Fluorescence in Insects

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ABSTRACT

Fluorescent molecules are much in demand for biosensors, solar cells, LEDs and VCSEL diodes, therefore, considerable efforts have been expended in designing and tailoring fluorescence to specific technical applications. However, naturally occurring fluorescence of diverse types has been reported from a wide array of living organisms: most famously, the jellyfish *Aequorea victoria,* but also in over 100 species of coral and in the cuticle of scorpions, where it is the rule, rather than the exception.

Despite the plethora of known insect species, comparatively few quantitative studies have been made of insect fluorescence. Because of the potential applications of natural fluorescence, studies in this field have relevance to both physics and biology. Therefore, in this paper, we review the literature on insect fluorescence, before documenting its occurrence in the longhorn beetles *Sternotomis virescens, Sternotomis variabilis var. semi rufescens, Anoplophora elegans* and *Stellognatha maculata*, the tiger beetles *Cicindela maritima and Cicindela germanica* and the weevil *Pachyrrhynchus gemmatus purpureus.* Optical features of insect fluorescence, including emitted wavelength, molecular ageing and naturally occurring combinations of fluorescence with bioluminescence and colour-producing structures are discussed.

1. INTRODUCTION- SIGNIFICANCE OF BIOLOGICAL FLUORESCENCE

In 2008, the Nobel Prize in Chemistry was awarded to three US-based scientists for their work isolating and characterizing a green pigment from an obscure jellyfish. However, these bald facts neglect the huge and transformative role that the pigment, green fluorescent protein (hereafter, "GFP"), has played as a tool for molecular cell biology. In 2005, it was estimated that over 100 papers per month were being published that utilized "GFP and its variants" and that more than 6000 papers had been published up to that point using GFP (and allied compounds) as a label[1]. Fluorescent substances are also in great demand for physics and engineering applications, such as biosensors, solar cells, LEDs and VCSEL diodes. Consequently, the possibility of using or adapting the molecules and optical tricks employed by living organisms to produce and enhance their own natural fluorescence is tantalizing.

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1.1 Overview of Fluorescence in the living world

Fluorescence is known to occur in a disparate array of living organisms. From plants[2], to animals and algae (symbiotic zooxanthellae- see e.g. Oswald *et al*.[3]). Whilst most documented examples of animal fluorescence have come from sea creatures (especially corals[3-5]) and from scorpions[6-8], the phenomenon has also been described in vertebrates, such as marine fish and birds.

Fluorescent invertebrates include Amphioxus (Lancelets), sea anemones, hydroid polyps, jellyfish and a plethora of corals, as noted above. Among the corals, the fluorescent "sea pansy", *Renilla reniformis* is particularly well known. Among jellyfish, examples of fluorescence most famously include *Aequoria victoria,* but also *Phialidium sp*.[9-12], a deep-water siphonophore of the genus *Erenna*[13] and at least one species of bioluminescent ctenophore (S. H. D. Haddock and N. Mastroianni, unpublished paper cited by Haddock *et al*.[14]).

Amongst arthropods, fluorescence has also been documented in a spider in the genus *Harpactira*[7], various scorpions[6-8], five species of copepod[12, 15, 16], the isopod *Cubaris burnupi*[7], the mantis shrimp (stomatopod) *Lysioquillina glabriuscula*[17], the millipedes *Doratogonus setosus* and *Sphaerotherium giganteum,* the centipedes *Cormocephalus nitidus* and *C. multispinus*[7] and many insects.

1.2 Overview of the Study of Insect Fluorescence

The study of insect fluorescence has been considerably less exhaustive than that in certain other groups, beginning in earnest in 1924, with the publication of a study of the distribution of fluorescent pigments in butterflies[18]. Further qualitative studies followed, particularly in the 1950s – notably Phillips's 1959 paper on Lepidopteran fluorescence[19], Lawrence's 1954 study of fluorescence in Arthropoda[7] and Willis and Roth's 1956 work on fluorescence in cockroaches, in which they dissected the animals and examined their internal organs, bodily fluids and secretions for fluorescence[20]. Although John Huxley provided an early quantitative (and chemical) analysis of the phenomenon in the African butterfly *Papilio zalmoxis*[21], the bulk of quantitative studies of insect fluorescence have been far more recent (e.g. Kumazawa and Tabata's 2001 study of *Morpho sulkowskyi* and *Papilio xuthus*[22], Vukusic and Hooper's 2005 study of butterflies[23], Israelowitz *et al*. 2007 study of *Melanophila acuminata*[24] and Vigneron *et al*. 2008 study of the fluorescence of *Troïdes magellanus*[25] discovered by Lawrence *et al*.[26]).

