



TAMIL NADU FOREST DEPARTMENT



Advanced Institute for Wildlife Conservation
(Research, Training & Education)
Vandalur - 600 048.

Report

**FIREFLIES: DIVERSITY,
DISTRIBUTION, HABITATS
AND THEIR ECOLOGY IN
ANAMALAI TIGER RESERVE**

**Annual Plan of Operations
2023-24**

March 2025

Tamil Nadu Forest Department



ADVANCED INSTITUTE FOR WILDLIFE CONSERVATION
(Research, Training & Education)
Vandalur, Chennai – 600 048



Project Completion Report
on

FIREFLIES: DIVERSITY, DISTRIBUTION, HABITATS AND THEIR ECOLOGY IN ANAMALAI TIGER RESERVE

Project Sanctioned under

Annual Plan of Operations (APO) 2023-24

Researcher

K. Ragavendran, Project Associate II

Guided by

Dr. M. Gabriel Paulraj, AIWC
Dr. S. Siva Ranjani, FVAS, AIWC
Thiru. S. Selvakumar, FRO, AIWC

CENTRE FOR CONSERVATION ECOLOGY, AIWC

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Office of the PCCF & Director: 044-29372331.

For correspondence: aiwcrte@tn.gov.in

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ABBREVIATIONS AND SYMBOLS

AIWC – Advanced Institute for Wildlife Conservation
ALAN – Artificial Light at Night
ATL – Alkyl Transferase-like
ATR – Anamalai Tiger Reserve
BLAST – Basic Local Alignment Search Tool
bp - Base pair
cm - Centimetre
DNA – Deoxyribonucleic Acid
EPI – Excess Proportion Index
h - Hour
km - Kilometre
LED – Light-emitting diode
m – Metre
MFC – Meta Femoral Comb
min- Minutes
ml- Millilitre
mt COI – Mitochondrial Cytochrome C Oxidase subunit 1
mph – Miles Per Hour
MPP – Median Posterior Projection
NC – Number in the control side
NCBI – National Centre for Biotechnology Information
nm – Nanometre
NT – Number in the treatment side
PCR – Polymerase Chain Reaction
PM – Post Meridiem
PVC – Polyvinyl chloride
sec - Seconds
sp. - Species
spp. – Multiple species
V5 – Fifth ventrite
V7 – Seventh Ventrite
µl - Microlitre
°C – Degree Celsius
% - Percentage

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Project Completion Report

Title of the Project	: ‘Fireflies: Diversity, Distribution, Habitats and their Ecology in Anamalai Tiger Reserve’
Project Category	: Annual Plan of Operations (APO) Project 2023-24
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Project Associate	: K. Ragavendran
Technical Guidance	: Dr. M. Gabriel Paulraj
Project Coordinators	: Dr. S. Siva Ranjani, FVAS and Thiru. S. Selvakumar, FRO

1. ABSTRACT

A study was conducted from May 2024 to March 2025 in 10 different locations within Anamalai Tiger Reserve (ATR), Tamil Nadu, to understand the firefly species assemblage, seasonal changes in their population, environmental conditions in their habitats and their genetic diversity. Their phototactic movement behaviour towards different wavelengths of light was studied to understand the impact of light on their behaviour. Monthly collection of different firefly species was carried out by the sweeping net method along 100 m transect lines in three ranges, namely Pollachi, Ulandy and Manambolly in ATR. In these three ranges, fireflies were collected from 10 different locations: Anaikundhi viewpoint, Kannadi Bungalow, Kozhikamuthi, Manambolly, Manthirimattam, Saralapathi, Savamalai Estate, Topslip, Urulikkalcheckpost and Varagalayar. In Saralapathy, the firefly sampling was done in agroecosystems. The intensity of light, wind velocity, atmospheric temperature and humidity in their habitat were recorded during specimen collection.

Eight different species of fireflies, namely *Absconditaperplexa*, *Ab. terminalis*, *Asymmetricatahumeralis*, *Curtos* sp. 1, *Curtos* sp. 2, *Lamprigera* sp., *Pyrocoelia* sp. and one unidentified sp., were collected during the study period. The phototactic movement behaviour of *A. perplexa* was studied and the results clearly showed that fireflies preferred darkness and avoided six different colours, namely blue, green, yellow, orange, pink and red. White light was neither preferred nor avoided by the fireflies. DNA barcoding of different species was performed by amplifying a partial sequence of the mitochondrial Cytochrome C oxidase subunit 1 (mt COI) gene using the Folmer primer. DNA barcoding helped to confirm the identification of morphologically distinct species. The evolutionary relationships of the firefly genera were studied by constructing phylogenetic tree. The neighbor-joining approach clearly showed a distinct, separate group of clade for each genus.

In a questionnaire survey 48.7% respondents stated that firefly population was at its peak during summer and 51.2% of the respondents were aware of the declining population of fireflies in the region. Studies on firefly habitats, preferred environmental conditions, and their interactions with other organisms help us understand ecosystem health and develop strategies to conserve firefly habitats and maintain ecological balance.

Keywords: firefly, bioluminescence, types of pollution, artificial light at night (ALAN), morphological identification, phototactic study, molecular analysis

2. INTRODUCTION

Fireflies, the lightning bugs, are one of the most celebrated insects worldwide. They are beetles (Order: Coleoptera) that belong to the family Lampyridae. They possess the unique ability to produce bioluminescence due to the presence of specialised light-producing organs called lanterns. Both larval and adult fireflies are bioluminescent in all firefly species. Bioluminescence in fireflies plays a crucial role in sexual communication and also serves as a warning signal to escape from their predators. Globally, nearly 2,400 firefly species have been discovered. Fireflies inhabit various ecosystems, including forests, wetlands, grasslands, and traditional agricultural fields. They are commonly found near water sources like ponds, streams and marshes, which support their prey and mating activities (Martin *et al.*, 2019; Ghosh *et al.*, 2023a; Powell *et al.*, 2022; Lewis *et al.*, 2024).

Fireflies play a significant role in natural ecosystems and food chains. The lifespan of different life stages of fireflies varies. Larvae may live for months to years, depending on the species and adults live for only a few weeks to two months. The firefly larvae are carnivorous, feeding on soft-bodied prey such as snails, earthworms, and other small invertebrates. The larvae feed on some snail species that are causing damage to agricultural crops and thus act as a biocontrol agent. Adult fireflies have more varied diets and consume nectar or pollen from flowers (Tathawee *et al.*, 2020).

Firefly fauna includes both diurnal and nocturnal species. Diurnal species generally do not communicate with the opposite sex using their light during the daytime, and they mainly produce pheromone signals. However, light-emitting adult fireflies are primarily active at night, using their characteristic flashing signals for mating communication (Ming and Lewis, 2010; Stanger-Hall *et al.*, 2018). Ideal habitats for mating and reproduction include tree canopies, herbaceous plants, and grasses along

riverbanks(Jusoh *et al.*, 2011; Faust and Faust, 2014; Sartsanga *et al.*, 2018).

Fireflies serve as important bioindicators of ecosystem health due to their sensitivity to environmental changes. Their presence reflects the availability of fresh air, unpolluted water, healthy soil (vegetation) and balanced ecosystems. A decline in firefly populations often results from habitat degradation caused by pollution, excessive pesticide use, exposure to bright high-voltage LED lights, urbanisation, and changing climate patterns. Protecting fireflies contributes to broader conservation efforts for biodiversity and ecosystem stability (Fallon *et al.*, 2021; Lewis *et al.*, 2024).

Habitat destruction is the most significant factor contributing to the decline in firefly diversity and distribution worldwide. As habitat specialists, fireflies depend on distinct environments for different life stages – larval development and adult mating. The widespread loss of natural habitats due to commercial development and agricultural conversion, notably the transition from traditional to modern farming practices and increasing urban expansion, poses a serious threat to the survival of fireflies (Wagner *et al.*, 2021; Vivian *et al.*, 2023; Lewis *et al.*, 2024).

The use of harmful agrochemicals, including organophosphates, carbamates and neonicotinoids, is hazardous to all fauna. These long-lasting chemicals persist in the air, soil, and water, disrupting the ecological balance of a wide range of fauna (Sánchez-Bayo, 2012; Pisa *et al.*, 2015; Lewis *et al.*, 2024).

Light pollution is also a significant threat to firefly populations. Studies have shown that artificial lighting interferes with firefly mating communications and their expected behaviour (Kyba, 2018; Owens and Lewis, 2021). Artificial lights disturb their sensory systems and affect interactions with the opposite sex, interrupting reproductive activities and population decline (Fobert *et al.*, 2019; Maggi *et al.*, 2020). Researchers found that blue and green (480 nm & 560 nm) light wavelengths negatively impact firefly light patterns, but red (above 600 nm) light did not

significantly affect their signaling behaviour (Biggley *et al.*, 1967; Owens *et al.*, 2018). Habitat protection, habitat restoration and species restoration plans will support the life cycle and encourage the firefly population (Lewis *et al.*, 2024).

