CHAPTER 4
PHYLOGENETIC ANALYSIS

4.1 INTRODUCTION

Phylogenetics involves reconstruction and depiction of evolutionary relationships. The process of estimation of these relationships is known as phylogenetic analysis and the inferred results are depicted as branched trees. Relationships among molecules, organisms or both can be resolved by this method. Phylogenetics is also known as Cladistics, rooted from the word 'clade' which denotes a set of descendants from a common ancestor. The results of phylogenetic analysis are commonly represented in the form of phylogenetic trees (Brinkman and Leipe, 2001).

A phylogenetic tree is an inference of the relationships among taxa (or sequences) and their presumed common ancestors (Nei and Kumar, 2000; Felsenstein, 2004; Hall, 2013). In the modern world majority of phylogenetic analyses are done based on molecular data. Tree construction based on molecular sequences was first put forward by Emile Zuckerkandl and Linus Pauling (Gonnet, 2012). Either coding DNA or amino acid sequences are used for the construction of phylogenetic trees.

The terms frequently used in interpretation of phylogenetic analysis are as follows: -

1. Clade- The word 'Clade' was derived from the Greek word '*kaldos*' which means branch or twig. A clade is a group of biological taxa that includes the recent common ancestor and all the descendants of that ancestor.
2. Node- Node is the point of a phylogenetic tree from which branches arise.
3. Root- Root represents the ancestral population from which other species evolve.
4. Monophyletic- In a monophyletic group, all species that originated from a common ancestor are grouped together.
5. Paraphyletic- In a paraphyletic group, all species share a common ancestor, but not all descendants of a common ancestor are included in the grouping.
6. Polyphyletic- In the polyphyletic group, species do not have an immediate common ancestor.

Phylogenetic trees are of two types; rooted trees and unrooted trees

a) Unrooted phylogenetic tree

   Unrooted phylogenetic trees depict the relatedness among organisms. These trees are without a root and do not show ancestry.

b) Rooted phylogenetic tree

   Rooted phylogenetic trees depict the relatedness among organisms along with the ancestry. The trees start from a unique node which represents the recent common ancestor.

4.1.1 Construction of Phylogenetic tree

The molecular phylogenetic analysis results can be depicted through diagrams called phylogenetic trees. Detailed analysis of a phylogenetic tree provides information about the species involved and their interrelationships. It is possible to construct both rooted and unrooted trees. If the number of selected taxa is 'n', the possible number of rooted trees N = (2n-3)! 2n-2(n-s)! and possible number of unrooted trees N=(2n-5)!/2n-3(n-3)!.

So, there is the possibility of millions of tree topologies even for fewer taxa. It is important to apply appropriate methods for the selection of an optimal tree. The trees are of two types cladogram and phylogram. Cladogram represents the interrelationship between organisms with respect to a common ancestor and having equal branch length. Phylogram also represents organisms' interrelationship but have unequal branch lengths according to the amount of evolutionary change (Patwardhan et al., 2014).

The method of phylogenetic tree building is comprised of four steps:-

1) Identify and obtain a group of nucleotide or protein sequences

The first step requires intellectual and practical caution but it is often done with the least attention. The lack of precision results in tree invalidity or failure in interpretation. The accuracy in the first step leads to a well-resolved and robust tree.

Often, the investigator is interested in a certain gene or protein that has been studied and wants to know how that gene or protein is related to its homologs. Homologous sequences are required to align using alignment programs. The aligned

sequences are then used to create trees with tree-building software. The tree will be meaningless and maybe deceptive if the sequences are not actually descended from a common ancestor. A Basic Local Alignment Search Tool (BLAST) search is the most reliable approach to find sequences that are homologous to the sequence of interest. The sequence of interest should be used as a query in the BLAST search.

2) Align the sequences

Proper alignment of sequences is crucial for phylogenetic reconstruction. It is possible to recognize the evolutionarily conserved sequence patterns and the ancestral relationships among organisms. Sequence alignment can be done across the entire length (global alignment) or in specific regions (local alignment). Clustal series of programs (Des Higgins and Sharp, 1988) and MUSCLE (Edgar,2004) are the popular MSA software in use (Chenna et al., 2003; Hall, 2013).

3) Estimate the tree

Various approaches for estimating phylogenetic trees are extensively in use in the modern world viz. Neighbor-joining, UPGMA, Maximum Parsimony, Bayesian Inference, and Maximum Likelihood [ML].

4) Precise interpretation of the tree

Precise interpretation of the tree is very crucial. It is upto the investigator to ensure that the information presented is accurate. A phylogenetic tree has the following parts; external nodes that represent the sequences involved, internal nodes that represent the hypothetical ancestor and branches that link between nodes. The branch length between a pair of nodes corresponds to the change occurred.

A variety of tree building methods are available presently. The conventionally used methods are coming under two categories. 1) Character based methods and 2) Distance based methods.

1) Character based methods: These methods rely on the mutational events occurred on the sequences and gives an overall idea about the homoplasy and ancestral characters. More reliable trees can be produced by these methods as the loss of data is prevented. The well accepted methods under this category are maximum parsimony and maximum likelihood methods.

2) Distance based methods: In these methods, evolutionary distance is inferred from the dissimilarity or the distance between sequences (Patwardhan et al., 2014). Pair wise distances between sequences are estimated and the obtained distances are used for tree building. UPGMA and Neighbour joining are the commonly used methods under this category.

Russo (1996) compared the resolving power of different tree building methods. According to him while using considerably longer sequence of appropriate gene in analysis, a well resolved tree is produced despite the method used. A good tree is produced by all tree building methods while a complete set of gene (entire genome) is used.

4.2 REVIEW OF LITERATURE

PHYLOGENETIC ANALYSIS

Phylogeny is the evolutionary history of a species or a group which comprises the branching order and also the time of divergence. The term "Phylogeny" was evolved from two Greek words, "*Phylos*" means "tribe" or "race" and "*geneia*" means "Origin". The foundation stone of phylogenetic studies was laid by Aristotle who classified marine organisms by using morphological and embryological data. The first phylogenetic tree was drawn by Carl Linnaeus, the father of modern taxonomy, who formalised the binomial system of nomenclature. The second milestone was made possible by Charles Darwin, who emphasized phylogenetic branching and divergence (Patwardhan et al., 2014).

The unravelling of phylogenetic relationships among organisms of the world is a mammoth task because of the limitless diversity of nature. There are millions of organisms yet to be described. It is very important to find out the evolutionary history of organisms as they are the results of the evolutionary process (Patwardhan et al., 2014). For classifying species into groups, phylogenetic studies were well utilised from history. Long before the emergence of molecular techniques, the phylogenetic trees rely on the morphological characteristics of organisms. There are lots of phylogenetic studies based on morphology available in the literature (Pfau, 1991; Trueman,1996; Fleck, 2011). Earlier works on phylogeny were mainly based on adult morphology, particularly the wing venation (Needham, 1903; Carle, 1982; Munz, 1919; Trueman, 1996).

O'Grady (2003) used morphological characters for analysing the phylogeny of subfamilies of Coenagrionidae. He used both traditionally accepted characters as well as formerly unstudied characters. The current classification of Coenagrionidae was not supported by cladistics analysis using specific and consistent morphological features. Through this work, he pointed out that the classification of the family Coenagrionidae based on traditional methods is defective. Most of the traits are indistinct, and some clearly defined characters show inconsistency within taxa. Increased percentage of homoplasy also makes a big hurdle in the resolution of phylogenetic relationships using morphological traits.

Several recent works have revealed that morphological traits may fail to resolve close relationships because certain venation features have evolved several times (Dijkstra and Vick, 2006; Fleck et al., 2008; Pilgrim and von Dohlen, 2008). Certain works based on morphological characters were unsuccessful in resolving interfamily relationships (Pfau, 1991; Carle, 1995; Lohmann, 1996; Trueman, 1996; Bechly et al., 1998). Also, some morphological works are even contradictory in family level phylogenetic relationships of Anisoptera (Pfau, 1991; Carle, 1995; Lohmann,1996; Trueman,1996; Bechly et al.,1998; Misof et al., 2001). As adult morphology, especially wing venation, often resulted from homoplasious evolution, much of the previous works require revision (Fleck et al., 2008). Classification based only on plesiomorphic characters is unreliable in the modern world (Vick, 2000; Dijkstra and Vick, 2006). Combining other traits such as anal appendages or larval features (Fleck et al., 2008a; Rehn, 2003) or genetic studies (Bybee et al., 2008; Dumont et al., 2010) with venation data may solve this problem.

Rehn (2003) incorporated skeletal morphology and wing venation of adults with larval characters and found 122 phylogenetically significant features. He used 85 genera referable to 45 families and subfamilies for analysis. Parsimony analysis has resulted in Anisoptera and Zygoptera as two monophyletic clades. This was a contradiction to the well accepted paraphyletic position of Zygoptera. There were two sister clades revealed within Zygoptera, one consists of Calopterygoidea without Amphipterygidae. Amphypterygidae remained within Calopterygidae traditionally however the author found it within the second clade which comprises both Lestinoidea and Coenagrionoidea. Anisozygoptera and Anisoptera grouped into a single clade.

Since the discovery of molecular techniques, molecular data has been routinely employed in taxonomic research for species delineation and the formulation of more reliable species hypotheses (Pimenta et al., 2019). Despite the fact that there have been numerous studies combining data from various branches with morphological data, the use of molecular data in phylogeny is the most widely acknowledged, trustworthy, and practical way. In phylogenetic investigations, the use of molecular techniques has resulted in more accurate conclusions.

The percentage of resemblance among organisms can be calculated by analyzing homologous gene sequences and the information is used to construct the phylogenetic tree. (Patwardhan et al., 2014). The percentage of genetic divergence can be calculated by the variation among the gene sequences of organisms which is resulted from molecular evolution. Development of an enormous quantity of sequence data along with strong tools for statistical analysis for resolving phylogenetic relationships cast light on molecular systematics. Although molecular phylogeny is a branch of biology, it is more related to statistical science as it requires simulation experiments which rely on complicated computations for deducing phylogenetic trees from sequence data (Patwardhan et al., 2014). Although many of the existing hypotheses on morphology-based phylogeny are not supported by molecular data, certain works are in agreement with the traditional phylogeny to some extent (Chippindale et al., 1999).

Molecular phylogenetic analyses are carried out using single or multiple marker genes. Single gene phylogenetic analysis is weaker when compared to multiple gene-based trees in inferring phylogenetic relationships (Chippindale et al., 1999). A phylogenetic tree deduced from a single marker gene will represent only single gene evolution. This may create problems in analysis as other genes may vary both in evolutionary rate and evolutionary history. Variation in evolutionary history usually occurs due to horizontal gene transfer. While vertical gene transfer occurs from parent to offspring, horizontal gene transfer occurs between organisms other than parent and offspring. Despite this phenomenon is more common in prokaryotes it is also seen in eukaryotes and causes difficulties in phylogenetic analysis. So, the results of phylogenetic analysis become more convincing when multiple marker genes are included in the study. The mutation rate of different genes varies according to the tolerance capacity of each gene to perform its function without failure (Patwardhan et al., 2014).

Mitochondrial genes have been considered as the well accepted marker genes since the advent of molecular phylogeny. These genes possessed many qualities which made them ideal for phylogenetic studies. First among them is the easiness in gene amplification and availability of primers. Also, introns are absent in the mitochondrial genes while they are ordinarily seen in nuclear gene sequences. Mitochondrial genes exhibit maternal inheritance, non-recombination and higher

evolutionary rate than nuclear genes (Lin and Danforth, 2004). The tempo of nucleotide substitutions in mitochondrial genome is 5-10 times faster than that of nuclear genome (Brown et al., 1982). Higher evolutionary rate is favourable for species level discrimination (Lin and Danforth, 2004).

Despite these advantages, mitochondrial genes also possess some disadvantages. Nuclear genes can provide more unbiased results because they are unlinked, than the mitochondrial genes which are linked as they are situated on single chromosome (Harrison, 1989). Although higher substitution rate is appropriate for shorter time scale, it become unsuitable for longer time span, particularly more than 10 million years. i.e. the higher mutation rate makes the mitochondrial genes unsuitable for the resolution of deeper branches. Misof et al. (2001) and Misof and Fleck (2003) failed in resolving deeper braches in order Odonata by using mitochondrial markers (Hasegawa and Kasuya, 2006).

There is also an increased chance of homoplasy by mitochondrial genes when analysing phylogeny (Frati et al., 1997; Mooers and Holmes, 2000). Nowadays, nuclear genes are also well accepted for phylogenetic studies since they have some advantages over mitochondrial genes, especially in resolution of deeper divergences. The base composition of nuclear genes shows little bias when compared to that of mitochondrial genes (Tarrio et al., 2001). The rate of evolution is slower than that of mitochondrial genes. They possess two different regions one is slowly evolving and the other is fastly evolving (Brower and DeSalle, 1994). Despite these advantages, sometimes analysis using nuclear genes becomes hard due to the difficulties in PCR amplification and the occurrence of two or more loci which affect the quality of resolution of phylogenetic analysis (Lin and Danforth, 2004).

Studies conducted by using both nuclear and mitochondrial genes revealed the peculiarities of the former one such as higher resolution, lesser homoplasy and better bootstrap support than the latter one (Brady, 2002; Danforth et al., 2003; Leys et al., 2000; 2002; Morris et al., 2002; Reed and Sperling, 1999). Further studies also supported that nuclear genes are advantageous over mitochondrial genes (Baker et al., 2001; Caterino et al., 2000; Lin and Danforth, 2004). Nuclear genes evolve at a slower rate than mitochondrial genes. Slowly evolving nuclear genes are ideal for

the resolution of deeper branches (Hasegawa and Kasuya, 2006; Dumont et al., 2010).

Phylogenetic study by combining both nuclear and mitochondrial data has become an ordinary process recently. These two genes have different evolutionary histories and are unlinked too. By comparing the nuclear and mitochondrial sequences, it is possible to study the substitution patterns of both (Lin and Danforth, 2004).

The ordinarily sequenced mitochondrial marker genes are Cytochrome oxidase subunits I and II (COI & COII), ribosomal RNAs (12S and 16S), Cytochrome b(Cytb), tRNA and NADH Dehydrogenase subunit 1(ND1) and the commonly using nuclear genes in odonate phylogeny are the ribosomal RNA (5.8S, 18S and 28S), the nuclear elongation factor subunit 1 alpha (EF1A), Histone3, Internal Transcribed Spacer-1 and 2 (ITS-1 and ITS-2).

Cytochrome oxidase subunit I (COI) gene, is a crucial protein coding gene in mitochondrial DNA and it is one of the most accepted marker gene for animal species identification for barcoding studies, molecular evolution studies and in analyzing inter and intraspecific diversity (Tallei et al., 2017). Even the closely related species can be easily differentiated by the CO1 sequence divergence (Hebert et al., 2003). The nuclear gene 28S and 18S rRNAs are apt for deep branch resolution because of their highly conserved sequences and are also not suitable for species level discrimination. In contrast, ITS 1&2 nuclear genes and COI, COII, 16S mitochondrial genes are suitable for species level classification (Yong et al., 2014). Ferreira et al. (2014) proposed five new polymorphic nuclear DNA markers which can be used as complementary to the existing marker genes in phylogeny. The five markers are, cell division cycle 5 protein (CDC5), arginine methyltransferase (PRMT), acetylglucosaminyl-transferase (AgT), myosin light chain (MLC) and phosphoglucose isomerase (PGI).

Chippindale et al. (1999) inferred the relationships among North American members of the genus *Ischnura* by using three mitochondrial genes cytochrome b, cytochrome oxidase II and 12S ribosomal DNA. Kambhampati and Charlton (1999) used 16S rRNA mitochondrial gene to identify the taxonomic positions of two Libellulid taxa - *Ladona* and *Plathemis*. They analysed the phylogeny using

parsimony, maximum likelihood and neighbour-joining analyses and reached the conclusion that *Ladona* and *Plathemis* should be incorporated as either genera or subgenera within the family Libellulidae. This result was supported by another study based on two marker genes mitochondrial COI and 16S ribosomal RNA sequences (Artiss et al., 2001). A study using the ribosomal spacers (ITS1 and ITS2) and the intervening 5.8S rDNA gene supported the morphological data partially. The main objective of the study was to deduce biogeographical patterns using sequence data and phylogeny (Weekers et al., 2001).

Dumont et al. (2005) produced a well resolved phylogenetic hypothesis of the calopterygoid superfamily on a combination of molecular phylogeny using the ribosomal 18S and 5.8S genes and internal transcribed spacers (ITS1, ITS2), geographic analysis and fossil data. They selected 62 species for sequencing and phylogenetic analysis belonging to Calopterygidae and Hetaerinidae and other outgroup families such as Polythoridae, Dicteriadidae, Amphipterygidae, Euphaeidae, Chlorocyphidae, Megapodagrionidae, Protoneuridae, Platycnemidae, and Diphlebiidae. The authors tried to find out the phylogenetic relationships and correlate with geographical and geological data. The study resulted in a strongly supported phylogenetic reconstruction which partially supported traditional taxonomy and denoted patterns of distribution. Monophyly of Calopterygidae was revealed and Hetaerinidae was found as sister clade to Calopterygidae. In addition to this, clade of seven subfamilies was also found under Calopterygidae.

Phylogenetic reconstruction of the three suborders of order Odonata using two independent marker genes, the mitochondrial 16S rRNA gene and the nuclear 28S rRNA gene was done by Hasegawa and Kasuya (2006). By analysing sequences, they found that evolutionary rate of 28S rRNA sequences is much slower than 16S rRNA sequences. So 28S rRNA gene is suitable for resolution of deeper branches of phylogenetic tree. The results indicated the paraphyly of Zygoptera. Also, the phylogenetic position of species of Anisozygoptera was in between Anisoptera and Zygoptera.

A well resolved phylogeny of Libelluloidea was generated by using two independent gene fragments, the 16S(mitochondrial) and 28S rRNA (Ware et al., 2007). 28S marker gene fragments of 93 ingroup and 6 out group taxa and 16S

marker gene fragments of 78 ingroup and 5 outgriup taxa were selected for amplification. The authors carried out a combined analysis of both marker genes by using Bayesian, Maximum likelihood and Maximum parsimony analyses. All analyses supported most of the formerly proposed monophyletic groups. Based on the results it was found that Macromiinae, Corduliidae (only one subfamily Corduliinae) and Libellulidae are monophyletic clades. The remaining subfamilies of Corduliidae (Synthemistinae, Gomphomacromiinae, and Idionychinae) form another monophyetic clade. So, the authors suggested these subfamilies along with Cordulephyinae into family Gomphomacromiidae and thus proposed four families under Libelluloidea (Gomphomacromiidae, the Macromiidae, the Corduliidae, and the Libellulidae) in agreement with Fraser (1957) and Davies and Tobin, (1985). Only three formerly proposed subfamilies of Libellulidae were supported along with five additional groups. The study pointed out the problems while using plesiomorphic characters like wing venation in phylogeny. Also, the requirement of focusing on adult evolution and larval morphological features was also studied.

The odonate family level relationships were well scrutinized by Carle et al. (2008) and inferred the families Lestidae and Synlestedae as sister to other Zygopteran families. They used Bayesian methods for analysing 28S and 18S nuclear ribosomal RNAs, EF1α and 12S and 16S mitochondrial rRNAs. Fleck et al. (2008) applied larval morphology and molecular data for the classification of subfamilies of family Libellulidae. The work suggested that certain species of subfamily Tetrathemistinae shows close similarity to the species of subfamily Libellulinae. A combined study using COI barcode data, male genitalia, wing venation and geometrical variation was done on four populations of a single species *Polythore procera.* Two reciprocal monophyly and a high barcode divergence of 3% were observed and this pointed out the possibility of cryptic speciation (Herrera et al., 2010). Dumont et al. (2010) documented odonate phylogeny using the nuclear ribosomal genes 5.8S, 18S and intergenic spacers ITS1 and ITS2. 18S analysis helped in the resolution of deep relations and has brought Zygoptera and Epiprocta as monophyletic. While analysis of all the genes mentioned above resolved recent branches better.

Froufe et al. (2014) selected the *Cordulegaster* genus for molecular phylogeny using COI and ITS-1 gene fragments which is the first record of the same

genus from Europe. The molecular data supported the traditional major groups – *boltonii* and *bidentata*. But there was also noted little genetic variation between 2 subspecies- *Cordulegaster bidentata bidentata* and *Cordulegaster bidenta sicilica*. Phylogeny and systematics of dragonflies of the genus *Orthetrum* was studied by Yong et al. (2014) using 28S rRNA, ITS1 & 2 nuclear genes and COI, COII and 16S rRNA mitochondrial genes. Cryptic speciation between *O. pruinosum schneideri* and *O. pruinosum neglectum* could be observed as a result of this study.

Dijkstra et al. (2014) carried out a vast phylogenetic reconstruction of damselflies including 59% of all the known genera and all families except Hemiphlebiidae by using 16S and COI mitochondrial and 28S nuclear marker genes. Both individual and combined analyses of these genes were done using maximum parsimony, maximum likelihood and Bayesian inference methods. Families Calopterygidae, Chlorocyphidae, Euphaeidae, Isostictidae, Lestidae, Lestoideidae, Platystictidae and Polythoridae were evolved as strongly supported monophyletic clades. The authors proposed a partial reclassification. This includes the restructuring of the superfamily Coenagrionoidea to comprise the three families Isostictidae, Platycnemididae and Coenagrionidae. The genera *Archboldargia, Hylaeargia, Palaiargia, Papuargia* and *Onychargia* were previously placed in Coenagrionidae, and were moved to Platycnemididae. Also, the genera *Leptocnemis, Oreocnemis* and *Thaumatagrion* were transferred from Platycnemididae to Coenagrionidae. Well supported clades of Platycnemididae were considered as subfamilies. As a result, Disparoneurinae was added and three subfamilies Allocnemidinae, Idiocnemidinae, Onychargiinae and one tribe Coperini were described. Another one, Calicnemiinae has been restricted. Most of the larger genera didn't show monophyly requiring a detailed revision of the suborder. Many of the well accepted families like Calopterygidae, Euphaeidae and Platycnemididae were devoid of clear morphological apomorphies. Consistency of certain morphological features, particularly wing venation characters with molecular data was very low. Family Protoneuridae was divided into six clades in five families- Platystictidae, Lestoideidae, Isostictidae, Platycnemididae, Coenagrionidae. The study results pointed out the requirement of revision of the traditional taxonomy based on fossil data which relies mainly on wing venation with the help of molecular data.

Hamalainen et al. (2015) used molecular and morphological methods for the revision of genus *Dysphaea*. Phylogenetic analysis was done by using three marker genes COI, 16S and 28S rRNA genes. Casas et al. (2018) collected 36 species of 19 genera and 10 families from Mindanao island and produced 134 COI barcodes. Out of 36 species records, 31 species were first barcode records. The observed barcode divergence gap was negligible within species and also between species. A great number of islands facilitated fast species formation and this may be the reason for the above condition. Mitochondrial 12S rRNA gene sequence was used to deduce odonate phylogeny. The study revealed Anisoptera as monophyletic while Zygoptera as paraphyletic and family Lestidae was found more closer to Anisoptera than Zygoptera. Pimenta et al. (2019) used molecular markers COI, 16S rRNA and PRMT (the gene encoding arginine methyltransferase) for the first phylogenetic analysis of the 7 species of the genus Forcepsioneura. PMRT was also suggested by Ferreira et al. (2014).

A comparative study of traditional and molecular methods of phylogeny was conducted by Huang et al. (2020) to scrutinise the compatibility between the two. Mitochondrial COI gene and the nuclear genes 18S, 28S rRNA and ITS gene markers were used for molecular phylogeny of 10 Libellulid species. Wing morphology and migratory behaviour were selected for morphology based analysis. The close relationship between wing morphology and migratory capacity was proved. The phylogenetic significance of forewings and species-specific variation of dragonfly wing structure are also described. The shape of forewing bears only limited phylogenetic data and hind wing shape bears not worthy phylogenetic data when compared to molecular information.

Chavarria and Carpenter (1994) put forward the combined analysis method (total evidence method) for combining the sequence data from different marker genes and to construct well supported phylogenetic relationships. Phylogenetic hypotheses based on combined data analyses would be stronger and more reliable (Artiss et al., 2001; Chippindale et al., 1999; Flook et al., 1999). The bootstrap and decay index values are higher for the resulting trees, and the unsettled polytomies are rare.

But the combined analysis was not always successful. Controversial results made the results of separate analyses unclear. This condition occurs when separate analyses of components show contradictory results (Lecointre and Deleporte, 2005). Comprehensible signals from one marker gene data set are concealed by phylogenetically misleading characters from another marker gene (Hasegawa and Kasuya, 2006). The effectiveness of this method can be fully achieved when data from different sources are congruent. If data from multiple sources show incongruence the resolution power of combined analysis will lessen. Literature strongly recommends a method of doing separate analysis first followed by combined analysis (Farris et al., 1994; Hasegawa and Kasuya, 2006). So separate analysis should be the first preference of every phylogenetic study.

4.3 MATERIALS AND METHODS

Phylogenetic analyses were performed for resolving relationships under two suborders, selected families and genera. The partial COI and 18S rRNA gene sequences obtained were used for the analyses. The suborder trees were constructed using the sequences generated during the present study. For family and genus tree construction, supplementary sequences were retrieved from GenBank.

4.3.1 Retrieval of supplementary sequences

For the construction of a particular family tree, all the genera of the corresponding family were noted and searched for the sequences of the species that belong to these genera in databases. Priority was given to the genera present in India or Asian continent. From the available sequences, sequence having good product size and quality was selected. These sequences were used along with the sequences generated during the study. Both mitochondrial COI gene and the nuclear 18S rRNA gene were used and separate trees were generated for each marker gene. The selected sequences were saved in MS Word file along with the generated sequences of the corresponding family. The sequences were aligned using the Clustal Omega tool (Sievers and Higgins, 2014) and trimmed manually. The trimmed sequences were saved as MS Notepad file and used for further analyses.

For construction of genus trees, BLAST search on generated sequences was done and conspecific (if available) and congeneric sequences were retrieved from GenBank. As nuclear 18S rRNA gene has highly conserved regions, only mitochondrial COI gene was incorporated in genus trees. From the available sequences, sequences with good product size and quality were selected and saved along with the generated sequences as separate MS Word documents. Sequences of each genus were aligned using the tool Clustal Omega and manually trimmed. The aligned sequences were saved as MS Notepad files.

4.3.2 Construction of Phylogenetic tree

The tree construction at different taxonomic levels was carried out using the Molecular Evolutionary Genetics Analysis version 11 (MEGA 11) software (Tamura et al. 2021). In the first step, sequences of a single file were aligned once again in MEGA 11 and exported the file into MEGA format and saved. Model selection was done prior to the tree construction. The model with lowest BIC

(Bayesian Information Criterion) value was considered for tree construction. General Time Reversible (GTR), Tamura–Nei, Hasegawa–Kishino–Yano, Tamura Three-Parameter, Kimura Two-Parameter, Tajima–Nei, Jukes–Cantor are the substitution models in MEGA (Tamura et al. 2011). The tree was constructed based on the Maximum likelihood method (Hasegawa et al. 1991) and the best fit model by bootstrap analyses over 500 replicates (Felsenstein, 1985).

4.3.3 Calculation of genetic divergence

The intraspecific and interspecific divergence values of the sequences used for phylogenetic tree construction were calculated using the best fit model (the model with lowest BIC value) and presented as tables.

4.3.4 Estimation of nucleotide composition

Nucleotide composition of COI and 18S rRNA gene sequences involved in the analyses were calculated. The AT and GC percentages were estimated and compared between both marker genes.

4.4 RESULTS

Phylogenetic analyses were carried out at different taxonomic levels of order Odonata. Phylogenetic works on odonates of Kerala are meagre, particularly studies based on more than one marker gene that have not been conducted so far. So the present study based on dual maker genes is a novel work in this category. Analyses using mitochondrial (COI) and nuclear (18S rRNA) marker genes were carried out for comparing the efficiency and accuracy of these genes in resolving relationships at different taxonomic levels. In the first step, relationships within suborders were studied. This was followed by the phylogenetic analysis of selected families. Finally, phylogenetic relationships among the members of 27 genera based on COI gene sequences were resolved. As the 18S rRNA gene sequence has more conserved regions, it was excluded from the species level analyses. There was no COI and 18S rRNA gene sequences of the genus *Onychothemis* were available in the GenBank for comparison and analysis, as the current sequences of *Onychothemis testacea* are the pioneer records of the genus. So phylogenetic analysis of the corresponding genus was not carried out.

All the trees were constructed using Mega 11 software and the best fit model. The genetic divergence and nucleotide composition were calculated by the same tool. 68 sequences generated during the present work were used along with sequences retrieved from the GenBank database for the tree construction.

4.4.1 PHYLOGENY OF THE SUBORDER ZYGOPTERA

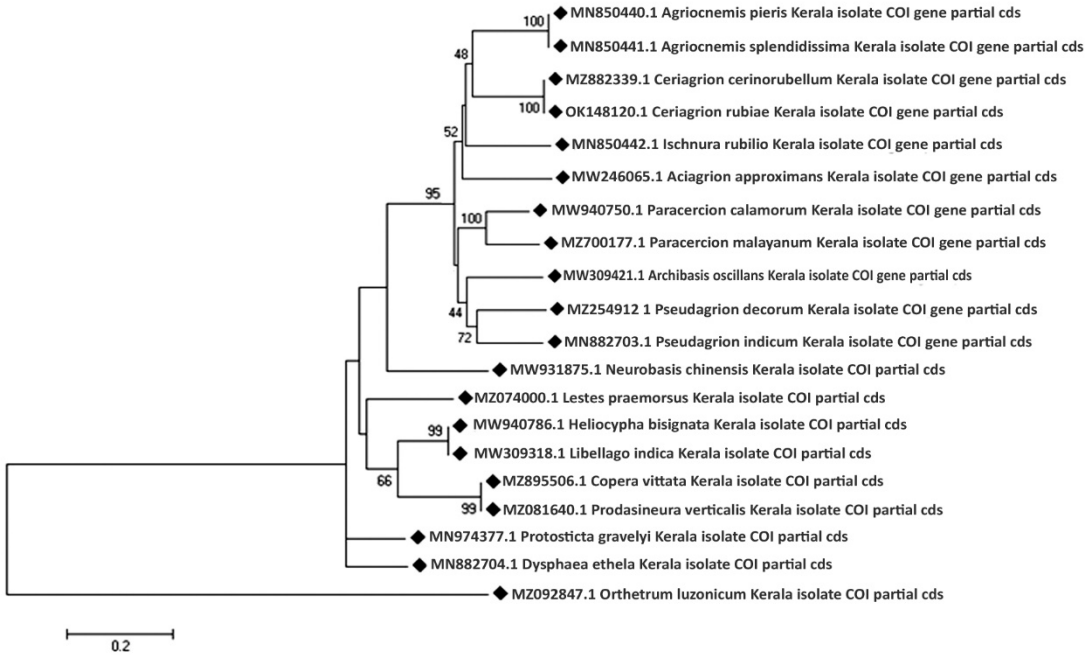

Figure 4.4.1: Inferred phylogenetic tree based on COI gene sequences of suborder Zygoptera

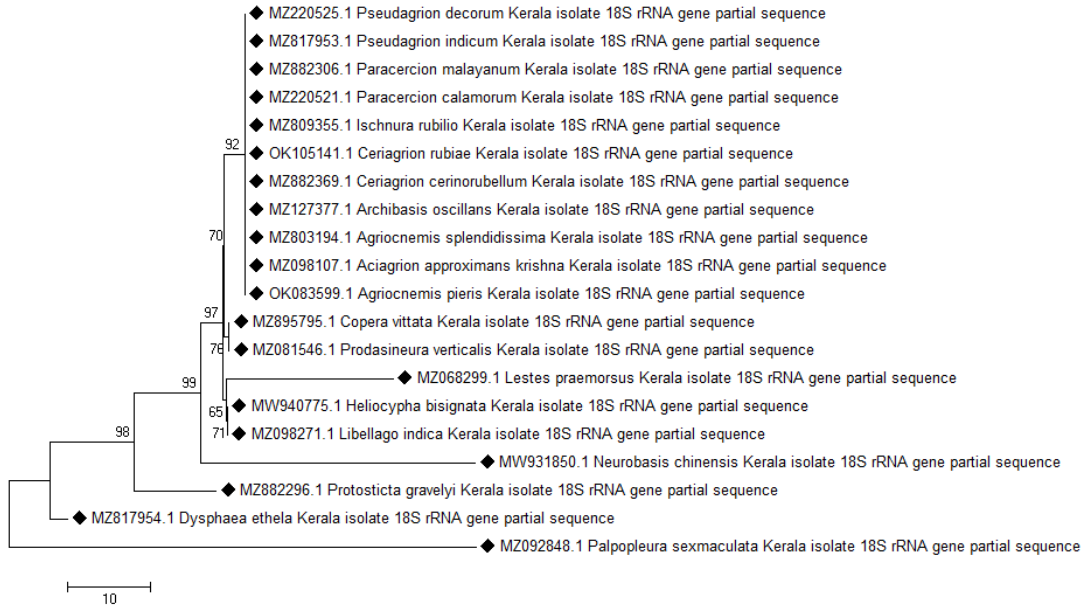

Figure 4.4.2: Inferred phylogenetic tree based on 18S rRNA gene sequences of suborder Zygoptera

Phylogenetic analysis

Phylogeny of the species belonging to the suborder Zygoptera based on partial COI and 18S rRNA gene sequence were resolved. The analysis involved 19 Zygopteran sequences generated during the present study and a species of suborder Anisoptera as out group. A total of 20 sequences were involved in the analysis.

a) Based on partial COI gene sequence

The analysis (Figure 4.4.1) showed the monophyly of families Coenagrionidae, Calopterygidae, Lestidae, Chlorocyphidae, and Platycnemididae and was found as a distinct clade. The remaining families Platystictidae and Euphaeidae were polyphyletic to the former clade showing more genetic divergence. Family Coenagrionidae was monophyletic (bootstrap 95%) and Calopterygidae shared common ancestry with Coenagrionidae but genetically diverged. Chlorocyphidae and Platycnemididae were sister clades and Lestidae was paraphyletic to them. Genera such as *Agriocnemis, Paracercion* and *Ceriagrion* were formed separate clusters with bootstrap value of 100.

b) Based on partial 18S rRNA gene sequence

All the species were grouped into distinct clusters according to the family they belonging to (Figure 4.4.2). Species of the family Euphaeidae was found as highly diverged from the common ancestor followed by the family Platystictidae (*Protosticta gravelyi*) and Calopterygidae (*Neurobasis chinensis*). From the common ancestor, a monophyletic clade of Coenagrionidae, Platycnemididae, Lestidae and Chlorocyphidae was evolved. Euphaeidae, Platystictidae and Calopterygidae were polyphyletic.

4.4.2 PHYLOGENY OF THE SUBORDER ANISOPTERA

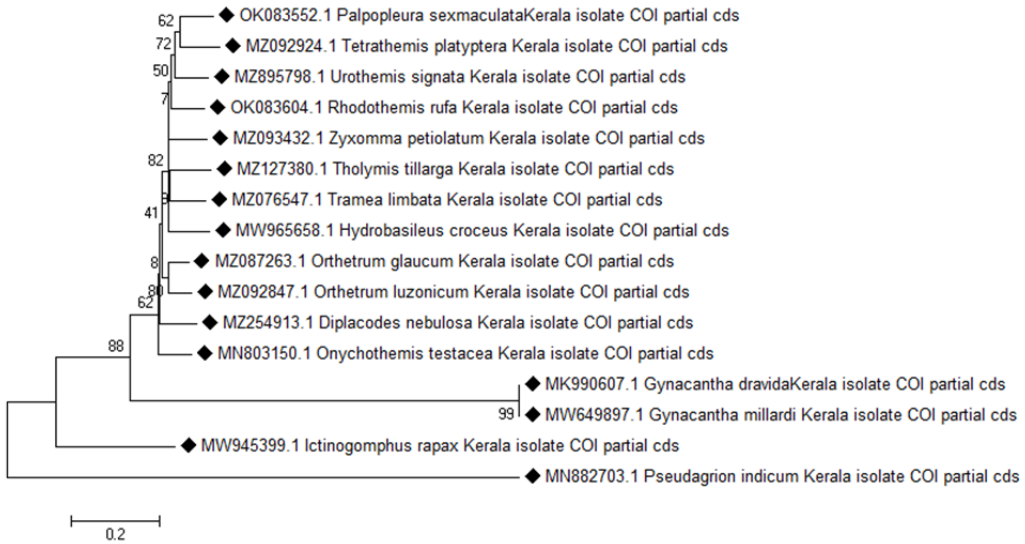

Figure 4.4.3: Inferred phylogenetic tree based on COI gene sequences of suborder Anisoptera

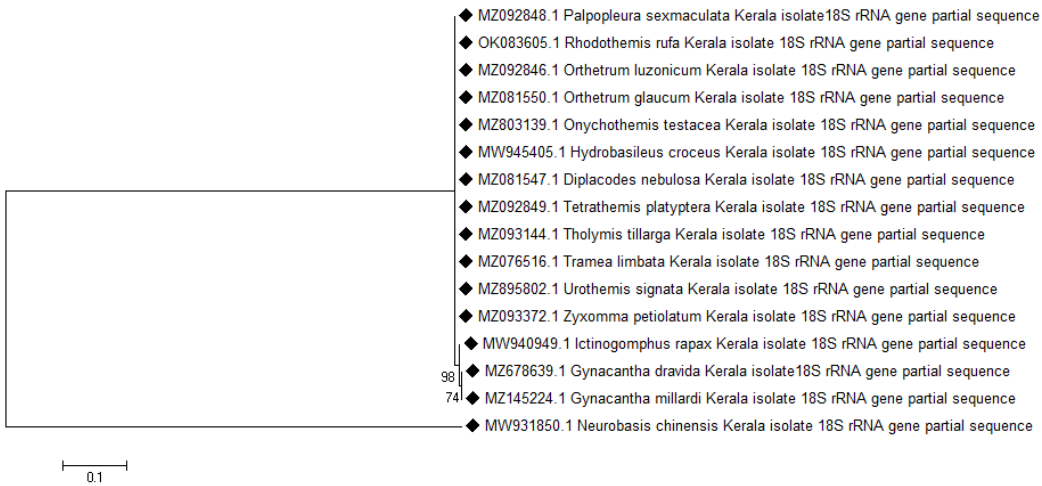

Figure 4.4.4: Inferred phylogenetic tree based on 18S rRNA gene sequences of suborder Anisoptera

Phylogenetic analysis

Phylogenetic relationship among the members of suborder Anisoptera based on partial COI and 18S rRNA gene sequences were resolved. Fifteen species of suborder Anisoptera and a species of suborder Zygoptera as out group which were sequenced during the present work were used for the analyses. The well resolved 16 sequence phylogenies were presented in Figures 4.4.3 and 4.4.4.

a) Based on partial COI gene sequence

The phylogenetic tree showed that species of three families were clustered into distinct monophyletic clades. Family Aeshnidae and family Gomphidae were polyphyletic to the family Libellulidae. Family Gomphidae was found as sister to the remaining families (bootstrap 88%). Relationships up to species level were resolved by the COI analysis.

b) Based on partial 18S rRNA gene sequence

The result indicated the monophyly of family Aeshnidae. The other families Libellulidae and Gomphidae were polyphyletic. Relationships between species were not resolved by the 18S analysis.

4.4.3 PHYLOGENY OF SELECTED FAMILIES

1) Resolution of phylogenetic relationships within Family Lestidae

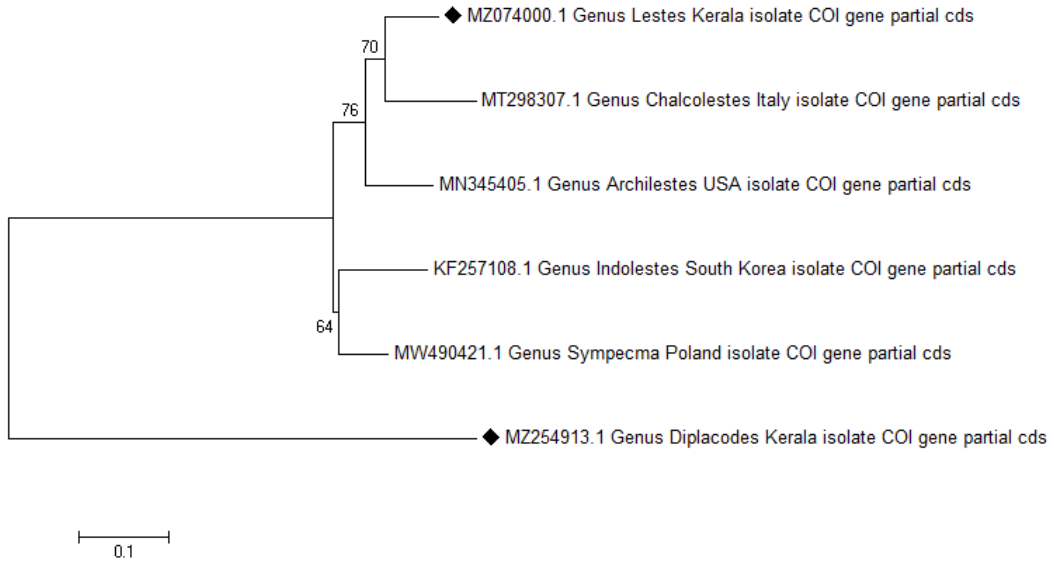

Figure 4.4.5: Inferred phylogenetic tree based on COI gene sequences of family Lestidae, rooted by outgroup

Phylogenetic analysis and genetic divergence

Phylogenetic reconstruction of the genera of family Lestidae was carried out by COI and 18S rRNA gene sequence of *Lestes praemorsus* along with sequences of

4 genera downloaded from GenBank and sequence of *Diplacodes nebulosa* was included as out group.

a) Based on partial COI gene sequence

The COI analysis (Figure 4.4.5) indicated the presence of two distinct clades in the phylogeny of family Lestidae (Clade 1: *Lestes, Chalcolestes & Archilestes*; Clade 2: *Indolestes & Sympecma*). Genus *Lestes* + *Chalcolestes* and *Indolestes+Sympecma* formed sister clades. *Archilestes* was paraphyletic to *Lestes* and *Chalcolestes* and shared a common ancestry.

