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Observations on a Luminous Apogonid Fish, *Siphamia versicolor*, and on Others of the Same Genus[1]

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(with 4 Plates)

ヒカリイシモチ *Siphamia versicolor* とその近縁種の発光について

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Introduction

The first discovery of a luminous apogonid fish was made by KATO (1947). He found

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that *Apogon marginatus* possesses three organs at the bend of the intestine and on either side of the rectum immediately before the anus. IWAI and ASANO (1958) found similar luminous organs in *Apogon ellioti*. *Apogon marginatus* is identical with and predated by *A. ellioti*. HANEDA, JOHNSON and SIE (1958) studied the luminous organs of this fish and demonstrated a second example of the luciferin-luciferase reaction among fishes; these extracts were found to produce a light-emitting cross-reaction with partially purified luciferase of the ostracod crustacean, *Cypridina hilgendorfii*.

Apogon ellioti was the only species of apogonid fish known to possess luminous organs until the luminous organ of *Siphamia* was discovered.

Siphamia, a genus of the family Apogonidae, is characterized by having a silvery area extending from the isthmus to the lower corner of the caudal peduncle along the ventral contour of the body (WEBER and de BAUFORT 1929, SHULTZ 1940, LACHNER 1953). The general outer appearance of this fish resembles ordinary, non-luminous fishes; however, the silvery area of this fish resembles that of *Acropoma*, which possesses a luminous duct inside the area.

IWAI (1958) discovered that *Siphamia versicolor* has a luminous organ on the inner surface of the body wall immediately above the pelvic girdle. According to him a curious organ within the body wall immediately before the base of the ventral fin is probably luminescent.

It consists of a compact mass of polyhedral cells with granular cytoplasm, and lacks openings to the outside of the body or to the alimentary canal. He also reported that silvery gland lying along the ventral contour of the body is composed of longitudinal muscle bundles sheathed with a fine stratum of fibrous connective tissue. This may act as an important element for diffusing the light emitted from the luminous organ. Again in 1959, he reported in a new species of this genus, *Siphamia majimai*, a peculiar organ, possibly luminescent, which lies in the abdominal cavity immediately below the liver. This organ opens into the intestine by a slender duct.

However only preserved specimens of the above mentioned two species of *Siphamia* were studied, so that their luminescence in a living condition could not observe.

Thus it was still uncertain whether these fish were luminous or not.

During my collecting expedition around the world from April 1959 to May 1960, I had a chance to visit the U. S. National Museum in Washington, D. C., and had the privilege of examining all the specimens of the genus *Siphamia* of the Museum collection.

In April 1960 I collected a single fresh specimen of *Siphamia versicolor* from the miscellaneous fishes in the Market of Cebu, Philippines. I observed a weak light being emitted from the organ, but obtained negative results in an attempt to cultivate luminous bacteria from the organ.

Due to the shortage of material, the results were inconclusive.

I was introduced to Mr. T. MATSUOKA of the Hamanako Branch Institute of the Shizuoka Prefectural Fisheries Institution, through the courtesy of Dr. Y. TOMINAGA of the Misaki Marine Biological Station. Mr. MATSUOKA found that *Siphamia versicolor* lives symbiotically among the spines of *Diadema setosum*, a long spined sea urchin in the

waters of Okinawa. He gave me very useful suggestions for collecting live *Siphamia*. In April, and September 1964 I had a chance to visit Okinawa Island, and there obtained enough specimens of *Siphamia versicolor* to study. I also observed the light emission of live specimens in the dark.

Material and Methods

The specimens of *Siphamia versicolor* were obtained among the *Diadema setosum*, long spined sea urchins, at the sandy bottom of the subtidal zone of coral reefs, about 4~5 meters deep, off Itoman and Minatogawa Beach, Okinawa Island. Usually these fish live symbiotically among the long spines of the sea urchins. If the sea urchin were moved with a hook, the *Siphamia* also moved together, and, so they were collected together with the sea urchin in a hand net.

The materials were brought to laboratory and kept alive in a small aerated aquarium 2 or 3 days in order to observe their coloration and light emission at night. Some specimens were fixed in 10% sea water formalin and dissected.

The lower portion of the body was sectioned by the celloidin method and stained with Haemotoxylin eosin and Azocarmin G, Anilin blue orange G. The material were also stained with acridin orange for observation by ultra-violet rays under the fluorescent microscope.

Three other species of the genus *Siphamia* were studied: *Siphamia ovalis*, *S. fuscolineata*, and *S. elongata*, all of which were presented as exchange specimens to the Yokosuka City Museum for the study from the division of fishes, Smithsonian Institution on September 25, 1959 through the courtesy of Dr. L. P. Schults of the said Institution. The specimens number are as follow: *S. elongata*, U. S. N. M. No. 112099, March 11, 1909, Canmahala Bay, Luzon, Philippines, by Albatross; *S. versicolor*, U. S. N. M. No. 112269, April 18, 1908, Philippines, by Albatross; and *S. fuscolineata*, U. S. N. M. No. 142281, April 25, 1946, Marshalls, Bikini Atoll, by Morrison and Ladd.

Results

1. Color when alive and in formalin.

Siphamia versicolor is a small apogonid fish attaining a standard length of 30 mm. Due to the expansion or contraction of melanophores scattered all over the body, at least 3 color patterns were recognized. While the fish is among the spines of sea urchins, its body is uniformly dark or blackish brown, the same color as the sea urchin. While the fish is swimming over a white sandy bottom away from the sea urchin, the color becomes dusky silver with three longitudinal brown stripes. After death the fish usually becomes a light-colored silver with black and pink dots; sometimes the head remains black. It is possible that this color change serves as a mechanism for screaning the light emitted from the luminous body.

