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Moth diversity of Guindy, Chennai, India and DNA barcoding of selected erebid moths

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Abstract: In this study, diversity of moths has been documented from Chennai, the capital city of Tamil Nadu. During the study, over 100 specimens were collected from which 59 moth species were identified from the commercial hub of Chennai, Guindy. The species identified belonged to 52 genera, 11 families, and 25 subfamilies. Erebiidae was a front runner, followed by Crambidae, Geometridae, Sphingidae, and Noctuidae. Furthermore, Eupterotidae, Uraniidae, Nolidae, Lasiocampidae, Pterophoridae, and Thyrididae were the least recorded families. Among 26 erebids, 14 species were subjected for identification through mitochondrial cytochrome oxidase subunit 1 gene to resolve the ambiguity. The sequences resulted were deposited in GenBank and BOLD system where they received accession numbers and process IDs. Further, phylogenetic analysis categorized *Metanastria hyrtaca* Cramer, 1782 in a separate clade.

Keywords: Barcode, biodiversity, conservation, Erebiidae, moths.

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Author contributions: S. Bhuvargavan: Conceptualization, sample collection, investigation, interpretation, preparation of manuscript draft and funding acquisition. M. Meenakumari: Investigation and data curation. R. Nivetha: Interpretation of results, data curation and preparation of manuscript draft. S. Janarthanan: Project administration, supervision, funding acquisition, review and edit of manuscript.

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INTRODUCTION

With about 1.2 million species, arthropods continue to be a dominant group in the earth's biodiversity. Their significance in sustaining the health of an ecosystem by furnishing livelihood and nutrition to human communities is far-reaching (Chakravarthy & Sridhara 2016). Nevertheless, insects are contemplated to be a potential group for understanding the effects of habitat attributes and environmental gradients on faunal diversity (Watt et al. 1997; Humphrey et al. 1999; Dey et al. 2017). Lepidoptera, which encompasses butterflies and moths, constitutes one of the three most species-rich insect orders and the largest evolutionary radiation of herbivorous animals comprising around 175,000 described species (Cover & Bogan 2015). However, another 125,000 to 150,000 species are thought to await description (Goldstein 2017). It exhibits close association with vegetation, their depletion and ensuing regeneration and is accordingly regarded as an indicator taxon (Summerville et al. 2004; Dey et al. 2015). Moths, being the most prominent terrestrial invertebrates, represent the majority of the order Lepidoptera consisting 158,570 described species (Zhang 2013). An estimation of about 15000 species of Lepidoptera belonging to 84 families are reported from India (Chandra et al. 2019). They form a critical facet of the terrestrial ecosystem by serving as nocturnal pollinators, herbivores of crops and prey for numerous species (Wagner et al. 2021). Many angiospermous plants that largely depend on animal-assisted pollination are critically associated with moth species (Wahlberg et al. 2013). Erebidae is the most prominent moth family consisting of 24,569 species belonging to 18 subfamilies (Nieukerken et al. 2011). Most of them are phytophagous as larvae and few are nectar suckers as adults (Terra & Ferreira 2020). The economic importance of family Erebidae can be attributed to the fact that it includes a significant number of major and minor pest species, and therefore their distributional knowledge is highly significant for the economy of any country (Bin-Cheng 1994). Furthermore, exploring the changes in the pattern associated with moth distribution and abundance in different local habitats constitutes a significant element of global biodiversity monitoring and conservation (Dennis et al. 2019).

Classification of organisms is a prerequisite for understanding their distribution and diversity in any habitat. Classification of closely related lepidopteran species based on wing patterns and other morphological attributes poses difficulties and imprecision those are amenable to change as a function of environment and

prevalence of several biotypes. Over the last few years, DNA barcodes are known to answer elemental ecological questions that govern community assemblage, processes of macroevolution, species conservation and incorporation of molecular tools along with morphology, which can add value to the existing information on moth diversity (Dey et al. 2019). A cytochrome oxidase subunit 1 (COI) gene identification system is contemplated to be more reliable, economical and a quick fix to the problems involved in species identification (Hebert et al. 2003). Since Hebert et al. (2003), order Lepidoptera has been regarded as a model group for DNA-barcoding studies (Goldstein 2017). Several studies have been carried out to investigate the moth diversity in peninsular India, yet Tamil Nadu has only fewer studies especially minuscule information in Chennai metropolitan, as follows. Reports of 154 species of noctuid moths from the Tamil Nadu part of Western Ghats, 67 species of erebid moths and 105 moth species from Maruthamalai hills are notable among them (Sivasankaran & Ignacimuthu 2014). Close to 135 species have been recorded in Valmiki Nagar, Chennai (Nagarajan et al. 2021). Besides being an ecologically significant group, they are less explored, finding their way into the present biodiversity conservation scenario (Dey et al. 2015). Despite rich lepidopteran diversity existing in India, attempts that are made to generate DNA barcode data of moths in India are very scarce (Dey et al. 2019; Kumar et al. 2019). Urban areas are considered significant drivers of biodiversity change due to expressively transformed landscape changes and rapid anthropogenic actions (Zari 2018). Declines in the diversity and abundance of moth population are reported over the past few years due to explicit factors like loss of habitat, fragmentation, pollution, urbanization and other related anthropogenic practices (Dennis et al. 2019; Hallmann et al. 2020). There is a research gap in knowledge of how the aforementioned explicit factors impact the diversity and abundance of population of moths in an urban environment. Consequently, an attempt was made to generate a preliminary checklist of moth fauna from Guindy, a commercial hub in Chennai and further species authentication of selected erebid moths to resolve ambiguity in identification using mitochondrial COI gene.

