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Some Observations on Photophores, especially a Transparent Cheek Area in the Toadfishes, *Porichthys*.[1]

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(with 2 plates)

カルフォルニアの発光魚 *Porichthys* の発光器 特に眼の下部の透明部について

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1. Introduction

The fishes belonging to the genus *Porichthys* are shallow water teleosts possessing numerous photophores on the body surface, embedded in th dermis. The genus, containing several species, is found off the coasts of North, Central, and South America. In California these fishes are called "midshipman", because the shining photophores on the skin are supposed to resemble the buttons on a midshipman's uniform. They are also called "singing fish" or "canary bird fish" because of a curious humming noise they make with the air bladder (JORDAN & EVERMAN)[1].

Porichthys notatus, the Californian form, is the best known of the group. HUBBS and SCHULTZ (1939)[2] have published a bibliography for the group, recognizing a new genus *Nautopaedium* JORDAN instead of *Porichthys* for *porosissimum*, the only Atlantic species. *Porichthys porosus*, a non-luminous form, has also been separated as *Aphos porosus*.

During a collecting trip around the world in 1959–1960, I acquired several specimens of *Porichthys myriaster* at the fishermen's wharf of San Pedro, Los Angeles from an Italian fisherman. I collected several *Porichthys margaritatus* at Guaimas, Mexico, and also was able to study the preserved specimens of these fishes in the United States National Museum and in the Scripps Institution of Oceanography, in the Museum of Stanford

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University. Some observation on luminescence were performed on freshly caught material, and the rest of the material was preserved in formalin and sent to the laboratory of the Yokosuka City Museum for anatomical and histological study.

The main purpose of this study was to observe the photophores of these fishes under a fluorescent microscope in addition to the ordinary microscopical examination, and to discuss the structure and function of the transparent area situated under either eye.

The arrangement of the luminous organs of the genus *Porichthys* differs slightly according to species, but the general pattern is alike in all. According to GREENE (1899)[3], *Porichthys* possesses four lateral lines, and the photophores are disposed in rows. These photophores can be separated into a cephalic series and a trunk series. The cephalic series includes the branchiostegal, mandibular, and opercular rows. The trunk series comprises the lateral, dorsal, pleural, anal, gastral, ventral and gular rows. As many as 840 photophores have been counted on *Porichthys notatus*, some one hundred of which are rudimentary. At least 750 are well developed.

GREENE and GREENE (1926)[4] studied *Porichthys notatus* specimens caught during the spawing season in Montorey Bay, California. No luminescence was obseved in free swimming fish, but light would appear after strong mechanical or electrical stimulation. The light was slow to appear after electrical stimulation. According to GREENE, when injections of adrenalin were given, luminescence appeared near the point of injection and gradually spread to other regions until the whole body was glowing brilliantly. NICOL (1957)[5] studied the effects of electrical stimulation and of the injection of hormonal preparation on the luminescence of the photophores of *Porichthys myriaster*, and concluded that they are under nervous control. SHOEMAKER (1957)[6] observed the luminescence of the Atrantic species, *Nautopaedium porosissimum*. In California, *Porichthys* is not a rare fish. The construction and luminescence of its photophores were studied by GREENE, GREENE and GREENE, HARVEY (1952), NICOL, SHOEMAKER, and others, but no one recognized or discussed the transparent area under the eye.

2. Material and Methods

Ten fresh specimens of *P. myriaster* were used for observations of luminescence and ten specimens were used for test reactions to luciferin-luciferase. Five preserved specimens were used for anatomical and histological studies. For microscopic examination, pieces of skin containing photopheres and a part of the transparent area under the eye were immersed in Bouin's solution and formaldehyde. The material was sectioned in celloidin, and stained with Heidenhain's haematoxylin eosin. Unstained material and material stained with Acrydin orange also studied under the fluorescent microscope.

