

Light-mimicking cockroaches indicate Tertiary origin of recent terrestrial luminescence

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Abstract Bioluminescence is a common feature of the communication and defence of marine organisms, but this phenomenon is highly restricted in the terrestrial biota. Here, we present a geographical distribution of only the third order of luminescent insects—luminescent cockroaches, with all 13 known and/or herein reported new living species (based on deposited specimens). We show that, for the first time, photo-characteristics of three examined species are nearly identical with those of toxic luminescent click beetles, which they mimic. These observations are the evidence for the mimicry by light—a new type of defensive, Batesian and interordinal mimicry. Our analysis surprisingly reveals an evolutionary novelty of all living luminescent insects, while in the sea (and possibly in the soil) luminescence is present also phylogenetically in very primitive organisms.

Keywords Evolutionary ecology · Luminescence · Batesian mimicry · Endangered species · Cockroaches

Introduction

Bioluminescence in marine habitats is widespread, and is also present in a freshwater New Zealand stream snail, *Latia neritoides* (Gray 1850; Ohmiya et al. 2005). On land, in addition to rare bacteria (including those isolated from human wounds; Wilson and Hastings 1998) and mushrooms (Isobe et al. 1994), terrestrial animals emitting light are represented only by the snail, *Dyakia striata* (Gray 1834; Copeland and Daston 1989), a few earthworms, collembolan *Anurida granaria* (Nicolet 1847) and some millipedes (Shimomura 2006). If we do not take into consideration the obscure staphilinid beetle larvae (Costa et al. 1986), only three insect groups produce light (Redfort 1982, Meyer-Rochow 2007): (1) elateroid beetles including alight click beetles, fireflies and peculiar whole termite nests illuminated by predatory *Pyrearinus termitillumians* larvae; (2) troglobitic fly larvae, with alight insect-capturing webs and (3) virtually unstudied, the recently discovered adult cockroaches of the genus *Lucihormetica*.

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Many groups of organisms are known to emit light that can have multiply adaptive functions. In literature, single alight insects known for ages were fireflies. A surprising discovery took place in 1999, when luminescent cockroaches were discovered in the canopy forests of South America by Oliver Zompro with the second junior author (Zompro and Fritzsche 1999). This discovery literally brought new light and also a unity to the Latin expression *Blatta*, which, from the twenty-second century BP up to the fourteenth century, ambiguously identified two very different insect groups—dark cockroaches and alight fireflies (Vidlička 2001).

The study of the diversity of luminescent cockroaches is in rapid progress: 13 years of investigations in the field, as well as in collections, have revealed 13 species sparsely distributed in South America. These species have consistently non-overlapping geographic ranges, possibly implying parapatric speciation with the rapid and contemporaneous adaptive radiation of the genus (Fig. 1f, see below for details). The herein studied new species (Figs. 1a–e, k, l, 3 and 5), is the only known species that occurs west of the Cordilleras and the single representative of this lineage of luminescent cockroaches from Ecuador (Peru is the second country from which we report luminescent cockroaches for the first time). Ironically, the type locality was damaged December 5, 2010, during the eruption of Tungurahua—a volcano, along with the adjacent Chimborazo, representing the most remote places from the Earth's centre. While no one was able to collect this species for 70 years, we propose an “Endangered” status of this possibly extinct species.

For context, there are 121 described cockroach species living in Ecuador (Vidlička 2012) and 644 catalogued species (the estimation is five to six times higher) are reported from Brazil (Pellens and Grandcolas 2008). For a temporal context, earliest living cockroach genera are reported from the Palaeocene (60 Ma), with most genera known from the Eocene (Vršanský et al. 2012a, b). Nevertheless, the modern cockroach American fauna radiated only after the recently recognised mass extinction during the time between the deposition of the Mexican Chiapas and Dominican Republic ambers, earliest at 22 Ma (Vršanský et al. 2011, 2012a, b).

It is not incidental, that for the perception of largely visually oriented organisms, alight organisms are somewhat aesthetically preferred: two luminescent organisms were among the top ten species (International Institute for Species Exploration) in the last 3 years. A polychaete releasing illuminated “bombs” (Osborn et al. 2009) functionally analogical to aircraft defence systems designed to fool heat-seeking missiles, appears peculiar.

The sparse diversity of luminescent insects is surprising as firefly flashing increases metabolic rates only by 37 % above resting values (Wilson and Hastings 1998). It can be considered of high values, but the rate rises 57 % during walking (Wilson and Hastings 1998) and so the direct visual contact

can save a lot of energy for seeking a partner at the end (without considering an aposematic meaning of this signal).

Until recently discovered fossil nematodes containing luminophores (Poinar 2011), parasitic Metazoans producing light were unknown (Harvey 1956).

Based on new investigations on luminescent cockroaches, we summarise systematic (including extant *Lucihormetica luckae* sp.n. described herein), geographical and photobiological knowledge about the enigmatic group, which allowed us to assess their ecological and phylogenetical significance. In this manuscript, our aims are to (1) evaluate these significances, (2) compare luminescence attributes to those of other insects with some general indications of evolution of luminescence patterns on land and (3) compare appearance times among terrestrial and marine clades.

Hypotheses tested (and supported) in this manuscript were that the luminescent cockroaches are rare and morphologically similar which indicates their close relation and recent origin (even when compared to the Tertiary origin of their family, the Blaberidae); photobiological properties of the content of luminescent lanterns in cockroaches are similar to those of their mimic model—the click beetle *Pyrophorus*; and finally, that all living lineages of luminescent insect (in contrast to marine bioluminescent clades) are of Tertiary origin. The discovery of occurrence of all *Lucihormetica* spp. in areas without light pollution was unexpected.

