 14 forwards (3).

LIFE CYCLE

The development of the larvae of *Chaetopterus variopedatus* has been studied in detail and could be the subject of a separate review. Part of the reason for a heightened interest in chaetopterid larvae is their size, as they can reach 2.5 mm, making them one of the largest larval forms among polychaetes (4,14). The fundamental process of reproduction and development of the larvae into adults has been described primarily by Enders (10,31), and several later publications (28,32-36). Both male and female individuals are found in the collections of tubes in the sediment (10), and mature worms are identified by swollen and opaque parapodia on their posterior sections due to the accumulation of gametes (14). When mature adults are fecund, the eggs and sperm are released from these segments and fertilization occurs in the water column (11,37). By cutting off parapodia from mature adults, it is possible to replicate this process *in vitro* to produce large amounts of larvae (34). After 18–72 h of fertilization light-emitting photocytes, located ventrolateral to the intestine are present, and luminescence can be detected from the free-swimming larvae (34). From this stage on, the larvae secrete a parachute-like mucus network they hang onto from their posterior end that provides buoyancy as well as aiding feeding (32). Sixty-day-old larvae undergo metamorphosis, transitioning from free-swimming to creeping life (10,33,36) when sediment is available for settlement (32). At this time, the larvae are 1–2 mm long with well-developed anterior and middle section. Both sections consist of the same number of segments as those found in juvenile and adult individuals. At the end of metamorphosis, the body elongates and the structures associated with pumping and feeding expand considerably. Once settled, the worm starts to make horizontal mucus coated tunnels into the sediment to build its U-shaped tube (10). The first tube formed is about 1 mm in diameter and 18–22 mm long. After a couple of days, as this dwelling becomes too small, the worm enlarges the tube by splitting it from the inside at a portion where it starts to curve upward (10). This expands the tube laterally, and similar excavations are performed as the worm increases in size, shifting it deeper into the sediment, which continues until the worm reaches maturity and can repeat the cycle of reproduction (10) (Fig. 2).

HABITAT AND DISTRIBUTION

After settlement, the juvenile of *Chaetopterus variopedatus* stays within a U-shaped parchment-like tube buried in marine soft sediments for the remainder of its life (10). The larvae of these animals can settle in many coastal regions of the world, generally in intertidal zones (14,27,28). In these regions, the animals are more abundant in calmer waters and soft, fine sediment, rather than areas with larger wave action and coarse sands, for example in protected bays and harbors. The tube of *C. variopedatus* can

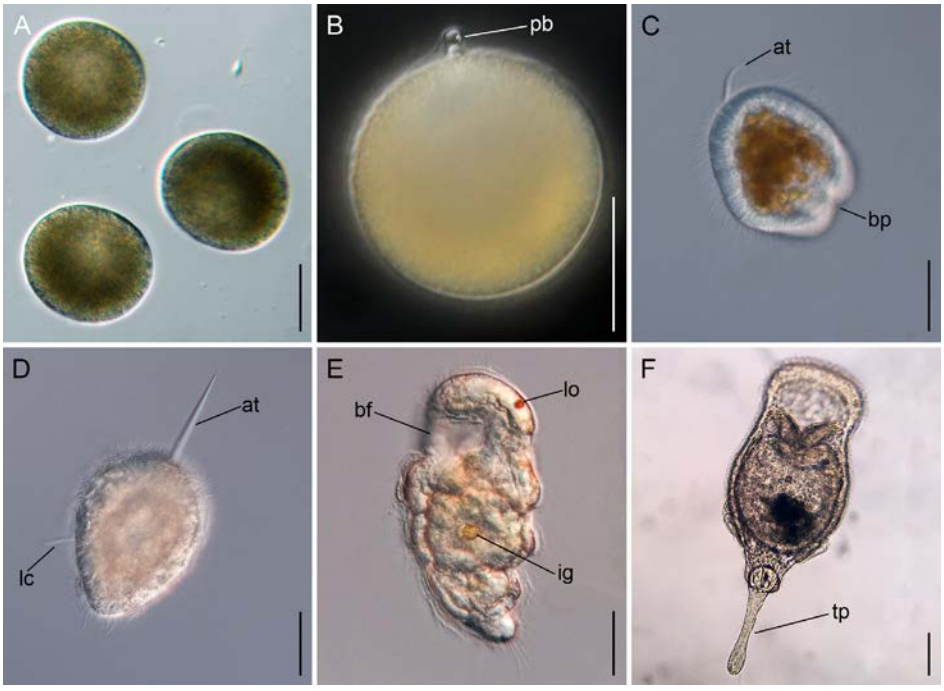

Figure 2. Photographs taken under light microscopy of different stages of larval development following *in vitro* fertilization of *Chaetopterus variopedatus*. (A) Unfertilized eggs; (B) fertilized egg with polar bodies (pb), 20–30 min after insemination; (C) free-swimming larva at the beginning of gastrulation, *c.* 24 h after insemination, note apical tuft of cilia (at) and region of blastopore (bp); (D) 2-day-old larva, note apical tuft (at) and lateral cilia (lc); (E) left-lateral view of a 7-day-old larva, with large buccal funnel (bf), unicellular ingested algae (ig), left-lateral ocellus (lo); (F) ventral view of a 10-day-old larva showing long terminal papilla (tp). Scale bars: 50 μm.

be identified by the two conical openings that protrude from the sediment surface. The location and depth in which *C. variopedatus* establish its populations varies depending on the environment and conditions (4,10-11,14). For instance in Brazil, the worms' tubes can be exposed at low tides, whereas along the coast of France, it is found at 10 m depth due to high mobility of sediment closer to the shore, which does not allow the settlement of larvae (11) (Fig. 3).

