MOLECULAR CHARACTERIZATION AND MOLECULAR PHYLOGENETIC ASSESSMENT OF SELECTED FAMILIES OF ODONATES IN NORTH KERALA

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By

JISHA KRISHNAN E. K.

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

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DECLARATION

I do hereby declare that the work entitled "**MOLECULAR CHARACTERIZATION AND MOLECULAR PHYLOGENETIC ASSESSMENT OF SELECTED FAMILIES OF ODONATES IN NORTH 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.

JISHA KRISHNAN E. K.

CERTIFICATE

This is to certify that the thesis entitled "**MOLECULAR CHARACTERIZATION AND MOLECULAR PHYLOGENETIC ASSESSMENT OF SELECTED FAMILIES OF ODONATES IN NORTH KERALA**" submitted to the University of Calicut in partial fulfillment of the Degree of Doctor of Philosophy in Zoology ,in the record of the original work done by **Ms. Jisha Krishnan E.K.**, in the Department of Zoology under my supervision and guidance, and it has not formed on the basis for the award of any degree / diploma or other similar title to any candidate of any University.

Calicut University
November, 2018

Dr. SEBASTIAN C.D.
Supervising teacher

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CONTENTS

INTRODUCTION

Insects are the abundant invertebrate animals categorized under the the most diversed and largest class of Insecta under the Phylum Arthropoda.The word Insecta came from a Latin word "*Insectum*" meaning "notched or divided body". They are characterised by outer bilateral chitionous exoskeleton all over the body, three pairs of jointed appendages, a pair of compound eyes and a pair of antennae. The number of insects were known to be 6-8 million species which represents 90 % of total animal life on earth (Chapman, 2006; Novotny, 2002; Erwin, 1982). They known to be existed in almost all environments with their highest abundance seen in tropics. As they have short life span and high fecundity, they are widely used in many research areas like Genetics, Evolution, Ethology, developmental biology, Forensic biology, physiology....etc and play an important role in research field. There are 30 insect orders coming under this class and among them the Order Odonata represents most ancient aquatic as well as very primitive winged insects existing today.

As the time progresses, Earth biota has been continuously changing due to certain modification in the environment. Hence it is very essential to know about the proper managing of sustainability of biodiversity. Taxonomy is the best suited scientific field of biology helping for biodiversity studies. This branch is mainly concerned with identifying, describing and naming different organisms and there by providing a universal accessibility to every organism. Literally it means "arrangement with laws" and it is mainly concerned with categorising each organism in a coherent manner for reflecting their evolution and relatedness. For classifying each organism , there exists a perfect ranking system consisting of Domain, Kingdom, Phylum, Class, Order, Genus and Species. Thus helping to facilitate the

communication between specialists working in similar areas to understand the relationship between various group of insects.

Molecular techniques became a more common procedure for the species identification of many organisms in the field of Entomology (Roques et al., 2009). About 20 years back onwards, there has been widely used DNA sequences for predicting and comparing evolutionary relationships among different organisms. Here DNA sequences themselves act as a reference system for comparing and confirming taxonomic identity. A particular gene of interest whose sequences that differs each other by maximum number of base pairs are taken as for comparing and confirming identity in DNA taxonomy. According to Floyd et al. (2002), these sequences are termed as Molecular operational taxonomic units (MOTU). In DNA taxonomy, molecular data inputs are analysed and interpreted by many statistical innovations present in the concerned software tool we are used. As it provide a better result on the base of DNA sequences there exist a wide accessibility to molecular systematic than traditional taxonomy. Thus the DNA sequences available on the online database helps to enhance our knowledge about earth biodiversity biota in a more easier way and thereby helping to unweil the hidden relationship among different organisms. Thus it is very essential to make a need to stimulate and advance taxonomy in terms of investment and popularity at the species and population level.

Generally the molecular systematics utilises DNA, RNA and protein sequences for predicting evolution on the basis of changes in their sequences. This method has a lot of advantages over traditional taxonomy because it is more numerous and based on gene level, it is easy to obtain and no need of sampling method. Traditional method generally based on fossil record for the prediction of evolution make a great problem if the sample is damaged. Molecular taxonomy actually helps to reconstruct phylogeny at phyla, class,

order and family level as it became inconvenient to distinguish between two organisms merely on their morphological data. As this is mainly working on the basis of gene level, it helps to predict the evolution of a gene as time progresses and to know about how a single change in the gene sequence can lose its function.

Mitochondrial DNA became a popular phylogenetics tool for population studies because of its easy isolation, use of restriction enzymes to detect nucleotide differences, development of PCR methodologies and applicability of universal primers for amplification of DNA (Brown et al., 1982). It is a powerful tool for the species level phylogenies of many organisms as the arrangement of genes are variable which are separated by many noncoding regions of genetic DNA. (Anand,et al., 2014). Mitochondrial cytochrome oxidase I (COI) and cytochrome oxidase II (COII) are the energy transfer enzymes in the respiratory chain. They represents the common candidates used in the phylogenetic problems to resolve many taxonomic hierarchial levels in insects from closely related species to genera, subfamily, family and even Orders. They are popularly known as the "Molecular fossils" of systematic studies and widely used for the comparative analysis of many related organism. The COI gene is a slowly evolving gene compared to other protein coding genes and is a good performer in recovering an expected tree (Zardoya et al.,1996).

DNA barcoding is a new innovative research in the field of modern systematics. It is the easiest tool for taxonomic identification using a universal standard gene region among different organisms. This technique was first described by Paul Hebert in 2003 and this method provides a unique "barcode" to every organism in the world for easy identification. The main advantage of this technique is the easy diagnosis of species irrespective of their life stages (as larvae, nymph, adult etc), damages and body decay therby

helping for accurate identification and taxonomic relatedness. Generally morphology based taxonomic (classical or traditional) method uses fossil records to reveal phylogenetic ancestry and if it is damaged make a great problem. Theoretically the DNA sequences of approximately 600bp mitochondrial cytochrome oxidase I gene contains more than enough information to distinguish millions of species and it has been widely accepted a universal "barcode " region. The usual methodology involves extracting DNA from any sample and compare those sequenced DNA against the barcode library for identification thus helps to predict the origin, evolution and evolutionary relationships.

Even though molecular based systematic studies are popular now, morphology based phylogenetic studies are essential to make a "reality check" to molecular results (Doyle, 1992). Only experts such as taxonomists and grand technicians can identify taxa accurately as it requires special skills acquired through extensive experience. Most of the laboratory based morphology studies are essential not only for taxonomy studies but also other fields like ecolgy, behaviour and physiology (Maddisson, 1996). For the better understanding of evolution and systematic, it is essential for understanding relationship between different groups of population at their species level.

The insect order Odonata represents the most primitive winged insects existing today and known to be here in the universe about 250 million years ago since the caboniferous period. The term Odonata came from a greek word "*odontos*" having a meaning of "toothed flies or teeth on mandibles" and it was Fabricius who coined the term Odonata (Mickel, 1934). They are known as "primitive winged ones" because their wings cannot folded backward due to the articulation of wing muscles and hence known as 'Palaeoptera'. Taxonomically this order is very close to mayflies and hence both were

placed under this category.Generally there are two subdivisions under this order as Anisoptera (dragonflies) and Zygopter (damselflies). Most of the species are habitat specialists and generally found associated with aquatic ecosystems like ponds, streams, rivers etc. Most of the species are seen in tropical areas but odonates of both the major suborders occur in every faunal region except Antarctica. A third suborder, Anisozygoptera, largely known from fossils, is represented by one extant species in Japan and one in the Himalayas only.

GLOBAL DIVERSITY OF ODONATA

Extant Odonata has been divided into 3 suborders on the basis of morphological differences. They are Anisoptera (dragonflies), Zygoptera (damselflies) and a third suborder Anisozygoptera. Globally there is an estimate of 6256 species distributed in 39 families under 686 genera (Subramanian and Babu, 2017). The 39 families falling into 3 distinct suborders consisting of 27 families under Suborder Zygoptera and 11 families under Suborder Anisoptera and only one family under Suborder Anisozygoptera. The representing 27 families under Zygoptera are the following: Hemiphlebiidae (Genera: 1; Species: 1); Perilestidae (Genera: 2; Species: 19); Synlestidae (Genera: 9; Species: 38); Lestidae (Genera: 9; Species: 153); Platystctidae (Genera: 9; Species: 262); Amphipterygidae (Genera: 1; Species: 5); Argiolestidae (Genera: 20; Species: 114); Calopterygidae (Genera: 21; Species: 180); Chlorocyphidae (Genera: 20; Species: 156); Devadattidae (Genera: 1; Species: 13); Dicteriadidae (Genera: 2; Species: 2); Euphaeidae (Genera: 9; Species: 75); Heteragrionidae (Genera: 2; Species: 56); Hypolestidae (Genera: 1; Species: 3); Lestoideidae (Genera: 2; Species: 9); Megapodagrioniiidae (Genera:3; Species: 29); Pentaphlebiidae (Genera:1; Species:3); Philogangiidae (Genera:1; Species:4); Philoginiidae (Genera: 2; Species: 40); Philosinidae (Genera: 2; Species: 12); Polythoridae

(Genera: 7; Species: 6); Pseudolestidae (Genera: 1; Species: 1); Rimanellidae (Genera: 1; Species: 1); Thaumatoneuridae (Genera: 2; Species: 1); Isostictidae (Genera:12; Species:45); Placticnemidae (Genera:43; Species:455); Coenagrionidae (Genera:121; Species:1351). The 11 families of the concerned Suborder Anisoptera includes the following: Austropetaliidae (Genera:4; Species:1); Aeshnnidae (Genera:54; Species:480); Petaluridae (Genera:5; Species:11); Gomphidae (Genera:101; Species:1010); Chlorogomphidae (Genera:3; Species:52); Cordulegatridae (Genera:3; Species:55); Neopataliidae (Genera:1; Species:1); Synthemestidae (Genera:26; Species:147); Macromiidae (Genera:4; Species:125); Corduliidae (Genera:21; Species:165); Libellulidae (Genera:144; Species:1035). The third suborder Anisozygoptera is represented by only one family Epiophlebiidae (Genera:1; Species:3). (Subramanian and Babu, 2017).

DIVERSITY IN INDIA

About 488 species and 27 subspecies distributed in 154 genera and 18 families are known to be existing in India.This insect order is told to be abundantly found in Western Ghats, Eastern Himalyas and Andaman Nicobar island in India (Subramanian and Babu, 2017). The suborder Zygoptera consists of about 211 species falling under 59genera and 9 families. The representing families are Lestidae (Genera: 5; Species: 25); Synlestidae (Genera:1; Species: 6); Platystictidae (Genera:3; Species:15); Coenagrionidae (Genera:12; Species: 60); Calopterygidae (Genera: 6; Species: 9); Chlorocyphidae (Genera: 8; Species: 22); Euphaeidae (Genera: 6; Species:19); Philogangiidae (Genera:1; Species:1) and Placticnemiididae (Genera:15; Species:53). The ssuborder Anisoptera consists of about 276 species categorized in 94 genera and 8 families. The representing families are Aeshnidae (Genera: 13; Species: 49); Gomphidae (Genera: 29; Species: 85); Cordulegastridae (Genera:3; Species: 9); Chlorogomphidae (Genera:3;

Species: 8); Corduliidae (Genera: 2; Species: 2); Libellulidae (Genera: 40; Species: 91); Macromiidae (Genera: 2; Species: 17); Synthemestidae (Genera: 2; Species: 15). The third suborder Anisozygoptera is represented by only one family Epiophlebiidae (Genera: 1; Species: 1).(Subramanian and Babu, 2017).

DIVERSITY IN KERALA

About 142 species spreading in 74 genera and 13 families are distributed in Kerala.(Kiran and Raju, 2011). The suborder Anisoptera consists of about 48 genera and 85 species falling under 6 families. The representing families are Aeshnidae (Genera: 3; Species: 7); Gomphidae (Genera: 13; Species: 19); Chlorogomphidae (Genera:1; Species: 2); Corduliidae (Genera: 2; Species: 4); Libellulidae (Genera: 27; Species: 47); Macromiidae (Genera: 2; Species: 6). The suborder Zygoptera consists of 26 genera and 57 species under 7 families.The representing families are Lestidae (Genera: 2; Species: 5); Platystictidae (Genera: 2; Species: 10); Coenagrionidae (Genera:10; Species: 21); Calopterygidae (Genera: 2; Species: 3); Euphaeidae (Genera: 2; Species: 4); and Placticnemiididae (Genera:1; Species:1); Protoneuridae (Genera: 7; Species: 13).Recently the same authors has reported about 154 species from Kerala.

Odonates are medium to large sized amphipterygote insects which are hemimetabolous and carnivorous. Both larvae and adults are voracious predators of many insects and hence ecologically important as indicators of healthy ecosystem. They are always associated wth many agroecosystems as it feeds on wide variety of crop pests and also in aquatic ecosystem for feeding various larvae. Adults feed a lot of mosquitos larvae and hence in some countries they are rearing for this purpose also. Life cycle of these insects are strictly correlated with water since adults are generally oviposit near the aquatic vegetations.Egg directly hatches into nymphs after one week

of ovipostion they directly develops into adults and have an average life span of 4 months to one years depending on the species. Dragonflies is the common name given to notify this order. There exists a clear difference in the morphology of both dragonflies and damselflies for theit adult and larval stages. Dragonflies are more prominent and dominating over damselflies and even some dragonfly species are cannibalic to damselflies. Dragonflies are strong and heavly bodied with rounded head,eyes meet on the front with wings always placed horizontally on rest. Damselflies are unlikely small bodied ones, widely separated eyes on either side and with their wings placed vertically at rest. The distribution of Odonates among different families are considering to be the drifting apart of southern continent Gondwana occurred at an earlier time. Some species are cosmopolitous in nature, some are locally distributed and some in cool streams, rivers, ponds, stagnant waterbodies and also to marshy lands. Among Anisopterans, Libellulidae represent the most wide spread and species rich family while Coenagrionidae is the dominant one in Zygoptera. The most restricted one is monotypic Hemiphlebiidae (Zygoptera), only known from six or so small reedy pools in south-eastern Australia.

Odonates are considered as the indicator of ecosystem quality because their local faunal composition is strongly affected by changes in water flow, turbidity etc, or in aquatic or waterside vegetations. Those animals which are generally found at the lower position of food chain causes a great change in the health of ecosystem than do in the top. As Odonates are placed in the low aquatic food chain, they can be used as an indicator for determining the health of ecosystem. Inland fishermen may know dragonfly larvae as "mud-eyes" and use them as bait. Adult dragonflies are a minor food item in some countries, and the larvae sometimes have been used to control pest insects (eg. mosquitos in domestic water tanks). Their main attraction for humans is aesthetic. The present study aims to employ DNA barcode technique using

partial cytochrome oxidase subunit I gene to provide estimates of provisional species diversity in the study areas.

The state of Kerala lies mainly in the tropical region which experiences humid tropical wet climate by Earth's rain forest. It receives an average rainfall of 3107mm with an average of about 120-140 rainy days per year. Odonates are strictly correlated with aquatic ecosystems and this state is blessed with 44 rivers, it makes a suitable habitat for many more Odonata species. Most of the Odonata works from Kerala contributed by Fraser (1933, 1934, 1936), Peters (1981), Rao and Lahiri (1982), Emiliyamma and Radhakrishnan (2000) as well as Kiran and Raju (2013).

From Kerala there are 154 species reports (Kiran and Raju, 2013) but there was no barcode data available for this order till to date. In Kerala the molecular aspects of odonata fauna are scanty and hence the present work is mainly based upon the molecular phylogenetic analysis of odonates from Northern Kerala. As Northern Kerala is a part of Western Ghats, it is significantly important as a species diversed area. This COI gene based study in Kerala is a pioneer molecular taxonomic work in the field of Odonatology. The present study has surveyed and collected Odonates from 7 districts of Northern Kerala. The cytochrome oxidase I gene of the specimens were amplified using specifically designed primers and then sequenced. The COI genes used for phylogenetic studies and delineate its phylogenetic relationships. Genetic divergence and nucleotide composition of 20 different Odonata specimens were described. The major objectives of the present study includes:

- To compliment the classification of odonates using molecular systematics.

- To evaluate the relationship between the different species of Odonata.
- To generate a database for the partial COI gene sequence of these species.
- To delineate their phylogenetic relationships with other related insect groups.
- To evaluate the direction of the evolution of Odonata species.

REVIEW OF LITERATURE

Taxonomy

Taxonomy is the basic science deals with the scientific study of identifying, naming, and classifying different organisms existing in the world. A biologist who is working in any scientific field would be incapable to interpret their findings without prior information regarding their target organisms. Thus this field help to classify these millions of organisms existing in the planet into different categories like family, genus, species etc. for their easy study and proper understanding. Wilson et al. (2003) reported that there about 5 -100 million species are awaiting for their discovery and description and hence there exists an immediate urge to augment taxonomy in terms of need (Godfrey, 2002; Hebert, 2003). It help us to understand what type of characters are present in an organisms, its position in the evolutionary history of organisms, how each animal are different in their physical and mental development, their geographical distribution etc. It also makes a baseline data available for conservation and ecology studies, and affords humans the possibility to take advantage of the underutilized resources offered by the earths' biodiversity (Wilson, 2004). Generally Taxonomy has divided into 2 categories- Classical taxonomy and Molecular taxonomy.

Classical Taxonomy and its limitations

The branch of taxonomy in which members have categorised in specific groups on the basis of their own similar morphological and anatomical characters is called classical taxonomy or traditional taxonomy. Here each species are mainly classified on the basis of observable similarities. Appropriate taxonomic keys have used for the species identification and also for the proper management of biological collections. There exists a perfect

hierarchal system for the classification of every organism starting from kingdom, domain, phylum, class, order, family, genus and finally to the species level. The main drawback of this method is its inability to identify immature, damaged or incomplete specimens and also to predict phenomenon like cryptic morphology and polymorphism existing among different species. Hence traditional taxonomy requires high levels of expertise in any given group and is therefore restricted to specialists.

Identification using conventional taxonomy is not easy due to the morphological changes in the organisms caused by seasonal and geographical variations. They alter themselves physiologically and morphologically due to certain unfavourable conditions in the environment. These morpho variations gets accumulate in the species concerned leading to a drastic change in the outlook or appearance. This in turn causes the misidentification of species (Pushparaj et al., 2012). Actually the traditional taxonomic methods make a intractable problem for cryptic and polymorphic species.

Cryptic speciation is a common phenomenon existing among metazoan taxa and this is often observed in all sorts of habitats and biogeographic zones (Bickford et al., 2007; Pfenninger et al., 2007; Trontelj et al., 2009). Those groups which are subjected to poor dispersal abilities are greater prone to cryptic speciation (Neusser and Schrodl, 2011; Casu et al., 2004). But this kind of morphological similarities which exists in the same species make it difficult for traditional taxonomist to reveal their prompt identity. There is no specific taxonomic key to resolve species exhibiting cryptic speciation because it is continually changing over time due to environmental impact. But uncovering this phenomenon one could clearly understood the processes of evolution, historical biogeography, ecology and also their conservation approaches (Bickford et al., 2007; Pfenninger et al., 2007; Trontelj et al., 2009). So it represents one of the complicated scenarios of taxonomic

incompleteness. Teo et al. (2017) analyzed the importance of cryptic speciation for conservation by scrutinizing the South European cryptic complex of the subterranean amphipod (*Niphagus stygius sensu lato*) using uni locus and multi locus delineation method. Egea (2016) states that cryptic species which are evolutionary young from population to species level and their ancestors are not getting diverged indicate that they have little importance of conservation. But those species which are phylogenetically old and reproductively isolated through strong biological barriers needs high needs of conservation (Trontelj et al., 2009).

The process of polymorphism is another thing challenging to traditional taxonomists. Due to the natural selection, recent evolutionary radiation has generated diverse colour patterns and other morphometric differences among animals. The evolution of biological species diversity has often been accompanied by a corresponding expression of morphological variations, colour patterns, mouth and beak shapes (Givnish et al., 2000). Most of the polymorphic species exhibits spatial and temporal heterogeneity and the morphological differences are actually due to the sexual dimorphism (Larvae and adults), epigenetic development and geographic variation (Xiao et al., 2010). It becomes a very difficult task for a traditional taxonomist when either of the sex became difficult to collect (Roff, 1986). Fig wasps are well known for exhibiting both polymorphism and cryptic speciation. Xio et al. (2010) differentiated six polymorphic males and four extremely sexually dimorphic species of fig wasps using COI gene and ITS molecular markers when traditional taxonomy fails to work over it unambiguously.

Geographic variation among individuals is another taxonomic problem confronted by conventional taxonomists. It is the latitudinal, longitudinal and altitudinal variations. Certain environmental conditions may additionally influences the selective gain to them at a particular stage in their life cycle

which persists and leads to the formation of new species. So the knowhow about each species regarding their relationship to their close relative's traditional line of taxonomy is not at all an easy task.

Among insects, sexual dimorphism and mimicry often leads to the misidentification of the original species. Sexual variation represents one of the best morphological variations exhibited by animals. It is the difference in physical appearance of both sexes other than the distinction in sex organs. It includes difference in colouration, size and body structures between sexes. It permits not only in the larval and juvenile periods, but even in their adult stage also. According to Shine (1989), the prime ecological cause for the sexual dimorphism is the competition between sexes for existence and the evolution of foraging specialization is discovered to be the most essential cause of sexual dimorphism.

Thus the adoption of manual taxonomy, on the basis of the above mentioned limitations, leads to misidentification of the species in between. This trouble has thus influenced the emergence of the molecular taxonomic frame work studies for the conformation and the betterment in the identification of species.

Emergence of Molecular Taxonomy

Molecular systematics is one of the most unexpectedly expanding fields in modern biology, but our grasp of sample of molecular evolution remains relatively superficial. The theoretical framework for molecular systematics used to be laid in the 1960's mainly on the works of Emile Zuckerkandl, Emanuel Margoliash, Linus Pauling and Walter M. Fitch. Analysis of molecular statistics has verified to be essential for perception of phylogenetic relationship, examining population structure within a species and assigning unknown specimens. The use of molecular characters for fast

identification of unknown organisms has been proved to be useful and pretty effective. The genes encoded in the mitochondrial DNA (mt DNA) have dominated in the field of molecular systematic because of their maternal inheritance, restrained recombination and speedy evolution.

The major steps used in most systematic studies include taxon sampling, choice of appropriate markers and analytical studies. One of the key elements in designing a molecular systematic study is selecting ingroup and outgroup taxa. Most studies agree that sampling can significantly affect phylogenetic inference. Sequencing is generally most appropriate for studies at inter specific levels or even higher. Other methods like restriction fragment length polymorphism (RFLP), single-stranded conformational polymorphism (SSCP), random amplification of polymorphic DNA (RAPD) *etc* are also used nowadays. DNA sequencing has become dominant technique for generating molecular data for comparative analysis. The DNA sequences exhibit certain properties like inherent comparability of sequence data that facilitates the connectivity and unique insight towards evolutionary processes deriving diversification of DNA itself.

The use of molecular data in taxonomy has several advantages. First and foremost, the classification schemes for groups such as Fungi, whose phylogeny has long confounded many taxonomists who rely upon more traditional morphological characters, can now be determined more easily. Secondly, organisms typically have many thousands of different genes, so that there is a potential data base of characters which is virtually unlimited in size. Third, as the changes in DNA form the basis for all other evolutionary changes such as changes in morphology, comparison of gene sequences allow study of evolution at most basic level. Comparative studies of morphology will continue to play an important role in taxonomy but gene sequences are becoming more widely used for easy comparison in taxonomy.

Molecular techniques provides powerful tool for the study of insect systematics. Similar morphology and high genetic diversity poses problems in phylogenetic studies of insects. To solve these problems, mitochondrial based markers have been adopted and are increasingly used as molecular markers for phylogenetic studies. Varied markers have been used for different species of insects, viz. markers for 16S rRNA, 12S rRNA, ND (1-6 genes), ATPase and control regions. Molecular phylogenetics uses the structure and function of molecules and how they change over time to infer these evolutionary relationships.

Mitochondrial DNA markers

Mitochondrial markers considered as promising instrument for Insect systematics (Cameron et al., 2014). It is a highly conserved 15-18 kbp long DNA span containing 37 functional genes comprising 13 protein coding genes, 2 rRNA genes and 22 tRNA genes (Boore et al., 1999). Among these techniques, the analysis of mitochondrial DNA is particularly useful in discriminating between closely related species. The fact that mitochondrial DNA has maternal inheritance, its high mutation rate due to limited repair system, high nucleotide substitution rate (5-10 times more rapidly than nuclear) and relatively simple conserved structure makes it suitable for examining population and subpopulation structures among related taxa (Brown et al., 1979). Also the robustness of mtDNA against degradation makes them ideal markers for many species level questions. Mutation hotspots or adaptive substitution are known to exist in mtDNA causing heterogeneous evolutionary rates across genes.

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 et al., 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 et al., 1996).

DNA barcoding and its applications

DNA barcoding is a novel system designed to provide rapid, accurate and automatable species identification by combining taxonomy, genetics and computer science that automates the process of obtaining expert species identification. It differs from molecular phylogeny in that barcoding is not used to determine classification but to identify an unknown sample in terms of a known classification. Thus this technique does not require any taxonomist for identification process DNA barcoding technique is mainly based upon idea that sequence diversity of standard gene region amongst different organisms which can serve as a tool to identify specimens to known species

and potentially discover new species (Hebert et al., 2003). It provides a universally accessible format across the widespread scientific community.

The public consortia for DNA sequences include International Nucleotide Sequence Database Collaboration (INSDC) comprised of NCBI (National Centre for Biotechnology Information), EMBL (European Molecular Biology Laboratory) and DDBJ (DNA Data Bank of Japan) as well as BOLD (Barcode of Life Data system) (Ratnasingam and Hebert, 2007).

The COI gene is used for barcoding as it is the largest gene among the three mitochondrial genes encoding cytochrome oxidase subunit and has high insertion deletion events. Hebert et al. (2003) described a 648 bp region in the mitochondrial COI gene for animal barcoding because it showed high efficiency for the identification of bird, fish, flies and other animals. Also it has a high rate of nucleotide substitution helping to discriminate cryptic species also. Hence this region is considered as the universally accepted barcode region in molecular studies.

DNA barcoding essentially provide the case of specimen identification using simple molecular protocol irrespective of specimen's life stages, sex, location of collection as well as non availability of taxonomic expertisation (Teletchea et al., 2010). It has a lot of application in various ways like invasive species, identification of botanicals, detection of specific substitution in sea foods and also biomonitoring of ecosystem health (Adamowicz, 2015). Creer et al. (2010) says that DNA barcoding can revitalize traditional taxonomy in conjunction with ecological, morphological and other genetic studies.

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 CO I 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 mutations consists of 56 transition, 66 transversion and 20 mutation with both transition and transversion.

DNA barcoding methods in human and veterinary helped to understand pathogen life cycle more easily. A eukaryotic universal primer to amplify 758 bp of vertebrate COI gene was designed for the identification of blood sucking arthropod. They were amplified in PCR. The analysis of mosquito, phlebetomic, sucking bugs and ticks revealed upto 40 vertebrate hosts, 23 avian, 16 mammalian and 1 reptilian species (Alcaide et al., 2009).

Non coding satellite DNA (satDNA) usually has a high turnover rate frequently leading to species specific patterns. Some sat DNA families evolve more slowly and can be found in several related species. Martinsen et al. (2009) analyzed the mode of evolution of PDO500 satDNA family using 12 Dolichoda

cave crickets. 199 genomic or PCR amplified satDNA repeats of PDO500 family from these species were sequenced. The PD0500 satDNA exhibits the molecular evolution at a gradual rate that is only slightly faster than mitochondrial COI sequences. The PDO50 phylogeny was basically congruent with mtDNA phylogenies. Thus PDO500 satDNA was found informative and could be used as one of the phylogenetic marker.

The internal transcriber spacer 2 (ITS-2) which separates nuclear ribosomal genes 5.8S and 28S constitute a rapidly evolving nuclear DNA fragment and proved very useful in inferring phylogenetic relationships of closely related species in plants and fungi. In the butterfly family Lycaenidae, ITS-2 structure was analyzed to align sequences of different sub tribes in *Polyommatini* and produce a phylogenetic tree of their tribe which was obtained with COI and COII. The usage of ITS-2 marker and character based phylogenetic method can improve versatility of ITS marker and characterize phylogenetic studies (Weimees et al., 2009).

DNA barcoding is a challenge in the field of bioinformatics. Virgilio et al., (2010) compared the performance of DNA based barcoding identification among insect orders and concluded that distance based criterion showed higher and more robust performance than the tree based character. Character based identification provides more accurate result since it directly uses nucleotide variation in each base position as a diagnostic character and better than distance based method. Dasmahapatra et al. (1985) emphasized that AFLP marker can be used as an effective tool to check the results of DNA barcoding experiments.

ORDER ODONATA

Odonata is one of the ancient groups of winged insects, dating back to the permian period (Grimaldi and Engel, 2005). They can be easily recognised by their long, slender abdomen, large globular eyes often making

upon large head, short antennae, long wings with conspicuous nodus and have pterostigma. Extant dragonflies are divided into 2 suborders Zygoptera (Damselflies) and the Anisoptera (Dragonflies) and a third suborder Anisozygoptera under the new name Epiprocta. Zygoptera have a broad head with widely separated eyes, similar sized fore and hindwings. The larvae are slender and having 2-3 caudal gills for respiration. Anisoptera are little larger than Zygoptera and are robust bodied ones. Their hind wings are broader at their base than the forewings and in most of the families with their eyes touch on the top of the head. Larvae are stouter than Zygoptera are lacking caudal gills. Oxygen is absorbed through rectal gills. This order has been divided into 2 suborders in which Anisoptera (12 families) and Zygoptera (24 families). About 6000 species of Odonata and subspecies belonging to 652 genera have been documented worldwide (Schorr et al., 2010).

Odonates are aquatic carnivorous and hemimetabolous insect with their pupal stage is wanting during their life cycle. Life cycle begins as eggs and oviposition takes mostly on plants found above or below water surface. Their hatching period found to be 7-9 days upto several months depending on species. Larvae moult about 30 times and have a life span of 4 months to 10 years. Adults are spending most of their time as aerial predators and well known as aerodynamic fliers in the insect world. They generally feeds upon whatever prey is abundant in the nature and mainly as mosquitoes, bees, wasps, butterflies and other insects.

The taxonomy of Indian Odonata is well worked and descriptions are available for all the reported species (Fraser, 1933, 1934, 1936; Prasad and Varshney, 1995; Subramanian, 2014). Introduction of field guides from Emiliyamma et al. (2005), Subramanian (2005), Nair (2011) and Kiran and Raju (2013) have recently accelerated the process of data collection in odonates. Information regarding the number of odonates was derived mainly

from the Global species database for the catalogue of Life. Emiliyamma et al. (2007) reported 137 species and sub species of Odonata spread over 79 genera, 12 families and 31 subfamilies from Kerala.

The suborder Anisoptera consists of dragonflies which existed 250 million years ago in the carboniferous period. This group composed of 3012 species in 348 genera in 11 families (Suhling, 2015). They are characterised by robust stout body, prothorax covered by pronotum, fused meso and meta thorax (Synthorax), stout abdomen having 10 segments, a pair of compound eyes, legs and different sized forewing and hindwing having pterostigma. The characteristic feature is the differently sized forewing and hindwing, anal appendage consists of both cerci and epiproct for holding the prey, cubital vein forms the basal side of discoidal cell, anal vein forms anal loop which is differently shaped (Silsby, 2011). Coenagrionidae and Libellulidae represents the most dominant families of these two suborders which are known to be recently originated (Rehn, 2003; Jisha and Sebastian, 2015a). Dragonflies are the most recognizable of insects and their progenitors are known to date in the Carboniferous period and probably the most widely known extinct insects. They are widely used in the studies of morphology, behaviour, ecology and evolution (Corbet et al., 1999).

The suborder Zygoptera represents one of the ancient suborder commonly called as ‘Damselflies’. They existed 250 million years ago with primitive proto odonates existed in the Mesozoic Era (Grimaldi and Engel, 2005). This group have 2942 extant species listed in 309 genera categorised in 28 families (Suhling, 2015). They are known to be geographically distributed in all biological realms except Antartica (Nilsson, 1997). This suborder characteristically have widely separated eyes, fused thorax, a pair of tiny antennae, legs and long slender abdomen having 10 segments. The tenth abdominal segment characteristically has cerci and paraprocts (Paulson and

Dennis, 2011). Their wings are similar in size, coloured or uncoloured and supported by many cross veins filled with Haemolymph (Silsby, 2001). About 6256 species exist till now and out of which 487 species in 152 genera and 18 families are existed in India. About 12 families out of 31 are mostly found in running waters within the tropical forest habitat. This suborder characteristically have widely separated eyes, fused thorax, a pair of tiny antennae, legs and long slender abdomen having 10 segments. The tenth abdominal segment characteristically has cerci and paraprocts (Paulson and Dennis, 2011). Their wings are similar in size, coloured or uncoloured and supported by many cross veins filled with Haemolymph. The characteristic feature of this group is that the cubital vein forms the posterior margin of discoid cell and the anal vein generally fused at the wing border (Silsby, 2001). The shape of quadrilateral cell (discoidal cell) is a distinguishing feature for resolving different families of this suborder. It is a skewed trapezium in Coenagrionidae and Lestidae, triangle in Lestidae and fewer triangles in Platycnemidae. This structure is altogether absent in Calopteryygidae and they composed of many rectangular cells (Silsby, 2001).

ECOLOGICAL IMPORTANCE OF ODONATES

Most of the species are found in the temperate region have a dramatic decline in the distribution and abundance because half of the species are reduced due to habitat destruction, eutrophication, acidification and pollution of aquatic habitats. These carnivorous insects have been looked as biocontrol agent against mosquitoes (Andrew et al., 2008). In some countries they have been widely used as food and as magical or modified resource at a local scale. They features as the natures management in the temperate region of the world (Westfall et al., 1996) also used as an indicator of environment health and conservation management. They are very much sensitive to structural habitat quality such as forest cover and water chemistry and amphibious habitat

makes them well suited for environmental changes in long term and short term, above and below the water surface (Clark & Samways 1996; Sahlen and Ekestubbe, 2001; Claushizer, 2003).

Agricultural fields provide a unique ecosystem for certain odonate species in order to complete their life cycle. Besides larvae and adults of odonates are regarded as important predators of paddy fields, they are also well abundant because of the aquatic nature and availability of prey species that are major pests of crops ((Bambaradeniya et al., 2004).

MOLECULAR PHYLOGENETIC STUDIES OF ODONATES

The most notable pre-cladistic studies of Odonata were mostly based on wing venation (Needham, 1903; Munz, 1919). Here ancestral states are classified as their forewings and hindwings are alike and derived states as forewings and hindwings are differentiated. Modern dragonflies are a well supported monophyletic group (Rehn, 2003; Kristensen, 1975; Wheeler, 2001). They share several unique characters most notably the secondary male genitalia and the prehensile labial mask of the larvae. Some controversy has existed within the order Odonata regarding which suborder is monophyletic and which is paraphyletic. According to Needham (1903) there existing a dichotomy between Anisoptera and Zygoptera. Anisoptera is divided into Libellulidae and Aeshnidae with Aeshnidae representing a primitive branch. Zygoptera are in turn divided into Calopterygidae and Agrionidae. Munz (1919) argued for dichotomy between Zygoptera and Anisoptera where the Agrionidae are a grade including monophyletic Coenagrionidae. Zygoptera are seen as being derived from Anisopzygotera. There are two convergent phylogenetic theories that are at the centre of the debate. One phylogenetic theory was put forth by Handlirsch (Trueman et al., 2001) that states Anisozygoptera is a paraphyletic group from which the monophyletic groups Anisoptera and Zygoptera were derived. Tillyard (Trueman et al., 2001)

regards Zygoptera as the paraphyletic group from which the monophyletic groups Anisoptera and Anisozygoptera were derived. This debate has been going on for years and will likely continue until evidence from fossils or DNA will reveals, the true phylogeny.

Cytochrome oxidase (CO) gene and Nitrogen dehydrogenase (ND) gene represents 2 effective molecular marker genes used for resolving phylogeny among dragonflies. Cytochrome oxidase I (COI) gene serve as a core of a global bio-identification system for all animal phyla. The Nitrogen dehydrogenase sequence analysis provides strong interspecific and intraspecific differences in the population structure of all species that have been shown to be highly informative at different taxonomic levels in dragonflies (Hebert et al., 2003).

Laltanpuii et al. (2017) used COI and NDI gene sequences to reveal the phylogenetic relationships between different members of Libellulidae family. It was inferred using Maximum likelyhood, Parsimony and Neighbour joining methods of phylogenetic tree construction. Among the 18 genera analysed, *Trithemis*, *Neurothems*, *Tramea* and *Orthetrum* were resolved as monophyletic. The nucleotide distance between *Tramea limbata* and *Tramea basilaris* were found to be lowest and it was highest for *Potamrcha congener* and *Neurothemis tulllia*. Their study also states that there exists a significant correlation between species richness with temperature and humidity was found but rainfall did not significantly affect the species richness.

Ricardo et al. (2017) studied about the DNA barcoding of 38 Neotropical odonate species using COI gene sequences. The 130 cytochrome oxidase I genes sequenced from the collected specimens showed a distinct gap between 0-2% intraspecific and more than 15% intraspecific variations. Joan et al. (2017) resolved the DNA sequence divergence among the genus complex of *Sympatrum* using COI gene and ITS gene. The complex involved

4 species namely *Sympatrum vulgatum vulgatum, Sympatrum vulgatum decoloranum* and *Sympatrum vulgatum ibericum* in the west palaeartic. They have differentiated parapatric distribution and noticeable morphological differences in colour and body size to debate their taxonomic status. The phylogenetic tree based on the mitochondrial COI marker gene inferred that all sequences from *Sympatrum vulgatum* exhibit a highly supported clade. *S. vulgatum ibericum* and *S. vulgatum decoloration* were recovered as monophyletic. The maximum genetic distance of *Sympatrum vulgatum ibernicum* with respect to *S. vulgaticum decoloratum* is (3 mutation, 0.4 % uncorrected P distance) much lower than the typical divergence between generally accepted sister or closely related species of odonates. Based on the ITS sequence analysis also it was proved that *Sympatrum vulgatum* is the highly supported clade. It was observed that the COI gene sequences of *Sympatrum striolatum* were highly diverged (6.3%) from that of *Sympatrum vulgatum* and further remarkable sequence divergence (more than 10%) between *S. vulgatum* and *S. striolatum*.

Princess et al. (2018) generated around 134 COI barcodes from 36 morphologically identified species of odonates representing 10 families in 19 genera from the islands of Philippines archipelago. Their intraspecific sequence divergence ranges from 0 to 6.7 % with four species showing more than 2% while intraspecific sequence divergences from 0.5 to 23.3% with seven species show less than 2%. The geographic isolation between the islandes might have facilitated rapid speciation and resulted in low interspecies sequence divergences among closely related group of species.

The molecular phylogenetic relationships among members of different genera of Libellulidae were studied using 735bp of mitochondrial COI and 416bp of 16S ribosomal RNA gene sequences by Thomas et al. (2011). *Ladona* and *Plathemis* were often placed as subgenera of *Libellula* genus. Here

parsimony and maximum likelihood analysis of the separate and combined data sets indicated that *Plathemis* is a basal clade and *Ladona* is a sister clade to the remaining Libellulidae genera. Jessica et al. (2007) performed a well sustained phylogeny of the Libellulidae from 2 gene fragments of 16S and 28S rRNA. A total of 93 ingroup taxa and 6 outgroup taxa were amplified for 28S rRNA fragment and 78 ingroup taxa and 5 outgroup taxa were amplified for 16S rRNA fragment. Bayesian, likelyhood and parsimony analysis of the combined data produced well resolved phylogenetic hypothesis with the conclusion that the Macromiinae, Cordullidae and Libellulidae families of odonates are monophyletic. This study showed the inherent problem of using poorly developed inaccurately scored characters like wing venation for taxonomic identification.

Relationships of North American damselflies of the genus *Ischnura* (Coenagrionidae) were investigated using a total of 1205 bp portion of three mitochondrial genes cytochrome b, cytochrome oxidase II and 12S rDNA (Paul et al., 1999). Protein coding genes exhibited the greatest number of changes in the third codon position and the fewest at the 2^{nd} position. Cyt b is strongly towards transition at first and third positions with divergence between 0.4% to 16.9% and transversion accounts for second position substitution. Estimated number of transition and transversion substitutions for 12S rDNA appears to be equal and showed a divergence from 0 to 14.6%. The COII gene sequence examination showed that 131 of the 363 position were variable and of these 84 were parsimony informative. Phylogenetic analysis indicated that several species of *Ischnura* form monophyletic group within North America that likely is of recent origin. The analysis of taxa ranging throughout the Caribbean and the rest of American areas suggested that the North American Ischnuran fauna is having a Neotropical ancestry.

DNA barcoding approaches can be character based, where species are identified through the presence or absence of discrete nucleotide substitution within a DNA sequence. Potential of character based DNA barcodes were demonstrated by analyzing 833 Odonate specimens from 103 localities belonging to 64 species (Hadrys et al., 2006). Here mitochondrial NADH dehydrogenase I (NDI) gene region was explored for finding character based DNA barcodes for taxonomic units in odonates. The ND I has been successfully applied to phylogenetic and population genetic studies in odonates and found that it is well suited as alternative or compliment to COI sequencing. Similar reports were generated through the studies on 54 species and 22 genera of odonates by Rach et al. (2008).

Mitochondrial DNA barcode gene COI and morphological traits were used to reveal the relationship among four population of the Neotropical damselfly *Polythore procera* in the Colombian Andes foot hills. The lack of morphological differentiation coupled with 3% genetic divergence at the molecular level showed the phenomenon of cryptic speciation in this species (Mellisa et al., 2010*)*. Sandra et al. (2010) used character based DNA barcoding data method in a dragonfly model system and discovered two visually cryptic species *Trithemis stictica*. Deciphering NDI and COI gene sequences, three genetically distinct clusters of *Trithemis* species were discovered. Lin et al. (2010) studied the first complete mitochondrial genome structure of a damselfly, *Euphaea formosa* and reconstructed a phylogeny based on 13 protein coding genes of mitochondrial genomes in 25 representative hexapods to examine the relationships among the basal Pterygota. The gene arrangement, nucleotide composition and codon usage pattern of the mitochondrial gene arrangement are similar across these three odonate species suggested a conserved genome evolution within the Odonata members.

Sexual dimorphism is particularly high in the Libellulidae and Aeshnidae families of Odonates. Pushparaj et al. (2012) carried out DNA barcoding method using COI gene for the accurate identification of selected dragonfly species of the family of Libellulidae and Aeshnidae along with three other evident species retrieved from NCBI GenBank. The phylogenetic tree was created using NJ (Neighbour Joining) method to determine the origin and evolutionary relationships of the species. The GenBank results showed maximum identity of 100% for *Diplacodes trivialis*, 98% for *Bradinopygea geminata,* and 87% for *Anaciaeschna jaspidea*. Study concluded that the DNA barcoding is a valuable tool for the authentication of the species. Thus DNA barcoding provides crucial information in the cryptic species discovery and also to analyse the relationship among the dragonflies even up to sub species level.

Migratory behaviour is relatively unrevealed among the odonates species (Russel et al., 1998). Their migration is known to occur only in one direction and it is relevant to intraspecific phylogeographic studies because extensive gene flow will homogenize phylogenetic pattern. Artiss studied the phylogeography of a facultative migratory dragonfly, *Libellulla quadricacus* using COI gene in Asia, Europe and North America and proved that there is only limited genetic distance of 1-2% between populations and does not influence the phylogenetic relationships of population between continents (Artiss et al., 2001).

The identification of odonate larvae is a major challenge to scientists (Rach et al., 2008). It is always make difficulties for some morphologically similar species. But DNA barcoding allows for consistent and reliable results which compliments traditional morphological identification (Rach et al., 2008). Bedjanic et al. (2016) studied about the taxonomy and molecular phylogeny of the Platystictidae of Srilanka using molecular characters. About

five new species have been identified and described and all members showed monophyletic ancestry. One of the South Indian species *Platysticta deccanensis* was found not been placed under Srilankan Clade.

ODONATES OF KERALA

The Kerala state represents a narrow stretched land found in between Southern Western Ghats and Arabian sea. This geographical area is well known for vertebrate fauna and having a latitude of 8^0 18'-12^0 48' N. As this state is blessed with 44 rivers and having a average rainfall of 3107mm, it make a suitable habitat for many odonate species as they are being aquatic insects. A total of 154 species spreading in 79 genera and 12 families are known from Kerala (Kiran and Raju, 2013; Emiliyamma, 2005). In Kerala most of the information regarding odonate fauna was based on the works of Fraser (1933, 1934, 1936), Peters (1981), Rao and Lahiri (1982), Mathavan et al. (1989), Emiliyamma and Radhakrishnan (2000, 2002), Kiran and Raju (2011), Kiran and Kakkasery (2007) and Kakkasery (2005).

Shuan and Kakkassery (2013) conducted taxonomic and diversity studies of odonate nymphs by using their exuviae. According to them, exuvia can be used for the identification of nymphs at the species level without disturbing the live specimens and reported five new species belonging to 3 different families in Thrissur district, Kerala.

Odonates are ecologically important as the predators of many rice field pests (Fraser, 1933; Krishnaswamy, 1984; Gunathilagaraj et al., 1999). A survey conducted on the odonate diversity among the rice field of Palakkad district found that their maximum abundance was observed during harvesting stage (Palot et al., 2005). According to them paddy fields are continuously changing microhabitat and most of the species are voracious predators of rice pests resulting the maximum odonate diversity during cultivation season.

MATERIALS AND METHODS

1. Insect collection, identification and preservation

The adult specimens of odonates were collected from seven major districts of Northern Kerala. Collections were done mainly by hand net sweeping method and preliminary morphological identification was done by using authentic identification keys and guides. Expert taxonomist in the field of odontology, Dr. K. G. Emiliyamma, Scientist-D, Western Ghat Field Research Centre, Zoological Survey of India, Calicut was consulted for species confirmation. The identified specimens were photographed using different cameras such as Canon EOS 1200D and Nickond d40x. These specimems were stored at $^{-}20^{\circ}C$ 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 thoracic 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 provides 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 are eluted under low strength by Tris-EDTA buffer (TE buffer) or distilled water for permanent storage of DNA

(Esser, 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 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.

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, 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 at 50° C anneal and 45 sec 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 (Sambrook and Russell, 2001) and documented using a gel documentation system. A 1Kb DNA Ladder (Thermo Scientific GeneRuler, #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 JETTM PCR Purification Kit (Fermentas Life Science), 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 SciGenom 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 chromatogram 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) of INSDC (International Nucleotide Sequence Database Collaboration) and BOLD (Barcode of Life Data system) (Ratnasingam and Hebert, 2007).

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.

RESULTS AND DISCUSSION

The order Odonata, consisting of dragonflies and damselflies, are the most popular insects in the public figure. They are known to be existed in the carboniferous period along with mayflies and well known as the enchanting "Charismatic fauna" of the insect world due to the existence of variety of colours. They are the living representatives of primitive winged insects found in all biological realms except Antarctica. Historical studies proved that most of the species exists today are truly the descendants of proto-odonates and their fossils resembles to those existed in the Mesozoic era (Emiliyamma et al., 2005). Scientists still have a controversy for the correct phylogenetic position of dragonflies due to their unique flight and mating behaviour. As both life stages are tightly correlated with aquatic habitat, they are widely used for studying ecological, behavioural, biochemical and evolutionary aspects (Corbet, 1999).

Generally, most of the members have freely articulating head, narrow neck, short inconspicuous 3-7 segmented filiform antennae, biting and chewing mouth parts, reduced prothorax, fused meso and metathorax, thin long legs, membranous wings and long slender abdomen. Order Odonata has been divided mainly into 2 suborders due to the difference in morphology as Anisoptera (Dragonflies) and Zygoptera (Damselflies). Anisopterans have difference in the size of their forewings and hindwings while Zygopterans have similar sized. This is the crucial diagnostic feature for differentiating these two suborders.

The systematic and phylogeny based studies of Odonates were pioneered during 1980s. As insects are exposed to different ecological niche, they exhibit a great deal of morphological variation. Also their ability of metamorphosis from egg to adult makes a lot of variation in their morphology

causing superficial differences. This made the incorporation of molecular methods too in systematic studies. Molecular studies of the Order Odonata has been widely used since 2002 onwards and it is the most effortless, reliable and faster method for interpreting phylogeny. So the combined use of morphological and molecular methods became a more powerful and essential methodology for the prediction of phylogeny. The present study analyzed the morphological and molecular characterisation of 31 different odonate species under 4 superfamilies: Coenagrionoidea, Calopterygoidea, Gomphoidae, Aeshnoidea and Libelluloidea. The specimens were collected from the study area spanning the seven major districts of North Kerala.

STUDY AREA

The present study investigates the morphological identification, molecular and phylogenetic analysis of odonates from Northern Kerala. The Northern Kerala or Malabar area has been selected for the present study as it represents the western side of Western Ghats, blessed with abundant annual rainfall due to south west monsoon and thereby making a perfect homage for many odonate species. The study area consists of seven districts viz. Kasaragod, Kannur, Wayanad, Kozhikode, Malappuram, Palakkad and Thrissur (Table 1). Each district was represented by three different ecosystems such as Agro-ecosystem, Forest ecosystem and Riparian Ecosystem. The selection of adopting these three ecosystems was due to the high occurrence of odonates for various reasons such as social developmental activities, pest management strategies, food source availability and also their role in ecosystem nutrient recycling.

1. Riparian ecosystems

Riparian ecosystems are the transitional zone between aquatic ecosystem and terrestrial ecosystem. As it possesses the characteristics of

both these ecosystems, a good habitat is available for a variety of organisms. This ecosystem generally depends upon various climatic, hydrological and ecological environments and hence the biodiversity of each area will also changes. Also different latitudes and longitudes cause changes in the precipitation and temperature supporting differences in the riparian communities in different areas. Due to the above statements and odonates are being semi aquatic insects with their development strictly correlated with water habitat, Riparian ecosystems had selected as one of the study area for the present study. The selected riparian ecosystems specified in each district include Kasaragod: Periya (11.500º N 75 º50"E), Kannur: Koothuparamba (11.8319º N 75.655º E), Wayanad: Vythiri (11.5517º N 76.0403º E), Kozhikode: Beypore (11.1736º N 75.8040º E), Malappuram: Tirur (10.9146º N 75.9221º E), Palakkad: Ottapalam (10.7723º N 76.3695º E) and Thrissur: Peramangalam (10.5303º N 76.214º E). The location details were represented in Table 1 and site photographs were represented in Figure 1.

2. Forest ecosystems

Forest ecosystem includes biotic component of forest area. As these ecosystems have rich biodiversity they have unique exciting and fascinating features. Biota is changing over different seasons in forest and so the associated biodiversity will also be changes. Most of the adult dragonflies are often seen in forest ecosystem. Their ecology and special behaviour makes them unique to adapt into the forest ecosystem. In forest ecosystem, visual observation and selective catches with sweeping net are generally used for specimen collection. The significance of selecting forest ecosystem as study area are as follows. It offers abundant food sources mainly dipterans and hymenopterans that constitute a major food item for adult dragonflies. Hence they are incorporated into the trophic chains or webs of forest ecosystem for regulating the abundance of many insect species. Also they frequently

observed these areas in search of finding mate, mating and also the protection of home range. The selected forest ecosystems specified in each district include Kasaragod: Parappa (12.36745º N 75.22535º E), Kannur: Aaralam (11.9676º N 75.7720º E), Wayanad: Sulthan's Bathery (11.6656º N 76.2627º E), Kozhikode: Thusharagiri (11.473022º N 76.052896º E), Malappuram: Nilambur (11.2794º N 76.3695º E), Palakkad: Attapadi (11.114893º N 76.6180º E) and Thrissur: Peechi (10.5270382º N 76.36083 º E). The location details were represented in Table 1 and site photographs were represented in Figure 2.

3. Agro-ecosystems

Agro-ecosystem is an artificial ecosystem managed by humans for the production of plants and animals in accordance with their needs. This represents a highly dynamic ecosystems and nowadays monoculture is practicing everywhere. As this ecosystem is performing for getting economically beneficial crop yield, the pesticides are always practicing. This in turn alters the biodiversity of plants and animals. As odonates are general predators of a wide variety of crop pests and used in the crop management strategy, this ecosystem has been selected for the present study. Agro-ecosystems are used not only for the production of food but also the recycling of nutrients, regulation of microclimate, local hydrological process suppression of undesirable organisms and detoxification of noxious chemicals. The selected agro-ecosystems specified in each district include Kasaragode: Kangangad (12.332º N 75.096º E), Kannur: Payyanur (12.1051º N 75.2058º E), Wayanad: Pulpally (11.7923º N 76.1663º E), Kozhikode: Ramanattukara (11.1785º N 75.865º E), Malappuram: Villunniyal (11.1340º N 75.895º E), Palakkad: Thrithala (10.803º N 76.1349º E) and Thrissur: Kunnamkulam (10.601º N 76.202º E). The location details were represented in Table 1 and site photographs were represented in Figure 3.

The list and taxonomic key prepared for all the species selected under present study as per the suitable identification guides and their molecular characterization and phylogenetic analysis were also done based on mitochondrial cytochrome oxidase subunit I gene sequence is as follows:.

Systematic position of species selected for the present study

1. Suborder: Zygoptera

1.1. Super family: Coenagrionoidea (closed wings)

1.1.1. Family: Coenagrionidae (Pond damselflies)

1.1.1.1 Subfamily: Coenagrioninae

1. *Ceriagrion coromendelianum* (Fabricius, 1798)

1.1.1.2. Subfamily: Agriocnemidinae

2. *Agriocnemis pygmaea* (Rambur, 1842)

3. *Agriocnemis keralensis* Peters, 1981

1.1.1.3. Subfamily: Ischnurinae

4. *Ishnura aurora* (Brauer, 1865)

5. *Ishnura senegalensis* (Rambur, 1842)

6. *Aciagrion occidentale* Laidlaw, 1919

1.1.2. Family: Platycnemididae (Brook damselfly)

1.1.2.1. Subfamily: Platycnemidinae

7. *Copera marginipes* Rambur, 1842

1.2. Superfamily: Calopterygoidea

1.2.1. Family: Calopterygidae

1.2.1.1. Subfamily: Calopteryginae

8. *Vestalis apicalis* Selys, 1873

9. *Vestalis gracilis* (Rambur, 1842)

2. Suborder: Anisoptera

2.1. Superfamily: Aeshnoidea

2.1.1. Family: Gomphidae

2.1.1.1. Subfamily: Onychogomphinae

1. *Onychogomphus malabarensis* Fraser, 1924

2.1.1.2. Subfamily: Aeshninae

2. *Anaciaeschna jaspidea* (Burmeister, 1839)

3. *Anax parthenope* (Selys, 1839)

2.2. Superfamily: Libelluloidea

2.2.1. Family: Libellulidae (common skimmers)

2.2.1.1. Subfamily: Libellulinae

4. *Orthetrum sabina* (Drury, 1770)

5. *Neurothemis intermedia* (Rambur, 1842)

6. *Potamarcha congener* (Rambur, 1842)

7. *Brachydiplax chlybea* Brauer, 1868

8. *Trithemis aurora* (Burmeister, 1839)

9. *Neurothemis fulvia* (Drury, 1773)

10. *Crocothemis servilia* (Drury, 1770)

11. *Trithemis pallidinervis* (Kirby, 1889)

12. *Trithemis festiva* (Rambur, 1842)

13. *Brachythemis contaminata* Fabricius, 1793

14. *Diplacodes trivialis* (Rambur, 1842)

15. *Bradinopyga geminata* (Rambur, 1842)

16. *Rhyothemis variegata* Linneus, 1763

17. *Pantala flavescence* (Fabricius, 1798)

18. *Acisoma panorpoides* Rambur, 1842

19. *Neurothemis tullia* (Drury, 1773)

20. *Lathresia asiatica* (Rambur, 1842)

21. *Aethriamanta brevipennis* (Rambur, 1842)

22. *Brachydiplax sobrina (Rambur, 1842*)

Key to the suborders of Odonata

1. The forewings and hindwings are of the same shape and breadth, petiolate; eyes are well separated; slender body, male with two superior and two inferior anal appendages ..... Zygoptera
- The forewings and hindwings are of variable shape and hindwing usually broad at base, not petiolate; eyes are usually confluent across the middle line or separated (as in Gomphidae); stout body, male with two superior and one inferior anal appendage ..... Anisoptera

Key to the superfamilies of Zygoptera

1. Wings with two antenodal nervures, petiolate; postnodal nervures are in line with the cross veins below; pterostigma always present .... Coenagrionoidea
- Wings with more than two antenodal nervures; slightly petiolated; postnodals are not in line with the cross veins below ..... Calopterygoidea (Family: Calopterygidae); pterostigma absent (Genus: *Vestalis* Selys)

Key to the families of Superfamily Coenagrionoidea

- Discoidal cell elongate, the costal or anterior side slightly shorter than the basal, the distal end subacute..... Platycnemididae [genus *Copera* Kirby: two hind pairs of tibiae bright orange to dull reddish, moderately dilated; superior anal appendage only one fourth the length of inferiors (sp. *C. marginipes* (Rambur)]
- Discoidal cell short, the anterior side much shorter than the basal, the distal end very acute. ..... Coenagrionidae

Key to the genus of Family Platycnemididae

- Genus *Copera* Kirby :2nd segment of antennae as long as or even longer than the 3rd segment (sp. *C. marginipes* (Rambur)] two hind pairs of tibiae bright orange to dull reddish, moderately dilated; superior anal appendage only one fourth the length of inferiors

Key to the genera of Family Coenagrionidae

1. Arc situated at the level of the distal antenodal nervure 2
- Arc situated away from the distal antenodal nervure ....*Agriocnemis* Selys
2. *ab* (anal bridge) arising at the level of *ac;* a prominent ridge on the frons; pterostigma similar in shape and size in both wings of male; segment 10 without apical tubercles..... *Ceriagrion* Selys [sp. *coromandelianum* (Fabr.): abdomen bright citron yellow, without markings]
- *ab* arising at the level or well proximal to *ac*; no ridge on the frons; pterostigma different in shape and size in both wings of male; segment 10 of male with a pair of dorsal apical tubercles 3
3. *ab* arising well proximal to the level of *ac*; anterior border of thorax usually with a pair of small horseshoe shaped hooks; postnodal nervures 8 to 10 in the forewings ..... *Ischnura* Charpentier
- *ab* arising at the point where *ac* meets hinder border of wing; anterior border of thorax simple, without hooks; postnodal nervures 10 to 13 in forewings……..*Aciagrion* Selys [*A.occidentale* Laidlaw: very slender specimen; blue coloured with black markings on head, thorax and abdomen; abdominal segment 8 with a black elongate triangular mark]

Key to the species of genus *Agriocnemis* Selys

1. Labrum metallic blue; segment 2 without eyespots like spectacles; ....*pygmaea* (Rambur)

- Labrum non-metallic; segment 2 of abdomen with broad black marking along with a pair of small greenish eyespots like spectacles on the hood of a cobra .....*keralensis* Peters

Key to the species of genus *Ischnura* Charpentier

1. Abdominal segments 1 to 7 bright yellow, 8 to10 azure blue; pterostigma in forewing rose red, that in hindwing uniform pale grey ... . *aurora* (Brauer)

- Abdomen black, marked with yellow and blue, pterostigma in forewing black, that in hindwing uniform pale brown .... *senegalensis* (Rambur)

Key to the species of Genus *Vestalis* Selys

1. Tips of wings black; two rows of cells between origins of Cuii and IA-. .....*apicalis* Selys

- Tips of wings hyaline; two rows of cells between origins of Cuii and IA .....*gracilis* (Rambur)

Key to the super families of suborder Anisoptera

1. Discoidal cell in fore and hind wings more or less similar in shape..... .....Aeshnoidea (Family: Aeshnidae)

- Discoidal cell in fore and hind wings differs in shape 2

2. Eyes broadly confluent on vertex ...Libelluloidea (Family: Libellulidae)

- Eyes widely separated .....Gomphoidea (Family: Gomphidae)

Key to the genera of family Gomphidae

1. Discoidal cell, hypertrigone and subtrigone of both wings entire, anal loop present; anal triangle usually 4 celled; abdominal segment 8 and 9 without or with pseudo lateral dilations; superior anal appendage much straighter and curled only at tips; inferiors very closely apposed and curled strongly upto meet the superiors ....*Onychogomphus* Selys [sp. *O. malabarensis* Fraser

- Labrum yellow, with black border; frons black, with a broad yellow stripe; vertex and occiput black, with a yellow spot at the middle of occiput, which is raised into a small tubercle; only one row of cells between *Rii* and *IRii* ....*malabarensis* Fraser] (sp. *C. marginipes* (Rambur)]

Key to the genera of family Aeshnidae

1. Base of hind wing without a notch; tornus of hind wing rounded in both sexes; anal triangle absent; IRiii not forked; segments 4 to8 of abdomen with longitudinal supplementary ridges on the sides; superior anal appendages of male obtuse at apex..... *Anax* Leach [*A. parthenope* (Selys): thorax pale brown; three – fourth of wings tinted with yellow; frons with pale blue stripe]

- Base of hind wing deeply notched; tornus of hind wing angulated in the male, rounded in the female; anal triangle always present; *IRiii* forked into two equal branches near inner end of pterostigma; superior anal appendages with apex prolonged and curled downwards abruptly. ----------*Anaciaeschna* Selys [*A.jaspidea* (Burmeister): thorax reddish brown with two greenish yellow stripes; wings partly tinted with yellow]

Key to the genera of Family Libellulidae

1. Distal antenodal nervure in forewing complete.....2
- Distal antenodal nervure in forewing incomplete 3
2. Upper surface of frons metallic; wings with 6-9 antenodal nervures..... *Brachydiplax* Brauer [sp. *B.chalybea* (Brauer): bases of all wings and abdomen with burnt brown or golden brown colour]
- Upper surface of frons non-metallic; wings with 9-16 antenodal nervures ..... *Orthetrum* Newman [sp. *O.sabina* (Drury): abdomen extremely swollen at base, then very slim and narrow, at the end dilated and compressed; green with pale yellow markings on thorax and abdomen]
3. Sectors of arc lying between 2nd and 3rd antenodal nervure; upper surface of frons black, never metallic; wing base without black or reddish brown spot; thorax and abdomen with blue and yellow colour .. *Potamarcha* Karsch [P.congener (Rambur): adults with bluish black thorax and base of abdomen; remaining segments of abdomen bright yellowish, laterally black stripes, enclosed with yellow colour]
- Sectors of arc lying between 1st and 2nd antenodal nervure; upper surface of frons metallic violet, reddish or reddish brown; wing base with black or reddish brown spot 5
5. Wings with several cubital nervures in forewings and hindwings; reticulation very close; wing base black or reddish brown .... *Neurothemis* Brauer
- Wings with 1 cubital nervure in both fore and hind wings; reticulation not very close; wing base never black or reddish brown 6
6. Discoidal field strongly convergent at wing border in forewing; upper surface of frons metallic violet ..... *Trithemis* Brauer
- Discoidal field widely divergent at wing border in forewing; upper surface of frons bright blood red .....

Crocothemis Brauer [sp. C.servilia (Drury): Eyes blood red above; labrum, face, frons and vesicle bright blood-red; thorax bright reddish brown,; abdomen blood-red, a black mid dorsal stripe present along the whole length of abdomen; wing base yellowish brown]

Key to the species of Genus *Neurothemis* Brauer

1. Wings dark reddish brown from base to about middle of pterostigma, tips of wings also narrowly reddish brown to partly enclosing a clear and uncoloured area ..... *fulvia* (Drury)
- Base of all wings tinted with pale yellow, and with a broad basal amber yellow marking; costal and subcostal spaces also tinted with yellow and extend up to pterostigma.....*intermedia* (Rambur)

Key to the species of Genus *Trithemis* Brauer

1. Pterostigma bicoloured, black with creamy white ends; body yellow marked with black ..... *pallidinervis* (Kirby)
- Pterostigma uniclorous; body colour variable.....2
2. Thorax and abdomen violaceous crimson coloured; base of wings with a broad amber yellow fascia, with dark yellowish brown rays in subcostal and cubital spaces; neuration crimson..... *aurora* (Burmeister)
- Thorax and abdomen violaceous black; base of hindwing with a dark brown spot with dark rays in subcostal and cubital spaces; neuration black.....*festiva* (Rambur)

1. SUBORDER: ZYGOPTERA

Zygoptera represents one of the ancient suborder commonly called as “Damselflies”. They existed 250 million years ago with primitive proto Odonates existed in the Mesozoic Era (Grimaldi and Engel, 2005). This group have 2942 extant species listed in 309 genera categorised in 28 families

(Suhling et al., 2015). They are known to be geographically distributed in all biological realms except Antartica (Nilsson, 1997).

This suborder characteristically have widely separated eyes, fused thorax, a pair of tiny antennae, legs and long slender abdomen having 10 segments. The tenth abdominal segment characteristically has cerci and paraprocts (Paulson and Dennis, 2011). Their wings are similar in size, coloured or uncoloured and supported by many cross veins filled with Haemolymph (Silsby, 2001).

1.1. Superfamily: Coenagrionoidea (closed wings)

The superfamily Coenagrionoidea consists of most of the smallest and largest damselflies of varying colours. It includes 6 families and they are characterized by uncoloured closed petiolate wings consists of two antenodal nervures, uncrossed quadrilateral vein, fused anal vein at the base of wing and postnodal nervures are in line with the cross vein below. Pterostigma is always present.

1.1.1. Family: Coenagrionidae (Pond damselflies)

This is the largest and dominant damselfly family distributed globally. About 1100 species existing in this group and about 90 genera are currently accepted till to date. This family consists of six subfamilies which are: Agriocneminae, Coenagrioninae, Ischnurinae, Leptobasinae, Argininae and Pseudogrioninae. They are characteristically have black pattern, green, blue, yellow, orange or purple coloured body, colourless wings and small slender abdomen. Female members often exhibit polymorphism. The characteristic feature of this family are narrow stalked body, colourless and clear wings, two antenodal cross veins and vein M3 arising nearer to nodus than arculus (Kirby, 1890). The discoid cell of the wing is short in which the anterior side much shorter than the basal and the distal end is very acute.

1.1.1.1. Subfamily: Coenagrioninae

This dominant subfamily is geographically distributed in temperate and tropical regions. They can be easily diagnosed by having petiolate wings composed mostly of rectangular, trapezoidal and discoidal cells with separate anal vein, and sharp acute distal angle of the wing (Silsby, 2001).

The description on the morphological identification, molecular barcoding and molecular phylogeny analysis of each specimen under Zygoptera are as follows:

***Ceriagrion coromandelianum* Fabricius, 1798**

Ceriagrion coromandelianum is a medium sized green coloured damselfly having olive green thorax, bright yellow abdomen, yellow with black spined legs and transparent wings with golden yellow spot (Fig. 4). Female have golden brown thorax, olivaceous abdomen and wings with pale yellow spot (Subramanian, 2009). This species is always seen associated with ponds with male species specifically found in grasses besides aquatic habitat.This is widely distributed in Oriental regions (Subramanian, 2009).

The partial coding sequence of mitochondrial COI gene of *Ceriagrion coromendelianum,* collected from Palakkad district (10.4621° N 76.3950° E) of Kerala state has been amplified using the primer JCC (Table 2). The PCR amplification yielded a product of 573 bp amplified DNA. The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the figures 4 (a) to 4 (g) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KT222949 and Barcode of Life Data System BIN Cluster ID – BOLD: AA25825 with Specimen ID – GBMIN88578-17 (Table 65).

The COI sequence of *Ceriagrion coromendelianum* showed bias to nucleotide AT, with following composition of nucleotides T = 34.0%, C = 17.1%, A = 32.1% and G = 16.8% (Table : 3). This high AT content of 66.1% over 33.9% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The BLAST analysis showed that this species is having 100% sequence similarity to the same species reported from Belgium and Karnataka with respective accession numbers KU220871 and KT879897. The analysis involved 9 nucleotide sequences retrieved from NCBI and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 573 positions in the final dataset after eliminating all positions containing gaps and missing data. The phylogenetic tree plotted by Neighbour joining method depicts that the common ancestor of all the *Ceriagrion* members were spitted into 2 clades at an earlier time with one clade contains *Ceriagrion coromendelianum* and *Ceriagrion olivaceum* as sister clades while the other contains *Ceriagrion glabrum* and *Ceriagrion suave* (Fig. 4g). On the basis of COI gene similarity, *C. coromendelianum* species from Belgium and Karnataka were more closely related as they are seems to be sister taxa than compared with those species from Kerala. As all the 3 *Coromendelianum* sp. were found in one clade, we can confirm the molecular taxonomic identity of this species. The percentage of divergence table plotted by maximum likely hood method showed that the nearest neighbour of this species is found to be *C. olivaceum* followed by *Ceriagrion glabrum* and *Ceriagram suave* with a respective divergence of 0.18%, 9.24% and 11.53% (Table 4). The sequence has also submitted in BOLD system, another database to confirm the species authenticity. The analysis showed 98-99.82% sequence similarity to out of 20 different similar matches already reported in BOLD system. The line diagram of this species for the confirmation of above statement is shown in (Fig. 4f). The close matching BIN of the species is found to be 3% and the most similar species

was found to be *C. coromendelianum* reported from Mizoram having the accession number AAZ5825. The average and maximum nucleotide distance to this species is found to be 0.43% (p-distance) and 1.61% (p-distance) respectively. Here also the nearest neighbour was *Ceriagrion olivaceum* (BOLD: ADC4050) with an average and maximum nucleotide distance of 1.63% and 0.64% (p-distance) respectively. Thus this morphologically identified species was confirmed with their molecular taxonomic identity due to its high sequence similarity with the same species on various locations and also inferred its phylogeny.

DISCUSSION

Ceriagrion coromendelianum is a widely distributed Coenagrionidae species known from India (Prasad and Varshney, 1995), Sreelanka, Nepal, Pakistan and also certain records from China (Needham, 1931). This is one of the dominating species in the paddy fields as it feeds on various varieties of paddy pests like leafhoppers, plant hoppers, midges and flies (Krishnasamy et al., 1984). Hence this species is an ecologically beneficial insect due to its pest management strategy. Here we have done both the morphological and molecular identification of *C. coromendelianum* using conventional taxonomic keys and modern DNA based taxonomy. Both the result confirmed that the molecular identification method is strictly correlated with classical taxonomy. The cytochrome oxidase I gene of *Ceriagrion coromendelianum* yielded a product having 573bp amplified DNA and the correspondent 191bp long translated amino acid sequence. Both the resultant nucleotide and protein BLAST analysis from NCBI states that *Ceriagrion coromendelianum* found in Belgium and Karnataka were more closely related than from Kerala. As the branch length of a phylogram was strictly correlated with a specific trait like gene sequences and hence their difference showed a divergence in the evolution of time. The shorter branch length showed slower evolution

while the longer branch represents many sequence changes and faster evolution. The phylogenetic tree of *C. cormendelianum* showed that *C. coromendeliaum* from Kerala has a longer branch and may be having faster evolution than those compared from Belgium and Karnataka. Even though Karnataka and Kerala are adjacent states, the species in Begium and Karnataka were found to be closeier related each other. The Kerala species showed only 0.18% divergence to the same species reported from the above two. According to the BOLD system, if there existed a deep divergence of > 2% from the existing reports, we can confirm it as a new species. But here there was only a slight difference and hence it was confirmed strictly as *Ceriagrion coromendelianum*.

The phylogenetic tree constructed by Neighbour Joining method clearly showed that during an earlier period of evolutionary process the ancestors of *Ceriagrion* members had spitted into two different main clades, one contains *Ceriagrion coromendelianum* and *Ceriagrion olivaceum* as sister clades while the other contains *Ceriagrion glabrum* and *Ceriagrion suave*. The branch length of the tree suggesting that *C. coromendelianum* is phylogenetically more close to *C. olivaceum* followed by *C. glabrum* and *C. suave* (Jisha and Sebastian, 2015f). The percentage of divergence table plotted using Maximum Composite Likelihood model supported the above statement because of the respective divergence of 9.24, 11.53 and 11.81 respectively. Thus the results of both BOLD and NCBI database strictly confirmed the taxonomic identity of this species as *Ceriagrion coromendelianum*.

1.1.1.2. Subfamily: Agriocnemidinae

This subfamily encompasses the smallest damselfly group consists of only 5 genera of 63 species. Most members are geographically distributed in all tropical zones of the world. Their wings are characterized by having short

stems, scanty wing venation and differently shaped pterostigmas on fore and hind wing (Silsby, 2001).

***Agriocnemis pygmaea,* Rambur 1842**

This species popularly called as “Wandering midget “or ‘Pygmy darlet’ (Subramanian, 2014). These are green striped black coloured (males) or brick red coloured (females) sexually dimorphic Coenagrionidae member. Males have specifically black coloured eyes above and pale green coloured below. Thorax and abdomen were black in colour but provided with pale green coloured stripes on the sides of abdomen and the tip of the abdomen was found to be orange in colour (Fig. 5). Its wings were transparent having yellow coloured wingspot at forewing and black coloured in hindwing. Female species were differently coloured, some were similar to male and some members were rusty red in colour. Eyes specifically have blue coloured cap above with black colour and on dorsal region and green colour on ventral region. Its wings were also transparent but the wingspot was yellowish in both forewing and hindwing. It is usually observed during morning time.This species is widely distributed in Oriental regions.

The partial coding sequence of mitochondrial CO I gene of *Agriocnemis pygmaea* was PCR amplified using JAF as primer (Table 2). The PCR amplification of partial COI sequence of *Agriocnemis pygmaea* collected from Malappuram (11.0300° N 76.0500° E) district Kerala, India yielded a product having 567bp. The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST ,line diagram and molecular phylogenetic tree were presented in the figures 5(a) to 5(g) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KU871002 and Barcode of Life Data System BIN Cluster ID – BOLD: ADC3017 with Specimen ID – GBMIN88575-17 (Table 65).

The COI sequence of *Agriocnemis pygmaea* showed bias to nucleotide AT, with following composition of nucleotides T = 33.5%, C = 17.8%, A = 30.5% and G = 18.2% (Table 5). This high AT content of 64 % over 36% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The BLAST analysis showed that this species was very close to *Agriocnemis minima* reported from Thailand (KT957464). The analysis involved 12 nucleotide sequences retrieved from NCBI and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 567 positions in the final dataset after eliminating all positions containing gaps and missing data. The phylogenetic tree constructed by Neighbour joining method interprets that *Agriocnemis pygmaea* is having 100% sequence similarity to *Agriocnemis minima* reported from Thailand. The number of base substitutions per site between sequences is shown in Table 6 using the Maximum Composite Likelihood model. The above statement is strictly correlated to the percentage of divergence table plotted by maximum likely hood method (Table 5). The tree also confirmed the taxonomy of *Agriocnemis* genera as all the retrieved sequences of *Agriocnemis* sp were found in one clade. On the basis of COI gene similarity, this species is very close to *Agriocnemis minima*. Morphological characters also showed that this is very close to *Agriocnemis minima* than other members. Phylogenetic analysis and divergence analysis confirmed the genus taxonomy of this species as *Agriocnemis.* The sequence has also submitted in BOLD system, another database to confirm the species authenticity. The analysis showed 98-99 % sequence similarity to out of 20 different similar matches already reported in BOLD system. The line diagram of this species for the confirmation of above statement is shown in (Fig. 5f). The close matching BIN of the species is found to be 3% (BOLD: ADC3017) and the most similar species was found to be *Agriocnemis minima* reported from Thailand having the accession number

KT957464. The average and maximum nucleotide distance to this species is found to be 0.95% (p-distance) and 2.29% (p-distance) respectively. Here also the nearest neighbour was *Agriocnemis minima* (BOLD: ADC3017) with an average and maximum nucleotide distance of 0.61% and 1.22% (p-distance) respectively. Thus the above result confirmed that this morphologically identified providing a molecular id to easily spot and also to infer phylogenetic relationship with other Agriocnemidae members.

***Agriocnemis keralensis* Peter, 1981**

Agriocnemis keralensis represents one of the endemic species of Western Ghats known to be distributed only in five locations of Kerala and Goa (Kakassery, 2011). It has a dark coloured thorax with pale green stripes, pale green eyes, reddish orange abdomen, bluish white legs and wings with pale yellow spot. The second abdominal segment characteristically has a spectacle mark which is the easy diagnostic character. Female members exceptionally have an orange thorax and reddish orange eyes (Fig. 6). This species was often seen along with *Agriocnemis pygmaea* as small groups and usually found in grassy areas besides paddy fields and small streams.

The partial coding sequence of mitochondrial COI gene of *Agriocnemis keralensis* from Kannur district (12.8700° N 74.9000° E) of Kerala was PCR amplified using JPF as the primer (Table 2). The PCR product yielded about 628bp amplified DNA and 209 amino acid sequence. The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the figures 6 (a) to 6 (f) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KU133367 and Barcode of Life Data System BIN Cluster ID – BOLD: ACF9984 with Specimen ID – GBMIN88574-17 (Table 65).

The COI sequence of *Agriocnemis keralensis* showed bias to nucleotide AT, with following composition of nucleotides T = 30.3%, C = 19.3%, A = 31.7% and G = 18.8% (Table 7). This high AT content of 61.9% over 38.1% of GC was mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The nucleotide and peptide BLAST analysis of NCBI showed that it is 100% similar to *Agriocnemis forcipata* reported from Netherland (KF369284). The analysis involved 9 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 560 positions in the final dataset after eliminating all positions containing gaps and missing data. The phylogenetic tree constructed by Neighbour joining method interprets that *Agriocnemis keralensis* has been rooted from the ancestral clade which has spitted into two clades, one contains *Agriocnemis pygmae* and *Agriocnemis femina* as sister clades while the other contains *Agriocnemis keralensis* and *Agriocnemis femina*. These two species were evolutionarily very much related with each other. However tree depicts monophyletic ancestry to this *Agriocnemis* genus as all members were found in one clade. Molecular taxonomic relationship of this species to others was in the order as *Agriocnemis forcipata* followed by *Agriocnemis femina* and *Agriocnemis pygmeae*. This result was fully supported by the percentage of divergence table plotted by Maximum Likelihood method (Table 8). The table showed respective dibvergence of *Agriocnemis keralensis* to other members as 0% for *Agriocnemis forcipata*, 3.83% for *Agriocnemis femina* and 4.44% for *Agriocnemis pygmeae*. The sequence has also submitted to BOLD system database for ensuring the taxonomic conformity. The analysis also showed that this species has 100% sequence similarity to *Agriocnemis forcipata* having the BIN cluster ID (KF369284) and sequence ID (ODOPH089-13 COI -5P). It also showed around 99-100% sequence similarity to different *Agriocnemis* members reported in BOLD system.The above statement is truly

correlated to the line diagram plotted in BOLD system over 15 similar matches (Figure 6i). Thus for both NCBI and BOLD system, the barcode generated is a new report can be used to easily spot the specimen.

DISCUSSION

Agriocnemis keralensis is a small damselfly species known to be endemic to Western Ghats (Kakkasseri, 2011). As we know that odonates are indicators of healthy aquatic ecosystem, this species is known to be threatened in water polluted areas due to the continued use of pesticides on paddy fields (Kakkassery, 2011). Morphologically this species is very similar to *Agriocnemis pygmeae* and always seen associated with it grassy areas near paddy fields. Even though this species is morphologically similar to *Agriocnemis pygmeae*, it is phylogenetically more related to *Agriocnemis forcipata* on the basis of nucleotide sequences. The average high content of AT base pair over GC content is mainly due to the mutation pressure on the third position of nucleotide sequence. All the database analysis finally confirmed that this report is a novel one and confirmed the monophyletic ancestry to all *Agriocnemidae* members. As this species is endemic to Southern India, no other taxonomic work has been reported till date. The tree depicted that *Agriocnemis femina* and *Agriocnemis pygmeae* were rooted many years ago and *Agriocnemis keralensis* phylogenetically more close to *Agriocnemis femina* followed by *Agriocnemis pygmeae*. Thus the present work concluded that this morphologically identified species in Kerala has been provided with a molecular id to easily spot the specimen and also to infer phylogeny.

1.1.1.3. Subfamily: Ischnurinae

This widely distributed subfamily consists of 29 genera.Most of the species are characterised by having petiolate wings, a pair of spot or bands on the top of the occipit, different sized pterostigma in male and female wings.Female species often exhibits polymorphism and males are usually Andromorphs (Silsby, 2001)

Ishnura aurora Brauer, 1865

Ishnura aurora commonly called as 'Aurora blue tail' (Theischinger, 2006). Males are characterised by having black thorax with green stripes, greenish white legs, transparent wings with pterostigma is rose red in forewing and pale grey in hind wing (Fig. 7). Their second and seventh segments have upper narrow and broad black marks. The abdominal segments 1 to 7 are bright yellow, segments 8-10 are entirely azure blue and 10^{th} segment is having an upper black spot (Subramanian, 2009). Females are less bright coloured than males and do not have blue markings on abdomen (Manoj, 2011). This species usually observed besides ponds and rivers.

The partial coding sequence of mitochondrial COI gene of *Ishnura aurora* collected from Malappuram (11.0300° N 76.0500° E) district was PCR amplified using OCM as primer (Table 2). The PCR amplification of partial COI yielded a product of 628bp amplified DNA and 209bp translated amino acid sequence. The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the figures 7 (a) to 7 (f) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KR149808 and Barcode of Life Data System BIN Cluster ID – BOLD: AAH6873 with Specimen ID – GBMH0673-15 (Table 65).

The COI sequence of *Ischnura aurora* showed bias to nucleotide AT, with following composition of nucleotides T=34.3%, C=16.2%, A=31.8% and G=17.6% (Table: 9). This high AT content of 66.1% over 33.8% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The nucleotide and protein BLAST analysis of *Ischnura aurora* showed 100 % sequence similarity to the same species reported from Netherland (KF369414). The evolutionary history was inferred using the Neighbor Joining method and the analysis involved 10 nucleotide sequences and the codon positions included were 1st+2nd+3rd. All positions containing gaps and missing data were eliminated. There were a total of 487 positions in the final dataset and the evolutionary analyses were conducted in MEGA6. The phylogenetic tree constructed by Neighbour joining method showed that this species showed a sister taxa relationship to those those reported from Netherland. However tree analysis clearly shown that all Ischnura *aurora* members were found in one main clade arranged as sister taxa with each other indicating monophyletic ancestry. All the members were diverged from the main clade having the confidence value of 100 clearly supporting the above statement. Phylogenetically this species is more close to *Ischnura delicata* followed by *Ischnura verticalis* and *Ischnura asiatica* (Fig. 7g). This was supported by the percentage of divergence table plotted by Maximum likely hood method (Table 9). The result proved that this species has no sequence divergence to those reported from geographically different areas like Netherland and France and its close relatives were found to be respectively as *Ischnura delicate*, *Ischnura verticalis* and *Ischnura asiatica* with respective divergence of 0.01%, 0.05% and 0.54% (Table 10). This sequence has been submitted to BOLD system and found that about 99.82 – 100% sequence similar to the same species reported from various geographically isolated regions. This is supported by the line diagram of 25 different matched

sequences already reported in BOLD system (Fig. 7f). The close matching BIN of the species is found to be 3% and the most similar species is found to be *Ischnura aurora* reported having the accession number AAH6873 with an average distance of 0.34% (p-distance) and maximum distance of 1.18% (p-distance). The nearest member of this species is found to be *I. delicata* with an average distance of 0.04 % and 0.44 % maximum distance (AB22396).

