MOLECULAR BARCODING AND PHYLOGENETIC ANALYSIS OF THE INSECT PESTS OF CUCURBITACEAE FROM SELECTED DISTRICTS OF KERALA

Thesis
Submitted to the Faculty of Science, University of Calicut
For the Award of the degree of
DOCTOR OF PHILOSOPHY IN ZOOLOGY

By

PRIYA BHASKARAN K. P.

Under the supervision of

Dr. SEBASTIAN C. D.
Associate Professor
Department of Zoology
University of Calicut

DIVISION OF MOLECULAR BIOLOGY
DEPARTMENT OF ZOOLOGY
UNIVERSITY OF CALICUT
KERALA, INDIA
2019

കാലിക്കറ്റ് സർവ്വകലാശാല
ജന്തുശാസ്ത്ര പഠനവിഭാഗം
കാലിക്കറ്റ് സർവ്വകലാശാല (പി.ഒ.), മലപ്പുറം (ജില്ല),
കേരള, ഇന്ത്യ - 673 635

UNIVERSITY OF CALICUT
DEPARTMENT OF ZOOLOGY
Calicut University (P.O.), Malappuram (Dt.)
Kerala India - 673 635
Tel: 0494 - 2407420 (Head), 0494 - 2407419 (Off.)

Re-Accredited by NAAC with "A" Grade

Dr. Sebastian C.D.
Associate Professor

No:..................................

Date:....5 June, 2019....

CERTIFICATE

This is to certify that the thesis entitled "MOLECULAR BARCODING AND PHYLOGENETIC ANALYSIS OF THE INSECT PESTS OF CUCURBITACEAE FROM SELECTED DISTRICTS OF KERALA" submitted to the University of Calicut for the award of the degree of Doctor of Philosophy in Zoology, is the record of the original work done by **Ms. Priya Bhaskaran K.P.** in the Molecular Biology Laboratory, Department of Zoology under my supervision and guidance, and that it has not formed the basis for the award of any degree/diploma or other similar title to any candidate of any University.

Dr. Sebastian C.D.
Supervising Teacher

DECLARATION

I do hereby declare that the work entitled “MOLECULAR BARCODING AND PHYLOGENETIC ANALYSIS OF THE INSECT PESTS OF CUCURBITACEAE FROM SELECTED DISTRICTS OF KERALA” is an authentic record of the work carried out by me under the supervision and guidance of Dr. Sebastian C.D., Associate Professor, Division of Molecular Biology, Department of Zoology, University of Calicut and that no part of this has been published previously or submitted to the award of any other degree / diploma.

PRIYA BHASKARAN K. P.

Acknowledgements

It is a great pleasure to thank the people who made this thesis possible.

I would like to thank my research supervisor Dr. Sebastian C. D., Associate Professor, Department of Zoology, University of Calicut, for his constant encouragement and valuable guidance throughout this work. I feel very fortunate to have his support and to experience his work ethic which I do always admire.

My sincere thanks goes to the Head of the Department of Zoology, Dr. E. Pushpalatha, for being so helpful and approachable and also for providing the technical helps and facilities required to complete this work. I am grateful to the former Heads of the Department- Dr. M . Nasser, Dr. V.M. Kannan and Dr. Y. Shibu Vardhanan.

I express my thanks to Mr. Akhilesh V.P., Dr. Vidya P., Dr. Rukhsana K, Ms. Bindu P.U., Ms. Jisha Krishnan E.K., Ms. Surya P., Ms. Sreevidya P., Mr. Muhammed Ali V.C., Ms.Remya P.K., Ms.Reshma ., Ms. Greeshma ., Ms. Thara Paul., Ms. Aleena .,Ms. Nasrin and Ms. Sameena of Molecular Biology Laboratory , Department of Zoology, University of Calicut, for their help rendered for the successful execution of the thesis work.

I am grateful to Dr. Sreejith Kanholi, Assistant Curator and Mr. Santhosh K.V., Librarian, Department of Zoology, University of Calicut, for their help.

I wish to thank my friends, Mr. Rajesh K., Mr. Vijesh Varghese ., Ms. Prameela., and all other Research Scholars from other Laboratories of Zoology Department for their constant inspiration, support and for providing a stimulatingand pleasant environment in the Department.

I would like to record my thanks to M.Sc and M.Phil students, for making the Department alive.

I would like to thank all the teaching faculties and non teaching staff of the Department of Zoology at University of Calicut for all their possible help extended to carry out the work successfully.

I would like to express my thanks to Mr. Prasannan, Agricultural Officer, at Parappanangadi for his great support for the specimen collection.

I express my thanks to Ms. Rashmi, Finance Section, Mr. Prajosh, Mr. Sasi, Mr. Balachandran and Ms. Sindhu, DOR, University of Calicut for making the administrative part smooth.

I would like to thank all the academic and administrative members of the University of Calicut.

The financial assistance by Kerala State Council for Science, Technology and Environment (KSCSTE- BLP), under Women Scientist Division is greatly acknowledged.

I am very much grateful to Dr. K.R. Lekha., Head, Women Scientists Division, KSCSTE, for all the support and encouragements.

My sincere thanks to Vision Scientific and Vision Scientific Services, Ankamaly for their consistent support for the successful completion of the work.

I also thank Mr. Balu and Vinesh, Bina Photostat, Chenakkal for their help in typesetting the thesis.

I am indebted to my parents and parents-in-law and to my siblings. Their unlimited support will never be forgotten and my deepest respect goes to them.

I have no words to express my heart-felt gratitude to my husband, Dr. Vinod Kumar P., for his love, support, and encouragement in every step of my life.

My biggest thanks goes to my daughter, Pooja Vinod, for giving me unlimited happiness and pleasure and for being a strong motivation to go on.

Finally, I offer my regards and wishes to all, those who supported me in any respect during the completion of the project.

Above all I am thankful to God for His immeasurable blessings showered upon my life.

Priya Bhaskaran K. P.

CONTENTS

Chapter	Title	Page
1.	Introduction	1 – 6
2.	Review of Literature	7 – 27
3.	Materials and Methods	28 – 31
4.	Coleoptera	32 – 151
	Introduction	32
	Systematic Position	34
	Taxonomic Key	35
	Result and Discussion	
	▪ *Mantura chrysanthemi*	45
	▪ *Mantura rustica*	52
	▪ *Aulacophora foveicollis*	59
	▪ *Aulacophora frontalis*	67
	▪ *Aulacophora lewisii*	74
	▪ *Sphenoraia bicolor*	81
	▪ *Paria thoracica*	88
	▪ *Rhabdopterus praetextus*	95
	▪ *Oocassida pudibunda*	102
	▪ *Dactylispa carinata*	109
	▪ *Henosepilachna vigintioctopunctata*	116
	▪ *Henosepilachna septima*	123
	▪ *Epilachna septima*	130
	▪ *Carpophilus marginellus*	137
	▪ *Epuraea luteolus*	144
	Consolidated Phylogenetic Tree	151

Chapter	Title	Page
5.	Hemiptera	152 – 249
	Introduction	152
	Systematic Position	153
	Taxonomic Key	154
	Result and Discussion	
	▪ *Nezara viridula*	165
	▪ *Spermatodes variolosa*	172
	▪ *Agonoscelis nubilis*	179
	▪ *Carbula scutellata*	186
	▪ *Halymorpha halys*	193
	▪ *Zicrona caerulea*	200
	▪ *Oncopeltus nigriceps*	207
	▪ *Geocoris varius*	214
	▪ *Paraplesius unicolor*	221
	▪ *Cletus schmidti*	228
	▪ *Dysdercus ocreatus*	235
	▪ *Campylomma vendicarina*	242
	Consolidated Phylogenetic Tree	249
6.	Lepidoptera	250 – 347
	Introduction	250
	Systematic Position	251
	Taxonomic Key	253
	Result and Discussion	
	▪ *Ophiusa coronate*	263
	▪ *Mocis proverai*	270
	▪ *Asota orbona*	277
	▪ *Asota caricae*	284
	▪ *Eudocima cocalus*	291

Chapter	Title	Page
	▪ *Hypocala deflorata*	298
	▪ *Polytela gloriosae*	305
	▪ *Acosmeryx anceus subdentata*	312
	▪ *Diaphania indica*	319
	▪ *Glypholes bicolor*	326
	▪ *Melanitis leda*	333
	▪ *Cryptophasa atecmarta*	340
	Consolidated Phylogenetic Tree	347
7.	Diptera	348 – 389
	Introduction	348
	Systematic Position	349
	Taxonomic Key	350
	Result and Discussion	
	▪ *Spaniocelyphus falcatus*	354
	▪ *Spaniocelyphus pilosus*	361
	▪ *Celyphus obtectus*	368
	▪ *Bactrocera tau*	375
	▪ *Pegomya circumpolaris*	382
	Consolidated Phylogenetic Tree	389
8.	Conclusion	390 – 392
	References	393 – 418
	Publications	

1

Introduction

Insects represent the most successful groups among the described animal fauna. Out of 5.57–9.8 million estimated animals in the world, about 4–8 million species are insects, which implies that three-fourths of the total described living organisms present on earth are insects (Lokeshwari and Shantibala, 2010). Insects show remarkable uniqueness in both species diversity and abundance. The origin of insects was dated to the Early Ordovician period (~479 million years ago), insect flight to the Early Devonian period (~406 Ma), the major extant lineages belong to the Mississippian period (~345 Ma) and the major diversification of the holometabolous insects to the Early Cretaceous period (Misof, 2014; Mayer et al., 2014).

In every terrestrial ecosystem, insects play key ecological roles in functionally various ecological processes including nutrient cycling, seed dispersal, bioturbation, pollination, and pest control. Insects, as drivers of ecosystem functions, play a pivotal role in agro-ecology, the management of agricultural systems in an ecologically sound and sustainable way by encouraging the existing ecosystem services (ES) (Jankielsohn, 2018). Terrestrial insects are typical in contributing to the biodiversity to a great extent.

Insects have always been predominantly considered as competitors in the race for their own survival. The dominant biotic relationship found to be existing in the ecosystem is the insect-plant relationship. Crop production possesses a direct proportion with ecosystem functions provided by insects, thus proving insects to be vital part of human survival. Globally, the insect pollination services estimated to contribute 9.5% to the total yield of crop production in the agricultural sector. Insects also improve fertility of agricultural soil by increasing nitrogen, phosphorous, potassium, calcium and magnesium or total protein content which significantly elevates the crop yield.

Along with the development in agricultural practices, pests became co-evolved as an unavoidable fauna. Due to the incidence of pests, the crop productivity grown for human consumption is at great risk (Oerke, 2006). As a result the insect pest has become the major constraints for agricultural crop sector. Among the pest fauna, the frequent visitors affecting the vegetables include different species under the orders Lepidoptera, Homoptera, Hemiptera, Coleoptera, Orthoptera, Diptera, Thynanoptera and Acarina. The pests are really susceptible to seeds, roots, stems, leaves and fruit. The range of insect damage usually ranges from reduced plant vigor to plant death hence causing heavy crop loss. The mode of insect damage is by chewing foliages, sucking juices, laying eggs and transmitting diseases. The proper identification of insect pests is an essential step in their proper management and control of successful vegetable farming, hence led to the beginning of a successful integrated pest management (IPM) strateegies. A regular pest screening provides early warning of problems allowing favourable economic, ecological, and social consequences.

Cucurbits contribute an important group of vegetable crops, extensively cultivated in both subtropical and tropical countries. The subfamily Cucurbitoideae specifically includes all the cultivable species. The fruit of *Cucurbita maxima* is the largest known fruit among all flowering plants category. In the world, the prime and popular cucurbit crop is watermelon, followed by cucumber produced from the leading producing countries China and Turkey. This family incorporates about 130 genera and 800 species. The important genera included are *Trichosanthes, Lagenaria, Luffa, Benincasa, Momordica, Cucumis, Citrullus, Cucurbita, Bryonopsis* and *Corallocarpus*. Cucurbit plants were used as traditional herbal remedies against various diseases as anti-inflammatory, antitumor, hepatoprotective, cardiovascular and immunoregulatory agents. The members of this group have always been considered as rich source of proteins, with many biological activities like anti-fungal, anti-bacterial, antiviral, anti-diabetic, anti-tumor and anti-AIDS. They also reported to incorporate several bioactive compounds such as cucurbitacins, triterpenes, sterols and alkaloids. Herbivorous insects cause 18% damage of world agricultural production.

As the insects forms an important and diverse group in the field of agricultural entomology, their accurate species identification becomes difficult. The systematics and IPM are invariably interdependent and interconnected. Thus the advancement in the area of taxonomic knowledge leads to better pest management. As the basic foundation of all the meaningful biological research is confined to biosystematics, taxonomy plays an important role in pest management programmes (Narendran, 2001).

The Classical Taxonomy is the conventional or traditional taxonomy method based mainly on the external morphology which is thus supported and supplemented by ethological and ecological data (Sheeba and Narendran, 2008). The availability of taxonomical and identification experts has reduced in recent times. Hence, the alternative sources for accurate and prompt identification which a non expert can perform are in requisite. Systematics includes taxonomy, identification, nomenclature and classification. The basic foundation of biological research is the biosystematics of the organisms. Better control of insect pest is directly proportional to enhancement of taxonomic knowledge. Molecular markers are considered as the indispensable tools used for determining the genetic variation and biodiversity with high levels of accuracy and reproducibility. These molecular markers can be classified as mitochondrial and nuclear markers. The mitochondrial DNA markers commonly used are 12S rDNA > 16S rDNA > cytochrome b > control region (CR) in their decreasing order of conserved sequences; thus the highly conserved sequences is 12S rDNA and highly variable is CR. The nuclear markers commonly used for DNA fingerprinting include random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) and microsatellites.

The properties of ideal molecular marker are: (a) A single-copy gene used which is satisfied by the mitochondrial and nuclear functional genes; (b) easy alignment (c) optimum substitution rate (d) easy availability; (e) minimum base variation among the taxa (Arif and Khan, 2009). Molecular phylogenetics is one aspect of molecular systematics, comprehending the use of molecular data in taxonomy and biogeography. There exist a positive correlation between molecular phylogenetics and molecular evolution. The mutations at molecular levels, mainly in gene sequences and proteins, during various phases of evolution lead to molecular evolution. Molecular phylogenetics makes inferences after confirmation of the evolutionary relationships of molecular evolution hence resulting in the construction of a phylogenetic tree (Patwardhan et al., 2014).

The identification of insects based on the traditional perspective is quite difficult because their morphological changes may cause due to seasonal and geographic variation. Organisms alter themselves physiologically and morphologically to thrive in the adverse conditions. These mutations accumulate leading to the changes in the species outlook hence, resulting in the misleading towards species identification. The molecular data are comparatively more numerous and consistent than fossil records, easier to obtain, have no sampling bias, hence helps to correct the gaps in real fossil records leading to construct a robust phylogenetic tree. The molecular data are generally based on nucleic acids and, each nucleotide position, considered as a character which is independent. Normally the morphological adaptations of organisms are mirrored in its cellular biomolecules and vice versa. In the absence of correct identification of the pest organisms, it is quite difficult to chart their proper pest management strategy.

DNA sequences help in establishing the species diversity, allow analyzing evolutionary relationships within groups, identifying specimens and resolving species boundaries in populations of exhibiting apparently similar organisms. A short standardized sequence of DNA fragment (400–800 bp) generated in characterization of almost all species is the basic principle of DNA barcodes. DNA barcoding shows users to efficiently recognize known species for the speedy discovery of species found in nature. The main criteria for the DNA barcoding is to use the information of specific or a few gene regions to identify all life.

The site-specific nucleotide or the domain specific amino acid substitution models and the phylogenetic analyses of both nucleotide and amino acid sequences directs to statistically robust and congruent results hence resolving controversial phylogenetic relationships. The closely related species are likely to have higher rate of accumulation of new mutations. DNA sequence analysis of a uniform target gene to enable species identification has been termed *DNA barcoding*, similar with the Uniform Product Code barcodes on manufactured goods. The analysis of mitochondrial cytochrome *c* oxidase subunit I (COI) sequences among closely related species across diverse phyla in the animal kingdom help to distinguish better species discrimination (Hebert et al., 2003a). Eukaryotic nuclear genes encoding for the 18S rRNA, the 5.8S rRNA and the 28S rRNA subunits are organized in tandemly repeated units, separated by transcribed (ETS, ITS1 and ITS2) and non-transcribed (IGS) spacers. The largest subunit, 28S subunit is composed of a mosaic of core regions and hypervariable expansion segments, also known as Divergent Domains (Hassouna et al., 1984; Bennet-Clark, 1984).

Metazoan mitochondrial DNA (mtDNA) are double-stranded, circular with size ranging from approximately 14–39 kb, encoding 13 protein-coding genes (COI – III, Cytb, ND1 – 6, ND4L, ATP6, and ATP8), 2 rRNA genes (16S and 12S rRNAs), and 22 tRNA genes. Additionally, it also possesses the adenine (A) + thymine (T)-rich region, which serve as the origin of heavy-strand mtDNA replication in case of vertebrates (Gholamzadeh and Incekara, 2016). The popularity of mtDNA in both phylogenetics and population genetic studies is based upon i) developments in methodology of mtDNA isolation, ii) nucleotide differences detection using restriction enzymes iii) PCR developments and iv) DNA amplification using universal primers.

Molecular systematic studies proved that the genes (COI and COII) evolved at an appropriate rate for the phylogenetic reconstruction at the generic level. Both the COI and COII genes codes for two out of seven polypeptide subunits in the cytochrome c oxidase complex. The COI gene size almost 1500 bp. Both are applied in phylogenetic problems at a wide range of hierarchical levels in insects, in closely related species to genera and subfamilies, families, and orders. The most slowly evolving COI gene and 12S rRNA is most appropriate in fulfiling the putative phylogenetic accuracy in distinguishing the taxa at different taxonomic level.

The DNA barcodes and molecular taxonomic tools for insect pests of Cucurbitaceae of Kerala remain unexplored. Further, morphological identification of eggs and early instar larvae, damaged specimens, or fragments of specimens are difficult and limited taxonomic expertise promoted DNA-based identification system. They act as important tool for species identification in biomonitoring. Therefore the identification of insects in the non-adult stage using conventional taxonomy is a wearisome process. Therefore the correct identification of insect fauna helps in better control measures and IPM.

In the present study, molecular barcoding and phylogenetic assessment of insect pest of Cucurbitaceae from selected districts of Kerala using COI gene sequences were performed. The mitochondrial partial COI sequences were used for the construction of phylogenetic tree and to analyse phylogenetic relationships. The genetic divergence and nucleotide composition of the entire insect pest is also well studied.

Objectives of the Present Study

- To study the genetic structure of mitochondrial cytochrome oxidase subunit I gene in the common insect pests of Cucurbitaceae from selected districts of Kerala.
- To analyze the genetic divergence within and between different pest species.
- To develop mitochondrial cytochrome oxidase subunit I gene sequence based barcode for the proper identification of these insect pests.
- To determine the evolution and phylogenic status of the insect pests of Cucurbitaceae of Kerala.

2

Review of Literature

Insects are the fellow inhabitants of our Earth (Gullan and Cranston, 2014). They are the most exuberant and diverse group among the animal fauna. They are economically and ecologically important and are capable to exploit every conceivable environment (Chandra, 2011; Raman et al., 2014). They play very crucial role that ensures numerous ecosystem services which are important aspects of human livelihood (Raman et al., 2014). Almost three fourth of the total living creatures on earth are identified as insects (Pedigo, 2002). Out of the 5.51 – 9.8 million estimated animals in the world, 4-8 millions are filled up by the Class Insecta (May, 1990; Hammond, 1995). Even though a very precise check listing of Indian fauna are not yet been done accurately, approximately 0.1 million insect species belong to Indian sub continent (Venkatesha, 2007). Recent data estimated that the number of described insect species is already crossed one million (current status ~1,060,704). Insects represent 61 – 71% among 1.50 – 1.74 million described eukaryotic species (May, 1990; Costello et al., 2011).

Insects are important because of their diversity, ecological niche and their influence on environment, agriculture, human health and natural resources. Insects create a biological foundation for all terrestrial ecosystems. They are promising in cycling nutrients, pollinate plants, disperse seeds, maintain soil structure and fertility, control populations of other organisms and provide a major food source for other taxa. Generally insects are habitat specific in nature and hence, highly sensitive to disturbances, particularly habitat fragmentation and pollution (Kearns et al., 1998; Aizen and Feinsinger, 1994; Ashworth et al., 2004). Especially the terrestrial insects are the most different gatherings of creatures on earth and contribute to the biodiversity to a huge degree (Althaf et al., 2016).

Most of the insect radiations likely rely on the small size, along with short generation time, sensory and neuromotor complexity, evolutionary interaction with plants and organisms, metamorphosis and versatile winged adults. The substantial time taken since the beginning of each significant insect group has permitted numerous opportunities for ancestry diversification. Present day species decent variation results from either higher rates of speciation or lower rate of species elimination from the pool (Gullan and Cranston, 2014). Insects are fundamental and accompany various ecosystem functions, such as nutrient supplement reusing, plant propagation counting pollination and seed dispersal. Their ecologies are unimaginably variable. Maintenance of plant community structure and composition through phytophagy, including seed sustaining, nourishment for insectivorous vertebrates, maintenance of animal community structure, through transmission of malady of vast creatures, predation and paratization of smaller ones (Gullan and Cranston, 2014).

Most of the described insect species are very robust and hence it necessitates efficient and appropriate sampling techniques for their collection (Russo et al., 2011). The accurate and prompt documentation of insects is the most important aspect for the study of their biodiversity and population dynamics (Althaf et al., 2016). Certain coleopteran insects like beetles, the ground dwelling insects like ants (formicidae), soil insects etc can be sampled through pitfall trapping method. The diurnal insects that are low flying can be collected specifically through various traps like Malaise trap and Sweep net method (Marinoni and Dutra, 1997; Aguiar and Santos, 2010; Malaise, 1937; Mazon and Bordera, 2008). There are thousands of diverse nocturnal insect species that cannot be simply collected by these conventional methods and in such cases light trapping is the best method (Szentkiralyi, 2002).

The present insect fauna consisted of representatives from the four important stages of evolution. The first evolutionary stage includes wingless insects, exemplified in the existing fauna by two orders, the Thysanura (silver fish) and the Entotrophi. This phylogenetic assemblage of wingless insects is termed the Apterygota. The second stage in the evolution of insects is the development of wing during the lowest Carboniferous strata. The first winged insects or Paleoptera had a simple wing articulation and were capable of flexing them back and forth even at rest. The third stage in insect evolution began with the modification of certain plates of wing articulation in order to permit wing flexing and these insects are called Neoptera. Later, the Paleopterous insects which found to be dominant during the Carboniferous and Permian, began to wane and the Neoptera started to flourish. This trend in insect evolution continued to the present time, 90% of the existing orders including 97% species are now Neoptera (Carpenter, 1953). The development of a complex metamorphosis is the fourth stage of evolution, where the larvae show slight resemblance with the adult.

One of the most challenging issues before the biologist is the disentangling evolutionary background of the arthropods. Arthropods are grouped mainly into five principal general gatherings *viz*. Trilobites (extinct since the end of the Paleozoic; ~4,000 species described); Cheliceriformes (~75,000 described living species); Crustaceans (~50,000 described living species); Myriapods (~14,000 described living species) and Hexapods (~ 8,78,000 to 1.5 million living species). There reported a close sister group relationship between the two phyla namely Tardigrada and Arthropoda (Garey et. al., 1996). About 1.02 to 1.64 million described arthropod species are found in practically all environmental condition over the earth.

Among the 150,000 described species, the ranking of the insect orders is Coleoptera, Lepidoptera, Diptera and Hymenoptera on their abundance (Grimaldi and Engel, 2005; Beutel and Pohl, 2006). The phylogenetic courses of action of the higher gatherings of insects have always been argumentative since Linnaeus. The 32 (approximately) surviving hexapod orders contain maybe the most assorted, diverse and universal creatures on earth. Great advance has been made through the examination of anatomical highlights exemplified by Snodgrass (1933) and the epistemological unrest realized by Hennings (1966). This convention proceeds through the basic and mechanical work decribed by Kristensen (1975, 1981, 1991, 1995 and 1998). Most recently molecular data on late sub-atomic grouping information have offered extra data on occasion in tremendous (Kristensen and Skalski, 1998). This investigation mainly endeavors to coordinate novel sub-atomic information with the anatomical and conduct highlights analyzed in the course of recent hundreds of years (Wheeler et al., 2001).

The described members of the Class Insecta are grouped in 29 orders. About 80% of all the described living species are coming under the orders Coleoptera, Diptera, Hymenoptera and Lepidoptera of which the coleopterans are far leading ahead followed by Lepidoptera and Diptera (Polaszek, 2005). Approximately 4,00,000 species of the insect fauna are well known as plant feeders. According to Strong et al. (1984), the phytophagous insects make up nearly 25% of living species on the earth. The members of the orders Hemiptera, Lepidoptera and Orthoptera are almost entirely phytophagous (Brues et al., 1954). The effect of insects, as plant feeding organisms exceeds that of all other animal fauna (Grimaldi and Engel, 2005). Since history, humans practiced agricultural crop production; pests became inevitable part of their crops (Jankielsohn, 2018). Almost all the insects predominantly perceived certain competition in their race for survival. The insect-plant relationship is one of the dominant biotic interactions in the nature (Samways, 1993). Approximately half of described insect species are categorized as herbivorous, with most of the species feeding on plants in one or some related plant families (Schoonhoven et al., 2005). Herbivorous insects together cause 18% damage of world agricultural production (Losey and Vaughan, 2006). Insects are vital for human survival, because crops can only be produced with a balanced ecosystem functions provided by insects. Around 72% of the world's crops are dependent on insects for pollination directly or indirectly (Dicke, 2017). Pollinating insects improve and stabilize the yield of one third of all types of crop production by volume globally (Schwagerl, 2016).

Insects as Pests

Phytophagous insects can be potential pests that can have deadly effect on the host, but only less than 2% have reached the status of being minor pest. The insect defoliators have devastating effect on the growth (Mott et al., 1957) and survival of the forest trees (Morris, 1951), and can alter the forest-ecosystem balance function (Naiman, 1988; Carson et al., 2008). Few ecologists have the argument that the world’s most destructive insects are the plant hoppers (Nault, 1994). They cause more than $1.23 billion losses to rice every year mainly from feeding injury and by transmission of plant viruses reported from Southeast Asia (Herdt, 1987). According to Baron (1972), the desert locusts have severe effect on African crops. Pfadt (1962) reported various pests from corn, cotton, fruits, households, legumes, livestock, poultry, small grains, stored products, and vegetable crops.

Major insect pests in agriculture are usually introduced species without their natural biological control agents (Pimentel, 2014). Introduced insect species are responsible for 5-8 billion USD annual damage and control costs (Pimentel, 2014). The transmission of plant-diseases through insect has been known since long (Leach, 1940). For instance, sugarcane (*Saccharum officinarum* L.) is the most important cash-cum-industrial crops of Bangladesh (Ahad et al., 1987; Begum et al., 2004, 2006). In the world it is ranked second in sugar production but in Bangladesh it ranks first (Ahad et al., 1987). Insect pests alone cause damage ranging from 20-60% of productivity (Alam, 1967). Among the recorded factors of yield reduction; insect pests inflicts considerable losses, estimated to be nearly 20% in cane yield and 15% in sugar recovery (Avasthi and Shafee, 1983). However, according to various studies, about 70 species of the insect pests have been identified to feed on sugarcane in Bangladesh.

Among the insect pests of Black pepper, the king of spices, biting black ants (*Tetramorium* species), black pepper flea beetle (*Longitarsus* species), leaf gall thrips (*Liothrips* species) and stink bugs (Pentatomidae) were recorded relatively with high infestation and damage level from almost all surveyed areas (Girma et al., 2008; Purseglove et al., 1981). The pepper is infested by about 56 general species of insects damaging various parts of plant such as root, stem, shoot, leaves, spikes and berries (Devasahayam, 2000). Among them, the *pollu* beetle is considered as major pest depending on the severity and extent of damage followed by top shoot borer, leaf gall thrips and scale insects.

Almost twenty species of insect pests were considered as prominently destructors in paddy fields of Jaffna, Sri Lanka (Venugoban and Ramanan, 2014). Thirteen insect species were recorded from grain storehouses of Northern Cyprus during the study. It was determined that among them, seven species were primary, four species were secondary, and two species were relatively insignificant (Gozuacik et al., 2016). Some insect pests initiate damage at the crop ripening stage and continue their damage throughout till storage. Major sources of infestations include old bags, storage structure, old containers, and cross over infestation (Hem and Mohan, 1950; Srivastava and Subramanian, 2016).

The insect pests of stored grain are categorized as major or minor pests based on the severity of damage. On the basis of their feeding preference, these are further grouped as external and internal feeders. External feeders are the insects feeding on germ and endosperm from outside. Hence the whole seed and germinal portion are damaged. These insect pests or any of their developing stages are generally visible among the seeds e.g. rice weevil, pulse beetle, granary weevil, *Angoumois* moth etc. On the other hand internal feeders mostly lay eggs inside or on the surface of grains, spending a part or entire larval and pupal life inside the grains and emerge as an adult. They contribute significant loss of germination which is not detectable from outside. Secondary feeders include insects and mites which develop after the primary infestation by other pests and they feed on cut and broken seeds, moulds and detritus, dead insects, animal wastes e.g., common grain mite, cheese mites, and psocids. These damage result in ultimate loss of germination, contamination such as webbing and ball formation and inconspicuous deterioration of grains or seeds. The damage by these also invites fungal activity, hotspot formation and moisture migration across the stored grains (Srivastava and Subramanian, 2016).

The dipterans, true flies or two winged flies, are the most diverse insect order with a current record nearly 1,60,000 extant species (Pape et al., 2011). They are highly diverse in structure, life habits, habitat exploitation and interactions with human (Henning et al., 1982; McAlpine et al., 1981; Darvas and Papp, 2000; Brown et al., 2001; Skevington and Dang 2002; Pape et al., 2009; Marshall and Sinclair, 2012). The Diptera have colonized almost all sort of habitat leaving open sea and glaciers. The group includes approximately 40 families and more than 55,000 species worldwide (Pape et al., 2011). A large number of fruit flies belongs to Tephritidae are even capable of causing damage to both fruits and vegetables, hence making the dipterans important to the agriculture (Dowell and Wange, 1986; Norrbom et al., 2007). They mostly include plant-miners, gall midges, plant part feeders and so on.

The suborder Heteroptera has been categorized mainly into seven infraorders and 24 superfamilies (Schuh and Slater 1995; Henry, 2009). Hemiptera is generally considered to be fifth largest suborder, comprising half of the estimated 90,000 species (Cassis et al., 2006). They exhibit both phytophagous and zoophagous feeding habits (Schuh and Slater, 1995). The huge crop losses caused by Heteroptera every year, add to the single most important reason to study this diverse suborder. The Heteroptera fall into broad feeding regimes: plant feeders and predators (Schaefer and Panizzi, 2000; Wheeler et al., 2000, Wheeler, 2001); blood sucking parasites or water quality indicators.

Beetles occupy a major portion of the known insect fauna and they are the most diverse group on the Earth. The estimated number of described insect species is between 3,00,000 and 4,50,000 (Nielsen and Mound, 2000). The development of the forewing into sclerotized elytra (Lawrence and Britton, 1994) and the close historical association of most diverse groups of beetles with the flowering plants during their own period of diversification adds to the beetles diversification (Barraclough et al., 2007; Mckenna et al., 2015). The true coleopterans appeared in the Triassic period (Lawrence and Slipinski, 2013) and they played pivotal role in agricultural–crop sector.

The Lepidoptera – moths, butterflies, and skippers – represents the three most species rich and the largest evolutionary radiation of herbivorous animals (Scoble, 1992; Wahlberg et al., 2013). The Jurassic origin of the Lepidoptera makes them the youngest of the five mega diverse orders. They account for economically and agriculturally significant example towards forest, agriculture and stored product pests (Boettner et al., 2000).

The insects and mites cause serious damage to vegetables during all developmental stages of growth mainly aphid, thrip, whitefly, leafhopper, two spotted spider mite, squash bug, pumpkin beetles, flea beetles, hadda or *Epilachna* beetles, eggplant shoot and fruit borer, cutworms, hornworms, tomato fruit borer, tobacco caterpillar and melon fruit fly. Almost all vegetables are susceptible to pest attack, and so also their roots, stems, leaves as well as fruits are all subjected to injury. Purrington et al., (2017) reported various garden pests commonly found on vegetable cultivations and recommended the use of insecticides for their better control. Moran (1983) reviewed about the phytophagous arthropods of cultivated plants in South Africa. The characterization of the fauna was done based on taxon, relative pest status, and original domicile and according to host plant choice, feeding habits and plant damage. The analyses were focused on 472 species of insects and mites, many of which was generalist in infestation, and that warrant attention because of their agricultural importance. As a result, it was noticed that there were eleven orders of insects and mites of agricultural importance in South Africa which included, twenty-five most important families of insects and mites. Lepidoptera (31.0%), Homoptera (29.8%) and Coleoptera (14.2%) account for 75% of the pest problems on cultivated plants (Moran, 1983). However, it was added that indigenous and introduced species of insects and mites are about equally important on alien crops. Among the pest insects, more than 78%, are polyphagous species. These results are quite similar when the analysis of host choice is done in accordance with the pest status.

Cucurbits

The name, Cucurbitaceae, is likely to be derived from Latin word *corbis* meaning basket or bottle (Prazak, 1941), reflecting the usage of their fruits. Cultivation of cucurbits as the vegetable source began roughly 3000 years ago. From India, the cucumber was carried to Greece and Italy, where it played a pivotal role throughout the Roman Empire. From Rome, it spread to China and southern Russia and then to Europe by the Romans. The first records of cucumber cultivation were in France during the 9th century, then Great Britain (14th century), followed by Caribbean (late 15th century) and North America (mid 16th century) (Dhiman and Chawla, 2005). The world's largest producers of cucurbits are China, India, Iran, Turkey, Egypt and USA. Among them China is the world's leading producer of the major cucurbits, exporter of fresh fruits, watermelon, and squash seeds (Maynard et al., 2001).

The Cucurbitaceae, the largest family of vegetable crops originated from tropical parts of the world (Jeffrey, 2005) but most of its known genera were originated from Asia, America and largely Africa. Cucurbits are categorized mainly into two groups: Zanonioideae and Cucurbitoidea. The subfamily Cucurbitoideae mainly consists of crop plants, particularly vegetables (Maynard and Maynard, 2000). The actual number of the genera of Cucurbits is still in controversy. According to Chakravarthy (1982) Cucurbitaceae include 117 genera and 825 species and Yamaguchi (1983) reported 100 genera and more than 750 species. Subrahmanyam (2004) reported 100 genera and 800 species along with Mabberley (2008) who confirmed 122 genera and 940 species distributed along tropical and warm temperate regions of the world (Fapohunda et al., 2018).

Asia dominates the world production of cantaloupe along with other melons with more than 71% of the total tonnage followed by Europe, North America and Central America, which accounts for more than 20% of the total global production (Esquinas-Alcazara, 1983). According to the available checklists of the Cucurbitaceae, India possess 94 described species (of which 10 are endemic) varieties included under in 31 genera. Among them, *Trichosanthes* with 22 species, *Cucumis* with 11 (all but two wild), *Momordica* with 8, and *Zehneria* with 5 are the most species-rich genera. From evolutionary point of view, India has special status because it harbors a wide range of lineages, including relatively old and phylogenetically isolated.

The cucumber is believed to be originated in India where it has been grown for thousands of years (Zeven and Zhukovsky, 1975). The ancient Egyptians also cultivated cucumbers. Later, cucumbers spread to China and Greece about 2,000 years ago (Whitaker and Davis, 1962; Robinson and Decker-Walters, 1997). Phytogeographically the north eastern and peninsular regions in India have the richest species diversity, while the Jammu Kashmir and Himachal regions have very few Cucurbitaceae (Renner and Pandey, 2013). Cucurbitaceae is highly specialized, medium sized and largest family of summer climbing plants. Cucurbitaceae family contains about 100 genera with 800 species which are mostly tropical or subtropical in distribution, with a few species in temperate climate (Subrahmanyam, 2004). The cultivated species under investigation belong to the genera *Benincasa, Cucurbita, Citrullus, Cucumis, Lagenaria, Momordica, Luffa* and *Trichosanthes*. These have considerable economic value and are used as fruits and vegetables, (Purseglove, 1968). Most members of the family Cucurbitaceae are monoecious in nature, and a few are dioecious too. A number of hermaphrodite and andromonoecious cultivars are also reported. These are all summer season crops and are extremely susceptible to frost (Choudhury, 1967).

Among the family, the genera *Cucumis, Cucurbita* and *Citrullus* are considered to have the highest economic importance. Of about 3000 plant species commonly used for human consumption, only 150 species are cultivated extensively, and the elite genus, *Citrullus* is ranked twenty-fourth (Raven et al., 1993). A striking feature in cucurbits cultivation is their adaptation to a wide variety of agricultural environments (Bates et al., 1990). Cucurbits were reported from both New and Old World and were listed as important plant families that supply human with edible products and useful fibers. Cucurbits are classified mainly into five sub-families: Fevilleae, Melothrieae, Cucurbitaceae, Sicyoideae and Cyclanthereae.

Morphological characteristics include:

a) Vines, usually annuals, with five-lobed or palatably divided leaves with long petioles; leaves arranged alternately.

b) Tendrils are spring-like

c) Monoecious

d) Flowers with five fused petals and five stamens (male) and an inferior ovary (female).

e) Fruits large and fleshy, usually with a hard outer covering (a special type of berry termed a pepo).

f) Parietal placentation

g) Many large, fairly flat seeds with two very large cotyledons

h) Hard exocarp

The most important cultivated genera include

- *Cucurbita* – squash, pumpkin, zucchini, some gourds
- *Lagenaria* – mostly inedible gourds
- *Citrullus* – watermelon (*C. lanatus, C. colocynthis*) and others
- *Cucumis* – cucumber (*C. sativus*), various melons
- *Luffa* – the common name is also luffa, sometimes spelled loofah (when fully ripened, two species of this fibrous fruit are the source of the loofah scrubbing sponge)

The Cucurbitaceae is a remarkable plant family, having economic, aesthetic, cultural, medicinal and botanical significance. In the Old and New Worlds, cucurbits were associated with human nutrition and agriculture for more than 12,000 years (Brothwell and Brothwell, 1969; Lira-Saade, 1995). Thus, the Cucurbitaceae, along with Brassicaceae and Asteraceae, are of extraordinary importance to humans as they follow cereals and legumes in their economic significance to human economy (Whitaker and Davis, 1962; Nayar and More, 1998). Shrivatsava and Roy (2013) have highlighted the ethno-medicinal importance of cucurbits and to improve productivity on sustainable basis as it comprises both wild and cultivable varieties (Shrivatsava and Roy, 2013). Cucurbitaceae seeds and fruits possess purgatives, emetics and antihelmintics properties due to the presence of cucurbitacin, a secondary metabolite. Cucurbitacins constitute a group of triterpenoid, well known for its bitterness and toxicity (Dhiman et al., 2012).

Family Cucurbitaceae possess tremendous medicinal properties including anti-HIV, anxiolytic, antipyretic, anti-diarrhoeal, carminative, antioxidant, antidiabetic, antibacterial, laxative, anthelmintic, antitubercular, purgative and hepatoprotective. They are also employed as an abortificient, diuretic and cardiotonic agent. Added to the above, they show strong anti-inflammatory, antitussive, cytotoxic, and expectorant properties (Rajasree et al., 2016).

Insect Pests of Cucurbits

The major insect pests of cucurbits are Fruit fly, Red pumpkin beetle, Flea beetle, spotted beetle, Green stinky bug, blister bee and the major diseases are bacterial wilt, Downey mildew, Cucumber mosaic virus and Anthracnose etc (Marwaha et al., 1998; Neupane, 2002). Red pumpkin beetle (*Raphidopalpa foveicollis*), a very destructive pest of cucurbitaceous vegetable crops, defoliate the cucurbit leaves and cause severe damage in the early stages of the crop. Two species of fruit flies namely *Bactrocera cucurbitae* and *Bactrocera tau* flies lay eggs inside the developing fruit or flower and the developing larvae cause fruit rotting (Neupane, 2002). Striped cucumber beetle, *Acalymma vittatum* Fab found exclusively feeding on cucurbits are considered to be the most important cucurbit pest in the United States (Gould, 1943). The spotted cucumber beetle, *Diabroticaundecim punctata howardi* (Barber), possesses a wide host range of over 200 plants (Radin and Drummond, 1994). They cause damage to cucurbits either by feeding on seedlings, roots, flower or even foliage (Isley, 1927, 1929). The most troublesome insects of cucumber include cucumber beetles (*Diabrotica* and *Acalymma*, vector of bacterial wilt), *Epilachna* beetles, greasy worm (*Agrotis ipsilon*), the melon fruit fly (*Dacus*) and aphids (Welbaum et al., 2014).

In India, *Aulacophora foveicollis and A. attripennis* are the most common cucurbit pests. Their larvae found feeding mostly on roots and stem and the adults are confined to the foliage, petals and fruits causing severe damage. *A. foveicollis* shows the highest preference for cucumber, then pumpkin, bottle gourd, snake gourd and least towards ridge and bitter gourd. The genus *Raphidopalpa* was a synonym of *Aulacophora*, which includes the most important cucurbit pest in India (Sohi and Mann, 1987). Khan and Hajela (1987) reported that the insect pest shows preferences towards many cucurbitaceae species. The choice of preference accordingly was *Cucurbita maxima* followed by Cucumber, *Citrullus vulgaris* Var.*fistulosus*, Loffah and Bottle gourd in the decreasing order. The bitter gourd showed highest resistant against chrysomelids, whereas cucumber muskmelon and watermelon showed moderate and round melon is the susceptible cucurbit (Mehta and Sandhu, 1992).

The insect pests of cucurbit plants cause damage of about 20 to 100 percent of its gross productivity. It was observed that > 50 percent loss in cruciferous fruits was merely due to the pest attack. Sagarika and Suvadip (2017) reported that the devasting major insect pest on Bottle gourd along gangetic plains of West Bengal include *Bactrocera tau* (Melon fruit fly), *Aulacophora foveicollis* (Red pumpkin beetle), *Aphis gossypii* (Melon aphid), *Diaphania indica* (pumpkin caterpillar), *Henosepilachna vigitioctopunctata*, *Epilachna dodecastigma*, *Spenarches caffer* (Bottle gourd plume moth), *Thrips palmi* (Melon thrips) and *Bemisia tabaci* (white fly).

The susceptibility of cucurbits to Tephritids based on fruit damage rates and infestation rates, at different agroecological localities in Southern Cameroon was assessed and found that losses were more on *Sechium edule* and *Cucurbita moschata* at Yaounde and on *S. edule*, *Citrullus lanatus* (watermelon), *Cucumis melo* (sweet melon), *Cucumis sativus* and *Cucurbita moschata* at Koutaba. *Cucumis melo* var. *agrestis* was the least susceptible species at both sites (Mokam et al., 2014).

The winter and summer squash cucumber and watermelon are found to be invariably attacked by striped and spotted cucumber beetles. The larvae of cucumber beetle generally feeds on cucurbit roots and adult beetles cause economically important damage. The striped cucumber beetles are strictly specialists on cucurbits while spotted cucumber beetles are quite generalist, feeding on other plants in addition to cucurbits. In North American cucurbit crops, two species of cucumber beetles, the striped cucumber beetle (*Aclymma vittatum* in the eastern US and *A. trivittatum* in the western US) and the spotted cucumber beetle (*Diabrotica undecimpunctata*) caused destructive effects (Williams et al., 2015).

Cucurbits are generally infested by a variety of insect pests right from the germination till the harvesting stage, but a few of them like red pumpkin beetle, fruit flies and hadda beetle are categorized as serious pest (Gupta and Verma, 1992). Red pumpkin beetle, *Aulacophora foveicollis* (Lucas), a polyphagous pest of cucurbit crops in India (Butani and Jotwani, 1984), attacks cucumber, bitter gourd and sponge gourd equally (Khan and Hajela, 1987; Mehta and Sandhu, 1992). Cucurbitacins are widely distributed in cucurbits, which may play a major role in host attraction. A feeding deterrent was found in bitter gourd, which is a triterpenoid, Glucoside (Chandravadana and Pal, 1983).

The beetles cause the retardation of growth of the seedlings due to severe foliar damage. The beetle resumes its activity during March and continues till October with the peak being April to June and the population declines from September onwards (Butani and Jotwani, 1984). The hadda beetle, *Henosepilachna vigintioctopunctata* (Fabricius), is another serious pest having agricultural importance in Asia, Australia, Africa, East Indies and Japan (Rajgopal and Trivedi, 1989; Hirano, 1994). Its preference was proved towards solanaceous and cucurbitaceous crops of mid-hills and plains in India.

Both the larval and adult beetles scrap and feed voraciously on the green matter of the leaf resulting in skeletonizing it in a characteristic manner leaving the upper epidermal tissue intact. The adults are active fliers and are capable of damaging large crop areas during their peak infestation. The adults usually confined to the upper leaf surface whereas the grubs confined to the lower surface of leaves (Chaudhuri et al., 2001). Hence on severe pest infestation there happens the plant defoliation.

The Tephritidae, a dipteran family consists of nearly 4000 species, of which 700 species belong to the Dacine fruit flies (Fletcher, 1987). Fruit flies are polyphagus and are ranked as one among ten most serious problems of the entire agriculture. Of 207 described species of fruit flies found in India, nine were found to be major pests and economically important (Sardana et al., 2005). The two species namely *Bactrocera cucurbitae* (Coquillett) and *Bactrocera tau* (Walker), commonly known as melon fruit flies, mostly infest the cucurbits.

The findings evidenced that the 45 pests of cucurbits were recorded in Bangladesh, of which 21 arthropod pests (that included 12 insect pests and a mite pests); 19 disease causing pathogens and 5 weeds. Among these insect pests of cucurbits, cucurbit fruit fly was the most damaging compared to others.

Insect Taxonomy

In the Eastern world, earliest pharmacopoeias were written by Shen Nung, Emperor of China around 3000 BC. During 1500 BC, the medicinal plants were illustrated on wall paintings in Egypt. Among the Greeks and Romans, Aristotle (384–322 BC) classified all living things while Theophrastus (370–285 BC) classified all plants; Dioscorides (40–90 AD) attempted on medicinal plants and Plinius (23–79 AD) wrote many books important one include *Naturalis Historia*, a work elaborated in 160 volumes. One of the earliest taxonomic authors was Caesalpino (1519–1603) of Italy, who referred to as the first taxonomist. During Linnaean era, the starting point of modern taxonomy, Carl Linnaeus (1707–1778) is regarded as the man of modern botanical and zoological taxonomy. The initiation of a zoological code started even somewhat later. In 1842, a British ornithologist Hugh Edwin Strickland (1811–1853) elaborated the first nomenclatural laws for Zoology, the Strickland Code. From phenetics to phylogenetics, Charles Darwin (1809–1822) and Alfred Russel Wallace (1823–1913) introduced the evolutionary theory in 1858. Later, Ernst Haeckel (1834–1919) and August Wilhelm Eichler (1839–18878), the two German biologists laid the foundation for the construction of evolutionary trees (Manktelow and Nyberg, 2005).

The taxonomic studies originated in the eighteenth century with Carolous Linneaus work on *Systema Naturae*, first published in 1735. Systematics, in addition to classification and naming, also deals with the relationships and environmental adaptations, thus drawing attention to the evolution as well as phylogeny. The term biodiversity was coined by Wilson (1989) as a miniature of biological diversity, represents the diversity of life at all taxonomic levels including genetic, species, and ecosystem diversity and is the core of natural resources for sustainable development and biotic capital for sustenance of the ongoing life-support system (Lu et al, 2006). Grimaldi and Engel (2005) has estimated about 9,25,000 species of living insects. Systematics Agenda 2000 (Jansen, 1993) clearly restated the taxonomy mission as to discover and describe the Earths species, to place them in a predictive, phylogenetic classification and to make the species data accessible (Janzen and Philips, 2006). The reasons to learn our world’s species diversity are largely self evident, sustainable ecosystem and even the evolutionary lineage is also comprised of species (Wheeler et al., 2004). The comparative morphological, paleontological and molecular studies have confirmed a great deal in insect systematics at higher taxa (Grimaldi and Engel, 2005). According to Wagele (2004), the complexity of characters is one of the most significant criteria of homology. The morphological characters are testable and transparent as they are potentially falsified by contradictory evidences (Rieppel, 2004).

Taxonomy is the branch of science dedicated with discovering, describing, naming, and identifying species or other taxa (Lee, 2002; DeCarvalho et al., 2008). In recent decades, the most significant advances in insect taxonomy are the development of molecular data techniques, specifically the DNA sequence and genomics data so as to analyse the relationships. They have provided evidence where morphological synapomorphies and yielded intact relationships, even overturning the long held ideas (Grimaldi and Engel, 2005). Later on, the Sanger sequencing technology has been replaced by next generation (Next-Gen) sequencing techniques. The next generation parallel sequencing method have resulted in new openings towards genomics and promise to revolutionize the knowledge of interaction between variation, both in the genome and phenotype (Pevsner, 2015).

Classical taxonomy is concerned with four principle orders, which includes generality or resemblance; composition or collectivity; hierarchy; genealogy or evolution (McGhee et al, 2004). Classical taxonomy is the conventional taxonomical method based on external morphological features supported and supplemented by ethological and ecological data. Some taxonomists classify based on phenetic similarities and maximum number of characters (morphological, behavioral, karyological and so on) with equal weightage called Numerical taxonomy. Molecular taxonomy, a relatively recent research branch, includes DNA barcoding, analyses of isosymes, molecular cytogenetics and a number of other such related techniques altogether. DNA barcoding is an accepted taxonomic method using a short genetic marker in the mitochondrial DNA (mtDNA) of an organism for prompt identification of species. Genomic barcoding is regarded as a supplementary tool to the conventional classical taxonomy (especially in differentiating sibling species) and not to replace it completely. The problem, as Grimaldi and Engel (2007) pointed out, is that high tech descriptions (such as molecular taxonomy) are seen by some as more scientific and this view is not acceptable to many scientists who believe that all branches of science is equally important especially when classical taxonomy is still making startling discoveries (for instance discovery of a new insect order recently viz. Matophasmatoidea) even now. As Ogura (1964) pointed out, classical taxonomy will continue to reign supreme complemented with DNA barcoding in many more future years to come (Narendran, 2008).

Hebert et al. (2003) incited the study of molecular diversity considering the inherent limitations of morphology, and the steady decrease in the number of taxonomic specialists. DNA barcoding is a wealth of computable data that in many ways are much easier to work compared to classical taxonomic descriptions.

Voucher specimens are a potential currency for both the taxonomic literature and sequence databases. An obstacle for classical approach is the lack of stable, resolvable specimen identifiers. The number of species on Earth is uncertain and inconsistent, and they show no signs of converging (Caley et al., 2014). According to taxonomic efforts it suggests that two-thirds of all species have already been described (Costello et al., 2011). Contrast to the widely scattered taxonomic knowledge, most of the genomic information is highly centralised and stored in the three separate components of the International Nucleotide Sequence Database Collaboration (INSDC), namely GenBank, EMBL and the DDBJ (Benson et al., 2012). DNA sequences are unique for each species; they can be viewed as genetic barcodes and have the potential to solve various problems inherent to the kind of taxonomy practiced. The mitochondrial cytochrome c oxidase I (COI) gene as barcode source was originally proposed so as to identify and delimit animal species (CBOL, Consortium for the Barcode of Life). The organization aims for eukaryotic mitochondrial COI, which was chosen for animal barcoding because of so many advantageous characteristic like insertions and deletions are very rare, availability of universal primers for amplification, and it possess a great range of phylogenetic information with fast rate of nucleotide substitution that enable the discrimination of extremely cryptic species (Hebert et al., 2003).

The contemporary systematics of insects, based on morphological traits is limited both by high variability and complications in comparisons of remote taxa having low number of common traits (Polukonova and Karmokov, 2013). The generally depending morphological data are often time consuming and need taxonomic specialists, whereas DNA barcoding techniques is a uniform and practical method of species identification in insects, irrespective of their developmental stages. Molecular systematic studies initiated since 1970, for the first time ribosomal RNA were used for the classification of bacteria (Fox et al., 1980). Over the time, the application of DNA sequence data both in taxonomy and species diagnosis has aroused a great deal of controversy, but the general agreement convince that genetic information is inevitable for associating different developmental stages of organisms and for identifying morphologically impotent or partially preserved specimens

(Vences et al., 2005; Wheeler et al., 2004; Will et al., 2005). Mitochondrial markers as well as DNA and protein markers reveal phylogenetic relationships among related groups and taxa. The mitochondrial DNA (mtDNA) is the most widely accepted molecule in phylogenetic analyses of eukaryotes. The mitochondrial genome is double stranded circular DNA molecule, which range around 14 kb to 19 kb in size (Beckenbach and Joy, 2009; Boulton-Lewis et al., 1995), present in multiple copies in each and every cell and is also maternally inherited. It possesses genes of different functions and hence have different rate of evolutionary divergence - quite conserved while the others more variable and most of the nucleotide substitutions occur at neutral sites. Mitochondria are under strong selection and evolve under unusual evolutionary rules when compared with the other genomes. Hurst and Jiggins (2005) suggested that selection can act directly on the mtDNA, or can also arise indirectly from disequilibrium with other maternally transmitted DNA sequences. The mitochondrial DNA, considered to be a neutral molecular marker that reflecting the history of the species, Ballard and Whitlock (2004) and Bazin et al. (2006).

Molecular traits of taxa, are also revealed by use of cytochrome-c-oxidase I (COI), these are less variable and more uniform. With respect to the genetic marker, the intra- and inter-phylogenetic relationships were analysed by using the sequence data obtained from the COI marker gene amplification. Relative homogeneity is maintained by concerted evolution, where the mutations rapidly spread to other members of the gene family located at different chromosomes (Arnheim, 1983; Gerbi, 1986; Tautz et al., 2002). The absence of introns, simple alignment, limited exposure to recombination and the availability of robust primer sites makes the COI as an ideal marker for species-identification in insects (Hebert et al., 2003a).

The COII is also a frequently used marker among mitochondrial genes in phylogenetic analyses of several arthropods especially in insects (Liu and Beckenbach, 1992; Simon et al., 1994). Extensive data on COII gene contributed in Collembola (Carapelli et al., 1995; Frati et al., 1997) is useful to reconstruct relationships between species and genera of Arthropleona. Various species among animal groups have been discriminated reliably using different fragments of the mitochondrial gene, cytochrome c oxidase 1 (COI) (Hebert et al., 2003, 2004; Hogg and Hebert, 2004).

Among insects, the mitochondrial genome is circular with size ranging from 15 to 20kbp approximately, and an A+T rich control region showing substantial length variation among taxa. Advances in method of data generation and analysis have led to accumulation of large amount of DNA sequence data from most major insects group. This helps easier comparison of relationship and evolution. The cytochrome oxidase I (COI), cytochrome oxidase II (COII), 16S rRNA, 18S rRNA and Elongation Factor-1 (EFI) genes are widely used and informative in wide range of mitochondrial divergence in insects. These are used as standards for insect molecular systematics. Insect mitochondria contains two rRNA genes encoding 12S and 16S ribosomal RNA in which the former is used for resolving diversity in phyla while the latter is used for families or genera. The phylogenetic status of *Dactylopus* of Mexico using 12S rRNA sequence and the phylogeny of termites, cockroaches as well as damselflies using 16S rRNA sequence are the classical examples (Kambhampati, 1995, 1996). Among the different marker genes in mitochondria, protein coding genes are known to be having faster evolutionary rates compared to rRNA gene sequences. They are classified into good (ND4, ND5, ND2, Cyt b and COI), medium (COII, COIII, ND1) and poor (ATPase 6, ND3, ATPase 8 and ND4) on the basis of resolving evolutionary relationships (Zardoya & Meyer, 1996). Recent advances in high-throughput DNA sequencing technology (Shendure et al., 2004) and reductions in costs (Hajibabaei et al., 2005) have made the generation of large volumes of DNA data much more straightforward (Jalali et al., 2015).

In animals, species boundaries are successfully established using barcode analysis. Variation in the divergence threshold is used to diagnosis species and detection percentage between congeneric species rapidly increases beyond 1-2% (Hebert et al., 2003). The COI barcoding is an effective tool for protistologists capable of differentiating closely related ciliate species. The COI barcoding for species identification of the genus *Tetrahymena* showed divergence by <1% belong to same species and >5% belong to different species (Chandini et al., 2011). The partial (780 bp) mitochondrial cytochrome oxidase I subunit (COI) and nuclear 18S rRNA (1780 bp) sequences were directly compared to assess their relative usefulness as markers for species identification and phylogenetic analysis of coccidian parasites (Phylum: Apicomplexa). The observations demonstrated that partial COI sequence provides more synapaomorphic characters at the species level than 18S rRNA sequences from the same taxa. It can be concluded that COI performs well as a marker for the identification of coccidian taxa (Eimeriorina) and will make an excellent DNA 'barcode' for coccidian (Ogedengbe et al., 2011). Two molecular identification techniques like PCR and RFLP were used for differentiating six Lepidopteran pests infesting apples in Korea (Shresta et al., 2009). A 489 bp sequence of COI showed variation in their DNA sequence with 142 mutation consists of 56 transition, 66 transversion and 20 mutation with both transition and transversion.

Extraction and amplification of specific DNA from insects, including eggs and larvae, has no technical challenge (Ball and Armstrong, 2006). Barcoding of water beetles was done by Monaghan and Haussmann (2006) using the genus *Copelatus* from Fiji. Four DNA markers (three mitochondrial regions namely COI, cytochrome b, and 16S rRNA and the nuclear histone 3 gene) used for sequencing for 118 specimens collected from 20 islands (Floyd, 2009). The accurate identification of mosquito species has been done through allozyme electrophoresis, DNA hybridization and restriction fragment length polymorphism (RFLP) (Fanello et al., 2002). Sequencing-based approaches have also been used extensively, focusing mainly on genes other than mitochondrial COI (Kent et al., 2004; Marrelli et al., 2005; Michel et al., 2005). However, recent studies have exerted that the standard COI barcode marker serves effectively for species-level discrimination in surveys of Canadian (Cywinska et al., 2006) and Indian (Kumar et al., 2007) mosquitoes. Foley et al (2007) constructed a molecular phylogeny data of the Australian *Anopheles annulipes* species complex based on four different loci, including both nuclear and mitochondrial (COI, COII, ITS2, and EF-1α). Molecular barcodes enabled the linking of the various life stages of the Lepidoptera, both the males and females of sexually dimorphic species (Janzen and Philips, 2006). This advancement is particularly very relevant for the identification of pest and invasive species (Balland Armstrong, 2006).

3

Materials and Methods

1. Insect collection, identification and preservation

The adult specimens of insect pests were collected from almost all the districts of Kerala on the basis of occurrence and reports. Collections were done using active collection methods mainly sweeping net method, hand picking and passive collection methods like colour trap, yellow pan trap, sticky trap, light trap (Jackman and Drees, 1998). The preliminary morphological identification was done by using authentic identification keys and guides. The identified specimens were photographed and stored at -20º C in the repository of Molecular Biology Laboratory, Department of Zoology, University of Calicut as voucher specimens for future references.

2. Mitochondrial DNA Extraction

The genomic DNA was extracted using commercially available genomic DNA preparation kit following manufacturer’s instructions. The insect specimens were taken out, washed primarily in running water and then 2 – 3 times in distilled water. One of the legs of each specimen was grounded using mortar and pestle and complete tissue lysis was done with Proteinase K, incubating the tissue at 56° C for 1-3 hours (Shere-Kharwar et al., 2013). This method provided a non-destructive way for extracting DNA that involves soaking samples in Guanidinium hydrochloride (GuHCl) with subsequent adsorption of DNA to silica (Rohland et al., 2004). Silica gel binds tightly towards the positively charged silica particles. After centrifugation process, DNA molecules were eluted under low strength by Tris-

EDTA buffer (TE buffer) or distilled water for permanent storage of DNA (Esser et al., 2006). The DNA isolated was confirmed using 1% agarose gel electrophoresis.

3. Primer designing

Primer designing is essential for a successful PCR reaction. It requires a brief sequence of dNTPs to the DNA polymerase to work on and additionally allows in restricting the amplification in the desired target regions. Usually the primers are 18-25 bases in length and are complementary to the end of the regions of DNA to be copied. The cocktails of specific forward and reverse primers were designed. Cytochrome oxidase subunit I gene (COI) sequences of various related groups of insects were fetched from GenBank using BLAST programme of NCBI and primers were designed using Primer 3 software (Untergasser et al., 2012). The details of the primers specifically designed and used for PCR amplification in the present study is represented in Table 1.

Table 1: The list of specific primers used for PCR amplification of the present study

Sl. No.	Name of the primer	Direction	Sequence description
1	LEP	Forward	5'- CATTGGAGATGACCAAATTTATAATG - 3'
		Reverse	5'- TGAAATTAATCCAAATCCAGGTAAA - 3'
2	BTL	Forward	5'- ATTCAACCAATCATAAAGATATTGG -3'
		Reverse	5'- TAAACTTCTGGATGTCCAAAAAATCA -3'
3	BUG	Forward	5'- GGAATAGTAGGATCAGCAATAG - 3'
		Reverse	5'- GGATCTCCTCCTCCTGAAGGATC - 3'
4	CP2	Forward	5' – GGTCAACAAATCATAAAGATATTGG – 3'
		Reverse	5'- TAAACTTCTGGATGTCCAAAAAATCA -3'

4. PCR amplification and DNA sequencing

The mitochondrial cytochrome oxidase subunit I (COI) gene of the collected specimen was amplified separately using the specific set of forward and reverse primer. The PCR reaction mixture consisted of 2ng of genomic DNA (1µl), 1µl each forward and reverse primers at a concentration of 10 µM, 2 µl 10X reaction buffer(with $MgCl_2$), 2 µl of dNTPs (2 mM), 0.20µl Taq polymerase (5 U/µl) and 12.8 µl distilled water. The PCR profile consisted of an initial denaturation step of 5 min at 95° C, followed by 30 cycles of 10 sec at 95° C melt, 1 min 50 sec at 50° C anneal and 1 min at 72° C extend, ending with a final extension phase at 72° C for 3 minutes. The PCR products were resolved on 2% Tris Acetate EDTA (TAE) – Agarose gel, stained with Ethidium Bromide (Russell and Sambrook, 2001) and documented using a gel documentation system. A 1Kb DNA Ladder (Thermo Scientific GeneRuler, Product No SM 0242) was used to determine the size of the product. The PCR amplified product was portrayed by different size of DNA band depending up on the set of primers used. The PCR product was column purified using Gene JET^{TM} PCR Purification Kit (Fermentas Life Science Inc), designed for rapid purification of single stranded or double stranded PCR amplification products from other components in the reactions such as the primers, dNTPs, unincorporated labelled nucleotides, enzymes and salts from the PCR products. The purified product was again resolved on 2% agarose gel to confirm the presence of amplified DNA.

The PCR amplified DNA was mixed with binding buffer and added to the purification column. The chaotropic agent in the binding buffer denatures proteins and promotes DNA binding to the silica membrane in the column. Binding buffer contained a colour indicator which allows for easy monitoring of the solution pH for optimal DNA binding (Boom et al., 1990). The impurities were removed by a simple wash step. Purified DNA was then eluted from the column with elution buffer. The sequencing of the purified DNA template from both ends using the Sanger’s dideoxy chain termination sequencing method (Sanger and Coulson, 1975) was done at Agrigenome Laboratories Ltd., Cochin with ABI 3730XL automated sequencer. By sequencing from each ends using forward and reverse primers it was feasible to urge longer sequences than by employing a primer in one direction.

5. Alignment and analyses of DNA sequences

The sequence information records containing Electropherogram were analysed with the aid of a reader-kind programme (Finch TV) for checking and annotation of forward and reverse primer sequences. Annotated sequences were imported and primer sequences had been removed from the beginning and the end of the obtained sequence and sequence ambiguities had been resolved. The COI sequences obtained were multiple aligned using ClustralW (Thompson et al., 1994) programme. The aligned COI sequences have been translated to amino acids to assess for the presence of premature stop codons that indicate the presence of nuclear pseudogenes or sequencing errors. The FASTA format of the final sequence was used to search for its similarity utilising the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1997) of NCBI (http://www.ncbi.nlm.nih.gov). The BLAST search identifies the sequences which are homologous to the query sequence acquired by the present study. The nucleotide sequences obtained in the study were deposited in the public databases and have been assigned with accession numbers in NCBI GenBank (National Centre for Biotechnology Information, USA) of INSDC (International Nucleotide Sequence Database Collaboration).

6. Phylogenetic analyses

Final nucleotide sequences were analyzed using the Molecular Evolutionary Genetics Analysis version 6 (MEGA6) software specifically designed for statistical analysis of sequence data (Tamura et al., 2013). The interspecific and intraspecific genetic diversity were generated using Kimura 2 parameter model, and a phylogenetic tree was generated using the Neighbor – Joining algorithm (Saitou and Nei, 1987). Bipartitions in the Neighbor – Joining tree were examined by bootstrap analyses over 500 replicates (Felsenstein, 1985). This bootstrap analysis was important for calculating the confidence interval of monophyletic groups within phylogenies. Percentage nucleotide distances calculation were performed using MEGA6 software. The results were depicted in the form of respective figures.

4

Coleoptera

The Coleoptera are endopterygotes (insects with complete metamorphosis), having mesothoracic wings modified into hard elytra or into haltere-like structures. They have biting type mouthparts and the head has a distinct gular region below. They represent the largest group of organisms on Earth, with over 3,50,000 described species. The word Coleoptera was originated from the Greek words *koleos* (means sheath), and *ptera* (means wings). Of the two pairs of wings, the first pair is enlarged and thickened into a pair of hard sheaths, or elytra, that cover the delicate, membranous and large hind wings. Their 2-5 segmented tarsi are the important morphological character for taxonomic identification (White, 1983). Adults are oligophagous with biting and chewing mouthparts. The feeding habits of beetles are extremely varied and, being holometabolous, adults and larvae may feed on different food resources. Many beetles play vital role in the decomposition of plant and animal matter, some act as predators of insects (hence important biological control agents) and others act as pests of crops, stored foods and other products (Bouchard et al., 2011).

The production of cucurbits is drastically affected by the onset of diseases and insect pests, inadequate availability of quality seeds, lack of maintenance of genetic varieties and naturally occurring biodiversities, and the lack of knowledge on the international standard of quality production and postharvest handling (Nath and Velu, 2006). The major insect pests included are Fruit fly, Red pumpkin beetle, Flea beetle, spotted beetle, Green stinky bug; and the major diseases are Bacterial wilt, Downey mildew, Cucumber mosaic virus and Anthracnose etc (Marwaha et al., 1998; Neupane, 2002). Red pumpkin beetle (*Raphidopalpa foveicollis*) is a very destructive and notorious pest of cucurbitaceous vegetables. Red and blue pumpkin beetles cause defoliation of the cucurbit leaves and can cause severe damage at early stage of the crop production. Striped cucumber beetle, *Acalymma vittatum* Fab. (Coleoptera: Chrysomelidae), potent pest of cucurbits, feeds exclusively on cucurbits, studies from United States (Gould, 1944). The spotted cucumber beetle, *Diabroticaundecim punctata howardi* (Barber) (Coleoptera: Chrysomelidae), known widely as the southern 8 corn rootworm, possesses a host range of about 200 plants (Radin and Drummond, 1994). The insect pests cause damages to cucurbits in four ways by: feeding on seedlings, feeding on roots, damage flower and foliage and transmission of *Erwinia tracheiphila* E.F.Sm., causing bacterial wilt (Isley, 1929). *Aulacophora foveicollis* Lucas (Coleoptera:Chrysomelidae) and *Epilachna dodecastigma* (Wied.)(Coleoptera: Coccinellidae) are herbivore pests of *Momordica cochinchinensis* Spreng (Cucurbitaceae) ever recorded. It is reported that *Aphtona* sp. (Coleoptera, Chrysomelidae) attacks cucurbits, *Empoasca decipiens,* Paoli (Homoptera, Cicadellidae) attack squash and cucurbits. Out of the harmful insect species *Bruchidius kamtshaticus,* Mast (Coleoptera, Curculionidae) was recorded for the first time from Turkey. Flea beetle attacks cucurbit crops. Flea beetles feeds up on cotyledons, stems, and foliage (Metcalf and Metcalf, 1993).

The systematic position of coleopteran pests of cucurbits collected and identified during the present study is given below:

Order: Coleoptera; Suborder: Polyphaga

1. **Superfamily: Chrysomeloidea**
    - **1.1. Family: Chrysomelidae**
        - **1.1.1 Subfamily: Galerucinae**
            - *Mantura chrysanthemi* (Koch, 1803)
            - *Mantura rustica* (Linnaeus, 1766)
            - *Aulacophora foveicollis* (Lucas,1849)
            - *Aulacophora frontalis* (Baly,1888)
            - *Aulacophora lewisii* (Baly,1888)
            - *Sphenoraia bicolor* (Hope 1831)
        - **1.1.2. Subfamily: Eumolpinae**
            - *Paria thoracica* (Wilcox, 1954)
            - *Rhabdopterus praetextus* (Say, 1824)
        - **1.1.3. Subfamily: Cassidinae**
            - *Oocassida pudibunda* (Boheman, 1856)
            - *Dactylispa carinata* (Chen and T'an, 1961)
2. **Superfamily: Coccinelloidea**
    - **2.1. Family: Coccinellidae**
        - **2.1.1. Subfamily: Epilachinae**
        - *Henosepilachna septima* (Dieke,1947)
        - *Henosepilachna vigintioctopunctata* (Motschulsky, 1857)
        - *Epilachna septima* (Dieke, 1947)
3. **Superfamily: Cucujoidea**
    - **3.1. Family: Nitidulidae**
        - **3.1.1. Subfamily: Carpophilinae**
        - *Carpophilus marginellus* (Motschulsky, 1858)
        - **3.1.2. Subfamily: Epuraeinae**
        - *Epuraea luteolus* (Erichson, 1843)

A brief description on the relevant higher taxa and the taxonomic key prepared for classification and morphological identification (with the help of suitable identification guides and expert consultation) of all the coleopteran pests of cucurbits collected during the present study are given below:

Key to the suborders of Order Coleoptera

1. Hind coxae large, immovably articulated to metasternum, tarsi nearly always filiform and 5-segmented; completely dividing first visible abdominal sternite; first three visible abdominal sternites connate; prothorax with notopleural sutures usually distinct; antennae nearly always filiform and 11-segmented, maxillary galea 2-segmented, palp-like; length never less than 1.5mm. .... **ADEPHAGA**

– Hind coxae movably articulated to metasternum, tarsi various; non-completely dividing first abdominal sternite; prothorax rarely with distinct noto-pleural suture; antennae structure various, not filiform; maxillary galea not palp-like; length less than 1mm...**POLYPHAGA**

SUBORDER: POLYPHAGA

Polyphaga represents the largest and most diverse suborder among beetles. It comprises 144 families in 16 superfamilies, with over 300,000 described extant species. It displays an enormous variety in both specialization and adaptation of life. The name *polyphaga* is derived from two Greek words: *poly* (many) and *phagein* (to eat), meaning as the ‘eaters of many things’. The key characteristics of Polyphaga includes the hind coxa (base of the leg), non divided first and second abdominal/ventral plates (sternites) and the absence of the notopleural suture (found under the pronotal shield) in prothorax (Johnson et al., 2004).

Key to the superfamilies of Suborder Polyphaga

1. Tarsi pseudotetramerous on all legs, fourth true segment extremely minute; if antennae clubbed, head rostrate or basal abdominal sternites connate; front coxal cavities wholly or partially closed behind, hind coxae flat or slightly inclined; prothorax often without distinct side borders; abdomen rarely with more than five visible sternites **2**

– Tarsi pseudotrimerous or rarely pseudotetramerous, antennae clubbed, antennal club rarely involving more than three apical segments, head not roetrate; front coxae transverse or rounded; prothorax with distinct side borders; all abdominal sternites free, tergite of abdominal segment 8 usually hidden ....**Cucujoidea**

2. Antennae filiform or slightly thickened apically, not inserted in grooves or pits; head not or slightly rostrate; gular sutures distinct and separate; middle coxal cavities rarely closed outwardly by sterna, if so all abdominal sternites freely articulated .... **Chrysomeloidea**

– Antennae more or less clubbed, often geniculate, inserted in pits or grooves ; head more or less rostrate, gular sutures nearly always obsolete or confluent (fig. llO); middle coxal cavities almost always closed outwardly by sterna ; abdomen with at least two basal stemites more or less connate ....**Curculionoidea**

SUPERFAMILY: CHRYSOMELOIDEA

Superfamily Chrysomeloidea, with more than 63,000 described extant species; comprises the families Chrysomelidae, Cerambycidae, Megalopodidae, Vesperidae, Oxypeltidae, Lacordaire, Disteniidae and Orsodacnidae (Hunt et al., 2007). Chrysomeloidea is considered to be the sister group of superfamily Curculionoidea, the group of weevils (Marvaldi et al., 2009; McKenna et al., 2015). They together form a clade informally known as the Phytophaga, which is the largest and most diverse radiation of phytophagous beetles, with around 125,000 described extant species. It occupies second largest lineage of phytophagous animals next to the order Lepidoptera (Grimaldi and Engel, 2005). Superfamily Chrysomeloidea includes mostly wood or plant feeders with varied body shape and non-clubbed antennae beetles. Several species in this superfamily are important as pests of crop plants.

FAMILY: CHRYSOMELIDAE

Chrysomelidae (leaf beetles), which were formerly included under the family Bruchidae (presently Bruchinae). The word *Chrysomela* (pl. *Chrysomelidae*) was derived from Greek *khrusomelon* meaning ‘golden apple’. Among phytophagus families, the leaf beetles positioned the second in number to Curculionidae. There reported more than 35,000 species spans in 2,000 genera worldwide (Medvedev and Iwan, 2006; Hieke, 1968; Verma and Jolivet, 2008). The key characteristics defining the family include small, oval shaped body, variation in colour, short elbowed beak; adults having an apparent 4-4-4 tarsal formula, the third tarsal segment are bifid and notched for the terminal segment (Costa et al., 2000; Jolivet et al., 2008). It was known since the beginning of last century that the leaf beetles are serious pests of cereals (Vereschagin and Baryshnikov, 1989). A large number of the Chrysomelidae are the pest insects of both agricultural and forest fields. The damage caused rarely leads to the total loss of host plants but worsen the physiological condition consequently result in decreased plant growth, harvest and productivity. The larvae are voracious feeder on varied host parts and their attack to the plant is to various degree (Jurado et al., 2009; Garzia et al., 2008; Descampe et al., 2014). The most featured particularity of the leaf beetles is relatively limited trophic range, ie, every pest species determine solid trophic links to the damaged species and they possess extremely varied biology. Leaf beetles are the most destructive pests of crops and ornamental plants (Jolivet et al., 2008).

Key to the subfamilies of Family Chrysomelidae

1. Head vertical or pro-erect, Mouth anterior in position **2**

– Head bent inwards, mouth not anterior and hidden, maxillary palpi two segmented, rotundate or oval form with explanate margin all around; antennae and legs non-visible in repose ....**Cassidinae**

2. Antennae not widely separated at base, elytra more or less soft texture, anterior coxae conically prominent at apex................................. **Galerucinae**

– Antennae widely separated at base, elytra with hard texture, thorax with distinct lateral margins, eyes not prominent, prosternum broad, last joint of tarsi deeply bilobed, thorax narrower than elytra at base, legs not compressed ....**Eumolpinae**

SUBFAMILY: GALERUCINAE

Galerucinae (cucumber beetles), one of the largest groups of leaf beetles is a key group to study the phylogeny of polyphaga (Yang et al., 2017). There exists a special relationship between *Galerucinae* and its host plants, which makes the group as a good model to study the evolution of herbaceous beetles, the co-evolution of insect fauna with plants (Futuyma and McCafferty, 1990) and the evolutionary mechanisms of biodiversity (Farrell et al., 2001). Adult *Galerucinae* have oval to oblong body, with the head inserted into the prothorax. The mouthparts directed forwards and downwards; not flattened. The antenna has eleven segments; the antennal insertions are situated close together in front or between the eyes. Frontal tubercles well developed and the elytral sensilla patch is single (Nadein and Bezdek, 2014). The key morphological character used to distinguish *Galerucinae* is the metafemoral extensor tendon (MET) in the hind femora (also known as metafemoral spring, metafemoral apodeme, or Maulik’s organ), a structure that helps large jumps for predator evasion (Furth and Suzuki, 1990; Nadein and Betz, 2016). Both the adults and larvae of *Galerucinae* are herbivorous (on both monocotyledon and dicotyledon plants) and highly host specific. In addition, many species are used for biological control of weeds or are important pest species of agriculture (Morton and Vencl, 1998; Alonso-Zarazaga et al,2014).

Key to the species of pests collected from Subfamily Galerucinae

1. Elytra dark brown or blackish with metallic tinge, elytral tips black and brown, striated elytral punctures, base of pronotum as wide as front of elytra .... **2 *(Mantura)***

– Elytra black and shining, ventral surface black **3**

2. Dark brown with metallic bronze reflection or sometimes reddish brown with a weaker reflection; shiny, relatively rounded – oval, pronotum with two short grooves (sometimes faint) running forwards from base, 1.8-2.7mm size .... ***M. chrysanthemi***

– Metallic green or bluish head and pronotum, elytra dark and reddish brown, usually with the third apical except the suture, yellowish; sometimes entirely dark reddish brown, rarely dark blue or blue green with yellow tips, 2.0-2.8mm size .... ***M. rustica***

3. Anterior coxal cavities closed behind, Tarsal claws appendiculate or simple, Pronotum without a distinct fovea ....***Sphenoraia bicolor***

– Anterior coxal cavities open behind, Tarsal claws bifid, Pronotum with a transverse depression, Elytral epipleuron narrowed behind basal one-third .... **4 *(Aulacophora)***

4(1). Upper surface of elytra generally shinning, abdominal sternite black insect smaller in size; length 6.75 mm, breadth 3.5 mm, in the male humerus covered with erect hairs ....***A. foveicollis***

4(2). Whole elytron black, ventral surface black, antennomeres III to V triangular in case of males, antennomere III longer than wide and vertex having longitudinal grooves in males and in females antennomeres VII to X relatively slender...***A. frontalis***

4(3). Whole elytron black and shinning, ventral surface black, antennomeres III to V filiform in either sexes, antenna of male wider than that of females,apical margin of abdominal ventrite V sinuate in females............................***A. lewisii***

SUBFAMILY EUMOLPINAE

The Eumolpinae includes > 500 genera and 7000 species (Gomez et al., 2005; Jolivet et al., 2008). Eumolpinae can be recognized as oval, and convex in form, and measure up to 10 mm in size. And the coloration for this subfamily of beetles ranges from bright yellow to dark red with spotting. Many species are iridescent or brilliantly metallic blue or green in appearance (Bouchard et al., 2011).They can be recognized by their rounded thoraces, more or less spherical or bell-shaped, and significantly narrower compared to the mesothorax as covered by the and front coxae rounded and third tarsal segment bilobed beneath . Additional features include a small head set deeply into the thorax and well-developed legs. Adult and larvae both occurs during the crop growing season. The adult feeds voraciously on plant foliage which can be detected by the presence of characteristic ‘bored leaf’ damage. The soil inhabiting larvae are more destructive.

Key to the species of pests collected from Subfamily Eumolpinae

1. Anterior margin of prosternum arcuate forming postocular lobe, Pronotum not deeply, coarsely punctuate, Ocular grooves narrow above eyes; colour yellow, orange or black, Elytra entirely black..........................***Paria thoracica***

– Anterior margin of prosternum without lobe, Pronotum without distinct margins, Head without distinct grooves above eyes, Pronotal margin irregular or undulating, Prosternum wide, nearly parallel side..........................
....***Rhabdopterus praetextus***

SUBFAMILY CASSIDINAE

The Cassidinae, tortoise beetles or gold bugs comprised of >3,000 species and over 125 genera with greatest diversity in tropical latitudes (Arnett, 1968). The distinguishing features of cassidines are round outlined body with edges of the pronotum and elytra spreading out over the legs and head. They have colourful and metallic body, with the ability to change the colour which is present below the translucent cuticle. The mouthparts is reduced into a cavity in the head capsule and the legs have four segmented tarsi (Bottcher et al., 2009). The larvae are slow moving showing peculiar defense mechanisms. The concealment of leaf feeders and their defense is enhanced with the accumulation of faeces and exuviae on a coudal fork and used against predators (Eisner et al., 1967). The adults are conspicuous, diurnally active feeding on young and rapidly growing vegetation. The larval feeding leads to rolling of leaves while adult feed by scraping and producing linear scars on the leaves (Prathapan and Konstantinov, 2003).

Key to the species of pests collected from Subfamily Cassidinae

1. Body elongate and oval, colour dirty brown, with a faint greenish tinge, antennae are short; the third joint is a little longer than the second and almost equal to the fourth, pronotum have a groove on its side for antenna reception, the elytra possesses faint longitudinal red stripe all along the suture ....
....***Oocassida pudibunda***

– Body elongate and subconvex; colour yellowish to black; head small, vertex with medial sulcus, interocular space subequal to width of an eye; cleye; eye; clypeus large, generally setose, antennae filiform; reaches to humerus; with 11-antennomeres; antennomere 1 incrassate, without spine, pronotum transverse, lateral margin with 3 spines, anterior angle rounded, anterior margin curved, with branched spine on each side, elytral lateral margin straight with long spines..***Dactylispa carinata***

SUPERFAMILY COCCINELLOIDEA

Coccinelloidea are characterized by the following combination of anatomical features: adults with reduced tarsal formula (4-4-4 or 3-3-3), lack closed radial cell and reduced anal veins in hindwings, hind coxae separated by more than ⅓ coxal width, intercoxal process of abdominal ventrite 1 broadly rounded or truncate (most), when retracted aedeagus rest on side, and (phallobase) tegmen reduced (exception: Coccinellidae). Both the larvae and adult are economically important pest of agricultural fields and orchards (Perry et al., 2007)

FAMILY COCCINELLIDAE

Coccinellids are found worldwide, with over 6,000 species described (Allen et al., 2000; Seago et al., 2011). Coccinellidae is a widespread family of small beetles ranging in size from 0.8 to 18 mm (Seago et al., 2011). Most coccinellids have round to elliptical body with convex dorsum and flattened venter, clubbed antennae.There is usually the presence of a postcoxal line on the first abdominal ventrite. The tarsal formula of most species is 4-4-4 with the third tarsomere minute and tucked within the broad triangular second (cryptotetramerous or pseudotrimerous), only a few have the tarsomeres more equal (truly tetramerous), and some have tarsi reduced to 3-3-3 (truly trimerous). Dome-shaped bodies with six short black legs, head and antennae. Coccinellids are often prominently coloured with yellow, orange, or red and have small black spots on their wing covers. The majority of coccinellid species are generally useful predators, which prey on herbivorous homopterans like aphids or scale insects, which are regarded as important agricultural pests. These coccinellids lay their eggs directly in aphid and scale insect colonies and ensures immediate food source and supply (Verma and Jolivet, 2008).

SUBFAMILY EPILACHNINAE

However, some species are themselves pests, the most prominent are of the subfamily Epilachninae (include Mexican bean beetle), which are herbivorous themselves. The distinguishing feature is antenne inserted between rather than in front, anterior margins of compound eyes present and the tip of the mandibles is multidentate and without basal tooth (Li, 1961). Usually, epilachnines are regarded as minor agricultural pests, feeding the leaves of their host. Their numbers can explosively increase when their natural enemies (parasitoid wasps) number are few. That means there exists inverse proportionality between prey-predator relation.

Key to the species of pests collected from Subfamily Epilachninae

1. Elytral tips angular, brown-yellow, with 14 black spots on each elytron; male genitalia with basal knife, edge of the median lobe half the length of the latter, siphon slightly bent at the apex ....***Henosepilachna vignitioctopunctata***

– Elytral tips rounded, male genitalia not as above **2**

2. Elytral spot No. 4 generally not touching the external margin of the elytra; male genitalia with median lobe not dentulate dorsally; apex of siphon sharply pointed on one side like a nib ....***Henosepilachna septima***

– Elytral spots vary from six to fourteen on each elytron; male genitalia sipho bented near the basal third, apex sharply narrowed on one side and pointed; median lobe with a sudden curve at apex to form a large pointed hook, dorsal side with a blade or knife-edge of nearly half the length of median lobe .... ***Epilachna septima***

SUPERFAMILY CUCUJOIDEA

Cucujoidea families are characterized by the following features: adults with procoxal cavities internally opened (mostly), tarsal formula 5-5-5 in female and 5-5-5 or 5-5-4 in male (rarely 4-4-4), tergite VIII in female is dorsally concealed by tergite VII, tergite X (proctiger) found in male is completely membranous (Lawrence et al., 2011).

FAMILY NITIDULIDAE

Subfamily Nitidulidae, “sap beetles” have been found feeding on flowers, fruits, sap, fungi, decaying and fermenting plant tissues or dead animal tissue (Parsons, 1943, Higashi et al., 2006). They have also been recognized as vectors of fungi (Dowd, 2000). Adult sap beetles are variable in size, 0.9 to 15 mm in length, antennae are usually eleven segmented with the distal three segments forming a club. The clubbed portion, however, is quite variable and peculiar within species (Parsons, 1943). Presence of antennal grooves and the elytra or wing covers entire or sometimes shortened to expose two or three abdominal segments are the other characteristic feature of this family. The tarsal formula is 5-5-5 having five segmented tarsi with normal size first tarsal segment, small fourth and long fifth tarsal segment. The VIII abdominal segment of males is heavily sclerotized, well raised and large and in females it is reduced and submembranous (Dowd, 2000). Several species are well known minor agricultural pests of both field and stored products.

SUBFAMILY CARPOPHILINAE

Subfamily Carpophilinae, have free Labrum, which is usually clearly visible but may be retracted and partly hidden below clypeus; base of antenna is visible or not in dorsal aspect, front usually notched above its point of insertion, the elytra short exposing pygidium and preceding one or two segments. The evenly convex abdomen with sternites 2 and 3 shorter, while sternites 1, 4 and 5 longer (McNamara, 1991; Habeck, 2002).

SUBFAMILY EPURAEINAE

Subfamily Epuraeinae, are small sized species having fine decumbent setae in dorsal surface of body, elytron lacking sutural stria; ventral head with antennal grooves posteriorly convergent, triangular or conical shaped submentum; genital capsule visible along hind margin of pygidium in males; labrum with anterior margin deeply emarginate medially; lateral margins of pronotum and elytra without fringe of fimbriate setae.

Key to the species of pests collected from Family Nitidulidae

1. Body darker, dark rufous to black; length about 3mm, pronotum and elytra with or without setal fringe; Mesosternum without a raised medial line, with auxillary space larger.. ***Carpophilus marginellus***

– Body granulated above, bearing lanceolate or spatulate short setae; length about 4mm, frontal median setae present; Ninth abdominal segment with about 2 granules before each pregomphus, which, when viewed from above, are arranged in an arcuate row with pregomphus; asperities of cephalic portion of tergum irregularly strewn or connected to each other .... ***Epuraea luteolus***

The morphological description, distribution, nature of the damage caused to the crops, analyses on mitochondrial COI gene sequences, molecular evolutionary divergence and phylogenic status of each pest specimen collected under Order Coleoptera during the present study are as follows:

1. ***Mantura chrysanthemi*** (Koch, 1803)

Specimen details:

Voucher specimen	:	CUMC – 01 – A1
Date of collection	:	30-Aug-2017
Locality	:	Thiruvananthapuram: Neyyatinkara
Lat- Lon	:	8.4016° N, 77.0871° E
GenBank accessions	:	MH674105

Description and distribution:

Mantura chrysanthemi are leaf mining forms with a size ranging between 1.8 – 2.7mm without any spots on the body surface (Figure 4.1). They are generally dark brown or red brown with bronze reflection or faint metallic shell, hind femora may be darkened (Saroli et al., 2016). Sexual dimorphism is apparent in the posterior margin of the fifth abdominal ventrite, which is evenly rounded in case of females and lobate in males. The aedeagal length-width ratio is comparatively greater

(Konstantinov and Prathapan, 2008). The elytral punctuation is arranged in rows and the pronotum is with confused punctuation. Antennae of male and female *M. chrysanthemi* are of similar length. Protarsomeres exhibits sexual dimorphism. The first tarsomere in males is larger and broader than females. The ratio of the width of the first tarsomere to the second is 2:1 but in females it is 1:1 (Saroli et al., 2016). They are mainly palaearctic and tropical in distribution.

Figure 4.1: *Mantura chrysanthemi*

Damage:

The larvae bore into the underground stem of young plants / seedlings leading to the crop loss. The adult beetles are often contaminated with bacteria which cause bacterial wilt of cucurbits.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *M. chrysanthemi* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 582 bp and 597 bp long products for the specimens obtained from two different locations. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 4.2 – 4.5 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 4.1.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 4.2 and Figure 4.6 respectively.

>*Mantura chrysanthemi* CDS-2018 / 597 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUMC-01-A1

> *Mantura chrysanthemi*

```
GTTGGGACTTCATTAAGTATATTAATTCGTACAGAATTAGGAGCCCCTGGATCATTAATT
GGAAATGACCAAATTTATAATGTTATTGTAACAGCCCATGCATTCATTATAATTTTTTTC
ATAGTTATACCAATTATAATTGGGGGATTTGGAAATTGATTAGTCCCCTTAATAATCGGA
GCTCCAGATATAGCTTTTCCACGAATAAATAATATAAGATTTTGATTACTTCCTCCTTCC
TTATTTCTACTAATTATAAGTAGAGTGGTCGAAAGGGGTGCTGGAACAGGTTGAACAGTT
TATCCCCCTTTATCTTCTAATATTGCCCACGGAGGATCTTCAGTGGACTTAGCTATTTTT
AGCCTTCATTTAGCAGGAATTTCATCAATTTTGGGAGCAATTAATTTTATTACTACAGTT
ATTAATATACGACCTATAGGAATAACCTTAGATCGAATACCCTTATTTGTGTGAGCAGTA
GTAATCACTGCTATTTTACTTCTTCTATCCTTGCCTGTCTTAGCAGGAGCTATTACTATA
CTTTTAACAGATCGTAATCTAAATACATCATTTTTCGATCCTGCAGGAGGGGGTGAC
```

Figure 4.2: The partial DNA sequence of the mitochondrial COI gene of *Mantura chrysanthemi*

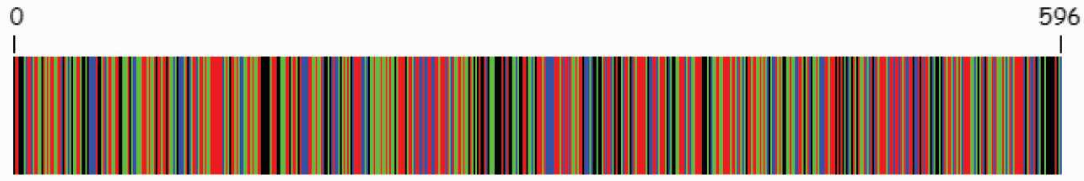


Figure 4.3: Molecular barcode of the mitochondrial COI gene of *Mantura chrysanthemi*

> *Mantura chrysanthemi* / 193AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUMC-01-A1

> *Mantura chrysanthemi*

```
MLIRTELGAPGSLIGNDQIYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMIGAPDMAF
PRMNNMSFWLLPPSLFLLIMSSVVESGAGTGWTVYPPLSSNIAHGGSSVDLAIFSLHLAG
ISSILGAINFITTVINMRPMGMTLDRMPLFVWAVVITAILLLLSLPVLAGAITMLLTDRN
LNTSFFDPAGGGD
```

Figure 4.4: The translation product of the mitochondrial COI gene of *Mantura chrysanthemi*

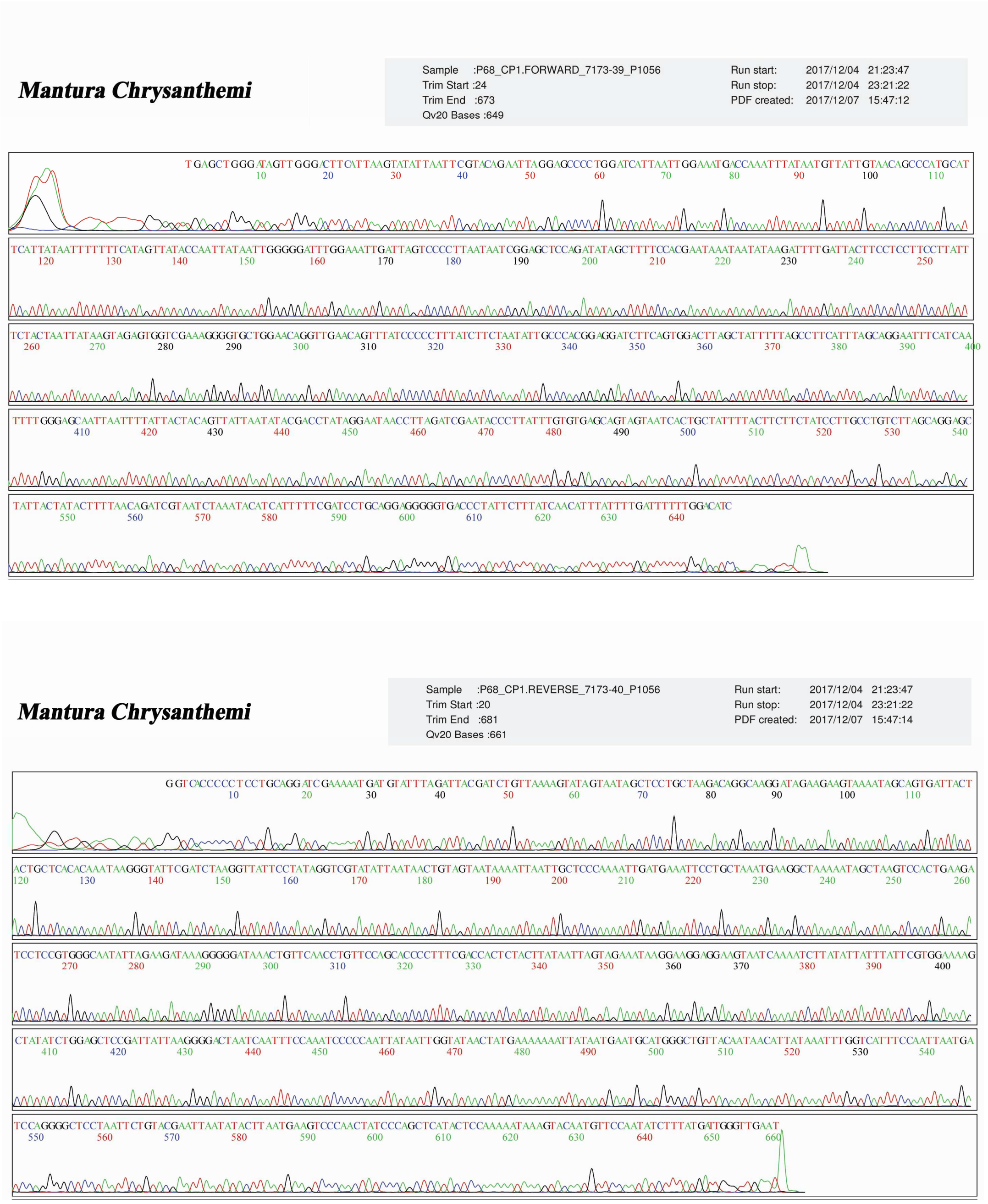


Figure 4.5: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Mantura chrysanthemi* using Forward and Reverse primers.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *M. chrysanthemi* voucher collected during the present study (Table 4.1). It showed bias to nucleotide AT, with nucleotide composition with T = 37.4%, C = 17.2%, A = 29.5% and G = 15.9%.

Table 4.1: Comparison of Nucleotide frequencies of COI gene sequence of *Mantura chrysanthemi* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**																
	T	C	A	G	Total	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH674105 Mantura chrysanthemi	37.4	17.2	29.5	15.9	553.0	37	22.2	20.0	20.5	46	13.0	32.6	8.7	29	16.3	35.9	18.5
KU557525 Mantura sp.	37.4	17.2	29.5	15.9	553.0	37	22.2	20.0	20.5	46	13.0	32.6	8.7	29	16.3	35.9	18.5
KJ677801 Galerucinae sp.	37.8	15.7	31.5	15.0	553.0	36	23.2	20.5	20.0	48	9.2	35.3	7.1	29	14.7	38.6	17.9
KU697451 Aphthona strigosa	37.6	15.6	32.0	14.8	553.0	37	22.2	21.1	20.0	45	9.8	38.0	7.6	32	14.7	37.0	16.8
KC185740 Paridea biplagiata	38.2	15.9	30.7	15.2	553.0	37	22.2	20.0	20.5	46	11.4	34.8	7.6	31	14.1	37.5	17.4
KF653209 Mantura chrysanthemi	36.9	15.4	32.7	15.0	553.0	37	22.2	20.5	20.5	41	10.3	42.9	6.0	33	13.6	34.8	18.5
KC185742 Paridea transversofasciata	38.2	16.1	29.7	16.1	553.0	37	22.7	20.0	20.5	48	10.9	32.6	8.2	29	14.7	36.4	19.6
MG587922 Aulacophora lewisii	37.4	15.7	31.3	15.6	553.0	36	22.7	21.1	20.0	43	10.9	37.5	8.2	33	13.6	35.3	18.5
AB794766 Monolepta sp.	38.2	16.1	29.5	16.3	553.0	37	22.7	20.0	20.5	47	9.8	34.8	8.7	31	15.8	33.7	19.6
LT991425 Ochthebius klapperichi	41.0	14.5	29.3	15.2	553.0	36	23.8	19.5	21.1	53	7.6	32.6	6.5	34	12.0	35.9	17.9
KM446929 Luperomorpha xanthodera	38.2	14.8	30.9	16.1	553.0	36	23.8	19.5	20.5	45	8.7	37.5	9.2	34	12.0	35.9	18.5
MG298995 Tachinidae sp.	40.3	13.4	30.9	15.4	553.0	37	23.8	19.5	20.0	48	7.1	38.0	6.5	36	9.2	35.3	19.6
KU917773 Longitarsus tabidus	38.3	14.5	32.7	14.5	553.0	37	22.2	20.5	20.0	43	10.3	41.3	5.4	35	10.9	36.4	17.9
KM448517 Olibrus liquidus	38.9	16.5	29.1	15.6	553.0	36	24.9	18.4	21.1	48	10.3	33.2	8.2	33	14.1	35.9	17.4
KM446101 Longitarsus anchusae	37.8	15.9	32.5	13.7	553.0	36	23.2	21.1	19.5	45	10.3	38.6	6.0	32	14.1	38.0	15.8

Table 4.2 Percentage of evolutionary divergence of *Mantura chrysanthemi* with its closely related species accessible from NCBI GenBank

Sl. No.	**Accession No**	**Organism**	**Percentage of divergence**
1.	MH674105	*Mantura chrysanthemi* (Kerala)	
2.	KU557525	*Mantura* sp.(Kerala)	0.00%
3.	KF653209	*Mantura chrysanthemi* (Spain)	9.21%
4.	KJ677801	*Galerucinae* sp.	12.96%
5.	KU697451	*Aphthona strigosa*	14.35%
6.	MG587922	*Aulacophora lewisii*	14.37%
7.	KC185742	*Paridea transversofasciata*	14.56%
8.	KC185740	*Paridea biplagiata*	14.76%
9.	KM446101	*Longitarsus anchusae*	15.69%
10.	AB794766	*Monolepta* sp.	15.77%
11.	KM448517	*Olibrus liquidus*	15.94%
12.	KU917773	*Longitarsus tabidus*	15.97%
13.	KM446929	*Luperomorpha xanthodera*	17.04%
14.	LT991425	*Ochthebius klapperichi*	17.21%
15.	MG298995	*Tachinidae* sp.	17.89%

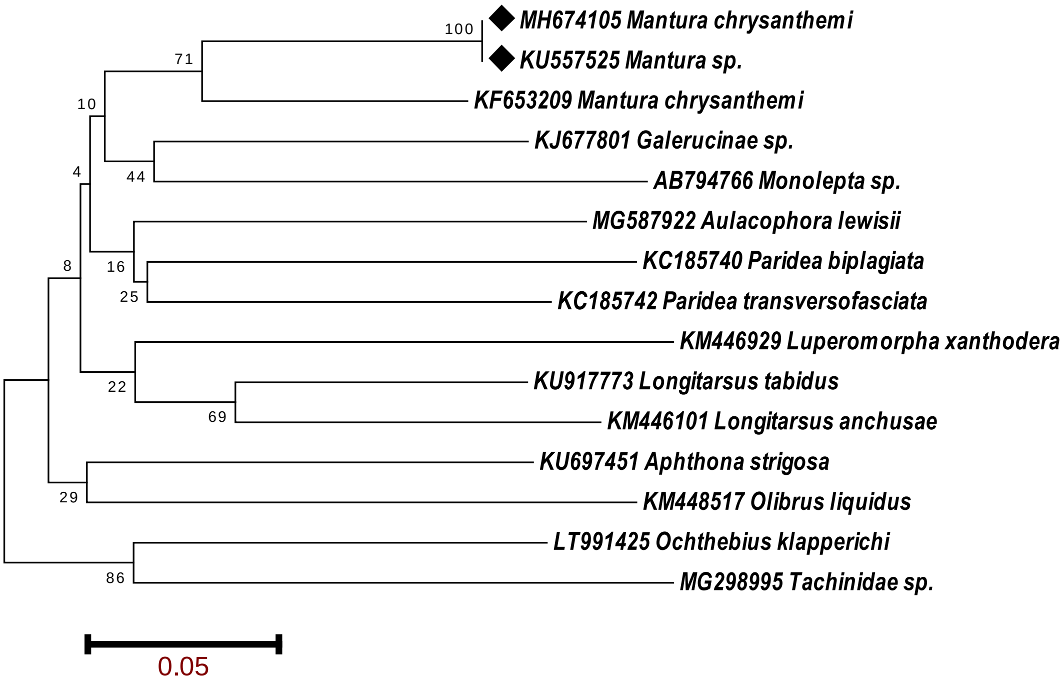


Figure 4.6: Phylogenetic relationship of *Mantura chrysanthemi* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that *Mantura chrysanthemi* (MH674105) has 100% sequence similarity to the *Mantura* species reported from Kerala (KU557525). This species has been found in various geographically isolated areas, their sequence showed variation of 9.21% with the same species from Spain. It has been found that this species represents one of the common species of Palearctic regions. Hence the present study stress that the barcode generated can be used to easily spot the specimen and also to analyse its phylogeny.

The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *M. chrysanthemi* showed bias to nucleotide AT, with following composition of nucleotides T = 37.4%, C = 17.2%, A = 29.5% and G = 15.9%. This greater AT content (66.9%) over GC content (33.1%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *M.*

chrysanthemi showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Even though the COI sequences has been reported from different geographical locations, it showed 0% to 17.89 % differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has no divergence (0%) those from Kerala while have 9.21% from Spain (Table 4.2). On the basis of the data observed this species may be rooted from those found in Spain which diverted into different clades due to geographical variation. Result thus concluded that this species doesn't have any major changes in India while possess changes from those reported from Spain during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *M. chrysanthemi* with related species. *M. chrysanthemi* isolated from Kerala (MH674105) showed 9.21% divergence with *Mantura chrysanthemi* (KF653209) from Spain and 17.89% divergence with *Tachinidae* sp (MG298995) from California. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *M. chrysanthemi* isolated from Kerala. Closest relative of *M. chrysanthemi* collected during the present study is *M. chrysanthemi* from Spain represented within the same clade.

2. ***Mantura rustica*** (Linnaeus, 1766)

Specimen details:

Voucher specimen	:	CUMR – 02 – A1
Date of collection	:	30-Aug-2017
Locality	:	Thiruvananthapuram: Neyyatinkara
Lat- Lon	:	8.4016° N, 77.0871° E
GenBank accession	:	MH674106

Description and distribution:

Mantura rustica are epidermal miners with size ranging between 2-2.8 mm having flattened body and head (Figure 4.7). They are dark reddish brown with rear third of elytra usually yellowish in colour, except suture. Metallic greenish or bluish coloured pronotoum is attached to head, orange to brown coloured leg with darkened hind femora (Cox, 1976). These are Euroasiatic species, distributed in whole Europe, Asia Minor eastwards reaching to Mongolia and China.

Figure 4.7: *Mantura rustica*

Damage:

The larvae are voracious leaf miners. The mines are whitish and serpentine, on the upper leaf surface, with frass in irregular dark lumps and strings . The adult pest feeding results in closely spaced, oval to elongate shaped holes penetrating the host plant leaf giving it a pitted, stippled appearance.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *M. rustica* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 431 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 4.8 – 4.11 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 4.3.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *M. rustica* voucher collected during the present study (Table 4.3). It showed bias to nucleotide AT, with nucleotide composition with T = 36.4%, C = 16.5%, A = 30.2% and G = 16.9%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 4.4 and Figure 4.12 respectively.

> *Mantura rustica* CDS-2018/ 431 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUMR-02-A1

> *Mantura rustica*

```
GAGCCCCTGGATCATTAATTGGAAATGACCAAATTTATAATGTTATTGTAACAGCCCATG
CATTCATTATAATTTTTTTCATAGTTATACCAATTATAATTGGGGGATTTGGAAATTGAT
TAGTCCCCTTAATAATCGGAGCTCCAGATATAGCTTTTCCACGAATAAATAATATAAGAT
TTTGATTACTTCCTCCTTCCTTATTTCTACTAATTATAAGTAGAGTGGTTGAAAGGGGTG
GTGGAACAGGTTGAACAGTTTATCCCCCTTTATCTTCTAATATGGCCCACGGAGGATCTT
CAGTGGAATTAGCTATTTTTAGCCTTCATTTAGCAGGAATTTCATCAATTTTGGGAGCAA
TTAATTTTATTACTACAGTTATTAATATACGACCTATAGGAATAACCTTAGATCGAATAC
CCTTATTTGTG
```

Figure 4.8: The partial DNA sequence of the mitochondrial COI gene of *Mantura rustica*

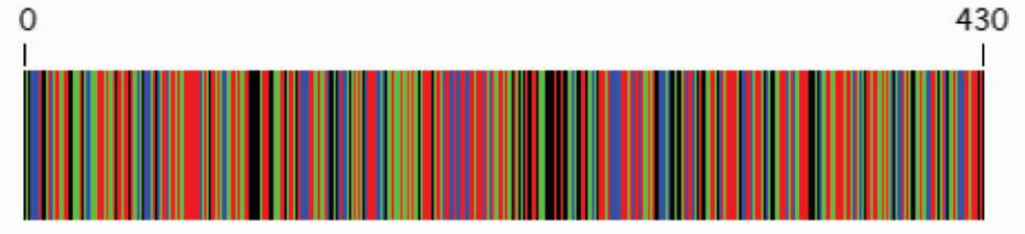


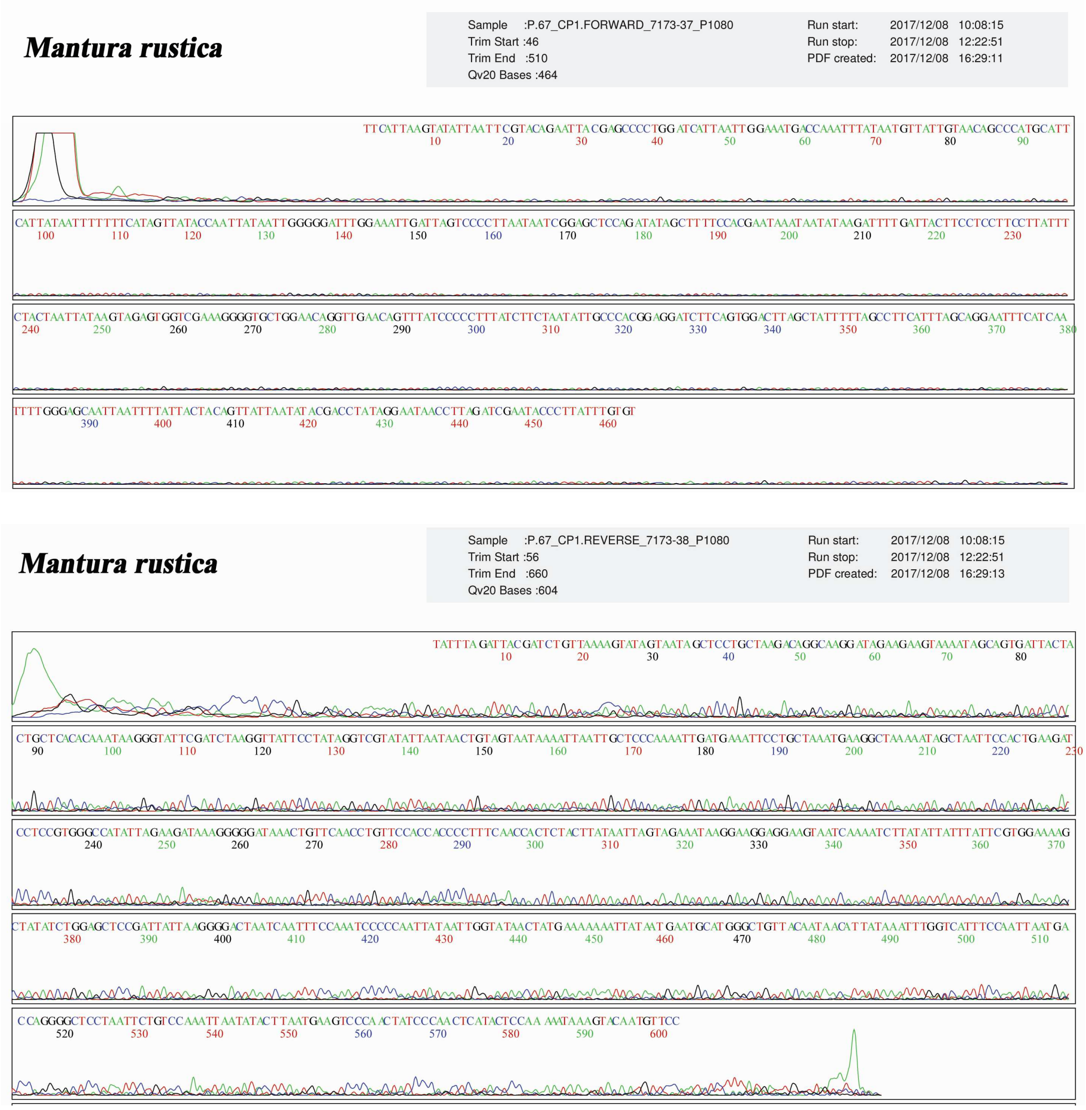
Figure 4.9: Molecular barcode of the mitochondrial COI gene of *Mantura rustica*

> *Mantura rustica* / 121AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUMR-02-A1

> *Mantura rustica*

```
MIFFMVMPIMIGGFGNWLVPLMIGAPDMAFPRMNNMSFWLLPPSLFLLIMSSVVESGGGT
GWTVYPPLSSNMAHGGSSVELAIFSLHLAGISSILGAINFITTVINMRPMGMTLDRMPLF
V
```

Figure 4.10: The translation product of the mitochondrial COI gene of *Mantura rustica*



Sample :P.67_CP1.FORWARD_7173-37_P1080
Trim Start :46
Trim End :510
Qv20 Bases :464
Run start: 2017/12/08 10:08:15
Run stop: 2017/12/08 12:22:51
PDF created: 2017/12/08 16:29:11

Sample :P.67_CP1.REVERSE_7173-38_P1080
Trim Start :56
Trim End :660
Qv20 Bases :604
Run start: 2017/12/08 10:08:15
Run stop: 2017/12/08 12:22:51
PDF created: 2017/12/08 16:29:13

Figure 4.11: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Mantura rustica* using Forward and Reverse primers.

Table 4.3: Comparison of Nucleotide frequencies of COI gene sequence of *Mantura rustica* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH674106 Mantura rustica	36.4	16.5	30.2	16.9	24	15.4	33.6	27.3	44	23.6	13.2	18.8	41	10.4	43.8	4.9
KU557525 Mantura sp.	36.9	16.2	30.2	16.7	24	13.9	34.0	27.8	44	24.5	13.3	18.2	42	10.4	43.1	4.2
KJ677800 Galerucinae sp.	39.0	14.2	30.2	16.7	26	13.9	31.3	29.2	44	23.1	12.6	20.3	47	5.6	46.5	.7
MG587922 Aulacophora lewisii	37.8	15.3	29.5	17.4	26	13.2	31.9	29.2	44	22.9	12.5	20.8	44	9.8	44.1	2.1
MF140520 Luperomorpha sp.	37.6	14.8	30.2	17.4	25	14.7	30.1	30.1	44	22.9	12.5	20.8	44	6.9	47.9	1.4
KF652714 Mantura chrysanthemi	36.2	14.8	31.6	17.4	27	12.5	31.3	29.2	43	23.1	12.6	21.0	38	9.0	50.7	2.1
KC185695 Aulacophora indica	36.6	14.8	30.8	17.8	25	13.9	31.9	29.2	42	21.5	15.3	20.8	42	9.0	45.1	3.5
KU917809 Galerucella lineola	38.3	15.3	29.5	16.9	24	15.3	31.3	29.2	44	23.1	12.6	20.3	47	7.6	44.4	1.4
KC185688 Apophylia epipleuralis	38.4	14.6	29.2	17.8	24	14.6	31.9	29.9	42	21.5	15.3	20.8	49	7.6	40.3	2.8
KC185740 Paridea biplagiata	36.8	15.5	29.9	17.8	25	13.9	31.9	29.2	42	21.5	15.3	20.8	43	11.1	42.4	3.5
KM842073 Tricholochmaea cavicollis	38.1	15.3	29.2	17.4	28	11.8	31.3	28.5	44	23.1	12.6	20.3	42	11.1	43.8	3.5
KM842407 Tricholochmaea cavicollis	38.1	15.3	28.8	17.7	28	11.8	31.3	28.5	44	23.1	12.6	20.3	42	11.2	42.7	4.2
KC185742 Paridea transversofasciata	37.0	15.3	30.1	17.6	26	13.2	32.6	28.5	42	21.5	16.0	20.1	43	11.1	41.7	4.2
KU697451 Aphthona strigosa	38.7	14.4	30.4	16.5	26	13.2	34.0	26.4	44	22.9	13.2	20.1	46	7.0	44.1	2.8
KU906966 Dasytes niger	36.9	13.7	32.9	16.5	30	13.2	29.9	27.1	43	23.8	14.7	18.2	38	4.2	54.2	4.2

Table 4.4: Percentage of evolutionary divergence of *Mantura rustica* with its closely related species accessible from NCBI GenBank

Sl. No.	**Accession No**	**Organism**	**Percentage of divergence**
1.	MH674106	*Mantura rustica* (Kerala)	
2.	KU557525	*Mantura* sp.(Kerala)	1.20%
3.	KF652714	*Mantura chrysanthemi*	9.70%
4.	KJ677800	*Galerucinae* sp.	11.58%
5.	KC185695	*Aulacophora indica*	13.13%
6.	MG587922	*Aulacophora lewisii*	13.33%
7.	KU917809	*Galerucella lineola*	13.82%
8.	MF140520	*Luperomorpha* sp.	14.66%
9.	KC185742	*Paridea transversofasciata*	14.74%
10.	KM842073	*Tricholochmaea cavicollis*	14.85%
11.	KC185740	*Paridea biplagiata*	14.88%
12.	KM842407	*Tricholochmaea cavicollis*	15.21%
13.	KU697451	*Aphthona strigosa*	15.95%
14.	KC185688	*Apophylia epipleuralis*	16.19%
15.	KU906966	*Dasytes niger*	18.18%

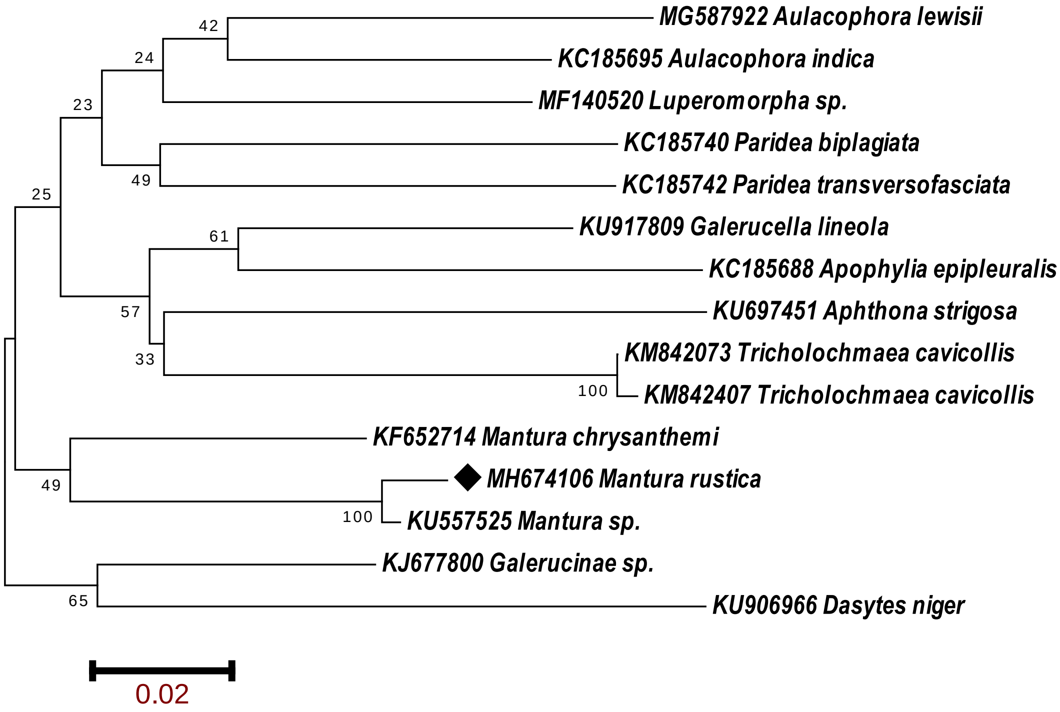


Figure 4.12: Phylogenetic relationship of *Mantura rustica* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that *Mantura rustica* (MH674106) has 98.8% sequence similarity to the same genus reported from Kerala, *Mantura* sp. (KU557525). Even though this species has been found in various geographically isolated areas, their sequence possesses slight variation. Hence the present study stress that the barcode generated can be used to easily spot the specimen and also to analyse its phylogeny.

The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *M. rustica* showed bias to nucleotide AT, with following composition of nucleotides T = 36.4%, C = 16.5%, A = 30.2% and G = 16.9%. This greater AT content (66.6%) over GC content (33.4%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *M. rustica* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed 1.20% to 18.18% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has 1.20% divergence with *Mantura species* from Kerala and 9.70% with *Mantura chrysanthemi* from Spain (Table 4.4). On the basis of the data observed this species may be rooted from those found in Spain which diverted into different clades due to geographical variation. Result thus concluded that this species have slight changes within India and possess changes from those reported from Spain during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *M. rustica* with related species. *M. rustica* isolated from Kerala (MH674106) showed 9.21% divergence with *Mantura sp* (KU557525) from Kerala and 18.18% divergence with (MG298995) *Mantura* sp. from Canada. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *M. chrysanthemi* isolated from Kerala. Closest relative of *M. rustica* is *M. species* from Kerala represented within the same clade.

3. ***Aulacophora* (*Raphidopalpa*) *foveicollis*** (Lucas, 1849)

Specimen details:

Voucher specimen	:	CUAF – 01 – A1
Date of collection	:	8-Dec-2017
Locality	:	Malappuram: Kondotty
Lat- Lon	:	11.1458° N, 75.9643° E
GenBank accession	:	MH590763

Description and distribution:

Figure 4.13: *Aulacophora foveicollis*

They are commonly called as red pumpkin beetles. The larval size range up to 15 mm, body is yellow – white coloured with dark-browned head (Figure 4.13). Generally adults are brown in colour, metasternum and abdominal sternites entirely or largely black. The body size of the adult is 7mm long and 3-3.75 mm wide. The colour of the elytra varies from pale orange-yellow to bright orange-red to medium brown, and the abdomen is black with soft white hairs (Lee and Beenen, 2015). The male humerus covered with erect hairs. From the egg to the imago life cycle varies from 32-55 days. *Aulacophora foveicollis* is polyphagous, attacking more than 80 plant species, preferring more Cucurbitaceae family. The grubs are found in the soil with size range up to 15 mm and are slender and creamy-yellow, with pale brown heads and prothorax. They are found throughout Asia, Pakistan, Africa and Mediterranean region (Agarval and Rastogi, 2008).

Damage:

Adult pumpkin beetles feed on the foliage and flowers of the host plants (members of Cucurbitaceae family); seedlings may be destroyed by its massive attacks and young plants may also be severely affected. Several beetles cluster on a single leaf and feed between the veins, often cutting and removing circular discs which they then eat, leaving other leaves untouched. Larvae feed on roots result in plant rot and withering and adult feeding on seedling may retard plant development (Islam et al., 2011; Muniappan, 2012).

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Aulacophora foveicollis* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 514 bp and 590 bp long products for the specimens obtained from two different locations. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 4.14 – 4.17 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 4.5.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Aulacophora foveicollis* voucher collected during the present study (Table 4.5). It showed bias to nucleotide AT, with nucleotide composition with composition T = 39.9%, C = 16.3%, A = 28.0 and G = 15.8%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 4.6 and Figure 4.18 respectively.

