Squid producing an abundant Luminous secretion found in Suruga Bay, Japan

Yata HANEDA*

(With 2 Plates)

INTRODUCTION

According to Dr. E. N. HARVEY (1952) luminous squid may be divided into three groups, depending on their method of light production : 1) Those associated with luminous bacteria ; 2) Those producing an abundant luminous secretion ; and 3) Those with well-developed photophores and intracellular luminescence. The first and second groups are all myopsid and the third group belong to all oegopsid squid.

Among these 3 groups the second is the most interesting. It is sometimes thought that perhaps the luminous secretions of deep sea squid have replaced the more familiar and so-colled "ink" ejections ; however no attempts have been made to confirm this.

Until recently, *Heteroteuthis dispar*, a deep sea luminous squid occasionally caught along the coasts of southern Italy, was the only species known to be of the second group. This squid has been studied by many investigators since its discovery about 50 years ago. MEYER (1906, 08) made the first studies concerning this squid, examining the morphology and histology of the light organ. When PIERANTONI, in 1918 found luminous bacteria in the organs of *Sepiola* and *Rondeletia*, he suggested that *Heteroteuthis* might also contain luminous bacteria ; and in 1924 after a study of the luminous gland of this species, PIERANTONI postulated this to be true. However, MORTARA (1922, 24) proved PIERANTONI to be mistaken, and conclusively showed that no luminous bacteria could be cultured from the light organ of this species. HERFURTH (1936) also studied the morphology and histology of this squid group.

The luminous organ of *Heteroteuthis* appears in both male and female, just posterior to the anus and surrounded by a small ink sac. In 1925 during a trip to Messina, Italy, Dr. HARVEY was able to carry out a number of biochemical experiments on the luminous secretion of *Heteroteuthis* along with Dr. SKOWLON. Some of the results of these experiments are contained in a report published by SKOWLON in 1926. Importantly, it was noted that the dried luminous gland of this squid would emit light when fresh water was added. Also Dr. HARVEY obtained negative result of Luciferin-Luciferase reaction.

*Director of the Yokosuka City Museum, Yokosuka, Japan.

Recently anather species of the same type of luminous squid, *Sepiolina nipponensis* was discovered in Suruga Bay, Japan.

MATERIAL

This squid, producing an abundant luminous secretion, was originally reported in the auther's paper in 1955 under the name of *Stoloteuthis leucoptera* VERILL ; howerer, Dr. S. Stillman BERRY later correctly identified this squid as *Sepiolina nipponensis* (BERRY).

In the winter season near the port of Heta on Suruga Bay, trawling boats draw their nets 200—300 fathoms deep. In these nets *Sepiolina* are sometimes caught. Also, from October to May deep-sea fishing boats set out from Yui and Kambara, also on Suruga Bay, in quest of deep-sea shrimp (*Sergestes prehensilis*). In these nets too *Sepiolina* appears sometimes. These nets are draw only at night, in comparatively shallow water, where many bathypelagic organisms fly to the surface water.

OBSERVATIONS

As shown in Fig, 1, the mantle of this squid is 10 to 20mm long, and upon it appears a pair of large white bands, which have been mistaken by some observers to be a luminous skin organ. However, disection reveals the round white luminous gland to be situated upon the ink sac and connected to the exterior by two openings.

Sepiolina is not luminous when freely swimming, but if touched, it will spurt through its funnel a beautiful and bright bluish secretion like that of the mollusc *Pholas* or *Rocellaria*. The luminous secretion is ejected through the openings of the luminous gland reservoir.

MORPHOLOGY AND HISTOLOGY

In his experimental observations of this squid, the auther preserved the whole body in a fixative of sea water prepared with formalin and Bouin's solution. The material was cut at 10μ transversely by celloidin, and stains used were haematoxylin-eosin and also alminium morine (500.000 times diluted solution) for observation under fluorescent microscope.

