

Proceedings of 2017 International Firefly Symposium

Timetable of 2017 International Firefly Symposium

	April 23
0930~1400	Pre-symposium treat (Eco-tea ceremony of IFS 2017) see more detail in website: http://firefly.hfu.edu.tw/index_en.html
1400~1500	International participants of IFS 2107 check in (Education center, Taipei zoo)
1500~1700	Firefly drama(Lecture room, Education center), ice break with light food (Education center)
1700~2000	Firefly watching in Taipei zoo
2000~	Back to hotel

	April 24
0830~0920	Registration
0920~1030	Opening, Firefly song, Seminar of Taipei firefly conservation(International meeting room, Panda house, Taipei zoo) Modulator: Pingshih Yang
1030~1100	Refresh time & Twinkles of Hope (TVBS)
1100~1120	Group photo and refresh time (International meeting room)
1120~1200	Keynote speech section I: Sara Lewis, Title: Emerging Directions in Firefly Research (Things We Don't Know but Should) (oral key-1) Modulator: Chiahsiung Wu Place: International meeting room, Panda house
1200~1300	Lunch time & Twinkles of Hope (TVBS)
1300~1420	Keynote speech section II: Mark Branham Title: The Function and Evolution of Bioluminescence in Beetles (oral key-2) Keynote speech section III: Lesley Ballantyne, Title: The firefly dilemma (oral key-3) Modulator: Minglun Jeng Place: International meeting room, Panda house
1420~1440	Tea time & Twinkles of Hope (TVBS)
1440~1600	Keynote speech section IV: Nobuyoshi Ohba Title: Characteristic features of Japanese aquatic fireflies (oral key-4) Keynote speech section V: Mitsuo Nakamura (Japanese-English translation) Title: Recovery aquatic firefly habitat in Kitakyushu Japan (without abstract) Modulator: Jenzon Ho Place: International meeting room, Panda house
1600~1654	Ecology section I: oral a1 a2 a3 (Lawrent L. Buschman) a1: B. Nada, Estimating the diversity of the elusive fireflies a2: Lawrent L. Buschman, Evolution and Function of Bioluminescent Behavior in Firefly Larvae (Coleoptera: Lampyridae) a3: Anchana Thancharoen, Relationship between Synchronous Fireflies, <i>Pteroptyx</i> spp. and Their Firefly Trees Place: International meeting room, Panda house
1654~1830	Dinner
1830~2030	Firefly visiting in Mucha and Yuanjian
2030~	Back to hotel

	April 25
0830~0924	Ecology section II: oral a4 a5 a6 (Mark Branham) a4: Zazil Ha Mucui Kac García Trujillo, Assessment of socio-economic aspects of tourism of fireflies in Nanacamilpa, Tlaxcala. a5: Zazil Ha Mucui Kac García Trujillo, Economic valuation of the Sanctuary of the Firefly in Nanacamilpa, Tlaxcala a6: Victor Benno Meyer-Rochow, What can the eyes of fireflies and other luminescent invertebrates teach us about their biology? Place: International meeting room, Panda house
0924~0935	Refresh time & Twinkles of Hope (TVBS)
0935~1047	Eco section II: oral a7 a8 a9 a10 (Anchana Thancharoen) a7: Lynn Faust, Honduran Fireflies and the Jungle Adventures in Catching Them a8: Avalon C.S. Owens, Short-wavelength artificial lighting influences <i>Aquatica ficta</i> flash signals (Coleoptera: Lampyridae) a9: Scott R. Smedley, Escape into Winter: Does a Phenological Shift by a Lucibufagin-Containing Firefly (<i>Ellychnia corrusca</i>) Shield It from a Specialist Predator (<i>Photuris</i>)? a10: Po-Hsiung Lin, Microscale meteorological monitor for ecological watch_a case study at firefly recovery site in Daan Park, Taipei Place: International meeting room, Panda house
1047~1100	Refresh time & Twinkles of Hope (TVBS)
1100~1230	Taxonomy section I: oral b1 b2 b3 b4 b5 (Lesley Ballantyne) b1: Channarong Sartsanga, Distribution of <i>Pteroptyx</i> Olivier (Coleoptera; Lampyridae) in Thailand b2: Jen-Zon Ho, Flash Diversity of Taiwanese Fireflies b3: Wan F. A. Jusoh, Species Identity Crisis in SE Asian Fireflies: Will the Real <i>Pteroptyx</i> Please Stand up? b4: Oliver Keller, Firefly (Coleoptera: Lampyridae) Diversity of Hispaniola b5: Gavin J. Martin, Anchored hybrid enrichment analysis of Lampyrid phylogeny Place: International meeting room, Panda house
1230~1330	Lunch time & Twinkles of Hope (TVBS)
1330~1500	Taxonomy and Physiology section I: oral b6 b7 oral c1 c2 c3 (Kathrin F. Stanger-Hall) b6: Kathrin F. Stanger-Hall, The evolution of sexual signal modes and associated sensor morphology in fireflies (Lampyridae, Coleoptera) b7: Vor Yiu, Prolonged adult life span of five firefly species in Hong Kong c1: Seth M. Bybee, Firefly Genomics: why it matters

	<p>c2: Stephen T. Deyrup, Advanced NMR and HPLC-MS Methods for Determination of Firefly Chemical Defenses</p> <p>c3: Timothy R. Fallon, Sulfoluciferin is Biosynthesized by a Specialized Luciferin Sulfotransferase in Fireflies</p> <p>Place: International meeting room, Panda house</p>
1500~1530	Tea time and poster introduction (Poster room)
1530~1700	<p>Physiology section I: oral c4 c5 c6 c7 c8 (Sara Lewis)</p> <p>c4: Yun-Ru Chen, Impacts of artificial light pollution on the transcriptome of <i>Aquatica ficta</i> larvae</p> <p>c5: Yuichi Oba, Dual-color Luminescence of Firefly Pupa</p> <p>c6: Sarah E. Lower, Genetic variation within <i>Photinus pyralis</i> reveals positive selection on luciferase, but not opsins</p> <p>c7: Soichi Osozawa, Quaternary vicariance of <i>Curtos</i> (Lampyridae; firefly) in the Ryukyu-Taiwan islands; Molecular and X-ray micro CT analyses</p> <p>Oral c8: Lu Yang, The evolution of Na⁺, K⁺ - ATPase in relation to predatory feeding and lucibufagin sequestration in fireflies</p> <p>Place: International meeting room, Panda house</p>
1700~1830	Tea time and poster introduction (Poster room)
1830~2030	Firefly visiting in Daan and Zongxing
2030~	Back to hotel

	April 26
0830~0924	<p>Conservation section I: oral d1 d2 d3 (Chiahsiong Wu)</p> <p>d1: Veronica Khoo, Artificial light: Consequences to the congregating firefly, <i>Pteroptyx tener</i> (Coleoptera: Lampyridae)</p> <p>d2: Mei Lee Wong, Observations From The Breeding of <i>Luciola ficta</i> in a Controlled Environment at Entopia, Malaysia</p> <p>d3: Norela Sulaiman, Eco-tourism based on fireflywatching in Malaysia</p> <p>Place: International meeting room, Panda house</p>
0924~0935	Refresh time & Twinkles of Hope (TVBS)
0935~1047	<p>Conservation section II: oral d4 d5 d6 d7 (Chiahsiong Wu)</p> <p>d4: Radim Schreiber, Firefly Experience (Art Presentation)</p> <p>d5: Soraya Jaikla, Biology of the Synchronous Firefly <i>Pteroptyx valida</i> Rearing in Artificial Media</p> <p>d6: Jia-Cong Chen, Study of Public Participation on Ecological Restoration for Firefly Habitat – Muzha Park’s Cui Lake in Taipei</p> <p>d7: Bing-Wen Wu, Study of Ecological Compensation of Firefly Ditch in Taipei Muzha</p> <p>Place: International meeting room, Panda house</p>
1047~1100	Refresh time & Twinkles of Hope (TVBS)
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1230~1330	Lunch time & Twinkles of Hope (TVBS)
1330~1406	<p>Conservation section IV: oral d13 d14 (Chiahsiong Wu)</p> <p>d13: Hsin-Chieh Tang, Taipei Zoo Breeding Plan of Fireflies in Kinmen</p> <p>d14: Tsutomu Sugita, Nature Restoration for the Genji Firefly, <i>Luciola cruciata</i> at Tama Zoological Park</p> <p>Place: International meeting room, Panda house</p>

1406~1600	Seminar (Modulator: Sara and Sonny Wong) and selecting for next host (Modulator: Chiahsiug Wu) Place: International meeting room, Panda house
1600~1830	Poster room closed, free time, all international participants will meet at Exit 5, Taipei MRT Dongman station before 1830
1830~2030	Dinner in Yuankan
2030~	Back to hotel

	4/27~4/28 Post-symposium tour (All international participants are invited, not just speaker)
0800~	Departure from Holiday inn and Taipei Fullon hotel, and go to Alishan area, see more detail at website: http://www.ifs2017taipei.org/ecotourism/

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oral key-1

Emerging Directions in Firefly Research (Things We Don't Know but Should)

Sara Lewis

Department of biology, Tufts University

Over the past few decades, our collective scientific studies have provided a rich tapestry of firefly knowledge. We've gained new insights into firefly evolution, biochemistry, behavior, biodiversity, and conservation. Yet many important topics remain unexplored, so this talk will highlight several of these crucial knowledge gaps. Now there is an urgent need for research to fill these gaps, as firefly populations are increasingly threatened by habitat loss and urbanization.

oral key-2

The Function and Evolution of Bioluminescence in Beetles

Marc A. Branham

Department of Entomology and Nematology, University of Florida

While bioluminescence in insects is not restricted to the order Coleoptera (beetles), beetles do represent the highest diversity of bioluminescent organisms found in terrestrial ecosystems. Bioluminescence occurs across multiple beetle families, serves multiple functions, and has been repeatedly gained and lost within certain lineages. One such lineage, the family Lampyridae, contains more bioluminescent species than any other family of organisms. This diverse and easily accessible group of beetles is well suited to studies focusing on the function and evolution of bioluminescence.

oral key-3

The firefly dilemma

Lesley Ballantyne

School of Agricultural and Wine Sciences, Charles Sturt University

SE Asian flashing fireflies will be addressed including their taxonomy, habitat and conservation, with an overview of current progress being made in the area in the taxonomic field. Many species will be introduced in a pictorial presentation.

Characteristic features of Japanese aquatic fireflies

Ohba Nobuyoshi

Ohba firefly research center

About 2000 species of fireflies are globally known, but about 10 species of aquatic fireflies, including three in Japan have been recorded from Asia. A typical firefly of Japan, known as Genji-botaru (*Luciola cruciata*), flies slowly around bodies of shallow water and glows. This fantastic spectacle is so attractive that it has been referred to in poems and songs. This species has been part of the local people's lives for centuries and is characteristic of Japan's rural landscape around paddy fields. The Kumejima-botaru (*Luciola owadai*) is very similar to the Genji-botaru, but the species occurs only on the Kumejima Island of Okinawa at the southern end of Japan. Colour of pronotum, activity, oviposition behaviour, life cycle and seasonal appearance are all clearly different from Genji-botaru. How these two species disperse and communicate are important questions, since both species are endemic to Japan.

The so-called Heike-firefly (*Luciola lateralis*) is smaller than Genji-botaru and the Kumejima-botaru. It occurs in paddy fields and wetlands and its flash communication system differs from that of the Genji-botaru. Heike-botaru is present throughout Japan with the exception of Okinawa Island, and it is therefore Japan's most common species. Its communication system is very similar to that of the Taiwanese *Luciola ficta* and the Chinese *L. leii*.

The larvae of the three Japanese aquatic fireflies possess gills as they live in water during their larval period. Because of Japan's abundance in forests and streams it can support aquatic firefly species particularly well.

The firefly rearing and protection in Kitakyushu city— the history and current situation

Nakamura mitsuo

The Kitakyushu firefly society

In 1960s, Japan had national pollution problems, Kitakyushu city is one of four main industry area in Japan, industry development made the air, river and ocean pollution, and the increasing of residential area decrease the nature habitat of different creature. The Murasaki River was polluted by household waste water and became a dirty ditch. The policy of agriculture department made the field and creek covering with the cement, beside that the use of pesticide made the aquatic animal population number decreasing, including the larvae of *Luciola cruciata* and *L. lateralis*. Firefly in the summer romance in Japanese culture. The decreasing of firefly rise people to care the environment pollution problems, and from 1980s, the people and government in Kitakyushu city began to protection firefly. In 1970s, river cleaning by local people, “River-lover group” began their activities. In 1980s, the reintroduction of *L. cruciata* larva in Ogumano river and 20 adults was found, this situation rise others residents caring fireflies problems. In 1990s, the firefly office of Kitakyushu city government establish, and host the 31th national firefly symposium. In 2000s, Kitakyushu firefly museum open. After that, the different units co-operate and organize activities for the purpose of firefly-protection and nature protection.