>*Aulacophora foveicollis* CDS-2016/ 590 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUAF-01-A1

> *Aulacophora foveicollis*

```
ATAGTAGGAACTTCCCTAAGAGTCCTAATTCGAACAGAATTAGGAAGCCCTGGATCTTTA
ATTGGAAATGATCAAATTTATAATGTAATTGTCACTGCCCATGCATTCATTATAATTTTT
TTTATAGTTATACCAATTATAATCGGAGGGTTTGGAAACTGATTAGTACCCTTAATAATT
GGAGCTCCTGATATAGCTTTCCCTCGTATAAATAATATAAGATTTTGATTACTTCCTCCT
TCTCTATTTTTATTAATTATAAGTAGAGTTGTTGAAAGAGGGGCTGGGACTGGTTGAACT
GTTTACCCTCCTCTTTCTTCAAATATTGCCCATGGAGGTTCTTCTGTTGATTTAGCAATT
TTCAGTTTACATTTAGCCGGAATTTCTTCAATTTTAGGAGCAATTAATTTTATCACAACC
GTAATTAATATGCGTCCTAAAGGAATAACACTAGACCGAATACCACTATTTGTATGAGCT
GTTGTTATTACAGCTGTATTATTATTATTATCTCTACCAGTTTTAGCTGGAGCCATTACA
ATATTATTAACAGATCGAAATCTAAATACTTCCTTTTTTGATCCTGCTGG
```

Figure 4.14: The partial DNA sequence of the mitochondrial COI gene of *Aulacophora foveicollis*

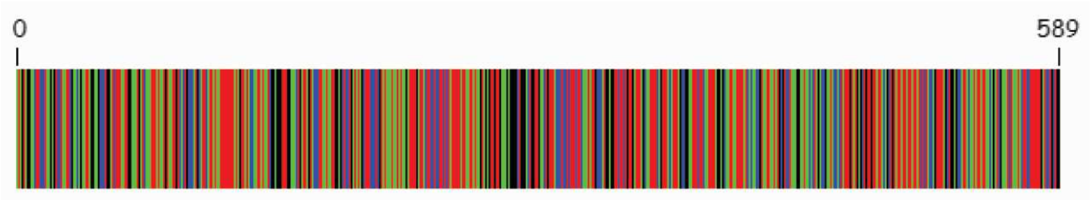


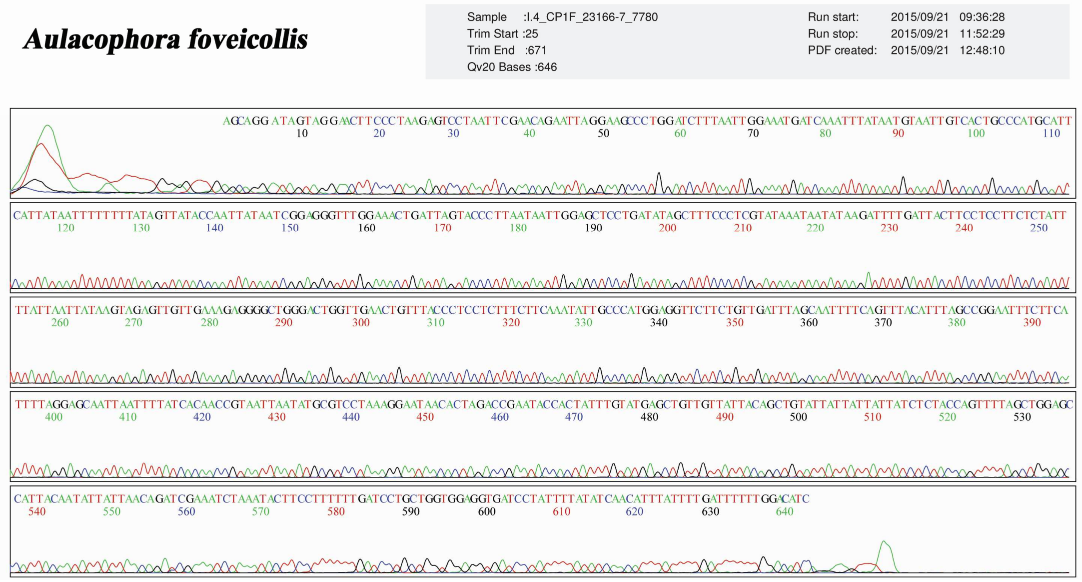
Figure 4.15: Molecular barcode of the mitochondrial COI gene of *Aulacophora foveicollis*

> *Aulacophora foveicollis* / 196AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUAF-01-A1

> *Aulacophora foveicollis*

```
MVGTSLSVLIRTELGSPGSLIGNDQIYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMI
GAPDMAFPRMNNMSFWLLPPSLFLLIMSSVVESGAGTGWTVYPPLSSNIAHGGSSVDLAI
FSLHLAGISSILGAINFITTVINMRPKGMTLDRMPLFVWAVVITAVLLLLSLPVLAGAIT
MLLTDRNLNTSFFDPA
```

Figure 4.16: The translation product of the mitochondrial COI gene of *Aulacophora foveicollis*



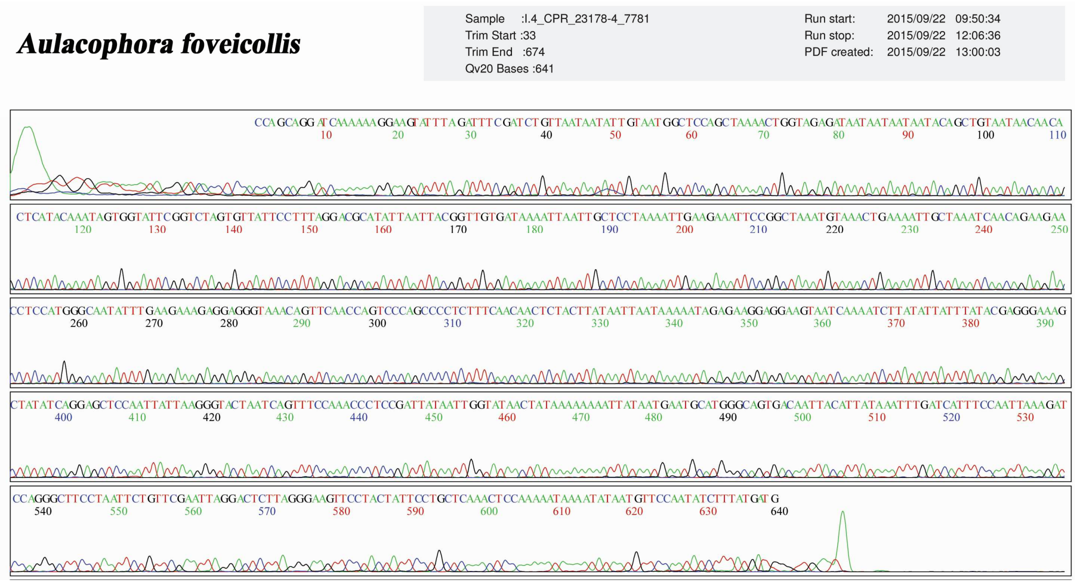


Figure.4.17 Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Aulacophora foveicollis* using Forward and Reverse primers.

Table 4.5: Comparison of Nucleotide frequencies of COI gene sequence of *Aulacophora foveicollis* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH590763 Aulacophora foveicollis (Kondotty)	39.9	16.3	28.0	15.8	27	16.4	29.2	27.5	45	25.0	12.8	17.4	48	7.6	42.1	2.3
KU557524 Aulacophora foveicollis	37.9	16.5	29.4	16.1	24	15.7	32.6	27.9	46	24.0	12.3	18.1	44	9.9	43.3	2.3
KY846455 Aulacophora indica	37.4	16.3	29.8	16.5	25	14.6	32.7	28.1	44	23.4	12.9	19.9	44	11.0	43.6	1.7
MG587922 Aulacophora lewisii	36.2	16.0	30.9	16.9	26	13.5	32.2	28.7	45	23.4	11.7	19.9	38	11.0	48.8	2.3
KC185685 Anadimonia potanini	35.9	15.3	32.0	16.7	23	14.0	36.6	26.7	42	22.7	15.1	19.8	43	9.4	44.4	3.5
AB794742 Monolepta sp.	39.9	13.8	29.6	16.7	26	12.8	33.1	28.5	44	23.4	12.3	19.9	50	5.3	43.3	1.8
KR425318 Galerucinae sp.	39.1	14.8	29.6	16.5	25	14.0	33.3	28.1	44	23.4	12.3	19.9	48	7.0	43.0	1.7
KC185733 Monolepta atrimarginata	36.9	14.0	33.0	16.1	24	12.8	34.9	27.9	42	23.3	14.5	19.8	44	5.8	49.7	.6
KF134566 Longitarsus atricillus	37.4	15.6	31.1	16.0	27	12.3	33.9	26.9	44	23.4	12.9	19.3	41	11.0	46.5	1.7
KU697451 Aphthona strigosa	36.8	15.2	31.9	16.1	24	14.0	35.5	26.7	44	24.0	12.9	19.3	43	7.6	47.4	2.3
KF134558 Longitarsus bedeli	37.9	15.0	31.3	15.8	27	11.7	33.9	26.9	44	23.4	12.9	19.3	42	9.9	47.1	1.2
KU915650 Longitarsus atricillus	37.0	16.0	31.5	15.6	27	12.3	34.5	26.3	44	24.0	12.9	19.3	40	11.6	47.1	1.2
MG058721 Glyptina abbreviate	35.6	15.4	32.1	16.9	24	14.0	33.7	27.9	44	23.4	12.3	19.9	38	8.8	50.3	2.9
KC185708 Euliroetis ornate	36.7	15.9	30.7	16.7	23	15.7	35.5	25.6	43	22.7	15.1	19.2	44	9.4	41.5	5.3
KU915449 Longitarsus gracilis	37.5	17.1	29.4	16.0	25	14.0	34.5	26.3	44	24.0	12.9	19.3	44	13.4	40.7	2.3

Table 4.6 Percentage of evolutionary divergence of *Aulacophora foveicollis* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH590763	*Aulacophora foveicollis* (Kondotty)	
2.	KU557524	*Aulacophora foveicollis* (Kerala)	0.00%
3.	KY846455	*Aulacophora indica* (Pakisthan)	2.25%
4.	MG587922	*Aulacophora lewisii*	16.22%
5.	KU697451	*Aphthona strigosa*	20.97%
6.	KU915449	*Longitarsus gracilis*	21.90%
7.	KF134566	*Longitarsus atricillus*	22.63%
8.	KF134558	*Longitarsus bedeli*	23.28%
9.	KU915650	*Longitarsus atricillus*	23.28%
10.	KR425318	*Galerucinae* sp.	25.87%
11.	KC185685	*Anadimonia potanini*	26.08%
12.	AB794742	*Monolepta* sp.	26.56%
13.	KC185733	*Monolepta atrimarginata*	27.51%
14.	MG058721	*Glyptina abbreviate*	28.54%
15.	KC185708	*Euliroetis ornate*	28.72%

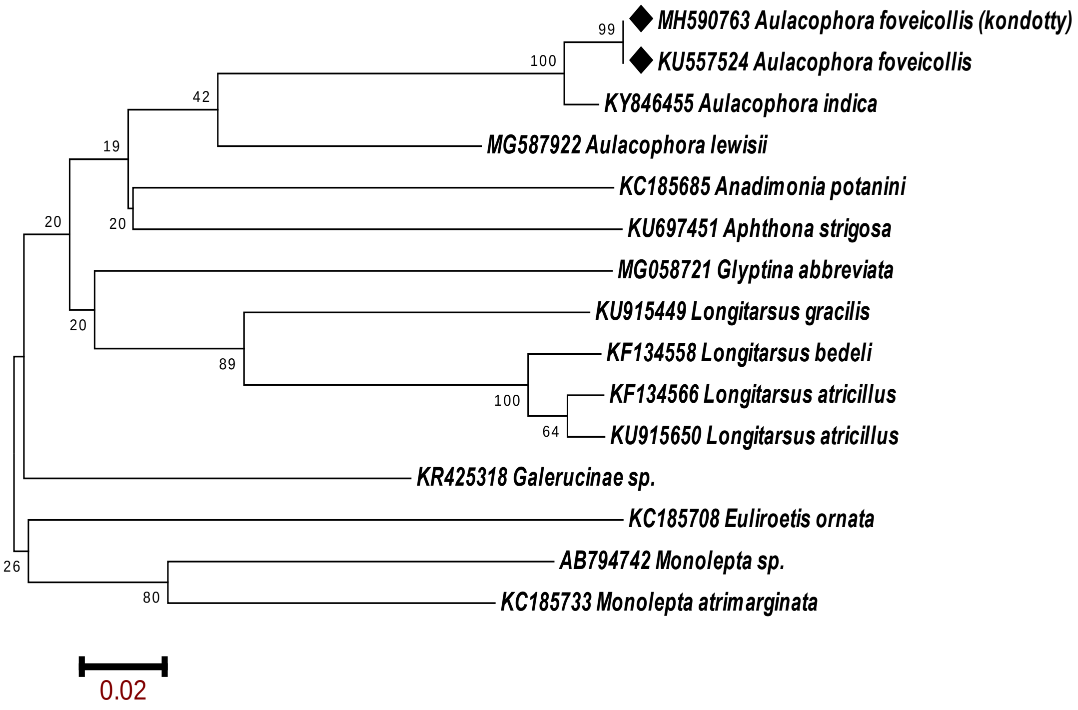


Figure 4.18: Phylogenetic relationship of *Aulacophora foveicollis* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that *Aulacophora foveicollis* (MH590763) has 100% sequence similarity to the same genus and species reported from Kerala (KU557524). This species has been found in sevsral geographically isolated areas, but their sequence doesn't have any kind of variation.

The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Aulacophora foveicollis* showed bias to nucleotide AT, with following composition of nucleotides T = 39.9%, C = 16.3%, A = 28.0% and G = 15.8%. This greater AT content (66.9%) over GC content (32.1%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Aulacophora foveicollis* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed 0% to 28.72% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has no divergence (0%) from Kerala while 28.72% *with Euliroetis ornate* (KC185708) from China (Table 4.6). On the basis of the data observed this species may be rooted from those found in China which diverted into different clades due to geographical variation. Result thus concluded that this species doesn't have any major changes in India while slightly changes from those reported from China during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Aulacophora foveicollis* with related species. *Aulacophora foveicollis* isolated from Kerala (MH590763) showed 0% divergence with *Aulacophora foveicollis* (KU557524) from Kerala and 28.72% *with Euliroetis ornate* (KC185708) from China. The phylogeny tree generated by using

NJ method reveals the phylogenetic status of *Aulacophora foveicollis* isolated from Kerala. Closest relative of *Aulacophora foveicollis* is *Aulacophora foveicollis* from Kerala represented within the same clade.

4. ***Aulacophora frontalis*** (Baly, 1888)

Specimen details:

Voucher specimen	:	CUAF – 03 – A1
Date of collection	:	8- Apr-2016
Locality	:	Eranakulam: Tripunithura
Lat- Lon	:	9.9487° N, 76.3464° E
GenBank accession	:	MH674107

Description and distribution:

Fig.4.19: *Aulacophora frontalis*

Aulacophora frontalis is generally brown coloured, elytra black cooured, in male ventral area of head on each side with a prominence, the upper edge of which is compressed and curved; antennae with the segment 3 not shorter than segment 4; length 5.75 mm (Lee and Beenen, 2015). *A. frontalis* is with modified antennomeres III to V in males and longitudinal grooves on the vertex (Figure 4.19). They are specialized having more slender antennomere III in males and more wider antennomere VII to X in females. *Aulacophora frontalis* is distributred in India, Indonesia, China, Cambodia, Laos, Malaysia, Taiwan, Thailand and Vietnam.

Damage:

These adult feed on the leaves of young seedlings makes shot holes on them (Sarwar, 2014; Lee and Beenen, 2015). Grubs bore into the roots, causing them to swell, discoloured and eventually retard the plant growth (Muniappan, 2012)

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *A. frontalis* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 601 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 4.20 – 4.23 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 4.7.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *A. frontalis* voucher collected during the present study (Table 4.7). It showed bias to nucleotide AT, with nucleotide composition with T = 37.9%, C = 17.1%, A = 29.8% and G = 15.2%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 4.8 and Figure 4.24 respectively.

>*Aulacophora frontalis* CDS-2018/ 601 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUAF-03-A1

> *Aulacophora frontalis*

```
ATAGTAGGGACATCTTTAAGAATCTTAATTCGGCTAGAATTAGGAACCCCAAGAGCATTA
ATTGGAAATGACCAAATTTACAATGTAATTGTTACCGCTCATGCTTTCATTATAATTTTT
TTCATAGTAATACCAATTATAATTGGAGGATTTGGAAATTGATTAGTCCCCTTAATAATT
GGGGCTCCAGATATAGCTTTTCCTCGATTAAATAATATAAGATTTTGATTGCTACCTCCT
GCTCTTTCTCTTTTAATTTTAAGAAGTTTAGTAGAAAGGGGAGCCGGTACTGGTTGAACA
GTATACCCACCTCTATCATCTAATATTGCCCATGGAGGTTCTTCTGTAGATTTAGCAATC
TTTAGTCTTCATTTAGCTGGAATTTCTTCTATTCTAGGAGCAGTTAATTTTATTACTACA
ATTTTTAACATACGTCCTTTTGGAATAACATTAGAAAAAATACCTTTGTTTGTTTGATCA
GTTTTGATTACTGCCGTTCTACTACTTCTATCTTTACCAGTTTTAGCAGGAGCTATCACC
ATACTATTAACAGACCGAAATATTAATACATCATTTTTTGACCCCGCAGGAGGAGGAGAT
C
```

Figure 4.20: The partial DNA sequence of the mitochondrial COI gene of *Aulacophora frontalis*

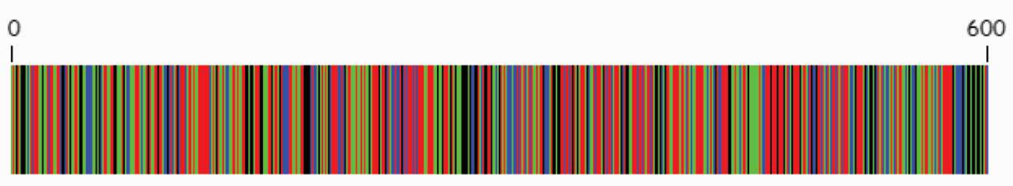


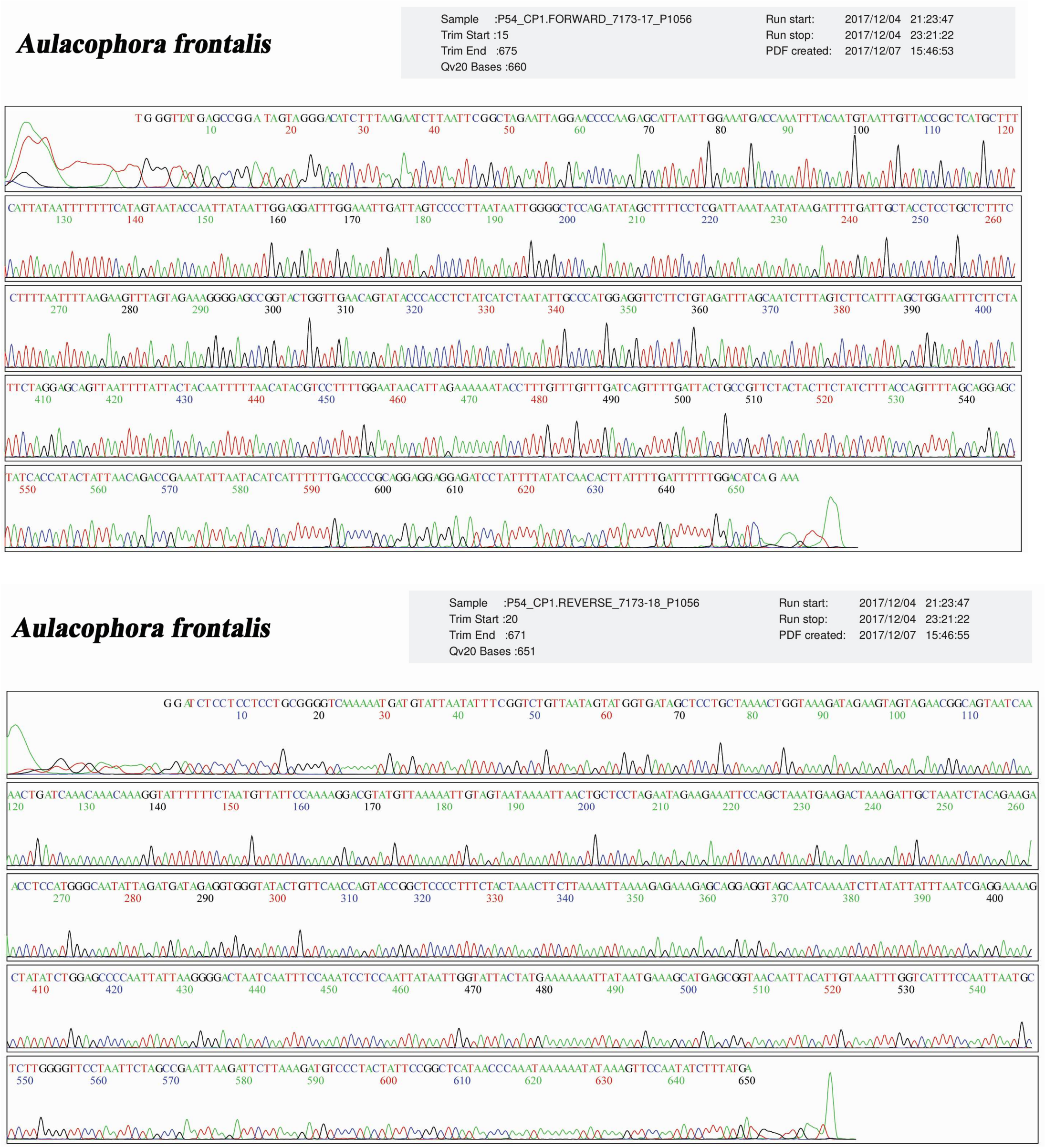
Figure 4.21: Molecular barcode of the mitochondrial COI gene of *Aulacophora frontalis*

> *Aulacophora frontalis* / 200AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUAF-03-A1

> *Aulacophora frontalis*

```
MVGTSLSILIRLELGTPSALIGNDQIYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMI
GAPDMAFPRLNNMSFWLLPPALSLLILSSLVESGAGTGWTVYPPLSSNIAHGGSSVDLAI
FSLHLAGISSILGAVNFITTIFNMRPFGMTLEKMPLFVWSVLITAVLLLLSLPVLAGAIT
MLLTDRNINTSFFDPAGGGD
```

Figure 4.22: The translation product of the mitochondrial COI gene of *Aulacophora frontalis*



Aulacophora frontalis

Sample :P54_CP1.FORWARD_7173-17_P1056
Trim Start :15
Trim End :675
Qv20 Bases :660

Run start: 2017/12/04 21:23:47
Run stop: 2017/12/04 23:21:22
PDF created: 2017/12/07 15:46:53

Aulacophora frontalis

Sample :P54_CP1.REVERSE_7173-18_P1056
Trim Start :20
Trim End :671
Qv20 Bases :651

Run start: 2017/12/04 21:23:47
Run stop: 2017/12/04 23:21:22
PDF created: 2017/12/07 15:46:55

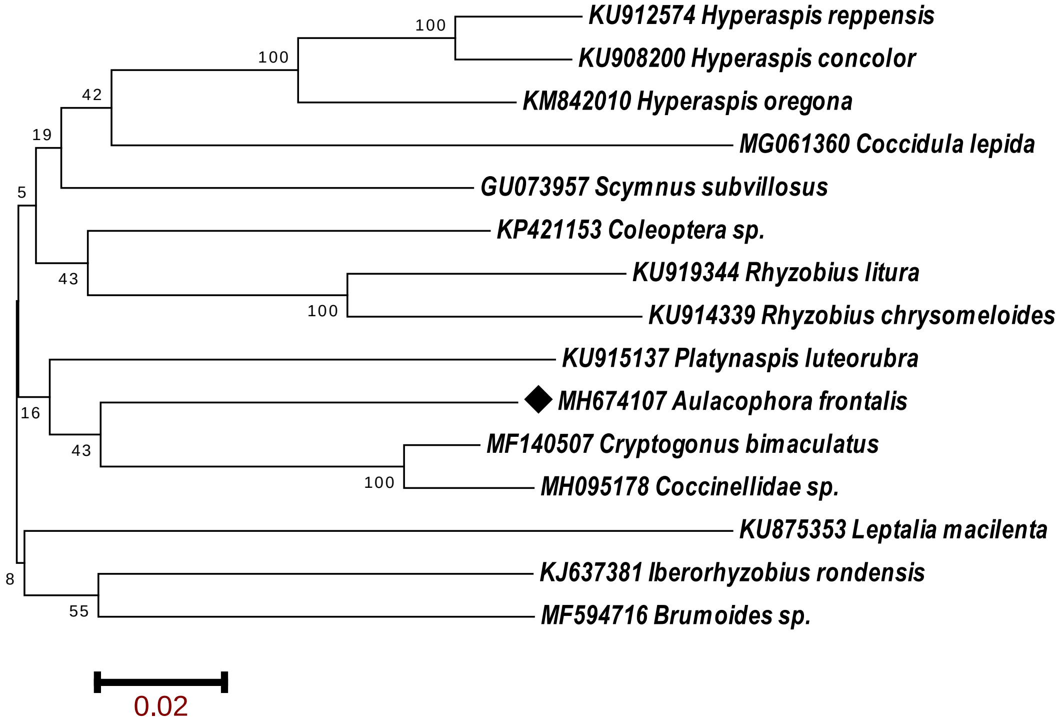
Figure 4.23: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Aulacophora frontalis* using Forward and Reverse primers

Table 4.7: Comparison of Nucleotide frequencies of COI gene sequence of *Aulacophora frontalis* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH674107 Aulacophora frontalis	37.9	17.1	29.8	15.2	42	9.4	44.8	3.6	25	16.6	32.1	25.9	46	25.4	12.4	16.1
MF140507 Cryptogonus bimaculatus	38.9	16.3	29.8	15.1	44	8.3	44.6	3.6	27	15.1	32.3	25.5	46	25.4	12.4	16.1
MH095178 Coccinellidae sp.	37.9	17.1	28.9	16.1	44	9.4	41.7	4.7	26	16.1	30.6	27.5	44	25.9	14.5	16.1
KU915137 Platynaspis luteorubra	38.9	14.2	31.6	15.4	41	5.2	50.8	2.6	28	13.0	32.6	25.9	47	24.4	11.4	17.6
KP421153 Coleoptera sp.	40.7	13.7	30.6	15.1	46	3.6	48.2	2.6	31	11.9	31.6	25.9	46	25.5	12.0	16.7
KU912574 Hyperaspis reppensis	38.0	15.2	32.1	14.7	41	5.7	52.3	1.0	27	14.5	32.1	25.9	46	25.4	11.9	17.1
KJ637381 Iberorhyzobius rondensis	40.7	15.1	28.9	15.4	49	6.3	43.2	1.6	27	14.0	32.1	26.9	46	24.9	11.4	17.6
KU919344 Rhyzobius litura	39.0	16.4	29.2	15.4	47	7.8	43.0	2.6	25	16.1	32.6	26.4	46	25.4	11.9	17.1
GU073957 Scymnus subvillosus	39.6	14.7	31.7	14.0	44	6.2	48.7	1.0	30	11.5	32.8	25.5	45	26.4	13.5	15.5
KU908200 Hyperaspis concolor	37.7	15.5	31.8	15.0	40	5.7	51.8	2.1	27	15.5	31.6	25.9	46	25.4	11.9	17.1
KU914339 Rhyzobius chrysomeloides	39.0	16.1	30.1	14.9	45	8.8	45.1	1.0	26	14.0	33.2	26.4	46	25.4	11.9	17.1
MG061360 Coccidula lepida	37.4	17.3	29.8	15.6	41	10.9	45.3	3.1	25	16.1	31.6	26.9	46	24.9	12.4	16.6
KU875353 Leptalia macilenta	39.4	15.2	29.7	15.7	47	4.7	46.6	2.1	26	15.0	30.1	28.5	45	25.9	12.4	16.6
MF594716 Brumoides sp.	38.2	14.2	32.5	15.2	42	3.6	51.3	3.1	27	14.0	34.2	24.9	46	24.9	11.9	17.6
KM842010 Hyperaspis oregona	38.3	15.0	32.1	14.5	41	5.2	52.8	.5	27	15.0	31.6	25.9	46	24.9	11.9	17.1

Table 4.8: Percentage of evolutionary divergence of *Aulacophora frontalis* with its closely related species accessible from NCBI GenBank

Sl. No.	**Accession No**	**Organism**	**Percentage of divergence**
1.	MH674107	*Aulacophora frontalis* (Kerala)	
2.	MF140507	*Cryptogonus bimaculatus* (Meghalaya)	12.12%
3.	MH095178	*Coccinellidae* sp.	13.81%
4.	KU919344	*Rhyzobius litura*	15.58%
5.	KU912574	*Hyperaspis reppensis*	15.71%
6.	KU915137	*Platynaspis luteorubra*	15.73%
7.	KU914339	*Rhyzobius chrysomeloides*	15.96%
8.	KU908200	*Hyperaspis concolor*	16.18%
9.	GU073957	*Scymnus subvillosus*	16.37%
10.	KJ637381	*Iberorhyzobius rondensis*	16.41%
11.	KP421153	*Coleoptera* sp.	16.44%
12.	MG061360	*Coccidula lepida*	16.72%
13.	KM842010	*Hyperaspis oregona*	17.12%
14.	MF594716	*Brumoides* sp.	17.55%
15.	KU875353	*Leptalia macilenta*	18.47%



Figuure 4.24 Phylogenetic relationship of *Aulacophora frontalis* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 87.88% sequence similarity to *Cryptogonus bimaculatus* reported from Meghalaya (MF140507). There is considerably high degree of variation in their COI gene sequences with their related species among those reported from various geographically isolated areas. The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Aulacophora frontalis* showed bias to nucleotide AT, with following composition of nucleotides T = 37.9%, C = 17.1%, A = 29.8% and G = 15.2%. This greater AT content (67.7%) over GC content (32.3%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Aulacophora frontalis* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed varied differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has (12.12%) divergence with those from Meghalaya (Table 4.8). On the basis of the data observed this species may be rooted from those found in Meghalaya which diverted into different clades due to geographical variation. Result thus concluded that this species doesn't have any major changes in India while slightly changes from those reported from Malaysia and Thailand during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Aulacophora frontalis* with related species. *Aulacophora frontalis* isolated from Kerala (MH674107) showed 12.12% with *Cryptogonus bimaculatus* (MF40507) from Meghalaya and 18.47% divergence with *Leptalia macilenta* (KU875353) from Canada. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Aulacophora frontalis* isolated from Kerala. Closest relative of *Aulacophora frontalis* is *Platynaspis luteorubra* (KU915137) of Germany and *Cryptogonus bimaculatus* (MF140507) of Meghalaya represented within the clade.

5. *Aulacophora lewisii* (Baly, 1886)

Specimen details:

Voucher specimen	:	CUAL – 01 – A1
Date of collection	:	14- Nov-2015
Locality	:	Kasaragod: Nileshwaram
Lat- Lon	:	12.2557° N, 75.1341° E
GenBank accession	:	MH590776

Description and distribution:

Aulacophora lewisii, is with bright yellowish brown to dark yellowish body along with black shinning elytron and easily identified by the filiform antenna in males and the sinuate apical margin of the abdominal ventrite V in females (Lee and Bennen, 2015). The antenna of male not modified but fairy stout, length 5.51 mm (Figure 4.25). The elytron slightly uneven, not very shiny having punctures mostly one-third to half as wide as interspaces (Lee, 2002). This pest species is distributed mainly in Bhutan, Cambodia, China, India, Indonesia, Japan, Laos, Taiwan, Thailand and Vietnam.

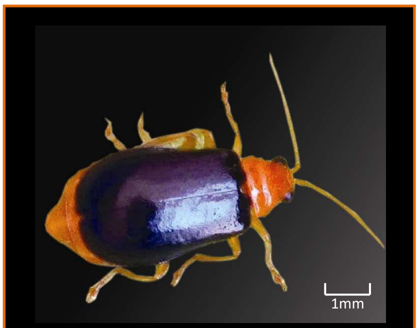


Fig.4.25: *Aulacophora lewisii*

Damage:

The adult feeds on flowers and leaves, thus causing serious damage. The seedling is heavily attacked till total destruction. Grubs boring to the roots lead to swelling, discolouration and misshappening. Ultimately, the plants growth gets retarded or the plant gets killed.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *A. lewisii* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 601 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 4.26 – 4.29 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 4.9.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *A. lewisii* voucher collected during the present study (Table 4.9). It showed bias to nucleotide AT, with nucleotide composition with T = 36.9%, C = 16.1%, A = 30.7% and G = 16.3%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 4.10 and Figure 4.30 respectively.

>*Aulacophora lewisii* CDS-2018/ 601 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUAL-01-A1

> *Aulacophora lewisii*

```
AGCTGGTATAGTTGGAACCTCTTTAAGAATACTAATTCGAACAGAGTTAGGAAGCCCAGG
GTCTTTAATTGGAAATGATCAAATTTATAATGTTATTGTAACAGCCCATGCATTCATTAT
AATTTTTTTCATAGTTATGCCAATTATGATTGGTGGCTTTGGAAATTGATTAGTTCCTTT
AATAATTGGAGCTCCAGATATAGCATTTCCTCGTATAAATAATATAAGATTTTGATTATT
ACCCCCTTCCTTATTTTTATTAATTATAAGAAGTGTTGTTGAAAGAGGCGCTGGAACAGG
ATGAACCGTTTATCCCCCACTATCTTCAAATATTGCCCATGGAGGATCCTCAGTTGATCT
AGCAATTTTTAGTCTTCATTTAGCAGGAATTTCTTCAATTTTAGGAGCAATTAATTTTAT
TACAACAGTTATTAATATGCGACCTAAAGGAATAACTTTAGACCGAATACCATTATTCGT
ATGAGCGGTAGTAATTACAGCAATTTTATTGCTTCTATCACTACCTGTTTTAGCTGGTGC
AATTACGATATTATTAACAGACCGAAATTTAAATACTTCCTTTTTTGATCCTGCAGGAGG
C
```

Figure 4.26: The partial DNA sequence of the mitochondrial COI gene of *Aulacophora lewisii*

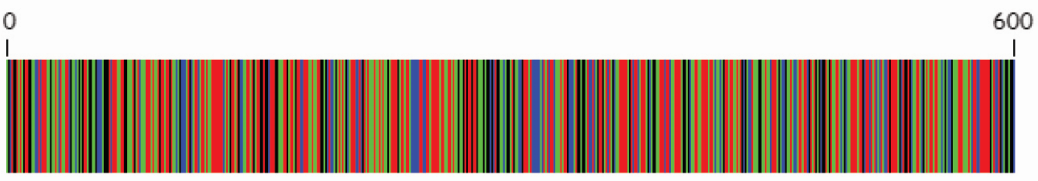


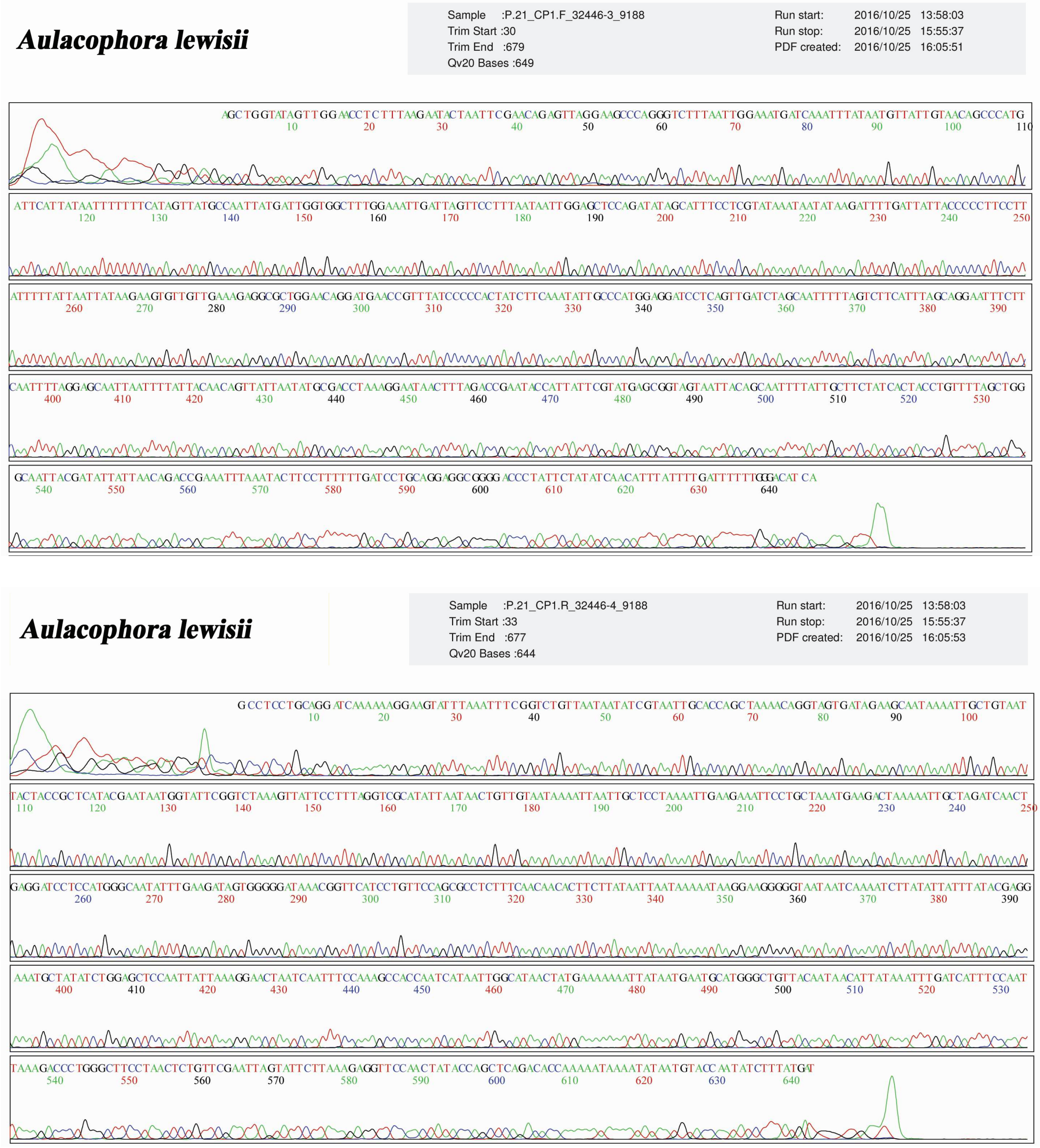
Figure 4.27: Molecular barcode of the mitochondrial COI gene of *Aulacophora lewisii*

> *Aulacophora lewisii* / 198AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUAL-01-A1

> *Aulacophora lewisii*

```
MVGTSLSMLIRTELGSPGSLIGNDQIYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMI
GAPDMAFPRMNNMSFWLLPPSLFLLIMSSVVESGAGTGWTVYPPLSSNIAHGGSSVDLAI
FSLHLAGISSILGAINFITTVINMRPKGMTLDRMPLFVWAVVITAILLLLSLPVLAGAIT
MLLTDRNLNTSFFDPAGG
```

Figure 4.28: The translation product of the mitochondrial COI gene of *Aulacophora lewisii* using Forward and Reverse primers



Sample :P.21_CP1.F_32446-3_9188
Trim Start :30
Trim End :679
Qv20 Bases :649

Run start: 2016/10/25 13:58:03
Run stop: 2016/10/25 15:55:37
PDF created: 2016/10/25 16:05:51

Sample :P.21_CP1.R_32446-4_9188
Trim Start :33
Trim End :677
Qv20 Bases :644

Run start: 2016/10/25 13:58:03
Run stop: 2016/10/25 15:55:37
PDF created: 2016/10/25 16:05:53

Figure 4.29: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Aulacophora lewisii* using Forward and Reverse primers

Table 4.9: Comparison of Nucleotide frequencies of COI gene sequence of *Aulacophora lewisii* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH590776 Aulacophora lewisii	36.9	16.1	30.7	16.3	25	14.7	33.0	27.4	45	25.0	12.8	17.3	41	8.6	46.2	4.1
KC185696 Aulacophora lewisii	37.4	15.6	30.5	16.6	24	14.2	33.0	28.4	45	23.9	13.2	18.3	43	8.6	45.2	3.0
MG587922 Aulacophora lewisii	37.3	15.4	30.8	16.4	25	14.2	33.5	27.4	46	23.9	12.2	18.3	41	8.2	46.9	3.6
KY833124 Chrysomelidae sp.	37.1	16.9	30.8	15.1	25	14.7	33.0	26.9	46	25.0	11.7	17.3	40	11.2	47.7	1.0
KF654042 Calomicrus fallax	38.3	14.4	31.4	15.9	25	13.7	32.5	28.4	46	24.5	11.2	18.4	44	5.1	50.3	1.0
KJ677807 Galerucinae sp.	40.0	14.4	30.0	15.6	25	13.7	33.0	27.9	46	24.5	12.2	16.8	48	5.1	44.7	2.0
KU914889 Longitarsus	37.3	17.5	30.2	15.1	27	13.2	33.0	26.9	46	25.0	11.7	17.3	39	14.2	45.7	1.0
KY846455 Aulacophora indica	38.0	16.3	29.7	16.1	25	14.2	31.5	28.9	46	24.5	11.7	17.9	43	10.2	45.7	1.5
KF653479 Calomicrus foveolatus	38.5	14.7	30.3	16.4	24	14.7	32.0	28.9	46	24.0	11.2	18.4	45	5.6	47.7	2.0
KR489849 Glyptina abbreviata	36.4	15.9	31.9	15.8	26	14.7	32.5	26.9	46	24.5	11.7	17.9	38	8.6	51.3	2.5
KU557524 Aulacophora foveicollis	37.8	16.9	29.3	15.9	24	15.7	32.0	28.4	45	25.4	12.7	17.3	45	9.7	43.4	2.0
KF654246 Mantura chrysanthemi	37.3	14.6	32.9	15.3	28	13.2	32.0	26.9	46	24.0	12.2	17.9	38	6.6	54.3	1.0
KT175583 Monolepta quadriguttata	39.0	13.2	32.0	15.8	26	13.2	34.5	26.4	46	23.9	11.7	18.8	45	2.6	50.0	2.0
KY769285 Paria thoracica	36.6	17.3	29.7	16.4	24	15.7	32.5	27.9	45	25.4	12.2	17.3	41	10.7	44.4	4.1

Table 4.10: Percentage of evolutionary divergence of *Aulacophora lewisii* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH590776	*Aulacophora lewisii* (Kerala)	
2.	KC185696	*Aulacophora lewisii* (China)	0.37%
3.	MG587922	*Aulacophora lewisii*	0.37%
4.	KY833124	*Chrysomelidae* sp.	13.51%
5.	KR489849	*Glyptina abbreviate*	14.00%
6.	KF654042	*Calomicrus fallax*	14.18%
7.	KU914889	*Longitarsus*	14.41%
8.	KF654246	*Mantura chrysanthemi*	14.50%
9.	KJ677807	*Galerucinae* sp.	14.70%
10.	KY769285	*Paria thoracica*	14.81%
11.	KF653479	*Calomicrus foveolatus*	14.88%
12.	KY846455	*Aulacophora indica*	15.12%
13.	KU557524	*Aulacophora foveicollis*	15.14%
14.	KT175583	*Monolepta quadriguttata*	15.45%

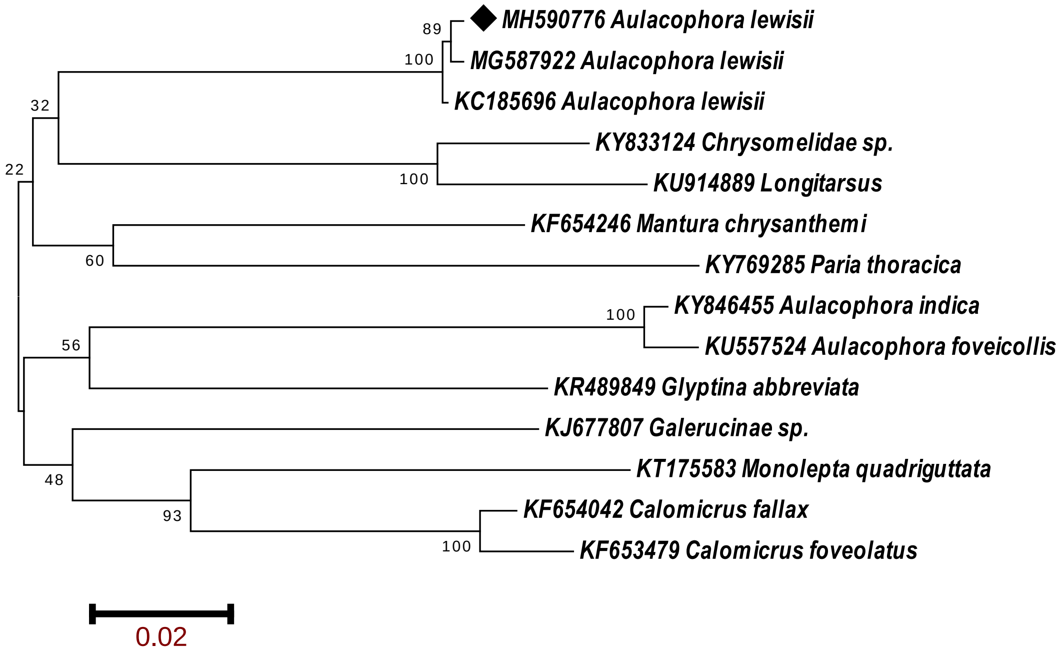


Figure 4.30: Phylogenetic relationship of *Aulacophora lewisii* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 99.7% sequence similarity to the same genus reported from China (KC185696). The COI gene sequence of this species doesn't have any kind of variation among those reported from various localities. The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Aulacophora lewisii* showed bias to nucleotide AT, with following composition of nucleotides T = 36.9%, C = 16.1%, A = 30.7% and G = 16.3%. This greater AT content (67.6%) over GC content (32.4%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Aulacophora lewisii* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species has a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 0.37% to 15.45% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has slight divergence (0.37%) those from China and Bangladesh while 15.45% Meghalaya (Table 4.10). On the basis of the data observed this species may be rooted from those found in China and Bangladesh which diverted into different clades due to geographical variation. Result thus concluded that this species doesn't have any major changes in India while slightly changes from those reported from China and Bangladesh during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Aulacophora lewisii* with related species. *Aulacophora lewisii* isolated from Kerala (MH590776) showed 0.37% divergence with *Aulacophora lewisii* (KC185696) from China and 17.89% divergence with *Monolepta quadriguttata* (KT175583) from Meghalaya. The phylogenetic tree generated by using NJ method reveals the phylogenetic status of *Aulacophora lewisii* isolated from Kerala. Closest relative of *Aulacophora lewisii* is *Aulacophora lewisii* from China represented within the same clade.

6. ***Sphenoraia bicolor*** (Hope, 1831)

Specimen details:

Voucher specimen	:	CUSB – 02 – A1
Date of collection	:	21- Mar-2016
Locality	:	Kannur: Azhikode
Lat- Lon	:	11.9171º N, 75.3354º E
GenBank accession	:	MH656688

Description and distribution:

Fig 4.31: *Sphenoraia bicolor*

The body is brown coloured with seven black spots on pronotum and each elytron (Basu et al., 2017) (Figure 4.31). General colour dark brown with black spots and patches on dorsal and ventral side in a great number of varities; but generally each elytron with seven spots (2:2:2:1) and pronotum with four spots; scutellum always black; vertical area of head, labrum and antennae (except three basal segments) always black; pronotum distinctly convex before middle; elytral punctrues more regularly arranged in rows; length 8.0-8.5 mm. They are active during rainy season, diapause during winter and continues it to summer (Kalaichelvan and Verma, 2005). They are distributed throughout India: West Bengal (Darjiling, Haora and 24 Parganas), Assam, Himachal Pradesh, Kerala, Maharashtra, Meghalaya, Sikkim, and Uttar Pradesh. Elsewhere: Bunna and Nepal.

Damage:

They are extensive leaf miners.The adult are external feeders of leaves. The larvae are gregarious on leaves (Basu et al., 2017).

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Sphenoraia bicolor* collected has been amplified using the primer CP2 (Table 3.1). The PCR amplification yielded 596 bp and 642 bp long products for the specimens obtained from two different locations. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 4.32 – 4.35 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 4.11.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Sphenoraia bicolor* voucher collected during the present study (Table 4.11). It showed bias to nucleotide AT, with nucleotide composition with T = 35.8%, C = 17.7%, A = 29.6% and G = 16.9%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 4.12 and Figure 4.36 respectively.

>*Sphenoraia bicolor* CDS-2018/ 642 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUSB-01-A1

> *Sphenoraia bicolor*

```
AAGGATCAAAAAATGAAGTATTTAAATTTCGGTCTGTTAATAATATTGTAATTGCTCCAG
CTAAAACTGGTAAAGATAAGAGTAGTAAGATAGCTGTAATTACCACAGATCAAACAAATA
GGGGTATTCGATCAAGTGTTATACCTTTAGGACGTATATTAATTACAGTTGTAATAAAAT
TAATTGCTCCTAAAATTGAAGAAATTCCAGCTAAATGAAGACTAAAAATAGCTAAATCAA
CTGAAGCTCCATTATGTGCAATATTGGCTGACAGTGGGGGATATACAGTTCAACCAGTTC
CTGCTCCTCTTTCTACTACTCTTCTTATAATGAGAAGAAAAAGAGAGGGGGGTAAAAGTC
AAAATCTTATGTTATTTAGTCGGGGGAATGCTATGTCAGGGGCACCGATTATTAAGGGGA
CTAATCAGTTTCCAAAACCTCCAATTATAATTGGCATTACTATGAAGAAAATCATAATAA
AAGCATGGGCTGTAACAATAACATTGTAAATTTGATCATTACCGATCAATGATCCTGGGC
TTCCCAATTCTACTCGAATTAAAATTCTTAGGGAAGTTCCTACTATACCTGCTCAGATTC
CAAAAATAAAATAAAGTGTACCAATATCTTTATGATTTGTTG
```

Figure 4.32: The partial DNA sequence of the mitochondrial COI gene of *Sphenoraia bicolor*

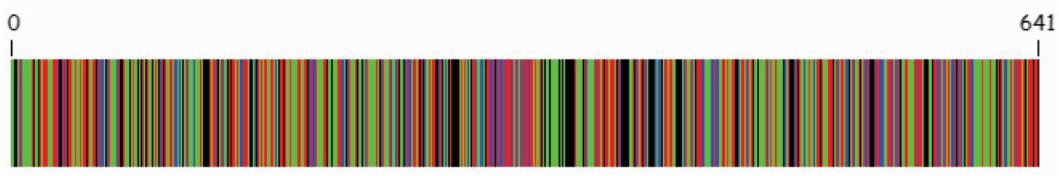


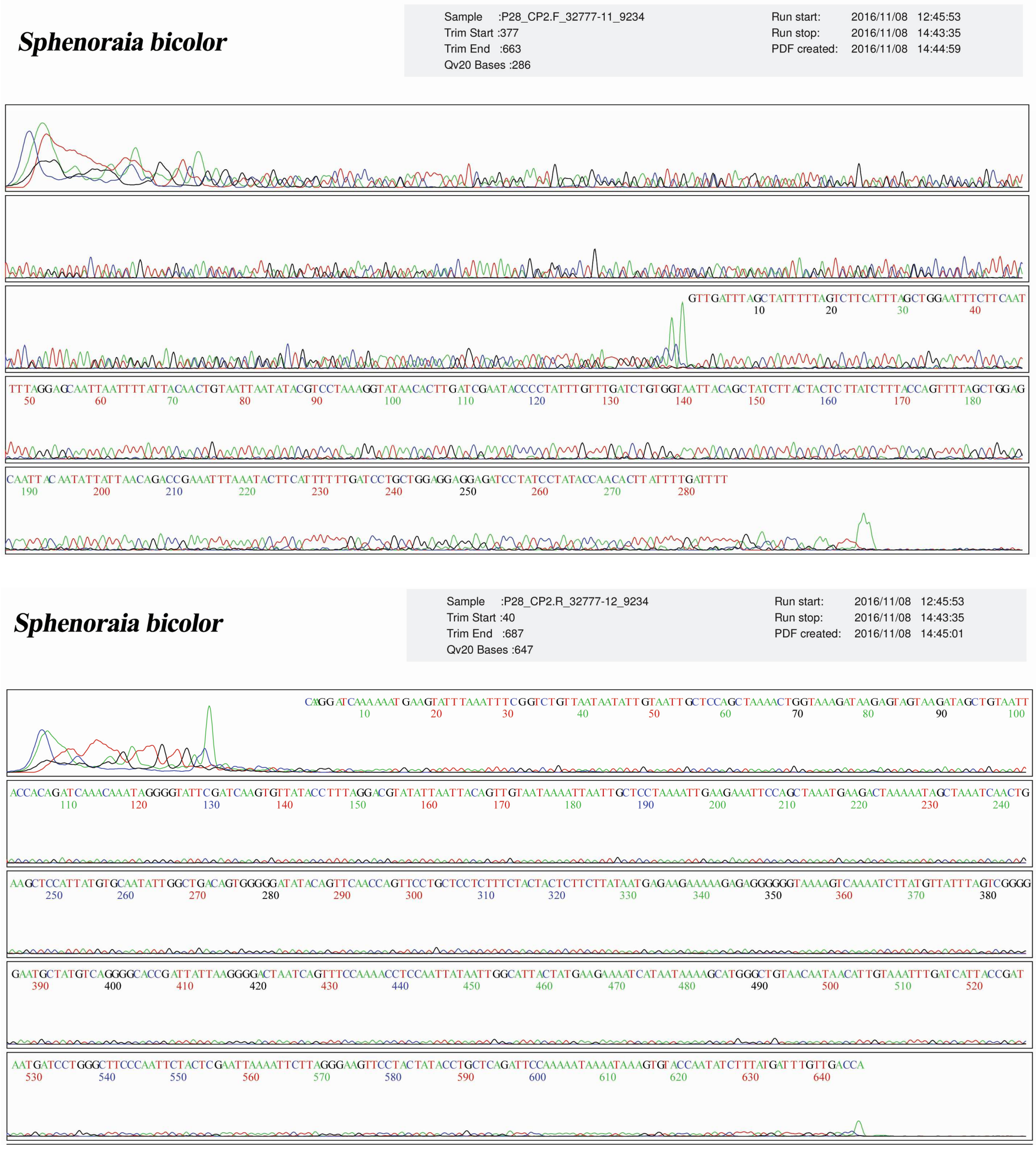
Figure 4.33: Molecular barcode of the mitochondrial COI gene of *Sphenoraia bicolor*

> *Sphenoraia bicolor* / 195AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUSB-01-A1

> *Sphenoraia bicolor*

```
MVGTSLSILIRVELGSPGSLIGNDQIYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMI
GAPDMAFPRLNNMSFWLLPPSLFLLIMSSVVESGAGTGWTVYPPLSANIAHNGASVDLAI
FSLHLAGISSILGAINFITTVINMRPKGMTLDRMPLFVWSVVITAILLLLSLPVLAGAIT
MLLTDRNLNTSFFDP
```

Figure 4.34: The translation product of the mitochondrial COI gene of *Sphenoraia bicolor*



Sphenoraia bicolor

Sample :P28_CP2.F_32777-11_9234
Trim Start :377
Trim End :663
Qv20 Bases :286

Run start: 2016/11/08 12:45:53
Run stop: 2016/11/08 14:43:35
PDF created: 2016/11/08 14:44:59

Sphenoraia bicolor

Sample :P28_CP2.R_32777-12_9234
Trim Start :40
Trim End :687
Qv20 Bases :647

Run start: 2016/11/08 12:45:53
Run stop: 2016/11/08 14:43:35
PDF created: 2016/11/08 14:45:01

Figure 4.35: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Sphenoraia bicolor* using Forward and Reverse primers

Table 4.11: Comparison of Nucleotide frequencies of COI gene sequence of *Sphenoraia bicolor* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH656688 Sphenoraia bicolor	35.8	17.7	29.6	16.9	40	14.0	40.5	5.6	22	18.6	33.0	26.0	45	20.5	15.3	19.1
KC255440 Sphenoraia bicolor	36.0	17.8	29.5	16.7	42	13.5	40.9	3.7	23	17.2	31.2	28.4	43	22.8	16.3	18.1
KY835900 Chrysomelidae sp.	38.7	15.9	29.0	16.4	46	8.6	42.4	3.3	26	15.3	31.1	27.3	44	23.9	13.4	18.7
KC185726 Macrima sp.	36.5	16.3	31.8	15.4	42	10.7	47.0	.5	24	15.3	32.1	28.4	43	22.9	16.4	17.3
KC573813 Phalera assimilis	38.5	15.0	31.6	14.9	46	6.0	47.2	.9	28	14.3	30.9	26.7	41	24.9	16.6	17.1
KC255426 Phaedon armoraciae	38.4	15.5	30.2	15.8	48	7.0	44.2	.9	25	16.3	29.8	28.8	42	23.3	16.7	17.7
KC185715 Gallerucida ornatipennis	37.6	16.5	29.7	16.3	43	12.1	39.7	4.7	27	14.4	33.0	26.0	43	22.8	16.3	18.1
KC255442 Hespera lomasa	36.7	15.7	30.5	17.1	43	7.4	46.5	3.3	25	15.8	30.2	29.3	43	23.7	14.9	18.6
KC185698 Brachyphora nigrovittata	37.2	15.2	31.0	16.6	43	7.4	47.0	2.3	26	14.4	31.2	28.4	42	23.7	14.9	19.1
MG061719 Acalymma vittatum	37.1	16.1	30.6	16.2	42	7.6	47.6	2.4	25	16.7	30.1	27.8	44	23.9	13.9	18.7
KC185707 Doryidomorpha fulva	37.2	14.1	32.2	16.4	43	5.1	51.2	.9	27	13.5	30.7	29.3	42	23.7	14.9	19.1
KF656383. Aphthona albertinae	36.3	18.7	29.7	15.3	38	16.3	43.5	1.9	27	16.3	31.1	25.8	44	23.6	14.4	18.3
KM286140 Dinoptera collaris	41.1	13.7	29.5	15.8	51	2.9	45.2	1.0	29	13.4	29.2	28.2	43	24.9	13.9	18.2
KF654912 Longitarsus cerinthes	38.2	15.7	30.4	15.8	44	9.1	45.0	1.9	27	14.4	31.6	27.3	44	23.6	14.4	18.3

Table 4.12: Percentage of evolutionary divergence of *Sphenoraia bicolor* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH656688	*Sphenoraia bicolor* (Kerala)	
2.	KC255440	*Sphenoraia bicolor* (China)	7.31%
3.	KY835900	*Chrysomelidae* sp.	24.34%
4.	KC185726	*Macrima* sp.	25.76%
5.	KF654912	*Longitarsus cerinthes*	26.00%
6.	KC185698	*Brachyphora nigrovittata*	26.10%
7.	KC255442	*Hespera lomasa*	26.12%
8.	KC185707	*Doryidomorpha fulva*	26.29%
9.	MG061719	*Acalymma vittatum*	26.32%
10.	KM286140	*Dinoptera collaris*	26.33%
11.	KF656383	*Aphthona albertinae*	26.36%
12.	KC185715	*Gallerucida ornatipennis*	26.80%
13.	KC573813	*Phalera assimilis*	26.86%
14.	KC255426	*Phaedon armoraciae*	27.87%

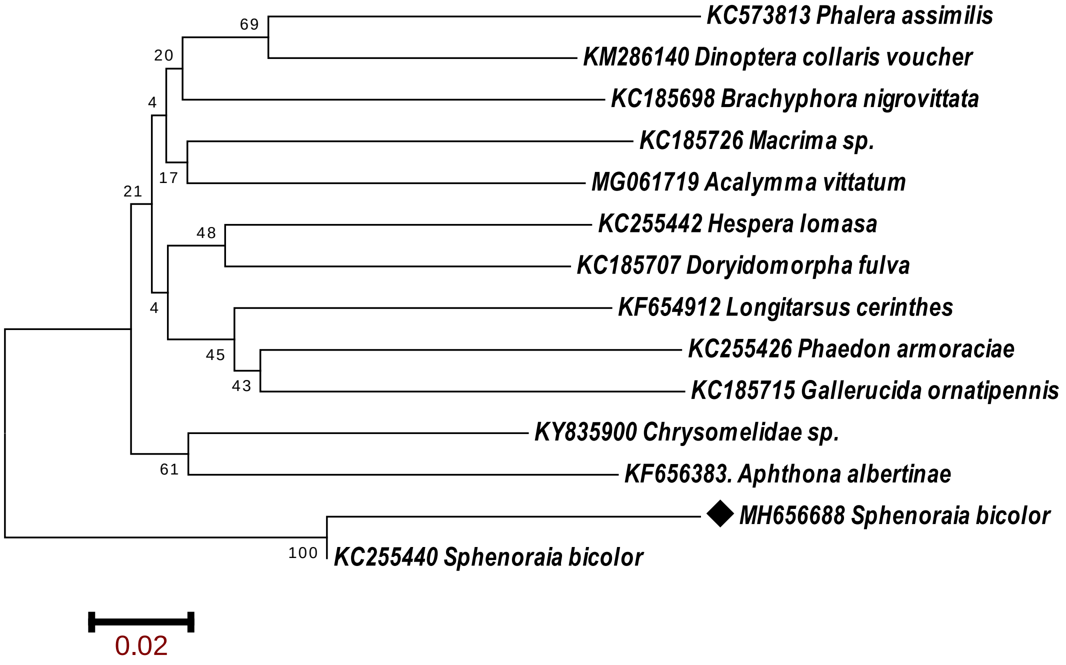


Figure 4.36: Phylogenetic relationship of *Sphenoraia bicolor* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 92.7% sequence similarity to the same genus reported from China (KC255440). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Sphenoraia bicolor* showed bias to nucleotide AT, with following composition of nucleotides T = 35.8%, C = 17.7%, A = 29.6% and G = 16.9%. This greater AT content (65.2%) over GC content (34.6%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Sphenoraia bicolor* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed 7.31% to 27.87% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has 7.31% divergence with those from China while 27.87% from Beijing (Table 4.12). On the basis of the data observed this species may be rooted from those found in Beijing which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while wide changes from those reported from China and Beijing during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Sphenoraia bicolor* with related species. *Sphenoraia bicolor* isolated from Kerala (MH656688) showed 7.31% divergence with *Sphenoraia bicolor* (KC255440) and 27.87% divergence with *Phaedon armoraciae* (KC255426) from Beijing. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Sphenoraia bicolor* isolated from Kerala.

Closest relative of *Sphenoraia bicolor* is the *Sphenoraia bicolor* from China represented within the same clade.

7. ***Paria thoracica*** (Wilcox, 1954)

Specimen details:

Voucher specimen	:	CUPT – 01 – A2
Date of collection	:	30-Apr-2016
Locality	:	Kasaragod: Chandragiri
Lat- Lon	:	12.4991° N, 74.9989° E
GenBank accession	:	MH590759

Description and distribution:

The body lenth is 3.5mm (Figure 4.37). The body entirely black but head and pronotum yellow coloured, punctuation fine or absent, front femur have no tooth, posterior claws with inner lobe long, three-quarters length of outer lobe, elytra black coloured and moderately punctuate, aedeagus with large lateral lobes nearly as long as broad median lobe, the median lobe moderately broad (James et al., 1995). Found in India, South and North America.

Fig: 4.37: *Paria thoracica*

Damage:

The larvae feed on the male flower head and adult preach on leaves (Krischik and Denno, 1990).

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Paria thoracica* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 610 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 4.38 – 4.41 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 4.13.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Paria thoracica* voucher collected during the present study (Table 4.13). It showed bias to nucleotide AT, with nucleotide composition with T = 36.3%, C = 17.4%, A = 29.6% and G = 16.8%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 4.14 and Figure 4.42 respectively.

>*Paria thoracica* CDS-2018/ 610 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUPT-01-A2

> *Paria thoracica*

```
TGAGCCGGGATAGTTGGGACTTCATTAAGTATATTAATTCGTACAGAATTAGGAGCCCCT
GGATCATTAATTGGAAATGACCAAATTTATAATGTTATTGTAACAGCCCATGCATTCATT
ATAATTTTTTTCATAGTTATACCAATTATAATTGGGGGATTTGGAAATTGATTAGTCCCC
TTAATAATCGGAGCTCCGGATATAGCTTTTCCACGAATAAATAATATAAGATTTTGATTA
CTTCCTCCTTCCTTATTTCTACTAATTATAAGTAGAGTGGTCGAAAGGGGTGCTGGAACA
GGTTGAACAGTTTATCCCCCTTTATCTTCTAATATTGCCCACGGAGGATCTTCAGTGGAC
TTAGCTATTTTTAGCCTTCATTTAGCAGGAATTTCATCAATTTTGGGAGCAATTAATTTT
ATTACTACAGTTATTAATATACGACCTATAGGAATAACCTTAGATCGAATACCCTTATTT
GTGTGAGCAGTAGTAATCACTGCTATTTTACTTCTTCTATCCTTGCCTGTCTTAGCAGGA
GCTATTACTATACTTTTAACAGATCGTAATCTAAATACATCATTTTTCGATCCTGCAGGA
GGGGGTGACC
```

Figure 4.38: The partial DNA sequence of the mitochondrial COI gene of *Paria thoracica*

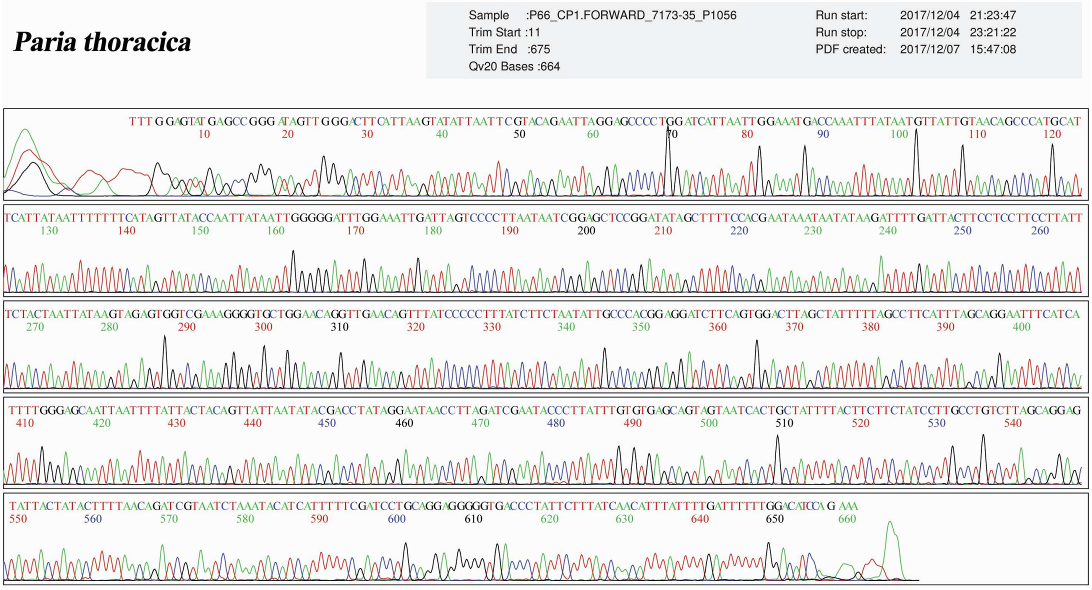
Figure.4.39. Molecular barcode of the mitochondrial COI gene of *Paria thoracica*

> *Paria thoracica* / 200AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUAF-01-A2

> *Paria thoracica*

```
MVGTSLSMLIRTELGAPGSLIGNDQIYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMI
GAPDMAFPRMNNMSFWLLPPSLFLLIMSSVVESGAGTGWTVYPPLSSNIAHGGSSVDLAI
FSLHLAGISSILGAINFITTVINMRPMGMTLDRMPLFVWAVVITAILLLLSLPVLAGAIT
MLLTDRNLNTSFFDPAGGGD
```

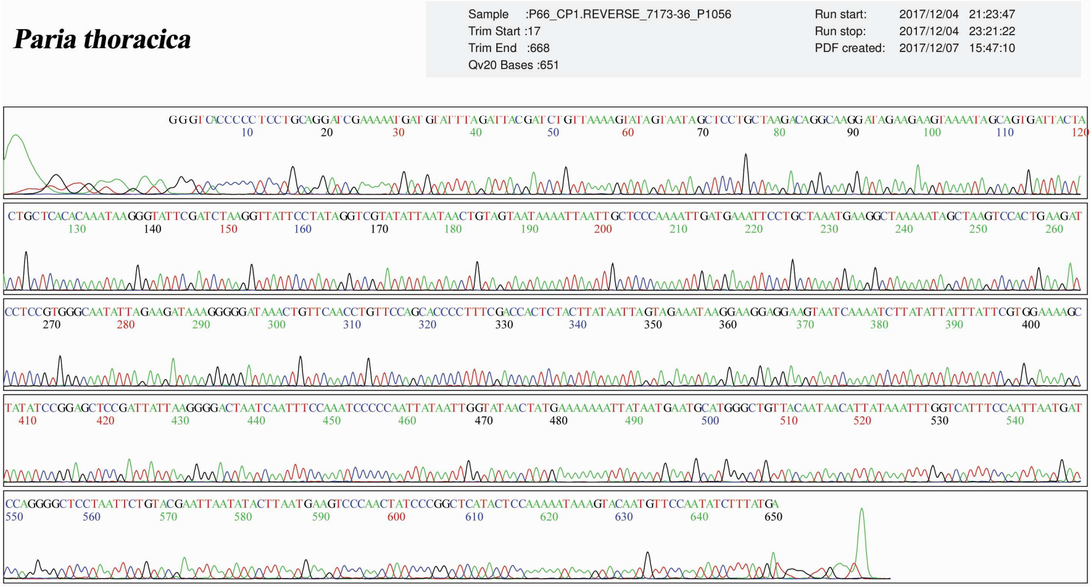
Figure 4.40: The translation product of the mitochondrial COI gene of *Paria thoracica*



Paria thoracica

Sample :P66_CP1.FORWARD_7173-35_P1056
Trim Start :11
Trim End :675
Qv20 Bases :664

Run start: 2017/12/04 21:23:47
Run stop: 2017/12/04 23:21:22
PDF created: 2017/12/07 15:47:08



Paria thoracica

Sample :P66_CP1.REVERSE_7173-36_P1056
Trim Start :17
Trim End :668
Qv20 Bases :651

Run start: 2017/12/04 21:23:47
Run stop: 2017/12/04 23:21:22
PDF created: 2017/12/07 15:47:10

Figure 4.41: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Paria thoracica* using Forward and Reverse primers

Table 4.13: Comparison of Nucleotide frequencies of COI gene sequence of *Paria thoracica* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH590759 Paria thoracica	36.3	17.4	29.6	16.8	24	15.5	33.0	27.8	45	25.8	11.9	17.5	40	10.8	43.8	5.2
KY769285 Paria thoracica	36.3	17.4	29.9	16.5	23	16.0	33.0	27.8	45	25.8	11.9	17.5	41	10.3	44.8	4.1
KU557525 Mantura sp.	37.5	16.8	29.4	16.3	24	15.5	32.5	27.8	46	24.2	12.4	17.0	42	10.8	43.3	4.1
KJ677801 Galerucinae sp.	38.3	15.3	30.6	15.8	23	16.5	32.5	28.4	46	24.2	11.3	18.0	46	5.2	47.9	1.0
KC185740 Paridea biplagiata	37.8	15.5	30.4	16.3	25	13.9	33.5	27.3	45	23.2	12.9	18.6	43	9.3	44.8	3.1
KU697451 Aphthona strigosa	37.3	15.6	31.4	15.6	24	15.5	34.0	26.3	45	24.2	12.4	18.0	42	7.2	47.9	2.6
KF652923 Mantura chrysanthemi	37.5	14.8	32.6	15.1	28	12.9	32.0	26.8	46	24.2	11.9	17.5	38	7.2	54.1	1.0
MG587922 Aulacophora lewisii	37.3	15.6	30.4	16.7	25	14.4	33.0	27.8	46	24.2	11.3	18.6	41	8.2	46.9	3.6
LT991425 Ochthebius klapperichi	41.1	14.9	27.8	16.2	27	13.9	29.9	28.9	46	25.3	11.3	17.5	50	5.7	42.3	2.1
MF140520 Luperomorpha sp.	37.5	15.6	30.6	16.3	24	15.5	31.4	28.9	46	24.2	11.3	18.6	42	7.2	49.0	1.5
KU917773 Longitarsus tabidus	38.0	15.3	31.6	15.1	27	12.9	32.5	27.8	47	24.2	11.3	17.5	40	8.8	51.0	.0
KM448517 Olibrus liquidus	38.8	16.7	27.8	16.7	24	18.0	29.9	28.4	45	25.8	11.3	17.5	47	6.2	42.3	4.1
KF737784 Gaurotes tuberculicollis	40.1	13.6	30.4	16.0	27	13.3	33.3	26.7	43	23.7	14.9	18.0	51	3.6	42.8	3.1
KC185762 Hespera brevipilosa	37.1	15.6	29.9	17.4	25	14.4	29.9	30.4	45	23.7	13.4	18.0	41	8.8	46.4	3.6
KF653323 Longitarsus minusculus	39.2	15.5	29.4	16.0	28	12.4	33.0	26.8	47	24.2	11.3	17.5	43	9.8	43.8	3.6

Table 4.14: Percentage of evolutionary divergence of *Paria thoracica* with its closely related species accessible from NCBI GenBank

Sl. No.	**Accession No**	**Organism**	**Percentage of divergence**
1.	MH590759	*Paria thoracica* (Kerala)	
2.	KY769285	*Paria thoracica* (Kerala)	0.32%
3.	KU557525	*Mantura* sp.	4.75%
4.	KJ677801	*Galerucinae* sp.	9.18%
5.	MG587922	*Aulacophora lewisii*	10.84%
6.	KF652923	*Mantura chrysanthemi*	10.91%
7.	KU697451	*Aphthona strigosa*	11.25%
8.	KC185762	*Hespera brevipilosa*	11.52%
9.	KU917773	*Longitarsus tabidus*	11.70%
10.	MF140520	*Luperomorpha* sp.	12.00%
11.	KC185740	*Paridea biplagiata*	12.12%
12.	KF737784	*Gaurotes tuberculicollis*	12.30%
13.	KF653323	*Longitarsus minusculus*	12.51%
14.	LT991425	*Ochthebius klapperichi*	12.53%
15.	KM448517	*Olibrus liquidus*	13.69%

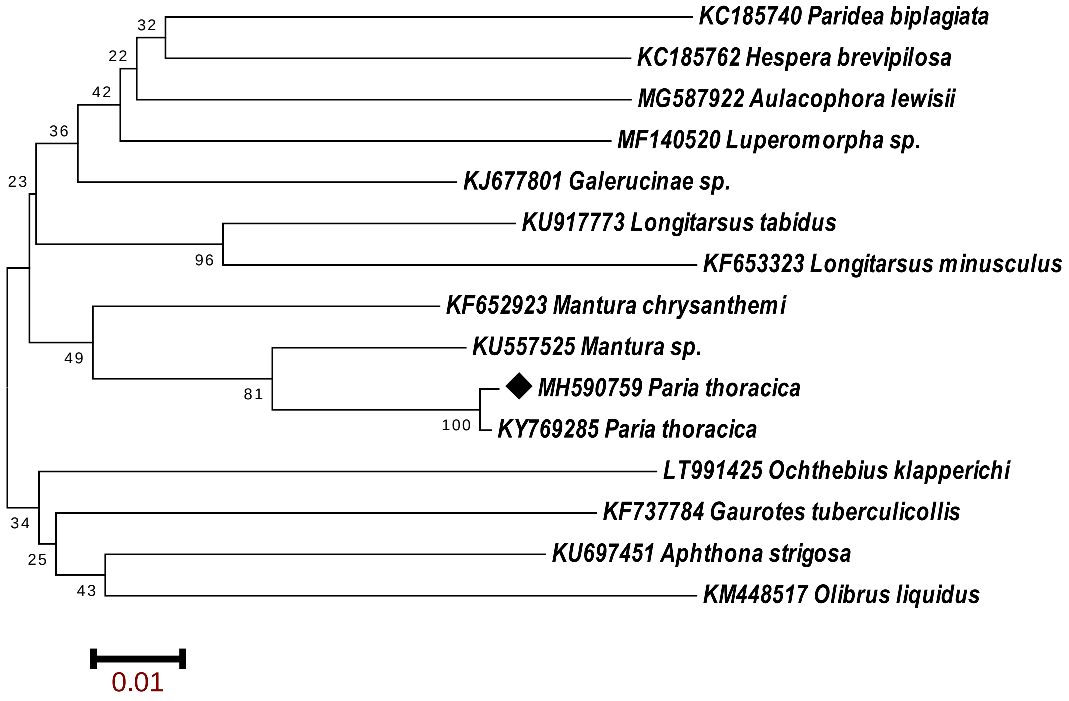


Figure 4.42: Phylogenetic relationship of *Paria thoracica* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 99.7% sequence similarity to the same genus reported from Kerala (KY769285). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Paria thoracica* showed bias to nucleotide AT, with following composition of nucleotides T = 36.3%, C = 17.4%, A = 29.6% and G = 16.8%. This greater AT content (65.9%) over GC content (34.2%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Paria thoracica* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 0.32% to 13.69% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (0.32%) to those from Kerala while 13.69% from Germany (Table 4.14). On the basis of the data observed this species may be rooted from those found in Germany which diverted into different clades due to geographical variation. Result thus concluded that this species doesn't have any major changes in India while slightly changes from those reported from Germany during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Paria thoracica* with related species. *Paria thoracica* isolated from Kerala (MH674105) showed 0.32% divergence with *Paria thoracica* (KY769285) from Kerala and 13.69% divergence with *Olibrus liquidus* (KM448517) from Germany. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Paria thoracica* isolated from Kerala. Closest relative of *Paria thoracica* is *Paria thoracica* from Kerala represented within the same clade.

8. *Rhabdopterus praetextus* (Say, 1824)

Specimen details:

Voucher specimen	:	CURP – 02 – A1
Date of collection	:	6- Apr-2016
Locality	:	Malappuram: Kadalundi
Lat- Lon	:	11.03º N, 76.05º E
GenBank accession	:	MH674108

Description and distribution:

They are small sized and possess whitish antennae and palpi with pale legs, eye emargination and pronotal lateral margin absent, prothorax cylindrical and transverse. Aedeagus oblong, sides slightly convergent, the apex are abruptly, sinuately truncate with a short median process (Figure 4.43). Apical tooth of aedeagus are short and rounded. The male have hind tibiae with lower carina obsolescent. They are phytophagus distributed in India, Vietnam, South and North America (Krischik and Denno,1990).

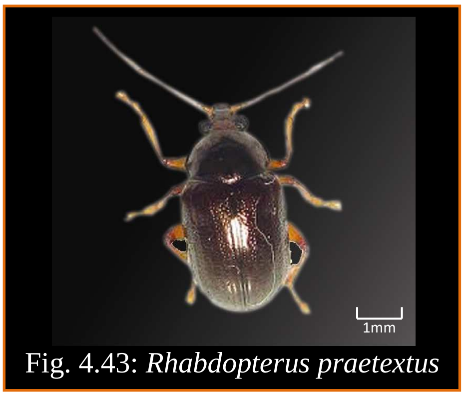


Fig. 4.43: *Rhabdopterus praetextus*

Damage:

The adults are regarded as a polyphagus leaf eaters. Curved holes or slits in the leaves indicate the voracious night time feeding of *Rhabdopterus* species especially *Rhabdopterus praetextus.*

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Rhabdopterus praetextus* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 601 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 4.44 – 4.47 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 4.15.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Rhabdopterus praetextus* voucher collected during the present study (Table 4.15). It showed bias to nucleotide AT, with nucleotide composition with T = 37.8%, C = 15.5%, A = 31.4% and G = 15.3%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 4.16 and Figure 4.48 respectively.

>*Rhabdopterus praetextus* CDS-2018/ 601 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CURP-02-A1

> *Rhabdopterus praetextus*

```
ATAGTTGGGACTTCTTTAAGAGTGTTAATTCGACTTGAACTTGGAACAACAAGAACTTTA
ATTGGAAACGACCAAATTTATAACGTAATTGTAACAGCCCATGCATTCATTATAATTTTT
TTTATAGTTATACCCATTATAATTGGAGGATTTGGAAATTGATTAGTGCCTTTAATAATT
GGGGCTCCAGATATAGCCTTTCCTCGATTAAATAATATAAGATTTTGATTATTACCTCCC
TCACTAACTTTTCTAATTTTAAGAAGAATTGTAGAAAGAGGGGCAGGAACAGGTTGGACA
GTCTACCCTCCTTTATCTTCAAATTTAGCCCATGGGGGATCTTCTGTAGATTTAGCTATT
TTTAGATTACATTTAGCTGGAATTTCTTCAATTTTAGGAGCAGTAAATTTCATTTCTACA
ATTATTAATATACGAACTTACGGAATAACCTTTGAAAAAATACCTTTATTTGTTTGATCA
GTTTTTATTACAGCCATTTTATTACTACTTTCACTACCAGTCTTAGCAGGTGCTATTACA
ATGCTATTAACAGATCGAAATATTAATACATCCTTTTTTGATCCTGCTGGAGGAGGAGAC
C
```

Figure 4.44: The partial DNA sequence of the mitochondrial COI gene of *Rhabdopterus praetextus*

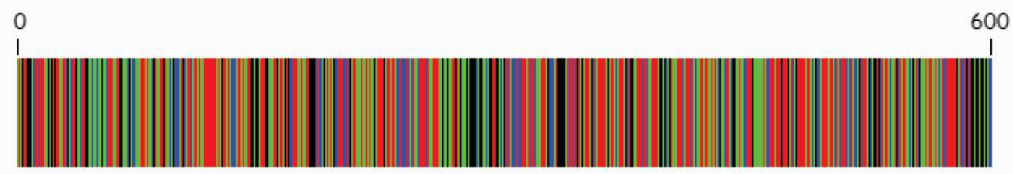


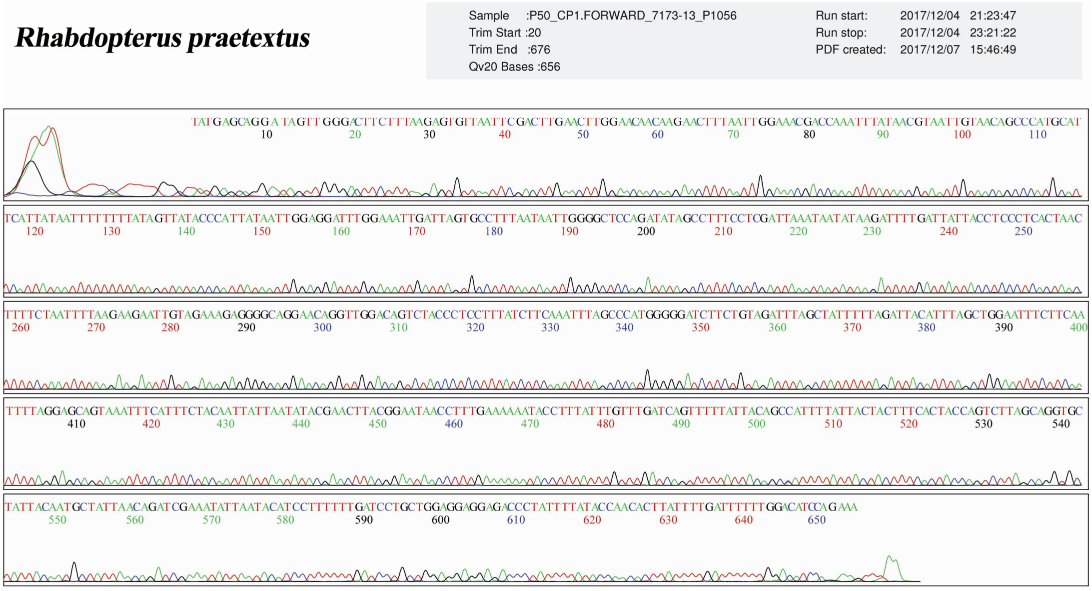
Figure 4.45: Molecular barcode of the mitochondrial COI gene of *Rhabdopterus praetextus*

> *Rhabdopterus praetextus* / 200AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CURP-02-A1

> *Rhabdopterus praetextus*

```
MVGTSLSVLIRLELGTTSTLIGNDQIYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMI
GAPDMAFPRLNNMSFWLLPPSLTFLILSSIVESGAGTGWTVYPPLSSNLAHGGSSVDLAI
FSLHLAGISSILGAVNFISTIINMRTYGMTFEKMPLFVWSVFITAILLLLSLPVLAGAIT
MLLTDRNINTSFFDPAGGGD
```

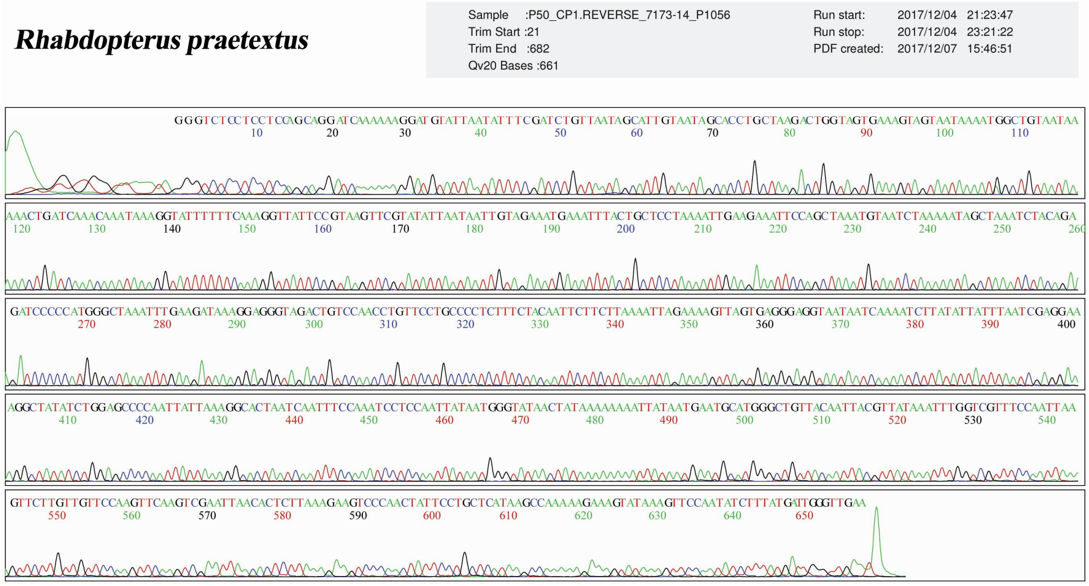
Figure 4.46: The translation product of the mitochondrial COI gene of *Rhabdopterus praetextus*



Rhabdopterus praetextus

Sample :P50_CP1.FORWARD_7173-13_P1056
Trim Start :20
Trim End :676
Qv20 Bases :656

Run start: 2017/12/04 21:23:47
Run stop: 2017/12/04 23:21:22
PDF created: 2017/12/07 15:46:49



Rhabdopterus praetextus

Sample :P50_CP1.REVERSE_7173-14_P1056
Trim Start :21
Trim End :682
Qv20 Bases :661

Run start: 2017/12/04 21:23:47
Run stop: 2017/12/04 23:21:22
PDF created: 2017/12/07 15:46:51

Figure 4.47: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Rhabdopterus praetextus* using Forward and Reverse primers

Table 4.15: Comparison of Nucleotide frequencies of COI gene sequence of *Rhabdopterus praetextus* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH674108 Rhabdopterus praetextus	37.8	15.5	31.4	15.3	46	24.4	12.8	16.9	40	8.7	47.7	4.1	28	13.4	33.7	25.0
KM440433 Scymnus impexus	38.3	14.5	31.1	16.1	44	23.8	13.4	18.6	43	6.4	46.8	3.5	27	13.4	33.1	26.2
JF887875 Didion longulum	40.4	13.3	30.9	15.3	45	23.8	12.8	18.6	49	2.9	47.4	1.2	28	13.4	32.6	26.2
KJ163825 Coccinellidae sp.	39.3	14.1	31.5	15.1	45	23.8	12.8	18.6	45	5.2	49.1	.6	28	13.4	32.6	26.2
MH094933 Coleoptera sp.	39.4	15.6	31.0	14.0	46	27.1	12.9	14.1	42	7.6	47.1	2.9	30	12.3	32.7	25.1
KU918863 Scymnus nigrinus	38.4	14.9	31.0	15.7	44	23.8	13.4	18.6	42	8.7	46.5	2.3	28	12.2	33.1	26.2
GU073957 Scymnus subvillosus	39.5	14.3	31.8	14.3	46	25.0	12.8	16.3	42	6.4	50.6	1.2	31	11.6	32.0	25.6
KM440972 Scymnus interruptus	38.5	15.1	30.9	15.5	44	23.8	13.4	18.6	44	8.1	46.2	1.7	27	13.4	33.1	26.2
KM441516 Scymnus limbatus	39.1	14.5	31.1	15.3	45	23.8	12.8	18.6	43	7.5	48.0	1.7	30	12.2	32.6	25.6
KM448896 Scymnus abietis	39.1	14.1	30.4	16.4	44	23.8	13.4	18.6	42	8.1	45.1	4.6	31	10.5	32.6	26.2
KM445762 Scymnus haemorrhoidalis	38.7	14.1	30.2	17.0	45	23.3	12.8	19.2	44	5.8	44.5	5.8	27	13.4	33.1	26.2
MF140494 Scymnus posticalis	38.8	15.3	31.0	14.9	46	24.4	12.8	16.9	42	8.1	46.5	2.9	28	13.4	33.7	25.0
KU918430 Scymnus ferrugatus	37.5	15.5	30.6	16.4	44	23.8	13.4	18.6	40	10.4	45.7	4.0	28	12.2	32.6	26.7
KR490680 Scymnus caurinus	38.9	13.2	32.1	15.8	44	23.3	13.4	19.2	43	5.8	49.7	1.8	30	10.5	33.3	26.3
KM444040 Scymnus mimulus	38.2	15.1	31.6	15.1	45	24.4	12.2	18.0	42	8.1	48.3	1.2	27	12.8	34.3	26.2

Table 4.16: Percentage of evolutionary divergence of *Rhabdopterus praetextus* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH674108	*Rhabdopterus praetextus* (Kerala)	
2.	MH094933	*Coleoptera* sp.(Germany)	7.59%
3.	KM440433	*Scymnus impexus*	10.85%
4.	GU073957	*Scymnus subvillosus*	10.94%
5.	KR490680	*Scymnus caurinus*	11.53%
6.	KU918430	*Scymnus ferrugatus*	11.79%
7.	KM441516	*Scymnus limbatus*	12.10%
8.	KM448896	*Scymnus abietis*	12.11%
9.	KU918863	*Scymnus nigrinus*	12.16%
10.	KJ163825	*Coccinellidae* sp.	12.65%
11.	JF887875	*Didion longulum*	12.73%
12.	KM440972	*Scymnus interruptus*	12.99%
13.	KM445762	*Scymnus haemorrhoidalis*	13.03%
14.	MF140494	*Scymnus posticalis*	13.20%
15.	KM444040	*Scymnus mimulus*	14.31%

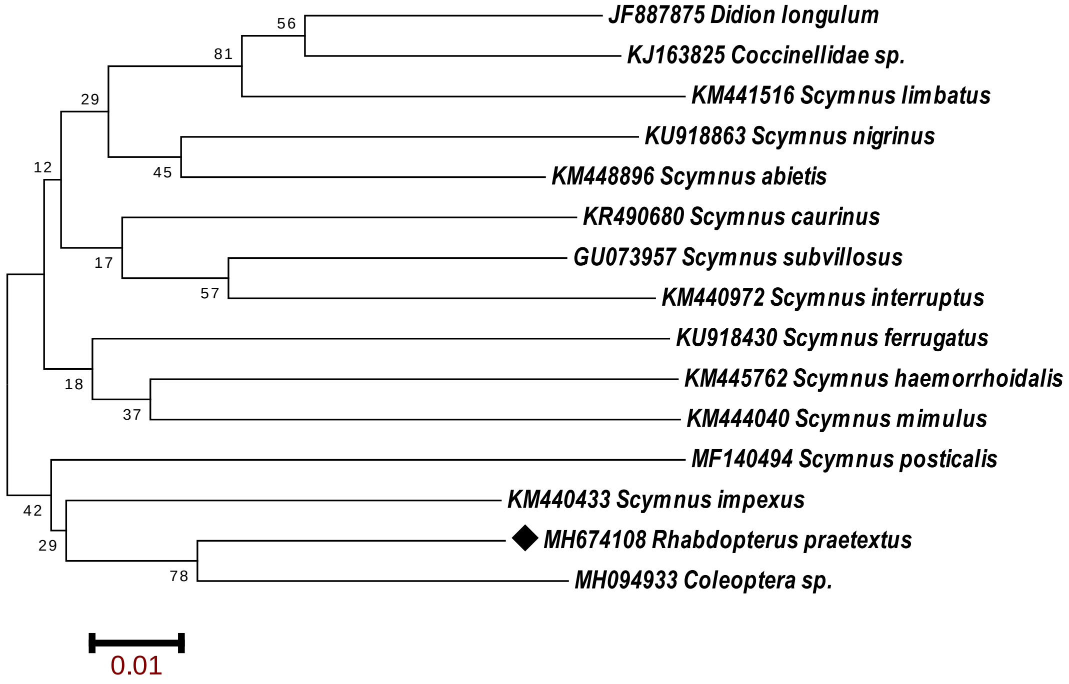


Figure 4.48: Phylogenetic relationship of *Rhabdopterus praetextus* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 92.5% sequence similarity to the same genus reported from Germany (MH094933). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Rhabdopterus praetextus* showed bias to nucleotide AT, with following composition of nucleotides T = 37.8%, C = 15.5%, A = 31.4% and G = 15.3%. This greater AT content (69.2%) over GC content (30.8%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Rhabdopterus praetextus* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 7.59% to 14.31% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (7.59%) those from Germany (Table 4.16). On the basis of the data observed this species may be rooted from those found in Germany which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from Germany during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Rhabdopterus praetextus* with related species. *Rhabdopterus praetextus* isolated from Kerala (MH674108) showed 7.59% divergence with *Coleoptera* sp. (MH094933) from Germany and 14.31% divergence with *Scymnus mimulus* (KM444040) from Germany. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Rhabdopterus praetextus* isolated from Kerala. Closest relative of *Rhabdopterus praetextus* is *Coleoptera* sp.from Germany represented within the same clade.

9. *Oocassida pudibunda* (Boheman, 1856)

Specimen details:

Voucher specimen : CUOP – 01 – A1
Date of collection : 6- Sep-2016
Locality : Malappuram: Kadalundi
Lat- Lon : 11.03º N, 76.05º E
GenBank accession : KX603663

Description and distribution:

Fig. 4.49: *Occassida pudibunda*

Length is 7 mm; breadth, 4 mm (Figure 4.49). Tarsal hair have no comb like structures at its base, pronotum have a groove on its side for antenna reception, the elytra possesses faint longitudinal red stripe all along the suture (Rawat and Modi, 1972). Body elongate to oval in shape. Colour dirty brown, with a faint greenish tinge (elytra green in life), the narrow explanate margins lighter, with a faint red stripe along the suture; the central part of the underside black, but not the legs. The antennae are short; the third joint is a little longer than the second and almost equal to the fourth, the fifth and sixth each shorter than the fourth, and the club more hairy and darker. Prothorax as broad as the elytra at the base, with the basal edge gently bisinuate, and the whole of the upper surface including the explanate margins uniformly and closely punctate. Scutellum is smooth and impunctate. Elytra strongly convex, with the suture raised. There is a short scutellar row of punctures and ten complete rows on each elytron, the centre of the punctures being black. Underside black, except the sides of the abdominal segments, smooth, shining and impunctate; the legs brown. These are found to be distributed throughout India.

Damage:

The larvae feed from the lower surface of the leaf lamina, while the adults feed on both sides of the leaf. Resting beetles often prefer the upper surface of the apical region of the leaf. The feeding of both larvae and the adults is in the form of small irregular scraping. They feed on the entire leaf (primary and secondary ribs and petiole) and changing to next leaf only when the previous was totally eaten.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Oocassida pudibunda* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 564 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 4.50 – 4.53 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 4.17.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Oocassida pudibunda* voucher collected during the present study (Table 4.17). It showed bias to nucleotide AT, with nucleotide composition with T = 34.8%, C = 15.4%, A = 34.6% and G = 15.2%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 4.18 and Figure 4.54 respectively.

>*Oocassida pudibunda* CDS-2016/ 564 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUOP-01-A1

> *Oocassida pudibunda*

CGTCATTAAGAATTTTAATTCGAGCTGAATTAGGAAGTCCAGGAACTTTGATCGGTAATG
ATCAAATTTATAATTCTATTGTAACTGCCCACGCATTTGTTATAATTTTTTTTATAGTAA
TACCTATTATAATTGGAGGATTTGGAAATTGATTAGTACCACTAATATTAGGAGCACCAG
ACATAGCATTCCCACGACTAAATAATATAAGATTTTGACTTTTACCACCCTCAATTAGAT
TTTTAGTATTAAGAAGAATTGTTGAAAGAGGGGTAGGAACAGGTTGAACAGTATACCCCC
CATTATCAAATAATATTGCCCATAGAGGATCATCAGTAGATCTAGCAATTTTCAGTTTAC
ATTTAGCAGGTATTTCATCAATTTTAGGGGCAATTAATTTTATTTCAACAATTATAAATA
TACGACCATCAGGAATAAATCTTGATAAAATAGCCTTATTTGTTTGATCAGTAATTATTA
CAGCTATTCTTTTACTACTATCACTGCCAGTATTAGCAGGGGCTATTACTATATTACTAA
CAGATCGTAATATAAATACATCTT

Figure 4.50: The partial DNA sequence of the mitochondrial COI gene of *Oocassida pudibunda*

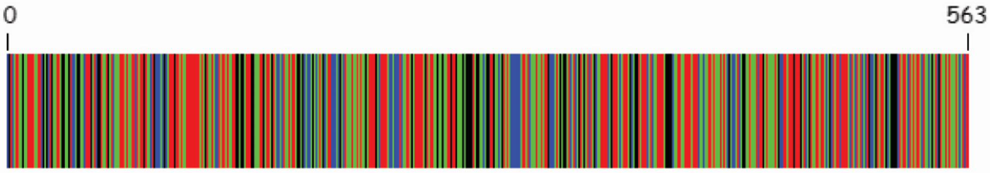


Figure 4.51: Molecular barcode of the mitochondrial COI gene of *Oocassida pudibunda*

> *Oocassida pudibunda* / 154AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUOP-01-A1

> *Oocassida pudibunda*

MIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSISFLVLSSIVESGVGT
GWTVYPPLSNNIAHSGSSVDLAIFSLHLAGISSILGAINFISTIMNMRPSGMNLDKMALF
VWSVIITAILLLLSLPVLAGAITMLLTDRNMNTS

Figure 4.52: The translation product of the mitochondrial COI gene of *Oocassida pudibunda*

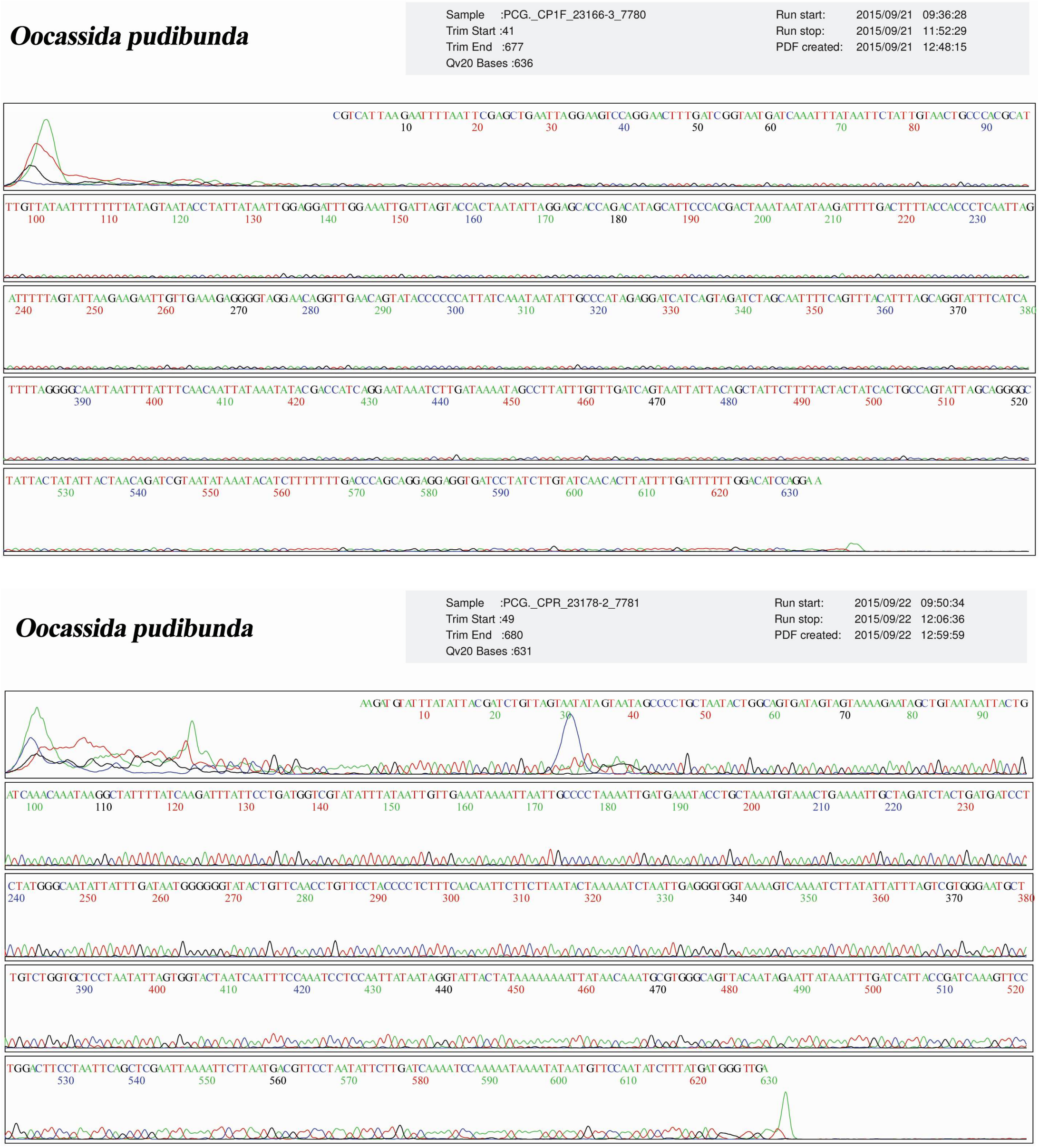


Figure 4.53: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Oocassida pudibunda* using Forward and Reverse primers

Table 4.17: Comparison of Nucleotide frequencies of COI gene sequence of *Oocassida pudibunda* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
KX603663 Oocassida pudibunda	34.8	15.4	34.6	15.2	36	5.9	55.1	3.2	24	15.6	34.9	25.3	44	24.6	13.9	17.1
KX051834 Cassida compuncta	34.7	16.5	33.6	15.2	35	9.1	53.2	2.7	25	15.5	33.2	26.2	44	24.7	14.5	16.7
KJ966318 Cassida murraea	35.0	16.3	33.2	15.5	37	7.0	51.3	4.3	24	15.6	34.9	25.8	44	26.2	13.4	16.6
KF654910 Hypocassida meridionalis	35.4	17.5	31.6	15.5	36	11.8	48.1	3.7	24	16.6	34.2	25.1	46	24.2	12.4	17.7
KM451640 Cassida rubiginosa	32.9	17.7	34.3	15.2	27	13.9	55.1	3.7	26	14.4	34.8	24.6	45	24.7	12.9	17.2
MH322762 Cassida sanguinolenta	36.8	15.5	32.7	15.0	41	6.4	49.7	3.2	25	15.5	35.3	24.6	45	24.7	12.9	17.2
KM451192 Cassida azurea	35.5	17.5	32.3	14.6	36	11.8	49.7	2.7	26	15.5	34.8	24.1	45	25.3	12.4	17.2
KU910819 Cassida stigmatica	35.0	16.8	32.1	16.1	37	9.1	48.1	5.9	23	17.1	34.8	25.1	45	24.2	13.4	17.2
KU917781 Cassida ferruginea	35.5	16.8	31.8	15.9	36	9.6	48.1	5.9	25	16.0	34.2	24.6	45	24.7	12.9	17.2
KJ963600 Cassida nebulosa	36.1	15.7	32.0	16.3	40	6.4	49.7	4.3	23	15.6	34.9	26.3	45	25.1	11.2	18.2
FJ819705 Laccophilus sp.	38.9	14.1	30.7	16.3	44	3.2	50.8	2.1	28	14.4	29.9	27.8	45	24.7	11.3	18.8
KM554912 Ectoedemia quadrinotata	38.9	13.6	32.5	15.0	41	4.3	52.9	1.6	30	12.3	31.0	26.2	45	24.2	13.4	17.2
JN201715 Ectoedemia haraldi	39.8	13.5	32.1	14.6	43	3.7	52.4	.5	31	11.8	30.5	26.7	45	25.1	13.4	16.6
KF397116 Philobota atrisignis	40.4	15.0	30.2	14.5	49	5.3	44.4	1.1	27	15.0	32.6	25.1	45	24.7	13.4	17.2
KJ195306 Chiridopsis ventralis	33.4	16.6	34.8	15.2	31	10.8	55.9	2.2	24	13.9	36.4	25.7	45	25.1	12.3	17.6

Table 4.18: Percentage of evolutionary divergence of *Oocassida pudibunda* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	KX603663	*Oocassida pudibunda* (Kerala)	
2.	KX051834	*Cassida compuncta* (France)	13.31%
3.	FJ819705	*Laccophilus* sp.	17.59%
4.	KJ966318	*Cassida murraea*	17.96%
5.	KF654910	*Hypocassida meridionalis*	19.08%
6.	KM554912	*Ectoedemia quadrinotata*	19.66%
7.	KM451640	*Cassida rubiginosa*	19.79%
8.	MH322762	*Cassida sanguinolenta*	19.96%
9.	KM451192	*Cassida azurea*	20.34%
10.	JN201715	*Ectoedemia haraldi*	20.43%
11.	KU917781	*Cassida ferruginea*	20.73%
12.	KJ195306	*Chiridopsis ventralis*	20.73%
13.	KF397116	*Philobota atrisignis*	20.87%
14.	KU910819	*Cassida stigmatica*	21.11%
15.	KJ963600	*Cassida nebulosa*	21.71%

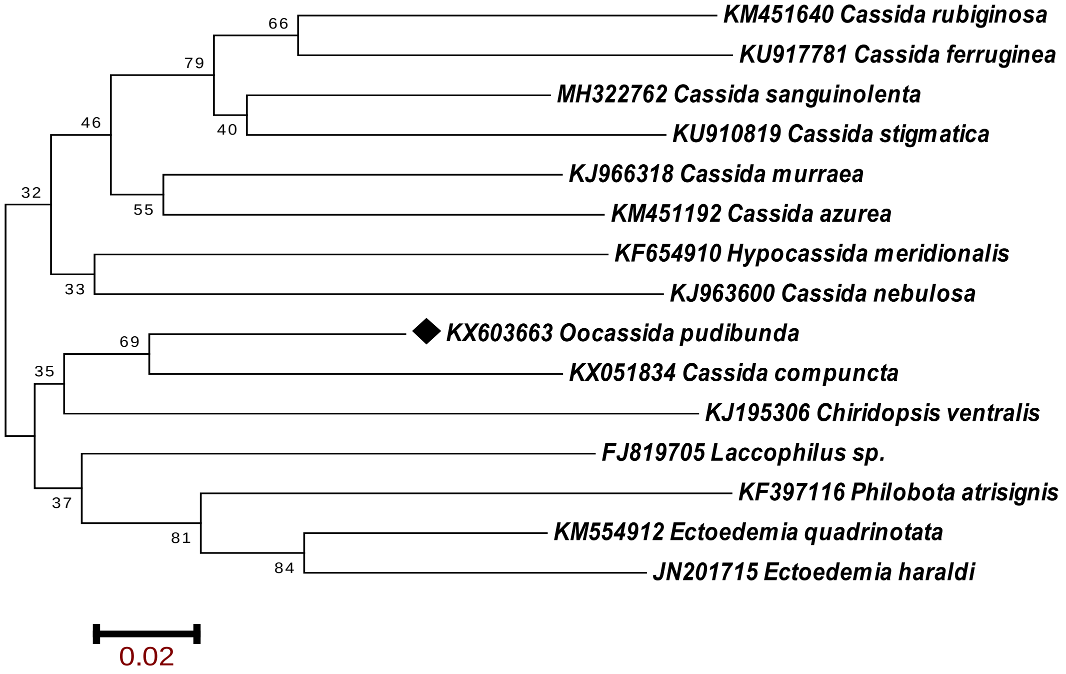


Figure 4.54: Phylogenetic relationship of *Oocassida pudibunda* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 86.7% sequence similarity to the related genus reported from France (KX051834). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *M. chrysanthemi* showed bias to nucleotide AT, with following composition of nucleotides T = 34.8%, C = 15.4%, A = 34.6% and G = 15.2%. This greater AT content (69.2%) over GC content (30.6%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Oocassida pudibunda* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed 13.31% to 21.71% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (13.31%) those from France while 21.71% from Finland (Table 4.18). On the basis of the data observed this species may be rooted from those found Finland which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from Finland during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Oocassida pudibunda* with related species. *Oocassida pudibunda* isolated from Kerala (KX603663) showed 13.31% divergence with *Cassida compuncta* (KX051834) from France and 21.71% divergence with *Cassida nebulosa* (KJ963600) from Finland. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Oocassida* *pudibunda* isolated from Kerala. Closest relative of *Oocassida pudibunda* is *Cassida compuncta* from France represented within the same clade.

10. ***Dactylispa carinata*** (Chen and T'an, 1961)

Specimen details:

Voucher specimen	:	CUDC – 02 – A1
Date of collection	:	20-Apr-2016
Locality	:	Kannur: Madayi
Lat- Lon	:	12.0308° N, 75.2395° E
GenBank accession	:	MH674110

Description and distribution:

D. carinata have shorter spines and body usually much more widened apically (Figure 4.55). The body is subparallel, lateral margin of pronotum and elytra with long, stiff spines; antennomere 1 without spine, pronotum with anterior margin with branched spine on each side; elytra with spines or tubercles on disc. These are found to be distributed in India, China, various parts of Neotropical regions (Chen et al., 2005).

Fig.4.55: *Dactylispa carinata*

Damage:

They are inhabitants of leaves of host plant (Zaitsev et al., 2014). Larvae of this *Dactylispa carinata* mine in leaves, while adults eat the cuticula parallel to the leaf veins.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Dactylispa carinata* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 612 bp long products for the specimens obtained from two different locations. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 4.56 – 4.59 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 4.19.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Dactylispa carinata* voucher collected during the present study (Table 4.19). It showed bias to nucleotide AT, with nucleotide composition with T = 33.9%, C = 17.6%, A = 33.1% and G = 15.4%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 4.20 and Figure 4.60 respectively.

> *Dactylispa carinata* CDS-2018/ 612 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUDC-02-A1

> *Dactylispa carinata*

TCTGATCTGGTATAGTAGGAACAGCTCTGAGAATTCTAATTCGAACAGAATTAGGAAACC
CTGGTACATTTATCGGAAATGATCAAATCTACAATGTTATTGTAACCGCTCATGCATTTA
TTATAATTTTTTTTATAGTAATACCAATTATAATTGGAGGATTTGGTAACTGATTAGTAC
CACTAATAATTGGAGCACCTGATATAGCATTCCCTCGTCTAAATAATATAAGATTCTGGC
TTCTTCCACCTTCAATTTTTCTTTTAATAATAAGAAGAATGATTGAAAGAGGGGCAGGAA
CCGGATGAACAGTATATCCTCCTTTATCATCAAATATTGCTCACAGGGGAGCCTCAGTTG
ATCTAGCAATTTTTAGATTACATTTAGCTGGTATTTCATCAATCTTAGGAGCTATTAATT
TTATCTCAACCATTATAAATATACAGCCAACAATAATAAAATTTGATAAAACACCTCTGT
TCGTTTGAGCAGTATTAATTACAGCAATCCTTCTATTACTATCACTACCGGTACTAGCAG
GAGCAATTACAATATTATTAACTGATCGAAACTTAAATACATCGTTTTTTGACCCAGCAG
GAGGAGGTGACC

Figure 4.56: The partial DNA sequence of the mitochondrial COI gene of *Dactylispa carinata*

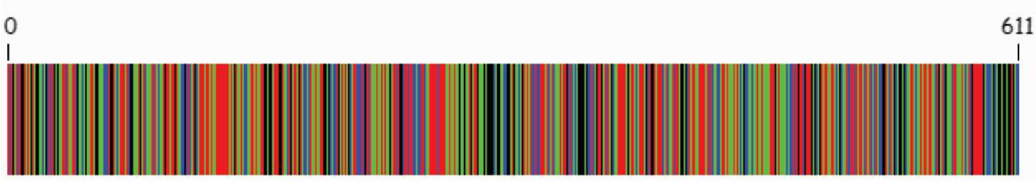


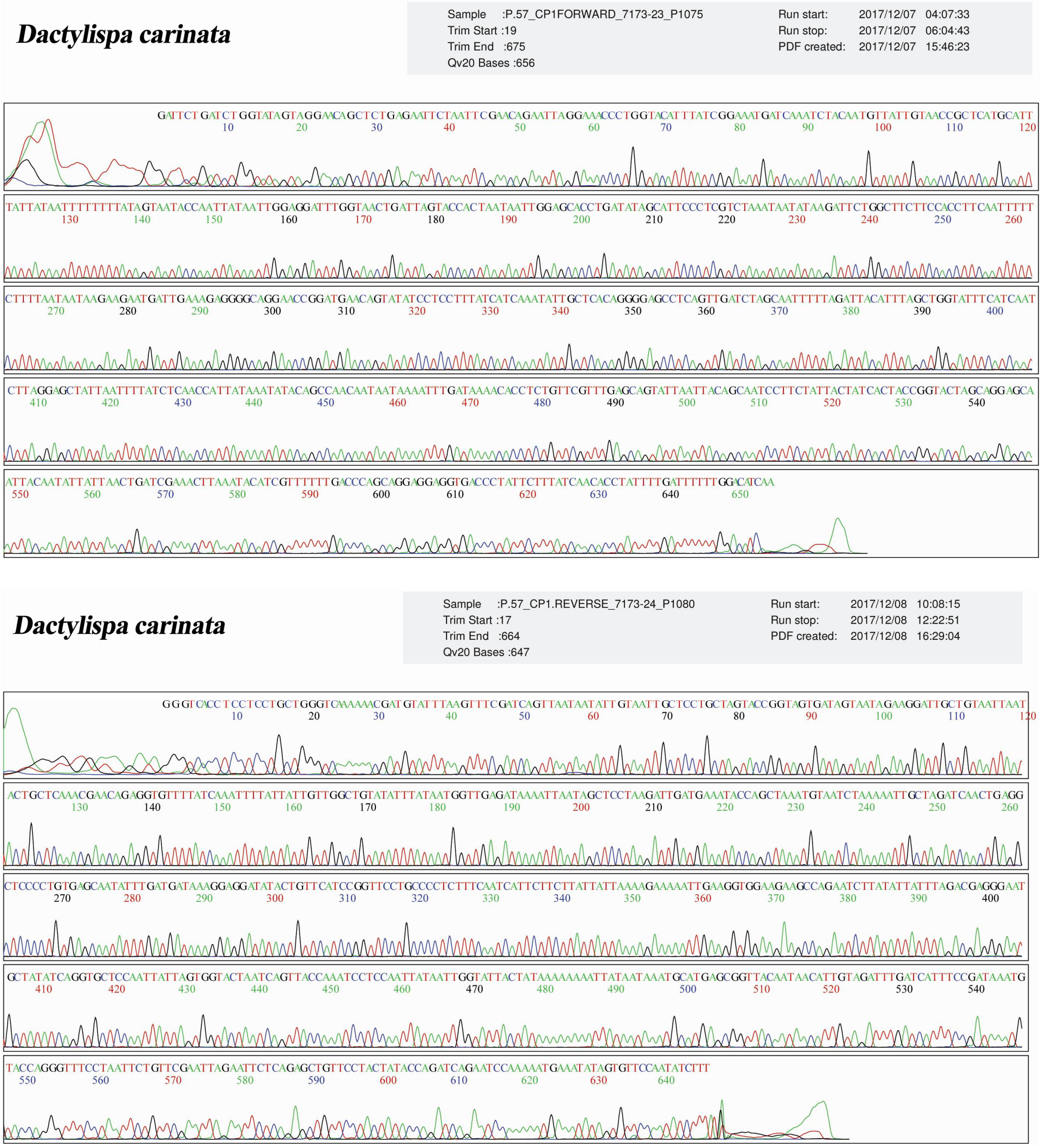
Figure 4.57: Molecular barcode of the mitochondrial COI gene of *Dactylispa carinata*

> *Dactylispa carinata* / 199AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUDC-02-A1

> *Dactylispa carinata*

VGTALSILIRTELGNPGTFIGNDQIYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMIG
APDMAFPRLNNMSFWLLPPSIFLLMMSSMIESGAGTGWTVYPPLSSNIAHSGASVDLAIF
SLHLAGISSILGAINFISTIMNMQPTMMKFDKTPLFVWAVLITAILLLLSLPVLAGAITM
LLTDRNLNTSFFDPAGGGD

Figure 4.58: The translation product of the mitochondrial COI gene of *Dactylispa carinata*



Sample :P.57_CP1FORWARD_7173-23_P1075
Trim Start :19
Trim End :675
Qv20 Bases :656

Run start: 2017/12/07 04:07:33
Run stop: 2017/12/07 06:04:43
PDF created: 2017/12/07 15:46:23

Sample :P.57_CP1.REVERSE_7173-24_P1080
Trim Start :17
Trim End :664
Qv20 Bases :647

Run start: 2017/12/08 10:08:15
Run stop: 2017/12/08 12:22:51
PDF created: 2017/12/08 16:29:04

Figure 4.59: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Dactylispa carinata* using Forward and Reverse primers

Table 4.19: Comparison of Nucleotide frequencies of COI gene sequence of *Dactylispa carinata* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH674110 Dactylispa carinata	33.9	17.6	33.1	15.4	36	9.4	50.6	4.4	21	18.3	35.6	25.6	46	25.0	13.3	16.1
KF655345 Hispa atra	39.4	14.1	31.5	15.0	46	5.0	45.6	3.9	26	15.0	35.0	24.4	47	22.2	13.9	16.7
KY833634 Dicladispa armigera	35.9	17.0	32.6	14.4	38	11.7	48.9	1.1	23	16.1	35.0	25.6	46	23.3	13.9	16.7
KM440095 Hippuriphila modeeri	36.2	16.7	32.3	14.8	37	12.3	48.6	2.2	26	15.0	35.0	24.4	46	22.8	13.3	17.8
KJ677801 Galerucinae sp.	38.9	14.4	30.7	15.9	48	5.0	46.7	.6	23	15.0	33.3	28.3	46	23.3	12.2	18.9
KM451450 Dicladispa testacea	32.6	18.1	34.1	15.2	31	13.3	53.3	2.8	22	17.8	35.0	25.0	45	23.3	13.9	17.8
KU918516 Chrysomela cuprea	36.1	17.2	30.7	15.9	41	11.7	45.0	2.8	23	16.7	32.8	27.8	45	23.3	14.4	17.2
AB794744 Monolepta sp.	40.2	13.7	30.0	16.1	48	5.0	45.0	1.7	26	12.8	33.3	27.8	46	23.3	11.7	18.9
KU697481 Lypnea pubipennis	39.6	14.6	31.1	14.6	47	6.7	44.4	2.2	26	13.9	36.1	23.9	46	23.3	12.8	17.8
KY833674 Dicladispa armigera	36.3	17.0	32.2	14.4	40	11.1	47.8	1.1	23	16.7	35.0	25.6	46	23.3	13.9	16.7
MH051936 Rhopalomesites proximus	34.3	18.1	32.8	14.8	37	11.7	48.3	2.8	23	17.2	35.6	23.9	42	25.6	14.4	17.8
KM843013 Hapalaraea megarthroides	38.9	13.7	31.9	15.6	45	2.8	51.7	.6	27	13.3	31.7	27.8	44	25.0	12.2	18.3
MF495677 Longitarsus nigripennis	38.5	15.2	30.7	15.6	46	5.6	46.1	2.8	24	14.4	34.4	26.7	46	25.6	11.7	17.2
KM285766 Colydium elongatum	38.7	14.1	31.5	15.7	45	4.4	50.0	.6	26	13.9	31.7	28.3	45	23.9	12.8	18.3

Table 4.20: Percentage of evolutionary divergence of *Dactylispa carinata* with its closely related species accessible from NCBI GenBank

Sl. No.	**Accession No**	**Organism**	**Percentage of divergence**
1.	MH674110	*Dactylispa carinata* (Kerala)	
2.	KF655345	*Hispa atra* (Spain)	15.34%
3.	KY833674	*Dicladispa armigera*	17.01%
4.	KY833634	*Dicladispa armigera*	17.48%
5.	KM440095	*Hippuriphila modeeri*	18.91%
6.	KM451450	*Dicladispa testacea*	18.92%
7.	KU918516	*Chrysomela cuprea*	19.52%
8.	MF495677	*Longitarsus nigripennis*	19.94%
9.	KJ677801	*Galerucinae* sp.	20.39%
10.	AB794744	*Monolepta* sp.	21.07%
11.	MH051936	*Rhopalomesites proximus*	21.58%
12.	KU697481	*Lypnea pubipennis*	21.89%
13.	KM285766	*Colydium elongatum*	22.88%
14.	KM843013	*Hapalaraea megarthroides*	23.47%

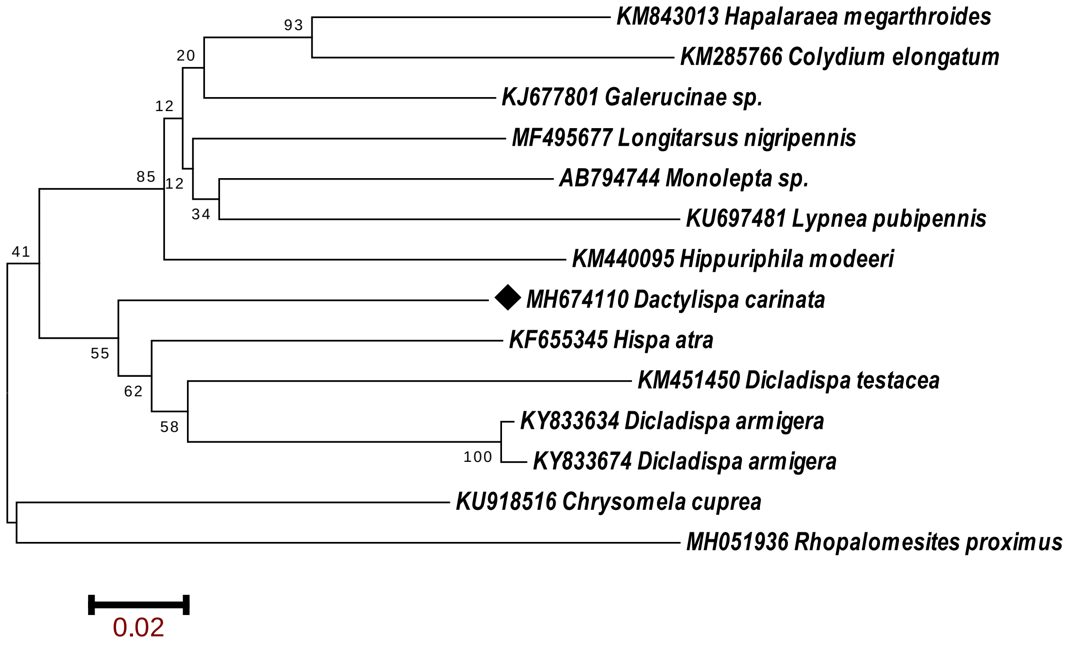


Figure 4.60: Phylogenetic relationship of *Dactylispa carinata* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 84.7% sequence similarity to the related reported from Spain (KF655345). Even though this species has been found in various geographically isolated areas, their sequence doesn't have any kind of variation. The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Dactylispa carinata* showed bias to nucleotide AT, with following composition of nucleotides T = 33.9%, C = 17.6%, A = 33.1% and G = 15.4%. This greater AT content (67.0%) over GC content (33.0%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Dactylispa carinata* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 15.34% to 23.47% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (15.34%) from Spain while 23.47% from Canada (Table 4.20). On the basis of the data observed this species may be rooted from those found in Canada which diverted into different clades due to geographical variation. Result thus concluded that this species doesn't have any major changes in India while slightly changes from those reported from Spain and Canada during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Dactylispa carinata* with related species. *Dactylispa carinata* isolated from Kerala (MH674105) showed 15.34% divergence with *Hispa atra* (KF655345) from Spain and 23.47% divergence with *Hapalaraea megarthroides* (KM843013) from Canada. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Dactylispa carinata* isolated from

Kerala. Closest relative of *Dactylispa carinata* is *Hispa atra* from Spain represented within the same clade.

11. ***Henosepilachna vigintioctopunctata*** (Motschulsky, 1857)

Specimen details:

Voucher specimen : CUHV – 01 – A1
Date of collection : 26-Nov-2017
Locality : Palakkad: Ottapalam
Lat- Lon : 10.7723° N, 76.3695° E
GenBank accession : MH590761

Description and distribution:

These are commonly known as the 28-spotted potato ladybird or the Hadda beetles (Figure 4.61). The body of the beetle is nearly 7mm in length, round, convex and glossy. The colour is reddish brown with 13 black spots on each elytron and one or more is found on either side of the thorax. The newly emerged adult is straw or cream yellow in colour, the 28 spot of variable size appear on the dorsal side. The whole body is covered with fine short hairs. The adult darken on with age and attain orange brown colour with a bronze tinge. The adult males are smaller than females. *H. vigintioctopunctata* are distributed over a wide range from Japan (Abbas et al., 1985) to South Asia and Australia (Kalashoven, 1981; Richards, 1983).

Fig.4.61: *Henosepilachna vigintioctopunctata*

Damage:

Both the adult and larvae (grubs) are found on lower surfaces of the leaves, scrapping and feeding gregariously on the parenchyma and the lower epidermis between the veins and skleletonize leaving intact upper epidermis and tougher tissue in the form of “windows”.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Henosepilachna vigintioctopunctata* collected has been amplified using the primer LEP (Table 3.1). The PCR amplification yielded 512 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 4.62 – 4.65 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 4.21.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Henosepilachna vigintioctopunctata* voucher collected during the present study (Table 4.21). It showed bias to nucleotide AT, with nucleotide composition with T = 38.5%, C = 18.0%, A = 27.7% and G = 15.8%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 4.22 and Figure 4.66 respectively.