The luminous organ of this squid situated, in both male and female, on the rather small inc sac, from which, even though they live in total darkness, they do eject a small amount of ink whenever disturbed. This organ is not luminous, even when stimulated; only the secretion which is spurted through the gland openings is luminous.

According to PIERANTONI the luminous orgon of *Heteroteuthis dispar* consists of a luminous gland, lenses, reflector, a reservoir with two discharge openings indicating its paired origin, and muscles to squeeze out the secretion. The luminous organ of the *Sepiolina nipponensis* is the same as that of the *Heteloteuthis*, but only in construc-

tion. An illustration of the transverse section of *Sepiolina* is shown in Fig. 3.

The organ consists of a luminous gland, a reservoir with paired discharge openings (OP), and muscles (M_1, M_2) to squeeze out the luminous secretion. However, as this organ manifests no luminous function in itself, but rather merely serves as a reservoir of the luminous masses of the gland, it is difficult to accept PIERANTONI'S contention that the organ itself is luminous, for the muscles (M_1, M_2) are not transparent, and evidence no other lense or reflector functions.

As is seen in the Fig. 2, the luminous reservoir lies situated upon the ink sac (TN). Blood vessels (V) enter through the muscles. The muscles (M_1, M_2) are eosinophilic: they stain with eosin, the gland cell stain selectively with Haematoxylin, and the luminous masses of gland stain with eosin. Under microscopic observation in the compartments of the gland (G) appear as clumps of both round and bacillus-like granules. Under the fluorescent microscope, these granules shine with a yellowish-green when stained with alminum-morine.

It seems that these granules tend to remove from the luminous gland reservoir to the mantle cavity, where they become luminous through contact with the sea water contained in the cavity, and from where they are ejected through the funnel into the surrounding water.

This would be difficult to establish by direct observation, however the construction of the squid body the nature of the luminous ejection, and non-luminous character of the organ (reservoir of the luminous masses) itself would seem to validate this conclusion.

BIOCHEMISTRY

A number of luminous organ (reservoir) were removed from their bodies and thoroughly dried. They were then ground in a mortar and moistend with water; the luminescence was recovered.

A negative Lueiferin-Luciferase reaction was obtained by mixing hot water and cold water extracts of the crushed luminous organ. There was also no light when *Cypridina* luciferin was mixed with *Sepiolina* extracts or on mixing *Cypridina* Luciferase with *Sepiolina* luciferin. If the cold water extract is allowed to stand until the light disappears, it will not recover its light when ATP is added.

Acknowlegement:

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Explanation of Plate V.

Fig. 1. *Sepiolina nipponensis* (BERRY) ×2.5

Fig. 2. Luminous gland reservoir of *Sepiolina nipponensis* ×6

Explanation of Plate VI.

Fig. 3. Transverse section of the luminous gland reservoir. ×18

Fig. 4. Transverse sections of the luminous gland reservoir ×800

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抄　　錄

発光液を噴出する駿河湾の深海イカ *Sepiolina nipponensis* に就て

羽根田弥太

Dr. HARVEY によると発光イカをその発光形式より次の三つに分けている。即ち 1) 発光バクテリアを特殊な器官の中に培養して光るもの。2) 発光性の化学物質を噴出するもの。3) よく発達した発光器を具えたもので、発光は細胞内で起るもの。この中第2の発光液を噴出する深海イカは特に興味深い。浅海のイカは黒い墨をはくが、暗黒の深海に棲むイカは光る液をはいて敵から逃れると言われる。このような解釈がはたして妥当であるかどうかは別として、ともかくこのようなイカの墨汁嚢は退化している。イタリアのメツシナ海峡の深海イカの1種 ***Heteroteuthis dispar*** は発光液を噴出するので可なり古くより知られている。