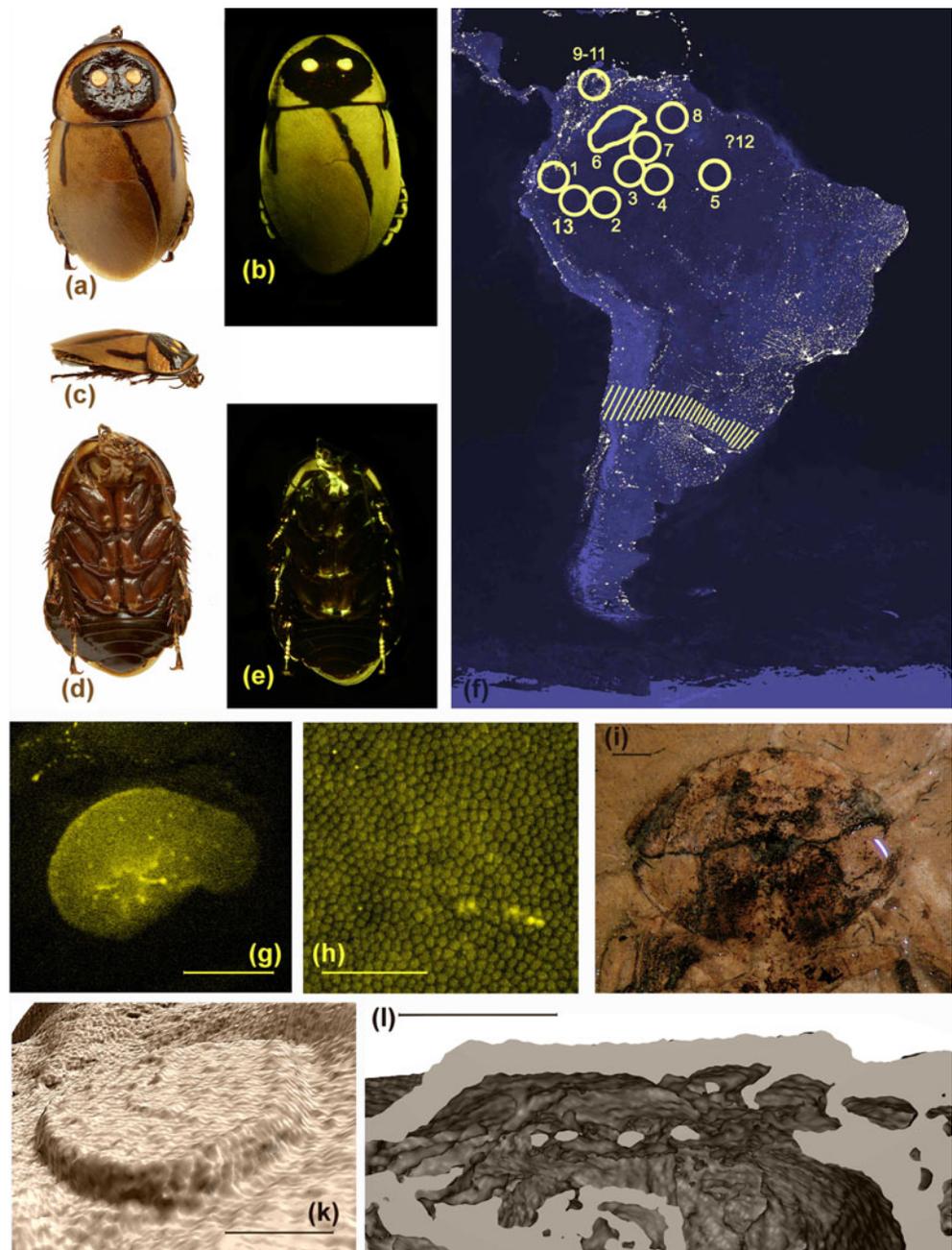
Material and methods

Adult male holotype. Banos, Tungurahua, Ecuador, collected 5.5. 1939 by Mr. MacIntyre. Deposited in the Department of Entomology, National Museum of Natural History, Washington D.C., USA.

Photographs were made using Canon EOS D60 digital camera and custom optical system for fluorescence imaging. Illumination was provided by 150 W halogen fibre optic illuminator Optem International (Schott-Fostec, LLC Auburn, NY, USA), excited by violet light using 390–470 nm band-pass filter, fluorescence emission was detected through 525-nm long-pass (LP) filter. Artworks were made using CorelDraw X3 and Adobe Photoshop 6.0 programs.

Optical properties of the specimen bioluminescent organs were characterised by confocal microscopy and fluorescence spectroscopy. The confocal laser scanning microscopy measurements were performed by a LSM510 META scanning head attached to an Axiovert 200 microscope stage (both Zeiss, Germany). For imaging we used 2.5×/0.075 Plan-Neofluar (Fig. 1g) and 20×/0.75 Plan Apochromat (Fig. 1h) objectives, 477 nm laser excitation, LP 505 nm and LP 560 nm emission filters. The fluorescence emission properties of the holotype luminescent lanterns were compared to lanterns of other bioluminescent species, and to

Fig. 1 Luminescent cockroaches. **a–e, k, l** Holotype of a new, possibly extinct species, *Lucihormetica luckae* sp.n. Locality (Ecuador) damaged by Tungurahua volcano eruption, December 5, 2010. Total length 24 mm. **a, c, d** Daylight; **b, e** fluorescence imaging; **k, l** non-destructive 3D μ CT reconstruction and section of luminescent lantern; **g, h** lantern of *L. verrucosa*; **i** lantern of *L. verrucosa*; **j** extinct (Jurassic) nocturnal *L. lativalvata* (Latiblattidae) from Kazakhstan with structures resembling luminescent lanterns. **f** Distribution of 13 bioluminescent cockroach species plotted over light pollution—all in non-photopolluted zones. Interrupted line is the southern margin of occurrence of *Pyrophorus*. For map credits, details on geography, geology and ecology see “Materials and methods”. Scales 1 mm (**h** 50 μ m)



isolated purified luciferin (Invitrogen Molecular Probes, Eugene, OR, USA), used as a reference. Luminescence properties were investigated by steady-state and time-resolved fluorescence spectroscopy. Emission spectra (Fig. 2a) were detected by fiberoptic spectrometer Maya (Ocean Optics, USA) following excitation by 473 nm laser (BDL-473, Becker and Hickl, Germany). Laser emission was filtered out from emitted light by 495-nm long-pass filter (Standa, Lithuania) and guided to the detector by 1-m long optical fibre (fused silica, 600 μ m diameter, Ocean Optics, USA). We used an integration time of 2 s and a boxcar smoothing of 5 points, three successive spectra were averaged to obtain the final plot.

