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Morphological and Molecular identification of firefly (*Abscondita* sp.) from Nsukka Nigeria

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Abstract
The beetle family Lampyridae (fireflies) are fascinating group of insects known for their characteristic lighting patterns. The light of firefly is produced from their abdomen by a chemical reaction of the organic compound luciferase. There is lack of information on the species composition and biodiversity of fireflies in Africa despite the speculations that Africa is the richest continent in firefly species diversity. To date, there is no taxonomic key for morphological identification of fireflies of Africa. In addition, there is no reference DNA barcodes for African fireflies in GenBank to enhance species identification using DNA barcoding. These knowledge gaps hamper the study and harnessing the economic and ecological benefits of fireflies in the ecosystem in the continent. The objective of this study was to identify a species of firefly collected in Nsukka using both morphological and molecular methods to encourage and kickstart research on fireflies in Africa. The firefly was collected in the study area using direct capture with the aid of insect net. The species was identified using picture vouchers and keys from Canada and South East Asia. The firefly was identified as Abscondita anceyi using morphological method. Molecular method employed amplified and sequenced 658 bp of the barcode region of the COI gene of the firefly samples. DNA barcoding showed the firefly has a similarity of 89.06% with Abscondita chinensis. Molecular phylogeny based on the COI gene clustered the samples with Abscondita chinensis but in a different branch of the tree. The firefly thus belongs to the genus Abscondita but its specific name is yet to be determined. This is the first barcode of fireflies of the subfamily Luciolinae in Africa. We call for a follow up in documenting fireflies of Africa to harness their benefits in the environment.

Keywords: Barcoding, Firefly, Identification, Molecular, and Taxonomy

INTRODUCTION
Fireflies are species of high social interests as they captivate individuals from all works of life because of their aesthetic value (Takeda et al., 2006). They are exceptional species of insects as they influence many people's culture (Kobori & Primack 2003; Takeda *et al*., 2006).

Fireflies are a fascinating group of animals that most exhibit bioluminescence. They belong to the Order Coleoptera and the Family Lampyridae (Ghosh *et al*., 2021). Beetles in the family Lampyridae are very interesting species and the prominent feature of this family is the ability to produce light (Ineichen, 2016). Among animals, the Order Coleoptera has the most

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abundance and varied bioluminescence species (Ghosh *et al*., 2021). The family Lampyridae consists of the true fireflies and are cosmopolitan in distribution with the exception of New Zealand and parts of Australia (Jeng *et al*., 2003). The characteristics lightening play a role in sexual communication among the species (Harvey, 1940).

There are about 2000 - 2200 known species of fireflies with over 90 genera globally (Martin *et al*., 2019; Lewis *et al*., 2021) but many await discovery and formal description (Faust, 2004). The genus *Abscondita* belongs to the subfamily Luciolinae. Males and females in this subfamily are bioluminescence and consists of about 235 described species in 30 genera (Ballantyne *et al*., 2016; 2019). Although the genus *Abscondita* has been reported to be exclusively present in South East Asia being widely distributed in China, Loas, Thailand and India (Ghosh *et al*., 2021), this assertion might not be correct given the poor knowledge of the species composition and distribution of fireflies especially in Africa. As at April 6, 2023, there is no single barcode of firefly in the subfamily Luciolinae in Africa available in the Barcode of Life Data (BOLD). Environmental degradation and climate change are causing redistribution and loss of species and some species might go extinct before they are discovered.

The importance of fireflies to science and the ecosystem led to the Selengor Declaration 2010. The Selengor Declaration 2010 arose from the 2nd International Firefly Symposium and urged the world leaders to protect and conserve fireflies to effectively harness the ecological and economic benefits of the species (Wong & De Cock, 2014). However, to date, there is no publication on the firefly species composition and diversity in any African region. Knowledge of the species diversity of fireflies is crucial in the field of taxonomy, conservation, behavioural ecology, and use in biological control of other pests (Lloyd, 2003). The disappearance of this species in the ecosystem is indicative of abrupt changes in landscape use, light pollution and threat to biodiversity (Lewis, 2016).