著者は十数年前より駿河湾の深海発光動物を調べているが、多種の発光イカの中、この形式の発光イカを研究する機会を得た。

このイカは外套膜の長さ 10～20mm の小形で外套膜上に左右に白いバンドが縦にあり、このものが皮膚発光器と考えていた人もあつた。然し、このものは単なる白い筋繊維であつて、発光器は外套膜を切開すると退化した小さい墨汁嚢の上にみられ、白色真珠様光沢の直径 4mm 位の円形の器官で左右にやや隆起した二つの開孔が見られる。ミミイカやダンゴイカのように発光バクテリアの力で光るイカは総て Myopsida（閉眼族）に属し、発光器には外界との通路があり、開孔式と言われている。

このイカもやはり Myopsida に属し、開孔式の発光器であるから著者は、その発光体は発光バクテリアであろうと考えて、発光バクテリアの培養試験を行つたが、いづれの場合も陰性であつた。1953年11月静岡県由比の桜エビ網漁舟に便乗する機会を得、夜間このイカの生きた状態を観察することが出来た。自由に游泳しているときはこのイカは少しも発光しないが、何かで刺戟をすると強く光る液を漏斗より噴出し、海水中に光る雲を作り、その光は 1，2 分後に除々に消えて行くのを見た。このイカは Dr. S. Stillman BERRY の同定によると ***Sepiolina nipponensis*** (BERRY) であつた。

このイカのセロイヂン切片を見ると第2図及び第3図のように墨汁嚢（TN）に接して筋肉（M_2）があり、前方にも筋肉（M_1）がある。PIERANTONI は ***Heteroteuthis dispar*** について、前者を反射器、後者をレンズと考えたが ***Sepiolina*** に於てはレンズに相当する部の筋肉（M_1）は不透明でレンズの役をしていない。墨汁嚢に接した筋肉（M_2）は透明で反射の役をするとは考え難い。むしろこの器官は発光物質（G）を貯蔵する腺構造の器官で、この器官の外殻をなす筋肉はむしろ発光液を開孔（OP）から出すため収縮する筋肉と考えた方がいい。この筋肉を通じて血管（V）が中に入つておる。開孔より押し出された顆粒は外套膜内の海水と混合して発光液となり、この液が漏斗より噴出されるものと思われる。発光器の外殻をなす筋肉組織はエオヂンに染り、内部の腺細

胞は紫に、腺内の発光顆粒は紫紅色に染る。発光顆粒は非常に密に集まつた球状又は桿状の顆粒である。この円形の器官は発光器と言うよりはむしろ、発光顆粒を貯蔵しておく器官であると考えた方が妥当かもしれない。この発光顆粒を乾燥して、これに水を加えると再び発光するがLuciferin-Luciferase 反応は陰性、又このイカの Luciferin（或は Luicferase）に海螢 *Cypridina* の Luicferase（或は Luciferin）を加えたが発光は見なかつた。又、この発光顆粒の浸出液に ATP (Adenosine-Tri-Phosphate) を加えたが発生は再現しなかつた。

摘　　要

1) 駿河湾の深海発光イカの1種 *Sepiolina nipponensis* (BERRY) は外套膜上に縦に白いバンドがあり、これが皮膚発光器のように考えられていたが、このものは発光には無関係である。

2) 発光器は墨汁嚢の上にある白色真珠様光沢の丸い器官で、外套膜の長さ 20mm のものでは直径 5 mm。10mm のもので 4 mm 位、左右に1対のやや膨隆した点があり、その中央に開孔がある。

3) このイカは Myopsida に属し、発光器は開孔式であるから、発光バクテリアの共棲により光るのではないかと誤解されやすいが、培養試験の結果は陰性であつた。

4) 発光器官の内部は球形、桿状形の顆粒が充満し、構造に於て腺である。この発光器官の前方及び後方に筋肉組織があり、これの収縮によつて発光物質を外套腔の中に押し出すものと考える。

