APMB - Atti della Accademia Peloritana dei Pericolanti
Classe di Scienze Medico Biologiche
Vol. 109(2) 2021

ISSN 1828-6550

DOI: 10.13129 / 1828-6550 / APMB.109.2.2021.OS2

Original Study **OS.2(1-7)**

Morphological and immunohistochemical study on photophores of *Gonostoma denudatum* Rafinesque, 1810 (Fam: Gonostomatidae)

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Abstract

A preliminary contribution to the knowledge of the photophore structure of the mesopelagic fish *Gonostomadenudatum* from the central Mediterranean Sea (Strait of Messina) is given by means of a structural and immunohistochemical study, to describe the component structures of these luminescent organs.Photophores of *G. denudatum* are made by different functional parts: the tank with photogenic cells, lens-filter and reflector surrounded the entire organ.

Key Words: bioluminescence, lanternfish, luminous organ, structure, ultrastructure

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Introduction

Bioluminescence is light produced by a chemical reaction within a living organism. Bioluminescence is a type of chemiluminescence, which is simply the term for a chemical reaction where light is produced. The Oceans as well as the Mediterranean sea present the deep scattering layer consisting of a variety of marine animals including small mesopelagic fishes [1]. This layer of deep water plays a fundamental role in regulating nocturnal migrations which in their face trigger very important trophic mechanisms along the water column. In particular, the Gonostomatids, do not migrate to the epipelagic waters, being partly responsible for the permanent acoustic response at the 400–600 m, i.e., the Deep Scattering Layer (DSL) reported in the Mediterranean continental slope [2]. The studies on the biology and ecology of these mesopelagic species and their light emission mechanisms, which our research group has been dealing with for several years [3-8], are therefore fundamental to deepen the complicated mechanisms of ecological regulation of deep environments.

Photophores are glandular luminous organs present in the body surface of numerous species of mesopelagic fish belonging to different families (Gonostomatidae, Myctophidae, Sternoptychidae, Stomidae) [9] that regularly stranding along the Strait of Messina . Their structure and shape vary according to the species they belong to and can represent 12% of the body surface and up to 15% of the volume [10]. These bioluminescent fishes are well adapted for living in the twilight zone

and possess several photophores and other light organs [10-13], which produce intrinsic bioluminescence[14,15]. The photophores show, at a macroscopic level, similar structural organization, but different morphology when observed in microscopic analysis.

This study provides important data on the structure of the photophores of *Gonostomadenudatum*Rafinesque, 1810 , in order to clarify some aspects of the biology of this species. The genus Gonostoma is represented with two valid species around the worldwide. In Mediterranean Sea commonly is present only *G. denudatum*[16], distributed from the subtropical to temperate North Atlantic and the Mediterranean [17]. This species is widespread throughout the Eastern Central and Western Central Atlantic [18] and is important which plays increasingly important trophic roles in the food web, especially for large pelagics[19].

Materials and Methods

A total of 5 specimens of *G. denudatum* were collected along the Sicilian coast of the Strait of Messina (central Mediterranean Sea), during the sampling activity aimed to assess the phenomenon of mesopelagic fish stranding in the study area (Battaglia et al., 2017). Their photophores were still active at the time of collection. Samples were fixed immediately using different methods for structural, ultrastructural and immunohistochemical analysis, respectively.

In order to carry out a structural study on the morphology of skin photophores of D. holti, the specimens were fixed in 4% paraphormaldehyde in phosphate-buffered saline (PBS) 0.1 mol/L (pH 7.4) for 24 hr. After dehydration in an increasing alcohol series, the samples were embedded in paraffin wax. Thin sections (5 μm) were dried in an oven for 12 hr and then deparaffined in xylene and rehydrated in a decreasing alcohol series. Sections were stained using haematoxylin–eosinand. Sections were observed by means of a Zeiss Axioskop2 binocular microscope, equipped with an Image-ProPlus 3.1 software for image acquisition.

Immunofluorescence staining was performed on the ventral–lateral body of G. denudatum, where the photophores are mostly distributed. Thick sections (10 μm) on sagittal planes were obtained by paraffin-embedded samples. Serial sections were deparaffined and rehydrated, rinsed several times in PBS and blocked in 2.5% bovine serum albumin (BSA) for 1 hr. Sections were incubated overnight at 4°C in a humid chamber. with the primary antibodies Polyclonal Rabbit Anti-nNOS/NOS Type I used at 1/250 in PBS/0.3% triton X-100, according to the manufacturer's directions (BD Transductions Laboratories TM cat. n. 610310). After incubation, sections were washed in the same buffer and incubated for 2 hr at room temperature with Alexa Fluor® 594 goat anti-rabbit IgG antibody (Molecular Probes, Invitrogen A11012, diluted 1:100). After washing, sections were mounted with Vectashield (Vector Labs) to prevent photobleaching and were cover slipped. Each image was rapidly acquired in order to minimize photodegradation. Sections were

