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Red Fluorescence of European Hedgehog (*Erinaceus europaeus*) Spines Results from Free-Base Porphyrins of Potential Microbial Origin

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Abstract

Bioluminescence has been recognized as an important means for inter- and intra-species communication. A growing number of reports of red fluorescence occurring in keratinaceous materials have become available. The fluorophore(s) in these cases were shown to be, or suspected to be, free base porphyrins. The red fluorescence found in the downs of bustards was associated with inter-species signaling in mate selection. First reported in 1925, we confirm that spines of the European hedgehog (*Erinaceus europaeus*) when irradiated with UV (365–395 nm) light display red fluorescence localized in the light-colored sections of their proximal ends. Using reflectance fluorescence spectroscopy, we confirmed that the fluorophores responsible for the emission are free-base porphyrins, as suspected in the original report. Base-induced degradation of the spine matrix and subsequent HPLC, UV-vis, and ESI+ mass spectrometry analysis revealed the presence of a mixture of coproporphyrin III and uroporphyrin III as predominant porphyrins and a minor fraction of protoporphyrin IX. Investigation of the spine microbiome uncovered the abundant presence of bacteria known to secrete and/or interconvert porphyrins and that are not present on the non-fluorescing quills of the North American porcupine (*Erethizon dorsatum*). Given this circumstantial evidence, we propose the porphyrins could originate from commensal bacteria. Furthermore, we hypothesize that the fluorescence may be incidental and of no biological function for the hedgehog.

Keywords Biofluorescence · Hedgehogs · Porphyrins · Commensal Bacteria · Microbiome

Introduction

Porphyrins are found in most organisms as their iron complexes, serving as prosthetic groups in numerous heme-based enzymes (Dolphin 1978). Free-base porphyrins — porphyrins lacking a coordinated metal ion in their central cavity — are intermediates in the biosynthesis of heme and other tetrapyrrolic 'pigments of life' (Battersby 1994, Kadish et al. 2003; Frigaard et al. 2006; Scott et al. 2003). As a consequence of their π-aromatic chromophores, porphyrins are deeply colored and many are strongly fluorescent, emitting red (~630–660 nm) light when irradiated with UV-A, blue, or green light.

The biological occurrence of free-base porphyrins outside of heme biosynthesis pathways (or their pathologies) (Eales 1979; Hift and Meissner 2003) is relatively rare (Gorchein 2003; Gorchein et al. 2005; Payne 1994). In addition, Nature utilizes (metallo)porphyrins only sparingly as coloring agents or fluorophores (Gorchein et al. 2005; Payne 1994). For instance, turacin ([uroporphyrinatoIII]copper(II) **1Cu**; Fig. 1), a deep-red metalloporphyrin feather pigment, is found only in the wing patches of a single avian family: the turacos (*Musophagidae*) (McGraw 2006; Nicholas and Rimington 1951). Other examples include protoporphyrin IX (**2**), uroporphyrin I, and uroporphyrin III (**1**) serving as red-brown pigments in the shells of marine snails (*Trochoidea*) (Kennedy 1979; Williams et al. 2016), as well as the corallistins, porphyrins found