Estimating the diversity of the elusive fireflies

B. Nada^{1,2*}, A.P. Beckerman¹, L.A. Ballantyne³, K.L. Evans¹

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Large scale patterns in the structure of firefly assemblages are very rarely described, and the factors driving these patterns are thus very poorly understood. One of the reasons for this is because of the difficulty of obtaining complete sampling of firefly assemblages, due to their high diversity, and the pragmatic difficulties of working in remote locations at night. Numerous statistical methods are available to provide species richness estimates despite incomplete sampling. Here, we use a recently developed technique by Hsieh *et al.* 2016 that is implemented in the iNEXT package in R version 2.0.12. This tool kit estimates species richness by unifying sample-size and coverage-based rarefaction and extrapolation with the effective number of species, known as Hill numbers. We test the reliability of this method for estimating firefly species richness using a series of increasingly conservative tests that assess repeatability of richness estimates from sampling conducted in the same location: i) on consecutive nights, ii) five months apart, and iii) three years apart. Our samples are primarily derived from intensive sampling of firefly communities along five elevational gradients (200-1900 m above sea level) on the main mountain range in Peninsular Malaysia (Banjaran Titiwangsa) in 2015 with additional samples from two locations in 2016. This additional two locations were also sampled in 2013 and sampled twice in 2016 at 5 months apart. This sampling programme comprises the first systematic assessment of south-east Asian firefly assemblages along elevational gradients. All fireflies were classified into morphospecies, and all Luciolinae fireflies (which comprised 50.2% of collected individuals) were identified to species level. We use our results to explore the validity of the technique for reliably estimating firefly species richness. This enables numerous ecological questions that focus on the factors determining the structure of firefly assemblages to be addressed despite the difficulties of completely sampling firefly assemblages. We also use our data for a preliminary analysis of elevational patterns in firefly diversity, including using the Sorensen dissimilarity index to assess spatial turnover and nestedness of firefly composition along each elevational gradient and between transects. In addition, within the 15 species of Luciolinae fireflies that were identified we discovered 6 previously undescribed species, which includes members of a previously undescribed genera. These findings provide additional evidence for the vastly under-recorded diversity of fireflies in south-east Asia.

Evolution and Function of Bioluminescent Behavior in Firefly Larvae (Coleoptera: Lampyridae)

Lawrent L. Buschman

The function of bioluminescent behavior of lampyrid larvae has fascinated biologists for centuries and there are hundreds of comments and suggestions on its function. In the past the function of bioluminescence has been confusing because it was considered one entity. Here I suggest that bioluminescence is actually several different entities with different functions and behaviors. Bioluminescent behavior can best be understood in the context of the evolution of bioluminescence. Initially, the larvae had chemical reactions that released photons to reduce the energy in certain species of molecules that could damage tissues (like peroxides). These photons then resulted in—"incidental bioluminescence". Then there could have been an increase in these chemical reactions as the larvae adapted to new (extreme) conditions that required more energy and therefore more of these detoxification reactions. This would lead to the release of more photons creating a brighter bioluminescence. This bioluminescence was then bright enough to take on a physiological function, like adaptation to extreme conditions or even immunity to pathogens. This bioluminescence was now bright enough that it could be seen by humans so we have—"faint continuous glowing". Bioluminescence was now bright enough that it attracted predators. Larvae that had also sequestered toxic/repellant chemicals would have had a selective advantage. Soon the remaining bioluminescent population would have had these sequestered chemicals. At this point bioluminescence becomes a marker for larvae that have the sequestered chemicals and bioluminescence has taken on an aposematic function. Now there would have been selection for more and better chemicals. There would also be selection for brighter bioluminescence and so we would have—"bright continuous glowing". Some predators would become resistant to the chemicals and would again target bioluminescent larvae. There would have been selection for larvae with nervous control of their bioluminescence so it could be turned off and larva could hide. Nervous control would have allowed the larvae to light up only/specifically when there was danger (movement nearby) so we have—"response glowing". Response glowing is most useful for larvae that are hiding. Response glowing can also be enhanced by other defensive behaviors and functions including: startle, blinding, false surfacing and eye-spot/false head. Larvae also need to move about as when they need to hunt for food. They are particularly vulnerable at this time so they need an even more effective aposematic signal so we have—"periodic glowing". Periodic glows are shorter glows that are repeated over time. Periodic glowing can be enhanced by other defensive behaviors/functions including: distraction and enhanced visibility. All of these functions are still active in lampyrid larvae, although the brighter forms get most of the attention. I have found no evidence supporting the following suggested functions: prey attraction, illumination and larval communication.

Relationship between Synchronous Fireflies, *Pteroptyx* spp. and Their Firefly Trees

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Fireflies in genus *Pteroptyx* inhabit restrictedly in brackish water ecosystem and mangrove forest. They have congregating behavior on mangrove trees especially Sonneratiaceae family that leads to question in the relationship between the fireflies and their host plants. Besides for sexual communication, firefly flashes are aposematic signals that probably act as insect herbivore protection for the host plants. The aim of this study is to prove the hypothesis of +, + relationship between *Pteroptyx* fireflies and firefly trees by surveying species diversity and specificity of firefly trees in central and eastern Thailand. The leaf samples of top five species were collected for investigating leaf structure by using peeling technique and paraffin section technique. Moreover, the experiment plots of *Sonneratia* sp. were set up to prove the herbivore repellent mechanism of the fireflies' flashes. The rhythmic LED lights were randomly laid on the trees to represent artificial fireflies. From the 158 firefly trees in 4 locations, top five host plants are *Sonneratia caseolaris*, *S. alba*, *Avicennia officinalis*, *Rhizophora apiculata*, and *R. mucronata*. All plants have glands and/or secretory ducts on leaf surface which have possibility to release some volatile chemical compounds to attract the fireflies or label themselves. The artificial lighting experiment showed that the trees with lighting have significantly lower numbers of damaged leaves than the control group. To conclude, the relationship between *Pteroptyx* fireflies and firefly trees were probably +, +, fireflies receive mating site while their flashes help plants defense nocturnal herbivores.

Key words: Lampyridae, firefly tree, host plant relationship

Assessment of socio-economic aspects of tourism of fireflies in Nanacamilpa, Tlaxcala

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The study aimed at analysing the social and economic impact the arrival of tourists had during the firefly watching months in Nanacamilpa, Tlaxcala due to the natural phenomenon of mating and to determine if the tourist activity which attracts thousands of visitors during the season is contributing to the sustainable development of the community. This research is a transversal design and mixed approached case study including interviews of key actors and surveying 100 people of the general population and 47 local businesses. Concluding that, there are negative social impacts to the community such as an increase in violence and garbage. The positive aspects are the improvements to the road and street infrastructure. However, the population in general does not perceive tourism as a source of development as the benefits reach only a small sector and the season is too short. It is necessary to include all the actors in the planning and sustainable development of the tourism activity.

Key words: Sustainable tourism, Economic Valuation, Travel Cost, Sanctuary of the firefly

Economic valuation of the Sanctuary of the Firefly in Nanacamilpa, Tlaxcala

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The economic valuation of a natural resource is not an easy topic, as it is believed that these resources do not have monetary value, however, it is necessary to carry out economic exercises that help to plan and reduce the social and environmental impacts as well as provide guidance in the economic development of rural communities. As a way of lowering the environmental impact in the forest area the present research is carried out to measure the economic impact of firefly tourism in Nanacamilpa and proposes an increase in the entrance fee based on the tourists' willingness to pay according to the Zonal Travel Cost methodology as a proposal to support the sustainable tourism development. It concludes that the tourism arriving to Nanacamilpa exceeds the capacity of services and attention currently on offer, and the impact in the forest during the firefly's reproduction stage affects the sustainability of the species. The increase of the entrance fee would reduce the number of tourists visiting the area but can be compensated with the improvement in the services offered in both the sanctuaries and the settlement.

Key words: Ecotourism, sustainable development, social impact, economic impact

What can the eyes of fireflies and other luminescent invertebrates teach us about their biology?

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It could be shown in a comparison between equally sized luminescent and non-luminescent phylogenetically related species of gastropods (*Latia neritoides* and *Ancylus fluviatilis*) that the luminescent species *L. neritoides* possessed a larger eye and considerably more voluminous retina (Meyer-Rochow and Bobkova 2001). The same held true when strongly *Luciola lateralis*) and weakly luminescent firefly species (*Lucidina biplagiata*) had their ommatidial dioptric structures compared. A comparison between larval eyes of the brightly luminescent *Arachnocampa luminosa* and the weakly luminescent *Keroplatus nipponicus* showed the same trend (Meyer-Rochow 2016).

However, when 13 different Japanese species of fireflies differing in body lengths, life styles (nocturnal versus noctodiurnal and diurnal) and communication patterns had their ommatidial organization analyzed, generalizations failed to support the notion that brighter species would inevitably possess larger and more visually capable eyes. It seems that before any generalizations can be formulated, what needs to be considered are additionally the distances flown by the males and females of the various species, sexual dimorphism, longevity as adults and communication patterns.

Acknowledgment: The author is indebted to the late Professor Dr Eisuke EGUCHI (Yokohama City University, Japan) for having made available data on Japanese fireflies.

Meyer-Rochow V.B. and Bobkova M.V. 2001. Anatomical and ultrastructural comparisons of the eyes of two species of aquatic, pulmonate gastropods: the bioluminescent *Latia neritoides* and the non-luminescent *Ancylus fluviatilis*. *New Zealand Journal of Marine and Freshwater Research* 35: 739-750.

Meyer-Rochow V.B. 2016. Eyes of two keroplatid dipterans: the luminescent *Arachnocampa luminosa* and the non-luminescent *Neoditomyia farri* plus comments on luminescent and non-luminescent beetles and gastropods. *Entomologie heute* 28: 117-126.

Honduran Fireflies and the Jungle Adventures in Catching Them

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This photographic presentation seeks to enlighten the viewers on the extreme variety of the often poorly understood Honduran fireflies. Little published information exists and few firefly surveys have occurred in the past 100 years in Central American Honduras which is centered in the biologically rich, species diverse Mesoamerican Biological Corridor. From 2005 to 2017 at 35+ sites, repeated surveys of fireflies were made by Hugh Faust, traveling primarily by foot or raft in extremely remote, often trail-less areas. Because Hugh had to carry all he needed on his back for weeks at a time, he used his hat as a net to catch thus far over 280+ specimens. Limited use of light traps and nets was possible in more inhabited areas. Sampled habitats in the Rios Copan, Cangrejal and Platano watersheds, spanning six (of 18) Honduran Departments, included primary and secondary rainforests, a cloud forest, coffee plantation, inhabited areas and coastal lagoons; plus the Stann Creek district of Belize. Site elevations ranged from sea level to 1276 m. Habitat photos, GPS coordinates, seasonality, time of display and preliminary flash data were recorded. Descriptions and over 7,000 photographs of 30+ species were taken of these fresh specimens in the wild and later the lab to document their living colors and appearance. Dissection revealed unique aedeagi in several otherwise “*Photinus* looking” fireflies. Eight genera (tentative species #s in parenthesis) have been identified: *Photinus* (11) Green’s Division 1 and Division 2; *Photuris* (4); *Bicellonycha* (6); *Pyropyga* (1); *Aspisoma* (1); *Cratomorphus* (2); *Phaenolis* (1); *Lucidota* (2) and *incertae sedis*. Specimens are contributing to ongoing studies at several universities and the CDC. Although representing a fraction of the extreme diversity of Lampyridae likely present, it is hoped this preliminary presentation will inspire future studies and spotlight the vast potential of the under-appreciated biological richness of Honduras.