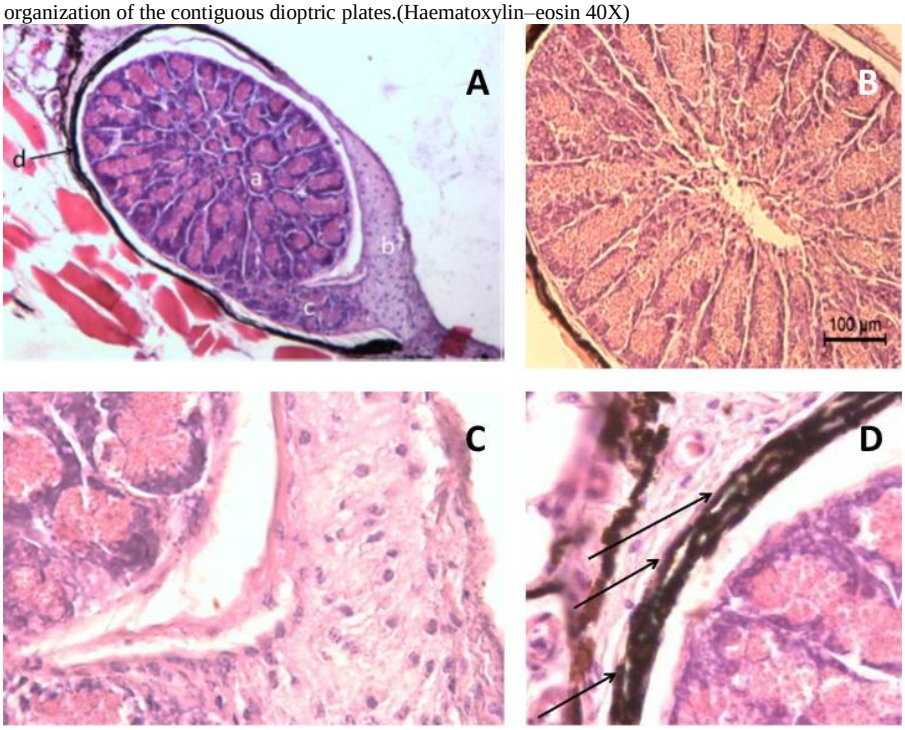
analysed and images acquired using a Zeiss LSMDUO confocal laser scanning microscope with META module (Carl Zeiss Micro Imaging GmbH) microscope LSM700 Axio Observer. Control experiments excluding incubation with primary antibody were performed (data not shown).

Results

The photogenous system of adult *G. denudatum* is constituted by: 1 Supra Orbital (SO), 1 Orbital (ORB), 3 Opercular (OP), 9 Branchiostegal (BR), 6 + 10Ventral series anterior to pelvic-fin base (IV), 5 (6) Ventral series between pelvic-fin base and origin of anal fin (VAV), (19) 20 Ventral series posterior to anal-fin origin (AC), 13Lateral series (OA); 14-15 AC over anal fin, 3-4 between last anal and first procurrent rays; VAV 4 over anus; last OA over VAV 3; 1 supra-caudal and 2 infra-caudal glands; no glandular masses associated with SO, ORB or OA. (Check-list UNESCO in 1973;[20])

In the photophore of *G. denudatum* different functional parts were observed. Particularly, in the tank photogenic cells, filter lens, reflector and a pigmented layer were found. (Fig 1a)

Fig. 1 Morphological analysis of the *G. denudatum* photophore.
A)Longitudinal section of the photophore of *G. denudatum*: photocites (a); lens (b); collector tank-lens (c); reflector (d). (Haematoxylin–eosin 10X)
B) Longitudinal section of the photophore's tank of G. denudatum containing photogenic cells. (Haematoxylin–eosin 20X)
C) Longitudinal section of the photophore's lens of *G. denudatum*. (Haematoxylin–eosin 40X)
D) Longitudinal section of the reflector of the photophore of *G. denudatum*. The arrows indicate the organization of the contiguous dioptric plates.(Haematoxylin–eosin 40X)



The tank showed a voluminous size with a labyrinthine feature traced by subtle connective textures. The morphological observation has evidenced a typical structure similar to the acinar glandular. These berries appeared almost spherical in shape, sometimes elongated. The photogenic cells, representing the secreting units of the grape, were regular in shape and arrayed in cords arranged radially towards the centre of the chamber.