When the fish were put alive into formalin solution, there appeared at least 3 color patterns: A uniform blackish brown, an intermediate color pattern with longitudinal

black stripes, and a whitish light colored silver.

2. Luminous organ.

The luminous organ of this fish consists of the following components: A luminous body a tube connecting it to the intestine and an accessory structure of translucent muscle bundles.

The luminous body lies just below the antero-ventral surface of the liver at the level of the base of the ventral fin.

It is a small spherical body with two distinct elements, outer and inner. This spherical organ measures 2.0 mm. in length, 1.5 mm. in width and 0.3 mm. in thickness in a specimen measuring 26.0 mm. in length. Dorsally, the main structure is completely covered by an opaque stratum. The dorsal surface is exposed to the abdominal cavity. This structure is the same as the reflector of the luminous body of *Apogon ellioti*. The inner element is a luminous duct with a well-developed plexus of blood capillaries. In the duct a great number of luminous bacteria live symbiotically, as in *Paratrachychthys*, *Leiognathus*, *Acropoma*, *Macrouridae* and *Monocentris*.

IWAI (1958) mentioned that the curious organ consisted of three elements, and that perhaps the most functional was a compact mass of polyhedral cells lying in the middle layer of the organ. The transparent muscle bundles that he considered to be a third element are in reality not part of the luminous body itself, but merely a bunch of transparent muscle fibers passing between the luminous organ and the translucent muscle bundles.

If the lower portion of the fish is cut away and the lower part of the intestine is slowly pulled out, the spherical luminous body can be seen at the end of a slender tube connected to the intestine. On further pulling, the spherical body separates from the ventral muscle. This spherical body is made up of the two components, luminous body and the reflector above. The slender tube connects the luminous body with the intestine. From the dorsal region of the reflector of the luminous body, the conducting tube begins and ends at the intestine. IWAI (1958) could not find this tube in *S. versicolor*, but he mentioned it in *S. majimai* (1959). He mentioned that eight canaliculi were observed in cross section, and all canaliculi extend backward within the reflector to the posterior end of the luminous body.

Along the ventral border of the third stripe a silvery band lies between the isthmus and the caudal peduncle. On the ventral aspect the band originates on either side of the brownish midline keel of the isthmus, and extends on each side posteriorly to the base of the ventral fin. The outer appearance of these silvery regions is similar to that of *Paratrachychthys prosthemius* or *Apogon japonicum* or *A. hanedai*.

Histological examination revealed that this region consists of longitudinal muscle bundles covered with a thin black pigment sheath. If transverse section of fresh material is observed these muscle bundles appear to be of translucent opaque tissue quite similar to that of *Acropoma*, *Paratrachychthys*, *Leiognathus* and *Parapriacanthus*. These peculiar muscles diffuse the light emitted from the luminous body. Accordingly it is certain that the muscle bundles also serve as an accessory structure to the luminous

body.

A great number of branched chromatophores are scattered throughout the outer surface of the fish, especially on the surface of muscle bundles. It is possible that these chromatophores serve as a mechanism for screening the light emitted from the luminous body.

3. Observations on the luminescence in the dark.

Since the luminous body is not visible on the surface of the body, this fish, externally and in daylight, has the appearance of an ordinary non-luminous fish, except for the distinguishing muscle bundles of the thoracic area.

Several fishes were put into aquarium of sea water in a dark room and their luminescence was studied. No luminescence could be seen when the fish were viewed laterally. A diffuse weak bluish-white luminescence from the keel muscle and the silvery muscle bundle could be seen. If the fish received a strong stimulus or was removed from the water the keel muscle and the lower part of the thoracic region lighted up. The intensity of luminescence increases owing to the contraction or expansion of the chromatophoreres, scattered in the skin of the ventral area.

4. Comparative observation of the emulsion of luminous body of *S. versicolor* and *Apogon, ellioti.*

For the purpose of comparing the contents of the luminous body of *Siphamia versicolor* and *Apogon ellioti*, emulsions of the contents of the luminous body were made in sea water and in distilled water and the luminescence of these preparations was observed in the dark.

A. Effect of salt.

If the emulsion was made in sea water, the whole of the emulsion of *S. versicolor* would be luminescent, but if it was allowed to stand, only the upper layer exposed to the atmosphere would glow while the deeper lower layer became non-luminous. When shaken up, luminescence was again uniformly distributed in the tube. In *Apogen ellioti* the whole of the emulsion was uniformly luminous much longer.

If it was centrifuged, the luminosity was concentrated in the sediment at the bottom of the tube; the fluid above was clear and not luminous. If the precipitate was well mixed with sea water, the whole mixture became uniformly luminous, but if it was mixed with distilled water, it failed to show any luminosity.

If the emulsion of the luminous substance of *Apogon ellioti* was centrifuged, the whole emulsion continued uniformly luminous, i. e., both the sediment and the fluid above.

B. Effect of temperature.

Luminescence of the emulsion of *S. versicolor* is greatest at a temperature of 20~26°C. When the temperature is raised luminescence decreases and finally disappears at 45°C. If the emulsion was heated above this point, luminescence ceased completely and could not be reactivated when cooled again to 20°C. If the emulsion was cooled below 5°C, luminescence ceased completely but could be reactivated when heated again to above 5°C.

The luminescence of the emulsion of *Apogon ellioti*, did not show such distinct differences at different temperatures.