Key words: Honduras, fireflies, Lampyridae, Central America, Rio Platano Biosphere Reserve

Short-wavelength artificial lighting influences *Aquatica ficta* flash signals (Coleoptera: Lampyridae)

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Urbanization can radically disrupt natural ecosystems through the introduction of chemical, noise, and light pollution. Such extreme habitat disturbances are predicted to favor behaviorally flexible species capable of rapidly adapting to changed environments. Artificial night lighting (ANL) in urban areas has the potential to impede the reproduction of local firefly populations by obscuring their bioluminescent courtship signals. However, while the distinctive signals of different species in the field are well described, we do not know if individuals can modify their signals to maintain visibility against an illuminated background. In this study, we explored the ways in which Taiwanese *Aquatica ficta* respond to artificial light. We exposed adult males to LEDs of varying wavelength and intensity, and recorded their flash rate and intensity. When exposed to short wavelengths (≤ 533 nm), male *A. ficta* emitted brighter signals with diminished frequency; however, long wavelengths (≥ 597 nm) did not affect signal morphology, likely because *A. ficta* cannot perceive these colors. Further research may reveal whether the observed increases in signal brightness are sufficient to ensure courtship success in illuminated areas. The results of this study can inform future decisions about artificial lighting in firefly habitats. By considering the emission spectra of different lighting types, we can minimize the impact of ANL on the rich biodiversity and thriving firefly ecotourism industry of Taiwan.

Key words: firefly, light pollution, light signal, signal plasticity, action spectrum

Escape into Winter: Does a Phenological Shift by a Lucibufagin-Containing Firefly (*Ellychnia corrusca*) Shield It from a Specialist Predator (*Photuris*)?

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Although lucibufagins (LBGs) have been documented from several genera of fireflies, the North American genus *Ellychnia* has yet to be investigated for these defensive steroids. Our chemical analysis (NMR and HPLC-MS) of the winter firefly (*Ellychnia corrusca*) detected a mixture of eight LBGs, including four novel compounds, both in whole body extracts of adults and in their defensive reflex bleeding. Furthermore, this was the first study to examine the defensive chemistry of all four life stages of a firefly species: LBGs were found throughout the life cycle. The high concentration (>500 µg/beetle) of these compounds, with demonstrated anti-predatory activity against birds, likely protects the adult *E. corrusca* as they are exposed on tree trunks throughout the winter, prior to mating in early spring. To consider whether this phenological shift by *E. corrusca* might serve as a ploy to avoid predation by LBG-seeking, summer-active *Photuris*, we conducted two laboratory experiments (behavioral assays) bringing together these fireflies which, although sympatric, are asynchronous as adults in nature. Both showed that female *Photuris* selectively consumed *E. corrusca* versus a non-LBG control. Chemical analysis of *Photuris* offered either *E. corrusca* or the control demonstrated sequestration of winter firefly LBGs. Thus *E. corrusca* is indeed vulnerable to *Photuris*, and consequently its seasonal shift may represent a means of temporal escape from this specialist predator.

Key words: *Ellychnia corrusca*, *Photuris*, lucibufagin, chemical defense, phenological escape

**Microscale meteorological monitor for ecological watch
— a case study at firefly recovery site in Daan Park, Taipei**

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Intensive meso-scale weather network over some urban & rural region/countries is delivered for many years in meteorological society. Microscale bio-meteorological study will be the next target worldwide. This study originates from the MOSPC (Meteorological Observation by Smart Phone Citizen Scientists) project (August, 2015 to July 2017) in National Taiwan University (NTU). Swiss-made micro-size meteorological sensor, Skywatch-Windoo, which is connected to the headphone jack of smart-phone is tested in MOSPC project. We also made a small data logger to replace smartphone for stationary measurement to approach possible ecological application. Ten sets of WINDOO mini weather station (short for WMWS) are installed temporarily surrounding the firefly pod in Daan Park, Taipei from February to April of 2017. The Windoo sensors of WMWS were closed to firefly habitat and the height range of sensors are from 23 cm to 44 cm over ground and water. The near-surface feature of air temperature, humidity, wind speed and solar intensity among different locations (tall grass, pool and warehouse) will be analyzed and discussed. This microscale environmental information might be helpful for firefly activity survey.

Distribution of *Pteroptyx* Olivier (Coleoptera; Lampyridae) in Thailand

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Pteroptyx Olivier is a firefly genus with a distinct bent-wing characteristic and synchronous flashing behavior that's unique to members of this genus. These fireflies are often spotted aggregating on vegetation along mangroves and estuary areas in great quantities, providing mesmerizing night time displays to passing observers. As such they are a primary attractant for ecotourism in many countries, particularly in Southeast Asia. In this study, the distribution of *Pteroptyx* Olivier was studied across Thailand. The survey was conducted during 2012-2016 from the mangroves in both Andaman and the Thai gulf coast. A total of four *Pteroptyx* species (*P. asymmetria* Ballantyne, *P. malacca* Gorham, *P. tener* Olivier, *P. valida* Olivier) were identified based on morphological characters and DNA barcoding. *P. malacca* Gorham was the most common species presented in all sampling sites. While *P. tener* Olivier was very scarce. In addition, *P. asymmetria* Ballantyne has been identified for the first time in the South of Thailand, though in small quantities across localities. Phylogenetic analyses of a partial mitochondrial *COI* genes showed that the *P. tener* Olivier and *P. asymmetria* Ballantyne were always grouped in the same clade when the tree was analyzed with different methods; however, a relationship of other species was uncertain shown by a low bootstrap support. The results suggest that although barcode sequences are very useful for species identification, a molecular phylogenetic delineation requires more data.

Key words: firefly, phylogenetic, Thailand, *Pteroptyx*

Flash Diversity of Taiwanese Fireflies

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The flash pattern is one of important ways of communication of adult firefly. The firefly flash pattern in Taiwan had been slowly studied before due to the costly photographic equipment and difficulties in filming skills and find the right place to shoot firefly. Because of the recent progress of digital single lens reflex camera and video equipment, researchers nowadays are able to shoot the flash pattern of firefly in an extremely dark wild environment, facilitating the study of firefly luminescence. According to the study of Dr. Nobuyoshi Ohba, there are six types of the flash communication systems of Japanese fireflies. Here in Taiwan, the flash patterns of Luciolinae showed the highest diversity. We classified male's in-flight flash pattern and investigated their phylogeny by COI sequence. We compared the flash characteristic of Taiwanese fireflies and suggested eight types of flash systems for Taiwanese fireflies with two of them new to the systems.

Key words: firefly, flash communication systems, biodiversity, COI, Luciolinae

Species Identity Crisis in SE Asian Fireflies: Will the Real *Pteroptyx* Please Stand up?

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The status and composition of the genus *Pteroptyx* Olivier 1902 has changed significantly since it was first described. The genus name *Pteroptyx* (*Ptero-* and *-ptyx*) is an epithet in Greek for “folded wings” denoting the deflexed elytral apex in males. This and the trisinate or trilobed terminal abdominal ventrite were the defining and only characters for taxonomic identification given by Olivier. *Pteroptyx* was erected for two species, *Luciola malacca* Gorham and *Luciola testacea* Motschulsky, which was misidentified by Olivier. However, our recent phylogenetic analyses using combined morphological and molecular data for 158 taxa revealed a distinct *Pteroptyx* clade as a morphologically variable genus with *Poluninius selangoriensis* Ballantyne being synonymised with *L. testacea*, the type species, which is redescribed from the type series. An expanded generic description of *Pteroptyx* now divides the genus into three groups: (a) a group of *Pteroptyx* species with the metafemoral comb (MFC), bipartite light organ in ventrite 7 (V7) but lacking lobes arising along the posterior margin of tergite 8 (T8) and including *P. malacca*; (b) a group of *Pteroptyx* species with characters as (a) but with presence of lobes arising along the posterior margin of T8 including *P. tener*; and (c) a group of *Pteroptyx* species which have either entire or bipartite LO in V7, with or without MFC and are without deflexed elytra. In this study, we particularly highlight species from (a) which presents a new species *Pteroptyx balingiana* Jusoh sp. nov. and confirms the identity of *Pteroptyx gelasina* Ballantyne as a distinct species. We also further discuss the three morphological subdivisions within *Pteroptyx malacca* (Gorham), which brings us back to the question of will the real *Pteroptyx* please stand up?

Key words: *Pteroptyx*, bent-winged, Southeast Asia

oral b4

Firefly (Coleoptera: Lampyridae) Diversity of Hispaniola

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Over 2400 specimens from twelve museum and private collections were examined to establish a baseline knowledge about the firefly biodiversity of Hispaniola. We calculated the theoretical species richness of Hispaniola using Darlington's Rule, and estimated 124 expected firefly species based on the size of the island using the well documented firefly fauna of Jamaica as a baseline. Specimens were then pre-sorted into morphotypes, and then identified with the help of currently available keys. This resulted in the detection of 64 undescribed species across 10 genera. Previously only 69 species were reported from Hispaniola, and with the new records the total number of species is now at 133. Based on this revised number of documented species we used the Chao1 Species Richness Estimator to calculate new firefly biodiversity estimates for Hispaniola. Chao1 estimated a species richness between 151 and 238 species with a mean of 176. This data suggests that some 43 more species could be awaiting discovery, indicating that we currently have documented approximately 80% of the hypothesized firefly biodiversity of Hispaniola.

Key words: West Indies, Hispaniola, firefly, biodiversity

ANCHORED HYBRID ENRICHMENT ANALYSIS OF LAMPYRID PHYLOGENY

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Fireflies are an exciting beetle family with a rich but conflicted taxonomic history. This is true not only within the family, but also extends to the sister lineages of fireflies. At various times the sister group to Lampyridae has been considered either Lycidae, Elateridae, Phengodidae, Cantharidae, Rhagophthalmidae, or a combination of these families. Resolving these sister group relationships is essential for examining character evolution, including bioluminescence, sexual signals and morphological traits, across not only Lampyridae, but also the greater Elateroidea. We used Anchored Hybrid Enrichment (AHE) to illuminate the sister group relationships of Lampyridae with probes that were designed using genomes and transcriptomes from 15 firefly species, including *Photinus pyralis*, and the distantly related flour beetle *Tribolium castaneum*. These probes captured 400-500 genes for each taxon representing ~80 species from seven outgroup families (Lycidae, Elateridae, Phengodidae, Rhagophthalmidae, Cantharidae, Eucnemidae, & Telegeusidae), as well as ~30 lampyrid genera representing eight subfamilies. Based on these data, all families were inferred to be monophyletic, and a Phengodidae & Rhagophthalmidae clade was found to be the sister group of Lampyridae. All subfamilies included in the analysis except Lampyrinae were monophyletic. In support of our previous work, *Ellychnia* was reconstructed within the *Photinus* clade. This phylogeny provides a new framework for the generation of a comprehensive worldwide phylogeny of fireflies. As additional taxa are added to this data set it will allow us to revise and update the taxonomic classification for both extant and fossil taxa, and it also provides a more robust framework for the description of new taxa.

Key words: firefly, probes, *Ellychnia*, outgroup

The evolution of sexual signal modes and associated sensor morphology in fireflies (Lampyridae, Coleoptera)

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Animals employ different sexual signal modes in different environments and behavioural contexts. If production of sensory structures is costly, then evolutionary shifts in primary signal mode should be associated with changes in sensor morphology. Sex differences are expected if signaling behavior differs between males and females. Fireflies are known for their spectacular light signals during mate search, but many species communicate exclusively with pheromones and are active during the day. Phylogenetic analyses of male eye and antenna size (46 North American species) showed that signal mode (light vs. pheromones) is significantly correlated with both eye and antenna size, and that eyes change faster than antennae when signal mode changes. Worldwide male data (100 taxa in 30 genera) support this as a general pattern for fireflies, and suggest that both signal mode and mate search behavior are associated with differences in male and female eye size (27 taxa in 12 genera): males have relatively larger eyes than their females, but only unlighted males have significantly longer antennae than their females. A phylogenetic discriminant analysis of sensor traits correctly predicted the signal mode of fireflies with “unknown” signals, which is promising for including fossils and museum specimen into analyses of signal evolution.

Key words: light signals, pheromones, antenna length, eye size, communication

oral b7

Prolonged adult life span of five firefly species in Hong Kong

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Larvae of three species of firefly namely *Pyrocoelia* sp., *Diaphanes lampyroides* and *Lamprigera* sp., were reared in captivity and emerged adults were kept until they died naturally. A female adult of *Rhagophthalmus motschulskyi* and another *Rhagophthalmus* sp. collected in the field were kept in captivity until they died naturally. Very long adult life spans were recorded, ranging from 20 to 111 days. Possible associations of the very long adult life spans with sexuality, seasonality of flight periods, body morphology, light producing behaviour, courtship behaviour and locomotion are discussed.