> *Henosepilachna vigintioctopunctata* CDS-2018/ 512 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUHV-01-A1

> *Henosepilachna vigintioctopunctata*

```
GATTTTTTTTATAGTAATACCAATTTTAATTGGAGGTTTTGGAAATTGACTAGTACCTTT
AATAATTGGGGCTCCCGATATAGCTTTCCCTCGACTAAATAATATAAGATTTTGACTTCT
TCCCCCAGCTTTAACATTCTTACTTTTAAGAAGAATAGTAGAAAGAGGGGCAGGAACAGG
CTGAACAGTTTACCCGCCTCTTTCAGCTAATATTGCCCATAGAGGTCCTTCTGTTGATTT
AGCTATTTTTAGATTACATTTAGCTGGTGTATCATCAATTTTAGGGGCAATTAATTTTAT
TACTACCATGATTAATATACGACCTATTGGTATACAATTAGATAAACTTCCTTTATTTGC
TTGGTCAGTTTTAATTACTGCTATTTTACTTCTTCTTTCCCTCCCTGTATTAGCAGGAGC
AATCACTATGCTTTTAACAGATCGAAATATTAATACTTCATTTTTTGACCCTGCAGGAGG
TGGGGATCCTATTTTATACCAACACTTATTTT
```

Figure 4.62: The partial DNA sequence of the mitochondrial COI gene of *Henosepilachna vigintioctopunctata*

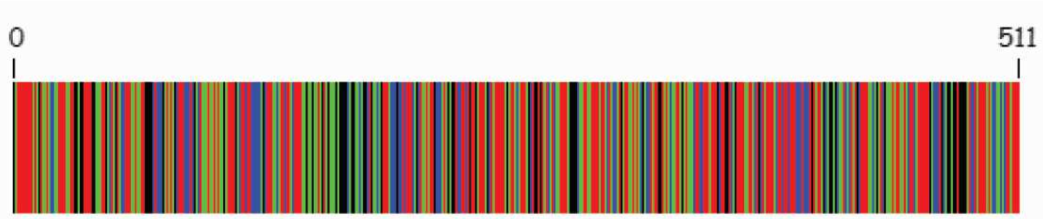


Figure 4.63: Molecular barcode of the mitochondrial COI gene of *Henosepilachna vigintioctopunctata*

> *Henosepilachna vigintioctopunctata* / 167AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUHV-01-A1

> *Henosepilachna vigintioctopunctata*

```
MVMPILIGGFGNWLVPLMIGAPDMAFPRLNNMSFWLLPPALTFLLLSSMVESGAGTGWTV
YPPLSANIAHSGPSVDLAIFSLHLAGVSSILGAINFITTMINMRPIGMQLDKLPLFAWSV
LITAILLLLSLPVLAGAITMLLTDRNINTSFFDPAGGGDPILYQHLF
```

Figure 4.64: The translation product of the mitochondrial COI gene of *Henosepilachna vigintioctopunctata*

Figure 4.65: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Henosepilachna vigintioctopunctata* using Forward and Reverse primers.

Table 4.21: Comparison of Nucleotide frequencies of COI gene sequence of *Henosepilachna vigintioctopunctata* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH590761 Henosepilachna vigintioctopunctata	38.5	18.0	27.7	15.8	25	19.9	29.2	25.7	44	25.9	13.5	16.5	46	8.2	40.4	5.3
KU234200 Henosepilachna vigintioctopunctata	37.9	15.2	30.1	16.8	27	14.0	32.7	25.7	43	23.4	14.6	18.7	43	8.2	42.9	5.9
AB002180 Henosepilachna vigintioctopunctata	37.9	15.2	30.1	16.8	27	14.0	32.7	25.7	43	23.4	14.6	18.7	43	8.2	42.9	5.9
KY842764 Coccinellidae sp.	38.1	15.8	29.7	16.4	27	15.8	31.0	26.3	44	24.1	13.5	18.8	44	7.6	44.4	4.1
AB002232 Henosepilachna yasutomii	38.5	15.8	30.5	15.2	25	15.8	33.3	25.7	43	23.4	14.6	18.7	47	8.2	43.5	1.2
AB002222 Henosepilachna niponica	38.5	15.6	30.7	15.2	25	15.8	33.3	25.7	43	23.4	14.6	18.7	47	7.6	44.1	1.2
LC228588 Henosepilachna pustulosa	38.0	15.6	30.8	15.6	24	15.8	33.9	26.3	43	23.4	14.6	19.3	47	7.6	43.9	1.2
KU234209 Henosepilachna vigintioctopunctata	38.7	15.6	30.5	15.2	25	15.8	33.3	25.7	43	23.4	14.6	18.7	48	7.6	43.5	1.2
AB002229 Henosepilachna yasutomii	38.7	15.6	30.5	15.2	25	15.8	33.3	25.7	43	23.4	14.6	18.7	48	7.6	43.5	1.2
AB002209 Henosepilachna pustulosa	38.5	15.8	30.5	15.2	25	15.8	33.3	25.7	43	23.4	14.6	18.7	47	8.2	43.5	1.2
AB002184 Henosepilachna yasutomii	38.5	15.4	30.7	15.4	26	15.2	33.3	25.1	43	23.4	15.2	18.7	46	7.6	43.5	2.4
AB002231 Henosepilachna yasutomii	38.5	15.8	30.3	15.4	25	15.8	32.7	26.3	43	23.4	14.6	18.7	47	8.2	43.5	1.2
AB002181 Henosepilachna vigintioctopunctata	38.5	14.8	30.1	16.6	25	15.8	33.3	25.7	43	23.4	15.2	18.7	48	5.3	41.8	5.3
AB002195 Henosepilachna vigintioctomaculata	38.1	16.0	29.9	16.0	25	16.4	33.3	25.7	43	23.4	14.6	18.7	46	8.2	41.8	3.5

Table 4.22: Percentage of evolutionary divergence of *Henosepilachna vigintioctopunctata* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH590761	*Henosepilachna vigintioctopunctata* (Kerala)	
2.	KU234200	*Henosepilachna vigintioctopunctata* (Tamilnadu)	1.11%
3.	AB002180	*Henosepilachna vigintioctopunctata*	1.11%
4.	KY842764	*Coccinellidae* sp.	1.87%
5.	AB002181	*Henosepilachna vigintioctopunctata*	5.84%
6.	AB002222	*Henosepilachna niponica*	5.85%
7.	AB002232	*Henosepilachna yasutomii*	5.87%
8.	AB002195	*Henosepilachna vigintioctomaculata*	6.06%
9.	LC228588	*Henosepilachna pustulosa*	6.08%
10.	KU234209	*Henosepilachna vigintioctopunctata*	6.08%
11.	AB002229	*Henosepilachna yasutomii*	6.08%
12.	AB002209	*Henosepilachna pustulosa*	6.08%
13.	AB002184	*Henosepilachna yasutomii*	6.08%
14.	AB002231	*Henosepilachna yasutomii*	6.10%

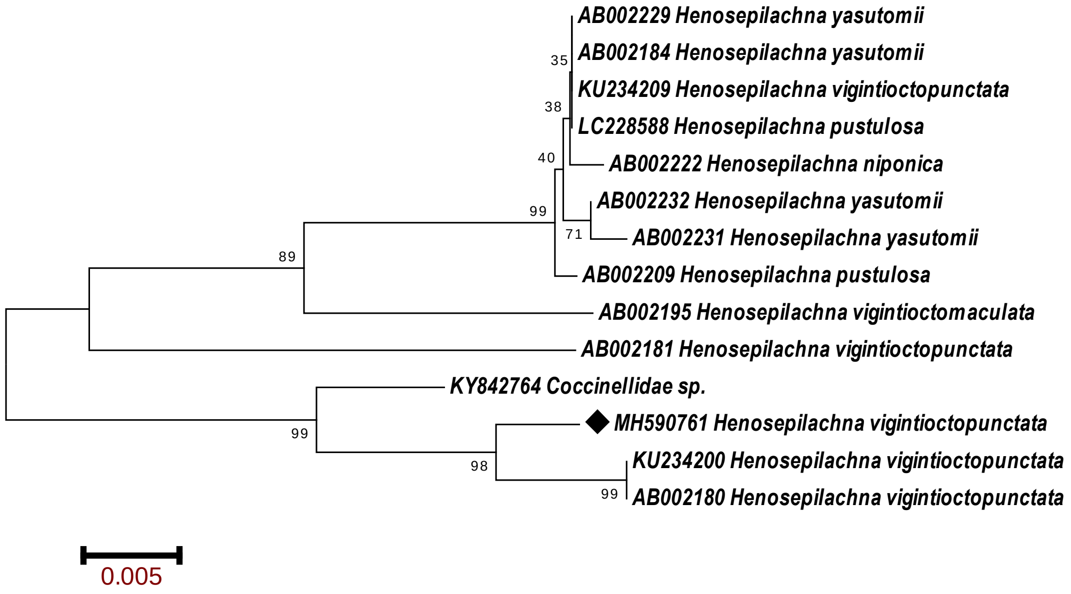


Figure 4.66: Phylogenetic relationship of *Henosepilachna vigintioctopunctata* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 98.9% sequence similarity to the same genus reported from Tamilnadu (KU234200). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Henosepilachna vigintioctopunctata* showed bias to nucleotide AT, with following composition of nucleotides T = 38.5%, C = 18.0%, A = 27.7% and G = 15.8%. This greater AT content (66.2%) over GC content (33.8%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. ` *Henosepilachna vigintioctopunctata* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 1.11% to 6.10% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (1.11%) from Tamilnadu while 6.10% from Japan (Table 4.22). On the basis of the data observed this species may be rooted from those found in Japan which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from Japan during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Henosepilachna vigintioctopunctata* with related species. *Henosepilachna vigintioctopunctata* (MH590761) isolated from Kerala showed 1.11% divergence from Tamilnadu (KU234200) and showed 6.10% divergence with *Henosepilachna yasutomii* (AB002231) from Japan. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Henosepilachna vigintioctopunctata* isolated from Kerala. Closest relative of

Henosepilachna vigintioctopunctata is *Henosepilachna vigintioctopunctata* from Tamilnadu and Japan represented within the same clade.

12. ***Henosepilachna septima*** (Dieke,1947)

Specimen details:

Voucher specimen : CUHS – 04 – A1
Date of collection : 25- July-2016
Locality : Malappuram: Parappanangadi
Lat- Lon : 11.06º N, 75.85º E
GenBank accession : KX503056

Description and distribution:

Newly emerged adult are purely yellow in colour, without any markings/spots and are inactive. Within few hours it turns darker and the black spots on the elytra appears (Figure 4.67). These beetles are oval in shape having convex body, elytra dark brown in colour with 12 black spots (6 on each elytra) (Ghule et al., 2014). Usually females are bigger than males. This species is distributed throughout Asia and Australia.

Fig.4.67: *Henosepilachna septima*

Damage:

Both the adult and the larvae feed on the epidermal tissues of leaves and fruits, resulting in considerable economic losses. Both grub and adult feeds on leaf lamina and leaving papery epidermis intact (Ghule et al., 2014). They prefer more the leaves and fruits of the host plants.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Henosepilachna septima* collected has been amplified using the primer BTL (Table 3.1). The PCR

amplification yielded 553 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 4.68 – 4.71 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 4.23.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Henosepilachna septima* voucher collected during the present study (Table 4.23). It showed bias to nucleotide AT, with nucleotide composition with T = 36.7%, C = 16.4%, A = 29.9% and G = 17.0%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 4.24 and Figure 4.72 respectively.

> *Henosepilachna septima* CDS-2018/ 553 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUHS-04-A1

> *Henosepilachna septima*

```
CTCCAGGATCATTAATTGGAAATGACCAAATTTACAATGCAATTGTAACAGCTCATGCTT
TCATTATGATTTTTTTTATGGTTATGCCTATTTTAATTGGGGGATTCGGAAACTGACTAA
TTCCTTTAATAATTGGAGCTCCTGATATAGCTTTCCCTCGTTTAAACAATATAAGATTTT
GACTTTTACCTCCAGCATTAACTTTTCTTCTTTTAAGAAGACTGGTTGAGATGGAGCTGG
GATGATGGATGAACAGTTACCCCCCCCTTTCTCAAATATTGCTCATGGAGGACCTTCTGT
AGATTTAGCTATTTTTAGTCACATTTAGCTGGATTTCCTCTCTATTTAGGAGCAATTAAT
TTTATTACTACAATGTTAATAATACGACGACCAAAGGGATACTTTGGATAAATTCCCTTA
TTTGCTGACAGTTGTAATTACAGCATTTTACTTCTTCTTTCTCTTCCTGTATTAGCAGGA
GCCATTACTATGCTATTAACTGACCGAAATAAAATACTTCTTTTTTGACCCAGCAGGAGG
AGGAGACCCAATC
```

Figure 4.68: The partial DNA sequence of the mitochondrial COI gene of *Henosepilachna septima*

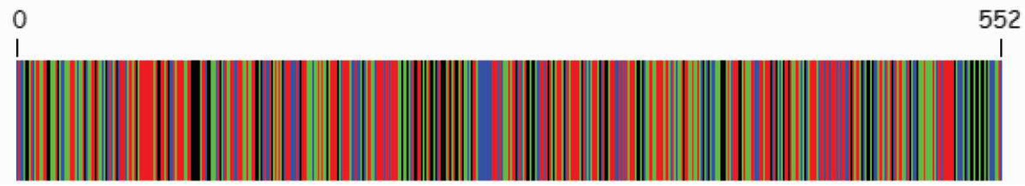


Figure 4.69: Molecular barcode of the mitochondrial COI gene of *Henosepilachna septima*

> *Henosepilachna septima* / 150AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUHS-04-A1

> *Henosepilachna septima*

```
MIGSSLSILIRMELGTPSSLIGNDQIYNVIVTAHAFIMIFCTVMPILIGGFGNWLIPLMI
GAPDMAFPRLNNMSFWLLPPALTFLLLSSLVESGAGTGWTVYPPLSANIAHSGPSVDLAI
FSLHLAGISSILGAINFITTMINMRPKGMN
```

Figure 4.70: The translation product of the mitochondrial COI gene of *Henosepilachna septima*

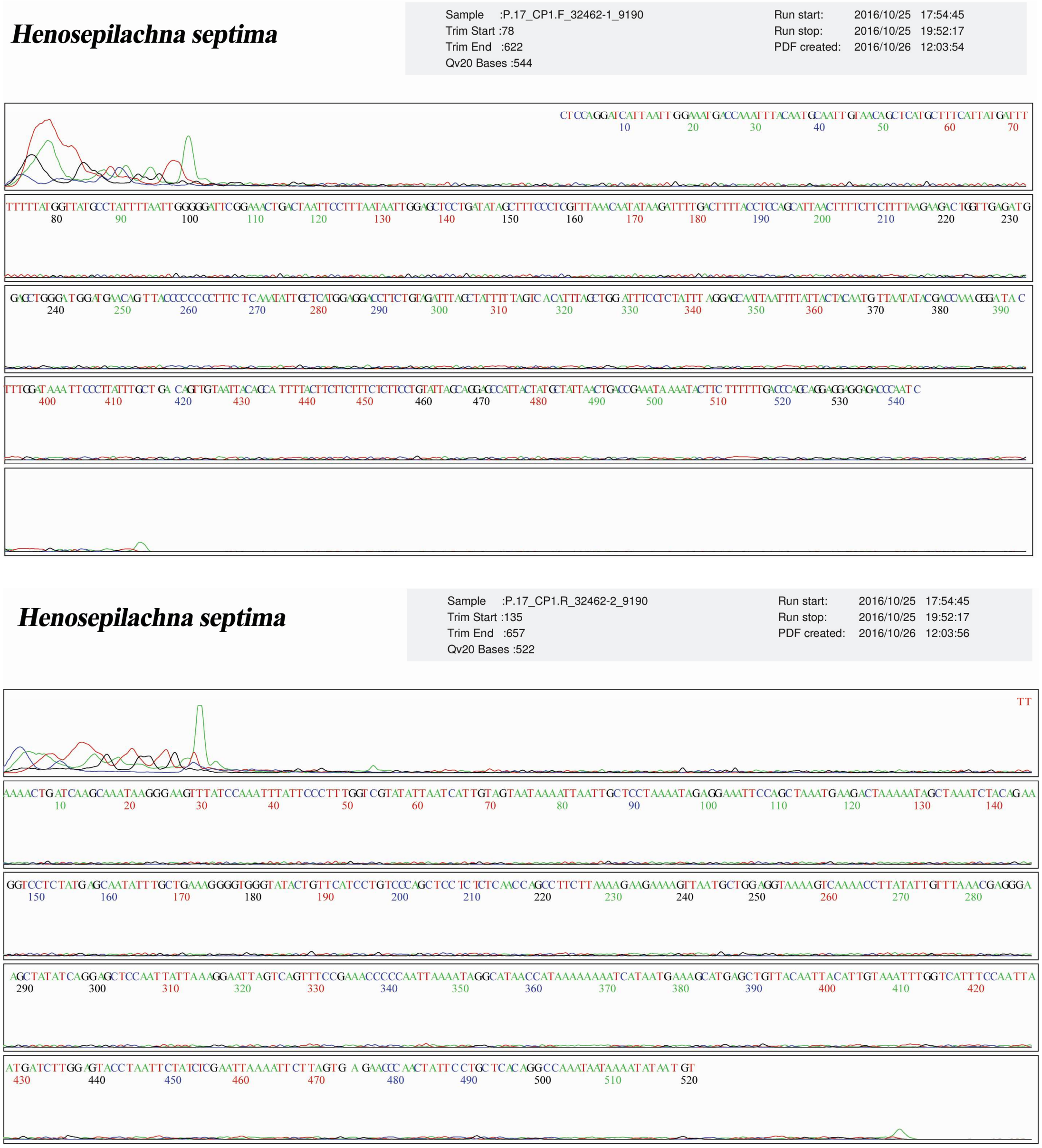


Figure 4.71: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Henosepilachna septima* using Forward and Reverse primers

Table 4.23: Comparison of Nucleotide frequencies of COI gene sequence of *Henosepilachna septima* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH656689 Henosepilachna septima	37.6	17.7	28.1	16.6	29	22.7	26.7	21.3	46	15.9	25.2	12.6	37	14.6	32.5	15.9
AB002176 Henosepilachna septima	37.4	16.2	29.2	17.3	28	19.2	31.1	21.9	45	17.3	21.3	16.0	39	11.9	35.1	13.9
KX503056 Henosepilachna septima	36.7	16.4	29.9	17.0	27	21.3	30.0	21.3	46	16.7	22.0	15.3	37	11.2	37.5	14.5
EU392441 Epilachna anhweiana	36.1	17.0	31.2	15.7	32	18.8	28.9	20.8	44	17.2	23.8	14.6	32	15.1	40.8	11.8
AB002173 Henosepilachna enneasticta	38.7	15.0	30.5	15.7	30	15.2	31.8	22.5	48	18.7	17.3	16.0	38	11.3	42.4	8.6
KF523749 Autosticha modicella	40.5	14.4	29.9	15.3	29	18.7	28.7	24.0	49	18.0	18.7	14.0	43	6.6	42.1	7.9
HQ923098 Haereta cryphimaea	38.3	14.6	31.9	15.3	29	18.7	29.3	23.3	45	19.3	22.0	14.0	41	5.9	44.1	8.6
HQ921150 Scaptesyle dictyota	40.9	14.8	29.2	15.0	28	19.3	28.7	24.0	50	17.3	18.7	14.0	45	7.9	40.1	7.2
KF393430 Macrobathra monostadia	40.9	15.0	28.5	15.5	27	20.7	28.7	24.0	47	18.7	20.7	14.0	49	5.9	36.2	8.6
MH415593 Pioneabathra olesialis	39.8	13.7	31.0	15.5	30	17.3	29.3	23.3	47	18.7	19.3	14.7	42	5.3	44.1	8.6
KR661971 Hylemyza partita	41.2	13.5	29.0	16.4	28	19.3	27.3	25.3	50	16.0	20.0	14.0	45	5.3	39.5	9.9
KP189249 Lespesia aletiae	39.8	13.7	30.1	16.4	28	18.7	29.3	24.0	50	15.2	19.9	15.2	42	7.3	41.1	9.9
HQ923099 Haereta cryphimaea	38.3	14.6	31.6	15.5	29	18.7	29.3	23.3	45	19.3	22.0	14.0	41	5.9	43.4	9.2
KF492075 Rachiplusia virgula	40.5	14.6	29.6	15.3	29	18.0	28.7	24.0	49	18.0	19.3	14.0	43	7.9	40.8	7.9
EU392444 Afissula kambaitana	36.5	15.9	32.3	15.3	29	19.5	30.2	21.5	44	16.6	23.8	15.2	36	11.8	42.8	9.2

Table 4.24: Percentage of evolutionary divergence of *Henosepilachna septima* with its closely related species accessible from NCBI GenBank

Sl. No.	**Accession No**	**Organism**	**Percentage of divergence**
1.	KX503056	*Henosepilachna septima* (Kerala)	
2.	AB002176	*Henosepilachna septima* (Japan)	1.30%
3.	EU392441	*Epilachna anhweiana*	22.64%
4.	AB002173	*Henosepilachna enneasticta*	27.67%
5.	HQ921150	*Scaptesyle dictyota*	30.77%
6.	EU392444	*Afissula kambaitana*	31.00%
7.	KF523749	*Autosticha modicella*	31.28%
8.	KF492075	*Rachiplusia virgule*	34.20%
9.	HQ923098	*Haereta cryphimaea*	34.30%
10.	KP189249	*Lespesia aletiae*	34.54%
11.	HQ923099	*Haereta cryphimaea*	35.01%
12.	KR661971	*Hylemyza partita*	35.05%
13.	KF393430	*Macrobathra monostadia*	36.05%
14.	MH415593	*Pioneabathra olesialis*	40.82%

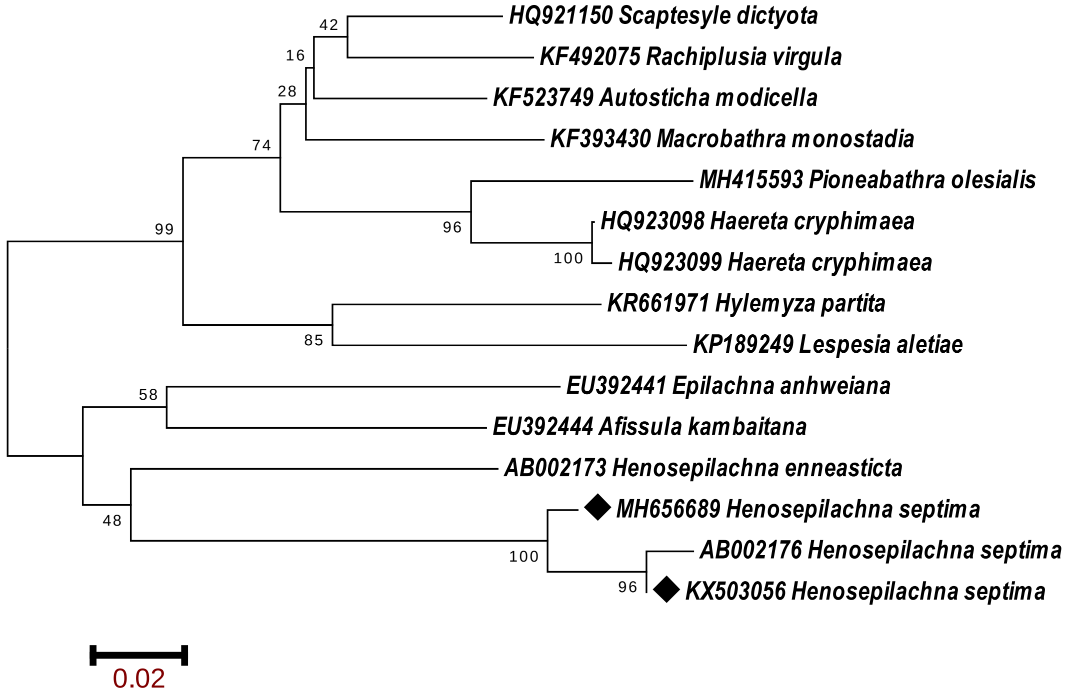


Figure 4.72: Phylogenetic relationship of *Henosepilachna septima* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 98.7% sequence similarity to the same genus reported from Japan (AB002176). Even though this species has been found in various geographically isolated areas, their sequence shows mild kind of variation. The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *H. septima* showed bias to nucleotide AT, with following composition of nucleotides T = 36.7%, C = 16.4%, A = 29.9% and G = 17.0%. This greater AT content (66.6%) over GC content (33.4%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Henosepilachna septima* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 1.30% to 40.82% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (1.30%) with those from Japan while 40.82% Madagascar (Table 4.24). On the basis of the data observed this species may be rooted from those found in Madagascar which diverted into different clades due to geographical variation. Result thus concluded that this species doesn't have any major changes in India while slightly changes from those reported from Madagascar during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Henosepilachna septima* with related species. *Henosepilachna septima* isolated from Kerala (KX503056) showed 1.30% divergence with *Henosepilachna septima* (AB002176) from Japan and *Pioneabathra olesialis* (MH415593) from Madagascar. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Henosepilachna septima* isolated from

Kerala. Closest relative of *Henosepilachna septima* is *Henosepilachna septima* from Japan represented within the same clade.

13. ***Epilachna septima*** (Dieke, 1947)

Specimen details:

Voucher specimen	:	CUES – 02 – A3
Date of collection	:	25- July-2016
Locality	:	Malappuram: Parappanangadi
Lat- Lon	:	11.06º N, 75.85º E
GenBank accession	:	MH656689

Description and distribution:

The general body size range from 6.1 to 7.1mm in males and 7 to 7.6mm in females. The shape of the elytral apex rounded. Head is spotless, pronotum with 2-6 spots (Figure 4.73). Each elytron is with 8-15 spots. Sipho of the male genitalia is sharply narrowed down and pointed, females have the inner margin of the genital plate deeply notched. The genital plate of the female of *E. septima* was deeply notched with the notched parts overlapping. In the male genitalia, the apex of the sipho is sharply narrowed on one side and pointed. Notorious pest of Asia and Africa (Nakano and Katakura, 1999).

Fig.4.73: *Epilachna septima*

Damage:

E. septima is an oligophagus, multivoltine pest of the class leaf miners. Adult and grub, both, scrape and skeletonize the leaves, hence resulting in drying up of the leaves and causing debilitation to the crops throughout the stages.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Epilachna septima* collected has been amplified using the primer CP2 (Table 3.1). The PCR amplification yielded 505 bp long products for the specimens obtained from two different locations. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram and are exhibited in Figures 4.74 – 4.77 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 4.25.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Epilachna septima* voucher collected during the present study (Table 4.25). It showed bias to nucleotide AT, with nucleotide composition with T = 36.9%, C = 19.2%, A = 29.0 and G = 14.9%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 4.26 and Figure 4.78 respectively.

> *Epilachna septima* CDS-2018/ 505 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUES-02-A3

> *Epilachna septima*

```
GTTACAGCCCATGCTTTTATTATGATTTTCTTCATAGTAATGCCAATTATAATTGGAGGT
TTTGGAAACTGATTAGTCCCCTTAATAATCGGTGCCCCTGACATAGCATTCCCCCGACTA
AATAACATAAGATTTTGACTTTTACCCCCCTCTCTTTTTCTTCTCATTATAAGAAGAGTA
GTAGAAAGAGGAGCAGGAACTGGTTGAACTGTATATCCCCCACTGTCAGCCAATATTGCA
CATAATGGAGCTTCAGTTGATTTAGCTATTTTTAGTCTTCATTTAGCTGGAATTTCTTCA
ATTTTAGGAGCAATTAATTTTATTACAACTGTAATTAATATACGTCCTAAAGGTATAACA
CTTGATCGAATACCCCTATTTGTTTGATCTGTGGTAATTACAGCTATCTTACTACTCTTA
TCTTTACCAGTTTTAGCTGGAGCAATTACAATATTATTAACAGACCGAAATTTAAATACT
TCATTTTTTGATCCTG
```

Figure 4.74: The partial DNA sequence of the mitochondrial COI gene of *Epilachna septima*

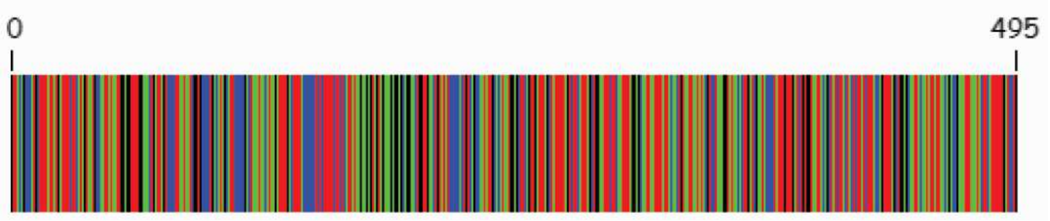


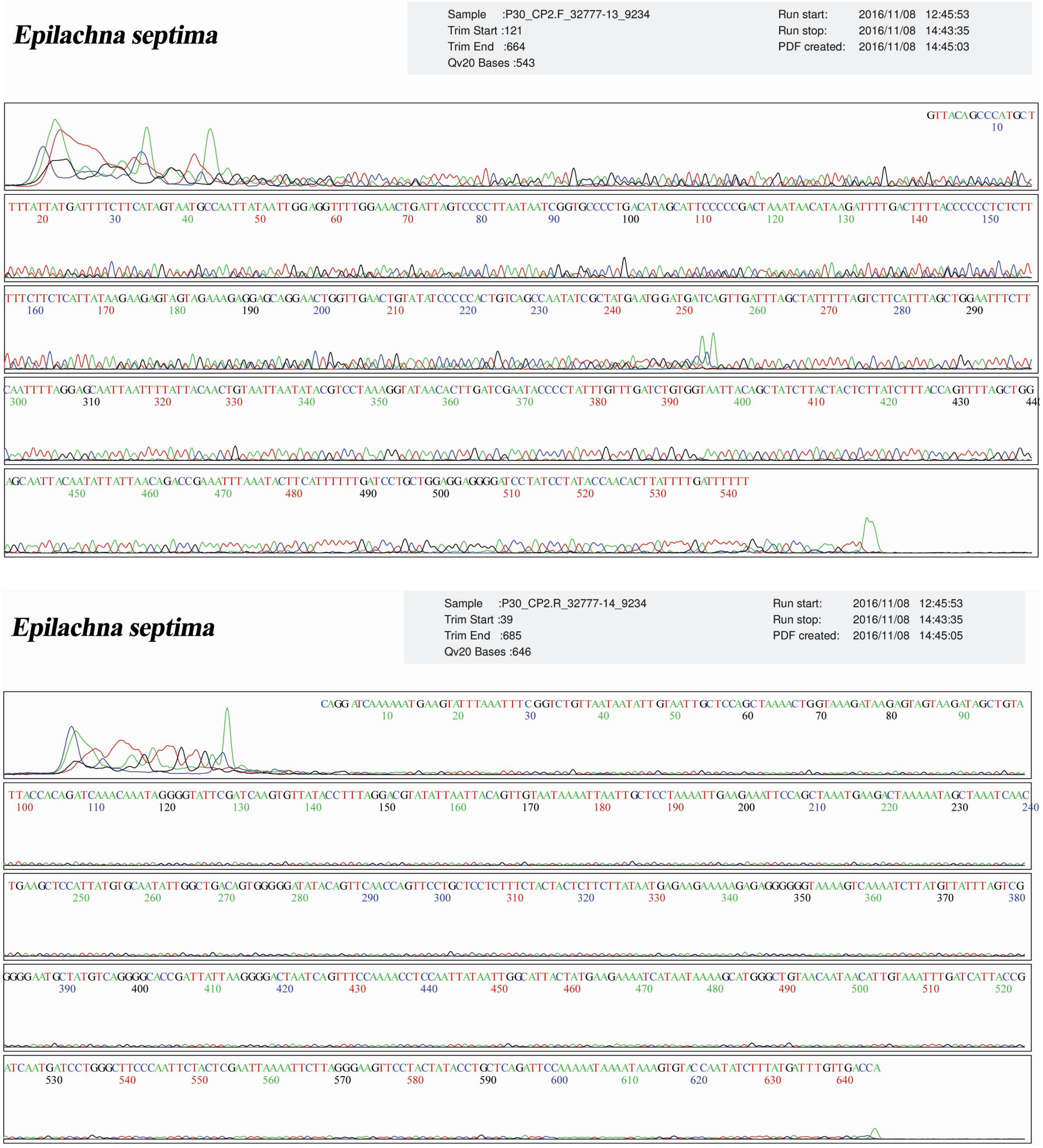
Figure 4.75: Molecular barcode of the mitochondrial COI gene of *Epilachna septima*

> *Epilachna septima* / 158AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUES-02-A3

> *Epilachna septima*

```
MIFFMVMPIMIGGFGNWLVPLMIGAPDMAFPRLNNMSFWLLPPSLFLLIMSSVVESGAGT
GWTVYPPLSANIAHNGASVDLAIFSLHLAGISSILGAINFITTVINMRPKGMTLDRMPLF
VWSVVITAILLLLSLPVLAGAITMLLTDRNLNTSFFDP
```

Figure 4.76: The translation product of the mitochondrial COI gene of *Epilachna septima*



Epilachna septima

Sample :P30_CP2.F_32777-13_9234
Trim Start :121
Trim End :664
Qv20 Bases :543

Run start: 2016/11/08 12:45:53
Run stop: 2016/11/08 14:43:35
PDF created: 2016/11/08 14:45:03

Epilachna septima

Sample :P30_CP2.R_32777-14_9234
Trim Start :39
Trim End :685
Qv20 Bases :646

Run start: 2016/11/08 12:45:53
Run stop: 2016/11/08 14:43:35
PDF created: 2016/11/08 14:45:05

Figure 4.77: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Epilachna septima* using Forward and Reverse primers

Table 4.25: Comparison of Nucleotide frequencies of COI gene sequence of *Epilachna septima* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**																
	T(U)	C	A	G	Total	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH656689 Epilachna septima	36.9	19.2	29.0	14.9	496.0	23	18.1	31.9	27.1	46	26.7	12.1	15.2	42	12.7	43.0	2.4
KU578138 Epilachna sp.	35.3	18.1	29.2	17.3	496.0	21	17.6	32.7	29.1	45	22.9	13.3	19.3	41	13.9	41.8	3.6
KC255440 Sphenoraia bicolor	35.1	17.5	30.0	17.3	496.0	21	15.7	34.9	28.3	43	21.8	15.8	19.4	41	15.2	39.4	4.2
KY835900 Chrysomelidae sp.	36.9	15.7	30.8	16.5	496.0	24	15.2	35.2	26.1	44	23.0	13.3	20.0	43	9.0	44.0	3.6
KF656634 Aphthona albertinae	36.1	18.3	30.2	15.3	496.0	24	15.8	34.5	25.5	44	23.0	13.9	19.4	40	16.3	42.2	1.2
KM286367 Acmaeops marginatus	40.5	14.1	28.8	16.5	496.0	26	13.9	32.1	27.9	43	23.6	13.9	19.4	52	4.8	40.4	2.4
KF654908 Longitarsus candidulus	37.3	15.1	32.7	14.9	496.0	25	13.9	35.2	25.5	44	23.0	13.3	19.4	42	8.4	49.4	.0
MG061719 Acalymma vittatum	37.1	14.3	32.1	16.5	496.0	24	13.9	34.5	27.3	45	22.4	12.7	20.0	42	6.6	48.8	2.4
MG057938 Psylliodes cucullatus	35.1	16.1	30.8	17.9	496.0	23	13.9	34.9	27.7	44	23.6	12.7	19.4	38	10.9	44.8	6.7
KU697467 Euphitrea piceicollis	40.3	14.7	29.2	15.7	496.0	25	14.5	34.9	25.9	45	23.0	12.1	19.4	51	6.7	40.6	1.8
KC185777 Pallasiola absinthii	34.5	16.1	31.9	17.5	496.0	22	15.1	36.1	27.1	43	21.8	15.2	20.0	39	11.5	44.2	5.5
MH020282 Prionus coriarius	40.7	14.1	29.8	15.3	496.0	27	13.3	32.5	27.1	43	25.5	13.3	18.2	52	3.6	43.6	.6
KU917112 Dinoptera collaris	40.1	13.1	30.8	15.9	496.0	28	12.1	32.7	27.3	42	24.2	13.9	19.4	50	3.0	45.8	1.2
KF655810 Longitarsus exsoletus	37.3	15.5	32.5	14.7	496.0	26	13.9	35.2	24.8	44	23.0	13.9	18.8	42	9.6	48.2	.6
KF653719 Aphthona depressa	36.3	17.5	30.2	15.9	496.0	27	14.5	33.9	24.8	44	23.0	13.3	20.0	39	15.1	43.4	3.0

Table 4.26: Percentage of evolutionary divergence of *Epilachna septima* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH656689	*Epilachna septima* (Kerala)	
2.	KU578138	*Epilachna* sp.(Kerala)	0.00%
3.	KC255440	*Sphenoraia bicolor*	3.45%
4.	KU697467	*Euphitrea piceicollis*	23.07%
5.	KF655810	*Longitarsus exsoletus*	23.31%
6.	KY835900	*Chrysomelidae* sp.	24.00%
7.	KC185777	*Pallasiola absinthii*	25.49%
8.	KF656634	*Aphthona albertinae*	26.16%
9.	MH020282	*Prionus coriarius*	26.84%
10.	KU917112	*Dinoptera collaris*	26.84%
11.	KM286367	*Acmaeops marginatus*	26.87%
12.	KF654908	*Longitarsus candidulus*	27.02%
13.	MG061719	*Acalymma vittatum*	27.02%
14.	KF653719	*Aphthona depressa*	28.31%
15.	MG057938	*Psylliodes cucullatus*	32.60%

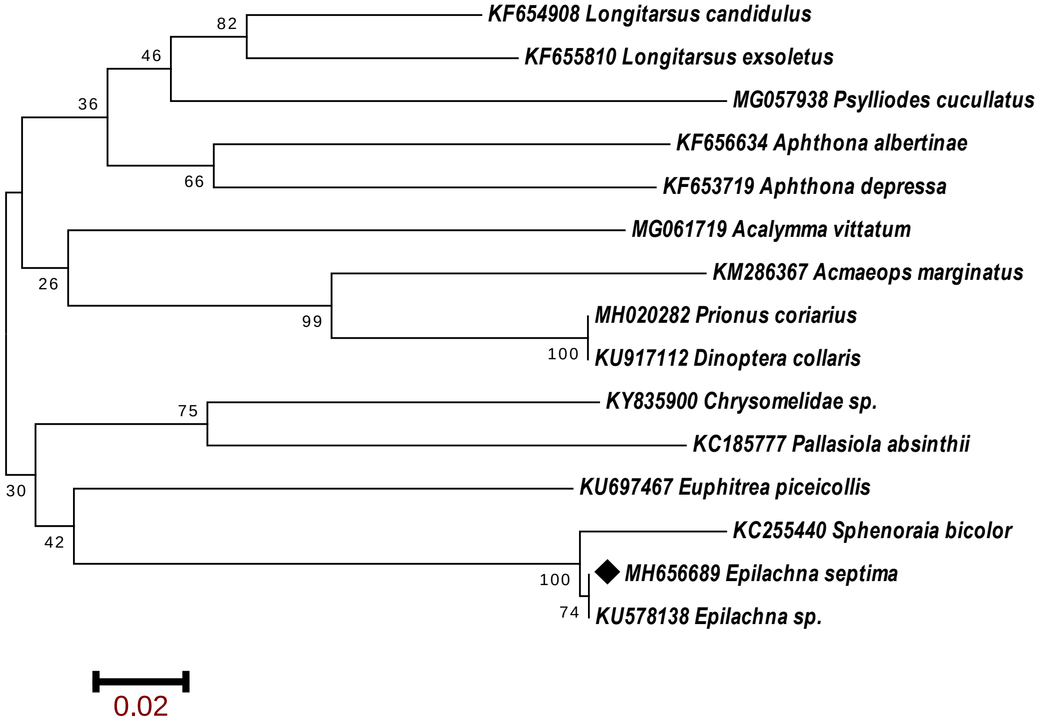


Figure 4.78: Phylogenetic relationship of *Epilachna septima* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 100% sequence similarity to the same genus reported from Kerala (KU578138). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Epilachna septima* showed bias to nucleotide AT, with following composition of nucleotides T = 36.9%, C = 19.2%, A = 29.0% and G = 14.9%. This greater AT content (65.9%) over GC content (34.1%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Epilachna septima* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 0% to 32.60% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has no divergence (0%) those from Kerala while 32.60% from Canada (Table 4.26). On the basis of the data observed this species may be rooted from those found Canada which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from Canada during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Epilachna septima* with related species. *Epilachna septima* isolated from Kerala (MH656689) showed 0% divergence with *Epilachna* sp. (KU578138) from Kerala and 32.60 % divergence with *Psylliodes cucullatus* (MG057938) from Canada. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Epilachna septima* isolated from Kerala. Closest relative of *Epilachna septima* is *Epilachna septima* from Kerala represented within the same clade.

14. ***Carpophilus (Semocarpolus) marginellus*** (Motschulsky, 1858)

Specimen details:

Voucher specimen : CUCM – 02 – A1
Date of collection : 23-Nov-2017
Locality : Wayanad: Sulthan Bathery
Lat- Lon : 11.6656° N, 76.2627° E
GenBank accession : MH590768

Description and distribution:

Fig. 4.79: *Carpophilus marginellus*

Carpophilus spp. is minute beetles with body 2-4 mm in length and short truncate elytra that partially cover the abdomen (Figure 4.79). The adults of *Carpophilus marginellus* are brown, black-brown, or black with yellow-red coloured legs, short elytra are generally yellow-brown and the antennae are 11-segmented. The larvae are whitish or yellowish with a brown head, final length 5-7 mm. Elytron darker, dark rufous to black, some specimens with faint to distinct pale spots; pronotum and elytra with or without setal fringe; usually in decaying plant material. Mesosternum without a raised medial line, its surface not divided, or if a weak line is present, elytra not distinctly patterned; prosternal process without a median ridge; elytron color various but without sharply defined spots as above; punctation various, less coarse. It is found distributed in Australia, Europe and Northern Asia (excluding China), India, North America and Oceania.

Damage:

They are polyphagus pest and infest ripening fruits on the tree and on the ground. The adult enter the fruit, usually through the calyx end, feeding on the pulp and thereby causing damage.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Carpophilus marginellus* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 593 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 4.80 – 4.83 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 4.27.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Carpophilus marginellus* voucher collected during the present study (Table 4.27). It showed bias to nucleotide AT, with nucleotide composition with T = 36.2%, C = 17.8%, A = 29.3% and G = 16.7%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 4.28 and Figure 4.84 respectively.

> *Carpophilus marginellus* CDS-2018/ 593 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUCM-02-A1

> *Carpophilus marginellus*

```
GATCAGGAATAGTGGGTACCTCATTAAGAATTCTTATTCGAACAGAATTAGGATCCCCCG
GATCTTTAATTGGTAATGATCAAATTTATAATGTAATTGTTACAGCCCACGCATTTATTA
TAATTTTTTTTATGGTAATACCTTTTATAATTGGAGGATTTGGAAATTGATTGGTACCAC
TAATATTAGGAGCCCCAGATATGGCCTTCCCTCGAATAAACAATATAAGATTTTGATTAC
TTCCCCCCTCATTATCTTTACTTTTAATAAGAAGAATTGTTGAAAGAGGGGCAGGCACTG
GATGAACAGTATACCCCCCTCTGTCATCTAATATCGCTCATGGTGGATCATCAGTAGATT
TAGCCATTTTTAGCCTTCATTTAGCAGGTATTTCTTCAATTTTAGGAGCAGTAAATTTTA
TTACTACTGTAATTAATATACGACCAACAGGAATAACGTTTGATCGAATACCCCTATTCG
TTTGAGCTGTTGTAATTACTGCTGTTTTATTACTTTTATCCCTTCCTGTATTAGCAGGAG
CTATTACTATATTATTAACAGATCGAAATTTAAATACTACTTTTTTCGATCCT
```

Figure 4.80: The partial DNA sequence of the mitochondrial COI gene of *Carpophilus marginellus*

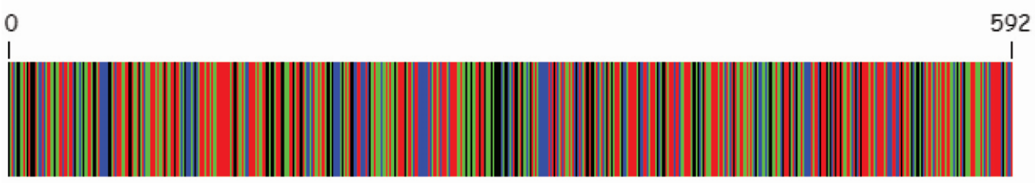


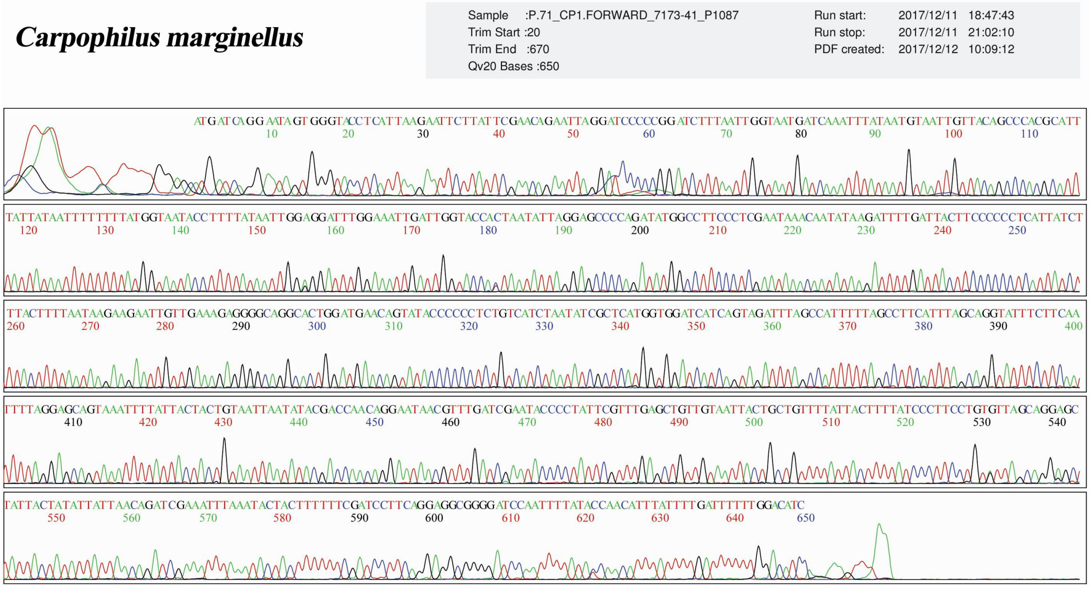
Figure 4.81: Molecular barcode of the mitochondrial COI gene of *Carpophilus marginellus*

> *Carpophilus marginellus* / 195AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUCM-02-A1

> *Carpophilus marginellus*

```
MVGTSLSILIRTELGSPGSLIGNDQIYNVIVTAHAFIMIFFMVMPFMIGGFGNWLVPLML
GAPDMAFPRMNNMSFWLLPPSLSLLLMSSIVESGAGTGWTVYPPLSSNIAHGGSSVDLAI
FSLHLAGISSILGAVNFITTVINMRPTGMTFDRMPLFVWAVVITAVLLLLSLPVLAGAIT
MLLTDRNLNTTFFDP
```

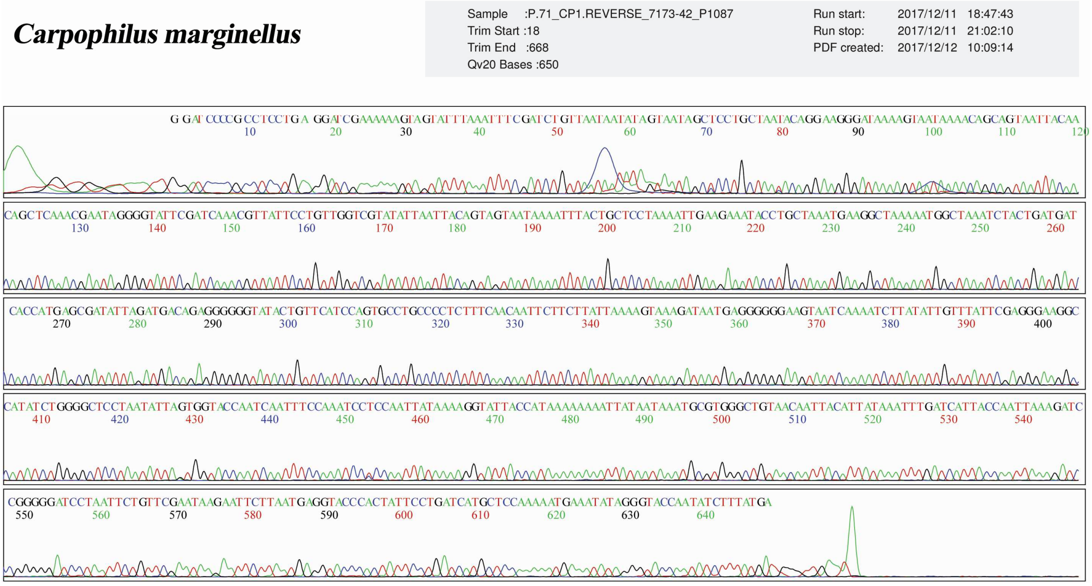
Figure 4.82: The translation product of the mitochondrial COI gene of *Carpophilus marginellus*



Carpophilus marginellus

Sample :P.71_CP1.FORWARD_7173-41_P1087
Trim Start :20
Trim End :670
Qv20 Bases :650

Run start: 2017/12/11 18:47:43
Run stop: 2017/12/11 21:02:10
PDF created: 2017/12/12 10:09:12



Carpophilus marginellus

Sample :P.71_CP1.REVERSE_7173-42_P1087
Trim Start :18
Trim End :668
Qv20 Bases :650

Run start: 2017/12/11 18:47:43
Run stop: 2017/12/11 21:02:10
PDF created: 2017/12/12 10:09:14

Figure 4.83: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Carpophilus marginellus* using Forward and Reverse primers

Table 4.27: Comparison of Nucleotide frequencies of COI gene sequence of *Carpophilus marginellus* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH590768 Carpophilus marginellus	36.2	17.8	29.3	16.7	39	10.9	46.4	3.8	25	15.8	30.6	28.4	45	26.6	10.9	17.9
KU916283 Carpophilus marginellus	36.6	17.9	29.2	16.3	39	11.5	46.4	3.1	26	15.6	30.2	28.1	45	26.6	10.9	17.7
MG060352 Carpophilus sp.	36.6	17.9	28.8	16.7	39	11.5	45.3	4.2	26	15.6	30.2	28.1	45	26.6	10.9	17.7
KU914959 Carpophilus marginellus	37.0	17.7	29.0	16.3	40	10.9	46.4	3.1	27	15.6	29.7	28.1	45	26.6	10.9	17.7
KU918768 Carpophilus sexpustulatus	35.8	18.2	28.3	17.7	36	13.5	43.2	7.3	27	15.1	30.7	27.6	45	26.0	10.9	18.2
KM850647 Carpophilus pallipennis	36.1	20.1	26.2	17.5	39	16.7	37.0	7.3	24	17.2	30.7	27.6	45	26.6	10.9	17.7
KR484408 Carpophilus brachypterus	37.5	18.2	27.4	16.8	42	12.0	41.1	4.7	26	16.1	30.2	28.1	45	26.6	10.9	17.7
GU217467 Carpophilus obsoletus	38.6	15.9	29.7	15.7	44	5.8	47.4	2.3	27	15.1	30.2	27.9	45	26.7	11.6	16.9
KU919608 Omosita discoidea	37.2	16.7	30.4	15.8	39	10.9	47.4	3.1	28	13.5	31.3	27.1	45	25.5	12.5	17.2
KR488443 Carpophilus sp.	37.3	18.6	27.1	17.0	42	12.5	40.1	5.2	25	16.7	30.2	28.1	45	26.6	10.9	17.7
KR481234 Aleochara curtula	41.0	13.4	29.9	15.8	49	1.6	48.4	1.0	29	13.0	29.2	29.2	45	25.5	12.0	17.2
GU217499 Carpophilus davidsoni	34.4	21.2	26.8	17.7	38	15.8	38.0	8.2	20	20.9	30.8	27.9	45	26.7	11.6	16.9
KU874983 Epuraea terminalis	34.5	20.3	28.1	17.0	34	17.2	43.2	5.7	24	17.7	30.2	27.6	45	26.0	10.9	17.7
KR483076 Epuraea linearis	36.3	19.1	28.5	16.1	40	13.0	44.3	3.1	24	18.2	30.2	27.6	45	26.0	10.9	17.7
MG988541 Cymatodera linsleyi	38.8	15.3	29.7	16.2	45	6.3	47.1	2.1	27	13.5	31.3	28.1	45	26.0	10.9	18.2

Table 4.28: Percentage of evolutionary divergence of *Carpophilus marginellus* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH590768	*Carpophilus marginellus* (Kerala)	
2.	KU916283	*Carpophilus marginellus* (Germany)	0.44%
3.	MG060352	*Carpophilus* sp.	0.88%
4.	KU914959	*Carpophilus marginellus*	0.89%
5.	KR481234	*Aleochara curtula*	34.11%
6.	GU217499	*Carpophilus davidsoni*	36.41%
7.	KU918768	*Carpophilussexpustulatus*	37.56%
8.	GU217467	*Carpophilus obsoletus*	39.01%
9.	KM850647	*Carpophilus pallipennis*	39.99%
10.	KU874983	*Epuraea terminalis*	40.31%
11.	KR483076	*Epuraea linearis*	40.56%
12.	KU919608	*Omosita discoidea*	42.68%
13.	KR484408	*Carpophilusbrachypterus*	43.96%
14.	KR488443	*Carpophilus* sp.	45.17%

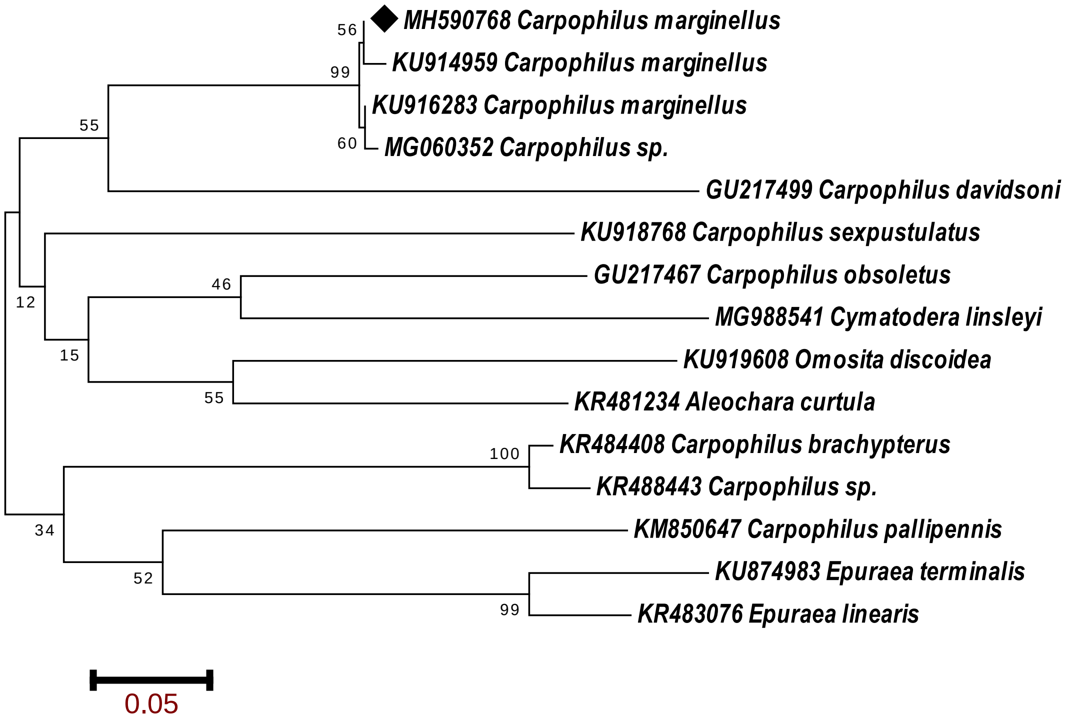


Figure 4.84: Phylogenetic relationship of *Carpophilus marginellus* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 99.6% sequence similarity to the same genus reported from Germany (KU916283). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Carpophilus marginellus* showed bias to nucleotide AT, with following composition of nucleotides T = 36.2%, C = 17.8%, A = 29.3% and G = 16.7%. This greater AT content (65.5%) over GC content (34.5%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Carpophilus marginellus* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 0.4% to 45.17% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has least divergence (0.4%) from Germany while 45.17% from Canada (Table 4.28). On the basis of the data observed this species may be rooted from those found in Canada which diverted into different clades due to geographical variation. Result thus concluded that this species doesn't have any major changes in India while slightly changes from those reported from Canada during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Carpophilus marginellus* with related species. *Carpophilus marginellus* isolated from Kerala (MH590768) showed 0.4% divergence with *Carpophilus marginellus* (KU916283) from Germany and 45.17% divergence with *Carpophilus* sp. from Canada. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Carpophilus marginellus* isolated from Kerala. Closest relative of *Carpophilus marginellus* is *Carpophilus marginellus* from Germany represented within the same clade.

15. ***Epuraea (Haptoncus) luteola*** (Erichson, 1843)

Specimen details:

Voucher specimen : CUEL – 01 – A1
Date of collection : 23-Nov-2017
Locality : Kasaragod: Chandragiri
Lat- Lon : 12.4991° N, 74.9989° E
GenBank accession : MH674104

Description and distribution:

Fig.4.85: *Epuraea (Haptoncus) luteola*

These are often referred as sap feeding beetles. *E. luteola* is oblong ovate, subdepressed. *Epuraea luteola* are somewhat shiny, yellowish brown coloured. The anterior margin of pronotum is with trapezium like emargination (Figure 4.85). The male hind tibia is curved and dilated at the middle, have finely punctuated dorsum, the cuticle is with moderately dense, and decumbent pubescence. Body granulated above, bearing lanceolate or spatulate short setae. Body length about 4 mm. Head-capsule about 0.46 mm in breadth; frontal median setae present. Relative lengths of 1st to 3rd antennal joints are in the ratio 6:11:9. Setae of thoracic and abdominal terga are shorter. Ninth abdominal segment with about 2 granules before each pregomphus, which, when viewed from above, are arranged in an arcuate row with pregomphus; asperities of cephalic portion of tergum irregularly strewn or connected to each other as illustrated. Widely distributed in Asia, Australia and Oceania.

Damage:

Both direct and indirect damage can be caused by *Carpophilus* sp., sap beetle adults, larvae and their damage on varieties of fruits and vegetables. The sap beetle attacks ripe, nearly ripe or decaying strawberry fruit by boring into the berry and devouring a portion in the field. *Epuraea luteolus* apparently feeds on decomposing fruit material. The presence of *Epuraea luteolus* on fruit may be more of an issue of contamination by beetles possibly by larvae leading to bacterial attack.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Epuraea luteola* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 603 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 4.86 – 4.89 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 4.29.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Epuraea luteola* voucher collected during the present study (Table 4.29). It showed bias to nucleotide AT, with nucleotide composition with T = 38.9%, C = 16.1%, A = 28.4% and G = 16.6%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 4.30 and Figure 4.75 respectively.

> *Epuraea luteola* CDS-2018/ 603 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUEL-01-A1

> *Epuraea luteola*

ATAGTAGGAACTTCATTAAGAATTTTGATTCGAACTGAATTGGGCTCCCCCGGCTCTCTA
ATTGGTAATGATCAAATTTATAATGTTATTGTGACTGCTCACGCTTTTATTATAATTTTT
TTTATAGTTATACCTTTTATAATTGGAGGATTTGGAAATTGATTAGTCCCTTTAATACTA
GGAGCCCCTGATATAGCATTCCCTCGAATAAATAATATGAGATTTTGATTACTTCCCCCA
TCTTTATCTCTTTTAATTATAAGAAGAATTGTAGAAAGAGGGGCTGGAACAGGTTGAACT
GTTTATCCACCTCTTTCATCTAATATTGCTCATGGAGGTTCTTCCGTAGATTTAGCTATT
TTTAGACTTCATTTAGCTGGAATTTCTTCAATTCTTGGGGCTGTAAATTTTATTACTACT
GTAATTAATATACGACCAACTGGAATAAGGTTAGATCGAATACCACTATTTGTTTGAGCT
GTAATTATTACAGCCGTTTTACTACTCTTATCATTACCAGTATTAGCAGGAGCAATTACA
ATACTACTTACTGATCGAAATTTAAATACTACCTTTTTTGACCCCTCAGGAGGAGGAGAC
CCA

Figure 4.86: The partial DNA sequence of the mitochondrial COI gene of *Epuraea luteola*

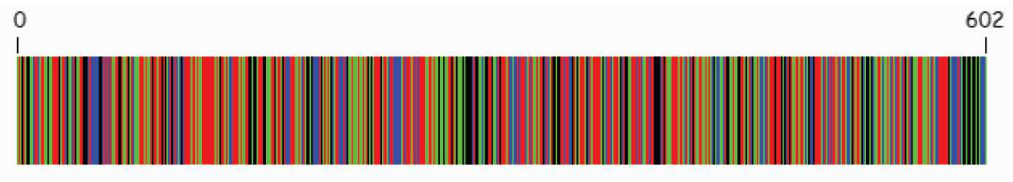


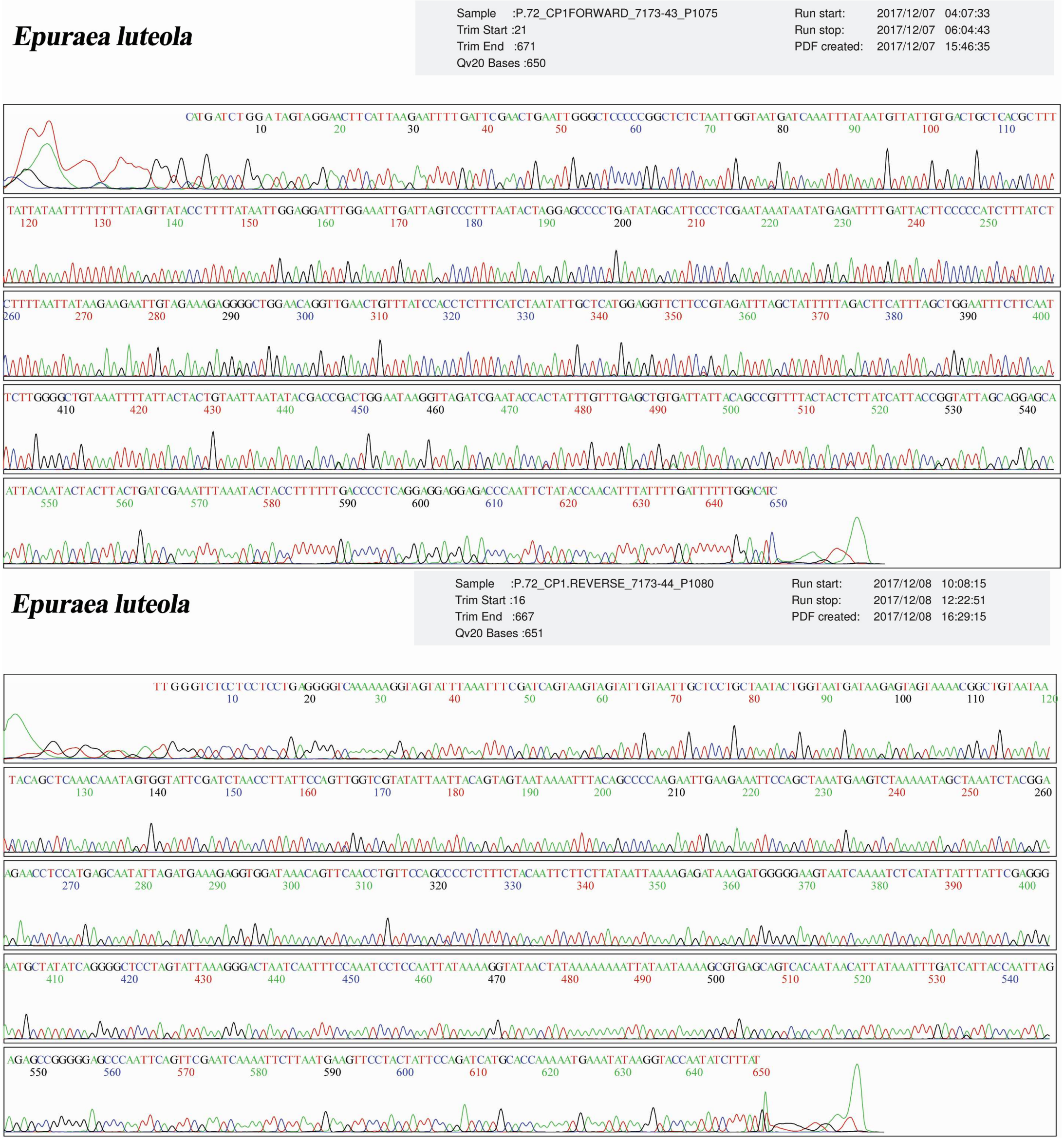
Figure 4.87: Molecular barcode of the mitochondrial COI gene of *Epuraea luteola*

> *Epuraea luteola* / 201AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUEL-01-A1

> *Epuraea luteola*

MVGTSLSILIRTELGSPGSLIGNDQIYNVIVTAHAFIMIFFMVMPFMIGGFGNWLVPLML
GAPDMAFPRMNNMSFWLLPPSLSLLIMSSIVESGAGTGWTVYPPLSSNIAHGGSSVDLAI
FSLHLAGISSILGAVNFITTVINMRPTGMSLDRMPLFVWAVIITAVLLLLSLPVLAGAIT
MLLTDRNLNTTFFDPSGGGDP

Figure 4.88: The translation product of the mitochondrial COI gene of *Epuraea luteola*



Epuraea luteola

Sample :P.72_CP1FORWARD_7173-43_P1075
Trim Start :21
Trim End :671
Qv20 Bases :650

Run start: 2017/12/07 04:07:33
Run stop: 2017/12/07 06:04:43
PDF created: 2017/12/07 15:46:35

CATGATCTGGATAGTAGGAACTTCATTAAGAATTTTGATTCGAACTGAATTGGGCTCCCCCGGCTCTCTAATTGGTAATGATCAAATTTATAATGTTATTGTGACTGCTCACGCTTT
TATTATAATTTTTTTTATAGTTATACCTTTTATAATTGGAGGATTTGGAAATTGATTAGTCCCTTTAATACTAGGAGCCCCTGATATAGCATTCCCTCGAATAAATAATATGAGATTTTGATTACTTCCCCCATCTTTATCT
CTTTTAATTATAAGAAGAATTGTAGAAAGAGGGGCTGGAACAGGTTGAACTGTTTATCCACCTCTTTCATCTAATATTGCTCATGGAGGTTCTTCCGTAGATTTAGCTATTTTTAGACTTCATTTAGCTGGAATTTCTTCAAT
TCTTGGGGCTGTAAATTTTATTACTACTGTAATTAATATACGACCGACTGGAATAAGGTTAGATCGAATACCACTATTTGTTTGAGCTGTGATTATTACAGCCGTTTTACTACTCTTATCATTACCGGTATTAGCAGGAGCA
ATTACAATACTACTTACTGATCGAAATTTAAATACTACCTTTTTTGACCCCTCAGGAGGAGGAGACCCAATTCTATACCAACATTTATTTTGATTTTTTGGACATC

Epuraea luteola

Sample :P.72_CP1.REVERSE_7173-44_P1080
Trim Start :16
Trim End :667
Qv20 Bases :651

Run start: 2017/12/08 10:08:15
Run stop: 2017/12/08 12:22:51
PDF created: 2017/12/08 16:29:15

TTGGGTCTCCTCCTCCTGAGGGGTCAAAAAGGTAGTATTTAAATTTCGATCAGTAAGTAGTATTGTAATTGCTCCTGCTAATACTGGTAATGATAAGAGTAGTAAAACGGCTGTAATAA
TACAGCTCAAACAAATAGTGGTATTCGATCTAACCTTATTCCAGTTGGTCGTATATTAATTACAGTAGTAATAAAATTTACAGCCCCAAGAATTGAAGAAATTCCAGCTAAATGAAGTCTAAAAATAGCTAAATCTACGGA
AGAACCTCCATGAGCAATATTAGATGAAAGAGGTGGATAAACAGTTCAACCTGTTCCAGCCCCTCTTTCTACAATTCTTCTTATAATTAAAAGAGATAAAGATGGGGGAAGTAATCAAAATCTCATATTATTTATTCGAGGG
AATGCTATATCAGGGGCTCCTAGTATTAAAGGGACTAATCAATTTCCAAATCCTCCAATTATAAAAGGTATAACTATAAAAAAAATTATAATAAAAGCGTGAGCAGTCACAATAACATTATAAATTTGATCATTACCAATTAG
AGAGCCGGGGGAGCCCAATTCAGTTCGAATCAAAATTCTTAATGAAGTTCCTACTATTCCAGATCATGCACCAAAAATGAAATATAAGGTACCAATATCTTTAT

Figure 4.89: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Epuraea luteola* using Forward and Reverse primers

Table. 4.29. Comparison of Nucleotide frequencies of COI gene sequence of *Epuraea luteola* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH674104 Epuraea luteola	38.9	16.1	28.4	16.6	43	25.5	12.8	18.8	50	6.7	38.9	4.0	23	16.1	33.6	26.8
KX054052 Nitidulidae sp.	39.8	15.7	28.2	16.3	46	26.8	9.4	17.4	48	2.7	45.0	4.0	25	17.4	30.2	27.5
KR480683 Epuraea rufa	37.8	16.8	28.0	17.4	43	24.8	12.8	19.5	40	12.8	42.3	4.7	30	12.8	28.9	28.2
JN290428 Coleoptera sp.	37.8	16.8	27.5	17.9	43	24.8	12.8	19.5	40	12.8	40.9	6.0	30	12.8	28.9	28.2
KU913042 Epuraea melina	36.7	17.7	28.0	17.7	43	24.8	12.8	19.5	38	14.1	41.6	6.0	29	14.1	29.5	27.5
KU907805 Epuraea terminalis	36.5	17.9	28.6	17.0	43	24.8	12.8	19.5	40	12.8	43.6	4.0	27	16.1	29.5	27.5
KJ961742 Epuraea placida	36.2	17.2	30.0	16.6	43	24.8	12.8	19.5	42	10.1	44.3	3.4	23	16.8	32.9	26.8
MH322728 Arima marginata	39.8	13.2	30.4	16.6	44	22.8	13.4	19.5	48	4.7	44.3	2.7	27	12.1	33.6	27.5
KU919592 Longitarsus pratensis	37.6	15.0	31.1	16.3	44	22.8	13.4	19.5	42	10.1	45.6	2.7	27	12.1	34.2	26.8
KR485404 Longitarsus scutellaris	37.6	15.0	31.1	16.3	44	22.8	13.4	19.5	42	10.1	45.6	2.7	27	12.1	34.2	26.8
HQ983161 Carpophilus brachypterus	37.4	17.9	27.3	17.4	43	24.8	12.8	19.5	42	13.4	39.6	5.4	28	15.4	29.5	27.5
KP193938 Leucophenga spilossoma	37.8	15.2	29.8	17.2	41	24.2	15.4	19.5	48	5.4	45.6	.7	24	16.1	28.2	31.5
KU913847 Omosita colon	38.5	17.0	29.1	15.4	43	24.2	13.4	19.5	43	14.1	42.3	.7	30	12.8	31.5	26.2
MF639827 Epuraea avara	36.5	18.8	28.0	16.8	43	25.5	12.1	19.5	42	13.4	40.9	3.4	24	17.4	30.9	27.5
KU916489 Vincenzellus ruficollis	39.6	15.2	28.2	17.0	43	24.2	13.4	19.5	51	6.0	42.3	.7	25	15.4	28.9	30.9

Table 4.30: Percentage of evolutionary divergence of *Epuraea luteola* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH674104	*Epuraea luteola* (Kerala)	
2.	KX054052	*Nitidulidae* sp.(France)	0.00%
3.	KU913042	*Epuraea melina*	2.14%
4.	HQ983161	*Carpophilus brachypterus*	2.14%
5.	KR480683	*Epuraea rufa*	2.70%
6.	JN290428	*Coleoptera* sp.	2.70%
7.	MH322728	*Arima marginata*	2.70%
8.	KU907805	*Epuraea terminalis*	3.23%
9.	KJ961742	*Epuraea placida*	3.23%
10.	KU913847	*Omosita colon*	3.23%
11.	KU919592	*Longitarsus pratensis*	3.27%
12.	KR485404	*Longitarsus scutellaris*	3.27%
13.	MF639827	*Epuraea avara*	3.78%
14.	KU916489	*Vincenzellus ruficollis*	4.96%
15.	KP193938	*Leucophenga spilossoma*	8.75%

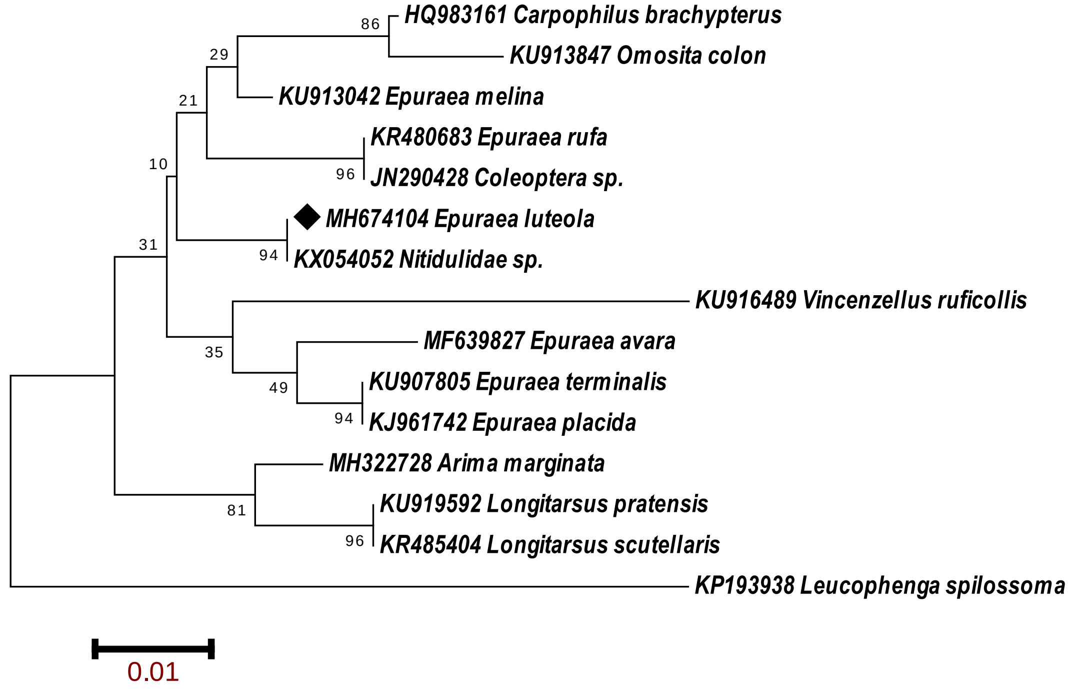


Figure 4.90: Phylogenetic relationship of *Epuraea luteola* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 100% sequence similarity to the related genus reported from France (KX054052). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Epuraea luteola* showed bias to nucleotide AT, with following composition of nucleotides T = 38.9%, C = 16.1%, A = 28.4% and G = 16.6%. This greater AT content (67.3%) over GC content (32.7%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Epuraea luteola* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 0% to 8.75% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has no divergence (0%) those from France while 8.75% from China (Table 4.30). On the basis of the data observed this species may be rooted from those found in China which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from China during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Epuraea luteola* with related species. *Epuraea luteola* isolated from Kerala (MH674104) showed 0% divergence with *Nitidulidae* sp (KX054052) from France and 8.75% divergence with *Leucophenga spilossoma* (KP193938) from China. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Epuraea luteola* isolated from Kerala. Closest relative of *Epuraea luteola* is *Nitidulidae* sp from France.

Consolidated Phylogenetic Tree

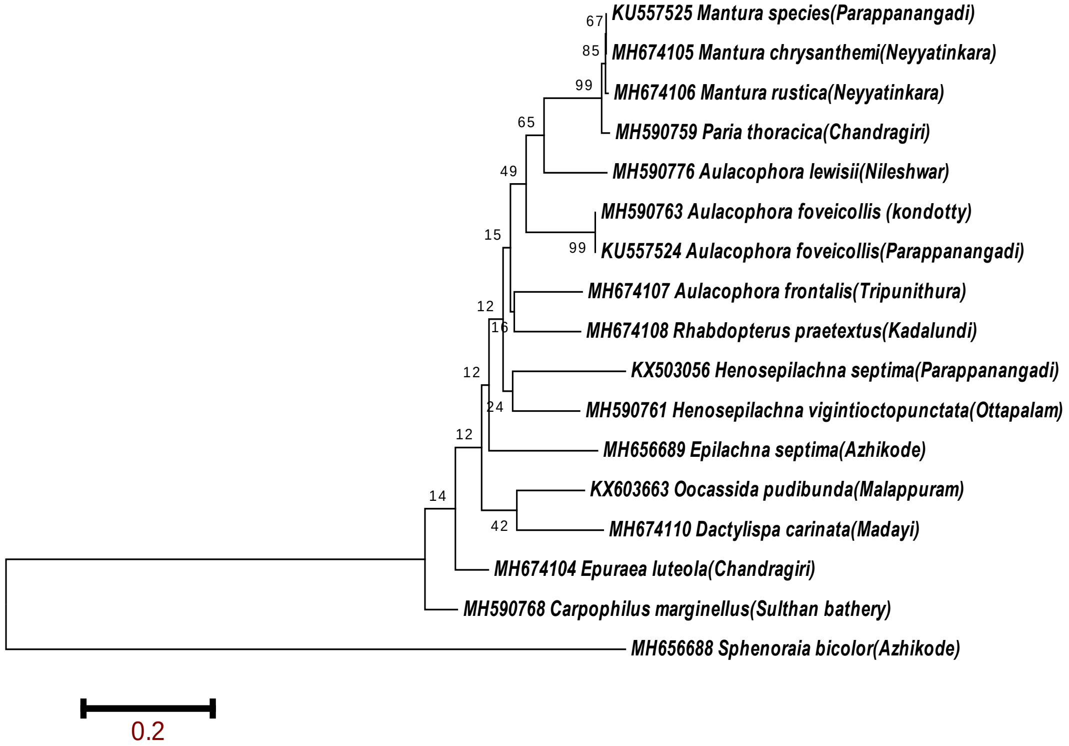


Figure 4.91: Phylogenetic relationship of different coleopteran pests of cucurbitaceae isolated from selected districts of Kerala

The evaluation of the composition of nucleotides within the COI sequences of Coleopteran pest of cucurbits of Kerala has revealed that the nucleotides found to be varied within the every third position of the codon. The analysis involved 15 species nucleotide sequences (Figure 4.91). The phylogenetic relationships among them was analysed by NJ tree method. Families included in the superfamily Chrysomeloidea is Chrysomelidae, superfamily Coccinelloidea is Coccinellidae and superfamily Cucujoidea is Nitidulidae. The species from these three families are aligned nearly in the phylogenetic tree with a monophyletic origin. Species from the family Chrysomelidae are originated from a main clade and various species from the genus *Mantura* and *Aulacophora* are aligned in a single clade and then branches to subclades. *Oocassida pudibunda* and *Dactylispa carinata* are aligned in a same clade and they are included in the Cassidinae subfamily. *Carpophilus marginellus* and *Epuraea luteolus* are aligned in a same clade and they are included in the Nitidulidae subfamily. *Sphenoraia bicolor* is situated as an outgroup to all other pests in the phylogenetic tree.

5

Hemiptera

Hemiptera is the largest and very diverse order among hemimetabolous insects, not undergo complete metamorphosis (Gullan and Cook, 2007)), containing > 75,000 named species (Coulson et al, 1993). They are economically important in agriculture, known to cause direct damage to plants by herbivory and indirectly by transporting diseases (Popov, 1990). Predatory hemipterans are used as biological control systems against control agricultural pests (Coll, 1998).

The defining feature of hemipterans is "stylet", modified mandibles and maxillae, sheathed within a modified labium (Gullan et al, 1994). The stylet is capable of piercing tissues and sucking liquids, supported by the labium. The tubular proboscis at front of head, X-pattern formed by front wings on back, triangular scutellum in centre of back leathery front wings and Segments 8 and 9 are modified to form the genitalia (Slater and Baranowski, 1978) are other supporting characters.

According to Kaur and Sharma (2017), Cucurbits are infested with a wide variety of insect pests right from the primordial stages till the harvest of the crop products. *Empoasca decipiens* Paoli (Homoptera, Cicadellidae) are pest on squash and cucurbit. Squash bug, *Anasa tristis* DeGeer (Hemiptera: Coreidae), sap feeding endemic pest species and is one among the major pest of squash and pumpkins in the United States. Squash bugs attacks plants in the genus Cucurbita especially Squash and pumpkins. Over 50% of the total squash bug eggs collected statewide were parasitized (Wilson and Kuhar, 2017). Report states that all stages of *Megymenum brevicorne*, F., attacks grenadilla (*Passiflora quadrangularis*), pumpkin (*Cucurbita maxima*), and snake gourd (*Trichosanthes anguina*) in Malaya (Miller, 1929). Family Pentatomidae are represented by *Nezara viridula* Linn., which is pest upon tomato, potato, bringal, cucurbits .

The systematic position of hemipteran pests of cucurbits collected and identified during the present study is given below:

Order: Hemiptera; Suborder: Heteroptera

1. **Superfamily: Pentatomoidea**
    - **1.1. Family: Pentatomidae**
        - **1.1.1 Subfamily: Pentatominae**
            - *Nezara viridula* (Linnaeus, 1758)
            - *Spermatodes variolosa* (Walker, 1867)
            - *Agonoscelis nubilis* (Fabricius, 1775)
            - *Carbula scutellata* (Distant, 1887)
            - *Halymorpha halys* (Stahl, 1855)
        - **1.1.2. Subfamily: Asopinae**
            - *Zicrona caerulea* (Linnaeus, 1758)
2. **Superfamily: Lygaeoidea**
    - **2.1. Family: Lygaeidae**
        - **2.1.1. Subfamily: Lygaeinae**
            - *Oncopeltus nigriceps* (Distant, 1903)
    - **2.2. Family: Geocoridae**
        - **2.2.1. Subfamily: Geocorinae**
            - *Geocoris varius* (Uhler, 1860)
3. **Superfamily: Coreoidea**
    - **3.1. Family: Alydidae**
        - **3.1.1. Subfamily: Micrelytrinae**
            - *Paraplesius unicolor* (Scott, 1874)
    - **3.2. Family: Coreidae**
        - **3.2.1. Subfamily: Coreinae**
            - *Cletus schmidti* (Kiritshenko, 1916)

4. **Superfamily: Pyrrhocoroidea**
    4.1. **Family: Pyrrhocoridae**
        4.1.1. **Subfamily: Pyrrhocorinae**
            - *Dysdercus ocreatus* (Uhler, 1886)

5. **Superfamily: Miroidea**
    5.1. **Family: Miridae**
        5.1.1. **Subfamily: Phylinae**
            - *Campylomma vendicarina* (Carapezza, 1991)

A brief description on the relevant higher taxa and the taxonomic key prepared for classification and morphological identification (with the help of suitable identification guides and expert consultation) of all the hemipteran pests of cucurbits collected during the present study are given below:

Key to the suborders of Order Hemiptera

1. Rostrum touches sternum in between fore coxae; hemelytra placed slanting and side by side upon abdomen, uniformly coriaceous................................... ....**AUCHENORRHYNCHA**

– Rostrum never touches sternum on repose; hemelytra are always overlapping with proximal membranous and distal coriaceous.............**HETEROPTERA**

SUBORDER: HETEROPTERA

Heteroptera comprises the so called true bugs. They have complex and important roles in the balance of nature. There also are heteropterans that act as carriers of disease. Antennae are slender with 4-5 segments; proboscis 3-4 segmented and arising from front of head and usually curving below the body; pronotum large, trapezoidal or rounded; presence of triangular scutellum present behind pronotum; 2 or 3 segmented tarsi (Wheeler, 2001).

Key to the superfamilies of Suborder Heteroptera

1. Ocelli present...**2**

– Ocelli absent **4**

2. Antennae 5 segmented, scutellum enlarged to cover the abdomen .... **Pentatomoidea**

– Antennae 4 segmented, scutellum small, not extending beyond half of the abdomen.. **3**

3. Antennae inserted on the upper side of the head above the line drawn from the eyes to the base of the rostrum; front wing having many veins; hind tibiae in some species expanded giving leaf like appearance.................... **Coreoidea**

– Antennae inserted below a line drawn through centre of the eyes; front wing in lygaeids have only four to five veins.. **Lygaeoidea**

4. Hemelytra with broad corium without appendix, bright coloration, predominantly red and black .... **Pyrrhocoroidea**

– Hemelytra with distinct corium with appendix, variable coloration, triangular heads, four segmented beak and antennae, forewings are tilted at the distinct angle along posterior of abdomen.. **Miroidea**

SUPERFAMILY: PENTATOMOIDEA

The Pentatomoidea are most commonly referred to as shield bugs, chust bugs, and stink bugs. There are ~7000 species under Pentatomoidea , in 14 or 15 families (Chinery, 1993). Since they belong to Hemiptera, share a common arrangement of sucking mouthparts. The Pentatomoidea are characterized by a highly developed scutellum, the hardened extension of the thorax over the abdomen; five segmented antennae; tarsi having two or three segments. Shield bugs have defensive glands located in the thoraces of first and second pair of legs that produce a foul-smelling liquid, which is used to deter potential predators (Grazia et al., 2008).

FAMILY: PENTATOMIDAE

Pentatomidae are a family of insects under the order Hemiptera, which are generally called stink bugs or shield bugs. The scutellum body is half of an inch long, green or brown coloured, usually trapezoidal in shape, giving this family the name "shield bug". The tarsi are 3-segmented. The forewings of stink bugs are called hemelytra, with the basal half thickened and membranous apex (as are the hindwings) (Chinery, 1993). Sternite viii concealed in males; Pendergrast's organs absent in case of females; trichobothria single and short frena present.

SUBFAMILY: PENTATOMINAE

Pentatominae features include paired trichobothria; trichobothrium nearest spiracle on sternite vii is lateral of imaginary line which is tangential to spiracular opening on sternites vi and vii by distance equal to greatest diameter of spiracular opening; base of abdominal venter mesial tubercle and metasternum produced flattened; frena one third or more length of scutellum and scutellum not reaching apex of abdomen (Grazia et al., 2008). Species on this subfamily are phytophages and several are considered agricultural pests.

Key to the species of pests collected from Subfamily Pentatominae

1. Body usually not pilose, if pilose hind tibiae sulcate; bucculae usually angulate or with tooth; abdominal sternites without median longitudinal furrow, if present, deeply furrowed 2

– Body with long, erect, rather sparse pilosity, hind tibiae not sulcate, bucculae smooth, rounded, not angulate or toothed anteriorly; abdominal sternites usually with shallow, median longitudinal furrow …………………..

…………………………………………………………***Agonoscelis nubilis***

2. Scutellum triangular, elongate or U-shaped exposing greater part of hemelytra 3

– Scutellum ampliated from the very base, without frena and covering greater part of hemelytra and abdomen 4

3. Humeral angles of pronotum spined or sometimes with blunt spine, peritreme either elongate, groove-shaped and reaching only middle of metapleuron or short, spout-shaped and not reaching middle of metapleuron; scutellum variable in colour, sometimes with spots both at basal angles and at apex of scutellum, lateral and inner margins of mandibular plates not reflexed and head slightly narrowing in front of eyes; females with rounded ovipositor, posterior margin of third, fourth and fifth visible abdominal sternites with row of hairs in males and females sometimes with sparse hairs .... ***Carbula scutellata***

– Humeral angles of pronotum lobe-like or rounded or subprominent, peritreme elongate either reaching or extending beyond middle of metapleuron; anterolateral margins of pronotum smooth or rarely with slight crenulations; labium shorter, reaching at most fourth visible abdominal sternite; second segment of labium longest; variously coloured bugs with brownish or transparent veins on hemelytral membrane, Head shorter than pronotum, anterior margin of pronotum not reflexed or callose, anterolateral margins of pronotum weakly reflexed, shar ; abdominal sternites with punctae either absent or with brown or black coloured punctae .... ***Halymorpha halys***

4. Lateral margins of head and pronotum densely and uniformly punctate; head distinctly longer than broad between eyes, metasternal carina with or without notch posteriorly, punctation present on metapleural region laterad to metepimeral pseudosuture, peritreme short, not extending beyond middle of metapleuron .... ***Nezara viridula***

– Lateral margins of head and pronotum not explanate, anterolateral margins of pronotum smooth or crenulate; antennal segments cylindrical; ventrolateral area of abdominal sternites devoid of longitudinal band of striations; body generally ovoid, never conspicuously elongate ....***Spermatodes variolosa***

SUBFAMILY: ASOPINAE

The subfamily Asopinae comprises about 300 described species in 69 genera worldwide. They are predatory stink bugs or soldier bugs are of moderate to large size, ranging in length from 7 to 25 mm, and are broadly elliptical in shape. The piercing-sucking mouthparts with a four-segmented rostrum or beak (labium) forms a sheath that encloses a pair of mandibular and maxillary stylets. In asopines, the first segment of the rostrum is markedly thickened and free, which enables the rostrum to swing forward fully (Bonte and Clercq, 2008).

From this subfamily, only a single specimen is collected during the present study, *Zicrona caerulea*.

SUPERFAMILY: LYGAEOIDEA

The Lygaeoidea is the second largest super family in the infraorder Pentatomomorpha, consisting about 700 genera and more than 4,200 species in the world (Sweet, 2000). These bugs are known as 'seed bugs', with 1.2-12 mm long, have variability in shape and color and shows aposematic coloration (Baranowski and Slater, 2005). Sweet (2000) reviewed them as major and minor pest species of economic concern.

Key to the families of Superfamily Lygaeoidea

1. Head narrower with eyes smaller, if convex and protuberant not extending posterior of anterior lateral angle of pronotum, abdominal spiracle on segment 2nd to 7th located dosally, apical margin of corium straight, brightly coloured .... **Lygaeidae**

– Head very broad with eyes large and protuberant and extending posteriorly to envelope anterior angles of pronotum, abdominal spiracle of segment 5th to 7th located ventrally, antennal segment 1 shorter than combined length of segments 2 and 3, and shorter than head length .... **Geocoridae**

FAMILY: LYGAEIDAE

Lygaeidae are typically medium sized insects, ranging in size ~1 to 12mm. Aposematism is widespread within the family, particularly within the subfamily Lygaeidae (Aldrich et al. 1997) also majority of them are cryptically colored (Schuh and Slater 1995). They are oval in shape and generally resemble the shape of the seeds they feed on (Schuh and Slater 1995). Most possess four segmented antennae, although some Lygaeidae have only three. They can be distinguished from their close relatives, the Coreidae, by the number of veins present on the forewing. Lygaeidae have five or fewer, while coreids have six of more. The Lygaeidae also closely resemble the Miridae but, unlike the mirids, they lack a distinctive cuneus and possess ocelli. The identification of key morphological features of Lygaeidae is problematic due to their polyphyletism (Aldrich et al., 1997).

SUBFAMILY: LYGAEINAE

Accoding to Slater and Baranowski (1990), there are 58 genera under Lygaeinae. The Lygaeinae features include impunctate pronotum and scutellum; black coloured body with red markings; claval regions of hemelytra forming median commissure; apical margin of corium. Pronotum in lateral aspect with anterior lateral angle impressed and concave for reception of eye; general color mainly black, often with red or orange markings; legs fuscous to black; coarse punctation reduced, dorsally restricted to anterior submarginal and medial transverse areas of pronotum, and ventrally on prosternum and propleuron; setae various, small and appressed to short and recurved, or some specimens with erect longer bristles on head and pronotum. straight; dark coloured elytral membrane.

From this subfamily, only a single specimen is collected during the present study, *Oncopeltus nigriceps.*

FAMILY: GEOCORIDAE

Geocoridae are commonly called as Big-eyed bugs. A small family (<300 species), composed of common generalist predatory Heteroptera. They are being assayed for controlling turf grass pests and occasionally feed on plants by sucking the sap. Adults are small (approximately 1/4 inch); wide head that gives the big-eyed appearance; antennae arising close together in the lower middle of the "face"; lack of triangular plates ("cuneus") on the front wings; antennae have 4 segments (Torres and Boyd, 2009).

SUBFAMILY: GEOCORINAE

The members of this subfamily are large sized and kidney-shaped. They are easily recognized by protruded eyes, reniform projecting caudolateral around anterior corner of pronotum, femore of anterior legs are not incrassate or provided with a row of spines, dorsoventrally flattened prothorax and abdomen. Pronotum is large and fronto-laterally without hairs. Body is stout and oval. Clavus is tapered at the distal part of scutellum. Spiracles are dorsal at abdominal segments 2-4, and ventral at the 5-7th abdominal segments. They are partially or entirely predators in many species. Thirteen genera are known in this subfamily, and are distributed in all of the major zoogeographic regions of the world (Slater and Baranowski, 1990).

From this subfamily, only a single specimen is collected during the present study, *Geocoris varius*

SUPERFAMILY: COREOIDEA

Superfamily Coreoidea, true bugs belongs to the infraorder Pentatomomorpha, comprises of leaf-footed bugs and allies. The Coreoidea as a whole represent a part of a close-knit group with the Lygaeoidea and Pyrrhocoroidea and hence these three superfamilies are paraphyletic. The families include: Alydidae (broad-headed bugs), Coreidae (leaf-footed bugs and squash bugs), Hyocephalidae, Rhopalidae (scentless plant bugs), Stenocephalidae.

Key to the families of Superfamily Coreoidea

1. Head broad and triangular, last antennal segments are elongated and curved, hind femur bearing spines, trichobothria of abdominal sternum five (visible sternum 3) arranged in a row lateral or anterior to spiracle, compound eyes globular and protruding, and they also have ocelli, tarsus three segmented .... **Alydidae**

– Head longitudinally impressed near central lobe; Abdominal spiracle dorsal, Metathoracic scent gland auricles large and conspicuous, never bristly, ovipositor usually flattened and plate like, if laciniate then tibiae not sulcate and abdominal pore bearing organs lacking, tibiae sulcate, posterior angles of dorsal surface of sixth abdominal segment in male rounded, obtuse, or straight, or somewhat prominently recurved; tibiae above generally sulcate or dilated .... **Coreidae**

FAMILY: ALYDIDAE

The broad-headed bugs (alydids) are small to medium sized, slender, with a triangular head. They are divided into two subfarnilies, Alydinae and Micrelytrinae Schaefer (2004), having slight modifications. The farnily Alydidae has over 50 genera with around 250 species; in the neotropics there are over 20 genera. The alydids of economic importance in the world were reviewed by Panizzi et al. (2006).

These are broad-headed bugs up to 10–12 millimetres (0.4–0.5 in) long, and have slender bodies with very thin legs of dusky or blackish coloration. The most notable characteristics of the family are that the head is broad, often similar in length and width to the pronotum and the scutellum, and that the last antennal segments are elongated and curved. The compound eyes are globular and protruding, and they also have ocelli. The femora of the hindlegs bear several strong spines; the tarsus has three segments. Most species have well-developed hemelytra (forewings), allowing them to fly well, but in some cases hemelytra are vestigial. The membranous parts of the hemelytra are with several closely spaced long veins (Oliveira, 1985). They have scent glands that produce a stink with smell and worser than that of other true stink bugs (Schaefer, 1999).

SUBFAMILY: MICRELYTRINAE

Micrelytrinae is distinguished with pronotum slightly wider and longer than head, second rostral segment usually distinctly longer compared to posterior two together, hind femora without spines, pygophore having a median posterior spine and aedeagus with dorsal pair of thecal appendages. Hind femur without spines, trichobothria of abdominal sternum five arranged in a triangle posterior to spiracle

From this subfamily, only a single specimen is collected during the present study, *Paraplesius unicolor*

FAMILY: COREIDAE

The coreids, known as leaf-footed bugs or squash bugs, are, generally, medium to very large in size, with strong robust bugs and strikingly colorful, presenting expansion of femora, tibiae, humeral angles, or antennae. They are distributed worldwide, but are found more abundant in the tropics (Packauskas, 2010). Coreids are polyphagous plant feeders on gymnosperms and angiosperms, monocots and dicots (Schaefer and Mitchell, 1983). Mitchell (2000) reviewed the coreids for its econornic importance.

SUBFAMILY: COREINAE

Coreinae (Stal, 1867), hind wing cell without a hamus; head in front of eyes with a median sulcus; tibiae sulcate on outer surface. Large subfamily comprising a great diversity of forms classified into many tribes and of world-wide distribution. This subfamily is classified into many tribes and of world-wide distribution. Includes numerous species of economic importance

From this subfamily, only a single specimen is collected during the present study, *Cletus schmidti*

SUPERFAMILY: PYRRHOCOROIDEA

China and Miller (1959) used the following characters to typify the Pyrrhocoroidea. Presence of abdominal trichobothria, small scutellum small, shorter than the clavus, a distinct claval commisure present, visible Antennophores, Antennae four segmented, absence of ocelli absent, Membrane of the hemelytra usually with two basal cells, from which 7 - 8 branching longitudinal veins extend to the apical margin, medium to large, brightly colored, usually phytophagous bug.

FAMILY: PYRRHOCORIDAE

Pyrrhocoridae (Red bugs or Stainers) are elongate oval bugs. Show warning colouration. They are brightly marked with red and black. Membrane having more branched veins and cells. Feeding injury caused by these bugs leads to the contamination and destruction by the fungus, Nematospora hence resulting in yellowish brown discolouration of the lint.

SUBFAMILY: PYRRHOCORINAE

Pyrrhocorinae are characterized as robust insects with bright coloration (predominantly red and black), absence of ocelli, broad corium without appendix. The key distinguishing feature is sixth ventral segment of female entire.

From this subfamily, only a single specimen is collected during the present study, *Dysdercus ocreatus*

SUPERFAMILY: MIROIDEA

Miroidea includes at least 4,800 described species. These are characterized with triangular heads. They have varied colouration and are devoid of ocelli. The antennae are four segmented and hemelytra with distinct corium with appendix. The forewings are tilted at the distinct angle along posterior of abdomen.

FAMILY: MIRIDAE

They form one of the most diverse and speciose family within the Heteroptera, comprising about 1,300 genera and nearly 11,000 described species throughout the world. Mirids are known as plant bugs or capsids and are usually small to medium sized, elongated to ovoid bugs having triangular heads; they show variable coloration, being omnivorous. Distinguished feature include four segmented beak and antennae, hemelytra with distinct corium, clavus and cuneus; the forewings are tilted at the distinct angle along posterior of abdomen. Nymphs and adults feed on plant juice and some species cause phytotoxemia due to the injection of toxic saliva (Schuh et al., 2009). They are important pests in agricultural crops, and the predatory species show potential towards biological control programs. This is the largest family in the order. Most species are plant feeders, but many are predaceous on other insects. Some of the plant feeding species is pests of cultivated plants.

The mirids are soft bodied bugs, mostly 4-10 mm long, which may be variously coloured. Some species are brightly marked with red, orange, green or white. Members can be recognized by the presence of a cuneus and only one or two closed cells at the base of the membrane. The antennae and beak are four segmented, the ocelli are lacking.

SUBFAMILY: PHYLINAE

Phylinae is a subfamily of insects in the family Miridae, the plant bugs (Mazon et al., 2016). The Phylinae are diagnosed primarily by the structure of male genitalia, which are distinctive in their possession and pretarsal structure.

From this subfamily, only a single specimen is collected during the present study, *Campylomma vendicarina*

The morphological description, distribution, nature of the damage caused to the crops, analyses on mitochondrial COI gene sequences, molecular evolutionary divergence and phylogenic status of each pest specimen collected under Order Hemiptera during the present study are as follows:

1. ***Nezara viridula*** (Linnaeus, 1758)

Specimen details:

Voucher specimen : CUNV – 01 – A4
Date of collection : 24-Aug-2016
Locality : Kerala: Parappanangadi
Lat- Lon : 11.0510° N, 76.0711° E
GenBank accession : KX603657

Description and distribution:

Fig. 5.1: *Nezara viridula*

Nezara viridula, plant sap feeders, are commonly known as the Southern green stink bug (Figure 5.1). The adult males body length (from front to elytral apex) is about 12.1 millimetres, while females are bigger, having a size of about 13.1 millimetres. The body is bright green and shield-shaped and the eyes are usually reddish or sometimes blackish. Stink bugs of Pentatomidae are recognized by their shield-shape, five-segmented antennae, and their malodorous scent (Buschman, 1980). They have distinct shape for their scent gland openings, which are short and wide. They are highly polyphagous herbivore (Yukava et al., 2007). *Nezara viridula* are widely distributed all over India, Southeast Asia, Ethiopea, and Australasia.

Damage

Both larvae and adult attack the host by inserting their piercing sucking mouthparts into tissue and introducing the digestive enzymes (Jones and Caprio, 1994). Feeding on flower buds by adult causes premature abscission, and damage to seed pods. The bug attacks many important major horticultural, vegetable and field crops, with a preference for crucifers.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Nezara viridula* collected has been amplified using the primer BUG (Table 3.1). The PCR amplification yielded 462 bp, 449 bp, 427 bp long products for the specimens obtained from different locations. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 5.2 – 5.5 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 5.1.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Nezara viridula* voucher collected during the present study (Table 5.1). It showed bias to nucleotide AT, with nucleotide composition with T = 33.7%, C = 18.7%, A = 31.6% and G = 15.9%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 5.2 and Figure 5.6 respectively.

> *Nezara viridula* CDS-2016/ 462 bp / cytochrome oxidase subunit I (COI) gene,partial cds; mitochondrial / voucher CUNV-01-A4

>*Nezara viridula*

```
AAGTGTTTGGGGGTTGTGTAAATTTTTTACCCTTTGGGGGGACCGGTGCTTTAGAGGTGT
CATTCCGGTCGAATAATTTTTTCCGATTCTGACAAAAACCCCCATCATTAACCCTTTTAA
TAGTAAGAAGATTAGCAGAATCTGGAGCAGGGACAGGATGAACTGTTTATCCTCCTTTAT
CTAGTAACTTATCCCATAGAGGAGCTTCAGTGGATTTAGCTATTTTTTCATTACATCTAG
CAGGAGTATCATCAATTTTAGGTGCAGTAAATTTCATTTCAACTATTATTAATATACGAC
CAACAGGTATAACTCCAGAACGAGTGCCACTATTTGTTTGATCAGTTGGAATCACAGCAC
TATTATTACTACTTTCATTACCTGTACTAGCAGGGGCAATTACAATATTATTAACAGATC
GAAACTTTAATACATCATTCTTTGACCCTTCAGGAGGGGGAG
```

Figure 5.2: The partial DNA sequence of the mitochondrial COI gene of *Nezara viridula*

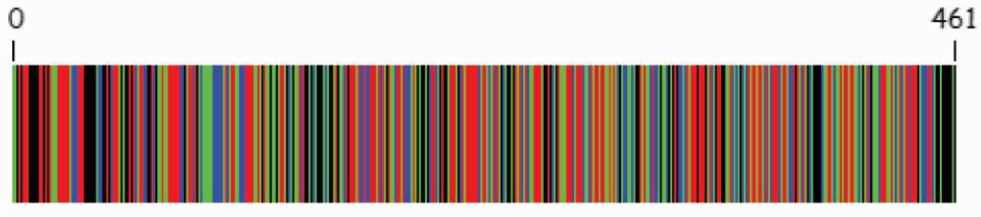


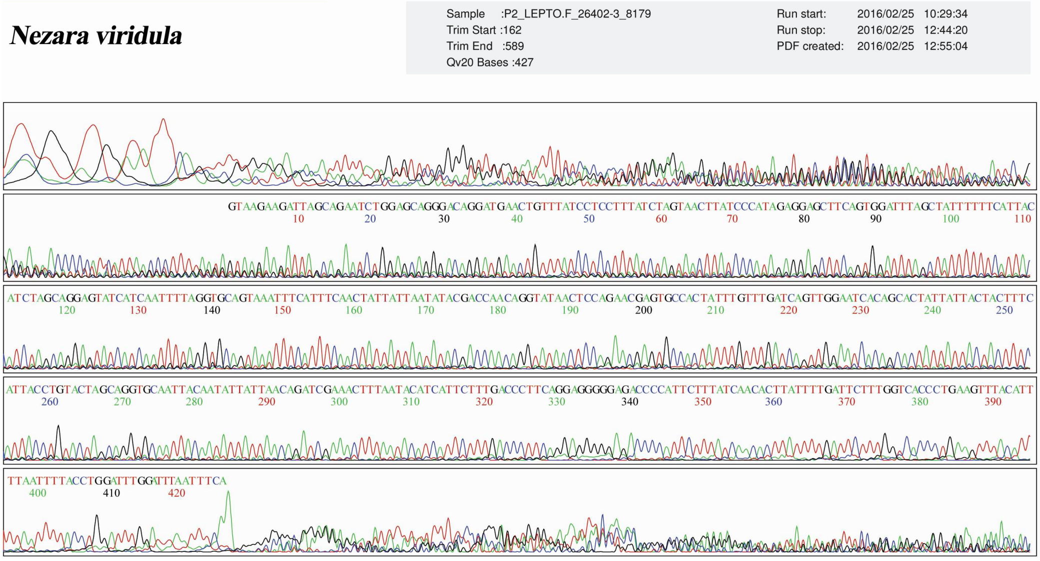
Figure 5.3: Molecular barcode of the mitochondrial COI gene of *Nezara viridula*

> *Nezara viridula* / 121AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUNV-01-A4

> *Nezara viridula*

```
MVSSLAESGAGTGWTVYPPLSSNLSHSGASVDLAIFSLHLAGVSSILGAVNFISTIINMR
PTGMTPERVPLFVWSVGITALLLLLSLPVLAGAITMLLTDRNFNTSFFDPSGGGXXXXXX
X
```

Figure 5.4: The translation product of the mitochondrial COI gene of *Nezara viridula*

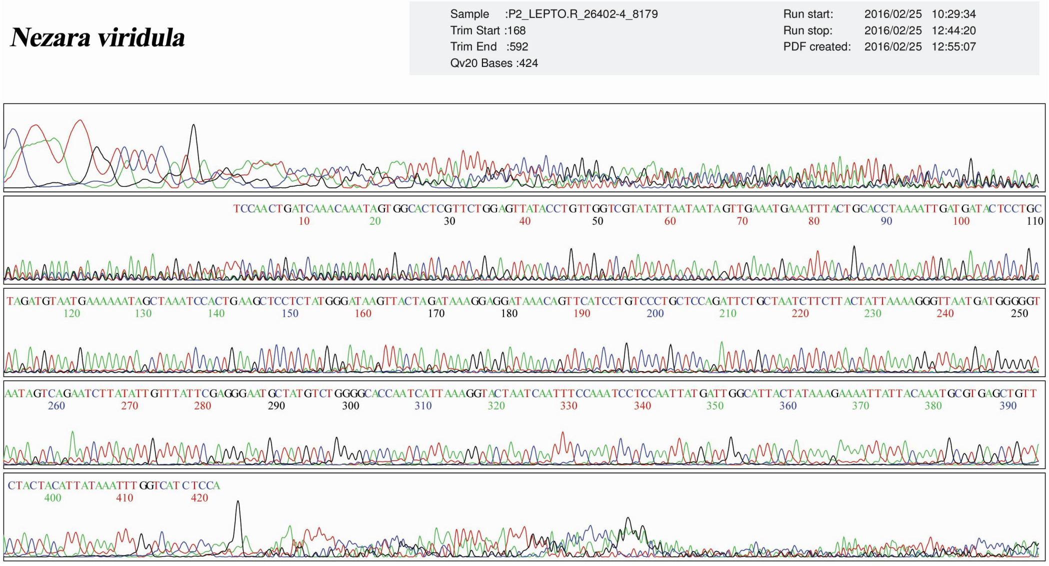


Nezara viridula

Sample :P2_LEPTO.F_26402-3_8179
Trim Start :162
Trim End :589
Qv20 Bases :427

Run start: 2016/02/25 10:29:34
Run stop: 2016/02/25 12:44:20
PDF created: 2016/02/25 12:55:04

GTAAGAAGATTAGCAGAATCTGGAGCAGGGACAGGATGAACTGTTTATCCTCCTTTATCTAGTAACTTATCCCATAGAGGAGCTTCAGTGGATTTAGCTATTTTTTCATTAC
ATCTAGCAGGAGTATCATCAATTTTAGGTGCAGTAAATTTCATTTCAACTATTATTAATATACGACCAACAGGTATAACTCCAGAACGAGTGCCACTATTTGTTTGATCAGTTGGAATCACAGCACTATTATTACTACTTTC
ATTACCTGTACTAGCAGGTGCAATTACAATATTATTAACAGATCGAAACTTTAATACATCATTCTTTGACCCTTCAGGAGGGGGAGACCCCATTCTTTATCAACACTTATTTTGATTCTTTGGTCACCCTGAAGTTTACATT
TTAATTTTACCTGGATTTGGATTTAATTTCA



Nezara viridula

Sample :P2_LEPTO.R_26402-4_8179
Trim Start :168
Trim End :592
Qv20 Bases :424

Run start: 2016/02/25 10:29:34
Run stop: 2016/02/25 12:44:20
PDF created: 2016/02/25 12:55:07

TCCAACTGATCAAACAAATAGTGGCACTCGTTCTGGAGTTATACCTGTTGGTCGTATATTAATAATAGTTGAAATGAAATTTACTGCACCTAAAATTGATGATACTCCTGC
TAGATGTAATGAAAAAATAGCTAAATCCACTGAAGCTCCTCTATGGGATAAGTTACTAGATAAAGGAGGATAAACAGTTCATCCTGTCCCTGCTCCAGATTCTGCTAATCTTCTTACTATTAAAAGGGTTAATGATGGGGGT
AATAGTCAGAATCTTATATTGTTTATTCGAGGGAATGCTATGTCTGGGGCACCAATCATTAAAGGTACTAATCAATTTCCAAATCCTCCAATTATGATTGGCATTACTATAAAGAAAATTATTACAAATGCGTGAGCTGTT
CTACTACATTATAAATTTGGTCATCTCCA

Figure 5.5: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Nezara viridula* using Forward and Reverse primers.

.

Table 5.1: Comparison of Nucleotide frequencies of COI gene sequence of *Nezara viridula* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
KX603658 Nezara viridula (Kerala)	33.7	18.7	31.6	15.9	27	16.9	28.2	27.5	44	28.9	9.9	16.9	29	10.5	56.6	3.5
KX587504 Nezara viridula (Kerala)	33.7	18.5	31.6	16.2	27	16.8	28.0	28.0	44	28.9	9.9	16.9	30	9.9	57.0	3.5
KX603657 Nezara viridula (Kerala)	34.7	19.0	31.6	14.8	27	17.5	29.4	26.6	45	28.4	12.1	14.2	32	11.2	53.1	3.5
KX351397 Nezara viridula	33.0	15.9	33.0	18.0	26	14.8	28.2	31.0	43	23.2	13.4	20.4	30	9.8	57.3	2.8
KU578143 Coccinella transversalis	32.1	16.6	33.3	18.0	25	14.8	28.9	31.7	42	25.2	13.3	19.6	30	9.9	57.7	2.8
KX503049 Riptortus pedestris	33.3	18.5	31.9	16.4	25	17.6	28.2	28.9	45	28.0	10.5	16.8	30	9.9	57.0	3.5
KU163626 Acrosternum gramineum	32.1	16.6	33.3	18.0	26	14.0	28.7	31.5	42	24.6	13.4	19.7	28	11.3	57.7	2.8
KC135971 Nezara antennata	33.5	16.6	32.1	17.8	26	13.4	28.2	32.4	44	24.6	13.4	17.6	30	11.9	54.5	3.5
JN087429 Plagiodera versicolora	32.1	16.9	33.3	17.8	25	14.0	29.4	31.5	42	25.4	13.4	19.0	29	11.3	57.0	2.8
GQ415599 Anthocoris expansus	31.6	17.8	35.6	15.0	24	16.8	34.3	25.2	42	26.1	14.1	17.6	29	10.6	58.5	2.1
KM023126 Zicrona caerulea voucher	36.3	15.7	30.4	17.6	25	15.5	28.9	30.3	43	23.9	13.4	19.7	41	7.7	49.0	2.8
KY492344 Tolumnia basalis	36.5	15.0	31.4	17.1	29	12.0	28.9	30.3	43	23.9	13.4	19.7	38	9.1	51.7	1.4
KY206850 Bagrada qinlingensis	34.0	14.3	35.8	15.9	25	13.3	34.3	27.3	43	19.7	20.4	16.9	34	9.9	52.8	3.5
KJ541515 Eysarcoris ventralis	34.2	14.8	34.7	16.4	27	14.1	30.3	28.9	43	23.9	13.4	19.7	33	6.3	60.1	.7
MF934944 Chlorochroa ligata	34.0	16.6	32.1	17.3	27	14.8	28.2	30.3	43	23.9	13.4	19.7	32	11.2	54.5	2.1

Table 5.2: Percentage of evolutionary divergence of *Nezara viridula* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	KX603657	*Nezara viridula* (Kerala)	
2.	KX603658	*Nezara viridula* (Kerala)	0.00%
3.	KX587504	*Nezara viridula* (Kerala)	1.09%
4.	KX351397	*Nezara viridula* (Meghalaya)	1.09%
5.	KU578143	*Coccinella transversalis*	1.09%
6.	KX503049	*Riptortus pedestris*	1.09%
7.	KU163626	*Acrosternum gramineum*	12.14%
8.	KC135971	*Nezara antennata*	12.14%
9.	GQ415599	*Anthocoris expanses*	29.91%
10.	MF934944	*Chlorochroa ligata*	22.53%
11.	KM023126	*Zicrona caerulea*	26.46%
12.	KY206850	*Bagrada qinlingensis*	27.21%
13.	KJ541515	*Eysarcoris ventralis*	29.91%
14.	GQ415599	*Anthocoris expanses*	29.91%
15.	KY492344	*Tolumnia basalis*	29.91%

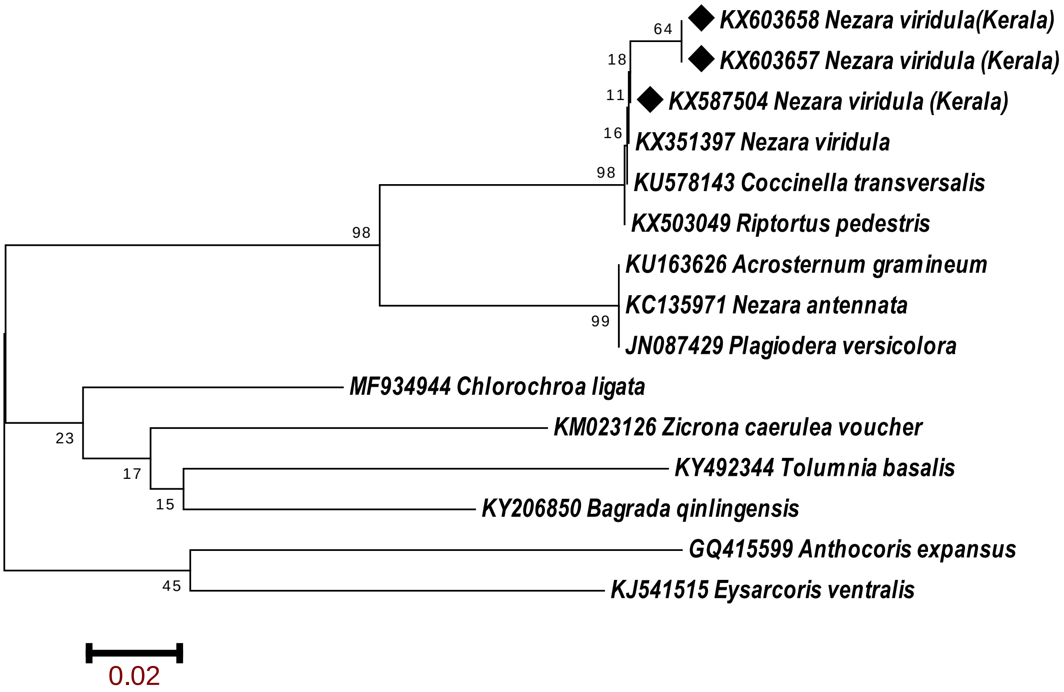


Figure 5.6: Phylogenetic relationship of *Nezara viridula* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 100% sequence similarity to the same genus reported from Kerala (KX603658). Even though this species has been found in various geographically isolated areas, their sequence doesn't have any kind of variation. The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Nezara viridula* showed bias to nucleotide AT, with following composition of nucleotides T = 33.7%, C = 18.7%, A = 31.6% and G = 15.9%. This greater AT content (65.3%) over GC content (34.6%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Nezara viridula* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Even though the COI sequences has been reported from different geographical locations, it showed only 0% to 29.91% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has no divergence (0%) with those from Kerala while 29.91% China (Table 5.2). On the basis of the data observed this species may be rooted from those found in Karnataka which diverted into different clades due to geographical variation. Result thus concluded that this species doesn't have any major changes in India while slightly changes from those reported from China during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Nezara viridula* with related species. *Nezara viridula* isolated from Kerala (KX603657) showed 0% divergence with *Nezara viridula* (KX603658) of kerala and 29.91% divergence with *Anthocoris expanses* (GQ415599) of China. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Nezara viridula* isolated from Kerala. Closest relative of *Nezara viridula* is *Nezara viridula* from Kerala represented within the same clade.

2. ***Spermatodes variolosa*** (Walker, 1867)

Specimen details:

Voucher specimen	:	CUSV – 01 – A1
Date of collection	:	26-Mar-2016
Locality	:	Malappuram: Nilambur
Lat- Lon	:	11.2794° N, 76.2398° E
GenBank accession	:	MH590762

Description and Distribution

Spermatodes variolosa are very small, ochraceour, thickly punctate, head anterior area of pronotum, basl margin of scutellum and body beneath brassy green, a sub triangular discal spot to scutellum casteous, two central spots on anterior area of pronotum, three spots at basal margin of scutellum. The body is piceous beneath and thickly punctuated (Figure 5.7). The lateral margins and a series of segmental spots present near the lateral margins luteous. Antennal formula: 1<2<3<4<5. The scutellum is large and extending to apex of abdomen. The eyes are prominent and extending beyond the anterior margin of pronotum (Mathew and Koshy, 1986). They are present throughout India.

Fig.5.7: *Spermatodes variolosa*

Damage

They are serious pest and they suck the plant sap (Mathew and Koshy, 1986). Adults and larval stages feed on the flowers and cause damage.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Spermatodes variolosa* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 591 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 5.8 – 5.11 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 5.3.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Spermatodes variolosa* voucher collected during the present study (Table 5.3). It showed bias to nucleotide AT, with nucleotide composition with T = 31.3%, C = 20.8%, A = 31.5% and G = 16.4%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 5.4 and Figure 5.10 respectively.

>*Spermatodes variolosa* CDS-2018/ 591 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUSV-01-A1

> *Spermatodes variolosa*

```
CGCTATAAGATTAATTATTCGTATTGAAGTAGGACAACCTGGGAGATTTATTGGAGATGA
TCAAATTTATAATGTAATAGTAACAGCTCATGCATTTGTAATAATTTTCTTTATAGTAAT
ACCAATTATAATTGGAGGATTCGGTAACTGGCTAGTGCCTTTAATAATTGGAGCCCCTGA
TATAGCATTCCCTCGAATAAATAATATAAGATTCTGACTTCTACCCCCCTCATTAACATT
ATTAATAATTAGAAGATTAACAGAATCAGGAGCCGGAACCGGATGAACAGTTTATCCCCC
TCTATCAAGCAACCTCTCCCACAGAGGAATATCAGTTGACTTAGCCATTTTTTCACTTCA
CCTGGCCGGTGTATCATCAATCCTAGGTGCAGTAAATTTCATCTCAACTATTATAAATAT
ACGACCTGAAGGAATAACCCCTGAACGAACTCCTTTATTTGTATGATCAGTAGGTATCAC
AGCCTTATTGTTACTTCTATCTTTACCAGTACTAGCCGGAGCTATTACAATATTACTCAC
AGACCGAAATTTTAACACATCTTTTTTCGACCCATCGGGGGGAGGAGACCC
```

Figure 5.8: The partial DNA sequence of the mitochondrial COI gene of *Spermatodes variolosa*

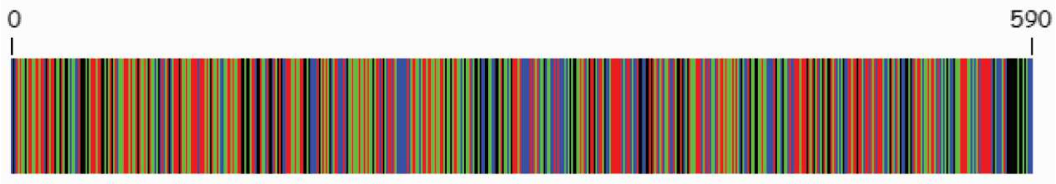


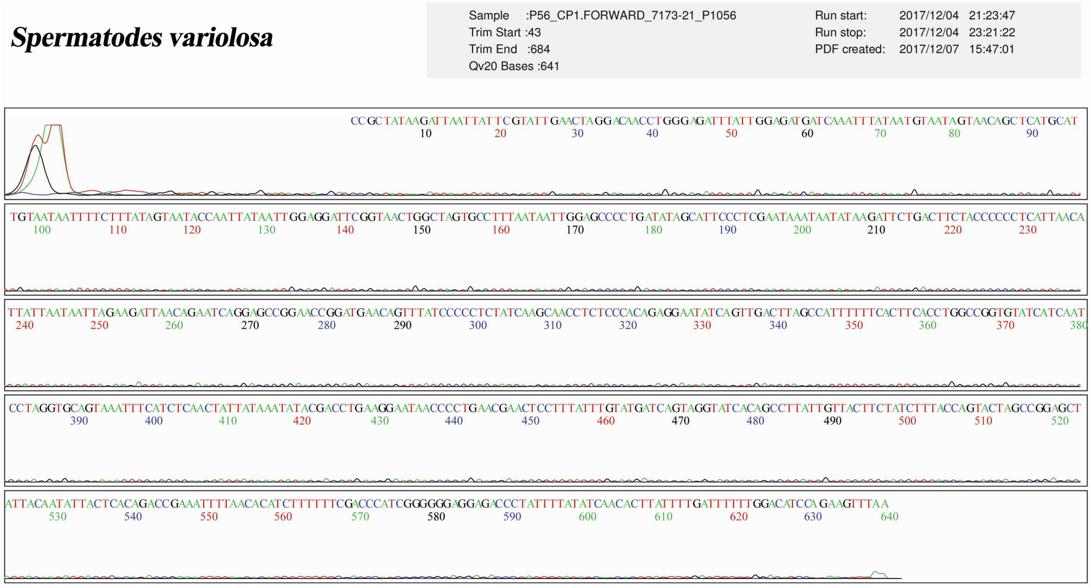
Figure 5.9: Molecular barcode of the mitochondrial COI gene of *Spermatodes variolosa*

> *Spermatodes variolosa* / 195AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUSV-01-A1

> *Spermatodes variolosa*

```
MSLIIRIEVGQPGSFIGDDQIYNVMVTAHAFVMIFFMVMPIMIGGFGNWLVPLMIGAPDM
AFPRMNNMSFWLLPPSLTLLMISSLTESGAGTGWTVYPPLSSNLSHSGMSVDLAIFSLHL
AGVSSILGAVNFISTIMNMRPEGMTPERTPLFVWSVGITALLLLLSLPVLAGAITMLLTD
RNFNTSFFDPSGGGD
```

Figure 5.10: The translation product of the mitochondrial COI gene of *Spermatodes variolosa*

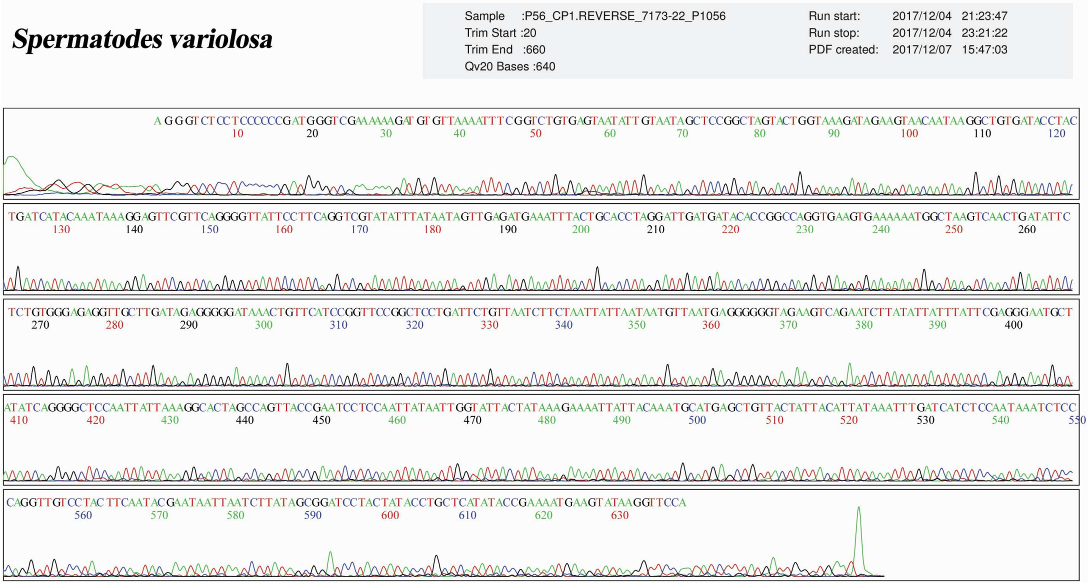


Spermatodes variolosa

Sample :P56_CP1.FORWARD_7173-21_P1056
Trim Start :43
Trim End :684
Qv20 Bases :641

Run start: 2017/12/04 21:23:47
Run stop: 2017/12/04 23:21:22
PDF created: 2017/12/07 15:47:01

```
CCGCTATAAGATTAATTATTCGTATTGAACTAGGACAACCTGGGAGATTTATTGGAGATGATCAAATTTATAATGTAATAGTAACAGCTCATGCAT
TGTAATAATTTTCTTTATAGTAATACCAATTATAATTGGAGGATTCGGTAACTGGCTAGTGCCTTTAATAATTGGAGCCCCTGATATAGCATTCCCTCGAATAAATAATATAAGATTCTGACTTCTACCCCCCTCATTAACA
TTATTAATAATTAGAAGATTAACAGAATCAGGAGCCGGAACCGGATGAACAGTTTATCCCCCTCTATCAAGCAACCTCTCCCACAGAGGAATATCAGTTGACTTAGCCATTTTTTCACTTCACCTGGCCGGTGTATCATCAAT
CCTAGGTGCAGTAAATTTCATCTCAACTATTATAAATATACGACCTGAAGGAATAACCCCTGAACGAACTCCTTTATTTGTATGATCAGTAGGTATCACAGCCTTATTGTTACTTCTATCTTTACCAGTACTAGCCGGAGCT
ATTACAATATTACTCACAGACCGAAATTTTAACACATCTTTTTTCGACCCATCGGGGGGAGGAGACCCTATTTTATATCAACACTTATTTTGATTTTTTGGACATCCAGAAGTTTAA
```



Spermatodes variolosa

Sample :P56_CP1.REVERSE_7173-22_P1056
Trim Start :20
Trim End :660
Qv20 Bases :640

Run start: 2017/12/04 21:23:47
Run stop: 2017/12/04 23:21:22
PDF created: 2017/12/07 15:47:03

```
AGGGTCTCCTCCCCCCGATGGGTCGAAAAAGATGTGTTAAAATTTCGGTCTGTGAGTAATATTGTAATAGCTCCGGCTAGTACTGGTAAAGATAGAAGTAACAATAAGGCTGTGATACCTAC
TGATCATACAAATAAAGGAGTTCGTTCAGGGGTTATTCCTTCAGGTCGTATATTTATAATAGTTGAGATGAAATTTACTGCACCTAGGATTGATGATACACCGGCCAGGTGAAGTGAAAAAATGGCTAAGTCAACTGATATTC
TCTGTGGGAGAGGTTGCTTGATAGAGGGGGATAAACTGTTCATCCGGTTCCGGCTCCTGATTCTGTTAATCTTCTAATTATTAATAATGTTAATGAGGGGGGTAGAAGTCAGAATCTTATATTATTTATTCGAGGGAATGCT
ATATCAGGGGCTCCAATTATTAAAGGCACTAGCCAGTTACCGAATCCTCCAATTATAATTGGTATTACTATAAAGAAAATTATTACAAATGCATGAGCTGTTACTATTACATTATAAATTTGATCATCTCCAATAAATCTCC
CAGGTTGTCCTACTTCAATACGAATAATTAATCTTATAGCGGATCCTACTATACCTGCTCATATACCGAAAATGAAGTATAAGGTTCCA
```

Figure 5.11: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Spermatodes variolosa* using Forward and Reverse primers.