The tubes are formed and reinforced by mucus secreted from the worm's body. The animal reinforces the inner part of its tube

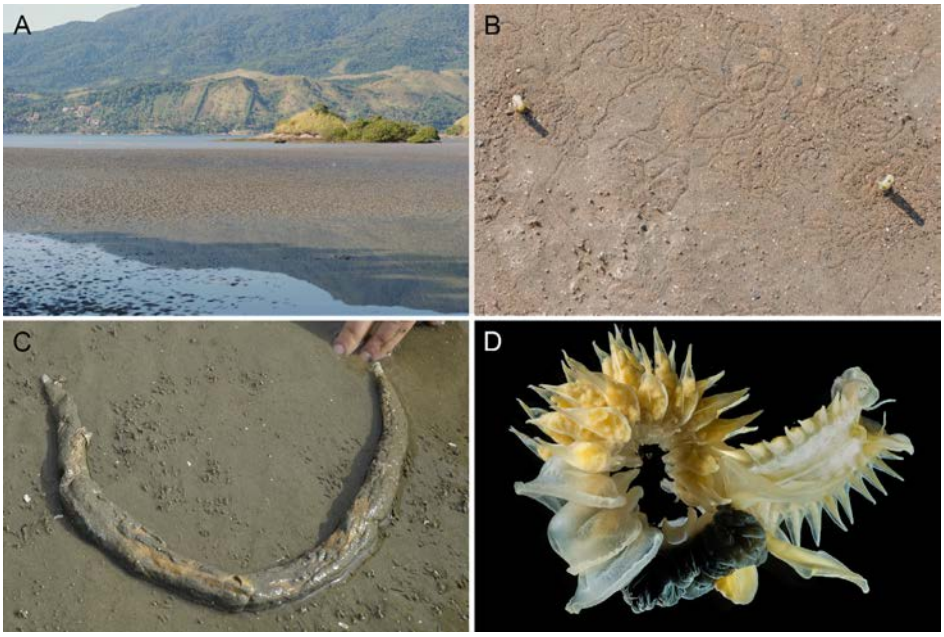

Figure 3. The bristle worm *Chaetopterus variopedatus* from the São Sebastião Channel, Brazil. (A) View at the site where the worm is frequently found, during low tide. (B) The two conical openings of the tube protruding from the surface of the sediment, during low tide, seen from above. (C) The U-shaped tube after being dug out from the sediment. (D) A live specimen removed from its tube.

by positioning its head at one opening to the tube and then moving it along the tube to the other opening, thus spreading mucus along the inner surface (3,31). As a result, the tube is structurally stable, with the material showing thermal stability and elasticity from −75°C up to 250°C (38). If undisturbed, the animal maintains a constant flow of water through the tube providing both aeration and food supply.

In the family Chaetopteridae (3,4), which according to Britayev and Martin (39) includes 73 species, 16 within *Chaetopterus*, 16 within *Mesochaetopterus*, 24 within *Phyllochaetopterus* and 17 within *Spiochaetopterus*, the morphology of the tube and its position on the substrate vary. Besides the typical U-shaped tube of several species of *Chaetopterus*, usually the other genera of the family construct less regular tubes, that could be straight or curved, branched or forming aggregations, completely buried within the sediment or not. The shape of the tube may also differ within the genus *Chaetopterus*. Previous researchers have identified two clear groups of *Chaetopterus* based on their tube building, consisting of an infaunal group that build regular tubes in soft sediment and an epifaunal group that construct irregular shaped tubes affixed to hard surfaces (1,6-7,40). The tube of *Chaetopterus charlesdarwinii*, for example, is "irregularly curved and attached to rocks or aggregated and entangled into a mass," while in *C. aduncus* it is J-shaped (40).

The building of U-shaped tubes by several morphologically similar species has potentially led to them being classified as one variable species, *C. variopedatus* (41,42). Despite being abundant along the coasts of several continents, the taxonomy and systematics of *C. variopedatus* have not been reviewed, leading to confusion as to what exactly represents this species (4). Additionally, the capacity of the larval stage to stay in the water column long enough to be dispersed across ocean basins caused all 25 previously identified *Chaetopterus* species to be grouped into one (43). From previous studies (6-9), *C. variopedatus* has been considered a species complex comprising of up to 18 species, and according to the most recent WoRMS database in 2019, at least 17 species exist within this complex (40,44). Despite the variation in morphology, all lineages within this species complex exhibit bioluminescence, although the luminescent sections of the body and the intensity of light emission differ among some of them, based on the observations of different researchers (24,45).

