Fur glowing under UV: a widespread consequence of porphyrin accumulation in mammals

Séverine Toussaint
Humboldt Universität zu Berlin
Jasper Ponstein
Museum für Naturkunde Berlin, Leibniz Institute for Evolution and Biodiversity Science
Mathieu Thoury
CNRS
Rémi Métivier
Université Paris-Saclay, ENS Paris-Saclay, CNRS
Daniela Kalthoff
Swedish Museum of Natural History
Benoît Habermeyer
PorphyChem SAS
Roger Guilard
Université de Bourgogne Franche-Comté
Steffen Bock
Museum für Naturkunde Berlin, Leibniz Institute for Evolution and Biodiversity Science
Peter Mortensen
Swedish Museum of Natural History
Sverre Sandberg
Haukeland University Hospital
Pierre Gueriau
University of Lausanne
Eli Amson (eli.amson@smns-bw.de)
Staatliches Museum für Naturkunde Stuttgart https://orcid.org/0000-0003-1474-9613

Article

Keywords: UV Photoluminescence, Fluorescence, Pelage, Mammal, Spectroscopy, Multispectral imaging, Porphyrin

Posted Date: May 6th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-499384/v1

Abstract

Spectacular photoluminescence (PL) phenomena have been increasingly reported in various organisms from diverse ecosystems. However, the chemical basis of this PL remains poorly defined, and its potential ecological function is still blurry, especially in mammals. Here we used state-of-the-art spectroscopy and multispectral imaging techniques to document new cases of mammalian ultraviolet-induced PL (UV-PL) and to identify free-base porphyrins and natural derivatives as the organic compounds responsible for the reddish luminescence observed in the hairs and spines of distantly related species. We argue that pink to red UV-PL is predominantly observed in crepuscular and nocturnal mammals because porphyrins are photodegradable, and that this phenomenon might not have a specific function in intra- or interspecific communication but consists of a byproduct of a widespread physiological condition, overlooked in mammals.

Co-first authors: Séverine Toussaint and Jasper Ponstein

Introduction

Ultraviolet-induced photoluminescence (UV-PL) has been observed in the external organs of plants[1], 'invertebrates'[2] and in diverse vertebrates including 'fishes', lissamphibians, squamates, birds, and mammals[3]. Concerning mammals, a handful of predominantly opportunistic observations have been reported, notably in the platypus[4], some marsupials (opossums[5,6] and bandicoots[7]), and rodents (flying squirrels[8] and springhares[9]).

Interestingly, this UV-PL can occur in different tissues, and the observed emitted colors vary a lot within the visible spectrum depending on the species. Reported observations include shades of dark blue from bone[10–13], of light blue and green from skin and hairs[3,4,6,8,13], of yellow from birds' feathers[14–16], and shades of pink to red from feathers as well as from the pelage of most of the photoluminescent mammals[5–9, 17]. This high variability implies that it is not the same UV-reactive molecular or structural compounds that are at the origin of this phenomenon depending on the species, suggesting probable different mechanisms at play. For instance, biological fluids like urine are known to be photoluminescent[18,19], resulting in blue to yellow UV-PL observed. Recent studies found iridophores as responsible for greenish UV-PL in the dermis of a gecko [3]. The reddish UV-PL observed on the plumage of some birds was identified as caused by coproporphyrin III and protoporphyrin IX[20–25], which are common molecules playing a crucial role in various biological processes and notably in heme biosynthesis[26]. A recent analysis on springhare pelage[9] identified a mixture of other porphyrins, usually found in urine or feces of mammals[27], along with an unidentified molecule. However, the question remains whether these compounds are also responsible for the reddish UV-PL observed in other mammals. Moreover, the precise spatial and spectral repartitions of these compounds within the pelage are still unknown, as well as their inter- and intraspecific variability. Answering these questions would greatly help improving our knowledge on the biological origin and potential function of such photoluminescent mechanism in animals.

In addition to molecular identification, it remains unclear whether PL is actually seen and used by the organisms in their natural environment[1,28]. So far, two cases of sexual selection using PL have been experimentally demonstrated in budgerigars[14] and in jumping spiders[29]. In mammals, based on the fact that UV-PL was predominantly observed in crepuscular or nocturnal animals, it was recently hypothesized that this may serve

ecological functions in light-deprived or snow-covered environments such as intraspecific communication or antipredator behavior[4,8,9]. These hypotheses have not been tested experimentally. Moreover, as the associated species are adapted to different ecosystems and lifestyles, from the semi-aquatic platypus to the terrestrial springhares and the arboreal flying squirrels, it remains unclear whether UV-PL has indeed a specific ecological significance in nocturnal animals, or is simply a byproduct of a physiological condition such as pigmentation[1,28].

Here we document a new selection of mammals exhibiting luminescence under UV, including hedgehogs (*Erinaceus* spp.) and ermine (*Mustela erminea*), substantiating the widespread occurrence of this phenomenon across this group. We focused on the reddish UV-PL observed in most of the species' pelage and used customized spectroscopy techniques to precisely identify *in-situ* the organic compounds at play as metal-free porphyrins. Furthermore, we used multispectral luminescence imaging to describe the spatial and spectral distributions of these porphyrinic compounds, demonstrating their specific localization within hairs and spines along with their variable concentration across species. These results suggest a more pragmatic scenario in which this erratic accumulation of porphyrins into mammalian pelage, resulting in an observable photoluminescence under UV illumination, may not result from a specific selection for nocturnal communication as previously argued, but from the non-pathological degradation process of porphyrins.