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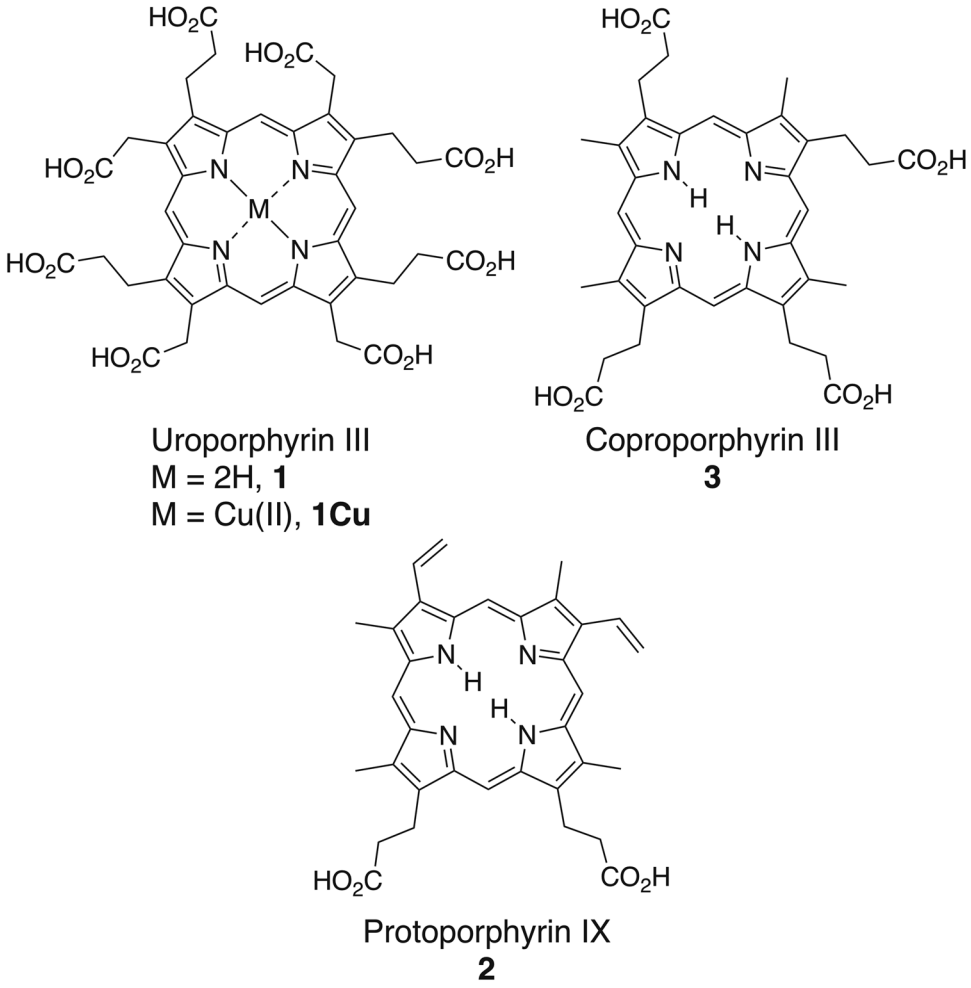


Fig. 1 Structures of select porphyrins found in Nature

in the purple New Caledonian marine demosponge, *Corallistes sp*. (D'Ambrosio et al. 1993). Purple-brown protoporphyrin IX (**2**) is, together with its catabolites, also utilized as a pigment in the colored eggshells of birds (Cassey et al. 2010; Gosler et al. 2005; Hamchand et al. 2020; Ishikawa et al. 2010; Kennedy and Vevers 1976; Kilner 2006; Moreno et al. 2006; Moreno and Osorno 2006).

Important in the context of this work, free-base uroporphyrin III (**1**), protoporphyrin IX (**2**), and coproporphyrin III (**3**) have also been found as red-fluorescing pigments in the light-colored plumulaceous barbs of the contour feathers of turacos (*Musophagidae*), the feather stems of young domestic pigeons (*Columbiformes*) (Derrien and Turchini 1925), and in the downy barbs under the wings of young owls (*Strigiformes*) (Weidensaul et al. 2011; With 1978), nightjars (*Caprimulgiformes*) (Völker 1938), bustards (*Gruiformes*) (Galván et al. 2016; With 1978), and a kite (*Elanus caeruleus*) (Negro et al. 2009). The function of these porphyrins is unclear (With 1978). A hypothesis was advanced that the light-pink porphyrin coloring at the base of the contour body feathers in bustards serves as an ephemeral — because photodegradable — optical signal of virginity in mate selection (Galván et al. 2016).

Within Mammalia, biofluorescent markings, in general, and porphyrin-based coloration, in particular, have not been widely documented. Fluorescence was described to be visible on the hair, skin, and skin excretions of the Virginia opossum (*Didelphis virginianus*) (Meisner 1983) and other didelphid marsupials (Pine et al. 1985). UV light-induced red fluorescence was observed on the underbelly of nocturnal New World flying squirrels (*Glaucomys*), but not in diurnal species (Olson et al. 2019). While the fluorescent dye(s) were not identified in these cases, their described colors fit the characteristics of porphyrins. Most recently, vivid and patterned free base porphyrin-based biofluorescence was described for the pelage of the nocturnal springhares (*Pedetidae*) (Olson et al. 2021).

