Histological evidence for secretory bioluminescence from pectoral pockets of the American Pocket Shark (Mollisquama mississippiensis)

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The function of pocket shark pectoral pockets has puzzled scientists over decades. Here, we show that the pockets of the American Pocket Shark (Mollisquama mississippiensis) contain a brightly fluorescent stratified cubic epithelium enclosed in a pigmented sheath and in close contact with the basal cartilage of the pectoral fins; cells of this epithelium display a centripetal gradient in size and a centrifuge gradient in fluorescence. These results strongly support the idea that pocket shark’s pockets are exocrine holocrine glands capable of discharging a bioluminescent fluid, potentially upon a given movement of the pectoral fin. Such capability has been reported in many other marine organisms and is typically used as a close-range defensive trick. In situ observations would be required to confirm this hypothesis.

Pocket sharks (Mollisquama spp., Squaliformes) are among the most enigmatic cartilaginous fish species. Extremely rare —only one mature female and one immature male have been collected so far, from the Nazca Submarine Ridge in the southeast Pacific Ocean (M. parini discovered in 1984)1 and the central Gulf of Mexico (M. mississippiensis, discovered in 2010 and described in 2019)2, respectively—, these oceanic deepwater sharks also display an extraordinary combination of morphological traits including several cranial synapomorphies4, a strong diphagic heterodonty possibly reflecting an ectoparasitic feeding strategy, a peculiar denticle morphology, putative photogenic organ (photophore) aggregations on the ventral side and a lateral conspicuous slit above each of the pectoral fin bases2. These slits form the opening of the ‘pockets’ from which pocket sharks received their name. Representing a unique feature among sharks, these side pockets have puzzled scientists since their discovery, which led to many speculations about their functional significance. While Dolganov considered these glands to produce sexual pheromones1, more recent work suggests they might be analogous to the abdominal pouch of the Taillight Shark (Eurytomomiroides zantedeschia), another shark species related to Mollisquama species, that has been observed to secrete and eject a blue bioluminescent fluid upon mechanical stimulation2,5–7. This pouch on the taillight shark, located immediately in front of the cloaca of both male and female specimens, shares indeed several morphological similarities with pocket shark pectoral pockets, including the presence of voluminous unscaled skinfolds raised above surrounding tissue along the slit margin and internal tightly packed villiform projections. Besides, the inner cavity of this pouch is also dark grey with a lighter bluish-grey color close to the opening. The secretory tissue of the Taillight Shark pouch is pseudostratified, made of tall columnar cells (containing small cytoplasmic granules and a large distal inclusion) separated and topped by flattened cells5.

In this work, we used light and fluorescence microscopy to analyze several histological sections through the right pocket of the holotype specimen of the American Pocket Shark (M. mississippiensis, male, 142 mm TL) as an attempt to elucidate their biological function.

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Results

Sloping ~ 45° posteriorly to just below right pectoral fin base, the right pocket gland’s orifice had a length of ~ 4 mm (Fig. 1a) and was bordered with a series of bluish dermal folds. The interior of the gland was made of numerous darker villiform structures, which encompasses 95% of the gland volume (~ 16 mm³ or 0.1% of total shark volume) and its blind end was pointing anterodorsally (towards the head). No fluid was observed into the lumen of the gland but a frozen milky white fluid, potentially coming from the pocket, was observed surrounding the specimen before fixation.

Histological sections demonstrated that villiform structures reached a length of 50–150 μm and consisted in a stratified cuboidal epithelium composed of over 50 cell layers—colored in red by Masson’s trichrome (Fig. 1b,c). This epithelium was in close contact with the basal cartilage of the pectoral fin (Fig. 1b) and enclosed in a pigmented sheath surrounded by connective tissue (Fig. 1b,c). Fluorescence microscopy revealed intense green autofluorescence from villiform structures (Fig. 1d) and highlighted two cell populations: (i) small cells (2–4 μm) present close to the basal lamina and displaying a bright, homogeneously fluorescent cytoplasm (Fig. 1e) and larger cells (4–8 μm) showing only peripheral fluorescence (but with an overall larger quantity of fluorescent material) and situated between basal cells and the gland’s lumen (Fig. 1f). Due to the presence of a continuum between these two cell populations, cells composing the villiform structures displayed a centripetal gradient in size (Fig. 1g, Supplementary Spreadsheet S1) and a centrifuge gradient in fluorescence (Fig. 1h, Supplementary Spreadsheet S1).

