GENETIC DIVERSITY OF THE COMMON MOTHS OF NORTH KERALA

Thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Zoology

By

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CERTIFICATE

This is to certify that the thesis entitled “Genetic diversity of the common moths of North Kerala” is a bonafide record of research work done by Ms. Seema Jayaprakash I.K. in the Department of Zoology under my supervision and guidance, in partial fulfilment of the requirement of the Degree of Doctor of Philosophy under the Faculty of Science of the University of Calicut. I also certify that no part of this thesis has been presented before for any other Degree.

Calicut University,
November 4, 2019

Dr. K.V.Lazar

DECLARATION

I, Seema Jayaprakash I.K., hereby declare that this thesis entitled "Genetic Diversity of the common moths of North Kerala" is an authentic record of the work carried out by me under the supervision and guidance of Dr. K.V. Lazar, Professor (Retd.), Department of Zoology, University of Calicut and that no part of this has been published previously or submitted for the award of any Degree, Diploma or Title of recognition before. Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

C.U. Campus
November 4, 2019

Seema Jayaprakash I.K.

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Seema Jayaprakash I.K.

This thesis is dedicated to my parents

FOREWORD

Insects are the most diverse forms of life adapted to exploit all terrestrial and aquatic environments on the planet earth. They form a major component of the biodiversity of any area forming the biological foundation for all terrestrial ecosystems. They play diverse roles in an ecosystem by cycling nutrients, pollinating plants, dispersing seeds, maintaining soil structure and fertility, controlling populations of other organisms and providing a major food source for other taxa (Major, 1987). They are a prime factor in regulating the abundance of all plants particularly flowering plants which in turn are the corner stones of all food chains. The process of insect pollination is believed to be the basis for the evolutionary history of flowering plants, spanning about 135 million years (Crepet, 1979). Approximately 85% of angiosperms are pollinated by insects (Grimaldi and Engel, 2005). Insects are important supplementary food source of calories and protein and hence they are consumed in many parts of the world. Hence their documentation is very important for all scientific studies and conservation programmes.

Moths are a group of insects belonging to the order Lepidoptera. Moths are abundant in almost all parts of the world. The ability to utilize a wide variety of food sources has allowed moths to survive in virtually every habitat on Earth (Kendrick, 2002). They are major players at the bottom of the food chain. Many of the rare and endangered butterflies and moths have restricted habitat requirements (Fowles et al., 2004, Howe et al., 2004). By understanding the habitat requirements of rare or endangered species the chances of their survival can be increased by manipulation of habitats.

Moths are important pollinators. Most moths, particularly their caterpillars are major agricultural pests in most parts of the world, eg., corn borers, bollworms, gypsy moth, etc. Moths of the family Tineidae are regarded as pests as their larvae eat fabric. Moths are great mimics. To avoid being eaten some moths have evolved to look like palatable insects, some mimic bird droppings. Some moths are farmed, eg., *Bombyx mori* (silk worm). They are important food for many animals like bats, owls and other birds, lizards, cats, rodents and bears.

Moths play an important role in giving us information about the health of our environment as they are so widespread and found in different habitats, and are very sensitive to environmental changes and hence are useful as indicator species. By monitoring their numbers and ranges we get vital information about the changes in our environment such as the effects of new farming practices, pesticides, air pollution and climate change. In this context, conservation of moths have great relevance in the natural sustainability of all life forms. Hence study of the biodiversity of moths is very important. Moth assemblages are powerful indicators of forest disturbance (Kitching et al., 2000).

Genetic diversity of the mots of Kerala has little been studied despite the fact that it is part of the Western Ghats which is one of the biodiversity hotspots of the world. In the present study partial sequence of the mitochondrial cytochrome oxidase subunit I gene is employed to study the genetic diversity of 28 common moths of North Kerala. They belong to 6 families, viz., Noctuidea, Erebidae, Geometridae, Crambidae, Sphingidae, and Lasiocampidae. Of these 16 moths were novel genotypes.

The phylogenetic relationship, evolutionary divergence and origin of each species under study were also described. The DNA barcodes generated in the present study can be used for their species identification. The sequences generated in the study were deposited in GenBank.

The phylogenetic analysis of the various moths revealed that the North Kerala moths showed a close relationship to the moth fauna of South East Asia, Africa and Australia which were part of the erstwhile Gondwana. Divergences might have occurred due to geographical isolation when the land masses separated by continental drift. Nucleotide polymorphisms are the main cause for genetic variation in most of the species.

The dissertation commences with a brief review of literature on the subject. This is followed by materials and methods employed in the study. The results of genotyping of each insect and its discussion are presented in the results. A brief summary of the findings is also given at the end of results. The dissertation ends with a bibliography.

REVIEW OF LITERATURE

Introduction

Biodiversity is the foundation for sustainable development as it is the biological wealth of a nation. It is richest in the tropics as it contains about 90% of the world's species. The planet earth is going through the greatest ever biodiversity crisis because of over-exploitation. United Nations has designated 2011-2020 as the United Nations Decade on Biodiversity and 2021- 2030 as the United Nations Decade on Ecosystem Restoration. The Convention on Biological Diversity (CBD) held at Rio de Janeiro in 1992, has three main goals which comprises the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising from genetic resources.

Biological surveys, inventories and monitoring provide the basic knowledge required to enhance local scientific and technical expertise and to initiate sound conservation strategies. Insects form the major proportion of biodiversity of any area as they make up over three-fourths of all currently known living and fossil organisms. Moths are insects which belong to the order Lepidoptera. The word `Lepidoptera' is derived from the Greek words, viz., `*lepis* 'meaning scales and `*pteron*' meaning wings. Lepidoptera are studied as an effective tool for detecting environmental changes. Lepidoptera are important in biodiversity studies because they are the major group of phytophagous insects and it is the largest order that is almost entirely associated with angiosperms (Scoble, 1992).

Lepidoptera are found in many habitats and niches, and hence their study helps in ecological comparisons and they can indicate areas of endemism (Solis, 1997). The order Lepidoptera includes two sub orders – Rhopalocera comprising butterflies and Heterocera comprising moths. It is the most diverse order of insects associated mainly with angiosperm plants and is one of the largest insect orders. As moths play a prominent role in the biodiversity of any region inventorying is the first step towards their conservation.

Lepidoptera comprises the butterflies (some 20,000 species in two or three super families) and moths (the great majority of species, spread among some 30 super families) (Kristensen and Skalski, 1999). Lepidoptera have a significant effect on human survival

as they play vital role in plant pollination, biological control of weeds, and as a part of human diet in many parts of the tropics. Lepidopteran species are restricted by their larval host specificity. For significant progress in the study of Lepidopteran biodiversity, inventorying and monitoring of ecologically sensitive areas of conservation is a must.

Moths are thought to have evolved along with angiosperms which is a classic example of co-evolution. Recent fossil studies have revealed that primitive moths known as Glossata evolved even before the appearance of flowering plants, emerging during the Jurassic period about 145 million years ago. They developed sucking proboscis to draw nutrition from gymnosperm seeds (Timo *et al*., 2018).

Evolutionary success of insects

The evolution of herbivory has likely played a particularly important role in the evolutionay success of insects which constitute the most species-rich order of multicellular organisms on the planet (Gloss *et al*, 2016). Herbivorous insect clades display faster rates of net diversification and higher species richness compared to clades of non-herbivorous insects and nearly half of all extant insect species are herbivorous (Mitter *et al*., 1988; Wiens *et al*., 2015). Species radiations of plants and insect herbivores evolved from reciprocal, antagonistic interactions between the two groups (Fraenkel, 1959). Herbivorous insects are largely dependent on plants for habitat and food (Price 1980) and hence selective pressures on insects are strongly influenced by their host plants. Plants protect themselves against herbivores by means of structural characters (e.g., trichomes) and defensive chemicals. Production of these defences occurs in response to an attack, regulated through a complex signalling network (Thaler *et al*., 2012).

Plant defensive traits and herbivore counter-adaptations are typically polymorphic and are considered as a result of co-evolutionary dynamics (Flor, 1956; Karasov *et al*., 2014). Hence insect evolution is driven by pressure to overcome specific plant defensive chemicals, and lineages that develops mechanisms to overpower these chemicals diversify as they spread across new niches. Those insects which are host plant-specialists, either gain the ability to sequester plant-produced chemicals to defend themselves against attack by the third trophic level, through aposematic coloration or they evolve detoxification mechanisms and evasive strategies such as camouflage or mimicry (Farkas *et al*., 2013). Insect diversification is closely linked to evolutionary shifts between host plant species

(Janz *et al*., 2011). Predators and parasitoids, which drive the evolution of plant defensive chemical sequestration and crypsis in herbivores, play comparatively major roles in driving divergence among insect populations (Bernays and Graham 1988).

Decline of moths – need for conservation

Habitat loss, degradation or deterioration in quality of habitats and fragmentation by human interference, use of chemical pesticides, light pollution and climate change are the major causes for the decline of moths in all parts of the world. Fragmentation have isolation effects (Fox, 2013). Alarming decrease in the overall abundance of widespread British macro-moths have been reported by Conrad (Conrad *et al*., 2006). *Laelia coenosa*, reed tussock moth and *Lymantria dispar*, gypsy moth became extinct because of wetland drainage, and *Emmelia trabealis*, spotted sulphur moth, as a result of afforestation and agricultural intensification (Majerus, 2002). Intensive agriculture, reduces habitat area, quality and heterogeneity by the impacts of increased use of pesticides, changes in tilling and grazing practices and larger cropped areas and is widely recognized as a major driver of decline in biodiversity (Benton *et al*., 2002, 2003; Kleijn *et al*., 2009). The International Union for Conservation of Nature (IUCN) has listed about 19 extinct moths.

The decline in moth populations all over the world due to human interference is of great concern because moths are important primary consumers and prey items for a wide range of other taxa, and also play a very important role in the ecosystem as pollinators. Hence there documentation and assessment of their diversity is of prime importance.

In the present context of increasing habitat destruction which threaten the existence of many moth species, there is an urgent need for their detection and documentation to evolve conservation strategies. This can be achieved by using gene sequences as molecular markers which make the identification and documentation of species easy and efficient within a short span of time and effort in comparison to the time consuming and elaborate traditional morphological methods of identifications (Godfray, 2002).

Historical background

Moths are included in over 55 different families (Hampson, 1891). There are approximately 160000 named species of moths in the world (Kristensen & Skalski, 1999). Nearly 12000 species of moths belonging to 41 families have been found in India

(Chandra, 2007). The largest families of moths are the Noctuidae, ca. 35000 species; and the Geometridae, ca. 21000 species. These families are found worldwide. Insects, particularly the moths, have taken advantage of vast number of rare small niches and this is the reason for such huge diversity. The larval stages of moths successfully occupy a wide array of small niches (Kendrick, 2002).

P. Cramer and C. Stoll were the pioneers in the study of moths. They published the studies on the Lepidoptera of Asia, Africa and America in 1775 (*De uitlandsche kapellen, voorkomende in de drie waereld-deelen, Asia, Africa en America,* Amsteldam, Chez S. J. Baalde; 1779-1782.). In 1775, J. C. Fabricius published many works on Lepidoptera of which the most significant one was *Systema entomologiæ* in which he used the form of mouthparts to discriminate the orders. *Illustrations of Exotic Entomology* was published in 1837 by D. Drury with figures of exotic moths and butterflies. *General history and illustrations of the Lepidoptera and caterpillars of Northern America* was published by J. B. Boisduval in 1837.

F. Walker catalogued insects for the British Museum (1848-1873). He gave lot of synonyms for the same species. From 1855 to 1866 he published many works on the major families of moths in his *List of the Specimens of Lepidopterous Insects in the Collection of the British Museum of* Natural History, London.

The Rothamsted Insect Survey (RIS), a network monitoring moth population of UK operated by Rothamsted Research since 1968 provides one of the longest-running and most extensive data of a species-rich insect taxon anywhere in the world (Conrad *et al*., 2007).

Studies on Indian moths

F. Moore in 1865 published the lepidopteron insects of Bengal in the Proceedings of Zoological Society of London. He published six volumes of *Lepidoptera Indica* (1890–1913), a major work on the butterflies of the South Asia, which was completed after his death by Charles Swinhoe. Hampson G.F. has given a good description on the moths of Nilgiris carried out at all different elevations and on each of the several slopes (Hampson, 1892).

The major work on Indian moths was by Sir George Francis Hampson. His works were *The Lepidoptera of the Nilgiri District* (1891) and *The Lepidoptera Heterocera of Ceylon* (1893) as part of *Illustrations of Typical Specimens of Lepidoptera Heterocera of the British Museum (Part 8 and 9)*. His work on The Fauna of British India, Including Ceylon and Burma: Moths (4 volumes 1892-1896) was a very elaborative work on Indian moths. Hampson recorded about 611 species of moths particularly from Maharashtra. The moths of North West Himalaya were collected by the Rev. J. H. Hocking in 1890 which is now in the British museum (Hampson, 1892). T.R.D. Bell and F.B. Scott published the V volume of Fauna of British India, on Sphingidae in 1937.

Srivastava (2002) studied Noctuid moths from Himachal Pradesh (*Taxonomy of moths in India*). The moths from Sanjay Gandhi National park, Boriwali, Mumbai were studied by V. Subhalaxmi (2003). Ghosh (2003) studied the Geometrid moths of Sikkim and reported 525 species of Geometrid moths, 460 from Meghalaya and 260 from West Bengal. Rose & Pooni (2004-2005) recorded 18 species of moths belonging to superfamily Pterophoroidea and 16 species belonging to superfamily Tortricoidea from North western part of India. Chandra and Nema (2007) studied moth diversity of Madhya Pradesh and recorded 313 species of moths belonging to 221 genera and 25 families. They also studied the moth fauna of Jabalpur and reported 42 species belonging to 38 genera under 6 families. Gurule *et al*., (2010) catalogued 70 moth species belonging to the family Noctuidae from Nashik District of Maharashtra. Sidhu *et al*., (2010) documented 109 species micro- lepidopteran moths from the family Pterophoridae.

Studies from Kerala

In Kerala prominent works on moth biodiversity studies were done by Mathew, G & Rahamathulla, K. (1995). They reported 318 species of moths belonging to 19 families from the Silent Valley National Park (Western Ghats). Maximum number of moths collected belonged to the families Pyralidae, Noctuidae, Geometridae and Arctiidae. Some families like Lasiocampidae, Bombycidae and Gelechidae were only poorly represented. Their findings were that, in general, the fauna bears a close resemblance to that of Sri Lanka, although it is characterised by the presence of several endemic species having affinities with the Malayan elements. Sudheendrakumar, V.V. and Mathew, G. also

studied the moth fauna of Parambikulam (1999) and identified 277 species of moths of which the dominant families were Noctuidae, Geometridae, Pyralidae and Arctidae.

An inventory of Indian Pyralids comprising about 1646 species was a significant work of Mathew, G. (2006). The moths of Noctuidae, Pyralidae, Saturnidae and Spingidae were reported from Palakkad by Praveen, K (2017). Inventory of moth fauna of Malabar region comprising 267 species of moths from 22 families belonging to 10 superfamilies was presented by Rajan, R. and Shamsudheen, R.S.M. (2018). Sondhi, *et al*., (2018) reported a checklist of 282 species of moths from Shendurney and Ponmudi in Agastyamalai Biosphere Reserve, Kerala. An extensive study of moths of Vagamon hills (Western Ghats), Idukki district, were carried out by Pratheesh, *et al*., (2018). 112 species from 16 families and eight super families were reported in this study. The highest species richness was shown by the family Erebidae and the least by the families Lasiocampidae, Uraniidae, Notodontidae, Pyralidae, Yponomeutidae, Zygaenidae and Hepialidae with one species each.

Importance of moths in conservation

Moths play an important role in giving us information about the health of our environment as they are so widespread and found in different habitats, and are very sensitive to environmental changes and hence are useful as indicator species. By monitoring their numbers and ranges we get vital information about the changes in our environment such as the effects of new farming practices, pesticides, air pollution and climate change. In this context, conservation of moths have great relevance in the natural sustainability of all life forms. Hence study of the biodiversity of moths is very important. Moth assemblages are powerful indicators of forest disturbance (Kitching, *et al*., 2000).

Importance of studies on genetic diversity in conservation

The genome is continually subjected to modification by the forces of evolution. The genetic variations seen in organisms represents their ultimate identity. Hundreds of millions of years of trial and error efforts have created today's biosphere of animal, plant and microbial species. A complete understanding of genome function needs a parallel

understanding of the sequence difference across species and the fundamental processes that have made their genomes into the modern-day forms. The evaluation of inter-species sequence comparisons is essential for identifying functional elements in the genome. It also provides insight into the distinct anatomical, physiological and developmental features of various organisms that will help to define the genetic basis for speciation and will facilitate the characterization of mutational processes (Collins *et al*., 2003). Moths are thought to have evolved 190 million years ago in the early Jurassic Period.

Genetic variation is fundamental to Darwin's theory of evolution through natural selection. Selection favours some phenotypes over others. Decrease in genetic variation may lead to extinction. Increasing genetic variance enhances the survival of populations. Fitness is the reproductive success of the individual by which it contributes to the gene pool of the next generation. It may be different in different environments. The fittest ones will leave the most copies of itself in successive generations (Roderick & Navajas, 2009). Loss of biodiversity may be viewed as species loss from an ecosystem or even the entire biosphere.

Diversity among organisms is an outcome of variations in DNA sequences and of environmental effects. Each individual of a species have a unique DNA sequence. DNA variations are mutations resulting from substitution of single nucleotides (single nucleotide polymorphisms – SNPs), insertion or deletion of DNA fragments of various lengths or duplication or inversion of DNA fragments. DNA variations are considered as "neutral" when they do not cause any change in the metabolic or phenotypic traits, and hence are not subjected to positive, negative, or balancing selection. Mutations in key nucleotides of a coding sequence may change the amino acid composition of a protein, and lead to new functional variants. Such variants may have an increased or decreased metabolic efficiency compared to the original "wild type", or may lose their functionality completely, or even gain a novel function.

Methods for studying genetic diversity of species

Assessment of genetic diversity can be based on morphological, biochemical, and molecular types of information (Mohammadi & Prasanna, 2003). However, molecular markers have advantages over other methods as they show genetic differences on a more detailed level and provide fast results (Garcia *et al*., 2004; Avise, 1994). The molecular

markers are used by a taxonomist as indicators of levels of reproductive isolation, gene flow between different groups and to determine how far they get dissimilar (Tautz *et al*., 2003; Blaxter, 2004). Molecular markers become very useful in identifying cryptic species which are otherwise unrecognized (Hebert *et al*., 2004).

Species delimitation based on morphology and their host preferences is quite difficult as there may be some host specific variants among same species with slight morphological differences. These host specific variants of a species are called 'host races' or 'biotypes' (Thorpe, 1930).

As moths belongs to a species rich order Lepidoptera, and considering their formidable contribution to eukaryotic diversity and ecologic function (Godfray *et al*., 1999) accelerated methods of species discovery and identification is needed. DNA-based methods may help overcome these problems by providing a readily assessed character system (Tautz *et al*., 2003). Sequence-based species delimitation could allow quick biodiversity assessment in critical geographical areas or poorly known taxa (Smith *et al*., 2005).

Molecular markers – tools for exploring genetic diversity

Molecular diagnostic tools provide valuable support for the rapid and accurate identification of morphologically indistinct alien species thereby ensuring biosecurity against any risk through 'biological harm', apart from the economic impact from the spread of pest insects. Various types of molecular data provide a plethora of information with which to address problems at all taxonomical levels. These recent advances in nucleic acid technology have been used in the taxonomic studies of living organisms (Claridge *et al*., 1997).

Alloenzymes

Allozymes are protein products of genes that are encoded by a single gene locus. As they represent genes of known function, they are considered to be Type I markers (Liu and Cordes, 2004). Allozymes are the different allelic forms of the same enzymes encoded at the same locus (Hunter and Market, 1957). They represent different allelic forms of the same gene. The variation detected in allozymes may be the result of point mutations, insertions, or deletions (indels). Allozyme electrophoresis helps to detect genetic variation

in natural populations. Individual genotypes at each locus are inferred from the banding patterns observed on the gels. Allozymes exhibit high levels of functional evolutionary conservation throughout specific phyla and kingdoms and serve as molecular markers which help to gauge evolutionary histories and relationships between different species.

Restriction Fragment Length Polymorphism (RFLP)

In RFLP analysis differences in homologous DNA sequences are detected by identifying DNA fragments of different lengths after digesting DNA samples with specific restriction endonuclease enzymes. Restriction endonucleases recognize and cut specific nucleotide motifs in a DNA sequence producing a population of fragments with discrete sizes. To analyse the DNA restriction pattern, the fragments are separated according to size by gel electrophoresis and, after transfer to a membrane by Southern blotting, fragments of interest are identified by hybridization to probes which are labelled with radioisotopes. A polymorphism in a restriction pattern occurs when the mutation of a single base-pair results in the loss, or creation, of a new restriction site, or when, by insertion/ deletion, the size of a restriction fragment is altered. These alterations are detected on an autoradiograph, when these fragments bind the hybridization probe. Such polymorphism in a specific gene locus can be used to distinguish different species.

Random Amplified Polymorphic DNA (RAPD)

RAPD was the first PCR based molecular marker technique developed and it is by far the simplest (Williams *et al*., 1990). Short PCR primers of 10 bp long are randomly selected to amplify random DNA segments throughout the genome. The resulting amplification product is generated at the region flanking a part of the 10 bp priming sites in the appropriate orientation. RAPD products are then visualized on agarose gels stained with ethidium bromide. Most of the RAPD markers are dominant and hence heterozygous individuals cannot be distinguished from homozygotes. This is in contrast with RFLP markers which are co-dominant and therefore, can distinguish between heterozygotes and homozygotes. Thus, relative to standard RFLP markers, and especially VNTR loci, RAPD markers generate less information per locus examined. Poor reproducibility between different runs due to the short primer length and low annealing temperature is another disadvantage (Al-barrak *et al*., 2004).

Amplified Fragment Length Polymorphism (AFLP)

AFLP based genomic DNA fingerprinting is a technique used to detect DNA polymorphism. It has been reliably used for determining genetic diversity and phylogenetic relationship between closely related genotypes. AFLP analysis combines both the reliability of restriction fragment length polymorphism (RFLP) and the convenience of PCR-based fingerprinting methods. AFLP is a DNA fingerprinting technique that detects genomic restriction fragments as RFLP technique, but employs PCR amplification instead of Southern hybridisation for detection of restriction fragments. AFLP markers, can be used to construct high density genetic maps of genomes or genome segments (Vos *et al*., 1995). AFLP markers are generally dominant and hence do not require prior knowledge of the genomic composition. The AFLP is applicable to all species giving very reproducible results. AFLP markers can also be used to assess host associated differentiation (HAD) in insects (Antwi *et al*., 2015).

Microsatellite markers

Microsatellites are polymorphic regions within a genome composed of short tandem nucleotide repeats (2-7 base pairs in length). Mutation occurs more frequently in repetitive DNA, as a result of a phenomenon known as slipped-strand mispairing. Slipped-strand mispairing occurs during DNA replication which result in the loss or addition of an entire repeating unit, or several repeating units, leading to polymorphism at that locus. The number of individual repeating units in microsatellite regions may range from a few to 50 or more, resulting in alleles that are highly variable in length.

Microsatellites or simple sequence repeat (SSR) markers, are bounded by single copy sequences used to design primers to amplify across a defined locus by PCR and are inherited as Mendelian co-dominant traits. These characteristics makes microsatellites the genetic marker of choice in insect genetic studies such as (i) genome mapping, (ii) identification of quantitative trait loci, marker-assisted selection (MAS), (iii) genetic diversity and phylogenetic relationships, and (iv) population and evolutionary studies. Compared to mtDNA, microsatellite markers are much easier to use because they are highly abundant, multi-allelic and more versatile as they are encoded in the nuclear genome. Hence microsatellites are the most powerful molecular marker used extensively by insect population geneticists and ecologists. There are certain limitations to SSR

markers that DNA slippage may occur during DNA amplification due to encountering repeat sequences or may fail to amplify due to primer template mismatch (Wang*, et al*., 2009). It is difficult to isolate microsatellite markers for most of the Lepidoptera species assayed (Antony *et al*., 2001). This is due to flanking region similarity among loci (Meglécz *et al*., 2007), microsatellite association with transposable elements (Tay *et al*., 2010) and high frequencies of null alleles (Mikheyev *et al*., 2010).

Single Nucleotide Polymorphism (SNP)

SNPs are variations at single nucleotides which do not change the overall length of the DNA sequence in the region. SNPs occur throughout the genome. They are highly abundant, mutationally stable and are present at one SNP in every 1000 bp in the human genome (Sachinandam *et al*., 2001). Most SNPs are located in non-coding regions, and have no direct impact on the phenotype of an individual. However, some introduce mutations in expressed sequences or regions influencing gene expression (promoters, enhancers), and may induce changes in protein structure or regulation. These SNPs have the potential to detect functional genetic variation. SNPs close to particular gene acts as a marker for that gene. SNPs can be applied to a wide range of population studies, from individual identification to population structure and taxonomy (Kuhner *et al*., 2000). The advantage of using SNPs relative to other nuclear markers such as microsatellites include ease and efficiency of discovery and genotyping (Elfstrom *et al*., 2006) and ability to identify variation in random genomic regions or known genes (Aitken *et al*., 2004).

DNA barcoding

DNA barcoding system is based upon sequence diversity in cytochrome c oxidase subunit 1 (COI). The diversity in the amino acid sequences coded by the 5' section of the mitochondrial gene helps to place species into higher taxonomic categories (from phyla to orders) (Herbert *et al*., 2003). The analysis of the variations in the genetic makeup of a species by examining the DNA sequence is called 'Genotyping'. It is less erroneous compared to other traditional practices in taxonomy. The individual sequences are compared with the related or unrelated sequences using molecular tools, for the definite identification and comparison of genetic variation. Any variation in the DNA sequence of species is observed as their 'molecular barcodes' (Hajibabaei *et al.,* 2006). DNA barcoding

libraries can identify and distinguish different organisms which belong to different taxonomic positions. Congeneric species of animals regularly possess substantial sequence divergence in their COI genes.

DNA sequences are important source of information for greater understanding of evolutionary and genetic relationships. Barcoding helps to discover cryptic species (Herbert *et al*., 2004). The COI is a better target for analysis because it lacks introns, has limited exposure to recombination and has haploid mode of inheritance (Saccone *et al*., 1999). Universal primers for this gene are very robust (Folmer, *et al*., 1994). The evolution of this gene is rapid which allows discrimination of both closely allied species as well as phylogenetic groups within a single species (Cox & Hebert, 2001). The establishment of the DNA barcoding libraries can identify and distinguish various organisms which belong to different taxonomic positions.

DNA barcoding employs short DNA sequences from a standardized region of the genome as a tool which facilitates identification of known species and helps to discover new ones. It is based on the principle that sequence diversity within a short standardized region of the genome can present a "biological barcode" that helps identification at the species level. The DNA barcode as proposed by Herbert *et al*., (2003a) is a small region from the 5' –end of the cytochrome oxidase I (COI) mitochondrial DNA gene which consists of 648 bp. These sequences act as genetic "barcodes" that are embedded in all cells. DNA barcoding helps to identify species without taxonomic expertise. DNA barcoding is very useful in conservation studies. It provides valuable insight into the role of historical habitat fragmentation, in species diversification and to identify priority areas for conservation. Barcoding can be used to optimize diversity assessments and unravel hidden biological diversity (Swartz *et al*., 2008)

The mitochondrial genome of organisms is a better target for analysis than the nuclear genome because of several reasons. Mitochondrial genome lacks introns. It is less exposed to recombination and its mode of inheritance is haploid (Saccone *et al*., 1999). The cytochrome c oxidase I gene (COI) has two important advantages. The universal primers for this gene are very robust, which enables the recovery of its 5' end from representatives of most animal phyla (Folmer *et al*., 1994; Zhang & Hewitt, 1997).

COI possess a wide range of phylogenetic signals than any other mitochondrial gene. Its third-position nucleotides show a high incidence of base substitutions. Hence the rate of molecular evolution in COI is about three times greater than that of 12S or 16S r DNA (Knowlton & Weigt, 1998). The evolution of COI is rapid which helps in the discrimination of not only closely allied species, but also phylogeographic groups within a single species (Cox & Herbert, 2001; Wares & Cunningham, 2001). Changes in the amino acid sequence of COI occur more slowly than those in any other mitochondrial gene (Lynch & Jarrel, 1993). Thus by examining amino acid substitutions, any unidentified organism can be assigned to a higher taxonomic group before analysing the nucleotide substitutions to determine its species identity. DNA barcoding will circumvent the complexities involved in the morphological identification of species and helps to establish a simple system of identification based on DNA sequence similarity. Insect molecular systematics has complemented and enhanced the value of morphological and ecological data, making significant contributions to evolutionary biology in the process.

Next Generation Sequencing (NGS)

NGS is a powerful programme that allows the sequencing of thousands to millions of DNA molecules simultaneously. This powerful tool is revolutionizing fields such as medicine, genetic diseases, and clinical diagnostics by offering a high throughput option with the capability to sequence multiple individuals at the same time. The next-generation technologies is used for standard sequencing applications, such as genome sequencing and re-sequencing, and for novel applications previously unexplored by Sanger sequencing. NGS rapidly generates huge amounts of sequence data in a very cost-effective way. NGS aids in nucleotide variation profiling and large-scale discovery of genetic markers, which in turn will be useful in tracking the genetic basis of ecologically important phenotypic variation. Information about specific genes of interest can be unearthed from NGS transcriptome or genome data of non-model organisms using coding nucleotide or protein sequence information from genomic reference species. NGS has great potential to open up conservation genetics to more species and include analyses of a larger number of potentially important genes.

Barcoding of Lepidoptera

Lepidoptera are one of the most taxonomically diverse orders of animals and they show low sequence divergences. Diversity in nucleotide sequences of the 5' region of the

mitochondrial gene COI permits the discrimination of closely allied species of lepidopterans, which shows modest rates of molecular evolution and high species diversity. The high degree of sexual dimorphism exhibited by some Lepidopterans causes a problem in species identification by means of morphological characters. This can be solved by employing DNA barcoding. Specimens of the sympatric (or fine-scale parapatric) and morphologically identifiable species in three families of Lepidoptera– Hesperiidae (skipper butterflies), Sphingidae (sphinx moths), and Saturniidae (wild silk moths) were unambiguously distinguished by DNA barcoding (Hajibabaei *et al*., 2006). Their barcode sequences formed distinct, non -overlapping clusters in a neighbor-joining (NJ) analysis which helped in their identification. Developing of barcode data for moths will assist in the easy, rapid and accurate identification of various species of moths.

DNA barcoding studies of moths in India

Not much reports of DNA barcoding studies on moths are available from India. DNA barcoding of some Indian species of hawk moths based on COI gene (Lepidoptera: Sphingidae) were done by Devinder Singh and Navneet Kaur (2017). Molecular phylogenetic analysis of two species of *Asota* genus (Erebidae) was done by Priya and Sebastian (2017). Sinha, *et al*., (2018) did DNA barcoding of the moth *Antoculeora ornatissima* (Walker, 1858) from Himalayan region of India. Barcoding of Geometridae moths of Eastern Himalayas were done by Kumar, *et al*., (2019).

MATERIALS AND METHODS

Collection of insects

The moths for the study were collected at random from different places in North Kerala. The specimens were collected using light traps from indoors and outdoors. The moths were stored at -20^{o} C in freezer till the extraction of DNA.

Extraction of DNA

Total genomic DNA was extracted from the thoracic legs of the experimental insects using GenElute^{TM} Mammalian Genomic DNA Miniprep Kit (Merck-Millipore) following the manufacturer's instructions. The quality of the DNA extracted was analysed by agarose gel electrophoresis in 1% agarose gel. The gel was stained, visualized under a UV transilluminator and photographed by gel documentation system.

Polymerase chain reaction

About 5ng of genomic DNA from each moth specimen was amplified separately for cytochrome oxidase subunit I (COI) gene using the forward and reverse primers (Folmer *et al*., 2004) given below:

Primer name	**Sequence**
Forward primer	5'-GGTCAACAAATCATAAAGATATTGG-3'
Reverse primer	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'

The PCR reaction mixture consisted of 5 ng of genomic DNA (2 µl), 2.5µl each of 10mM forward and reverse primers, 5µl of 2mM dNTPs, 5µl 10X reaction buffer, 0.50µl of 5 U/µl Taq polymerase and 32.5µl of water. The PCR profile consisted of an initial denaturation step at 95^{o} C for 3 min, followed by 35 cycles of 95^{o}C for 10 sec, 50^{o}C for 30 sec, and 72^{o}C for 45 sec and ending with a final phase at 72^{o}C for 3 min. The reaction products were stored at 4^{o}C.

Electrophoresis of PCR product

5µl of PCR product was loaded onto a 2% agarose gel stained with Ethidium Bromide. 100bp DNA ladder was used as marker. Electrophoresis was done at a constant voltage of 100V for 1 hour. After the run is completed, it was visualized on UV trans-illuminator and photographed by gel documentation system. The gel picture of the PCR products are given in Figures A and B.

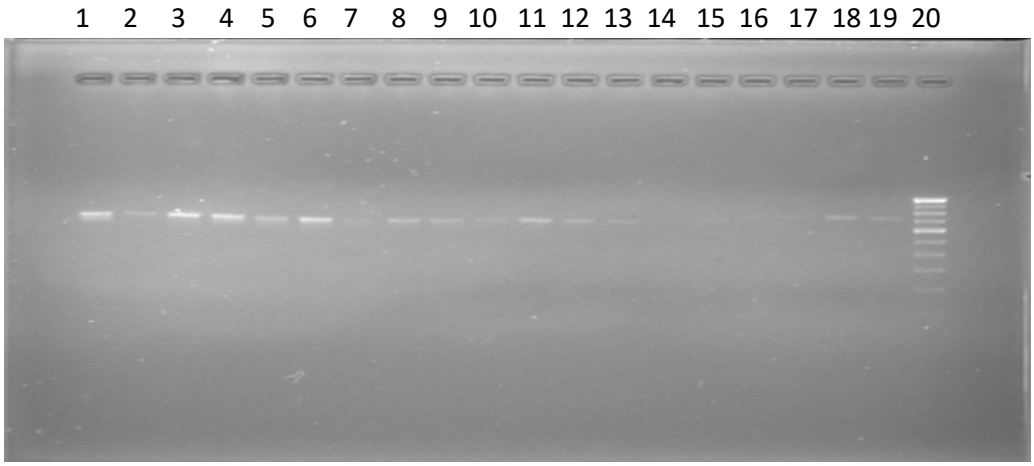

Figure A: Gel picture showing PCR products of mitochondrial cytochrome oxidase subunit I obtained from moth samples. Lane 1-19: PCR products amplified from moth samples 1-19; lane 20: 100bp DNA ladder.

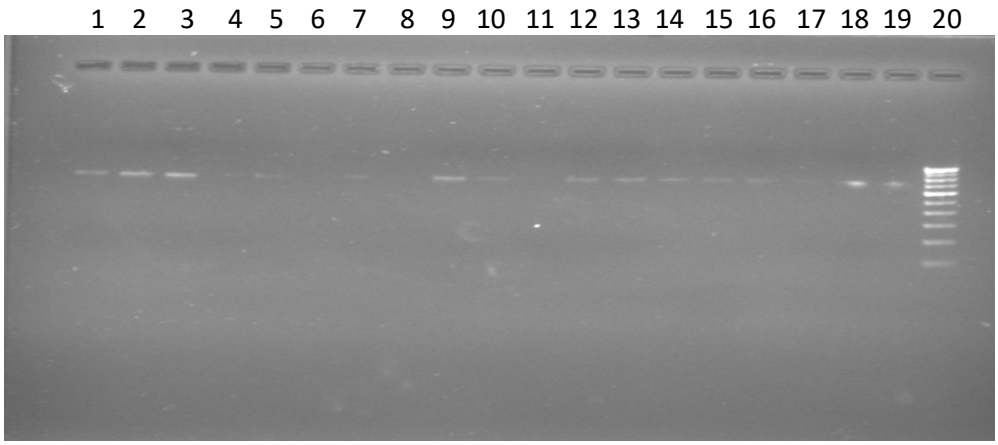

Figure B: Gel picture showing PCR products of mitochondrial cytochrome oxidase subunit I obtained from moth samples. Lane 1-19: PCR products amplified from moth samples 20-37, 40, 41; lane 20: 100bp DNA ladder.

Purification of PCR product

The PCR products were column purified using GenElute^{TM}PCR Clean-Up (MERCK-MILLIPORE) as per the manufacturer's instructions. The purified PCR products were sequenced from both ends using forward and reverse primers by Sanger's sequencing at SciGenom laboratories Ltd., Cochin. The forward and reverse sequences obtained were trimmed off the primer sequences and assembled using ClustalW and the consensus sequence was taken for analysis.

Nucleotide BLAST

The sequence similarities of the consensus sequence obtained was searched using the BLASTn programme of NCBI (https://www.ncbi.nlm.nih.gov/). The BLAST results provides information about the similarities and differences with the sequences deposited in the nucleotide database. Sequences with close similarity were aligned with the query sequence using the ClustalW. The conceptual translation of the DNA sequences were obtained using EMBOSS Transeq of EMBL-EBI (https://www.ebi.ac.uk/).

Phylogenetic Analysis

The phylogenetic analysis was done using MEGA 7.0 (https://www.megasoftware.net/). The phylogenetic tree was plotted by Neighbor-Joining (NJ) method. Multiple sequence alignments were done in ClustalW (https://www.genome.jp/tools-bin/clustalw) for identifying variations in nucleotides between the samples.

1. *Condica sp.* SJIK5

The specimen SJIK5 was identified as *Condica sp.* Walker, 1856 referring to the morphological features described by Walker 1856.

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Noctuidae; Amphipyrinae; *Condica*.

Condica sp. is found in India, China, Japan, Australia, Sri Lanka, Mayanmar, Sundaland and Fiji. In India it is seen in Maharashtra and South Andamans (Shubhalaxmi *et al*., 2011). *Condica sp.* belongs to the family Noctuidae and subfamily Amphipyrinae.

Identifying characters: Female: stout body; moderately long proboscis; palpi ascending to the vertex; third joint cylindrical, full half the length of the second; simple antennae, more than half the length of the body; abdomen not extending beyond the hind wings; stout legs; hind tibiae with four very long spurs; moderately broad wings; fore wings straight in front, somewhat rounded at the tips, hardly oblique or denticulated along the exterior border; 1st, 2nd, and 3rd inferior veins contiguous at the base; 4th moderately remote.

Results and discussion

The PCR of the COI gene fragment of *Condica sp.* SJIK5 from Kerala yielded a product of 658bp long. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 2 – 6. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190676).

The DNA isolated from the sample *Condica sp.* SJIK5 from Kerala gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 1 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

Fig. 1. *Condica sp.* SJIK5 (dorsal and ventral view)

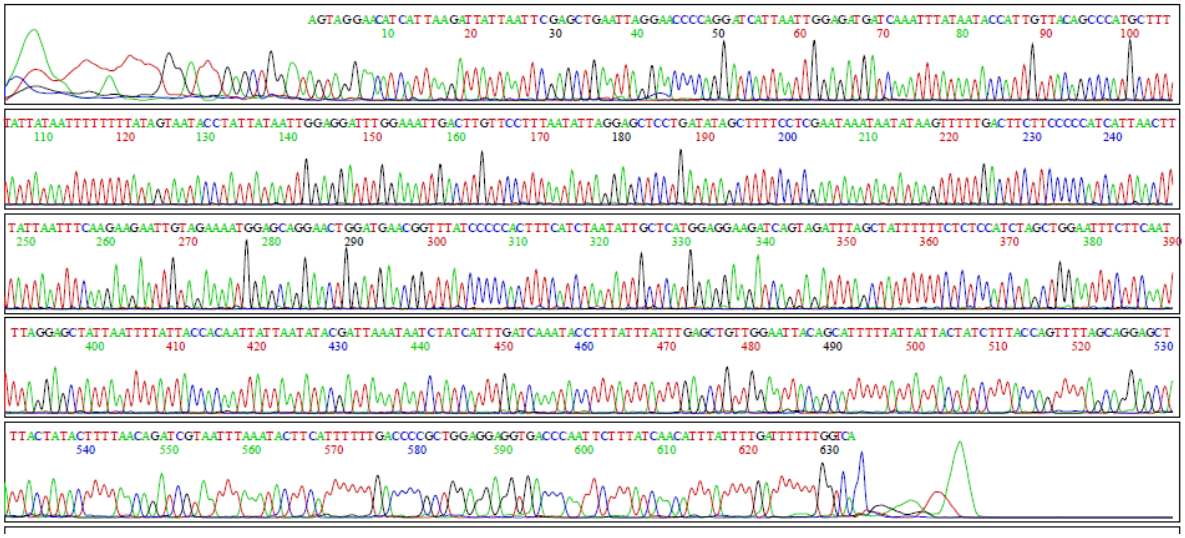

Fig. 2. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *Condica sp.* SJIK5.

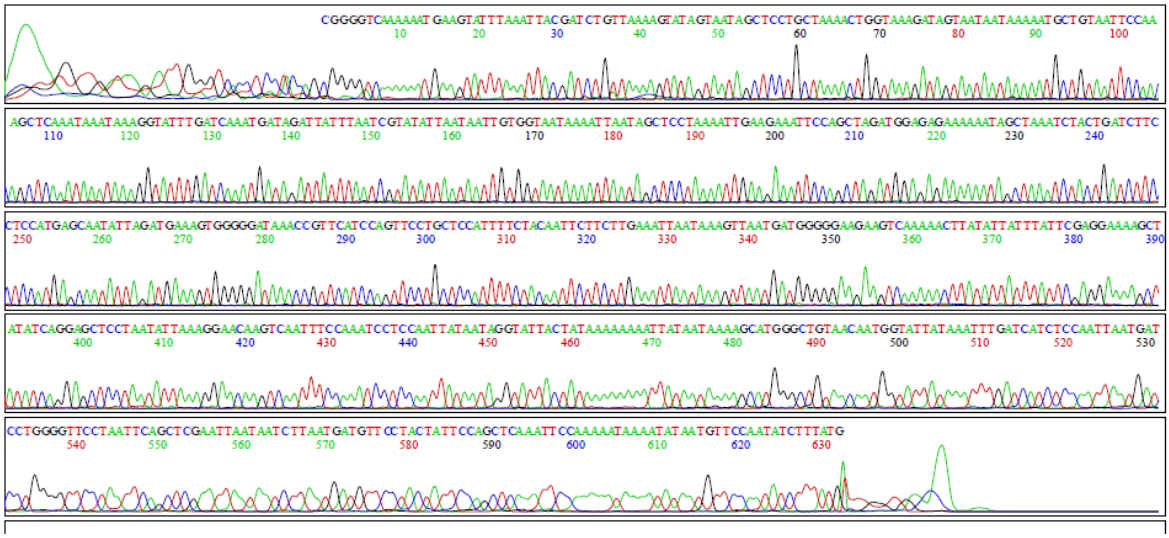

Fig. 3. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *Condica sp.* SJIK5.

> *Condica sp.* Voucher SJIK5 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACATTATATTTTATTTTTGGAATTTGAGCTGGAATAGTAGGAACATCATTAAGATTATTAATTCGAGCTGAATTAGGAACCCC
AGGATCATTAATTGGAGATGATCAAATTTATAATACCATTGTTACAGCCCATGCTTTTATTATAATTTTTTTTATAGTAATACC
TATTATAATTGGAGGATTTGGAAATTGACTTGTTCCTTTAATATTAGGAGCTCCTGATATAGCTTTTCCTCGAATAAATAATAT
AAGTTTTTGACTTCTTCCCCCATCATTAACTTTATTAATTTCAAGAAGAATTGTAGAAAATGGAGCAGGAACTGGATGAACGGT
TTATCCCCCACTTTCATCTAATATTGCTCATGGAGGAAGATCAGTAGATTTAGCTATTTTTTCTCTCCATCTAGCTGGAATTTC
TTCAATTTTAGGAGCTATTAATTTTATTACCACAATTATTAATATACGATTAAATAATCTATCATTTGATCAAATACCTTTATT
TATTTGAGCTGTTGGAATTACAGCATTTTTATTATTACTATCTTTACCAGTTTTAGCAGGAGCTATTACTATACTTTTAACAGA
TCGTAATTTAAATACTTCATTTTTTGACCCCGCTGGAGGAGGTGACCCAATTCTTTATCAACATTTATTT
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Fig. 4. Partial coding sequence of *Condica sp.* SJIK5 COI gene.