C. Effect of water.

Dried luminous body of *S. versicolor* did not recover luminescence if water was poured on it in the dark, but the dried luminous body of *Apogon ellioti* emited light when wet.

D. Microscopical and bacteriological observation.

Microscopically the emulsion of the luminous body of *S. versicolor* is made up of disintegrated particles of gland cells with innumerable bacteria. Isolation experiments were carried out by the usual method of culture, and obtained the same kind of luminous bacteria from the different specimens of *S. versicolor*. On the contrary, the emulsion of *A. ellioti* is made up of particles of the luminous substance itself, and culture experiments showed negative results.

E. Luciferin-Luciferase reaction.

In *Apogon ellioti*, a light emitting "Luciferin-luciferase" reaction resulted on mixing two aqueous extracts, one prepared by boiling for 1–2 minutes, followed by rapid cooling with ice (luciferin or substrate solution), and the other by grinding the minced ducts in cold water in a mortar (Luciferase, or enzyme solution). On the other hand no reaction was noted on mixing the two extracts from *S. versicolor*. Concluding from the results of the above mentioned experiments, the luminous source of *S. versicolor* is luminous bacteria, which live symbiotically in the duct of the luminous body. The luminous body was not contain any luminous substance. On the other hand, the luminous source of *Apogon ellioti* is a luminous chemical substance, and there are no luminous bacteria.

5. Comparative anatomy of the luminous organ of the different species of Genus *Siphamia*.

The comparative anatomy of the luminous organ of the different species of genus *Siphamia*, *S. ovalis*, *S. fuscolineata*, and *S. elongata* was studied. No remarkable difference was noted. Recently TOMINAGA (1964) stated that *S. cuprea*, *S. ovalis*, *S. fuscolineata* and *S. argentea* are synonyms of *S. versicolor*, and that *S. elongata* is a slender variety of *S. versicolor*.

6. Origin of the symbiotic luminous bacteria in the luminous duct of *Siphamia*.

BUCHNER (1921, 26) and PIERANTONI (1914, 18) published their so-called intracellular luminous symbiosis theory, describing the special relationship existing between luminous bacteria and animals. They described the luminous bacteria as always occurring within the cell and transmitted to offspring by means of the egg, in this manner infecting the second generation. However, it has been concluded that this is not the case; rather it is believed that the luminous bacteria are present externally, and pass through external openings into the light organs to settle there during the larval stage of the fish. In other words, the bacteria infection is secondary and not transmitted by means of the egg. However, there have been no experimental demonstrations of how the symbiotic luminous bacteria enter the luminous organs of fish.

In the majority of apogonid fishes, the adult males protect the eggs in their mouth.

Siphamia versicolor also keep their eggs and newly hatched larvae in their mouth.

Fortunately, when I visited Okinawa Island in April, the breeding season of *Siphamia versicolor* had started. I obtained a large amount of eggs and newly born larvae, and was able to observe them in the dark. Even a faint light could not be seen from the mass of living eggs and larvae.

If the symbiotic luminous bacteria are transmitted to the offspring by means of the egg, as BUCHER or PIERANTONI postulated, the mass of living eggs or new-born larvae must be luminous in the dark.

The fact that neither the mass of eggs or larvae emitted no light is a clear demonstration that the bacterial infection is secondary and not transmitted by means of the egg.

Discussion

The luminous body of *Siphamia versicolor* is a small spherical body which is composed of two distinct elements, outer and inner. As in the report by IWAI, the outer element consists of an opaque stratum of fibrous connective tissue. It is virtually the same as the reflector of the thoracic luminous body of *Apogon ellioti*. From the dorsal region of the outer element of the luminous body, a slender connecting tube runs to the intestine. IWAI's "compact mass of polyhedral cells lying in the middle layer" is the inner element. It is a duct, in which a great number of symbiotic luminous bacteria live, similar to *Paratrachychthys*, *Acropoma*, *Leiognathus*, *Macrouridae*, and *Monocentris*.

The longitudinal muscle bundles located on the ventral side of the inner element, which IWAI described as a third element, are not a portion of the luminous body, but instead are bundles of transparent muscles running between the luminous body and the accessory translucent muscle bundles. The cross section of these muscle bundles is elliptical in shape. These transparent muscle bundles are a lens to strengthen the light of the luminous body, which light is then diffused through the translucent muscle bundles.

The genus *Siphamia* is well characterized among the family Apogonidae by the presence of longitudinal muscle bundles along each side of the anal base. The function of these translucent muscle bundles is to diffuse the light emitted from the luminous body, as in *Acropoma*, *Paratrachychthys*, and *Leioguatus*.

IWAI thought that the luminescence of this fish was intracellular, but the actual luminous source is symbiotic luminous bacteria which live in the duct. BUCHNER and PIERANTONI supposed that the luminous bacteria entered the luminous organ by infection of the egg. Instead I propose that the bacteria enter by secondary infection, that is, through the opening of the duct during the larval stage.

Although no practical demonstration was hitherto possible, I observed no luminescence in newly hatched larvae of *Siphamia versicolor* and therefore concluded that infection was secondary and not via the egg.

Although *Siphamia versicolor* is closely related to *Apogon ellioti* of the same family

Apogonidae, and although the structure of the luminous organ is very similar, the light source is entirely different. The light source of *Apogon ellioti* is a chemical substance with a positive luciferin luciferase reaction, while that of *S. versicolor* is symbiotic luminous bacteria. This contrast to be extremely interesting biologically.