Noteworthy chemical studies of insect fluorescence include the work of Umebachi[27] and Umebachi and Yoshioka[28] on the papiliochrome pigments found in the wings of *Papilio spp*. Butterflies,whilst Tabata *et al*.[29] and Kumazawa and Tabata[22] investigated the fluorescence of *Morpho Adonis* and *M. sulkowskyi* butterflies and showed the roles played by biopterin, pterin and isoxanthopterin in producing their fluorescence.

Table 1 summarizes the quantitative studies of insect fluorescence, whilst Table 2 provides an extensive but not completely exhaustive lists of insects in which fluorescence has been described. From these, notably table 2, it is evident that a sizable proportion of the data in this subject comes from fairly few papers and that the study of insect fluorescence is something of a neglected field, especially when compared with that of marine fluorescence. Additionally, whilst some papers on insect fluorescence prioritize documenting its mere occurrence in various taxa, others consider either its chemical origin or optical characterization (or sometimes both). Therefore, the relevant literature is very much scattered between disciplines (chemistry, biology and physics) and journals, as well as being somewhat dispersed chronologically. Finally, although certain insect taxa: namely butterflies (Lepidoptera) and cockroaches (Blattodea) have been the subject of sizeable studies (multiple studies in the case of the Lepidoptera), others- notably Coleoptera, Diptera and all Hymenoptera except for *Formica sp*. Ants[30] have been conspicuously neglected by comparison.

2. QUESTIONS OF EVOLUTION AND FUNCTION *IN VIVO*

Apparently non-functional "(auto)-fluorescence" has been reported from certain common biological, and biologically-derived, substances- such as dental enamel and paper. Consequently, all instances of fluorescence cannot be assumed to be functional. The fluorescence described from the internal organs of cockroaches[20] for example can almost certainly be considered non-functional, albeit the fluorescent molecules in those cockroaches may well have other important internal functions that are unrelated to their appearance. Moreover, those same molecules on the outside of the cockroach do modify the appearance of the organism, as would an ordinary pigment and they are therefore subject to the same natural selection pressures as any other pigment.

Salih *et al*.[5] hypothesized that the function of the fluorescent pigments found in corals and in their endosymbiotic zooxanthellae was to protect the corals from damage by ultra violet light. The importance of this screening function was subsequently evaluated and downplayed by Gilmore *et al*.[31], although it is conceivable that fluorescent proteins may afford insects bearing them some protection from ultra violet light. Further, insects living at high altitudes might be expected to incur more potentially harmful ultra violet radiation than organisms at sea level. However, in many known cases, insect fluorescence is not dispersed uniformly across the dorsal and/or ventral surface of the animal, but instead, only observed from parts of the organism: often those same areas that have markings coloured with more conventional natural pigments. For example, *Pachyrrhynchus gemmatus purpuerus* displays fluorescence from its ordinarily green abdominal and thoracic spots, whilst *Cicindela maritima* displays a white fluorescence from its light abdominal markings (Figure1).

Cockayne noted that many Papilio butterflies exhibit sexual dimorphism, with brightly fluorescent males and non-fluorescent females[18]. Philips likewise noted that several butterfly species exhibited sexual dimorphism with regard to their fluorescence[19]: observations that imply that the fluorescent markings have a role in sexual reproduction in some species. For example, Philips notes that *Glaucopshyche columbia* is a butterfly in which the females have a red-purple fluorescence but the males have none, whereas *Pieris melete agaope* is a species in which females have a "Rich, bright red-purple burgundy" fluorescence from parts of their wings, whilst the males remain "dull".