Terminological clarifications

Photoluminescence (PL) is a physical phenomenon in which a material reemits photons after a photonic excitation. It gathers two distinct phenomena, namely fluorescence, or phosphorescence that can be differentiated by the difference in the electronic states involved in the radiative de-excitation pathway. Fluorescence and phosphorescence occur when the molecule in an excited state returns to the electronic ground state (essentially S_0 for most organic molecules), from the excited singlet or triplet state, respectively, by emission of a photon. The fluorescence process occurs within a few tens of picoseconds to a few hundreds of nanoseconds, whereas phosphorescence, which corresponds to the forbidden transition between the excited and ground states, takes place on a timescale up to seconds, even minutes or more. UV-induced photoluminescence (UV-PL) is the process whereby absorbed UV photons trigger a radiative deexcitation at longer wavelengths. In contrast, bioluminescence (BL) corresponds to an emission of light generated by a living organism via chemical reactions.

Multiple cases of UV-PL and BL have been observed in various organisms either in their natural habitat or from preserved specimens. However, UV-PL is often referred to as "biofluorescence" in the literature, which might confuse either the scientific community and the general public. Therefore, UV-PL is the appropriate technical term to use in this context, because it is neither proven whether it is fluorescence or phosphorescence, and nor does it relate to bioluminescence. Moreover, although both phenomena result in spectacular luminous or glowing organisms, they consist in quite different mechanisms and are not observable under the same conditions. In practice, the UV-PL phenomenon is only observable to the human eye by using an artificial source of ultraviolet light, associated with an optical filter to ensure error-free visual interpretation. Importantly, another noticeable difference is that the energetically demanding BL phenomenon can be correlated to identifiable functions, while PL and UV-PL phenomena are more difficult to decipher[28].

Results And Discussion

UV-induced photoluminescence is widespread in mammals

Our investigation reveals that multiple species of mammals, representing the three major mammalian clades (monotremes, marsupials, and placentals), indeed show conspicuous photoluminescence when observed under long-wave UV illumination (365–395 nm; Fig. 1, Table 1). Also, we hereby document cases of UV-PL observed in two eutherian genera hitherto overlooked: the hedgehog (*Erinaceus* spp., Erinaceomorpha) which appears reddish, and the common ermine (*Mustela erminea*, Carnivora) which appears lavender to the human eye.

Table 1. Specimens analyzed. Collection abbreviation: ZMB= Museum für Naturkunde of Berlin; JAGUARS= Cayenne collection; MNHN= Muséum national d'Histoire naturelle of Paris; NRM= Swedish Museum of Natural History of Stockholm; AH= Anthony Herrel personal collection; NKMP= Naturkundemuseum of Potsdam. Type of analysis conducted: UVL= macro-observation under UV lamp, EmS= emission spectroscopy, Ex-MI= excitation spectroscopy & multispectral imaging. Visible color of their fur observed under UV lamp: NF= no luminescent color detected with the eye. Porphyrin detection obtained with spectroscopy analysis: 0= no, 1= yes.

Mammal class	Order	Species	Specimen number	Type of analysis	Fur under UV	Porph.
Prototheria (egg-laying mammals)	Monotremata	*Ornithorhynchus anatinus* (Platypus)	ZMB_Mam_35991	UVL, EmS	green/cyan	0
Metatheria (marsupials)	Didelphimorphia	*Caluromys lanatus* (Brown-eared Woolly Opossum)	ZMB_Mam_47995	UVL	pink	-
		Marmosa murina (Linnaeus's Mouse Opossum)	JAGUARS-M535	UVL, EmS, Ex-MI	pink/red	1
		Marmosa murina (Linnaeus's Mouse Opossum)	MNHN 2001-1966	UVL	pink	-
		Metachirus nudicaudatus (Brown Four-eyed Opossum)	MNHN 1988-68	UVL	pink	-
		Monodelphis brevicaudata (Guyanan short-tailed Opossum)	JAGUARS-M2962	UVL, EmS, Ex-MI	pink/red	1
		Monodelphis brevicaudata (Guyanan short-tailed Opossum)	JAGUARS-M2838	UVL, EmS, Ex-MI	pink/red	1
		Monodelphis brevicaudata (Guyanan short-tailed Opossum)	MNHN 1995-3216	UVL	pink	-
Eutheria (placentals)	Erinaceomorpha	*Erinaceus europaeus* (West European Hedgehog)	NRM_594242	UVL, EmS, Ex-MI	pink/red	-
		Erinaceus roumanicus (Northern White-breasted Hedgehog)	ZMB_Mam_48212	UVL	pink/red	1
	Carnivora	*Mustela erminea* (Beringian ermine)	NKMP-unnumbered	UVL	lavender	-
		Mustela erminea	NRM_20175124	UVL, EmS	lavender	0

	(Beringian ermine)				
	Vulpes lagopus (Arctic Fox)	NRM_710029	UVL, EmS	NF	0
Soricomorpha	*Crocidura russula* (Greater white-toothed Shrew)	AH, unnumbered	UVL, EmS	NF	0
	Talpa europaea (European Mole)	AH, unnumbered	UVL, EmS	NF	0
Lagomorpha	*Lepus timidus* (Mountain Hare)	NRM_588837	UVL, EmS	NF	0
Rodentia	*Glaucomys volans* (Southern Flying Squirrel)	ZMB_Mam_60634	UVL, EmS, Ex-MI	pink	1
	Glaucomys volans (Southern Flying Squirrel)	MNHN 1939-707	UVL	pink	-
	Glaucomys sabrinus (Northern Flying Squirrel)	NRM_875246	UVL, EmS, Ex-MI	pink	1
	Hylopetes spadiceus (Red-cheeked Flying Squirrel)	MNHN 1979-368	UVL	pink	-
	Pteromyscus pulverulentus (Smoky Flying Squirrel)	MNHN - 1979-376	UVL	pink	-
Chiroptera	*Plecotus auritus* (Brown Long-eared Bat)	AH, unnumbered	UVL, EmS	NF	0
Primate	*Mico argentatus* (Silvery Marmoset)	NRM_617493	UVL, EmS	NF	0