DISCUSSION

Ischnura aurora was popularly known as 'aurora blue tail' (Theischninger and Endersby 2009) due to the presence two blue colour markings on the 8^{th} and 9^{th} abdominal segment. They are geographically distributed in Australia, Pacific Islands, East Asia and South East Asia (Dow et al., 2013). According to Westfall and May (1996), *Ischnura aurora* is considered to be a cosmopolitan genus consisting of 69 species lists widely distributed in North America, Eurassia, India and South China. This species is found to be originated during Oligocene period during 25-45 million years ago (Bechley, 2000).

In the present work, the morphological identification of this species was done using available keys (Emiliyamma et al., 2005) and molecular identification and phylogeny using cytochrome oxidase I gene analysis. This is a pioneer work from India and found that this species doesn't have any sequence divergence till now compared with those seen in different geographically isolated countries. The tree depicts that all *Ischnura aurora* members were having a common ancestry or they are more specifically monophyletic in origin. This was supported by the analysis of molecular taxonomy of *Ischnura* genus done by Dumont (2013) who made the phylogeny of different *Ischnura* members with special emphasis on the old world taxa. Here the result made an assumption that this species doesn't have any sequence divergence as time progresses and strictly it is a monophyletic

species with its nearest members were found to be *Ischnura delicate* followed by *Ischnura verticalis* and *Ischnura asiatica* (Jisha and Sebastian, 2015b). Even though there exists a slight difference in the morphology of different Ischnidae members no classical taxonomic work has been reported till now. But it was shown that classical taxonomy moves hand in hand with molecular taxonomy as no sequence divergence has been reported in almost all phylogeny based works. Hence the present study helped to confirm the taxonomy and the barcode generated can be used to easily spot the specimen and also for interpreting phylogenetic relationships.

***Ishnura senegalensis* (Rambur, 1842)**

Ischnura senegalensis have black to bronze black thorax, black legs, hyaline wings with diamond shaped pterostigma which is black in forewing and uniform pale green in hindwing (Dawn, 2018). The tibiae and tarsi are yellow coloured and its abdomen is black in colour marked with yellow and blue (Fig. 8). *Ischnura senegalensis* represents one of the most wide spread species of Ishnurinae subfamily commonly observed in slow stagnant waters and forests free areas (Dayakrishna, 2015; Manoj, 2011).

The partial coding sequence of mitochondrial COI gene of *Ishnura senegalensis* collected from Thrissur district (10.5200° N 76.2100° E) was PCR amplified using JAP as primer (Table 2). The PCR product yielded a 603bp amplified COI DNA segment. The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the figures 8 (a) to 8 (f) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KT305961 and Barcode of Life Data System BIN Cluster ID – BOLD: ABW0501 with Specimen ID – AGIR1303-17 (Table 65).

The COI sequence of *Ischnura senegalensis* showed bias to nucleotide AT, with following composition of nucleotides T=34.2%, C=18.1%, A=30.3% and G=17.4% (Table 11). This high AT content of 64.5% over 35.5% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The BLAST analysis of both nucleotide and protein sequences showed that this species is having 100% sequence similarity to the same species reported from Japan having the accession number (AB758088). The evolutionary history was inferred using the Neighbor-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 10 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 603 positions in the final dataset after eliminating all positions containing gaps and missing data. Here also the tree depicts that all *Ischnura senegalensis* members have a monophyletic origin with the indication of the common ancestry. The percentage of divergence table plotted by Maximum likelihood also states that no sequence divergence to the species with those reported from various geographically isolated areas (Table 12). The sequence has also submitted for BOLD system to confirm the species authenticity. The analysis showed 99.83-100% sequence similarity to out of 23 different matches of the same species already reported in BOLD system. The line diagram of this species for the confirmation of above statement is shown in (Fig. 8h). The close matching BIN of the species was found to be 3% and the most similar species was found to be having average and maximum nucleotide distance as 0.43% (p-distance) and 1.3%(p-distance) respectively. The nearest neighbour (BOLD ID: ADC4050) was found to be having 0.27% (p-distance) and 1.22% (p-distance).

DISCUSSION

Ischnura senegalensis is popularly known as ‘Senegal golden dartlet’. They are widely distributed in India, Oriental and Ethiopian regions (Manoj, 2011). This species is a highly ubiquitous member seems to be salt and pollution tolerant as it is seen in many stagnant and slow water bodies. This species is known to be having high survival chance even in the unfavourable condition because of short adult pre-reproductive phase (Kadoya et al., 2008). This species was having a habit of migration reported in Cape Verde. Here the morphological identification was done using available keys (Emiliyamma et al., 2005) and molecular identification with phylogenetic status also with cytochrome oxidase I gene marker. This is a pioneer work from India and the barcode generated can be used to identify and analyse the relationship of this species to other geographically isolated areas.On the basis of certain morphological features like vulvar spine (Hovmoller, 2006) there existed a monophylety for all Ischnurinae members having good genetic support (Sharma and Clausnitzer, 2016). However all the database analysis clearly showed that there is no sequence divergence to this species reported, even from the geographically isolated areas, confirming the conserved nature of COI gene sequences during its evolution. Thus allopatric speciation doesn’t work here during the course of evolution. Hence the species identity was confirmed in both morphological and molecular level.

Aciagrion occidentale Laidlaw, 1919

Aciagrion occidentale is a Coenagrionidae member widely distributed in India, Srilanka, Vietnam and Thailand. The body is long and slender provided with pale green stripes on black thorax with long slender blackish abdomen tipped with black spot on the last abdominal segment (Fig. 9). They occur as loose groups. It is commonly seen in marshy land and usually distributed in India, Vietnam, Sreelanka and Thailand (Mitra, 2010).

The partial coding sequence of mitochondrial COI gene of *Aciagrion occidentale* collected from Malappuram (11.0300° N 76.0500° E) district of Kerala was PCR amplified using AOD as primer (Table 2). The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the Figures 9 (a) to 9 (f) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KM096996 and Barcode of Life Data System BIN Cluster ID – BOLD: ACG1133 (Table 65).

The COI sequence of *Aciagrion occidentale* showed bias to nucleotide AT, with following composition of nucleotides T = 34.4%, C = 17.5%, A = 29.2% and G = 19.0% (Table 13). This high AT content of 63.6% over 36.4% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The BLAST analysis of both nucleotide and protein sequences showed that this species is having 100% sequence similarity to the same species reported from Netherland having the accession number (KF369275). The evolutionary history was inferred using the Neighbor-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 13 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 522 positions in the final dataset after eliminating all positions containing gaps and missing data. Here also the tree depicts that Aciagrion occidentale have a sister clade relationship with *Aciagrion boorenense* and this clade is sister to other damselfly members like *Enallagma* sp, *Ischnura asiatica*, *Africallagma elongatum* with the indication of confirming Zygoptran phylogeny. The above statement is confirmed by the percentage of divergence table plotted by Maximum likelihood (Table 14).

The sequence has also submitted in BOLD system, another database to confirm the species authenticity. The analysis showed 98-99.82% sequence similarity to out of 12 different similar matches already reported in BOLD system. The line diagram of this species for the confirmation of above statement is shown in (Fig. 9f). The close matching BIN of the species is found to be 3% and the most similar species was found to be *Aciagrion occidentale* (BOLD: ACG1133*).* The average and maximum nucleotide distance to this species is found to be 0.3% (p-distance) and 1.37% (p-distance) respectively. Here also the nearest neighbour was found to be *Aciagrion hispa* (BOLD: ADC4230) with an average and maximum nucleotide distance of 0.95% and 1.68% (p-distance) respectively. Thus the above result confirmed species identity by providing a unique molecular id and also to infer its phylogenetic relationship.

DISCUSSION

Aciagrion occidentale is popularly known as “Green striped slender darlet”. As this species is very small in size, it is well known for being migration (Fraser, 1933). Morphological identification done with available taxonomic keys and expert consultation confirmed its species identity. Molecular identification done using cytohrome oxidase I gene analysis by both BOLD and NCBI confirmed its generic taxonomy. Phylogenetically this species is close to *A. boorneense* by NCBI analysis while *A. hispa* by BOLD. This is a pioneer molecular work from Kerala and the barcode generated can be used to easily spot the specimen and also for resolving its phylogeny (Jisha and Sebastian, 2015a).

1.1.2. Family: Platycnemididae (Brook damselfly)

This family is commonly known as 'white legged damselflies' consisting of 42 genera including 400 species. They often found among long grasses bordering brooks and streams. They are characterised by laterally expanded heads with shallow labial cleft and tibiae with long dense spines (Rehn, 2003; Carle et al., 2008). Members of this old family can be easily identified by having dilation of the tibiae in males and sometimes in female (Silsby, 2001). The two hind pairs of tibiae are bright orange to dull reddish, moderately dilated and the superior anal appendage are found only one fourth of the length of inferiors. The discoidal cell of wing is elongate with its costal or anterior side is slightly shorter than the basal and its distal end seems to be subacute.

Copera marginipes Rambur, 1842

Copera marginipes is characterised by having bronze black colour with yellow lines on thorax, bright yellowish orange legs, transparent wings with brown wing spot and bronze black coloured abdomen in males (Fig.10). Female members are brown coloured thorax, brownish legs, transparent wings with pale brown coloured wing spot and brown coloured abdomen (Subramanian, 2009). This species is usually observed in streams. It is widely distributed in Asia and Newguinea.

The partial coding sequence of mitochondrial COI gene of *Copera marginipes* collected from Kasaragod (12.5000° N 75.000° E) district was PCR amplified using OCM as primer (Table 2) and yielded a product having 616bp and translated sequence of 205bp. The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the figures 10(a) to 10(g) respectively. The sequence was

deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KR149804 and Barcode of Life Data System BIN Cluster ID – BOLD: ABA1480 with Specimen ID – GBMH0650-15 (Table 65).

The COI sequence of *Copera marginipes* showed bias to nucleotide AT, with following composition of nucleotides T =3 4.6%, C = 15.8%, A = 31.4% and G = 18.3% (Table 15). This high AT content of 66.0% over 34.1% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time. The BLAST analysis showed that this species is 100% sequence similar to the same species reported from Netherland (KF369351).

The evolutionary history was inferred using the Neighbour-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 8 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 602 positions in the final dataset after eliminating all positions containing gaps and missing data. The phylogentic tree confirmed the taxonomic identity of this species as *Copera marginipes* due to sister taxa relationship with the same species. Phylogenetically this clade is sister to the clade possessing *Copera nyansana* and *Copera silikkassoeni*s indicating genus level taxonomy and monophyletic origin (Fig. 10g). The percentage of divergence table plotted by Maximum Composite Likelihood model confirmed the above statement. The nucleotide sequence showed respective divergence of 16.02-17.18% with *Copera nyansana* and *Copera sikassoensis* (Table 16). The sequence has also submitted for BOLD system, another database to confirm the species authenticity. The analysis showed 99.82-100% sequence similarity to out of 15 different similar matches already reported in BOLD system. The line diagram of this species for the confirmation of above statement is shown in

figure: 10f. The close matching BIN of the species was found to be 3% and its average and maximum distance was respectively as 0.88% and 2.5% (BOLD: ABA1480). The average and maximum nucleotide distance to the nearest member was found to be 0.43% (p-distance) and 1.61% (p-distance) respectively (BOLD: ADC5413). The 100% similar species was found to be reported from Gujarat and Malaysia having accession numbers (BOLD: ABA1480 and KF369351).

DISCUSSION

Copera marginipes is popularly known as 'Yellow bush dart'. The highest diversity of this species has been reported from tropical Asia, Southeastern Asia and New Guinea (Lim et al., 2013). Morphological identification of the species was done using taxonomic key (Emiliyamma et al., 2005) and with online photographs. The wing venation and other morphological characters of this species clearly described that it is a Plactinimidinae member having the unique features of *Copera marginipes.* Morphological features confirmed a monophyletic assemblage to all Platicnemidae members due to its characteristic feather like tibiae (Dijkstra et al., 2014). Most of the male members of this family are having white, yellow, orange, red, blue or black tibiae. Molecular identification and phylogenetic status of this species clearly states that the conserved sequence of cytochrome oxidase I gene doesn't have major evolutionary change as time progresses. Those species reported from Gujarat and Malaysia are 100% sequence similar indicating no means of sympatric speciation. The phylogenetic tree says that the ancestors of *Copera* genus were splitted at an earlier time with *Copera nyansana* and *Copera sikassoensis* were found in one clade as sister taxa and *Copera marginipes* from Kerala and Malaysia were found in another clade as sister taxa. Phylogenetically its nearest member was found to be *Copera nyansana* and *Copera sikassoensis* with

respective divergence of 16.02 and 17.18%. The above result is confirmed by the previous works done by Lim et al. (2013). *Copera marginipes* was found to be evolved for the first time followed by *Copera sikassoensis* and *Copera nyansana.* Thus the result confirmed that all the genera have splitted from one clade indicating monophyletic origin. Most of the morphological unique features indicated phylogeny shown that this genus has been originated in Eastern Asia (Indonesia to Japan) and it is strictly a Palaearctic representative (Dijkstra et al., 2014). Thus both morphology and molecular analysis provided a unique result and its molecular taxonomic id can be used to easily spot the species and also to infer evolutionary relationships.

1.2. Super family: Calopterygoidea

The Superfamily Calopterygoidea is characterised by slightly petiolated broad winged damselflies possessing two antenodal nervures on wings. The postnodals are not in line with the cross veins below.

1.2.1. Family: Calopterigidae (Broad winged damselfly)

These are broad winged, 1.5 to 2.5 long small damselflies commonly observed as metallic green or black coloured ones. They have long antennae, long and slender abdomen, broad blue coloured wings in males and brownish to green coloured wings in females. Their wings are heavenly veined consists of 18 or more antenodal veins. This family constitute about 16 genera consisting of 161species (Cordoba and Adolfo, 2005). They are often seen associated with forests, streams and rivers.

***Vestalis apicalis* Selys, 1873**

This species has an emerald coloured body with green coloured head, thorax and abdomen, dark brown eyes, brown coloured legs and amber

coloured wings having black tip (Fig. 10). This species is observed in both forest and also certain streamy areas.

The partial coding sequence of mitochondrial COI gene of *Vestalis apicalis* collected from Malappuram (11.0300° N 76.0500° E) district was PCR amplified using JAG as primer (Table 2). The PCR amplification yielded a product having 561bp amplified COI segment of DNA. The sequence obtained, DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the figures 11 (a) to 11 (g) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KU510326 and Barcode of Life Data System BIN Cluster ID – BOLD: ACS6273 with Specimen ID – GBMIN88573-15 (Table 65).

The COI sequence of *Vestalis apicalis* showed bias to nucleotide AT, with following composition of nucleotides T = 30.3%, C = 22.1%, A = 27.5% and G = 20.1% (Table: 17). This high AT content of 57.8% over 42.2% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The 561bp sequence obtained by amplification process yielded 187bp translated amino acid sequence.Both nucleotide and protein BLAST analysis showed that this species is 100% sequence similar to the same species reported from Kerala (KM675770). The evolutionary history was inferred using the Neighbour-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 10 nucleotide sequences and the codon positions included were 1st+2nd+3rd. All positions containing gaps and missing data were eliminated and there were a total of 555 positions in the final dataset. Evolutionary analyses were conducted using MEGA7 software and the phylogenetic tree constructed by Neighbour joining method showed a

sister clade relationship to the same species reported from Kerala indicating no divergence. The phylogenetic tree interprets that it showed a closer relationship with *V. gracilis* and its nearest neighbour is found to be *V. ambalis* (KF369576). The percentage of evolutionary divergence table confirmed this result due to the divergence of 0%and 21.83% with *Vestalis gracilis* and *Vestalis ambicalis* (Table 18). The sequence has also submitted for BOLD system, another database to confirm the species authenticity. The analysis showed 99-100% sequence similarity to out of 4 different similar matches already reported in BOLD system. The line diagram of this species for the confirmation of above statement is shown (Fig. 11f).

DISCUSSION

Vestalis apicalis is popularly known as 'Black tipped forest glory' and found to be geographically distributed in India and Sreelanka (Dow, 2009). Morphological identification of this species has done with available keys (Emiliyamma et al., 2005) and also with online photographs. Morphologically this species seems very close to *Vestalis gracilis* and they were always seen flying together in forest habitat. These 2 species can be easily distinguished by looking at its wings; *V. apicalis* is having a black spot at the extreme end of the transparent wing while the other (*V. gracillis*) is without the marking. They were usually seen associated aquatic habitat in the forest ecosystem (Manoj, 2011). Molecular identification method in NCBI and BOLD database showed the conformity of this species as *Vestalis apicalis*. Here also the phylogenetic relationship showed that it was very close to *Vestalis gracilis* morphologically. Phylogenetic tree interprets that all *Vestalis* genera have a common ancestry as all are bifurcated from one clade. It showed that the ancestor has been diverted into 2 clades at an earlier time with one clade contains *Vestalis apicalis* and *V. gracilis* as sister taxa while all other closely related damselflies on another clade. Thus result confirmed the *Vestalis*

genera and also Zygopteran phylogeny. All the concerned species in the tree may have evolved from their common ancestor at different period of time and found in separate clades in relation with littile differences in the nucleotide sequences. Thus both classical taxonomy and DNA barcoding technique provided a better taxonomic tool for confirming the taxonomic identity and prediction of evolutionary relationships.

***Vestalis gracilis* (Rambur, 1842)**

Vestalis gracilis is characterised by iridescent emerald coloured thorax and abdomen, dark brown coloured legs, dark brown eyes and transparent with a blue sheen wings in male (Fig. 12). Female members are exceptionally having dull coloured abdomen (Subramanian, 2009). This Calopterygidae species is often seen in shady forest paths and edges of streams.

The partial coding sequence of mitochondrial COI gene of *Vestalis gracilis* collected from Kozhikode district (11.1352° N 75.8933° E) was PCR amplified using JAG as primer (Table 2). The PCR amplification product yielded 587bp long amplified DNA. The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the figures 12(a) to 12(g) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KX503058 and Barcode of Life Data System BIN Cluster ID – BOLD: ACS6273 with Specimen ID – GBMIN88573-17 (Table 65).

The COI sequence of *Vestalis gracilis* showed bias to nucleotide AT, with following composition of nucleotides T = 32.1%, C = 21.1%, A = 27.0% and G = 19.9% (Table: 19). This high AT content of 59.1% over 41% of GC

is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The 561bp sequence obtained by amplification process yielded 187bp translated amino acid sequence. Both nucleotide and protein analysis showed this species is having 100% sequence similar to the same species. The evolutionary history was inferred using the Neighbour-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 10 nucleotide sequences and the codon positions included were 1st+2nd+3rd. The phylogenetic tree showed that all *Vestalis gracili*s were found in one clade with a sister clade relationship with *Vestalis apicalis.* This is again supported by the divergence table plotted by Maximum Likelihood (ML) method in which the conserved sequence doesn't have any kind of sequence variation as it showed 0% divergence to all *Vestalis* members (Table 20). The sequence has also submitted for BOLD system for confirming species authenticity. The analysis showed 99-100% sequence similarity to the same species by analysing 4 different similar matches reported in BOLD system. The line diagram of this species for the confirmation of above statement is shown in (Fig. 12f). The close matching BIN of the species is found to be 3% with 100% sequence similarity (BOLD: AC S6273) having the average and maximum distance of 0.89% (p-distance) and 3.07% (p-distance) respectively. The closest neighbour is found to be *Vestalis ambalis* having the distance of 17.18% (p-distance) with 84.38% sequence similarity (Table 20)

DISCUSSION

Vestali gracilis is popularly known as 'Clearwinged forest glory'. This species is geographically distributed in South East Asia (Dow, 2009). Morphological identification clearly proved that it is strictly *Vestalis gracilis* species as per the taxonomic key (Emiliyamma et al., 2005). Cytochrome oxidase I gene analysis also showed the same result due to similarities in the

conserved nucleotide sequence. Both NCBI and BOLD analysis confirmed its taxonomic identity and the unique barcode generated can be used to spot the specimen very easily. Phylogenetic tree showed that the nearest member is *Vestalis apicalis* and nearest genera is *Vestalis ambicalis.* The tree also depicts the close relation of this species to other odonate families like Aeshnidae (*Anax*) and Libellulidae (*Aethriamanta*) (Fig. 12g). Thus the result showed that zygopteran members are having a closer relationship with other anisopteran members indicating different period of time of origin. Thus it can be confirmed the result that all odonate members are interrelated on the basis of nucleotide sequences and may have evolved at different period of time.

PHYLOGENETIC STATUS OF ZYGOPTERAN MEMBERS

Zygoptera represents small slimmer bodied odonates with their ancestors known to be existed in Eocene period over 311-30 million years ago, called protozygopterans. There are about 2942 extant species of damselflies spreading in 309 genera were reported. They are distributed in almost all biological region with maximum diversity found in Oriental region (Suhling, 2015). The families like Coenagrionidae, Platycnemidae and Platystictidae were the dominant ones across world. Zygopterans are characterised by having similar sized hindwing and forewing, widely separated eyes and wings are placed vertically at rest (Needham, 1903). They generally feed on flies, mosquitos and other small insects and existed in a variety of habitats. Most of the damselflies are indicators of ecosystem quality since their larval development depends on water depth, water movement and pH (Katherine, 2009).

The mitochondrial genome is known to be evolved considerably faster than nuclear gene and hence it has many merits for predicting phylogenetic divergence. Also the nucleotide substitutions were considered to be lower in Nuclear DNA compared to mitochondrial DNA (Brown et al., 1979; 1982).

The commonly used markers for resolving phylogeny from species to family level were 16S and 28S and COI gene. The present study is a pioneer work from Kerala and it confirmed the taxonomic identity and phylogenetic relationship of different damselfly species found in Kerala. Here different species of damselflies from 7 major districts of Northern Kerala were identified morphologically by the available taxonomic keys (Emiliyamma et al., 2005; Subramanian, 2009; Fraser, 1936) and examined for molecular analysis to confirm its taxonomic identity and also for the analysis of its phylogenetic relationships. Here 3 different families of Zygopteran suborder have been taken to analyse the above taxonomic assessment.

Platycnemididae

Platicnemididae represents one of the family under study consisting of about 400 species worldwide which usually seen associated with streams and rivers. Adults often have laterally expanded heads with shallow labial cleft and no trace of postfrontal suture and tibiae are provided with dense long spines. Platycnemidinae seems to be a sister clade to Coenagrionoidea. The genus *Copera* is limited to the palaeartic region and the species *Copera marginipes* has unique larval characters and adults are provided with feather like tibiae with dense spines (Rehn, 2003; Carle et al., 2008). Eventhough the barcode sequence has also be reported from Netherland, this conserved sequences doesn't have any kind of sequence divergence indicating neutral speciation. The generic status of this species confirmed monophyly indicating *Copera marginipes* (India and Netherland) were found in one clade and *Copera nyasana* and *Copera silikkasosis* in another clade. This monophyly of *Copera marginipes* were also supported by the previous work of Lim et al. (2013). *Copera marginipes* showed closer relationship to other Coenagrionidae members like *Agriocnemis* sp and *Ceriagrion coromendelianum* in the phylogenetic tree. This indicates a closer relation of

Platicnemididae and *Coenagrionidae* families (Fig. 13). This result was well supported by the previous works of Dumont et al. (2010) and Bybee et al. (2008).

Coenagrionidae

Coenagrionidae represents one of the largest damselfly family found in all biological realms .It consists of about 1100 species distributed cosmopolitically. It represents the most dominant damselfly family in every checklist studies of odonate population (Jisha and Sebastian, 2015c). It consists of 3 families and 6 subfamilies from which Agriocnemidae, Coenagrionidae and Ishnurinae subfamilies were selected for the present taxonomic studies. The phylogenetic tree plotted by using Neighbour joining method confirmed monogeneric status of the respective individuals in the concerned subfamily (Fig. 13). All members in the Ischnurinidae subfamily are having monophyletic ancestry (Jisha and Sebastian, 2015b; Hovmoller, 2006) because all species were found in one clade and it is sister to the monophyletic *Agriocnemis* members. The tree also depicts that *Copera marginipes* in Platycnemidae family is found sister to *Agriocnemis* species indicating a closer relation with Coenagrionidae family. Thus the phylogenetic tree plotted for all zygopteran members showed a monophyletic relationship with a closer relationship of Coenagrionidae and Platicnemididae. The above statement found well supported by the previous works of Dumont et al. (2010) and Bybee et al. (2008).

Calopterygidae

Calopterygidae is also another Zygopteran family with most of the species are confined to forest ecosystems. Two species of *Vestalis* viz. *V. apicalis* and *V. gracilis* were taken in the present study to make a taxonomic conformity through morphological validation and DNA sequence analysis.

The result confirmed a monophyletic ancestry to this genus. Most of the previous works done in this family provided a monophyletic ancestry with good support (Rehn, 2003; Bybee et al., 2008). The *Vestalis ambalis* and *Vestalis smaragdina* were found as sister clades (Klass et al., 2014). Taxonomically this family is found outer to the clade containing Ischnurinae and Coenagrionidae.

There exist a lot of controversies whether the suborder is coming under Zygoptera and also whether this suborder is monophyletic or paraphyletic. According to Bechley (1996) and Truemann (1996), Zygoptera generally have a paraphyletic ancestry on the basis of many morphological features and molecular characters (Saux et al., 2003). But Rehn (2003) produced a controversial result that this suborder has monophyletic ancestry on the basis of morphological features only because they used detailed analysis of merely the skeletal morphology and wing venation characters. Kjer et al. (2004) produced the same results on the basis of 18S rRNA sequences. Both morphological and molecular analysis of the concerned families was strictly correlated to those reports already done in various locations. The phylogenetic tree plotted by using morphological features and molecular method strongly suggested the closer relationship of Coenagriondae and Calopterygidae (Pfau, 1991; Bechly, 1996; Rehn, 2003; Carle, 1982; Fleck et al., 2008) while the combined analysis of both of these showed that there is existing a monophyly to this suborder with a close relationship of Coenagrionidae and Platycnemididae family as sister taxa and Calopterygidae family seems to be separate to this clade (Dumont et al., 2010; Bybee et al., 2008). Phylogenetic tree by Neighbour joining method showed monogeneric clade to some families (Calopterygidae) and Coenagrionidae. Klass et al. (2014) studied about the comprehensive molecular phylogeny of Zygopterans in which all traditional families recovered are monophyletic but reorganised the superfamily Coenagrionidae into 3 families: Isosctictidae, Platycnemididae

and Coenagrionoidea. They have proved COI, 16S rRNA and 28S rRNA genes as the most accurate molecular markers in Odonata and established that it provides well resolved and supported trees from species to family level. The findings of Silsby (2001) seem better applicable for the definition of representing families as it is strictly on the basis of traditional classification. Calopterygidae families were well studied by Dumont et al. (2005). The COI, 16S rRNA and 28S rRNA gene sequence data provided the best phylogenetic trees from species to family level. Thus the present study confirmed the taxonomic identity of all species both morphologically and also at the molecular level and the closer association of the 3 families was established as Coenagrionidae and Platicnemididae in the sister clades with Calopterygidae found outer to this clade.

II. SUBORDER: ANISOPTERA

This suborder consists of dragonflies existed 250 million years ago in the carboniferous period. This group composed of 3012 species in 348 genera and 11 families (Suhling, 2015). They are characterised by robust stout body, prothorax covered by pronotum, fused meso and metathorax (synthorax), stout abdomen having 10 segments, a pair of compound eyes and legs and different sized forewing and hindwing having pterostigma. The characteristic feature is the differently sized forewing and hindwing, anal appendage consists of both cerci and epiproct for holding the prey, cubital vein forms the basal side of discoidal cell, anal vein forms anal loop which is differently shaped (Silsby, 2001).

2.1. Super family: Aeshnoidea

This primitive group of dragonflies are fast fliers. They are characterised by similar shaped triangles in both forewing and hindwing. Base

of the hindwing of male are usually angulated and primary antenodals are evident.

2.1.1. Family: Gomphidae

This family consist of 90 genera and 900 species. The members of this family have a club like swelling at their abdomen and hence the name. They are characterised by widely separated eyes, green colored eyes and black with yellow or green marking on thorax. They have 40-70 mm total length and often seen in streams and rivers. This group possess subtriangles in which the discoidal cell of the hindwing is more elongated than forewing.

2.1.1.1. Subfamily: Onychogomphinae

This subfamily consists of about 19 genera which are widely distributed in Palaearctic, Ethiopian and Oriental regions. Hindwing is provided with second primary antenodal cross vein nearer to first primary antenodal cross vein and provided with a intermedian cross vein. The abdominal segment 8 and 9 without or with pseudo lateral dilations; superior anal appendage much straighter and curled only at tips; inferiors very closely apposed and curled strongly up to meet the superiors.

***Onychogomphus malabarensis* Fraser, 1924**

This species is commonly known as ‘Pincertails’. It is characterised by yellow labrum with black border; black colored frons with a broad yellow stripe; vertex and occiput black in colour having a yellow spot at the middle of occiput and it is raised into a small tubercle (Fig. 14). Further one row of cells found between Rii and IRii (Fraser, 1936). It is commonly seen in terrestrial and freshwater habitat.

The partial coding sequence of mitochondrial COI gene of *Onychogomphus malabarensis* collected from Palakkad district (10.4621°N

76.3950° E) was PCR amplified using JCC as the primer (Table 2). The PCR amplification product yielded 602bp amplified segment of cytochrome oxidase I gene. The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST ,line diagram and molecular phylogenetic tree are presented in the figures 14 (a) to 14 (g) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KU133368 and Barcode of Life Data System BIN Cluster ID – BOLD: AAA4278 with Specimen ID – GBMIN88722-17 (Table 65).

The COI sequence of *Onychogomphus malabarensis* showed bias to nucleotide AT, with following composition of nucleotides T = 34.6%, C = 17.6%, A = 31.7% and G = 16.1% (Table: 21). This high AT content of 66.3% over 33.7% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The 602bp sequence obtained by amplification process yielded 200bp translated amino acid sequence. Both nucleotide and protein BLAST analysis showed closer match to *Ophiogomphus anomalus* reported from America (KX890962). The evolutionary history was inferred using the Neighbour-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 10 nucleotide sequences and the codon positions included were 1st+2nd+3rd. All positions containing gaps and missing data were eliminated and there were a total of 602 positions in the final dataset. Evolutionary analyses were conducted using MEGA7 software. The phylogenetic tree says that this species is strictly a Gomphidae member due to 100% sequence similarity to other Gomphidae members like *Ophiogomphus anomalus* and *Ophiogomphus mainensis.* The number of base substitutions per site between sequences is shown in Table 21 using the Maximum Composite Likelihood model. It showed 0% divergence to even other

members of Gomphidae family indicating conserved gene sequences among all Gomphidae members during the course of evolution (Table 22). The sequence has also submitted for BOLD system to confirm species authenticity. The analysis showed 100 % sequence similarity to *Ophiogomphus anomalus* and *Ophiogomphus mainensis* out of 20 different matches reported in BOLD system. The line diagram of this species for the confirmation of above statement is shown in Figure 14f.

DISCUSSION

Onychogomphus malabarensis is an endemic species known to be reported only from the Palakkad district of Kerala, India (Subramanian and Dow, 2010). Morphological identification was done by available keys (Emiliyamma et al., 2005) and also with online photographs. As this species is a pioneer report to the available databases, the nucleotide BLAST, Protein BLAST percentage of divergence table and BOLD system clearly demarcated this as a Gomphidae member because it doesn't have major changes in the nucleotide sequences. The average nucleotide frequencies are A = 31.73%, T = 34.55%, C = 17.61% and G = 16.11% indicating high AT content. This was supported by the reports of Chippindale et al. (1999) who stated that the overall A + T content were high among the order Odonata. The present study provided novel report to all databases and its unique barcode can be easily spot and analyze the phylogenetic position of this species based on DNA sequences.

2.1.2. Family: Aeshnidae

This family represents the largest and fast flying anisopterans commonly known as 'Darners'. They generally have blue or green coloured body, compound eyes, biting mouth parts and long slender abdomen. Most of the species are 2-3 inches in length and moves in water by squirting water

through abdomen. Female's abdomen looks like a sewing needle and hence the name "darner".

2.1.2.1. Subfamily: Aeshninae

This subfamily possesses most of the largest dragonflies consisting about 20 genera. Members are characterised by triangles on the wings having cross veins and R4 and anterior median gradually converge.

***Anaciaeschna jaspidea* (Burmeister, 1839)**

Anaciaeschna jaspidea have bluish grey eyes, reddish brown thorax, black legs, reddish brown abdomen and transparent wings with bright ochreous wing spot (Fig. 15). Females have deep amber or brownish coloured wings different to male. It was observed as a crecuspular species and often seen in dense vegetation. This is often seen associated with brackish waters.

The partial coding sequence of mitochondrial COI gene of *Anaciaeschna jaspidea* collected from Kasaragod district (12.500° N 75.0000° E) was PCR amplified using FOM as primer (Table 23). The PCR amplification yielded a product having 591bp. The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST and molecular phylogenetic tree are presented in the Figures 15(a) to 15(g) respectively. The sequence was deposited in the GenBank having the accession number KR149806 (Table 65).

The COI sequence of *Anaciaeschna jaspidea* showed bias to nucleotide AT, with following composition of nucleotides T = 32.9%, C = 16.5%, A = 32.9% and G = 17.7% (Table 23). This high AT content of 65.8% over 34.2% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The 591bp sequence obtained by amplification process yielded 200bp translated amino acid sequence. Both nucleotide and protein BLAST analysis showed that this species is 100% sequence similar to the same species reported from Tamil Nadu (JX306649). The evolutionary history was inferred using the Neighbour-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 9 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 334 positions in the final dataset after eliminating all positions containing gaps and missing data. The phylogenetic tree confirmed that this species showed a sister clade relationship to the same species from Tamil Nadu. This was supported by the divergence table plotted by Maximum Composite Likelihood model (Table 24). This result showed that this species has been rooted from *Orthetrum glacum* which divides the main clade into two separate clades having *Anaciaeschna jaspidea* and *Rhyothemis Phyllis* in one clade and *Rhyothemis variegata* in another one. Phylogenetically *Anaciaeschna jaspidea* and *Rhothemis phyllis* are very close together.

DISCUSSION

Anaciaeschna jaspidea is popularly known as 'Australian duskhawker' and it is widely distributed in Australia, India, Nepal, China and Japan (Theischinger & Hawking, J. 2006*).*This Aeshnidae member is morphologically identified with the available keys of taxonomic experts along with online photographs. The molecular id developed for this species is a pioneer work from Kerala and it showed a closer relationship with the same species reported from Tamil Nadu. This result confirmed the closer association of Aeshnidae and Libellulidae members due to its similarities with other Libellulidae members like *Rhyothemis variegate* and *Rhyothemis phyllis.*

***Anax parthenope* (Selys, 1839)**

This Aeshnidae member, commonly known as 'Lesser emperor', is geographically distributed over Southern Europe, North Africa and Asia (Mitra, 2010a). This species can be easily diagnosed by having a pale brown coloured thorax; three – fourth of wings tinted with yellow and the frons possess pale blue stripe (Fig. 16). They are usually seen in ponds, lakes and still waters.

The partial coding sequence of mitochondrial COI gene of *Anax parthenope* collected from Thrissur district (10.5200° N 76.2100° E) of the Kerala state was PCR amplified using FOM as primer (Table 2). The PCR amplification yielded a product having 607bp. The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the figures 16(a) to 16(g) respectively. The sequence was deposited in the GenBank having the accession number KR149805. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KR149805 and Barcode of Life Data System BIN Cluster ID – BOLD: ABX6596 with Specimen ID – GBMH0633-15 (Table 65).

The COI sequence of *Anax parthenope* showed bias to nucleotide AT, with following composition of nucleotides T = 35.8%, C = 15.4%, A = 31.9% and G = 16.8% (Table 25). This high AT content of 67.7% over 32.2% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The 607bp sequence obtained by amplification process yielded 202bp translated amino acid sequence.The nucleotide and protein BLAST analysis showed that this species is 100% sequence similar to the same species

reported from South Korea (KC13589). The evolutionary history was inferred using Neighbour-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 8 nucleotide sequences and the codon positions included were 1st+2nd+3rd. All positions containing gaps and missing data were eliminated and there were a total of 607 positions in the final dataset. Evolutionary analyses were conducted using MEGA7 software. Phylogenetic tree constructed by Neighbour joining method says that this species is strictly as *Anax parthenope* due to its sister clade relationship with the same species reported from South Korea. This clade is sister to the clade contains *Anax imperator*. So the phylogenetic tree interprets the mpnophyly of *Anax* genus and its nearest neighbour is seems to be *Anax imperator* due to sister taxa arrangement and it is followed by *Anax junius* and other Aeshnidae member (Fig. 16g). This is supported by the evolutionary divergence table plotted by maximum likely hood method with respective evolutionary divergence of 0% with *Anax parthenope*, 0.89% -1.42% with *Anax imperator* and 2.09 % with *Anax junious* (Table 26). The sequence has also submitted for BOLD system to confirm the species authenticity. The analysis showed 99-100% sequence similarity to out of different similar matches reported in BOLD system. The line diagram of this species for the confirmation of above statement is shown in (Fig. 16f). The average and maximum nucleotide distance to this species is found to be 0.64% (p-distance) and 2.38% (p-distance) respectively and the nearest neighbour was found to be *Anax junius* (BOLD: AAC9113) having a nucleotide distance of 2.2 %.

DISCUSSION

Anax parthenope is popularly known as ‘Lessor emperor’. This species is geographically distributed in South East Europe, North Africa to Japan and South to Australia. This morphologically identified specimen by taxonomic keys also confirmed its molecular taxonomic identity as *Anax parthenope* on

the basis of nucleotide sequences. This species showed a monophyletic ancestry because most of the *Anax* genera found in one clade and it was splitted into 2 clades in which *Anax imperator* and *Anax parthenope* were found as sister clades and this main clade is sister to those clade possessing *Anax junious* and other Aeshnidae members. The nucleotide composition showed difference in the composition of bases with other closely related individuals showed high AT content ratio. This is also supporting the above fact. Thus the database analysis showed same result and also the result is being the pionner work from Kerala, its molecular id can be easily spot the specimen and also for inferring phylogeny.

2.2. SuperFamily: Libelluloidea

This is the most dominant superfamily of the Suborder Anisoptera. The most diagnostic feature is the presence of foot shaped anal loop in the hind wing with differently sized triangles in forewing and hindwings. Eyes are broadly confluent on vertex.

2.2.1. Family: Libellulidae (common skimmers)

This cosmopolitan family includes brightly coloured medium sized dragonflies consisting of 1000 species. The most diagonistic key of this group is the presence of foot shaped anal loop in the hind wing, notch found on the posterior side of compound eye and triangles in the wings dissimilar in size and orientation. They are usually seen associated with ponds, lakes and still waters with their highest peak seen in April to September.

***Orthetrum sabina* (Drury, 1770)**

Orthetrum sabina species can be easily identified by having a greenish yellow with black stripes on thorax, black coloured legs, transparent wings having black reddish brown spot and green coloured abdomen with broad

black rings swollen at the base (Fig. 17). This species was observed besides ponds, tanks and grassy vegetation.

The partial coding sequence of mitochondrial COI gene of *Orthetrum sabina* collected from Kozhikode district (11.1352° N 75.8933° E) was PCR amplified using JOS as primer (Table 2). The PCR amplification yielded a product having 500 bp amplified segment of DNA. The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the figures 17 (a) to 17 (g) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KP938529 and Barcode of Life Data System BIN Cluster ID – BOLD: AAH6870 with Specimen ID – GBMIN88805-17 (Table 65).

The number of base substitutions per site between sequences were analysed using the Maximum Composite Likelihood model. The COI sequence of *Orthetrum sabina* showed bias to nucleotide AT, with following composition of nucleotides T = 35.5%, C = 17.6%, A = 29.7% and G = 17.2% (Table 27). This high AT content of 65.2% over 34.8% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The 500 bp sequence obtained by amplification process yielded 166bp translated amino acid sequence (Fig. 17c and Fig. 17e). Both nucleotide and peptide BLAST analysis showed that this species is 100% sequence similar to the same species reported from Mizoram and Punjab (KC12234, KT961626). The evolutionary history was inferred using the Neighbour-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 8 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 499 positions in the final

dataset after eliminating all positions containing gaps and missing data. 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 1.63% differences in the nucleotides sequences. The divergence table plotted by maximum likely hood method clearly showed that it has no divergence (0%) those from Punjab, Mizoram and Thailand while 1.63% to Indonesia and 1.01% to Malaysia (Table 28). On the basis of the data observed this species may be rooted from those found in Malaysia and Thailand 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 Malaysia and Thailand during the course of evolution. The analysis in BOLD system showed 97-100% sequence similarity to out of 30 different similar matches already reported in BOLD database. The line diagram of this species for the confirmation of above statement is shown in (Fig: 17f). The close matching BIN of the species is found to be 3% and the most similar species was found to be *Orthetrum sabina* reported from Mizoram having the BIN Cluster ID accession number BOLD: AAH6870. The average and maximum nucleotide distance to this species was found to be 1.63% (p-distance) and 3.08% (p-distance) respectively. This species is found to be more close to *O. sabina* (BOLD: ADC4050) with an average distance of 1.63% and 0.64% (p-distance) respectively.

DISCUSSION

Orthetrum sabina is commonly known as 'Slender skimmer' (Mitra, 2013). This species is known to be observed in Ethiopian, Oriental and Australian regions (Subramanian, 2009). Morphological identification done using wing venation and other superficial characters strictly confirm it as

Orthetrum sabina. Molecular identification done using cytochrome oxidase I gene also confirmed it taxonomy. About 14 species of *Orthetrum* have been reported from India (Subramanian, 2014). It is known to cannibalic on other odonate members having size greater than its own (Silsby, 2001).The present study confirmed a monophyletic ancestry to all *Orthetrum* members and this result is supported by the phylogenetic studies of *Orthetrum* genera already done in Mizoram (Lal□anpuii, 2014). Eventhough this species has been found in various geographically isolated areas, their sequence doesn't have any kind of variation. It has been told that this species represents one of the Asia's dominant species and gets migrated into Northern Africa, Turkey and Europe (Dilkstra et al., 2014). Hence the present study stress that the barcode generated can be used to easily spot the specimen and also to analyse its phylogeny.

***Neurothemis intermedia* Rambur, 1842**

Neurothemis intermedia have rusty brown thorax, reddish brown legs and reddish brown face. Their wings are transparent and have yellow patch in all four wings with a reddish brown spot and a bright red colour abdomen (Fig. 18). This libellulidae member commonly observed in streams and also besides paddy fields. This species is known to be widely distributed in Asian countries (Subramanian, 2010b).

The partial coding sequence of mitochondrial COI gene of *Neurothemis intermedia* collected from Kozhikode district (11.1352° N 75.8933° E) using JNT as the primer (Table 2). The PCR amplification yielded a product having 612bp. The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the Fig. 18 (a) to 18 (g) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession

No. KU052672, KP835514 and Barcode of Life Data System BIN Cluster ID – BOLD: ADJ7302 with Specimen ID – GBMIN8879-17 (Table 65).

The number of base substitutions per site between sequences was analysed using the Maximum Composite Likelihood model. The COI sequence of *Neurothemis intermedia* showed bias to nucleotide AT, with following composition of nucleotides T = 33.8%, C = 20.5%, A = 27.3% and G = 18.5% (Table 29). This high AT content of 61.1% over 39% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The 527bp sequence obtained by amplification process yielded 204bp translated amino acid sequenc. Both the nucleotide and protein BLAST analysis showed that this species has 100% sequence similarity to the same species reported from Mizoram (KC122227). The evolutionary history was inferred using the Neighbour - Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 7 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 352 positions in the final dataset after eliminating all positions containing gaps and missing data. The phylogenetic tree constructed by Neighbour joining method showed that this species is having a sister taxa relationship with those reported from Mizoram (Fig. 18g). Also the tree depicts that it infers a monophyletic ancestry with all *Neurothemis* sp. were diverged from one clade and it is phylogenetically more close to *Neurothemis fluctans*. The respective divergences are 1.44% and 1.74% to *N. intermedia* and *N. fluctans* (Table 30). The sequence has also submitted for BOLD system inorder to confirm the species authenticity. The analysis showed 99.48 % sequence similarity to the same species, 95.02% to *Neurothemis intermedia atlanta* and 94.67% to *Neurothemis fluctans*. The line diagram of this species for the confirmation of above statement is shown in (Fig. 18f).

DISCUSSION

Neurothemis intermedia is popularly known as 'Paddy field parasol' (Subramanian, 2010b; Fraser, 1936). This is widely distributed in Asian countries (Subramanian, 2010b). Morphological identification done using available keys helps to confirm its taxonomic identity. *Neurothemis,* the commonly called 'Red dragonflies' is a libellulidae member commonly found in drains, ditches, shallow streams, paddy fields etc. There are about 18 species are known to exist and out of which 3 species are commonly found in Kerala. Most of the species looks similar in terms of their appearance, behaviour and other notable characteristics but in a close look and detailed study, they all found to be reproductively isolated (Dow and Clausnitzer, 2012). Most of the genus exhibits female-limited polymorphism with a clear difference in the wing and body coloration (Schorr et al., 2010). Phylogenetic analysis showed that this genus is having a monophyletc ancestry which was supported by the evolutionary study made in different *Neurothemis* species in Kerala (Jisha and Sebastian, 2015d). The close relative of this species is found to be *Neurothemis fluctans* with a divergence of 1.74%. Thus both morphological and molecular helps for providing a better taxonomic tool to identify and analyse phylogenetic relationships.

Potamarcha obscura (Rambur, 1842)

The male species have bluish black thorax, hyaline wings, brown eyes, and black with orange striped abdomen. Wings are hyaline with brown tips and dark reddish brown coloured pterostigma.Abdominal appendages are black in colour (Fig. 19). This species is commonly observed besides small weedy ponds.

The partial coding sequence of mitochondrial COI gene of *Potamarcha obscura* collected from Palakkad district (10.5200° N 76.2100° E) of Kerala

has been done using JPC as primer (Table 2). The PCR amplification yielded a product having 633bp. The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST and molecular phylogenetic tree are presented in the Fig. 19 (a) to 19 (g) respectively. The sequence was deposited in the GenBank having the accession number KX503060 (Table 65).

The COI sequence of *Potamarcha obscura* showed bias to nucleotide AT, with following composition of nucleotides T =36.7%, C = 16.0%, A = 32.7% and G = 14.6% (Table: 31). This high AT content of 69.4 % over 30.4% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The 633bp sequence obtained by amplification process yielded 211bp translated amino acid sequences.Both nucleotide and protein BLAST analysis showed that this species is 100% sequence similarity to the same species reported from Mizoram (KC122230). The evolutionary history was inferred using the Neighbour-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 10 nucleotide sequences and the codon positions included were 1st+2nd+3rd. The analysis involved 10 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 611 positions in the final dataset after eliminating all positions containing gaps and missing data. The phylogenetic tree constructed by Neighbour joining method confirmed the taxonomic identity of this species as *Potamarcha obscura* due to sister clade relationships with the same species from Mizoram. This result is supported by the evolutionary divergence table plotted by Maximum likely hood method (Table 32).

DISCUSSION

Potamarcha obscura is popularly known as 'Yellow tailed ashy skimmer' (Theischinger et al., 2006). This species is widely distributed in Asian countries (Mitra and Dow, 2017). The morphological identification of the specimen did using the available keys uphoded the unique features of *Potamarcha obscura* (Emiliyamma et al., 2005). All the database analysis showed that the conserved sequence of this species doesn't have any kind of sequence change. This is a pioneer molecular work from Kerala and hence its molecular id can be used to easily spot the specimen and can be used for further research.Evolutionary relationship shows that this family is very close to Lepidopterans as its sequence similarity to butterflies (*Pieris candida*).

Brachydiplax chalybea Brauer, 1868

It is commonly called as 'Yellow patched lieutenant' (Cheong et al., 2008). Most of the male members are characterised by having powdery bluish colour body, light bown on sides and dark tip on abdomen. Wings are transparent and brown colour at the base (Figure 20). They are generally found in disturbed habit.

The partial coding sequence of mitochondrial COI gene of *Brachydiplax chalybea* collected from Wayanad district (11.6050° N 76.0830° E) was PCR amplified using JPF as primer (Table 2). The PCR amplification yielded a product having 574bp.The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the figures 20 (a) to 20 (g) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KT372721 and Barcode of Life Data System BIN

Cluster ID – BOLD: ACD4364 with Specimen ID – GBMIN88778-17 (Table 65).

The COI sequence of *Brachydiplax chalybea* showed bias to nucleotide AT, with following composition of nucleotides T = 32.9%, C = 18.8%, A = 30.7% and G = 17.6% (Table 33). This high AT content of 63.6% over 36.4 % of GC is mainly due to the mutational pressure on a single nuceotide substitution during the evolutionary period of time.

The 574bp sequence obtained by amplification process yielded 191 long translated amino acid sequence. Both nucleotide and protein BLAST analysis showed that this species is 100% sequence similar to the same species reported from Mizoram (KC287156). The evolutionary history was inferred using the Neighbour-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 9 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 423 positions in the final dataset after eliminating all positions containing gaps and missing data. The phylogenetic tree constructed by Neighbour joining method clearly says that this is *Brachydiplax chalybeae* due to its sister clade relationship with the same species from Mizoram. The above result is confirmed by the divergence table plotted by Maximum Composite Likelihood model (Table 34). Phylogenetically this species is very close to the other Libellulidae members such as *Acisoma inflatum and Acisoma attenboroughi*. The analysis showed 97-100% sequence similarity to out of 20 different matches of different species reported in BOLD system. The line diagram of this species for the confirmation of above statement is shown in (Fig. 20f). The close matching BIN of the species is found to be 3% and the most similar species is found to be reported *Brachydiplax chalybeae* (BOLD: ACD4364) from Mizoram. It showed an average and maximum nucleotide distance of 0.73% (p-distance) and 2.27% (p-distance) respectively.

DISCUSSION

Brachydiplax chalybeae is popularly known as 'Yellow patched lieutendant' (Cheong et al., 2008). This species has been widely distributed in Eastern Asia including India, Japan and Indonesia (Dow, 2010). Morphological identification was done using taxonomic keys (Emiliyamma et al., 2005) by observing its wing venation characters and other superficial characters. The cytochrome oxidase I gene analysis of this species is a pioneer work from Kerala and its phylogenetic analysis confirmed the taxonomic identity of this species due to the conserved sequence. No other taxonomic work of this species has been reported till now. Here the database analysis confirmed the above result and its closer relationship with other Libelluidae members (*Acisoma* sp.) indicating confirmed family relationships. Result thus depicted that it doesnt have any sequence divergence during the course of evolution.

Trithemis aurora (Burmeister, 1839)

Trithemis aurora is characterised by having reddish brown face, crimson eyes, black legs, red with purple pruinescence thorax, violet coloured abdomen and transparent wing with dark reddish brown spot (Fig. 21). Female has bright reddish brown face, dark grey legs, olivaceous thorax with characteristic median black lateral stripes, dark grey with yellow striped legs and reddish abdomen. Their wings are transparent with brown tips and have bright yellow coloured venation. It is often seen on vegetation near to water bodies.

The partial coding sequence of mitochondrial COI gene of *Trithemis aurora* collected from Kasargode district (12.5000° N 75.0000° E) was PCR amplified using JPF as primer (Table 2). The PCR amplification yielded a product having 606bp. The DNA sequence interpret, representative molecular

barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the Figures 21 (a) to 21 (g) respectively. The sequence was deposited in the GenBank having the accession number KT305963. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession Nos. KT305962, KT305963 and Barcode of Life Data System BIN Cluster ID – BOLD: AAQ0253 with Specimen ID – GBMIN88911-17 (Table 65).

The COI sequence of *Trithemis aurora* showed bias to nucleotide AT, with following composition of nucleotides T = 32.6%, C = 18.0%, A = 32.8% and G = 16.6% (Table 35). This high AT content of 65.4% over 34.6% of GC is mainly due to the mutational pressure on a single nuceotide substitution during the evolutionary period of time.

The 606bp sequence obtained by amplification process yielded 202 long translated amino acid sequence.The BLAST analysis showed that this species was 100% sequence similar to the same species reported from Mizoram (JN817428). The evolutionary history was inferred using the Neighbour-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 9 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 451 positions in the final dataset after eliminating all positions containing gaps and missing data. Phylogenetic tree depicted a monophyletic ancestry to *Trithemis aurora* because all similar species distributed in various geographical areas were found in one clade. Phylogenetically it is very near to *Trithemis festiva* than other members (Fig. 21g). This is shown in the divergence table plotted by the Maximum Composite Likelihood model (Table 36). The tree depicts that this species doesn’t have any sequence divergence to the species found in Mizoram while have 1.5% to 2% divergence to the same species reported from Punjab and Japan.

Taxonomically this species seems to be very close to *T. festiva, T. grouti* and *T. werneri* with respective divergence of 13.33%, 13.55% and 14.35% respectively. As the sequence has been reported from Mizoram, Punjab and Japan, it showed showed greater divergence to those reported from Japan. Hence geographical barrier acted as an evolutionary tool for the sequence divergence. The sequence has also be deposited in BOLD system for the confirmation of taxonomic identity which reveals that the species is 100% sequence similar to the same species reported from Mizoram (BOLD: AAQO253) while 98.83% to those reported from Punjab. The line diagram of this species also confirmed the above statement (Fig: 21f). The close matching BIN of the species is found to be 3% and the most similar species showed an average and maximum distance of 1.18% (p-distance) and 3.21% (p-distance) respectively. The distance to the nearest neighbour (*Trithemis festiva*) is found to be having a distance of 10.27% (p-distance).

DISCUSSION

Trithemis aurora is commonly known as 'Crimson marsh glider' (Subramanian and Dow, 2010). It is widely distributed in Oriental region (Subramanian, 2009; Kiran and Kakassery, 2007). The morphological identification was done using available taxonomic keys through observing wing venation characters and other superficial characteristics confrmed its morphotaxonomy (Emiliyamma et al., 2005). Both NCBI and BOLD system showed that this is strictly *Trithemis aurora* due to its high sequence similaity in the conserved COI region even though they are found in various geographically isolated areas. Thus the phylogenetic tree depicts that their common ancestor has been divided into two main clades in which *Trithemis aurora* and *Trithemis festiva* were found in one clade while *Trithemis grouti* and *Trithemis wernerii* were foud in another clade indicating *Trithemis aurora* is taxonomically very close to *Trithemis festiva.*However tree says that

all *Trithemis aurora* are having a monophyletic orgin as all are descendent from a single clade. Hence both analysis confirmed the taxonomic identity to this species and inferred it's the phylogenetic status.

***Neurothemis fulvia* (Drury, 1773)**

Neurothemis fulvia is a rusty colored dragonfly species diagnosed by reddish brown coloured head, thorax and abdomen in males. Their wings are dark reddish and opaque having reddish brown pterostigma and also a transparent triangular area at the tip (Fig. 22). The female members are paler and rusty brown in colour with their wings are amber yellow in colour. This rusty coloured dragonfly commonly observed as large colonies in almost all dense vegetation areas.

The partial coding sequence of mitochondrial COI gene of *Neurothemis fulvia* collected from Malappuram district (11.0300° N 760500° E) was PCR amplified using JNT as primer (Table 2). The PCR amplification yielded a product having 600bp length. The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the Figures 22 (a) to 22 (g) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KP835515 and Barcode of Life Data System BIN Cluster ID – BOLD: ACD6379 with Specimen ID – GBMIN88796-17 (Table 65).

The COI sequence of *Neurothemis fulvia* showed bias to nucleotide AT, with following composition of nucleotides T = 33.7%, C = 20.0%, A = 27.7% and G = 18.6% (Table 37). This high AT content of 61.4% over 38.6% of GC is mainly due to the mutational pressure on a single nuceotide substitution during the evolutionary period of time.

The 600bp sequence obtained by amplification process yielded 200 long translated amino acid sequence. Both nucleotide and protein BLAST analysis showed that this species is 100% sequence similarity to the same species reported from Mizoram (JN817427). The evolutionary history was inferred using the Neighbour-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 10 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 583 positions in the final dataset after eliminating all positions containing gaps and missing data. The phylogenetic tree constructed by Neighbour-Joining method clearly showed that this species doesn't have any sequence divergence as time progressed. Phylogenetically this species is very close to close to *N. intermedia, N. fluctans* and *N. tullia* with respective the divergence of 17.6%, 18.10% and 18.55% respectively. The above result is confirmed by the divergence table plotted by Maximum Composite Likelihood model (Table 38).The BOLD database analysis showed 99.82-100% similarity sequences already reported in BOLD system. The line diagram of this species for the confirmation of above statement is shown in (Fig. 22 f). The close matching BIN of the species is found to be 3% and the most similar species is found to be reported from Mizoram having the accession number BOLD ACD6379. The average and maximum nucleotide distance to this species is found to be 0.43% (p-distance) and 1.61% (p-distance) respectively. The generic close neighbour was *Neurothemis tullia* having a divergence of 1% (BOLD: ADK3152).

DISCUSSION

Neurothemis fulvia is commonly known as 'Fulvous forest skimmer' and is geographically distributed in Asian countries (Mitra, 2010b). Morphological identification was done using the authentic taxonomic keys (Emiliyamma et al., 2005). This species is always seen associated with

agroecosystem and also in ponds and other aquatic habitat. This is a pioneer molecular work from Kerala and its sequence was found to be conserved during the evolutionary period of time. This sequence doesn't have any kind of sequence divergence to the same species.Phylogenetically this species is very close to Neurothemis tullia by NCBI and BOLD system. However all *Neurothemis* genus are having a monophyletic ancestry as all members were splitted from a single node (Jisha and Sebastian, 2015e). Hence the barcode generated helped to easily spot the specimen very and also to infer phylogenetic status.

***Crocothemis servillia* Drury, 1770**

It is medium sized bloods coloured species with males characteristically bear red eye, red face, ferrogeneous to orange colored thorax, red colored abdomen and hyaline wings with amber colored base provided with dark brown wingspot (Fig. 23). Female members are pale yellow in color with their eyes are brown above and olivaceous below, dark brown coloured thorax and legs and transparent wing with pale yellow wingspot. Abdomen is yellowish brown with a characteristic mid dorsal black stripe.They are commonly observed in ponds, wells, tanks, ditches and paddy fields.

The partial coding sequence of mitochondrial COI gene of *Crocothemis servillia* collected from Wayanad district (11.6050° N 76.0830° E) was PCR amplified using OTF as primer (Table 2). The PCR amplification yielded a product having 603bp. The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the figures 23 (a) to 23 (g) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession

No. KR149807 and Barcode of Life Data System BIN Cluster ID – BOLD: AAQ0252 with Specimen ID – GBMH0652-15 (Table 65).

The COI sequence of *Crocothemis servillia* showed bias to nucleotide AT, with following composition of nucleotides T = 34.8%, C = 17.1%, A = 31.3% and G = 16.8% (Table 38). This high AT content of 66.1% over 33.9% of GC is mainly due to the mutational pressure on a single nuceotide substitution during the evolutionary period of time.

The 603bp sequence obtained by amplification process yielded 201 long translated amino acid sequence.Both the nucleotide and protein BLAST analysis showed this species is having 100% sequence similarity to the same species reported from Mizoram (JN817425). The evolutionary history was inferred using the Neighbour-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 10 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 537 positions in the final dataset after eliminating all positions containing gaps and missing data. The phylogenetic tree depicts that this species is having a sister taxa arrangement to the same species reported from Mizoram and thereby confirmed its taxonomic identity. The tree also depicts that it has a monophyletic ancestry due to the splitting from a common ancestry. This was supported by the divergence table plotted by Maximum Composite Likelihood model (Table 40). Phylogenetically this species is very close to close to *Crocothemis erythraea* having a divergence of 2.71. The analysis showed 98.02-100% sequence similarity to the same species reported in BOLD system. The line diagram of this species for the confirmation of above statement is shown in (Fig. 23f). The close matching BIN of the species is found to be 3% and the most similar species was found to be reported from Mizoram having the BIN cluster ID: AAQ0252. The average and maximum

nucleotide distance to this species is found to be 0.82% (p-distance) and 2.32% (p-distance) respectively.

DISCUSSION

Crocothemis servillia is popularly known as 'Scarlet skimmer' and it is geographically distributed in East and South East Asia (Dow et al., 2013). This is commonly seen in man made and disturbed habitat and considered to be an oppurtunitic species (Dow, 2017). Morphological identification was done using taxonomic keys (Emiliyamma et al., 2005) by observing its wing venation characters and other superficial characters. This is a pioneer molecular work from Kerala and phylogenetic tree interprets a monophyletic ancestry to this genus due to closer relationship with other species of the same genus. Most of the database results showed 99% of sequence similarity to the same species reported from various geographically isolated areas. Phylogenetic tree also interprets such a monophyly to this genus and those species from Mizoram is found to be closer than other areas. Phylogenetically this species is very close to *Crocothemis erythreae*. This species was formely treated as a subspecies of *Crocothemis erythreae* and later described as *C. servillia* on the basis of its unique morphological features.

***Trithemis pallidinervis* (Kirby, 1889)**

Trithemis pallidinervis is a medium sized yellowish brown dragonfly having reddish brown eyes, olivaceous brown thorax, black legs, bright yellow abdomen and transparent wings with reddish venation provided with black coloured pterostigma (Fig 24). It is commonly observed in weedy ponds.

The partial coding sequence of mitochondrial COI gene of *Trithemis pallidinervis* collected from Kannur district (12.8700° N 74.9000° E) was PCR amplified using OTF as primer (Table 2), yielded a product having

580bp size. The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, Line diagram, peptide BLAST and molecular phylogenetic tree are presented in the figures 24 (a) to 24 (g) respectively.The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KR149803 and Barcode of Life Data System BIN Cluster ID – BOLD: AAQ0251 with Specimen ID – GBMH0999-15 (Table 65).

The COI sequence of *Trithemis pallidinervis* showed bias to nucleotide AT, with following composition of nucleotides T = 37.8%, C = 16.2%, A = 29.8% and G = 16.2% (Table 41).This high AT content of 67.6% over 32.4 % of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The 580bp sequence obtained by amplification process yielded 193 long translated amino acid sequence.Both the BLAST analysis showed that this species is having 100% sequence similarity to the same species reported from Mizoram and Thailand (KJ499455 and KT957508). The evolutionary history was inferred using the Neighbour-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 8 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 580 positions in the final dataset after eliminating all positions containing gaps and missing data. Phylogenetic tree interprets that this species is having a sister clade relationship to the same species repoted from Mizoram. This clade is sister to the clade containing *Trithemis pallidiervis* from Thailand. This result is supported by the divergence table plotted by Maximum Composite Likelihood model (Table 42). The analysis showed 99.61-100% sequence similarity to different similar matches already reported in BOLD system. The line diagram of this species for the confirmation of above statement is shown

in (Fig. 24f). The close matching BIN of the species is found to be 3% and the most similar species is found to be *Trithemis pallidinervis* reported from Mizoram having the BIN cluster ID: AAQ0251. The average and maximum nucleotide distance to this species is found to be 0.2% (p-distance) and 0.92% (p-distance) respectively. The genetic distance to the nearest member is found to be 11.11%. The phylogenetic tree constructed by Neighbour joining method clearly showed that this species doesn't have any sequence divergence to the species reported from Mizoram and Thailand and phylogenetically this species is very close to close to *T. glacum* with a respective divergence of 14.51.

DISCUSSION

Trithemis pallidinervis is popularly known as 'Long legged marsh glider' and is widely distributed throughout the Asian countries (Subramanian, 2010a). Morphological identification was done using taxonomic keys (Emiliyamma et al., 2005) by observing its wing venation characters and other morphological differences. This Libellulidae member have also confirmed the species identity by the analysis of conserved cytochrome oxidase I gene analysis. This is a pionner molecular work from Kerala and those species which are reported from Mizoram and Thailand doesn't have major sequence divergence indicating neutral evolution. However this speces have a monophyletic ancestry due to the divergence of similar genera from one clade. Thus the above result is used for easily spot the specimen by using cytochrome oxidase I gene and also to infer its phylogeny.

Trithemis festiva (Rambur, 1842)

This libellulidae member commonly called as 'Black stream glider' (Fraser, 1936). Male species characteristically have dark brown eyes, black

with deep purple coloured thorax, black legs and transparent wing with a brown mark at the base of the wing. Abdomen seen as black in colour and fully covered by blue pruiniscence (Fig. 25). Females have a dirty brown face, dark olive brown thorax, black legs and dark yellow abdomen characteristically having medial and lateral black stripes.

The partial coding sequence of mitochondrial COI gene of *Trithemis festiva* collected from Wayanad district (11.6050° N 76.0830° E) was PCR amplified using OTF as primer (Table 2). The PCR amplification yielded a product of 567bp amplified segment of Cytochrome oxidase I gene. The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the figures 25 (a) to 25 (g) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KR149802 and Barcode of Life Data System BIN Cluster ID – BOLD: AAQ0247 with Specimen ID – GBMH0998-15 (Table 65).

The COI sequence of *Trithemis festiva* showed bias to nucleotide AT, with following composition of nucleotides T = 37%, C = 16.3%, A = 29.0% and G = 17.6% (Table 43). This high AT content of 66% over 33.9% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The 567bp sequence obtained by amplification process yielded 189 long translated amino acid sequences. Both nucleotide and protein BLAST analysis showed that this species is having 100% sequence similarity to the same species reported from Mizoram (JN817429). The evolutionary history was inferred using the Neighbour-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 9 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding.

There were a total of 551 positions in the final dataset after eliminating all positions containing gaps and missing data. The phylogenetic tree constructed by Neighbour joining method clearly showed that this species is a sister clade to the same species reported from Mizoram and this main clade is sister to those reported from Punjab. The above result is confirmed by the divergence analysis table plotted by Maximum likelihood method (Table 44). There was only 1.10% divergence to those reported in Punjab. Phylogenetically this species seems to be very close to *Trithemis stictica*, *Trithemis werneri*, *Trithemis furva* and *Trithemis grouti* with respective divergence as 11.60%, 12.00%, 12.10% and 12.20%. The analysis showed 97.16-100% sequence similarity to different similar matches reported in BOLD system. The line diagram of this species for the confirmation of above statement is shown in (Fig. 25f). The close matching BIN of the species is found to be 3% and the most similar species is found to be reported from Mizoram having the BIN cluster ID: AAQ0247. The average and maximum nucleotide distance to this species is found to be 1.23% (p-distance) and 3.05% (p-distance) respectively. The nearest neighbour of this species is found to be *Trithemis stictica* (BOLD: ABA9530).

DISCUSSION

Trithemis festiva, popularly known as 'Black stream glider' is geographically distributd from Asia to New Guinea (Dow, 2009a). Morphological identification was done using taxonomic keys by observing wing venation characters and other morphological features.This is a pioneer molecular work from Kerala and its phylogeny depicts monophyletic ancestry with a closer relationship with *Trithemis stictica*. Morphologically *Trithemis festiva* is very similar to *Trithemis sticticta* in most of the features with only a little difference in eye colour. The DNA sequence analysis showed 11.60% divergence with *Trithemis stictica* species and found in a separate clade sister

to the branch having many *Trithemis festiva* members.Thus we can confirm the result that phylogenetically this species is very close to *Trithemis sticticta.* Hence the barcode generated can be used to easily spot the specimen.

***Brachythemis contaminata* Fabricius, 1793**

Brachythemis contaminate is one of the dominant Libellulidae member in Asian countries. They generally inhabit on weedy ponds, lakes and streams, sewage canal and ditches (Sharma, 2010). Males have olivaceous face,brown colored eyes, olivaceous brown to reddish brown thorax provided with two lateral reddish brown stripes and dark brown coloured legs. Abdomen is bright red in colour and the transparent wings are having reddish venation and rusty wing spot. A broad orange patch is seen extending from wing base to wing spot in both wings. Females have yellwish white face, brown to bluish grey eyes, greenish yellow thorax, with a brown dorsal spine and brown coloured legs. Wings are transparent without wing patches. Its hindwings are tinted with yellow and have rusty wing spot.Abdomen is pale olivaceous brown with a black middorsal sripe (Fig. 26).

The partial coding sequence of mitochondrial COI gene of *Brachythemis contaminata* collected from Palakkad district (10.4621° N 76.3950° E) of Kerala was PCR amplified using JOS as primer (Table 2). The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the Figures 26 (a) to 26 (g) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KP938531 and Barcode of Life Data System BIN Cluster ID – BOLD: ADC3495 with Specimen ID – GBMIN8878--17 (Table 65).

The COI sequence of *Brachythemis contaminata* showed bias to nucleotide AT, with following composition of nucleotides T = 32.8%, C = 16.9%, A = 31.2% and G = 19.1% (Table: 45). This high AT content of 64% over 36% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The BLAST analysis of both nucleotide and protein sequences showed that this species is having 100% sequence similarity to the same species reported from Karnataka having the accession number KC287157. The evolutionary history was inferred using the Neighbour-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 13 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 383 positions in the final dataset after eliminating all positions containing gaps and missing data. Here also the tree depicts that all *Brachythemis contaminate species* from various geographical areas are separated from one common clade with the indication of common ancestry. The percentage of divergence table plotted by Maximum likelihood also confirmed the above statement and showed only that only 0.02% sequence divergence to the same species from various geographically isolated areas such as Karnataka, Mizoram and China (Table 46).

The sequence has also submitted in BOLD system, another database to confirm the species authenticity. The analysis showed 98-99.82% sequence similarity to similar matches already reported in BOLD system. The line diagram of this species for the confirmation of above statement is shown in (Fig. 26h). The close matching BIN of the species is found to be 3% and the most similar species was found to be *Brachythemis contaminate* reported from Bengaluru having the BIN cluster ID ADC3495 and it showed an average and maximum nucleotide distance of 0.92% (p-distance) and 0.92%

(p-distance) respectively.Thus the above result confirmed the taxonomic identity to this species as *Brachythemis contaminata* on the basis of DNA sequence.

DISCUSSION

Brachythemis contaminate is commonly known as “ditch jewel”, widely distributed in Asian countries. The present study investigated to confirm the taxonomic identity of this species by both morphological identification and molecular characterisation.The morphological features is strictly correlated with the previously identified specimens by taxonomic keys and online photographs.This is a pioneer molecular work from Kerala but other reports were also reported in Mizoram,Bengaluru and China.Even though this species is reported from various locations,it showed more closer relationship with those reported from Mizoram. But BOLD analysis says that it is more close to those from Karnataka. Anyhow the unique molecular id produced can be used to easily spot the specimen.Phylogenetic relationship was also determined on the basis of conserved COI gene sequence, which showed and its nearest neighbour is found to be *Diplacodes trivalis* than other Libellulidae members.

***Diplacodes trivialis* (Rambur, 1842)**

Diplacodes trivalis is one of the commonest libellulid dragonfly species found in garden, paddy fields, and playground and it is commonly known as ‘Ground skimmer’ or ‘Blue percher’.This Libellulidae species is widely distributed in Oriental region and Pacific islands. This species showed an extreme case of sexual dimorphism with clear distinct morphological differences in both the sexes.Male has beautiful blue eyes, pale azure blue face with reddish brown coloured eye above and pale bluish or yellowish colour below. Thorax is greenish yellow or olivaceous. The dorso-lateral area

is violet brown and is speckled with minute dots. Legs are greenish yellow marked with black and the wings are transparent. Abdominal segments 1-7 are greenish yellow with middorsal and subdorsal black stripes and the remaining segments are black in colour (Fig. 27). They usually breed in muddy puddles, tanks and ponds (Subramanian, 2009).

The partial coding sequence of mitochondrial COI gene of *Diplacodes trivialis* collected from Kozhikode (11.1352° N 75.8933° E), Kasargode (12.5000° N 75.000° E), Kannur (12.8700° N 74.900° E) and Thrissur (10.5200° N 76.2100° E) districts of Kerala were PCR amplified using ODT as primer (Table 2). The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the Figures 27 (a) to 27 (g) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KP835512 and Barcode of Life Data System BIN Cluster ID – BOLD: AAH6874with Specimen ID – GBMH065-15 (Table 65).

The COI sequence of *Diplacodes trivialis* showed bias to nucleotide AT, with following composition of nucleotides T = 35.8%, C = 16.7%, A = 29.4% and G = 18% (Table 47). This high AT content of 65.2% over 34.8% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The BLAST analysis of both nucleotide and protein sequences showed that this species is having 100% sequence similarity to the same species reported from other locations of Kerala and Mizoram having the accession numbers KP087931, KP087932, KP087933, KP087934, KP835513 and JX306647. The evolutionary history was inferred using the Neighbour-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 18 nucleotide sequences and the codon positions included

were 1st+2nd+3rd+Noncoding. There were a total of 466 positions in the final dataset after eliminating all positions containing gaps and missing data. Here also the tree depicts that all *Diplacodes trivalis* members have a monophyletic origin with the indication of the common ancestry. The percentage of divergence table plotted by Maximum likelihood also states that no major sequence divergence to the species has been reported from various geographically isolated areas (Table 48).

The sequence has also submitted in BOLD system, another database to confirm the species authenticity. The analysis showed 99.82-100% sequence similarity to out of 34 different similar matches already reported in BOLD system. The line diagram of this species for the confirmation of above statement is shown in (Fig. 27f). The close matching BIN of the species is found to be 3% having the BIN accession number AAH6894. The average and maximum nucleotide distance of this species is found to be 0.37% (p-distance) and 0.95% (p-distance) respectively. Thus the above result confirmed the molecular taxonomic identity to this species by providing a unique molecular id and also its phylogenetic relationship with other *Diplacodes* members.

DISCUSSION

Diplacodes trivalis is commonly known as “Blue ground skimmer”, widely distributed in Oriental region and Pacific islands. This species is well known being sexually dimorphic and Morphological difference between male and female *D. trivalis* could be externally identified using authentic reference guides. The molecular phylogenetic analysis of both male and female members of the same species showed the confirmation of the sequence and conserved gene evolution with those reported from various geographically isolated areas (Jisha Krishnan and Sebastian, 2015e). In the present study *Diplacodes trivalis* from various districts of the Kerala state produced same

sequence similarity with 0% sequence divergence and evolutionary more related to those reported from Mizoram than other geographically different areas. Phylogenetic analysis and divergence table showed closer relationship with *Acisoma panorpoides* than other members. This is also a pioneer work from Kerala and hence the unique barcode generated can be used to easily spot and evaluate its phylogeny.

***Bradinopyga geminata* (Rambur, 1842)**

Bradinopyga geminate is a medium sized Libelllulidae member commonly observed besides rocky pools, walls of granite and rocky substratum.Male species have dirty pale yellow or white marbled thorax, dirty black or grey coloured abdomen and pale creamy white appendages (Fig. 28). Wings are transparent with black wing spot and brown eyes. It is a widespread species all over the world (Mitra, 1991).

The partial coding sequence of mitochondrial COI gene of *Bradinopyga geminata* collected from Malappuram district (11.0300° N 760500° E) of Kerala was PCR amplified using FOM as primer (Table 2). The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the Figures 28 (a) to 28 (g) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KM096995 and Barcode of Life Data System BIN Cluster ID – BOLD: ABY3063 with Specimen ID – GBMIN22799-13 (Table 65).

The COI sequence of *Bradinopyga geminata* showed bias to nucleotide AT, with following composition of nucleotides T = 37.4%, C = 15.5%, A = 30.1% and G = 17.0% (Table: 49). This high AT content of 67.5% over

32.5% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The BLAST analysis of both nucleotide and protein sequences showed that this species is having 100% sequence similarity to the same species reported from Tamilnadu having the accession number JX306648. The evolutionary history was inferred using the Neighbor-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 14 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 469 positions in the final dataset after eliminating all positions containing gaps and missing data. Here also the tree depicted that this species is having a monophyletic origin as all members of the differently geographically isolated areas are closely related each other..All members were found in one cladeand it is supported by the percentage of divergence table plotted by Maximum likelihood (Table 50). This species showed 0.04, 0.08 to 0/.11 % of sequence divergence to those reported from Tamilnadu and China respectively.

The sequence has also submitted in BOLD system, another database to confirm the species authenticity.The analysis showed 98.5-99% sequence similarity matches already reported in BOLD system. The line diagram of this species for the confirmation of above statement is shown in (Fig. 28f). The close matching BIN of the species is found to be 3% and the most similar species was found to be *Bradinopyga geminata* reported from Tamilnadu having BIN cluster id ABY3063. The average and maximum nucleotide distance of this species is found to be 1.64% (p-distance) and 2.76% (p-distance) respectively. Here also the nearest neighbour was *Bradinopyga geminata* (BOLD: CBL014-12). Thus the above result confirmed the molecular taxonomic identity to this species by providing a unique molecular id and also its phylogenetic relationship with other *Libellulidae* members.

DISCUSSION

Bradinopyga geminate is commonly known as “Granite ghost” and this species is well known for being predators of mosquito larvae of *Aedes agypti* (Venkatesh and Tyagi, 2013). Morphological identification done with taxonomic keys and expert consutation confirmed its taxonomy as *Bradinipyga geminate.* Eventhough this species has been reported from various geographical areas, this species showed closer relationship to those reported from Mizoram.Phylogenetically this species is very close to *Orthemis cultriformis*, another Libellulidae member and hence the present study concluded a conserved gene evolution as time progresses.

Rhyothemis variegata Linneus, 1763

It is a wide spread Libellulidae member in South Asia which often seen in marshes, ponds and paddy fields (Subramanian, 2010c). It is a medium sized dragonfly with golden wings variegated with black and yellow patches. Males are characterised by irridiscent green thorax, black legs, black abdomen, transparent and golden yellow wing. The hindwing is characteristically have a “w”shaped brown mark with a black coloured wing spot.Also the tip of the hindwing possesss a clear yellow spot (Fig. 29).

The partial coding sequence of mitochondrial COI gene of *Rhyothemis variegata* collected from Malappuram (11.0300° N 76.0500° E) districtof Kerala was PCR amplified using JRV as primer (Table 1). The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the Figures 29 (a) to 29 (g) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KP938530 and Barcode of Life Data System BIN Cluster ID – BOLD: ABX8023 (Table 65).

The COI sequence of *Rhyothemis variegata* showed bias to nucleotide AT, with following composition of nucleotides T = 34.7%, C = 15.8%, A = 32.2% and G = 17.3% (Table 50). This high AT content of 66.9% over 33.1% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The BLAST analysis of both nucleotide and protein sequences showed that this species is having 100% sequence similarity to the same species reported from Mizoram having the accession number KC287151. The evolutionary history was inferred using the Neighbor-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 13 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 450 positions in the final dataset after eliminating all positions containing gaps and missing data. Here also the tree depicts that *Rhyothemis variegate* is having a close relationship to the same species from variously geographically distant areas and thereby confirmed the species taxonomy.Also the result confirmed its genus taxonomy as its close relationship to *R. phyllis.* The above statement is strictly correlated to the percentage of divergence table plotted by Maximum likelihood (Table 51). It showed also states that no sequence divergence to the species with those reported from various geographically isolated areas.

The sequence has also submitted in BOLD system, another database to confirm the species authenticity. The analysis showed 99.82% sequence similarity to out of 20 different similar matches already reported in BOLD system. The line diagram of this species for the confirmation of above statement is shown in Fig: 29f. The close matching BIN of the species is found to be 3% and the most similar species was found to be *Rhyothemis variegate* reported from Mizoram having the BIN cluster ID: ABX8023. The average and maximum nucleotide distance of this species is found to be

0.61% (p-distance) and 2.07% (p-distance) respectively.Thus the above result confirmed the molecular taxonomic identity to this species by providing molecular id and also its phylogenetic relationship with other *Rhyothemis* members.

DISCUSSION

Rhyothemis variegate is commonly known as “picture wing” or “variegated flutter”, widely distributed in South Asian countries. Morphologically this species is often mistaken with butterflies (Fraser, 1936). About 24 different species are coming under this genus.Here also both morphological and molecular taxonomic analysis confirmed the taxonomic identity of this species strictly as *Rhyothemis variegate*.This species doesn’t have any kind of major evolutionary change during the course of evolution and phylogenetically this is very similar to those reported from Mizoram followed by those reported from Japan.As this species is phylogenetically very close to *R. phyllis,* confirmed its genus taxonomy. Thus the present study confirmed the taxonomic identity of this species by both morphological identification and by the molecular characterization.

Pantala flavescence **(Fabricius, 1798)**

Pantala flavescence is a wide spread Libellulidae member in all continents except Antartica and rarely in Europe. It is a medium sized one having rusty thorax, reddish brown abdomen, reddish brown eye and transparent wing with reddish brown spot. Males have bright yellow or orange colored face, olivaceous or rusty thorax coated with yellowish hair and black legs. This species can be observed in all habitats except forest (Fig. 30).

The partial coding sequence of mitochondrial COI gene of *Pantala flavescence* collected from Kozhikode district (11.1352° N 75.8933° E) was PCR amplified using JRV as primer (Table 2). The DNA sequence interpret,

representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the Figures 30 (a) to 30 (g) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KR11198 and Barcode of Life Data System BIN Cluster ID – BOLD: AAH6890 (Table 65).

The COI sequence of *Pantala flavescence* showed bias to nucleotide AT, with following composition of nucleotides T = 37.0%, C = 19.4%, A = 26.6% and G = 16.9% (Table: 53). This high AT content of 63.6% over 36.4% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The BLAST analysis of both nucleotide and protein sequences showed that this species is having 100% sequence similarity to the same species reported from Malaysia having the accession number KR080077. The evolutionary history was inferred using the Neighbor-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 14 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 444 positions in the final dataset after eliminating all positions containing gaps and missing data. Here also the tree depicts that this species have a monophyletic origin with the indication of the common ancestry. The percentage of divergence table plotted by Maximum likelihood also supported the above result as it has only a slight sequence divergence of 0.09 - 0.011% sequence divergence to the same species with those reported from various geographically isolated areas (Table 53).