Like feathers, spines are inert integumentary structures primarily colored by melanins. A 1925 report first mentioned that the spines of the European hedgehog (*Erinaceus europaeus*) contain a fluorescent component that was, based on the red color of the emission that is extinguished upon treatment with strong acid, suspected to be a porphyrin

(Derrien and Turchini 1925). The only other independent works that mention fluorescent spines in hedgehogs are the case study of a sole inbred African pigmy hedgehog (*Atelerix albiventris*) diagnosed with a congenital porphyria (Wolff et al. 2005), and an internet post by an African pigmy hedgehog pet keeper (Blarg_King 2012).

To our knowledge, the routine, non-pathological presence of porphyrins in hedgehog spines has never been confirmed since the 1925 report, nor have the specific porphyrin(s) responsible for the fluorescence been identified. This aspect is the primary aim of this report. Additionally, we will present circumstantial evidence that the source of the porphyrins in the spines and, by extension, in other keratinaceous matrixes, is of microbiological origin. We further posit that the red fluorescence serves no signaling purpose for the hedgehog.

Methods and Materials

Samples The European hedgehog (*Erinaceus europaeus)* spines were collected from fully grown, fresh road kills (in April 2013, city of Königswusterhausen, State of Brandenburg, and in November 2018, the town of Eching, State of Bavaria, both Germany) of unknown sex, and were gently washed with a mild dish soap, air dried, and kept in the dark. An additional sample, from a British specimen, was sourced from a commercial supplier (Etsy.com). The crested porcupine (*Hystrix cristata*) quills were from a commercial source (Etsy.com). The North American porcupine (*Erethizon dorsatum*) quills were collected from road kill (in June 2017, near the town of Digby, southern Nova Scotia, Canada). Standard porphyrins were purchased from Frontier Scientific Ltd. (Logan, UT, USA).

Instrumentation Solution UV-Vis spectra were recorded with a Cary 50 spectrophotometer, fluorescence spectra with a Cary Eclipse fluorimeter, both Varian, Inc. Diffuse reflectance spectra were recorded on a Fluorolog-3. High-resolution mass spectra were recorded on a Thermo Scientific Q ExactiveTM HF mass spectrometer in the ESI+ mode.

Diffuse Reflectance Fluorescence Spectra The unpigmented sections of several hedgehog spines were trimmed off and placed vertically in a 3 mm ID cylindrical quartz tube. Emission spectra were then recorded using front-face detection at the excitation wavelengths indicated.

Chemicals All solvents (HPLC grade) and reagents for the extractions were used as received from Fisher Scientific.

Porphyrin Extraction Procedure 20 spines (~ 500 mg) that exhibited strong red fluorescence under 365 nm 'black light' were selected for extractions. The whole spines were placed in a porcelain mortar alongside crushed dry ice and the mixture was ground with a porcelain pestle until a course-grained sample was achieved. Once all the dry ice had sublimed, the crushed spines were transferred to a 20 mL scintillation vial containing 2 mL of 0.1 M aq. NaOH. The mixture was sonicated for 20 min in a sonicated water bath after which all remaining solids were removed through gravity filtration via a Pasteur pipette plugged with cotton wool. The filtered solution was kept away from light and, using a stream of nitrogen, dried into a film. The film was dissolved in 100 µL of MeOH and subjected to LC-MS. This workflow was also applied to ~500 mg of porcupine quills.

LC-MS Analysis For analytical analysis, hedgehog spine or porcupine quill extracts were analyzed via an Agilent 1200 Series high-performance liquid chromatograph utilizing DAD and single quadrupole mass detectors and equipped with a Phenomenex Kinetex C18 reverse-phase column (250 × 4.6 mm, 5 µM diameter). Mobile phase: linear gradient of 1:9 H_2O + 0.1% formic acid and ACN + 0.1% formic acid to 100 ACN + 0.1% formic acid over 30 min. DAD detection wavelength set to 400 nm. For ESI+ high-resolution mass detection, the extracts were analyzed via an iFunnel 6550 Quadrupole Time-of-Flight (QTof) mass spectrometer utilizing the same column and elution gradient detailed directly above.