Emission spectroscopy reveals that all analyzed mammal specimens emit broadly in the blue region with varying intensities (see the broad band at 450–480 nm in Fig. 1b). This blue UV-PL is particularly visible to the human eye on the poorly pigmented pelage in hedgehogs, ermines, and flying squirrels (Fig. 1a). We also confirm a visible blueish to greenish UV-PL on the pelage of the platypus, as previously reported[4]. As low pigmented human hair is also known to be visibly photoluminescent under UV in these specific ranges due to their keratin fibers[30], this phenomenon appears broadly shared in mammals.

Interestingly, a pink to red UV-PL is exhibited on the skin appendages (here hairs and spines) of phylogenetically distant species, such as marsupials (opossums) and placental mammals, the latter including flying squirrels

(Euarchontoglires) and hedgehogs (Laurasiatheria) (Table 1, Fig. 1). Emission spectroscopy reveals in these mammals a series of peaks (often three) in the red region, between 600 and 690 nm, with variable intensity and position depending on the specimens (Fig. 1b), suggesting potential differences in the compounds at play or differences of the binding sites of the same compound[31]. We additionally observed that the associated specimens exhibited variable intensity of observable reddish UV-PL. Notably, the older preserved specimens investigated appeared less intense, suggesting that the responsible compounds are degradable probably by light exposure and might be sensitive to storage condition. Furthermore, we observed that the localization of this reddish UV-PL on the pelage seems to vary within species. In our specimens, the belly was the most luminescent body region of opossum marsupials and flying squirrels, while it was the dorsal spines in hedgehogs (Fig. 1a). Lastly, the red UV-PL is not homogeneously distributed within individuals, as also reported in flying squirrels and springhares[8,9], suggesting potential biological differences within individuals.

Porphyrin accumulation into mammalian pelage is ubiquitous

We used excitation spectroscopy at 700 nm to probe, directly on solid samples, the absorption properties of the compounds responsible for the pink to red UV-PL observed in mammals' pelages. Spectra collected on hairs or spines show an intense band between 390 and 430 nm (so-called Soret band), followed by a series of bands, ten to a hundred times less intense, between 480 and 660 nm (so-called Q-bands) characteristic of free-base (non-metalated) porphyrins (Fig. 2a). Porphyrins are tetrapyrroles, macrocyclic organic compounds including heme and chlorophyll (metalated) that are essential for life on Earth. Q-bands of decreasing intensity at 504.5, 539.6, 578.4 and 630.1 nm are undoubtedly indicative of protoporphyrin IX (PPIX) in the hedgehog's spines (Fig. 2a). However, the position and number of bands is different for the hairs of opossums and flying squirrels, indicating the presence of other porphyrins. Following ref.[32]'s database, the position of Q-bands at 501.4, 535.4, 570.5 and 619.6 nm in the opossums' hairs (Fig. 2a) is consistent with uroporphyrin I or III, and to a lesser extent with porphyrin c. The fifth band observed at 638.4 nm most likely indicates that the uroporphyrin is protonated, as also suggested by the shoulder on the Soret band, two modifications illustrated by our protonated protoporphyrin IX (PPIX2H) reference spectrum. For the flying rodents' hairs, the position of Q-bands at ~ 506, ~550, 578 and 640–646 nm only matches that of porphyrin S-411[32], an analogue of coproporphyrin[33].

As part of the heme biosynthesis pathway, porphyrins are associated with all aerobic and anaerobic metabolisms and are ubiquitous in the cells of many organisms[26,34]. Protoporphyrins and their natural derivatives, such as uroporphyrins and protoporphyrin S-411, are known to be photoluminescent and also involved in coloration, especially in birds feathers[20–25]. Interestingly, although protoporphyrins are normally excreted through feces in mammals, their overproduction and tissue accumulation are known but often associated with diseases called erythropoietic protoporphyrias. Other inherited metabolic porphyria disorders – described in humans with accumulation of δ-aminolevulinic acid and porphobilinogen – cause neurovisceral attacks (acute hepatic porphyrias), or/and skin lesions (cutaneous porphyrias with accumulation of porphyrin ring structures) depending on the affected step of the heme biosynthetic pathway[35,36]. In addition to humans, this condition has been described in a few other mammals such as ruminants, horses, and cats[27,37]. Interestingly, several cases of protoporphyrin accumulation not causing any disease were also reported in mammals. Notably, all members of the fox squirrel *Sciurus niger* are known to accumulate the free-base uroporphyrin I in their internal organs and skin without showing detrimental symptoms, as a result of the low activity of the enzyme uroporphyrinogen III synthase[34]. Other free-base porphyrins were recently identified as causing a non-pathological conspicuous reddish

UV-PL on the pelage of several springhare species[9]. Non-pathological porphyrin accumulation has also been described in a pet hedgehog[38].