The percentage of divergence was maximum (18.5%) between *Chalcolestes* and *Indolestes* and minimum (12.9%) between *Archilestes* and *Lestes* (Table 4.4.1).

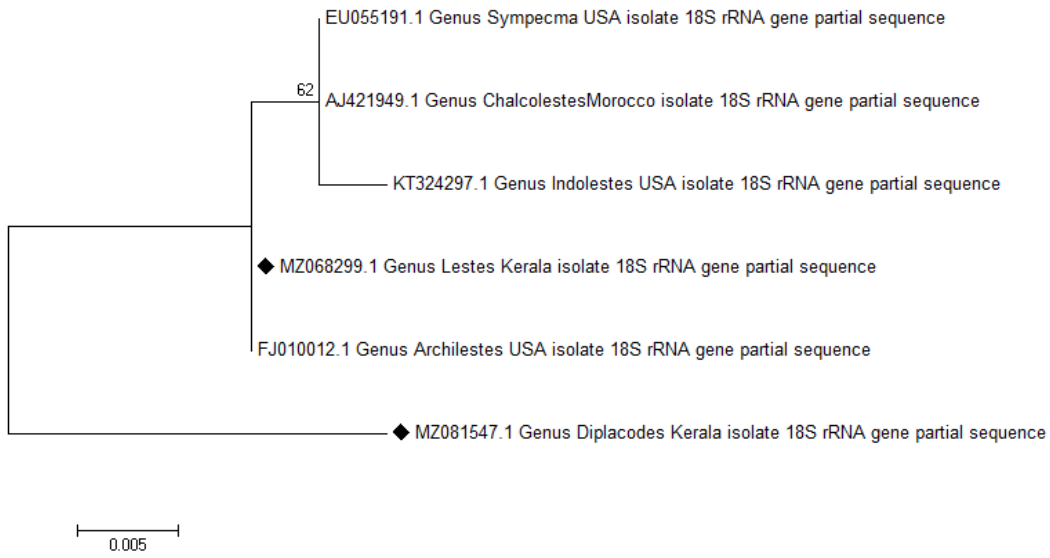

Figure 4.4.6: Inferred phylogenetic tree based on 18S rRNA gene sequences of family Lestidae, rooted by outgroup

b) Based on partial 18S rRNA gene sequence

The 18S analysis suggested that, genera *Sypecma, Chalcolestes* and *Indolestes* were monophyletic to each other. *Lestes* and *Archilestes* were polyphyletic and genetically more diverged from the other genera (Figure 4.4.6).

According to the calculated divergence values, no divergence was observed between *Lestes* and *Archilestes ; Chalcolestes* and *Sympecma*. The divergence values ranged from 0% to 0.7% (Table 4.4.3).

Nucleotide composition

The nucleotide composition of six COI partial gene sequences were 39.37% (A), 27.98% (T/U), 16.41% (C) and 16.24% (G). AT content was high (67.35%) over the GC content (32.65%). The nucleotide frequencies of six 18S rRNA partial gene sequnces were 18.51% (A), 29.59% (T/U), 20.47% (C) and 31.43% (G) and the distribution of nucleotides was balanced (AT content 48.1% ; GC content 51.9%) (Table 4.4.2 and 4.4.4).

Table 4.4.1 Estimates of genetic divergence of the COI gene sequences of family Lestidae and out group

	Genus COI	**1**	**2**	**3**	**4**	**5**
1.	MZ074000.1_Genus_*Lestes*_Kerala					
2.	KF257108.1_Genus_*Indolestes*_South_Korea	0.167				
3.	MN345405.1_Genus_*Archilestes*_USA	0.129	0.162			
4.	MW490421.1_Genus_*Sympecma*_Poland	0.136	0.136	0.147		
5.	MT298307.1_Genus_*Chalcolestes*_Italy	0.144	0.185	0.159	0.154	
6.	MZ254913.1_Genus_*Diplacodes*_Kerala	0.558	0.553	0.548	0.548	0.571

Table 4.4.2 Nucleotide base composition of COI gene sequences of family Lestidae and out group

Domain: Data COI																
	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
MZ074000.1 Genus *Lestes* Kerala	33.0	18.9	30.1	18.0	33	8.0	49.3	10.0	21	20.7	28.7	30.0	46	28.2	12.1	14.1
KF257108.1 Genus *Indolestes* South Korea	29.6	20.3	30.5	19.6	23	12.0	50.7	14.0	21	20.0	28.7	30.7	45	28.9	12.1	14.1
MN345405.1 Genus *Archilestes* USA	33.0	19.2	31.2	16.7	30	11.3	52.7	6.0	23	18.0	28.7	30.0	46	28.2	12.1	14.1
MW490421.1 Genus *Sympecma* Poland	32.7	18.0	30.3	18.9	29	8.7	50.0	12.7	24	17.3	28.7	30.0	46	28.2	12.1	14.1
MT298307.1 Genus *Chalcolestes* Italy	32.7	19.2	29.4	18.7	32	9.3	48.0	10.7	21	20.0	28.7	30.7	46	28.2	11.4	14.8
MZ254913.1 Genus *Diplacodes* Kerala	11.8	3.1	80.5	4.6	14	1.5	81.5	3.1	5	3.8	83.1	7.7	16	3.9	76.7	3.1
Avg.	29.2	16.7	37.7	16.4	27	8.6	54.8	9.5	19	16.9	36.7	26.9	41	24.7	21.5	12.6

Table 4.4.3 Estimates of genetic divergence of the 18S rRNA gene sequences of family Lestidae and out group

	Genus 18S	**1**	**2**	**3**	**4**	**5**
1.	MZ068299.1_Genus_*Lestes*_Kerala					
2.	KT324297.1_Genus_*Indolestes*_USA	0.007				
3.	EU055191.1_Genus_*Sympecma*_USA	0.003	0.003			
4.	FJ010012.1_Genus_*Archilestes*_USA	0.000	0.007	0.003		
5.	AJ421949.1_Genus_*Chalcolestes*Morocco	0.003	0.003	0.000	0.003	
6.	MZ081547.1_Genus_*Diplacodes*_Kerala	0.030	0.037	0.034	0.030	0.034

Table 4.4.4 Nucleotide base composition of 18S rRNA gene sequence of family Lesidae and out group

Domain: Data 18S																
	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
MZ068299.1 Genus *Lestes* Kerala	29.4	20.7	18.4	31.4	36	20.0	18.0	26.0	27	21.0	22.0	30.0	25	21.2	15.2	38.4
KT324297.1 Genus *Indolestes* USA	29.8	20.4	18.7	31.1	36	20.0	19.0	25.0	28	20.0	22.0	30.0	25	21.2	15.2	38.4
EU055191.1 Genus *Sympecma* USA	29.8	20.4	18.4	31.4	36	20.0	18.0	26.0	28	20.0	22.0	30.0	25	21.2	15.2	38.4
FJ010012.1 Genus *Archilestes* USA	29.4	20.7	18.4	31.4	36	20.0	18.0	26.0	27	21.0	22.0	30.0	25	21.2	15.2	38.4
AJ421949.1Genus *Chalcolestes* Morocco	29.8	20.4	18.4	31.4	36	20.0	18.0	26.0	28	20.0	22.0	30.0	25	21.2	15.2	38.4
MZ081547.1 Genus *Diplacodes* Kerala	28.9	21.5	18.5	31.2	35	20.2	18.2	26.3	26	22.0	23.0	29.0	25	22.2	14.1	38.4
Avg.	29.5	20.7	18.5	31.3	36	20.0	18.2	25.9	27	20.7	22.2	29.8	25	21.4	15.0	38.4

2) Resolution of phylogenetic relationships within Family Platystictidae

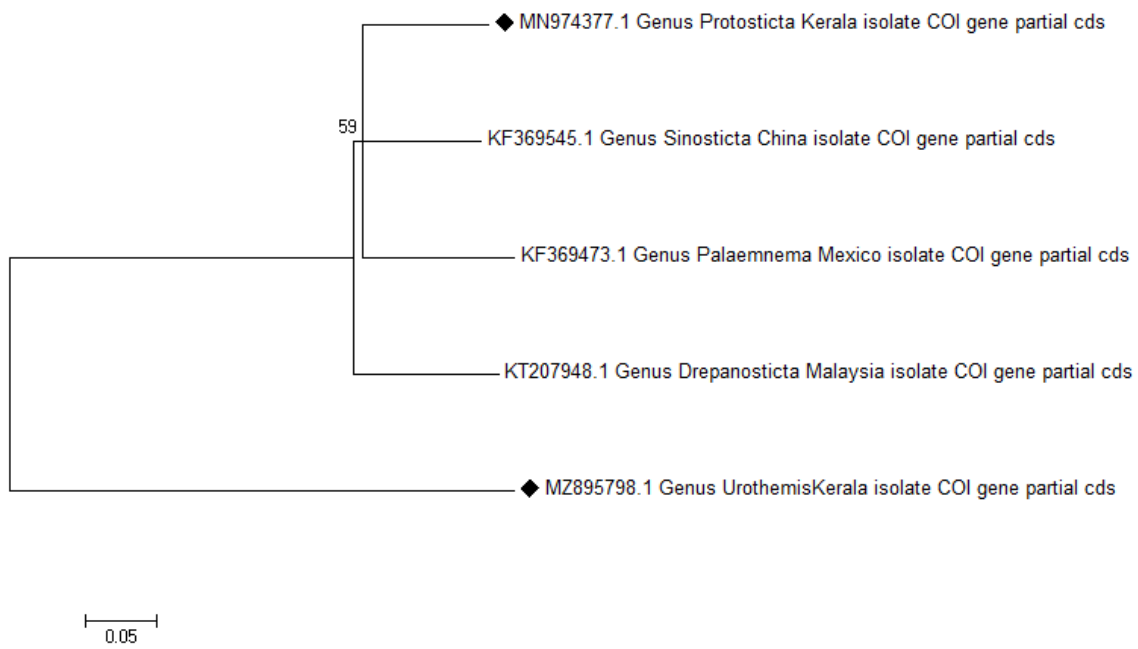

Figure 4.4.7: Inferred phylogenetic tree based on COI gene sequences of family Platystictidae, rooted by outgroup

Table 4.4.5: Estimates of genetic divergence of the COI gene sequences of family Platystictidae and out group

	Genus (COI gene sequence)	**1**	**2**	**3**	**4**
1.	MN974377.1_Genus_*Protosticta*_Kerala				
2.	KF369473.1_Genus_*Palaemnema*_Mexico	0.192			
3.	KF369545.1_Genus_*Sinosticta*_China	0.170	0.187		
4.	KT207948.1_Genus_*Drepanosticta*_Malaysia	0.196	0.208	0.192	
5.	MZ895798.1_Genus_*Urothemis* Kerala	0.682	0.705	0.674	0.690

Table 4.4.6 : Nucleotide base composition of COI gene sequences of family Platystictidae and out group

Domain: Data COI																
	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
MN974377.1 Genus *Protosticta* Kerala	31.2	20.2	30.2	18.4	38	26.7	24.1	11.5	26	13.2	43.4	17.5	30	20.5	23.2	26.3
KF369473.1 Genus *Palaemnema* Mexico	30.9	19.8	31.1	18.2	36	25.1	26.2	12.6	25	15.3	42.9	16.4	31	18.9	24.2	25.8
KF369545.1 Genus *Sinosticta* China	31.6	19.3	29.6	19.5	37	25.1	24.6	13.1	26	13.8	41.3	18.5	31	18.9	23.2	26.8
KT207948.1 Genus *Drepanosticta* Malaysia	32.1	19.8	29.1	18.9	40	26.2	20.9	13.1	26	13.2	43.4	17.5	31	20.0	23.2	26.3
MZ895798.1 Genus *Urothemis*Kerala	13.9	7.2	10.6	68.3	18	9.1	8.5	64.8	11	1.7	17.7	69.1	13	10.9	5.7	70.9
Avg.	28.2	17.4	26.4	28.0	34	22.7	21.1	22.3	23	11.6	38.0	27.2	27	18.0	20.1	34.7

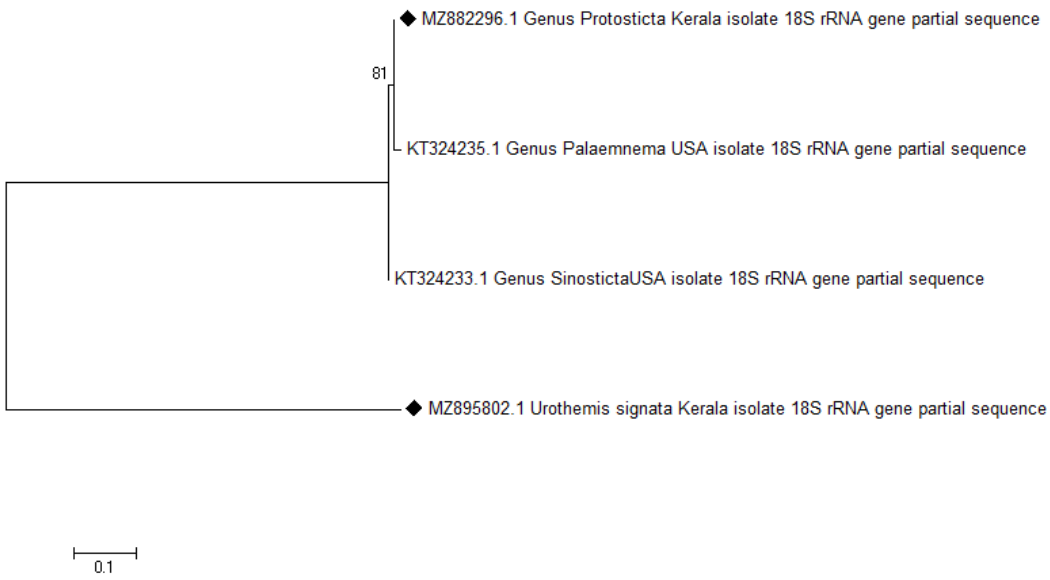

Figure 4.4.8: Inferred phylogenetic tree based on 18S rRNA gene sequences of family Platystictidae, rooted by outgroup

Phylogenetic analysis and genetic divergence

Resolution of phylogeny based on partial COI and 18S rRNA gene sequences of the genera of family Platystictidae was performed.

a) Based on partial COI gene sequence

The sequence data involved were the sequence of genus *Protosticta*, sequences of three genera retrieved from GenBank and included the dragonfly genus *Urothemis* as out group. The result showed that genera *Protosticta, Palaemnema* and *Sinosticta* were clustered into a monophyletic clade. *Drepanosticta* was paraphyletic and more diverged from the other three (Figure 4.4.7).

The genetic divergence value (Table 4.4.5) was minimum between *Sinosticta* and *Protosticta* (17%) and maximum between *Drepanosticta* and *Palaemnema* (20.8%).

b) Based on partial 18S rRNA gene sequence

The sequence of genus *Protosticta* and sequence of other 2 genera downloaded from GenBank were used for analysis and genus *Urothemis* was included as out group. According to the result, the three genera analysed were found to be monophyletic and *Protosticta* and *Palaemnema* were closer and formed sister clades (Figure 4.4.8).

The genetic divergence value was maximum (Table 4.4.7) between *Palaemnema* and *Sinosticta* (0.8%). *Protosticta* showed equal and minimum divergence from other two genera (0.4%).

Nucleotide composition

Nucleotide composition of four partial COI gene sequences were 28.2%(T),17.4%(C), 26.4%(A), 28.0%(G). The observed AT content was 54.6% and GC content was 45.4% (Table 4.4.6).The nucleotide composition of four 18S rRNA partial gene sequence were 32.11 % (A), 23.33% (T/U), 18.41% (C) and 26.15% (G). The AT content was 55.44% and GC content was 44.56% (Table 4.4.8).

Table 4.4.7: Estimates of genetic divergence of the 18S rRNA gene sequences of family Platystictidae and out group

	Genus 18S	**1**	**2**	**3**
1	MZ882296.1_Genus_*Protosticta*_Kerala			
2	KT324235.1_Genus_*Palaemnema*_USA	0.004		
3	KT324233.1_Genus_*Sinosticta*USA	0.004	0.008	
4	MZ895802.1_*Urothemis_signata*_Kerala	0.469	0.473	0.469

Table 4.4.8: Nucleotide base composition of 18S rRNA gene sequences of family Platystictidae and out group

Domain: Data 18S																
	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
MZ882296.1 Genus *Protosticta* Kerala	27.6	21.8	20.5	30.1	24	23.8	16.3	36.3	30	20.0	20.0	30.0	29	21.5	25.3	24.1
KT324235.1 Genus *Palaemnema* USA	27.2	22.2	20.5	30.1	24	23.8	16.3	36.3	30	20.0	20.0	30.0	28	22.8	25.3	24.1
KT324233.1 Genus *Sinosticta*USA	28.0	21.3	20.5	30.1	25	22.5	16.3	36.3	30	20.0	20.0	30.0	29	21.5	25.3	24.1
MZ895802.1 *Urothemis signata* Kerala	10.2	8.2	67.8	13.9	7	9.8	65.9	17.1	10	8.5	64.6	17.1	14	6.2	72.8	7.4
Avg.	23.2	18.3	32.5	26.0	20	19.9	28.9	31.4	25	17.1	31.4	26.7	25	17.9	37.4	19.8

3) Resolution of phylogenetic relationships within Family Calopterygidae

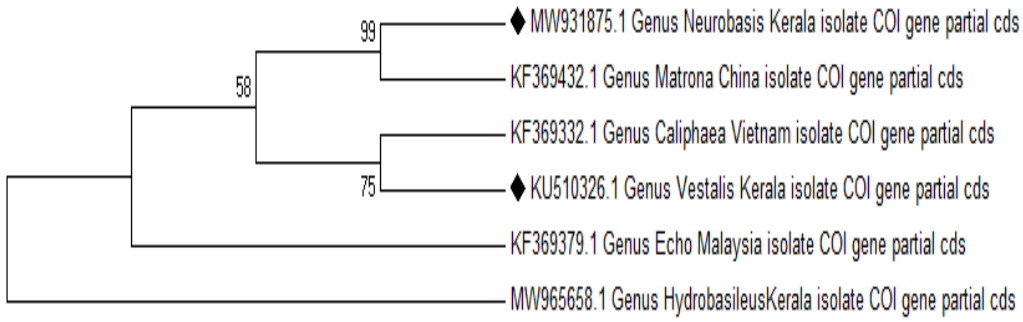

Figure 4.4.9: Inferred phylogenetic tree based on COI gene sequences of family Calopterygidae, rooted by outgroup

Phylogenetic analysis and genetic divergence

Phylogenetic relationships within the family Calopterygidae were resolved and genetic divergence values were calculated.

a) Based on partial COI gene sequence

Phylogeny of genera under the family Calopterygidae were resolved by using partial COI gene sequences of *Neurobasis chinenesis* and sequences of 4 genera which were retrieved from GenBank. Sequence of *Hydrobasileus croceus* was included as out group. Sister clade relationship of *Neurobasis+Matrona* and *Caliphaea +Vestalis* were revealed from the result. Genus *Echo* was found genetically more diverged from the other four genera (Figure 4.4.9).

The calculated divergence value was minimum between *Matrona* and *Neurobasis* (16.2%) and maximum between *Caliphaea* and *Neurobasis* (25.1%) as shown in Table 4.4.9.

b) Based on partial 18S rRNA gene sequence

Phylogeny of the Calopterygid genera were resolved using partial 18S rRNA gene sequence of *Neurobasis chinenesis* and sequences of 4 genera retrieved from GenBank. 18S rRNA gene sequence of *Ictinogomphus rapax* was used as out group. The relationship among the genera of family Calopterygidae was not clearly discriminated by 18S rRNA phylogeny. All the genera were clustered into a single monophyletic clade (Figure 4.4.10).

The genetic divergence value was 0% between the genera except the out-group genus (Table 4.4.11).

Nucleotide composition

The nucleotide frequencies of six COI partial gene sequences are 38.68% (A), 26.20% (T/U), 18.03% (C) and 17.08% (G). High AT bias was observed with AT content of 64.88% over GC content of 35.11% (Table 4.4.10). The nucleotide frequencies of 18S rRNA gene sequences are 21.45% (A), 29.46% (T/U), 20.46% (C) and 28.63% (G). The analysis involved 6 nucleotide sequences (Table 4.4.12). Nucleotides were evenly distributed and no AT bias was observed (AT content 50.91%; GC content 49.09%).

Table 4.4.9: Estimates of genetic divergence of the COI gene sequences of family Calopterygidae and out group

	Genus (COI gene sequence)	**1**	**2**	**3**	**4**	**5**
1	MW931875.1_Genus_*Neurobasis*_Kerala					
2	KF369332.1_Genus_*Caliphaea*_Vietna	0.251				
3	KF369379.1_Genus_*Echo*_Malaysia	0.212	0.219			
4	KF369432.1_Genus_*Matrona*_China	0.162	0.225	0.205		
5	KU510326.1_Genus_*Vestalis*_Kerala	0.228	0.217	0.217	0.210	
6	MW965658.1_Genus_*Hydrobasileus* Kerala	0.567	0.586	0.551	0.567	0.601

Table 4.4.10: Nucleotide base composition of COI gene sequence of family Calopterygidae and out group

Domain : Data COI																
	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
MW931875.1 Genus *Neurobasis* Kerala	31.0	18.2	31.2	19.6	22	17.1	29.4	31.6	42	26.2	14.4	17.1	29	11.2	49.7	10.2
KF369332.1 Genus *Caliphaea* Vietnam	29.2	22.1	28.3	20.3	20	19.3	28.9	31.6	43	25.7	14.4	17.1	25	21.4	41.7	12.3
KF369379.1 Genus *Echo* Malaysia	29.6	22.1	31.0	17.3	20	18.7	30.5	30.5	42	26.7	15.0	16.0	26	20.9	47.6	5.3
KF369432.1 Genus *Matrona* China	29.2	19.6	31.6	19.6	19	19.3	28.9	32.6	42	26.7	14.4	17.1	27	12.8	51.3	9.1
KU510326.1 Genus *Vestalis* Kerala	30.1	21.9	27.3	20.7	20	19.8	27.3	33.2	42	27.8	14.4	16.0	29	18.2	40.1	12.8
MW965658.1 Genus *Hydrobasileus*Kerala	8.0	4.3	82.7	5.0	4	4.3	81.8	9.6	9	6.4	80.7	4.3	11	2.1	85.6	1.1
Avg.	26.2	18.0	38.7	17.1	18	16.4	37.8	28.2	37	23.3	25.6	14.6	24	14.4	52.7	8.5

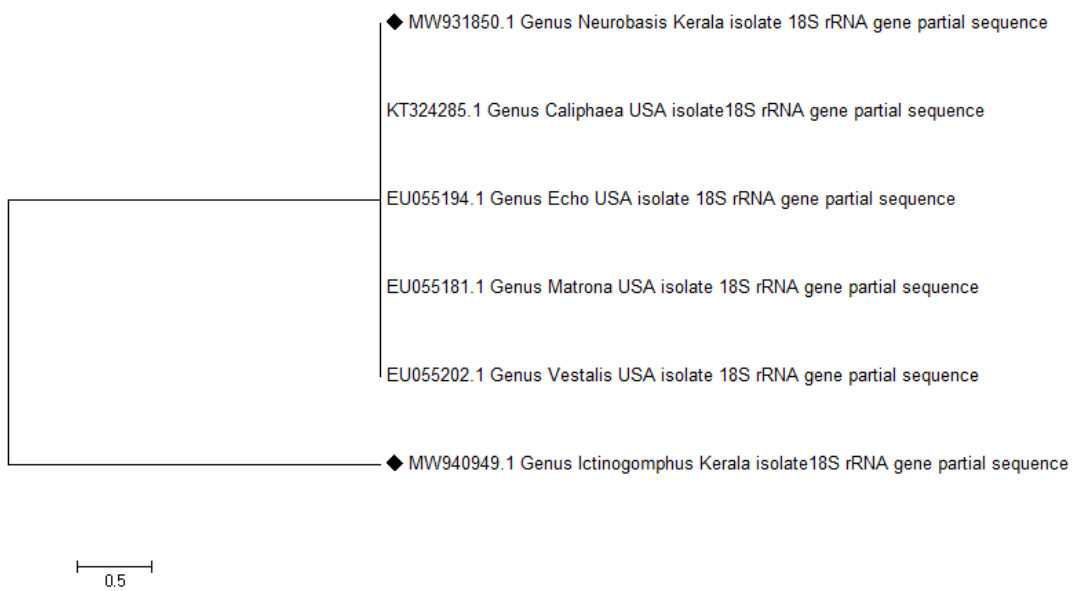

Figure 4.4.10: Inferred phylogenetic tree based on 18S rRNA gene sequences of family Calopterygidae, rooted by outgroup

Table 4.4.11: Estimates of genetic divergence of the 18S rRNA gene sequences of family Calopterygidae and out group

	Genus 18S	**1**	**2**	**3**	**4**	**5**
1	MW931850.1_Genus_*Neurobasis*_Kerala					
2	KT324285.1_Genus_*Caliphaea*_USA	0.000				
3	EU055194.1_Genus_*Echo*_USA	0.000	0.000			
4	EU055181.1_Genus_*Matrona*_USA	0.000	0.000	0.000		
5	EU055202.1_Genus_*Vestalis*_USA	0.000	0.000	0.000	0.000	
6	MW940949.1_Genus_*Ictinogomphus*_Kerala	0.025	0.025	0.025	0.025	0.025

Table 4.4.12: Nucleotide base composition of 18S rRNA gene sequence of family Calopterygidae and out group

Domain: Data 18S																
	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
MW931850.1 Genus *Neurobasis* Kerala	29.2	20.8	21.3	28.7	29	20.6	17.6	32.4	30	19.4	22.4	28.4	28	22.4	23.9	25.4
KT324285.1 Genus *Caliphaea* USA	29.2	20.8	21.3	28.7	29	20.6	17.6	32.4	30	19.4	22.4	28.4	28	22.4	23.9	25.4
EU055194.1 Genus *Echo* USA	29.2	20.8	21.3	28.7	29	20.6	17.6	32.4	30	19.4	22.4	28.4	28	22.4	23.9	25.4
EU055181.1 Genus *Matrona* USA	29.2	20.8	21.3	28.7	29	20.6	17.6	32.4	30	19.4	22.4	28.4	28	22.4	23.9	25.4
EU055202.1 Genus *Vestalis* USA	29.2	20.8	21.3	28.7	29	20.6	17.6	32.4	30	19.4	22.4	28.4	28	22.4	23.9	25.4
MW940949.1 Genus *Ictinogomphus* Kerala	30.7	18.8	22.3	28.2	32	16.2	19.1	32.4	31	17.9	23.9	26.9	28	22.4	23.9	25.4
Avg.	29.5	20.5	21.5	28.6	30	19.9	17.9	32.4	30	19.2	22.6	28.1	28	22.4	23.9	25.4

4) Resolution of phylogenetic relationships within Family Chlorocyphidae

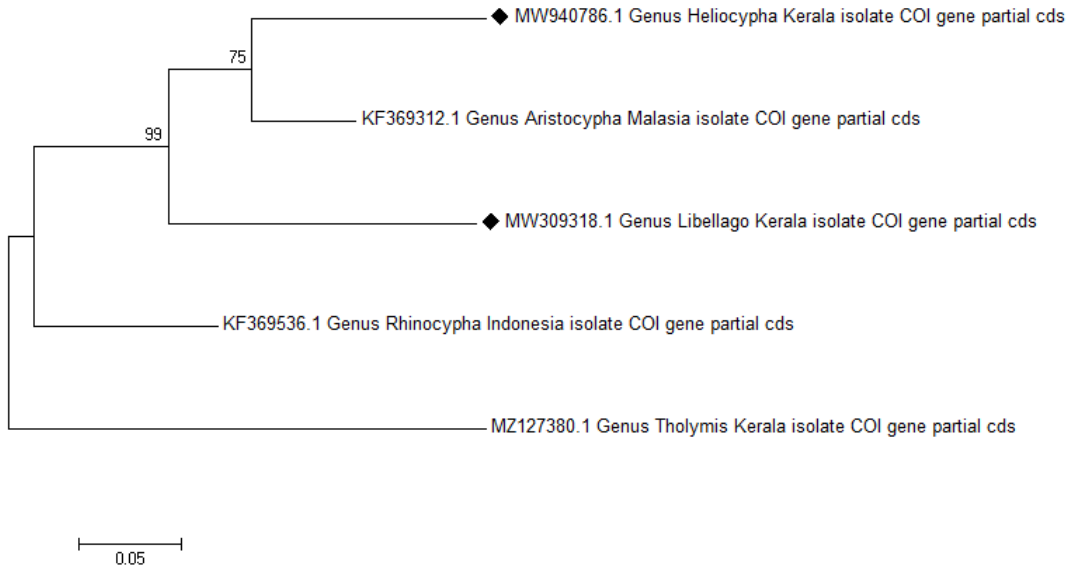

Figure 4.4.11: Inferred phylogenetic tree based on COI gene sequences of family Chlorocyphidae, rooted by outgroup

Table 4.4.13: Estimates of genetic divergence of the COI gene sequences of family Chlorocyphidae and out group

	Genus COI sequence	**1**	**2**	**3**	**4**
1	MW940786.1_Genus_*Heliocypha*_Kerala				
2	MW309318.1_Genus_*Libellago*_Kerala	0.174			
3	KF369312.1_Genus_*Aristocypha*_Malasia	0.125	0.149		
4	KF369536.1_Genus_*Rhinocypha*_Indonesia	0.176	0.190	0.161	
5	MZ127380.1_Genus_*Tholymis*_Kerala	0.217	0.222	0.205	0.188

Table 4.4.14: Nucleotide base composition of COI gene sequences of family Chlorocyphidae and out group

Domain: Data COI																
	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
MW940786.1 Genus *Heliocypha* Kerala	31.8	18.8	30.9	18.5	30	11.8	49.2	8.7	22	17.4	29.7	30.8	43	27.2	13.8	15.9
MW309318.1 Genus *Libellago* Kerala	32.8	19.5	29.7	17.9	34	13.3	45.1	7.2	21	17.9	30.3	30.8	43	27.2	13.8	15.9
KF369312.1 Genus *Aristocypha* Malasia	30.6	19.7	32.5	17.3	28	13.3	53.8	5.1	21	18.5	29.7	30.8	43	27.2	13.8	15.9
KF369536.1 Genus *Rhinocypha* Indonesia	31.1	20.0	30.4	18.5	29	15.9	47.2	7.7	21	17.4	30.3	31.3	43	26.7	13.8	16.4
MZ127380.1 Genus *Tholymis* Kerala	33.0	18.1	30.4	18.5	36	9.2	47.7	7.2	19	19.0	29.7	31.8	44	26.2	13.8	16.4
Avg.	31.9	19.2	30.8	18.1	31	12.7	48.6	7.2	21	18.1	29.9	31.1	43	26.9	13.8	16.1

Phylogenetic analysis and genetic divergence

Phylogenetic reconstruction of the family Chlorocyphidae based on partial COI and 18S rRNA gene sequence was carried out by using sequences of genus *Heliocypha* and *Libellago* and sequences of other genera retrieved from GenBank.

a) Based on partial COI gene sequence

Genus *Tholymis* was included as out group in the COI analysis (Figure 4.4.11). Genera *Heliocypha*, *Aristocypha* and *Libellago* were monophyletic with 99% bootstap support. *Heliocypha* and *Aristocypha* showed close similarity and formed sister clades. *Rhinocypha* was paraphyletic to the other genera.

Minimum value of genetic divergence was observed between *Heliocypha* and *Aristocypha*(12.5%). Divergence was maximum between *Rhinocypha* and *Heliocypha* (19%). The values are given in Table 4.4.13.

b) Based on partial 18S rRNA gene sequence

The tree was rooted by an outgroup sequence of genus *Orthetrum* . In the obtained tree all the 4 genera were grouped as sister clades. The relationship was not clearly discriminated (Figure 4.4.12).

The divergence values showed the close resemblance between *Libellago, Aristocypha* and *Rhinocypha* (0%) and a divergence of 1.4% from *Heliocypha* to other genera (Table 4.4.15).

Nucleotide composition

The nucleotide composition of five partial COI gene sequences are 30.80% (A), 31.86% (T/U), 19.21% (C) and 18.12% (G). High AT bias was observed with an AT content of 62.66% over the GC content of 37.33% (Table 4.4.14). The nucleotide frequencies of five 18S rRNA gene sequences are 25.99% (A), 27.89% (T/U), 17.01% (C) and 29.12% (G) with an AT content of 53.88% over GC content of 46.13% (Table 4.4.16).

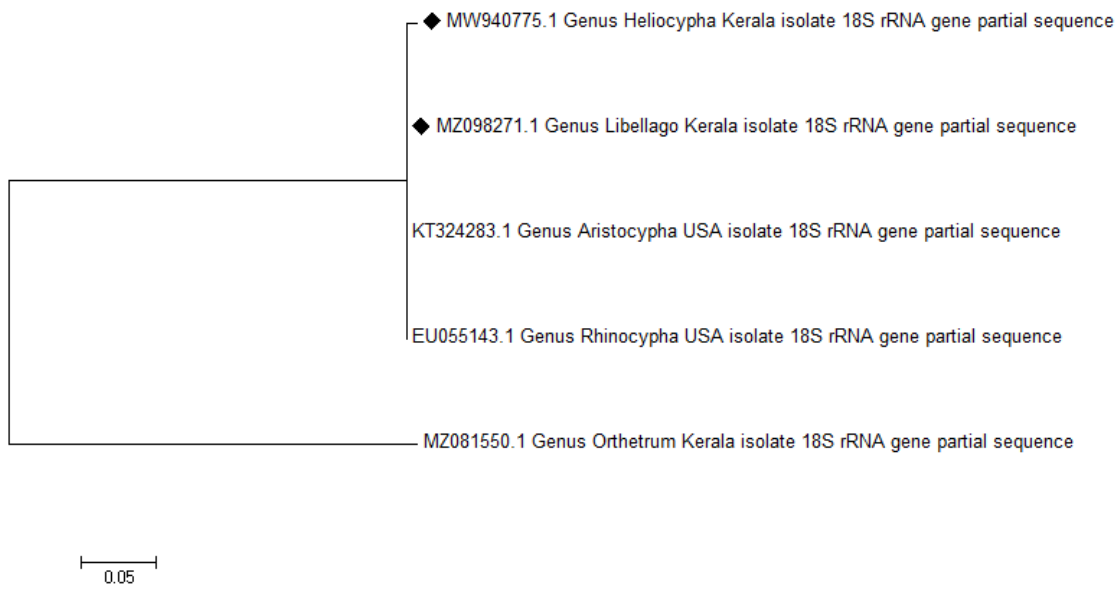

Figure 4.4.12: Inferred phylogenetic tree based on 18S rRNA gene sequences of family Chlorocyphidae, rooted by outgroup

Table 4.4.15: Estimates of genetic divergence of the18S rRNA gene sequences of family Chlorocyphidae and out group

	Genus 18S	**1**	**2**	**3**	**4**
1	MW940775.1_Genus_*Heliocypha*_Kerala				
2	MZ098271.1_Genus_*Libellago*_Kerala	0.014			
3	KT324283.1_Genus_*Aristocypha*_USA	0.014	0.000		
4	EU055143.1_Genus_*Rhinocypha*_USA	0.014	0.000	0.000	
5	MZ081550.1_Genus_*Orthetrum*_Kerala	0.020	0.007	0.007	0.007

Table 4.4.16: Nucleotide base composition of 18S rRNA gene sequences of family Chlorocyphidae and out group

Domain: Data 18S																
	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
MW940775.1 Genus *Heliocypha* Kerala	27.7	16.9	25.7	29.7	27	16.3	22.4	34.7	28	14.0	28.0	30.0	29	20.4	26.5	24.5
MZ098271.1 Genus *Libellago* Kerala	27.7	16.9	25.7	29.7	27	16.3	22.4	34.7	28	16.0	28.0	28.0	29	18.4	26.5	26.5
KT324283.1 Genus *Aristocypha* USA	27.7	16.9	25.7	29.7	27	16.3	22.4	34.7	28	16.0	28.0	28.0	29	18.4	26.5	26.5
EU055143.1 Genus *Rhinocypha* USA	27.7	16.9	25.7	29.7	27	16.3	22.4	34.7	28	16.0	28.0	28.0	29	18.4	26.5	26.5
MZ081550.1 Genus *Orthetrum* Kerala	28.4	16.9	26.4	28.4	29	16.3	22.4	32.7	28	16.0	28.0	28.0	29	18.4	28.6	24.5
Avg.	27.8	16.9	25.8	29.5	27	16.3	22.4	34.3	28	15.6	28.0	28.4	29	18.8	26.9	25.7

5) Resolution of phylogenetic relationships within Family Euphaeidae

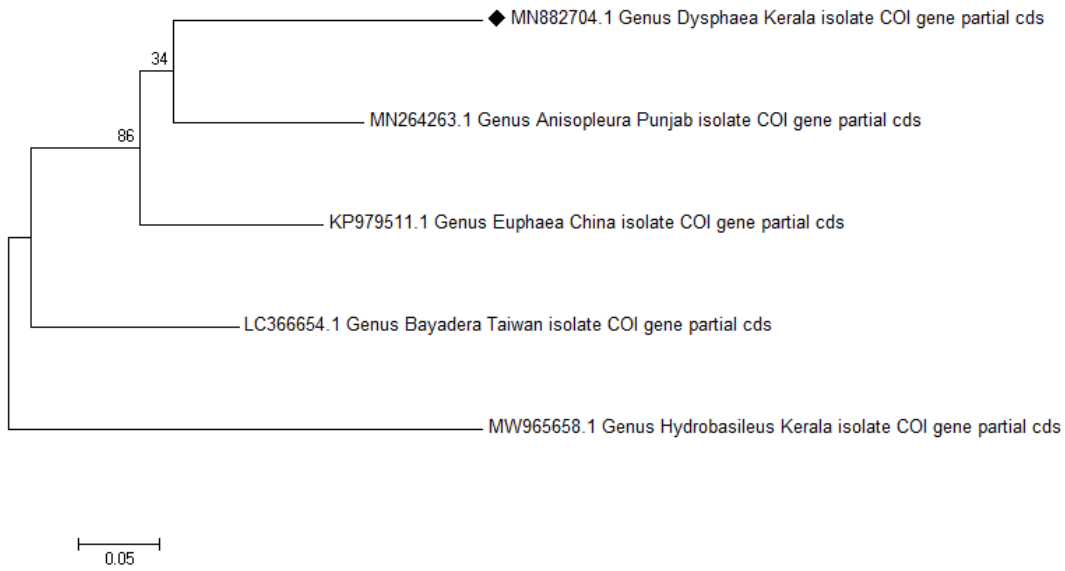

Figure 4.4.13: Inferred phylogenetic tree based on COI gene sequences of family Euphaeidae, rooted by outgroup

Phylogenetic analysis and genetic divergence

Phylogenetic analysis based on partial COI gene sequences of family Euphaeidae was done by using the sequences of genus *Dysphaea* and other genera downloaded from GenBank. Five sequences were involved in the sequence data.

a) Based on partial COI gene sequence

The dragonfly genus *Hydrobasileus* was used as out group in the analysis. The result indicated the monophyly of *Dysphaea, Anisopleura* and *Euphaea* with bootstrap support of 86%. Genus *Bayadera* was paraphyletic (Figure 4.4.13).

The calculated divergence values (Table 4.4.17) suggested that minimum divergence value was observed between *Euphaea* and *Anisopleura* (13.5%). Divergence value was maximum between *Dysphaea* and *Bayadera* (19.1%).

b) Based on partial 18S rRNA gene sequence

Sequence of dragonfly genus *Tetrathemis* was considered as out group in the 18S analysis. In the result all the four genera were grouped into a monophyletic clade (Figure 4.4.14).

There was no genetic divergence observed among *Bayadera, Euphaea* and *Dysphaea* in the 18S rRNA gene sequence. 0.5% divergence was found between *Anisopleura* and other genera (Table 4.4.19).