BIOLUMINESCENCE

Bioluminescence, a biochemical process resulting in light emission, is a widespread phenomenon that has evolved independently many times (46). It is observed in many marine and terrestrial organisms such as fungi, cnidarians, comb jellies, crustaceans, fireflies, squids, echinoderms, fishes (46-49) and among polychaete worms. In the Class Polychaeta, bioluminescence is found in different colors and in numerous species belonging to several families, such as Acrocirridae, Chaetopteridae, Cirratulidae, Flabelligeridae, Polynoidae, Syllidae, Tomopteridae and Terebellidae (18,46). Within Chaetopteridae, the genera *Chaetopterus* and *Mesochaetopterus* were reported to have blue light-emitting representatives (e.g. *Chaetopterus pugaporcinus* and *C. variopedatus*) (26,50), the most common marine bioluminescence color (46). However, within the Class, the light emission range is also represented by yellow, a rare color among marine organisms, and green. The first is found in most tomopterids (e.g. *Tomopteris helgolandica*) (18,51), and the second color in many syllids (e.g. *Odontosyllis enopla*), (18,52) and some cirratulids (e.g. *Chaetozone caputesocis*) (18).

Studies of many luminescent species, including *C. variopedatus,* go back decades or even centuries, yet the complexity of their light emission mechanisms and instability of reaction components hindered the elucidation of their bioluminescence mechanisms. Aspects of luminescence of *Chaetopterus* have been observed and studied for over a century with some of the first descriptions of light emission being noted by Panceri (19) and Joyeux-Laffuie (11). Following on from this, biochemical studies from several research groups provided insight into different aspects of the reaction culminating in the partial characterization of the light emission reaction (20-22,53-56). The physiology and control of light emission was reported in the adults (12,23). Moreover, observations of physiology and development in regard to bioluminescence were noted in studies of the larvae (34,35). Finally, nearly 50 yr after the research of Shimomura and Johnson (22,56), a series of new publications have begun to re-analyze the biochemistry and components of the reaction (24-25,57,58). This is a summation of the key findings of these papers, along with a discussion of what is still unknown regarding the bioluminescence of *C. variopedatus* in terms of physiology, biochemistry and ecological functions of the light emission reaction.

PHYSIOLOGICAL STUDIES OF BIOLUMINESCENCE

The first observations of *C. variopedatus* provided a brief description of the light emitted by the animal. Bioluminescence was shown to have two forms: a flash-type emission from the photogenic tissue within the body and a glowing mucus secreted into the water column (10-11,19). A series of papers (20,53,54) were some of the first to examine the physiology of the body tissue bioluminescence in greater detail. They investigated the effect of different salts (e.g. NaCl, KCl, $CaCl_2$ and $MgCl_2$) on light emission in *C. variopedatus* in order to explain the possible mechanism of stimulation. These results showed that NaCl and KCl induced bright light emission, while $CaCl_2$ caused irritation to the worm leading to faint light emission following gentle mechanical stimulation. Overall, they concluded that the addition of these salts changed the ionic composition of the environment around the worms and induced depolarization of nervous and photogenic tissue, leading to bioluminescence (20).

Several years later, Sie et al. (21) examined the influence of pressure, temperature and organic compounds on *Chaetopterus* bioluminescence. They studied both the cell-free luminescent mucus in addition to the luminogenic tissue of *C. variopedatus*. This study showed that the luminescence reaction occurring in *C. variopedatus* was sensitive to both temperature and pressure (21). At lower temperatures or in the presence of urethane, the duration of the blue flash of light from *C. variopedatus* body segments became prolonged. Additionally, luminescence from both the body and the mucus could be reversibly inhibited by an increase in hydrostatic pressure, with light emission reoccurring upon the release of pressure (21). While this and previous studies had shown the effects of different conditions on light emission, none had provided information on the key components of the bioluminescence reaction: the enzyme and the luciferin.

Other studies focussed more on physiological regulation and control of luminescence in *C. variopedatus* and analyzed the microscopic structures of the light-emitting tissue (12,23). An epithelial luminescent gland and several types of cells that appeared to be associated with luminescence were identified in the wing-like parapodia (12). Orthochromatic cells were observed in these regions and proposed to contain the photoprotein involved in light emission (12). These cells synthesize and expel large quantities of secreted products through pores and could potentially be a source of luminescence for both the light emission observed throughout *C. variopedatus'* body and in the mucus it secretes. In additional studies, electrical and mechanical stimulation was used to investigate the neurological mechanisms of regulating bioluminescence in the worms (23). Mechanical stimulation of the worm's tube caused it to release a cloud of mucus from one of the holes in its tube and then move its position to the other end of the tube. Based on this observation, it was proposed that the discharges of luminescent mucus are a defensive strategy. Finally, Martin and Anctil showed that luminescence from the worm's body segments is coordinated and controlled by its ventral nerve cord (23).