DNA Extraction, PCR Amplification, and Sequencing of Taxonomic Marker DNA was extracted from 0.1 g of spine samples using the MoBio PowerMag Soil 96 well kit (MoBio Laboratories, Inc) according to the manufacturer's protocol for the Eppendorf epMotion liquid handling robot. DNA extracts were quantified using the Quant-iT PicoGreen kit (Invitrogen, ThermoFisher Scientific). Partial bacterial 16S rRNA genes (V4, 0.8 picomole each 515F and 806R with Illumina adapters and 8 basepair (bp) dual indices (Kozich et al. 2013) were amplified in triplicate 15 µl reactions using GoTaq (Promega) with the addition of 10 µg BSA (New England BioLabs). To overcome initial primer binding inhibition because the majority of the primers do not match the template priming site, we added 0.1 fmole 515F and 806R that do not have the barcodes and adapters. The PCR reaction was incubated at 95 °C for 2 min, then 30 cycles of 30 s at 95.0 °C, 60 s at 50.0 °C and 60 s at 72.0 °C, followed by final extension as 72.0 °C for 10 min. PCR products were pooled for quantification and visualization using the QIAxcel DNA Fast Analysis (Qiagen). PCR products were normalized based on the concentration of DNA from 350 to 420 bp then pooled using the QIAgility liquid handling robot. The pooled PCR products were cleaned using the Mag-Bind RxnPure Plus (Omega Bio-Tek) according to the manufacturer's protocol. The cleaned pool was sequenced

on the MiSeq using v2 2×250 base pair kit (Illumina, Inc) (Caporaso et al. 2012). Negative controls from the extraction and PCR were sequenced along with the samples.

Sequence Data Processing and Statistical Analyses. Sequences were demultiplexed using onboard bcl2fastq. The sequences were then processed using the DADA2 package (Callahan et al. 2016) in R version 3.6.0 (Team 2016); DADA2 trims low quality bases from the ends of the reads, merges paired-end sequences, and then calls each unique amplicon sequence variant (ASV) as an operational taxonomic unit. Bacterial taxonomy was assigned by aligning the reads to the Silva reference database (Pruesse et al. 2007; Quast et al. 2012) and classified with the RDP's Naive Bayesian Classifier (Cole et al. 2013; Wang et al. 2007).

Results

We began our study with a verification of the observations made in the report by Derrien and Turchini (Derrien and Turchini 1925). Thus, inspection of two fresh adult European hedgehog (*Erinaceus europaeus*) carcasses under hand-held UV light (395 nm) confirmed the presence of a pink to red fluorescence on the third generation-type, adult spines with no clear discernable pattern across the entire dorsal side; no fluorescence was observed on the ventral side which did not carry any spines (Reeve 1994).

Under laboratory conditions, the fluorescence intensity varied between individual spines (from random portions of the body). While some were comparably bright, others were dull or even dark when irradiated with UV light (λ = 365 nm) (Fig. 2). When present, the fluorescence is most visible in the light sections at their proximal ends. For comparison, we also inspected the spines of two rodents, the American porcupine (*Erethizon dorsatum*) and the African crested porcupine (*Hystrix cristata*), but neither possessed visible or measurable fluorescence (ESI).

We recorded diffuse reflectance fluorescence emission and excitation spectra of the light and dark sections of the spines. The results for the non-fluorescing dark-colored sections are consistent with the presence of melanins only (Gallas and Eisner 1987). Excitation of the fluorescing light-colored sections at wavelengths ranging from 400 to 500 nm generated two emission bands, one strongly wavelength-dependent broad emission between 450 and 600 nm, attributed to brown eumelanins (Gallas and Eisner 1987), and a more narrow band at ~650 nm that displayed strong intensity modulation with excitation wavelength, but a small variation of the emission maxima (Fig. 3a). An excitation emission scan (Fig. 3b) returned a spectrum with a strong absorbance feature at 406 nm, known as the Soret band, and a series of side bands (Q-bands) that are diagnostic for