Table 5.3: Comparison of Nucleotide frequencies of COI gene sequence of *Spermatodes variolosa* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH590762 Spermatodes variolosa (Kerala)	31.3	20.8	31.5	16.4	29	17.3	50.3	3.6	23	18.8	30.5	27.9	42	26.4	13.7	17.8
KY492346 Spermatodes variolosa	32.0	20.0	31.5	16.6	28	16.2	51.3	4.1	23	18.3	31.0	27.4	44	25.4	12.2	18.3
KX960067 Graphosoma lineatum	31.3	18.6	34.3	15.7	26	12.2	59.9	1.5	24	16.8	30.5	28.4	43	26.9	12.7	17.3
MF673705 Pentatomidae sp.	32.5	19.3	32.0	16.2	27	17.3	53.8	2.0	26	14.7	30.5	28.4	44	25.9	11.7	18.3
GQ292256 Zicrona caerulea	36.5	17.3	30.1	16.1	41	8.6	47.7	2.5	25	16.8	29.9	28.4	44	26.4	12.7	17.3
MF673706 Pentatomidae sp.	32.5	19.3	31.8	16.4	27	17.3	53.3	2.5	26	14.7	30.5	28.4	44	25.9	11.7	18.3
KY492352 Acesines bambusana	35.7	15.9	33.0	15.4	36	7.6	56.3	.5	27	14.7	30.5	27.9	45	25.4	12.2	17.8
KF273395 Halyomorpha halys	34.7	17.3	31.6	16.4	32	11.7	52.3	4.1	28	14.2	31.0	26.9	44	25.9	11.7	18.3
KR034008 Melanaethus robustus	33.0	17.9	32.7	16.4	28	13.2	55.8	2.5	26	15.2	30.5	28.4	45	25.4	11.7	18.3
KJ866504 Carbula biguttata	34.5	16.6	32.5	16.4	34	9.1	55.3	1.5	25	15.2	29.9	29.4	44	25.4	12.2	18.3
HQ333534 Carbula insocia	34.7	16.9	32.1	16.2	35	9.6	54.3	1.5	26	15.2	29.4	29.4	44	25.9	12.7	17.8
MG298985 Neohalys acuticornis	33.3	18.1	32.5	16.1	31	12.2	54.8	2.0	24	17.3	31.0	27.4	45	24.9	11.7	18.8
HQ236459 Catacanthus incarnatus	33.7	17.1	33.7	15.6	30	11.7	56.9	1.5	27	13.7	32.0	27.4	44	25.9	12.2	17.8
JQ218463 Oebalus poecilus	33.0	18.8	31.6	16.6	30	13.7	52.3	3.6	25	16.2	29.9	28.4	43	26.4	12.7	17.8

Table 5.4: Percentage of evolutionary divergence of *Spermatodes variolosa* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH590762	*Spermatodes variolosa* (Kerala)	
2.	KY492346	*Spermatodes variolosa* (Karnataka)	1.11%
3.	KX960067	*Graphosoma lineatum*	20.29%
4.	MF673705	*Pentatomidae* sp.	21.25%
5.	MF673706	*Pentatomidae* sp.	21.25%
6.	KJ866504	*Carbula biguttata*	22.04%
7.	HQ333534	*Carbula insocia*	22.47%
8.	KF273395	*Halyomorpha halys*	23.18%
9.	JQ218463	*Oebalus poecilus*	23.35%
10.	KY492352	*Acesines bambusana*	23.43%
11.	GQ292256	*Zicrona caerulea*	24.13%
12.	KR034008	*Melanaethus robustus*	24.56%
13.	HQ236459	*Catacanthus incarnates*	24.83%
14.	MG298985	*Neohalys acuticornis*	25.04%

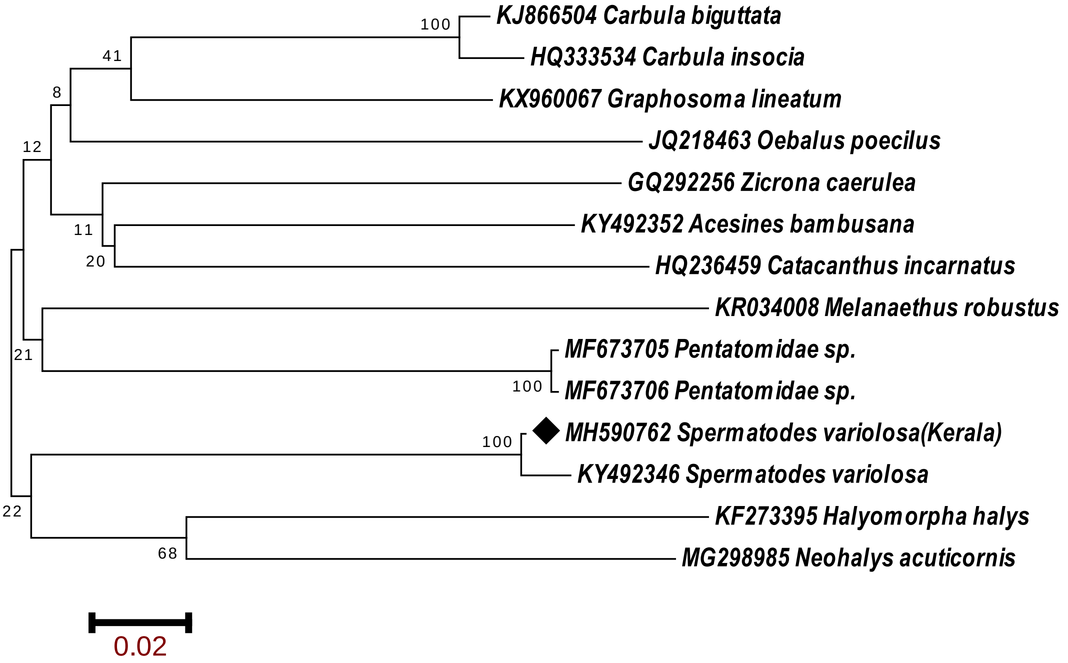


Figure 5.12: Phylogenetic relationship of *Spermatodes variolosa* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 98.9% sequence similarity to the same genus reported from Karnataka (KY492346). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Spermatodes variolosa* showed bias to nucleotide AT, with following composition of nucleotides T = 31.3%, C = 20.8%, A = 31.5% and G = 16.4%. This greater AT content (62.8%) over GC content (37.2%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Spermatodes variolosa* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. The COI sequences has been reported from different geographical locations, it showed 1.11% to 25.04% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (1.11%) those from Karnataka while 25.04% from Canada (Table 5.4). On the basis of the data observed this species may be rooted from those found in Canada was diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from Canada during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Spermatodes variolosa* with related species. *Spermatodes variolosa* isolated from Kerala (MH590762) showed 1.11% divergence with *Spermatodes variolosa* (KY492346) of Karnataka and 25.04% divergence with *Neohalys acuticornis* (MG298985) of Canada. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Spermatodes variolosa* isolated from Kerala. Closest relative of *Spermatodes variolosa* is *Spermatodes variolosa* from Karnataka represented within the same clade.

3. ***Agonoscelis nubilis*** (Fabricius, 1775)

Specimen details:

Voucher specimen : CUAN – 01 – A1
Date of collection : 10-Feb-2016
Locality : Kasaragod: Cheemeni
Lat- Lon : 12.2425° N, 75.2333° E
GenBank accession : MH590760

Description and Distribution

Fig. 5.13: *Agonoscelis nubilis*

Agonoscelis nubilis possess elongated oval shape with remote and long pilosity, overall brownish creamy body with coarse black punctures, central faint blackish band on pronotum which is posteriorly continued on to the scutellum, connexivum orange yellow with minute black spots at the posterio lateral apex, antennae black, tibia, tarsi and apices of femora black, a series of lateral black spots on sternum and abdomen, underneath body mostly yellow but lateral margins of sternum and abdomen with a tinge of orange (Figure 5.13). Head: head slightly narrowed at front but rounded at apex, median and lateral lobes are of equal length. Thorax: lateral margins of pronotum entire not serrate. Scutellum long and triangular. Mesosternum with a central carina. In hemelytra, membrane smoky with dark veins. Abdomen moderately sulcate (Alan, 2004). These are distributed all over India, Sri Lanka, Burma, also found in China, Japan, Malay peninsula and several islands of the Malayan archipelago.

Damage

These are plant feeders and suck the sap of leaves, stem and fruits (Nath and Arnowitt, 1975). Adults and nymphs suck the sap from the host plants, hence heavily infested plants shows light defoliation resulting in stunting.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Agonoscelis nubilis* collected has been amplified using the primer BUG (Table 3.1). The PCR amplification yielded 495 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 5.14 – 5.17 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 5.5.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Agonoscelis nubilis* voucher collected during the present study (Table 5.5). It showed bias to nucleotide AT, with nucleotide composition with T = 32.1%, C = 20.0%, A = 30.9 and G = 17.0%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 5.6 and Figure 5.18 respectively.

> *Agonoscelis nubilis* CDS-2018/ 495 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUAN-01-A1

> *Agonoscelis nubilis*

```
TTATTGGGGATGATCAAATTTATAACGTAATCGTTACAGCCCACGCATTTATTATAATCT
TTTTTATAGTTATGCCAATTATAATTGGAGGATTCGGAAATTGATTAGTACCCTTAATAA
TTGGAGCACCCGATATAGCATTCCCTCGAATAAATAATATAAGATTCTGGCTACTGCCCC
CCTCATTGACCCTTTTAATAATAAGAAGACTTACAGAAGCAGGGGCTGGGACAGGATGAA
CAGTTTACCCCCCTCTATCAAGAAACATCTCTCACAGAGGAGCATCAGTTGATCTAGCAA
TTTTCTCACTGCATCTAGCAGGAGTATCTTCAATTTTGGGTGCAGTAAACTTTATCTCAA
CCATTATTAATATACGCCCAGTTGGAATAACACCAGAGCGAATTCCATTGTTTGTCTGAT
CAGTTGGAATTACAGCTCTATTGTTATTATTATCTTTACCTGTACTAGCAGGAGCTATTA
CAATACTATTAACAG
```

Figure 5.14: The partial DNA sequence of the mitochondrial COI gene of *Agonoscelis nubilis*

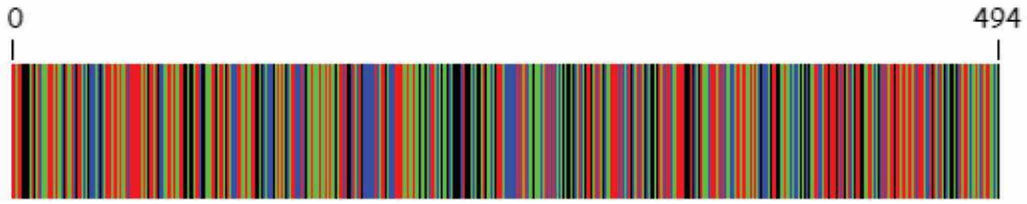


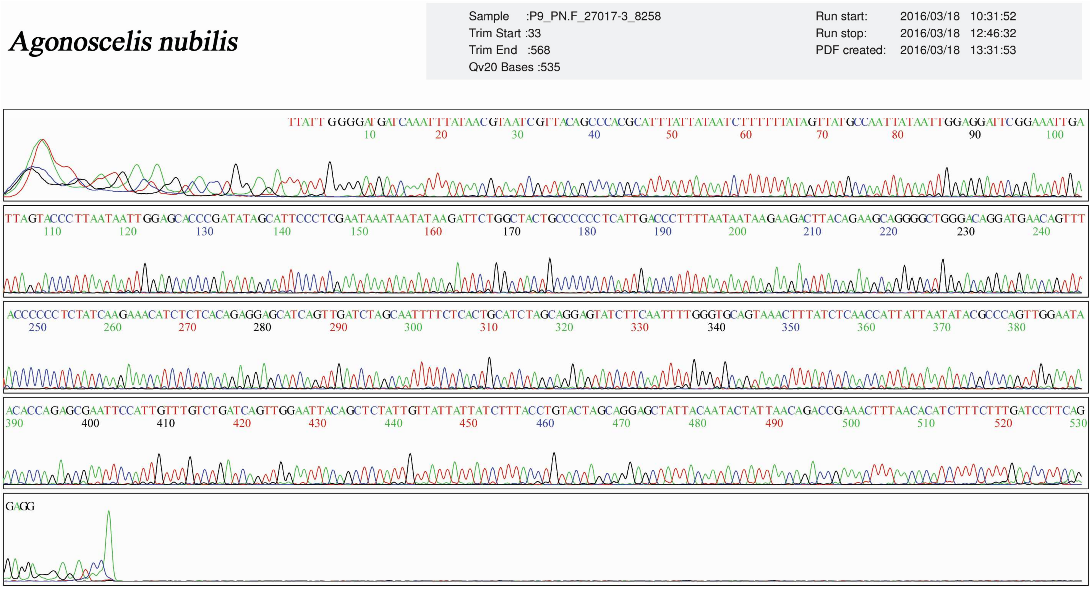
Figure 5.15: Molecular barcode of the mitochondrial COI gene of *Agonoscelis nubilis*

> *Agonoscelis nubilis* / 147AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUAN-01-A1

> *Agonoscelis nubilis*

```
MIFFMVMPIMIGGFGNWLVPLMIGAPDMAFPRMNNMSFWLLPPSLTLLMMSSLTEAGAGT
GWTVYPPLSSNISHSGASVDLAIFSLHLAGVSSILGAVNFISTIINMRPVGMTPERIPLF
VWSVGITALLLLLSLPVLAGAITMLLT
```

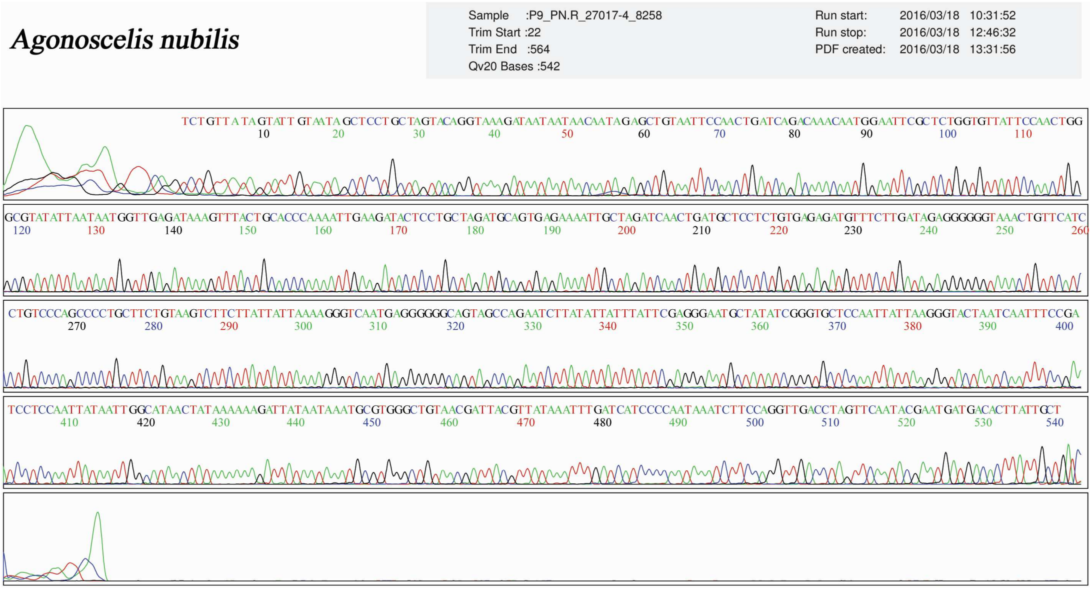
Figure 5.16: The translation product of the mitochondrial COI gene of *Agonoscelis nubilis*



Agonoscelis nubilis

Sample :P9_PN.F_27017-3_8258
Trim Start :33
Trim End :568
Qv20 Bases :535

Run start: 2016/03/18 10:31:52
Run stop: 2016/03/18 12:46:32
PDF created: 2016/03/18 13:31:53



Agonoscelis nubilis

Sample :P9_PN.R_27017-4_8258
Trim Start :22
Trim End :564
Qv20 Bases :542

Run start: 2016/03/18 10:31:52
Run stop: 2016/03/18 12:46:32
PDF created: 2016/03/18 13:31:56

Figure 5.17: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Agonoscelis nubilis* using Forward and Reverse primers.

Table 5.5: Comparison of Nucleotide frequencies of COI gene sequence of *Agonoscelis nubilis* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH590760 Agonoscelis nubilis (Kerala)	32.1	20.0	30.9	17.0	22	18.2	31.5	27.9	45	27.3	11.5	15.8	28	14.5	49.7	7.3
MG838334 Agonoscelis sp.	31.5	20.4	31.5	16.6	22	18.8	31.5	27.9	45	27.3	12.1	15.8	28	15.2	50.9	6.1
KY492355 Agonoscelis nubilis	31.1	19.0	32.1	17.8	20	18.2	33.3	28.5	43	24.2	13.3	19.4	30	14.5	49.7	5.5
LC099273 Acanthosoma forficula	31.9	19.2	31.7	17.2	25	13.9	32.1	28.5	42	24.8	12.7	20.0	28	18.8	50.3	3.0
KY206850 Bagrada qinlingensis	34.3	14.9	34.3	16.4	25	13.9	32.7	27.9	39	22.4	20.0	18.2	38	8.5	50.3	3.0
KU873983 Aelia americana	32.3	18.4	32.3	17.0	23	15.8	31.5	29.7	42	25.5	12.7	20.0	32	13.9	52.7	1.2
GQ292101 Eurystylus coelestialium	33.3	15.2	34.3	17.2	23	14.5	33.9	28.5	42	24.8	13.9	18.8	35	6.1	55.2	4.2
KY508434 Pentatoma sp.	34.5	15.8	33.7	16.0	25	13.3	33.9	27.3	42	25.5	12.7	20.0	36	8.5	54.5	.6
KP753723 Cletus punctiger	34.9	18.4	29.9	16.8	24	17.6	31.5	26.7	43	25.5	13.3	18.2	38	12.1	44.8	5.5
JQ888560 Castolus ferox	33.7	17.6	31.1	17.6	22	15.8	32.7	29.1	42	25.5	12.7	20.0	37	11.5	47.9	3.6
KM022889 Neottiglossa pusilla	34.3	16.2	33.9	15.6	25	13.9	35.2	25.5	43	24.2	13.3	19.4	35	10.3	53.3	1.8
KM022520 Rhaphigaster nebulosa	35.4	16.0	31.9	16.8	25	13.9	32.1	28.5	42	24.8	13.3	19.4	38	9.1	50.3	2.4
KU601554 Chlorochroa persimilis	31.3	18.8	31.3	18.6	23	17.0	30.3	29.7	42	24.8	13.3	20.0	29	14.5	50.3	6.1
KP753788 Cletus punctiger	35.2	18.2	29.7	17.0	24	17.6	31.5	26.7	43	25.5	13.3	18.2	38	11.5	44.2	6.1
KJ541549 Carpocoris fuscispinus	31.5	17.8	32.7	18.0	22	16.4	32.7	29.1	43	24.2	13.3	19.4	30	12.7	52.1	5.5

Table 5.6: Percentage of evolutionary divergence of *Agonoscelis nubilis* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH590760	*Agonoscelis nubilis (*Kerala)	
2.	MG838334	*Agonoscelis* sp. (Maharashtra)	0.36%
3.	KY492355	*Agonoscelis nubilis*	0.72%
4.	KY508434	*Pentatoma* sp.	17.14%
5.	KP753723	*Cletus punctiger*	17.58%
6.	KP753788	*Cletus punctiger*	17.58%
7.	LC099273	*Acanthosoma forficula*	18.66%
8.	GQ292101	*Eurystylus coelestialium*	19.12%
9.	KU873983	*Aelia Americana*	19.34%
10.	KM022889	*Neottiglossa pusilla*	20.08%
11.	KY206850	*Bagrada qinlingensis*	20.70%
12.	KJ541549	*Carpocoris fuscispinus*	20.73%
13.	KM022520	*Rhaphigaster nebulosa*	21.69%
14.	JQ888560	*Castolus ferox*	22.02%
15.	KU601554	*Chlorochroa persimilis*	22.06%

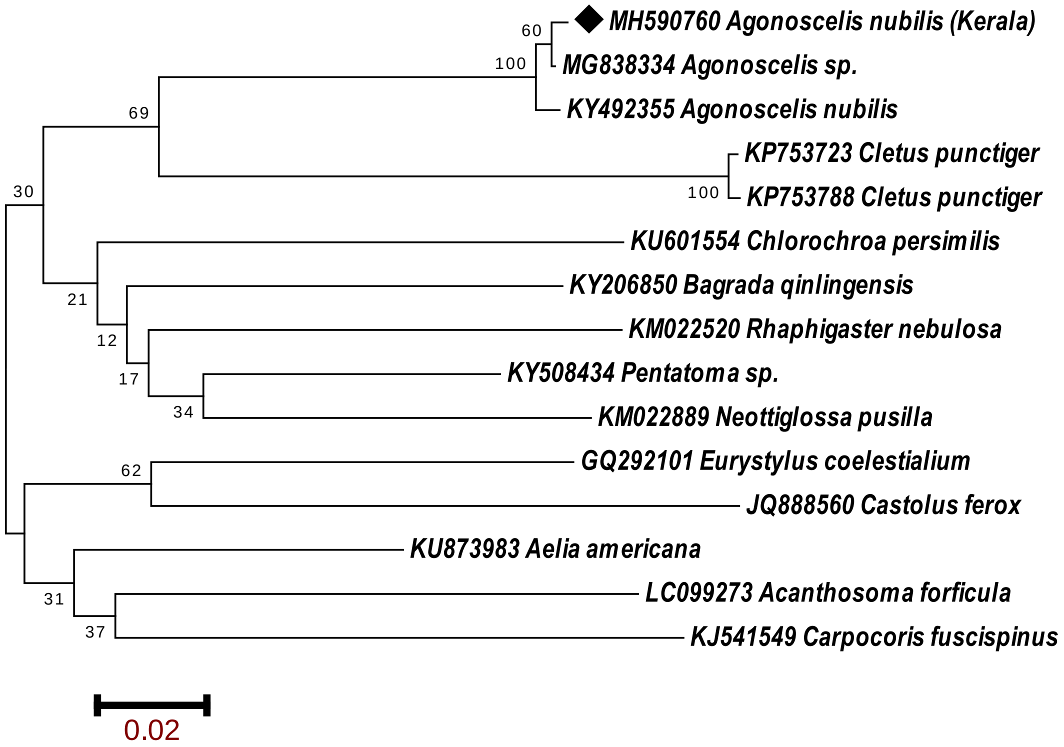


Figure 5.18: Phylogenetic relationship of *Agonoscelis nubilis* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 99.7% sequence similarity to the same genus reported from Maharashtra (MG838334). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Agonoscelis nubilis* showed bias to nucleotide AT, with following composition of nucleotides T = 32.1%, C = 20.0%, A = 30.9% and G = 17.0%. This greater AT content (63.0%) over GC content (37.0%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Agonoscelis nubilis* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 0.36% to 22.06% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has no divergence (0.36%) with those from Maharashtra while 22.06% from USA (Table 5.6). On the basis of the data observed this species may be rooted from those found in USA which diverted into different clades due to geographical variation. Result thus concluded that this species doesn't have any major changes in India while slightly changes from those reported from USA during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Agonoscelis nubilis* with related species. *Agonoscelis nubilis* isolated from Kerala (MH590760) showed 0.36% divergence with *Agonoscelis* sp. (MG838334) from Maharashtra and 22.06% divergence with *Chlorochroa persimilis* (KU601554) from USA. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Agonoscelis nubilis* isolated from Kerala. Closest relative of *Agonoscelis nubilis* is *Agonoscelis* sp. of Maharashtra *and Agonoscelis nubilis* of Karnataka represented within the same clade.

4. ***Carbula scutellata*** (Distant, 1887)

Specimen details:

Voucher specimen	:	CUCS – 11 – A1
Date of collection	:	14-Jan-2016
Locality	:	Kannur: Payyanur
Lat- Lon	:	12.1051° N, 75.2058° E
GenBank accession	:	MH590777

Description and Distribution

Fig.5.19: *Carbula Scuttellata*

The head and dorsum of *Carbula scutellata* shinning greenish brown, mottled with presence of ochraceous spots, antennae pale white mainly first three segments, presence of triangular pale white area on scutellar disk (Figure. 5.19). Peritreme short, anterolateral margins of pronotum crenulated and posterior margin of third, fouth and fifth visible, abdominal sternite with a row of hairs, humeral angles of pronotum lobe like, triangular scutellum, head is flattened dorsally (Knight, 1917). Distributed in India, Thailand and Taiwan.

Damage

Adults pierce the plant tissue and suck the juice (Knight, 1941).

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Carbula scutellata* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 593 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 5.20 – 5.23 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 5.7.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Carbula scutellata* voucher collected during the present study (Table 5.7). It showed bias to nucleotide AT, with nucleotide composition with T = 33.3%, C = 19.9%, A = 29.4% and G = 17.3%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 5.8 and Figure 5.24 respectively.

>*Carbula scutellata* CDS-2018/ 593 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUCS-11-A1

> *Carbula scutellata*

```
GCTGGAATAGTGGGGTCCGCAATAAGCCTAATTATTCGAATTGAACTAGGGCAACCTGGA
AGATTTATTGGAGATGACCAAATCTACAATGTAGTAGTCACAGCCCACGCTTTTGTAATA
ATTTTCTTCATAGTTATGCCAATCATAATTGGTGGATTCGGCAATTGACTAGTTCCTTTA
ATAATTGGAGCCCCTGATATAGCATTCCCCCGTATAAATAATATAAGATTCTGATTATTA
CCCCCCTCACTAACACTATTAATAGTAAGTAGACTTGCTGAATCGGGAGCCGGAACTGGA
TGAACTGTATATCCCCCTCTATCAAGAAACATCTCCCATAGAGGGGCATCTGTTGATTTA
GCTATTTTCTCATTACACTTAGCAGGAGTATCATCCATTTTAGGTGCAGTAAATTTTATT
TCAACTATCATCAATATGCGACCAGAAGGGATAACTCCTGAACGAATTCCATTATTTGTG
TGATCCGTAGGTATTACTGCTCTTTTATTATTATTATCCTTGCCAGTATTAGCTGGTGCA
ATTACAATATTATTAACTGACCGAAATTTCAATACCTCTTTCTTTGATCCTTC
```

Figure 5.20: The partial DNA sequence of the mitochondrial COI gene of *Carbula scutellata*

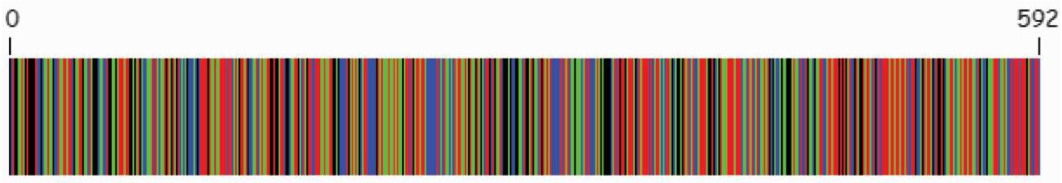


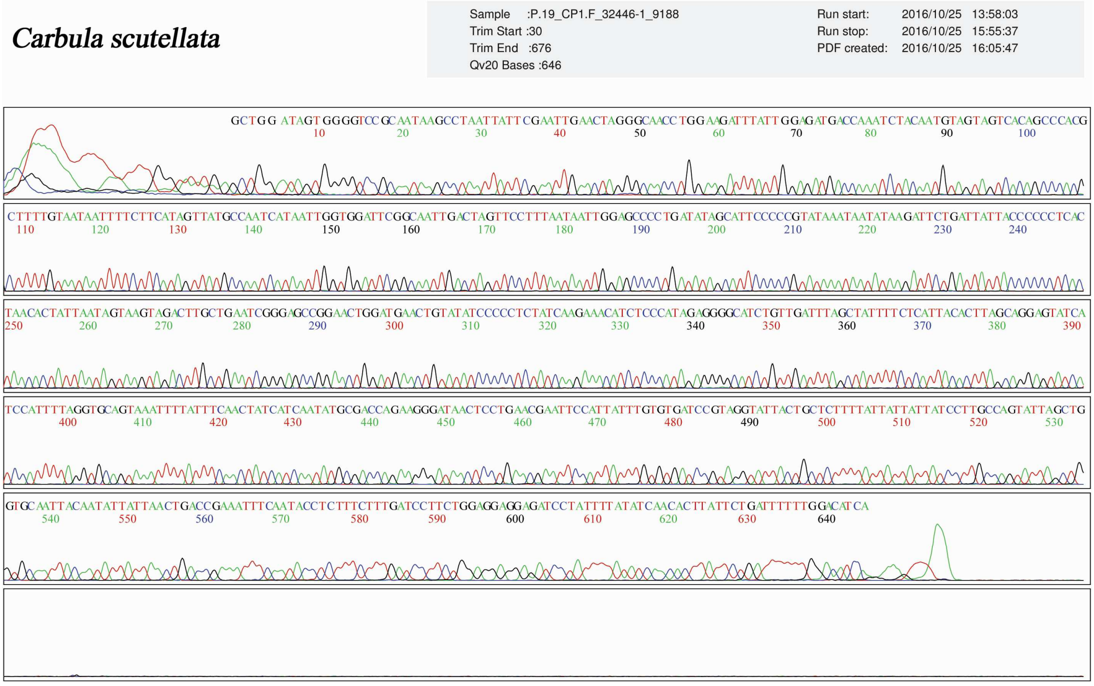
Figure 5.21: Molecular barcode of the mitochondrial COI gene of *Carbula scutellata*

> *Carbula scutellata* / 195AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUCS-11-A1

> *Carbula scutellata*

```
MVGSAMSLIIRIELGQPGSFIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMI
GAPDMAFPRMNNMSFWLLPPSLTLLMVSSLAESGAGTGWTVYPPLSSNISHSGASVDLAI
FSLHLAGVSSILGAVNFISTIINMRPEGMTPERIPLFVWSVGITALLLLLSLPVLAGAIT
MLLTDRNFNTSFFDP
```

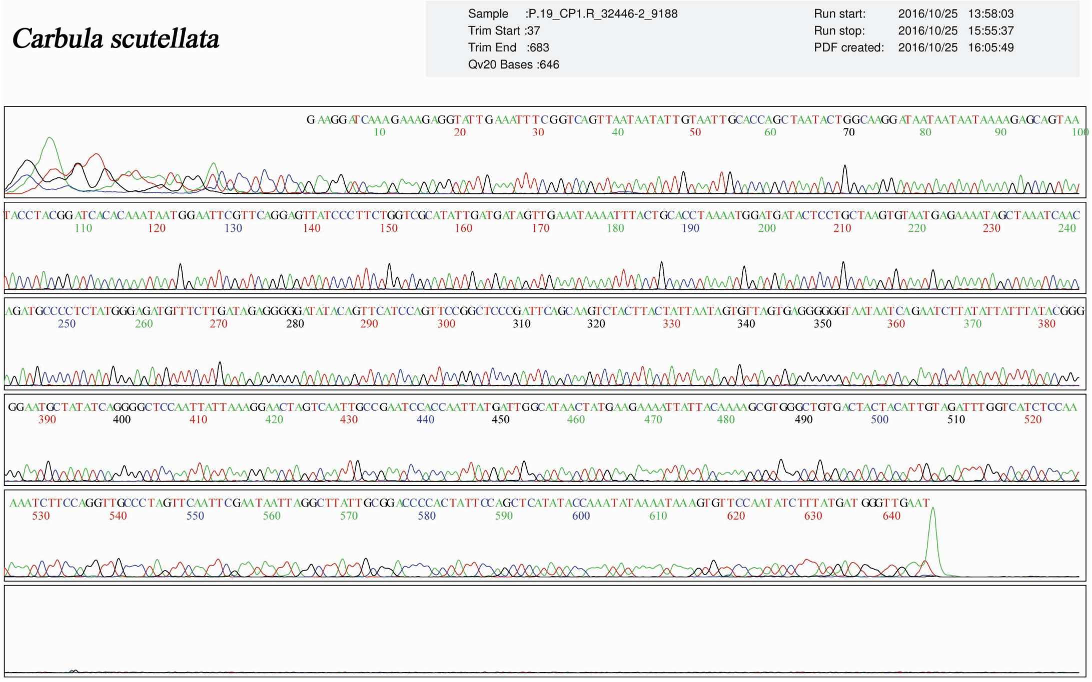
Figure 5.22: The translation product of the mitochondrial COI gene of *Carbula scutellata*



Carbula scutellata

Sample :P.19_CP1.F_32446-1_9188
Trim Start :30
Trim End :676
Qv20 Bases :646

Run start: 2016/10/25 13:58:03
Run stop: 2016/10/25 15:55:37
PDF created: 2016/10/25 16:05:47



Carbula scutellata

Sample :P.19_CP1.R_32446-2_9188
Trim Start :37
Trim End :683
Qv20 Bases :646

Run start: 2016/10/25 13:58:03
Run stop: 2016/10/25 15:55:37
PDF created: 2016/10/25 16:05:49

Figure 5.23: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Carbula scutellata* using Forward and Reverse primers.

Table 5.7: Comparison of Nucleotide frequencies of COI gene sequence of *Carbula scutellata* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH590777 Carbula scutellata (Kerala)	33.3	19.9	29.4	17.3	32	17.9	45.4	5.1	25	15.8	29.6	29.6	43	26.0	13.3	17.3
GU247473 Carbula scutellata	30.4	20.1	31.1	18.4	35	14.3	42.3	8.7	19	17.4	36.4	27.2	38	28.4	14.7	19.3
MG676483 Carbula insocia	34.0	18.2	31.8	16.0	32	13.8	53.6	.5	26	15.3	29.6	29.6	44	25.5	12.2	17.9
KR032364 Chlorochroa uhleri	34.0	17.9	31.6	16.5	32	11.7	53.6	2.6	26	16.3	29.1	29.1	44	25.5	12.2	17.9
LC209910 Eysarcoris guttigerus	35.5	16.5	32.1	15.8	37	8.7	52.6	2.0	28	14.3	30.6	27.6	42	26.5	13.3	17.9
KR037941 Chlorochroa granulosa	34.0	17.9	31.5	16.7	32	11.7	53.1	3.1	26	16.3	29.1	29.1	44	25.5	12.2	17.9
KR582065 Chlorochroa ligata	34.7	17.0	32.1	16.2	32	11.2	55.1	1.5	28	14.3	29.1	29.1	44	25.5	12.2	17.9
LC209923 Eysarcoris annamita	34.0	17.2	32.5	16.3	32	11.7	54.6	2.0	28	13.8	29.6	29.1	43	26.0	13.3	17.9
LC209891 Eysarcoris guttigerus	35.4	16.8	32.1	15.6	36	10.2	52.6	1.5	28	14.3	30.6	27.6	43	26.0	13.3	17.9
KJ866504 Carbula biguttata	34.5	16.5	32.7	16.3	34	9.2	55.6	1.5	26	14.8	30.1	29.6	44	25.5	12.2	17.9
JX231168 Epicephala mirivalvata	34.7	16.5	32.1	16.7	31	12.2	54.6	2.0	28	12.8	29.6	29.6	45	24.5	12.2	18.4
KY492344 Tolumnia basalis	36.2	15.8	31.6	16.3	34	10.2	53.6	2.0	30	11.7	29.6	29.1	45	25.5	11.7	17.9
KU874601 Chlorochroa rossiana	34.9	17.0	31.8	16.3	33	11.2	53.6	2.6	28	14.3	29.6	28.6	44	25.5	12.2	17.9

Table 5.8: Percentage of evolutionary divergence of *Carbula scutellata* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH590777	*Carbula scutellata* (Kerala)	
2.	MG676483	*Carbula insocia* (Punjab)	11.87%
3.	LC209910	*Eysarcoris guttigerus*	22.42%
4.	LC209923	*Eysarcoris annamita*	22.99%
5.	JX231168	*Epicephala mirivalvata*	23.49%
6.	LC209891	*Eysarcoris guttigerus*	23.65%
7.	KY492344	*Tolumnia basalis*	24.43%
8.	KJ866504	*Carbula biguttata*	25.70%
9.	GU247473	*Carbula scutellata*	25.83%
10.	KR032364	*Chlorochroa uhleri*	26.08%
11.	KR037941	*Chlorochroa granulose*	26.51%
12.	KR582065	*Chlorochroa ligata*	26.55%
13.	KU874601	*Chlorochroa rossiana*	27.05%

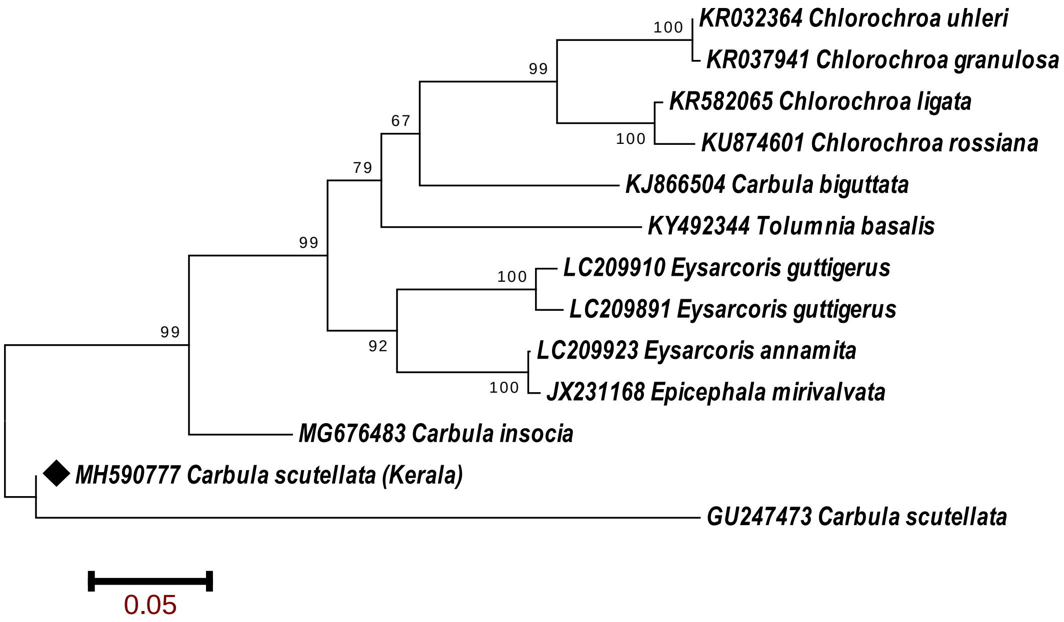


Figure 5.24: Phylogenetic relationship of *Carbula scutellata* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 88.2% sequence similarity to the same genus reported from Punjab (MG676483). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Carbula scutellata* showed bias to nucleotide AT, with following composition of nucleotides T = 33.3%, C = 19.9%, A = 29.4% and G = 17.3%. This greater AT content (62.7%) over GC content (37.2%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Carbula scutellata* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 11.87% to 27.05% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (11.87%) with those from Punjab while 27.05% from USA (Table 5.8). On the basis of the data observed this species may be rooted from those found in USA which diverted into different clades due to geographical variation. Result thus concluded that this species doesn't have any major changes in India while slightly changes from those reported from USA during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Carbula scutellata* with related species. *Carbula scutellata* isolated from Kerala (MH590777) showed 11.87% divergence with (MG676483) *Carbula insocia* from Punjab and 27.05% divergence with *Chlorochroa rossiana* (KU874601) from USA. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Carbula scutellata* isolated from

Kerala. Closest relative of *Carbula scutellata* is *Carbula insocia* from Punjab represented within the same clade.

5. ***Halyomorpha halys*** (*Stahl*, 1855)

Specimen details:

Voucher specimen : CUHH – 02 – A1
Date of collection : 28- Mar-2016
Locality : Eranakulam: Aluva
Lat- Lon : 10.1076º N, 76.3457º E
GenBank accession : MH674109

Description and Distribution

The adult *Halyomorpha halys* are larger than other native stink bug species, ranging from 12 to 17 mm in length (Figure. 5.25). The base colour is a mixture of brown, dark red and black on the dorsal surface, with a beige or cream colored ventral surface punctuated with metallic green markings on the ventral thorax. Key identification features of the adult include white bands on antennae and legs, green arrows representing the banding on the antennae, absence of humeral spines and alternating dark and light bands on the margin of the abdomen (Aldrich et al., 2009). The brown marmorated stink bug, *Halyomorpha halys* is native to eastern Asia, including China, Taiwan, Korea, and Japan (Lee et al., 2013).

Fig. 5.25: *Halyomorha halys*

Damage

Halyomorpha halys is highly polyphagous in both its native and invaded ranges. *H. halys* , both nymph and adult causes direct damage to plants, importantly to many commercial crops, through its feeding activities and can cause indirect damage through the transmission of plant diseases including Paulownia witches' broom (Joseph et al., 2016).

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Halyomorpha halys* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 596 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 5.26 – 5.29 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 5.9.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Halyomorpha halys* voucher collected during the present study (Table 5.9). It showed bias to nucleotide AT, with nucleotide composition with T = 33.3%, C = 19.9%, A = 29.4% and G = 17.3%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 5.10 and Figure 5.25 respectively.

>*Halyomorpha halys* CDS-2018/ 596 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUHH-02-A1

> *Halyomorpha halys*

```
ATAGTAGGGTCATCATTAAGATGAATTATCCGGATTGAATTAGGTCAACCAGGACCCTTC
ATTAACGATGACCAAATTTATAATGTAGTAGTAACAGCTCATGCTTTTATTATAATTTTC
TTTATAGTTATGCCAATCATAATTGGAGGATTTGGTAATTGATTAGTACCCTTAATAATT
GGAGCCCCTGATATAGCATTCCCCCGACTTAACAATATAAGATTTTGACTTTTACCTCCT
TCATTAACATTATTAATCACTAGAAGTATAGTTGAAAAGGGAGCTGGAACAGGATGAACA
GTATACCCGCCTTTATCAAGCAACATTTCCCACAGAGGAGCCTCAGTAGACCTAGCTATC
TTTTCATTACACTTAGCAGGAGTATCATCAATCTTGGGAGCAATTAACTTCATCTCAACC
ATTTCAAATATACGACCAAGAGGAATAATCCCAGAACGAATTCCTTTATTCGTATGATCA
GTAGGAATTACCGCAATTCTTCTACTTACATCACTACCAGTACTAGCAGGAGCTATTACA
ATACTTTTAACTGATCGAAACCTAAATACATCATTCTTTGATCCAACAGGAGGAGG
```

Figure 5.26: The partial DNA sequence of the mitochondrial COI gene of *Halyomorpha halys*

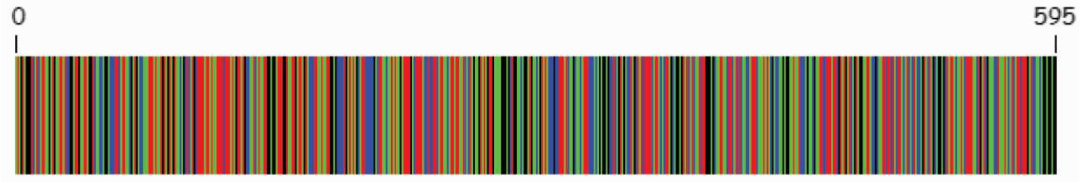


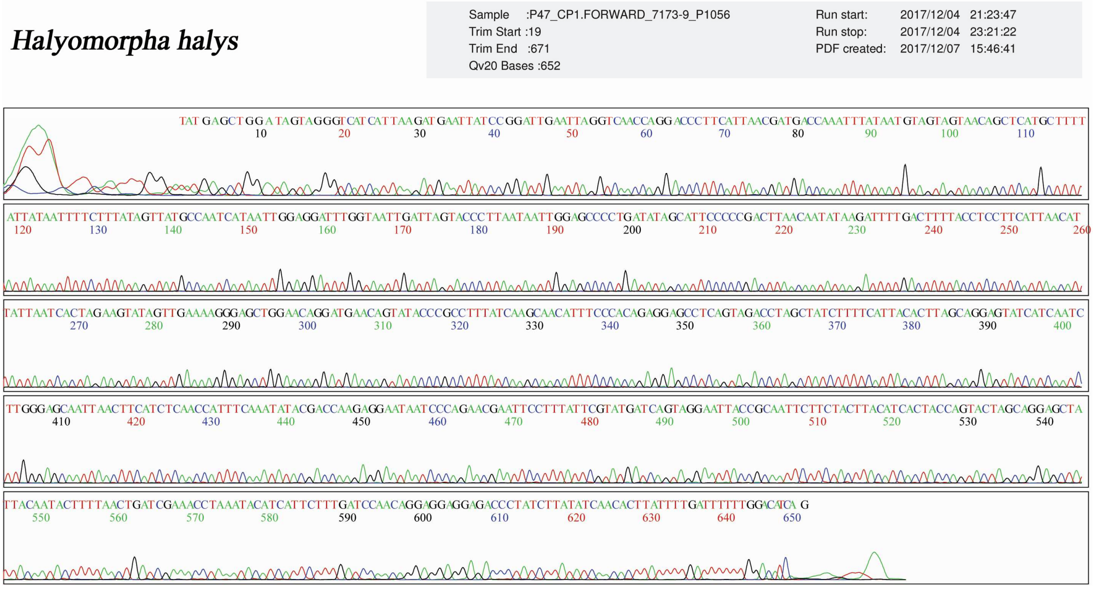
Figure 5.27: Molecular barcode of the mitochondrial COI gene of *Halyomorpha halys*

> *Halyomorpha halys* / 198AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUHH-02-A1

> *Halyomorpha halys*

```
MVGSSLSWIIRIELGQPGPFINDDQIYNVVVTAHAFIMIFFMVMPIMIGGFGNWLVPLMI
GAPDMAFPRLNNMSFWLLPPSLTLLITSSMVEKGAGTGWTVYPPLSSNISHSGASVDLAI
FSLHLAGVSSILGAINFISTISNMRPSGMIPERIPLFVWSVGITAILLLTSLPVLAGAIT
MLLTDRNLNTSFFDPTGG
```

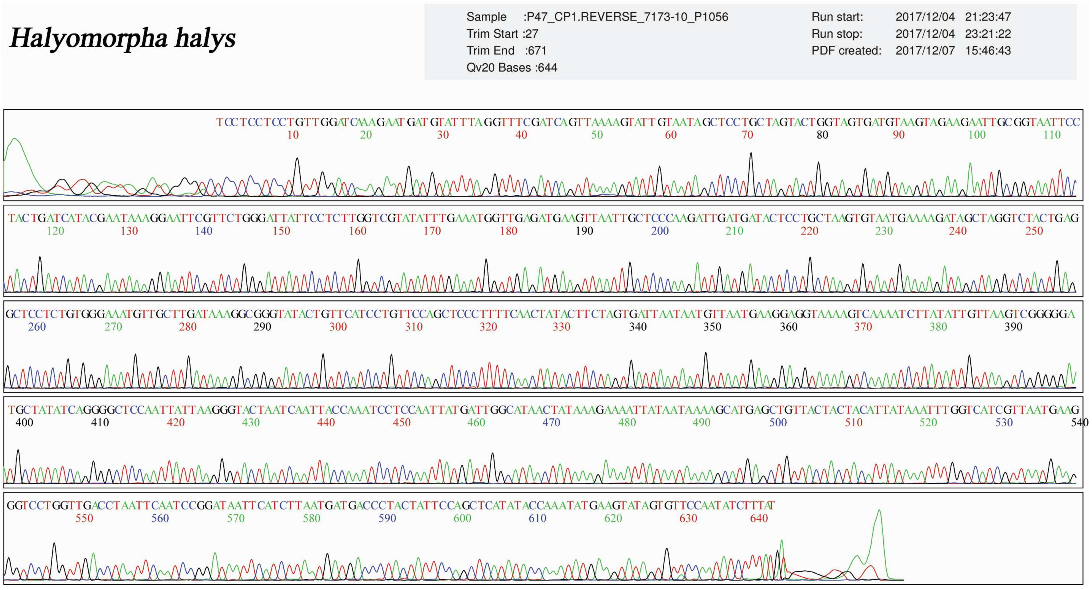
Figure 5.28: The translation product of the mitochondrial COI gene of *Halyomorpha halys*



Halyomorpha halys

Sample :P47_CP1.FORWARD_7173-9_P1056
Trim Start :19
Trim End :671
Qv20 Bases :652

Run start: 2017/12/04 21:23:47
Run stop: 2017/12/04 23:21:22
PDF created: 2017/12/07 15:46:41



Halyomorpha halys

Sample :P47_CP1.REVERSE_7173-10_P1056
Trim Start :27
Trim End :671
Qv20 Bases :644

Run start: 2017/12/04 21:23:47
Run stop: 2017/12/04 23:21:22
PDF created: 2017/12/07 15:46:43

Figure 5.29: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Halyomorpha halys* using Forward and Reverse primers.

Table 5.9: Comparison of Nucleotide frequencies of COI gene sequence of *Halyomorpha halys* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH674109 Halyomorpha halys	30.9	20.1	33.1	15.9	24	17.6	32.2	26.6	42	26.6	13.6	18.1	27	16.2	53.5	3.0
HQ105989 Neoneides muticus	36.6	14.3	33.9	15.3	29	14.1	31.7	25.6	44	24.6	13.6	18.1	37	4.0	56.6	2.0
GQ292219 Acanthocoris sordidus	33.1	18.0	31.5	17.4	27	15.1	32.2	26.1	43	26.1	12.6	18.6	30	12.6	50.0	7.6
KR036557 Drymus unus	30.9	18.3	36.6	14.3	23	17.6	35.2	24.6	44	25.3	13.6	16.7	26	12.1	60.8	1.5
KM021994 Phytocoris dimidiatus	33.1	17.1	34.6	15.1	28	13.6	31.7	27.1	44	25.3	13.1	17.2	27	12.6	59.1	1.0
KU242572 Macrocheraia grandis	31.2	18.6	35.1	15.1	23	19.1	31.7	26.6	44	26.1	12.1	18.1	27	10.6	61.6	.5
MF673706 Pentatomidae sp.	32.4	19.3	32.0	16.3	27	14.6	30.7	28.1	44	25.8	12.1	18.2	27	17.6	53.3	2.5
KY835820 Coreidae sp.	36.7	15.4	32.7	15.1	29	15.1	29.6	26.6	45	25.3	12.1	17.7	37	6.0	56.3	1.0
KX523454 Probergrothius sexpunctatus	30.4	18.6	35.2	15.8	22	19.1	32.7	26.1	43	25.8	12.6	18.7	26	11.1	60.3	2.5
KR041581 Orius tristicolor	33.1	16.4	34.9	15.6	25	16.6	31.2	27.6	44	25.8	12.6	17.7	31	7.0	60.8	1.5
KM022490 Berytinus crassipes	33.2	17.4	33.7	15.6	26	16.6	30.7	26.6	44	24.2	13.1	18.2	29	11.6	57.3	2.0
KM021972 Neides tipularius	37.6	13.6	32.9	15.9	30	12.6	31.7	26.1	44	25.8	11.6	18.2	39	2.5	55.3	3.5
KR579718 Emblethis vicarius	28.9	22.5	33.1	15.6	23	19.1	32.2	26.1	44	26.3	12.6	17.2	20	22.1	54.3	3.5
KR035951 Tropidosteptes amoenus	32.4	16.8	35.9	14.9	26	14.6	33.2	26.6	45	25.3	12.6	17.2	27	10.6	61.8	1.0

Table 5.10: Percentage of evolutionary divergence of *Halyomorpha halys* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH674109	*Halyomorpha halys* (Kerala)	
2.	KR036557	*Drymus unus* (Canada)	26.60%
3.	GQ292219	*Acanthocoris sordidus*	26.65%
4.	KM021994	*Phytocoris dimidiatus*	27.32%
5.	HQ105989	*Neoneides muticus*	27.97%
6.	KR035951	*Tropidosteptes amoenus*	29.04%
7.	MF673706	*Pentatomidae* sp.	30.30%
8.	KM022490	*Berytinus crassipes*	30.42%
9.	KY835820	*Coreidae* sp.	30.62%
10.	KR041581	*Orius tristicolor*	30.69%
11.	KU242572	*Macrocheraia grandis*	31.29%
12.	KX523454	*Probergrothius sexpunctatus*	31.49%
13.	KM021972	*Neides tipularius*	31.49%
14.	KR579718	*Emblethis vicarious*	31.62%

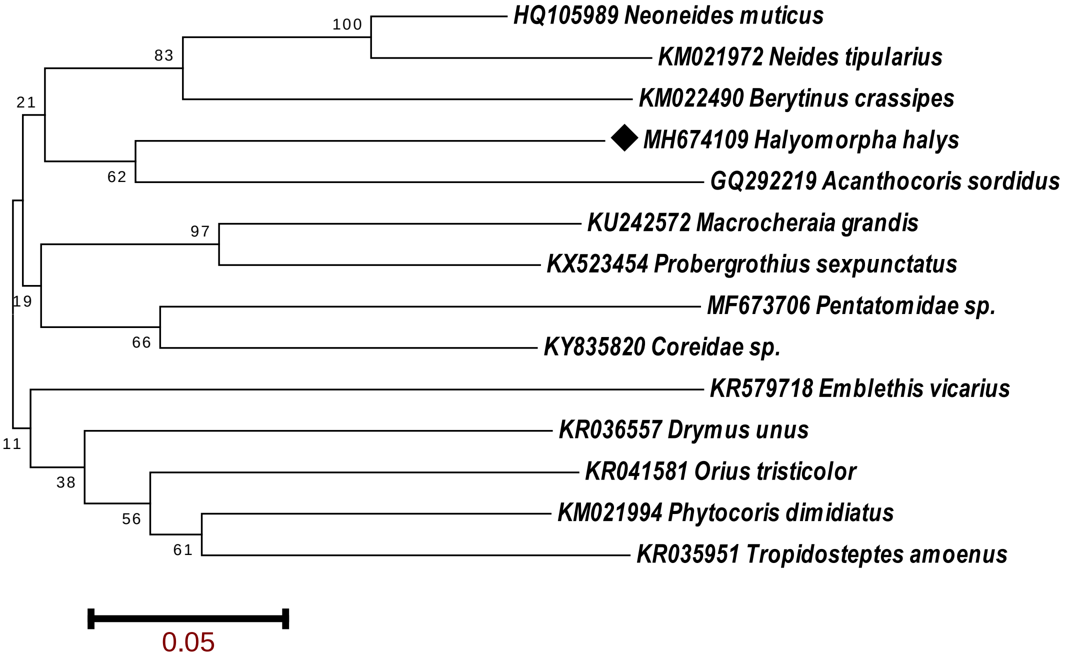


Figure 5.30: Phylogenetic relationship of *Halyomorpha halys* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 73.4% sequence similarity to the related genus reported from Canada (KR036557). Even though this species has been found in various geographically isolated areas, their sequence shows wide variation.

The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Halyomorpha halys* showed bias to nucleotide AT, with following composition of nucleotides T = 30.9%, C = 20.1%, A = 33.1% and G = 15.9%. This greater AT content (64.0%) over GC content (36.0%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Halyomorpha halys* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Even though the COI sequences has been reported from different geographical locations, it showed only 26.60% to 31.62% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (26.60%) with those from Canada while 31.62% from Canada (Table 5.10). On the basis of the data observed this species may be rooted from those found in Canada which diverted into different clades due to geographical variation. Result thus concluded that this species doesn't have any major changes in India while slightly changes from those reported from Canada during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Halyomorpha halys* with related species. *Halyomorpha halys* isolated from Kerala (MH674109) showed 26.60% divergence with *Drymus unus* (KR036557) from Canada and 31.62% divergence with *Emblethis vicarious* (KR579718) from Canada. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Halyomorpha halys* isolated from Kerala. Closest relative of *Halyomorpha halys* is *Acanthocoris sordidus* from Korea represented within the same clade.

6. ***Zicrona caerulea*** (Linnaeus, 1758)

Specimen details:

Voucher specimen : CUZC – 01 – A1
Date of collection : 03- Feb-2016
Locality : Malappuram: Parappanangadi
Lat- Lon : 11.0605º N, 75.8508º E
GenBank accession : KU201287

Description and Distribution

Zicrona caerulea, belonging to are commonly named as Blue shield-bug (Figure. 5.31). Adult size range about 5–8 mm (0.20–0.31 in). The dorsal body is uniformly metallic blue-green and in the immatures the abdomen is red with black markings. The distinguished features include absence of subapical spine on forefemora. This species is present in Eurasia and in North America.

Fig. 5.31: *Zicrona caerulea*

Damage

These bugs are known to be useful predators of leaf beetles of the genus *Altica*, but it also feeds upon plants. The adult feeds on the leaves and stem of the plant (Lee et al., 2013).

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Zicrona caerulea* collected has been amplified using the primer BUG (Table 3.1). The PCR amplification yielded 490 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 5.32 – 5.35 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 5.11.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Zicrona caerulea* voucher collected during the present study (Table 5.11). It showed bias to nucleotide AT, with nucleotide composition with T = 38.0%, C = 16.7%, A = 29.2% and G = 16.1%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 5.12 and Figure 5.36 respectively.

>*Zicrona caerulea* CDS-2016/ 490 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUZC-01-A1

> *Zicrona caerulea*

```
TTTATTGGAGATGACCAAATTTATAATGTGGTAGTTACAGCTCATGCCTTTGTTATAATT
TTCTTTATAGTTATACCAATTATAATTGGAGGATTTGGGAATTGACTAGTTCCTTTAATA
ATTGGAGCCCCTGATATAGCATTTCCTCGAATAAATAATATAAGATTTTGACTGTTACCC
CCTTCATTAACACTCCTAATAATTAGTAGATTAACAGAAGCAGGGGCCGGAACTGGGTGA
ACAGTTTATCCTCCTTTATCTAGTAATCTTTCCCATAGAGGAGCTTCAGTTGATTTAGCT
ATTTTTTCATTACATTTAGCAGGAGTATCTTCTATTTTAGGAGCTGTAAATTTCATTTCT
ACGATTATTAATATACGACCAGCAGGAATAATTCCTGAACGAATTCCTTTATTCGTTTGA
TCAGTTGGAATTACAGCATTATTATTACTTCTTTCATTACCTGTACTAGCAGGAGCTATT
ACTATACTAT
```

Figure 5.32: The partial DNA sequence of the mitochondrial COI gene of *Zicrona caerulea*

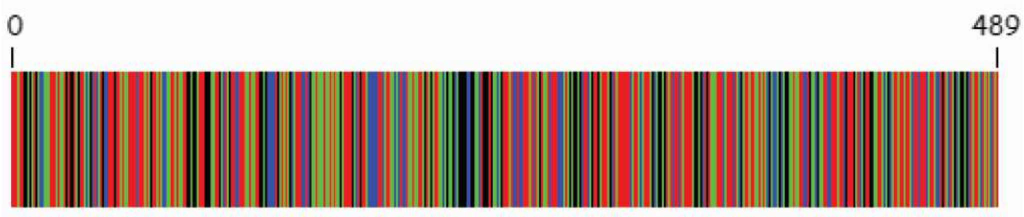


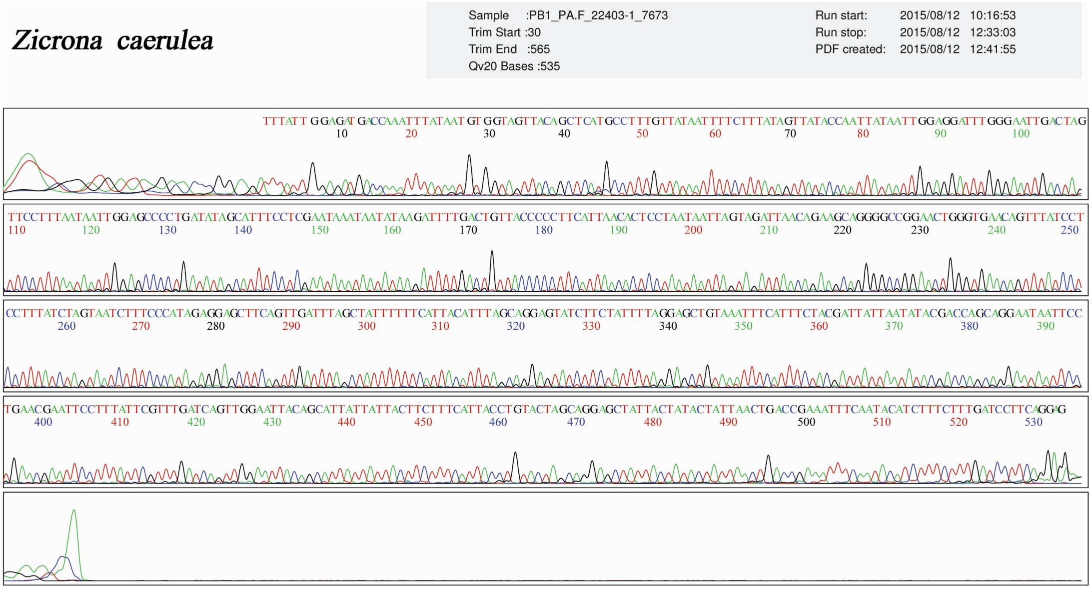
Figure 5.33: Molecular barcode of the mitochondrial COI gene of *Zicrona caerulea*

> *Zicrona caerulea* / 145AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUZC-01-A1

> *Zicrona caerulea*

```
MIFFMVMPIMIGGFGNWLVPLMIGAPDMAFPRMNNMSFWLLPPSLTLLMISSLTEAGAGT
GWTVYPPLSSNLSHSGASVDLAIFSLHLAGVSSILGAVNFISTIINMRPAGMIPERIPLF
VWSVGITALLLLLSLPVLAGAITML
```

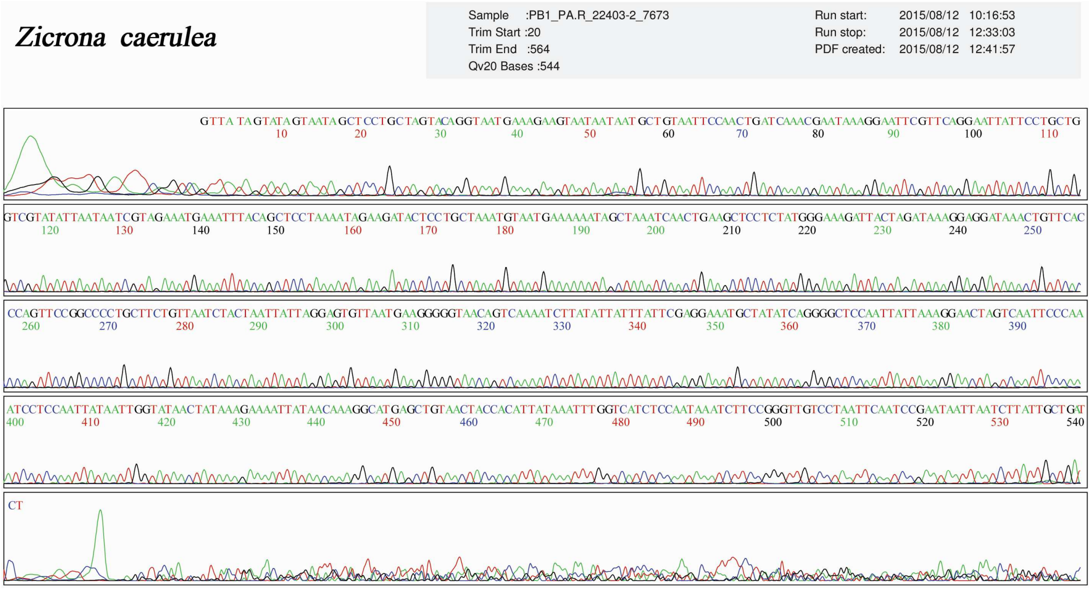
Figure 5.34: The translation product of the mitochondrial COI gene of *Zicrona caerulea*



Zicrona caerulea

Sample :PB1_PA.F_22403-1_7673
Trim Start :30
Trim End :565
Qv20 Bases :535

Run start: 2015/08/12 10:16:53
Run stop: 2015/08/12 12:33:03
PDF created: 2015/08/12 12:41:55



Zicrona caerulea

Sample :PB1_PA.R_22403-2_7673
Trim Start :20
Trim End :564
Qv20 Bases :544

Run start: 2015/08/12 10:16:53
Run stop: 2015/08/12 12:33:03
PDF created: 2015/08/12 12:41:57

Figure 5.35: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Zicrona caerulea* using Forward and Reverse primers.

Table 5.11: Comparison of Nucleotide frequencies of COI gene sequence of *Zicrona caerulea* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
KU201287 Zicrona caerulea (Kerala)	38.0	16.7	29.2	16.1	25	17.1	29.3	28.7	45	27.0	11.7	16.0	44	6.1	46.6	3.7
GQ292256 Zicrona caerulea	36.3	15.7	30.4	17.6	23	15.9	31.1	29.9	43	24.5	12.9	19.6	43	6.7	47.2	3.1
KY063087 Arma chinensis	35.1	15.7	31.0	18.2	25	14.0	29.3	31.7	42	23.9	14.7	19.0	38	9.2	49.1	3.7
KC155929 Arma custos	35.1	15.7	31.2	18.0	25	14.0	29.9	31.1	42	23.3	15.3	19.0	38	9.8	48.5	3.7
KY063105 Arma maculata	35.5	14.9	31.6	18.0	26	12.8	30.5	30.5	42	23.9	15.3	19.0	39	8.0	49.1	4.3
KY063102 Arma ferruginea	35.5	15.1	31.8	17.6	26	13.4	30.5	30.5	42	23.9	15.3	18.4	39	8.0	49.7	3.7
KY063104 Arma koreana	34.7	16.1	33.1	16.1	27	16.5	29.9	26.8	41	21.5	19.6	17.8	36	10.4	49.7	3.7
KM022180 Rhacognathus punctatus	37.1	14.5	31.6	16.7	25	12.3	31.3	31.3	42	25.8	13.5	19.0	45	5.5	50.0	.0
KC155930 Arma tubercula	35.1	15.9	31.0	18.0	24	15.2	29.9	31.1	42	23.3	15.3	19.0	39	9.2	47.9	3.7
KY206884 Eurydema maracandica	37.3	14.1	33.3	15.3	31	14.6	28.7	25.6	41	21.5	19.6	17.8	40	6.1	51.5	2.5
KR346365 Podisus brevispinus	36.3	16.3	29.2	18.2	26	13.5	30.1	30.7	43	24.5	13.5	19.0	40	11.0	43.9	4.9
KR816890 Thyrinteina leucoceraea	33.5	18.8	30.4	17.3	21	17.2	30.1	31.3	43	24.5	12.9	19.6	36	14.6	48.2	1.2
KR042272 Podisus brevispinus	36.1	16.5	28.8	18.6	26	13.5	30.1	30.7	43	24.5	13.5	19.0	40	11.6	42.7	6.1
KY206854 Eurydema liturifera	34.5	14.5	35.3	15.7	26	15.2	34.8	24.4	43	19.6	20.9	16.6	35	8.6	50.3	6.1
KX262839 Eurydema ventralis	35.3	15.5	33.9	15.3	27	17.7	29.9	25.6	41	21.6	19.8	17.9	38	7.3	51.8	2.4

Table 5.12: Percentage of evolutionary divergence of *Zicrona caerulea* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	KU201287	*Zicrona caerulea* (Kerala)	
2.	GQ292256	*Zicrona caerulea* (Korea)	1.80%
3.	KY063087	*Arma chinensis*	17.09%
4.	KC155929	*Arma custos*	17.09%
5.	KX262839	*Eurydema ventralis*	18.37%
6.	KR042272	*Podisus brevispinus*	18.65%
7.	KY206854	*Eurydema liturifera*	19.19%
8.	KR346365	*Podisus brevispinus*	19.36%
9.	KY063104	*Arma koreana*	19.45%
10.	KM022180	*Rhacognathus punctatus*	19.79%
11.	KY063105	*Arma maculate*	20.49%
12.	KY063102	*Arma ferruginea*	20.49%
13.	KC155930	*Arma tubercula*	21.04%
14.	KY206884	*Eurydema maracandica*	21.28%
15.	KR816890	*Thyrinteina leucoceraea*	21.70%

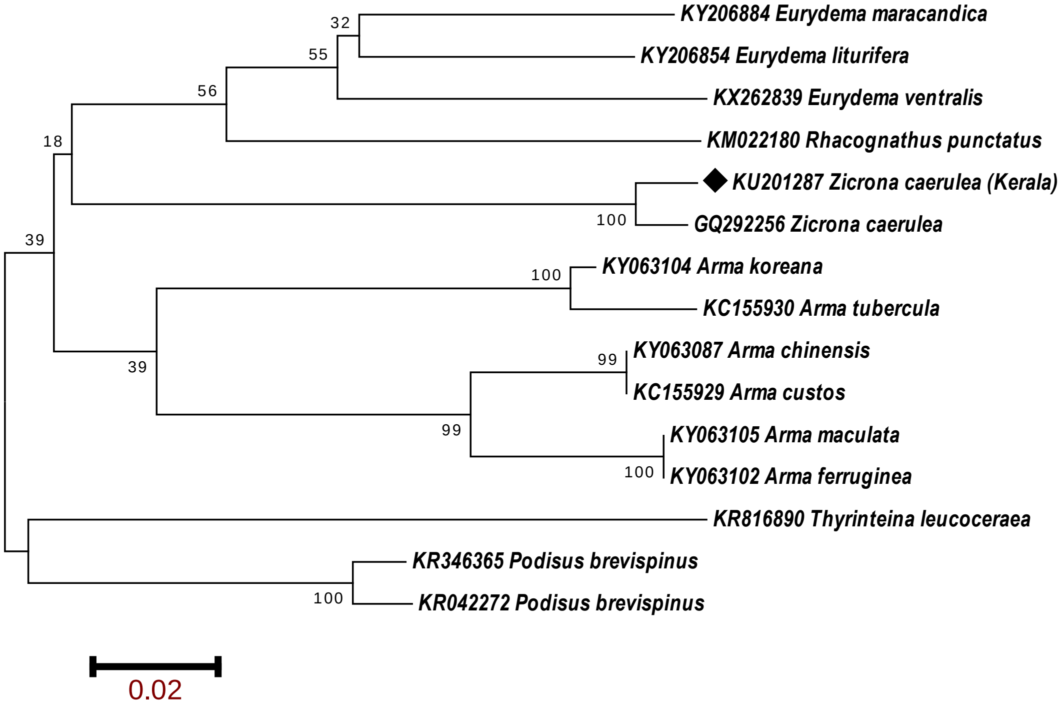


Figure 5.36: Phylogenetic relationship of *Zicrona caerulea* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 98.2% sequence similarity to the same genus reported from Korea (GQ292256). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Zicrona caerulea* showed bias to nucleotide AT, with following composition of nucleotides T = 38.0%, C = 16.7%, A = 29.2% and G = 16.1%. This greater AT content (67.2%) over GC content (32.8%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Zicrona caerulea* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 1.80% to 21.70% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (1.80%) with those from Korea while 21.70% from Netherlands (Table 5.12). On the basis of the data observed this species may be rooted from those found in Netherlands which diverted into different clades due to geographical variation. Result thus concluded that this species doesn't have any major changes in India while slightly changes from those reported from Netherlands during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Zicrona caerulea* with related species. *Zicrona caerulea* isolated from Kerala (KU201287) showed 1.80% divergence with *Zicrona caerulea* (GQ292256) from Korea and 21.70% divergence with *Thyrinteina leucoceraea* (KR816890) from Netherlands. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Zicrona caerulea* isolated from Kerala.

Closest relative of *Zicrona caerulea* is *Zicrona caerule* from Korea represented within the same clade.

7. ***Oncopeltus nigriceps*** (Distant, 1903)

Specimen details:

Voucher specimen : CUON – 01 – A1
Date of collection : 30-Nov-2017
Locality : Kannur: Payyanur
Lat- Lon : 12.1051° N, 75.2058° E
GenBank accession : MH590766

Fig. 5.37: *Oncopeltus nigriceps*

Description and Distribution

According to Harry (1993) the key features of *Oncopeltus nigriceps* include - Head and pronotum pilose (Figure 5.37), body red, with head, pronotum anteriorly, base of scutellum, transverse band across middle of corium and the membrane, black; the last with basal angle and discal spot, white. Pleurites with broad black bands, anteriorly. Scutellum is tumid and swollen with a weak median longitudinal carina, lateral areas not excavated, lacks a subbasal transverse carina, apical corial margin straight and hind wing possessing a subcosta but lacking intervanals The majority of Lygaeidae are lacerate-flush feeders (Schuh and Slater, 1995). Similar to all Hemiptera, they possess piercing–sucking mouth parts and feed through a flexible feeding tube, proboscis. They are distributed in India, China and Japan (Slater, 1972).

Damage

Both the adult and nymph are found usually on the pods of the plants, where they feed on the developing seeds within the pods.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Oncopeltus nigriceps* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 596 bp and 599 bp long products for the specimens obtained from two different locations. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 5.38 – 5.41 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 5.13.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Oncopeltus nigriceps* voucher collected during the present study (Table 5.13). It showed bias to nucleotide AT, with nucleotide composition with T = 34.2%, C = 17.0%, A = 32.8% and G = 15.9%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 5.14 and Figure 5.42 respectively.

> *Oncopeltus nigriceps* CDS-2018/ 599bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUON-01-A1

> *Oncopeltus nigriceps*

```
AGGCATAGTAGGATCATCCATAAGATGAATTATTCGAATTGAATTAGGACAGCCAGGAAT
ATTCATTGGAAATGATCAAATTTATAATGTAATCGTAACAGCCCATGCCTTTATTATAAT
TTTTTTTATAGTAATACCTATTATAATTGGAGGGTTCGGAAATTGGTTAGTACCTTTAAT
AATTGGGGCTCCTGACATGGCATTCCCACGAATAAATAATATAAGATTTTGATTGCTACC
ACCTTCATTAACCTTGTTATTATCTAGAAGCATTACAGAAAGAGGGGCTGGAACAGGATG
AACTGTTTATCCTCCTCTATCAAATAGAATCTTTCATAGAGGGGCTTCTGTAGATATAAC
AATTTTTTCCTTACACCTAGCAGGTATCTCATCAATTCTAGGTGCTATTAATTTTATCTC
AACCATTATTAATATACGACCAACAGGTATGACCTTAGAAAAAATCCCACTATTTGTATG
ATCTGTAGGAATTACAGCCTTACTTCTACTATTATCATTACCAGTATTAGCAGGAGCTAT
TACTATATTATTAACTGATCGAAACTTCAATACTTCATTTTTTGACCCTACAGGGGGAG
```

Figure 5.38: The partial DNA sequence of the mitochondrial COI gene of *Oncopeltus nigriceps*

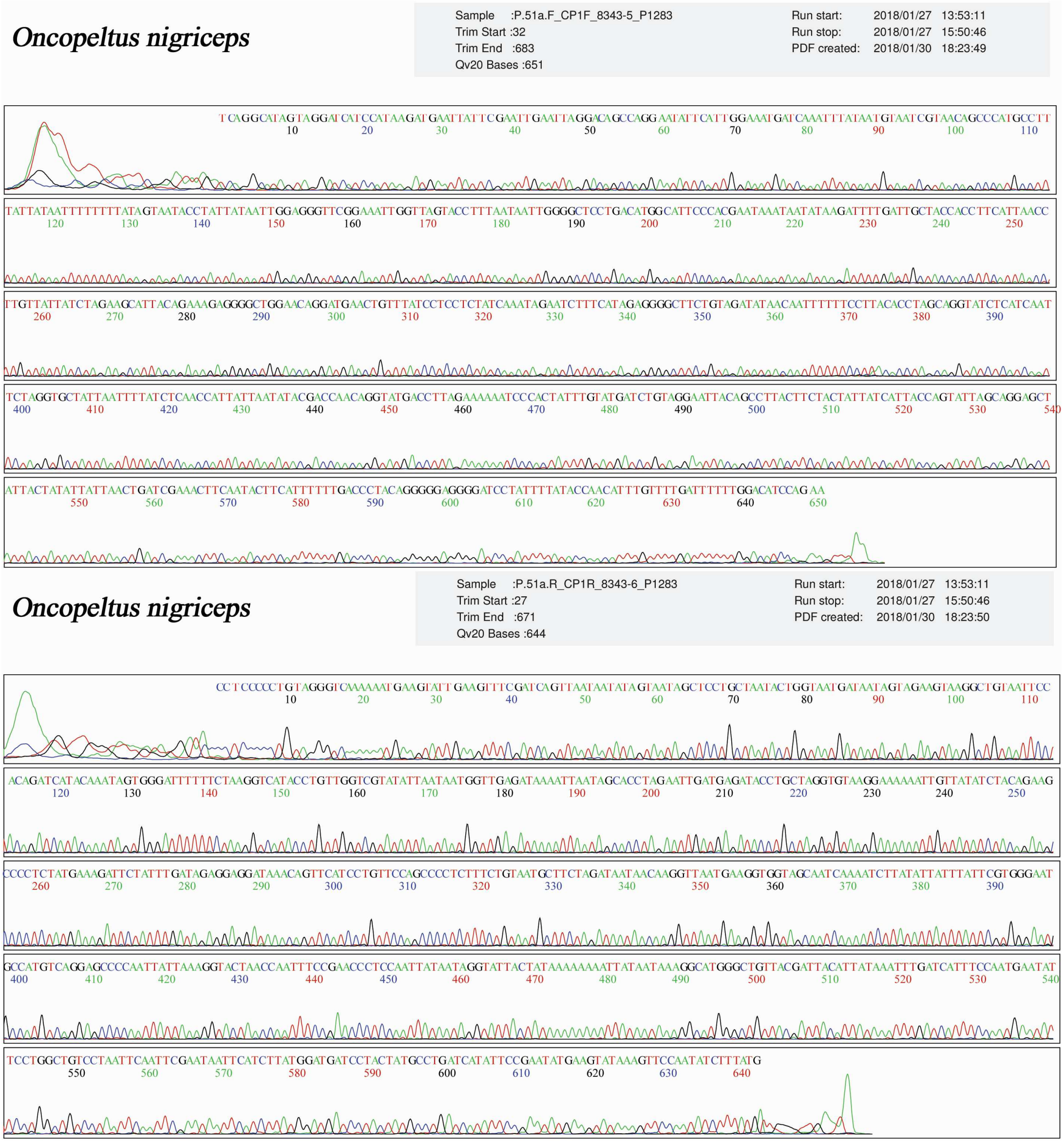
Figure 5.39: Molecular barcode of the mitochondrial COI gene of *Oncopeltus nigriceps*

> *Oncopeltus nigriceps* / 198AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUON-01-A1

> *Oncopeltus nigriceps*

```
MVGSSMSWIIRIELGQPGMFIGNDQIYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMI
GAPDMAFPRMNNMSFWLLPPSLTLLLSSSITESGAGTGWTVYPPLSNSIFHSGASVDMTI
FSLHLAGISSILGAINFISTIINMRPTGMTLEKIPLFVWSVGITALLLLLSLPVLAGAIT
MLLTDRNFNTSFFDPTGG
```

Figure 5.40: The translation product of the mitochondrial COI gene of *Oncopeltus nigriceps* using Forward and Reverse primers.



Oncopeltus nigriceps

Sample :P.51a.F_CP1F_8343-5_P1283
Trim Start :32
Trim End :683
Qv20 Bases :651

Run start: 2018/01/27 13:53:11
Run stop: 2018/01/27 15:50:46
PDF created: 2018/01/30 18:23:49

TCAGGCATAGTAGGATCATCCATAAGATGAATTATTCGAATTGAATTAGGACAGCCAGGAATATTCATTGGAAATGATCAAATTTATAATGTAATCGTAACAGCCCATGCCTT
TATTATAATTTTTTTTATAGTAATACCTATTATAATTGGAGGGTTCGGAAATTGGTTAGTACCTTTAATAATTGGGGCTCCTGACATGGCATTCCCACGAATAAATAATATAAGATTTTGATTGCTACCACCTTCATTAACC
TTGTTATTATCTAGAAGCATTACAGAAAGAGGGGCTGGAACAGGATGAACTGTTTATCCTCCTCTATCAAATAGAATCTTTCATAGAGGGGCTTCTGTAGATATAACAATTTTTTCCTTACACCTAGCAGGTATCTCATCAAT
TCTAGGTGCTATTAATTTTATCTCAACCATTATTAATATACGACCAACAGGTATGACCTTAGAAAAAATCCCACTATTTGTATGATCTGTAGGAATTACAGCCTTACTTCTACTATTATCATTACCAGTATTAGCAGGAGCT
ATTACTATATTATTAACTGATCGAAACTTCAATACTTCATTTTTTGACCCTACAGGGGGAGGGGATCCTATTTTATACCAACATTTGTTTTGATTTTTTGGACATCCAG AA

Oncopeltus nigriceps

Sample :P.51a.R_CP1R_8343-6_P1283
Trim Start :27
Trim End :671
Qv20 Bases :644

Run start: 2018/01/27 13:53:11
Run stop: 2018/01/27 15:50:46
PDF created: 2018/01/30 18:23:50

CCTCCCCCTGTAGGGTCAAAAAATGAAGTATTGAAGTTTCGATCAGTTAATAATATAGTAATAGCTCCTGCTAATACTGGTAATGATAATAGTAGAAGTAAGGCTGTAATTCC
ACAGATCATACAAATAGTGGGATTTTTTCTAAGGTCATACCTGTTGGTCGTATATTAATAATGGTTGAGATAAAATTAATAGCACCTAGAATTGATGAGATACCTGCTAGGTGTAAGGAAAAAATTGTTATATCTACAGAAG
CCCCTCTATGAAAGATTCTATTTGATAGAGGAGGATAAACAGTTCATCCTGTTCCAGCCCCTCTTTCTGTAATGCTTCTAGATAATAACAAGGTTAATGAAGGTGGTAGCAATCAAAATCTTATATTATTTATTCGTGGGAAT
GCCATGTCAGGAGCCCCAATTATTAAAGGTACTAACCAATTTCCGAACCCTCCAATTATAATAGGTATTACTATAAAAAAAATTATAATAAAGGCATGGGCTGTTACGATTACATTATAAATTTGATCATTTCCAATGAATAT
TCCTGGCTGTCCTAATTCAATTCGAATAATTCATCTTATGGATGATCCTACTATGCCTGATCATATTCCGAATATGAAGTATAAAGTTCCAATATCTTTATG

Figure 5.41: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Oncopeltus nigriceps* using Forward and Reverse primers.

Table 5.13: Comparison of Nucleotide frequencies of COI gene sequence of *Oncopeltus nigriceps* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH590766 Oncopeltus nigriceps (Kerala)	34.2	17.0	32.8	15.9	33	10.8	50.5	5.4	25	15.1	35.5	24.7	45	25.3	12.4	17.7
MH590767 Oncopeltus nigriceps (Kerala)	34.4	17.2	32.6	15.8	34	10.8	50.0	5.4	25	15.6	35.5	24.2	45	25.3	12.4	17.7
AB619246 Oncopeltus nigriceps	34.2	17.9	32.3	15.6	35	11.3	48.4	5.4	25	16.1	34.4	24.7	43	26.3	14.0	16.7
KU188489 Margarinotus niponicus	36.2	15.8	32.1	15.9	35	9.7	50.0	5.4	28	13.4	33.9	24.2	45	24.2	12.4	18.3
GQ292265 Ninomimus flavipes	35.1	16.3	32.6	15.9	34	10.2	49.5	6.5	27	13.4	34.9	24.7	45	25.3	13.4	16.7
KM023017 Tropidothorax leucopterus	36.7	14.7	33.9	14.7	35	7.5	55.4	1.6	30	12.4	33.9	24.2	45	24.2	12.4	18.3
KC425122 Oncopeltus fasciatus	35.1	15.8	35.5	13.6	39	12.3	47.6	1.6	28	15.6	36.0	20.4	39	19.5	22.7	18.9
KC425139 Lygaeus kalmii kalmia	35.8	15.2	35.3	13.6	39	12.3	47.1	2.1	30	13.4	36.0	20.4	39	20.0	22.7	18.4
KC425117 Neacoryphus nigrinervis	36.2	14.3	35.5	14.0	39	10.7	47.6	2.7	30	13.0	36.8	20.5	40	19.4	22.0	18.8
KC425110 Lygaeus reclivatus	36.0	15.1	35.1	13.8	39	11.8	46.5	2.7	30	13.4	36.0	20.4	39	20.0	22.7	18.4
KR037952 Melanopleurus pyrrhopterus	36.6	15.4	33.0	15.1	38	7.5	50.5	4.3	26	15.1	35.5	23.1	46	23.7	12.9	17.7
KR032820 Lygaeospilus brevipilus	37.3	15.2	33.0	14.5	39	7.0	52.7	1.6	28	14.5	33.9	23.7	45	24.2	12.4	18.3
KR562526 Lygaeus reclivatus	37.1	15.1	33.7	14.2	38	7.5	54.3	.5	28	13.4	34.4	23.7	45	24.2	12.4	18.3
MF140487 Spilostethus pandurus militaris	37.1	15.9	32.3	14.7	38	9.7	50.5	1.6	27	13.4	34.4	24.7	46	24.7	11.8	17.7
KM021453 Arocatus longiceps	37.1	16.1	32.3	14.5	36	11.3	51.1	1.6	31	12.4	33.3	23.7	45	24.7	12.4	18.3

Table 5.14: Percentage of evolutionary divergence of *Oncopeltus nigriceps* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH590766	*Oncopeltus nigriceps* (Kerala)	
2.	MH590767	*Oncopeltus nigriceps* (Kerala)	0.00%
3.	AB619246	*Oncopeltus nigriceps*	1.37%
4.	KC425122	*Oncopeltus fasciatus*	16.89%
5.	KR032820	*Lygaeospilus brevipilus*	18.35%
6.	GQ292265	*Ninomimus flavipes*	18.84%
7.	KC425139	*Lygaeus kalmia kalmia*	18.97%
8.	KU188489	*Margarinotus niponicus*	19.54%
9.	KC425110	*Lygaeus reclivatus*	19.55%
10.	KR037952	*Melanopleurus pyrrhopterus*	19.66%
11.	KR562526	*Lygaeus reclivatus*	20.82%
12.	KM023017	*Tropidothorax leucopterus*	21.53%
13.	KM021453	*Arocatus longiceps*	21.70%
14.	MF140487	*Spilostethus pandurusmilitaris*	22.64%
15.	KC425117	*Neacoryphus nigrinervis*	23.11%

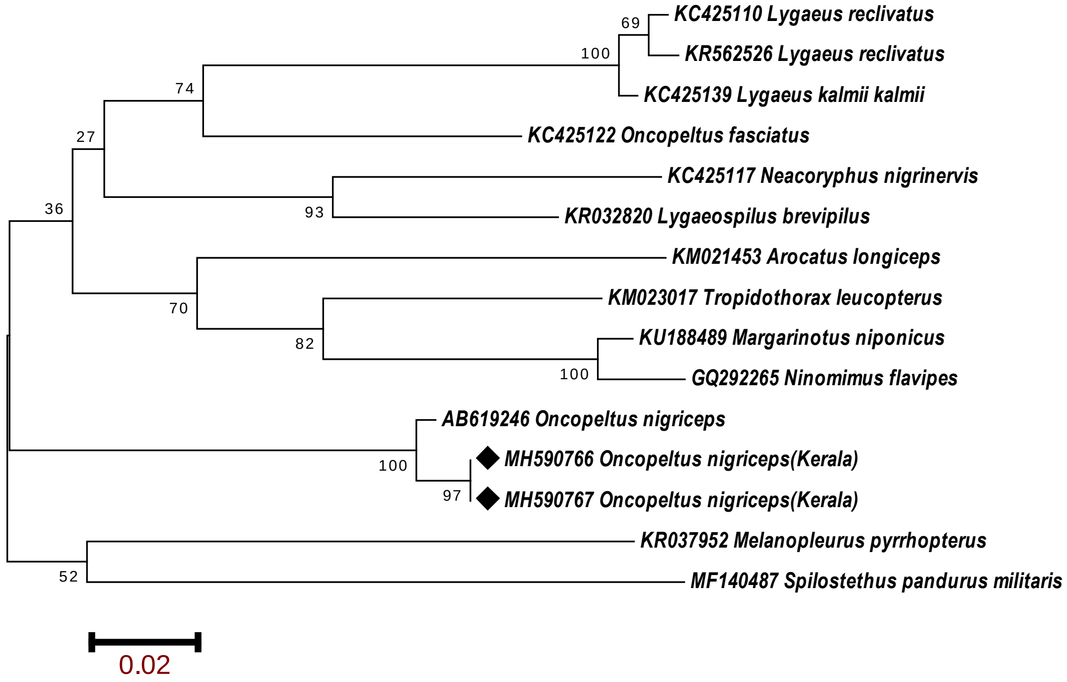


Figure 5.42: Phylogenetic relationship of *Oncopeltus nigriceps* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 100% sequence similarity to the same genus reported from Kerala (MH590767). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Oncopeltus nigriceps* showed bias to nucleotide AT, with following composition of nucleotides T = 34.2%, C = 17.0%, A = 32.8% and G = 15.9%. This greater AT content (67.0%) over GC content (32.9%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Oncopeltus nigriceps* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed 0 % to 23.11% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has no divergence (0%) with those from Kerala while 23.11% from USA (Table 5.14). On the basis of the data observed this species may be rooted from those found in USA which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from USA during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Oncopeltus nigriceps* with related species. *Oncopeltus nigriceps* isolated from Kerala (MH590767) showed 0% divergence with *Oncopeltus nigriceps* (MH590767) from Kerala and 23.11% divergence with *Neacoryphus nigrinervis* (KC425117) from USA. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Oncopeltus nigriceps* isolated from Kerala. Closest relative of *Oncopeltus nigriceps* is

Oncopeltus nigriceps from Kerala and *Oncopeltus nigriceps* from Japan represented within the same clade.

8. ***Geocoris varius*** (Uhler, 1860)

Specimen details:

Voucher specimen : CUGV – 01 – A1
Date of collection : 28-Nov-2017
Locality : Kannur: Payyanur
Lat- Lon : 12.1051° N, 75.2058° E
GenBank accession : MH590772

Description and Distribution

Fig. 5.43: *Geocoris varius*

Geocoris species are commonly known as big-eyed bugs (Figure. 5.43), are generalist omnivores insect occurring naturally worldwide (Tamaki and Weeks, 1972). *Geocoris* species prey on a variety of insects, including economically important agricultural pests (Eastop, 1970) but are also been reported to feed upon plant material (Tillman and Mullinex, 2003), mostly attacking the seeds (Sweet, 1960). According to Kobor (2018) only extreme base of head black, never exceeding the level of ocelli. Antennal segment I ochraceous, segments II-IV brownish, segment II-III with apex ochraceous. Humeral angles with irregular ochraceous spot of various extent. Punctuation of hemelytra fine, dense in M-R region. Compound eye large, without stalk; compound eye curved backward and reaching to the pronotum. Body glossy and dorsal exoskeleton coarsely punctate. Species of Geocoris predate also small arthropods, although they primarily eat plant. Found distributed in India, China, Formosa and Japan (Slater, 1972).

Damage

Geocoris varius is an omnivorous generalist insect. The adult feed up on plant material particularly on seedlings and young plants (Schuman et al., 2013).

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Geocoris varius* collected has been amplified using the primer LEP (Table 3.1). The PCR amplification yielded 515 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 5.44 – 5.47 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 5.15.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Geocoris varius* voucher collected during the present study (Table 5.15). It showed bias to nucleotide AT, with nucleotide composition with T = 35.1%, C = 18.3%, A = 31.5% and G = 15.1%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 5.16 and Figure 5.48 respectively.

> *Geocoris varius* CDS-2018/ 515 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUGV-01-A1

> *Geocoris varius*

```
AATTTTTTTTATAGTTATACCAATTATAATTGGAGGATTTGGCAATTGATTAGTACCTTT
AATAATTGGAGCCCCAGATATAGCATTCCCCCGAATAAATAATATATCATTTTGACTTTT
ACCCCCTTCTTTAACATTATTATTATCAAGTAGAATAGTAGAAATAGGGGCAGGAACCGG
ATGAACAGTATATCCTCCTCTATCAAATAGATTATTTCATAGAGGAGCATCAGTAGATAT
AGCAATCTTTTCATTACAT1CTAGCAGGAGTTTCATCAATTCTTGGGGCTATTAATTTTA
TTTCCACTATTATAAATATACGACCAGAAGGAATATCATCTGAACGAATTCCATTATTTG
TTTGATCAGTAGGGATCACTGCATTACTTTTACTCCTATCCTTACCCGTTTTAGCTGGGG
CTATCACTATATTATTAACAGACCGTAATATTAATACAACATTCTTTGACCCTACAGGAG
GGGGAGACCCTATCTTGTATCAACACTTATTCTGAT
```

Figure 5.44: The partial DNA sequence of the mitochondrial COI gene of *Geocoris varius*

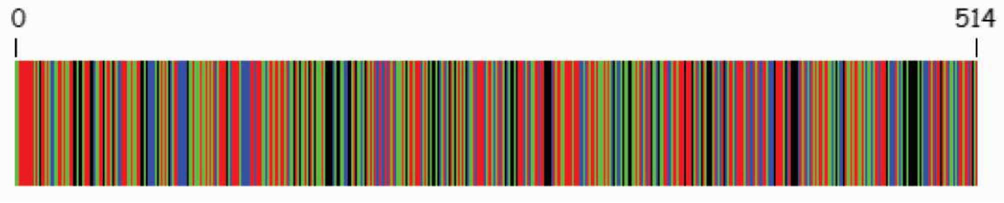


Figure 5.45: Molecular barcode of the mitochondrial COI gene of *Geocoris varius*

> *Geocoris varius* / 168AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUGV-01-A1

> *Geocoris varius*

```
MVMPIMIGGFGNWLVPLMIGAPDMAFPRMNNMSFWLLPPSLTLLLSSSMVEMGAGTGWTV
YPPLSNSLFHSGASVDMAIFSLHLAGVSSILGAINFISTIMNMRPEGMSSERIPLFVWSV
GITALLLLLSLPVLAGAITMLLTDRNINTTFFDPTGGGDPILYQHLFW
```

Figure 5.46: The translation product of the mitochondrial COI gene of *Geocoris varius*

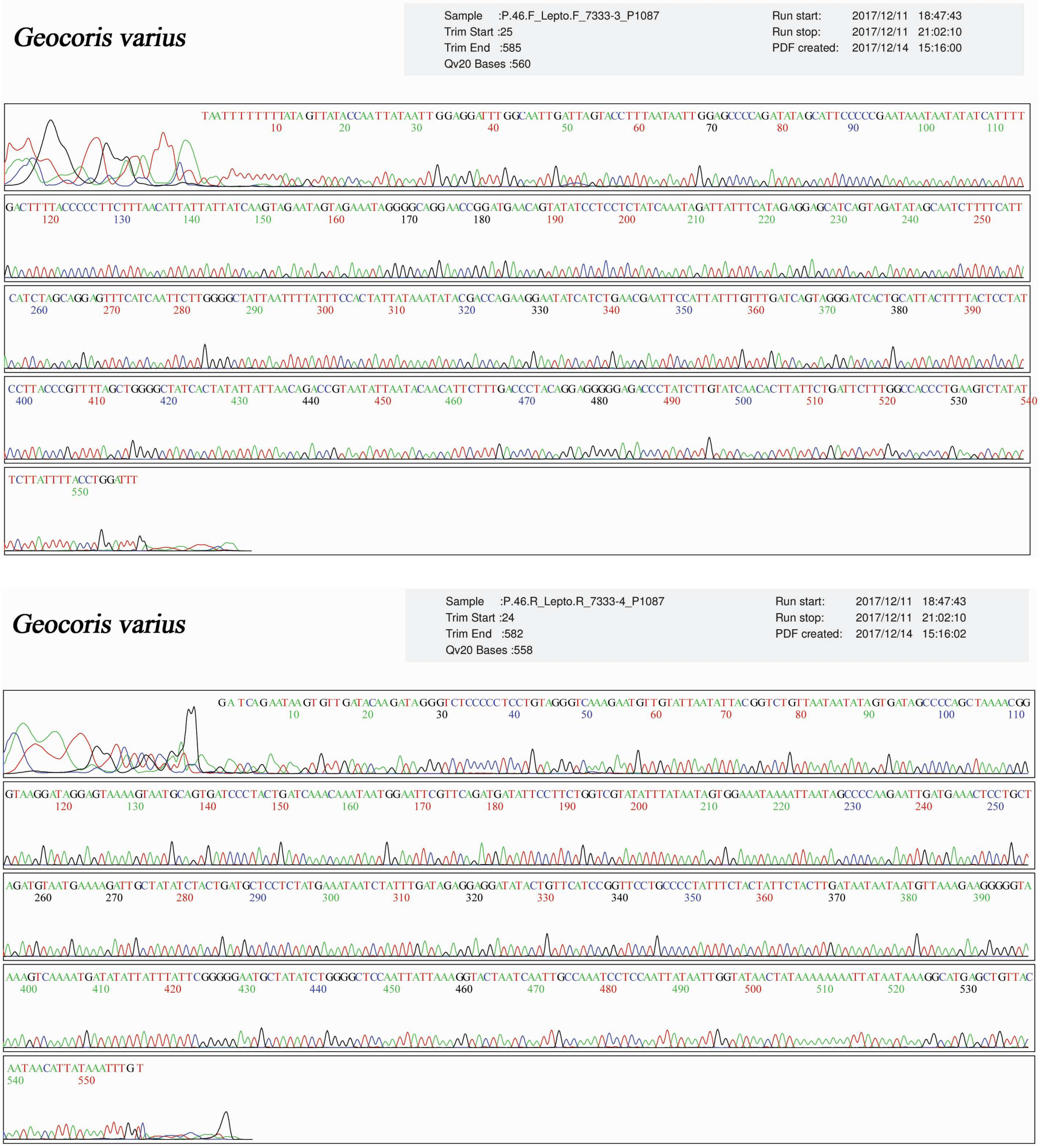


Figure 5.47: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Geocoris varius* using Forward and Reverse primers.

Table 5.15: Comparison of Nucleotide frequencies of COI gene sequence of *Geocoris varius* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH590772 Geocoris varius (Kerala)	35.1	18.3	31.5	15.1	33	11.6	52.3	3.5	30	16.3	29.1	25.0	43	26.9	12.9	17.0
GQ292262 Geocoris varius	34.0	17.9	31.7	16.5	33	9.9	52.0	4.7	24	18.6	30.2	26.7	44	25.0	12.8	18.0
KM022381 Geocoris grylloides	34.2	16.5	34.2	15.1	33	8.7	57.6	1.2	27	16.3	30.8	26.2	43	24.6	14.0	18.1
HQ978682 Geocoris atricolor	36.1	15.3	32.8	15.7	39	5.8	52.9	2.3	26	16.3	31.4	26.2	43	24.0	14.0	18.7
HQ105701 Geocoris pallens	36.3	15.1	32.4	16.1	38	6.4	51.7	3.5	27	15.1	31.4	26.2	43	24.0	14.0	18.7
HQ105688 Geocoris bullatus	35.7	15.5	33.2	15.5	37	7.0	54.1	1.7	27	15.7	31.4	26.2	43	24.0	14.0	18.7
KR039107 Neoneides muticus	36.3	14.2	33.2	16.3	38	2.9	55.8	2.9	27	14.5	31.4	26.7	43	25.1	12.3	19.3
KU875103 Geocoris howardi	35.9	15.5	32.8	15.7	38	7.0	52.9	2.3	27	15.7	31.4	26.2	43	24.0	14.0	18.7
KU875099 Geocoris discopterus	36.5	15.0	32.2	16.3	39	5.8	51.2	4.1	27	15.1	31.4	26.2	43	24.0	14.0	18.7
KR034338 Jalysus wickhami	36.9	14.6	32.8	15.7	41	4.1	54.7	.6	27	15.7	30.8	26.7	43	24.0	12.9	19.9
KM022291 Geocoris dispar	33.8	17.7	33.2	15.3	32	11.6	55.2	1.2	26	16.9	30.2	26.7	43	24.6	14.0	18.1
HQ105784 Jalysus wickhami	37.3	15.5	32.2	15.0	41	5.2	52.3	1.2	26	15.7	32.0	26.7	45	25.7	12.3	17.0
KR037560 Peritrechus convivus	35.9	15.1	34.2	14.8	36	7.6	55.2	1.2	28	13.4	33.7	24.4	43	24.6	13.5	18.7
KM022926 Geocoris ater	35.5	16.1	32.8	15.5	35	10.5	52.3	1.7	28	14.0	32.0	26.2	43	24.0	14.0	18.7
HQ106089 Peritrechus convivus	36.9	14.2	34.4	14.6	38	5.2	55.8	.6	29	12.8	33.7	24.4	43	24.6	13.5	18.7

Table 5.16: Percentage of evolutionary divergence of *Geocoris varius* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH590772	*Geocoris varius* (Kerala)	
2.	GQ292262	*Geocoris varius* (Korea)	5.92%
3.	KM022381	*Geocoris grylloides*	13.03%
4.	KR039107	*Neoneides muticus*	15.06%
5.	KR034338	*Jalysus wickhami*	15.14%
6.	HQ105784	*Jalysus wickhami*	15.14%
7.	HQ978682	*Geocoris atricolor*	16.61%
8.	KM022926	*Geocoris ater*	17.12%
9.	KR037560	*Peritrechus convivus*	17.13%
10.	HQ106089	*Peritrechus convivus*	17.17%
11.	KM022291	*Geocoris dispar*	17.51%
12.	HQ105701	*Geocoris pallens*	18.69%
13.	KU875099	*Geocoris discopterus*	18.71%
14.	HQ105688	*Geocoris bullatus*	19.22%
15.	KU875103	*Geocoris howardi*	19.27%

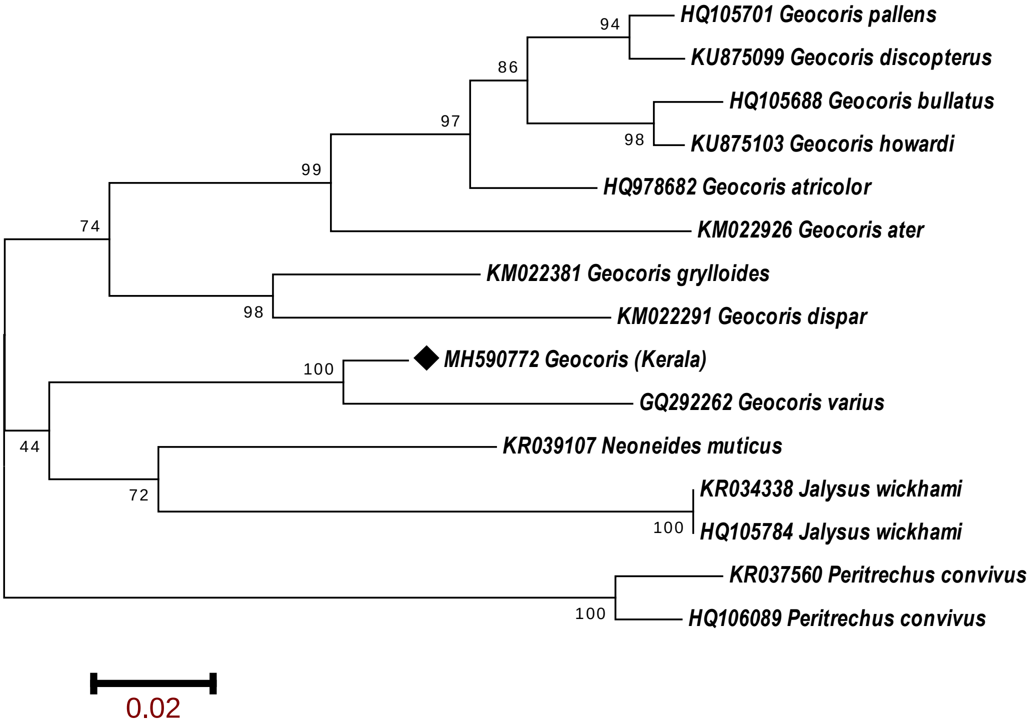


Figure 5.48: Phylogenetic relationship of *Geocoris varius* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 94.1% sequence similarity to the same genus reported from Korea (GQ292262). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Geocoris varius* showed bias to nucleotide AT, with following composition of nucleotides T = 35.1%, C = 18.3%, A = 31.5% and G = 15.1%. This greater AT content (66.6%) over GC content (33.4%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Geocoris varius* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 5.92% to 19.27% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (5.92%) with those from Korea while 19.27% from USA (Table 5.16). On the basis of the data observed this species may be rooted from those found in USA which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from USA during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Geocoris varius* with related species. *Geocoris varius* isolated from Kerala (MH590772) showed 5.92% divergence with *Geocoris varius* (GQ292262) from Korea and 19.27% divergence with *Geocoris howardi* (KU875103) from USA. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Geocoris varius* isolated from Kerala. Closest relative of *Geocoris varius* is *Geocoris varius* from Korea represented within the same clade.

9. ***Paraplesius unicolor*** (Scott, 1874)

Specimen details:

Voucher specimen : CUPU – 01 – A1
Date of collection : 30-Apr-2016
Locality : Malappuram: Nilambur
Lat- Lon : 11.2794° N, 76.2398° E
GenBank accession : MH590772

Description and Distribution

The plant feeding, *Paraplesius unicolor*, has a body length range is 12-14mm (Figure. 5.49). They possess green and light yellowish brown colour with scattered black spot; the length of the head and front chest, back and stomach is identical; the bottom of the body is yellowish brown and black center front, as a whole body is bar shaped (Sun et al., 2012). Distributed in Temperate Asia, Eastern Asia and Japan.

Fig.5.49: *Paraplesius unicolor*

Damage

The adult and nymph either feed on the phloem or bore into the stem causing complete death of the host plant (Sun et al., 2012).

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Paraplesius unicolor* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 608 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram and are exhibited in Figures 5.50 – 5.53 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 5.17.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Paraplesius unicolor* voucher collected during the present study (Table 5.17). It showed bias to nucleotide AT, with nucleotide composition with T = 34.3%, C = 18.7%, A = 29.3% and G = 17.7%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 5.18 and Figure 5.54 respectively.

> *Paraplesius unicolor* CDS-2018/ 608bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUPU-01-A1

> *Paraplesius unicolor*

```
GCTGGGATAGTGGGTACATCCTTAAGATGAATTATTCGTATTGAATTAGGACAACCAGGA
TCTTTTATTGGTGATGACCAAATTTACAATACTATTGTAACTGCACATGCCTTTGTAATA
ATTTTCTTTATAGTTATACCTATTATAATTGGGGGGTTTGGTAACTGATTAGTACCGTTA
ATAATCGGTGCTCCTGATATAGCATTCCCACGAATAAACAATATAAGATTTTGACTACTT
CCTCCTTCTCTAACACTATTATTAACTAGTAGAATTGTTGAAAAGGGAGTTGGTACAGGA
TGAACCGTTTACCCTCCTCTAGCAAGTAATATTGCCCATAGAGGGCCTGCAGTAGATTTA
GCAATTTTTAGCCTTCACTTAGCAGGAGTATCTTCAATTTTAGGGGCTGTAAACTTCATT
TCAACTATTATCAATATACGTCCCAAGGGAATAATTCCTGAACGAATTCCCTTATTTGTA
TGATCAGTAGGGATTACTGCCTTATTATTATTATTATCCCTCCCTGTACTAGCAGGAGCT
ATTACTATACTATTAACCGATCGAAACTTTAATACCTCATTCTTTGACCCAGCAGGGGGA
GGGGATCC
```

Figure 5.50: The partial DNA sequence of the mitochondrial COI gene of *Paraplesius unicolor*

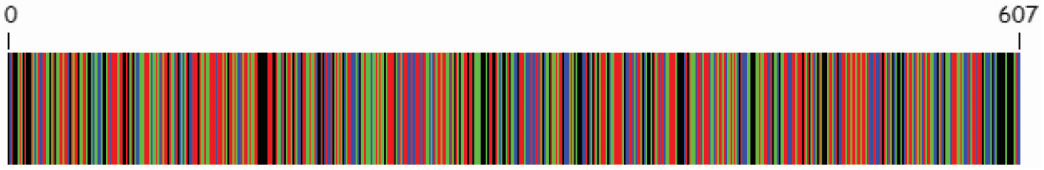


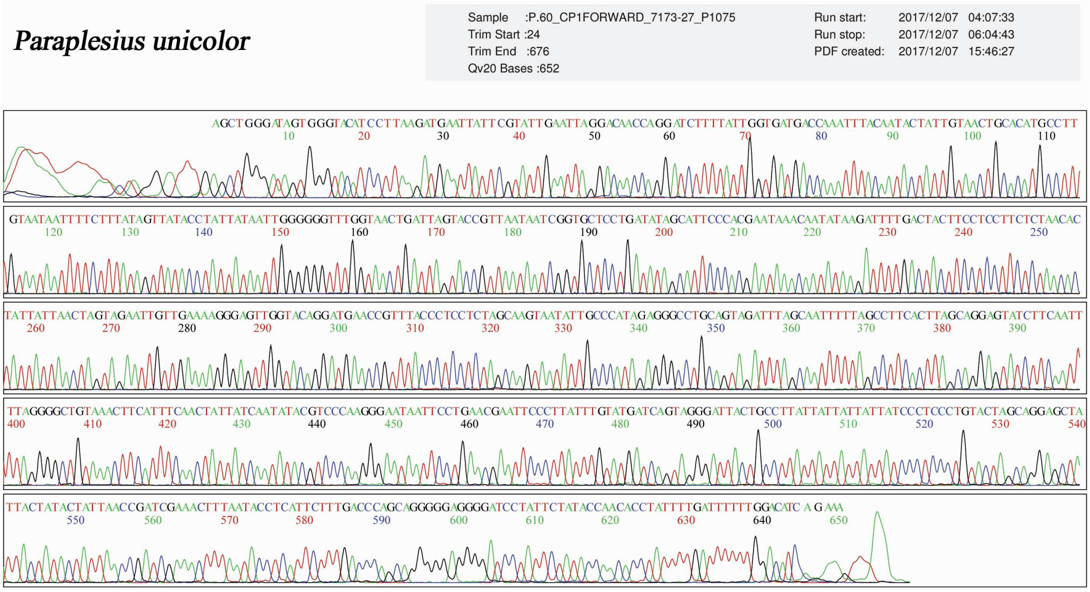
Figure 5.51: Molecular barcode of the mitochondrial COI gene of *Paraplesius unicolor*

> *Paraplesius unicolor* / 200AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUPU-01-A1

> *Paraplesius unicolor*

```
MVGTSLSWIIRIELGQPGSFIGDDQIYNTIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMI
GAPDMAFPRMNNMSFWLLPPSLTLLLTSSIVEKGVGTGWTVYPPLASNIAHSGPAVDLAI
FSLHLAGVSSILGAVNFISTIINMRPKGMIPERIPLFVWSVGITALLLLLSLPVLAGAIT
MLLTDRNFNTSFFDPAGGGD
```

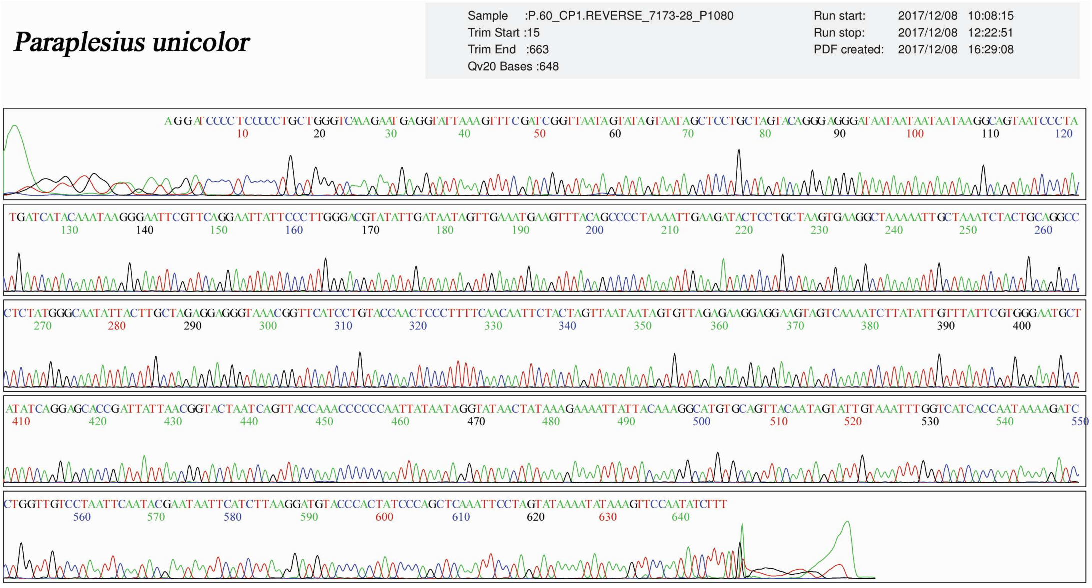
Figure 5.52: The translation product of the mitochondrial COI gene of *Paraplesius unicolor*



Paraplesius unicolor

Sample :P.60_CP1FORWARD_7173-27_P1075
Trim Start :24
Trim End :676
Qv20 Bases :652

Run start: 2017/12/07 04:07:33
Run stop: 2017/12/07 06:04:43
PDF created: 2017/12/07 15:46:27



Paraplesius unicolor

Sample :P.60_CP1.REVERSE_7173-28_P1080
Trim Start :15
Trim End :663
Qv20 Bases :648

Run start: 2017/12/08 10:08:15
Run stop: 2017/12/08 12:22:51
PDF created: 2017/12/08 16:29:08

Figure 5.53: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Paraplesius unicolor* using Forward and Reverse primers.

Table 5.17: Comparison of Nucleotide frequencies of COI gene sequence of *Paraplesius unicolor* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH590770 Paraplesius unicolor (Kerala)	34.3	18.7	29.3	17.7	38	13.5	43.0	5.5	23	17.0	31.5	29.0	43	25.5	13.5	18.5
GQ292200 Paraplesius unicolor	32.7	19.0	31.2	17.2	34	15.0	47.5	3.5	21	18.0	32.5	29.0	44	24.0	13.5	19.0
KJ541631 Stictopleurus crassicornis	40.2	14.5	28.8	16.5	47	6.0	43.0	4.5	30	13.0	30.5	27.0	45	24.5	13.0	18.0
MG924623 Himacerus apterus	35.0	15.5	33.2	16.3	34	6.5	57.0	3.0	27	15.5	29.5	28.5	45	24.5	13.0	17.5
KM022743 Stictopleurus abutilon	37.7	15.5	31.2	15.7	39	8.5	50.5	2.5	30	13.5	30.5	26.5	45	24.5	12.5	18.0
LC099269 Lindbergicoris hochii	37.3	17.3	28.7	16.7	41	11.5	43.5	4.5	28	14.5	30.0	27.5	44	26.0	12.5	18.0
MG924567 Himacerus nodipes	36.5	15.3	32.3	15.8	36	9.0	54.0	1.0	29	12.5	30.0	28.5	45	24.5	13.0	18.0
MG924534 Nabicula flavomarginata	33.2	19.2	31.3	16.3	34	13.0	51.0	2.0	21	20.5	29.5	29.5	45	24.0	13.5	17.5
LC099268 Lindbergicoris gramineus	37.7	17.0	28.5	16.8	41	11.5	42.5	5.5	29	13.5	30.5	27.0	44	26.0	12.5	18.0
KM021640 Nabis flavomarginatus	33.3	19.0	31.5	16.2	35	12.5	51.5	1.5	21	20.5	29.5	29.5	45	24.0	13.5	17.5
KR032818 Notonecta kirbyi	35.7	15.8	33.3	15.2	35	6.5	57.5	1.0	29	14.5	29.5	27.5	44	26.5	13.0	17.0
KM022180 Rhacognathus punctatus	38.2	14.5	31.3	16.0	43	5.5	51.5	.0	28	12.0	30.0	30.0	44	26.0	12.5	18.0
HQ106088 Pentacora signoreti	34.8	15.2	34.2	15.8	35	5.5	58.5	1.5	26	15.5	31.0	27.5	44	24.5	13.0	18.5
HQ106390 Stictopleurus punctiventris	39.7	14.2	29.8	16.3	45	5.0	46.0	4.0	30	13.0	30.5	27.0	45	24.5	13.0	18.0

Table 5.18: Percentage of evolutionary divergence of *Paraplesius unicolor* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH590770	*Paraplesius unicolor* (Kerala)	
2.	GQ292200	*Paraplesius unicolor* (Korea)	9.15%
3.	KM022180	*Rhacognathus punctatus*	21.18%
4.	LC099269	*Lindbergicoris hochii*	21.20%
5.	MG924623	*Himacerus apterus*	21.27%
6.	KJ541631	*Stictopleurus crassicornis*	22.03%
7.	LC099268	*Lindbergicoris gramineus*	22.24%
8.	MG924567	*Himacerus nodipes*	22.61%
9.	KM022743	*Stictopleurus abutilon*	22.89%
10.	HQ106088	*Pentacora signoreti*	23.86%
11.	KM021640	*Nabis flavomarginatus*	24.17%
12.	MG924534	*Nabicula flavomarginata*	24.17%
13.	HQ106390	*Stictopleurus punctiventris*	24.51%
14.	KR032818	*Notonecta kirbyi*	24.92%

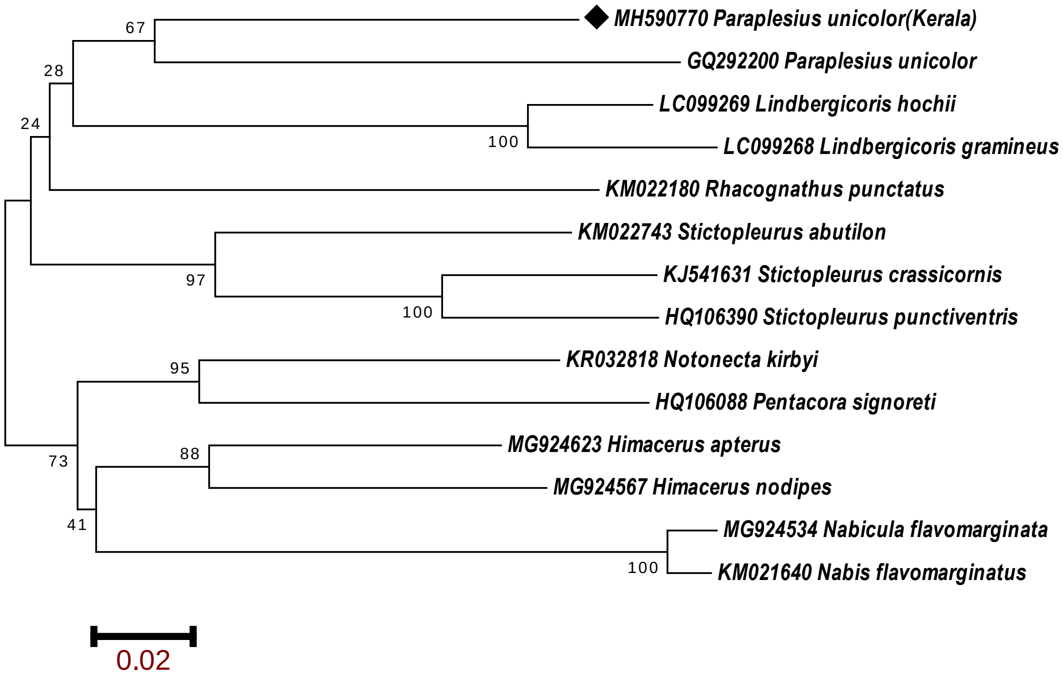


Figure 5.54: Phylogenetic relationship of *Paraplesius unicolor* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 90.9% sequence similarity to the same genus reported from Korea (GQ292200). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Paraplesius unicolor* showed bias to nucleotide AT, with following composition of nucleotides T = 34.3%, C = 18.7%, A = 29.3% and G = 17.7%. This greater AT content (63.6%) over GC content (36.4%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Paraplesius unicolor* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed 9.15% to 24.92% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (9.15%) with those from Korea while 24.92% from Canada (Table 5.18). On the basis of the data observed this species may be rooted from those found in Canada which diverted into different clades due to geographical variation. Result thus concluded that this species doesn't have any major changes in India while slightly changes from those reported from Canada during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Paraplesius unicolor* with related species. *Paraplesius unicolor* isolated from Kerala (MH590770) showed 9.15% divergence with *Paraplesius unicolor* (GQ292200) from Korea and 24.92% divergence with *Notonecta kirbyi* (KR032818) from Canada. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Paraplesius* *unicolor* isolated from Kerala. Closest relative of *Paraplesius unicolor* is *Paraplesius unicolor* from Korea represented within the same clade.

