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Luminescent fish with open-type glands containing luminous bacteria or luminous substances of ingested crustaceans*

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(With 5 text-figures)

発光細菌または甲殻類の発光素を光源とする発光魚

羽 根 田 弥 太**

発光魚の大多数はワニトカゲギス亜目とハダカイワシ亜目に属する深海魚にみられる。これとは別の型の発光魚は消化管と連絡し体内深くに埋設している発光腺が体外または消化管内に開孔している。発光体が発光腺内の発光細菌である場合と摂取した甲殻類の発光体ルシフェリンである場合とにわけられる。発光器の構造は両者共全く同じであるのに発光体だけが全く異っている。従って両者は同じカテゴリーに属すると考えられる。その理由は，両者共に極めて原始的な発光器と中間型のものと高度に発達した発光器とがみられるからである。発光能力のない魚は，消化管内で発育した発光細菌の光または摂取した発光甲殻類の光で消化管内が絶えず刺戟をされてるいる。これらの発光魚は長い進化の過程で消化管の一部に発光器ができて，その光を強めるための装置，反射器や，光の点滅装置ができ，発光魚へと変化をしたと考えることが可能である。

Most of the known luminous fish are found in deep oceanic waters and belong to the suborders Stomiatina and Myctophina. The luminous organs of these fish are distributed over the head and trunk; in some the organs are found on barbels. The organs often vary greatly in size, shape, and arrangement.

In contrast, other luminescent fish are known which lack these external organs and resemble ordinary, non-luminous fish. In these fish, the luminous organs or ducts are located in the body cavity or opening and cannot be seen from the exterior. The light of the organ is reflected internally and then into the ventral abdominal muscles, which serve to diffuse the light over the ventral surface of the fish, producing a bluish glow.

Fish luminescent systems may be classified into four types in which:

1. the photophores are well developed and luminescence is intracellular,
2. a luminous secretion is produced and luminescence is extracellular, e.g., *Himantolophus* (HANEDA, 1968a) and *Searsia* (HERRING, 1972),

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3. the luminous organ contains symbiotic luminous bacteria and is open to the outside,
4. the luminous organ contains luminous substances of ingested crustaceans and is open to the outside.

The report deals largely with the fourth type of luminescent system. The third type is found in a wide range of unrelated, deep and shallow water, fish, as shown in Figure 1. The luminous bacteria of this type of organ system are easily culturable on artificial media, except for bacteria from the luminous organ of fish belonging to the family Anomalopidae and a species belonging to the family *Leiognathus*, viz., *Leiognathus elongatus* (HANEDA and TSUJI, 1976). Although *Anomalops* and *Photoblepharon* (HANEDA and TSUJI, 1977) are quite rare, their existence has been known for a long time from the Banda Island, Indonesia.

In 1969, during the Alpha Helix Expedition to New Guinea, I went to Banda Island and made numerous attempts to culture luminous bacteria from the luminous organs of these fish, but without success. The emulsions of the light organs prepared in sea water behaved as an emulsion of luminous bacteria in sea water, but when the emulsion was prepared in distilled water, the light was immediately extinguished. Air-dried organs were non-luminous when moistened with water. Hot (luciferin) and cold (luciferase) water extracts of the organs were also prepared and tested. When mixed, no light was observed, indicating the absence of the luciferin-luciferase reaction. Electron micrographs of sections of the light organs are shown in Figure 3. Nemerous bacteria are seen to be present. These results suggest that the light produced is due to symbiotic luminous bacteria that are non-culturable. These findings agree with the results of HARVEY (1952), who earlier studied the luminescence systems of these fish. (Figs. 2, 3)

There has been much speculation regarding the origin of symbiotic luminous bacteria in the luminous organ of fish. According to BUCHNER and PIERANTONI, who proposed the so-called intracellular luminous symbiosis theory, luminous bacteria always occur within the cell and are transmitted to the offspring through the egg, thus causing the next generation to be infected. However, it is now believed that infection of the luminous organ takes place during the early larval stage and the bacteria enter from the outside through external openings. However, this mode of infection has never been experimentally demonstrated except in the case of *Siphamia versicolor* (family Apogonidae).

