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Widespread fluorescence in terrestrial and marine samples investigated from India

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Abstract

The discovery of green fluorescent protein (GFP) from jellyfish *Aequorea victoria* has provoked numerous studies to unveil the myriads of biomedical applications. Consequently, several studies also revealed the prevalence of fluorescence in different marine and terrestrial organisms. However, since GFP's discovery or the Nobel prize award on GFP, the fluorescence has not been explored so far from India. The current study presents the widespread fluorescent organisms resources available in India for biomedical and toxicological applications. Fluorescence emission from different plant and animal components were examined by direct observations using UV torchlight. Investigation revealed that blue light excited fluorescence in several organisms. For the first time, this study observed GFP like fluorescence in a cockroach *Neostylopyga rhombifolia* from India. Observations are indicating that fluorescent proteins have essential ecological functions that are yet to be determined. The examined plant and animal components were not bioluminescent. In comparison, the potential untouched research areas on fluorescence aspects are detailed. A thorough review of fluorescent organisms reported hitherto is also provided to spread the current knowledge on fluorescence.

Keywords: Photoproteins, fluorescent proteins, green fluorescent protein, fluorescent assays, India.

Introduction

Fluorescence is a bio-optical phenomenon in which light is absorbed in one wavelength and re-emitted at another longer wavelength. Fluorescence disappears when the excitation source ceases, but phosphorescence remains for a while even after removing of the excitation source. Fluorescent proteins (FPs) have become an indispensable component of the biomedical imaging toolbox for cellular imaging applications [1,2]. In 1852, George Gabriel Stokes, “Father of Fluorescence,” Lucasian Professor at Cambridge University, first introduced fluorescence as a refrangibility of light [3]. Later, pioneers on bioluminescent organisms have observed fluorescence coupled with luminescence [4]. The first representative of fluorescent protein-coupled to bioluminescence was the green fluorescent protein (GFP). For the first time, Osamu Shimomura and colleagues discovered and extracted the GFP from the jellyfish *Aequorea victoria* [5]. It took about 30 years from GFP’s discovery to find GFP’s potential application as a genetically encoded fluorescence marker. The tremendous impact of GFP technology on life science research was recognized by the award of the 2008 Nobel Prize in Chemistry to Osamu Shimomura, Martin Chalfie, and Roger Tsien [6]. A recent study suggested that fluorescence can be used as a biosignature to observe life in other planets [7]. Applications of FPs include the labeling of cells, cellular compartment or proteins, studies of gene activity, sensor studies, and taxonomic markers. This has increased the requisite demand for investigating novel and potential FPs other than GFP for different biomedical and industrial applications.

Invertebrates and vertebrates are the most promising sources of novel marker proteins because of the vast diversification of photoactive and photo-inactivated FPs within the tree of life [6,8]. These glowing FP markers allow researchers to visualize the tagged protein movements, positions, and interactions in live cells. Therefore investigating FPs from marine organisms- from ecological to application-level would fetch discoveries in evolutionary and industrial applications. Since 1999, FPs have increased commercial potential as tagging markers in bioscience applications. Dr. Zhiyuan Gong and his colleagues developed a patented brand of GloFish for detecting pollution through selectively fluorescing in the presence of environmental toxins. It is the first genetically modified animal to become publicly available as a pet, and later Alba, the fluorescent rabbit [9]. Since then, GFP has revolutionized industries and biomedical institutions worldwide by enabling a wide range of applications. Recent studies have focused on FP’s from different marine organisms such as sea anemones [10], corals [11], sharks, rays, turtles [12], shells [13,14], different marine fishes, such as eels, lizardfishes, blennies, scorpionfishes, gobies, goatfish and flatfishes [8], bristle worms, anemone hermit crab, nudibranchs and tunicates.

Among the vast array of marine pigments [15,16], FPs have gained more attention due to their applications in neurological studies and toxicological assays to visualize cancer cells *in vivo*. Therefore, the exploitation of natural FP's from marine organisms is increasing in recent years. Natural FPs such as GFP, RFP (red fluorescent protein), and YFP (yellow fluorescent protein) have numerous applications in research [17]. FPs are being used more extensively in biotechnology as genetically encoded labels and reporters [6]. Many model organisms such as Rats, Rabbits, and Pigs, Mouse & Mice Models, *Drosophila*, Adenovirus, Vaccinia virus, yeasts, *Caenorhabditis elegans*, *Bradyrhizobium*, *Escherichia coli*, *Salmonella typhimurium*, *Vibrio cholerae*, *Shigella*, Zebrafish, White Leghorn Chick Embryo, African clawed frog *Xenopus laevis*, cynomolgus monkey *Macaca fascicularis* and various plants (e.g., *Arabidopsis*) have been developed to study the properties of FPs for multiple applications including the development of cells, disease propagation and toxicity [18–25]. In this context, the present study is focused on geographically distinct and unexplored Andaman and Nicobar Islands to study marine biodiversity as a prospective source of natural FPs for industrial applications.

Andaman and Nicobar Islands have located about 1500 km from the mainland, consisting of a chain of 572 Islands. These islands have rich marine biodiversity and erratic weather conditions, making these islands different from other geographic places. Diversity of marine fluorescent organisms on these islands are prevalent, and GFP possessing jellyfish *Aequorea victoria*, other species of jellyfishes, and some fishes are existing in these Islands (author Unpublished data). Fluorescence from marine and terrestrial organisms has not been documented anywhere in India, especially from Andaman & Nicobar Islands. This has necessitated the present study to explore the fluorescent organisms for ecological and biomedical applications.

Material and methods

Standard methods are performed throughout this research [6,8,10,11] and being implemented for future applications.

Study area, field surveys, and identification of specimens

Andaman and Nicobar Islands are rich in marine biodiversity but least studied in India. The field surveys were mostly conducted at different locations along the Burmanallah coast of Andaman Islands (11°33'36.24" N, 92°43'49.73" E). In addition, some field surveys were conducted in Barrankula, Andhra Pradesh on the east coast (15°53'46.51" N, 80°57'22.02" E), and Goa (15°29'48.39" N, 73°49'40.17" E) on the west coast of India. Different specimens of invertebrates (e.g., sponges, sea slugs, shrimps, molluscans, and sea stars) were handpicked; vertebrates (e.g.,

fishes, sharks, rays) from the tide pools in Burmanallah were collected with a hand net. Some specimens, such as corals and sea anemones, were photographed during SCUBA diving at night dives. Terrestrial specimens such as frogs, snakes, bird feathers, scorpions were investigated during night fields at Barrankula, Andhra Pradesh. After careful observations and photography, live specimens were released back into their habitats for conservation. All the living and non-living fluorescent components were identified with identification keys and previous literature.