> *Condica sp.* Voucher SJIK5

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TLYFIFGIWAGMVGTSLSLLIRAELGTPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWLVPLM
LGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHGGSSVDLAIFSLHLAGIS
SILGAINFITTIINMRLNNLSFDQMPLFIWAVGITAFLLLLSLPVLAGAITMLLTDRNLNTSFFDPAGGG
DPILYQHLF
```

Fig. 5. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *Condica sp.* SJIK5.

Table 1. The BLAST hit table of the partial coding DNA sequence of COI gene of *Condica sp.* SJIK5.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Query end	Geogra. location
1	Condica illecta KX863280.1	99.85	658	1	0	1	658	Pakistan
2	Condica illecta KX862962.1	99.85	658	1	0	1	658	Pakistan
3	Condica illecta KX862659.1	99.85	658	1	0	1	658	Pakistan
4	Condica illecta KX862571.1	99.85	658	1	0	1	658	Pakistan
5	Condica illecta KX861678.1	99.85	658	1	0	1	658	Pakistan
6	Condica illecta KX861632.1	99.85	658	1	0	1	658	Pakistan
7	Condica illecta KX861515.1	99.85	658	1	0	1	658	Pakistan
8	Condica illecta KX861347.1	99.85	658	1	0	1	658	Pakistan
9	Condica sutor JN262083.1	96.51	658	23	0	1	658	USA
10	Condica circuita JQ564403.1	96.05	658	26	0	1	658	Costa Rica
11	Chaograptis rhaptina HQ949235.1	95.44	658	30	0	1	658	Australia

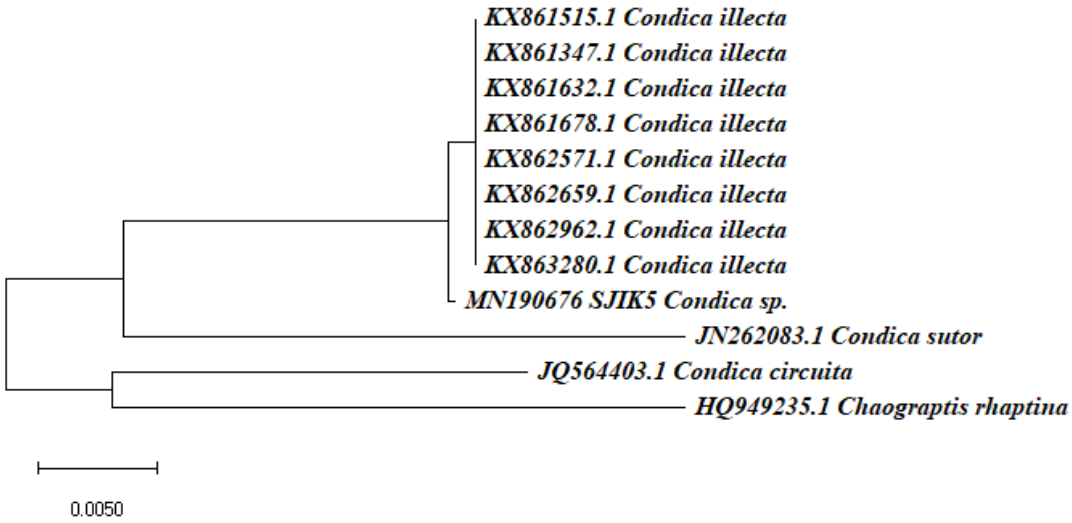

Fig. 6. The NJ tree showing phylogenetic relationships of *Condica sp.* SJIK5.

The COI sequence of *Condica sp.* SJIK5 from Kerala showed a similarity of 99.85% to 8 samples of *Condica illecta* sequences in the database viz., KX863280, KX862962, KX862659, KX862571, KX861678, KX861632, KX861515 and KX861347 from Pakistan. There is single nucleotide difference with the species from Pakistan. They are polymorphic novel variants of the species and were placed in adjacent clades. The species SJIK5 is a novel one. The phylogeny of *Condica sp.* SJIK5 was derived from NJ-tree developed from the similar sequences obtained from database. The NJ-tree distance data revealed that the species was diverged from its closely related species *Condica sutor* from USA about 20000 years ago. The divergence might have occurred by geographical isolation after the species reached the North American Continent through land bridges from South America which was once part of Gondwana.

2. *Grammodes sp.* SJIK7

The specimen SJIK7 was identified as *Grammodes sp.* Guenee, 1852 referring to the morphological features described by Hampson, 1894.

Synonyms: *Colbusa* Walker, 1865

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Noctuidae; Catocalinae; *Grammodes*.

Grammodes sp. is seen in India, Australia, South America, Caribbean Islands, Mexico, China, Korea, Taiwan, Ethiopia and Japan. In India it is found in Tamil Nadu (W. Ghats), Assam, N. Maharashtra, Kerala (Vagamon and Ponmudi), and Himalayas (Shubhalaxmi *et al*., 2011, Singh et al., 2018, Arandhara *et al.,* 2018, Sondhi *et al*., 2018, Shah *et al*., 2018). *Grammodes sp.* belongs to the family Noctuidae and subfamily Catocalinae. It has a wide range of host plants such as Euphorbiaceae, Gramineae, Leguminosae, etc.

Identifying characters: Greyish brown in colour; palpi upturned, reaching just above vertex of head; the third joint minute; simple antennae in male; thorax and abdomen smoothly scaled and somewhat slender; tibiae covered with long hair and the mid tibiae spined; fore wing short and broad; the apex somewhat acute; fore wing with a large black patch occupying the whole wing except the basal, costal and outer areas, its outer edge waved.

Results and discussion

The PCR of the COI gene fragment of *Grammodes* sp. SJIK7 from Kerala yielded a product of 658bp long. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 8 - 12. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190662).

Fig. 7. *Grammodes* sp. SJIK7 (dorsal and ventral view)

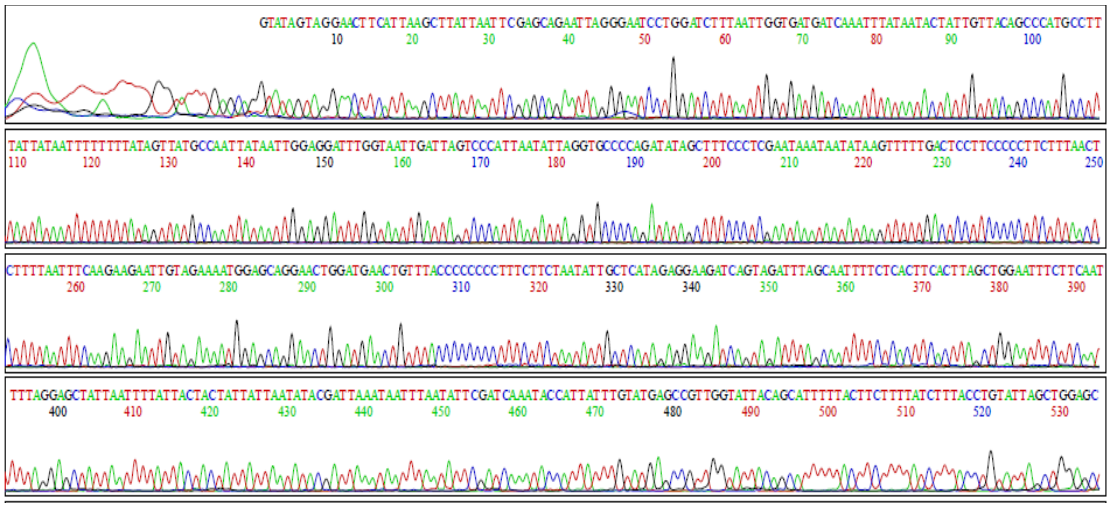

Fig. 8. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *Grammodes* sp. SJIK7.

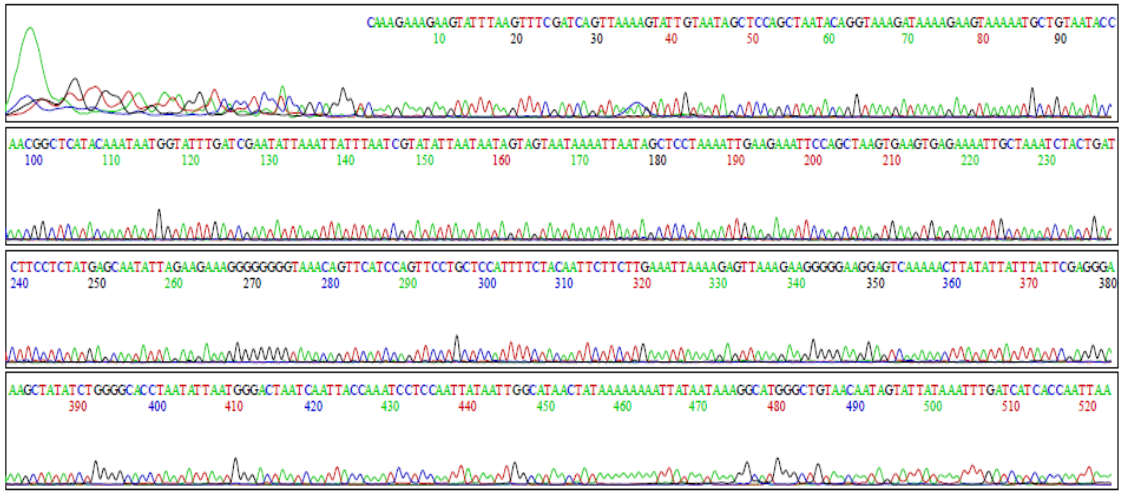

Fig. 9. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *Grammodes* sp. SJIK7.

> *Grammodes sp.* Voucher SJIK7 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACATTATATTTTATTTTTGGTATTTGAGCAGGTATAGTAGGAACTTCATTAAGCTTATTAATTCGAGCAGAATTA
GGGAATCCTGGATCTTTAATTGGTGATGATCAAATTTATAATACTATTGTTACAGCCCATGCCTTTATTATAATTT
TTTTTATAGTTATGCCAATTATAATTGGAGGATTTGGTAATTGATTAGTCCCATTAATATTAGGTGCCCCAGATAT
AGCTTTCCCTCGAATAAATAATATAAGTTTTTGACTCCTTCCCCCTTCTTTAACTCTTTTAATTTCAAGAAGAATT
GTAGAAAATGGAGCAGGAACTGGATGAACTGTTTACCCCCCCCTTTCTTCTAATATTGCTCATAGAGGAAGATCAG
TAGATTTAGCAATTTTCTCACTTCACTTAGCTGGAATTTCTTCAATTTTAGGAGCTATTAATTTTATTACTACTAT
TATTAATATACGATTAAATAATTTAATATTCGATCAAATACCATTATTTGTATGAGCCGTTGGTATTACAGCATTT
TTACTTCTTTTATCTTTACCTGTATTAGCTGGAGCTATTACAATACTTTTAACTGATCGAAACTTAAATACTTCTT
TCTTTGACCCTGCTGGAGGAGGTGATCCTATTCTTTACCAACATCTATTT
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Fig. 10. Partial coding sequence of *Grammodes sp.* SJIK7 COI gene.

> *Grammodes sp.* Voucher SJIK

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TLYFIFGIWAGMVGTSLSLLIRAELGNPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNW
LVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHSGSSVDLA
IFSLHLAGISSILGAINFITTIINMRLNNLMFDQMPLFVWAVGITAFLLLLSLPVLAGAITMLLT
DRNLNTSFFDPAGGGDPILYQHLF
```

Fig. 11. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *Grammodes sp.* SJIK7.

Table 2. The BLAST hit table of the partial coding DNA sequence of COI gene of *Grammodes sp.* SJIK7.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Query end	Geogra. location
1	Grammodes sp. HQ950267.1	93.16	658	45	0	1	658	Australia
2	Bastilla sp. HQ950320.1	93.16	658	43	2	2	658	Australia
3	Bastilla sp. HQ950319.1	93.16	658	43	2	2	658	Australia
4	Grammodes sp. HQ950266.1	93.01	658	46	0	1	658	Australia
5	Bastilla sp. HQ950321.1	93.01	658	44	2	2	658	Australia
6	Bastilla infractafinis HQ950317.1	92.86	658	47	0	1	658	Australia
7	Grammodes diagarmma HQ949248.1	92.86	658	47	0	1	658	Australia
8	Bastilla hicanora HQ950295.1	92.71	658	48	0	1	658	Australia
9	Bastilla propyrrha HQ950310.1	92.71	658	48	0	1	658	Australia
10	Bastilla frontinus HQ950311.1	92.71	658	48	0	1	658	Australia
11	Bastilla latizona HQ950314.1	92.55	658	49	0	1	658	Australia
12	Bastilla hercodes HQ950279.1	92.40	658	50	0	1	658	Australia
13	Grammodes sp. HQ950275.1	92.40	658	50	0	1	658	Australia
14	Grammodes sp. HQ950262.1	92.40	658	50	0	1	658	Australia
15	Bastilla joviana HQ950301.1	92.40	658	50	0	1	658	Australia

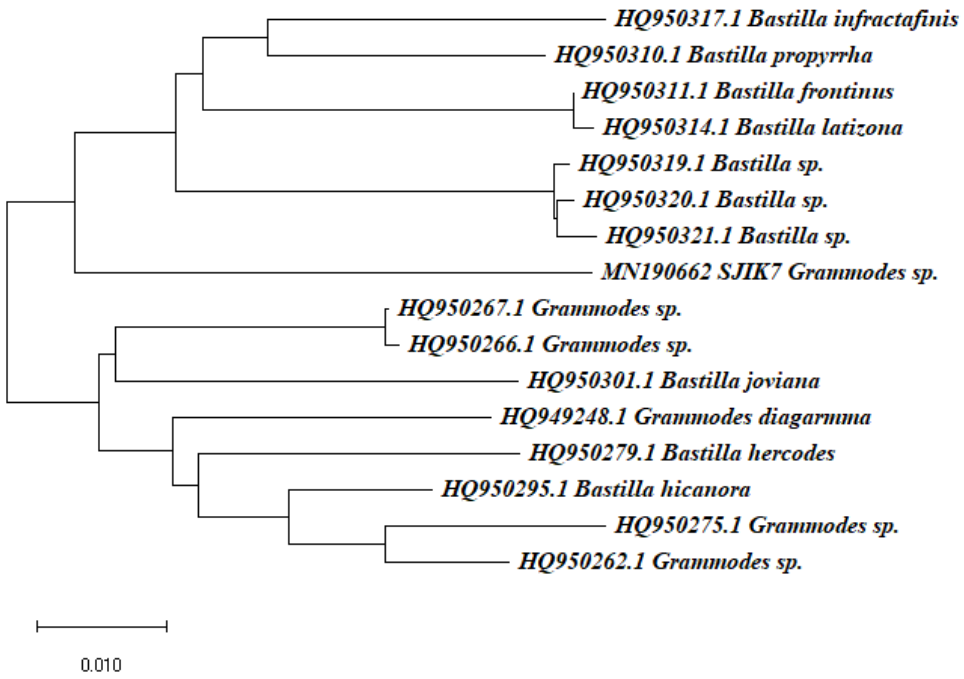

Fig. 12. The NJ tree showing phylogenetic relationships of *Grammodes sp.* SJIK7.

The DNA isolated from the sample *Grammodes sp.* SJIK7 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 2 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

The COI sequence of SJIK7 BLAST result showed a maximum similarity of 93.16% to *Grammodes sp.* HQ950267 from Australia. Hence the sequence is a novel one. It also showed a similarity of 93.16% to *Bastilla sp.* HQ950320 and HQ950319 from Australia. The NJ- tree showed the similarity of *Grammodes sp.* SJIK7 to the genus *Bastilla* both of which belong to the subfamily Catocalinae. They are placed in adjacent clades reflecting the divergence from a common ancestor. The NJ tree showed that the species diverged from a common ancestor, as a result of the break- up of the Indo-Australian plate induced by the collision of the Indo- Australian plate with Eurasia.

3. *Spirama retorta* SJIK10

The specimen SJIK10 was identified as *Spirama retorta* (Clerck, 1764) referring to the morphological features described by Hampson, 1894.

Synonyms: *Phalaena retorta* Clerck, 1764
Noctua spiralis Fabricius, 1775
Erebus chimista Kollar, 1844
Spirama isabella Guenee, 1852

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Noctuidae; Catocalinae; *Spirama.*

S. retorta is seen in India, Japan, China, Sri Lanka, Myanmar, Borneo and Java. In India it is seen in Kerala (Ponmudi), Jharkhand, Maharashtra, Tamil Nadu, Sunderbans, Himalayas and West Bengal (Shubhalaxmi et al., 2011, Singh et al., 2018, Sondhi *et al*., 2018, Bharmal, 2015, Shah *et al*., 2018). *S. retorta* belongs to the family Noctuidae and subfamily Catocalinae. It is major pest of *Albizia* in nurseries and plantations in Central India. *Acacia mangium* is also a host plant.

Identification characters: Antennae is minutely fasciculate in male, tibiae devoid of hairs and the mid tibiae with spines; head and collar dark chestnut-brown; thorax paler with dark bands; abdomen crimson with triangular black dorsal patches; wings fuscous brown; fore wing with the costal and outer areas more or less suffused with purplish and sometimes with an olive tinge; an ante-medial line excurved below costa, oblique to inner margin; a large inverted-comma mark beyond end of cell, with ochreous and black edges and some white on inner edge of tail, the center fuscous-black; a post-medial curved line passing round the stigma or interrupted by it; another post-medial line excurved below costa and slightly sinuous; two crenulate sub-marginal lines and two more prominent lines within the margin; hind wing with indistinct ante-medial, medial and traces of two post-medial and a sub-marginal line; underside suffused with dull red with two medial lines and one post medial to each wing.

Results and discussion

The PCR of the COI gene fragment of *S. retorta* SJIK10 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence

Fig. 13. *S. retorta* SJIK10 (dorsal and ventral view)

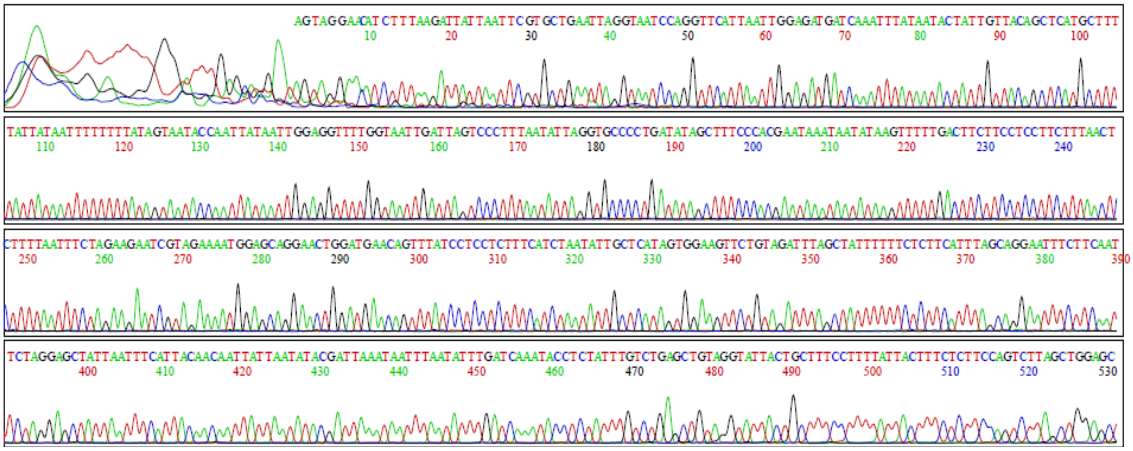

Fig. 14. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *S. retorta* SJIK10.

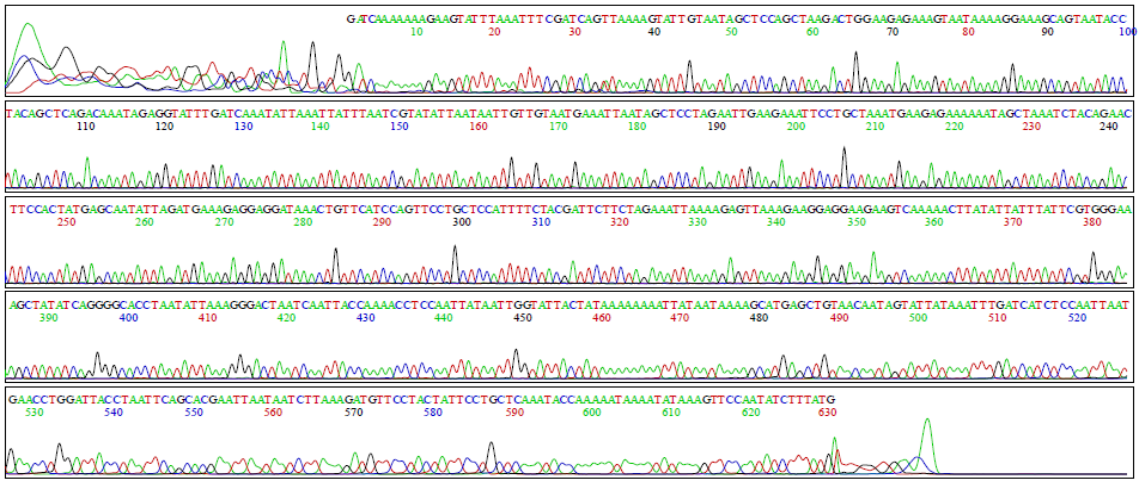

Fig. 15. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *S. retorta* SJIK10.

obtained, its conceptual translation product and NJ tree are presented in Figures 14-18. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190645).

The DNA isolated from the sample *S. retorta* SJIK10 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 3 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

The BLAST result of SJIK10 isolated from Kerala showed a maximum similarity of 99.85% to *S. retorta* MG783875 from Maharashtra. The multiple sequence alignment showed a single nucleotide polymorphism between them (C in SJIK10 being replaced by G). It showed 99. 61% similarity to that from Kerala KU257552, 99.49% to KJ380867 from Western Ghats (India). They are polymorphic variants of SJIK10 being placed in the adjacent clade. The Kerala isolate *S. retorta* SJIK10 is placed in a separate clade showing the novelty of the sequence. *S. helicina* KX862166 from Pakistan with 99.7% similarity is the closely related species. There is single nucleotide difference (C in SJIK10 replaced by G). *S. recessa* HQ950476 from Australia with 96.66% similarity is placed in the adjacent clade. The phylogeny tree distance data reveals that the species diverged from its closely related species about 20,000 years ago. The NJ tree shows that SJIK10 is close to the genera *Pindara*, *Pindara illibata* KF924010 from Tamil Nadu showing 95.46% similarity. The phylogenetic tree also shows that the *S. retorta* species from China, Japan and South Korea together occupying a different clade might have diverged from the Indian species due to the rise of Himalayas which formed a barrier. 4 novel bp of COI were added to the database.

> *S. retorta* Voucher SJIK10 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACTTTATATTTTATTTTTGGTATTTGAGCAGGAATAGTAGGAACATCTTTAAGATTATTAATTCGTGCTG
AATTAGGTAATCCAGGTTCATTAATTGGAGATGATCAAATTTATAATACTATTGTTACAGCTCATGCTTTT
ATTATAATTTTTTTTATAGTAATACCAATTATAATTGGAGGTTTTGGTAATTGATTAGTCCCTTTAATATT
AGGTGCCCCTGATATAGCTTTCCCACGAATAAATAATATAAGTTTTTGACTTCTTCCTCCTTCTTTAACTC
TTTTAATTTCTAGAAGAATCGTAGAAAATGGAGCAGGAACTGGATGAACAGTTTATCCTCCTCTTTCATCT
AATATTGCTCATAGTGGAAGTTCTGTAGATTTAGCTATTTTTTCTCTTCATTTAGCAGGAATTTCTTCAAT
TCTAGGAGCTATTAATTTCATTACAACAATTATTAATATACGATTAAATAATTTAATATTTGATCAAATAC
CTCTATTTGTCTGAGCTGTAGGTATTACTGCTTTCCTTTTATTACTTTCTCTTCCAGTCTTAGCTGGAGCT
ATTACAATACTTTTAACTGATCGAAATTTAAATACTTCTTTTTTTGATCCAGCACGAGGAGGTGATCCTAT
TTTATACCAACATTTATTT
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Fig. 16. Partial coding sequence of *S. retorta* SJIK10 COI gene.

> *S. retorta* Voucher SJIK10

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TLYFIFGIWAGMVGTSLSLLIRAELGNPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWLV
PLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHSGSSVDLAIFSL
HLAGISSILGAINFITTIINMRLNNLMFDQMPLFVWAVGITAFLLLLSLPVLAGAITMLLTDRNLNT
SFFDPARGGDPILYQHLF
```

Fig. 17. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *S. retorta* SJIK10.

Table 3. The BLAST hit table of the partial coding DNA sequence of COI gene of *S. retorta* SJIK10.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Query end	Geographical location
1	Spirama retorta MG783875.1	99.85	654	1	0	2	655	India, Maharashtra
2	Spirama helicina KX862166.1	99.70	658	2	0	1	658	Pakistan
3	Spirama retorta KU257552.1	99.61	511	2	0	148	658	India, Kerala
4	Spirama retorta KJ380867.1	99.49	593	3	0	35	627	India, W. Ghats
5	Spirama recessa HQ950476.1	96.66	658	22	0	1	658	Australia
6	Spirama retorta KF492136.1	95.59	658	29	0	1	658	Japan
7	Spirama retorta JN087379.1	95.59	657	29	0	2	658	S. Korea
8	Spirama retorta JN263994.1	95.44	658	30	0	1	658	China
9	Pindara illibata KF924010.1	95.46	638	29	0	21	658	India, TN
10	Spirama retorta KF924015.1	95.12	615	30	0	44	658	India, TN
11	Hypopyra vespertilio MG783881.1	93.43	654	43	0	2	655	India, Maharashtra
12	Donuca orbigera HQ950467.1	93.03	660	42	2	1	658	Australia

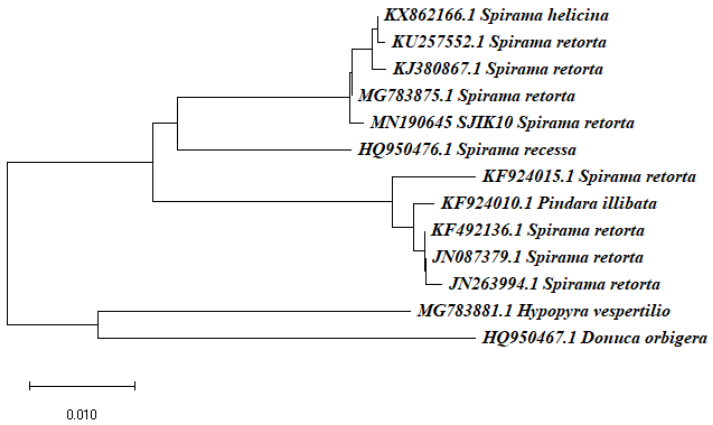

Fig. 18. The NJ tree showing phylogenetic relationships of *S. retorta* SJIK10.

4. *Argina astrea* SJIK11

The specimen SJIK11 was identified as *Argina astrea* (Drury, 1773) referring to the morphological features described by Drury, 1773.

Synonyms: *Phalaena astrea* Drury, 1773
Phalaena cribaria Clerck, 1764
Bombyx pylotis Fabricius, 1775
Deiopea dulcis Walker, 1854

Systematic position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Erebidae; Arctiinae; *Argina.*

Argina astrea is found in India, Sri Lanka, Pacific Islands, Australia and Tahiti. In India it has been reported from North Bengal, Sunderbans, Assam, Maharashtra, Tamil Nadu and Himalayas (Singh *et al*., 2014, Arandhara *et al.,* 2018, Shah *et al*., 2018). *Argina astrea* belongs to the family Erebidae. It is also called as crotalaria pod borer. It is a pest of tea. Host plants are species of *Crotalaria* (Leguminosae).

Head deep yellow; eyes dark; antennae thread-like and dark brown; neck and thorax yellow with two small black spots on the neck and four on the thorax; abdomen yellow; both the fore wings and hind wings are deep yellow; fore wing nearly orange coloured and have several rows of irregular and uneven black spots, the number of spots nearly 40. The hind wings are spotted with black, but much larger than those on the fore wings, except three that run along the external edges; the number being eleven; palpi are yellow, tipped with black; legs, breast and abdomen are yellow, abdomen spotted with black; under side of the wings are deep yellow; the edges of the wings are plain.

Results and discussion

The PCR of the COI gene fragment of *A. astrea* SJIK11 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 20 - 24. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190646).

Fig. 19. *Argina astrea* SJIK11 (dorsal and ventral view)

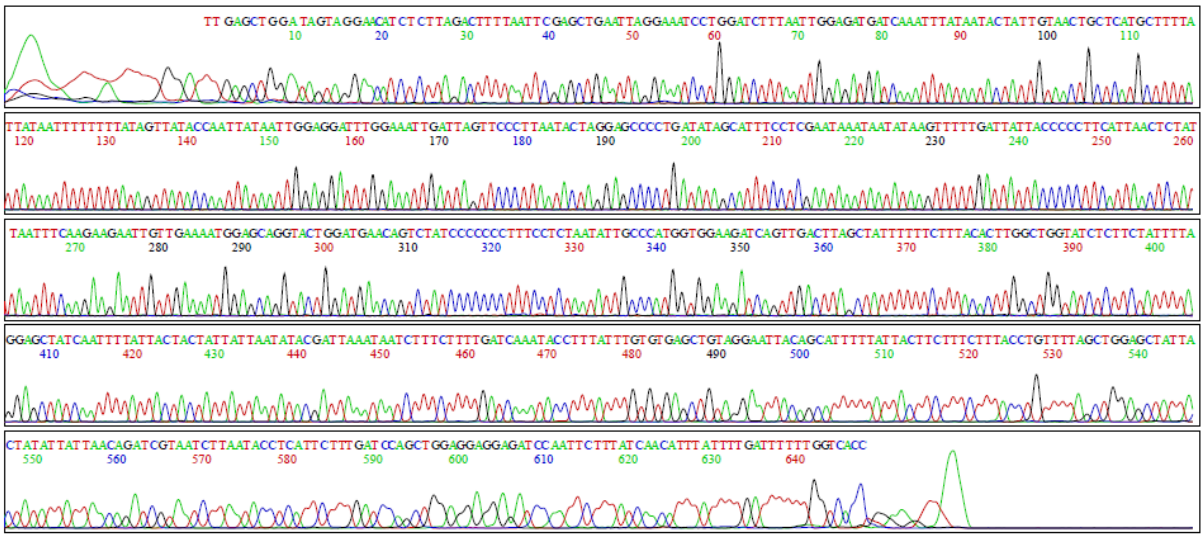

Fig. 20. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *A. astrea* SJIK11.

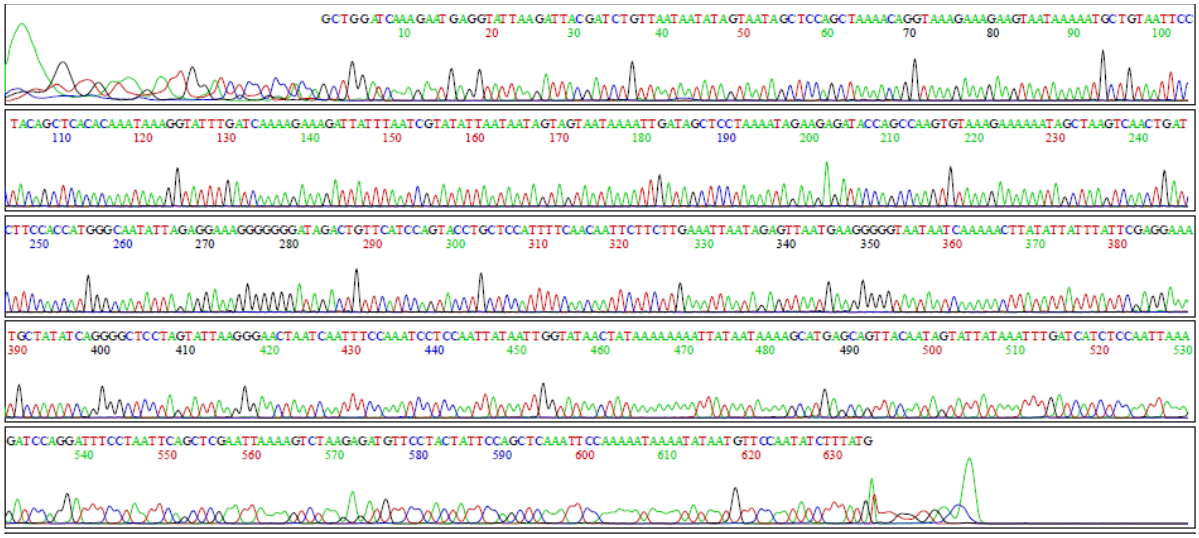

Fig. 21. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of of *A. astrea* SJIK11.

> *A. astrea* Voucher SJIK11 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACATTATATTTTATTTTTGGAATTTGAGCTGGAATAGTAGGAACATCTCTTAGACTTTTAATTCGAGCTGAATTAG
GAAATCCTGGATCTTTAATTGGAGATGATCAAATTTATAATACTATTGTAACTGCTCATGCTTTTATTATAATTTTT
TTTATAGTTATACCAATTATAATTGGAGGATTTGGAAATTGATTAGTTCCCTTAATACTAGGAGCCCCTGATATAGC
ATTTCCTCGAATAAATAATATAAGTTTTTGATTATTACCCCCTTCATTAACTCTATTAATTTCAAGAAGAATTGTTG
AAAATGGAGCAGGTACTGGATGAACAGTCTATCCCCCCCTTTCCTCTAATATTGCCCATGGTGGAAGATCAGTTGAC
TTAGCTATTTTTTCTTTACACTTGGCTGGTATCTCTTCTATTTTAGGAGCTATCAATTTTATTACTACTATTATTAA
TATACGATTAAATAATCTTTCTTTTGATCAAATACCTTTATTTGTGTGAGCTGTAGGAATTACAGCATTTTTATTAC
TTCTTTCTTTACCTGTTTTAGCTGGAGCTATTACTATATTATTAACAGATCGTAATCTTAATACCTCATTCTTTGAT
CCAGCTGGAGGAGGAGATCCAATTCTTTATCAACATTTATTT
```

Fig. 22. Partial coding sequence of *A. astrea* SJIK11 COI gene

> *A. astrea* Voucher SJIK11

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TLYFIFGIWAGMVGTSLSLLIRAELGNPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGF
GNWLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHGG
SSVDLAIFSLHLAGISSILGAINFITTIINMRLNNLSFDQMPLFVWAVGITAFLLLLSLPVL
AGAITMLLTDRNLNTSFFDPAGGGDPILYQHLF
```

Fig. 23. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *A. astrea* SJIK11.

Table 4. The BLAST hit table of the partial coding DNA sequence of COI gene of *A. astrea* SJIK11.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Query end	Geogra. location
1	Argina astrea HQ921235.1	100	658	0	0	1	658	Australia
2	Argina astrea KJ380862.1	98.65	593	8	0	35	627	India, W.Ghats
3	Argina astrea HQ921234.1	98.33	658	11	0	1	658	Australia
4	Melese sp. JQ557778.1	92.31	663	41	10	1	658	Costa Rica
5	Haploa contigua KJ380354.1	91.95	658	53	0	1	658	Canada
6	Rifargia xylinoides HQ568696.1	91.67	660	51	4	1	658	Brazil
7	Bertholdia soror JN262711.1	91.64	658	55	0	1	658	Brazil
8	Nystalea squamosa HQ567876.1	91.64	658	55	0	1	658	Brazil
9	Acontia thapsina HQ949187.1	91.50	659	52	4	2	658	Australia
10	Carteris oculatalis MF131611.1	91.34	658	57	0	1	658	USA

The DNA isolated from the sample *A. astrea* SJIK11 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 4 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

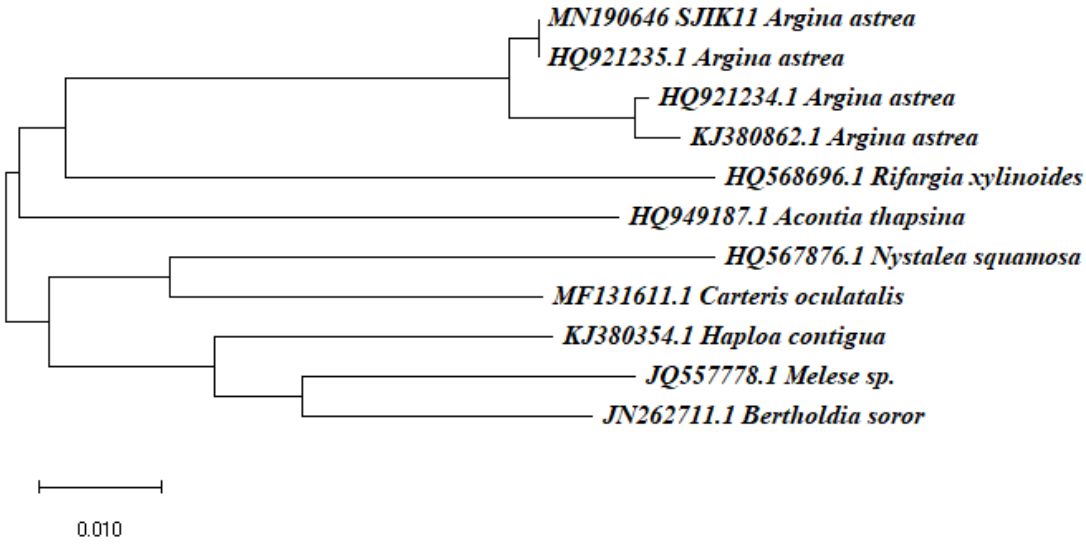

Fig. 24. The NJ tree showing phylogenetic relationships of *A. astrea* SJIK11.

A. astrea SJIK11 (MN190646) from Kerala showed 100% similarity to *A. astrea* HQ921235 from Australia, occupying the same clade and hence it can be used as a molecular barcode for species identification. It showed 98.65% similarity to *A. astrea* KJ380862 from India (W. Ghats) and 98.33% to *A. astrea* HQ921234 from Australia in the adjacent clade. The NJ tree revealed that all the four *Argina* species in the database shares a common ancestor. They are polymorphic variants of SJIK11. Its closest out-group is *Rifargia xylinoides* of the subfamily Notodontidae. The NJ tree distance data revealed that the species had diverged from its closely related species about 50000 years ago. The distribution pattern shows that the *Argina* genus has not traversed much across continents and has remained relatively isolated.

5. *Asota caricae* SJIK25

The specimen SJIK25 was identified as *Asota caricae* **(**Fabricius, 1775) referring to the morphological features described by Gurule, 2013.

Synonyms: *Noctua caricae* Fabricius, 1775
Psephea alciphron Cramer, 1777
Asota euroa Rothschild, 1897
Asota anwa Swinhoe, 1903

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Erebidae; Aganainae; *Asota*.

A. caricae is seen in India, Sri Lanka, China, Malaysia, Philippines, Borneo, Hong Kong, Indonesia, Java, Ireland, New Guinea and Australia. In India it is seen in Assam, Maharashtra, Jharkhand, Vagamon (Kerala), Ponmudi (Kerala), West Bengal, Himalayas, Chattisgarh and Tamil Nadu (Shubhalaxmi *et al*., 2011, Singh et al., 2018, Mathew *et al.,* 2018, Arandhara *et al.,* 2018, Sondhi *et al*., 2018, Shah *et al*., 2018). *Asota caricae* is known as tropical tiger moth. It belongs to the family Erebidae and subfamily Aganainae. Host plants are Sunhemp, *Ficus spp*., *Broussonetia sp*., *Mesua sp*., *Shorea robusta*. It is a pest of tea, teak, etc.

Identification characters: Palpi upturned; in male moths the antennae are fasciculate and ciliated in female; head, thorax and abdomen orange coloured; palpi with a black spot on 1st and 2nd joints; a black spot on tegulae; a dorsal series of black spots on abdomen often expanding into bands; fore wings are brownish fuscous; a basal orange patch with one basal and two sub-basal black spots and series of three on its outer edge; the veins streaked with white; a white spot at lower angle of cell; hind wing orange yellow; a black spot at end of cell, one beyond, one below vein 2, a sub-marginal irregular series which sometimes become a nearly complete marginal band, the veins crossing it yellow.

Results and discussion

The PCR of the COI gene fragment of *A. caricae* SJIK25 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 26-30. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190659).

Fig. 25. *Asota caricae* SJIK25 (dorsal and ventral view)

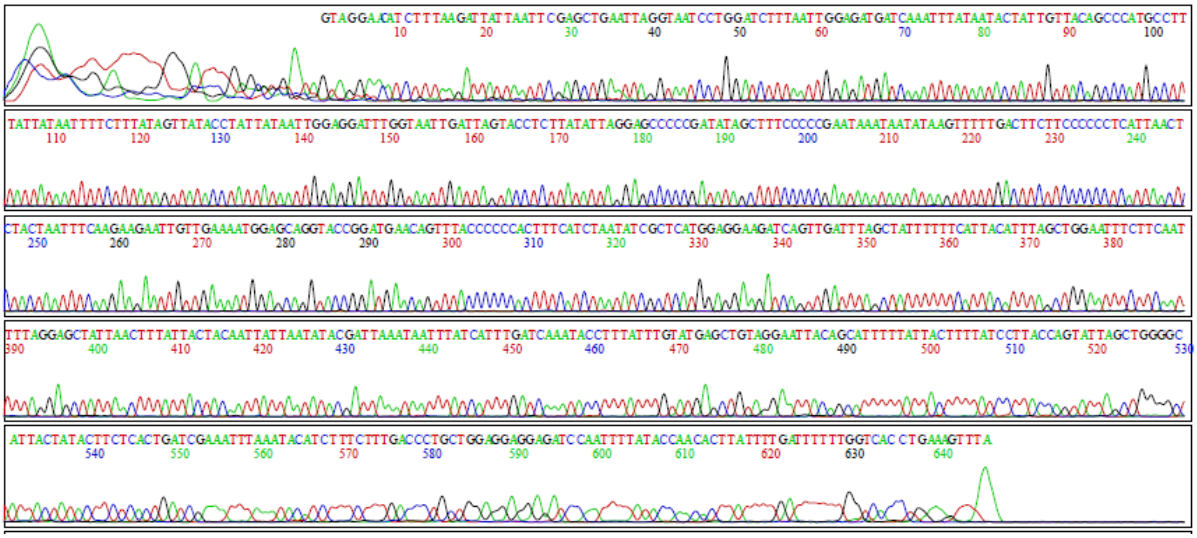

Fig. 26. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *A. caricae* SJIK25.

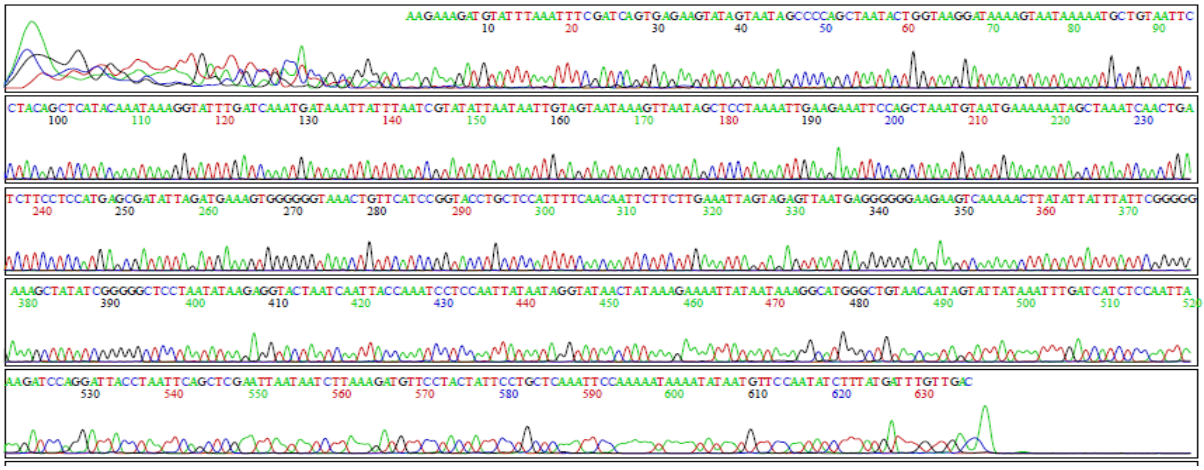

Fig. 27. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *A. caricae* SJIK25.

The DNA isolated from the sample *A. caricae* SJIK25 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 5 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

> *A. caricae* Voucher SJIK25 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

AACATTATATTTTATTTTTGGAATTTGAGCAGGAATAGTAGGAACATCTTTAAGATTATTAA
TTCGAGCTGAATTAGGTAATCCTGGATCTTTAATTGGAGATGATCAAATTTATAATACTATT
GTTACAGCCCATGCCTTTATTATAATTTTCTTTATAGTTATACCTATTATAATTGGAGGATT
TGGTAATTGATTAGTACCTCTTATATTAGGAGCCCCCGATATAGCTTTCCCCCGAATAAATA
ATATAAGTTTTTGACTTCTTCCCCCCTCATTAACTCTACTAATTTCAAGAAGAATTGTTGAA
AATGGAGCAGGTACCGGATGAACAGTTTACCCCCCACTTTCATCTAATATCGCTCATGGAGG
AAGATCAGTTGATTTAGCTATTTTTTCATTACATTTAGCTGGAATTTCTTCAATTTTAGGAG
CTATTAACTTTATTACTACAATTATTAATATACGATTAAATAATTTATCATTTGATCAAATA
CCTTTATTTGTATGAGCTGTAGGAATTACAGCATTTTTATTACTTTTATCCTTACCAGTATT
AGCTGGGGCTATTACTATACTTCTCACTGATCGAAATTTAAATACATCTTTCTTTGACCCTG
CTGGAGGAGGAGATCCAATTTTATACCAACACTTATTT

Fig. 28. Partial coding sequence of *A. caricae* SJIK25 COI gene.

> *A. caricae* Voucher SJIK25

TLYFIFGIWAGMVGTSLSLLIRAELGNPGSLIGDDQIYNTIVTAHAFIMIFFMVMPI
MIGGFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYP
PLSSNIAHGGSSVDLAIFSLHLAGISSILGAINFITTIINMRLNNLSFDQMPLFVWA
VGITAFLLLLSLPVLAGAITMLLTDRNLNTSFFDPAGGGDPILYQHLF

Fig. 29. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *A. caricae* SJIK25.

Asota caricae SJIK25 from Kerala showed 100% similarity to 4 species of *A. caricae* KX862642, KX861434 and KX861239 from Pakistan, MG783846 from India (Maharashtra). HQ921356 from Australia with 99.7 % similarity is a polymorphic variant of SJIK25 occupying the same clade. All these share a common ancestor. Some subspecies of *A. caricae* like *A. caricae caricae* GU662336 from Thailand (100% similarity), KC499393 from China (99.85% similarity) also occupies the same clade which might have evolved by geographical isolation.

Table 5. The BLAST hit table of the partial coding DNA sequence of COI gene of *A. caricae* SJIK25.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Q start	Q end	Geogra. location
1	Asota caricae KX862642.1	100	658	0	0	1	658	Pakistan
2	Asota caricae KX861434.1	100	658	0	0	1	658	Pakistan
3	Asota caricae KX861239.1	100	658	0	0	1	658	Pakistan
4	Asota caricae MG783846.1	100	654	0	0	2	655	India, Maharashtra
5	Asota caricae caricae GU662336.1	100	658	0	0	1	658	Thailand
6	Asota caricae caricae KC499393.1	99.85	658	1	0	1	658	China
7	Asota caricae HQ921356.1	99.70	658	2	0	1	658	Australia
8	Asota plana plana HQ569734.1	98.48	658	10	0	1	658	Indonesia
9	Asota paliura HQ569654.1	98.18	658	12	0	1	658	China
10	Asota heliconia venalba GU662357.1	98.02	658	13	0	1	658	India, Andaman Islands
11	Asota caricae caricae GU662335.1	98.02	658	13	0	1	658	Thailand
12	Asota plana albifera GU662391.1	98.02	658	13	0	1	658	Indonesia
13	Asota heliconia heliconia GU662345.1	98.02	658	13	0	1	658	Thailand
14	Asota plaginota plaginota HQ569790.1	97.87	658	14	0	1	658	India, Andaman Islands
15	Asota paliura HQ569661.1	97.87	658	14	0	1	658	Vietnam
16	Asota albiformis ternatensis HM395494.1	97.87	658	14	0	1	658	Indonesia
17	Asota darsania KC499401.1	97.72	658	15	0	1	658	Indonesia
18	Asota albivena GU662387.1	97.72	658	15	0	1	658	Indonesia
19	Asota albiformis albiformis KC499379.1	97.57	658	16	0	1	658	Philippines
20	Asota sulawesiensis GU662399.1	97.26	658	18	0	1	658	Indonesia

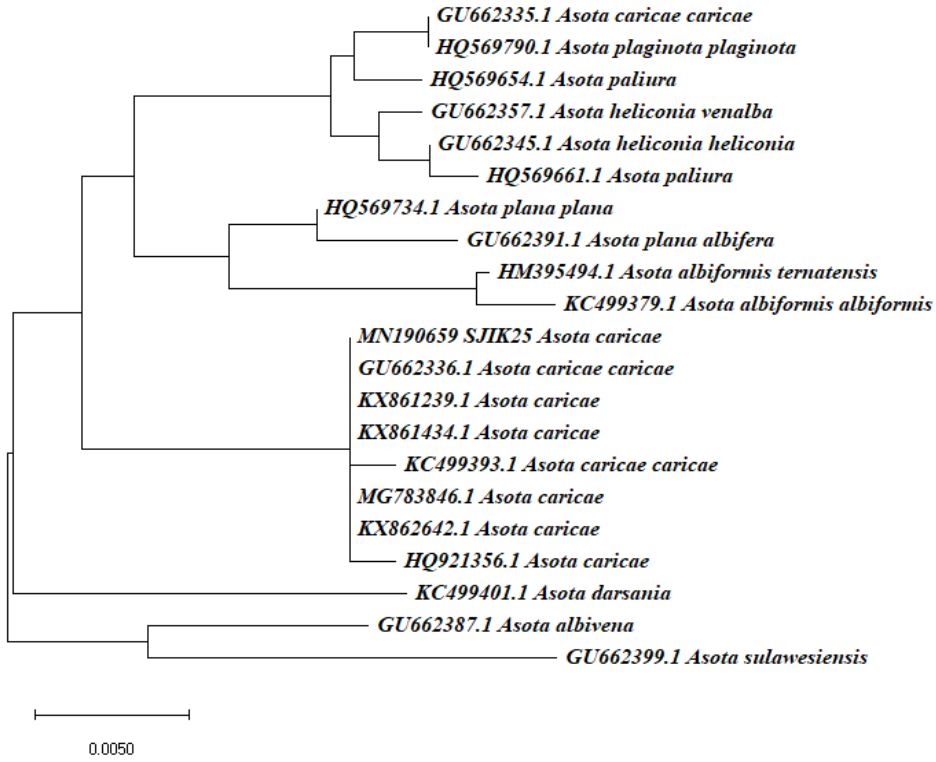

Fig. 30. The NJ tree showing phylogenetic relationships of *A. caricae* SJIK25.

The NJ- tree shows that *A. paliura* is the most distant relative of SJIK25. *A. darsania* KC499401 with 97.72% similarity, placed in a different clade, is a different species of the genus which remain close to SJIK25. The distance data revealed that the species originated from its closely related species *A. darsania* about 15000 years ago. The geographical distribution pattern shows the common origin of the various species of *Asota caricae* from the Gondwana. It showed a South East Asian lineage. The COI sequence of *Asota caricae* SJIK25 BLAST results showed 100% similarity to that of 4 isolates of *Asota caricae* deposited in the database. Therefore, the sequence isolated from SJIK25 can be used as a molecular barcode for identification of the species.

6. *Pandesma quenavadi* SJIK16.

The specimen SJIK16 was identified as *Pandesma quenavadi* (Guenee, 1852) referring to the morphological features described by Hampson, 1894.

Synonyms: *Pandesma jubra*, Swinhoe, 1889

Thria quenavadi

Systematic position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Noctuidae; Catocalinae; *Pandesma*. *P. quenavadi* is found in DR Congo, Egypt, Gambia, Kenya, Madagascar, Malawi, Namibia, Somalia, South Africa, Sudan, Tanzania, Uganda, Zambia, Zimbabwe, Kenya, Sudan, Srilanka, Bangladesh, Pakistan Myanmar, Philippines Japan and Australia. In India it is reported from Himachal Pradesh, Karnataka, Nilgiri biosphere, Vagamon (Kerala) and Maharashtra (Mathew *et al*., 2018). *P. quenavadi* belongs to the family Noctuidae and subfamily Catocalinae. Larval food plants include *Vachellia (Acacia) karroo, Acacia mollissima, Albiza chinensis, Albiza lebbeck, etc.*

The moths are brownish grey in colour; fore wing with sub-basal, ante-medial, medial, excurved post-medial and sub-marginal waved lines; the orbicular and renifrom indistinct; a marginal series of specks are seen; basal part of hind wing whitish; the outer area black, with post-medial and sub-marginal indistinct waved lines; underside white, with a broad sub-marginal fuscous band and marginal series of black specks to each wing; ochreous on head and collar; abdomen ringed with ochreous; fore wing with a black speck on ante-medial line; a brown diffused sub-marginal band.

Larval food plants include *Vachellia (Acacia) karroo, Acacia mollissima, Albiza chinensis, Albiza lebbeck,*

Results and discussion

The PCR of the COI gene fragment of *P. quenavadi* SJIK16 from Kerala yielded a product of 658bp long. The forward and reverse sequence chromatograms, COI gene sequence obtained, BLASTn result, conceptual translation product result are presented in Figures 32- 36 . The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190650).

Fig. 31. *P. quenavadi* SJIK16 (dorsal and ventral view)

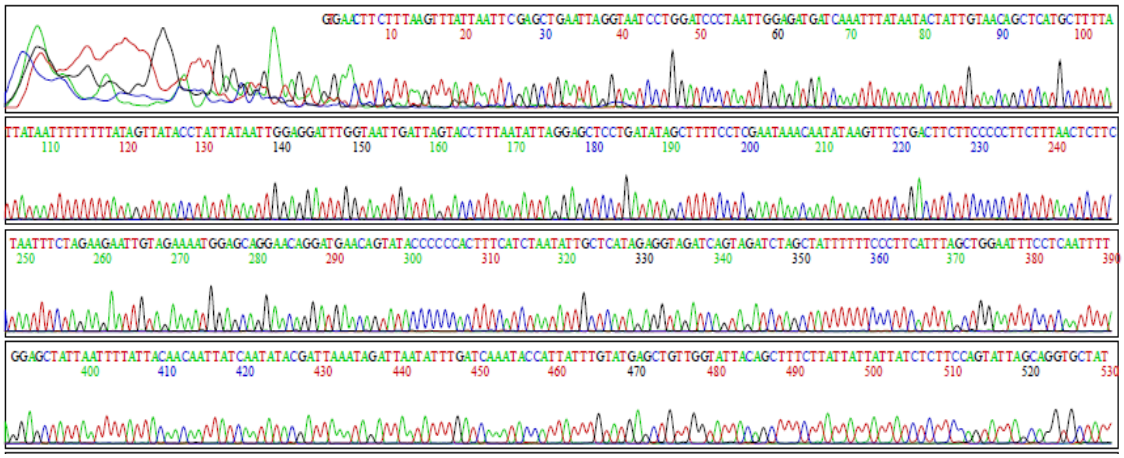

Fig. 32. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *P. quenavadi* SJIK16.

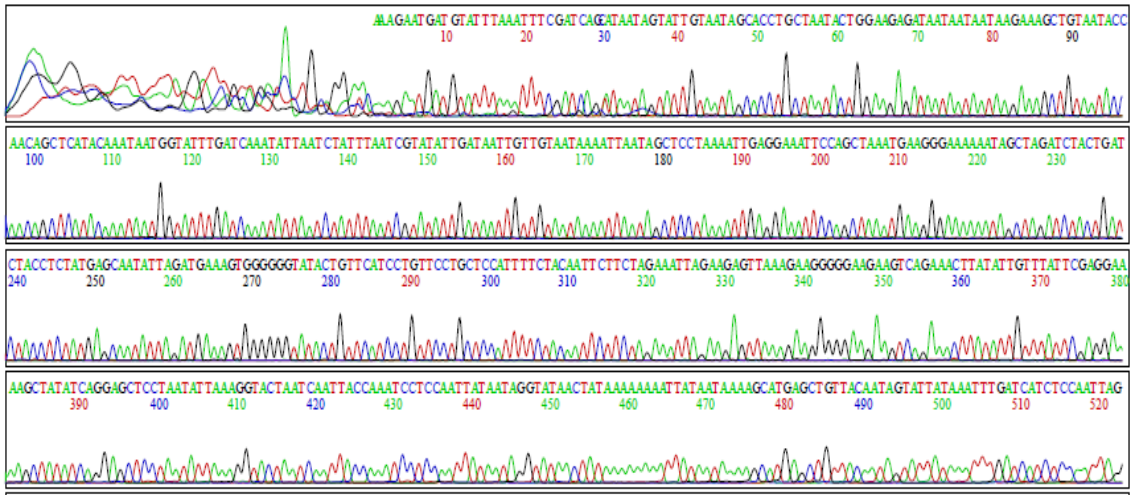

Fig. 33 Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *P. quenavadi* SJIK16.

> *P. quenavadi* Voucher SJIK16 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACTTTATATTTTATTTTTGGTATTTGAGCAGGAATAGTAGGAACTTCTTTAAGTTTATTAATTCGAGCT
GAATTAGGTAATCCTGGATCCCTAATTGGAGATGATCAAATTTATAATACTATTGTAACAGCTCATGCTT
TTATTATAATTTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGTAATTGATTAGTACCTTTAAT
ATTAGGAGCTCCTGATATAGCTTTTCCTCGAATAAACAATATAAGTTTCTGACTTCTTCCCCCTTCTTTA
ACTCTTCTAATTTCTAGAAGAATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTATACCCCCCACTTT
CATCTAATATTGCTCATAGAGGTAGATCAGTAGATCTAGCTATTTTTTCCCTTCATTTAGCTGGAATTTC
CTCAATTTTAGGAGCTATTAATTTTATTACAACAATTATCAATATACGATTAAATAGATTAATATTTGAT
CAAATACCATTATTTGTATGAGCTGTTGGTATTACAGCTTTCTTATTATTATTATCTCTTCCAGTATTAG
CAGGTGCTATTACAATACTATTAACTGATCGAAATTTAAATACATCATTCTTTGATCCTGCAGGAGGTGG
TGATCCTATTTTATATCAACATTTATTT
```