As our analyses were conducted on preserved collection specimens, we could not determine whether they exhibited porphyria symptoms before their death, and the causes of porphyrin accumulation in the mammals that we have sampled remain to be identified. Nonetheless, previous observations on living individuals suggest that this condition is predominantly not harmful for most mammal species. Also, as dermal lesions in humans are found in the skin where hairs are sparse[35], it is possible that porphyrin accumulation occurring in the inert hair or spine tissues is naturally less problematic than the one occurring in living tissues such as those of the skin. Therefore, we suppose that most species may exhibit physiological solutions to adapt to the potential toxicity of porphyrin overproduction[34]. This suggests that porphyrin accumulation in the pelage of mammals, resulting in a reddish UV-PL phenomenon, is likely a much more common trait than previously assumed.

The platypus and ermine likely have other photoluminescent compounds in their pelage that remain to be identified. The analysis for these specific UV-PL colors (blue-green and lavender) would require further investigation, because contrary to porphyrins they are not associated with characteristic spectra.

Does pelage photoluminescence have an ecological function?

Our multispectral imaging analysis reveals that the porphyrinic compounds responsible for the reddish UV-PL are located inside the spine and hair fibers of mammals (Fig. 2b), as previously seen in springhares[9]. This is particularly visible in the hedgehog' spines, where the PPIX is located within the inner lumen (Fig. 2b). This suggests that these porphyrinic compounds are excreted within the skin appendages, and throughout the natural process of continuous pelage growth. Also, we found that the spatial and spectral repartition of these compounds is not homogeneously distributed along the hairs and spines, and neither between individuals nor species. Indeed, in the hedgehog's spines, PPIX is coating the walls of the inner lumen from base to apex (Fig. 2b). In the hairs of marsupials and rodents, the porphyrins are present either throughout the total hair length (marsupials) or restricted to a more basal region (flying squirrels). Additionally, we found that the intensity of UV-PL is also lower in areas that are otherwise less pigmented and in hairs that are thinner (*e.g.*, flying squirrels) compared to the thicker and more pigmented spines of hedgehogs (Fig. 2b). Additionally, we found that alcohol-preserved specimens (e.g., marsupial from JAGUARS collections) show higher intensity of UV-PL, compared to the older and dry-preserved specimens of hedgehog or flying squirrels that we analyzed. This variation in degrees of reddish UV-PL intensity were also previously noted in museum specimens[6,8,9]. Because porphyrins are photodegradable compounds[39], it appears thus likely that the preservation of the reddish UV-PL on specimens' pelage may vary depending on both the preservation method employed and the amount of light they have been exposed to through time. This entails that one cannot draw the conclusion that a given species is not accumulating porphyrins in its pelage based on the apparent lack of UV-PL in museum specimens.

As this reddish UV-PL is predominantly found in crepuscular and nocturnal species, this phenomenon has been interpreted as potentially related to visual functions for intraspecific communication or antipredator behavior in light-deprived environments[4,8,9,24]. However, in showing that the reddish UV-PL of mammals' pelage is induced by the accumulation of photodegradable protoporphyrins, it appears more likely that this phenomenon might simply be overrepresented in crepuscular and nocturnal specimens mainly because in their case the porphyrinic compounds are less degraded than in diurnal species. Furthermore, it should be kept in mind that it is far from

evident that natural UV illuminating sources, even at dusk or dawn, are sufficient for this UV-PL phenomenon to be perceived and contrasted from the reflected visible light by co-specifics or predators[11]. Indeed, for the UV-PL to have a visual function, it would require that the animals are exposed, voluntarily or not, to sufficient UV illumination so that the photoluminescence reemitted is seen by other individuals or species. Additionally, one should keep in mind that when it comes to UV-PL, the reemitted light is in the “visible spectrum” (400–700 nm), so whether the animals have an improved UV light vision (200–400 nm) does not provide them with any advantage to perceive such photoluminescence.

Given these results, we thus suggest an alternative hypothesis to interpret the accumulation of PPIX derivatives in the pelage of mammals, resulting in this observable UV-PL. We suggest that this phenomenon is a byproduct of the heme biosynthesis with no other function than to be excreted from the organism by being naturally degraded through the pelage[1,28]. Storing these residual molecules of the heme pathway – which might become toxic when produced in too high quantities[26,35,36] – in skin appendages that are composed of inert tissues such as hair or spines and that are likely to be exposed to light, could hence help excreting these compounds without further energetically demanding metabolic processes. Given its distribution in the phylogeny, this perhaps represents a mechanism that was acquired early in the evolution of mammals. Finally, further inquisition on the functional significance of UV-induced photoluminescence in animals and plants should include a more thorough and comprehensive approach, incorporating both the ecology and physiology of the organisms exhibiting these phenotypes[1,28].

Methods

Specimens studied. We examined a variety of specimens from various species of monotreme, marsupial, and placental mammals with preserved furs in either alcohol or as dry preparations, from museum collections of the Museum für Naturkunde of Berlin, the Swedish Museum of Natural History of Stockholm, the Muséum national d’Histoire naturelle of Paris, the JAGUARS collection of Cayenne, personal collection from Dr. Anthony Herrel, and the Naturkundemuseum of Potsdam. A selection of some specimens and associated methods of analysis are presented in Table 1. To proceed with photoluminescent spectroscopy and multispectral imaging, we collected several samples of complete hairs and spines of known orientation and spatial localization on the pelage of several selected specimens (Table 1, Fig. 1a., Fig. 2b.). Spectroscopy and multispectral imaging measurements were also carried out on two complete specimens preserved in alcohol (JAGUARS-M535 and JAGUARS-M2838).

