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Not All Ctenophores Are Bioluminescent: *Pleurobrachia*

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Abstract. The traditional view has been that all species of the phylum Ctenophora are capable of producing light. Our inability to elicit luminescence from members of the well-known genus *Pleurobrachia,* as well as a lack of published documentation, led to an effort to determine whether this genus is truly bioluminescent. Physical and chemical assays of several species from the family Pleurobrachiidae produced no evidence of bioluminescence capability, although all other species of ctenophores tested gave positive results. Some of the historical misperception that *Pleurobrachia* can produce light might be attributable to confusion with similar luminous genera.

Introduction

Planktonic marine invertebrates are noted for their ability to produce light (Herring, 1987; Haddock and Case, 1994), but even among these organisms, the phylum Ctenophora is remarkable for the extent of bioluminescence expression. Because there have been no systematic investigations, speculation about the true extent of bioluminescence ability in ctenophores comes mainly from secondary sources. According to Ruppert and Barnes (1994), "Ctenophores are noted for their luminescence, which is characteristic of all species." Others agree that "all ctenophores" (MacGintie and MacGintie, 1968) or "probably all species" (Harvey, 1940) are bioluminescent, and Dahlgren (1916) goes so far as to state that "all the ctenophores have been known for a long time to be light producing."

Pleurobrachia, perhaps the best-known and most studied ctenophore genus, has long been considered capable of bioluminescence (Gadeau de Kerville, 1890; Herring, 1987). However most authors who mention bioluminescence in *Pleurobrachia* proceed to give details of the luminescent system of *Mnemiopsis* or some other species. The published records of luminescent spectra contain no measurements from *Pleurobrachia* (Nicol, 1958; Young, 1981; Herring, 1983; Widder *et al.,* 1983; Latz *et al.,* 1988), even though this genus is one of the most frequently encountered. Despite 'conventional wisdom', we know of no credible accounts of luminescence in the family Pleurobrachiidae—either in the genus *Pleurobrachia* or *Hormiphora.*

It is often difficult to evaluate an early report that a species is bioluminescent. Results can be confounded by the luminescence of a contaminating organism or by external light causing reflection or refraction (Herring, 1987). In some cases the taxonomy of a group of organisms has changed so much that it is not possible to determine which species was investigated by early researchers. Furthermore, once an organism has been reported as luminous, there is considerable resistance to removing it from the list of luminous species (*e.g.,* sponges). To an extent this resistance is understandable, because the ability to luminesce may vary within a population on a sexual, ontogenic, seasonal, or diel basis (Herring, 1987). Variation may also occur between subpopulations, as in the midshipman fish, which is luminous off California but not when found further north (Warner and Case, 1980).

With these caveats in mind, we have attempted to rigorously demonstrate that *Pleurobrachia* is a notable exception to the dogma that all ctenophores are bioluminescent.

Materials and Methods

Various species of *Pleurobrachia* were sampled in the Santa Barbara Channel (*P. bachei,* throughout the year), the Alboran Sea (*P. rhodopis,* spring), the Gulf of Maine (*P. pileus,* summer), at Santa Catalina Island, California (*P. bachei,* summer), and at Friday Harbor, Washington