Fig. 34. Partial coding sequence of *P. quenavadi* SJIK16 COI gene.

> *P. quenavadi* Voucher SJIK16

```
TLYFIFGIWAGMVGTSLSLLIRAELGNPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFG
NWLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHSGSS
VDLAIFSLHLAGISSILGAINFITTIINMRLNSLMFDQMPLFVWAVGITAFLLLLSLPVLAGA
ITMLLTDRNLNTSFFDPAGGGDPILYQHLF
```

Fig. 35. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *P. quenavadi* Voucher SJIK16.

Table 6. The BLAST hit table of the partial coding DNA sequence of COI gene of *P. quenavadi* SJIK16.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Query end	Geographical location
1	Pandesma quenavadi JN988583.1	100	658	0	0	1	658	Pakistan
2	Pandesma quenavadi HQ949897.1	97.26	658	18	0	1	658	Australia
3	Pandesma quenavadi HQ949895.1	97.11	658	19	0	1	658	Australia
4	Pandesma partita KF391129.1	95.44	658	30	0	1	658	Australia
5	Pandesma submurina HQ949900.1	94.83	658	34	0	1	658	Australia
6	Pandesma submurina HQ949898.1	94.68	658	35	0	1	658	Australia
7	Pandesma submurina HQ949899.1	94.53	658	36	0	1	658	Australia
8	Pandesma robusta KX860367.1	94.07	658	39	0	1	658	Pakistan
9	Pandesma robusta KY370624.1	93.92	658	40	0	1	658	Spain
10	Pandesma robusta JN988585.1	93.92	658	40	0	1	658	Pakistan
11	Pandesma robusta KX861569.1	93.77	658	41	0	1	658	Pakistan
12	Pandesma robusta KX860854.1	93.77	658	41	0	1	658	Pakistan
13	Pandesma robusta KX860341.1	93.75	656	41	0	1	656	Pakistan
14	Palyna metagona JQ550606.1	93.47	658	43	0	1	658	Costa Rica
15	Bastilla joviana HQ950301.1	93.31	658	44	0	1	658	Australia

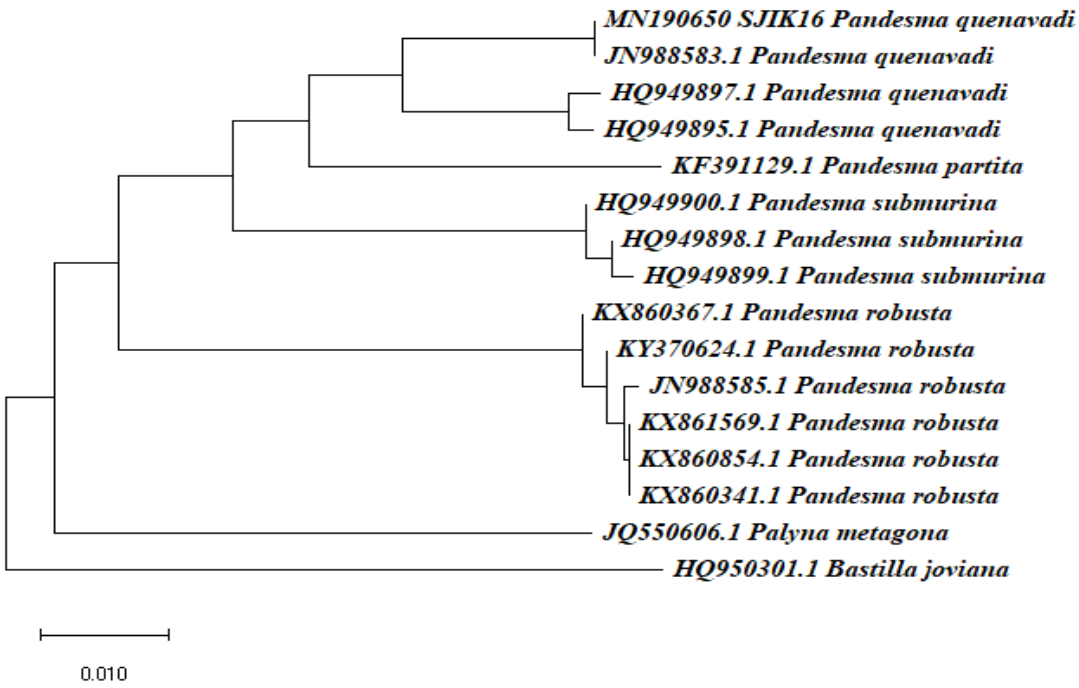

Fig. 36. The NJ tree showing phylogenetic relationships of *P. quenavadi* SJIK16.

The DNA isolated from the sample *P. quenavadi* SJIK16 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 6 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

SJIK16 isolated from Kerala showed 100% similarity to *P. quanevadi* JN988583 from Pakistan occupying the same clade. Hence it can be used as barcode for species identification. SJIK 16 showed 97.26% similarity to *P. quanevadi* HQ949897 and 97.11% to HQ949895 from Australia placed in the adjacent clade. They are polymorphic variants of SJIK16 evolved from a common ancestor. The Kerala isolate showed 95.44% similarity to *P. partita* KF391129 from Australia which is the closest relative of the species occupying the adjacent clade. The distance data shows that the species originated from its closest relative about 25000 years ago and it is comparatively of recent origin. The phylogenetic tree also shows that *P. quanevadi, P. partita, P. submurina* and *P. robusta* diverged from a common ancestor.

7. *Heteropalpia sp.* SJIK19

The specimen SJIK19 was identified as *Heteropalpia sp.* (Berio, 1939) referring to the morphological features described by Berio, 1960 and Wiltshire, 1970.

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Noctuidae; Catocalinae; *Heteropalpia.*

Heteropalpia sp. is seen in Iran, Egypt, Jordan and countries of the Arabian Peninsula. No *Heteropalpia sp.* has been reported from India. This is the first record of the species from India. It belong to the family Noctuidae and subfamily Catocalinae. These moths are multivoltine. Host plant is *Acacia sp.*

Identifying characters: Smoky coloured with dark brown spots on the upper side of the wings; underside of the wings creamy; lower edges of the wings are wavy; femur without spines, male genitalia with complex scaphium (uncus), androconial groove more or less developed on the second tibia; uncus rigid, thick and short, without gnathos; in females posterior apophyses much shorter than anterior; genital plate sclerotized, irregular, often asymmetrical; ductus sclerotized, wide, asymmetrical, well demarcated from bursa and longer than it; bursa globular, without signum; abdomen without dorsal crests.

Results and discussion

The PCR of the COI gene fragment of *Heteropalpia sp.* SJIK19 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 38 - 42. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190655).

The DNA isolated from the sample *Heteropalpia sp.* SJIK19 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 7 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

Fig. 37. *Heteropalpia sp.* SJIK19 (dorsal and ventral view)

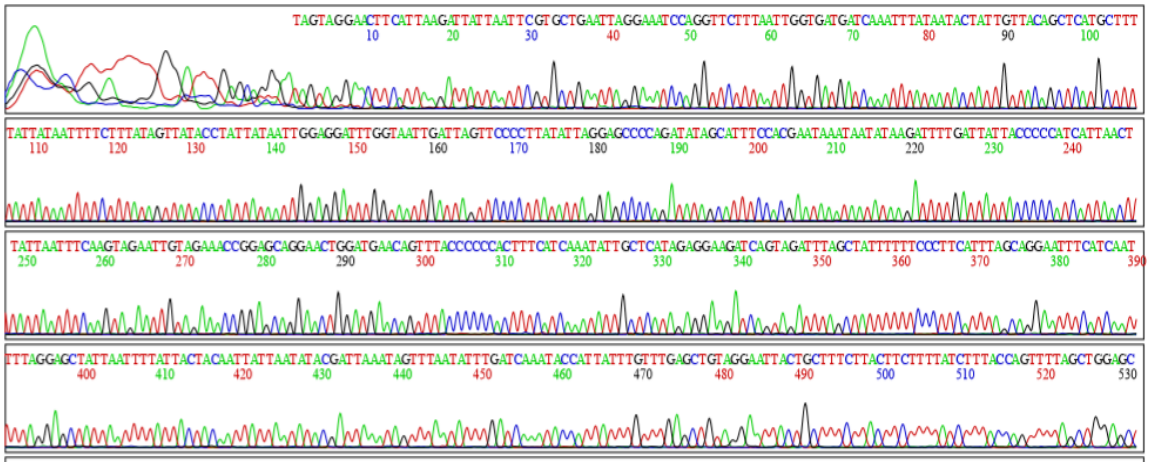

Fig. 38. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *Heteropalpia sp.* SJIK19.

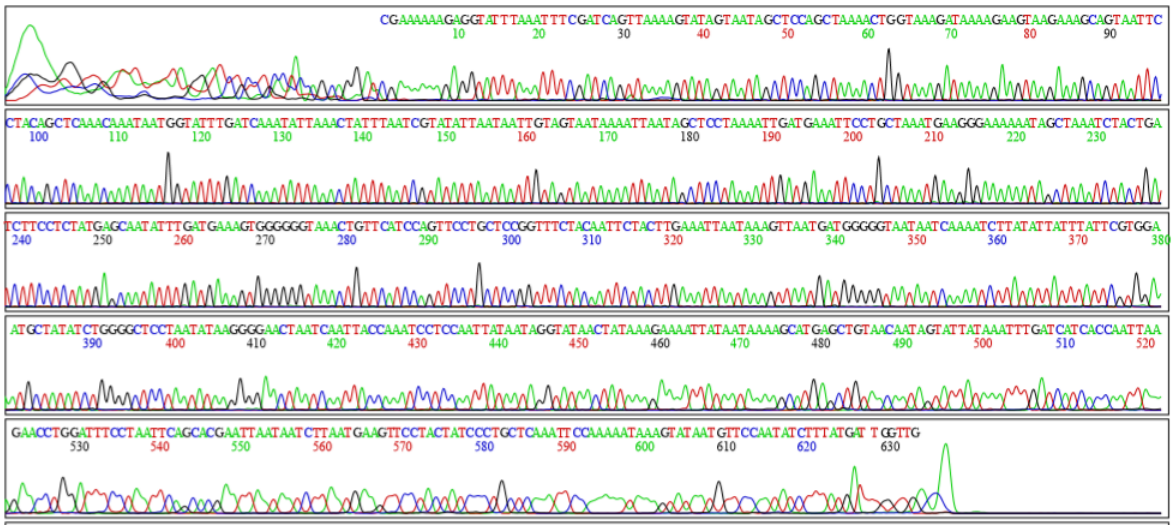

Fig. 39. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *Heteropalpia sp.* SJIK19.

> *Heteropalpia sp.* Voucher SJIK19 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACATTATACTTTATTTTTGGAATTTGAGCAGGGATAGTAGGAACTTCATTAAGATTATTAATTCGTGCTGAATTA
GGAAATCCAGGTTCTTTAATTGGTGATGATCAAATTTATAATACTATTGTTACAGCTCATGCTTTTATTATAATTT
TCTTTATAGTTATACCTATTATAATTGGAGGATTTGGTAATTGATTAGTTCCCCTTATATTAGGAGCCCCAGATAT
AGCATTTCCACGAATAAATAATATAAGATTTTGATTATTACCCCCATCATTAACTTTATTAATTTCAAGTAGAATT
GTAGAAACCGGAGCAGGAACTGGATGAACAGTTTACCCCCCACTTTCATCAAATATTGCTCATAGAGGAAGATCAG
TAGATTTAGCTATTTTTTCCCTTCATTTAGCAGGAATTTCATCAATTTTAGGAGCTATTAATTTTATTACTACAAT
TATTAATATACGATTAAATAGTTTAATATTTGATCAAATACCATTATTTGTTTGAGCTGTAGGAATTACTGCTTTC
TTACTTCTTTTATCTTTACCAGTTTTAGCTGGAGCTATTACTATACTTTTAACTGATCGAAATTTAAATACCTCTT
TTTTCGATCCTGCTGGAGGAGGAGATCCTATTTTATATCAACATTTATTT
```

Fig. 40. Partial coding sequence of *Heteropalpia sp.* SJIK19 COI gene.

> *Heteropalpia sp.* Voucher SJIK19

```
TLYFIFGIWAGMVGTSLSLLIRAELGNPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWLVPLM
LGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVETGAGTGWTVYPPLSSNIAHSGSSVDLAIFSLHLAGIS
SILGAINFITTIINMRLNSLMFDQMPLFVWAVGITAFLLLLSLPVLAGAITMLLTDRNLNTSFFDPAGGG
DPILYQHLF
```

Fig. 41.The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *Heteropalpia sp.* SJIK19.

Table 7. The BLAST hit table of the partial coding DNA sequence of COI gene of *Heteropalpia sp.* SJIK19.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Query end	Geographical location
1	Heteropalpia acrosticta HQ006186.1	95.28	657	31	0	2	658	N.A.
2	Rhabdophera robusta KX862755.1	93.62	658	42	0	1	658	Pakistan
3	Asota speciosa GU662443.1	93.31	658	44	0	1	658	Nigeria
4	Catocala hermia MF130755.1	93.17	659	43	2	1	658	USA
5	Catocala jair MF130674.1	93.03	660	42	4	1	658	USA
6	Catocala californica MF129043.1	93.02	659	44	2	1	658	USA
7	Catocala semirelicta hippolyta MF132208.1	92.87	659	45	2	1	658	USA
8	Catocala allusa MF126563.1	92.87	659	45	2	1	658	USA

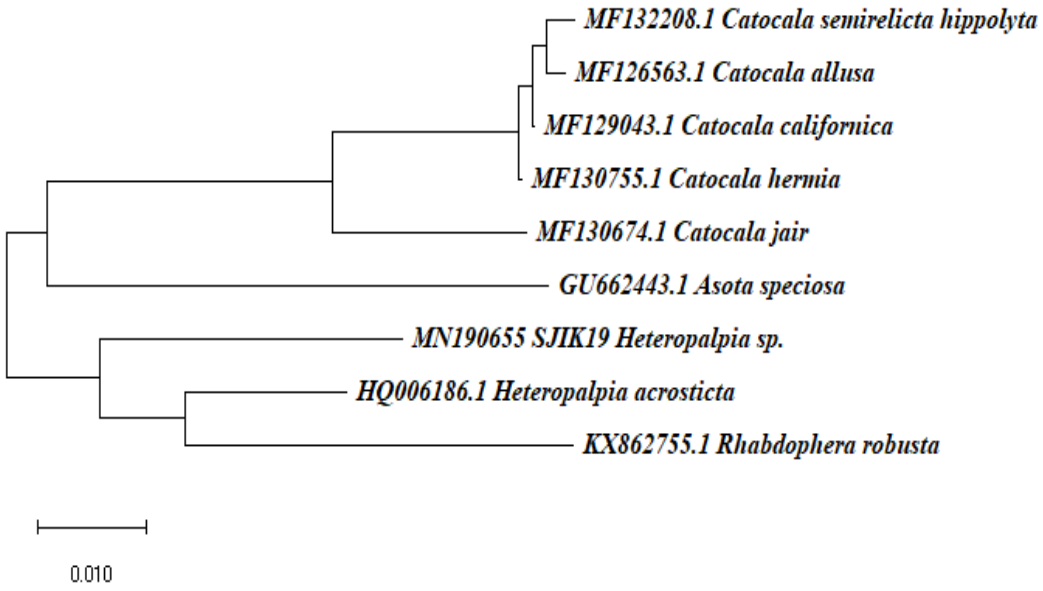

Fig. 42. The NJ tree showing phylogenetic relationships of *Heteropalpia sp.* SJIK19.

The sequence blast of the COI sequence of SJIK19 isolated from Kerala showed a maximum similarity of 95.28% to *H. acrosticta* HQ006186 in the database and was placed in a separate clade. Hence the sequence of SJIK19 is a novel one. The species is being reported for the first time from India.The NJ tree distance data revealed that the species had diverged from its closely related species *H. acrosticta* about 22000years ago. The phylogenetic tree also shows that the genus is close to the genus *Asota* of the family Erebidae showing a similarity of 93.31%.

8. *Biston suppressaria* SJIK20

The specimen SJIK20 was identified as *Biston suppressaria*, (Guenee, 1858) referring to the morphological features described by Hampson, 1895 and Gurule, 2013.

Synonyms: *Amphidasys suppressaria* Guenee, 1858
Buzura suppressaria
Buzura multipuctaria Walker, 1863
Biston luculentus Inoue, 1992
Buzura strigaria Moore, 1879

Sytematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Geometroidea; Geometridae; Ennominae; *Biston*.

B. suppressaria is found in India, China, Myanmar, Nepal, Thailand, Japan and Srilanka (Letchner, 2011). In India it is reported from Maharashtra, Jharkhand, Assam, Himachal Pradesh, Ponmudi (Kerala), West Bengal, Sikkim and Himalayas (Shubhalaxmi *et al*., 2011, Singh et al., 2018, Arandhara *et al.,* 2018, Kumar *et al*., 2018, Sondhi *et al*., 2018, Shah *et al*., 2018). *B. suppressaria* is known as tea looper. It belongs to the family Geometridae and subfamily Ennominae. It is a pest of field crops like castor and plantation crops like arecanut and tea. Larve feed on *Cassia auriculata*.

Identifying features: Grey, irrorated with black; the head ochreous; thorax and abdomen with yellow bars; fore wing with waved yellow ante-medial band; both wings with irregularly sinuous and indistinct yellow medial line excurved beyond cell of fore wing; an ill-defined post medial maculate band angled at vein 5 of both wings with some yellow spots beyond it and some black suffusion at the middle of outer margin of fore wing; a marginal series of yellow spots; palpi are short and hairy; bipectinate antennae in male; thorax is stout and clothed with thick pile; legs are hairy; fore wings with rounded apex; a marginal series of yellow and black spots.

Results and discussion

The PCR of the COI gene fragment of *B. suppressaria* SJIK20 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 44 - 48. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190653).

Fig. 43. *Biston suppressaria* SJIK20 (dorsal view & ventral view)

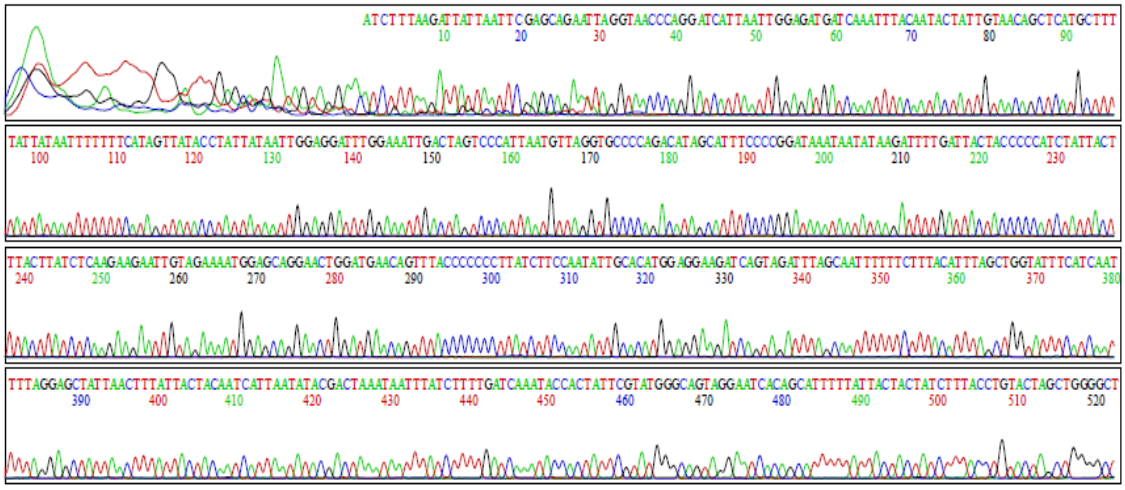

Fig. 44. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *B. suppressaria* SJIK20.

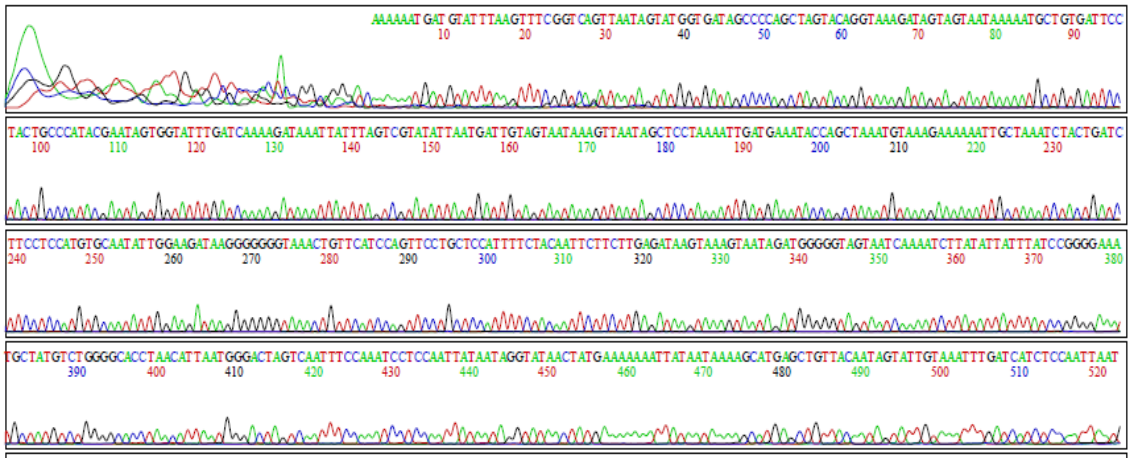

Fig. 45. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *B. suppressaria* SJIK20.

> *B. suppressaria* Voucher SJIK20 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACATTATATTTTATTTTTGGTATTTGAGCAGGAATAGTAGGAACATCTTTAAGATTATTAATTCGAGCAGAATTAGGTA
ACCCAGGATCATTAATTGGAGATGATCAAATTTACAATACTATTGTAACAGCTCATGCTTTTATTATAATTTTTTTCATA
GTTATACCTATTATAATTGGAGGATTTGGAAATTGACTAGTCCCATTAATGTTAGGTGCCCCAGACATAGCATTTCCCCG
GATAAATAATATAAGATTTTGATTACTACCCCCATCTATTACTTTACTTATCTCAAGAAGAATTGTAGAAAATGGAGCAG
GAACTGGATGAACAGTTTACCCCCCCTTATCTTCCAATATTGCACATGGAGGAAGATCAGTAGATTTAGCAATTTTTTCT
TTACATTTAGCTGGTATTTCATCAATTTTAGGAGCTATTAACTTTATTACTACAATCATTAATATACGACTAAATAATTT
ATCTTTTGATCAAATACCACTATTCGTATGGGCAGTAGGAATCACAGCATTTTTATTACTACTATCTTTACCTGTACTAG
CTGGGGCTATCACCATACTATTAACTGACCGAAACTTAAATACATCATTTTTTGACCCTGCCGGAGGGGGAGACCCAATT
CTTTATCAACATTTATTT
```

Fig. 46. Partial coding sequence of *B. suppressaria* SJIK20 COI gene.

> *B. suppressaria* Voucher SJIK20

```
TLYFIFGIWAGMVGTSLSLLIRAELGNPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWL
VPLMLGAPDMAFPRMNNMSFWLLPPSITLLISSSIVENGAGTGWTVYPPLSSNIAHGGSSVDLAIF
SLHLAGISSILGAINFITTIINMRLNNLSFDQMPLFVWAVGITAFLLLLSLPVLAGAITMLLTDRN
LNTSFFDPAGGGDPILYQHLF
```

Fig. 47. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *B. suppressaria* SJIK20.

Table 8. The BLAST hit table of the partial coding DNA sequence of COI gene of *B. suppressaria* SJIK20.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Query end	Geograph. location
1	*Biston suppressaria* KX861510.1	97.26	658	18	0	1	658	Pakistan
2	*Biston suppressaria* KX860660.1	97.26	658	18	0	1	658	Pakistan
3	*Biston suppressaria* KX860493.1	97.26	658	18	0	1	658	Pakistan
4	*Biston suppressaria* KX860330.1	97.26	658	18	0	1	658	Pakistan
5	*Biston suppressaria* KF748228.1	97.26	658	18	0	1	658	China
6	*Biston suppressaria* KX862514.1	97.11	658	19	0	1	658	Pakistan
7	*Biston suppressaria* KF748229.1	97.11	658	19	0	1	658	China
8	*Biston suppressaria* KX860646.1	97.06	646	19	0	1	646	Pakistan
9	*Biston suppressaria* KX862586.1	96.66	658	22	0	1	658	Pakistan

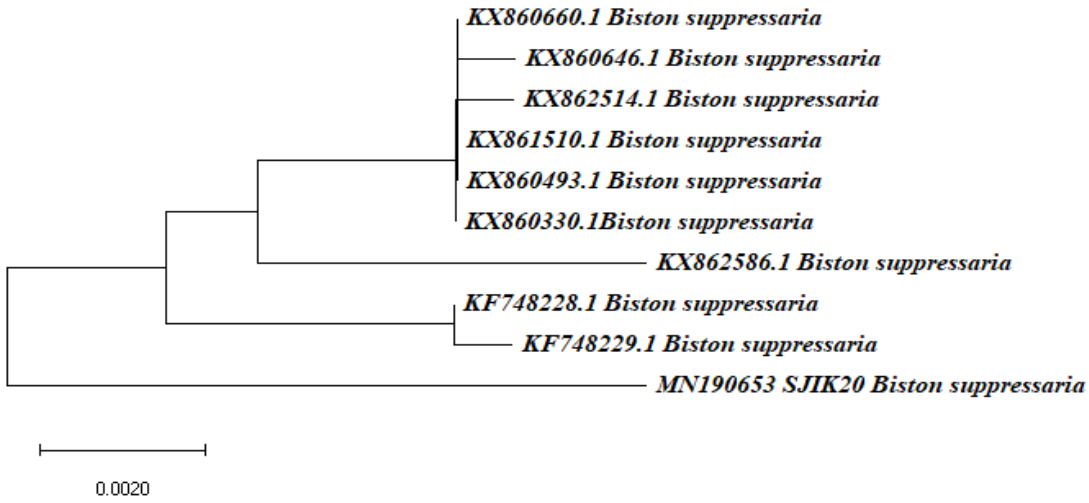

Fig. 48. The NJ tree showing phylogenetic relationships of *B. suppressaria* SJIK20.

The DNA isolated from the sample *B. suppressaria* SJIK20 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 8 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

The BLAST result of SJIK20 isolated from Kerala showed a maximum similarity of 97.26% to *B. suppressaria* KX861510, KX860660, KX860493 and KX860330 from Pakistan and KF748228 from China. Hence the sequence obtained is a novel one and it is placed in a separate clade. It shows 97.11 % similarity to *B. suppressaria* KX862514 from Pakistan and KF748229 from China, 97.06 % to KX860646 from Pakistan and 96.66% to KX862586 also from Pakistan. All are geographical variants of the species occupying separate clades, diverged from a common ancestor. The NJ tree shows that SJIK20 isolated from Kerala and all other *Biston* species considered for constructing the NJ tree have evolved from a common ancestor but SJIK20 diverged from its Chinese variant about 8000 years ago occupying a separate clade. The species showed an Asian lineage.

9. *Nyctemera coleta* SJIK21

The specimen SJIK21 was identified as *Nyctemera coleta* (Stoll, 1782) referring to the morphological features described by Hampson, 1894.

Synonyms: *Phalaena coleta* Stoll, 1782

Nyctemera nigrovenosa Moore, 1879

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Holometabola; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Erebidae; Arctiinae; *Nyctemera*.

N. coleta is found in India, Srilanka, Myanmar, Philippines, Japan and Papua New Guinea. In India it is reported from Parambikulam (Kerala), Assam and Nicobar Islands (Sudheendrakumar, 1999). *Nyctemera coleta* is a day flying moth which belongs to the family Erebidae and subfamily Arctiinae. It is known as marbled white moth or white tiger moth. It is a pest of some medicinal plants like *Gynura procumbens* used as analgesic and antimicrobial. It eats up the leaves leaving only the petiole. The alkaloids present in the plant helps the moth to escape from predators as it renders the insect non palatable.

Identifying characters: palpi porrectly upturned; antennae bipectinate in both sexes, the branches shorter in females; fore wing with vein 3 from before the angle of cell, 5 from above it; 6 from upper angle, 7 and 10 from the short areole which is formed by the anastomosis of 8 and 9; hind wing with vein 3 from before end of cell, 5 from angle or from above it; 6 and 7 stalked or from upper angle; 8 from before middle of cell; lower three spots of the post-medial band of fore wing separated and have another spot below them towards outer angle; cilia white below the apex and in most specimens at anal angle.

Results and discussion

The PCR of the COI gene fragment of *N. coleta* SJIK21 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 50- 54. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190654).

Fig. 49. *Nyctemera coleta* SJIK21 (dorsal and ventral view)

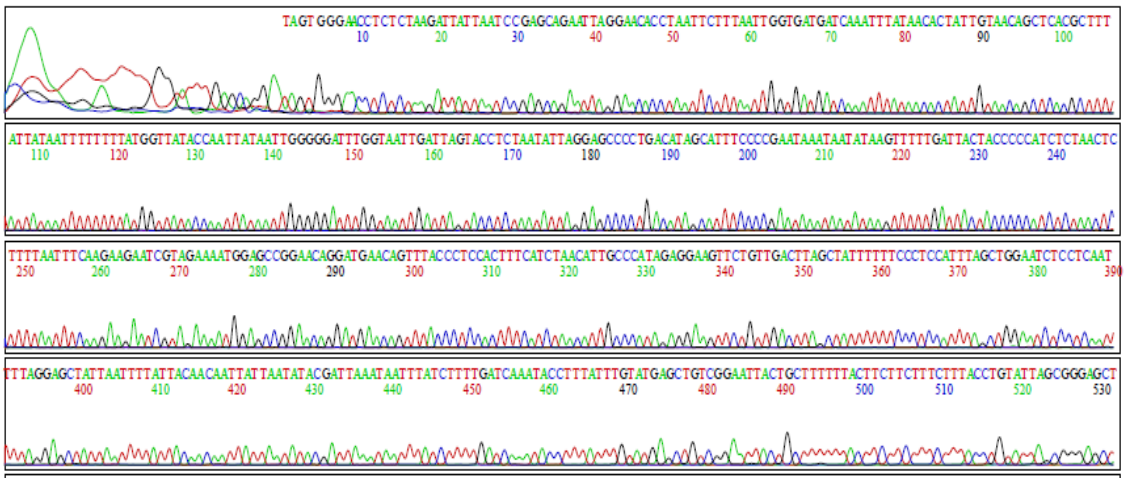

Fig. 50. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *N. coleta* SJIK21.

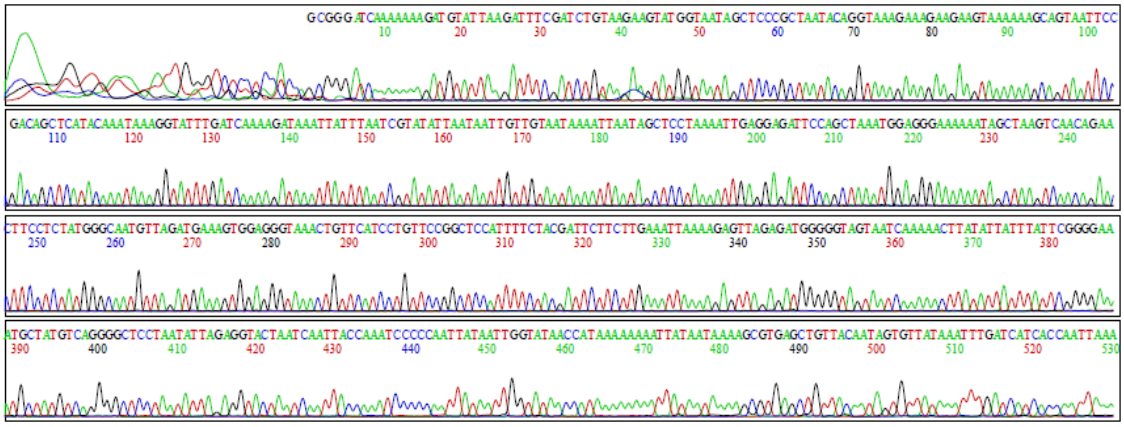

Fig. 51. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *N. coleta* SJIK21.