Firefly Genomics: why it matters

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The sequencing of genomes, entirely or partially, is becoming possible and fairly routine beyond the traditional model organisms due to emerging technologies and bioinformatics tools. Firefly beetles (Lampyridae) represent a worldwide and diverse adaptive radiation that lends itself to addressing evolutionary questions of broad interest. However, the generation of genomic resources for fireflies has lagged behind other insect groups, in large part, because they are not economically important. Due to their importance to medical and evolutionary sciences as well as their strong appeal to the general public, fireflies are emerging as important study organisms with respect to biotechnology, evolution of communication systems, behavioral ecology, responses to climate change, and conservation biology. These contexts, among others, illuminate exciting applications for genomic tools in the study of fireflies. We highlight the importance of fireflies as focal organisms for genomics research and outline where they can serve as potential models for evolutionary and ecological biology.

Key words: Genomics, evolution, ecology, genomic tools

Advanced NMR and HPLC-MS Methods for Determination of Firefly Chemical Defenses

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Firefly chemical defenses play vital roles in the ecology and evolution of this fascinating group of insects. The discovery of lucibufagins (LBGs) as the primary defensive compounds of *Photinus* spp. in the 1970s opened the door to this interesting and enlightening field. However, since then, only two other North American firefly genera have had species studied in-depth for their defensive chemistry. Herein, results of our recent chemical investigations of several genera of North American fireflies will be described, focusing on the chemical methodology used. Topics will include: NMR spectroscopic techniques and analysis that made possible the discovery of eight LBGs from *Ellychnia corrusca*, including four new compounds; NMR spectroscopy for quantitation of LBGs; NMR spectroscopic analysis to determine the presence or absence of LBGs in a single *Photuris* individual; HPLC-MS techniques allowing the determination of the presence or absence of LBGs in hemolymph samples taken from individual *Photuris* subjects without sacrificing them. Additionally, the secondary metabolite profile of *Pyrractomena borealis* will be used as an example to demonstrate the necessity of using NMR spectroscopy when studying the chemical defenses of lampyrids.

Key words: *Ellychnia*, *Photuris*, NMR, HPLC-MS, lucibufagin, chemical defense

Sulfoluciferin is Biosynthesized by a Specialized Luciferin Sulfotransferase in Fireflies

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Firefly luciferin is a specialized metabolite restricted to fireflies (family Lampyridae) and other select families of beetles (order Coleoptera). Firefly luciferin undergoes luciferase-catalyzed oxidation to produce light, thereby enabling the luminous mating signals essential for reproductive success in many bioluminescent beetle species. Although firefly luciferin and luciferase have become widely used biotechnological tools, questions remain regarding the physiology and biochemistry of firefly bioluminescence. Here we report sulfoluciferin to be an in vivo derivative of firefly luciferin in fireflies, and report the cloning of luciferin sulfotransferase (LST) from the North American firefly *Photinus pyralis*. LST catalyzes the production of sulfoluciferin from firefly luciferin and the sulfo-donor 3'-phosphoadenosine-5'-phosphosulfate (PAPS). Sulfoluciferin is abundant in several surveyed firefly genera, as well as the bioluminescent elaterid beetle *Pyrophorus luminosus* at a low level. LST is one of the most highly expressed genes in the *Photinus pyralis* photophore. Molecular phylogenetics of the LST gene combined with analyses of gene loci in the draft *Photinus pyralis* genome demonstrate that LST arose from a tandem duplication of an ancestral sulfotransferase gene, which we dub ST0. We propose that the LST gene arose early in the evolution of the Lampyridae by gene duplication and subsequently specialized to catalyze luciferin sulfonation. We hypothesize that sulfoluciferin may serve as a luciferin storage molecule in fireflies, and that LST may find use as a new tool to enhance existing biotechnological applications of the firefly bioluminescent system.

Key words: Lampyridae, sulfotransferase, natural products, LC/MS, de-novo transcriptomics, genomics

Impacts of artificial light pollution on the transcriptome of *Aquatica ficta* larvae

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The artificial light pollution is considered as one of the factors cause declines in firefly population. To elucidate the effect of light pollution on firefly larvae from molecular perspective, we used NGS (Next Generation Sequencing) method to profile the transcriptome of firefly larvae, *Aquatica ficta*, under artificial light pollution. More than 2G of RNA-seq reads were generated from artificial light exposed as well as regular light/dark cycle firefly larvae for transcriptome assembly. Approximately 208,672 transcripts (unigenes) were obtained, and 60,410 unigenes were annotated. Comparing to the control, we identified 1,450 DEGs (Differentially Expressed Genes) from the larvae with the artificial light treatment during the dark cycle. From them, 58 and 1,392 unigenes were activated / up-regulated or inactivated / down-regulated, respectively. We then performed GO (Gene Ontology) analysis and found that activated / up-regulated unigenes are enriched in terms relative to regulation of hormone levels and oxidation-reduction process, while inactivated / down-regulated are enriched in terms relative to ribonucleoprotein complex biogenesis, primary metabolic process, macromolecule metabolic process, cellular biosynthetic process, organic substance biosynthetic process, and response to osmotic stress. We then repeat the experiment and found that death rate of light treated larvae is 71.4% (n=28) while the control is 25% (n=28), suggesting a strong effect of light pollution on firefly larvae during dark cycle. More research is needed to confirm whether the effect could continue to adult stage.

Key words: *Aquatica ficta*, light pollution, transcriptome

Dual-color Luminescence of Firefly Pupa

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Railroad worms (Phengodidae) emit red light from head / yellow light from abdomen. Fire beetles (Elateridae) emit green light from dorsal photophores / orange light from ventral photophore. They have two different luciferase genes and these gene products generate different colors of light in the presence of same luciferin substrate. In fireflies (Lampyridae), on the other hand, it has been considered that the species emit single color of light with a few exceptions, like sexual dimorphism of luminescence color in the Chinese *Pygoluciola qingyu* and the Papua New Guinean *Pteroptyx effulgens*. Recently, we found that some lampyrid species exhibit dual-color luminescence at pupal stage and the duality is caused by the presence of two different luciferase genes.

Key words: luciferase, light color, evolution

GENETIC VARIATION WITHIN *PHOTINUS PYRALIS* REVEALS POSITIVE SELECTION ON LUCIFERASE, BUT NOT OPSINS

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Natural selection is expected to favor signals and receptors that maximize signal detection. Consequently, when a signal changes, the genes underlying signal production and reception should both show evidence of positive selection. Fireflies are an excellent system for testing this prediction because the primary genes underlying signal production and reception are known. Amino acid sequence variation in luciferase, the enzyme that catalyzes the light reaction, is thought to cause the flash color differences between species. Likewise, sequence variation in opsins, the protein components of visual reception, may underlie variation in visual sensitivity. Here we tested the hypothesis that light color variation *within* species is due to variation in luciferase protein sequence and three resultant predictions: (1) amino acid sequence variation in luciferase is correlated with variation in signal color, (2) amino acid sequence variation in long-wavelength (LW), but not ultraviolet (UV), opsin is correlated with variation in signal color (and thus, inferred visual sensitivity), and (3) that genetic signatures of positive selection are evident in both genes. We sequenced the luciferase and both the LW and UV opsin genes of 192 *Photinus pyralis* fireflies from 12 populations in North America, which vary in flash color. We also genotyped these individuals at 716 single nucleotide polymorphisms to allow us to account for population structure when testing for selection. We found no variation at the protein level in either luciferase or opsins, despite population differences in signal color. However, silent variation in luciferase, but not opsins, showed high level of differentiation among populations, strongly suggesting that selection is acting at this locus. The absence of protein variation rejects the hypothesis that variation in light color is due to variation in luciferase protein sequence within *P. pyralis*. Instead, natural selection appears to target luciferase regulation.

Key words: luciferase, opsin, selection, signal color, *Photinus pyralis*, RADseq

**Quaternary vicariance of *Curtos* (Lampyridae; firefly)
in the Ryukyu-Taiwan islands; Molecular and
X-ray micro CT analyses**

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Yuichi OBA, John WAKABAYASHI

Two *Curtos* species were reported on the Ryukyu islands, with different elytral coloration: dark yellow and brownish black. The former is *C. costipennis* distributed on the Amami-Tokara islands (north) and also on the Yaeyama islands (south), and the latter is *C. okinawanus* distributed on the Okinawa islands (center). However, we showed that *C. costipennis* on each islands group (northern Amami-Tokara and southern Yaeyama, and also Taiwan) is genetically different, and the coloration is not an important indicator for the systematics of *Curtos*. Newly obtained X-ray micro CT (computed tomography) images showed that male genitalia are distinct between these specimens of *C. costipennis*. We drew ML and BI trees of *Curtos* fireflies in the Ryukyu Islands and Taiwan using raxmlGUI and BEAST, based on the COI and 28SrRNA genes. In BEAUti we calibrated the splitting age of the MRCA of *Curtos* endemic species as 1.55 ± 0.15 Ma, a date that the Okinawa trough began to rift and isolate the Ryukyu-Taiwan islands from the Chinese continent. The physical isolation of these islands generated the allopatric speciation and six endemic *Curtos* species (three species are from Taiwan). Taiwan yields an additional four uncollected species, reflecting adaptive radiation within the island. Each Ryukyu population was further differentiated to form local populations constituting each island, reflecting the low stand of sea level at the last glacial time (ca. 0.02 Ma). By these minor genetic differences, it was possible to detect the southern Okinawa-jima origin of human-introduced *C. okinawanus* in Kagoshima, Kyushu, Japan. The base substitution rate was estimated as 0.0452 substitutions/site/myr for the COI gene, and 0.0147 s/s/myr for the 28SrRNA gene.

THE EVOLUTION OF Na^+ , K^+ - ATPASE IN RELATION TO PREDATORY FEEDING AND LUCIBUFAGIN SEQUESTRATION IN FIREFLIES

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Although conspicuous in the dark, fireflies are usually avoided by predators because they contain a group of potent bitter-tasting and toxic chemical compounds called lucibufagins. Intriguingly, *Photuris* fireflies have lost the ability to make toxin. Driven by the exigency of survival in an insect-eating world, *Photuris* females have evolved the ability to lure *Photinus* males by mimicking the mating flash signal of *Photinus* females, prey on them and sequester the toxin for their own defense. However, it is unknown how the *Photuris* females are not affected by lucibufagin toxicity, which inhibits Na^+ , K^+ - ATPase, a key enzyme used in a variety of cellular processes including neural signal transduction and muscle contraction. Here, we found the gene of Na^+ , K^+ - ATPase has duplicated three times in *Photuris* and the duplicates bear amino acid substitutions at key toxin-binding sites, which renders these fireflies insensitivity to lucibufagins. Furthermore, our results show a tissue-specific expression pattern of the duplicates. The most resistant copy is upregulated in the gut, the tissue exposed to the highest concentrations of lucibufagins, but not in head, where the toxin is blocked by the blood-brain barrier. This divergent expression pattern helps preserve the duplicates and lower fitness costs from negative pleiotropy. This study is an example of how next-generation sequencing can help us better understand ecology and behavior in firefly research.

Key words: firefly, toxin resistance, adaptation, predation, *Photuris*, NGS

**ARTIFICIAL LIGHT: CONSEQUENCES TO
THE CONGREGATING FIREFLY, *PTEROPTYX TENER*
(COLEOPTERA: LAMPYRIDAE)**

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Males of the firefly *Pteroptyx tener* (Coleoptera: Lampyridae) flash synchronously in communal groups to attract mates. One well-known population that attracts ecotourism occurs along the Selangor River estuary in Malaysia. With increasing artificial light penetration from villages, canals and jetties around their habitat, there is concern that the behaviour and, consequently, population of the fireflies may be affected. To investigate this possibility, field studies involving short- and long-term exposure to artificial light were carried out, using digital night photography to detect changes in the number of flashes. When a spotlight directed at display trees across the riverbank was switched on for 90 minutes and switched off briefly at intervals to capture images, numbers of flashes recorded in the images were much reduced compared to an unilluminated control site. Number of flashes rebounded initially beyond the control site when illumination ceased completely. When the spotlight was switched off for a longer period of one minute at each interval for three intervals, a sharp rebound could be seen within these short periods of darkness. However, when the display trees were illuminated continuously for three nights, there was a drop in the number of flashes and little recovery was seen seven days after illumination ceased. A similar result was obtained when the test and control sites were swapped a month later. The findings suggest that short-term illumination has an immediate but reversible effect on the flashing behaviour of the fireflies, and that long-term illumination causes fireflies to leave their display trees.

Key words: Fireflies, *Pteroptyx tener*, light pollution, artificial light, behaviour, conservation

Observations From The Breeding of *Luciola ficta* in a Controlled Environment at Entopia, Malaysia

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Entopia (by Penang Butterfly Farm), Penang, Malaysia

Fireflies have become increasingly distant from humans as their populations continue to decline from habitat encroachment and urbanisation. Fireflies used as an educational tool to enhance environmental and conservation awareness is imperative to highlight these blinking wonders and the efforts involved in their conservation. The experimental breeding of *Luciola ficta* in a laboratory setup with controlled environmental parameters and an artificial water system is presented. Observations on the annual adult emergence rate, sex ratio and matters arising from the breeding of this species is discussed. Fireflies as a showcase species in a display setup is also presented.