Accurate identification of fireflies to species level are very difficult (Luk *et al*., 2011). This is because most taxonomic keys for fireflies are poorly described, incomplete and inaccessible (Luk *et al*., 2011). With the current concern on the increasing light pollution and habitat degradation on fireflies, it has been suggested to develop a user-friendly tool for identification of fireflies for accessing the faunal composition and effective conservation of the species (Luk *et al*., 2011). A few of the recent studies on fireflies in Africa include a description of new genus *Afrodiaphanes* Geisthardt (2007) and a description of a new species of *Afrodiaphanes pulcher* (Fanti & Pankowski, 2022).

There is paucity of knowledge on the firefly faunal composition and species diversity in Africa. In addition, there is no identification keys and DNA barcodes for indigenous species of African fireflies. These knowledge gaps hamper the study of fireflies in Africa. The objectives of this study are thus to apply DNA barcoding to identify a species of firefly collected from Nsukka Nigeria.

MATERIALS AND METHODS

The Study Area

The research was carried out in University of Nigeria, Nsukka in Nsukka Local Government Area (LGA), Enugu State, Nigeria (Fig. 1). Nsukka lies on latitude 6°56'N and longitude 7°20'E (The world Gazette, 2004) and on an elevation of 419 m above sea level (Agwu *et al*., 2004). Nsukka is bordered by Igbo-Eze South, Uzo- Uwani, Igbo-Etiti, Isi-Uzo and Udenu LGAs.

Nsukka climate is tropical with mean monthly temperature fluctuating between 24°C and 29°C (Inyang, 1978). The area has two distinct seasons in the year – the wet season which is April – October and the dry season which runs from November to March (Agwu & Osibe, 1992). The annual rainfall ranges from 986 to 2098 mm (Inyang, 1978). The natural day length for Nsukka is 12–13 hours and the average annual maximum and minimum temperatures are 29.7˚C and 21.0˚C respectively. The relative humidity ranges from 34 to 78% (Monanu, 1975). Nsukka Agroecological zone has an area of approximately 4618 km² (NPC, 2006). The vegetation consists chiefly of woodland with about 53% phanerophytes, hence called phanerophytic plant climate (Nwadinigwe & Onyekwelu, 2006).

