Observations on the Luminescence of the Shallow-Water Squid, *Uroteuthis bartschi*[1]

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(with 3 text-figures)

浅海イカ *Uroteuthis bartschi* の発光に就て

羽根田弥太

1. Introduction

In the deep sea, especially among the oegopsids squids, there are many luminous species. Some have simple photophores, othere highly complex ones. These photophores occur on every conceivable region of the body—the head, eyeball, around the eye openings, the mantle, arms, tentacles, fins, and in the intrapallial cavity. The number of photophores and their distribution varies with the species, and the arrangement also varies, slightly. These photophores shine with intracellular luminescence, and do not give off any luminous secretion. In deep water, *Heteroteuthis dispar*, a mediterranean species, and *Sepiolina nipponensis*, a Japanese species, among the myopsids squid have a special luminous organ which produces light by secretion. The light-emitting secretion of those two species is a form of mucus which comes from a gland on the ink sack. The gland stores the luminous secration, of which there is an abudant supply.

In shallow water, there some luminous species among the myopsids squids.

In the myosid squids except for above two species, there is no doubt that the light comes from symbiotic luminous bacteria. PIERANTONI (1914, 1918) and BUCHNER (1914, 1921) have pointed out that the symbiotic luminous bacteria are transmitted from generation to generation through the egg, and live in the photogenic cells. However, KISHITANI (1928), MEISSNER (1926), and HERFURTH (1936) found that the symbiotic luminous bacteria live in the open-type luminous organ, not in the photogenic cells.

Workers on symbiotic luminous bacteria are ZIRPOLO (1917-24), MORTARA (1922, 24), MEISSNER (1926), KISHITANI (1928), and GETZEL (1934). Many workers have been able to cultivate luminous bacteria from the luminous organ of myopsid squids, but the differ in

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their opinions. ZIRPOLO and MEISSNER followed PIERANTONI and BUCHNER in believing that the bacteria are transmitted through the egg, while MORTARA and KISHITANI believed that the bacteria entered through the opening of the luminous organ and settled there after the young squid hatched. According to HARVEY (1952), the distribution of luminescence among the myopsid squids is as follows:

Loliginidae—*Doryteuthis* and *Loligo*

Sepiolidae —*Heteroteuthis*, *Nectoteuthis*, *Iridoteuthis*, *Stoloteuthis*, *Euprymna*, *Sepiola*, *Inioteuthis*, and *Rondeletia*

Spirulidae —*Spirula*.

HARVEY pointed out that forms of luminous bacteria have been isolated from the genus *Loligo*, but have not been claimed as symbionts. KISHITANI cultivated *Coccobacillus loligo* n. sp. from the luminous gland of *Loligo edulis*, but was unable to observe the luminescence of these squids in life.

During my collecting expedition of luminous organism around the world from 1959 to 1960, I had opportunities to observe luminescence and collect luminous squids of both the Oegopsida and Myopsida in the straits of Messin, Sicily, and several parts of South East Asia. In this report I wish to describe the luminescence of the squid *Uroteuthis bartschi* REHDER, which I collected off Pipi Beach, Dumaguete Island, in the Philippines. Until now the luminescence of this squid has been entirely unknown.

2. Material

The material was collected in April, 1960, 1000 meters off Pipi beach, Dumaguete Island, P.I. The squids came floating up from the depths of a coral reef in clear sea water, attracted by a gas light shone from a native canoe. They were elongate and transparent. The light from the luminous organ above the ink sac shone out through the transparent body. After observing the luminescence in living specimens, 20 of them were fixed in Bouin's solution and formaldehyde and sent to the laboratory of Yokosuka City Museum for microscopic study.

The material was sectioned in Celloidin. HEIDENHAIN'S haematoxylin eosin was employed for staining, and the material was also observed under the fluorescent microscope. In order to isolate the luminous bacteria from the luminous organ the following procedure was carried out: the body of the squid was washed carefully in distilled water; the luminous organ with the ink sac and rectum were then taken out and put into sterilized water. The organ was cut with a sterilized knife and the luminous substance inside was put on an agar agar culture medium containing 3% NaCl. This was kept at room temperature.