The sequence has also submitted in BOLD system, another database to confirm the species authenticity. The analysis showed 99.82% sequence similarity to out of 20 different similar matches already reported in BOLD

system. The line diagram of this species for the confirmation of above statement is shown in Figure 30f. The close matching BIN of the species is found to be 3% and the most similar species was found to be *Pantala flavescence* reported from Gujarat having the BOLD accession number BOLD: ANGEN067. The average and maximum nucleotide distance of this species is found to be 1% (p-distance) and 2.89% (p-distance) respectively. Here also the nearest neighbour was *Acisoma inflatum* with an average and maximum nucleotide distance of 1.52% and 2.75% (p-distance) respectively.Thus the above result confirmed the molecular taxonomic identity and also inferred its phylogeny.

DISCUSSION

Pantala flavescence is commonly known a “globe skimmer” or” globe wanderer”. This is a ubiquiotous and migratory across oceans before and after monsoon as huge swarm. This genus has high rate of gene flow among all geographic regions and can be considerd as a global panmictic populations (Daniel et al., 2016). This species is often observed during daytime and cosmopolitan in distribution and always seen as groups. The morphological identification strictly correlated with the available keys and guides. This molecular work is the pioneer work from India but doesn’t show major line of evolutionary sequence divergence to the same species from various geographically different areas. It shows only a slight variation in the sequence and hence indicated a neutral evolution. Thus the barcode generated can be used to identify and analyse its phylogenetic status at the molecular level. Phylogenetically the nearest neighbour was found to be *Acisoma inflatum* than other Libellulidae member by BOLD analysis.

***Acisoma panorpoides* Rambur, 1842**

This medium sized dragonfly is a common Libellulidae member often seen in weeded tanks and lakes.This is geographically distributed in almost all

Indian subcontinent and usual inhabitance of swampy and marshy habitats.The characteristic feature of this species is the “trumpet” like structure of Abdomen as its 5th abdominal segment is very dilated and hence the name “trumpet tail”. Male have blue face and blue eyes, azure blue colored thorax ,black legs ,transparent wings and azure blue colored abdomen.Its 1-5 abdominal segments are dilated, 6-10 are cylindrical, 3-5 consists of large lateral spots while 6-7 have azure blue spot (Fig. 31).

The partial coding sequence of mitochondrial COI gene of Acisoma panorpoides collected from Malappuram (11.0300° N 76.0500° E) district was PCR amplified using JAP as primer (Table 2). The sequence obtained, DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the Figures 31 (a) to 31 (g) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KT222947 and Barcode of Life Data System BIN Cluster ID – BOLD: ADL6242 (Table 65).

The COI sequence of *Acisoma panorpoides* showed bias to nucleotide AT, with following composition of nucleotides T = 37.2%, C = 16.5%, A = 30.3% and G = 16.1% (Table 55). This high AT content of 67.5% over 32.5% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The BLAST analysis of both nucleotide and protein sequences showed that this species is having 100% sequence similarity to the same species reported from Mizoram having the accession number (KC122228). The evolutionary history was inferred using the Neighbor-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 21 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 473 positions in the final

dataset after eliminating all positions containing gaps and missing data. Here also the tree depicts that all *Acisoma panorpoides* members have a monophyletic origin with the indication of the common ancestry. This species has also be reported from Mizoram and Karnataka but the present species showed closer relationship to those from Mizoram than Karnataka with respective divergence of to 0.04 % and 0.018%. The percentage of divergence table plotted by Maximum likelihood states only a slight sequence divergence to the species with those reported from various geographically isolated areas (Table 56).

The sequence has also submitted in BOLD system, another database to confirm the species authenticity. The analysis showed 99.82% sequence similarity to out of 20 different similar matches already reported in BOLD system. The line diagram of this species for the confirmation of above statement is shown in Figure: 31f). The close matching BIN of the species is found to be 3% and the most similar species was found to be *Acisoma panorpoides* reported from Bengaluru having the accession number BOLD: ACK2137. The average and maximum nucleotide distance of this species is found to be 0.63% (p-distance) and 1.12% (p-distance) respectively. Here also the nearest neighbour was *Acisoma panorpoides* (BOLD: ACK2137) with an average and maximum nucleotide distance of 1.07% and 1.93% (p-distance) respectively.Thus the above result confirmed the molecular taxonomic identity to this species by providing a molecular id and also for inferring its phylogenetic relationships.

DISCUSSION

Acisoma panorpoides is commonly known as “trumpet tail” (Mens et al., 2016). This libellulidae member is taxonomically identified by running keys and also from experts. Both morphological and molecular analysis confirmed the taxonomic identity of this species as *Acisoma panorpoides.* As

similar sequences have been reported from Mizoram and Karnataka, phylogenetically this species is very close to those sequences reported from Mizoram. The nearest generic neighbour of this species is *A. inflatum* followed by *A. variegatum* and *A. attenborooughi.* Thus the molecular work helped to easily identify *Acisoma panorpoides* species by providing a barcode and to infer its phylogeny.

***Neurothemis tullia* (Drury, 1773)**

Neurothemis tullia is a dragonfly species commonly called as "Pied Paddy Skimmer", widely distributed in south and Southeast Asia. It is a small black dragonfly with black and white (male) or brown and black (female) wings. They abundantly seen as large colonies in swamps and heavily-weeded tanks, aquatic weeds. They usually have two or more generation per year and its nymphal stage is usually seen associated with rice fields (Fig. 32).

The partial coding sequence of mitochondrial COI gene of *Neurothemis tullia* collected from Malappuram (11.0300° N 76.0500° E) district was PCR amplified using JNT as primer (Table: 1). The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the Figures 32 (a) to 32 (g) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KP835513 and Barcode of Life Data System BIN Cluster ID – BOLD: ABX8024 (Table 65).

The COI sequence of *Neurothemis tullia* showed bias to nucleotide AT, with following composition of nucleotides T = 33.8%, C = 17.4%, A = 28.3% and G = 20.5% (Table: 57). This high AT content of 62.1% over 37.9% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The BLAST analysis of both nucleotide and protein sequences showed that this species is having 100% sequence similarity to the same species reported from Mizoram having the accession number KC12229. The evolutionary history was inferred using the Neighbor-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 21 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 383 positions in the final dataset after eliminating all positions containing gaps and missing data. Here also the tree depicts that all *Neurothemis tullia* members have a monophyletic origin with the indication of the common ancestry. The percentage of divergence table plotted by Maximum likelihood also states that no sequence divergence to the species with those reported from various geographically isolated areas (Table 58). It showed the respective divergence of 0.12, 0.10 and 0.09 % divergence to the same species reported from Mizoram, Thailand and Japan.

The sequence has also submitted in BOLD system, another database to confirm the species authenticity. The analysis showed 99-100% sequence similarity to out of 10 different similar matches already reported in BOLD system.The line diagram of this species for the confirmation of above statement is shown in Figure: 32f. The close matching BIN of the species is found to be 3% and the most similar species was found to be *Neurothemis tullia* reported having the BIN cluster id as BOLD: ABX8024. The average and maximum nucleotide distance of this species is found to be 0.63% (p-distance) and 1.28% (p-distance) respectively. Here also the nearest neighbour was *Neurothemis* fluctans having the BOLD ID: ADC4643 with an average and maximum nucleotide distance of 0.75% and 1.68.64% (p-distance) respectively. Thus the above result confirmed the molecular taxonomic identity of this species by providing a molecular id and also for inferring its phylogenetic relationship with other *Neurothemis* members.

DISCUSSION

Neurothemis tullia is popularly known as “Pied paddy skimmer” known to be widely distributed in South and South East Asia. They have 2 generation per year and its nymphal stage is found in stagnant water around rice fields.They are ecologically important as the predators of rice pests such as Plant hoppers, leaf hoppers and stem borers (Che et al., 2000). The female polymorphism is one of the mechanisms exhibited by *Neurothemis tullia* and it has been known to be reported from North eastern sides of India (Kumar, 1988; Mitra, 1991). In the preent study morphological keys strictly says its morphotaxonomy as Neurothemis tullia.Eventhough it exhibits polymorphism the male, female and andromorphic female (polymorphic female) showed similar DNA sequences when its cytochrome oxidase I gene was PCR amplified and analysed (Jisha and Sebastian, 2015d). Even though this species has been reported from various geographical areas, phylogenetically this species is found to those reported in the following manner Mizoram, Thailand and Japan. Phylogenetically this species is very close to *Neurothemis fluctans* than other *Neurothemis* genera by both NCBI and BOLD analysis. Thus the present study concluded that both morphological and molecular analysis showed similar result that helped to resolve its phylogeny.

Lathresia asiatica (Rambur, 1842)

Body is metallic bluish black in colour having reddish brown eys, dark brown colour thorax on the dorsal and bright yellow on lateral sides with two black “Y” shaped markings with narrow black stripes (Fig. 33). This species is sparingly distributed in India exept in dry zones and usually seen in colonies.Their breeding is known to be occur in pools (Emiliyamma et al., 2005).

The partial coding sequence of mitochondrial COI gene of *Lathresia asiatica* collected from Kozhikode district (11.1352° N 75.8933° E) of Kerala state was PCR amplified using JPF as primer (Table:1). The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the Figures 33 (a) to 33 (g) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KU052671 and Barcode of Life Data System BIN Cluster ID – BOLD: ADJ7302 with Specimen ID – GBMIN88787-17 (Table 65).

The COI sequence of *Lathresia asiatica* showed bias to nucleotide AT, with following composition of nucleotides T = 33.7%, C = 20.7%, A = 27.2% and G = 18.4% (Table 59). This high AT content of 60.9% over 39.1% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The BLAST analysis of both nucleotide and protein sequences showed that this species is having 100% sequence similarity to *Neurothemis intermedia* reported from Kerala having the accession numbe KU052672.The evolutionary history was inferred using the Neighbor-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 16 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 407 positions in the final dataset after eliminating all positions containing gaps and missing data. Here the tree depicted that *Lathresia* sp is having close relationship to Neurothemis members than other Libellulidae members. This is also supported by the percentage of divergence table plotted by Maximum likelihood method. This showed 0 to 0.11% sequence divergence to other *Neurothemis* species reported from various geographically isolated areas (Table 60).

The sequence has also submitted in BOLD system, another database to confirm the species authenticity. The analysis showed 97-99.82% sequence similarity to out of 10 different similar matches already reported in BOLD system. The line diagram of the species for the confirmation of above statement is shown in Figure 34h. The close matching BIN of the species is found to be 3% having the accession number BOLD: ADJ7302. The distance to the nearest neighbour is found to be 5.33 %.Thus the above result confirmed the molecular taxonomic identity to this species by providing molecular id and also for inferring its phylogenetic relationship with other *Neurothemis* members.

DISCUSSION

Lahresia asiatica is a common Libellulidae member found during rainy season. This is a pioneer molecular work from India and its COI gene analysis showed its closer relationship with *Neurothemis intermedia*, another Libellulidae member. This species showed closer relationhip with *Neurothemis* genus than other Odonata species. There is no taxonomic work has been reported for this species till now and hence the barcode generated can be used to easily spot the specimen and also to infer its phylogeny.

Aethriamanta brevipennis (Rambur, 1842)

Aethriamanta brevipennis is a small dragonfly having black thorax and scarelet abdomen.They are usually seen in many Asian countries and consist of face which is covered with short and stiff black hairs, dark chocolate thorax, black legs and transparent wings with bright golden yellow venation (Fig. 34). The common habitat of this species is found to be weedy lakes, ponds and lakes (Manoj, 2011).

The partial coding sequence of mitochondrial COI gene of *Aethriamanta brevipennis* collected from Malappuram (11.0300° N 76.0500°

E) district was PCR amplified using JAT as primer (Table 2). The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the Figures 34 (a) to 34 (g) respectively. The sequence was deposited in nucleic acid database for public accession having NCBI GenBank Accession No. KU510325 (Table 65).

The COI sequence of *Aethriamanta brevipennis* showed bias to nucleotide AT, with following composition of nucleotides T = 31.1%, C = 21.3%, A = 27.4% and G = 20.3% (Table 61). This high AT content of 58.5% over 41.5% of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The BLAST analysis of both nucleotide and protein sequences showed that this species is having 99 % sequence similarity to *Anax speratus* reprted from Netherland having the accession numbers KU565929 and KU565930. The evolutionary history was inferred using the Neighbor-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 12 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 508 positions in the final dataset after eliminating all positions containing gaps and missing data. Here the tree depicts that this species showed a sister clade relationships to Aeshidae members than other Libellulidae members on the basiss of sequence similarity. The above statement is also supported by the divergence table plotted in maximul likelyhod method. It showed 0.17 % sequence divergence to *Anax speratus* on the basis of COI gene analysis.

DISCUSSION

Aethriamanta brevipennis is commonly known as “Scarelet marsh hawk” widely distributed in Asian countries. Morphological identication done

with online photographs and taxonomic keys strictly says it as *Aethriamanta brevipennis*. This is the pionner molecular report from India and its unique id developed by PCR method strictly says it as a Libellulidae member by Nucleotide and Protein analysis. Phylogenetically this libellulidae member showed closer relationship with Gomphidae members (*Anax parthenope)* indicating a closer relationship of Libellulidae and Gomphidae.

***Brachydiplax sobrina* Rambur, 1842**

Brachydiplax sobrina is a Libellulidae member widely distributed in India, Myanmar, Bangladesh, Nepal, Sreelanka and Thailand (Subramanian, 2009). Males have yellowish white and black with metallic blue green head, dark brown thorax, black legs and transparent wings with brown base. Abdomen is black with bluish white in colour. They are commonly observed in marshes, ponds and rivers (Subramanian and Sivaramkrishnan, 2005).

The partial coding sequence of mitochondrial COI gene of *Brachydiplax sobrina* collected from Kasargode district (12.5000° N 75.0000° E) of Kerala was PCR amplified using JPF as primer (Table 2). The DNA sequence interpret, representative molecular barcode, conceptual translation product, nucleotide BLAST, peptide BLAST, line diagram and molecular phylogenetic tree are presented in the Figures 35 (a) to 35 (g) respectively. The sequence was deposited in nucleic acid databases for public accession having NCBI GenBank Accession No. KT372720 and Barcode of Life Data System BIN Cluster ID – BOLD: ACD4364 with Specimen ID – GBMIN88787-17 (Table 65).

The COI sequence of *Brachydiplax sobrina* showed bias to nucleotide AT, with following composition of nucleotides T = 31.2%, C = 20.0%, A = 30.9% and G = 17.9% (Table 63). This high AT content of 62.1% over 37.9%

of GC is mainly due to the mutational pressure on a single nucleotide substitution during the evolutionary period of time.

The BLAST analysis of both nucleotide and protein sequences showed that this species is having 100% sequence similarity to *Brachydiplax chalybae* reported from kerala having the accession number (KT372721). The evolutionary history was inferred using the Neighbor-Joining method and the evolutionary history of the taxa was analyzed. The analysis involved 15 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 423 positions in the final dataset after eliminating all positions containing gaps and missing data. Here the tree depicted the *Brachydiplax* genus taxonomy, which also confirmed by the distance table analysis.The distance table analysis showed 0.13 – 0.16% divergence to *Brachydiplax chalybeae* species reported from Kerala and Mizoram respectively in MEGA analysis. The percentage of divergence table plotted by Maximum likelihood also showed the respective divergence to the species with those reported from various geographically isolated areas (Table 64).

The sequence has also submitted in BOLD system, another database to confirm the species authenticity. The analysis showed 98-99% sequence similarity to out of 20 different similar matches already reported in BOLD system. The line diagram for the confirmation of above statement is shown in Figure 35f). The close matching BIN of the species is found to be 3% and the most similar species was found to be reported from Kerala having the accession number BOLD ACD4364. The average and maximum nucleotide distance to this species is found to be 0.73% distance) and 2.27% (p-distance) respectively. Here also the nearest neighbour was is found to be having 10.27% of divergence. Thus the above result confirmed the molecular

taxonomic identity to this species by providing a molecular id and also to infer its phylogenetic relationships.

DISCUSSION

Brachydiplax sobrina is popularly known as "Little Blue Marsh Hawk". Morphological identification done by running keys and expert consultation confirmed it as *Brachidiplax sobrina*. Molecular identification method in NCBI and BOLD database showed the conformity of this species as in *Brachydiplax genera.* Here the phylogenetic relationship showed that it was very close to *Brachydiplax chalybaea*. Phylogenetic tree interprets that all *Brachydiplax* genus have a common ancestry as all are bifurcated from one clade. As this is a pioneer molecular work from Kerala, the barcode generated can be used to easily spot the specimen and also to infer phylogeny.

PHYLOGENETIC ANALYSIS OF ANISOPTERA

Anisoptera represents one of the suborders of the Order Odonata known to be evolved during Triassic period about 250-200 million years ago (Grimaldi and Engel, 2005). They are characterised by differently sized forewing and hindwings, large sized, brightly coloured and stout bodied ones. The present study investigates the taxonomic relationship of Libellulidae, Aeshnidae and Gomphidae family of the Superfamily Libelluloidea. Libelluidae represents one of the most successful and recently differentiated Anisopteran families. They are cosmopolitan in distribution and these ubiquitous members are usually seen in all lentic habitats. This is the most dominant family of Anisoptera in all diversity studies (Jisha and Sebastian, 2015a; Dayakrishna, 2015; Khan, 2018). It has been held that Libellulidae were evolved during cretaceous period about 142-65million years ago (Jarzembowski and Nel, 1996). It consists of 143 genera and 969 species. This is considered to be the most recently originated dragonfly family. This

family can be easily recognised by having brightly coloured or patterned wing with a boot shaped series of veins (anal loop) in the hindwing. The adult reproductive behaviour, feeding behaviour, ecology and biogeography of all these members are varying and hence it made in intensive study by various workers (Carle, 1982; Corbet, 1999). Most of the morphological studies of this family were based on wing venation characters (Kirby, 1890; Needham, 1903; 1931; Fraser. 1936; Bechly, 1996; Carle et al., 2008; Rehn, 2003). Some studies are mainly based upon egg, genitalia, flight musculature, colour, and larval characteristics (Theischinger et al., 2009). Several recent molecular studies were also reported (Kambanpati and Charlton, 1999; Artiss et al., 2001; Jisha and Sebastian, 2015c) which were mainly based upon single marker gene analysis. However all morphology based and molecular based studies produced a monophyletic ancestry to this family. Gomphidae represent another family taken for analysis. Species diversity of Gomphidae is likely higher than of any other Anisopteran suborder except Libellulidae but no phylogenetic studies of Gomphidae has been reported till now. Carle (1982) provided the most recent and comprehensive classification based on morphological synapomorphies but it did not provided phylogenetc analysis. Jessica et al. (2007) made the first molecular phylogenetic analysis of Gomphidae. She classified all members in this family into 4 divisions in which the subdivision Lindenia possess the subfamily Onychogomphinae. *Onychogomphus malabarensis* is an Onychogomphinae member and here the morphological identification, its molecular taxonomic conformity and phylogenetic analysis has been done. The phylogenetic tree confirmed its taxonomic identity and phylogeny showed its closer relationship with *Ophiogomphus* species indicating Gomphidae genus. Another family Aeshnidae has taken represented by two species such as *Anax parthenope* and *Anaciaeshna jaspidea* which also showed closer relation ship as sister taxa. This family was found to be closer to Libellulidae.

The phylogenetic tree constructed by Neighbur joining method showed the phylogenetic relationship of all Anisopteran members in the representing families such as Libellulidae, Aeshnidae and Gomphidae (Fig. 36). In the present study among the 28 genera analysed *Trithemis*, *Neurothemis, Brachydiplax* and *Agriocnemis* genera were represented by more than one species. *Trithemis*, *Agriocnemis* and *Neurothemis* were represented by three and *Brachydiplax* by two voucher specimens. Both these genera showed a monophyletic ancestry. As *Trithemis* genera is represented by three species in which *Trithemis festiva* and *Trithemis aurora* are found sister taxa with each other while *Trithemis pallidinervis* was not made a conclusive result since the support value is lower and found outer to this clade. Also among *Neurothemis* genus, *Neurothemis fulvia* and *Neurothemis intermedia* were arranged as sister taxa and *N. tullia* found outer to this clade. This conclusion is strongly supported by the previous study of Laltanpuii et al. (2017). The nucleotide frequencies of all these members are A = 30.3%, T = 34.1%, C = 18.3%, G = 17.4% with the codon positions including 1^{st} + 2^{nd} + 3^{rd} + non coding. The nucleotide frequencies are high in AT (64.4%) and low GC (35.7%) which is typical for Arthropods in many previous studies.

A consolidated list of nucleic acid database accession details for the species analysed during the present study is presented in Table 65.

CONCLUSION

The insect order Odonata along with mayflies represents the most basal group of primitive winged insect commonly known as Palaeoptera. They were known to be diverged during Jurassic period and widely used to trace out the history of the entire insect fauna as being the primitive winged ones. The ancestors of the modern odonates were known to be existed during the middle Carboniferous period (325 million years ago) and these proto-odonates were very similar to the modern ones with respect to their ability for fast flight and the habit of voracious feeding. The first fossil record appeared dates from Permian period (250 million years ago) exhibited the characteristics of either Protoanisoptera or Protozygoptera. The Odonata of India is represented by 488 species and 27 subspecies in 154 genera and 18 families. The Suborder Zygoptera comprises of 211 species under 59 genera and 9 families; Anisozygoptera consist one species under one genera and one family while Anisoptera has 276 species under 94 genera and 18 families. Out of this abundance, 154 species were authentically reported from Kerala so far.

Morphological studies

During the present study, the odonates were collected from seven major districts of Northern Kerala by selecting each district with three different ecosystems on the basis of the observed specimen abundance. The morphological identification of each species was done with the aid of available keys and was confirmed from taxonomic experts. Based on the morphologically identified characters, the collected specimens were classified into 4 super families falling to 6 different families such as Coenagrionidae (6 sp), Plactinemididae (1 sp) and Calopterygidae (2sp) (Zygoptera: Super families Coenagrionoidea and Calopterygoidea); Libellulidae (25 sp),

Aeshnidae (2 sp) and Gomphidae (1 sp) (Anisoptera: Super families Libelluloidea and Aeshnoidea).

Molecular studies

Molecular studies based on cytochrome oxidase I (COI) gene analysis confirmed the taxonomic position and phylogenetic status of the representative members selected under the study. This is a pioneer molecular work from Kerala and the COI gene sequences (barcode sequences) of 37 voucher specimens has been submitted to public nucleotide databases for confirming their species identity. The submission data includes five new reports globally, means two endemic species from Kerala (*Onychogomphus malabarensis* and *Agriocnemis keralensis*), two Coenagrionidae members (*Ceriagrion coromandelianum* and *Aciagrion occidentale*), one Libellulidae member (*Lathresia* sp.) along with 5 other pioneer reports from India (Table 66).

There existed a lot of controversies for the prediction of interfamily relationship among the concerned suborders. It was over last 45 years, phylogenetic hypothesis have been made to resolve the phylogenetic relationship based on the morphological features like wing venation characters, flight apparatus and also copulatory structures. But most of these phylogenetic studies showed different outcomes when considered either morphological features or molecular characteristics as the tool.

The previous hypotheses of odonate phylogeny based on the analysis of morphological features can be explained as follows: Among Anisopterans; Libellulidae and Aeshnidae are sister clades with each other with Gomphidae as outgroup. At the same time there were other reports that Gomphidae and Libellulidae are sister clades with Aeshnidae family as outer. Among Zygopterans; Coenagrionidae and Calopterygidae are sister clades.

Phylogenetic analysis done using molecular methods were also supported the above interfamily relationship of Anisoptera and Zygptera to certain extend.

The phylogenetic analysis done using MEGA software by Neighbour joining method in the present study revealed a close relationship of all members under study and the respective families also. It was established that a phylogenetic tree with sum of branch length 1.82 (Jukes cantor method) as evolutionary distance only can be taken into account as an optimal evolutionary tree. The generic taxonomy of *Trithemis, Neurothemis, Ischnura*, *Agriocnemis Brachydiplax* and *Vestalis* has been confirmed by the present phylogenetic tree analysis. Further the monophyly of both suborders (Anisoptera and Zygoptera) were confirmed as it seems diverged from a single ancestral clade. The interfamily relationships among these orders were also proved.

Among Anisopterans, interfamily relationships are exhibited by three families (Libellulidae, Gomphidae and Aeshnidae) with a closer relationship of Aeshnidae + Libellulidae to Gomphidae. This relationship was supported by the morphological studies of Carle (1982), Truemann (1996) and Rehn (2003) and also the molecular study reported by Saux et al. (2003).

Among Zygopterans, interfamily relationships are established among three families (Coenagrionidae, Platicnemididae and Calopterygidae) with a close relationship of Coenagrionidae + Platicnemididae to Calopterygidae. This relationship was also supported by Fleck et al. (2008), Dument et al. (2010) and Bybee et al. (2008) through their combined analysis based on both molecular and morphological characters. Thus it can be confirmed that among Anisoptera; Aeshnidae and Libellulidae families are more related with each other whereas in in Zygoptera; Plactinemidae and Coenagrionidae families. Thus cytochrome oxidase I gene sequence provided a better molecular tool

for confirming the taxonomic identity and also the phylogenetic relationship of the different members of the order Odonata.

In order to understand how the order Odonata has been related to other insect groups, one each representative species were taken from different insect families that are closely related to this order, namely Ephemeroptera, Coleoptera and Lepidoptera, for the construction of phylogenetic tree. The result showed that damselflies are more closely related to Ephemeroptera whereas dragonflies to Coleoptera and Lepidoptera. This indicates different rate of divergence of insect families during their evolution over time. Further on the basis of the COI gene nucleotide substitution analysis, it can be attributed that the odonate members from Kerala doesn't have any major sequence divergence to those reported from various other geographical areas indicating their neutral evolution.

BIOGEOGRAPHY OF ODONATES

The geographical distribution of the odonates existing today is known to be attributed to the continental drift forces coupled with natural dispersal and adaptive radiation over 300 million years. This in turn leads to the speciation and endemism in tropical regions as most of them, especially anisopterans, are strong fliers and some species are migratory in nature. It can be concluded that most of the anisopterans exhibit vicariance and dispersal events while speciation among zygopterans are on the basis of adaptation to climatic variations. In short it can be established that this insect order has dispersal ability mainly influenced by biogeographic patterns.

BIOGEOGRAPHY OF ZYGOPTERA

These small sized damselflies' distributions coincide with climatologically distinct zones and known to be more diverse along equator where they experienced high temperature. A limited number of studies have

explored the effects of key biogeographical events on individual damselfly taxa (De Marmels, 2001; Dumont et al., 2005; Groeneveld et al., 2007; Polhemus, 1997; Turgeon et al., 2005). Tropical regions hold the greatest number of species, and it has been suggested that this high diversity can be explained by the abundance of aquatic habitat in tropical forest (Orr, 2006) and as tropical mountains provide a diverse niche and regional refugia (Kalkman et al., 2008).

Coenagrionidae represents the most diverse and abundant family and it known to be existed in all continents except Antartica due to its high capacity for colonization. In the present study, there are seven Coenagrionidae species from Kerala has taken for phylogenetic assessment, which doesn't show much more sequence variation from its individual species in the geographically isolated areas by molecular analysis. Calopterygidae represents another family and here two species (*Vestalis apicalis* and *Vestalis gracilis*) from Kerala was taken for inferring the phylogeny through molecular analysis. This family, which is known to be distributed in all continents except Australian region possessing similar habitat, morphology and mating displays, showed very less sequence variation from its counterparts in the other geographically isolated areas. The tree indicated a monophyletic ancestry which is in tune with the previous works of Bybee et al. (2008) and Dumont et al. (2005) and clearly rejects the postulates of Mullen and Andres (2007) on geographic pattern of distribution.

Plactinemidae is the other family studied, which was represented by only one species, *Copera marginipes.* Here also the COI gene sequence comparison has confirmed the taxonomic identity and inferred the monophyletic evolution of this species. This is a pioneer study from India and this species is very similar to those reported from Netherlands indicating no geographical pattern of divergence.

Thus the Zygopteran phylogeny analysis during the present study revealed a conserved sequence evolution among the representative members and can be concluded that this suborder doesn't have any major evolutionary changes in the geographically different areas.

BIOGEOGRAPHY OF ANISOPTERA

Anisoptera represents the active fast flying geographically vast individuals. The phylogeny of this suborder is still unclear due to contradictory outcome of results from various studies. There exist a lot of disagreements for the prediction of interfamily relationships. Here there are 28 species distributed in 3 families (Libellulidae: 25sp, Aeshnidae: 2sp and Gomphidae: 1sp) has been taken for phylogenetic analysis. Libellulidae was the dominant and most abundant family throughout the entire duration of study represented with 25 species and most of the species showed similarity in the concerned sequences showing conserved evolution. It has been proved that the superfamily Libelluloidea was diverged during the Jurassic (Thomas et al., 2011) and Early Cretaceous periods (Jarzembowski and Nel, 1996; Fleck et al., 2008). At that time the Pangaea land mass begun to split creating southwest Indian Ocean rift which separated South America and Africa from East Gondwanaland as well as India from Antartica (Dietz and Holden, 1970). Most of the previous works had shown that Gondwanaland begun to apart during cretaceous period leading to dispersal and isolated population (Veevers, 2004). Thus the phylogenetic study of Libellulidae represents the conserved gene sequence in the geographically isolated regions of all similar species under study and their taxonomic relationship produced monophyletic divergence. Gomphidae is another family represented with only 1 species (*Onychogomphus malabarensis),* which also emphasised the family conformity. This family showed a close relation to Libellulidae than other family members in the suborder. Still, there exists a disagreement to where

this family has to be placed unambiguously. Evolutionarily it has been told that these two families are closely related on the basis of possessing exophytic oviposition behaviour and reduced vestigial ovipositor (Mellisa, 2010). In the present molecular based analysis also they were shown to be arranged very close to each other confirming their taxonomic relatedness. Aeshnidae family is represented by two species, *Anax parthenope* and *Anaciaeshna jaspidea*, which also showed no difference in their COI gene sequences with their counterparts from the geographically different areas. Thus the present study proved that even though Libellulidae has been related to Gomphidae, it is more close to Aeshnidae as more members are found near its clade on the basis of nucleotide sequences.

The maximum likelihood tree generated showed monophyly for both suborders. Here the phylogenetic tree can be divided into two distinct clades (A1 and A2), in which A1 clade represents Anisopteran families while A2 clade represents Zygopteran families (Fig. 38). This monophyly is supported by the previous molecular works done by Rehn (2003), Truemann (1996) and Pfu (1991). Thus the present study concluded that even though the representing members of each families are distributed in various geographically isolated areas by continental shift, there is no much considerable variations has been observed on the basis of COI gene sequence analysis and hence can predict the neutral evolution and monophyletic origin for odonate fauna.

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Table 23: The Nucleotide substitution table of *Anaciaeschna jaspidea*

	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
KR149806.1 *Anaciaeschna jaspidea* (Kerala)	32.9	16.5	32.9	17.7	33	3.6	58.0	5.4	25	14.4	27.9	32.4	41	31.5	12.6	15.3
AB709110.1 *Rhyothemis phyllis*	32.9	16.5	32.9	17.7	33	3.6	58.0	5.4	25	14.4	27.9	32.4	41	31.5	12.6	15.3
AB709113.1 *Rhyothemis variegata*	32.6	16.8	32.9	17.7	32	4.5	58.0	5.4	25	14.4	27.9	32.4	41	31.5	12.6	15.3
JX306649.1 *Anaciaeschna jaspidea*	32.9	16.5	32.9	17.7	33	3.6	58.0	5.4	25	14.4	27.9	32.4	41	31.5	12.6	15.3
KC287151.1 *Rhyothemis variegata*	32.6	16.5	33.2	17.7	32	3.6	58.9	5.4	25	14.4	27.9	32.4	41	31.5	12.6	15.3
KP938530.1 *Rhyothemis variegata*	32.9	16.5	32.9	17.7	33	3.6	58.0	5.4	25	14.4	27.9	32.4	41	31.5	12.6	15.3
KT957511.1 *Rhyothemis phyllis phyllis*	32.9	16.5	32.9	17.7	33	3.6	58.0	5.4	25	14.4	27.9	32.4	41	31.5	12.6	15.3
KU361232.1 *Orthetrum glaucum*	35.0	17.1	32.0	15.9	41	2.7	55.4	.9	23	17.1	27.9	31.5	41	31.5	12.6	15.3
KU496893.1 *Orthetrum glaucum*	35.0	17.1	32.0	15.9	41	2.7	55.4	.9	23	17.1	27.9	31.5	41	31.5	12.6	15.3
Avg.	33.3	16.6	32.8	17.3	35	3.5	57.5	4.4	25	15.0	27.9	32.2	41	31.5	12.6	15.3

Table 39: The Nucleotide substitution table of *Crocothemis servilia*

Domain: Data																
	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
KR149807.1 *Crocothemis servilia* (Kerala)	34.8	17.1	31.3	16.8	23	16.8	29.1	31.3	44	26.3	14.5	15.1	37	8.4	50.3	3.9
JN817425.1 *Crocothemis servilia*	34.8	17.1	31.3	16.8	23	16.8	29.1	31.3	44	26.3	14.5	15.1	37	8.4	50.3	3.9
KY847585.1 *Crocothemis erythraea*	35.0	16.6	31.3	17.1	23	16.8	28.5	31.3	44	26.3	14.5	15.1	37	6.7	50.8	5.0
KY847584.1 *Crocothemis erythraea*	34.8	16.8	31.1	17.3	23	16.8	28.5	31.3	44	26.3	14.5	15.1	37	7.3	50.3	5.6
KY847583.1 *Crocothemis erythraea*	35.0	16.6	31.3	17.1	23	16.8	28.5	31.3	44	26.3	14.5	15.1	37	6.7	50.8	5.0
KY847582.1 *Crocothemis erythraea*	35.0	16.6	31.3	17.1	23	16.8	28.5	31.3	44	26.3	14.5	15.1	37	6.7	50.8	5.0
KC912240.1 *Crocothemis erythraea*	35.0	16.6	31.3	17.1	23	16.8	28.5	31.3	44	26.3	14.5	15.1	37	6.7	50.8	5.0
KC912241.1 *Crocothemis erythraea*	35.0	16.6	31.3	17.1	23	16.8	28.5	31.3	44	26.3	14.5	15.1	37	6.7	50.8	5.0
KC912238.1 *Crocothemis erythraea*	34.8	16.8	31.1	17.3	23	16.8	28.5	31.3	44	26.3	14.5	15.1	37	7.3	50.3	5.6
KY847581.1 *Crocothemis erythraea*	34.8	16.8	31.3	17.1	23	16.8	28.5	31.3	44	26.3	14.5	15.1	37	7.3	50.8	5.0
Avg.	34.9	16.7	31.2	17.1	23	16.8	28.6	31.3	44	26.3	14.5	15.1	37	7.2	50.6	4.9

Table 15: The Nucleotide substitution table of *Copera marginipes*

Domain: Data																
	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
KR149804.1 *Copera marginipes (*KERALA)	34.6	15.8	31.4	18.1	24	15.9	28.4	31.8	43	27.0	13.5	16.5	37	4.5	52.5	6.0
KF369351.1 *Copera marginipes*	34.6	15.8	31.4	18.1	24	15.9	28.4	31.8	43	27.0	13.5	16.5	37	4.5	52.5	6.0
KT879906.1 *Elattoneura vittata*	35.1	15.6	31.9	17.3	25	12.9	29.9	31.8	43	27.0	13.0	17.0	37	7.0	53.0	3.0
KF369352.1 *Copera nyansana*	34.8	16.3	30.3	18.6	23	15.4	28.9	32.3	43	27.0	13.0	17.0	38	6.5	49.0	6.5
KX890965.1 *Ophiogomphus smithi*	35.1	16.5	31.6	16.8	23	15.4	28.9	32.3	43	27.5	13.5	16.0	39	6.5	52.5	2.0
KX890938.1 *Ophiogomphus smithi*	35.1	16.5	31.6	16.8	23	15.4	28.9	32.3	43	27.5	13.5	16.0	39	6.5	52.5	2.0
KX890940.1 *Ophiogomphus westfalli*	34.8	16.8	31.6	16.8	23	15.9	28.9	32.3	43	27.5	13.5	16.0	39	7.0	52.5	2.0
KF369353.1 *Copera sikassoensis*	34.4	16.3	31.1	18.1	25	14.4	28.4	32.3	43	27.0	13.0	17.0	36	7.5	52.0	5.0
Avg.	34.8	16.2	31.4	17.6	24	15.2	28.8	32.2	43	27.2	13.3	16.5	38	6.3	52.1	4.1

Table 33: The Nucleotide substitution table of *Brachydiplax chalybaea*

Domain: Data																
	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
KT372721.1 *Brachydiplax chalybea (*KERALA)	33.0	18.9	30.7	17.4	44	27.4	14.2	14.7	32	12.6	50.5	4.7	23	16.8	27.4	32.6
KC287156.1 *Brachydiplax chalybea*	33.0	18.9	30.7	17.4	44	27.4	14.2	14.7	32	12.6	50.5	4.7	23	16.8	27.4	32.6
KX281798.1 *Acisoma inflatum*	36.8	16.3	30.5	16.3	43	27.4	14.2	15.3	44	4.2	49.5	2.1	23	17.4	27.9	31.6
KX281797.1 *Acisoma attenboroughi*	35.8	17.0	31.2	16.0	43	27.4	14.2	15.3	41	6.3	51.6	1.1	23	17.4	27.9	31.6
KX281792.1 *Acisoma attenboroughi*	35.4	17.4	31.1	16.1	43	27.4	14.2	15.3	40	7.4	51.1	1.6	23	17.4	27.9	31.6
KX281796.1 *Acisoma attenboroughi*	35.6	17.2	31.1	16.1	43	27.4	14.2	15.3	41	6.8	51.1	1.6	23	17.4	27.9	31.6
KX281795.1 *Acisoma attenboroughi*	35.4	17.4	31.2	16.0	43	27.4	14.2	15.3	40	7.4	51.6	1.1	23	17.4	27.9	31.6
KX281794.1 *Acisoma attenboroughi*	35.4	17.4	31.2	16.0	43	27.4	14.2	15.3	40	7.4	51.6	1.1	23	17.4	27.9	31.6
KX281793.1 *Acisoma attenboroughi*	35.4	17.4	31.2	16.0	43	27.4	14.2	15.3	40	7.4	51.6	1.1	23	17.4	27.9	31.6
Avg.	35.1	17.5	31.0	16.4	43	27.4	14.2	15.1	39	8.0	51.0	2.1	23	17.3	27.8	31.8

Table 43: The Nucleotide substitution table of *Trithemis festiva*

Domain: Data																
	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
KR149802.1 *Trithemis festiva* (KERALA)	36.3	15.1	31.8	16.9	24	15.2	28.3	32.6	43	27.7	14.1	15.2	42	2.2	53.0	2.7
FJ358469.1 *Trithemis stictica*	37.0	16.3	29.0	17.6	22	16.8	28.8	32.6	43	27.2	14.1	15.8	46	4.9	44.3	4.4
FJ358477.1 *Trithemis grouti*	36.7	16.7	29.0	17.6	22	16.8	28.3	32.6	43	27.2	14.1	15.8	45	6.0	44.8	4.4
FJ358481.1 *Trithemis furva*	36.7	16.0	30.5	16.9	23	15.8	28.3	32.6	43	27.2	14.1	15.8	44	4.9	49.2	2.2
JN817429.1 *Trithemis festiva*	36.3	15.1	31.8	16.9	24	15.2	28.3	32.6	43	27.7	14.1	15.2	42	2.2	53.0	2.7
KT961629.1 *Trithemis festiva*	36.7	14.9	31.0	17.4	24	15.2	28.3	32.6	43	27.7	14.1	15.2	43	1.6	50.8	4.4
KU566418.1 *Trithemis anomala*	35.2	17.6	29.9	17.2	20	18.5	28.8	32.6	43	27.2	14.1	15.8	43	7.1	47.0	3.3
KU566456.1 *Trithemis stictica*	36.8	16.5	29.2	17.4	22	16.3	28.8	32.6	43	27.2	14.1	15.8	45	6.0	44.8	3.8
KU566458.1 *Trithemis werneri*	35.9	15.6	31.2	17.2	23	15.8	28.3	32.6	43	27.7	14.1	15.2	42	3.3	51.4	3.8
Avg.	36.4	16.0	30.4	17.2	23	16.2	28.4	32.6	43	27.4	14.1	15.5	44	4.3	48.7	3.5

Table 27: The Nucleotide substitution table of *Orthetrum sabina*

Domain: Data																
	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
KP938529.1 *Orthetrum sabina* (Kerala)	35.4	17.6	29.8	17.2	44	27.5	13.2	15.0	39	8.4	47.9	4.8	23	16.9	28.3	31.9
KT961626.1 *Orthetrum sabina*	35.4	17.6	29.8	17.2	44	27.5	13.2	15.0	39	8.4	47.9	4.8	23	16.9	28.3	31.9
KT957506.1 *Orthetrum sabina*	35.4	17.6	29.8	17.2	44	27.5	13.2	15.0	39	8.4	47.9	4.8	23	16.9	28.3	31.9
KT957505.1 *Orthetrum sabina*	35.4	17.6	29.8	17.2	44	27.5	13.2	15.0	39	8.4	47.9	4.8	23	16.9	28.3	31.9
KC122234.1 *Orthetrum sabina*	35.4	17.6	29.8	17.2	44	27.5	13.2	15.0	39	8.4	47.9	4.8	23	16.9	28.3	31.9
KT957507.1 *Orthetrum sabina*	35.2	17.8	29.8	17.2	44	27.5	13.2	15.0	38	9.0	47.9	4.8	23	16.9	28.3	31.9
KU361234.1 *Orthetrum sabina*	34.9	18.0	29.7	17.4	44	27.5	13.2	15.0	37	9.6	47.6	5.4	23	16.9	28.3	31.9
KX670387.1 *Orthetrum sabina*	34.8	18.2	30.0	17.0	44	27.5	13.2	15.0	37	10.2	48.5	4.2	23	16.9	28.3	31.9
Avg.	35.2	17.8	29.8	17.2	44	27.5	13.2	15.0	38	8.8	47.9	4.8	23	16.9	28.3	31.9

Table 17: The Nucleotide substitution table of *Vestalis apicalis*

Domain: Data																
	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
KU510326.1 *Vestalis apicalis* (KERALA)	30.3	22.1	27.5	20.1	22	21.5	25.8	31.2	41	25.9	16.8	16.8	29	18.8	39.8	12.4
KM675770.1 *Vestalis apicalis*	30.3	22.1	27.5	20.1	22	21.5	25.8	31.2	41	25.9	16.8	16.8	29	18.8	39.8	12.4
KM675768.1 *Vestalis gracilis*	30.3	22.1	27.5	20.1	22	21.5	25.8	31.2	41	25.9	16.8	16.8	29	18.8	39.8	12.4
KX503058.1 *Vestalis gracilis*	30.3	22.1	27.5	20.1	22	21.5	25.8	31.2	41	25.8	16.7	16.7	29	18.9	40.0	12.4
KJ493064.1 *Metagrion fornicatum*	30.9	20.3	31.1	17.6	23	19.4	29.6	28.5	41	24.9	16.8	17.3	29	16.8	47.0	7.0
FJ812855.1 *Nesobasis selysi*	30.0	21.2	31.2	17.6	22	19.9	27.4	30.6	42	25.4	16.8	16.2	26	18.3	49.5	5.9
FJ812847.1 *Nesobasis selysi*	30.0	21.2	31.2	17.6	22	19.9	27.4	30.6	42	25.4	16.8	16.2	26	18.3	49.5	5.9
KT879907.1 *Euphaea fraseri*	30.7	19.2	33.6	16.5	21	19.9	30.6	28.5	41	24.9	16.8	17.3	30	12.9	53.2	3.8
KJ493062.1 *Metagrion* sp	29.6	21.4	32.0	17.1	22	20.4	30.6	27.4	41	25.4	16.8	17.3	27	18.3	48.4	6.5
KF369576.1 *Vestalis amabilis*	28.9	22.6	28.9	19.6	22	18.3	28.5	31.2	41	25.4	16.8	16.8	24	24.2	41.4	10.8
Avg.	30.1	21.4	29.8	18.6	22	20.4	27.7	30.2	41	25.5	16.7	16.8	28	18.4	44.8	8.9

Table 21: The Nucleotide substitution table of *Onychogomphus malabarensis*

Domain: Data																
	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
KU133368.1 *Onychogomphus malabarensis CUOM*	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5
KX890962.1 *Ophiogomphus anomalus*	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5
KX890932.1 *Ophiogomphus mainensis*	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5
JN420156.1 *Ophiogomphus mainensis*	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5
JN420133.1 *Ophiogomphus mainensis*	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5
JN420085.1 *Ophiogomphus mainensis*	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5
JN420057.1 *Ophiogomphus sp.*	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5
JN420056.1 *Ophiogomphus sp.*	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5
JN420053.1 *Ophiogomphus sp.*	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5
JN420024.1 *Ophiogomphus sp*	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5
Avg.	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5

Table 5: The Nucleotide substitution table of *Agriocnemis pygmaea*

Domain: Data																
	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
KU871002.1 *Agriocnemis pygmaea* (KERALA)	33.6	17.9	30.4	18.2	23	17.1	27.3	32.6	42	27.8	14.4	16.0	36	8.6	49.5	5.9
KT957464.1 *Agriocnemis minima*	33.0	19.3	30.5	17.1	21	18.7	27.3	32.6	42	27.8	14.4	16.0	36	11.3	50.0	2.7
KT957463.1 *Agriocnemis minima*	33.2	19.3	29.8	17.7	21	18.7	27.3	32.6	42	27.8	14.4	16.0	37	11.3	47.8	4.3
KT957465.1 *Agriocnemis minima*	33.2	19.3	30.0	17.5	21	18.7	27.3	32.6	42	27.8	14.4	16.0	37	11.3	48.4	3.8
KT957462.1 *Agriocnemis minima*	33.0	19.5	30.2	17.3	21	18.7	27.3	32.6	42	27.8	14.4	16.0	36	11.8	48.9	3.2
KT957461.1 *Agriocnemis minima*	32.9	19.6	30.0	17.5	21	18.7	27.3	32.6	42	27.8	14.4	16.0	35	12.4	48.4	3.8
KF369578.1 *Xanthagrion erythroneurum*	33.4	17.7	32.0	17.0	24	16.0	27.3	32.6	42	28.3	14.4	15.5	34	8.6	54.3	2.7
KF369446.1 *Metaleptobasis mauritia*	33.8	16.3	33.2	16.8	25	15.0	27.3	32.6	42	27.3	14.4	16.0	34	6.5	58.1	1.6
KU566458.1 *Trithemis werneri*	35.5	15.9	31.3	17.3	23	16.6	27.8	32.6	42	27.8	14.4	15.5	41	3.2	51.6	3.8
KF369562.1 *Teinobasis rufithorax*	33.4	16.8	32.7	17.1	25	16.0	26.2	33.2	42	27.3	14.4	16.0	33	7.0	57.5	2.2
AB757983.1 *Ischnura senegalensis*	33.9	18.2	29.8	18.0	23	17.1	27.3	32.6	42	27.8	14.4	15.5	37	9.7	47.8	5.9
AB758091.1 *Ischnura senegalensis*	34.1	18.0	30.2	17.7	23	17.1	27.3	32.6	42	27.8	14.4	15.5	37	9.1	48.9	4.8
Avg.	33.6	18.1	30.8	17.4	23	17.4	27.2	32.7	42	27.8	14.4	15.9	36	9.2	50.9	3.7

Table 7: The Nucleotide substitution table of *Agriocnemis keralensis*

Domain: Data																
	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
KU133367.1 *Agriocnemis keralensis* (KERALA)	30.3	19.3	31.7	18.8	20	18.4	28.2	33.5	42	27.7	13.6	17.0	29	11.7	53.4	5.8
KF369284.1 *Agriocnemis forcipata*	30.3	19.3	31.7	18.8	20	18.4	28.2	33.5	42	27.7	13.6	17.0	29	11.7	53.4	5.8
KT957459.1 *Agriocnemis femina*	33.7	16.3	30.3	19.7	23	16.0	27.2	34.0	42	27.2	13.6	17.5	36	5.8	50.0	7.8
KF369283.1 *Agriocnemis femina*	33.7	16.3	30.1	19.9	22	16.5	27.2	34.0	42	27.2	13.6	17.5	37	5.3	49.5	8.3
KT957460.1 *Agriocnemis femina*	33.8	16.2	30.3	19.7	23	16.0	27.2	34.0	42	27.2	13.6	17.5	37	5.3	50.0	7.8
KT957458.1 *Agriocnemis pygmaea*	31.1	19.3	30.4	19.3	21	18.0	27.2	34.0	42	27.2	13.6	17.5	31	12.6	50.5	6.3
KT957456.1 *Agriocnemis pygmaea*	31.2	19.1	30.6	19.1	21	17.5	27.2	34.0	42	27.2	13.6	17.5	31	12.6	51.0	5.8
KT957453.1 *Agriocnemis pygmaea*	31.2	19.1	30.4	19.3	21	17.5	27.2	34.0	42	27.2	13.6	17.5	31	12.6	50.5	6.3
Avg.	31.9	18.1	30.7	19.3	21	17.3	27.4	33.9	42	27.3	13.6	17.4	33	9.7	51.0	6.7

Table 25: The Nucleotide substitution table of *Anax parthenope*

Domain: Data																
	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
KR149805.1 *Anax parthenope* (Kerala)	35.8	15.4	31.9	16.8	42	3.2	51.6	3.7	22	16.3	30.5	31.6	44	26.8	13.7	15.3
KC135891.1 *Anax parthenope*	35.8	15.4	31.9	16.8	42	3.2	51.6	3.7	22	16.3	30.5	31.6	44	26.8	13.7	15.3
KX161841.1 *Anax imperator*	35.8	15.6	31.9	16.7	42	3.7	51.6	3.2	22	16.3	30.5	31.6	44	26.8	13.7	15.3
KU565916.1 *Anax imperator*	35.4	16.0	31.9	16.7	41	4.2	51.6	3.2	21	16.8	30.5	31.6	44	26.8	13.7	15.3
KX781748.1 *Aeshnidae sp*.	36.5	15.3	31.8	16.5	43	3.2	51.1	2.6	22	15.8	30.5	31.6	44	26.8	13.7	15.3
KR143134.1 *Anax junius*	37.0	14.9	31.6	16.5	45	2.1	50.5	2.6	22	15.8	30.5	31.6	44	26.8	13.7	15.3
AY555548.1 *Anax junius*	36.5	15.1	31.9	16.5	43	2.6	51.6	2.6	22	15.8	30.5	31.6	44	26.8	13.7	15.3
KF584974.1 *Anax imperator*	36.0	15.4	31.9	16.7	42	3.2	51.6	3.2	22	16.3	30.5	31.6	44	26.8	13.7	15.3
Avg.	36.1	15.4	31.9	16.6	42	3.2	51.4	3.1	22	16.2	30.5	31.6	44	26.8	13.7	15.3

Table 29: The Nucleotide substitution table of *Neurothemis intermedia*

Domain: Data																
	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
KU052672.1 *Neurothemis intermedia* (KERALA)	33.8	20.5	27.3	18.5	42	32.2	11.9	13.6	35	12.0	41.9	11.1	24	17.1	28.2	30.8
KT222948.1 *Neurothemis intermedia*	33.8	20.5	27.3	18.5	42	32.2	11.9	13.6	35	12.0	41.9	11.1	24	17.1	28.2	30.8
KC122227.1 *Neurothemis intermedia*	34.7	19.6	27.6	18.2	42	32.2	11.9	13.6	38	9.4	42.7	10.3	24	17.1	28.2	30.8
KT957504.1 *Neurothemis fluctuans*	33.8	20.5	27.0	18.8	42	32.2	11.9	13.6	35	12.0	41.0	12.0	24	17.1	28.2	30.8
AB709004.1 *Neurothemis fluctuans*	34.4	20.2	27.3	18.2	42	32.2	11.9	13.6	37	11.1	41.9	10.3	24	17.1	28.2	30.8
KT372719.1 *Neurothemis intermedia*	33.8	20.5	27.3	18.5	42	32.2	11.9	13.6	35	12.0	41.9	11.1	24	17.1	28.2	30.8
KP835514.1 *Neurothemis intermedia*	34.7	19.6	27.6	18.2	42	32.2	11.9	13.6	38	9.4	42.7	10.3	24	17.1	28.2	30.8
AB709003.1 *Neurothemis fluctuans*	35.8	19.0	26.7	18.5	42	32.2	11.9	13.6	38	10.3	40.2	11.1	26	14.5	28.2	30.8
Avg.	34.3	20.0	27.2	18.4	42	32.2	11.9	13.6	36	11.0	41.8	10.9	24	16.8	28.2	30.8

Table 41: The Nucleotide substitution table of *Trithemis pallidinervis*

Domain: Data																
	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
KR957509.1 *Trithemis pallidinervis* (KERALA)	37.8	16.2	29.8	16.2	23	16.5	28.9	31.4	44	27.5	14.0	14.5	46	4.7	46.6	2.6
KT957508.1 *Trithemis pallidinervis*	37.8	16.2	29.8	16.2	23	16.5	28.9	31.4	44	27.5	14.0	14.5	46	4.7	46.6	2.6
KT149803.1 Trithemis pallidinervis	37.8	16.2	29.8	16.2	23	16.5	28.9	31.4	44	27.5	14.0	14.5	46	4.7	46.6	2.6
KJ499455.1 Trithemis pallidinervis v	37.8	16.2	29.8	16.2	23	16.5	28.9	31.4	44	27.5	14.0	14.5	46	4.7	46.6	2.6
KT957510.1 Trithemis pallidinervis	37.6	16.4	29.7	16.4	23	16.5	28.9	31.4	44	27.5	14.0	14.5	46	5.2	46.1	3.1
KU496892.1 Orthetrum glaucum	37.2	16.4	30.7	15.7	22	17.5	28.9	31.4	44	27.5	14.0	14.5	46	4.1	49.2	1.0
KU496893.1 Orthetrum glaucum	37.4	16.4	30.5	15.7	23	17.0	28.9	31.4	44	27.5	14.0	14.5	46	4.7	48.7	1.0
Avg.	37.6	16.3	30.0	16.1	23	16.7	28.9	31.4	44	27.5	14.0	14.5	46	4.7	47.2	2.2

Table 11: The Nucleotide substitution table of *Ischnura senegalensis*

Domain: Data																
	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
KT305961.1 *Ischnura senegalensis* (KERALA)	34.2	18.1	30.3	17.4	37	10.4	47.3	5.5	24	17.9	27.4	30.8	42	25.9	16.4	15.9
AB758088.1 *Ischnura senegalensis*	34.2	18.0	30.4	17.4	37	10.4	47.5	5.4	24	17.8	27.2	30.7	42	25.9	16.4	15.9
AB758084.1 *Ischnura senegalensis*	34.2	18.0	30.4	17.4	37	10.4	47.5	5.4	24	17.8	27.2	30.7	42	25.9	16.4	15.9
AB758083.1 *Ischnura senegalensis*	34.2	18.0	30.4	17.4	37	10.4	47.5	5.4	24	17.8	27.2	30.7	42	25.9	16.4	15.9
AB758082.1 *Ischnura senegalensis*	34.2	18.0	30.4	17.4	37	10.4	47.5	5.4	24	17.8	27.2	30.7	42	25.9	16.4	15.9
AB758081.1 *Ischnura senegalensis*	34.2	18.0	30.4	17.4	37	10.4	47.5	5.4	24	17.8	27.2	30.7	42	25.9	16.4	15.9
AB758080.1 *Ischnura senegalensis*	34.2	18.0	30.4	17.4	37	10.4	47.5	5.4	24	17.8	27.2	30.7	42	25.9	16.4	15.9
AB758079.1 *Ischnura senegalensis*	34.2	18.0	30.4	17.4	37	10.4	47.5	5.4	24	17.8	27.2	30.7	42	25.9	16.4	15.9
AB758078.1 *Ischnura senegalensis*	34.2	18.0	30.4	17.4	37	10.4	47.5	5.4	24	17.8	27.2	30.7	42	25.9	16.4	15.9
AB758077.1 *Ischnura senegalensis*	34.2	18.0	30.4	17.4	37	10.4	47.5	5.4	24	17.8	27.2	30.7	42	25.9	16.4	15.9
AB758076.1 *Ischnura senegalensis*	34.2	18.0	30.4	17.4	37	10.4	47.5	5.4	24	17.8	27.2	30.7	42	25.9	16.4	15.9
AB758075.1 *Ischnura senegalensis*	34.2	18.0	30.4	17.4	37	10.4	47.5	5.4	24	17.8	27.2	30.7	42	25.9	16.4	15.9
AB758074.1 *Ischnura senegalensis*	34.2	18.0	30.4	17.4	37	10.4	47.5	5.4	24	17.8	27.2	30.7	42	25.9	16.4	15.9
Avg.	34.2	18.0	30.4	17.4	37	10.4	47.5	5.4	24	17.8	27.2	30.7	42	25.9	16.4	15.9

Table 31: The Nucleotide substitution table of *Potamarcha obscura*

Domain: Data																
	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
KX503060.1 *Potamarcha obscura* (KERALA)	36.7	16.0	32.7	14.6	26	15.2	32.4	26.0	43	26.0	14.7	16.2	40	6.9	51.2	1.5
KC122230.1 *Potamarcha obscura*	36.7	16.0	32.7	14.6	26	15.2	32.4	26.0	43	26.0	14.7	16.2	40	6.9	51.2	1.5
KT175605.1 *Pieris canidia*	36.7	16.2	32.6	14.6	26	15.2	32.4	26.0	43	26.0	14.7	16.2	40	7.4	50.7	1.5
LC090563.1 *Pieris canidia*	36.8	16.0	32.6	14.6	26	15.2	32.4	26.0	43	26.0	14.7	16.2	41	6.9	50.7	1.5
JQ965750.1 *Pieris canidia*	36.7	16.0	32.7	14.6	26	15.2	32.4	26.0	43	26.0	14.7	16.2	40	6.9	51.2	1.5
GU372552.1 *Pieris canidia kaolicola*	36.5	16.5	32.2	14.7	26	15.2	32.4	26.0	43	26.0	14.7	16.2	40	8.4	49.8	2.0
KJ423050.1 *Pieris canidia*	36.8	16.0	32.6	14.6	26	15.2	32.4	26.0	43	26.0	14.7	16.2	41	6.9	50.7	1.5
JX242477.1 *Pieris canidia*	36.5	16.4	32.6	14.6	26	15.2	32.4	26.0	43	26.0	14.7	16.2	40	7.9	50.7	1.5
KJ423047.1 *Pieris canidia*	36.7	16.2	32.6	14.6	26	15.2	32.4	26.0	43	26.0	14.7	16.2	40	7.4	50.7	1.5
KJ423043.1 *Pieris canidia*	36.7	16.2	32.6	14.6	26	15.2	32.4	26.0	43	26.0	14.7	16.2	40	7.4	50.7	1.5
Avg.	36.7	16.2	32.6	14.6	26	15.2	32.4	26.0	43	26.0	14.7	16.2	40	7.3	50.8	1.5

Table 61 : The Nucleotide substitution table of *Aethriamanta brevipennis*

Domain: Data																
	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
KU510325.1 *Aethriamanta brevipennis* (KERALA)	31.1	21.2	27.5	20.2	21	19.2	28.1	31.7	43	29.3	12.0	16.2	30	15.0	42.5	12.6
KU565929.1 *Anax speratus*	34.8	17.8	30.4	17.0	23	16.8	28.7	31.7	43	29.3	12.0	15.6	39	7.2	50.6	3.6
KU565930.1 *Anax speratus*	34.6	17.8	30.6	17.0	23	16.8	28.7	31.7	43	29.3	12.0	15.6	38	7.2	51.2	3.6
KU565923.1 *Anax speratus*	34.6	17.8	30.4	17.2	23	16.8	28.7	31.7	43	29.3	12.0	15.6	38	7.2	50.6	4.2
KU565925.1 *Anax speratus*	34.8	17.6	30.8	16.8	23	16.8	28.7	31.7	43	29.3	12.0	15.6	39	6.6	51.8	3.0
KU566457.1 *Trithemis tropicana*	36.2	16.4	30.4	17.0	25	16.8	27.5	31.1	44	28.7	12.0	15.6	40	3.6	51.8	4.2
KY773653.1 *Telebasis digiticollis*	35.4	18.4	29.0	17.2	25	16.8	26.9	31.7	44	28.1	12.0	16.2	38	10.2	48.2	3.6
KR080108.1 *Pantala flavescens*	38.0	18.2	26.2	17.6	23	17.4	27.5	31.7	44	28.7	12.0	15.6	47	8.4	39.2	5.4
KR080127.1 *Pantala flavescens*	38.2	18.0	26.6	17.2	23	17.4	27.5	31.7	44	28.7	12.0	15.6	48	7.8	40.4	4.2
KX890927.1 *Ophiogomphus colubrinus*	34.8	18.2	30.0	17.0	25	16.2	28.1	31.1	43	29.3	12.0	15.6	37	9.0	50.0	4.2
KU566453.1 *Trithemis osvaldae*	35.4	18.4	29.6	16.6	25	16.2	28.1	31.1	44	28.7	12.0	15.6	38	10.2	48.8	3.0
MF174502.1 *Orthetrum caledonicum*	36.8	15.8	30.2	17.2	26	15.0	28.1	31.1	44	28.7	12.0	15.6	41	3.6	50.6	4.8
MF174500.1 *Orthetrum caledonicum*	37.0	15.6	30.4	17.0	26	14.4	28.1	31.1	44	28.7	12.0	15.6	41	3.6	51.2	4.2
KX890937.1 *Ophiogomphus severus*	35.4	18.0	30.0	16.6	25	16.2	28.1	31.1	43	29.3	12.0	15.6	39	8.4	50.0	3.0
KU566454.1 *Trithemis osvaldae*	35.6	18.2	29.4	16.8	25	16.2	28.1	31.1	44	28.7	12.0	15.6	39	9.6	48.2	3.6
KU566452.1 *Trithemis osvaldae*	35.6	18.2	29.4	16.8	25	16.2	28.1	31.1	44	28.7	12.0	15.6	39	9.6	48.2	3.6
KU566423.1 *Anectothemis apicalis*	35.6	18.2	29.4	16.8	25	16.2	28.1	31.1	44	28.7	12.0	15.6	39	9.6	48.2	3.6
KU566422.1 *Anectothemis apicalis*	35.6	18.2	29.4	16.8	25	16.2	28.1	31.1	44	28.7	12.0	15.6	39	9.6	48.2	3.6
Avg.	35.5	17.9	29.4	17.2	24	16.5	28.1	31.4	43	28.9	12.0	15.6	39	8.2	48.3	4.3

Table 13 : The Nucleotide substitution table of *Aciagrion occidentale*

Domain: Data																
	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
KM096996.1 *Aciagrion occidentale* (KERALA)	34.6	17.4	29.7	18.2	25	13.5	30.4	31.0	44	27.1	13.5	15.3	35	11.8	45.3	8.2
KF369275.1 *Aciagrion borneense*	34.4	17.6	30.3	17.6	25	14.0	31.0	30.4	45	27.1	13.5	14.7	34	11.8	46.5	7.6
KU565891.1 *Africallagma quingentum*	35.0	16.4	33.1	15.5	24	14.6	30.4	31.0	45	27.1	14.1	14.1	36	7.6	54.7	1.2
KU565890.1 *Africallagma quingentum*	35.0	16.4	33.1	15.5	24	14.6	30.4	31.0	45	27.1	14.1	14.1	36	7.6	54.7	1.2
KU565889.1 *Africallagma quingentum*	35.0	16.4	33.1	15.5	24	14.6	30.4	31.0	45	27.1	14.1	14.1	36	7.6	54.7	1.2
KU565888.1 *Africallagma quingentum*	35.0	16.4	33.1	15.5	24	14.6	30.4	31.0	45	27.1	14.1	14.1	36	7.6	54.7	1.2
KU565887.1 *Africallagma quingentum*	35.0	16.4	33.1	15.5	24	14.6	30.4	31.0	45	27.1	14.1	14.1	36	7.6	54.7	1.2
KF369279.1 *Africallagma elongatum*	34.8	16.4	32.3	16.4	24	14.6	30.4	31.0	45	27.1	14.1	14.1	36	7.6	52.4	4.1
KF369280.1 *Africallagma vaginale*	34.6	16.6	32.5	16.2	22	16.4	30.4	31.0	45	27.1	14.1	14.1	37	6.5	52.9	3.5
JN419694.1 *Enallagma sp*	34.8	16.8	31.9	16.4	25	14.6	29.8	31.0	45	27.1	13.5	14.7	35	8.8	52.4	3.5
KC135957.1 *Ischnura asiatica*	35.4	16.8	31.5	16.2	24	15.2	29.8	31.0	45	27.1	13.5	14.7	38	8.2	51.2	2.9
LC101610.1 *Ischnura asiatica*	35.4	16.8	31.7	16.0	24	15.2	29.8	31.0	45	27.1	13.5	14.7	38	8.2	51.8	2.4
LC101585.1 *Ischnura asiatica*	35.6	16.6	31.5	16.2	24	15.2	29.8	31.0	45	27.1	13.5	14.7	38	7.6	51.2	2.9
Avg.	35.0	16.7	32.1	16.2	24	14.8	30.3	30.9	45	27.1	13.8	14.4	36	8.4	52.1	3.2

Table 55 : The Nucleotide substitution table of *Acisoma panorpoides*

Domain: Data																
	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
KT222947.1 *Acisoma panorpoides* (KERALA)	37.2	16.5	30.3	16.1	46	28.1	11.9	14.4	43	4.4	49.4	3.1	23	17.0	29.6	30.8
KC122228 *Acisoma panorpoides*	37.2	16.5	30.3	16.1	46	28.1	11.9	14.4	43	4.4	49.4	3.1	23	17.0	29.6	30.8
KT879899.1 *Acisoma panorpoides*	37.6	16.3	30.3	15.9	46	28.1	11.9	14.4	44	3.8	49.4	3.1	23	17.0	29.6	30.2
KX281820.1 *Acisoma panorpoides*	37.6	16.7	29.4	16.3	46	28.1	11.9	14.4	44	4.4	46.9	4.4	23	17.6	29.6	30.2
KX281827.1 *Acisoma panorpoides*	38.0	16.3	29.2	16.5	46	28.1	11.9	14.4	46	3.1	46.3	5.0	23	17.6	29.6	30.2
KX281825.1 *Acisoma panorpoides*	38.0	16.3	29.2	16.5	46	28.1	11.9	14.4	46	3.1	46.3	5.0	23	17.6	29.6	30.2
KX281824 *Acisoma panorpoides*	38.0	16.3	29.2	16.5	46	28.1	11.9	14.4	46	3.1	46.3	5.0	23	17.6	29.6	30.2
KX281818 *Acisoma panorpoides*	37.6	16.7	29.4	16.3	46	28.1	11.9	14.4	45	3.8	46.9	4.4	22	18.2	29.6	30.2
KX281813.1 *Acisoma panorpoides*	37.4	16.7	29.9	16.1	46	28.1	11.9	14.4	44	4.4	48.1	3.8	23	17.6	29.6	30.2
KX281812.1 *Acisoma panorpoides*	37.4	16.7	29.9	16.1	46	28.1	11.9	14.4	44	4.4	48.1	3.8	23	17.6	29.6	30.2
KT957514.1 *Acisoma panorpoides*	37.8	16.3	29.4	16.5	46	28.1	11.9	14.4	45	3.1	46.9	5.0	23	17.6	29.6	30.2
KX281823.1 *Acisoma panorpoides*	37.4	16.9	29.4	16.3	46	28.1	11.9	14.4	44	4.4	46.9	4.4	22	18.2	29.6	30.2
KX281814.1 *Acisoma panorpoides*	37.4	16.9	29.6	16.1	46	28.1	11.9	14.4	44	5.0	47.5	3.8	23	17.6	29.6	30.2
KT957515.1 *Acisoma panorpoides*	37.6	16.7	29.6	16.1	46	28.1	11.9	14.4	44	4.4	46.9	4.4	23	17.6	30.2	29.6
KX281804.1 *Acisoma inflatum*	37.4	16.9	30.3	15.4	46	28.1	11.9	14.4	44	5.0	49.4	1.9	23	17.6	29.6	30.2
KX281810.1 *Acisoma inflatum*	37.6	16.5	30.7	15.2	46	28.1	11.9	14.4	43	5.0	50.6	1.3	24	16.4	29.6	30.2
KX281841.1 *Acisoma variegatum*	37.4	16.7	30.7	15.2	46	28.1	11.9	14.4	43	5.0	50.6	1.3	23	17.0	29.6	30.2
KX281839.1 *Acisoma variegatum*	37.4	16.7	30.7	15.2	46	28.1	11.9	14.4	43	5.0	50.6	1.3	23	17.0	29.6	30.2
KX281797.1 *Acisoma attenboroughi*	36.7	16.9	31.1	15.2	46	28.1	11.9	14.4	42	5.0	51.9	1.3	23	17.6	29.6	30.2
KX281796.1 *Acisoma attenboroughi*	36.3	17.3	31.1	15.2	46	28.1	11.9	14.4	41	6.3	51.9	1.3	23	17.6	29.6	30.2
KX281830.1 *Acisoma trifidum*	37.0	16.7	29.6	16.7	46	28.1	11.9	14.4	40	6.3	48.1	5.6	25	15.7	28.9	30.2
Avg.	37.4	16.6	30.0	16.0	46	28.1	11.9	14.4	44	4.4	48.5	3.4	23	17.4	29.6	30.2

Table 59 : The Nucleotide substitution table of *Lathrecista sp*

Domain: Data																
	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
KU052671.1 *Lathrecista sp* (KERALA)	32.9	20.1	28.0	18.9	40	30.9	13.2	15.4	35	11.8	42.6	10.3	23	17.8	28.1	31.1
KT372719.1 *Neurothemis intermedia*	33.2	20.4	26.9	19.6	41	32.0	12.5	14.8	36	11.7	41.4	10.9	23	17.3	26.8	33.1
KT222948.1 *Neurothemis intermedia*	33.7	20.6	27.9	17.8	41	31.3	13.3	14.1	36	12.5	41.4	10.2	24	18.1	29.1	29.1
AB709004.1 *Neurothemis fluctuans*	32.9	20.4	28.3	18.4	40	30.9	13.2	15.4	35	12.5	43.4	8.8	23	17.8	28.1	31.1
KT957504.1 *Neurothemis fluctuans*	32.7	20.4	27.8	19.2	40	30.9	13.2	15.4	35	12.5	41.9	11.0	23	17.8	28.1	31.1
KC122227.1 *Neurothemis intermedia*	33.7	19.4	28.3	18.7	40	30.9	13.2	15.4	38	9.6	43.4	9.6	23	17.8	28.1	31.1
KP835514.1 *Neurothemis intermedia*	34.3	19.2	28.6	17.8	42	30.6	12.9	14.5	37	9.8	43.1	9.8	24	17.1	30.1	29.3
AB709003.1 *Neurothemis fluctuans*	34.6	18.9	27.3	19.2	40	30.9	13.2	15.4	38	10.3	40.4	11.0	25	15.6	28.1	31.1
KC122229.1 *Neurothemis tullia*	34.4	19.4	27.3	18.9	40	30.9	13.2	15.4	42	7.4	40.4	10.3	21	20.0	28.1	31.1
KT957502.1 *Neurothemis tullia*	33.4	19.8	28.0	18.8	40	31.1	13.3	15.6	40	8.1	42.2	9.6	20	20.1	28.4	31.3
KT957494.1 *Neurothemis tullia*	32.9	20.0	28.2	18.8	40	31.1	13.3	15.6	39	8.9	43.0	9.6	20	20.1	28.4	31.3
AB709007 *Neurothemis ramburii*	33.8	18.7	30.3	17.2	40	30.6	13.4	15.7	38	8.2	49.3	4.5	23	17.2	28.4	31.3
KT957503.1 *Neurothemis tullia*	33.4	19.8	27.7	19.1	40	31.1	13.3	15.6	40	8.1	41.5	10.4	20	20.1	28.4	31.3
KT957501.1 *Neurothemis tullia*	33.4	19.8	27.7	19.1	40	31.1	13.3	15.6	40	8.1	41.5	10.4	20	20.1	28.4	31.3
KT879900.1 *Neurothemis tullia*	32.9	20.3	27.2	19.6	40	31.1	13.3	15.6	39	9.6	40.0	11.9	20	20.1	28.4	31.3
Avg.	33.4	19.8	28.0	18.7	40	31.0	13.2	15.3	38	10.0	42.4	9.9	22	18.4	28.3	31.1

Table 49: The Nucleotide substitution table of *Bradinopyga geminate*

Domain: Data																
	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
KM096995.1 *Bradinopyga geminate* (Kerala)	37.5	15.4	30.2	16.9	26	13.5	27.6	32.7	45	26.9	14.1	14.1	41	5.8	49.0	3.9
JX306648.1 *Bradinopyga geminata*	37.8	15.2	30.0	17.0	26	13.5	27.6	32.7	45	27.1	13.5	14.2	42	5.2	49.0	3.9
KM245283.1 *Bradinopyga geminata*	37.3	15.7	30.3	16.7	26	13.5	28.2	32.1	45	27.1	13.5	14.2	41	6.5	49.0	3.9
JN817424.1 *Bradinopyga geminata*	37.7	15.3	30.4	16.6	26	13.5	27.7	32.3	45	27.1	13.5	14.2	42	5.2	50.0	3.2
MF774498.1 *Bradinopyga geminata*	36.5	15.0	31.1	17.4	26	13.5	28.8	32.1	45	27.7	13.5	14.2	39	3.9	51.0	5.8
KY947476.1 *Orthemis cultriformis*	36.3	16.8	31.0	15.9	25	15.4	26.9	32.7	45	27.1	13.5	14.2	39	7.8	52.6	.6
KY947477.1 *Orthemis cultriformis*	36.3	17.0	30.9	15.9	25	15.4	26.9	32.7	45	27.1	13.5	14.2	39	8.4	52.3	.6
KY947422.1 *Orthemis cultriformis*	36.3	17.0	30.9	15.9	25	15.4	26.9	32.7	45	27.1	13.5	14.2	39	8.4	52.3	.6
KY947421.1 *Orthemis cultriformis*	36.3	17.0	30.9	15.9	25	15.4	26.9	32.7	45	27.1	13.5	14.2	39	8.4	52.3	.6
KY947420.1 *Orthemis cultriformis*	36.3	17.0	30.9	15.9	25	15.4	26.9	32.7	45	27.1	13.5	14.2	39	8.4	52.3	.6
KU980966.1 *Libellulidae sp*	33.7	17.6	32.2	16.5	23	17.3	26.9	32.7	45	27.1	13.5	14.2	33	8.4	56.1	2.6
KY947386.1 *Orthemis discolor*	34.3	17.4	31.5	16.7	22	17.9	26.9	32.7	45	27.1	13.5	14.2	35	7.1	54.2	3.2
KX055147 *Tramea limbata*	37.1	16.3	28.8	17.8	26	14.1	27.6	32.7	45	27.1	12.9	14.8	41	7.7	45.8	5.8
KX055146.1 *Tramea limbata*	37.1	16.3	28.8	17.8	26	14.1	27.6	32.7	45	27.1	12.9	14.8	41	7.7	45.8	5.8
Avg.	36.5	16.3	30.6	16.6	25	14.8	27.4	32.6	45	27.1	13.5	14.3	39	7.1	50.8	3.0

Table 47: The Nucleotide substitution table of *Diplacodes trivialis*

Domain: Data																
	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
KT879902.1 *Diplacodes trivialis*(KERALA)	35.8	16.7	29.4	18.0	38	5.8	48.7	7.1	24	16.8	26.5	32.9	45	27.7	12.9	14.2
KP835512.1 *Diplacodes trivialis* (KERALA)	35.8	16.7	29.4	18.0	38	5.8	48.7	7.1	24	16.8	26.5	32.9	45	27.7	12.9	14.2
KP087934.1 *Diplacodes trivialis* (KERALA)	35.8	16.7	29.4	18.0	38	5.8	48.7	7.1	24	16.8	26.5	32.9	45	27.7	12.9	14.2
KP087933.1 *Diplacodes trivialis* (KERALA)	35.8	16.7	29.4	18.0	38	5.8	48.7	7.1	24	16.8	26.5	32.9	45	27.7	12.9	14.2
KP087932.1 *Diplacodes trivialis* (KERALA)	35.8	16.7	29.4	18.0	38	5.8	48.7	7.1	24	16.8	26.5	32.9	45	27.7	12.9	14.2
KP087931.1 *Diplacodes trivialis* (KERALA)	35.8	16.7	29.4	18.0	38	5.8	48.7	7.1	24	16.8	26.5	32.9	45	27.7	12.9	14.2
KC287153.1 *Diplacodes trivialis*	35.8	16.7	29.4	18.0	38	5.8	48.7	7.1	24	16.8	26.5	32.9	45	27.7	12.9	14.2
JX306647.1 *Diplacodes trivialis*	35.8	16.7	29.4	18.0	38	5.8	48.7	7.1	24	16.8	26.5	32.9	45	27.7	12.9	14.2
KT957542.1 *Diplacodes trivialis*	35.8	16.7	29.6	17.8	38	5.8	49.4	6.4	24	16.8	26.5	32.9	45	27.7	12.9	14.2
KT957540.1 *Diplacodes trivialis*	35.8	16.7	29.6	17.8	38	5.8	49.4	6.4	24	16.8	26.5	32.9	45	27.7	12.9	14.2
KT957538.1 *Diplacodes trivialis*	35.8	16.7	29.6	17.8	38	5.8	49.4	6.4	24	16.8	26.5	32.9	45	27.7	12.9	14.2
KT957537.1 *Diplacodes trivialis*	35.8	16.7	29.6	17.8	38	5.8	49.4	6.4	24	16.8	26.5	32.9	45	27.7	12.9	14.2
KT957536.1 *Diplacodes trivialis*	35.8	16.7	29.6	17.8	38	5.8	49.4	6.4	24	16.8	26.5	32.9	45	27.7	12.9	14.2
KT957535.1 *Diplacodes trivialis*	35.8	16.7	29.6	17.8	38	5.8	49.4	6.4	24	16.8	26.5	32.9	45	27.7	12.9	14.2
KT957533.1 *Diplacodes trivialis*	35.8	16.7	29.6	17.8	38	5.8	49.4	6.4	24	16.8	26.5	32.9	45	27.7	12.9	14.2
KT957532.1 *Diplacodes trivialis*	35.8	16.7	29.6	17.8	38	5.8	49.4	6.4	24	16.8	26.5	32.9	45	27.7	12.9	14.2
KT957531.1 *Diplacodes trivialis*	35.8	16.7	29.6	17.8	38	5.8	49.4	6.4	24	16.8	26.5	32.9	45	27.7	12.9	14.2
KC122228.1 *Acisoma panorpoides*	37.4	16.1	30.1	16.3	45	3.9	49.0	2.6	23	17.4	27.7	32.3	45	27.1	13.5	14.2
KY947419.1 *Orthemis discolor*	34.5	17.2	31.8	16.5	35	7.1	54.5	3.2	23	17.4	27.1	32.3	45	27.1	13.5	14.2
KY947454.1 *Telebasis willinki*	37.0	15.7	29.2	18.1	38	7.1	48.4	6.5	28	13.5	25.8	32.9	45	26.5	13.5	14.8
KU566497.1 *Zygonyx flavicosta*	35.3	18.5	29.2	17.0	40	9.0	47.7	3.2	21	19.4	26.5	33.5	45	27.1	13.5	14.2
Avg.	35.9	16.8	29.6	17.7	39	6.0	49.2	6.2	24	16.8	26.5	32.9	45	27.6	13.0	14.2

Table 57: The Nucleotide substitution table of *Neurothemis tullia*

Domain: Data																
	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
KP835513 *Neurothemis tullia* (KERALA)	32.5	19.5	27.2	20.7	20	19.5	27.4	32.7	42	31.0	12.4	15.0	36	8.0	42.0	14.3
KT957503.1 *Neurothemis tullia*	32.5	19.5	27.5	20.4	20	19.5	27.4	32.7	42	31.0	12.4	15.0	36	8.0	42.9	13.4
KT957501.1 *Neurothemis tullia*	32.5	19.5	27.5	20.4	20	19.5	27.4	32.7	42	31.0	12.4	15.0	36	8.0	42.9	13.4
KT957499.1 *Neurothemis tullia*	32.5	19.5	27.5	20.4	20	19.5	27.4	32.7	42	31.0	12.4	15.0	36	8.0	42.9	13.4
KT879900.1 *Neurothemis tullia*	32.2	19.8	27.2	20.7	20	19.5	27.4	32.7	42	31.0	12.4	15.0	35	8.9	42.0	14.3
KC122229.1 *Neurothemis tullia*	32.5	19.5	26.9	21.0	20	19.5	27.4	32.7	42	31.0	12.4	15.0	36	8.0	41.1	15.2
KT957502.1 *Neurothemis tullia*	32.5	19.5	27.8	20.1	20	19.5	27.4	32.7	42	31.0	12.4	15.0	36	8.0	43.8	12.5
KT957500.1 *Neurothemis tullia*	32.5	19.5	27.8	20.1	20	19.5	27.4	32.7	42	31.0	12.4	15.0	36	8.0	43.8	12.5
KT957497.1 *Neurothemis tullia*	32.2	19.8	27.5	20.4	20	19.5	27.4	32.7	42	31.0	12.4	15.0	35	8.9	42.9	13.4
KT957495.1 *Neurothemis tullia*	32.2	19.8	27.5	20.4	20	19.5	27.4	32.7	42	31.0	12.4	15.0	35	8.9	42.9	13.4
KT957494.1 *Neurothemis tullia*	32.2	19.5	27.8	20.4	20	19.5	27.4	32.7	42	31.0	12.4	15.0	35	8.0	43.8	13.4
KT957498.1 *Neurothemis tullia*	32.5	19.8	27.2	20.4	20	19.5	27.4	32.7	42	31.0	12.4	15.0	36	8.9	42.0	13.4
KT957496.1 *Neurothemis tullia*	32.5	19.2	27.5	20.7	20	19.5	27.4	32.7	42	31.0	12.4	15.0	36	7.1	42.9	14.3
AB709007.1 *Neurothemis ramburii*	33.5	18.7	30.3	17.5	22	17.9	27.7	32.1	42	31.0	12.4	15.0	37	7.1	50.9	5.4
AB709005.1 *Neurothemis ramburii*	33.2	19.0	30.3	17.5	22	17.9	27.7	32.1	42	31.0	12.4	15.0	36	8.0	50.9	5.4
KC122227.1 *Neurothemis intermedia*	32.5	19.8	27.8	19.8	22	17.7	27.4	32.7	42	31.0	12.4	15.0	34	10.7	43.8	11.6
AB709003.1 *Neurothemis fluctuans*	34.4	18.7	27.0	19.9	23	17.0	27.7	32.1	42	31.0	12.4	15.0	38	8.0	41.1	12.5
AB709004.1 *Neurothemis fluctuans*	33.2	19.6	27.6	19.6	22	17.9	27.7	32.1	42	31.0	12.4	15.0	36	9.8	42.9	11.6
KT957504.1 *Neurothemis fluctuans*	32.6	20.2	27.0	20.2	22	17.9	27.7	32.1	42	31.0	12.4	15.0	34	11.6	41.1	13.4
KU052672.1 *Neurothemis intermedia*	32.5	20.3	27.2	20.0	22	17.9	27.7	32.1	41	31.3	12.5	15.2	34	11.7	41.4	12.6
KT222948.1 *Neurothemis intermedia*	32.5	20.3	27.2	20.0	22	17.9	27.7	32.1	41	31.3	12.5	15.2	34	11.7	41.4	12.6
AB709009.1 *Neurothemis terminata*	31.4	20.1	28.4	20.1	23	17.1	27.9	32.4	41	31.3	12.5	15.2	31	11.7	45.0	12.6
AB709010.1 *Neurothemis terminata*	31.4	20.1	28.4	20.1	23	17.1	27.9	32.4	41	31.3	12.5	15.2	31	11.7	45.0	12.6
KP835514.1 *Neurothemis intermedia*	33.2	18.6	28.8	19.3	23	15.3	29.6	31.6	40	30.3	13.1	16.2	36	10.2	43.9	10.2
Avg.	32.6	19.6	27.8	20.0	21	18.5	27.6	32.5	41	31.0	12.4	15.1	35	9.1	43.4	12.4