Firefly diversity studies in the Anamalai Tiger Reserve (ATR) are scanty. Veetil *et al.* (2015) have reported the large congregation of fireflies and synchronous flashing in Varagaliar at ATR. Sriram *et al.* (2023) have recorded three firefly species and documented the synchronous flashing of fireflies at ATR. Except for these studies, no other studies, particularly on firefly species diversity, are available in the literature for ATR. Hence, the present study was conducted in ATR to survey the diversity of firefly species. Fireflies were collected from 10 different locations of ATR, namely Anaikundhi viewpoint, Kannadi Bungalow, Kozhikamuthi, Manambolly, Manthirimattam, Saralapathi, Savamalai Estate, Topslip, Urulikkal checkpoint and Varagalayar.

3. OBJECTIVES

1. To collect and document the firefly species in Kozhikamuthi, Varagalayar, and Topslip areas within the Anamalai Tiger Reserve (ATR).
2. To study the diversity of fireflies in ATR and adjacent agroecosystems, including coconut farms, tea and coffee plantations and sugarcane fields.
3. To investigate the effect of light on firefly distribution and species composition.
4. To identify the wavelength of light which is preferred by fireflies.
5. To conduct DNA barcoding analysis on the collected firefly species for species confirmation.
6. To document the organisms (insects, small mammals, etc.) associated with the life stages of fireflies.

4. REVIEW AND STATUS OF RESEARCH AND DEVELOPMENT IN THE SUBJECT

4.1. International status

As fireflies are fascinating organisms, they attract researchers worldwide. Their light-emitting behaviour, science behind the bioluminescence and applications of the bioluminescent chemicals in human welfare activities have been well studied by researchers. Owens and Lewis (2022) conducted a study on the mating success of *Photinus* sp. in North America and found that artificial light prevented mating success in both laboratory and field studies. Studies about the effect of artificial light exhibit the firefly lifecycle of immature *Photuris* sp. and *Photinus obscurellus* fireflies. The dim light exposure did not affect development stages, but rapid weight gain during early instars and light exposure during late larval stages negatively impacted adult reproductive fitness (Owens and Lewis, 2021). Long *et al.* (2012) also reported that jumping spiders, *Phidippus princeps* and *Phidippus audax*, react to firefly *Photuris* sp. flashing, which may increase spider attacks but also aid learning about unpalatable prey, depending on the prevalence of unpalatable fireflies.

In North America, differences in signalling methods and mate search behaviour help explain variations in eye and antenna size among fireflies. When they shift from using light signals to relying on pheromones, their eye size changes faster than their antenna (Stanger-Hall *et al.*, 2018). However, recent field experiments reported the impact of artificial light at night (ALAN) on fireflies during courtship and predation. The results showed that both male and female fireflies flash less when exposed to artificial light (Hillón Salas *et al.*, 2024). Firebaugh and Haynes (2016) conducted field experiments to test the impact of light pollution on fireflies, using bioluminescent flashes for mating. Artificial light reduced female courtship flashes and prevented mating, though local abundance was not immediately

affected. However, prolonged exposure to ALAN could result in population declines due to repeated mating failures.

Additionally, Hagen *et al.* (2015) investigated the impact of artificial illumination of firefly activity in Brazil, and they reported significant negative impacts. They proposed that fireflies could serve as bioindicators for light pollution. Overall, these studies suggested that fireflies prefer darker environments and are negatively affected by artificial lighting.

4.2. National status

Among the studies conducted in India, Ghosh *et al.* (2023) documented nine genera of fireflies and their distribution in two Indian states, Odisha and West Bengal. A population study on *Abscondita chinensis* was conducted in paddy fields and domestic areas of Barrankula, Andhra Pradesh, India, where the field investigations clearly highlighted the population decline caused by pesticides (Chatragadda, 2020). Some studies have observed differences in the light emission spectra between males and females. The *Luciola praeusta*, the female's emission wavelength exceeds that of the male, particularly as the temperature approaches the species' optimum. (Rabha *et al.*, 2017). Ghosh *et al.* (2024) conducted the first experimental study on the effects of artificial light at night (ALAN) in India. Using three different *Abscondita* species, they analysed the impact of low, medium and high ALAN exposure levels. The study displayed that fireflies could be used as indicators to measure light pollution.

Synchronous flashing pattern of fireflies was also documented in Varagalayar (ATR). Understanding the specific species involved, their synchronous behaviour and the unique ecosystem characteristics is essential for conserving such behaviour. This approach ensures the preservation of this unique phenomenon for future generations (Veetil *et al.*, 2015). In their habitat study conducted between 2017 and 2022, Ghosh *et al.* (2023) also documented that six different firefly species showed a strong preference for plants of the Fabaceae family. Previously, Sriram and his co-workers recorded three firefly species, namely *Abscondita perplexa*,

Asymmetricata humaralis and *Curtos* sp., from the Ulandy range of the Anamalai Tiger Reserve ATR (Sriram *et al.*, 2023; Karuppasamy and Arunachalam, 2023). Firefly faunal studies were very scanty in Tamil Nadu, especially in diverse protected areas like Anamalai Tiger Reserve. Hence, the present study was undertaken to further explore the diversity of fireflies within ATR. Fireflies were collected and documented from three different ranges of ATR, namely Pollachi, Ulandy and Manambolly. Additionally, DNA barcoding was conducted to help in the accurate identification of firefly species.

5. MATERIALS AND METHODS

5.1. Place of Study

A firefly survey was conducted at ten different locations within the Anamalai Tiger Reserve (ATR) (Fig. 1). ATR was declared as a Tiger Reserve in 2007. The study sites within ATR are situated in the Southwestern Ghats of India (Latitude: 10° 13.2' N to 10° 33.3'N; Longitude: 76° 49.3' E to 77° 21.4' E) in the Coimbatore district of Tamil Nadu. The area of the core/critical tiger habitat is 958.59 sq. km and buffer/peripheral area of ATR is 521.28 sq. km (National Tiger Conservation Authority, 2017). The reserve encompasses a range of diverse ecosystems, including deciduous, tropical evergreen forests, grasslands, and Shola forests.

The firefly specimens were collected from 10 locations, namely Anaikundhi viewpoint, Kannadi Bungalow, Kozhikamuthi, Manambolli, Manthirimattam, Saralpathi, Savamalai Estate, Topslip, Urulikkalcheckpost and Varagalayarin the Pollachi, Ulandy, and Manambolli ranges of ATR. The geographic coordinates(latitude and longitude) and altitudes of the study places are given in Table 1.

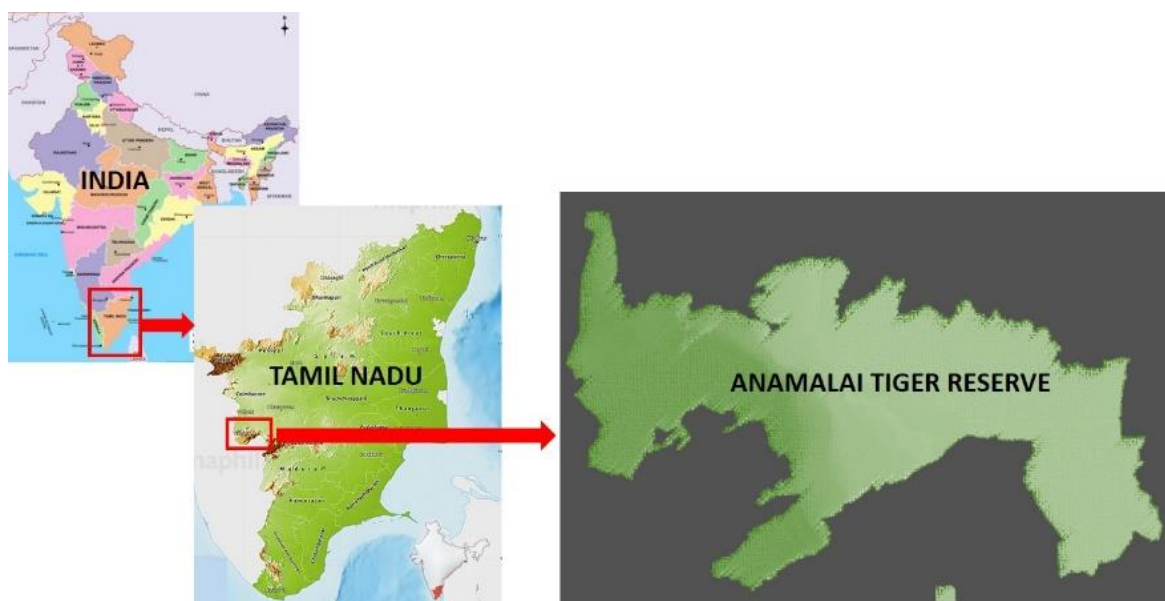


Figure 1. Location map of Anamalai Tiger Reserve (ATR) in Tamil Nadu

Table 1. Locations of Firefly collection in Anamalai Tiger Reserve

Sl. No	Location	Range	Latitude	Longitude	Altitude
1	Anaikundhi View Point	Ulandy	10.47329	76.84085	756m
2	Kannadi Bungalow	Ulandy	10.450604	76.828622	673m
3	Kozhikamuthi	Ulandy	10.443925	76.849092	656m
4	Manambolly	Manambolly	10.35731	76.87824	583m
5	Manthirimattam	Manambolly	10.37542	76.86102	554m
6	Saralapathi	Pollachi	10.53656	76.86372	357m
7	Savamalai Estate	Manambolly	10.35635	76.8952	792m
8	Topslip	Ulandy	10.4711	76.84441	710m
9	Urulikkal Checkpost	Manambolly	10.343	76.90313	1097m
10	Varagalayar	Ulandy	10.41755	76.86588	654m

5.2. Firefly sampling

Firefly specimens were collected between 6:00 PM and 10:00 PM. Flying adult fireflies were collected along a 100 m transect line using a sweeping net (25 cm diameter). Larvae were collected manually from their habitats. All collected adult and larval specimens were preserved in 70% ethanol, labelled appropriately, and transported to the AIWC laboratory for morphological and molecular studies. The number of individuals per species was also recorded.