Photography of fluorescent organisms

Images of fluorescent organisms under natural illumination and UV blue light were captured with Samsung Galaxy grand2 smartphone or underwater digital cameras GoPro Hero 6 and Nikon Cool Pix using yellow filters. Fluorescence of different plant and animal components was excited using underwater blue LED torchlight (Tonelife Fluoro-Diving Fluo Night Dive Underwater UV Light torch) (450 nm) or UV blue torch lights (THINK3 21 LED UV Ultra Blacklight Pocket Flashlight and WOLFWILL 100 LED Ultraviolet Blacklight Flashlight) (390 to 395 nm).

Results

During the night, field observations have unveiled the widespread of fluorescence in different plants, animals, and non-living components under UV blue flashlight (Fig. 1-5). Red fluorescence is more prevalent in plant components, including marine filamentous algae, seaweed, seagrass, and several other terrestrial plants. Scientifically, it is evident that marine and terrestrial plants display red fluorescence due to chlorophyll-a pigment; however, not many plants displayed red fluorescence (data not shown). In this study, fluorescent components are alone represented from the application's point of view. Interestingly, the development of brown spots on exterior fruit parts of the banana plant *Musa paradisiaca* was obvious under UV blue light. Similarly, the internally damaged flower part of the false-bird paradise *Heliconia rostrata* displayed green fluorescence. In rose periwinkle *Catharanthus roseus*, flower buds alone showed green fluorescence. Various species of wood-rotting fungi such as *Trametes* sp. and *Coriolopsis* sp. have exhibited greenish-yellow fluorescence, which is not demonstrated by any plant investigated in this study (Fig. 1a-x).

In contrast, the majority of the animal components displayed mostly blue-green fluorescence. Most of the cnidarian groups of animals, such as corals, sea anemones, and zoanthids, showed green fluorescence (Fig. 2a-o). Exceptionally, mouth regions of sea anemone, *Phymanthus buitendijki*, displayed yellow and orange fluorescence besides their whole body green fluorescence (Fig. 2e-h). In a polychaete worm, *Marphysia* sp., parapodia alone found to fluoresce in green. Likewise, some part of the crab, *Thalamita integra* carapace, revealed green fluorescence (Fig. 2q-t). Blue-green

fluorescence commonly seen in spiders, cockroach, and flies; red fluorescence was detected in a butterfly and praying mantis (Fig. 3). Red fluorescence is highly prevalent in gastropods than in other groups (Fig. 4). Orange-red fluorescence was perceived in sea slugs *Haminoea ovalis* and *Pteraeolidia ianthina*, and sea urchin *Temnopleurus toreumaticus* (Fig. 2p & 2u-x). Green fluorescence is prevalent in the epidermis of scorpion *Hottentotta tamulus* and tarsal segments and thoracic segments of the cockroach *Neostylopyga rhombifolia* (Fig. 2y-a2). Though red fluorescence is dominant in gastropods, gastropod's fluorescence pattern is area-specific, but the whole shell fluorescence is not typical in all cases (Fig. 4a-a6). The common hermit crab, *Diogenes* sp. displayed green fluorescence (Fig. 4a3).

The emission color of fluorescence varied among higher animals like birds, amphibians, reptiles, and fishes (Fig. 5a-l). The eggshells of country chicken *Gallus gallus domesticus* showed red fluorescence, while newly hatched chick feathers displayed green fluorescence in the hackle region (Fig. 4a-d). In amphibians like the Indian burrowing frog *Sphaerotheca breviceps* and common tree frog *Polypedates maculatus*, blue-green fluorescence was detected in their eyes (Fig. 5e-h). The white cross bands on the nonvenomous Indian wolf snake, *Lycodon aulicus*, emits yellow fluorescence (Fig. 5i&j). The Indian mud moray eel, *Gymnothorax pictus* is seen in large numbers during August month at near freshwater channels connected to the Burmanallah coast, where this species comes for breeding and spawning. During this period, investigations revealed whole body yellow fluorescence in *G. pictus* (Fig. 5k&l).

In non-living components, several chemical components are involved in the emission of varied colors of fluorescence (Fig. 6). Often, phosphorescence is mistaken as fluorescence by several people. Fluorescence is seen only under source light exposure like blue light and remains up to light exposure, but phosphorescence lasts up to several minutes even after removing inducing light (Fig. 6a-d). The macro fecal excretions from the herbivore fish guts displayed red fluorescence due to chlorophyll-a intake via plant components (Fig. 6i&j). Fluorescence phenomena are utilized in the currency for security from duplicated currency (Fig. 6e&f), but photoluminescence is utilized to direct the paths (Fig. 6g&h). The presence of fluorescent compounds in other products such as color naphthalene balls and yogurt, and their functions are unknown (Fig. 6k-n). The methanol extractions of several invertebrates and bacteria have displayed red fluorescence; however, these fluorescent molecules are yet to be elucidated.

Discussion

The tiny blue planet earth is filled with mesmerizing and unexplored living and non-living components with novel and new chemistry. Among all the known animals and plants from terrestrial and marine environments, only some creatures have attained special attention due to their extraordinary biochemistry of fluorescence, bioluminescence, and iridescence. Primarily, FP's were well documented in bioluminescent [26] and non-bioluminescent Anthozoa species [22,27]. In hydromedusan like *Olindias formosus* and other marine fishes, fluorescent proteins function as prey attractants [28], and significantly, many reef fishes used fluorescence for visual function [8]. In corals, the photoconvertible red fluorescent proteins (pcRFPs) transform the prevalent blue light into orange-red light, penetrating into zooxanthellae containing coral tissues to facilitate homogenous distribution of electrons [29]. This mechanism helps corals to adapt and acclimatize to shallow to deep water environments through coral–*Symbiodinium* association [29]. Significantly, the fluorescence of corals also demonstrated to attract symbiotic photosynthetic dinoflagellates of the genus *Symbiodinium* [30]. Several other hypothesized functions of fluorescence in scleractinian corals are photoacclimation [31], visual contrast [32], antioxidation [33,34], coral health [35], photoprotective from excessive sunlight and light enhancers under low light and resistant to heat stress [36], photoprotection of zooxanthellae [37] and regulation of symbiotic dinoflagellates [38]. Many fishes possess the yellow intraocular filters that act as long-pass filters to perceive fluorescence in the surrounding milieus [39–41]. Several other hypothesizes were also inferred from the presumed functions of fluorescence in fish [42]. A recent study also reported the application of FPs to detect coral recruitment, coral health, tissue damage, and disease propagation and identify organisms not visible to naked eyes [43].