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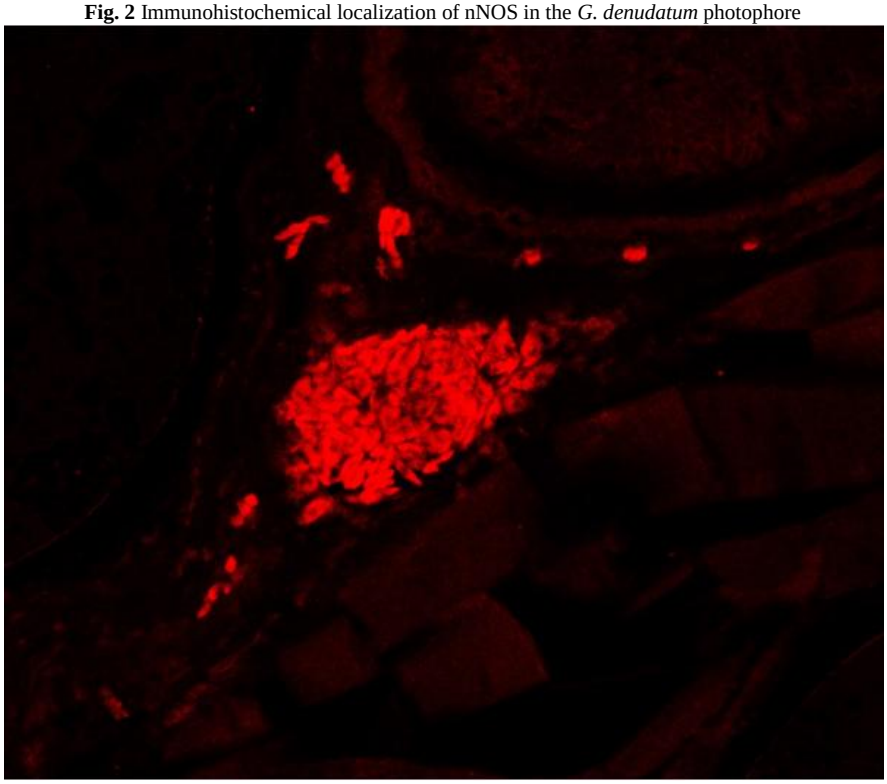
Fig. 2 Immunohistochemical localization of nNOS in the *G. denudatum* photophore



Moreover, these cells exhibited an intense basophilic cytoplasm in the basal part while the apical portion displayed the occurrence of granules of different origin. In this view the secretum is conveyed inside a central lumen of the photogenic chamber to be subsequently channelled towards the lens in accordance with Bassot (1960 a, b) (Fig. 1b). The lens - filter, was arranged as a shield to cover the lower part of the photophore and it was formed by cells, with rounded nuclei localized in an abundant extracellular matrix of a gelatinous nature (Fig. 1c). The reflector displayed a contiguous layer that envelops the entire organ consisting of a layer organized in pigmented dioptric plates, stacked compactly to each other. This morphological aspect could be related to the prevention of the light wave dispersion (Fig. 1d).

Moreover, a intense and specif immunoreaction for nNOS was found in the lens (Fig. 2).

Discussion

This study investigated for the first time the structure of the photophore of the G. denudatum, confirming the glandular nature of its bioluminescence in accordance with previous studies performed in other species of mesopelagic fishes presents in the Strait of Messina [8,12,21-24].

The istomorphological investigation of the photophore shows a particular tissue organization

different from the bioluminescence organs studied in other species. Specifically, the tank and the two dioptric annexes: the lens-filter and the reflector are connected by a collector in which the photocites convey their secretion product.

The tank containing elongated cells, organized in a radiated laminar system, converging towards the centre of the photogenic chamberThe microscopic analysis revealed the presence of several granules in each cell suggesting the presence of an evident exocytosis activity in cells. The secretum is accumulated in granules and carried towards the filter lens, thanks across the collector.

The morphological analysis of the lens confirms its function to collection of substrate for its chemical re-processing. Indeed, the lens is linked to the photogenic chamber by a collector, where the secretum produced by the photocytes is transported. Moreover, it has been confirmed the presence of the reflector and the pigmented layer in photophores oh *G. denudatum* having a dioptric and protective function. A recent study on the lanternfish D. holti[5] showed that in lanternfish the reflector is constituted by a thin pigmented layer, made up from a single bundle of thin dense and intricate filaments with melanin granules, which surrounds and covers its external surface.The melanin granules that surround the entire organ are involved in the dioptric function, avoiding the light dispersion and conveying it outside the photophore.

According to Denton et al. [21], the reflective features of the reflector have an important effect upon the spectral quality of the emitted light. As found in other species [21], the reflector of the ventral photophores in *G. denudatum* is involved in the countershading mechanism.

Results of the immunohistochemical analysis show the immunoreactivity in the cytoplasm of cells of the lens. This result may be related to the activity of the membranes of Golgi apparatus, for the synthesis of the nNos enzyme. The NO (nitric oxide), synthesized by the cytosolic protein named nitric oxide synthase (NOS), acts as a neurotransmitter and/or modulator of the adrenergic control of bioluminescence [25,26]. The n-NOS sequence is extremely well conserved among species suggesting that n-NOS has maintained the same biological functions in fish and mammalian species [27]. Our results showed that the synthesis of NO occurs in the entire body of lens, whereas no reactions were observed in the photogenic chamber. These results lead to deepen the ultrastructure studies of the photophores, in order to better understand the fundamental role of these organs in the ecological equilibrium of these mesopelagic species.

Conflicts of Interest: There is no potential conflict of interest, and the authors have nothing to disclose. This work was no supported by any grant.

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Communicated and Received November 9, 2021, revised and accepted November 9, 2021, published on line November 15, 2021