Table 51: The Nucleotide substitution table of *Rhyothemis variegata*

Domain: Data																
	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
KP938530.1 *Rhyothemis variegata* (KERALA)	32.9	16.3	33.2	17.5	26	14.7	27.5	32.1	41	30.6	13.0	15.7	32	3.7	59.3	4.6
KC287151.1 *Rhyothemis variegata*	32.6	16.3	33.5	17.5	26	14.7	27.5	32.1	41	30.6	13.0	15.7	31	3.7	60.2	4.6
LC366724.1 *Rhyothemis variegata*	32.9	16.3	33.2	17.5	26	14.7	27.5	32.1	41	30.6	13.0	15.7	32	3.7	59.3	4.6
AB709110.1 *Rhyothemis phyllis*	32.9	16.3	33.2	17.5	26	14.7	27.5	32.1	41	30.6	13.0	15.7	32	3.7	59.3	4.6
AB709113.1 *Rhyothemis variegata*	32.6	16.6	33.2	17.5	26	14.7	27.5	32.1	41	30.6	13.0	15.7	31	4.6	59.3	4.6
KC912256.1 *Orthetrum brachiale*	33.6	17.3	31.5	17.6	25	17.6	25.9	31.5	41	30.6	13.0	15.7	35	3.7	55.6	5.6
KC912258.1 *Orthetrum brachiale*	33.6	17.3	31.5	17.6	25	17.6	25.9	31.5	41	30.6	13.0	15.7	35	3.7	55.6	5.6
LC366850.1 *Rhyothemis phyllis*	32.6	16.3	34.5	16.6	26	14.7	27.5	32.1	41	30.6	13.0	15.7	31	3.7	63.0	1.9
LC366849.1 *Rhyothemis phyllis*	32.6	16.3	34.5	16.6	26	14.7	27.5	32.1	41	30.6	13.0	15.7	31	3.7	63.0	1.9
KU361232.1 *Orthetrum glaucum*	35.1	16.9	32.0	16.0	24	17.4	27.5	31.2	41	30.6	13.0	15.7	41	2.8	55.6	.9
KU496893.1 *Orthetrum glaucum*	35.1	16.9	32.0	16.0	24	17.4	27.5	31.2	41	30.6	13.0	15.7	41	2.8	55.6	.9
KU496892.1 *Orthetrum glaucum*	34.5	17.2	32.3	16.0	23	18.3	27.5	31.2	41	30.6	13.0	15.7	40	2.8	56.5	.9
KU496891.1 Orthetrum glaucum	35.5	16.7	31.5	16.4	24	17.6	26.9	31.5	41	30.6	13.0	15.7	42	1.9	54.6	1.9
Avg.	33.6	16.7	32.8	17.0	25	16.1	27.2	31.8	41	30.6	13.0	15.7	35	3.4	58.2	3.3

Table 63: The Nucleotide substitution table of *Brachydiplax sobrina*

Domain: Data																
	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
KT372720.1 *Brachydiplax sobrina* (KERALA)	31.5	19.6	31.0	17.9	40	31.2	13.8	15.2	32	10.9	50.7	6.5	23	16.8	28.5	32.1
KT372721.1 *Brachydiplax chalybea*	31.5	19.6	31.0	17.9	40	31.2	13.8	15.2	32	10.9	50.7	6.5	23	16.8	28.5	32.1
KC287156.1 *Brachydiplax chalybea*	31.5	19.6	31.0	17.9	40	31.2	13.8	15.2	32	10.9	50.7	6.5	23	16.8	28.5	32.1
MF358746.1 *Brachydiplax chalybea*	30.5	20.6	30.8	18.2	40	31.2	13.8	15.2	30	12.3	50.7	6.5	21	18.2	27.7	32.8
AB708947.1 *Brachydiplax chalybea*	30.8	20.6	31.2	17.4	40	31.9	13.0	15.2	32	10.9	52.9	4.3	20	19.0	27.7	32.8
MF358747.1 *Libellula quadrimaculata*	31.6	20.1	30.6	17.7	40	31.9	13.0	15.2	34	10.2	51.1	5.1	21	18.2	27.7	32.8
MF358748.1 *Libellula quadrimaculata*	33.5	18.7	30.3	17.5	40	31.9	13.0	15.2	38	7.3	50.4	4.4	23	16.8	27.7	32.8
AB708628.1 *Planaeschna ishigakiana*	32.5	17.7	32.0	17.7	39	31.9	13.8	15.2	33	7.3	54.0	5.8	26	13.9	28.5	32.1
KY947485.1 *Dythemis multipunctata*	34.5	18.9	29.6	17.0	40	31.2	13.8	15.2	42	7.3	47.4	3.6	22	18.2	27.7	32.1
KY947366.1 *Dythemis multipunctata*	34.5	18.9	29.6	17.0	40	31.2	13.8	15.2	42	7.3	47.4	3.6	22	18.2	27.7	32.1
KU980970.1 *Libellulidae sp.*	34.2	19.2	29.4	17.2	40	31.2	13.8	15.2	41	8.0	46.7	4.4	22	18.2	27.7	32.1
KY947486.1 *Dythemis multipunctata*	34.2	19.2	29.6	17.0	40	31.2	13.8	15.2	41	8.0	47.4	3.6	22	18.2	27.7	32.1
KY947365.1 *Dythemis multipunctata*	34.2	19.2	29.6	17.0	40	31.2	13.8	15.2	41	8.0	47.4	3.6	22	18.2	27.7	32.1
KY947364.1 *Dythemis multipunctata*	34.2	19.2	29.6	17.0	40	31.2	13.8	15.2	41	8.0	47.4	3.6	22	18.2	27.7	32.1
KY947363.1 *Dythemis multipunctata*	34.2	19.2	29.6	17.0	40	31.2	13.8	15.2	41	8.0	47.4	3.6	22	18.2	27.7	32.1
Avg.	32.9	19.4	30.3	17.4	40	31.4	13.6	15.2	37	9.0	49.5	4.8	22	17.6	27.9	32.3

Table 9: The Nucleotide substitution table of *Ischnura aurora*

Domain: Data																
	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
KR149808.1 *Ischnura aurora* (KERALA)	34.3	16.2	31.8	17.6	36	7.9	52.7	3.0	24	15.2	28.7	32.3	43	25.6	14.0	17.7
KF369414.1 *Ischnura aurora*	34.3	16.2	31.8	17.6	36	7.9	52.7	3.0	24	15.2	28.7	32.3	43	25.6	14.0	17.7
KX053528.1 *Ischnura aurora*	34.3	16.2	31.6	17.8	36	7.9	52.1	3.6	24	15.2	28.7	32.3	43	25.6	14.0	17.7
KX053526.1 *Ischnura aurora*	34.3	16.2	31.6	17.8	36	7.9	52.1	3.6	24	15.2	28.7	32.3	43	25.6	14.0	17.7
KX053524.1 *Ischnura aurora*	34.3	16.2	31.6	17.8	36	7.9	52.1	3.6	24	15.2	28.7	32.3	43	25.6	14.0	17.7
KX053532.1 *Ischnura aurora*	34.1	16.4	31.6	17.8	36	8.5	52.1	3.6	24	15.2	28.7	32.3	43	25.6	14.0	17.7
KX053525.1 *Ischnura aurora*	34.1	16.4	31.6	17.8	36	8.5	52.1	3.6	24	15.2	28.7	32.3	43	25.6	14.0	17.7
KX053531.1 *Ischnura aurora*	34.1	16.4	31.6	17.8	36	8.5	52.1	3.6	24	15.2	28.7	32.3	43	25.6	14.0	17.7
KX053529.1 *Ischnura aurora*	34.1	16.4	31.8	17.6	36	8.5	52.1	3.6	24	15.2	29.3	31.7	43	25.6	14.0	17.7
KX053530.1 *Ischnura aurora*	34.3	16.2	31.6	17.8	36	7.9	52.1	3.6	24	15.2	28.7	32.3	43	25.6	14.0	17.7
KY843451.1 *Ischnura delicate*	33.9	16.2	32.5	17.4	35	8.5	54.5	2.4	24	14.6	28.7	32.3	43	25.6	14.0	17.7
KY844428.1 *Ischnura delicata*	33.9	16.2	32.5	17.4	35	8.5	54.5	2.4	24	14.6	28.7	32.3	43	25.6	14.0	17.7
KY838304.1 *Ischnura delicata*	34.1	16.1	32.4	17.3	35	8.5	54.3	2.4	25	14.7	28.8	31.9	43	25.2	14.1	17.8
KY832433.1 *Ischnura delicata*	34.1	16.3	32.3	17.3	35	8.5	53.9	2.4	25	14.7	28.8	31.9	43	25.6	14.0	17.7
KM535165.1 *Ischnura verticalis*	33.9	15.8	31.6	18.7	36	6.1	52.1	6.1	23	15.9	28.7	32.3	43	25.6	14.0	17.7
KM532708.1 *Ischnura verticalis*	33.9	15.8	31.6	18.7	36	6.1	52.1	6.1	23	15.9	28.7	32.3	43	25.6	14.0	17.7
KM536053.1 *Ischnura verticalis*	34.0	15.9	31.4	18.7	36	6.1	51.8	6.1	23	16.0	28.2	32.5	43	25.6	14.0	17.7
KC135957.1 *Ischnura asiatica*	33.1	16.4	32.3	18.3	33	8.5	54.5	4.2	24	15.2	28.7	32.3	43	25.6	13.4	18.3
Avg.	34.0	16.2	31.9	17.9	36	7.9	52.8	3.7	24	15.2	28.7	32.2	43	25.6	14.0	17.7

Table 3: The Nucleotide substitution table of *Ceriagrion coromandelianum*

Domain: Data																
	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
KT222949.1 *Ceriagrion coromandelianum* (KERALA)	34.0	17.1	32.1	16.8	32	9.9	55.0	2.6	26	15.2	27.2	31.9	44	26.2	14.1	15.7
KU220871.1 *Ceriagrion coromandelianum*	34.0	17.1	31.9	16.9	32	9.9	54.5	3.1	26	15.2	27.2	31.9	44	26.2	14.1	15.7
KT879897.1 *Ceriagrion coromandelianum*	33.9	17.3	32.1	16.8	32	10.5	55.0	2.6	26	15.2	27.2	31.9	44	26.2	14.1	15.7
KU220869.1 *Ceriagrion olivaceum*	33.9	16.6	33.2	16.4	32	8.4	58.1	1.6	26	15.2	27.2	31.9	44	26.2	14.1	15.7
KU566000.1 *Ceriagrion suave*	34.7	15.5	32.8	16.9	35	4.7	57.1	3.1	25	15.7	27.2	31.9	44	26.2	14.1	15.7
KU565956.1 *Ceriagrion glabrum*	34.9	15.4	32.8	16.9	36	4.2	57.1	3.1	25	15.7	27.2	31.9	44	26.2	14.1	15.7
KU565947.1 *Ceriagrion glabrum*	34.9	15.4	32.8	16.9	36	4.2	57.1	3.1	25	15.7	27.2	31.9	44	26.2	14.1	15.7
KU565990.1 *Ceriagrion suave*	34.2	16.2	32.6	16.9	35	5.8	56.5	3.1	24	16.8	27.2	31.9	44	26.2	14.1	15.7
KU565964.1 *Ceriagrion glabrum*	34.7	15.5	32.8	16.9	35	4.7	57.1	3.1	25	15.7	27.2	31.9	44	26.2	14.1	15.7
Avg.	34.4	16.2	32.6	16.8	34	6.9	56.4	2.9	25	15.6	27.2	31.9	44	26.2	14.1	15.7

Table 19: The Nucleotide substitution table of *Vestalis gracilis*

Domain: Data																
	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
KM675768 *Vestalis gracilis* (KERALA)	30.3	22.1	27.5	20.1	22	21.5	25.8	31.2	41	25.9	16.8	16.8	29	18.8	39.8	12.4
KM675770.1 *Vestalis apicalis*	30.3	22.1	27.5	20.1	22	21.5	25.8	31.2	41	25.9	16.8	16.8	29	18.8	39.8	12.4
KU510326.1 *Vestalis apicalis*	30.3	22.1	27.5	20.1	22	21.5	25.8	31.2	41	25.9	16.8	16.8	29	18.8	39.8	12.4
KX503058.1 *Vestalis gracilis*	30.3	22.1	27.5	20.1	22	21.5	25.8	31.2	41	25.8	16.7	16.7	29	18.9	40.0	12.4
KJ493064.1 *Metagrion fornicatum*	30.9	20.3	31.1	17.6	23	19.4	29.6	28.5	41	24.9	16.8	17.3	29	16.8	47.0	7.0
FJ812855.1 *Nesobasis selysi*	30.0	21.2	31.2	17.6	22	19.9	27.4	30.6	42	25.4	16.8	16.2	26	18.3	49.5	5.9
FJ812847.1 *Nesobasis selysi*	30.0	21.2	31.2	17.6	22	19.9	27.4	30.6	42	25.4	16.8	16.2	26	18.3	49.5	5.9
KT879907.1 *Euphaea fraseri*	30.7	19.2	33.6	16.5	21	19.9	30.6	28.5	41	24.9	16.8	17.3	30	12.9	53.2	3.8
KJ493062.1 *Metagrion sp*	29.6	21.4	32.0	17.1	22	20.4	30.6	27.4	41	25.4	16.8	17.3	27	18.3	48.4	6.5
KF369576.1 *Vestalis amabilis*	28.9	22.6	28.9	19.6	22	18.3	28.5	31.2	41	25.4	16.8	16.8	24	24.2	41.4	10.8
Avg.	30.1	21.4	29.8	18.6	22	20.4	27.7	30.2	41	25.5	16.7	16.8	28	18.4	44.8	8.9

Table 21: The Nucleotide substitution table of *Onychogomphus malabarensis*

Domain: Data																
	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
KU133368.1 *Onychogomphus malabarensis* (KERALA)	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5
KX890962.1 *Ophiogomphus anomalus*	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5
KX890932.1 *Ophiogomphus mainensis*	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5
JN420156.1 *Ophiogomphus mainensis*	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5
JN420133.1 *Ophiogomphus mainensis*	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5
JN420085.1 *Ophiogomphus mainensis*	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5
JN420057.1 *Ophiogomphus sp.*	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5
JN420056.1 *Ophiogomphus sp.*	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5
JN420053.1 *Ophiogomphus sp.*	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5
JN420024.1 *Ophiogomphus sp*	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5
Avg.	34.6	17.6	31.7	16.1	43	26.4	15.4	15.4	38	8.5	51.2	2.5	23	18.0	28.5	30.5

Table 35: The Nucleotide substitution table of *Trithemis aurora*

Domain: Data																
	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
KT305963.1 *Trithemis aurora* (KERALA)	32.6	18.0	32.8	16.6	32	5.3	58.9	3.3	24	18.0	26.0	32.0	41	30.7	13.3	14.7
JN817428.1 *Trithemis aurora*	32.6	18.0	32.8	16.6	32	5.3	58.9	3.3	24	18.0	26.0	32.0	41	30.7	13.3	14.7
MF358779.1 *Trithemis aurora*	32.8	18.2	32.6	16.4	33	5.3	58.3	3.3	24	18.7	26.0	31.3	41	30.7	13.3	14.7
MF358791.1 *Trithemis aurora*	32.4	18.4	32.8	16.4	32	6.0	58.9	3.3	24	18.7	26.0	31.3	41	30.7	13.3	14.7
KT961627.1 *Trithemis aurora*	33.0	18.2	32.4	16.4	34	5.3	57.6	3.3	24	18.7	26.0	31.3	41	30.7	13.3	14.7
AB709237.1 *Trithemis* aurora	32.4	18.4	33.0	16.2	32	6.0	59.6	2.6	24	18.7	26.0	31.3	41	30.7	13.3	14.7
AB709236.1 *Trithemis aurora*	32.6	18.2	32.8	16.4	32	5.3	58.9	3.3	24	18.7	26.0	31.3	41	30.7	13.3	14.7
KU566458.1 *Trithemis werneri*	34.4	17.1	32.2	16.4	37	3.3	56.3	3.3	25	17.3	26.7	31.3	41	30.7	13.3	14.7
FJ358477.1 *Trithemis grouti*	35.9	18.0	29.0	17.1	42	6.0	47.0	4.6	24	18.0	26.7	31.3	41	30.0	13.3	15.3
FJ358478.1 *Trithemis grouti*	35.9	18.0	28.8	17.3	42	6.0	46.4	5.3	24	18.0	26.7	31.3	41	30.0	13.3	15.3
Avg.	33.5	18.0	31.9	16.6	35	5.4	56.1	3.6	24	18.3	26.2	31.5	41	30.5	13.3	14.8

Table 37: The Nucleotide substitution table of *Neurothemis fulvia*

Domain: Data																
	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
KP835515.1 *Neurothemis fulvia* (KERALA)	33.7	20.0	27.7	18.6	21	20.0	27.2	31.8	43	26.7	14.9	15.4	37	13.3	41.0	8.7
JN817427.1 *Neurothemis fulvia*	33.7	20.0	27.7	18.6	21	20.0	27.2	31.8	43	26.7	14.9	15.4	37	13.3	41.0	8.7
KC122229.1 *Neurothemis tullia*	34.4	19.3	26.0	20.2	20	20.0	28.2	31.8	43	26.7	14.9	15.4	40	11.3	35.1	13.4
KT957504.1 *Neurothemis fluctuans*	34.2	18.8	27.4	19.7	22	17.9	28.2	31.8	43	26.7	14.9	15.4	37	11.8	39.0	11.8
KC122227.1 *Neurothemis sp*	34.2	18.6	27.5	19.7	22	17.9	28.2	31.8	43	26.7	14.9	15.4	37	11.3	39.5	11.8
KU566458.1 *Trithemis werneri*	36.2	15.4	31.5	16.9	24	16.4	28.2	31.8	43	26.7	14.9	15.4	42	3.1	51.3	3.6
KU566447.1 *Trithemis legrandi*	33.8	17.8	30.9	17.4	22	19.0	27.7	31.8	43	26.7	14.9	15.4	37	7.7	50.3	5.1
KU566445.1 *Trithemis legrandi*	33.8	17.8	30.9	17.4	22	19.0	27.7	31.8	43	26.7	14.9	15.4	37	7.7	50.3	5.1
KU566444.1 *Trithemis legrandi*	33.9	17.8	30.8	17.5	22	19.0	27.7	31.8	43	26.8	14.4	15.5	37	7.7	50.3	5.1
KU566446.1 *Trithemis legrandi*	34.0	17.6	30.9	17.4	22	19.0	27.7	31.8	43	26.7	14.9	15.4	37	7.2	50.3	5.1
Avg.	34.2	18.3	29.1	18.3	22	18.8	27.8	31.8	43	26.7	14.8	15.4	38	9.4	44.8	7.9

Table 53: The Nucleotide substitution table of *Pantala flavescens*

Domain: Data																	
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	Pos #2	T-3	C-3	A-3	G-3
KR011198.1 *Pantala flavescens* (KERALA)	36.9	19.4	26.6	17.1	22	19.6	27.0	31.1	41	31.1	13.5	14.9	148.0	48	7.4	39.2	5.4
LC366762.1 *Pantala flavescens*	36.7	19.8	26.4	17.1	22	20.3	26.4	31.1	41	31.1	13.5	14.9	148.0	47	8.1	39.2	5.4
KR080114.1 *Pantala flavescens*	37.4	19.5	26.0	17.2	23	20.1	26.2	30.9	41	30.9	13.4	14.8	149.0	48	7.4	38.3	6.0
KR080089.1 *Pantala flavescens*	37.8	19.0	26.4	16.8	24	18.8	26.2	30.9	41	30.9	13.4	14.8	149.0	48	7.4	39.6	4.7
KR080079.1 *Pantala flavescens*	37.1	19.7	26.2	17.0	23	20.1	26.2	30.9	41	30.9	13.4	14.8	149.0	48	8.1	38.9	5.4
KR080077.1 *Pantala flavescens*	37.1	19.5	26.4	17.0	23	20.1	26.2	30.9	41	30.9	13.4	14.8	149.0	48	7.4	39.6	5.4
KR080131.1 *Pantala flavescens*	38.0	18.8	26.4	16.8	24	18.8	26.2	30.9	41	30.9	13.4	14.8	149.0	49	6.7	39.6	4.7
KR080120.1 *Pantala flavescens*	37.8	19.0	26.2	17.0	24	18.8	26.2	30.9	41	30.9	13.4	14.8	149.0	48	7.4	38.9	5.4
KR080112.1 *Pantala flavescens*	37.4	19.5	26.2	17.0	23	20.1	26.2	30.9	41	30.9	13.4	14.8	149.0	48	7.4	38.9	5.4
KR080110.1 *Pantala flavescens*	37.1	19.7	26.0	17.2	23	20.1	26.2	30.9	41	30.9	13.4	14.8	149.0	48	8.1	38.3	6.0
KR080108.1 *Pantala flavescens*	37.8	19.0	26.2	17.0	24	18.8	26.2	30.9	41	30.9	13.4	14.8	149.0	48	7.4	38.9	5.4
KR080100.1 *Pantala flavescens*	37.8	19.0	26.2	17.0	24	18.8	26.2	30.9	41	30.9	13.4	14.8	149.0	48	7.4	38.9	5.4
KR080095.1 *Pantala flavescens*	37.8	19.0	26.2	17.0	24	18.8	26.2	30.9	41	30.9	13.4	14.8	149.0	48	7.4	38.9	5.4
KR080076.1 *Pantala flavescens*	37.8	19.0	26.2	17.0	24	18.8	26.2	30.9	41	30.9	13.4	14.8	149.0	48	7.4	38.9	5.4
Avg.	37.5	19.3	26.2	17.0	23	19.4	26.2	30.9	41	30.9	13.4	14.8	148.9	48	7.5	39.0	5.4

Table 45: The Nucleotide substitution table of *Brachythemis contaminata*

Domain: Data																
	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
KP938531.1 *Brachythemis contaminate* (KERALA)	32.8	16.9	31.5	18.8	22	15.3	29.0	33.9	42	25.8	12.9	19.4	35	9.7	52.4	3.2
KT879898.1 *Brachythemis contaminata*	32.8	16.9	31.5	18.8	22	15.3	29.0	33.9	42	25.8	12.9	19.4	35	9.7	52.4	3.2
KM658172.1 *Brachythemis contaminata*	32.5	17.2	31.2	19.1	21	16.1	29.0	33.9	42	25.8	12.9	19.4	35	9.7	51.6	4.0
KC287157.1 *Brachythemis contaminata*	32.8	16.9	30.9	19.4	22	16.9	27.4	33.9	42	25.8	12.9	19.4	35	8.1	52.4	4.8
KU566425.1 *Trithemis donaldsoni*	33.2	15.9	31.3	19.7	22	16.1	28.2	33.9	42	25.0	12.9	20.2	36	6.5	52.8	4.9
KT957542.1 *Diplacodes trivialis*	33.2	15.9	30.5	20.5	21	16.9	27.4	34.7	42	25.8	12.9	19.4	37	4.9	51.2	7.3
KT957540.1 *Diplacodes trivialis*	33.2	15.9	30.5	20.5	21	16.9	27.4	34.7	42	25.8	12.9	19.4	37	4.9	51.2	7.3
KT957538.1 *Diplacodes trivialis*	33.2	15.9	30.5	20.5	21	16.9	27.4	34.7	42	25.8	12.9	19.4	37	4.9	51.2	7.3
KT957537.1 *Diplacodes trivialis*	33.2	15.9	30.5	20.5	21	16.9	27.4	34.7	42	25.8	12.9	19.4	37	4.9	51.2	7.3
KT957527.1 *Diplacodes trivialis*	33.2	15.9	30.5	20.5	21	16.9	27.4	34.7	42	25.8	12.9	19.4	37	4.9	51.2	7.3
KT957526.1 *Diplacodes trivialis*	33.2	15.9	30.5	20.5	21	16.9	27.4	34.7	42	25.8	12.9	19.4	37	4.9	51.2	7.3
KU566468.1 *Urothemis venata*	33.4	17.5	29.4	19.7	21	16.9	27.4	34.7	42	25.8	12.9	19.4	37	9.8	48.0	4.9
KU566466.1 *Urothemis venata*	33.4	17.5	29.4	19.7	21	16.9	27.4	34.7	42	25.8	12.9	19.4	37	9.8	48.0	4.9
Avg.	33.1	16.5	30.6	19.8	21	16.6	27.9	34.4	42	25.7	12.9	19.4	36	7.1	51.2	5.7

Table 1: The locations selected for the collection during present study

Sl. No.	*District*	*Ecosystem*	*Location*	*GPS coordinates*
1	Kasaragod	Agro-ecosystem	Kangangad	12.3324º N 75.0963º E
		Forest ecosystem	Parappa	12.3674º N 75.2253º E
		Riparian ecosystem	Periya	12.3957º N 75.0965º E
2	Kannur	Agro-ecosystem	Payyanur	12.1051º N 75.2058º E
		Forest ecosystem	Aaralam	11.9676º N 75.7720º E
		Riparian ecosystem	Koothuparamba	11.8319º N 75.6556º E
3	Wayanad	Agro-ecosystem	Pulpally	11.7923º N 76.1663º E
		Forest ecosystem	Sulthan's Bathery	11.6656º N 76.2627º E
		Riparian ecosystem	Vythiri	11.5517º N 76.0403º E
4	Kozhikode	Agro-ecosystem	Ramanattukara	11.1785º N 75.8652º E
		Forest ecosystem	Thusharagiri	11.4730º N 76.0529º E
		Riparian ecosystem	Beypore	11.1736º N 75.8040º E
5	Malappuram	Agro-ecosystem	Villunniyal	11.1340º N 75.8954º E
		Forest ecosystem	Nilambur	11.2794º N 76.3695º E
		Riparian ecosystem	Tirur	10.9146º N 75.9221º E
6	Palakkad	Agro-ecosystem	Thrithala	10.8033º N 76.1349º E
		Forest ecosystem	Attapadi	11.1149º N 76.6180º E
		Riparian ecosystem	Ottapalam	10.7723º N 76.3695º E
7	Thrissur	Agro-ecosystem	Kunnamkulam	10.6014º N 76.2023º E
		Forest ecosystem	Peechi	10.5270º N 76.3608º E
		Riparian ecosystem	Peramangalam	10.5303º N 76.2148º E

Table 2: The list of specific primers used for PCR amplification of the present study

Sl. No.	*Name of the primer*	*Direction*	*Sequence description*
1	FOM	Forward	5' – GGTCAACAAATCATAAAGATATTGG – 3'
		Reverse	5'– TAAACTTCAGGGTGACCAAAAAATCA – 3'
2	JAF	Forward	5' – GGTCAACAAATCATAAAGATATTGG – 3'
		Reverse	5' – TAAACTTCAGGGTGACCAAAAAATCA – 3'
3	JAG	Forward	5' – GATATTGGAACCCTTTACCTG – 3'
		Reverse	5' – GTTGATAAAGGATTGGCAGGGTGACC – 3'
4	JAP	Forward	5' – AGGTCAACCTGGATCTTTAATTGGA – 3'
		Reverse	5' – AGAATAGGGTCTCCTCCTCCG – 3'
5	JCC	Forward	5' – TCGGTGCATGAGCAGGTATAGTAGGTAC – 3'
		Reverse	5' – AATAGGATCTCCTCCACCTGCTG – 3'
6	JNT	Forward	5' – ACTGCCCACGCCTTTGTAATAATTTTC – 3'
		Reverse	5' – GCTATTACTATACTATTAACTGA – 3'
7	JOS	Forward	5' – ATTAGTGCCGTTAATACTTGGTGCTCC – 3'
		Reverse	5' – AGATAGGATCTCCTCCTCCCG – 3'
8	JPC	Forward	5' – CGGAATTTGATCAGGAATAGTAGGA – 3'
		Reverse	5' – GATCTCCTCCTCCAGCTGGG – 3'
9	JPF	Forward	5' – ATTAGTGCCGTTAATACTTGGTGCTCC – 3'
		Reverse	5' – AAAATTGGATCTCCTCCCCCTGC – 3'
10	OCM	Forward	5' – TTTTCTACTAACCACAA – 3'
		Reverse	5' – TTTTCCTCTTTCTTGGG – 3'
11	OTF	Forward	5' – TAATACGACTCACTATAGGGGG – 3'
		Reverse	5' – ATTAACCCTCACTAAAGTAAA – 3'
12	JRV	Forward	5' – TTGAACTGGGACAACCTGGA – 3'
		Reverse	5' –GGCTCCAGCAAGAACAGGT – 3'
13	ODT	Forward	5' – GGAACAGCATTAAGAGTTTTAATTCGA – 3'
		Reverse	5' – GACCCGGCAGGTGGTGGAGATC– 3'
14	AOD	Forward	5' – CATTGGAGATGACCAAATTTA– 3'
		Reverse	5' – ATTGGATCTCCACCACCTGC– 3'

Table 65: List database accession details in NCBI GenBank and Barcode of Life Data System Index Number (BIN) of the species selected for the present study

Sl. No.	Species	NCBI Accession Number	BOLD Accession Number	
			BIN Cluster ID	Specimen ID
	Suborder: Zygoptera			
	Family: Coenagrionidae			
1	*Ceriagrion coromendelianum*	KT222949	BOLD: AA25825	GBMIN88578-17
2	*Agriocnemis pygmeae*	KU871002	BOLD: ADC3017	GBMIN88575-17
3	*Agriocnemis keralensis*	KU133367	BOLD: ACF9984	GBMIN88574-17
4	*Ischnura aurora*	KR149808	BOLD: AAH6873	GBMH0673-15
5	*Ischnura senegalensis*	KT305961	BOLD: ABW0501	AGIR1303-17
6	*Aciagrion occidentale*	KM096996	BOLD: ACG1133	
	Family: Placticnemidae			
7	*Copera marginipes*	KR149804	BOLD: ABA1480	GBMH0650-15
	Family: Calopterygidae			
8	*Vestalis gracilis*	KX503058	BOLD: ACS6273	GBMIN88573-17
9	*Vestalis apicalis*	KU510326	BOLD: ACS6273	GBMIN88573-15
	Suborder: Anisoptera			
	Family: Gomphidae			
10	*Onychogomphus malabarensis*	KU133368	BOLD: AAA4278	GBMIN88722-17
	Family: Aeshnidae			
11	*Anax parthenope*	KR149805	BOLD: ABX6596	GBMH0633-15
12	*Anaciaeshna jaspidea*	KR149806		
	Family: Libellulidae			
13	*Orthetrum sabina*	KP938529	BOLD: AAH6870	GBMIN88805-17
14	*Neurothemis intermedia*	KU052672	BOLD: ADJ7302	GBMIN8879-17
15	*Neurothemis intermedia*	KP835514	BOLD: ADJ7302	GBMIN8879-17
16	*Trithemis aurora*	KT305963	BOLD: AAQ0253	GBMIN88911-17
17	*Trithemis aurora*	KT 305962	BOLD: AAQ0253	GBMIN88911-17
18	*Brachydiplax chalybaea*	KT372721	BOLD: ACD4364	GBMIN88778-17
19	*Neurothemis fulvia*	KP835515	BOLD: ACD6379	GBMIN88796-17
20	*Crocothemis servillia*	KR149807	BOLD: AAQ0252	GBMH0652-15
21	*Trithemis festiva*	KR149802	BOLD: AAQ0247	GBMH0998-15
22	*Trithemis pallidinervis*	KR149803	BOLD: AAQ0251	GBMH0999-15
23	*Potamarcha obscura*	KX503060		
24	*Brachythemis contaminata*	KP938531	BOLD: ADC3495	GBMIN8878--17
25	*Diplacodes trivialis*	KP835512	BOLD: AAH6874	GBMH065-15
26	*Diplacodes trivialis*	KP835513	BOLD: AAH6874	GBMH065-15
27	*Diplacodes trivialis*	KP087931	BOLD: AAH6874	GBMH065-15
28	*Diplacodes trivialis*	KP087932	BOLD: AAH6874	GBMH065-15
29	*Diplacodes trivialis*	KP087933	BOLD: AAH6874	GBMH065-15
30	*Bradinopyga geminata*	KM096995	BOLD: ABY3063	GBMIN22799-13
31	*Rhyothemis variegata*	KP938530	BOLD: ABX8023	
32	*Pantala flavescence*	KR11198	BOLD: AAH6890	
33	*Acisoma panorpoides*	KT222947	BOLD: ADL6242	
34	*Neurothemis tullia*	KP835513	BOLD: ABX8024	
35	*Lathresia asiatica*	KU052671	BOLD: ADJ7302	GBMIN88787-17
36	*Aethriamanta brevipennis*	KU510325		
37	*Brachydiplax sobrina*	KT372720	BOLD:ACD4364	

Table 66: The database submission status of COI gene sequences of the species studied

PIONEER REPORT IN THE DATABASE			
Sl. No.	***Organism***	***Sl. No.***	***Organism***
1	*Agriocnemis keralensis*	2	*Onychogomphus malabarensis*
3	*Ceriagrion coromendelianum*	4	*Aciagrion occidentale*
5	*Lathresia sp.*		
PIONEER REPORT FROM INDIA			
Sl. No.	***Organism***		
1	*Anax parthenope*		
2	*Agriocnemis pygmeae*		
3	*Copera marginipes*		
4	*Ischnura aurora*		
5	*Ischnura senegalensis*		
PIONEER REPORT FROM KERALA			
Sl. No.	***Organism***	***Sl. No.***	***Organism***
1	*Acisoma panorpoides*	9	*Neurothemis intermedia*
2	*Anaciaeshna jaspidea*	10	*Neurothemis tullia*
3	*Brachydiplax chalybaea*	11	*Orthetrum sabina*
4	*Brachythemis contaminata*	12	*Potamarcha obscura*
5	*Bradinopyga geminata*	13	*Rhyothemis variegata*
6	*Crocothemis servillia*	14	*Trithemis aurora*
7	*Diplacodes trivialis*	15	*Trithemis festiva*
8	*Neurothemis fulvia*	16	*Trithemis pallidinervis*

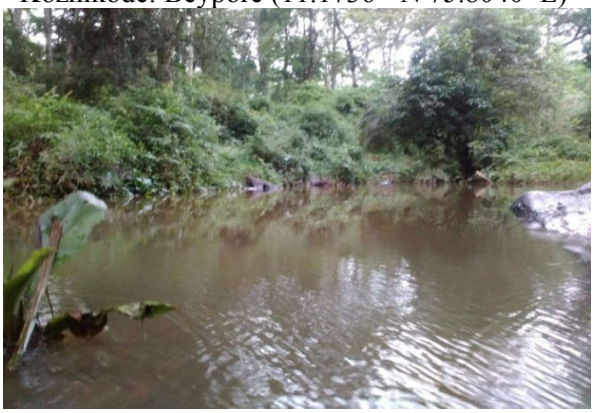
Thrissur: Peramangalam (10.5303º N 76.214 ºE)

Palakkad : Ottapalam (10.7723º N 76.3695ºE)

Malappuram: Tirur (10.9146º N 75.9221ºE

Kozhikode: Beypore (11.1736º N 75.8040º E)

Wayanad : Vythiri (11.5517º N 76.0403º E)



Kannur: Koothuparamba (11.8319º N 75.655º E)

Kasargode: Periya (11.500º N 75.50º E)

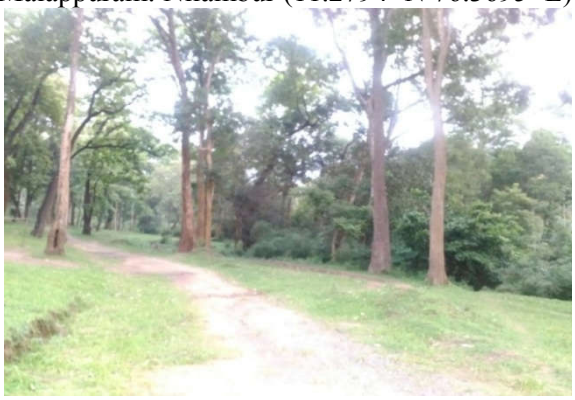
Figure 1: Study Area – Riparian Ecosystems

Thrissur: Peechi (10.5270382ºN 76.36083º E)

Palakkad : Attapadi (11.114893ºN76.6180º E)

Malappuram: Nilambur (11.2794º N 76.3695 ºE)

Kozhikode: Thusharagiri (11.4730º N 76.0529º E)



Wayanad : Sulthan Batheri (11.666 ºN76.2627ºE)

Kannur: Aaralam (11.9676º N 75.7720 º E)

Kasargode: Parappa (12.36745º N 75.22535º E)

Figure 2: Study Area – Forest Ecosystems

Thrissur: Kunnamkulam (10.601º N 76.202º E)

Palakkad : Thrithala (10.803º N 76.1349º E)

Malappuram: Villunniyal (11.1340º N 75.895º E))

Kozhikode: Ramanattukara (11.1785º N 75.865º E)

Wayanad : Puplally (11.7923ºN 76.1663º E)

Kannur: Payyanur (12.1051º N 75.2058ºE)

Kasargode: Kangangad (12.352º N 75.096º E)

Figure 3: Study Area – Agroecosystems

Figure 4: *Ceriagrion coromandelianum*

> KT222949 *Ceriagrion coromandelianum* |cytochrome oxidase subunit I gene |voucher CUCC 01-A1|partial cds, mitochondrial|573bp

TAGTATATTAATTCGAGTTGAATTAGGTCAACCAGGATCCCTCATTGGAGATGACCAAATTTATAATGTAGTAGTA
ACAGCACATGCATTTGTAATAATTTTTTTCATAGTTATACCAATTATAATTGGAGGATTCGGAAATTGATTAGTTC
CCTTGATATTAGGGGCACCTGATATAGCTTTCCCACGATTAAATAATATGAGATTTTGACTTTTACCTCCTTCATT
AACACTACTATTAGCAAGAAGTTTAGTAGAAAGAGGAGCAGGTACTGGTTGAACAGTATATCCACCCCTTGCAGGA
GCAATCGCACATGCAGGAGGATCTGTTGATTTAACAATTTTCTCATTACACTTAGCTGGAGTATCATCCATTTTAG
GTGCAATTAATTTTATTACCACTGTAATTAATATAAAATCCCCAGGAATAAAATTAGACCAATTACCACTATTTGT
ATGGGCAGTAGTAATTACTGCAGTTTTATTGTTACTATCATTACCAGTATTAGCTGGTGCTATTACCATATTATTA
ACTGATCGAAACATCAATACATCATTCTTTGATCCAGCAGG

Figure 4a: The DNA sequence interpret of COI gene of *Ceriagrion coromandelianum*

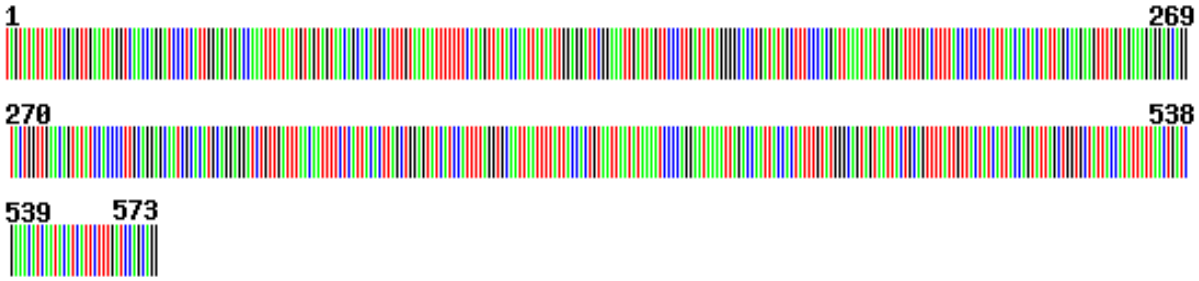


Figure 4b: Representatvie molecular barcode of COI gene of *Ceriagrion coromandelianum*

> AKV16034 *Ceriagrion coromandelianum* |cytochrome oxidase subunit I gene |voucher CUCC 01-A1|partial cds, mitochondrial|191 bp

```
SMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSL
TLLLASSLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMKLDQLPLFV
WAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAG
```

Figure 4c: The conceptual translation product of the COI gene of *Ceriagrion coromandelianum*

Ceriagrion coromandelianum voucher CUCC 01-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID: KT222949. Length: 573Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand	Frame
1059 bits(573)	0.0()	573/573(100%)	0/573(0%)	Plus/Plus	

```
Query  1    TAGTATATTAATTCGAGTTGAATTAGGTCAACCAGGATCCCTCATTGGAGATGACCAAAT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TAGTATATTAATTCGAGTTGAATTAGGTCAACCAGGATCCCTCATTGGAGATGACCAAAT  60

Query  61TTATAATGTAGTAGTAACAGCACATGCATTTGTAATAAttttttCATAGTTATACCAAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   TTATAATGTAGTAGTAACAGCACATGCATTTGTAATAATTTTTTTCATAGTTATACCAAT  120

Query  121  TATAATTGGAGGATTCGGAAATTGATTAGTTCCCTTGATATTAGGGGCACCTGATATAGC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  TATAATTGGAGGATTCGGAAATTGATTAGTTCCCTTGATATTAGGGGCACCTGATATAGC  180

Query  181  TTTCCCACGATTAAATAATATGAGATTTTGACTTTTACCTCCTTCATTAACACTACTATT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  TTTCCCACGATTAAATAATATGAGATTTTGACTTTTACCTCCTTCATTAACACTACTATT  240

Query  241  AGCAAGAAGTTTAGTAGAAAGAGGAGCAGGTACTGGTTGAACAGTATATCCACCCCTTGC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  AGCAAGAAGTTTAGTAGAAAGAGGAGCAGGTACTGGTTGAACAGTATATCCACCCCTTGC  300

Query  301  AGGAGCAATCGCACATGCAGGAGGATCTGTTGATTTAACAATTTTCTCATTACACTTAGC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  AGGAGCAATCGCACATGCAGGAGGATCTGTTGATTTAACAATTTTCTCATTACACTTAGC  360

Query  361  TGGAGTATCATCCATTTTAGGTGCAATTAATTTTATTACCACTGTAATTAATATAAAATC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  TGGAGTATCATCCATTTTAGGTGCAATTAATTTTATTACCACTGTAATTAATATAAAATC  420

Query  421  CCCAGGAATAAAATTAGACCAATTACCACTATTTGTATGGGCAGTAGTAATTACTGCAGT  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  CCCAGGAATAAAATTAGACCAATTACCACTATTTGTATGGGCAGTAGTAATTACTGCAGT  480

Query  481  TTTATTGTTACTATCATTACCAGTATTAGCTGGTGCTATTACCATATTATTAACTGATCG  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  TTTATTGTTACTATCATTACCAGTATTAGCTGGTGCTATTACCATATTATTAACTGATCG  540

Query  541  AAACATCAATACATCATTCTTTGATCCAGCAGG  573
            ||||||||||||||||||||||||||||||||
Sbjct  541  AAACATCAATACATCATTCTTTGATCCAGCAGG  573
```

Figure 4d: Nucleotide BLAST output of *Ceriagrion coromandelianum* showing its nearest match subject

Cytochrome oxidase subunit I, partial (mitochondrion) [*Ceriagrion coromandelianum*]

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps
372 bits(954)	4e-130()	Compositional matrix adjust	191/191(100%)	191/191(100%)	0/191(0%)

Sequence ID: AKV16034. Length: 191Number of Matches: 1

```
Features:
Query  1    SMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMA  60
            SMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMA
Sbjct  1    SMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMA  60
Query  61   FPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIFSLHLA  120
            FPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIFSLHLA
Sbjct  61   FPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIFSLHLA  120
Query  121  GVSSILGAINFITTVINMKSPGMKLDQLPLFVWAVVITAVLLLLSLPVLAGAITMLLTDR  180
            GVSSILGAINFITTVINMKSPGMKLDQLPLFVWAVVITAVLLLLSLPVLAGAITMLLTDR
Sbjct  121  GVSSILGAINFITTVINMKSPGMKLDQLPLFVWAVVITAVLLLLSLPVLAGAITMLLTDR  180
Query  181  NINTSFFDPAG  191
            NINTSFFDPAG
Sbjct  181  NINTSFFDPAG  191
```

Figure 4e: Peptide BLAST output of COI gene of *Ceriagrion coromandelianum*

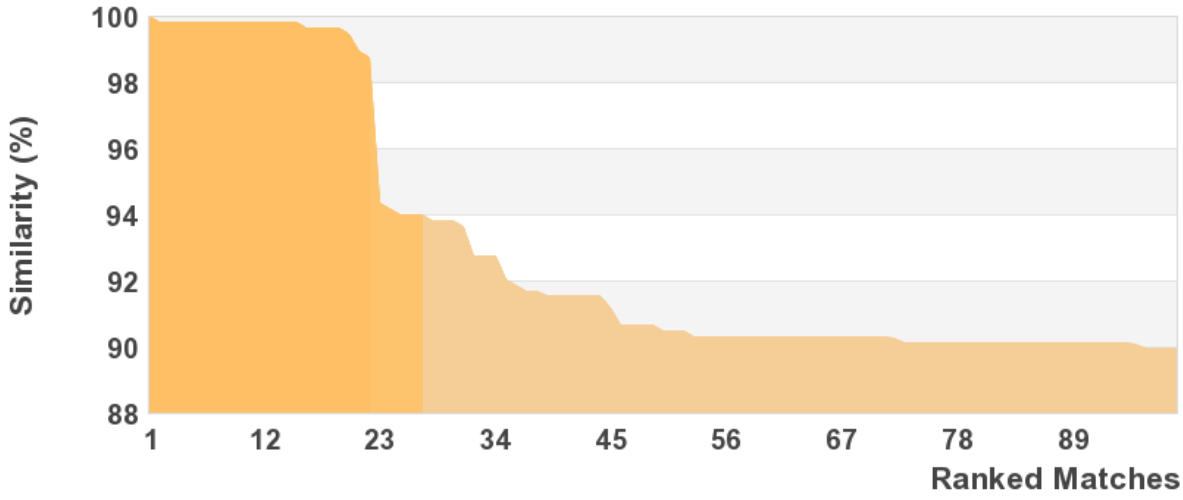


Figure 4f: The line diagram of *Ceriagrion coromendelianum*(Kerala) with more than 99 % match to other retrieved sequences from BOLD system.

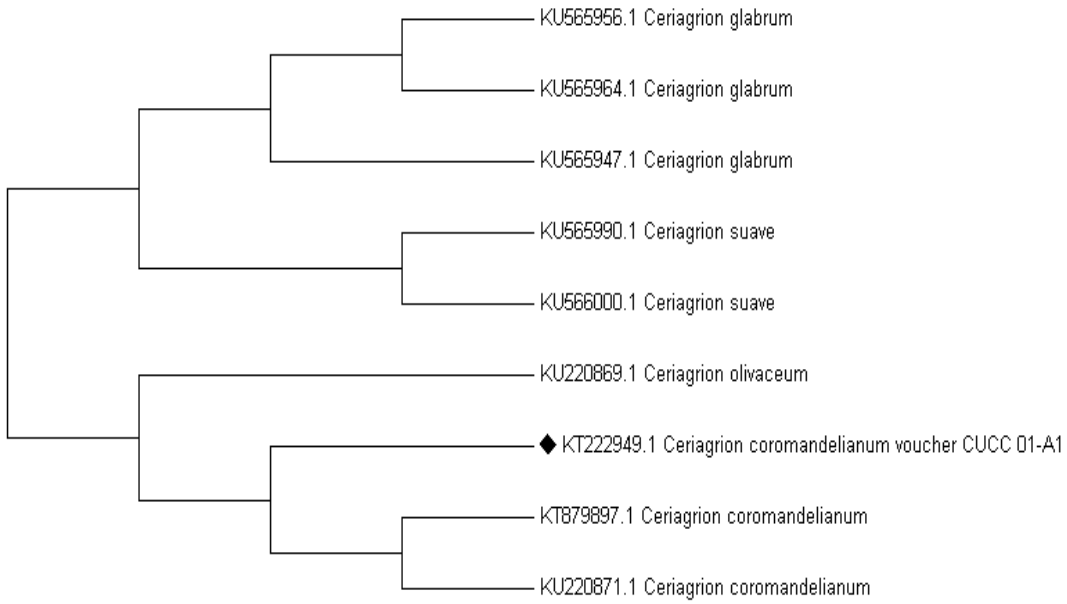


Figure 4g: Molecular phylogenetic tree of *Ceriagrion coromandelianum* inferred by NJ tree method

Table 4: Percentage of evolutionary divergence of *Ceriagrion coromendelianum* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	KT222949	*Ceriagrion coromendelianum* (Kerala)	
2.	KU220871	*Ceriagrion coromendelianum* (Belgium)	0.18
3.	KT879897	*Ceriagrion coromendelianum* (Karnataka)	0.18
4.	KU220869	*Ceriagrion olivaceum*	9.24
5.	KU566000	*Ceriagrion suave*	11.53
6.	KU565964	*Ceriagrion glabrum*	11.53
7.	KU565947	*Ceriagrion glabrum*	11.53
8.	KU565990	*Ceriagrion suave*	11.81
9.	KU565964	*Ceriagrion glabrum*	11.76

Figure 5: *Agriocnemis pygmeae*

> KU871002.1 *Agriocnemis pygmaea* |cytochrome oxidase subunit I gene |voucher CUAP-03-A1 partial cds, mitochondrial|567bp

AGATTAGGACAACCAGGCTCTCTTATTGGTGATGACCAAATTTATAACGTAGTTGTGACAGCACACGCCTTCGTAA
TAATTTTTTTTATAGTTATACCAATTATAATTGGTGGATTTGGAAACTGACTAGTACCATTAATGCTTGGAGCACC
CGATATAGCTTTCCCACGATTAAATAATATAAGATTTTGATTACTCCCCCCTTCATTAACACTTTTACTCGCAAGT
AGATTAGTAGAAAGTGGAGCAGGAACCGGATGAACAGTTTATCCTCCATTAGCAGGAGCAATTGCTCACGCTGGGG
GATCTGTTGATTTAACAATTTTTTCACTTCATTTGGCAGGGGTATCTTCAATTTTAGGGGCAATCAATTTTATTAC
AACTACAATTAATATAAAATCACCAGGAATAAAACTGGAACAAATGCCATTATTTGTATGAGCAGTTGTAATTACT
GCTGTATTACTATTATTATCATTACCTGTATTAGCAGGAGCTATTACTATATTACTTACTGACCGTAATATTAATA
CTTCATTTTTTGATCCGGCAGGGGGGGGAGATCCC

Figure 5a: DNA sequence interpret of COI gene of *Agriocnemis pygmaea*

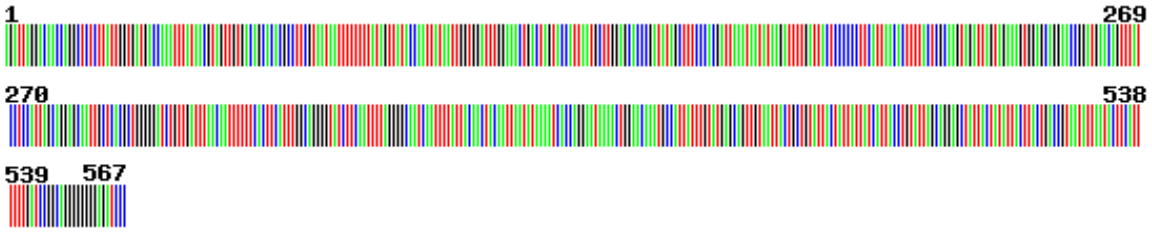


Figure 5b: The representative molecular barcode of the COI gene of *Agriocnemis pygmaea*

> AMR58407 *Agriocnemis pygmaea* |cytochrome oxidase subunit I gene |voucher CUAP-03-A1 partial cds, mitochondrial| 189bp

SLGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSLTLLLAS
SLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIFSLHLAGVSSILGAINFITTTINMKSPGMKLEQMPLFVWAVVIT
AVLLLLSLPVLAGAITMLLTDRNINTSFFDPAGGGDP

Figure 5c: The conceptual translation product of the COI gene of *Agriocnemis pygmaea*

Agriocnemis pygmaea voucher CUAP-03-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

Sequence ID: KU871002.Length: 567Number of Matches: 1

Alignment statistics for match

Score	Expect	Identities	Gaps	Strand
1048 bits(567)	0.0()	567/567(100%)	0/567(0%)	Plus/Plus

```
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Query  1    AGATTAGGACAACCAGGCTCTCTTATTGGTGATGACCAAATTTATAACGTAGTTGTGACA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    AGATTAGGACAACCAGGCTCTCTTATTGGTGATGACCAAATTTATAACGTAGTTGTGACA  60

Query  61   GCACACGCCTTCGTAATAAtttttttATAGTTATACCAATTATAATTGGTGGATTTGGA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GCACACGCCTTCGTAATAATTTTTTTTATAGTTATACCAATTATAATTGGTGGATTTGGA  120

Query  121  AACTGACTAGTACCATTAATGCTTGGAGCACCCGATATAGCTTTCCCACGATTAAATAAT  180
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Sbjct  121  AACTGACTAGTACCATTAATGCTTGGAGCACCCGATATAGCTTTCCCACGATTAAATAAT  180

Query  181  ATAAGATTTTGATTACTCCCCCCTTCATTAACACTTTTACTCGCAAGTAGATTAGTAGAA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  ATAAGATTTTGATTACTCCCCCCTTCATTAACACTTTTACTCGCAAGTAGATTAGTAGAA  240

Query  241  AGTGGAGCAGGAACCGGATGAACAGTTTATCCTCCATTAGCAGGAGCAATTGCTCACGCT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  AGTGGAGCAGGAACCGGATGAACAGTTTATCCTCCATTAGCAGGAGCAATTGCTCACGCT  300

Query  301  GGGGGATCTGTTGATTTAACAATTTTTTCACTTCATTTGGCAGGGGTATCTTCAATTTTA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  GGGGGATCTGTTGATTTAACAATTTTTTCACTTCATTTGGCAGGGGTATCTTCAATTTTA  360

Query  361  GGGGCAATCAATTTTATTACAACTACAATTAATATAAAATCACCAGGAATAAAACTGGAA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  GGGGCAATCAATTTTATTACAACTACAATTAATATAAAATCACCAGGAATAAAACTGGAA  420

Query  421  CAAATGCCATTATTTGTATGAGCAGTTGTAATTACTGCTGTATTACTATTATTATCATTA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  CAAATGCCATTATTTGTATGAGCAGTTGTAATTACTGCTGTATTACTATTATTATCATTA  480

Query  481  CCTGTATTAGCAGGAGCTATTACTATATTACTTACTGACCGTAATATTAATACTTCATTT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  CCTGTATTAGCAGGAGCTATTACTATATTACTTACTGACCGTAATATTAATACTTCATTT  540

Query  541TTTGATCCGGCAgggggggAGATCCC  567
            |||||||||||||||||||||||||
Sbjct  541  TTTGATCCGGCAGGGGGGGGAGATCCC  567
```

Figure 5d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Agriocnemis pygmaea* showing its nearest match subject

Cytochrome oxidase subunit 1, partial (mitochondrion) [*Agriocnemis pygmaea]*
Sequence ID: AMR58407 Length: 219Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps
366 bits(939)	2e-127()	Compositional matrix adjust	186/189(98%)	188/189(99%)	0/189(0%)

Features:

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            LGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNN
Sbjct  24   ELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNN  83

Query  61   MSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIFSLHLAGVSSIL  120
            MSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIFSLHLAGVSSIL
Sbjct  84   MSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIFSLHLAGVSSIL  143

Query  121  GAINFITTTINMKSPGMKLEQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSF  180
            GAINFITTTINMKSPGMK+EQ+PLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSF
Sbjct  144  GAINFITTTINMKSPGMKMEQLPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSF  203

Query  181  FDPAGGGDP  189
            FDPAGGGDP
Sbjct  204  FDPAGGGDP  212
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Figure 5e: Peptide BLAST output of the mt DNA COI gene of *Agriocnemis pygmaea*

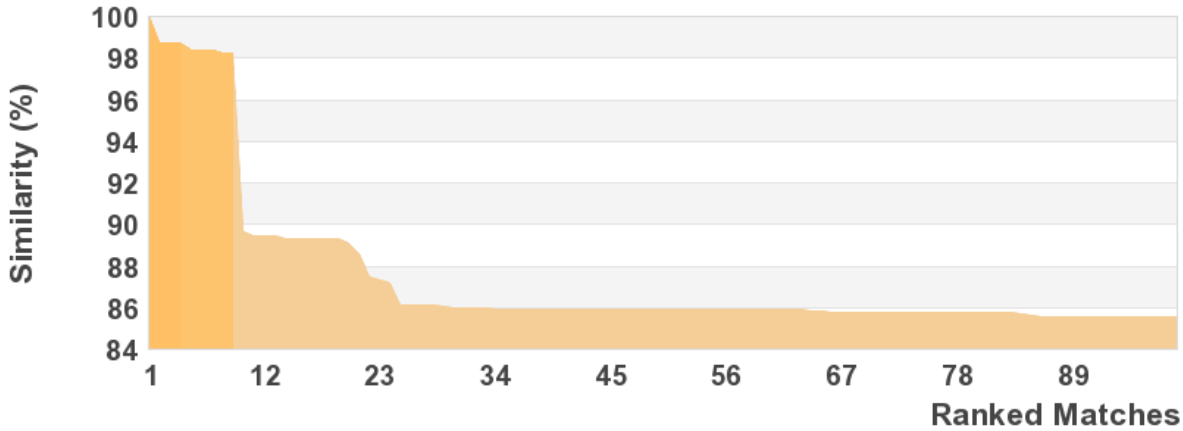

Figure 5f: The line diagram of *Agriocnemis pygmeae* over more than 98% match to other retrieved sequences from BOLD system

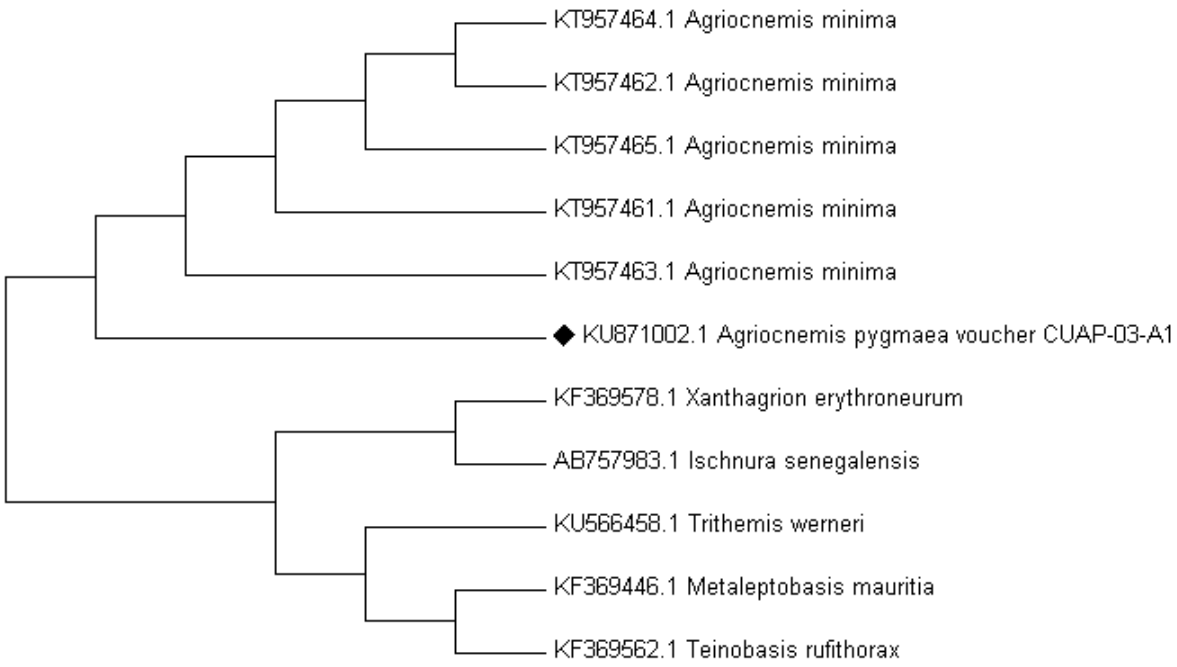

Figure 5g: Molecular phylogenetic tree of *Agriocnemis pygmaea* inferred by NJ tree method

Table 6: Percentage of evolutionary divergence of *Agriocnemis pygmaea* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KU871002	*Agriocnemis pygmaea* (Kerala)	
2	KT957464	*Agriocnemis minima* (Thailand)	0.11
3	KT957463	*Agriocnemis minima*(Thailand)	0.11
4	KT957465	*Agriocnemis minima* (Thailand)	0.11
5	KT957462	*Agriocnemis minima* (Thailand)	0.11
6	KT957461	*Agriocnemis minima* (Thailand)	0.12
7	KF369578	*Xanthagrion erythroneurum*	0.16
8	KF369446	*Metaloptobasis mauritia*	0.16
9	KF369562	*Teinobasis rufithorax*	0.17
10	KU220894	*Rhodischnura nursei*	0.17
11	KT879911	*Lestes elatus*	0.17
12	KU566458	*Trithemis werneri*	0.16

Figure 6: *Agriocnemis keralensis*

> KU135367 *Agriocnemis keralensis* |cytochrome oxidase subunit I gene |voucher CUAK-01-A1 partial cds, mitochondrial|628bp

CGGAGCATGGGCAGGAATAGTAGGAACTGCCCTAAGTATATTAATTCGAGTAGAACTTGGACAACCAGGATCTCTA
ATTGGAGATGATCAAATCTACAATGTAGTAGTGACTGCGCACGCTTTTGTAATAATTTTTTTCATAGTAATACCAA
TTATGATTGGAGGGTTTGGAAATTGACTCGTACCCTTAATACTAGGAGCACCAGACATAGCTTTCCCACGACTTAA
TAACATAAGATTTTGACTATTACCCCCTTCATTAACATTATTGATAGCAAGATCCCTAGTAGAAAGAGGGGCCGGT
ACTGGATGAACAGTCTATCCTCCTTTAGCAGGAGCCATTGCTCATGCAGGAGGGTCAGTAGACCTTACAATTTTTT
CACTACATTTAGCAGGAGTTTCATCCATCTTAGGGGCAATCAACTTTATTACAACTACAATTAATATGAAATCCCC
TGGTATAAAAATAGAACAAATACCTCTATTTGTATGGGCTGTAGTAATTACAGCAATCCTACTTCTATTATCATTA
CCTGTATTAGCAGGTGCAATTACTATACTATTAACAGACCGTAATATTAATACATCATTTTTTGATCCTGCAGGGG
GAGGAGACCCAGTACTATAC

Figure 6a: The DNA sequence interpret of COI gene of *Agriocnemis keralensis*

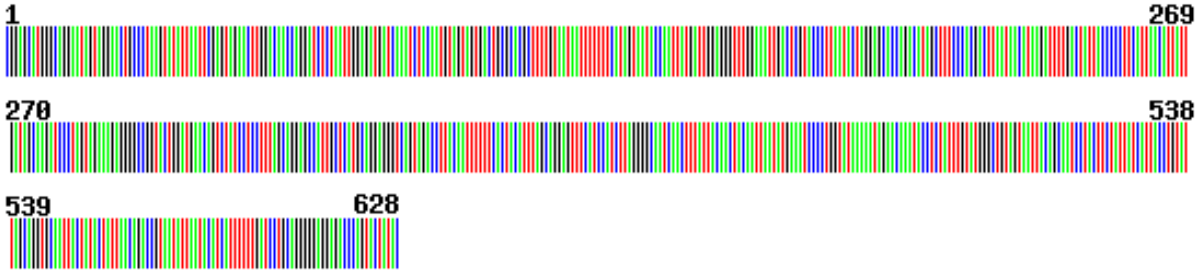

Figure 6b: Representative molecular barcode of COI gene of *Agriocnemis keralensis*

> ALQ75278 *Agriocnemis keralensis* |cytochrome oxidase subunit I gene |voucher CUAK-01-A1 partial cds, mitochondrial|209 bp

GAWAGMVGTALSMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLN
NMSFWLLPPSLTLLMASSLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIFSLHLAGVSSILGAINFITTTINMKSP
GMKMEQMPLFVWAVVITAILLLLSLPVLAGAITMLLTDRNINTSFFDPAGGGDPVLY

Figure 6c: The conceptual translation product of the COI gene of *Agriocnemis keralensis*

Agriocnemis keralensis voucher CUAK-01-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID: KU135367 Length: 628Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
1160 bits(628)	0.0()	628/628(100%)	0/628(0%)	Plus/Plus

```
Features:
Query  1    CGGAGCATGGGCAGGAATAGTAGGAACTGCCCTAAGTATATTAATTCGAGTAGAACTTGG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    CGGAGCATGGGCAGGAATAGTAGGAACTGCCCTAAGTATATTAATTCGAGTAGAACTTGG  60

Query  61   ACAACCAGGATCTCTAATTGGAGATGATCAAATCTACAATGTAGTAGTGACTGCGCACGC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   ACAACCAGGATCTCTAATTGGAGATGATCAAATCTACAATGTAGTAGTGACTGCGCACGC  120

Query  121TTTTGTAATAAttttttCATAGTAATACCAATTATGATTGGAGGGTTTGGAAATTGACT  180
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Sbjct  121  TTTTGTAATAATTTTTTTCATAGTAATACCAATTATGATTGGAGGGTTTGGAAATTGACT  180

Query  181  CGTACCCTTAATACTAGGAGCACCAGACATAGCTTTCCCACGACTTAATAACATAAGATT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CGTACCCTTAATACTAGGAGCACCAGACATAGCTTTCCCACGACTTAATAACATAAGATT  240

Query  241  TTGACTATTACCCCCTTCATTAACATTATTGATAGCAAGATCCCTAGTAGAAAGAGGGGC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  TTGACTATTACCCCCTTCATTAACATTATTGATAGCAAGATCCCTAGTAGAAAGAGGGGC  300

Query  301  CGGTACTGGATGAACAGTCTATCCTCCTTTAGCAGGAGCCATTGCTCATGCAGGAGGGTC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  CGGTACTGGATGAACAGTCTATCCTCCTTTAGCAGGAGCCATTGCTCATGCAGGAGGGTC  360

Query  361  AGTAGACCTTACAATTTTTTCACTACATTTAGCAGGAGTTTCATCCATCTTAGGGGCAAT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  AGTAGACCTTACAATTTTTTCACTACATTTAGCAGGAGTTTCATCCATCTTAGGGGCAAT  420

Query  421  CAACTTTATTACAACTACAATTAATATGAAATCCCCTGGTATAAAAATAGAACAAATACC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  CAACTTTATTACAACTACAATTAATATGAAATCCCCTGGTATAAAAATAGAACAAATACC  480

Query  481  TCTATTTGTATGGGCTGTAGTAATTACAGCAATCCTACTTCTATTATCATTACCTGTATT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  TCTATTTGTATGGGCTGTAGTAATTACAGCAATCCTACTTCTATTATCATTACCTGTATT  540

Query  541  AGCAGGTGCAATTACTATACTATTAACAGACCGTAATATTAATACATCATTTTTTGATCC  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  AGCAGGTGCAATTACTATACTATTAACAGACCGTAATATTAATACATCATTTTTTGATCC  600

Query  601  TGCAGGGGGAGGAGACCCAGTACTATAC  628
            |||||||||||||||||||||||||||
Sbjct  601  TGCAGGGGGAGGAGACCCAGTACTATAC  628
```

Figure 6d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Agriocnemis keralensis* showing its nearest match subject

Cytochrome oxidase subunit I, partial (mitochondrion) [*Agriocnemis keralensis*]
Sequence ID: ALQ75278 Length: 209Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps
408 bits(1048)	8e-144()	Compositional matrix adjust.	209/209(100%)	209/209(100%)	0/209(0%)

```
Features:
Query  1    GAWAGMVGTALSMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWL  60
            GAWAGMVGTALSMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWL
Sbjct  1    GAWAGMVGTALSMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWL  60

Query  61   VPLMLGAPDMAFPRLNNMSFWLLPPSLTLLMASSLVESGAGTGWTVYPPLAGAIAHAGGS  120
            VPLMLGAPDMAFPRLNNMSFWLLPPSLTLLMASSLVESGAGTGWTVYPPLAGAIAHAGGS
Sbjct  61   VPLMLGAPDMAFPRLNNMSFWLLPPSLTLLMASSLVESGAGTGWTVYPPLAGAIAHAGGS  120

Query  121  VDLTIFSLHLAGVSSILGAINFITTTINMKSPGMKMEQMPLFVWAVVITAILLLLSLPVL  180
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Sbjct  121  VDLTIFSLHLAGVSSILGAINFITTTINMKSPGMKMEQMPLFVWAVVITAILLLLSLPVL  180

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            AGAITMLLTDRNINTSFFDPAGGGDPVLY
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Figure 6e: Peptide BLAST output of COI gene of *Agriocnemis keralensis.*

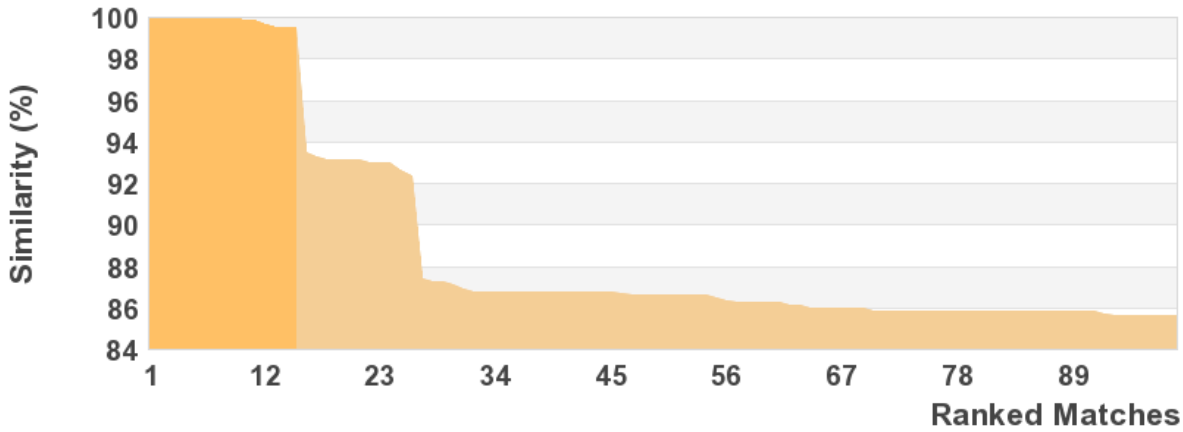

Figure 6f: The line diagram of *Agriocnemis keralensis* over more than 98 % match to other retrieved sequences from BOLD system.

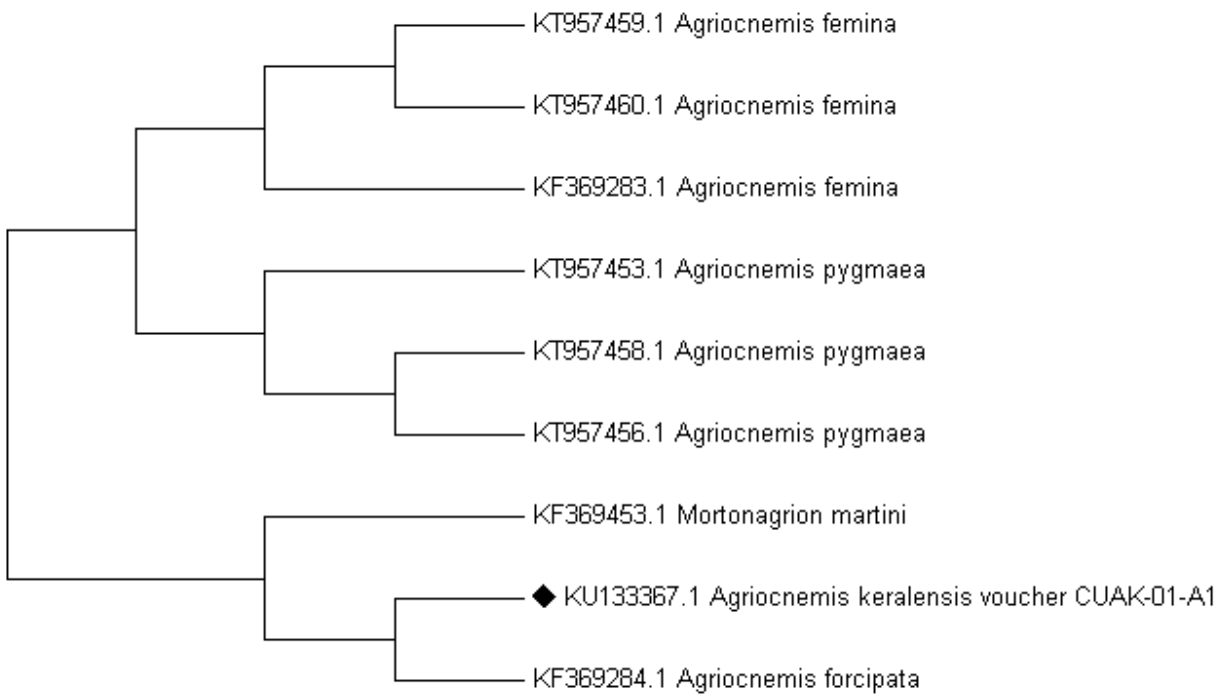

Figure 6g: The molecular phylogenetic tree of *Agriocnemis keralensis* inferred by NJ tree method

Table 8: Percentage of evolutionary divergence of *Agriocnemis keralensis* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KU133367	*Agriocnemis keralensis* (Kerala)	
2	KF369284	*Agriocnemis forcipata* (Netherland)	0.00
3	KT957459	*Agriocnemis femina* (Thailand)	3.83
4	KF369283	*Agriocnemis femina* (Netherland)	3.81
5	KT957460	*Agriocnemis femina* (Thailand)	4.18
6	KT957458	*Agriocnemis pygmaea*	4.44
7	KT957456	*Agriocnemis pygmaea*	4.44
8	KT957456	*Agriocnemis pygmaea*	4.43

Figure 7: *Ischnura aurora*

> KR149808 *Ischnura aurora* |cytochrome oxidase subunit I gene |voucher CUIA 01-A1 partial cds, mitochondrial| 628bp

AATGTTTGGAGCATGGGCTGGAATAGTAGGAACTGCTTTAAGAATATTAATTCGAGTTGAACTAGGACAACCAGGA
TCTCTTATTGGAGATGACCAAATTTATAATGTAGTAGTAACTGCACACGCTTTTGTTATAATTTTTTTTATAGTAA
TACCTATTATAATTGGAGGGTTCGGAAATTGATTAGTACCTTTAATATTAGGAGCACCAGATATAGCTTTCCCTCG
ATTAAATAATATAAGATTCTGACTTCTACCACCATCATTAACATTATTACTAGCAAGTAGTTTAGTAGAAAGAGGA
GCTGGAACGGGATGAACTGTTTACCCTCCACTAGCAGGTGTTATTGCTCACGCTGGAGCTTCTGTTGATTTAACAA
TTTTCTCTTTACACTTAGCAGGAGTATCTTCTATTTTAGGTGCAATTAATTTCATTACCACCACAATTAATATAAA
GTCACCAGGAATAAATATAGACCAATTACCTTTATTTGTATGAGCTGTAGTTATTACAGCGGTATTACTTTTATTA
TCATTACCAGTTCTGGCTGGTGCTATTACTATACTTTTAACTGATCGTAATATTAATACGTCCTTCTTTGATCCGG
CAGGAGGAGGAGACCCTATT

Fig 7a: The DNA sequence interpret of the COI gene of *Ischnura aurora.*

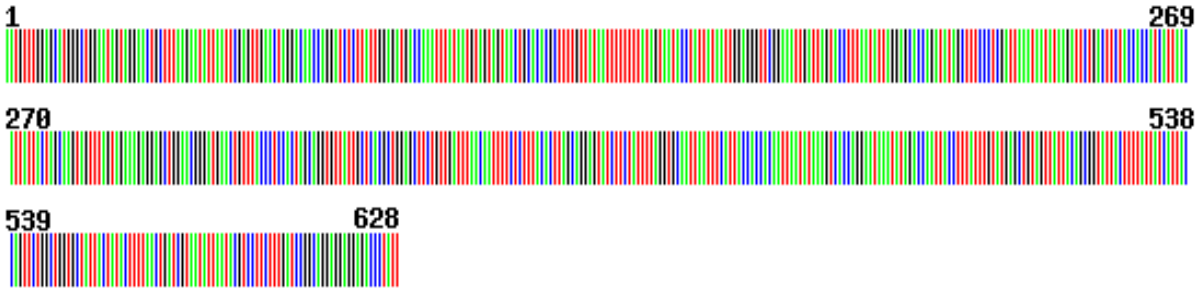

Figure 7b: Representative molecular barcode of the COI gene of *Ischnura aurora.*

> AKL82322 *Ischnura aurora* |cytochrome oxidase subunit I gene |voucher CUIA 01-A1 partial cds, mitochondrial|209 bp

MFGAWAGMVGTALSMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPR
LNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGVIAHAGASVDLTIFSLHLAGVSSILGAINFITTTINMK
SPGMNMDQLPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAGGGDPI

Figure 7c : The conceptual translation product of the COI gene of *Ischnura aurora*

Ischnura aurora voucher CUIA 01-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID KR149808 Length: 628Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand	Frame
1160 bits(628)	0.0()	628/628(100%)	0/628(0%)	Plus/Plus	

Features:

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           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    AATGTTTGGAGCATGGGCTGGAATAGTAGGAACTGCTTTAAGAATATTAATTCGAGTTGA  60

Query  61   ACTAGGACAACCAGGATCTCTTATTGGAGATGACCAAATTTATAATGTAGTAGTAACTGC  120
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   ACTAGGACAACCAGGATCTCTTATTGGAGATGACCAAATTTATAATGTAGTAGTAACTGC  120

Query  121  ACACGCTTTTGTTATAAtttttttttATAGTAATACCTATTATAATTGGAGGGTTCGGAAA  180
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  ACACGCTTTTGTTATAATTTTTTTTTATAGTAATACCTATTATAATTGGAGGGTTCGGAAA  180

Query  181  TTGATTAGTACCTTTAATATTAGGAGCACCAGATATAGCTTTCCCTCGATTAAATAATAT  240
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Sbjct  181  TTGATTAGTACCTTTAATATTAGGAGCACCAGATATAGCTTTCCCTCGATTAAATAATAT  240

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Sbjct  241  AAGATTCTGACTTCTACCACCATCATTAACATTATTACTAGCAAGTAGTTTAGTAGAAAG  300

Query  301  AGGAGCTGGAACGGGATGAACTGTTTACCCTCCACTAGCAGGTGTTATTGCTCACGCTGG  360
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Sbjct  361  AGCTTCTGTTGATTTAACAATTTTCTCTTTACACTTAGCAGGAGTATCTTCTATTTTAGG  420

Query  421  TGCAATTAATTTCATTACCACCACAATTAATATAAAGTCACCAGGAATAAATATAGACCA  480
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Sbjct  421  TGCAATTAATTTCATTACCACCACAATTAATATAAAGTCACCAGGAATAAATATAGACCA  480

Query  481  ATTACCTTTATTTGTATGAGCTGTAGTTATTACAGCGGTATTACTTTTATTATCATTACC  540
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  ATTACCTTTATTTGTATGAGCTGTAGTTATTACAGCGGTATTACTTTTATTATCATTACC  540

Query  541  AGTTCTGGCTGGTGCTATTACTATACTTTTAACTGATCGTAATATTAATACGTCCTTCTT  600
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  AGTTCTGGCTGGTGCTATTACTATACTTTTAACTGATCGTAATATTAATACGTCCTTCTT  600

Query  601  TGATCCGGCAGGAGGAGGAGACCCTATT  628
           ||||||||||||||||||||||||||||
Sbjct  601  TGATCCGGCAGGAGGAGGAGACCCTATT  628
```

Figure 7d: Nucleotide BLAST output of COI gene of *Ischnura aurora* showing its nearest match subject

Cytochrome oxidase subunit 1, partial (mitochondrion) [*Ischnura aurora*]
Sequence ID: AGY95143 Length: 219Number of Matches: 1

Score	Expect	Method	Identities	Positives	Gaps	Frame
405 bits(1042)	9e-143()	Compositional matrix adjust.	209/209(100%)	209/209(100%)	0/209(0%)	

Features:

```
Query  1    MFGAWAGMVGTALSMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGN  60
            MFGAWAGMVGTALSMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGN
Sbjct  5    MFGAWAGMVGTALSMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGN  64

Query  61   WLVPLMLGAPDMAFPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGVIAHAG  120
            WLVPLMLGAPDMAFPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGVIAHAG
Sbjct  65   WLVPLMLGAPDMAFPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGVIAHAG  124

Query  121  ASVDLTIFSLHLAGVSSILGAINFITTTINMKSPGMNMDQLPLFVWAVVITAVLLLLSLP  180
            ASVDLTIFSLHLAGVSSILGAINFITTTINMKSPGMNMDQLPLFVWAVVITAVLLLLSLP
Sbjct  125  ASVDLTIFSLHLAGVSSILGAINFITTTINMKSPGMNMDQLPLFVWAVVITAVLLLLSLP  184

Query  181  VLAGAITMLLTDRNINTSFFDPAGGGDPI  209
            VLAGAITMLLTDRNINTSFFDPAGGGDPI
Sbjct  185  VLAGAITMLLTDRNINTSFFDPAGGGDPI  213
```