3. Photophores

As described by GREENE and NICOL, the photophores of *Porichthys* are deeply embedded in the dermis. The photophores appear outwardly as shining pearl-white dots. Each one is made up of the following components ; a luminous cell, a lens, a reflecting layer,

and melanophores. As shown in Fig. 2, the lens consists of polygonal cells. They stain deeply with eosin, appearing dark reddish violet. This lens at first appears as if it were made of luminous tissue, but actually the luminous tissue is between the lens and the reflecting layer.

The luminous tissue consists of a single epithelial layer. The cytoplasm stains very poorly, becoming pale violet pink. The light of the luminous cells passes downward through the lens.

As described by NICOL, the connective tissue sheath or reflecting layer extends downwards and inwards from the dermis, and is continuous around the sides of the lens and luminous cells. It is made up of parallel fibers which stain poorly, becoming pale blue. The reflecting effect is due to guanine.

Outside the reflecting layer is another layer that is loosely reticular.

GREENE was unable to show the existance of any nerves, but he indicated a rich supply of blood vessels. NICOL instead demonstrated that both nerves and blood vessels extend from the dermis proper to the luminous cells. GREENE believed that the photophores of *Porichthys* were not innervated, and that the occasional fibers seen entering the luminous cells were branches of the nerve. NICOL, however, showed clearly that the luminescence is under nervous control.

Under the fluorescent microscope, the unstained lens showed pale, greenish yellow fluorescence, while the luminous cells and the reflecting layer showed none at all. When the material was stained with Acrydin orange diluted 1:100,000, the lens structure showed very strong greenish fluorescence, and the luminous cells showed pale greenish flurescence. In material stained with haematoxylin eosin, the blood vessels which run from the dermis to the luminous cells showed a beautiful golden fluorescence.

4. The Large Transparent Area under the Eye

The six following species possessing photophores have also a large transparent area under either eye : *Porichthys notatus. P. myriaster, P. greenei, P. margaritatus, P. analis, Nautopaedium porosissimum. Aphos porosus*, which is closely related to *Porichthys*, has neither photophores nor the transparent area under the eye. As shown in Fig. 1, *P. myriaster* has 16 photophores at the bottom of the large transparent area. These have been generally overlooked up to now. Moreover, no one up to now has reported on the function of this transparent area. Suspecting that it might be part of a luminous organ, I observed some fresh material in the dark. In this material the photophores, especially those of the ventral, gastral, and pleural rows showed a continuously dim light, while the 16 photophores below the eye refleced a very dim light up through the transparent area. A section of the eye with the transparent area is shown in Figs. 3 and 4. The transparent area is a loose reticular layer. At its bottom arranged the 16 photophores, pointing downward, each emitting its light through a lens. However, the guanin content of the reflecting layer of these 16 photophores is very poor, so that the reflecting layer is nearly transparent. Thus the light of the luminous cells passes up through the reflecting layer into the tarnsparent area under the eye. Measurements of the transpa-