5) 押し出された発光顆粒は海水と混合して光る液となり、漏斗より噴出される。

6) この円形の器官は発光器官と考えるよりもむしろ、発光顆粒を貯蓄しておく器官と考えた方が適当である。

7) 発光顆粒を乾燥し、これに水を加えると又発光する。Luciferin-Luiferase 反応、ATP 反応は陰性であつた。

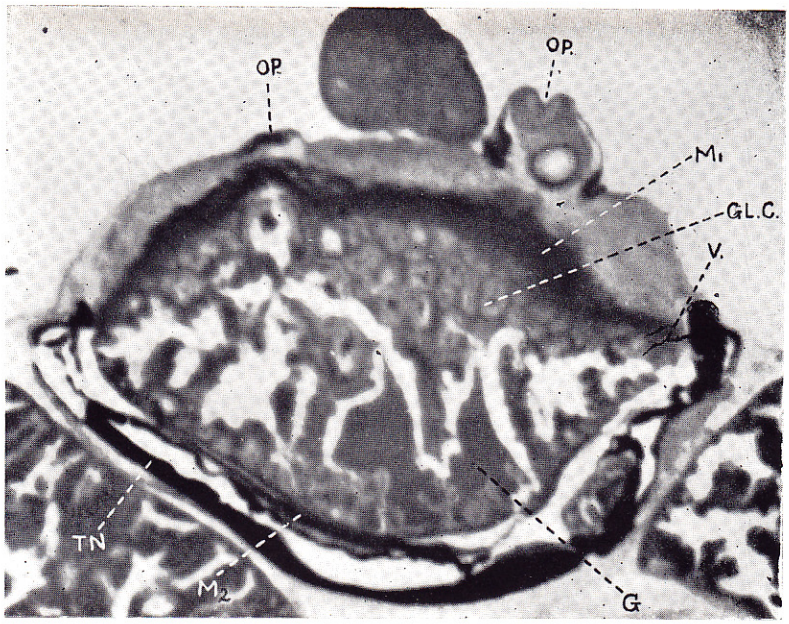

Fig. 3. Transvers section of the luminous gland reservoir. ×18
G, in compartments of the gland. GL. C., gland cell. OP, discharge openings. M_1,M_2, muscles to squeeze out the luminous secletion. TN, inc sac. V, blood vessels.

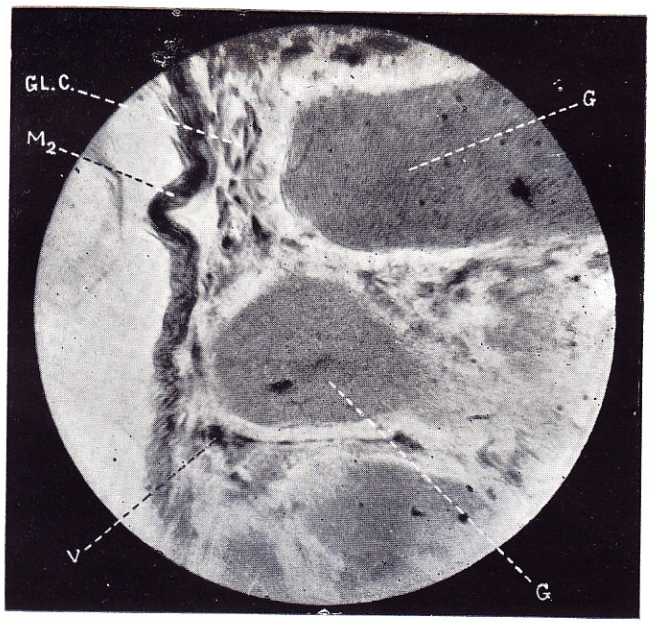

Fig. 4. Transverse section of luminous gland reservoir. ×800
Lettering same as in Fig. 3.
In compartments of the gland appears as clumps of both round and bacillus form glanules.

