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BRIEF COMMUNICATION

The first evidence of intrinsic epidermal bioluminescence within ray-finned fishes in the linebelly swallower *Pseudoscopelus sagamianus* (Chiasmodontidae)

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Abstract

External and histological examination of the photophores of the linebelly swallower *Pseudoscopelus sagamianus* reveal three epidermal layers of cells that form the light-producing and light-transmitting components of the photophores. Photophores among the examined photophore tracts are not significantly different in structure but the presence of mucous cells in the superficial layers of the photophore suggest continued function of the epidermal photophore in contributing to the mucous coat. This is the first evidence of intrinsic bioluminescence in primarily epidermal photophores reported in ray-finned fishes.

KEYWORDS

bioluminescence, deep-sea, histology, integument, photophores, *Pseudoscopelus sagamianus*, Scombriformes

Bioluminescent organs evolved at least 27 times in teleosts and include multiple instances of the evolution of both bacterial and intrinsic bioluminescent organs (Davis *et al*., 2014, 2016; Hastings, 1983; Herring, 1987). Known bacterial bioluminescent organs in fishes include a folded epithelial chamber derived from the alimentary tract or the epidermis, such as organs derived from the oesophagus (Chakrabarty *et al*., 2011), the intestine (Dunlap & Nakamura, 2011; Poulsen *et al*., 2016), the perianal proctodeum (Ghedotti *et al*., 2018; Haneda, 1957; Somiya, 1977) and the epidermis (Bassot, 1968; Munk, 1999; Okada, 1926). Cases where the bioluminescent bacteria are primarily intracellular are not known in fishes (Labella *et al*., 2017). Intrinsic bioluminescence evolved in a more diverse range of tissues of origin, including muscle (Johnston & Herring, 1985; Merrett *et al*., 1973), the hepatopancreas (Ghedotti *et al*., 2015), the intestine (Herring, 1977) and the dermis (Hansen & Herring, 1977; Lawry, 1973; Mallefet *et al*., 2019; Nichol, 1957, 1958; Poulsen, 2019). In a few clades of fishes, the anatomical basis of their bioluminescence has not been established (Davis *et al*., 2016).

The photophores of *Pseudoscopelus* Lütken 1892 were first described by Lütken (1892) as serial mucous pores. Beebe (1932) was the first to recognise these as bioluminescent when he observed blue-green light in fresh specimens. Haneda (1950) recognised *Pseudoscopelus* as having photophores that are similar to those in the Myctophiformes, Stomiiformes and the batrachoidiform genus *Porichthys* Girard 1854 in being serial and emitting light directly, but he did not discuss the anatomical structure of the photophores in *Pseudoscopelus* spp. The patterns of the serial photophores in *Pseudoscopelus* spp. subsequently have been important in the taxonomy of the group (Melo, 2010; Melo *et al*., 2007; Prokofiev, 2014; Spitz *et al*., 2007), but their anatomical structure has not been determined and whether bioluminescence in *Pseudoscopelus* spp. is intrinsic or bacterial has not been established (Davis *et al*., 2016). The fact that they have cutaneous, serial photophores has led to the assumption that their bioluminescence is intrinsic as it is in other well-studied taxa with directly-emitting serial photophores (Herring, 1987; Paitio *et al*., 2016; Priede, 2017).

The purpose of this study was to determine the structure of the bioluminescent organs in *Pseudoscopelus* spp. In particular, we seek to test the hypothesis that they produce light intrinsically using tissues in the dermis as in those other taxa with serial photophores that have been closely studied anatomically (Lawry, 1973; Mallefet *et al*., 2019; Nichol, 1957). We used preserved museum specimens and did not collect or work with live animals. We borrowed specimens from the ichthyological collections of the University of Washington, Burke Museum of Natural History (UW) and the University of Minnesota, Bell Museum of Natural History (JFBM) that included *Chiasmodon braueri* Weber 1913 (JFBM 49398), *Chiasmodon subniger* Garman 1899 (UW 45303; UW 48713 cleared and stained), *Kali kerberti* (Weber 1913) (UW 47237) and *Pseudoscopelus sagamianus* Tanaka 1908 (UW 115214). We grossly examined photophores and the homologous skin in non-bioluminescent taxa with a Leica MZ 12.5 stereomicroscope (www.leica-microsdystems.com). We used the photophore terminology of Prokofiev and Kukuev (2005) as modified by Melo (2010). We photographed whole specimens using a Canon EOS Rebel T3i camera with macro lens (www.canon.com).