Figure 7e: Peptide BLAST output of the mt DNA COI gene of *Ischnura aurora*

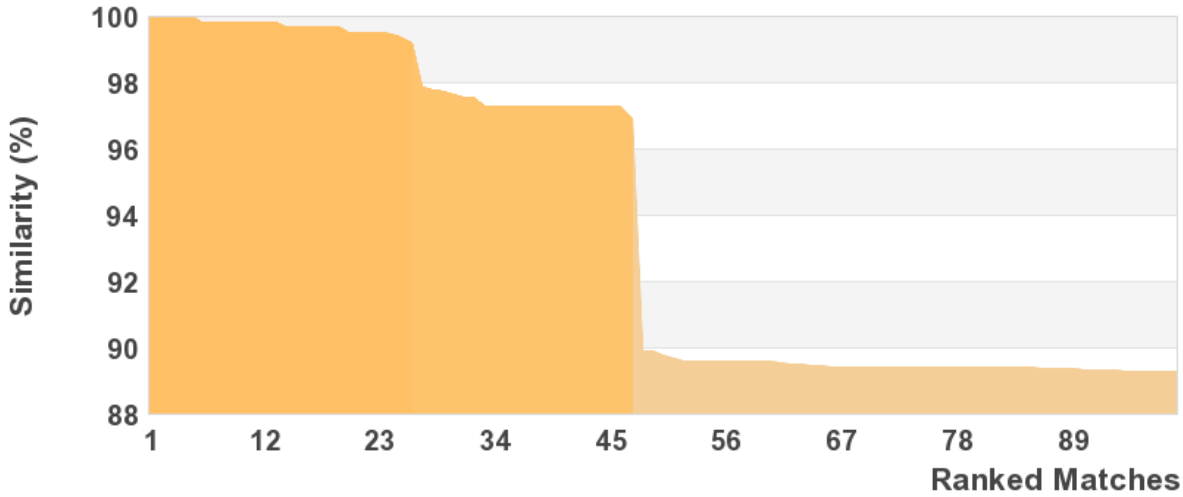

Figure 7f: The line diagram of *Ischnura aurora* with more than 98 % match to other retrieved sequences (BOLD SYSTEM)

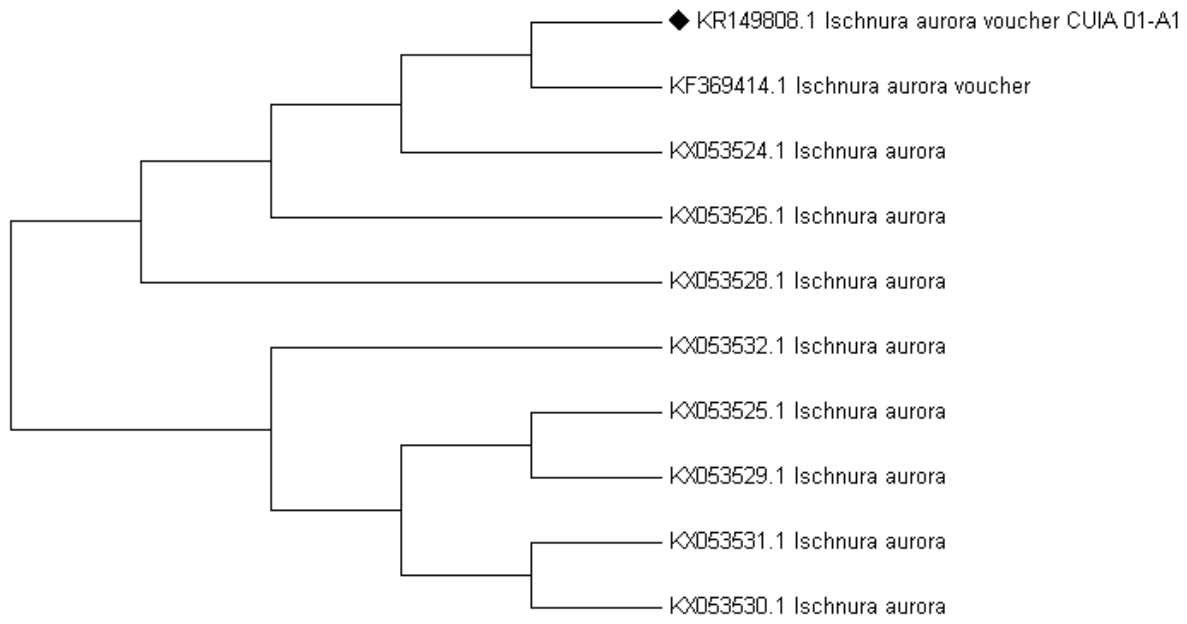

Figure 7g: Molecular Phylogenetic tree of *Ischnura aurora* inferred by NJ tree method

Table 10: Percentage of evolutionary divergence of *Ischnura aurora* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KR149808	*Ischnura aurora* (Kerala)	0.00
2	KF369414	*Ischnura aurora* (Netherland)	0.00
3	KX053528	*Ischnura aurora* (France)	0.16
4	KX053526	*Ischnura aurora* (France)	0.16
5	KX053529	*Ischnura aurora* (France)	0.16
6	KX053530	*Ischnura aurora* (France)	0.16
7	KX053524	*Ischnura aurora* (France)	0.16
8	KX053532	*Ischnura aurora* (France)	0.16
9	KX053525	*Ischnura aurora* (France)	0.32
10	KX053531	*Ischnura aurora* (France)	0.32

Figure 8 : *Ischnura senegalensis*

> KT305961 *Ischnura senegalensis* |cytochrome oxidase subunit I gene |voucher CUSI 01-A1 partial cds, mitochondrial|603 bp

ACCAGATATAGCTTTCCCCCGATTAAATAATATAAGATTTTGACTTCTACCTCCCTCATTAACTTTACTTTTAGCA
AGAAGCTTAGTAGAAAGAGGAGCGGGAACTGGATGAACAGTTTATCCTCCACTAGCAGGGGTAATTGCTCATGCTG
GAGCGTCCGTTGACTTAACTATTTTTTCATTACACTTGGCAGGAGTATCCTCAATTTTAGGAGCAATTAATTTTAT
TACCACTACAATTAATATAAAGTCTCCTGGGATAAATATAGACCAACTACCTCTATTTGTCTGAGCTGTAGTTATT
ACTGCAGTATTACTTTTATTATCACTACCAGTATTAGCTGGTGCTATTACTATATTACTGACAGATCGTAACATCA
ATACATCATTTTTTGACCCTGCAGGAGGGGGAGACCCTATTCTATATCAACATTTATTTTGATTCTTTGGCCACCC
CGAAGTGTACATTTTAATTTTACCAGGATTTGGTATAATTTCACATATTATTGCACAAGAAAGAGGTAAAAAGGAA
ACATTTGGAGTACTAGGTATAATTTATGCTATAATTGCAATTGGAATTCTAGGATTTGTAGTATGGGCCCA

Figure 8a: The DNA sequence interpret of the COI gene of *Ischnura senegalensis*

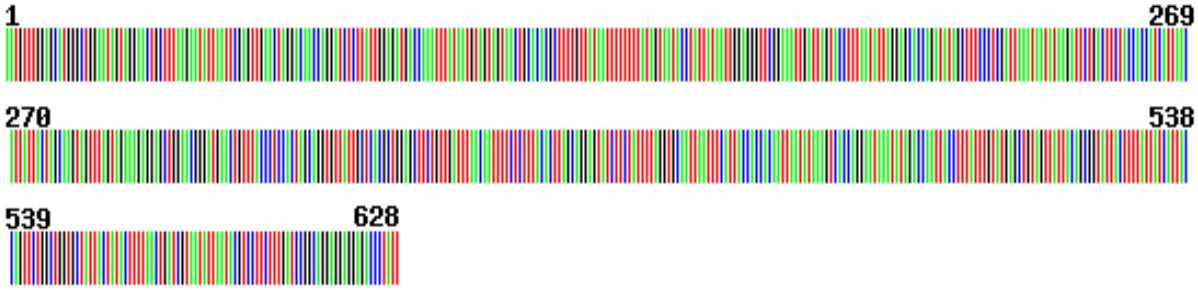

Figure 8b: The representative molecular barcode of COI gene of *Ischnura senegalensis.*

> ALT31504 *Ischnura senegalensis* |cytochrome oxidase subunit I gene |voucher CUSI 01-A1 partial cds, mitochondrial| 200 bp

PDMAFPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGVIAHAGASVDLTIFSLHLAGVSSILGAINFI
TTTINMKSPGMNMDQLPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAGGGDPILYQHLFWFFGHP
EVYILILPGFGMISHIIAQESGKKETFGVLGMIYAMIAIGILGFVVWA

Figure 8c: The conceptual translation product of the COI gene of *Ischnura senegalensis*

Ischnura senegalensis voucher CUSI 01-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID: KT305961 Length: 603Number of Matches: 1

```
             Alignment statistics for match #1
    Score      Expect  Identities     Gaps     Strand  Frame
1114 bits(603) 0.0()  603/603(100%) 0/603(0%) Plus/Plus
Features:
Query  1    ACCAGATATAGCTTTCCCCCGATTAAATAATATAAGATTTTGACTTCTACCTCCCTCATT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    ACCAGATATAGCTTTCCCCCGATTAAATAATATAAGATTTTGACTTCTACCTCCCTCATT  60

Query  61   AACTTTACTTTTAGCAAGAAGCTTAGTAGAAAGAGGAGCGGGAACTGGATGAACAGTTTA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   AACTTTACTTTTAGCAAGAAGCTTAGTAGAAAGAGGAGCGGGAACTGGATGAACAGTTTA  120

Query  121  TCCTCCACTAGCAGGGGTAATTGCTCATGCTGGAGCGTCCGTTGACTTAACTATTTTTTC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  TCCTCCACTAGCAGGGGTAATTGCTCATGCTGGAGCGTCCGTTGACTTAACTATTTTTTC  180

Query  181  ATTACACTTGGCAGGAGTATCCTCAATTTTAGGAGCAATTAATTTTATTACCACTACAAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  ATTACACTTGGCAGGAGTATCCTCAATTTTAGGAGCAATTAATTTTATTACCACTACAAT  240

Query  241  TAATATAAAGTCTCCTGGGATAAATATAGACCAACTACCTCTATTTGTCTGAGCTGTAGT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  TAATATAAAGTCTCCTGGGATAAATATAGACCAACTACCTCTATTTGTCTGAGCTGTAGT  300

Query  301  TATTACTGCAGTATTACTTTTATTATCACTACCAGTATTAGCTGGTGCTATTACTATATT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  TATTACTGCAGTATTACTTTTATTATCACTACCAGTATTAGCTGGTGCTATTACTATATT  360

Query  361  ACTGACAGATCGTAACATCAATACATCATTTTTTGACCCTGCAGGAGGGGGAGACCCTAT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  ACTGACAGATCGTAACATCAATACATCATTTTTTGACCCTGCAGGAGGGGGAGACCCTAT  420

Query  421  TCTATATCAACATTTATTTTGATTCTTTGGCCACCCCGAAGTGTACATTTTAATTTTACC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  TCTATATCAACATTTATTTTGATTCTTTGGCCACCCCGAAGTGTACATTTTAATTTTACC  480

Query  481  AGGATTTGGTATAATTTCACATATTATTGCACAAGAAAGAGGTAAAAAGGAAACATTTGG  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  AGGATTTGGTATAATTTCACATATTATTGCACAAGAAAGAGGTAAAAAGGAAACATTTGG  540

Query  541  AGTACTAGGTATAATTTATGCTATAATTGCAATTGGAATTCTAGGATTTGTAGTATGGGC  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  AGTACTAGGTATAATTTATGCTATAATTGCAATTGGAATTCTAGGATTTGTAGTATGGGC  600

Query  601  CCA  603
            |||
Sbjct  601  CCA  603
```

Figure 8d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Ischnura senegalensis* showing its nearest match subject

Cytochrome oxidase subunit I, partial (mitochondrion) [*Ischnura senegalensis*]
Sequence ID: ALT31504 Length: 200Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps	Frame
393 bits(1010)	2e-138()	Compositional matrix adjust.	200/200(100%)	200/200(100%)	0/200(0%)	

Features:

```
Query  1    PDMAFPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGVIAHAGASVDLTIFS  60
            PDMAFPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGVIAHAGASVDLTIFS
Sbjct  1    PDMAFPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGVIAHAGASVDLTIFS  60

Query  61   LHLAGVSSILGAINFITTTINMKSPGMNMDQLPLFVWAVVITAVLLLLSLPVLAGAITML  120
            LHLAGVSSILGAINFITTTINMKSPGMNMDQLPLFVWAVVITAVLLLLSLPVLAGAITML
Sbjct  61   LHLAGVSSILGAINFITTTINMKSPGMNMDQLPLFVWAVVITAVLLLLSLPVLAGAITML  120

Query  121  LTDRNINTSFFDPAGGGDPILYQHLFWFFGHPEVYILILPGFGMISHIIAQESGKKETFG  180
            LTDRNINTSFFDPAGGGDPILYQHLFWFFGHPEVYILILPGFGMISHIIAQESGKKETFG
Sbjct  121  LTDRNINTSFFDPAGGGDPILYQHLFWFFGHPEVYILILPGFGMISHIIAQESGKKETFG  180

Query  181  VLGMIYAMIAIGILGFVVWA  200
            VLGMIYAMIAIGILGFVVWA
Sbjct  181  VLGMIYAMIAIGILGFVVWA  200
```

Figure 8e: Peptide BLAST output of the COI gene of *Ischnura senegalensis*

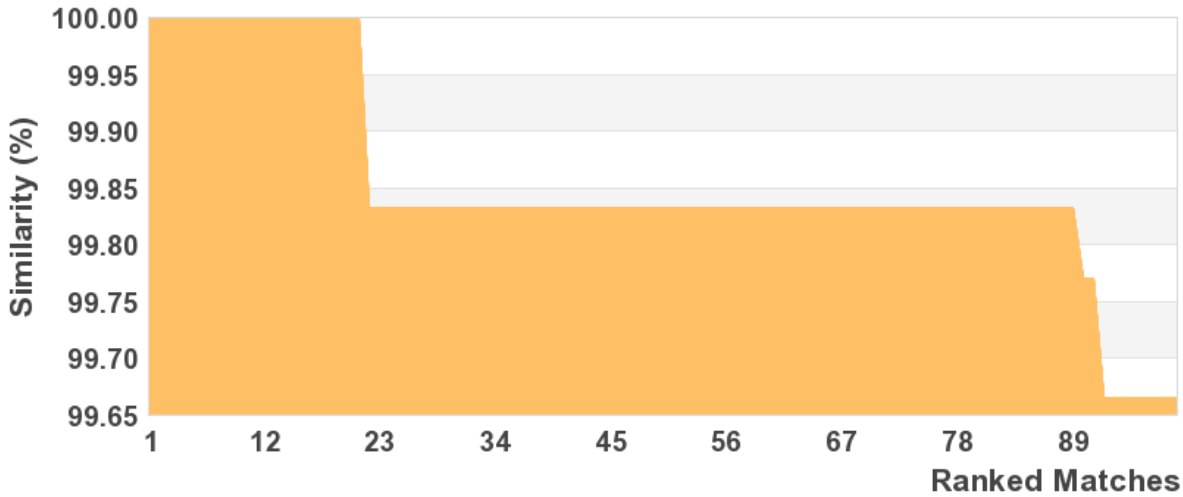

Figure 8f: The line diagram of *Ischnura senegalensis* with more than 99 % match to other retrieved sequences (BOLD SYSTEM)

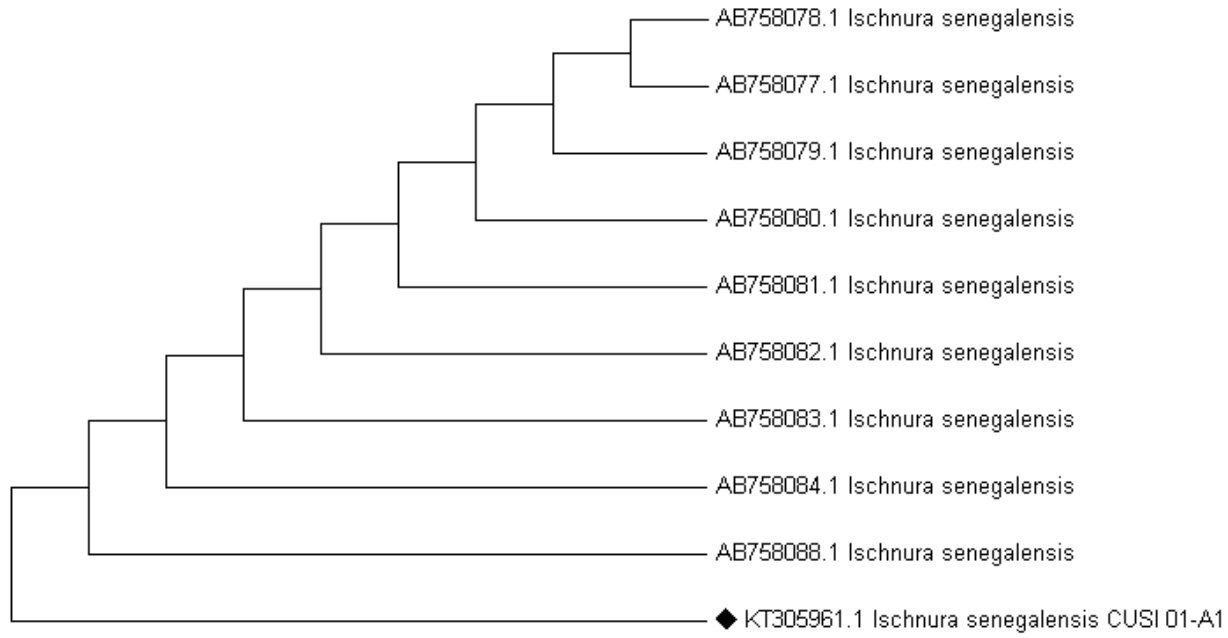

Figure 8g: Phylogenetic relationship of *Ischnura senegalensis* inferred by NJ tree method

Table 12: Percentage of evolutionary divergence of *Ishnura senegalensis* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KT30596	*Ischnura senegalensis* (Kerala)	
2	AB758088	*Ischnura senegalensis* (Japan)	0.00
3	AB758084	*Ischnura senegalensis* (Japan)	0.00
4	AB758081	*Ischnura senegalensis* (Japan)	0.00
5	AB758080	*Ischnura senegalensis* (Japan)	0.00
6	AB758079	*Ischnura senegalensis* (Japan)	0.00
7	AB758078	*Ischnura senegalensis* (Japan)	0.00
8	AB758077	*Ischnura senegalensis* (Japan)	0.00
9	AB758082	*Ischnura senegalensis* (Japan)	0.00

Figure 9: *Aciagrion occidentale*

>KM096996 *Aciagrion occidentale* voucher JO5 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

```
ATTGGAGATGACCAAATTTATAATGTAGTAGTAACTGCGCATGCATTTGTTATAATTTTCTTCATAGTTATACCCA
TCATAATTGGGGGATTTGGAAACTGGCTGGTTCCATTAATGTTAGGTGCACCAGATATTGCTTTCCCTCGATTAAA
TAATATAAGATTTTGACTTCTACCACCATCCTTAACACTTCTATTAGCAAGAAGATTAGTAGAAAGAGGGGCCGGA
ACTGGTTGGACTGTCTACCCCCCATTGGCAGGAGTAATTGCCCATGCTGGAGCATCAGTAGATTTAACTATTTTCT
CTTTACATTTAGCAGGGGTATCCTCAATTTTAGGGGCTATTAATTTCATCACAACCACTATTAATATAAAATCTCC
GGGTATAAGTATAGATCAAATACCATTATTTGTGTGAGCTGTAGTTATTACAGCAGTTTTATTATTATTATCATTA
CCTGTATTGGCAGGTGCCATCACTATGTTATTAACTGATCGAAATAGTAATACATTGGGGACTCGG
```

Figure 9a: The DNA sequence interpret of COI gene of *Aciagrion occidentale*

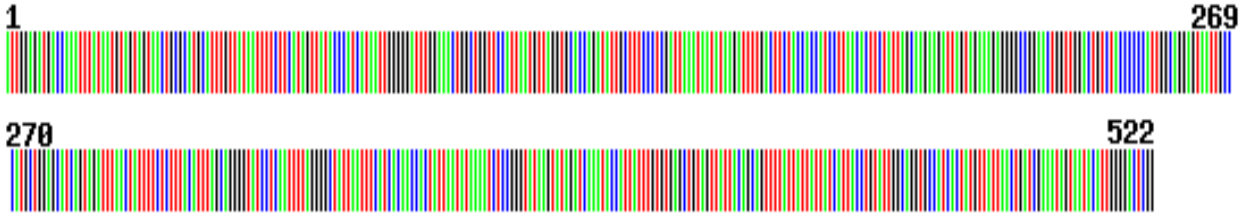

Figure 9b: Representative molecular barcode of COI gene of *Aciagrion occidentale.*

> AIT71755 *Aciagrion occidentale* |cytochrome oxidase subunit I gene |voucher CUAC-01-A1 partial cds, mitochondrial|174 bp

```
IGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDIAFPRLNNMSFWLLPPSLTLLLASSLVESGAG
TGWTVYPPLAGVIAHAGASVDLTIFSLHLAGVSSILGAINFITTTINMKSPGMSMDQMPLFVWAVVITAVLLLLSL
PVLAGAITMLLTDRNSNTLGTR
```

Figure 9c: The conceptual translation product of the COI gene of *Aciagrion occidentale*

Aciagrion occidentale voucher JO 5 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

Sequence ID: KM096996.1Length: 522Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
965 bits(522)	0.0	522/522(100%)	0/522(0%)	Plus/Plus

```
Query  1    ATTGGAGATGACCAAATTTATAATGTAGTAGTAACTGCGCATGCATTTGTTATAATTTTC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    ATTGGAGATGACCAAATTTATAATGTAGTAGTAACTGCGCATGCATTTGTTATAATTTTC  60

Query  61   TTCATAGTTATACCCATCATAATTGGGGGATTTGGAAACTGGCTGGTTCCATTAATGTTA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   TTCATAGTTATACCCATCATAATTGGGGGATTTGGAAACTGGCTGGTTCCATTAATGTTA  120

Query  121  GGTGCACCAGATATTGCTTTCCCTCGATTAAATAATATAAGATTTTGACTTCTACCACCA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  GGTGCACCAGATATTGCTTTCCCTCGATTAAATAATATAAGATTTTGACTTCTACCACCA  180

Query  181  TCCTTAACACTTCTATTAGCAAGAAGATTAGTAGAAAGAGGGGCCGGAACTGGTTGGACT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  TCCTTAACACTTCTATTAGCAAGAAGATTAGTAGAAAGAGGGGCCGGAACTGGTTGGACT  240

Query  241  GTCTACCCCCCATTGGCAGGAGTAATTGCCCATGCTGGAGCATCAGTAGATTTAACTATT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  GTCTACCCCCCATTGGCAGGAGTAATTGCCCATGCTGGAGCATCAGTAGATTTAACTATT  300

Query  301  TTCTCTTTACATTTAGCAGGGGTATCCTCAATTTTAGGGGCTATTAATTTCATCACAACC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  TTCTCTTTACATTTAGCAGGGGTATCCTCAATTTTAGGGGCTATTAATTTCATCACAACC  360

Query  361  ACTATTAATATAAAATCTCCGGGTATAAGTATAGATCAAATACCATTATTTGTGTGAGCT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  ACTATTAATATAAAATCTCCGGGTATAAGTATAGATCAAATACCATTATTTGTGTGAGCT  420

Query  421  GTAGTTATTACAGCAGTTTTATTATTATTATCATTACCTGTATTGGCAGGTGCCATCACT  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  GTAGTTATTACAGCAGTTTTATTATTATTATCATTACCTGTATTGGCAGGTGCCATCACT  480

Query  481  ATGTTATTAACTGATCGAAATAGTAATACATTGGGGACTCGG  522
            ||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  ATGTTATTAACTGATCGAAATAGTAATACATTGGGGACTCGG  522
```

Figure 9d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Aciagrion occidentale* showing its nearest match subject

Sequence ID: AIT71755.1Length: 174Number of Matches: 1

Score	Expect	Method	Identities	Positives	Gaps
358 bits(867)	2e-117	Compositional matrix adjust.	174/174(60%)	174/174(60%)	0/174(0%)

```
Query  1    IGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDIAFPRLNNMSFWLLPP  60
            IGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDIAFPRLNNMSFWLLPP
Sbjct  1    IGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDIAFPRLNNMSFWLLPP  60

Query  61   SLTLLLASSLVESGAGTGWTVYPPLAGVIAHAGASVDLTIFSLHLAGVSSILGAINFITT  120
            SLTLLLASSLVESGAGTGWTVYPPLAGVIAHAGASVDLTIFSLHLAGVSSILGAINFITT
Sbjct  61   SLTLLLASSLVESGAGTGWTVYPPLAGVIAHAGASVDLTIFSLHLAGVSSILGAINFITT  120

Query  121  TINMKSPGMSMDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNSNTLGTR  174
            TINMKSPGMSMDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNSNTLGTR
Sbjct  121  TINMKSPGMSMDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNSNTLGTR  174
```

Figure 9e: Peptide BLAST output of COI gene of *Aciagrion occidentale.*

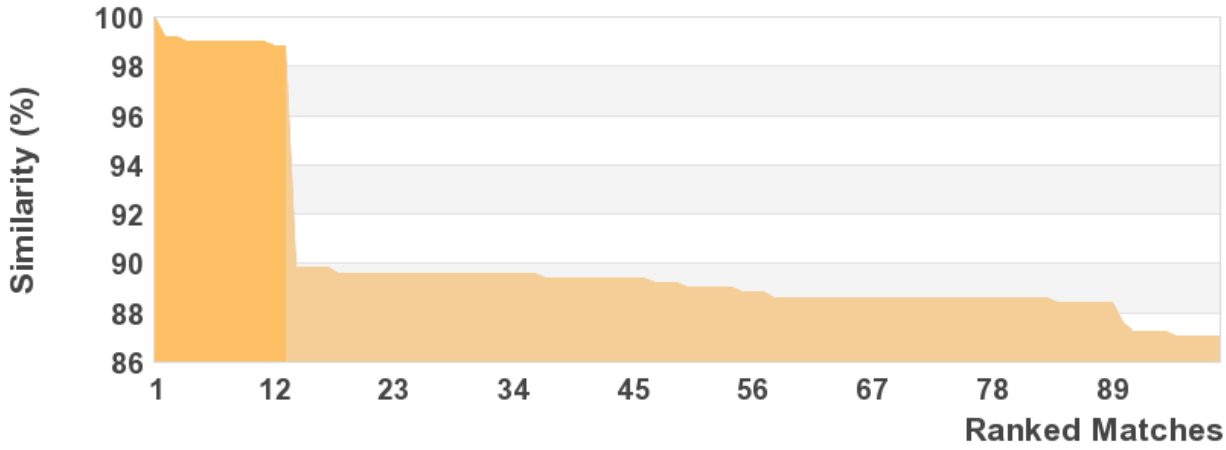

Figure 9f: The line diagram of *Aciagrion occidentale* over more than 98 % match to other retrieved sequences (BOLD SYSTEM)

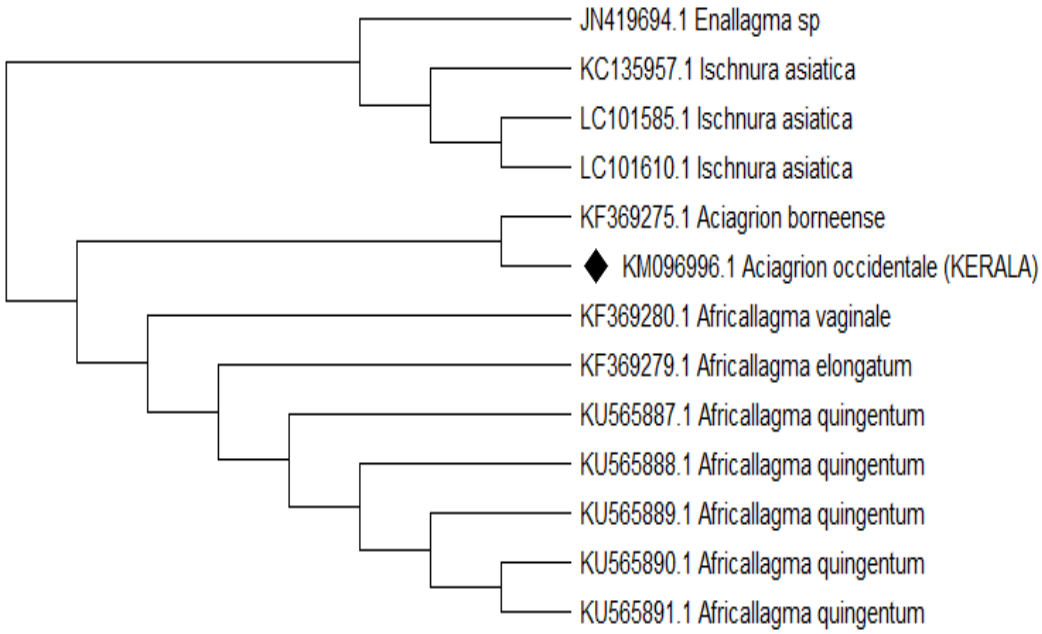

Figure 9g: Molecular phylogenetic relationship of *Aciagrion occidentale* inferred by NJ tree method

Table 14: Percentage of evolutionary divergence of *Aciagrion occidentale* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KM096996	*Aciagrion occidentale* (Kerala)	
2	KF369275	*Aciagrion borneense*	0.12
3	JN419694	*Enallagma* sp	0.12
4	KC135957	*Ischnura asiatica*	0.12
5	KF369279	*Africallagma elongatum*	O.13
6	KF369280	*Africallagma vaginale*	0.14
7	KU565887.	*Africallagma quingentum*	0.11
8	KU565888	*Africallagma quingentum*	0.11
9	KU565889.	*Africallagma quingentum*	0.11
10	KU565890	*Africallagma quingentum*	0.11
11	KU565891	*Africallagma quingentum*	0.11
12	LC101585	*Ischnura asiatica*	0.13
13	LC101610.	*Ischnura asiatica*	0.12

Fig 10: *Copera marginipes*

> KR149804 *Copera marginipes* |cytochrome oxidase subunit I gene |voucher CUCM 01-A1 partial cds, mitochondrial| 616 bp

AGCTGGAATAGTAGGAACAGCTTTAAGAATATTAATTCGAATTGAATTAGGACAACCAGGGTCATTAATCGGAGAT
GATCAAATTTATAACGTTGTGGTTACAGCACACGCTTTCGTTATAATTTTTTTTATAGTTATACCTATTATAATTG
GAGGATTTGGTAACTGGCTAGTACCTTTAATACTAGGAGCCCCAGATATAGCATTCCCACGACTTAATAATATAAG
ATTTTGGTTACTACCTCCCTCATTAACTCTTTTACTATCAAGTAGATTAGTAGAAAGAGGGGCGGGTACTGGATGA
ACTGTTTATCCTCCATTAGCTGGAGCTATTGCTCATTCAGGAGGGTCAGTTGATCTAACTATTTTTTCTCTTCATT
TGGCAGGAGTATCATCAATTTTAGGGGCAATTAATTTTATTACTACAACTATTAATATAAAATCACCAGGTATAAA
ATTAGATCAAATACCATTATTTGTATGAGCAGTGGTAATTACAGCAGTGTTACTATTATTATCTTTGCCAGTACTT
GCTGGAGCAATTACAATATTATTAACAGATCGAAATATTAATACATCATTCTTTGATCCAGCAGGTGGAGGGGACC
CAATCCTA

Figure 10a: The DNA sequence interpret of COI gene of *Copera marginipes*

Figure 10b: Representative molecular barcode of the COI gene of *Copera marginipes*

> KR149804 *Copera marginipes* |cytochrome oxidase subunit I gene |voucher CUCM 01-A1 partial cds, mitochondrial| 616 bp

AGMVGTALSMLIRIELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMS
FWLLPPSLTLLLSSSLVESGAGTGWTVYPPLAGAIAHSGGSVDLTIFSLHLAGVSSILGAINFITTTINMKSPGMK
LDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAGGGDPIL

Figure 10c: The conceptual translation product of the COI gene of *Copera marginipes*

Copera marginipes voucher CUCM 01-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID: KR149804. Length: 616Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
1138 bits(616)	0.0()	616/616(100%)	0/616(0%)	Plus/Plus

Features:

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Query  1    AGCTGGAATAGTAGGAACAGCTTTAAGAATATTAATTCGAATTGAATTAGGACAACCAGG  60
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Sbjct  1    AGCTGGAATAGTAGGAACAGCTTTAAGAATATTAATTCGAATTGAATTAGGACAACCAGG  60

Query  61   GTCATTAATCGGAGATGATCAAATTTATAACGTTGTGGTTACAGCACACGCTTTCGTTAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GTCATTAATCGGAGATGATCAAATTTATAACGTTGTGGTTACAGCACACGCTTTCGTTAT  120

Query  121  AAtttttttATAGTTATACCTATTATAATTGGAGGATTTGGTAACTGGCTAGTACCTTT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  AATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGTAACTGGCTAGTACCTTT  180

Query  181  AATACTAGGAGCCCCAGATATAGCATTCCCACGACTTAATAATATAAGATTTTGGTTACT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  AATACTAGGAGCCCCAGATATAGCATTCCCACGACTTAATAATATAAGATTTTGGTTACT  240

Query  241  ACCTCCCTCATTAACTCTTTTACTATCAAGTAGATTAGTAGAAAGAGGGGCGGGTACTGG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  ACCTCCCTCATTAACTCTTTTACTATCAAGTAGATTAGTAGAAAGAGGGGCGGGTACTGG  300

Query  301  ATGAACTGTTTATCCTCCATTAGCTGGAGCTATTGCTCATTCAGGAGGGTCAGTTGATCT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  ATGAACTGTTTATCCTCCATTAGCTGGAGCTATTGCTCATTCAGGAGGGTCAGTTGATCT  360

Query  361  AACTATTTTTTCTCTTCATTTGGCAGGAGTATCATCAATTTTAGGGGCAATTAATTTTAT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  AACTATTTTTTCTCTTCATTTGGCAGGAGTATCATCAATTTTAGGGGCAATTAATTTTAT  420

Query  421  TACTACAACTATTAATATAAAATCACCAGGTATAAAATTAGATCAAATACCATTATTTGT  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  TACTACAACTATTAATATAAAATCACCAGGTATAAAATTAGATCAAATACCATTATTTGT  480

Query  481  ATGAGCAGTGGTAATTACAGCAGTGTTACTATTATTATCTTTGCCAGTACTTGCTGGAGC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  ATGAGCAGTGGTAATTACAGCAGTGTTACTATTATTATCTTTGCCAGTACTTGCTGGAGC  540

Query  541  AATTACAATATTATTAACAGATCGAAATATTAATACATCATTCTTTGATCCAGCAGGTGG  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  AATTACAATATTATTAACAGATCGAAATATTAATACATCATTCTTTGATCCAGCAGGTGG  600

Query  601  AGGGGACCCAATCCTA  616
            ||||||||||||||||
Sbjct  601  AGGGGACCCAATCCTA  616
```

Figure 10d: Nucleotide BLAST output of the COI gene of *Copera marginipes* showing its nearest match subject

Cytochrome oxidase subunit I, partial (mitochondrion) [*Copera marginipes*]
Sequence ID: AKL82318 Length: 205Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps
397 bits(1021)	6e-140()	Compositional matrix adjust.	205/205(100%)	205/205(100%)	0/205(0%)

Features:

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Query  1    AGMVGTALSMLIRIELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPL  60
            AGMVGTALSMLIRIELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPL
Sbjct  1    AGMVGTALSMLIRIELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPL  60

Query  61   MLGAPDMAFPRLNNMSFWLLPPSLTLLLSSSLVESGAGTGWTVYPPLAGAIAHSGGSVDL  120
            MLGAPDMAFPRLNNMSFWLLPPSLTLLLSSSLVESGAGTGWTVYPPLAGAIAHSGGSVDL
Sbjct  61   MLGAPDMAFPRLNNMSFWLLPPSLTLLLSSSLVESGAGTGWTVYPPLAGAIAHSGGSVDL  120

Query  121  TIFSLHLAGVSSILGAINFITTTINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGA  180
            TIFSLHLAGVSSILGAINFITTTINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGA
Sbjct  121  TIFSLHLAGVSSILGAINFITTTINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGA  180

Query  181  ITMLLTDRNINTSFFDPAGGGDPIL  205
            ITMLLTDRNINTSFFDPAGGGDPIL
Sbjct  181  ITMLLTDRNINTSFFDPAGGGDPIL  205
```

Figure 10e: The Peptide BLAST output of the mt DNA COI gene of *Copera marginipes*

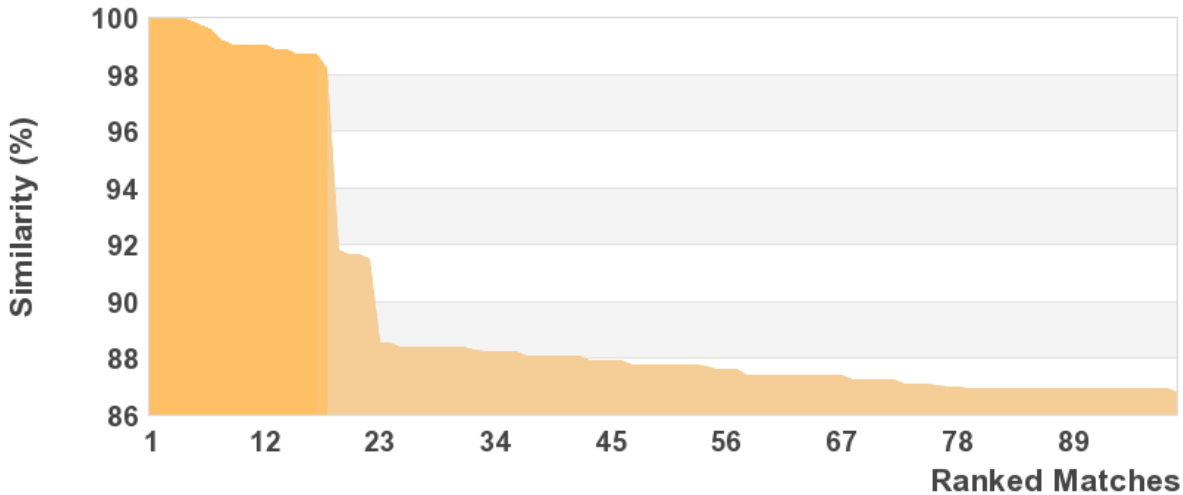

Figure 10f: The line diagram of *Copera marginipes* with more than 99% match to other retrieved sequences (BOLD SYSTEM)

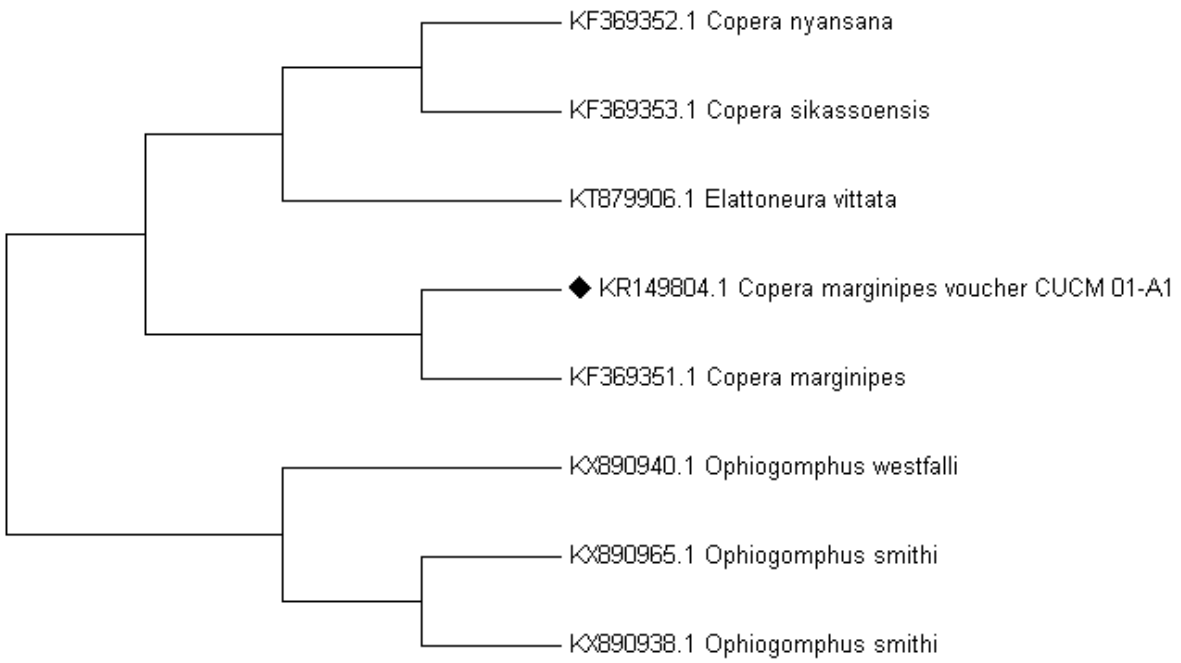

Figure 10g: The molecular phylogenetic tree of *Copera marginipes* inferred by NJ tree method

Table 16: Percentage of evolutionary divergence of *Copera marginipes* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KR149804	*Copera marginipes* (Kerala)	
2	KF369351	*Copera marginipes* (Netherland)	0.00
3	KF369352	*Copera nyansana* (Netherland)	16.02
4	KF369353	*Copera sikassoensis* (Netherland)	17.18
5	KT879906	*Elattoneura vittata*	16.02
6	KX890965	*Ophiogomphus smithi*	18.15
7	KX890938	*Ophiogomphus smithi*	18.15
8	KX890940	*Ophiogomphus westfalli*	18.74

Figure 11: *Vestalis apicalis*

> KU510326 *Vestalis apicalis* |cytochrome oxidase subunit I gene |voucher CUVA-01-A1 partial cds, mitochondrial| 561bp

GAACTAGGACAACCGGGATCCCTTATTGGAGACGACCAAATCTACAACGTAGTAGTCACCGCCCATGCATTTGTAA
TAATCTTTTTTATAGTAATACCTATTATAATTGGGGGATTTGGAAATTGGCTTGTCCCACTAATGTTAGGGGCCCC
TGATATGGCTTTCCCTCGACTAAACAACATGAGATTTTGACTTCTGCCCCCAGCATTAACTCTTCTATTAACAAGA
AGTTTAGTAGAAAGAGGGGCTGGGACAGGTTGAACCGTATACCCTCCTCTAGCGGGGGCTATTGCTCACGCAGGAG
GATCAGTAGATTTAACTATTTTCTCGCTTCACCTAGCAGGCGTATCCTCGATTTTAGGTGCCGTTAATTTCATTAC
TACAACAATTAATATAAAATCCCCTGGAATGAAGGCAGAGCAACTACCATTATTTGTTTGAGCAGTAGTAATTACA
GCCATTTTGTTGCTATTATCATTACCCGTTCTGGCTGGAGCCATCACTATACTTTTAACAGACCGTAACATAAATA
CATCGTTCTTTGACCCTGCTGGGGGGGGG

Figure 11a: The DNA sequence interpret of the COI gene of *Vestalis apicalis*

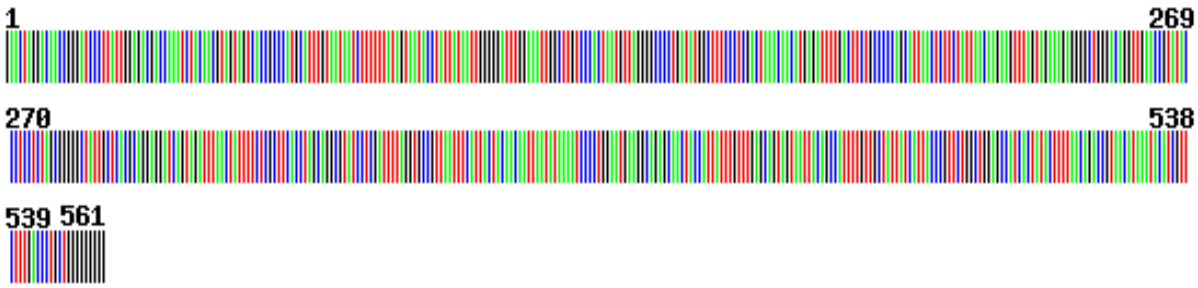

Figure 11b: The representative molecular barcode of the COI gene of *Vestalis apicalis*

> ALX71652 *Vestalis apicalis* |cytochrome oxidase subunit I gene |voucher CUVA-01-A1 partial cds, mitochondrial| 187 bp

ELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPALTLLLTS
SLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIFSLHLAGVSSILGAVNFITTTINMKSPGMKAEQLPLFVWAVVIT
AILLLLSLPVLAGAITMLLTDRNMNTSFFDPAGGG

Figure 11c: The conceptual translation product of the COI gene of *Vestalis apicalis*

Vestalis apicalis voucher CUVA-01-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID: KU510326 Length: 561Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand	Frame
1037 bits(561)	0.0()	561/561(100%)	0/561(0%)	Plus/Plus	

Features:

```
Query  1    GAACTAGGACAACCGGGATCCCTTATTGGAGACGACCAAATCTACAACGTAGTAGTCACC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GAACTAGGACAACCGGGATCCCTTATTGGAGACGACCAAATCTACAACGTAGTAGTCACC  60

Query  61   GCCCATGCATTTGTAATAATCTTTTTTATAGTAATACCTATTATAATTGGGGGATTTGGA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GCCCATGCATTTGTAATAATCTTTTTTATAGTAATACCTATTATAATTGGGGGATTTGGA  120

Query  121  AATTGGCTTGTCCCACTAATGTTAGGGGCCCCTGATATGGCTTTCCCTCGACTAAACAAC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  AATTGGCTTGTCCCACTAATGTTAGGGGCCCCTGATATGGCTTTCCCTCGACTAAACAAC  180

Query  181  ATGAGATTTTGACTTCTGCCCCCAGCATTAACTCTTCTATTAACAAGAAGTTTAGTAGAA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  ATGAGATTTTGACTTCTGCCCCCAGCATTAACTCTTCTATTAACAAGAAGTTTAGTAGAA  240

Query  241  AGAGGGGCTGGGACAGGTTGAACCGTATACCCTCCTCTAGCGGGGGCTATTGCTCACGCA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  AGAGGGGCTGGGACAGGTTGAACCGTATACCCTCCTCTAGCGGGGGCTATTGCTCACGCA  300

Query  301  GGAGGATCAGTAGATTTAACTATTTTCTCGCTTCACCTAGCAGGCGTATCCTCGATTTTA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  GGAGGATCAGTAGATTTAACTATTTTCTCGCTTCACCTAGCAGGCGTATCCTCGATTTTA  360

Query  361  GGTGCCGTTAATTTCATTACTACAACAATTAATATAAAATCCCCTGGAATGAAGGCAGAG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  GGTGCCGTTAATTTCATTACTACAACAATTAATATAAAATCCCCTGGAATGAAGGCAGAG  420

Query  421  CAACTACCATTATTTGTTTGAGCAGTAGTAATTACAGCCATTTTGTTGCTATTATCATTA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  CAACTACCATTATTTGTTTGAGCAGTAGTAATTACAGCCATTTTGTTGCTATTATCATTA  480

Query  481  CCCGTTCTGGCTGGAGCCATCACTATACTTTTAACAGACCGTAACATAAATACATCGTTC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  CCCGTTCTGGCTGGAGCCATCACTATACTTTTAACAGACCGTAACATAAATACATCGTTC  540

Query  541TTTGACCCTGCTgggggggggg  561
           ||||||||||||||||||||||
Sbjct  541  TTTGACCCTGCTGGGGGGGGG  561
```

Figure 11d: Nucleotide BLAST output of the COI gene of *Vestalis apicalis* showing its nearest match subject

Cytochrome oxidase subunit I, partial (mitochondrion) [*Vestalis apicalis*]
Sequence ID: ALX71652 Length: 187Number of Matches: 1

Alignment statistics for match #1						
Score	Expect	Method	Identities	Positives	Gaps	Frame
365 bits(936)	1e-127()	Compositional matrix adjust	187/187(100%)	187/187 (100%)	0/187(0%)	

Features:

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Query  1    ELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNN  60
           ELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNN
Sbjct  1    ELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNN  60

Query  61   MSFWLLPPALTLLLTSSLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIFSLHLAGVSSIL  120
           MSFWLLPPALTLLLTSSLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIFSLHLAGVSSIL
Sbjct  61   MSFWLLPPALTLLLTSSLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIFSLHLAGVSSIL  120

Query  121  GAVNFITTTINMKSPGMKAEQLPLFVWAVVITAILLLLSLPVLAGAITMLLTDRNMNTSF  180
           GAVNFITTTINMKSPGMKAEQLPLFVWAVVITAILLLLSLPVLAGAITMLLTDRNMNTSF
Sbjct  121  GAVNFITTTINMKSPGMKAEQLPLFVWAVVITAILLLLSLPVLAGAITMLLTDRNMNTSF  180

Query  181  FDPAGGG  187
           FDPAGGG
Sbjct  181  FDPAGGG  187
```

Figure 11e: Peptide BLAST output of the mt DNA COI gene of *Vestalis apicalis*

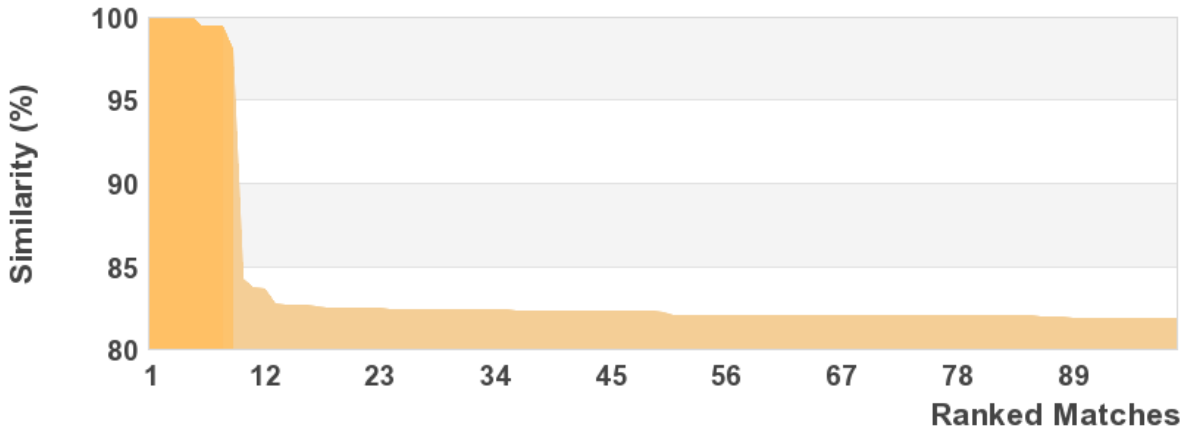

Figure 11f: The line diagram of *Vestalis apicalis* with more than 99 % match to other retrieved sequences (BOLD SYSTEM)

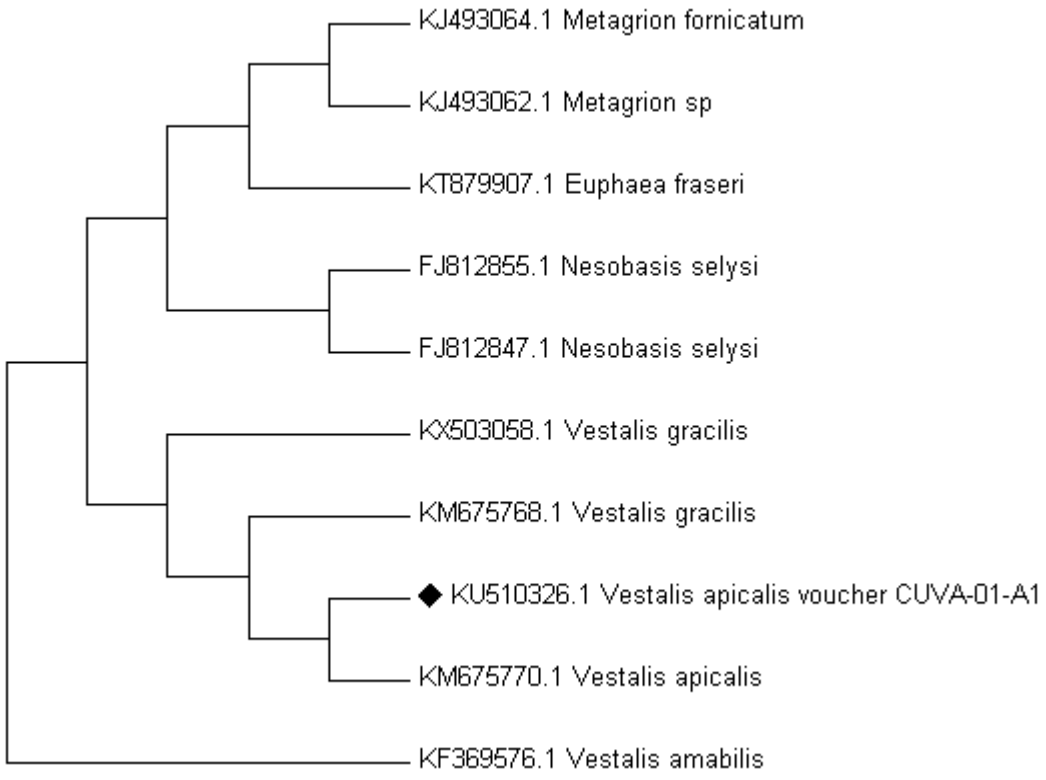

Figure 11g: The molecular phylogenetic tree of *Vestalis apicalis* inferred by NJ tree method

Table 18: Percentage of evolutionary divergence of *Vestalis apicalis* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KU510326	*Vestalis apicalis* (Kerala)	
2	KM675770	*Vestalis apicalis* (Kerala)	0.00
3	KM675768	*Vestalis gracilis* (Kerala)	0.00
4	KM503058	*Vestalis gracilis* (Kerala)	0.00
5	KF369576	*Vestalis ambalis* (Netherland)	21.83
6	KJ493064	*Metagrion forcipatum*	20.98
7	FJ812855	*Nesobasis selysi*	21.17
8	KJ493062	*Metagrion sp*	21.17
9	KT879907	*Euphaea fraseri*	21.49

Figure 12: *Vestalis gracilis*

> KX503058 *Vestalis gracilis* |cytochrome oxidase subunit I gene |voucher CUVG-01-A1 partial cds, mitochondrial|587bp

ACGGCCCTAAGAATGCTAATTCGAATTGAACTAGGACAACCGGGATCCCTTATTGGAGACGACCAAATCTACAACG
TAGTAGTCACCGCCCATGCATTTGTAATAATCTTTTTTATAGTAATACCTATTATAATTGGGGGATTTGGAAATTG
GCTTGTCCCACTAATGTTAGGGGCCCCTGATATGGCTTTCCCTCGACTAAACAACATGAGATTTTGACTTCTGCCC
CCAGCATTAACTCTTCTATTAACAAGAAGTTTAGTAGAAAGAGGGGCTGGGACAGGTTGAACCGTATACCCTCCTC
TAGCGGGGGCTATTGCTCACGCAGGAGGATCAGTAGATTTAACTATTTTCTCGCTTCACCTAGCAGGCGTATCCTC
GATTTTAGGTGCCGTTAATTTCATTACTACAACAATTAATATAAAATCCCCTGGAATGAAGGCAGAGCAACTACCA
TTATTTGTTTGAGCAGTAGTAATTACAGCCATTTTGTTGCTATTATCATTACCCGTTCTGGCTGGAGTCCATCACT
ATACTTTAACAGACCGTAACATAAATACATCGTTCTTTGACCCTGCTGGGGGGGG

Figure 12a: The DNA sequence interpret of COI gene of *Vestalis gracilis*

Figure 12b: Representative molecular barcode of COI gene of *Vestalis gracilis*

> ANU39518 *Vestalis gracilis* |cytochrome oxidase subunit I gene |voucher CUVG-01-A1 partial cds, mitochondrial 196bp

TALSMLIRIELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPALTLLLT
SSLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIFSLHLAGVSSILGAVNFITTTINMKSPGMKAEQLPLFVWAVVITAILLLLS
LPVLAGVHHYTLTDRNMNTSFFDPAGGG

Figure 12c: The conceptual translation product of the COI gene of *Vestalis gracilis*

Vestalis gracilis voucher CUVG-01-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

Sequence ID: KX503058 Length: 587Number of Matches: 1

```
      Alignment statistics for match #1
    Score     Expect  Identities     Gaps     Strand  Frame
1085 bits(587) 0.0()  587/587(100%) 0/587(0%) Plus/Plus
Features:
Query  1    ACGGCCCTAAGAATGCTAATTCGAATTGAACTAGGACAACCGGGATCCCTTATTGGAGAC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    ACGGCCCTAAGAATGCTAATTCGAATTGAACTAGGACAACCGGGATCCCTTATTGGAGAC  60

Query  61   GACCAAATCTACAACGTAGTAGTCACCGCCCATGCATTTGTAATAATCTTTTTTATAGTA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GACCAAATCTACAACGTAGTAGTCACCGCCCATGCATTTGTAATAATCTTTTTTATAGTA  120

Query  121  ATACCTATTATAATTGGGGGATTTGGAAATTGGCTTGTCCCACTAATGTTAGGGGCCCCT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  ATACCTATTATAATTGGGGGATTTGGAAATTGGCTTGTCCCACTAATGTTAGGGGCCCCT  180

Query  181  GATATGGCTTTCCCTCGACTAAACAACATGAGATTTTGACTTCTGCCCCCAGCATTAACT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  GATATGGCTTTCCCTCGACTAAACAACATGAGATTTTGACTTCTGCCCCCAGCATTAACT  240

Query  241  CTTCTATTAACAAGAAGTTTAGTAGAAAGAGGGGCTGGGACAGGTTGAACCGTATACCCT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  CTTCTATTAACAAGAAGTTTAGTAGAAAGAGGGGCTGGGACAGGTTGAACCGTATACCCT  300

Query  301  CCTCTAGCGGGGGCTATTGCTCACGCAGGAGGATCAGTAGATTTAACTATTTTCTCGCTT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  CCTCTAGCGGGGGCTATTGCTCACGCAGGAGGATCAGTAGATTTAACTATTTTCTCGCTT  360

Query  361  CACCTAGCAGGCGTATCCTCGATTTTAGGTGCCGTTAATTTCATTACTACAACAATTAAT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  CACCTAGCAGGCGTATCCTCGATTTTAGGTGCCGTTAATTTCATTACTACAACAATTAAT  420

Query  421  ATAAAATCCCCTGGAATGAAGGCAGAGCAACTACCATTATTTGTTTGAGCAGTAGTAATT  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  ATAAAATCCCCTGGAATGAAGGCAGAGCAACTACCATTATTTGTTTGAGCAGTAGTAATT  480

Query  481  ACAGCCATTTTGTTGCTATTATCATTACCCGTTCTGGCTGGAGTCCATCACTATACTTTA  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  ACAGCCATTTTGTTGCTATTATCATTACCCGTTCTGGCTGGAGTCCATCACTATACTTTA  540

Query  541ACAGACCGTAACATAAATACATCGTTCTTTGACCCTGCTgggggggg  587
           ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  ACAGACCGTAACATAAATACATCGTTCTTTGACCCTGCTGGGGGGGG  587
```

Figure 12d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Vestalis gracilis* showing its nearest match subject

Cytochrome oxidase subunit I, partial (mitochondrion) [*Vestalis gracilis*]
Sequence ID: ANU39518 Length: 196Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps
385 bits(989)	2e-135()	Compositional matrix adjust.	196/196(100%)	196/196(100%)	0/196(0%)

Features:

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Query  1    TALSMLIRIELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAP  60
            TALSMLIRIELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAP
Sbjct  1    TALSMLIRIELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAP  60

Query  61   DMAFPRLNNMSFWLLPPALTLLLTSSLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIFSL  120
            DMAFPRLNNMSFWLLPPALTLLLTSSLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIFSL
Sbjct  61   DMAFPRLNNMSFWLLPPALTLLLTSSLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIFSL  120

Query  121  HLAGVSSILGAVNFITTTINMKSPGMKAEQLPLFVWAVVITAILLLLSLPVLAGVHHYTL  180
            HLAGVSSILGAVNFITTTINMKSPGMKAEQLPLFVWAVVITAILLLLSLPVLAGVHHYTL
Sbjct  121  HLAGVSSILGAVNFITTTINMKSPGMKAEQLPLFVWAVVITAILLLLSLPVLAGVHHYTL  180

Query  181  TDRNMNTSFFDPAGGG  196
            TDRNMNTSFFDPAGGG
Sbjct  181  TDRNMNTSFFDPAGGG  196
```

Figure 12e: Peptide BLAST output of the mt DNA COI gene of *Vestalis gracilis*

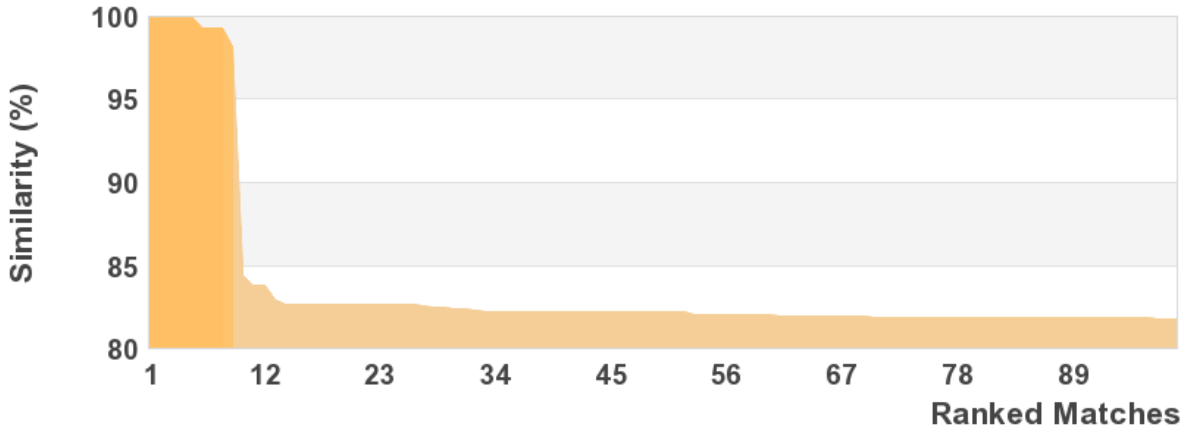


Figure 12f: The line diagram of *Vestalis gracilis* with more than 99 % match to other retrieved sequences (BOLD SYSTEM)

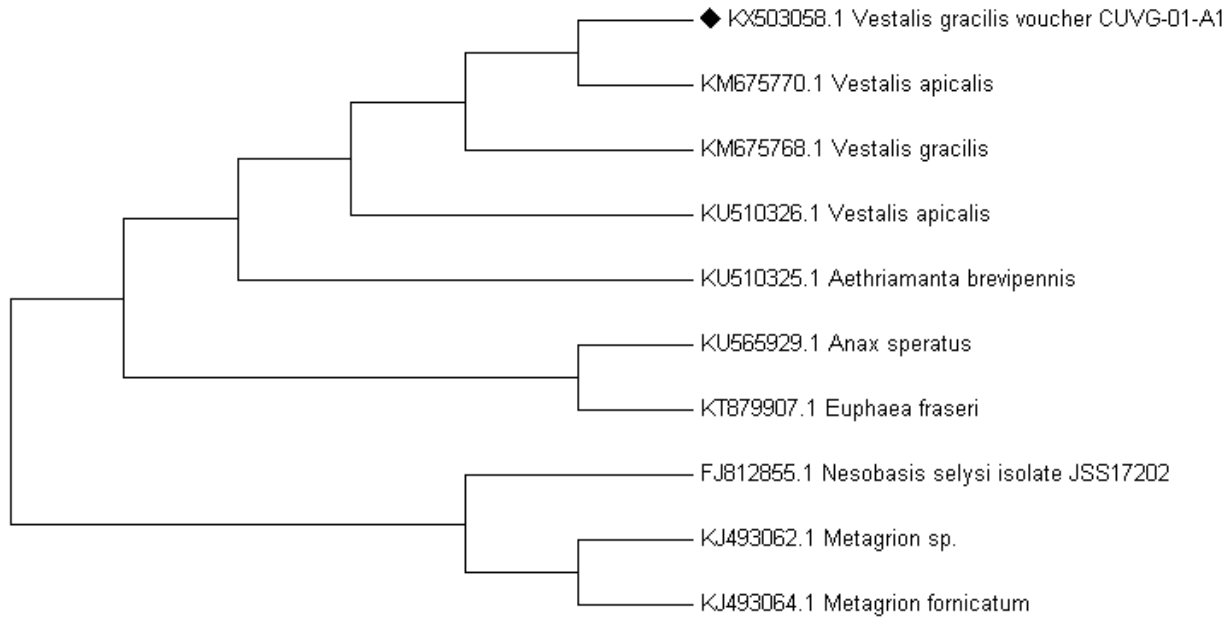


Figure 12g: Molecular Phylogenetic tree of *Vestalis gracilis* inferred by NJ tree method

Table 20: Percentage of evolutionary divergence of *Vestalis gracilis* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KX503058	*Vestalis gracillis* (Kerala)	
2	KM675770	*Vestalis apicalis* (Kerala)	0.00
3	KM675768	*Vestalis gracillis* (Kerala)	0.00
4	KU510326	*Vestalis apicalis* (Kerala)	0.00
5	KU510325	*Aethriamanta brevipennis*	0.61
6	KU565929	*Anax speratus*	20.49
7	KT879907	*Euphaea fraseri*	21.18
8	KJ493062	*Metagrion sp.*	21.77
9	KJ493064	*Metagrion fornicatum*	20.94
10	FJ8128551	*Nesobasis selys*	21.69

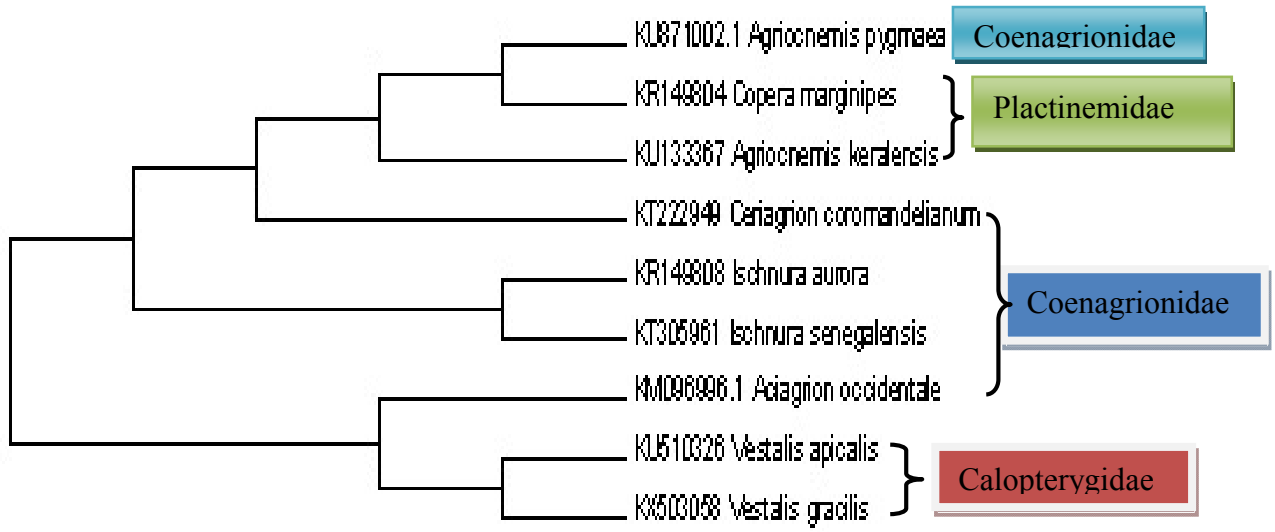


Figure 13: Phylogenetic tree of all Zygopteran members plotted by Neighbour joining method

Fig 14: *Onychogomphus malabarensis*

> KU135368 *Onychogomphus malabarensis* |cytochrome oxidase subunit I gene |voucher CUOM-01-A1 partial cds, mitochondrial|602bp

TAAGAATATTAATTCGAATTGAATTAGGACAGCCAGGTTCATTAATTGGAGATGATCAAATTTATAATGTTATTGT
AACTGCTCATGCATTTGTAATAATTTTCTTTATAGTTATACCTATTATAATTGGAGGATTTGGAAATTGACTAGTA
CCTTTAATATTAGGAGCACCAGATATAGCATTCCCACGACTTAATAATATAAGATTTTGATTACTACCACCCTCAT
TAACTTTACTACTAGCCAGTAGTTTAGTAGAAAGAGGAGCCGGAACAGGATGAACTGTTTACCCTCCACTTGCAGG
AGCTATTGCCCATGCAGGAGCATCAGTTGATCTTACCATTTTTTCATTACACTTGGCAGGGGTATCTTCAATTCTA
GGAGCAATTAATTTTATTACTACAACAATTAATATAAAGTCTCCTGGAATAAAATTAGATCAAATACCTTTATTTG
TTTGAGCTGTCGTTATTACTGCCGTGTTACTTTTATTATCTCTACCAGTTTTAGCAGGAGCAATTACTATACTATT
AACTGACCGAAATATTAATACATCATTCTTCGACCCCGCTGGAGGAGGAGATCCAATTTTATACCAACAT

Figure 14a: DNA sequence interpret of COI gene of *Onychogomphus malabarensis*

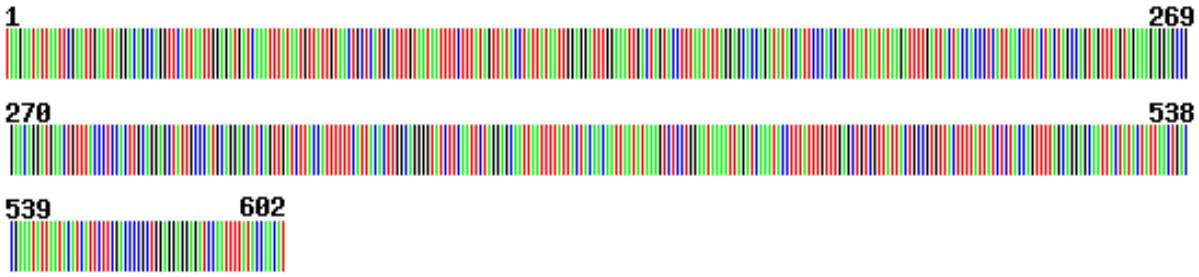


Figure 14b: Representative molecular barcode of COI gene of *Onychogomphus malabarensis*

> ALQ75279 *Onychogomphus malabarensis* |cytochrome oxidase subunit I gene |voucher CUOM-01-A1 partial cds, mitochondrial|200bp

SMLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSL
TLLLASSLVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTTINMKSPGMKLDQMPLFV
WAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAGGGDPILYQH

Figure 14c: The conceptual translation product of the COI gene of *Onychogomphus malabarensis*

Onychogomphus malabarensis voucher CUOM-01-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

```
Sequence ID: KU135368 Length: 602Number of Matches: 1

            Alignment statistics for match #1
     Score     Expect  Identities     Gaps     Strand  Frame
1112 bits(602) 0.0()  602/602(100%) 0/602(0%) Plus/Plus
Features:
Query  1    TAAGAATATTAATTCGAATTGAATTAGGACAGCCAGGTTCATTAATTGGAGATGATCAAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TAAGAATATTAATTCGAATTGAATTAGGACAGCCAGGTTCATTAATTGGAGATGATCAAA  60

Query  61   TTTATAATGTTATTGTAACTGCTCATGCATTTGTAATAATTTTCTTTATAGTTATACCTA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   TTTATAATGTTATTGTAACTGCTCATGCATTTGTAATAATTTTCTTTATAGTTATACCTA  120

Query  121  TTATAATTGGAGGATTTGGAAATTGACTAGTACCTTTAATATTAGGAGCACCAGATATAG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  TTATAATTGGAGGATTTGGAAATTGACTAGTACCTTTAATATTAGGAGCACCAGATATAG  180

Query  181  CATTCCCACGACTTAATAATATAAGATTTTGATTACTACCACCCTCATTAACTTTACTAC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CATTCCCACGACTTAATAATATAAGATTTTGATTACTACCACCCTCATTAACTTTACTAC  240

Query  241  TAGCCAGTAGTTTAGTAGAAAGAGGAGCCGGAACAGGATGAACTGTTTACCCTCCACTTG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  TAGCCAGTAGTTTAGTAGAAAGAGGAGCCGGAACAGGATGAACTGTTTACCCTCCACTTG  300

Query  301  CAGGAGCTATTGCCCATGCAGGAGCATCAGTTGATCTTACCATTTTTTCATTACACTTGG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  CAGGAGCTATTGCCCATGCAGGAGCATCAGTTGATCTTACCATTTTTTCATTACACTTGG  360

Query  361  CAGGGGTATCTTCAATTCTAGGAGCAATTAATTTTATTACTACAACAATTAATATAAAGT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  CAGGGGTATCTTCAATTCTAGGAGCAATTAATTTTATTACTACAACAATTAATATAAAGT  420

Query  421  CTCCTGGAATAAAATTAGATCAAATACCTTTATTTGTTTGAGCTGTCGTTATTACTGCCG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  CTCCTGGAATAAAATTAGATCAAATACCTTTATTTGTTTGAGCTGTCGTTATTACTGCCG  480

Query  481  TGTTACTTTTATTATCTCTACCAGTTTTAGCAGGAGCAATTACTATACTATTAACTGACC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  TGTTACTTTTATTATCTCTACCAGTTTTAGCAGGAGCAATTACTATACTATTAACTGACC  540

Query  541  GAAATATTAATACATCATTCTTCGACCCCGCTGGAGGAGGAGATCCAATTTTATACCAAC  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  GAAATATTAATACATCATTCTTCGACCCCGCTGGAGGAGGAGATCCAATTTTATACCAAC  600

Query  601  AT  602
            ||
Sbjct  601  AT  602
```

Figure 14d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Onychogomphus malabarensis* showing its nearest match subject

Cytochrome oxidase subunit 1, partial (mitochondrion) [*Ophiogomphus mainensis*]
Sequence ID: AEO19446 Length: 211Number of Matches: 1

Score	Expect	Method	Identities	Positives	Gaps
393 bits(1009)	4e-138()	Compositional matrix adjust.	200/200(100%)	200/200(100%)	0/200(0%)

Features:

```
Query 1   SMLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMA  60
          SMLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMA
Sbjct 10  SMLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMA  69

Query 61  FPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLA  120
          FPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLA
Sbjct 70  FPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLA  129

Query 121 GVSSILGAINFITTTINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDR  180
          GVSSILGAINFITTTINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDR
Sbjct 130 GVSSILGAINFITTTINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDR  189

Query 181 NINTSFFDPAGGGDPILYQH  200
          NINTSFFDPAGGGDPILYQH
Sbjct 190 NINTSFFDPAGGGDPILYQH  209
```

Figure 14e: Peptide BLAST output of the mt DNA COI gene of *Onychogomphus malabarensis*

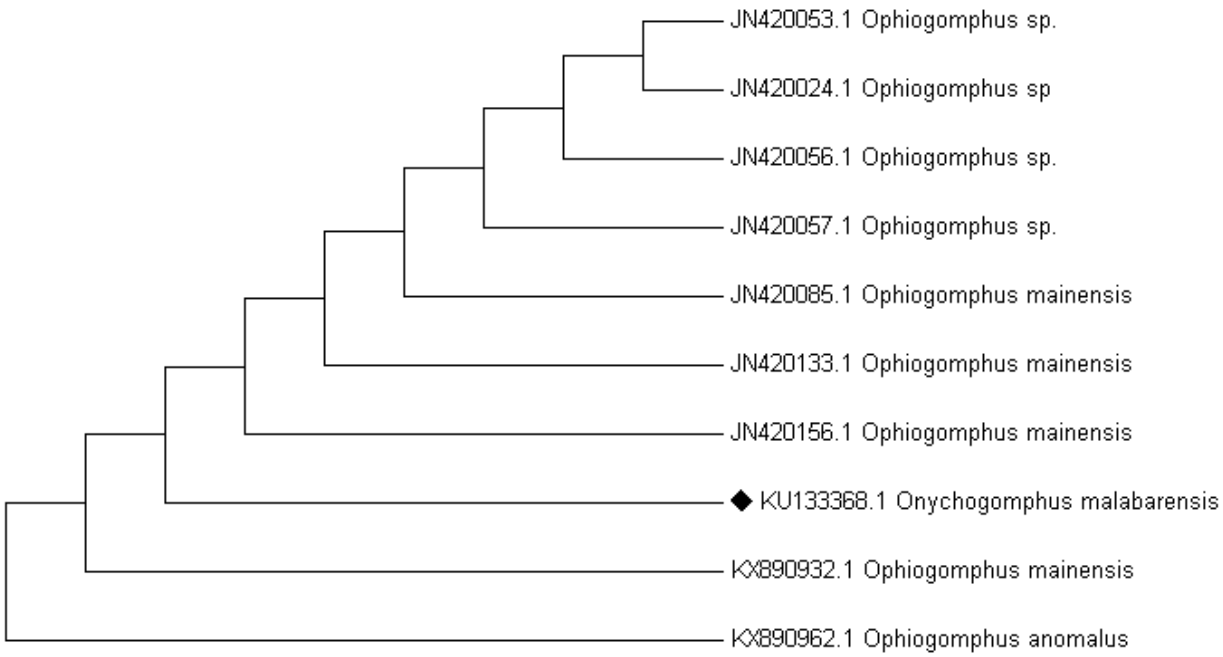


Figure 14g: Phylogenetic relationship of *Onychogomphus malabarensis* inferred by NJ tree metho

Table 22: Percentage of evolutionary divergence of *Onychogomphus malabarensis* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KU133368	*Onychogomphus malabarensis* (Kerala)	
2	KX890962	*Ophiogomphus anomalus* (America)	0.00
3	KX890932	*Ophiogomphus mainensis*	0.00
4	JN420156	*Ophiogomphus mainensis*	0.00
5	JN420133	*Ophiogomphus mainensis*	0.00
6	JN420085	*Ophiogomphus mainensis*	0.00
7	JN420057	*Ophiogomphus* sp	0.0
8	JN420056	*Ophiogomphus* sp	0.00
9	JN420053	*Ophiogomphus* sp	0.00
10	JN420024	*Ophiogomphus* sp	0.00

Figure 15: *Anaciaeschna jaspidea*

> KR149806.1 *Anaciaeschna jaspidea* |cytochrome oxidase subunit I gene |voucher CUAJ 01-A1 partial cds, mitochondrial|591 bp

TTAATTCGAATTGAACTGGGACAACCTGGATCTCTAATTGGAGATGATCAAATTTATAATGTAATTGTTACTGCAC
ATGCCTTCGTTATAATTTTCTTCATAGTAATACCTATTATAATTGGAGGATTTGGTAATTGGCTTGTGCCATTAAT
ATTAGGAGCACCAGATATGGCTTTCCCACGACTAAATAATATAAGATTTTGATTATTACCTCCCTCATTCACTTTA
TTACTTGCAAGAAGAGTAGTAGAAAGAGGGGCAGGAACAGGATGAACTGTATATCCACCATTAGCAGGAGCTATTG
CTCATGCTGGAGCATCTGTAGATTTAACTATTTTTTCTTTACACTTAGCTGGAGTATCATCAATTTTAGGGGCAAT
TAATTTTATTACTACAGTAATTAATATAAAGTCACCAGGAATAAAAATAGATCAAATACCTTTATTTGTATGAGCT
GTAGTAATTACTGCAGTGTTATTATTATTATCTCTACCTGTTCTTGCTGGAGCCATTACTATACTTTTAACTGATC
GAAATATTAATACATCCTTCTTTGACCCAGCAGGAGGAGGAGATCCAATTCTTTATTCA

Figure 15a: The DNA sequence interpret of the COI gene of *Anaciaeschna jaspidea*

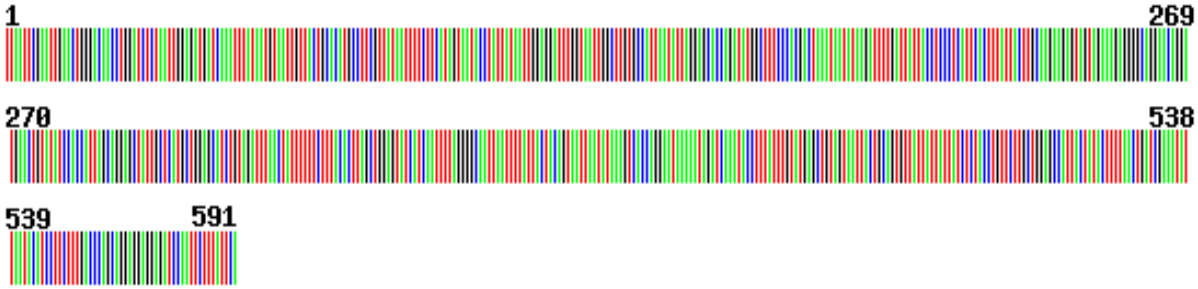


Figure 15b: Representative molecular barcode of COI gene of *Anaciaeschna jaspidea*

> AFP19427 *Anaciaeschna jaspidea* |cytochrome oxidase subunit I gene |voucher CUAJ 01-A1 partial cds, mitochondrial|200 bp

LIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSFTL
LLASSVVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMKMDQMPLFVWA
VVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAGGGDPILYS

Figure 15c: The conceptual translation product of the COI gene of *Anaciaeschna jaspidea*