The present study infers that the widespread fluorescence in different components is not in correlation with emission color. Coelenterates have a distinct fluorescence than molluscans (shells), where the former group of animals exhibits blue-green, orange, red, and pink, while the latter displayed red fluorescence. The fluorescent molecules in coral tissues regulate light exposure; thus, coral tissues are protected from sunlight [37]. The red fluorescent proteins present in silkworm, *Bombyx Mori* L., has displayed potential antiviral [44] and antimicrobial activities [45]. Insects may use fluorescence to attract prey or warn predatory creepers. However, further experimental studies are required to understand the actual functions. A study reported the fluorescent fecal excretion by German cockroach, *Blattella germanica*, upon feeding orange fluorescent bait [46]. In the present study, the cockroach, *N. rhombifolia* did not show any fluorescence in fecal pellets or eggs. One live specimen of cockroach, *P. americana*, investigated from Andaman did not show fluorescence, but

the same species from Goa did fluoresce, suggesting the evolutionary adaptation in this species. Fluorescence of the internal organs of cockroaches may be considered non-functional [47,48], but needed to be confirmed with experiments. High nitrogen content containing molecules like resilin may be responsible for external fluorescence in many insects [49].

A review has detailed various chemicals responsible for fluorescence in different plants and animals [50]. The primary light-harvesting pigments of photosystem II of algae are water-soluble red fluorescent phycobiliproteins. While, chlorophyll-a and phycoerythrin are among the well-studied fluorescent molecules with red and orange emission, respectively, and are used as fluorescent probes or antibodies in different applications. The autofluorescence of plant components is non-function. In addition to chlorophyll, most algae and plants also possess orange-fluorescent pigment phycoerythrin [51]. The green fluorescence in *Heliconia rostrata* and *Catharanthus roseus* might be due to the presence of betaxanthins [52]. In crustaceans, exoskeletons display blue fluorescence due to chitin. Based on the fluorescent color emitted from fishes' excretions under the UV blue light, fish can be differentiated as herbivore or carnivore or omnivore. The gut contents of herbivore fish display red or orange fluorescence. In contrast, the carnivore fish gut contents exhibit blue fluorescence, and omnivore fish shows mixed fluorescence of blue, orange, and red colors. Some experimental studies demonstrated that predator animals' fluorescent proteins act as lures to prey [28,41,53]. This happens when prey or predator can perceive fluorescence through special retinal cones. In animals like cnidarians, GFP-like molecules are primary chemical agents responsible for fluorescence (Table 1). Several other fluorescent compounds responsible for fluorescence in birds, millipedes, scorpions, cockroaches, and other marine organisms are detailed in a review [50]. The exterior fruit part of the banana showing brownish bruises or spots indicates that the entire fruit acid content is converted to sugars, and the fruit is ripe. These brown to black spots are highly accumulated with TNF-cytokines, which regulate and direct immune cells to infection areas. Peripheries of these black spots display cream to white fluorescence caused by tyrosinase enzyme and TNF-cytokines accumulation (Fig. 1p). While phosphorescence is mostly due to chemical formulations of strontium aluminate or zinc sulphide mixed with copper.

The present study raises various questions on the existence of fluorescence in specific parts of animal bodies like mouth rings of sea anemones, cockroach tarsal segments, wolf snake yellow bands, frog eyes, eggshells, gastropods, and parapodia of polychaete worm (Fig 2-5). Why only some parts of the body display fluorescence rather than whole-body fluorescence? and why fluorescence color remains the same in different gastropods, and it varies in other groups of

animals?. These questions need to be addressed in further research to understand the functions of fluorescence.

Prospects of this work

The present study findings reveal new avenues in the field of fluorescence from different ecosystems in India. The various visual colors of fluorescence observed in other organisms will be extracted, purified, and maintained as a baseline data depository for future reference. They will be readily used for further applications in biomedical research. The discovery and development of novel fluorescent molecules with potential fluorescence ability will be given special attention for further analysis.

Conclusion

Fluorescence is widespread in tropical environments than in Polar Regions and vastly distributed in marine than in terrestrial ecosystem. Numerous fluorescent animals, plants, and minerals have been documented from freshwater, marine, and terrestrial environments. Based on the evidences, does the same phenomenon exist in other planets? If so, this could assist in resolving evolutionary lineages. This manuscript provides a detailed preliminary data on some fluorescent resources of flora and fauna available in India. Further, the immense biodiversity potential hotspot areas viz. the Andaman & Nicobar Islands, Lakshadweep, Gulf of Mannar, Gulf of Kachchh, Malavan, Western Ghats, and North-Eastern Hills of India are yet to be explored for a variety of fluorescent organisms to address evolutionary aspects as well as to explore novel proteins for various applications. Especially, the exploration of fluorescent proteins and minerals from special and extreme environments such as the deep sea, glaciers, hydrothermal vents, and other planets will be of great interest to delineate the evolutionary lineages and improve the fluorescence-based assays. In addition, detailed reviews are required on natural, synthetic, and recombinant fluorescent proteins to understand the comparative chemical properties and their applicability in different fields. Spectral analysis and chemical molecules responsible for fluorescence in these organisms need to be elucidated. This study infers that fluorescence can be utilized as an important proxy to identify life on other planets.

Conflict of interest

The author does not have any conflict of interest in this manuscript.