UV-induced photoluminescence macro-analysis and photography. Specimens were observed and photographed first under natural or standard white light conditions. Then they were placed in the dark and illuminated using specific UV light setups emitting at a distance of about 20 cm from the specimens. We initially used a 395 nm LED torch (100 LED flashlight, Youthink) following ref.[8] and associated with a yellow filter (K&F Concept) to annul the purple light emitted by this torch. Nevertheless, this setup may leave out some wavelengths that are not captured by the yellow filter and which can be misinterpreted as UV-PL. We therefore used a professional 365 nm compact lamp (4 W, UVL-21, UVP) associated with a strict UV black filter (Filter Band U-360 2IN SQ, Edmund Optics Ltd). Photographs presented in Fig. 1.a. were obtained using the second setup, when the filter was placed directly on the UV emitting surface of the lamp. Photographs were taken using a Canon EOS 5D Mark III equipped with a 50mm macro lens and a UV blocking filter (UV390 Protect Filter, hama). White balance was systematically readjusted for each specimen.

Photoluminescence spectroscopy. Emission and excitation spectroscopy were performed using a spectrofluorometer Fluorolog 3–22 from HORIBA Jobin Yvon. Spectra were collected using a bundle of optical fiber equipped with a focusing optics allowing to perform measurements out of analysis compartment. Entrance and exit slits widths of the monochromators were set at 10 nm in order to ensure a balance between the collection of spectra with good signal-to-noise and a spectral resolution compatible with the detection of Q bands. The emission spectra were collected using an excitation at 400 nm. To record the excitation spectra, a 732 ± 34 nm bandpass filters (from Semrock) was placed at the entrance of the emission monochromator in order to collect the signal at 700 nm without any interference due to the second order diffraction of excitation light or due to stray light.

Photoluminescence multispectral imaging. False color luminescence images were assembled using black and white images collected with a home-made setup which consists of a low-noise 4-megapixel Si C-MOS camera (ORCA flash V4.0 V2 - Hamamatsu) with a sensitivity ranging from 200 to 1100 nm. The camera is fitted with a UV-VIS-IR 60 mm 1:4 Apo Macro lens (CoastalOptics) in front of which is positioned a filter wheel holding 8 Interference band-pass filters (Semrock) to collect images in specific spectral ranges. Illumination was provided by 16 LED lights ranging from 365 up to 700 nm (CoolLED pE-4000), coupled to a liquid light-guide fiber fitted with a fiber-optic ring light-guide, allowing homogeneous illumination of the region of interest. False color images were generated by assigning red, green and blue color to specific emission ranges using ImageJ[40] software.

Declarations

Data availability statement

The dataset generated for this study, *i.e.*, the raw multispectral images and emission and excitation spectra, are available on Figshare (https://doi.org/10.6084/m9.figshare.XXX).

Code availability

The code used in this study can be accessed on Figshare (https://doi.org/10.6084/m9.figshare.XXX).

Acknowledgments

We thank the Museum für Naturkunde of Berlin, especially C. Funk, A. Rosemann, F. Mayer and D. Willborn; and the Naturkundemuseum of Potsdam, especially I. Pokorny, for lending us various specimens of mammals (Germany). We are grateful to B. de Thoisy and A. Herrel for lending us fresh marsupial specimens from the JAGUARS collection (French Guyana). We thank the Dortmund Zoo, especially M. Stawinoga and I. Schappert for their support with data acquisition. Finally, we thank M. Jansen for helpful advice in data interpretation. E.A. was supported by the German Research Council (Deutsche Forschungsgemeinschaft; grant number AM 517/1-1). J.P. was supported by the Elsa-Neumann-Stipendium (Humboldt-Universität zu Berlin). S.L.D.T. was supported by the Fyssen Foundation and the Alexander Von Humboldt Foundation.

Author contributions

E.A., J.P., S.L.D.T., P.G., and M.T. conceived this study. S.L.D.T., J.P., and E.A. conducted the macro-analysis and photography of collection specimens. D.C.K. helped in macro-analysis. P.G., M.T., B.H., R.G., and R.M. conducted the multispectral imaging and spectroscopy analyses and processing and identified the porphyrins. D.C.K., S.B., P.M.

and S.S. equally helped with data interpretation. S.L.D.T, J.P. and E.A. drafted the manuscript with inputs from M.T. and P.G. All authors reviewed the final version of the manuscript.

Competing interests

We have no competing interest to declare.

Materials & Correspondence

Material and correspondence requests should be addressed to J.P., E.A. and S.L.D.T.