Anaciaeschna jaspidea voucher CUAJ 01-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID: KR149806. length: 591Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
1092 bits(591)	0.0	591/591(100%)	0/591(0%)	Plus/Plus

```
Query  1    TTAATTCGAATTGAACTGGGACAACCTGGATCTCTAATTGGAGATGATCAAATTTATAAT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TTAATTCGAATTGAACTGGGACAACCTGGATCTCTAATTGGAGATGATCAAATTTATAAT  60
Query  61   GTAATTGTTACTGCACATGCCTTCGTTATAATTTTCTTCATAGTAATACCTATTATAATT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GTAATTGTTACTGCACATGCCTTCGTTATAATTTTCTTCATAGTAATACCTATTATAATT  120
Query  121  GGAGGATTTGGTAATTGGCTTGTGCCATTAATATTAGGAGCACCAGATATGGCTTTCCCA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  GGAGGATTTGGTAATTGGCTTGTGCCATTAATATTAGGAGCACCAGATATGGCTTTCCCA  180
Query  181  CGACTAAATAATATAAGATTTTGATTATTACCTCCCTCATTCACTTTATTACTTGCAAGA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CGACTAAATAATATAAGATTTTGATTATTACCTCCCTCATTCACTTTATTACTTGCAAGA  240
Query  241  AGAGTAGTAGAAAGAGGGGCAGGAACAGGATGAACTGTATATCCACCATTAGCAGGAGCT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  AGAGTAGTAGAAAGAGGGGCAGGAACAGGATGAACTGTATATCCACCATTAGCAGGAGCT  300
Query  301  ATTGCTCATGCTGGAGCATCTGTAGATTTAACTATTTTTTCTTTACACTTAGCTGGAGTA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  ATTGCTCATGCTGGAGCATCTGTAGATTTAACTATTTTTTCTTTACACTTAGCTGGAGTA  360
Query  361  TCATCAATTTTAGGGGCAATTAATTTTATTACTACAGTAATTAATATAAAGTCACCAGGA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  TCATCAATTTTAGGGGCAATTAATTTTATTACTACAGTAATTAATATAAAGTCACCAGGA  420
Query  421  ATAAAAATAGATCAAATACCTTTATTTGTATGAGCTGTAGTAATTACTGCAGTGTTATTA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  ATAAAAATAGATCAAATACCTTTATTTGTATGAGCTGTAGTAATTACTGCAGTGTTATTA  480
Query  481  TTATTATCTCTACCTGTTCTTGCTGGAGCCATTACTATACTTTTAACTGATCGAAATATT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  TTATTATCTCTACCTGTTCTTGCTGGAGCCATTACTATACTTTTAACTGATCGAAATATT  540
Query  541  AATACATCCTTCTTTGACCCAGCAGGAGGAGGAGATCCAATTCTTTATTCA  591
            ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  AATACATCCTTCTTTGACCCAGCAGGAGGAGGAGATCCAATTCTTTATTCA  591
```

Figure 15d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Anaciaeschna jaspidea* showing its nearest match subject

Cytochrome c oxidase I, partial (mitochondrion) [*Anaciaeschna jaspidea*]

Sequence ID: AFP19427.1Length: 208Number of Matches: 1

Alignment statistics for match #1					
Score	**Expect**	**Method**	**Identities**	**Positives**	**Gaps**
386 bits(991)	2e-135	Compositional matrix adjust.	197/197(100%)	197/197(100%)	0/197(0%)

```
Query  1    LIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFP  60
            LIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFP
Sbjct  7    LIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFP  66

Query  61   RLNNMSFWLLPPSFTLLLASSVVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGV  120
            RLNNMSFWLLPPSFTLLLASSVVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGV
Sbjct  67   RLNNMSFWLLPPSFTLLLASSVVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGV  126

Query  121  SSILGAINFITTVINMKSPGMKMDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNI  180
            SSILGAINFITTVINMKSPGMKMDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNI
Sbjct  127  SSILGAINFITTVINMKSPGMKMDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNI  186

Query  181  NTSFFDPAGGGDPILYS  197
            NTSFFDPAGGGDPILYS
Sbjct  187  NTSFFDPAGGGDPILYS  203
```

Figure 15e: Peptide BLAST output of the mt DNA COI gene of *Anaciaeschna jaspidea*

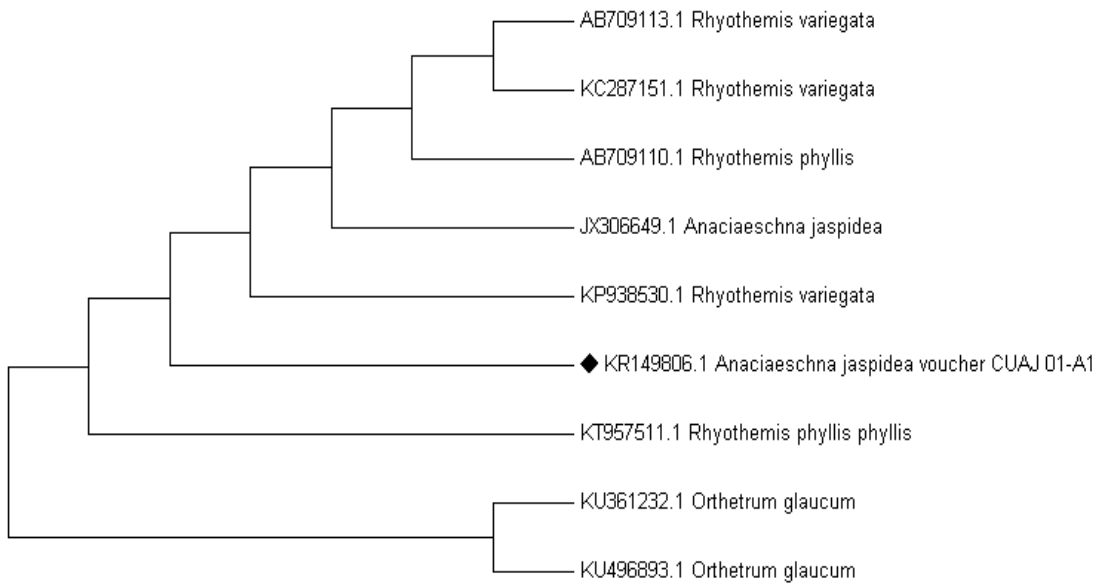


Figure 15f: Molecular Phylogenetic tree of *Anaciaeschna jaspidea* inferred by NJ tree method

Table 24: Percentage of evolutionary divergence of *Anaciaeschna jaspidea* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KR149806	*Anaciaeshna jaspidea* (Kerala)	
2	JX306649	*Anaciaeshna jaspidea* (Tamil nadu)	0.00
3	AB709113	*Rhyothemis variegata*	0.30
4	AB709110	*Rhyothemis phyllis*	0.00
5	KP938530	*Rhyothemis variegata*	0.30
6	KT957511	*Rhyothemis phyllis phyllis*	0.37
7	KU361232	*Orthetrum glacum*	15.15
8	KC287151	*Rhyothemis variegata*	0.35
9	KU496893	*Orthetrum glacum*	15.15

Figure 16: *Anax parthenope*

> KR149805 *Anax parthenope* |cytochrome oxidase subunit I gene |voucher CUAP 01-A1 partial cds, mitochondrial|607 bp

AATGGTAGGAACTGCTCTAAGAGTTTTAATTCGAATTGAATTAGGACAACCAGGATCATTAATTGGAGATGATCAA
ATTTATAATGTAATTGTAACAGCTCATGCTTTTGTTATAATTTTCTTTATAGTAATACCTATTATAATTGGAGGAT
TTGGAAATTGATTAGTGCCACTAATATTAGGAGCACCCGATATAGCTTTCCCACGATTAAATAATATAAGATTTTG
ATTACTACCACCTTCTCTAACACTTTTATTAGCAGGAAGTATAGTTGAAAGAGGTGCAGGAACAGGATGAACAGTT
TATCCTCCTCTTGCTGGTGCAATTGCCCATGCAGGAGCATCTGTAGATTTAACTATTTTTTCTCTTCATTTGGCTG
GAGTATCTTCAATTTTAGGTGCTATTAATTTTATTACTACAACAATTAATATAAAGTCACCGGGAATAAAGATAGA
TCAAATACCACTATTTGTATGAGCCGTAGTAATTACAGCCGTATTATTATTATTATCTCTTCCTGTTCTTGCTGGT
GCAATTACAATGTTATTAACAGATCGAAATATTAATACATCATTCTTTGATCCTGCAGGAGGGGGTGATCCAATT

Figure 16a: The DNA sequence interpret of COI gene of *Anax parthenope*

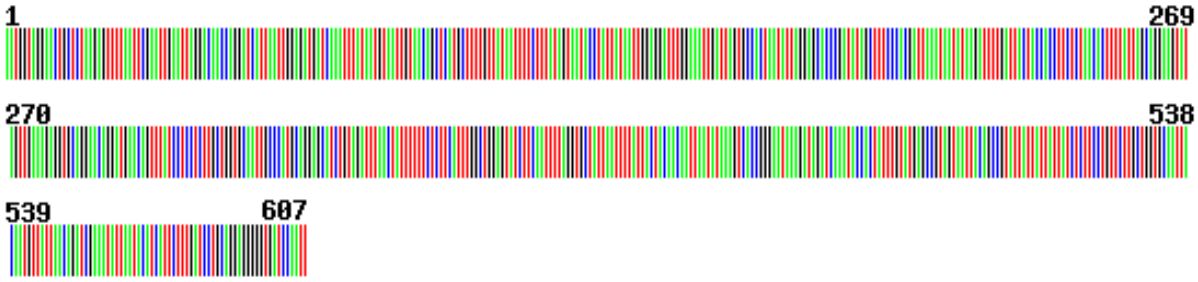


Figure 16b: Representative molecular barcode of COI gene of *Anax parthenope*

> AKL82319 *Anax parthenope* |cytochrome oxidase subunit I gene |voucher CUAP 01-A1 partial cds, mitochondrial| 202 bp

MVGTALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFW
LLPPSLTLLLAGSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTTINMKSPGMKMD
QMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAGGGDPI

Figure 16c: The conceptual translation product of the COI gene of *Anax parthenope*

Anax parthenope voucher CUAP 01-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Mitochondrial
Sequence ID: KR149805.1Length: 607Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
1122 bits(607)	0.0	607/607(100%)	0/607(0%)	Plus/Plus

```
Query  1    AATGGTAGGAACTGCTCTAAGAGTTTTAATTCGAATTGAATTAGGACAACCAGGATCATT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    AATGGTAGGAACTGCTCTAAGAGTTTTAATTCGAATTGAATTAGGACAACCAGGATCATT  60
Query  61   AATTGGAGATGATCAAATTTATAATGTAATTGTAACAGCTCATGCTTTTGTTATAATTTT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   AATTGGAGATGATCAAATTTATAATGTAATTGTAACAGCTCATGCTTTTGTTATAATTTT  120
Query  121  CTTTATAGTAATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTGCCACTAATATT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CTTTATAGTAATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTGCCACTAATATT  180
Query  181  AGGAGCACCCGATATAGCTTTCCCACGATTAAATAATATAAGATTTTGATTACTACCACC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  AGGAGCACCCGATATAGCTTTCCCACGATTAAATAATATAAGATTTTGATTACTACCACC  240
Query  241  TTCTCTAACACTTTTATTAGCAGGAAGTATAGTTGAAAGAGGTGCAGGAACAGGATGAAC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  TTCTCTAACACTTTTATTAGCAGGAAGTATAGTTGAAAGAGGTGCAGGAACAGGATGAAC  300
Query  301  AGTTTATCCTCCTCTTGCTGGTGCAATTGCCCATGCAGGAGCATCTGTAGATTTAACTAT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  AGTTTATCCTCCTCTTGCTGGTGCAATTGCCCATGCAGGAGCATCTGTAGATTTAACTAT  360
Query  361  TTTTTCTCTTCATTTGGCTGGAGTATCTTCAATTTTAGGTGCTATTAATTTTATTACTAC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  TTTTTCTCTTCATTTGGCTGGAGTATCTTCAATTTTAGGTGCTATTAATTTTATTACTAC  420
Query  421  AACAATTAATATAAAGTCACCGGGAATAAAGATAGATCAAATACCACTATTTGTATGAGC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  AACAATTAATATAAAGTCACCGGGAATAAAGATAGATCAAATACCACTATTTGTATGAGC  480
Query  481  CGTAGTAATTACAGCCGTATTATTATTATTATCTCTTCCTGTTCTTGCTGGTGCAATTAC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  CGTAGTAATTACAGCCGTATTATTATTATTATCTCTTCCTGTTCTTGCTGGTGCAATTAC  540
Query  541  AATGTTATTAACAGATCGAAATATTAATACATCATTCTTTGATCCTGCAGGAGGGGGTGA  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  AATGTTATTAACAGATCGAAATATTAATACATCATTCTTTGATCCTGCAGGAGGGGGTGA  600
Query  601  TCCAATT  607
            |||||||
Sbjct  601  TCCAATT  607
```

Figure 16d: Nucleotide BLAST output of COI gene of *Anax parthenope* showing its nearest match subject

Cytochrome oxidase subunit I, partial (mitochondrion) [*Anax parthenope*]
Sequence ID: AKL82319.Length: 202Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps
93 bits(1009)	4e-138()	Compositional matrix adjust.	202/202(100%)	202/202(100%)	0/202(0%)

Features:

```
Query  1    MVGTALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLML  60
           MVGTALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLML
Sbjct  1    MVGTALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLML  60

Query  61   GAPDMAFPRLNNMSFWLLPPSLTLLLAGSMVESGAGTGWTVYPPLAGAIAHAGASVDLTI  120
           GAPDMAFPRLNNMSFWLLPPSLTLLLAGSMVESGAGTGWTVYPPLAGAIAHAGASVDLTI
Sbjct  61   GAPDMAFPRLNNMSFWLLPPSLTLLLAGSMVESGAGTGWTVYPPLAGAIAHAGASVDLTI  120

Query  121  FSLHLAGVSSILGAINFITTTINMKSPGMKMDQMPLFVWAVVITAVLLLLSLPVLAGAIT  180
           FSLHLAGVSSILGAINFITTTINMKSPGMKMDQMPLFVWAVVITAVLLLLSLPVLAGAIT
Sbjct  121  FSLHLAGVSSILGAINFITTTINMKSPGMKMDQMPLFVWAVVITAVLLLLSLPVLAGAIT  180

Query  181  MLLTDRNINTSFFDPAGGGDPI  202
           MLLTDRNINTSFFDPAGGGDPI
Sbjct  181  MLLTDRNINTSFFDPAGGGDPI  202
```

Figure 16e: Peptide BLAST output of COI gene of *Anax parthenope*

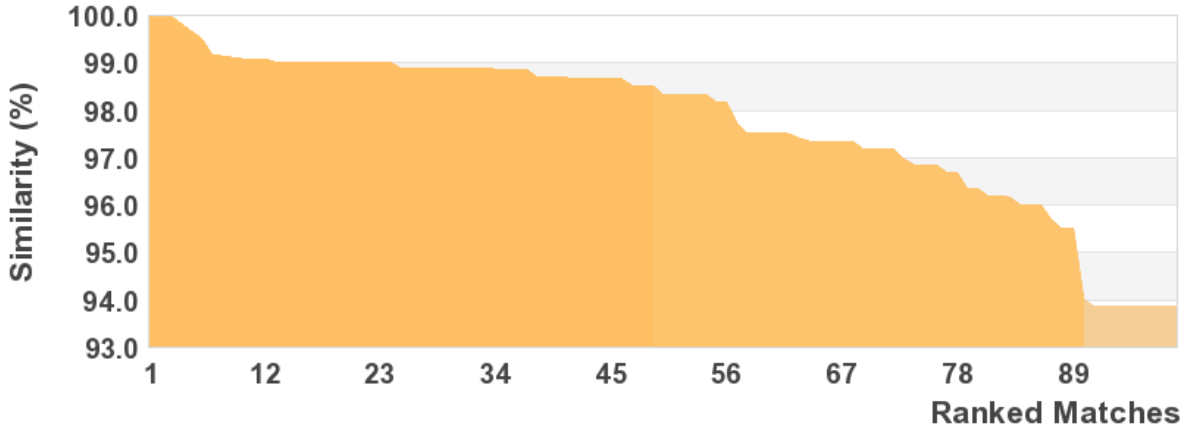


Figure 16f: The line diagram of *Anax parthenope* with more than 99 % match to other retrieved sequences (BOLD SYSTEM)

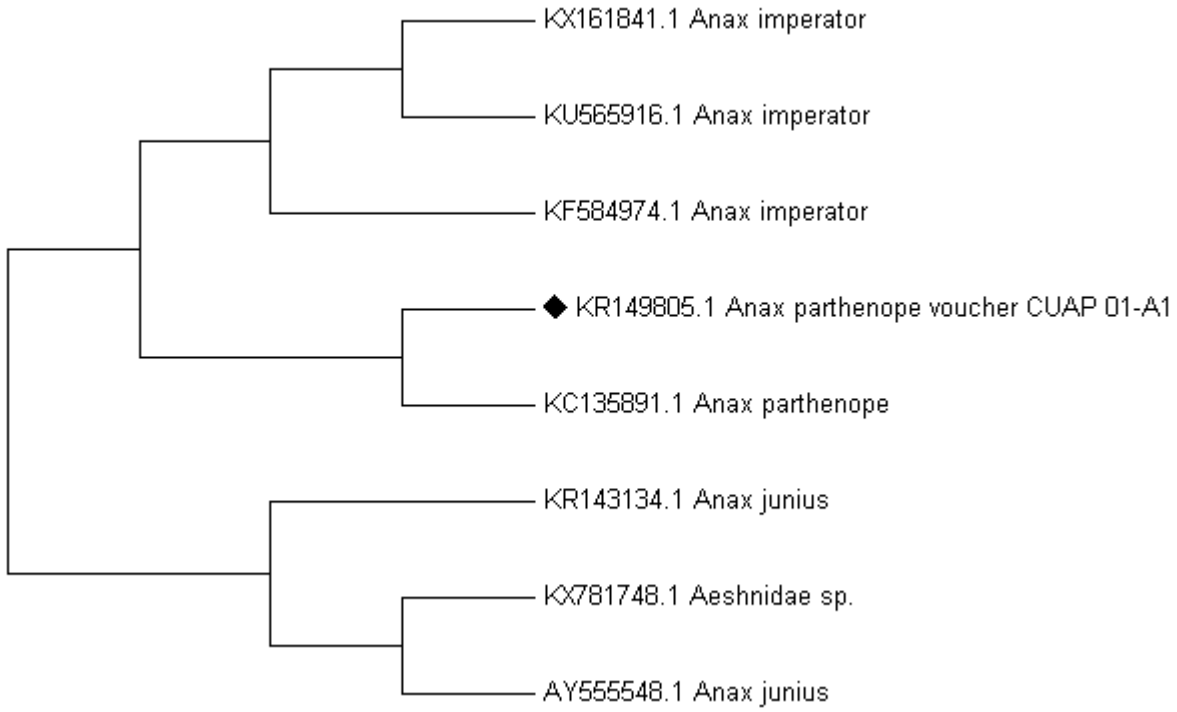


Figure 16g: Molecular phylogenetic tree of *Anax parthenope* inferred by NJ tree method

Table 26: Percentage of evolutionary divergence of *Anax parthenope* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KR149805	*Anax parthenope* (Kerala)	
2	KC13589	*Anax parthenope* (South Korea)	0.00
3	KX161841	*Anax imperator*	1.06
4	KU565916	*Anax imperator*	1.42
5	KX781748	*Aeshnidae sp*	2.90
6	KR143134	*Anax junius*	2.90
7	AY555548	*Anax junius*	2.90
8	KF584974	*Anax imperator*	0.89

Fig 17: *Orthetrum sabina*

> KP938529 *Orthetrum sabina* |cytochrome oxidase subunit I gene |voucher CUOS 01-A1 partial cds, mitochondrial|500bp

GTCAGCCCGGTTCTTTAATTGGAGATGACCAAATTTATAATGTAATTGTTACTGCACATGCATTTGTAATAATTTT
CTTCATAGTAATACCTATTATAATTGGTGGATTCGGAAATTGACTTGTACCATTAATACTAGGGGCACCAGATATA
GCATTCCCACGACTTAATAATATAAGTTTTTGACTTTTACCTCCTTCATTCACCCTTTTATTAGCAAGTAGAATGG
TTGAAAGTGGGGCAGGTACTGGATGAACTGTATACCCTCCTCTTGCAGGAGCAATTGCCCACGCAGGAGCATCAGT
AGATTTAACAATTTTCTCACTACATTTAGCAGGGGTATCTTCTATTTTAGGAGCAATTAATTTTATCACTACAGTA
ATTAATATAAAGTCACCTGGGATAAAGCTTGATCAAATACCTTTATTTGTATGAGCAGTAGTAATTACTGCAGTTT
TATTACTATTATCCTTACCAGTTTTAGCAGGTGCTATTACTATA

Figure 17a： The DNA sequence interpret of COI gene of *Orthetrum sabina*

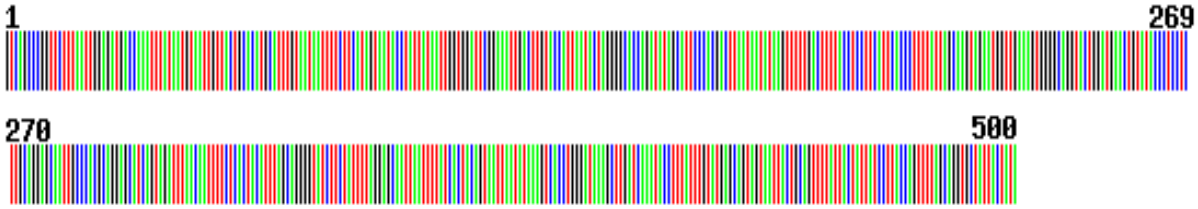


Figure 17b: Representative molecular barcode of COI gene of *Orthetrum sabina*

> ALC74204 *Orthetrum sabina* |cytochrome oxidase subunit I gene |voucher CUOS 01-A1 partial cds, mitochondrial|166bp

QPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMV
ESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVL
LLLSLPVLAGAITM

Figure 17c: The conceptual translation product of the COI gene of *Orthetrum sabina*

Orthetrum sabina isolate 140314 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID: KT961626. Length: 606Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
924 bits(500)	0.0	500/500(100%)	0/500(0%)	Plus/Plus

```
Query  1    GTCAGCCCGGTTCTTTAATTGGAGATGACCAAATTTATAATGTAATTGTTACTGCACATG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  17   GTCAGCCCGGTTCTTTAATTGGAGATGACCAAATTTATAATGTAATTGTTACTGCACATG  76
Query  61   CATTTGTAATAATTTTCTTCATAGTAATACCTATTATAATTGGTGGATTCGGAAATTGAC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  77   CATTTGTAATAATTTTCTTCATAGTAATACCTATTATAATTGGTGGATTCGGAAATTGAC  136
Query  121  TTGTACCATTAATACTAGGGGCACCAGATATAGCATTCCCACGACTTAATAATATAAGTT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  137  TTGTACCATTAATACTAGGGGCACCAGATATAGCATTCCCACGACTTAATAATATAAGTT  196
Query  181  TTTGACTTTTACCTCCTTCATTCACCCTTTTATTAGCAAGTAGAATGGTTGAAAGTGGGG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  197  TTTGACTTTTACCTCCTTCATTCACCCTTTTATTAGCAAGTAGAATGGTTGAAAGTGGGG  256
Query  241  CAGGTACTGGATGAACTGTATACCCTCCTCTTGCAGGAGCAATTGCCCACGCAGGAGCAT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  257  CAGGTACTGGATGAACTGTATACCCTCCTCTTGCAGGAGCAATTGCCCACGCAGGAGCAT  316
Query  301  CAGTAGATTTAACAATTTTCTCACTACATTTAGCAGGGGTATCTTCTATTTTAGGAGCAA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  317  CAGTAGATTTAACAATTTTCTCACTACATTTAGCAGGGGTATCTTCTATTTTAGGAGCAA  376
Query  361  TTAATTTTATCACTACAGTAATTAATATAAAGTCACCTGGGATAAAGCTTGATCAAATAC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  377  TTAATTTTATCACTACAGTAATTAATATAAAGTCACCTGGGATAAAGCTTGATCAAATAC  436
Query  421  CTTTATTTGTATGAGCAGTAGTAATTACTGCAGTTTTATTACTATTATCCTTACCAGTTT  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  437  CTTTATTTGTATGAGCAGTAGTAATTACTGCAGTTTTATTACTATTATCCTTACCAGTTT  496
Query  481  TAGCAGGTGCTATTACTATA  500
            ||||||||||||||||||||
Sbjct  497  TAGCAGGTGCTATTACTATA  516
```

Figure 17d: Nucleotide BLAST output of COI gene of *Orthetrum sabina* showing its nearest match subject

Cytochrome oxidase subunit I, partial (mitochondrion) [Orthetrumsabina]
Sequence ID: ALC74204.1_Length: 166Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps
323 bits(827)	1e-111	Compositional matrix adjust.	166/166 (100%)	166/166 (100%)	0/166 (0%)

```
Query  1    QPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSF  60
            QPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSF

Sbjct  1    QPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSF  60

Query  61   WLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAI  120
            WLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAI

Sbjct  61   WLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAI  120

Query  121  NFITTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITM  166
            NFITTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITM

Sbjct  121  NFITTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITM  166
```

Figure 17e: Peptide BLAST output of COI gene of *Orthetrum sabina*

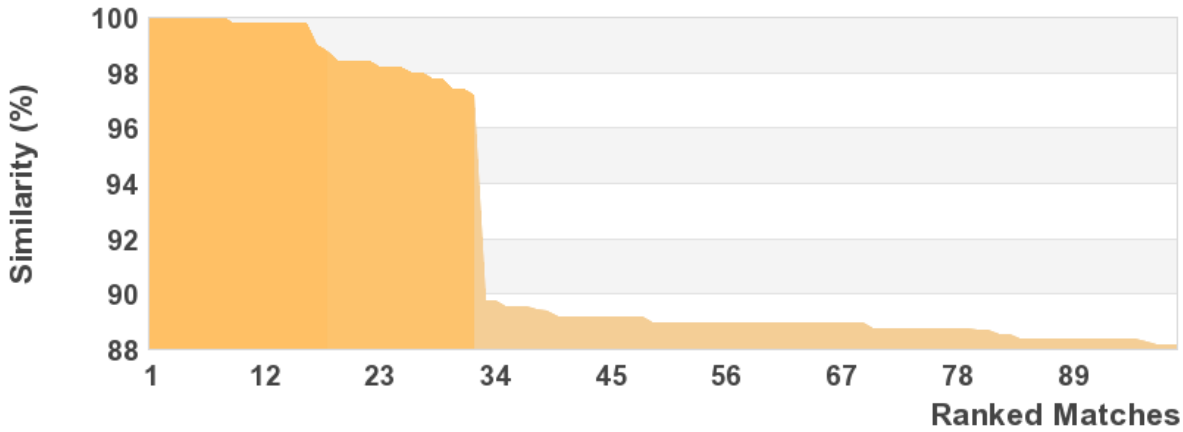


Figure 17f: The line diagram of *Orthetrum Sabina* with more than 99% match to other retrieved sequences (BOLD SYSTEM)

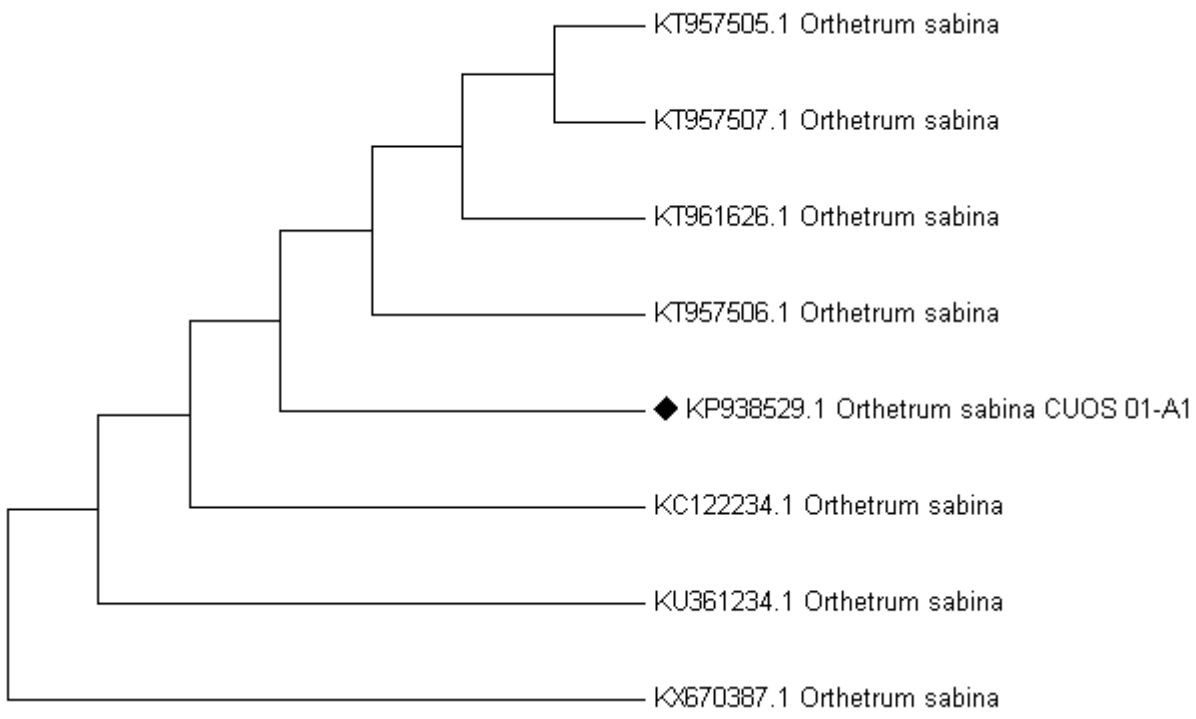


Figure17g: Molecular phylogenetic tree of *Orthetrum sabina* inferred by NJ tree method

Table 28: Percentage of evolutionary divergence of *Orthetrum sabina* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KP938529	*Orthetrum sabina* (Kerala)	
2	KT961626	*Orthetrum sabina* (Punjab)	0.00
3	KT957506	*Orthetrum sabina* (Thailand)	0.00
4	KT957505	*Orthetrum sabina* (Thailand)	0.00
5	KC12234	*Orthetrum sabina* (Mizoram)	0.00
6	KT957507	*Orthetrum sabina* (Thailand)	0.20
7	KU361234	*Orthetrum sabina* (Malaysia)	1.01
8	KX670387	*Orthetrum sabina* (Indonesia)	1.63

Fig 18: *Neuorothemis intermedia*

> KT222948 *Neurothemis intermedia* |cytochrome oxidase subunit I gene |voucher CUNI 01-A2 partial cds, mitochondrial|527bp

TTGTTACTGCACATGCTTTTGTAATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGTTTTGGTAATTGACT
TGTACCTTTAATACTAGGAGCTCCAGATATAGTGCCGTTAATACTTGGTGCTCCAGATATGGCCTTTCCACGACTC
AATAATATAAGATTTTGACTTTTACCCCCTTCTTTCACCTTACTGTTAGCCAGAAGTATAGTTGAAAGAGGGGCAG
GAACAGGATGAACAGTTTATCCCCCTCTAGCAGGGGCCATTGCACATGCCGGAGCATCTGTAGACTTAACAATTTT
TTCTCTTCATTTGGCGGGTGTTTCATCAATTTTAGGAGCAATTAATTTTATTACAACAGTAATTAATATGAAGTCT
CCTGGCATAAAGTTAGATCAGATACCCTTATTTGTATGGGCGGTAGTAATCACTGCAGTACTCCTATTATTATCCC
TGCCAGTTCTTGCTGGGGCTATTACTATACTATTAACTGACCGAAATATTAATACATCATTCTTTGATCCT

Figure 18a: The DNA sequence interpret of the COI gene of *Neurothemis intermedia*

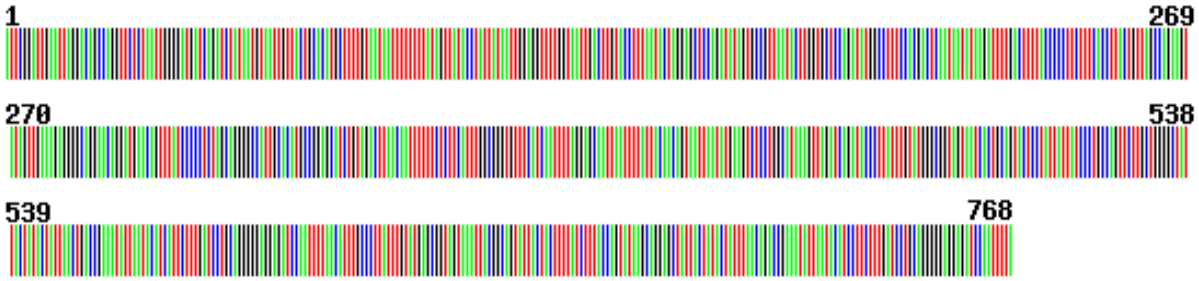


Figure18b: The Representative molecular barcode of the COI gene of *Neurothemis intermedia*

> ALQ35273 *Neurothemis intermedia* |cytochrome oxidase subunit I gene |voucher CUAP-03-A1 partial cds, mitochondrial| 204bp

IRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMVPLMLGAPDMAFPR
LNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITT
VINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAGGGDPIL

Figure 18c: The conceptual translation product of the COI gene of *Neurothemis intermedia*

Neurothemis intermedia voucher CUNI-01-A3 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

Sequence ID: KU052672. Length: 612Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
1131 bits(612)	0.0	612/612(100%)	0/612(0%)	Plus/Plus

```
Query  1    ATTCGGATTGAATTAGGACAGCCAGGTTCTCTAATTGGGGATGATCAGATCTATAATGTA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    ATTCGGATTGAATTAGGACAGCCAGGTTCTCTAATTGGGGATGATCAGATCTATAATGTA  60
Query        61ATTGTTACTGCACATGCTTTTGTAATAAtttttttATAGTTATACCTATTATAATTGGA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   ATTGTTACTGCACATGCTTTTGTAATAATTTTTTTTATAGTTATACCTATTATAATTGGA  120
Query  121  GGTTTTGGTAATTGACTTGTACCTTTAATACTAGGAGCTCCAGATATAGTGCCGTTAATA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  GGTTTTGGTAATTGACTTGTACCTTTAATACTAGGAGCTCCAGATATAGTGCCGTTAATA  180
Query  181  CTTGGTGCTCCAGATATGGCCTTTCCACGACTCAATAATATAAGATTTTGACTTTTACCC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CTTGGTGCTCCAGATATGGCCTTTCCACGACTCAATAATATAAGATTTTGACTTTTACCC  240
Query  241  CCTTCTTTCACCTTACTGTTAGCCAGAAGTATAGTTGAAAGAGGGGCAGGAACAGGATGA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  CCTTCTTTCACCTTACTGTTAGCCAGAAGTATAGTTGAAAGAGGGGCAGGAACAGGATGA  300
Query  301  ACAGTTTATCCCCCTCTAGCAGGGGCCATTGCACATGCCGGAGCATCTGTAGACTTAACA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  ACAGTTTATCCCCCTCTAGCAGGGGCCATTGCACATGCCGGAGCATCTGTAGACTTAACA  360
Query  361  ATTTTTTCTCTTCATTTGGCGGGTGTTTCATCAATTTTAGGAGCAATTAATTTTATTACA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  ATTTTTTCTCTTCATTTGGCGGGTGTTTCATCAATTTTAGGAGCAATTAATTTTATTACA  420
Query  421  ACAGTAATTAATATGAAGTCTCCTGGCATAAAGTTAGATCAGATACCCTTATTTGTATGG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  ACAGTAATTAATATGAAGTCTCCTGGCATAAAGTTAGATCAGATACCCTTATTTGTATGG  480
Query  481  GCGGTAGTAATCACTGCAGTACTCCTATTATTATCCCTGCCAGTTCTTGCTGGGGCTATT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  GCGGTAGTAATCACTGCAGTACTCCTATTATTATCCCTGCCAGTTCTTGCTGGGGCTATT  540
Query  541  ACTATACTATTAACTGACCGAAATATTAATACATCATTCTTTGATCCTGCAGGGGGAGGA  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  ACTATACTATTAACTGACCGAAATATTAATACATCATTCTTTGATCCTGCAGGGGGAGGA  600
Query  601  GATCCAATTTTA  612
            ||||||||||||
Sbjct  601  GATCCAATTTTA  612
```

Figure 18d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Neurothemis intermedia* showing its nearest match subject

Cytochrome oxidase subunit I, partial (mitochondrion) [*Neurothemis intermedia]*
Sequence ID: ALQ35273. Length: 204Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps
396 bits (1017)	2e-139	Compositional matrix adjust.	204/204 (100%)	204/204 (100%)	0/204 (0%)

```
Query  1    IRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMVPLM  60
            IRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMVPLM

Sbjct  1    IRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMVPLM  60

Query  61   LGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLT  120
            LGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLT

Sbjct  61   LGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLT  120

Query  121  IFSLHLAGVSSILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAI  180
            IFSLHLAGVSSILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAI

Sbjct  121  IFSLHLAGVSSILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAI  180

Query  181  TMLLTDRNINTSFFDPAGGGDPIL  204
            TMLLTDRNINTSFFDPAGGGDPIL
Sbjct  181  TMLLTDRNINTSFFDPAGGGDPIL  204
```

Figure 18e: Peptide BLAST output of the mt DNA COI gene of *Neurothemis intermedia*

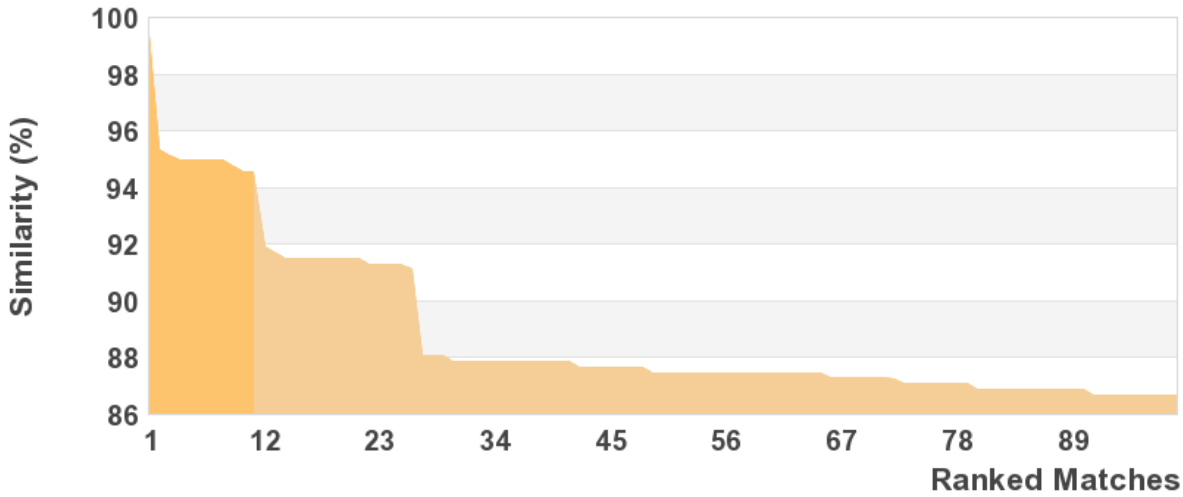


Figure 18f: The line diagram of *Neurothemis intermedia* with more than 99 % match to other retrieved sequences(BOLD SYSTEM)

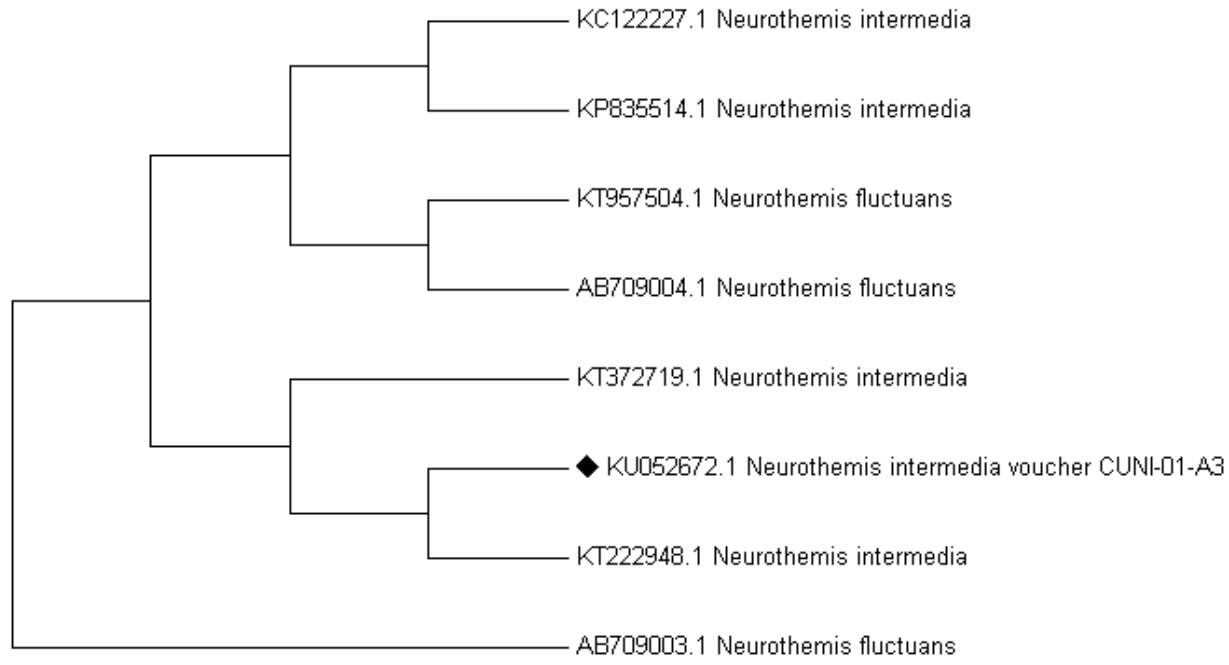


Figure 18g: Molecular phylogenetic tree of of *Neurothemis intermedia* inferred by NJ tree method

Table 30: Percentage of evolutionary divergence of *Neurothemis intermedia* with closely related species

Sl. No.	Accession No	Species name	% of divergence
1	KU052672.	*Neurothemis intermedia CUNI (Kerala)*	0.00%
2	KT222948	*Neurothemis intermedia (Kerala)*	0.00%
3	KP835514	*Neurothemis intermedia(Kerala)*	0.00%
4	KT372719	*Neurothemis intermedia (Kerala)*	0.00%
5	KC122227	*Neurothemis intermedia (Mizoram)*	1.74%
6	KT957504	*Neurothemis fluctans*	1.44%
7	AB709004	*Neurothemis fluctans*	1.15%
8	AB709003	*Neurothemis fluctans*	6.11%

Figure 19: *Potamarcha obscura*

> KX503060 *Potamarcha obscura* |cytochrome oxidase subunit I gene |voucher CUPO-01-A1 partial cds, mitochondrial|635bp

ATAGTAGGAACATCTTTAAGATTACTAATTCGAACTGAATTAGGAAACCCAGGATTTCTAATTGGAGACGATCAAA
TTTATAATACTATTGTAACAGCTCATGCTTTTATTATAATTTTTTTTATAGTAATACCTATTATAATTGGAGGATT
CGGAAATTGATTAGTGCCTTTAATATTAGGAGCTCCTGATATAGCTTTCCCACGAATAAATAATATAAGATTTTGA
TTACTCCCTCCTTCATTAACTTTATTAATTTCAAGAAGAATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTGT
ACCCCCCACTTTCATCTAACATTGCTCATAGAGGTTCTTCAGTAGATTTAGCCATTTTTTCTTTACATTTAGCTGG
AATTTCTTCAATTTTAGGAGCCATTAATTTTATTACAACTATTATTAATATACGTATTAGAAATATATCATTTGAC
CAAATACCATTATTTGTATGAGCTGTAGGAATCACAGCTTTACTTTTATTACTATCATTGCCAGTTCTAGCAGGTG
CAATTACAATACTTTTAACAGACCGAAATTTAAATACTTCATTTTTTGACCCAGCTGGAGGAGGAGATCCAATTCT
TTACCAACACTTGTTTTGATTTTTT

Figure 19a: The DNA sequence interpret of the COI gene of *Potamarcha obscura*

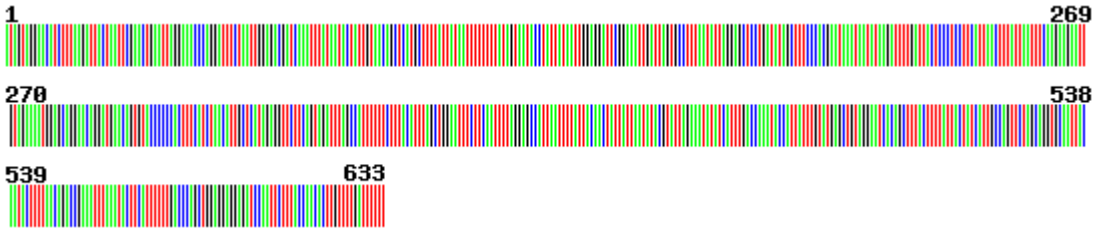


Figure 19b: Representative molecular barcode of COI gene of *Potamarcha obscura*

> ANU39520 *Potamarcha obscura* |cytochrome oxidase subunit I gene |voucher CUPO-01-A1 partial cds, mitochondrial|210bp

MVGTSLSLLIRTELGNPGFLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRM
NNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHSGSSVDLAIFSLHLAGISSILGAINFITTIINMR
ISNMSFDQMPLFVWVGITALLLLLSLPVLAGAITMLLTDRNLNTSFFDPAGGGDPILYQHLFWFF

Figure 19c: The conceptual translation product of the COI gene of *Potamarcha obscura*

Potamarcha obscura voucher CUPC-01-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

Sequence ID: KX503060.1 Length: 635 Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
1170 bits(635)	0.0	635/635(100%)	0/635(0%)	Plus/Plus

```
Query  1    ATAGTAGGAACATCTTTAAGATTACTAATTCGAACTGAATTAGGAAACCCAGGATTTCTA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    ATAGTAGGAACATCTTTAAGATTACTAATTCGAACTGAATTAGGAAACCCAGGATTTCTA  60
Query  61ATTGGAGACGATCAAATTTATAATACTATTGTAACAGCTCATGCTTTTATTATAAttttt  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   ATTGGAGACGATCAAATTTATAATACTATTGTAACAGCTCATGCTTTTATTATAATTTTT  120
Query  121tttATAGTAATACCTATTATAATTGGAGGATTCGGAAATTGATTAGTGCCTTTAATATTA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  TTTATAGTAATACCTATTATAATTGGAGGATTCGGAAATTGATTAGTGCCTTTAATATTA  180
Query  181  GGAGCTCCTGATATAGCTTTCCCACGAATAAATAATATAAGATTTTGATTACTCCCTCCT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  GGAGCTCCTGATATAGCTTTCCCACGAATAAATAATATAAGATTTTGATTACTCCCTCCT  240
Query  241  TCATTAACTTTATTAATTTCAAGAAGAATTGTAGAAAATGGAGCAGGAACAGGATGAACA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  TCATTAACTTTATTAATTTCAAGAAGAATTGTAGAAAATGGAGCAGGAACAGGATGAACA  300
Query  301GTGTACCCCCCACTTTCATCTAACATTGCTCATAGAGGTTCTTCAGTAGatttagccatt  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  GTGTACCCCCCACTTTCATCTAACATTGCTCATAGAGGTTCTTCAGTAGATTTAGCCATT  360
Query  361ttttctttacatttagctggaatttcttcaattttaggagccattaattttaTTACAACT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  TTTTCTTTACATTTAGCTGGAATTTCTTCAATTTTAGGAGCCATTAATTTTATTACAACT  420
Query  421  ATTATTAATATACGTATTAGAAATATATCATTTGACCAAATACCATTATTTGTATGAGCT  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  ATTATTAATATACGTATTAGAAATATATCATTTGACCAAATACCATTATTTGTATGAGCT  480
Query  481  GTAGGAATCACAGCTTTACTTTTATTACTATCATTGCCAGTTCTAGCAGGTGCAATTACA  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  GTAGGAATCACAGCTTTACTTTTATTACTATCATTGCCAGTTCTAGCAGGTGCAATTACA  540
Query  541  ATACTTTTAACAGACCGAAATTTAAATACTTCATTTTTTGACCCAGCTGGAGGAGGAGAT  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  ATACTTTTAACAGACCGAAATTTAAATACTTCATTTTTTGACCCAGCTGGAGGAGGAGAT  600
Query  601  CCAATTCTTTACCAACACTTGTTTTGATTTTTT  635
            ||||||||||||||||||||||||||||||||
Sbjct  601  CCAATTCTTTACCAACACTTGTTTTGATTTTTT  635
```

Figure 19d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Potamarcha obscura* showing its nearest match subject

Cytochrome oxidase subunit I, partial (mitochondrion) [*Potamarcha obscura*]

Sequence ID: ANU39520.1 Length: 211 Number of Matches: 1

Score	Expect	Method	Identities	Positives	Gaps
411 bits (1057)	4e-145	Compositional matrix adjust.	211/211 (100%)	211/211 (100%)	0/211 (0%)

```
Query  1    MVGTSLSLLIRTELGNPGFLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWLVPLML  60
            MVGTSLSLLIRTELGNPGFLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWLVPLML
Sbjct  1    MVGTSLSLLIRTELGNPGFLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWLVPLML  60

Query  61   GAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHSGSSVDLAI  120
            GAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHSGSSVDLAI
Sbjct  61   GAPDMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHSGSSVDLAI  120
```

Figure 19e: Peptide BLAST output of the mt DNA COI gene of *Potamarcha obscura*

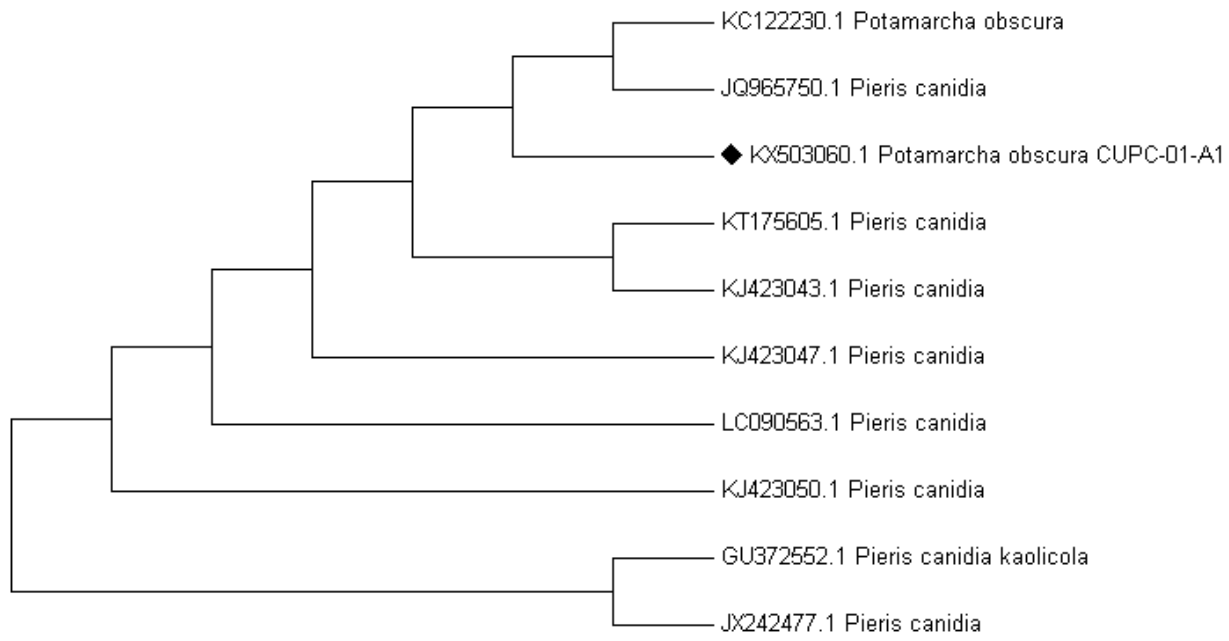


Figure 19f: Moleular Phylogenetic tree of *Potamarcha obscura* inferred by NJ tree method

Table 32: Percentage of evolutionary divergence of *Potamarcha obscura* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KX503060	*Potamarcha obscura* (Kerala)	
2	KC122230	*Potamarcha obscura* (Mizoram)	0.00
3	KT175605	*Pieris canidia*	0.33
4	LC090563	*Pieris canidia*	0.16
5	JQ965750	*Pieris canidia*	0.00
6	GU372552	*Pieris canidia kaolicola*	1.16
7	KJ423050	*Pieris canidia*	0.16
8	JX242477	*Pieris canidia*	0.83
9	KJ423047	Pieris canidia	0.33
10	KJ423043	Pieris canidia	0.33

Figure 20: *Brachydiplax chalybaea*

> KT372721 *Brachydiplax chalybaea* |cytochrome oxidase subunit I gene |voucher CUBC 02-A1 partial cds, mitochondrial|574 bp

GAGTTAGGACAACCTGACTCATTAATCGGAGATGTTCAAGTTTATAATGTAATTGTCACAGCACATGCATTTGTCA
TAATTTTCTTTATAGTTTACCAATCATAATTGGAGGATTCGGCAACTGACTTGTACCTTTAATATTAGGAGCTCCA
GATATAGCATTCCCACGTTTAAATAACATAAGATTTTGACTTTTACCACCATCATTCACTTTATTATTAGCAAGAA
GAATGGTTGAAAGAGGGGCAGGAACAGGATGAACCGTTTATCCTCCACTAGCGGGAGCTATTGCTCATGCAGGAGC
ATCCGTTGATTTAACAATTTTTTCTCTTCATTTAGCAGGAGTATCCTCAATTCTAGGTGCAATTAACTTTATTACA
ACAGTAATCAATATAAAGTCACCTGGGATAAAAATAGATCAAATACCCCTATTTGTATGGGCAGTAGTAATTACCG
CCGTACTTCTTTTGTTATCACTTCCGGTATTAGCTGGAGCAATTACTATACTATTAACCGATCGAAATATTAATAC
CTCATTCTTTGATCCCGCAGGAGGGGGAGATCCTATTTTAT

Figure 20a: The DNA sequence interpret of COI gene of *Brachydiplax chalybea*

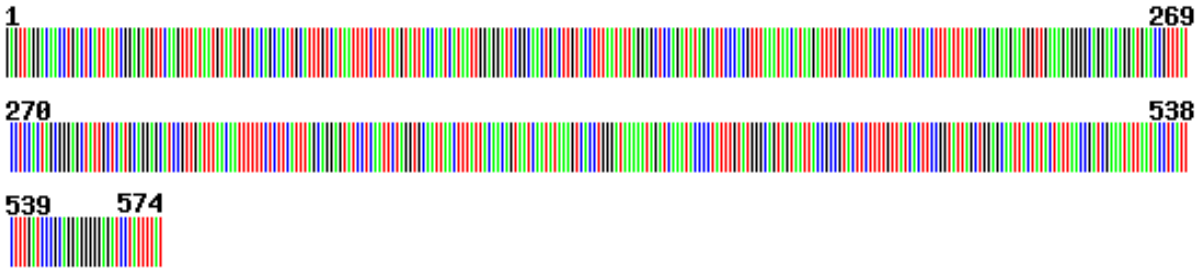


Figure 20b: Representative molecular barcode of the mt DNA COI gene of *Brachydiplax chalybea*

> AGD98696 *Brachydiplax chalybea*|cytochrome oxidase subunit I gene |voucher CUBC 02-A1 partial cds, mitochondrial|191 bp

ELGQPDSLIGDVQVYNVIVTAHAFVMIFFMVLPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLAS
SMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMKMDQMPLFVWAVVIT
AVLLLLSLPVLAGAITMLLTDRNINTSFFDPAGGGDPIL

Figure 20c: The conceptual translation product of the COI gene of *Brachydiplax chalybe*
Brachydiplax chalybea isolate voucher CUBC 02-A1 cytochrome oxidase I subunit gene, partial cds; mitochondrial
Sequence ID: KT372721.1Length: 574Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
1061 bits(574)	0.0	574/574(100%)	0/574(0%)	Plus/Plus

```
Query  1    GAGTTAGGACAACCTGACTCATTAATCGGAGATGTTCAAGTTTATAATGTAATTGTCACA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GAGTTAGGACAACCTGACTCATTAATCGGAGATGTTCAAGTTTATAATGTAATTGTCACA  60
Query  61   GCACATGCATTTGTCATAATTTTCTTTATAGTATTACCAATCATAATTGGAGGATTCGGC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GCACATGCATTTGTCATAATTTTCTTTATAGTATTACCAATCATAATTGGAGGATTCGGC  120
Query  121  AACTGACTTGTACCTTTAATATTAGGAGCTCCAGATATAGCATTCCCACGTTTAAATAAC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  AACTGACTTGTACCTTTAATATTAGGAGCTCCAGATATAGCATTCCCACGTTTAAATAAC  180
Query  181  ATAAGATTTTGACTTTTACCACCATCATTCACTTTATTATTAGCAAGAAGAATGGTTGAA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  ATAAGATTTTGACTTTTACCACCATCATTCACTTTATTATTAGCAAGAAGAATGGTTGAA  240
Query  241  AGAGGGGCAGGAACAGGATGAACCGTTTATCCTCCACTAGCGGGAGCTATTGCTCATGCA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  AGAGGGGCAGGAACAGGATGAACCGTTTATCCTCCACTAGCGGGAGCTATTGCTCATGCA  300
Query  301  GGAGCATCCGTTGATTTAACAATTTTTTCTCTTCATTTAGCAGGAGTATCCTCAATTCTA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  GGAGCATCCGTTGATTTAACAATTTTTTCTCTTCATTTAGCAGGAGTATCCTCAATTCTA  360
Query  361  GGTGCAATTAACTTTATTACAACAGTAATCAATATAAAGTCACCTGGGATAAAAATAGAT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  GGTGCAATTAACTTTATTACAACAGTAATCAATATAAAGTCACCTGGGATAAAAATAGAT  420
Query  421  CAAATACCCCTATTTGTATGGGCAGTAGTAATTACCGCCGTACTTCTTTTGTTATCACTT  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  CAAATACCCCTATTTGTATGGGCAGTAGTAATTACCGCCGTACTTCTTTTGTTATCACTT  480
Query  481  CCGGTATTAGCTGGAGCAATTACTATACTATTAACCGATCGAAATATTAATACCTCATTC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  CCGGTATTAGCTGGAGCAATTACTATACTATTAACCGATCGAAATATTAATACCTCATTC  540
Query  541  TTTGATCCCGCAGGAGGGGGAGATCCTATTTTAT  574
            ||||||||||||||||||||||||||||||||||
Sbjct  541  TTTGATCCCGCAGGAGGGGGAGATCCTATTTTAT  574
```

Figure 20d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Brachydiplax chalybaea* showing its nearest match subject.

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps

```
373 bits (957)  2e-130   Compositional matrix adjust.   191/191 (100%)   191/191 (100%)   0/191 (0%)

Query  1    ELGQPDSLIGDVQVYNVIVTAHAFVMIFFMVLPIMIGGFGNWLVPLMLGAPDMAFPRLNN  60
            ELGQPDSLIGDVQVYNVIVTAHAFVMIFFMVLPIMIGGFGNWLVPLMLGAPDMAFPRLNN
Sbjct  4    ELGQPDSLIGDVQVYNVIVTAHAFVMIFFMVLPIMIGGFGNWLVPLMLGAPDMAFPRLNN  63

Query  61   MSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSIL  120
            MSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSIL
Sbjct  64   MSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSIL  123

Query  121  GAINFITTVINMKSPGMKMDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSF  180
            GAINFITTVINMKSPGMKMDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSF
Sbjct  124  GAINFITTVINMKSPGMKMDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSF  183

Query  181  FDPAGGGDPIL  191
            FDPAGGGDPIL
Sbjct  184  FDPAGGGDPIL  194

Query  121  FSLHLAGISSILGAINFITTIINMRISNMSFDQMPLFVWAVGITALLLLLSLPVLAGAIT  180
            FSLHLAGISSILGAINFITTIINMRISNMSFDQMPLFVWAVGITALLLLLSLPVLAGAIT
Sbjct  121  FSLHLAGISSILGAINFITTIINMRISNMSFDQMPLFVWAVGITALLLLLSLPVLAGAIT  180

Query  181  MLLTDRNLNTSFFDPAGGGDPILYQHLFWFF  211
            MLLTDRNLNTSFFDPAGGGDPILYQHLFWFF
Sbjct  181  MLLTDRNLNTSFFDPAGGGDPILYQHLFWFF  211
```

Figure 20e: Peptide BLAST output of the mt DNA COI gene of *Brachydiplax chalybea*

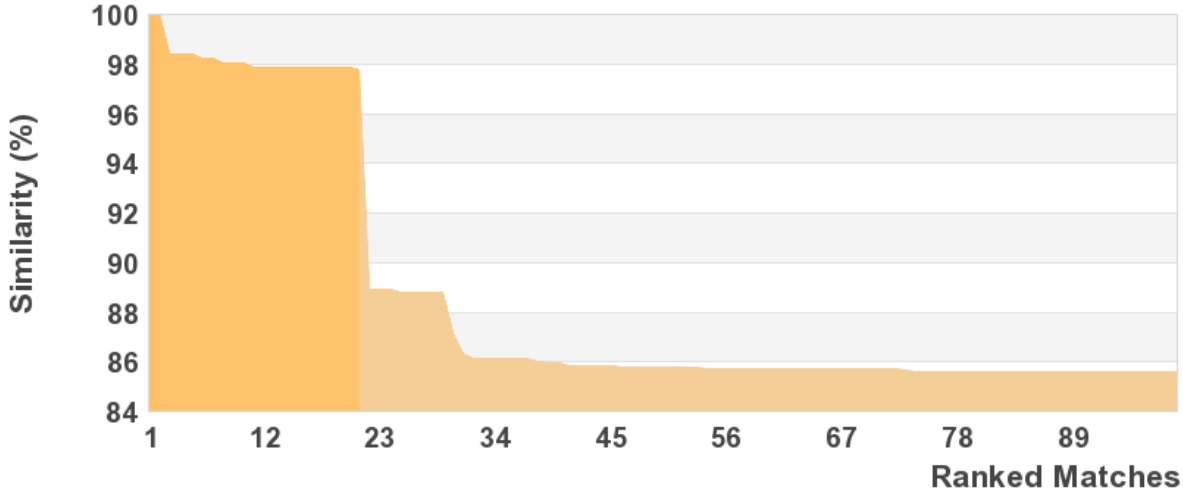


Figure 20f: The line diagram of *Brachydiplax chalybaea* with more than 99% match to other retrieved sequences (BOLD SYSTEM)

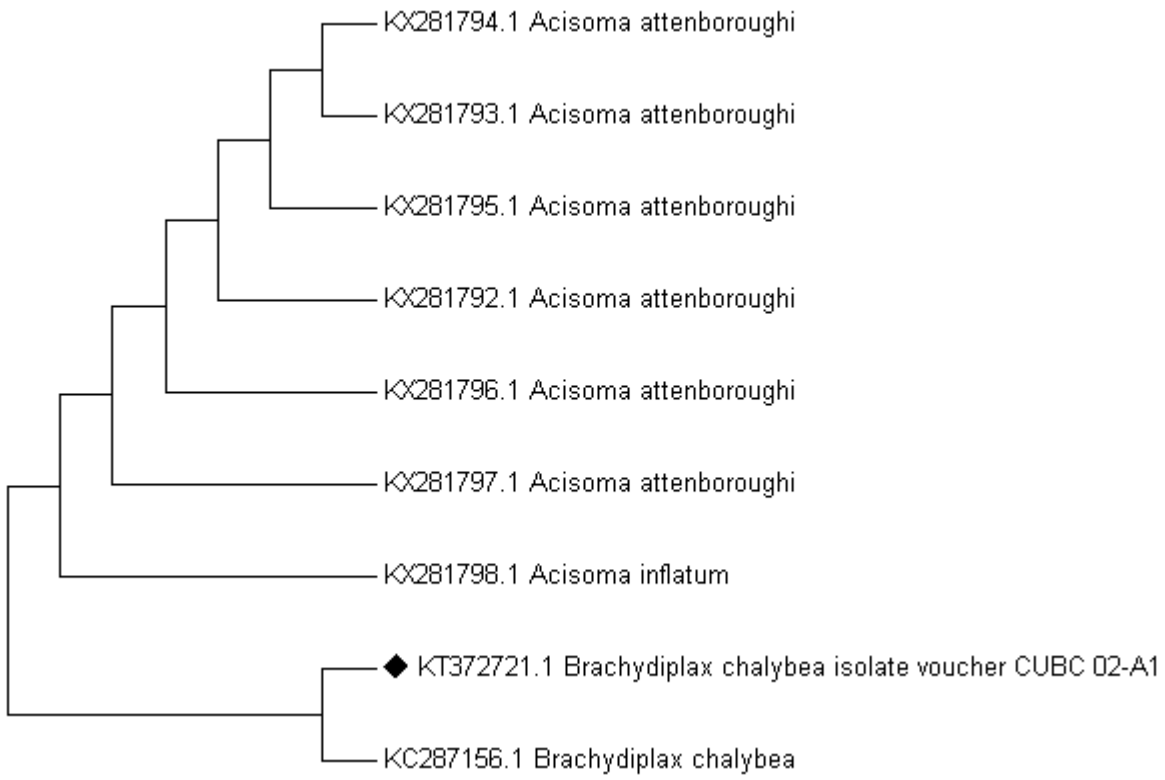


Figure 20g: Molecular Phylogenetic tree of *Brachydiplax chalybaea* inferred by NJ tree method

Table 34: Percentage of evolutionary divergence of *Brachydiplax chalybeae* with itsclosely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KT372721	*Brachdiplax chalybea* (Kerala)	
2	KC287156	*Brachdiplax chalybea* (Mizoram)	0.00
3	KC281798	*Acisoma inflatum*	21.44
4	KC281797	*Acisoma attenboroughi*	21.35
5	KC281792	*Acisoma attenboroughi*	21.43
6	KC281796	*Acisoma attenboroughi*	21.70
7	KC281795	*Acisoma attenboroughi*	21.79
8	KC281794	*Acisoma attenboroughi*	21.79
9	KC281793	*Acisoma attenboroughi*	21.79

Figure 21: *Trithemis aurora*

> KT305963 *Trithemis aurora* |cytochrome oxidase subunit I gene |voucher CUTA-01-A3 partial cds, mitochondrial|606bp

GTCCTAATTCGAATTGAATTGGGACAGCCAGGGTCACTAATTGGTGATGACCAAATTTATAATGTTATTGTAACAG
CACACGCATTTGTAATAATTTTCTTTATAGTTATACCAATCATAATTGGTGGATTTGGTAATTGATTAGTGCCATT
AATATTAGGGGCACCAGATATAGCATTCCCACGTCTAAATAATATAAGATTTTGACTTCTCCCACCATCATTCACG
TTATTACTAGCAAGAAGAATAGTAGAAAGAGGAGCAGGAACAGGATGAACAGTTTATCCTCCTCTTGCAGGAGCAA
TTGCTCATGCTGGAGCATCTGTAGACTTAACTATTTTTTCTTTACATCTTGCAGGAGTTTCATCAATTTTAGGTGC
TATCAATTTTATTACAACAGTAATTAATATAAAATCCCCAGGAATAAAACTAGATCAATTACCTGTATTTGTATGA
GCCGTAGTAATTACAGCAGTACTATTATTATTATCACTACCAGTACTAGCGGGGGCAATTACAATATTATTAACAG
ATCGTAATATTAATACATCATTCTTTGATCCTGCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTCTGA

Fig 21a: The DNA sequence interpret of the mitochondrial COI gene of *Trithemis aurora*

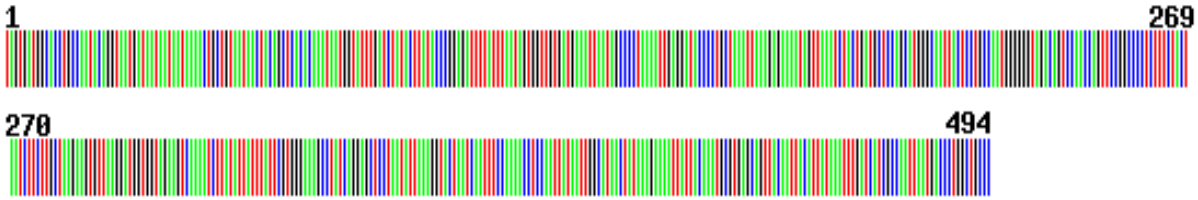


Figure 21b: Representative molecular barcode of the COI gene of *Trithemis aurora*

> AFI62049 *Trithemis aurora* |cytochrome oxidase subunit I gene |voucher CUTA-01-A3 partial cds, mitochondrial|202bp

VLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSFT
LLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMKLDQLPVFVW
AVVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAGGGDPILYQHLFW

Figure 21c: The conceptual translation product of the COI gene of *Trithemis aurora*

Trithemis aurora voucher CUTA 01-A3 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID: KT305963. Length: 606Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
1120 bits(606)	0.0	606/606(100%)	0/606(0%)	Plus/Plus

```
Query  1    GTCCTAATTCGAATTGAATTGGGACAGCCAGGGTCACTAATTGGTGATGACCAAATTTAT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GTCCTAATTCGAATTGAATTGGGACAGCCAGGGTCACTAATTGGTGATGACCAAATTTAT  60
Query  61   AATGTTATTGTAACAGCACACGCATTTGTAATAATTTTCTTTATAGTTATACCAATCATA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   AATGTTATTGTAACAGCACACGCATTTGTAATAATTTTCTTTATAGTTATACCAATCATA  120
Query  121  ATTGGTGGATTTGGTAATTGATTAGTGCCATTAATATTAGGGGCACCAGATATAGCATTC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  ATTGGTGGATTTGGTAATTGATTAGTGCCATTAATATTAGGGGCACCAGATATAGCATTC  180
Query  181  CCACGTCTAAATAATATAAGATTTTGACTTCTCCCACCATCATTCACGTTATTACTAGCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CCACGTCTAAATAATATAAGATTTTGACTTCTCCCACCATCATTCACGTTATTACTAGCA  240
Query  241  AGAAGAATAGTAGAAAGAGGAGCAGGAACAGGATGAACAGTTTATCCTCCTCTTGCAGGA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  AGAAGAATAGTAGAAAGAGGAGCAGGAACAGGATGAACAGTTTATCCTCCTCTTGCAGGA  300
Query  301  GCAATTGCTCATGCTGGAGCATCTGTAGACTTAACTATTTTTTCTTTACATCTTGCAGGA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  GCAATTGCTCATGCTGGAGCATCTGTAGACTTAACTATTTTTTCTTTACATCTTGCAGGA  360
Query  361  GTTTCATCAATTTTAGGTGCTATCAATTTTATTACAACAGTAATTAATATAAAATCCCCA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  GTTTCATCAATTTTAGGTGCTATCAATTTTATTACAACAGTAATTAATATAAAATCCCCA  420
Query  421  GGAATAAAACTAGATCAATTACCTGTATTTGTATGAGCCGTAGTAATTACAGCAGTACTA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  GGAATAAAACTAGATCAATTACCTGTATTTGTATGAGCCGTAGTAATTACAGCAGTACTA  480
Query  481  TTATTATTATCACTACCAGTACTAGCGGGGGCAATTACAATATTATTAACAGATCGTAAT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  TTATTATTATCACTACCAGTACTAGCGGGGGCAATTACAATATTATTAACAGATCGTAAT  540
Query  541  ATTAATACATCATTCTTTGATCCTGCAGGTGGTGGAGATCCAATTTTATATCAACATTTA  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  ATTAATACATCATTCTTTGATCCTGCAGGTGGTGGAGATCCAATTTTATATCAACATTTA  600
Query  601  TTCTGA  606
            ||||||
Sbjct  601  TTCTGA  606
```

Figure 21d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Trithemis aurora* showing its nearest match subject

Cytochrome oxidase subunit 1, partial (mitochondrion) [*Trithemis aurora*]
Sequence ID: AFI62049.1 Length: 205Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps
396 bits (1017)	3e-139	Compositional matrix adjust.	202/202 (100%)	202/202 (100%)	0/202 (0%)

```
Query  1    VLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAF  60
            VLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAF
Sbjct  2    VLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAF  61
Query  61   PRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAG  120
            PRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAG
Sbjct  62   PRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAG  121
Query  121  VSSILGAINFITTVINMKSPGMKLDQLPVFVWAVVITAVLLLLSLPVLAGAITMLLTDRN  180
            VSSILGAINFITTVINMKSPGMKLDQLPVFVWAVVITAVLLLLSLPVLAGAITMLLTDRN
Sbjct  122  VSSILGAINFITTVINMKSPGMKLDQLPVFVWAVVITAVLLLLSLPVLAGAITMLLTDRN  181
Query  181  INTSFFDPAGGGDPILYQHLFW  202
            INTSFFDPAGGGDPILYQHLFW
Sbjct  182  INTSFFDPAGGGDPILYQHLFW  203
```

Figure 21e : Peptide BLAST output of the mt DNA COI gene of *Trithemis aurora*

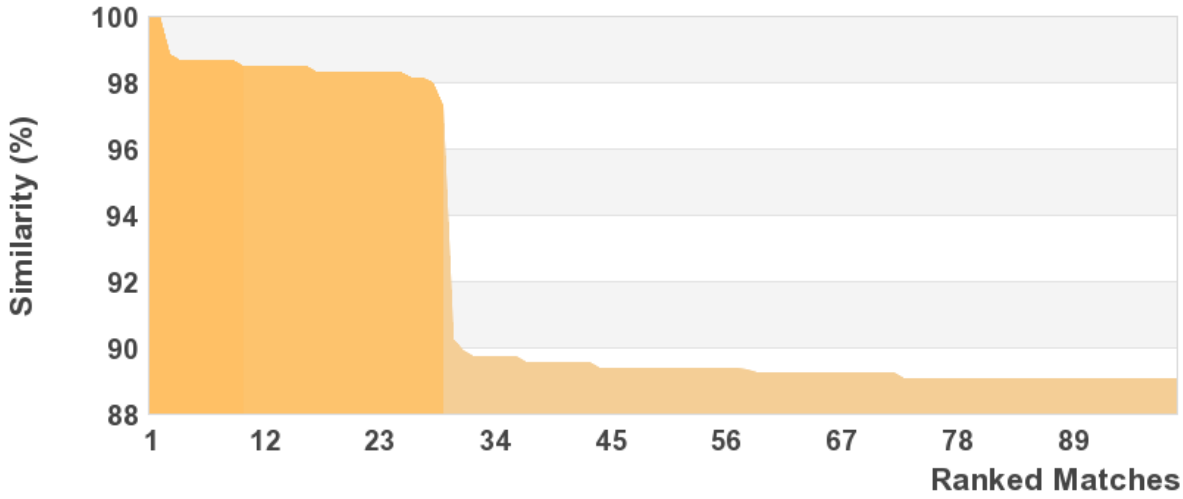


Figure 21f: The line diagram of *Trithemis aurora* with more than 99% match to other retrieved sequences (BOLD SYSTEM)

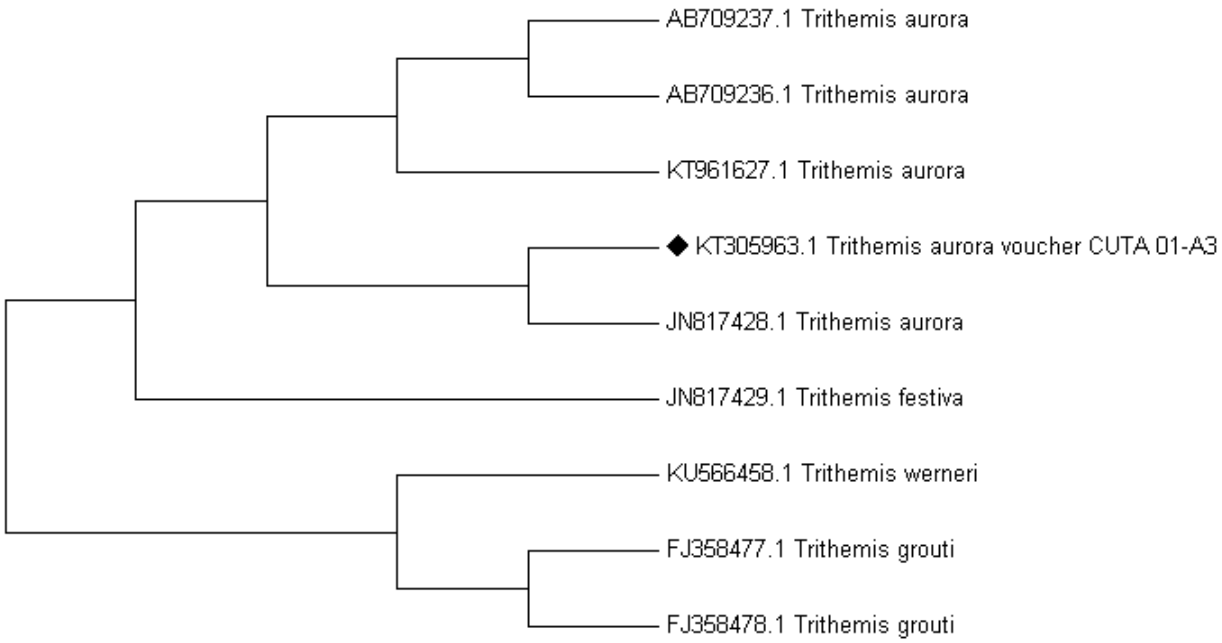


Figure 21g: Molecular Phylogenetic tree of *Trithemis aurora* inferred by NJ tree method

Table 36: Percentage of evolutionary divergence of *Trithemis aurora* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KT305963	*Trithemis aurora* (Kerala)	
2	JN817428	*Trithemis aurora* (Mizoram)	0.00
3	KT961627	*Trithemis aurora* (Punjab)	1.30
4	AB709236	*Trithemis aurora* (Japan)	2.00
5	AB709237	*Trithemis aurora* (Japan)	1..5 0
6	KU566458	*Trithemis werneri*	14.35
7	FJ358477	*Trithemis grouti*	13.41
8	FJ358478	*Trithemis grouti*	13.69
9	JN817429	*Trithemis festiva*	13.33

Fig 22: *Neurothemis fulvia*

>KP835515.1 Neurothemis fulvia voucher CUNF 01-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

ATTCGCATTGAATTAGGACAACCCGGATCATTAATTGGGGATGACCAGATTTATAATGTAATTGTCACTGCCCACG
CTTTTGTAATAATTTTCTTCATGGTAATGCCCATTATAATTGGTGGTTTCGGTAACTGGCTAGTCCCACTGATACT
CGGAGCACCTGACATGGCTTTCCCGCGACTTAATAACATAAGATTTTGACTTCTACCACCCTCTTTTACTTTATTA
TTAGCTAGAAGTTTAGTAGAAAGAGGAGCAGGAACGGGGTGAACAGTATATCCCCCCCTAGCAGGAGCCATTGCAC
ATGCCGGGGCATCTGTAGATTTAACAATTTTTTCACTTCATCTGGCAGGGGTTTCATCAATTCTGGGTGCTATTAA
TTTTATTACCACAGTAATTAATATAAAGTCTCCTGGAATAAAACTAGATCAATTACCCTTATTTGTATGGGCAGTA
GTAATTACTGCAGTACTCCTACTATTGTCTTTACCAGTTCTTGCTGGTGCTATTACAATACTATTAACCGACCGAA
ATATTAATACATCATTTTTTGATCCTGCAGGAGGTGGTGATCCAATTTTATATCAACATTTATTCTGA

Figure 22a: The DNA sequence interpret of the COI gene of *Neurothemis fulvia*

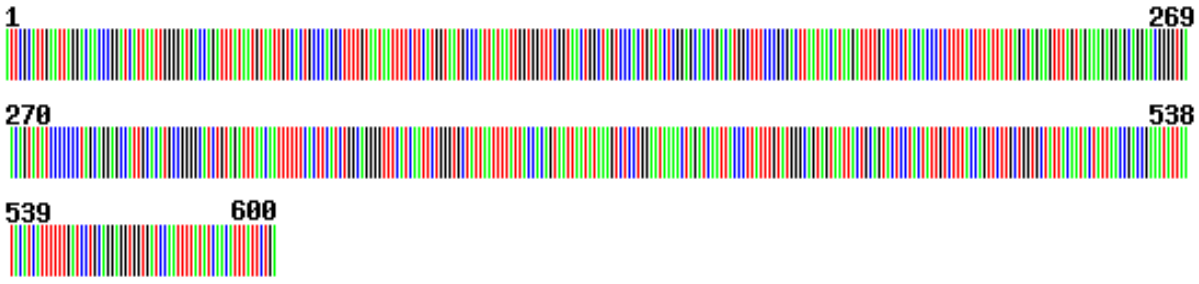


Figure 22b: Representative molecular barcode of the COI gene of *Neurothemis fulvia*

>AF162048 *Neurothemis fulvia* |cytochrome oxidase subunit I gene |voucher CUNF 01-A1 partial cds, mitochondrial|200bp

IRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSFTLL
LASSLVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMKLDQLPLFVWAV
VITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAGGGDPILYQHLFW

Figure 22c: The conceptual translation product of the COI gene of *Neurothemis fulvia*

Neurothemis fulvia voucher CUNF 01-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID: KP835515.1Length: 600Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
1109 bits(600)	0.0	600/600(100%)	0/600(0%)	Plus/Plus

```
Query  1    ATTCGCATTGAATTAGGACAACCCGGATCATTAATTGGGGATGACCAGATTTATAATGTA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    ATTCGCATTGAATTAGGACAACCCGGATCATTAATTGGGGATGACCAGATTTATAATGTA  60
Query  61   ATTGTCACTGCCCACGCTTTTGTAATAATTTTCTTCATGGTAATGCCCATTATAATTGGT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   ATTGTCACTGCCCACGCTTTTGTAATAATTTTCTTCATGGTAATGCCCATTATAATTGGT  120
Query  121  GGTTTCGGTAACTGGCTAGTCCCACTGATACTCGGAGCACCTGACATGGCTTTCCCGCGA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  GGTTTCGGTAACTGGCTAGTCCCACTGATACTCGGAGCACCTGACATGGCTTTCCCGCGA  180
Query  181  CTTAATAACATAAGATTTTGACTTCTACCACCCTCTTTTACTTTATTATTAGCTAGAAGT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CTTAATAACATAAGATTTTGACTTCTACCACCCTCTTTTACTTTATTATTAGCTAGAAGT  240
Query  241TTAGTAGAAAGAGGAGCAGGAACGGGGTGAACAGTATATccccccTAGCAGGAGCCATT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  TTAGTAGAAAGAGGAGCAGGAACGGGGTGAACAGTATATCCCCCCCTAGCAGGAGCCATT  300
Query  301  GCACATGCCGGGGCATCTGTAGATTTAACAATTTTTTCACTTCATCTGGCAGGGGTTTCA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  GCACATGCCGGGGCATCTGTAGATTTAACAATTTTTTCACTTCATCTGGCAGGGGTTTCA  360
Query  361  TCAATTCTGGGTGCTATTAATTTTATTACCACAGTAATTAATATAAAGTCTCCTGGAATA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  TCAATTCTGGGTGCTATTAATTTTATTACCACAGTAATTAATATAAAGTCTCCTGGAATA  420
Query  421  AAACTAGATCAATTACCCTTATTTGTATGGGCAGTAGTAATTACTGCAGTACTCCTACTA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  AAACTAGATCAATTACCCTTATTTGTATGGGCAGTAGTAATTACTGCAGTACTCCTACTA  480
Query  481  TTGTCTTTACCAGTTCTTGCTGGTGCTATTACAATACTATTAACCGACCGAAATATTAAT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  TTGTCTTTACCAGTTCTTGCTGGTGCTATTACAATACTATTAACCGACCGAAATATTAAT  540
Query  541  ACATCATTTTTTGATCCTGCAGGAGGTGGTGATCCAATTTTATATCAACATTTATTCTGA  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  ACATCATTTTTTGATCCTGCAGGAGGTGGTGATCCAATTTTATATCAACATTTATTCTGA  600
```