MATERIALS AND METHODS

Study area

The study was conducted in Guindy, one of the largest Southern neighbourhoods of Chennai, Tamil

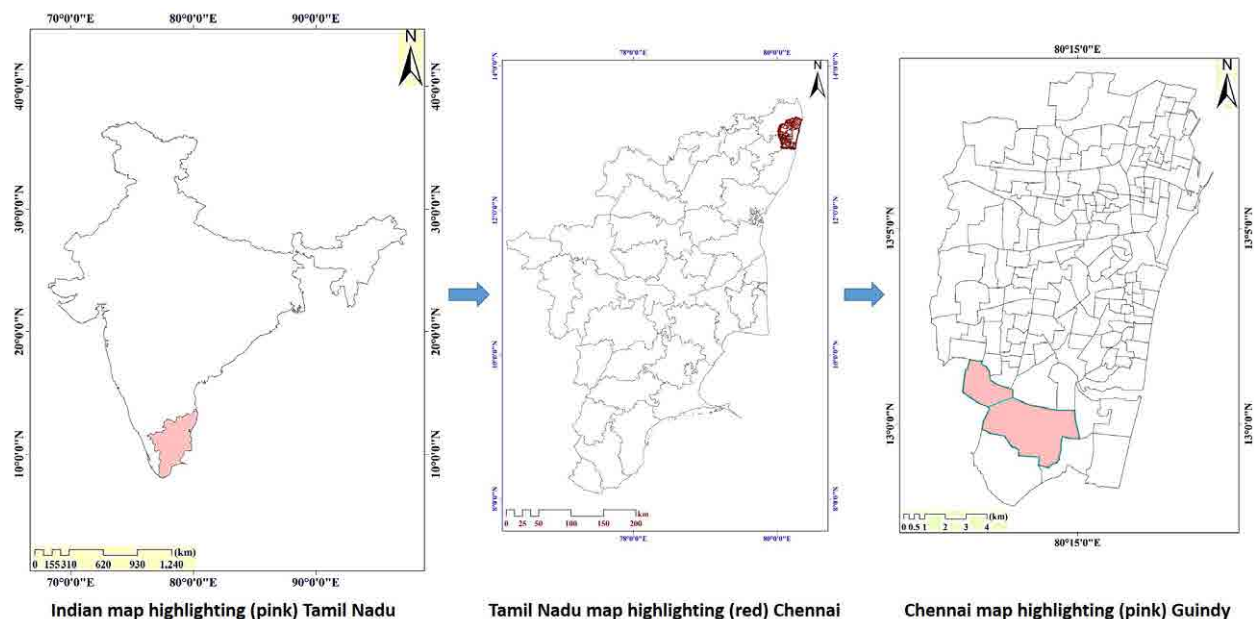


Figure 1. Map depicting the study area (Guindy, Chennai, Tamil Nadu) generated using ArcGIS (v10.8) software.

Nadu (Figure 1). It is located between 13.010236° N latitude and 80.215652° E longitude. Guindy National Park is situated inside the city covering an area of 2.70 km² lies between 12.99° N, 80.23° E and 13.00° N, 80.21° E consisting of single habitat type, dry evergreen woodland.

Sample collection and identification

Moth species were collected using traps consisting of light source (Mercury vapour light) during night from places in and around Guindy, Chennai. The collected specimens were identified by their morphological characters using manuals of Bell & Scott (1937) and Hampson (1892, 1895, 1896). They were killed using chloroform, pinned using entomological pins and stretched on spreading board. Later, they were oven-dried at 52°C and were preserved in the insect box. The stretched specimens were photographed using Nikon camera after drying.

Genomic DNA extraction, PCR amplification (COI gene) and sequencing

Species authentication was carried out using the mitochondrial COI gene to resolve ambiguity in identifying 14 selected *Erebidae* individuals. Total genomic DNA from individual species was extracted from the legs using the phenol-chloroform method. DNA extracted were then resuspended in Tris-ethylenediaminetetraacetic acid (EDTA) buffer (TE buffer) and stored at -20°C until further use. The lepidopteran specific COI

primers of Hebert et al. (2003) [Forward primer - F: 5'-ATTCAACCAATCATAAAGATATTGG-3'; Reverse primer - R: 5'-TAAACTTCTGGATGTCCAAAAATCA-3'] were used to amplify regions of COI from 14 species of moths belonging to the *Erebidae* family that exhibited uncertainty in their identification using taxonomic keys. PCR amplification was carried out in a total volume of 10 µl consisting of Ampliqon-Taq DNA Polymerase 2x Master Mix RED, lepidopteran specific COI primers of Hebert et al. (2003), template DNA and sterile water (MyGene Series, Peltier Gradient Thermal Cycler). The reaction mixture was initially denatured for 5 min at 94°C followed by 35 cycles of denaturation at 94°C for 1 min, annealing of 56°C for 1 min, extension of 72°C for 1 min and a final extension cycle of 72°C for 7 min. It was then stored at 4°C. A control reaction was prepared without template DNA. A 1.2% agarose gel stained with ethidium bromide was used to examine the amplified gene product. It was then gel purified and sequenced using the Sanger dideoxynucleotide sequencing protocol (AgriGenome Labs, Kochi). Sequences were then analysed with the National Centre for Biotechnology Information (NCBI) Blast Server and submitted in NCBI GenBank and Barcode of Life Data (BOLD) system to obtain corresponding accession numbers and process IDs.