Table 1. Measurement of the Transparent Area of *Porichthys*

Species	Body length	Diameter of Eye	Transparent Area		Remarks
			Length	Height	
Porichthys myriaster	280 mm	8.0 mm	10.0 mm	3.0 mm	Yokosuka City Mus. Gr.No. LF.30, Loc. San Pedro, Apr. 1959. Coll. Y. Haneda
	145	5.0	7.0	2.5	〃 Gr. No. LF. 31 〃
	120	4.5	7.0	2.5	〃 〃 32 〃
	98	3.0	4.0	2.0	〃 〃 33 〃
	110	4.5	7.0	2.5	S.U-N.S.M. Gr.No. 63, Cat. No. 15238 Loc. Baja Calif. Mexico
P. notatus	105	4.0	4.0	1.2	Stanford Univ· Nat. Sci. Mus. Gr. No. 63, Cat. No. 15822 Loc. Coast South of Santa Catalina Is. Coll. "Zacca"
	160	7.0	6.0	2.2	S.U.N.S.M. Gr. No. 63. Cat. No. 15825 Loc. Santa Barbara. Coll. "Zacca" Sept. 1958
P. greenei	80	3.8	2.0	1.2	S.U.N.S.M. Cat. No. 6512 Loc. Panama. Coll. C.H. Gilbert et al.
P. analis	105	3.0	4.0	1.8	S.U.N.S.M. Gr. No. 63, Cat No. 19397 Loc. Baja Calif. Mexico. Coll. Shrimp trawl, March 1, 1951
P. margaritata	75	4.0	3.0	1.8	Yokosuka City Mus. Gr. No. LF. 35, Loc. Guaimas, Mexico Coll. Y. Haneda
	170	7.0	6.0	2.0	S.U.N.S.M. Gr. No. 63, Cat. No. 46739 Loc. Manzanill, Mexico
Nautopaedium porosissimum	120	4.0	4.0	1.6	S.U.N.S.M. Gr. No. 63, Cat No. 40535 Loc. Freeport, Texas, Coll. May 12, 1940 J. L. Baughman
	130	5.0	4.2	2.0	S.U.N.S.M. Gr. No. 63, Cat. No. 40536
	260	8.0	10.0	3.5	S.U.N.S.M. Gr. No. 63, Cat. No. 52348 Santos, Brazil.
	100	2.2	3.0	1.0	Leland Stanford Junior Univ. Mus. No. 227 Loc. Galapagos.
Aphos porosus	130	5.0	None		S.U.N.S.M. Gr. No. 291, Cat. No. 22680 Loc. Tome, Chil. Coll. Albatros. Non - Luminous Form

rent area in several species are shown in Table 1. It is generally thought that this fish buries itself in the sand at the bottom of the sea, with only the eyes showing. If this is so, it can be surmised that the fish leaves also the transparent area under each eye exposed, causing it to light up under stimulation. In this the function of these area would be similar that of the cheek organs of *Anomalops*, *Photobrepharon* and other deep sea luminous fish.

5. Biochemistry

A number of photophores of *Porichthys myriaster* were cut from their bodies and thoroughly dried. They were ground in a mortar and moistened with tap of water in the dark, luminescence did not appear. A negative luciferin luciferase reaction was obtained by mixing hot and cold water extracts of the crushed photophores.

Summary

1) This report deals with the photophores of *Porichthys*, which were observed under a fluorescent microscope. In unstained material, the lens of the photophores showed autofluorescence, but the luminous cells and the reflecting layer did not.

2) The six species of toadfish which possess photophores, viz. *P. notatus P. myriaster*, *P. greenei*, *P. margaritatus*, *P. analis* and *Nautopaedium porosissimum*, have also a large transparent area under either eye. The related species *Aphos porosus*, which does not have photophores, does not have this transparent area. Therefore, it is surmised that this area functions as a part, namely as the lens, of a large luminous organ, This function would be similar to that of the cheek organs of *Anomalops*, *Photobrepharon* and other deep sea luminous fish.

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カルフォルニアからメキシコ，中，南米にかけて，比較的浅い海底に住む *Porichthys* 属の魚は腹面に，真珠様光沢の発光器が並び，丁度，海軍士官候補生の制服のボタンを思わせるので，カリフォルニアでは士官候補生ミッドシップマンの名で知られている。発光器の数は850を数え，外観は大体同じようであるが種類によって少しずつ配列が異っている。HUBBS 等は大西洋岸の *P. porosissimum* を *Nautopaedium*，発光器のない *P. porosus* を *Aphos* なる新属とした。