3. Luminescence

The body of the squid is slender as shown in Fig. 1. The diameter of an individual 200 mm long measured 6 mm. Another individual 150 mm long was 5 mm in diameter. The light of the luminous organ, which is situated above the ink sac, can be seen through

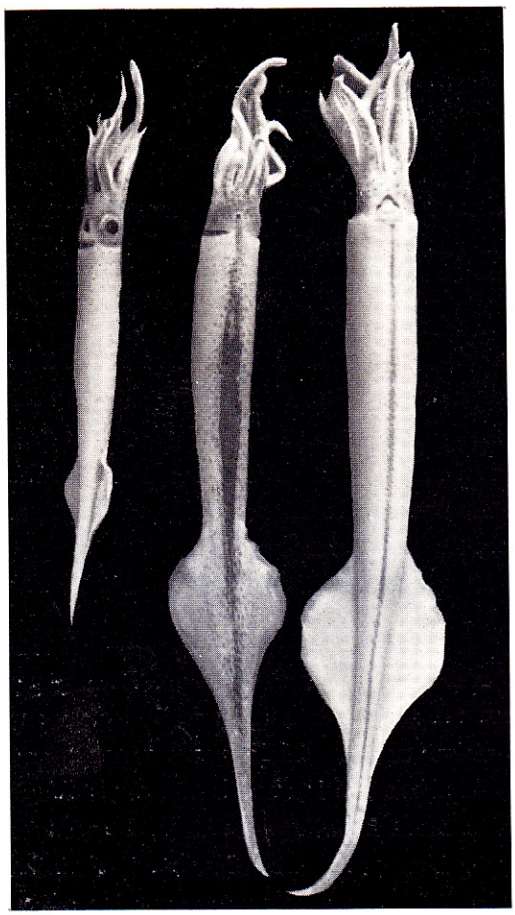

Fig. 1. Luminous shallow-water squid, *Uroteuthis bartschi*.

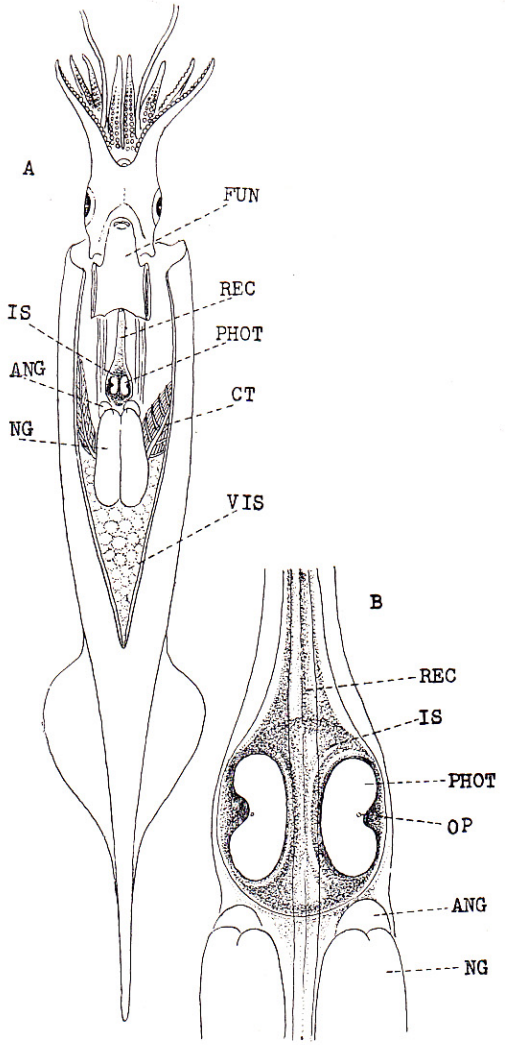

Fig. 2. A and B. Diagramatic figure of *Uroteuthis bartschi*, showing the position of luminous organ. PHOT, Pair of lemon colored lens of the luminous organ; IS, Inc sac; NG, Nidamental gland; ANG, Accessory nidamental gland; CT, Ctenidium; VIS, Visceral sac; FUN, Funnel.

the transparent mantle. If a squid caught in the net was picked up, the light would alternately appear and disappear. At this time the mantle of a living squid was cut open and the luminous organ observed. As is Fig. 2, to the right and left of the rectum above the ink sac were two organs like pale lemon beans. These were surrounded by a thin film of ink, which, by contraction and expansion, similar to the working of the pupil of the eye, would allow the light emitted to increase or decrease. If the ink sac was flushed out with water, then the light shine continuously. Since the source of light

is not luminous cells, but symbiotically cultivated bacteria, the light shines continuously, As shown in Fig. 2, the light which passes through the lens is controlled by the expansion and contraction of a film of ink. KISHITANI described the luminous organ of *Euprymna*, in which the light is controlled by a thin film of ink between the lens and the luminous body. In this squid, however, the ink film envelops both the luminous body and the lens.