> *N. coleta* Voucher SJIK21 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACATTATATTTTATTTTTGGTATTTGAGCAGGAATAGTGGGAACCTCTCTAAGATTATTAATCCGAGCAGAATTAGGA
ACACCTAATTCTTTAATTGGTGATGATCAAATTTATAACACTATTGTAACAGCTCACGCTTTTATTATAATTTTTTTTA
TGGTTATACCAATTATAATTGGGGGATTTGGTAATTGATTAGTACCTCTAATATTAGGAGCCCCTGACATAGCATTTCC
CCGAATAAATAATATAAGTTTTTGATTACTACCCCCATCTCTAACTCTTTTAATTTCAAGAAGAATCGTAGAAAATGGA
GCCGGAACAGGATGAACAGTTTACCCTCCACTTTCATCTAACATTGCCCATAGAGGAAGTTCTGTTGACTTAGCTATTT
TTTCCCTCCATTTAGCTGGAATCTCCTCAATTTTAGGAGCTATTAATTTTATTACAACAATTATTAATATACGATTAAA
TAATTTATCTTTTGATCAAATACCTTTATTTGTATGAGCTGTCGGAATTACTGCTTTTTTACTTCTTCTTTCTTTACCT
GTATTAGCGGGAGCTATTACCATACTTCTTACAGATCGAAATCTTAATACATCTTTTTTTGATCCCGCTGGAGGAGGAG
ACCCAATTCTTTATCAACACTTATTT
```

Fig. 52. Partial coding sequence of *N. coleta* SJIK21 COI gene.

> *N. coleta* Voucher SJIK21

```
TLYFIFGIWAGMVGTSLSLLIRAELGTPNSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGG
FGNWLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAH
SGSSVDLAIFSLHLAGISSILGAINFITTIINMRLNNLSFDQMPLFVWAVGITAFLLLLSL
PVLAGAITMLLTDRNLNTSFFDPAGGGDPILYQHLF
```

Fig. 53. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *N. coleta* SJIK21.

Table 9. The BLAST hit table of the partial coding DNA sequence of COI gene of *N. coleta* SJIK21.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Q start	Q end	Geographical location
1	Nyctemera coleta MH165322.1	99.52	630	2	1	10	639	Malaysia
2	Nyctemera regularis GU696117.1	90.76	660	57	4	1	658	Malaysia
3	Euchromia polymena KR063169.1	90.58	658	62	0	1	658	India
4	Amata naderii HQ682508.1	90.58	658	62	0	1	658	Iran
5	Amata leucacma HQ921315.1	90.58	658	62	0	1	658	Australia
6	Nyctemera arctata albofasciata HM377826.1	90.58	658	62	0	1	658	Taiwan
7	Nyctemera leuconoe KF491936.1	90.44	659	61	2	1	658	Democratic Republic of Congo
8	Nyctemera baulus HQ921250.1	90.44	659	61	2	1	658	Australia
9	Nyctemera secundiana KF388629.1	90.44	659	61	2	1	658	Australia
10	Nyctemera arctata albofasciata KF491934.1	90.44	659	61	2	1	658	Thailand
11	Pseudorthodes vecors GU090155.1	90.44	659	61	2	1	658	USA
12	Nyctemera baulus KF391567.1	90.29	659	62	2	1	658	Australia
13	Nyctemera baulus HQ921245.1	90.29	659	62	2	1	658	Australia
14	Nyctemera secundiana KF389372.1	90.14	659	63	2	1	658	Australia
15	Nyctemera secundiana HQ921246.1	90.14	659	63	2	1	658	Australia
16	Nyctemera secundiana HQ921248.1	90.14	659	63	2	1	658	Australia
17	Acronicta atristrigatus JF846732.1	89.97	658	66	0	1	658	USA

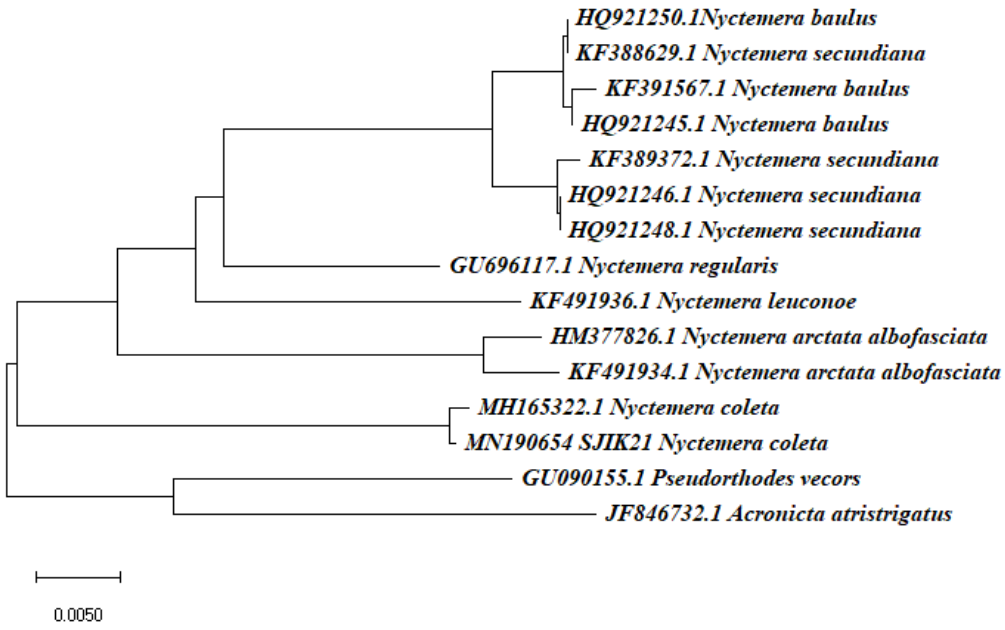

Fig. 54. The NJ tree showing phylogenetic relationships of *N. coleta* SJIK21.

The DNA isolated from the sample *N. coleta* SJIK21 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 9 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

The BLAST result of SJIK21 showed a similarity of 99.52% to *Nyctemera coleta* MH165322 from Malaysia occupying the same clade. Hence the sequence obtained is a novel one and it also shows the South East origin of the species. The adjacent clade shows the close relationship with two subspecies *Nyctemera arctata albofaciata* from Taiwan and Thailand, which might have diverged from the species due to geographic isolation. The distance data revealed that the various species of *Nyctemera* genus evolved from a common ancestor about 30000 years ago and spread over Asia-Pacific region, the SJIK21 from Kerala being the closest relative of the species isolated from Malaysia. The nearest match from Malaysia differed by two nucleotides. In the Malaysian sp. G is replaced by A and T is replaced by C. The pattern of distribution of *Nyctemera* genus in the countries of the erstwhile Gondwana viz., Africa (Congo), India, Australia and the nearby regions of Taiwan, Thailand and Malaysia shows the divergence from a common ancestor. *Nyctemera coleta* SJIK21, showed a South East Asian lineage.

10. *Nausinoe sp.* SJIK31

The specimen SJIK31 was identified as *Nausinoe sp.* (Hubner, 1825) referring to the morphological features described by Hampson, 1896.

Synonyms: *Lepyrodes* Gunee, 1854

Phalangiodes Gunee, 1854

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Pyraloidea; Crambidae; Spilomelinae; *Nausinoe.*

Nausinoe sp. is seen India, Japan, China, Korea, Australia and Taiwan. *Nausinoe geometralis* has been reported from India from U.P., Maharashtra, Kerala (Vellayani), Tamil Nadu (Nilgiris), Bihar, West Bengal and Assam (Shubhalaxmi *et al*., 2011, Shah *et al*., 2018). *N. neptis* has been reported from Chattisgarh. *Nausinoe sp.* belong to the family Crambidae and subfamily Spilomelinae. Some members of the genus like *N. geometralis* is a serious pest of jasmine causing severe damage to the plant.

Identifying characters : Palpi are obliquely upturned; the 2nd joint is very broadly scaled in front; the 3rd porrect; maxillary palpi are filiform; antennae longer than the forewing and almost simple; legs are long and slender; the outer spurs about two-thirds length of inner; fore wing with veins 3, 4, 5 normally from angle of cell; 7 straight and well separated from 8, 9 to which 10 is closely approximated; Hind wing with the cell very short; the disco cellulars straight; veins 3, 4, 5 normally from angle of cell; 6, 7 shortly stalked, 7 anastomosing with 8; fore legs of male with thick tufts of long hair on the tibiae; the first joint of tarsus fringed with hair on both sides; mid and hind tibiae fringed on both sides with short hair.

Results and discussion

The PCR of the COI gene fragment of *Nausinoe sp.* SJIK31 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 56-78. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190664).

Fig. 55. *Nausinoe sp.* SJIK31

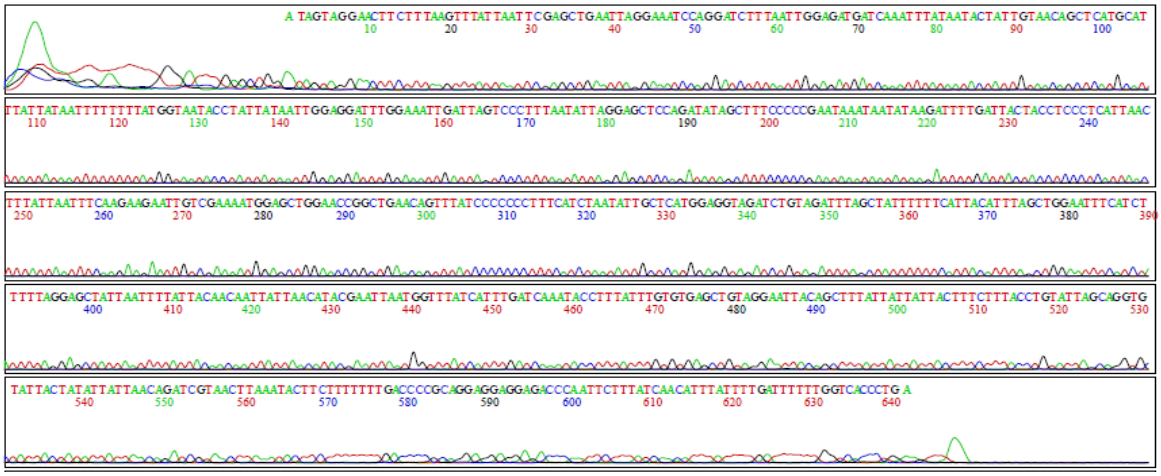

Fig. 56. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *Nausinoe sp.* SJIK31.

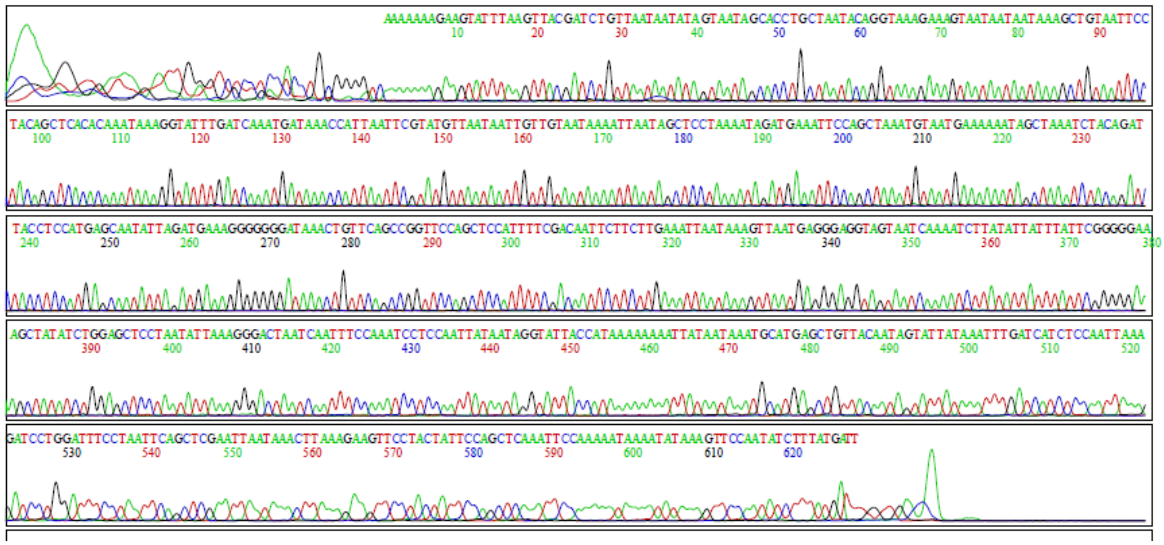

Fig. 57. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *Nausinoe sp.* SJIK31.

> *Nausinoe sp.* Voucher SJIK31 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACTTTATATTTTATTTTTGGAATTTGAGCTGGAATAGTAGGAACTTCTTTAAGTTTATTAATTCGAGCTGAATT
AGGAAATCCAGGATCTTTAATTGGAGATGATCAAATTTATAATACTATTGTAACAGCTCATGCATTTATTATAAT
TTTTTTTATGGTAATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTCCCTTTAATATTAGGAGCTCCAGA
TATAGCTTTCCCCCGAATAAATAATATAAGATTTTGATTACTACCTCCCTCATTAACTTTATTAATTTCAAGAAG
AATTGTCGAAAATGGAGCTGGAACCGGCTGAACAGTTTATCCCCCCCTTTCATCTAATATTGCTCATGGAGGTAG
ATCTGTAGATTTAGCTATTTTTTCATTACATTTAGCTGGAATTTCATCTATTTTAGGAGCTATTAATTTTATTAC
AACAATTATTAACATACGAATTAATGGTTTATCATTTGATCAAATACCTTTATTTGTGTGAGCTGTAGGAATTAC
AGCTTTATTATTATTACTTTCTTTACCTGTATTAGCAGGTGCTATTACTATATTATTAACAGATCGTAACTTAAA
TACTTCTTTTTTTGACCCCGCAGGAGGAGGAGACCCAATTCTTTATCAACATTTATTT
```

Fig. 58. Partial coding sequence of *Nausinoe sp.* SJIK31 COI gene.

> *Nausinoe sp.* Voucher SJIK31

```
TLYFIFGIWAGMVGTSLSLLIRAELGNPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNW
LVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHGGSSVDLA
IFSLHLAGISSILGAINFITTIINMRINGLSFDQMPLFVWAVGITALLLLLSLPVLAGAITMLLT
DRNLNTSFFDPAGGGDPILYQHLF
```

Fig. 59. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *Nausinoe sp.* SJIK31.

Table 10. The BLAST hit table of the partial coding DNA sequence of COI gene of *Nausinoe sp.* SJIK31.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Query end	Geograph. location
1	*Nausinoe neptis* KJ380848.1	99.66	593	2	0	35	627	India, W. Ghats
2	*Nausinoe pueritia* HQ952798.1	97.87	658	14	0	1	658	Australia
3	*Nausinoe pueritia* HQ952797.1	97.72	658	15	0	1	658	Australia
4	*Nausinoe geometralis* KX862160.1	93.69	650	41	0	1	650	Pakistan
5	*Eulepte sp.* JQ572382.1	93.62	658	42	0	1	658	Costa Rica
6	*Nausinoe geometralis* HQ952793.1	93.47	658	43	0	1	658	Australia
7	*Herpetogramma* sp. HQ990748.1	93.31	658	44	0	1	658	Pakistan
8	*Maruca vitrata* HQ953023.1	93.31	658	44	0	1	658	Australia
9	*Hymenia lophoceralis* MK020081.1	93.30	657	44	0	2	658	Papua New Guinea
10	*Cnaphalocrocis poeyalis* KX052273.1	93.17	659	43	2	1	658	French Polynesia

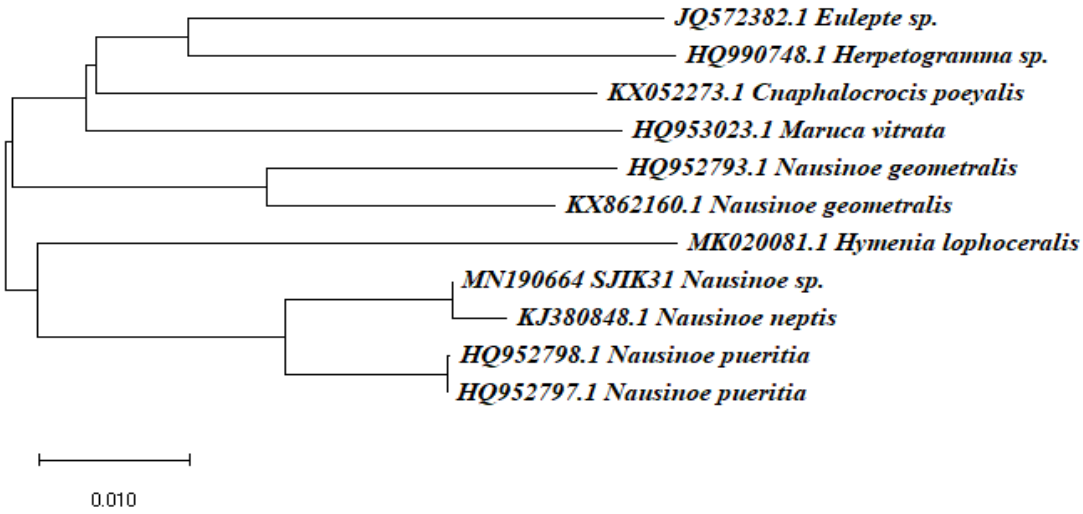

Fig. 60. The NJ tree showing phylogenetic relationships of *Nausinoe sp.* SJIK31.

The DNA isolated from the sample *Nausinoe sp.* SJIK31 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 10 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

Nausinoe sp. SJIK31 isolated from Kerala showed a maximum similarity of 99.66% to *N. neptis* KJ380848 from India (Western Ghats). The nearest match from India *Nausinoe neptis* differs by a T-A in SJIK31 being replaced by A-T. The NJ- tree shows that they are monophyletic. It showed 97.87 % similarity to *N. peuritia* HQ952798 and 97.72% to HQ952797 from Australia. They are polymorphic geographic variants of SJIK31 sharing a common ancestor. But it showed only 93.69% similarity to *N. geometralis* from Pakistan (KX862160) and 93.47% from Australia (HQ952793) though they were from the same genus.

The NJ – tree shows that the closest species to SJIK31 is *N. neptis* from India which occupies the same clade. *N. peuritia* species from Australia, *N. neptis* from India and SJIK31 from Kerala are monophyletic having descended from a common ancestor. But SJIK31 and *N. geometralis* species are polyphyletic diverging from the common ancestor and occupying a different clade. The geographic pattern of distribution showed a common origin and a later divergence due to geographical isolation. The *Nausinoe sp.* isolated from Kerala SJIK31 showed a maximum similarity of 99.66% to that in the database and hence the isolate from Kerala is a novel one. 65 novel bp of COI were added to the database.

11. *Bastilla sp.* SJIK32

The specimen SJIK32 was identified as *Bastilla sp.* (Swinhoe, 1918) referring to the morphological features described by Hampson, 1894.

Synonyms: *Caranilla* Moore, 1885
Naxia Guenee, 1852
Xiana Nye, 1975

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Noctuidae; Catocalinae; *Bastilla*.

Bastilla sp. is seen in India, Australia, South America, Caribbean Islands, Mexico, China, Korea, Taiwan, Ethiopia and Japan. In India it is found in Tamil Nadu (W. Ghats), Assam, N. Maharashtra, Kerala (Vagamon and Ponmudi), Himachal Pradesh and Himalayas (Shubhalaxmi *et al*., 2011, Mathew *et al*., 2018, Arandhara *et al.,* 2018, Sondhi *et al*., 2018). *Bastilla sp.* belongs to the family Noctuidae and subfamily Catocalinae. The host plants belong to Euphorbiaceae particularly *Phyllanthus*.

Identifying characters: Bronze brown coloured wings; triangular in shape; palpi upturned and smoothly scaled, the 2nd joint reaching vertex of head, the 3rd variable in length and longer in the female; thorax and abdomen smoothly scaled; mid tibiae spined; tibiae fringed with long hair in male; fore wing with apex somewhat acute; the outer margin nearly straight; hind wing with the outer margin slightly angled at vein 2.

Results and discussion

The PCR of the COI gene fragment of *Bastilla sp.* SJIK32 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 62 - 66. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190665).

Fig. 61. *Bastilla sp.* SJIK32 (dorsal and ventral view)

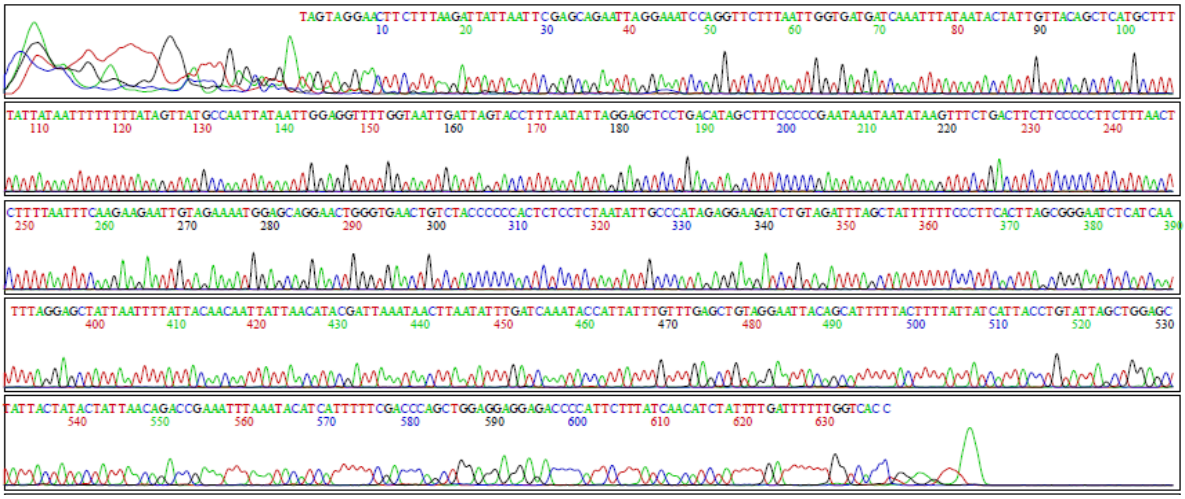

Fig. 62. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *Bastilla sp.* SJIK32.

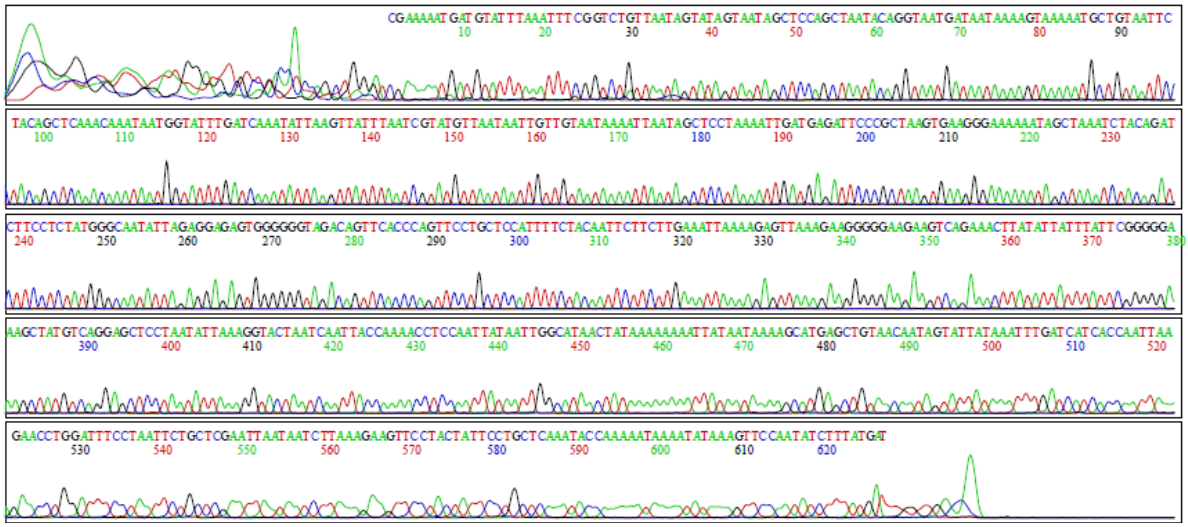

Fig. 63. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *Bastilla sp.* SJIK32.

> *Bastilla sp.* Voucher SJIK32 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACTTTATATTTTATTTTTGGTATTTGAGCAGGAATAGTAGGAACTTCTTTAAGATTATTAATTCGAGCAGAATTA
GGAAATCCAGGTTCTTTAATTGGTGATGATCAAATTTATAATACTATTGTTACAGCTCATGCTTTTATTATAATTT
TTTTTATAGTTATGCCAATTATAATTGGAGGTTTTGGTAATTGATTAGTACCTTTAATATTAGGAGCTCCTGACAT
AGCTTTCCCCCGAATAAATAATATAAGTTTCTGACTTCTTCCCCCTTCTTTAACTCTTTTAATTTCAAGAAGAATT
GTAGAAAATGGAGCAGGAACTGGGTGAACTGTCTACCCCCCACTCTCCTCTAATATTGCCCATAGAGGAAGATCTG
TAGATTTAGCTATTTTTTCCCTTCACTTAGCGGGAATCTCATCAATTTTAGGAGCTATTAATTTTATTACAACAAT
TATTAACATACGATTAAATAACTTAATATTTGATCAAATACCATTATTTGTTTGAGCTGTAGGAATTACAGCATTT
TTACTTTTATTATCATTACCTGTATTAGCTGGAGCTATTACTATACTATTAACAGACCGAAATTTAAATACATCAT
TTTTCGACCCAGCTGGAGGAGGAGACCCCATTCTTTATCAACATCTATTT
```

Fig. 64. Partial coding sequence of *Bastilla sp.* SJIK32 COI gene.

> *Bastilla sp.* Voucher SJIK32

```
TLYFIFGIWAGMVGTSLSLLIRAELGNPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIG
GFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNI
AHSGSSVDLAIFSLHLAGISSILGAINFITTIINMRLNNLMFDQMPLFVWAVGITAFLLL
LSLPVLAGAITMLLTDRNLNTSFFDPAGGGDPILYQHLF
```

Fig. 65. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *Bastilla sp.* SJIK32.

Table 11. The BLAST hit table of the partial coding DNA sequence of COI gene of *Bastilla sp.* SJIK32.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Query end	Geograph. location
1	*Bastilla sp. 'purpurata'* JF854969.1	93.47	658	43	0	1	658	Brazil
2	*Grammodes oculata* HQ950258.1	93.47	658	43	0	1	658	Australia
3	*Parallelia arctotaenia* HM377874.1	93.57	653	42	0	6	658	Taiwan
4	*Grammodes oculata* HQ950259.1	93.31	658	44	0	1	658	Australia
5	*Grammodes oculicola* HQ950253.1	93.31	658	44	0	1	658	Australia
6	*Bastilla sp. 'purpurata'* HQ571048.1	93.31	658	44	0	1	658	Brazil
7	*Bastilla sp. 'purpurata'* JN806521.1	93.31	658	44	0	1	658	Costa Rica
8	*Grammodes oculata* HQ950257.1	93.16	658	45	0	1	658	Australia
9	*Grammodes oculicola* HQ950254.1	93.16	658	45	0	1	658	Australia
10	*Bastilla absentimacula* HM906295.1	93.16	658	45	0	1	658	Papua New Guinea
11	*Bastilla absentimacula* KF391112.1	93.01	658	46	0	1	658	Australia
12	*Bastilla absentimacula* KF390776.1	93.01	658	46	0	1	658	Australia
13	*Bastilla absentimacula* KC158229.1	93.01	658	46	0	1	658	Papua New Guinea
14	*Grammodes pulcherrima* HQ950250.1	93.01	658	46	0	1	658	Australia
15	*Grammodes sp.* HQ950267.1	93.01	658	46	0	1	658	Australia

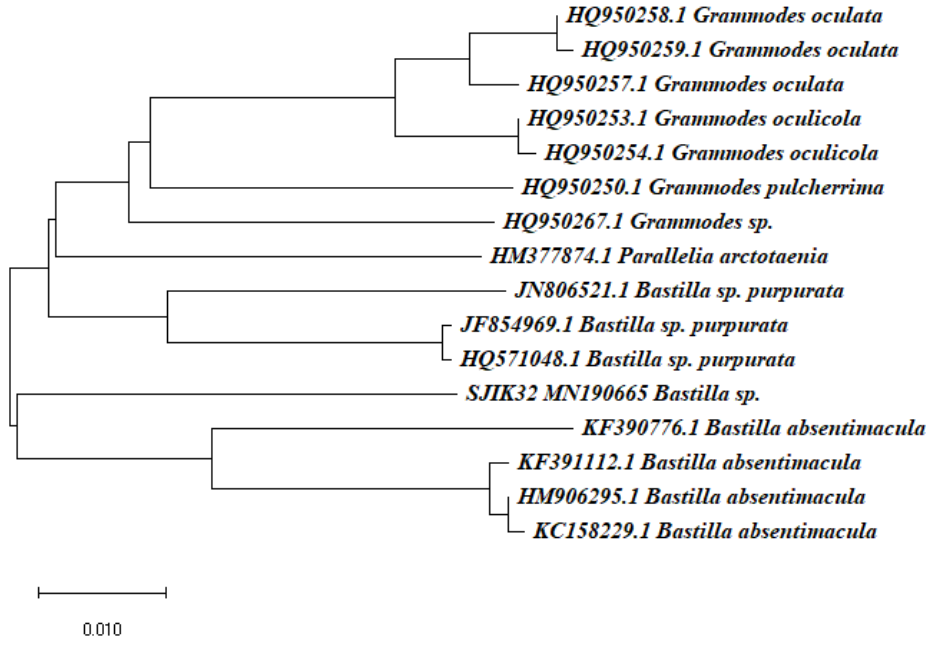

Fig. 66. The NJ tree showing phylogenetic relationships of *Bastilla sp.* SJIK32.

The DNA isolated from the sample *Bastilla sp.* SJIK32 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 11 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

The SJIK32 BLAST result showed a maximum similarity of 93.47% to *Bastilla sp. purpurata* JF854969 from Brazil. The NJ tree also shows the similarity of the isolate from Kerala SJIK32 to the two Brazilian species *Bastilla sp. purpurata* JF854969 and HQ571048 (93.31%) and to JN806521 from Costa Rica. The sequence isolated from SJIK32 is a novel one as it is placed in a separate clade. *B. absentimacula* HM906295 with 93.16% and KC158229 with 93.01% similarities from Papua New Guinea, and KF391112 with 93.01% from Australia were different species of the genus and they formed a separate clade. The phylogenetic tree also showed the relationship of the genus to various species of the genera *Grammodes* which belonged to same subfamily. The pattern of distribution of the *Bastilla sp.* shows the common origin of the species. The species also shows a South American lineage.

12. *Hyperythra lutea* SJIK1

The specimen SJIK1 was identified as *Hyperythra lutea* (Stoll, 1781) referring to the morphological features described by Hampson, 1895.

Synonyms: *Phalaena lutea* Stoll, 1787
Phalaena flavaria Fabricius, 1787
Phalaena flavata Fabricius, 1794
Hyperythra ennomaria Gunee, 1857

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Holometabola; Lepidoptera; Glossata; Ditrysia; Geometroidea; Geometridae; Ennominae; *Hyperythra.*

The *Hyperythra lutea* is found in India, Sri Lanka and South East Asia extending to Sundaland. In India it has been reported from Jharkhand, Eastern Ghats (Southern Andhra Pradesh), West Bengal, Maharashtra and Assam (Gurule *et al*., 2013, Shubhalaxmi *et al*., 2011, Singh *et al*., 2018, Harinath *et al*., 2014, Arandhara *et al.,* 2018, Shah *et al*., 2018). *H. lutea* belongs to the family Geometridae and subfamily Ennominae. Host plants in India *Ziziphus oenoplia* and *Gouania leptostachya* (Rhamnaceae).

Identifying characters: males are yellow suffused with pink and striated with fuscous; some white on palpi and shaft of antennae; Fore wing with indistinct ante medial line angled below costa; medial and post medial ill-defined, slightly curved pinkish bands; Hind wings with similar narrow ante medial and broad post medial bands, the latter with one or two black marks on it below costa; Underside bright yellow, with the area behind the post medial line more or less completely coloured pink; fore wing with a whitish patch below apex; the pink suffusion of upper and undersides varies in extent; females are much brighter yellow with three lines on the fore wing and two on the hind wing usually prominent.

Results and discussion

The PCR of the COI gene fragment of SJIK1 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 68-72. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190673).

Fig. 67. *Hyperythra lutea* SJIK1

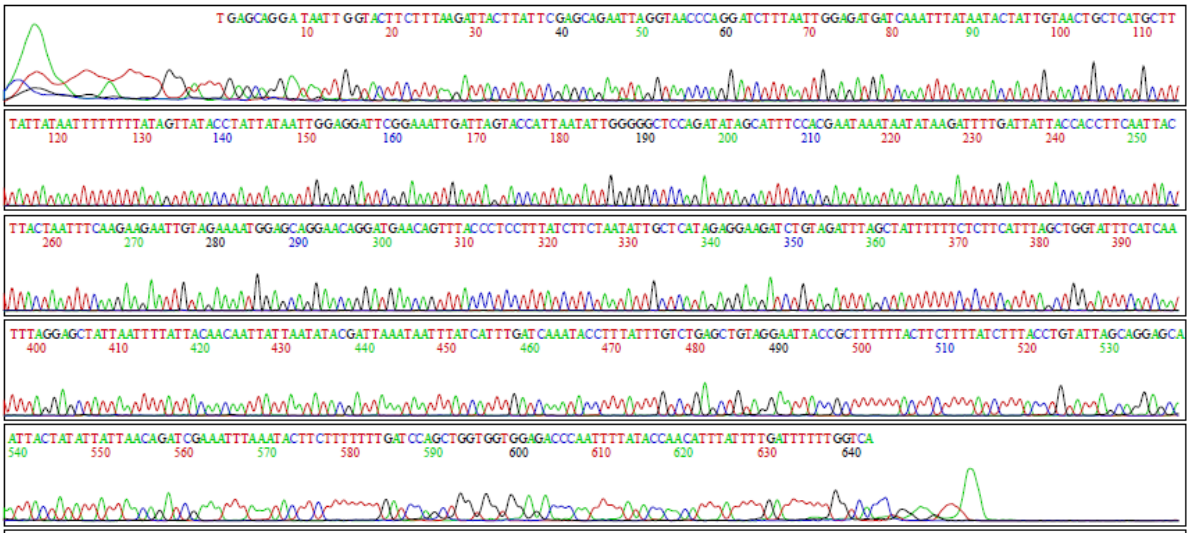

Fig. 68. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *H. lutea* SJIK1.

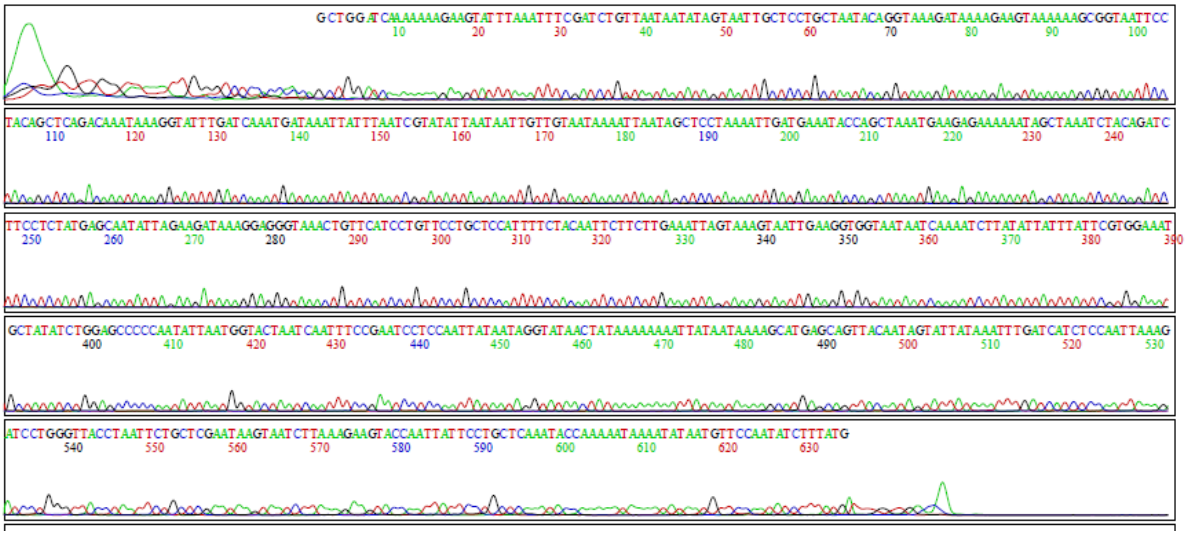

Fig. 69. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *H. lutea* SJIK1.

> *H. lutea* Voucher SJIK1 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

AACATTATATTTTATTTTTGGTATTTGAGCAGGAATAATTGGTACTTCTTTAAGATTACTTATTCGAGCAGAATTA
GGTAACCCAGGATCTTTAATTGGAGATGATCAAATTTATAATACTATTGTAACTGCTCATGCTTTTATTATAATTT
TTTTTATAGTTATACCTATTATAATTGGAGGATTCGGAAATTGATTAGTACCATTAATATTGGGGGCTCCAGATAT
AGCATTTCCACGAATAAATAATATAAGATTTTGATTATTACCACCTTCAATTACTTTACTAATTTCAAGAAGAAT
TGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCTCCTTTATCTTCTAATATTGCTCATAGAGGAAGA
TCTGTAGATTTAGCTATTTTTTCTCTTCATTTAGCTGGTATTTCATCAATTTTAGGAGCTATTAATTTTATTACAAC
AATTATTAATATACGATTAAATAATTTATCATTTGATCAAATACCTTTATTTGTCTGAGCTGTAGGAATTACCGCTT
TTTTACTTCTTTTATCTTTACCTGTATTAGCAGGAGCAATTACTATATTATTAACAGATCGAAATTTAAATACTTCT
TTTTTTGATCCAGCTGGTGGTGGAGACCCAATTTTATACCAACATTTATTT

Fig. 70. Partial coding sequence of *H. lutea* SJIK1 COI gene.

> *H. lutea* Voucher SJIK1

TLYFIFGIWAGMIGTSLSLLIRAELGNPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWLVP
LMLGAPDMAFPRMNNMSFWLLPPSITLLISSSIVENGAGTGWTVYPPLSSNIAHSGSSVDLAIFSL
HLAGISSILGAINFITTIINMRLNNLSFDQMPLFVWAVGITAFLLLLSLPVLAGAITMLLTDRNLNTSF
FDPAGGGDPILYQHLF

Fig. 71. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *H. lutea* SJIK1.

Table 12. The BLAST hit table of the partial coding DNA sequence of COI gene of *H. lutea* SJIK1.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Query end	Geograph. location
1	Hyperythra lutea KJ380856.1	100	593	0	0	35	627	India, Western Ghats
2	Fascellina chromataria KJ380877.1	99.66	593	2	0	35	627	India, Western Ghats
3	Hyperythra rubricata KF389104.1	95.44	658	30	0	1	658	Australia
4	Hyperythra rubricata KF389744.1	95.29	658	31	0	1	658	Australia
5	Hyperythra rubricata KF388085.1	95.14	658	32	0	1	658	Australia
6	Arhodia lasiocamparia HQ923354.1	93.78	659	39	2	1	658	Australia
7	Oenochroma barcodificata FJ863287.1	93.31	658	44	0	1	658	Australia, Tasmania
8	Phallaria ophiusaria HQ923544.1	93.16	658	43	2	2	658	Australia
9	Capusa senilis JN267178.1	92.87	659	45	2	1	658	Australia
10	Rucana bisecta HM432120.1	92.72	659	46	2	1	658	Ecuador

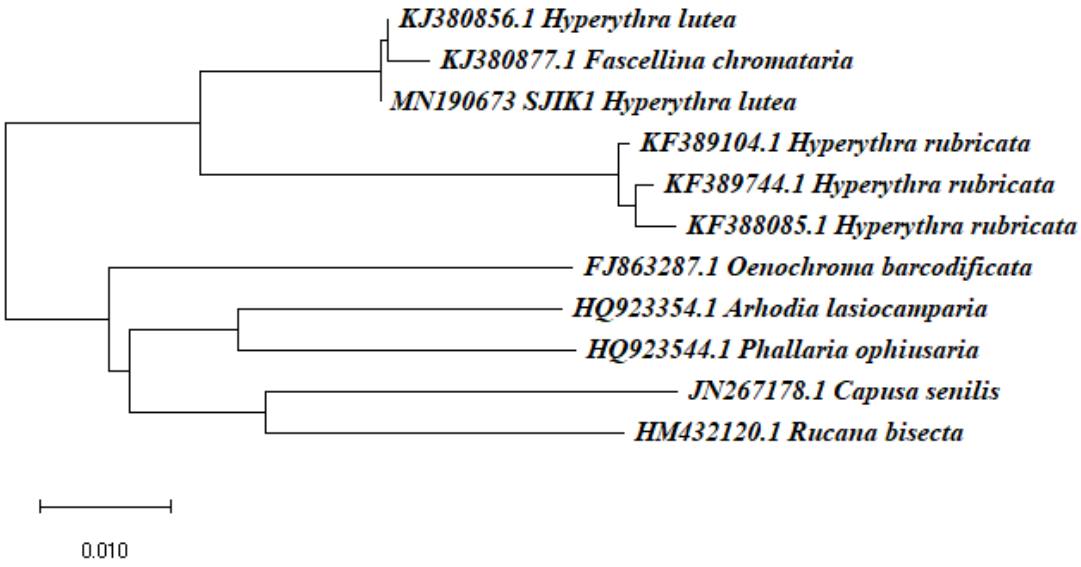

Fig. 72. The NJ tree showing phylogenetic relationships of *H. lutea* SJIK1.

The DNA isolated from the sample *H. lutea* SJIK1 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 12 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

The BLAST results showed the 100% similarity of SJIK1 from Kerala to *H. lutea* KJ380856 isolated from Western Ghats. Hence the sequence can be used as barcode for species identification. It showed 99.66% similarity to *Fascellina chromataria* KJ380877 from Western Ghats which shows the close relation of the species to the genus *Fascellina* occupying the adjacent clade in the NJ tree. It also shows that they evolved from a common ancestor. SJIK1 showed 95.44% similarity with *H. rubricata* KF389104, 95.29 % to KF389744 and 95.14 % to KF388085 isolated from Australia which occupied the adjoining clade showing common ancestry. The distance data revealed that the species diverged from SJIK1 about 25000 years ago. The NJ tree shows that *H. rubricata* from Australia is of recent origin when compared to *H. lutea* from India. The divergence might have occurred because of the separation of the Australian continent from the Indian subcontinent during the breakup of the Gondwana. 65 novel bp of COI were added to the database.

13. *Pygospila tyres* SJIK6

The specimen SJIK6 was identified as *Pygospila tyres* (Cramer, 1780) referring to the morphological features described by Hampson, 1896.

Synonyms: *Phalaena tyres* Cramer, 1780
Pygospila thyralis Hubner, 1825
Pygospila tyresalis Guenee, 1854

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Pyraloidea; Crambidae; Spilomelinae; *Pygospila*.

Pygospila tyres is found India, Sri Lanka, Nepal, Burma, Vietnam, China, Japan, Java, Borneo, Philippines, New Guinea and Australia. In India it is seen in Northern Maharashtra, Kerala (Parambikulam and Ponmudi) and West Bengal (Shubhalaxmi et al., 2011, Sudheendrakumar, 1999, Sondhi *et al*., 2018, Shah *et al*., 2018). *Pygospila tyres* belongs to the family Crambidae and subfamily Spilomelinae. It is seen exclusively in moist deciduous forests.

Identification Marks: Palpi white from below, upturned, the 2nd joint broadly scaled in front, the 3rd porrect and lying on the hair of 2nd joint; maxillary palpi filiform and as long as the labial; frons with lateral white line; thorax and patagia striped with white; tibiae with the outer spurs half the length of the inner; abdomen long with paired dorsal and lateral series of white spots, male with the large anal tuft. Fore wing with the costa arched towards apex; the outer margin oblique; the inner margin lobed before middle and somewhat excised towards outer angle. Wings are black shot with purple; Fore wing with two oblique whitish sub basal lines; an oblique ante medial series of three white spots, the two below the cell nacreous hyaline; a speck in the cell; a nacreous spot in end of cell and larger spot below the end; a bidentate spot beyond the cell and another towards apex. Hind wing with nacreous streaks in and below the cell; the cilia white towards anal angle. Both the wings with a pair of spots between origin of veins 3 and 5, three sub marginal smaller spots and a spot below vein 2.

Results and discussion

The PCR of the COI gene fragment of *P. tyres* SJIK6 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 74-78. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190677).

Fig. 73. *Pygospila tyres* SJIK6

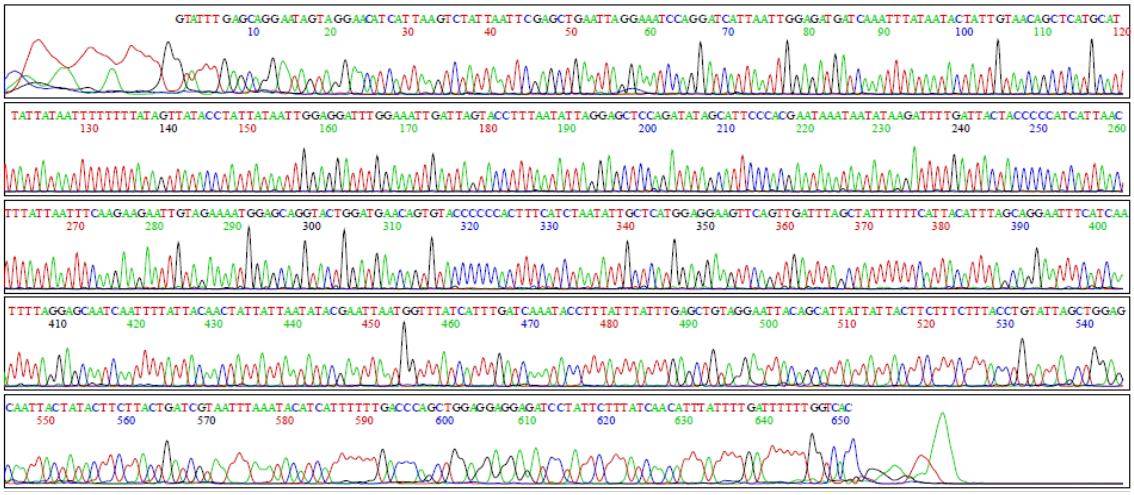

Fig. 74. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *P. tyres* SJIK6.

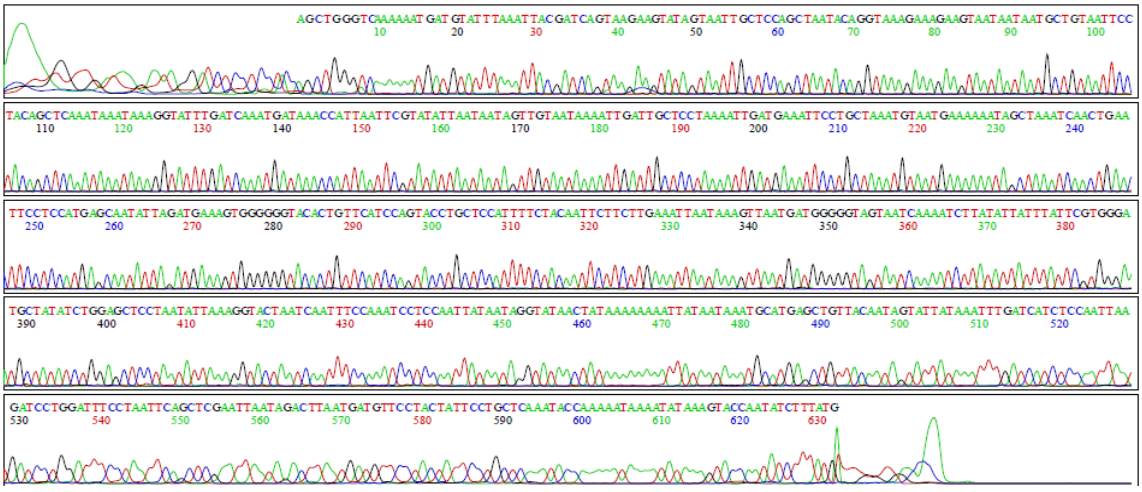

Fig. 75. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene *P. tyres* SJIK6.

> *P. tyres* Voucher SJIK6 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
TACTTTATATTTTATTTTTGGTATTTGAGCAGGAATAGTAGGAACATCATTAAGTCTATTAATTCGAGCTG
AATTAGGAAATCCAGGATCATTAATTGGAGATGATCAAATTTATAATACTATTGTAACAGCTCATGCATTT
ATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTACCTTTAATATT
AGGAGCTCCAGATATAGCATTCCCACGAATAAATAATATAAGATTTTGATTACTACCCCCATCATTAACTT
TATTAATTTCAAGAAGAATTGTAGAAAATGGAGCAGGTACTGGATGAACAGTGTACCCCCCACTTTCATCT
AATATTGCTCATGGAGGAAGTTCAGTTGATTTAGCTATTTTTTCATTACATTTAGCAGGAATTTCATCAAT
TTTAGGAGCAATCAATTTTATTACAACTATTATTAATATACGAATTAATGGTTTATCATTTGATCAAATAC
CTTTATTTATTTGAGCTGTAGGAATTACAGCATTATTATTACTTCTTTCTTTACCTGTATTAGCTGGAGCA
ATTACTATACTTCTTACTGATCGTAATTTAAATACATCATTTTTTGACCCAGCTGGAGGAGGAGATCCTAT
TCTTTATCAACATTTATTT
```

Fig. 76. Partial coding sequence of *P. tyres* SJIK6 COI gene.