著者は1959年から60年にかけてのアメリカ，欧州，東南アジアへの採集旅行の折，カルフォルニア，メキシコに於て，その発光を観察すると共に，スクリップス海洋研究所，スタンフォード大学，ワシントンの U. S. 国立博物館の材料を検査する機会を得た。この属の魚の発光器に就ては既に古くから知られ，その構造，発光の状態等についても多くの報告があるが，著者はロサンゼルス郊外サンピドロにて漁獲された *P. myriaster* の発光を観察中，眼の直下に淡黄色透明の部分のあるのを見つけた。この部分は Fig. 1. に示すように，両眼の左右にかなり大きな面積を占め，下部は黒色色素層 (PG) にておおわれ，その色素層の下に *P. myriaster* では今まで見落されていた16個の発光器 (PHOT) が一列に並んでいるのを見た。この透明の部を眼を含めて横断すると Figs. 3 及び 4 に示すように，一大発光器を思わせる。即ち，16 個の発光群を大発光器の発光体と見なすと，眼の下の透明部はレンズと黒色色素層である。この魚は海底の砂中に体を埋めて，眼だけを出していると言われるが，眼と共にこの透明部も出して，発光しているのではなかろうか。

個々の発光器は Fig. 2 に示すように皮下に埋れた真珠様小発光器でエオジンに染るレンズ (LENS) と，エオジンに染りにくい，発光体 (LC)，その発光体を包む銀白色不透明の反射器 (REFL)，黒色色素斑 (PG) よりなり，発光体へは毛細管，神経が分布している。反射層はグアニンを含み，不透明で反射の役目をしているが，眼下の透明部の下部に並んだ16個の発光器では反射層内にグアニンの含有量が少く，殆んど透明で発光体の光は下方だけでなく，反射器を通して，透明部に達する。従って，16個の発光器の光は総合して，透明部を通って両眼の左右から見られるわけである。

実際にサンピドロで採集された *P. myriaster* を観察した処，腹面の発光器が連続的に光るのを見たが，観察した 30 個体の中，5 個体では明らかに眼の下の透明部から，かすかな光を認めることが出来た。

従って，今まで見過されて来た眼の下の透明部は腹側の個々の発光器とは別に，16 個の個々の発光器の光を光源とする大発光器であると考える。丁度，*Anomalops* や *Photobrepharon* に見られる眼下の大発光器と同じような役目をする発光器ではないかと思われる。