5.3. Morphological Identification

Morphological characteristics of the collected specimens were examined using a stereo zoom microscope, and individuals were sorted to species or genus level. Species identification was done using all available identification keys, taxonomic revisions and original descriptions. Morphological characteristics used for the species identification were: body size, colour and patterns in the dorsal side of the elytra, size and structure of the pronotum, colour of the ventral side, colour of the legs, structure of head and compound eyes, antennal structure, and the shape of light-producing organs on the ventral side (Fig. 2). Identification was supported by taxonomic keys and literature provided by Ballantyne and McLean (1970),

Ballantyne (1987), Ballantyne (2001), Ballantyne and Lambkin (2009), Ballantyne and Lambkin (2013), Ballantyne *et al.*, (2013, 2016 & 2019), Jusoh *et al.* (2018 & 2021) and Wijekoon *et al.*, (2021).



Figure 2. Parts of a typical firefly species

5.4. DNA Barcoding

DNA barcoding study was done for the species identification and/or confirmation of a morphologically identified species. The mitochondrial cytochrome C oxidase subunit 1 (COI) gene was selected as the standard barcode region. DNA was extracted from a single leg of an individual adult specimen from each species using Qiagen DNEasy Blood and Tissue extraction Kit, following the manufacturer's protocol. Each leg sample was kept in a microcentrifuge tube (1.5ml) containing 200µl ATL buffer and 20-30 µl Proteinase K and incubated at 56°C, and DNA was extracted. The DNA barcode region (COI) was amplified by Folmer primers, LCO14090 (forward) and HCO2198 (Reverse) (Folmer *et al.*, 1994) following standard Polymerase Chain Reaction (PCR) protocol. The PCR steps included denaturation at

95°C(3 min.), extension at 55.7°C (30 sec.) and annealing at 72°C (40 sec.). The amplified PCR products from different individuals were run on a 2% agarose gel and visualised under ultraviolet light. The successfully amplified PCR products were purified using the Qiagen gel extraction kit protocol and sequenced by Sanger sequencing on an ABI 3500 Genetic Analyser.

The resulting 22 barcode sequences were compared against existing sequences in the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST) to identify the matching species. All COI sequences were submitted to GenBank and accession numbers were received.

5.5. Phylogenetic analysis

Twenty-two COI sequences were aligned by ClustalW and the evolutionary history was inferred using the Neighbour-Joining approach (Saitou & Nei, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) and are expressed in units of the number of base substitutions per site. The pairwise deletion option was applied to all ambiguous positions for each sequence pair, resulting in a final data set comprising 778 positions. *Agriotes lineatus*(click beetle) COI sequence was used as an outgroup. Evolutionary analyses were conducted in MEGA 12 (Kumar *et al.*, 2024) utilizing up to 4 parallel computing threads.

5.6. Phototactic movement behaviour

The phototactic movement behaviour of *Abscondita perplexa* adults was studied to find out their orientation behaviour when exposed to different colours of light. A cross-shaped chamber was used to study the phototactic movement behaviour. This type of device was reported by Yang *et al.* (2024) and it was slightly modified according to needs of the present study.

Device structure: The device has a central cubic chamber of 10 cm × 10 cm × 10 cm with a 2 cm diameter opening at the top middle for introduction of

specimens (Fig. 3). The four sides of the cube extend into four rectangle arms, each measuring 20cm × 10cm × 10 cm and the distal ends of each arm were sealed with transparent glass. The top portion of each arm was also fitted with glass to view the movement of the firefly. The four sides of the central cubic chamber were equipped with sliding door mechanisms that could be raised to allow the fireflies to move into the arms.

Testing method: Five pairs of active adult fireflies (*A. perplexa*) were released into the central cubic chamber through the top middle opening, which was closed immediately to prevent escape. The outer ends of two arms were fitted with colour lights and other two arms were not fitted with light source and considered as dark controls. After a two-minute acclimation period, the sliding doors on the four sides of the central cubic chamber were lifted simultaneously to allow the fireflies to move from the central chamber. After every 30 minutes, the number of fireflies present near the light source and on the dark sides was counted and recorded. The experiment was conducted for a total period of 2 h. Each colour light treatment was replicated three times with new insect specimens for each trial. A total of six different colour lights, such as yellow, green, red, orange, pink and white, were tested using this method.

The preference or avoidance of fireflies to light or dark was assessed using the Excess Proportion Index formula as described by Sakuma and Fukami (1985):

$$EPI = (NT - NC) / (NT + NC)$$

Where NT=number of insects found in the test side (light source) and NC=number of insects found in the control (dark) side.

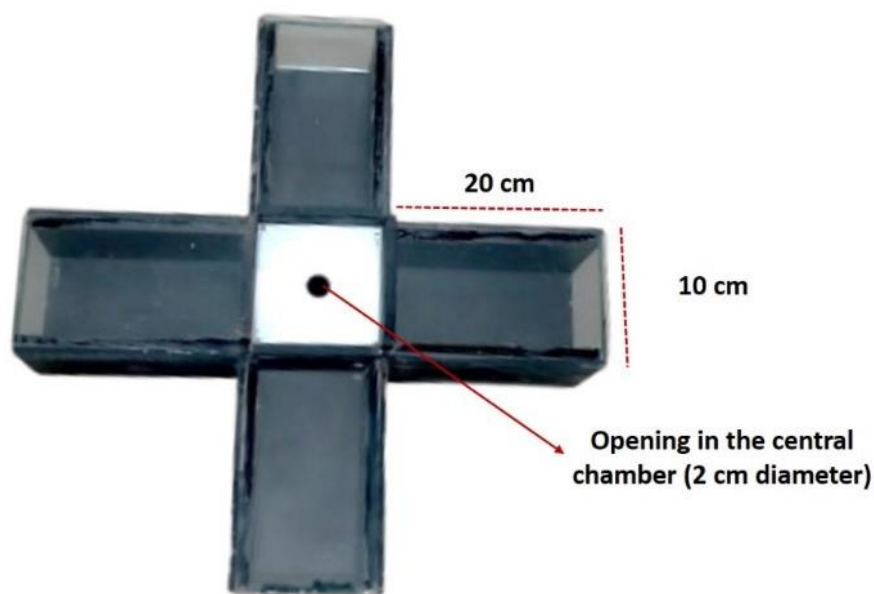


Figure 3. Phototactic movement behaviour study chamber

5.7. Quadrat Study

The quadrat method is a widely used ecological technique for estimating the abundance and distribution of organisms within a study area. A quadrat (0.5 m × 0.5 m) was constructed using PVC pipes and placed on the soil at random sites in the study area. The presence of firefly larvae within each quadrat was observed and counted. Other insect species that were found inside the quadrat were also counted and recorded. This method decreases the impact of organisms moving away from the survey site during the study (Cherilland Brown, 1990; Ausden, 1996; Gardiner and Hill, 2006).

5.8. Measurement of physical parameters of the atmosphere

Atmospheric temperature (°C), humidity, wind velocity and light intensity were measured using appropriate instruments (Fig. 4). A combined hygrometer and thermometer (Brand: Testo) was used to record temperature and humidity. Wind velocity was measured using an anemometer (Brand: Testo), and light intensity was measured using a lux meter (Brand: Testo).

Lux level was measured at night hours in the firefly habitat during sampling.



Figure 4. Lux meter (a), Anemometer (b), GPS (c), and temperature-humidity measurement device (d) used to measure the light intensity, wind velocity, location of the study place and temperature & humidity respectively during field work

5.9. Questionnaire Survey

A survey in the form of a questionnaire was carried out among local residents and field personnel of the forest department to evaluate their understanding of fireflies, their habitats, seasonal distribution and the effects of light pollution on fireflies. The villages included in the survey were mainly populated by indigenous groups such as the Malasar, MalaiMalasar, Kaadar and Irulas. The survey was conducted in the villages of T. Saralopathy, Topslip, Erumaiparai, Kozhikamuthi, Manambolli and Koomatti within the ATR region of Pollachi.

6. RESULTS

6.1. Firefly species assemblage

Fireflies were collected from 10 different places at ATR during night hours. At each site, adult fireflies were captured randomly using a sweep net along 100 m transect lines. Larval instars were manually collected whenever visible, guided by their bioluminescent flashes.

A total of eight different firefly species namely *Abscondita terminalis*, *Abscondita perplexa*, *Asymmetricata humeralis*, *Curtos* sp. 1, *Curtos* sp. 2, *Lamprigera* sp., *Pyrocoelia* sp. and one unidentified species were collected (Fig. 5-17). Table 2 shows the total number of individuals collected from 10 different places. All eight species showed variations in size, colour and other morphological characteristics, although they shared similar habitat types with overlapping distribution zones.