References

1. Lagorio, M., Cordon, G. & Iriel, A. Reviewing the relevance of fluorescence in biological systems. *Photochem. Photobiol. Sci.***14**, 1538–1559 (2015).
2. Jeng, M.-L. Biofluorescence in Terrestrial Animals, with Emphasis on Fireflies: A Review and Field Observation. in *Bioluminescence - Analytical Applications and Basic Biology* 1–15 (IntechOpen, 2019). doi:10.5772/intechopen.86029.
3. Prötzel, D., Heß, M., Schwager, M., Glaw, F. & Scherz, M. D. Neon - green fluorescence in the desert gecko *Pachydactylus rangei* caused by iridophores. *Sci. Rep.* 1–10 (2021) doi:10.1038/s41598-020-79706-z.
4. Anich, P. S. *et al.* Biofluorescence in the platypus (*Ornithorhynchus anatinus*). *Mammalia* (2020) doi:10.1515/mammalia-2020-0027.
5. Meisner, D. H. Psychedelic opossums: fluorescence of the skin and fur of *Didelphis virginiana*. *Ohio J. Sci.***83**, 4 (1983).
6. Pine, R. H., Rice, J. E., Bucher, J. E., Tank, D. H. & Greenhall, A. M. Labile pigments and fluorescent pelage in didelphid marsupials. *Mammalia***49**, 249–256 (1985).
7. Reinhold, L. Mammals with fluorescent fur : Observations from the Wet Tropics. *North Queensl. Nat.***51**, (2021).
8. Kohler, A. M., Olson, E. R., Martin, J. G. & Anich, P. S. Ultraviolet fluorescence discovered in New World flying squirrels (*Glaucomys*). *J. Mammal.***100**, 21–30 (2019).
9. Olson, E. R. *et al.* Vivid biofluorescence discovered in the nocturnal Springhare (Pedetidae). *Sci. Rep.* 1–8 (2021) doi:10.1038/s41598-021-83588-0.
10. Bachman, C. H. & Ellis, E. H. Fluorescence of bones. *Nature* 1328–1331 (1965).
11. Goutte, S. *et al.* Intense bone fluorescence reveals hidden patterns in pumpkin toadlets. *Sci. Rep.***9**, 5388 (2019).
12. Prötzel, D. *et al.* Widespread bone-based fluorescence in chameleons. *Sci. Rep.***8**, 698 (2018).
13. Taboada, C. *et al.* Naturally occurring fluorescence in frogs. *Proc. Natl. Acad. Sci.***114**, 3672–3677 (2017).
14. Arnold, K. E., Owens, I. P. F. & Marshall, N. J. Fluorescent signaling in parrots. *Science***295**, 92 (2002).
15. McGraw, K. J. *et al.* A description of unique fluorescent yellow pigments in penguin feathers. *Pigment Cell Res.***20**, 301–304 (2007).
16. Pearn, S. M., Bennett, A. T. D. & Cuthill, I. C. Ultraviolet vision, fluorescence and mate choice in a parrot, the budgerigar Melopsittacus undulatus. *Proc. R. Soc. B Biol. Sci.***268**, 2273–2279 (2001).

17. Weidensaul, C. S., Colvin, B. A., Brinker, D. F. & Huy, J. S. Use of ultraviolet light as an aid in age classification of owls. *Wilson J. Ornithol.***123**, 373–377 (2011).
18. Kellie, A., Dain, S. J. & Banks, P. B. Ultraviolet properties of Australian mammal urine. *J. Comp. Physiol. A Neuroethol. Sensory, Neural, Behav. Physiol.***190**, 429–435 (2004).
19. Leiner, M. J. P., Hubmann, M. R. & Wolfbeis, O. S. The total fluorescence of human urine. *Anal. Chim. Acta***198**, 13–23 (1987).
20. Church, A. H. & Oxon, M. A. Researches on Turacin, an animal pigment containing copper. *Philos. Trans. R. Soc. London* 627–636 (1869).
21. Rimington, C. A reinvestigation of turacin, the copper porphyrin pigment of certain birds belonging to the Musophagidae. *Natl. Inst. Med. Res.* 106–120 (1938).
22. Negro, J. J., Bortolotti, G. R., Mateo, R. & García, I. M. Porphyrins and pheomelanins contribute to the reddish juvenal plumage of black-shouldered kites. *Comp. Biochem. Physiol. - B Biochem. Mol. Biol.***153**, 296–299 (2009).
23. Okazaki, T. & Imamura, S. Distribution of protoporphyrin □ in bird feathers. *Int. J. Anal. bio-science***7**, 41–48 (2019).
24. Camacho, C., Negro, J. J., Redondo, I., Palacios, S. & Sáez-Gómez, P. Correlates of individual variation in the porphyrin-based fluorescence of red-necked nightjars (*Caprimulgus ruficollis*). *Sci. Rep.***9**, 1–9 (2019).
25. Galván, I., Camarero, P. R., Mateo, R. & Negro, J. J. Porphyrins produce uniquely ephemeral animal colouration: A possible signal of virginity. *Sci. Rep.***6**, 1–9 (2016).
26. Ajioka, R. S., Phillips, J. D. & Kushner, J. P. Biosynthesis of heme in mammals. *Biochim. Biophys. Acta - Mol. Cell Res.***1763**, 723–736 (2006).
27. Rimington, C. & Moore, M. R. Porphyria in animals. *Clin. Dermatol.***3**, 144–155 (1985).
28. Marshall, J. & Johnsen, S. Fluorescence as a means of colour signal enhancement. *Philos. Trans. R. Soc. B Biol. Sci.***372**, (2017).
29. Lim, M. L. M., Land, M. F. & Li, D. Sex-specific UV and fluorescence signals in jumping spiders. *Science***315**, 481 (2007).
30. Millington, K. R. & Marsh, J. M. Anomalous Fluorescence of White Hair Compared to Other Unpigmented Keratin Fibres. *Int. J. Cosmet. Sci.* 0–3 (2019) doi:10.1111/ics.12596.
31. Sandberg, S., Talstad, I., Hovding, G. & Bjelland, N. Light-induced release of protoporphyrin, but not of zinc protoporphyrin, from erythrocytes in a patient with greatly elevated erythrocyte protoporphyrin. *Blood***62**, 846–851 (1983).
32. DiNello, R. K. & Chang, C. K. Isolation and modification of natural porphyrins. in *The Porphyrins* (ed. David, D.) 289–339 (1978).
33. French, J., Nicholson, D. C. & Rimington, C. Identification of the acrylate porphyrin S-411 from meconium. *Biochem. J.***120**, 393–397 (1970).
34. de Oliveira Neves, A. C. & Galván, I. Models for human porphyrias: Have animals in the wild been overlooked?: Some birds and mammals accumulate significant amounts of porphyrins in the body without showing the injurious symptoms observed in human porphyrias. *BioEssays***42**, 1–9 (2020).
35. Karim, Z. *et al.* Porphyrias: A 2015 update. *Clin. Res. Hepatol. Gastroenterol.***39**, 412–425 (2015).
36. Kauppinen, R. Porphyrias. *Lancet***365**, 241–252 (2005).