Only the luminescent lantern area was illuminated by the laser spot of diameter \sim 1 mm. For time-resolved spectroscopy (Fig. 2c), we used a setup analogous to the one described (Chorvat and Chorvatova 2006). For a short period, a time-correlated single-photon counting board SPC-830 was used with a PML-SPEC detector and 473-nm picosecond laser diode as an excitation source (all Becker-Hickl, Germany). The pulse repetition rate of the laser was 50 MHz, average power $<$ 1 mW and pulse duration below 100 ps. The excitation geometry and laser blocking optics were the same as in the case of steady-state spectroscopy. Light was collected by optical fibre, focused with a quartz lens and dispersed by a

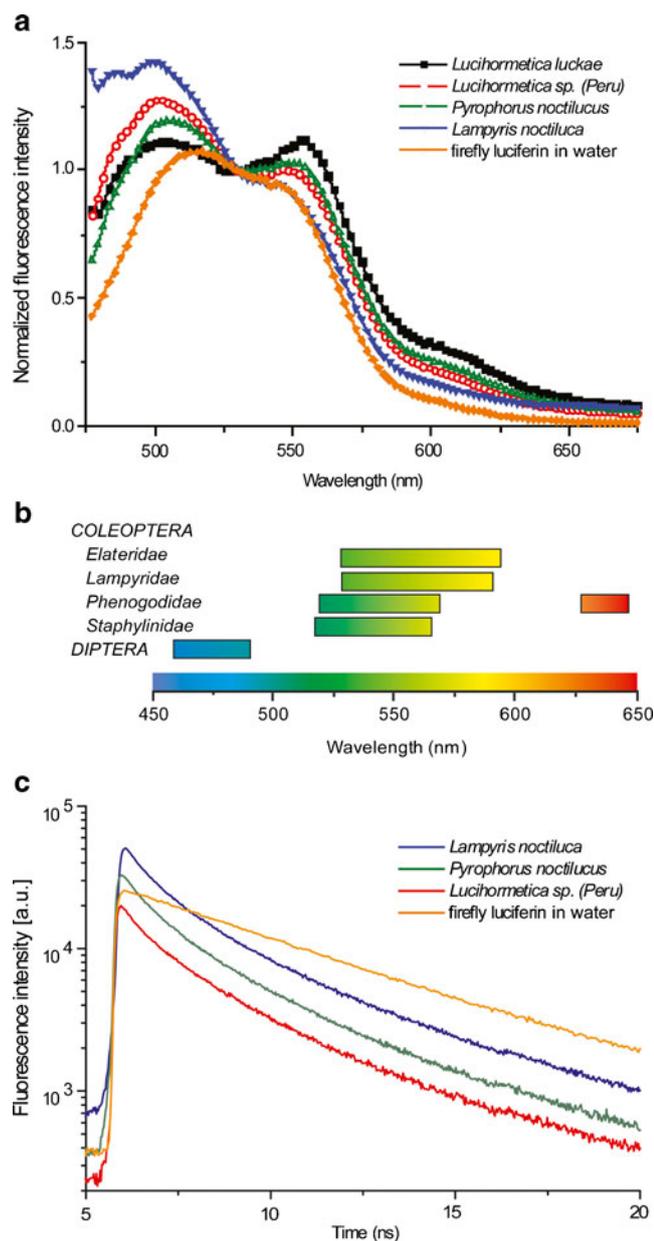


Fig. 2 Optical properties of a luciferine in alight insects. **a** Steady-state fluorescence spectra of *L. luckae* sp. luminescent lantern in comparison to the spectra from lanterns of genus *Lampyris*, *Pyrophorus* and *Lucihormetica*, and isolated purified luciferin from *L. noctiluca*. Spectral profiles were normalized with respect to intensity at 530 nm. **b** Reviewed and observed ranges of insect bioluminescent emission maxima. **c** Fluorescence decays of luciferin from the lanterns of species described in **a** after pulsed laser excitation

160-mm optical spectrograph (Oriel M-125, Germany), fitted with a 16-channel photomultiplier array (PML-Spec, Becker-Hickl, Germany). The spectrograph had 600 line-pairs per millimetre grating, providing a dispersion of 13 nm per channel within the spectral region of 400–600 nm. For fluorescence decay analysis we used only the data recorded in the spectral channel corresponding to 520–530 nm. The emission was detected in 25 ns window using 1,024 temporal channels.

Light pollution and geography credit: (DMSP (OLS): data courtesy of Marc Imhoff (NASA/GSFC) and Christopher Elvidge (NOAA/NGDC). Image by Craig Mayhew (NASA/GSFC) and Robert Simmon (NASA/GSFC)). (1) *L. luckae* (Jurassic–Cretaceous sediments below active volcano Tungurahua, 2,000–3,000 m asl, deforested zone, originally mountain rainforest); (2–5) *Lucihormetica seabrai*, *Lucihormetica tapurucuara*, *Lucihormetica amazonica*, *Lucihormetica fenestrata* (Paleogene–Neogene sediments, Amazon river basin, ≤ 300 m asl, canopy lowland forest); (6–7) *Lucihormetica verrucosa*, *Lucihormetica subscincta* (Quaternary, Tertiary, Jurassic; mostly ≤ 300 m asl, Savanna–Llanos); (8) *Lucihormetica grossei* (Middle Proterozoic rocks (1.6–1.9 Ga), Guayana upland, $\leq 5,000$ m asl, Canopy Gallery forest); (9–11) *Lucihormetica cerdai*, *Lucihormetica zomproi*, *Lucihormetica osunai* (Quaternary sediments, $\leq 1,000$ m asl, oases in semideserts of Salinas—forested mountain slopes of East Cordillera). (12) *Lucihormetica interna* (unknown locality in Brazil; unlabelled circle), undetermined species from lowland forest in Peru (13).

μ CT 3D reconstructions were based on X-ray microtomograph GE Phoenix|X-Ray nanotom 180, with tungsten target in the X-ray tube. Accelerating voltage $U=70$ kV; beam current $I=50$ μ A; 1,800 projections; 500 ms detector timing acquired and consequently used for 3D volume reconstruction by filtered back projection. Magnification 4.46, binning 2×2 , final voxel size 22.4 μ m. Post-processing by VGStudio MAX 2.1 software for contrast and plasticity enhancement, surface detection, segmentation; for noise and artefacts reduction the adaptive Gauss filter was used with smoothing (2) and edge threshold (0.1).