Figure 22d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Neurothemis fulvia* showing its nearest match subject

Cytochrome oxidase subunit 1, partial (mitochondrion) [*Neurothemis fulvia*]
Sequence ID: AFI62048Length: 205Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps	Frame
392 bits(1006)	1e-137()	Compositional matrix adjust.	200/200(100%)	200/200(100%)	0/200(0%)	

Features:

```
Query  1    IRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPR 60
            IRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPR

Sbjct  4    IRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPR 63

Query  61   LNNMSFWLLPPSFTLLLASSLVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVS120
            LNNMSFWLLPPSFTLLLASSLVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVS

Sbjct  64   LNNMSFWLLPPSFTLLLASSLVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVS123

Query  121  SILGAINFITTVINMKSPGMKLDQLPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNIN180
            SILGAINFITTVINMKSPGMKLDQLPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNIN

Sbjct  124  SILGAINFITTVINMKSPGMKLDQLPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNIN183

Query  181  TSFFDPAGGGDPILYQHLFW  200
            TSFFDPAGGGDPILYQHLFW

Sbjct  184  TSFFDPAGGGDPILYQHLFW  203
```

Figure 22e: Peptide BLAST output of the mt DNA COI gene of *Neurothemis fulvia*

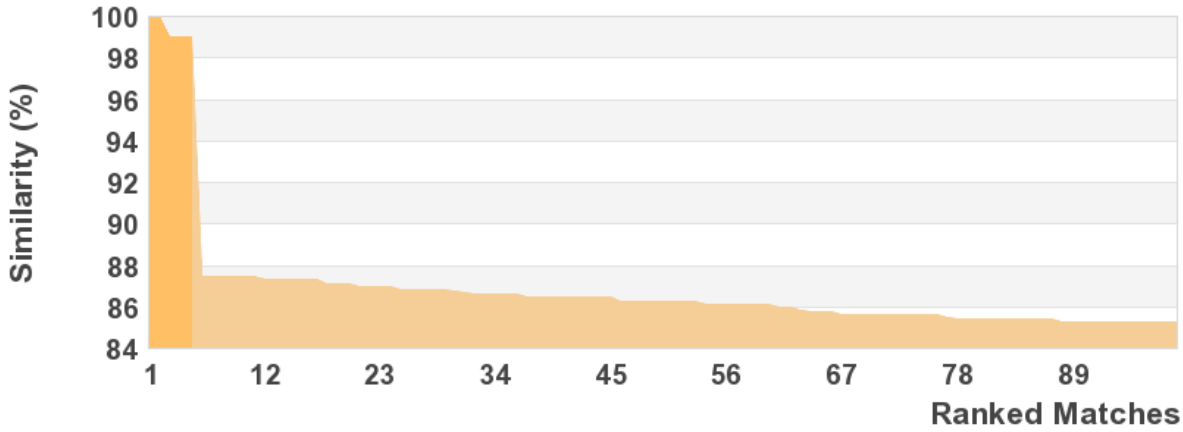


Figure 22f: The line diagram of *Neurothemis fulvia* with more than 99 % match sequences (BOLD SYSTEM)

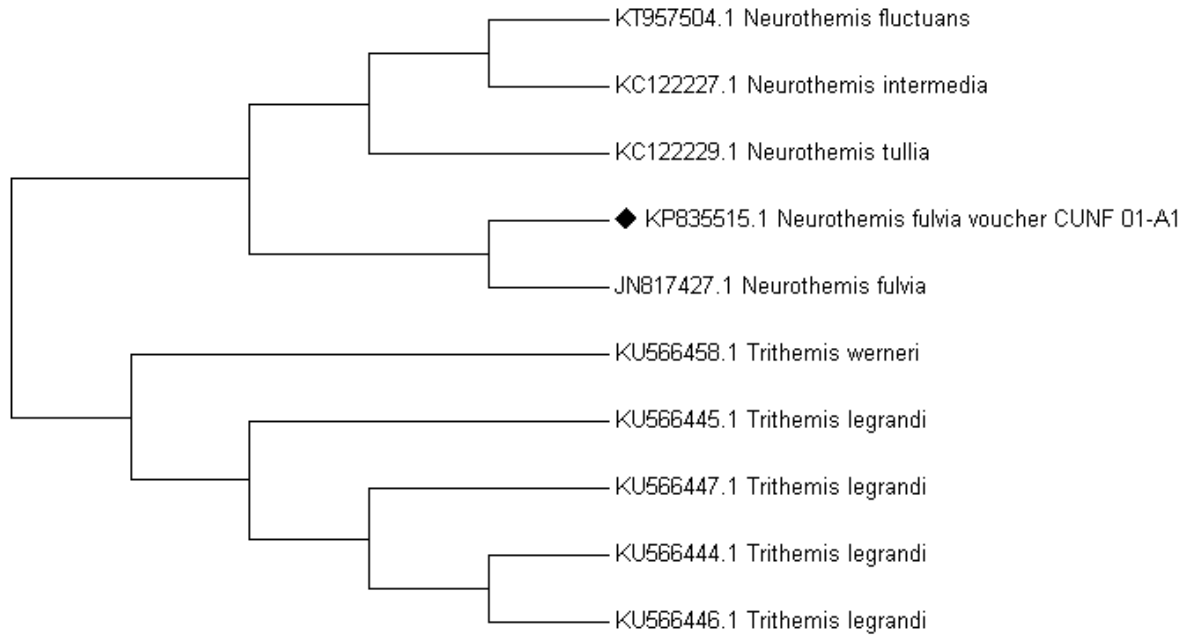


Figure 22g: Molecular phylogenetic tree of *Neurothemis fulvia* inferred by NJ tree method

Table 38: Percentage of evolutionary divergence of *Neurothemis fulvia* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KP835515	*Neurothemis fulvia (Kerala)*	
2	JN817427	*Neurothemis fulvia (Mizoram)*	0.00
3	KC12229	*Neurothemis tullia*	18.55
4	KT957504	*Neurothemis fluctans*	18.10
5	KC12227	*Neurothemis intermedia*	17.67
6	KU566458	*Trithemis werneri*	23.14
7	KU566447	*Trithemis legrandi*	23.92
8	KU566444	*Trithemis legrandi*	23.92
9	KU566446	*Trithemis legrandi*	23.92

Figure 23: Crocothemis servillia

> KR149807 *Crocothemis servilia* |cytochrome oxidase subunit I gene |voucher CUCS 02 A1 partial cds, mitochondrial|603b

CGAATTGAATTAGGTCAACCAGGATCACTAATTGGAGATGATCAAATTTATAATGTTATTGTGACCGCCCATGCAT
TTGTCATAATTTTCTTTATAGTAATACCTATTATAATTGGTGGATTTGGAAATTGATTAGTACCACTAATACTAGG
AGCACCTGATATAGCATTCCCACGATTAAATAATATAAGATTTTGACTTTTACCTCCTTCATTCACCCTACTATTA
GCAAGAAGTATAGTAGAAAGAGGAGCAGGAACTGGATGAACAGTCTACCCACCCTTAGCTGGTGCAATTGCTCACG
CAGGGGCTTCTGTAGATTTAACCATCTTTTCATTACACTTAGCTGGAGTATCATCAATTTTAGGAGCAATTAATTT
TATCACTACAGTAATTAATATAAAGTCTCCTGGTATAAAGTTGGATCAAATACCTTTATTTGTATGAGCAGTAGTA
ATTACTGCAGTATTACTTTTGTTATCTTTACCAGTTTTAGCGGGTGCTATTACTATACTTCTAACAGATCGTAATA
TTAATACATCATTCTTTGATCCAGCAGGAGGGGGGGATCCAATTTTATATCAACACTTATTTTGATTTTTT

Figure 23a: The DNA sequence interpret of COI gene of *Crocothemis servilia*

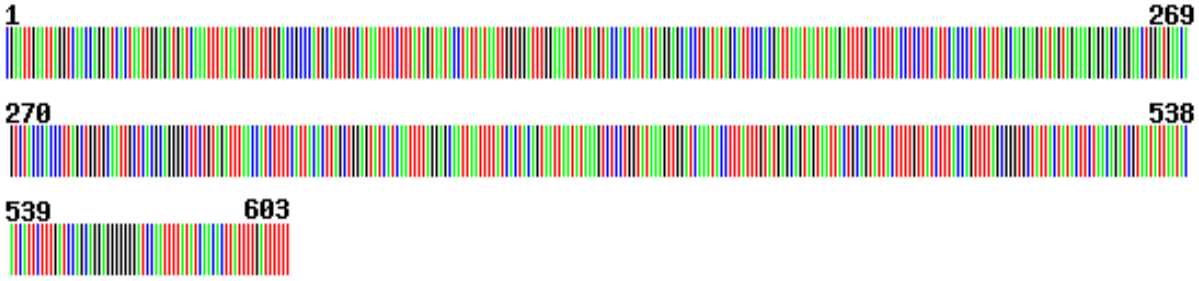


Figure 23b: Representative molecular barcode of the COI gene of *Crocothemis servilia*

> AFI62046 *Crocothemis servilia*|cytochrome oxidase subunit I gene |voucher CUCS 02 A1 partial cds, mitochondrial|201 bp

RIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLL
ASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMKLDQMPLFVWAVV
ITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAGGGDPILYQHLFWFF

Figure 23c: The conceptual translation product of the COI gene of *Crocothemis servilia*

Crocothemis servilia voucher CUCS 02-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID: KR149807.1Length: 603Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
1114 bits(603)	0.0	603/603(100%)	0/603(0%)	Plus/Plus

```
Query  1   CGAATTGAATTAGGTCAACCAGGATCACTAATTGGAGATGATCAAATTTATAATGTTATT  60
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1   CGAATTGAATTAGGTCAACCAGGATCACTAATTGGAGATGATCAAATTTATAATGTTATT  60
Query  61  GTGACCGCCCATGCATTTGTCATAATTTTCTTTATAGTAATACCTATTATAATTGGTGGA  120
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61  GTGACCGCCCATGCATTTGTCATAATTTTCTTTATAGTAATACCTATTATAATTGGTGGA  120
Query  121 TTTGGAAATTGATTAGTACCACTAATACTAGGAGCACCTGATATAGCATTCCCACGATTA  180
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121 TTTGGAAATTGATTAGTACCACTAATACTAGGAGCACCTGATATAGCATTCCCACGATTA  180
Query  181 AATAATATAAGATTTTGACTTTTACCTCCTTCATTCACCCTACTATTAGCAAGAAGTATA  240
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181 AATAATATAAGATTTTGACTTTTACCTCCTTCATTCACCCTACTATTAGCAAGAAGTATA  240
Query  241 GTAGAAAGAGGAGCAGGAACTGGATGAACAGTCTACCCACCCTTAGCTGGTGCAATTGCT  300
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241 GTAGAAAGAGGAGCAGGAACTGGATGAACAGTCTACCCACCCTTAGCTGGTGCAATTGCT  300
Query  301 CACGCAGGGGCTTCTGTAGATTTAACCATCTTTTCATTACACTTAGCTGGAGTATCATCA  360
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301 CACGCAGGGGCTTCTGTAGATTTAACCATCTTTTCATTACACTTAGCTGGAGTATCATCA  360
Query  361 ATTTTAGGAGCAATTAATTTTATCACTACAGTAATTAATATAAAGTCTCCTGGTATAAAG  420
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361 ATTTTAGGAGCAATTAATTTTATCACTACAGTAATTAATATAAAGTCTCCTGGTATAAAG  420
Query  421 TTGGATCAAATACCTTTATTTGTATGAGCAGTAGTAATTACTGCAGTATTACTTTTGTTA  480
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421 TTGGATCAAATACCTTTATTTGTATGAGCAGTAGTAATTACTGCAGTATTACTTTTGTTA  480
Query  481 TCTTTACCAGTTTTAGCGGGTGCTATTACTATACTTCTAACAGATCGTAATATTAATACA  540
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481 TCTTTACCAGTTTTAGCGGGTGCTATTACTATACTTCTAACAGATCGTAATATTAATACA  540
Query  541 TCATTCTTTGATCCAGCAGGAggggggATCCAATTTTATATCAACACTTATTTTGATTT  600
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541 TCATTCTTTGATCCAGCAGGAGGGGGGGATCCAATTTTATATCAACACTTATTTTGATTT  600
Query  601 TTT  603
           |||
Sbjct  601 TTT  603
```

Figure 23d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Crocothemis servilia* showing its nearest match subject

Cytochrome oxidase subunit 1, partial (mitochondrion) [*Crocothemis servilia*]
Sequence ID: AFI62046. Length: 205Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps
395 bits (1016)	3e-139	Compositional matrix adjust.	201/201 (100%)	201/201 (100%)	0/201 (0%)

```
Query  1    RIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRL  60

            RIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRL

Sbjct  5    RIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRL  64

Query  61   NNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSS  120

            NNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSS

Sbjct  65   NNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSS  124

Query  121  ILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINT  180

            ILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINT

Sbjct  125  ILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINT  184

Query  181  SFFDPAGGGDPILYQHLFWFF  201

            SFFDPAGGGDPILYQHLFWFF

Sbjct  185  SFFDPAGGGDPILYQHLFWFF  205
```

Figure 23e: Peptide BLAST output of the mt DNA COI gene of *Crocothemis servilia*

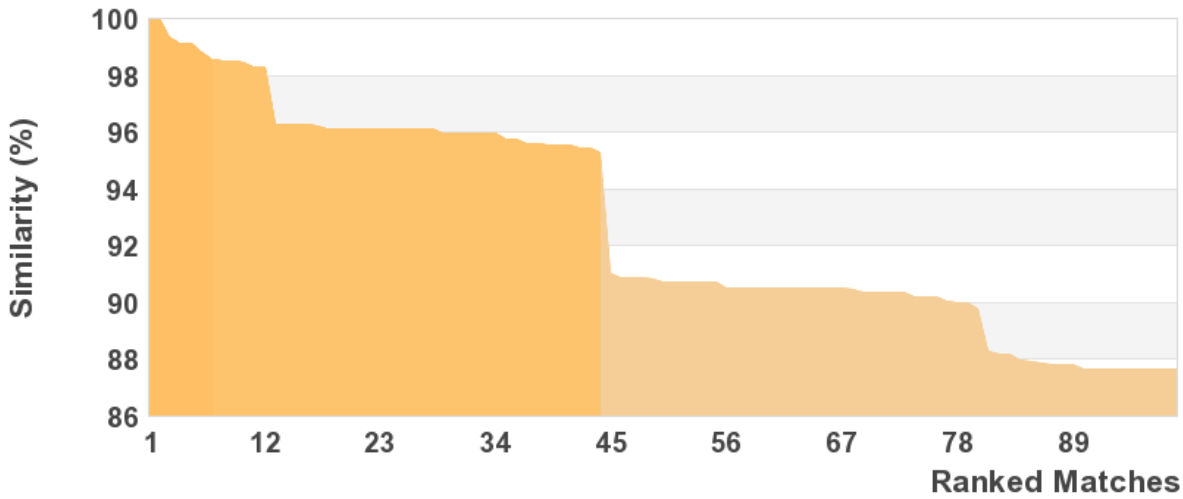


Figure 23f: The line diagram of *Crocothemis servilia* with more than 99 % match to other sequences retrieved (BOLD SYSTEM)

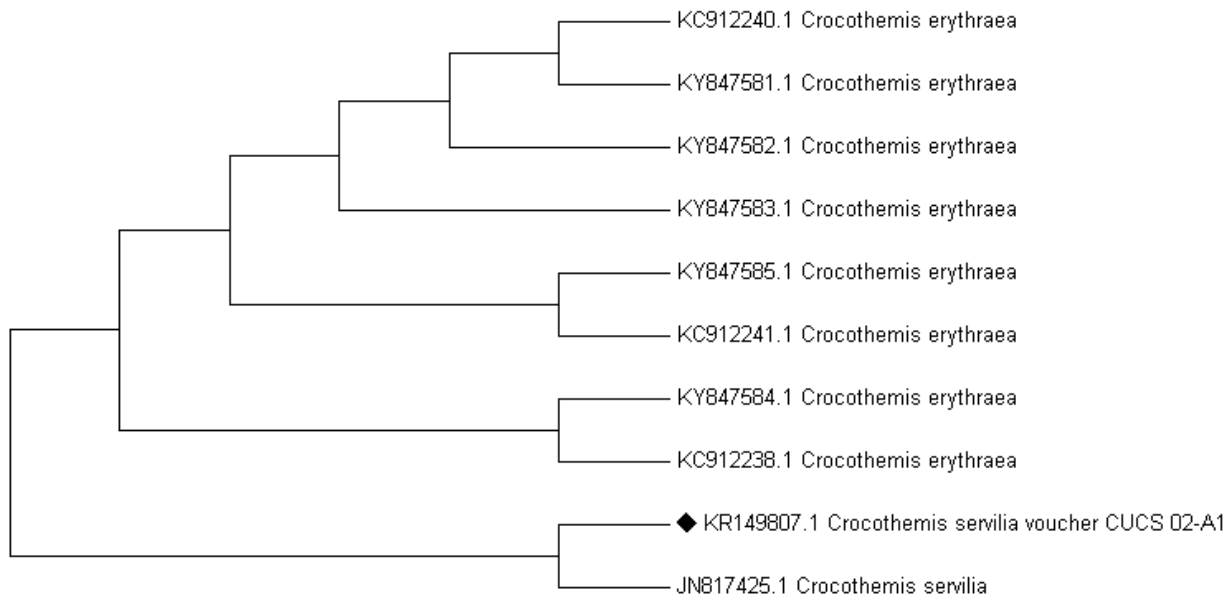


Figure 23g: Molecular Phylogenetic tree of *Crocothemis servilia* inferred by NJ tree method

Table 40: Percentage of evolutionary divergence of *Crocothemis servillia* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KR149807	*Crocothemis servillia* (Kerala)	
2	JN817425	*Crocothemis servillia* (Mizoram)	0.00
3	KC912338	*Crocothemis erythraea*	2.71
4	KC912240	*Crocothemis erythraea*	2.71
5	KY847581	*Crocothemis erythraea*	2.71
6	KY847582	*Crocothemis erythraea*	2.71
7	KY847583	*Crocothemis erythraea*	2.71
8	KY847584	*Crocothemis erythraea*	2.71
9	KY847585	*Crocothemis erythraea*	2.71
10	KC912238	*Crocothemis erythraea*	2.71

Figure 24: *Trithemis pallidinervis*

> KR149803 *Trithemis pallidinervis* |cytochrome oxidase subunit I gene |voucher CUTP 01-A1 partial cds, mitochondrial| 580bp

ACTGCTCTAAGTGTTTTAATTCGAATTGAATTAGGTCAACCTGGATCTCTAATTGGAGATGATCAAATTTATAATG
TTATTGTAACTGCCCATGCATTTGTAATAATTTTCTTCATGGTTATACCTATTATAATTGGTGGATTTGGTAATTG
ACTAGTGCCATTAATGTTAGGTGCACCAGATATAGCATTTCCACGACTTAATAATATAAGTTTTTGATTATTACCT
CCTTCATTTACACTTCTTCTAGCTAGAAGTATAGTTGAAAGTGGAGCAGGAACAGGATGAACTGTTTATCCTCCTC
TAGCTGGAGCTATTGCCCATGCAGGAGCATCCGTAGATTTAACTATTTTCTCATTACATTTGGCTGGAGTATCTTC
CATTTTAGGGGCTATTAATTTTATTACTACAGTAATTAATATAAAATCTCCTGGAATAAAATTAGATCAAATACCA
TTATTTGTATGAGCTGTAGTAATTACAGCAGTTCTATTATTATTATCATTACCAGTATTAGCAGGTGCTATTACCA
TACTATTAACTGATCGTAATATTAATACATCATTTTTTGACCCTGCAG

Figure 24a: The partial DNA sequence of the mitochondrial COI gene of *Trithemis pallidinervis*

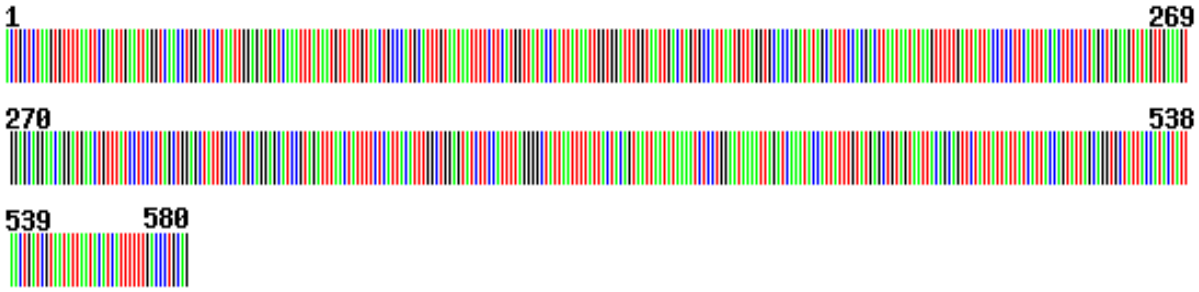


Figure 24b: Representative molecular barcode of the COI gene of *Trithemis pallidinervis*

> AKL82317 *Trithemis pallidinervis* |cytochrome oxidase subunit I gene |voucher CUAK-01-A1 partial cds, mitochondrial|193 bp

TALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLP
PSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMKLDQMP
LFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPA

Figure 24c: The conceptual translation product of the COI gene of *Trithemis pallidinervis*

Trithemis pallidinervis isolate RB11_F cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrialSequence ID: KT957509.1Length: 657Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
1072 bits(580)	0.0	580/580(100%)	0/580(0%)	Plus/Plus

```
Query 1    ACTGCTCTAAGTGTTTTAATTCGAATTGAATTAGGTCAACCTGGATCTCTAATTGGAGAT  60
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  43  ACTGCTCTAAGTGTTTTAATTCGAATTGAATTAGGTCAACCTGGATCTCTAATTGGAGAT  102
Query  61  GATCAAATTTATAATGTTATTGTAACTGCCCATGCATTTGTAATAATTTTCTTCATGGTT  120
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  103 GATCAAATTTATAATGTTATTGTAACTGCCCATGCATTTGTAATAATTTTCTTCATGGTT  162
Query  121 ATACCTATTATAATTGGTGGATTTGGTAATTGACTAGTGCCATTAATGTTAGGTGCACCA  180
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  163 ATACCTATTATAATTGGTGGATTTGGTAATTGACTAGTGCCATTAATGTTAGGTGCACCA  222
Query  181 GATATAGCATTTCCACGACTTAATAATATAAGTTTTTGATTATTACCTCCTTCATTTACA  240
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  223 GATATAGCATTTCCACGACTTAATAATATAAGTTTTTGATTATTACCTCCTTCATTTACA  282
Query  241 CTTCTTCTAGCTAGAAGTATAGTTGAAAGTGGAGCAGGAACAGGATGAACTGTTTATCCT  300
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  283 CTTCTTCTAGCTAGAAGTATAGTTGAAAGTGGAGCAGGAACAGGATGAACTGTTTATCCT  342
Query  301 CCTCTAGCTGGAGCTATTGCCCATGCAGGAGCATCCGTAGATTTAACTATTTTCTCATTA  360
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  343 CCTCTAGCTGGAGCTATTGCCCATGCAGGAGCATCCGTAGATTTAACTATTTTCTCATTA  402
Query  361 CATTTGGCTGGAGTATCTTCCATTTTAGGGGCTATTAATTTTATTACTACAGTAATTAAT  420
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  403 CATTTGGCTGGAGTATCTTCCATTTTAGGGGCTATTAATTTTATTACTACAGTAATTAAT  462
Query  421 ATAAAATCTCCTGGAATAAAATTAGATCAAATACCATTATTTGTATGAGCTGTAGTAATT  480
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  463 ATAAAATCTCCTGGAATAAAATTAGATCAAATACCATTATTTGTATGAGCTGTAGTAATT  522
Query  481 ACAGCAGTTCTATTATTATTATCATTACCAGTATTAGCAGGTGCTATTACCATACTATTA  540
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  523 ACAGCAGTTCTATTATTATTATCATTACCAGTATTAGCAGGTGCTATTACCATACTATTA  582
Query  541 ACTGATCGTAATATTAATACATCATTTTTTGACCCTGCAG  580
           ||||||||||||||||||||||||||||||||||||||||
Sbjct  583 ACTGATCGTAATATTAATACATCATTTTTTGACCCTGCAG  622
```

Figure 24d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Trithemis pallidinervis* showing its nearest match subject

Cytochrome oxidase subunit I, partial (mitochondrion) [*Trithemis pallidinervis*]
Sequence ID: AKL82317.1Length: 193 Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps	Frame
377 bits(967)	5e-132()	Compositional matrix adjust.	193/193(100%)	193/193(100%)	0/193(0%)	

Features:

```
Query  1    TALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAP  60
            TALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAP
Sbjct  1    TALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAP  60

Query  61   DMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSL  120
            DMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSL
Sbjct  61   DMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSL  120

Query  121  HLAGVSSILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLL  180
            HLAGVSSILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLL
Sbjct  121  HLAGVSSILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLL  180

Query  181  TDRNINTSFFDPA  193
            TDRNINTSFFDPA
Sbjct  181  TDRNINTSFFDPA  193
```

Figure 24e: Peptide BLAST output of the mt DNA COI gene of *Trithemis pallidinervis*

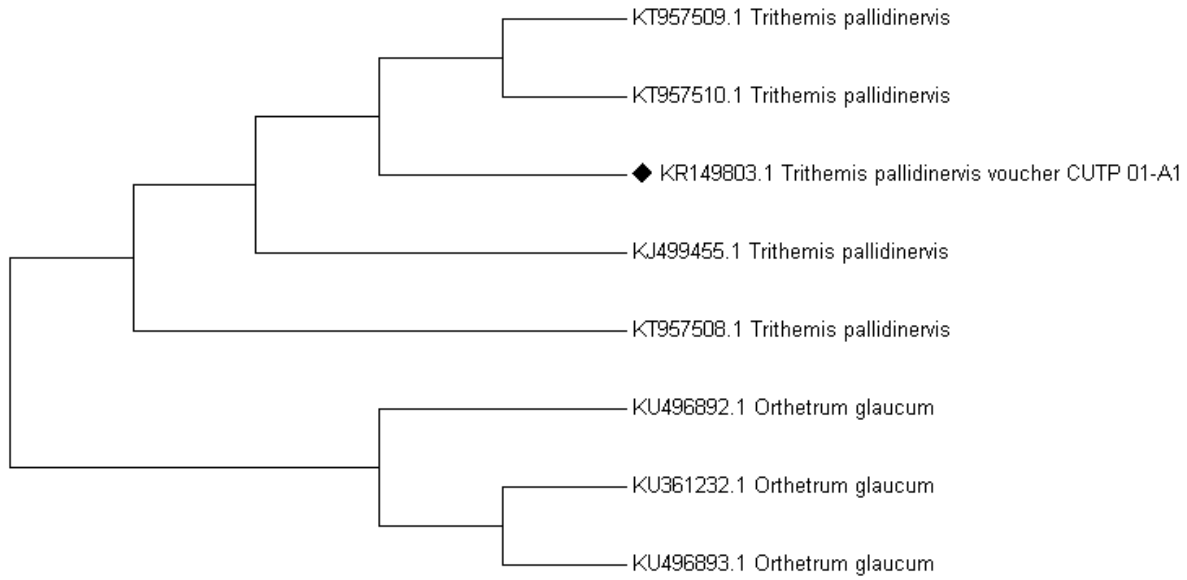


Figure 24f: Molecular Phylogenetic tree of *Trithemis pallidinervis* inferred by NJ tree method

Table 42: Percentage of evolutionary divergence of *Trithemis pallidinervis* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KR149803	*Trithemis pallidinervis* (Kerala)	
2	KJ499455	*Trithemis pallidinervis* (Mizoram)	0.00
3	KT957508	*Trithemis pallidinervis* (Thailand)	0.00
4	KT957509	*Trithemis pallidinervis* (Thailand)	0.00
5	KT957510	*Trithemis pallidinervis* (Thailand)	0.35
6	KU361232	*Trithemis glacum*	14.51
7	KU496892	*Trithemis glacum*	13.73
8	KU496893	*Trithemis glacum*	14.51

Figure 25: *Trithemis festiva*

> KR149802 *Trithemis festiva* |cytochrome oxidase subunit I gene |voucher CUTF 01-A1 partial cds, mitochondrial|567bp

GGATCTCTTATTGGAGATGATCAAATTTATAATGTTATTGTTACAGCACATGCATTTGTAATAATTTTTTTTATAG
TAATACCTATTATAATTGGTGGATTTGGTAATTGATTAGTACCTTTAATATTAGGAGCACCAGATATAGCATTTCC
ACGACTTAATAATATAAGATTCTGATTATTACCTCCTTCATTCACTCTATTATTAGCAAGAAGTATAGTAGAAAGA
GGTGCAGGAACAGGATGAACCGTATATCCTCCTCTAGCTGGAGCAATTGCTCATGCTGGAGCATCTGTAGACTTAA
CAATTTTTTCTCTTCATCTTGCAGGAGTATCATCAATTTTAGGAGCGATTAATTTTATTACAACAGTAATTAATAT
GAAATCACCTGGAATAAATCTAGATCAAATACCATTGTTTGTATGAGCTGTAGTAATTACTGCAGTATTATTATTA
TTATCACTTCCAGTTTTAGCAGGAGCTATTACAATATTATTGACAGATCGTAATATTAATACATCATTTTTTGATC
CTGCGGGAGGAGGAGATCCAATTTTATATCAGCAC

Figure 25a:The DNA sequence interpret of the COI gene of *Trithemis festiva*

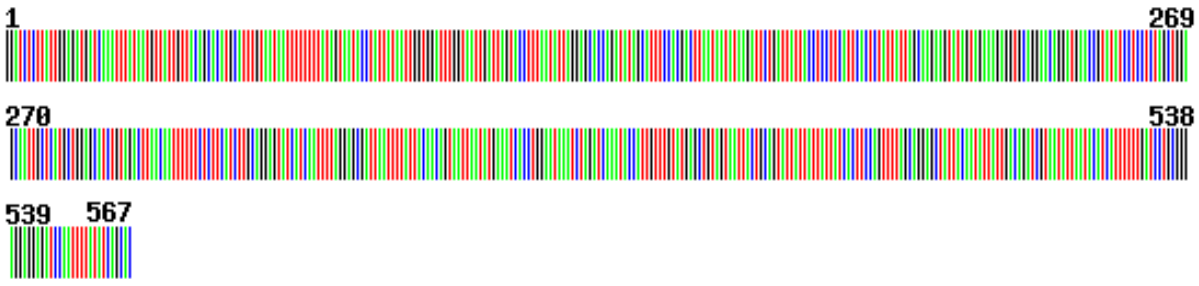


Figure 25b: Representative molecular barcode of the mt DNA COI gene of *Trithemis festiva*

> AKL82316 *Trithemis festiva* |cytochrome oxidase subunit I gene |voucher CUTF 01-A1 partial cds, mitochondrial|189bp

GSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVES
GAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMNLDQMPLFVWAVVITAVLLL
LSLPVLAGAITMLLTDRNINTSFFDPAGGGDPILYQH

Figure 25c: The conceptual translation product of the COI gene of *Trithemis festiva*

Trithemis festiva voucher CUTF 01-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

Sequence ID: KR149802.1Length: 567Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
1048 bits(567)	0.0	567/567(100%)	0/567(0%)	Plus/Plus

```
Query  1   GGATCTCTTATTGGAGATGATCAAATTTATAATGTTATTGTTACAGCACATGCATTTGTA  60
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1   GGATCTCTTATTGGAGATGATCAAATTTATAATGTTATTGTTACAGCACATGCATTTGTA  60
Query  61  ATAAtttttttATAGTAATACCTATTATAATTGGTGGATTTGGTAATTGATTAGTACCT  120
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61  ATAATTTTTTTTATAGTAATACCTATTATAATTGGTGGATTTGGTAATTGATTAGTACCT  120
Query  121 TTAATATTAGGAGCACCAGATATAGCATTTCCACGACTTAATAATATAAGATTCTGATTA  180
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121 TTAATATTAGGAGCACCAGATATAGCATTTCCACGACTTAATAATATAAGATTCTGATTA  180
Query  181 TTACCTCCTTCATTCACTCTATTATTAGCAAGAAGTATAGTAGAAAGAGGTGCAGGAACA  240
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181 TTACCTCCTTCATTCACTCTATTATTAGCAAGAAGTATAGTAGAAAGAGGTGCAGGAACA  240
Query  241 GGATGAACCGTATATCCTCCTCTAGCTGGAGCAATTGCTCATGCTGGAGCATCTGTAGAC  300
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241 GGATGAACCGTATATCCTCCTCTAGCTGGAGCAATTGCTCATGCTGGAGCATCTGTAGAC  300
Query  301 TTAACAATTTTTTCTCTTCATCTTGCAGGAGTATCATCAATTTTAGGAGCGATTAATTTT  360
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301 TTAACAATTTTTTCTCTTCATCTTGCAGGAGTATCATCAATTTTAGGAGCGATTAATTTT  360
Query  361 ATTACAACAGTAATTAATATGAAATCACCTGGAATAAATCTAGATCAAATACCATTGTTT  420
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361 ATTACAACAGTAATTAATATGAAATCACCTGGAATAAATCTAGATCAAATACCATTGTTT  420
Query  421 GTATGAGCTGTAGTAATTACTGCAGTATTATTATTATTATCACTTCCAGTTTTAGCAGGA  480
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421 GTATGAGCTGTAGTAATTACTGCAGTATTATTATTATTATCACTTCCAGTTTTAGCAGGA  480
Query  481 GCTATTACAATATTATTGACAGATCGTAATATTAATACATCATTTTTTGATCCTGCGGGA  540
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481 GCTATTACAATATTATTGACAGATCGTAATATTAATACATCATTTTTTGATCCTGCGGGA  540
Query  541  GGAGGAGATCCAATTTTATATCAGCAC  567
             |||||||||||||||||||||||||||
Sbjct  541  GGAGGAGATCCAATTTTATATCAGCAC  567
```

Figure 25d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Trithemis festiva* showing its nearest match subject

Cytochrome oxidase subunit I, partial (mitochondrion) [*Trithemisfestiva*]

Sequence ID: AKL82316Length: 189Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps
369 bits (947)	4e-129	Compositional matrix adjust.	189/189 (100%)	189/189 (100%)	0/189 (0%)

```
Query  1    GSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWL  60
            GSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWL
Sbjct  1    GSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWL  60

Query 61   LPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINF  120
           LPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINF
Sbjct 61 LPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINF  120

Query 121 ITTVINMKSPGMNLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAG  180
           ITTVINMKSPGMNLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAG
Sbjct 121 ITTVINMKSPGMNLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAG  180

Query  181  GGDPILYQH  189
             GGDPILYQH
Sbjct  181  GGDPILYQH  189
```

Figure 25e: Peptide BLAST output of the mt DNA COI gene of *Trithemis festiva*

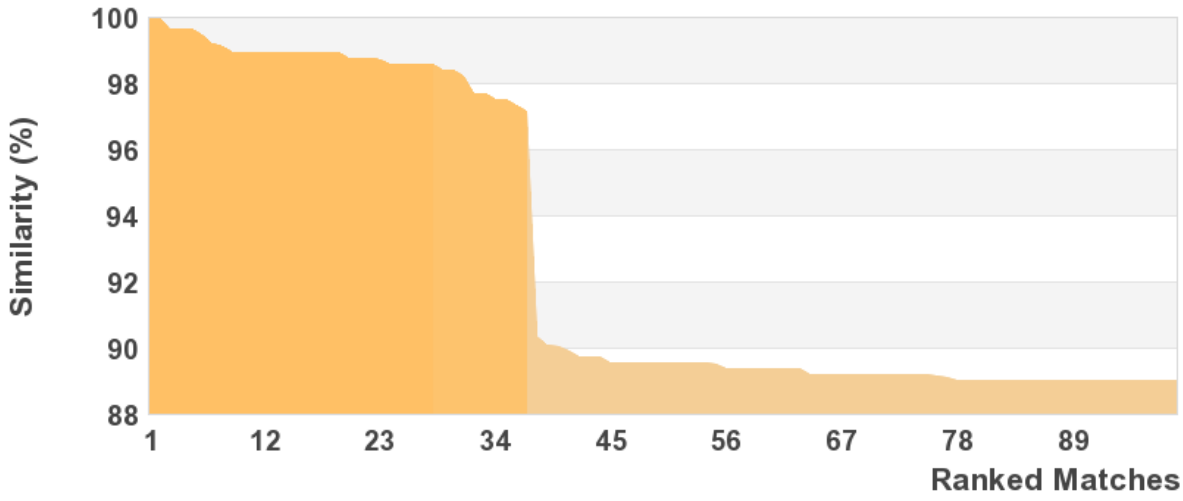


Figure 25f: The line diagram of *Trithemis festiva* with more than 99 % match to other retrieved sequences (BOLD SYSTEM)

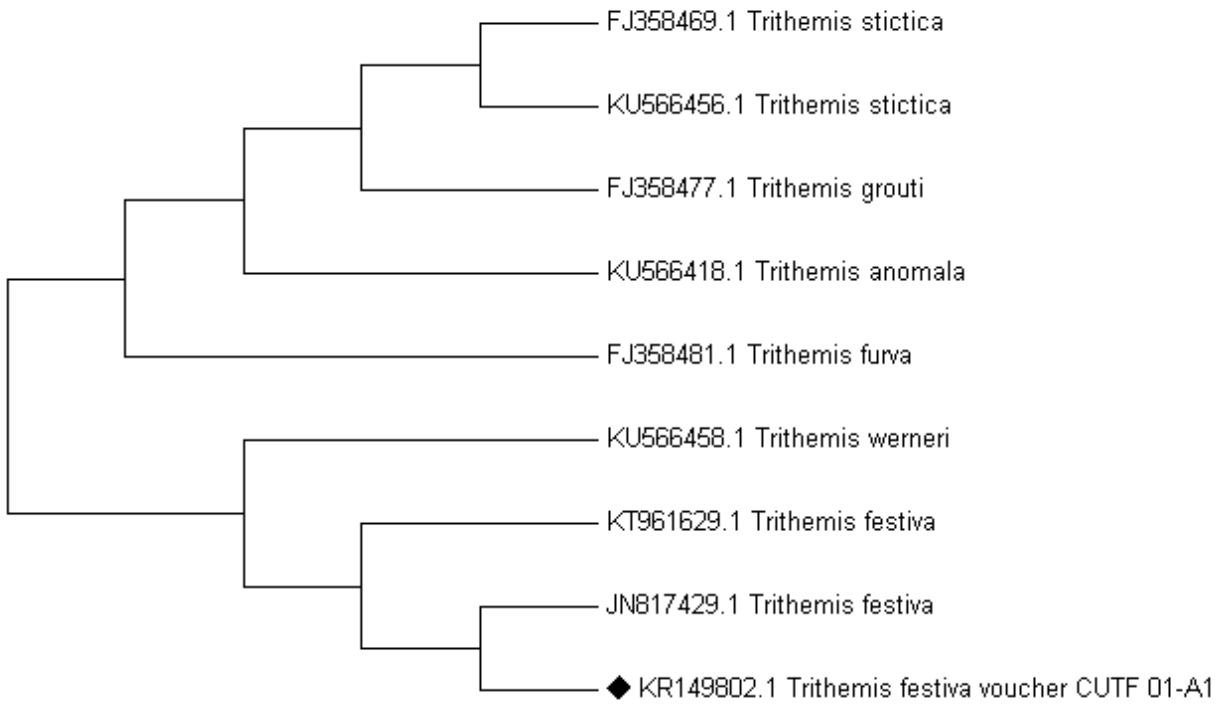


Figure 25g: Molecular Phylogenetic tree of *Trithemis festiva* inferred by NJ tree method

Table 44: Percentage of evolutionary divergence of *Trithemis festiva* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	JN149802	*Trithemis festiva* (Kerala)	
2	JN817429	*Trithemis festiva* (Mizoram)	0.00
3	KT961629	*Trithemis festiva* (Punjab)	1.10
4	FJ358477	*Trithemis stictica*	11.60
5	KU566456	*Trithemis grouti*	12.20
6	KU566456	*Trithemis stictica*	11.80
7	FJ358481	*Trithemis furva*	12.10
8	KU56645	*Trithemis werneri*	12.00

Figure 26: *Brachythemis contaminata*

>KP938531.1 *Brachythemis contaminata* voucher CUBC 01-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

GGGCAGGAATAATTGGTACAGCTTTAAGAGTATTAATTCGTATTGAATTAGGACAACCCGGATCCATAAT
TGGAGACGATCAAATTTATAATGTTATTGTAACAGCTCATGCATTTGTAATAATTTTCTTCATAGTAATA
CCAATTATAATTGGTGGTTTCGGAAATTGATTAGTACCATTAATATTAGGGGCACCTGATATGGCTTTCC
CCCGACTTAATAATATAAGATTTTGATTACTACCACCATCATTTACTTTACTTCTTGCAAGAAGTATAGT
TGAAAGAGGGGCAGGAACAGGATGAACAGTTTACCCACCATTAGCAGGGGCTATTGCCCATGCCGGTGCA
TCAGTTGATTTAACAATTTTCTCATTGCACCTA

Figure 26a: The DNA sequence interpret of COI gene of *Brachythemis contaminata*

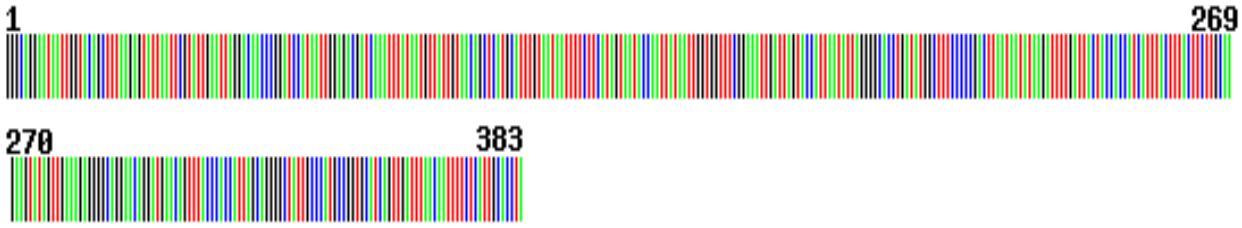


Figure 26b: Representative molecular barcode of COI gene of *Brachythemis contaminata.*

> AIT71754 *Brachythemis contaminata* |cytochrome oxidase subunit I gene |voucher CUAC-01-A1 partial cds, mitochondrial|155 bp

DDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTG
WTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPV
LAG

Figure 26c: The conceptual translation product of the COI gene of *Brachythemis contaminata*

Sequence ID: KP938531.1Length: 383Number of Matches: 1

Related Information

Range 1: 1 to 383GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
708 bits(383)	0.0	383/383(100%)	0/383(0%)	Plus/Plus

```
Query  1    GGGCAGGAATAATTGGTACAGCTTTAAGAGTATTAATTCGTATTGAATTAGGACAACCCG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GGGCAGGAATAATTGGTACAGCTTTAAGAGTATTAATTCGTATTGAATTAGGACAACCCG  60

Query  61   GATCCATAATTGGAGACGATCAAATTTATAATGTTATTGTAACAGCTCATGCATTTGTAA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GATCCATAATTGGAGACGATCAAATTTATAATGTTATTGTAACAGCTCATGCATTTGTAA  120

Query  121  TAATTTTCTTCATAGTAATACCAATTATAATTGGTGGTTTCGGAAATTGATTAGTACCAT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  TAATTTTCTTCATAGTAATACCAATTATAATTGGTGGTTTCGGAAATTGATTAGTACCAT  180

Query  181  TAATATTAGGGGCACCTGATATGGCTTTCCCCCGACTTAATAATATAAGATTTTGATTAC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  TAATATTAGGGGCACCTGATATGGCTTTCCCCCGACTTAATAATATAAGATTTTGATTAC  240

Query  241  TACCACCATCATTTACTTTACTTCTTGCAAGAAGTATAGTTGAAAGAGGGGCAGGAACAG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  TACCACCATCATTTACTTTACTTCTTGCAAGAAGTATAGTTGAAAGAGGGGCAGGAACAG  300

Query  301  GATGAACAGTTTACCCACCATTAGCAGGGGCTATTGCCCATGCCGGTGCATCAGTTGATT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  GATGAACAGTTTACCCACCATTAGCAGGGGCTATTGCCCATGCCGGTGCATCAGTTGATT  360

Query  361  TAACAATTTTCTCATTGCACCTA  383
            |||||||||||||||||||||||
Sbjct  361  TAACAATTTTCTCATTGCACCTA  383
```

Figure 26d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Brachythemis contaminata* showing its nearest match subject

cytochrome oxidase subunit I, partial (mitochondrion) [Brachythemis contaminata]
Sequence ID: ALC74206.1Length: 127Number of Matches: 1
Related Information

Range 1: 1 to 127GenPeptGraphicsNext MatchPrevious Match

Score	Expect	Method	Identities	Positives	Gaps
250 bits(638)	5e-84	Compositional matrix adjust.	127/127(100%)	127/127(100%)	0/127(0%)

```
Query  1    AGMIGTALSVLIRIELGQPGSMIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPL  60
            AGMIGTALSVLIRIELGQPGSMIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPL
Sbjct  1    AGMIGTALSVLIRIELGQPGSMIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPL  60

Query  61   MLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDL  120
            MLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDL
Sbjct  61   MLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDL  120

Query  121  TIFSLHL  127
            TIFSLHL
Sbjct  121  TIFSLHL  127
```

Figure 26e: Peptide BLAST output of COI gene of *Brachythemis contaminata*

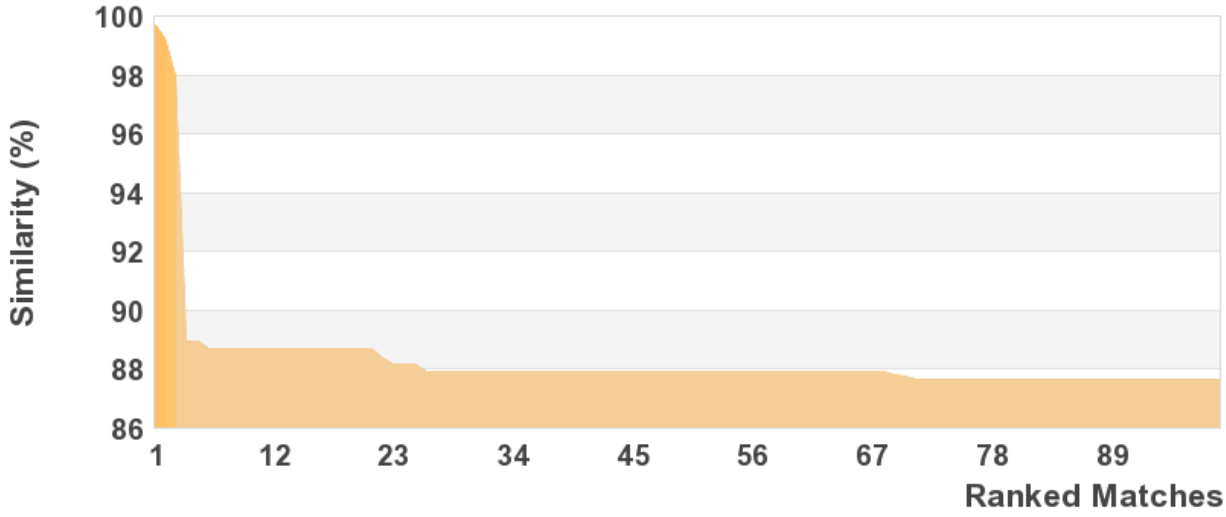


Figure 26f: The line diagram of *Brachythemis contaminata* over more than 98% match to other retrieved sequences (BOLD SYSTEM)

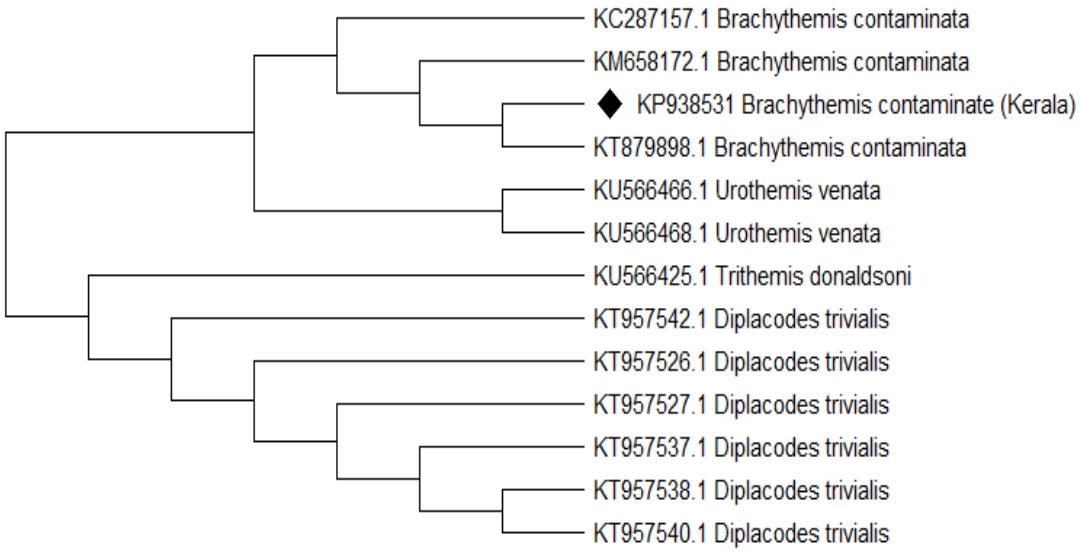


Figure 26g: Molecular phylogenetic tree of *Brachythemis contaminata* inferred by NJ tree method.

Table 46: Percentage of evolutionary divergence of *Brachythemis contaminata* with itsclosely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	KP938531	*Brachythemis contaminate(Kerala)*	
2	KC287157	*Brachythemis_contaminata(Karnataka)*	0.02
3	KM658172	*Brachythemis contaminate (china)*	0.02
4	KT879898	*Brachythemis contaminate(Mizoram)*	0.02
5	KT957526	*Diplacodes trivialis*	0.13
6	KT957527	*Diplacodes trivialis*	0.13
7	KT957537	*Diplacodes trivialis*	0.13
8	KT957538.	*Diplacodes trivialis*	0.13
9	KT957540	*Diplacodes trivialis*	0.13
10	KT957542	*Diplacodes trivialis*	0.13
11	KU566425	*Trithemis donaldsoni*	0.14
12	KU566466	*Urothemis venata*	0.13
13	KU566468	*Urothemis venata*	0.13

Figure 27: *Diplacodes trivalis*

>KP835512 *Diplacodes trivialis voucher CUDT 01-B1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial*

AGATGATCAAATTTATAATGTTGTTGTAACAGCCCATGCATTTGTAATAATTTTTTTTATAGTAATGCCT
ATTATAATTGGGGGGTTTGGTAATTGGTTAGTTCCTTTAATATTAGGAGCACCAGATATGGCCTTCCCAC
GACTAAATAATATAAGATTTTGATTATTACCTCCATCATTTACACTACTTTTAGCAAGAAGAATAGTAGA
AAGAGGGGCAGGAACAGGATGAACGGTTTATCCACCCTTAGCTGGGGCTATTGCCCATGCAGGGGCCTCT
GTTGATCTAACAATTTTTTCATTACATCTTGCAGGGGTTTCATCTATTCTTGGTGCAATCAATTTTATTA
CCACAGTAATTAATATAAAATCTCCAGGTATAACACTAGATCAGTTACCACTATTTGTATGAGCAGTAGT
AATTACAGCTGTTTTACTTTTATTATCTTTACCCGTATTAGCAGGT

Figure 27a: The DNA sequence interpret of COI gene of *Diplacodes trivalis*

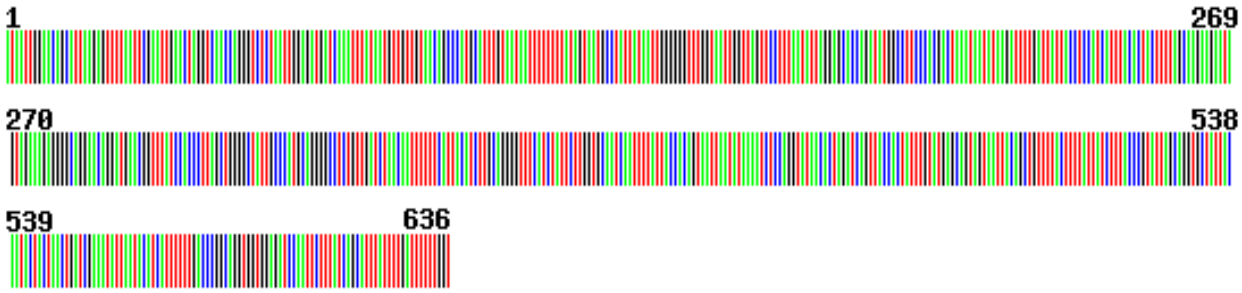


Figure 27b: Representative molecular barcode of COI gene of *Diplacodes trivalis*

> AKU75050 *Diplacodes trivalis* |cytochrome oxidase subunit I gene |voucher CUAD-01-A1 partial cds, mitochondrial|155 bp

DDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVE
SGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMTLDQLPLFVWAVV
ITAVLLLLSLPVLAG

Figure 27c: The conceptual translation product of the COI gene of *Diplacodes trivalis*

Sequence ID: KT879902.1Length: 658Number of Matches: 1
Related Information

Range 1: 100 to 565GenBank Graphics Next Match Previous Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
861 bits(466)	0.0	466/466(100%)	0/466(0%)	Plus/Plus

```
Query  1    AGATGATCAAATTTATAATGTTGTTGTAACAGCCCATGCATTTGTAATAAtttttttttAT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  100  AGATGATCAAATTTATAATGTTGTTGTAACAGCCCATGCATTTGTAATAATTTTTTTTAT  159
Query  61   AGTAATGCCTATTATAATTGGGGGGTTTGGTAATTGGTTAGTTCCTTTAATATTAGGAGC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  160  AGTAATGCCTATTATAATTGGGGGGTTTGGTAATTGGTTAGTTCCTTTAATATTAGGAGC  219
Query  121  ACCAGATATGGCCTTCCCACGACTAAATAATATAAGATTTTGATTATTACCTCCATCATT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  220  ACCAGATATGGCCTTCCCACGACTAAATAATATAAGATTTTGATTATTACCTCCATCATT  279
Query  181  TACACTACTTTTAGCAAGAAGAATAGTAGAAAGAGGGGCAGGAACAGGATGAACGGTTTA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  280  TACACTACTTTTAGCAAGAAGAATAGTAGAAAGAGGGGCAGGAACAGGATGAACGGTTTA  359
Query  241  TCCACCCTTAGCTGGGGCTATTGCCCATGCAGGGGCCTCTGTTGATCTAACAATTTTTTC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  340  TCCACCCTTAGCTGGGGCTATTGCCCATGCAGGGGCCTCTGTTGATCTAACAATTTTTTC  399
Query  301  ATTACATCTTGCAGGGGTTTCATCTATTCTTGGTGCAATCAATTTTATTACCACAGTAAT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  400  ATTACATCTTGCAGGGGTTTCATCTATTCTTGGTGCAATCAATTTTATTACCACAGTAAT  459
Query  361  TAATATAAAATCTCCAGGTATAACACTAGATCAGTTACCACTATTTGTATGAGCAGTAGT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  460  TAATATAAAATCTCCAGGTATAACACTAGATCAGTTACCACTATTTGTATGAGCAGTAGT  519
Query  421  AATTACAGCTGTTTTACTTTTATTATCTTTACCCGTATTAGCAGGT  466
            |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  520  AATTACAGCTGTTTTACTTTTATTATCTTTACCCGTATTAGCAGGT  565
```

Figure 27d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Diplacodes trivalis* showing its nearest match subject

cytochrome oxidase subunit I, partial (mitochondrion) [Diplacodes trivialis]
Sequence ID: AJL35340.1Length: 181Number of Matches: 1
Related Information
Range 1: 22 to 176GenPeptGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps
302 bits(773)	3e-103	Compositional matrix adjust.	155/155(100%)	155/155(100%)	0/155(0%)

```
Query  1    DDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSF  60
            DDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSF
Sbjct  22   DDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSF  81

Query  61   TLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVI  120
            TLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVI
Sbjct  82   TLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVI  141

Query  121  NMKSPGMTLDQLPLFVWAVVITAVLLLLSLPVLAG  155
            NMKSPGMTLDQLPLFVWAVVITAVLLLLSLPVLAG
Sbjct  142  NMKSPGMTLDQLPLFVWAVVITAVLLLLSLPVLAG  176
```

Figure 27e: Peptide BLAST output of COI gene of *Diplacodes trivalis*

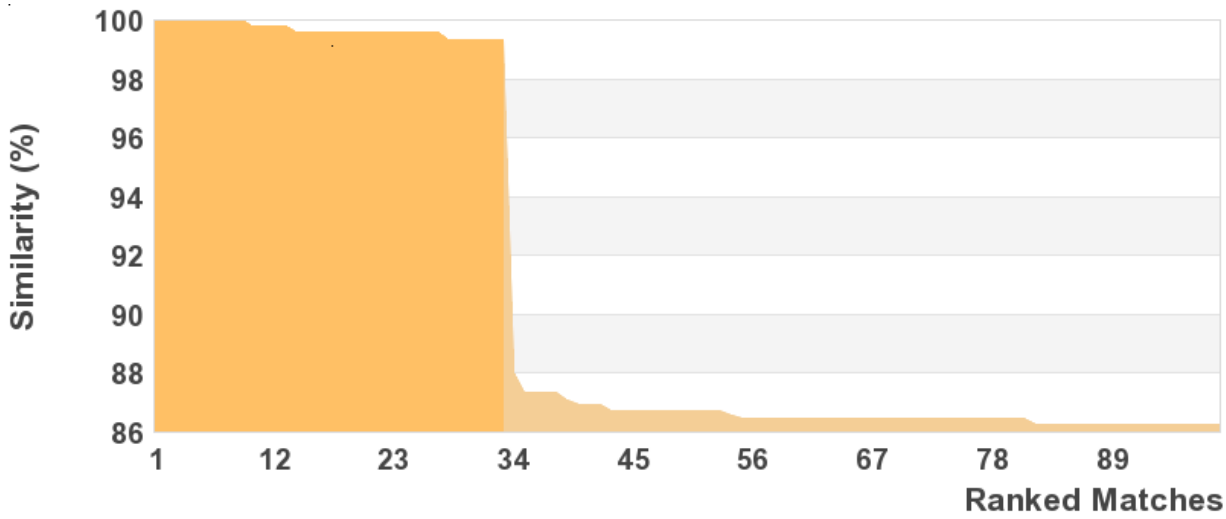


Figure 27f: The line diagram of *Diplacodes trivalis* over more than 98 % match to other retrieved sequences (BOLD SYSTEM)

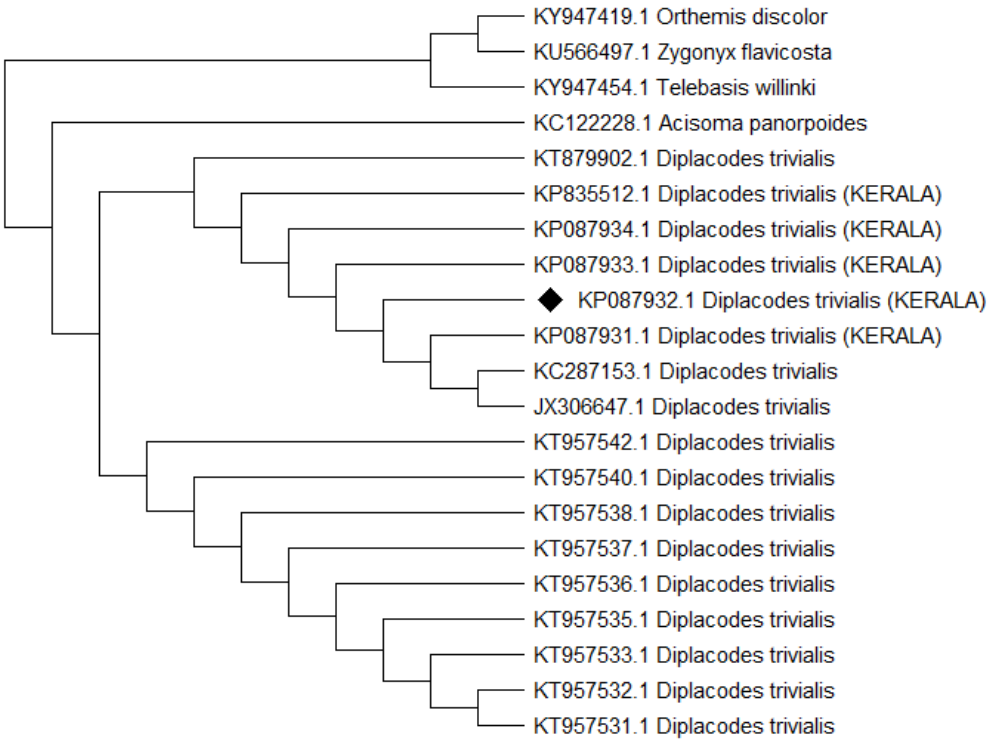


Figure 27g: Molecular phylogenetic relationship of *Diplacodes trivialis* inferred by NJ tree method

Table 48: Percentage of evolutionary divergence of *Diplacodes trivalis* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	KP835512	*Diplacodes trivialis* (Kerala)	
2	KP087934.	*Diplacodes trivialis* (Kerala)	0
3	KP087933.	*Diplacodes trivialis* (Kerala)	0
4	KP087932.	*Diplacodes trivialis* (Kerala)	0
5	KP087931.	*Diplacodes trivialis* (Kerala)	0
6	JX306647.	*Diplacodes trivialis*(Mizoram)	0
7	KC287153.	*Diplacodes trivialis*	0
8	KT957542.	*Diplacodes trivialis*	0.002
9	KT957540.	*Diplacodes trivialis*	0.002
10	KT957538.	*Diplacodes trivialis*	0.002
11	KT957537.	*Diplacodes trivialis*	0.002
12	KT957536.	*Diplacodes trivialis*	0.002
13	KT957535.	*Diplacodes trivialis*	0.002
14	KT957533	*Diplacodes trivialis*	0.002
15	KT957532	*Diplacodes trivialis*	0.002
16	KT957531.	*Diplacodes trivialis*	0.002
17	KC122228	*Acisoma panorpoides*	0.13
18	KY947419.	*Orthemis discolor*	0.14

Figure 28: *Bradinopyga geminata*

>KM096995.1 *Bradinopyga geminata* voucher JK 1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

GCGATGATCAAATTTATAATGTAATTGTAACTGCTCACGCATTTGTAATAATTTTCTTTATAGTTATGCC
AATTATAATTGGAGGTTTTGGAAATTGATTAGTACCTTTAATATTAGGAGCTCCTGATATAGCATTTCCT
CGACTTAATAATATAAGATTTTGGTTATTACCTCCTTCATTTACCTTACTTTTAGCAAGAAGTATAGTAG
AAAGAGGGGCAGGTACTGGATGAACAGTTTACCCCCCTCTAGCTGGAGCTATTGCACATGCAGGGGCTTC
AGTAGATTTAACTATTTTCTCCTTACATTTAGCAGGTGTATCTTCAATTTTAGGTGCAATCAATTTTATC
ACTACTGTAATTAATATAAAGTCACCTGGAATAAAATTAGATCAAATACCTTTATTTGTATGAGCAGTAG
TAATTACTGCAGTATTATTATTGTTATCACTTCCAGTATTAGCTGGTGA

Figure 28a: The DNA sequence interpret of COI gene of *Bradinopyga geminata*

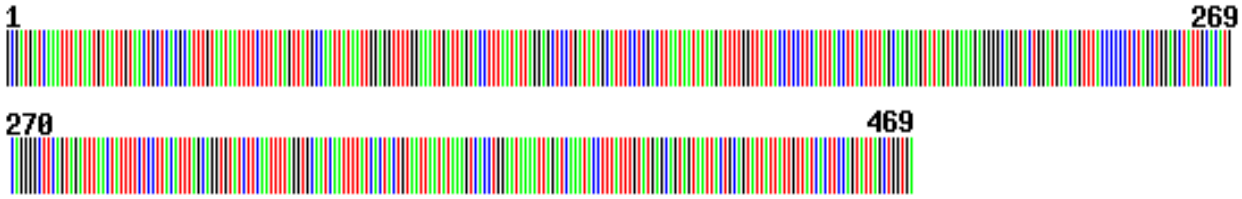


Figure 28b: Representative molecular barcode of COI gene of *Bradinopyga geminata*

> AIT71754 *Bradinopyga geminata* |cytochrome oxidase subunit I gene |voucher CUBG-01-A1 partial cds, mitochondrial|209 b

DDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTG
WTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPV
LAG

Figure 28c: The conceptual translation product of the COI gene of *Bradinopyga geminata*

Sequence ID: KM096995.1Length: 469Number of Matches: 1
Related Information
Range 1: 1 to 469GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
867 bits(469)	0.0	469/469(100%)	0/469(0%)	Plus/Plus

```
Query  1    GCGATGATCAAATTTATAATGTAATTGTAACTGCTCACGCATTTGTAATAATTTTCTTTA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GCGATGATCAAATTTATAATGTAATTGTAACTGCTCACGCATTTGTAATAATTTTCTTTA  60
Query  61   TAGTTATGCCAATTATAATTGGAGGTTTTGGAAATTGATTAGTACCTTTAATATTAGGAG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   TAGTTATGCCAATTATAATTGGAGGTTTTGGAAATTGATTAGTACCTTTAATATTAGGAG  120
Query  121  CTCCTGATATAGCATTTCCTCGACTTAATAATATAAGATTTTGGTTATTACCTCCTTCAT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CTCCTGATATAGCATTTCCTCGACTTAATAATATAAGATTTTGGTTATTACCTCCTTCAT  180
Query  181  TTACCTTACTTTTAGCAAGAAGTATAGTAGAAAGAGGGGCAGGTACTGGATGAACAGTTT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  TTACCTTACTTTTAGCAAGAAGTATAGTAGAAAGAGGGGCAGGTACTGGATGAACAGTTT  240
Query  241  ACCCCCCTCTAGCTGGAGCTATTGCACATGCAGGGGCTTCAGTAGATTTAACTATTTTCT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  ACCCCCCTCTAGCTGGAGCTATTGCACATGCAGGGGCTTCAGTAGATTTAACTATTTTCT  300
Query  301  CCTTACATTTAGCAGGTGTATCTTCAATTTTAGGTGCAATCAATTTTATCACTACTGTAA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  CCTTACATTTAGCAGGTGTATCTTCAATTTTAGGTGCAATCAATTTTATCACTACTGTAA  360
Query  361  TTAATATAAAGTCACCTGGAATAAAATTAGATCAAATACCTTTATTTGTATGAGCAGTAG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  TTAATATAAAGTCACCTGGAATAAAATTAGATCAAATACCTTTATTTGTATGAGCAGTAG  420
Query  421  TAATTACTGCAGTATTATTATTGTTATCACTTCCAGTATTAGCTGGTGA  469
            ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  TAATTACTGCAGTATTATTATTGTTATCACTTCCAGTATTAGCTGGTGA  469
```

Figure 28d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Bradinopyga geminate*

cytochrome oxidase subunit I, partial (mitochondrion) [Bradinopyga geminata]
Sequence ID: AIT71754.1Length: 155Number of Matches: 1
Related Information
Range 1: 1 to 155GenPeptGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps
301 bits(772)	2e-103	Compositional matrix adjust.	155/155(100%)	155/155(100%)	0/155(0%)

```
Query  1    DDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSF  60
            DDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSF
Sbjct  1    DDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSF  60
Query  61   TLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVI  120
            TLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVI
Sbjct  61   TLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVI  120
Query  121  NMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAG  155
            NMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAG
Sbjct  121  NMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAG  155
```

Figure 28e: Peptide BLAST output of COI gene of *Bradinopyga geminata*

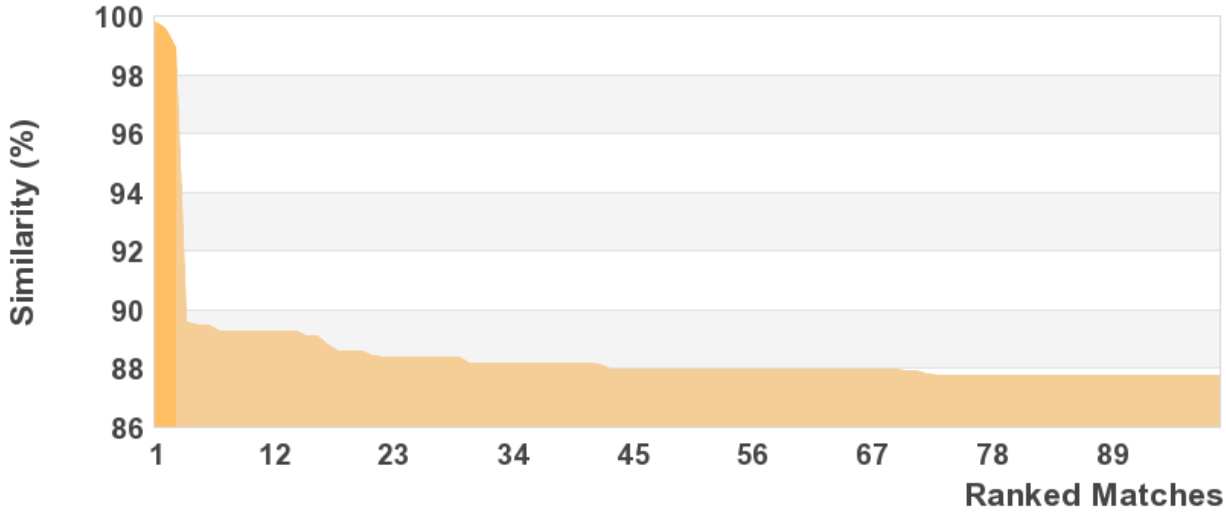


Figure 28f: The line diagram of *Bradinopyga geminata* over more than 98% match to other retrieved sequences (BOLD SYSTEM)

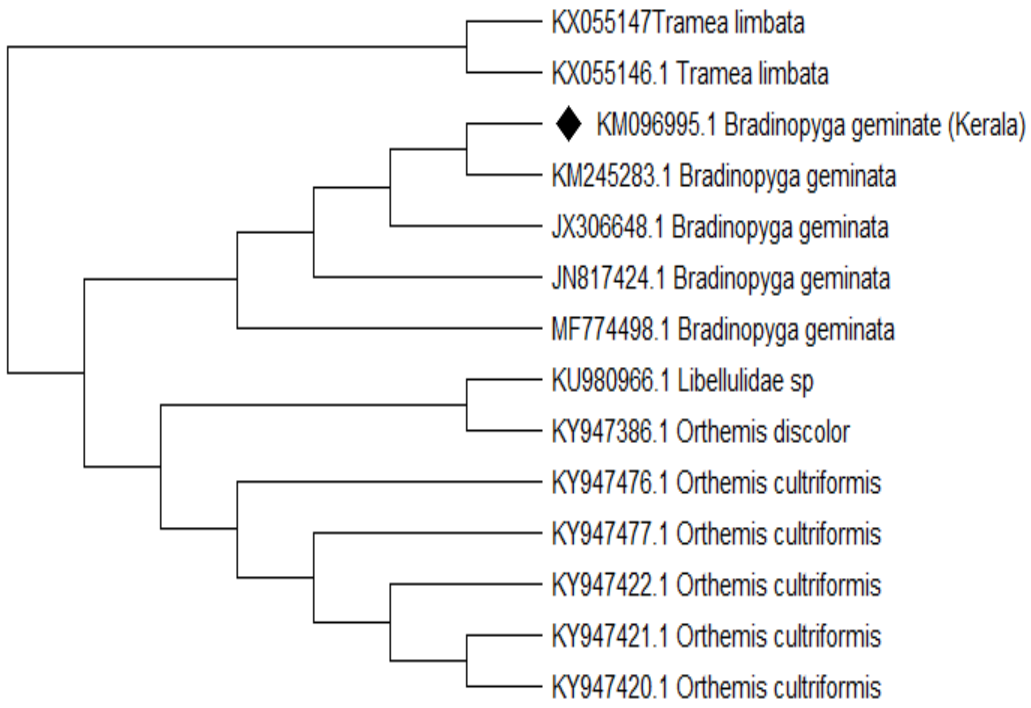


Fig 28h: Molecular phylogenetic relationship of *Bradinopyga geminata* inferred by NJ tree method

Table 50: Percentage of evolutionary divergence of *Bradinopyga geminata* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KM096995	*Bradinopyga geminate* (Kerala)	
2	JX306648	*Bradinopyga geminate* (Tamilnadu)	0.04
3	KM245283	*Bradinopyga geminata* (Tamilnadu)	0.08
4	JN817424.	*Bradinopyga geminate* (Mizoram)	0.08
5	MF774498	*Bradinopyga geminate* (China)	0.11
6	KY947476	*Orthemis cultriformis*	0.11
7	KY947477	*Orthemis cultriformis*	0.11
8	KY947422	*Orthemis cultriformis*	0.11
9	KY947421	*Orthemis cultriformis*	0.11
10	KY947420	*Orthemis cultriformis*	0.11
11	KU980966	*Libellulidae* sp	0.12
12	KY947386	*Orthemis discolour*	0.12
13	KX055147	*Tramea limbata*	0.12
14	KX055146	*Tramea limbata*	0.12

Figure 29: *Rhyothemis variegata*

>KP938530.1 *Rhyothemis variegata* voucher CURV 01-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

```
CAACCTGGATCTCTAATTGGAGATGATCAAATTTATAATGTAATTGTTACTGCACATGCCTTCGTTATAATTTTCT
TCATAGTAATACCTATTATAATTGGAGGATTTGGTAATTGGCTTGTGCCATTAATATTAGGAGCACCAGATATGGC
TTTCCCACGACTAAATAATATAAGATTTTGATTATTACCTCCCTCATTCACTTTATTACTTGCAAGAAGAGTAGTA
GAAAGAGGGGCAGGAACAGGATGAACTGTATATCCACCATTAGCAGGAGCTATTGCTCATGCTGGAGCATCTGTAG
ATTTAACTATTTTTTCTTTACACTTAGCTGGAGTATCATCAATTTTAGGGGCAATTAATTTTATTACTACAGTAAT
TAATATAAAGTCACCAGGAATAAAAATAGATCAAATACCTTTATTTGTATGAGCTGTAGTAATTACTGCA
```

Figure 29a: The DNA sequence interpret of COI gene of *Rhyothemis variegata*

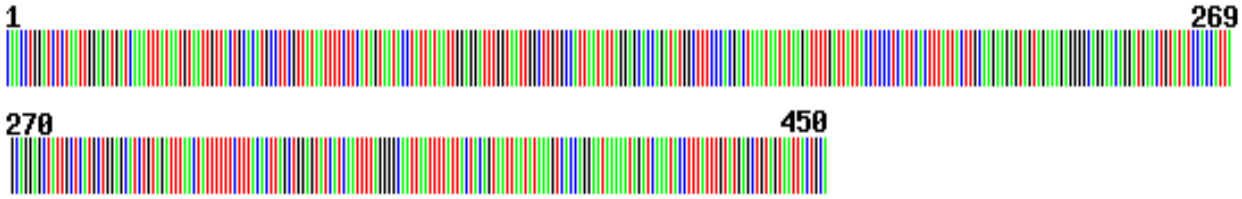


Figure 29b: Representative molecular barcode of COI gene of *Rhyothemis variegata.*

> ALC74205 *Rhyothemis variegata* |cytochrome oxidase subunit I gene |voucher CURV-01-A1 partial cds, mitochondrial| 150 bp

```
QPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSVV
ESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMKMDQMPLFVWAVVITA
```

Figure 29c: The conceptual translation product of the COI gene of *Rhyothemis variegata*

Sequence ID: KP938530.1Length: 450Number of Matches: 1

Related Information

Range 1: 1 to 450GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
832 bits(450)	0.0	450/450(100%)	0/450(0%)	Plus/Plus

```
Query  1    CAACCTGGATCTCTAATTGGAGATGATCAAATTTATAATGTAATTGTTACTGCACATGCC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    CAACCTGGATCTCTAATTGGAGATGATCAAATTTATAATGTAATTGTTACTGCACATGCC  60

Query  61   TTCGTTATAATTTTCTTCATAGTAATACCTATTATAATTGGAGGATTTGGTAATTGGCTT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   TTCGTTATAATTTTCTTCATAGTAATACCTATTATAATTGGAGGATTTGGTAATTGGCTT  120

Query  121  GTGCCATTAATATTAGGAGCACCAGATATGGCTTTCCCACGACTAAATAATATAAGATTT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  GTGCCATTAATATTAGGAGCACCAGATATGGCTTTCCCACGACTAAATAATATAAGATTT  180

Query  181  TGATTATTACCTCCCTCATTCACTTTATTACTTGCAAGAAGAGTAGTAGAAAGAGGGGCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  TGATTATTACCTCCCTCATTCACTTTATTACTTGCAAGAAGAGTAGTAGAAAGAGGGGCA  240

Query  241  GGAACAGGATGAACTGTATATCCACCATTAGCAGGAGCTATTGCTCATGCTGGAGCATCT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  GGAACAGGATGAACTGTATATCCACCATTAGCAGGAGCTATTGCTCATGCTGGAGCATCT  300

Query  301  GTAGATTTAACTATTTTTTCTTTACACTTAGCTGGAGTATCATCAATTTTAGGGGCAATT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  GTAGATTTAACTATTTTTTCTTTACACTTAGCTGGAGTATCATCAATTTTAGGGGCAATT  360

Query  361  AATTTTATTACTACAGTAATTAATATAAAGTCACCAGGAATAAAAATAGATCAAATACCT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  AATTTTATTACTACAGTAATTAATATAAAGTCACCAGGAATAAAAATAGATCAAATACCT  420

Query  421  TTATTTGTATGAGCTGTAGTAATTACTGCA  450
            ||||||||||||||||||||||||||||||
Sbjct  421  TTATTTGTATGAGCTGTAGTAATTACTGCA  450
```

Figure 29d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Rhyothemis variegata*

cytochrome oxidase subunit 1, partial (mitochondrion) [Rhyothemis variegata]
Sequence ID: AGD98691.1Length: 193Number of Matches: 1
Related Information
Range 1: 16 to 165GenPeptGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps
298 bits(763)	1e-101	Compositional matrix adjust.	150/150(100%)	150/150(100%)	0/150(0%)

```
Query  1    QPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSF  60
            QPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSF
Sbjct  16   QPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSF  75

Query  61   WLLPPSFTLLLASSVVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAI  120
            WLLPPSFTLLLASSVVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAI
Sbjct  76   WLLPPSFTLLLASSVVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAI  135

Query  121  NFITTVINMKSPGMKMDQMPLFVWAVVITA  150
            NFITTVINMKSPGMKMDQMPLFVWAVVITA
Sbjct  136  NFITTVINMKSPGMKMDQMPLFVWAVVITA  165
```

Figure 29e: Peptide BLAST output of COI gene of *Rhyothemis variegata*

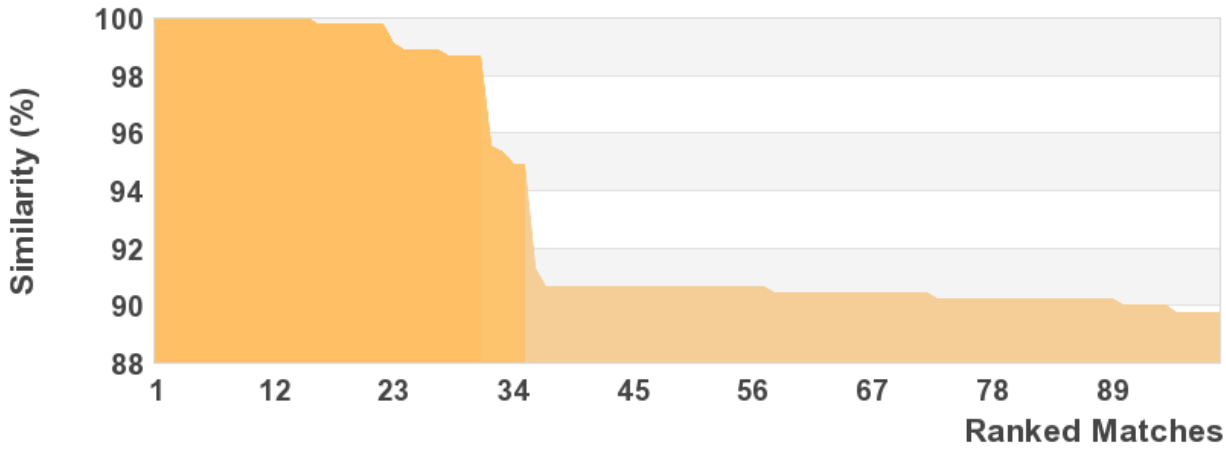


Figure 29f: The line diagram of *Rhyothemis variegata* over more than 98 % match to other retrieved sequences (BOLD SYSTEM)

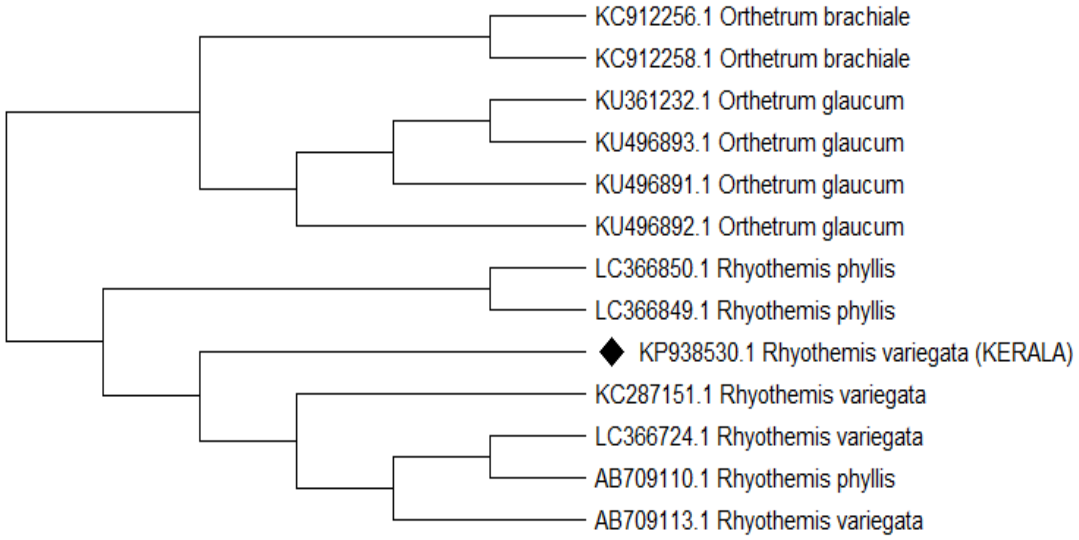


Figure 29g: The molecular phylogenetic tree of *Rhyothemis variegata* inferred by NJ tree method

Table 52: Percentage of evolutionary divergence of *Rhyothemis variegata* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KP938530	*Rhyothemis variegata* (KERALA)	
2	KC287151	*Rhyothemis variegate* (Mizoram)	0.002
3	LC366724	*Rhyothemis variegate* (Japan)	0.00
4	AB709110	*Rhyothemis phyllis* (Japan)	0.00
5	AB709113	*Rhyothemis variegata*	0.003
6	KC912256	*Orthetrum brachiale*	0.09
7	KC912258	*Orthetrum brachiale*	0.09
9	LC366849	*Rhyothemis phyllis*	0.0185
10	KU361232	*Orthetrum glaucum*	0.109
11	KU496893	*Orthetrum glaucum*	0.109
12	KU496892	*Orthetrum glaucum*	0.107
13	KU496891	*Orthetrum glaucum*	0.109

Figure 30: *Pantala flavescence*

>KR011198.1 *Pantala flavescens* voucher CUPF 02-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

GCCGTTAATACTTGGTGCTCCAGATATGGCTTTCCCTCGACTAAATAATATAAGATTTTGACTTTTACCT
CCATCTTTTACTCTTCTTTTAGCTAGAAGTATAGTTGAAAGAGGAGCTGGAACAGGATGAACTGTTTACC
CTCCTTTAGCAGGGGCTATTGCTCACGCTGGAGCATCAGTTGATCTCACAATTTTCTCTCTCCACTTAGC
TGGTGTTTCTTCCATTTTAGGAGCTATTAATTTTATTACAACTGTAATTAATATGAAGTCCCCAGGAATA
AAGCTTGATCAATTACCATTATTTGTATGAGCAGTAGTAATTACTGCTGTTCTTCTACTTTTATCTTTAC
CTGTATTAGCTGGAGCTATTACTATACTTTTAACAGATCGAAATATTAATACCTCTTTCTTTGATCCTGC
AGGGGGAGGAGATCCAATTTTAAA

Fig 30a: The DNA sequence interpret of COI gene of *Pantala flavescence*

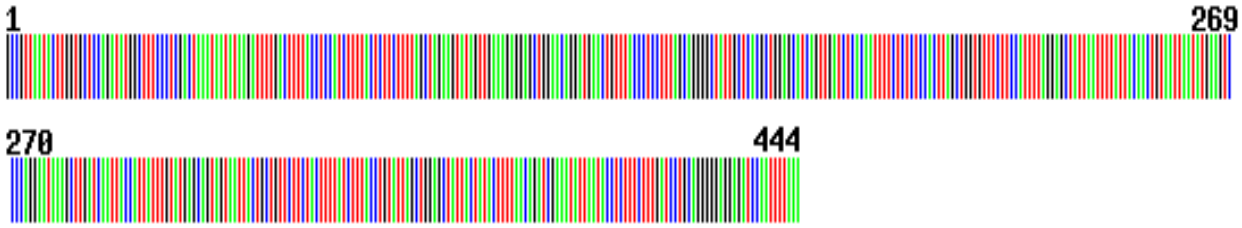


Figure 30b: Representative molecular barcode of COI gene of *Pantala flavescence.*

> ALD10377 *Pantala flavescence* |cytochrome oxidase subunit I gene |voucher CUPF-01-A1 partial cds, mitochondrial| 147 bp

PLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSIL
GAINFITTVINMKSPGMKLDQLPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAGGGDPIL

Figure 30c: The conceptual translation product of the COI gene of *Pantala flavescence*

Sequence ID: KR011198.1Length: 444Number of Matches: 1
Related Information
Range 1: 1 to 444GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
821 bits(444)	0.0	444/444(100%)	0/444(0%)	Plus/Plus

```
Query  1    GCCGTTAATACTTGGTGCTCCAGATATGGCTTTCCCTCGACTAAATAATATAAGATTTTG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GCCGTTAATACTTGGTGCTCCAGATATGGCTTTCCCTCGACTAAATAATATAAGATTTTG  60

Query  61   ACTTTTACCTCCATCTTTTACTCTTCTTTTAGCTAGAAGTATAGTTGAAAGAGGAGCTGG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   ACTTTTACCTCCATCTTTTACTCTTCTTTTAGCTAGAAGTATAGTTGAAAGAGGAGCTGG  120

Query  121  AACAGGATGAACTGTTTACCCTCCTTTAGCAGGGGCTATTGCTCACGCTGGAGCATCAGT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  AACAGGATGAACTGTTTACCCTCCTTTAGCAGGGGCTATTGCTCACGCTGGAGCATCAGT  180

Query  181  TGATCTCACAATTTTCTCTCTCCACTTAGCTGGTGTTTCTTCCATTTTAGGAGCTATTAA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  TGATCTCACAATTTTCTCTCTCCACTTAGCTGGTGTTTCTTCCATTTTAGGAGCTATTAA  240

Query  241  TTTTATTACAACTGTAATTAATATGAAGTCCCCAGGAATAAAGCTTGATCAATTACCATT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  TTTTATTACAACTGTAATTAATATGAAGTCCCCAGGAATAAAGCTTGATCAATTACCATT  300

Query  301  ATTTGTATGAGCAGTAGTAATTACTGCTGTTCTTCTACTTTTATCTTTACCTGTATTAGC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  ATTTGTATGAGCAGTAGTAATTACTGCTGTTCTTCTACTTTTATCTTTACCTGTATTAGC  360

Query  361  TGGAGCTATTACTATACTTTTAACAGATCGAAATATTAATACCTCTTTCTTTGATCCTGC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  TGGAGCTATTACTATACTTTTAACAGATCGAAATATTAATACCTCTTTCTTTGATCCTGC  420

Query  421  AGGGGGAGGAGATCCAATTTTAAA  444
            ||||||||||||||||||||||||
Sbjct  421  AGGGGGAGGAGATCCAATTTTAAA  444
```

Figure 30d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Pantala flavescence*

cytochrome oxidase subunit I, partial (mitochondrion) [Pantala flavescens]
Sequence ID: ALD10377.1Length: 147Number of Matches: 1
Related Information
Range 1: 1 to 147GenPeptGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps
304 bits(726)	1e-96	Compositional matrix adjust.	147/147(100%)	147/147(100%)	0/147(0%)

```
Query  1    PLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASV  60
            PLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASV
Sbjct  1    PLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASV  60

Query  61   DLTIFSLHLAGVSSILGAINFITTVINMKSPGMKLDQLPLFVWAVVITAVLLLLSLPVLA  120
            DLTIFSLHLAGVSSILGAINFITTVINMKSPGMKLDQLPLFVWAVVITAVLLLLSLPVLA
Sbjct  61   DLTIFSLHLAGVSSILGAINFITTVINMKSPGMKLDQLPLFVWAVVITAVLLLLSLPVLA  120

Query  121  GAITMLLTDRNINTSFFDPAGGGDPIL  147
            GAITMLLTDRNINTSFFDPAGGGDPIL
```

Figure 30e: Peptide BLAST output of COI gene of *Pantala flavescence*

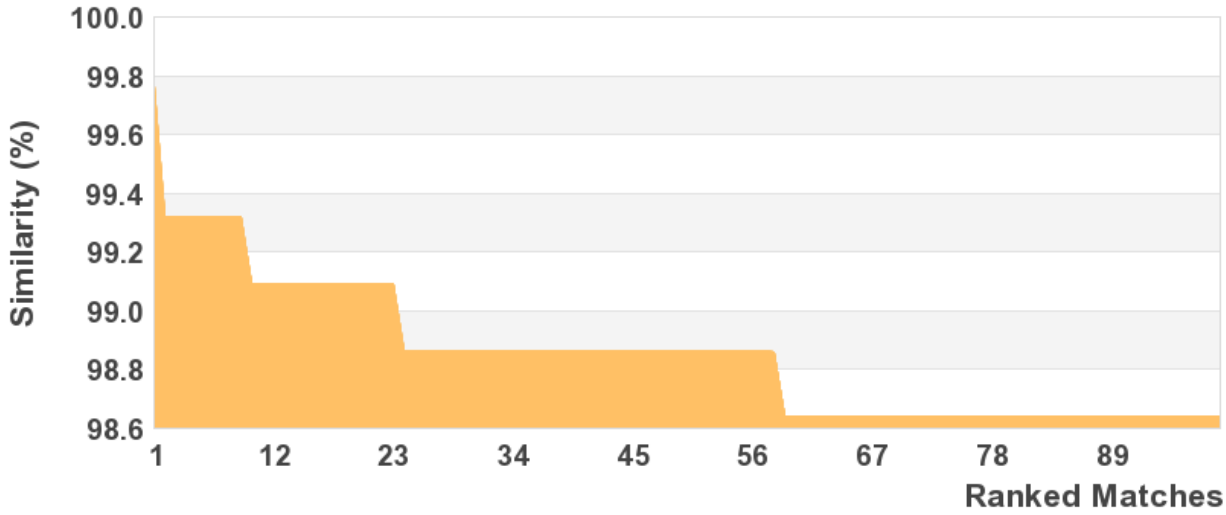


Figure 30f: The line diagram of over more than 98 % match to other retrieved sequences (BOLD SYSTEM)

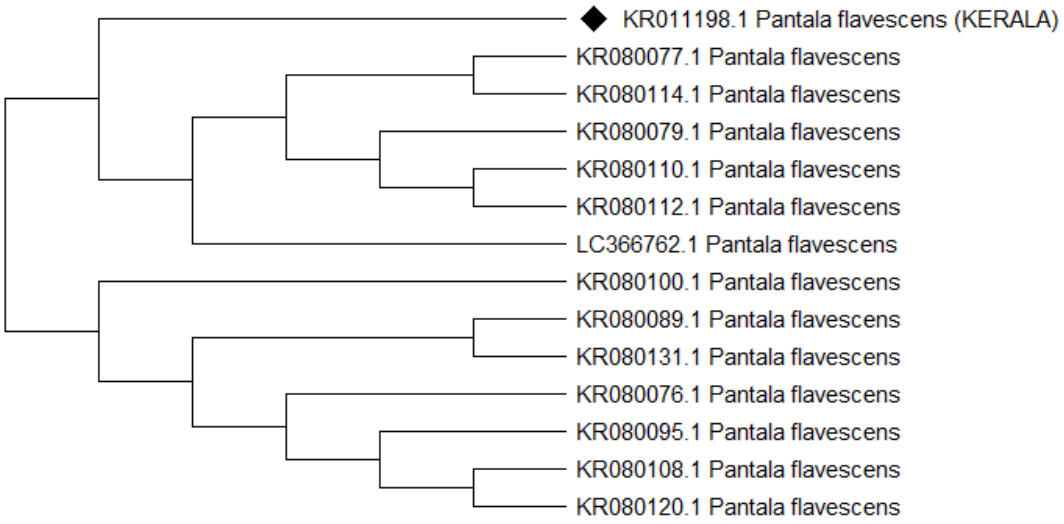


Figure 30g: The molecular phylogenetic tree of *Pantala flavescence* inferred by NJ tree method.