Fluorescence is known to influence pairing in the King Penguin, *Aptenodytes patagonicus*[32], but the role of fluorescence in insect sexual reproduction remains essentially unstudied. Both Cockayne and Philips described examples of non-sex-based fluorescence dimorphisms: in the case of the latter, one pair of examples was found in the butterflies *Papilio polyxenes asterius* and *Papilio palamedes,* where it was noted that, in both cases, some individuals produce a yellow fluorescence under UV from areas that are ordinarily yellow; yet, in others, the same areas fluoresce orange[18, 19].

Philips also draws attention to the phenomenon of "false fluorescence" from the eyes of certain dead insects: i.e., whilst he notes that a bright green fluorescence is visible in the eyes of all the North American moth species that he examined, he records that this fluorescence is not visible from the eyes of a living or freshly dead *Cecropia sp*. moth, but that it appears as the sample dries and that it can be reversibly removed by placing the moth in a moist chamber. Clearly, this fluorescence is an artifact of desiccation.

Multiple attempts have been made to classify insects on the basis of their fluorescence or the precise fluorescent compounds they contain and these have met with variable, but always qualified, success (see e.g. papers by Cockayne[18], Ford[33, 34], Rawson[35], Waywell and Corey[36], Wilkerson and Lloyd[37]). The role of environmental conditions in driving the evolution of fluorescence in insects is another currently unexplored avenue of research.

It has been stated that fluorescence is under represented in land animals compared with marine organisms; this may well be true, but it should be noted that the vast majority of extant insect species have never been examined for fluorescence

and that a more comprehensive examination of insect (rather than butterfly) fluorescence might change our picture of its abundance and distribution significantly. With such an incomplete data-set, it is not possible to draw conclusions about the overall abundance of fluorescence in insects; however, data on the abundance and distribution of fluorescence within groups of butterflies certainly does exist. For example, Philips stated that he found fluorescence in only three out of 203 species of Geometridae he examined[19]. Ford noted that he found fluorescence in ten of 92 species of *Graphium*[33] (N.B. in the same paper, he subsequently describes a total of eleven species from this genus as fluorescent).

3. OPTICAL PROPERTIES, CHEMISTRY, SYNERGY AND APPLICATIONS

Insect fluorescence has been described of all hues- for example, Wilkerson and Lloyd[37] described fireflies as having fluorescent compounds that range in hue from purple and dark blue to green, yellow and pink, whilst butterfly fluorescence has been described to be of all shades from "brown-lavender", to yellow, white and red[18, 19, 33, 34]. Measured peaks of fluorescence emission from entire insects range from 465nm to 625nm, whereas measured peak excitation wavelengths of intact insects range from around 340nm to 480nm (Figure 1). Emission peaks from fluorescent compounds in vivo can be markedly different-from those which the same substances produce in solution, with emission peaks in vitro being dependent upon solution pH, amongst other things[21, 22]. Finally, Cockayne noted that none of the many butterfly species he examined showed any fluorescence when exposed to X-rays instead of ultra violet radiation[18].

With regard to intensity, the fact that some insect species fluoresce more brightly than others has been noted qualitatively by Ford[33] and Philips[19] and is borne out by our own studies; that said, this interspecific variation in intensity has not yet been quantitatively investigated and it is further complicated by two factors. Firstly, insect fluorescence sometimes appears to occur in conjunction with optically active nano-structured components[38]- such as natural photonic band gap materials- and, secondly, because there is evidence that certain natural fluorescent molecules "decay" or "age" and either emit less light following prolonged exposure to UV or emit light of a different wavelength from that originally emitted.

With regard to the first of these factors, in 2005, Vukusic and Hooper discovered a two-dimensionally periodic photonic crystal slab in the wings of the butterfly *Papilio nireus* that was infused with a highly fluorescent pigment[23]. Band gap calculations indicated that the observed 2-D photonic crystal inhibits the emission of fluorescent light in the crystal plane and "thereby increases its out-of-plane emission, enhancing the intensity of the observed fluorescence from the butterfly's wing. More recently, Van Hooijdonk *et al*., described fluorescent-pigment-impregnated three-dimensionally periodic photonic crystals in the scales of two longhorn beetles: *Celosterna pollinosa sulfurea* and *Phosphorus virescens*[39].