Phylogenetic analysis

A phylogenetic tree was constructed using MEGA X: Molecular Evolutionary Genetics Analysis across

computing platforms to study the evolutionary relationship among various species identified (Kumar et al. 2018). The Neighbour-Joining method was used to infer the evolutionary history, and the Kimura 2-parameter method was used to compute evolutionary distances (Kimura 1980). Bootstrap analysis was also performed using MEGA X (10000 replicates). The available (database) mitochondrial COI gene sequences of morphologically-identified species (38) (among the 45 species) were retrieved from NCBI for constructing phylogenetic tree along with COI gene-based identified species (14) in this study. Multiple sequence alignment was carried out before the construction of the phylogenetic tree using CLUSTALW multiple alignment available as accessory application in BioEdit software. All the sequences were then subjected to evolutionary analysis by phylogenetic tree construction using neighbour-joining method mentioned above.

RESULTS

Distribution profile of moth fauna from Guindy, Chennai

59 species were identified, and a checklist was constructed along with their scientific name, common name, family and subfamily (Table 1, Image 1–7). The 59 species identified belonged to 52 genera and 11 families such as Erebidae, Crambidae, Geometridae, Sphingidae, Noctuidae, Eupterotidae, Lasiocampidae, Nolidae, Pterophoridae, Thyrididae and Uraniidae (Figure 2). As a result of the comparative distribution, family Erebidae was higher in numbers with a total of 26 species (21 genera and 25 species), followed by the families such as Crambidae with 10 species (9 genera and 10 species), Geometridae with 8 species (7 genera and 6 species), Sphingidae with 5 species (5 genera and 5 species) and Noctuidae with 4 species (4 genera and 3 species); while families viz. Eupterotidae, Lasiocampidae, Nolidae, Pterophoridae, Thyrididae and Uraniidae accounted for single species each. The Family Erebidae was observed to be a species-rich group in Guindy, Chennai.

Mitochondrial COI gene amplification

The lepidopteran specific COI primers of Hebert et al. (2003) did amplify COI gene from all the 14 erebid species. The product was then gel purified, sequenced, and analysed. To resolve ambiguity in identification of Erebid moths, the DNA barcoding was adopted and the sequence results identified 14 different species of Erebidae which includes *Achaea janata* (Linnaeus,

1758), *Achaea mercatoria* (Fabricius, 1775), *Amata passalis* (Fabricius, 1781), *Asota caricae* (Fabricius, 1775), *Cretonotos gangis* (Linnaeus, 1763), *Erebus caprimulgus* (Fabricius, 1781), *Erebus macrops* (Linnaeus, 1768), *Eudocima materna* (Linnaeus, 1767), *Eudocima phalonia* (Linnaeus, 1763), *Hypocala deflorata* (Fabricius, 1794), *Olepa schleini* (Witt et al. 2005), *Perina nuda* (Fabricius, 1787), *Sphingomorpha chlorea* (Cramer, 1777) and *Utetheisa pulchelloides* (Hampson, 1907). The representative amplified COI gene is presented in Figure 3. The nucleotide sequences of mitochondrial COI gene from all the 14 species were deposited in GenBank and BOLD system where they received individual accession numbers and process IDs, respectively (Table 2).

Phylogenetic analysis

MEGA X: Molecular Evolutionary Genetics Analysis was used to construct a phylogenetic tree to infer the evolutionary relationship among various identified species of moths. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10000 replicates) was shown next to the branches. The Neighbourhood joining method was used instead of maximum parsimony or maximum likelihood approaches because of its accuracy, rapidity and optimum assumptions (Hong et al. 2021). The results of the phylogenetic analysis are shown in Figure 4, with *Apis mellifera* being the outgroup. *Metanastria hyrtaca* (Cramer, 1782) formed a separate clade, and all other species were clustered in another clade.

DISCUSSION

Species identification is a prerequisite in estimating biodiversity in an area and perceiving knowledge on species ecology. Thus, explicit identification is obligatory to gain insights into any species' diversity and distribution profile in any place under study. Morphological identification and taxonomic keys are important methods used extensively (Sviridov & Leuschner 1986). Notably, among the various moths collected in this study, moths belonging to Erebidae family dominated others. Presumably, their polyphagous nature could be the impetus for their wide distribution, making them fit to survive in any resource condition (Zahiri et al. 2012). A similar domination pattern of erebid moths was also observed in the Northern part of the Western Ghats (Shubhalaxmi et al. 2011; Gurule & Nikam 2013). These are then accompanied by species belonging to the family Crambidae, the second most prominent family,

Table 1. Checklist of moth fauna from Guindy, a commercial hub in Chennai.