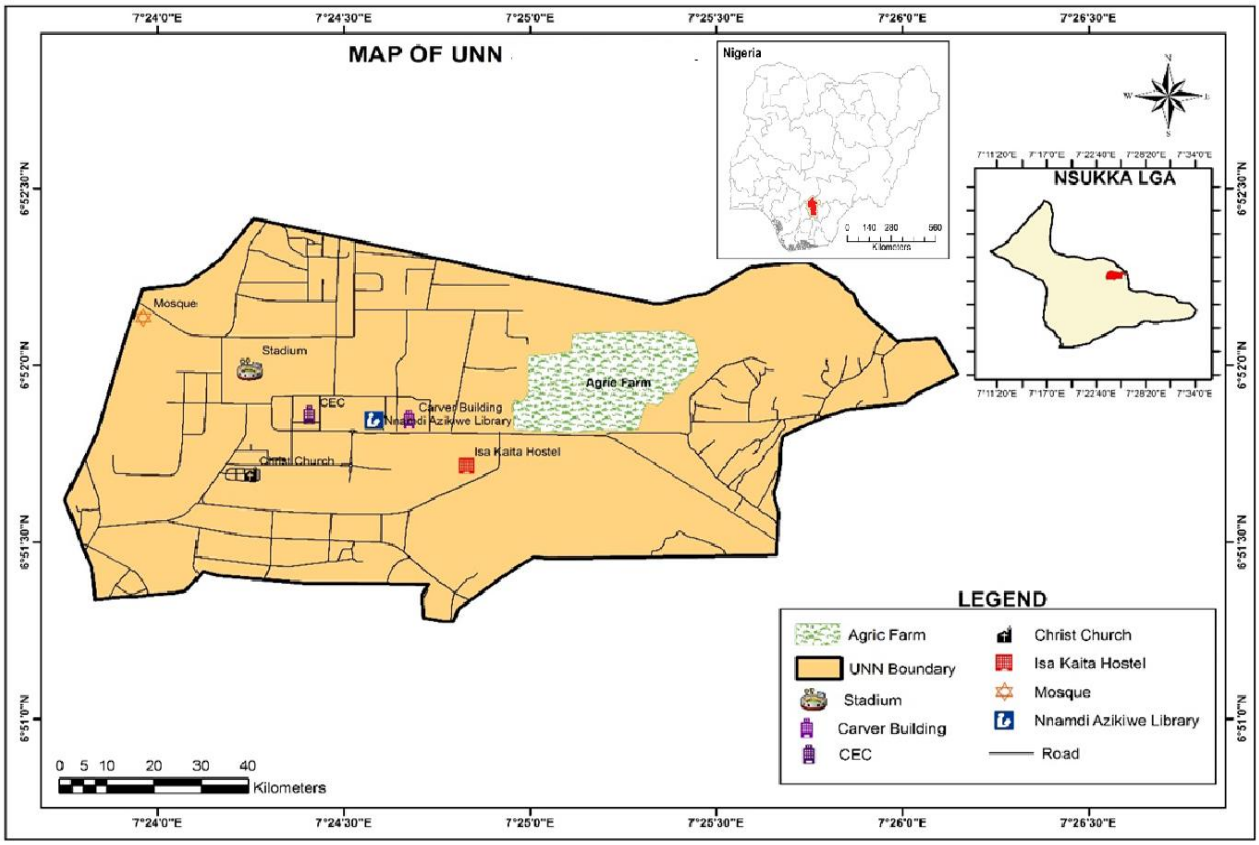

Fig. 1: Map showing University of Nigeria Nsukka

Collection of Firefly Samples

Firefly samples were collected at night within the University of Nigeria, Nsukka for a period of four weeks. The fireflies were collected using insect net. The collected samples were preserved in 70% ethanol for morphological identification while samples for DNA barcoding were preserved in absolute ethanol. Absolute ethanol is used to preserve sample for DNA analysis because DNA do not degrade in absolute ethanol while samples meant for morphological identification are preserved in 70% ethanol to make it flexible while examining the various morphological features.

Morphological Identification of the Firefly

The firefly was identified morphologically using vouchers and keys by Luk *et al*. (2011) and Ballantyne *et al*. (2013).

DNA Barcoding of the Firefly

Genomic DNA was extracted from 5 firefly samples using Zymo Research Quick-DNA™ Miniprep Plus Kit. The barcode region of the COI gene of the DNA extracts were amplified using the primers LCO1490 5′ GGTCAACAAATCATAAAGATATTGG 3′ and HCO2198 5′ TAAACTTCAGGGTGACCAAAAAATCA 3′ (Folmer *et al*., 1994). Each reaction was performed in a total volume of 25µl. The master mix was made up of 12.5µl Taq DNA Polymerase, 2x Master Mix Red-Ampliqon, 0.3µl of each primer and 0.5µl Magnesium chloride. Additionally, 0.8µl of 3.2% bovine serum albumin (BSA) was added to all PCR reactions, as well as 0.5µl of 4.5% dimethyl sulphoxide (DMSO). Finally, 1µl of DNA was added to the tube.

PCR amplification was performed in an Applied Biosystems Proflex™ PCR System (Thermo Fisher Scientific) using the following programmes: pre-melt at 94°C for 3 min, denaturation for 1 min at 94°C, annealing at 48°C for 1 min, extension at 72°C for 3 min (for 28 cycles), followed by a final extension at 72°C for 7 min. The PCR products were purified using ExoSAP-ITTM (GRiSP, Lda, Portugal) reagent protocol (1.8 µl H2O, 0.20 µl Exo1, 0.40 µl FastAP) – incubation at 37˚C for 30 min followed by 80˚C for 15 min. Cycle sequencing reactions were carried out using BigDye TMv.3.2 Terminator Kit (Thermo Fisher Scientific, Massachusetts, USA). The purified PCR amplicon were sequenced in both forward and reverse directions using their respective forward and reverse primer. The cycle sequencing thermal programme consisted of 26 cycles for 10 seconds of denaturation at 96°C, 5 seconds annealing at 50°C and 4 minutes extension at 60°C. Cycle sequencing products were precipitated in ethanol and sodium acetate to remove excess dye terminators before capillary electrophoresis on an ABI3130xl genetic analyser. DNA barcoding was done in the African Centre for DNA Barcoding, Department of Botany, University of Johannesburg, South Africa.