With regard to molecular "ageing", Van Hooijdonk *et al*., additionally observed that the intensity of fluorescence in both longhorns decreased exponentially over the course of several hours, in response to protracted UV-exposure. This echoes anecdotal evidence from zoo keepers of captive scorpions losing their fluorescence if they are kept under ultra violet lighting for prolonged periods[8]. Similarly, Ando *et al*. and Wiedenmann *et al*. both report that ultra-violet light exposure can lead to photoconversion of coral fluorescent proteins and alter the wavelength of light they emit- from green to red[40, 41]. In 2005, Andresen *et al*., proposed exploiting a reversible version of such UV-induced photoconversion to create a photoswitch for optical circuits and data storage[42].

In marine organisms, fluorescence is commonly found in species which also have bioluminescence - the two phenomena are even correlated physiologically in *Renilla reniformis* and, probably, functionally in the siphonophore *Erenna sp*.[13], since the latter attracts prey with a bioluminescent lure that is surrounded by fluorescent tissue. The association of bioluminescence with fluorescence is not ubiquitous, though–there are examples of fluorescence from marine organisms in which bioluminescence is lacking e.g. certain non-bioluminsecent sea anemones[43]. In insects, the association of

bioluminescence and fluorescence breaks down even further, since, with the exception of fluorescence in fireflies[37], all insects so far described as possessing fluorescence lack bioluminescence.

Although the best known natural fluorescent molecule is GFP (and its many natural and artificial variants), it has not been reported from insects. Instead, instances of insect fluorescence have been attributed to several other compounds that work either in isolation or in different combinations and concentrations. Specifically, insect fluorescence has been attributed –variously- to Papiliochrome II[22, 28] , pterin[22, 29], biopterin[22, 29], isoxanthopterin[22, 29] and kynurine[21, 28]. The molecular basis of insect fluorescence is a complex subject, despite being one that is very much in its infancy; moreover, there are still numerous cases where the molecular cause of a known example of insect fluorescence has not been investigated at all

4. CONCLUSIONS

Fluorescence is well known in the living world and in recent years has been thoroughly examined in marine organisms- especially corals. The biological applications of the best known natural fluorescent compound- GFP- have been extensively exploited; however, GFP is just one of many fluorescent compounds produced by living organisms. Certain insects also exhibit fluorescence – these include beetles, ants, many butterflies and at least one grasshopper and one dragonfly. In many cases the molecular cause of this fluorescence has not been determined, nor has a quantitative spectrum of light emission been taken. The physics, engineering and optical applications of insect fluorescence remain to be explored.

5. ACKNOWLEDGEMENTS

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Table 1. Quantitative data of insect fluorescence collected/published to date.

Taxon	Type of Animal/ Common name	Species Studied	Excitation wavelength (nm), Peak Emission wavelength(s) (nm), if known	Reference
- COLEOPTERA	**(Beetles)**			
Carabidae-Cicindelinae	**(Tiger Beetles)**	*Cicindela germanica*	Bright fluorescence. emission peak at circa 466nm and a lesser peak at circa 548nm when illuminated with source peaking at 365nm	Figure 1
		Cicindela martima	Bright, whiteish fluorescence. emission peak at circa 465nm and a lesser peak at circa 547nm when illuminated with source peaking at 365nm	Figure 1
		Pachyrrhynchus gemmatus purpureus	Fluorescence from the (ordinarily green) thoracic and abdominal spots. Emission peak at circa 469nm and a lesser peak at circa 541nm when illuminated with source peaking at 365nm	Figure 1
	"The Firechaser Beeetle"	*Melanophila acuminata*	Peak excitation wavelength is 480nm, peak emission at 570nm, evidence of possible secondary emission peak at 625nm (Yellow colour observed)	Israelowitz *et al*.[24]
Cerambycidae	**(Longhorn beetles)**	*Celosterna pollinosa sulfurea*	Peak emission at 535nm for excitation wavelengths of 340nm to 420nm. 420nm generates maximal intensity of emission. (Yellow colour observed)	Van Hooijdonk *et al*.[39]
		Phosphorus virescens	Peak emission at 550nm 535nm for excitation wavelengths of 340nm to 420nm. 420nm generates maximal intensity of emission. (Yellow colour observed)	