	Family	Subfamily	Species (Common name)	Author & year
1	Crambidae	Pyraustinae	<i>Maruca vitrata</i> (Bean pod borer)	Fabricius, 1787
2	Crambidae	Pyraustinae	<i>Omphisa anastomosalis</i> (Sweetpotato vineborer)	Guenée, 1854
3	Crambidae	Pyraustinae	<i>Spoladea recurvalis</i> (Beet Webworm Moth)	Fabricius, 1775
4	Crambidae	Spilomelinae	<i>Botyodes asialis</i>	Guenée, 1854
5	Crambidae	Spilomelinae	<i>Cnaphalocrocis medinalis</i> (Rice leaf roller)	Guenée, 1854
6	Crambidae	Spilomelinae	<i>Cnaphalocrocis poeyalis</i> (Lesser rice- leafroller)	Boisduval, 1833
7	Crambidae	Spilomelinae	<i>Diaphania indica</i> (Cucumber Moth)	Saunders, 1851
8	Crambidae	Spilomelinae	<i>Haritalodes derogata</i> (Cotton leaf roller)	Fabricius, 1775
9	Crambidae	Spilomelinae	<i>Isocentris filalis</i>	Guenée, 1854
10	Crambidae	Spilomelinae	<i>Palpita vitrealis</i> (Jasmine Moth)	Rossi, 1794
11	Erebidae	Aganainae	<i>Asota caricae</i> (Tropical Tiger Moth)	Fabricius, 1775
12	Erebidae	Arctiinae	<i>Amata passalis</i> (Sandalwood defoliator)	Fabricius, 1781
13	Erebidae	Arctiinae	<i>Cretonotos gangis</i> (Baphomet Moth)	Linnaeus, 1763
14	Erebidae	Arctiinae	<i>Olepa schleini</i>	Witt et al. 2005
15	Erebidae	Arctiinae	<i>Utetheisa pulchelloides</i> (Heliotrope Moth)	Hampson, 1907
16	Erebidae	Calpinae	<i>Eudocima materna</i> (Dot-underwing Moth)	Linnaeus, 1767
17	Erebidae	Calpinae	<i>Eudocima phalonia</i> (Common fruit-piercing Moth)	Linnaeus, 1763
18	Erebidae	Catocalinae	<i>Achaea janata</i> (Castor semi-looper)	Linnaeus, 1758
19	Erebidae	Erebinae	<i>Achaea mercatoria</i>	Fabricius, 1775
20	Erebidae	Erebinae	<i>Dysgonia stuposa</i>	Fabricius, 1794
21	Erebidae	Erebinae	<i>Erebus caprimulgus</i>	Fabricius, 1781
22	Erebidae	Erebinae	<i>Erebus macrops</i> (Common Owl Moth)	Linnaeus, 1768
23	Erebidae	Erebinae	<i>Lacera noctilio</i>	Fabricius, 1794
24	Erebidae	Erebinae	<i>Ophiura tirhaca</i> (Green Drab)	Cramer, 1777
25	Erebidae	Erebinae	<i>Pericyma cruegeri</i> (Poinciana looper)	Butler, 1886
26	Erebidae	Erebinae	<i>Sphingomorpha chlorea</i> (Sundowner Moth)	Cramer, 1777
27	Erebidae	Hypeninae	<i>Hypena obacerralis</i>	Walker, 1859
28	Erebidae	Hypocalinae	<i>Hypocala deflorata</i>	Fabricius, 1794
29	Erebidae	Lymantriinae	<i>Artaxa digramma</i>	Boisduval, 1844
30	Erebidae	Lymantriinae	<i>Euproctis scintillans</i> (Lymantriid Moth)	Walker, 1856
31	Erebidae	Lymantriinae	<i>Euproctis similis</i> (Yellow-tail Moth)	Fuessly, 1775
32	Erebidae	Lymantriinae	<i>Laelia exclamationis</i>	Kollar, 1848
33	Erebidae	Lymantriinae	<i>Laelia litura</i> (Tussock Moth)	Walker, 1855
34	Erebidae	Lymantriinae	<i>Olene mendosa</i> (Brown Tussock Moth)	Hübner, 1823
35	Erebidae	Lymantriinae	<i>Perina nuda</i> (Clearwing Tussock Moth)	Fabricius, 1787
36	Erebidae	Scoliopteryginae	<i>Anomis</i> spp.	Hübner, 1821
37	Eupterotidae	Eupterotinae	<i>Eupterote bifasciata</i> (Giant Lappet Moth)	Kishida, 1994
38	Geometridae	Ennominae	<i>Iridopsis larvaria</i> (Bent-lined Gray)	Guenée, 1858
39	Geometridae	Ennominae	<i>Chiasmia eleonora</i>	Cramer, 1780
40	Geometridae	Ennominae	<i>Chiasmia</i> spp.	Cramer, 1780
41	Geometridae	Ennominae	<i>Macaria multilineata</i> (Many-lined Angle)	Packard, 1873
42	Geometridae	Ennominae	<i>Cleora</i> spp.	Curtis, 1825
43	Geometridae	Geometrinae	<i>Thalassodes veraria</i>	Guenée, 1858
44	Geometridae	Geometrinae	<i>Nemoria bistriaria</i> (Red-fringed Emerald)	Hübner, 1818