> *P. tyres* Voucher SJIK2

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TLYFIFGIWAGMVGTSLSLLIRAELGNPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWL
VPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHGGSSVDLAIF
SLHLAGISSILGAINFITTIINMRINGLSFDQMPLFIWAVGITALLLLLSLPVLAGAITMLLTDRN
LNTSFFDPAGGGDPILYQHLF
```

Fig. 77. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *P. tyres* SJIK6.

Table 13. The BLAST hit table of the partial coding DNA sequence of COI gene of *P. tyres* SJIK6.

SN	Subject IDs	% Identity	Align. length	Mis-match	Query start	Quer y end	Geogra. location
1	Pygospila tyres KX862292.1	100	658	0	1	658	Pakistan
2	Pygospila tyres HQ953034.1	100	658	0	1	658	Australia
3	Pygospila tyres HQ953033.1	100	609	0	1	609	Australia
4	Pygospila tyres KF392550.1	100	550	0	1	550	Australia
5	Pygospila tyres HQ990824.1	99.85	658	1	1	658	Pakistan
6	Pycnarmon sp. KY370922.1	94.37	657	37	2	658	Papua New Guinea
7	Pygospila hyalotypa HQ953030.1	93.93	659	38	1	658	Australia
8	Pygospila bivittalis HQ953029.1	93.62	658	42	1	658	Australia
9	Omiodes odontosticta HQ952909.1	93.62	658	40	2	658	Australia
10	Cnaphalocrocis poeyalis KX052247.1	93.47	658	41	2	658	French Polynesia
11	Cnaphalocrocis trapezalis KF147312.1	93.32	659	42	1	658	Nigeria

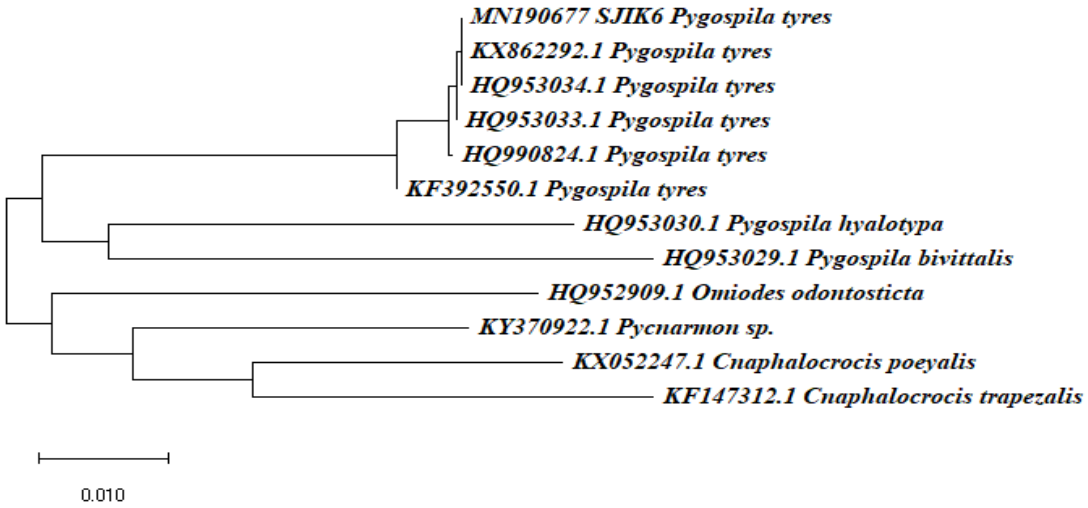

Fig. 78. The NJ tree showing phylogenetic relationships of *P. tyres* SJIK6.

The DNA isolated from the sample *P. tyres* SJIK6 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 13 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

P. tyres SJIK6 isolate from Kerala showed 100% similarity to *P. tyres* from Pakistan KX862292 and HQ953034, HQ953034, HQ953033 and KF392550 from Australia. Hence it can be used as barcode for species identification. It also showed 99.85% similarity to HQ990824 from Pakistan which is a polymorphic variant. A single nucleotide change (C in SJIK6 changed to T) was the difference observed. The NJ tree shows that all the species of *P. tyres* in the BLAST result are monophyletic and are in adjacent clades. It depicts the common origin of all these species. *P. hyalotypa* HQ953030 from Australia showing 93.93% similarity is the closest relative and *P. bivittalis* HQ953029 also from Australia showing 93.62 % similarity are placed in the adjacent clade. The NJ tree showed that the two groups and SJIK6 have diverged from a common ancestor, as a result of the break- up of the Indo-Australian plate due to the stresses induced by the collision of the Indo-Australian plate with Eurasia. The NJ tree distance data reveals that the species was diverged from their closely related species *P. hyalotypa* about 35000 years ago. The phylogenetic tree shows that *P. tyres* is closely related to the species of the genera *Cnaphalocrocis* viz., *C. poeyalis* and *C. trapezalis.*

14. *Pycnarmon sp.* SJIK9

The specimen SJIK9 was identified as *Pycnarmon* sp. (Lederer, 1863) referring to the morphological features described by Hampson, 1896.

Synonyms: *Entephria* Lederer, 1863
Aripana Moore, 1886
Satanastra Meyrick, 1890
Eutrichotis Swinhoe, 1900

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Holometabola; Lepidoptera; Glossata; Ditrysia; Pyraloidea; Crambidae; Spilomelinae; *Pycnarmon*.

Pycnarmon sp. are seen in India, Australia, Japan, China, Korea and Taiwan. In India *Pycnarmon sp.* like *P. cribata*, *P. lactiferalis*, *P. meritalis* and *P. virgatalis* are reported from West Bengal, and *P. alboflavalis* from Assam, Maharashtra and Kerala (Parambikulam) (Shubhalaxmi et al., 2011, Sudheendrakumar, 1999, Shah *et al*., 2018). *Pycnarmon sp.* belong to the family Crambidae and subfamily Spilomelinae. Host plants are lamiaceae and apocynaceae.

Identifying characters: palpi upturned; the second joint broadly scaled in front and reaching vertex of head; the 3rd long and acuminate; maxillary palpi minute and filiform; frons rounded; tibiae with the outer spurs about half the length of inner; abdomen with lateral tufts on terminal segments; Fore wing with veins 3, 4,5 from angle of cell; 7 well separated from 8, 9 to which 10 is approximated; Hind wing with veins 3,4,5 from angle of cell, which is short; 6, 7 from upper angle, 7 anastomosing with 8. Antennae of male with the shaft thickened to about one-third length, where there is a cleft fringed with hair on each side.

Results and discussion

The PCR of the COI gene fragment of *Pycnarmon sp.* SJIK9 from Kerala yielded a product of 658bp long. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 80-84. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190644).

Fig. 79. *Pycnarmon sp.* SJIK9

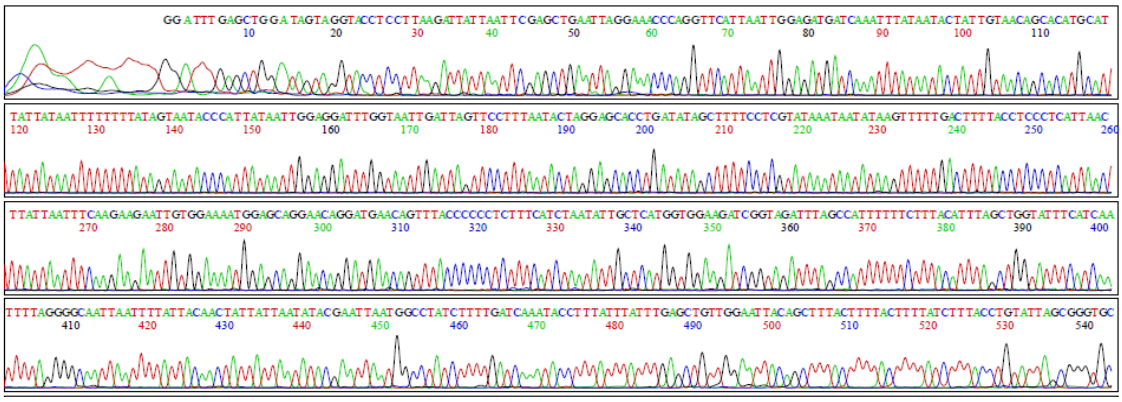

Fig. 80. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *Pycnarmon sp.* SJIK9.

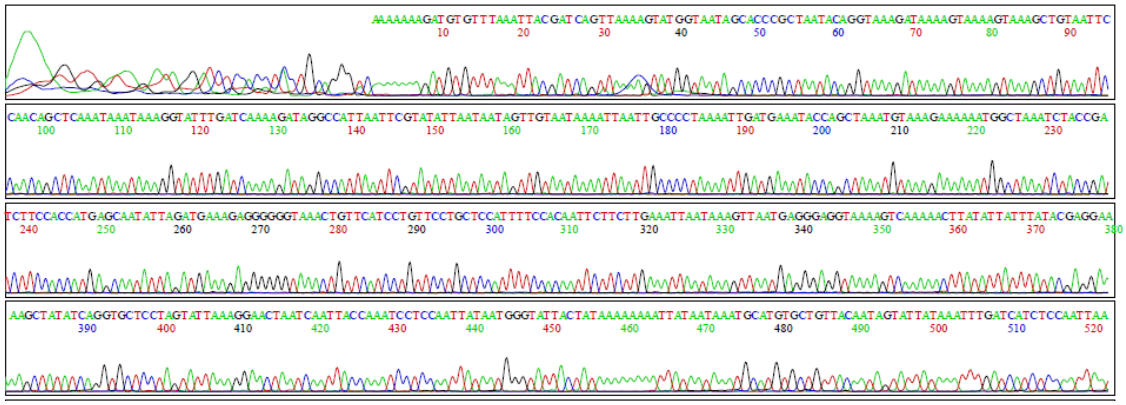

Fig. 81. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene *Pycnarmon sp.* SJIK9.

The DNA isolated from the sample *Pycnarmon sp.* SJIK9 from Kerala gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 14 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

> *Pycnarmon sp.* Voucher SJIK9 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACTTTATATTTTATTTTTGGAATTTGAGCTGGAATAGTAGGTACCTCCTTAAGATTATTAATTCGAGCTGAATTA
GGAAACCCAGGTTCATTAATTGGAGATGATCAAATTTATAATACTATTGTAACAGCACATGCATTTATTATAATTT
TTTTTATAGTAATACCCATTATAATTGGAGGATTTGGTAATTGATTAGTTCCTTTAATACTAGGAGCACCTGATAT
AGCTTTTCCTCGTATAAATAATATAAGTTTTTGACTTTTACCTCCCTCATTAACTTTATTAATTTCAAGAAGAATT
GTGGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCCTCTTTCATCTAATATTGCTCATGGTGGAAGATCGG
TAGATTTAGCCATTTTTTCTTTACATTTAGCTGGTATTTCATCAATTTTAGGGGCAATTAATTTTATTACAACTAT
TATTAATATACGAATTAATGGCCTATCTTTTGATCAAATACCTTTATTTATTTGAGCTGTTGGAATTACAGCTTTA
CTTTTACTTTTATCTTTACCTGTATTAGCGGGTGCTATTACCATACTTTTAACTGATCGTAATTTAAACACATCTT
TTTTTGACCCTGCAGGAGGTGGAGATCCTATTCTTTATCAACATTTATTT
```

Fig. 82. Partial coding sequence of *Pycnarmon sp.* SJIK9 COI gene.

> *Pycnarmon sp.* Voucher SJIK9

```
TLYFIFGIWAGMVGTSLSLLIRAELGNPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGN
WLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHGGSSVD
LAIFSLHLAGISSILGAINFITTIINMRINGLSFDQMPLFIWAVGITALLLLLSLPVLAGAITM
LLTDRNLNTSFFDPAGGGDPILYQHLF
```

Fig. 83. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *Pycnarmon sp.* Voucher SJIK9.

Table 14. The BLAST hit table of the partial coding DNA sequence of COI gene of *Pycnarmon sp.* SJIK9

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Query end	Geograph. location
1	*Pycnarmon sp.* KY370922.1	93.31	658	44	0	1	658	Papua New Guinea
2	*Apogeshna stenialis* JQ572403.1	92.39	657	50	0	2	658	Costa Rica
3	*Phostria metalobalis* JQ526494.1	92.10	658	52	0	1	658	Costa Rica
4	*Phostria metalobalis* JQ541875.1	91.95	658	53	0	1	658	Costa Rica
5	*Phostria metalobalis* JQ541872.1	91.95	658	53	0	1	658	Costa Rica
6	*Phostria metalobalis* JQ533139.1	91.95	658	53	0	1	658	Costa Rica
7	*Pycnarmon sp.* MK019996.1	91.64	658	55	0	1	658	Papua New Guinea
8	*Pycnarmon cribrata* HQ953171.1	93.73	606	38	0	1	606	Australia

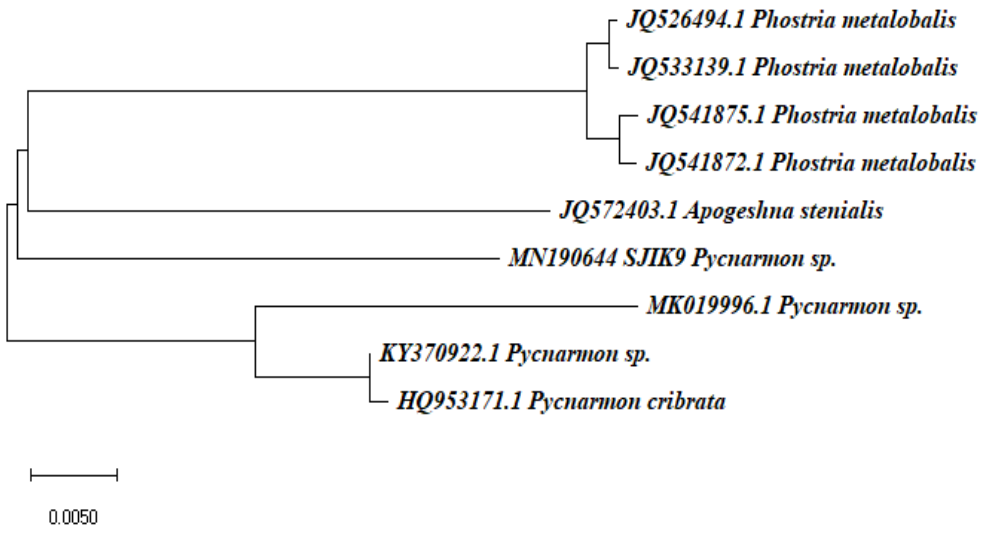

Fig. 84. The NJ tree showing phylogenetic relationships of *Pycnarmon* sp. SJIK9.

The SJIK9 BLAST result showed a maximum similarity of 93.31% to *Pycnarmon sp.* KY370922 and a similarity of 91.64% to *Pycnarmon sp.* MK019996 from Papua New Guinea. *P. cribata* HQ953171 from Australia showed 93.73% similarity to SJIK9. Hence the sequence is a novel one. The NJ tree showed the relationship of the species to the genus *Apogeshna,* being placed in adjacent clades. The phylogenetic tree showed that it might have diverged from the nearest related species about 25000 years ago. The distribution of the various species of *Pycnarmon* in Australia, India and Papua New Guinea shows the common origin from the Gondwana.

15. *Pingasa sp.* SJIK12

The specimen SJIK12 was identified as *Pingasa sp.* **(**Moore, 1887) referring to the morphological features described by Hampson, 1895.

Synonym: *Skorpisthes* Lucas, 1900

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Geometroidea; Geometridae; Geometrinae; *Pingasa.*

Pingasa sp. are seen in India, China, Japan, Malaysia, Indonesia, Philippines, Borneo, Australia and Thailand (Abang et al., 2002). In India is seen in Ponmudi (Kerala), Assam, Maharashtra (W. Ghats), Himalayas, Chattisgarh, Tripura and West Bengal (Shubhalaxmi *et al*., 2011, Sondhi *et al*., 2018, Shah *et al*., 2018). *P. chlora* has been reported from Ponmudi and W. Bengal and *P. ruginaria* from W. Bengal. *Pingasa sp.* belong to the family Geometriae and subfamily Geometrinae. It is a pest of pigeon pea.

Identifying characters: palpi porrect; the 2nd joint hairy, reaching beyond the sharp frontal tuft, the 3rd naked and varying in length; hind tibiae of male usually dilated ending in a slight process on upper side and with a fold containing a tuft of long hair; the two pairs of spurs short; abdomen with short spreading dorsal tufts on medial segments; both wings with crenulated margins; fore wing with vein 3 from near angle of cell; vein 5 from below upper angle; 6 from angle; 7, 8, 9 and 10 stalked; 11 free or anastomosing with 12; long hind wing; vein 3 from angle of cell; 5 from near upper angle; 7 from before angle; antennae of male bipectinate; hind wing with some tufts of long hair and below end of cell on upper side.

Results and discussion

The PCR of the COI gene fragment of *Pingasa sp.* SJIK12 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 86 - 90. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190647).

Fig. 85. *Pingasa sp.* SJIK12 (dorsal and ventral view)

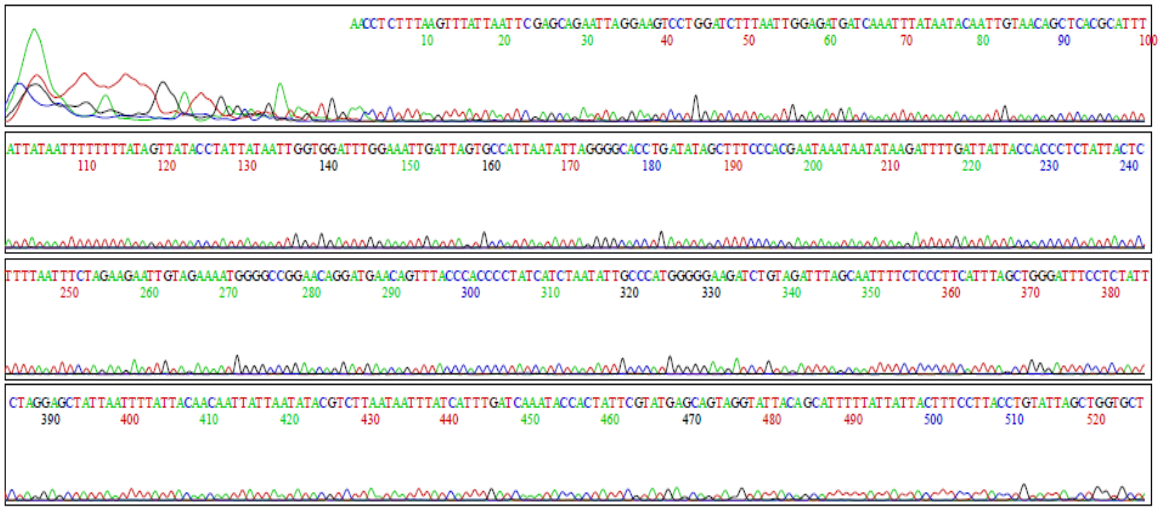

Fig. 86. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *Pingasa sp.* SJIK12.

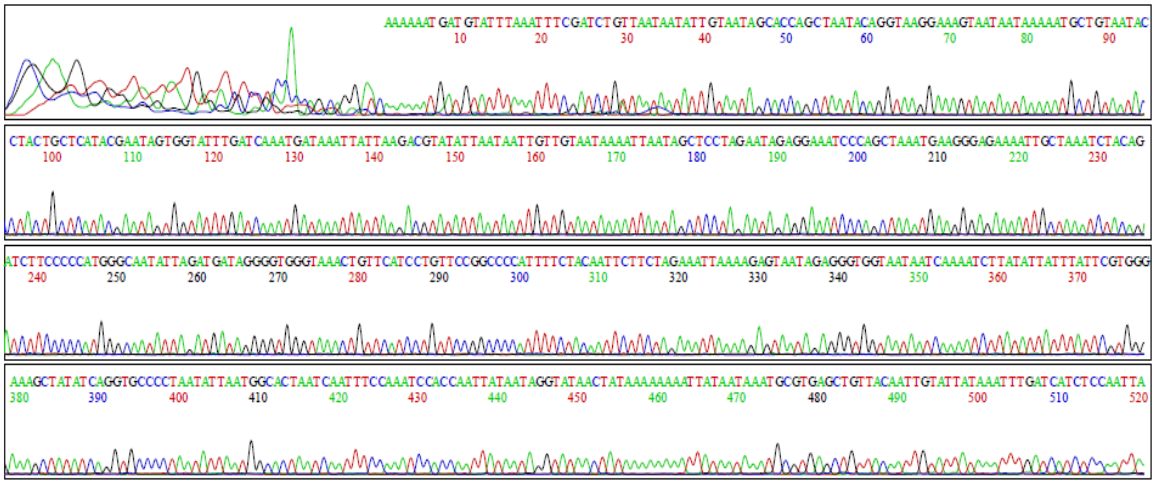

Fig. 87. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *Pingasa sp.* SJIK12.

> *Pingasa sp*. Voucher SJIK12 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACATTATATTTTATTTTTGGAATTTGAGCAGGAATAATTGGAACCTCTTTAAGTTTATTAATTCGA
GCAGAATTAGGAAGTCCTGGATCTTTAATTGGAGATGATCAAATTTATAATACAATTGTAACAGCTC
ACGCATTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGTGGATTTGGAAATTGATTAGT
GCCATTAATATTAGGGGCACCTGATATAGCTTTCCCACGAATAAATAATATAAGATTTTGATTATTA
CCACCCTCTATTACTCTTTTAATTTCTAGAAGAATTGTAGAAAATGGGGCCGGAACAGGATGAACAG
TTTACCCACCCCTATCATCTAATATTGCCCATGGGGGAAGATCTGTAGATTTAGCAATTTTCTCCCT
TCATTTAGCTGGGATTTCCTCTATTCTAGGAGCTATTAATTTTATTACAACAATTATTAATATACGT
CTTAATAATTTATCATTTGATCAAATACCACTATTCGTATGAGCAGTAGGTATTACAGCATTTTTAT
TATTACTTTCCTTACCTGTATTAGCTGGTGCTATTACAATATTATTAACAGATCGAAATTTAAATAC
ATCATTTTTTGATCCTGCTGGTGGAGGAGATCCAATTCTTTACCAACATTTATTC
```

Fig. 88. Partial coding sequence of *Pingasa sp.* SJIK12 COI gene.

> *Pingasa sp.* Voucher SJIK12

```
TLYFIFGIWAGMIGTSLSLLIRAELGSPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNW
LVPLMLGAPDMAFPRMNNMSFWLLPPSITLLISSSIVENGAGTGWTVYPPLSSNIAHGGSSVDLA
IFSLHLAGISSILGAINFITTIINMRLNNLSFDQMPLFVWAVGITAFLLLLSLPVLAGAITMLLT
DRNLNTSFFDPAGGGDPILYQHLF
```

Fig. 89. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *Pingasa sp.* SJIK12.

Table 15. The BLAST hit table of the partial coding DNA sequence of COI gene of *Pingasa sp*. SJIK12.

SN	Subject IDs	% Identity	Align. length	Mis-match	Quer ystart	Query end	Geograph. location
1	Pingasa sp. MG014827.1	98.71	618	8	41	658	China
2	Pingasa ruginaria pacifica, KF522486.1	94.37	657	37	1	657	Japan
3	Pingasa nobilis JN271280.1	93.62	658	40	1	657	Australia
4	Pingasa nobilis KR070782.1	93.31	658	42	1	657	Papua New Guinea
5	Pingasa nobilis JN271279.1	93.16	658	43	1	657	Australia
6	Pingasa lariaria KY370925.1	93.89	638	39	20	657	Papua New Guinea
7	Pingasa chlora KF389293.1	92.25	658	51	1	658	Australia
8	Pingasa lahayei GU655395.1	92.11	659	50	1	658	Ethiopia
9	Pingasa chlora KY370881.1	92.09	657	52	1	657	Papua New Guinea
10	Pingasa sp. HM892136.1	91.93	657	53	1	657	Gabon
11	Nemoria bistriaria KM551015.1	91.79	658	52	1	657	Canada
12	Pingasa sp. HM891932.1	91.63	657	55	1	657	Gabon
13	Calamodes subscudularia GU686574.1	91.64	658	53	1	657	Italy
14	Epitausa dilina JN304550.1	91.63	657	55	1	657	French Guiana
15	Chloeres citrolimbaria JN271243.1	91.48	657	56	1	657	Australia
16	Pingasa commutata MG767854.1	91.34	658	55	1	657	Estonia
17	Pingasa distensaria HM422793.1	91.34	658	55	1	657	Ethiopa
18	Pingasa rufofasciata MG014826.1	92.71	617	45	41	657	China

The DNA isolated from the sample *Pingasa sp*. SJIK12 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 15 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

SJIK12 blast results showed a maximum similarity of 98.71% to *Pingasa sp.* MG014827 from China. Hence the Kerala isolate is a novel one. They are monophyletic having a common ancestor occupying the same clade. The NJ tree shows that *P. nobilis* JN271280 from Australia with 93.62% similarity, KR070782 from Papua New Guinea with 93.31% similarity and JN271279 from Australia with 93.16% similarity were found in the adjacent clade. *P. chlora* KF389293 from Australia with 92.25% and KY370881 from Papua New Guinea with 92.09% similarities remained close to *Pingasa sp.* SJIK12 being placed in the adjoining clade. The NJ tree distance data revealed that the species diverged from its closely related species *P. nobilis* JN271279 from Australia with 93.16% similarity about 30000 years ago. The pattern of geographic distribution of the species shows the common origin of the various species of the genus *Pingasa* and the later divergence to the various species distributed across Gabon, Ethiopia, India, China, Papua New Guinea and Australia, all being part of the erstwhile Gondwana. . 40 novel bp of COI were added to the database.

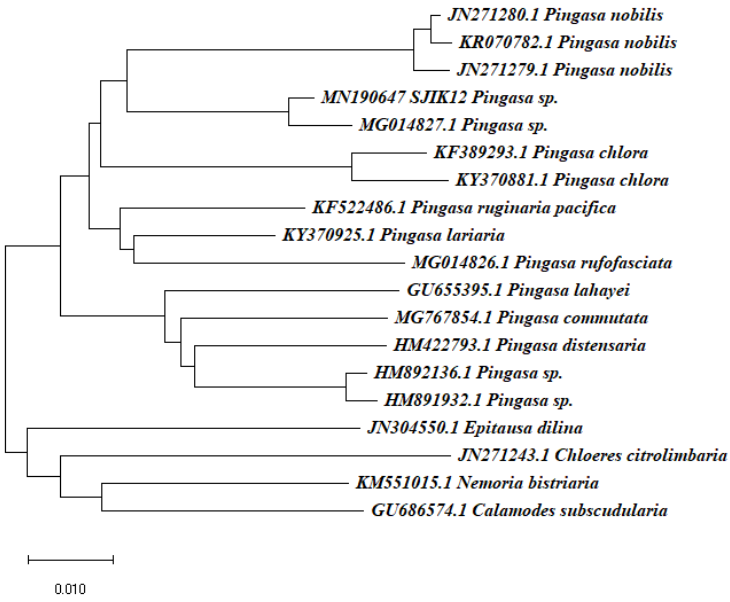

Fig. 90. The NJ tree showing phylogenetic relationships of *Pingasa sp*. SJIK12.

16. *Helicoverpa armigera* (SJIK15)

The specimen SJIK15 was identified as *Helicoverpa armigera* (Hubner, 1808) referring to the morphological features described by Hampson, 1894.

Synonyms: *Chloridea armigera* Hubner
Chloridea obsoleta Duncan & Westwood, 1841
Helicoverpa commoni Hardwick, 1965
Helicoverpa obsoleta Auctorum
Heliothis conferta Walker, 1857
Heliothis armigera Hubner, 1805

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Noctuidae; Heliothinae; *Helicoverpa*.

H. armigera is universally distributed. It is seen in Africa, Europe, Mauritius, India, Sri Lanka, Burma, Nepal, Japan, China, Hong Kong, Malaysia, Borneo, Java and Korea. In India it has been reported from Maharashtra, Chattisgarh and Tamil Nadu (Bharmal, 2015). *H. armigera* or cotton bollworm belongs to the family Noctuidae and subfamily Heliothinae. It is also known as cutworm moth. It is a major pest of cotton. It is one of the most polyphagus and cosmopolitan pest species. It infests vegetable crops like tomato, bitter gourd, okra, potato, chillies, rice, sorghum, cowpea, peach and many fruit trees, grape vine, tobacco, ornamental plants like rose and chrysanthemum and field crops like ragi, pigeon pea, chick pea, pigeon pea, green and black gram, castor, groundnut, sunflower, etc. The larva feeds on rose buds.

Identifying features: The moth has naked eyes without lashes; palpi porrect, a short frontal tuft; head, thorax and abdomen ochreous with pale brown, olive or red-brown tinge; thorax and abdomen without tufts; fore tibia with a pair of slender terminal spines; mid and hind tibia spined. Fore wings are ochreous with a pale brown olive, or red-brown tinge; indistinct double waved ante-medial lines; a dark speck representing the orbicular; an indistinct curved medial line; the reniform indistinct; post-medial and sub-marginal waved lines; hind wing white; the veins fuscous; under side of fore wing with the orbicular and reniform stigmata conspicuously black; a broad blackish band beyond the post-medial line; the apices of both wings and outer area of fore wing pinkish.

Results and discussion

The PCR of the COI gene fragment of *H. armigera* SJIK15 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 92-96. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190649).

Fig. 91. *H. armigera* SJIK15 (dorsal and ventral view)

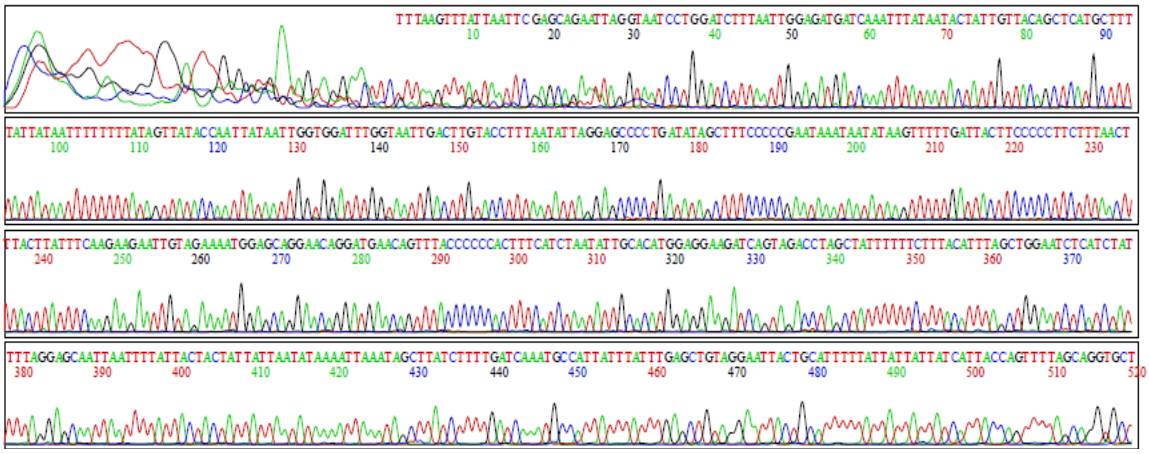

Fig. 92. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *H. armigera* SJIK15.

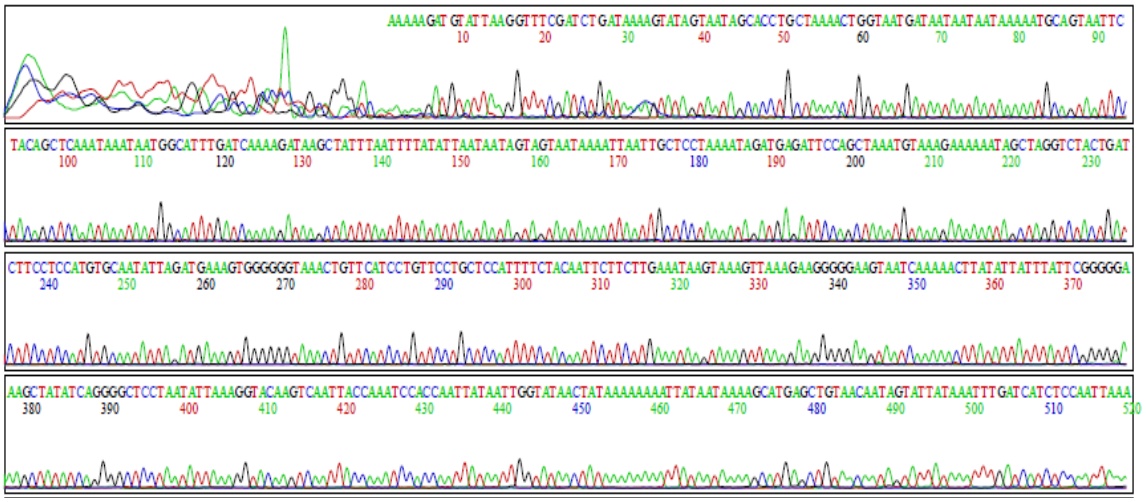

Fig. 93. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *H. armigera* SJIK15.

> *H. armigera* Voucher SJIK15 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACATTATATTTTATTTTTGGAATTTGAGCAGGAATAGTAGGAACTTCTTTAAGTTTATTAATTCGAGCAGAATT
AGGTAATCCTGGATCTTTAATTGGAGATGATCAAATTTATAATACTATTGTTACAGCTCATGCTTTTATTATAAT
TTTTTTTATAGTTATACCAATTATAATTGGTGGATTTGGTAATTGACTTGTACCTTTAATATTAGGAGCCCCTGA
TATAGCTTTCCCCCGAATAAATAATATAAGTTTTTGATTACTTCCCCCTTCTTTAACTTTACTTATTTCAAGAAG
AATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCCACTTTCATCTAATATTGCACATGGAGGAAG
ATCAGTAGACCTAGCTATTTTTTCTTTACATTTAGCTGGAATCTCATCTATTTTAGGAGCAATTAATTTTATTAC
TACTATTATTAATATAAAATTAAATAGCTTATCTTTTGATCAAATGCCATTATTTATTTGAGCTGTAGGAATTAC
TGCATTTTTATTATTATTATCATTACCAGTTTTAGCAGGTGCTATTACTATACTTTTATCAGATCGAAACCTTAA
TACATCTTTTTTTGACCCTGCTGGAGGAGGTGATCCTATTTTATATCAACATTTATTT
```

Fig. 94. Partial coding sequence of *H. armigera* SJIK15 COI gene.

> *H. armigera* Voucher SJIK15

```
TLYFIFGIWAGMVGTSLSLLIRAELGNPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWLV
PLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHGGSSVDLAIFSL
HLAGISSILGAINFITTIINMKLNSLSFDQMPLFIWAVGITAFLLLLSLPVLAGAITMLLSDRNLNT
SFFDPAGGGDPILYQHLF
```

Fig. 95. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *H. armigera* SJIK15.

Table 16. The BLAST hit table of the partial coding DNA sequence of COI gene of *H. armigera* SJIK15.

SN.	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Query end	Geogra. location
1	Helicoverpa armigera MH190450.1	99.85	658	1	0	1	658	Kenya
2	Helicoverpa armigera MF673566.1	99.85	658	1	0	1	658	Senegal
3	Helicoverpa armigera KY411345.1	99.85	658	1	0	1	658	China
4	Helicoverpa armigera KY411327.1	99.85	658	1	0	1	658	China
5	Helicoverpa armigera KY411311.1	99.85	658	1	0	1	658	China
6	Helicoverpa armigera KY411310.1	99.85	658	1	0	1	658	China
7	Helicoverpa armigera KY411299.1	99.85	658	1	0	1	658	China
8	Helicoverpa armigera KY411298.1	99.85	658	1	0	1	658	China
9	Helicoverpa armigera KY411297.1	99.85	658	1	0	1	658	China
10	Helicoverpa armigera MG954446.1	99.70	658	2	0	1	658	China
11	Helicoverpa armigera MH190453.1	99.70	658	2	0	1	658	Kenya
12	Helicoverpa armigera MH190451.1	99.70	658	2	0	1	658	Kenya
13	Helicoverpa armigera KY411354.1	99.70	658	2	0	1	658	China
14	Helicoverpa armigera KY411353.1	99.70	658	2	0	1	658	China
15	Helicoverpa armigera KY411352.1	99.70	658	2	0	1	658	China

The DNA isolated from the sample *H. armigera* SJIK15 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 16 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

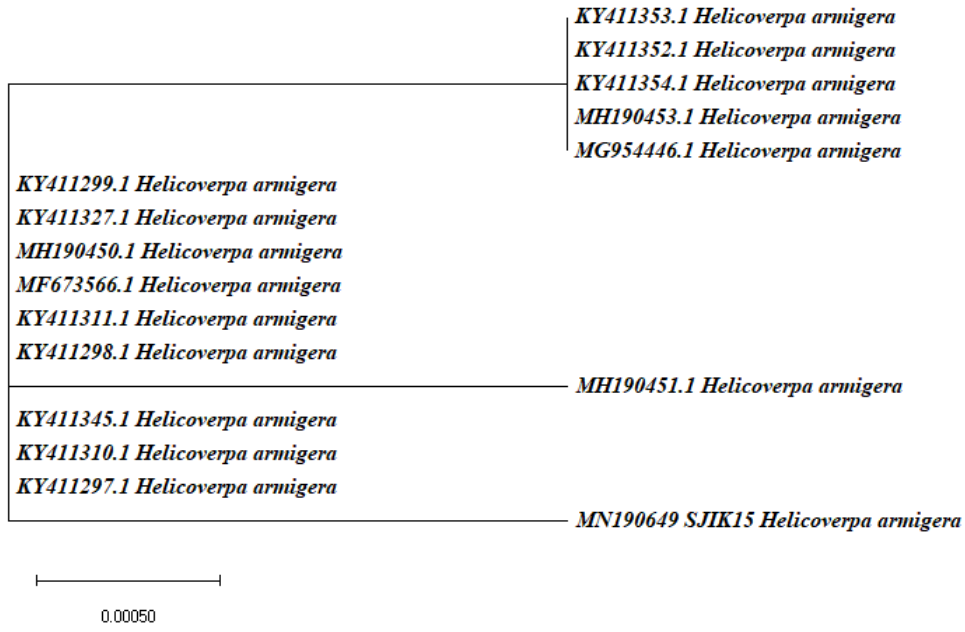

Fig. 96. The NJ tree showing phylogenetic relationships of *H. armigera* SJIK15.

The SJIK15 isolate from Kerala showed a maximum similarity of 99.85% to *H. armigera* MH190450 from Kenya, MF673566 from Senegal, KY411345, KY411327, KY411311, KY411310, KY411299, KY411298 and KY411297 from China all being polymorphic variants of SJIK15. Therefore the sequence of SJIK15 is novel. The nearest match from Kenya showed a single nucleotide change (T in SJIK15 changed to A). It showed 99.7% similarity to MG954446, KY411354 and KY411352 from China and to HM190453 and MH190451 from Kenya. These are also polymorphic variants. The NJ-tree shows a common ancestry for all the *H. armigera* species considered for the construction of the tree and that SJIK15 diverged from its closely related species *Helicoverpa armigera* KY411297 about 1700 years ago and hence it is of comparatively recent origin. The distribution pattern also confirms the common origin.

17. *Xanthodes transversa* SJIK17.

The specimen SJIK17 was identified as *Xanthodes transversa* **(**Gunee, 1852) referring to the morphological features described by Gurule, 2013.

Synonyms: *Xanthodes migrator* Walker, 1858

Trileuca dentalis Smith, 1891

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Noctuidae; Bagisarinae; *Xanthodes*.

X. transversa is found in India, Sri Lanka, Burma, China, Hong Kong, Japan, Ryukyu Is., Singapore, Indonesia, Java and Australia. In India it is seen in Nicobar Islands, Maharashtra, Assam, Jharkhand and Ponmudi (Shubhalaxmi *et al*., 2011, Singh et al., 2018, Sondhi *et al.,* 2018). *X. transversa* belong to the family Noctuidae and subfamily Bagisarinae. It is known as hibiscus caterpillar. It is a mutivoltine moth species having more than two generations per year. This moth enters facultative diapause in the pre-pupal stage by responding to environmental cues. *X. transversa* SJIK17 is pest of cotton and *Malvaceae* plants and vegetable crops like brinjal and okra.

Identification Marks: Palpi are reddish brown, long and porrect; head, thorax and abdomen bright canary yellow; vertex of the thorax tinged with rufous; legs are red brown; the tibia clothed with long hairs; fore wings are bright canary yellow; ante-medial and post-medial highly angulated rufous lines, which are sometimes waved, the post-medial touching a sub-marginal angled line; a large bright rufous triangular patch occupying the whole outer area, and sometimes produced backwards along median nervure to the base, or occasionally almost obsolete; a black sub-apical speck; cilia rufous; hind wing slightly suffused with red- brown, the outer margin rufous.

Results and discussion

The PCR of the COI gene fragment of *X. transversa* SJIK17 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 98 - 102. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190651).

Fig. 97. *Xanthodes transversa* SJIK17 (dorsal & ventral view)

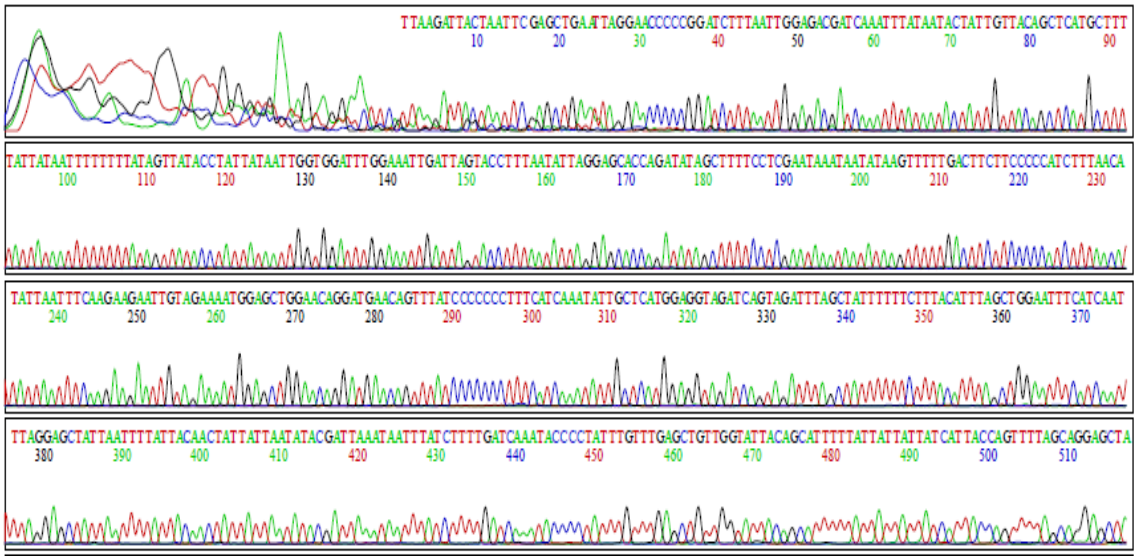

Fig. 98. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *X. transversa* SJIK17.

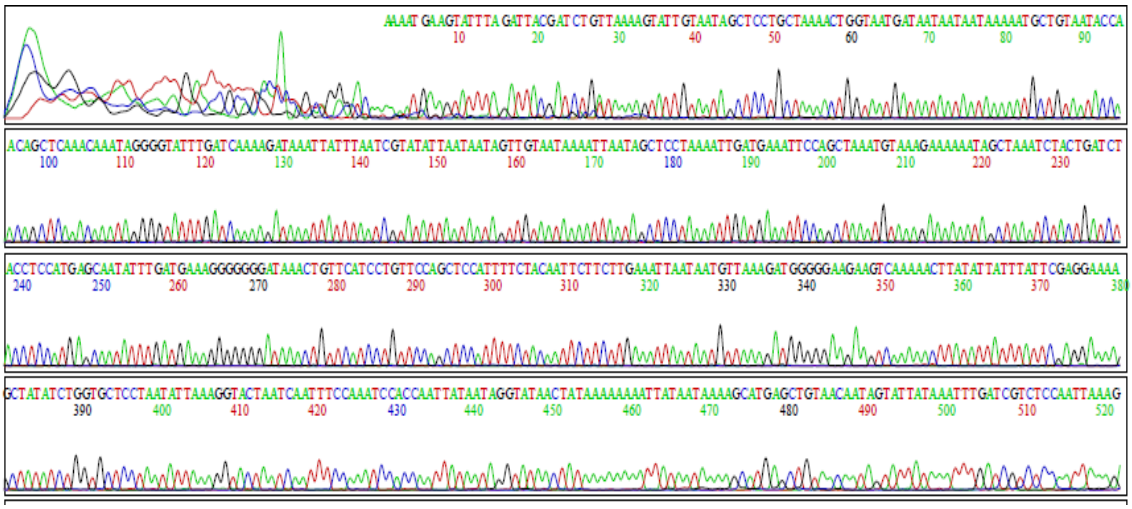

Fig. 99. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *X. transversa* SJIK17.