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References

- [1] M. Zimmer, *Glowing genes: a revolution In biotechnology*, Prometheus Books, Amherst, 2005.
- [2] V. Pieribone, D.F. Gruber, *A glow in the dark: the revolutionary science of biofluorescence*, Harvard University Press, USA, 2005.
- [3] D.M. Jameson, *Introduction to fluorescence*, CRC Press, Taylor & Francis Group, 2014. <https://doi.org/10.1201/b16502>.
- [4] J. Wiedenmann, C. D'Angelo, G.U. Nienhaus, Fluorescent proteins: nature's colorful gifts for live cell imaging, in: G. Jung (Ed.), *Fluoresc. Proteins II*, Springer Berlin Heidelberg, Heidelberg, 2011: pp. 3–33. https://doi.org/10.1007/4243_2011_21.
- [5] O. Shimomura, I. Yampolsky, *Bioluminescence Chemical Principles and Methods*, 3rd ed., World Scientific, 2019.
- [6] E.G. Smith, C. D'Angelo, F. Oswald, G.U. Nienhaus, J. Wiedenmann, Fluorescent proteins from the oceans: marine macromolecules as advanced imaging tools for biomedical research, in: E. Fattorusso, W.H. Gerwick, O. Tagliabue-Scafati (Eds.), *Handb. Mar. Nat. Prod.*, Springer Netherlands, 2012: pp. 1231–1257. https://doi.org/10.1007/978-90-481-3834-0_24.
- [7] J.T. O'Malley-James, L. Kaltenecker, Biofluorescent worlds - II. Biological fluorescence induced by stellar UV flares, a new temporal biosignature, *Mon. Not. R. Astron. Soc.* 488 (2019) 4530–4545. <https://doi.org/10.1093/mnras/stz1842>.
- [8] M.G. Meadows, N. Anthes, S. Dangelmayr, M.A. Alwany, T. Gerlach, G. Schulte, D. Sprenger, J. Theobald, N.K. Michiels, Red fluorescence increases with depth in reef fishes, supporting a visual function, not UV protection, *Proc. R. Soc. B Biol. Sci.* 281 (2014) 20141211. <https://doi.org/10.1098/rspb.2014.1211>.
- [9] C.N. Stewart, Go with the glow: fluorescent proteins to light transgenic organisms, *Trends Biotechnol.* 24 (2006) 155–162. <https://doi.org/10.1016/j.tibtech.2006.02.002>.
- [10] C.E. Schnitzler, R.J. Keenan, R. McCord, A. Matysik, L.M. Christianson, S.H.D. Haddock, Spectral diversity of fluorescent proteins from the anthozoan *Corynactis californica*, *Mar. Biotechnol.* 10 (2008) 328–342. <https://doi.org/10.1007/s10126-007-9072-7>.
- [11] N.K. Michiels, N. Anthes, N.S. Hart, J. Herler, A.J. Meixner, F. Schleifenbaum, G. Schulte, U.E. Siebeck, D. Sprenger, M.F. Wucherer, Red fluorescence in reef fish: A novel signalling mechanism?, *BMC Ecol.* 8 (2008) 16. <https://doi.org/10.1186/1472-6785-8-16>.
- [12] D.F. Gruber, J.S. Sparks, First observation of fluorescence in marine turtles, *Am. Museum Novit.* 3845 (2015) 1–8. <https://doi.org/10.1206/3845.1>.
- [13] A.A. Prudkovsky, V.N. Ivanenko, M.A. Nikitin, K.A. Lukyanov, A. Belousova, J.D. Reimer, M.L. Berumen, Green fluorescence of *Cyrtopsis* hydroids living in association with *Nassarius* gastropods in the Red Sea, *PLoS One.* 11 (2016) e0146861. <https://doi.org/10.1371/journal.pone.0146861>.

- [14] J.R. Hendricks, Glowing Seashells: Diversity of Fossilized Coloration Patterns on Coral Reef-Associated Cone Snail (Gastropoda: Conidae) Shells from the Neogene of the Dominican Republic, PLoS One. 10 (2015) e0120924. <https://doi.org/10.1371/journal.pone.0120924>.
- [15] W.M. Bandaranayake, The nature and role of pigments of marine invertebrates, Nat. Prod. Rep. 23 (2006) 223–55. <https://doi.org/10.1039/b307612c>.
- [16] S.T. Williams, Molluscan shell colour, Biol. Rev. 92 (2016) 1039–1058. <https://doi.org/10.1111/brv.12268>.
- [17] S. Daunert, S.K. Deo, Photoproteins in bioanalysis, Wiley-VCH Verlag GmbH & Co. KGaA, 2006. <https://doi.org/10.1002/3527609148>.
- [18] Y. Shiba, T. Gomibuchi, T. Seto, Y. Wada, H. Ichimura, Y. Tanaka, T. Ogasawara, K. Okada, N. Shiba, K. Sakamoto, D. Ido, T. Shiina, M. Ohkura, J. Nakai, N. Uno, Y. Kazuki, M. Oshimura, I. Minami, U. Ikeda, Allogeneic transplantation of iPS cell-derived cardiomyocytes regenerates primate hearts, Nature. 538 (2016) 388–391. <https://doi.org/10.1038/nature19815>.
- [19] F.D. Dip, T. Ishizawa, N. Kokudo, R.J. Rosenthal, Fluorescence imaging for surgeons concepts and applications, Springer, Switzerland, 2015. <https://doi.org/10.1007/978-3-319-15678-1>.
- [20] R.M. Hoffman, In vivo cellular imaging using fluorescent proteins methods and protocols, Human Press, Springer, New York, 2012.
- [21] S.-K. Ko, X. Chen, J. Yoon, I. Shin, Zebrafish as a good vertebrate model for molecular imaging using fluorescent probes, Chem. Soc. Rev. 40 (2011) 2120–2130. <https://doi.org/10.1039/c0cs00118j>.
- [22] D.M. Chudakov, M. V Matz, S. Lukyanov, K.A. Lukyanov, Fluorescent proteins and their applications in imaging living cells and tissues, Physiol. Rev. 90 (2010) 1103–1163. <https://doi.org/10.1152/physrev.00038.2009>.
- [23] D. Shcherbo, E.M. Merzlyak, T. V Chepurnykh, A.F. Fradkov, G. V Ermakova, E.A. Solovieva, K.A. Lukyanov, E.A. Bogdanova, A.G. Zaraisky, S. Lukyanov, D.M. Chudakov, Bright far-red fluorescent protein for whole-body imaging, Nat. Methods. 4 (2007) 741–746. <https://doi.org/10.1038/nmeth1083>.
- [24] M. Chalfie, S.R. Kain, Green fluorescent protein properties, applications, and protocols, 2nd ed., John Wiley & Sons, Inc., New Jersey, 2006.
- [25] R. Bhatia, R.C. Dogra, P.K. Sharma, Construction of green fluorescent protein (GFP)-marked strains of *Bradyrhizobium* for ecological studies, J. Appl. Microbiol. 93 (2002) 835–839. <https://doi.org/10.1046/j.1365-2672.2002.01768.x>.
- [26] O. Shimomura, Bioluminescence: chemical principles and methods, World Scientific, Singapore, 2006.
- [27] M. V Matz, A.F. Fradkov, Y.A. Labas, A.P. Savitsky, A.G. Zaraisky, M.L. Markelov, S.A. Lukyanov, Fluorescent proteins from nonbioluminescent Anthozoa species, Nat. Biotechnol. 17 (1999) 969–973.