37. Tennant, B. C. Lessons from the porphyrias of animals. *Clin. Dermatol.***16**, 307–315 (1998).
38. Wolff, C. F., Corradini, P. R. & Galaxia, C. L. M. V. Congenital erythropoietic porphyria in an African hedgehog (*Atelerix albiventris*). *J. Zoo Wildl. Med.***36**, 323–325 (2005).
39. Poh-Fitzpatrick, M. B. & DeLeo, V. A. Rates of plasma porphyrin disappearance in fluorescent vs. red incandescent light exposure. *J. Invest. Dermatol.***69**, 510–512 (1977).
40. Schindelin J, . *et al.* Fiji: an open-source platform for biological-image analysis. *Nat. Methods.* **9**, 676–682 (2012).
41. Kumar, S., Stecher, G., Suleski, M. & Hedges, S. B. TimeTree: A Resource for Timelines, Timetrees, and Divergence Times. *Mol. Biol. Evol.***34**, 1812–1819 (2017).

Figures

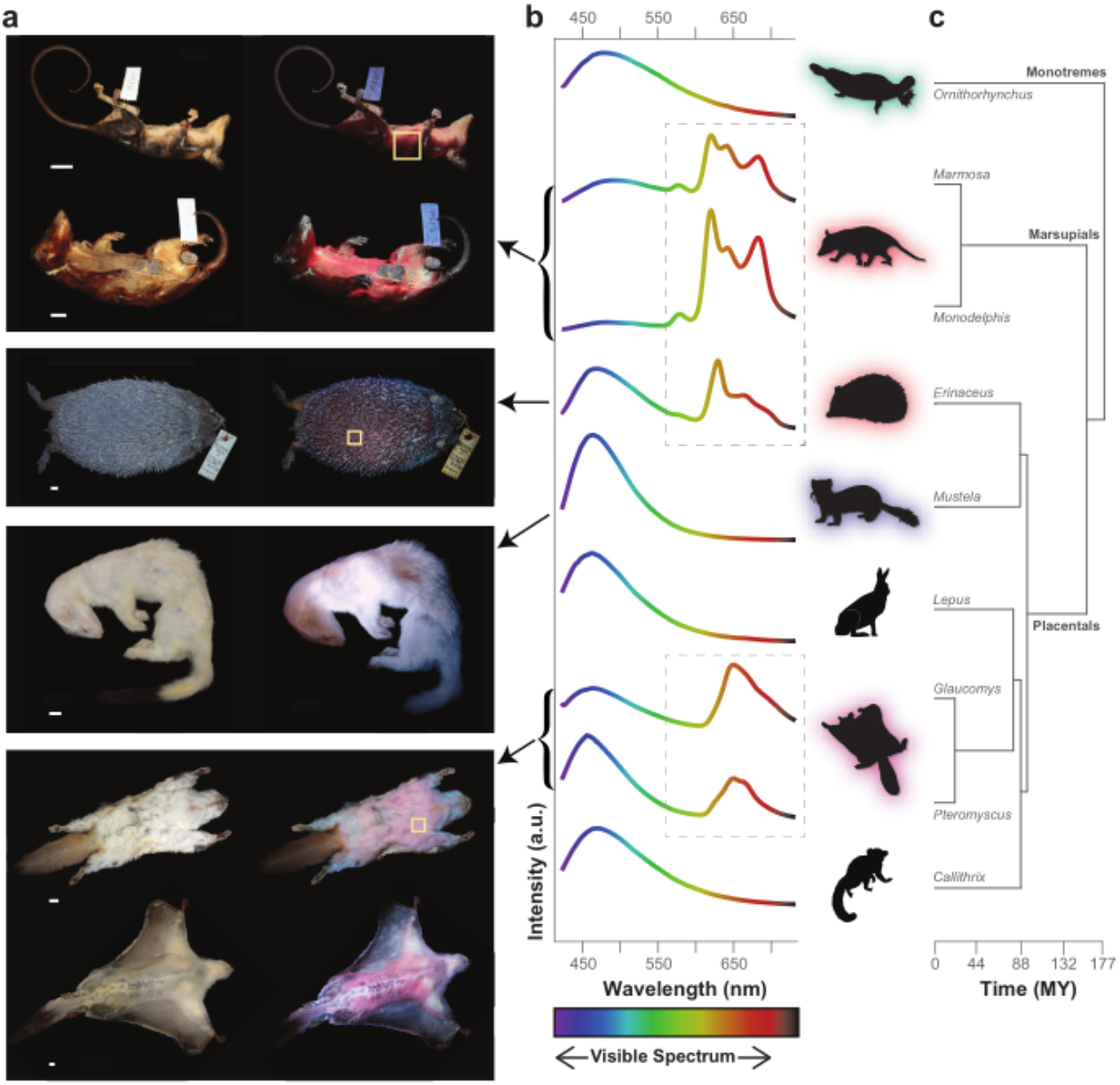

Figure 1

Illustration of UV-based photoluminescence of fur and spines in various mammal species. a. Photographs of, from top to bottom: Marmosa murina JAGUARS-M535 (opossum), Monodelphis brevicaudata JAGUARS-M2962 (opossum), Erinaceus roumanicus ZMB_Mam_48212 (hedgehog), Mustela erminea NKMP-unnumbered (ermine), Glaucomys volans MNHN 1939-707 (flying squirrel), and Pteromyscus pulverulentus MNHN - 1979-376 (flying squirrel), under standard white light (left) and under controlled UV illumination (right). Scale bars = 1 cm. The yellow squares represent the localization of further multispectral imaging presented in Figure 2. b. Emission spectra obtained with luminescence spectroscopy technique on fur samples of, from top to bottom: Ornithorhynchus anatinus ZMB_Mam_35991 (platypus), Marmosa murina JAGUARS-M535, Monodelphis brevicaudata JAGUARS-M2962, Erinaceus europaeus NRM_594242, Mustela erminea NRM_20175124, Lepus timidus NRM_588837 (hare), Glaucomys volans ZMB_Mam_60634, Glaucomys sabrinus NRM_875246, and Mico argentatus NRM_617493 (primate). Glowing halos surrounding black silhouettes indicate the visible photoluminescent color of the fur only, observed from macro-analysis (see also Table 1). Black silhouettes are all public domain and from phylopic.org c. Phylogenetic relationships of the associated specimens (from timetree.org41) showing the widespread repartition of UV-based photoluminescence across mammals.