	Family	Subfamily	Species (Common name)	Author & year
45	Geometridae	Sterrhinae	<i>Idaea sylvestraria</i> (Dotted Border Wave)	Hübner, 1799
46	Lasiocampidae	Pinarinae	<i>Metanastria hyrtaca</i> (Hairy caterpillar)	Cramer, 1782
47	Noctuidae	Hadeninae	<i>Chasmina candida</i>	Walker, 1865
48	Noctuidae	Heliethinae	<i>Helicoverpa armigera</i> (Cotton Bollworm)	Hübner, 1808
49	Noctuidae	Noctuinae	<i>Spodoptera litura</i> (Tobacco Cutworm)	Fabricius, 1775
50	Noctuidae	Noctuinae	<i>Mythimna</i> spp.	Ferdinand Ochsenheimer, 1816
51	Nolidae	Nolinae	<i>Nola analis</i>	Wileman & West, 1928
52	Pterophoridae	Pterophorinae	<i>Geina periscelidactyla</i> (Grape Plume Moth)	Fitch, 1855
53	Sphingidae	Macroglossinae	<i>Hippotion boerhaviae</i> (Hippotion Sphinx Moth)	Fabricius, 1775
54	Sphingidae	Macroglossinae	<i>Nephele hespera</i> (Crepuscular Hawkmoth)	Fabricius, 1775
55	Sphingidae	Sphinginae	<i>Acherontia lachesis</i> (Greater death's head Hawkmoth)	Fabricius, 1798
56	Sphingidae	Sphinginae	<i>Agrius convolvuli</i> (Convolvulus Hawkmoth)	Linnaeus, 1758
57	Sphingidae	Sphinginae	<i>Psilogamma increta</i> (Plain grey Hawkmoth)	Walker, 1864
58	Thyrididae	Striglinae	<i>Strigina scitaria</i> (Daincha leaf webber)	Walker, 1862
59	Uraniidae	Microniinae	<i>Micronia aculeata</i> (Asian Spotted Swallowtail Moth)	Guenée, 1857

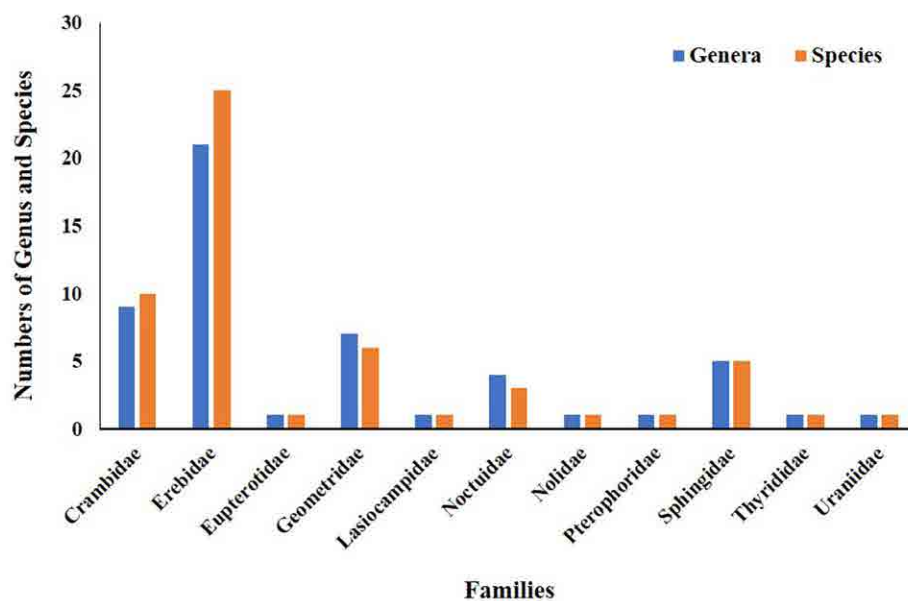


Figure 2. The species richness of moth fauna in relation to their families from Guindy, Chennai.

which is attributed to the phytophagous, detritivorous, coprophagous, parasitic habits of their larvae and ability to feed on roots, stems or grasses (Nayak & Ghosh 2020). This is followed by the distribution of Geometridae, the next abundant moth family. Comparatively, the least documented families were Eupterotidae, Uraniidae, Nolidae, Lasiocampidae, Pterophoridae and Thyrididae. Twenty-six species belonging to 18 genera of family Pterophoridae were identified and examined from the Shiwalik hills of North-West India (Pooni et al. 2019).