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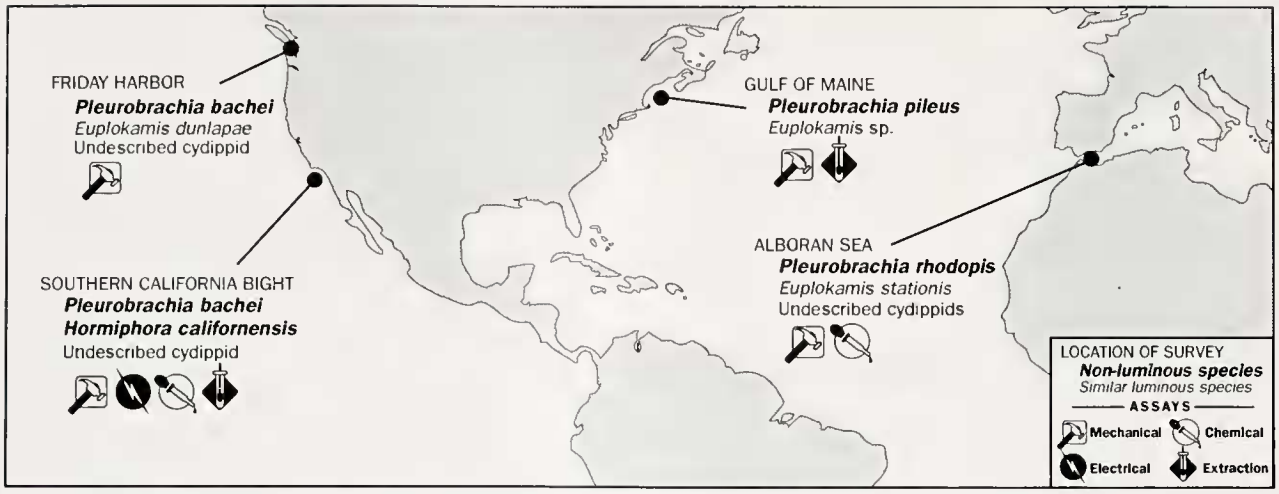

Figure 1. Four types of assays were conducted to determine whether ctenophores were bioluminescent or bore any light-producing chemicals. At each site where specimens were collected, we also found luminous genera which could have been mistaken for *Pleurobrachia*.

(*P. bachei*, fall). To ensure that the ctenophores were not prestimulated or damaged during collection, specimens used in these studies were hand-collected in jars by blue-water divers, except at Friday Harbor, where they were collected from the surface in beakers. Because some ctenophores lose their luminescence upon exposure to light (Ward and Seliger, 1976), specimens were dark-adapted for a minimum of 30 min prior to experiments. After this recovery period, *Pleurobrachia* were subjected to mechanical, electrical, and a variety of chemical stimuli (Fig. 1). To ensure that the assay techniques were effective, we also tested the luminescence of other ctenophore species found at the same locations.

Physical stimulation

The most commonly applied test for luminescence was physical stimulation by a dark-adapted observer. This technique was used at all sites where ctenophores were collected. For quantitative tests of mechanical stimulation, five specimens of *P. pileus* collected in the Gulf of Maine (northwest Atlantic Ocean) were transferred to filtered seawater, allowed to dark-adapt, and stimulated by stirring in a photon-counting chamber for at least five seconds. This test was repeated three times with five or more *P. bachei* collected in the Santa Barbara Channel (eastern temperate Pacific Ocean). For comparison, luminous species were placed in the same apparatus and induced to luminesce by stirring or brief prodding. Because some organisms may be resistant to physical stimulation, additional specimens were exposed to KCl, ddH_2O, $CaCl_2$, and H_2O_2, which can bypass normal control processes and act directly on light-producing cells or chemicals (Herring, 1981).

Photoprotein extraction

Calcium-activated photoproteins have been identified as the light-producing agents in all luminous ctenophores examined (Ward and Seliger, 1974; unpub. results). To test for the presence of active photoproteins in *Pleurobrachia*, dark-adapted specimens were extracted in a Ca^{2+}-chelating buffer as follows.

In the Santa Barbara Channel, five specimens of *Pleurobrachia bachei* were collected at depths between 5 and 20 m on a blue-water dive. Several small ctenophores from three other families (one *Haeckelia beehleri*, one *Beroë cucumis*, and three *Velamen parallelum*) were collected at the same time and used as positive controls. Specimens were sorted into filtered seawater and maintained in the dark for 7 h (until 2100) to allow recovery from potential photodegradation of their luminescence ability (Ward and Seliger, 1976; Anctil and Shimomura, 1984) and to account for the possibility of a diel cycle of luminescence, which is present in some luminous organisms, but has never been reported for ctenophores. These specimens were homogenized in 200 m*M* Tris, 40 m*M* EDTA, pH 8.8, and a 400 μl subsample was assayed by adding 100 μl of 360 m*M* $CaCl_2$.

This experiment was repeated three times using up to 50 *P. bachei* in the extraction, once with *P. bachei* frozen directly in liquid nitrogen, and once using *P. pileus* collected on dives in the Gulf of Maine, with various local luminous species used as positive controls.