Summary

1. *Siphamia versicolor* is a small apogonid fish which lives symbiolically among the long spines of *Diadema setosum*, a long-spined sea urchin. Thus was observed in living conditions both in daylight and in the dark at Okinawa Island.
2. The luminous organ of this fish consists of a luminous body with a connecting tube to the intestine and an accessory structure of translucent muscle bundles. The luminous body is inbeded in the thoracic muscle and is a small spherical body composed of two distinct elements, outer and inner. From the dorsal region of the outer elements, the connecting tube begins and runs to the intestine.
3. The inner element of the luminous body is a luminous duct, a well developed plexus of blood capillaries. In the duct a great number of luminous bacteria live symbiotically, similar to *Acropoma, Paratrachychthys.*
4. Along the ventral border of the third stripe, a silvery band lies between the isthmus and the caudal peduncle.

   The outer appearance of this silvery region is similar to that of *Paratrachychthys prosthemius* or *Acropoma japonicum.*

   This region is the longitudinal muscle bundles, covered with a thin black pigment sheath. These peculiar muscle bundles diffuse the light emitted from the luminous body and also serve as an accessory structure to the luminous body.
5. In the dark, no luminescence could be seen when the fish was viewed laterally. A diffuse weak bluish luminescence of the keel muscle and the silvery region could be seen. If the fish received a strong stimulus or was removed from the water, the intensity of luminescence increased.
6. The fact that the mass of living eggs or newly hatched larvae of this fish emitted no light, it is a clear demonstration that the bacterial infection in the luminous duct is secondary and not transmitted by means of the egg.

抄　　録

Apogonidae の中，*Apogon* 属で発光器を持つものはツマグロイシモチ *Apogon ellioti* が唯一つのものであるが，*Siphamis* 属では，すべての種類が発光器を持ち，発光器が属の特徴となっている。最初に *Siphamia* 属の発光器について報告したのは岩井保氏 (1958) の行なった *Siphamia versicolor* の組織，解剖学的研究であって，ハリダシエビス *Paratrachichthys prosthemius* やホタルジャコ *Acropoma japonicus* のように，発光体が胸部の筋肉中に埋まっていて，その光は，胸部および腹部の半透明乳白色の筋肉を通して見られることを明かにした。その後，同氏は *Siphamia majimai* (1959) に就ても同様の構造の発光器のあることを明かにし，前回 *S. versicolor* において見落していた発光体 (PHOT) と消化管 (INT) とが細い管で連結していることを報告した。しかしこの 2 つの報告は，固定した材料についての研究であったため，生きた材料が真に光

を放つか否かは確認されていなかった。著者は 1959 年より 60 年にかけての採集旅行の際ワシントンの国立博物館の材料を見る機会を与えられ，*Siphamia* 属の他の種類も，同様な発光器を持つことを知り，帰路 1960 年 4 月，フィリッピンのセブ島にて採集中，雑魚の中から僅か一尾の *Siphamia versicolor* を得て，その発光を確認すると共に，発光器の内容について小実験を行なった (1962) 然し，材料不足のため確実な結論が出ないままであったが，たまたま三崎の東大臨海実験所の富永義昭氏より沖縄で採集された *Siphamia versicolor* のフォルマリン漬標本の分譲を受け，採集者である静岡県水産試験場，浜名湖分場の松岡玳良氏を紹介された。松岡氏は沖縄で，本魚が，ガンガセと共生していることを発見，採集場所，採集方法，採集者の紹介までして下さった。そこで昨年 5 月と 10 月の 2 回にわたり沖縄を訪れ研究に充分な材料と，数日間生かしたまま発光状況などを観察することができた。本魚と同じ科のツマグロイシモチ *Apogon ellioti* の発光体がウミボタルの発光物質とルチフェリン，ルチフェラーゼ，交叉反応のある化学物質であるから，本魚の発光体ももちろん，化学物質であろうとの推定のもとに，この魚を大量に採集しようと計画して，東京教育大学の杉山登教授も著者より一週間おくれて沖縄を訪れた。ところが，意外にも，発光内容の検査の結果，本魚の発光体は発光化学物質を全く含まず，ハリダシエビスやホタルジヤコなどと同じく発光腺内に共生する発光バクテリアであることがわかった。

同じ科の魚で発光器の構造が根本的に同様であるにもかかわらず属が異るだけで，一方はその内容が，発光化学物質であり，一方は発光バクテリアであることは生物学的にみて，きはめて興味あることである。

本魚は体長 40～45 mm. の小形のイシモチで，沖縄本島，港川，糸満の沖，水深 4～5 米の珊瑚礁原に群生しているガンガセの長い棘の間に共生している，ガンガセを移動すると魚も長い棘の間にかくれて移動するので，採集は容易である。魚は小形のエヤーポンプを用ひて，3～4 日間小さい水槽内で生しておくことも容易である。

発光器の組織切片はセロイジン法を用ひ，染色は Haematoxylin eosin，および Azocarmin Anilin blue, Orange G を用ひ，組組内のバクテリア，発光体のエムルジョンの染色には，カルボールフクシンを用ひた。発光体内よりのバクテリアの培養は，3% 食塩加寒天培養基，3% 食塩加ブイヨン，ペプトン水などを用ひた。発光器内容が，化学物質であるか発光バクテリアであるかの判段は特に慎重にして，ツマグロイシモチと比較をして行なった。