While several studies of *C. variopedatus* have looked into adult luminescence, very few have discussed luminescence in the larvae (35,36). One study by Henry (34) described the propagation of luminescence in larvae following fertilization. The *C. variopedatus* larvae were shown to develop luminogenic organs located on either side of the mouth (59), and light emission was observable as early as 48–72 h after fertilization (34). Following fertilization, the embryo developed unequally even after the first division into two cells called blastomeres. Following the second division after fertilization, it was possible to separate these blastomeres (referred to as AB and CD) and both pairs of cells still went on to develop into larvae (34). Observations of these larvae after 72 h showed that only those derived from CD blastomeres could emit light (34). This study showed that luminescence is differentiated in larvae as early as the second cell division in the embryo; however, the function of luminescence in the free-swimming form has not been investigated in detail.

The physiology of *C. variopedatus* luminescence, occurring both in the adult and larval stages of the worm, has been described in numerous publications. The fact that *C. variopedatus* luminescence is differentiated after the second cell division of the blastomeres and can be observed in the organism as early as 48 h after fertilization strongly suggests that all the components required for light emission are inherent, rather than acquired through diet. Stimulation of light emission has been attainable through chemical means or by mechanical or electrical stimulation. This stimulatory effect demonstrates that light emission is controlled and regulated by the nervous system, whether it is light emission observed intrinsically within the tissue or from the secreted glowing mucus.

BIOCHEMISTRY OF THE BIOLUMINESCENT REACTION

The light emission reaction generally involves three components, a substrate (small organic molecule customarily called luciferin) that is oxidized by oxygen, in the presence of an enzyme (luciferase) (45). This reaction produces an unstable intermediate (usually a cyclic peroxide), rapid decomposition of which results in a compound called oxyluciferin and releases a large amount of energy in the form of cold visible light (60). In several bioluminescent systems, the luciferase and luciferin form an enzyme–substrate complex and require an additional cofactor for light emission to occur. These enzyme–substrate complexes are generally referred to as a photoproteins (45).

Further studies by Harvey (26) demonstrated that the reaction taking place in the animal's body required molecular oxygen, while the mechanism underlying light emission in the luminescent slime did not resemble a luciferin–luciferase type reaction (26), and, therefore, is an example of what is now known as a photoprotein. Attempts at *in vitro* reconstruction of the *Chaetopterus* bioluminescence system were unsuccessful, thus rendering the elucidation of the light emission reaction components a difficult task. The basic technique for luciferin and luciferase separation, developed by Dubois (61-63), is termed "hot-cold extract." In this method, two water extracts of luminogenic tissue are prepared. The use of cold extract allows for the preserving of activity for the enzyme (luciferase), which consumes substrate before *in vitro* tests, while the heated fraction destroys the proteins and yields the luciferin (45,63). The two extracts give an *in vitro* luminescence when mixed together. This procedure can often be used to purify both the luciferin and the luciferase from luminescent organisms; however in several cases such as that of *C. variopedatus*, this method does not work, and alternate protocols need to be developed to purify the components of the reaction.

Certain luminescent systems do not exhibit a typical luciferin–luciferase reaction, and instead, these two components form an enzyme–substrate complex that requires an additional cofactor, such as a metal ion or peroxide, for light emission to occur (45,60). Such systems are referred to as photoproteins, and research by Shimomura and Johnson (22,56) determined that the bioluminescent system of *C. variopedatus* is of this type. While it was not possible to identify the luciferin involved in the reaction, they showed that this photoprotein emitted light in the presence of H_2O_2 and Fe^{2+}. A light-emitting extract was prepared by homogenizing the 12^{th} body segment of *C. variopedatus* containing the wing-like parapodia. By adding H_2O_2 and Fe^{2+} to the active fraction obtained after several stages of ammonium sulfate precipitation, dialysis and ionic exchange chromatography, they were able to observe light emission. Further purification of this photoprotein was then achieved by chromatography using a high-resolution size exclusion column. The purified photoprotein was then crystallized using ammonium sulfate (22,56).

After purifying the photoprotein, Shimomura and Johnson (22,56) were able to investigate the properties of the luminescent reaction in *C. variopedatus*. They observed that the intensity of the light emission decreased during purification and noted that there were other cofactors in addition to molecular oxygen, H_2O_2 and Fe^{2+}. By adding partially purified fractions to the *in vitro* luminescence assay, they were able to identify two additional cofactors that led to an increase in light activity. One of these cofactors was identified as a macromolecule resembling a protein, while the other was a lipid-like substance (22).

In the same study, Shimomura and Johnson (22) determined the photoprotein to have a molecular weight of 128–130 kDa before crystallization. When crystallized, the photoprotein appeared to have a molecular weight of 184 kDa. By comparing these two differing weights, they proposed that the actual monomer subunit of the photoprotein could be $\sim$ 60 kDa, with the noncrystallized and crystallized forms being a dimer and trimer, respectively. In terms of spectral properties, the photoprotein was

shown to emit blue luminescent light with an emission peak between 453–455 nm. The optimal wavelength for fluorescence absorption was 375 nm, which led to a fluorescent emission peak at 453–455 nm, matching the luminescent spectra.