Discussion

Bioluminescence—e.g., the ability for an organism to produce a visible light thanks to a chemical reaction—is common in marine organisms, and especially in the permanent darkness of the deep-sea where most taxa use light to evade predators, acquire food or communicate with conspecifics10,9. In sharks, only two families are known to be endowed with bioluminescence capability: the Etmopteridae (Squaliformes; 53 species) and the Dalatiidae (Squaliformes; 10 species including pocket sharks and the Taillight Shark)10,11: phylogenetic analyses and putative photogenic organs found in Zameus squamulosus suggests that Somniosidae (Squaliformes) might also contain bioluminescent species1 but observation of light-emission displays would be needed to confirm this hypothesis. Catsharks (Scyliorhinidae, Carcharhiniformes) and carpet sharks (Orectolobidae, Orectolobiformes), on the other hand, show biofluorescence, a phenomenon distinct from bioluminescence, which results from the absorbance of the ambient down-welling light and its re-emittance at longer, lower-energy wavelengths12.

Wing-shaped structure supports the hypothesis that these peculiar organs secrete and discharge a light-producing substance to the exterior via a folded aperture and closely associated with the basal cartilage of the pectoral fin. This structure supports the hypothesis that these peculiar organs secrete and discharge a light-producing substance to the exterior in response to a specific movement of the pectoral fins (upon a given stimulation). Indeed, (1) bioluminescent compounds often display autofluorescence13–16, (2) photogenic cells (including secretory glands) from mesopelagic animals are often enclosed in light-tight pigmented sheaths to avoid unwanted bioluminescence detection17–18 and (3) secretory photogenic glands are sometimes associated with structures allowing fast discharge of luminous compounds19,20.

The ring-shaped fluorescent pattern of the cells situated at the apex of the villiform structures, which contrasts with the homogeneously fluorescent cytoplasm of basal cells, might result from a progressive accumulation of cytoplasmic inclusions in the center of the cell. Cytoplasmic inclusions have been observed in both the photocytes and secretory photogenic cells of dalatiid sharks and might be linked to luminescence competence21,22. The histological organisation of the villiform structures is consistent with a holocrine secretion mode, where small basal cells, accumulating fluorescent material and developing a cytoplasmic inclusion, would progressively turn into larger apical cells when approaching the lumen of the gland, finally becoming the product of the secretion23.

Holocrine secretions are rare among secretory photophores from marine animals, which appear—based on the morphology of secretory cells and/or the examination of secretion products—to be mostly apocrine (secretion via the breaking up of the apical part of the secretory cell, resulting in the formation of extracellular membrane-bound vesicles)21,24–27 or merocrine (secretion via exocytosis from secretory cells)28,29 glands. Interestingly, the only other reported case of holocrine photogenic glands is found in tubeshoulder fishes (Sebastidae), which also possess two light-producing glands opening in the close vicinity of pectoral fins30; such similar structural organization likely represents a case of convergent evolution. Furthermore, piscine venom glands—which are also holocrine structures lacking associated musculature—, rely on a mechanical coupling with skeleton structures (e.g., grooved spines) to discharge their secretion31–33, potentially representing another convergent evolution case. Surprisingly, however, the stratified cubic and putatively holocrine epithelium of the pocket shark pockets appears dramatically different from the pseudostratified columnar and putatively apocrine (based on morphology) epithelium of the pelvic photogenic pouch of the Taillight Shark3, suggesting an independent origin of the two organs in these closely related species. Reaching close to 10 μm in diameter, apical cells of the villiform structures reach a similar size as photocytes of juvenile dalatiid (Squaliolus aliciae; I. M. Claes, personal observation) and etmopterid sharks (Etmopterus spinax)34. In the Slendertail Lantern Shark (Etmopterus mulleri), photophores are known to form enlarged photogenic structure at the base of the dorsal fin35. Similarly, one can suppose the pocket shark pockets to be modified photophores, that merged and invaginated themselves to become a secretory gland. The photogenic position of M. mississippiensis among Dalatiidae21 supports the pocket glands to be a derived character, posterior to the presence of epidermal ventral photophores.