		Sternotomis variabilis var. semi rufescens	Fluorescence from the light spots on the ordinarily-green part of the abdomen peaks at 477nm (with a secondary peak at 550nm); white/very desaturated orange fluorescence from the light spots on the ordinarily-orange part of the insect peaks at 480nm (with a secondary peak at 550nm) and a low level of orange fluorescence (too low to measure accurately) from the areas that are orange in daylight. All measurements made when illuminated with source peaking at circa 365nm	Figure 1
		Sternotomis virescens	Emission peak around 470nm and a lesser peak around 540nm, when illuminated with source peaking at 365nm	
		Anoplophora elegans	Emission peak around 468nm and a lesser peak around 546nm when illuminated with source peaking at 365nm	
		Stellognatha maculata	Emission peak around 469nm with a lesser peak around 550nm when illuminated with source peaking at 365nm	
-LEPIDOPTERA	**(Butterflies and moths)**			
		Papilio nireus "group"	Peak excitation 420nm, Peak emission circa 505nm ("depending upon species"),	Vukusic and Hooper[23]
		Papilio xuthus-Male	Excitation wavelength circa 400 nm, emission wavelength circa 470 nm.	Kumazawa and Tabata[22]
		Papilio zalmoxis	Emission peak circa 473nm when illuminated with source peaking at 370nm (blue colour observed)	Huxley[21]
		Troïdes magellanus -male	Excitatory peak circa 350nm, emission peak circa 540nm (Yellow colour observed)	Vigneron *et al*.[25]
		Morpho sulkowskyi –male	Excitatory peak circa 325 nm, emission peak 410 nm	Kumazawa and Tabata[22]

Table 2- Partial List of Insect species in which fluorescence has been documented (spectra have been taken of those species that are underlined).

Taxon	Type of Animal/ Common name	Species Studied	Reference
-BLATTODEA	**(Cockroaches)**		
Blattidae, Blaberinae		*Blaberus craniifer and Blaberus giganteus.*	Willis and Roth[20]
Blattinae		*Blatta orientalis, Eurycotis floridana, Neostylopyga rhombifolia, Periplaneta Americana, Periplaneta australasiae, Periplaneta brunnea* and *Periplaneta fuliginosa.*	
Ectobiinae		*Ectobius livens,*	
Epilamprinae		*Leucophaea maderae and Nauphoeta cinerea.*	
Pseudomopinae		*Pycnoscelus surinamensis, Blatella germanica, Blatella vaga, Loboptera decipiens, Parcoblatta pensylvanica, Supella supellectilium*	
Diplopteriidae, Diplopteriinae		*Diploptera dytiscoides*	
- COLEOPTERA	**(Beetles)**		
Carabidae-Cicindelinae	**(Tiger Beetles)**	*Cicindela germanica,* *Cicindela martima*	Figure 1
Curculionidae	**(Weevils)**	*Hipporhinus furvus*	Lawrence[7]
		Pachyrrhynchus gemmatus purpureus	Figure 1
Scarabaeidae	**(Scarab beetles)**	*Ceratorhynchus derbiana, Genyodonta flavomaculata*	Lawrence[7]
Buprestidae	**(Buprestid beetles)**	*Sternocera Orissa*	Lawrence[7]
		Melanophila acuminata ("The Firechaser Beeetle")	Israelowitz *et al*.[24]
Lampyridae	**(Fire-flies)**	*Photuris congener*[α]*, plus four unidentified/new Photuris species*[α]*- designated Photuris "A", "C", "D", and "BR", Pyractomena lucifera*[α]*, Pyractomena angulata*[α]*, Micronaspis floridana*[α]*, Pyropyga nigricans*[α]*, Pyropyga minuta*[α]*, Photinus umbratus*[α]*, Photinus pyralis*[α]*, Photinus floridanus*[α]*.*	Wilkerson and Lloyd[37 α]