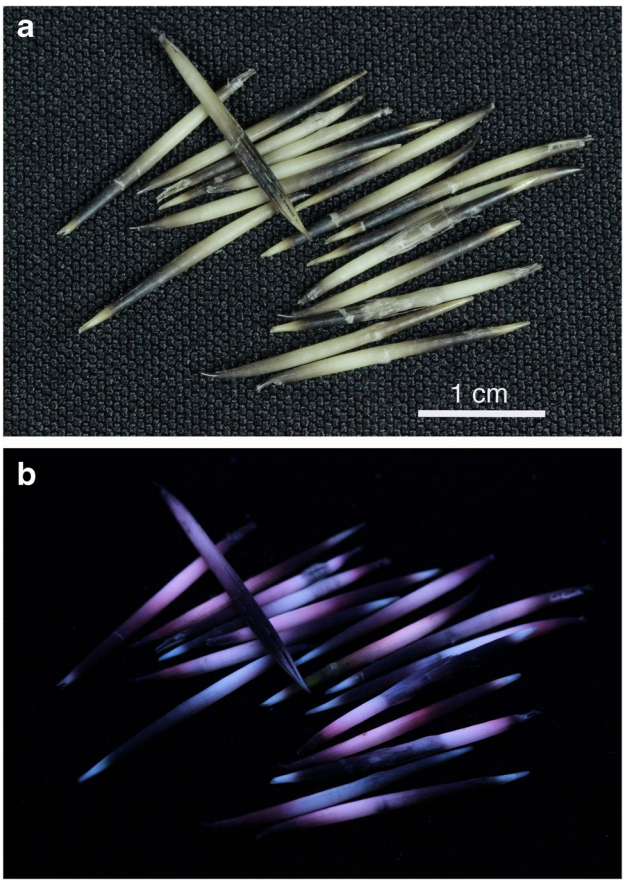


Fig. 2 Photographs of European hedgehog spines selected for their fluorescence under white (**a**) and UV (**b**) (365 nm) light

free-base porphyrins (Gouterman 1978). In comparison, the porcupine quills showed only the presence of melanins (Figure S3) (Gallas and Eisner 1987).

As many naturally occurring free-base porphyrins have similar optical spectra (Gouterman 1978), the spectra cannot identify the specific porphyrin present or establish whether (non-emissive) metalloporphyrins or other pigments are also present. We therefore chemically extracted the spine pigments from the keratinaceous matrix by sonication in an aqueous NaOH solution. Reverse-phase HPLC of the extract revealed the presence of several porphyrinic pigments in the spine that are identical in retention time to genuine uroporphyrin III (**1**), protoporphyrin IX (**2**), and coproporphyrin III (**3**), as well as the presence of some ill-defined, non-porphyrinic components (Fig. 4).

ESI+ HR-MS data from the porphyrin peaks (Experimental *m/z* = 831.2347, 563.2650, and 655.2763 Da for **1**, **2**, and **3**, respectively) are also consistent with the composition expected for each porphyrin ($C_{40}H_{39}N_4O_{16}$, $C_{34}H_{35}N_4O_4$, and $C_{36}H_{39}N_4O_8$ for $[M{\cdot}H^+]$ for **1**, **2**, and **3**, respectively) (for details, see Figure S4). Through comparison with genuine standards, we estimate the quantity of all porphyrins