KISHITANI reported the presence of symbiotic luminous bacteria above the ink sac of *Loligo*, *Euprymna*, and *Sepiola*, but never observed the light in living specimens. HAMABE and SHIMIZU (1957) reported detailed observation on living *Loligo breekeri*, especially during copulation time. They observed the copulation behavior during March, 1955, in Urago Bay, Oki Island, Japan Sea. Mating is carried on at the surface at about sunset. When courting the male squid turns white and glimmers and approaches the female. The female also turns white and emits light when touched by the male. During copulation the male emits light continuously, while the female remains dark. Immediately after copulation, the male ceases to shine, and the female sinks down to the bottom, turning white and glimmering. I was unable to observe the copulation behaviour of *Utoteuthis bartschi*, but since the structure and light emission of the luminous organ is similar to that of *Loligo*, I think that the luminescence behaviour would likewise be similar.

4. Structure of the Luminous Organ

As shown in Fig. 3, the structure of the luminous organ of *Uroteuthis bartschi* is similar to that of *Euprymna*, *Sepiola* and *Loligo*.

The organ is a pair of bean-shaped bodies, opaque and lemon yellow in color, which are situated on either side of the ink sac; each of these consists of a luminous sac and its opening, a lens, a reflector and an ink layer as a light control mechanism which covers the outside of the reflector and part of the lens. The length of the lens from a specimen 200 mm in mantle length was 2 mm, and that of a specimen 150 mm in mantle length was 1.8 mm. The luminous body is composed of gland tissue which lies in the center of the organ. In the gland tissue appear numerous bacteria. These bacteria are present in clusters or else are attached to the surface of the gland cells. The external opening of the luminous sac of *Loligo edulis* opens into the accessory nidamental duct through a long canal, but that of *Uroteuthis bartschi* opens directly to the lens near the edge of the ink film, The situation of this opening is similar to that of *Euprimna* and *Sepiola*.

As I mentioned before, BUCHNER, PIERANTONI, MORTARA, OKADA SKOWLON, KISHITANI, MEISSNER, etc. held varying opinions on the relations of the accessory gland and the luminous organ. SAITO (1952) attempted an explation of this relation through an anatomical, histological, and bacteriological study of the nidamental duct, but was unable to do so satisfactorily. In *Utoteuthis bartschi* there is apparently no relation between the luminous organ and the accessory nidamental duct.

The inner substance of the luminous body stained very well with Ziehl's solution (carbol fuchsin solution), and poorly with Haematoxylin eosin. A rich supply of blood vessels is found in the gland tissue.