Key words: *Luciola ficta*, breeding, education, display

ECO-TOURISM BASED ON FIREFLY WATCHING IN MALAYSIA

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Land use changes and prawn aquaculture ponds on both sides of the river banks have threatened the community of synchronous firefly species (*Pteroptyx tener*) in Malaysia. This, in turn, has affected the ecotourism activities of firefly watching operated by the local community. Firefly conservation efforts have been initiated by the expert group of experienced researchers from Universiti Kebangsaan Malaysia (UKM) through rehabilitation of the firefly habitat. The intervention programme involves replanting of the host trees of the mating fireflies, i.e. Berembang (*Sonneratia caseoralis*) and teaching the local community to select ripe fruits, sorting the best seeds for germination in polybags containing the river sediments and caring for the growing seedlings until they reach one-meter height. The villagers were also trained to transplant the Berembang seedling in cages along the eroded and degraded river banks, regular training sessions were conducted to enhance the skills, knowledge, and awareness of the eco-tourism operators. These community outreach activities were supported by various stakeholders such as District Council, Forestry Department, Tourism Agency, NGOs and local school children. A few thousands of Berembang seedlings have been replanted along the riverbanks and more Berembang trees are scheduled to be replanted in future. Furthermore, to facilitate sustainable eco-tourism, the boatmen were trained to become good, well-informed tour guides, fluent in the Malay language and a smattering of English. The result of regular training and several stakeholders' consultations have succeeded in raising the awareness of the village community, empowering them to engage in small home-based enterprises such as operating food outlets and gift stalls at the jetty area. Through media coverage by the local newspapers and televised broadcast of the Berembang replanting ceremony attended by dignitaries, tourist arrivals have steadily increased. The increase in visitor arrivals has indirectly enhanced the income of the local community and positively impacted the local socioeconomics.

Key words: *Pteroptyx tener*, *Sonneratia caseoralis*, rehabilitation, tourist arrivals, local socioeconomic

Firefly Experience (Art Presentation)

Radim Schreiber

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Firefly Experience is an art project by award winning photographer and cinematographer Radim Schreiber. Radim has been specializing in photographing the magical glow of fireflies in their natural environment without digital manipulation since year 2008. Radim's presentation is a multimedia presentation focusing on the beauty and magic of fireflies.

Radim's Notable Awards:

2015 - 1st place, Smithsonian Magazine 13th Annual Photo Contest

2015 - Short Film Award Creative Edge Film Festival 2015 Beauty Award!

2012 - Finalist, National Geographic photo contest

2011 - 1st place, National Wildlife Federation 41st annual Photo Contest

2011 - 1st place, Smithsonian Magazine 8th Annual Photo Contest

2011 - 2nd place, International Garden Photographer of the Year Photography Contest

2009 - Grand Prize, The Rainforest Alliance's annual Picture Sustainability Photo Contest

2008 - Grand Prize, The Rainforest Alliance's first annual Picture Sustainability Photo Contest

Key words: firefly, art, photography, photos, movie, cinematography

Biology of the Synchronous Firefly *Pteroptyx valida* Rearing in Artificial Media

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The rearing of *Pteroptyx* spp. is important information for firefly conservation and implication for firefly reintroduction in mangroves with decreasing firefly populations. Thus, the objectives of this study were to develop a rearing technique and to study biology of *P. valida*. The fireflies were reared under laboratory condition by using an artificial substance to support immature stages. The technique could support whole life cycle of *P. valida* with the duration of 161.76 ± 7.42 days (157.60 ± 7.60 days for male and 160.49 ± 7.54 days for female) with 44% mortality rate. The females laid 202.92 ± 61.39 eggs with the hatching rate of 83.88%. The developmental time of egg, six larval instars, pupa and adult were 20.73 ± 2.02 , 30.76 ± 0.43 , 22.36 ± 6.06 , 24.26 ± 8.41 , 32.62 ± 8.40 , 26.67 ± 5.93 , 31.33 ± 4.93 , 9.25 ± 2.36 and 20.53 ± 8.54 days, respectively. Interestingly, the variation of larval instar numbers was examined. The 30% of male and 52.63% of female fireflies had five larval instars, while only females (15.78%) have six larval instars.

Key words: synchronous firefly, life cycle, rearing

Study of Public Participation on Ecological Restoration for Firefly Habitat – Muzha Park’s Cui Lake in Taipei

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Public participation is one of the topics promoted by Agenda 21. As a result of the urban development, many original landscapes have been altered, leading to species disappearance. Firefly is an obvious example. Fortunately, it still retains the native communities of fireflies in Taipei Muzha Park’s Cui Lake. And with the help of Wenshan Community College, Taipei City and NGOs, local people participated in the fireflies’ restoration, environmental remediation, sludge and exotic species removal, and wetland construction. In our process, there were 461 people participated in planting aquatic plants to create biodiversity and removing nonnative fishes around 4,775. The study was researched the efficiency of public participation on ecological restoration for firefly habitat, and the experiment were water temperature, pH, EC, DO, turbidity, SS, nitrate nitrogen, orthophosphate, total phosphorus, ammonia nitrogen, Kjeldahl nitrogen content, and flow rate. The dissolved oxygen in the newly constructed wetland (4.4 mg/L) was higher than in the Cui Lake (2.6 mg/L), and the effect of water quality optimization was obvious. After the habitat construction, the average number of fireflies’ increased from 10±0 in June 2015 to 97±134 in April, 80±20 in May and 35±9 in October 2016, showing the effect of public participation on improving fireflies’ habitat was positive. However, the relationship between the habitat restoration and the fireflies’ population variation needs further discussion, and more experiments are expected to be conducted.

Key words: firefly, public participation, ecological restoration, habitat construction

Study of Ecological Compensation of Firefly Ditch in Taipei Muzha

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Ecological restoration in suburban shallow mountain area is one of the issues of modern ecological engineering. In Taipei Muzha, a wetland with an area of 1788.23 m², was filled because of engineering constructions. In order to restore the fireflies, the ecological compensation was done nearby the wetland. Three waters of constructed ecological grass ditches were connected with a total length of 125.1 m, an area of 225 m², altitude from 18.66 m to 15.22 m, and water mainly came from the daily pumping groundwater. The grass ditches were constructed with stone materials and provided biological concealments and slope protections with *Alocasia macrorrhizos*, *Bolbitis subcordata*, and other ferns and plants. The construction was divided into two parts, fireflies restoration and water quality control. The total number of *Aquatica ficta* (Olivier, 1909) was increased from 256 in 2014 to 332 in 2016, and the month of firefly was April to September, mainly in April and July. The distribution range of fireflies became wider, showing the effectiveness of fireflies' restoration was positive. In water quality control, dissolved oxygen 6.48±3.62 mg/L and ammonia nitrogen 0.21±0.62 mg/L were suitable for fireflies' restoration. With lots of ferns and plants, the fireflies were observed more when the plant cover was 81.66±13.77%, which was also conducive to create a high relative humidity environment. The fireflies appeared in the range of temperature 33.47±3.03°C and humidity 83.17±3.92%, showing that maximum air temperature and relative humidity were positively correlated with the number of fireflies in this area.

Key words: *Aquatica ficta* (Olivier), firefly, ditch, wetland

***Aquatica ficta* (Olivier) Habitat Conservation in Daan Forest Park of Taipei**

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The Daan Forest Park is the largest metropolitan park in Taipei, covering an area of 25.894 hectares and creating the habitat of the firefly *Aquatica ficta* (Olivier, 1909) in October 2015. This study explored the effect of *Aquatica ficta* (Olivier) habitat conservation in Daan Forest Park, and its environmental impact factors. The research were done on a regular basis for hydrology, water quality, microclimate surveys, and the number of the firefly observed. The habitat conservation of *Aquatica ficta* (Olivier) was divided into two stages. The first stage was completed in November 2015, with an area of 375 m²; and the second stage, the expansion of the area to 2555 m², was completed in September 2016. With the observation from April to December in 2016, the fireflies appeared in April, May, August, September and November. The distribution range of fireflies was mainly in grass ditches and grass strips, with a small part on the lawn, showing their geographical distribution was extremely uneven. According to the surveys, the average water depth was 17.03±3.72 cm, and the corresponding dissolved oxygen (DO) to the appearance of the firefly was 5.17±1.48 mg/L. The temperature of microclimatic factors was 25.93±2.40°C, humidity 86.85±9.77%, illumination 2.14±0.64 lux, wind speed 0.27±0.51 m/s. The results show that the use of natural water, shallow water, low light and low wind speed in the environment are the necessary constructions for the firefly habitat conservation in the city.

Key words: *Aquatica ficta* (Olivier), habitat, conservation, park

攜手打造螢火蟲社區－九年成果分享
Sharing of the 9-year education work in building a firefly community

Mark Mak

Firefly Conservation Foundation

香港是一個人口超過 700 萬的大都會，由於社會的急速發展，在地少人多的情況下，香港每天都面對著不同的破壞、污染等問題，亦有很多生態環境出現危機。螢火蟲對環境變化高度敏感，故首當其衝成為城市發展的犧牲品。為喚醒了香港市民、政府對保育螢火蟲及保護環境的重視，本會在 2008 年起落實多項的公眾教育項目，深入各個階層領域推動螢火蟲的保育，包括：螢火蟲棲地統計、品種調查研究、大型戶外展覽、學界巡迴講座、生態教育項目、復修環境工程等，另在大嶼山設立了香港首個螢火蟲館。九年過去，各項計劃成果出眾及豐碩，本會藉著今次國際螢火蟲研討會，分享多年來推動螢火蟲保育之進程與心得經驗，與研討會上諸位互惠學習。

Hong Kong is a metropolitan city with over 7 million population. With the rapid development and overpopulated situation, the environment in Hong Kong is facing various adverse impact on habitat destruction, pollution etc. Firefly is highly sensitive to the environment, thus becoming the direct victim of the city development. To arouse the public awareness of Hong Kong citizens and the government on firefly conservation, The Firefly Conservation Foundation started a number of public education programmes since 2008. The programmes include firefly habitat and species survey, outdoor exhibition, school talks, education projects, habitat restoration projects etc. The Foundation also establishes the Hong Kong first Firefly Museum on Lantau Island. Over the past 9 years, the results from these projects are fruitful. In this International Firefly Symposium, we would like to share our experience and exchange ideas in promoting firefly conservation.

Key word: Firefly, community conservation, Hong Kong

How to design and maintain an aquatic firefly eco-pond in conservation biology, ecology and engineering principles

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The guidelines of designing and maintaining a firefly-suitable eco-pond includes the principles of conservation biology, ecology and realistic considerations of engineering process. In eco-pond design stage, the main guidelines are the classic protection areas design principles and ecology factors. The shape, size, corridor of eco-pond, original water area type and water quality, invasive alien species, original fauna and flora, these ecology parameters are all factors for designing a firefly eco-pond. Combining the construction of waterproof layer and the choice of source water supply, a small-scale eco-pond is the best solution, and the link of small-eco-pond will make a large-scale, firefly-suitable eco-pool. In the beginning of eco-pond construction work, the important things are durability and built-in reliability of waterproof layer, and in end of eco-pond construction, planting high-density aquatic flora is the best way to avoid water eutrophication. After eco-pond construction finishing, the shadow and spaces of trees and emergent vegetation are the micro-habitat for adult firefly. For declining light pollution, the streetlamp must be closed or using special streetlamp. A regular biota census of firefly eco-pond will need for monitoring invasive alien species and the population number of firefly. A maintain calendar is also needed, the regular maintain work includes tree pruning, trimming of emergent and submerged vegetation, remove any animals that will make the damage of waterproof layer, such as crab and shrimp, or lobster. An extra mud also need to remove to keep the status of wetland. The aquatic firefly is the flagship species for wetland conservation, and the releasing activity of firefly larvae is a good environment education to increase the emotion of human-firefly relationship.