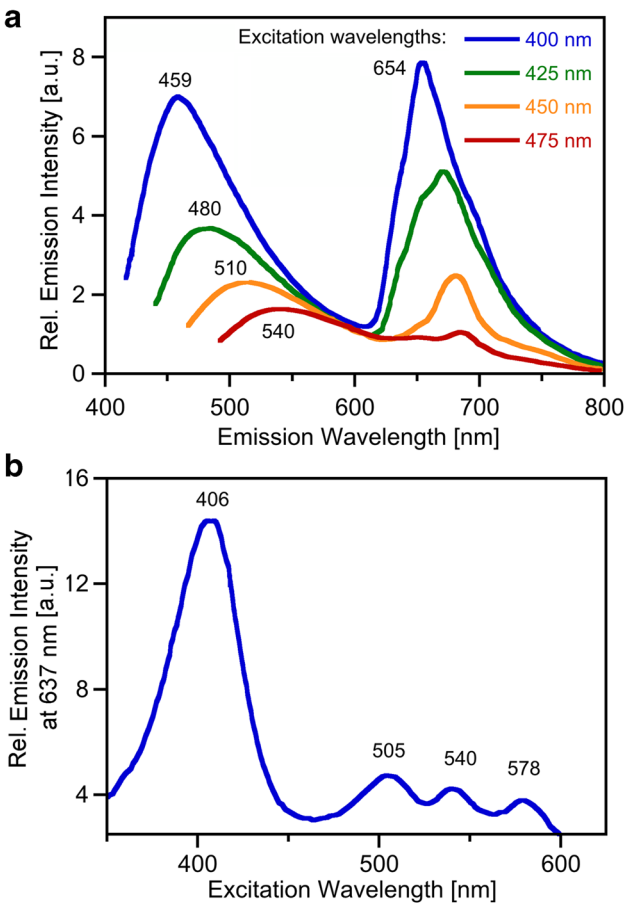


Fig. 3 **a** Diffuse reflectance fluorescence emission spectra and (**b**) diffuse reflectance fluorescence excitation spectrum (recorded at $\lambda_{emission}$ at 637 nm) of the light bands of hedgehog spines; the fourth λ_{max} band expected near 630 nm was not recorded because of its proximity to the excitation wavelength

to be in the low ng/g spine range in the spines selected for their fluorescence, with some batch-to-batch variations of their relative ratios. We note that the fluorescence varied significantly between individual spines, as well as within sections of a single spine; thus, more quantitative assessments of the porphyrin contents and ratios could not be made. A synonymous analysis of the American porcupine quills showed no indications for the presence of porphyrins.

We are unaware of a previous assessment of the bacterial microbiome of the integument of hedgehogs. We thus subjected European hedgehog spines — and American porcupine quills as a comparison — to genomic sequencing to identify the organisms present (Tringe and Hugenholtz 2008). This analysis provided a detailed overview of the bacterial taxa in the spine microbiome of the specimen investigated (Fig. 5). Importantly, the bacterial microbiomes of the whole vs ground-up spines/quills (i.e. microbiomes on the surface of the spines/quills vs. outside as well as inside) were qualitatively similar, indicating that the intrinsic microbiomes were only minimally distorted by external contamination.

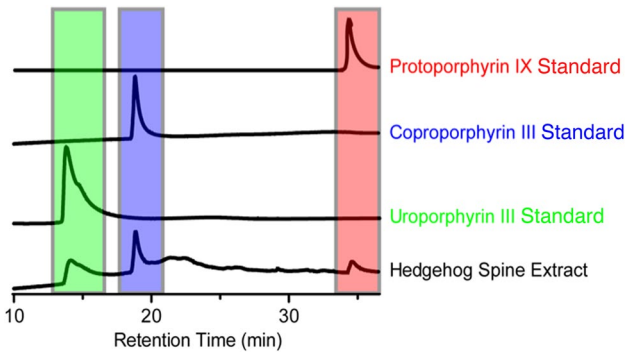
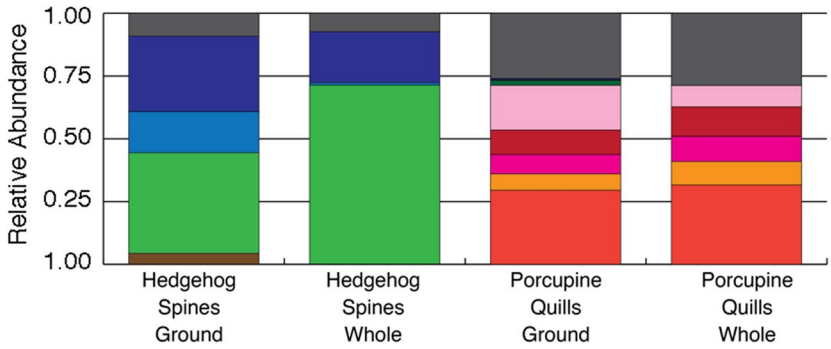


Fig. 4 Reverse-phase HPLC chromatograms of the hedgehog spine extract and the porphyrin standards listed; detection wavelength 400 nm. See Methods and Materials section for experimental details

Discussion

Biofluorescence has been observed in an ever-increasing number of vertebrate animals (Anich et al. 2021; Derrien and Turchini 1925; Galván et al. 2016; Gruber and Sparks 2015; Negro et al. 2009; Olson et al. 2019, 2021; Park et al. 2019; Prötzel et al. 2021; Sparks et al. 2014; Völker 1938; Weidensaul et al. 2011; With 1978). While the function of bioluminescence in spectrally-shifted marine environments is well-documented (Gruber and Sparks 2015; Park et al. 2019; Sparks et al. 2014), only scant information exists regarding the biological functions of terrestrial fluorescence. Particularly for nocturnal species (owls, nightjars, flying squirrels, springhares, opossums, hedgehogs, etc.), it is difficult to envision a physiological role for red fluorescence that requires UV or blue to green light to become visible and especially since the fluorescence is typically found in areas that are well-hidden (feather stems at ventral sections, underbellies, base of the spines).