> *X. transversa* Voucher SJIK17 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACTTTATATTTTATTTTTGGAATTTGAGCAGGAATAGTAGGAACTTCATTAAGATTACTAATTCG
AGCTGAATTAGGAACCCCCGGATCTTTAATTGGAGACGATCAAATTTATAATACTATTGTTACAGC
TCATGCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGTGGATTTGGAAATTGATT
AGTACCTTTAATATTAGGAGCACCAGATATAGCTTTTCCTCGAATAAATAATATAAGTTTTTGACT
TCTTCCCCCATCTTTAACATTATTAATTTCAAGAAGAATTGTAGAAAATGGAGCTGGAACAGGATG
AACAGTTTATCCCCCCCTTTCATCAAATATTGCTCATGGAGGTAGATCAGTAGATTTAGCTATTTT
TTCTTTACATTTAGCTGGAATTTCATCAATTTTAGGAGCTATTAATTTTATTACAACTATTATTAA
TATACGATTAAATAATTTATCTTTTGATCAAATACCCCTATTTGTTTGAGCTGTTGGTATTACAGC
ATTTTTATTATTATTATCATTACCAGTTTTAGCAGGAGCTATTACAATACTTTTAACAGATCGTAA
TCTAAATACTTCATTTTTTGACCCTGCTGGAGGAGGAGATCCAATTTTATATCAACATTTATTT
```

Fig. 100. Partial coding sequence of *X. transversa* SJIK17 COI gene.

> *X. transversa* Voucher SJIK17

```
TLYFIFGIWAGMVGTSLSLLIRAELGTPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWLVP
LMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHGGSSVDLAIFSLHL
AGISSILGAINFITTIINMRLNNLSFDQMPLFVWAVGITAFLLLLSLPVLAGAITMLLTDRNLNTSFF
DPAGGGDPILYQHLF
```

Fig. 101. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *X. transversa* SJIK17.

The DNA isolated from the sample *X. transversa* SJIK17 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 18 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

The BLAST result of SJIK17 isolate from Kerala was 100% similar to *X. transversa* MH686470 from Thailand sharing the same clade and showing the South East Asian origin of the species. It showed 99.85% similarity to *X. transversa* MG250706 from India, 98.63% to HQ951631 & HQ951632 from Australia and 98.18% to HM906230 from Papua New Guinea all being polymorphic variants of the species and occupying adjoining clades. The closely related species from India MG250706 showed a single nucleotide change (A in SJIK17 changed to G). *X. intersepta* MG783851 from Maharashtra with 97.71% similarity is the closest relative of the species. The Kerala isolate appears to have evolved from the closest species *X. intersepta* about 15000 years ago. *Xanthodes transversa*, SJIK17, showed a Gondwana origin being distributed in India, Thailand, Papua New Guinea and Australia which diverged at various stages. The COI sequence of *X. transversa* SJIK17 obtained in the present study can be used for the accurate taxonomic identification of the species as it shows 100% match to that in the database.

Table 18. The BLAST hit table of the partial coding DNA sequence of COI gene of *X. transversa* SJIK17.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Q start	Q end	Geographical location
1	Xanthodes transversa MG250706.1	99.85	658	1	0	1	658	India
2	Xanthodes transversa MH686470.2	100	635	0	0	2	636	Thailand
3	Xanthodes transversa HQ951631.1	98.63	658	9	0	1	658	Australia
4	Xanthodes transversa HQ951632.1	98.48	658	10	0	1	658	Australia
5	Xanthodes transversa HM906230.1	98.18	658	12	0	1	658	Papua New Guinea
6	Pardoxia graellsi KX046091.1	97.57	658	16	0	1	658	France
7	Xanthodes intersepta MG783851.1	97.71	654	15	0	2	655	India, Maharashtra
8	Xanthodes emboloscia HQ951628.1	96.96	658	20	0	1	658	Australia
9	Xanthodes congenita HQ951634.1	96.96	658	20	0	1	658	Australia
10	Xanthodes amata HQ951638.1	96.35	658	24	0	1	658	Australia
11	Xanthodes albago KF388534.1	94.83	658	34	0	1	658	Australia
12	Xanthodes emboloscia HQ951629.1	96.88	609	19	0	1	609	Australia
13	Zanclognatha laevigata MF128227.1	93.62	658	42	0	1	658	Canada
14	Condica illecta KX052357.1	93.62	658	42	0	1	658	French Polynesia
15	Xanthodes albago GU828844.1	93.31	658	44	0	1	658	Finland

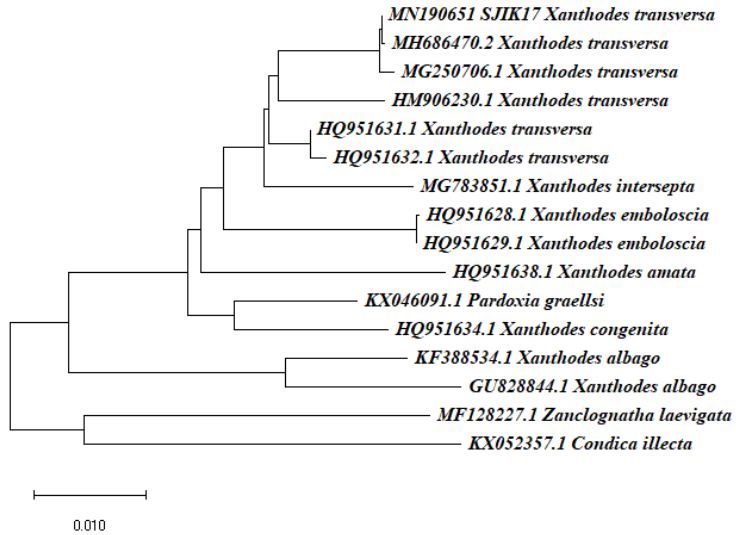

Fig. 102. The NJ tree showing phylogenetic relationships of *X. transversa* SJIK17.

18. *Condica illecta* SJIK18

The specimen SJIK18 was identified as *Condica illecta* (Walker, 1865) referring to the morphological features described by Walker 1865.

Synonyms: *Perigea illecta* Walker, 1865

Hadena funesta Walker, 1865

Hadena spargens Walker, 1865

Platysenta illecta

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Noctuidae; Amphipyrinae; *Condica*

C. illecta is found in India, Srilanka, Mayanmar, China, Japan, Australia, Sundaland and Fiji. In India it is reported from Maharashtra (Shubhalaxmi *et al*., 2011). *C. illecta* belongs to the family Noctuidae and subfamily Amphipyrinae. It is a pest of soybean. Larval food plants- *Acasia* (Leguminosae) and *Acanthads.*

Identifying characters: Palpi stout, smooth, applied to the head, rising higher than the vertex in female; third joint lanceolate; abdomen cinereous; extending a little beyond the hind wings; smooth legs; tarsi brown; hind wings cinereous; fuscous brown coloured wings; fore wing with indistinct sub-basal, ante, post-medial and sub-marginal lines; the orbicular and reniform indistinct, the latter edged with white specks; some white specks on costa towards apex; and a series on outer margin; hind wing slightly paler at base; pale cilia; underside with an obscure post-medial line.

Results and discussion

The PCR of the COI gene fragment of *C. illecta* SJIK18 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 104 - 108. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190652).

Fig. 103. *Condica illecta* SJIK18

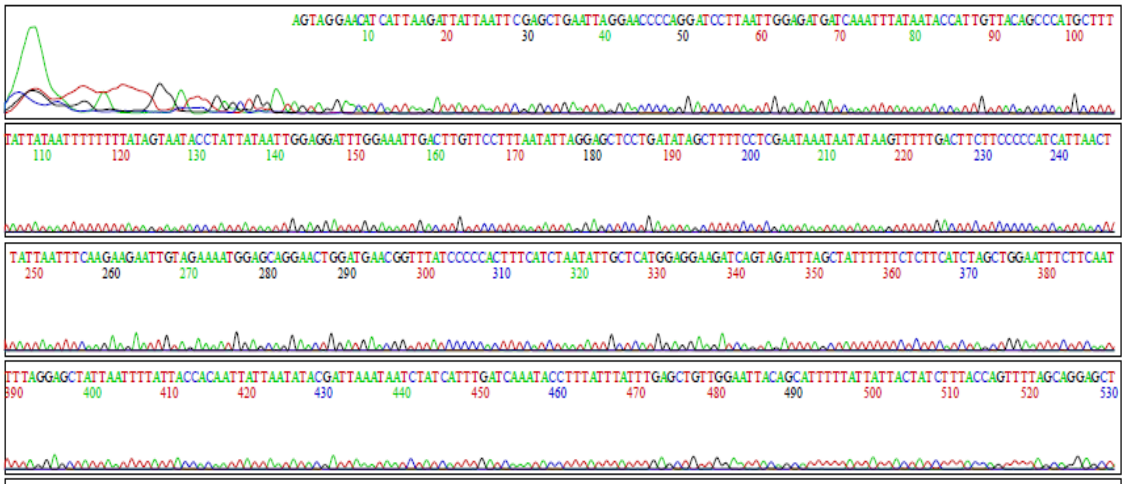

Fig. 104. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *C. illecta* SJIK18.

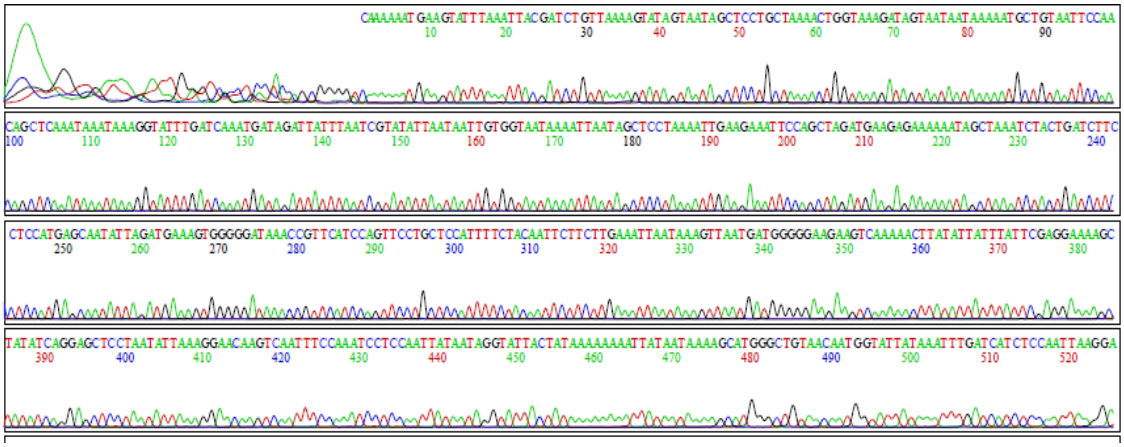

Fig. 105. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *C. illecta* SJIK18.

> *C. illecta* Voucher SJIK18 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACATTATATTTTATTTTTGGAATTTGAGCTGGAATAGTAGGAACATCATTAAGATTATTAATTCGAGCTGAATTA
GGAACCCCAGGATCCTTAATTGGAGATGATCAAATTTATAATACCATTGTTACAGCCCATGCTTTTATTATAATTT
TTTTTATAGTAATACCTATTATAATTGGAGGATTTGGAAATTGACTTGTTCCTTTAATATTAGGAGCTCCTGATAT
AGCTTTTCCTCGAATAAATAATATAAGTTTTTGACTTCTTCCCCCATCATTAACTTTATTAATTTCAAGAAGAATT
GTAGAAAATGGAGCAGGAACTGGATGAACGGTTTATCCCCCACTTTCATCTAATATTGCTCATGGAGGAAGATCAG
TAGATTTAGCTATTTTTTCTCTTCATCTAGCTGGAATTTCTTCAATTTTAGGAGCTATTAATTTTATTACCACAAT
TATTAATATACGATTAAATAATCTATCATTTGATCAAATACCTTTATTTATTTGAGCTGTTGGAATTACAGCATTT
TTATTATTACTATCTTTACCAGTTTTAGCAGGAGCTATTACTATACTTTTAACAGATCGTAATTTAAATACTTCAT
TTTTTGACCCCGCTGGAGGAGGTGACCCAATTCTTTATCAACATTTATTT
```

Fig. 106. Partial coding sequence of *C. illecta* SJIK18 COI gene.

> *C. illecta* Voucher SJIK18

```
TLYFIFGIWAGMVGTSLSLLIRAELGTPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWLVPLM
LGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHGGSSVDLAIFSLHLAGIS
SILGAINFITTIINMRLNNLSFDQMPLFIWAVGITAFLLLLSLPVLAGAITMLLTDRNLNTSFFDPAGGG
DPILYQHLF
```

Fig. 107. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *C. illecta* SJIK18.

The DNA isolated from the sample *Condica illecta* SJIK18 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 18 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

The BLAST result showed that SJIK18 from Kerala is 100% similar to *C. illecta* KX862760 from Pakistan occupying the same clade. Hence it can be used as a molecular barcode for species identification. It showed 99.85% similarity to *C. illecta* MK019877 and MK019292 from Papua New Guinea, KX863280 and KX862962 from Pakistan all being polymorphic geographical variants of SJIK18 and placed in the adjacent clade. The closest species from Papua New Guinea showed a single nucleotide difference (G in SJJIK18 changed to A). *C. sutor* JN262083 from USA with 96.51% similarity, placed in a different clade, is a different species of the genus which remain close to SJIK18. The phylogenetic distance data revealed that the species was originated from its closely related species *C. sutor* about 20000 years ago.

Table 18. The BLAST hit table of the partial coding DNA sequence of COI gene of *C. illecta* SJIK18.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Query end	Geograph. location
1	Condica illecta KX862760.1	100	658	0	0	1	658	Pakistan
2	Condica illecta MK019877.1	99.85	658	1	0	1	658	Papua New Guinea
3	Condica illecta MK019292.1	99.85	658	1	0	1	658	Papua New Guinea
4	Condica illecta KX863280.1	99.85	658	1	0	1	658	Pakistan
5	Condica illecta KX862962.1	99.85	658	1	0	1	658	Pakistan
6	Condica sutor JN262083.1	96.51	658	23	0	1	658	USA
7	Condica circuita JQ564403.1	96.20	658	25	0	1	658	Costa Rica
8	Chaographis rhaptina HQ949235.1	95.44	658	30	0	1	658	Australia
9	Condica aroana HQ950407.1	95.44	658	30	0	1	658	Australia
10	Condica cupentia GU679082.1	95.45	659	28	2	1	658	USA
11	Condica mobilis JQ568020.1	95.29	658	31	0	1	658	Costa Rica
12	Chaographis crystallodes HQ949230.1	95.14	658	32	0	1	658	Australia
13	Condica dolorosa HQ950408.1	95.14	658	32	0	1	658	Australia
14	Condica confederata GU679081.1	95.14	658	32	0	1	658	USA
15	Condica funerea GU163108.1	95.14	658	32	0	1	658	Costa Rica

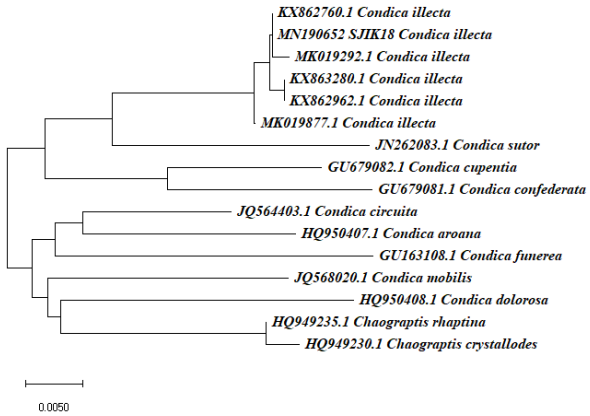

Fig. 108. The NJ tree showing phylogenetic relationships of *C. illecta* SJIK18.

The NJ tree shows that SJIK18 and the 5 samples viz., KX862760 from Pakistan, MK019877 and MK019292 from Papua New Guinea, KX863280 and KX862962 from Pakistan have a common ancestor. The most distant relative is *C. dolorosa* HQ950408 from Australia showing 95.14% similarity. The data shows that some species of the genus *Condica* has a Gondwana origin and later diverged and separated by continental drift reaching South America and through land bridges might have traversed to North America.

19. *Trabala sp.* SJIK22

The specimen SJIK22 was identified as *Trabala sp.* (Walker, 1856) referring to the morphological features described by Hampson, 1892.

Synonym: *Amydona* Walker, 1855

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Bombycoidea; Lasiocampidae; Pinarinae; *Trabala*.

Trabala sp. are found in China, India, Sri Lanka, Myanmar, Borneo and Java. In India it is seen in Maharashtra and Assam (Shubhalaxmi *et al*., 2011, Abang et al., 2002, Arandhara *et al.,* 2018, Bharmal, 2010). *Trabala sp.* belongs to the family Lasiocampidae and subfamily Pinarinae. It is a pest of fruit crops like pomegranate and jamun, ornamental plants like rose and trees like sandal.

Identifying characters: Palpi somewhat short and slight; antennae with branches shorter in females; mid and hind tibiae with terminal pair of spurs; broad fore wings, outer margin rounded and the cell open; hind wing with cell open.

Results and discussion

The PCR of the COI gene fragment of *Trabala sp.* SJIK22 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 110-114. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190656).

The DNA isolated from the sample *Trabala sp.* SJIK22 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 19 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

Fig. 109. *Trabala sp.* SJIK22 (dorsal and ventral view)

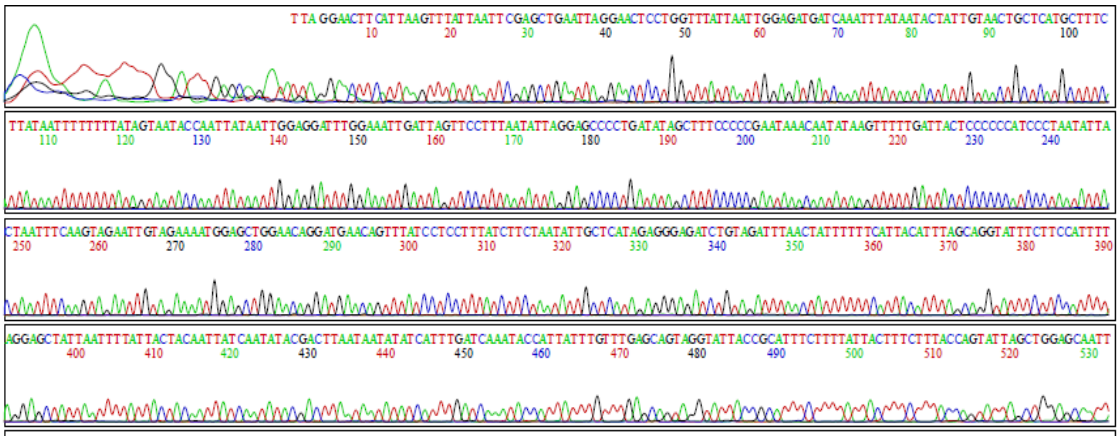

Fig. 110. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *Trabala sp.* SJIK22.

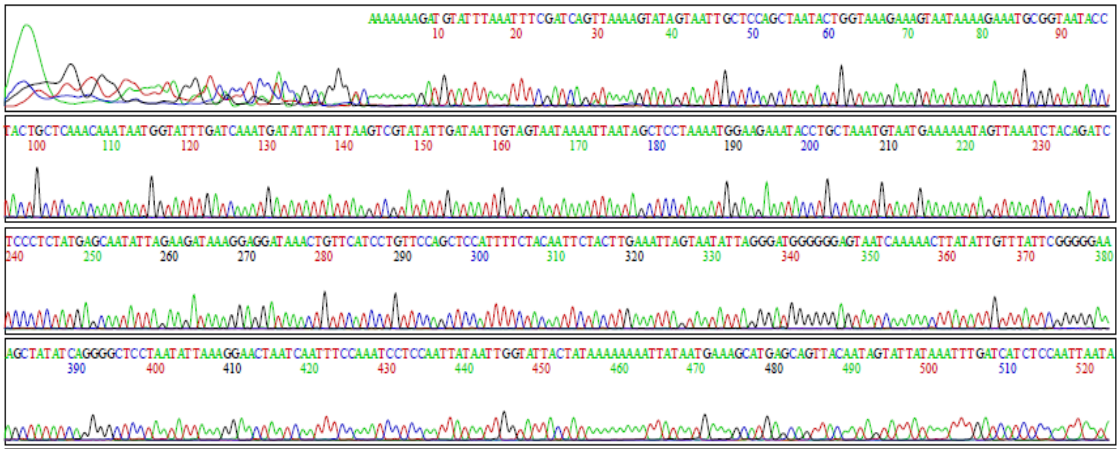

Fig. 111. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *Trabala sp.* SJIK22.

> *Trabala sp.* Voucher SJIK22 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACATTATATTTTATTTTTGGAATTTGAGCAAGAATATTAGGAACTTCATTAAGTTTATTAATTCGAGCTGAATT
AGGAACTCCTGGTTTATTAATTGGAGATGATCAAATTTATAATACTATTGTAACTGCTCATGCTTTCATTATAAT
TTTTTTTATAGTAATACCAATTATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCCTGA
TATAGCTTTCCCCCGAATAAACAATATAAGTTTTTGATTACTCCCCCCATCCCTAATATTACTAATTTCAAGTAG
AATTGTAGAAAATGGAGCTGGAACAGGATGAACAGTTTATCCTCCTTTATCTTCTAATATTGCTCATAGAGGGAG
ATCTGTAGATTTAACTATTTTTTCATTACATTTAGCAGGTATTTCTTCCATTTTAGGAGCTATTAATTTTATTAC
TACAATTATCAATATACGACTTAATAATATATCATTTGATCAAATACCATTATTTGTTTGAGCAGTAGGTATTAC
CGCATTTCTTTTATTACTTTCTTTACCAGTATTAGCTGGAGCAATTACTATACTTTTAACTGATCGAAATTTAAA
TACATCTTTTTTTGACCCTGCTGGAGGAGGTGATCCTATTTTATACCAACACTTATTT
```

Fig. 112. Partial coding sequence of *Trabala sp.* SJIK22 COI gene.

> *Trabala sp.* Voucher SJIK22

```
TLYFIFGIWASMLGTSLSLLIRAELGTPGLLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWLV
PLMLGAPDMAFPRMNNMSFWLLPPSLMLLISSSIVENGAGTGWTVYPPLSSNIAHSGSSVDLTIFSL
HLAGISSILGAINFITTIINMRLNNMSFDQMPLFVWAVGITAFLLLLSLPVLAGAITMLLTDRNLNT
SFFDPAGGGDPILYQHLF
```

Fig. 113. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *Trabala sp.* SJIK22.

Table 19. The BLAST hit table of the partial coding DNA sequence of COI gene of *Trabala sp.* SJIK22.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Query end	Geographi-cal location
1	Trabala sp. KP662056.1	96.46	593	21	0	19	611	China
2	Trabala vishnou KP662048.1	96.30	621	23	0	1	621	China
3	Trabala vishnou guttata JN305952.1	96.20	658	25	0	1	658	China
4	Trabala vishnou guttata KF492154.1	96.05	658	26	0	1	658	Taiwan
5	Trabala vishnou guttata JN305929.1	96.05	658	26	0	1	658	China
6	Trabala vishnou KP662049.1	95.90	658	27	0	1	658	China
7	Trabala vishnou KP233788.1	95.90	658	27	0	1	658	India, H.P.
8	Trabala vishnou JF858109.1	95.90	658	27	0	1	658	Pakistan
9	Trabala vishnou JF858105.1	95.90	658	27	0	1	658	Pakistan
10	Trabala vishnou JF858111.1	95.75	658	28	0	1	658	Pakistan
11	Trabala vishnou JF858107.1	95.75	658	28	0	1	658	Pakistan
12	Trabala gautama JN305950.1	93.60	656	42	0	1	656	Malaysia
13	Trabala vishnou KJ183618.1	95.43	612	28	0	47	658	China
14	Sphinx vashti HM866982.1	91.19	658	58	0	1	658	Canada

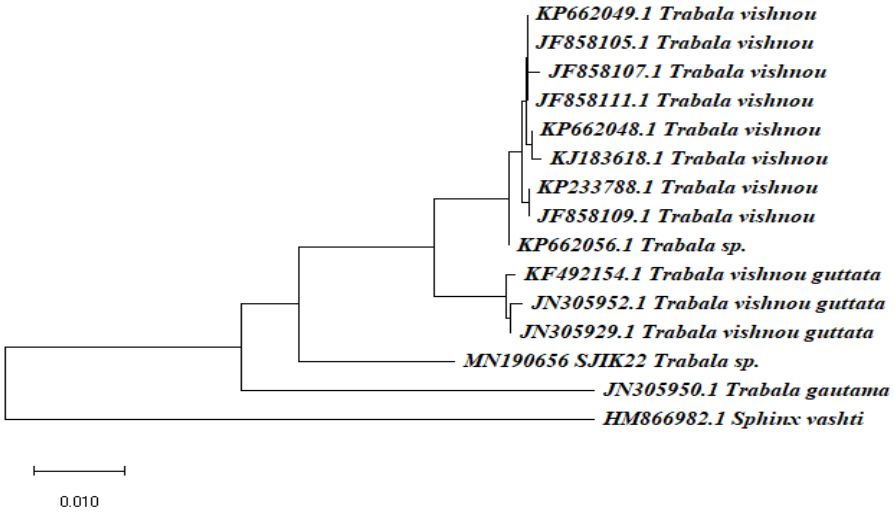

Fig. 114. The NJ tree showing phylogenetic relationships of *Trabala sp.* SJIK22.

The BLAST result showed that SJIK22 isolated from Kerala showed 96.46% similarity to its nearest match from China, *Trabala sp.* KP662056. *Trabala gautama* JN305950 from Malaysia with a similarity of 93.6%, placed in the adjacent clade is a different species of the genus which remain close to SJIK22. The distance data showed that the species originated from its closely related species about 35000 years ago. The adjoining clade shows its relationship to the subspecies *Trabala vishnou guttata* from China and Taiwan which might have evolved by geographical isolation. The NJ tree shows that SJIK22 from Kerala is a novel species as it showed only 96.46% similarity to the nearest match *Trabala sp*. in the database occupying a separate clade. The most distant relative is *T. vishnou* KP662049 from China. The phylogenetic tree shows that the Kerala isolate diverged from other *Trabala* species from China and Taiwan as a result of vicariance events like the rise of Himalayas, disappearance of the Tethys Sea and associated climatic changes. 65 novel bp of COI were added to the database.

20. *Stemorrhages sp.* SJIK24.

The specimen SJIK24 was identified as *Stemorrhages sp*. **(**Lederer, 1863) referring to the morphological features described by Hampson, 1896.

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Pyraloidea; Crambidae; Spilomelinae; *Stemorrhages*.

Stemorrhages sp. is found in India, DR Congo, Ghana, Madagascar, Mauritius, South Africa, Sudan, Tanzania, Australia, Zimbabwe and Mali (Mathew, 2006). The *Stemorrhages sp.* belongs to the family Crambidae and subfamily Spilomelinae. Host plants are Rubiaceae, viz., *Gardenia jasminoides*.

Identifying characters : Palpi upturned, the second joint broadly scaled in front, the 3rd porrect and lying along the hair on the 2nd joint; maxillary palpi triangularly scaled; frons rounded; antennae of male nearly simple; tibiae with the outer spurs less than half the length of inner; male with the anal tuft large; fore wing with the costa highly arched towards apex; veins 3, 4, 5 from angle of cell, 7 closely approximated to 8, 9; hind wing with vein 3 from angle of cell; 4, 5 closely approximated for a short distance; the disco-cellulars slightly angled and almost erect; 6, 7 from upper angle, or shortly stalked, 7 anastomosing with 8.

Results and discussion

The PCR of the COI gene fragment of *Stemorrhages sp*.SJIK24 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 116-120. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190658).

The DNA isolated from the sample *Stemorrhages sp*. SJIK24 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 20 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

Fig. 115. *Stemorrhages sp*.SJIK24

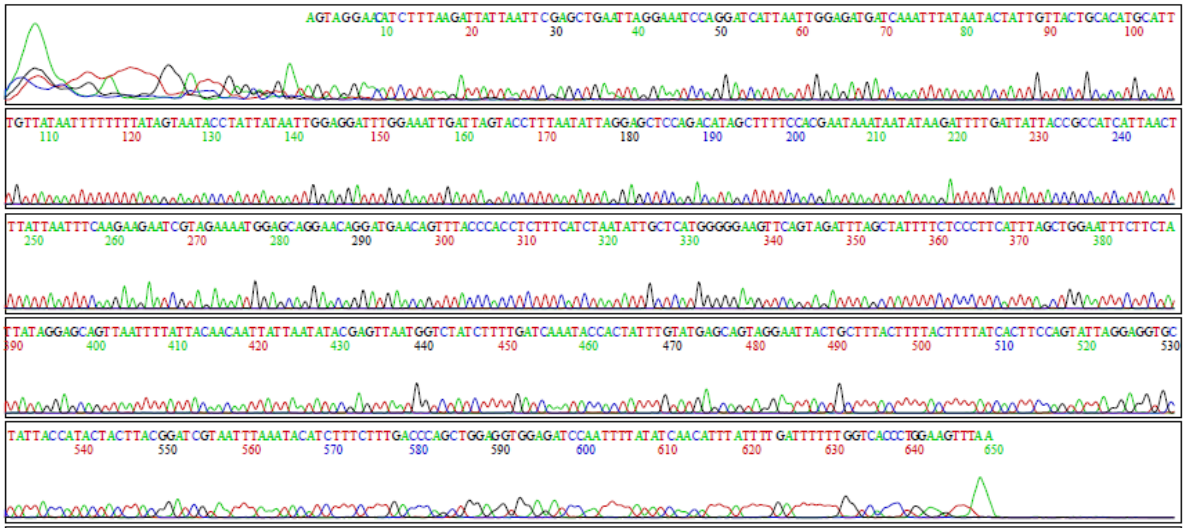

Fig. 116. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *Stemorrhages sp*.SJIK24.

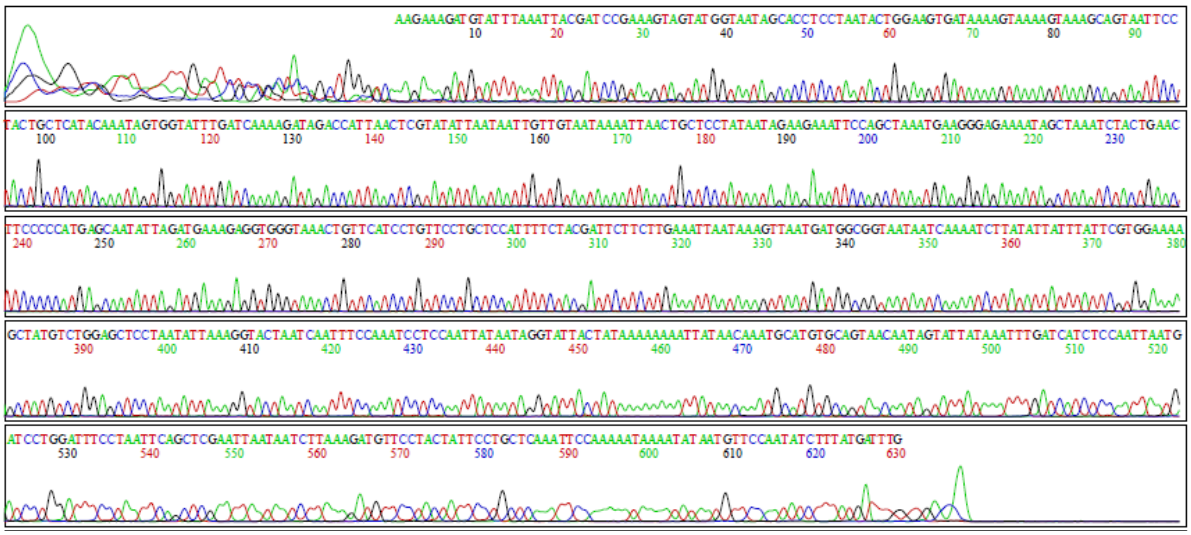

Fig. 117. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *Stemorrhages sp*.SJIK24.

> *Stemorrhages sp*. Voucher SJIK24 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACATTATATTTTATTTTTGGAATTTGAGCAGGAATAGTAGGAACATCTTTAAGATTATTAATTCGAGCTG
AATTAGGAAATCCAGGATCATTAATTGGAGATGATCAAATTTATAATACTATTGTTACTGCACATGCATTT
GTTATAATTTTTTTTATAGTAATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTACCTTTAATATT
AGGAGCTCCAGACATAGCTTTTCCACGAATAAATAATATAAGATTTTGATTATTACCGCCATCATTAACTT
TATTAATTTCAAGAAGAATCGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCACCTCTTTCATCT
AATATTGCTCATGGGGGAAGTTCAGTAGATTTAGCTATTTTCTCCCTTCATTTAGCTGGAATTTCTTCTAT
TATAGGAGCAGTTAATTTTATTACAACAATTATTAATATACGAGTTAATGGTCTATCTTTTGATCAAATAC
CACTATTTGTATGAGCAGTAGGAATTACTGCTTTACTTTTACTTTTATCACTTCCAGTATTAGGAGGTGCT
ATTACCATACTACTTACGGATCGTAATTTAAATACATCTTTCTTTGACCCAGCTGGAGGTGGAGATCCAAT
TTTATATCAACATTTATTT
```

Fig. 118. Partial coding sequence of *Stemorrhages sp*. SJIK24 COI gene.

> *Stemorrhages sp*. Voucher SJIK24

```
TLYFIFGIWAGMVGTSLSLLIRAELGNPGSLIGDDQIYNTIVTAHAFVMIFFMVMPIMIGGFGNWLV
PLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHGGSSVDLAIFSL
HLAGISSIMGAVNFITTIINMRVNGLSFDQMPLFVWAVGITALLLLLSLPVLGGAITMLLTDRNLNT
SFFDPAGGGDPILYQHLF
```

Fig. 119. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *Stemorrhages sp*. SJIK24.

Table 20. The BLAST hit table of the partial coding DNA sequence of COI gene of *Stemorrhages sp*. SJIK24.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Query end	Geograph. location
1	Stemorrhages marthesiusalis HQ952940.1	98.33	658	11	0	1	658	Australia
2	Stemorrhages marthesiusalis HQ952939.1	98.18	658	12	0	1	658	Australia
3	Stemorrhages sericea HM892565.1	93.31	658	44	0	1	658	Gabon
4	Stemorrhages sp. MH417265.1	93.16	658	45	0	1	658	Madagascar
5	Stemorrhages sericea HM892414.1	93.16	658	45	0	1	658	Gabon
6	Dichocrocis tlapalis JQ539014.1	91.95	658	53	0	1	658	Costa Rica
7	Rhectocraspeda periusalis JQ539670.1	91.78	657	54	0	2	658	Costa Rica
8	Diastictis ventralis KT143799.1	91.64	658	55	0	1	658	Canada
9	Rehimena leptophaes KF389795.1	91.64	658	55	0	1	658	Australia
10	Stemorrhages sericea HM893281.1	92.81	626	45	0	33	658	Gabon

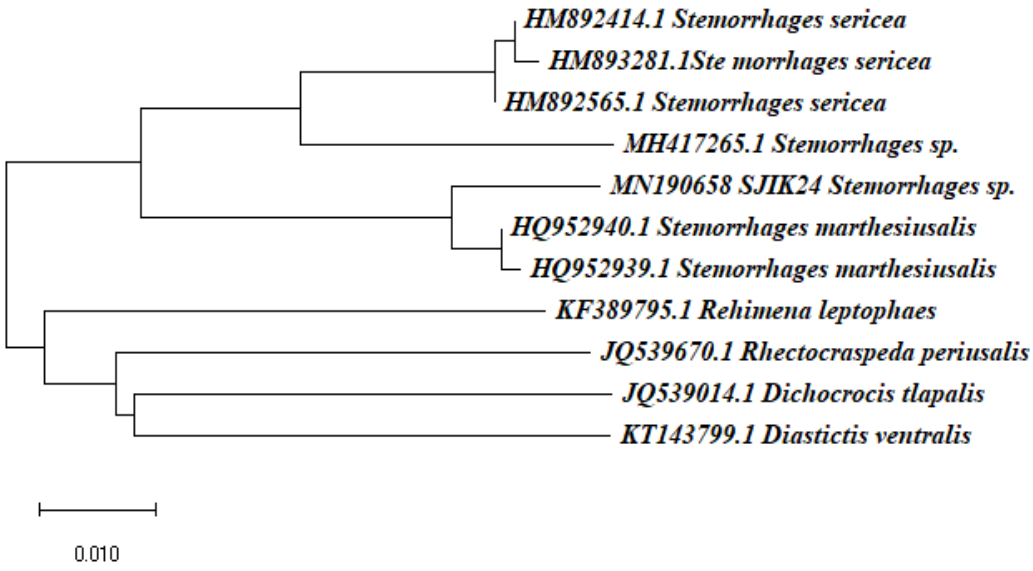

Fig. 120. The NJ tree showing phylogenetic relationships of *Stemorrhages sp*. SJIK24.

The BLAST results of *Stemorrhages sp.* SJIK24 showed a maximum similarity of 98.33% with *S. marthesiusalis* HQ952940 from Australia and 98.18% similarity to HQ952939 also from Australia. Hence the *Stemorrhages sp.* isolated from Kerala SJIK24 is a novel one. It is being reported for the first time from India. The NJ tree showed that the Australian and Indian species diverged from a common ancestor, occupying adjacent clades. The phylogenetic tree reveals that the species of *Stemorrhages* isolated from Gabon, viz., *S. sericea* and that from Madagascar also had a common origin with SJIK24 from the Indian subcontinent. They were placed in the adjacent clades. The geographical distribution pattern confirms the common ancestry and they might have diverged due to geographical isolation when the continents separated.

21. *Godonela* sp. SJIK27

The specimen SJIK27 was identified as *Godonela* sp. (Boisduval, 1840) referring to the morphological features described by Walker, 1861.

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Geometroidea; Geometridae; Ennominae; *Godonela*.

The *Godonela sp*.is found in India and South East Asia (Abang *et al*., 2002). In India it has been reported from Maharashtra, Kerala (Ponmudi), Assam and Himalayas (Shubhalaxmi *et al*., 2011, Sondhi *et al*., 2018). *Godonela sp*. belongs to the family Geometridae and subfamily Ennominae.

Identifying characters: Body slender and squamous; frons slightly villose; papli rostriform and very short; third joint obtuse and short; minutely speckled and long abdomen; hind tibiae incrassated and with tuft of hairs; wings oblong; fore wings prolonged at the tips; exterior border notched; hind wings quadrate and dentate; the moth is variegated black and dark grey in colour and has broad white bands medially on each wing; tinges of yellow on the hind wing, abdomen and basal zone of the undersurface of both wings.

Results and discussion

The PCR of the COI gene fragment of *Godonela sp.* SJIK27 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 122- 126. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190660).

The DNA isolated from the sample *Godonela sp.* SJIK27 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 21 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

Fig. 121. *Godonela sp.* SJIK27

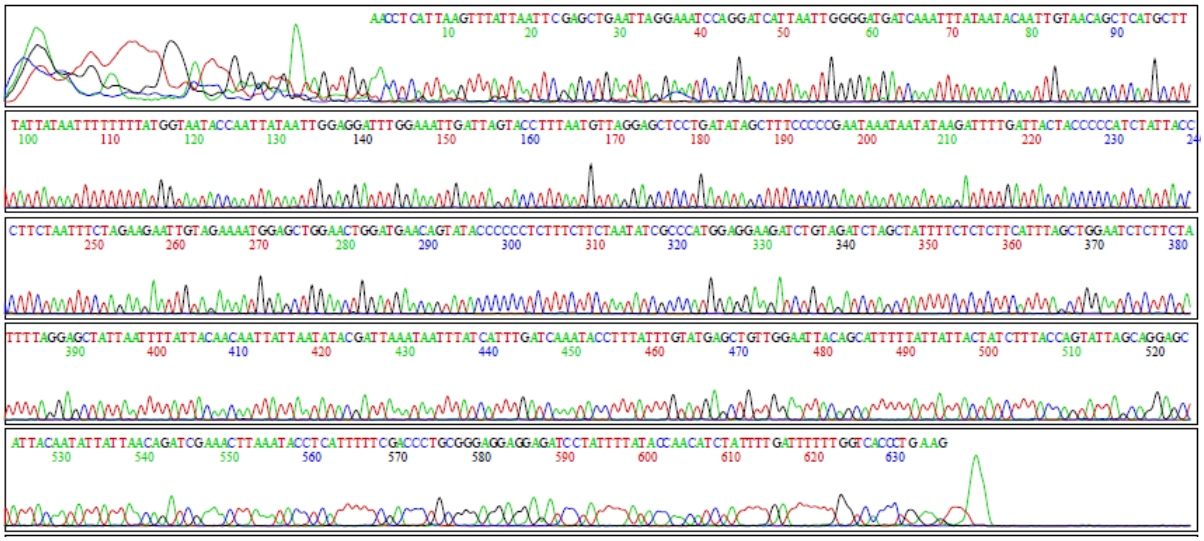

Fig. 122. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *Godonela sp.* SJIK27.

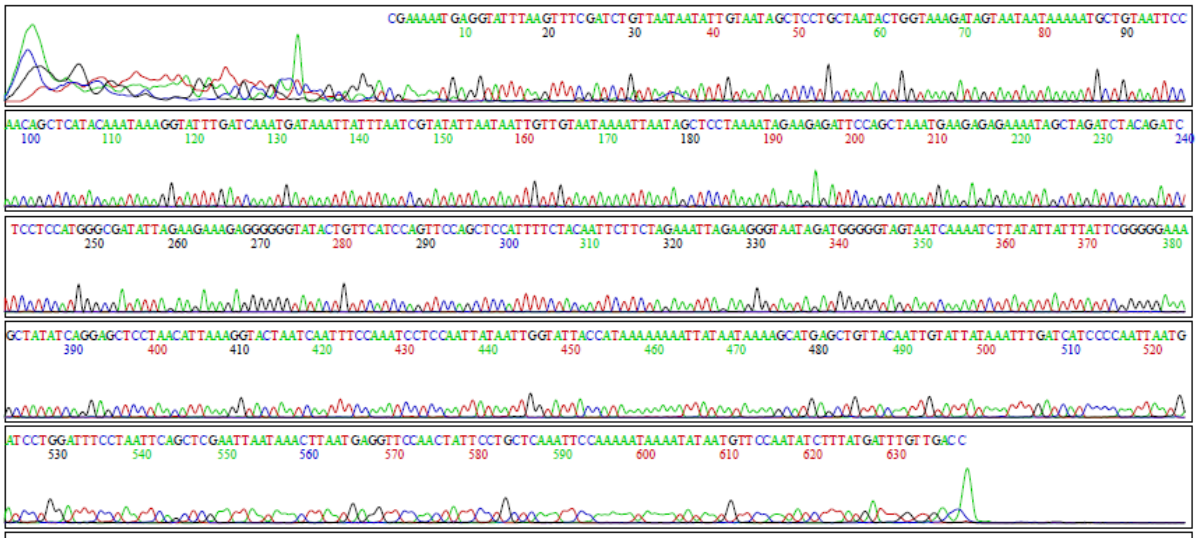

Fig. 123. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *Godonela sp.* SJIK27.

> *Godonela sp.* Voucher SJIK27 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACATTATATTTTATTTTTGGAATTTGAGCAGGAATAGTTGGAACCTCATTAAGTTTATTAATTCGAGCTGAATTA
GGAAATCCAGGATCATTAATTGGGGATGATCAAATTTATAATACAATTGTAACAGCTCATGCTTTTATTATAATTT
TTTTTATGGTAATACCAATTATAATTGGAGGATTTGGAAATTGATTAGTACCTTTAATGTTAGGAGCTCCTGATAT
AGCTTTCCCCCGAATAAATAATATAAGATTTTGATTACTACCCCCATCTATTACCCTTCTAATTTCTAGAAGAATT
GTAGAAAATGGAGCTGGAACTGGATGAACAGTATACCCCCCTCTTTCTTCTAATATCGCCCATGGAGGAAGATCTG
TAGATCTAGCTATTTTCTCTCTTCATTTAGCTGGAATCTCTTCTATTTTAGGAGCTATTAATTTTATTACAACAAT
TATTAATATACGATTAAATAATTTATCATTTGATCAAATACCTTTATTTGTATGAGCTGTTGGAATTACAGCATTT
TTATTATTACTATCTTTACCAGTATTAGCAGGAGCTATTACAATATTATTAACAGATCGAAACTTAAATACCTCAT
TTTTCGACCCTGCGGGAGGAGGAGATCCTATTTTATACCAACATCTATTT
```

Fig. 124. Partial coding sequence of *Godonela sp.* SJIK27 COI gene.

> *Godonela sp.* Voucher SJIK27

```
TLYFIFGIWAGMVGTSLSLLIRAELGNPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWLVPLM
LGAPDMAFPRMNNMSFWLLPPSITLLISSSIVENGAGTGWTVYPPLSSNIAHGGSSVDLAIFSLHLAGIS
SILGAINFITTIINMRLNNLSFDQMPLFVWAVGITAFLLLLSLPVLAGAITMLLTDRNLNTSFFDPAGGG
DPILYQHLF
```

Fig.125.The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *Godonela sp.* SJIK27.

Table 21. The BLAST hit table of the partial coding DNA sequence of COI gene of *Godonela sp.* SJIK27.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Query end	Geogra. location
1	Godonela sp. KJ380857.1	100	593	0	0	35	627	India,Western Ghats
2	Chiasmia nora HQ990988.1	99.85	658	1	0	1	658	Pakistan
3	Chiasmia sp. MH197465.1	97.26	658	18	0	1	658	India
4	Chiasmia sp. KF391332.1	95.44	658	30	0	1	658	Australia
5	Chiasmia goldiei KF390006.1	93.62	658	42	0	1	658	Australia
6	Chiasmia goldiei KF388771.1	93.62	658	42	0	1	658	Australia
7	Eois ambarilla KU380809.1	92.72	659	46	2	1	658	Ecuador
8	Eurranthis plummistaria MK739459.1	92.55	658	49	0	1	658	Sweden
9	Iridopsis clivinaria HQ648597.1	92.55	658	49	0	1	658	USA
10	Eusarca sp. crameraria JQ561249.1	92.41	659	48	2	1	658	Costa Rica

The BLAST result of SJIK27 isolated from Kerala showed 100% similarity to that isolated from Western Ghats and hence it is useful as a barcode for species identification.

It showed 99.85% similarity to *Chiasmia nora* HQ990988 from Pakistan, 97.26% to *Chiasmia sp.* MH19465 from India and 95.44% to *Chiasmia sp.* KF391332 from Australia. *Chiasma goldiei* species KF390006 and KF388771 from Australia showed 93.62% similarity to SJIK27.

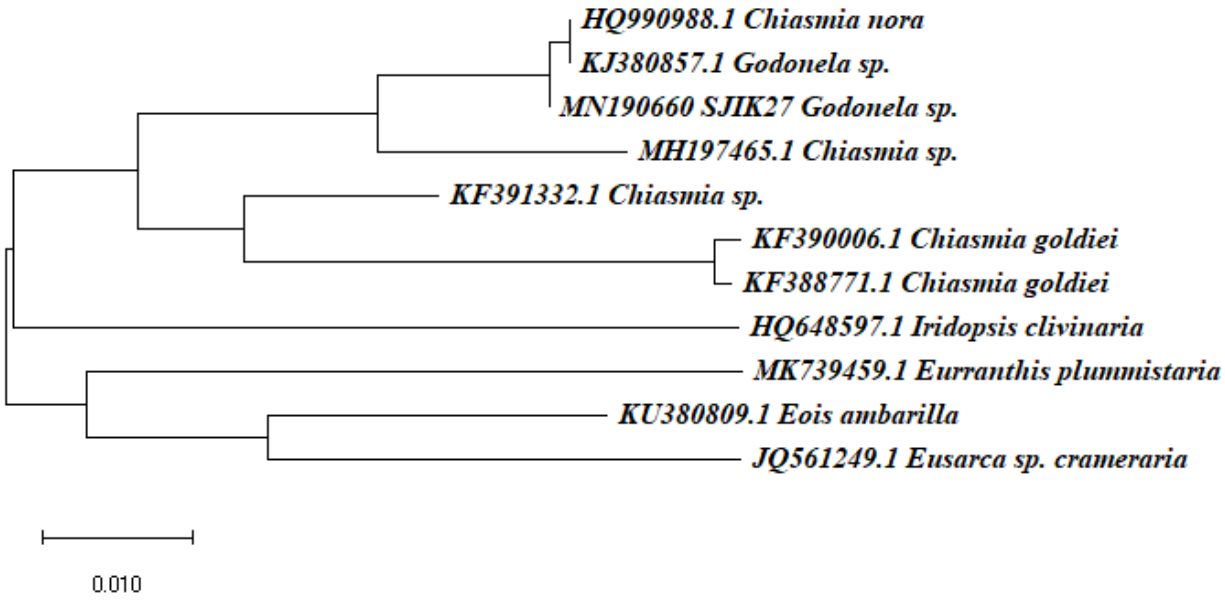

Fig. 126. The NJ tree showing phylogenetic relationships of *Godonela sp.* SJIK27.

All these data shows the close relationship of *Godonela sp.* to the genus *Chiasma*. The NJ tree also shows the close relationship between SJIK27 and *Chiasma* genus and that the *Godonela sp*. and *Chiasmia sp.* have evolved from a common ancestor and diverging later on. 65 novel bp of COI were added to the database.

22. *Nagia sp.* SJIK28

The specimen SJIK28 was identified as *Nagia sp.* (Walker, 1858) referring to the morphological features described by Hampson, 1896.

Synonyms: *Phryganodes* Guenee, 1854
Omiodes, Guenee
Coenostola Lederer, 1863
Condiga Moore, 1886
Charema Moore, 1888

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Erebidae; Erebidae incertae sedis; *Nagia*.

Nagia sp. is found in India, Sri Lanka, Myanmar and Australia and African countries. In India *Nagia sp.* is found in Tamil Nadu, Assam and Vagamon (Kerala). *Nagia linteola* has been reported from Tamil Nadu (W. Ghats), Jharkhand and Vagamon (Mathew *et al*., 2018). *Nagia sp.* belongs to the family Erebidae and subfamily Lymantriinae (incertae sedis).

Identifying characters: Palpi upturned and reaching vertex of head, the 2nd and 3rd joints conically scaled and tapering to the apex, maxillary palpi filiform; frons rounded; antennae as long as fore wing and minutely ciliated; tibiae with the outer spurs about half the length of inner; abdomen long; fore wing with the costa arched towards apex which is produced, the outer margin obliquely rounded, the inner margin somewhat lobed towards base; veins 3, 4, 5 from angle of cell, 7 anastomosing to 8, 9 for about one-third length; 10 closely approximated to 8, 9; hind wing with the costa arched at middle; the cell short.

Results and discussion

The PCR of the COI gene fragment of *Nagia. Sp.* SJIK28 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 128 - 132. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190661).

Fig. 127. *Nagia. Sp.* SJIK28

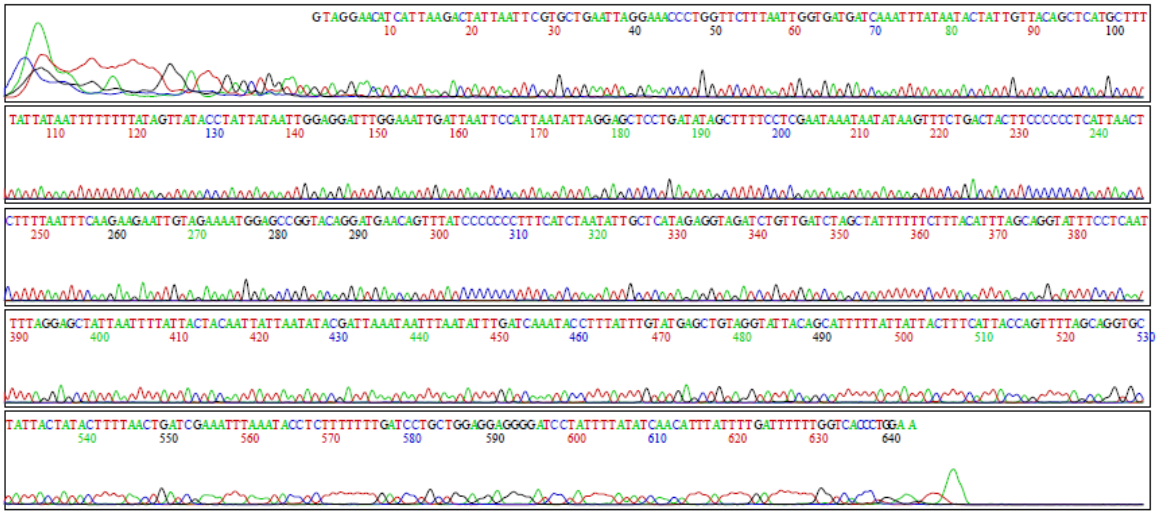

Fig. 128. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *Nagia. Sp.* SJIK28.

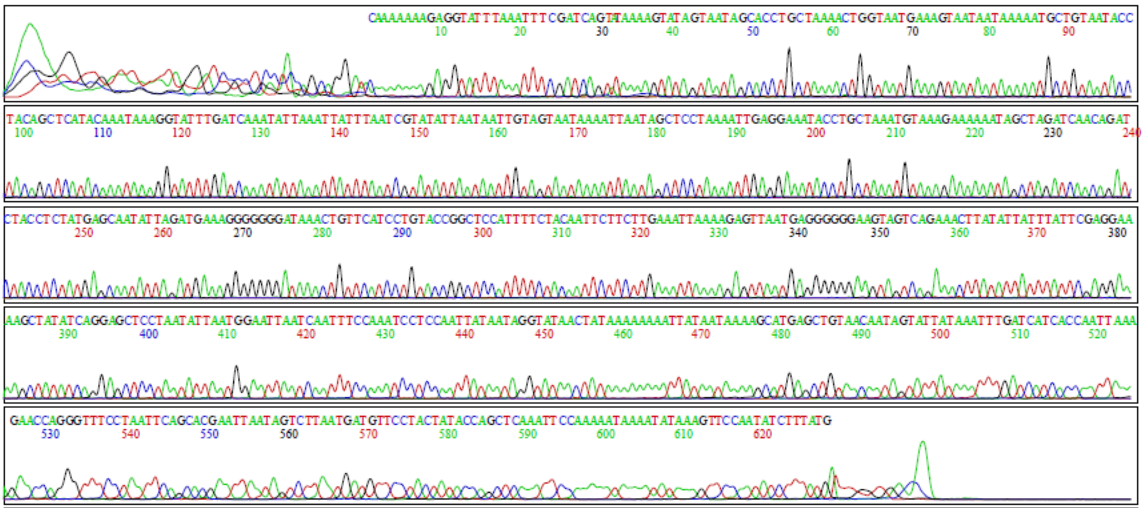

Fig. 129. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *Nagia. Sp.* SJIK28.

> *Nagia. Sp.* Voucher SJIK28 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACTTTATATTTTATTTTTGGAATTTGAGCTGGTATAGTAGGAACATCATTAAGACTATTAATTCGTGCTGAATT
AGGAAACCCTGGTTCTTTAATTGGTGATGATCAAATTTATAATACTATTGTTACAGCTCATGCTTTTATTATAAT
TTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGAAATTGATTAATTCCATTAATATTAGGAGCTCCTGA
TATAGCTTTTCCTCGAATAAATAATATAAGTTTCTGACTACTTCCCCCCTCATTAACTCTTTTAATTTCAAGAAG
AATTGTAGAAAATGGAGCCGGTACAGGATGAACAGTTTATCCCCCCCTTTCATCTAATATTGCTCATAGAGGTAG
ATCTGTTGATCTAGCTATTTTTTCTTTACATTTAGCAGGTATTTCCTCAATTTTAGGAGCTATTAATTTTATTAC
TACAATTATTAATATACGATTAAATAATTTAATATTTGATCAAATACCTTTATTTGTATGAGCTGTAGGTATTAC
AGCATTTTTATTATTACTTTCATTACCAGTTTTAGCAGGTGCTATTACTATACTTTTAACTGATCGAAATTTAAA
TACCTCTTTTTTTGATCCTGCTGGAGGAGGGGATCCTATTTTATATCAACATTTATTT
```

Fig. 130. Partial coding sequence of *Nagia. Sp.* SJIK28 COI gene.