Regeneration

To test the hypothesis that *Pleurobrachia* contains an inactive photoprotein but lacks the luciferin necessary to

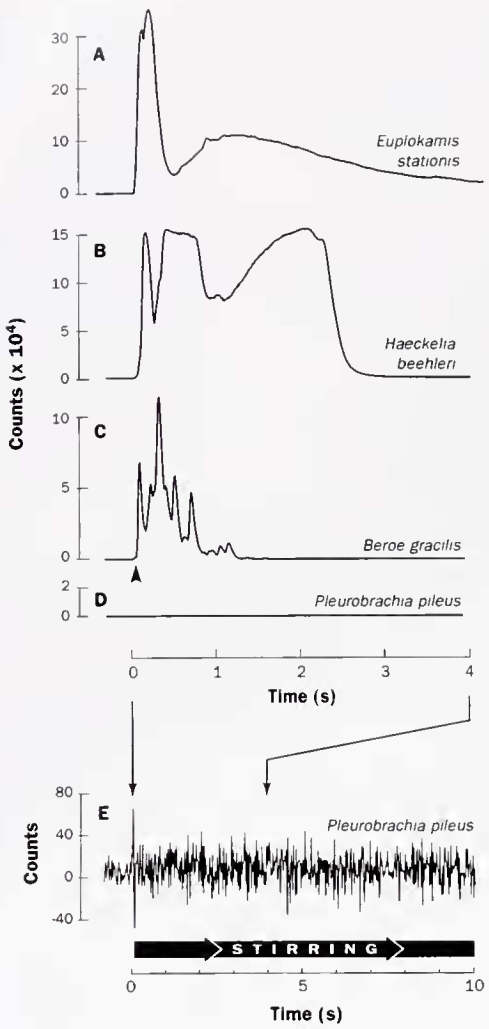

Figure 2. Responses of ctenophores to physical stimulation. Luminous ctenophores produced bright flashes when disturbed (A–C), even if only briefly touched (arrowhead). In contrast, *Pleurobrachia pileus* showed no light emission even during continuous stirring (D, E). The *y*-axis shows counts per 20-ms bin.

produce light, we attempted to regenerate extracts with synthesized coelenterazine (provided by O. Shimomura), the luciferin found in luminous ctenophores and cnidarians (Ward and Cormier, 1975; Shimomura, 1985).

Specimens were homogenized in 100 m*M* Tris, 50 m*M* EDTA, 500 m*M* NaCl, pH 7.5, filtered through a Whatman GF/C glass-fiber filter to remove debris, and centrifuged for 30 min at 35,000 × *g*. Photoprotein present in one ml of supernatant was triggered by the addition of 50 m*M* $CaCl_2$ until no further light was produced (typically 250 μl was sufficient, although no light was emitted by *Pleurobrachia* preparations). This was followed by 250 μl of 200 m*M* EDTA to chelate the added Ca^{2+}, and the solution was saturated with ammonium sulfate to precipitate the reacted protein. For the regeneration, one ml of the saturated solution was centrifuged at 15,000 RPM in an Eppendorf minicentrifuge for 15 min. The pellet of precipitate was resuspended in 200 μl of 10 m*M* Tris, 5 m*M* EDTA, 500 m*M* NaCl, and 5 m*M* β-mercaptoethanol (techniques based on Campbell and Herring, 1990). Each treatment was incubated for 6 h at 4°C with 2 μl methanol either containing coelenterazine or with no luciferin for the negative controls. The light produced upon final addition of $CaCl_2$ indicated the extent of regeneration.

This experiment was conducted using the hydromedusa *Haliscera conica* as a positive control. We replicated this experiment once using *Haliscera*, the hydroid *Obelia* sp., and an undescribed luminous ctenophore; and again using the ctenophores *Beroë cucumis*, *Velamen parallelum*, and *Haeckelia beehleri* with 0.1% gelatin present in the regeneration solution to increase the stability of regenerated photoproteins (Campbell and Herring, 1990).

Results

At no time during these experiments did we detect any bioluminescence produced by *Pleurobrachia* or by the closely related genus *Hormiphora*. Every one of more than forty other ctenophore species tested produced luminescence that was easily detected using our methods.