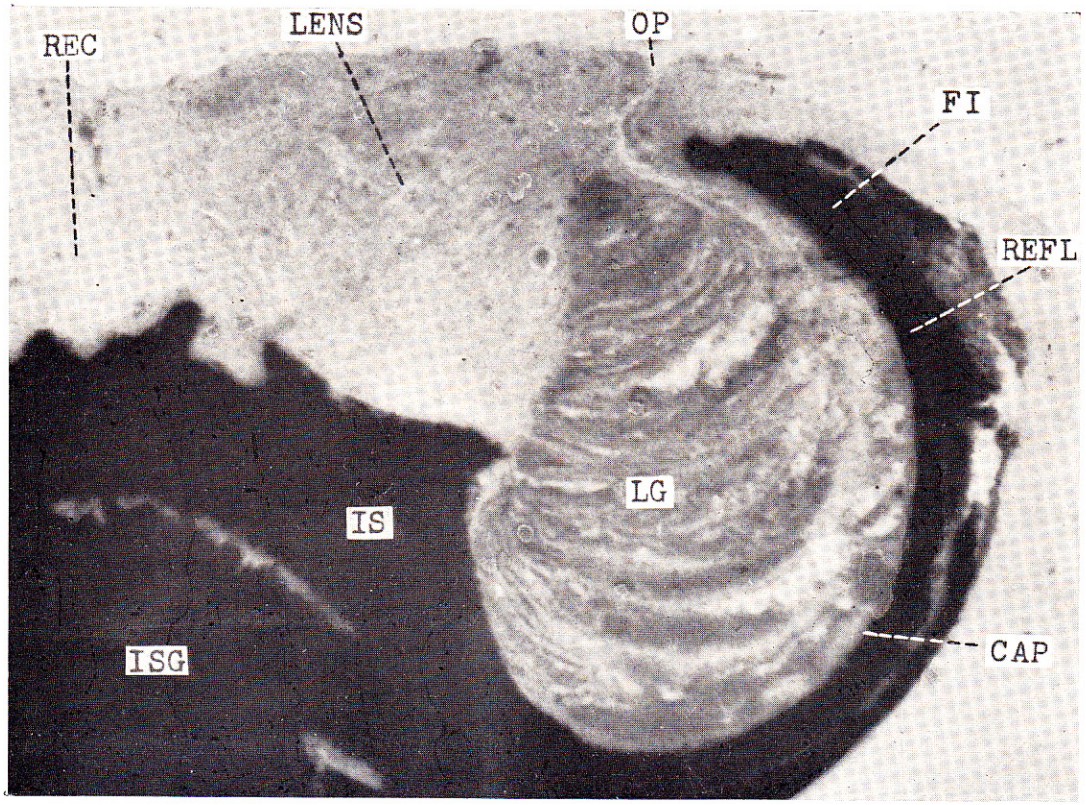

Fig. 3. Transverse section of the luminous organ of *Uroteuthis bartschi* LG, Luminous gland; CAP, Capsel of Luminous gland; REFL, Reflector; OP, Opening of luminous gland; LENS, lens; FI, Film of ink; IS, Ink sac; ISG, Ink sac gland; REC, Rectum.

The lens tissue is transparent and bean-like in shape, situated on either side of the rectum.

Since luminous bacteria are the source, the light is continuous. However, when irritated, the squid alternately emits a bright light and extinguishes it. This is caused by the contraction and expansion of a thin film of ink, which exposes or shuts off the light source like the pupil of an eye.

Pure culture

The pure culture of the bacteria was easily obtained. All the 20 strains of pure culture emitted strong bluish light at room temperature after 12 hours, and all showed the same morphological and cultural characteristics.

Summary

1. The possession of a luminous organ and the manner of light emmission was observed in the shallow water squid *Uroteuthis bartschi* REHDER. This was a fact hitherto unknown, The observations were carried out in April, 1960, off Pippi Beach, Dumagrete Island, Philippine Islands.

2. The luminous organ appears as a pearl-like lemon yellow lens situated above the ink sac to the right and left of the rectum.

3. A thin film of ink envelops the lens, and by its contraction and expansion controls the emission of light like the pupil of an eye.

4. Luminous bacteria exist symbiotically inside the luminous body. Both the right and left body has an external opening. In *Loligo edulis* this opens through a long canal into the accessory nidamental gland, but in *U. bartschi* this opening is near the center of either lens. Many explanations have been given in the past about the relation between the accessory nidamental gland and the opening of the luminous body, but in this squid there is apparently no direct relation.

References

BUCHNER, P. 1914: Sind die Leuchtorgane Pilzorgane? Zool. Anz. 45: 17-21.

BUCHNER, P. 1921, 1930: Symbiosen bei Leuchtenden Tiersen. Tier und Pflanze in Symbiose. Berlin, 2nd ed.: 664-736; 1st ed. 1921,: 340-400.

BUCHNER, P. 1926: Tierisches Leuchten und Symbiose. Berlin: 58.

GETZEL, D. 1934: I microbi della glandola nidamentale accessoria in *Sepia officinalis*. Priva nota. Arch. Zool. (ital.) Napoli 20: 33-43.

HAMABE, and SHIMIZU 1957: The copulation behavior of Yariika, *Loligo Bleekeri* K. Ann. Rept. Japan Sea Regional Fish. Research Lab. No. 3: 131-136.

HANEDA, Y. 1956: Squid producing an abundant luminous secretion found in Suruga Bay, Japan. Sci. Rep. Yokosuka City Mus. No. 1: 27-32.

HARVEY, E. N. 1952: Bioluminescence. Academic Press, Inc. New York: 270.

HERFURTH, A. H. 1936: Beiträge zur Kenntnis der Bakteriensymbiose der Cephalopoden. Zeit. Morph. Ökol. Tiere. 31: 561-607.

KISHITANI, T. 1928: Über das Leuchten von *Euprymna morsei* VERRILL und die symbiontishe Leuchtbakterien. Proc. Imp. Acad. Japan, 4: 306-309.

KISHITANI, T. 1928: Preliminary report on the luminous symbiosis in *Sepiola birostrata* SASAKI Proc. Imp. Acad. Japan, 4: 393-396.

KISHITANI, T. 1932: Studien über Leuchtsymbiose von japanishen Sepien. Folia anat. Japan, 10: 315-418. and Stud. Tokugawa Inst. 2: 315-418.

MEISSNER, G. 1926: Bakteriologische Untersuchungen über die symbiontischen Leuchtbakterien von Sepien aus dem Golf von Neapel. Zbl. Bakt. (Abt. 2) 67: 194-238.

MEIYER, W. T. 1906: Ueber das Leuchtorgan der Sepiolini. Zool. Anz. 30: 388-392.