Table 54: Percentage of evolutionary divergence of *Pantala flavescence* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KR011198	*Pantala flavescens* (KERALA)	
2	KR080076	*Pantala flavescens* (Malaysia)	0.009
3	KR080077	*Pantala flavescens* (Malaysia)	0.009
4	KR080079	*Pantala flavescens* (Malaysia)	0.009
5	KR080089	*Pantala flavescens*	0.009
6	KR080095	*Pantala flavescens*	0.011
7	KR080100	*Pantala flavescens*	0.011
8	KR080108	*Pantala flavescens*	0.011
9	KR080110	*Pantala flavescens*	0.011
10	KR080112	*Pantala flavescens*	0.011
11	KR080114	*Pantala flavescens*	0.009
12	KR080120	*Pantala flavescens*	0.011
13	KR080131	*Pantala flavescens*	0.011
14	LC366762	*Pantalaflavescens*	0.009

Figure 31: *Acisoma panorpoides*

>KT223147 *Acisoma panorpoides* voucher CUAP 02-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

```
TTGTTACTGCACATGCTTTTGTAATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGTTTTGGTAATTGACT
CGTACCTTTAATACTAGGAGCTCCAGATATAGCATTCCCACGATTAAATAATATAAGATTTTGATTATTACCTCCT
TCTTTTACATTACTTTTAGCTAGTAGTATAGTAGAAAGAGGAGCAGGAACAGGTTGAACTGTTTATCCACCATTAG
CAGGGGCAATTGCTCATGCAGGTGCATCAGTAGATCTAACAATTTTCTCACTTCATTTAGCTGGGGTTTCCTCAAT
TCTAGGAGCTATTAATTTTATTACAACAGTAATTAATATAAAATCACCTGGAATAAAGCTAGATCAAATACCTCTT
TTTGTATGAGCAGTAGTAATTACTGCTGTCCTTCTTTTATTATCTTTACCCGTATTGGCAGGAGCAATTACAATAT
TATTGACTGATCGAAATATCAAT
```

Figure 31a: The DNA sequence interpret of COI gene of *Acisoma panorpoides*

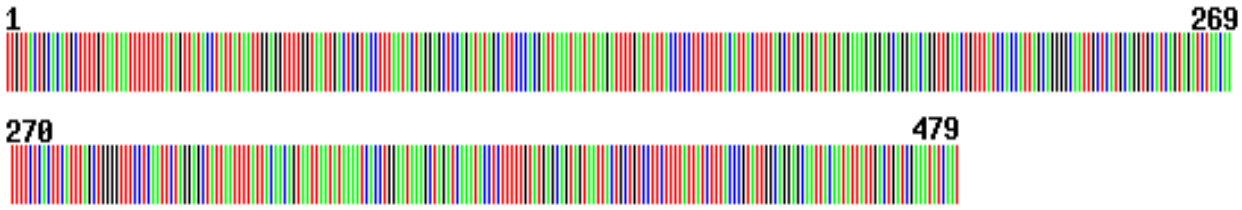


Figure 31b: Representative molecular barcode of COI gene of *Acisoma panorpoides.*

> AKV16032 *Acisoma panorpoides* |cytochrome oxidase subunit I gene |voucher CUPF-01-A1 partial cds, mitochondrial| bp

```
VTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLA
GAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITML
LTDRNIN
```

Figure 31c: The conceptual translation product of the COI gene of *Acisoma panorpoides*

Sequence ID: KT223147.1Length: 479Number of Matches: 1
Related Information
Range 1: 1 to 479GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
885 bits(479)	0.0	479/479(100%)	0/479(0%)	Plus/Plus

```
Query  1    TTGTTACTGCACATGCTTTTGTAATAAtttttttttATAGTTATACCTATTATAATTGGAG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TTGTTACTGCACATGCTTTTGTAATAATTTTTTTTATAGTTATACCTATTATAATTGGAG  60

Query  61   GTTTTGGTAATTGACTCGTACCTTTAATACTAGGAGCTCCAGATATAGCATTCCCACGAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GTTTTGGTAATTGACTCGTACCTTTAATACTAGGAGCTCCAGATATAGCATTCCCACGAT  120

Query  121  TAAATAATATAAGATTTTGATTATTACCTCCTTCTTTTACATTACTTTTAGCTAGTAGTA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  TAAATAATATAAGATTTTGATTATTACCTCCTTCTTTTACATTACTTTTAGCTAGTAGTA  180

Query  181  TAGTAGAAAGAGGAGCAGGAACAGGTTGAACTGTTTATCCACCATTAGCAGGGGCAATTG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  TAGTAGAAAGAGGAGCAGGAACAGGTTGAACTGTTTATCCACCATTAGCAGGGGCAATTG  240

Query  241  CTCATGCAGGTGCATCAGTAGATCTAACAATTTTCTCACTTCATTTAGCTGGGGTTTCCT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  CTCATGCAGGTGCATCAGTAGATCTAACAATTTTCTCACTTCATTTAGCTGGGGTTTCCT  300

Query  301  CAATTCTAGGAGCTATTAATTTTATTACAACAGTAATTAATATAAAATCACCTGGAATAA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  CAATTCTAGGAGCTATTAATTTTATTACAACAGTAATTAATATAAAATCACCTGGAATAA  360

Query  361  AGCTAGATCAAATACCTCTTTTTGTATGAGCAGTAGTAATTACTGCTGTCCTTCTTTTAT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  AGCTAGATCAAATACCTCTTTTTGTATGAGCAGTAGTAATTACTGCTGTCCTTCTTTTAT  420

Query  421  TATCTTTACCCGTATTGGCAGGAGCAATTACAATATTATTGACTGATCGAAATATCAAT  479
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  TATCTTTACCCGTATTGGCAGGAGCAATTACAATATTATTGACTGATCGAAATATCAAT  479
```

Figure 31d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Acisoma panorpoides*

cytochrome oxidase subunit I, partial (mitochondrion) [Acisoma panorpoides]
Sequence ID: AKV16032.1Length: 159Number of Matches: 1
Related Information
Range 1: 1 to 159GenPeptGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps
308 bits(788)	8e-106	Compositional matrix adjust.	159/159(100%)	159/159(100%)	0/159(0%)

```
Query  1    VTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSM  60
            VTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSM
Sbjct  1    VTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSM  60

Query  61   VESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMK  120
            VESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMK
Sbjct  61   VESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMK  120

Query  121  LDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNIN  159
            LDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNIN
Sbjct  121  LDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNIN  159
```

Figure 31e: Peptide BLAST output of COI gene of *Acisoma panorpoides*

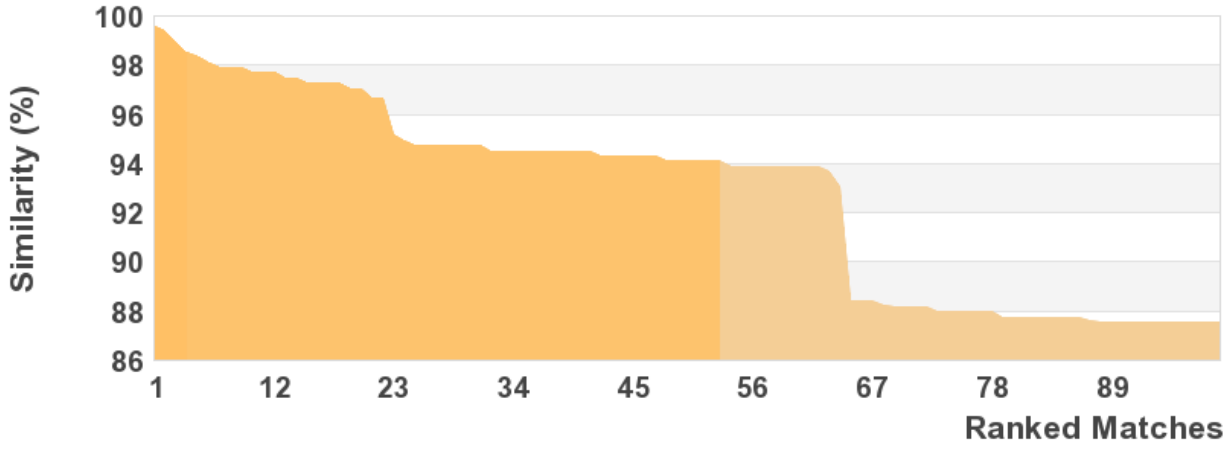


Figure 31f: The line diagram of *Acisoma panorpoides* over more than 98 % match to other retrieved sequences (BOLD SYSTEM)

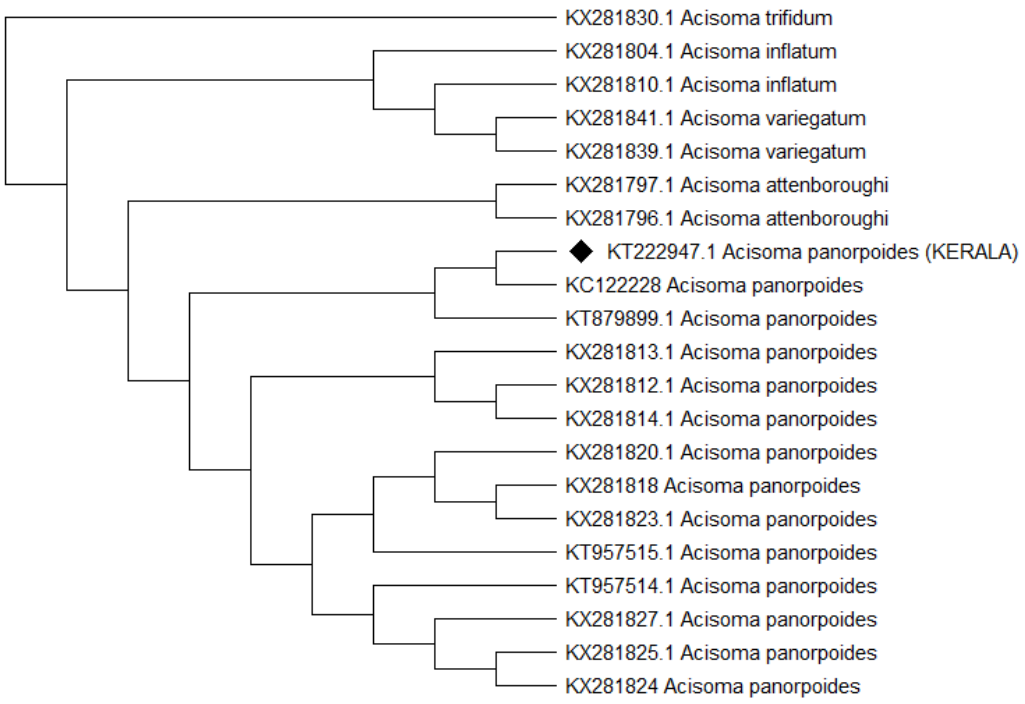


Figure 31g: The molecular phylogenetic tree of *Acisoma panorpoides* inferred by NJ tree method.

Table 56: Percentage of evolutionary divergence of *Acisoma panorpoides* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KT222947	*Acisoma panorpoides* (Kerala)	
2	KC122228	*Acisoma panorpoides* (Mizoram)	0.004
3	KT879899	*Acisoma panorpoides* (Karnataka)	0.018
4	KX281820	*Acisoma panorpoides*	0.020
5	KX281827	*Acisoma panorpoides*	0.020
6	KX281825	*Acisoma panorpoides*	0.020
7	KX281824	*Acisoma panorpoides*	0.020
8	KX281818	*Acisoma panorpoides*	0.025
9	KX281813	*Acisoma panorpoides*	0.025
10	KX281812	*Acisoma panorpoides*	0.025
11	KT957514	*Acisoma panorpoides*	0.025
12	KX281823	*Acisoma panorpoides*	0.025
13	KX281814	*Acisoma panorpoides*	0.025
14	KT957515	*Acisoma panorpoides*	0.025
15	KX281804	*Acisoma inflatum*	0.04
16	KX281810	*Acisoma inflatum*	0.05
17	KX281841	*Acisoma variegatum*	0.05
18	KX281839	*Acisoma variegatum*	0.05
19	KX281797	*Acisoma attenboroughi*	0.060
20	KX281796	*Acisoma attenboroughi*	0.060
21	KX281830	*Acisoma trifidum*	0.12

Figure 32: *Neurothemis tullia*

>KP835513.1 *Neurothemis tullia* voucher CUNT 01-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

GTTCGGTAACTGGCTGGTCCCATTAATGCTTGGGGCACCAGACATGGCCTTCCCACGACTTAATAATATAAGATTT
TGACTTCTACCTCCTTCATTCACTTTACTTTTAGCTAGAAGTATAGTTGAAAGAGGGGCAGGGACAGGGTGAACAG
TTTATCCACCTCTAGCGGGGGCTATTGCACATGCAGGAGCATCTGTAGATTTAACAATTTTTTCTCTTCATTTGGC
GGGGGTTTCCTCAATTTTAGGTGCTATCAATTTTATTACAACAGTAATTAATATAAAGTCCCCCGGGATGAAGTTA
GATCAAATACCTCTATTTGTATGAGCAGTAGTAATTACTGCAGTACT

Fig 32a: The DNA sequence interpret of COI gene of *Neurothemis tullia*

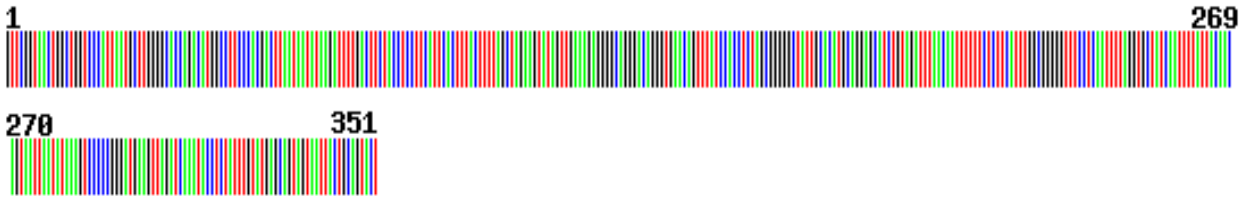


Figure 32b: Representative molecular barcode of COI gene of *Neurothemis tullia.*

> *Neurothemis tullia* |cytochrome oxidase subunit I gene |voucher CUPF-01-A1 partial cds, mitochondrial|117bp

FGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTI
FSLHLAGVSSILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVL

Figure 32c: The conceptual translation product of the COI gene of *Neurothemis tullia*

Neurothemis tullia voucher CUNT 01-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

Sequence ID: KP835513.1Length: 351Number of Matches: 1

Related Information

Range 1: 1 to 351GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
959 bits(351)	0.0	351/351(100%)	0/351(0%)	Plus/Plus

```
Query  1    GTTCGGTAACTGGCTGGTCCCATTAATGCTTGGGGCACCAGACATGGCCTTCCCACGACT  90
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GTTCGGTAACTGGCTGGTCCCATTAATGCTTGGGGCACCAGACATGGCCTTCCCACGACT  90

Query  91   TAATAATATAAGATTTTGACTTCTACCTCCTTCATTCACTTTACTTTTAGCTAGAAGTAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  91   TAATAATATAAGATTTTGACTTCTACCTCCTTCATTCACTTTACTTTTAGCTAGAAGTAT  120

Query  121  AGTTGAAAGAGGGGCAGGGACAGGGTGAACAGTTTATCCACCTCTAGCGGGGGCTATTGC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  AGTTGAAAGAGGGGCAGGGACAGGGTGAACAGTTTATCCACCTCTAGCGGGGGCTATTGC  180

Query  181  ACATGCAGGAGCATCTGTAGATTTAACAATTTTTTCTCTTCATTTGGCGGGGGTTTCCTC  260
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  ACATGCAGGAGCATCTGTAGATTTAACAATTTTTTCTCTTCATTTGGCGGGGGTTTCCTC  260

Query  261  AATTTTAGGTGCTATCAATTTTATTACAACAGTAATTAATATAAAGTCCCCCGGGATGAA  320
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  261  AATTTTAGGTGCTATCAATTTTATTACAACAGTAATTAATATAAAGTCCCCCGGGATGAA  320

Query  321  GTTAGATCAAATACCTCTATTTGTATGAGCAGTAGTAATTACTGCAGTACT  351
            |||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  321  GTTAGATCAAATACCTCTATTTGTATGAGCAGTAGTAATTACTGCAGTACT  351
```

Figure 32d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Neurothemis tullia*

Figure 32d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Neurothemis tullia*

cytochrome oxidase subunit I, partial (mitochondrion) [Neurothemis tullia]
Sequence ID: AKU75051.1Length: 117Number of Matches: 1
Related Information
Range 1: 1 to 117GenPeptGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps
229 bits(583)	7e-76	Compositional matrix adjust.	117/117(100%)	117/117(100%)	0/117(0%)

```
Query  1    FGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIA  60
            FGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIA
Sbjct  1    FGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIA  60

Query  61   HAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVL  117
            HAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVL
Sbjct  61   HAGASVDLTIFSLHLAGVSSILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVL  117
```

Figure 32e: Peptide BLAST output of COI gene of *Neurothemis tullia*

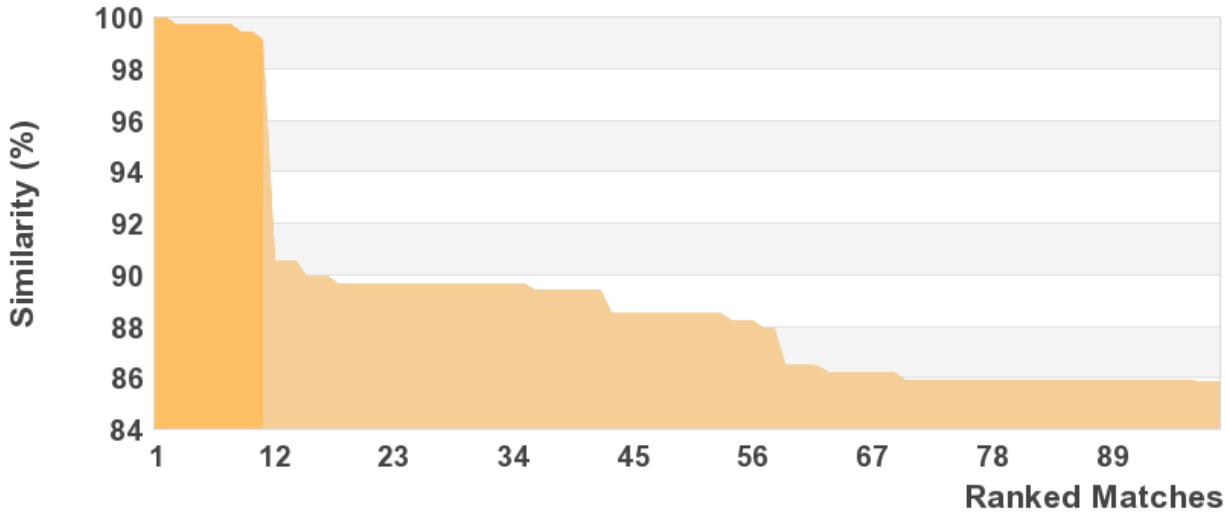


Figure 32f: The line diagram of *Neurothemis tullia* over more than 98 % match to other retrieved sequences (BOLD SYSTEM)

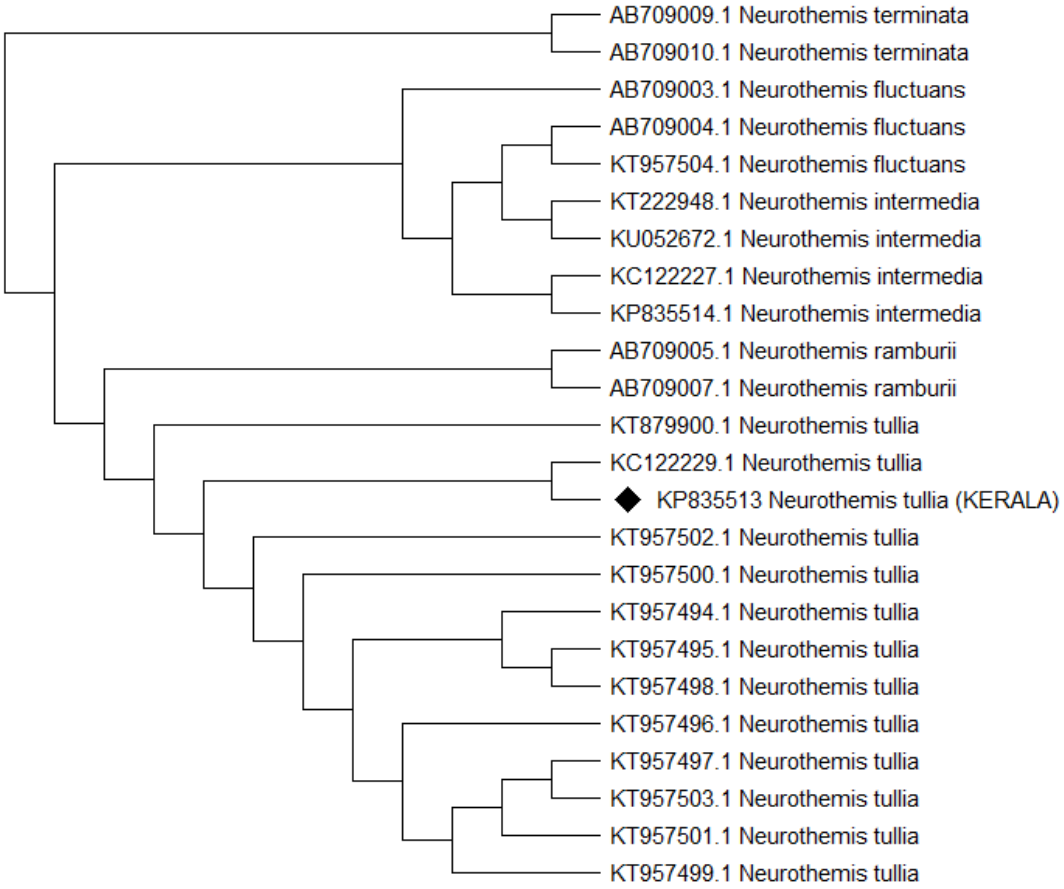


Figure 32g: The molecular phylogenetic tree of *Neurothemis tullia* inferred by NJ tree method.

Table 58: Percentage of evolutionary divergence of *Neurothemis tullia* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	*Organism*	Percentage of divergence
1	KP835513	*Neurothemis tullia* (Kerala)	
2	AB709004	*Neurothemis fluctuans*	
3	AB709005	*Neurothemis ramburii*	0.06
4	AB709007	*Neurothemis ramburii* (Japan)	0.09
5	AB709009	*Neurothemis terminata*	0.09
6	AB709010	*Neurothemis terminata*	0.12
7	KC122227	*Neurothemis intermedia*	0.12
8	KC122229	*Neurothemis tullia* (Mizoram)	0.05
9	KP835514	*Neurothemis intermedia*	0.10
10	KT222948	*Neurothemis intermedia*	0.05
11	KT879900	*Neurothemis tullia*	0.05
12	KT957494	*Neurothemis tullia*	0.10
13	KT957495	*Neurothemis tullia*	0.10
14	KT957496	*Neurothemis tullia*	0.10
15	KT957497	*Neurothemis tullia*	0.10
16	KT957498	*Neurothemis tullia*	0.11
17	KT957499	*Neurothemis tullia*	0.10
18	KT957500	*Neurothemis tullia* (Thailand)	0.10
19	KT957501	*Neurothemis tullia* (Thailand)	0.10
20	KT957502	*Neurothemis tullia* (Thailand)	0.10
21	KT957503	*Neurothemis tullia*	0.10
22	KT957504	*Neurothemis fluctuans*	0.10
23	KU052672	*Neurothemis intermedia*	0.06

Figure 33: *Lathresia asiatica*

>KU052671.1 Lathrecista sp. CDS-2015 voucher CULA-01-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

```
CACGACTCAATAATATAAGATTTTGACTTTTACCCCCTTCTTTCACCTTACTGTTAGCCAGAAGTATAGT
TGAAAGAGGGGCAGGAACAGGATGAACAGTTTATCCCCCTCTAGCAGGGGCCATTGCACATGCCGGAGCA
TCTGTAGACTTAACAATTTTTTCTCTTCATTTGGCGGGTGTTTCATCAATTTTAGGAGCAATTAATTTTA
TTACAACAGTAATTAATATGAAGTCTCCTGGCATAAAGTTAGATCAGATACCCTTATTTGTATGGGCGGT
AGTAATCACTGCAGTACTCCTATTATTATCCCTGCCAGTTCTTGCTGGGGCTATTACTATACTATTAACT
GACCGAAATATTAATACATCATTCTTTGATCCTGCAGGGGGAGGAGATCCAATTTTA
```

Figure 33a: The DNA sequence interpret of COI gene of *Lathresia asiatica*

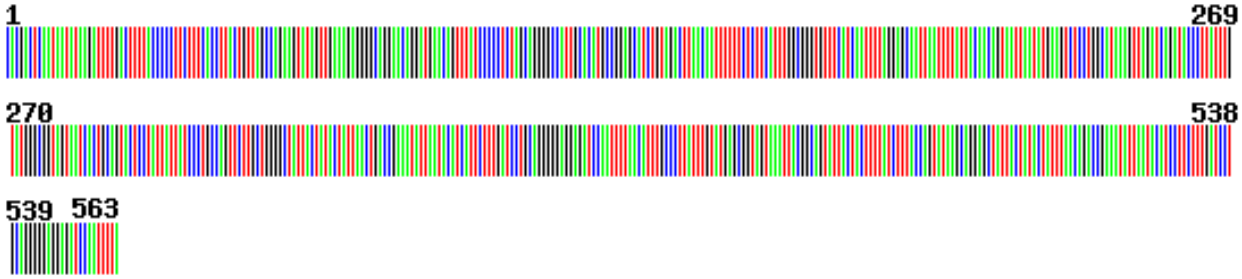


Figure 33b: Representative molecular barcode of COI gene of *Lathresia asiatica.*

> ALQ35272 *Lathresia asiatica* |cytochrome oxidase subunit I gene |voucher CULA-01-A1 partial cds, mitochondrial| 136bp

```
RLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFI
TTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAGGGDPIL
```

Figure 33c: The conceptual translation product of the COI gene of *Lathresia asiatica*

Lathrecista sp. CDS-2015 voucher CULA-01-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

Sequence ID: KU053371.1Length: 407Number of Matches: 1

Related Information

Range 1: 1 to 407GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
794 bits(407)	0.0	407/407(100%)	0/407(0%)	Plus/Plus

```
Query  1    CACGACTCAATAATATAAGATTTTGACTTTTACCCCCTTCTTTCACCTTACTGTTAGCCA  90
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    CACGACTCAATAATATAAGATTTTGACTTTTACCCCCTTCTTTCACCTTACTGTTAGCCA  90

Query  91   GAAGTATAGTTGAAAGAGGGGCAGGAACAGGATGAACAGTTTATCCCCCTCTAGCAGGGG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  91   GAAGTATAGTTGAAAGAGGGGCAGGAACAGGATGAACAGTTTATCCCCCTCTAGCAGGGG  120

Query  121  CCATTGCACATGCCGGAGCATCTGTAGACTTAACAATTTTTTCTCTTCATTTGGCGGGTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CCATTGCACATGCCGGAGCATCTGTAGACTTAACAATTTTTTCTCTTCATTTGGCGGGTG  180

Query  181  TTTCATCAATTTTAGGAGCAATTAATTTTATTACAACAGTAATTAATATGAAGTCTCCTG  260
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  TTTCATCAATTTTAGGAGCAATTAATTTTATTACAACAGTAATTAATATGAAGTCTCCTG  260

Query  261  GCATAAAGTTAGATCAGATACCCTTATTTGTATGGGCGGTAGTAATCACTGCAGTACTCC  330
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  261  GCATAAAGTTAGATCAGATACCCTTATTTGTATGGGCGGTAGTAATCACTGCAGTACTCC  330

Query  331  TATTATTATCCCTGCCAGTTCTTGCTGGGGCTATTACTATACTATTAACTGACCGAAATA  390
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  331  TATTATTATCCCTGCCAGTTCTTGCTGGGGCTATTACTATACTATTAACTGACCGAAATA  390

Query  391  TTAATACATCATTCTTTGATCCTGCAGGGGGAGGAGATCCAATTTTA  407
            |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  391  TTAATACATCATTCTTTGATCCTGCAGGGGGAGGAGATCCAATTTTA  407
```

Figure 33d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Lathresia asiatica*

cytochrome oxidase subunit I, partial (mitochondrion) [Lathrecista sp. CDS-2015]
Sequence ID: ALQ35272.1Length: 135Number of Matches: 1
Related Information
Range 1: 1 to 135GenPeptGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps
262 bits(670)	1e-88	Compositional matrix adjust.	135/135(100%)	135/135(100%)	0/135(0%)

```
Query  1    RLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGV  60
            RLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGV
Sbjct  1    RLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGV  60

Query  61   SSILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNI  120
            SSILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNI
Sbjct  61   SSILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNI  120

Query  121  NTSFFDPAGGGDPIL  135
            NTSFFDPAGGGDPIL
Sbjct  121  NTSFFDPAGGGDPIL  135
```

Figure 33e: Peptide BLAST output of COI gene of *Lathresia asiatica*

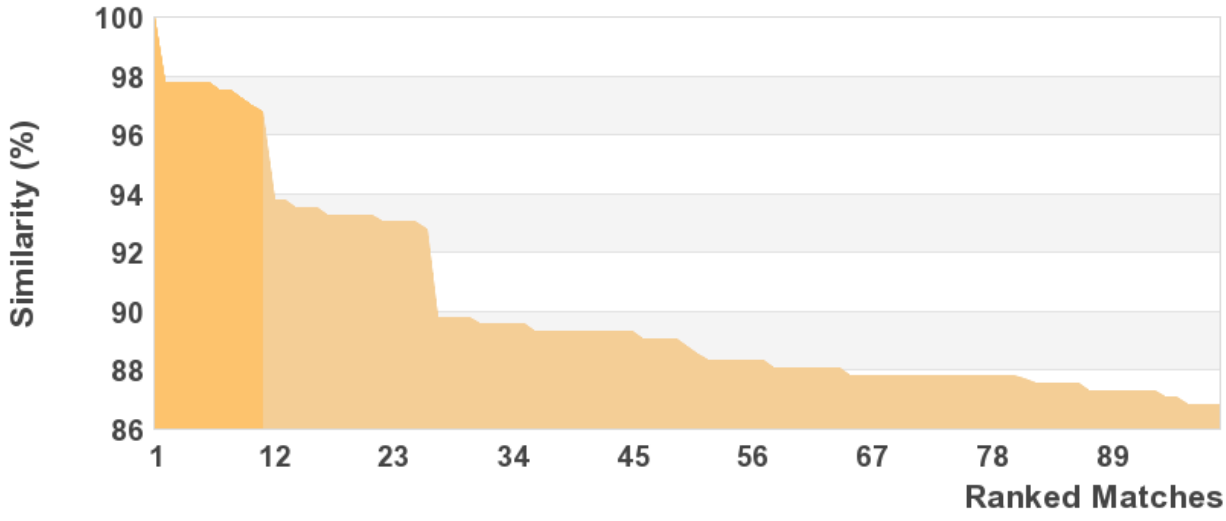


Figure 33f: The line diagram of *Lathresia asiatica* over more than 98 % match to other retrieved sequences (BOLD SYSTEM)

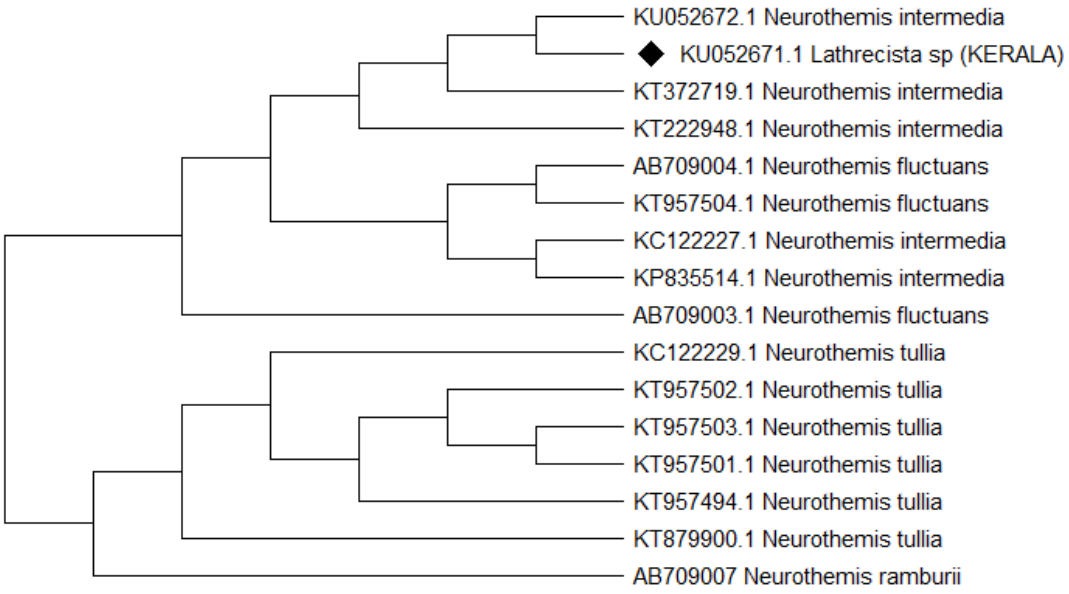


Figure 33g: The molecular phylogenetic tree of *Lathresia asiatica* inferred by NJ tree method.

Table 60: Percentage of evolutionary divergence of *Lathresia asiatica* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KU052671	*Lathrecista* sp. (Kerala)	
2	KU052672	*Neurothemis intermedia* (Kerala)	0.00
3	KT372719	*Neurothemis intermedia* (Kerala)	0.00
4	KT222948	*Neurothemis intermedia* (Kerala)	0.00
5	AB709004	*Neurothemis fluctuans* (Japan)	0.014
6	KT957504	*Neurothemis fluctuans*	0.01
7	KC122227	*Neurothemis intermedia*	0.01
8	KP835514	*Neurothemis intermedia*	0.01
9	AB709003	*Neurothemis fluctuans*	0.05
10	KC122229	*Neurothemis tullia*	0.01
11	KT957502	*Neurothemis tullia*	0.10
12	KT957494	*Neurothemis tullia*	0.10
13	AB709007	*Neurothemis ramburii*	0.11
14	KT957503	*Neurothemis tullia*	0.10
15	KT957501	*Neurothemis tullia*	0.10
16	KT879900	*Neurothemis tullia*	0.10

Figure 34: *Aethriamanta brevipennis*

>KU510345.1 *Aethriamanta brevipennis* voucher CUAB-02-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial

```
ATGCATTTGTAATAATCTTTTTTATAGTAATACCTATTATAATTGGGGGATTTGGAAATTGGCTTGTCCC
ACTAATGTTAGGAGCCCCTGATATGGCATTCCCTCGACTAAACAACATGAGATTTTGACTTCTGCCCCCA
GCATTAACTCTTCTATTAACAAGAAGTTTAGTAGAAAGAGGGGCTGGGACAGGTTGAACCGTATACCCTC
CTCTAGCGGGGGCTATTGCTCACGCAGGAGGATCAGTAGATTTAACTATTTTCTCGCTTCACCTAGCAGG
CGTATCCTCGATTTTAGGTGCCGTTAATTTCATTACTACAACAATTAATATAAAATCCCCTGGAATGAAG
GCAGAGCAACTACCATTATTTGTTTGAGCAGTAGTAATTACAGCCATTTTGTTGCTATTATCATTACCCG
TTCTGGCTGGAGCCATCACTATACTTTTAACAGACCGTAACATAAATACATCGTTCTTTGACCCTGCAGG
GGGAGGAGATCCCGGCTG
```

Fig 34a: The DNA sequence interpret of COI gene of *Aethriamanta brevipennis*

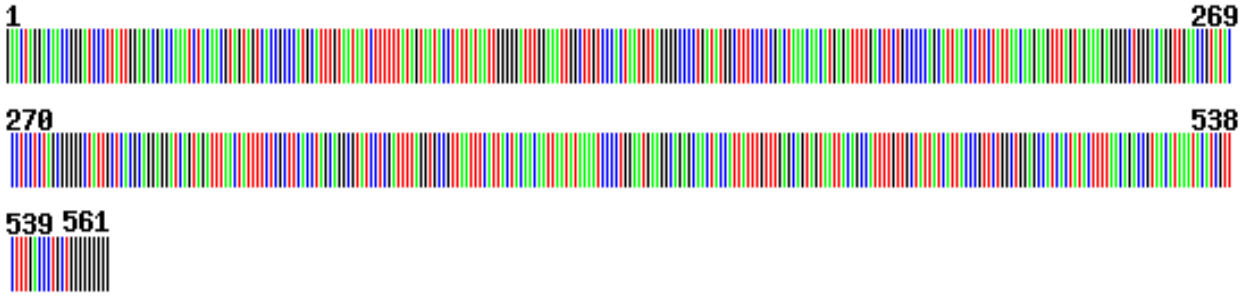


Figure 34b: Representative molecular barcode of COI gene of *Aethriamanta brevipennis*.

> ALX71651 *Aethriamanta brevipennis* |cytochrome oxidase subunit I gene |voucher CUAB-01-A1 partial cds, mitochondrial| 167 bp

```
AFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPALTLLLTSSLVESGAGTGWTVYPPLAGAIA
AGGSVDLTIFSLHLAGVSSILGAVNFITTTINMKSPGMKAEQLPLFVWAVVITAILLLLSLPVLAGAITMLLTDRN
MNTSFFDPAGGGDPG
```

Figure 34c: The conceptual translation product of the COI gene of *Aethriamanta brevipennis*

Aethriamanta brevipennis voucher CUAB-02-A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID: KU510349.1Length: 508Number of Matches: 1
Related Information
Range 1: 1 to 508GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
939 bits(508)	0.0	508/508(100%)	0/508(0%)	Plus/Plus

```
Query  1   ATGCATTTGTAATAATCTTTTTTATAGTAATACCTATTATAATTGGGGGATTTGGAAATT  90
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1   ATGCATTTGTAATAATCTTTTTTATAGTAATACCTATTATAATTGGGGGATTTGGAAATT  90
Query  91  GGCTTGTCCCACTAATGTTAGGAGCCCCTGATATGGCATTCCCTCGACTAAACAACATGA  120
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  91  GGCTTGTCCCACTAATGTTAGGAGCCCCTGATATGGCATTCCCTCGACTAAACAACATGA  120
Query  121 GATTTTGACTTCTGCCCCCAGCATTAACTCTTCTATTAACAAGAAGTTTAGTAGAAAGAG  180
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121 GATTTTGACTTCTGCCCCCAGCATTAACTCTTCTATTAACAAGAAGTTTAGTAGAAAGAG  180
Query  181 GGGCTGGGACAGGTTGAACCGTATACCCTCCTCTAGCGGGGGCTATTGCTCACGCAGGAG  260
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181 GGGCTGGGACAGGTTGAACCGTATACCCTCCTCTAGCGGGGGCTATTGCTCACGCAGGAG  260
Query  261 GATCAGTAGATTTAACTATTTTCTCGCTTCACCTAGCAGGCGTATCCTCGATTTTAGGTG  340
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  261 GATCAGTAGATTTAACTATTTTCTCGCTTCACCTAGCAGGCGTATCCTCGATTTTAGGTG  340
Query  341 CCGTTAATTTCATTACTACAACAATTAATATAAAATCCCCTGGAATGAAGGCAGAGCAAC  390
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  341 CCGTTAATTTCATTACTACAACAATTAATATAAAATCCCCTGGAATGAAGGCAGAGCAAC  390
Query  391 TACCATTATTTGTTTGAGCAGTAGTAATTACAGCCATTTTGTTGCTATTATCATTACCCG  420
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  391 TACCATTATTTGTTTGAGCAGTAGTAATTACAGCCATTTTGTTGCTATTATCATTACCCG  420
Query  421 TTCTGGCTGGAGCCATCACTATACTTTTAACAGACCGTAACATAAATACATCGTTCTTTG  480
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421 TTCTGGCTGGAGCCATCACTATACTTTTAACAGACCGTAACATAAATACATCGTTCTTTG  480
Query  481 ACCCTGCAGGGGGAGGAGATCCCGGCTG  508
           ||||||||||||||||||||||||||||
Sbjct  481  ACCCTGCAGGGGGAGGAGATCCCGGCTG  508
```

Figure 34d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Aethriamanta brevipennis*

cytochrome oxidase subunit I, partial (mitochondrion) [Aethriamanta brevipennis]
Sequence ID: ALX71651.1Length: 168Number of Matches: 1
Related Information
Range 1: 1 to 168GenPeptGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps
344 bits(830)	7e-112	Compositional matrix adjust.	168/168(100%)	168/168(100%)	0/168(0%)

```
Query  1    AFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPALTLLLTSSLVESG  60
            AFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPALTLLLTSSLVESG
Sbjct  1    AFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPALTLLLTSSLVESG  60

Query  61   AGTGWTVYPPLAGAIAHAGGSVDLTIFSLHLAGVSSILGAVNFITTTINMKSPGMKAEQL  120
            AGTGWTVYPPLAGAIAHAGGSVDLTIFSLHLAGVSSILGAVNFITTTINMKSPGMKAEQL
Sbjct  61   AGTGWTVYPPLAGAIAHAGGSVDLTIFSLHLAGVSSILGAVNFITTTINMKSPGMKAEQL  120

Query  121  PLFVWAVVITAILLLLSLPVLAGAITMLLTDRNMNTSFFDPAGGGDPG  168
            PLFVWAVVITAILLLLSLPVLAGAITMLLTDRNMNTSFFDPAGGGDPG
Sbjct  121  PLFVWAVVITAILLLLSLPVLAGAITMLLTDRNMNTSFFDPAGGGDPG  168
```

Figure 34e: Peptide BLAST output of COI gene of *Aethriamanta brevipennis*

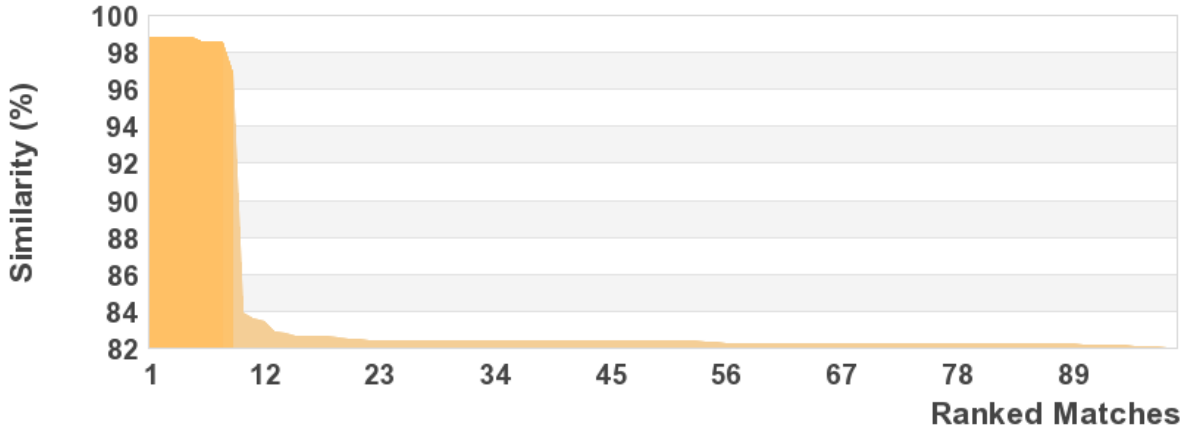
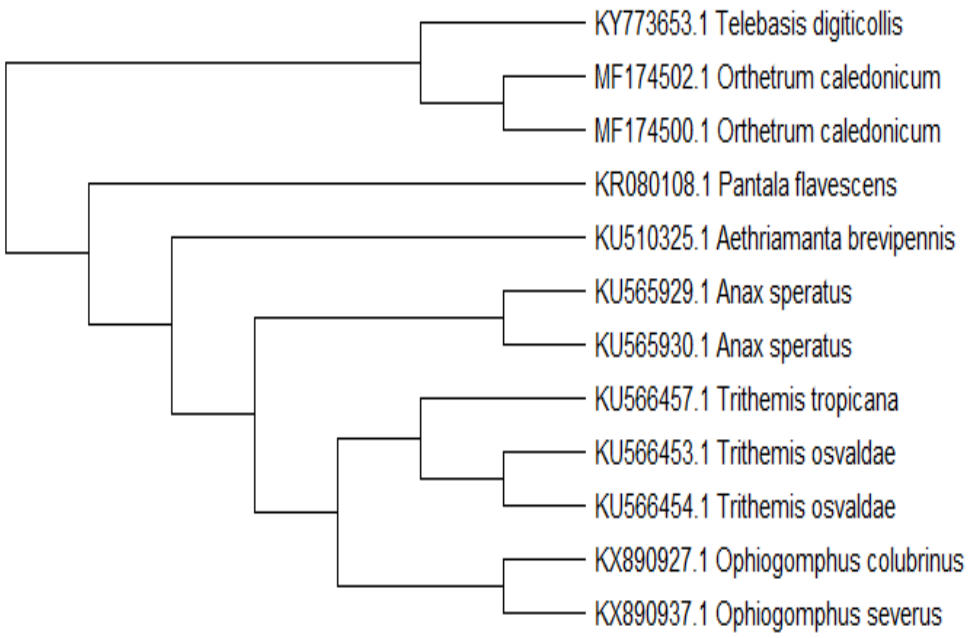


Figure 34f: The line diagram of *Aethriamanta brevipennis* over more than 98 % match to other retrieved sequences (BOLD SYSTEM)



34g: The molecular phylogenetic tree of *Aethriamanta brevipennis* inferred by NJ tree method.

Table 62: Percentage of evolutionary divergence of *Aethriamanta brevipennis* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1	KU510325	*Aethriamanta brevipennis (Kerala)*	
2	KU565929	*Anax speratus (Netherland)*	0.17
3	KU565930	*Anax speratus (Netherland)*	0.17
4	KU566457	*Trithemis tropicana*	0.18
5	KY773653	*Telebasis digiticollis*	0.74
6	KR080108	*Pantala flavescens*	0.18
7	KX89092	*Ophiogomphus colubrinus*	0.18
8	KU566453	*Trithemis osvaldae*	0.18
9	MF174502	*Orthetrum caledonicum*	0.73
10	MF174500	*Orthetrum caledonicum*	0.73
11	KX890937	*Ophiogomphus severus*	0.18
12	KU566454	*Trithemis osvaldae*	0.18

Figure 35: *Brachydiplax sobrina*

>KT372720 *Brachydiplax sobrina* isolate voucher CUBS 01-A1 cytochrome oxidase I subunit gene, partial cds; mitochondrial

```
CTCCAGATATAGCATTCCCACGTTTAAATAACATAAGATTTTGACTTTTACCACCATCATTCACTTTATT
ATTAGCAAGAAGAATGGTTGAAAGAGGGGCAGGAACAGGATGAACCGTTTATCCTCCACTAGCGGGAGCT
ATTGCTCATGCAGGAGCATCCGTTGATTTAACAATTTTTTCTCTTCATTTAGCAGGAGTATCCTCAATTC
TAGGTGCAATTAACTTTATTACAACAGTAATCAATATAAAGTCACCTGGGATAAAAATAGATCAAATACC
CCTATTTGTATGGGCAGTAGTAATTACCGCCGTACTTCTTTTGTTATCACTTCCGGTATTAGCTGGAGCA
ATTACTATACTATTAACCGATCGAAATATTAATACCTCATTCTTTGATCCCGCAGGAGGGGGAGATCCTA
TTT
```

Figure 35a: The DNA sequence interpret of COI gene of *Brachydiplax sobrina*

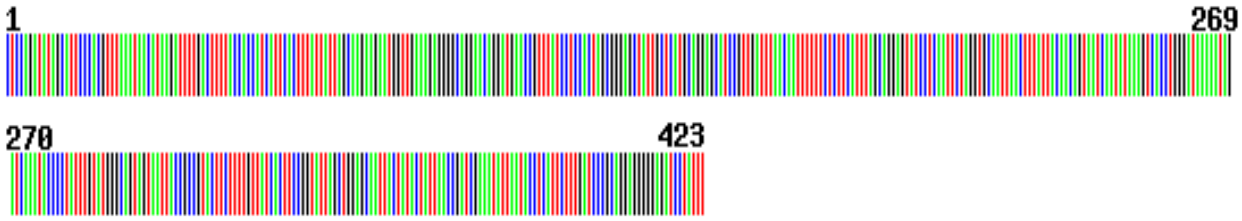


Figure 35b: Representative molecular barcode of COI gene of *Brachydiplax sobrina.*

> ALY11021 *Brachydiplax sobrina* |cytochrome oxidase subunit I gene |voucher CUBS-01-A1 partial cds, mitochondrial|140 bp

```
PDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAINFI
TTVINMKSPGMKMDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAGGGDPI
```

Figure 35c: The conceptual translation product of the COI gene of *Brachydiplax sobrina*

Brachydiplax sobrina isolate voucher CUBS 01-A1 cytochrome oxidase I subunit gene, partial cds; mitochondrial
Sequence ID: KT372720.1Length: 423Number of Matches: 1
Related Information
Range 1: 1 to 423GenBankGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
782 bits(423)	0.0	423/423(100%)	0/423(0%)	Plus/Plus

```
Query  1    CTCCAGATATAGCATTCCCACGTTTAAATAACATAAGATTTTGACTTTTACCACCATCAT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    CTCCAGATATAGCATTCCCACGTTTAAATAACATAAGATTTTGACTTTTACCACCATCAT  60

Query  61   TCACTTTATTATTAGCAAGAAGAATGGTTGAAAGAGGGGCAGGAACAGGATGAACCGTTT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   TCACTTTATTATTAGCAAGAAGAATGGTTGAAAGAGGGGCAGGAACAGGATGAACCGTTT  120

Query  121  ATCCTCCACTAGCGGGAGCTATTGCTCATGCAGGAGCATCCGTTGATTTAACAATTTTTT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  ATCCTCCACTAGCGGGAGCTATTGCTCATGCAGGAGCATCCGTTGATTTAACAATTTTTT  180

Query  181  CTCTTCATTTAGCAGGAGTATCCTCAATTCTAGGTGCAATTAACTTTATTACAACAGTAA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CTCTTCATTTAGCAGGAGTATCCTCAATTCTAGGTGCAATTAACTTTATTACAACAGTAA  240

Query  241  TCAATATAAAGTCACCTGGGATAAAAATAGATCAAATACCCCTATTTGTATGGGCAGTAG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  TCAATATAAAGTCACCTGGGATAAAAATAGATCAAATACCCCTATTTGTATGGGCAGTAG  300

Query  301  TAATTACCGCCGTACTTCTTTTGTTATCACTTCCGGTATTAGCTGGAGCAATTACTATAC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  TAATTACCGCCGTACTTCTTTTGTTATCACTTCCGGTATTAGCTGGAGCAATTACTATAC  360

Query  361  TATTAACCGATCGAAATATTAATACCTCATTCTTTGATCCCGCAGGAGGGGGAGATCCTA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  TATTAACCGATCGAAATATTAATACCTCATTCTTTGATCCCGCAGGAGGGGGAGATCCTA  420

Query  421  TTT  423
            |||
Sbjct  421  TTT  423
```

Figure 35d: Nucleotide BLAST output of the mitochondrial DNA COI gene of *Brachydiplax sobrina*

cytochrome oxidase subunit I, partial (mitochondrion) [Brachydiplax sobrina]
Sequence ID: ALY11021.1Length: 140Number of Matches: 1
Related Information
Range 1: 1 to 140GenPeptGraphicsNext MatchPrevious Match

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps
273 bits(699)	8e-93	Compositional matrix adjust.	140/140(100%)	140/140(100%)	0/140(0%)

```
Query  1    PDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFS  60
            PDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFS
Sbjct  1    PDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFS  60

Query  61   LHLAGVSSILGAINFITTVINMKSPGMKMDQMPLFVWAVVITAVLLLLSLPVLAGAITML  120
            LHLAGVSSILGAINFITTVINMKSPGMKMDQMPLFVWAVVITAVLLLLSLPVLAGAITML
Sbjct  61   LHLAGVSSILGAINFITTVINMKSPGMKMDQMPLFVWAVVITAVLLLLSLPVLAGAITML  120

Query  121  LTDRNINTSFFDPAGGGDPI  140
            LTDRNINTSFFDPAGGGDPI
Sbjct  121  LTDRNINTSFFDPAGGGDPI  140
```

Figure 35e: Peptide BLAST output of COI gene of *Brachydiplax sobrina*

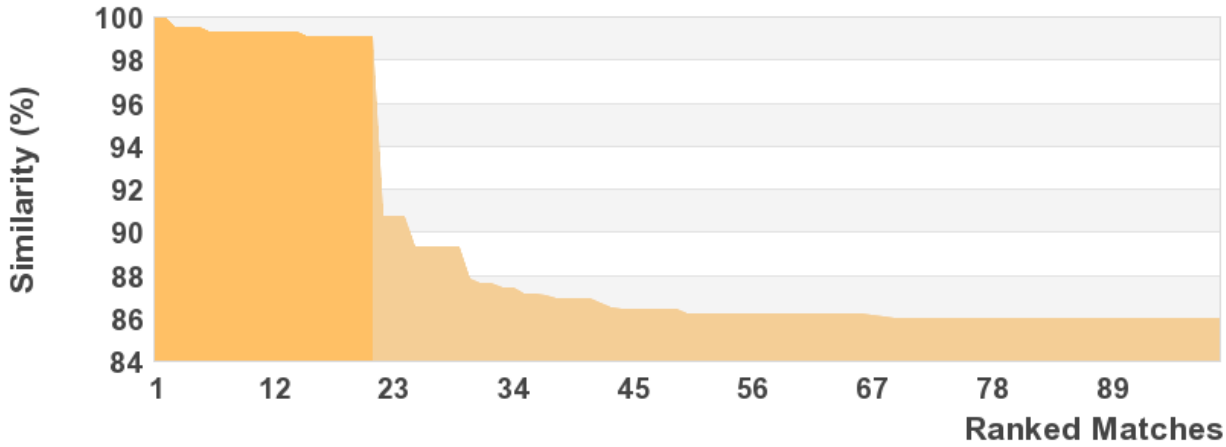


Figure 35f: The line diagram of *Brachydiplax sobrina* over more than 98% match to other retrieved sequences (BOLD SYSTEM)

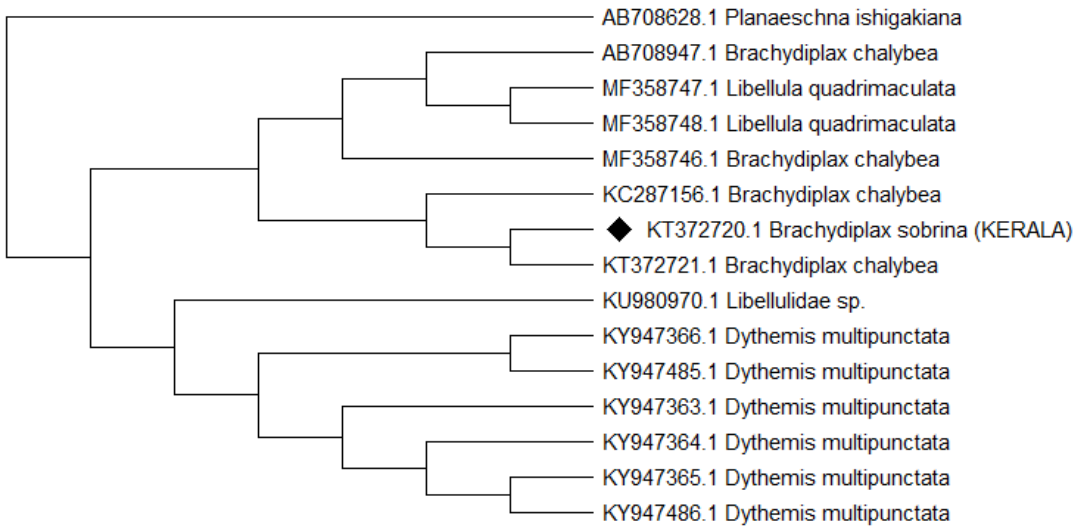


Figure 35g: The molecular phylogenetic tree of *Brachydiplax sobrina* inferred by NJ tree method.

Table 64: Percentage of evolutionary divergence of *Brachydiplax sobrina* with its closely related species accessible from NCBI GenBank

Sl. No.	Accession No	Organism	Percentage of divergence
1.	KT372720	*Brachydiplax sobrina* (Kerala)	
2	KT372721	*Brachydiplax chalybea* (Kerala)	0.13
3	KC287156	*Brachydiplax chalybea* (Mizoram)	0.13
5	AB708947	*Brachydiplax chalybea* (Japan)	0.16
6	KU980970	*Libellulidae* sp.	0.17
7	KY947363	*Dythemis multipunctata*	0.17
8	KY947364	*Dythemis multipunctata*	0.17
9	KY947365	*Dythemis multipunctata*	0.17
10	KY947366	*Dythemis multipunctata*	0.17
11	KY947485.	*Dythemis multipunctata*	0.17
12	KY947486	*Dythemis multipunctata*	0.17
13	MF358746	*Brachydiplax chalybea*	0.14
14	MF358747	*Libellula quadrimaculata*	0.17
15	MF358748.	*Libellula quadrimaculata*	0.17

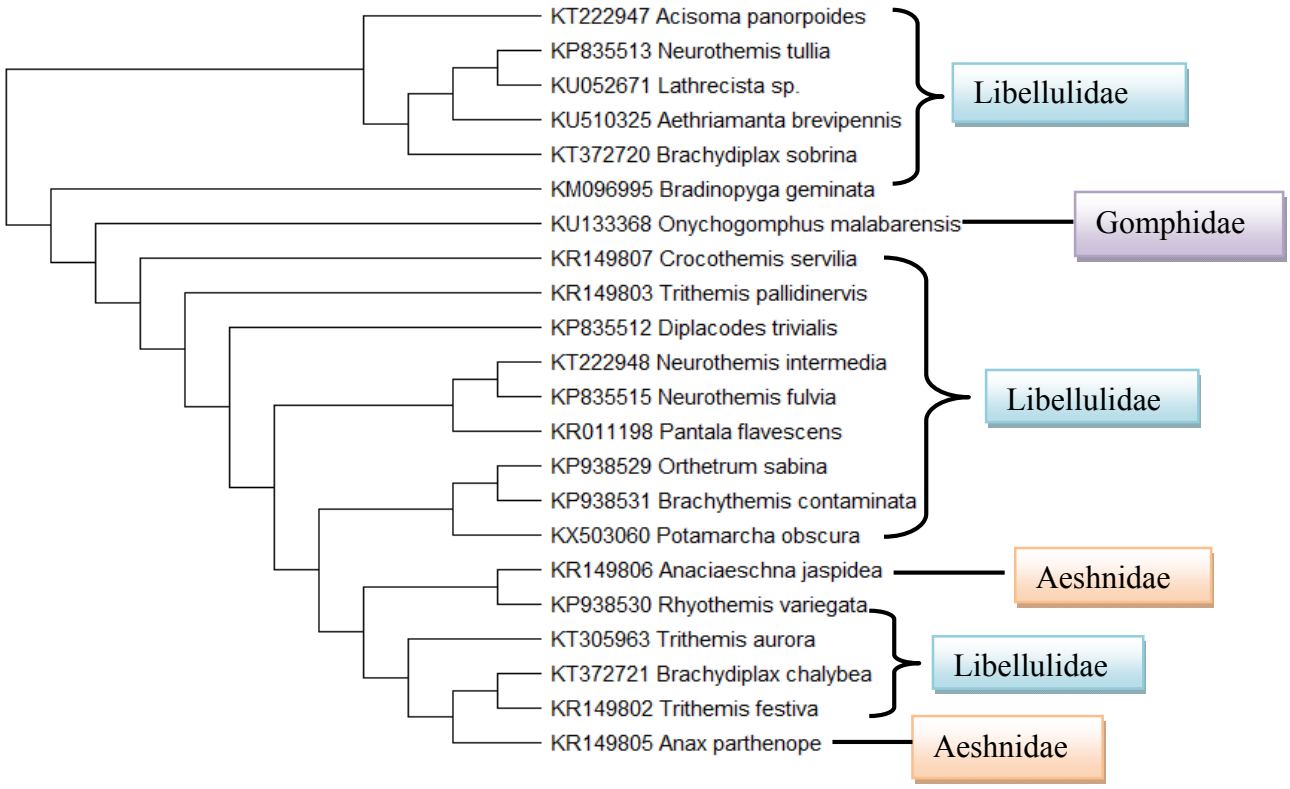


Figure 36: Molecular phylogenetic tree of all Anisopterans in the present study

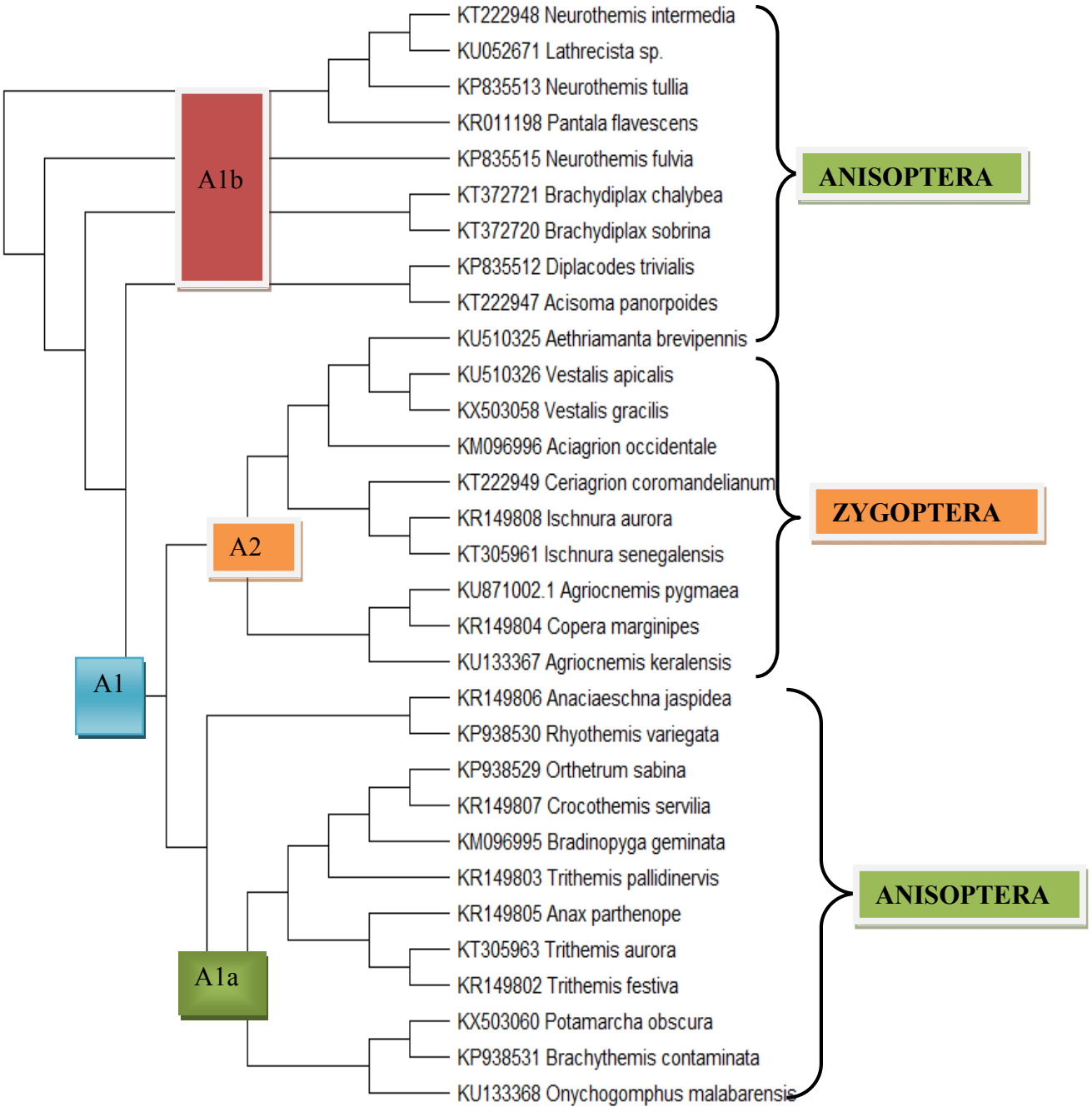


Figure 37: Molecular phylogenetic tree of all Odonata members under present study

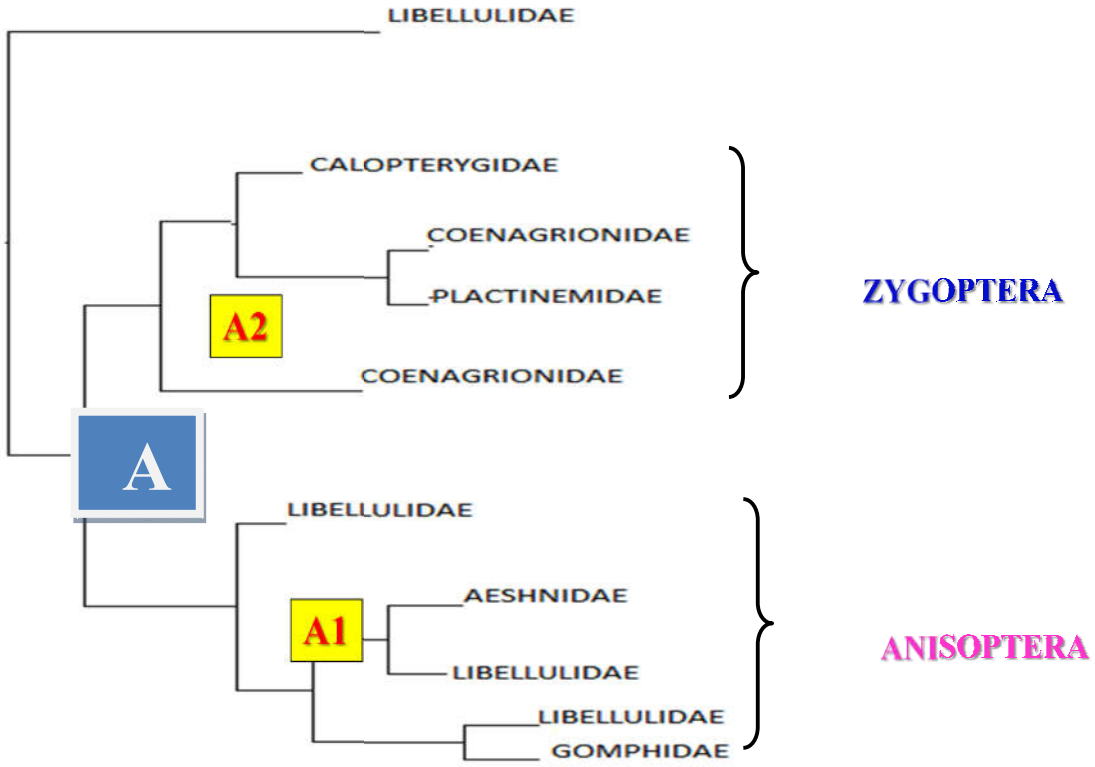


Figure 38: Phylogenetic tree showing inter-familian relationship of odonates

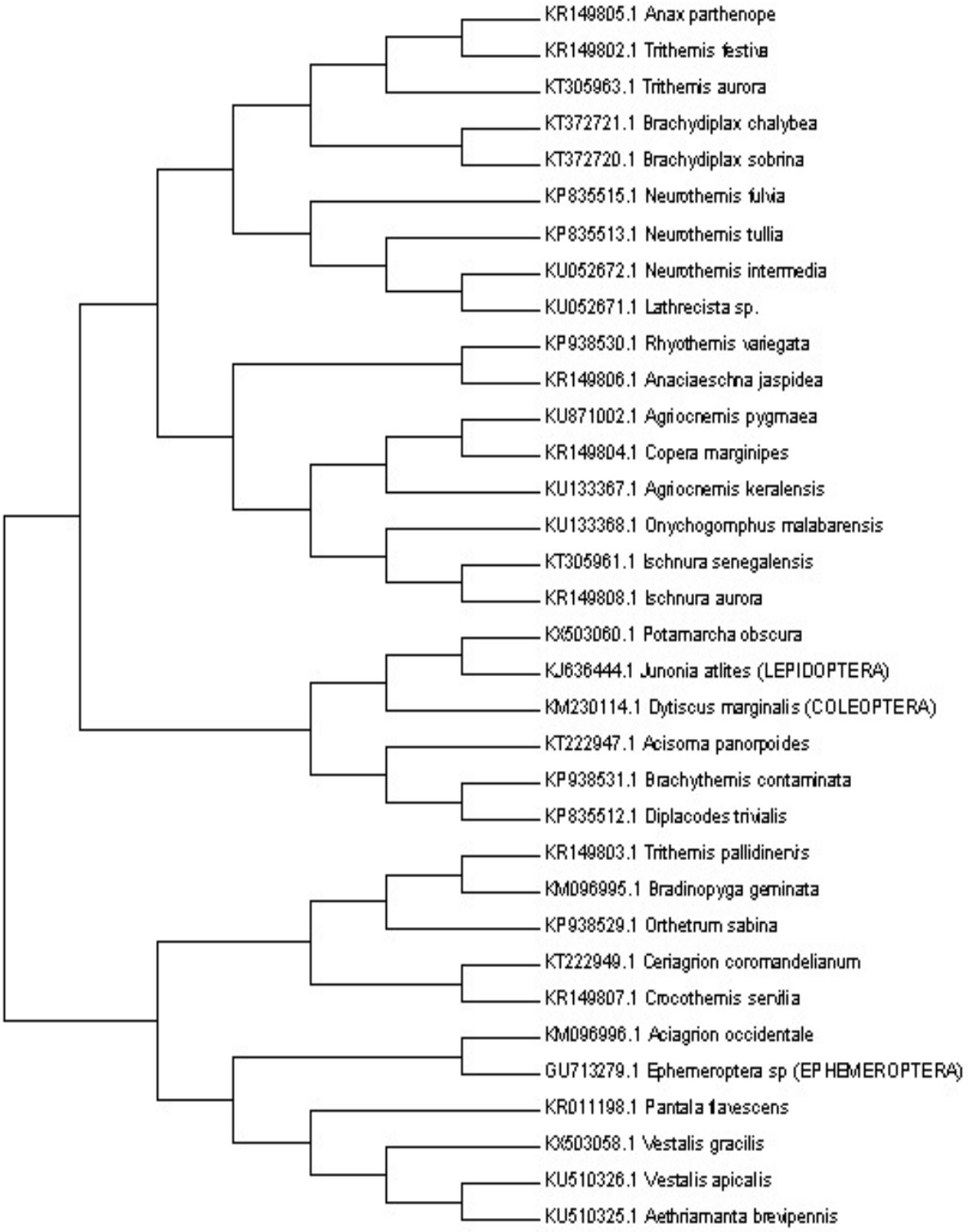


Figure 39: Phylogenetic tree showing relationship of odonates with other insect groups