Physical stimulus

Repeated attempts at mechanical stimulation failed to elicit luminescence from *Pleurobrachia pileus* (Figs. 2D, E). The five specimens run during these trials were negative, as were ten *Pleurobrachia bachei* collected from the Pacific Ocean and run in an identical experiment (not

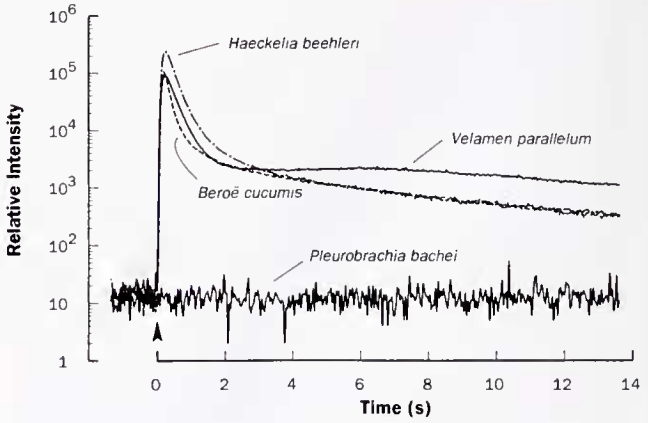

Figure 3. Photoproteins extracted from luminous ctenophores using a calcium-chelating buffer can be triggered to produce light upon the addition of excess calcium. Species from three families shown here illustrate typical flashes produced by extracts of luminous species. In contrast, *Pleurobrachia bachei* and *P. pileus* showed no photoprotein activity in any assays.

shown). Light was not produced by *Pleurobrachia* collected at any of the locations included in this study (Fig. 1). Another member of Pleurobrachiidae, *Hormiphora californensis*, which was collected from the Santa Barbara Channel, also consistently failed to produce light. For comparison, other luminescent ctenophores tested at the same time produced luminescence for the duration of the stirring. Even when given only a single brief stimulus, luminous species produced bright flashes (Fig. 2A–C), with peak intensities of more than 1.75×10^7 counts/s (3.5×10^5 counts in 20 ms).

Chemical extraction

Assays of calcium-free extracts of *Pleurobrachia bachei* from the Santa Barbara Channel (Fig. 3) and *P. pileus* from the Gulf of Maine (not shown) were indistinguishable from the background signal. All extracts of *Pleurobrachia* were inert, while in every case positive control extracts from the ctenophores *Haeckelia beehleri, Beroë cucumis, Velamen parallelum* (Fig. 3), *Bolinopsis infundibulum, Beroë gracilis, Kiyohimea aurita, Bathocyroë fosteri*, and *Bathyctena chuni*, and from the hydrozoans *Haliscera conica* and *Obelia* sp. (not shown), produced light both during extraction and upon the addition of $CaCl_2$, at intensities up to 2.6×10^6 counts/s.

Photoprotein regeneration

Extracts of *Pleurobrachia bachei* incubated with luciferin were not significantly different from those incubated with methanol only, nor were they different from the negative control treatment, which contained only buffer and luciferin (Fig. 4). Regeneration was noted in the positive controls treatments of *Haliscera conica, Haeckelia beehleri*, and *Obelia* sp. However one positive control replicate (*Beroë cucumis*) showed no luminescence activity after the regeneration, and in some replications, the luminous species used as positive controls (undescribed Mertensiid, *Velamen parallelum*) gave inconclusive results, since residual activity remained in luminescent extracts which had been depleted by $CaCl_2$ and then incubated without luciferin.