Nucleotide composition

The nucleotide composition of five COI nucleotide sequences are 32.81% (A), 31.35% (T/U), 20.38% (C) and 15.46% (G) with a high AT content (64.16%) over GC content (35.84%). The nucleotide frequencies of five 18S rRNA gene sequences are 36.50% (A), 23.17% (T/U), 13.99% (C) and 26.34% (G) . The observed AT content was 59.67% and GC content was 40.33%. The values are given in Tables 4.4.18 and 4.4.20.

Table 4.4.17 Estimates of genetic divergence of the COI gene sequences of family Euphaeidae and out group

	Genus COI	**1**	**2**	**3**	**4**
1	MN882704.1_Genus_*Dysphaea*_Kerala				
2	MN264263.1_Genus_*Anisopleura*_Punjab	0.149			
3	LC366654.1_Genus_*Bayadera*_Taiwan	0.191	0.158		
4	KP979511.1_Genus_*Euphaea*_China	0.154	0.135	0.154	
5	MW965658.1_Genus_*Hydrobasileus* Kerala	0.208	0.189	0.180	0.206

Table 4.4.18 Nucleotide base composition of COI gene sequence of family Euphaeidae and out group

Domain: Data COI																
	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
MN882704.1 Genus *Dysphaea* Kerala	29.1	22.0	33.1	15.8	24	14.9	56.0	5.0	21	20.6	30.5	27.7	42	30.5	12.8	14.9
MN264263.1 Genus *Anisopleura* Punjab	29.6	19.4	35.7	15.4	25	7.8	63.8	3.5	22	19.9	30.5	27.7	42	30.5	12.8	14.9
LC366654.1 Genus *Bayadera* Taiwan	31.9	21.0	32.2	14.9	31	12.1	53.9	2.8	23	20.6	29.8	27.0	42	30.5	12.8	14.9
KP979511.1 Genus *Euphaea* China	31.2	20.1	32.9	15.8	29	10.6	56.0	4.3	23	19.1	29.8	28.4	42	30.5	12.8	14.9
MW965658.1 Genus *Hydrobasileus* Kerala	35.0	19.4	30.3	15.4	38	7.1	51.8	2.8	24	19.1	27.0	29.8	43	31.9	12.1	13.5
Avg.	31.3	20.4	32.8	15.5	30	10.5	56.3	3.7	23	19.9	29.5	28.1	42	30.8	12.6	14.6

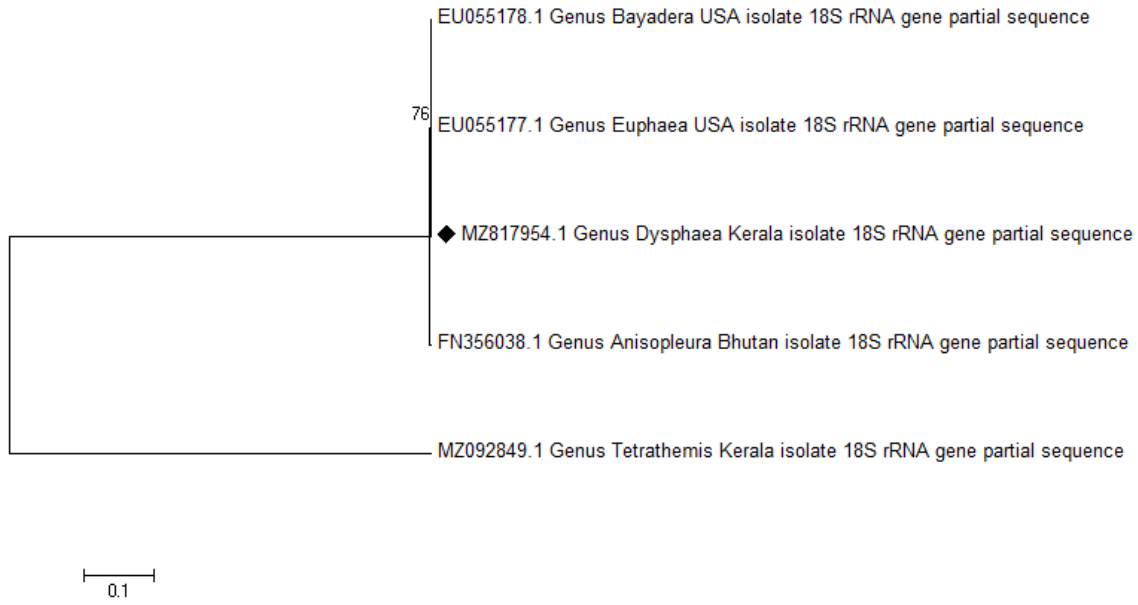

Figure 4.4.14: Inferred phylogenetic tree based on 18S rRNA gene sequences of family Euphaeidae, rooted by outgroup

Table 4.4.19 Estimates of genetic divergence of the 18S rRNA gene sequences of family Euphaeidae and out group

	Genus 18S	**1**	**2**	**3**	**4**
1	MZ817954.1_Genus_*Dysphaea*_Kerala				
2	FN356038.1_Genus_*Anisopleura*_Bhutan	0.005			
3	EU055178.1_Genus_*Bayadera*_USA	0.000	0.005		
4	EU055177.1_Genus_*Euphaea*_USA	0.000	0.005	0.000	
5	MZ092849.1_Genus_*Tetrathemis*_Kerala	0.568	0.568	0.568	0.568

Table 4.4.20: Nucleotide base composition of 18S rRNA gene sequence of family Euphaeidae and out group

Domain: Data 18S																
	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
MZ817954.1 Genus *Dysphaea* Kerala	27.3	16.4	25.1	31.1	25	18.0	27.9	29.5	28	19.7	19.7	32.8	30	11.5	27.9	31.1
FN356038.1 Genus *Anisopleura* Bhutan	27.9	15.8	25.1	31.1	25	18.0	27.9	29.5	28	19.7	19.7	32.8	31	9.8	27.9	31.1
EU055178.1 Genus *Bayadera* USA	27.3	16.4	25.1	31.1	25	18.0	27.9	29.5	28	19.7	19.7	32.8	30	11.5	27.9	31.1
EU055177.1 Genus *Euphaea* USA	27.3	16.4	25.1	31.1	25	18.0	27.9	29.5	28	19.7	19.7	32.8	30	11.5	27.9	31.1
MZ092849.1 Genus *Tetrathemis* Kerala	5.8	4.7	82.7	6.8	5	6.3	82.8	6.3	3	6.3	81.3	9.4	10	1.6	84.1	4.8
Avg.	23.0	13.9	37.1	26.1	20	15.6	39.3	24.7	23	16.9	32.5	27.9	26	9.1	39.4	25.7

6) Resolution of phylogenetic relationships within Family Platycnemididae

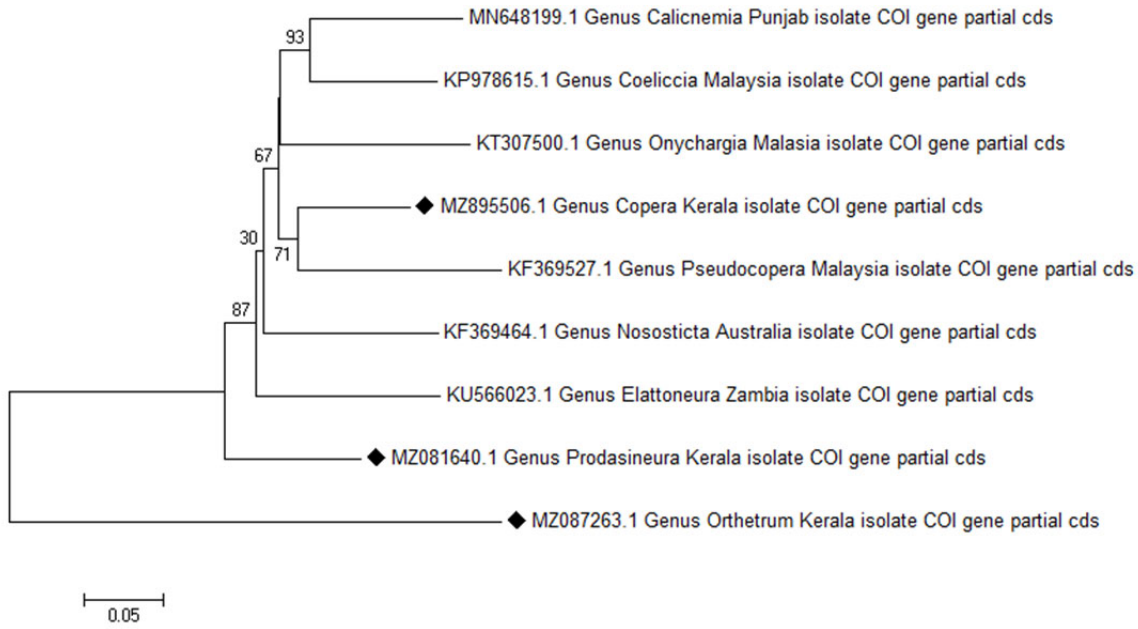

Figure 4.4.15: Inferred phylogenetic tree based on COI gene sequences of family Platycnemididae, rooted by outgroup

Phylogenetic analysis and genetic divergence

a) Based on partial COI gene sequence

Phylogeny of the family Platycnemididae based on partial COI gene sequence was resolved using sequences of genera *Copera* and *Prodasineura* and sequences of 6 genera retrieved from GenBank. Sequence of the dragonfly genus *Orthetrum* was included as out group. A total of 9 sequences were included in the analysis (Figure 4.4.15).

All the Platycnemidid genera except *Prodasineura* were grouped into a monophyletic clade well supported by a bootstrap value of 87%. *Prodasineura* was paraphyletic. *Calicnemia*+*Coeliccia* and *Copera* + *Pseudocopera* clustered to form sister clades.

The divergence values ranged from 14.3% to 20.2%. The minimum divergence was between *Calicnemia* and *Coeliccia* and the maximum value was possessed between *Pseudocopera* and *Elattoneura* (Table 4.4.21).

b) Based on partial 18S rRNA gene sequence

Phylogenetic analysis of the family Platycnemididae based on partial 18S rRNA gene sequence was carried out with 8 sequences including the sequences of *Copera* and *Prodasineura* and sequences of 5 genera retrieved from GenBank. The sequence of the dragonfly genus *Palpopleura* was included as outgroup (Figure 4.4.16).

The phylogenetic tree showed that all the genera of family Platycnemididae involved in the current analysis were monophyletic to each other except the genus *Prodasineura*. But the relationship within the monophyletic clade was not clearly discriminated by this analysis and showed less sequence diversion.

The genetic divergence was zero among the members of monophyletic clade. *Prodasineura* showed 1.5% divergence from the other genera (Table 4.4.23).

Nucleotide composition

The nucleotide composition of nine COI nucleotide sequences were 32.57% (A), 31.53% (T/U), 18.00% (C) and 17.91% (G). High AT bias was observed (AT content 64.1%; GC content 35.91%). The nucleotide frequencies of eight 18S rRNA gene sequences were 30.11% (A), 25.00% (T/U), 15.51% (C) and 29.38 % (G) (AT content= 55.11%; GC content=44.89%). The values are given in Tables 4.4.22 and 4.4.24 respectively.

Table 4.4.21: Estimates of genetic divergence of the COI gene sequences of family Platycnemididae and out group

	Genus COI	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1.	MZ895506.1_Genus_*Copera*_Kerala								
2.	MZ081640.1_Genus_*Prodasineura*_Kerala	0.169							
3.	MN648199.1_Genus_*Calicnemia*_Punjab	0.149	0.173						
4.	KP978615.1_Genus_*Coeliccia*_Malaysia	0.146	0.181	0.143					
5.	KF369527.1_Genus_*Pseudocopera*_Malaysia	0.157	0.186	0.202	0.185				
6.	KU566023.1_Genus_*Elattoneura*_Zambia	0.172	0.175	0.180	0.180	0.201			
7.	KF369464.1_Genus_*Nososticta*_Australia	0.156	0.167	0.189	0.177	0.183	0.170		
8.	KT307500.1_Genus_*Onychargia*_Malasia	0.162	0.177	0.178	0.172	0.185	0.186	0.178	
9.	MZ087263.1_Genus_*Orthetrum*_Kerala	0.318	0.311	0.331	0.313	0.345	0.326	0.332	0.343

Table 4.4.22: Nucleotide base composition of COI gene sequences of family Platycnemididae and out group

Domain: Data COI																
	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
MZ895506.1 Genus *Copera* Kerala	35.0	15.6	31.6	17.8	37	6.8	53.1	3.4	26	13.0	28.8	32.2	42	26.9	13.0	17.8
MZ081640.1 Genus *Prodasineura* Kerala	31.5	19.7	30.0	18.8	33	13.5	48.3	5.3	19	19.2	28.4	33.2	42	26.4	13.5	17.8
MN648199.1 Genus *Calicnemia* Punjab	32.7	17.7	31.1	18.5	35	9.2	50.2	5.3	21	16.8	29.8	32.7	42	26.9	13.5	17.3
KP978615.1 Genus *Coeliccia* Malaysia	34.7	16.2	31.9	17.2	38	7.7	52.7	1.9	24	13.9	29.8	32.2	42	26.9	13.5	17.3
KF369527.1 Genus *Pseudocopera* Malaysia	32.3	19.3	28.9	19.6	33	12.6	46.4	7.7	21	17.8	27.4	33.7	42	27.4	13.0	17.3
KU566023.1 Genus *Elattoneura* Zambia	31.3	19.9	29.5	19.3	30	15.9	47.3	6.8	22	16.8	27.9	33.7	42	26.9	13.5	17.3
KF369464.1 Genus *Nososticta* Australia	31.6	19.3	29.2	19.9	31	13.5	46.4	8.7	21	17.3	27.9	33.7	42	26.9	13.5	17.3
KT307500.1 Genus *Onychargia* Malasia	30.2	21.3	29.7	18.8	27	19.8	47.8	5.3	21	17.3	27.9	33.7	42	26.9	13.5	17.3
MZ087263.1 Genus *Orthetrum* Kerala	24.3	12.3	51.3	12.0	27	3.5	65.7	3.9	17	13.5	47.0	22.6	29	20.0	41.3	9.6
Avg.	31.4	17.9	32.8	17.9	32	11.3	51.1	5.4	21	16.2	30.7	31.8	41	26.1	16.7	16.5

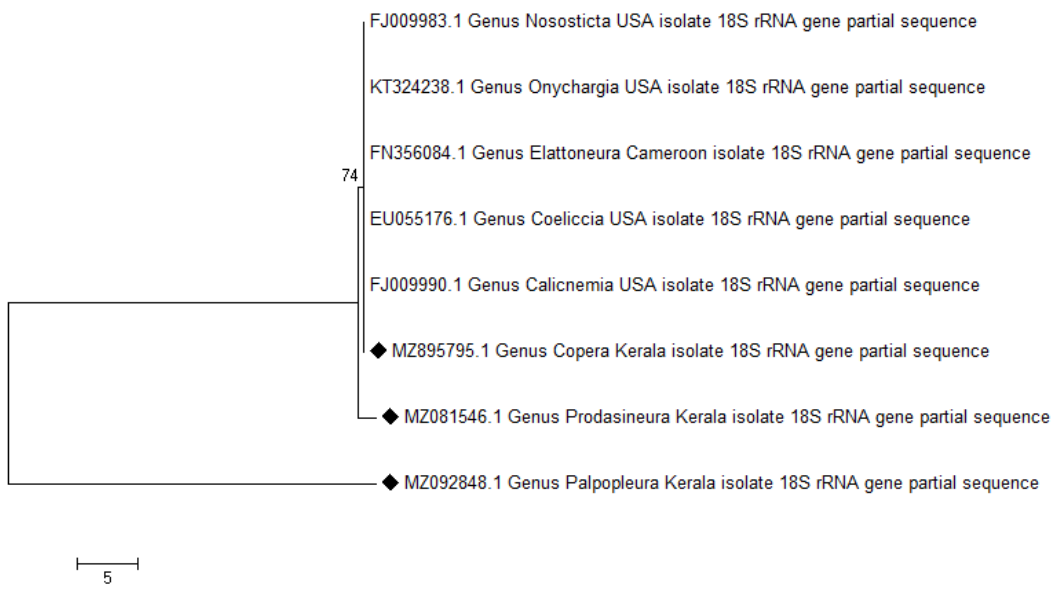

Figure 4.4.16: Inferred phylogenetic tree based on 18S rRNA gene sequences of family Platycnemididae, rooted by outgroup.

Table 4.4.23: Estimates of genetic divergence of the 18S rRNA gene sequences of family Platycnemididae and out group

Genus 18S	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1. MZ895795.1_Genus_*Copera*_Kerala							
2. MZ081546.1_Genus_*Prodasineura*_Kerala	0.015						
3. FJ009990.1_Genus_*Calicnemia*_USA	0.000	0.015					
4. EU055176.1_Genus_*Coeliccia*_USA	0.000	0.015	0.000				
5. FN356084.1_Genus_*Elattoneura*_Cameroon	0.000	0.015	0.000	0.000			
6. FJ009983.1_Genus_*Nososticta*_USA	0.000	0.015	0.000	0.000	0.000		
7. KT324238.1_Genus_*Onychargia*_USA	0.000	0.015	0.000	0.000	0.000	0.000	
8. MZ092848.1_Genus_*Palpopleura*_Kerala	0.431	0.438	0.431	0.431	0.431	0.431	0.431

Table 4.4.24: Nucleotide base composition of 18S rRNA gene sequence of family Platycnemididae and out group

Domain: Data 18S																
	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
MZ895795.1 Genus *Copera* Kerala	27.5	16.7	24.6	31.2	26	17.4	28.3	28.3	30	17.4	19.6	32.6	26	15.2	26.1	32.6
MZ081546.1 Genus *Prodasineura* Kerala	27.5	16.7	23.9	31.9	28	14.9	27.7	29.8	30	19.6	17.4	32.6	24	15.6	26.7	33.3
FJ009990.1 Genus *Calicnemia* USA	27.5	16.7	24.6	31.2	26	17.4	28.3	28.3	30	17.4	19.6	32.6	26	15.2	26.1	32.6
EU055176.1 Genus *Coeliccia* USA	27.5	16.7	24.6	31.2	26	17.4	28.3	28.3	30	17.4	19.6	32.6	26	15.2	26.1	32.6
FN356084.1 Genus *Elattoneura* Cameroon	27.5	16.7	24.6	31.2	26	17.4	28.3	28.3	30	17.4	19.6	32.6	26	15.2	26.1	32.6
FJ009983.1 Genus *Nososticta* USA	27.5	16.7	24.6	31.2	26	17.4	28.3	28.3	30	17.4	19.6	32.6	26	15.2	26.1	32.6
KT324238.1 Genus *Onychargia* USA	27.5	16.7	24.6	31.2	26	17.4	28.3	28.3	30	17.4	19.6	32.6	26	15.2	26.1	32.6
MZ092848.1 Genus *Palpopleura* Kerala	10.4	7.6	66.7	15.3	10	6.3	70.8	12.5	13	8.3	64.6	14.6	8	8.3	64.6	18.8
Avg.	25.3	15.5	30.0	29.2	24	15.6	33.7	26.4	28	16.5	25.1	30.3	24	14.4	31.2	30.9

7) Resolution of phylogenetic relationships within Family Coenagrionidae

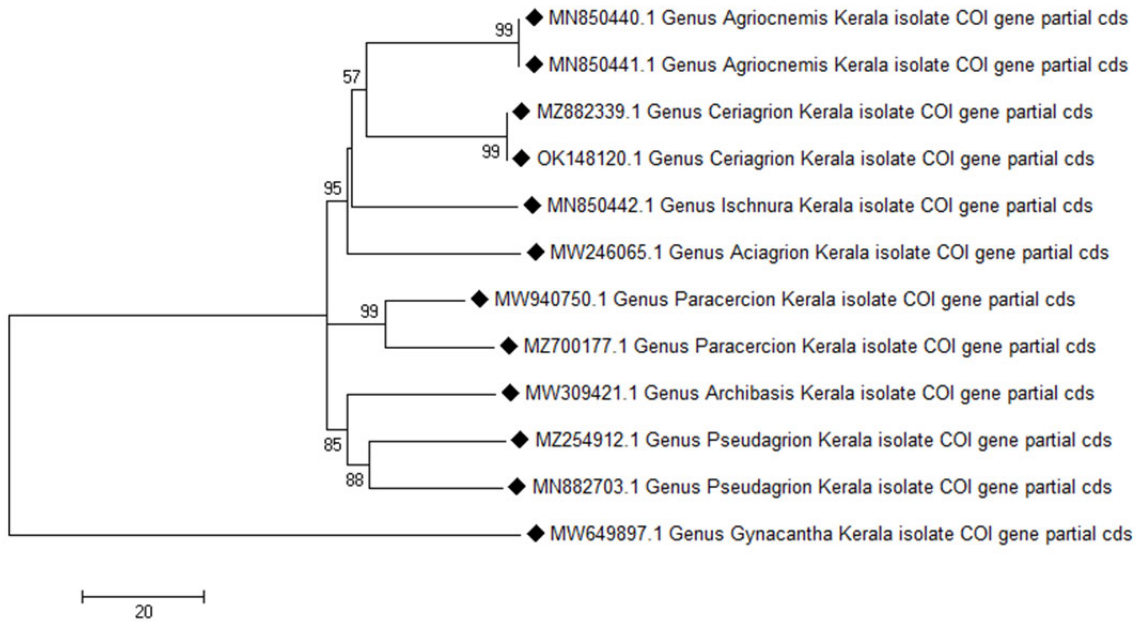

Figure 4.4.17: Inferred phylogenetic tree based on COI gene sequences of family Coenagrionidae, rooted by outgroup

Phylogenetic analysis and genetic divergence

a) Based on partial COI gene sequence

Phylogeny of the genera of family Coenagrionidae was resolved using the twelve partial COI gene sequences of the current study. Sequence of genus *Gynacantha* was considered as out group (Figure 4.4.17).

All branches of the tree were well supported with boot strap values ranging from 85% to 99% except one node with value 57%. The tree was branched into three monophyletic clades. The first clade was formed by the monophyly of *Agriocnemis, Ceriagrion, Ischnura* and *Aciagrion*. The second clade was formed by *Paracercion* and the third clade was formed by the grouping of *Archibasis* and *Pseudagrion. Agriocnemis* and *Ceriagrion* formed sister clades and *Ischnura* and *Aciagrion* were polyphyletic to the former genera.

The divergence values were ranged from 9.7% to 23.1%. The maximum divergence was observed between *Pseudagrion* and *Agriocnemis* (Table 4.4.25).

b) Based on partial 18S rRNA gene sequence

Phylogeny of the Coenagrionid genera based on 18S rRNA partial gene sequence was resolved using sequences of the seven genera and one out group sequenced during the current work. The dragonfly genus *Tramea* was included as out group (Figure 4.4.18).

The result suggested that Coenagrionid genera were grouped into two main clades. One clade was formed by *Archibasis* and *Pseudagrion* genera and the other clade was formed by the clustering of remaining genera. *Paracercion* + *Agriocnemis* and *Aciagrion*+ *Ischnura* formed separate sister clades. *Ceriagrion* was paraphyletic.

The divergence values were ranged from 0.7% to 2.9% (Table 4.4.27).

Nucleotide composition

The nucleotide composition of 12 COI partial gene sequences were 31.07% (A), 33.15% (T/U), 17.73% (C) and 18.05% (G). High AT bias was observed with an AT content of 64.22% over the GC content of 35.78% (Table 4.4.26). The nucleotide frequencies of twelve 18S rRNA partial gene sequences were 27.70% (A), 27.57% (T/U), 24.94% (C) and 19.79% (G). The AT content was slightly higher (55.27%) than the GC content (44.73%) as shown in Table 4.4.28.

Table 4.4.25: Estimates of genetic divergence of the COI gene sequences of family Coenagrionidae and out group

	Genus COI	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1.	MW246065.1_Genus_*Aciagrion*_Kerala											
2.	MW309421.1_Genus_*Archibasis*_Kerala	0.197										
3.	MN850440.1_Genus_*Agriocnemis*_Kerala	0.172	0.184									
4.	MN850441.1_Genus_*Agriocnemis*_Kerala	0.188	0.203	0.200								
5.	MZ882339.1_Genus_*Ceriagrion*_Kerala	0.172	0.181	0.150	0.209							
6.	MN850442.1_Genus_*Ischnura*_Kerala	0.184	0.178	0.178	0.163	0.156						
7.	MW940750.1_Genus_*Paracercion*_Kerala	0.169	0.147	0.150	0.213	0.166	0.181					
8.	MZ254912.1_Genus_*Pseudagrion*_Kerala	0.197	0.156	0.194	0.231	0.175	0.191	0.175				
9.	MN882703.1_Genus_*Pseudagrion*_Kerala	0.178	0.156	0.209	0.203	0.175	0.184	0.169	0.138			
10.	MZ700177.1_Genus_*Paracercion*_Kerala	0.188	0.163	0.184	0.200	0.194	0.166	0.097	0.188	0.169		
11.	OK148120.1_Genus_*Ceriagrion*_Kerala	0.181	0.188	0.150	0.206	0.109	0.169	0.172	0.188	0.163	0.172	
12.	MW649897.1_Genus_*Gynacantha*_Keral	0.197	0.203	0.200	0.225	0.181	0.181	0.175	0.200	0.200	0.188	0.172

Table 4.4.26: Nucleotide base composition of the COI gene sequence of family Coenagrionidae and out group

Domain: Data COI																
	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
MW246065.1 Genus *Aciagrion* Kerala	35.3	16.3	30.9	17.5	38	7.5	47.7	6.5	26	10.3	31.8	31.8	42	31.1	13.2	14.2
MW309421.1 Genus *Archibasis* Kerala	31.3	19.7	30.3	18.8	31	14.0	45.8	9.3	22	14.0	31.8	31.8	41	31.1	13.2	15.1
MN850440.1 Genus *Agriocnemis* Kerala	35.0	15.0	31.9	18.1	38	3.7	52.3	5.6	25	12.1	29.9	32.7	42	29.2	13.2	16.0
MN850441.1 Genus *Agriocnemis* Kerala	31.6	20.6	28.8	19.1	31	16.8	41.1	11.2	23	13.1	31.8	31.8	41	32.1	13.2	14.2
MZ882339.1 Genus *Ceriagrion* Kerala	35.0	16.3	30.6	18.1	36	7.5	50.5	5.6	27	11.2	28.0	33.6	42	30.2	13.2	15.1
MN850442.1 Genus *Ischnura* Kerala	35.9	17.8	29.4	16.9	41	10.3	44.9	3.7	25	12.1	29.9	32.7	42	31.1	13.2	14.2
MW940750.1 Genus *Paracercion* Kerala	32.8	17.8	32.2	17.2	37	5.6	53.3	3.7	21	16.8	29.9	32.7	41	31.1	13.2	15.1
MZ254912.1 Genus *Pseudagrion* Kerala	31.3	17.2	32.2	19.4	30	10.3	53.3	6.5	23	12.1	29.9	34.6	41	29.2	13.2	17.0
MN882703.1 Genus *Pseudagrion* Kerala	29.1	19.7	31.9	19.4	26	12.1	51.4	10.3	21	15.9	30.8	32.7	41	31.1	13.2	15.1
MZ700177.1 Genus *Paracercion* Kerala	31.3	19.1	32.5	17.2	31	11.2	54.2	3.7	22	15.0	29.9	32.7	41	31.1	13.2	15.1
OK148120.1 Genus *Ceriagrion* Kerala	34.4	15.9	32.5	17.2	36	5.6	56.1	2.8	26	12.1	28.0	33.6	42	30.2	13.2	15.1
MW649897.1 Genus *Gynacantha* Kerala	35.0	17.5	29.7	17.8	42	6.5	45.8	5.6	22	14.0	29.9	33.6	41	32.1	13.2	14.2
Avg.	33.2	17.7	31.1	18.0	35	9.3	49.7	6.2	24	13.2	30.1	32.9	41	30.8	13.2	15.0

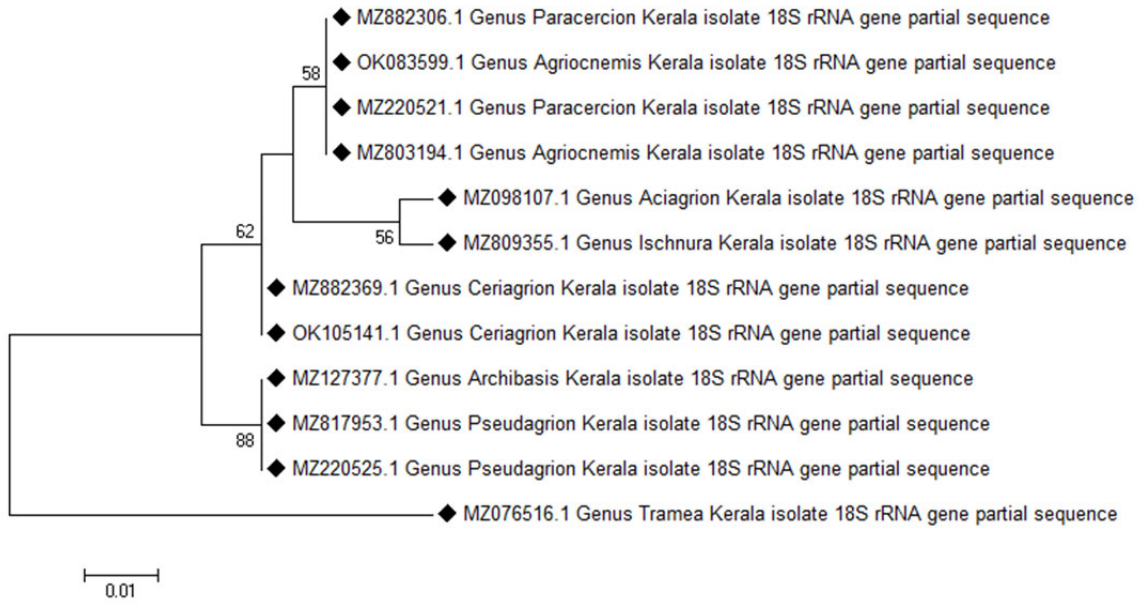

Figure 4.4.18: Inferred phylogenetic tree based on 18S rRNA gene sequences of family Coenagrionidae, rooted by outgroup.

Table 4.4.27: Estimates of genetic divergence of the 18S rRNA gene sequences of family Coenagrionidae and out group

	Genus 18S	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1.	MZ098107.1_Genus_*Aciagrion*_Kerala											
2.	MZ127377.1_Genus_*Archibasis*_Kerala	0.029										
3.	MZ803194.1_Genus_*Agriocnemis*_Kerala	0.015	0.022									
4.	MZ882369.1_Genus_*Ceriagrion*_Kerala	0.015	0.015	0.007								
5.	MZ809355.1_Genus_*Ischnura*_Kerala	0.007	0.029	0.015	0.015							
6.	MZ220521.1_Genus_*Paracercion*_Kerala	0.015	0.022	0.000	0.007	0.015						
7.	MZ882306.1_Genus_*Paracercion*_Kerala	0.015	0.022	0.000	0.007	0.015	0.000					
8.	MZ817953.1_Genus_*Pseudagrion*_Kerala	0.029	0.000	0.022	0.015	0.029	0.022	0.022				
9.	MZ220525.1_Genus_*Pseudagrion*_Kerala	0.029	0.000	0.022	0.015	0.029	0.022	0.022	0.000			
10.	OK105141.1_Genus_*Ceriagrion*_Kerala	0.015	0.015	0.007	0.000	0.015	0.007	0.007	0.015	0.015		
11.	OK083599.1_Genus_*Agriocnemis*_Kerala	0.015	0.022	0.000	0.007	0.015	0.000	0.000	0.022	0.022	0.007	
12.	MZ076516.1_Genus_*Tramea*_Kerala	0.074	0.066	0.066	0.066	0.066	0.066	0.066	0.066	0.066	0.066	0.066

Table 4.4.28: Nucleotide base composition of 18S rRNA gene sequence of family Coenagrionidae and out group

Domain: Data 18S																
	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
MZ098107.1 Genus *Aciagrion* Kerala	27.5	24.6	26.8	21.0	15	30.4	30.4	23.9	41	26.1	15.2	17.4	26	17.4	34.8	21.7
MZ127377.1 Genus *Archibasis* Kerala	29.0	23.9	28.3	18.8	17	28.3	32.6	21.7	43	26.1	15.2	15.2	26	17.4	37.0	19.6
MZ803194.1 Genus *Agriocnemis* Kerala	27.5	25.4	27.5	19.6	15	30.4	30.4	23.9	41	28.3	15.2	15.2	26	17.4	37.0	19.6
MZ882369.1 Genus *Ceriagrion* Kerala	28.3	24.6	27.5	19.6	15	30.4	30.4	23.9	43	26.1	15.2	15.2	26	17.4	37.0	19.6
MZ809355.1 Genus *Ischnura* Kerala	27.5	24.6	27.5	20.3	15	30.4	30.4	23.9	41	26.1	17.4	15.2	26	17.4	34.8	21.7
MZ220521.1 Genus *Paracercion* Kerala	27.5	25.4	27.5	19.6	15	30.4	30.4	23.9	41	28.3	15.2	15.2	26	17.4	37.0	19.6
MZ882306.1 Genus *Paracercion* Kerala	27.5	25.4	27.5	19.6	15	30.4	30.4	23.9	41	28.3	15.2	15.2	26	17.4	37.0	19.6
MZ817953.1 Genus *Pseudagrion* Kerala	29.0	23.9	28.3	18.8	17	28.3	32.6	21.7	43	26.1	15.2	15.2	26	17.4	37.0	19.6
MZ220525.1 Genus *Pseudagrion* Kerala	29.0	23.9	28.3	18.8	17	28.3	32.6	21.7	43	26.1	15.2	15.2	26	17.4	37.0	19.6
OK105141.1 Genus *Ceriagrion* Kerala	28.3	24.6	27.5	19.6	15	30.4	30.4	23.9	43	26.1	15.2	15.2	26	17.4	37.0	19.6
OK083599.1 Genus *Agriocnemis* Kerala	27.5	25.4	27.5	19.6	15	30.4	30.4	23.9	41	28.3	15.2	15.2	26	17.4	37.0	19.6
MZ076516.1 Genus *Tramea* Kerala	25.5	23.4	32.1	19.0	15	28.3	32.6	23.9	41	23.9	23.9	10.9	20	17.8	40.0	22.2
Avg.	27.9	24.6	28.0	19.5	16	29.7	31.2	23.4	42	26.6	16.1	15.0	26	17.4	36.8	20.1

8) Resolution of phylogenetic relationships within family Aeshnidae

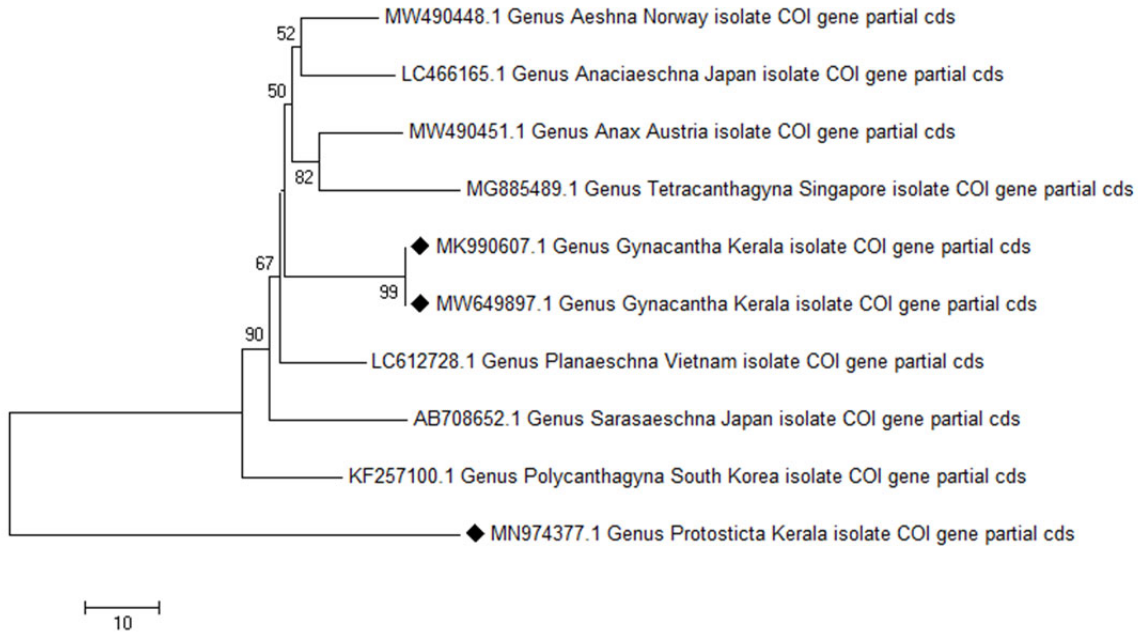

Figure 4.4.19: Inferred phylogenetic tree based on COI gene sequences of family Aeshnidae, rooted by outgroup.

Table 4.4.29: Estimates of genetic divergence of the COI gene sequences of family Aeshnidae and out group

Genus COI	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1. MK990607.1_Genus_*Gynacantha*_Kerala									
2. MW649897.1_Genus_*Gynacantha*_Kerala	0.090								
3. MW490448.1_Genus_*Aeshna*_Norway	0.126	0.126							
4. LC466165.1_Genus_*Anaciaeschna*_Japan	0.130	0.157	0.103						
5. MW490451.1_Genus_*Anax*_Austria	0.148	0.135	0.099	0.121					
6. LC612728.1_Genus_*Planaeschna*_Vietnam	0.126	0.112	0.108	0.126	0.126				
7. KF257100.1_Genus_*Polycanthagyna*_South_Korea	0.148	0.143	0.143	0.152	0.179	0.121			
8. AB708652.1_Genus_*Sarasaeschna*_Japan	0.161	0.161	0.148	0.157	0.157	0.148	0.170		
9. MG885489.1_Genus_*Tetracanthagyna*_Singapore	0.184	0.161	0.157	0.184	0.135	0.157	0.184	0.197	
10. MN974377.1_Genus_*Protosticta*_Kerala	0.520	0.502	0.516	0.498	0.502	0.489	0.471	0.498	0.511

Table 4.4.30: Nucleotide base composition of COI gene sequences of family Aeshnidae and out group

Domain: Data COI																
	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
MK990607.1 Genus *Gynacantha* Kerala	38.2	18.7	26.7	16.4	45	8.0	41.3	5.3	24	14.7	26.7	34.7	45	33.3	12.0	9.3
MW649897.1 Genus *Gynacantha* Kerala	38.2	18.7	26.7	16.4	45	8.0	41.3	5.3	24	14.7	26.7	34.7	45	33.3	12.0	9.3
MW490448.1 Genus *Aeshna* Norway	36.9	17.8	28.0	17.3	43	5.3	44.0	8.0	23	14.7	28.0	34.7	45	33.3	12.0	9.3
LC466165.1 Genus *Anaciaeschna* Japan	38.7	18.2	28.4	14.7	48	6.7	44.0	1.3	24	13.3	29.3	33.3	44	34.7	12.0	9.3
MW490451.1 Genus *Anax* Austria	38.2	17.8	27.6	16.4	49	2.7	42.7	5.3	20	17.3	28.0	34.7	45	33.3	12.0	9.3
LC612728.1 Genus *Planaeschna* Vietnam	35.6	17.3	31.6	15.6	37	5.3	54.7	2.7	24	13.3	28.0	34.7	45	33.3	12.0	9.3
KF257100.1 Genus *Polycanthagyna* South Korea	35.6	17.3	31.6	15.6	37	5.3	54.7	2.7	24	13.3	28.0	34.7	45	33.3	12.0	9.3
AB708652.1 Genus *Sarasaeschna* Japan	33.3	21.3	29.8	15.6	35	13.3	50.7	1.3	20	17.3	26.7	36.0	45	33.3	12.0	9.3
MG885489.1 Genus *Tetracanthagyna* Singapore	36.4	17.8	30.2	15.6	40	6.7	50.7	2.7	24	13.3	28.0	34.7	45	33.3	12.0	9.3
MN974377.1 Genus *Protosticta* Kerala	12.6	7.2	71.3	9.0	13	2.7	80.0	4.0	8	6.8	68.9	16.2	16	12.2	64.9	6.8
Avg.	34.4	17.2	33.1	15.3	39	6.4	50.4	3.9	21	13.9	31.8	32.8	42	31.4	17.2	9.1

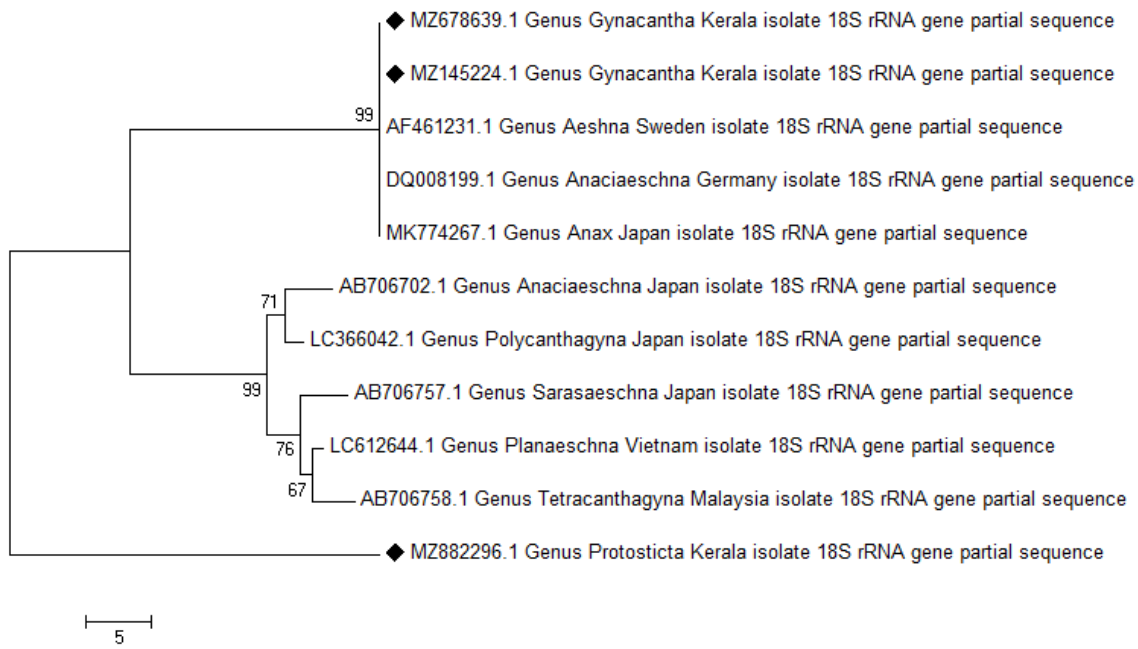

Figure 4.4.20: Inferred phylogenetic tree based on 18S rRNA gene sequences of family Aeshnidae, rooted by outgroup.