In an attempt to document the moth fauna of Goa, *Collinsa decoratalis* (Warren, 1986), a thyridid moth, was reported as a new record from the Western Ghats. In addition to this, the uraniid moth *Pseudhyria rubra* (Hampson, 1891) was also reported for the first time from Goa (Gurule & Brookes 2021). Estimated diversity and distribution of moths in Nanda Devi Biosphere Reserve, Shendurney and Ponmudi in Agastyamalai Biosphere Reserve, Tawang district (Arunachal Pradesh) recorded that the most abundant family was Geometridae

(Chandra & Sambath 2013; Dey et al. 2015; Sondhi et al. 2018). Geometrid moths were found in abundance at tea plantations of North-East India (Sinu et al. 2013). However, Erebiidae was the most profusely distributed family in Vagamon hills (Western Ghats), Dehradun and Devalsari, North East Jharkhand, Midnapore town (West Bengal) and Banaras Hindu University, Varanasi (Sondhi & Sondhi 2016; Singh et al. 2017; Nayak & Ghosh 2020; Nayak & Sasmal 2020).

Family Erebiidae is copiously found in a diverse habitat, which includes predominantly polyphagous species and pests. The discovery of the species *Asota paliura* (Swinhoe 1893) belonging to the family Erebiidae from India was also reported (Rajan & Shamsudeen 2020). A tentative list of Erebiidae from the Tamil Nadu part of Western Ghats is documented as well (Sivasankaran & Ignacimuthu 2014). In addition, based on the survey made in Tamil Nadu at different localities, the genus *Othreis* (Synonym *Eudocima*) (Linnaeus, 1763) was one among the two genera of predominant fruit piercers, which is by far the most harmful and a severe pest on citrus, guava, pomegranate, grapes, fig, sapota, mango, papaya, and tomato in India (Ramkumar et al. 2010). An endemic Indian moth, *Gurna indica* (Moore, 1879) of the Erebiidae family, was rediscovered after 125 years (Kalawate et al. 2019). An attempt has been made to document the species of Erebid moths from Aligarh, Uttar Pradesh, India (Farooqui et al. 2020). In addition, the discovery of *Asota paliura* (Swinhoe, 1893) (Lepidoptera: Erebiidae) represents a new record from India (Rajan & Shamsudeen 2020). Similarly, *Pericyma cruegeri* (Butler, 1886) was also reported for the first time in India (Singh & Ranjan 2016). New additions of eight species to the known Indian fauna of the family Erebiidae was also accounted (Kirti et al. 2017). Recently, moth diversity and preliminary checklist of moths from different regions of Rajasthan including Sariska Tiger Reserve were reported (Dar et al. 2021a,b; Jamal 2021). Additionally, there is also first report of Oleander Hawkmoth, *Daphnis nerii* (Linnaeus, 1758) from India (Dar et al. 2022).

DNA barcoding is a proven tool used for expeditious and unambiguous identification of species, thus circumventing the problems associated with morphology-based identification of species (Hebert & Gregory 2005). PCR amplification of short fragments within the barcoding region of the COI gene has been comprehensively used to identify different species. Sustainable identification relies mainly upon the construction of a system that utilizes DNA sequences as taxon barcodes. The mitochondrial COI gene was

Table 2. GenBank accession numbers and BOLD process IDs for erebid species authenticated using mitochondrial COI gene.

	Species	GenBank accession number	BOLD Process ID
1	<i>Achaea Janata</i>	MW421768	DBEM007-21
2	<i>Achaea mercatoria</i>	MW425700	DBEM008-21
3	<i>Amata passalis</i>	MW425697	DBEM002-21
4	<i>Asota caricae</i>	MW425696	DBEM001-21
5	<i>Cretonotos gangis</i>	MW425695	DBEM014-21
6	<i>Erebus caprimulgus</i>	MW435024	DBEM009-21
7	<i>Erebus macrops</i>	MW425705	DBEM010-21
8	<i>Eudocima materna</i>	MW425702	DBEM005-21
9	<i>Eudocima phalonia</i>	MW425701	DBEM006-21
10	<i>Hypocala deflorata</i>	MW407951	DBEM012-21
11	<i>Olepa schleini</i>	MW425704	DBEM003-21
12	<i>Perina nuda</i>	MW425699	DBEM013-21
13	<i>Sphingomorpha chlorea</i>	MW425703	DBEM011-21
14	<i>Utetheisa pulchellodes</i>	MW425698	DBEM004-21