Figure 5. *Abscondita* sp. adults on the underside of plant leaf during day time

Brief descriptions of each species are given below:

***Abscondita terminalis* (Olivier, 1883):** Body length of adult beetle ranges from 8.8 to 13.2 mm. The pronotum and elytra are yellowish-orange, with

the elytral tips distinctly black. Elytral punctuation is dense, non-linear and the elytral apices are not deflexed; V7 (seventh ventrite) has a broadly rounded posterior margin; In males, the light organ nearly occupies the entire flattened surface of ventrite 7, which features a very short and wide median posterior projection (MPP); meta femoral comb (MFC) is absent (Fig. 6&7).



Figure 6.*Absconditaterminalis*– Male: Dorsal and ventral views



Figure 7.*Abscondita terminalis*- Female: Dorsal and ventral views

***Abscondita perplexa*:** The head is black, while the antennae and palpi are dark brown. The elytra are brownish-yellow, with a distinctly dark brown apical part. The pronotum is brownish-yellow in colour with small pits or dots. The ventral side of the abdomen is also brownish-yellow in colour. The legs are orange-yellow with brown-tipped femora; tibiae and tarsi are dark brown. The fifth ventral segment (V5) has lateral dark markings (Fig. 8 & 9).

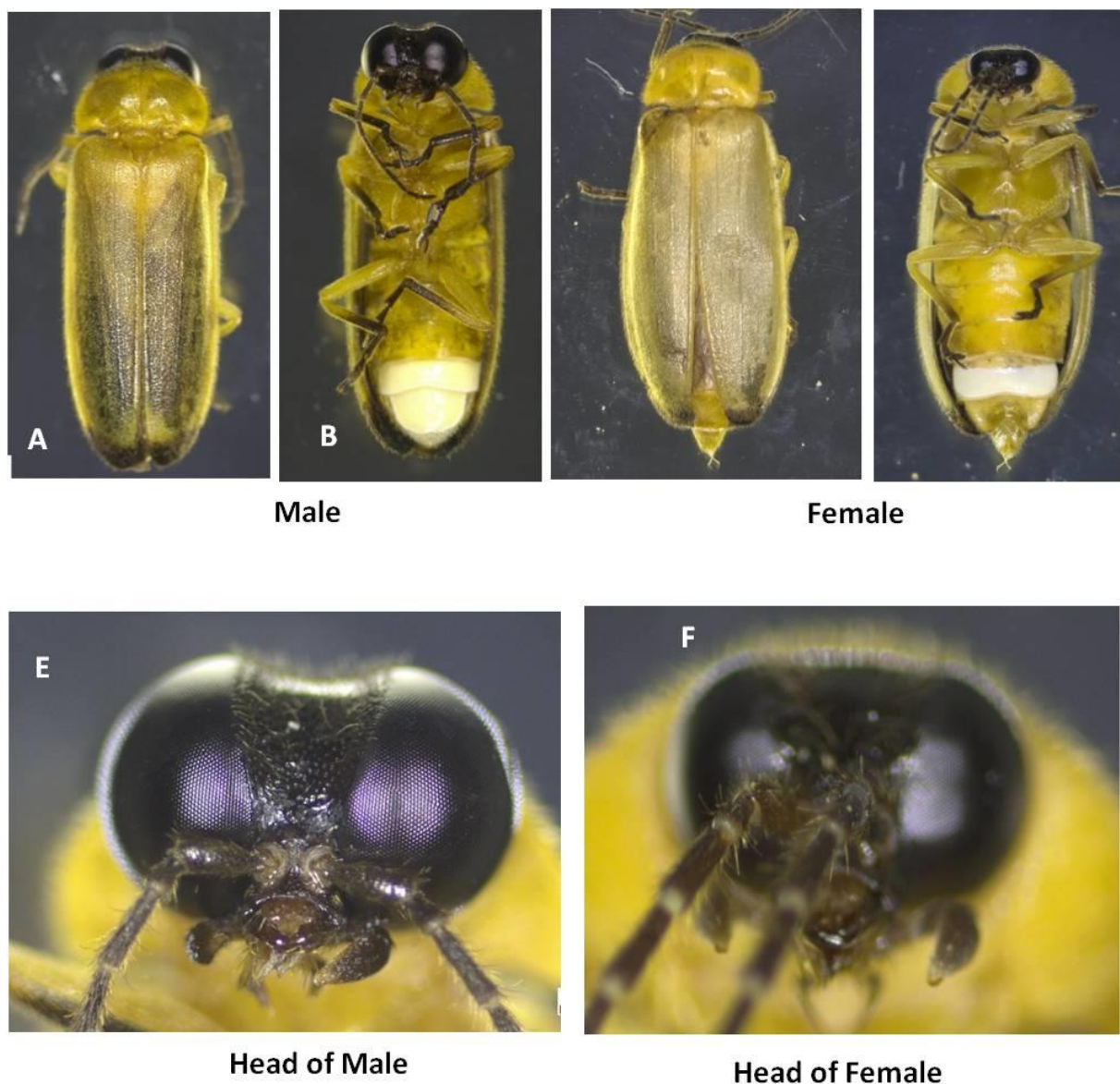


Figure 8. *Abscondita perplexa* male and female: Dorsal and ventral views (above) and head structures (below)



Figure 9. *Abscondita perplexa*- Legs and antennal structure

***Curtos* sp.:** In the present study, three morphologically different *Curtos* spp., were collected, which are the smallest firefly species among all the collected species. They have maxillary palps with a non-toothed apex; the carina (ridge or keel) on each elytron is well-developed (Fig. 10-12). There was an unidentified adult specimen shown in figure 13. The species identification of this specimen based on the morphological features is ongoing.



Figure 10. *Curtos* sp.1 (male):Dorsal (A) and ventral (B) views



Figure 11. *Curtos* sp.1 (female):Ventral (A) and dorsal (B) views



Figure 12. *Curtos* sp.2 (male). Dorsal (A) and ventral (B) sides



Figure 13. Unidentified species. Ventral (A), abdomen ventral (B) and head (C)

***Asymmetricata humeralis* (Ho, 2019):** Pronotum and mesoscutellum are orange in colour; elytra are black with narrow pale margins; legs are orange in colour; basal abdominal ventrites are black in colour (Figure 14& 15)



Figure 14. *Asymmetricata humeralis* male dorsal side, ventral side, head and light organs



Figure 15. *Asymmetricata humeralis* female dorsal side, ventral side

***Lamprigera* sp.:** The genus *Lamprigera* is a poorly studied group of fireflies. In the present study, a *Lamprigera* sp. was collected, which was the largest in size among all the firefly specimens documented in the present study (Fig. 16).

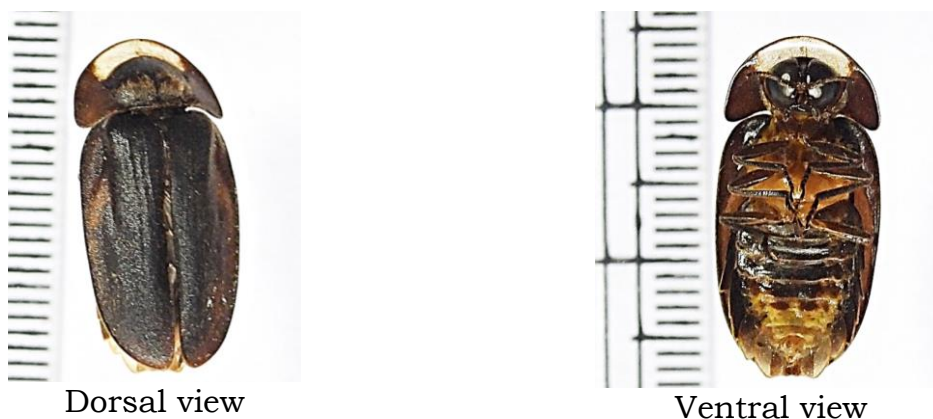


Figure 16. *Lamprigera* species male

***Pyrocoelia* sp.:** *Pyrocoelia* belongs to the family Lampyridae. It is a terrestrial firefly. The pronotum covers the head dorsally, and this is a characteristic feature of many fireflies in this genus (Fig. 17). Sexual dimorphism is seen in *Pyrocoelia* where males are alate (winged) and females have vestigial elytra and lack hind wings.



Figure 17. *Pyrocoelia* sp. adult male ventral side (A) and dorsal side (B)

Figure 18 shows the size of three different firefly genera namely *Abscondita*, *Asymmetricata* and *Curtos*. Species of *Curtos* are the smallest-sized fireflies. *Abscondita* is slightly larger than *Asymmetricata*.



Figure 18. Size comparison between *Abscondita* sp., *Asymmetricata* sp. and *Curtos* sp.

Larval forms of fireflies

The larval forms of fireflies showed morphological differences (Fig. 19 & 20). Among the larvae of different species, *Lamprigera* is the largest in size ranging from 7.5 to 8.0 cm in length.



Lamprigera sp. (Dorsal view)



Lamprigera sp. (Ventral view)



Larva of *Lamprigera* sp. emitting light



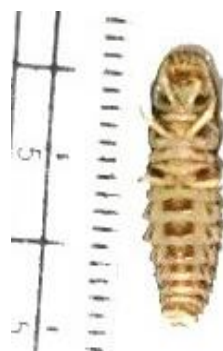
Pyrocoelia sp. (Dorsal view)



Pyrocoelia sp. (Ventral view)



Larva of unidentified species 1 (Dorsal side), collected near Parambikulam dam)



Larva of unidentified species 1 (Ventral side), collected near Parambikulam dam)



Larva of unidentified species 2 (Dorsal side)



Larva of unidentified species 2 (Ventral side)

Figure 19. Morphology of the larval stages of different firefly genera collected from the study area



Figure 20. Larval forms of different Firefly genera and their size. 1) *Lamprigera* sp., 2) Unidentified sp., 3) Unidentified species and 4) *Pyrocoelia* sp.