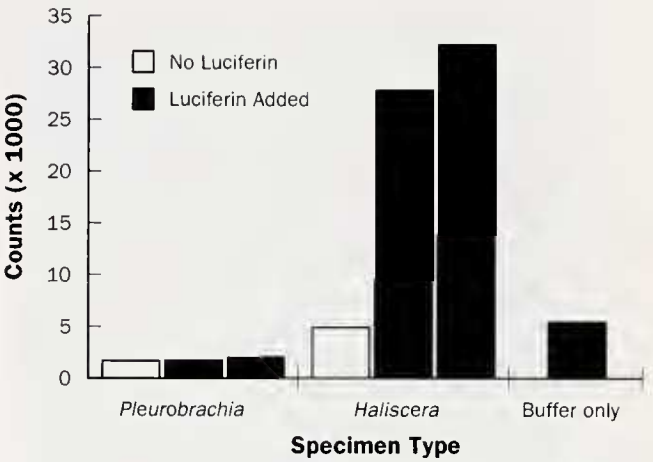

Figure 4. Photoprotein regeneration assays. Even when incubated with an excess of luciferin, extracts of *Pleurobrachia bachei* did not become luminescent, indicating an absence of inactive photoprotein. Extracts from the hydromedusa *Haliscera conica* show the regeneration that typically occurs when an exhausted photoprotein is combined with coelenterazine.

Discussion

Past research

The published record regarding the luminescence of *Pleurobrachia* is sparse, consisting mostly of anecdotal nineteenth-century reports. We have not found any published photographs, spectra, or unequivocal quantitative measurements of bioluminescence from *Pleurobrachia*.

Of the early accounts, the report of Dahlgren (1916) is most explicit in describing bioluminescence in *Pleurobrachia*. Although most of the text concerns *Beroë* and the lobate ctenophore *Mnemiopsis*, there are drawings of *Pleurobrachia* swimming about in "the lighted and unlighted state" (also reproduced in Nicol, 1967). There are also drawings of low-power sections through the gastrovascular canal of a *Pleurobrachia:* one professing to show the "layer of luminous cells covering ovary and testis," and one showing a closer view of the "probable luciferine-secreting cells." Dahlgren supposed that these were luminous cells because of their "highly-vacuolated and glandular nature." Subsequent work on the ultrastructure of the luminous system of *Mnemiopsis leidyi* has shown that these vacuolar cells are not those responsible for light production (Freeman and Reynolds, 1973; Anctil, 1985). Therefore, the cells depicted by Dahlgren are not evidence for light-production in *Pleurobrachia*.

We have found only one quantitative account of *Pleurobrachia* bioluminescence. For this study, Hardy and Kay (1964) placed "a large number of very small *Pleurobrachia*" in unfiltered seawater and left them undisturbed in a light-measuring device to monitor "spontaneous" luminescence. Their records show many brief flashes during several hours of experimentation. To establish that dinoflagellates in the seawater were not producing the flashes, the authors sieved the ctenophores from the container and measured the light again, this time noting no flashes. However, by removing the ctenophores they also removed the stimulation that would have been caused by their actively beating comb plates. The authors themselves noted this effect in a later experiment testing the stimulation of dinoflagellates by mysids. The number and intensity of flashes recorded during the *Pleurobrachia* experiment are more similar to the dinoflagellate