Key words: *Aquatica ficta*, Aquatic firefly, Firefly eco-pond

The Key Role of the Fireflies Restoration in Taipei City – The Government Department

Liyuan Huang

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“Eco-city” is one of the main objectives of the municipal administration in Taipei City. Restoration of the native biota is an urgent task now, among them, yellow-rimmed firefly, *Aquatica ficta* (Olivier), is the indicator in good water quality, thus The Parks and Street Lights Office (PSLO) cooperates with firefly experts, non-government organization (NGO) and industry members by selecting Daan Forest Park, Rongxing Park and Mucha Park as firefly restoration site. Firefly experts suggest that the firefly restoration goals of these three parks are as follows: habitat quality improving in Rongxing Park, habitat expanding in Mucha Park and habitat restoration in Daan Forest Park. During the restoration of Rongxing Park, the Government established bid project to seek experts and ecological investigator team for the basic ecological data in the assessment of the eco firefly pond. PSLO also convenes the information meeting for local community to earn the public support. For the design of firefly eco-pond, the review conference convening by PSLO is the best way for communication between the firefly experts and the consultant company to ensure the firefly eco pond fulfill the ecology and conservation principles. During construction stage, supervision, inspection and acceptance mechanism of PSLO ensures the construction quality of the firefly eco pond. And after construction, PSLO establish a platform for the communication of firefly experts, the maintenance unit and the local community in maintaining the firefly habitat quality through the effort done by the local community. In Mucha Park, PSLO cooperates with NGO and firefly experts to expand the firefly habitat as the model in Rongxing Park, seek good contractor to remove the hard pavement, establish the eco pond, restore wetland appearance, and create a firefly habitat. The firefly restoration in Daan Forest Park, is the tripartite cooperation of PSLO, firefly experts and industry in overcoming the light pollution for the firefly by using special LED street lights, through the professional planning and surveying, Daan Forest Park become a successful example of firefly restoration in this high-density urban area. By this successful experience, PSLO could share and promote the firefly restoration or conservation experience to other parks and let Taipei City to become a renowned “Eco-city”.

Key words: firefly, *Aquatica ficta*, Eco-city, habitat restoration, Parks and Street Lights Office

Designing a Learner / Visitor Oriented Firefly Exhibition: Mobile Taipei Zoo in Kinmen

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Zoos, as free-choice learning institutes, play an important role in promoting conservational knowledge/ attitudes and environmental literacy. However, in some remote area, zoos and other free-choice learning institutes are difficult to be accessed. In this project, we will design a learner-oriented exhibit in Kinmen with the idea of mobile zoo in which converting an exhibit of firefly in Taipei Zoo into another display and an outreach educational program for junior high school students. Meanwhile, we will apply a more visitor/ learner oriented methodology instead of the traditional expert/ scientist/ educator oriented way in designing these educational materials. In the early stages of the project, we conducted a front-end evaluation in Kinmen to explore general public's conservational awareness, attitude and behavior, as well as their understanding of and interest in firefly conservational. This front-end evaluation helps us to understand what public have known, want to know and preferred ways to be communicated in such conservational programs. We then design and modify the exhibits, invite public to visit and conduct formative evaluation, which helps us to understand public's learning about the subject in order to modify the exhibit to facilitate visitors' learning. After the exhibit is formally on, a summative evaluation will be conducted in order to assess visitors' learning outcomes and see whether our educational objectives achieved. The idea of learner-centered exhibit design and all three evaluations could hopefully be considered to apply in such free-choice learning program in the future.

Key words: Learner-oriented exhibit design, front-end evaluation, formative evaluation, summative evaluation, mobile zoo

Taipei Zoo Breeding Plan of Fireflies in Kinmen

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Taipei Zoo cooperated with the Chinese Nature Resources Conservation Association in a breeding in captivity plan of two firefly species of Kinmen in 2016. We collected 100 larvae of *Pyrocoelia formosana*, 37 adults and 59 larvae of *Sclerotia flavida* from Kinmen and transferred to Taipei Zoo for the breeding plan. We have set up a firefly breeding facility, established *standard operating procedures* for breeding in captivity and suitable habitat assessment for future re-introduction in Kinmen Botanic Garden. The offspring of the two species will be released to their suitable habitats in Kinmen this year.

Key words: firefly, captive breeding, Kinmen

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Nature Restoration for the Genji Firefly, *Luciola cruciata* at Tama Zoological Park

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Tama Zoological Park has been breeding Genji fireflies since 1962, and we had held the events to observe fireflies at the insectarium and the artificial waterway. In 2009, as a part of nature restoration, the streams running through the lush natural environment of our zoo were improved. The reason was to translocate and establish Genji fireflies (mitochondrial DNA analyzed) that have been inhabiting the neighboring areas. Adult emergence was confirmed from 2012, and since June of 2014 we have held observational exhibitions. These activities and observation events are part of our ongoing efforts to improve our environment for the lives of fireflies.

Key words: firefly, nature restoration

A KEY TO DISTINGUISH THE SPANISH GLOW-WORM FIREFLIES BASED ON MACROSCOPIC VISUAL TRAITS

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Most of the ca. 2000 described lamproid species are distributed in tropical regions. In regions with more temperate climates however, we deal with much smaller numbers of species. Especially in Europe where the species numbers are quite low, probably due to difficulties in a prehistoric past for species to migrate along a North-South axis to follow changing climate during the last Glaciations and the Mediterranean Sea acting as a barrier. Although, we deal with much smaller species numbers, often we still face a lot of difficulties in discerning the correct taxonomic status of specimens, especially in citizen science surveys because of a lack of updated, recent, detailed and suitable references, guides and keys. Moreover, in many occasions the scientific references to support the identification are hard to find and the species specific characteristics taken into account in species descriptions are usually based solely on male specimens and often not very useful for the local firefly (citizen) scientist.

In very recent years, this situation has fortunately changed in some countries thanks to the increasing interest in firefly study and (citizen science) research. In some Mediterranean countries, revision of Portuguese, Italian and France *lamproid* fauna with accompanied keys have been developed recently. In the case of Spain, the citizen science project “¿Has visto una luciérnaga?” (www.gusanosdeluz.com) has enabled us to prepare a photo-base taxonomic key to support the identification of males, females and even larvae of the most common species of Spanish fireflies.

Key words: lamproid species identification, citizen science, glow-worm survey, Spain

A MORE DETAILED STUDY ON THE DISTRIBUTION OF SPANISH GLOW-WORM FIREFLIES

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In past Firefly Symposia we have presented the combined results from the Spanish web-survey “Have you seen a glow-worm?” (<http://gusanosdeluz.com>) and from the photo-biodiversity database “BiodiversidadVirtual” (<http://biodiversidadvirtual.org>). These data have enabled us now to update the Spanish distribution of lampyrid species at a much more detailed provincial (subregional) scale. Species recorded in Spain since 2009 are: *Lampyris iberica* and *L. noctiluca*, *Lamprohiza mulsantii* and *L. paulinoi*, *Phosphaenus hemipterus*, *Phosphaenopterus metzneri*, *Nyctophila heydeni* and *N. reichii*.

In this poster we show more detailed distribution maps of the Spanish species at a county scale. This approach emphasises some features of interest as the isolated presence of *Lampyris iberica* in some humid and temperate areas in the South, far from the standard distribution area of this species, or the apparently separated distribution of the two *Lamprohiza* species, *L. mulsantii* and *L. paulinoi*. Nevertheless, these new, more accurate species distribution maps introduce some new challenging questions about the ecology of these insects.

Key words: citizen science project, survey, species distribution, Spain

**PRESENCE OF EVERISIBLE ORGANS IN
TWO NYCTOPHILA SPECIES FROM SPAIN:
N. REICHII AND *N. HEYDENI***

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Fireflies are well known to present a range of defensive mechanisms to avoid predation. Among them, some species possess the ability to reflex bleed when disturbed by emitting droplets of hemolymph, usually from the elytra or the pronotum. The knowledge about defensive eversible organs in larvae has been expanded by Tyler (2001), who described them in *Lampyris noctiluca* Linnaeus 1767 and subsequently by Trice, Tyler and Day (2004), who compared the larvae of three genera of fireflies (*L. noctiluca*, *Luciola cruciata* Motschulsky 1854 and a *Nyctophila* species from Amol forest, in Iran) and by Fu *et al* (2009), who studied these organs in *Luciola cruciata*, *L. lateralis* Motschulsky 1860, *L. leii* Fu et Ballantyne 2006, *Lampyris noctiluca* Linnaeus 1767, *Pyrocoelia analis* Fabricius 1801, *Pyrocoelia pectoralis* Oliver 1883, *Pyrocoelia sp.* and two *Diaphanes* species. Thanks to the Spanish photo-biodiversity database “Biodiversidad Virtual” and the Spanish glow-worm survey “¿Has visto una luciérnaga?” (www.gusanosdeluz.com), we could record the presence of such defence mechanisms in two different species of the genus *Nyctophila* from Spain: *Nyctophila reichii* (Jacquelin du Val, 1859) and *N. heydeni* (Olivier, 1884).
<http://www.biodiversidadvirtual.org/insectarium/Nyctophila-reichii-img424576.html>
<http://www.biodiversidadvirtual.org/insectarium/Nyctophila-heydeni-img791656.html>

Key words: *Nyctophila reichii*, *Nyctophila heydeni*, defensive eversible organs, Spain

'Firefly' brand name survey

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The survey is to find out why the 'firefly' was chosen as their product and services brand name or for their organisation's name and how does it relates to their product or services and do they perceive that the 'firefly' brand name is more competitive than their competitors or their own similar products or services with different brand names. With the 'firefly' as a brand name whether they are aware of the firefly shrinking habitat and willingness to help in conserving the fireflies. A total of 140 companies of various products and services were randomly picked from the internet and were asked to do the survey in the SurveyMonkey website.

Key words: firefly, brand name, perception, competitiveness, survey, conservation

Developing a Data Pipeline (workflow) to Digitally Record and Analyze Firefly Flash Patterns

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Bioluminescent flash patterns are used by many firefly species as courtship signals. As these signals are also species specific, an accurate knowledge of their signal characteristics can inform studies of mate choice as well as species identification. Historically, stopwatches were used to quantify the various timing parameters of firefly flash patterns. Subsequently, video (VHS) was used to record and then analyze flash patterns through frame-by-frame analysis. In an attempt to record and analyze firefly flash patterns with increased precision beyond analog video technology, we have developed a data pipeline using a combination of digital video, Adobe Premiere, TiLIA (Time-Lapse Image Analysis) software, and Microsoft Excel to generate comprehensive visual representations of firefly flash patterns that quantify both flash duration and flash intensity; facilitating detailed analysis. Specific needs and their associated technological constraints, such as data storage, file conversion, data organization, and video encoding were also explored.

Key words: firefly, digital, video, flash pattern

**LIGHT IN THE DARKNESS I:
SOME INSIGHTS IN LAMPYRIDAE FROM THE BALKANS FROM
THE ZAGREB MUSEUM OF (CROATIA): SPECIES OF
THE GENUS *LUCIOLA* LAPORTE, 1833**

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To this day there is still some degree of taxonomy-related uncertainty prevailing within the genus *Luciola* Laporte, 1833 in Europe. Thanks to the extensive phenotypic plasticity, the numerous synonyms have been finally grouped into three currently recognized species. These are *Luciola italica* Linnaeus, 1767, *Luciola lusitanica* Charpentier, 1825 and enigmatic and long forgotten *Luciola novaki* G. Müller, 1946. *Luciola mingrelica* Motshulsky, 1854 represents a special case being recognised by Russian authors as a valid species, but currently synonymised with *L. lusitanica*. We have visited the Museum of Natural History in Zagreb to study the collections of *Luciola* species from Apennine and Balkan Peninsulas to provide a simple overview of local distributions of the abovementioned species and hopefully shine a bit of light on the problematics of *Luciola* species and their phenotypes from a morphological point of view. We have examined the collections of R. Mikšić, P. Novak, K.I. Igalffy, V. Redenšek and G.J. Koča to find six groups of morphologically different *Luciola* males. We based our comparisons on morphological features such as presence/absence of black dot on the pronotum, shape of the pronotum, presence/absence of lighter rim on the inner/outer edge of the elytra, colouration of head, thorax, legs, pronotum and scutellum and the shape of the lantern where possible. From what is now considered *L. italica*, we found two forms; 1) Italian specimens and 2) specimens from the outskirts of Zagreb (sometimes labelled as *L. lusitanica* var. *illyrica*). From what is now considered *L. lusitanica*, we found 3) *L. lusitanica* (Italy), 4) *L. lusitanica minuta* spp. (Italy) and 5) *L. mingrelica* (Balkans), which is quite different from the previous two. Last group is 6) *L. novaki*. In the work presented, we describe and compare the individual groups, present images as well as dates and places of their findings.