Fossil cockroach, nocturnal *Latiblatta lativalvata* (PIN 2904/29)—collected by the Paleontological Institute RAS, Moscow in the locality of Karatau in Kazakhstan (Vishniakova 1968), Karabastau Formation, Kimmeridgian Upper Jurassic (150.8–155.7 Ma according to Gradstein et al. (2004)).

Results

Systematic entomology

Order Blattaria Latreille, 1810=Blattodea Brunner von Wattenwyl, 1882

Family Blaberidae Brunner von Wattenwyl, 1865

Tribe Brachycolini *sensu* Roth, 1970

Genus *Lucihormetica* Zompro et Fritzsche 1999

Composition of the genus L. amazonica: (Rocha e Silva, 1987); *L. cerdai* (Ramirez-Pérez, 1992); *L. fenestrata* Zompro and Fritzsche 1999; *L. grossei* Fritzsche, 2003; *L. interna* (Walker, 1868); *L. osunai* (Ramirez-Pérez, 1992); *L.*

seabrai (Rocha e Silva, 1987); *L. subscincta* (Walker, 1868); *L. tapurucuara* (Rocha e Silva, 1979); *L. verrucosa* (Brunner von Wattenwyl, 1865); *L. zomproi* Fritzsche, 2008. All species are limited to South America (for details, see Fig. 1f and “Materials and methods” section).

Lucihormetica luckae Vršanský, Fritzsche et Chorvát sp.n.

Holotype Banos, Tungurahua, 5.5. 1939 (col. Mr. MacIntyre). Deposited in NMNH, Washington D.C., USA.

Differential diagnosis The present species can be categorised as belonging to the genus *Lucihormetica* based on the presence of two distinct luminescent organs on the pronotum.

It differs from all representatives of the genus except *L. grossei* and *L. amazonica* in having a high arcuate body with long forewings covering the whole body, and from its sister species *L. amazonica* in a less triangular head with less distinct pronotal tubercles, and with less distinct colouration. *L. grossei* has more dark colouration covering the pronotum (with two pale basal macula) and the base of the forewings. The species is unique in being nearly twice as small as all other *Lucihormetica* spp., and in having male terminalia with hla and sclerotised L3 round in section (Klass 1997).

Description Total individual length ca. 24 mm. Head hypognathous, of medium relative size (4.5/4.1 mm), with slender antenna (0.25–0.38 mm wide, first three segments comparatively short (ca. 0.9/0.2/0.6 mm)). Maxillary palp short and strong (third–fifth palpomere 0.9/0.6/0.9 mm long), with numerous short sensilla chaetica. Luminescent organs on the head are absent.

Pronotal length/width 8.8/12.3 mm. Luminescent fenestra ca. 1.8 mm in diameter, small asymmetrical (right) luminescent fenestrum ca. 0.25 mm in diameter.

Length/width of the left tegmen: 17.8/9.4 mm, clavus nearly 0.7 mm long. Body width, ca. 14 mm. Extremities are short and massive, heavily carinated with strong spurs. Coxae with numerous sense mechanoreceptors (*sensilla chaetica*) on the ventral side, with posterior long *setae* (derived from *sensillae*) and with dense small *chaetica* on contact with another coxa. All trochantera are very large with a distinct separated area centrally and with posterior *setae*. Femora with numerous lateral long and fine *setae* and with a very short but strong terminal spur. Tibiae heavily carinated with spurs very strong and long up to 1.3 mm. Tarsi robust, with long basitarsus and claw and three short segments. Pulvili distinct, transparent (fluorescent and possibly luminescent).

Fore coxa forming a very robust and distinct shield (5.3/2.7 mm), with distinct lobe partly covering the trochanter and femur. Trochanter (2.7/0.9 mm). More than half of the fore femur (4.9/1.1 mm) is covered by a coxal shield. Fore

tibia is extremely short (under 3/0.8 mm), tarsus is also short (1.3/0.4/0.4/0.4/1.2 mm).

Mid coxa is very long (4.8 mm), trochanter is long (3.3/0.9 mm), femur with arcuate anterior margin (5.3/2.5 mm), tibia is short (3.8/1.2 mm) and tarsus is short (1.7/0.5/0.5/0.5/1.5 mm).

Hind leg is massive, and comparatively short. Coxa is short and massive (3.6 mm—shorter than mid coxa); trochanter is very distinct (3.1/1.0 mm) femur (5.6/2.6 mm); tibia is massive (6.5/1.4 mm) with extremely long spines of red colour with a dark tip. Hind tarsus is comparatively long (ca. 4.95 mm: 2.3/0.4/0.4/0.4/1.8 mm),

Ecology. unknown

Derivation of name: *luckae*, after Lucka (Lucia), daughter of the senior author.

Structures (including lanterns)

The studied *L. luckae* Vršanský, Fritzsche et Chorvát, sp.n. (a new species by the present designation, holotype (Figs. 1a–e, 1, k; 3, 5) deposited in the NMNH, Washington D.C.; for details, see description) is unique in being almost half as small as the rest of the species, is extremely asymmetrical in body structures, has a unique reproductive tract (*hla* with sclerotised L3 (*sensu* Klass 1997) round in section—Fig. 3d), and its luminescent lanterns are present only in pronotum, while they are present also on the head of some species. The head lanterns are important for illumination of the nearby environment in phengodid beetles (Viviani and Bechara 1997) and due to the position on the anterior margin of the head also in advanced alight cockroaches. The asymmetry in this new species is expressed not only as frequent deformations, but also in a dark diagonal stripe apparent in the general habitus. Asymmetry and intraspecific variability in cockroaches do not correlate (Liang *et al.* 2012), but the presence of these structures suggests that this species has different adaptive advantages and can survive even with aberrations. Also, the present species has luminescent tissues out of the main lanterns incompletely covered by a cuticle, which results in the presence of an asymmetrical small lantern on the right side (Fig. 1b), on the place of a narrowed cuticle in one of the depressions.