In summation, Shimomura's research (22,56) afforded the first insight into the biochemistry of *Chaetopterus* luminescence. A photoprotein was identified along with five cofactors (molecular oxygen, H_2O_2, Fe^{2+}, an additional protein and a lipid-like substance) necessary for light emission. Moreover, this protein was purified, crystallized and shown to produce a blue luminescent light *in vitro*. No information on the structure of the photoprotein involved in the reaction was acquired despite successful crystallization. Moreover, the *Chaetopterus* luminescence mechanism remained unclear as the luciferin involved in the reaction had not been identified. Despite there being a wide array of questions to be answered regarding the chemistry of *C. variopedatus* luminescence, this would not be revisited for over 50 yr, possibly due to the difficulty in studying this system further.

Based on the previous studies, it appears that *C. variopedatus* mechanism of light emission is unique. Following the work of Shimomura and Johnson (22,56), it had not been studied in great depth for over half a century until several recent studies reanalyzed light emission in the mucus (24-25,57,58). These studies agreed with Shimomura and Johnson's work (56), confirming that the light emission reaction was of the photoprotein type rather than a luciferin–luciferase reaction (24). However, Branchini and colleagues suggested that H_2O_2 when added to luminescent mucus secreted by the polychaete caused inhibition of light instead of enhancement (24). While measuring light emission in the mucus, they observed a green fluorescent signal that increased as the light emission reaction progressed, which was proposed to be associated with the oxyluciferin product of the reaction. LC/MS analysis of the luminescent mucous showed the presence of riboflavin, a compound normally associated with bacterial bioluminescence, though no luminescent bacteria were found in *C. variopedatus* (24). Based on these findings, Branchini's group have hypothesized that riboflavin or a structurally related compound could act as the light emitter in this bioluminescent system (24). Additionally, they proposed that light emission might be produced by a variant of the same mechanism as bacterial bioluminescence, involving a photoprotein rather than a luciferase enzyme (24).

Further research by this group stated that Fe^{2+} did not have a strong enhancing effect on light emission in the mucus (57), which contradicted previous studies on the luminogenic tissue of *C. variopedatus* (22,56). Additionally, H_2O_2 increased the viscosity of the luminescent mucus, while causing inhibition. The luminescent mucus showed fluorescent activity and could be secreted from all over the body (57). Bioluminescence and fluorescence emission spectra for *C. variopedatus* mucus were both blue in color, with emission maxima coinciding at 455 nm. The observed spectra were similar to those recorded in previous studies of the luminescent tissue by Shimomura and Johnson (22,56). They showed that increased density of mucus caused dimming and then inhibition of luminescence, which is similar to the inhibitory effect of pressure described previously by Sie et al. (21). Moreover, it was possible to preserve the mucus in form so that it could still fluoresce using high-temperature treatments, thus allowing further isolation and characterization of the reaction components for *C. variopedatus* bioluminescence (57).

These findings showed that the luminescence of *C. variopedatus'* mucus involves a photoprotein, iron and flavins, but further investigation was hindered by the viscosity of the mucus causing inhibition of light emission (58). Using two chromatographic steps (high-resolution anionic exchange followed by size exclusion chromatography), it became possible to isolate several proteins associated with light emission in the mucus (58). The luminescent mucus had two phases, one with spontaneous luminescence, the other phase had no spontaneous light activity but could be stimulated with H_2O_2 (58). Fractions that showed luminescent activity had only a few proteins present, one with similarities to ferritin. The protein was confirmed to be a ferritin following cloning, sequencing and confirmation of ferroxidase activity and was named "Chaetopterus Ferritin" (ChF). Based on this, it was proposed that this could be a source of iron and a catalyst in *C. variopedatus* bioluminescence (58).

ChF was successfully crystallized in order to further characterize its structure along with other biochemical characteristics (25). Compared to other ferritin-like proteins, ChF exhibits faster catalytic performance in terms of ferroxidase activity and is unique in that it is secreted as part of the luminescent mucus of *C. variopedatus* (25). The catalytic site's crystal structure of ChF was shown to be similar to other known eukaryotic ferritins. Differences were observed in parts of the ChF structure that could potentially allow for a more efficient pathway for Fe^{2+} ion transfer to the enzyme's active site. It is possible that this could have an impact on the ferroxidase activity of ChF, and therefore bioluminescence of *Chaetopterus*, though a lot more research into this is still required (25). However, while this research has noted several new observations about *C. variopedatus* luminescence, a lot remains unclear. For instance, while ChF was proposed to be involved in this reaction based on its effect on Fe^{2+}, no luminescence assay was established as an attempt to confirm this. Thus, while it is possible this enzyme could be involved in luminescence, the current published works do not yet sufficiently clarify this.