発光器を全く持たない *Apos porosus* には，この眼下の透明部を欠くことからしてこの透明部は発光器の一部と考えるべきであろう。

腹面の個々の発光器を切りとり乾燥した後暗所で水をかけたが，発光は恢復しなかった。又，発光体のルチフエリン，ルチフェラーゼ反応も陰性であった。

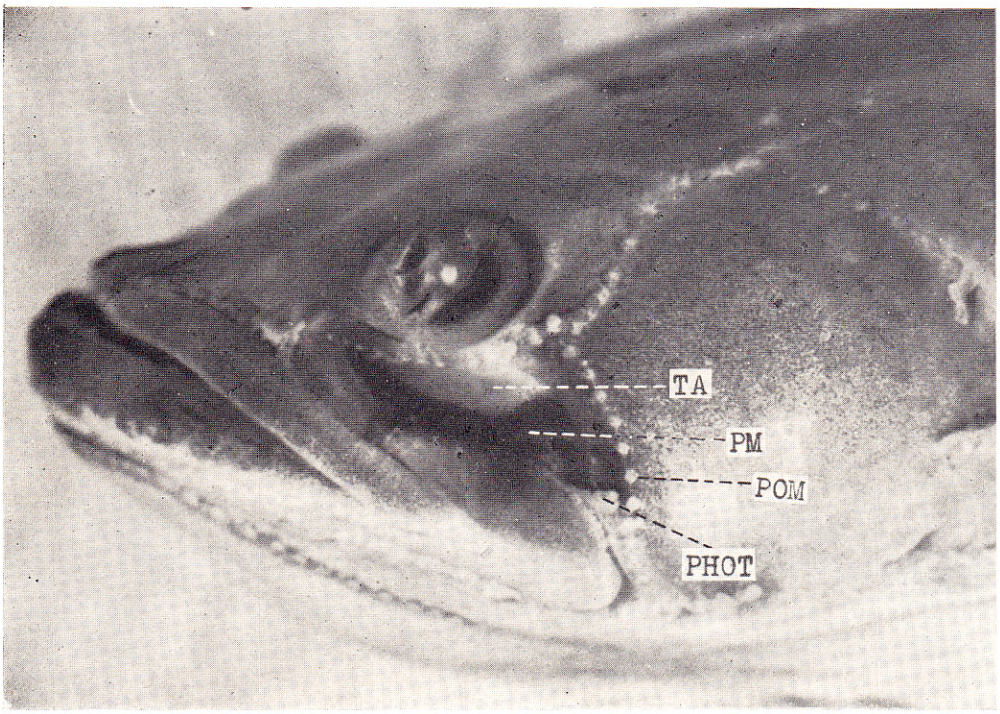

Fig. 1. *Porichthys myriaster*. showing large transparent area under the eye. TA : Large transparent area; PM : Pigment membrane; PHOT : Photophore ; POM, Mucous pore.

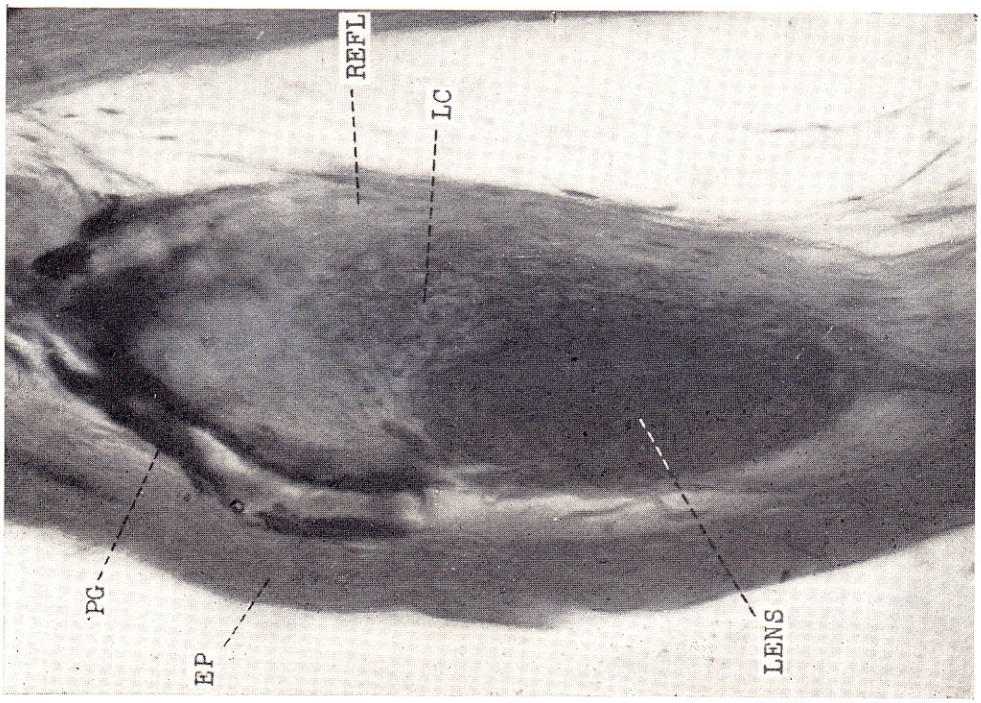

Fig. 2. Transverse section of ordinary photophore of *P. myriaster*, LC, Luminous cell; LENS, Lens; REFL, Reflector; PG, Pigment; EP, Epithelium