In the majority of apogonid fish, including *Siphamia versicolor,* the adult males keep the eggs and newly hatched larvae in their mouth. During the breeding season at Okinawa Island, Japan, in May, I obtained a large number of living eggs and newly hatched larvae and observed them in the dark. Even a faint light could not be detected from the mass of eggs and larvae. If symbiotic luminous bacteria are transmitted to the offspring via the egg, the mass of eggs and larvae should have been luminescent. The absence of any light is a clear indi-

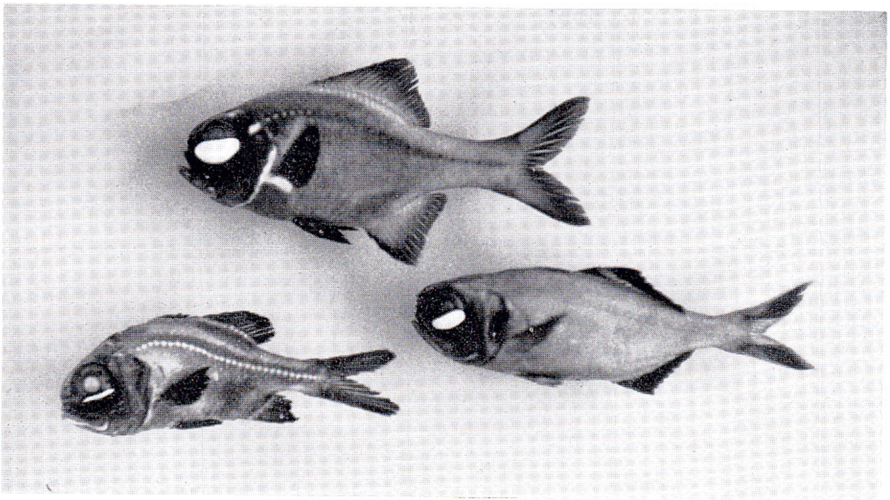

Fig. 2. Three species of luminous fish belonging to the family Anomalopidae. From above *Photoblepharon palpebratus*, *Anomalops katoptron* and *Kryptophanaron alfredi*. (×1/2).

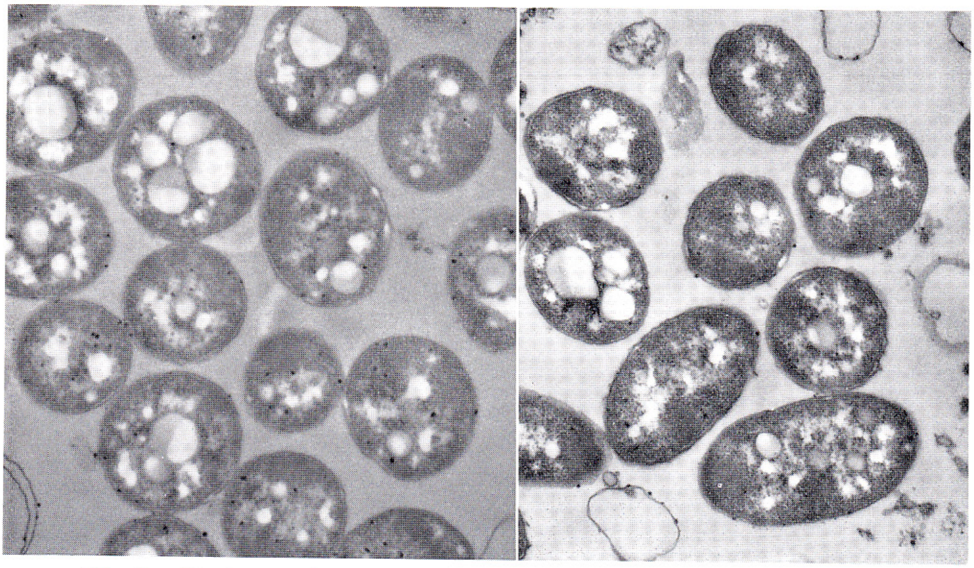

Fig. 3. Electron micrographs of sections of the luminous organs of *Anomalops* (left) and *Photoblepharon* (right). Both are 17,000 times.

cation that bacterial infection is a secondary process and not due to transmission via the egg.

Other widely different and unrelated luminescent fish with an open-type of gland containing symbiotic luminous bacteria are *Monocentris* (YASAKI, 1928), *Cleidopus* (HANEDA, 1966), *Acropoma* (HANEDA, 1950), *Paratrachichthys* (HANEDA, 1957), *Physiculus* (KISHITANI, 1930), *Macrouridae* (HANADA, 1951), *Steindach-*