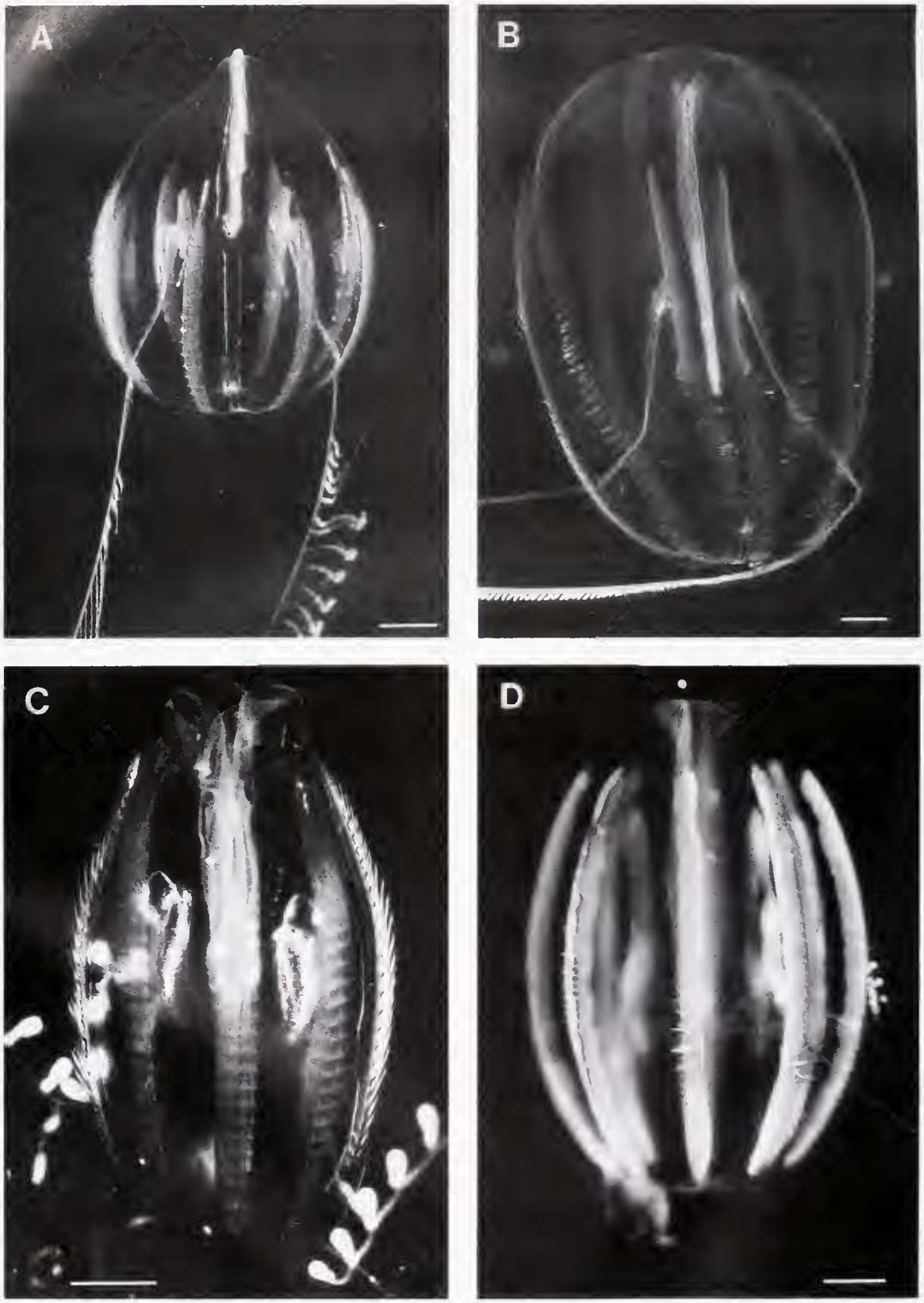

Figure 5. Non-luminous and luminous cydippid ctenophores. Reports of bioluminescence from non-luminous species like *Pleurobrachia bachei* (A) and *Hormiphora californensis* (B) may be attributed to confusion with similar luminous genera. *Euplokamis* (C) and other undescribed species (D) are brightly luminescent and are found at the same locations as *Pleurobrachia* (see Fig. 1). Scale bars: 2 mm.

experiments than to a *Beroë* experiment which produced fewer, but brighter, flashes (Hardy and Kay, 1964: figs 1, 2, 14–16). Because the authors did not see the ctenophore luminesce and did not sufficiently rule out the possibility of dinoflagellate flashes, this account of *Pleurobrachia* luminescence remains unconvincing.

Considering that *Pleurobrachia* is one of the most widely distributed and best-known of the ctenophore genera, it is remarkable that we have been unable to find any substantiated reports of its bioluminescence, especially in the recent literature.

Unpublished research

The unpublished observations indicating that *Pleurobrachia* is non-luminous are as convincing as the void in the published literature. In nearly 30 years of observations on luminous plankton, workers from this laboratory have never encountered a luminescent specimen. Similarly, other researchers who have studied bioluminescence in ctenophores from around the world have been unable to observe luminescence in this genus [P. J. Herring, Y. A. Labas (White Sea), B. H. Robison, E. A. Widder, pers. comm.]. Because these negative results have never seen their way into print, apocryphal accounts persist.