Otherwise, the structure of the main lantern of all species of *Lucihormetica* is extremely sophisticated, and the cuticle forms a bilayer of chitin net with a strong supporting function, visible on the non-invasive section (Fig. 11). The environment of the reaction, which produces light, is completely isolated from the rest of the body. The surface of the lantern is extremely modified, covered by a film of transparent reflector-like structure. Even more surprising, the rest of the luminescent groups, such as fireflies, do not have such a specialised, distinct and isolated luminescent organ.

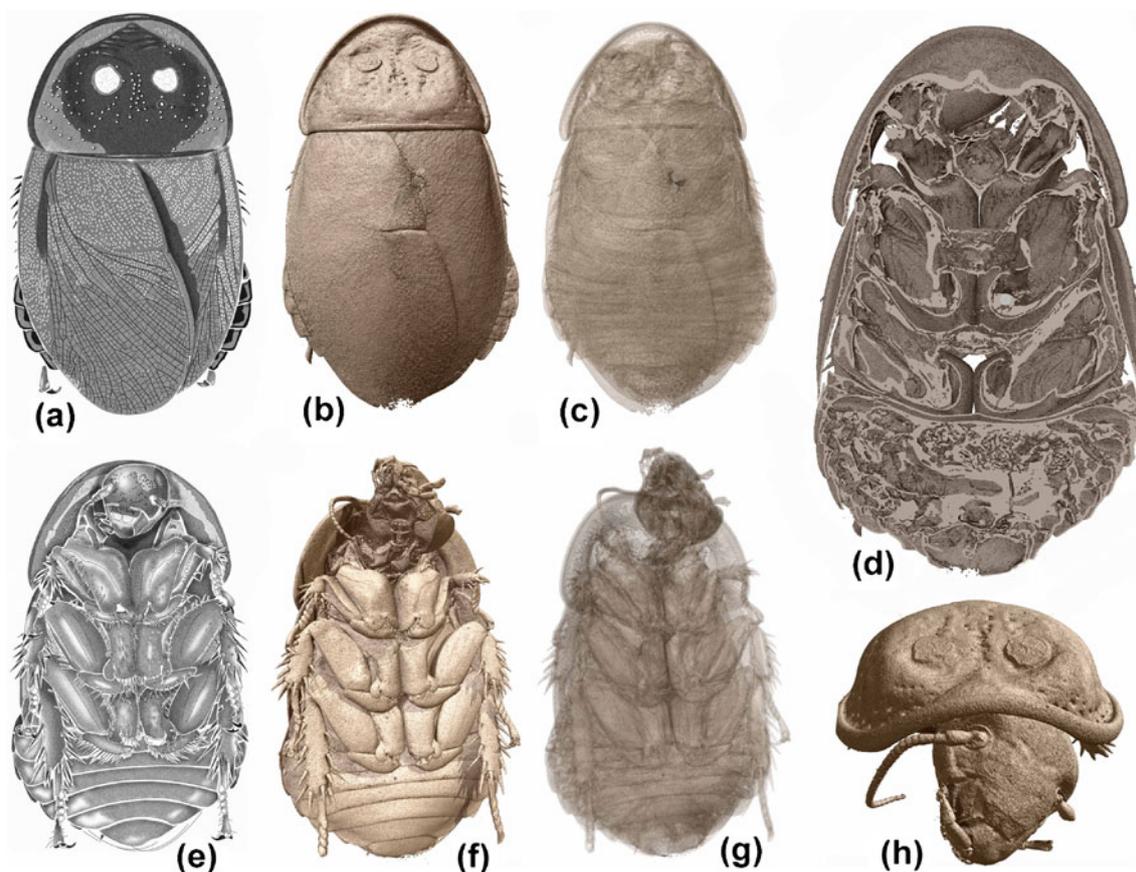


Fig. 3 New luminescent cockroach, *L. luckae* sp.n. Total length 24 mm. **a** Reconstruction based on dorsal views; **b** 3D μ CT reconstruction of dorsal view; **c** 3D μ CT reconstruction with 20 % transparency; **d** non-invasive section of 3D μ CT reconstruction in plane of

copulatory organs; **e** reconstruction based on ventral views; **f** 3D μ CT reconstruction based on ventral view; **g** 3D μ CT reconstruction with 20 % transparency; **h** frontal view

Optical properties

L. luckae's holotype was studied by a confocal laser scanning microscopy, steady-state, and time-resolved fluorescence spectroscopy. Since the biochemical extraction of the luminescence substances was not possible to reveal from the examined holotype, instead, the fluorescence emission properties of its lanterns were measured and compared to other bioluminescent groups (*Lampyris*, *Pyrophorus*, living *Lucihormetica*, as well as isolated purified firefly luciferin, were used as a reference—Fig. 2). Both emission spectra and fluorescence decay characteristics of *L. luckae* are apparently similar to the other examined species and to the click beetle luciferin, which also gives us strong supporting evidence that molecular origin of lantern luminescence substances—a luminophore in the new species—are closely related among different insects. In this respect, another important finding was that the spectra and decays detected from other fluorescing parts of the body, such as legs or wings (Fig. 1b, e), were much dimmer and different in shape from the lanterns, and do not contain luciferin. The spectrum

of the bio- or chemiluminescence matches the fluorescence spectrum of the reaction product, and this provides a clue as to the identity of this product (Wilson and Hastings 1998). These are important inferences as there are still 30 extant independent systems (Wilson and Hastings 1998) and respective luciferases are non-homological, unrelated proteins from unrelated genes and categorised based on a single common characteristic—the production of light.

Lucihormetica is hidden not only beneath its own light, but also from the light—no species have been recorded in the photopolluted area. The locality of *L. luckae* (Fig. 1f–1) is located in the dark area, but surrounded by a light polluted area at the scale of dozens of kilometres. Seventy-three years ago, when the last specimen was collected, the sky above Tuhurangua was clear, thus light pollution as the case of extinction cannot be definitively excluded. The locality shared by *L. cerdai*, *L. zomproi* and *L. osunai* (Figs. 1f and 3) is the only dark area within a strongly lit agglomeration. The remaining occurrences are present in extensively dark areas.