6.2. Firefly occurrence at different seasons

Abscondita perplexa was observed throughout all seasons in ATR. In contrast, *Abscondita terminalis* was exclusively found in the farm lands within the Pollachi range. Among the collected species, *Curtos* spp. were the smallest (size ranging from 5 mm to 6 mm) and *Lamprigera* sp. was the largest (23 mm).

Out of the 10 study sites, five sites viz., Manthirimattam, Varagalayar, Kozhikamuthi, Topslip and Savamalai estate recorded the highest firefly population in the southwest monsoon season. During this season, *Abscondita perplexa* was the dominant species, followed by *Asymmetricata humeralis*. The summer season witnessed a higher number of fireflies in Anaikundhi view point, and Topslip (Fig. 21). At Saralpathi, the place which is dominated by agricultural ecosystem, firefly population was abundant during the northeast monsoon season and declined in the winter season. The firefly species recorded in Saralpathi was *A. terminalis*.

The highest number of firefly specimens (93 individuals) was collected at Saralapathi, (in agroecosystems) where *A.terminalis* was the only species. At Saralapathi, the wind velocity was 0 mph during the firefly collection and the atmospheric temperature ranged from 27.8°C to 28.6 °C and the humidity ranged from 52 to 81.8% (Table 3).

Manthirimattam and Varagalyar also recorded a higher firefly populations, with 67 individuals collected from each site, and all specimens were identified as *Abscondita perplexa*. The total number of specimens from the genus *Abscondita*, collected from all the study sites throughout the study period, was 197 males and 136 females (Table 2).

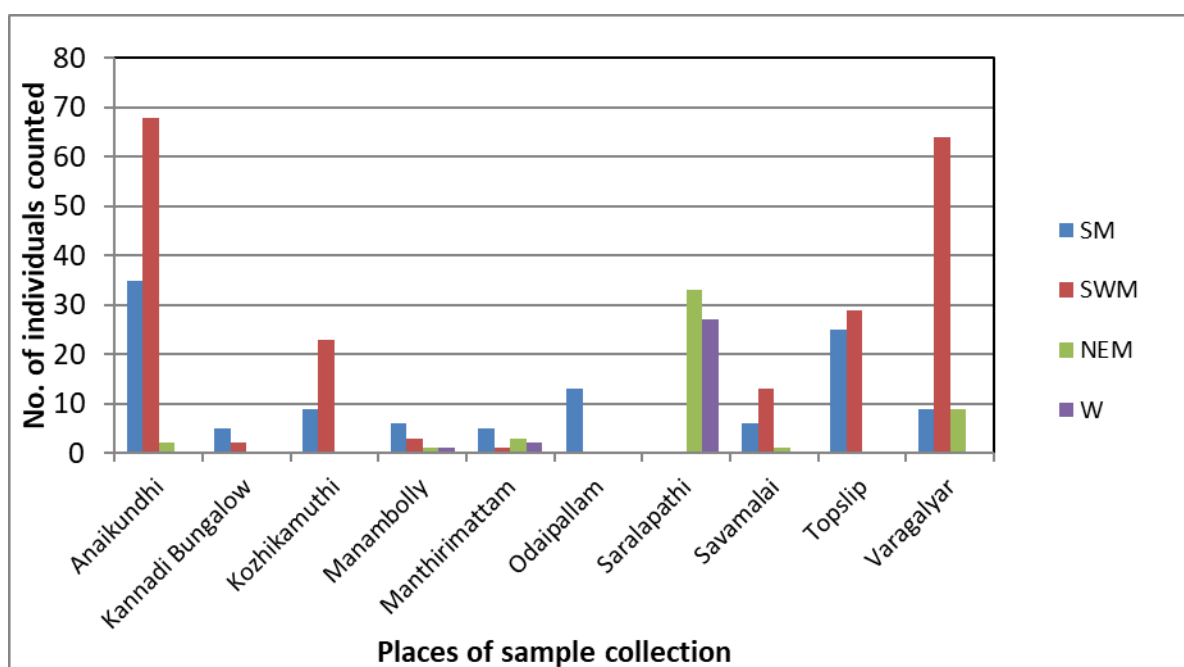


Figure 21. Total number of firefly individuals sampled at different seasons, such as summer (SM), southwest monsoon (SWM), northeast monsoon (NEM) and winter (W) in 10 different places in Anamalai Tiger Reserve

6.3. Colours of light production by different species

Abscondita spp., *Asymmetricata* sp., and *Curtos* spp. emitted yellow colored light. *Lamprigera* sp. and *Pyrocoelia* sp. produced pale greenish color light.

Table 2. Number of specimens of different firefly species collected by sweep net along different transect lines at different locations in ATR.

Sl. No	Place	<i>Abscondita</i> spp.		<i>Asymmetricata</i> spp.		<i>Curtos</i> spp.		<i>Lamprigera</i> sp.	<i>Pyrocoelia</i> sp.	Unidentified sp.	Total
		Male	Female	Male	Female	Male	Female	Male	Male	Grub	
1	Anaikundhi View Point	29	13	5	1	2	1	0	0	2	53
2	Kannadi Bungalow	3	5	0	3	0	0	0	0	0	11
3	Kozhikamuthi	13	13	5	1	0	0	0	0	2	34
4	Manambolly	2	5	0	0	0	0	1	1	3	12
5	Manthirimattam	32	31	0	0	1	0	0	0	3	67
6	Odaipallam	0	5	0	0	0	0	0	0	8	13
7	Saralopathy	*82	11	0	0	0	0	0	0	0	93
8	Savamalai Estate	6	5	0	0	0	0	0	0	9	20
9	Topslip	8	18	3	2	0	0	0	0	0	31
10	Varagalayar	22	30	12	1	1	1	0	0	0	67
Total		197	136	25	8	4	2	1	1	27	401

*Among the 82 specimens, 60 were *Absconditaterminalis*

Table 3. Wind velocity, atmospheric temperature, humidity and Lux level in the firefly collection sites.

Sl. No.	Date	Place	Wind velocity (mph)	Atmospheric Temperature (°C)	Humidity (%)	Lux level
1	15-04-2024	Anaikundhi	1.5	29.3	73.4	0
2	17-05-2024		1.0	27.3	84.9	0
3	11-06-2024		1.6	26.8	88	0
4	23-07-2024		0.0	28.1	74.4	0
5	23-08-2024		0.0	27.8	87.6	0
6	20-10-2024		0.0	30.2	98.4	0
7	26-01-2025		0.0	19.4	61	0
8	20-02-2025		0.0	26.6	40.6	0
9	14-05-2024	Kannadi bungalow	2.6	26.9	95.4	0
10	12-06-2024		0.0	27.8	91.2	0
11	19-10-2024		0.0	26.2	85	0
12	26-01-2025		0.0	26.6	61.4	0
13	19-02-2025		0.0	27.7	61.4	0
14	24-04-2024	Manamboly	0.0	27.5	88.0	0
15	20-07-2024		1.2	27.5	91.3	0
16	20-08-2024		0.0	25.5	88.1	0
17	23-10-2024		0.0	31.0	91.3	0
18	28-01-2025		0.0	24.7	67.1	0
19	24-02-2025		0.0	28.7	53.7	0
20	25-04-2024	Manthirimattam	1.2	29.3	75.0	0
21	19-07-2024		0.0	26.8	92.7	0
22	19-08-2024		0.0	26.2	82.1	0
23	22-10-2024		0.0	27.2	95.4	0
24	22-02-2025		0.0	26.2	63.7	0
25	13-05-2024	Kozhikamuthi	0.7	26.1	93.5	0
26	13-06-2024		0.0	28.2	88	0
27	22-07-2024		0.0	26.5	77.7	0
28	22-08-2024		0.0	28.3	85.1	0
29	19-10-2024		0.0	37.0	81.6	0
30	25-01-2025		0.0	24.3	56	0
31	18-02-2025		0.0	25.4	47.1	0
32	22-11-2024	Saralapathy	0.0	28.6	81.8	0
33	23-01-2025		0.0	27.8	67.9	0
34	17-02-2025		0.0	28.4	52	0
35	21-07-2024	Savamalai Estate	1.7	27.2	91.9	0
36	21-08-2024		0.0	27.3	85.3	0
37	24-10-2024		0.0	28.0	87.7	0
38	29-01-2025		0.0	26.3	81.7	0
39	25-02-2025		0.0	32.1	42.3	0
40	16-05-2024	Topslip	1.1	26.1	83.9 - 85.4	0
41	14-06-2024	Vinayagarkovil	0.9	25.6	86.7	0

42	18-10-2024	road	0.0	26.6	86.3	0
43	24-01-2025		0.3	26.5	86.7	0
44	26-04-2024	Urulikal	0.0	26.3	84.5	0
45	15-05-2024	Varagalayar	1.2	27.9	87.5	0
46	13-06-2024		0.0	29.3	86.3	0
47	23-07-2024		0.0	24.3	88.3	0
48	23-08-2024		0.0	28.3	83.6	0
49	20-10-2024		0.0	30.3	77.1	0
50	26-01-2025		0.0	33.9	73.5	0
51	20-02-2025		0.0	29.4	36.1	0

6.4. DNA barcoding

Mitochondrial Cytochrome C Oxidase subunit-1 (COI) gene of the firefly DNA was successfully amplified using Folmer primers (LCO14090 and HCO2198), yielding a 710bp of the barcode region in the firefly samples collected (Fig. 22). The amplified products were sequenced. A similarity search was performed using the BLAST tool. The percentage similarities of *Abscondita* and *Lamprigera* sequences with the NCBI sequences were 91-100% and 86-100%, respectively, confirming their genus-level identities. The *Asymmetricata* and *Curtos* sequences highlighted cross-genus relationships with similarity values ranging from 83%-100% and 85%-100%, respectively. This method proved to be one of the most effective and straightforward methods for species identification (Fig. 23-26). This DNA barcoding assisted in species identification complementing morphological species identification. A total of ten firefly mitochondrial barcode region (COI) sequences were submitted to the GenBank genetic sequence database (Table 4).