We took *c*. 1.0 × 0.25 cm histological samples of the maxillary, pectoral and anal-fin photophore series (Figure 1a) with adjacent skin from the right side of *P. sagamianus* (UW 115214, 52 mm standard length; L_S) and similar skin samples from *C. braueri* (JFBM 49398, 121 mm L_S). We dehydrated samples in an ethanol series, followed by clearing in xylene, embedding in paraffin wax, sectioning every 10 μm on a rotary microtome and mounting on slides (Humason, 1979). We stained every other slide using a standard haematoxylin-eosin procedure modified with alcian blue staining (HE+A) to identify acidic polysaccharides (Charman & Reid, 1972) and the Masson's trichrome (MT) procedure to differentiate muscle and collagen (Bancroft & Stevens, 1982; Sheehan & Hrapchak, 1980). We examined slides with a Leica DM 2500 compound microscope and took digital images with an attached Q Imaging MicroPublisher 5.0 RTV photodocumentation system (www.qimaging.com). We prepared photos by increasing brightness and contrast evenly across the images and eliminating discoloration in the mounting medium outside the tissues using image-editing software.

Examination of the photophore tracts in *P. sagamianus* reveals regular photophores that are morphologically similar among tracts. The tracts are composed of irregularly arranged, small, round to oval photophores *c*. 0.05–0.20 mm in width and height (Figure 1b,c). The centre of each photophore is white to light tan in colour and the margins are darkly coloured, in some cases with the ventral margin appearing lighter with dark colour extending ventrally from the anterior and posterior margins of the photophore. The photophores did not differ substantially among tracts except that those in the maxillary and pectoral tract were more likely to be arranged in a single row than the other tracts where multiple rows in tracts were more common.

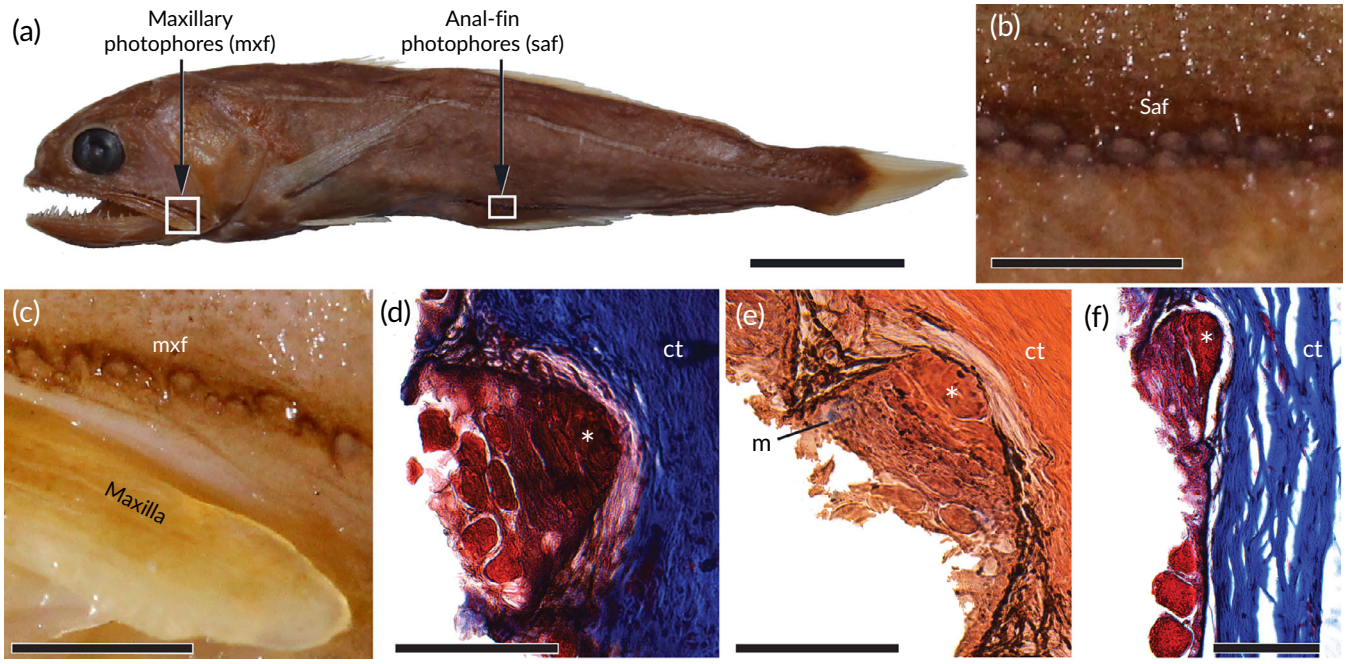

FIGURE 1 Anatomical structure of maxillary and anal-fin photophores. (a) *Pseudoscopelus sagamianus* (UW 115214; 53 mm standard length) showing the location of external photos of portions of labelled tracts in white boxes. Scale bar = 1 cm. (b) Anal-fin photophore tract showing irregular arrangement of photophores, often with more than one in dorsal-ventral stack. Scale bar = 1 mm. (c) Maxillary photophore tract showing irregular arrangement of photophores, more frequently in a single row. Scale bar = 1 mm. (d) Maxillary photophore cross section; Masson's trichrome (MT) stain. Scale bar = 0.1 mm. (e) Maxillary photophore cross section; haematoxylin-eosin procedure modified with alcian blue staining (HE+A). Scale bar = 0.1 mm. (f) Anal-fin photophore and adjacent skin cross section; MT stain. Scale bar = 0.1 mm. Pectoral-fin photophore structure is similar to the maxillary photophores and is not depicted. For histological sections, lateral at left: *, probable photogenic cells; ct, collagen-rich connective tissue; m, acidic-polysaccharide containing mucous cell; mxf, maxillary photophore series; s, likely protein-containing serous mucous cell; saf, anal-fin photophores. Standard photophore abbreviations from Prokofiev and Kukuev (2005) as modified by Melo (2010)

Examination of the same areas in non-bioluminescent chiasmodontids revealed scaleless skin with scattered dark chromatophores and an irregular surface with small prominences that is similar to the skin adjacent to the photophores in *P. sagamianus* (Figure 1b,c).