Key words: *Luciola italica*, *Luciola lusitanica*, *Luciola novaki*, *Luciola mingrelica*, morphology, taxonomy

**LIGHT IN THE DARKNESS II:
SOME INSIGHTS IN LAMPYRIDAE FROM THE BALKANS FROM
THE CROATIAN NATURAL HISTORY MUSEUM: SPECIES OF
THE GENERA *LAMPYRIS* GEOFFR., 1762, *PHOSPHAENUS* CAST.,
1833 AND *LAMPROHIZA* MOTSCH., 1858**

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Due to increased efforts of local researchers, enthusiasts and (online) citizen science survey projects, blogs and internet forums, we are getting a quite good image of the distribution of lampyrid species present in North, Central, South and Southwest Europe (Portugal, Spain, France, British isles and Ireland, Belgium, Netherlands, Germany, Switzerland, Austria, Italy, Scandinavia, Baltic states, Poland, North-West Russia, Czech republic, Slovakia). However, our knowledge of lampyrid biodiversity and distribution in South-eastern Europe (Slovenia, Croatia, Bosnia & Herzegovina, Serbia, Montenegro, Albania, Rep. of Macedonia, Hungary, Romania, Moldavia, Ukraine, Bulgaria, Greece, European part Turkey, Southwest Russia and Caucasus) remains quite vague and chaotic. This is probably due to a lack of local specialists and the general lagging behind what concerns citizen science or perception, awareness and enjoyment of nature by the general public, or the disclosure thereof. Yet, given its complex geographical composition and situation touching the Asian continent, especially the Balkans with its many mountain chains and Greek and Croatian islands, this region might actually represent a hotspot for lampyrid biodiversity. For instance 13 endemic species and subspecies have been reported for Greece alone. But details and recent confirmation are still lacking. We visited the Croatian Natural History Museum in order to examine the lampyrid collection of eminent entomologist René Mikšić which was not disclosed since decades. In addition we revised the collections of I. & P. Novak, Weingärtner and Perović. The studied collections cover the ex-Yugoslavian countries; Croatia, Slovenia, Bosnia, Montenegro and Serbia. Here we present some insights on the distribution of species of the genera *Lampyrus* Geoffr., 1762, *Phosphaenus* Cast., 1833 and *Lamprohiza* Motsch., 1858 and some detailed notes on the characteristics and distinction of males and females of less well known species *Lampyrus zenkeri* Germar, 1817 and, *Lampyrus germariensis* Jacquelin du Val, 1860 (syn. *lusitanica*, *soror*).

Key words: *Lampyrus*, *Lamprohiza*, *Phosphaenus*, ex-Yugoslavia, biodiversity, distribution

**LIGHT IN THE DARKNESS III:
SOME INSIGHTS IN LAMPYRIDAE FROM THE BALKANS FROM
THE CROATIAN NATURAL HISTORY MUSEUM:
GENETIC RESULTS AND NOTES ON THE MONTENEGRIAN
ENDEMIC *LUCIOLA NOVAKI* MÜLLER, 1946**

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Among the three currently recognized species of European *Luciola*, the coastal Montenegro endemic *Luciola novaki* Müller, 1946 has been neglected for decades. No work describing its morphology, or behaviour exists after its first description in 1946 and the last reference comes from 1967. The last specimens to be collected were from the 1950's and consequently the species was thought to be extinct. We have found *Luciola novaki* in collections of the Croatian Natural History Museum in Zagreb. It differs from *L. italica* and *L. lusitanica* by its black thorax, abdomen and legs. The pronotum is longer, flatter, and its shape is different compared to the other *Luciola* species of Europe. The dorsal surface of the body is matt, which is especially prominent on the pronotum. Shape of the lantern is different in Balkan specimens to that of currently named *L. italica* and *L. lusitanica*. We found a single larval specimen collected in 1936 and assigned to *L. novaki* in the collection. The larva clearly belongs to the *Luciola* genus, but its colouration seems to be unique, having a pair of lateral dots on each segment of the body as seen in typical *Lampyrus* sp. Nonetheless, a fresh specimen would be needed to avoid dubious conclusions, especially considering pigmentation. Finally, we can report good news considering the possible extinction of this species. Thanks to entomology enthusiasts, we have discovered specimens of *L. novaki* males in a collection from 2013. Consequently, further studies of this beautiful firefly and its other life forms should be feasible in the near future. In our work, we present diagnostics added to existing description of *L. novaki*, discrimination from other European *Luciola* as well as finding dates and places together with image documentation. Genetic research is currently on going and we present preliminary findings during the IFS-2017.

Key words: *Luciola novaki*, Montenegro endemic, firefly, morphology, distribution, DNA barcoding

**CITIZEN SCIENCE AND FIREFLIES IN FLANDERS,
BELGIUM. RESULTS FROM WAARNEMINGEN. BE AN ONLINE
SURVEY OF THE NATURE ORGANISATION NATUURPUNT**

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Thanks to efforts of local researchers, enthusiasts and citizen science survey projects, we are getting a quite good image of the distribution of lampyrid species in Flanders, Belgium. First attempts for a lampyrid survey started from 1997 on. Since then various random calls were posted by radio, newspapers and the internet, in order to ask the general public to send in sightings of lampyrids (1997-2016). Since 2014 the first author approached Natuurpunt with the request to found the “Glow-worm workgroup” (in Dutch: Glimwormenwerkgroep) in order to have a much wider platform and professional back-up team in order to assist in spreading calls for firefly surveys, divulge species information and especially to store, manage and analyse the gathered data by means of Natuurpunt’s survey website and database www.waarnemingen.be. Natuurpunt is a Flemish independent volunteer organization with over 100,000 members that protects and manages vulnerable and endangered nature in Flanders. Through more than 120 study workgroups and the citizen science survey website waarnemingen.be (since 2005), Natuurpunt keeps an eye on the health of the Flemish countryside and provides the scientific basis for the government’s nature management. The glow-worm workgroup is one such a region-wide workgroup. Here we present results on the distribution of glow-worm firefly species present in our country between 1945-2016: *Lampyris noctiluca* (3.932 observations; 15.125 individuals), *Lamprohiza splendidula* (436 observations; 15,319 individuals) and *Phosphaenus hemipterus* (155 observations; 676 individuals). The survey showed that *L. splendidula* is much rarer and vulnerable than previously thought and it might candidate as a Red Data Book species. We show and discuss commented distribution maps, the phenology of adults and larvae, information on (micro) habitat and detailed statistics on finding data (seasonal peak; earliest and latest sightings; numbers of sightings per year).

Key words: *Lampyris noctiluca*, *Lamprohiza splendidula*, *Phosphaenus hemipterus*, Flanders, citizen science, survey

FIREFLIES' NIGHTS - A PROGRAM OF ENVIRONMENTAL EDUCATION

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For more than 20 years, the Biological Park of Gaia, in Portugal, has reserved the month of June to open its doors at night in order for the public to participate in the “Noites dos Pirlampos” (Fireflies’ nights). For about 18 nights the Park is host to more than 200 visitors who travel in groups led by a Guide without using artificial lighting to enjoy the observation of the light of hundreds of Fireflies either resting or in flight. The popularity of this activity and phenomenon means that places - about 3,600 a year - are rapidly fully-booked and the Firefly Evenings become a particular means of making people of the region appreciate the need for Conservation of the Biodiversity and the problems of pollution. To address this point, the visits are preceded with a short briefing and end with Astronomical Observations and to see the “Fireflies in the sky” where the visitor can see the effects of light pollution that is also a problem to Astronomers. Within the Park six species of Firefly have been observed: *Luciola lusitanica*, *Lamprohiza paulinoi*, *Lamprohiza mulsantii*, *Lampyrus noctiluca*, *Lampyrus iberica*, and *Phosphaenus hemipterus*. The Park is dominated by an Oak forest, Alders and Willow trees and is crossed by the river Febros. Owned by the Municipality of Vila Nova de Gaia, the main objective of the Biological Park of Gaia is Environmental Education. Parque Biológico de Gaia (Biological Park of Gaia) is the oldest permanent Environment Education Centre in Portugal and also one of the oldest in Europe.

Key words: firefly, glow-worm, environmental education, biological park

Monitoring Death of an Aquatic Firefly Larvae, *Aquatica ficta* Caused by Decreasing Soil Water Content

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The global climate change makes extreme drought events frequently, and it directly affect on the surviving of an aquatic firefly larvae, *Aquatica ficta*. In the drought environment, the mature larvae would crawl for finding moist soil to respiration. The middle-instar larvae (n=10; body length=11.52±0.02 mm) and mature larvae (n=10; body length=11.52±0.02 mm) were kept individually in the plastic container under laboratory conditions of soil water content which decreased from 60%. Their death had been observed and monitored respectively for modified drought environment. Results showed that mean lethal water content (LWC) of middle-instar larvae treatment was the 2.72±1.01% (max. 4%, and min. 1.6%) and the mature larvae treatment was 2.48±0.8% (max. 3.2%, and min. 0.8%). Median lethal dose was 2.4% for each treatment with no significant difference by *t*-test ($p < 0.05$).

Key words: aquatic firefly, *Aquatica ficta*, drought, lethal water content, median lethal dose

Composition and Endogenous Biosynthesis of the Chemical Defense of the Firefly *Pyractomena borealis*

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Chemical investigations of several genera of North American fireflies have shown the presence of steroidal pyrones known as lucibufagins (LBGs). These compounds have been shown to have potent anti-predator activity. Lucibufagins are a subclass of bufadienolides, toxins that affect sodium-potassium ATPase, that includes molecules known from toads, a snake, and at least six families of plants. Due to the wide variety of taxa that contain bufadienolides and the compounds' medically-relevant bioactivity, there is great interest in their biosynthesis. Here we describe the presence of LBGs from *Pyractomena borealis* and show evidence of endogenous biosynthesis of these compounds. Analysis of NMR spectra of extracts of *P. borealis* adults indicated the presence of several LBGs, including one previously known from *Lucidota atra*. HPLC-MS studies using doubly ¹³C-labeled cholesterol were performed and indicated that *P. borealis* can produce LBGs using cholesterol to provide the steroid ring-system.

Key words: *Pyractomena*, HPLC-MS, lucibufagin, biosynthesis, chemical defense

Sequencing and de novo assembly of the *Photinus pyralis* genome

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Photinus pyralis is one of the most widely distributed firefly species in North America, and was the species used for foundational studies on the biochemistry of firefly bioluminescence by McElroy and colleagues. To provide a high-quality reference genome for studying the molecular basis of firefly traits, we sequenced DNA from *P. pyralis* individuals collected from the eastern United States using short-read Illumina and long-read Pacific Biosciences sequencing platforms. We also sequenced RNA across diverse tissues to provide a complete transcriptome for *P. pyralis*. This sequencing enabled the *de novo* assembly of the *P. pyralis* mitochondrial genome, nuclear genome, and tissue-specific transcriptomes, including that of the photophore tissue. Preliminary analyses reveal that the adult luciferase gene is clustered with non-luciferase acyl-CoA ligases, whereas the luciferase paralog and other closely related luciferase homologs are not within this cluster. This result suggests the luciferase loci arose by tandem gene duplication from an adjacent gene within the cluster, whereas the luciferase paralog descended from luciferase by duplication to a distant site. Analysis of the *P. pyralis* genome is ongoing and the finalized genome and transcriptome datasets will be a useful community resource for the study of firefly traits, conservation, and evolution.

Key words: *Photinus pyralis*, Lampyridae, de novo genomics, de novo transcriptomics

Biology of Winter Firefly, *Diaphanes niveus* in Taiwan

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Diaphanes is a highly diverse genus of Lampyridae with unique biological characteristics. Both sexes can emit green light and females are flightless. A few species of *Diaphanes*, which occur from November to March and are active between 5-15°C at various altitudes from 800-3,000 m, are called winter fireflies. Five winter species of *Diaphanes* in Taiwan have been recorded including: *D. niveus*, *D. cheni*, *D. lampyridies*, *D. flavilateralis*, and *D. nubilus*. Winter fireflies, more specifically adult females, are seldom investigated because of remote habitats and harsh phenological conditions. Recently however, the life cycles of *D. lampyridies*, and *D. nubilus* have been studied successively. This study further surveyed the life cycle of *D. niveus*, and compared results with other winter fireflies. Moreover, larvae diet, larvae morphology and adult luminous behavior were analyzed. In recent years, winter firefly watching eco-tourism has prospered. The establishment of basic research can facilitate the making of effective habitat management and conservation strategies.

Key words: Taiwan, winter fireflies, *Diaphanes niveus*, life cycle, biology

Fireflies as Herbalists: Does Access to Phytosterols Influence the Lucibufagin Content of *Photuris*?