- **CHONDRICHTHYES**
  - Squalidae
    - *Etmopterus lucifer*, *E. pusillus*
    - *Squaliolus laticaudus*
    - *Centroscyllium ritteri*
    - *Spinax niger*
    - *Isistius brasiliensis*
- **OSTEICHTHYES**
  - Clupeiformes
    - Alepocephalidae — *Searsia*
    - Argentinidae
      - *Opisthoproctus grimaldi**
      - *Winteria telescopa**
    - Gonostomidae — *Gonostoma*
    - Sternoptychidae — *Polyipnus*, *Sternoptyx*, *Argyropelecus*
    - Chauliodontidae — *Chauliodus*
    - Stomiatidae — *Stomias*
    - Astronesthidae — *Astronesthes*
    - Idiacanthidae — *Idiacanthus*
    - Melanostomiatidae — *Melanostomias*
  - Myctophiformes
    - Chlorophthalmidae — *Chlorophthalmus albatrossis**
    - Myctophidae
      - Neoscopelinae — *Neoscopelus*
      - Myctophinae
        - *Myctophum*
        - *Diaphus*
        - *Lampanyctus*
    - Paralepididae — *Lestidium*
  - Beryciformes
    - Trachichthyidae — *Paratrachichthys prosthemius**
    - Anomalopidae
      - *Photobrepharon palpebratus**
      - *Anomalops katoptron**
      - *Kryptophanaron alfredi**
    - Monocentridae
      - *Monocentris japonicus**, *M. reedi**
      - *Cleidopus gloriamaris**
  - Gadiformes
    - Moridae — *Physiculus japonicus**
    - Macrouridae
      - *Steindachneria argentea**
      - *Nezumia**
      - *Coryphaenoides**
      - *Coelorhynchus**
      - *Hymenocepharus**
      - *Malacocephalus**
      - *Abyssicola**
  - Perciformes
    - Leiognathidae
      - *Gazza minuta**
      - *Leiognathus rivulatus**, *L. elongatus**
      - *Secutor insidiator**, *S. ruconius**
    - Pempheridae
      - *Parapriacanthus ransonneti***
      - *Pempheris klunzingeri***
    - Apogonidae
      - *Epigonus macrops***
      - *Apogon ellioti***, *A. striata***
      - *Archamia lineolata***, *A. fucata***
      - *Rhabdamia cypselura***
      - *Siphamia versicolor**
    - Acropomidae — *Acropoma japonicus**, *A. hanedai**
  - Batrachoidiformes
    - Batrachoididae — *Porichthys notatus*, *P. myriaster*
  - Lophiiformes
    - Linophrynidae
    - Oneirodidae — *Oneirodes acanthias**
    - Himantolophidae — *Himantolophus groenlandicus*

Fig. 1. Taxonomic classification of luminous fishes.
* Fish with open glands containing luminous bacteria. ** Fish with open glands containing ingested crustacean luminous substances.

neria (macrourid fish) (HANEDA, 1968b), *Opisthoproctus solealus* (BERTELSEN and MUNK, 1964), *Linophryne* (PIETSCH, 1974), *Oneirodes* (O'DAY, 1974), and *Chlorophthalmus* (SOMIYA, 1977).

The fourth type of luminescent organ system is found in *Parapriacanthus ransonneti* (family Pempheridae) (HANEDA and JOHNSON, 1958) and *Apogon ellioti* (family Apogonidae) (HANEDA *et al.*, 1966). It is interesting to note that in the family Apogonidae, all species of the genus *Siphamia* possess the third type of luminescent system, whereas several species of the genera *Apogon*, *Archamia*, *Rhabdamia* (HANEDA *et al.*, 1969), and *Epigonus* (MAYER, 1974) possess the fourth type of organ system. I will now consider the latter in detail.

The family Apogonidae is represented by a large number of small, shallow water species widely distributed in the Pacific, Indian, and Australian waters. In all species of the genus *Siphamia* (Fig. 4), luminescence appears to be due to symbiotic luminous bacteria. The luminescent system consists of a luminous

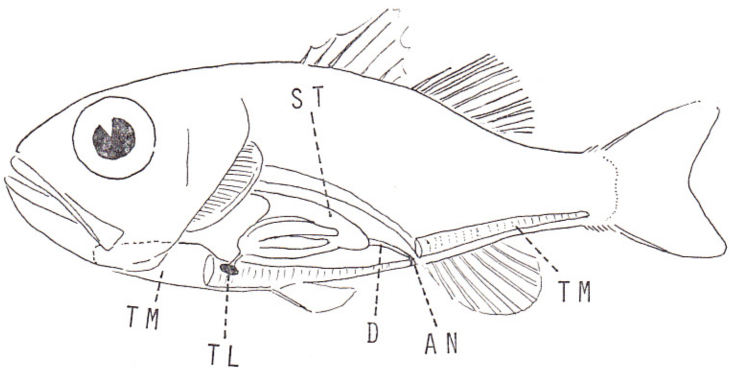

Fig. 4. Diagram of the photogenic organ system of *Siphamia versicolor*. TL, thoracic luminous duct; ST, stomach; D, duodenum; AN, anus; TM, translucent muscle bundle.