Phylogenetic analysis and genetic divergence

a) Based on partial COI gene sequence

Phylogenetic reconstruction of the genera of family Aeshnidae based on COI gene sequence was done by using the gene sequences of genus *Gynacantha* and sequences of other genera downloaded from GenBank. Sequence of the damselfly genus *Protosticta* was included as out group. Phylogeny of 10 genera were analysed (Figure 4.4.19).

The result showed that *Aeshna, Anaciaeshna, Anax* and *Tetracanthagyna* were monophyletic and remaining genera including *Gynacantha* were polyphyletic. *Polycanthagyna* was diverged from the common ancestor at an earlier time period.

The genetic divergence values ranged from 9.9% to 19.7%. Minimum divergence was observed between *Anax* and *Aeshna* and maximum value was found between *Tetracanthagyna* and *Sarasaeschna* (Table 4.4.29).

b) Based on partial 18S rRNA gene sequence

Phylogenetic relationship among the genera of family Aeshnidae based on 18S rRNA gene sequence was resolved by the maximum likelihood method and the best fit model. Sequence of the genus *Gynacantha* was used along with the sequences of eight genera retrieved from GenBank and sequence of the damselfly genus *Protosticta* was included as out group (Figure 4.4.20).

The obtained tree showed, the genera *Aeshna, Anaciaeshna, Anax* and *Gynacantha* were clustered into a monophyletic clade, all the five were in sister clade relationship with 99% boot strap support. The remaining genera were grouped into another monophyletic clade.

The genetic divergence values were ranged from 0% to 60.7% (Table 4.4.31).

Nucleotide composition

The nucleotide frequencies of ten COI partial gene sequences were 33.41% (A), 34.26% (T/U), 16.95% (C) and 15.38% (G) with a high AT bias (AT content 67.67%; GC content 32.33%). The nucleotide composition of 11 partial gene sequences of 18S rRNA were 19.23% (A), 22.21% (T/U), 25.78% (C) and 32.79% (G). AT content was lower (41.44%) than GC content (58.57%). The values are given in Tables 4.4.30 and 4.4.32 respectively.

Table 4.4.31: Estimates of genetic divergence of the 18S rRNA gene sequences of family Aeshnidae and out group

Genus 18S	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1. MZ678639.1_Genus_*Gynacantha*_Kerala										
2. MZ145224.1_Genus_*Gynacantha*_Kerala	0.000									
3. AF461231.1_Genus_*Aeshna*_Sweden	0.000	0.000								
4. DQ008199.1_Genus_*Anaciaeschna*_Germany_	0.000	0.000	0.000							
5. AB706702.1_Genus_*Anaciaeschna*_Japan	0.574	0.574	0.574	0.574						
6. LC612644.1_Genus_*Planaeschna*_Vietnam	0.541	0.541	0.541	0.541	0.131					
7. MK774267.1_Genus_*Anax*_Japan	0.000	0.000	0.000	0.000	0.574	0.541				
8. LC366042.1_Genus_*Polycanthagyna*_Japan	0.508	0.508	0.508	0.508	0.082	0.131	0.508			
9. AB706757.1_Genus_*Sarasaeschna*_Japan	0.574	0.574	0.574	0.574	0.180	0.098	0.574	0.180		
10. AB706758.1_Genus_*Tetracanthagyna*_Malaysia	0.607	0.607	0.607	0.607	0.148	0.066	0.607	0.164	0.115	
11. MZ882296.1_Genus_*Protosticta*_Kerala	0.918	0.918	0.918	0.918	0.852	0.885	0.918	0.852	0.869	0.836

Table 4.4.32: Nucleotide base composition of 18S rRNA gene sequence of family Aeshnidae and out group

Domain: Data 18 S																
	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
MZ678639.1 Genus *Gynacantha* Kerala	29.0	25.8	8.1	37.1	38	28.6	4.8	28.6	33	23.8	14.3	28.6	15	25.0	5.0	55.0
MZ145224.1 Genus *Gynacantha* Kerala	29.0	25.8	8.1	37.1	38	28.6	4.8	28.6	33	23.8	14.3	28.6	15	25.0	5.0	55.0
AF461231.1 Genus *Aeshna* Sweden	29.0	25.8	8.1	37.1	38	28.6	4.8	28.6	33	23.8	14.3	28.6	15	25.0	5.0	55.0
DQ008199.1 Genus *Anaciaeschna* Germany	29.0	25.8	8.1	37.1	38	28.6	4.8	28.6	33	23.8	14.3	28.6	15	25.0	5.0	55.0
AB706702.1 Genus *Anaciaeschna* Japan	24.2	29.0	14.5	32.3	14	33.3	14.3	38.1	24	38.1	9.5	28.6	35	15.0	20.0	30.0
LC612644.1 Genus *Planaeschna* Vietnam	19.4	35.5	11.3	33.9	14	33.3	9.5	42.9	24	38.1	9.5	28.6	20	35.0	15.0	30.0
MK774267.1 Genus *Anax* Japan	29.0	25.8	8.1	37.1	38	28.6	4.8	28.6	33	23.8	14.3	28.6	15	25.0	5.0	55.0
LC366042.1 Genus *Polycanthagyna* Japan	21.0	29.0	14.5	35.5	10	38.1	14.3	38.1	24	33.3	9.5	33.3	30	15.0	20.0	35.0
AB706757.1 Genus *Sarasaeschna* Japan	19.4	33.9	12.9	33.9	5	33.3	9.5	52.4	24	38.1	14.3	23.8	30	30.0	15.0	25.0
AB706758.1 Genus *Tetracanthagyna* Malaysia	16.1	33.9	16.1	33.9	10	38.1	9.5	42.9	19	33.3	19.0	28.6	20	30.0	20.0	30.0
MZ882296.1 Genus *Protosticta* Kerala	.0	.0	100.0	.0		.0	100.0	.0		.0	100.0	.0		.0	100.0	.0
Avg.	22.2	26.3	19.4	32.1	22	28.9	16.8	32.3	25	27.2	21.6	25.9	19	22.6	19.9	38.5

9) Resolution of phylogenetic relationships within the family Gomphidae

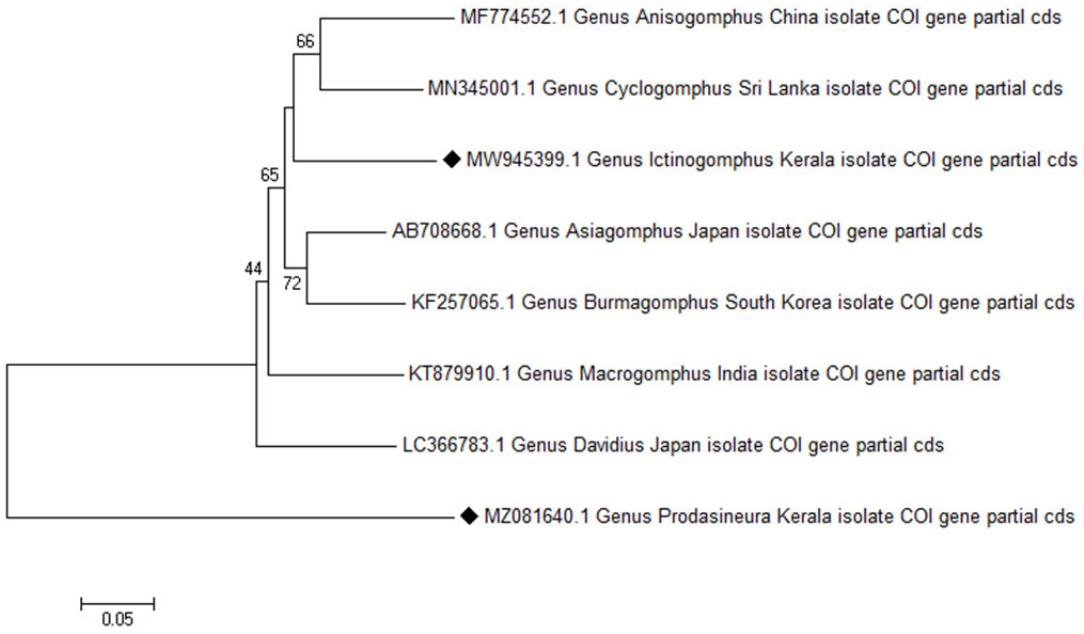

Figure 4.4.21: Inferred phylogenetic tree based on COI gene sequences of family Gomphidae, rooted by outgroup.

Table 4.4.33: Estimates of genetic divergence of the COI gene sequences of family Gomphidae and out group

	Genus COI	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	MW945399.1_Genus_*Ictinogomphus*_Kerala							
2	MF774552.1_Genus_*Anisogomphus*_China	0.200						
3	AB708668.1_Genus_*Asiagomphus*_Japan	0.184	0.176					
4	KF257065.1_Genus_*Burmagomphus*_South_Korea	0.184	0.200	0.120				
5	MN345001.1_Genus_*Cyclogomphus*_Sri_Lanka	0.192	0.160	0.152	0.176			
6	LC366783.1_Genus_*Davidius*_Japan	0.240	0.216	0.184	0.192	0.192		
7	KT879910.1_Genus_*Macrogomphus*_India	0.200	0.248	0.160	0.176	0.192	0.208	
8	MZ081640.1_Genus_*Prodasineura*_Kerala	0.568	0.600	0.576	0.584	0.608	0.568	0.560

Table 4.4.34: Nucleotide base composition of COI gene sequences of family Gomphidae and out group

Domain: Data COI																
	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
MW945399.1 Genus *Ictinogomphus* Kerala	28.8	20.8	32.8	17.6	26	9.5	57.1	7.1	20	24.4	31.7	24.4	40	28.6	9.5	21.4
MF774552.1 Genus *Anisogomphus* China	36.8	16.8	28.0	18.4	43	.0	47.6	9.5	27	22.0	26.8	24.4	40	28.6	9.5	21.4
AB708668.1 Genus *Asiagomphus* Japan	32.8	20.0	31.2	16.0	31	9.5	57.1	2.4	27	22.0	26.8	24.4	40	28.6	9.5	21.4
KF257065.1 Genus *Burmagomphus* South Korea	32.0	20.8	31.2	16.0	29	14.3	54.8	2.4	27	19.5	29.3	24.4	40	28.6	9.5	21.4
MN345001.1 Genus *Cyclogomphus* Sri Lanka	33.6	19.2	28.8	18.4	33	9.5	47.6	9.5	27	19.5	29.3	24.4	40	28.6	9.5	21.4
LC366783.1 Genus *Davidius* Japan	28.8	23.2	31.2	16.8	26	14.3	54.8	4.8	20	26.8	29.3	24.4	40	28.6	9.5	21.4
KT879910.1 Genus *Macrogomphus* India	28.8	20.0	32.8	18.4	24	4.8	61.9	9.5	22	26.8	26.8	24.4	40	28.6	9.5	21.4
MZ081640.1 Genus *Prodasineura* Kerala	4.4	3.7	83.1	8.8	4	2.2	91.3	2.2	2	2.2	82.2	13.3	7	6.7	75.6	11.1
Avg.	28.0	17.9	37.9	16.2	27	7.9	59.4	5.9	21	20.2	35.8	22.9	36	25.7	18.3	20.1

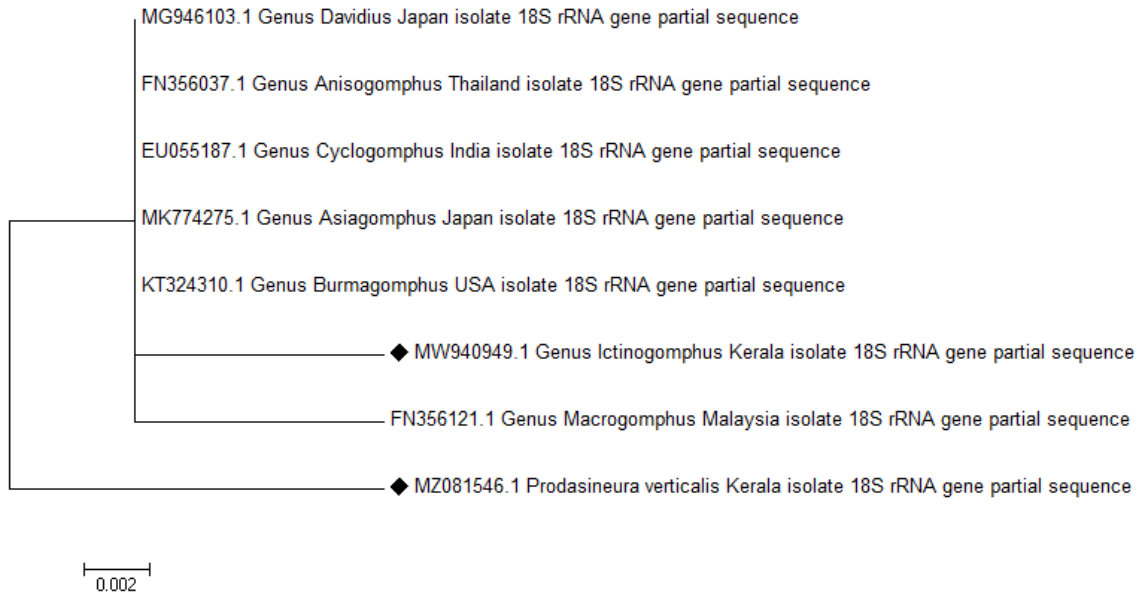

Figure 4.4.22: Inferred phylogenetic tree based on 18S rRNA gene sequences of family Gomphidae, rooted by outgroup.

Phylogenetic analysis and genetic divergence

a) Based on partial COI gene sequence

Phylogenetic relationship among the genera of family Gomphidae based on partial COI gene sequences were resolved. The sequence data was composed of the sequence of genus *Ictinogomphus* and sequences of other genera retrieved from GenBank. Sequence of genus *Prodasineura* was included as out group. A total of 8 sequences were included in the analysis (Figure 4.4.21).

The result showed that *Anisogomphus* and *Cyclogomphus* were sister clades and *Ictinogomphus* was paraphyletic. *Asiagomphus* and *Burmagomphus* also formed sister clades. Genus *Davidius* diverged from the common ancestor long ago.

The divergence values were ranged from 12.0% to 24.8%. Minimum divergence was observed between *Burmagomphus* and *Asiagomphus*. Maximum value of genetic divergence was possessed by *Macrogomphus* and *Anisogomphus* (Table 4.4.33).

b) Based on partial 18S rRNA gene sequence

Phylogeny of the genera of family Gomphidae based on partial 18S rRNA gene sequence was resolved. In addition to the sequence of the genus *Ictinogomphus*, 6 more sequences of other genera of the corresponding family were retrieved from GenBank and the sequence of the damselfly genus *Prodasineura* was included as out group. Phylogeny of the 8 genera was resolved and presented in Figure 4.4.22.

All the genera were grouped into a monophyletic clade in the phylogenetic tree obtained. Genus *Ictinogomphus* and *Macrogomphus* showed variation from the remaining genera.

The divergence values were ranged from 0% to 1.5%. Maximum value of divergence was observed between *Macrogomphus* and *Ictinogomphus* (Table 4.4.35).

Nucleotide composition

The nucleotide frequencies of eight partial COI gene sequences were 37.20% (A), 28.30% (T/U), 18.10% (C) and 16.40% (G) with high AT content(65.5%) over GC content(34.5%).The nucleotide composition of eight partial 18S rRNA gene sequences were 24.24% (A), 27.46% (T/U), 17.33% (C) and 30.97% (G) with balanced AT content (51.5%) and GC content (48.3%). The values are showed in Tables 4.4.34 and 4.4.36 respectively.

Table 4.4.35: Estimates of genetic divergence of the 18S rRNA gene sequences of family Gomphidae and out group

	Genus 18S	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	MW940949.1_Genus_*Ictinogomphus*_Kerala							
2	MK774275.1_Genus_*Asiagomphus*_Japan	0.008						
3	KT324310.1_Genus_*Burmagomphus*_USA	0.008	0.000					
4	EU055187.1_Genus_*Cyclogomphus*_India	0.008	0.000	0.000				
5	MG946103.1_Genus_*Davidius*_Japan	0.008	0.000	0.000	0.000			
6	FN356121.1_Genus_*Macrogomphus*_Malaysia	0.015	0.008	0.008	0.008	0.008		
7	FN356037.1_Genus_*Anisogomphus*_Thailand	0.008	0.000	0.000	0.000	0.000	0.008	
8	MZ081546.1_*Prodasineura_verticalis*_Kerala	0.023	0.015	0.015	0.015	0.015	0.023	0.015

Table 4.4.36: Nucleotide base composition of 18S rRNA gene sequences of family Gomphidae and out group

Domain: Data 18S																
	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
MW940949.1 Genus *Ictinogomphus* Kerala	28.6	16.5	24.1	30.8	27	17.8	28.9	26.7	32	15.9	18.2	34.1	27	15.9	25.0	31.8
MK774275.1 Genus *Asiagomphus* Japan	27.8	17.3	24.1	30.8	27	17.8	28.9	26.7	30	18.2	18.2	34.1	27	15.9	25.0	31.8
KT324310.1 Genus *Burmagomphus* USA	27.8	17.3	24.1	30.8	27	17.8	28.9	26.7	30	18.2	18.2	34.1	27	15.9	25.0	31.8
EU055187.1 Genus *Cyclogomphus* India	27.8	17.3	24.1	30.8	27	17.8	28.9	26.7	30	18.2	18.2	34.1	27	15.9	25.0	31.8
MG946103.1 Genus *Davidius* Japan	27.8	17.3	24.1	30.8	27	17.8	28.9	26.7	30	18.2	18.2	34.1	27	15.9	25.0	31.8
FN356121.1 Genus *Macrogomphus* Malaysia	27.8	17.3	24.8	30.1	27	17.8	28.9	26.7	30	18.2	18.2	34.1	27	15.9	27.3	29.5
FN356037.1 Genus *Anisogomphus* Thailand	27.8	17.3	24.1	30.8	27	17.8	28.9	26.7	30	18.2	18.2	34.1	27	15.9	25.0	31.8
MZ081546.1 *Prodasineura verticalis* Kerala	28.6	17.3	23.3	30.8	29	15.6	28.9	26.7	31	20.0	15.6	33.3	26	16.3	25.6	32.6
Avg.	28.0	17.2	24.1	30.7	27	17.5	28.9	26.7	30	18.1	17.8	34.0	27	16.0	25.4	31.6

10) Resolution of phylogenetic relationships within the family Libellulidae

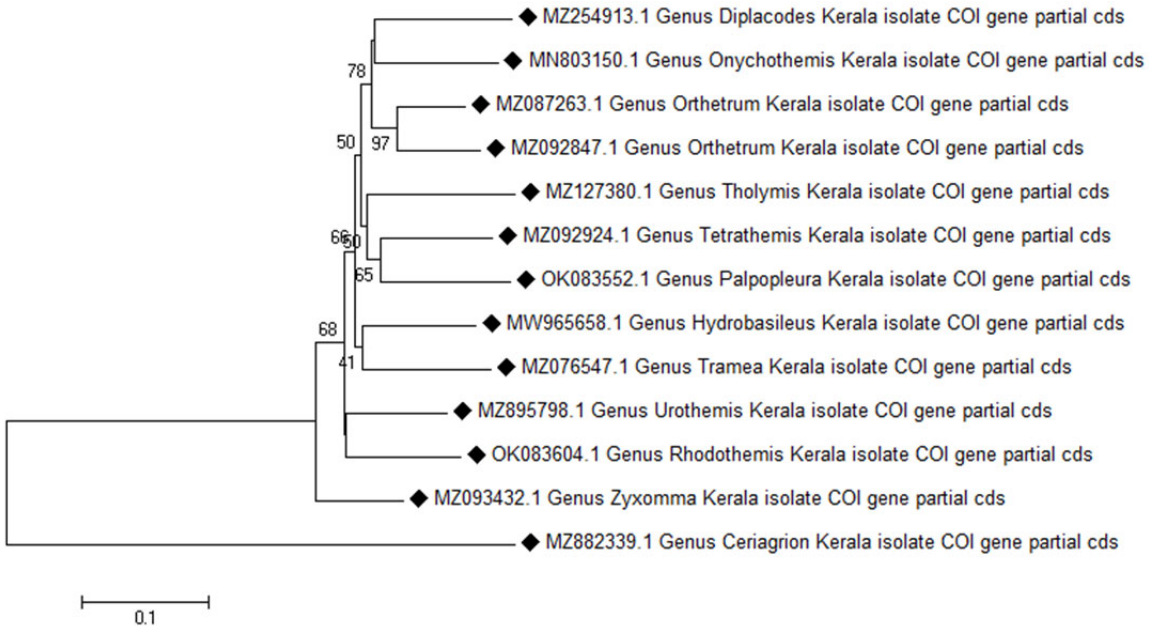

Figure 4.4.23: Inferred phylogenetic tree based on COI gene sequences of family Libellulidae, rooted by outgroup.

Phylogenetic analysis and genetic divergence

a) Based on partial COI gene sequence

Phylogeny of family Libellulidae, based on partial COI gene sequence were resolved (Figure 4.4.23). Species of 11 genera sequenced during the current study were utilized for the analysis along with damselfly genus *Ceriagrion* as out group.

The result showed that genus *Zyxomma* was paraphyletic to the remaining genera. The other genera formed a monophyletic clade including sister clades of *Diplacodes*+ *Onychothemis, Tetrathemis* + *Palpopleura, Hydrobasileus*+ *Tramea* and *Urothemis*+ *Rhodothemis.*

The divergence values were ranged from 13.7% to 18.1%. The maximum value of divergence (18.1%) was observed between *Tholymis* and *Urothemis* (Table 4.4.37).

b) Based on partial 18S rRNA gene sequence

Phylogenetic reconstruction of the genera of family Libellulidae based on 18S rRNA gene sequence was carried out by using the 11 genera of family Libellulidae sequenced during the study and damselfly genus *Ceriagrion* as out group. Phylogeny of 12 genera were resolved and presented in Figure 4.4.24.

According to the result *Rhodothemis* was paraphyletic to the other Libellulid genera. The remaining genera formed a monohyletic clade in which *Hydrobasileus* and *Tramea* formed a distinct clade.

The divergence values were ranged from 0% to 1.4% (Table 4.4.39).

Nucleotide composition

The nucleotide frequencies 13 partial COI nucleotide sequences were 29.80 % (A), 35.67 % (T/U), 16.69 % (C) and 17.85% (G) with AT content (65.47%) over GC content (34.54%). The nucleotide composition of 13 partial 18S rRNA gene sequences were 29.17 % (A), 21.21 % (T/U), 22.36 % (C) and 27.26% (G) with balanced nucleotide content (AT content 50.38%; GC content 49.62%). The obtained values are presented in Tables 4.4.38 and 4.4.40 respectively.

Table 4.4.37: Estimates of genetic divergence of the COI gene sequences of family Libellulidae and out group

Genus COI	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1. MZ254913.1_Genus_*Diplacodes*_Kerala												
2. MW965658.1_Genus_*Hydrobasileus*_Kerala	0.172											
3. MZ087263.1_Genus_*Orthetrum*_Kerala	0.150	0.137										
4. MZ092847.1_Genus_*Orthetrum*_Kerala	0.150	0.141	0.103									
5. MN803150.1_Genus_*Onychothemis*_Kerala	0.161	0.154	0.145	0.145								
6. MZ076547.1_Genus_*Tramea*_Kerala	0.177	0.152	0.157	0.168	0.172							
7. MZ092924.1_Genus_*Tetrathemis*_Kerala	0.179	0.166	0.150	0.157	0.177	0.166						
8. MZ127380.1_Genus_*Tholymis*_Kerala	0.172	0.170	0.148	0.159	0.195	0.170	0.165					
9. MZ895798.1_Genus_*Urothemis*_Kerala	0.168	0.157	0.148	0.146	0.157	0.148	0.148	0.181				
10. MZ093432.1_Genus_*Zyxomma*_Kerala	0.168	0.157	0.154	0.139	0.170	0.156	0.165	0.161	0.152			
11. OK083604.1_Genus_*Rhodothemis*_Kerala	0.177	0.170	0.157	0.156	0.156	0.165	0.143	0.175	0.139	0.154		
12. OK083552.1_Genus_*Palpopleura*_Kerala	0.165	0.177	0.163	0.157	0.166	0.174	0.152	0.177	0.161	0.172	0.170	
13. MZ882339.1_Genus_*Ceriagrion*_Kerala	0.197	0.174	0.156	0.190	0.175	0.157	0.177	0.183	0.150	0.161	0.174	0.183

Table 4.4.38: Nucleotide base composition of COI gene sequences of family Libellulidae and out group

Domain: Data COI																
	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
MZ254913.1 Genus *Diplacodes* Kerala	34.9	17.7	28.4	19.0	45	25.9	13.0	16.2	38	8.7	44.6	8.7	22	18.5	27.7	32.1
MW965658.1 Genus *Hydrobasileus* Kerala	35.3	16.3	30.9	17.5	45	26.5	12.4	16.2	37	5.4	53.8	3.8	24	16.8	26.6	32.6
MZ087263.1 Genus *Orthetrum* Kerala	37.3	15.7	30.6	16.5	45	25.9	13.0	16.2	43	4.9	51.1	1.1	24	16.3	27.7	32.1
MZ092847.1 Genus *Orthetrum* Kerala	35.8	16.3	30.9	17.0	45	25.9	13.0	16.2	39	6.5	51.1	3.3	23	16.3	28.8	31.5
MN803150.1 Genus *Onychothemis* Kerala	35.8	17.4	29.7	17.2	45	25.9	13.0	16.2	39	8.7	49.5	3.3	24	17.4	26.6	32.1
MZ076547.1 Genus *Tramea* Kerala	36.5	16.5	28.2	18.8	45	25.9	12.4	16.8	40	8.2	44.6	7.6	25	15.2	27.7	32.1
MZ092924.1 Genus *Tetrathemis* Kerala	35.1	17.5	29.8	17.5	45	25.9	13.0	16.2	38	9.2	48.9	3.8	22	17.4	27.7	32.6
MZ127380.1 Genus *Tholymis* Kerala	33.3	17.7	30.2	18.8	45	24.9	13.0	17.3	34	9.8	48.9	7.1	21	18.5	28.8	32.1
MZ895798.1 Genus *Urothemis* Kerala	36.0	16.8	29.5	17.7	45	25.9	12.4	16.8	40	7.1	49.5	3.8	23	17.4	26.6	32.6
MZ093432.1 Genus *Zyxomma* Kerala	34.0	15.9	32.5	17.5	45	25.4	13.0	16.8	31	9.2	57.1	2.7	26	13.0	27.7	33.2
OK083604.1 Genus *Rhodothemis* Kerala	36.9	16.3	29.1	17.7	45	25.9	13.0	16.2	40	6.5	48.9	4.3	26	16.3	25.5	32.6
OK083552.1 Genus *Palpopleura* Kerala	37.6	16.3	27.7	18.4	45	25.9	12.4	16.8	43	5.4	42.9	8.2	24	17.4	27.7	30.4
MZ882339.1 Genus *Ceriagrion* Kerala	35.3	16.6	29.8	18.3	45	25.4	13.0	16.8	35	9.2	51.1	4.9	26	15.2	25.5	33.2
Avg.	35.7	16.7	29.8	17.8	45	25.8	12.8	16.5	38	7.6	49.4	4.8	24	16.6	27.3	32.2

Figure 4.4.24: Inferred phylogenetic tree based on 18S rRNA gene sequences of family Libellulidae, rooted by outgroup.

Table 4.4.39: Estimates of genetic divergence of the 18S rRNA gene sequences of family Libellulidae and out group

	Genus 18S	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	MZ081547.1_Genus_*Diplacodes*_Kerala												
2	MW945405.1_Genus_*Hydrobasileus*_Kerala	0.007											
3	MZ081550.1_Genus_*Orthetrum*_Kerala	0.000	0.007										
4	MZ092846.1_Genus_*Orthetrum*_Kerala	0.000	0.007	0.000									
5	MZ803139.1_Genus_*Onychothemis*_Kerala	0.002	0.009	0.002	0.002								
6	MZ092848.1_Genus_*Palpopleura*_Kerala	0.000	0.007	0.000	0.000	0.002							
7	MZ076516.1_Genus_*Tramea*_Kerala	0.012	0.005	0.012	0.012	0.014	0.012						
8	MZ092849.1_Genus_*Tetrathemis*_Kerala	0.000	0.007	0.000	0.000	0.002	0.000	0.012					
9	MZ093144.1_Genus_*Tholymis*_Kerala	0.000	0.007	0.000	0.000	0.002	0.000	0.012	0.000				
10	MZ895802.1_Genus_*Urothemis*_Kerala	0.000	0.007	0.000	0.000	0.002	0.000	0.012	0.000	0.000			
11	MZ093372.1_Genus_*Zyxomma*_Kerala	0.000	0.007	0.000	0.000	0.002	0.000	0.012	0.000	0.000	0.000		
12	OK083605.1_Genus_*Rhodothemis*_Kerala	0.005	0.012	0.005	0.005	0.007	0.005	0.012	0.005	0.005	0.005	0.005	
13	MZ817954.1_Genus_*Dysphaea*_Kerala	0.479	0.486	0.479	0.479	0.481	0.479	0.484	0.479	0.479	0.479	0.479	0.477

Table 4.4.40: Nucleotide base composition of 18S rRNA gene sequences of family Libellulidae and out group

Domain: Data 18S																
	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
MZ081547.1 Genus *Diplacodes* Kerala	22.4	24.2	25.1	28.3	20	20.8	27.8	31.3	19	31.0	20.0	29.7	28	20.7	27.6	24.1
MW945405.1 Genus *Hydrobasileus* Kerala	22.1	24.2	25.1	28.6	20	20.1	27.8	31.9	19	31.7	20.0	29.0	27	20.7	27.6	24.8
MZ081550.1 Genus *Orthetrum* Kerala	22.4	24.2	25.1	28.3	20	20.8	27.8	31.3	19	31.0	20.0	29.7	28	20.7	27.6	24.1
MZ092846.1 Genus *Orthetrum* Kerala	22.4	24.2	25.1	28.3	20	20.8	27.8	31.3	19	31.0	20.0	29.7	28	20.7	27.6	24.1
MZ803139.1 Genus *Onychothemis* Kerala	22.6	24.0	25.1	28.3	20	20.8	27.8	31.3	19	31.0	20.0	29.7	28	20.0	27.6	24.1
MZ092848.1 Genus *Palpopleura* Kerala	22.4	24.2	25.1	28.3	20	20.8	27.8	31.3	19	31.0	20.0	29.7	28	20.7	27.6	24.1
MZ076516.1 Genus *Tramea* Kerala	21.9	24.4	25.3	28.3	20	20.1	28.5	31.3	19	31.7	20.0	29.0	26	21.4	27.6	24.8
MZ092849.1 Genus *Tetrathemis* Kerala	22.4	24.2	25.1	28.3	20	20.8	27.8	31.3	19	31.0	20.0	29.7	28	20.7	27.6	24.1
MZ093144.1 Genus *Tholymis* Kerala	22.4	24.2	25.1	28.3	20	20.8	27.8	31.3	19	31.0	20.0	29.7	28	20.7	27.6	24.1
MZ895802.1 Genus *Urothemis* Kerala	22.4	24.2	25.1	28.3	20	20.8	27.8	31.3	19	31.0	20.0	29.7	28	20.7	27.6	24.1
MZ093372.1 Genus *Zyxomma* Kerala	22.4	24.0	25.3	28.3	20	20.7	28.3	31.0	19	31.0	20.0	29.7	28	20.1	27.8	24.3
OK083605.1 Genus *Rhodothemis* Kerala	22.1	24.4	25.3	28.1	20	20.8	28.5	30.6	19	31.0	20.0	29.7	27	21.4	27.6	24.1
MZ817954.1 Genus *Dysphaea* Kerala	10.0	7.5	73.0	9.6	8	7.1	76.6	8.5	10	11.1	67.4	11.1	12	4.2	75.0	9.0
Avg.	21.4	22.9	28.8	26.9	19	19.7	31.6	29.5	19	29.6	23.6	28.1	26	19.4	31.2	23.1

4.4.4 Phylogeny of selected genera

Out of the 28 genera sequenced, representatives of 27 genera were used for the phylogenetic reconstruction based on COI sequences. The genus *Onychothemis* was excluded, as sequences of the same were not available at GenBank. Nuclear 18S rRNA gene sequences are composed of highly conserved regions and they are not suitable for resolving species level phylogenetic relationships (Dumont et al., 2010) hence this marker gene was excluded from genus trees. Genetic divergence between sequences were also calculated.

1) Phylogenetic analysis of the genus *Lestes*

For the phylogenetic reconstruction of the genus *Lestes* based on COI gene, in addition to the sequence of *Lestes preamorsus*, 9 more sequences of the corresponding genus were retrieved from GenBank and sequence of *Gynacantha dravida* was included as out group. Phylogeny of 11 species were resolved (Table 4.4.41; Figure 4.4.25).

Table 4.4.41: Details of COI gene sequences selected for phylogenetic analysis of genus *Lestes*

Sl No.	Accession Number	Scientific Name	Product size
1.	MZ074000.1	*Lestes praemorsus*, Kerala	671bp
2.	KF369423.1	*Lestes praemorsus*, Malaysia	658bp
3.	KM536082.1	*Lestes congener*, Canada	658bp
4.	KM531462.1	*Lestes congener*, Canada	658bp
5.	KM528476.1	*Lestes dryas*, Canada	658bp
6.	KM534143.1	*Lestes dryas*, Canada	658bp
7.	KM537254.1	*Lestes dryas*, Canada	658bp
8.	KM534772.1	*Lestes disjunctus*, Canada	658bp
9.	HM413470.1	*Lestes forcipatus*, Canada	658bp
10.	KM536047.1	*Lestes rectangularis*, Canada	658bp
11.	MK990607.1	*Gynacantha dravida*, Kerala	631bp

The findings revealed from the result are as follows: *Lestes praemorsus* from Kerala and Malaysia were phylogenetically very closer and well supported with 99%

bootstrap. *Lestes dryas* samples and *Lestes congener* samples from Canada formed separate monophyletic clades with strong support and *Lestes congener* formed sister clade with *Lestes praemorsus*. The remaining three species *Lestes disjunctus, Lestes forcipatus* and *Lestes rectangularis* are clustered together found to be monophyletic to each other and polyphyletic to *Lestes praemorsus*.

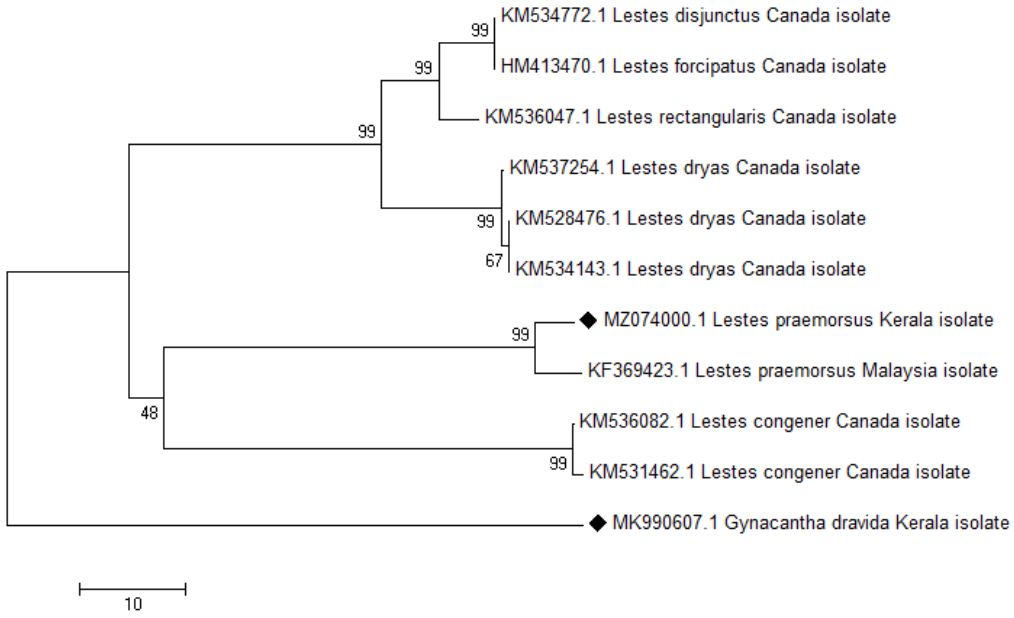

Figure 4.4.25: Inferred phylogenetic tree of the genus *Lestes*, rooted by outgroup.

Intraspecific and interspecific divergence

The intraspecific and interspecific divergence were calculated and presented in Table 4.4.42. Conspecifics of *Lestes Preamorsus* exhibited 1.3% divergence between Kerala and Malaysia specimen. 0.2% divergence was found between *Lestes congener* specimens. *Lestes dryaas* specimens showed 0% to 0.2% divergence. Interspecific divergence values ranged from 1.5% to 13.3%.

Nucleotide composition

The estimated nucleotide frequencies were 30.90 % (A), 33.90% (T/U), 16.82 % (C) and 18.38 % (G). Codon positions included were 1^{st}+2^{nd}+3^{rd}+noncoding. All positions containing gaps and missing data were eliminated. There was a high AT bias observed in the gene sequence of *Lestes praemorsus* (T=31.8%, C=18.2%, A=30.8%, G=19.1%) (Table 4.4.43).