> *Nagia. Sp.* Voucher SJIK28

```
TLYFIFGIWAGMVGTSLSLLIRAELGNPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGG
FGNWLIPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAH
SGSSVDLAIFSLHLAGISSILGAINFITTIINMRLNNLMFDQMPLFVWAVGITAFLLLLSL
PVLAGAITMLLTDRNLNTSFFDPAGGGDPILYQHLF
```

Fig. 131. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *Nagia. Sp.* SJIK28.

The DNA isolated from the sample *Nagia. Sp.* SJIK28 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 22 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

Table 22. The BLAST hit table of the partial coding DNA sequence of COI gene of *Nagia. Sp.* SJIK28.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Quer y end	Geograph. location
1	Nagia linteola HQ949904.1	99.09	658	6	0	1	658	Australia
2	Zanclognatha laevigata KJ377166.1	93.92	658	40	0	1	658	Canada
3	Zanclognatha pedipilalis KJ376680.1	93.92	658	40	0	1	658	Canada
4	Chytolita sp. MF132992.1	93.77	658	41	0	1	658	USA
5	Hemeroblemma sp. GU163280.1	93.77	658	41	0	1	658	Costa Rica
6	Chytolita morbidalis MG364464.1	93.47	658	43	0	1	658	Canada

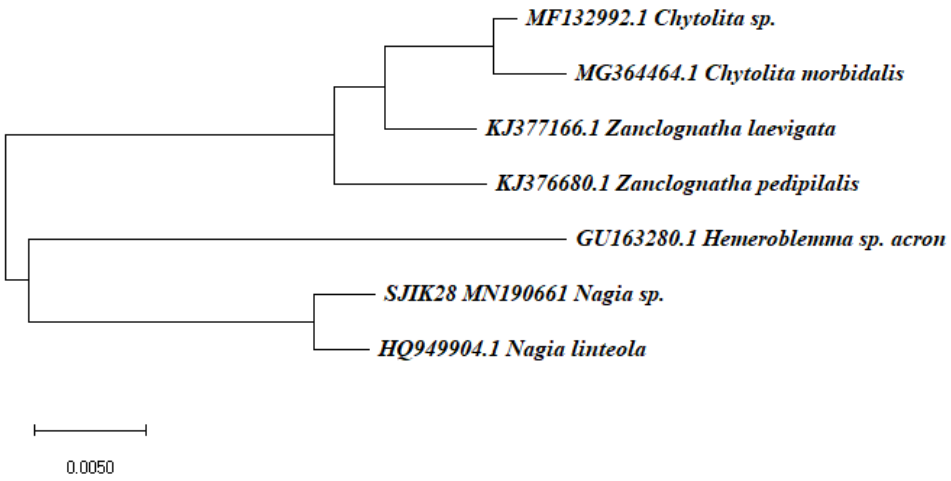

Fig. 132. The NJ tree showing phylogenetic relationships of *Nagia. Sp.* SJIK28.

The BLAST result of the consensus sequence of SJIK28 showed a maximum similarity of 99.09% to *Nagia linteola* HQ949904 from Australia. Hence the sequence is a novel one. The NJ tree shows that the *Nagia sp.* SJIK28 isolated from Kerala and *N. linteola* from Australia are monophyletic occupying the same clade and having a common origin. The genus *Hemeroblemma* in the adjacent clade showing a similarity of 93.77 is the closest related genus belonging to the same family. The divergence from the common ancestor might have occurred about 15000 years ago.

23. *Stenhypena sp.* SJIK29

The specimen SJIK29 was identified as *Stenhypena sp.* (Hampson, 1895) referring to the morphological features described by Hampson, 1895.

Synonyms: *Parhypena* Bethune-Baker, 1908

Consobrambus Berio, 1977

Systematic Position

Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Erebidae; Herminiinae; *Stenhypena*.

Moths of the genus *Stenhypena* are seen in Srilanka. No reports of *Stenhypena sp.* has been from India. This is first record of the species from India. *Stenhypena sp.* belongs to the family Erebidae and subfamily Herminiinae.

Identifying characters: Narrow fore wings, and of almost even width throughout, the outer margin nearly erect; areole very small, vein 10 given off far beyond it; raised specks in and at end of cell; hind wing with veins 3, 4 and 6, 7 stalked; palpi with the second joint of moderate length and fringed with hair above; the third upturned and hairy, with the apex naked; head, thorax and fore wing ochreous brown, suffused and irrorated with fuscous; hind wing and abdomen pale fuscous.

Results and discussion

The PCR of the COI gene fragment of *Stenhypena sp.* SJIK29 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 134- 138. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190663).

The DNA isolated from the sample *Stenhypena sp.* SJIK29 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 23 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

Fig. 133. *Stenhypena sp.* SJIK29

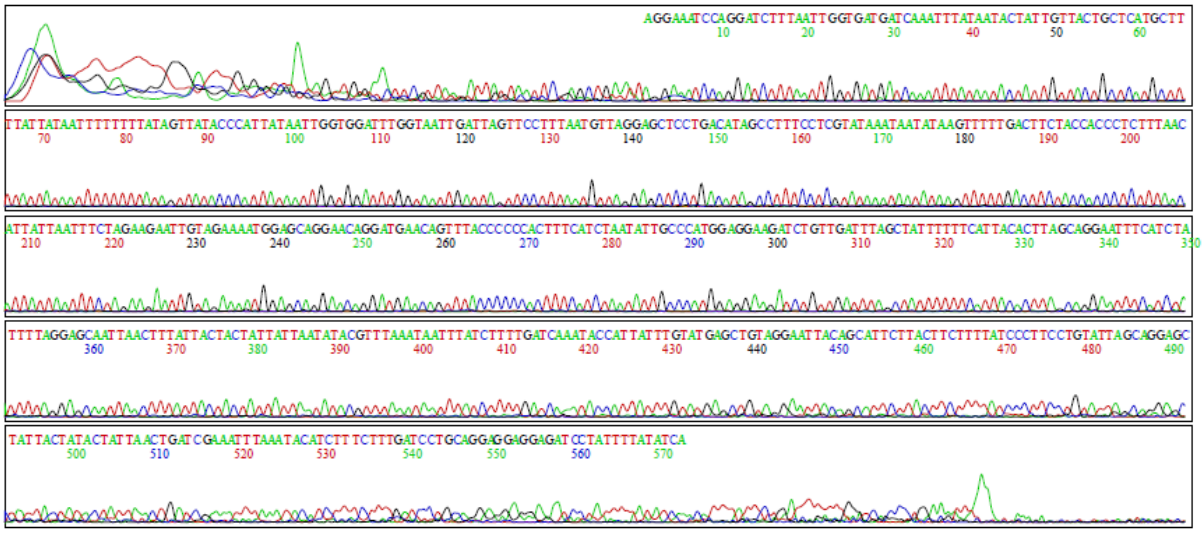

Fig. 134. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *Stenhypena sp.* SJIK29.

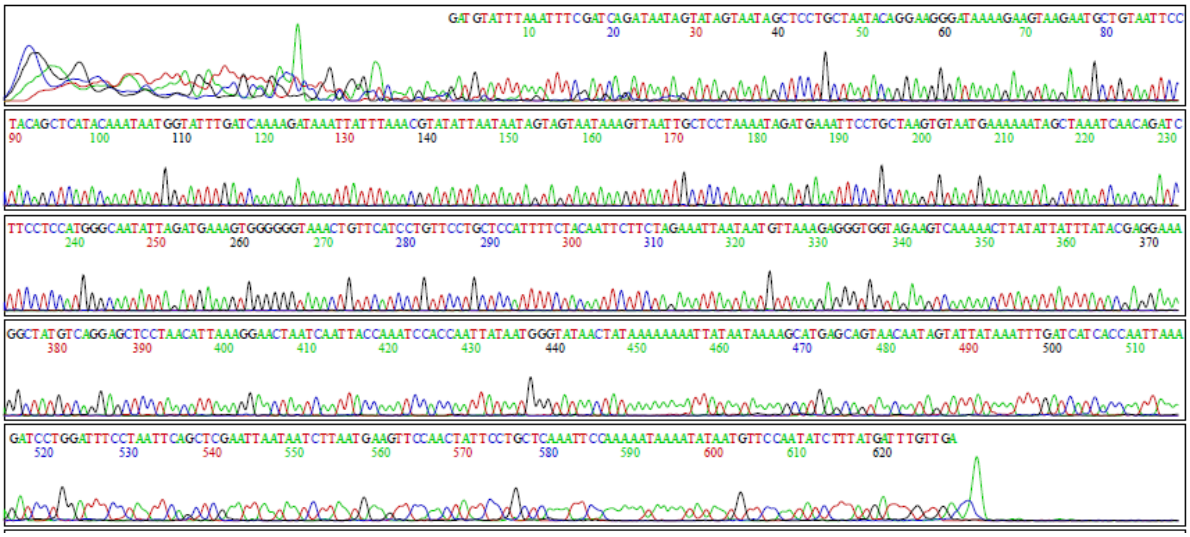

Fig. 135. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *Stenhypena sp.* SJIK29.

> *Stenhypena sp.* Voucher SJIK29 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACATTATATTTTATTTTTGGAATTTGAGCAGGAATAGTTGGAACTTCATTAAGATTATTAATTCGAGCTGA
ATTAGGAAATCCAGGATCTTTAATTGGTGATGATCAAATTTATAATACTATTGTTACTGCTCATGCTTTTAT
TATAATTTTTTTTTATAGTTATACCCATTATAATTGGTGGATTTGGTAATTGATTAGTTCCTTTAATGTTAGG
AGCTCCTGACATAGCCTTTCCTCGTATAAATAATATAAGTTTTTGACTTCTACCACCCTCTTTAACATTATT
AATTTCTAGAAGAATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCCACTTTCATCTAATAT
TGCCCATGGAGGAAGATCTGTTGATTTAGCTATTTTTTCATTACACTTAGCAGGAATTTCATCTATTTTAGG
AGCAATTAACTTTATTACTACTATTATTAATATACGTTTAAATAATTTATCTTTTGATCAAATACCATTATT
TGTATGAGCTGTAGGAATTACAGCATTCTTACTTCTTTTATCCCTTCCTGTATTAGCAGGAGCTATTACTAT
ACTATTAWCTGATCGAAATTTAAATACATCTTTCTTTGATCCTGCAGGAGGAGGAGATCCTATTTTATATCA
ACATTTATTC
```

Fig. 136. Partial coding sequence of *Stenhypena sp.* SJIK29 COI gene.

> *Stenhypena sp.* Voucher SJIK29

```
TLYFIFGIWAGMVGTSLSLLIRAELGNPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWL
VPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHGGSSVDLAIF
SLHLAGISSILGAINFITTIINMRLNNLSFDQMPLFVWAVGITAFLLLLSLPVLAGAITMLLXDRN
LNTSFFDPAGGGDPILYQHLF
```

Fig. 137. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *Stenhypena sp.* SJIK29.

Table 23. The BLAST hit table of the partial coding DNA sequence of COI gene of *Stenhypena sp.* SJIK29.

SN	Subject IDs	% Identity	Align. length	Mismatch	Gap opens	Query start	Query end	Geogra. location
1	*Stenhypena albopunctata HQ921579.1*	93.15	657	45	0	1	657	Australia
2	*Stenhypena albopunctata HQ921580.1*	93.00	657	46	0	1	657	Australia
3	*Lacinipolia cuneata KJ383303.1*	93.01	658	44	2	1	657	Canada
4	*Chytolita petrealis MF132651.1*	92.86	658	45	2	1	657	USA
5	*Chytolita morbidalis KJ375711.1*	92.86	658	45	2	1	657	Canada
6	*Zanclognatha laevigata MF132347.1*	92.71	658	46	2	1	657	USA
7	*Zanclognatha pedipilalis KJ376680.1*	92.55	658	47	2	1	657	Canada
8	*Rejectaria niciasalis JN807206.1*	92.54	657	49	0	1	657	Costa Rica

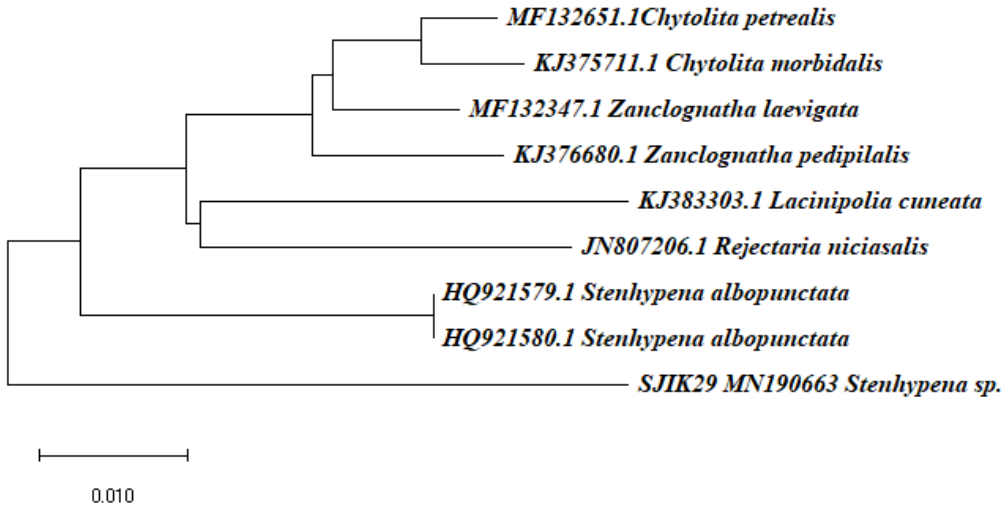

Fig. 138. The NJ tree showing phylogenetic relationships of *Stenhypena sp.* SJIK29.

The nucleotide blast analysis of COI sequence of SJIK29 in the database showed 93.15% similarity to *S. albopunctata* HQ921579 and 93% similarity to HQ921580 from Australia. Hence SJIK29 isolate from Kerala is a novel species. The NJ tree shows that SJIK29 is placed in a separate clade showing the novelty of the sequence. The two species of *Stenhypena*, viz., *S. albopunctata* from Australia are in the adjacent clade. The distance data of the phylogenetic tree reveals that the species diverged from its closely related species about 40000 years ago. The NJ tree also shows that the genus closest to SJIK29 species is genus *Rejectaria* viz., *Rejectaria niciasalis* JN807206.1 (92.54%) from Costa Rica. One novel bp of COI is added to the database. *Stenhypena sp.* is being reported for the first time from India.

24. *Ceryx sp.* SJIK33

The specimen SJIK33 was identified as *Ceryx sp.* (Wallengren, 1863) referring to the morphological features described by Hampson, 1892.

Synonyms: *Agaphthora* Meyrick, 1886

Syntomoides Hampson, 1892

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Erebidae; Arctiinae; *Ceryx*.

Ceryx sp. is seen in India, Malaysia, Indonesia, Sri Lanka, Myanmar, Malacca, Sumatra, Australia, South and West Africa. In India it is reported from Assam, Meghalaya, Sikkim, West Bengal (Sunderbans), Kashmir, Himachal Pradesh, Maharashtra, Tamil Nadu, Himalayas and Andaman Islands (Shah *et al*., 2018). *Ceryx sp.* is known as orange spotted tiger moth. It is a pest of mulberry and sorghum sp.

Identifying characters: proboscis well-developed; antennae filiform, in males shortly ciliated; labial palpi short, porrect and loosely scaled and not extending beyond frons; spurs very short; fore wings with vein 5 curved; 6 from, or from below upper angle; 7, 8, 9, 10, 11 stalked; hind wing with vein 2 from well before angle of cell; 3, 4 and 7 absent; mid and hind tibia each with a minute terminal pair of spurs, hind tibia rarely with two pairs; thorax smoothly scaled below; meta thorax with a yellow streak; abdomen with the first yellow band sometimes obsolescent; fore wing with large hyaline patches.

Results and discussion

The PCR of the COI gene fragment of *Ceryx sp.* SJIK33 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 140- 144. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190666).

The DNA isolated from the sample *Ceryx sp.* SJIK33 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 24 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

Fig. 139. *Ceryx sp.* SJIK33 (dorsal and ventral view)

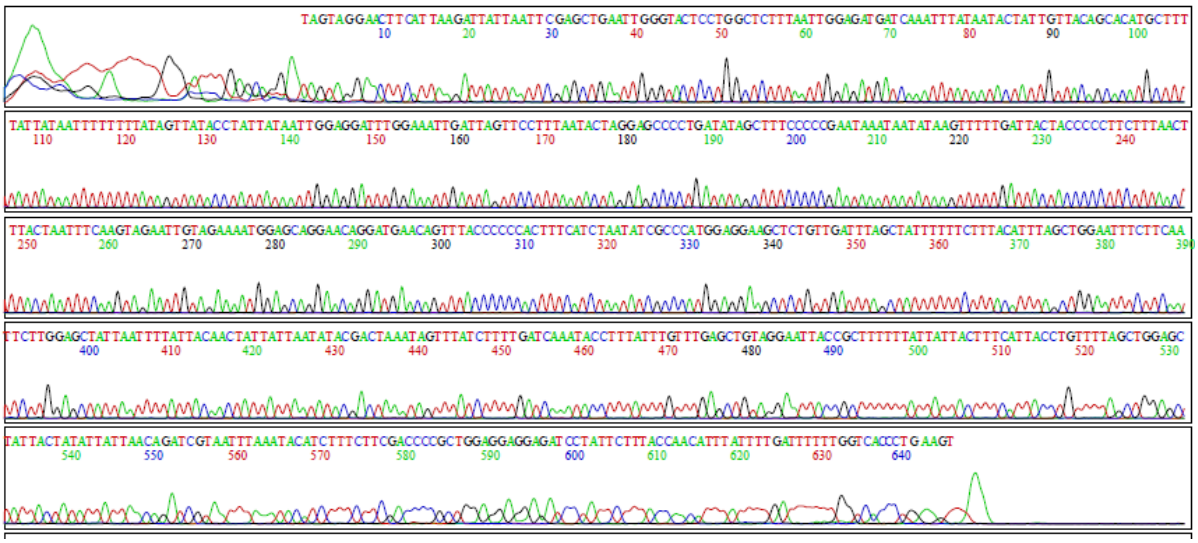

Fig. 140. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *Ceryx sp.* SJIK33.

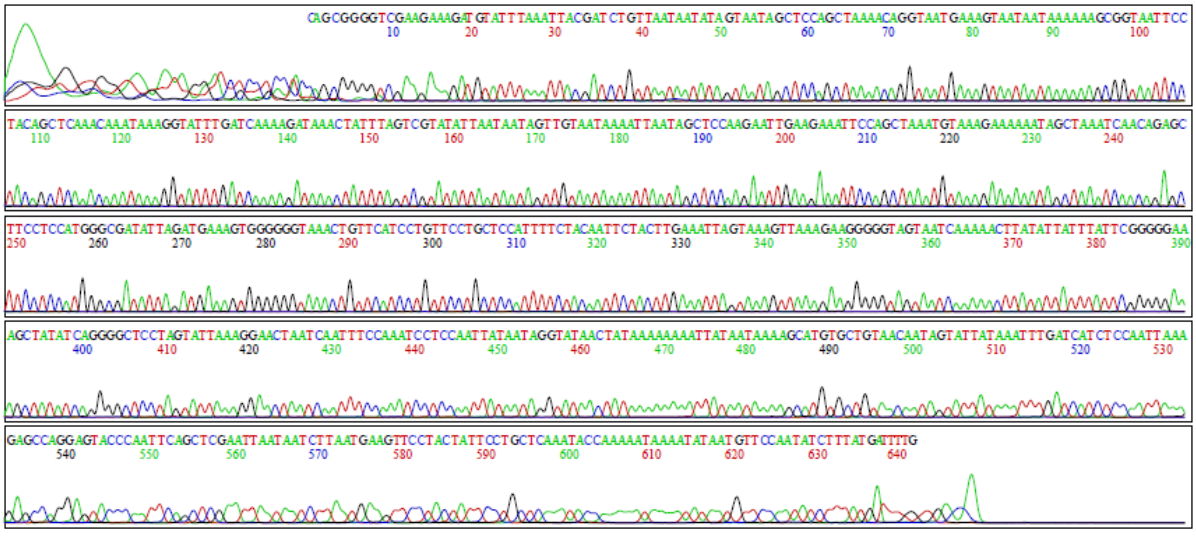

Fig. 141. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *Ceryx sp.* SJIK33.

> *Ceryx sp.* Voucher SJIK33 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACATTATATTTTATTTTTGGTATTTGAGCAGGAATAGTAGGAACTTCATTAAGATTATTAATTCGAGCTG
AATTGGGTACTCCTGGCTCTTTAATTGGAGATGATCAAATTTATAATACTATTGTTACAGCACATGCTTTT
ATTATAATTTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATACT
AGGAGCCCCTGATATAGCTTTCCCCCGAATAAATAATATAAGTTTTTGATTACTACCCCCTTCTTTAACTT
TACTAATTTCAAGTAGAATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCCACTTTCATCT
AATATCGCCCATGGAGGAAGCTCTGTTGATTTAGCTATTTTTTCTTTACATTTAGCTGGAATTTCTTCAAT
TCTTGGAGCTATTAATTTTATTACAACTATTATTAATATACGACTAAATAGTTTATCTTTTGATCAAATAC
CTTTATTTGTTTGAGCTGTAGGAATTACCGCTTTTTTATTATTACTTTCATTACCTGTTTTAGCTGGAGCT
ATTACTATATTATTAACAGATCGTAATTTAAATACATCTTTCTTCGACCCCGCTGGAGGAGGAGATCCTAT
TCTTTACCAACATTTATTT
```

Fig. 142. Partial coding sequence of *Ceryx sp.* SJIK33 COI gene.

> *Ceryx sp.* Voucher SJIK33

```
TLYFIFGIWAGMVGTSLSLLIRAELGTPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWLVP
LMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHGGSSVDLAIFSLHL
AGISSILGAINFITTIINMRLNSLSFDQMPLFVWAVGITAFLLLLSLPVLAGAITMLLTDRNLNTSFF
DPAGGGDPILYQHLF
```

Fig. 143. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *Ceryx sp.* SJIK33.

Table 24. The BLAST hit table of the partial coding DNA sequence of COI gene of C*eryx sp.* SJIK33.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Quer y end	Geograph. location
1	Amata sp. JF858087.1	99.70	658	2	0	1	658	Pakistan
2	Amata sp. JF858088.1	99.54	658	3	0	1	658	Pakistan
3	Amata sp. HM377803.1	99.09	658	6	0	1	658	Taiwan
4	Ceryx guttulosa MG250707.1	99.51	612	3	0	47	658	India
5	Ceryx transitiva HM377802.1	94.07	658	39	0	1	658	Malaysia
6	Ceryx guttulosa HQ921333.1	93.92	658	40	0	1	658	Australia
7	Amata sp. MF804552.1	93.77	658	41	0	1	658	Myanmar
8	Ceryx guttulosa HQ921332.1	93.77	658	41	0	1	658	Australia
9	Ceryx sphenodes HQ921330.1	93.62	658	42	0	1	658	Australia
10	Ceryx sphenodes HQ921331.1	93.47	658	43	0	1	658	Australia
11	Melese sixola JQ534177.1	93.47	659	41	2	1	658	Costa Rica

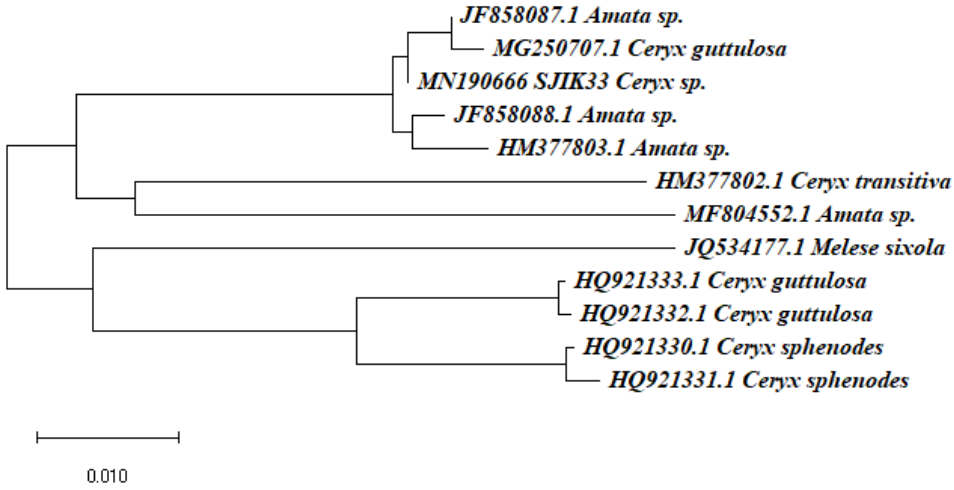

Fig. 144. The NJ tree showing phylogenetic relationships of C*eryx sp.* SJIK33.

The BLAST results of *Ceryx sp.* SJIK33 from Kerala showed 99.51% similarity to *Ceryx guttulosa* MG250707 from India. It is a polymorphic novel variant of SJIK33. It is placed in the adjacent clade separately. It showed 3 nucleotide differences (T for C, A for G and T for C). *Ceryx transitiva* HM377802 from Malaysia with 94.07% similarity, placed in the adjoining clade is a different species of the genus which remain close to SJIK33. The NJ tree distance data revealed that the species originated from its closely related species *Ceryx transitiva* about 20000 years ago. The NJ tree shows that SJIK33 has close relationship with genus *Amata* JF858088 (99.54%) and JF858087 (99.70%) from Pakistan which is in the adjacent clade. They share a common ancestor and the distribution pattern also points to the common origin from the erstwhile Gondwana. 46 novel bp of COI were added to the database.

25. *Hippotion boerhaviae* SJIK2

The specimen SJIK2 was identified as *Hippotion boerhaviae* Fabricius, 1775 referring to the morphological features described by Bell & Scott, 1937.

Synonyms: *Sphinx boerhaviae* Fabricius, 1775
Sphynx vampyrus Fabricius, 1787
Sphinx octopunctata Gmelin, 1790

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Bombycoidea; Sphingidae; Macroglossinae; Macroglossini; *Hippotion*.

Hippotion boerhaviae is found across India, Sri Lanka, Pakistan, Nepal, Thailand, Indonesia, Australia and Solomon Islands. In India it has been reported from Kerala (Palakkad), Jharkhand, Andhra Pradesh, North Karnataka, Maharashtra, Madhya Pradesh, Nicobar Islands, Gujarat, Orissa, West Bengal and Himalayas (Shah *et al*., 2018). *Hippotion boerhaviae*, the pale striated hawk moth, belongs to the family Sphingidae. It is a pest of colocasia, yam and grape wine. The food-plants belong to *Geraniaceae*, *Nyctaginaceae*, *Rubiaceae*, *Scrophulariaceae*, etc.

Identifying characters: First segment of palpus paler; a clayish sub anal patch; apical hook of the sternite long; process of harpe stout, rounded at end, with a long dorso-apical tooth curved towards the clasper; penis-funnel elongated and triangular; adults have striped brown forewings; hind wings are red with dark outer margins and pale brown hind margins. The wing span is nearly 6 cm. Under side of the abdomen has a narrow, pale median stripe; juxta are short in males.

Results and discussion

The PCR of the COI gene fragment of *H. borhaviae* SJIK2 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree, are presented in Figures 146-150. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190674).

Fig. 145. *Hippotion borhaviae* SJIK2 (dorsal view)

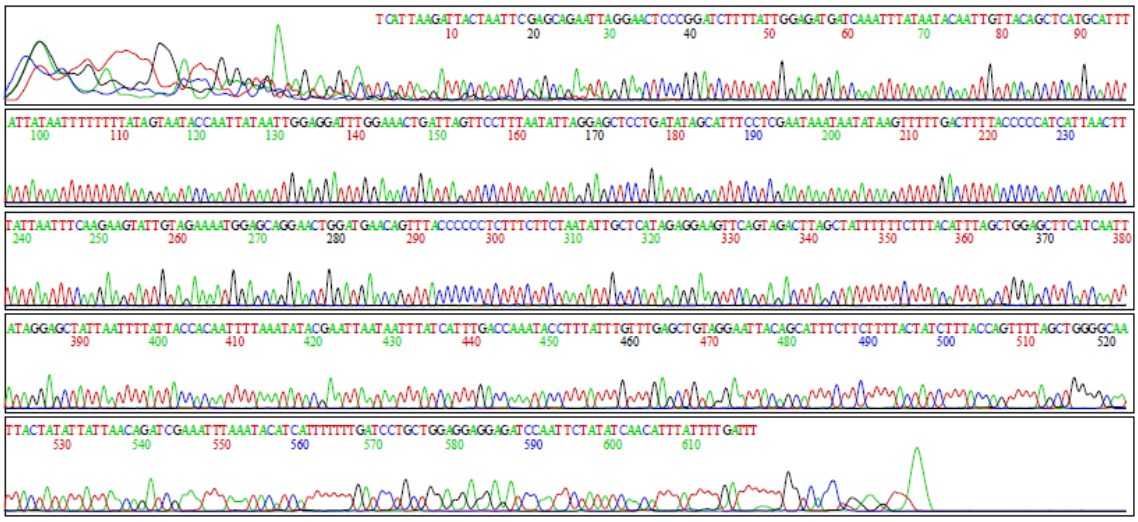

Fig. 146. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *H. borhaviae* SJIK2.

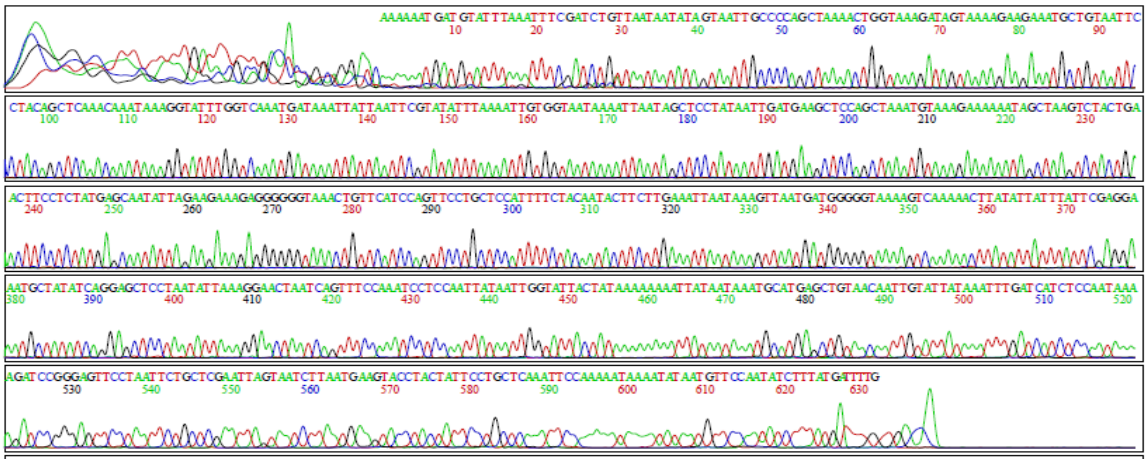

Fig. 147. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *H. borhaviae* SJIK2.

> *H. borhaviae* Voucher SJIK2 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACATTATATTTTATTTTTGGAATTTGAGCAGGAATAGTAGGTACTTCATTAAGATTACTAATTCGAGCAG
AATTAGGAACTCCCGGATCTTTTATTGGAGATGATCAAATTTATAATACAATTGTTACAGCTCATGCATTT
ATTATAATTTTTTTTTATAGTAATACCAATTATAATTGGAGGATTTGGAAACTGATTAGTTCCTTTAATATT
AGGAGCTCCTGATATAGCATTTCCTCGAATAAATAATATAAGTTTTTGACTTTTACCCCCATCATTAACTT
TATTAATTTCAAGAAGTATTGTAGAAAATGGAGCAGGAACTGGATGAACAGTTTACCCCCCTCTTTCTTCT
AATATTGCTCATAGAGGAAGTTCAGTAGACTTAGCTATTTTTTCTTTACATTTAGCTGGAGCTTCATCAAT
TATAGGAGCTATTAATTTTATTACCACAATTTTAAATATACGAATTAATAATTTATCATTTGACCAAATAC
CTTTATTTGTTTGAGCTGTAGGAATTACAGCATTTCTTCTTTTACTATCTTTACCAGTTTTAGCTGGGGCA
ATTACTATATTATTAACAGATCGAAATTTAAATACATCATTTTTTGATCCTGCTGGAGGAGGAGATCCAAT
TCTATATCAACATTTATTT
```

Fig. 148. Partial coding sequence of *H. borhaviae* SJIK2 COI gene.

> *H. borhaviae* Voucher SJIK2

```
TLYFIFGIWAGMVGTSLSLLIRAELGTPGSFIGDDQIYNTIVTAHAFIMIFFM
VMPIMIGGFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGA
GTGWTVYPPLSSNIAHSGSSVDLAIFSLHLAGASSIMGAINFITTILNMRINN
LSFDQMPLFVWAVGITAFLLLLSLPVLAGAITMLLTDRNLNTSFFDPAGGGDP
ILYQHLF
```

Fig. 149. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *H. borhaviae* SJIK2.

The DNA isolated from the sample *H. borhaviae* SJIK2 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 25 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

The BLAST result of *H. boerhaviae* SJIK2 isolated from Kerala showed 100% similarity to *H. boerhaviae,* GU704520 and KJ168258 from Maharashtra. Hence it can be used as barcode for species identification. SJIK2 showed 99.85% similarity to *H. boerhaviae* JN281154 from India.It showed single nucleotide difference (G changed to A). SJIK2 showed 99.7% similarity to GU704518, and 99.67% to MF882908 from India, placed in the adjacent clade sharing a common ancestor and 99.16% to KJ380863 from Western Ghats. *H. rosetta* MG783972 from India showed a close similarity of 99.85% which is a different species of the genus.

Table 25. The BLAST hit table of the partial coding DNA sequence of COI gene of *H. borhaviae* SJIK2.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Query End	Geographical location
1	Hippotion boerhaviae GU704520.1	100	658	0	0	1	658	India
2	Hippotion rosetta MG783972.1	99.85	658	1	0	1	658	India, Maharashtra
3	Hippotion boerhaviae JN281154.1	99.85	658	1	0	1	658	India
4	Hippotion boerhaviae GU704518.1	99.70	658	2	0	1	658	India
5	Hippotion boerhaviae JN281151.1	99.24	658	5	0	1	658	Indonesia
6	Hippotion echeclus JN678020.1	97.72	658	15	0	1	658	Philippines
7	Hippotion boerhaviae KJ168258.1	100	603	0	0	7	609	India, Maharashtra
8	Hippotion boerhaviae MF882908.1	99.67	603	2	0	5	607	India
9	Hippotion brennus KJ168437.1	96.81	658	21	0	1	658	Indonesia
10	Hippotion brunnea JN678016.1	96.66	658	22	0	1	658	Indonesia
11	Hippotion joiceyi KJ168349.1	96.20	658	25	0	1	658	Papua New Guinea
12	Hippotion boerhaviae KJ380863.1	99.16	593	5	0	35	627	India, Western Ghats
13	Hippotion rafflesii dyokeae JN678031.1	95.90	658	27	0	1	658	Indonesia
14	Hippotion scrofa KJ168776.1	95.59	658	29	0	1	658	Australia
15	Hippotion balsaminae MK187996.1	94.99	658	33	0	1	658	Gabon
16	Hippotion eson MK187645.1	94.68	658	35	0	1	658	Gabon
17	Hippotion celerio MG200178.1	94.53	658	36	0	1	658	India
18	Hippotion rebeli JN678032.1	94.53	658	36	0	1	658	Yemen

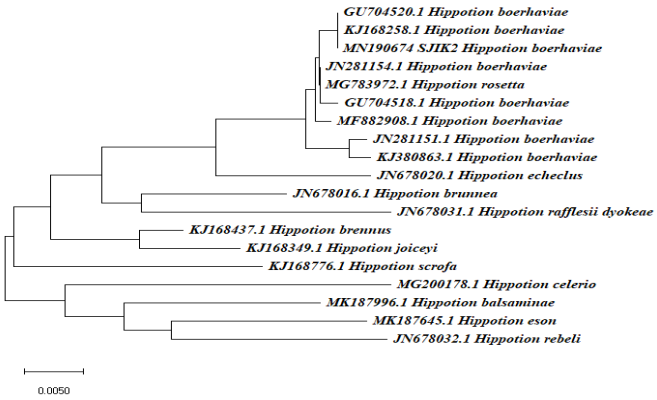

Fig. 150. The NJ tree showing phylogenetic relationships of *H. borhaviae* SJIK2.

The NJ tree shows that *H. boerhaviae* GU704520 and KJ168258, JN281154, GU704518, MF882908 and KJ380863 from India, JN281151 from Indonesia are polymorphic variants of SJIK2. The Kerala species might have diverged from the closely related species *H. echeclus* from Philippines about 15000 years ago. The phylogenetic tree shows that the closest species is *H. rosetta* and the most distant relative is *H. rebeli*. It clearly depicts the common origin of the three species *H. boerhaviae*, *H. rosetta* and *H. echeclus*. The geographical distribution pattern also confirms the common origin of the various *Hippotion* species. It also showed a South East Asian lineage.

26. *Spodoptera litura* (SJIK34)

The specimen SJIK34 was identified as *Spodoptera litura* (Fabricius, 1775) referring to the morphological features described by Hampson, 1909.

Synonyms: *Noctua litura* Fabricius, 1775
Noctua histrionica Fabricius, 1775
Noctua elata Fabricius, 1781
Prodenia ciligera Guenee, 1852
Prodenia tasmanica Guenee, 1852
Prodenia subterminalis Walker, 1856

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Noctuidae; Amphipyrinae; *Spodoptera*.

Spodoptera litura is seen in India, China, Australian, Pacific Islands, Hong Kong and Hawaii. In India it is seen in Jharkhand, South Andhra Pradesh, Kerala (Parambikulam & Palakkad), Maharashtra, West Bengal, and Tamil Nadu (Shubhalaxmi *et al*., 2011, Singh et al., 2018, Harinath *et al*., 2014, Sudheendrakumar, 1999, Bharmal, 2015, Shah *et al*., 2018). *S. litura* is known as cotton leafworm or tobacco cutworm. It belongs to the family Noctuidae and subfamily Amphipyrinae. It is a pest of cotton, maize, ragi, soybean, castor, groundnut, brinjal, colocasia, tomato, crucifers, guava, banana, tobacco, etc.

Identifying characters: Head and thorax whitish suffused with rufous; palpi with blackish marks at sides of joints; frons with brown bar above; tegulae with some brown at base, slight medial line and brown tips; mid tibiae streaked with black; abdomen ochreous tinged with rufous; fore wing ochreous mostly suffused with brown, the medial area below the cell ochreous tinged with rufous; some silvery grey suffusion before ante medial line; claviform elongate, slightly defined by black scales; orbicular narrow, oblique; reniform whitish slightly defined by black and with some brown in centre; a white subterminal line from the fascia to submedian fold, excurved at middle; a fine white line before termen slightly defined by black on outer side; a terminal series of slight black lunules; cilia brown intersected with white and with fine white line at base followed by a brown line; hind wing white, the apex slightly tinged with brown.

Results and discussion

The PCR of the COI gene fragment of *S. litura* SJIK34 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its

Fig. 151. *S. litura* SJIK34 (dorsal and ventral view)

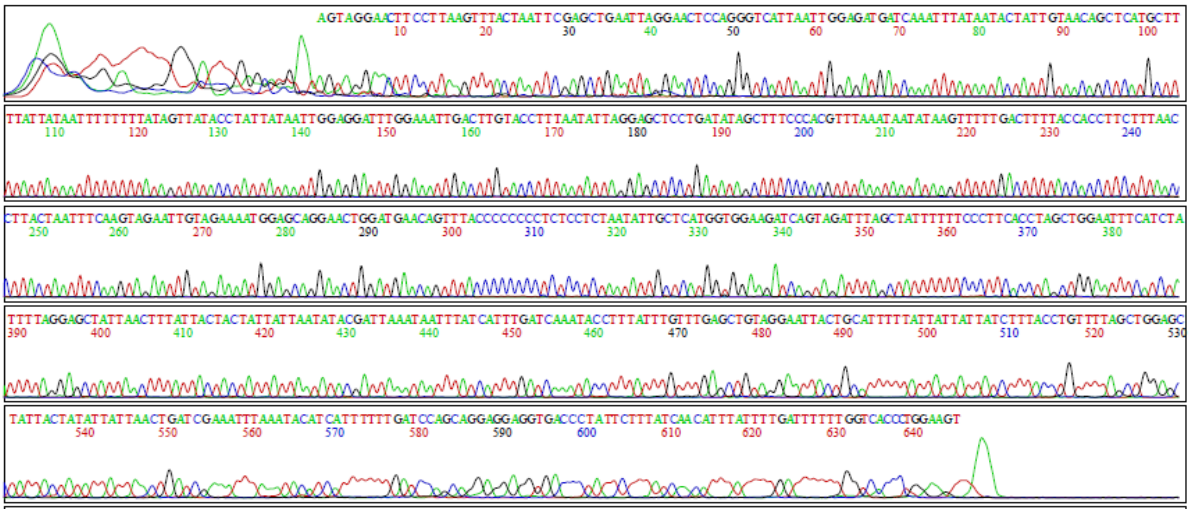

Fig. 152. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *S. litura* SJIK34.

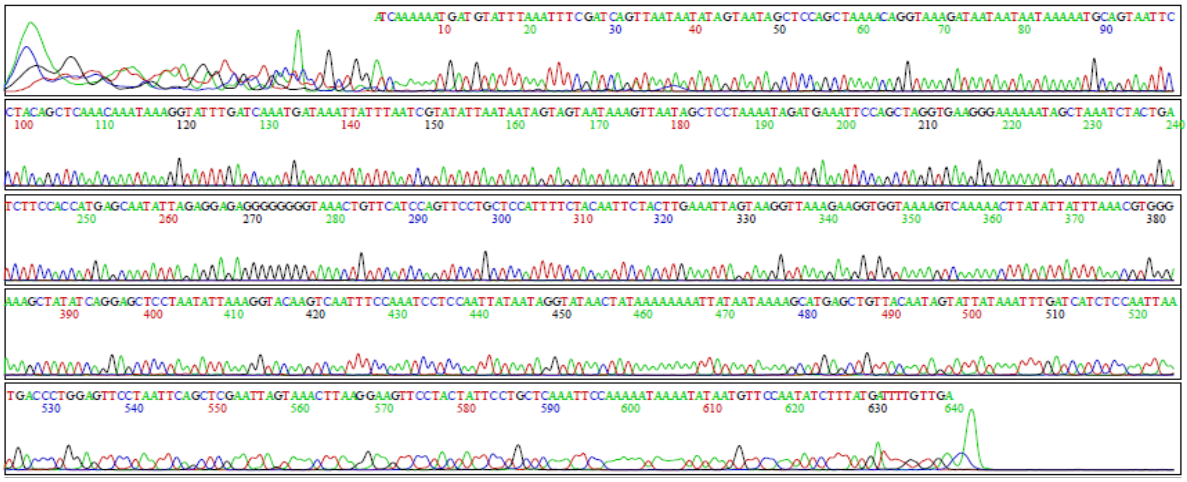

Fig. 153. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *S. litura* SJIK34.

> *S. litura* Voucher SJIK34 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

```
AACATTATATTTTATTTTTGGAATTTGAGCAGGAATAGTAGGAACTTCCTTAAGTTTACTAATTCG
AGCTGAATTAGGAACTCCAGGGTCATTAATTGGAGATGATCAAATTTATAATACTATTGTAACAGC
TCATGCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGAAATTGACT
TGTACCTTTAATATTAGGAGCTCCTGATATAGCTTTCCCACGTTTAAATAATATAAGTTTTTGACT
TTTACCACCTTCTTTAACCTTACTAATTTCAAGTAGAATTGTAGAAAATGGAGCAGGAACTGGATG
AACAGTTTACCCCCCCCTCTCCTCTAATATTGCTCATGGTGGAAGATCAGTAGATTTAGCTATTTT
TTCCCTTCACCTAGCTGGAATTTCATCTATTTTAGGAGCTATTAACTTTATTACTACTATTATTAA
TATACGATTAAATAATTTATCATTTGATCAAATACCTTTATTTGTTTGAGCTGTAGGAATTACTGC
ATTTTTATTATTATTATCTTTACCTGTTTTAGCTGGAGCTATTACTATATTATTAACTGATCGAAA
TTTAAATACATCATTTTTTGATCCAGCAGGAGGAGGTGACCCTATTCTTTATCAACATTTATTT
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Fig. 154. Partial coding sequence of *S. litura* SJIK34 COI gene.