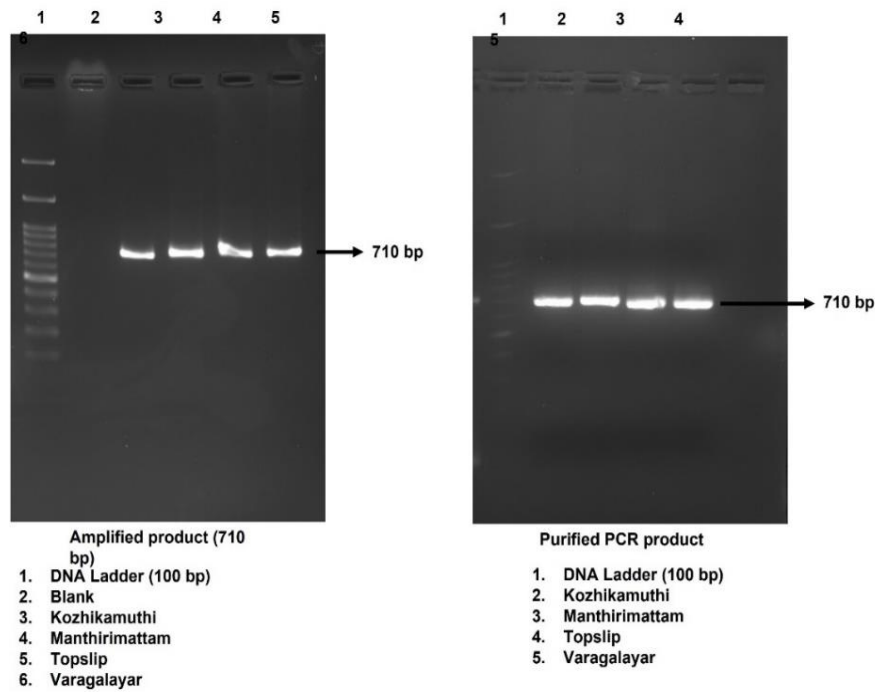


Figure 22. Amplified PCR product of COI region (710 bp) of mitochondrial DNA of fireflies

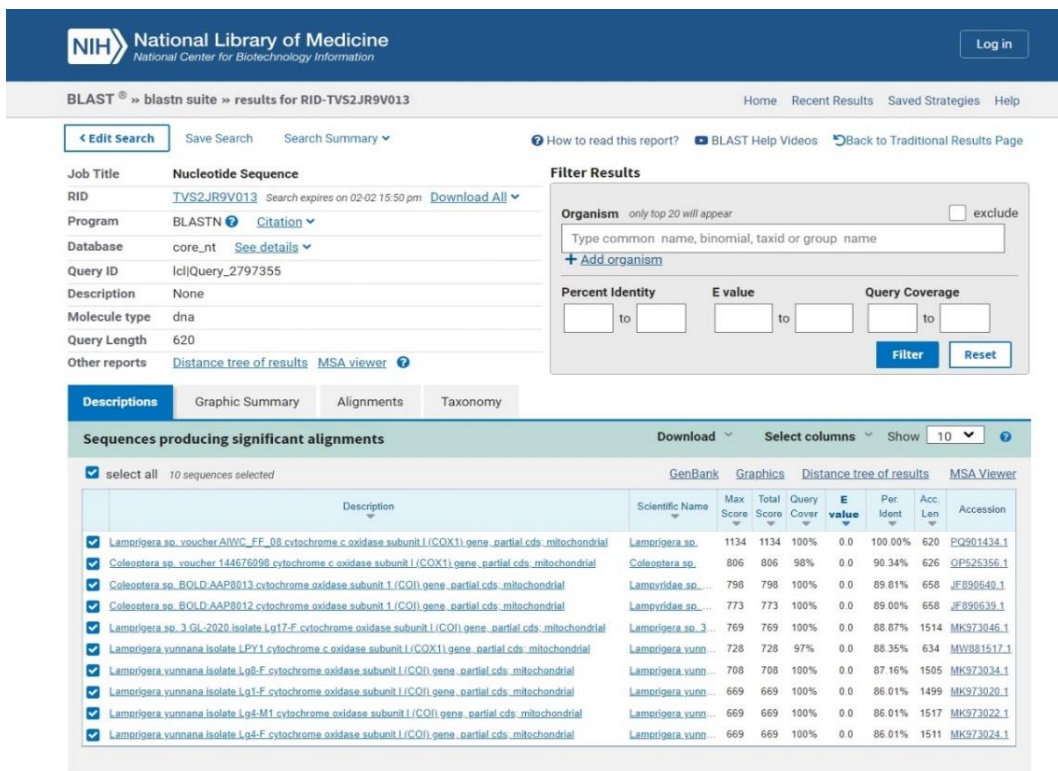


Figure 23. The COI sequence similarity search for *Lamprigera* sp. in NCBI.

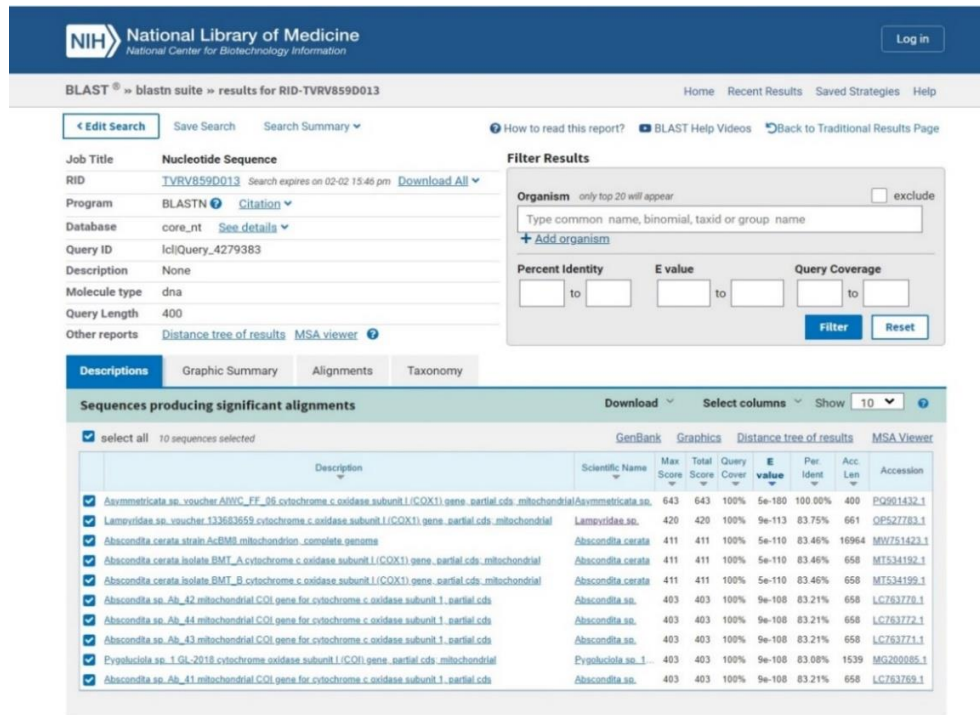


Figure 24. The COI sequence similarity search for *Asymmetricata sp.* in NCBI.