Sequence Data Analysis

Raw sequence data was BLAST Search in GenBank, and the sequences with the highest percentage score and query cover were downloaded and used to align the sequences. The sample sequences were aligned with *Abscondita chinensis* (Accession Numbers: MT534196 and ON209457) in MEGA7 (Kumar *et al*., 2016). The aligned sequences were edited in Microsoft Word by removing the primer sequences at the extremes and crosschecking the Genetic Analyzer chromatograms where the sequences did not match and replacing the appropriate bases. The sequences were deposited in GenBank under the accession numbers (LC763769-LC763773).

Phylogenetic Analysis based on COI Gene

The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model (Nei & Kumar, 2000). The tree with the highest log likelihood (-2222.98) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying the Maximum Parsimony method. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 60.49% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 17 nucleotide sequences. Codon positions included were 1st+2nd+3rd. All positions containing gaps and missing data were eliminated. There were 658 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al*., 2016).

RESULTS

The species of firefly identified in the study is *Abscondita* sp. It has morphological similarities with *Lucolia anceyi* which is now *Abscondita anceyi*.

The primers successfully amplified and sequenced 658 bp of the barcode region in the firefly samples collected. The sequence similarity showed 89.06% with *Abscondita chinensis* in GenBank. There was no variation in the 658 bp sequences of the barcode region for the 5 samples sequenced in this study. Phylogenetic analysis based on COI gene clustered the samples with the genus *Abscondita* and shares the same branch with *Abscondita chinensis* (Fig. 2). The firefly belongs to the genus *Abscondita* but the specific name is not yet known. Both morphology and molecular data narrowed the firefly to the genus *Abscondita* however, morphological features identified the firefly as *Abscodnita anceyi* (*Locolia anceyi*) but molecular data showed that it is not *Abscondita anceyi* but a close relative of *Abscondita chinensis*.