Table 4.4.42: Estimates of genetic divergence among the COI gene sequences of genus *Lestes* and out group

	Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1.	MZ074000.1_*Lestes_praemorsus*_Kerala										
2.	KF369423.1_*Lestes_praemorsus*_Malaysia	0.013									
3.	KM536082.1_*Lestes_congener*_Canada	0.128	0.130								
4.	KM536047.1_*Lestes_rectangularis*_Canada	0.126	0.128	0.125							
5.	KM534772.1_*Lestes_disjunctus*_Canada	0.126	0.126	0.126	0.015						
6.	KM531462.1_*Lestes_congener*_Canada	0.130	0.131	0.002	0.126	0.128					
7.	KM528476.1_*Lestes_dryas*_Canada	0.126	0.126	0.131	0.035	0.038	0.133				
8.	HM413470.1_*Lestes_forcipatus*_Canada	0.126	0.126	0.126	0.015	0.000	0.128	0.038			
9.	KM534143.1_*Lestes_dryas*_Canada	0.126	0.126	0.131	0.035	0.038	0.133	0.000	0.038		
10.	KM537254.1_*Lestes_dryas*_Canada_	0.128	0.128	0.130	0.033	0.037	0.131	0.002	0.037	0.002	
11.	MK990607.1_*Gynacantha_dravida*_Kerala	0.183	0.189	0.173	0.159	0.168	0.173	0.169	0.168	0.169	0.168

Table 4.4.43: Nucleotide base composition of COI gene sequences of genus *Lestes* and out group

Species																
	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
MZ074000.1 *Lestes praemorsus* Kerala	31.8	18.2	30.8	19.1	31	9.0	49.8	10.0	20	18.9	29.4	31.3	44	26.9	13.4	15.9
KF369423.1 *Lestes praemorsus* Malaysia	31.7	18.1	30.7	19.6	30	9.0	49.3	11.4	21	18.4	29.4	31.3	44	26.9	13.4	15.9
KM536082.1 *Lestes congener* Canada	33.3	18.1	29.7	18.9	35	9.0	46.3	9.5	21	18.4	29.4	31.3	44	26.9	13.4	15.9
KM536047.1 *Lestes rectangularis* Canada	34.7	15.8	31.2	18.4	35	6.0	50.7	8.0	25	14.4	29.4	31.3	44	26.9	13.4	15.9
KM534772.1 *Lestes disjunctus* Canada	34.4	15.8	31.6	18.3	35	6.0	51.7	7.5	25	14.5	29.5	31.5	44	26.9	13.4	15.9
KM531462.1 *Lestes congener* Canada	33.3	18.1	29.5	19.1	35	9.0	45.8	10.0	21	18.4	29.4	31.3	44	26.9	13.4	15.9
KM528476.1 *Lestes dryas* Canada	34.5	16.3	31.5	17.7	35	7.5	51.2	6.5	25	14.4	29.9	30.8	44	26.9	13.4	15.9
HM413470.1 *Lestes forcipatus* Canada	34.3	15.9	31.5	18.2	35	6.0	51.7	7.5	24	14.9	29.4	31.3	44	26.9	13.4	15.9
KM534143.1 *Lestes dryas* Canada	34.5	16.3	31.5	17.7	35	7.5	51.2	6.5	25	14.4	29.9	30.8	44	26.9	13.4	15.9
KM537254.1 *Lestes dryas* Canada	34.5	16.3	31.3	17.9	35	7.5	50.7	7.0	25	14.4	29.9	30.8	44	26.9	13.4	15.9
MK990607.1 *Gynacantha dravida* Kerala	35.8	17.2	30.0	16.9	42	7.5	47.8	3.0	22	17.4	28.9	31.8	44	26.9	13.4	15.9
Avg.	33.9	16.9	30.9	18.4	35	7.6	49.7	7.9	23	16.2	29.5	31.3	44	26.9	13.4	15.9

2) Phylogenetic analysis of the genus *Protosticta*

Phylogeny of the species of genus *Protosticta* based on partial COI gene sequences were resolved by using the sequence of *Protosticta gravelyi* and the sequences of 4 species retrieved from GenBank. Sequence of the dragonfly *Gynacantha dravida* was included as out group (Table 4.4.44; Figure 4.4.26). As partial COI gene sequence of *Protosticta gravelyi* is the first record in GenBank, sequence of the conspecific was not available for comparison.

Table 4.4.44: Details of COI gene sequences involved in the phylogenetic analysis of genus *Protosticta*

Sl No.	Accession Number	Scientific Name	Product size
1.	MN974377.1	*Protosticta gravelyi*, Kerala	593bp
2.	KF369523.1	*Protosticta satoi*, Vietnam	658bp
3.	KF369522.1	*Protosticta plicata*, Philippines	658bp
4.	KF369521.1	*Protosticta linnaei*, Vietnam	658bp
5.	KF369520.1	*Protosticta grandis*, Vietnam	658bp
6.	MK990607.1	*Gynacantha dravida*, Kerala	631bp

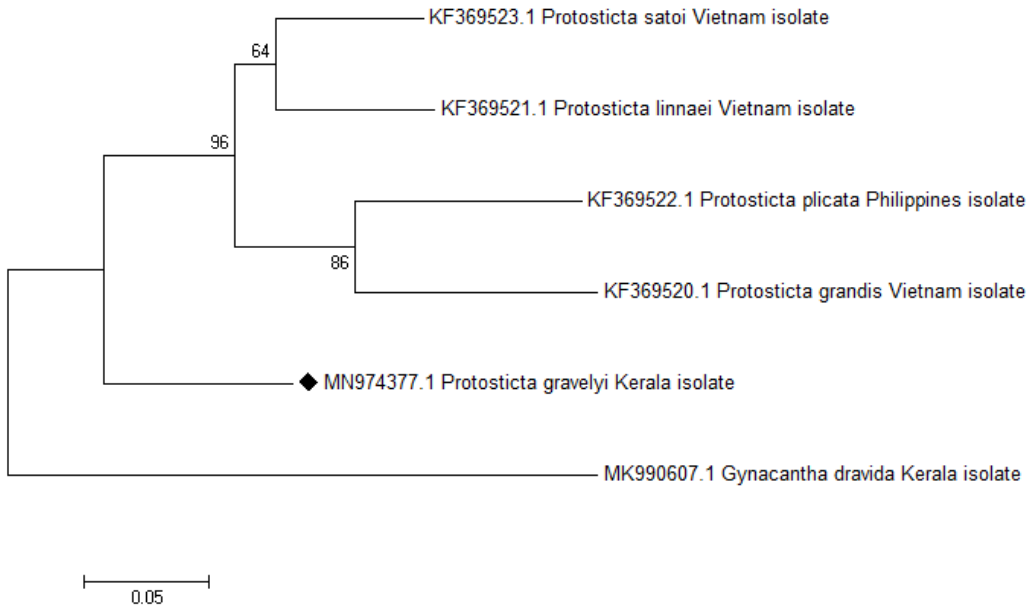

Figure 4.4.26: Inferred phylogenetic tree of the genus *Protosticta*, rooted by outgroup.

The phylogenetic analysis result showed that *Protosticta gravelyi* shared a common ancestor with *Protosticta* members but showed high sequence diversion. As a result the tree was divided into two main clades and *Protosticta gravelyi* was paraphyletic to others. The other *Protosticta* species were evolved from the second clade in which *Protosticta satoi* and *Protosticta linnaei* formed sister clades. Also, *Protosticta plicata* and *Protosticta grandis* exihibit sister clade relationship.

Intraspecific and interspecific divergence

The intraspecific and interspecific divergence were calculated and presented in Table 4.4.45. Interspecific divergence over COI gene sequences among the *Protosticta* species ranges from 11.1% to 20.9%.

Nucleotide composition

The nucleotide frequencies were 30.94 % (A), 32.39% (T/U), 19.33 % (C) and 17.33% (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The observed nucleotide compositon in the gene sequence of *Protosticta gravelyi* was T=31.2%, C=20.2%, A=30.2%, G=18.4%. High AT bias was observed (AT content- 61.4%, GC content-38.6%). The obtained values are given in Table 4.4.46.

Table 4.4.45: Estimates of genetic divergence among COI gene sequences of genus *Protosticta*.

Species	**1**	**2**	**3**	**4**	**5**
1. MN974377.1_*Protosticta_gravelyi*_Kerala					
2. KF369523.1_*Protosticta_satoi*_Vietnam	0.155				
3. KF369522.1_*Protosticta_plicata*_Philippines	0.184	0.169			
4. KF369521.1_*Protosticta_linnaei*_Vietnam	0.165	0.111	0.162		
5. KF369520.1_*Protosticta_grandis*_Vietnam	0.209	0.167	0.165	0.162	
6. MK990607.1_*Gynacantha_dravida*_Kerala	0.268	0.278	0.301	0.282	0.308

Table 4.4.46: Nucleotide base composition of COI gene sequence of genus *Protosticta*

Species																
	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
MN974377.1 *Protosticta gravelyi* Kerala	31.2	20.2	30.2	18.4	36	16.8	42.4	5.2	18	19.6	33.9	28.6	40	24.2	14.2	21.6
KF369523.1 *Protosticta satoi* Vietnam	33.0	18.9	29.3	18.8	37	15.7	39.8	7.3	22	15.9	33.9	28.0	39	25.3	14.2	21.1
KF369522.1 *Protosticta plicata* Philippines	34.4	18.1	29.5	18.1	40	15.7	38.7	5.8	23	14.3	35.4	27.5	41	24.2	14.2	21.1
KF369521.1 *Protosticta linnaei* Vietnam	32.3	18.6	29.6	19.5	35	16.8	39.8	8.4	21	14.8	34.9	29.1	41	24.2	14.2	21.1
KF369520.1 *Protosticta grandis* Vietnam	29.8	21.1	29.5	19.6	30	23.0	37.2	9.4	19	15.3	37.6	28.6	41	24.7	13.7	21.1
MK990607.1 *Gynacantha dravida* Kerala	29.4	16.6	39.2	14.7	38	9.0	50.6	2.6	19	14.6	42.7	23.6	31	26.3	24.4	17.9
Avg.	31.8	19.0	31.0	18.3	36	16.4	41.1	6.6	20	15.8	36.2	27.7	39	24.8	15.6	20.7

3) Phylogenetic analysis of the genus *Neurobasis*

For the resolution of phylogenetic relationships of the members of *Neurobasis* based on COI gene sequence, 7sequence samples of the species belonging to the corresponding genus were downloaded from GenBank in addition to the current sequence of *Neurobasis chinensis.* The COI sequence of the dragonfly *Gynacantha dravida* was considered as out group (Table 4.4.47). The inferred phylogenetic tree of 9 sequences, the estimates of genetic divergence and the nucleotide base composition are given in Figure 4.4.27, Table 4.4.48 and Table 4.4.49 respectively.

Table 4.4.47: Details of COI gene sequences involved in the phylogenetic analysis of genus *Neurobasis*

Sl No.	**Accession Number**	**Scientific name**	**Product size**
1.	MW931875.1	*Neurobasis chinensis*, Kerala	642bp
2.	MN392926.1	*Neurobasis chinensis*, Tamil Nadu	680bp
3.	MN231300.1	*Neurobasis chinensis*, Punjab	619bp
4.	MN264264.1	*Neurobasis chinensis*, Punjab	614bp
5.	MT266925.1	*Neurobasis chinensis*, Malaysia	638bp
6.	MG518624.1	*Neurobasis chinensis*, Punjab	582bp
7.	KF369461.1	*Neurobasis longipes* Malaysia	658bp
8.	KF369460.1	*Neurobasis ianthinipennis*, Indonesia	658bp
9.	MK990607.1	*Gynacantha dravida*, Kerala	631bp

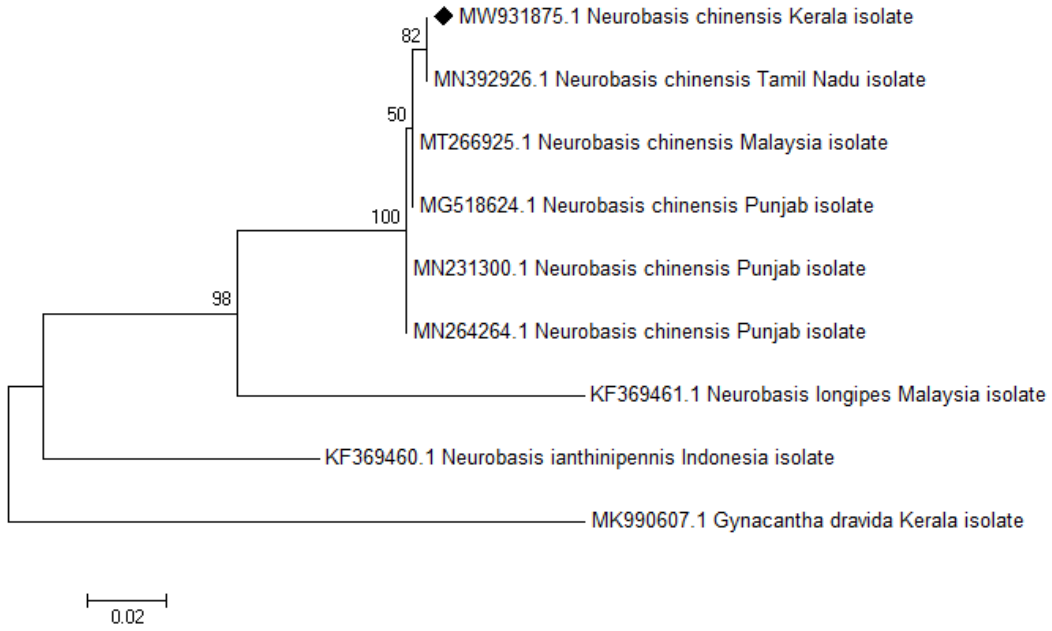

Figure 4.4.27: Inferred phylogenetic tree of the genus *Neurobasis*, rooted by outgroup.

Most branches of the tree were well supported with bootstrap values ranging from 82% to 100% except 50% at one node. Six specimens of *Neurobasis chinensis* from different locations were selected for analysis as only 3 species of *Neurobasis* are available in the GenBank. *Neurobasis ianthinipennis* indicated highest sequence diversion from the common ancestor of *Neurobasis* species and found as a distinct clade. *Neurobasis chinensis* was phylogenetically closer to *Neurobasis longipes*. Among the 6 sequences, 5 sequences were from India and one from Malaysia. All *Neurobasis chinensis* members were monophyletic and found as sister taxa. Here could be found a monophyletic ancestry with bootstrap value of 100. The *Neurobasis chinensis* individual from Kerala was more closely related to the individual from Tamil Nadu than Punjab and Malaysia specimens. However, there was only slight variation among individuals of same species from different locations. The phylogenetic tree was supported by the genetic divergence values.

Intraspecific and interspecific divergence

Intraspecific divergence ranges from 0% to 0.5%. There is no genetic divergence is observed between samples of *Neurobasis chinensis* from Kerala and Tamil Nadu. This indicated that the sequence of *Neurobasis chinensis* has not been

subjected to any major change by geographical isolation and by the course of evolution (Table 4.4.48).

Nucleotide composition

The nucleotide frequencies of the selected COI sequences were 31.92 % (A), 31.12 % (T/U), 18.51 % (C) and 18.45% (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The nucleotide composition of *Neurobasis chinensis* sample of the current study were T=31.1%, C=18.2%, A=32.2%, G=18.6%. The high AT content (63.3%) was observed over GC content (36.8%) and the values were given in Table 4.4.49.

Table 4.4.48: Estimates of genetic divergence among COI gene sequences of genus *Neurobasis*

	Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1.	MW931875.1_*Neurobasis_chinensis*_Kerala								
2.	MN392926.1_*Neurobasis_chinensis*_Tamil_Nadu	0.000							
3.	MN231300.1_*Neurobasis_chinensis*_Punjab	0.005	0.005						
4.	MN264264.1_*Neurobasis_chinensis*_Punjab	0.005	0.005	0.000					
5.	MT266925.1_*Neurobasis_chinensis*_Malaysia	0.004	0.004	0.002	0.002				
6.	MG518624.1_*Neurobasis_chinensis*_Punjab	0.004	0.004	0.002	0.002	0.000			
7.	KF369461.1_*Neurobasis_longipes*_Malaysia	0.117	0.117	0.117	0.117	0.117	0.117		
8.	KF369460.1_*Neurobasis_ianthinipennis*_Indonesia	0.147	0.147	0.141	0.141	0.143	0.143	0.157	
9.	MK990607.1_*Gynacantha_dravida*_Kerala	0.180	0.180	0.182	0.182	0.184	0.184	0.223	0.191

Table 4.4.49: Nucleotide base composition of COI gene sequence of the genus *Neurobasis*

Name of species																
	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
MW931875.1 *Neurobasis chinensis* Kerala	31.1	18.2	32.2	18.6	20	17.5	31.7	30.7	44	25.4	13.8	16.9	29	11.7	51.1	8.0
MN392926.1*Neurobasis chinensis* TamilNadu	31.1	18.2	32.2	18.6	20	17.5	31.7	30.7	44	25.4	13.8	16.9	29	11.7	51.1	8.0
MN231300.1 *Neurobasis chinensis* Punjab	31.1	18.4	32.0	18.6	20	17.5	31.7	30.7	44	25.4	13.8	16.9	29	12.2	50.5	8.0
MN264264.1 *Neurobasis chinensis* Punjab	31.1	18.4	32.0	18.6	20	17.5	31.7	30.7	44	25.4	13.8	16.9	29	12.2	50.5	8.0
MT266925.1 *Neurobasis chinensis* Malaysia	31.1	18.2	32.2	18.6	20	17.5	31.7	30.7	44	25.4	13.8	16.9	29	11.7	51.1	8.0
MG518624.1 *Neurobasis chinensis* Punjab	31.1	18.2	32.2	18.6	20	17.5	31.7	30.7	44	25.4	13.8	16.9	29	11.7	51.1	8.0
KF369461.1 *Neurobasis longipes* Malaysia	28.4	20.5	31.4	19.6	17	19.6	31.7	31.2	44	25.4	13.8	16.9	24	16.5	48.9	10.6
KF369460.1 *Neurobasis ianthinipennis* Indonesia	29.0	19.6	32.9	18.6	18	18.5	32.8	30.7	44	25.4	13.8	16.9	25	14.9	52.1	8.0
MK990607.1 *Gynacantha dravida* Kerala	36.0	17.0	30.4	16.6	21	17.5	29.6	31.7	44	27.0	13.8	15.3	43	6.4	47.9	2.7
Avg.	31.1	18.5	31.9	18.5	20	17.8	31.6	30.9	44	25.6	13.8	16.8	30	12.1	50.5	7.7

4) Phylogenetic analysis of Genus *Heliocypha*

Phylogenetic relationship of the species of genus *Heliocypha* based on partial COI gene sequence were resolved using 12 sequences. In addition to the sequence of *Heliocypha bisignata*, sequences of 10 species were retrieved from GenBank and the sequence of the dragonfly *Gynacantha millardi* was included as out group (Table 4.4.50; Figure 4.4.28).

Table 4.4.50: Details of COI gene sequences selected for phylogenetic analysis of genus *Heliocypha*

Sl No.	**Accession Number**	**Scientific Name**	**Product size**
1.	MW940786.1	*Heliocypha bisignata*, Kerala	676bp
2.	KM675769.1	*Rhinocypha bisignata*, Kerala	691bp
3.	MK955887.1	*Heliocypha bisignata*, Punjab	665bp
4.	MN271677.1	*Heliocypha bisignata*, Punjab	659bp
5.	MN240303.1	*Heliocypha bisignata*, Punjab	605bp
6.	KF369393.1	*Heliocypha fenestrata cornelli*, Indonesia	658bp
7.	MN231297.1	*Heliocypha biforata*, Punjab	542bp
8.	MN387796.1	*Heliocypha biforata*, Punjab	541bp
9.	MN271678.1	*Heliocypha biforata*, Punjab	539bp
10.	MN387792.1	*Heliocypha perforata*, Punjab	640bp
11.	MN271680.1	*Heliocypha perforata*, Punjab	581bp
12.	MW649897.1	*Gynacantha millardi*, Kerala	615bp

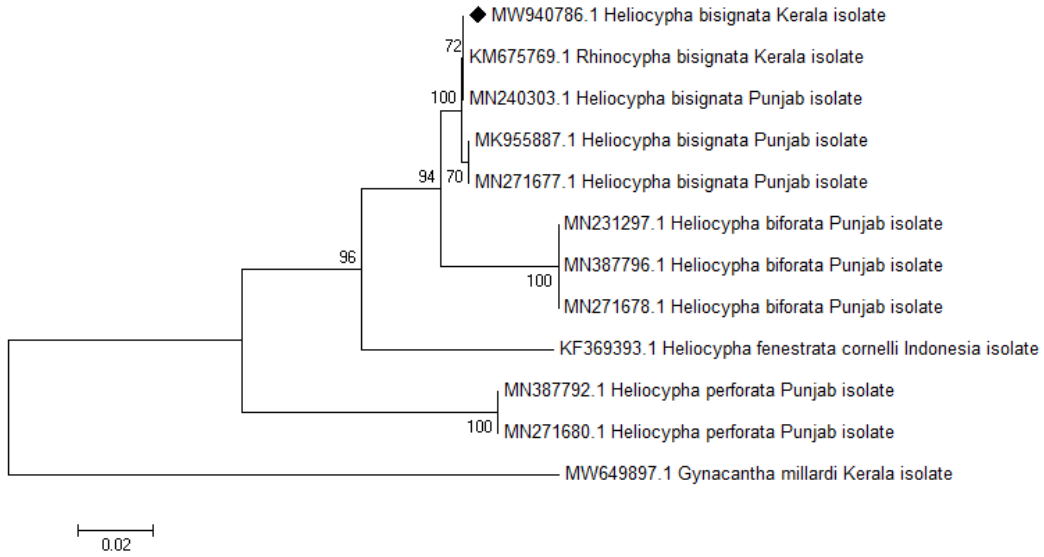

Figure 4.4.28: Inferred phylogenetic tree of the genus *Heliocypha*, rooted by outgroup.

The obtained phylogenetic tree branches are well supported with bootstrap values ranging from 94-100 except two nodes with values 72 and 70. All the four members of *Heliocypha bisignata* and *Rhinocypha bisignata* were monophyletic with 100% bootstrap support. The specimens of *Heliocypha biforata* also formed distinct monophyletic clade(bootstrap 100%). *Heliocypha biforata* was more closely related to *Heliocypha bisignata* (bootstrap 94%). *Heliocypha perforata* indicated highest sequence diversion and clustered into monophyletic clade (bootstrap 100%).

Intraspecific and interspecific divergence

The intraspecific divergence between the *Heliocypha bisignata* specimens was ranged from 0% to 0.2%. No divergence was observed between conspecifics of *Heliocypha biforata* and *Heliocypha perforata*. Maximum interspecific divergence value was 13.1% (Table 4.4.51).

Nucleotide composition

The nucleotide composition of the sequences is as follows; 31.53 % (A), 31.84% (T/U), 19.25 % (C) and 17.38% (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The nucleotide frequencies of *Heliocypha bisignata* sequenced during

the current study were T=31.8%, C=19.3%, A=30.8%, G=18.1%. The AT content and GC content are 62.6% and 37.4% respectively (Table 4.4.52).

Table 4.4.51: Estimates of genetic divergence among COI gene sequences of the genus *Heliocypha*

	Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	MW940786.1_*Heliocypha_bisignata*_Kerala											
2	KM675769.1_*Rhinocypha_bisignata*_Kerala	0.000										
3	MK955887.1_*Heliocypha_bisignata*_Punjab	0.002	0.002									
4	MN271677.1_*Heliocypha_bisignata*_Punjab	0.002	0.002	0.000								
5	MN240303.1_*Heliocypha_bisignata*_Punjab	0.000	0.000	0.002	0.002							
6	KF369393.1_*Heliocypha_fenestrata_cornelli* Indonesia	0.073	0.073	0.075	0.075	0.073						
7	MN231297.1_*Heliocypha_biforata*_Punjab	0.036	0.036	0.037	0.037	0.036	0.097					
8	MN387796.1_*Heliocypha_biforata*_Punjab	0.036	0.036	0.037	0.037	0.036	0.097	0.000				
9	MN271678.1_*Heliocypha_biforata*_Punjab	0.036	0.036	0.037	0.037	0.036	0.097	0.000	0.000			
10	MN387792.1_*Heliocypha_perforata*_Punjab	0.114	0.114	0.112	0.112	0.114	0.131	0.127	0.127	0.127		
11	MN271680.1_*Heliocypha_perforata*_Punjab	0.114	0.114	0.112	0.112	0.114	0.131	0.127	0.127	0.127	0.000	
12	MW649897.1_*Gynacantha_millardi*_Kerala	0.204	0.204	0.206	0.206	0.204	0.211	0.217	0.217	0.217	0.221	0.221

Table 4.4.52: Nucleotide base composition of COI gene sequence of the genus *Heliocypha*

Name of species																
	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
MW940786.1 *Heliocypha bisignata* Kerala	31.8	19.3	30.8	18.1	43	27.4	14.5	15.1	30	12.9	47.8	9.6	22	17.4	30.3	29.8
KM675769.1 *Rhinocypha bisignata* Kerala	31.8	19.3	30.8	18.1	43	27.4	14.5	15.1	30	12.9	47.8	9.6	22	17.4	30.3	29.8
MK955887.1 *Heliocypha bisignata* Punjab	31.6	19.4	30.8	18.1	43	27.4	14.5	15.1	29	13.5	47.8	9.6	22	17.4	30.3	29.8
MN271677.1 *Heliocypha bisignata* Punjab	31.6	19.4	30.8	18.1	43	27.4	14.5	15.1	29	13.5	47.8	9.6	22	17.4	30.3	29.8
MN240303.1 *Heliocypha bisignata* Punjab	31.8	19.3	30.8	18.1	43	27.4	14.5	15.1	30	12.9	47.8	9.6	22	17.4	30.3	29.8
KF369393.1 *Heliocypha fenestrata cornelli* Indonesia	32.7	18.7	31.6	17.0	43	27.4	14.5	15.1	33	10.7	49.4	6.7	22	18.0	30.9	29.2
MN231297.1 *Heliocypha biforata* Punjab	31.4	18.9	32.7	17.0	43	26.8	16.2	14.0	29	12.4	49.4	9.6	22	17.4	32.6	27.5
MN387796.1 *Heliocypha biforata* Punjab	31.4	18.9	32.7	17.0	43	26.8	16.2	14.0	29	12.4	49.4	9.6	22	17.4	32.6	27.5
MN271678.1 *Heliocypha biforata* Punjab	31.4	18.9	32.7	17.0	43	26.8	16.2	14.0	29	12.4	49.4	9.6	22	17.4	32.6	27.5
MN387792.1 *Heliocypha perforata* Punjab	30.3	21.1	32.1	16.4	43	27.4	14.5	15.1	26	17.4	51.7	4.5	21	18.5	30.3	29.8
MN271680.1 *Heliocypha perforata* Punjab	30.3	21.1	32.1	16.4	43	27.4	14.5	15.1	26	17.4	51.7	4.5	21	18.5	30.3	29.8
MW649897.1 *Gynacantha millardi* Kerala	36.1	16.8	30.1	17.0	43	27.9	14.5	14.5	42	6.2	46.6	5.6	24	16.3	29.2	30.9
Avg.	31.8	19.3	31.5	17.4	43	27.3	14.9	14.8	30	12.9	48.9	8.1	22	17.6	30.9	29.3

5) Phylogenetic analysis of the genus *Libellago*

The phylogenetic relationships among the species of genus *Libellago* were resolved by 8 sequences, including sequence of *Libellago indica*, with 6 sequences of the corresponding genus retrieved from GenBank. The sequence of the dragonfly *Ictinogomphus rapax* was considered as out group (Table 4.4.53; Figure 4.4.29).

Table 4.4.53: Details of COI gene sequences involved in the phylogenetic analysis of genus *Libellago*

Sl No.	Accession No.	Scientific Name	Product size
1.	MW309318.1	*Libellago indica*, Kerala	585bp
2.	MN387797.1	*Libellago lineata*, Punjab	648bp
3.	MN271674.1	*Libellago lineata*, Punjab	651bp
4.	MN231298.1	*Libellago lineata*, Punjab	593bp
5.	KF369426.1	*Libellago aurantiaca*, Malaysia	658bp
6.	KF369427.1	*Libellago celebensis orientalis*, Indonesia	658bp
7.	KF369428.1	*Libellago hyalina*, Thailand	658bp
8.	MW945399.1	*Ictinogomphus rapax*, Kerala	582bp

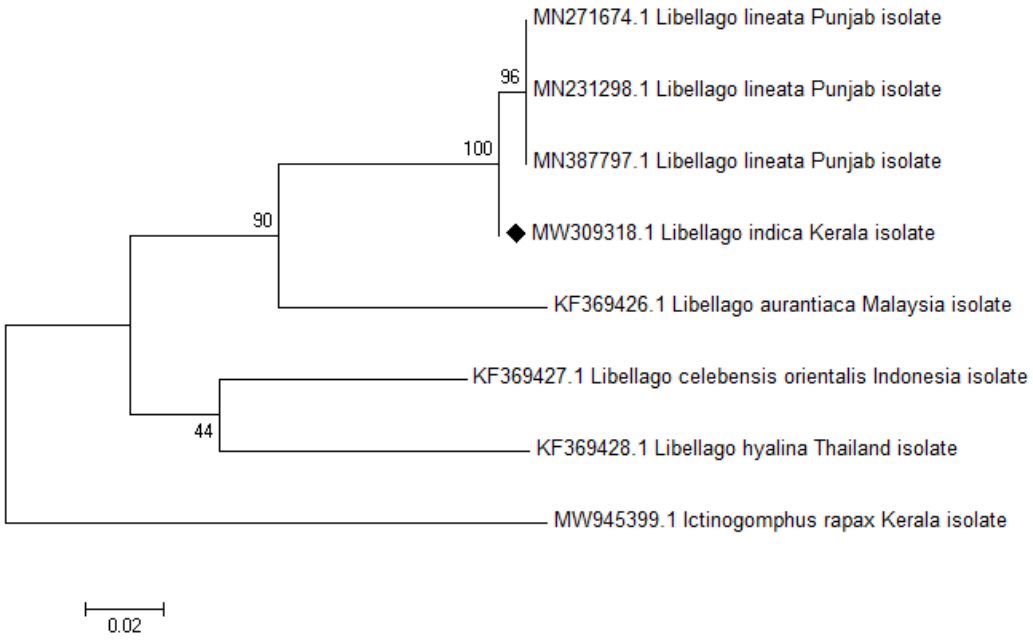

Figure 4.4.29: Inferred phylogenetic tree of the genus *Libellago*, rooted by outgroup.

All the nodes of the tree were supported with bootstrap values ranging from 90 to 100, except value of 44 for one node. As *Libellago indica* partial COI gene sequence is the first record in GenBank, conspecific sequence was not available for comparison. *Libellago indica* and *Libellago lineata* clustered into a single monophyletic clade with a bootstrap value of 100 in which *Libellago lineata* formed a separate group. The common ancestor of *Libellago* species was split to form two main clades one comprising *Libellago aurantiaca*, *Libellago lineata* and *Libellago indica* and the other clade which clustered *Libellago celebensis* and *Libellago hyalina*.

Intraspecific and interspecific divergence

Intraspecific and interspecific divergence were calculated and presented in Table 4.4.54. The intraspecific divergence of *Libellago lineata* specimens from Punjab was 0%. 0.7% divergence was found between *Libellago indica* and *Libellago lineata*. The maximum interspecific divergence was observed between *Libellago aurantiaca* and *Libellago hyalina* (16.1%).

Nucleotide composition

The nucleotide frequencies of the sequences used for phylogenetic reconstruction are 30.25% (A), 33.20% (T/U), 19.42 % (C) and 17.12 % (G). High percentage of A and T bases in all the eight COI sequences were observed. The nucleotide composition of *Libellago indica* was T=33.3%, C=19.4%, A=29.9%, G=17.4% (Table 4.4.55).

Table 4.4.54: Estimates of genetic divergence among COI gene sequences of genus *Libellago*

	Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1.	MW309318.1_*Libellago_indica*_Kerala							
2.	MN387797.1_*Libellago_lineata*_Punjab	0.007						
3.	MN271674.1_*Libellago_lineata*_Punjab	0.007	0.000					
4.	MN231298.1_*Libellago_lineata*_Punjab	0.007	0.000	0.000				
5.	KF369426.1_*Libellago_aurantiaca*_Malaysia	0.115	0.113	0.113	0.113			
6.	KF369427.1_*Libellago_celebensis_orientalis*_Indonesia	0.141	0.141	0.141	0.141	0.142		
7.	KF369428.1_*Libellago_hyalina*_Thailand	0.151	0.153	0.153	0.153	0.161	0.128	
8.	MW945399.1_*Ictinogomphus_rapax*_Kerala	0.207	0.210	0.210	0.210	0.212	0.208	0.203

Table 4.4.55: Nucleotide base composition of COI gene sequence of genus *Libellago*

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
MW309318.1 *Libellago indica* Kerala	33.3	19.4	29.9	17.4	35	13.0	44.8	7.3	21	18.2	30.7	29.7	44	27.1	14.1	15.1
MN387797.1 *Libellago lineata* Punjab	33.0	19.8	29.9	17.4	34	14.1	44.8	7.3	21	18.2	30.7	29.7	44	27.1	14.1	15.1
MN271674.1 *Libellago lineata* Punjab	33.0	19.8	29.9	17.4	34	14.1	44.8	7.3	21	18.2	30.7	29.7	44	27.1	14.1	15.1
MN231298.1 *Libellago lineata* Punjab	33.0	19.8	29.9	17.4	34	14.1	44.8	7.3	21	18.2	30.7	29.7	44	27.1	14.1	15.1
KF369426.1 *Libellago aurantiaca* Malaysia	34.9	17.9	30.4	16.8	37	10.9	46.4	5.7	24	15.6	30.7	29.7	44	27.1	14.1	15.1
KF369427.1 *Libellago celebensis orientalis* Indonesia	34.0	18.2	30.6	17.2	38	9.4	45.8	7.3	21	18.2	31.8	29.2	44	27.1	14.1	15.1
KF369428.1 *Libellago hyalina* Thailand	32.8	20.1	30.2	16.8	33	15.1	44.8	6.8	21	18.2	31.8	28.6	44	27.1	14.1	15.1
MW945399.1 *Ictinogomphus rapax* Kerala	31.6	20.3	31.4	16.7	30	14.6	50.0	5.7	21	19.3	30.7	29.2	44	27.1	13.5	15.1
Avg.	33.2	19.4	30.3	17.1	34	13.2	45.8	6.8	22	18.0	31.0	29.4	44	27.1	14.0	15.1

6) Phylogenetic analysis of the genus *Dysphaea*

Phylogenetic reconstruction of the genus *Dysphaea* based on COI gene sequence was carried out using 14 sequences including the sequence of *Dysphaea ethela* and the sequences of the corresponding genus retrieved from GenBank. Twelve sequences of conspecifics and non-conspecifics were retrieved from GenBank. The dragonfly species *Ictinogomphus rapax* was included as out group (Table 4.4.56; Figure 4.4.30).

Table 4.4.56: Details of COI gene sequences involved in the phylogenetic analysis of genus *Dysphaea*

Sl No.	Accession Number	Scientific Name	Product size
1.	MN882704.1	*Dysphaea ethela*, Kerala	677bp
2.	MN264262.1	*Dysphaea ethela*, Punjab	530bp
3.	MN387794.1	*Dysphaea ethela*, Punjab	527bp
4.	MN271676.1	*Dysphaea ethela*, Punjab	526bp
5.	KP979481.1	*Dysphaea basitincta*, China	613bp
6.	KP979502.1	*Dysphaea ulu*, Malaysia	613bp
7.	KP979506.1	*Dysphaea ulu*, Malaysia	613bp
8.	KP979500.1	*Dysphaea gloriosa*, China	613bp
9.	KP979508.1	*Dysphaea vanida*, Thailand	613bp
10.	KP979484.1	*Dysphaea dimidiata*, Indonesia	613bp
11.	KP979496.1	*Dysphaea dimidiata*, Malaysia	613bp
12.	KP979499.1	*Dysphaea dimidiata*, Malaysia	613bp
13.	MN498288.1	*Dysphaea walli*, Punjab	527bp
14.	MW945399.1	*Ictinogomphus rapax*, Kerala	582bp

Phylogenetic tree based on 14 COI sequence data depicted 4 nodes with 100% bootstrap support. All the 4 specimens of *Dysphaea ethela* were monophyletic. But the specimen from Kerala and Punjab were separated into two sister clades. The common ancestor of *Dysphaea* species split into two to give rise to two main clades one comprising the *Dysphaea ethela* species and the other containing the remaining species. *Dysphaea walli* and *Dysphaea ulu* were

monophyletic to each other. *Dysphaea dimidiata* formed a monophyletic clade (bootstrap 99%). *Dysphaea dimidiata* and *Dysphaea vanida* grouped together and *Dysphaea basitincta* and *Dysphaea gloriosa* were found as sister clades and paraphyletic to the former.

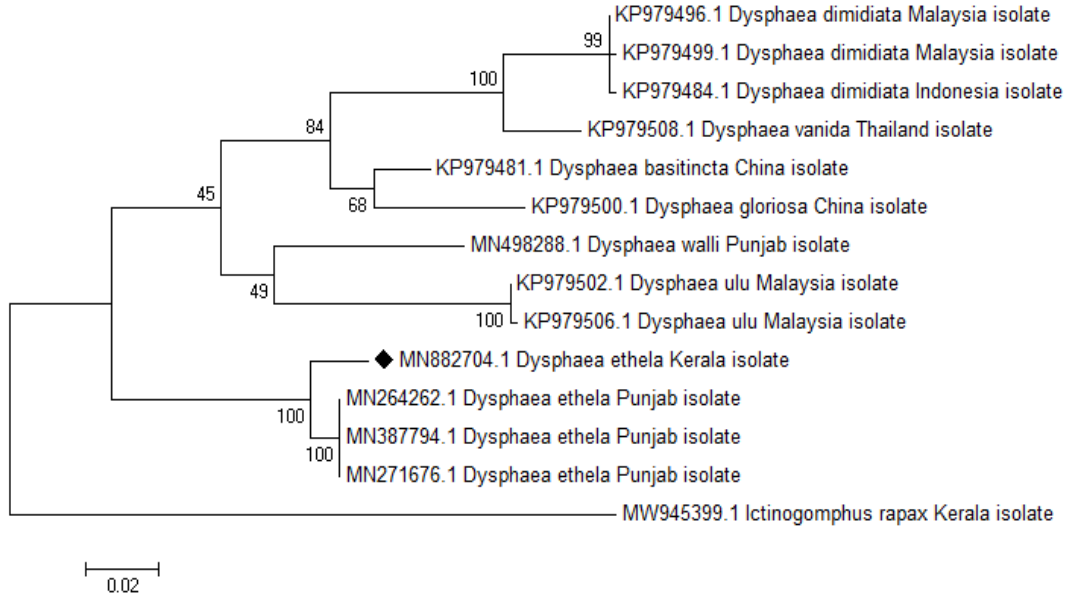

Figure 4.4.30: Inferred phylogenetic tree of the genus *Dysphaea*, rooted by outgroup.

Intraspecific and interspecific divergence

There was no intraspecific divergence between Punjab specimens of *Dysphaea ethela*. But exhibited a divergence value of 2.3% between Kerala and Punjab specimens. The other conspecifics of *Dysphaea ulu* and *Dysphaea dimidiata* showed divergence ranging from 0.2% to 0.4% (Table 4.4.57).

Nucleotide composition

The nucleotide composition of the 14 sequences were 31.66% (A), 30.97% (T/U), 20.18% (C) and 17.19% (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The base composition of *Dysphaea ethela* was (T=29.9%, C=20.5%, A=33.2%, G=16.3%). High AT bias was observed with an AT content of 63.1% and GC content of 36.8% (Table 4.4.58).

Table 4.4.57: Estimates of genetic divergence among COI gene sequences of genus *Dysphaea*

	Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	MN882704.1_*Dysphaea_ethela*_Kerala													
2	MN264262.1_*Dysphaea_ethela*_Punjab	0.023												
3	MN387794.1_*Dysphaea_ethela*_Punjab	0.023	0.000											
4	MN271676.1_*Dysphaea_ethela*_Punjab	0.023	0.000	0.000										
5	KP979481.1_*Dysphaea_basitincta*_China	0.121	0.113	0.113	0.113									
6	KP979502.1_*Dysphaea_ulu*_Malaysia	0.129	0.125	0.125	0.125	0.111								
7	KP979506.1_*Dysphaea_ulu*_Malaysia	0.131	0.127	0.127	0.127	0.113	0.002							
8	KP979500.1_*Dysphaea_gloriosa*_China	0.131	0.125	0.125	0.125	0.054	0.127	0.129						
9	KP979508.1_*Dysphaea_vanida* Thailand	0.136	0.136	0.136	0.136	0.084	0.132	0.134	0.094					
10	KP979484.1_*Dysphaea_dimidiata* Indonesia	0.140	0.132	0.132	0.132	0.090	0.131	0.129	0.098	0.050				
11	KP979496.1_*Dysphaea_dimidiata* Malaysia	0.142	0.134	0.134	0.134	0.092	0.132	0.131	0.100	0.048	0.002			
	KP979499.1_*Dysphaea_dimidiata*_Malaysia	0.144	0.136	0.136	0.136	0.094	0.134	0.132	0.102	0.050	0.004	0.002		
	MN498288.1_*Dysphaea_walli*_Punjab	0.132	0.127	0.127	0.127	0.104	0.104	0.106	0.119	0.136	0.125	0.127	0.129	
	MW945399.1_*Ictinogomphus_rapax*_Kerala	0.203	0.203	0.203	0.203	0.196	0.196	0.198	0.194	0.205	0.209	0.211	0.209	0.203

Table 4.4.58: Nucleotide base composition of COI gene sequence of genus *Dysphaea*

Name of species																
	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
MN882704.1 *Dysphaea ethela* Kerala	29.9	20.5	33.2	16.3	21	18.4	31.0	29.9	44	27.0	14.4	14.9	25	16.2	54.3	4.0
MN264262.1 *Dysphaea ethela* Punjab	30.3	19.8	33.2	16.7	21	17.8	31.0	30.5	44	27.0	14.9	14.4	27	14.5	53.8	5.2
MN387794.1 *Dysphaea ethela* Punjab	30.3	19.8	33.2	16.7	21	17.8	31.0	30.5	44	27.0	14.9	14.4	27	14.5	53.8	5.2
MN271676.1 *Dysphaea ethela* Punjab	30.3	19.8	33.2	16.7	21	17.8	31.0	30.5	44	27.0	14.9	14.4	27	14.5	53.8	5.2
KP979481.1 *Dysphaea basitincta* China	31.7	19.2	32.1	17.1	23	16.1	31.0	29.9	44	27.0	14.4	14.9	28	14.5	50.9	6.4
KP979502.1 *Dysphaea ulu* Malaysia	31.9	20.2	31.5	16.5	22	17.2	31.0	29.9	44	27.0	14.4	14.9	30	16.2	49.1	4.6
KP979506.1 *Dysphaea ulu* Malaysia	31.9	20.2	31.3	16.7	22	17.2	31.0	29.9	44	27.0	14.4	14.9	30	16.2	48.6	5.2
KP979500.1 *Dysphaea gloriosa* China	31.1	20.2	32.2	16.5	24	15.5	31.0	29.9	44	27.0	14.4	14.9	26	17.9	51.4	4.6
KP979508.1 *Dysphaea vanida* Thailand	31.7	20.0	29.9	18.4	24	14.9	31.0	29.9	44	27.0	14.4	14.9	27	17.9	44.5	10.4
KP979484.1 *Dysphaea dimidiata* Indonesia	30.9	20.7	29.9	18.4	24	14.9	31.0	29.9	44	27.0	14.4	14.9	25	20.2	44.5	10.4
KP979496.1 *Dysphaea dimidiata* Malaysia	30.9	20.7	29.8	18.6	24	14.9	31.0	29.9	44	27.0	14.4	14.9	25	20.2	43.9	11.0
KP979499.1 *Dysphaea dimidiata* Malaysia	30.7	20.9	29.8	18.6	24	15.5	31.0	29.9	44	27.0	14.4	14.9	25	20.2	43.9	11.0
MN498288.1 *Dysphaea walli* Punjab	30.5	20.3	32.1	17.1	20	19.0	31.0	29.9	44	27.0	14.4	14.9	28	15.0	50.9	6.4
MW945399.1 *Ictinogomphus rapax* Kerala	31.5	20.3	31.9	16.3	22	17.8	31.0	29.3	44	27.6	13.8	14.4	28	15.6	50.9	5.2
Avg.	31.0	20.2	31.7	17.2	22	16.8	31.0	30.0	44	27.1	14.4	14.8	27	16.7	49.6	6.8

7) Phylogenetic analysis of the genus *Copera*

Phylogenetic reconstruction of the genus *Copera* was conducted using the sequences of 10 specimens. In addition to the current sequence of *Copera vittata*, 8 sequence samples were retrieved from GenBank and the sequence of the dragonfly *Hydrobasileus croceus* was involved as out group (Table 4.4.59; Figure 4.4.31).