Cerambycidae	**(Longhorn beetles)**	*Celosterna pollinosa sulfurea* *Phosphorus virescens*	Van Hooijdonk *et al.*[39]
		Sternotomis variabilis var. semi rufescens, *Sternotomis virescens,* *Anoplophora elegans,* *Stellognatha maculata*	Figure 1
- LEPIDOPTERA	**(Butterflies)**		
Papilonidae	**(Swallowtail butterflies)**	*Papilio alphenor* *Papilio semperinus* *Papilio antiphulus philippus*	Phillips[19]
		"Papilio rhadamantus" (*Troides rhadamantus* $^{\beta}$) – male (=The golden birdwing butterfly), "*Papilio (Orthoptera) helena hephæstus*"$^{\gamma}$ – *male,Papilio helenus nicconicolens* $^{\gamma}$, *Papilio euchenor euchenor* $^{\gamma}$, *Papilio ambrax egipius* $^{\gamma}$, *Papilio polytes cyrus* $^{\gamma}$, *Papilio cynorta* $^{\gamma}$, *Papilio gallienus* $^{\gamma}$, *Papilio demodocus* $^{\gamma}$, *Papilio mackinnoni* $^{\gamma}$, *Papilio phorcas ansorgei* $^{\gamma}$ – male, *Papilio nireus lyæus* $^{\gamma}$ -male, *Papilio bromius bromius* $^{\gamma}$ - male, *Papilio rex* $^{\gamma}$, *Papilio menestheus lormieri* $^{\gamma}$, *Papilio dardanus polytrophus* $^{\gamma}$ - male, *Papilio nobilis* $^{\gamma}$ - male *Papilio polyxenes asterius,* *Papilio palamedes,* *Papilio cresphontes,* *Papilio zelicaon,* *Papilio troilus,* *Papilio troilus ilioneus,* *Papilio glaucus glaucus,* *Papilio glaucus Canadensis,* *Papilio rutulus,* *Papilio marcellus,* *Papilio eurymedon,* *Parnassius clodius,*	Phillips[19]
		Papilio nireus "group"	Vukusic and Hooper[23]
		Papilio xuthus- *Male*	Kumazawa and Tabata[22]
		Papilio zalmoxis	Huxley[21]
		Troïdes magellanus -male	Vigneron *et al.*[25]
	(Sword-tail-butterflies)	*Graphium zonaria, Graphium philolaus,*	Cockayne[18], Ford[33]
		Graphium asius, Graphium arcesilius, Graphium epidaus, Graphium agesilaus, Graphium gyas ,	Ford[33]

		Graphium idaeoides, Graphium podalirius , Graphium lysithous, Graphium celadon	
Nymphalidae			
-Morphinae	**"The Morphos"**	*Morpho sulkowskyi* –male	Kumazawa and Tabata[22]
-Satyrinae	"The browns"/ "The satyrid butterflies"	*Cercyonis p. pegala,* *Cœenonympha ampelos,* *Cœenonympha ochracea,* *Cœenonympha california galactinus,*	Phillips[19]
Lycaenidae		*Plebius* (=*Plebejus*)*acmon,* *Glaucopsyche columbia*	Phillips[19]
Pieridae		*Eurema lisa (albino specimen),* *Pieris melete agaope ,*	Phillips[19]
Hesperiidae	"The skippers"	*Anclyoxypha numitor,* *Polites vibex,* *Poanes viator,* *Copœodes minima,* *Catocala Sappho,* *Catocala relicta,*	Phillips[19]
Saturiniidae	(Saturnid moths)	*Hyalophora (Platysamia) cecropia,* *Actinas luna,* *Eacles imperialis,*	
Arctiidae		*Diacrisia virginica*	
Geometridae	(Geometer moths)	*Sabulodes lorata,* *Mesoleuca gratulata,* *Xanthotype crocataria*	
- ORTHOPTERA	**(Grasshoppers, Crickets and Locusts)**		
Gryllidae		*Liogryllus bimaculata*	Lawrence[7]
- ODONATA	**(Dragonflies and damselflies)**		
"Aeschnidae" (Aeshnidae)	**(The hawkers / darners: large dragonflies)**	*Cordulegaster sp.*	Lawrence[7]

[α]This quantitative study examined the emittance of mechanically and chemically extracted fluorescent compounds from these organisms.

