Short note

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Biofluorescence in the platypus (*Ornithorhynchus anatinus*)

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Abstract: The occurrence of biofluorescence across Mammalia is an area of active study. We examined three specimens of the platypus (*Ornithorhynchus anatinus*) from Tasmania and New South Wales, Australia, housed in the Field Museum of Natural History (Chicago, Illinois, USA) and the University of Nebraska State Museum (Lincoln, Nebraska, USA) under visible light and ultraviolet (UV) light. The pelage of the animals appeared uniformly brown under visible light and green or cyan under UV light, due to fluoresced wavelengths that peaked around 500 nm. Our observations are the first report of biofluorescence in a monotreme mammal.

Keywords: biofluorescence; monotremata; museum specimens; nocturnal–crepuscular; *Ornithorhynchus anatinus*.

Biofluorescence, in which short wavelengths of light are absorbed and longer wavelengths are re-emitted by living organisms, has been observed in a wide range of fishes (Sparks et al. 2014), reptiles and amphibians (Gruber and Sparks 2015; Lamb and Davis 2020) and birds (Pearn et al. 2001; Weidensaul et al. 2011). Within mammals, biofluorescence of the pelage under ultraviolet (UV) light has been previously documented in nocturnal–crepuscular New World taxa including marsupial opossums (Meisner 1983; Pine et al. 1985) and placental flying squirrels (Kohler et al. 2019). Here we document the discovery of fluorescence of the pelage of the platypus (*Ornithorhynchus*

anatinus)—to our knowledge, the first report of biofluorescence in a monotreme mammal under UV light.

Living monotremes represent an ancient mammalian lineage with a long independent evolutionary history. Platypuses are semi-aquatic monotremes that inhabit streams, lakes, and lagoons across eastern Australia, ranging from Queensland to Victoria and Tasmania (Pasitschniak-Arts and Marinelli 1998). Platypuses are typically nocturnal–crepuscular and use a suite of unique phenotypic traits to exploit low-light aquatic environments at dawn, dusk, overnight, and in murky water (Pasitschniak-Arts and Marinelli 1998; Pettigrew et al. 1998; Bethge et al. 2009; Bino et al. 2018). Swimming with their eyes closed, they rely on mechanoreception and electroreception to locate prey underwater (Pettigrew et al. 1998). Platypus fur is uniform in color, extremely dense (providing insulation in water), and covers the body except for the bill, feet, and the underside of the tail (Pasitschniak-Arts and Marinelli 1998).

Of the species of mammals previously known to have biofluorescent pelage under UV light, all are nocturnal–crepuscular (Meisner 1983; Pine et al. 1985; Kohler et al. 2019) and only the water opossum (*Chironectes minimus*), which biofluoresces purple, is semi-aquatic (Pine et al. 1985). The fur of other species biofluoresces in shades of red, orange, yellow, blue, purple, lavender, and pink (Meisner 1983; Pine et al. 1985; Kohler et al. 2019). While most past research on mammalian biofluorescence has been based on the study of museum specimens, live animals with biofluorescent pelage have been observed, validating collection-based research (Meisner 1983; Kohler et al. 2019). We used museum specimens, photography, and fluorescence spectroscopy to examine platypus pelage under UV light.

We first observed platypus biofluorescence at the Field Museum of Natural History (FMNH) in Chicago, Illinois, USA. We examined two stuffed museum specimens of the platypus collected in Tasmania, Australia: FMNH 55559, a female collected in 1889; and FMNH 16612, a specimen we inferred to be a male based on ankle spurs, with no collection date listed. In a dark room, we photographed the dorsal and

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ventral sides of each specimen (Canon EOS 50D, Canon USA Inc., Melville, NY, USA; Sigma 17–70 mm f 2.8–4 DC Macro) under visible light (Canon Speedlite 430EX) and then separately under a 385–395 nm UV light (LED UV flashlight, iLumen8 100 LED) with and without a 470 nm longpass filter (K&F Concept, Guangdong Sheng, China) that blocked short wavelengths (including reflected UV and blue wavelengths) to allow greater visibility of longer biofluoresced wavelengths. In addition, we captured UV reflectance images using a Nurugo SmartUV camera (Unioncommunity Co., Ltd., Seoul, Republic of Korea). In a dark chamber that excluded ambient light, we used an Ocean Optics Flame-S-UV-VIS-ES spectrometer in fluorescence mode (integration time = 1 s, and five scans per spectrum) and an Ocean Optics DH-2000-BAL deuterium light source to take fluorescence spectra. We examined five different points on the ventral surface of each specimen. At each location, we placed the probe holder directly on the specimen with the probe at a 45° angle to the sample. To create an average spectrum for each specimen, we combined the five spectra taken. The light source spectra were taken against a polytetrafluoroethylene (PTFE) diffuse reflectance standard.