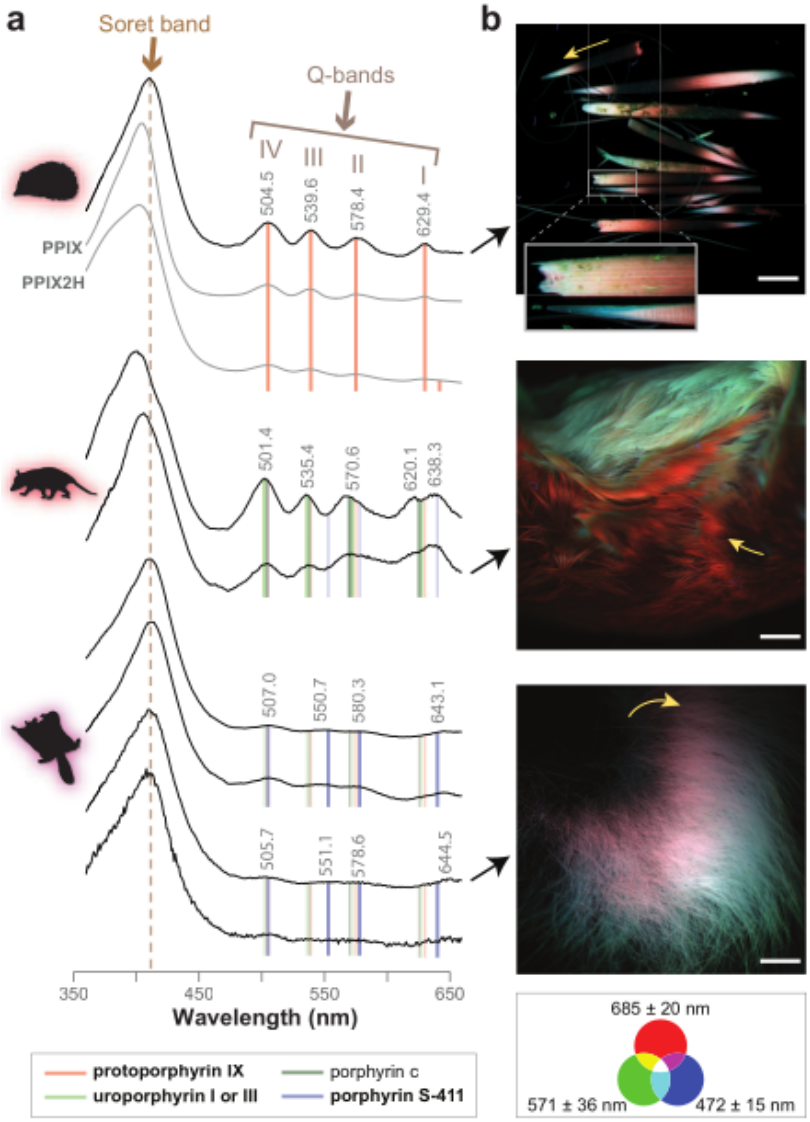

Figure 2

Characterization of the reddish photoluminescence of mammalian skin appendages. a. Excitation spectra of, from top to bottom: Hedgehog's dorsal spines (Erinaceus europaeus NRM_594242), protoporphyrin IX (PPIX) and protonated protoporphyrin IX (PPIX2H) reference spectra, opossums' ventral fur from complete alcohol-preserved specimen (Monodelphis brevicaudata JAGUARS-M2838 above, and Marmosa murina JAGUARS-M535 below), a flying squirrel's ventral hair samples (Glaucomys volans ZMB_Mam_60634) which appeared correspondingly glowing (above) and non-glowing (below) to the human eye under macro-imaging, and another flying squirrel's ventral and dorsal hair samples (Glaucomys sabrinus NRM_875246) which appeared correspondingly glowing (above) and non-glowing (below) to the human eye under macro-imaging. Position of the Q-bands is characteristic of PPIX for the hedgehog, uroporphyrin for the marsupials, and porphyrin S-411 for the flying squirrels. Position of the Q-bands of porphyrin c, close to that of uroporphyrin, are also displayed for reference. b. False color multispectral images under 385 nm excitation of spines and hairs analyzed using spectroscopy, revealing a non-homogeneous repartition of photoluminescent compounds into and along the spines of hedgehogs (including a magnified inset) and pelage and hairs of marsupials and flying squirrels. Sampling localizations are indicated by yellow squares in Fig. 1a. Arrows represent the direction of spine or hair growth. Scale bars = 3 mm. False colors correspond to the detected wavelengths (see the three-color scale for correspondences).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ToussaintetalSpectra.xls
- RawMultispectralImages.zip
- ToussaintetalRscript.txt