Discussion

Some species within the cockroach genus *Lucihormetica* (such as the here analysed *L. verrucosa*) are common and even available in legal culture and are easily taken care of. Nevertheless, unlike the rest alight groups, in the culture they do not produce light and stay completely in the shadows. *Pyrophorus* does glow in captivity which seems to be strictly associated to the light conditions and/or life cycles controlled with pheromones. It is important as light serves as aposematic signals for generalist predators, but attracts parasites (Sivinski 1998) and specialist predatory fireflies (Lewis and Cratsley 2008)—roaches thus need to stay alight most likely only during courtship, similarly to the case of some fireflies.

Character of illumination

In contrast to continual glowing, discrete flashes as those of the presently studied cockroaches contain information encoded in temporal features of the signal (Lewis and Cratsley 2008) and are more difficult to imitate. The temperature dependent character of flash timing (Tamura *et al.* 2005) may provide a clue to the limited geographic range of bioluminescent cockroaches in respect to click beetles.

For the mimicking of alight organisms, colour representing the spectral composition of light is another important property. In addition to the necessity of mimicking the blitz frequency and intensity of light, correlated evolutionary tempo of colour change is important as well. In this respect, it is noteworthy that the chemistry of luminescent insects is the most diverse relative to other luminescent organisms (Viviani 2002). Such a diverse biochemistry in respect to the emitted wavelength would practically disallow adaptation to colour change. Surprisingly, the emission colour of luminescing substrate—luciferin—can be simply changed by variation of its molecular surroundings, present in the reaction chain. A single amino-acid substitution can shift the peak of emission significantly (H433Y changes, 562 to 612 nm) (Wilson and Hastings 1998). The simplicity of the colour change is advantageous for imitators. *Aequorea aequorea* reaction is carried out *in vitro* reveal cyan (486 nm), whereas the living organism emits green light (508 nm; Wilson and Hastings 1998). Luciferin purified from *Lampyrus noctiluca* shows a typical green emission (500–530 nm) while dissolved in water with its molecular structure corresponding to the phenolate form. In DMSO or alcohol, it turns into a phenol derivate and its fluorescence shifts to blue (400–450 nm). This photophysical property could serve as a preadaptation, which allows for biochemical change in the remote time horizons. Traces of diverse colours in cockroach spectra contrasting with homogenous

firefly light support this. Namely, the colour change appears to reflect a response to environmental conditions. Simple temperature and/or humidity fluctuation (and other factors) can definitely alter the colour and intensity of emitted light in ectothermous insects, which would be very difficult to adapt to, even for non-mimicking light-emitters.

To summarise, the optical properties of the *Lucihormetica* lanterns shows (1) optical characteristics with (2) distance among lanterns nearly identical to *Pyrophorus* luciferin and with (3) traces of diverse colour in contrast to firefly homogenous light.

Light as mimicry

Potentially alight are the whole organisms, not only their lanterns. Even traditionally known fireflies contain luciferin throughout the body and can produce light locally in their organs due to the controlled expression of luciferase enzymes. Larvae and pupas of the Brazilian fireflies, *Aspisoma lineatum* and *Cratomorphus* spp., emit a continuous weak glow throughout their entire bodies during all stages (Viviani *et al.* 2008). It is thus not surprising that *Orfelia fultoni* (Mycetophilidae) bears two translucent lanterns at the extremities of its body, and the millipede *Luminodesmus sequoiae* produces light from the cuticle, legs and antennas (Shimomura 2006).

The function of luminescence in cockroaches as a simple (although multimodal, *i.e.*, combined with steroidal pyrones—lucibufagins (Fu *et al.* 2007)) aposematic (warning) signal can be excluded because luminescent cockroaches are rare (*L. amazonica*, *L. cerdai*, *L. interna*, *L. osunai*, *L. seabrai*, *L. tapurucuara*, *L. zomproi*, *L. luckae*, *Lucihormetica* sp. N. (Peru)—each is known by a single individual) and also too phylogenetically young, Miocene at earliest (all known species are morphologically similar, morphotypes are not stabilised, geographical ranges are associated; see also above for origin of modern American entomofauna) to trigger the learned and/or inherited response reaction of birds and other predators.

Each group has its own lighting colour, ranging from the blue-violet (460 nm) of flies, up to the red (640 nm) of railroad worms (Viviani *et al.* 2006). The flashing colour of cockroaches is identical with the light of luminescent click beetles of the genus *Pyrophorus* (Fig. 2a). These narrow insects have luminescent lanterns and the very marginal parts of the pronotum, but in more robust cockroaches these are located in the middle part—so the total size and distance of their lanterns is identical with lanterns of *Pyrophorus*. (Click beetles are additionally not sexually dimorphic and during flight expose an additional lantern.) Cockroaches are fat, and therefore we suggest that their luminescence would not prevent predators from eating the non-alight fat parts of

the body, but they simply mimic highly toxic luminescent beetles. *Pyrophorus*, is phylogenetically slightly older, and overlaps (Fig. 1f; in the north it reaches the northern margin of the southern states of the USA) with the geographic range of *Lucihormetica*. Based on the overlapping geographic range (Fig. 1f), same habitats, absence of known predators, identical colour of light (see below), the same structure of protein (Fig. 2) and comparable structure (lanterns of other luminescent insects are diverse) and distance among lanterns (in otherwise different insects), this light signal likely corresponds to mimicry.