Secretory luminescence is relatively uncommon among marine organisms, i.e., concerning ~ 10% of marine bioluminescent genera. However, ‘bioluminescent cloud’ emitters are found in most marine taxonomic groupings (Fig. 2a), which probably results from multiple independent origins of the cloud-emitting capability and highlights its paramount ecological significance. Although secretory luminescence is involved in intraspecific
sexual courtship in ostracods\textsuperscript{36} and in Odontosyllis worms\textsuperscript{37,38}, probably because of the energy costs associated
with the production of the secretory products, the primary function of bioluminescent cloud emission appears to be last-minute short-distance defensive behaviors to facilitate escape8. This and the fact that the investigated specimen is not sexually mature let us hypothesize that the pocket shark pockets (and the Taillight Shark pouch) are used in similar ‘smoke screen’ predation avoidance contexts, potentially blinding approaching predators (Fig. 2b). In situ observations and additional biological material would be needed to confirm this hypothesis, even if this might require some patience given the extreme rarity of pocket sharks.

Methods

Histology. The holotype (TU 203676; 142.0 mm in total length and 14.6 g in total weight) of *M. mississippiensis* (which represents the unique specimen of this species) was collected in the central Gulf of Mexico on 4 February 2010 by the NOAA Ship *PISCES* (survey 7620101001, station 053) and immediately frozen in water after capture for about 3 years before being preserved in 20% formalin for 3 weeks and gradually transferred to a final solution of 70% ethanol for storage. The right pocket of this specimen was excised, cut in several subsamples and dehydrated in a graded ethanol series, with some of the subsamples fixed in Bouin’s fluid beforehand. Subsamples were then embedded in paraffin using a routine method39. Sections (10 µm in thickness) were cut using a Microm HM340E microtome and mounted on clean glass slides (SuperFrost Ultra Plus, Menzel GmbH & Co. KG, Germany). Sections obtained from ethanol-fixed samples were analyzed using the FITC-filter of a Zeiss Axioskop A1 microscope equipped with an AxioCam ICc3 camera (Zeiss, Oberkochen, Germany) for

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**Figure 2.** (a) Systematic distribution of ‘bioluminescent cloud’ emitters among 566 bioluminescent marine genera. Names in capital letters represent “phylum-level” groups or broader. Circles scale to the number of marine bioluminescent genera in a given taxonomic grouping while blue color indicates the proportion of genera with ‘bioluminescent cloud’ emitters. Blue taxonomic grouping contains at least one genus known to discharge intrinsic bioluminescent secretions outside of their body. (b) Artistic illustration of a putative defensive cloud-emitting behavior in an American Pocket Shark (left) following the attack of a Goblin Shark (*Mitsukurina owstoni*; right) in the darkness of the deep-sea (illustration by J. M. Claes).
Scientific Reports

Received: 20 July 2020; Accepted: 8 October 2020
Published online: 30 October 2020

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Acknowledgements
J.M.C. is a Scientific Collaborator of the Catholic University of Louvain (Marine Biology Laboratory). J.D. is postdoctoral researcher from the F.R.S.–FNRS (Ref nº34761044). L.D. is a postdoctoral researcher from the Catholic University of Louvain. J.M. is Research Associate to F.R.S.–FNRS. This paper is a contribution to the Earth and Life Institute – Biodiversity (ELIB) and the Centre Interuniversitaire de Biologie Marine (CIBIM). We deeply thank Henry L. Bart from Tulane University Biodiversity Research Institute for giving us access to the holotype of M. mississippiensis, as well as the two anonymous reviewers whose valuable suggestions allowed to significantly improve the manuscript.

Author contributions
M.A.G. and M.H.D. collected the specimen and provided the pocket tissue for histological analysis. J.M.C., J.D. and L.D. performed the histological analyses. J.D. took light and fluorescent microscopy images. J.M.C. analyzed the results, wrote the article and created the exhibits. All authors reviewed the final version of the manuscript.
Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41598-020-75656-8.

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