Finally, a recent study by Purtov and colleagues has begun to analyze the components of the chemical reaction for *Chaetopterus* bioluminescence (64). They argued that the previously described *in vitro* chemiluminescent reaction for *C. variopedatus* (22,56) did not resemble a typical photoprotein system. With this in mind, they were able to successfully perform a cross-reaction between two extracts of *C. variopedatus* prepared using either phosphate buffer or ethanol (64). These results supported the idea that *C. variopedatus* may utilize a classical luciferin–luciferase type reaction instead of the previously proposed reaction using a photoprotein to facilitate light emission (22,56,64). Using a series of chromatographic steps, they were able to partially purify the luciferase enzyme and detect light emission *in vitro* via the addition of the luciferin that was prepared and extracted on ethanol. They demonstrated a direct correlation between the volume of the luciferin extract added and the intensity of bioluminescence observed, when increasing volumes of the luciferin extract were added to the partially purified luciferase (64). Moreover, they noted that Fe^{2+} was a critical cofactor for the reaction, as the addition of a prepared solution of iron sulfate resulted in an increase in light intensity over 100 times greater than in the absence of Fe^{2+} (64). This supported previous studies by Shimomura and Johnson (22,56), that had previously identified Fe^{2+} as being involved in the reaction. Studies by these researchers and others are likely ongoing in order to isolate and purify the

luciferin and luciferase components of *Chaetopterus* bioluminescence.

Based on the literature, light emission in the tissue appears to behave differently to light emission in the mucus, both in terms of the kinetics of the reaction and in terms of the effect of different cofactors, namely Fe^{2+} and H_2O_2. Given these differences in the reaction mechanisms, it is reasonable to assume that either the components of the reaction are already present in the mucus, or potentially that the exact chemical reaction for luminescence in the tissue is different to that in the mucus. While it is possible to speculate over this, the luciferin substrate of the reaction is still unknown and the actual chemical mechanism for light emission remains unclear. In order to further study this system, further experiments aiming to identify the true structure of the luciferin involved along with obtaining the sequence and structure of the enzymatic component involved in the reaction; whether it resembles the photoprotein that was purified and crystallized over 50 yr ago, or the luciferase that was only recently identified and partially purified.

In summation, decades of research on *Chaetopterus variopedatus* have yielded conflicting data regarding the number and function of the reaction components involved in *Chaetopterus* light emission (Table 1). Early studies of the worm's 12^{th} segment by Shimomura and Johnson revealed the presence of photoprotein requiring 5 cofactors (oxygen, H_2O_2, Fe^{2+}, a lipid-like substance and an additional protein) to produce visible light. The function of Fe^{2+} and H_2O_2 was later contested in the experiments with *Chaetopterus* secreted luminous mucus, which showed that these cofactors either have no stimulatory effect or even inhibit the mucus luminescence. Further research has suggested the involvement of ferritin and riboflavin in *Chaetopterus* photoprotein luminescence reaction, but did not confirm the presence of other low-molecular-weight components in this bioluminescence system.

In the latest study, a new method for the separation of the protein and low-molecular-weight components of the *Chaetopterus variopedatus* bioluminescent system was designed. None of the separated compounds displayed independent light emission, but an intense *in vitro* luminescence was triggered upon the addition of Fe^{2+} to the reaction mixture containing the purified protein and the separated low-molecular-weight component. Furthermore, the luminescence intensity showed a linear dependence on the amount of the low-molecular-weight compound, suggesting that it belongs to the luciferin–luciferase type, rather than a photoprotein.

Analysis of the data presented in the bioluminescence studies by Shimomura, Branchini and Purtov might seem contradictory. However, a plausible explanation of biochemical mechanism of bioluminescence may still be drawn from these results. The *in vitro* bioluminescence kinetics presented in the studies by Shimomura and Deheyn are too slow, which is not typical for the photoprotein reactions, while the described changes in the luminescence intensity in response to the addition of H_2O_2 could be explained by the contribution of chemiluminescence to the Fenton reaction. Nonetheless, these studies have highlighted several key components necessary for the *Chaetopterus* bioluminescence reaction. An enzyme, which is most probably luciferase based on the previously described kinetics, oxidizes a luciferin, which is likely unstable; hence, it has not yet been isolated. All of the studies concur that iron is an important cofactor in the reaction, and it is feasible that ferritin may be its source according to research conducted by Branchini's group.

It is also important to note that all the studies mentioned above were performed on distinct populations of *Chaetopterus variopedatus* in addition to using different parts of the body (12^{th} segment, entire body or mucus). Therefore, the seemingly different conclusions drawn from these investigations might not be contradictory and could imply separate evolutionary origins of bioluminescence in various species of the vast *Chaetopterus* complex. Further isolation and structural characterization of the *Chaetopterus* bioluminescence system components will hopefully shed light on the true nature of this bioluminescence reaction.