また，解剖および，組織の検鏡には螢光顕微鏡による観察もなし，自家螢光の有無，オーラミン，アクリジンなどの螢光色素の染色の結果も比較した。

実 験 結 果

1. 体　　色

本魚は体表に分布する黒色色素斑の伸縮によって急激に体色を変化させ，通常，ガンガセの長い棘の間に入っている時はガンガセの暗褐色の色と全く同様であるが，魚がガンガセから離れて白い珊瑚礁の砂の上に来ると Fig. 2, (3) のように三本の黒褐色のシマができる。魚をこの状態で撮影しようと水から上げている間に黒色，あるいは淡紅色となり，死後はいづれも明るい淡紅色となり，時に頭部だけ暗黒色に残る個体もある。したがって，魚を固定する時の状態によって，著しい体色の差を来し，全く別種の魚のように思はれる。

2. 発 光 状 態

発光状態は，ハリダシエビス，ホタルジャコ，ヒイラギ，キンメモドキ，ツマグロイシモチなどと同様で，体内の発光体の光が乳白色半透明の胸部竜骨筋，腹部の両側を走る筋肉の中を通って外部に出るため，魚を上部から見ると光は見えないが，魚を横または下からみると明かに魚の下半部が，全体光っている。魚を水から上げて，刺激をすると光が強くなり，特に胸部が強くなる。多くは，死後，光が消えて外部から見えなくなるが，胸部を切開して発光体をとり出すと強い青色の光がみられる。この光の明滅は体表に分布する黒色色素斑と，発光体を包む透明なカプセル (Fig. 3, 4 CAP) の中に分布する黒色色素斑 (PG) の伸縮によるものである。

3. 発光器の構造

発光器の構造は，ハリダシエビス，ツマグロイシモチ，ホタルジヤコなどと根本的に同様であって，胸部

または腹部筋肉中に埋もれた発光体と，その光を強め，拡散する半透明，乳白色の筋肉 (Fig. 3, TLM) とからなっている。発光体は発光腺細胞よりなる発光部 (Fig. 3, 4, PHOT) と反射器 (RF) 発光体を包むカプセル (CAP) よりなる，発光腺細胞は毛細管によって反射層を通り，反射層の背面に細い導管 (Fig. 6, CT) となり，この導管は消化管に連結している。生きた魚の胸部竜骨筋を切って胸部，腹部の筋肉を静かに引っぱると腸管につながる細い管の先に発光体が胸部の筋肉と離れてついて来る。この有様はツマグロイシモチの胸部発光器と同様である。急激な光の明滅は，発光体を包む透明なカプセル内の黒色色素班 (PG) の伸縮によるものである。

発光体の染色の結果は Fig. 5. では全体が紅色に染るが，腺細胞 (LD) はオレンヂ G でオレンヂ色に染る。紅色に染る部 (LB) は発光バクテリアの集団である。

螢光顕微鏡による所見では自家螢光を放つ物質も，また，螢光染色による螢光も認められなかった，一方ツマグロイシモチでは，発光体の美しい青緑色の自家螢光が見られた。これは発光体内のルチフエリンの螢光である。

本魚の発光器も消化管内に孔する開孔式発光器である。

4. 発光内容の観察

発光体の内容について，ツマグロイシモチと比較した。

(A) 塩 と 酸 素

発光体を水および海水でエムルジオンとして，発光状態をみるに，本魚では海水の場合は強く液全体が光るが，水の場合は消光した。また海水エムルジオンを静置すると液の表面だけが光って管底は消光する，これをふると再び液全体が光るが再び静置すると発光は空気にふれる液の表面だけとなる。ツマグロイシモチでは海水，真水共によく光つて，液全体が長く光つている。

(B) 温 度

本魚では 20～26°C 位が光は最も強く，45°C 以上，5°C 以下にすると消光する。低温で消光したものを再び温度を上ると発光を恢復するが，高温の場合は 45°C 以上に長く置き，再び温度を下げても光を恢復しない。ツマグロイシモチの場合は，温度に対するこのような反応はない。

(C) 水 の 影 響

発光体を切りとり，日光で乾燥した場合，暗所で水をかけると，ツマグロイシモチでは再び発光力を恢復し，乾燥状態に保てば数ケ月も発光能力を保たせることが出来るが，本魚では乾燥したものは再び水をかけても発光しない。

(D) 検鏡所見と培養試験

顕微鏡下では本魚は，無数のバクテリアと腺細胞の小片がみられるが，ツマグロイシモチでは全くバクテリアを認めることが出来ない，3% 食塩加培養基上に，法にしたがって，培養試験を行なったが，本魚では，すべて，10～15 時間後に，無色円形の発光するコロニーが出来，検鏡するに，発光体のエムルジョンに見るバクテリアと一致する。培養したバクテリアはいずれも同一種のバクテリアであって，本魚を宿主とするバクテリアである。

(E) エムルジョンを遠心沈澱すると，本魚では，発光物質が管底に沈澱し，上澄液は透明で発光力がない，ツマグロイシモチでは，管底に発光する物質が沈澱するが，液全体が光つて，上澄液が消光することはない。

(F) ルチフェリン，ルチフェラーゼ反応において，ツマグロイシモチは強い反応を示し，ウミボタル，キンメモドキの発光体の間でも強い交叉反応がみられたが，本魚の発光体ではこのような反応は見られなかった。

以上のような実験結果から，本魚の発光体内容は，腺内に共生する発光バクテリアである。

5. *Siphamia* 属の他の種類の発光器について

Siphamia 属の他の種類については LACHNER (1953) が，多くの種類，*S. versicolor*, *S. cuprea*, *S. ovalis*, *S. fuscolineata*, *S. argentea*, *S. elogato* などを区別しており，著者自身，これらの標本を見る機