- [28] S.H.D. Haddock, C.W. Dunn, Fluorescent proteins function as a prey attractant: experimental evidence from the hydromedusa *Olindias formosus* and other marine organisms, *Biol. Open.* 4 (2015) 1094–1104. <https://doi.org/10.1242/bio.012138>.
- [29] E.G. Smith, C. D'Angelo, Y. Sharon, D. Tchernov, J. Wiedenmann, Acclimatization of symbiotic corals to mesophotic light environments through wavelength transformation by fluorescent protein pigments, *Proc. R. Soc. B Biol. Sci.* 284 (2017) 20170320. <https://doi.org/10.1098/rspb.2017.0320>.
- [30] Y. Aihara, S. Maruyama, A.H. Baird, A. Iguchi, S. Takahashi, J. Minagawa, Green fluorescence from cnidarian hosts attracts symbiotic algae, *Proc. Natl. Acad. Sci.* 116 (2019) 2118–2123. <https://doi.org/10.1073/pnas.1812257116>.
- [31] M.S. Roth, M.I. Latz, R. Goericke, D.D. Deheyn, Green fluorescent protein regulation in the coral *Acropora yongei* during photoacclimation, *J. Exp. Biol.* 213 (2010) 3644–3655. <https://doi.org/10.1242/jeb.040881>.
- [32] D.F. Gruber, H.-T. Kao, S. Janoschka, J. Tsai, V.A. Pieribone, Patterns of fluorescent protein expression in scleractinian corals, *Biol. Bull.* 215 (2008) 143–154. <https://doi.org/10.2307/25470695>.
- [33] C. V Palmer, C.K. Modi, L.D. Mydlarz, Coral fluorescent proteins as antioxidants, *PLoS One.* 4 (2009) e7298. <https://doi.org/10.1371/journal.pone.0007298>.
- [34] F. Bou-Abdallah, N.D. Chasteen, M.P. Lesser, Quenching of superoxide radicals by green fluorescent protein, *Biochim. Biophys. Acta.* 1760 (2006) 1690–1695. <https://doi.org/10.1016/j.bbagen.2006.08.014>.
- [35] M.S. Roth, D.D. Deheyn, Effects of cold stress and heat stress on coral fluorescence in reef-building corals, *Sci. Rep.* 3 (2013) 1421. <https://doi.org/10.1038/srep01421>.
- [36] A. Salih, A. Larkum, G. Cox, M. Kühl, O. Hoegh-Guldberg, Fluorescent pigments in corals are photoprotective, *Nature.* 408 (2000) 850–853. <https://doi.org/10.1038/35048564>.
- [37] E.G. Smith, C. D'Angelo, A. Salih, J. Wiedenmann, Screening by coral green fluorescent protein (GFP)-like chromoproteins supports a role in photoprotection of zooxanthellae, *Coral Reefs.* 32 (2013) 463–474. <https://doi.org/10.1007/s00338-012-0994-9>.
- [38] S.F. Field, M.Y. Bulina, I. V Kelmanson, J.P. Bielawski, M. V Matz, Adaptive evolution of multicolored fluorescent proteins in reef-building corals, *J. Mol. Evol.* 62 (2006) 332–339. <https://doi.org/10.1007/s00239-005-0129-9>.
- [39] J.S. Sparks, R.C. Schelly, W.L. Smith, M.P. Davis, D. Tchernov, V.A. Pieribone, D.F. Gruber, The covert world of fish biofluorescence: a phylogenetically widespread and phenotypically variable phenomenon, *PLoS One.* 9 (2014) e83259. <https://doi.org/10.1371/journal.pone.0083259>.
- [40] P.H. Heinemann, Yellow intraocular filters in fishes, *Exp. Biol.* 43 (1984) 127–147.
- [41] T. Gerlach, D. Sprenger, N.K. Michiels, Fairy wrasses perceive and respond to their deep red fluorescent coloration, *Proc. R. Soc. B Biol. Sci.* 281 (2014) 20140787. <https://doi.org/10.1098/rspb.2014.0787>.

- [42] N. Anthes, J. Theobald, T. Gerlach, M.G. Meadows, N.K. Michiels, Diversity and ecological correlates of red fluorescence in marine fishes, *Front. Ecol. Evol.* 4 (2016) 126. <https://doi.org/10.3389/fevo.2016.00126>.
- [43] C.H. Ramesh, S. Koushik, T. Shunmugaraj, M. V. Ramana Murthy, A rapid in situ fluorescence census for coral reef monitoring, *Reg. Stud. Mar. Sci.* 28 (2019) 100575. <https://doi.org/10.1016/j.rsma.2019.100575>.
- [44] A. Marathe, Purification and characterization of an antiviral protein from the silkworm *Bombyx mori* L., University of Agricultural Sciences, 2012.
- [45] Manjunatha, A. Peter, M.B.N. Naika, P. Niranjana, P. Shamprasad, Identification of *in-vitro* red fluorescent protein with antipathogenic activity from the midgut of the silkworm (*Bombyx mori* L.), *Protein Pept. Lett.* 25 (2018) 302–313. <https://doi.org/10.2174/0929866525666180115121853>.
- [46] Z. Varadínová, D. Frynta, R. Aulický, V. Stejskal, Detection of cockroach faeces: consumption of fluorescent bait and production of UV-light-detectable faeces from German cockroach, *Blattella germanica*, *Entomol. Exp. Appl.* 155 (2015) 167–175. <https://doi.org/10.1111/eea.12301>.
- [47] V.L. Welch, E. Van Hooijdonk, N. Intrater, J.-P. Vigneron, Fluorescence in insects, *Nat. Light Light Nat. IV. Proc. SPIE.* 8480 (2012) 1–15. <https://doi.org/10.1117/12.929547>.
- [48] E.R. Willis, L.R. Roth, Fluorescence in cockroaches, *Ann. Entomol. Soc. Am.* 49 (1956) 495–497.
- [49] W.D. Wiesenborn, UV-excited fluorescence on riparian insects except hymenoptera is associated with nitrogen content, *Psyche (London)*. 2011 (2011) 875250. <https://doi.org/10.1155/2011/875250>.
- [50] M.G. Lagorio, G.B. Cordon, A. Iriel, Reviewing the relevance of fluorescence in biological systems, *Photochem. Photobiol. Sci.* 14 (2015) 1538–1559. <https://doi.org/10.1039/c5pp00122f>.
- [51] D.J. Suggett, O. Prášil, M.A. Borowitzka, Chlorophyll-a fluorescence in aquatic sciences: methods and applications, Springer Netherlands, Heidelberg, 2010. <https://doi.org/10.1007/978-90-481-9268-7>.
- [52] M.A. Guerrero-Rubio, J. Escribano, F. García-Carmona, F. Gandía-Herrero, Light emission in betalains: from fluorescent flowers to biotechnological applications, *Trends Plant Sci.* 25 (2020) 159–175. <https://doi.org/10.1016/j.tplants.2019.11.001>.
- [53] S.H.D. Haddock, C.W. Dunn, P.R. Pugh, C.E. Schnitzler, Bioluminescent and red-fluorescent lures in a deep-sea siphonophore, *Science (80-.)*. 309 (2005) 263.