ECOLOGICAL FUNCTIONS OF BIOLUMINESCENCE

Despite all the years of research, little progress has been made in understanding why the animal produces light. Bioluminescence is generally used for predation, defensive purposes or intra-specific communication for either migration or courtship (46). At first

Table 1. Physiological and biochemical characteristics of *Chaetopterus variopedatus* bioluminescence

Population	Year of study	Source of luminescence	Protein type	Cofactors/ Coenzymes	BL stimulation	Citation
Plymouth, UK	1952 1954	Entire animal	Unknown	Unknown	NaCl, KCl	(20,53,54)
Massachusetts, USA	1958	Entire animal and secreted mucus	Unknown	Unknown	Mechanical stimulation	(21)
Venice, California, USA	1966 1968	12^{th} body segment	Photoprotein 128–130 kDa (184 kDa crystal)	H_2O_2, Fe^{2+} Lipid-like substance Additional protein	Mechanical stimulation	(22,56)
Venice, California, USA	1979 1984	Entire animal and secreted mucus	Unknown	Unknown	Electrical and mechanical stimulation	(12,23)
Massachusetts, USA	1986	Larvae	Unknown	Unknown	Electrical stimulation	(34)
San Diego, California, USA	2013 2016 2017	Secreted mucus	Photoprotein	ChF Riboflavin H_2O_2	KCl	(24-25,57,58)
Sao Sebastião, Brazil	2019	Entire animal	Luciferase 80 kDa	Fe^{2+}	Mechanical stimulation, KCl	(64)

glance, few of the above functions appear to have potential roles in this organism due to the sedentary lifestyle of the adult animals living in an opaque tube burrowed into the sediment. However, a number of hypotheses regarding the possible roles of light emission in *C. variopedatus* have been postulated in several of the previously mentioned studies (23-24,57). Therefore, using this information, along with our own observations of *Chaetopterus variopedatus* from Brazil, it is worth discussing the potential functions of this light emission reaction.

Based on the previous articles and observations of Brazilian *C. variopedatus*, the animal displays at least two forms of luminescence, one from the body tissue (22,23) and another from a glowing mucus secreted by the animal (24,57). Different researchers have described these forms of light emission in almost contradictory manners, as if the intensity of light emission observed in each experiment for either the body or the mucus was different. Considering that *Chaetopterus* is a cosmopolitan species complex, it is possible that some variants possess comparatively brighter luminescence in the mucus, while the others show an intense light display from the luminogenic tissue.

The Brazilian population of *C. variopedatus* was observed to produce both a flash from different body sections and a glowing mucus that it secretes into the water column. The light emission in both cases is bright blue and can be induced by mechanical and electrical stimulation or by addition of KCl (See Video S1). Mucus secretion is more difficult to stimulate and requires a lot more force to generate light emission. It was observed that gently grabbing the polychaete with forceps and shaking it can induce the secretion of luminous mucus from a channel in the anterior section near to its mouth. The potential functions for both forms of light emission in *C. variopedatus* will be discussed in relation to their viable applications.

According to several reports, the luminescent mucus can be expelled from the worm's tubes if it is agitated leading to the production of a bright glowing cloud of mucus that can be observable in the water column (24,57). Unlike these, the Brazilian specimens that were observed produced luminescent globules of mucus that could easily stick to the forceps used for mechanical stimulation, without the loss of luminescence. It is more likely that this form of light emission has a defensive purpose, as suggested by previous authors (23). Specifically, the glowing mucus could act as a warning signal to dissuade predators from disturbing or disrupting the tube. Analogous aposematic signaling has been proposed for other organisms, although toxins are not known to be present and aposematism has not been investigated in this polychaete (46,65). Luminescent discharges have been described as having defensive purposes in several animals such as ctenophores, copepods and the vampire squid, which emits a cloud of luminescence instead of ink to startle predators (46,66,67). Moreover, as the mucus can stick to inanimate objects it is logical that it could stick to predators that were in the act of attacking the worm, while still emitting a bright blue luminescent glow. This would tag the would-be predator causing it irritation or leading it to move away from the tube in order to avoid predation by higher trophic level organisms.

Observations of Brazilian *C. variopedatus* showed that in addition to the glowing mucus, it emits brief flashes of blue light from its body segments when agitated using mechanical stimulation, electricity or the addition of KCl (Video S1). In particular, the tail region and wing-like parapodia emit a large amount of luminescent activity on stimulation. It is probable that the body luminescence also has a defensive function in *C. variopedatus,* with flashes either used to startle predators in the dark or to distract predators from the vital parts of the polychaete as seen in other light-emitting animals (46,68). A couple of early studies observed that the worms are not completely sedentary in their behavior, and on occasion will protrude either their anterior or posterior sections out of their tubes (3,10). Adult polychaetes have been observed to be regenerating lost body segments both from the tail and the head; however, the 13th segment containing the intestines was never regenerated (3). Coincidentally, this region emits little to no light compared with other regions of the body; therefore, it is possible that other parts of the body could emit light to act as a sacrificial tag as seen in other bioluminescent organism (46,69). This tactic would allow the worm to survive a partial destruction, as the vital components for regenerating body tissue would remain intact.