Histological cross sections demonstrate consistent similarity of the photophores among all three examined tracts, with the photogenic and overlying cells derived from and continuous with the epidermis. The photophores are conical to cup shaped with a cluster of dense, roughly cuboidal cells with granular inclusions at the deepest point. There are no identifiable chambers containing bacterial cells in the bioluminescent organ. The cuboidal cells at the deepest point of the photophore are overlain by a layer of compressed to squamous cells that are themselves more superficially overlain by another layer of compressed to cuboidal cells that include acidic polysaccharide-containing mucous cells and serous cells (Figure 1d,f). The mucous cells stained blue in the HE+A preparations (Figure 1e) and are represented by the clear oval areas in this same layer on the MT-stained slides. The serous cells are large, consistently-staining cells with granular inclusions in the MT preparation that are not stained by alcian blue in the HE+A sections. The photophore serous cells stain and appear generally similar to, though usually smaller than, the serous cells in the contiguous epidermis outside the photophores (Figure 1f) and the serous cells in the non-bioluminescent taxa. The surface of the photophore in many preparations appears to be shedding multiple large cells, but this is probably an artefact due to surface abrasion during capture or histological processing. The only dermal component appears to be the dark chromatophores that form the pigmented cup deep to the rest of the photophore in a capillary-rich region of the superficial dermis. If photophores in *P. sagamianus* are structured analogously to the photophores in other taxonomic groups with serial photophores (Lawry, 1973; Mallefet *et al*., 2019; Nichol, 1957), then, from deep to superficial, the dermal chromatophores form the pigment cup or reflector, followed by epidermal photocytes, filter and lens.

This structure of the bioluminescent organs of *P. sagamianus* and the absence of bacteria-containing chambers indicate that these organs are probably intrinsic with the photocytes and overlying cells derived from and continuous with the epidermis. This epidermal continuity was contrary to our initial expectation that the photophores would be composed primarily of structures in the dermis. The photophores exhibit a structure typical of many intrinsically bioluminescent taxa with overlying tissues serving as a filter or lens (Ghedotti *et al*., 2015; Hansen & Herring, 1977; Lawry, 1973; Mallefet *et al*., 2019; Nichol, 1957, 1958; Poulsen, 2019). The photophores also lack the obvious bacteria-containing chambers present in all other known bacterially bioluminescent fish taxa (Bassot, 1968; Chakrabarty *et al*., 2011; Dunlap & Nakamura, 2011; Ghedotti *et al*., 2018; Haneda, 1957; Munk, 1999; Okada, 1926; Poulsen *et al*., 2016; Somiya, 1977) (Figure 1d,f). The continuity of the likely photogenic and overlying cells with the epidermis and the absence of any possible photogenic cells in the collagen-rich dermis indicate an epidermal identity (Figure 1f).

The epidermal structure of the bioluminescent organ suggests potentially fewer static roles for at least some of the component cells. The typical transit of cells from basal to apical positions within the epithelium followed by slow shedding in the typical stratified teleost epidermis (Fishelson, 1996; Henrikson & Matoltsy, 1968a, 1968b) suggests either a much less actively dividing epithelium or a more dynamic involvement of cells within this type of photophore. In this case, function may vary over a cell's lifespan or a more regionalised pattern of division of the basal layer occurs. The presence of mucous and serous cells in the photophore also suggest that the photophores, like the epidermis generally, contribute to the mucous coat. This also is consistent with Lütken's (1892) initial identification of the photophores as mucous pores.

Epidermal involvement in photophore structure is more typical of bacterial bioluminescent organs where bacteria must be acquired from the environment, but in these cases the epidermal epithelium forms a chamber to house obvious groups of bacteria (Chakrabarty *et al*., 2011; Ghedotti *et al*., 2018; Munk, 1999; Somiya, 1977). Although serial photophores in other taxa may also have a developmental origin from generalised ectoderm like the epidermis, in the adult these structures are restricted to the dermis (Lawry, 1973; Mallefet *et al*., 2019; Nichol, 1957). This study provides evidence of a bioluminescent photophore that is probably intrinsic in a continuous epidermal tissue in ray-finned fishes, it expands the range of tissues that probably function in intrinsic bioluminescence and it increases the number of inferred independent acquisitions of intrinsic bioluminescence to 11 (Davis *et al*., 2016).

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