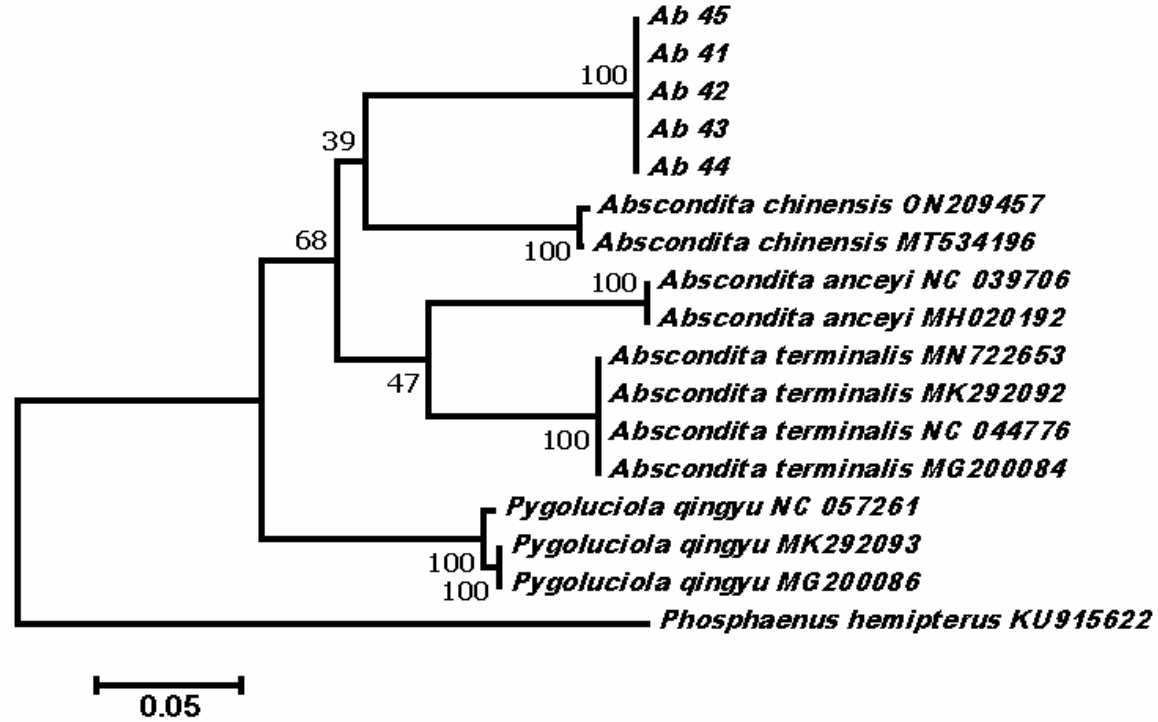

Fig. 1: Maximum Likelihood phylogenetic tree identification of *Abscondita* sp. based on COI gene.

DISCUSSION

In this study we employed morphological and molecular methods to identify a species of firefly collected in Nsukka. Although we could not identify it to the species level, we were able to identify it to the genus level and to establish that the genus *Abscondita* is present in Africa contrary to the assertion of Ghosh *et al*. (2021) that the genus *Abscondita* is exclusively present in South East Asia. The species recorded in Nigeria is in the genus *Abscondita* as shown by the DNA barcoding and the phylogenetic analysis based on the COI gene. This could mean that the species are also present in Nigeria or might be invasive in Nigeria. According to Fanti & Pankowski (2022) who described a new species of firefly in Central Africa Republic based on the morphological features, the greatest diversity of fireflies is in Africa but a large number of them are undescribed and the taxonomy of fireflies in Africa has not been a subject of serious study. In Nsukka, Nigeria we chose to identify *Abscondita* sp. because it is the one that caught our attention and we decided to establish its identity as a base for further studies not

that it is the only species of firefly in Nsukka. Identification of an existing species or determination of a new species of fireflies can be very difficult (Fu & Ballantyne, 2008). Integrative taxonomy remains the best approach in species identification (Jusoh *et al*., 2014). Morphological identification alone would have identified the species as *Lociola anceyi* = *Abscondita anceyi* however, DNA barcoding and molecular phylogeny based on COI gene clearly showed that the sample is not *Abscondita anceyi* although it belongs to the genus *Abscondita*. DNA barcoding is a powerful tool in species identification and has been employed in identification of fireflies in Southeast Asian countries (Jusoh *et al*., 2014; 2020; Zhu *et al*., 2022).

In Asia where there is extensive study of fireflies, they are still calling for an increase in the geographical and taxonomic sampling of fireflies to understand their biodiversity (Jusoh *et al*., 2020). This should be a motivation for African scientists to delve into firefly research. This study thus advocates for an intensified effort to document the firefly faunal composition, species diversity, and establish a comprehensive identification key for African species of fireflies. DNA barcodes for fireflies are lacking for many species and making identification using DNA barcoding very difficult (Riley *et al*., 2021). In Africa, DNA barcodes for fireflies are altogether lacking and there is urgent need to use DNA barcoding to document African species of fireflies before some of them go extinct and for effective identification, conservation and further scientific studies and harnessing the economic and ecological benefits of the species in the ecosystem.

CONCLUSION

There is paucity of knowledge on the firefly fauna of Africa as is evidenced by scarce journal articles on the species in the continent and the lack of DNA barcodes for African species in GenBank and Barcode of Life Data. Identification of fireflies based on morphological features alone is very difficult and a species identity could be missed if not complemented by molecular identification method. Thus, an integrative approach in the identification of the species is recommended.

Africa could be one of the richest continents in firefly's species diversity however, strenuous efforts should be made to document the species and have a taxonomic key for their identification. The DNA barcoding of a species of firefly in this study is the first of its kind in Africa and we advocate for further research to document the fireflies of Nigeria and Africa in general. In addition, the DNA barcodes for identified African species of fireflies should be generated and deposited as reference sequences in GenBank to enhance the use of DNA barcoding in identification of the species which will open up more research in ecology, conservation and other studies on the economic benefits of the species.

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REFERENCES

Agwu, C. O., & Osibe, C. E. (1992). Airborne polymorphs in Nsukka during the month of February to April 1990. *Nigerian Journal of Botany*, 5: 177-187.