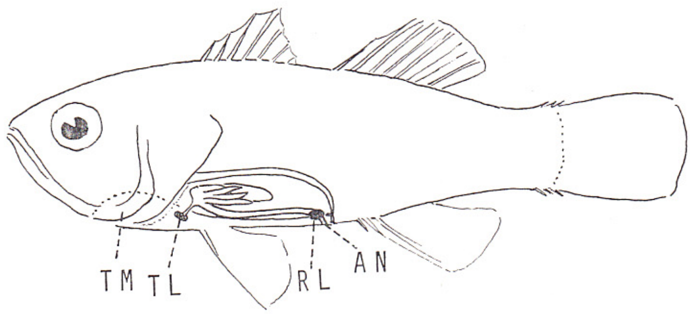

Fig. 5. Diagram of the photogenic organ system of *Apogon ellioti*. TL, thoracic luminous duct; RL, rectum luminous duct; TM, translucent muscle; RL, rectum luminous duct; AN, anus.

gland, a tube connecting the gland to the intestine, and an accessory structure made up of translucent abdominal muscle bundles. The luminous bacteria are easily culturable on artificial media. The luminescent system of *Apogon ellioti* (Fig. 5) consists of an oval-shaped, lemon-yellow organ which is connected to the second loop of the intestine by a duct. This organ lies in the translucent thoracic keel muscle through which the light passes to the outside. Posteriorly, two small organs, one on each side of the rectum, are found connected to the rectum by ducts. Each organ contains luciferin and luciferase and emits blue light continuously. When dark cold and hot water extracts of the organ are mixed, blue light is produced. The luciferin and luciferase also give reciprocal light-emitting cross-reactions with the luciferin and luciferase of *Parapriacanthus ransonneti* and with the luciferin and luciferase of the ostracod crustacean, *Cypridina hilgendorfii*. Thus, cross-reactions occur among the luciferins and luciferases of all three systems. The chemical properties of *Cypridina* luciferin are closely related to or identical with those of *Apogon* luciferin and *Parapriacanthus* luciferin.

The luminescent system of fishes belonging to the family Apogonidae may be divided roughly into four types:

1. *Siphamia* type,
2. *Apogon ellioti* type (including *A. striata*),
3. *Archamia lineolata* type (including *A. fucata, A. zosterophora,* and *A. poecilopterus*),
4. *Rhabdamia cypselura* and *Epigonus* types.

The luminous organ, containing the luciferin and luciferase, of *Archamia* is made up of the second loop of the intestine and the pyloric caeca. The light emitted by the second loop of the intestine is the brightest and is directed into the translucent abdominal muscles which act as a lens and diffuse the light. This type of organ system seems to be the most primitive in terms of evolutionary development. No luminous organ is present at the anus. The morphology and arrangement of the luminous organs appear to be similar in *Archamia fucata, A. zosterophora,* and *A. poecilopterus*. The luminous organ of *Rhabdamia cypselura* is substantially different in that the distal ends of the first pair of pyloric caeca are transformed into luminous bodies or glands. A pair of transparent lens-like organs, encircled by black pigment, are situated in the ventral-lateral wall of the body cavity. The luminous bodies are attached to the lens-like organs and the light is transmitted to the outside through them.

These two distinct types of organ systems each differ markedly from the system found in *Apogon ellioti* and, to an even greater extent, from that of *Siphamia,* which involves symbiotic luminous bacteria. According to MAYER (1974), the luminescent system of *Epigonus macrops* (family Apogonidae), with its luminous pyloric caeca and luminous window, is similar to that of *Rhabdamia*.

Accordingly, *Rhabdamia cypselura* and *Epigonus macrops* possess the most highly developed type of organ system among fish which have evolved from non-luminous to luminous species.

The crude luciferin and luciferase of the luminous apogonid fish were prepared in the following manner. Soon after collecting the specimens, the luminous organs were removed by dissection, air dried, and stored over $CaCl_2$; the glands were then dried further under vacuum. Luciferase was prepared by grinding the glands in distilled water, centrifuging, and using the supernatant directly. Luciferin was prepared by homogenizing the light organs in boiling distilled water for one minute, cooling rapidly in an ice-bath, centrifuging, and using the supernatant immediately. When the luminous glands were first ground in distilled water, the extract exhibited a blue luminescence that was readily visible in the dark. The luminescence lasted for several minutes and dark extracts again became luminous when fresh luciferin (hot water extract) was added. The luciferin-luciferase test was carried out using these crude preparations or luciferin and luciferase.

The crude luciferin and luciferase extracts of *Apogon, Archamia,* and *Rhabdamia* produced light immediately when the extracts were mixed. The luminescent cross-reactions observed among the luciferins and luciferases of *Apogon, Archamia, Rhabdamia, Parapriacanthus,* and the ostracod crustaceans, *Cypridina hilgendorfii* and *Cypridina dentata* offer further support for the theory that luciferin and luciferase originate from the same source.

The third and fourth types of luminescent systems may belong to a single category since non-luminous species may have evolved into luminous species as a result of long pressures of the digestive organs to light of luminous bacteria or luminous substances released by the digestion of luminescent crustaceans.

In conclusion, I might say that many more different kinds of live fish should be examined in the dark. If this is done, the discovery of additional luminous fish may be anticipated.

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