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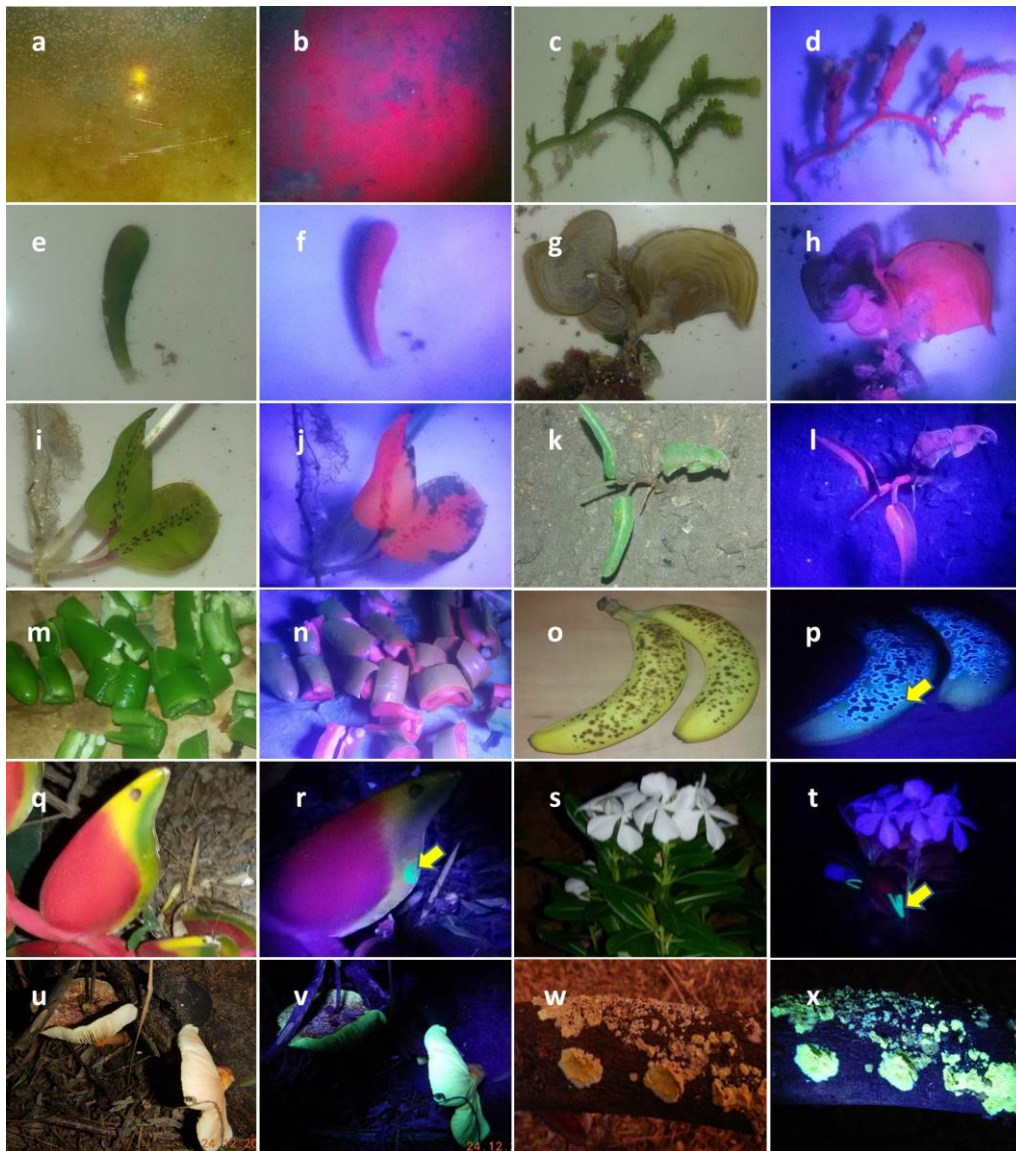


Fig. 1. Red and green fluorescence in different plant components under daylight and UV blue light. Red fluorescence in biofilm-forming algae growing on glass aquarium (**a, b**), green algae *Caulerpa serrulata* (**c, d**), green algae *Bornetella nitida* (**e, f**), brown algae *Padina gymnospora* (**g, h**), seagrass *Halophila ovalis* (**i, j**), water spinach *Ipomoea aquatica* (**k, l**), and green chilli *Capsicum annum* (**m, n**); cream-colored fluorescence around brown spots on exterior fruit part of banana plant *Musa paradisiaca* (**o, p**); green fluorescence in false-bird paradise *Heliconia rostrata* (**q, r**), green fluorescent flower buds of rose periwinkle *Catharanthus roseus* (**s, t**), common wood rotting fungi *Trametes* sp. (**u, v**) and wood rotting fungi *Coriopsis* sp. (**w, x**).