10. ***Cletus schmidti*** (Kiritshenko, 1916)

Specimen details:

Voucher specimen : CUCS – 09 – A1
Date of collection : 07-Sep-2016
Locality : Malappuram: Calicut University
Lat- Lon : 11.1340º N, 75.8952º E
GenBank accession : KX603656

Description and Distribution

Cletus schmidti (Figure. 5.55) are sap feeders with small elongate body, dorsal reddish brown coloration, pale yellowish at some places, ventrally pale cream in color, legs of the same color as abdomen ventrally, antennae reddish brown; head somewhat triangular, eyes large relative to the size of head, ocelli slightly closer to eyes than to each other, entire antenna granular and setose, Rostrum long and extending beyond the mid coxae. Third joint of antenna not foliaceously dilated on each side, pronotum with lateral angles not dilated, abdomen profoundly ampliated; pronotal angles acutely produced; abdominal segmental angles not exteriorly acutely produced. They are found to be distributed along Asia-Temperate, Eastern Asia, Korea, between Kannvya and Utibeni (Kiritshenko, 1916).

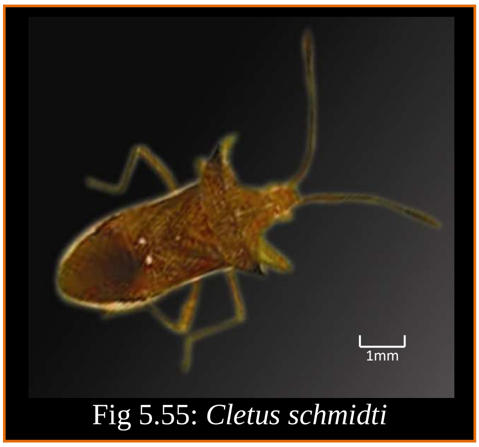


Fig 5.55: *Cletus schmidti*

Damage

The bugs are sap feeders, usually seen feeding on leaves and fruits of the host plants. It suck the skin of immature fruits eventually influencing the seed germination (Ding et al., 2004).

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Cletus schmidti* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 463 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 5.56 – 5.59 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 5.19.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Cletus schmidti* voucher collected during the present study (Table 5.19). It showed bias to nucleotide AT, with nucleotide composition with T = 37.7%, C = 16.1%, A = 30.7% and G = 15.5%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 5.20 and Figure 5.60 respectively.

> *Cletus schmidti* CDS-2017/ 463 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUCS-09-A1

> *Cletus schmidti*

```
AATTTATAATGTCATCGTTACAGCTCATGCATTCATTATAATTTTTTTTATAGTAATACC
TATTATAATTGGTGGGTTTGGAAACTGACTTGTACCATTAATAATTGGGGCACCTGATAT
AGCATTCCCACGTATAAATAATATAAGATTTTGATTATTACCACCTTCACTAACCCTTTT
ATTGACTAGTAGAATAGTAGAAAAAGGTGCTGGAACTGGTTGAACAGTTTATCCCCCTCT
ATCAAGTAATTTATCACATAGAGGTGCATCAGTAGATTTAGCAATTTTCTCATTACATTT
AGCAGGAGTATCCTCGATTTTAGGGGCTGTTAACTTTATTTCTACTATTATTAATATACG
CCCAGTAGGAATGATCCCCGAGCGTACCCCATTATTCGTTTGATCTGTAGGAATTACAGC
ACTTTTATTATTATTATCACTGCCTGTGTTAGCAGGAGCAATC
```

Figure 5.55: The partial DNA sequence of the mitochondrial COI gene of *Cletus schmidti*

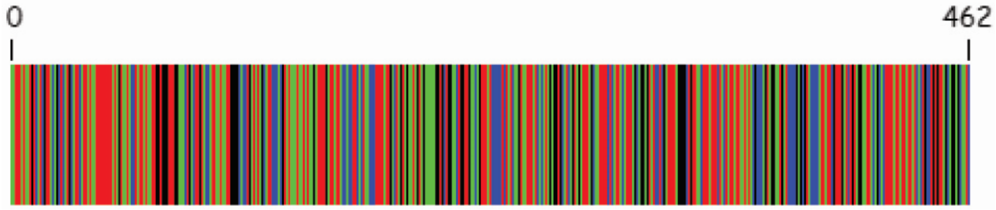


Figure 5.56: Molecular barcode of the mitochondrial COI gene of *Cletus schmidti*

> *Cletus schmidti* / 142AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUCS-09-A1

> *Cletus schmidti*

```
MIFFMVMPIMIGGFGNWLVPLMIGAPDMAFPRMNNMSFWLLPPSLTLLLTSSMVEKGAGT
GWTVYPPLSSNLSHSGASVDLAIFSLHLAGVSSILGAVNFISTIINMRPVGMIPERTPLF
VWSVGITALLLLLSLPVLAGAI
```

Figure 5.57: The translation product of the mitochondrial COI gene of *Cletus schmidti*

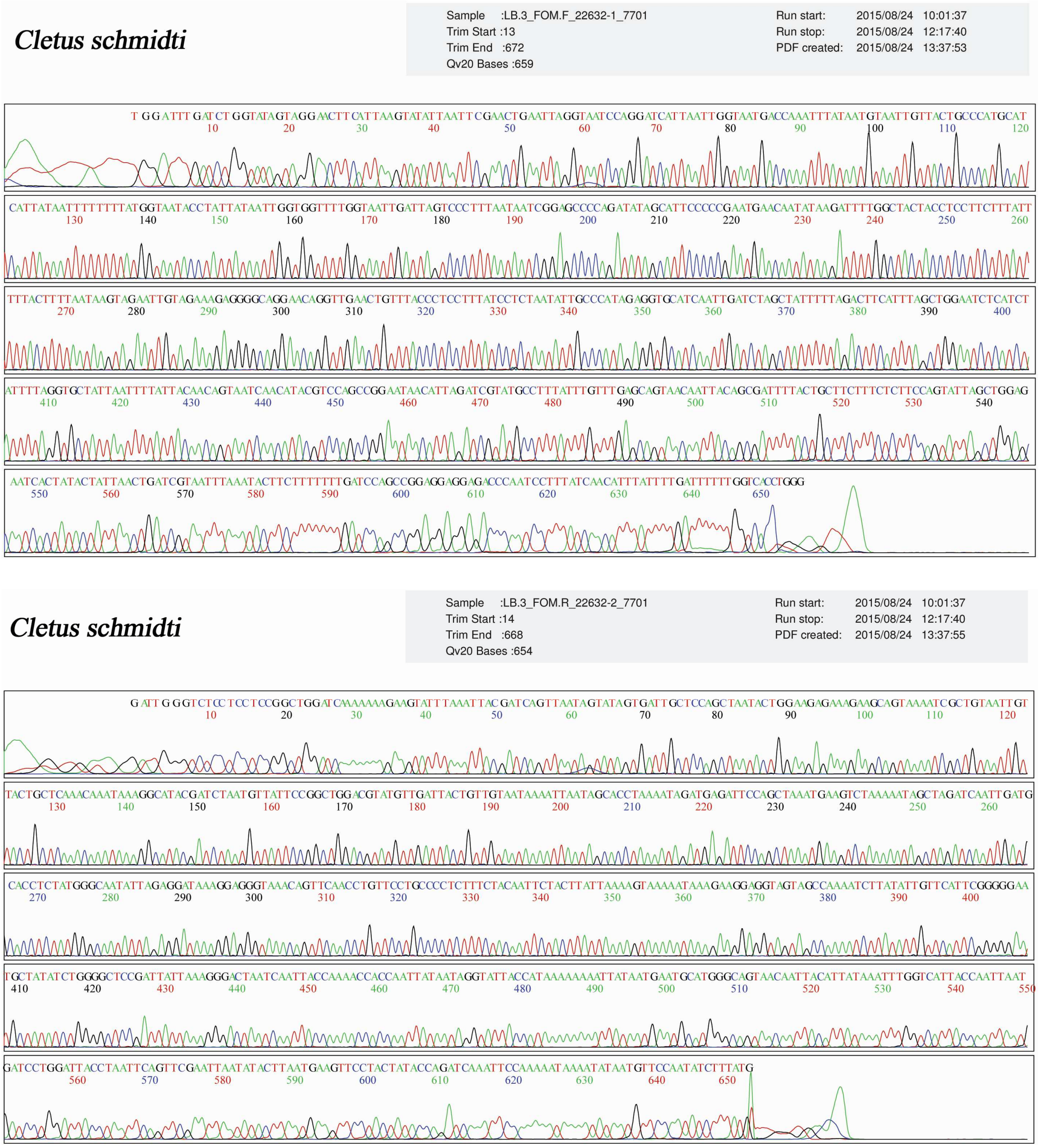


Figure 5.59: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Cletus schmidti* using Forward and Reverse primers.

Table 5.19: Comparison of Nucleotide frequencies of COI gene sequence of *Cletus schmidti* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
KX603656 Cletus schmidti (Kerala)	37.7	16.1	30.7	15.5	43	8.4	44.5	4.2	26	13.6	33.9	26.3	44	26.3	13.6	16.1
KP753681 Cletus trigonus	36.2	16.8	30.9	16.1	41	10.8	44.6	3.6	25	15.1	33.1	26.6	42	24.5	15.1	18.0
KP753680 Cletus trigonus	36.2	16.8	30.9	16.1	41	10.8	44.6	3.6	25	15.1	33.1	26.6	42	24.5	15.1	18.0
KP753671 Cletus trigonus	36.0	17.0	30.7	16.3	40	11.5	43.9	4.3	25	15.1	33.1	26.6	42	24.5	15.1	18.0
KP753566 Cletus pugnator	35.3	18.2	30.2	16.3	39	14.4	42.4	4.3	24	15.8	33.1	26.6	42	24.5	15.1	18.0
KP753564 Cletus pugnator	35.7	17.7	30.0	16.5	40	12.9	41.7	5.0	24	15.8	33.1	26.6	42	24.5	15.1	18.0
KP753563 Cletus pugnator	35.5	18.0	30.2	16.3	40	13.7	42.4	4.3	24	15.8	33.1	26.6	42	24.5	15.1	18.0
KP753626 Cletus schmidti	38.6	15.1	30.0	16.3	48	5.8	41.7	4.3	25	15.1	33.1	26.6	42	24.5	15.1	18.0
MG299062 Cletus punctulatus	35.6	16.4	30.7	17.3	38	11.0	45.2	5.8	26	14.3	32.5	27.3	43	24.0	14.3	18.8
KP753604 Cletus punctulatus	35.5	17.0	31.9	15.6	40	10.8	47.5	2.2	24	15.8	33.1	26.6	42	24.5	15.1	18.0
KX351382 Cletus rubidiventris	34.7	18.0	30.9	16.5	37	13.4	45.5	4.5	25	15.8	31.6	27.8	43	24.6	15.7	17.2
KP753538 Cletus graminis	34.5	17.7	31.4	16.3	36	13.7	46.0	4.3	25	15.1	33.1	26.6	42	24.5	15.1	18.0

Table 5.20: Percentage of evolutionary divergence of *Cletus schmidti* with its closely related species accessible from NCBI GenBank

Sl. No.	**Accession No**	**Organism**	**Percentage of divergence**
1.	KX603656	*Cletus schmidti* (Kerala)	
2.	KP753681	*Cletus trigonus* (China)	0.22%
3.	KP753680	*Cletus trigonus*	0.22%
4.	KP753671	*Cletus trigonus*	0.67%
5.	KP753566	*Cletus pugnator*	5.96%
6.	KP753564	*Cletus pugnator*	6.26%
7.	KP753626	*Cletus schmidti*	6.43%
8.	KP753563	*Cletus pugnator*	6.53%
9.	KP753604	*Cletus punctulatus*	7.86%
10.	MG299062	*Cletus punctulatus*	8.18%
11.	KX351382	*Cletus rubidiventris*	8.22%
12.	KP753538	*Cletus graminis*	8.51%

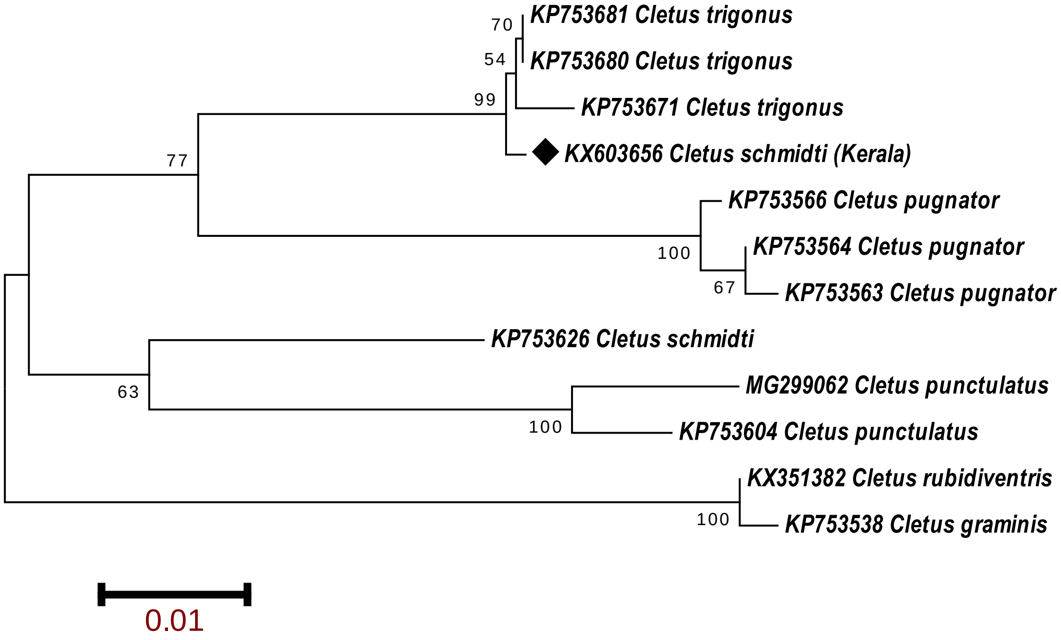


Figure 5.60: Phylogenetic relationship of *Cletus schmidti* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 99.8% sequence similarity to the same genus reported from China (KP753681). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Cletus schmidti* showed bias to nucleotide AT, with following composition of nucleotides T = 37.7%, C = 16.1%, A = 30.7% and G = 15.5%. This greater AT content (68.4%) over GC content (31.6%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Cletus schmidti* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 0.22% to 8.51% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (0.22%) with those from China while 8.51% from China (Table 5.20). On the basis of the data observed this species may be rooted from those found in China which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from China during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Cletus schmidti* with related species. *Cletus schmidti* isolated from Kerala (KX603656) showed 0.22% divergence with *Cletus trigonus* (KP753681) from China and 8.51% divergence with *Cletus graminis* (KP753538) from China. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Cletus schmidti* isolated from

Kerala. Closest relative of *Cletus schmidti* is *Cletus trigonus* from China represented within the same clade.

11. *Dysdercus ocreatus* (Uhler, 1886)

Specimen details:

Voucher specimen : CUDO – 01 – A1
Date of collection : 03-Dec-2017
Locality : Palakkad: Parli
Lat- Lon : 10.7977° N, 76.5627° E
GenBank accession : MH674102

Description and Distribution

Dysdercus ocreatus are small to medium sized(9.5-12.5 mm); ground colour red; posterior and lateral margins of pronotum and a transverse fascia on hemelytra usually black; abdomen concolorous red (Vandoesburg, 1968). Adult causes severe damage to plants resulting in fungal attack. Head not strongly bent down in front of the eyes. The adults have various sizes and colours, usually not brachypterous. Apex of corium is acute. Larger and brighter coloured insects, (12 - 20 mm). General color black and red or brown. The ‘cotton stainers’, Venter usually with contrasting stripes. They are found to be distributed throughout Eurasian countries (Figure. 5.61).

Fig.5.61: *Dysdercus ocreatus*

Damage

The adult and the nymph feed on the flower buds causing it eventually to shed.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Dysdercus ocreatus* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 561 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 5.62 – 5.65 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 5.21.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Dysdercus ocreatus* voucher collected during the present study (Table 5.21). It showed bias to nucleotide AT, with nucleotide composition with T = 37.8%, C = 16.6%, A = 29.8% and G = 15.9%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 5.22 and Figure 5.66 respectively.

> *Dysdercus ocreatus* CDS-2018/ 561 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUDO-01-A1

> *Dysdercus ocreatus*

```
TTCCTTAAGATTCCTAATTCGTGCAGAACTTGGAAGGCCAGGCTCTCTTATTGGAGACGA
TCAAATTTATAATGTTATTGTAACAGCCCACGCTTTTGTAATAATTTTTTTTATAGTTAT
ACCAATTATAATTGGAGGATTTGGCAACTGATTAGTACCTTTAATGTTAGGAGCACCAGA
TATAGCTTTCCCTCGTATAAATAATATAAGATTTTGGTTATTACCCCCTTCTTTAACACT
CCTTTTAATAAGAAGAATGGTAGAAAGAGGCGCAGGAACTGGTTGAACAGTATATCCTCC
TTTATCTTCTAATATTGCTCATGGAGGATCTTCAGTTGATTTAGCTATTTTTAGTCTTCA
TTTAGCAGGAATTTCCTCAATTCTAGGAGCTGTAAATTTTATTACTACAGTTATTAATAT
ACGCCCAATTGGTATAACTTTTGATCGTATACCATTATTTGTATGAGCAGTAGTAATTAC
TGCTGTACTTCTTTTATTATCTTTACCTGTTTTAGCAGGAGCAATTACAATATTATTAAC
AGACCGAAATTTAAATACATC
```

Figure 5.62 The partial DNA sequence of the mitochondrial COI gene of *Dysdercus ocreatus*

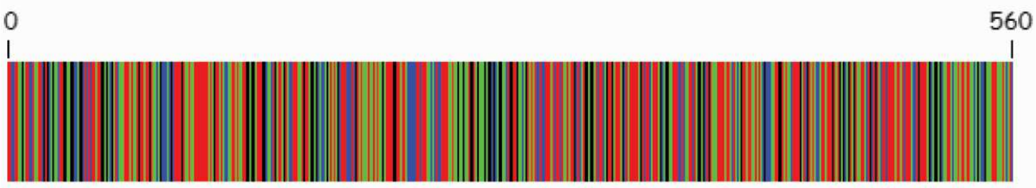


Figure 5.63: Molecular barcode of the mitochondrial COI gene of *Dysdercus ocreatus*

> *Dysdercus ocreatus* / 153AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUDO-01-A1

> *Dysdercus ocreatus*

```
MIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLLMSSMVESGAGT
GWTVYPPLSSNIAHGGSSVDLAIFSLHLAGISSILGAVNFITTVINMRPIGMTFDRMPLF
VWAVVITAVLLLLSLPVLAGAITMLLTDRNLNT
```

Figure 5.64: The translation product of the mitochondrial COI gene of *Dysdercus ocreatus*

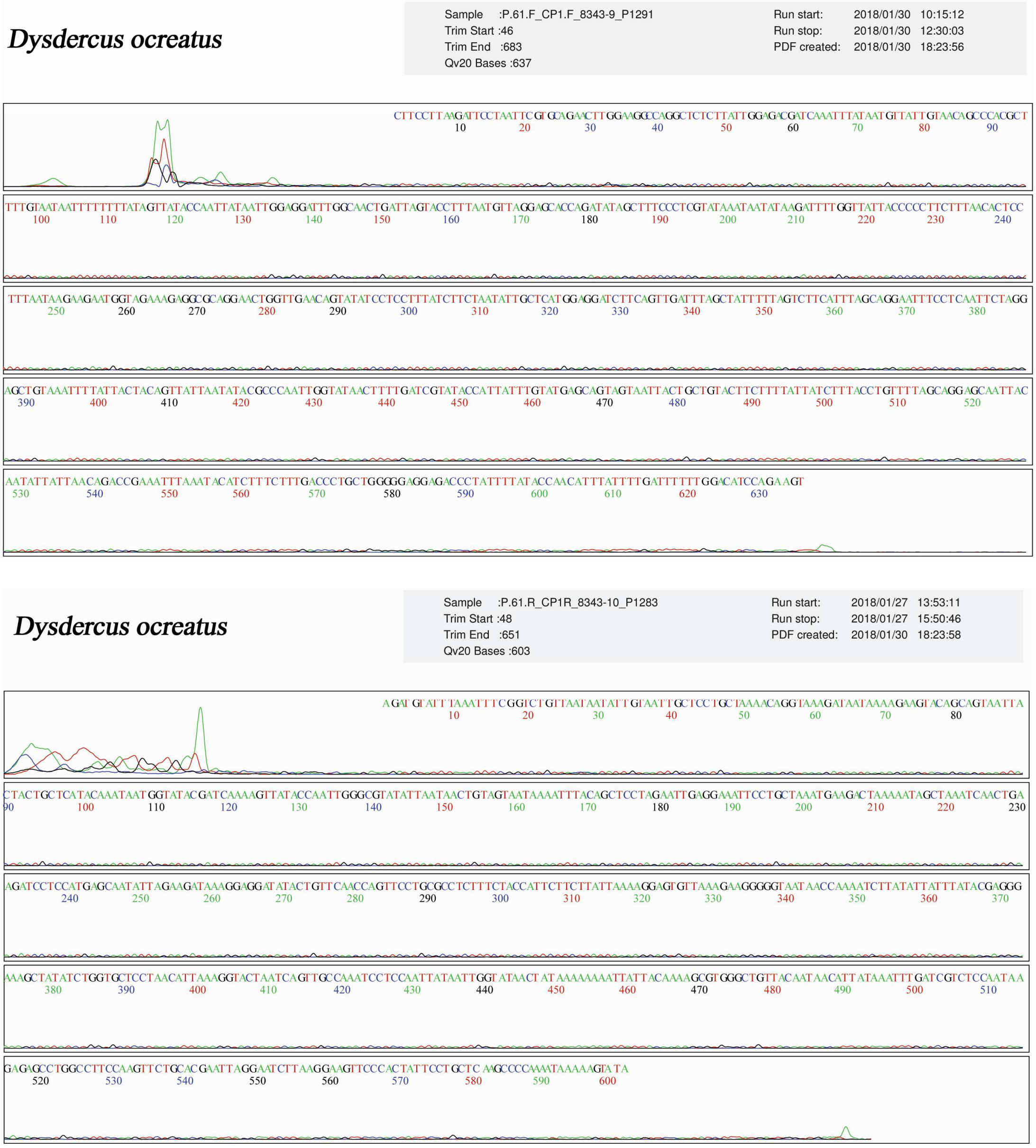


Figure 5.65: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Dysdercus ocreatus* using Forward and Reverse primers.

Table 5.21: Comparison of Nucleotide frequencies of COI gene sequence of *Dysdercus ocreatus* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH674102 Dysdercus ocreatus	37.8	16.6	29.8	15.9	43	8.0	46.5	2.1	25	16.0	30.5	28.3	45	25.7	12.3	17.1
KJ204201 Agabus arcticus	36.0	15.3	29.2	19.4	37	5.9	47.6	9.6	26	15.0	28.9	29.9	45	25.1	11.2	18.7
KC491230 Brachypeplus glaber	39.4	14.8	30.5	15.3	48	3.2	47.6	1.6	26	16.0	30.5	27.3	44	25.1	13.4	17.1
LT991366 Ochthebius arefniae	41.0	14.1	28.5	16.4	49	4.8	43.9	2.1	28	12.3	29.9	29.4	45	25.1	11.8	17.6
JF888119 Eusphalerum fenyesi	36.2	15.2	33.2	15.5	37	4.3	57.8	.5	26	16.0	29.9	28.3	45	25.1	11.8	17.6
LT991393 Ochthebius viridescens	39.8	14.6	29.4	16.2	45	6.4	47.1	1.1	28	12.3	29.4	29.9	45	25.1	11.8	17.6
KU918952 Pterostichus strenuus	39.9	13.9	29.8	16.4	47	3.2	48.7	1.6	28	12.8	29.4	29.4	45	25.7	11.2	18.2
KU918208 Pogonus chalceus	39.6	13.2	31.0	16.2	45	1.1	52.9	1.1	29	12.8	28.9	29.4	45	25.7	11.2	18.2
EU162478 Onthophagus xanthomerus	39.8	15.5	27.8	16.9	50	5.3	42.2	2.7	26	13.9	29.9	29.9	43	27.3	11.2	18.2
KJ371161 Pogonus reticulates	38.0	13.9	32.3	15.9	42	2.7	55.1	.5	27	13.4	30.5	28.9	45	25.7	11.2	18.2
KM447323 Pogonus iridipennis	39.0	13.5	30.5	16.9	44	1.6	51.9	2.7	28	13.4	28.3	29.9	45	25.7	11.2	18.2
KM445212 Laccophilus poecilus	39.9	12.8	31.4	15.9	45	1.6	52.4	1.1	30	11.8	30.5	27.8	45	25.1	11.2	18.7
MF638533 Pseudopsis montoraria	37.4	15.9	31.0	15.7	42	7.0	49.7	1.6	26	15.5	31.6	27.3	45	25.1	11.8	18.2
KY683690 Leptura annularis	38.9	15.2	29.6	16.4	45	5.3	48.7	1.1	27	15.0	28.3	29.9	45	25.1	11.8	18.2

Table 5.22: Percentage of evolutionary divergence of *Dysdercus ocreatus* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH674102	*Dysdercus ocreatus* (Kerala)	
2.	KU918208	*Pogonus chalceus*	17.88%
3.	LT991366	*Ochthebius arefniae*	17.89%
4.	KJ204201	*Agabus arcticus*	18.18%
5.	KM447323	*Pogonus iridipennis*	18.21%
6.	KM445212	*Laccophilus poecilus*	18.26%
7.	KC491230	*Brachypeplus glaber*	18.26%
8.	MF638533	*Pseudopsis montoraria*	18.32%
9.	JF888119	*Eusphalerum fenyesi*	19.00%
10.	KJ371161	*Pogonus reticulates*	19.02%
11.	KU918952	*Pterostichus strenuous*	19.05%
12.	KY683690	*Leptura annularis*	19.05%
13.	LT991393	*Ochthebius viridescens*	19.06%
14.	EU162478	*Onthophagus xanthomerus*	19.46%

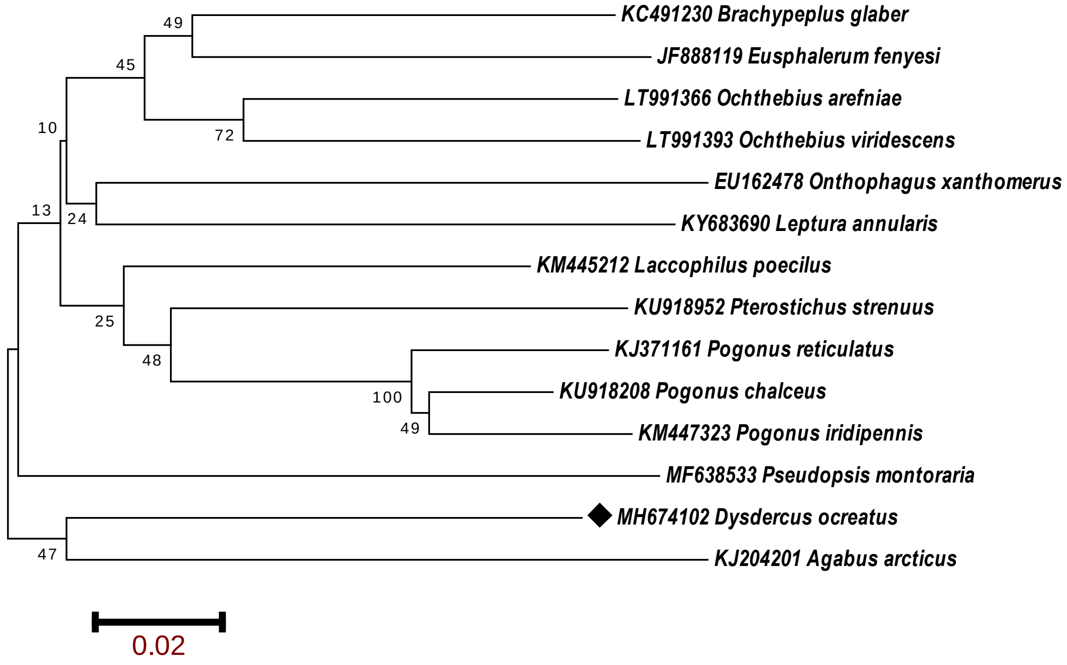


Figure 5.66: Phylogenetic relationship of *Dysdercus ocreatus* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 82.2% sequence similarity to the same genus reported from Kerala (KU918208). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Dysdercus ocreatus* showed bias to nucleotide AT, with following composition of nucleotides T = 37.8%, C = 16.6%, A = 29.8% and G = 15.9%. This greater AT content (67.6%) over GC content (32.5%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Dysdercus ocreatus* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed 17.88% to 19.46% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (17.88%) with those from Germany while 19.46% from USA (Table 5.22). On the basis of the data observed this species may be rooted from those found in USA and Germany which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from Germany and USA during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Dysdercus ocreatus* with related species. *Dysdercus ocreatus* isolated from Kerala (MH674102) showed 17.88% divergence with *Pogonus chalceus* (KU918208) from Germany and 19.46% divergence with *Onthophagus xanthomerus* (EU162478) from USA. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Dysdercus* *ocreatus* isolated from Kerala. Closest relative of *Dysdercus ocreatus* is *Agabus arcticus* from Canada represented within the same clade.

12. ***Campylomma vandicarinum*** (Carapezza, 1991)

Specimen details:

Voucher specimen : CUCV – 01 – A1
Date of collection : 20-Dec-2017
Locality : Kannur: Edat
Lat- Lon : 12.1006° N, 75.2306° E
GenBank accession : MH674101

Description and Distribution

Fig. 5.67: *Campylomma vandicarina*

Campylomma vandicarina is the only representative of the genus feeding on Juniperinus (Carapezza, 1991) (Figure. 5.67). The specimens differs from the typical form in the basis of following characters: ocular index in male 1.3-1.6, in female 1.6-1.8, length of third tarsal segment of posterior tarsus with second tarsal segment in ratio is 1.1-1.35, vesica with absence of spines and teeth on the anterior blade (Yee and Alston, 2012). Totally black appearance and first two antennal segments in both sexes make this species easily distinguished from all the other species of the genus *Campylomma*. This species is distributed in India, Sicily, Tunusia and East Mediterranean region.

Damage

The *Campylomma* bug causes extensive damage. Damage is inflicted by the nymphs, which feed on developing fruits causing dimpling and fruit distortion (Alston and Redding, 2011).

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Campylomma vandicarinum* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 587 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 5.68 – 5.71 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 5.23.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Campylomma vandicarinum* voucher collected during the present study (Table 5.23). It showed bias to nucleotide AT, with nucleotide composition with T = 29.6%, C = 21.2%, A = 34.4% and G = 14.8%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 5.24 and Figure 5.72 respectively.

> *Campylomma vendicarina* CDS-2018/ 587 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUCV-01-A1

> *Campylomma vendicarina*

```
ATTAGGCACATCACTAAGATGAATCATTCGTATTGAATTAGGTATACCAGGATCATTTAT
TGGAGATGATCAAACATATAATGTTGTAGTAACCGCACACGCATTCATCATAATTTTCTT
TATAGTAATACCAGTTATAATTGGAGGATTCGGAAACTGACTAGTACCATTAATAATTGG
AGCTCCCGATATAGCATTCCCCCGAATAAATAATATAAGATTCTGATTACTACCACCATC
AATTACACTTTTAATTATAAGAAGAATTGTAGAAAACGGTGCAGGCACAGGATGAACTGT
ATACCCACCCCTATCAACAACAATCTCCCATAATGGAGCATCAGTAGACCTCGCAATTTT
TTCCCTACACCTAGCCGGTGTATCATCAATCTTAGGGGCTGTAAACTTTATCTCAACCAT
TATAAATATACGATCAATCGGAATAACAATAGAACGAATCCCTTTATTCGTCTGATCAGT
AGGAATTACAGCCCTACTACTATTATTATCACTACCCGTGTTAGCAGGTGCAATCACTAT
ATTATTAACCGACCGAAATTTCAATACATCATTTTTTGACCCTTCAG
```

Figure 5.68: The partial DNA sequence of the mitochondrial COI gene of *Campylomma vendicarina*

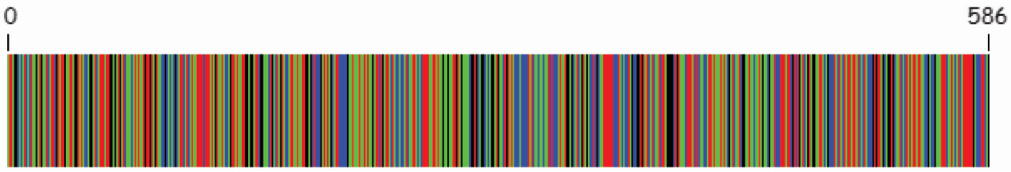


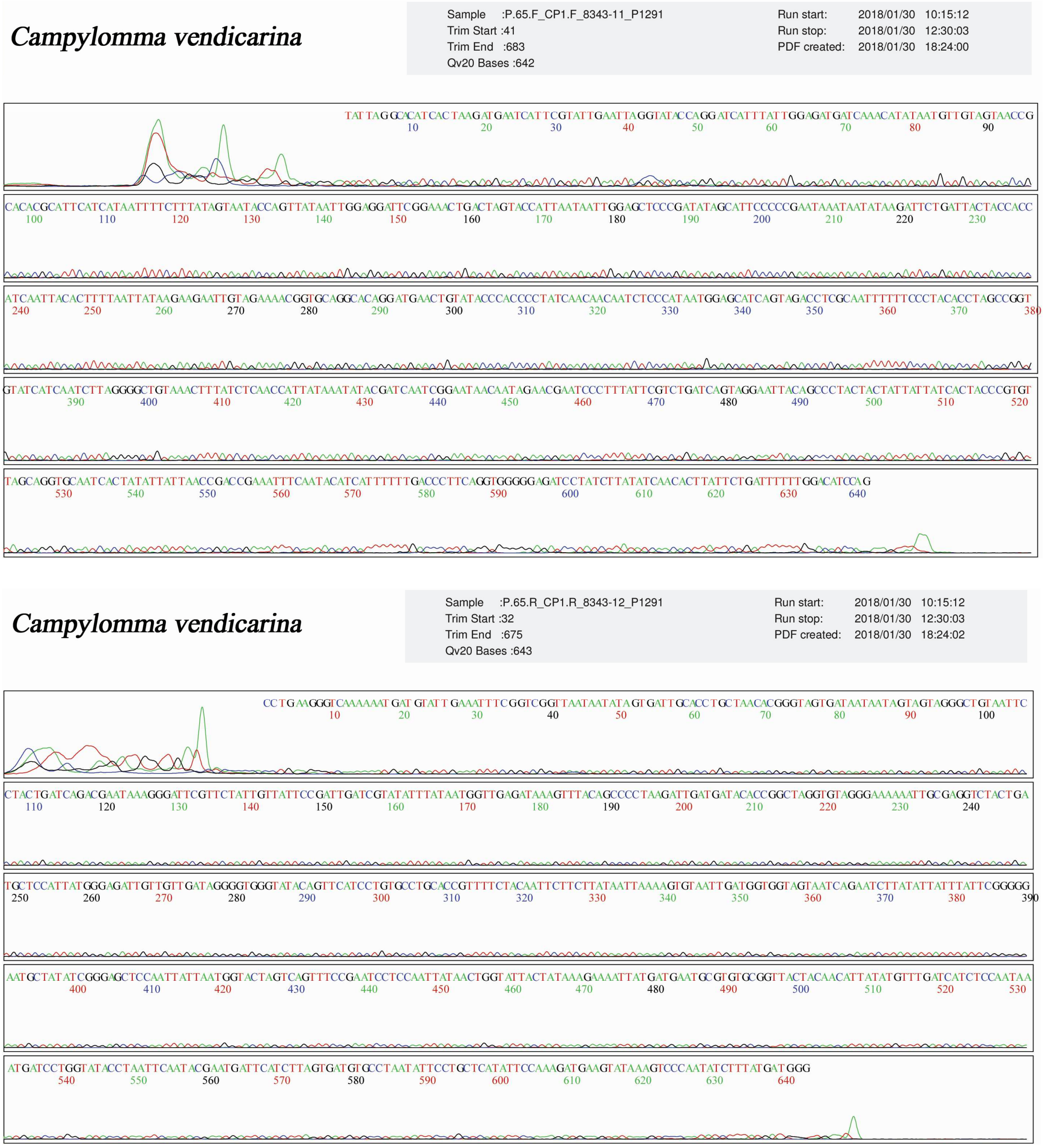
Figure 5.69: Molecular barcode of the mitochondrial COI gene of *Campylomma vendicarina*

> *Campylomma vendicarina* / 181AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUCV-01-A1

> *Campylomma vendicarina*

```
MPGSFIGDDQTYNVVVTAHAFIMIFFMVMPVMIGGFGNWLVPLMIGAPDMAFPRMNNMSF
WLLPPSITLLIMSSIVENGAGTGWTVYPPLSTTISHNGASVDLAIFSLHLAGVSSILGAV
NFISTIMNMRSIGMTMERIPLFVWSVGITALLLLLSLPVLAGAITMLLTDRNFNTSFFDP
S
```

Figure 5.70: The translation product of the mitochondrial COI gene of *Campylomma vendicarina*



Campylomma vendicarina

```
Sample      :P.65.F_CP1.F_8343-11_P1291       Run start:     2018/01/30   10:15:12
Trim Start  :41                               Run stop:      2018/01/30   12:30:03
Trim End    :683                              PDF created:   2018/01/30   18:24:00
Qv20 Bases  :642
```

```
TATTAGGCACATCACTAAGATGAATCATTCGTATTGAATTAGGTATACCAGGATCATTTATTGGAGATGATCAAACATATAATGTTGTAGTAACCG
10 20 30 40 50 60 70 80 90
CACACGCATTCATCATAATTTTCTTTATAGTAATACCAGTTATAATTGGAGGATTCGGAAACTGACTAGTACCATTAATAATTGGAGCTCCCGATATAGCATTCCCCCGAATAAATAATATAAGATTCTGATTACTACCACC
100 110 120 130 140 150 160 170 180 190 200 210 220 230
ATCAATTACACTTTTAATTATAAGAAGAATTGTAGAAAACGGTGCAGGCACAGGATGAACTGTATACCCACCCCTATCAACAACAATCTCCCATAATGGAGCATCAGTAGACCTCGCAATTTTTTCCCTACACCTAGCCGGT
240 250 260 270 280 290 300 310 320 330 340 350 360 370 380
GTATCATCAATCTTAGGGGCTGTAAACTTTATCTCAACCATTATAAATATACGATCAATCGGAATAACAATAGAACGAATCCCTTTATTCGTCTGATCAGTAGGAATTACAGCCCTACTACTATTATTATCACTACCCGTGT
390 400 410 420 430 440 450 460 470 480 490 500 510 520
TAGCAGGTGCAATCACTATATTATTAACCGACCGAAATTTCAATACATCATTTTTTGACCCTTCAGGTGGGGGAGATCCTATCTTATATCAACACTTATTCTGATTTTTTGGACATCCAG
530 540 550 560 570 580 590 600 610 620 630 640
```

Campylomma vendicarina

```
Sample      :P.65.R_CP1.R_8343-12_P1291       Run start:     2018/01/30   10:15:12
Trim Start  :32                               Run stop:      2018/01/30   12:30:03
Trim End    :675                              PDF created:   2018/01/30   18:24:02
Qv20 Bases  :643
```

```
CCTGAAGGGTCAAAAAATGATGTATTGAAATTTCGGTCGGTTAATAATATAGTGATTGCACCTGCTAACACGGGTAGTGATAATAATAGTAGTAGGGCTGTAATTC
10 20 30 40 50 60 70 80 90 100
CTACTGATCAGACGAATAAAGGGATTCGTTCTATTGTTATTCCGATTGATCGTATATTTATAATGGTTGAGATAAAGTTTACAGCCCCTAAGATTGATGATACACCGGCTAGGTGTAGGGAAAAAATTGCGAGGTCTACTGA
110 120 130 140 150 160 170 180 190 200 210 220 230 240
TGCTCCATTATGGGAGATTGTTGTTGATAGGGGTGGGTATACAGTTCATCCTGTGCCTGCACCGTTTTCTACAATTCTTCTTATAATTAAAAGTGTAATTGATGGTGGTAGTAATCAGAATCTTATATTATTTATTCGGGGG
250 260 270 280 290 300 310 320 330 340 350 360 370 380 390
AATGCTATATCGGGAGCTCCAATTATTAATGGTACTAGTCAGTTTCCGAATCCTCCAATTATAACTGGTATTACTATAAAGAAAATTATGATGAATGCGTGTGCGGTTACTACAACATTATATGTTTGATCATCTCCAATAA
400 410 420 430 440 450 460 470 480 490 500 510 520 530
ATGATCCTGGTATACCTAATTCAATACGAATGATTCATCTTAGTGATGTGCCTAATATTCCTGCTCATATTCCAAAGATGAAGTATAAAGTCCCAATATCTTTATGATGGG
540 550 560 570 580 590 600 610 620 630 640
```

Figure 5.71: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Campylomma vendicarina* using Forward and Reverse primers.

Table 5.23: Comparison of Nucleotide frequencies of COI gene sequence of *Campylomma vendicarina* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH674101 Campylomma vendicarina	29.6	21.2	34.4	14.8	24	18.0	37.6	20.1	39	24.9	16.4	19.6	25	20.6	49.2	4.8
MF673679 Campylomma sp.	31.0	20.1	33.3	15.5	23	19.6	36.0	21.7	39	24.9	16.9	19.6	32	15.9	47.1	5.3
AY253050 Neurocolpus arizonae	31.6	18.0	34.9	15.5	25	15.9	39.2	20.1	39	23.4	16.5	20.7	31	14.7	48.9	5.8
HQ106147 Phytocoris sewardi	31.6	18.5	34.0	15.9	26	16.9	36.0	20.6	38	23.8	18.0	20.1	30	14.8	48.1	6.9
KR044534 Neurocolpus nubilus	32.3	17.8	34.2	15.7	25	17.5	38.1	19.6	39	23.3	16.9	20.6	33	12.7	47.6	6.9
MG165718 Psallus confusus	29.5	20.8	33.9	15.9	23	21.2	34.4	21.2	38	24.9	16.4	20.6	27	16.4	50.8	5.8
KM022015 Adelphocoris seticornis	31.9	17.5	34.6	16.0	26	14.8	37.6	21.2	38	23.8	17.5	20.6	31	13.8	48.7	6.3
KR038177 Phytocoris sulcatus	31.0	18.9	34.0	16.0	26	15.3	37.6	20.6	39	23.3	17.5	20.6	28	18.0	47.1	6.9
KR032651 Taedia scrupea	32.3	18.2	33.7	15.9	28	14.3	37.6	20.1	39	23.3	17.5	20.6	30	16.9	46.0	6.9
KM021994 Phytocoris dimidiatus	33.0	17.1	34.3	15.5	28	15.4	35.6	21.3	39	22.8	18.0	20.1	32	13.2	49.2	5.3
GQ292102 Proboscidocoris varicornis	31.7	18.9	33.5	15.9	26	17.6	36.2	20.7	37	26.5	16.4	20.1	33	12.6	47.9	6.8
KM022238 Oncotylus punctipes	33.0	17.6	33.0	16.4	27	15.3	36.5	21.2	39	24.9	16.4	20.1	33	12.7	46.0	7.9
EF016736 Creontiades pacificus	31.0	19.9	33.3	15.7	25	17.6	35.6	21.8	38	25.9	16.9	19.6	31	16.3	47.4	5.8
EF016735 Creontiades pacificus	30.9	20.1	33.2	15.9	25	17.6	35.6	21.8	38	25.9	16.9	19.6	30	16.8	46.8	6.3

Table 5.24: Percentage of evolutionary divergence of *Campylomma vandicarinum* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH674101	*Campylomma vendicarina* (Kerala)	
2.	MF673679	*Campylomma* sp. (France)	7.61%
3.	KR044534	*Neurocolpus nubilus*	22.23%
4.	AY253050	*Neurocolpus arizonae*	22.60%
5.	EF016736	*Creontiades pacificus*	22.99%
6.	GQ292102	*Proboscidocoris varicornis*	23.01%
7.	EF016735	*Creontiades pacificus*	23.45%
8.	KM022015	*Adelphocoris seticornis*	23.68%
9.	MG165718	*Psallus confuses*	23.83%
10.	HQ106147	*Phytocoris sewardi*	24.04%
11.	KM021994	*Phytocoris dimidiatus*	24.09%
12.	KR038177	*Phytocoris sulcatus*	24.19%
13.	KM022238	*Oncotylus punctipes*	25.15%
14.	KR032651	*Taedia scrupea*	26.02%

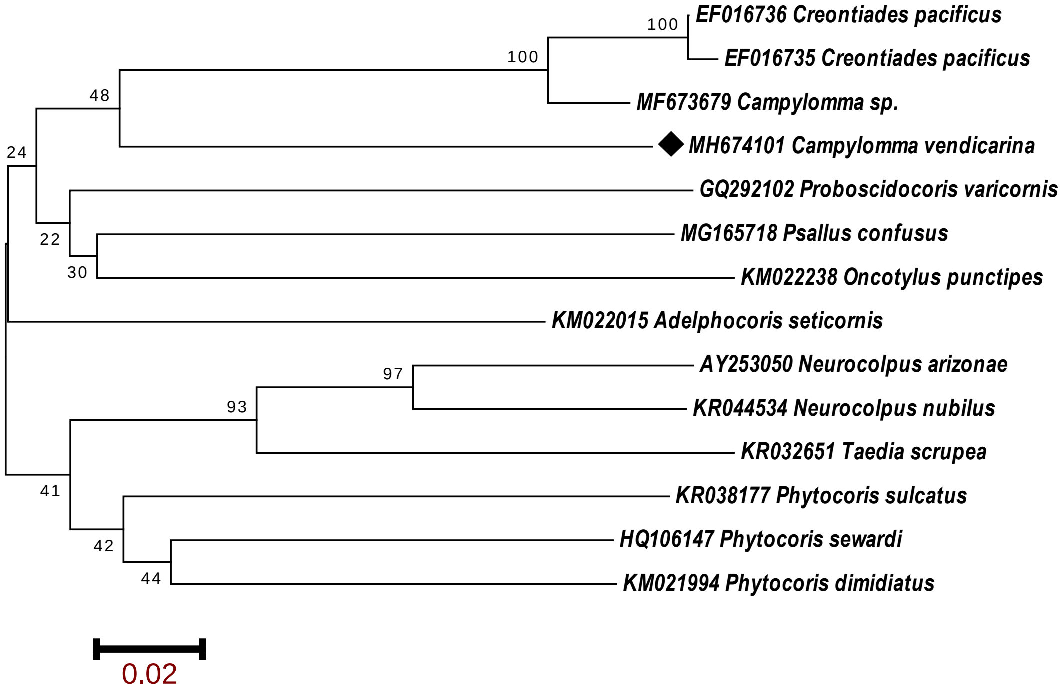


Figure 5.72: Phylogenetic relationship of *Campylomma vendicarina* inferred by NJ tree method

DISCUSSION

Both the nucleotide and peptide BLAST analysis showed that this species has 92.4% sequence similarity to the same genus reported from France (MF673679). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Campylomma vendicarina* showed bias to nucleotide AT, with following composition of nucleotides T = 29.6%, C = 21.2%, A = 34.4% and G = 14.8%. This greater AT content (64.0%) over GC content (36.0%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Campylomma vendicarina* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed 7.61% to 26.02% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (7.61%) with those from France while 26.02% from Canada (Table 5.24). On the basis of the data observed this species may be rooted from those found in Canada which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from France and Canada during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Campylomma vendicarina* with related species. *Campylomma vendicarina* isolated from Kerala (MH674101) showed 7.61% divergence with *Campylomma* sp. (MF673679) from France and 26.02% divergence with *Taedia scrupea* (KR032651) from Canada. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Campylomma vendicarina* isolated from Kerala. Closest relative of *Campylomma vendicarina* is *Campylomma* sp from France represented within the same clade.

Consolidated Phylogenetic Tree

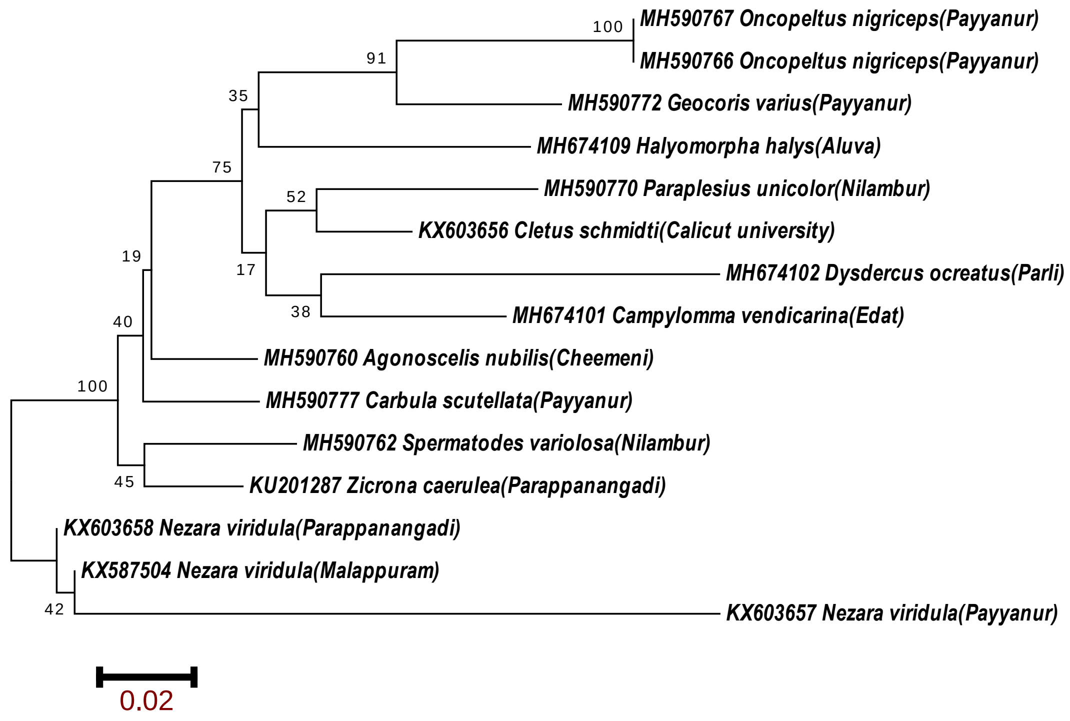


Figure 5.73: Phylogenetic relationship of different Hemipteran pests of cucurbitaceae isolated from selected districts of Kerala.

The analysis on the nucleotide composition of the COI gene sequences of Hemipteran pest of cucurbits of Kerala found to be varied within the every third position of codon. The analysis involved 12 species nucleotide sequence comparison (Figure 5.73). The phylogenetic relationships among them was analysed by NJ tree method. Families included in the analysis are Pentatomidae (superfamily Pentatomoidea), Lygaeidae and Geocoridae (superfamily Lygaeoidea), Alydidae and Coridae (superfamily Coreoidea), Pyrrhocoridae (superfamily Pyrrhocoroidea) and Miridae (superfamily Miroidea). The species from these five families are aligned nearly in the phylogenetic tree and monophyletic in origin. Species from the family Pentatomidae are originated from a main clade and then branches to subclades. *Nezara viridula* species are aligned in a same clade and they are included in the Pentatominae subfamily. *Geocoris varius* are aligned as an outgroup to *Oncopeltus nigriceps* and they are included in the Lygaeinae subfamily.

6

Lepidoptera

Lepidoptera is an order of insects including butterflies and moths. Among 10 per cent of the total described living species, about 180,000 species are lepidopterans (Capinera, 2008). It is considered to be one of the most widespread and widely recognizable insect order. Lepidoptera are characterized mainly by three derived features. The most apparent among them is the presence of scales covering the bodies, wings, and a proboscis. The scales are modified, flattened "hairs", and giving them their wide variety of colors and patterns. Almost all species have some form of membranous wings, except a few having reduced wings or are wingless. Lepidopteran species undergo holometabolism or "complete metamorphosis". The larvae of most lepidopteran species are major agricultural pests. Lepidopteran species are soft bodied, fragile, and almost defenseless, while the immature stages move slowly or are immobile, so all the stages are exposed to predation (Powell, 2009). Lepidoptera of Indian subregion belongs to five major families, Papilionidae, Pieridae, Nymphalidae, Lycaenidae and Hesperiidae classified under two superfamilies, Papilionoidea and Hesperioidea. The largest representative from India are under two families Nymphalidae with 450 species and Noctuidae with 1500 species respectively. Even though most butterflies and moths have negative affect on the economy, some species are a valuable economic resource. Some of the major Lepidopteran pests are included under Tortricidae, Noctuidae, and Pyralidae (Kristensen, 1999).

Among the various pest species, *Aphis gossypii* Glov. (Homoptera, Aphididae) cause maximum destruction to watermelon (Akkaya and Uygun, 1997).

Pickleworms, the larvae of *Diaphania nitidalis* Cramer (Lepidoptera: Crambidae), are a pest of cucurbits found in Hawaii, North America (primarily in the Southeastern United States), Central and South America, and the West Indies (Quaintance, 1898; Smith et al., 2013; Dupree et al., 1955; Van Balen, 1976; Heu et al., 2016). They are found feeding up on the fruits, buds, flowers, and stems of cucurbits (Quaintance, 1898; Smith et al., 2013; Van Balen, 1976). Newly-hatched larvae concentrate mainly on vegetative tissues, buds, and flowers, while older larvae tend to feed on fruits or tunnel the stems in the unavailability of former (Dupree et al., 1955; Smith et al., 2013; Liu et al., 2016). *Diaphania indica* (Saunders) (Lepidoptera: Pyralidae) also known as Pumpkin caterpillar, is the major pest of Cucurbitaceae (Ferguson and Opler, 2006). Melonworm, *Diaphania hyalinata* Linnaeus, occurs throughout most of Central and South America and the Caribbean (Medina Gaud et al., 1989). The attack of green semilooper (*Trichoplusia ni*) is during prime vegetative growth stage of cucurbit crop production and caused about 7.5 – 19.2% foliage damage. The cucumber moth, *Diaphania indica* (Saunders) (Lepidoptera: Crambidae), often known as Pumpkin caterpillar is a polyphagous pest and is particularly affecting cucurbits. During their outbreak larvae mainly attacks the leaves, infest flowers and fruits, causing considerable yield loss. They are also reffered as the cotton caterpillar and pumpkin caterpillar (Clavijo et al., 1995; Pandy, 1997). The incidence of *Diaphania nitidalis* (Stoll) as major pest in bitter gourd crop from Suriname is well reported (Nagaraju et al., 2010).

The systematic position of Lepidopteran pests of cucurbits collected and identified during the present study is given below:

Order: Lepidoptera; Suborder: Glossata

1. Superfamily: Noctuoidea

1.1. Family: Erebidae

1.1.1 Subfamily: Erebinae

- *Ophiusa coronate* (Fabricius, 1775)
- *Mocis proverai* (Zilli, 2000)

1.1.2. Subfamily: Aganainae

        - *Asota orbona* (Vollenhoven, 1863)
        - *Asota caricae* (Fabricius, 1775)

1.1.3. Subfamily: Calpinae

        - *Eudocima cocalus* (Cramer, 1780)

1.1.4. Subfamily: Hypocalinae

        - *Hypocala deflorata* (Fabricius, 1794)

1.2. Family: Noctuidae

1.1.2 Subfamily: Noctuinae

        - *Polytela gloriosae* (Fabricius, 1781)

2. Superfamily: Bombycoidea

2.1. Family: Sphingidae

2.1.1. Subfamily: Macroglossinae

        - *Acosmeryx anceus subdentata* (Rothschild and Jordan, 1903)

3. Superfamily: Pyraloidea

3.1. Family: Crambidae

3.1.1. Subfamily: Spilomelinae

        - *Diaphania indica* (Saunders, 1851)
        - *Glypholes bicolor* (Swainson, 1821)

4. Superfamily: Papilionoidea

4.1. Family: Nymphalidae

4.1.1. Subfamily: Satyrinae

        - *Melanitis leda* (Linnaeus, 1758)

5. Superfamily: Gelechioidea

5.1. Family: Xyloryctidae

5.1.1. Subfamily: Xyloryctinae

        - *Cryptophasa atecmarta* (Turner, 1917)

A brief description on the relevant higher taxa and the taxonomic key prepared for classification and morphological identification (with the help of suitable identification guides and expert consultation) of all the lepidopteran pests of cucurbits collected during the present study are given below:

SUBORDER: GLOSSATA

Glossata includes a majority of the species, with the most obvious difference - non-functioning mandibles, and elongated maxillary galeae or the proboscis. The retension of ancestral features of the wings including fore and hindwings are with relatively complete venation. Glossata contains the division Ditrysia, which contains 98% of all described species in Lepidoptera (Resh and Carde, 2009). Hindwings Rs with 3 o r4 branches, forewing almost always with jugal lobe marked produced. Maxillary galeae forming a proboscis; usually spirally coiled in repose; sometimes secondarily reduced or absent; mandibles often strongly reduced; articulations with head capsule undeveloped.

Key to the superfamilies of Suborder Glossata

1. Antennae pectinate, apically dilate, or simple; Chaetosema absent **2**

– Antennae clavate, apically dilate, or simple; Chaetosema absent **3**

2. Tympanal organs present in metathorax, frenulum nearly always present, small or medium sized; forewings with M2 and M3 approximated at origin ... . **Noctuoidea**

– Tympanal organs absent, frenulum nearly always lost, large and stout species; wings non-aculeate, with heteroneuran venation and amplexiform coupling ....**Bombycoidea**

3. Proboscis scaled; sternum II with paired anterior apodemes, wing membrane devoid of microtrichia; female genital system with copulatory orifice (on VIII) separate from more posterior ovipore..**4**

– Proboscis not scaled; antennae close together at bases and knobbed at tips; wings nonaculeate with heteroneuran venation and amplexiform coupling; hind pair with R 1 coincident with Sc forming Rs and Cu present, vinculum V -shaped produced into saccus; fore wings with R3 and R4 stalked............... .... **Papilionoidea**

4. Labial palps porrect, beak like or ascending; tympanal organs present at base of abdomen; sternum II of tortricoid type...................................... **Pyraloidea**

– Labial palps recurved, apical segment may exceed vertex, usually tapering; hindwing vein Rs unbranched, forewing with jugal lobe............ **Gelechioidea**

SUPERFAMILY: NOCTUOIDEA

Noctuoidea is one of the largest Lepidopteran superfamily, called night owl or owlet moths, with more than 70000 described species. The monophyly of Noctuoidea is well developed by the apomorphic presence of metathoracic tympanal organs and associated abdominal structures (Kitching and Rawlins, 1999).

Key to the families of Superfamily Noctuoidea

1. Basal abdominal brushes absent, direct articulation between the tegumen and vinculum, pleural sclerite fused to anteroventral margin of tegument; if tympanum present, bar or flat plate sclerite defines posterior margin of the tympanum proper, tympanal pocket 4 open, tympanal bullae separated; heavily sclerotised scaphium .... **Erebidae**

– Basal abdominal brushes present, no direct articulation between the tegumen and vinculum, pleural sclerite not fused to anteroventral margin of tegumen, nodular sclerite defines posterior margin of the typanum proper, tympanal pocket 4 closed, tympanal bullae fused, membranous scaphium .... **Noctuidae**

FAMILY: EREBIDAE

According to Fibiger and Lafontaine, 2005 Erebidae are characterized by the features such as spinneret with apical flaps, scaphlum sclerotised, larvae mainly on fungi, lower frons bare, possess double tympanal hood, female frenulum to single setae.

Key to the subfamilies of Family Erebidae

1. Tympanal organ absent; chaetosemata present..**2**

– Tympanal organ present; chaetosemata absent..**3**

2. Forewing without orbicular stigma in discal cell, hind wing plesiomorphic, tegmen longer than vinculum; juxta not fused with a heavily sclerotised anellus.. **Erebinae**

– Fore wing with orbicular stigma in discal cell, hind wing vein M2 located near the bottom of the cell adjacent to veins M3, Cu1, and Cu2antennae ciliate, filiform or slightly bipectinate; lower clypeofrons with distinct scaleless area..**Aganainae**

3. Scale tuft along the inner margin forewing, forewing variable in shape, sometimes with prominent bulge in middle of outer margin, pattern and colour variable; hindwing rounded to squared, pattern usually dull, rarely very contrasting .... **Calpinae**

– Scale tuft absent on forewing, forewing slightly scalloped on outer margin, sometimes with bulge in middle of outer margin, variable in pattern, or grey with a series of prominent white dots composing the lower part of the postmedial line; hindwing squared to rounded, black with yellow streaks .... **Hypocalinae**

SUBFAMILY: EREBINAE

Erebinae, larvae normally found and feed on vascular plants.They possess palpi beak, pleurite fused to tegument and vein M2 adjacent to M3 (Mitter et al., 2017).

Key to the species of pests collected from Subfamily Erebinae

1. Head and thorax pale reddish-brown; abdomen orange, segments fringed with black; forewings irrorated with dark specks; a short sub-basal dark line;

hindwing yellow coloured with two broad brown arcs .... ***Ophiusa coronate***

– Head and thorax light yellowish brown; abdomen beige. Forewing light beige, with sparse brown scales; elements of pattern dark brown; postmedial defined internally by pale yellowish beige .... ***Mocis proverai***

SUBFAMILY: AGANAINAE

Large sized and brightly coloured moths, antennae ciliate, filiform or slightly bipectinate, the pectinations slender, strongly curved and invested with a basal fan of fine cilia, tymbal organ absent, fore wing with an orbicular stigma in discal cell (Kitching and Rawlins, 1999). Male retinaculum elongate and narrow and is more than two times as long as wide ("barshaped"). Male retinaculum wider than long, lower part of clypeofrons with a small but distinct scaleless area.

Key to the species of pests collected from Subfamily Aganainae

1. Hindwing yellow or white without black spots; forewing with contrast veins and with discal and antemedial yellow to yellow orange spots .... ***Asota orbona***

– Hindwing yellow with dark spots or bands; forewing gray-brown or dark brown without white coastal fascia, one white small spot at centre .... ***Asota caricae***

SUBFAMILY: CALPINAE

These are usually moderate-sized erebids, often boldly patterned, with distinct scale tufts on inner margin of forewing. On head ocelli present, chaetosemata absent, head scales usually rough. Proboscis naked and heavily sclerotized which is sharply pointed apically, armed with tearing hooks and with basiconic sensilla modified into erectile barbs. Labial palps are ascending, long, slender or tufted. Antennae are filiform or pectinate, sometimes with long sensillae, with two scale rows per segment, half forewing the length or less. Thorax with dorsal scale tuft, with metathoracic tympanum; wings are heteroneurous, forewing variable in shape, sometimes with prominent bulge in middle of outer margin, with scale tuft along inner margin, pattern and colour variable; hindwing rounded to squared, pattern usually dull, rarely very contrasting; hind tibial spurs short, hind tarsal spines present. Abdomen smooth to hairy, rarely with dorsal scale tuft, rarely brightly coloured.

From this subfamily, only a single specimen is collected during the present study, *Eudocima cocalus* Cramer, 1780.

SUBFAMILY: HYPOCALINAE

Medium sized, often boldly patterned, with distinct scale tufts on inner margin of forewing. On head ocelli present, chaetosemata absent, head scales usually rough. Proboscis naked and heavily sclerotized which is sharply pointed apically, armed with tearing hooks and with basiconic sensilla modified into erectile barbs. Labial palps are ascending, long, slender or tufted. Thorax with metathoracic tympanum; wings heteroneurous, forewing slightly scalloped on outer margin, sometimes with bulge in middle of outer margin, variable in pattern, or grey with a series of prominent white dots composing the lower part of the postmedial line; hindwing squared to rounded, black with prominent yellow streaks or drab brown; hind tibial spurs long, hind tarsal spines present. Abdomen smooth, sometimes boldly patterned, sometimes with dorsal scale tuft (Handfield, 1999).

From this subfamily, only a single specimen is collected during the present study, *Hypocala deflorata* Fabricius, 1794.

FAMILY: NOCTUIDAE

Noctuidae are commonly known as owl moths, cutworms and armyworms. The family name was derived from the fact that this nocturnal species have eyes, which on reflection gives an orange glow. They are small to large moths with a wing span ranging 10 to 170 millimetres in size depending on the species. Majority of them are grey to brown in colour, often with dark and light areas on forewings. Most of the species are nocturnal and are light attracted (Kitching and Rawlins, 1999).

SUBFAMILY: NOCTUINAE

The members of subfamily Noctuinae, are characterised by having stoutly built bodies covered with long dense scales. The larvae, are known as cutworms and feed on a wide variety of low growing agricultural and ornamental plants. The name cutworm came from the behavioural pattern of the larvae, cutting off plant parts during the night which they drag back to their burrows in the soil so as to feed upon. Resting posture with forewings overlapping so as costae appearing parallel to the long axis of the body is a distinguished feature of Noctuinae.

From this subfamily, only a single specimen is collected during the present study, *Polytela gloriosae* Fabricius, 1781

SUPERFAMILY: BOMBYCOIDEA

Bombycoidea is an ecologically diverse and speciose superfamily of the order Lepidoptera. This superfamily includes many model organisms, even then taxonomy and classification of the superfamily has remained largely in disarray. Following Zwick (2008) and Zwick et al. (2011), ten families were recognized: Anthelidae, Apatelodidae, Bombycidae, Brahmaeidae, Carthaeidae, Endromidae, Eupterotidae, Phiditiidae, Saturniidae and Sphingidae. Bombycoidea is characterized by having stout species with heteromorphic antennae, vestigial proboscis, pectinate antennae, absence of tympanal organs, frenulum nearly always lost (Kitching et al., 2018).

FAMILY: SPHINGIDAE

The Sphingidae family of moths, commonly known as hawk moths, sphinx moths, and hornworms; it includes about 1,450 species. The family name was given by French Zoologist Pierre Andre Latreille in 1802 (Scoble et al., 1995). It is best represented mainly in the tropics, but species are almost cosmopolitan. They are basically moderate to large sized species and are distinguished among moths for their rapid, sustained flying ability (van Nieukerken et al., 2011). Their narrow wings and streamlined abdomens are adaptations for their rapid flight.

SUBFAMILY: MACROGLOSSINAE

The Macroglossinae are a sub-family of Sphingidae moths in the order Lepidoptera. The subfamily is divided into three tribes: Dilophonotini, Macroglossini and Philampelini. Most of species are crepuscular or nocturnal, but some of them are sometimes diurnal. Both males and females are relatively long lived (10 to 30 days). Prior to flight, most of the species shiver their flight muscles to warm them up, and, during flight, it was noticed that body temperatures may surpass 40 °C.

From this subfamily, only a single specimen is collected during the present study, *Acosmeryx anceus subdentata* Rothschild and Jordan, 1903**.**

SUPERFAMILY: PYRALOIDEA

The superfamily Pyraloidea comprises more than 15,576 described species worldwide (van Nieukerken et al., 2011). Among Lepidoptera, pyraloids encompasses very varied life history adaptations. Their distinguished features are Sternum II with paired anterior apodemes, wing membrane devoid of microtrichia; female genital system with copulatory orifice (on VIII) separate from more posterior ovipore. Due to their diverse feeding habits, many species feed either internally or externally on plants as leaf rollers, webbers, leaf miners, borers, root feeders, and some as seed feeders in their imago stage.

FAMILY: CRAMBIDAE

The Crambidae belong to the Superfamily Pyraloidea. More than 11,500 species have been described worldwide. Crambids are diverse and variable in morphology and biology. Habits and morphology of larvae are also highly variable among subfamilies, and include agricultural pest species. A large family containing the narrow-winged Crambinae 'grass-moths' such as *the Crambus* and *Agriphila* spp. as well as broad-winged species such as Pyraustinae which includes the colourful *Pyrausta* sp. The Crambidae possess a structure in the 'ears' called the *praecinctorium*, which joins two tympanic membranes, which is absent from the Pyralidae.

SUBFAMILY: SPILOMELINAE

Spilomelinae is a very large subfamily of the lepidopteran family Crambidae, the crambid snout moths. They were formerly included under Pyraustinae as tribe Spilomelini; Spilomelinae is usually treated as a separate subfamily within Pyralidae. They are characterized with well developed wings, hind wing veins Rs is unbranched; forewing with jugal lobe having not marked produced. Wing membrane usually with more or less extensive cover of microtrichia; sternum II without anterior apodemes; female with a single genital opening.

Key to the species of pests collected from Subfamily Spilomelinae

1. Forewings without white marks; head not patterned, without pigmented spot at genal angle; mandible with a projection on lateral margin; a pair of transverse plates posterior to dorsal pinacula on mesothorax; prothoracic shield without dark reniform spot; abdominal segments without conical black chalazae.. ***Diaphania indica***

– Forewings with two white marks on basal part of inner margin; a black-edged semihyaline white medial band not reaching the costa, often with a spot in the cell before it; a large oval black-edged hyaline white postmedial patch between the subcostal and vein 2, with a small white triangular spot on costa; hindwing with the basal area hyaline white, outer area blackbrown, with a black line on inner edge and a fine marginal black line; the cilia white; black-brown colour, head, collar, and patagia mostly white; palpi black, white below; anal tuft black.. ***Glypholes bicolor***

SUPERFAMILY: PAPILIONOIDEA

The superfamily Papilionoidea contains major butterflies except for the skippers, which were classified in superfamily Hesperioidea . Butterflies are considered as being the best group of insects for analyzing the patterns and the distribution of terrestrial biotic diversity (Robbins and Opler, 1997; Boriani et al., 2005). The Papilionoidea are medium to large, rarely small lepidopterans. They are among the most specialized Lepidopterans both in terms of behavior and ecology and also display aposematic or warning coloration and engage in mimicry relationships (Daly and Buntin, 2005). The superfamily Papilionoidea comprises five families that including Nymphalidae, Lycaenidae, Papilionidae, Pieridae and Satyridae. The largest families among Papilionoidea are Lycaenidae and Nymphalidae (Daly and Buntin, 2005; Ayberk et al., 2007).

FAMILY: NYMPHALIDAE

Nymphalidae, are characterized with brush-footed butterflies having the ventral surface of the antennae tricarinate and the species are with four walking legs. Head is mostly angular or covered with spines. Abdominal prolegs with triordinal crochlets usually. Secondary setae fairly equal in length or larvae with distinct warts or spines. It was reported that there are about 6,000 species worldwide.

SUBFAMILY: SATYRINAE

The Satyrinae, otherwise known as satyrines or satyrids, commonly known as the browns, are a subfamily of the Nymphalidae (brush-footed butterflies), formerly considered a distinct family, Satyridae. This group contains nearly half of the known diversity of brush-footed butterflies. There are about more than 2400 of the Satyrinae species worldwide.They prefer moist and semishaded habitats and are generally weak fliers and often shun bright sunlight. The caterpillars feed chiefly on monocotyledonous plants such as palms, grasses, bamboos and small agricultural plants.

From this subfamily, only a single specimen is collected during the present study, *Melanitis leda* Linnaeus, 1758.

SUPERFAMILY: GELECHIOIDEA

Gelechioidea (the name originated from the type genus *Gelechia*, "keeping to the ground") is the superfamily of moths that includes the case-bearers, twirler moths, and relatives, also simply called curved-horn moths or gelechioid moths. The name "curved-horn moths" refers to one of the few conspicuous features found in (almost) every Gelechioidea, and, at least in the more extreme developments, uniqueness include the well developed labial palps, though not thickened, and form more or less gently curved protrusions with the drawn-out, pointed tip. Their proboscis is generally well-developed, allowing for long-lived imagines (adults); the proximal part of the proboscis is scaly. The Gelechioidea vary extensively in their habitus; most have small hindwings with long, hairy fringes, .are not easily seen in the living animal as they are tucked under the forewings at rest. The body is usually compressed, either dorsoventrally or laterally.

FAMILY: XYLORYCTIDAE

Xyloryctidae is a family of moths under the superfamily Gelechioidea described by Edward Meyrick in 1914. Most genera are found in the Indo-Australian region. While many of these moths are tiny, but some members of the family grow to a wingspan of up to 66 mm, making them giants among the micromoths. The larvae of most members of this family are arboreal, whether they burrow into branches, bore into flower heads, tunnel under bark, or feed on lichens. Moths of the genus are pests and have crossed over from their wild host plant to become serious pests even for the cultivated stone fruit trees, particularly cherries.

SUBFAMILY: XYLORYCTINAE

The Xyloryctinae are a subfamily of Gelechioid moths which were first identified by examining the habits of some of their larvae, which make residential tunnels in wood, collecting leaves by night which they secure with silk to the entrance of the burrow, feeding on them as they dry out. Although most feed on leaves, some feed on bark, cambium, or lichens. Internationally, Xyloryctines are pests of commercial tree-grown crops, including tea, coffee, cocoa, coconut, and macadamia.

From this subfamily, only a single specimen is collected during the present study, *Cryptophasa atecmarta* Turner, 1917**.**

1. *Ophiusa coronata* (Fabricius, 1775)

Specimen details:

Voucher specimen	:	CUOC – 01 – A1
Date of collection	:	25-July-2016
Locality	:	Malappuram: Parappanangadi
Lat- Lon	:	11.0605° N, 75.8508° E
GenBank accession	:	KX503057

Description and distribution:

Fig 6.1.: Ophiusa coronata

Ophiusa coronata have wing expansion 90 mm (Figure 6.1). Head and thorax is pale reddish-brown; abdomen orange, segments fringed with black. Fore wing irrorated with dark specks with a short sub-basal dark line. Hind wing orange, with broad and sub-marginal fuscous black bands not reaching inner margin, the latter widest towards costa. The adult moths are characterized by having brown forewings with a dark spot at the centre, the hindwings bright yellow coloured with two broad brown arcs, the wingspan is about 6 cms. The caterpillars are the brown loopers, with a black head that having two white stripes on each side and have two black and white knobs on the tail (Richardson, 2015). The species occur commonly in countries from India to the islands of the Pacific, including Myanmar, Sri Lanka, Java, Hong Kong, Tahiti, Thailand and Australia.

Damage

This moth is an important agricultural pest, causing damage by introducing to fruit by piercing the fruit with its strong proboscis into the fruits to suck its juice. The caterpillars are voracious feeders mainly on the trees (Richardson, 2015).

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Ophiusa coronata* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 462 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 6.2 – 6.5 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 6.1.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Ophiusa coronata* voucher collected during the present study (Table 6.2). It showed bias to nucleotide AT, with nucleotide composition with T = 40.2%, C = 14.9%, A = 31.0% and G = 13.9%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 6.2 and Figure 6.6 respectively.

>*Ophiusa coronata* CDS-2016 / 552 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUOC-01-A1

> *Ophiusa coronata*

```
AGATTGTGAACTTCATTAAGTTTATTAATTCGAGCAGAATTAGGTAATCCTGGATCATTA
ATTGGAGATGATCAAATCTATAATACCATTGTTACAGCTCATGCTTTTATTATAATTTTT
TTTATAGTAATACCAATTATAATTGGAGGATTTGGAAATTGATTAGTTCCCTTAATATTA
GGAGCTCCTGATATAGCTTTCCCTCGAATAAATAATATAAGTTTTTGACTTCTTCCCCCT
TCTTTAACATTATTAATTTCAAGTAGAATTGTAGAAAATGGAGCAGGAACTGGATGAACT
GTTTACCCCCCTTTATCATCAAATATTGCTCACAGTGGAAGATCAGTAGATTTAGCTATT
TTTTCTCTACATTTAGCAGGTATTTCTTCAATTTTAGGAGCTATTAATTTTATTACTACA
ATTATTAATATACGATTAAATAATTTAATGTTTGATCAAATACCTTTATTTGTTTGAGCT
GTAGGAATTACTGCATTCTTACTATTATTATCTTTACCTGTTTTAGCAGGAGCTATTACC
ATACTTCTAACT
```

Figure 6.2: The partial DNA sequence of the mitochondrial COI gene of *Ophiusa coronata*

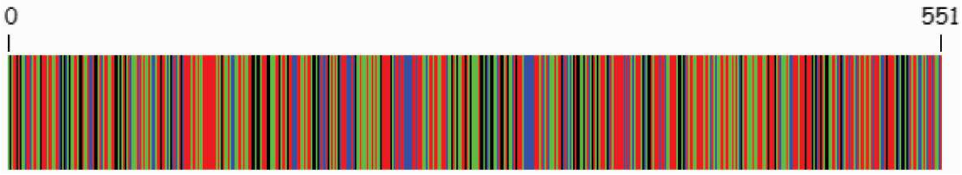


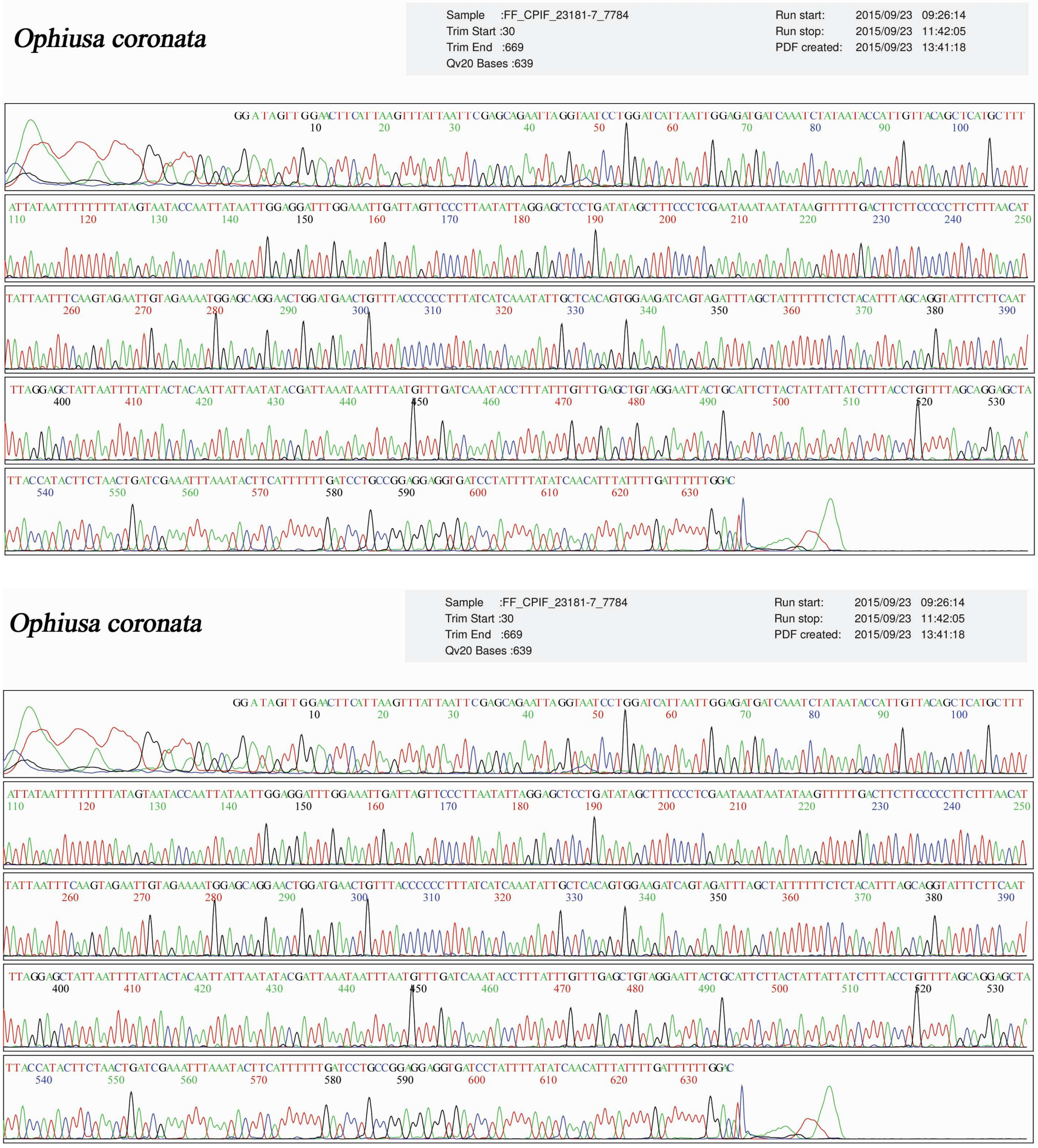
Figure 6.3: Molecular barcode of the mitochondrial COI gene of *Ophiusa coronata*

> *Ophiusa coronata* / 147AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUOC-01-A1

> *Ophiusa coronata*

```
MIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGT
GWTVYPPLSSNIAHSGSSVDLAIFSLHLAGISSILGAINFITTIINMRLNNLMFDQMPLF
VWAVGITAFLLLLSLPVLAGAITMLLT
```

Figure 6.4: The translation product of the mitochondrial COI gene of *Ophiusa coronata*


Ophiusa coronata

Sample :FF_CPIF_23181-7_7784
Trim Start :30
Trim End :669
Qv20 Bases :639

Run start: 2015/09/23 09:26:14
Run stop: 2015/09/23 11:42:05
PDF created: 2015/09/23 13:41:18

Ophiusa coronata

Sample :FF_CPIF_23181-7_7784
Trim Start :30
Trim End :669
Qv20 Bases :639

Run start: 2015/09/23 09:26:14
Run stop: 2015/09/23 11:42:05
PDF created: 2015/09/23 13:41:18

Figure 6.5: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Ophiusa coronata* using Forward and Reverse primers.

Table 6.1: Comparison of Nucleotide frequencies of COI gene sequence of *Ophiusa coronate* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
KX503057 Ophiusa coronate (Kerala)	40.2	14.9	31.0	13.9	47	5.4	46.2	1.1	29	13.6	33.2	24.5	45	25.5	13.6	16.3
KF491953 Ophiusa coronata	40.6	14.3	31.0	14.1	48	5.4	46.2	.5	29	13.0	32.6	25.0	45	24.5	14.1	16.8
HQ950440 Ophiusa coronata	40.6	14.3	30.8	14.3	48	5.4	45.7	1.1	29	13.0	32.6	25.0	45	24.5	14.1	16.8
HQ950439 Ophiusa hituense	40.4	15.0	30.4	14.1	48	7.1	44.6	.5	29	13.6	32.6	25.0	45	24.5	14.1	16.8
HQ950444 Ophiusa microrrhaea	41.5	13.6	30.8	14.1	53	1.1	45.7	.5	27	15.2	32.6	25.0	45	24.5	14.1	16.8
JN304502 Noctuidae sp.	41.8	14.5	29.7	13.9	52	5.4	42.4	.0	29	13.6	32.6	25.0	45	24.5	14.1	16.8
HQ950445 Ophiusa microrrhaea	41.5	13.6	30.8	14.1	53	1.1	45.7	.5	27	15.2	32.6	25.0	45	24.5	14.1	16.8
MF132708 Mimophisma delunaris	40.4	14.3	31.3	13.9	47	6.0	47.3	.0	30	12.5	32.6	25.0	45	24.5	14.1	16.8
HQ950424 Ophiusa discriminans	39.3	14.7	32.1	13.9	44	6.5	49.5	.0	29	13.0	32.6	25.0	45	24.5	14.1	16.8
KX862493 Clytie illunaris	40.8	13.4	31.5	14.3	47	3.8	48.4	.5	30	12.0	32.6	25.0	45	24.5	13.6	17.4
KP083431 Ophiusa tirhaca	40.8	13.4	31.7	14.1	47	3.8	48.9	.0	30	12.0	32.6	25.0	45	24.5	13.6	17.4
MF131783 Euclystis guerini	40.9	15.2	29.9	13.9	53	4.3	42.4	.5	26	15.8	33.2	25.0	44	25.5	14.1	16.3
MF131814 Catocala californica	41.8	14.5	29.9	13.8	53	4.3	42.4	.0	28	14.7	33.2	24.5	45	24.5	14.1	16.8
MF130755 Catocala hermia	42.0	14.3	29.9	13.8	54	3.8	42.4	.0	28	14.7	33.2	24.5	45	24.5	14.1	16.8
KJ380868 Ophiusa triphaenoides	40.0	13.9	32.1	13.9	47	2.7	49.5	.5	28	13.6	33.2	25.5	45	25.5	13.6	15.8

Table 6.2: Percentage of evolutionary divergence of *Ophiusa coronata* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	KX503057	*Ophiusa coronate* (Kerala)	
2.	KF491953	*Ophiusa coronate* (USA)	0.00%
3.	HQ950440	*Ophiusa coronate*	0.30%
4.	HQ950439	*Ophiusa hituense*	8.23%
5.	HQ950444	*Ophiusa microrrhaea*	9.54%
6.	JN304502	*Noctuidae* sp.	9.92%
7.	HQ950445	*Ophiusa microrrhaea*	9.93%
8.	HQ950424	*Ophiusa discriminans*	10.31%
9.	MF131783	*Euclystis guerini*	11.03%
10.	KX862493	*Clytie illunaris*	11.44%
11.	KP083431	*Ophiusa tirhaca*	11.52%
12.	MF130755	*Catocala hermia*	11.59%
13.	KJ380868	*Ophiusa triphaenoides*	11.97%
14.	MF132708	*Mimophisma delunaris*	11.98%
15.	MF131814	*Catocala californica*	12.00%

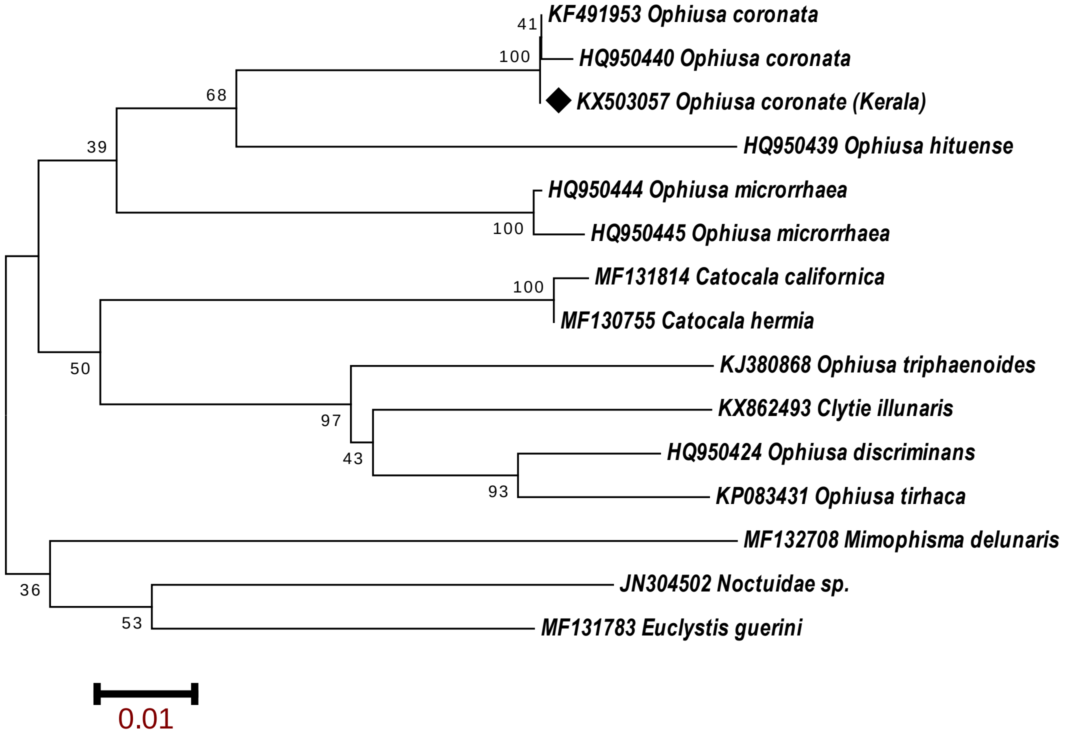


Figure 6.6: Phylogenetic relationship of *Ophiusa coronata* inferred by NJ tree method

DISCUSSION

Both nucleotide and peptide BLAST analysis showed that this species has 100% sequence similarity to the same genus reported from USA (KF491953). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Ophiusa coronata* showed bias to nucleotide AT, with following composition of nucleotides T = 40.2%, C = 14.9%, A = 31.0% and G = 13.9%. This greater AT content (71.2%) over GC content (28.2%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Ophiusa coronata* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 0% to 12% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has no divergence (0%) with those from USA while 12% to North America (Table 6.2). On the basis of the data observed this species may be rooted from those found in North America which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from North America during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Ophiusa coronata* with related species. *Ophiusa coronata* isolated from Kerala (KX503057) showed 0% divergence with *Ophiusa coronate* (KF491953) from USA and 12% divergence with *Catocala californica* (MF131814) from North America. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Ophiusa coronata* isolated from Kerala. Closest relative of *Ophiusa coronata* is *Ophiusa coronata* from USA represented within the same clade.

2. ***Mocis proverai*** (Zilli, 2000)

Specimen details:

Voucher specimen : CUMP – 01 – A1
Date of collection : 24-Apr-2016
Locality : Malappuram: Nilambur
Lat- Lon : 11.2794º N, 76.2398º E
GenBank accession : MH590771

Description and distribution:

Mocis proverai belongs to Erebidae family (Figure. 6.7). According to Zilli and Pavesi (2015), the background of *M. proverai* is often light coloured, particularly in the distal half of the medial field of the forewing, hence producing a contrasting pattern with dark markings. The male possess unequal superior and inferior processes of the left clasper, the superior are usually more than twice as long as the inferior, there by conferring a pronounced asymmetry. In the female genitalia, the appendix bursae of *M. proverai* is weakly sclerotized and poorly defined, consisting of some posterolateral ribs lying on the flank of corpus like a pouch, but practically becoming part of the corpus when this is fully extended. The length of the forewings ranges between 18–21 mm (0.71–0.83 in). *M. proverai* is found to be distributed in Asia, Africa and the Arabian Peninsula.

Fig. 6.7: *Mocis proverai*

Damage:

The larvae feed on various grasses (Zilli and Pavesi, 2015). The young larvae scrape the top surface of the leaf, the latter instars feed on the entire leaves. They feed at night to avoid predation and spend the day in a shelter at the base of the leaf (Davis, 1967).

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Mocis proverai* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 603 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 6.8 – 6.11 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 6.3.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Mocis proverai* voucher collected during the present study (Table 6.3). It showed bias to nucleotide AT, with nucleotide composition with T = 39.0%, C = 15.5%, A = 31.2% and G = 14.3%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 6.4 and Figure 6.12 respectively.

> *Mocis proverai* CDS-2018/ 603 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUMP-01-A2

> *Mocis proverai*

```
AGCTGGTATAGTAGGAACTTCATTAAGATTACTAATTCGAGCTGAATTAGGAAACCCTGG
ATCTTTAATTGGAGATGATCAAATTTATAATACTATTGTTACTGCTCACGCTTTTATTAT
AATTTTTTTTATAGTTATACCAATTATAATTGGGGGATTTGGAAACTGATTAGTACCTTT
AATATTAGGAGCTCCTGATATAGCATTCCCACGAATAAATAATATAAGTTTTTGACTTTT
ACCACCCTCATTAACTCTTTTAATTTCAAGTAGAATTGTAGAAAACGGAGCAGGTACTGG
ATGAACTGTTTATCCCCCACTTTCATCTAATATTGCTCATAGAGGTAGTTCAGTTGATTT
AGCTATTTTTTCATTACATTTAGCCGGAATTTCTTCAATTTTAGGAGCTATTAATTTCAT
TACAACAATTATTAATATACGACTTAATAACTTGATATTTGATCAAATACCTTTATTTGT
ATGAGCAGTAGGAATTACTGCTTTTCTATTACTCCTTTCTTTACCAGTATTAGCAGGAGC
TATTACTATATTATTAACAGATCGAAATTTAAATACATCTTTTTTCGATCCAGCAGGAGG
AGG
```

Figure 6.8: The partial DNA sequence of the mitochondrial COI gene of *Mocis proverai*

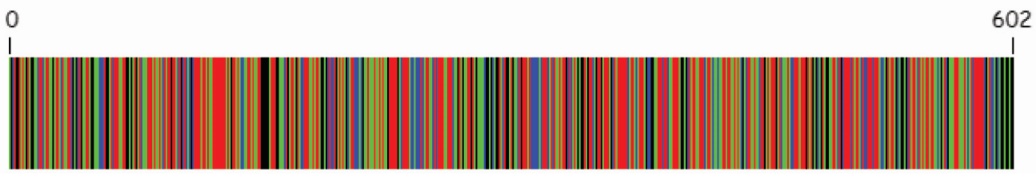


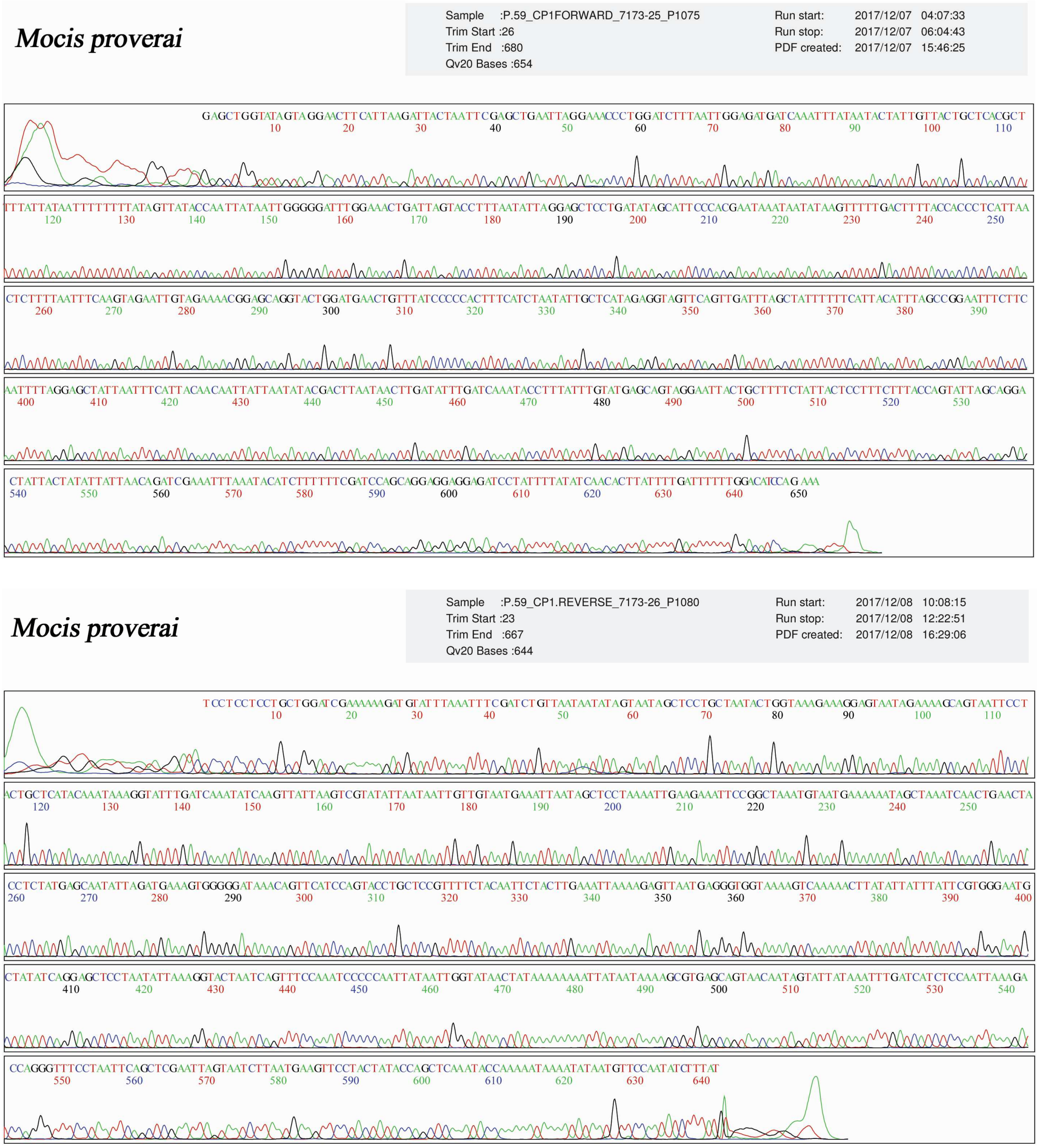
Figure 6.9: Molecular barcode of the mitochondrial COI gene of *Mocis proverai*

> *Mocis proverai* / 198AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUAF-01-A1

> *Mocis proverai*

```
MVGTSLSLLIRAELGNPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWLVPLML
GAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHSGSSVDLAI
FSLHLAGISSILGAINFITTIINMRLNNLMFDQMPLFVWAVGITAFLLLLSLPVLAGAIT
MLLTDRNLNTSFFDPAGG
```

Figure 6.10: The translation product of the mitochondrial COI gene of *Mocis proverai*



Mocis proverai

Sample :P.59_CP1FORWARD_7173-25_P1075
Trim Start :26
Trim End :680
Qv20 Bases :654
Run start: 2017/12/07 04:07:33
Run stop: 2017/12/07 06:04:43
PDF created: 2017/12/07 15:46:25

Mocis proverai

Sample :P.59_CP1.REVERSE_7173-26_P1080
Trim Start :23
Trim End :667
Qv20 Bases :644
Run start: 2017/12/08 10:08:15
Run stop: 2017/12/08 12:22:51
PDF created: 2017/12/08 16:29:06

Figure 6.11: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Mocis proverai* using Forward and Reverse primers.

Table 6.3: Comparison of Nucleotide frequencies of COI gene sequence of *Mocis proverai* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH590771 Mocis proverai (Kerala)	39.0	15.5	31.2	14.3	46	6.1	46.5	1.0	27	14.6	32.3	26.3	44	25.9	14.7	15.7
KX860613 Mocis proverai	39.6	14.8	31.0	14.5	47	5.6	46.5	1.0	27	14.1	32.8	25.8	45	24.9	13.7	16.8
KX861467 Mocis proverai	39.8	14.7	31.0	14.5	47	5.1	46.5	1.0	27	14.1	32.8	25.8	45	24.9	13.7	16.8
KJ380874 Mocis frugalis	39.0	15.2	31.4	14.5	47	5.1	47.2	1.0	27	14.6	32.3	26.3	43	25.8	14.6	16.2
JQ344624 Lepidoptera sp.	39.6	14.8	31.0	14.5	47	5.6	46.5	1.0	27	14.1	32.8	25.8	45	24.9	13.7	16.8
KJ380850 Noctuidae sp.	38.6	15.5	31.0	14.8	46	6.1	46.2	2.0	27	14.6	32.3	26.3	43	25.8	14.6	16.2
KJ380681 Mocis latipes	40.6	14.2	30.9	14.3	49	4.5	46.0	.5	28	13.6	32.3	25.8	45	24.4	14.2	16.8
KJ374994 Mocis latipes	40.5	14.2	30.9	14.5	49	4.0	46.0	1.0	28	13.6	32.8	25.8	45	24.9	13.7	16.8
HQ555235 Lepidoptera sp.	40.3	14.3	31.0	14.3	48	4.5	46.5	.5	28	13.6	32.8	25.8	45	24.9	13.7	16.8
MF130206 Mocis disseverans	39.5	15.0	31.4	14.2	46	6.1	47.5	.0	27	14.1	32.8	25.8	45	24.9	13.7	16.8
MF131097 Mocis disseverans	39.3	15.2	30.9	14.7	46	6.6	46.0	1.5	27	14.1	32.8	25.8	45	24.9	13.7	16.8
MF133168 Mocis texana	40.3	13.7	31.7	14.3	47	3.5	48.5	.5	29	12.6	32.8	25.8	45	24.9	13.7	16.8
MF126702 Mocis texana	40.3	13.7	31.7	14.3	47	3.5	48.5	.5	29	12.6	32.8	25.8	45	24.9	13.7	16.8
GU336767 Mocis diffluens	40.1	14.7	31.0	14.2	49	4.5	46.5	.0	27	14.6	32.8	25.8	45	24.9	13.7	16.8

Table 6.4: Percentage of evolutionary divergence of *Mocis proverai* with its closely related species accessible from NCBI GenBank

Sl. No.	**Accession No**	**Organism**	**Percentage of divergence**
1.	MH590771	*Mocis proverai* (Kerala)	
2.	KX860613	*Mocis proverai* (Pakisthan)	0.00%
3.	JQ344624	*Lepidoptera* sp.	0.00%
4.	KX861467	*Mocis proverai*	0.14%
5.	KJ380874	*Mocis frugalis*	0.27%
6.	KJ380850	*Noctuidae* sp.	0.69%
7.	KJ380681	*Mocis latipes*	3.25%
8.	HQ555235	*Lepidoptera* sp.	3.25%
9.	KJ374994	*Mocis latipes*	3.26%
10.	MF130206	*Mocis disseverans*	3.41%
11.	MF131097	*Mocis disseverans*	3.74%
12.	MF133168	*Mocis texana*	3.85%
13.	MF126702	*Mocis texana*	3.85%
14.	GU336767	*Mocis diffluens*	4.13%

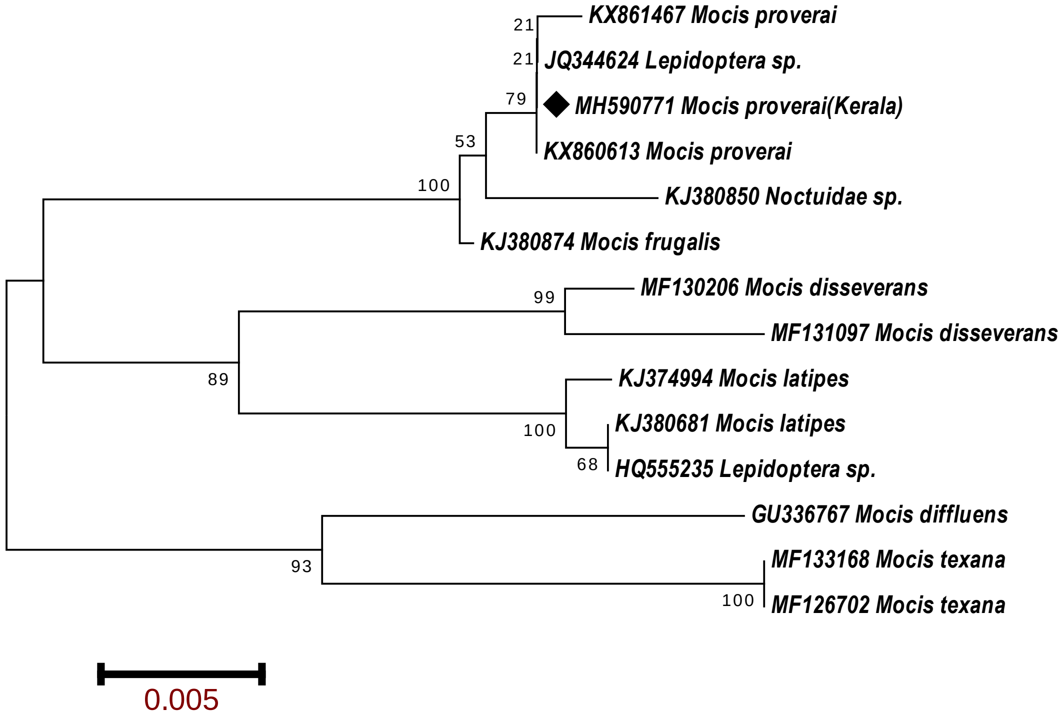


Figure 6.12: Phylogenetic relationship of *Mocis proverai* inferred by NJ tree method

DISCUSSION

Both nucleotide and peptide BLAST analysis showed that this species has 100% sequence similarity to the same genus reported from Kerala (KX860613). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Mocis proverai* showed bias to nucleotide AT, with following composition of nucleotides T = 39.0%, C = 15.5%, A = 31.2% and G = 14.3%. This greater AT content (70.2%) over GC content (29.8%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Mocis proverai* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 0% to 4.13% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has no divergence (0%) with those from Pakisthan while 4.13% to Canada (Table 6.4). On the basis of the data observed this species may be rooted from those found in Canada which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from Canada during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Mocis proverai* with related species. *Mocis proverai* isolated from Kerala (MH590771) showed 0% divergence with *Mocis proverai* (KX860613) from Pakisthan and 4.31% divergence with *Mocis diffluens* (GU336767) from Canada. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Mocis proverai* isolated from

Kerala. Closest relative of *Mocis proverai* is *Mocis proverai* from Pakisthan represented within the same clade.

3. ***Asota orbona*** (Vollenhoven, 1863)

Specimen details:

Voucher specimen	:	CUAO – 01 – A1
Date of collection	:	07-Sep-2016
Locality	:	Malappuram: Parappanangadi
Lat- Lon	:	11.0605° N, 75.8508° E
GenBank accession	:	KX603654

Description and distribution:

Asota orbona moth belongs to Noctuidae family (Figure. 6.13). They are characterized by the yellowish coloured wing with brown shading, forewing possess four spots with black dots on the thorax and the bases of the forewing. Hindwing with yellow ground colour, without black spots. Forewing with yellow to yellow orange spots, with contrast veins and with a yellow discal spots and with discal and antemedial spots. The wings are patterned in two different ways: wings close to the body are orange with small black dots, further along the wing they are orange and brown with two large creamy coloured markings on either of them. It has been reported from India, Indonesia, New Guinea and Queensland.

Fig.6.13.: *Asota orbona*

Damage:

Final instar larvae forage voraciously on pioneer host leaves, which is active at night but elusive during day light (van Nieukerken et al., 2011).

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Asota orbona* collected has been amplified using the primer LEP (Table 3.1). The PCR

amplification yielded 525 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 6.14 – 6.17 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 6.5.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Asota orbona* voucher collected during the present study (Table 6.5). It showed bias to nucleotide AT, with nucleotide composition with T = 39.2%, C = 17.5%, A = 29.5% and G = 13.7%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 6.6 and Figure 6.18 respectively.

> *Asota orbona* CDS-2016/ 525bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUAO-01-A1

> *Asota orbona*

```
CCTTTATTATAATTTTCTTTATAGTTATACCTATTATAATTGGAGGATTTGGTAATTGAT
TAGTACCTCTTATATTAGGAGCCCCCGATATAGCTTTCCCCCGAATAAATAATATAAGTT
TTTGACTTCTTCCCCCCTCATTAACTCTACTAATTTCAAGAAGAATTGTTGAAAATGGAG
CAGGTACCGGATGAACAGTTTACCCCCCACTTTCATTTAATATCGCTCATGGAGGAAGAT
CAGTTGATTTAGCTATTTTTTTCATTACATTTAGCTGGAATTTCTTCAATTTTAGGAGCTA
TTAACTTTATTACTACAATTATTAATATACGATTAAATAATTTATCATTTGATCAAATAC
CTTTATTTGTATGAGCTGTAGGAATTACAGCATTTTTATTACTTTTATCCTTACCAGTAT
TAGCTGGGGCTATTACTATACTTCTCACTGATCGAAATTTAAATACATCTTTCTTTGACC
CTGCTGGAGGAGGAGATCCAATTTTATACCAACACTTATTTTGAT
```

Figure 6.14: The partial DNA sequence of the mitochondrial COI gene of *Asota orbona*

Figure 6.15: Molecular barcode of the mitochondrial COI gene of *Asota orbona*

> *Asota orbona* / 172AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUAO-01-A1

> *Asota orbona*

```
MIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGT
GWTVYPPLSFNIAHGGSSVDLAIFSLHLAGISSILGAINFITTIINMRLNNLSFDQMPLF
VWAVGITAFLLLLSLPVLAGAITMLLTDRNLNTSFFDPAGGGDPILYQHLFW
```

Figure 6.16: The translation product of the mitochondrial COI gene of *Asota orbona*

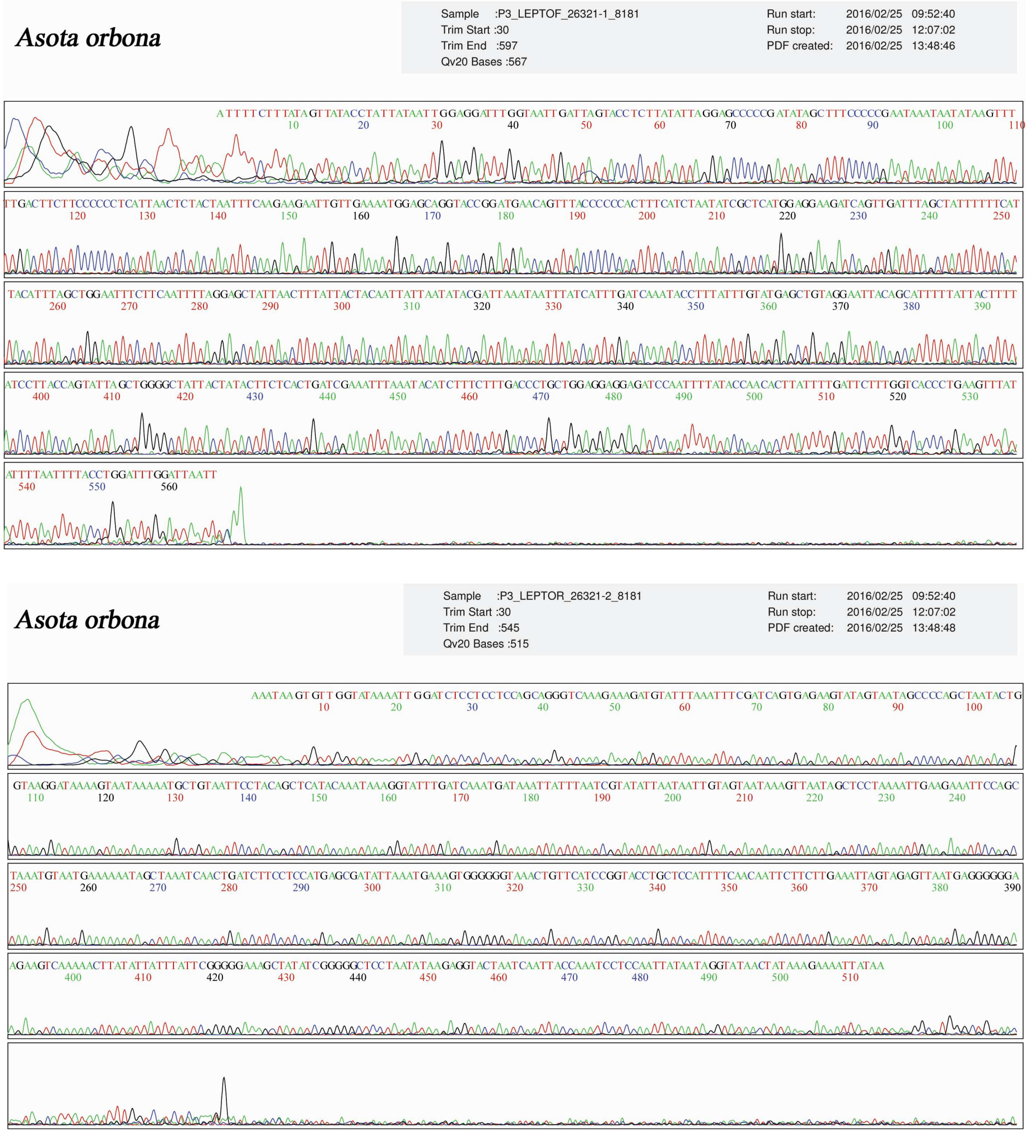


Figure 6.17: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Asota orbona* using Forward and Reverse primer.

Table 6.5: Comparison of Nucleotide frequencies of COI gene sequence of *Asota orbona* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
KX603654 Asota orbona	39.2	17.5	29.5	13.7	44	10.9	44.6	.6	29	16.6	29.7	24.6	45	25.1	14.3	16.0
KX862642 Asota caricae	38.7	15.4	31.2	14.7	46	8.0	45.7	.0	27	13.7	33.1	26.3	43	24.6	14.9	17.7
HQ569734 Asota plana plana	38.5	15.8	31.0	14.7	46	9.1	45.1	.0	27	13.7	33.1	26.3	43	24.6	14.9	17.7
HQ569654 Asota paliura	38.5	15.8	31.0	14.7	46	8.6	45.1	.0	26	14.3	33.1	26.3	43	24.6	14.9	17.7
GU662357 Asota heliconia venalba	38.5	15.8	31.0	14.7	46	8.6	45.1	.0	26	14.3	33.1	26.3	43	24.6	14.9	17.7
HQ569790 Asota plaginota plaginota	38.3	16.0	31.0	14.7	46	8.6	45.1	.0	26	14.9	33.1	26.3	43	24.6	14.9	17.7
KC499401 Asota darsania	38.3	15.6	31.0	15.0	45	9.1	44.6	1.1	27	13.1	33.7	26.3	43	24.6	14.9	17.7
HQ569723 Asota plana plana	38.5	15.8	30.9	14.7	45	9.7	45.1	.0	28	13.2	32.8	26.4	43	24.6	14.9	17.7
HM395494 Asota albiformis ternatensis	38.7	15.6	31.0	14.7	46	8.6	45.1	.0	27	13.7	33.1	26.3	43	24.6	14.9	17.7
KF549916 Asota eusemioides	38.3	16.8	30.7	14.3	43	9.1	46.9	.6	27	15.4	30.9	26.3	44	25.7	14.3	16.0
GU662399 Asota sulawesiensis	38.3	16.4	30.7	14.7	45	10.3	44.6	.0	27	13.7	33.1	26.3	43	25.1	14.3	17.7
KJ013116 Asota trinacria	37.3	17.0	30.5	15.2	43	11.4	44.0	1.7	26	14.3	33.1	26.3	43	25.1	14.3	17.7
KC499398 Asota clara donatana	38.1	16.0	31.0	14.9	45	9.7	45.1	.6	27	13.7	33.1	26.3	43	24.6	14.9	17.7
KU722730 Asota sp. *floresiensis*	37.7	16.6	30.7	15.0	45	10.3	44.0	1.1	26	14.9	33.1	26.3	43	24.6	14.9	17.7

Table 6.6: Percentage of evolutionary divergence of *Asota orbona* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	KX603654	*Asota orbona* (Kerala)	
2.	KX862642	*Asota caricae* (Pakisthan)	0.39%
3.	HQ569734	*Asota plana plana*	1.98%
4.	KC499398	*Asota clara donatana*	2.37%
5.	GU662357	*Asota heliconia venalba*	2.39%
6.	HQ569723	*Asota plana plana*	2.39%
7.	HQ569654	*Asota paliura*	2.80%
8.	HQ569790	*Asota plaginota plaginota*	2.80%
9.	KC499401	*Asota darsania*	3.21%
10.	HM395494	*Asota albiformis ternatensis*	3.21%
11.	KF549916	*Asota eusemioides*	3.62%
12.	KU722730	*Asota floresiensis*	3.64%
13.	GU662399	*Asota sulawesiensis*	3.65%
14.	KJ013116	*Asota trinacria*	4.98%

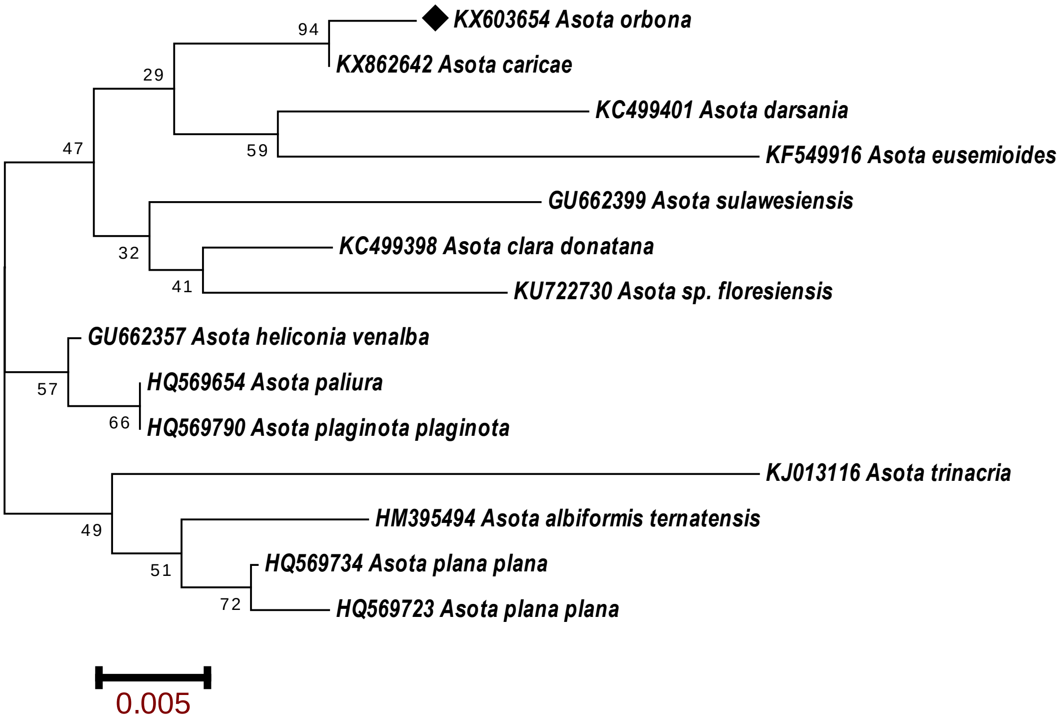


Figure 6.18: Phylogenetic relationship of *Asota orbona* inferred by NJ tree method

DISCUSSION

Both nucleotide and peptide BLAST analysis showed that this species has 99.7% sequence similarity to the same genus reported from Kerala (KX862642). Eventhough this species has been found in various geographically isolated areas, their sequence doesn’t have any kind of variation. Hence the present study stress that the barcode generated can be used to easily spot the specimen and also to analyse its phylogeny.

The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Asota orbona* showed bias to nucleotide AT, with following composition of nucleotides T = 39.2%, C = 17.5%, A = 29.5% and G = 13.7%. This greater AT content (68.7%) over GC content (31.2%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Asota orbona* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 0.39% to 4.98% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (0.39%) with those from Pakisthan while 4.98% to Philippines (Table 6.6). On the basis of the data observed this species may be rooted from those found in Philippines which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from Philippines during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Asota orbona* with related species. *Asota orbona* isolated from Kerala (KX603654) showed 0.39% divergence with *Asota caricae* (KX862642) from Pakisthan and 4.98% divergence with (KJ013116) *Asota* *trinacria* from Philippines. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Asota orbona* isolated from Kerala. Closest relative of *Asota orbona* is *Asota caricae* from Pakisthan represented within the same clade.

4. ***Asota caricae*** (Fabricius, 1775)

Specimen details:

Voucher specimen	:	CUAC – 02 – A1
Date of collection	:	24-Aug-2016
Locality	:	Malappuram: Parappanangadi
Lat- Lon	:	11.0605° N, 75.8508° E
GenBank accession	:	KX587505

Description and distribution:

The marked identification of *Asota caricae* include upturned palpi; antennae fasciculated in male and ciliated in female (Figure. 6.19). Head, thorax and abdomen orange coloured ; palpi having a black spot on 1st and 2nd joints; a black spot on tegulae; a dorsal series of black spots on abdomen expanding into bands. Fore wings are fuscous brownish ; 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 submarginal irregular series which sometimes become a nearly complete marginal band, the veins crossing it is yellow (Hampson, 1892). *Asota caricae* is distributed in India, Philippines and Australia.

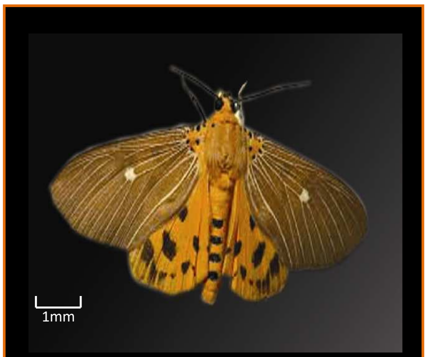


Fig.6.19: *Asota caricae*

Damage:

The folivorous caterpillars feed nocturnally and pupate on folded leaves or down in the earth. Adult ecloses during the dusk light.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Asota caricae* collected has been amplified using the primer LEP (Table 3.1). The PCR amplification yielded 532 bp and 532bp long products for the specimens obtained from two different locations. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 6.20 – 6.23 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 6.7.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Asota caricae* voucher collected during the present study (Table 6.7). It showed bias to nucleotide AT, with nucleotide composition with T = 40.2%, C = 16.9%, A = 28.8% and G = 14.1%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 6.8 and Figure 6.24 respectively.