MEIYER, W. T. 1908: Ueber das Leuchtorgan der Sepiolini. II. Das Leuchtorgan von *Heteroteuthis*. Zool. Anz. 32: 505-508.

MORTARA, S. 1922: È accettabile la teoria simbiotica della fotogenesi animale? Rivista Biol. 4: 1-2.

MORTARA, S. 1924: Sulla biofotogenesi e su alcuni batteri fotogeni. Riv. Biol. 6: 323-342.

OKADA, Yo. K. 1927: Contribution à l'étude des céphalopodes lumineux. Notes preliminaries. I. and II. Bull. Inst. océanogr. Monaco. No. 494, 1-16; No. 499, 1-5.

PIERANTONI, U. 1914: La luce degli insetti luminosi e la simbiosi ereditaria. R. C. Accad. Napoli 20: 15-21.

PIERANTONI, U. 1918: Organi luminosi, organi simbiotici e glandola nidamentale accessori nei Cefalopodi. Boll. Soc. Nat. Napoli 30: 30-36.

PIERANTONI, U. 1918: Gli organi simbiotici e la luminescenza batterica dei Cefalopodi. Pubbl. Sta. Zool. Napoli 2: 105-146.

SKOWRON, S. 1926: On the luminescence of some cephalopods (*Sepiola* and *Heteroteuthis*) Riv. Biol. 8: 236-240.

SAITO, S. 1952: Studies on a accessory nidamental gland of some Japanese squids. Reports of the Shimane University (Natural Science) Vol. 2: 41-47.

ZIRPOLO, G. 1917: Richerche su di un bacillo fosforescente che si svillupa sulla *Sepia officinalis*. Boll. Soc. Nat. Napoli 30: 47-77.

ZIRPOLO, G. 1918: I Batteri fologeni deli organi luminosi dè *Sepiola intermedia*, NAEF. (*Bacillus Pierantonii* n. sp.). Bull. Soc. Nat. Napoli 30: 206-220.

ZIRPOLO, G. 1924, 1926: Ancora sui batteri fotogeni. Riv. Biol. 4: 1-4; 8: 244-248.

抄　　録

深海発光イカの中特に Oegopsida に属する種類では極めて簡単な皮膚発光器から高度に発達した発光器を具えた種々の発光イカが知られているが，いずれもその発光は細胞内発光でイカ自身の造り出す発光細胞によるものである。Myopsida に属する発光イカは，墨汁嚢の上に発光液を分泌する器管を具えた地中海の *Heteroteuthis dispar* と，駿河湾の *Sepiolina nipponensis* を除いては浅海性であって発光器は墨汁嚢の上に左右一対あり，その器管内に共棲する発光バクテリアが棲息，器管は開口を通じて外界に開いている。

PIERANTONI, BUCHNER 等はこの共棲バクテリアは卵を通じて次代に遺伝すると考えたがこれらの共棲発光バクテリアは開孔を通じて孵化後感染したと考えた方が妥当である。*Loligo edulis* に於ては，その開孔が長い導管を通じて，副纏卵腺，accessory nidamental gland の近くに開孔していることから，発光バクテリアとこの腺との関係に就て，種々検討されて来た。

著者はフィリッピン，Dumagueti 島，pipi 海岸に於て，夜間，灯火に集って来た Myopside に属する浅海性のイカ *Uroteuthis bartschi* の発光を観察し，発光器の構造，組織をしらべ，共棲発光バクテリアを培養した。従来まで *Uroteuthis bartschi* の発光に就ては全く知られていなかった。

発光器は墨汁嚢の上，直腸の両側にあり，その横断面をみると *Loligo edulis* に似ているが，外界との開孔は直接レンズ組織を貫通して開孔している。この点 *Euprymna morsei* の場合と似ている。発光器と，副纏卵腺との関係について，従来，論議されて来たが，このイカに於ては全く関係がない。

このイカは生時，殆んど透明で，発光器の光は透明な外套膜を通してみられるが，外套膜を切開して，墨汁嚢の上の発光器をみると左右にレモン色の透明なレンズと，そのレンズが，丁度，瞳のように或は又写真器の絞のように急激に拡大したり収縮するのが認められた，これは発光体とレンズの一部を墨汁の薄い層が包んでいるのであって，筋肉の伸縮によって墨汁の層に墨汁が流れ込むのをコントロールし，光の急激な明滅を司るものである。

浜部及び清水両氏は *Loligo breekeri* の生殖時に於ける発光を詳細に観察しているが，恐らく，このイカも同様にその構造上よりして急激な明滅をし，実生活に役立っているものと考えられる。