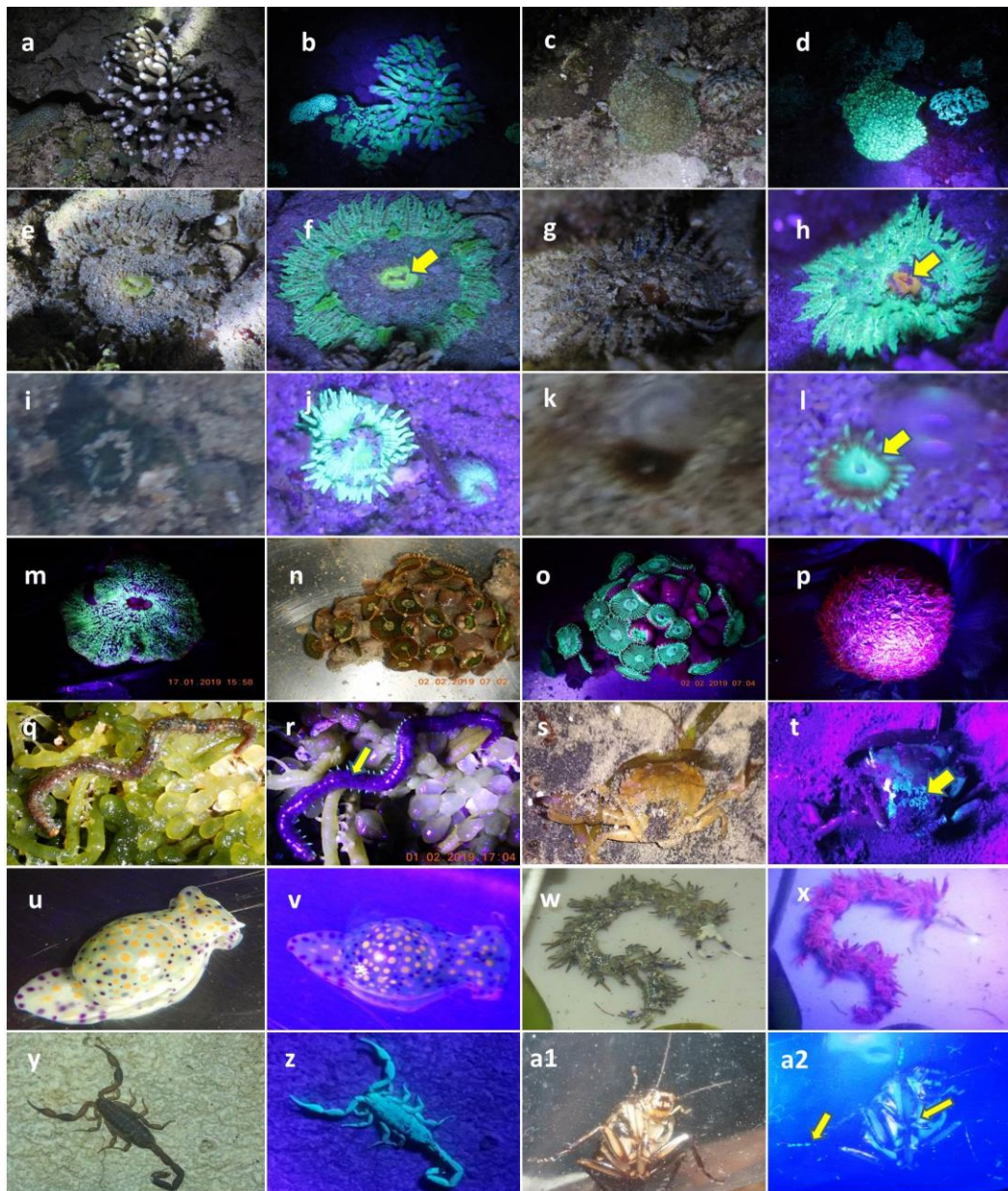


Fig. 2. Green in different marine cnidarians, polychaete, crustaceans, and terrestrial arachnids and insects, and red fluorescence molluscans and echinoderms. Green fluorescence in coral *Acropora millepora* (**a, b**), *Montastraea* sp. (**c, d**), sea anemone *Phymanthus buitendijki* green variant (**e, f**), *P. buitendijki* orange variant (**g, h**), unidentified sea anemone green fluorescence (**i, j**), *Telmatactis* sp. with red fluorescent ring (**k, l**), *Stichodactyla haddoni* (**m**), zoanthids *Palythoa mutuki* (**n, o**), red fluorescence in sea urchin *Temnopleurus toreumaticus* (**p**), green fluorescent parapodia of polychaete worm *Marphysia* sp. (**q, r**), carapace of crab *Thalamita integra* (**s, t**), red fluorescence in sea slugs *Haminoea ovalis* (**u, v**) and *Pteraeolidia ianthina* (**w, x**), green fluorescence in Indian brown scorpion *Hottentotta tamulus* (**y, z**) and cockroach *Neostylopyga rhombifolia* (**a1, a2**).

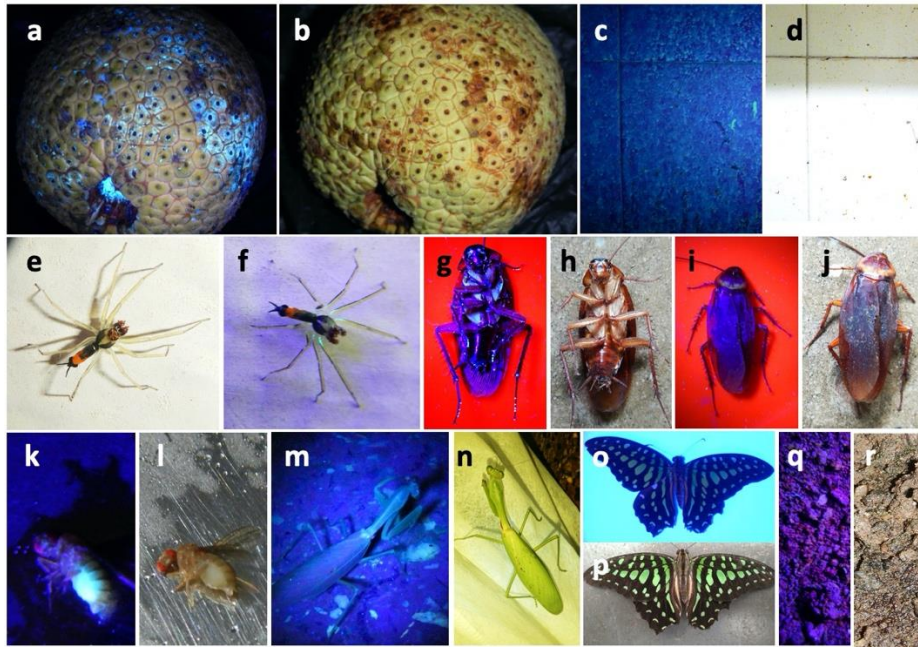


Fig. 3. Samples under UV blue and white light. Blue fluorescent fungal species spreading over the epidermis of breadfruit, *Artocarpus altilis* (**a**, **b**), blue-green fluorescence of cooked sunflower oil vapors on kitchen tiles (**c**, **d**), green fluorescence in basal femur region of jumping spider, *Asemonea cf. tenuipes* (**e**, **f**), green fluorescence on ventral body segments (**g**, **h**) and on head (dorsal) of cockroach, *Periplaneta americana* (**i**, **j**), abdominal green fluorescence in fruit fly, *Drosophila melanogaster* (**k**, **l**), red fluorescence on dorsal wings of praying mantis, *Mantis religiosa* subsp. *inornata* (**m**, **n**), red fluorescence on basal hindwing of tailed jay, *Graphium agamemnon* (**o**, **p**), and red fluorescence in an algal mat (**q**, **r**).