Table 4.4.59: Details of COI gene sequences involved in the phylogenetic analysis of genus *Copera*

Sl No.	Accession Number	Scientific Name	Product size
1	MZ895506.1	*Copera vittata*, Kerala	691bp
2	MN442124.1	*Copera vittata*, Punjab	618bp
3	MN447532.1	*Copera vittata*, Punjab	600bp
4	MN640593.1	*Copera vittata*, Punjab	578bp
5	KF369353.1	*Copera sikassoensis*, Africa	658bp
6	KF369352.1	*Copera nyansana*, Africa	658bp
7	KF369351.1	*Copera marginipes*, Malaysia	658bp
8	MN648196.1	*Copera marginipes*, Punjab	600bp
9	KF966553.1	*Copera annulata*, South Korea	609bp
10	MW965658.1	*Hydrobasileus croceus*, Kerala	671bp

The inferred phylogenetic tree suggested that all the 4 members of *Copera vittata* have clustered into a monophyletic clade with 99% bootstrap support. The Kerala specimen was formed a separate branch and was found as sister clade to the conspecifics from Punjab. *Copera marginipes* from Malaysia and Punjab were found to be monophyletic, well supported by 99% bootstrap. *Copera vittata* and *Copera marginipes* were polyphyletic. *Copera sikkassoensis* and *Copera nyansana* were monophyletic to each other. *Copera annulata* was paraphyletic to all the remaining species of *Copera* in the present study.

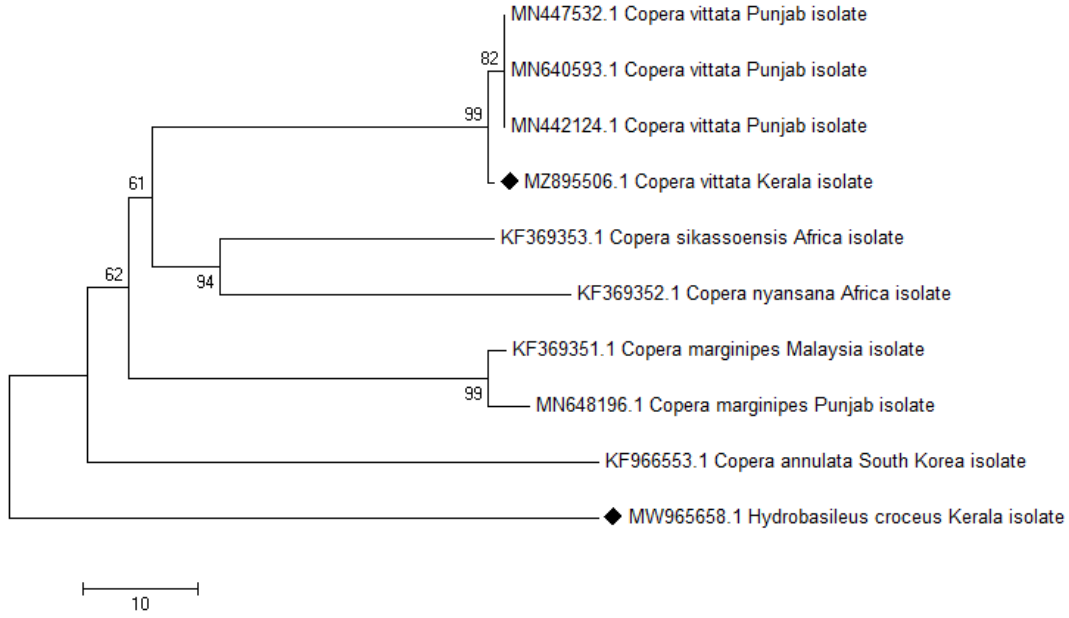

Figure 4.4.31: Inferred phylogenetic tree of the genus *Copera*, rooted by outgroup.

Intraspecific and interspecific divergence

The intraspecific divergence between Kerala and Punjab specimens of *Copera vittata* was observed as 0.4%. No divergence was found among Punjab specimens. The conspecifics of *Copera marginipes* from Punjab and Malaysia showed 0.9% divergence. Both divergences can be the result of geographical isolation. The interspecific divergence ranges from 10.2% to 19.2% (Table 4.4.60).

Nucleotide composition

The nucleotide composition of the 10 sequences were 31.15% (A), 35.34% (T/U), 16.91% (C) and 16.60% (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The base composition of *Copera vittata* was T=36.2%, C=16.2%, A=31.7%, G=15.8%. High AT bias was found with an AT content of 67.9% and GC content of 32.0% (Table 4.4.61).

Table 4.4.60: Estimates of genetic divergence among COI gene sequences of genus *Copera*

	Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1.	MZ895506.1_*Copera_vittata*_Kerala_isolate									
2.	MN442124.1_*Copera_vittata*_Punjab_isolate	0.004								
3.	MN447532.1_*Copera_vittata*_Punjab_isolate	0.004	0.000							
4.	MN640593.1_*Copera_vittata*_Punjab_isolate	0.004	0.000	0.000						
5.	KF369353.1_*Copera_sikassoensis*_Africa_isolate	0.106	0.109	0.109	0.109					
6.	KF369352.1_*Copera_nyansana*_Africa_isolate	0.128	0.130	0.130	0.130	0.102				
7.	KF369351.1_*Copera_marginipes*_Malaysia_isolate	0.121	0.123	0.123	0.123	0.130	0.125			
8.	MN648196.1_*Copera_marginipes*_Punjab_isolate	0.125	0.126	0.126	0.126	0.132	0.132	0.009		
9.	KF966553.1_*Copera_annulata*_South_Korea_isolate	0.149	0.147	0.147	0.147	0.153	0.160	0.158	0.162	
10.	MW965658.1_*Hydrobasileus_croceus*_Kerala_isolate	0.179	0.181	0.181	0.181	0.174	0.187	0.172	0.174	0.192

Table 4.4.61: Nucleotide base composition of COI gene sequence of genus *Copera*

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
MZ895506.1 *Copera vittata* Kerala	36.2	16.2	31.7	15.8	27	13.6	29.9	29.4	44	27.7	13.6	15.3	38	7.4	51.7	2.8
MN442124.1 *Copera vittata* Punjab	36.0	16.4	31.5	16.0	27	13.6	29.9	29.4	44	27.7	13.6	15.3	38	8.0	51.1	3.4
MN447532.1 *Copera vittata* Punjab	36.0	16.4	31.5	16.0	27	13.6	29.9	29.4	44	27.7	13.6	15.3	38	8.0	51.1	3.4
MN640593.1 *Copera vittata* Punjab	36.0	16.4	31.5	16.0	27	13.6	29.9	29.4	44	27.7	13.6	15.3	38	8.0	51.1	3.4
KF369353.1 *Copera sikassoensis* Africa	35.1	17.0	31.1	16.8	26	15.3	28.8	29.9	44	27.7	13.6	15.3	36	8.0	51.1	5.1
KF369352.1 *Copera nyansana* Africa	35.3	17.2	30.2	17.4	24	16.4	29.4	29.9	44	27.7	13.6	15.3	38	7.4	47.7	6.8
KF369351.1 *Copera marginipes* Malaysia	35.5	16.4	31.1	17.0	25	16.9	28.8	29.4	44	27.7	14.1	14.7	38	4.5	50.6	6.8
MN648196.1 *Copera marginipes* Punjab	35.3	16.8	30.9	17.0	25	16.9	28.8	29.4	44	27.7	14.1	14.7	38	5.7	50.0	6.8
KF966553.1 *Copera annulata* South Korea	32.5	18.7	30.6	18.3	21	18.6	29.4	30.5	44	27.7	14.1	14.7	32	9.7	48.3	9.7
MW965658.1 *Hydrobasileus croceus* Kerala	35.5	17.5	31.3	15.7	23	17.5	28.8	30.5	44	28.2	13.6	14.1	39	6.8	51.7	2.3
Avg.	35.3	16.9	31.2	16.6	25	15.6	29.4	29.7	44	27.7	13.7	15.0	37	7.3	50.5	5.1

8) Phylogenetic analysis of the genus *Prodasineura*

The phylogeny of the genus *Prodasineura* based on COI gene sequence was resolved based on the current sequence of *Prodasineura verticalis*, along with 11 sequences downloaded from GenBank and sequence of the dragonfly species *Onychothemis testacea* was involoved as out group. A total of 13 COI sequences were involved in the analysis (Table 4.4.62; Figure 4.4.32).

Table 4.4.62: Details of COI gene sequences involved in the phylogenetic analysis of genus *Prodasineura*

Sl No.	Accession Number	Scientific Name	Product size
1.	MZ081640.1	*Prodasineura verticalis*, Kerala	701bp
2.	MN304942.1	*Prodasineura verticalis*, Punjab	633bp
3.	MN389528.1	*Prodasineura verticalis*, Punjab	627bp
4.	MN401308.1	*Prodasineura verticalis*, Punjab	605bp
5.	KF369511.1	*Prodasineura dorsalis*, Malaysia	658bp
6.	KF369513.1	*Prodasineura vittata*, Cameroon	658bp
7.	KF369512.1	*Prodasineura sita*, Sri Lanka	658bp
8.	MG885045.1	*Prodasineura notostigma*, Singapore	313bp
9.	MG885302.1	*Prodasineura notostigma*, Singapore	313bp
10.	MG885287.1	*Prodasineura collaris,* Singapore	313bp
11.	MG885288.1	*Prodasineura humeralis*, Singapore	313bp
12.	MG885296.1	*Prodasineura interrupta*, Singapore	313bp
13.	MN803150.1	*Onychothemis testacea*, Kerala	632bp

The tree indicated that 3 distinct clades were present in the phylogeny of genus *Prodasineura*. *Prodasineura sita* which is an endemic to Sri Lanka (Kalkman et al. 2020) was formed a separated monophyletic clade. The remaining species were grouped into two clusters. All the 4 specimens of *Prodasineura verticalis* and *Prodasinura humeralis* were monophyletic to each other supported by a boot strap value of 98%. *Prodasineura interrupta* was paraphyletic to the cluster.

Prodasineura dorsalis and *Prodasineura collaris* formed sister clades. *Prodasineura vittata* was paraphyletic to *Prodasineura notostigma*.

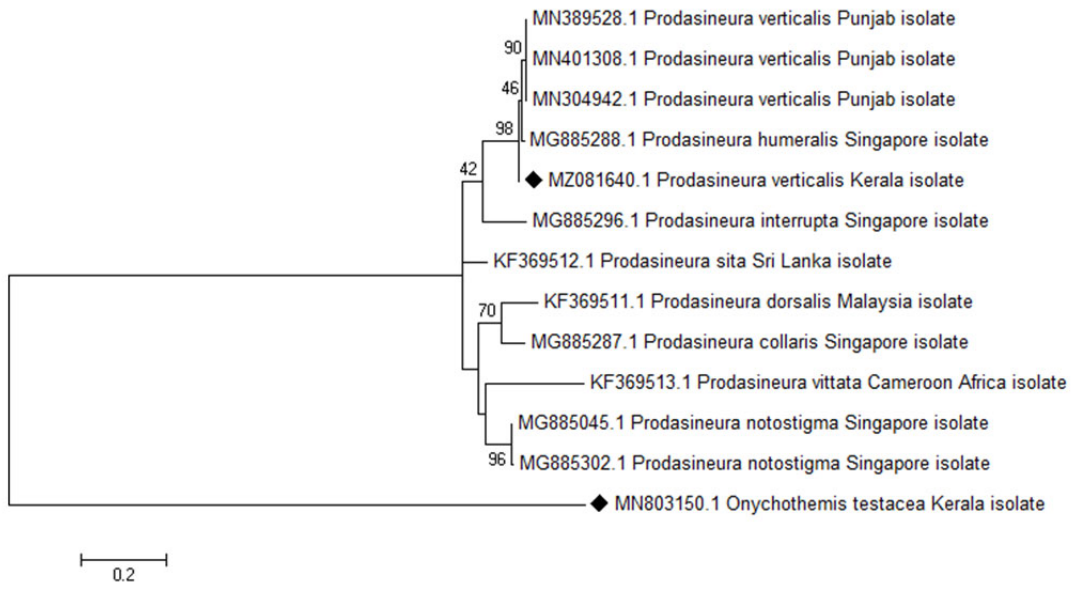

Figure 4.4.32: Inferred phylogenetic tree of the genus *Prodasineura*, rooted by outgroup.

Intraspecific and interspecific divergence

Conspecifics of *Prodasineura verticalis* from Kerala and Punjab showed 1.5% divergence. There was no divergence between Punjab specimens. *Prodasinura humeralis* from Singapore was closer to *Prodasineura verticalis* from Kerala and possessed only 1.2 % divergence. The geographical isolation has made significant changes in the gene sequence of specimens from the three locations. *Prodasineura notostigma* specimens from Singapore possessed 0.4% divergence each other. The interspecific divergence ranged from 11.2% to 21.6% (Table 4.4.63).

Nucleotide composition

The nucleotide composition of 13 sequences were 35.14 % (A), 31.01% (T/U), 19.10 % (C) and 14.76% (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The base composition of *Prodasineura verticalis* was T=32.8%, C=18.7%, A=32.8%, G=15.6%. High AT bias was found with an AT content of 65.6% and GC content of 34.3% (Table 4.4.64).

Table 4.4.63: Estimates of genetic divergence among COI gene sequences of genus *Prodasineura*

	Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	MZ081640.1_*Prodasineura_verticalis*_Kerala												
2	MN304942.1_*Prodasineura_verticalis*_Punjab	0.015											
3	MN389528.1_*Prodasineura_verticalis*_Punjab	0.015	0.000										
4	MN401308.1_*Prodasineura_verticalis*_Punjab	0.015	0.000	0.000									
5	KF369511.1_*Prodasineura_dorsalis*_Malaysia	0.139	0.147	0.147	0.147								
6	KF369513.1_*Prodasineura_vittata* Africa	0.208	0.212	0.212	0.212	0.216							
7	KF369512.1_*Prodasineura_sita*_Sri_Lanka	0.127	0.131	0.131	0.131	0.158	0.178						
8	MG885045.1_*Prodasineura_notostigma*_Singapore	0.143	0.135	0.135	0.135	0.147	0.193	0.116					
9	MG885287.1_*Prodasineura_collaris*_Singapore	0.154	0.154	0.154	0.154	0.112	0.205	0.135	0.131				
10	MG885288.1_*Prodasineura_humeralis*_Singapore	0.012	0.012	0.012	0.012	0.143	0.208	0.127	0.139	0.151			
11	MG885302.1_*Prodasineura_notostigma*_Singapore	0.147	0.139	0.139	0.139	0.143	0.189	0.120	0.004	0.135	0.143		
12	MG885296.1_*Prodasineura_interrupta*_Singapore	0.131	0.139	0.139	0.139	0.162	0.197	0.127	0.166	0.143	0.135	0.170	
13	MN803150.1_*Onychothemis_testacea*_Kerala	0.448	0.452	0.452	0.452	0.463	0.463	0.452	0.459	0.486	0.444	0.463	0.471

Table 4.4.64: Nucleotide base composition of COI gene sequence of genus *Prodasineura*

Name of species																
	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
MZ081640.1 *Prodasineura verticalis* Kerala	32.8	18.7	32.8	15.6	38	17.0	38.6	6.8	24	10.3	40.2	25.3	37	28.7	19.5	14.9
MN304942.1 *Prodasineura verticalis* Punjab	32.1	19.5	32.8	15.6	36	18.2	38.6	6.8	23	11.5	40.2	25.3	37	28.7	19.5	14.9
MN389528.1 *Prodasineura verticalis* Punjab	32.1	19.5	32.8	15.6	36	18.2	38.6	6.8	23	11.5	40.2	25.3	37	28.7	19.5	14.9
MN401308.1 *Prodasineura verticalis* Punjab	32.1	19.5	32.8	15.6	36	18.2	38.6	6.8	23	11.5	40.2	25.3	37	28.7	19.5	14.9
KF369511.1 *Prodasineura dorsalis* Malaysia	28.2	23.9	32.0	15.8	29	24.7	38.8	7.1	20	17.2	37.9	25.3	36	29.9	19.5	14.9
KF369513.1 *Prodasineura vittata* Cameroon Africa	34.7	20.6	28.2	16.4	40	19.3	35.2	5.7	25	14.9	29.9	29.9	39	27.6	19.5	13.8
KF369512.1 *Prodasineura sita* Sri Lanka	34.0	18.3	32.4	15.3	39	18.2	38.6	4.5	23	11.5	39.1	26.4	40	25.3	19.5	14.9
MG885045.1 *Prodasineura notostigma* Singapore	32.4	20.6	32.8	14.1	34	21.6	39.8	4.5	25	12.6	39.1	23.0	38	27.6	19.5	14.9
MG885287.1 *Prodasineura collaris* Singapore	30.5	21.0	32.4	16.0	32	20.5	39.8	8.0	20	17.2	37.9	25.3	40	25.3	19.5	14.9
MG885288.1 *Prodasineura humeralis* Singapore	32.1	19.5	33.2	15.3	35	19.3	39.8	5.7	24	10.3	40.2	25.3	37	28.7	19.5	14.9
MG885302.1 *Prodasineura notostigma* Singapore	32.4	20.6	32.4	14.5	34	21.6	39.8	4.5	25	12.6	37.9	24.1	38	27.6	19.5	14.9
MG885296.1 *Prodasineura interrupta* Singapore	32.4	19.8	32.8	14.9	35	20.5	37.5	6.8	22	13.8	41.4	23.0	40	25.3	19.5	14.9
MN803150.1 *Onychothemis testacea* Kerala	14.1	10.0	68.8	7.1	21	10.0	66.7	2.2	10	5.6	73.3	11.1	11	14.6	66.3	7.9
Avg.	30.7	19.3	35.2	14.8	34	19.0	40.9	5.9	22	12.3	41.4	24.2	36	26.7	23.2	14.3

9) Phylogenetic analysis of the genus *Aciagrion*

Phylogenetic analysis of the genus *Aciagrion* was carried out based on 11 partial COI gene sequences. The analysis included the sequence of *Aciagrion approximans krishna*, 9 sequnce samples retrieved from GenBank and the dragonfly species *Orthetrum glaucum* was included as out group (Table 4.4.65; Figure 4.4.33).

Table 4.4.65: Details of COI gene sequences involved in the phylogenetic analysis of genus *Aciagrion*

Sl No.	Accession Number	Scientific Name	Product size
1.	MW246065.1	*Aciagrion approximans krishna*; Kerala	670bp
2.	MW812349.1	*Aciagrion migratum*; Punjab isolate	525bp
3.	LC490098.1	*Aciagrion migratum*; Japan	451bp
4.	LC490102.1	*Aciagrion migratum*; Japan	451bp
5.	MT229961.1	*Aciagrion occidentale*; Punjab	545bp
6.	MH881303.1	*Aciagrion pallidum* ;Thailand	591bp
7.	KU565886.1	*Aciagrion bapepe*, Africa	658bp
8.	KM096996.1	*Aciagrion occidentale*, Kerala	522bp
9.	KF369276.1	*Aciagrion brosseti*, Africa	641bp
10.	KF369275.1	*Aciagrion borneense*, Malaysia	658bp
11.	MZ087263.1	*Orthetrum glaucum* Kerala	696bp

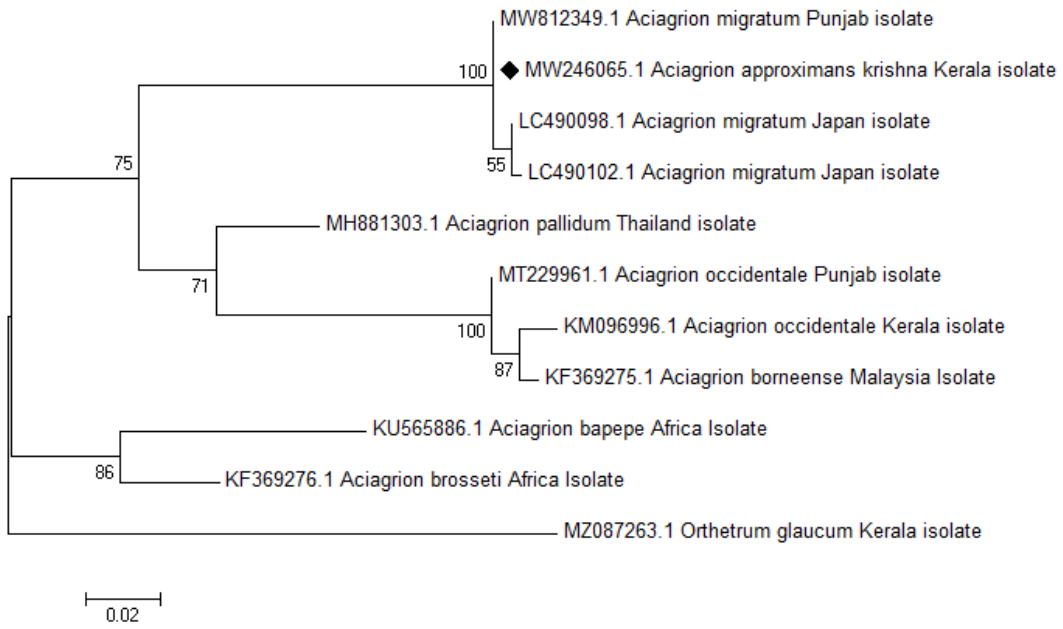

Figure 4.4.33: Inferred phylogenetic tree of the genus *Aciagrion*, rooted by outgroup.

The inferred phylogenetic tree branches were supported with bootstrap values ranging from 71- 100 except one node in which the value was 55. According to the tree, the common ancestor of *Aciagrion* was split into two main clades. In one clade *Aciagrion bapepe* and *Aciagrion brosseti* were found in sister clade relationships. The other clade was formed by the grouping of remaining species. *Aciagrion approximans krishna* was closely related with *Aciagrion migratum* from India and was found as sister clade with 100% bootstrap support. But *Aciagrion migratum* is not found in India (Kalkman et al. 2020). The geo coordinates (lat_lon="8.6080 N 77.0046 E") of the specimen (Accession number MW812349.1) indicated that this specimen was collected from Kerala. However this species is absent in Kerala odonate list (Nair et al., 2021; Gopalan et al., 2022). So *Aciagrion approximans krishna* might be wrongly identified as *Aciagrion migratum* and submitted in GenBank by the authors. Here we can consider it as conspecific with *Aciagrion approximans krishna* and this may be the reason for the close similarity. However, *Aciagrion approximans krishna* was monophyletic with *Aciagrion migratum* from Japan. *Aciagrion occidentale* from Kerala formed sister clade with *Aciagrion borneense* and Punjab specimen of the former one is paraphyletic. The phylogenetic tree is in congruence with calculated genetic divergence values.

Intraspecific and interspecific divergence

The calculated intraspecific divergence values showed that there is no divergence between *Aciagrion approximans krishna* and *Aciagrion migratum* from Punjab. There is only negligible divergence from *Aciagrion migratum* from Japan (0.5% to 0.7%). The intraspecific divergence between the conspecifics of *Aciagrion occidentale* from Kerala and Punjab is 1.7%. *Aciagrion borneese* showed 1.5% divergence from *Aciagrion occidentale* specimen from Kerala and only 1.2% from Punjab specimen. The maximum value of interspecific divergence was 21.2% (Table 4.4.66).

9.5 Nucleotide composition

The nucleotide composition of the 11 sequences were 30.97% (A), 34.12% (T/U), 18.45% (C) and 16.46% (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The base composition of *Aciagrion approximans krishna* is (T=33.9%, C=19.0%, A=30.7%, G=16.5%). High AT bias was observed with an AT content of 64.6% and GC content of 35.5% (Table 4.4.67).

Table 4.4.66: Estimates of genetic divergence among COI gene sequences of genus *Aciagrion*

	Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
	MW246065.1_*Aciagrion_approximans_krishna* Kerala											
	KM096996.1_*Aciagrion_occidentale*_Kerala	0.167										
	MW812349.1_*Aciagrion_migratum*_Punjab	0.000	0.167									
	LC490098.1_*Aciagrion_migratum*_Japan	0.005	0.167	0.005								
	LC490102.1_*Aciagrion_migratum*_Japan	0.007	0.165	0.007	0.002							
	MT229961.1_*Aciagrion_occidentale*_Punjab	0.157	0.017	0.157	0.157	0.155						
	KT879901.1_*Aciagrion_olympicum*_Karnataka	0.204	0.197	0.204	0.209	0.212	0.190					
	MH881303.1_*Aciagrion_pallidum*_Thailand	0.122	0.107	0.122	0.122	0.120	0.095	0.172				
	KU565886.1_*Aciagrion_bapepe*_Africa	0.162	0.167	0.162	0.162	0.165	0.160	0.202	0.147			
	KF369276.1_*Aciagrion_brosseti*_Africa	0.147	0.147	0.147	0.152	0.150	0.140	0.200	0.112	0.087		
	KF369275.1_*Aciagrion_borneense*_Malaysia	0.165	0.015	0.165	0.165	0.162	0.012	0.197	0.105	0.165	0.145	
	MZ087263.1_*Orthetrum_glaucum*_Kerala	0.190	0.207	0.190	0.195	0.197	0.195	0.190	0.172	0.182	0.175	0.200

Table 4.4.67: Nucleotide base composition of COI gene sequence of genus *Aciagrion*

Species																
	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
MW246065.1 *Aciagrion approximans krishna* Kerala isolate	33.9	19.0	30.7	16.5	24	17.2	29.9	29.1	43	30.6	12.7	14.2	35	9.0	49.6	6.0
KM096996.1 *Aciagrion occidentale* Kerala isolate	34.2	18.5	29.7	17.7	27	14.2	29.1	29.9	42	30.6	11.9	15.7	34	10.5	48.1	7.5
MW812349.1 *Aciagrion migratum* Punjab isolate	33.9	19.0	30.7	16.5	24	17.2	29.9	29.1	43	30.6	12.7	14.2	35	9.0	49.6	6.0
LC490098.1 *Aciagrion migratum* Japan isolate	34.2	19.0	30.2	16.7	24	17.2	29.9	29.1	43	30.6	12.7	14.2	36	9.0	48.1	6.8
LC490102.1 *Aciagrion migratum* Japan isolate	34.2	19.0	29.9	17.0	24	17.2	29.9	29.1	43	30.6	12.7	14.2	36	9.0	47.4	7.5
MT229961.1 *Aciagrion occidentale* Punjab isolate	33.9	19.0	30.4	16.7	27	14.2	29.1	29.9	43	30.6	11.9	14.9	32	12.0	50.4	5.3
KT879901.1 *Aciagrion olympicum* Karnataka Isolate	29.4	21.2	32.2	17.2	20	20.9	29.9	29.1	42	30.6	12.7	14.9	26	12.0	54.1	7.5
MH881303.1 *Aciagrion pallidum* Thailand isolate	34.7	17.2	31.7	16.5	28	13.4	29.1	29.9	43	30.6	12.7	14.2	34	7.5	53.4	5.3
KU565886.1 *Aciagrion bapepe* Africa Isolate	33.7	18.2	32.7	15.5	25	16.4	29.9	29.1	43	30.6	12.7	14.2	34	7.5	55.6	3.0
KF369276.1 *Aciagrion brosseti* Africa Isolate	34.4	17.5	32.2	16.0	25	16.4	29.1	29.9	43	30.6	12.7	14.2	36	5.3	54.9	3.8
KF369275.1 *Aciagrion borneense* Malaysia Isolate	33.7	19.0	30.4	17.0	26	14.9	29.9	29.1	43	30.6	11.9	14.9	32	11.3	49.6	6.8
MZ087263.1 *Orthetrum glaucum* Kerala isolate	34.7	18.0	32.2	15.2	23	17.9	28.4	30.6	43	30.6	12.7	14.2	38	5.3	55.6	.8
Avg.	33.7	18.7	31.1	16.5	25	16.4	29.5	29.5	42	30.6	12.5	14.5	34	9.0	51.4	5.5

10) Phylogenetic analysis of the genus *Agriocnemis*

Phylogenetic reconstruction of genus *Agriocnemis* based on COI partial gene sequence was done by using the sequences of *Agriocnemis splendidissima* and *Agriocnemis pieris* and sequences of 9 species downloaded from GenBank. Sequence of the dragonfly species *Orthetrum glaucum* was included as out group. A total of 12 sequences were involved in the phylogenetic reconstruction (Table 4.4.68; Figure 4.4.34).

Table 4.4.68: Details of COI gene sequences involved in the phylogenetic analysis of genus *Agriocnemis*

Sl No.	Accession Number	Scientific Name	Product size
1.	MN850440.1	*Agriocnemis pieris*, Kerala	627bp
2.	MN850441.1	*Agriocnemis splendidissima*, Kerala	647bp
3.	MW819848.1	*Agriocnemis pieris*, Punjab	533bp
4.	KT957464.1	*Agriocnemis minima*, Thailand	657bp
5.	KT957463.1	*Agriocnemis minima*, Thailand	657bp
6.	MW807205.1	*Agriocnemis splendidissima*, Punjab	639bp
7.	MK506260.1	*Agriocnemis femina*, Thailand	658bp
8.	KU565901.1	*Agriocnemis canuango*, Africa	658bp
9.	KU133367.1	*Agriocnemis keralensis*, Kerala	628bp
10.	KF369284.1	*Agriocnemis forcipata*, Africa	658bp
11.	MK506261.1	*Argiocnemis rubescens*, Thailand	658bp
12.	MZ087263.1	*Orthetrum glaucum*, Kerala	696bp

The conspecifics of *Agriocnemis pieris, Agriocnemis minima* and *Agriocnemis splendidissima* formed separate monophyletic clades with 100% boot strap support. *Agriocnemis pieris* and *Agriocnemis minima* were found as sister clades supported by a bootstrap value of 83%. *Agricnemis rubens* showed close similarity with *Agriocnemis splendidissima* (bootstrap 92%). The remaining 4 species were clustered to form another monophyletic clade. The species *Agriocnemis*

keralensis which is endemic to the Western Ghats was closely similar to *Agriocnemis forcipata* from Africa.

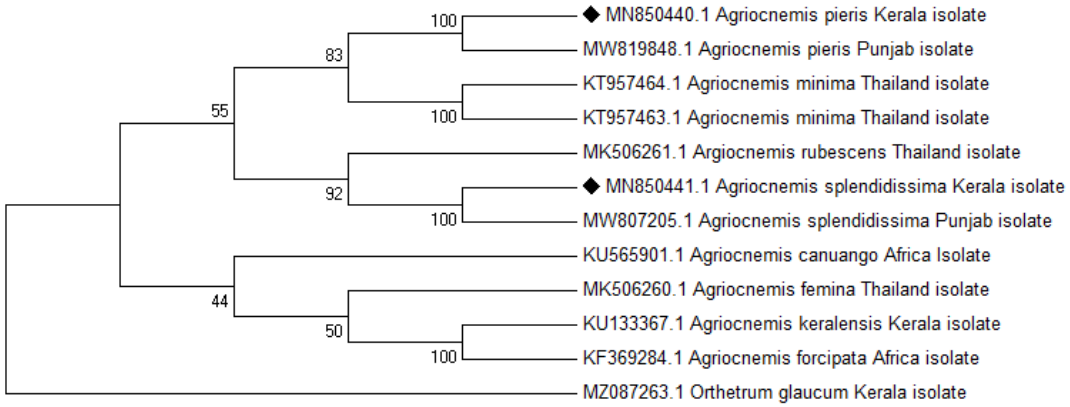

Figure 4.4.34: Inferred phylogenetic tree of the genus *Agriocnemis*, rooted by outgroup.

Intraspecific and interspecific divergence

The calculated genetic divergence values suggested that there is 1.1 % divergence between the conspecifics of *Agriocnemis pieris* from Kerala and Punjab. 0.4% divergence is observed between the conspecifics of *Agriocnemis splendidissima* from Kerala and Punjab. The genetic divergence is zero between *Agriocnemis keralensis* and *Agriocnemis forcipata*. This close similarity is well supported by the phylogenetic tree. The intraspecific divergence of *Agriocnemis minima* is 1.5%. The highest value of genetic divergence (19.8%) was observed between *Agriocnemis splendidissima* and *Agriocnemis femina* (Table 4.4.69).

Nucleotide composition

The nucleotide composition of the 12 sequences were 31.34% (A), 33.13% (T/U), 18.90% (C) and 16.63% (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The base composition of *Agriocnemis pieris* was T=35.8%, C=16.4%, A=31.1%, G=16.6% with high AT content (66.9%) over GC content (33%) and that of *Agriocnemis splendidissima* was T=33.0%, C=20.5%, A=29.2%, G=17.3% also possessed high AT bias (AT content 62.2%, GC content 37.8%). The values are presented in Table 4.4.70.

Table 4.4.69: Estimates of genetic divergence among COI gene sequences of genus *Agriocnemis*

	Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
	MN850440.1_*Agriocnemis_pieris*_Kerala											
	MN850441.1_*Agriocnemis_splendidissima* Kerala	0.185										
	MW819848.1_*Agriocnemis_pieris*_Punjab	0.011	0.179									
	KT957464.1_*Agriocnemis_minima*_Thailand	0.115	0.168	0.105								
	KT957463.1_*Agriocnemis_minima*_Thailand	0.115	0.174	0.105	0.015							
	MW807205.1_*Agriocnemis_splendidissima* Punjab	0.181	0.004	0.174	0.166	0.172						
	MK506260.1_*Agriocnemis_femina*_Thailand	0.144	0.198	0.139	0.157	0.159	0.196					
	KU565901.1_*Agriocnemis_canuango*_Africa	0.163	0.179	0.155	0.150	0.153	0.179	0.124				
	KU133367.1_*Agriocnemis_keralensis*_Kerala	0.170	0.194	0.166	0.179	0.176	0.190	0.135	0.161			
	KF369284.1_*Agriocnemis_forcipata*_Africa	0.170	0.194	0.166	0.179	0.176	0.190	0.135	0.161	0.000		
	MK506261.1_*Argiocnemis_rubescens* Thailand	0.185	0.172	0.179	0.166	0.168	0.172	0.187	0.183	0.190	0.190	
	MZ087263.1_*Orthetrum_glaucum*_Kerala	0.240	0.283	0.240	0.240	0.240	0.283	0.255	0.240	0.266	0.266	0.270

Table 4.4.70: Nucleotide base composition of COI gene sequence of genus *Agriocnemis*

Species																
	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
MN850440.1 *Agriocnemis pieris* Kerala	35.8	16.4	31.1	16.6	29	13.5	36.5	21.2	40	22.3	15.9	21.7	38	13.5	41.0	7.1
MN850441.1 *Agriocnemis splendidissima* Kerala	33.0	20.5	29.2	17.3	25	16.7	35.9	22.4	39	24.8	15.9	20.4	35	19.9	35.9	9.0
MW819848.1 *Agriocnemis pieris* Punjab	35.8	17.1	30.9	16.2	29	13.5	35.9	21.2	39	23.6	15.9	21.0	38	14.1	41.0	6.4
KT957464.1 *Agriocnemis minima* Thailand	34.5	18.8	31.3	15.4	28	17.3	34.0	21.2	39	23.6	15.9	21.0	37	15.4	44.2	3.8
KT957463.1 *Agriocnemis minima* Thailand	34.8	18.8	30.5	16.0	28	17.3	34.0	21.2	39	23.6	15.9	21.0	37	15.4	41.7	5.8
MW807205.1 *Agriocnemis splendidissima* Punjab	33.0	20.5	29.2	17.3	25	16.7	35.9	22.4	39	24.8	15.9	20.4	35	19.9	35.9	9.0
MK506260.1 *Agriocnemis femina* Thailand	36.2	16.4	29.9	17.5	28	13.5	37.2	21.8	39	23.6	15.9	21.0	42	12.2	36.5	9.6
KU565901.1 *Agriocnemis canuango* Africa	33.7	18.1	32.2	16.0	26	14.7	37.2	21.8	39	23.6	16.6	20.4	35	16.0	42.9	5.8
KU133367.1 *Agriocnemis keralensis* Kerala	32.4	19.6	32.0	16.0	26	14.1	37.8	21.8	38	26.1	16.6	19.7	33	18.6	41.7	6.4
KF369284.1 *Agriocnemis forcipata* Africa	32.4	19.6	32.0	16.0	26	14.1	37.8	21.8	38	26.1	16.6	19.7	33	18.6	41.7	6.4
MK506261.1 *Agriocnemis rubescens* Thailand	30.5	22.6	30.1	16.8	24	17.3	36.5	21.8	38	26.1	15.3	21.0	29	24.4	38.5	7.7
MZ087263.1 *Orthetrum glaucum* Kerala	29.4	16.6	39.2	14.7	22	14.1	43.6	20.5	28	24.8	27.4	19.7	38	10.9	46.8	3.8
Avg.	33.5	18.7	31.5	16.3	26	15.2	36.9	21.6	38	24.4	17.0	20.6	36	16.6	40.7	6.7

11) Phylogenetic analysis of the genus *Archibasis*

The phylogenetic reconstruction of the genus *Archibasis* was conducted based on 9 sequences, including the sequence of *Archibasis oscillans*, sequences of the corresponding genus retrieved from GenBank and sequence of the dragonfly *Orthetrum luzonicum* as out group (Table 4.4.71; Figure 4.4.35).