会を得たが，発光器については著しい差違を認め得なかった，然し，富永 (1964) は *S. cuprea*, *S. ovalis*, *S. fuscolineata*, *S. argentea* を *S. versicolor* の Synonym とし，僅かに，*S. elongata* を別種としているので，発光器の構造に差違のないのは当然である。*S. elongata* との間にも著しい発光器の相違は認められなかった。

6. 発光共生バクテリアの由来

発光体内のバクテリアが，どのようにして定着したかは従来，多くの説があり，卵を通して，発光バクテリアが次代に伝えられたとの説と，発光バクテリアは，魚が孵化した後，発光器が出来て，その小さい開孔を通って，海水中のバクテリアが浸入，定着したという説とがある。著者らは後者の説を信じていたが，さて，これを証明する材料がなかった。幸にも今回，多数の孵化直後の生きた幼魚を暗室にて観察し，発光の有無をしらべることが出来た。この魚は 5 月頃，沖縄では産卵期であり，口腔内に幼魚を入る習性があるので，捕獲した魚は，非常に多くの幼魚をはき出した。もし，発光バクテリアが卵を通して次の世代に遺伝されるものとすれば，生きた卵，孵化直後の幼魚は発光する筈である。たとえ個々の光が弱くとも非常に多く集まれば光は認められる筈である。ところが，暗室で注意深く観察したが，孵化直後の幼魚には全く光は認められなかった，魚が，どの位，生長して，発光するようになるかは，今後の研究課題である。もしこの微細な幼魚の発光器が出来上るまで飼育出来るとすれば興味ある問題が解決されるわけである。

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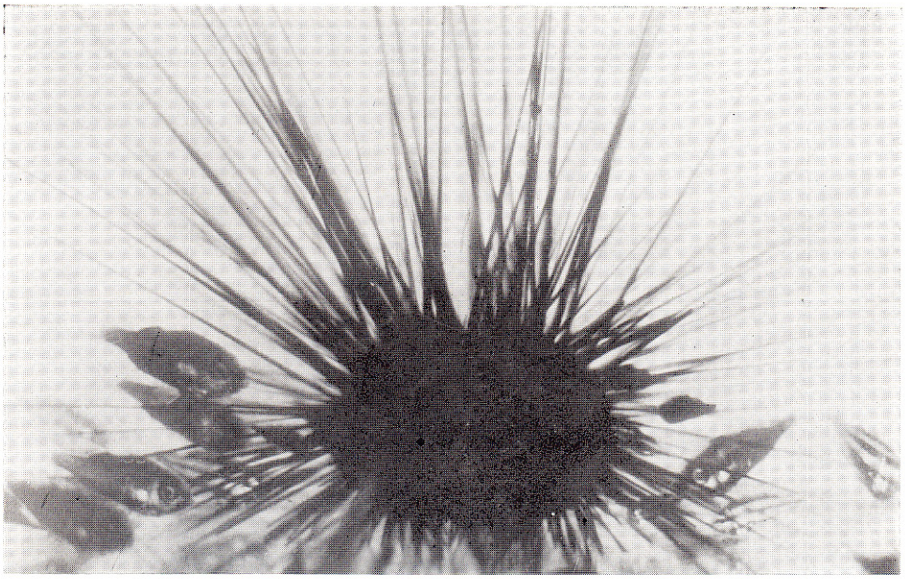

Fig. 1. *Siphamia versicolor* in the long spines of *Diadema setosum*, a long spined sea urchin.

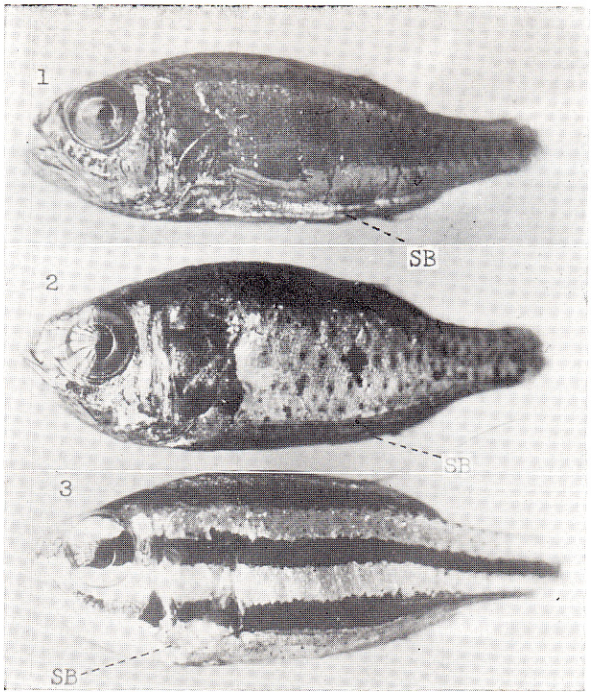

Fig. 2. Three specimens of *Siphamia versicolor*, showing three different color patterns.

1, uniformly dark or dark brown; 2, light colored or pale pink; 3, dusky silver with three longitudinal brown stripes; SB, silvery band.