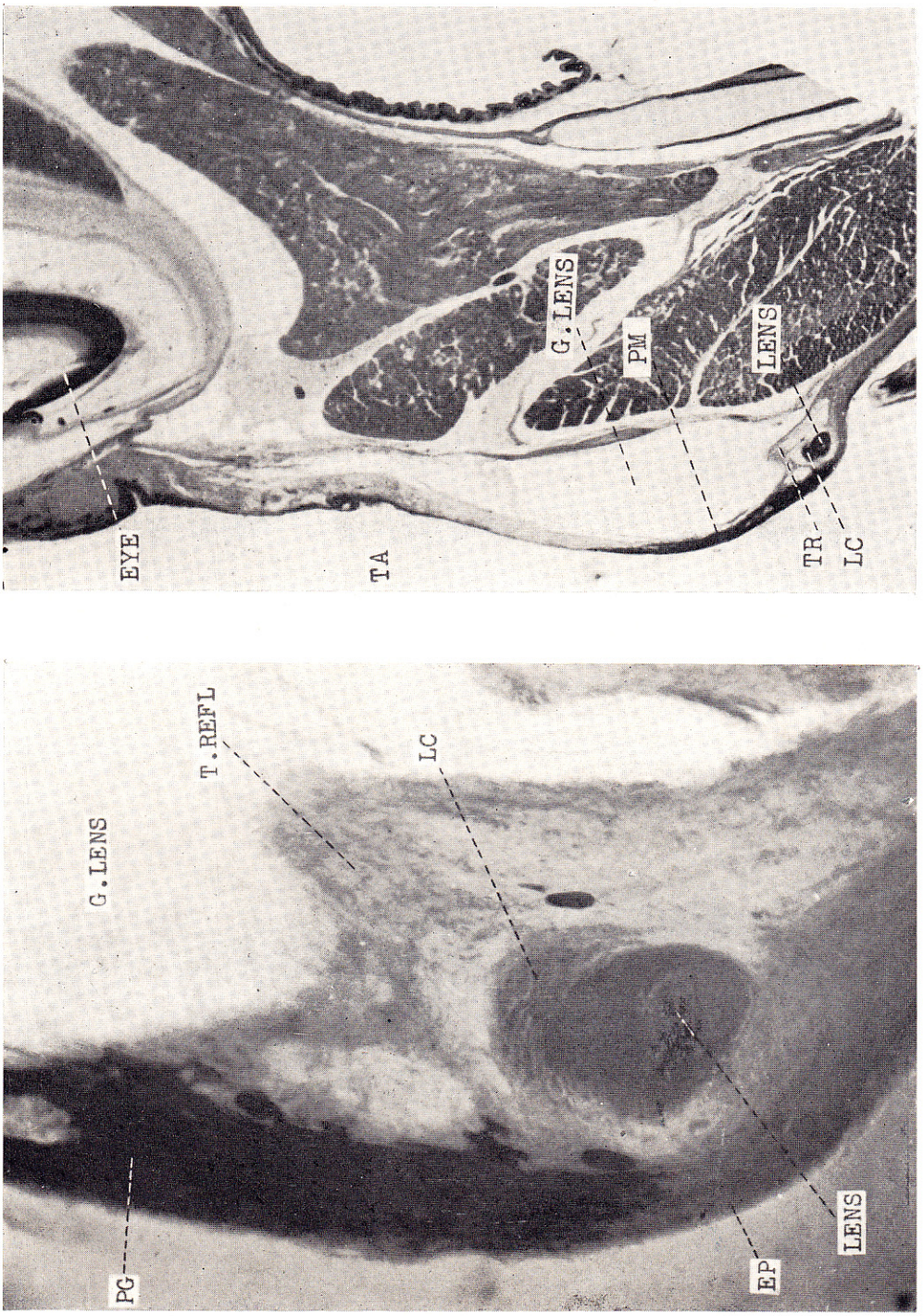

Fig. 3 and 4, Transverse section of a large transparent area unde the eye. LC, Luminous cell; LENS, Lens; T. REFL, Transparent Reflector; G. LENS; giant lens, PG, Pigment; EYE, Eye., PM, Pigment membrane.