Table 4.4.71: Details of COI gene sequences involved in the phylogenetic analysis of genus *Archibasis*

Sl No.	Accession Number	Scientific Name	Product size
1.	MW309421.1	*Archibasis oscillans*, Kerala	617bp
2.	KF369305.1	*Archibasis melanocyana*, Malaysia	658bp
3.	MG885231.1	*Archibasis viola*, Singapore	313bp
4.	MG885181.1	*Archibasis viola*, Singapore	313bp
5.	MG885044.1	*Archibasis viola*, Singapore	313bp
6.	MG884649.1	*Archibasis viola*, Singapore	313bp
7.	MG884648.1	*Archibasis viola*,Singapore	313bp
8.	MG884647.1	*Archibasis melanocyana*, Singapore	313bp
9.	MZ092847.1	*Orthetrum luzonicum*, Kerala	692bp

The current submission of *Archibasis oscillans* sequence is the first in GenBank records of this species so sequences for intraspecific analysis were not available. Only three species could be incorporated in the phylogenetic analysis because of the scarcity of records of the corresponding genus. The common ancestor of *Archibasis viola* and *Archibasis melanocyana* was diverged from the ancestor of *Archibasis oscillans* at an earlier stage. *Archibasis oscillans* formed a distinct monophyletic clade, well differentiated from other two species and paraphyletic to them. The other two clustered into separate monophyletic clades

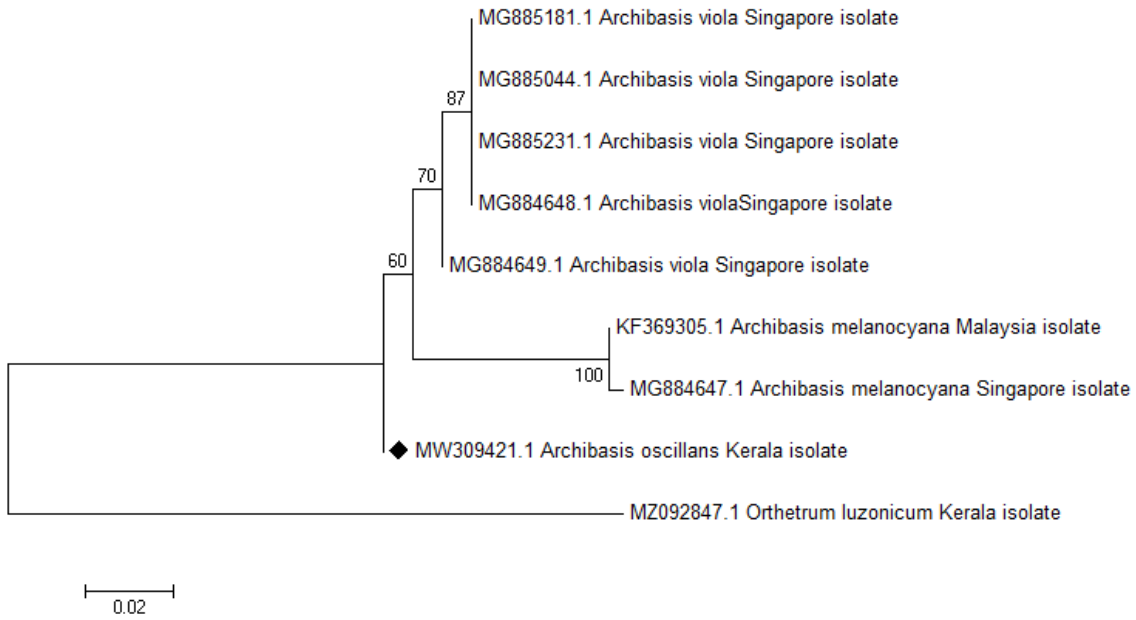

Figure 4.4.35: Inferred phylogenetic tree of the genus *Aciagrion*, rooted by outgroup.

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Intraspecific and interspecific divergence

The intraspecific divergence among *Archibasis viola* specimens ranged from 0% to 0.7%. The divergence between conspecifics of *Archibasis melanocyana* was 0.3%. The interspecific divergence values ranged from 1.3% to 5.3% (Table 4.4.72).

Nucleotide composition

The nucleotide composition of the 9 sequences were 30.26% (A), 32.74% (T/U), 19.63% (C) and 17.37% (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated.The base composition of *Archibasis oscillans* was T=32.3%, C=20.0%, A=30.3%, G=17.3% with an AT content of 62.6% over GC content of 37.3% (Table 4.4.73).

Table 4.4.72: Estimates of genetic divergence among COI gene sequences of genus *Archibasis*

	Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1.	MW309421.1_*Archibasis_oscillans*_Kerala								
2.	KF369305.1_*Archibasis_melanocyana*_Malaysia	0.050							
3.	MG885231.1_*Archibasis_viola*_Singapore	0.020	0.050						
4.	MG885181.1_*Archibasis_viola*_Singapore	0.020	0.050	0.000					
5.	MG885044.1_*Archibasis_viola*_Singapore	0.020	0.050	0.000	0.000				
6.	MG884649.1_*Archibasis_viola*_Singapore	0.013	0.050	0.007	0.007	0.007			
7.	MG884648.1_*Archibasis_viola* Singapore	0.020	0.050	0.000	0.000	0.000	0.007		
8.	MG884647.1_*Archibasis_melanocyana*_Singapore	0.053	0.003	0.053	0.053	0.053	0.053	0.053	
9.	MZ092847.1_*Orthetrum_luzonicum*_Kerala	0.190	0.213	0.200	0.200	0.200	0.200	0.200	0.213

Table 4.4.73: Nucleotide base composition of COI gene sequence of genus *Archibasis*

Species																
	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
MW309421.1 *Archibasis oscillans* Kerala	32.3	20.0	30.3	17.3	32	14.0	46.0	8.0	23	15.0	30.0	32.0	42	31.0	15.0	12.0
KF369305.1 *Archibasis melanocyana* Malaysia	33.0	20.0	28.7	18.3	32	16.0	41.0	11.0	25	13.0	30.0	32.0	42	31.0	15.0	12.0
MG885231.1 *Archibasis viola* Singapore	32.3	19.7	30.7	17.3	31	14.0	47.0	8.0	24	14.0	30.0	32.0	42	31.0	15.0	12.0
MG885181.1 *Archibasis viola* Singapore	32.3	19.7	30.7	17.3	31	14.0	47.0	8.0	24	14.0	30.0	32.0	42	31.0	15.0	12.0
MG885044.1 *Archibasis viola* Singapore	32.3	19.7	30.7	17.3	31	14.0	47.0	8.0	24	14.0	30.0	32.0	42	31.0	15.0	12.0
MG884649.1 *Archibasis viola* Singapore	32.3	19.7	30.7	17.3	31	14.0	47.0	8.0	24	14.0	30.0	32.0	42	31.0	15.0	12.0
MG884648.1 *Archibasis viola* Singapore	32.3	19.7	30.7	17.3	31	14.0	47.0	8.0	24	14.0	30.0	32.0	42	31.0	15.0	12.0
MG884647.1 *Archibasis melanocyana* Singapore	33.0	20.0	28.3	18.7	32	16.0	40.0	12.0	25	13.0	30.0	32.0	42	31.0	15.0	12.0
MZ092847.1 *Orthetrum luzonicum* Kerala	34.7	18.3	31.7	15.3	39	8.0	52.0	1.0	22	16.0	28.0	34.0	43	31.0	15.0	11.0
Avg.	32.7	19.6	30.3	17.4	32	13.8	46.0	8.0	24	14.1	29.8	32.2	42	31.0	15.0	11.9

12) Phylogenetic analysis of the genus *Ceriagrion*

Phylogeny of the genus *Ceriagrion* based on partial coding COI gene sequence was resolved by using the sequences of *Ceriagrion cerinorubellum* and *Ceriagrion rubiae* and sequences of 11 species including conspecifics and non-conspecifics were downloaded from GenBank. Sequence of the dragonfly *Orthetrum glaucum* was included as out group. A total of 14 sequences were involved in the phylogenetic reconstruction (Table 4.4.74; Figure 4.4.36).

Table 4.4.74: Details of COI gene sequences involved in the phylogenetic analysis of genus *Ceriagrion*

Sl No.	Accession Number	Scientific Name	Product size
1.	MZ882339.1	*Ceriagrion cerinorubellum*, Kerala	690bp
2.	OK148120.1	*Ceriagrion rubiae*, Kerala	346bp
3.	KU220868.1	*Ceriagrion cerinorubellum*, Malaysia	641bp
4.	KU220867.1	*Ceriagrion cerinorubellum*, India	641bp
5.	MF784361.1	*Ceriagrion cerinorubellum*, Bangladesh	640bp
6.	KU566000.1	*Ceriagrion suave*, Africa	658bp
7.	KU565956.1	*Ceriagrion glabrum*, Tanzania	658bp
8.	KU565935.1	*Ceriagrion bakeri*, Liberia	658bp
9.	KU220869.1	*Ceriagrion olivaceum*, Thailand	641bp
10.	MN867589.1	*Ceriagrion coromandelianum*, Punjab	654bp
11.	KU220871.1	*Ceriagrion coromandelianum*, India	641bp
12.	AB860041.1	*Ceriagrion chaoi*, Malaysia	451bp
13.	KX263700.1	*Ceriagrion fallax*, China	550bp
14.	MZ087263.1	*Orthetrum glaucum*, Kerala	696bp

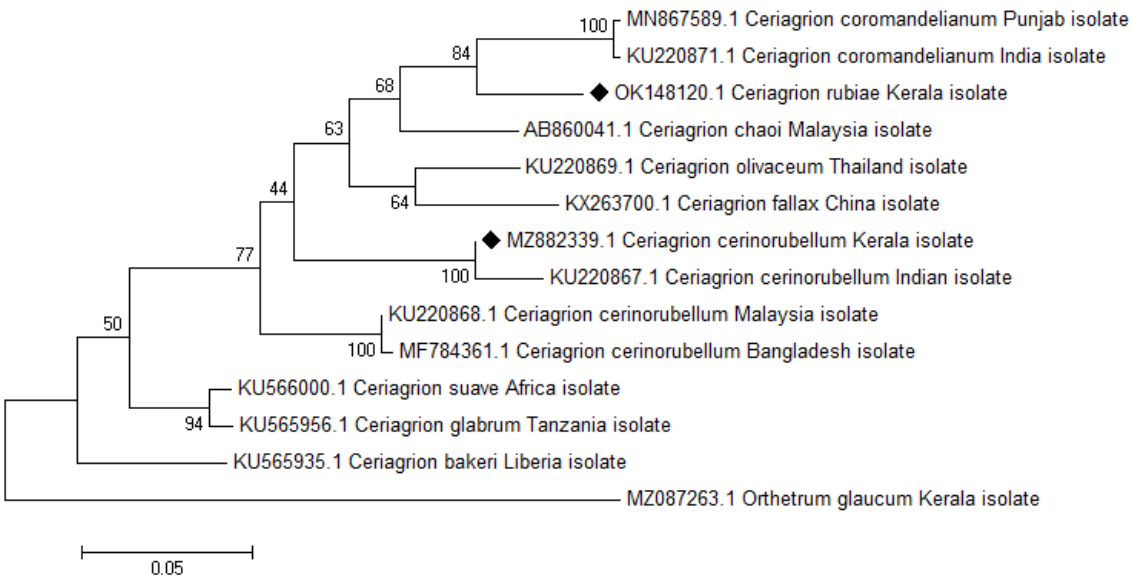

Figure 4.4.36: Inferred phylogenetic tree of the genus *Ceriagrion*, rooted by outgroup.

The current submission of *Ceriagrion rubiae* is the first record of GenBank of this species so intraspecific analysis was not carried out because of the lack of conspecific sequences. According to the phylogenetic tree, *Ceriagrion rubiae* was in sister clade relationship with *Ceriagrion coromandelianum*. *Ceriagrion cerinorubellum* specimens from Kerala and another location from India formed sister clades. However, the Indian samples of *Ceriagrion cerinorubellum* were distantly placed from Malaysia and Bangladesh samples. They were polyphyletic. The ancestor of *Ceriagrion bakeri* and ancestor of *Ceriagrion suave* and *Ceriagrion glabrum* were diverged earlier from the ancestor of other *Ceriagrion* species.

Intraspecific and interspecific divergence

Intraspecific divergence between *Ceriagrion cerinorubellum* from Kerala and another Indian specimen was 2%. The divergence values between Indian and Malaysian specimens ranged from 8.8% to 10.7%. The divergence between Indian and Bagladesh specimens ranged from 8.5% to 10.4%. The intraspecific divergence among the specimens of *Ceriagrion coromandelianum* was 0.3%. The highest interspecific divergence value was 14% between *Ceriagrion coromandelianum* and *Ceriagrion cerinorubellum* specimens from India (Table 4.4.75).

Nucleotide composition

The nucleotide composition of the 14 sequences were 32.13% (A), 33.09% (T/U), 17.08% (C) and 17.71% (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The base composition of *Ceriagrion cerinorubellum* was T=33.5%, C=16.8%, A=30.3%, G=19.4% with a high AT content of 63.8% over GC content of 35.5% (Table 4.4.76). The base composition of *Ceriagrion rubiae* was T=32.4%, C=16.8%, A=32.7%, G=18.2% and a high AT bias was observed (AT content= 65.1%, GC content= 35%).

Table 4.4.75: Estimates of genetic divergence among COI gene sequences of genus *Ceriagrion*

	Name of Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	MZ882339.1_*Ceriagrion_cerinorubellum*_Kerala													
2	OK148120.1_*Ceriagrion_rubiae*_Kerala	0.117												
3	KU220868.1_*Ceriagrion_cerinorubellum*_Malaysia	0.088	0.111											
4	KU220867.1_*Ceriagrion_cerinorubellum*_Indian	0.020	0.137	0.107										
5	MF784361.1_*Ceriagrion_cerinorubellum*_Bangladesh	0.085	0.114	0.003	0.104									
6	KU566000.1_*Ceriagrion_suave*_Africa	0.111	0.104	0.094	0.124	0.098								
7	KU565956.1_*Ceriagrion_glabrum*_Tanzania	0.111	0.111	0.094	0.124	0.098	0.013							
8	KU565935.1_*Ceriagrion_bakeri*_Liberia	0.104	0.124	0.111	0.117	0.114	0.072	0.078						
9	KU220869.1_*Ceriagrion_olivaceum*_Thailand	0.101	0.078	0.091	0.114	0.094	0.098	0.091	0.124					
10	MN867589.1_*Ceriagrion_coromandelianum*_Punjab	0.121	0.065	0.121	0.140	0.124	0.107	0.107	0.137	0.094				
11	KU220871.1_*Ceriagrion_coromandelianum*_India	0.121	0.065	0.121	0.140	0.124	0.107	0.107	0.137	0.094	0.003			
12	AB860041.1_*Ceriagrion_chaoi*_Malaysia	0.091	0.081	0.098	0.111	0.101	0.114	0.114	0.130	0.091	0.085	0.085		
13	KX263700.1_*Ceriagrion_fallax*_China	0.101	0.091	0.098	0.121	0.101	0.117	0.117	0.117	0.068	0.107	0.107	0.098	
14	MZ087263.1_*Orthetrum_glaucum*_Kerala	0.248	0.238	0.225	0.254	0.228	0.208	0.208	0.208	0.215	0.238	0.238	0.235	0.225

Table 4.4.76: Nucleotide base composition of COI gene sequence of genus *Ceriagrion*

Species																
	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
MZ882339.1 *Ceriagrion cerinorubellum* Kerala	33.5	16.8	30.3	19.4	27	10.4	40.0	22.6	33	21.7	19.1	26.1	41	18.1	31.9	9.5
OK148120.1 *Ceriagrion rubiae* Kerala	32.4	16.8	32.7	18.2	23	12.2	43.5	20.9	32	22.6	19.1	26.1	41	15.5	35.3	7.8
KU220868.1 *Ceriagrion cerinorubellum* Malaysia	33.5	15.9	31.5	19.1	26	8.7	41.7	23.5	32	22.6	19.1	26.1	42	16.4	33.6	7.8
KU220867.1 *Ceriagrion cerinorubellum* Indian	31.6	16.4	34.5	17.5	24	10.6	43.4	22.1	32	21.1	24.6	22.8	39	17.4	35.7	7.8
MF784361.1 *Ceriagrion cerinorubellum* Bangladesh	32.9	16.5	31.5	19.1	25	9.6	41.7	23.5	32	22.6	19.1	26.1	41	17.2	33.6	7.8
KU566000.1 *Ceriagrion suave* Africa	32.4	15.6	33.2	18.8	30	6.1	42.6	21.7	31	23.5	19.1	26.1	36	17.2	37.9	8.6
KU565956.1 *Ceriagrion glabrum* Tanzania	32.4	15.6	33.2	18.8	30	6.1	42.6	21.7	31	23.5	19.1	26.1	36	17.2	37.9	8.6
KU565935.1 *Ceriagrion bakeri* Liberia	31.5	17.6	33.2	17.6	25	12.2	41.7	20.9	32	22.6	19.1	26.1	37	18.1	38.8	6.0
KU220869.1 *Ceriagrion olivaceum* Thailand	31.2	16.8	33.8	18.2	23	10.4	44.3	21.7	31	23.5	19.1	26.1	39	16.4	37.9	6.9
MN867589.1 *Ceriagrion coromandelianum* Punjab	31.8	17.3	32.7	18.2	27	9.6	40.9	22.6	31	23.5	19.1	26.1	37	19.0	37.9	6.0
KU220871.1 *Ceriagrion coromandelianum* India	31.8	17.6	32.4	18.2	27	9.6	40.9	22.6	31	23.5	19.1	26.1	37	19.8	37.1	6.0
AB860041.1 *Ceriagrion chaoi* Malaysia	33.8	15.9	32.1	18.2	30	7.8	40.0	22.6	32	22.6	19.1	26.1	40	17.2	37.1	6.0
KX263700.1 *Ceriagrion fallax* China	31.5	17.9	31.8	18.8	23	12.2	41.7	22.6	30	24.3	19.1	26.1	41	17.2	34.5	7.8
MZ087263.1 *Orthetrum glaucum* Kerala	29.4	16.6	39.2	14.7	21	14.2	43.9	20.6	28	24.8	27.4	19.7	39	10.8	46.5	3.8
Avg.	32.1	16.7	33.2	18.1	26	10.1	42.1	22.1	31	23.1	20.3	25.3	39	16.8	37.1	7.1

13) Phylogenetic analysis of the genus *Ischnura*

Phylogenetic reconstruction of the genus *Ischnura* was done by using 12 sequences which include, sequence of *Ischnura rubilio*, 10 COI sequences of the species of the genus *Ischnura* downloaded from GenBank and sequence of the dragonfly *Orthetrum luzonicum* as out group (Table 4.4.77; Figure 4.4.37).

Table 4.4.77: Details of COI gene sequences involved in the phylogenetic analysis of genus *Ischnura*

Sl No.	Accession Number	Scientific Name	Product size
1.	MN850442.1	*Ischnura rubilio*, Kerala	670bp
2.	MH450006.1	*Ischnura aurora,* Thailand	692bp
3.	KR149808.1	*Ischnura aurora*, Kerala	628bp
4.	KY844428.1	*Ischnura delicata*, Pakistan	567bp
5.	MH450000.1	*Ischnura senegalensis*, Yemen	683bp
6.	MG449768.1	*Ischnura kellicotti*, Canada	658bp
7.	MH449996.1	*Ischnura rufostigma*, China	667bp
8.	KX053536.1	*Ischnura taitensis*, France	658bp
9.	KY127433.1	*Ischnura elegans*, Cyprus	675bp
10.	MG379400.1	*Ischnura verticalis*, Canada	658bp
11.	MH449986.1	*Ischnura nursei*, Iran	702bp
12.	MZ092847.1	*Orthetrum luzonicum*, Kerala	692bp

Mainly three clades could be found in the phylogeny of genus *Ischnura*. In the first clade *Ischnura senegalensis* and *Ischnura elegans* formed sister clades and *Ischnura rufostigma* and *Ischnura nursei* were polyphyletic (boot strap 99%). The second clade was formed by the monophyly of *Ischnura kellicotti* and *Ischnura verticalis* (boot strap 99%). The last clade was the group of *Ischnura rubilio, Ischnura aurora* and *Ischnura delicata* as monophyletic and *Ischnura taitensis* as paraphyletic with a bootstrap value of 91%.

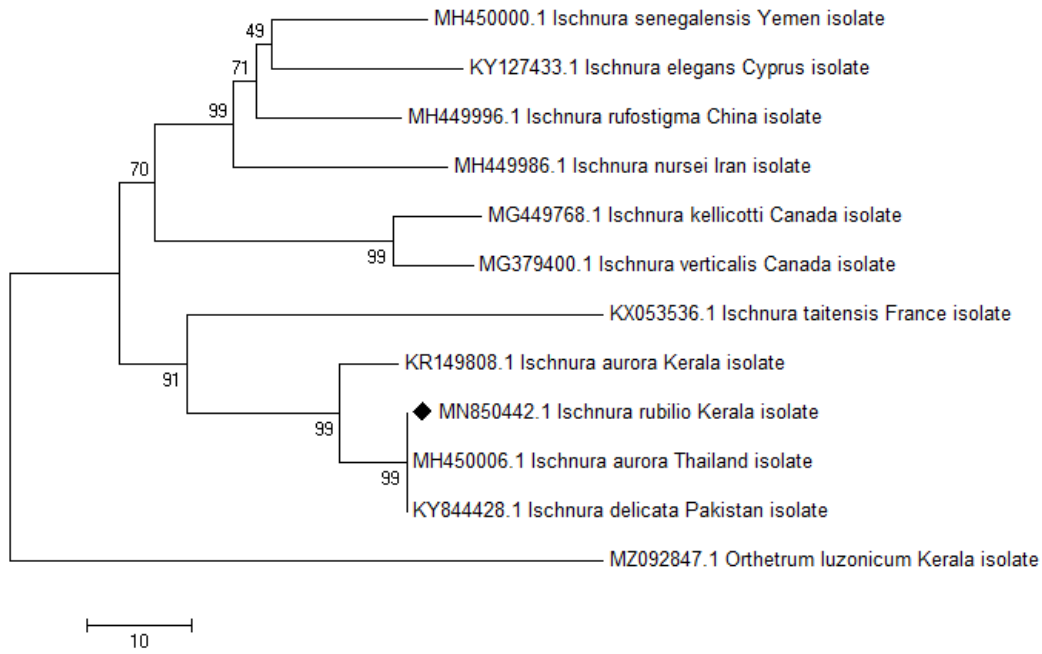

Figure 4.4.37: Inferred phylogenetic tree of the genus *Ischnura*, rooted by outgroup.

Intraspecific and interspecific divergence

The phylogenetic tree was well supported by observed intraspecific and interspecific divergence values. There was no genetic divergence between *Ischnura rubilio, Ischnura delicata* and *Ischnura aurora* from Thailand. But 2.1% divergence was shown by *Ishnura aurora* specimen from Kerala. Intraspecific divergence values ranged from 2.8% to 14.9%. The highest interspecific divergence values were found between *Ischnura taitensis* and *Ischura kellicotti* (Table 4.4.78).

Nucleotide composition

The nucleotide composition of the 12 sequences are 30.85 % (A), 34.58% (T/U), 16.43 % (C) and 18.15 % (G) as shown in Table 4.4.79. Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The base composition of *Ischnura rubilio* was T=34.8%, C=16.3%, A=31.4%, G=17.6% (AT content= 66.2%; GC content= 33.9%).

Table 4.4.78: Estimates of genetic divergence among COI gene sequences of genus *Ischnura*

	Name of Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	MN850442.1 *Ischnura rubilio* Kerala											
2	MH450006.1 *Ischnura aurora* Thailand	0.000										
3	KR149808.1 *Ischnura aurora* Kerala	0.021	0.021									
4	KY844428.1 *Ischnura delicata* Pakistan	0.000	0.000	0.021								
5	MH450000.1 *Ischnura senegalensis* Yemen	0.096	0.096	0.098	0.096							
6	MG449768.1 *Ischnura kellicotti* Canada	0.105	0.105	0.105	0.105	0.108						
7	MH449996.1 *Ischnura rufostigma* China	0.096	0.096	0.091	0.096	0.057	0.099					
8	KX053536.1 *Ischnura taitensis* France	0.107	0.107	0.107	0.107	0.131	0.149	0.130				
9	KY127433.1 *Ischnura elegans* Cyprus	0.110	0.110	0.105	0.110	0.059	0.108	0.055	0.135			
10	MG379400.1 *Ischnura verticalis* Canada	0.105	0.105	0.103	0.105	0.105	0.028	0.096	0.147	0.108		
11	MH449986.1 *Ischnura nursei* Iran	0.108	0.108	0.103	0.108	0.069	0.092	0.067	0.142	0.071	0.094	
12	MZ092847.1 *Orthetrum luzonicum* Kerala	0.169	0.169	0.174	0.169	0.169	0.188	0.160	0.185	0.171	0.183	0.172

Table 4.4.79: Nucleotide base composition of COI gene sequence of genus *Ischnura* species and out group

Name of species																
	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
MN850442.1 *Ischnura rubilio* Kerala	34.8	16.3	31.4	17.6	36	7.4	54.3	2.7	24	14.9	27.7	33.5	45	26.6	12.2	16.5
MH450006.1*Ischnura aurora* Thailand	34.8	16.3	31.4	17.6	36	7.4	54.3	2.7	24	14.9	27.7	33.5	45	26.6	12.2	16.5
KR149808.1 *Ischnura aurora* Kerala	35.3	16.1	30.7	17.9	37	6.9	52.1	3.7	24	14.9	27.7	33.5	45	26.6	12.2	16.5
KY844428.1*Ischnura delicata* Pakistan	34.8	16.3	31.3	17.6	36	7.5	54.0	2.7	24	14.9	27.7	33.5	45	26.6	12.2	16.5
MH450000.1 *Ischnura senegalensis* Yemen	35.3	15.8	30.3	18.6	37	5.9	51.1	5.9	24	14.9	27.7	33.5	45	26.6	12.2	16.5
MG449768.1 *Ischnura kellicotti* Canada	34.0	16.3	31.4	18.3	34	7.4	53.7	5.3	24	14.9	28.2	33.0	45	26.6	12.2	16.5
MH449996.1 *Ischnura rufostigma* China	34.6	16.0	31.2	18.3	34	8.0	53.7	4.8	26	13.3	27.7	33.5	45	26.6	12.2	16.5
KX053536.1 *Ischnura taitensis* France	33.3	18.4	30.0	18.3	32	11.7	50.0	5.9	23	16.5	27.7	32.4	44	27.1	12.2	16.5
KY127433.1 *Ischnura elegans* Cyprus	33.2	17.0	31.2	18.6	31	9.0	53.7	5.9	23	15.4	27.7	33.5	45	26.6	12.2	16.5
MG379400.1 *Ischnura verticalis* Canada	34.6	15.6	31.2	18.6	35	5.3	53.7	5.9	24	14.9	27.7	33.5	45	26.6	12.2	16.5
MH449986.1 *Ischnura nursei* Iran	34.4	16.5	30.3	18.8	34	8.5	51.1	6.4	24	14.4	27.7	33.5	45	26.6	12.2	16.5
MZ092847.1 *Orthetrum luzonicum* Kerala	35.5	16.1	30.9	17.6	40	5.9	50.5	3.7	22	16.0	29.8	32.4	45	26.6	12.2	16.5
Avg.	34.5	16.4	30.9	18.1	35	7.6	52.7	4.6	24	15.0	27.9	33.3	45	26.6	12.2	16.5

14) Phylogenetic analysis of the genus *Paracercion*

Phylogeny of the genus *Paracercion* was resolved by using 10 partial COI gene sequences of *Paracercion calamorum* and *Paracercion malayanum* and sequences of 7 conspecifics and non-conspecifics, downloaded from GenBank and sequence of the dragonfly *Tetrathemis platyptera*, included as out group (Table 4.4.80, Figure 4.4.38).

Table 4.4.80: Details of COI gene sequences involved in the phylogenetic analysis of genus *Paracercion*

Sl No.	Accession Number	Scientific Name	Product size
1.	MW940750.1	*Paracercion calamorum*, Kerala	668bp
2.	MZ700177.1	*Paracercion malayanum*, Kerala	689bp
3.	KF257111.1	*Paracercion calamorum*, South Korea	1147bp
4.	KX263714.1	*Paracercion calamorum*, China	550bp
5.	MW361799.1	*Paracercion v-nigrum*, China	1066bp
6.	KF257117.1	*Paracercion sieboldii*, South Korea	1147bp
7.	MW361550.1	*Paracercion barbatum*, China	1066bp
8.	MW361685.1	*Paracercion melanotum*, China	1066bp
9.	MW361592.1	*Paracercion hieroglyphicum*, China	1066bp
10.	MZ092924.1	*Tetrathemis platyptera*, Kerala	1066bp

The phylogenetic tree was composed of three distinct monophyletic clades. All the nodes of the resultant tree were well supported by bootstrap value of 97-100 except one node. Three specimens of *Paracercion calamorum* formed a monophyletic clade in which sample from Kerala showed sequence diversion from other two. *Paracercion malayanum* was monphyletic with *Paracercion melanotum* and *Paracercion hieroglyphicum*. *Paracercion barbatum, Paracercion v-nigrum* and *Paracercion sieboldii* were grouped to form another monophyletic clade.

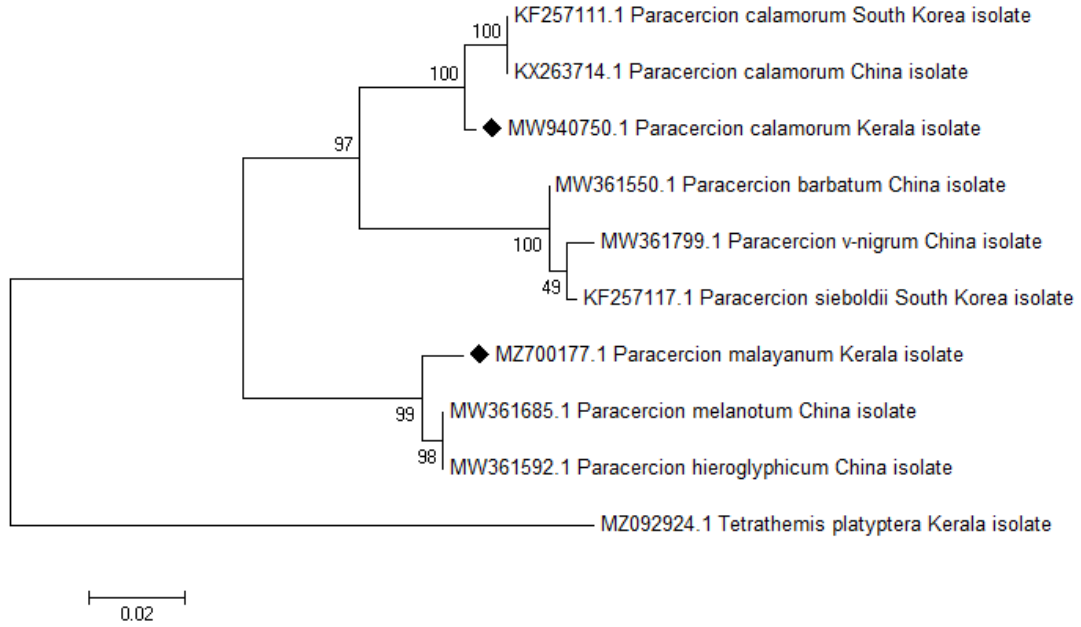

Figure 4.4.38: Inferred phylogenetic tree of the genus *Paracercion*, rooted by outgroup.

Intraspecific and interspecific divergence

The genetic divergence observed among the conspecifics of *Paracercion calamorum* ranged from 0- 1.1%. 1.1% divergence was shown by the Kerala specimen from South Korea and China specimens. The divergence between *Paracercion melanotum* and *Paracercion hieroglyphicum* was 0%. *Paracercion malayanum* showed 1.3% divergence from both. The interspecific divergence values ranged from 0.7% to 10.2% (Table 4.4.81).

Nucleotide composition

The nucleotide composition of the 10 sequences were 30.78 % (A), 33.75% (T/U), 19.07 % (C) and 16.40% (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The base composition of *Paracercion calamorum* was T=34.0%, C=18.7%, A=31.2%, G=16.1% with AT content of 65.2% and GC content of 34.8% (Table 4.4.82). The base composition of *Paracercion malayanum* was T=32.7%, C=20.2%, A=31.0%, G=16.1% which also possessed a high AT bias (AT content= 63.7%, GC content= 36.3%).

Table 4.4.81: Estimates of genetic divergence among COI gene sequences of genus *Paracercion*

	Name of Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
	MW940750.1 *Paracercion calamorum* Kerala									
	MZ700177.1 *Paracercion malayanum* Kerala	0.089								
	KF257111.1*Paracercion calamorum* South_Korea	0.011	0.093							
	KX263714.1*Paracercion calamorum* China	0.011	0.093	0.000						
	MW361799.1*Paracercion v-nigrum* China	0.056	0.096	0.065	0.065					
	KF257117.1 *Paracercion sieboldii* South Korea	0.063	0.100	0.069	0.069	0.007				
	MW361550.1 *Paracercion barbatum* China	0.059	0.095	0.065	0.065	0.009	0.006			
	MW361685.1 *Paracercion melanotum* China	0.080	0.013	0.083	0.083	0.098	0.102	0.096		
	MW361592.1*Paracercion hieroglyphicum* China	0.080	0.013	0.083	0.083	0.098	0.102	0.096	0.000	
	MZ092924.1*Tetrathemis platyptera* Kerala	0.182	0.180	0.180	0.180	0.186	0.191	0.189	0.186	0.186

Table 4.4.82: Nucleotide base composition of COI gene sequence of genus *Paracercion*

Species																
	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
MW940750.1 *Paracercion calamorum* Kerala	34.0	18.7	31.2	16.1	44	27.8	13.3	15.0	37	7.8	52.8	2.8	21	20.7	27.4	30.7
MZ700177.1 *Paracercion malayanum* Kerala	32.7	20.2	31.0	16.1	44	27.8	13.3	15.0	32	13.3	52.2	2.8	22	19.6	27.4	30.7
KF257111.1 *Paracercion calamorum* South Korea	33.8	19.1	30.8	16.3	44	27.8	13.3	15.0	36	8.9	51.7	3.3	21	20.7	27.4	30.7
KX263714.1 *Paracercion calamorum* China	33.8	19.1	30.8	16.3	44	27.8	13.3	15.0	36	8.9	51.7	3.3	21	20.7	27.4	30.7
MW361799.1 *Paracercion v-nigrum* China	34.1	18.6	30.6	16.7	44	27.8	13.3	15.0	37	7.2	51.1	4.4	21	20.7	27.4	30.7
KF257117.1 *Paracercion sieboldii* South Korea	34.3	18.4	30.4	16.9	43	28.3	13.3	15.0	38	6.7	50.6	5.0	22	20.1	27.4	30.7
MW361550.1 *Paracercion barbatum* China	34.5	18.0	30.8	16.7	44	27.8	13.3	15.0	38	6.1	51.7	4.4	22	20.1	27.4	30.7
MW361685.1 *Paracercion melanotum* China	33.0	19.9	30.8	16.3	44	27.8	13.3	15.0	33	12.2	51.7	3.3	22	19.6	27.4	30.7
MW361592.1 *Paracercion hieroglyphicum* China	33.0	19.9	30.8	16.3	44	27.8	13.3	15.0	33	12.2	51.7	3.3	22	19.6	27.4	30.7
MZ092924.1 *Tetrathemis platyptera* Kerala	34.3	18.9	30.6	16.1	44	27.8	13.3	14.4	36	11.1	50.0	3.3	23	17.9	28.5	30.7
Avg.	33.7	19.1	30.8	16.4	44	27.8	13.3	14.9	35	9.4	51.5	3.6	22	19.9	27.5	30.7

15) Phylogenetic analysis of the genus *Pseudagrion*

Phylogenetic analysis of the genus *Pseudagrion* based on 11 partial COI gene sequences. Sequences of *Pseudagrion decorum* and *Pseudagrion indicum* were used along with 8 sequences of the corresponding genus retrieved from GenBank. Sequence of the dragonfly *Tholymis tillarga* was included as out group (Table 4.4.83; Figure 4.4.39).

Table 4.4.83: Details of COI gene sequences involved in the phylogenetic analysis of genus *Pseudagrion*

Sl No.	Accession Number	Scientific Name	Product size
1.	MZ254912.1	*Pseudagrion decorum*, Kerala	628bp
2.	MN882703.1	*Pseudagrion indicum*, Kerala	649bp
3.	KT957467.1	*Pseudagrion australasiae*, Thailand	657bp
4.	MN967007.1	*Pseudagrion rubriceps*, Punjab	620bp
5.	MW856662.1	*Pseudagrion indicum*, Kerala	506bp
6.	MT251940.1	*Pseudagrion microcephalum*, Punjab	661bp
7.	MW361891.1	*Pseudagrion spencei*, China	1066bp
8.	MW361886.1	*Pseudagrion pruinosum*, China	1066bp
9.	JF839186.1	*Pseudagrion praetextatum*, Kenya	658bp
10.	KX447495.1	*Pseudagrion pilidorsum*, Indonesia	602bp
11.	MZ127380.1	*Tholymis tillarga*, Kerala	700bp

From the resultant tree it was clear that all the *Pseudagrion* species found in Kerala, viz. *Pseudagrion indicum, Pseudagrion australasiae, Pseudagrion decorum, Pseudagrion microcephalum, Psuedagrion rubriceps (Pseudagrion malabaricum* was not included because of the unavailability of sequence data) were evolved from one common ancestor. *Pseudagrion indicum* specimens from Kerala showed close similarity with 100% boot strap support. *Pseudagrion australasiae, Pseudagrion decorum* and *Pseudagrion microcephalum* were polyphyletic. *Pseudagrion decorum* is the only record of this species in GenBank so sequence of the same gene

was unavailable for analyzing intraspecific relationship. *Pseudagrion rubriceps* and *Pseudagrion spencei* formed sister clades but with low boot strap value.

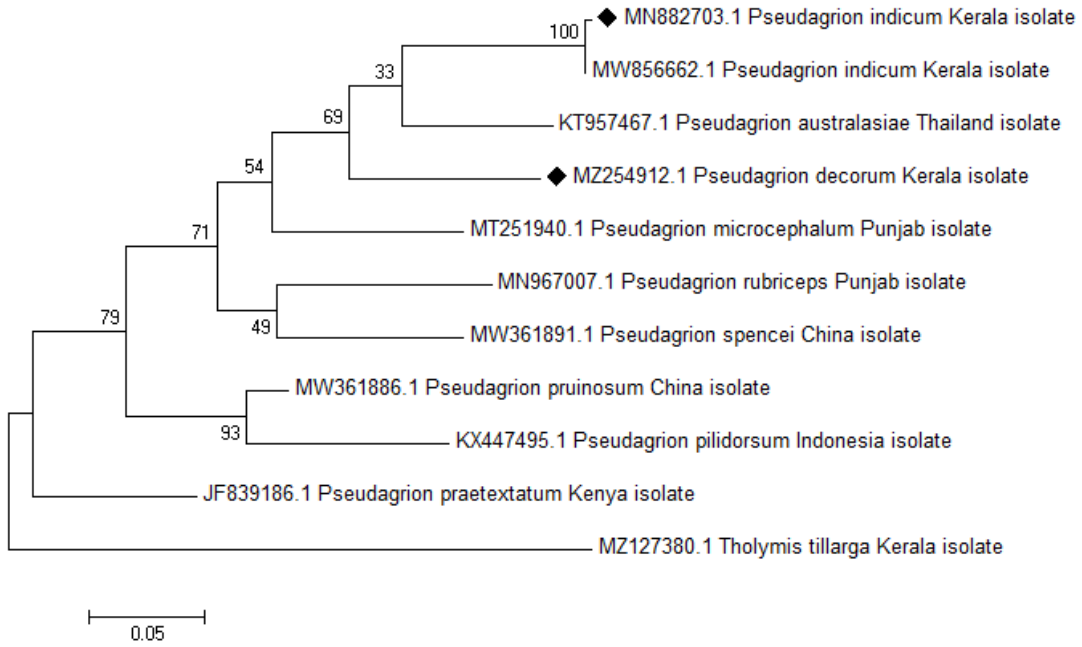

Figure 4.4.39: Inferred phylogenetic tree of the genus *Pseudagrion*, rooted by outgroup.

Intraspecific and interspecific divergence

The calculated intraspecific divergence between Kerala specimens of *Pseudagrion indicum* was 0.3% supporting the phylogenetic tree. The interspecific divergence values ranged from 9.9% to 20% (Table 4.4.84).

Nucleotide composition

The nucleotide composition of the 11 sequences are 30.75 % (A), 31.51% (T/U), 19.36 % (C) and 18.39% (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The base composition of *Pseudagrion decorum* was T=34.2%, C=17.3%, A=29.5%, G=19.0% with high AT bias (AT content= 63.7%, GC content= 36.3%). The nucleotide base composition of *Pseudagrion indicum* was T=30.2%, C=20.7%, A=29.5%, G=19.7% with an AT content of 59.7% over GC content of 40.4% (Table 4.4.85).