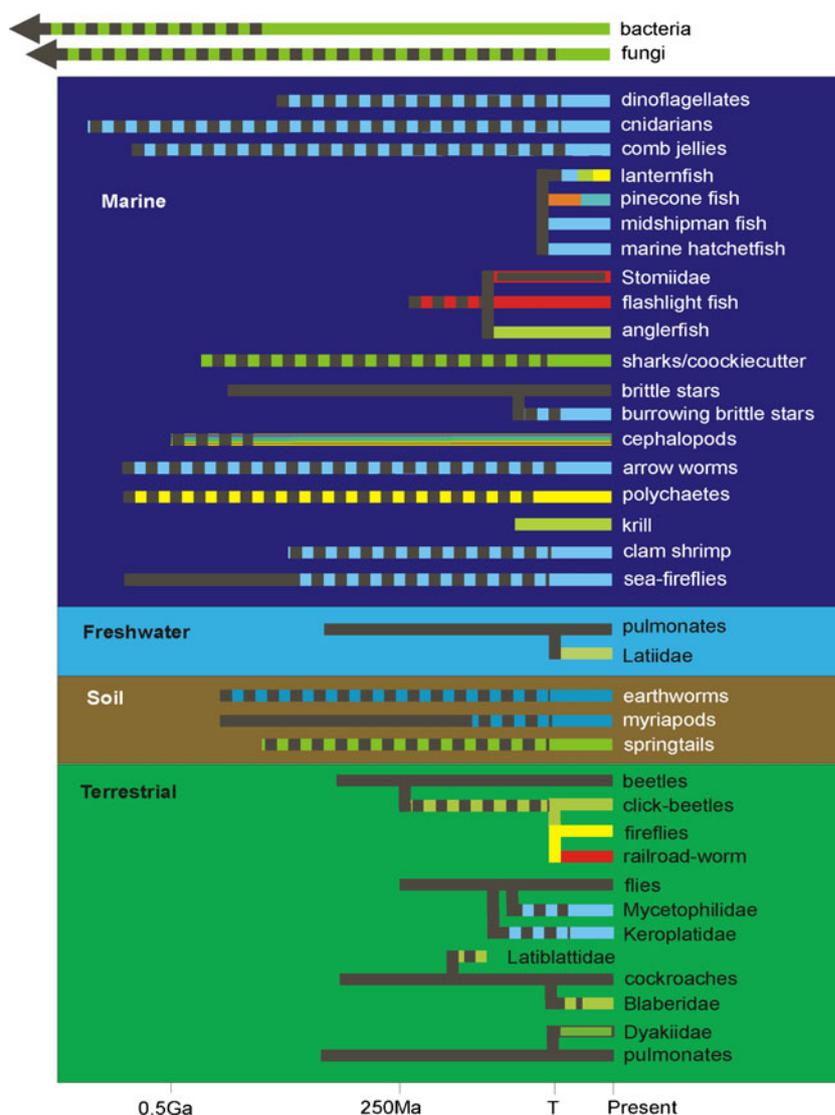
Ecoevolutionary context of luminescent cockroaches

More drastic and more rapid environmental changes on land may explain why there are no luminescent survivals among primitive lineages, while in the sea they persist and flourish.

All lineages of alight terrestrial organisms (a hypothetical exception is a soil-inhabiting collembolan) belong to phylogenetically younger clades (Fig. 4). Indigenous Late Jurassic cockroach *Latiblatta* as well as the Early Cretaceous luminescent nematode, *Proheterorhabditis burmanicus* (Poinar 2011), belong to extinct lineages.

It is indeed a very intriguing fact that is otherwise very difficult to explain; possibly suggesting that the phylogenetical meaning of luminescence on land may be different from that observed in aquatic animals (*e.g.*, lack of effective attenuation of toxic side products, lack of huge 3D space effective in distribution of optical information). Luminescence is found exclusively in post-Mesozoic groups of insects, in spite of the fact that the modification of proteins into luminescent products is extremely simple (and phylogenetically very primitive in marine animals). Fossil record is not biased in this respect (it is possible to trace these lineages) because there are roughly 2 million samples of fossil insects (Vršanský 2008), which is

Fig. 4 Luminescent organisms in respective habitats, plotted over the timescale, with colour of emitted light (Stomiidae emit infrared; plant cells emitting superweak high-energy ultraviolet light (Paiziev and Krakhmalev 2004) are excluded). *Dashed lines* are the earliest possible occurrence of luminescent organisms in each lineage, the *black line* is the occurrence of a group without a luminescent signal. Note the early (chronological and phylogenetical) occurrence of luminescence in marine environment (bacteria have recorded occurrences deep in the Palaeozoic due to preserved photophores in marine cephalopods; fish can have their own luminescence as well as with incorporated bacteria; Harvey 1956) while validated occurrence on land is as late as the Tertiary (T)



enough for comparative analysis. There are no indications why luminescent animals are absent in the history, so they perhaps faced regular extinctions (in this respect the fossil record is biased, because the colouration and lanterns is obscure to be traced among new lineages). Additionally, the reason for the extinction of earlier bioluminescence systems might be that light emission is not essential for life (Wilson and Hastings 1998). A first possible case of a luminescent extinct insect, a nocturnal Jurassic cockroach with structures resembling luminescent lanterns, is provided in Fig. 1i.

An alternative explanation for the recentness of luminescence is the structure of ecosystems: (1) luminescent insects can be present only in ecosystems with a dominance of vertebrate predators (advanced birds and mammals) capable of learning and thus avoiding a conspicuous alight insect; (2) luminescent organisms radiate during times when night life begins to be diverse (night life in terms of diversity and abundance was restricted in most known Mesozoic terrestrial ecosystems according to Liang *et al.* 2012); (3) only the complex trophic structure of advanced Oligocene ecosystems allows luminescence to spread. Evidence from recent heterogenic habitats with many obstacles (*e.g.*, modern forests in contrast to Carboniferous forest without dense ground cover including the lack of grasses) shows that they might select signalling systems that use a combination of pheromones for long-range attraction, and bioluminescence for better short-range localisation (Lewis and Cratsley 2008). Thus, all three hypotheses have some support.

In addition to the explanations above, it is possible that only the modern groups of insects are able to survive the side effect of luminescence—the toxicity of its by-products, which is more effectively attenuated in aquatic animals (the more constant temperature of marine environments (Harvey 1956) does not seem to cause this paradoxical state as many luminescent marine organisms are associated with temporally variable environments, and some reactions—such as those of ophiurids—are very tolerant to temperature changes (Bruggeman *et al.* 2001)). As luminescence is an oxidation reaction (luciferin originates as a random aberration in contrast to luciferases, which arose from AMP/CoA-ligases; Viviani 2004), it has been proposed that the primarily phylogenetical function of luciferin in insects was to be an antioxidant. Nevertheless, the superoxide dismutase protection against deleterious effects arising from the storage of molecular oxygen to sustain the bioluminescent reaction (Colepicolo Neto *et al.* 1986) suggests that it is a consequence rather than a cause. One of the products of proteogenesis of luminescence are toxic proteins, the causes of aposematic signals provided by marine (Grober 1988) and terrestrial (Lewis and Cratsley 2008; De Cock and Matthysen 2002; Branham and Wenzel 2001) luminescent animals. It is the toxicity which causes many non-luminescent groups of insects, namely diverse beetles, to mimic many hundreds

luminescent (some even aquatic (Yuma 2007)) fireflies species (common soldier beetles also mimic fireflies). The non-luminescent South American cockroach *Schultesia lampyridiformis* mimics fireflies as well. From this perspective it is understandable that toxicity can also eventually lead the evolution of luminescent animals on land to inevitable dead ends.