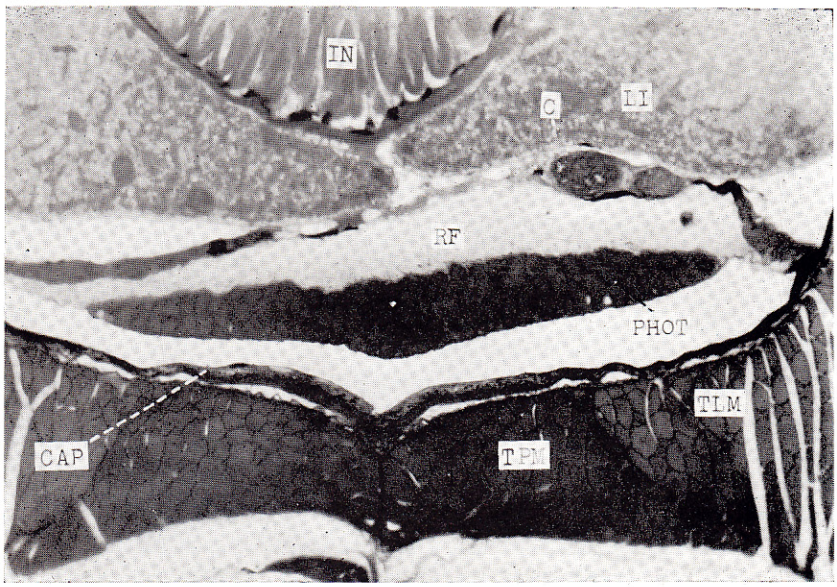

Fig. 3. Transverse section of the luminous body of *Siphamia versicolor*.
Photo, luminous body; RF, Refrector; C, Canaliculi; LI, liver; IN, intestine; CAP, capsel; PG, pigment; TPM, transparent muscle serve as a lens; TLM, translucent muscle bundles.

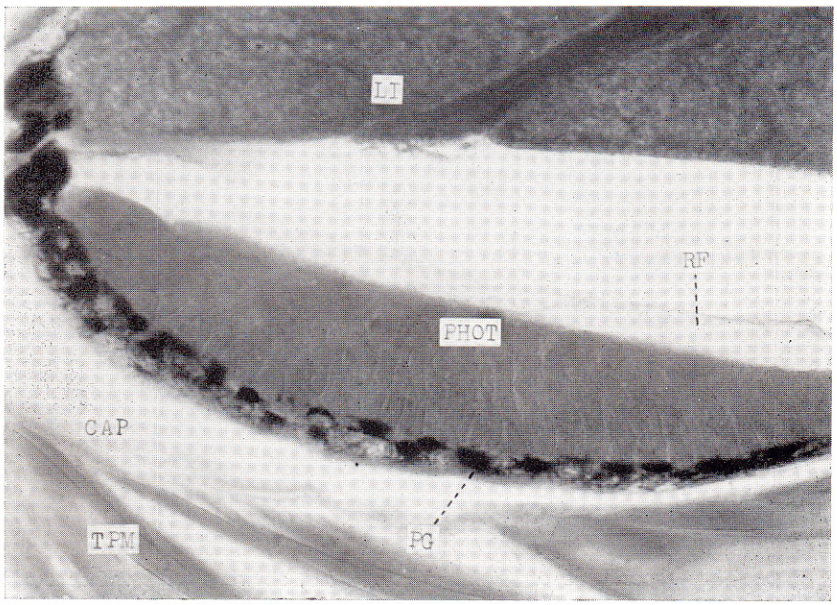

Fig. 4. Longitudinal section of the luminous body of *Siphamia versicolor*.
PHOT, luminous body; RF, refrector: CAP, capsel; PG. pigment; LI, liver; TPM, transparent muscle serve as a lens.

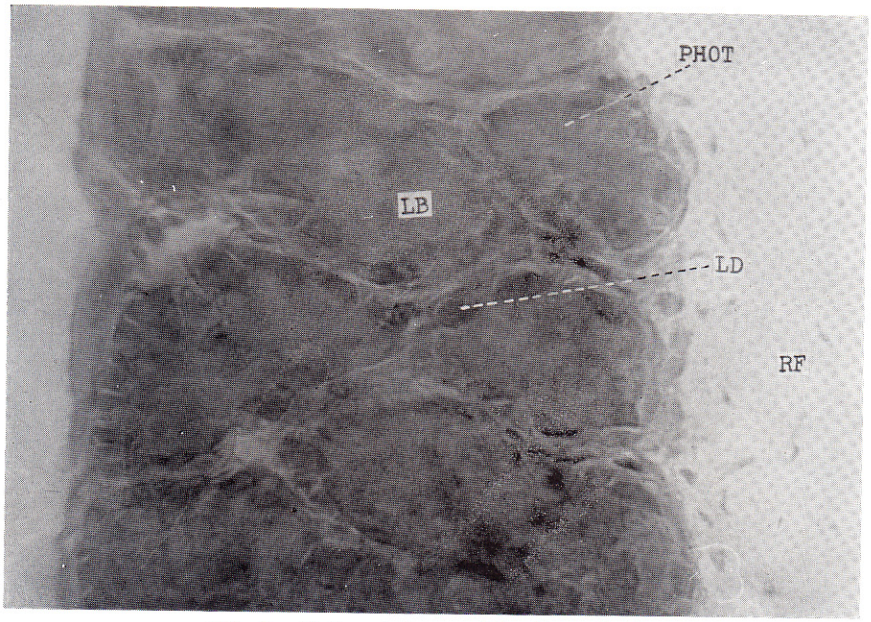

Fig. 5. Enlarged figure of luminous body.
PHOT, luminous body; RF, refrector; LD, luminous gland cell; LB, mass of luminous bacteria