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Much remains to be discovered about the ecology of firefly chemical defenses. While female *Photuris* are known to obtain defensive lucibufagins (LBGs) from prey through aggressive mimicry, the source of LBGs detected in field-collected males is unclear. Recent documentation of visitation to milkweed (*Asclepias*) by fireflies, including *Photuris*, has suggested that these sterol-containing plants could potentially influence firefly LBG content. We here report our preliminary tests of this hypothesis. Field-collected, adult male *Photuris* were randomly assigned to either milkweed (*Asclepias syriaca*, with cardenolide phytosterols as its main anti-herbivore defenses) or catmint (*Nepeta racemosa*, with terpenoid-based, rather than phytosterol-based, defenses). In one experiment, the fireflies received stems bearing both flowers and foliage, while in a second experiment they received stems with just foliage. Chemical analysis (NMR, HPLC-MS) of adult bodies from the flower/foliage experiment, found considerably higher LBG levels in *Photuris* with access to the milkweed compared to those given catmint. However, in the experiment presenting foliage alone, no difference in LBG content was found for beetles between the two plant species, suggesting that a nectar-based precursor from milkweed may be involved. Comparison of the structures of firefly LBGs and milkweed cardenolides indicates difficulty in directly deriving the LBG steroid core from that of a milkweed cardenolide. Thus, the *Photuris* may usurp an intermediary involved in milkweed cardenolide biosynthesis to serve as a precursor in constructing their own LBGs. Experiments providing adult *Photuris* with artificial nectars laced with various phytosterols are underway, as are experiments presenting larvae with roots from several plant species that are defended with cardenolides.

Key words: *Photuris*, *Asclepias*, lucibufagin, phytosterol, cardenolide, chemical defense

**Genetic Diversity of the Japanese Firefly,
*Lucidina biplagiata***

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Lucidina biplagiata (“Oba-botaru” in Japanese) is a diurnal firefly commonly distributed in Japan and Kuril Islands. Formerly this species was considered to be distributed also in Korea, but the Korean population was currently established as *Lucidina kotbandia*. We are presently carrying out the DNA barcoding project of world luminous organisms (LUMICODE) including fireflies. During the course of this project, we found that the genetic diversity of the Japanese *L. biplagiata* is relatively high compared to the other firefly species. We will discuss the population and genetic status of the Japanese *L. biplagiata* by comparing with the closely related species distributing in Asia.

Key words: *Lucidina biplagiata*, DNA barcoding, genetic diversity

FIREFLY DIVERSITY IN THAILAND: A SURVEY FROM 2012-2016

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Thailand has a tropical climate with average temperatures ranging from 22-36°C. It's comprised of differing terrain types, from open grasslands, rolling hills, high mountain ranges, rivers, mangroves to ocean fronts. This unique terrain and climate has resulted in rich flora and fauna including fireflies to flourish. Firefly research in Thailand has been conducted for many decades, however only a limited number of firefly species have been reported to date. In this paper, we report on a four-year study carried out across Thailand in which we observed over 40 unique species across differing firefly habitats, including wet lands, mangroves, lakes, farm lands, paddy fields and forests. Among the species observed, some are very common in urbanized areas, e.g., *Sclerotia aquatilis* and *Asymmetricata circumdata*, some are restricted to forests at high altitudes, e.g., *Pygoluciola spp.*, some prefer to dwell in mangroves, i.e., *Pteroptyx spp.* Of these 40 species, at least seven species haven't been described elsewhere. Most of these new species shares similar appearances, i.e. a small body size, orange pronotum, black elytra and trisinate ventrite 7. These characteristics are very similar to the widely distributed species *Inflata indica* Ballantyne, but can be distinguished by the shape of tergite 8 and genitalia. In addition, mitochondrial *COI* barcode was used to facilitate species identification and was proven to be very effective. These new species is proposed to be included in *Medeopteryx* Ballantyne, *Trisinuata* Ballantyne and *Australoluciola* Ballantyne.

Key words: *Australoluciola*, Lampyridae, *Medeopteryx*, new species, *Trisinuata*

**The First Study on Infectious Disease in
the Terrestrial Firefly, *Lamprigera tenebrosa* (Walker)**

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The Giant firefly, *Lamprigera tenebrosa* is a terrestrial firefly species that can be cultured under laboratory condition in Thailand. Besides their univoltine life cycle, infectious diseases result in high mortality rate in larval stage is a significant problem. To understand the disease symptom patterns, the pathology and behavior of fifteen infected larvae were morphologically observed in laboratory. From the observation, the infected larvae displayed no feeding behavior and abnormalities of leg joints. Additionally, swelling of soft tissue near head area, thorax and/or abdomen were observed, then the yellow sticky liquid inside was released from openings before dying in a few days. The duration of life cycle of disease was approximately 5-8 days.

Key words: disease, pathology, culture

Will orb web spiders use and manipulate firefly prey bioluminescence signal as a visual lure

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Many types of predators use visual signals to lure prey, such as spiders that use their own body color or that of other species as a visual cue to attract nocturnal prey. Some observations have shown that certain web-building spiders will catch male fireflies at night during firefly reproductive season. This suggests these spiders may use the bioluminescent signal from the firefly to lure prey into their webs. My proposed study will investigate whether web-building spiders use bioluminescent signals from captured fireflies to lure nocturnal prey, and whether the firefly's bioluminescent signal can be manipulated by the spider to enhance luring. This experiment will be separated into two parts: 1) spider eye-cover test, to determine if the spider can see the bioluminescence of the fireflies and manipulate the signal, 2) firefly control test, to examine how the visual signals from different sexes of the firefly respectively affect prey luring. Preliminary observations showed that fireflies can continue to release bioluminescent signals after being captured by web-building spiders for almost 50 minutes and frequently altered bioluminescent signals. I plan to continue performing field observations and conducting laboratory experiments to discern whether these spiders can use the visual signals of captured male fireflies to lure nocturnal prey into webs.

Key word: visual signal, orb web spider, firefly, bioluminescent signal, lure

Assessment of the Firefly Resources in Xitou Nature Education Area, Experimental Forest, NTU

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The Xitou Nature Education Area, a part of the Experimental Forest, National Taiwan University (NTU), is abundant in the firefly resources. To understand diversity and distribution of the firefly resources in this area, we conducted an investigation on the firefly populations within 6 sample plots for 4 successive years since 2011, including Conifer Arboretum (CA), Flux Tower Surroundings (FTS), Phoenix Logging Road (PLR), University Pond (UP), University Gulley Debris Park (UGD), and Shopping Street (SS). The results showed that the fireflies could appear all year-round in Xitou and that 35 firefly species within 12 genera comprised of the Lampyridae and Rhagophthalmidae (only one species) were recorded during the survey period. Among these, *Luciola*, *Drilaster* and *Diaphanes* were the most abundant genus which contained 5 species with a portion of 14%, respectively. Secondly, *Pyrocoelia* and *Curtos* both had 4 species with a portion of 11%, respectively. Then, *Abcondita* had 3 species with a portion of 6%. In addition, *Cyphonocerus* and *Lucidina* each had 2 species with a portion of 6%, respectively. Finally, another 5 genera with solitary species were *Aquatica*, *Stenocladus*, *Lamprigera*, *Vesta*, and *Rhagophthamus* (Rhagophthalmidae). Within the 6 sample plots, CA and PLR had the highest species richness hosting 24 firefly species, respectively, followed UGD with 16 species. Furthermore, SS, FTS, and UP had less species with 14, 8, and 6 ones in that order. In general, *Abcondita cerata* was dominant spring firefly species in Xitou, while *Diaphanes lamproides* was the primary species during the winter. Moreover, the horizontal distribution patterns of the investigated firefly species included central (C), northern and central (NC), southern and central (SC), and widely distributed (W), among which W and NC were the most common types. On the contrary, the vertical distribution patterns of the firefly could be classified as two types, submontane forest (SM, 500-1500 m) and Quercus upper zone of montane (MU, 2000-2500 m). This study clarifies the firefly species in Xitou Nature Education Area, and provided the basic information on firefly conservation and environment education.

Key words: Xitou, firefly, distribution pattern, conservation, environment education

Overview of Korean Firefly and Practical Use of Fireflies at Seoul Zoo

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The fireflies are winged beetles as Lampyridae family in Coleoptera order and they were commonly called as lightning bugs because of their conspicuous use of bioluminescence during twilight to attract mates or prey. However, their habitats and population sizes were quickly reduced with industry growth and urbanization in Korea. With their quick reduction of the species, their values as environmental indicator species were further increased in the water environment. There have been reported 8 species in 3 subfamily in Korea and recently, but we could find only four species of fireflies, *Hotaria papariensis*, *Hotaria unmunzana*, *Luciola lateralis*, *Pyrocoelia rufa*. In 1982, Korea government designate the fireflies and their habitats in Moojoo gun as a natural monument (No. 322). In those times, the firefly population sizes were abruptly reduced and many NGOs were organized to conserve the fireflies. One of NGOs, the Korean Society of Firefly was organized in October of 1998 and their survey activity has started and assigned the 6 firefly conservation sites since 2002. Seoul zoo has introduced an artificial growth system for mass proliferation of *Luciola lateralis* and has displayed the *Luciola cruciata* species in a large exhibition tank for a whole year since 1998. In addition, the Seoul zoo has designated the protection areas for the restoration of the *Luciola cruciata* habitat in the zoo, operated various *Luciola cruciata* conservation programs, and held a symposium with the some firefly experts to increase of the domestic *Luciola cruciata* awareness.

Key words: Conservation area, Firefly, Natural monument, The Korean Society of Firefly

Light Up the Zoo — How to arouse public awareness of environmental protection

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Taipei Zoo has been devoted to environmental education for over 20 years. Insects are excellent resource for environmental education due to its remarkable diversity and closely linkage with the environment. Thus, Taipei Zoo uses Insectarium as a stronghold of environmental education. The Insectarium is situated at the mouth of a valley, teeming with insects. Behind the Insectarium lies a ten hectares Insect Valley which can see fireflies on April to May annually. This makes the Insect Valley one of the best places in the zoo for educational tours and for visitors to feel immersed in nature. Fireflies are indicators of the health of the environment, and are declining across the world. The lightning of firefly produces mysterious impression to the public that promotes interest and enthusiasm of outdoor exploring. In order to arouse public's attention of environment, Taipei zoo has developed firefly camp for family during firefly season. At the camp, family get the chance to experience firefly and its habitation with their senses at the Insect Valley, hoping the participants generate environmental awareness through first-hand experience. The Insect Exploring Area at Insectarium displays themes that are inspired by diverse insects and changes display themes periodically. The theme of this year is firefly including the ecology, biodiversity and culture. Through the Insectarium, we are hoping visitors get a sense of environmental ethics and responsibility to reduce the occurrence of environmental protection violations in the future.

Key words: Environmental Education, Taipei Zoo, Firefly

Firefly habitat restoration, habitat quality improving and firefly surveying in Neishuangxi nature center

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Neishuangxi nature center located in mountain Dalunto, Taipei city, and the around environment is all secondary low land forests, *Ficus* tree is the most common plant, the attitude is 230 meter, and the climate parameters are 13.4°C in Jan., 29.8°C in August. The annual rainfall is 2,000 mm. Neishuangxi nature center has four area—teaching center, herb plant area, forest teaching area, geology teaching area, the landscape types are pool, glass land, nursery area and artificial building. The results of fireflies census are eight night-active fireflies, including *Abscondita cerata* (Olivier, 1911), *Abscondita anceyi* (Olivier, 1983), *Luciola kagiana* (Matsumura, 1928), *Curtos mundulus* (Olivier, 1913), *Diaphanes citrinus* (Olivier, 1911), *Pyrocoelia praetexta* (Olivier, 1911), *Lamprigera yunnana* (Fairmaire, 1897) and *Aquatica ficta* (Olivier, 1909). The most abundant fireflies are *A. cerata* in spring (Apr. to May) and *D. citrinus*, *P. praetexta* in autumn (Oct. to Nov.). “Forest classroom” is the area with most abundant fireflies’ populations, the landscape is fruit tree, forest and pool, and the management strange in the area is low-intensity management. For the purpose of firefly restoration, the habitat improving of eco-pool was constructed in autumn 2015, and the firefly larvae reintroduction was in autumn 2015 and 2016.

Key words: Neishuangxi nature center, *Abscondita cerata* (Olivier, 1911), *Diaphanes citrinus* (Olivier, 1911), *Pyrocoelia praetexta* (Olivier, 1911), *Aquatica ficta* (Olivier, 1909), Habitat restoration, habitat quality improving

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