Living fireflies are unique not only with illumination—in addition to 20 functions of bioluminescence (Buck 1978), their associated traits are nuptial gifts (Lewis *et al.* 2004) important for both sexes, but cells are uniquely also sexually undetermined prior to maturation.

Influence of photopollution

Photopollution is known to seriously influence the health of many organisms, and can also change the composition of associations and destabilise whole ecosystems (Davies *et al.* 2012). The light of the environment cannot hurt luminescent click beetles, which often occur in photopolluted areas and even cities, but once alight, predator can disclose and attack

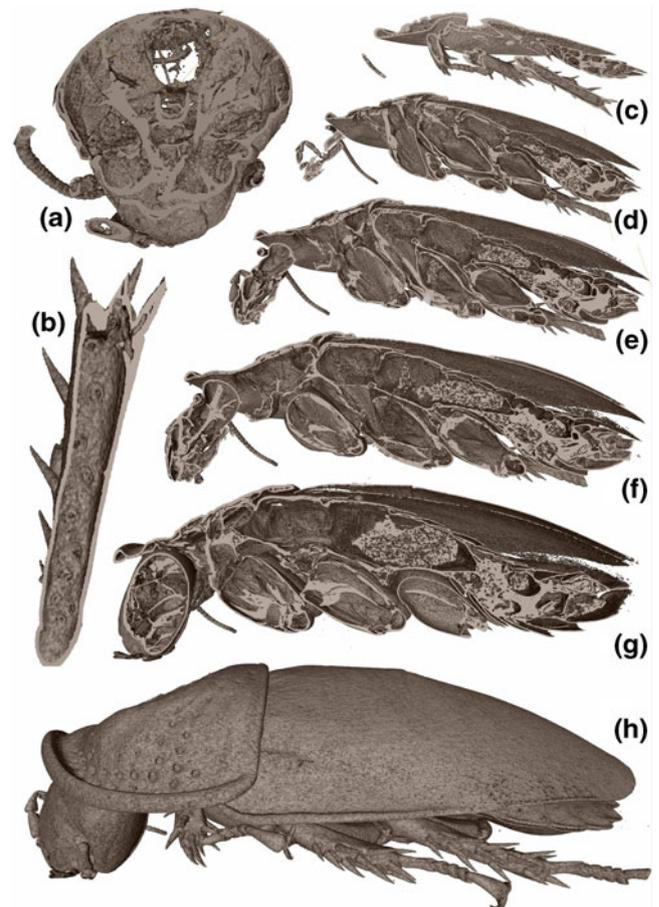


Fig. 5 Non-invasive sections through holotype of *L. luckae* sp.n. **c–h** Lateral views; **a** section through the head in frontal view; **b** hind leg tibia showing anchoring of the spurs (modified innervated *sensillae*)

mimic edible cockroaches. Some fireflies also fly only when the skylight is greatly reduced to avoid hawkers (Wing 1982) and represent additional support for the hypothetical communication of luminescent insects when emission reduces another firefly dark-adaptation (Lloyd 1983). It is pure speculation that light pollution influences luminescent cockroaches via their most frequent biochemical pathway of melatonin control, which is very important in long-living luminescent organisms. Nevertheless, the distribution map (Fig. 1f) shows this could be true, because ecological characteristic other than darkness do not seem to correlate with distribution of luminescent cockroaches because this group is not strictly restricted to remote undisturbed primaeval rainforests: no other geological (ranging from one of the Earth's oldest exposed Proterozoic rocks, through Jurassic sediments, up to active volcanoes and Quaternary sediments) or ecological (ranging from oases in semideserts of the Salinas, through the savannah-like Llanos, up to high elevated as well as lowland canopy forests) characteristics are shared by all respective species (see “Materials and methods” for details).

The herein proposed endangered status for the *L. luckae* sp.n. is particularly important in respect to conservation of the whole group as most species are known by a few individuals and even an individual collection may represent an actual risk to some species. In this particular case, threat is even more pronounced, the species is known by a single individual collected more than 70 years ago, and the type locality at Bános was completely destroyed by the Tungurahua eruption over a year ago. Moreover, to some extent, the locality is nowadays under influence of light and so might not appear any longer inhabitable for luminescent cockroach (Fig. 5).

Types of light mimicry

Finally, there is a plethora of ways of mimicking: defensive, aggressive, reproductive (including sound and odour), automimicry and behavioural. The first author who expected mimicry by light was Wallace (1878) himself, who erroneously supposed click beetles for firefly mimics. Nevertheless, their light is different and they also appeared inedible too (Harvey 1956). Cockroaches are fat and tasty, so the mimic is at the place.

One mimicry by light (aggressive, Batesian–Wallacian or Peckhamian) is actually known (Lloyd 1965, 1984): Predaceous fireflies *Photuris* (and also *Bicellychonia*) mimic the flash responses of females of other, up to five different (Lloyd 1983) species, attract males, and catch them, often during flight. Such predators may be expected to have had a strong evolutionary impact on both the signal coding (countermeasure trickery) of prey species and the flight paths and bioluminescent signals of mate-seeking males. Predaceous

females additionally obtain the defensive steroidal pyrones from prey and incorporate them into their own and their eggs' defences.

Prey itself also uses sophisticated patterns of “light counterespionage.” They partially mimic flashes of another prey species which causes the improper answering of a predator (Lloyd 1983).

Even more sophisticated is the mimicry of a mimicry of *Photinus macdermotti* males. They inject extra flashes into the coded patterns of rivals as they approach a contested female. The timing of these flashes matches the timing of similar flashes that two of their predators use when attracting them and they also can mimic prey to seek for their own hunting females (Lloyd 1980, 1981). Now, light as an ordinary (defensive) interordinal mimicry is also known—cockroaches are simply hidden beneath the mimicking light. Well, under the lamp, the densest dark.

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