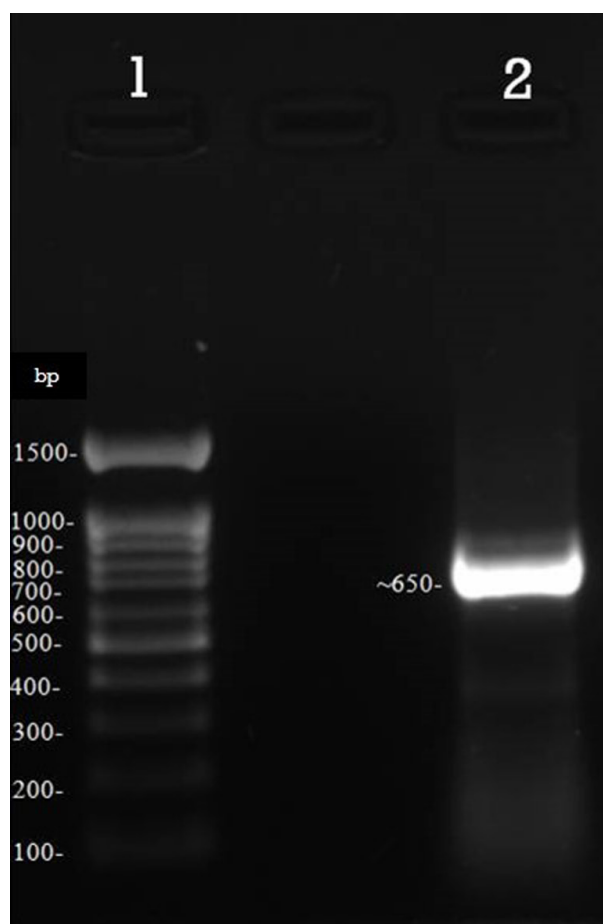


Figure 3. Electrophoresis of representative mtCOI gene: Lane 1—DNA ladder | Lane 2—Amplified product.

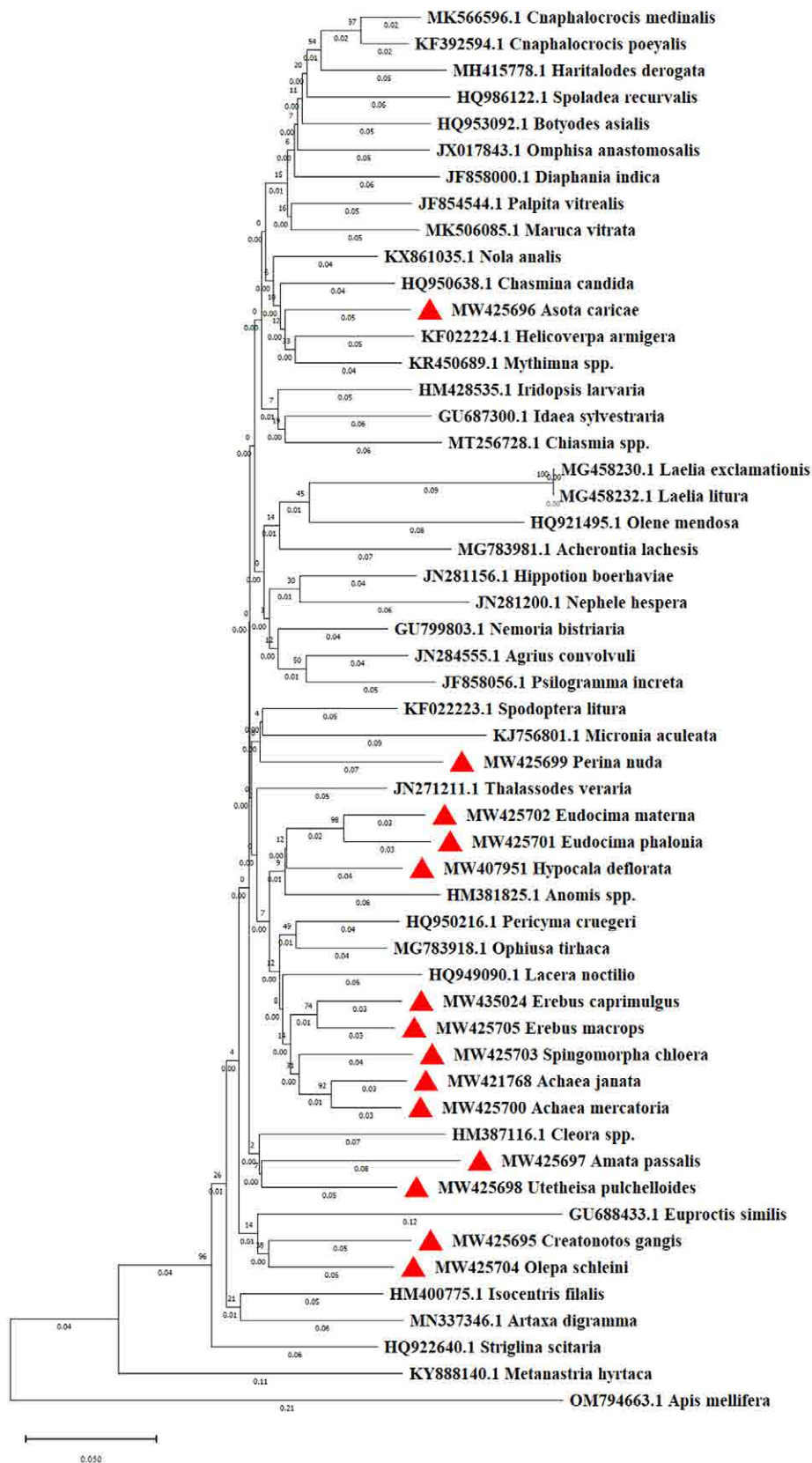


Figure 4. Phylogenetic tree based on mitochondrial COI gene sequences (MEGA X). The available (database) mitochondrial COI gene sequences of morphologically-identified species (38) (among the 45 species) were retrieved from NCBI for constructing phylogenetic tree along with COI gene-based identified species (14) (denoted in triangle) in this study.