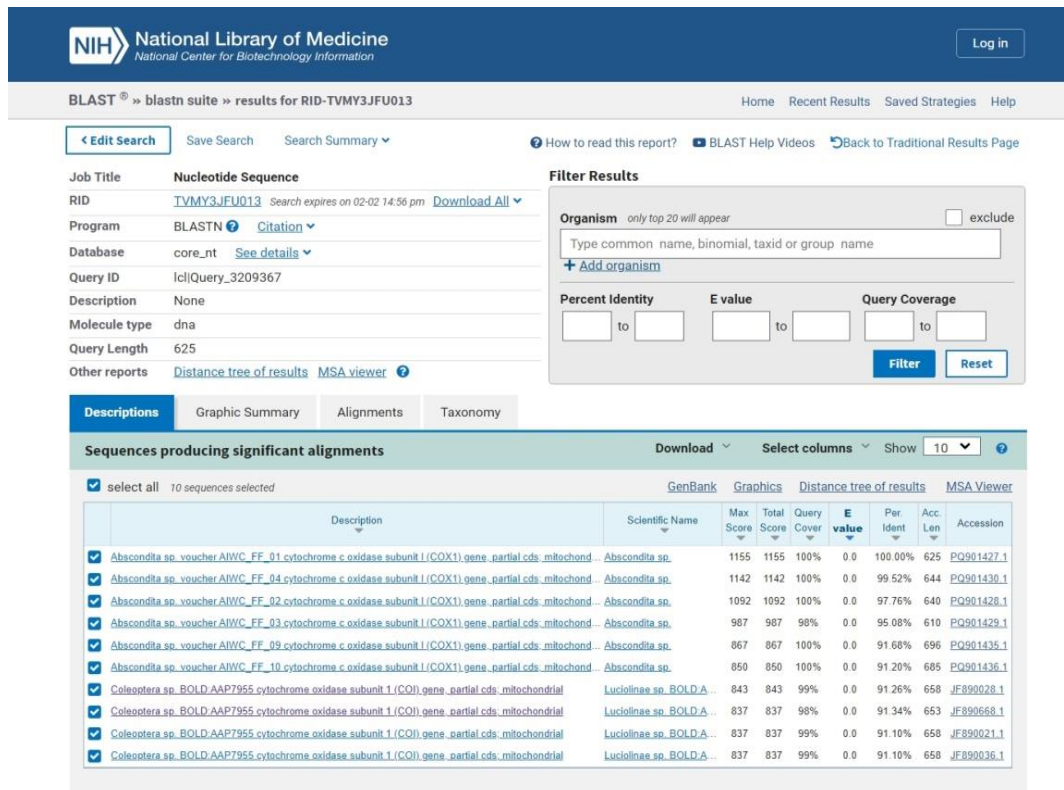


Figure 25. The COI sequence similarity search for *Abscondita sp.* in NCBI.

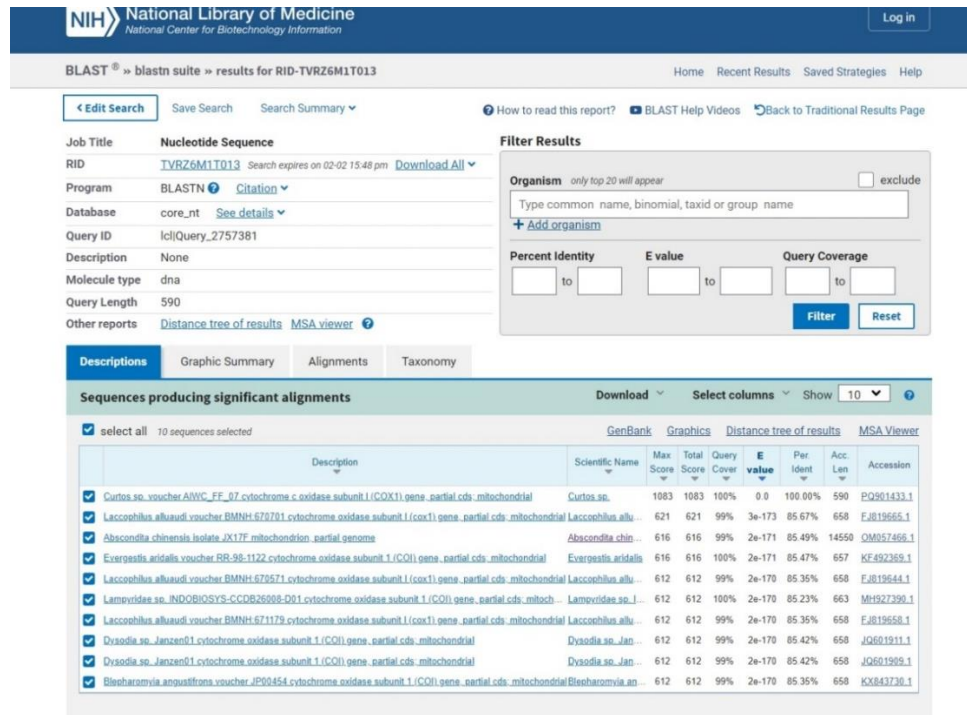


Figure 26. The COI sequence similarity search for *Curtos sp.* in NCBI.

Table 4. Firefly COI sequences submitted to GeneBank and their accession numbers

Sl. No.	Species	Place of collection	Accession No.
1	<i>Abscondita sp.</i>	Kozhikamuthi	PQ901427
2	<i>Abscondita sp.</i>	Manthirimattam	PQ901428
3	<i>Abscondita sp.</i>	Topslip	PQ901429
4	<i>Abscondita sp.</i>	Varagalayar	PQ901430
5	<i>Abscondita sp.</i>	Odaipallam	PQ901431
6	<i>Asymmetricata sp.</i>	Varagalayar	PQ901432
7	<i>Curtos sp.</i>	Anaikundhi view point	PQ901433
8	<i>Lamprigera sp.</i>	Manambolli	PQ901434
9	<i>Abscondita sp.</i>	Saralopathy	PQ901435
10	<i>Abscondita sp.</i>	Saralopathy	PQ901436

11	<i>Lamprigera</i> sp (Larva)	Manambolly	PV397448
12	<i>Pyrocoelia</i> sp (Larva)	Manambolly	PV397449
13	<i>Pyrocoelia</i> sp (Larva)	Manthirimattam	PV397450
14	<i>Abscondita perplexa</i>	Topslip	PV402670
15	<i>Abscondita perplexa</i>	Topslip	PV402671
16	<i>Abscondita</i> sp.	Vandalur	PV402672
17	<i>Abscondita</i> sp.	Vandalur	PV402673
18	<i>Abscondita</i> sp.	Vandalur	PV402674
19	<i>Abscondita terminalis</i>	Saralapathy	PV402675
20	<i>Abscondita terminalis</i>	Saralapathy	PV402676
21	<i>Abscondita terminalis</i>	Saralapathy	PV402677
22	<i>Pyrocoelia</i> sp	Manambolly	PV452788

6.5. Evolutionary relationships of 22 firefly sequences

A phylogenetic tree was constructed using the 22 COI sequences of five firefly genera (Fig. 27). The evolutionary history was inferred using the Neighbour-Joining method (Saitou and Nei, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). The neighbor-joining tree has five distinct clades, each occupied by different firefly genus. The largest clade was formed by *Abscondita* that comprised *A. perplexa* and *A. terminalis*.

The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) and are in the units of the number of base substitutions per site. The branch length of genus *Asymmetricata* is the longest which indicates that this genus is very primitive.

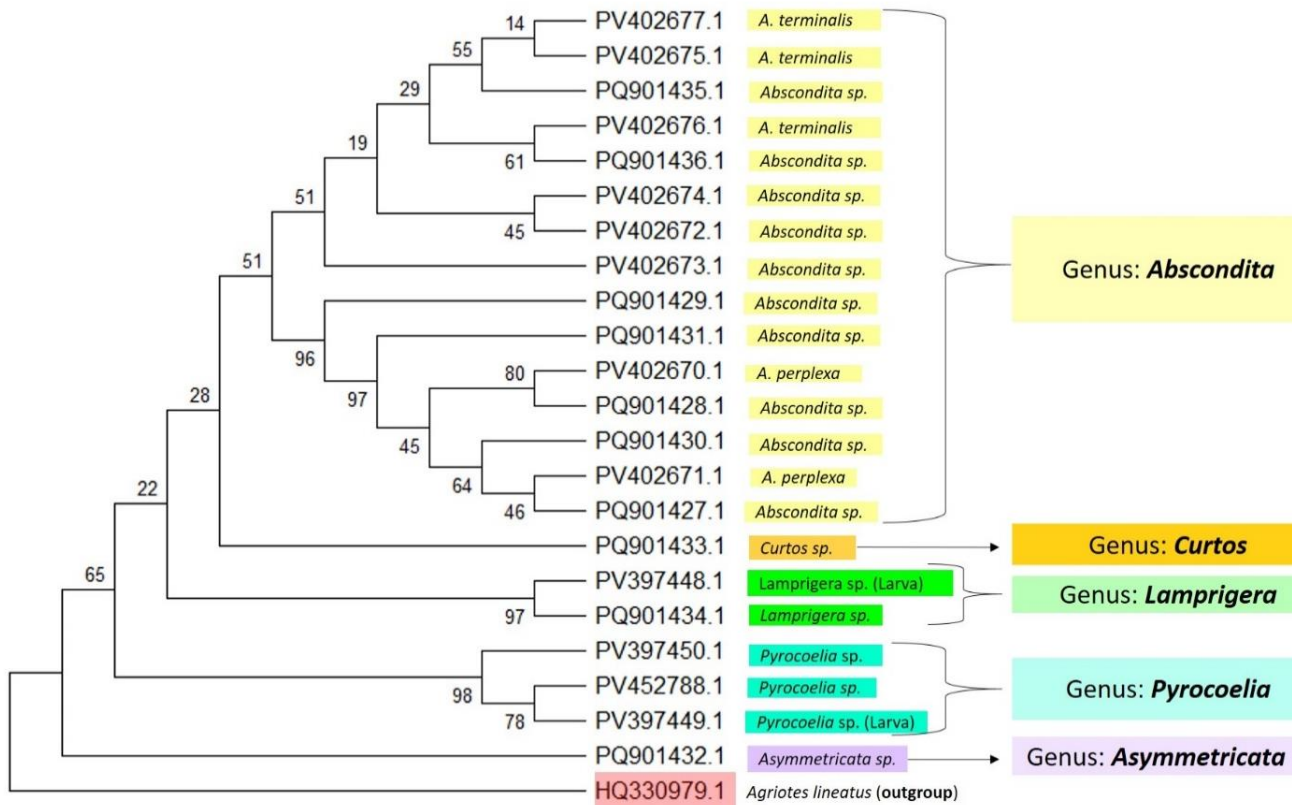


Figure 27. Neighbor-Joining tree to analyse the evolutionary relationships between different firefly species collected from ATR

6.6. Phototactic movement behaviour

The phototactic movement of adult firefly beetles in response to six different wavelengths of coloured light was studied using a specialized experimental setup. Six different colour lights, namely yellow, green, red, orange, pink and white, were used in the study. This experiment aimed to determine the colour light preferences or aversions of fireflies. To quantify this response, the Excess Proportion Index (EPI) was calculated to analyse and assess the extent to which each light colour influenced firefly distribution within the experimental chamber.