Additionally, light emission from the tissue may have a role in maintaining water circulation in *C. variopedatus'* tube, which is essential for its feeding strategies (70). In previous studies and our own observations, symbiotic commensal species of crabs (*e.g. Pinnixa chaetopterana and Polyonyx gibbesi)* have been found to dwell within the tubes of *C. variopedatus* (70). Moreover, under laboratory conditions, worms were shown to move water through their tubes at a higher rate when crabs were present (70). As light emission from the tissue can be stimulated by minimal mechanical abrasion, it is possible that luminescence could be induced by the worm bumping into the crabs as it is trying to cycle water through its tube. The brief flash of light produced could warn the crabs to stay at a particular distance within the tube, thus allowing them to co-habit it without disturbing the water circulation of *C. variopedatus.*

Like the sedentary adults, the larvae have the capacity to emit a blue bioluminescent light from their bodies (34). This can be observed as little as 48–72 h after fertilization, though the exact moment in development when luminescence occurs is not yet apparent (34). Luminescence in larvae may have similar defensive role to the adults, in that it can either startle or warn predators, preventing them from attacking. However, light emission may have different feeding-related functions in the larvae. *Chaetopterus variopedatus* larvae feed in the water column using mucous nets like those used by the sedentary adults (3,10,32). This net can capture organisms such as diatoms, copepods and other planktonic species, which are ingested by the larvae (32). As many zooplankton in the water column exhibit positive phototaxis (71), they are drawn toward points of light in the dark. It could be that luminescence is used as a lure to attract prey toward the mucous net, thus providing an easier means of predation and feeding for the larvae.

Finally, it should be considered that luminescence in Chaetopteridae may have no specific visual function and that this is merely a metabolic by-product of a reaction. For example, in bacteria it has been suggested that luminescence is a merely a side effect of oxidation reactions that were already occurring within cells (72,73). In fact, the first evolutions of luminescence may have had other nonlight-emitting functions as other luciferins, such as coelenterazine, have strong anti-oxidative properties that can prevent reactive oxidative species from damaging the cells (74). As Fe^{2+} and H_2O_2 are involved in the mechanism, it is possible that luminescence is merely a metabolic by-product of a reaction aiding in preventing damage to the animal from free radicals or peroxides, though as to how it may achieve this is still unclear.

The potential functions of light emission in both the larvae and the adult of *C. variopedatus* have been discussed. Bioluminescence may have different functions depending on the type of luminescent emission. In the adults, luminescent mucus can startle predators while flashes from body segments may distract attention away from the more vital body parts. In larvae, luminescence may act to aid in feeding, though it is still possible that in both adults and larvae that the primary function of the luminescent reaction is biochemical, not visual. Further study is required in order to validate these hypotheses, which in turn may aid in improving the understanding of how *C. variopedatus* emits light in addition to why.

SUMMARY

Polychaetes are an incredibly diverse lineage with some species such as *C. variopedatus* exhibiting high specialization to particular environments. This organism is cosmopolitan in distribution and has been described as a species complex, comprising of at least 17 species, and are generally dwell within U-shaped parchment-like tubes in soft sediments, normally around coastal, intertidal zones. All variants of *C. variopedatus* have the capacity to produce their own chemical light emission, through a process called bioluminescence. The objective of this review was to briefly describe the physiology, life history and habitat of this organism, and in greater detail elaborate on what is currently known about its bioluminescence. The animal is a filter feeder with a free-swimming larval stage and a sedentary tube-dwelling adult that reproduces by broadcast spawning. The light emission reaction has been studied in *C. variopedatus* in terms of chemistry; however, the ecological functions remain unclear. Moreover, a lot is still poorly understood regarding the chemistry of its luminescence, as the luciferin is still unknown, the sequence and structure of the luciferase or photoprotein involved have not been identified, and the pathway of the chemical reaction remains unclear. Despite this, there is great value to study this organism, as its luminescent system is unique among all known luminescent organisms based on the cofactors associated with this reaction. Finally, as it uses Fe^{2+} as a component of light emission, once characterized this luminescent system could be a useful bioanalytical or biomedical tool to detect and quantify amounts of the metal.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Video S1. Bioluminescence of *Chaetopterus variopedatus* (Annelida, Polychaeta). Light emission was recorded from a specimen kept within a U-shaped glass tube, after chemical stimulation with KCl crystals added directly on the top of the tube.

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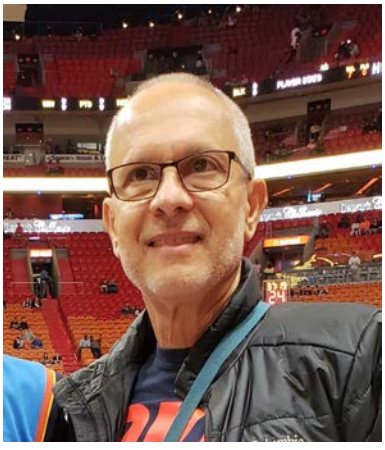

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