>*Asota caricae* CDS-2016/ 532 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUAC-02-A1

> *Asota caricae*

```
CATGCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGTAAT
TGATTAGTACCTCTTATATTAGGAGCCCCCGATATAGCTTTCCCCCGAATAAATAATATA
AGTTTTTGACTTCTCCCCCCCTCATTAACTCTTCTAATTTCAAGAAGAATTGTTGAAAAT
GGAGCAGGTACTGGATGAACAGTTTACCCCCCACTTTCATCTAATATTGCTCATGGGGGA
AGATCAGTTGATTTAGCTATTTTTTCACTGCATTTAGCTGGAATTTCTTCAATTTTAGGA
GCTATTAACTTTATTACTACAATTATTAATATACGATTAAATAATTTATCATTTGATCAA
ATACCTTTATTTGTATGAGCTGTAGGAATTACAGCATTTTTATTACTTTTATCTTTACCA
GTATTAGCTGGAGCTATTACTATACTTCTCACTGATCGAAATTTAAATACATCTTTTTTT
GACCCTGCTGGAGGGGGAGATCCAATTTTATACCAACACTTATTTTGATTTT
```

Figure 6.20: The partial DNA sequence of the mitochondrial COI gene of *Asota caricae*

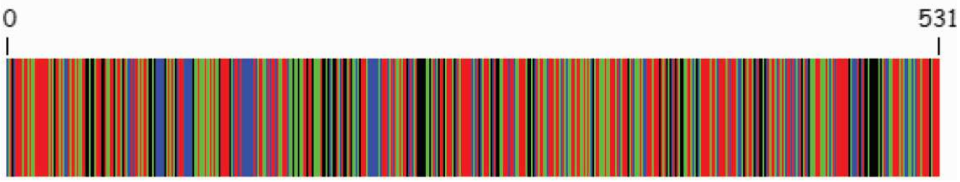


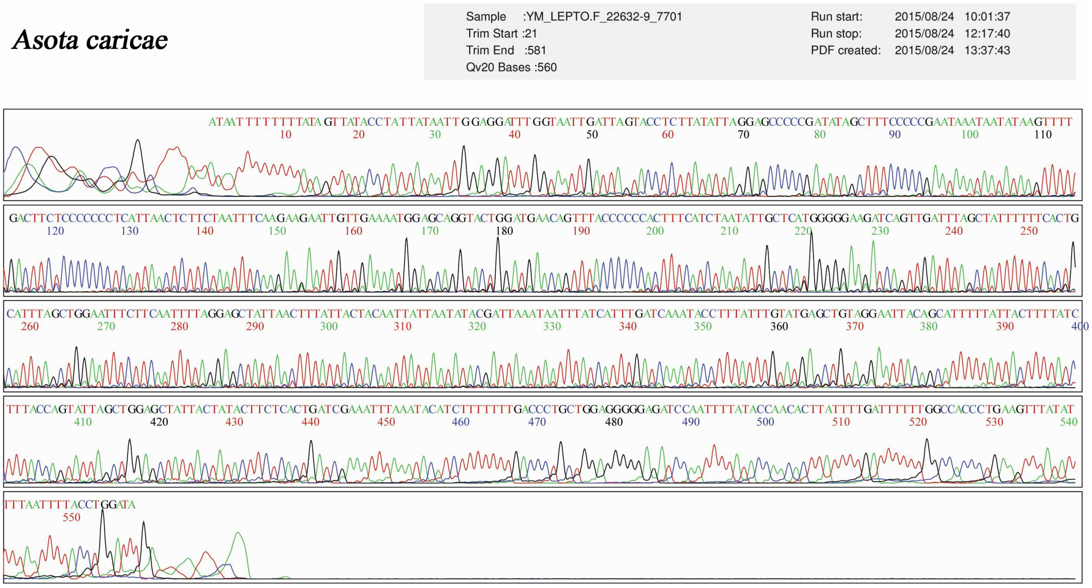
Figure 6.21: Molecular barcode of the mitochondrial COI gene of *Asota caricae*

> *Asota caricae* / 173AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUAC-02-A1

> *Asota caricae*

```
MIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGT
GWTVYPPLSSNIAHGGSSVDLAIFSLHLAGISSILGAINFITTIINMRLNNLSFDQMPLF
VWAVGITAFLLLLSLPVLAGAITMLLTDRNLNTSFFDPAGGGDPILYQHLFWF
```

Figure 6.22: The translation product of the mitochondrial COI gene of *Asota caricae*

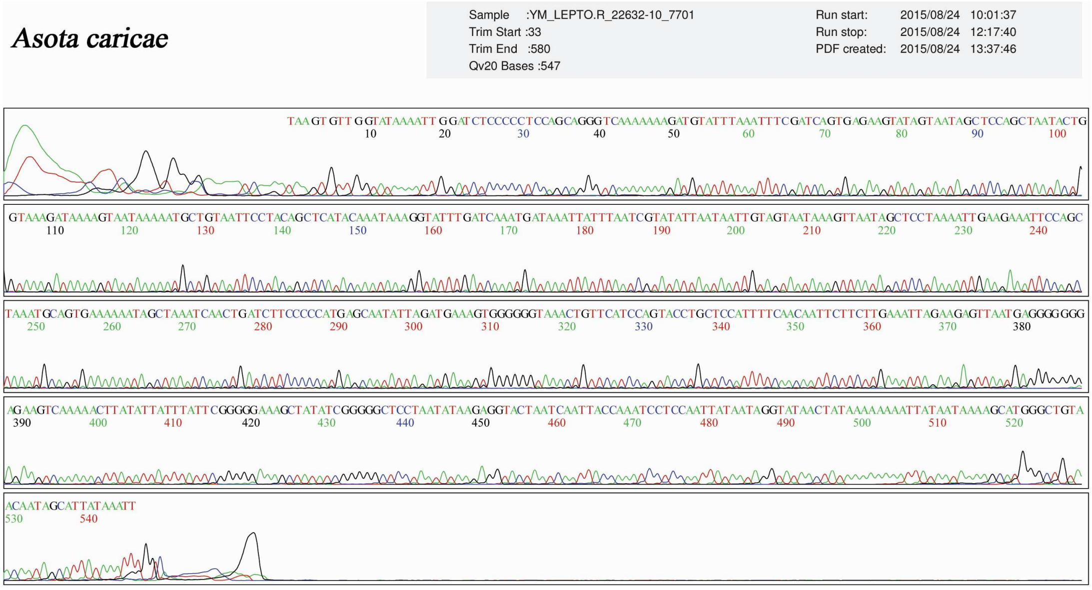


Asota caricae

Sample :YM_LEPTO.F_22632-9_7701
Trim Start :21
Trim End :581
Qv20 Bases :560

Run start: 2015/08/24 10:01:37
Run stop: 2015/08/24 12:17:40
PDF created: 2015/08/24 13:37:43

ATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGTAATTGATTAGTACCTCTTATATTAGGAGCCCCCGATATAGCTTTCCCCCGAATAAATAATATAAGTTTT
GACTTCTCCCCCCCTCATTAACTCTTCTAATTTCAAGAAGAATTGTTGAAAATGGAGCAGGTACTGGATGAACAGTTTACCCCCCACTTTCATCTAATATTGCTCATGGGGGAAGATCAGTTGATTTAGCTATTTTTTCACTG
CATTTAGCTGGAATTTCTTCAATTTTAGGAGCTATTAACTTTATTACTACAATTATTAATATACGATTAAATAATTTATCATTTGATCAAATACCTTTATTTGTATGAGCTGTAGGAATTACAGCATTTTTATTACTTTTATC
TTTACCAGTATTAGCTGGAGCTATTACTATACTTCTCACTGATCGAAATTTAAATACATCTTTTTTTGACCCTGCTGGAGGGGGAGATCCAATTTTATACCAACACTTATTTTGATTTTTTGGCCACCCTGAAGTTTATAT
TTTAATTTTACCTGGATA



Asota caricae

Sample :YM_LEPTO.R_22632-10_7701
Trim Start :33
Trim End :580
Qv20 Bases :547

Run start: 2015/08/24 10:01:37
Run stop: 2015/08/24 12:17:40
PDF created: 2015/08/24 13:37:46

TAAGTGTTGGTATAAAATTGGATCTCCCCCTCCAGCAGGGTCAAAAAAAGATGTATTTAAATTTCGATCAGTGAGAAGTATAGTAATAGCTCCAGCTAATACTG
GTAAAGATAAAAGTAATAAAAATGCTGTAATTCCTACAGCTCATACAAATAAAGGTATTTGATCAAATGATAAATTATTTAATCGTATATTAATAATTGTAGTAATAAAGTTAATAGCTCCTAAAATTGAAGAAATTCCAGC
TAAATGCAGTGAAAAAATAGCTAAATCAACTGATCTTCCCCCATGAGCAATATTAGATGAAAGTGGGGGGTAAACTGTTCATCCAGTACCTGCTCCATTTTCAACAATTCTTCTTGAAATTAGAAGAGTTAATGAGGGGGGG
AGAAGTCAAAAACTTATATTATTTATTCGGGGGAAAGCTATATCGGGGGCTCCTAATATAAGAGGTACTAATCAATTACCAAATCCTCCAATTATAATAGGTATAACTATAAAAAAAATTATAATAAAAGCATGGGCTGTA
ACAATAGCATTATAAATT

Figure 6.23: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Asota caricae* using Forward and Reverse primers.

Table 6.7: Comparison of Nucleotide frequencies of COI gene sequence of *Asota caricae* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
KX587505 Asota caricae (Kerala)	40.2	16.9	28.8	14.1	40	12.9	32.6	14.0	34	23.7	23.7	18.1	46	14.1	29.9	10.2
KU201286 Asota caricae (Kerala)	40.2	16.9	28.8	14.1	40	12.9	32.6	14.0	34	23.7	23.7	18.1	46	14.1	29.9	10.2
GU662335 Asota caricae caricae	39.3	17.9	29.1	13.7	39	13.0	34.5	13.6	33	25.8	22.5	18.5	46	14.7	30.5	9.0
HQ569651 Asota paliura	38.5	15.8	30.6	15.0	40	10.7	41.0	7.9	31	19.2	27.1	22.6	44	17.5	23.7	14.7
GU662357 Asota heliconia venalba	38.5	15.8	31.0	14.7	40	10.7	41.6	7.3	31	19.2	27.1	22.6	44	17.5	24.3	14.1
HQ569790 Asota plaginota plaginota	38.3	16.0	31.0	14.7	41	10.1	41.6	7.3	31	19.8	27.1	22.6	44	18.1	24.3	14.1
AB684348 Asota heliconia lanceolata	38.9	16.5	30.1	14.5	41	11.2	38.2	9.6	32	22.0	24.9	20.9	44	16.4	27.1	13.0
HQ569734 Asota plana plana	38.5	15.8	31.0	14.7	41	10.1	41.6	7.3	31	19.2	27.1	22.6	44	18.1	24.3	14.1
HM395494 Asota albiformis ternatensis	38.7	15.6	31.0	14.7	41	10.1	41.6	7.3	31	19.2	27.1	22.6	44	17.5	24.3	14.1
GU662388 Asota albivena	38.5	15.6	31.0	14.8	41	9.6	42.1	7.3	31	19.8	27.1	22.6	44	17.5	23.7	14.7
KC499398 Asota clara donatana	38.2	16.0	31.0	14.8	40	10.7	41.6	7.9	31	19.2	27.1	22.6	44	18.1	24.3	14.1
KJ013115 Asota trinacria	37.6	16.7	30.5	15.2	39	11.8	39.9	9.0	31	19.8	27.1	22.6	43	18.6	24.3	14.1
KU722730 Asota sp. *floresiensis*	37.8	16.5	30.6	15.0	40	10.7	40.4	8.4	30	20.3	27.1	22.6	43	18.6	24.3	14.1
KF549916 Asota eusemioides	38.2	16.7	30.5	14.7	40	10.7	38.8	10.1	32	21.5	24.9	21.5	42	18.1	27.7	12.4
HM862401 Asota clara clara	38.3	15.7	31.3	14.8	41	9.6	41.8	7.3	30	20.3	27.1	22.6	44	17.2	24.7	14.4
GU662410 Asota antennalis	38.5	15.8	30.8	14.8	42	9.6	41.6	7.3	31	19.2	27.1	22.6	43	18.6	23.7	14.7
AB684344 Asota australis sinuosa	37.6	17.7	30.3	14.5	40	12.4	38.2	9.6	33	21.5	24.9	20.9	40	19.2	27.7	13.0

Table 6.8: Percentage of evolutionary divergence of *Asota caricae* with its closely related species accessible from NCBI GenBank

Sl. No.	**Accession No**	**Organism**	**Percentage of divergence**
1.	KX587505	*Asota caricae* (Kerala)	
2.	KU201286	*Asota caricae* (Kerala)	0.00%
3.	GU662335	*Asota caricae caricae*	0.33%
4.	HQ569651	*Asota paliura*	0.33%
5.	GU662357	*Asota heliconia venalba*	1.01%
6.	HQ569790	*Asota plaginota plaginota*	1.01%
7.	HM862401	*Asota clara clara*	1.71%
8.	GU662388	*Asota albivena*	2.41%
9.	KC499398	*Asota clara donatana*	2.41%
10.	AB684348	*Asota heliconia lanceolata*	2.41%
11.	GU662410	*Asota antennalis*	2.43%
12.	HQ569734	*Asota plana plana*	2.76%
13.	HM395494	*Asota albiformis ternatensi*	2.76%
14.	KU722730	*Asota* sp. *Floresiensis*	3.14%
15.	KJ013115	*Asota trinacria*	4.25%
16.	KF549916	*Asota eusemioides*	4.98%
17.	AB684344	*Asota australis sinuosa*	5.71%

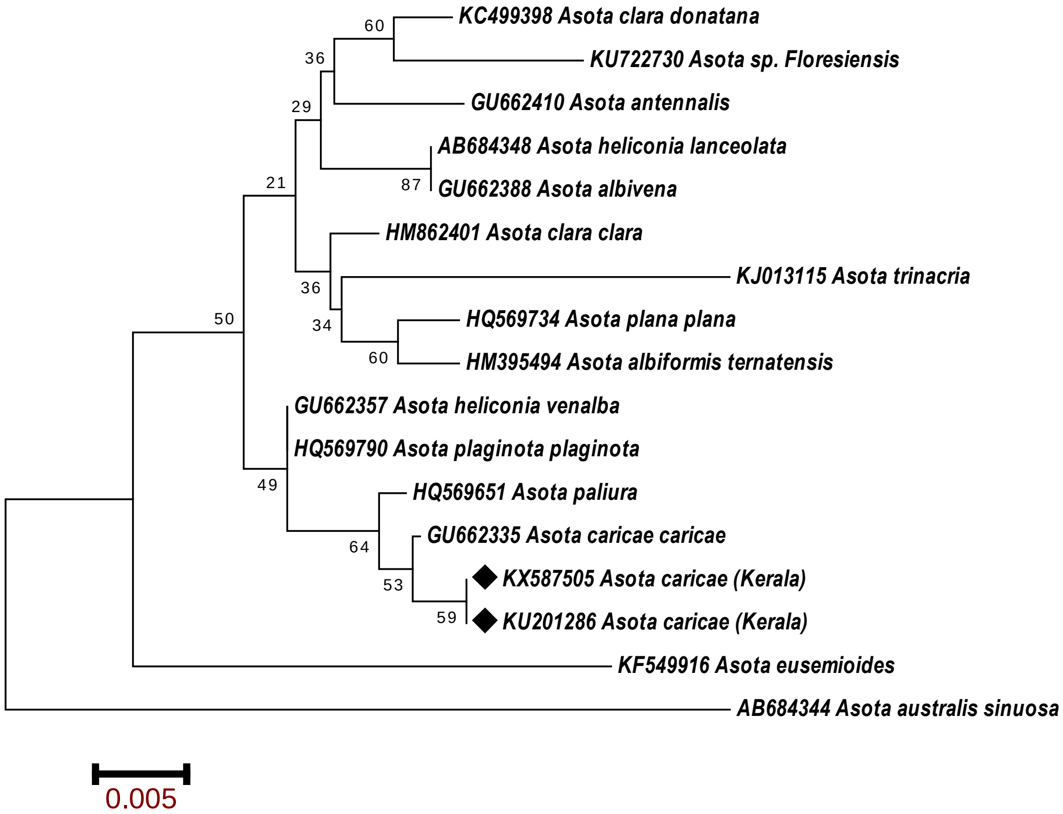


Figure 6.24: Phylogenetic relationship of *Asota caricae* inferred by NJ tree method

DISCUSSION

Both nucleotide and peptide BLAST analysis showed that this species has 100% sequence similarity to the same genus reported from Kerala (KU201286). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Asota caricae* showed bias to nucleotide AT, with following composition of nucleotides T = 40.2%, C = 16.9%, A = 28.8% and G = 14.1%. This greater AT content (69.0%) over GC content (31.0%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Asota caricae* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 0% to 5.71% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has no divergence (0%) with those from Kerala while 5.71% to Indonesia (Table 6.8). On the basis of the data observed this species may be rooted from those found in Indonesia which diverted into different clades due to geographical variation. Result thus concluded that this species doesn't have any major changes in India while slightly changes from those reported from Indonesia during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Asota caricae* with related species. *Asota caricae* isolated from Kerala (KX587505) showed 0% divergence with (KU201286) *Asota caricae* from Kerala and 5.71% divergence with *Asota australis sinuosa* (AB684344) from Indonesia. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Asota caricae* isolated from Kerala. Closest relative of *Asota caricae* is *Asota caricae* fr represented within the same clade.

5. ***Eudocima cocalus*** (Cramer, 1780)

Specimen details:

Voucher specimen	:	CUEC – 01 – A1
Date of collection	:	10-Nov-2015
Locality	:	Malappuram: Munniyoor
Lat- Lon	:	11.0748° N, 75.8921° E
GenBank accession	:	KX603659

Description and distribution:

Eudocima cocalus, the cocalus fruit piercing moth, is a moth of the family Erebidae family (Figure. 6.25). The wingspan is about 100 mm.The female adult moths of this species have forewings that are dark brown with several white spots. The hindwings are yellow with a broad black margin. The male moths have patchy brown forewings with no white spots, but the hindwings are orange with a broad black margin. For both sexes, each forewing has a hooked wingtip, and a concave hind margin. The head and thorax are dark brown, but the abdomen is bright orange. The wingspan is about 6 cms. It is found distributed in the north-eastern part of the Himalaya, to Sundaland and east to Queensland, along South India, Australia and the Solomons.

Fig. 6.25: *Eudocima cocalus*

Damage:

The larvae feed on *Cocculus* species. The adults are a pest in lychee, carambola orchards and polyphagus to agricultural crops. They pierce the fruit in order to suck the juice.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Eudocima cocalus* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 585 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 6.26 – 6.29 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 6.9.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Eudocima cocalus* voucher collected during the present study (Table 6.9). It showed bias to nucleotide AT, with nucleotide composition with T = 39.7%, C = 16.8%, A = 29.7% and G = 13.8%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 6.10 and Figure 6.30 respectively.

> *Eudocima cocalus* CDS-2017/ 585 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUEC-01-A1

> *Eudocima cocalus*

```
GGAACTTCTCTTAGTTTATTTAATTCGAGCTGAATTAGGAAATCCAGGATCATTAATTGG
AGATGATCAAATTTATAATACTATTGTCACAGCTCATGCTTTTATTATAATTTTTTTTAT
AGTAATACCTATTATAATTGGAGGATTCGGAAATTGATTAGTTCCTCTTATATTAGGAGC
CCCTGATATAGCTTTCCCTCGAATAAATAATATAAGTTTCTGACTCCTTCCCCCCTCTTT
AACTCTTCTTATTTCAAGAAGAATTGTAGAAAATGGAGCAGGAACTGGATGAACAGTTTA
CCCCCCACTTTCATCTAATATTGCACATGGAGGTAGCTCAGTTGATTTAGCTATTTTTTC
ATTACACTTAGCTGGTATTTCATCAATTTTAGGAGCTATTAATTTTATTACAACAATTAT
TAATATACGATTAAATAATTTATCATTTGATCAAATACCATTATTTATTTGAGCTGTTGG
AATTACTGCATTCTTATTACTTCTTTCTTTACCTGTATTAGCAGGTGCTATTACTATACT
TTTAACAGATCGAAATTTAAATACATCTTTTTTTGACCCCGCTGG
```

Figure 6.26: The partial DNA sequence of the mitochondrial COI gene of *Eudocima cocalus*

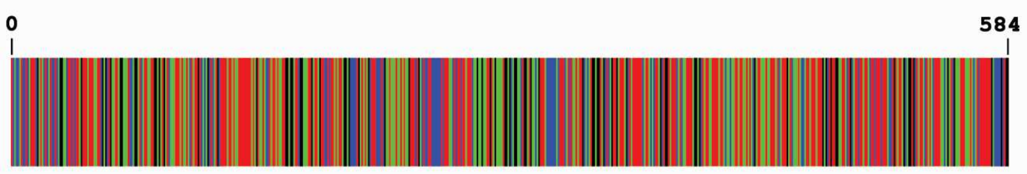


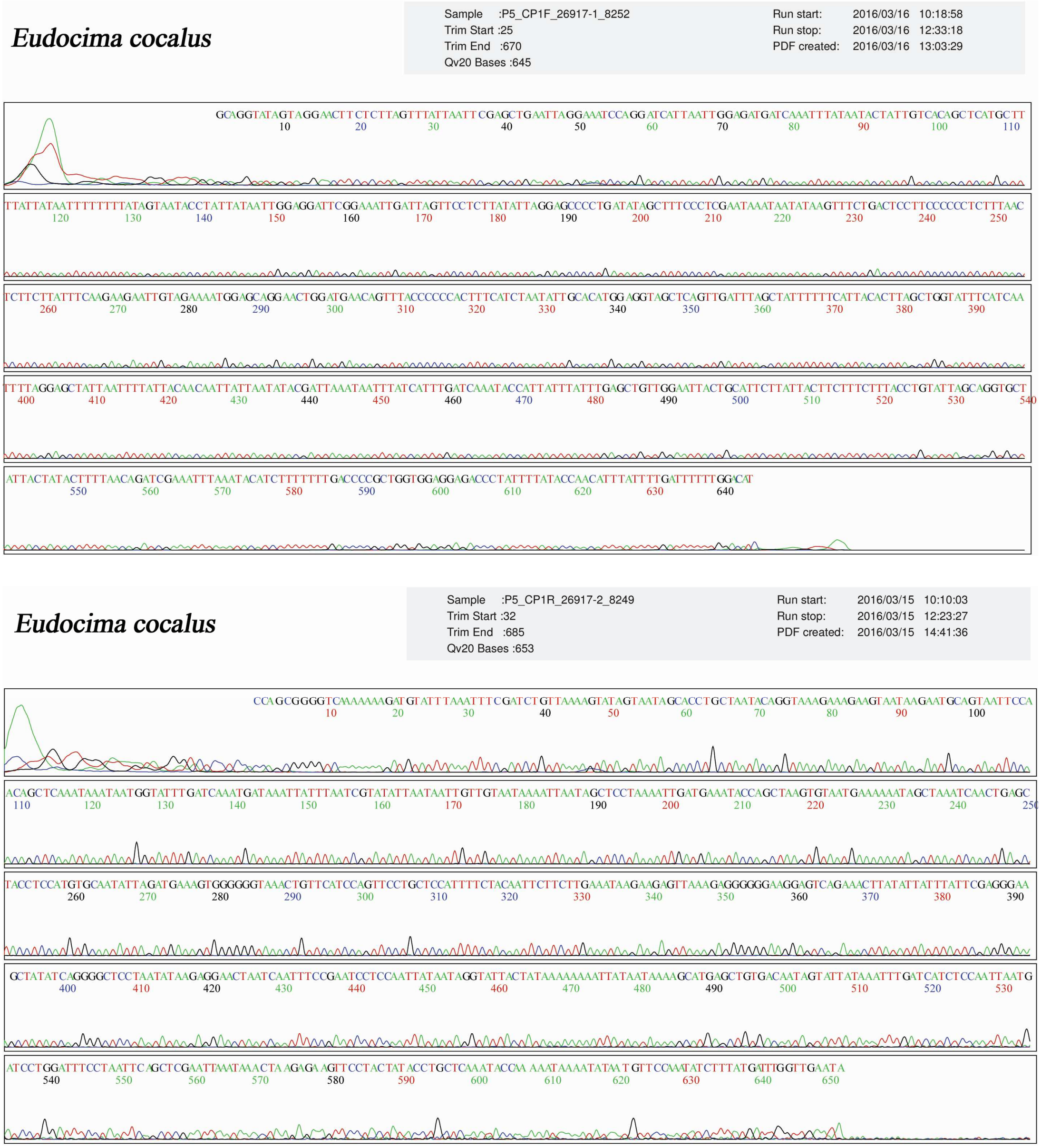
Figure 6.27: Molecular barcode of the mitochondrial COI gene of *Eudocima cocalus*

> *Eudocima cocalus* / 159AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUEC-01-A1

> *Eudocima cocalus*

```
MIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGT
GWTVYPPLSSNIAHGGSSVDLAIFSLHLAGISSILGAINFITTIINMRLNNLSFDQMPLF
IWAVGITAFLLLLSLPVLAGAITMLLTDRNLNTSFFDPA
```

Figure 6.28: The translation product of the mitochondrial COI gene of *Eudocima cocalus*


Eudocima cocalus

Sample :P5_CP1F_26917-1_8252
Trim Start :25
Trim End :670
Qv20 Bases :645
Run start: 2016/03/16 10:18:58
Run stop: 2016/03/16 12:33:18
PDF created: 2016/03/16 13:03:29

GCAGGTATAGTAGGAACTTCTCTTAGTTTATTAATTCGAGCTGAATTAGGAAATCCAGGATCATTAATTGGAGATGATCAAATTTATAATACTATTGTCACAGCTCATGCTT
TTATTATAATTTTTTTATAGTAATACCTATTATAATTGGAGGATTCGGAAATTGATTAGTTCCTCTTATATTAGGAGCCCCTGATATAGCTTTCCCTCGAATAAATAATATAAGTTTCTGACTCCTTCCCCCCTCTTTAAC
TCTTCTTATTTCAAGAAGAATTGTAGAAAATGGAGCAGGAACTGGATGAACAGTTTACCCCCCACTTTCATCTAATATTGCACATGGAGGTAGCTCAGTTGATTTAGCTATTTTTTCATTACACTTAGCTGGTATTTCATCAA
TTTTAGGAGCTATTAATTTTATTACAACAATTATTAATATACGATTAAATAATTTATCATTTGATCAAATACCATTATTTATTTGAGCTGTTGGAATTACTGCATTCTTATTACTTCTTTCTTTACCTGTATTAGCAGGTGCT
ATTACTATACTTTTAACAGATCGAAATTTAAATACATCTTTTTTTGACCCCGCTGGTGGAGGAGACCCTATTTTATACCAACATTTATTTTGATTTTTTGGACAT

Eudocima cocalus

Sample :P5_CP1R_26917-2_8249
Trim Start :32
Trim End :685
Qv20 Bases :653
Run start: 2016/03/15 10:10:03
Run stop: 2016/03/15 12:23:27
PDF created: 2016/03/15 14:41:36

CCAGCGGGGTCAAAAAAGATGTATTTAAATTTCGATCTGTTAAAAGTATAGTAATAGCACCTGCTAATACAGGTAAAGAAAGAAGTAATAAGAATGCAGTAATTCCA
ACAGCTCAAATAAATAATGGTATTTGATCAAATGATAAATTATTTAATCGTATATTAATAATTGTTGTAATAAAATTAATAGCTCCTAAAATTGATGAAATACCAGCTAAGTGTAATGAAAAAATAGCTAAATCAACTGAGC
TACCTCCATGTGCAATATTAGATGAAAGTGGGGGGTAAACTGTTCATCCAGTTCCTGCTCCATTTTCTACAATTCTTCTTGAAATAAGAAGAGTTAAAGAGGGGGGAAGGAGTCAGAAACTTATATTATTTATTCGAGGGAA
GCTATATCAGGGGCTCCTAATATAAGAGGAACTAATCAATTTCCGAATCCTCCAATTATAATAGGTATTACTATAAAAAAAATTATAATAAAAGCATGAGCTGTGACAATAGTATTATAAATTTGATCATCTCCAATTAATG
ATCCTGGATTTCCTAATTCAGCTCGAATTAAATAAACTAAGAGAAGTTCCTACTATACCTGCTCAAATACCAAAAATAAAATATAATGTTCCAAATATCTTTATGATTTGGTTGAATA

Figure 6.29: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Eudocima cocalus* using Forward and Reverse primers.

Table 6.9: Comparison of Nucleotide frequencies of COI gene sequence of *Eudocima cocalus* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
KX603659 Eudocima cocalus	39.7	16.8	29.7	13.8	44	25.6	15.9	14.9	49	8.2	42.6	.5	27	16.4	30.8	26.2
HQ949157 Eudocima cocalus	40.3	15.7	29.9	14.0	45	23.6	17.4	13.8	48	7.2	42.6	2.6	28	16.4	29.7	25.6
HQ949151 Eudocima phalonia	39.5	16.4	30.3	13.8	44	24.1	17.9	13.8	48	7.2	42.6	2.6	27	17.9	30.3	25.1
JN674870 Eudocima divitiosa	40.4	15.6	29.5	14.6	45	23.8	16.6	14.5	50	5.1	41.0	4.1	27	17.9	30.6	25.0
HQ949147 Eudocima iridescens	40.5	15.6	29.9	14.0	45	24.1	16.9	13.8	49	5.6	42.6	3.1	28	16.9	30.3	25.1
HQ949150 Eudocima jordani	40.0	15.7	30.4	13.8	45	23.6	17.9	13.8	48	6.2	43.1	2.6	27	17.4	30.3	25.1
KU205355 Eudocima hypermnestra	41.4	15.0	29.7	13.8	45	24.6	16.9	13.8	52	3.6	42.1	2.6	28	16.9	30.3	25.1
KP898255 Eudocima materna	41.4	15.0	29.7	13.8	45	24.6	16.9	13.8	52	3.6	42.1	2.6	28	16.9	30.3	25.1
KJ380871 Eudocima homaena	39.8	15.9	30.8	13.5	43	25.3	17.5	14.4	49	6.1	43.9	1.0	28	16.4	30.8	25.1
KT988573 Eudocima tyrannus	39.7	15.7	30.6	14.0	44	24.6	17.9	13.8	47	6.7	43.6	3.1	29	15.9	30.3	25.1
HQ571016 Eudocima apta	41.5	14.7	29.9	13.8	45	24.1	16.9	13.8	52	2.6	42.6	2.6	27	17.4	30.3	25.1
HM884517 Lepidoptera sp.	41.4	14.9	29.9	13.8	45	24.1	16.9	13.8	52	3.1	42.6	2.6	27	17.4	30.3	25.1
HM893264 Eudocima sp.	39.1	16.8	29.9	14.2	44	24.6	17.4	14.4	47	7.7	42.1	3.1	27	17.9	30.3	25.1
KF491730 Eudocima salaminia	40.0	15.9	29.9	14.2	45	23.6	17.4	13.8	48	6.2	42.6	3.1	27	17.9	29.7	25.6
MF126815 Eudocima serpentifera	40.9	15.0	29.9	14.2	45	24.6	16.4	13.8	49	4.1	43.1	3.6	28	16.4	30.3	25.1

Table 6.10: Percentage of evolutionary divergence of *Eudocima cocalus* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	KX603659	*Eudocima cocalus* (Kerala)	
2.	HQ949157	*Eudocima cocalus* (Canada)	0.00%
3.	KU205355	*Eudocima hypermnestra*	1.12%
4.	KP898255	*Eudocima maternal*	1.12%
5.	HQ571016	*Eudocima apta*	1.40%
6.	HM884517	*Lepidoptera* sp.	1.40%
7.	HQ949147	*Eudocima iridescens*	1.40%
8.	KF491730	*Eudocima salaminia*	1.40%
9.	HQ949150	*Eudocima jordani*	1.68%
10.	MF126815	*Eudocima serpentifera*	1.68%
11.	HQ949151	*Eudocima phalonia*	1.68%
12.	JN674870	*Eudocima divitiosa*	1.69%
13.	KJ380871	*Eudocima homaena*	1.69%
14.	KT988573	*Eudocima tyrannus*	1.97%
15.	HM893264	*Eudocima* sp.	1.97%

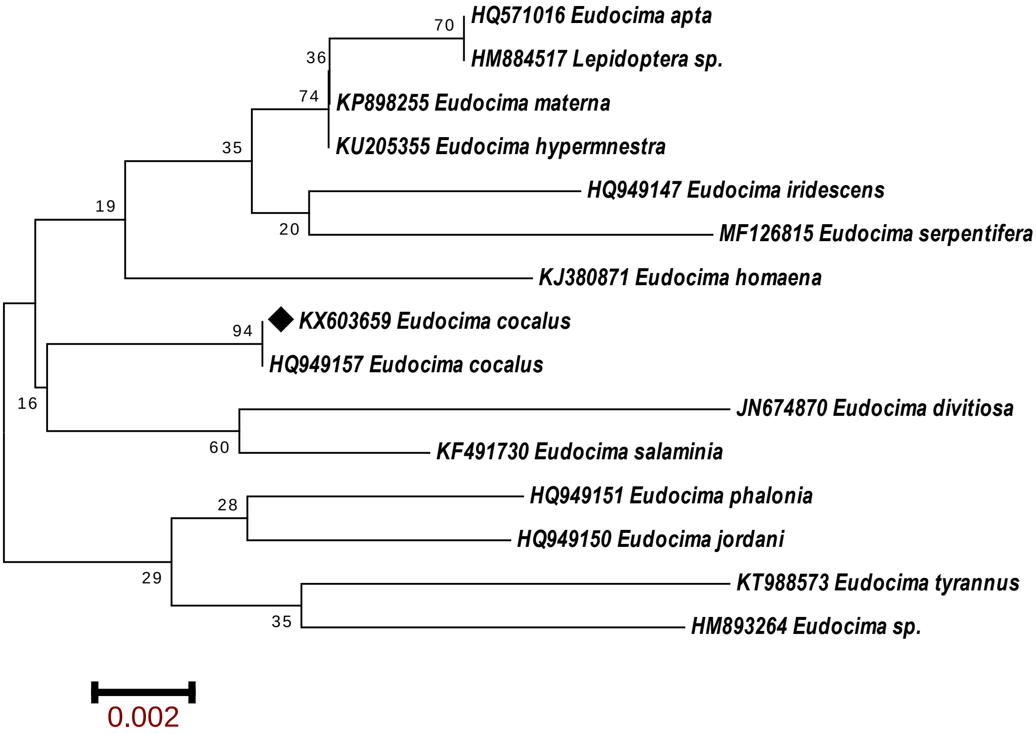


Figure 6.30: Phylogenetic relationship of *Eudocima cocalus* inferred by NJ tree method

DISCUSSION

Both nucleotide and peptide BLAST analysis showed that this species has 100% sequence similarity to the same genus reported from Kerala (HQ949157). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Eudocima cocalus* showed bias to nucleotide AT, with following composition of nucleotides T = 39.7%, C = 16.8%, A = 29.7% and G = 13.8%. This greater AT content (69.4%) over GC content (30.6%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Eudocima cocalus* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 0% to 1.97% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has no divergence (0%) with those from Canada while 1.97% to Ontario (Table 6.10). On the basis of the data observed this species may be rooted from those found in Ontario and Canada which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from Ontario during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Eudocima cocalus* with related species. *Eudocima cocalus* isolated from Kerala (KX603659) showed 0% divergence with *Eudocima cocalus* (HQ949157) from Canada and 1.97% divergence with *Eudocima* sp. (HM893264) from Ontario. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Eudocima cocalus* isolated from

Kerala. Closest relative of *Eudocima cocalus* is *Eudocima cocalus* from Canada represented within the same clade.

6. ***Hypocala deflorata*** (Fabricius, 1794)

Specimen details:

Voucher specimen : CUHD – 02 – A1
Date of collection : 21-Mar-2016
Locality : Malappuram: Parappanangadi
Lat- Lon : 11.0605° N, 75.8508° E
GenBank accession : KX603655

Description and distribution:

Hypocala deflorata have paler head, thorax, and fore wing (Figure. 6.31). Distinguished character include hind wing with the orange area larger; the marginal black area reduced and the two orange spots conjoined. Underside with the black on fore wing reduced to two bars and that on hind wing also much reduced (W.S. ♂ 38 mm) (Gurule, 2013). *Hypocala deflorata* is found to be distributed along Africa, India, Sri Lanka, Thailand, China, Japan; Australia, Hong Kong and Borneo (Subhalakshmi et al., 2017).

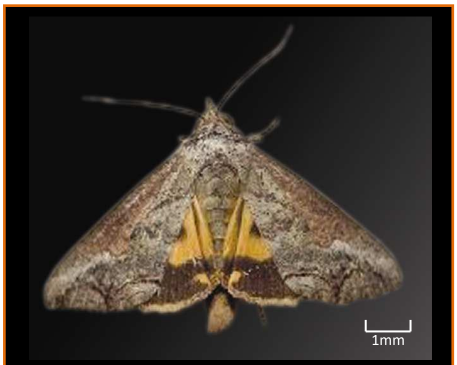


Fig.6.31: *Hypocala deflorata*

Damage:

The adult moth is a generalized feeder and attacks fruit, penetrating the skin to suck juice.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Hypocala deflorata* collected has been amplified using the primer LEP (Table 3.1). The PCR amplification yielded 434 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 6.32 – 6.35 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 6.11.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Hypocala deflorata* voucher collected during the present study (Table 6.12). It showed bias to nucleotide AT, with nucleotide composition with T = 40.1%, C = 15.7%, A = 30.4 % and G = 13.8%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 6.12 and Figure 6.36 respectively.

> *Hypocala deflorata* CDS-2016/ 434 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUHD-02-A1

> *Hypocala deflorata*

```
ATTGGAGGATTTGGAAATTGATTAGTTCCCTTAATATTAGGAGCTCCTGATATAGCTTTC
CCTCGAATAAATAATATAAGTTTTTGACTTCTTCCCCCTTCTTTAACATTATTAATTTCA
AGTAGAATTGTAGAAAATGGAGCAGGAACTGGATGAACTGTTTACCCCCCTTTATCATCA
AATATTGCTCACAGTGGAAGATCAGTAGATTTAGCTATTTTTTCTCTACATTTAGCAGGT
ATTTCTTCAATTTTAGGAGCTATTAATTTTATTACTACAATTATTAATATACGATTAAAT
AATTTAATGTTTGATCAAATACCTTTATTTGTTTGAGCTGTAGGAATTACTGCATTCTTA
CTATTATTATCTTTACCTGTTTTAGCAGGAGCTATTACCATACTTCTAACTGATCGAAAT
TTAAATACTTCATT
```

Figure 6.32: The partial DNA sequence of the mitochondrial COI gene of *Hypocala deflorata*

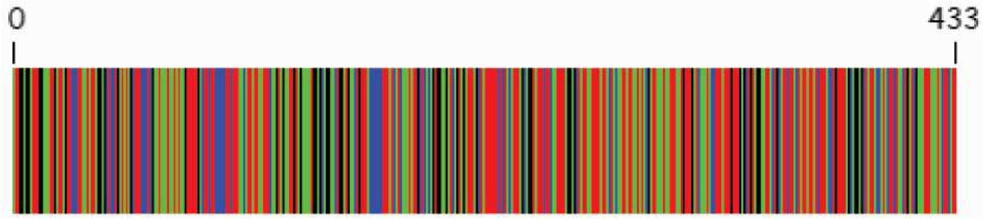


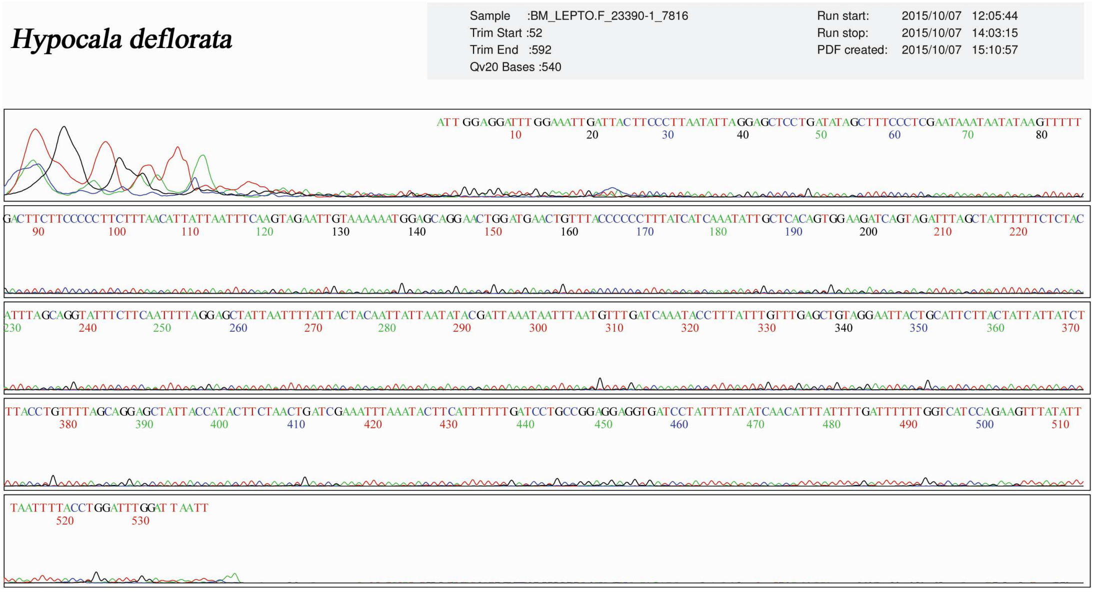
Figure 6.33: Molecular barcode of the mitochondrial COI gene of *Hypocala deflorata*

> *Hypocala deflorata* / 133AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUHD-01-A1

> *Hypocala deflorata*

```
MLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHSGSSVDL
AIFSLHLAGISSILGAINFITTIINMRLNNLMFDQMPLFVWAVGITAFLLLLSLPVLAGA
ITMLLTDRNLNTS
```

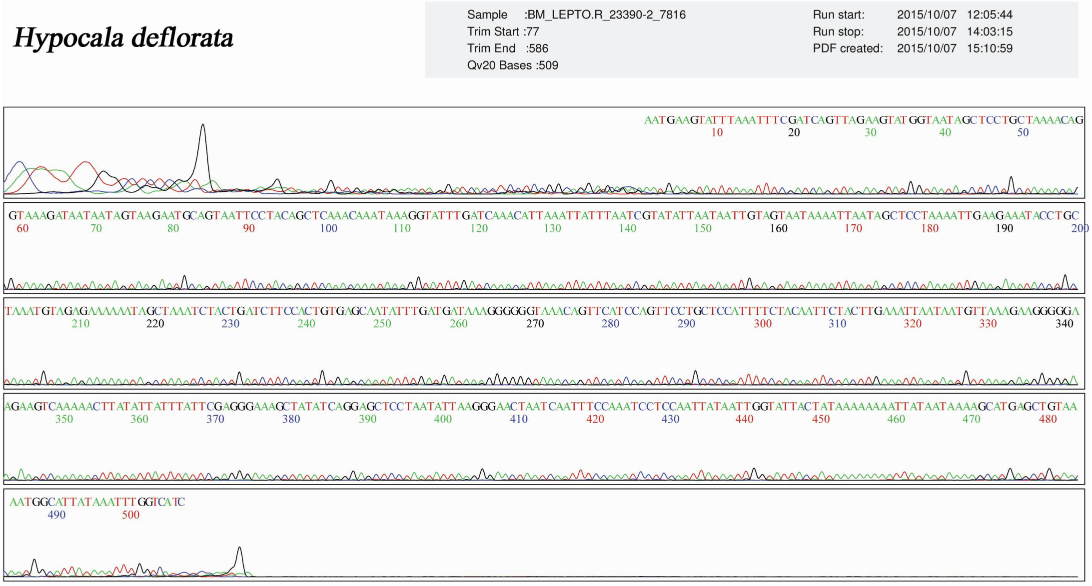
Figure 6.34: The translation product of the mitochondrial COI gene of *Hypocala deflorata*



Hypocala deflorata

Sample :BM_LEPTO.F_23390-1_7816
Trim Start :52
Trim End :592
Qv20 Bases :540

Run start: 2015/10/07 12:05:44
Run stop: 2015/10/07 14:03:15
PDF created: 2015/10/07 15:10:57



Hypocala deflorata

Sample :BM_LEPTO.R_23390-2_7816
Trim Start :77
Trim End :586
Qv20 Bases :509

Run start: 2015/10/07 12:05:44
Run stop: 2015/10/07 14:03:15
PDF created: 2015/10/07 15:10:59

Figure 6.35: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Hypocala deflorata* using Forward and Reverse primers.

Table 6.11: Comparison of Nucleotide frequencies of COI gene sequence of *Hypocala deflorata* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
KX603655 Hypocala deflorata (Kerala)	40.1	15.7	30.4	13.8	48	5.6	45.8	.7	30	14.5	31.7	24.1	43	26.9	13.8	16.6
KF924008 Hypocala deflorata	38.5	15.0	31.1	15.4	48	5.5	46.2	.0	26	13.2	33.3	27.1	41	26.2	13.8	19.3
MF131442 Euclystis guerini	40.3	14.7	29.0	15.9	52	4.8	42.1	.7	27	14.5	31.0	27.6	42	25.0	13.9	19.4
MF127030 Euclystis sp.	40.3	14.7	29.0	15.9	52	4.8	42.1	.7	27	14.5	31.0	27.6	42	25.0	13.9	19.4
JF844307 Lepidoptera sp.	40.6	14.5	28.8	16.1	53	4.1	41.4	1.4	27	14.5	31.0	27.6	42	25.0	13.9	19.4
HQ949292 Acantholipes zuboides	38.6	14.5	31.2	15.7	46	5.6	48.6	.0	28	13.1	31.0	27.6	42	25.0	13.9	19.4
HQ950435 Ophiusa hituense	38.9	15.2	30.2	15.7	47	7.6	45.5	.0	28	13.1	31.0	27.6	42	25.0	13.9	19.4
MF132708 Mimophisma delunaris	38.5	14.7	31.1	15.7	45	6.9	48.3	.0	29	12.4	31.0	27.6	42	25.0	13.9	19.4
KX862493 Clytie illunaris	39.6	13.4	31.1	15.9	48	3.4	48.3	.7	30	11.7	31.0	27.6	42	25.0	13.9	19.4
KP083432 Ophiusa tirhaca	38.7	13.8	33.4	14.1	48	4.2	47.9	.0	26	13.1	35.9	24.8	42	24.1	16.6	17.2
KY370722 Clytie illunaris	40.3	12.7	31.3	15.7	49	2.1	49.0	.0	30	11.0	31.0	27.6	42	25.0	13.9	19.4
MF131530 Mimophisma delunaris	38.5	14.7	30.9	15.9	45	6.9	47.6	.7	29	12.4	31.0	27.6	42	25.0	13.9	19.4
KJ380868 Ophiusa triphaenoides	38.2	13.8	33.2	14.7	47	2.8	50.0	.7	26	13.1	35.2	25.5	42	25.5	14.5	17.9
KF392463 Bastilla nielseni	39.9	14.5	29.7	15.9	51	4.1	44.1	.7	27	14.5	31.0	27.6	42	25.0	13.9	19.4

Table 6.12: Percentage of evolutionary divergence of *Hypocala deflorata* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	KX603655	*Hypocala deflorata* (Kerala)	
2.	KF924008	*Hypocala deflorata* (Tamilnadu)	0.00%
3.	HQ950435	*Ophiusa hituense*	9.06%
4.	HQ949292	*Acantholipes zuboides*	9.14%
5.	MF131442	*Euclystis guerini*	9.90%
6.	MF127030	*Euclystis* sp.	9.90%
7.	JF844307	*Lepidoptera* sp.	10.57%
8.	KF392463	*Bastilla nielseni*	12.16%
9.	KX862493	*Clytie illunaris*	12.89%
10.	MF132708	*Mimophisma delunaris*	12.96%
11.	KY370722	*Clytie illunaris*	12.96%
12.	MF131530	*Mimophisma delunaris*	12.96%
13.	KJ380868	*Ophiusa triphaenoides*	12.98%
14.	KP083432	*Ophiusa tirhaca*	13.02%

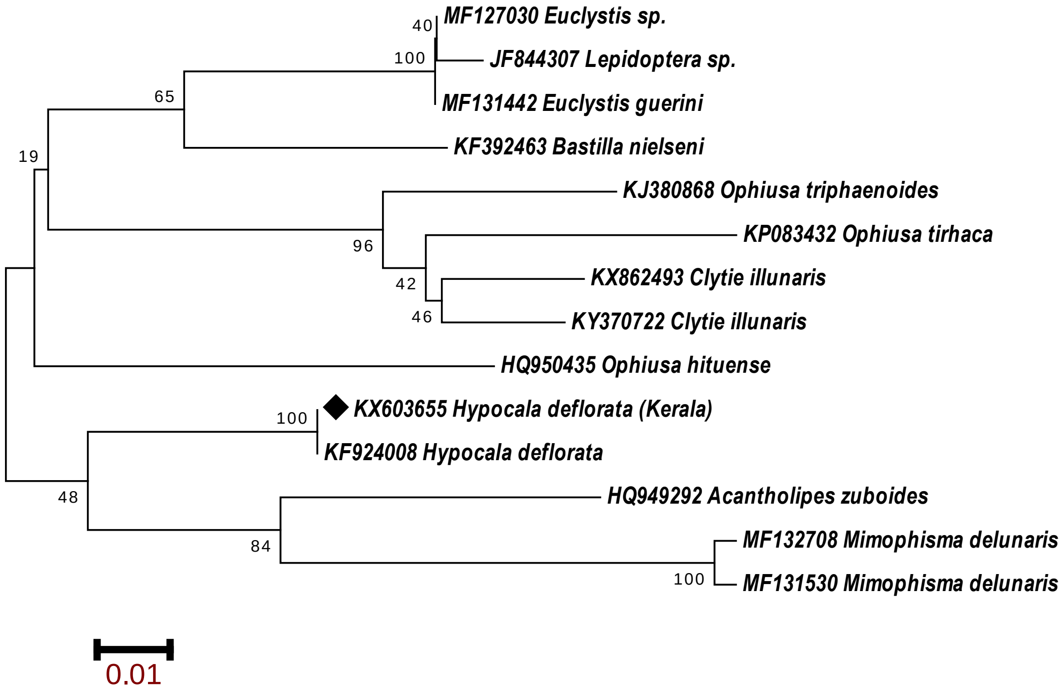


Figure 6.36: Phylogenetic relationship of *Hypocala deflorata* inferred by NJ tree method

DISCUSSION

Both nucleotide and peptide BLAST analysis showed that this species has 100% sequence similarity to the same genus reported from Tamilnadu (KF924008). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Hypocala deflorata* showed bias to nucleotide AT, with following composition of nucleotides T = 40.1%, C = 15.7%, A = 30.4% and G = 13.8%. This greater AT content (70.5%) over GC content (39.5%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Hypocala deflorata* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 0% to 13.02% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has no divergence (0%) those from Tamilnadu while 13.02% to South Africa (Table 6.12). On the basis of the data observed this species may be rooted from those found in South Africa which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from South Africa during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Hypocala deflorata* with related species. *Hypocala deflorata* isolated from Kerala (KX603655) showed 0% divergence with *Hypocala deflorata* (KF924008) from Tamilnadu and 13.02% divergence with *Ophiusa tirhaca* (KP083432) from South Africa. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Hypocala deflorata* isolated from

Kerala. Closest relative of *Hypocala deflorata* is *Hypocala deflorata* from Tamilnadu represented within the same clade.

7. ***Polytela gloriosae*** (Fabricius, 1781)

Specimen details:

Voucher specimen	:	CUPG – 01 – A1
Date of collection	:	21-Mar-2016
Locality	:	Malappuram: Parappanangadi
Lat- Lon	:	11.0605° N, 75.8508° E
GenBank accession	:	KX603662

Description and distribution:

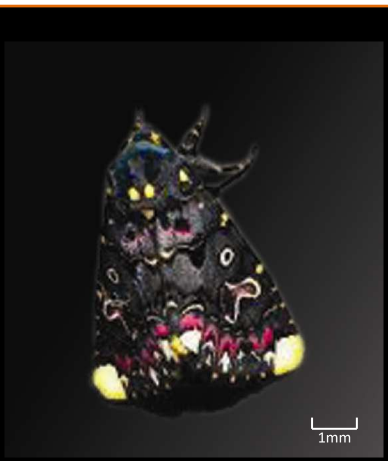


Fig. 6.37: *Polytela gloriosae*

Polytela gloriosae, identification include, Eyes hairy; proboscis fully developed; palpi porrect and roughly scaled, the 3rd joint short (Figure. 6.37). Head and thorax is blue-black; the basal joint of palpi and antennae orange; three orange specks on metathorax; abdomen black, the terminal segments orange. Fore wing blue-black; an orange speck at base; the subbasal and antemedial waved lines filled in with orange, with an orange speck between them in cell, and two pink and black lunules towards inner margin; the orbicular yellow ring-mark; the reniform yellow, with the upper part centred with pink, the lower with fuscous; some orange specks on costa towards apex; the curved lunulate double postmedial line filled in with yellow and with a series of pink and black lunules beyond it; large orange blotches at apex and outer angle; cilia black and orange. Hind wings are fuscous, with traces of an angled postmedial line; the cilia orange. (W.S.♂ 29 mm). It is found in Sri Lanka, India and probably in Indonesia.

Damage:

The caterpillar is considered as a minor pest on several plant families. The larvae feed voraciously on leaves. The later instar feeds on tissues and plants destroying it completely. This stage lasts for 2.5 days.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Polytela gloriosae* collected has been amplified using the primer LEP (Table 3.1). The PCR amplification yielded 465 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 6.38 – 6.41 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 6.13.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Polytela gloriosae* voucher collected during the present study (Table 6.13). It showed bias to nucleotide AT, with nucleotide composition with T = 41.9%, C = 16.1%, A = 28.4 % and G = 13.5%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 6.14 and Figure 6.42 respectively.

> *Polytela gloriosae* CDS-2018/ 465 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUPG-01-A1

> *Polytela gloriosae*

TCTAACTGTGAATGAAAAATTTTTTATGTTTACTGGGGGTTTTGGTAATTTCCTTGTTCC
TTTAATATTAGGAGCTCCTGATATAGCATTTCCTCGAATAAATAATATAAGTTTTTGACT
TCTTCCTCCTTCCTTAACTTTACTTATTTCAAGTAGAATTGTAGAAAATGGAGCAGGAAC
AGGATGAACAGTTTATCCCCCACTTTCATCTAATATTGzCTCATGGAGGTAGTTCAGTAG
ACTTAGCTATTTTTTCTCTTCATTTAGCTGGTATTTCCTCAATTCTCGGAGCTATTAATT
TTATTACTACAATTATTAATATACGATTAAATAATTTATCTTTCGATCAAATACCTTTAT
TTATTTGAGCTGTAGGAATTACTGCTTTTTTACTATTATTATCTTTACCTGTATTAGCTG
GAGCTATTACTATACTTTTAACAGACCGAAATTTAAATACATCTTT

Figure 6.38: The partial DNA sequence of the mitochondrial COI gene of *Polytela gloriosae*

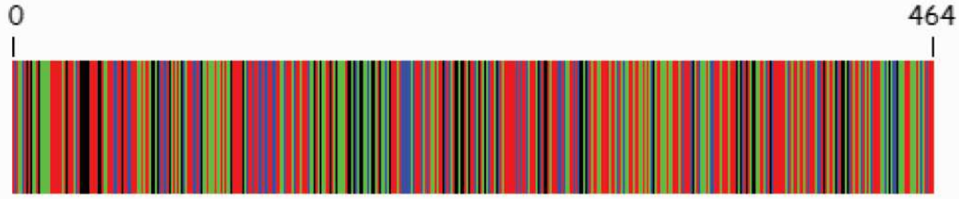


Figure 6.39: Molecular barcode of the mitochondrial COI gene of *Polytela gloriosae*

> *Polytela gloriosae* / 146AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUPG-01-A1

> *Polytela gloriosae*

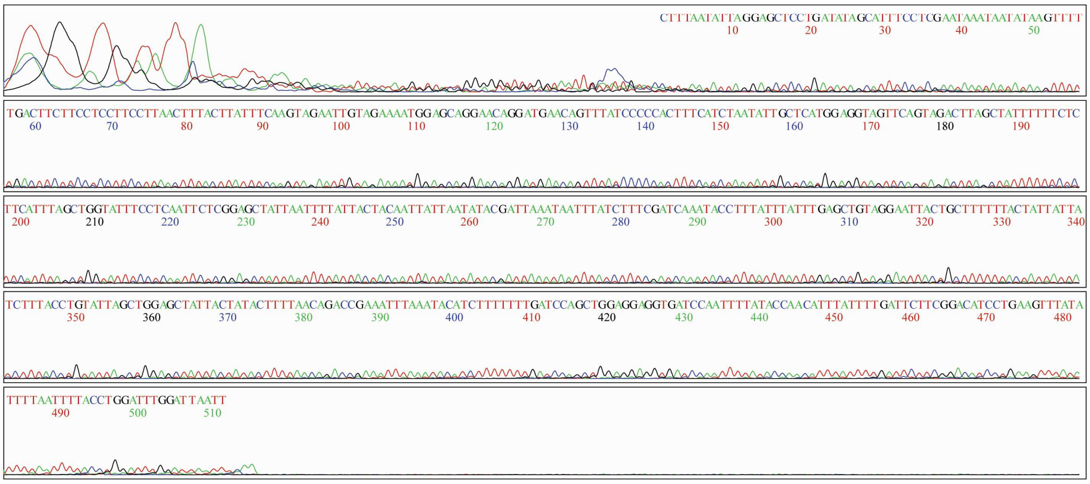
MFTGGFGNFLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPL
SSNIAHGGSSVDLAIFSLHLAGISSILGAINFITTIINMRLNNLSFDQMPLFIWAVGITA
FLLLLSLPVLAGAITMLLTDRNLNTS

Figure 6.40: The translation product of the mitochondrial COI gene of *Polytela gloriosae*

Polytela gloriosae

Sample :SM_LEPTO.F_23390-3_7816
Trim Start :81
Trim End :593
Qv20 Bases :512

Run start: 2015/10/07 12:05:44
Run stop: 2015/10/07 14:03:15
PDF created: 2015/10/07 15:10:53



Polytela gloriosae

Sample :SM_LEPTO.R_23390-4_7816
Trim Start :79
Trim End :590
Qv20 Bases :511

Run start: 2015/10/07 12:05:44
Run stop: 2015/10/07 14:03:15
PDF created: 2015/10/07 15:10:55

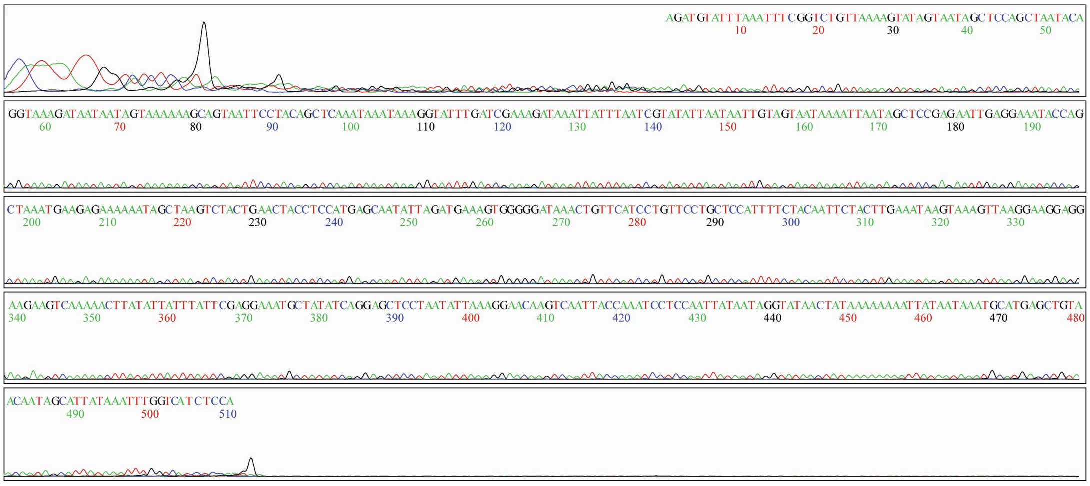


Figure 6.41: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Polytela gloriosae* using Forward and Reverse primers.

Table 6.13: Comparison of Nucleotide frequencies of COI gene sequence of *Polytela gloriosae* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
KX603662 Polytela gloriosae	41.9	16.1	28.4	13.5	35	20.0	24.5	20.0	52	15.5	26.5	6.5	39	12.9	34.2	14.2
KT879854 Polytela gloriosae	40.6	16.3	28.2	14.8	35	20.6	23.2	21.3	48	15.5	27.1	9.0	39	12.9	34.2	14.2
KX043776 Lacanobia oleracea	41.1	13.5	30.5	14.8	38	21.9	20.0	20.0	50	6.5	41.9	1.3	35	12.3	29.7	23.2
KP253339 Lacanobia splendens	40.4	14.2	30.5	14.8	38	21.9	20.0	20.0	49	7.7	41.9	1.3	34	12.9	29.7	23.2
GU828571 Diaphone sp.	39.0	14.7	31.0	15.3	38	21.2	19.9	20.5	47	9.1	42.2	1.3	31	13.6	31.2	24.0
KJ393988 Lacanobia nevadae	39.8	15.7	29.9	14.6	37	23.1	21.2	18.6	49	9.7	37.7	3.9	34	14.2	31.0	21.3
JF852847 Lacanobia radix	39.8	15.3	29.9	15.1	38	21.9	20.0	20.0	48	9.7	40.6	1.9	34	14.2	29.0	23.2
KX281210 Mamestra configurata	39.8	14.2	30.3	15.7	38	21.8	19.2	20.5	49	8.4	40.3	1.9	32	12.3	31.6	24.5
KX043003 Lacanobia suasa	39.4	15.7	30.8	14.2	37	23.2	21.3	18.7	48	9.7	39.4	2.6	33	14.2	31.6	21.3
KJ381520 Hada sutrina	39.6	15.5	30.1	14.8	38	22.6	19.4	20.0	47	9.7	41.9	1.3	34	14.2	29.0	23.2
HQ951038 Metopiora sanguinata	38.5	14.4	32.0	15.1	38	21.9	20.0	20.0	44	7.7	46.5	1.9	34	13.5	29.7	23.2
MG522740 Papestra biren	40.0	14.4	30.3	15.3	38	21.9	20.0	20.0	48	8.4	41.3	1.9	34	12.9	29.7	23.9
KT782674 Conisania leineri	39.6	14.8	30.5	15.1	38	21.9	20.0	20.0	46	9.7	41.9	1.9	34	12.9	29.7	23.2
KX045425 Anarta stigmosa	39.4	14.8	31.2	14.6	38	21.9	20.0	20.0	45	11.0	41.9	1.9	35	11.6	31.6	21.9

Table 6.14: Percentage of evolutionary divergence of *Polytela gloriosae* with its closely related species accessible from NCBI GenBank

Sl. No.	**Accession No**	**Organism**	**Percentage of divergence**
1.	KX603662	*Polytela gloriosae* (Kerala)	
2.	KT879854	*Polytela gloriosae* (Karnataka)	0.00%
3.	JF852847	*Lacanobia radix*	9.45%
4.	MG522740	*Papestra biren*	9.45%
5.	HQ951038	*Metopiora sanguinata*	9.50%
6.	KX043776	*Lacanobia oleracea*	10.60%
7.	KP253339	*Lacanobia splendens*	11.15%
8.	KT782674	*Conisania leineri*	11.15%
9.	GU828571	*Diaphone* sp.	11.20%
10.	KJ393988	*Lacanobia nevadae*	11.70%
11.	KX281210	*Mamestra configurata*	11.70%
12.	KX043003	*Lacanobia suasa*	12.82%
13.	KX045425	*Anarta stigmosa*	11.82%
14.	KX043003	*Lacanobia suasa*	12.82%

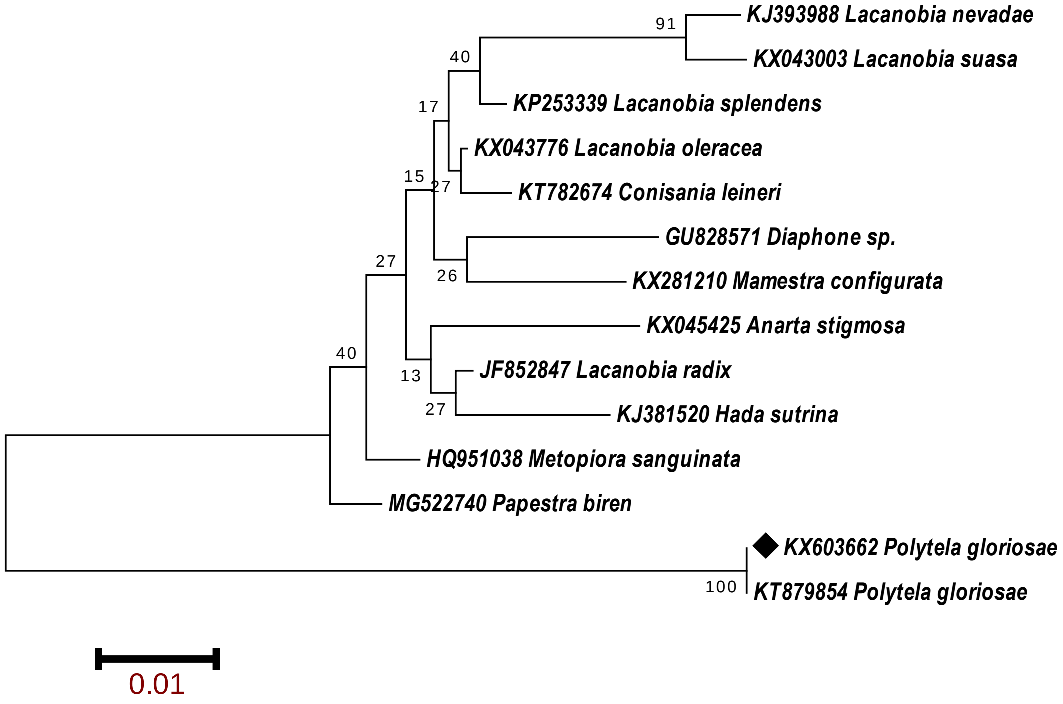


Figure 6.42: Phylogenetic relationship of *Polytela gloriosae* inferred by NJ tree method

DISCUSSION

Both nucleotide and peptide BLAST analysis showed that this species has 100% sequence similarity to the same genus reported from Karnataka (KT879854). Eventhough this species has been found in various geographically isolated areas, their sequence doesn't have any kind of variation. Hence the present study stress that the barcode generated can be used to easily spot the specimen and also to analyse its phylogeny.

The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Polytela gloriosae* showed bias to nucleotide AT, with following composition of nucleotides T = 41.9%, C = 16.1%, A = 28.4% and G = 13.5%. This greater AT content (70.3%) over GC content (29.6%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Polytela gloriosae* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 0% to 12.82% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has no divergence (0%) with those from Karnataka while 12.82% to England (Table 6.14). On the basis of the data observed this species may be rooted from those found in England which diverted into different clades due to geographical variation. Result thus concluded that this species doesn't have any major changes in India while slightly changes from those reported from England during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Polytela gloriosae* with related species. *Polytela gloriosae* isolated from Kerala (KX603662) showed 0% divergence with

Polytela gloriosae (KT879854) from Karnataka and 12.82% divergence with *Lacanobia suasa* (KX043003) from England. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Polytela gloriosae* isolated from Kerala. Closest relative of *Polytela gloriosae* is *Polytela gloriosae* from Karnataka represented within the same clade.

8. ***Acosmeryx anceus subdentata*** (Rothschild and Jordan, 1903)

Specimen details:

Voucher specimen : CUAA – 02 – A1
Date of collection : 04- DEC-2015
Locality : Palakkad: Parali
Lat- Lon : 18.8453º N, 76.5198º E
GenBank accession : MH656690

Description and distribution:

Acosmeryx anceus subdentata is a moth of the family Sphingidae (Figure. 6.43). They are the smallest species of the genus. The wingspan is 70-88 mm. Fore wing distinctly angulate at R3, often with traces of teeth. Upper side tawny cinnamon to chestnut brown in colour; an oblique distal band diffuse through posteriorly. Hind wing fuscous. *Acosmeryx anceus subdentata* was described by Caspar Stoll in 1781, and it is known from India, New Guinea, and Queensland (Australia).

Fig.6.43: *Acosmeryx anceus subdentata*

Damage:

The larvae are considered as a minor pest on several plant families. The larvae feed voraciously on leaves. The later instar feeds on tissues and plants causing massive destruction.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Acosmeryx anceus subdentata* collected has been amplified using the primer LEP (Table 3.1). The PCR amplification yielded 514 bp and 515 bp long products for the specimens obtained from two different locations. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 6.44 – 6.47 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 6.15.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Acosmeryx anceus subdentata* voucher collected during the present study (Table 6.15). It showed bias to nucleotide AT, with nucleotide composition with T = 41.9%, C = 16.1%, A = 28.4 % and G = 13.5%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 6.16 and Figure 6.48 respectively.

> *Acosmeryx anceus subdentata* CDS-2018/ 514 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUAA-02-A1

> *Acosmeryx anceus subdentata*

```
ATAATTTTTTTTATAGTAATACCAATTATAATTGGAGGATTTGGAAATTGATTAGTACCT
TTAATATTAGGAGCCCCTGATATAGCATTCCCACGAATAAATAATATAAGATTTTGACTT
CTCCCACCTTCATTAACATTATTAATTTCTAGAAGTATTGTAGAAAATGGAGCAGGTACT
GGATGAACAGTATACCCCCCTTTATCTTCTAATATTGCCCATAGAGGAAGCTCTGTTGAT
TTAGCAATTTTTTCCCTTCATTTAGCTGGTATTTCATCAATTATAGGAGCAGTTAATTTT
ATTACCACAATTATTAATATACGAATTAATAATTTATCATTTGATCAAATACCATTGTTT
GTATGAGCTGTAGGAATTACAGCTTTCCTTTTACTTTTATCTTTACCTGTATTAGCAGGA
GCAATTACCATACTATTAACTGACCGAAATTTAAATACATCATTTTTTGATCCTGCTGGA
GGAGGAGATCCTATTTTATATCAACATCTATCTG
```

Figure 6.44: The partial DNA sequence of the mitochondrial COI gene of *Acosmeryx anceus subdentata*

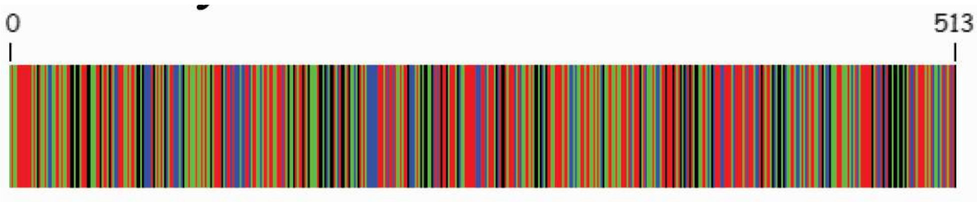


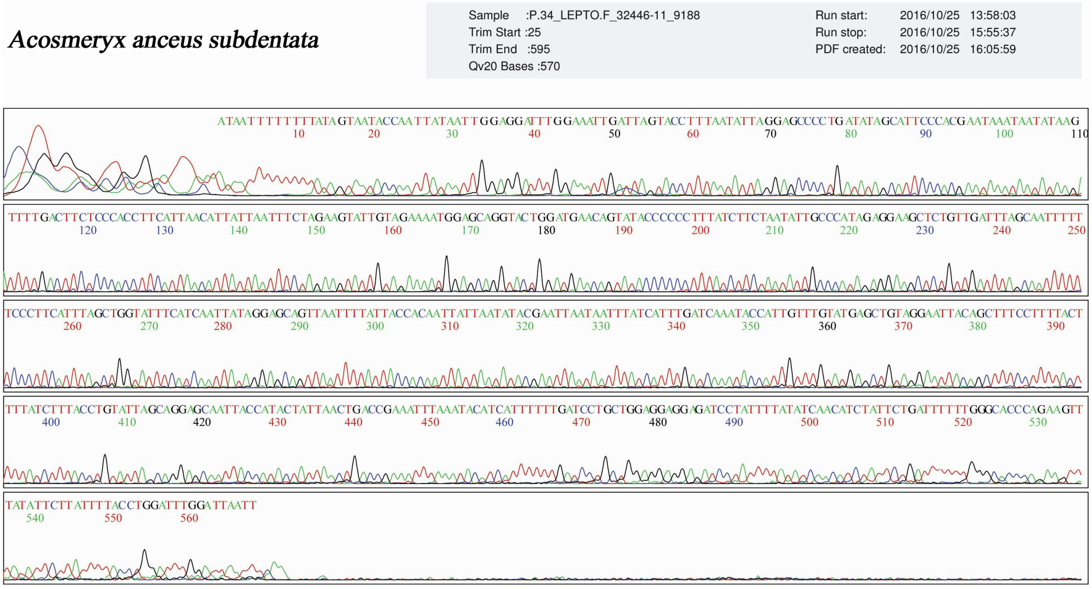
Figure 6.45: Molecular barcode of the mitochondrial COI gene of *Acosmeryx anceus subdentata*

> *Acosmeryx anceus subdentata* / 171AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUAA-02-A1

> *Acosmeryx anceus subdentata*

```
MIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGT
GWTVYPPLSSNIAHSGSSVDLAIFSLHLAGISSIMGAVNFITTIINMRINNLSFDQMPLF
VWAVGITAFLLLLSLPVLAGAITMLLTDRNLNTSFFDPAGGGDPILYQHLS
```

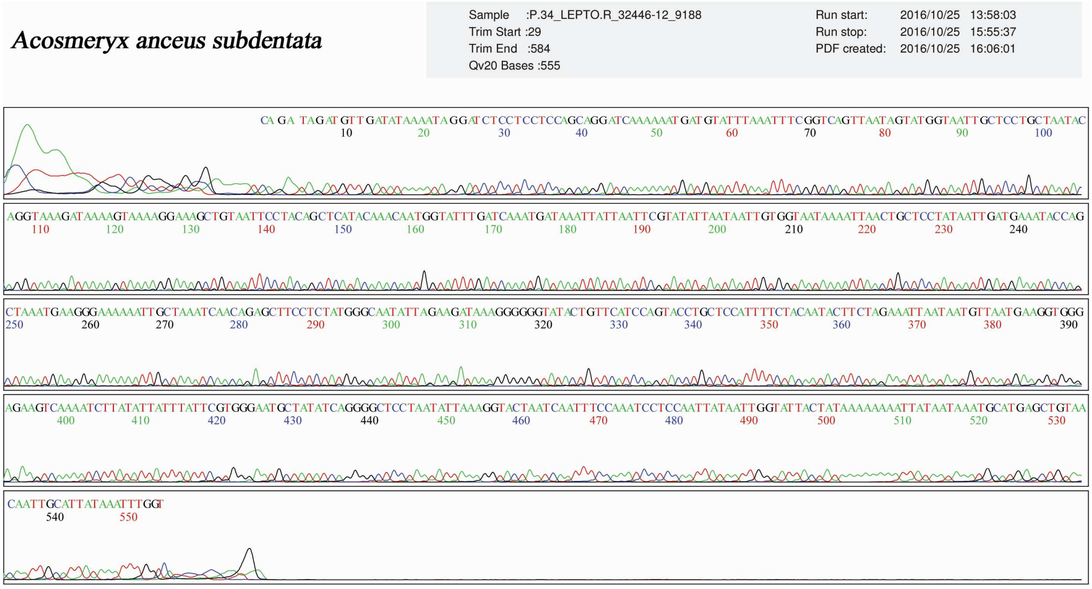
Figure 6.46: The translation product of the mitochondrial COI gene of *Acosmeryx anceus subdentata*



Acosmeryx anceus subdentata

Sample :P.34_LEPTO.F_32446-11_9188
Trim Start :25
Trim End :595
Qv20 Bases :570

Run start: 2016/10/25 13:58:03
Run stop: 2016/10/25 15:55:37
PDF created: 2016/10/25 16:05:59



Acosmeryx anceus subdentata

Sample :P.34_LEPTO.R_32446-12_9188
Trim Start :29
Trim End :584
Qv20 Bases :555

Run start: 2016/10/25 13:58:03
Run stop: 2016/10/25 15:55:37
PDF created: 2016/10/25 16:06:01

Figure 6.47: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Acosmeryx anceus subdentata* using Forward and Reverse primers.

Table 6.15: Comparison of Nucleotide frequencies of COI gene sequence of Acosmeryx anceus subdentata with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH656690 Acosmeryx anceus subdentata (Kerala)	38.1	16.3	31.5	14.0	43	7.0	49.1	.6	28	15.7	30.8	25.6	43	26.3	14.6	15.8
KJ168175 Acosmeryx anceus subdentata	37.7	15.1	32.4	14.8	43	7.0	48.8	1.2	27	13.4	33.7	25.6	43	25.1	14.6	17.5
KJ168113 Acosmeryx anceus subdentata	37.5	15.3	32.6	14.6	42	7.6	49.4	.6	27	13.4	33.7	25.6	43	25.1	14.6	17.5
KJ168151 Acosmeryx anceus	36.9	15.9	32.6	14.6	40	9.9	49.4	.6	28	12.8	33.7	25.6	43	25.1	14.6	17.5
KC182155 Acosmeryx naga hissarica	37.1	15.7	32.4	14.8	41	9.3	48.8	1.2	28	12.8	33.7	25.6	43	25.1	14.6	17.5
JN677661 Acosmeryx socrates	37.1	15.9	32.4	14.6	42	8.7	48.8	.6	27	14.0	33.7	25.6	43	25.1	14.6	17.5
JN677653 Acosmeryx formosana	37.9	15.5	32.0	14.6	44	7.6	47.7	.6	27	14.0	33.7	25.6	43	25.1	14.6	17.5
JN677652 Acosmeryx castanea	36.9	15.5	33.0	14.6	40	8.7	50.6	.6	28	12.8	33.7	25.6	43	25.1	14.6	17.5
JN677659 Acosmeryx shervillii	37.5	15.5	32.0	15.0	41	9.3	47.7	1.7	28	12.2	33.7	25.6	43	25.1	14.6	17.5
JN677660 Acosmeryx sinjaevi	37.7	15.7	32.0	14.6	44	7.6	47.7	.6	26	14.5	33.7	25.6	43	25.1	14.6	17.5
JN677656 Acosmeryx omissa	36.9	16.3	32.0	14.8	41	10.5	47.7	1.2	27	13.4	33.7	25.6	43	25.1	14.6	17.5
JN677865 Dahira rubiginosa	38.4	15.0	32.2	14.4	45	7.0	48.3	.0	28	12.8	33.7	25.6	43	25.1	14.6	17.5
JN677657 Acosmeryx pseudomissa	36.7	16.3	32.2	14.8	40	11.0	48.3	1.2	28	12.8	33.7	25.6	43	25.1	14.6	17.5
KJ168880 Acosmeryx miskini	36.7	16.1	32.8	14.4	41	9.3	50.0	.0	27	14.0	33.7	25.6	43	25.1	14.6	17.5
KJ168562 Acosmeryx miskinoides	36.7	16.5	32.2	14.6	40	11.0	48.3	.6	27	13.4	33.7	25.6	43	25.1	14.6	17.5

Table 6.16: Percentage of evolutionary divergence of *Acosmeryx anceus subdentata* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH656690	*Acosmeryx anceus subdentata* (Kerala)	
2.	KJ168175	*Acosmeryx anceus subdentata* (Myanmar)	0.41%
3.	KJ168113	*Acosmeryx anceus subdentata*	1.23%
4.	KJ168151	*Acosmeryx anceus*	5.06%
5.	KC182155	*Acosmeryx naga hissarica*	6.11%
6.	JN677660	*Acosmeryx sinjaevi*	6.98%
7.	JN677653	*Acosmeryx formosana*	6.98%
8.	KJ168880	*Acosmeryx miskini*	6.98%
9.	JN677865	*Dahira rubiginosa*	7.04%
10.	JN677656	*Acosmeryx omissa*	7.46%
11.	JN677652	*Acosmeryx castanea*	7.49%
12.	JN677661	*Acosmeryx Socrates*	7.56%
13.	KJ168562	*Acosmeryx miskinoides*	8.41%
14.	JN677659	*Acosmeryx shervillii*	8.91%
15.	JN677657	*Acosmeryx pseudomissa*	9.29%

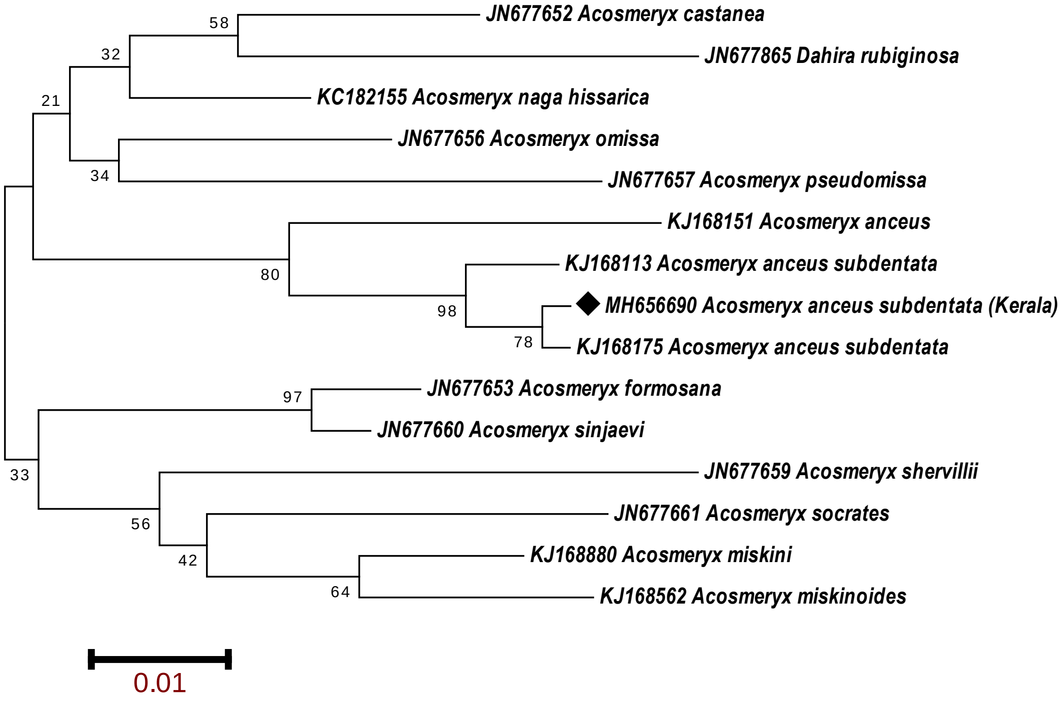


Figure 6.48: Phylogenetic relationship of *Acosmeryx anceus subdentata* inferred by NJ tree method

DISCUSSION

Both nucleotide and peptide BLAST analysis showed that this species has 99.6% sequence similarity to the same genus reported from Myanmar (KJ168175).

The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Acosmeryx anceus subdentata* showed bias to nucleotide AT, with following composition of nucleotides T = 38.1%, C = 16.3%, A = 31.5% and G = 14.0%. This greater AT content (69.6%) over GC content (30.3%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Acosmeryx anceus subdentata* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 0.41% to 9.29% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (0.41%) with those from Myanmar while 9.29% to Malaysia (Table 6.16). On the basis of the data observed this species may be rooted from those found in Malaysia which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from Malaysia during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Acosmeryx anceus subdentata* with related species. *Acosmeryx anceus subdentata* isolated from Kerala (MH656690) showed 0.41% divergence with *Acosmeryx anceus subdentata* (KJ168175) from Myanmar and 9.29% divergence with *Acosmeryx pseudomissa* (JN677657) from Malaysia. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Acosmeryx anceus subdentata* isolated from Kerala. Closest relative of *Acosmeryx anceus subdentata* is *Acosmeryx anceus subdentata* from Myanmar represented within the same clade.

9. ***Diaphana indica*** (Saunders, 1851)

Specimen details:

Voucher specimen : CUDI – 01 – A1
Date of collection : 12- Feb -2016
Locality : Kerala: Malappuram
Lat- Lon : 11.0510º N, 76.0711º E
GenBank accession : KX587508

Description and distribution:

Fig.6.49: *Diaphana indica*

The wingspan of the adult moth, *Diaphana indica* is 30 mm (Figure. 6.49). Adults have translucent whitish wings and having broad dark brown borders. The body is whitish below, and brown on top of head, thorax and the end of the abdomen with a tuft of light brown "hairs" on the tip of the abdomen, vestigial in the male but well developed in the case of females. It is formed by long scales, carried in a pocket on each side of the 7th abdominal segment, from where they can be everted to form the tufts. In the male, the clasper's harpe is twice as long as it is wide, with the costa and sacculus running parallel and being strongly sclerotized, and a rounded cucullus. The natural range of this moth seems to extend from South Asia to southern China and Taiwan, and south through Southeast Asia to the Maluku Islands of Indonesia.

Damage

The caterpillar larvae are a frequent visitor of agricultural pests, perhaps mostly noticed as a pest of cucumbers. *D. indica* is quite polyphagous and prefers other Cucurbitoideae to cucumbers. Typically, they feed upon the leaves of the food plants.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Diaphana indica* collected has been amplified using the primer LEP (Table 3.1). The PCR amplification yielded 525 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 6.50 – 6.53 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 6.17.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Diaphana indica* voucher collected during the present study (Table 6.17). It showed bias to nucleotide AT, with nucleotide composition with T = 40.0%, C = 15.6%, A = 29.9% and G = 14.5%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 6.18 and Figure 6.54 respectively.

> *Diaphania indica* CDS-2018/ 525 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUDI-01-A1

> *Diaphania indica*

```
TTTATTATAATTTTTTTTATAGTAATACCTATTATAATTGGAGGATTTGGTAATTGATTA
GTACCTTTAATATTAGGAGCACCTGATATAGCTTTCCCACGAATAAATAATATAAGTTTT
TGATTACTTCCACCCTCATTAACCTTACTAATTTCTAGAAGAATTGTTGAAAATGGGGCT
GGAACAGGATGAACAGTGTACCCCCCACTTTCATCTAATATTGCTCATGGAGGAAGATCA
GTAGATTTAGCTATTTTTTCATTACATTTAGCTGGAATTTCCTCTATTTTAGGAGCAATT
AATTTTATTACTACAATTATTAATATACGAATTAATGGACTTAATTTTGATCAAATACCT
TTATTTGTTTGAGCTGTAGGAATTACAGCCCTTCTTTTATTATTATCTCTACCAGTTTTA
GCTGGAGCTATTACCATACTTTTAACAGATCGAAATTTAAATACTTCTTTTTTTGACCCT
GCAGGTGGGGGAGATCCTATTCTATACCAACATTTATTTTGATTT
```

Figure 6.50: The partial DNA sequence of the mitochondrial COI gene of *Diaphania indica*

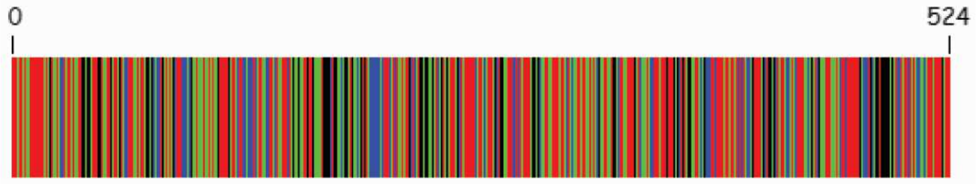


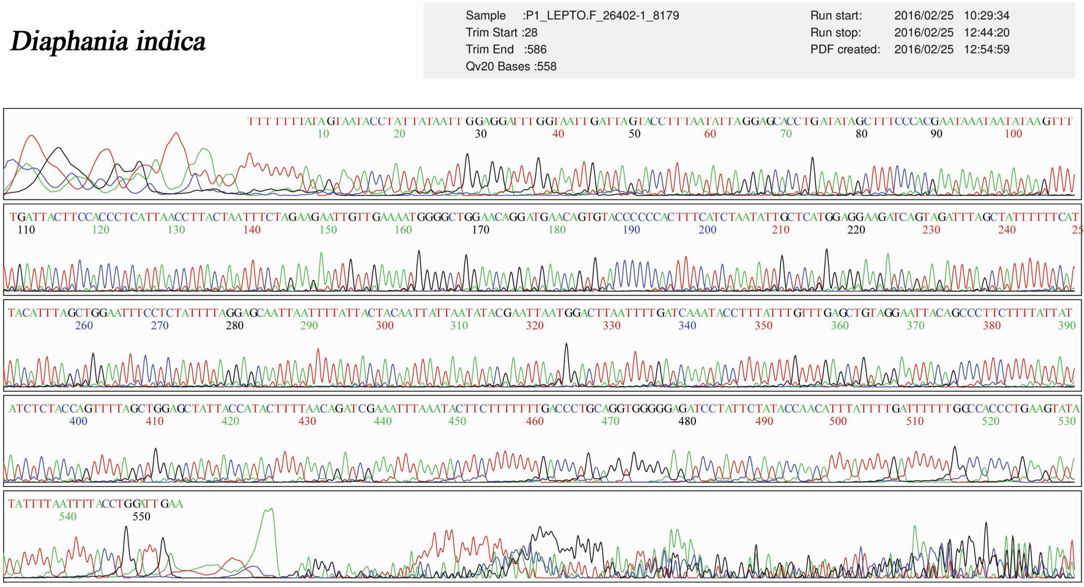
Figure 6.51: Molecular barcode of the mitochondrial COI gene of *Diaphania indica*

> *Diaphania indica* / 173AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUDI-01-A1

> *Diaphania indica*

```
MIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGT
GWTVYPPLSSNIAHGGSSVDLAIFSLHLAGISSILGAINFITTIINMRINGLNFDQMPLF
VWAVGITALLLLLSLPVLAGAITMLLTDRNLNTSFFDPAGGGDPILYQHLFWF
```

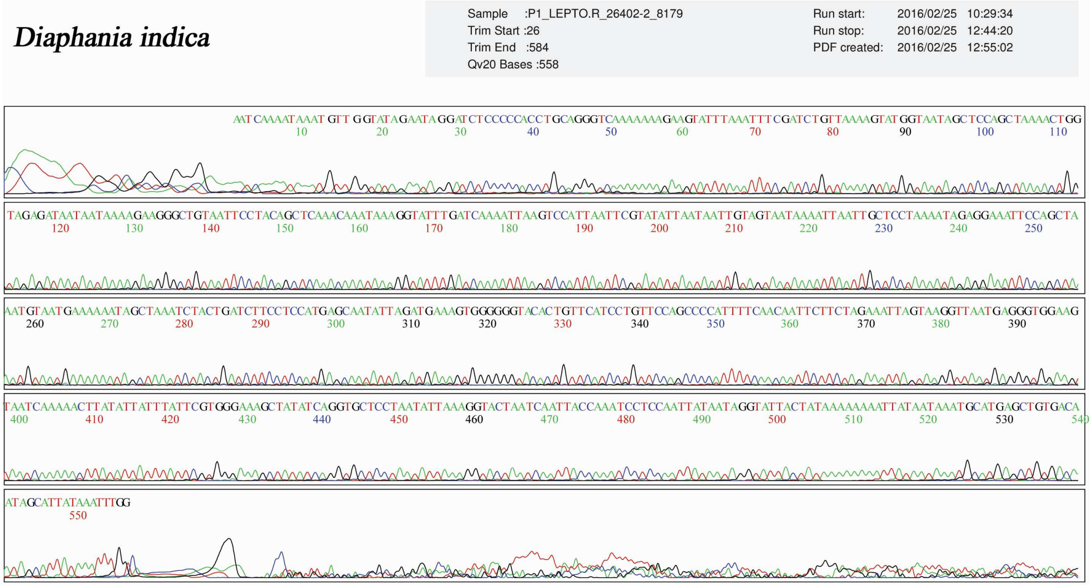
Figure 6.52: The translation product of the mitochondrial COI gene of *Diaphania indica*



Diaphania indica

Sample :P1_LEPTO.F_26402-1_8179
Trim Start :28
Trim End :586
Qv20 Bases :558

Run start: 2016/02/25 10:29:34
Run stop: 2016/02/25 12:44:20
PDF created: 2016/02/25 12:54:59



Diaphania indica

Sample :P1_LEPTO.R_26402-2_8179
Trim Start :26
Trim End :584
Qv20 Bases :558

Run start: 2016/02/25 10:29:34
Run stop: 2016/02/25 12:44:20
PDF created: 2016/02/25 12:55:02

Figure 6.53: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Diaphania indica* using Forward and Reverse primers.

Table 6.17: Comparison of Nucleotide frequencies of COI gene sequence of *Diaphania indica* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
KX587508 Diaphania indica	40.0	15.6	29.9	14.5	47	5.7	45.1	1.7	28	16.6	30.3	25.1	45	24.6	14.3	16.6
KR868762 Diaphania indica	39.0	15.0	31.0	14.9	47	5.1	46.9	1.1	26	14.9	32.0	26.9	44	25.1	14.3	16.6
KX052416 Crambidae sp.	38.5	13.5	32.2	15.8	46	4.0	47.4	2.3	26	12.6	34.3	26.9	43	24.0	14.9	18.3
JQ561266 Diaphania sp.	39.6	13.7	31.2	15.4	50	3.4	45.7	1.1	26	13.1	33.7	26.9	43	24.6	14.3	18.3
HQ934090 Lepidoptera sp.	39.8	13.5	31.2	15.4	50	2.9	45.7	1.1	26	13.1	33.7	26.9	43	24.6	14.3	18.3
JQ525706 Desmia sp.	39.4	14.3	31.2	15.0	47	6.3	46.3	.6	29	12.0	33.1	26.3	43	24.6	14.3	18.3
JQ553788 Diaphania elegans	39.2	13.3	32.2	15.2	47	3.4	48.6	.6	27	12.0	33.7	26.9	43	24.6	14.3	18.3
MF432558 Peridroma saucia	38.7	14.7	32.0	14.7	43	4.6	50.9	1.1	29	13.7	31.4	25.7	43	25.7	13.7	17.1
KF389795 Rehimena leptophaes	39.2	13.5	32.0	15.2	46	4.6	48.6	.6	29	11.4	33.1	26.9	43	24.6	14.3	18.3
KF388991 Scoparia pediopola	40.0	12.6	32.6	14.9	48	2.3	49.7	.0	29	10.9	33.7	26.3	43	24.6	14.3	18.3
HQ953158 Nosophora hypsalis	38.1	13.7	33.3	14.9	45	4.6	50.3	.6	27	12.0	34.9	26.3	43	24.6	14.9	17.7
HQ953057 Syllepte nigriscriptalis	39.2	12.6	33.3	14.9	46	2.9	51.4	.0	28	11.4	34.3	26.3	44	23.4	14.3	18.3
HQ952494 Hyalobathra minialis	38.3	13.7	32.8	15.2	45	4.6	49.7	.6	27	12.6	34.3	26.3	43	24.0	14.3	18.9

Table 6.18: Percentage of evolutionary divergence of *Diaphana indica* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	KX587508	*Diaphania indica* (Kerala)	
2.	KR868762	*Diaphania indica* (Rajasthan)	0.00%
3.	KX052416	*Crambidae* sp.	2.25%
4.	KF389795	*Rehimena leptophaes*	6.58%
5.	HQ953158	*Nosophora hypsalis*	7.82%
6.	HQ952494	*Hyalobathra minialis*	8.65%
7.	JQ553788	*Diaphania elegans*	8.65%
8.	KF388991	*Scoparia pediopola*	8.65%
9.	JQ525706	*Desmia* sp.	8.66%
10.	JQ561266	*Diaphania* sp.	9.06%
11.	HQ934090	*Lepidoptera* sp.	9.06%
12.	MF432558	*Peridroma saucia*	9.49%
13.	HQ953057	*Syllepte nigriscriptalis*	10.77%

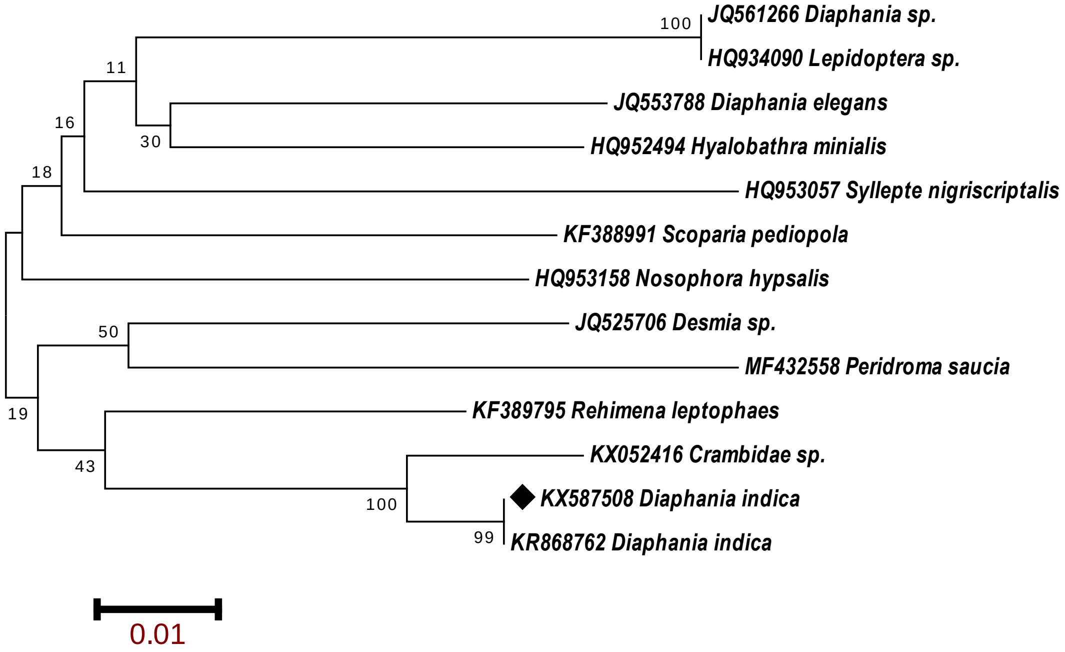


Figure 6.54: Phylogenetic relationship of *Diaphania indica* inferred by NJ tree method

DISCUSSION

Both nucleotide and peptide BLAST analysis showed that this species has 100% sequence similarity to the same genus reported from Rajasthan (KR868762). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Diaphana indica* showed bias to nucleotide AT, with following composition of nucleotides T = 40.0%, C = 15.6%, A = 29.9% and G = 14.5%. This greater AT content (69.9%) over GC content (30.1%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Diaphana indica* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 0% to 10.77% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has no divergence (0%) with those from Rajasthan while 10.77% to Australia (Table 6.18). On the basis of the data observed this species may be rooted from those found in Australia which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from Australia during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Diaphana indica* with related species. *Diaphana indica* isolated from Kerala (MH656690) showed 0% divergence with *Diaphana indica* (KR868762) from Rajasthan and 10.77% divergence with *Syllepte nigriscriptalis* (HQ953057) from Australia. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Diaphana indica* isolated from Kerala.

Closest relative of *Diaphana indica* is *Diaphana indica* from Rajasthan represented within the same clade.

10. ***Glyphodes bicolor*** (Swainson, 1821)

Specimen details:

Voucher specimen : CUGB – 01 – A1
Date of collection : 14- Nov-2014
Locality : Kasaragod: Cheruvathur
Lat- Lon : 12.2165º N, 75.1626º E
GenBank accession : MH674103

Description and distribution:

Glyphodes bicolor is small in size with the wing span of about 20 mm (Figure. 6.55). Adults are dark brown having translucent white patches on each wing. The wings fringed by long white hairs, forewing with translucent white coloured triangles, hind wings with basal white bands. These are commonly flushed from herbage beneath the vegetation. Distribution range includes Africa, India, Srilanka, Nepal, Thailand, China, New Guinea and Australia.

Fig.6.55: *Glyphodes bicolor*

Damage:

Since they are polyphagus larvae feed on the silken galleries on grasses and lower vegetations especially agricultural crops.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Glyphodes bicolor* collected has been amplified using the primer CP2 (Table 3.1). The PCR

amplification yielded 604 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 6.56 – 6.59 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 6.19.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Glyphodes bicolor* voucher collected during the present study (Table 6.19). It showed bias to nucleotide AT, with nucleotide composition with T = 38.6%, C = 14.2%, A = 32.1% and G = 15.1%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 6.20 and Figure 6.60 respectively.

> *Glyphodes bicolor* CDS-2018/ 604 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUGB-01-A1

> *Glyphodes bicolor*

```
ATAGTAGGAACATCTCTAAGTTTATTAATTCGAGCAGAATTAGGAAATCCAGGATCTTTA
ATTGGAGATGATCAAATTTATAATACTATTGTTACAGCTCATGCTTTTATTATAATTTTT
TTTATAGTTATGCCAATTATAATTGGAGGATTTGGTAACTGATTAGTACCATTAATACTT
GGAGCTCCAGATATAGCATTTCCTCGAATAAATAATATAAGATTCTGATTATTACCCCCA
TCATTAACTTTATTAATTTCTAGAAGAATTGTAGAAAATGGAGCTGGAACTGGTTGAACA
GTTTATCCCCCTCTTTCTTCTAATATTGCACATGGAGGAAGATCAGTTGATTTAGCAATT
TTTTCATTACATTTAGCTGGAATTTCATCAATTTTAGGAGCAATTAATTTTATTACAACT
ATTATTAATATACGAATTAATGGCTTATCTTTTGATCAAATACCTTTATTTGTATGAGCT
GTTGGAATTACAGCATTACTTCTTTTACTATCATTACCAGTTTTAGCAGGTGCTATTACT
ATATTATTAACAGATCGTAATTTAAATACATCATTTTTTGATCCTGCAGGAGGAGGAGAT
CCAA
```

Figure 6.56: The partial DNA sequence of the mitochondrial COI gene of *Glyphodes bicolor*

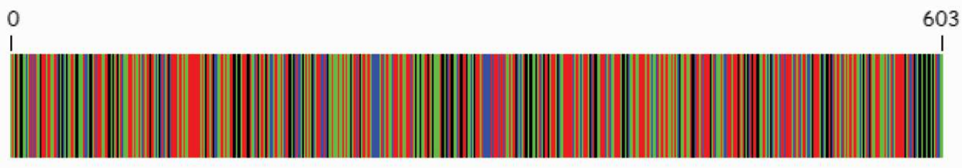


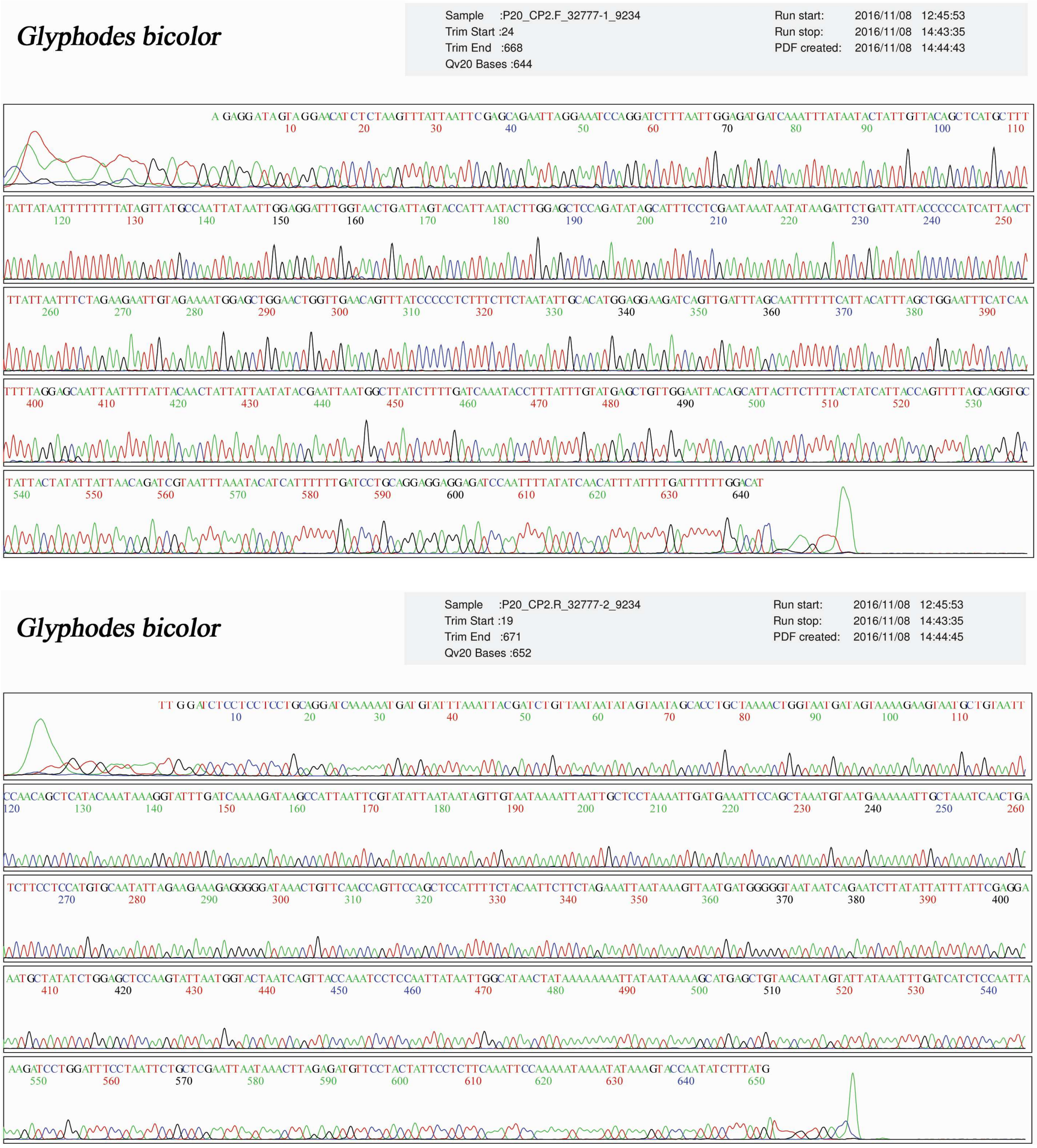
Figure 6.57: Molecular barcode of the mitochondrial COI gene of *Glyphodes bicolor*

> *Glyphodes bicolor* / 201AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUGB-01-A1

> *Glyphodes bicolor*

```
MVGTSLSLLIRAELGNPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWLVPLML
GAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHGGSSVDLAI
FSLHLAGISSILGAINFITTIINMRINGLSFDQMPLFVWAVGITALLLLLSLPVLAGAIT
MLLTDRNLNTSFFDPAGGGDP
```

Figure 6.58: The translation product of the mitochondrial COI gene of *Glyphodes bicolor*


Glyphodes bicolor

Sample :P20_CP2.F_32777-1_9234
Trim Start :24
Trim End :668
Qv20 Bases :644

Run start: 2016/11/08 12:45:53
Run stop: 2016/11/08 14:43:35
PDF created: 2016/11/08 14:44:43

Glyphodes bicolor

Sample :P20_CP2.R_32777-2_9234
Trim Start :19
Trim End :671
Qv20 Bases :652

Run start: 2016/11/08 12:45:53
Run stop: 2016/11/08 14:43:35
PDF created: 2016/11/08 14:44:45

Figure 6.59: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Glyphodes bicolor* using Forward and Reverse primers.

Table 6.19: Comparison of Nucleotide frequencies of COI gene sequence of *Glyphodes bicolor* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH674103 Glyphodes bicolor (Kerala)	38.6	14.2	32.1	15.1	27	13.9	31.2	27.7	42	26.4	14.4	16.9	46	2.5	50.7	.5
HQ952843 Glyphodes bicolor	38.4	14.6	32.3	14.7	28	12.9	32.8	25.9	44	24.9	13.9	17.4	43	5.9	50.0	1.0
AB158240 Glyphodes stolalis	40.4	12.7	32.1	14.7	29	12.4	32.2	26.7	42	24.9	15.4	17.4	50	1.0	48.8	.0
KF808403 Lepidoptera sp.	39.4	13.7	32.1	14.7	29	11.9	32.3	26.4	44	25.4	13.9	16.9	45	4.0	50.0	1.0
HQ952965 Cirrhochrista annulifera	40.4	13.6	31.5	14.6	29	12.4	32.3	26.4	44	25.4	13.9	16.9	49	3.0	48.0	.5
KX042561 Eudonia angustea	38.6	13.2	33.6	14.6	30	11.4	32.8	25.9	44	25.4	13.9	16.9	42	3.0	54.0	1.0
HQ952956 Palpita uedai	40.2	13.9	31.5	14.4	29	12.9	31.8	26.4	44	25.4	13.9	16.9	48	3.5	48.5	.0
HQ952846 Glyphodes microta	39.2	13.4	32.6	14.7	29	12.4	32.3	26.4	44	25.4	13.9	16.9	45	2.5	51.5	1.0
HQ952834 Glyphodes cosmarcha	40.4	13.6	31.6	14.4	28	12.9	32.3	26.4	44	25.4	13.9	16.9	49	2.5	48.5	.0
MH416568 Glyphodes shafferorum	39.7	13.7	32.1	14.4	28	12.9	32.3	26.4	44	25.4	13.9	16.9	47	3.0	50.0	.0
MH415901 Maruca fuscalis	39.2	13.9	32.3	14.6	30	11.4	31.8	26.4	44	25.4	13.9	16.9	44	5.0	51.0	.5
KX863217 Cirrhochrista annulifera	39.9	14.1	31.5	14.6	28	12.9	32.3	26.4	44	25.4	13.9	16.9	48	4.0	48.0	.5
HQ952833 Glyphodes cosmarcha	40.6	13.6	31.5	14.4	28	12.9	32.3	26.4	44	25.4	13.9	16.9	50	2.5	48.0	.0
HQ952819 Glyphodes onychinalis	39.6	13.4	32.8	14.2	30	11.4	32.8	25.9	44	25.4	13.9	16.9	45	3.5	51.5	.0
AB158241 Glyphodes multilinealis	40.4	13.2	31.5	14.9	29	11.9	31.7	27.2	42	24.9	15.4	17.4	50	3.0	47.3	.0

Table 6.20: Percentage of evolutionary divergence of *Glyphodes bicolor* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH674103	*Glyphodes bicolor* (Kerala)	
2.	HQ952843	*Glyphodes bicolor* (Australia)	2.11%
3.	HQ952956	*Palpita uedai*	6.40%
4.	KX042561	*Eudonia angustea*	11.95%
5.	AB158240	*Glyphodes stolalis*	12.22%
6.	KF808403	*Lepidoptera* sp.	12.67%
7.	HQ952965	*Cirrhochrista annulifera*	13.31%
8.	MH415901	*Maruca fuscalis*	13.31%
9.	HQ952834	*Glyphodes cosmarcha*	13.58%
10.	MH416568	*Glyphodes shafferorum*	13.81%
11.	KX863217	*Cirrhochrista annulifera*	13.95%
12.	HQ952833	*Glyphodes cosmarcha*	13.95%
13.	HQ952819	*Glyphodes onychinalis*	13.91%
14.	HQ952846	*Glyphodes microta*	14.27%
15.	AB158241	*Glyphodes multilinealis*	15.02%

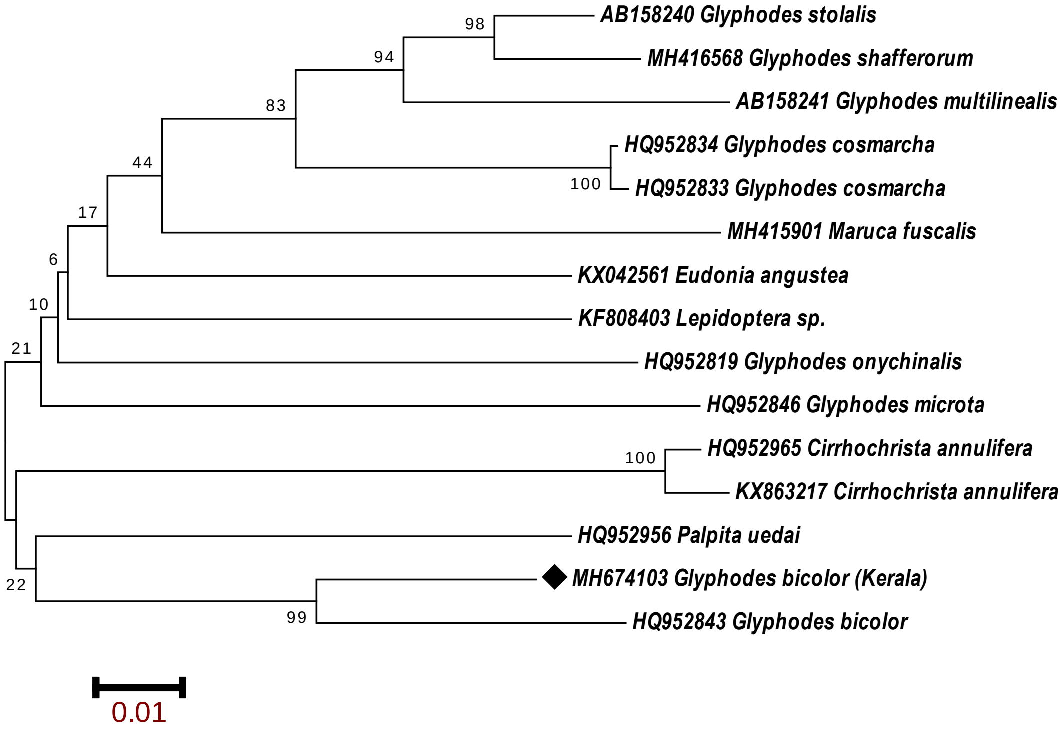


Figure 6.60: Phylogenetic relationship of *Glyphodes bicolor* inferred by NJ tree method

DISCUSSION

Both nucleotide and peptide BLAST analysis showed that this species has 97.9% sequence similarity to the same genus reported from Australia (HQ952843). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Glyphodes bicolor* showed bias to nucleotide AT, with following composition of nucleotides T = 38.6%, C = 14.2%, A = 32.1% and G = 15.1%. This greater AT content (70.7%) over GC content (29.3%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Glyphodes bicolor* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 2.11% to 15.02% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (2.11%) with those from Australia while 15.02% to Indonesia (Table 6.20). On the basis of the data observed this species may be rooted from those found in Indonesia which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from indonesia during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Glyphodes bicolor* with related species. *Glyphodes bicolor* isolated from Kerala (MH674103) showed 2.11% divergence with *Glyphodes bicolor* (HQ952843) from Australia and 15.02% divergence with *Glyphodes multilinealis* (AB158241) from Indonesia. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Glyphodes bicolor* isolated from Kerala. Closest relative of *Glyphodes bicolor* is *Glyphodes bicolor* from Australia represented within the same clade.

11. ***Melanitis leda*** (Linnaeus, 1758)

Specimen details:

Voucher specimen : CUML – 01 – A1
Date of collection : 20- Feb -2016
Locality : Kerala: Malappuram
Lat- Lon : 11.0510º N, 76.0711º E
GenBank accession : KX587506

Description and distribution:

Fig.6.61: *Melanitis leda*

Melanitis leda, the common evening brown, is a common species found flying at dusk (Figure. 6.61). They possess highly describing peculiarities. During wet season form: Forewing - apex subacute; termen slightly angulated just below apex, or straight. Upperside of the body is brown in appearance. Forewing with two large subapical black spots, each with a smaller spot outwardly of pure white, costal margin is narrowly pale. Hindwing with a dark, white-centred, fulvous-ringed ocellus subterminally in interspace two. Underside paler, densely covered with transverse dark brown striae; a discal curved dark brown narrow band is present on forewing. Dry-season form: Forewing - apex obtuse and more or less falcate; termen posterior to falcation straight or sinuous. Upperside is ground colour similar to that in the wet-season form, the markings, especially the ferruginous lunules inwardly bordering the black sub-apical spots on forewing, larger, more extended below and above the black costa. Hindwing: the ocellus in interspace 2 absent, posteriorly replaced by three or four small white subterminal spots. Underside varies in colour greatly. Antennae, head, thorax and abdomen in both seasonal forms brown or greyish brown: the antennae annulated with white, ochraceous at apex. They are found to be distributed in Africa, South Asia and South East Asia extending to parts of Australia (Wilson et al., 2010)

Damage:

The caterpillars are highly polyphagus and feed on a wide variety of agricultural crops and grasses. Adults feed mainly on nectar, and in rare cases visit even rotting fruits.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Melanitis leda* collected has been amplified using the primer CP2 (Table 3.1). The PCR amplification yielded 571 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 6.62 – 6.65 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 6.21.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Melanitis leda* voucher collected during the present study (Table 6.21). It showed bias to nucleotide AT, with nucleotide composition with T = 40.3%, C = 16.6%, A = 29.9% and G = 13.1%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 6.22 and Figure 6.66 respectively.

> *Melanitis leda* CDS-2016/ 571bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUML-01-A1

> *Melanitis leda*

```
AACATCCCTTAGTTTAATTATCCGTATAGAATTAGGAAATCCTGGATTTTTAATTGGAGA
TGATCAAATTTATAATACTATTGTTACTGCCCATGCTTTTATTATAATTTTTTTCATAGT
TATACCAATTATAATTGGAGGATTTGGAAATTGACTTGTTCCCTTAATATTAGGAGCTCC
TGATATAGCATTCCCTCGAATAAATAATATAAGTTTTTGACTTTTGCCCCCATCATTAGT
CTTATTAATTTCAAGTAGATTAGTAGAAAATGGAGCAGGAACTGGATGAACAGTCTATCC
CCCCCTTTCATCTAATATTGCTCACAGTGGTACTTCAGTTGATTTAGCAATTTTTTCTCT
TCATCTTGCTGGAATTTCTTCAATTTTAGGAGCTATTAACTTTATTACTACAATCATTAA
TATACGAATTAATAATATAACTTATGATCAAATACCACTATTTGTTTGAGCCGTCGGAAT
TACAGCTTTACTTCTTTTACTTTCATTACCTGTATTAGCTGGAGCTATTACTATATTATT
AACAGATCGTAATTTAAATACTTCCTTTTTT
```

Figure 6.62: The partial DNA sequence of the mitochondrial COI gene of *Melanitis leda*

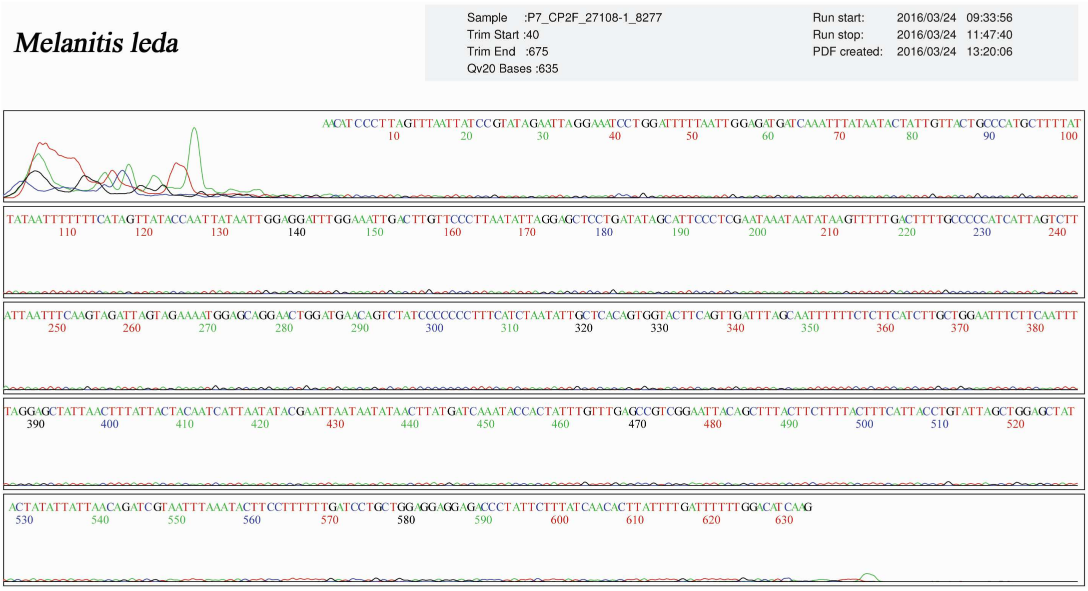
Figure 6.63: Molecular barcode of the mitochondrial COI gene of *Melanitis leda*

> *Melanitis leda* / 182AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUML-01-A1

> *Melanitis leda*

```
MELGNPGFLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMN
NMSFWLLPPSLVLLISSSLVENGAGTGWTVYPPLSSNIAHSGTSVDLAIFSLHLAGISSI
LGAINFITTIINMRINNMTYDQMPLFVWAVGITALLLLLSLPVLAGAITMLLTDRNLNTS
FF
```

Figure 6.6: The translation product of the mitochondrial COI gene of *Melanitis leda*

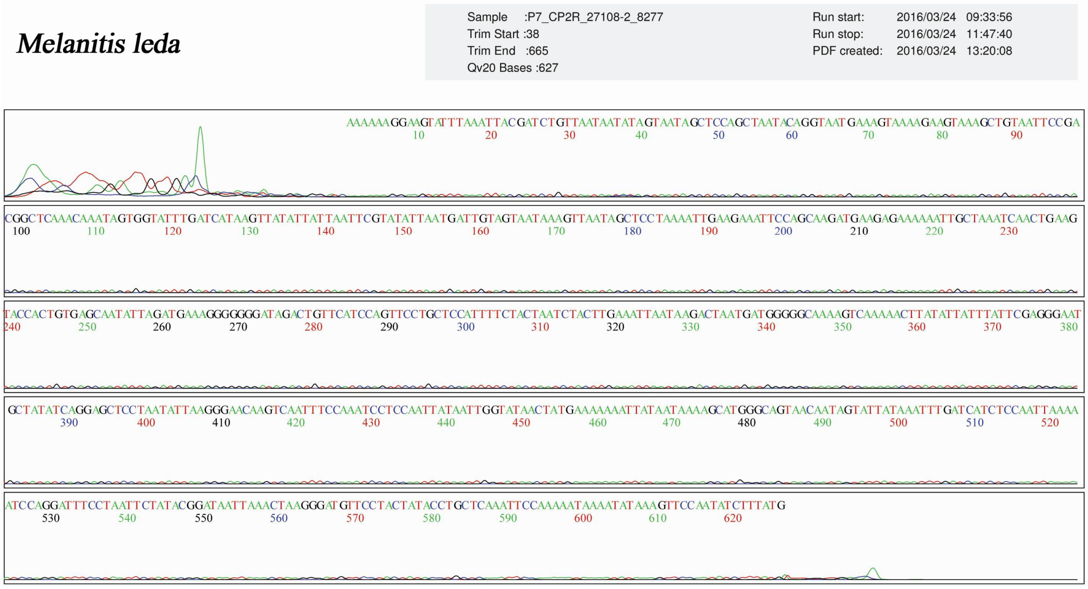


Melanitis leda

Sample :P7_CP2F_27108-1_8277
Trim Start :40
Trim End :675
Qv20 Bases :635

Run start: 2016/03/24 09:33:56
Run stop: 2016/03/24 11:47:40
PDF created: 2016/03/24 13:20:06

```
AACATCCCTTAGTTTAATTATCCGTATAGAATTAGGAAATCCTGGATTTTTAATTGGAGATGATCAAATTTATAATACTATTGTTACTGCCCATGCTTTTAT
TATAATTTTTTTCATAGTTATACCAATTATAATTGGAGGATTTGGAAATTGACTTGTTCCCTTAATATTAGGAGCTCCTGATATAGCATTCCCTCGAATAAATAATATAAGTTTTTGACTTTTGCCCCCATCATTAGTCTT
ATTAATTTCAAGTAGATTAGTAGAAAATGGAGCAGGAACTGGATGAACAGTCTATCCCCCCCTTTCATCTAATATTGCTCACAGTGGTACTTCAGTTGATTTAGCAATTTTTTCTCTTCATCTTGCTGGAATTTCTTCAATTT
TAGGAGCTATTAACTTTATTACTACAATCATTAATATACGAATTAATAATATAACTTATGATCAAATACCACTATTTGTTTGAGCCGTCGGAATTACAGCTTTACTTCTTTTACTTTCATTACCTGTATTAGCTGGAGCTAT
ACTATATTATTAACAGATCGTAATTTAAATACTTCCTTTTTTGATCCTGCTGGAGGAGGAGACCCTATTCTTTATCAACACTTATTTTGATTTTTTGGACATCAAG
```



Melanitis leda

Sample :P7_CP2R_27108-2_8277
Trim Start :38
Trim End :665
Qv20 Bases :627

Run start: 2016/03/24 09:33:56
Run stop: 2016/03/24 11:47:40
PDF created: 2016/03/24 13:20:08

```
AAAAAAGGAAGTATTTAAATTACGATCTGTTAATAATATAGTAATAGCTCCAGCTAATACAGGTAATGAAAGTAAAAGAAGTAAAGCTGTAATTCCGA
CGGCTCAAACAAATAGTGGTATTTGATCATAAGTTATATTATTAATTCGTATATTAATGATTGTAGTAATAAAGTTAATAGCTCCTAAAATTGAAGAAATTCCAGCAAGATGAAGAGAAAAAATTGCTAAATCAACTGAAG
TACCACTGTGAGCAATATTAGATGAAAGGGGGGATAGACTGTTCATCCAGTTCCTGCTCCATTTTCTACTAATCTACTTGAAATTAATAAGACTAATGATGGGGGCAAAAGTCAAAAACTTATATTATTTATTCGAGGGAAT
GCTATATCAGGAGCTCCTAATATTAAGGGAACAAGTCAATTTCCAAATCCTCCAATTATAATTGGTATAACTATGAAAAAAATTATAATAAAAGCATGGGCAGTAACAATAGTATTATAAATTTGATCATCTCCAATTAAAA
ATCCAGGATTTCCTAATTCTATACGGATAATTAAACTAAGGGATGTTCCTACTATACCTGCTCAAATTCCAAAAATAAAATATAAAGTTCCAATATCTTTATG
```

Figure 6.65: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Melanitis leda* using Forward and Reverse primers.