> *S. litura* Voucher SJIK34

```
TLYFIFGIWAGMVGTSLSLLIRAELGTPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIG
GFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNI
AHGGSSVDLAIFSLHLAGISSILGAINFITTIINMRLNNLSFDQMPLFVWAVGITAFLLL
LSLPVLAGAITMLLTDRNLNTSFFDPAGGGDPILYQHLF
```

Fig. 155. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *S. litura* SJIK34.

conceptual translation product and NJ tree are presented in Figures 152 - 156. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190667).

The DNA isolated from the sample *S. litura* SJIK34 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 26 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

The BLAST results showed the 100% similarity of *S. litura* SJIK34 from Kerala to S. litura MG954454, MG954453 and MG954452 from China, KX863232, KX862420, KX861832 and KX 860450 from Pakistan, JQ064568 and JQ064567 from Tamil Nadu and HQ950413 from Australia. Hence the sequence can be used as molecular barcode for species identification. *S. litura* MG954451 from China, KJ940206 and KC864790 from India (Punjab), JQ064566 from Tamil Nadu, HQ990979 from Pakistan and HQ950414 from Australia all with 99.85% similarity and KJ940209 and KC864791from India and GU695453 from Papua New Guinea which showed a similarity of 99.7% are polymorphic

geographical variants of SJIK34. The closest relative from China MG954451 with 99.85% similarity showed single nucleotide change (A changed to T).

Table 26. The BLAST hit table of the partial coding DNA sequence of COI gene of *S. litura* SJIK34.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Query end	Geograph. location
1	*Spodoptera litura* MG954454.1	100	658	0	0	1	658	China
2	*Spodoptera litura* MG954453.1	100	658	0	0	1	658	China
3	*Spodoptera litura* MG954452.1	100	658	0	0	1	658	China
4	*Spodoptera litura* KX863232.1	100	658	0	0	1	658	Pakistan
5	*Spodoptera litura* KX862420.1	100	658	0	0	1	658	Pakistan
6	*Spodoptera litura* KX861832.1	100	658	0	0	1	658	Pakistan
7	*Spodoptera litura* KX860450.1	100	658	0	0	1	658	Pakistan
8	*Spodoptera litura* JQ064568.1	100	658	0	0	1	658	India (TN)
9	*Spodoptera litura* JQ064567.1	100	658	0	0	1	658	India (TN)
10	*Spodoptera litura* HQ950413.1	100	658	0	0	1	658	Australia
11	*Spodoptera litura* MG954451.1	99.85	658	1	0	1	658	China
12	*Spodoptera litura* KJ940206.1	99.85	658	1	0	1	658	India (Punjab)
13	*Spodoptera litura* KC864790.1	99.85	658	1	0	1	658	India (Punjab)
14	*Spodoptera litura* JQ064566.1	99.85	658	1	0	1	658	India (TN)
15	*Spodoptera litura* HQ990979.1	99.85	658	1	0	1	658	Pakistan
16	*Spodoptera litura* HQ950414.1	99.85	658	1	0	1	658	Australia
17	*Spodoptera litura* KJ940209.1	99.70	658	2	0	1	658	India
18	*Spodoptera litura* KC864791.1	99.70	658	2	0	1	658	India (Punjab)
19	*Spodoptera litura* GU695453.1	99.70	658	2	0	1	658	Papua New Guinea
20	*Spodoptera littoralis* KJ634300.1	97.72	658	15	0	1	658	-
21	*Spodoptera picta* HQ950412.1	97.57	658	16	0	1	658	Australia
22	*Spodoptera praefica* HM867882.1	96.96	658	20	0	1	658	Canada

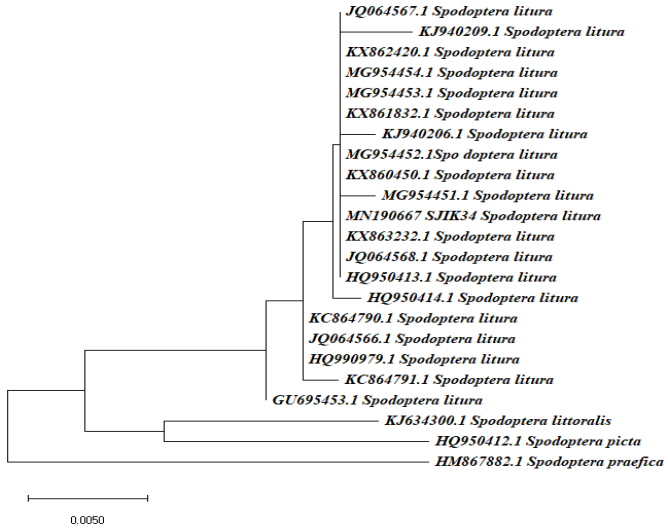

Fig. 156. The NJ tree showing phylogenetic relationships of *S. litura* SJIK34.

S. littoralis KJ634300 with 97.72% similarity is the closest related species. Phylogeny of SJIK34 was derived from the NJ tree developed from the sequences obtained from the blast hit results. *S. litura* SJIK34 was found to be placed in a clade with 14 samples viz., JQ064567, JQ064568, KJ940209, KJ940206 from India, KX862420, KX861832, KX860450, KX863232 from Pakistan, MG954454, MG954453, MG954452, MG954451 from China and HQ950413 and HQ950414 from Australia and were monophyletic having a common ancestor. However, *S. litura* KC864790 from Punjab, JQ064566 from Tamil Nadu, and HQ990979 from Pakistan with 99.85% similarity and KC864791 from Punjab with 99.7% similarity were placed in the adjacent clade. *S. littoralis* KJ634300 with 97.72% similarity, placed in the adjoining clade, is a different species of the genus which remain close to SJIK34. The NJ tree distance data revealed that the species was originated from its closely related species *S. littoralis* about 15000 years ago. *Spodoptera litura*, SJIK34 showed Gondwana origin being distributed in countries China, Pakistan, Australia and Papua New Guinea.

27. *Anticasia irrorata* SJIK36 (Owl moth)

The specimen SJIK36 was identified as *Anticarsia irrorata* (Fabricius, 1781) referring to the morphological features described by Hampson, 1894.

Synonyms: *Noctua irrorata* Fabricius, 1781
Noctua sordida Fabricius, 1794
Ophiusa rubricans Boisduval, 1833
Thermesia transducta Walker, 1865
Thermesia consueta Walker, 1869

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Holometabola; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Noctuidae; Ophiderinae; *Anticarsia*.

Anticarsia irrorata is seen in Africa, Madagascar, India, China, Japan, Hong Kong, Java and Pacific Island. In India it is reported from North Maharashtra, Jharkhand and Tamil Nadu (Singh et al., 2018). *Anticarsia irrorata* belongs to the family Noctuidae and subfamily Ophiderinae. It is also known as owl moth. It is a minor pest of leguminous plants like *Cicer*, *Phaseolus*, lablab, cowpea, etc.

Identifying characters: Head, thorax, abdomen and wings are rufous or grey-brown; palpi chestnut; fore wing with indistinct sub-basal curved line; a white speck in cell; the reniform very large, with two dark specks on it; a post-medial rufous line, very highly angled below the costa and joined by a dark apical streak; a sub-marginal series of dark specks; a rufous marginal line. Hind wing with rufous medial line, post-medial series of specks and arginal rufous line; underside much suffused with red; a white spot at end of cell, curved post-medial line and sub-marginal series of black and white lunules.

Results and discussion

The PCR of the COI gene fragment of *A. irrorata* SJIK36 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 158- 162. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190669).

Fig. 157. *A. irrorta* SJIK36

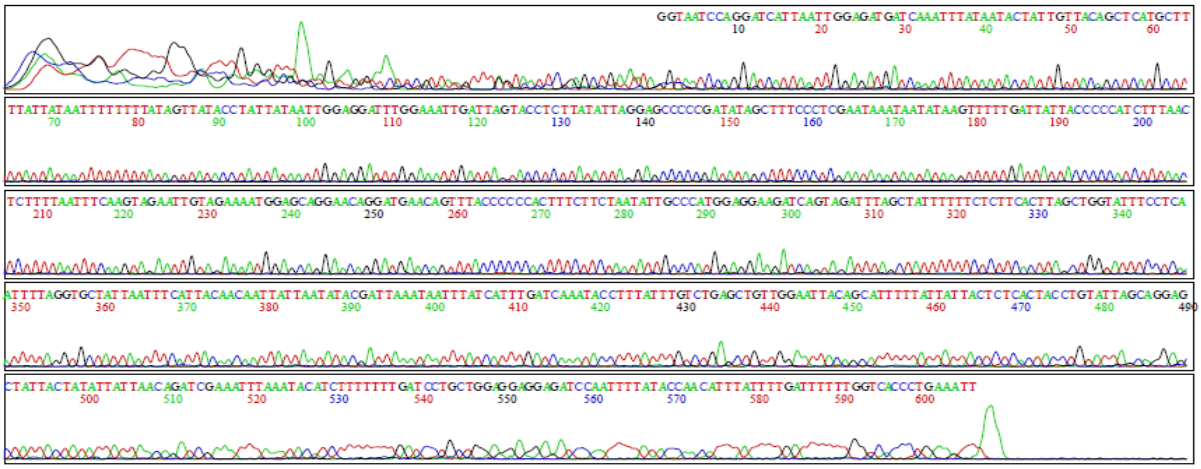

Fig. 158. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *A. irrorata* SJIK36.

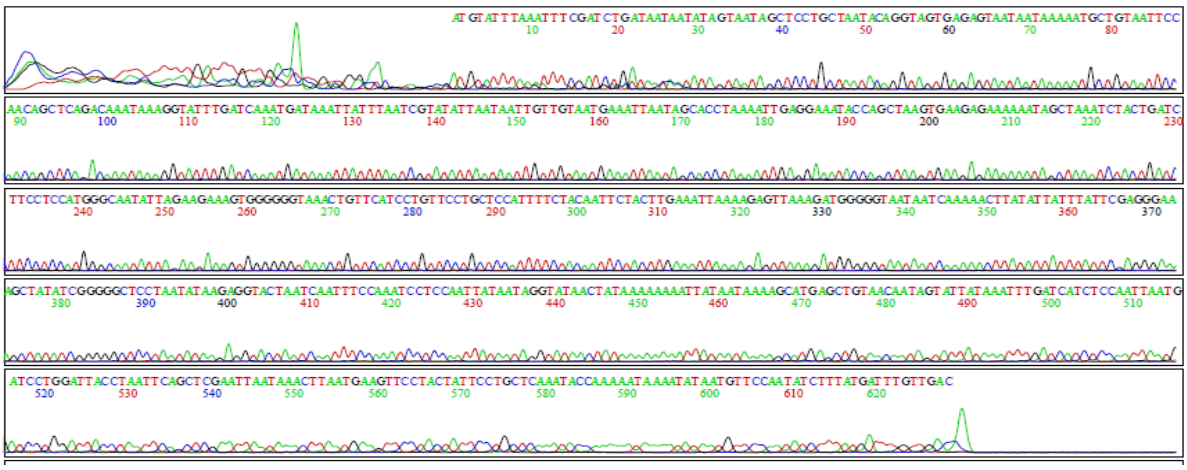

Fig. 159. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *A. irrorata* SJIK36.

> *A. irrorata* Voucher SJIK36 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

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AACATTATATTTTATTTTTGGTATTTGAGCAGGAATAGTAGGAACTTCATTAAGTTTATTAATTCGAGCTGAATTAGGTAA
TCCAGGATCATTAATTGGAGATGATCAAATTTATAATACTATTGTTACAGCTCATGCTTTTATTATAATTTTTTTTATAGT
TATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTACCTCTTATATTAGGAGCCCCCGATATAGCTTTCCCTCGAAT
AAATAATATAAGTTTTTGATTATTACCCCCATCTTTAACTCTTTTAATTTCAAGTAGAATTGTAGAAAATGGAGCAGGAAC
AGGATGAACAGTTTACCCCCCACTTTCTTCTAATATTGCCCATGGAGGAAGATCAGTAGATTTAGCTATTTTTTCTCTTCA
CTTAGCTGGTATTTCCTCAATTTTAGGTGCTATTAATTTCATTACAACAATTATTAATATACGATTAAATAATTTATCATT
TGATCAAATACCTTTATTTGTCTGAGCTGTTGGAATTACAGCATTTTTATTATTACTCTCACTACCTGTATTAGCAGGAGC
TATTACTATATTATTAACAGATCGAAATTTAAATACATCTTTTTTTGATCCTGCTGGAGGAGGAGATCCAATTTTATACCA
ACATTTATTT
```

Fig. 160. Partial coding sequence of *A. irrorata* SJIK36 COI gene.

> *A. irrorata* Voucher SJIK36

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TLYFIFGIWAGMVGTSLSLLIRAELGNPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIG
GFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNI
AHGGSSVDLAIFSLHLAGISSILGAINFITTIINMRLNNLSFDQMPLFVWAVGITAFLLL
LSLPVLAGAITMLLTDRNLNTSFFDPAGGGDPILYQHLF
```

Fig. 161. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *A. irrorata* SJIK36.

The DNA isolated from the sample *A. irrorata* SJIK36 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 27 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

Table 27. The BLAST hit table of the partial coding DNA sequence of COI gene of *A. irrorata* SJIK36.

S. No.	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Query end	Geograph. location
1	Anticarsia irrorata GU696094.1	100	658	0	0	1	658	Congo
2	Anticarsia irrorata JN401300.1	99.70	658	2	0	1	658	-
3	Mormoscopa sordescens HQ921557.1	94.37	657	37	0	2	658	Australia
4	Epitausa prona MF132774.1	94.23	658	38	0	1	658	Mexico
5	Ormetica ataenia JQ557229.1	94.07	658	39	0	1	658	Costa Rica
6	Ormetica iheringi KX300300.1	93.92	658	40	0	1	658	N.A.
7	Eois ambarilla KU380809.1	93.78	659	39	2	1	658	Ecuador
8	Idia lubricalis MF133118.1	93.77	658	41	0	1	658	USA
9	Idalus critheis HQ553485.1	93.77	658	41	0	1	658	Panama
10	Lithilaria anomozancla HQ921532.1	93.78	659	39	2	1	658	Australia

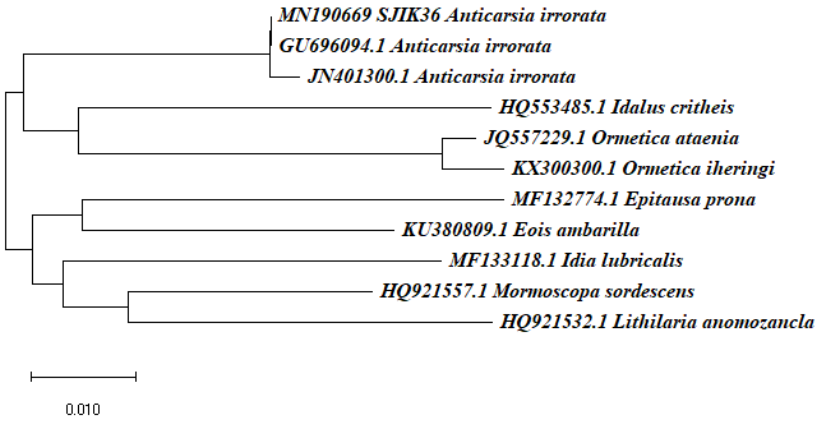

Fig. 162. The NJ tree showing phylogenetic relationships of *A. irrorata* SJIK36.

The *Anticarsia irrorata* SJIK36 iosalted from Kerala was 100% similar to *A. irrorata* from Congo GU696094 and hence can be used as molecular barcode for species identification. It showed 99.7% similarity to *A. irrorata* JN401300 in the database. It is a polymorphic variant of SJIK36 showing single nucleotide difference (T changed to C). The NJ tree shows that the three *Anticarsia sp.* are monophyletic, occupying the same clade and sharing a common ancestor from Africa.

28. *Psilogramma increta* SJIK40

The specimen SJIK40 was identified as *Psilogramma increta* **(**Walker, 1865) referring to the morphological features described by Walker 1865.

Synonyms: *Anceryx increta* Walker, 1865
Sphinx strobi Boisduval, 1868
Sphinx abietina Boisduval, 1875

Systematic Position

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Bombycoidea; Sphingidae; Sphinginae; Sphingini.

P. increta is found in India, Sri Lanka, Myanmar, Nepal, China, Thailand, Vietnam, Malaysia, Korea, Taiwan and Japan. In India it is seen in Jammu & Kashmir, Uttarakhand, Assam and Mahrashtra (Shubhalaxmi *et al*., 2011). *P. increta* belongs to the family Sphingidae and subfamily Sphinginae. It is known as the plain grey hawk moth. It is a pest of some ornamental trees. The larvae mostly feed on *Oleaceae*, *Scrophulariaceae* and *Verbenaceae* species.

Identifying characters: antennae short, hook short; the second segment of the palpus having a naked stripe over the inner surface; labrum very little raised in the middle; first segment of fore tarsus somewhat longer than segments 2 to 4 together; comb of mid-tarsus well developed; long spur of mid-tibia about half, the long apical one of hind tarsus nearly two-thirds the length of the respective first tarsal segment; pulvillus and paronychium present; in males clasper with patch of modified scales, the scales large, rounded, entire, multi-striate; harpe vestigial; process of penis-sheath short, forked; in females antenna sub-cylindrical, cilia not prolonged.

Results and discussion

The PCR of the COI gene fragment of *P. increta* SJIK40 from Kerala yielded a product of 658 bp. The forward and reverse sequence chromatograms, COI gene sequence obtained, its conceptual translation product and NJ tree are presented in Figures 164- 168. The sequence obtained is deposited in the GenBank (GenBank Accession Number: MN190670).

Fig. 163. *Psilogramma increta* SJIK40

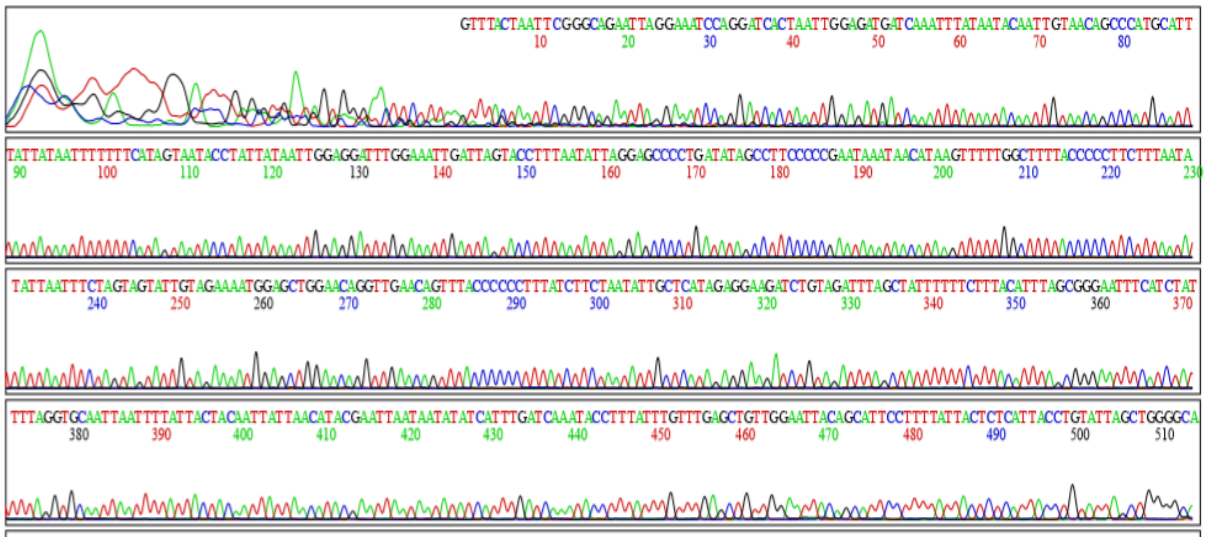

Fig. 164. Sequencing chromatogram (forward sequence) showing partial coding sequence of COI gene of *P. increta* SJIK40.

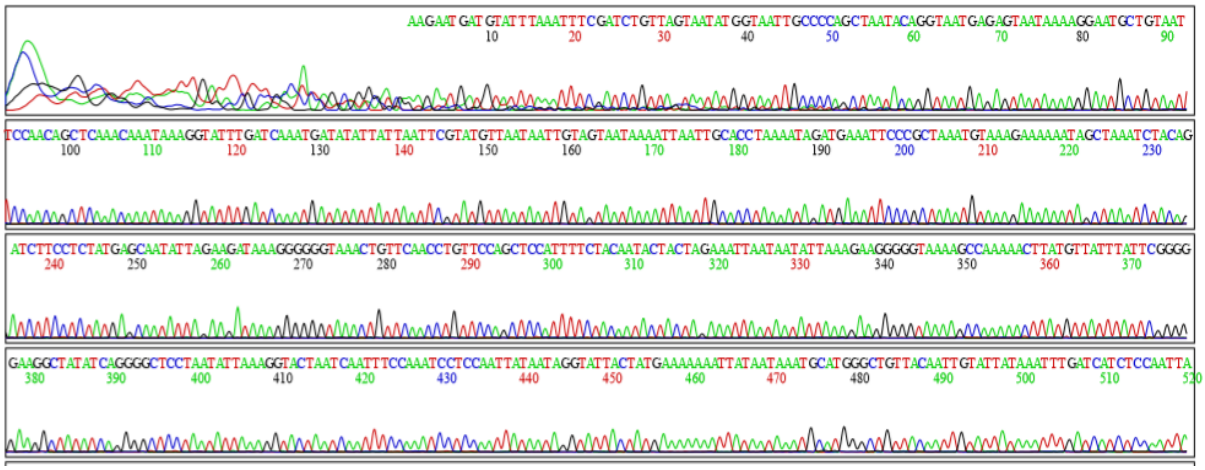

Fig. 165. Sequencing chromatogram (reverse sequence) showing partial coding sequence of COI gene of *P. increta* SJIK40.

> *P. increta* Voucher SJIK40 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 658 bases.

AACATTATATTTTATTTTTGGAATTTGAGCAGGAATAGTGGGAACTTCTTTAAGTTTACTAATTCGGGCAGAA
TTAGGAAATCCAGGATCACTAATTGGAGATGATCAAATTTATAATACAATTGTAACAGCCCATGCATTTATTAT
AATTTTTTTCATAGTAATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTACCTTTAATATTAGGAGCCCC
TGATATAGCCTTCCCCCGAATAAATAACATAAGTTTTTGGCTTTTACCCCCTTCTTTAATATTATTAATTTCTAGT
AGTATTGTAGAAAATGGAGCTGGAACAGGTTGAACAGTTTACCCCCCTTTATCTTCTAATATTGCTCATAGAG
GAAGATCTGTAGATTTAGCTATTTTTTCTTTACATTTAGCGGGAATTTCATCTATTTTAGGTGCAATTAATTTTA
TTACTACAATTATTAACATACGAATTAATAATATATCATTTGATCAAATACCTTTATTTGTTTGAGCTGTTGGAAT
TACAGCATTCCTTTTATTACTCTCATTACCTGTATTAGCTGGGGCAATTACCATATTACTAACAGATCGAAATTT
AAATACATCATTCTTTGACCCTGCTGGAGGGGGAGATCCAATTTTATACCAACACTTATTT

Fig. 166. Partial coding sequence of *P. increta* SJIK40 COI gene.

> *P. increta* Voucher SJIK40

TLYFIFGIWAGMVGTSLSLLIRAELGNPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNW
LVPLMLGAPDMAFPRMNNMSFWLLPPSLMLLISSSIVENGAGTGWTVYPPLSSNIAHSGSSV
DLAIFSLHLAGISSILGAINFITTIINMRINNMSFDQMPLFVWAVGITAFLLLLSLPVLAGAITMLL
TDRNLNTSFFDPAGGGDPILYQHLF

Fig. 167. The conceptual translation product of the partial coding DNA sequence of mitochondrial COI gene of *P. increta* SJIK40.

Table 28. The BLAST hit table of the partial coding DNA sequence of COI gene of *P. increta* SJIK40.

SN	Subject IDs	% Identity	Align. length	Mis-match	Gap opens	Query start	Quer y end	Geograph. location
1	Psilogramma increta KC182271.1	100	658	0	0	1	658	Pakistan
2	Psilogramma increta JF858056.1	100	658	0	0	1	658	Pakistan
3	Psilogramma vates MG783945.1	99.70	658	2	0	1	658	India, Maharashtra
4	Psilogramma increta JN678459.1	96.20	658	25	0	1	658	China
5	Psilogramma lukhtanovi GU704611.1	96.20	658	25	0	1	658	Thailand
6	Psilogramma yilingae GU704610.1	96.20	658	25	0	1	658	China
7	Psilogramma monastyrskii GU704617.1	96.05	658	26	0	1	658	Vietnam
8	Psilogramma increta JN087405.1	95.91	636	26	0	2	637	Indonesia
9	Psilogramma mandarina GU704616.1	95.90	658	27	0	1	658	China
10	Psilogramma gerstbergeri GU704607.1	95.75	658	28	0	1	658	Indonesia
11	Psilogramma menephron KJ168325.1	94.68	658	35	0	1	658	-
12	Psilogramma renneri GU704615.1	94.07	658	39	0	1	658	Sri Lanka
13	Psilogramma rupprechtorum GU704608.1	94.07	658	39	0	1	658	Indonesia
14	Psilogramma hainanensis GU704643.1	94.07	658	39	0	1	658	China

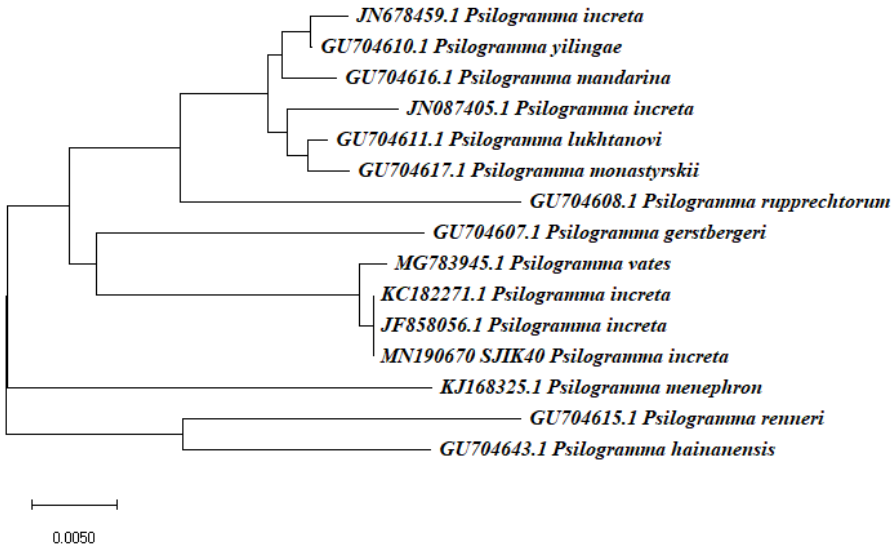

Fig. 168. The NJ tree showing phylogenetic relationships of *P. increta* SJIK40.

The DNA isolated from the sample *P. increta* SJIK40 gave a 658 nucleotide long consensus sequence of mitochondrial CO I gene. The BLAST hit table in Table 28 represents the % similarity and alignment details of the sequence with other related sequence accessions in the nucleotide database.

The BLAST result of *P. increta* SJIK40 from Kerala showed 100% similarity to *P. increta* KC182271 and JF858056 from Pakistan occupying a single clade and having a common ancestor. Hence the COI sequence of *P. increta* SJIK40 can be used as a molecular barcode for identifying the species. The NJ tree showed that *P. vates* MG783945 from India (Maharashtra) with 99.7% similarity is the closely related species of SJIK40 which also shares a common origin. It is a polymorphic variant of SJIK40. The distribution pattern of the various species of *Psilogramma* showed the South East Asian origin and the later divergence due to the separation of the Gondwana.

SUMMARY

Result of the study indicates that the genetic diversity of moths of Northern Kerala is characterized by the presence of species largely belonging to the families Noctuidae, Erebidae, Geometridae, Crambidae, Sphingidae and Lasiocampidae, which are among the most diverse families of moths and occurrence of other families is relatively rare. It also suggests that the moth fauna of North Kerala is highly diverse and a number of species are commonly encountered.

The moth samples for this study were collected from different localities of North Kerala. 658bp partial sequence of mitochondrial cytochrome oxidase subunit I gene of 28 species of moths belonging to various families of the order Lepidoptera were used for molecular barcoding. The sequences generated were deposited in GenBank, and the GenBank Accession Numbers with specimen voucher numbers are given in table 29.

Two moths were first records from India. They are 1) *Heteropalpia sp*. of family Noctuidea and subfamily Catocalinae and 2) *Stenhypena sp*. of family Erebidae and subfamily Herminiinae.

The maximum number of moths were from the family Noctuidae. Out of the 28 COI partial DNA molecular barcodes generated from moths in the present study 16 were novel genotypes. They are *Condica sp.* SKIJ5, *Grammodes sp.* SJIK7, *Pycnarmon sp.* SJIK9, *Spirama retorta* SJIK10, *Pinagsa sp.* SJIK12, *Helicoverpa armigera* SJIK15, *Heteropalpia sp.* SJIK19, *Biston suppressaria* SJIK20, *Nyctemera coleta* SJIK21, *Trabala sp.* SJIK22, *Stemorrhages sp.* SJIK24, *Nagia sp.* SJIK28, *Stenhypena sp.* SJIK29, *Nausinoe sp.* SJIK31, *Bastilla sp.* SJIK32 and *Ceryx sp.* SJIK33. 12 moth DNA sequences isolated were 100 % similar to that in the database. In these novel sequences are added in the case of *Hyperythra lutea* SJIK1, *Xanthodes transversa* SJIK17 and *Godonela sp.* SJIK27.

Hyperythra lutea SJIK1 showed 100 % similarity to that from Western Ghats, India KJ380856. 65 novel bp of COI were added to the database. It shares a common ancestory with *H. rubricata* KF389104 from Australia. The divergence might have occurred because of the separation of the Australian continent from the Indian subcontinent during the breakup of the Gondwana. The species showed an Asian lineage.

Table 29. The sequences generated in the present study with GenBank Accession Numbers.

SN	Specimen Voucher No.	GenBank Accession No.	Name of species	Family	DNA % similarity	Distribution of nearest species	Genotype: nove/known
1	SJIK1	MN190673	Hyperythra lutea	Geometridae	100	India	known
2	SJIK2	MN190674	Hippotion boerhaviae	Sphingidae	100	India	known
3	SJIK5	MN190676	Condica sp.	Noctuidae	99.85	Pakistan	novel
4	SJIK6	MN190677	Pygospila tyres	Crambidae	100	Pakistan & Australia	known
5	SJIK7	MN190662	Grammodes sp.	Noctuidae	93.16	Australia	novel
6	SJIK9	MN190644	Pycnarmon sp.	Crambidae	93.31	Papua New Guinea	novel
7	SJIK10	MN190645	Spirama retorta	Noctuidae	99.85	India	novel
8	SJIK11	MN190646	Argina astrea	Erebidae	100	Australia	known
9	SJIK12	MN190647	Pingasa sp.	Geometridae	98.71	China	novel
10	SJIK15	MN190649	Helicoverpa armigera	Noctuidae	99.85	Kenya	novel
11	SJIK16	MN190650	Pandesma quenavadi	Noctuidae	100	Pakistan	known
12	SJIK17	MN190651	Xanthodes transversa	Noctuidae	100	Thailand	known
13	SJIK18	MN190652	Condica illecta	Noctuidae	100	Pakistan	known
14	SJIK19	MN190655	Heteropalpia sp.	Noctuidae	95.28	-	novel
15	SJIK20	MN190653	Biston suppressaria	Geometridae	97.26	Pakistan	novel
16	SJIK21	MN190654	Nyctemera coleta	Erebidae	99.52	Malaysia	novel
17	SJIK22	MN190656	Trabala sp.	Lasiocampidae	96.46	China	novel
18	SJIK24	MN190658	Stemorrhages sp.	Crambidae	98.33	Australia	novel
19	SJIK25	MN190659	Asota caricae	Erebidae	100	Maharashtra, Pakistan	known
20	SJIK27	MN190660	Godonela sp.	Geometridae	100	India	known
21	SJIK28	MN190661	Nagia sp.	Erebidae	99.09	Australia	novel
22	SJIK29	MN190663	Stenhypena sp.	Erebidae	93.15	Australia	novel
23	SJIK31	MN190664	Nausinoe sp.	Crambidae	99.66	India	novel
24	SJIK32	MN190665	Bastilla sp.	Noctuidae	93.47	Brazil	novel
25	SJIK33	MN190666	Ceryx sp.	Erebidae	99.51	India	novel
26	SJIK34	MN190667	Spodoptera litura	Noctuidae	100	India, China, Pakistan	known
27	SJIK36	MN190669	Anticarsia irrorata	Noctuidae	100	Congo	known
28	SJIK40	MN190670	Psilogramma increta	Sphingidae	100	Pakistan	known

Hippotion boerhaviae SJIK2 showed a South East Asian lineage. The BLAST result showed 100% similarity to *H. boerhaviae,* GU704520 and KJ168258 from Maharashtra. SJIK2 showed 99.85% similarity to *H. boerhaviae* JN281154 from India which showed single nucleotide difference (G changed to A). The phylogeny clearly depicts the common origin of the three species *H. boerhaviae*, *H. rosetta* and *H. echeclus*.

The COI sequence of *Condica sp.* SJIK5 showed a similarity of 99.85% to 8 samples of *Condica illecta* sequences in the database from Pakistan. They are polymorphic novel variants of the species. *Condica sp.* SJIK5 is a novel one. There is single nucleotide difference with the species from Pakistan. The most nearest relative is *Condica sutor* from USA. The divergence might have occurred by geographical isolation after the species reached the North American Continent through land bridges from South America which was once part of Gondwana.

P. tyres SJIK6 isolate from Kerala showed 100% similarity to *P. tyres* from Pakistan KX862292 and HQ953034, HQ953034, HQ953033 and KF392550 from Australia. It also showed 99.85% similarity to HQ990824 from Pakistan which is a polymorphic variant. A single nucleotide change (C in SJIK6 changed to T) was the difference observed. The NJ tree shows that all the species of *P. tyres* in the BLAST result are monophyletic and are in adjacent clades. It depicts the common origin of all these species. *P. hyalotypa* HQ953030 from Australia showing 93.93% similarity is the closest relative and *P. bivittalis* HQ953029 also from Australia showing 93.62 % similarity are placed in the adjacent clade. The NJ tree showed that the two groups and SJIK6 have diverged from a common ancestor, as a result of the break- up of the Indo-Australian plate due to the stresses induced by the collision of the Indo- Australian plate with Eurasia.

Grammodes sp., SJIK7 is a novel one. The NJ- tree showed the similarity of *Grammodes sp.* SJIK7 to the genus *Bastilla* both of which belong to the subfamily Catocalinae. They are placed in adjacent clades reflecting the divergence from a common ancestor. The NJ tree showed that the species diverged from a common ancestor, as a result of the break- up of the Indo-Australian plate induced by the collision of the Indo-Australian plate with Eurasia.

Pycnarmon sp. SJIK9, is a novel one. The distribution of the various species of *Pycnarmon* in Australia, India and Papua New Guinea shows the common origin from the Gondwana.

Spirama retorta SJIK10 showed a maximum similarity of 99.85% to *S. retorta* MG783875 from Maharashtra. The multiple sequence alignment showed a single nucleotide polymorphism between them (C in SJIK10 being replaced by G). *S. helicina* KX862166 from Pakistan with 99.7% similarity is the closely related species. There is single nucleotide difference (C in SJIK10 replaced by G). *Spirama retorta* SJIK10, is a novel one. 4 novel bp of COI were added to the database. The phylogenetic analysis of *Spirama retorta*, SJIK10, showed that the species from China, Japan and South Korea together occupying a separate clade might have diverged from the Indian species due to the rise of Himalayas which formed a barrier.

The distribution pattern of some species of moths like *Argina astrea*, SJIK11, showed that the *Argina* genus has not traversed much across continents and has remained relatively isolated.

Pingasa sp. SJIK12 is a novel genotype. The pattern of geographic distribution of *Pingasa sp.*, showed the common origin of the various species of the genus *Pingasa* and the later divergence to the various species distributed across Gabon, Ethiopia, India, China, Papua New Guinea and Australia, all being part of the erstwhile Gondwana.

Helicoverpa armigera, SJIK15, is a novel genotype. It showed a common origin from the erstwhile Gondwana from the distribution pattern - African and Asia. The species showed a South East Asian and African lineage.

Pandesma quenavadi SJIK16, is mainly confined to the Indian subcontinent and Australia. The phylogenetic tree also shows that the various species of the genus *Pandesma* viz., *P. quanevadi, P. partita, P. submurina* and *P. robusta* diverged from a common ancestor.

Xanthodes transversa, SJIK17, showed a Gondwana origin being distributed in India, Thailand, Papua New Guinea and Australia which diverged at various stages. The closely related species from India MG250706 showed a single nucleotide change (A in SJIK17 changed to G).

Condica illecta SJIK18 from Kerala is 100% similar to *C. illecta* KX862760 from Pakistan occupying the same clade. The closest species from Papua New Guinea showed a single nucleotide difference (G in SJJIK18 changed to A). *Condica illecta* SJIK18 showed a Gondwana origin and later diverged from its closely related species *C. sutor* by continental drift reaching South America and through land bridges traversed to North America.

Heteropalpia sp. SJIK19, is being reported for the first time from India. It is a novel genotype.

Biston suppressaria, SJIK20, is a novel genotype. It showed an Asian lineage.

Nyctemera coleta SJIK21, showed a South East Asian origin. The nearest match from Malaysia differed by two nucleotides. In the Malaysian sp. G is replaced by A and T is replaced by C. The pattern of distribution of *Nyctemera* genus in the countries of the erstwhile Gondwana viz., Africa (Congo), India, Australia and the nearby regions of Taiwan, Thailand and Malaysia shows the divergence from a common ancestor.

Trabala sp., SJIK22, is a novel genotype. The phylogeny of *Trabala sp.,* showed that it diverged from other *Trabala sp.* from China and Taiwan as a result of vicariance events like the rise of Himalayas, disappearance of the Tethys Sea and associated climatic changes.

The *Stemorrhages sp.*, SJIK24 is a novel genotype and showed a Gondwana origin. It is being reported for the first time from India. The phylogenetic tree reveals that the species of *Stemorrhages* isolated from Gabon, viz., *S. sericea* and that from Madagascar also had a common origin with SJIK24 from the Indian subcontinent. The geographical distribution pattern confirms the common ancestry and they might have diverged due to geographical isolation when the continents separated.

The geographical distribution pattern of *Asota caricae,* SJIK25 shows the common origin of the various species of *Asota caricae* from the Gondwana. It showed a South East Asian lineage. Phylogeny of *Asota caricae,* SJIK25 showed that certain subspecies have evolved due to geographical isolation.

Godonela sp. SJIK27, showed 100% similarity to the Indian species in the database. 65 novel bp of COI were added to the database. The NJ tree showed the close relation to the genus *Chiasma*.

The *Nagia sp.,* SJIK28, is a novel one and it showed an Australian affinity. The NJ tree shows that the *Nagia sp.* SJIK28 isolated from Kerala and *N. linteola* from Australia are monophyletic occupying the same clade and having a common origin.

The *Stenhypena sp.* SJIK29, is a novel genotype and it showed Australian affinity. *Stenhypena sp.* is being reported for the first time from India.

The *Nausinoe sp.* SJIK31, is a novel genotype. The nearest match from India *Nausinoe neptis* differs by a T-A in SJIK31 being replaced by A-T. It showed Gondwana origin being distributed in Pakistan, Australia and India.

The *Bastilla sp.,* SJIK32 is a novel one. The phylogenetic tree also showed the relationship of the genus to various species of the genera *Grammodes* which belonged to same subfamily. The pattern of distribution of the *Bastilla sp.* shows the common origin of the species. The species also shows a South American lineage.

The *Ceryx sp.* SJIK33 from Kerala showed 99.51% similarity to *Ceryx guttulosa* MG250707 from India. It is a polymorphic novel variant of SJIK33. It is placed in the adjacent clade separately. It showed 3 nucleotide differences (T for C, A for G and T for C). SJIK33, is a novel one. 46 novel bp of COI were added to the database. The species showed Gondwana origin.

S. litura SJIK34 from Kerala showed 100% similarity to *S. litura* MG954454, MG954453 and MG954452 from China, KX863232, KX862420, KX861832 and KX 860450 from Pakistan, JQ064568 and JQ064567 from Tamil Nadu and HQ950413 from Australia. The closest relative from China MG954451 with 99.85% similarity showed single nucleotide change (A changed to T). *Spodoptera litura*, SJIK34 showed Gondwana origin being distributed in countries China, Pakistan, Australia and Papua New Guinea.

The *Anticarsia irrorata* SJIK36 iosalted from Kerala was 100% similar to *A. irrorata* from Congo GU696094. It showed 99.7% similarity to *A. irrorata* JN401300 in the database. It is a polymorphic variant of SJIK36 showing single nucleotide difference

(T changed to C). The NJ tree shows that the three *Anticarsia sp.* are monophyletic, occupying the same clade and sharing a common ancestor from Africa.

Psilogramma increta, SJIK40, showed South East Asian affinity. The distribution pattern of the various species of *Psilogramma* confirms the South East Asian origin and the later divergence due to the separation of the Gondwana.

The NJ tree constructed from COI sequences generated in the present study depicts the phylogenetic relationship of 28 moths (Figure 169, 170). Accordingly the abundance of 6 families of moths is in the following order: Noctuidae >Erebidae >Geometridae >Crambidae >Sphingidae >Lasiocampidae.

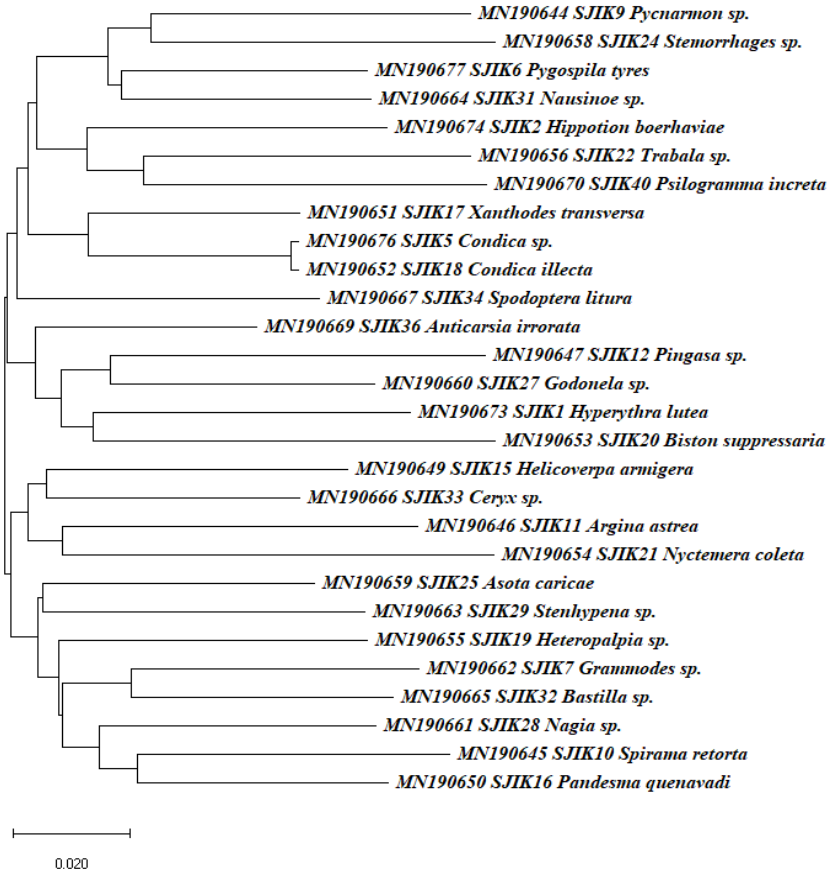

Fig. 169. The NJ–tree (rectangular format) constructed from COI sequences with phylogenetic relationships of the 28 species of moths from North Kerala.

Fig. 170. NJ tree (in curved format) constructed from the COI sequences of 28 moths in the present study.

The present study helps to narrate some aspects of the genetic diversity of moth fauna of North Kerala. The phylogenetic analysis of the various moths revealed that the North Kerala moths showed a close relationship to the moth fauna of South East Asia, Africa and Australia which were part of the erstwhile Gondwana. Divergences might have occurred due to geographical isolation when the land masses separated by continental drift. Nucleotide polymorphisms are the main cause for genetic variation in most of the species.

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