The excess proportion index ranges from +1 to -1. Plus values indicate that the organism shows preference, and negative values indicate avoidance behaviour. In the present study, the EPI values for yellow and orange lights were -1, which indicated the complete avoidance behaviour of the fireflies towards these two colours. Other colour lights such as green, blue, pink and red also showed negative EPI values, and it was understood that fireflies avoid these colours. Fireflies showed the highest preference towards dark places. Interestingly, it was observed that white light was neither preferred nor avoided by the fireflies. The number of fireflies which preferred the white LED light was equal to that of the control (dark side) and hence the EPI value was '0' for white light (Table 5).

Table 5. Excess Proportion Index (EPI) values of fireflies against different colour lights (Values are the mean of 5 replications)

Sl. No.	Test (T) / Control (C)	Colour light	Mean Number of Fireflies Present	(NT - NC)	(NT + NC)	EPI = (NT - NC) / (NT + NC)
1	Test (T)	White	2.8	0	5.6	0
2		Green	0.25	-2.55	3.05	-0.836
3		Blue	0.5	-2.3	3.3	-0.696
4		Pink	0.75	-2.05	3.55	-0.577
5		Red	0.5	-2.3	3.3	-0.696
6		Yellow	0	-2.8	2.8	-1
7		Orange	0	-2.8	2.8	-1
8	Control (C)	Dark (No light)	2.8			

NT = Number of fireflies in the Test; NC = Number of fireflies in the Control (Dark side)

6.7. Quadrat study

Random quadrat sampling was conducted at Kozhikamuthi, Varagalayar and Topslip for firefly larval survey. A total of 48 quadrats were sampled, but no firefly larvae were observed during the study.

6.8. Questionnaire survey

In the questionnaire survey, a total of 171 individuals (103 males and 68 females) participated. Among these participants, 92 individuals were from Tribal communities and 17 participants were employed in the Forest Department as Forest Range Officer (1 person), Foresters (4 persons), Forest Guards (4 persons), Forest Watchers (2 persons) and Anti-Poaching Watchers (6 persons).

Among the 171 participants, only 166 individuals reported having seen fireflies in ATR region. These 166 individuals were further involved in this survey. When asked about the season in which firefly activity was most prevalent, 3.6% of the participants were unable to provide a correct answer, while 48.7% indicated that firefly activity was observed during the summer season. Firefly activity was also noted in the rainy season, pre-monsoon and Post-monsoon seasons by 24%, 7.5% and 16.2% of individuals, respectively. The time of sighting was reported as between 6:00 PM and 9:00 PM by 84.8% of participants. Firefly habitats included trails (26.7% of respondents), followed by woody vegetation (19.6%), grassland (14%) and streams (13.8%). Among the total respondents, 51.2% indicated that the firefly population has decreased over the years, while 36.8% stated that it has increased. The remaining 12.1% of participants reported no change in the firefly population.

When asked about the reasons for the decline in firefly populations, 23% of participants cited pesticide use as the primary cause, while 10.3% attributed it to water pollution. Regarding the impact of light pollution on fireflies, 67.5% of participants believed that light would not affect fireflies, whereas 21.7% thought that light pollution would have an affect. Approximately 10.8% of participants did not respond to this question.

7. DISCUSSION

In the present study, firefly fauna from 10 different sites across ATR was documented. Species identification was done by both morphological and molecular methods. A total of eight species were documented from ATR, representing a significant increase in species richness compared to earlier studies, where only three species were recorded from the Ulandhy range by Sriram *et al.* (2023). Among the identified species, *Abscondita perplexa* and *A. terminalis* were the most commonly observed species in the study areas. In India, *Abscondita perplexa* has been recorded from Assam, Madhya Pradesh, Odisha and West Bengal (Ballantyne *et al.*, 2013; Ballantyne *et al.*, 2019; Ghosh *et al.*, 2023). However, *Abscondita terminalis* has been reported from West Bengal and Odisha (Ghosh *et al.* 2021, 2023a). *Asymmetricata humeralis* has been recorded in Goa, Maharashtra, Karnataka, Tamil Nadu, and Kerala (Ballantyne *et al.*, 2019).

The study may be the first of its kind since no other reports on firefly fauna are available in ATR. Two morphologically distinct species of *Curtos* and one species of *Lamprigera* have been documented in this study. Previously *Curtos* sp. has been reported from Karnataka (Gorham, 1895). The *Lamprigera* species has already been reported from India, Nepal, Burma, China, Taiwan, Indonesia, Western Indonesia and Sri Lanka. Especially *Lamprigera crassus* and *Lamprigera tenebrosa* were recorded in Pondicherry (Gorham, 1880; Walker, 1858; McDermott, 1966; Dang *et al.*, 2021).

DNA barcoding proved to be a reliable method for identifying species and has been utilized to identify fireflies in Southeast Asian nations (Jusoh *et al.*, 2020; Zhu *et al.*, 2022; OnahandAleke, 2023). Codon usage data from DNA barcoding can be utilized as a reliable taxonomic marker across insect species. It shows amino acids' functions, evolutionary patterns, and helps to trace the

unique evolutionary process at the subfamily or family level (Pentinsaari *et al.*, 2016). The barcoding technique helps to analyse phylogenetic relationships among different organisms and create accurate evolutionary trees (Karthika *et al.*, 2016). In the present study, the phylogenetic tree constructed by neighbor-joining approach clearly showed that each genus formed a distinct clade.

To complement field studies, a questionnaire survey was conducted among tribal communities and forest department staff in the region. The questionnaire gathered local ecological knowledge regarding firefly habitat, seasonal abundance, natural enemies and threats. The use of questionnaires for ecological research has historical precedence, with origins dating back to Sir Francis Galton in the late 1800s. In research fields, questionnaires are widely used to collect quantitative data from the public to identify problems (Roopa and Rani, 2012).

The study clearly showed that ATR provides a conducive habitat for nearly eight species of fireflies. Although this study was done only for ten months, a long-term study may bring out a more comprehensive diversity of fireflies at ATR.

Future study

In the future, the researchers may focus on the ecological significance of different firefly species and the environmental factors which are influencing their population dynamics within ATR. Understanding these crucial aspects will be vital for developing conservation strategies and in addition, outreach and awareness programs for local school students, and forest-dwelling communities may play a significant role in firefly conservation efforts, ensuring the protection of these ecologically important insects in their natural habitats.

8. SUMMARY

- Diversity and distribution of fireflies were studied at Anamalai Tiger Reserve (ATR) from April 2024 to March 2025.
- Firefly larvae and adults were sampled by sweep net and manual collection methods along 100 m transect lines from 10 different locations viz., Anaikundhi viewpoint, Kannadi Bungalow, Kozhikamuthi, Manambolly, Manthirimattam, Saralapathi, Savamalai Estate, Topslip, Urulikkal checkpoint and Varagalayar in 3 ranges namely Manambolly, Pollachi and Ulandy.
- Eight different species of fireflies, namely *Abscondita perplexa*, *Ab. terminalis*, *Asymmetricata humeralis*, *Curtos* sp. 1, *Curtos* sp. 2, *Lamprigera* sp., *Pyrocoelia* sp. and one unidentified sp., were collected during the study period.
- Phototactic movement behaviour study showed that *A. perplexa* preferred darkness and avoided six different colours, namely blue, green, yellow, orange, pink and red. White light was neither preferred nor avoided.
- DNA barcoding by amplifying a partial sequence of the mitochondrial Cytochrome C oxidase subunit 1 (mt COI) gene helped the confirmation of morphologically identified species.
- Phylogenetic analysis clearly showed a distinct, separate group of clade for each genus.
- Questionnaire survey in the ATR among the local people clearly showed that 97% of people have knowledge about fireflies and the decline of firefly population was mainly due to pesticide use and water pollution.
- Fireflies are incredibly sensitive to changes in their habitats, especially to pollution, habitat disruption, and light contamination. So, preserving forests, wetlands and meadows is essential for their conservation.

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CONTACT

Principal Chief Conservator of Forests & Director,
Advanced Institute for Wildlife Conservation,
Tamil Nadu Forest Department,
Vandalur, Chennai - 600 048.
E-mail: aiwcrte@tn.gov.in

Office of Director: 044-29372331
For any queries E-mail to: aiwcrte@tn.gov.in
Website: <https://www.aiwc.res.in>