The biological origin of porphyrins in hedgehog spines — in fact, in most keratinaceous tissues — has not been previously discussed. However, we note that bacteria such as *Cutibacterium acnes* (formerly *Propionibacterium acnes*) and *Corynibacterium acnes* have long been known to biosynthesize and excrete porphyrins (Ashkenazi et al. 2003; Lee et al. 1978). Porphyrin excretion from these, and other (Jones et al. 2020), bacteria produce a diagnostic coral-red fluorescence when colonized skin is irradiated with UV-light (known in the dermatological practice as Wood's lamp) (Asawanonda and Taylor 1999). These bacteria typically exist in commensal relationships with their hosts and live in and around the sweat and sebaceous glands of skin (Burkovski 2008; Madigan et al. 2018; Noble 1984).



Phylum	Class	Order	Family	Genus	Species
Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	*Kocuria*	
Actinobacteria	Actinobacteria	Micrococcales			
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*	
Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	*Fusobacterium*	*necrophorum*
All others					
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Janthinobacterium*	
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Klebsiella*	
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae		
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*	
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	

Fig. 5 The relative abundance of the most abundant taxa in the bacterial microbiomes of ground-up and whole European hedgehog (*Erinaceus europaeus*) spines (left two) and American porcupine (*Erethizon dorsatum*) quills (right two). Note that the "All others" category may contain additional taxa from higher taxonomic levels on this chart, at low abundance. For example, there may be additional Actinobacteria in the "All others" category from orders other than Micrococcales

Given the results from our spine extraction and microbiome analysis, we propose that the fluorescence of hedgehog spines may originate from epidermal microbes. Although the back skin of the hedgehog is free of sweat and sebaceous glands (Reeve 1994), an investigation of the lipid chemistry of the specialized epidermal structure of the related Indian hedgehog (*Hemiechinus collaris*) suggests the spines to be a lipid-rich environment (Poddar-Sarkar et al. 2011). It is conceivable, therefore, that the conditions in the follicle in which the spines are produced are suitable for porphyrin-excreting microbes. We cannot say whether the self-anointment behavior of hedgehogs (Reeve 1994; Tynes 2010), other (grooming) behavior of these animals, food, or environmental factors affect the porphyrin contents in the spines.

The microbiome analysis provides circumstantial support for a bacterial origin of the spine porphyrins. Inspection of Fig. 5 highlights a stark contrast between the bacterial microbiomes on the fluorescing spines and dark quills. The hedgehog spines were rich in Actinobacteria (48.2% of all bacteria), and nearly all of these were — as expected for a skin microbiome — from the order Micrococcales. While some Actinobacteria, such as Propionibacteriales and Corynebacteriales, are directly implicated in porphyrin secretion (Ashkenazi et al. 2003; Lee et al. 1978; Philipp-Dormston and Doss 1973; Wollenberg et al. 2014), we found these genera at low abundance in the ground Hedgehog spines (0.4% Propionibacteriales and 0.07% Corynebacteriales). However, bioinformatic analysis of porphyrin biosynthetic gene cluster of Propionibacteriales reveals the pathway to be broadly conserved among Actinobacteria and Micrococcales (Figure S5); Micrococcales made up 46.9% of the ground Hedgehog sample. In contrast, the porcupine quills are very poor in Actinobacteria (1.1% of all bacteria) and they contain very low Propionibacteriales and Corynebacteriales (0.01% and 0.19% of all bacteria, respectively). We additionally found the genus *Porphyromonas* (Phylum Bacteroidetes; order Bacteroidales), members of which are characterized by the production of porphyrin pigments (Gibson and Genco 2006), in the ground Hedgehog spine sample at very low abundance (~0.15% of the sample) and in none of the other samples. Thus, the differences in the presence of known porphyrin-secreting microbes may explain why the spines fluoresce while the quills do not.