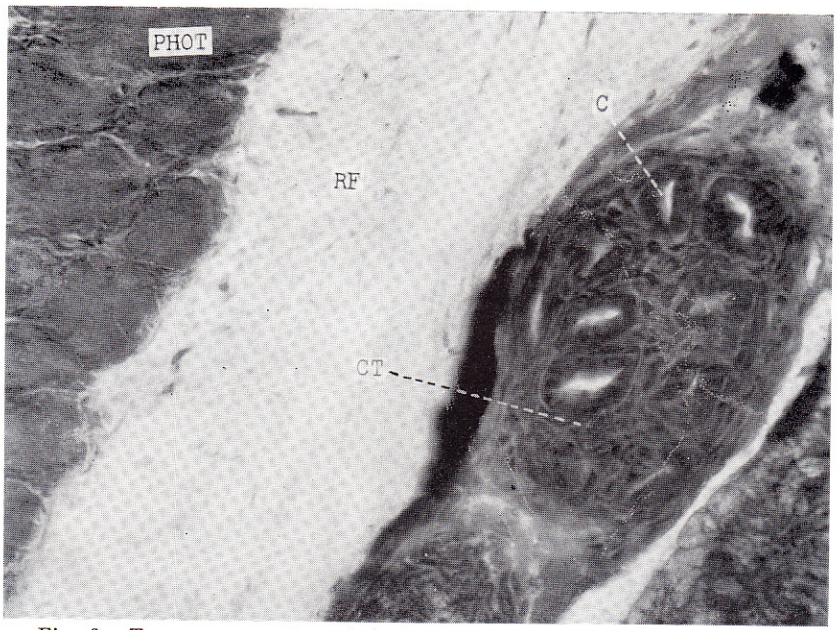

Fig. 6. Transverse section of the luminous body and connecting tube.
PHOT, luminous body; RF, refrector; CT, connecting tube; C, canaliculi.

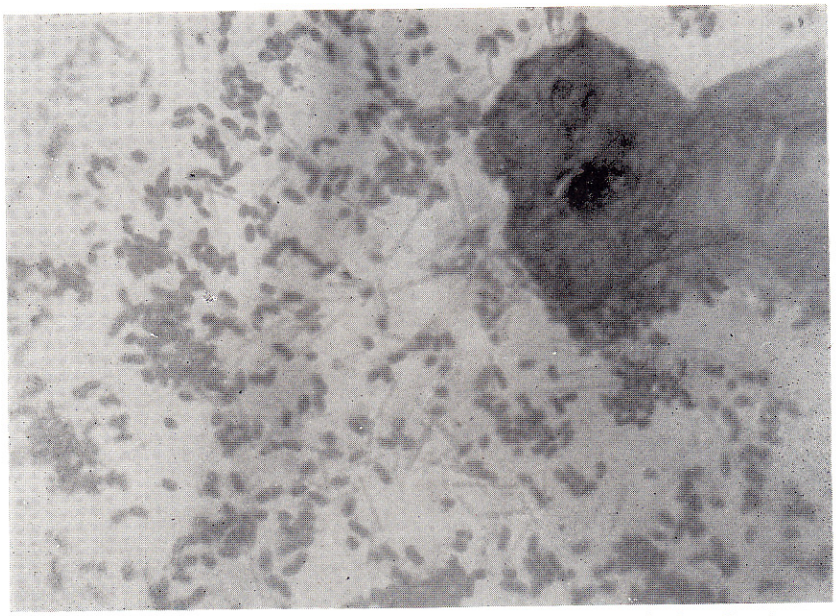

Fig. 7. Microscopic figure of the contents of luminous body, showing symbiotic luminous bacteria and a segments of gland cell.

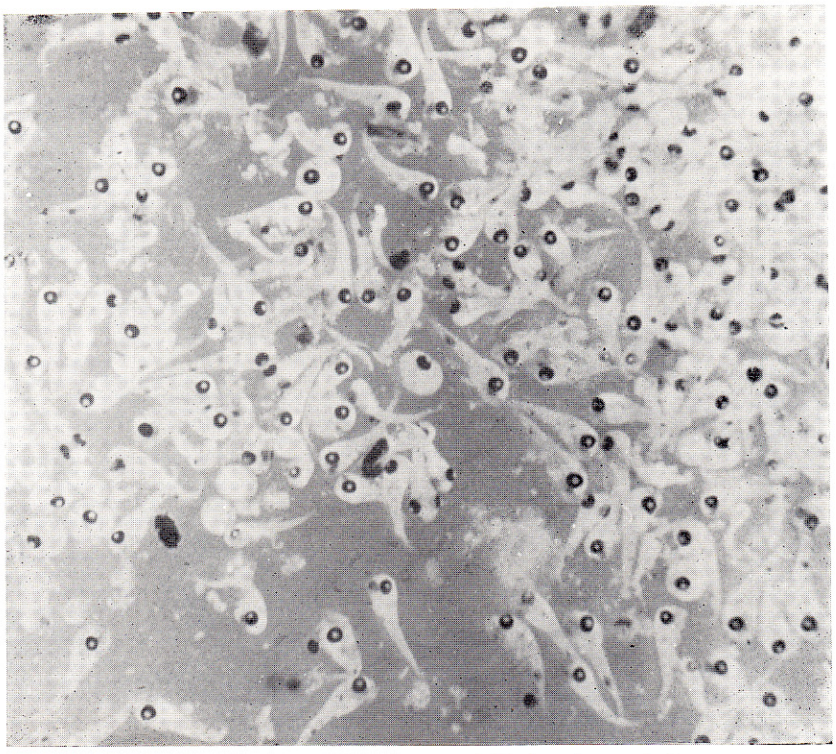

Fig. 8. Newly hatchet larvae of *Siphamia versicolor*.