Fig. 4. Different gastropods displaying red fluorescence. Chamaeleon nerite *Nerita insculpta* (a, b), dotted periwinkle *Littoraria undulata* (c, d), jingle shell clam *Enigmonia aenigmatica* (e, f), sea snail *Bulla ampulla* (g, h), common delphinula, *Angaria delphinus* (i, j), *Nerita rumphi* (k, l), *Nerita polita* (m, n), *Nerita* sp. (o-r), common button tops *Umbonium vestiarium* (s-v), *N. polita* (w, x), unidentified oyster shell (y, z), turban shell *Euchelus asper* (a1, a2), green fluorescence in hermit crab *Diogenes* sp. (a3), chiton *Acanthochitona* sp. (a4) and pearl oyster *Pinctada fucata* (a5, a6).

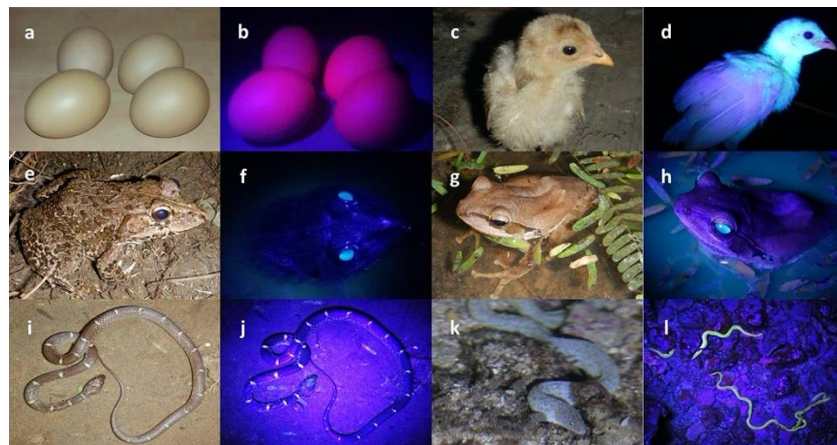


Fig. 5. Fluorescence in birds, amphibians, reptiles, and fish. Red fluorescence in eggshells of country chicken, *Gallus gallus domesticus* (a, b), blue-green fluorescence in feathers of domesticated chick, *Gallus gallus domesticus* (c, d), blue-green fluorescent eyes of Indian burrowing frog *Sphaerotheca breviceps* (e, f), and common tree frog *Polypedates maculatus* (g, h), yellow fluorescence in Indian wolf snake *Lycodon aulicus* (i, j), and yellow fluorescence in Indian mud moray, *Gymnothorax pictus* (k, l).

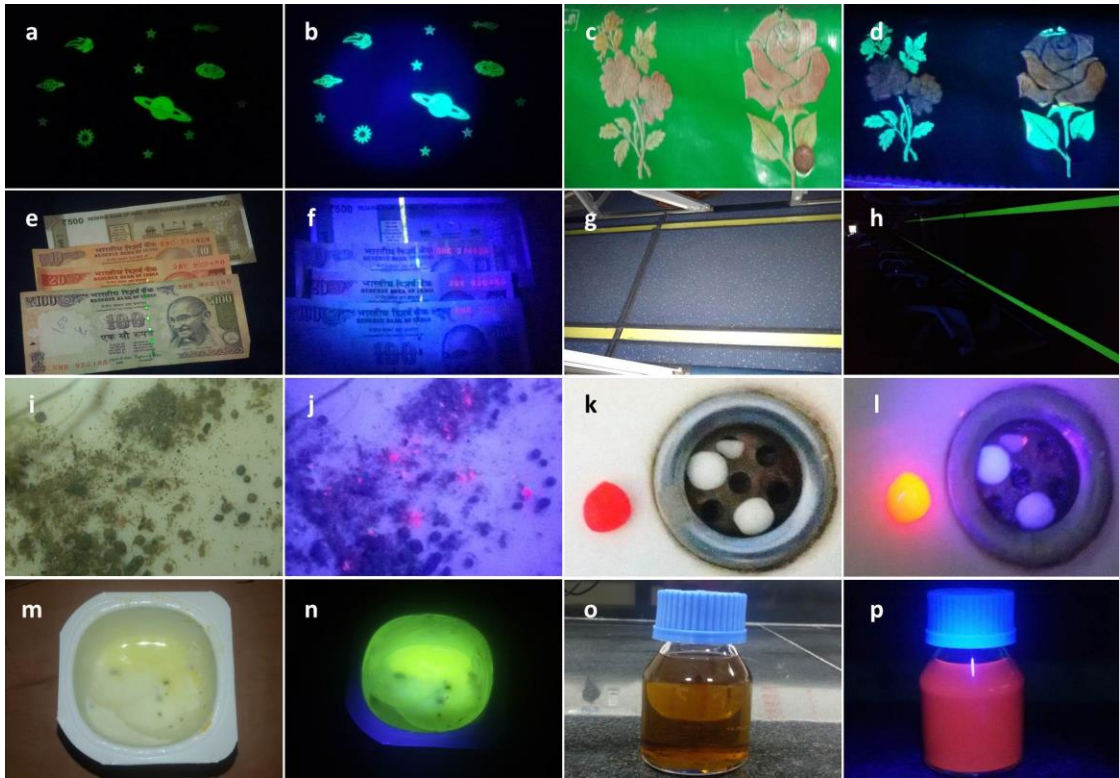


Fig. 6. Fluorescence in non-living components. Phosphorescence in glow in the dark Vinyl star stickers (**a, b**), fluorescent painting on a plastic sheet (**c, d**), fluorescent security threads in Indian currency (**e, f**), photoluminescent emergency floor path on aircraft (**g, h**), red fluorescence in the excretion of a herbivore fish (**i, j**), orange fluorescence of a red color naphthalene ball (**k, l**), yellow-green fluorescence of packed yogurt (**m, n**), and red fluorescence of methanol extract of a hydromedusan (**o, p**).