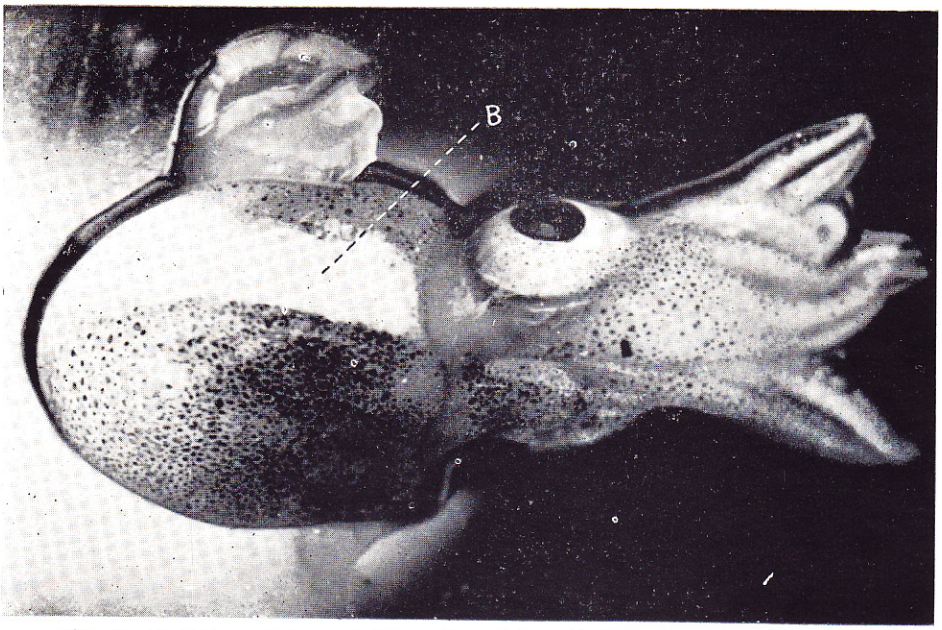

Fig. 1. *Sepiolina nipponensis* (BERRY) ×2.5 B, white band on the mantle.

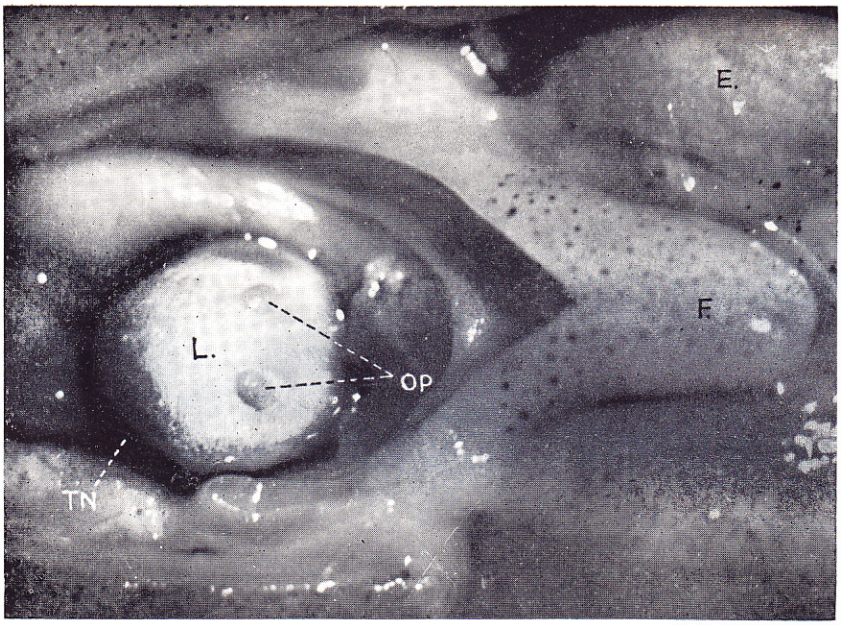

Fig. 2. Luminous gland reservoir of *Sepiolina nipponensis* ×6
L, luminous gland reservoir. TN, ink sac. OP, opening of luminous gland. F, funnel. E, eyeball.