Furthermore, the detection of specifically coproporphyrin III in the hedgehog quills supports a bacterial origin model. Bacteria produce porphyrins either by de novo biosynthesis or from exogenous uptake of heme followed by removal of iron (Philipp-Dormston and Doss 1973; Wollenberg et al. 2014). Bacterial de novo construction of porphyrins occurs through three distinct routes: the siroheme, coproporphyrin, and protoporphyrin pathways (Dailey et al. 2017). It is well-known that the coproporphyrin pathway can lead to the accumulation coproporphyrin III (**3**) — the most prevalent porphyrin in the spines, especially in the absence of iron (Dailey et al. 2015). Indeed, the coproporphyrin pathway is dominant within the porphyrin-excreting bacteria, such as the Actinobacteria, associated with this microbiome study (Ashkenazi et al. 2003).

Little is known about the biological role of the porphyrin secretion. While coproporphyrin III (**3**) produced by human-associated *Propionibacterium* spp. was recently implicated in biofilm formation (Wollenberg et al. 2014), the current understanding of the biological roles of free-base porphyrins, outside of being intermediates toward heme, is evolving and remains an active field of study (Cassey et al. 2010; D'Ambrosio et al. 1993; Galván et al. 2016; Gorchein 2003;

Gorchein et al. 2005; Gosler et al. 2005; Ishikawa et al. 2010; Kennedy 1979; Kennedy and Vevers 1976; Kilner 2006; McGraw 2006; Meisner 1983; Moreno et al. 2006; Moreno and Osorno 2006; Negro et al. 2009; Nicholas and Rimington 1951; Olson et al. 2019; Payne 1994; Pine et al. 1985; Völker 1938; Weidensaul et al. 2011; Williams et al. 2016; With 1978).

If the porphyrins uncovered in the spines are indeed of microbial origin, the resulting color and fluorescence of the hedgehog spines may be incidental to the presence of commensal bacteria, and possibly of no direct biological function to the host. Notably, the retina of hedgehogs contains only rods, suggesting that they lack color vision (Tynes 2010). This implies that the red fluorescence is, at a minimum, not involved in intra-species communication. In general, we assess the weak red fluorescence is likely neither useful in the day, under strong white light conditions, nor at night, where fluorescence is not induced. Other examples of such incidental fluorescence are known. For instance, the skin of mice shows a strong red fluorescence because the chlorophyll degradation products resulting from a green diet accumulate in their skin (and elsewhere) (Weagle et al. 1988).

Our proposed bacterial origin of spine fluorescence hypothesis can likely be extended to porphyrin fluorescence in the downy regions of feathers and fur. In this model, the suspected bacteria may live in or on the epidermis and flourish under the dark, humid, and warm conditions of, for example, nest rearing. In general, it is well-known that (micro)environmental and host factors influence the composition of the skin microflora (Madigan et al. 2018). We also note that the fluorescence in some hair and feather structures is associated only with young or adolescent animals and was shown (for owls and bustards) to be lost with age (Galván et al. 2016; Weidensaul et al. 2011; With 1978). Perhaps, as young animals fledge, the changing microclimates and/or prolonged light exposure may degrade the porphyrin(s). In nocturnal animals, the fluorescence may persist.

Conclusion

This study confirms that the red fluorescence of hedgehog spines results from a mixture of the free-base porphyrins coproporphyrin III, uroporphyrin III, and protoporphyrin IX. We thus provide molecular resolution to the phenomenon of hedgehog spine fluorescence. We also offer circumstantial evidence for a commensal bacteria-based origin theory of the fluorescence that possibly can also be extended to the red fluorescence in keratinaceous tissues of other terrestrial animals. Furthermore, since it is difficult to envision a behavioral signaling role of any red fluorescence for a nocturnal animal as it requires UV-visible light to be induced, and particularly for an animal likely not to be able to detect red light, we surmise that the fluorescence is incidental and of no direct importance to the hedgehog.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10886-021-01279-6.

Author Contributions The manuscript was written through contributions of all authors. Project inception and coordination by CB. Sample collection by CB and RB. Sample extraction and spectroscopic analysis by RH, AL, and HAF. Microbiome analysis by KRM and SMH; microbiome interpretation by SMH and MW; veterinarian context by RB. All authors have given approval to the final version of the manuscript.

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Data Availability Data used for this analysis can be found either in this article or the electronic supplementary material (ESI). Sequence data were submitted to the NCBI SRA (Accession: PRJNA720886).

Code Availability N/A

Declarations

Ethics Approval N/A

Conflict of Interest/Competing Interest The authors declare no conflicts of interest.

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