established to serve a crucial role in the global bio-identification system for animals (Hebert et al. 2003). DNA barcoding is considered a definitive method for identifying insects (Jalali et al. 2015). COI DNA barcodes were used to distinguish among species of three lepidopteran families in north-western Costa Rica (Hajibabaei et al. 2006). A DNA Barcoding reference library of about of 113 species of geometrid moths from Western Himalaya was constructed which can effectively provide information on geographical distribution and basis for their conservation (Dey et al. 2019). Another study in Namdapha National Park, East Himalaya, produced a DNA barcode sequence of 44 Geometridae moths (Kumar et al. 2018). Further, a study concluded that a two-step barcoding analysis pipeline could swiftly characterize insects' biodiversity and explicate species boundaries for taxonomic complexes (Jin et al. 2018). Thus, the DNA barcoding tool can be used to discriminate constructively among various species in the lepidopteran family (Hajibabaei et al. 2006). To resolve ambiguity in some erebids, we used mitochondrial COI gene for identification of species. This assisted in the precise identification of the 14 erbid species. Phylogenetic studies can provide clues on the evolutionary relatedness among various groups of organisms.

The collection site of this study also covers the area in the University of Madras. Many urban universities like Banaras Hindu University have developed many strategies to monitor, manage and conserve biodiversity (Nayak & Ghosh 2020). In addition, universities have an eccentric potentiality to embrace a biophilic design inside the campus which aids in reconfiguring urban residents to the biosphere and serve as an excellent source for biodiversity-based research in urban (Liu et al. 2021). Further, the study can be extended to cover many urban areas to comprehend the effect of urbanization on the distribution profile of moths.

The distribution profile of a species depends significantly on the biogeographical region in which they occur (Gaston 1994). Artificial light pollution due to the imprudent use of artificial light was reported to cause temporal and spatial disorientation, biorhythms desynchronization, and desensitization of visual systems, affecting the moth physiology and behaviour (Nayak & Ghosh 2020). In addition, LED lights have been found to lower the risk of urban areas becoming ecological traps (White et al. 2016). Spatial habitat heterogeneity is essential to sustain the gamma diversity of macro-moth species (de Miranda et al. 2019). Urban green areas were indicated in a finding to support a wide array of moths (Paul 2021). A maiden comprehensive annotated

checklist of moths of Delhi with 234 species that were not previously reported were added (Komal et al. 2021). Consequently, the number of described species may or may not constitute the definite number of species occurring in an area. Nevertheless, this documentation can provide particulars on their distribution and their conservation status.

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Crambidae

*Maruca vitrata**Omphisa anastomosalis**Spoladea recurvalis**Botyodes asialis**Cnaphalocrocis medinalis**Cnaphalocrocis poeyalis**Diaphania indica**Haritalodes derogata**Isocentris filalis**Palpita vitrealis*

Image 1. Moths of Guindy: Crambidae.

Erebidae

*Asota caricae*

GenBank accession no: MW425696

*Amata passalis*

GenBank accession no: MW425697

*Olepa schleini*

GenBank accession no: MW425704

*Utetheisa pulchelloides*

GenBank accession no: MW425698

*Eudocima materna*

GenBank accession no: MW425702

*Eudocima phalonia*

GenBank accession no: MW425701

*Achaea janata*

GenBank accession no: MW421768

*Achaea mercatoria*

GenBank accession no: MW425700

*Dysgonia stuposa*

Image 2. Moths of Guindy: Erebidae.



Erebus caprimulgus
GenBank accession no: MW435024



Erebus macrops
GenBank accession no: MW425705



Lacera noctilio



Ophiusa tirhaca



Pericyma cruegeri



Sphingomorpha chlorea
GenBank accession no: MW425703



Hypena obacerralis



Hypocala deflorata



Artaxa digramma

Image 3. Moths of Guindy: Erebiidae.



Euproctis scintillans



Euproctis similis



Laelia exclamationis



Laelia litura



Olene mendosa



Perina nuda
GenBank accession no: MW425699

Eupterotidae



Cretonotos gangis
GenBank accession no: MW425695



Anomis sp.



Eupterote bifasciata

Image 4. Moths of Guindy: Erebiidae and Eupterotidae.

Geometridae

*Iridopsis larvaria**Chiasmia eleonora**Chiasmia* sp.*Macaria multilineata**Cleora* sp.*Thalassodes veraria*
Lasiocampidae*Nemoria bistriaria**Idea sylvestriaria**Metanastria hyrtaca*

Image 5. Moths of Guindy: Geometridae and Lasiocampidae.

Noctuidae

*Chasmina candida**Helicoverpa armigera**Spodoptera litura**Mythimna* sp.

Nolidae

*Nola analis*

Pterophoridae

*Geina periscelidactyla*

Image 6. Moths of Guindy: Noctuidae, Nolidae and Pterophoridae.

Sphingidae

*Hippotion boerhaviae**Nephela hespera**Acherontia lachesis**Agrius convolvuli**Psilogramma increta*

Thyrididae

*Strigina scitaria*

Uraniidae

*Micronia aculeata*

Image 7. Moths of Guindy: Sphingidae, Thyrididae, Uraniidae.

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