The dorsal and ventral pelage of the platypus, which appear uniformly brown under visible light, were green to cyan under UV light (Figure 1). The male and female specimens were similar in appearance. Using fluorescence spectroscopy on the ventral surface, we detected a fluorescence peak around 500 nm (Figure 2). The decrease in intensity from 200 to 400 nm in the two sample spectra compared to the light source spectrum indicates that these specimens are absorbing some UV light (Figure 2). Correspondingly, the UV reflectance photography exhibited a low reflectance and potentially high absorbance of UV light by the pelage in some areas (Figure 1). Thus, the fur of the platypus was biofluorescent: absorbing short UV wavelengths (200–400 nm) and re-emitting visible light (500–600 nm).

In order to verify the results we obtained from the specimens housed at the FMNH, we examined a platypus specimen collected from a different locality and date that is housed in a different repository. We viewed a male platypus (UNSM 30375) collected in New South Wales, Australia, in 1909, curated at the University of Nebraska State Museum (UNSM), Lincoln, Nebraska, USA, under visible and UV light. The pelage of this specimen, which was uniformly brown under visible light, also biofluoresced green under UV light.

While biofluorescence in response to UV light was seen in all of the platypus specimens we examined, the small sample size limits our ability to draw conclusions about the ecological function of this trait. The male and

Figure 1: A male platypus (*Ornithorhynchus anatinus*) museum specimen (FMNH 16612) collected from Tasmania, Australia, photographed under visible light and 385–395 nm ultraviolet (UV) light without and with a yellow camera lens filter. Cyan to green biofluorescence of ~500 nm is seen in the middle panels. UV absorption is indicated by dark areas in the far right panel.

Figure 2: Fluorescence spectra of two platypus (*Ornithorhynchus anatinus*) museum specimens collected from Tasmania, Australia (FMNH 16612, blue; and FMNH 55559, red; intensity in arbitrary units). The peak starting at 500 nm is associated with the cyan/green biofluorescence. A diffuse reflectance standard was used to record the light source spectrum (black). In the range of 200–400 nm, the difference between the black and red and blue curves is due to absorbance of ultraviolet light by the specimens.

female specimens biofluoresced in the same patterns and intensity; therefore, it appears that the trait is not sexually dimorphic. We are confident that the fluorescence we

observed is not a property of museum specimens in general. We recently detected biofluorescence in New World flying squirrel museum specimens (confirmed with wild sighting), but not in specimens of diurnal squirrels housed in the same museum (Kohler et al. 2019). In addition, biofluorescence of the pelage of the Virginia opossum (*Didelphis virginiana*) was observed in museum specimens and living animals (Meisner 1983; Pine et al. 1985).

This new observation of biofluorescence in the platypus under UV light strengthens the hypothesis that the trait is adaptive in low-light environments (Kohler et al. 2019). Platypuses, like biofluorescent flying squirrels and opossums, are active at twilight and overnight (Bethge et al. 2009; Bino et al. 2018), when UV absorbance and fluorescence may be particularly important to mammals. Many nocturnal-crepuscular mammals appear to have UV-sensitive vision, further suggesting that UV light is ecologically important in low-light environments (Douglas and Jeffrey 2014). In the case of the platypus, a species that primarily navigates its world via mechanoreception and electroreception, we speculate that biofluorescence is less important for intra-specific interactions than it is for inter-species interactions. The absorbance of UV light and subsequent fluorescence of longer wavelengths, may reduce the visibility of the platypus to UV-sensitive predators. However, field-based research will be essential to document platypus biofluorescence and its ecological function in wild animals.

The discovery of biofluorescence in the platypus adds a new dimension to our understanding of this trait in mammals. Biofluorescence has now been observed in placental New World flying squirrels, marsupial New World opossums, and the monotreme platypus of Australia and Tasmania. These taxa, inhabiting three continents and a diverse array of ecosystems, represent the major lineages of Mammalia. Biofluorescence in mammals is not restricted to a few closely related specialists; instead, it appears across the phylogeny, which begs the question: Is biofluorescence an ancestral mammalian trait?

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