Agwu, C. O., Njukuocha, R. C., & Mezue, O. (2004). Study of air borne pollen and store Circulating at head level. *Bio-Research*, 2(2): 7-14.

Ballantyne, L. A., Lambkin, C. L., Ho, J. Z., Jusoh, W.F.A., Nada, B., Nak-Eiam, S., Thancharoen, A., Wattanachaiyingcharoen, W., & Yiu, V. (2019). The Luciolinae of S. E. Asia and the Australopacific region: a revisionary checklist (Coleoptera: Lampyridae) including description of three new genera and 13 new species. *Zootaxa*, 4687(1): 1e174. https://doi.org/10.11646/zootaxa.4687.1.1.

Ballantyne, L. A., Lambkin, C. L., Luan, X., Boontop, Y., Nak-Eiam, S., Pimpasalee, S., Silalom, S., & Thancharoen, A. (2016). Further studies on south eastern Asian Luciolinae: 1. Sclerotia Ballantyne, a new genus of fireflies with back swimming larvae. 2. Triangulara Pimpasalee, a new genus from Thailand (Coleoptera: Lampyridae). *Zootaxa*, 4170(2): 201e249. https://doi.org/10.11646/zootaxa.4170.2.1.

Ballantyne, L., Fu, X., Lambkin, C., Jeng, M.-L., Faust, L., Wijekoon, W. M. C. D., Li, D., & Zhu, T. (2013). Studies on South-east Asian fireflies: *Abscondita*, a new genus with details of life history, flashing patterns and behaviour of *Abs. chinensis* (L.) and *Abs. terminalis* (Olivier) (Coleoptera: Lampyridae: Luciolinae). *Zootaxa*, 3721(1): 001-048. http://dx.doi.org/10.11646/zootaxa.3721.1.1

Fanti, F., & Pankowski, M.G. (2022). A new species of *Afrodiaphanes* Geisthardt, 2007 (Coleoptera, Lampyridae), the first firefly described from the Central African Republic. *Zootaxa*, 5200(2): 191-195. https://doi.org/10.11646/zootaxa.5200.2.6

Faust, L. F. (2004). Fireflies as a catalyst for science education. *Integrated and Comparative Biology*, 44: 264-265.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for the amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3(5): 294-299.

Fu, X. H., & Ballantyne, L. (2008). Taxonomy and behaviour of lucioline fireflies (Coleoptera: Lampyridae: Luciolinae) with redefinition and new species of *Pygoluciola* Wittmer from mainland China and review of *Luciola* LaPorte. *Zootaxa*, 1733: 1-44.

Geisthardt, M. (2007). *Afrodiaphanes* a new genus from Africa (Coleoptera: Lampyridae: Lampyrini). *Mitteilungen des Internationalen Entomologischen Vereins e.V. Frankfurt a. M.*, 32 (1–2): 45-50.

Ghosh, S., Sarkar, S. K., & Chakraborty, S. K. (2021). Two new records of the subfamily Luciolinae Lacordaire, 1857 (Coleoptera: Lampyridae) with a checklist of genus *Abscondita* from India. *Journal of Asia-Pacific Biodiversity*, 14: 53-59. https://doi.org/10.1016/j.japb.2020.10.004

Harvey, E. N. (1940). Living Light. New Jersey: Princeton University Press.

Ineichen, S. (2016). Light into darkness: The significance of gowworms and fireflies in Western Culture. *Advances in Zoology and Botany*, 4(4), 54-58. DOI: 10.13189/azb.2016.040402

Inyang, P.E.B. (1978) The climate of Nsukka and environs. In: Ofomata GEK (Ed). *The Nsukka environment.* Fourth Dimension Publishers, Nigeria, pp. 86-97.

Jeng, M. L., Yang, P. S., & Lai, J. (2003). Notes on the genus Luciola (Coleoptera, Lampyridae, Luciolinae) of Taiwan. *Special Bulletin of the Japanese Society of Coleopterology*, 6: 247-262.