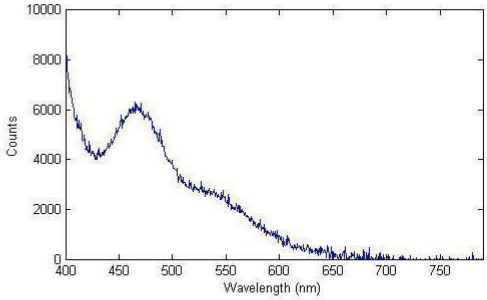
[β]Precise taxonomy of these organisms is either currently under debate/disputed.

[γ]Philips describes these species as "Ornthioptera" (Birdwing butterflies)- this classification has subsequently changed.

N. B. Cockayne published an account of his examination of every butterfly in the Natural History Museum and Oxford University's Hope Entomology Department for fluorescence[18]; it is not practical to reproduce it comprehensively here.

a

b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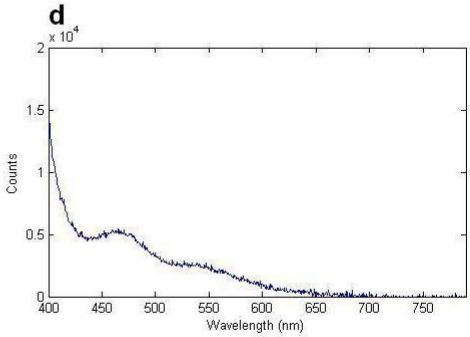
Wavelength (nm)

c

d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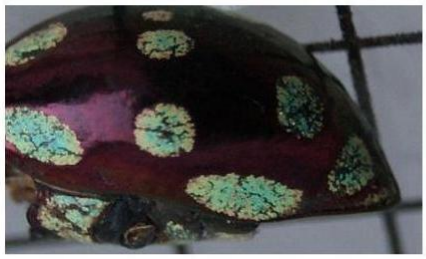
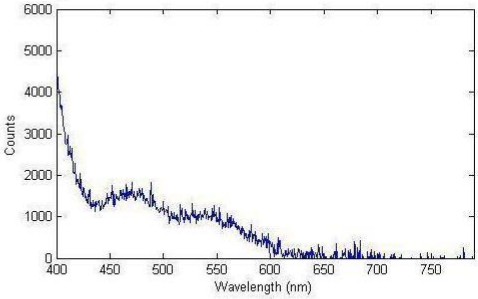
Counts
Wavelength (nm)

e

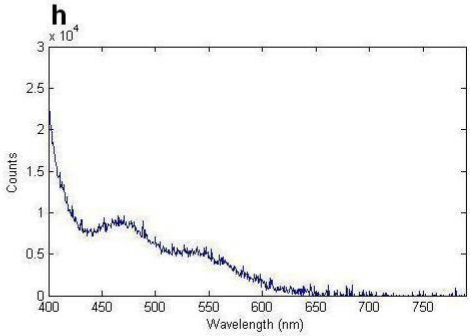


f


Counts
Wavelength (nm)

g

h


x 10^4
Counts
Wavelength (nm)