Results of our experiments

Because physical stimuli repeatedly failed to elicit light from *Pleurobrachia,* we attempted to determine whether the luminescent chemicals were present either as a calcium-activated photoprotein, or as a luciferin-deficient apophotoprotein. Extractions in calcium-chelating buffers have clearly demonstrated the presence of photoproteins in all other ctenophore species examined (Ward and Seliger, 1974; Shimomura, 1985; unpub. results). Based on the results of Tris-EDTA extractions, *Pleurobrachia* clearly lacks a conventional photoprotein, and because no luminescence was observed during homogenization, there is no evidence that another mechanism is employed.

At the chemical level, failure to detect an active photoprotein could be due to the lack of an appropriate protein, or to a lack of luciferin. Based on the negative results of regeneration experiments, it appears that there is not an apophotoprotein present that merely lacks luciferin. Extracts of *Pleurobrachia* never became luminous in any of the incubations in which coelenterazine was supplied. However, the results of attempted regenerations were sometimes ambiguous, because extracts from luminescent ctenophores used as positive controls could retain high levels of residual activity even after treatment with $CaCl_2$. In *Mnemiopsis* the regeneration of inactive photoproteins was originally found to occur only at pH 9.0 (Anctil and Shimomura, 1984), but it is now thought that the presence of gelatin in the regeneration buffer eliminates this pH sensitivity (Campbell and Herring, 1990; Campbell, pers. comm.). Nonetheless, it would be useful to repeat these experiments using recombinant apophotoprotein, so that discharging and recovering the positive control samples would not be required.

Although we have done most of our rigorous testing on *Pleurobrachia,* we have also been unable to find any luminescence in mechanical assays of *Hormiphora,* suggesting that this closely allied genus, which is abundant at depths around 100 meters off the coast of southern California, may also be unable to produce light.

Identification

Because historically any small cydippid was likely to be called *Pleurobrachia,* anecdotal accounts of luminescence may be due to confusion with similar lesser known genera (Figs. 1, 5). For example, *Euplokamis* (Fig. 5C) is commonly encountered in the north Pacific, the Gulf of Maine, and the Mediterranean Sea, yet this genus was grouped in the family Pleurobrachiidae until recent work by Mills (1987). The luminous species *Euplokamis dunlapae* (Mills) found off the coast of Washington has been alternately described as "*Pleurobrachia pileus*" (Freeman, 1977), "*P. ?pileus*" (Kozloff, 1974), and other *Pleurobrachia* species. (For a complete list, see Mills, 1987.) Prior to Mills's clarification it would not have been possible to know whether a ctenophore that was seen to luminesce was actually *Pleurobrachia.* Similarly, the widespread occurrence and 'pleurobrachioid' appearance (Fig. 5D) of an undescribed midwater ctenophore (Mills and Harbison, in prep.) may have led to other reports of luminescence attributed to *Pleurobrachia.* In light of recent taxonomic revision and the presence of several luminous genera that are easily confused with *Pleurobrachia,* it is not difficult to imagine how erroneous examples of bioluminescence might have been reported, even by knowledgeable researchers.

Conclusions

Pleurobrachia's inability to produce light raises questions about the role of bioluminescence for planktonic organisms: Is this 'deficiency' the handicap that it might seem, given the widespread occurrence of bioluminescence among marine plankton? If bioluminescence is serving a defensive role, it may not be important against non-visual predators such as the ctenophore *Beroë,* which is known to prey upon *Pleurobrachia.* Also of interest is what is missing in *Pleurobrachia* that makes it unable to produce light. Are the homologous genes present but inactive, or are cells equivalent to photocytes lacking altogether? A comparative study of the genetic relationships of ctenophores might help indicate when the ability to bioluminesce arose in this phylum.

The Pleurobrachiidae may not be the only non-luminescent group of ctenophores, because Platyctenida, a small order of non-planktonic ctenophores, has never been reported to be luminescent. However, we have not been able to assay specimens from this rarely studied order.

Despite examining hundreds of specimens of *Pleurobrachia*, collected under ideal conditions at a variety of locations and seasons, we have never observed light production in the genus, while over 40 other species of ctenophores produced luminescence during similar treatment. Furthermore, we have found no substantiated accounts of luminescence in the literature. Therefore, although we cannot say that no *Pleurobrachia* was *ever* luminous, it is clear that this genus is not generally luminescent. The burden of proof should be shifted to those who wish to show that *Pleurobrachia* is bioluminescent.

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