Jusoh, W. F. A., Hashim, N. R., Sääksjärvi, I. E., Adam, N.A., & Wahlberg, N. (2014). Species Delineation of Malaysian Mangrove Fireflies (Coleoptera: Lampyridae) Using DNA Barcodes. *The Coleopterists Bulletin*, 68(4): 703-711.

Jusoh, W. F. A., Ballantyne, L., & Onn Chan, K. (2020). DNA-based species delimitation reveals cryptic and incipient species in synchronous flashing fireflies (Coleoptera: Lampyridae) of Southeast Asia. *Biological Journal of the Linnean Society*, **130**: 520–532.

Kobori, H., & Primack, R. B. (2003). Participatory conservation approaches for Satoyama, the traditional forest and agricultural landscape of Japan. *Ambio*, 32: 307-311.

Kumar, S., Stecher, G., & Tamura, K. (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33:1870-1874.

Lewis, S. (2016). *Silent Sparks. The Wondrous World of Fireflies*. Princeton University Press, Princeton and Oxford.

Lewis, S. M., Thancharoen, A., Wong, C. H., López-Palafox, T., Santos, P. V., Wu, C., Faust, L., De Cock, R., Owens, A. C. S., Lemelin, R. H., Gurung, H., Jusoh, W. F. A., Trujillo, D., Yiu, V., López, P. J., Jaikla, S., Reed, J. M. (2021). Firefly tourism: Advancing a global phenomenon toward a brighter future. *Conservation Science and Practice,* 3: e391. https://doi.org/10.1111/csp2.391

Lloyd, J. E. (2003). On research and entomological education vi: firefly species and lists, old and now. *Florida Entomologist*, 86(2): 99-113.

Luk, S.P.L., Marshall, S.A., & Branham, M.A. (2011). The fireflies (Coleptera; Lampyridae) of Ontario. *Canadian Journal of Arthropod Identification*, 16. doi: 10.3752/cjai.2011.16

Martin, G. J., Stanger-Hall, K. F., Branham, M. A., da Silveira, L. F. L., Lower, S. E., Hall, D. W., Li, X.-Y., Lemmon, A. R., Lemmon, E. M., & Bybee, S. M. (2019). Higher-level phylogeny and reclassification of Lampyridae (Coleoptera: Elateroidea). *Insect Systematics and Diversity*, 3(6): 11. https://doi.org/10.1093/isd/ixz024

Monanu, P. C. (1975). *Nigeria in Maps: Eastern State*. Ethiope Publishing House, Benin City.

Nei, M., & Kumar, S. (2000). *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.

NPC (2006). *The Nigeria Population Census*. National Population Commission (NPC). http://www.population.gov.ng/index.php/censuses.

Nwadinigwe, A. O., & Onyekwelu, S. S. C. (2006). Ordination of a Nigeria Savanna woodland in Obollo Afor, Udenu local government area of Enugu State, Nigeria. *Nigeria Journal of Botany*, 19 (1): 1-16.

Riley, W. B., Rosa, S. P., & da Silveira L. F. (2021). A comprehensive review and call for studies on firefly larvae. *PeerJ*, 9:e12121. http://doi.org/10.7717/peerj.12121

Takeda, M., Amano, T., Katoh, K., & Higuchi, H. (2006). The habitat requirement of the Genji-firefly *Luciola cruciata* (Coleoptera: Lampyridae), a representative endemic species of Japanese rural landscapes. *Biodiversity and Conservation*, 15: 191-203. DOI 10.1007/s10531-004-6903-y

The World Gazette (2004). *Current Populations for Cities and Towns in Nigeria*. www.Gazette de/e/e/ng.htm.

Wong, C. H., & De Cock, R. (2014). A Quick Assessment on the Progress of the Selangor Declaration since 2010. *International Firefly Symposium 2014, Florida, USA*. (unpublished). https://conference.ifas.ufl.edu/firefly/Final_Report.pdf

Zhu, C.-Q., Xu, X.-D., & Zhen, Y. (2022). Systematic review of the firefly genus *Emeia* Fu, Ballantyne & Lambkin, 2012 (Coleoptera, Lampyridae) from China. ZooKeys, 1113: 153–166. https://doi.org/10.3897/zookeys.1113.79721