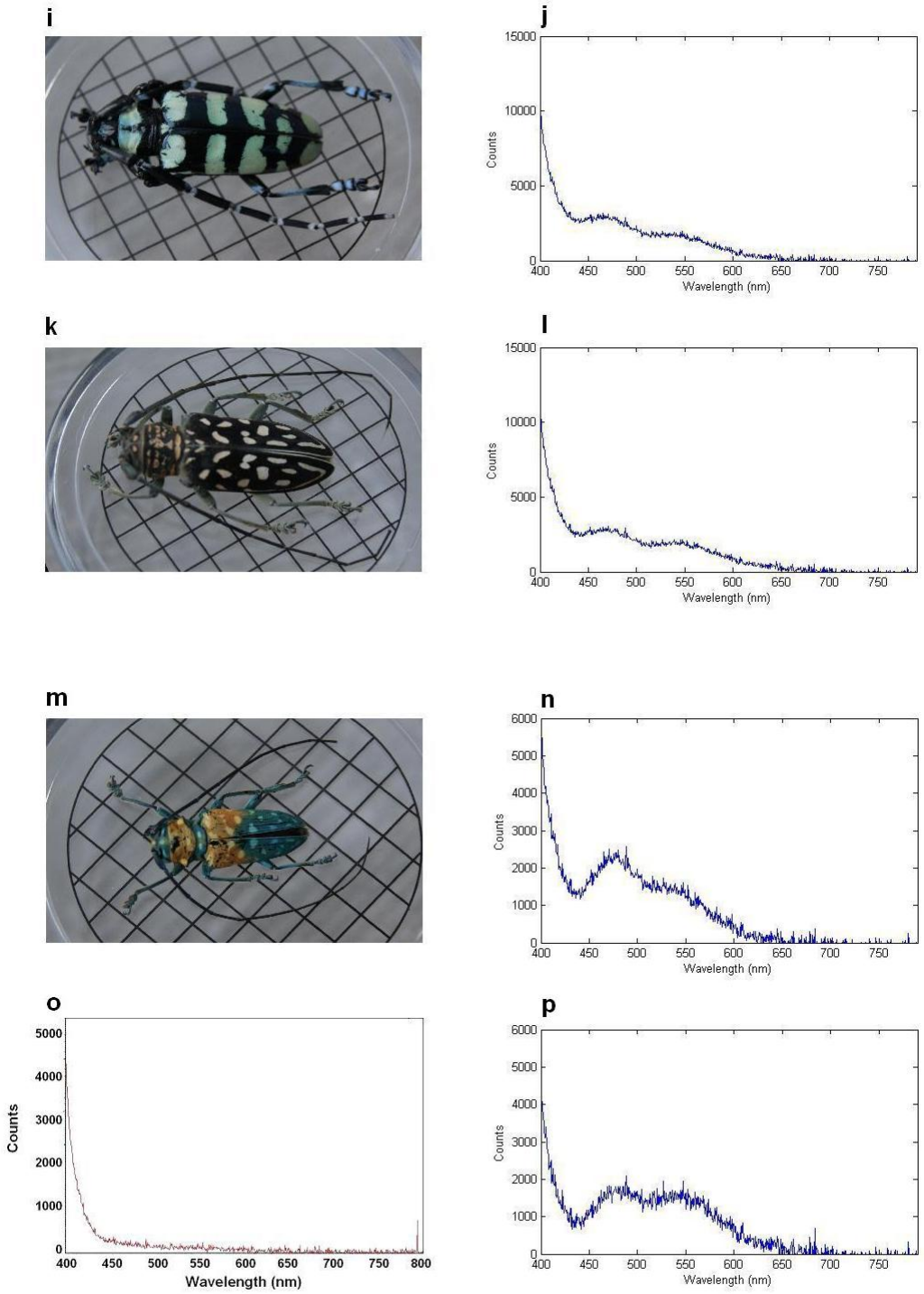


Figure 1. Previously undocumented examples of beetle fluorescence. a. - *Cicindela germanica* photographed in daylight, b. Fluorescence spectrum of *Cicindela germanica* measured with an aventes AvaSpec-2048 spectrometer and the manufacturer's recommended probe and software for detection of emitted light. (Measurements made in a dark-room using a UV source with the emittance spectrum shown in o), c. *Cicindela martima* photographed in daylight, d. Fluorescence spectrum of *Cicindela martima,* measured as described in b for *C. germanica,* e. *Pachyrrhynchus gemmatus*

purpureus photographed in daylight, f. Fluorescence spectrum of *Pachyrrhynchus gemmatus purpureus,* measured as described in b for *C. germanica,* g. *Sternotomis virescens* photographed in daylight, h. Fluorescence spectrum of *Sternotomis virescens,* measured as described in b for *C. germanica, i. Anoplophora elegans* photographed in daylight, j. Fluorescence spectrum of *Anoplophora elegans,* measured as described in b for *C. germanica,* k. *Stellognatha maculata* photographed in daylight, l. Fluorescence spectrum of *Stellognatha maculata,* measured as described in b for *C. germanica,* m. *Sternotomis variabilis var. semi rufescens* photographed in daylight, showing green and orange areas of body and light markings on these areas, n. Fluorescence spectrum of light markings in green area of *Sternotomis variabilis var. semi rufescens,* measured as described in b for *C. germanica,* o. Spectrum of UV light source that is greater than 400nm, p. Fluorescence spectrum of light markings in orange area of *Sternotomis variabilis var. semi rufescens,* measured as described in b for *C. germanica.*

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