Table 6.21: Comparison of Nucleotide frequencies of COI gene sequence of *Melanitis leda* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
KX587506 Melanitis leda	40.3	16.6	29.9	13.1	26	15.8	34.2	24.2	45	25.3	15.3	14.7	50	8.9	40.3	.5
KM111608 Melanitis leda	39.6	16.6	30.1	13.7	25	15.7	34.0	25.1	44	25.3	15.3	15.3	49	8.9	41.1	.5
KJ459747 Melanitis phedima	39.1	16.6	30.8	13.5	23	17.3	34.6	25.1	45	24.7	15.3	15.3	49	7.9	42.6	.0
DQ338759 Gnophodes chelys	40.1	14.4	31.2	14.4	27	14.1	33.5	25.7	44	24.7	14.7	16.3	49	4.2	45.3	1.1
KU219623 Gnophodes betsimena	37.3	16.8	31.3	14.5	26	15.7	33.5	25.1	43	23.2	16.8	17.4	44	11.6	43.7	1.1
MF172192 Calisto batesi	40.5	15.2	29.4	14.9	26	14.7	32.6	26.3	45	24.2	14.2	16.3	50	6.8	41.4	2.1
KM111653 Callerebia baileyi	41.2	15.9	29.4	13.5	25	16.2	34.0	24.6	44	25.3	15.3	15.3	54	6.3	38.9	.5
KF054340 Calisto archebates	38.7	16.5	30.3	14.5	25	15.8	33.7	25.8	44	24.7	14.2	16.8	47	8.9	42.9	1.0
GQ864761 Dynamine serina	41.5	14.7	29.9	13.8	28	14.2	32.6	25.3	45	24.2	14.7	16.3	52	5.8	42.4	.0
FJ663880 Paralasa hades	41.2	15.2	29.6	14.0	26	14.2	34.7	24.7	45	24.2	14.2	16.3	52	7.3	39.8	1.0
DQ176406 Pararge aegeria aegeria	38.7	15.9	31.2	14.2	25	15.7	34.0	25.1	44	25.3	14.7	16.3	47	6.8	44.7	1.1
GU659575 Eunica alcmena	39.9	14.7	30.8	14.5	28	13.2	32.1	26.3	45	25.3	13.7	16.3	47	5.8	46.6	1.0
GQ200968 Melanargia halimede	40.1	15.8	29.4	14.7	26	14.2	33.7	25.8	45	24.7	14.2	16.3	49	8.4	40.3	2.1
FJ663540 Kirinia eversmanni	41.7	14.0	30.1	14.2	27	13.7	33.7	25.3	44	25.3	14.2	16.3	53	3.1	42.4	1.0
MF172178 Calisto tragius	40.5	15.9	28.5	15.1	26	15.3	31.6	27.4	45	24.2	13.7	16.8	50	8.4	40.3	1.0

Table 6.22: Percentage of evolutionary divergence of *Melanitis leda* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	KX587506	*Melanitis leda* (Kerala)	
2.	KM111608	*Melanitis leda* (China)	0.00%
3.	KJ459747	*Melanitis phedima*	7.35%
4.	DQ338759	*Gnophodes chelys*	12.06%
5.	KU219623	*Gnophodes betsimena*	14.05%
6.	MF172192	*Calisto batesi*	15.51%
7.	KF054340	*Calisto archebates*	17.44%
8.	MF172178	*Calisto tragius*	17.71%
9.	FJ663540	*Kirinia eversmanni*	17.95%
10.	KM111653	*Callerebia baileyi*	18.15%
11.	GU659575	*Eunica alcmena*	18.57%
12.	GQ864761	*Dynamine serina*	18.67%
13.	DQ176406	*Pararge aegeriaaegeria*	18.78%
14.	FJ663880	*Paralasa hades*	18.97%
15.	GQ200968	*Melanargia halimede*	20.10%

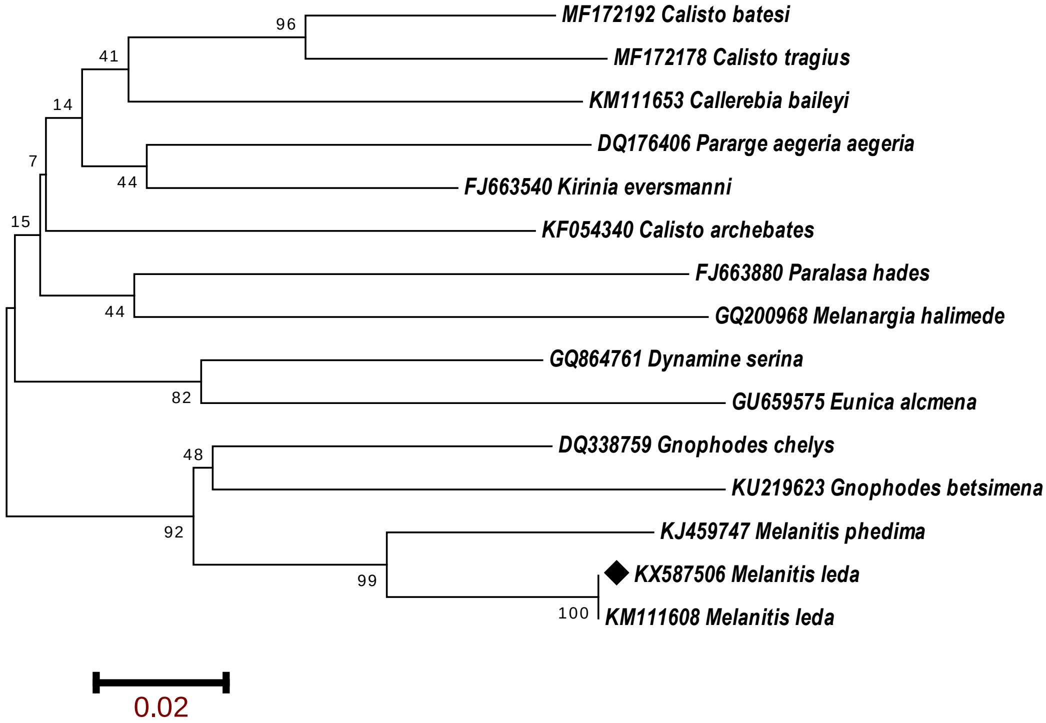


Figure 6.66: Phylogenetic relationship of *Melanitis leda* inferred by NJ tree method

DISCUSSION

This species is known to be observed mainly in Palaearctic and regions (Saroli, 2016). Larval stage is found to be greatly infectious. It mainly bores the underground part of the crop plant leading to the ultimate loss in productivity. Both nucleotide and peptide BLAST analysis showed that this species has 100% sequence similarity to the same genus reported from China (KM111608).

The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Melanitis leda* showed bias to nucleotide AT, with following composition of nucleotides T = 40.3%, C = 16.6%, A = 29.9% and G = 13.1%. This greater AT content (70.2%) over GC content (29.7%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Melanitis leda* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 0% to 20.10% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has no divergence (0%) with those from China while 20.10% to Ontario (Table 6.22). On the basis of the data observed this species may be rooted from those found in China which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from Ontario during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Melanitis leda* with related species. *Melanitis leda* isolated from Kerala (KX587506) showed 0% divergence with *Melanitis leda* (KM111608) from China and 20.10% divergence with *Melanargia* *halimede* (GQ200968) from Ontario. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Melanitis leda* isolated from Kerala. Closest relative of *Melanitis leda* is *Melanitis leda* from China represented within the same clade.

12. ***Cryptophasa atecmarta*** (Turner, 1917)

Specimen details:

Voucher specimen	:	CUCA – 01 – A1
Date of collection	:	11- Dec -2015
Locality	:	Kerala: Malappuram
Lat- Lon	:	11.0510º N, 76.0711º E
GenBank accession	:	KX587510

Description and distribution:

Cryptophasa atecmarta is a moth in the Xyloryctidae family (Figure. 6.67). It was described by Turner in 1917. The wingspan is of the adult moth is about 32 mm. The forewings are grey with sparse fuscous irroration and several indistinct markings, presence of a fuscous dot in the disc at one-fourth, a second in the middle and a third at three-fourths. This last dot forms the apex of a darker shape, the terminal area being paler grey (Puhringer and Kallies, 2004). The hindwings are pale fuscous. It is found to be distributed along Asia, Australia, New South Wales, Queensland and Western Australia.

Fig.6.67: *Cryptophasa atecmarta*

Damage:

The larvae feed on a wide variety of vegetations; they are highly adapted as stem borers and cause complete wilt of the host plant.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Cryptophasa atecmarta* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 600 bp long product. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 6.68 – 6.71 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 6.23.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Cryptophasa atecmarta* voucher collected during the present study (Table 6.23). It showed bias to nucleotide AT, with nucleotide composition with T = 40.3%, C = 16.6%, A = 29.9% and G = 13.1%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 6.24 and Figure 6.72 respectively.

> *Cryptophasa atecmarta* CDS-2018/ 600 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUCA-01-A1

> *Cryptophasa atecmarta*

```
ATAGTAGGTACATCTTTAAGATTATTAATTCGAGCTGAATTAGGTAATCCTGGATCTTTA
ATTGGAAATGATCAAATTTATAATACTATTGTAACTGCTCATGCATTTATTATAATTTTT
TTTATAGTAATACCAATTATAATTGGTGGATTTGGAAATTGATTAGTACCTTTAATACTT
GGTGCACCAGATATAGCCTTTCCACGAATAAATAATATAAGATTTTGATTATTACCCCCA
TCTTTAACTCTTTTAATTTCTAGAAGTATAGTAGAAAATGGAGCAGGAACTGGATGAACG
GTTTACCCCCCCCTTTCATCTAATATTGCCCATGGAGGAAGATCCGTAGATTTAGCTATT
TTTTCCCTTCATTTAGCTGGAATTTCATCTATTTTAGGAGCTATTAATTTTATTACTACA
ATTATTAATATACGATTAAGAAATTTATCTTTTGATCAAATACCCCTTTTTGTTTGAGCT
GTTGGTATTACAGCTTTCCTTTTACTTTTATCTTTACCTGTTTTAGCTGGAGCTATTACT
ATATTATTAACTGATCGAAATTTAAATACATCTTTCTTTGACCCTGCGGGAGGAGGAGAT
```

Figure 6.68: The partial DNA sequence of the mitochondrial COI gene of *Cryptophasa atecmarta*

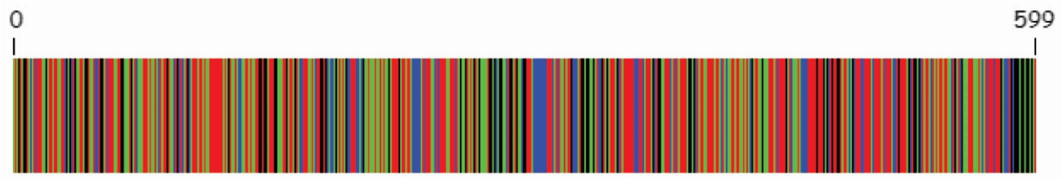


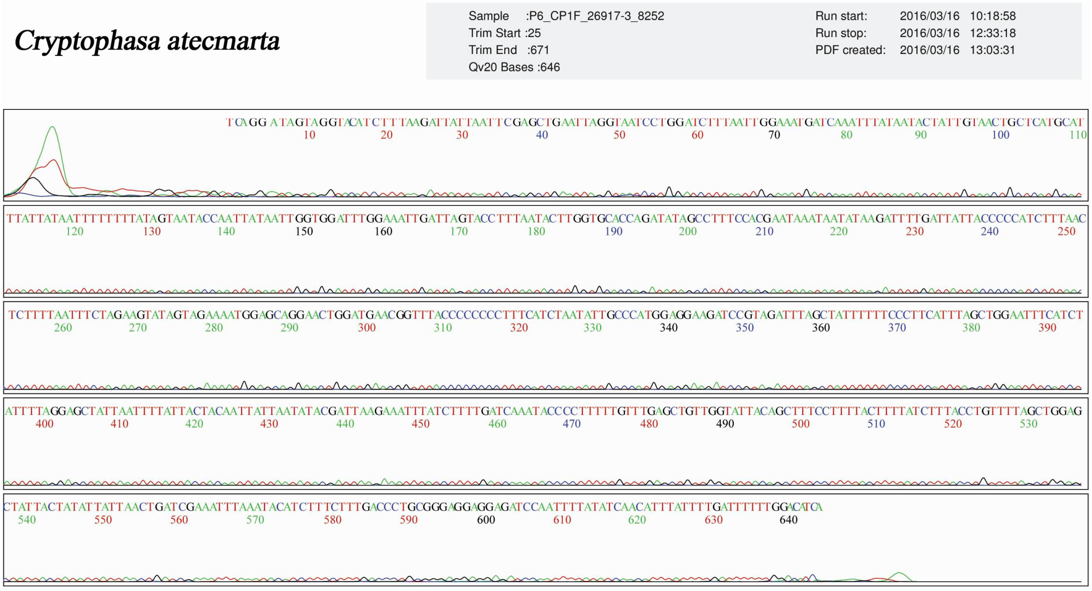
Figure 6.69: Molecular barcode of the mitochondrial COI gene of *Cryptophasa atecmarta*

> *Cryptophasa atecmarta* / 200AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUCA-01-A1

> *Cryptophasa atecmarta*

```
MVGTSLSLLIRAELGNPGSLIGNDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWLVPLML
GAPDMAFPRMNNMSFWLLPPSLTLLISSSMVENGAGTGWTVYPPLSSNIAHGGSSVDLAI
FSLHLAGISSILGAINFITTIINMRLSNLSFDQMPLFVWAVGITAFLLLLSLPVLAGAIT
MLLTDRNLNTSFFDPAGGGD
```

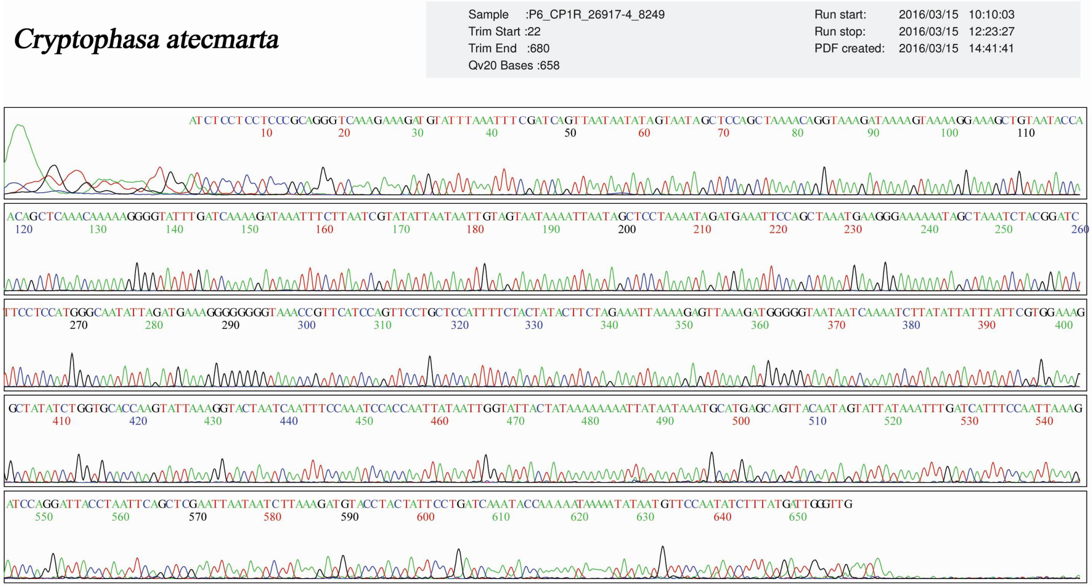
Figure 6.70: The translation product of the mitochondrial COI gene of *Cryptophasa atecmarta*



Cryptophasa atecmarta

Sample :P6_CP1F_26917-3_8252
Trim Start :25
Trim End :671
Qv20 Bases :646

Run start: 2016/03/16 10:18:58
Run stop: 2016/03/16 12:33:18
PDF created: 2016/03/16 13:03:31



Cryptophasa atecmarta

Sample :P6_CP1R_26917-4_8249
Trim Start :22
Trim End :680
Qv20 Bases :658

Run start: 2016/03/15 10:10:03
Run stop: 2016/03/15 12:23:27
PDF created: 2016/03/15 14:41:41

Figure 6.71: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Cryptophasa atecmarta* using Forward and Revers primers.

Table 6.23: Comparison of Nucleotide frequencies of COI gene sequence of *Cryptophasa atecmarta* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
KX587510 Cryptophasa atecmarta	39.8	15.3	29.8	15.0	50	6.0	43.5	1.0	28	14.0	31.5	27.0	43	26.0	14.5	17.0
KF398977 Cryptophasa atecmarta	39.1	14.8	31.8	14.3	47	4.5	48.8	.0	27	15.0	32.5	26.0	44	25.0	14.0	17.0
KF406000 Cryptophasa sp.	38.8	15.1	31.4	14.6	45	6.0	47.8	1.0	27	14.5	32.5	26.0	44	25.0	14.0	17.0
MF103614 Datana angusii	40.6	13.6	31.4	14.3	49	3.5	47.3	.5	29	12.5	32.5	26.0	44	25.0	14.5	16.5
KF406273 Lichenaula sp.	41.6	14.3	29.8	14.3	53	3.5	42.8	.5	28	14.5	32.0	26.0	44	25.0	14.5	16.5
MF103453 Datana diffidens	40.8	13.5	31.4	14.3	49	3.5	47.3	.5	30	12.0	32.5	26.0	44	25.0	14.5	16.5
KF405171 Cryptophasa sp.	38.4	15.5	31.6	14.5	44	7.0	47.8	1.0	27	14.5	33.0	25.5	44	25.0	14.0	17.0
JQ546934 Bolica sp.	40.6	14.1	31.3	14.0	50	3.0	47.3	.0	28	14.0	32.5	25.5	44	25.5	14.0	16.5
MF103603 Datana integerrima	39.7	14.5	31.3	14.5	47	5.0	47.0	1.0	28	13.5	32.5	26.0	44	25.0	14.5	16.5
MF102926 Datana diffidens	40.8	13.5	31.5	14.2	49	3.5	47.3	.5	30	12.0	32.5	26.0	44	25.1	14.6	16.1
MF103658 Datana major	40.8	13.5	31.4	14.3	49	3.0	47.3	.5	29	12.5	32.5	26.0	44	25.0	14.5	16.5
MF103651 Datana contracta	40.1	14.1	31.3	14.5	48	4.0	46.8	1.0	28	13.5	32.5	26.0	44	25.0	14.5	16.5
MF103302 Datana integerrima	39.8	14.5	31.3	14.5	47	5.0	46.8	1.0	28	13.5	32.5	26.0	44	25.0	14.5	16.5
MF103024 Datana ministra	40.3	14.0	31.6	14.1	48	4.5	47.8	.0	29	12.5	32.5	26.0	44	25.0	14.5	16.5
KX042384 Macaria carbonaria	39.9	14.3	31.3	14.5	48	4.0	46.8	1.0	28	14.0	32.5	26.0	44	25.0	14.5	16.5

Table 6.24: Percentage of evolutionary divergence of *Cryptophasa atecmarta* with its closely related species accessible from NCBI GenBank

Sl. No.	**Accession No**	**Organism**	**Percentage of divergence**
1.	KX587510	*Cryptophasa atecmarta* (Kerala)	
2.	KF398977	*Cryptophasa atecmarta* (Australia)	2.62%
3.	KF406000	*Cryptophasa* sp.	3.76%
4.	KF405171	*Cryptophasa* sp.	4.52%
5.	KX042384	*Macaria carbonaria*	12.41%
6.	MF103614	*Datana angusii*	13.04%
7.	KF406273	*Lichenaula* sp.	13.30%
8.	JQ546934	*Bolica* sp.	13.60%
9.	MF103651	*Datana contracta*	13.77%
10.	MF103658	*Datana major*	13.79%
11.	MF102926	*Datana diffidens*	13.81%
12.	MF103453	*Datana diffidens*	13.81%
13.	MF103024	*Datana ministra*	13.81%
14.	MF103603	*Datana integerrima*	14.18%
15.	MF103302	*Datana integerrima*	14.18%

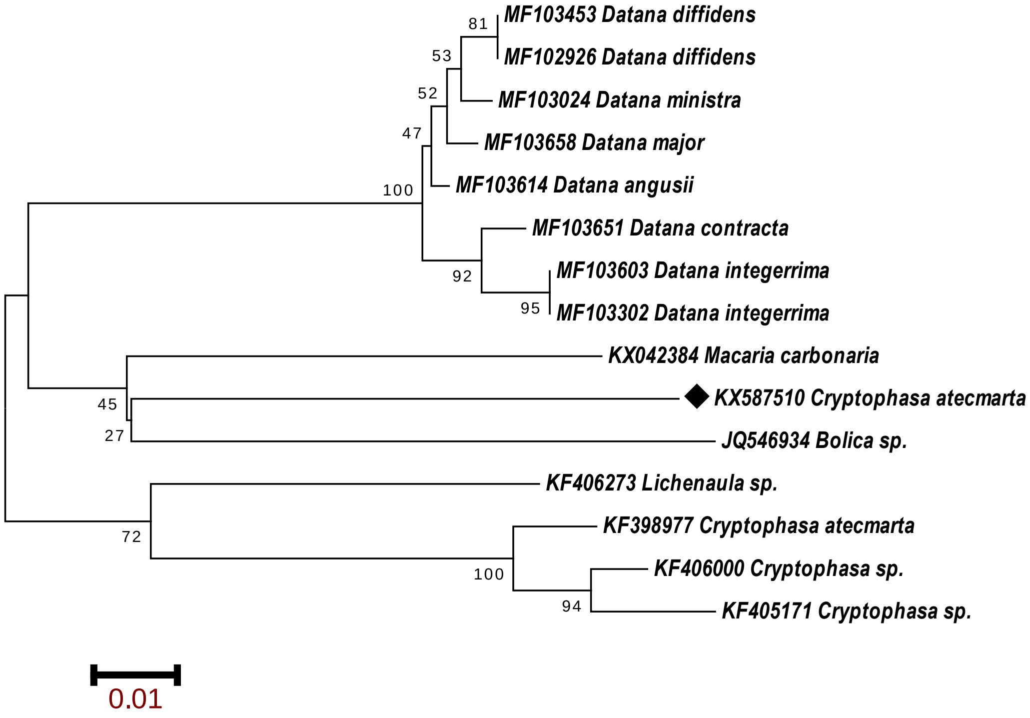


Figure 6.72: Phylogenetic relationship of *Cryptophasa atecmarta* inferred by NJ tree method

DISCUSSION

Both nucleotide and peptide BLAST analysis showed that this species has 97.4% sequence similarity to the same genus reported from Australia (KF398977). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Cryptophasa atecmarta* showed bias to nucleotide AT, with following composition of nucleotides T = 39.8%, C = 15.3%, A = 29.8% and G = 15.0%. This greater AT content (69.6%) over GC content (30.3%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Cryptophasa atecmarta* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 2.62% to 14.18% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has 2.62% with those from Australia while 14.18% to North America (Table 6.24). On the basis of the data observed this species may be rooted from those found in North America which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from North America during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Cryptophasa atecmarta* with related species. *Cryptophasa atecmarta* isolated from Kerala (KX587510) showed 2.62% divergence with *Cryptophasa atecmarta* (KF398977) from Australia and 14.18% divergence with *Datana integerrima* (MF103302) from North America. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Cryptophasa atecmarta* isolated from Kerala. Closest relative of *Cryptophasa atecmarta* is *Cryptophasa atecmarta* from Australia represented within the same clade.

Consolidated Phylogenetic Tree

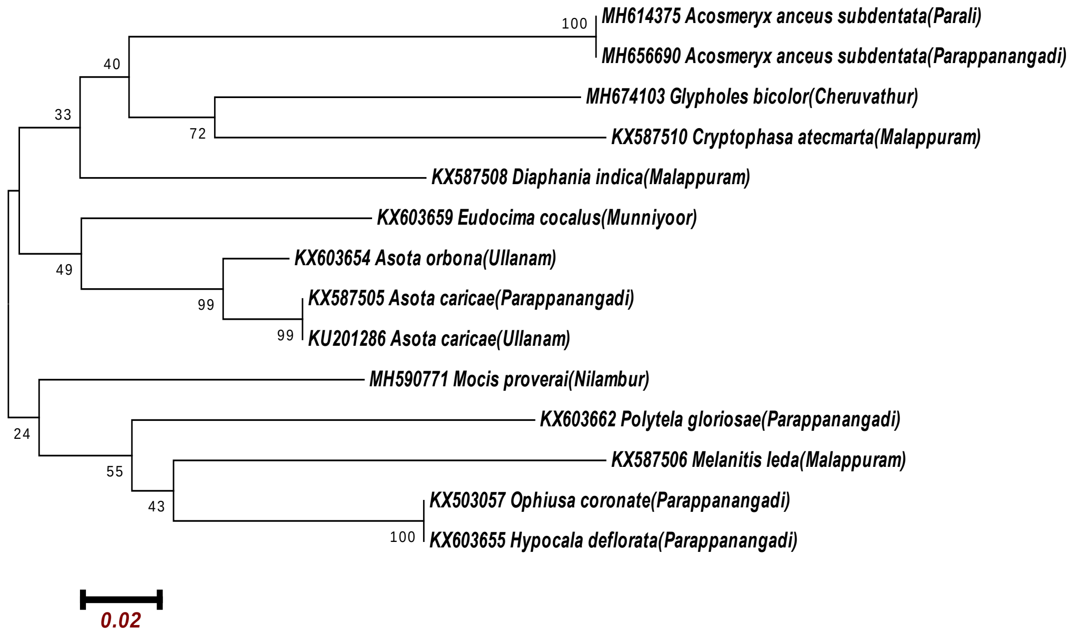


Figure 6.73: Phylogenetic relationship of different Lepidopteran pests of cucurbitaceae isolated from selected districts of Kerala.

The analysis of phylogenetic relationships among 12 species of Lepidopteran pest of cucurbits of Kerala by NJ tree method was done (Figure 6.73). Families included in the superfamily Noctuoidea are Erebidae and Noctuidae, superfamily Bombycoidea is Sphingidae, superfamily Pyraloidea is Crambidae, superfamily Papilionoidea is Nymphalidae, superfamily Gelechioidea is Xyloryctidae and Geometroidea are Geometridae. The species from these six families are aligned nearly in the phylogenetic tree and monophyletic in origin. Species from the family Erebidae are originated from a main clade and various species from the genus *Asota* are aligned in a single clade and then branches to subclades. *Ophiusa coronate* and *Hypocala deflorata* are aligned in a same clade. *Polytela gloriosae* and *Melanitis leda* are aligned in a same clade. *Carpophilus marginellus* and *Epuraea luteolus* are aligned in a same clade.

7

Diptera

The order Diptera, the ‘true’ or two-winged flies, or true flies have a single pair of wings. They are one among the most speciose insect orders, constituting over 160,000 described species globally. The remarkable habitat exploration, both terrestrial and aquatic helped them to flourish both in richness and diversity (Gregory et al, 2015). The key identifiable feature is the reduced hind wings with club-shaped structures called halters, the membranous front wings serve as aerodynamic surfaces (Courtney and Cranston, 2015). The halteres vibrate and help the insect in maintaining balance, thus acting like a gyroscope. The dipterans have greater economic impact on humans compared to other group of insects (DeMeyer, 2016).

The fruit flies *Bactrocera cucurbitae* and *Bactrocera tau* are important cucurbit pests. Adult flies cause fruit rotting by laying eggs inside the newly developing fruit or flower (Neupane, 2002). *Daucus cucurbitae* coq., fruit fly are attracted to both the flowers and fruit of pumpkin and bottlegourd. Fruit infestation by melon fruit fly in bitter gourd has been reported to vary from 41 to 89 percent (Gupta and Verma, 1992). According to report the cucurbit fruit fly infested 95 percent of bitter gourd fruits in Papua New Guinea, and 90 percent of snakegourd and 60 to 87 percent pumpkin fruits in Solomon Islands (Hollingsworth and Allwood, 2000). Fruit flies (*Dacus cucurbitae*) are distributed all over the world, causing destruction to almost all Cucurbitaceae members. *Myopardalis pardalina* Bigot (Diptera, Tephritidae) was reported on muskmelon.

The systematic position of dipteran pests of cucurbits collected and identified during the present study is given below:

Order: Diptera; Suborder: Brachycera

1. Superfamily: Lauxanioidea

1.1. Family: Celyphidae

1.1.1 Subfamily: Eurychoromyiinae

- *Spaniocelyphus falcatus* (Tenorio, 1972)
- *Spaniocelyphus pilosus* (Tenorio, 1972)
- *Celyphus obtectus* (Dalman 1818)

2. Superfamily: Tephritoidea

2.1. Family: Tephritidae

2.1.1. Subfamily: Dacinae

- *Bactrocera tau* (Walker, 1849)

3. Superfamily: Muscoidea

3.1. Family: Anthomyiidae

3.1.1. Subfamily: *Pegomy*inae

- *Pegomya circumpolaris* (Ackland and Griffiths, 1983)

A brief description on the relevant higher taxa and the taxonomic key prepared for classification and morphological identification (with the help of suitable identification guides and expert consultation) of all the dipteran pests of cucurbits collected during the present study are given below:

SUBORDER: BRACHYCERA

Brachycera constitute a monophyletic suborder and is one among the 58 fauna Europaea major. The most species rich families included are agromyzidae, dolichopodidae, empididae, syrphidae and tachinidae (Hovemeyer, 2000). Their most distinguishing characteristic features include reduced antennal segmentation, the maxillary palp with two or fewer segments, the larval head capsule extended into the prothorax, in males the epandrium and hypandrium of the genitalia are well separated and premandible absent. The venation configuration of wing in the CuA2 and A1 is marked distinctly (Thomas et al., 2001,2008)

Key to the superfamilies of Suborder Brachycera

1. Antennal flagellum not compact and the antennal arista is bare to long plumose; vertex not strongly excavate, but rounded or carinate; postocellar bristles distinctly convergent; eyes not prominently bulging and vertex not sunken; coasta unbroken, subcoasta complete and free from vein 1 (R1); anal cell (CuP) short not angularly produced; vein 6 (CuA +1A) discontinued well before wing margin; vibrissae absent and ovipositor of simple, flexible, tubular type .... **Lauxanioidea**

– Antennal flagellum not compact, very rarely more than 8 segments, apical segments often modified into a stylus or arista **2**

2. Greater ampulla rarely present or developed to varied degree; pedicel sometimes with dorsal seam; vibrissa present or absent; lower calypter absent or linear, its margin rarely weakly convex ....**Tephritoidea**

– Greater ampulla present as a distinct bulbous swelling below wing base and fused to the anepimeron. Vibrissae usually present. Antennal pedicel always with an entire dorsal seam; thorax not overly flattened hind coxa close together, tarsal claws small, somewhat curved, never toothed.......................... .... **Muscoidea**

SUPERFAMILY: LAUXANIOIDEA

Lauxaniidae are small flies with size range 2–7 mm in length. They are often categorized as plump, dull, or partly lustrous flies (Jindra et al, 2013). The body colour varies from yellow to brown or black, or their combination. The head have variable shape, the face either projecting or retreating, convex, flat or concave, usually without oral vibrissae. The frons are wide, with two pairs of frontal bristles, interfrontal bristles totally absent. The ocellar bristles are present or sometimes minute. The antennae are variable and the arista is plumose, pubescent to bare. The thorax has bristles, behind the suture. Except for the marginal bristles the scutellum is bare. All tibiae have a preapical bristle. The wings are marked (spots along the veins) or unmarked, complete wing venation and continuous costa, the subcosta entire and ends in costa. The apical cell usually widely opened and the second basal and anal cells short. The abdomen is oval, rarely elongated.

FAMILY: CELYPHIDAE

Celyphidae are easily recognizable small to medium-sized flies. The family name, Celyphidae is derived from the Greek word for pod or shell. Celyphidae are shiny or metallic in colour, enlarged scutellum, forming protective shell over the abdomen, giving them a beetle-like resemblance. The head consisted with a few bristles. At rest, the wings are usually folded beneath the scutellar "shell". The aristae of the antenna are flattened and leaf-like at the base. Male celyphids have paired gonapophyses for copulation and lack aedeagus. They have key taxonomic significance (Gaimari, 2017).

SUBFAMILY: EURYCHOROMYIINAE

This subfamily can be differentiated from other lauxaniids by the following characteristics. The head is always wider than the scutal width, with the vertex distinctly concave in dorsal view. The frons has very broad fronto-orbital plates, with the condition of fronto-orbital setae varying among species as follows: either with 2 reclinate pairs (anterior seta either diminutive or reaching 0.6 X the length of the posterior seta), or with 1 diminutive reclinate seta in the upper part of the fronto-orbital plate (this is sometimes so diminutive as to appear absent at first look). The ocellar triangle is small, slightly raised, and placed anterior to the vertex. The ocellar setae are either present (tiny or normal-sized), proclinate and diverging, or are absent. The postocellar setae are either present (tiny or normal-sized) and cruciate, or are absent. The eye is longer than high, and the height of the subgena and gena together approaches or exceeds the eye height. The subgena is enlarged, bulging and conspicuous. The lunule is straight. The face is at least slightly bulging and visible in profile.

Eurychoromyiinae include broad-flat-headed flies. According to Gaimari and Silva (2010), Eurychoromyiinae are characterized by face without spots but have reticulated pattern of brown and yellowish pruinosity, broad subgena, elongated wing, length 3 times height or greater; vein A1 long, almost reaching wing margin, fore tarsus is less than 1.5 times longer than hind tarsus.

Key to the species of pests collected from Subfamily Eurychoromyiinae

1. Vertex rounded, postvertical bristles absent or microscopic, artista very conspicuously widened on more than its basal half, leaf-like, its greatest width almost, or quite, as great as that of third antennal segment; distinct cross vein separating the discal and posterior basal cells of wing; species entirely testaceous in colour, blue mark below eye, thorax fulvous with a distinct blue tinge, scutellum metallic blue ....***Celyphus obtectus***

– Vertex sharp, postvertical bristles reduced or absent, arista leaf like at least basal half, palpi slender and not expanded at apices **2**

2. Scutellum rugose, with distinct depression ....***Spaniocelyphus falcatus***

– Scutellum smooth, with slight crinking at portions ....***Spaniocelyphus pilosus***

SUPERFAMILY: TEPHRITOIDEA

The Tephritoidea is a large group of acalyptrate flies including over 7300 described species arranged in eight families (Korneyev, 2006): the Lonchaeidae, Piophilidae, Pallopteridae, Richardiidae, Ulidiidae (D Otitidae, and D Pterocallidae), Platystomatidae, Pyrgotidae, and Tephritidae (including Tachiniscidae) (Han and Ro, 2005). They are very diverse not only in The Tephritoidea, includes flutter flies, fruit flies, skippers, signal flies. Body hairy, with weakly differentiated setae, frons with two pairs of orbital setae, males with 7 pairs of functional abdominal spiracle and females with 7th sternite and tergite separate.(Martin et al., 1980).

FAMILY: TEPHRITIDAE

Tephritids are small to medium-sized (2.5–10 mm), colourful flies having pictured wings. The subcostal vein curves forward at a right angle. The head is hemispherical and short. The face is vertical or retreating with broad frons with ocelli and cellar bristles. The post vertical bristles are parallel to divergent, two to eight pairs of frontal bristles present. The frontal bristles are inserted on a raised tubercle. True vibrissae are absent, but some genera have strong bristles near the vibrissal angle. The CuA2 vein is rarely straight or convex, the tibiae lacking a dorsal preapical bristle. The female has an oviscape (Merz, 1994; White, 1988).

SUBFAMILY: DACINAE

Dacinae are characterized with the following characters, dorsocentral seta usually absent, if present, then aligned with postsutural supra-alar seta ,wing predominantly hyaline with a costal band and narrow anal streak, wing variously marked with longitudinal and transverse bands; one or two scutellar setae; females have two spermathecae (David and Ramani, 2011).

From this subfamily, only a single specimen is collected during the present study, *Bactrocera tau* (Walker, 1849).

SUPERFAMILY: MUSCOIDEA

Muscoidea is a superfamily of flies under the subsection Calyptratae. Muscoidea, with approximately 7000 described species, includes 5% of the known species level diversity of the Diptera, the true flies. Most of them are saprophagous, coprophagous or necrophagous as larvae, but some species are parasitic, predatory, or phytophagous (Ding et al., 2015).

FAMILY: ANTHOMYIIDAE

Vein A1 reaching to wing-margin. One of the hind tarsomere has a distinct but short subbasal ventral seta. Scutellum surrounded with a group of soft and pale hairs on ventral surface at tip (Michelson, 2015).

SUBFAMILY: PEGOMYINAE

Pegomya is a large group of anthomyiid flies with poorly differentiated species complexes. The landmark study by Hennig (1973), characterized them based on differences in the male terminalia as a key in species recognition.

From this subfamily, only a single specimen is collected during the present study, *Pegomya circumpolaris*.

The morphological description, distribution, nature of the damage caused to the crops, analyses on mitochondrial COI gene sequences, molecular evolutionary divergence and phylogenic status of each pest specimen collected under Order Diptera during the present study are as follows:

1. ***Celyphus obtectus*** (Dalman, 1818)

Specimen details:

Voucher specimen	:	CUCO – 01 – A1
Date of collection	:	28- Mar-2016
Locality	:	Ernakulam: Aluva
Lat- Lon	:	10.1076º N, 76.3457º E
GenBank accessions	:	MH590775

Description and Distribution

The genera *Celyphus* is composed of small and medium sized beetles like insects, the peculiarity is caused by the immense sized scutellum, covering the whole abdomen and wings at rest (Sheets-Pyenson, 1981). They are usually

Fig.7.1: Celyphus obtectus

characterized by convex and long scutellum than mesonotum, having no scutellar bristles, hind tibia without terminal spur, arista broadened and leaf like (Tenorio, 1969). *Celyphus obtectus* (Figure 7.1) is one-sixth of an inch in length, head yellow, the last joint of antennae is black the thorax and scutellum are of bluish black colour with violet reflection, the former is broader than long, the abdomen is fawn colour, the legs and wings yellowish the latter with base brown (Sheets-Pyenson,1981). *C. obtectus* is distinguished by having the discal and 2nd basal cells distinct, the vertex rounded, post vertical bristles reduced or absent, arista thickened at its middle (Tenorio, 1969). They are found in India, China and Java.

Damage

They are polyphagus and highly generic insect pest. Larvae are saprophytic and adults are leaf miners (Tenorio, 1972).

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Celyphus obtectus* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 599 bp long products. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 7.2 – 7.5 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 7.1.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Celyphus obtectus* voucher collected during the present study (Table 7.1). It showed bias to nucleotide AT, with nucleotide composition with T = 36.7%, C = 16.9%, A = 28.9% and G = 16.9%

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 7.2 and Figure 7.6 respectively.

>*Celyphus obtectus* CDS-2018/ 599 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUCO-01-A1

> *Celyphus obtectus*

```
ATAGTAGGAACTTCCCTAAGAATCTTAATTCGTGCTGAATTAGGACACCCCGGAGCTTTA
ATTGGTGATGATCAAATTTATAATGTTATTGTTACAGCTCATGCCTTTGTTATAATTTTT
TTTATAGTAATACCTATTATAATTGGAGGGTTTGGAAATTGACTGGTTCCCTTAATATTA
GGAGCCCCAGATATAGCATTCCCTCGAATAAATAATATAAGATTTTGATTATTACCTCCT
TCATTAACCTTACTTTTAGTGAGAAGTATAGTTGAAAACGGAGCTGGAACAGGATGAACT
GTTTACCCCCCTCTTTCTTCAGGAATTGCTCACGGAGGAGCTTCAGTTGATCTTGCAATT
TTCAGTTTACATTTAGCCGGAATTTCTTCAATTTTAGGAGCAGTAAATTTTATCACAACA
GTTATTAATATACGATCGACAGGGATTACATTTGATCGAATACCATTATTTGTATGATCA
GTAGCTATTACTGCTCTACTTTTATTACTTTCTTTACCTGTACTAGCAGGAGCAATTACT
ATACTTTTAACGGATCGAAATTTAAATACTTCATTCTTTGACCCAGCAGGAGGAGGAGA
```

Figure 7.2: The partial DNA sequence of the mitochondrial COI gene of *Celyphus obtectus*

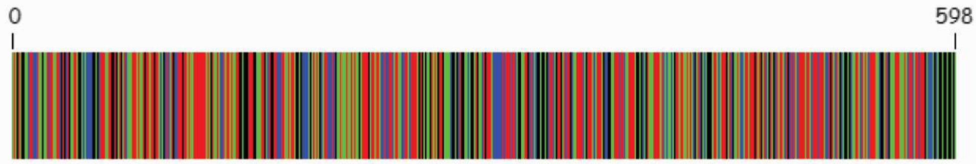


Figure 7.3: Molecular barcode of the mitochondrial COI gene of *Celyphus obtectus*

> *Celyphus obtectus* / 199AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUCO-01-A1

> *Celyphus obtectus*

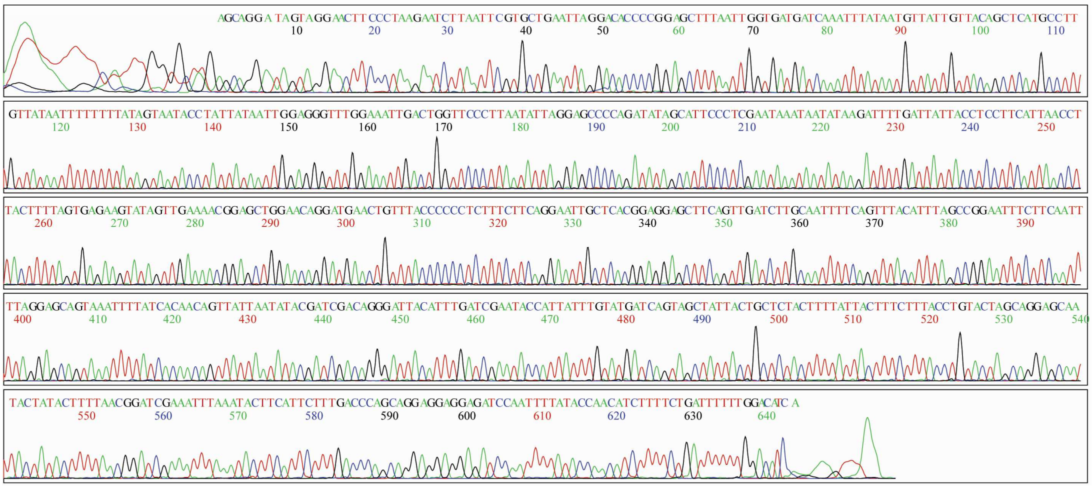
```
MVGTSLSILIRAELGHPGALIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLML
GAPDMAFPRMNNMSFWLLPPSLTLLLVSSMVENGAGTGWTVYPPLSSGIAHGGASVDLAI
FSLHLAGISSILGAVNFITTVINMRSTGITFDRMPLFVWSVAITALLLLLSLPVLAGAIT
MLLTDRNLNTSFFDPAGGG
```

Figure 7.4: The translation product of the mitochondrial COI gene of *Celyphus obtectus*

Celyphus obtectus

Sample :P41_CP1.FORWARD_7173-1_P1056
Trim Start :25
Trim End :672
Qv20 Bases :647

Run start: 2017/12/04 21:23:47
Run stop: 2017/12/04 23:21:22
PDF created: 2017/12/07 15:46:37



Celyphus obtectus

Sample :P41_CP1.REVERSE_7173-2_P1056
Trim Start :20
Trim End :671
Qv20 Bases :651

Run start: 2017/12/04 21:23:47
Run stop: 2017/12/04 23:21:22
PDF created: 2017/12/07 15:46:39

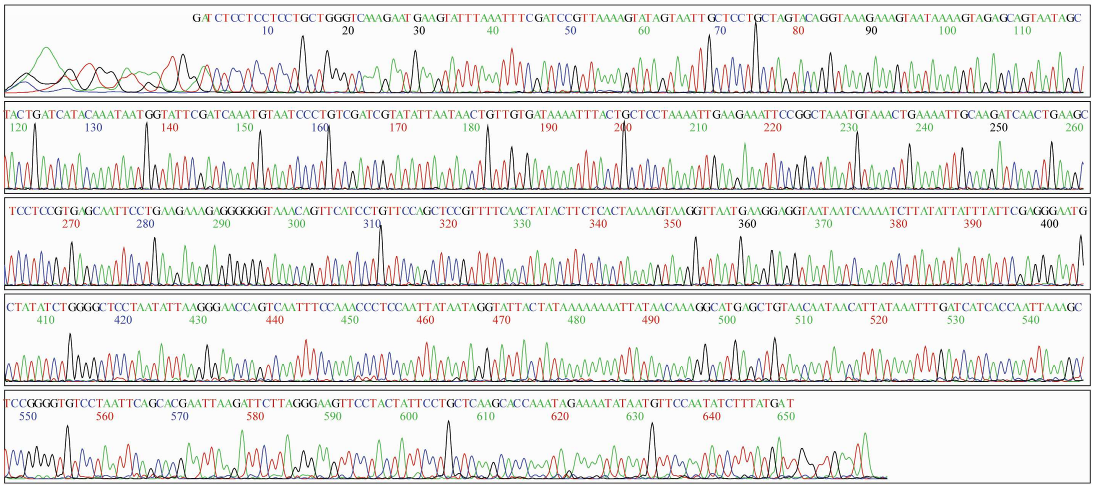


Figure 7.5: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Celyphus obtectus* using Forward and Reverse primers.

Table 7.1: Comparison of Nucleotide frequencies of COI gene sequence of *Celyphus obtectus* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH590775 Celyphus obtectus	37.3	16.9	28.9	16.9	44	22.1	18.6	15.7	40	12.3	38.6	9.4	28	16.3	29.7	25.6
MF818105 Spaniocelyphus falcatus	29.3	16.7	37.1	16.9	35	23.4	27.5	14.0	36	5.8	43.6	14.5	17	20.9	40.1	22.1
MF818104 Spaniocelyphus falcatus	29.2	16.7	37.2	16.9	35	23.4	27.5	14.0	36	5.8	43.9	14.6	17	20.9	40.1	22.1
EU435786 Dicranosepsis hamata	36.9	15.3	30.5	17.3	42	16.9	25.6	15.7	40	12.3	37.4	9.9	28	16.9	28.5	26.2
KR649705 Lonchaea sp.	39.8	14.8	28.2	17.3	47	16.9	20.3	15.7	43	12.3	35.7	9.4	30	15.1	28.5	26.7
KU876327 Tethina thula	37.9	14.2	31.8	16.1	42	16.9	25.6	15.1	41	10.5	39.8	8.8	30	15.1	30.2	24.4
KM626415 Sepsidae sp.	38.4	14.8	28.9	17.9	44	16.3	23.3	16.3	43	12.9	33.3	10.5	28	15.1	30.2	26.7
KR688930 Scatella stagnalis	37.1	15.0	29.9	18.1	43	16.9	23.8	16.3	42	12.9	36.3	8.8	26	15.1	29.7	29.1
EU435784 Dicranosepsis distincta	37.3	15.3	29.9	17.5	43	18.0	23.3	15.7	40	11.7	38.0	10.5	29	16.3	28.5	26.2
JN964757 Sarcophaga australis	36.5	15.3	31.7	16.5	45	16.9	22.1	15.7	39	11.7	41.5	8.2	26	17.4	31.4	25.6
JN860450 Dictya texensis	36.5	17.9	28.3	17.3	42	20.3	21.5	15.7	42	14.0	34.5	9.9	26	19.2	29.1	26.2
KR262649 Neosilba zadolicha	38.1	15.1	29.5	17.3	44	16.3	24.4	15.7	42	14.6	33.3	9.9	28	14.5	30.8	26.2
AY154408 Drosophila subquinaria	38.9	14.4	29.8	16.9	46	16.4	23.4	14.0	46	10.5	33.9	9.9	25	16.3	32.0	26.7
MF884189 Hydrotaea unispinosa	39.0	13.6	31.1	16.3	46	16.3	22.7	15.1	41	9.9	40.9	8.2	30	14.5	29.7	25.6

Table 7.2: Percentage of evolutionary divergence of *Celyphus obtectus* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH590775	*Celyphus obtectus* (Kerala)	
2.	EU435786	*Dicranosepsis hamata* (Singapore)	6.73%
3.	KM626415	*Sepsidae* sp.	7.07%
4.	JN860450	*Dictya texensis*	7.40%
5.	EU435784	*Dicranosepsis distinct*	7.52%
6.	AY154408	*Drosophila subquinaria*	8.24%
7.	KR688930	*Scatella stagnalis*	8.28%
8.	KR649705	*Lonchaea* sp.	8.66%
9.	KR262649	*Neosilba zadolicha*	8.66%
10.	MF884189	*Hydrotaea unispinosa*	9.05%
11.	JN964757	*Sarcophaga australis*	10.27%
12.	KU876327	*Tethina thula*	10.28%
13.	MF818105	*Spaniocelyphus falcatus*	20.63%
14.	MF818104	*Spaniocelyphus falcatus*	20.63%

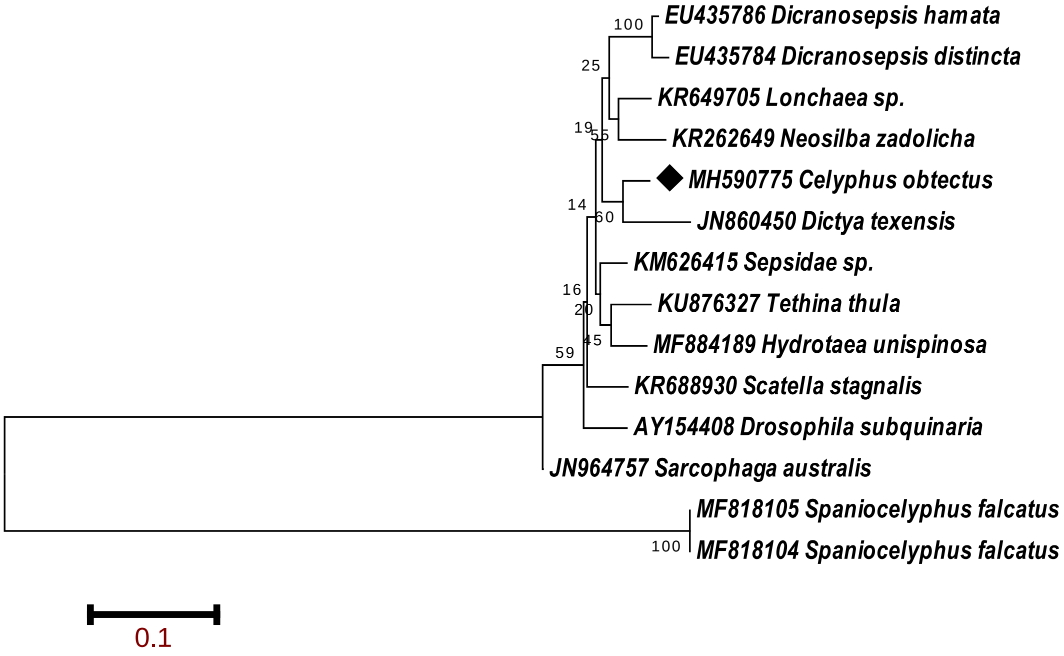


Figure 7.6: Phylogenetic relationship of *Celyphus obtectus* inferred by NJ tree method

DISCUSSION

Both nucleotide and peptide BLAST analysis showed that this species has 100% sequence similarity to the same genus reported from Singapore (EU435786). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Celyphus obtectus* showed bias to nucleotide AT, with following composition of nucleotides T = 36.7%, C = 16.9%, A = 28.9% and G = 16.9%. This greater AT content (65.9%) over GC content (33.8%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Celyphus obtectus* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 6.73% to 20.63% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (6.73%) with those from Singapore while 20.36% from Moscow (Table 7.2). On the basis of the data observed this species may be rooted from those found in Moscow and Singapore which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from Moscow during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Celyphus obtectus* with related species. *Celyphus obtectus* isolated from Kerala (MH590775) showed 6.73% divergence with *Dicranosepsis hamata* (EU435786) from Singapore and 20.64% divergence with (MF818104) *Spaniocelyphus falcatus* (MF818104) from Moscow. The phylogeny tree generated by using NJ method reveals the phylogenetic status of

Celyphus obtectus isolated from Kerala. Closest relative of *Celyphus obtectus* is *Dicranosepsis hamata* from Singapore represented within the same clade.

2. *Spaniocelyphus falcatus* (Tenorio, 1972)

Specimen details:

Voucher specimen	:	CUSF – 01 – A1
Date of collection	:	14- Nov-2014
Locality	:	Kasaragod: Cheruvathur
Lat- Lon	:	12.2165º N, 75.1626º E
GenBank accessions	:	MH590779

Description and Distribution

Spaniocelyphus falcatus (Figure 7.7) are characterized by having the head with carinate and sharp vertex margin, postvertical bristles minute, reduced or absent, a cross vein separating discal cell from 2nd basal cell, palpi slender and slightly expanded apically, antennal aristae flattened and widened leaf like at least on basal half, palpi slender and not expanded at apices. They are distinguished with abdominal tergites having lateral longitudinal sutures which divide each tergite into one dorsal and two lateral wide plates. Mesonotum is rugose at least medio apically, scutellum rugose, mesonotum or scutellum without pilosity (Datta and Parui, 1991). They are found distributed throughout in Asia.

Fig.7.7: *Spaniocelyphus falcatus*

Damage

This pest species cause severe damage to almost every type of agricultural crops. The adults are extensive leaf miners and feeds on sap of the vegetation. Larvae are saprophagus, feeds generally on litter but damage the vegetables and rootlets of seedlings.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Spaniocelyphus falcatus* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 578 bp, 578 bp long products for the specimens obtained from different locations. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 7.8 – 7.11 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 7.3.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Spaniocelyphus falcatus* voucher collected during the present study (Table 7.3). It showed bias to nucleotide AT, with nucleotide composition with T = 37%, C = 17.3%, A = 29.6% and G = 16.1%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 7.4 and Figure 7.12 respectively.

>*Spaniocelyphus falcatus* CDS-2018/ 578 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUSF-01-A1

> *Spaniocelyphus falcatus*

```
AGTAGGAACTTCCCTAAGAATCTTAATTCGTGCTGAATTAGGACACCCCGGAGCTTTAAT
TGGTGATGATCAAATTTATAATGTTATTGTTACAGCTCATGCCTTTGTTATAATTTTTTT
TATAGTAATACCTATTATAATTGGAGGATTTGGAAATTGACTGGTTCCCCTAATATTAGG
AGCCCCAGATATAGCATTCCCTCGAATAAATAATATAAGATTTTGACTATTACCACCTTC
ATTAACCTTACTTTTAGTGAGAAGTATAGTTGAAAATGGAGCTGGAACAGGATGAACTGT
TTACCCCCCTCTTTCTTCAGGAATTGCTCACGGAGGAGCTTCAGTTGATCTTGCAATTTT
CAGTTTACATTTAGCCGGAATTTCTTCAATTTTAGGAGCAGTAAATTTTATCACAACAGT
TATTAATATACGATCGACAGGAATTACATTTGACCGAATACCATTATTTGTATGATCAGT
AGCTATTACTGCTTTACTTTTATTACTTTCTTTACCTGTACTAGCTGGAGCAATTACTAT
ACTTTTAACTGATCGAAATTTAAATACTTCATTCTTTG
```

Figure 7.8: The partial DNA sequence of the mitochondrial COI gene of *Spaniocelyphus falcatus*

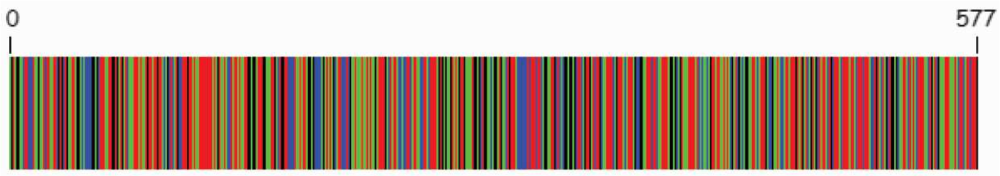


Figure 7.9: Molecular barcode of the mitochondrial COI gene of *Spaniocelyphus falcatus*

> *Spaniocelyphus falcatus* / 156AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUSF-01-A1

> *Spaniocelyphus falcatus*

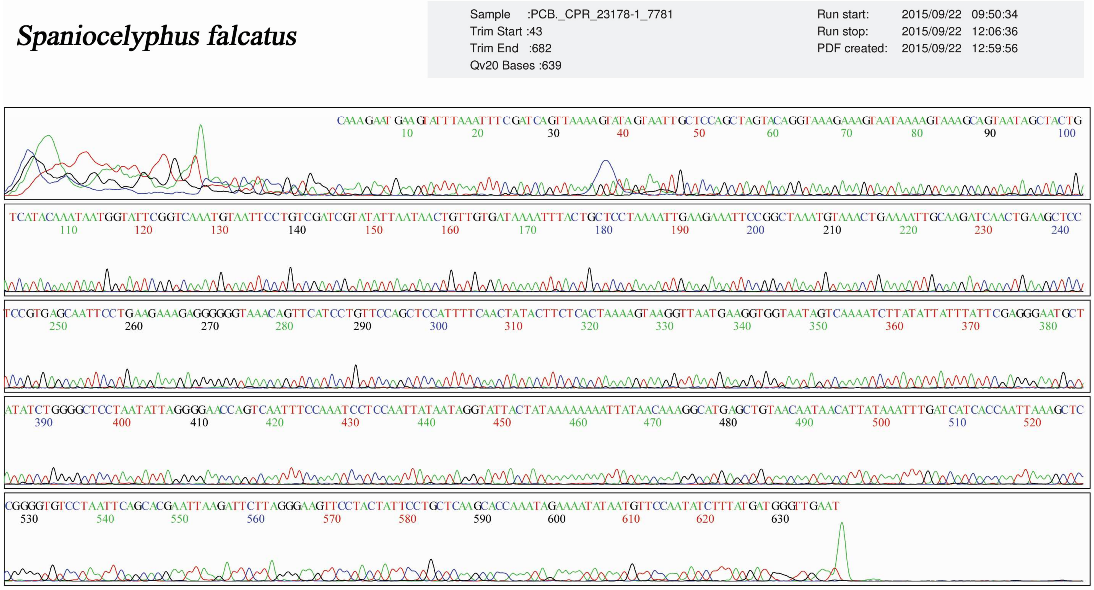
```
MIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLLVSSMVENGAGT
GWTVYPPLSSGIAHGGASVDLAIFSLHLAGISSILGAVNFITTVINMRSTGITFDRMPLF
VWSVAITALLLLLSLPVLAGAITMLLTDRNLNTSFF
```

Figure 7.10: The translation product of the mitochondrial COI gene of *Spaniocelyphus falcatus*

Spaniocelyphus falcatus

Sample :PCB._CP1F_23166-1_7780
Trim Start :37
Trim End :684
Qv20 Bases :647

Run start: 2015/09/21 09:36:28
Run stop: 2015/09/21 11:52:29
PDF created: 2015/09/21 12:48:12



Spaniocelyphus falcatus

Sample :PCB._CPR_23178-1_7781
Trim Start :43
Trim End :682
Qv20 Bases :639

Run start: 2015/09/22 09:50:34
Run stop: 2015/09/22 12:06:36
PDF created: 2015/09/22 12:59:56

Figure 7.11: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Spaniocelyphus falcatus* using Forward and Reverse primers.

Table 7.3: Comparison of Nucleotide frequencies of COI gene sequence of *Spaniocelyphus falcatus* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH590779 Spaniocelyphus falcatus (Cheruvathur)	37.0	17.3	29.6	16.1	44	26.6	12.5	16.7	42	8.8	47.2	1.6	24	16.6	29.0	30.1
JN964737 Sarcophaga australis	37.0	15.9	31.0	16.1	45	26.4	11.9	16.6	41	6.3	52.1	1.0	25	15.0	29.0	30.6
KM626415 Sepsidae sp.	39.1	15.1	28.7	17.1	45	26.6	11.5	17.2	45	5.7	46.6	2.6	27	13.0	28.0	31.6
MF885441 Lonchaea sp.	40.3	15.4	28.0	16.3	45	26.6	11.5	16.7	50	4.7	44.6	.5	25	15.0	28.0	31.6
KU876327 Tethina thula	38.9	14.2	31.3	15.6	45	27.1	11.5	16.7	43	3.6	52.8	.5	29	11.9	29.5	29.5
KR152335 Anopheles sergentii	38.4	16.6	28.2	16.8	44	27.1	12.0	17.2	47	7.8	43.5	2.1	25	15.0	29.0	31.1
DQ851706 Drosophila recens	38.2	16.1	29.2	16.4	45	26.0	11.5	17.2	42	8.8	48.2	.5	27	13.5	28.0	31.6
JN860450 Dictya texensis	37.4	18.9	27.2	16.6	45	26.6	11.5	17.2	49	7.8	41.5	1.6	18	22.3	28.5	31.1
HQ981398 Drosophila falleni	39.3	15.9	28.0	16.8	45	26.6	11.5	17.2	47	7.8	44.6	1.0	26	13.5	28.0	32.1
DQ851615 Drosophila subquinaria	39.8	14.7	29.1	16.4	45	26.0	11.5	17.2	46	5.7	47.7	.5	28	12.4	28.0	31.6
KM930426 Drosophila munda	39.1	15.2	28.5	17.1	45	26.6	11.5	17.2	46	6.2	45.6	2.6	27	13.0	28.5	31.6
KT894990 Chrysomya chani	38.8	14.4	31.5	15.4	44	25.4	14.0	16.6	44	5.2	51.3	.0	29	12.5	29.2	29.7
JF877925 Opacifrons convexa	40.5	13.5	29.4	16.6	45	26.6	11.5	17.2	48	2.1	48.7	1.6	29	11.9	28.0	31.1

Table 7.4: Percentage of evolutionary divergence of *Spaniocelyphus falcatus* with its closely related species accessible from NCBI GenBank

Sl. No.	**Accession No**	**Organism**	**Percentage of divergence**
1.	MH590779	*Spaniocelyphus falcatus* (Kerala)	
2.	JF877925	*Opacifrons convexa* (Canada)	3.56%
3.	JN860450	*Dictya texensis*	4.15%
4.	KM626415	*Sepsidae* sp.	4.18%
5.	HQ981398	*Drosophilafalleni*	4.20%
6.	MF885441	*Lonchaea* sp.	4.21%
7.	JN964737	*Sarcophaga australis*	4.22%
8.	DQ851615	*Drosophila subquinaria*	4.52%
9.	KM930426	*Drosophila munda*	4.83%
10.	DQ851706	*Drosophila recens*	4.83%
11.	KR152335	*Anopheles sergentii*	4.91%
12.	KU876327	*Tethina thula*	5.16%
13.	KT894990	*Chrysomya chain*	5.47%

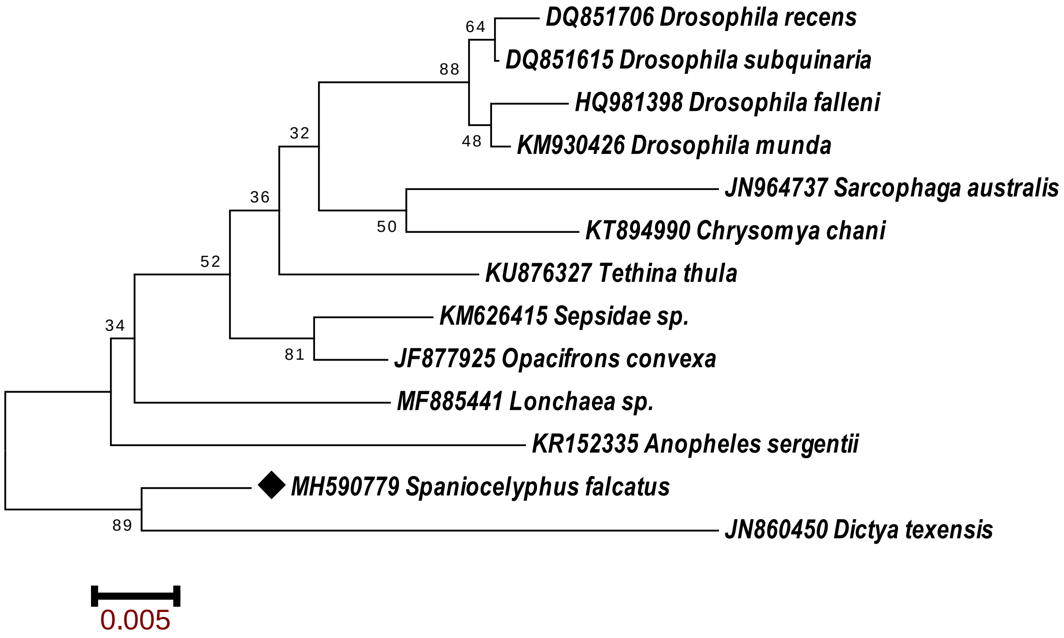


Figure 7.12: Phylogenetic relationship of *Spaniocelyphus falcatus* inferred by NJ tree method

DISCUSSION

Both nucleotide and peptide BLAST analysis showed that this species has 96.5% sequence similarity to the same genus reported from Canada (JF877925). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Spaniocelyphus falcatus* showed bias to nucleotide AT, with following composition of nucleotides T = 37.0%, C = 17.3%, A = 29.6% and G = 16.1%. This greater AT content (66.6%) over GC content (33.4%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Spaniocelyphus falcatus* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 3.56% to 5.47% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (3.56%) with those from Canada while 5.47% from Germany (Table 7.4). On the basis of the data observed this species may be rooted from those found in Canada and Germany was diverted into different clades due to geographical variation. Result thus concluded that this species doesn't have any major changes in India while slightly changes from those reported from Canada and Germany during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Spaniocelyphus falcatus* with related species. *Spaniocelyphus falcatus* isolated from Kerala (MH590779) showed 3.56% divergence with *Opacifrons convexa* (JF877925) of Canada and 5.47% divergence with (KT894990) *Chrysomya chain* of Germany. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Spaniocelyphus falcatus* isolated from Kerala. Closest relative of *Spaniocelyphus falcatus* is *Dictya texensis* from USA represented within the same clade.

3. ***Spaniocelyphus pilosus*** (Tenorio, 1972)

Specimen details:

Voucher specimen : CUSP – 01 – A1
Date of collection : 18-Apr-2016
Locality : Kannur: Payyannur
Lat- Lon : 12.0328º N, 75.2684º E
GenBank accessions : MH590773

Description and Distribution

Fig.7.13: *Spaniocelyphus pilosus*

Spaniocelyphus pilosus (Figure 7.13) may be distinguished by having a sharp vertex, reduced or absent postvertical bristles, and a cross vein separating discal cell from second basal cell, scutellum longer than broad, wing with discal and basal cells separated by a cross vein that may be incomplete, abdomen with segments 1-6 with terga divided into three parts, a dorsal plate and two large lateral plates These are leaf miners, are distinguished with slender palpi, which is not expanded at its apices. This species is distributed throughout Vietnam, Asia and Europe.

Damage

The adults are extensive leaf miners. The larvae damage the ripe fruits, vegetables, rootlets of seedlings (Lahiri and Mitra 2000).

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Spaniocelyphus pilosus* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 599 bp long products. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 7.14 – 7.17 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 7.5.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Spaniocelyphus pilosus* voucher collected during the present study (Table 7.5). It showed bias to nucleotide AT, with nucleotide composition with T = 36.7%, C = 16.5%, A = 29.0% and G = 17.7%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 7.6 and Figure 7.18 respectively.

>*Spaniocelyphus pilosus* CDS-2018/ 599 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUSP-01-A1

>*Spaniocelyphus pilosus*

```
ATAGTAGGGACTTCTCTAAGAATTTTAATTCGAGCAGAACTTGGACACCCTGGAGCACTA
ATTGGAGATGACCAAATTTATAATGTTATTGTAACAGCTCATGCTTTTGTAATAATTTTT
TTTATAGTTATGCCAATTATAATTGGAGGATTTGGAAATTGATTAGTTCCATTAATACTT
GGAGCTCCTGATATGGCTTTCCCTCGAATGAATAATATAAGATTTTGACTTTTACCTCCT
TCTCTTACACTATTATTAGTCAGTAGTATAGTGGAAAACGGAGCTGGAACCGGATGAACA
GTTTATCCACCTCTTTCTTCTGGAATTGCTCATGGAGGTGCATCAGTTGATTTGGCAATT
TTTAGACTTCATTTAGCAGGAATTTCTTCTATTCTAGGAGCAGTAAATTTTATTACTACA
GTAATTAATATACGATCTACAGGAATTACATTTGATCGAATGCCTTTATTCGTATGATCA
GTTGCTATTACTGCTTTATTATTGTTACTATCTCTTCCTGTCTTAGCTGGAGCAATCACT
ATATTATTAACAGATCGAAATTTAAACACTTCATTCTTTGACCCAGCAGGAGGAGGAGA
```

Figure 7.14: The partial DNA sequence of the mitochondrial COI gene of *Spaniocelyphus pilosus*

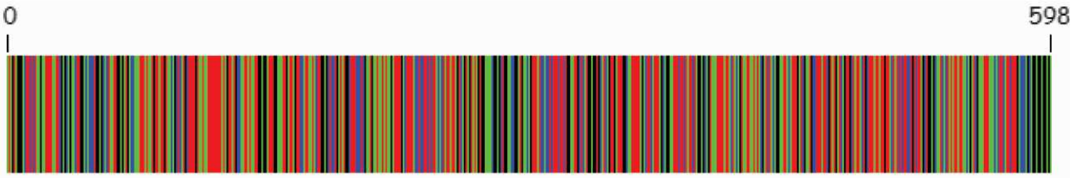


Figure 7.15: Molecular barcode of the mitochondrial COI gene of *Spaniocelyphus pilosus*

> *Spaniocelyphus pilosus* / 199AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUSP-01-A1

> *Spaniocelyphus pilosus*

```
MVGTSLSILIRAELGHPGALIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLML
GAPDMAFPRMNNMSFWLLPPSLTLLLVSSMVENGAGTGWTVYPPLSSGIAHGGASVDLAI
FSLHLAGISSILGAVNFITTVINMRSTGITFDRMPLFVWSVAITALLLLLSLPVLAGAIT
MLLTDRNLNTSFFDPAGGG
```

Figure 7.16: The translation product of the mitochondrial COI gene of *Spaniocelyphus pilosus*

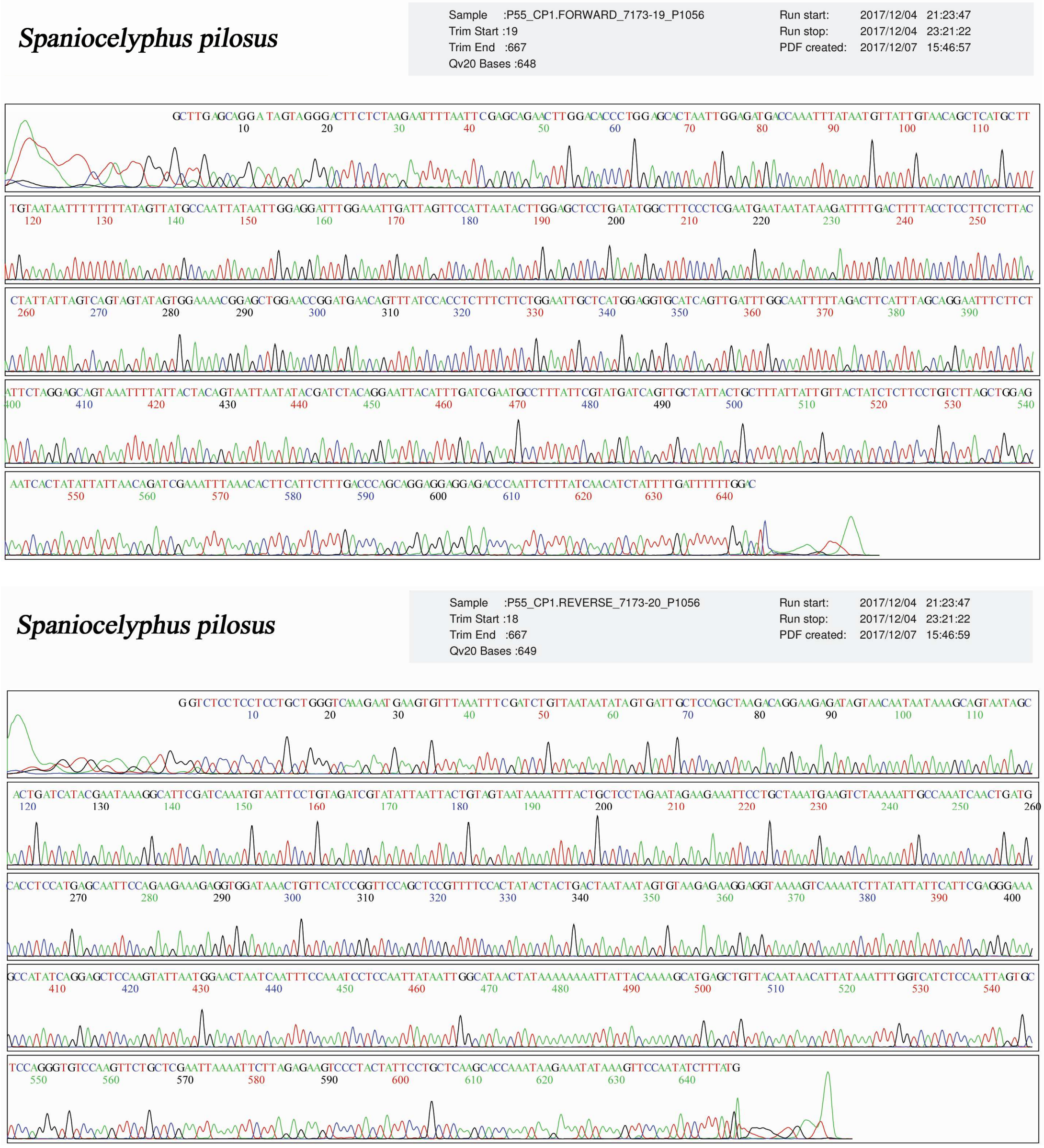


Figure 7.17: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Spaniocelyphus pilosus* using Forward and Reverse primers.

Table 7.5: Comparison of Nucleotide frequencies of COI gene sequence of *Spaniocelyphus pilosus* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH590773 Spaniocelyphus pilosus (Payyanur)	36.7	16.5	29.0	17.7	23	17.0	28.5	31.5	43	26.5	13.0	17.5	44	6.0	45.7	4.0
EU435802 Saltella bezzii	38.9	15.7	29.0	16.4	28	14.5	27.5	30.0	43	25.6	13.6	17.6	46	7.0	46.0	1.5
MG383395 Chrysomya chani	38.1	14.2	31.9	15.9	28	12.5	29.0	31.0	44	26.5	13.5	16.5	43	3.5	53.3	.0
MG673830 Pegoplata infirma	40.1	14.9	28.4	16.7	27	14.0	29.5	30.0	45	26.1	12.6	16.6	49	4.5	43.0	3.5
KC960755 Stomoxys uruma	41.1	14.0	28.7	16.2	26	14.5	29.5	30.0	45	26.0	13.0	16.5	53	1.5	43.7	2.0
GQ154221 Ceratitis fasciventris	39.1	16.5	28.2	16.2	25	16.6	27.1	31.2	45	26.5	12.0	16.5	47	6.5	45.5	1.0
AF295549 Compsomyiops callipes	38.7	14.7	30.7	15.9	29	14.0	29.0	28.0	43	25.5	14.5	17.0	44	4.5	48.7	2.5
KU565752 Leucophenga argentata	39.7	14.5	29.2	16.5	26	14.0	28.5	31.5	45	26.0	12.5	17.0	49	3.5	46.7	1.0
KU243345 Phortica longipenis	40.1	15.0	28.0	16.9	27	16.5	26.5	30.5	44	25.5	13.5	17.5	50	3.0	44.2	2.5
KU565747 Leucophenga argentata	40.7	13.9	29.0	16.4	26	14.0	28.5	31.5	45	26.0	12.5	17.0	52	1.5	46.2	.5
KR658315 Liohippelates bishoppi	37.7	14.0	32.4	15.9	28	13.0	29.0	30.5	45	26.6	12.1	16.6	41	2.5	56.0	.5
AB669755 Drosophila kanapiae	39.4	13.4	30.6	16.7	29	14.1	27.6	29.6	44	25.5	13.5	17.5	46	.5	50.5	3.0
MG967871 Compsomyiops callipes	38.9	14.5	30.9	15.7	28	13.0	29.5	29.5	45	26.1	12.6	16.6	44	4.5	50.5	1.0
KR678992 Anthomyiidae sp.	40.7	14.4	28.9	16.0	28	13.1	29.6	29.6	45	26.0	13.0	16.5	50	4.0	44.0	2.0
KR665987 Liohippelates bishoppi	37.9	13.9	32.4	15.9	28	13.0	29.0	30.5	45	26.6	12.1	16.6	42	2.0	56.0	.5

Table 7.6: Percentage of evolutionary divergence of *Spaniocelyphus pilosus* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH590773	*Spaniocelyphus pilosus* (Kerala)	
2.	MG383395	*Chrysomya chain* (Germany)	23.53%
3.	EU435802	*Saltella bezzii*	23.98%
4.	KU243345	*Phortica longipenis*	24.32%
5.	KC960755	*Stomoxys uruma*	24.76%
6.	MG673830	*Pegoplata infirma*	24.89%
7.	AF295549	*Compsomyiops callipes*	25.10%
8.	KU565752	*Leucophenga argentata*	25.26%
9.	KU565747	*Leucophenga argentata*	25.41%
10.	GQ154221	*Ceratitis fasciventris*	26.04%
11.	AB669755	*Drosophila kanapiae*	26.08%
12.	MG967871	*Compsomyiops callipes*	26.67%
13.	KR658315	*Liohippelates bishoppi*	27.25%
14.	KR665987	*Liohippelates bishoppi*	27.69%
15.	KR678992	*Anthomyiidae* sp.	27.94%

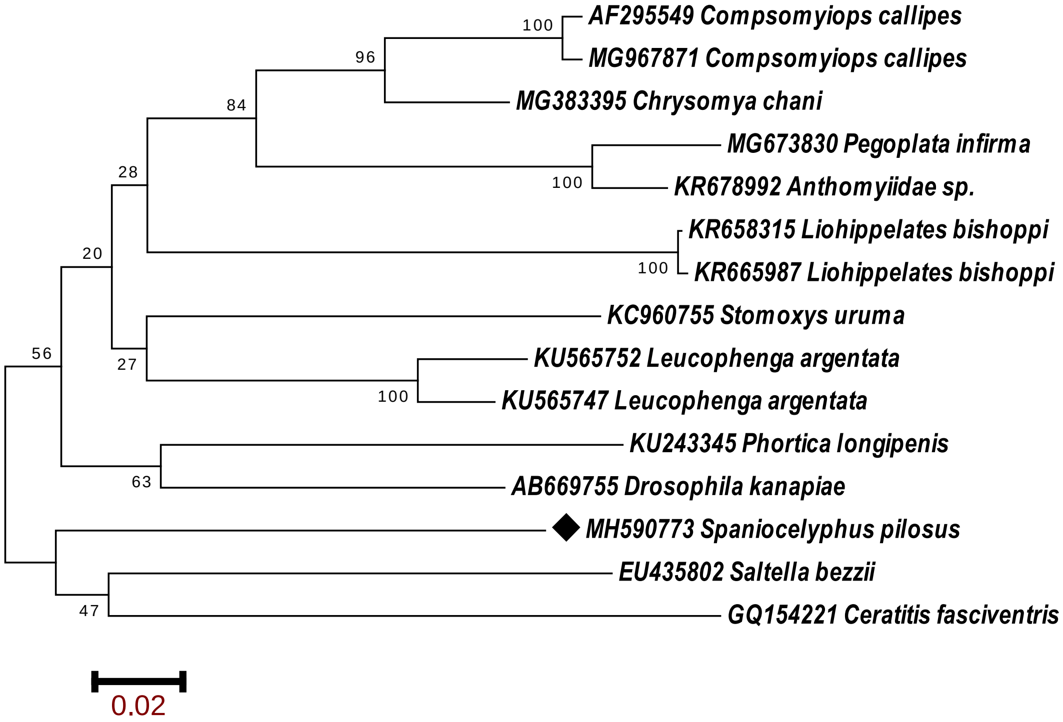


Figure 7.18: Phylogenetic relationship of *Spaniocelyphus pilosus* inferred by NJ tree method

DISCUSSION

Both nucleotide and peptide BLAST analysis showed that this species has 76.5% sequence similarity to the same genus reported from Kerala (MG383395). Eventhough this species has been found in various geographically isolated areas, their sequence have wide variation. Hence the present study stress that the barcode generated can be used to easily spot the specimen and also to analyse its phylogeny.

The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Spaniocelyphus pilosus* showed bias to nucleotide AT, with following composition of nucleotides T = 36.7%, C = 16.5%, A = 29.0% and G = 17.7%. This greater AT content (65.7%) over GC content (34.2%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Spaniocelyphus pilosus* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 23.53% to 27.94% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (23.53%) with those from Germany while 27.94% from Canada (Table 7.6). On the basis of the data observed this species may be rooted from those found in Canada which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from Canada during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Spaniocelyphus pilosus* with related species. *Spaniocelyphus pilosus* isolated from Kerala (MH590773) showed 23.53% divergence with *Chrysomya chain* (MG383395) from Thailand and 27.94%

divergence with *Anthomyiidae* sp. (KR678992) from Canada. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Spaniocelyphus pilosus* isolated from Kerala. Closest relative of *Spaniocelyphus pilosus* is *Saltella bezzii* of Singapore represented within the same clade.

4. ***Bactrocera tau*** (Walker,1849)

Specimen details:

Voucher specimen : CUBT – 01 – A1
Date of collection : 14- Nov-2015
Locality : Kerala: Malappuram
Lat- Lon : 11.0510º N, 76.0711º E
GenBank accessions : MH590778

Description and Distribution

Bactrocera tau (Figure 7.19) adult have orange brown scutellum marked with black and with lateral and medial yellow stripes, with facial spots, anterior supra alar setae, prescutellar setae, 4 scutellar setae,wing have coastal band overlapping vein R2+3 and expanded apically into a spot (Singh and Wells, 2013). According to Leblanc et al. (2015), wing of *B. tau* is with infuscations restricted to the costal band and anal streak, apex of costal band on wing not greatly expanded; have well developed yellow median and lateral postsutural vittae; Face fulvous with a pair of circular to oval black spots; apex of wing costal band expanded into an apical spot; males are attracted to cuelure. They are frugivorous (Singh et al., 2010). This species is distributed throughout Asia.

Fig.7.19: *Bactrocera tau*

Damage

Larval instars are apodous and frugivorous, larvae feed on the fruit tissue initially and later on rotten tissue.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Bactrocera tau* collected has been amplified using the primer BTL (Table 3.1). The PCR amplification yielded 598 bp, 598 bp long products for the specimens obtained from different locations s. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 7.20 – 7.23 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 7.7.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Bactrocera tau* voucher collected during the present study (Table 7.7). It showed bias to nucleotide AT, with nucleotide composition with T = 36.0%, C = 18.2%, A = 28.4% and G = 17.5%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 7.8 and Figure 7.24 respectively.

>*Bactrocera tau* CDS-2016/ 598 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUBT-01-A1

> *Bactrocera tau*

```
CAGGTATAGTAGGAACATCTCTTAGAATTTTAGTTCGGGCAGAACTAGGGCACCCAGGAG
CTTTAATCGGAGATGACCAAATCTATAATGTAATCGTAACAGCTCATGCATTTGTTATAA
TTTTTTTCATGGTAATGCCTATTATAATTGGAGGATTTGGAAATTGATTAGTACCTCTAA
TATTAGGAGCACCAGATATAGCGTTCCCTCGAATGAATAATATAAGATTTTGATTATTAC
CTCCCTCTCTTACATTACTTTTAGTGAGCAGTATAGTAGAAAACGGAGCTGGTACAGGTT
GAACTGTTTACCCTCCCCTTTCATCAATTATCGCTCATGGTGGAGCCTCAGTTGATTTAG
CTATTTTTTCTCTACATTTAGCTGGTATTTCATCAATTTTAGGGGCTGTAAATTTCATTA
CTACAGTAATTAATATACGATCAACAGGAATTACATTTGACCGAATACCTTTATTCGTTT
GAGCTGTAGTATTAACAGCTCTTCTTTTACTTCTATCTCTCCCAGTATTAGCTGGAGCTA
TTACTATACTTTTAACAGACCGAAACTTAAATACATCTTTCTTCGACCCAGCTGGTGG
```

Figure 7.20: The partial DNA sequence of the mitochondrial COI gene of *Bactrocera tau*

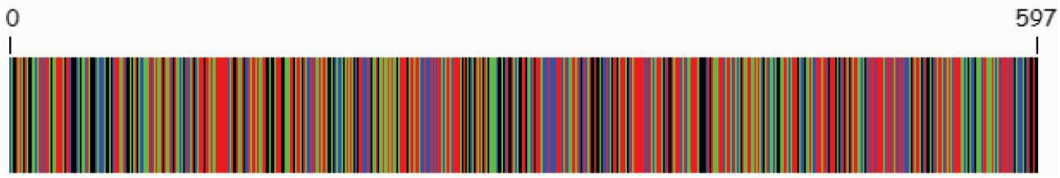


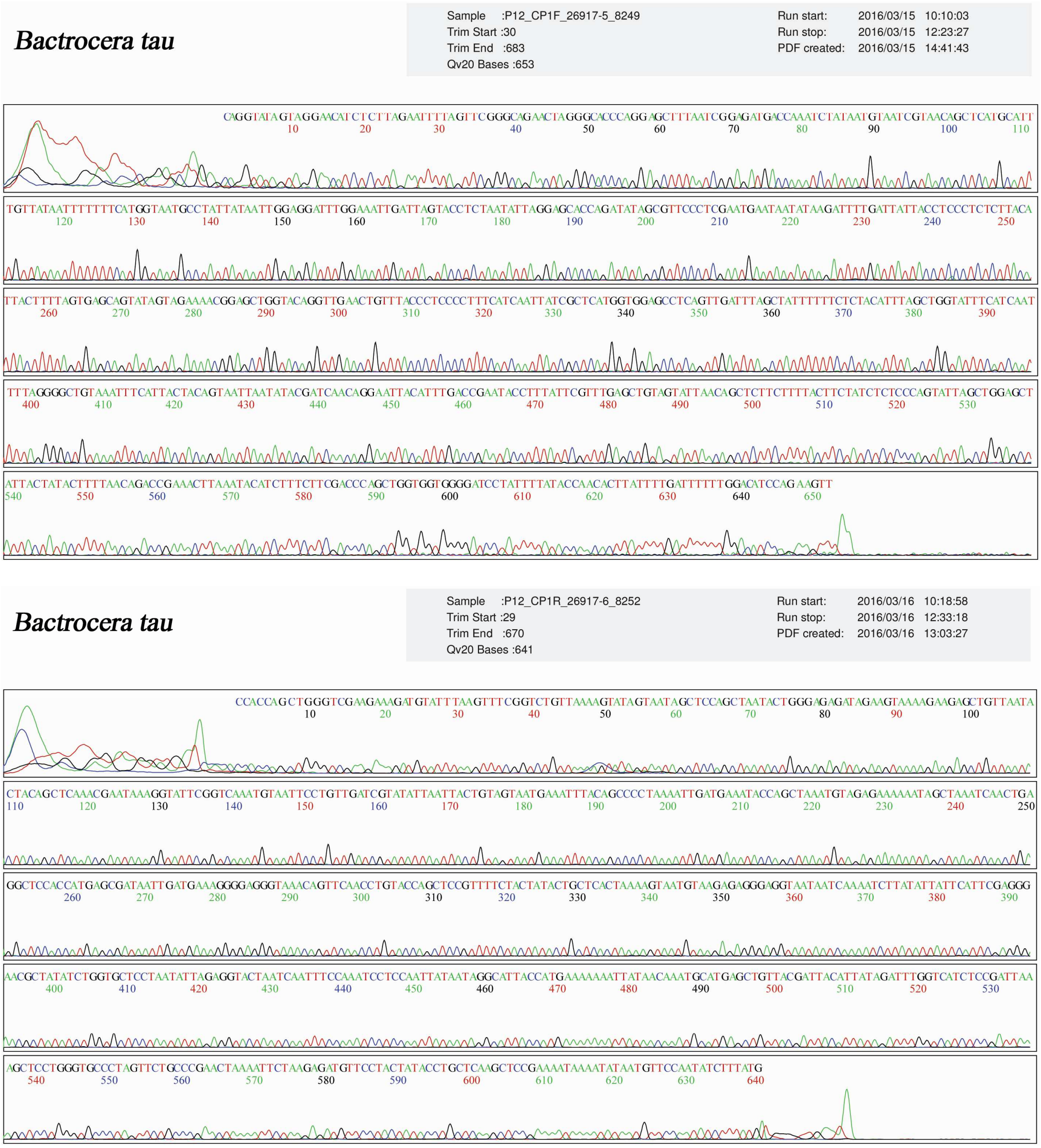
Figure 7.21: Molecular barcode of the mitochondrial COI gene of *Bactrocera tau*

> *Bactrocera tau* / 197AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUBT-01-A1

> *Bactrocera tau*

```
MVGTSLSILVRAELGHPGALIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLML
GAPDMAFPRMNNMSFWLLPPSLTLLLVSSMVENGAGTGWTVYPPLSSIIAHGGASVDLAI
FSLHLAGISSILGAVNFITTVINMRSTGITFDRMPLFVWAVVLTALLLLLSLPVLAGAIT
MLLTDRNLNTSFFDPAG
```

Figure 7.22: The translation product of the mitochondrial COI gene of *Bactrocera tau*



Bactrocera tau

Sample :P12_CP1F_26917-5_8249
Trim Start :30
Trim End :683
Qv20 Bases :653

Run start: 2016/03/15 10:10:03
Run stop: 2016/03/15 12:23:27
PDF created: 2016/03/15 14:41:43

Bactrocera tau

Sample :P12_CP1R_26917-6_8252
Trim Start :29
Trim End :670
Qv20 Bases :641

Run start: 2016/03/16 10:18:58
Run stop: 2016/03/16 12:33:18
PDF created: 2016/03/16 13:03:27

Figure 7.23: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Bactrocera tau* using Forward and Reverse primers.

Table 7.7: Comparison of Nucleotide frequencies of COI gene sequence of *Bactrocera tau* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH590778 Bactrocera tau (Kerala)	36.0	18.2	28.4	17.5	22	18.0	27.9	31.7	46	26.6	11.4	16.3	40	9.8	45.9	4.4
KX603660 Bactrocera tau (Kerala)	36.0	18.2	28.4	17.5	22	18.0	27.9	31.7	46	26.6	11.4	16.3	40	9.8	45.9	4.4
KT151119 Bactrocera tau	36.2	18.4	28.9	16.5	23	18.0	27.9	30.6	45	26.8	13.1	15.3	40	10.3	45.7	3.8
DQ116244 Bactrocera cucurbitae	36.2	18.4	29.1	16.4	23	18.0	27.9	30.6	45	26.6	13.0	15.2	40	10.4	46.4	3.3
MF095186 Zeugodacus tau	36.7	17.5	28.5	17.3	25	16.9	27.3	31.1	45	25.7	12.0	16.9	40	9.8	46.2	3.8
GQ154088 Bactrocera calumniata	36.7	17.5	28.5	17.3	25	16.9	27.3	31.1	45	25.7	12.0	16.9	40	9.8	46.2	3.8
KF660184 Bactrocera tau	37.1	17.8	28.5	16.5	25	18.6	27.9	28.4	46	25.1	12.6	15.8	40	9.8	45.1	5.4
DQ116243 Bactrocera cucurbitae	36.2	18.4	29.1	16.4	24	17.5	27.9	30.6	45	26.6	13.0	15.2	39	10.9	46.4	3.3
KF660137 Bactrocera tau	37.5	17.6	28.4	16.5	26	18.0	27.9	28.4	46	25.1	12.6	15.8	40	9.8	44.6	5.4
KT588405 Bactrocera tau	36.9	18.7	28.0	16.4	25	19.1	25.7	30.1	46	25.7	14.2	14.2	40	11.4	44.0	4.9
MF970789 Zeugodacus choristus	35.8	18.7	28.2	17.3	23	17.5	27.9	31.1	44	27.3	13.1	15.8	40	11.4	43.5	4.9
JQ671147 Bactrocera chorista	36.2	18.9	27.8	17.1	23	18.0	27.9	30.6	45	26.6	13.0	15.2	40	12.0	42.6	5.5
KM024432 Bactrocera vultus	35.8	17.3	28.2	18.7	24	15.8	28.3	32.1	44	25.1	13.1	17.5	39	10.9	43.2	6.6
KF660130 Bactrocera synnephes	36.4	18.0	27.5	18.2	25	16.9	27.3	31.1	45	25.7	12.0	16.9	39	11.4	42.9	6.5

Table 7.8: Percentage of evolutionary divergence of *Bactrocera tau* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH590778	*Bactrocera tau* (Kerala)	
2.	KX603660	*Bactrocera tau* (Kerala)	0.00%
3.	KT151119	*Bactrocera tau*	0.79%
4.	DQ116244	*Bactrocera cucurbitae*	1.19%
5.	MF095186	*Zeugodacus tau*	1.19%
6.	KF660137	*Bactrocera tau*	1.19%
7.	KT588405	*Bactrocera tau*	1.19%
8.	DQ116243	*Bactrocera cucurbitae*	1.59%
9.	GQ154088	*Bactrocera calumniate*	1.59%
10.	KF660184	*Bactrocera tau*	1.60%
11.	MF970789	*Zeugodacus choristus*	4.93%
12.	JQ671147	*Bactrocera chorista*	5.82%
13.	KM024432	*Bactrocera vultus*	5.82%
14.	KF660130	*Bactrocera synnephes*	5.82%

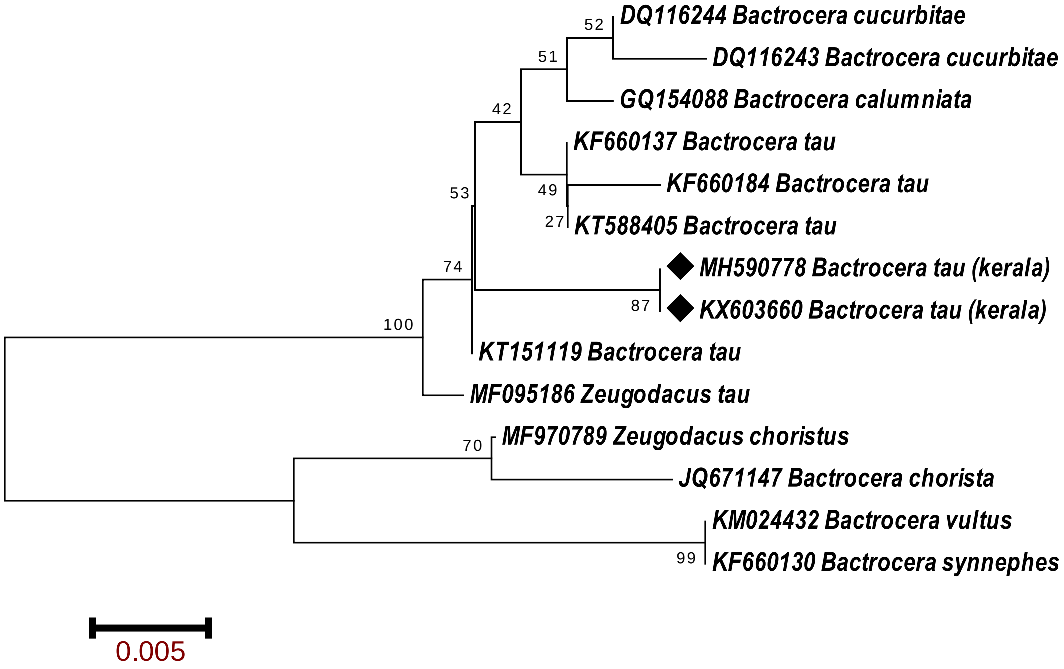


Figure 7.24: Phylogenetic relationship of *Bactrocera tau* inferred by NJ tree method

DISCUSSION

Both nucleotide and peptide BLAST analysis showed that this species has 100% sequence similarity to the same genus reported from Kerala (KX603660). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Bactrocera tau* showed bias to nucleotide AT, with following composition of nucleotides T = 36.0%, C = 18.2%, A = 28.4% and G = 17.5%. This greater AT content (64.4%) over GC content (35.7%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Bactrocera tau* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 0% to 5.82% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has no divergence (0%) with those from Kerala while 5.82% to China (Table 7.8). On the basis of the data observed this species may be rooted from those found in China which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from China during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Bactrocera tau* with related species. *Bactrocera tau* isolated from Kerala (MH590778) showed 0% divergence with *Bactrocera tau* (KX603660) from kerala and 5.82% divergence with *Bactrocera synnephes* (KF660130) of China. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Bactrocera tau* isolated from Kerala. Closest relative of *Bactrocera tau* is *Bactrocera tau* from Kerala represented within the same clade.

5. ***Pegomya circumpolaris*** (Ackland and Griffiths, 1983)

Specimen details:

Voucher specimen	:	CUPC – 02 – A1
Date of collection	:	30-Apr-2016
Locality	:	Kasaragod: Chandragiri
Lat- Lon	:	12.4991° N, 74.9989° E
GenBank accessions	:	MH590769

Description and Distribution

Pegomya circumpolaris (Figure 7.25) is a small sized pest having a wing length reach up to 5.5 mm. Males have subtle differences that include mid and hind femora distinctly yellow coloured at extreme basal portions. Hind femur on third basal with a row of fine setulae standing out from the surrounding ground setulae. Hind tibia with apical av seta long, terminalia differ most significantly by larger sternite V with stronger lateral setae, have surstylus broader in lateral view, and females with lower half of frontal vitta orange yellow coloured and apical av seta on hind tibia strong, oviscapt shorter than combined length of tergites III and V. Distributed along Asia and Alaska (Michelson, 2015).

Fig.7.25: Pegomya circumpolaris

Damage

They are polyphagus and larvae are often epidermal leaf miners.

Mitochondrial COI gene sequence analyses:

The partial coding sequence of mitochondrial COI gene of *Pegomya circumpolaris* collected has been amplified using the primer BTL (Table 3.1). The

PCR amplification yielded 605 bp long products. The DNA sequence interpret, representative molecular barcode, conceptual translation product and electropherogram are exhibited in Figures 7.26 – 7.29 respectively and the comparison of percentage of frequencies in the nucleotide composition with its kin species is represented in Table 7.9.

The mitochondrial COI gene nucleotide sequence was analysed for the nucleotide composition of *Pegomya circumpolaris* voucher collected during the present study (Table 7.9). It showed bias to nucleotide AT, with nucleotide composition with T = 37.9%, C = 15.2, A = 29.8% and G = 17.1%.

Evolutionary divergence and Phylogenic status:

The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree constructed with NJ method are exhibited in Table 7.10 and Figure 7.30 respectively.

>*Pegomya circumpolaris* CDS-2018/ 605 bp / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUPC-02-A1

> *Pegomya circumpolaris*

```
AGCAGGTATAGTAGGAACTTCATTAAGAATTTTAATTCGGGCTGAATTAGGGCATCCAGG
GGCATTAATTGGTGATGATCAAATTTATAATGTAATTGTCACTGCTCATGCTTTTATTAT
AATTTTTTTTATAGTTATACCTATCATAATTGGAGGGTTTGGAAATTGATTAGTTCCTTT
AATACTAGGAGCTCCAGATATAGCATTTCCACGAATAAATAATATAAGTTTTTGATTACT
ACCTCCTTCTTTAACTTTATTATTAGTCAGAAGCATAGTAGAAAATGGGGCTGGGACAGG
ATGAACCGTTTACCCCCCCTTATCATCAGTAATCGCTCATGGAGGAGCTTCAGTTGATTT
AGCTATTTTTTCTCTTCATTTAGCAGGAGCTTCTTCAATTTTAGGGGCAGTAAATTTTAT
TACTACAGTAATTAATATACGATCTACAGGAATTACTCTTGATCGAATACCTTTATTTGT
TTGAGCTGTAGTAATTACAGCATTTTTACTTTTATTATCTTTACCTGTATTAGCTGGAGC
AATTACTATACTATTAACAGATCGAAACTTAAATACTTCATTTTTTGATCCAGCTGGAGG
GGGAG
```

Figure 7.26: The partial DNA sequence of the mitochondrial COI gene of *Pegomya circumpolaris*

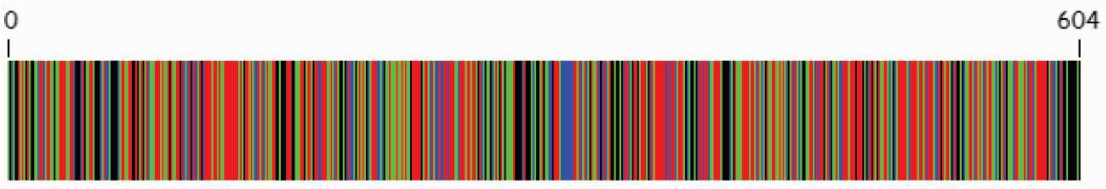


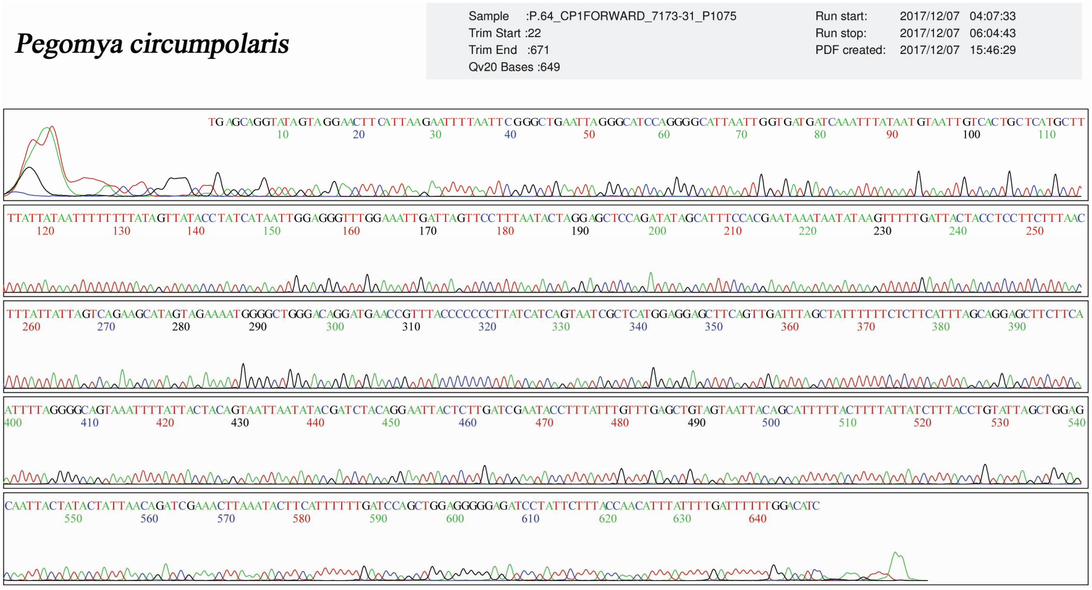
Figure 7.27: Molecular barcode of the mitochondrial COI gene of *Pegomya circumpolaris*

> *Pegomya circumpolaris* / 199AA / cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial / voucher CUAF-01-A1

> *Pegomya circumpolaris*

```
MVGTSLSILIRAELGHPGALIGDDQIYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLML
GAPDMAFPRMNNMSFWLLPPSLTLLLVSSMVENGAGTGWTVYPPLSSVIAHGGASVDLAI
FSLHLAGASSILGAVNFITTVINMRSTGITLDRMPLFVWAVVITAFLLLLSLPVLAGAIT
MLLTDRNLNTSFFDPAGGG
```

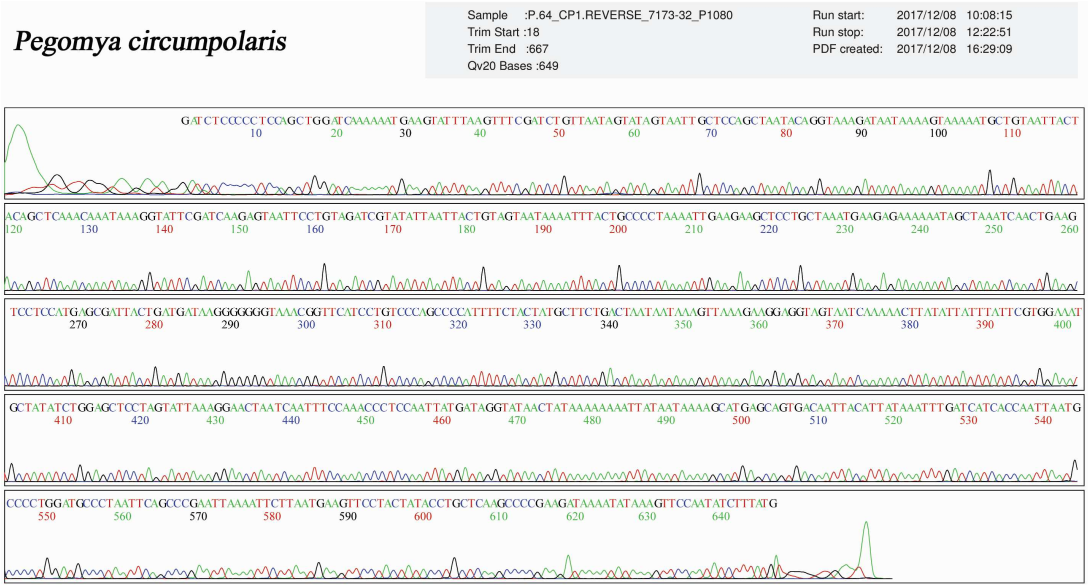
Figure 7.28: The translation product of the mitochondrial COI gene of *Pegomya circumpolaris*



Pegomya circumpolaris

Sample :P.64_CP1FORWARD_7173-31_P1075
Trim Start :22
Trim End :671
Qv20 Bases :649

Run start: 2017/12/07 04:07:33
Run stop: 2017/12/07 06:04:43
PDF created: 2017/12/07 15:46:29



Pegomya circumpolaris

Sample :P.64_CP1.REVERSE_7173-32_P1080
Trim Start :18
Trim End :667
Qv20 Bases :649

Run start: 2017/12/08 10:08:15
Run stop: 2017/12/08 12:22:51
PDF created: 2017/12/08 16:29:09

Figure 7.29: Electropherogram showing the nucleotide sequence of the mitochondrial COI gene of *Pegomya circumpolaris* using Forward and Reverse primers.

Table 7.9: Comparison of Nucleotide frequencies of COI gene sequence of *Pegomya circumpolaris* with its kin species

Name of the species	**NUCLEOTIDE FREQUENCIES (%)**															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
MH590769 Pegomya circumpolaris (Kerala)	37.9	15.2	29.8	17.1	27	13.5	27.4	31.7	43	27.4	12.5	17.3	44	4.8	49.3	2.4
KM861302 Pegomya circumpolaris	39.8	14.1	30.6	15.5	27	13.9	28.8	29.8	44	26.9	13.0	16.3	48	1.4	49.8	.5
MG115201 Pegomya sp.	39.8	14.1	30.6	15.5	27	13.9	28.8	29.8	44	26.9	13.0	16.3	48	1.4	49.8	.5
KT959818 Pegoplata tundrica	40.2	14.7	29.0	16.2	28	13.0	28.8	29.8	44	26.9	13.0	16.3	48	4.3	45.0	2.4
KM270848 Allobaccha sp.	41.3	13.1	29.9	15.7	28	12.5	29.3	30.3	44	25.8	13.9	16.7	52	1.0	46.6	.0
KR678992 Anthomyiidae sp.	40.2	14.6	28.5	16.8	28	13.0	28.4	30.8	44	26.3	12.9	17.2	49	4.3	44.2	2.4
KY775351 Amiota minufoliolata	39.5	13.9	30.6	16.0	27	13.4	28.2	31.1	44	26.0	13.9	16.3	48	2.4	49.5	.5
LT707503 Cheilosia vicina	39.5	14.6	30.2	15.7	28	13.9	27.9	30.3	43	26.9	13.5	16.3	47	2.9	49.3	.5
LT707502 Cheilosia impudens	39.5	14.6	30.2	15.7	27	14.9	27.9	30.3	43	26.9	13.5	16.3	48	1.9	49.3	.5
MG968248 Opsomeigenia xylota	40.2	14.6	30.6	14.7	29	13.5	29.8	27.4	44	27.4	12.5	16.3	47	2.9	49.3	.5
KM861538 Pegoplata nigroscutellata	40.5	14.2	29.6	15.7	29	12.0	28.8	29.8	44	26.9	13.0	16.3	48	3.8	46.9	1.0
HM412441 Phthitia plumosula	38.1	15.8	29.4	16.6	28	13.5	27.9	30.8	44	26.9	12.5	16.8	43	7.2	47.8	2.4
KP697245 Leucophenga nigriventris	39.5	14.4	29.9	16.2	27	13.0	28.8	30.8	44	26.9	12.5	16.8	47	3.3	48.3	1.0
KP697142 Leucophenga concilia	38.9	14.6	30.6	16.0	28	13.4	29.2	29.7	44	26.0	13.0	17.3	45	4.3	49.5	1.0
HM412730 Oscinisoma alienum	38.4	14.4	31.0	16.2	28	12.5	28.8	30.3	44	27.4	12.5	16.3	43	3.3	51.7	1.9

Table 7.10: Percentage of evolutionary divergence of *Pegomya circumpolaris* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	MH590769	*Pegomya circumpolaris* (Kerala)	
2.	KM861302	*Pegomya circumpolaris* (Canada)	11.30%
3.	MG115201	*Pegomya* sp.	11.30%
4.	KT959818	*Pegoplata tundrica*	15.05%
5.	KR678992	*Anthomyiidae* sp.	15.71%
6.	KM861538	*Pegoplata nigroscutellata*	15.99%
7.	HM412441	*Phthitia plumosula*	16.32%
8.	KY775351	*Amiota minufoliolata*	16.35%
9.	KP697245	*Leucophenga nigriventris*	16.74%
10.	KP697142	*Leucophenga concilia*	17.26%
11.	KM270848	*Allobaccha* sp.	18.15%
12.	MG968248	*Opsomeigenia xylota*	18.65%
13.	LT707502	*Cheilosia impudens*	18.83%
14.	LT707503	*Cheilosia vicina*	18.93%
15.	HM412730	*Oscinisoma alienum*	20.53%

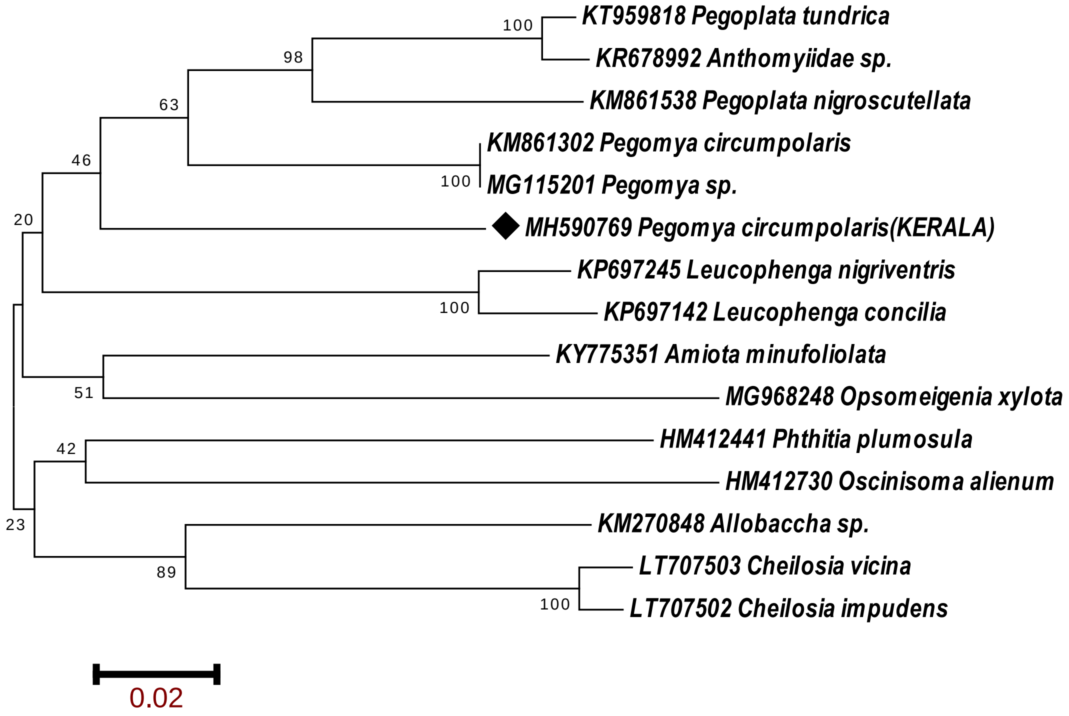


Figure 7.30: Phylogenetic relationship of *Pegomya circumpolaris* inferred by NJ tree method

DISCUSSION

Both nucleotide and peptide BLAST analysis showed that this species has 88.7% sequence similarity to the same genus reported from Canada (KM861302). The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Pegomya circumpolaris* showed bias to nucleotide AT, with following composition of nucleotides T = 37.9%, C = 15.2%, A = 29.8% and G = 17.1%. This greater AT content (67.7%) over GC content (32.3%) is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. *Pegomya circumpolaris* showed variation in the total composition of nucleotide in each of the position of codons in comparison with other related species isolated from different geographical locations.

The phylogenetic tree constructed by Neighbour joining method clearly says that this species have a monophyletic ancestry as all members were separated from one clade. Eventhough the COI sequences has been reported from different geographical locations, it showed only 11.30% to 20.53% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has divergence (11.30%) with those from Canada while 20.53% to Canada (Table 7.10). On the basis of the data observed this species may be rooted from Canada which diverted into different clades due to geographical variation. Result thus concluded that this species doesn’t have any major changes in India while slightly changes from those reported from Canada during the course of evolution.

The evolutionary divergence analysis depicts the percentage of divergence of geographically isolated species of *Pegomya circumpolaris* with related species. *Pegomya circumpolaris* isolated from Kerala (MH590769) showed 11.30% divergence with *Pegomya circumpolaris* (KM861302) from Canada and 20.53% divergence with *Oscinisoma alienum* (HM412730) from Canada. The phylogeny tree generated by using NJ method reveals the phylogenetic status of *Pegomya circumpolaris* isolated from Kerala. Closest relative of *Pegomya circumpolaris* is

Pegomya circumpolaris and *Pegomya* sp. from Canada represented within the same clade.

Consolidated Phylogenetic Tree

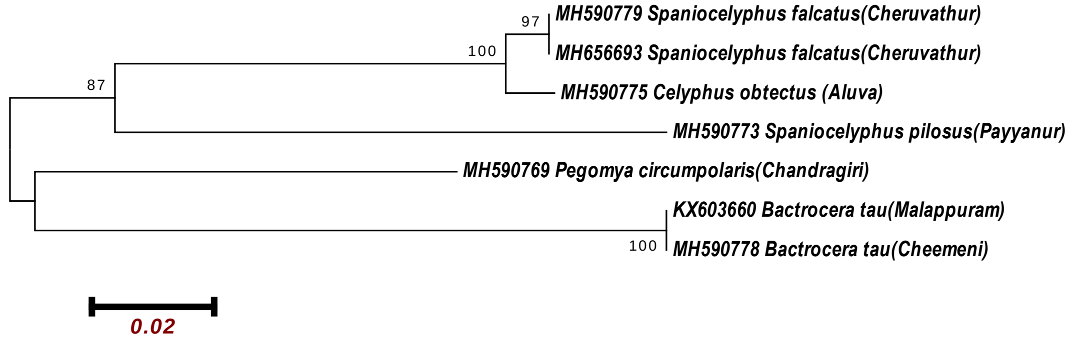
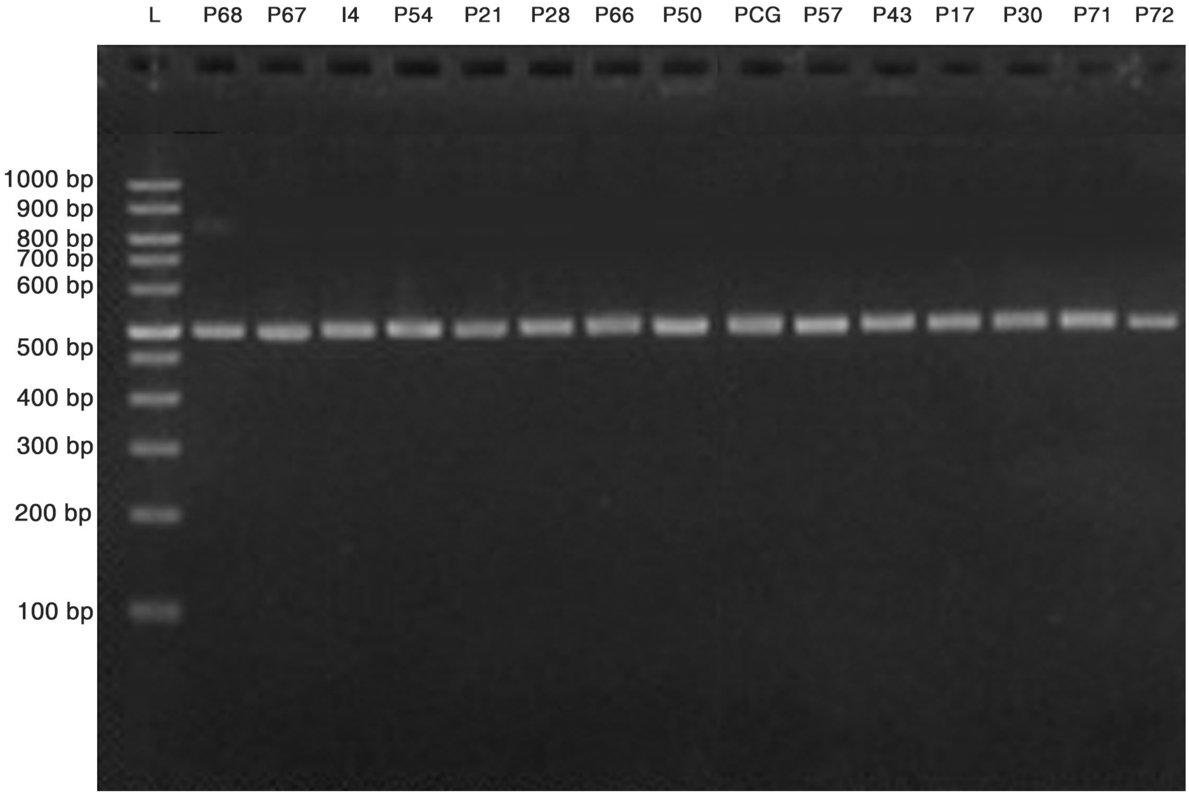
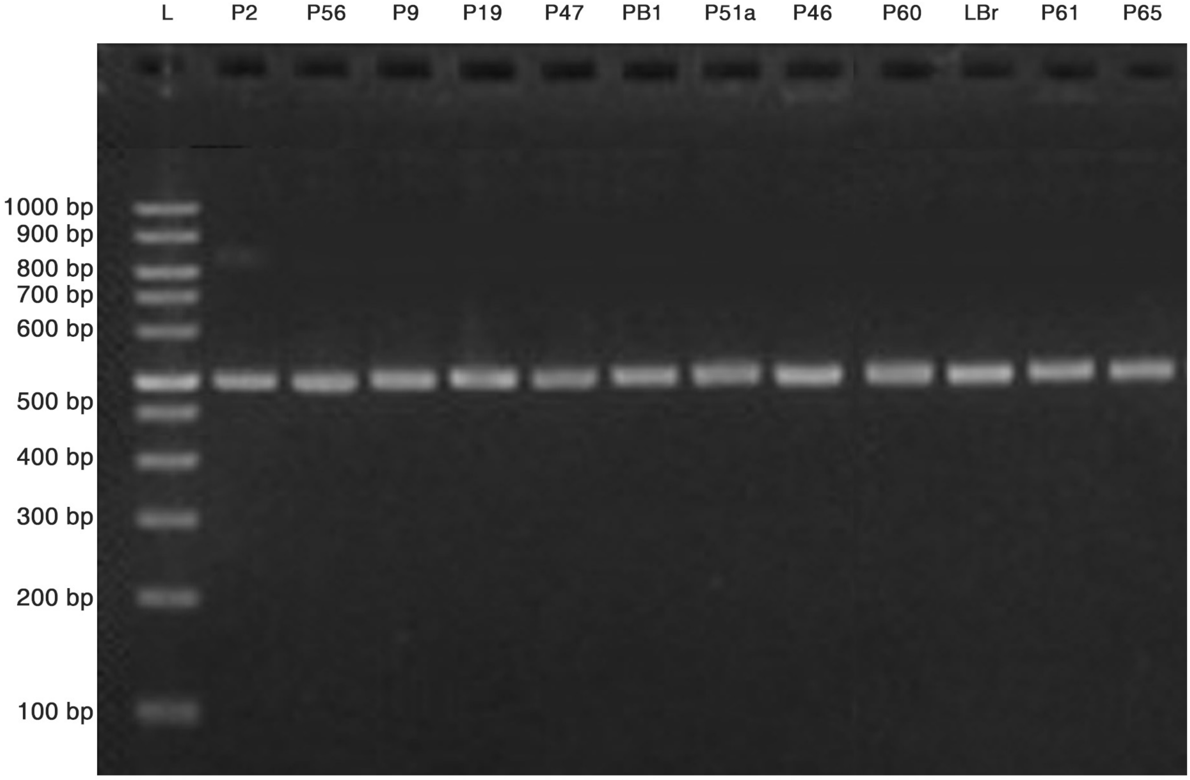


Figure 7.31: Phylogenetic relationship of different dippteran pests of cucurbitaceae isolated from selected districts of Kerala.

The evaluation of the composition of nucleotides within the COI sequences of Dipteran pest of cucurbits of Kerala revealed that the nucleotides are varied within the every third position of codon. The analysis involved 5 species nucleotide sequences (Figure 7.31). The phylogenetic relationships among them was analysed by NJ tree method. Families included in the superfamily Lauxanioidea is Celyphidae, superfamily Tephritoidea is Tephritidae and superfamily Muscoidea is Anthomyiidae. The species from these three families are aligned nearly in the phylogenetic tree and monophyletic in origin. Species from the family Chrysomelidae are originated from a main clade and various species from the genus *Spaniocelyphus* and *Celyphus* are aligned in a single clade and then branches to subclades. *Spaniocelyphus falcatus*, *Spanicelyphus pilosus* and *Celyphus obtectus* are aligned in a same clade and they are included in the Eurychoromyiinae subfamily. *Pegomya circumpolaris* is situated as an outgroup to *Bactrocera tau*.

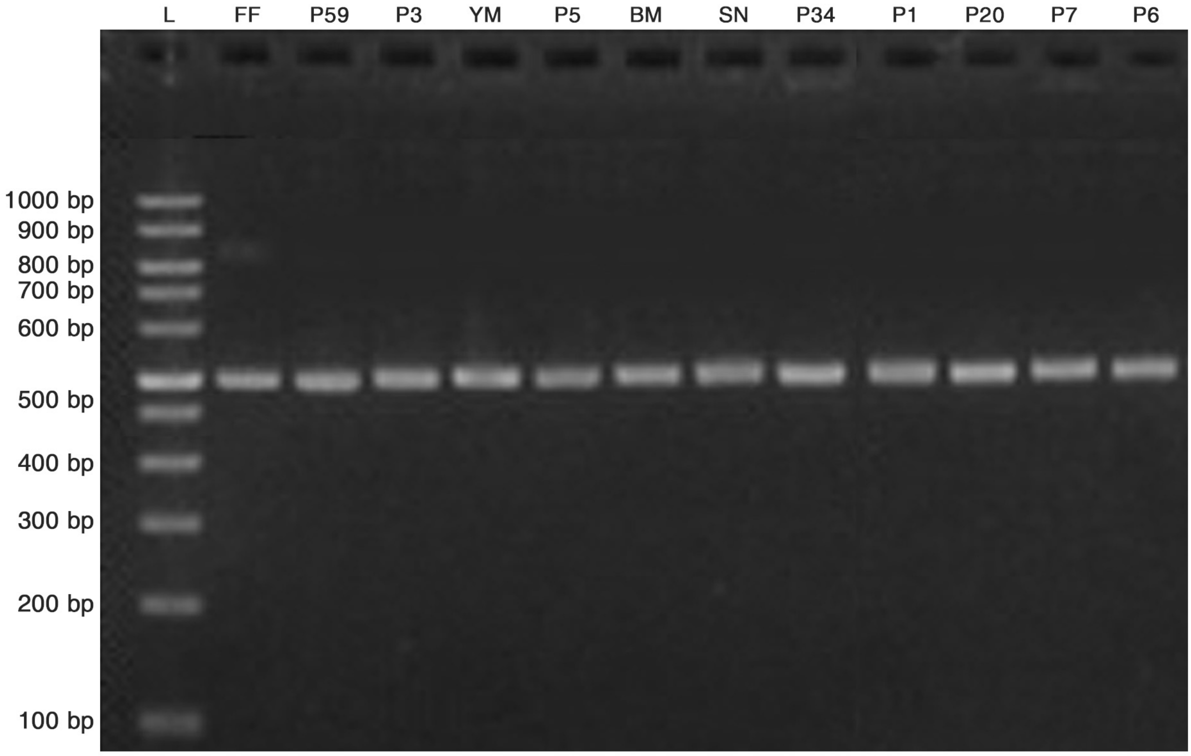


Gel electropherogram indicating PCR amplified product of 15 coleopteran insect pests of cucurbitaceae isolated from selected districts of Kerala, visualised by ethidium bromide staining marking.

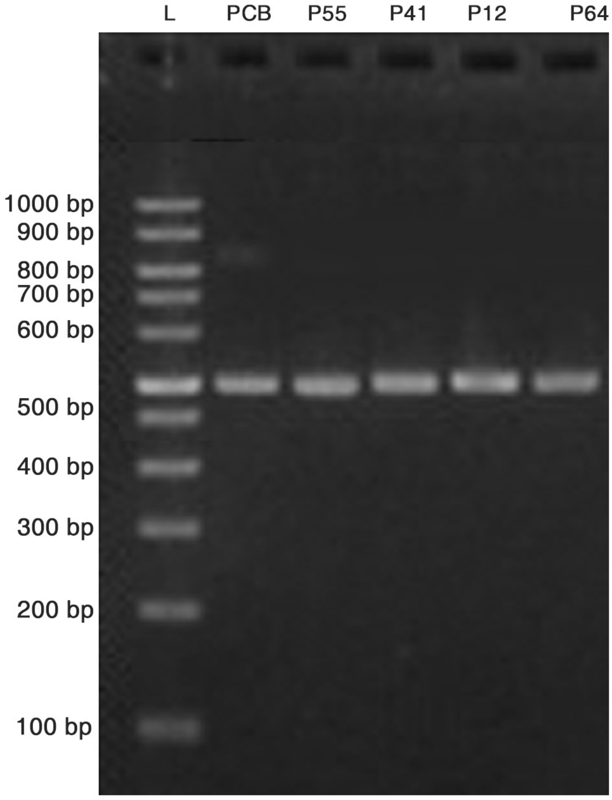


Gel electropherogram indicating PCR amplified product of 12 hemepteran insect pests of cucurbitaceae isolated from selected districts of Kerala, visualised by ethidium bromide staining marking.

Coleoptera	
Mantura chrysanthemi	P68
Mantura rustica	P67
Aulacophora foveicollis	I4
Aulacophora frontalis	P54
Aulacophora lewisii	P21
Sphenoraia bicolor	P28
Paria thoracica	P66
Rhabdopterus praetextus	P50
Oocassida pudibunda	PCG
Dactylispa carinata	P57
Henosepilachna vigintioctopunctata	P43
Henosepilachna septima	P17
Epilachna septima	P30
Carpophilus marginellus	P71
Epuraea luteolus	P72
Hemiptera	
Nezara viridula	P2
Spermatodes variolosa	P56
Agonoscelis nubilis	P9
Carbula scutellata	P19
Halymorpha halys	P47
Zicrona caerulea	PB1
Oncopeltus nigriceps	P51a
Geocoris varius	P46
Paraplesius unicolor	P60
Cletus schmidti	LBr
Dysdercus ocreatus	P61
Campylomma vendicarina	P65



Gel electropherogram indicating PCR amplified product of 12 lepidopteran insect pests of cucurbitaceae isolated from selected districts of Kerala, visualised by ethidium bromide staining marking.



Gel electropherogram indicating PCR amplified product of 5 dipteran insect pests of cucurbitaceae isolated from selected districts of Kerala, visualised by ethidium bromide staining marking.

Lepidoptera	
Ophiusa coronate	FF
Mocis proverai	P59
Asota orbona	P3
Asota caricae	YM
Eudocima cocalus	P5
Hypocala deflorata	BM
Polytela gloriosae	SM
Acosmeryx anceus subdentata	P34
Diaphania indica	P1
Glypholes bicolor	P20
Melanitis leda	P7
Cryptophasa atecmarta	P6
Diptera	
Spaniocelyphus falcatus	PCB
Spaniocelyphus pilosus	P55
Celyphus obtectus	P41
Bactrocera tau	P12
Pegomya circumpolaris	P64

List of cucurbitaceae insect pests used for CO1 gene sequencing in the present study

Sl. No.	Order	Family	Organism	GenBank Accession No.	Locality
1.	Coleoptera	Chrysomelidae	*Mantura chrysanthemi*	MH674105	Thiruvananthapuram
2.	Coleoptera	Chrysomelidae	*Mantura species*	KU557525	Parappanangadi
3.	Coleoptera	Chrysomelidae	*Mantura rustica*	MH674106	Thiruvananthapuram
4.	Coleoptera	Chrysomelidae	*Aulacophora foveicollis*	KU557524	Parappanangadi
5.	Coleoptera	Chrysomelidae	*Aulacophora foveicollis*	MH590763	Kondotty
6.	Coleoptera	Chrysomelidae	*Aulacophora frontalis*	MH674107	Thripunithura
7.	Coleoptera	Chrysomelidae	*Aulacophora lewisii*	MH590776	Nileshwar
8.	Coleoptera	Chrysomelidae	*Sphenoraia bicolor*	MH656688	Azhikode
9.	Coleoptera	Chrysomelidae	*Sphenoraia bicolor*	MH656692	Azhikode
10.	Coleoptera	Chrysomelidae	*Paria thoracica*	MH590759	Chandragiri
11.	Coleoptera	Chrysomelidae	*Rhabdopterus praetextus*	MH674108	kadalundi
12.	Coleoptera	Chrysomelidae	*Oocassida pudibunda*	KX603663	Malappuram
13.	Coleoptera	Chrysomelidae	*Dactylispa carinata*	MH674110	Madayi
14.	Coleoptera	Coccinellidae	*Henosepilachna septima*	KX503056	Parappanangadi
15.	Coleoptera	Coccinellidae	*Henosepilachna vigintioctopunctata*	MH590761	Ottapalam
16.	Coleoptera	Coccinellidae	*Epilachna septima*	MH656689	Parappanangadi
17.	Coleoptera	Nitidulidae	*Carpophilus marginellus*	MH590768	Sulthanbathery
18..	Coleoptera	Nitidulidae	*Epuraea luteolus*	MH674104	Chandragiri

Sl. No.	Order	Family	Organism	GenBank Accession No.	Locality
19..	Hemiptera	Pentatomidae	*Nezara viridula*	KX603658	Payyanur
20.	Hemiptera	Pentatomidae	*Nezara viridula*	KX603657	Parappanangadi
21.	Hemiptera	Pentatomidae	*Nezara viridula*	KX587504	Malappuram
22.	Hemiptera	Pentatomidae	*Spermatodes variolosa*	MH590772	Nilambur
23.	Hemiptera	Pentatomidae	*Agonoscelis nubilis*	MH590760	Cheemeni
24.	Hemiptera	Pentatomidae	*Carbula scutellata*	MH590777	Payyanur
25.	Hemiptera	Pentatomidae	*Halymorpha halys*	MH674109	Aluva
26.	Hemiptera	Pentatomidae	*Zicrona caerulea*	KU201287	Parappanangadi
27..	Hemiptera	Lygaeidae	*Oncopeltus nigriceps*	MH590767	Payyanur
28.	Hemiptera	Lygaeidae	*Oncopeltus nigriceps*	MH590766	Payyanur
29.	Hemiptera	Geocoridae	*Geocoris varius*	MH590762	Payyanur
30.	Hemiptera	Alydidae	*Paraplesius unicolor*	MH59070	Nilambur
31.	Hemiptera	Pyrrhocoridae	*Dysdercus ocreatus*	MH674102	Parali
32.	Hemiptera	Miridae	*Campylomma vendicarina*	MH674101	Edat
33.	Hemiptera	Coreidae	*Cletus schmidti*	KX603656	Calicut University
34.	Lepidoptera	Erebidae	*Ophiusa coronate*	KX503057	Parappanangadi
35.	Lepidoptera	Erebidae	*Mocis proverai*	MH590771	Nilambur
36.	Lepidoptera	Erebidae	*Asota orbona*	KX603654	Ullanam
37.	Lepidoptera	Erebidae	*Asota caricae*	KX587505	Ullanam

Sl. No.	Order	Family	Organism	GenBank Accession No.	Locality
38.	Lepidoptera	Erebidae	*Asota caricae*	KU201286	Parappanangadi
39.	Lepidoptera	Erebidae	*Eudocima cocalus*	KX603659	Munniyoor
40.	Lepidoptera	Erebidae	*Hypocala deflorata*	KX603655	Parappanangadi
41.	Lepidoptera	Noctuidae	*Polytela gloriosae*	KX603662	Parappanangadi
42.	Lepidoptera	Sphingidae	*Acosmeryx anceus subdentata*	MH614375	Parali
43.	Lepidoptera	Sphingidae	*Acosmeryx anceus subdentata*	MH656690	Parappanangadi
44.	Lepidoptera	Crambidae	*Diaphania indica*	KX587508	Malappuram
45.	Lepidoptera	Crambidae	*Glypholes bicolor*	MH674103	Cheruvathur
46.	Lepidoptera	Nymphalidae	*Melanitis leda*	KX587506	Malappuram
47.	Lepidoptera	Xyloryctidae	*Cryptophasa atecmarta*	KX587510	Malappuram
48.	Diptera	Celyphidae	*Spaniocelyphus falcatus*	MH656693	Cheruvathur
49.	Diptera	Celyphidae	*Spaniocelyphus falcatus*	MH590779	Cheruvathur
50.	Diptera	Celyphidae	*Spaniocelyphus pilosus*	MH590773	Payyanur
51.	Diptera	Celyphidae	*Celyphus obtectus*	MH590775	Aluva
52.	Diptera	Tephritidae	*Bactrocera tau*	KX603660	Malappuram
53.	Diptera	Tephritidae	*Bactrocera tau*	MH590778	Cheemeni
54.	Diptera	Anthomyiidae	*Pegomya circumpolaris*	MH590769	Chandragiri

8

Conclusion

- Family Cucurbitaceae is the largest family of climbing plants, contains about 100 genera with 800 species which are mostly tropical or subtropical in distribution, with a few species in temperate climate.

- Among this, the genera *Cucumis*, *Cucurbita*, and *Citrullus* are considered to have high economic importance.

- Cucurbits possess tremendous medicinal properties including anti-HIV, anxiolytic, antipyretic, anti-diarrhoeal, carminative, antioxidant, antidiabetic, antibacterial, laxative, antihelmintic, antitubercular, purgative and hepatoprotective.

- Insects are the most exuberant and diverse group of animal fauna, economically and ecologically important because of their diversity, ecological role, influence on agriculture, human health etc. and are capable to exploit every conceivable environment

- Insect-plant relationship is one of the most dominant and potent biotic interaction and a total 18% damage of world agricultural production are caused merely by the herbivorous insect pests.

- Deciphering the species composition of agricultural pests and accurate identification are crucial for their management strategies. An unambiguous molecular level identification of the pest fauna will facilitate the development of most appropriate IPM strategies for the better management of the insect pest.

- The accurate identification of insect pests in their infectious non-adult stage or morphologically damaged stage using conventional classical taxonomy is a wearisome process.
- The classical taxonomy of most of these pests was well described using morphological characteristics. But the proper identification of many of the Hemipteran and Lepidopteran pests are still tedious as there reported the existence of cryptic and sibling species.
- The most slowly evolving genes like mitochondrial COI, COII and 12S rRNA are the most appropriate markers in fulfilling the putative phylogenetic accuracy in distinguishing the taxa at different taxonomic level.
- The COI sequence of the insect pest is an effective identification tool at every stage of its life cycle. Also, pair-wise distance estimated during the study describes the taxonomic implications for the phylogeographic inferences.
- The present study targetted the collection, molecular level identification using mitochondrial COI gene sequences as marker and phylogenetic analysis of major insect pests from selected cucurbit cultivations in Kerala.
- Altogether 54 voucher specimens from 44 species of insect pests belonging to 4 orders were molecular barcoded using COI gene sequences and the resultant marker sequences were deposited in NCBI GenBank database for worldwide accession and retrieval.
- The universal barcode sequence data will help in discriminating the closely related species and constructing the phylogenetic tree for evaluation of evolutionary relationship between the species.
- The variation among the nucleotides within these collected voucher specimens proved that the nucleotide and amino acid variation are relatively comparable. It implies that the interspecies variation is large compared to intraspecies variation.

- The evaluation of the composition of nucleotides within the COI sequences of 15 species of Coleopteran, 12 species of Hemipteran, 12 species of Lepidopteran and 5 species of Dipteran pests during the present study has revealed that the nucleotides found to be varied within the every third position of the codon.

- The information on the genetic diversity and interspecies relationship of insect pests of the selected cucurbit cultivations from Kerala can be used for tracing its origin, evolutionary history and phylogenic relationship with such other groups from other parts of the world.

- The present study describes the mitochondrial COI gene sequences as the most potent tool for molecular identification and barcoding for the species accuracy and rectification of ambiguously identified insect pests.

References

Abbas, I., Nakamura, K., and Hasyim, A. (1985). Survivorship and fertility schedules of a Sumatran epilachnine" species" feeding on Solanum torvum under laboratory conditions (Coleoptera: Coccinellidae). *Applied entomology and zoology*, *20*(1), 50-55.

Agarwal, V. M., and Rastogi, N. (2008). Deterrent effect of a guild of extrafloral nectary-visiting ant species on Raphidopalpa foveicollis, a major insect pest of sponge gourd, Luffa cylindrica. *Entomologia Experimentalis et Applicata*, *128*(2), 303-311.

Aguiar, A. P., and Santos, B. F. (2010). Discovery of potent, unsuspected sampling disparities for Malaise and Möricke traps, as shown for Neotropical Cryptini (Hymenoptera, Ichneumonidae). *Journal of Insect Conservation*, *14*(2), 199-206.

Ahad, M. A., Roy, M. and Sardar, M. M. A. (1987). Agriculture Entomology (in Bengali). *BAU Campus, Mymensingh*. 201 .

Aizen, M. A., and Feinsinger, P. (1994). Habitat fragmentation, native insect pollinators, and feral honey bees in Argentine'Chaco Serrano'. *Ecological applications*, *4*(2), 378-392.

Akkaya, A., and Uygun, N. (1997, May). Faunistic studies on harmful and beneficial insects on cucurbit vegetables in the Southeastern Anatolian region of Turkey. In *I International Symposium on Cucurbits 492* (pp. 335-340).

Alam, M. Z. (1967). Insect pests of rice in East Pakistan. *Major Insect Pests of the Rice Plant*, 643-655.

Aldrich, J. R., Sant'Ana, J., Bruni, R., and Abdul-Baki, A. A., (1997). Pheromone-induced movement of nymphs of the predator, Podisus maculiventris (Heteroptera: Pentatomidae). *Biological Control*, *10*(2), 123-128.

Aldrich, J. R., Khrimian, A., Chen, X., and Camp, M. J. (2009). Semiochemically based monitoring of the invasion of the brown marmorated stink bug and unexpected attraction of the native green stink bug (Heteroptera: Pentatomidae) in Maryland. *Florida Entomologist*, *92*(3), 483-492.

Allan, J. D. (2004). Landscapes and riverscapes: the influence of land use on stream ecosystems. *Annu. Rev. Ecol. Evol. Syst.*, *35*, 257-284.

Allen, A. L., Wilf, P., Labandeira, C. C., Kress, W. J., Staines, C. L., Windsor, D. M., and Johnson, K. R. (2000). Timing the radiations of leaf beetles: Hispines on gingers from latest Cretaceous to recent. *Science*, *289*(5477), 291-294.

Alonso-Zarazaga, M. A., Anderson, R., Bartolozzi, L., Bezdek, J., Borowiec, L., Caldara, R., ... and Hespenheide, H. (2014). *Morphology and Systematics: Phytophaga*. Walter de Gruyter.

Alston, D. G., and Redding, M. E. (2011). Campylomma (Mullein Plant Bug).

Altaf Hussain Sheikh, Moni Thomas, Rita Bhandari and Khushboo Bunkar (2016) Light Trap and Insect Sampling: an overview *International Journal of Current Research. 8(11),40868-40873.*

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic acids research*, *25*(17), 3389-3402.

Arif, I. A., and Khan, H. A. (2009). Molecular markers for biodiversity analysis of wildlife animals: a brief review. *Animal biodiversity and conservation*, *32*(1), 9-17.

Arnett Jr, R. H. (1968). The beetles of the United States (a manual for identification). The American Entomological Institute. *Ann Arbor, Michigan*, *11*, 12.

Arnheim, R. (1983). *The power of the center: A study of composition in the visual arts*. Univ of California Press.

Ashworth, L., Aguilar, R., Galetto, L., and Aizen, M. A. (2004). Why do pollination generalist and specialist plant species show similar reproductive susceptibility to habitat fragmentation?. *Journal of Ecology*, *92*(4), 717-719.

Avasthi, R. K and Shafee, S. A., (1983). Chalcidoid parasites of pseudococcid pests (Coccoidea) in India. *Verh. SIEEC X. Budapest*, *1983*, 412-415.

Ayberk, H.,Akkuzu, E., and Inac, S. (2007). Hawk moths (Lepidoptera: Sphingidae) of Turkey and their zoogeographical distribution. *Journal of environmental biology*, *28*(4), 723.

Ball, S. L., and Armstrong, K. F. (2006). DNA barcodes for insect pest identification: a test case with tussock moths (Lepidoptera: Lymantriidae). *Canadian Journal of Forest Research*, *36*(2), 337-350.

Ballard, J. W. O., and Whitlock, M. C. (2004). The incomplete natural history of mitochondria. *Molecular ecology*, *13*(4), 729-744.

Baranowski, R. M., and Slater, J. A. (2005). *Lygaeidae of the West Indies*. Agricultural Experiment Station, Institute of Food and Agricultural Sciences, University of Florida.

Baron, S. (1972). The desert locust. *The desert locust.*

Barraclough, T.G.,Hunt, T., Bergsten, J., Levkanicova, Z., Papadopoulou, A., John, O. S., Wild, R., ... and Gómez-Zurita, J. (2007). A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation. *Science*, *318*(5858), 1913-1916.

Basu, P., Aditya, G., and Sanyal, A. K. (2017). Community structure of Coleoptera in Bethuadahari Wildlife Sanctuary, West Bengal, India. *Check List*, *13*(3), 2154.

Bates, D. M., Robinson, R. W., and Jeffrey, C., Eds.(1990) Biology and Utilization of the Cucurbitaceae, Ithaca, NY: Comstock.

Bazin, E., Glémin, S., and Galtier, N. (2006). Population size does not influence mitochondrial genetic diversity in animals. *Science*, *312*(5773), 570-572.

Beckenbach, A. T., and Joy, J. B. (2009). Evolution of the mitochondrial genomes of gall midges (Diptera: Cecidomyiidae): rearrangement and severe truncation of tRNA genes. *Genome biology and evolution*, *1*, 278-287.

Begum, M., Gurr, G. M., Wratten, S. D., and Nicol, H. I. (2004). Flower color affects tri-trophic-level biocontrol interactions. *Biological Control*, *30*(3), 584-590.

Begum, M., Gurr, G. M., Wratten, S. D., Hedberg, P. R., and Nicol, H. I. (2006). Using selective food plants to maximize biological control of vineyard pests. *Journal of Applied Ecology*, *43*(3), 547-554.

Bennet-Clark, H. C. (1984). A particle velocity microphone for the song of small insects and other acoustic measurements. *Journal of experimental biology*, *108*(1), 459-463.

Benson, D. A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., and Sayers, E. W. (2012). GenBank. *Nucleic acids research*, *41*(D1), D36-D42.

Beutel, R. G., and Pohl, H. (2006). Endopterygote systematics–where do we stand and what is the goal (Hexapoda, Arthropoda)? Review. *Systematic Entomology*, *31*(2), 202-219.

Boettner, G. H., Elkinton, J. S., and Boettner, C. J. (2000). Effects of a biological control introduction on three nontarget native species of saturniid moths. *Conservation Biology*, *14*(6), 1798-1806.

Bonte, M., and De Clercq, P. (2008). Developmental and reproductive fitness of Orius laevigatus (Hemiptera: Anthocoridae) reared on factitious and artificial diets. *Journal of Economic Entomology*, *101*(4), 1127-1133.

Boom, R. C. J. A., Sol, C. J., Salimans, M. M., Jansen, C. L., Wertheim-van Dillen, P. M., and Van der Noordaa, J. P. M. E. (1990). Rapid and simple method for purification of nucleic acids. *Journal of clinical microbiology*, *28*(3), 495-503.

Boriani, L., Burgio, G., Marini, M., and Genghini, M. (2005). Faunistic study on butterflies collected in Northern Italy rural landscape. *Bulletin of Insectology*, *58*(1), 49-56.

Bottcher, A., Zolin, J. P., Nogueira-de-Sá, F., and Trigo, J. R. (2009). Faecal shield chemical defence is not important in larvae of the tortoise beetle Chelymorpha reimoseri (Chrysomelidae: Cassidinae: Stolaini). *Chemoecology*, *19*(1), 63-66.

Bouchard, P., Bousquet, Y., Davies, A. E., Alonso-Zarazaga, M. A., Lawrence, J. F., Lyal, C. H., ... and Smith, A. B. (2011). Family-group names in Coleoptera (Insecta). *ZooKeys*, (88), 1.

Boulton-Lewis, G. M. (1995). The SOLO taxonomy as a means of shaping and assessing learning in higher education. *Higher Education Research and Development*, *14*(2), 143-154.

Brothwell, D. and Brothwell, P. (1969). "Patricia, Food in Antiquity: A Survey of the Diet of Early People". Thames and Hudson, London, UK.

Brown, V. K.Basset, Y., Charles, E. and Hammond, D. S. (2001). Short-term effects of canopy openness on insect herbivores in a rain forest in Guyana. *Journal of Applied Ecology*, *38*(5), 1045-1058.

Brues, C. T., Melander, A. L., and Carpenter, F. M. (1954). *Classification of insects*. Cambridge, Mass; USA.

Buschman, L. L., and Whitcomb, W. H. (1980). Parasites of Nezara viridula (Hemiptera: Pentatomidae) and other hemiptera in Florida. *Florida Entomologist*, 154-162.

Butani, D. K., and Jotwani, M. G. (1984). Insect in vegetables periodical. *Expert Book Agency D-42, Vivek Vihar, Dehli (India)*, *6*, 220-236.

Caley, M. J., Fisher, R., and Mengersen, K. (2014). Global species richness estimates have not converged. *Trends in Ecology and Evolution*, *29*(4), 187-188.

Capinera, J. L. (2008). Beet armyworm, Spodoptera exigua (Hübner)(Lepidoptera: Noctuidae). *Encyclopedia of Entomology*, 434-437.

Carapelli, A., Fanciulli, P. P., Frati, F., and Dallai, R. (1995). The use of genetic markers for the diagnosis of sibling species in the genus Isotomurus (Insecta, Collembola). *Italian Journal of Zoology*, *62*(1), 71-76.

Carapezza, A. (1991). Three new species of Miridae collected in Sicily (Insecta, Heteroptera). *Naturalista Siciliano*, *15*, 179-188.

Carpenter, F. M. (1953). The geological history and evolution of insects. *American Scientist*, *41*(2), 256-270.

Carson, W. P., Hovick, S. M., Baumert, A. J., Bunker, D. E., and Pendergast, T. H. (2008). Evaluating the post-release efficacy of invasive plant biocontrol by insects: a comprehensive approach. *Arthropod-Plant Interactions*, *2*(2), 77-86.

Cassis, G., Sanchez, J. A., Cenis, J. L., and Martinez-Cascales, J. I. (2006). Species identity of Macrolophus melanotoma (Costa 1853) and Macrolophus pygmaeus (Rambur 1839)(Insecta: Heteroptera: Miridae) based on morphological and molecular data and bionomic implications. *Insect Systematics and Evolution*, *37*(4), 385-404.

Chakravarty, H. L. (1982). Fascicles of Flora of India: Fascicle 2, Cucurbitaceae. *Howrah: Botanical Survey of India, Botanic Garden*, *36*.

Chandini, P., Kher, F., Paul, D., Jason, C., Pranvera, I., Undine, A., Frithjof, C., Kupper, Dennis, H. and Lynn (2011). Barcoding Tetrahymena: Discriminating Species and identifying unknown using COXI Barcode. *Protist,* 162: 2 - 13.

Chandra, K. (2011). Insect fauna of states and Union Territories in India. *ENVIS Bulletin: Wildlife and Protected Areas. Wildlife Institute of India, Dehradun*, *14*, 189-218.

Chandravadana, M. V., and Pal, A. B. (1983). Triterpenoid feeding deterrent of Raphidopalpa foveicollis L.(red pumpkin beetles) from Momordica charantia L. *Current Science*, *52*(2), 87-88.

Chaudhuri, N., Ghosh, S., Ghosh, J., and Senapati, S. K. (2001). Incidence of insect pests of cabbage in relation to prevailing climatic conditions of Terai region. *Indian journal of entomology*, *63*(4), 421-428.

Chen, H., Chen, Z., and Zhou, Y. (2005). Rice water weevil (Coleoptera: Curculionidae) in mainland China: invasion, spread and control. *Crop protection*, *24*(8), 695-702.

China, W. E., and Miller, N. C. E. (1959). *Check-list and keys to the families and subfamilies of the Hemiptera-Heteroptera*. British Museum (Natural History).

Chinery, M. (1993). *Insects of Britain and Northern Europe*. HarperCollins.

Choudhury, B. (1967, December). Sex modification in cucurbits as affected by gibberellin, auxin and other chemicals. In *Proc. Int. Symp. on Subtropical and Tropical Horticulture* (pp. 383-395).

Clavijo, A., Munroe, E., and Arias, C. (1995). The genus Diaphania Hübner (Lepidoptera: Cramibidae); key to the economically important species. *Agronomia Tropical (Maracay)*, *45*(3), 347-358.

Coll, M. (1998). Living and feeding on plants in predatory Heteroptera. *Predatory Heteroptera: their ecology and use in biological control*, 89-129.

Costa, S. D., Barbercheck, M. E., and Kennedy, G. G. (2000). Sublethal acute and chronic exposure of Colorado potato beetle (Coleoptera: Chrysomelidae) to the δ-endotoxin of Bacillus thuringiensis. *Journal of economic entomology*, *93*(3), 680-689.

Costello, M. J., Wilson, S., and Houlding, B. (2011). Predicting total global species richness using rates of species description and estimates of taxonomic effort. *Systematic Biology*, *61*(5), 871.

Coulson, S. J.,Strathdee, A. T., Bale, J. S., Block, W. C., Hodkinson, I. D., and Webb, N. R. (1993). Effects of temperature elevation on a field population of Acyrthosiphon svalbardicum (Hemiptera: Aphididae) on Spitsbergen. *Oecologia*, *96*(4), 457-465.

Courtney, G. W., and Cranston, P. S. (2015). Order Diptera. In *Thorp and Covich's Freshwater Invertebrates* (pp. 1043-1058). Academic Press.

Cox, M. L. (1976). *The taxonomy and biology of the British Chrysomelidae* (Doctoral dissertation, Newcastle University).

Cywinska, A., Hunter, F. F., and Hebert, P. D. (2006). Identifying Canadian mosquito species through DNA barcodes. *Medical and veterinary entomology*, *20*(4), 413-424.

Daly, T., and Buntin, G. D. (2005). Effect of Bacillus thuringiensis transgenic corn for lepidopteran control on nontarget arthropods. *Environmental Entomology*, *34*(5), 1292-1301.

Darvas, B., and Papp, L. (2000). Exotic dipteran pests in Europe. *Contributions to a Manual of Palaearctic Diptera*, *1*, 693-750.

Datta, M., and Parui, P. (1991). Diptera. *Animal Resources of India: Protozoa to Mammalia. Edited by Director, Zoological Survey of India*, 373-396.

David, K. J., and Ramani, S. (2011). An illustrated key to fruit flies (Diptera: Tephritidae) from Peninsular India and the Andaman and Nicobar Islands. *Zootaxa*, *3021*, 1-31.

Davis, D. R. (1967). A revision of the moths of the subfamily Prodoxinae (Lepidoptera: Incurvariidae).

De Carvalho, M. R., Bockmann, F. A., Amorim, D. S., and Brandao, C. R. F. (2008). Systematics must embrace comparative biology and evolution, not speed and automation. *Evolutionary Biology*, *35*(2), 150-157.

De Meyer, M., Mwatawala, M., Copeland, R. S., and Virgilio, M. (2016). Description of new Ceratitis species (Diptera: Tephritidae) from Africa, or how morphological and DNA data are complementary in discovering unknown species and matching sexes. *European Journal of Taxonomy*, (233).

Descampe, A., Meskens, C., Pasteels, J., Windsor, D., and Hance, T. (2014). Potential and realized feeding niches of neotropical hispine beetles (Chrysomelidae: Cassidinae, Cephaloleiini). *Environmental entomology*, *37*(1), 224-229.

Devasahayam, S. (2000). Insect pests of black pepper. In *Black Pepper* (pp. 336-362). CRC Press Venugoban, K., and Ramanan, A. (2014). Image classification of paddy field insect pests using gradient-based features. *International Journal of Machine Learning and Computing*, *4*(1), 1.

Dhiman, R. K., and Chawla, Y. K. (2005). Herbal medicines for liver diseases. *Digestive diseases and sciences*, *50*(10), 1807-1812.

Dhiman, K., Gupta, A., Sharma, D. K., Gill, N. S., and Goyal, A. (2012). A review on the medicinally important plants of the family Cucurbitaceae. *Asian Journal of Clinical Nutrition*, *4*(1), 16-26.

Dicke, M. (2017). Ecosystem services of insects. In *Insects as food and feed: from production to consumption* (pp. 61-76). Wageningen Academic Publishers.

Ding, J., Fu, W., Reardon, R., Wu, Y., and Zhang, G. (2004). Exploratory survey in China for potential insect biocontrol agents of mile-a-minute weed, Polygonum perfoliatum L., in eastern USA. *Biological Control*, *30*(2), 487-495.

Ding, S., Li, X., Wang, N., Cameron, S. L., Mao, M., Wang, Y., ... and Yang, D. (2015). The phylogeny and evolutionary timescale of muscoidea (Diptera: Brachycera: Calyptratae) inferred from mitochondrial genomes. *PloS one*, *10*(7), e0134170.

Dowd, P. F. (2000). Dusky sap beetles (Coleoptera: Nitidulidae) and other kernel damaging insects in Bt and non-Bt sweet corn in Illinois. *Journal of economic entomology*, *93*(6), 1714-1720.

Dowell, R. V., and Wange, L. K. (1986). Process analysis and failure avoidance in fruit fly programs. In *Pest control: operations and systems analysis in fruit fly management* (pp. 43-65). Springer, Berlin, Heidelberg.

Dupree, M., Bissell, T. L., and Beckham, C. M. (1955). *The pickleworm and its control*. University of Georgia College of Agriculture, Georgia Agricultural Experiment Stations.

Eastop, V. F. (1970). Hemiptera: Homoptera: Aphididae of South Georgia. *Pacific Insects Monograph*, *23*, 227.

Eisner, T., van Tassell, E., and Carrel, J. E. (1967). Defensive use of a" fecal shield" by a beetle larva. *Science*, *158*(3807), 1471-1473.

Esquinas-Alcazar, J. T. (1983). Genetic resources of Cucurbitaceae: a global report.

Esser, K. H., Marx, W. H., and Lisowsky, T. (2006). maxXbond: first regeneration system for DNA binding silica matrices. *Nature Methods*, *3*(1), 68.

Fanello, C., Santolamazza, F., and Della Torre, A. (2002). Simultaneous identification of species and molecular forms of the Anopheles gambiae complex by PCR-RFLP. *Medical and veterinary entomology*, *16*(4), 461-464.

Fapohunda, S. O., Adewumi, A. A., and Jegede, D. O. (2018). Cucurbitaceae-the family that nourishes and heals. *MicroMedicine*, *6*(2), 85-93.

Farrell, B. D., Sequeira, A. S., O'Meara, B. C., Normark, B. B., Chung, J. H., and Jordal, B. H. (2001). The evolution of agriculture in beetles (Curculionidae: Scolytinae and Platypodinae). *Evolution*, *55*(10), 2011-2027.

Felsenstein, J. (1985). Phylogenies and the comparative method. *The American Naturalist*, *125*(1), 1-15.

Ferguson, D. C., and Opler, P. A. (2006). Checklist of the Arctiidae (Lepidoptera: Insecta) of the continental United States and Canada. *Zootaxa*, *1299*(1), 1-33.

Fibiger, M., and Lafontaine, J. D. (2005). *A review of the higher classification of the Noctuoidea (Lepidoptera) with special reference to the Holarctic fauna*. Delta-Dr. und Verlag Peks.

Fletcher, B. S. (1987). The biology of dacine fruit flies. *Annual review of entomology*, *32*(1), 115-144.

Floyd, R. M., Wilson, J. J., and Hebert, P. D. (2009). DNA barcodes and insect biodiversity. *Insect Biodiversity: Science and Society. Oxford: Blackwell Publishing*, 417-431.

Foley, D. A., Sharpe, H. J. and Otte, S. (2007). Membrane topology of the endoplasmic reticulum to Golgi transport factor Erv29p. *Molecular Membrane Biology*, 24(4): 259 – 268

Fox, G. E.Brooks, B. W., Murray, R. G. E., Johnson, J. L., Stackebrandt, E., and Woese, C. R. (1980). Red-pigmented micrococci: a basis for taxonomy. *International Journal of Systematic and Evolutionary Microbiology*, *30*(4), 627-646.

Frati, F., Simon, C., Sullivan, J., and Swofford, D. L. (1997). Evolution of the mitochondrial cytochrome oxidase II gene in Collembola. *Journal of Molecular Evolution*, *44*(2), 145-158.

Furth, D. G., and Suzuki, K. (1990). The metatibial extensor and flexor tendons in Coleoptera. *Systematic Entomology*, *15*(4), 443-448.

Futuyma, D. J., and McCafferty, S. S. (1990). Phylogeny and the evolution of host plant associations in the leaf beetle genus Ophraella (Coleoptera, Chrysomelidae). *Evolution*, *44*(8), 1885-1913.

Gaimari, S. D., and Silva, V. C. (2010). Revision of the Neotropical subfamily Eurychoromyiinae (Diptera: Lauxaniidae). Revisión de la subfamilia Neotropical Eurychoromyiinae (Diptera: Lauxaniidae). *Zootaxa.*, (2342), 1-64.

Gaimari, S. D.,Shi, L. I., and Yang, D. (2017). Five new species of the genus Tetroxyrhina Hendel from China (Diptera, Lauxaniidae). *Zootaxa*, *4247*(3), 246-280.

Garey, J. R., Krotec, M., Nelson, D. R., and Brooks, J. (1996). Molecular analysis supports a tardigrade-arthropod association. *Invertebrate Biology*, 79-88.

Gerbi, S. A. (1986). The evolution of eukaryotic ribosomal DNA. *Biosystems*, *19*(4), 247-258.

Gholamzadeh, S., and Incekara, Ü. (2016). International Journal of Entomological Research. *Int. J. Entomol. Res*, *4*(01), 23-34.

Ghule, T. M., Devi, L. L., and Jha, S. (2014). Seasonal incidence of Epilachna beetle (Henosepilachna septima Dieke) in relation to weather parameters on pointed gourd (Tricosanthes dioica Roxb.). *Journal of Crop and Weed*, *10*(2), 430-432.

Girma, H. Sileshi, G., Schroth, G.and Rao, M. R. (2008). Weeds, diseases, insect pests and tri-trophic interactions in tropical agroforestry. *Ecological basis of agroforestry*, 73-94.

Gómez, J., Barrera, J. F., Rojas, J. C., Macias-Samano, J., Liedo, J. P., Cruz-Lopez, L., and Badii, M. H. (2005). Volatile compounds released by disturbed females of Cephalonomia stephanoderis (Hymenoptera: Bethylidae): A parasitoid of the coffee berry borer Hypothenemus hampei (Coleoptera: Scolytidae). *Florida Entomologist*, *88*(2), 180-188.

Gould, G. E. (1943). Insect pests of cucurbit crops in Indiana. In *Proceedings of the Indiana Academy of Science* (Vol. 53, pp. 165-171).

Gould, G.E., (1944). The biology and control of the striped cucumber beetle. Purdue University Agricultural Experiment Station, Lafayette, Indiana Bul. 490.

Gözüaçık, C., Güllü, M., Konuksal, A., Yücel, A., and Hekimhan, H. (2016, May). Insect Pests of Stored Cereal in Northern Cyprus. In *2nd International Symposium for Agriculture and Food, Ohrid, Former Yugoslav Republic of Macedonia. Accessed* (Vol. 28).

Grazia, J.Matesco, V. C., and Schwertner, C. F., (2008). Immature stages of Chinavia musiva (Berg, 1878): a unique pattern in the morphology of Chinavia Orian, 1965 (Hemiptera, Pentatomidae). *Journal of Natural History*, *42*(25-26), 1749-1763.

Gregory, T. R.,Hernández-Triana, L. M., Chaverri, L. G., Rodriguez-Perez, M. A., Prosser, S. W., Hebert, P. D., and Johnson, N. (2015). DNA barcoding of Neotropical black flies (Diptera: Simuliidae): species identification and discovery of cryptic diversity in Mesoamerica. *Zootaxa*, *3936*(1), 93-114.

Grimaldi, D. A., and Engel, M. S. (2007). Why descriptive science still matters. *BioScience*, *57*(8), 646-647.

Grimaldi, D., Engel, M. S., and Engel, M. S. (2005). *Evolution of the Insects*. Cambridge University Press.

Gullan, P. J.,Ting-Kui, Q., Beattie, G. A. C., Trueman, J. W., Cranston, P. S., Fletcher, M. J., and Sands, D. P. (1994). The current distribution and geographical origin of the scale insect pest Ceroplastes sinensis (Hemiptera: Coccidae). *Bulletin of entomological research*, *84*(4), 541-549.

Gullan, P. J., and Cook, L. G. (2007). Phylogeny and higher classification of the scale insects (Hemiptera: Sternorrhyncha: Coccoidea). *Zootaxa*, *1668*(1), 413-425.

Gullan, P. J., and Cranston, P. S. (2014). *The insects: an outline of entomology*. John Wiley and Sons.

Gupta D, Verma AK (1992). Population fluctuations of the maggots of fruit flies Dacus cucurbitae Coquillett and D. tau (Walker) infesting cucurbitaceous crops. Adv. Pl. Sci. 5: 518-523.

Gurule, S. A. (2013). *Taxonomic Study of Moths (Lepidoptera Heterocera) from North Maharashtra (India)* (Doctoral dissertation, Thesis submitted to University of Pune, Pune).

Habeck D.H. (2002) Nitidulidae, in: American Beetles, Vol. 2, Arnett R.H., Thomas M.C., Skelley P.E., Frank J.H. (Eds.), CRC Press, Boca Raton, 311–315.

Hajibabaei, M., dewaard, J. R., Ivanova, N. V., Ratnasingham, S., Dooh, R. T., Kirk, S. L., ... and Hebert, P. D. (2005). Critical factors for assembling a high volume of DNA barcodes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*(1462), 1959-1967.

Hammood, P.M. (1995). "The Current Magnitude of Biodiversity, in Global Biodiversity assessment", V.H. Heywood; Ed., pp. 113-138, Cambridge University Press, Cambridge, UK.

Hampson, G. F. (1892). The Fauna of British India including Ceylon and Burma-Moths Vol. 1,(eds.) Blanford, WT.

Han, H. Y., and Ro, K. E. (2005). Molecular phylogeny of the superfamily Tephritoidea (Insecta: Diptera): new evidence from the mitochondrial 12S, 16S, and COII genes. *Molecular Phylogenetics and Evolution*, *34*(2), 416-430.

Handfield, L. (1999). Le guide de papillons du Québec. Vol. 1. *Boucherville, QC: Broquet*.

Harry, M. (1993). Use of the median process of the pygophore in the identification of Rhodnius nasutus, R. neglectus, R. prolixus and R. robustus (Hemiptera: Reduviidae). *Annals of Tropical Medicine and Parasitology*, *87*(3), 277-282.

Hassouna, N., Mithot, B., and Bachellerie, J. P. (1984). The complete nucleotide sequence of mouse 28S rRNA gene. Implications for the process of size increase of the large subunit rRNA in higher eukaryotes. *Nucleic Acids Research*, *12*(8), 3563-3583.

Hebert, P. D., Cywinska, A., Ball, S. L., and Dewaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1512), 313-321.

Hebert, P. D., Cywinska, A., and Ball, S. L. (2003a). JR deWaard. 2003. Biological identifications through DNA barcodes. *Proc. R. Soc. Lond. B*, *270*(1512), 313-321.

Hebert, P. D., Stoeckle, M. Y., Zemlak, T. S., and Francis, C. M. (2004). Identification of birds through DNA barcodes. *PLoS biology*, *2*(10), e312.

Hem, S. P., and Mohán, S. (1950). Pests of stored Grain and their Control. *Indian Journal of Agricultural Science*, *18*(pt. 4).

Hennings, E. (1966). Zur histologie und funktion von einstichen der schwarzen bohnenlaus (Aphis Fabae Scop.) in Vicia Faba-Pflanzen. *Journal of Insect Physiology*, *12*(1), 65-76.

Henning, W. (1973). Diptera (Zweifliiger). *Handbuch der Zoologie. IV*, *4*(2), 2.

Henning, R. J., Allison, A. H., and Tripp, L. D. (1982). Cultural practices. *Peanut science and technology*, 123-138.

Henry, T. J. (2009). Biodiversity of Heteroptera. *Insect biodiversity: science and society*, *1*, 223-263.

Herdt, R. W. (1987). Equity considerations in setting priorities for Third World rice biotechnology research. *Dev. Seeds Change*, *4*, 19-24.

Heu, R.A., Hamasaki, R.T., Yalemar, J.A., Sugano, J.S. (2016). Pickleworm: *Diaphania nitidalis* Cramer.

Hieke F., (1968), Urania Tierreich. Berlin, S.267-271.

Higashi, T., Takeda, M., and Momoi, S. (2006). Inhibition and Termination of Larval Diapause and Low-Temperature'l'olerance in the Cigarette Beetle, Lasioderma serricorne (Coleoptera: Anobiidae).*17*(2), 45-51.

Hirano, M. C., Owusu, E. O.,and Horiike, (1994). Population parameters of the cotton aphid, Aphis gossypii, Glover (Homoptera: Aphididae) infesting cucumber. *Journal of horticultural science*, *69*(4), 731-734.

Hogg, I. D., and Hebert, P. D. (2004). Biological identification of springtails (Hexapoda: Collembola) from the Canadian Arctic, using mitochondrial DNA barcodes. *canadian Journal of Zoology*, *82*(5), 749-754.

Hollingsworth, R. G., and Allwood, A. J. (2000). *Melon fly*. Plant Protection Service, Secretariat of the Pacific Community.

Hövemeyer, K. (2000). Ecology of Diptera. *Contributions to a Manual of Palaearctic Diptera (with special reference to flies of economie importance)*, *1*, 437-489.

Hunt, T., Bergsten, J., Levkanicova, Z., Papadopoulou, A., John, O. S., Wild, R., ... and Gómez-Zurita, J. (2007). A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation. *Science*, *318*(5858), 1913-1916.

Hurst, G. D., and Jiggins, F. M. (2005). Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1572), 1525-1534.

Islam, K., Islam, M. S., and Ferdousi, Z. (2011). Control of Epilachna vigintioctopuntata Fab.(Coleoptera: Coccinellidae) using some indigenous plant extracts. *Journal of Life and Earth Science*, *6*, 75-80.

Isley, D., (1927). The striped cucumber beetle. University of Arkansas Agricultural Experiment Station, Fayetteville, Arkansas Bul. 216.

Isley, D., (1929). The southern corn rootworm. University of Arkansas Agricultural Experiment Station, Fayetteville, Arkansas Bul. 232

Jackman, J. A., and Drees, B. M. (1998). *A field guide to common Texas insects*. Taylor Trade Publishing.

Jalali, S., Kapoor, S., Sivadas, A., Bhartiya, D., and Scaria, V. (2015). Computational approaches towards understanding human long non-coding RNA biology. *Bioinformatics*, *31*(14), 2241-2251.

James, D. G., Faulder, R. J., and Bartelt, R. J. (1995). Fauna and Seasonal Abundance of Carpophilus spp.(Coleoptera: Nitidulidae) in Four Stone Fruit Growing Regions of Southeastern Australia as Determined by Pheromone-trapping. *Australian Journal of Entomology*, *34*(4), 327-333.

Jankielsohn, A. (2018). The Importance of Insects in Agricultural Ecosystems. *Advances in Entomology*, *6*(02), 62.

Jankielsohn, A. (2018). The Importance of Insects in Agricultural Ecosystems. *Advances in Entomology*, *6*(02), 62.

Jansen, R. C. (1993). Interval mapping of multiple quantitative trait loci. *Genetics*, *135*(1), 205-211.

Janzen, F. J., and Phillips, P. C. (2006). Exploring the evolution of environmental sex determination, especially in reptiles. *Journal of evolutionary biology*, *19*(6), 1775-1784.

Jeffrey, C. (2005). A new system of Cucurbitaceae. Botaniske Zhurnal. 90, 332-335.

Jindra, M., Palli, S. R., and Riddiford, L. M. (2013). The juvenile hormone signaling pathway in insect development. *Annual review of entomology*, *58*, 181-204.

Johnson, S. N., Gregory, P. J., Murray, P. J., Zhang, X., and Young, I. M. (2004). Host plant recognition by the root feeding clover weevil, Sitona lepidus (Coleoptera: Curculionidae). *Bulletin of entomological research*, *94*(5), 433-439.

Jolivet, P., Jolivet, P.,Beenen, R., Santiago-Blay, J., and Schmitt, M. (2008). Classification and habitat of brachelytrous Chrysomelidae (Coleoptera). *Research on Chrysomelidae*, *1*, 161-173.

Jones, V. P., and Caprio, L. C. (1994). Southern green stink bug (Hemiptera: Pentatomidae) feeding on Hawaiian macadamia nuts: the relative importance of damage occurring in the canopy and on the ground. *Journal of economic entomology*, *87*(2), 431-435.

Joseph, T.,Shanas, S., Job, J., and Anju Krishnan, G. (2016). First report of the invasive rugose spiraling whitefly, Aleurodicus rugioperculatus Martin (Hemiptera: Aleyrodidae) from the old world. *Entomon*, *41*(4), 365-368.

Jurado-Rivera, J. A., Reid, C. A., and Beatson, M. (2009). A new genus of Chrysomelinae from Australia (Coleoptera: Chrysomelidae). *Zootaxa*, *2207*, 53-66.

Kalaichelvan, T., and Verma, K. K. (2005). Checklist of leaf beetles (Coleoptera: Chrysomelidae) of Bhilai-Durg. *Zoos' Print Journal*, *20*(4), 1838-1842.

Kalshoven, L. G. E. (1981). Pests of Crops in Indonesia (PA van der Laan, rev. and transl.). 701pp. *PT lchtiar Barn-Van Hoeve, Jakarta*.

Kambhampati, S. (1995). A phylogeny of cockroaches and related insects based on DNA sequence of mitochondrial ribosomal RNA genes. *Proceedings of the National Academy of Sciences*, USA, 92: 2017 – 2020.

Kambhampati, S. (1996). Phylogenetic relationship among cockroach families inferred from mitochondrial 12SrRNA gene sequence. *Systematic Entomology*, 21: 89 – 98.

Kaur, H., and Sharma, K. (2017). COI-based DNA barcoding of some species of Pentatomidae from North India (Hemiptera: Heteroptera). *Mitochondrial DNA Part A*, *28*(5), 756-761.

Kearns, C. A., Inouye, D. W., and Waser, N. M. (1998). Endangered mutualisms: the conservation of plant-pollinator interactions. *Annual review of ecology and systematics*, *29*(1), 83-112.

Kent, W. J.,Bejerano, G., Pheasant, M., Makunin, I., Stephen, S., Mattick, J. S., and Haussler, D. (2004). Ultraconserved elements in the human genome. *Science*, *304*(5675), 1321-1325.

Khan, M. Z., and Hajela, K. P. (1987). Studies on Aulacophora foveicollis Lucas (Coleoptera: Chrysomelidae)-food preference and extent of damage. *Indian Journal of Entomology*, *49*(4), 457-459.

Kiritshenko, A. N. (1916). Coreidae: Coreinae. *Fauna Rossii i sopredel'nykh stran, Nasekomye poluzhestkokrylye (Insecta, Hemiptera)*, *6*(2).

Kitching, I. J., and Rawlins, J. E. (1999). The Noctuoidea,[in] NP Kristensen, Lepidoptera Moths and Butterflies, volume 1: Evolution, Systematics and Biogeography,[in] M. Fischer, Arthropoda: Insecta, 35, Volume IV, 355-401.

Kitching, I. J., Rougerie, R., Zwick, A., Hamilton, C. A., St Laurent, R. A., Naumann, S., ... and Kawahara, A. Y. (2018). A global checklist of the Bombycoidea (Insecta: Lepidoptera). *Biodiversity data journal*, (6).

Knight, H. H. (1917). A revision of the genus Lygus as it occurs in America north of Mexico, with biological data on the species from New York. *Bull. Cornell Univ. agric. Exp. Stn.*, *391*, 556-645.

Knight, H. H. (1941). The plant bugs, or Miridae, of Illinois. *Illinois Natural History Survey Bulletin; v. 022, no. 01*.

Kóbor, P. (2018). Geocoris margaretarum: description of a new species from the Oriental region with remarks on allied taxa (Heteroptera: Lygaeoidea: Geocoridae). *Raffles Bulletin of Zoology*, *66*, 580-586.

Konstantinov, A. S., and Prathapan, K. D. (2008). New generic synonyms in the Oriental flea beetles (Coleoptera: Chrysomelidae). *The Coleopterists Bulletin*, *62*(3), 381-419.

Korneyev, V. A. (2006). A revision of the quadratula group of the genus Terellia Robineau-Desvoidy (Diptera: Tephritidae). *Isr J Zool*, 35-36.

Krischik, V. A., and Denno, R. F. (1990). Patterns of growth, reproduction, defense, and herbivory in the dioecious shrub Baccharis halimifolia (Compositae). *Oecologia*, *83*(2), 182-190.

Kristensen, N. P. (1975). The phylogeny of hexapod “orders”. A critical review of recent accounts. *Journal of Zoological Systematics and Evolutionary Research*, *13*(1), 1-44.

Kristensen, N. P. (1981). Phylogeny of insect orders. *Annual review of entomology*, *26*(1), 135-157.

Kristensen, N. P. (1991). Phylogeny of extant hexapods. *The insects of Australia*, *2*, 125-140.

Kristensen, N. P. (1995). Forty years insect phylogenetic systematics. Hennig's. *Zool. Beitr. NF*, (36), 83-124.

Kristensen, N. P. (1998). 5. The homoneurous glossata.—Pp. 51–63 in: Kristensen, NP (ed.), Lepidoptera, moths and but ter flies, 1: Evolution, systematics, and biogeography. *Fi scher, M.(serial ed.), Handbook of Zoology*, *4*, 35.

Kristensen, N. P., and Skalski, A. W. (1998). Phylogeny and palaeontology. *Handbook of zoology*, *4*(Part 35), 7-25.

Kristensen, N. P. (1999). Lepidoptera, moths and butterflies. Vol. 1. Evolution, systematics and biogeography. *Handbook of zoology*, *4*, 1-491.

Kumar, M., Gromiha, M. M., and Raghava, G. P. (2007). Identification of DNA-binding proteins using support vector machines and evolutionary profiles. *BMC bioinformatics*, *8*(1), 463.

Lahiri, A. K., and Mitra, B. (2000). Insecta: Diptera. *Fauna of Gujarat: Invertebrates*, *2*, 127.

Lawrence, J. F., and Britton, E. B. (1994). *Australian beetles*. Melbourne University Press.

Lawrence, J. F., Ślipiski, A., Seago, A. E., Thayer, M. K., Newton, A. F., and Marvaldi, A. E. (2011, March). Phylogeny of the Coleoptera based on morphological characters of adults and larvae. In *Annales zoologici* (Vol. 61, No. 1, pp. 1-217). Museum and Institute of Zoology, Polish Academy of Sciences.

Lawrence, J., and Slipinski, A. (2013). *Australian beetles volume 1: morphology, classification and keys* (Vol. 1). CSIRO publishing.

Leach, J. G. (1940). Insect transmission of plant diseases. *Insect transmission of plant diseases.*

Leblanc, L., Fay, H., Sengebau, F., San Jose, M., Rubinoff, D., and Pereira, R. (2015). A survey of fruit flies (Diptera: Tephritidae: Dacinae) and their Opiine parasitoids (Hymenoptera: Braconidae) in Palau.

Lee, C. Y. (2002). Subterranean termite pests and their control in the urban environment in Malaysia. *Sociobiology*, *40*(1), 3-10.

Lee, W., Park, J., Lee, G. S., Lee, S., and Akimoto, S. I. (2013). Taxonomic status of the Bemisia tabaci complex (Hemiptera: Aleyrodidae) and reassessment of the number of its constituent species. *PLoS One*, *8*(5), e63817.

Lee, C. F., and Beenen, R. (2015). Revision of the genus Aulacophora from Taiwan (Coleoptera: Chrysomelidae: Galerucinae). *Zootaxa*, *3949*(2), 151-190.

Li, C. S. (1961). The Epilachninae of Taiwan (Col., Coccinellidae). *Pacif. Ins.*, *3*, 31-91.

Lira-Saade, R. (1995). *Estudios taxonomicos ecogeograficos de las Cucurbitaceae Latinoamericanas de importancia economica* (No. SB351. C8. L57 1995.). International Plant Genetic Resources Institute.

Liu, H., and Beckenbach, A. T. (1992). Evolution of the mitochondrial cytochrome oxidase II gene among 10 orders of insects. *Molecular phylogenetics and evolution*, *1*(1), 41-52.

Liu, Q. N., Xin, Z. Z., Bian, D. D., Chai, X. Y., Zhou, C. L., and Tang, B. P. (2016). The first complete mitochondrial genome for the subfamily Limacodidae and implications for the higher phylogeny of Lepidoptera. *Scientific reports*, *6*, 35878.

Lokeshwari, R. K., and Shantibala, T. (2010). A review on the fascinating world of insect resources: reason for thoughts. *Psyche: A Journal of Entomology*, *2010*.

Losey, J. E., and Vaughan, M. (2006). The economic value of ecological services provided by insects. *Bioscience*, *56*(4), 311-323.

Lu, J., Ho, D. M., Vogelaar, N. J., Kraml, C. M., Bernhard, S., Byrne, N., ... and Pascal, R. A. (2006). Synthesis, structure, and resolution of exceptionally twisted pentacenes. *Journal of the American Chemical Society*, *128*(51), 17043-17050.

Mabberley, D. J. (2008). *Mabberley's plant-book: a portable dictionary of plants, their classifications and uses* (No. Ed. 3). Cambridge University Press.

Malaise, R. (1937). A new insect trap. *Entomol. Tidskr*. 58:148-160

Manktelow, M., and Nyberg, K. (2005). Linnæus' apostles and the development of the Species Plantarum.

Marinoni, R. C., and Dutra, R. R. C. (1997). Famílias de Coleoptera capturadas com armadilha malaise em oito localidades do Estado do Paraná, Brasil. Diversidades alfa e beta. *Revista Brasileira de Zoologia*, *14*(3), 751-770.

Marrelli, M. T., Floeter-Winter, L. M., Malafronte, R. D. S., Tadei, W. P., Lourenço-de-oliveira, R., Flores-Mendoza, C., and Marinotti, O. (2005). Amazonian malaria vector anopheline relationships interpreted from ITS2 rDNA sequences. *Medical and veterinary entomology*, *19*(2), 208-218.

Marshall, K. E., and Sinclair, B. J. (2012). The impacts of repeated cold exposure on insects. *Journal of Experimental Biology*, *215*(10), 1607-1613.

Martin, J., Kuvangkadilok, C., Peart, D. H., and Lee, B. T. (1980). Multiple sex determining regions in a group of related Chironomus species (Diptera: Chironomidae). *Heredity*, *44*(3), 367.

Marvaldi, A. E., Duckett, C. N., Kjer, K. M., and Gillespie, J. J. (2009). Structural alignment of 18S and 28S rDNA sequences provides insights into phylogeny of Phytophaga (Coleoptera: Curculionoidea and Chrysomeloidea). *Zoologica Scripta*, *38*(1), 63-77.

Marwaha, K.K., Siddiqui, K.H, Singh, J.P. reprinted (1998). Handbook of crop pest control., 37-39.

Marwaha, K.K., Siddiqui, K.H, Singh, J.P. reprinted (1998). Handbook of crop pest control., 37-39.

Mason, J. M., Randall, T. A., and Frydrychova, R. C. (2016). Telomerase lost?. *Chromosoma*, *125*(1), 65-73.

Mathew and Koshy. (1986). On a collection of Pentatomidae (Hemiptera) from Silent Valley, Kerala, India. *Records of Zoological Survey of India*, *84*(1-4), 35-47.

May, R. M. (1990). How many species?. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *330*(1257), 293-304.

Mayer, C.,Peters, R. S., Meusemann, K., Petersen, M., Wilbrandt, J., Ziesmann, T., ... and Aberer, A. (2014). The evolutionary history of holometabolous insects inferred from transcriptome-based phylogeny and comprehensive morphological data. *BMC evolutionary biology*, *14*(1), 52.

Maynard, D. Maynard, D.N. (2000). Cucumbers, melons and watermelons. In: Kiple KF, Ornelas KC, eds. The Cambridge world history of food. Cambridge: Cambridge University Press, 298-313.

Maynard, G. V.Stanaway, M. A., Zalucki, M. P., Gillespie, P. S. and Rodriguez, C. M. (2001). Pest risk assessment of insects in sea cargo containers. *Australian journal of Entomology*, *40*(2), 180-192.

Mazón, M., and Bordera, S. (2008). Effectiveness of two sampling methods used for collecting Ichneumonidae (Hymenoptera) in the Cabañeros National Park (Spain). *European Journal of Entomology*, *105*(5), 879.

McAlpine, J. F., Peterson, B. V., Shewell, G. E., Teskey, H. J., Vockeroth, J. R., and Wood, D. M. (1981). Manual of Nearctic Diptera. Volume 1. *Manual of Nearctic Diptera. Volume 1.*, (27).

McGhee Jr, G. R., Sheehan, P. M., Bottjer, D. J., and Droser, M. L. (2004). Ecological ranking of Phanerozoic biodiversity crises: ecological and taxonomic severities are decoupled. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *211*(3-4), 289-297.

Mckenna, D. D., Wild, A. L., Kanda, K., Bellamy, C. L., Beutel, R. G., Caterino, M. S., ... and Leschen, R. A. (2015). The beetle tree of life reveals that C oleoptera survived end-P ermian mass extinction to diversify during the C retaceous terrestrial revolution. *Systematic Entomology*, *40*(4), 835-880.

McNamara, J. (1991). Family Scarabaeidae: scarab beetles. *Checklist of beetles of Canada and Alaska. Agriculture Canada, Ottawa*, 145-158.

Medina-Gaud, S., Abreu, E., Gallardo, F., and Franqui, R. A. (1989). Natural enemies of the melonworm, Diaphania hyalinata L.(Lepidoptera: Pyralidae), in Puerto Rico. *The Journal of Agriculture of the University of Puerto Rico*, *73*(4), 313-320.

Medvedev, G. S., and Iwan, D. (2006, December). Notes on the taxonomy of tenebrionid beetles (Coleoptera: Tenebrionidae). In *Annales Zoologici* (Vol. 56, No. 4, pp. 613-621). Museum and Institute of Zoology, Polish Academy of Sciences.

Mehta PK and Sandhu GS (1992) Influence of cucurbitacins on the feeding activity of red pumpkin beetle, *Aulacophora foveicollis* (Lucas). J Insect Sci 5:187–189

Merz, B. (1994). Diptera: Tephritidae Insecta Helvetica Fauna. 10, 1–198.

Metcalf, R. L., and Metcalf, R. A. (1993). Destructive and Useful Insects, 5th edn McGraw Hill: New York. Ślipiński, A., Yu, Y., Reid, C., Shih, C., Pang, H., and Ren, D. (2015). A new longhorn beetle (Coleoptera: Cerambycidae) from the Early Cretaceous Jehol Biota of western Liaoning in China. *Cretaceous Research*, *52*, 453-460.

Meyrick, E. (1914). Descriptions of New Zealand Lepidoptera. In *Transactions and proceedings of the New Zealand Institute*(Vol. 46, pp. 101-118).

Michel A. H., Kornmann, B., Dubrana, K. and Shore, D. (2005). Spontaneous rDNA copy number variation modulates Sir2 levels and epigenetic gene silencing. *Genes Development*, 19(10): 1199 – 210

Michelson, C. (2015). *Effects of agricultural land use on tree swallow (Tachycineta bicolor) reproduction, body condition and diet* (Doctoral dissertation, University of Saskatchewan).

Miller, N. C. E. (1929). Megymenum brevicorne F. Pentatomidae (Hemiptera-Heteroptera). A Minor Pest of Cucurbitaceae and Passifloraceae. *Malayan Agricultural Journal*, *17*(12), 421-430.

Misof, B.Blanke, A., Wipfler, B., Letsch, H., Koch, M., Beckmann, F., Beutel, R., (2012). Revival of Palaeoptera—head characters support a monophyletic origin of Odonata and Ephemeroptera (Insecta). *Cladistics*, *28*(6), 560-581.

Mitchell, P. L.(2000). Leaf-Footed Bugs (Coreidae). – In: Schaefer C., A. Panizzi (Eds.): Heteroptera of Economic Importance. CRC Press, Boca Raton, FL, 337-403.

Mitter, C., Davis, D. R., and Cummings, M. P. (2017). Phylogeny and evolution of Lepidoptera. *Annual review of entomology*, *62*, 265-283.

Mokam, D. G., Djiéto-Lordon, C., and Bilong Bilong, C. F. (2014). Patterns of species richness and diversity of insects associated with cucurbit fruits in the southern part of Cameroon. *Journal of Insect Science*, *14*(1).

Monaghan, P., and Haussmann, M. F. (2006). Do telomere dynamics link lifestyle and lifespan?. *Trends in Ecology and Evolution*, *21*(1), 47-53.

Moran, V. C. (1983). The phytophagous insects and mites of cultivated plants in South Africa: patterns and pest status. *Journal of Applied Ecology*, 439-450.

Morris, R. F. (1951). The importance of insect control in a forest management program. *The Canadian Entomologist*, *83*(7), 176-181.

Morton, T. C., and Vencl, F. V. (1998). Larval beetles form a defense from recycled host-plant chemicals discharged as fecal wastes. *Journal of chemical ecology*, *24*(5), 765-785.

Mott, D. G., Nairn, L. D., and Cook, J. A. (1957). Radial growth in forest trees and effects of insect defoliation. *For. Sci*, *3*(3), 286-304.

Muniappan, R. (2012). *Arthropod pests of horticultural crops in tropical Asia*. CABI.

Nadein, K., Bezděk, J. (2014). Galerucinae Latreille, 1802. In: Handbook of Zoology. Coleoptera, beetles. Morphology and systematics. Volume 3. (Leschen, R.A.B., Beutel, R.G. eds.), 251-259, Walter de Gruyter, Berlin/Boston.

Nadein, K., and Betz, O. (2016). Jumping mechanisms and performance in beetles. I. Flea beetles (Coleoptera: Chrysomelidae: Alticini). *Journal of Experimental Biology*, *219*(13), 2015-2027.

Nagaraju, D. K.,Jayanthi, P. D. K., Verghese, A., and Rani, B. J. (2010). Feeding attractants as a component for integrated management of fruit sucking moth, Eudocima (Othreis) materna (L.)(Lepidoptera: Noctuidae). *Entomon*, *35*(1), 17-22.

Naiman, R. J. (1988). Animal influences on ecosystem dynamics. *BioScience*, *38*(11), 750-752.

Nakano, S., and Katakura, H. (1999). Morphology and biology of a phytophagous ladybird beetle, Epilachna pusillanima (Coleoptera: Coccinellidae) newly recorded on Ishigaki Island, the Ryukyus. *Applied entomology and zoology*, *34*(1), 189-194.

Narendran, T. C. (2001). Taxonomic entomology: Research and education in India. *CURRENT SCIENCE-BANGALORE-*, *81*(5), 445-447.

Narendran, T. C. (2008). Taxonomy and its Relevance. *RESEARCH JOURNAL*, 9.

Nath, P., and Arnowitt, R. (1975). Generalized super-gauge symmetry as a new framework for unified gauge theories. *Physics Letters B*, *56*(2), 177-180.

Nath, P., and Velu, S. (2006). (9) Cucurbitaceous Crops in Asia. *HortScience*, *41*(4), 1016B-1016.

Nault, L. R. (1994). Transmission biology, vector specificity and evolution of planthopper-transmitted plant viruses. In *Planthoppers* (pp. 429-448). Springer, Boston, MA.

Nayar, N. M., and More, T. A. (1998). Cucurbits.

Neupane, F.P., (2002). Pests of crops and their management (Bali biruwaka shatruharu ra tinko roktham). Sajha prakashan. 4th edition,582.

Neupane, F. P., (2003). Status of botanical pesticides in Nepal. In: F. P. Neupane (ed.) Proceedings of NationalSeminar on Integrated Pest Management in Nepal, 25-26 September (2002.).

Nielsen, E. S., and Mound, L. A. (2000). Global diversity of insects: the problems of estimating numbers. *Nature and human society: The quest for a sustainable world*, 213-222.

Norrbom, A. L., Rowley, D. L., Coddington, J. A., Gates, M. W., Ochoa, R. A., Vandenberg, N. J., and Greenstone, M. H. (2007). Vouchering DNA-barcoded specimens: test of a nondestructive extraction protocol for terrestrial arthropods. *Molecular Ecology Notes*, *7*(6), 915-924.

Oerke, E. C. (2006). Crop losses to pests. *The Journal of Agricultural Science*, *144*(1), 31-43.

Ogedengbe, J. D., Hanner, R. H. and Barta, J. R. (2011). DNA barcoding identifies *Eimeria* species and contributes to the phylogenetics of coccidian parasites (Eimeriorina, Apicomplexa, Alveolata). International Journal of Parasitology, 41: 843 – 850

Ogura, Y. (1964). Comparative Morphology and Classification of Plants. Phytomorph 14,240-247.

Oliveira, P. S. (1985). On the mimetic association between nymphs of Hyalymenus spp.(Hemiptera: Alydidae) and ants. *Zoological Journal of the Linnean Society*, *83*(4), 371-384.

Packauskas, R. J. (2010). Catalog of the Coreidae, or leaf-footed bugs, of the New World.

Pandey, P. N. (1977). Host preference and selection of Diaphania indica Saunders (Lep., Pyralidae). *Deutsche Entomologische Zeitschrift*, *24*(1/3), 159-173.

Panizzi, A. R., Fortes, P., Magro, S. R., and Parra, J. R. (2006). Development of a dry artificial diet for Nezara viridula (L.) and Euschistus heros (Fabricius)(Heteroptera: Pentatomidae). *Neotropical Entomology*, *35*(5), 567-572.

Pape, T.,Courtney, G. W., Skevington, J. H., and Sinclair, B. J. (2009). Insect biodiversity: Science and society. *Biodiversity of Diptera*, 185-209.

Pape, T., Blagoderov, V., and Mostovski, M. B. (2011). Order Diptera Linnaeus, 1758. *Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness. Zootaxa*, *3148*(237), 222-229.

Parsons, C. T. (1943). *A revision of Nearctic Nitidulidae (Coleoptera)* (Vol. 92, No. 3). Museum of Comparative Zoölogy.

Patwardhan, A., Ray, S., and Roy, A. (2014). Molecular markers in phylogenetic studies-a review. *Journal of Phylogenetics and Evolutionary Biology*, *2014*.

Pedigo, L.P.,(2002) Entomology and pest management ,Prentice Hall, New york ,NY

Perry, J. N.,Bates, A. J., Sadler, J. P., and Fowles, A. P. (2007). The microspatial distribution of beetles (Coleoptera) on exposed riverine sediments (ERS). *European Journal of Entomology*, *104*(3), 479.

Pevsner, J. (2015). *Bioinformatics and functional genomics*. John Wiley and Sons.

Pfadt, R. E. (1962). Insect pest of corn. *Fundamentals of applied entomology. MacMillan, New York*, 267.

Pimentel, D. (2014). *Biological invasions: economic and environmental costs of alien plant, animal, and microbe species*. CRC press.

Polaszek, A. (2005). A universal register for animal names. *Nature*, *437*(7058), 477.

Polukonova, N. V., and Karmokov, M. K. (2013). Microevolutionary changes in populations of Chironomus nuditarsis Str.(Keyl, 1962)(Chironomidae, Diptera) from central caucasus. *Russian journal of genetics*, *49*(2), 151-157.

Popov, Y. A. (1990). Jurassic bugs (Hemiptera: Heteroptera) from the Museum of Natural History in Vienna/Die jurassischen Wanzen (Hemiptera: Heteroptera) des Naturhistorischen Museums in Wien. *Annalen des Naturhistorischen Museums in Wien. Serie A für Mineralogie und Petrographie, Geologie und Paläontologie, Anthropologie und Prähistorie*, 7-14.

Powell, J. A. (2009). Lepidoptera: moths, butterflies. In *Encyclopedia of insects* (pp. 559-587). Academic Press.

Prathappan, K.D. and A.S. Konstantinov (2003). The flea beetle genus Aphthona Chevrolat (Coleoptera: Chrysomelidae) of Southern India, with descriptions of seven new species. Proceeding of the Entomological Society of Washington 105(1): 154-179.

Pražák, V. (1941). Problém vzniku jednoposchodového domu v Cičmanoch. *Národopisný Sborník, Turócszentmárton*, 23-71.

Pühringer, F., and Kallies, A. (2004). Provisional checklist of the Sesiidae of the world (Lepidoptera: Ditrysia). *Mitteilungen der Entomologischen Arbeitsgemeinschaft Salzkammergut*, *4*, 1-85.

Purrington, F. F., Kendall, P. A., Bater, J. E., and Stinner, B. R. (2017). Alarm pheromone in a gregarious poduromorph collembolan (Collembola: Hypogastruridae). *The Great Lakes Entomologist*, *24*(2), 3.

Purseglove, J. W. (1968). Tropical crops. Dicotyledons 1 and 2. *Tropical crops. Dicotyledons 1 and 2.*

Purseglove, J. W., Brown, E. G., Green, C. L., and Robbins, S. R. J. (1981). *Spices Vol. 2* (pp. 447-813). Longman Group Ltd.

Quaintance, A. L. (1898). *Three injurious insects: bean leaf-roller, corn delphax, canna leaf-roller* (No. 45). Florida Agricultural Experiment Station.

Radin, A. M., and Drummond, F. A. (1994). An evaluation of the potential for the use of trap cropping for control of the striped cucumber beetle, Acalymma vittata (F.)(Coleoptera: Chrysomelidae). *Journal of agricultural entomology (USA)*.

Rajagopal, D., and Trivedi, T. P. (1989). Status, bioecology and management of Epilachna beetle, Epilachna vigintioctopunctata (Fab.)(Coleoptera: Coccinellidae) on potato in India: A review. *International Journal of Pest Management*, *35*(4), 410-413.

Rajasree, R. S., Sibi, P. I., Francis, F., and William, H. (2016). Phytochemicals of Cucurbitaceae family—A review. *IJPPR*, *8*, 113-123.

Raman Chandrasekar,B K Tyagi, Zhang Zheng Gui and Gerald R Reeck, (2014). Short views on Insect Biochemistry and Molecular Biology, I., First Edition, ISBN: 978-1-63315-205-2.

Raven, P. H., Berg, L. R., and Johnson, G. B.(1993). Environment, Ft. Worth, TX: Saunders College Publishing.

Rawat, R. R., and Modi, B. N. (1972). Preliminary study on the biology and natural enemies of tortoise-beetle, Oocassida pundibunda Boh.(Coleoptera: Chrysomelidae: Cassidinae) in Madhya Pradesh. *Indian J Agr Sci*.

Renner, S. S., and Pandey, A. K. (2013). The Cucurbitaceae of India: Accepted names, synonyms, geographic distribution, and information on images and DNA sequences. *PhytoKeys*, (20), 53.

Resh, V. H., and Cardé, R. T. (Eds.). (2009). *Encyclopedia of insects*. Academic press.

Richards, A. M. (1983). The Epilachna vigintioctopunctata complex (Coleoptera: Coccinellidae). *International Journal of Entomology*, *25*(1), 11-41.

Richardson, I. D. (Ed.) 2015. Practical Guide to the Study of Lepidoptera in Africa. Lepidopterists' Society of Africa, online publication.

Rieppel, O. (2004). The language of systematics, and the philosophy of 'total evidence'. *Systematics and Biodiversity*, *2*(1), 9-19.

Robbins, R. K., and Opler, P. A. (1997). Butterfly diversity and a preliminary comparison with bird and mammal diversity. *Biodiversity II, understanding and protecting our biological resources*, 68-82.

Robinson, R. W., and Decker-Walters, D. S. (1997). *Cucurbits*. Cab International.

Rohland, N., Siedel, H., and Hofreiter, M. (2004). Nondestructive DNA extraction method for mitochondrial DNA analyses of museum specimens. *Biotechniques*, *36*(5), 814-821.

Russell, D. W., and Sambrook, J. (2001). *Molecular cloning: a laboratory manual* (Vol. 1, p. 112). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Russo, L., Stehouwer, R., Heberling, J. M., and Shea, K. (2011). The composite insect trap: an innovative combination trap for biologically diverse sampling. *Plos one*, *6*(6), e21079.

Sagarika B, Suvadip S. (2017). Study on the pest complex of bottle gourd in the gangetic plains of West Bengal. *Journal of Entomology and Zoology studies*, 5(2), 725-727

Saitou, N., and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution*, *4*(4), 406-425.

Samways, M. J. (1993). Insects in biodiversity conservation: some perspectives and directives. *Biodiversity and Conservation*, *2*(3), 258-282.

Sanger, F., and Coulson, A. R. (1975). A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *Journal of molecular biology*, *94*(3), 441-448.

Sardana, H. R.,Jhala, R. C., Sisodiya, D. B., Tyagi, A., Patel, Z. P., Jagadale, V. S., ... and Verghese, A. (2005). Laboratory and field effectiveness of tephritid fruit fly baits in Gujarat and Elsewhere in India. *Pest Management in Horticultural Ecosystems*, *11*(2).

Saroli, J. A., Cutler, G. C., and Hillier, N. K. (2016). Morphological Comparison of Altica sylvia Malloch, 1919 and Mantura chrysanthemi (KOCH, 1803)(Coleoptera: Chrysomelidae: Galerucinae: Alticini), with a Focus on Sexual Dimorphism. *The Coleopterists Bulletin*, *70*(4), 892-903.

Sarwar, M. (2014). b. Some Insect Pests (Arthropoda: Insecta) of Summer Vegetables, Their Identification, Occurrence, Damage and Adoption of Management Practices. *International Journal of Sustainable Agricultural Research*, *1*(4), 108-117.

Schaefer, C. W. (1999). The higher classification of the Alydidae (Hemiptera: Heteroptera). *Entomological Society of Washington (USA).*

Schaefer, C. W. (2004). Key to the genera of new world Alydidae (Hemiptera: Heteroptera). *Proceedings of the Entomological Society of Washington*.

Schaefer, C. W., and Mitchell, P. L. (1983). Food plants of the Coreoidea (Hemiptera: Heteroptera). *Annals of the Entomological Society of America*, *76*(4), 591-615.

Schaefer, C. W., and Panizzi, A. R. (Eds.). (2000). *Heteroptera of economic importance*. CRC press.

Schoonhoven, L. M., Van Loon, B., van Loon, J. J., and Dicke, M. (2005). *Insect-plant biology*. Oxford University Press on Demand.

Schuh, R. T., and Slater, J. A. (1995). *True bugs of the world (Hemiptera: Heteroptera): classification and natural history*. Cornell University Press.

Schuh, R. T., Weirauch, C., and Wheeler, W. C. (2009). Phylogenetic relationships within the Cimicomorpha (Hemiptera: Heteroptera): a total-evidence analysis. *Systematic Entomology*, *34*(1), 15-48.

Schuman, M. C., Kessler, D., and Baldwin, I. T. (2013). Ecological observations of native Geocoris pallens and G. punctipes populations in the Great Basin Desert of southwestern Utah. *Psyche: A Journal of Entomology*, *2013*.

Schwägerl, C. (2016). What's Causing the Sharp Decline in Insects, and Why It Matters. *Yale Environment*, *360*(6).

Scoble, M. J. (1992). *The Lepidoptera. Form, function and diversity*. Oxford University Press.

Scoble, M. J.,Gaston, K. J., and Crook, A. (1995). Patterns in species description: a case study using the Geometridae (Lepidoptera). *Biological Journal of the Linnean Society*, *55*(3), 225-237.

Seago, A. E., Giorgi, J. A., Li, J., and Ślipiński, A. (2011). Phylogeny, classification and evolution of ladybird beetles (Coleoptera: Coccinellidae) based on simultaneous analysis of molecular and morphological data. *Molecular Phylogenetics and Evolution*, *60*(1), 137-151.

Sheeba, M., and Narendran, T. C. (2008). A new species of the genus Phanerotoma Wesmael (Braconidae: Cheloninae) with a key to Indian species. *Journal of Bio-Science*, *16*, 11-14.

Sheets-Pyenson, S. (1981). Darwin's data: His reading of natural history journals, 1837–1842. *Journal of the History of Biology*, *14*(2), 231-248.

Shendure, J., Mitra, R. D., Varma, C., and Church, G. M. (2004). Advanced sequencing technologies: methods and goals. *Nature Reviews Genetics*, *5*(5), 335.

Shere-Kharwar, A. S., Magdum, S., Khedkar, G. D., Gupta, S., and Zambare, V. (2013). Moth legs: Excellent source of tissue for DNA extraction (Lepidoptera: Noctuidae). *Indian Journal of Life Sciences*, *2*(2), 35.

Shrestha, S., Abdul, M. A., Sangwon, K., Minsoo, K., Dongkyun, L. and Yongyun (2009). Diagnostic molecular markers of six lepidopterans insect pests infesting apples Korea. *Journal of Asia-Pacific Entomology*, 12: 107 – 111.

Shrivastava, A., and Roy, S. (2013). Cucurbitaceae: A ethnomedicinally important vegetable family. *Journal of Medicinal Plants*, *1*(4).

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., and Flook, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the entomological Society of America*, *87*(6), 651-701.

Singh, S. K., Kumar, D., and Ramamurthy, V. V. (2010). Biology of Bactrocera (Zeugodacus) tau (Walker)(Diptera: Tephritidae). *Entomological Research*, *40*(5), 259-263.

Singh, B., and Wells, J. D. (2013). Molecular systematics of the Calliphoridae (Diptera: Oestroidea): evidence from one mitochondrial and three nuclear genes. *Journal of Medical Entomology*, *50*(1), 15-23.

Skevington, J. H., and Dang, P. T. (2002). Exploring the diversity of flies (Diptera). *Biodiversity*, *3*(4), 3-27.

Slater, J. A. (1972). Lygaeid bugs (Hemiptera: Lygaeidae) as seed predators of figs. *Biotropica*, 145-151.

Slater, J. A., and Baranowski, R. M. (1978). *How to know the true bugs (Hemiptera-Heteroptera)*. William C. Brown Co..

Slater, J. A., and Baranowski, R. M. (1990). Lygaeidae of Florida (Hemiptera: Heteroptera).

Smith, P., Rios, S., Atkinson, K., Mielke, O., and Casagrande, M. (2013). New Paraguayan Specimens and First Confirmed Phenological Data for Catharisa cerina Jordan, 1911 (Lepidoptera: Saturniidae). *Revista Brasileira de Biociências*, *11*(3).

Snodgrass, R. E. (1933). *Morphology of the insect abdomen: the genital ducts and the ovipositor* (No. 595.704 S56 v. 1).

Sohi, A. S. and Mann, J. S. (1987) Illustrated key for identification of Indian species of Empoascini (Cicadellidae, Typhlocybinae). Proceedings of the 6 th Auchenorrhyncha Meeting, 213-230. University of Turin Press.

Srivastava, C., and Subramanian, S. (2016). Storage insect pests and their damage symptoms: an overview. *Indian Journal of Entomology*, *78*(special), 53-58.

Stål, C. (1867) Bidrag till Hemipterernas Systematik. Öfversigt af Kongliga Vetenskaps-Akademiens Förhandlingar 24(7), 491–560.

Strong, D. R., Lawton, J. H., and Southwood, S. R. (1984). *Insects on plants. Community patterns and mechanisms*. Blackwell Scientific Publicatons.

Subbulakshmi, V., Yadava, N. D., Soni, M. L., Sheetal, K. R., and Renjith, P. S. (2017). Colophospermum mopane–A Potential Host for Rearing Wild Silk Worm (Gonometa rufobrunnea) in Arid Rajasthan.

Subrahmanyam, N. S. (2004). Modern plant taxonomy. Vikas Publishing House Pvt. Ltd. New Delhi, India,316-321.

Sun, J. T., Li, J. B., Yang, X. M., and Hong, X. Y. (2012). Development and characterization of nine polymorphic microsatellites for the small brown planthopper Laodelphax striatellus (Hemiptera: Delphacidae). *Genet. Mol. Res*, *11*, 1526-1531.

Sweet, M. H. (1960). The seed bugs: a contribution to the feeding habits of the Lygaeidae (Hemiptera: Heteroptera). *Annals of the Entomological Society of America*, *53*(3), 317-321.

Sweet, M. H. (2000). Seed and chinch bugs (Lygaeoidea). *Heteroptera of economic importance. CRC Press, Boca Raton, FL*, 143-264.

Szentkirályi, F. (2002). Fifty-year-long insect survey in Hungary: T. Jermy's contributions to light-trapping. *Acta Zoologica Academiae Scientiarum Hungaricae*, *48*(Suppl 1), 85-105.

Tamaki, G., and Weeks, R. E. (1972). Tech. Bull. US Dep. Agric. agric. Res. Serv.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular biology and evolution*, *30*(12), 2725-2729.

Tautz, D., Arctander, P., Minelli, A., Thomas, R. H., and Vogler, A. P. (2002). DNA points the way ahead in taxonomy. *Nature*, *418*(6897), 479.

Tenorio, J. M. (1969). *Diptera: Dolichopodiae: Appendix (Phoridae)..*. University of Hawaii Press.

Tenorio, J. M. (1972). A revision of the Celyphidae (Diptera) of the Oriental Region. *Transactions of the Royal Entomological Society of London*, *123*(4), 359-453.

Thomas, D. B., Holler, T. C., Heath, R. R., Salinas, E. J., and Moses, A. L. (2001). Trap-lure combinations for surveillance of Anastrepha fruit flies (Diptera: Tephritidae). *Florida Entomologist*, *84*(3), 344.

Thomas, D. B., Epsky, N. D., Serra, C. A., Hall, D. G., Kendra, P. E., and Heath, R. R. (2008). Ammonia formulations and capture of Anastrepha fruit flies (Diptera: Tephritidae). *Journal of entomological science*, *43*(1), 76-85.

Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic acids research*, *22*(22), 4673-4680.

Tillman, P. G., and Mullinix, B. G. (2003). Comparison of host-searching and ovipositional behavior of Cardiochiles nigriceps Viereck (Hymenoptera: Braconidae), a parasitoid of Heliothis virescens (Fabricius)(Lepidoptera: Noctuidae), in tobacco and cotton. *Journal of insect behavior*, *16*(4), 555-569.

Torres, J. B., and Boyd, D. W. (2009). Zoophytophagy in predatory Hemiptera. *Brazilian Archives of Biology and Technology*, *52*(5), 1199-1208.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., and Rozen, S. G. (2012). Primer3—new capabilities and interfaces. *Nucleic acids research*, *40*(15), e115-e115.

Van Balen, L. (1976). *The biology of the pickleworm (Diaphania nitidalis (Stoll), Lepidoptera: Pyralidae) in North Carolina* (Doctoral dissertation, North Carolina State University).

van Doesburg, P. H. (1968). *A Revision of the New World Species of Dysdercus Guérin Méneville:(Heteroptera, Pyrrhocoridae)* (Vol. 97). Brill Archive.

van Nieukerken, E. J., Kaila, L., Kitching, I. J., Kristensen, N. P., Lees, D. C., Minet, J., ... and Wahlberg, N. (2011). Order Lepidoptera Linnaeus, 1758. *Zootaxa*, *3148*, 212-221.

Vences, M., Thomas, M., Bonett, R. M., and Vieites, D. R. (2005). Deciphering amphibian diversity through DNA barcoding: chances and challenges. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*(1462), 1859-1868.

Venkatesha, M. G. (2007). Is entomology curriculum affecting insect biodiversity in India? 7. *Current Science*, *93*(8), 1047-1048.

Venugoban, K., and Ramanan, A. (2014). Image classification of paddy field insect pests using gradient-based features. *International Journal of Machine Learning and Computing*, *4*(1), 1.

Vereshchagin, N. K. and Baryshnikov, G. F. (1989). Quaternary mammalian extinctions in northern Eurasia. In Quaternary Extinctions. A Prehistoric Revolution, ed. P. S. Martin and R. G. Klein. Tucson: The University of Arizona Press, 483–516.

Verma, K., and Jolivet, P. (2008). Eumolpinae–a widely distributed and much diversified subfamily of leaf beetles (Coleoptera, Chrysomelidae). *Terrestrial Arthropod Reviews*, *1*(1), 3-37.

Wagele, J. W. (2004). Hennig's phylogenetic systematics brought up to date. *Systematics Association Special Volume*, *67*, 101-126.

Wahlberg, N., Wheat, C. W., and Peña, C. (2013). Timing and patterns in the taxonomic diversification of Lepidoptera (butterflies and moths). *PLOS one*, *8*(11), e80875.

Welbaum, G. E., Wilson, H. T.,and Khan, O. (2014). Chlorophyll Fluorescence in Developing'Top Mark'Cantaloupe (Cucumis melo) Seeds as an Indicator of Quality. *Seed Technology*, 103-113.

Wheeler, D. E., Tuchinskaya, I., Buck, N. A., and Tabashnik, B. E. (2000). Hexameric storage proteins during metamorphosis and egg production in the diamondback moth, Plutella xylostella (Lepidoptera). *Journal of Insect Physiology*, *46*(6), 951-958.

Wheeler, A. G. (2001). *Biology of the plant bugs (Hemiptera: Miridae): pests, predators, opportunists*. Cornell University Press.

Wheeler, W. C., Whiting, M., Wheeler, Q. D., and Carpenter, J. M. (2001). The phylogeny of the extant hexapod orders. *Cladistics*, *17*(2), 113-169.

Wheeler, Q. D., Raven, P. H., and Wilson, E. O. (2004). Taxonomy: impediment or expedient?.

Whitaker, T. W. and Davis, G. N. (1962). Cucurbits - botany, cultivation and utilization. Leonard Hill, London, United Kingdom. 249 pp.

Whitaker, T. W., and Davis, G. N. (1962). Cucurbits. Botany, cultivation, and utilization. *Cucurbits. Botany, cultivation, and utilization.*

White, F. (1983). *The vegetation of Africa* (Vol. 20).

White, I. M. (1988). Tephritid flies. *Handbooks for the identification of British insects*, *10*.

Will, K. W., Mishler, B. D., and Wheeler, Q. D. (2005). The perils of DNA barcoding and the need for integrative taxonomy. *Systematic biology*, *54*(5), 844-851.

Williams, M. A., Rojas, E. S., Batzer, J. C., Beattie, G. A., Fleischer, S. J., Shapiro, L. R.,... and Gleason, M. L. (2015). Bacterial wilt of cucurbits: resurrecting a classic pathosystem. *Plant disease*, *99*(5), 564-574.

Wilson, L. (1989) Introductory remarks of the chair. In *Biological Control: A Sustainable Solution to Crop Pests Problems in Africa*.

Wilson, J., Landry, J. F., Janzen, D., Hallwachs, W., Nazari, V., Hajibabaei, M., and Hebert, P. (2010). Identity of the ailanthus webworm moth (Lepidoptera, Yponomeutidae), a complex of two species: evidence from DNA barcoding, morphology and ecology. *ZooKeys*, *46*, 41.

Wilson, J. M., and Kuhar, T. P. (2017). A survey of the species of squash bug (Hemiptera: Coreidae) egg parasitoids in Virginia and their distribution. *Journal of economic entomology*, *110*(6), 2727-2730.

Yamaguchi, M. (1983). Yam. In *World Vegetables* (pp. 139-147). Springer, Dordrecht.

Yang, J. B., Wang, Y., Wang, J. F., Li, L. L., Wang, M., Yang, L. J., ... and Hou, Y. D. (2017). Development of the forensically important beetle Creophilus maxillosus (Coleoptera: Staphylinidae) at constant temperatures. *Journal of medical entomology*, *54*(2), 281-289.

Yee, W. L., and Alston, D. G. (2012). Behavioral responses, rate of mortality, and oviposition of western cherry fruit fly exposed to malathion, zeta-cypermethrin, and spinetoram. *Journal of Pest Science*, *85*(1), 141-151.

Zaitsev, A. S., Gongalsky, K. B., Persson, T., and Bengtsson, J. (2014). Connectivity of litter islands remaining after a fire and unburnt forest determines the recovery of soil fauna. *Applied soil ecology*, *83*, 101-108.

Zardoya, R. and Meyer, A. (1996). The complete nucleotide sequence of the mitochondrial genome of the lungfish (*Protopterus dolloi*) supports its phylogenetic position as a close relative of land vertebrates. *Genetics*, 142: 1249 – 1263.

Zeven, A. C., and Zhukovsky, P. M. (1975). *Dictionary of cultivated plants and their centres of diversity: excluding ornamentals, forest trees and lower plants*. Pudoc.

Zilli, A., and Pavesi, F. (2015). New or little known Pyraloids from Italy (Lepidoptera: Pyraloidea). *Phegea*, *43*(3), 81-96.

Zwick, A. (2008). Molecular phylogeny of Anthelidae and other bombycoid taxa (Lepidoptera: Bombycoidea). *Systematic Entomology*, *33*(1), 190-209.

Zwick, A.,Cho, S., Regier, J. C., Mitter, C., Cummings, M. P., Yao, J., ... and Davis, D. R. (2011). Can deliberately incomplete gene sample augmentation improve a phylogeny estimate for the advanced moths and butterflies (Hexapoda: Lepidoptera)?. *Systematic biology*, *60*(6), 782-796.

Publications

International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2015): 6.391

Molecular Phylogenetic Study of *Bactrocera tau* using Mitochondrial Cytochrome Oxidase Subunit I Gene Sequence

Priya Bhaskaran K. P[1], Sebastian C.D.[2]

Molecular Biology Laboratory, Department of Zoology, University of Calicut

Abstract: ***Diptera, order of winged insects is commonly known as flies. They are one of the most successful groups of organisms on Earth. They are very diverse biologically and occupies virtually every terrestrial niche. Many have co-evolved in association with plants and other invertebrates. Bactrocera tau is one of the most severe and economically important agricultural pest. B.tau, "devastating pest of cucurbits" is a serious pest in many of the Asian countries. The damage is done by the B.tau larvae that feeds in the fruit. We have developed the phylogenetic reconstruction and analysis of the B. tau (Genbank Accession No. KX603660.1) using mitochondrial cytochrome oxidase subunit I (COI) gene. The knowledge of the dipteran genomic structures will create new method of integrated pest management and will contribute for the sustainable agriculture and maintenance of biodiversity.***

Keywords: *B.tau*, COI, biodiversity, pest, dipteran, cucurbits

1. Introduction

Bactrocera (Zeugodacus) tau (Walker), is one of the serious pests of cucurbitaceae. The larvae are phytophagous. Females deposit eggs in living, healthy plant tissue using their telescopic ovipositors. The larvae find their food upon emerging. The larvae develop in leaves, stems, flowers, seeds, fruits, and roots of the host plant, depending on the species.

This species was found to be of regular occurrence and active during late summer. Pest management tools mainly rely on proper identification of arthropod species. However, keeping in mind the shortcomings and limitations of the conventional taxonomical identification methods of pest identification, DNA barcoding is used. A major feature of DNA barcoding is that it allows prompt identification of pest young instars, as well as of the fragmentary cuticular body parts. Partial DNA sequences of the mitochondrial gene such as Cytochrome oxidase I (COI) and other molecular markers have been used to identify and discover new species. The mitochondrial DNA has been extensively analysed [1] and proven to be an important tool in species delimitation as it possesses biological properties making it suitable as a marker for molecular biodiversity [2] and [3]. DNA barcoding has proved to be a versatile tool with a variety of applications, for example, by facilitating the association between different developmental stages in insects.

The molecular phylogenetic analysis using the mitochondrial COI gene sequences were carried out by many workers in varied group of organisms such as *Culex quinquefasciatus*[4], *Armigeres subalbatus*[5], green bottle fly, *Lucilia sericata*[6], cockroaches[7] and odonates[8].

2. Materials and Method

Bactrocera (Zeugodacus) tau (Walker) used in the present study was collected from Malappuram district in Kerala, India (Figure 1).

Figure 1: *Bactrocera tau*

Mitochondrial genomic DNA was extracted from the experimental insect. The tissue was homogenized using a glass pestle and mortar. The genomic DNA in the homogenate was extracted using a GeNei Ultrapure Mammalian Genomic DNA Prep Kit in accordance to the manufacturer's instructions. About 2 ng of genomic DNA was amplified for mitochondrial cytochrome oxidase subunit I (COI) gene using the forward primer with DNA sequence 5'- GGTCAACAAATCATAAAGATATTGG -3' and reverse primer with DNA sequence 5'- TAAACTTCAGGGTGACCAAAAAATCA -3'. The PCR reaction mixture consisted of 2 ng of genomic DNA, 1µl each forward and reverse primers at a concentration of 2.5 µM, 2.5 µl of dNTPs (2mM), 2.5 µl of 10X reaction buffer, 1.20 µl of Taq polymerase (3U/µl) and 11.8 µl H_2O. The PCR profile consisted of an initial denaturation step of 2 minutes at 95°C, followed by 30 cycles of 5s at 95°C, 45s at 50°C and 45s at 72°C and ending with a final phase of 72°C for 3 minutes. The PCR products were resolved on a 1% TAE-agarose gel, stained with Ethidium Bromide and photographed using a gel documentation system. After ascertaining the PCR amplification of the corresponding COI fragment, the remaining portion of the PCR products were column purified using Mo Bio Ultraclean PCR Clean-up Kit (Mo Bio Laboratories, Inc. California) as per the manufacturer's instructions. The purified PCR products were sequenced from both ends using the forward and reverse primers used for the PCR using Sanger's sequencing method (Sanger, 1975)[9]. The forward and reverse sequences obtained were trimmed for the primer sequences, assembled by using ClustalW and the consensus was taken for the analysis. The nucleotide sequence and peptide sequence were searched for its similarity using BLAST programme of NCBI (www.ncbi.nlm.nih.gov/) and Inter and intra specific genetic diversity were calculated using Kimura 2-parameter model with the pair wise deletion option and the difference in the nucleotide in codon usage partial COI sequence of *Bactrocera tau* using MEGA6 software.

3. Results and Discussion

The PCR of the COI gene fragment of *Bactrocera tau* yielded product size of 598 bp. The BLAST search using the sequences revealed that the sequences obtained in this study was novel. The evolutionarily close relative of *Bactrocera tau* is *Bactrocera cucurbitae* (Genbank Accession No. DQ116246.1) from Newzealand submitted to NCBI.

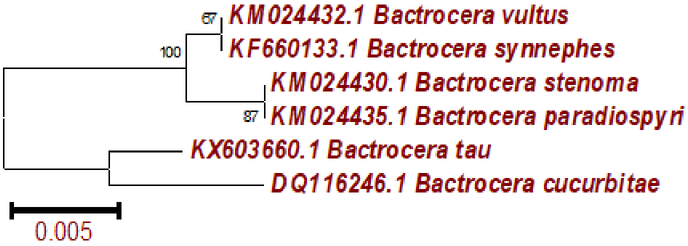


Figure 2: Comparison of phylogenetic status of *Bactrocera tau* using nucleotide

Table 1: The Evolutionary Nucleotide Divergence of *Bactrocera tau* with other related Species

KX603660.1 *Bactrocera tau*	
DQ116246.1 *Bactrocera cucurbitae*	1.1
KM024432.1 *Bactrocera vultus*	3.8
KF660133.1 *Bactrocera synnephes*	3.8
KM024430.1 *Bactrocera stenoma*	4.0
KM024435.1 *Bactrocera paradiospyri*	4.0

The evolutionary history was inferred using the Neighbor-joining method using COI partial sequence. The analysis of the evolutionary history of *Bactrocera tau* was done using the Neighbor- joining method (Figure 2).

4. Conclusions

Variation in the nucleotide is a fundamental property of all living organisms which can be used for the identification and phylogenetic status assessment. The COI sequence obtained in this study showed nucleotide variation between *Bactrocera tau* and *Bactrocera cucurbitae* (Genbank Accession No. DQ116246.1) to be 1.1% (Table. 1). BLAST result concludes that COI gene sequence of *B.tau* was found to be novel. The genetic level identification remains a prospect although COI divergence appear too low to regularity in enabling species diagnosis within the insects. Phylogeny analysis using NJ tree revealed the sharing of common ancestor for various species and is found to be in a diverged clade. The phylogenetically close species of *Bactrocera tau* is *Bactrocera cucurbitae* (Genbank Accession No. DQ116246.1). Inter specific divergence of partial coding fragment of COI gene is very efficient for species identification (Hebert *et al*., 2003)[10].

The present study suggests that the best phylogenetic inferences can be generated through moderately divergent nucleotide database from mitogenomes, among which the COI gene is best studied.

5. Acknowledgements

The financial assistance from Kerala State Council for Science Technology and Environment (KSCSTE) under BLP Research Project is gratefully acknowledged.

References

[1] J.W.O. Ballard, M.C. Whitlock. The incomplete natural history of mitochondria. Mol. Ecol. **13**: 729–744, 2004.

[2] W.S. Moore. Inferring phylogenies from mtDNA variation mitochondrial gene trees versus nuclear gene trees Evolution. **49**: 718–726, 1995.

[3] P.D.N. Hebert, A. Cywinska, S.L. Ball, J.R. de Waard. Biological identifications through DNA barcodes Proc. Biol. Sci. **270**: 313–321, 2003.

[4] K. Rukhsana , K.S. Ashitha, K. Mashoor, C.D. Sebastian. Molecular phylogeny analysis of Southern house mosquito, *Culex quinquefasciatus* (Diptera:Culicidae) derived from mitochondrial DNA sequences. International Journal of Research. **5**: 844-853, 2014.

[5] P.U. Bindu, C.D Sebastian. Genetic structure of mitochondrial cytochrome oxidase subunit I gene of the mosquito, *Armigeres subalbatus*. International Journal of Research. **10**: 49-56, 2014.

[6] K.P.P. Bhaskaran, C.D. Sebastian. Molecular Barcoding of green bottle fly, *Lucilia sericata* (Diptera: Calliphoridae) using CO I gene sequence. Journal of Entomology and Zoology studies, **3**:10-12, 2014.

[7] V.P. Akhilesh, M.P. Femida, C.D. Sebastian. Molecular phylogeny study of *Periplanata fuligimosa* from Lakshadweep Islands, India using cytochrome oxidase subunit I gene sequence, **6**: 382-385, 2015.

[8] E.K.J. Krishnan, C.D. Sebastian. Genetic Variation and Phylogeny Assessment of *Aciagrion occidentale* (Odonata: Coenagrionidae) using Mitochondrial cytochrome oxidase subunit I gene sequence.

International Journal of Science and Research, **4**: 1121-1123, 2015.

[9] F. Sanger, A. R. Coulson. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. Journal of Molecular Biology, **94 (3)**: 441–448, 1975.

[10] P.D.N. Hebert, S. Ratnasingham, J.D. Dewaard. Barcoding animal life, Cytochrome c oxidase subunit I divergences among closely related species. Proceedings of the Royal Society London Biological Science, **270**: 96-99, 2003.

INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

MOLECULAR PHYLOGENETIC ANALYSIS OF *ASOTA ORBONA* AND *ASOTA CARICAE* (LEPIDOPTERA: EREBIDAE) USING MITOCHONDRIAL COI GENE

Priya Bhaskaran K. P [1] and Sebastian C. D [2]*
[1]Research Scholar, Molecular Biology Laboratory, Department of Zoology, University of Calicut, Kerala, India
[2]Assistant Professor, Molecular Biology Laboratory, Department of Zoology, University of Calicut, Kerala, India
*Corresponding Author Email: drcdsebastian@gmail.com

Article Received on: 02/02/17 Approved for publication: 28/03/17

DOI: 10.7897/2230-8407.080446

ABSTRACT

DNA barcoding has become the method of choice for a rapid, reliable, and cost-effective identification of pest species. Since DNA barcoding have proven to be highly efficient in identifying both immatures and adults, it is used to differentiate invasive and native pests. It has been used in managing species complexes in agricultural, IPM systems and also in the cases unpredictable species. Recently, DNA barcoding of partial mitochondrial COI gene is very popular in DNA based identification of various agricultural pest species. The present study investigates the molecular evolution of the *Asota* species using COI gene and its usefulness for reconstructing phylogenetic relationships within and among different *Asota* species.

Keywords: DNA barcoding, phylogenetic evolution, pest, mitochondrial COI gene.

INTRODUCTION

Asota is a genus of noctuoid moths in the Erebidae family (Lepidoptera: Insecta). Insect pests are one of the major concerns for farmers across the world and more than 10,000 species of insects have been recorded damaging the agricultural crops[1]. Detection of pests belonging to different groups is required for the protection of horticultural crops since they cause serious damage to various vegetation's.

In *Asota* species, palpi are upturned, where the second joint reaching vertex of head and third joint slender in variable lengths. In males the antennae is fasciculate but ciliated in females. Forewings with vein 5 is from the lower angle of cell or slightly just above from it. The 6th vein starts from the upper angle or below it. Areole is absent throughout in both. Hindwings have vein 5 from just above lower angle of cell. Veins 6 and 7 emerge from the upper angle.

However, keeping the shortcomings and limitations of the conventional taxonomical identification methods of identification of the pest species, DNA barcoding is used. A major feature of DNA barcoding is that it allows prompt identification of pest during young instars[2,3.] The mitochondrial DNA has been extensively analysed and proven to be an important tool in species delimitation as it possesses biological properties making it suitable as a marker for molecular biodiversity. Fragment size of mitochondrial cytochrome oxidase subunit I (COI) gene has been shown to provide high resolution to identify cryptic species, thereby increasing taxonomy-based biodiversity estimates and its usefulness has been confirmed for identifying Coleoptera[4], Diptera[5,6], Odonata[7], Hemiptera[8,9], Hymenoptera[10] and Lepidoptera[11,12]. DNA barcoding has proved to be a versatile tool with a variety of applications, for example, by facilitating the association between different developmental stages in insects.

MATERIALS AND METHODS

The experimental organisms, *Asota orbona* and *Asota caricae*, were collected from Kannur and Malappuram districts (Kerala: India) respectively. These are morphologically identified by expert consultation and preserved in 70% alcohol.

The genomic DNA in the homogenate was extracted using a GeNei Ultrapure Genomic DNA Prep Kit in accordance to the manufacturer's instructions. About 2 ng of genomic DNA was amplified for mitochondrial cytochrome oxidase subunit I (COI) gene using the specifically designed forward primer with nucleotide sequence 5'-GGTCAACAAATCATAAAGATATTGG-3' and reverse primer with sequence 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'. The PCR reaction mixture consisted of 2ng of genomic DNA, 1µl each forward and reverse primers at a concentration of 2.5 µM, 2.5 µl of dNTPs (2mM), 2.5 µl of 10X reaction buffer, 1.20 µl of Taq polymerase (3U/µl) and 11.8 µl H_2O. The PCR profile consisted of an initial denaturation step of 2 minutes at 95 ^{0}C, followed by 30 cycles of 5s at 95 ^{0}C, 45s at 50 ^{0}C and 45s at 72 ^{0}C and ending with a final phase of 72 ^{0}C for 3 minutes. The PCR products were resolved on a 1% TAE-agarose gel, stained with Ethidium Bromide and photographed using a gel documentation system. After ascertaining the PCR amplification of the corresponding COI fragment, the remaining portion of the PCR products were column purified using Mo Bio Ultraclean PCR Clean-up Kit (Mo Bio Laboratories, Inc. California) as per the manufacturer's instructions. The purified PCR products were sequenced from both ends using the forward and reverse primers used for the PCR using Sanger's sequencing method[13]. The forward and reverse sequences obtained were trimmed for the primer sequences, assembled by using ClustalW and the consensus was taken for the analysis. The nucleotide sequence and peptide sequence were searched for its similarity using BLAST programme of NCBI (www.ncbi.nlm.nih.gov/) and Inter and intra specific genetic diversity were calculated using Kimura 2-parameter model with the pair wise deletion option and the difference in the nucleotide in codon usage partial COI sequence of *A. orbona* and *A. caricae* was analysed using MEGA6 software[14].

RESULTS AND DISCUSSION

The PCR of the COI gene fragment of *Asota orbona* (KX 603654) and *Asota caricae* (KU 201286) yielded product size of 525bp and 532 bp respectively. The BLAST search using the sequences revealed that the sequences obtained in this study was novel. The CO I gene in the mitochondrial genome has been proved to be an excellent source of information for the set of closely related families belonging to the order Lepidoptera. The evolutionary nucleotide divergence of *A. caricae* and *A. orbona* with various other *Asota* species is shown in Table 1.

Variation in the nucleotide is fundamental property of all living organisms which can be used for their identification and phylogenetic status. The average nucleotide frequencies for the species are A = 30.13%, T/U = 38.90%, C = 17.18% and G = 13.79%. The probability of substitution (r) from one base to another was calculated for 12 nucleotide sequences is shown in Table 2.

The evolutionary history was inferred using the Neighbor-joining method using COI partial sequence. The analysis of the evolutionary history of *A. orbona* and *A. caricae* was done using the Neighbor- joining method (Figure 1). Closely related species have 90% similarity in the standardized DNA sequence and distantly related species have less than 90% similarly in the same genus[15, 16].

Table 1: The evolutionary nucleotide divergence of *A. caricae* and *A. orbona* with various other *Asota* species

Organism with Accession No.	% of divergence
KX603654.1 *Asota orbona*	
KC499489.1 *Asota plana*	1.31%
HQ569654.1 *Asota paliura*	2.11%
HQ569644.1 *Asota heliconia clavata*	1.85%
KU201286.1 *Asota caricae*	2.68%
HQ569790.1 *Asota plaginota plaginota*	2.39%
KC499401.1 *Asota darsania*	2.39%
GU662416.1 *Asota albivena*	1.31%
HM395494.1 *Asota albiformis ternatensis*	2.11%
KF549916.1 *Asota eusemioides*	2.66%
GU662413.1 *Asota sulawesiensis*	2.40%
KJ013129.1 *Asota trinacria*	3.24%

Table 2: Maximum composite likelihood estimate of the pattern of nucleotide substitution. Each Entry shows the probability of substitution (r) from one base (Row) to another base (Column)

	A	T	C	G
A	-	2.0772	0.9176	5.2596
T	1.6092	-	22.2339	0.7364
C	1.6092	50.3319	-	0.7364
G	11.4940	2.0772	0.9176	-

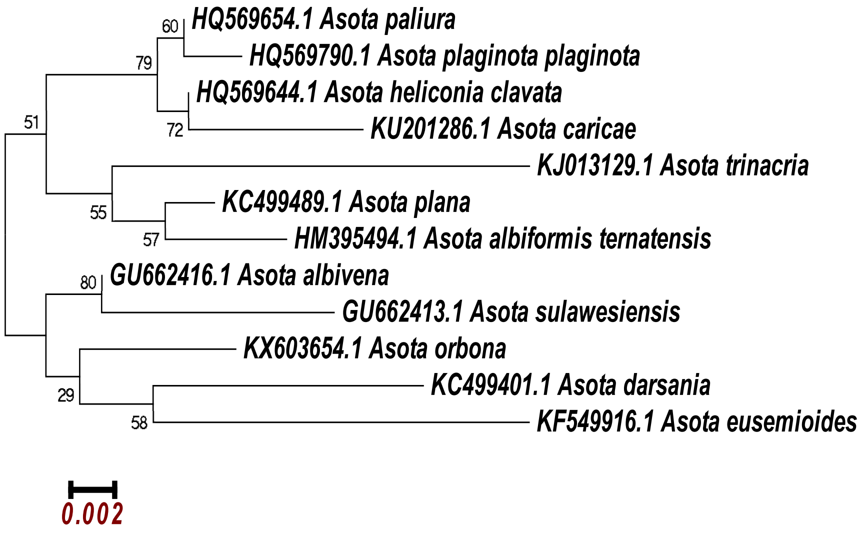


Figure 1: Phylogenetic status of *A. caricae* and *A. orbona* with various other *Asota* species using NJ tree method.

CONCLUSION

Variation within the genomic nucleotide sequence is one of the fundamental properties of all living organisms which can be used as the major criteria for unambiguous molecular level taxonomic identification and phylogenetic status analysis. The mitochondrial COI gene partial sequence obtained in this study showed nucleotide variation between the species *A. caricae* and *A. orbona* as 1.31%. Phylogeny analysis using NJ tree revealed the sharing of common ancestor for various *Asota* species and the two species *A. caricae* and *A. orbona*, is in a diverged clade. The phylogenetically close species of *A. caricae* and *A. orbona* are *A. trinaria* and *A. darsania* respectively. It is concluded that inter specific divergence of specific coding fragment of COI gene is very efficient for accurate species identification.

ACKNOWLEDGEMENT

The financial assistance from Kerala State Council for Science Technology and Environment, Thiruvananthapuram under BLP Research Project is gratefully acknowledged.

REFERENCES

1. Dhaliwal GS, Dhawan AK, Singh R. Biodiversity and ecological agriculture. Issues and Perspectives.Indian Journal of Ecology. 2007; 34(2): 100-109.
2. Foltan P, Sheppard SK, Konvicka M, Symondson WOC. The significance of facultative scavenging in generalist predator nutrition: detecting decayed prey in the guts of predators using PCR. Molecular Ecology. 2005; 14: 4147–4158.
3. Hayashi M, Sota T. Identification of Elmid larvae (Coleoptera: Elmidae) from Sanin District of Honshu, Japan, based on mitochondrial DNA sequences. Entomological Science, 2010; 13: 417–424.
4. Akhilesh VP, Sebastian CD. DNA based phylogenetic analysis of aquatic beetle *Dystiscus marginalis* isolated from north Kerala, using Mitochondrial COI marker. International journal of Current Research. 2015; 7(5): 16426-16429.
5. Rukhsana K, Akhilesh VP, Sebastian CD. Deciphering the molecular phylogenetics of the Asian honey bee, *Apis cerana* and inferring the phylogeographical relationship using DNA barcoding. Journal of Entomology and Zoology Studies, 2014; 2(4): 218- 220.
6. Priya Bhaskaran KP, Sebastian CD. Molecular barcoding of green bottle fly, *Lucilia sericata* (Diptera: Calliphoridae) using COI gene sequences. Journal of Entomology and Zoology Studies. 2014; 3(1): 10-12.
7. Jisha Krishnan EK, Sebastian CD. Analysis of evolutionary divergence of *Neurothemis tullia* (Odonata:Libellulidae)using cytochrome oxidase subunit I gene.International Journal of Advanced Life Sciences. 2015: 8(2), 110-114.E-ISSN:2217-758 X, P-ISSN.
8. Bindu PU, Sebastian CD. Genetic structure of mitochondrial cytochrome oxidase subunit I gene of the mosquito, *Armigeres subalbatus*. International Journal of Research, 2014; 1(10): 49- 56.
9. Sreejith K, Sebastian CD. Phylogenetic analysis and sequencing of the mitochondrial cytochrome oxidase subunit I (COI) of white backed plant hopper, *Sogatella furcifera* (Horvath). International Research Journal of Pharmacy, 2014; 5 (12): 887- 890. http://dx.doi.org/ 10.7897/2230-8407.0512180
10. Rukhsana K, Sebastian CD. Molecular barcoding and phylogeny analysis of Green Leafhopper, *Goniozus nephantidis* (Hymenoptera: Bethylidae), a larval parasitoid of coconut blackheaded caterpillar, Opisina arenosella (Lepidoptera: Oecophoridae). International Research Journal of Pharmacy, 2015; 6 (4), 239-241. http://dx.doi.org/10.7897/2230- 8407.06453
11. Pavana E, Sebastian CD. Genetic diversity and phylogenetic analysis of lepidopteran species by molecular barcoding using CO I gene sequences. International Journal of Science and Research, 2014; 3(5): 450-452.
12. Akhilesh VP, Sebastian CD. Molecular barcoding and phylogeny analysis of *Herpetogramma stultalis* (Lepidoptera: Crambidae) using cytochrome oxidase subunit I gene sequence.International Journal of Advanced Life Sciences, 2014; 7(3): 463-466.
13. Sanger F, Coulson AR. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. Journal of Molecular Biology, 1975; 94 (3): 441–448.
14. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution, 2013; 30(12): 2725- 2729. http://dx.doi.org/ 10.1093/molbev/mst197.
15. Gurney T, Elbel R, Ratnapradipa D, Brossard R. Introduction to the molecular phylogeny of insects. Tested studies for laboratory teaching. S. J. Karcher, Eds., Proceedings of the 21st Workshop/Conference of the Association for Biology Laboratory Education, 2000; 21: 63-79.
16. Hebert PDN, Ratnasingham S, Dewaard, JD. Barcoding animal life, Cytochrome c oxidase subunit I divergences among closely related species. Proceedings of the Royal Society London Biological Science, 2003; 270: 96-99.

Cite this article as:

Priya Bhaskaran K. P and Sebastian C. D. Molecular phylogenetic analysis of *Asota orbona* and *Asota caricae* (Lepidoptera: Erebidae) using mitochondrial COI gene. Int. Res. J. Pharm. 2017;8(4):41-43 http://dx.doi.org/10.7897/2230-8407.080446

Source of support: BLP Research Project, Conflict of interest: None Declared

Indian J.Sci.Res. 20(1): 50-52, 2018

ISSN: 0976-2876 (Print)
ISSN: 2250-0138(Online)

DNA BARCODING OF *Eudocima cocalus* FROM NORTH KERALA

K.P. PRIYA BHASKARAN[a] AND C.D. SEBASTIAN[b1]

[ab]Molecular Biology Laboratory, Department of Zoology, University of Calicut, Kerala, India

ABSTRACT

***Eudocima cocalus*, the *cocalus* fruit piercing moth, is a moth of the Erebidae family. The adult moths are one of the most severe and economically important agricultural pest among variety of fruits and vegetables. The moths pierce the fruit to suck the juice, thereby damaging the fruit and allowing the ingress of fungal spores and bacteria. Molecular approaches have led to species identification, which allowed rapid detection, discrimination, and identification of cryptic or sibling species based on DNA sequence data. We have developed the phylogenetic reconstruction and analysis of the *Eudocima cocalus* (GenBank Accession No. KX603659) using mitochondrial cytochrome oxidase subunit I (COI) gene. The knowledge of the *lepidopteran* genomic structures will create new method of integrated pest management which can contribute for the sustainable agriculture and maintenance of biodiversity.**

KEYWORDS: *Eudocima cocalus*, Erebidae, Cryptic Species, Sibling Species, Biodiversity.

Lepidoptera is a major order of insects that includes butterflies and moths and is the second most diverse insect pest order outnumbered only by the beetles. About 180,000 species of the Lepidoptera are described, in 126 families and 46 superfamilies [Capinera, 2008], 10% of the total described species of living organisms [Jim, 2007]. As pollinators of many plants, adult moths and butterflies are usually beneficial insects that feed on nectar using their siphoning proboscis. The caterpillars, almost always have chewing mouthparts that are suitable for feeding on various parts of a plant. Most caterpillars are defoliators or miners of succulent plant tissues. Most of the cultivated plants as well as the agricultural crops are attacked by at least one of the lepidopteran pest. Thus, pest insects have adverse and damaging impacts on agricultural crop production. Pest insects may cause problems by damaging crops and food production, parasitising livestock, or being a nuisance and health hazard to humans.

This beautiful looking moth is in fact a great pest, on account of the fact that it likes to make holes in fruits and also suck out the juices. Unfortunately, this can cause crop losses of more than 50% in many crops-by allowing microorganisms to enter-such as Lychee and Carambola.

The female adult moths of this species have forewings that are dark brown with several white spots. The hindwings are yellow with a broad black margin. The male moths have patchy brown forewings with no white spots, but the hindwings are orange with a broad black margin. For both sexes, each forewing has a hooked wingtip, and a concave inner margin. The head and thorax are dark brown, but the abdomen is bright orange. The wingspan is about 6cms.

DNA barcoding is a taxonomic method that uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species [Leong and Kueh, 2011]. Standardisation of a universal and sequenceable locus present in most of the taxa of interest for DNA barcoding that can be amplified with universal PCR primers is the best method to assess a large variation between species yet a relatively small amount of variation within a species [Hebert et.al., 2003]. Molecular phylogenetic analysis using DNA barcoding, especially mitochondrial COI gene sequences were adopted by several workers in various insect orders like Odonata [Jisha Krishnan and Sebastian, 2015], Diptera [Rukhsana et.al., 2014, Bindu and Sebastian, 2014 & Priya Bhaskaran and Sebastian, 2014], Hemiptera [Sreejith and Sebastian, 2014], Hymenoptera [Rukhsana et.al., 2014] and Lepidoptera [Akhilesh and Sebastian, 2014 & Pavana and Sebastian, 2014]. The result of a molecular phylogenetic analysis can be expressed in a phylogenetic tree and is one aspect of molecular systematics, a broader term that also includes the use of molecular data in taxonomy and biogeography.

MATERIALS AND METHODS

Collection and Identification of Specimen

The selected insect specimen *Eudocima cocalus* (Figure 1), was collected from Malappuram, Kerala. A diverse geographical status is spoken about the location, including lowland, highland and marshy areas, and the area exhibits a high diversity of organisms. The specimens were collected from the agricultural fields by employing the sweep net technique. Collected adult specimens were identified morphologically by consulting an expert. The collected specimens were stored at -20°C until the DNA is

---

[1]Corresponding Author

extracted and voucher specimen is restored.

Figure 1: ***Eudocima cocalus***

DNA Extraction, Amplification and Sequencing

DNA was extracted from the tissue of thoracic leg of the specimen, using Origin Kit as per the manufactures guidelines. The DNA isolated was confirmed using 1% agarose gel and 2ng was amplified for COI gene using the appropriate forward (5'- cattggagatgaccaaatttataatg -3') and reverse (5'-tgaaattaatccaaatccaggtaaa-3') primers. The PCR reaction mixture comprised of 2ng of genomic DNA (1µl), 1µl each of forward and reverse primer at a concentration of 5µM, 1µl of dNTPs (2.5mM), 2µl 10X reaction bufer, 0.20µl Taq polymerase (5U/µl) and 13.8µl H_2O. The PCR profile consisted of initial denaturation step of 5min at 95°C followed by 30 cycles of 10 sec at 95°C, 1 min at 50°C and 45 sec at 72°C and ending with a final phase of 72°C for 3 min. The PCR product was resolved on a 2% TAE-agarose gel, stained with ethidium bromide. To remove unincorporated primers and dNTPs the resultant PCR product was column purified using the nucleic acids purification kit of Gene JET of Fermentas Life Science. The purified PCR product was sequenced by Sanger's dideoxy chain termination method using an ABI 3730XL sequence analyser. The sequences were submitted to NCBI GenBank with accession number KX603659.

Alignment and Analyses

Chromatogram was analyzed for annotation with forward and reverse sequences. Annotated sequences were trimmed off primer sequences and any sequence ambiguity was resolved. The final sequence obtained were aligned using ClustalW programme [Rukhsana et.al., 2014].

Phylogenetic Analyses

Nucleotide sequences were analyzed using MEGA6 software [Bindu and Sebastian, 2014]. The matrix of corrected DNA distances was generated using Kimura Two parameter model and a phylogenetic tree was generated using the neighbor- joining algorithm [Priya Bhaskaran and Sebastian, 2014 & Sreejith and Sebastian, 2014].

RESULTS AND DISCUSSION

The PCR amplified sequences of mitochondrial cytochrome oxidase subunit I gene of *Eudocima cocalus* yielded a single product of 585 bp. The sequence has been deposited in the NCBI GenBank with Accession No. (KX603659). The phylogenetic tree plotted using Neighbor-joining method is presented in Figure 2. NJ clustering analysis showed that *Eudocima cocalus* belong to single monophyletic clade without any overlap. The evolutionary history of *Eudocima cocalus* clearly infers both the inter and intra species divergence. The *Eudocima cocalus* (KX603659) collected from Kerala exhibit 96% similarity to *Eudocima phalonia* (HQ949151) from Canada. Inter species divergence is found to be between 5-10%.

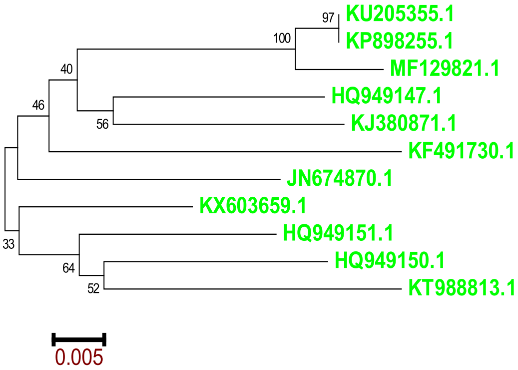


Figure 2: Phylogenetic relationship of *Eudocima cocalus* inferred by Neighbor-Joining method

CONCLUSION

The shortcomings and limitations of the conventional taxonomical identification methods highlighted need for new and simple methods of pest identification. The present study on molecular evolutionary analysis on partial mitochondrial cytochrome subunit I (COI) gene explicates phylogenetic relationships of *Eudocima cocalus*. The study emphasizes that the best phylogenetic studies and inferences can be created through moderately divergent nucleotide data from mitogenomes of which the CO I gene is best suited for deciphering the Lepidopteran taxonomic levels.

ACKNOWLEDGEMENT

The financial assistance provided by the Kerala State Council for Science, Technology and Environment, through WSD – BLP is greatly acknowledged. I express my thanks to the authorities of 27th Swadeshi Science Congress for giving the opportunity to publish my paper in IJSR.

REFERENCES

Capinera L.J., 2008. Butterflies and moths. Encyclopedia of Entomology, Springer, **4**(2): 626–672.

Jim M., 2007. Taxonomy of Lepidoptera: the scale of the problem. The Lepidoptera Taxome Project. University College, London. Retrieved 8 February 2011.

Leong S.C.T. and Kueh R.J.H., 2011. Seasonal abundance and suppression of fruit-piercing moth *Eudocima phalonia* (L.) in a citrus orchard in Sarawak. The Scientific World Journal, **11**:2330-2338.

Hebert P.D.N., Cywinska A., Ball S.L. and de Waard J.R., 2003. Biological identifications through DNA barcodes Proc. Royal Soc. London Biol. Sci., **270**: 313–321.

Jisha Krishnan E.K. and Sebastian C.D., 2015. Genetic variation and phylogeny assessment of *Aciagrion occidentale* (Odonata: Coenagrionidae) using mitochondrial cytochrome oxidase subunit I gene sequence. International Journal of Science and Research, **4**: 1121-1123.

Rukhsana K., Ashitha K.S., Mashoor K. and Sebastian C.D., 2014. Molecular phylogeny analysis of Southern house mosquito, *Culex quinquefasciatus* (Diptera: Culicidae) derived from mitochondrial DNA sequences. International Journal of Research, **1**(5): 844-853.

Bindu P.U. and Sebastian C.D., 2014. Genetic structure of mitochondrial cytochrome oxidase subunit I gene ofthe mosquito, *Armigeres subalbatus.* International Journal of Research, **1**(10): 49-56.

Priya Bhaskaran K.P. and Sebastian C.D., 2014. Molecular barcoding of green bottle fly, *Lucilia sericata* (Diptera: Calliphoridae) using COI gene sequences. Journal of Entomology and Zoology Studies, **3**(1): 10-12.

Sreejith K. and Sebastian C.D., 2014. Phylogenetic analysis and sequencing of the mitochondrial cytochrome oxidase subunit I (COI) of white backed plant hopper, *Sogatella furcifera* (Horvath). International Research Journal of Pharmacy, **5**(12): 887-890.

Rukhsana K., Akhilesh V.P. and Sebastian C.D., 2014. Deciphering the molecular phylogenetics of the Asian honey bee, *Apis cerana* (Hymenoptera: Apidae) and inferring the phylogeographical relationships using DNA barcoding, Journal of Entomology and Zoology Studies, **2(4)**: 218-220.

Akhilesh V.P. and Sebastian C.D., 2014. Molecular barcoding and phylogeny analysis of *Herpetogramma stultalis* (Lepidoptera: Crambidae) using cytochrome oxidase subunit I gene sequence. International Journal of Advanced Life Sciences, **7**: 463-466.

Pavana E. and Sebastian C.D., 2014. Genetic diversity and phylogenetic analysis of lepidopteran species by molecular barcoding using COI gene sequences. International Journal of Science and Research, **3**(5): 450-452.

Lahaye R., van der Bank M., Bogarin D., Warner J., Pupulin F., Gigot G., Maurin O., Duthoit S., Barraclough T.G. and Savolainen V., 2008. DNA Barcoding the floras of Biodiversity hotspots. Proc. Nat. Acad. Sci. USA, **105**(8): 2923-2928.

Thompson J.D., Higgins D.G., Gibson T.J. and Clustral W., 1994. Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nuc. Acids. Res., **22**: 4673-80.

Tamura K., Stecher G., Peterson D., Filipski A. and Kumar S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution, **30**: 2725-2729.

Tamura K., Nei M. and Kumar S., 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc. Nat. Acad. Sci. USA., **101**:11030-11035.