Table 4.4.84: Estimates of genetic divergence between COI gene sequences of genus *Pseudagrion*

	Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	MZ254912.1 *Pseudagrion decorum* Kerala										
2	MN882703.1 *Pseudagrion indicum* Kerala	0.142									
3	KT957467.1*Pseudagrion australasiae* Thailand	0.134	0.132								
4	MN967007.1 *Pseudagrion rubriceps* Punjab	0.177	0.175	0.149							
5	MW856662.1 *Pseudagrion indicum* Kerala	0.144	0.003	0.129	0.172						
6	MT251940.1 *Pseudagrion microcephalum* Punjab	0.165	0.147	0.154	0.182	0.149					
7	MW361891.1 *Pseudagrion spencei* China	0.162	0.154	0.177	0.147	0.154	0.147				
8	MW361886.1 *Pseudagrion pruinosum* China	0.182	0.192	0.175	0.157	0.190	0.167	0.149			
9	JF839186.1 *Pseudagrion praetextatum* Kenya	0.172	0.190	0.185	0.195	0.190	0.177	0.167	0.147		
10	KX447495.1 *Pseudagrion pilidorsum* Indonesia	0.200	0.177	0.180	0.190	0.180	0.172	0.180	0.099	0.190	
11	MZ127380.1 *Tholymis tillarga* Kerala	0.281	0.291	0.284	0.278	0.289	0.286	0.268	0.258	0.246	0.301

Table 4.4.85: Nucleotide base composition of COI gene sequence of genus *Pseudagrion*

Species																
	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
MZ254912.1 *Pseudagrion decorum* Kerala	34.2	17.3	29.5	19.0	45	22.0	12.8	20.6	36	13.5	43.3	7.1	22	16.5	32.4	29.5
MN882703.1 *Pseudagrion indicum* Kerala	30.2	20.7	29.5	19.7	41	27.0	12.8	19.1	28	17.7	41.1	12.8	21	17.3	34.5	27.3
KT957467.1 *Pseudagrion australasiae* Thailand	31.8	20.4	29.5	18.3	43	25.5	12.8	19.1	33	17.0	42.6	7.8	20	18.7	33.1	28.1
MN967007.1 *Pseudagrion rubriceps* Punjab	31.1	19.7	29.9	19.2	43	25.5	12.8	19.1	31	14.9	44.0	9.9	19	18.7	33.1	28.8
MW856662.1 *Pseudagrion indicum* Kerala	30.2	20.7	29.7	19.5	41	27.0	12.8	19.1	28	17.7	41.8	12.1	21	17.3	34.5	27.3
MT251940.1 *Pseudagrion microcephalum* Punjab	29.5	21.9	29.2	19.5	41	27.0	12.8	19.1	28	19.9	40.4	11.3	19	18.7	34.5	28.1
MW361891.1 *Pseudagrion spencei* China	30.2	20.7	30.9	18.3	41	27.0	12.8	19.1	31	14.9	46.1	7.8	18	20.1	33.8	28.1
MW361886.1 *Pseudagrion pruinosum* China	32.1	19.7	29.9	18.3	43	25.5	12.8	19.1	33	17.0	42.6	7.8	21	16.5	34.5	28.1
JF839186.1 *Pseudagrion praetextatum* Kenya	36.6	16.9	29.5	17.1	42	27.0	12.8	18.4	43	9.9	43.3	3.5	24	13.7	32.4	29.5
KX447495.1 *Pseudagrion pilidorsum* Indonesia	30.6	21.1	27.6	20.7	41	27.0	12.8	19.1	28	20.6	36.2	14.9	22	15.8	33.8	28.1
MZ127380.1 *Tholymis tillarga* Kerala	29.4	16.6	39.2	14.7	31	26.3	24.4	17.9	38	9.0	50.6	2.6	19	14.6	42.7	23.6
Avg.	31.4	19.6	30.5	18.5	41	26.1	13.9	19.1	33	15.6	43.0	8.8	21	17.1	34.6	27.8

16) Phylogenetic analysis of the genus *Gynacantha*

Phylogenetic relationships among the species of genus *Gynacantha* were resolved by using the sequences of *Gynacantha dravida* and *Gynacantha millardi*, sequences of six related species downloaded from GenBank and sequence of the damselfly *Lestes praemorsus* as out group. The sequence data was composed of nine COI sequences (Table 4.4.86; Figure 4.4.40)

Table 4.4.86: Details of COI gene sequences involved in the phylogenetic analysis of genus *Gynacantha*

Sl No.	**Accession Number**	**Scientific Name**	**Product size**
1.	MW649897.1	*Gynacantha millardi*, Kerala	615bp
2.	MK990607.1	*Gynacantha dravida*, Kerala	631bp
3.	MZ203544.1	*Gynacantha bayadera*, Punjab	603bp
4.	KU566127.1	*Gynacantha nigeriensis*, Liberia	658bp
5.	KU566118.1	*Gynacantha congolica*, Congo(Africa)	658bp
6.	KU566115.1	*Gynacantha bullata*, Gabon(Africa)	658bp
7.	KU566136.1	*Gynacantha usambarica*, South Africa	658bp
8.	KU566131.1	*Gynacantha pupillata*, Africa(Sierra Leone)	658bp
9.	MZ074000.1	*Lestes praemorsus*, Kerala	671bp

The current submission of *Gynacantha millardi* and *Gynacantha dravida* are the first and only records of these species in GenBank so no sequence of the conspecific was available for intraspecific comparison. The three species, *Gynacantha dravida, Gynacantha millardi* and *Gynacantha bayadera* were found to be monophyletic. *Gynacantha millardi* and *Gynacantha bayadera* were in sister clade relationship (bootstrap 99%) which denoted the close similarity between them. The other species of *Gynacantha* clustered together to form another monophyletic clade (bootstrap 91%).

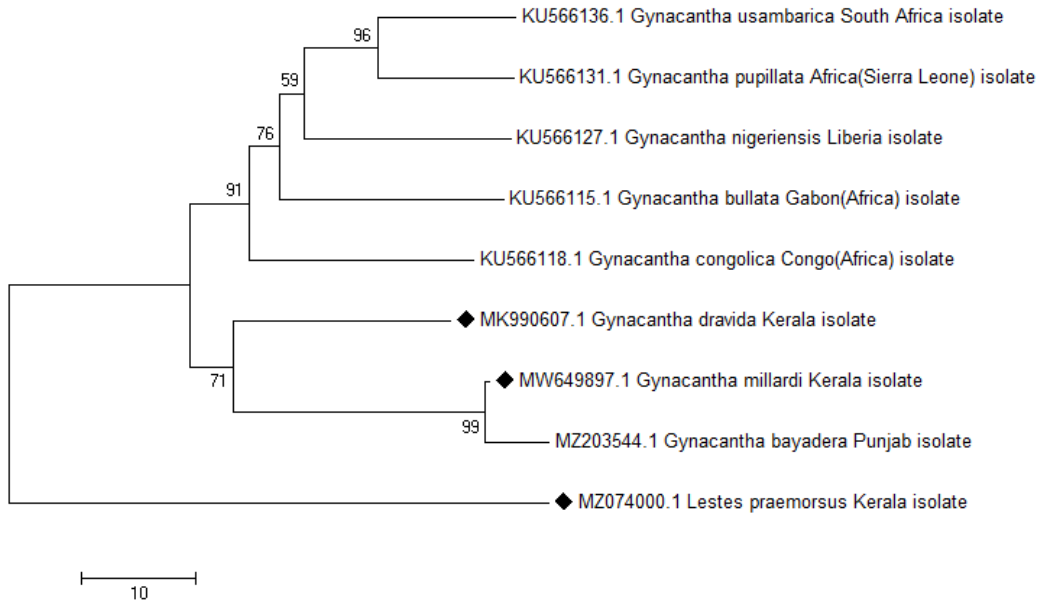

Figure 4.4.40: Inferred phylogenetic tree of the genus *Gynacantha*, rooted by outgroup.

Intraspecific and interspecific divergence

The interspecific divergence between *Gynacantha millardi* and *Gynacantha bayadera* was observed as 1.2%. The interspecific divergence values ranged from 1.2% to 12.3% (Table 4.4.87).

Nucleotide composition

The nucleotide frequencies of the 9 sequences are 31.23 % (A), 35.13% (T/U), 16.14 % (C) and 17.50% (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The base composition of *Gynacantha dravida* is T=36.2%, C=16.4%, A=30.4%, G=17.0% with AT content of 66.6% and GC content of 33.4%. The base composition of *Gynacantha millardi* is T=35.8%, C=16.0%, A=30.0%, G=18.2% with a high AT content (65.8%) over GC content (34.2%). The estimated values are given in Table 4.4.88.

Table 4.4.87: Estimates of genetic divergence among COI gene sequences of *Gynacantha* species and out group

	Name of Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	MW649897.1_*Gynacantha_millardi*_Kerala								
2	MK990607.1_*Gynacantha_dravida*_Kerala	0.081							
3	MZ203544.1_*Gynacantha_bayadera*_Punjab	0.012	0.093						
4	KU566127.1_*Gynacantha_nigeriensis*_Liberia	0.101	0.099	0.113					
5	KU566118.1_*Gynacantha_congolica*_Congo(Africa)	0.099	0.095	0.109	0.089				
6	KU566115.1_*Gynacantha_bullata*_Gabon(Africa)	0.111	0.087	0.121	0.087	0.087			
7	KU566136.1_*Gynacantha_usambarica*_South_Africa	0.111	0.101	0.123	0.063	0.083	0.079		
8	KU566131.1_*Gynacantha_pupillata*_Africa(Sierra_Leone)	0.113	0.105	0.123	0.081	0.077	0.071	0.047	
9	MZ074000.1_*Lestes_praemorsus*_Kerala	0.174	0.180	0.180	0.180	0.178	0.178	0.186	0.174

Table 4.4.88: Nucleotide base composition of COI gene sequence of genus *Gynacantha*

Species																
	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
MW649897.1 *Gynacantha millardi* Kerala	35.8	16.0	30.0	18.2	22	16.0	29.0	32.5	44	26.0	13.6	16.0	40	6.0	47.6	6.0
MK990607.1 *Gynacantha dravida* Kerala	36.2	16.4	30.4	17.0	21	17.2	29.0	32.5	44	26.0	13.6	16.0	43	6.0	48.8	2.4
MZ203544.1 *Gynacantha bayadera* Punjab	35.8	16.0	30.0	18.2	22	16.0	29.0	32.5	44	26.0	13.6	16.0	40	6.0	47.6	6.0
KU566127.1 *Gynacantha nigeriensis* Liberia	35.0	16.8	31.2	17.0	21	17.2	29.6	32.5	44	26.0	13.6	16.0	40	7.1	50.6	2.4
KU566118.1 *Gynacantha congolica* Congo	35.0	16.0	31.8	17.2	22	16.0	29.6	32.5	44	26.0	13.6	16.0	39	6.0	52.4	3.0
KU566115.1 *Gynacantha bullata* Gabon	35.2	15.4	32.0	17.4	22	15.4	29.6	32.5	44	26.0	13.6	16.0	39	4.8	53.0	3.6
KU566136.1 *Gynacantha usambarica* South Africa	35.2	16.0	32.6	16.2	22	16.0	29.6	32.5	44	26.0	13.6	16.0	39	6.0	54.8	.0
KU566131.1 *Gynacantha pupillata* Africa	36.0	15.0	32.2	16.8	23	14.8	29.6	32.5	44	26.0	13.6	16.0	40	4.2	53.6	1.8
MZ074000.1 *Lestes praemorsus* Kerala	32.2	17.6	30.6	19.6	20	18.9	29.0	32.0	44	26.0	13.6	16.0	32	7.7	49.4	10.7
Avg.	35.1	16.1	31.2	17.5	22	16.4	29.3	32.5	44	26.0	13.6	16.0	39	6.0	50.9	4.0

17) Phylogenetic analysis of the genus *Ictinogomphus*

The phylogenetic reconstruction of the genus *Ictinogomphus* was carried out based on 8 COI gene sequences. The sequence of *Ictinogomphus rapax*, sequences of the corresponding genus retrieved from GenBank and sequence of the damselfly *Heliocypha bisignata* were involved in the analysis (Table 4.4.89; Figure 4.4.41).

Table 4.4.89: Details of COI gene sequences involved in the phylogenetic analysis of genus *Ictinogomphus*

Sl No.	**Accession Number**	**Scientific Name**	**Product size**
1	MW945399.1	*Ictinogomphus rapax*, Kerala	582bp
2	MF358743.1	*Ictinogomphus rapax*, China	651bp
3	KX891024.1	*Ictinogomphus rapax*, USA	655bp
4	MN344903.1	*Ictinogomphus decoratus melaenops*	387bp
5	AB708703.1	*Ictinogomphus pertinax*, Thaiwan	451bp
6	AB708702.1	*Ictinogomphus pertinax*, Japan	451bp
7	AB860039.1	*Ictinogomphus decoratus*, Malaysia	451bp
8	MW940786.1	*Heliocypha bisignata*, Kerala	676bp

The result indicated that 3 species of *Ictinogomphus* involved in the analysis were grouped into three distinct monophyletic clades in which *Ictinogomphus decoratus* and *Ictinogomphus pertinax* were found as sister clades. *Ictinogomphus rapax* was paraphyletic. The three specimens of *Ictinogomphus* were monophyletic to each other (boot strap 98%). Specimens from China and USA were more close but low bootstrap support. The divergence values also supported the same (1.6% divergence).

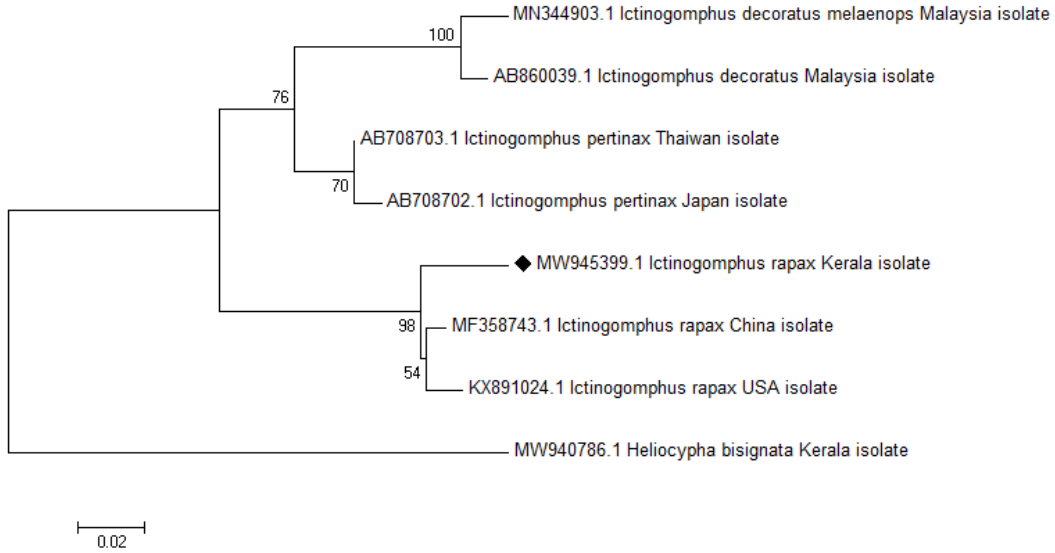

Figure 4.4.41: Inferred phylogenetic tree of the genus *Ictinogomphus*, rooted by outgroup.

Intraspecific and interspecific divergence

The intraspecific divergence values between Kerala, China and USA specimens ranged from 1.6% to 3.5%. This high percentage of divergence may be the result of changes accumulated in the gene sequence by geographical isolation. The conspecifics of *Ictinogomphus decoratus* showed 2.2% divergence and only 0.8% divergence was observed between conspecifics of *Ictinogomphus pertinax*. The interspecific divergence values ranged from 6.8% to 14.2% (Table 4.4.90).

Nucleotide composition

The nucleotide composition of the eight sequences were 30.38 % (A), 30.25 % (T/U), 22.31 % (C) and 17.06% (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The base composition of *Ictinogomphus rapax* was T=31.3%, C=21.5%, A=31.1%, G=16.1%. High AT bias was observed with an AT content of 62.4% and GC content of 37.6% (Table 4.4.91).

Table 4.4.90: Estimates of genetic divergence among COI gene sequences of genus *Ictinogomphus*

	Name of Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	MW945399.1_*Ictinogomphus_rapax*_Kerala							
2	MF358743.1_*Ictinogomphus_rapax*_China	0.030						
3	KX891024.1_*Ictinogomphus_rapax*_USA	0.035	0.016					
4	MN344903.1_*Ictinogomphus_decoratus_melaenops*_Malaysia	0.136	0.128	0.112				
5	AB708703.1_*Ictinogomphus_pertinax*_Thaiwan	0.112	0.095	0.101	0.076			
6	AB708702.1_*Ictinogomphus_pertinax*_Japan	0.120	0.104	0.109	0.074	0.008		
7	AB860039.1_*Ictinogomphus_decoratus*_Malaysia	0.142	0.128	0.112	0.022	0.071	0.068	
8	MW940786.1_*Heliocypha_bisignata*_Kerala	0.218	0.221	0.223	0.221	0.199	0.207	0.213

Table 4.4.91: Nucleotide base composition of COI gene sequence of genus *Ictinogomphus*

Species																
	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
MW945399.1 *Ictinogomphus rapax* Kerala	31.3	21.5	31.1	16.1	42	31.7	11.4	14.6	27	14.8	52.5	5.7	25	18.0	29.5	27.9
MF358743.1 *Ictinogomphus rapax* China	30.4	23.2	29.9	16.5	41	32.9	11.4	14.3	27	16.5	50.4	6.5	23	20.1	28.1	28.8
KX891024.1 *Ictinogomphus rapax* USA	30.0	23.2	29.5	17.4	42	32.4	11.5	14.4	26	16.7	48.6	8.7	22	20.4	28.5	29.2
MN344903.1 *Ictinogomphus decoratus melaenops* Malaysia	29.4	22.6	30.0	18.0	42	31.7	11.4	14.6	23	15.6	50.8	10.7	23	20.5	27.9	28.7
AB708703.1 *Ictinogomphus pertinax* Thaiwan	30.2	22.6	30.2	16.9	42	31.7	11.4	14.6	25	15.6	51.6	7.4	23	20.5	27.9	28.7
AB708702.1 *Ictinogomphus pertinax* Japan	30.0	22.9	29.7	17.4	42	31.7	11.4	14.6	25	16.4	50.0	9.0	23	20.5	27.9	28.7
AB860039.1 *Ictinogomphus decoratus* Malaysia	28.1	24.0	30.2	17.7	42	31.7	11.4	14.6	20	18.0	51.6	9.8	21	22.1	27.9	28.7
MW940786.1 *Heliocypha bisignata* Kerala	32.2	19.9	30.5	17.4	41	31.7	12.2	14.6	28	11.5	51.6	9.0	27	16.4	27.9	28.7
Avg.	30.2	22.5	30.1	17.2	42	32.0	11.5	14.6	25	15.7	50.8	8.3	23	19.8	28.2	28.7

18) Phylogenetic analysis of the genus *Diplacodes*

Phylogenetic analysis of the genus *Diplacodes* based on 8 partial coding COI gene sequence was conducted. Sequence of *Diplacodes nebulosa*, sequences of conspecifics and non-conspecifics retrieved from GenBank and sequence of the damselfly *Heliocypha bisignata* as out group were involved in the phylogenetic analysis (Table 4.4.92; Figure 4.4.42).

Table 4.4.92: Details of COI gene sequences involved in the phylogenetic analysis of genus *Diplacodes*

Sl No.	**Accession Number**	**Scientific Name**	**Product size**
1.	MZ254913.1	*Diplacodes nebulosa* isolate, Kerala	555bp
2.	KT879902.1	*Diplacodes trivialis*, Karnataka	658bp
3.	KT957513.1	*Diplacodes nebulosa*, Thailand	657bp
4.	MT298406.1	*Diplacodes lefebvrei*, Italy	658bp
5.	MN345740.1	*Diplacodes luminans*, Malawi(Africa)	658bp
6.	JF839456.1	*Diplacodes haematodes*, Australia	658bp
7.	AB708966.1	*Diplacodes bipunctata*, Japan	451bp
8.	MW940786.1	*Heliocypha bisignata*, Kerala	676bp

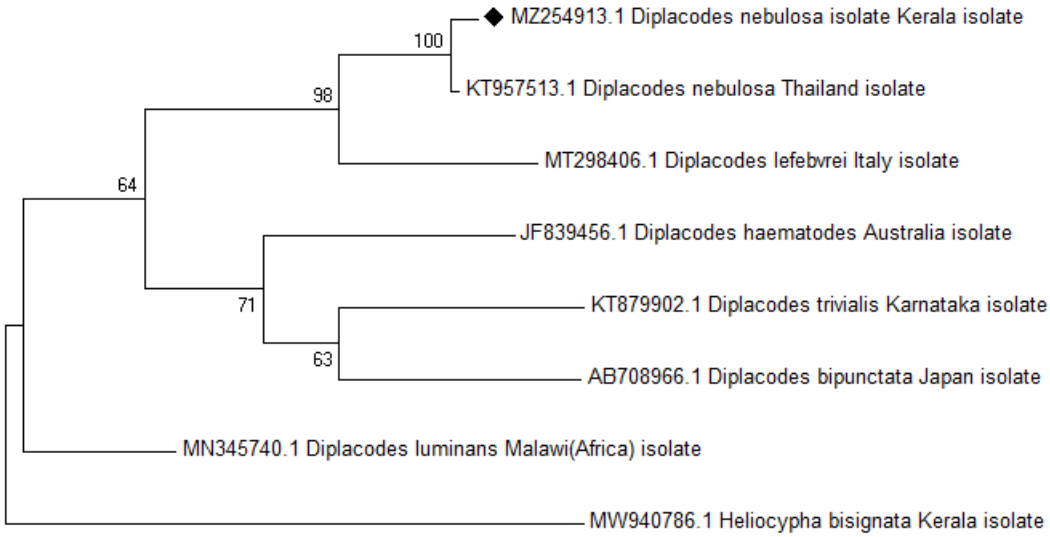

Figure 4.4.42: Inferred phylogenetic tree of the genus *Diplacodes*, rooted by outgroup

The result indicated that *Diplacodes luminans* diverged from the common ancestor at an earlier stage and it was paraphyletic to others. The remaining species were grouped into two distinct clusters. *Diplacodes lefebvrei* and *Diplacodes nebulosa* clustered together (98% bootstrap). Specimens of *Diplacodes nebulosa* from Kerala and Thailand exhibited close similarity with 100% boot strap support. The other clade was formed by *Diplacodes haematodes, Diplacodes trivialis* and *Diplacodes bipunctata* in which the latter two formed sister clades.

Intraspecific and interspecific divergence

The calculated divergence value between the conspecifics of *Diplacodes nebulosa* was 1.1%. The interspecific divergence values ranged from 9.1% to 17.5% (Table 4.4.93).

Nucleotide composition

The nucleotide frequencies of the eight sequences were 29.78% (A), 32.89% (T/U), 19.77% (C) and 17.56% (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The base composition of *Diplacodes nebulosa* were T=32.4%, C=20.2%,

A=29.4%, G=18.0%. High AT bias was observed with an AT content of 61.8% and GC content of 38.2% (Table 4.4.94).

Table 4.4.93: Estimates of genetic divergence among COI gene sequences of genus *Diplacodes*

	Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	MZ254913.1_*Diplacodes_nebulosa*_Kerala							
2	KT879902.1_*Diplacodes_trivialis*_Karnataka	0.175						
3	KT957513.1_*Diplacodes_nebulosa*_Thailand	0.011	0.169					
4	MT298406.1_*Diplacodes_lefebvrei*_Italy	0.097	0.166	0.091				
5	MN345740.1_*Diplacodes_luminans*_Malawi	0.152	0.172	0.147	0.152			
6	JF839456.1_*Diplacodes_haematodes*_Australia	0.163	0.144	0.152	0.172	0.163		
7	AB708966.1_*Diplacodes_bipunctata*_Japan	0.169	0.133	0.163	0.175	0.169	0.147	
8	MW940786.1_*Heliocypha_bisignata*_Kerala	0.235	0.222	0.235	0.224	0.199	0.227	0.235

Table 4.4.94: Nucleotide base composition of COI gene sequence of genus *Diplacodes*

Species																
	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
MZ254913.1 *Diplacodes nebulosa* Kerala	32.4	20.2	29.4	18.0	32	8.3	50.4	9.1	22	21.7	25.8	30.8	43	30.8	11.7	14.2
KT879902.1 *Diplacodes trivialis* Karnataka	34.1	19.1	29.6	17.2	34	6.6	52.9	6.6	25	19.2	25.0	30.8	43	31.7	10.8	14.2
KT957513.1 *Diplacodes nebulosa* Thailand	32.4	20.2	30.5	16.9	32	8.3	53.7	5.8	22	21.7	25.8	30.8	43	30.8	11.7	14.2
MT298406.1 *Diplacodes lefebvrei* Italy	31.6	21.6	29.6	17.2	32	9.9	51.2	6.6	19	24.2	25.8	30.8	43	30.8	11.7	14.2
MN345740.1 *Diplacodes luminans* Malawi	34.6	17.5	31.3	16.6	33	5.0	56.2	5.8	28	16.7	25.8	30.0	43	30.8	11.7	14.2
JF839456.1 *Diplacodes haematodes* Australia	32.4	20.2	29.4	18.0	31	7.4	52.1	9.1	23	21.7	25.0	30.8	43	31.7	10.8	14.2
AB708966.1 *Diplacodes bipunctata* Japan	34.1	19.7	28.5	17.7	32	9.9	49.6	8.3	27	17.5	25.0	30.8	43	31.7	10.8	14.2
MW940786.1 *Heliocypha bisignata* Kerala	31.6	19.7	29.9	18.8	27	9.1	51.2	12.4	25	19.2	26.7	29.2	43	30.8	11.7	15.0
Avg.	32.9	19.8	29.8	17.6	32	8.1	52.2	8.0	24	20.2	25.6	30.5	43	31.1	11.4	14.3

19) Phylogenetic analysis of the genus *Hydrabasileus*

The phylogeny of genus *Hydrobasileus* based on partial coding COI gene sequence was resolved by using 6 sequences including the sequence of *Hydrobasileus croceus* and sequence of the conspecifics and non-conspecifics downloaded from GenBank. Sequence of damselfly *Prodasineura verticalis* was used as out group (Table 4.4.95; Figure 4.4.43).

Table 4.4.95: Details of COI gene sequences involved in the phylogenetic analysis of genus *Hydrobasileus*

Sl No.	**Accession Number**	**Scientific Name**	**Product size**
1	MW965658.1	*Hydrobasileus croceus*, Kerala	671bp
2	MN344380.1	*Hydrobasileus brevistylus,* Solomon island	658bp
3	MG885137.1	*Hydrobasileus croceus*, Singapore	313bp
4	KM207068.1	*Hydrobasileus croceus*, China	658bp
5	AB708968.1	*Hydrobasileus croceus*, Japan	451bp
6	MZ081640.1	*Prodasineura verticalis*, Kerala	701bp

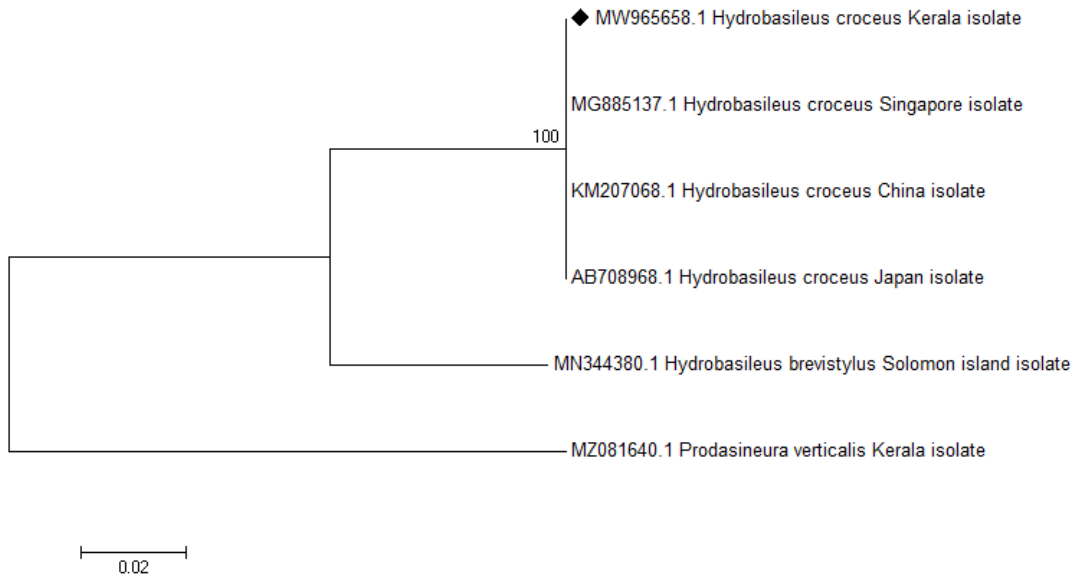

Figure 4.4.43: Inferred phylogenetic tree of the genus *Hydrobasileus*, rooted by outgroup

The phylogeny indicated that *Hydrobasileus* species samples from 4 geographically different locations were highly similar with a bootstrap value of 100. *Hydrobasileus brevistylus* was in paraphyletic relationship with *Hydrobasileus croceus*.

Intraspecific and interspecific divergence

The intraspecific divergence among the specimens of *Hydrobasileus croceus* from Kerala, Singapore, China and Japan was only 0%. This strongly suppoted the phylogenetic tree and confirmed the species authenticity of *Hydrobasileus croceus*. The interspecific divergence between *Hydrobasileus croceus* and *Hydrobasileus brevistylus* was 8% (Table 4.4.96).

Nucleotide composition

The nucleotide composition of the 6 sequences are 28.74 % (A), 37.04 % (T/U), 18.55 % (C) and 15.67% (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The base composition of *Hydrobasileus croceus* was T=37.9%, C=18.6%, A=27.9%, G=15.6% .The AT content was 65.8% and GC content was 34.2% (Table 4.4.97).

Table 4.4.96: Estimates of genetic divergence among COI gene sequences of genus *Hydrobasileus*

	Name of Species	**1**	**2**	**3**	**4**	**5**
1.	MW965658.1 *Hydrobasileus croceus* Kerala					
2.	MN344380.1 *Hydrobasileus brevistylus* Solomon Island	0.080				
3.	MG885137.1 *Hydrobasileus croceus* Singapore	0.000	0.080			
4.	KM207068.1 *Hydrobasileus croceus* China	0.000	0.080	0.000		
5.	AB708968.1 *Hydrobasileus croceus* Japan	0.000	0.080	0.000	0.000	
6.	MZ081640.1 *Prodasineura verticalis* Kerala	0.176	0.176	0.176	0.176	0.176

Table 4.4.97: Nucleotide base composition of COI gene sequence of genus *Hydrobasileus*

Species																
	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
MW965658.1 *Hydrobasileus croceus* Kerala	37.9	18.6	27.9	15.6	48	6.9	43.6	2.0	23	17.0	26.0	34.0	43	32.0	14.0	11.0
MN344380.1 *Hydrobasileus brevistylus* Solomon island	37.5	17.6	29.9	15.0	46	5.0	49.5	.0	24	16.0	26.0	34.0	43	32.0	14.0	11.0
MG885137.1 *Hydrobasileus croceus* Singapore	37.9	18.6	27.9	15.6	48	6.9	43.6	2.0	23	17.0	26.0	34.0	43	32.0	14.0	11.0
KM207068.1 *Hydrobasileus croceus* China	37.9	18.6	27.9	15.6	48	6.9	43.6	2.0	23	17.0	26.0	34.0	43	32.0	14.0	11.0
AB708968.1 *Hydrobasileus croceus* Japan	37.9	18.6	27.9	15.6	48	6.9	43.6	2.0	23	17.0	26.0	34.0	43	32.0	14.0	11.0
MZ081640.1 *Prodasineura verticalis* Kerala	33.2	19.3	30.9	16.6	36	11.9	47.5	5.0	22	16.0	30.0	32.0	42	30.0	15.0	13.0
Avg.	37.0	18.5	28.7	15.7	45	7.4	45.2	2.1	23	16.7	26.7	33.7	43	31.7	14.2	11.3

20) Phylogenetic analysis of the genus *Orthetrum*

Phylogeney of genus *Orthetrum* was resolved by using the sequence samples of *Orthetrum glaucum* and *Orthetrum luzonicum*, the 15 COI sequence samples retrieved from GenBank and out group sequence of the damselfly *Ceriagrion cerinorubellum*. The total sequence data was comprised of 18 sequences (Table 4.4.98; Figure 4.4.44).

Table 4.4.98: Details of COI gene sequences involved in the phylogenetic analysis of genus *Orthetrum*

Sl No.	Accession Number	Scientific Name	Product size
1.	MZ087263.1	*Orthetrum glaucum*, Kerala	696bp
2.	MZ092847.1	*Orthetrum luzonicum*, Kerala	692bp
3.	KU496893.1	*Orthetrum glaucum*, Malaysia	658bp
4.	MW208380.1	*Orthetrum cancellatum*, Austria	1607bp
5.	MT298551.1	*Orthetrum albistylum*, Italy	658bp
6.	MF774515.1	*Orthetrum testaceum*, China	691bp
7.	KU496887.1	*Orthetrum borneense*, Malaysia	658bp
8.	MT298569.1	*Orthetrum chrysostigma*, Morocco	658bp
9.	KX670387.1	*Orthetrum sabina*, Indonesia	700bp
10.	KU496894.1	*Orthetrum luzonicum*, Malaysia	658bp
11.	MW490473.1	*Orthetrum coerulescens*, Germany	658bp
12.	KC122236.1	*Orthetrum pruinosum*, Mizoram	654bp
13.	MN961328.1	*Orthetrum melania melania*, Japan	658bp
14.	MN609568.1	*Orthetrum japonicum*, South Korea	657bp
15.	MW490175.1	*Orthetrum brunneum*, Germany	658bp
16.	AB781568.1	*Orthetrum triangulare*, Malaysia	451bp
17.	KU496890.1	*Orthetrum chrysis*, Malaysia	658bp
18.	MZ882339.1	*Ceriagrion cerinorubellum*, Kerala	690bp

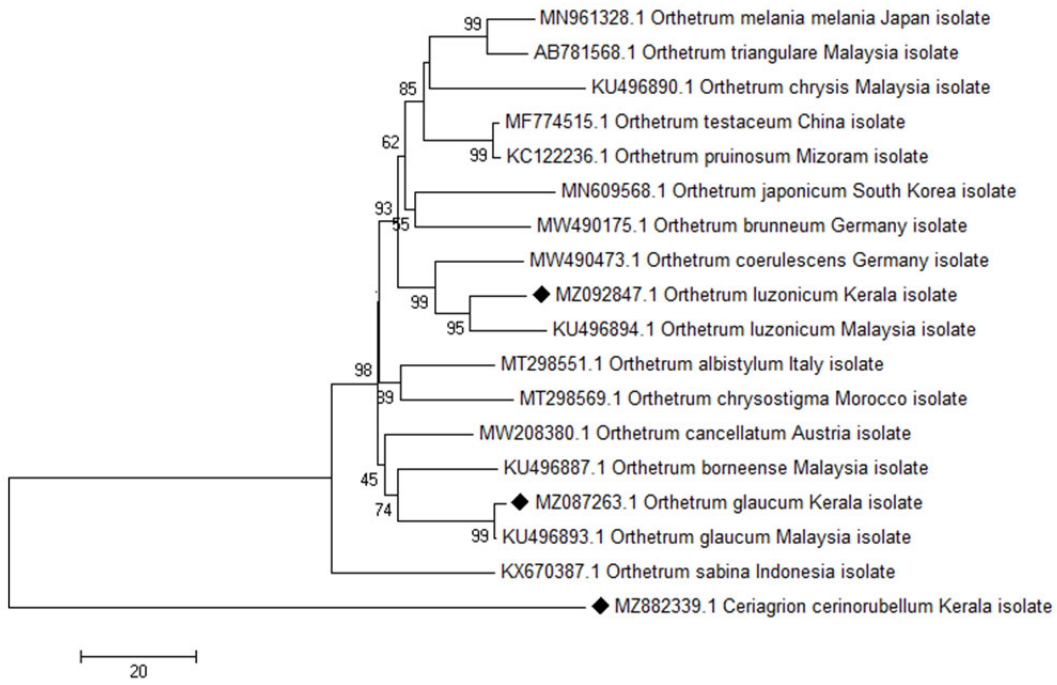

Figure 4.4.44: Inferred phylogenetic tree of the genus *Orthetrum*, rooted by outgroup

All the 6 species of genus *Orthetrum* found in Kerala except *Orthetrum taeniolatum* was included in the phylogenetic analysis. As the records of *Orthetrum taeniolatum* was unavailable it was excluded from the analysis. The result indicated that the 6 species of *Orthetrum* found in Kerala were polyphyletic and they were distantly placed in phylogenetic tree. *Orthetrum sabina* was diverged at an earlier stage from the common ancestor of *Orthetrum* species and it was paraphyletic to the remaining species. *Orthetrum glaucum* from Kerala showed high similarity with Malaysia specimen (99% bootstrap). The specimens of *Orthetrum luzonicum* from Kerala and Malaysia clustered with a boot strap support of 95%. However, similarity between them was less.

Intraspecific and interspecific divergence

The genetic divergence observed between the conspecifics of *Orthetrum glaucum* was 0.4% and this along with the phylogenetic tree result corroborated the authenticity of this species. 5.1% intraspecific divergence was observed between *Orthetrum luzonicum* samples from Kerala and Malaysia. *Orthetrum testaceum* was found to be very closer to *Orthetrum pruinosum* (0.4% divergence). The interspecific divergence values ranged from 0.4% to 15.5% (Table 4.4.99).

Nucleotide composition

The nucleotide composition of the 18 nucleotide sequences were 32.51 % (A), 33.37% (T/U), 18.50 % (C) and 15.62% (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The base composition of *Orthetrum glaucum* was T=35.0%, C=18.4%, A=30.8%, G=15.7% with a high AT bias (AT content= 65.8%, GC content= 34.1%). The base composition of *Orthetrum luzonicum* was T=33.7%, C=18.8%, A=31.7%, G=15.7% (AT content= 65.4%; GC content= 34.5%). The obtained values are presented in Table 4.4.100.

Table 4.4.99: Estimates of genetic divergence among COI gene sequences of genus *Orthetrum*

	Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
1	MZ087263.1 _*O glaucum*_																	
2	MZ092847.1_*O luzonicum*_	0.106																
3	KU496893.1_*O glaucum*_	0.004	0.102															
4	MW208380.1_*O cancellatum*	0.078	0.098	0.073														
5	MT298551.1_*O albistylum*_	0.082	0.102	0.078	0.071													
6	MF774515.1_*O testaceum*_	0.098	0.091	0.093	0.084	0.095												
7	KU496887.1_*O borneense*_	0.080	0.111	0.075	0.084	0.095	0.082											
8	MT298569.1_*O chrysostigma*	0.100	0.102	0.100	0.091	0.080	0.100	0.113										
9	KX670387.1_*O sabina*_	0.122	0.142	0.118	0.120	0.115	0.129	0.126	0.135									
10	KU496894.1_*O luzonicum*_	0.104	0.051	0.104	0.106	0.106	0.098	0.118	0.115	0.146								
11	MW490473.1_*O coerulescens*	0.104	0.055	0.100	0.104	0.100	0.082	0.111	0.093	0.140	0.091							
12	KC122236.1_*O pruinosum*_	0.098	0.086	0.093	0.084	0.095	0.004	0.086	0.104	0.124	0.093	0.086						
13	MN961328.1_*O melania*_	0.120	0.102	0.115	0.093	0.115	0.064	0.098	0.109	0.155	0.106	0.100	0.064					
14	MN609568.1_*O japonicum*_	0.122	0.113	0.120	0.100	0.109	0.089	0.118	0.104	0.137	0.115	0.113	0.089	0.104				
15	MW490175.1_*O brunneum*_	0.113	0.098	0.111	0.098	0.115	0.075	0.089	0.100	0.142	0.118	0.100	0.080	0.095	0.098			
16	AB781568.1_*O triangulare*_	0.106	0.100	0.102	0.086	0.104	0.071	0.089	0.118	0.142	0.106	0.093	0.075	0.033	0.113	0.095		
17	KU496890.1_*O chrysis*_	0.131	0.124	0.126	0.111	0.124	0.093	0.111	0.131	0.155	0.124	0.118	0.098	0.095	0.146	0.118	0.102	
18	MZ882339.1_*C cerinorubellum*	0.412	0.415	0.410	0.399	0.415	0.419	0.419	0.424	0.408	0.417	0.417	0.417	0.417	0.426	0.424	0.410	0.439

Table 4.4.100: Nucleotide base composition of COI gene sequence of genus *Orthetrum*

Species																
	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
MZ087263.1_*O glaucum* Kerala	35.0	18.4	30.8	15.7	40	6.0	52.3	1.3	23	18.7	26.7	31.3	41	30.7	13.3	14.7
MZ092847.1 _*O luzonicum* Kerala	33.7	18.8	31.7	15.7	36	7.9	53.6	2.0	23	18.0	28.0	30.7	41	30.7	13.3	14.7
KU496893.1 _*O glaucum* Malaysia	35.3	18.2	31.0	15.5	41	5.3	53.0	.7	23	18.7	26.7	31.3	41	30.7	13.3	14.7
MW208380.1_*O cancellatum* Austria	34.8	17.7	31.9	15.5	39	4.6	55.6	.7	24	18.0	26.7	31.3	41	30.7	13.3	14.7
MT298551.1_*O albistylum* Italy	33.0	19.1	31.3	16.6	35	7.3	53.6	4.0	23	19.3	26.7	31.3	41	30.7	13.3	14.7
MF774515.1_*O testaceum* China	35.9	18.4	30.2	15.5	43	6.0	50.3	.7	23	18.7	26.7	31.3	41	30.7	13.3	14.7
KU496887.1_*O borneense* Malaysia	33.9	19.7	30.4	16.0	38	8.6	51.0	2.0	22	20.0	26.7	31.3	41	30.7	13.3	14.7
MT298569.1_*O chrysostigma* Morocco	35.0	18.8	29.0	17.1	40	6.6	48.3	5.3	24	19.3	25.3	31.3	41	30.7	13.3	14.7
KX670387.1_*O sabina* Indonesia	33.7	19.3	30.4	16.6	36	9.3	51.0	4.0	24	18.0	26.7	31.3	41	30.7	13.3	14.7
KU496894.1_*O luzonicum* Malaysia	33.9	18.8	31.0	16.2	36	8.6	51.7	3.3	24	17.3	28.0	30.7	41	30.7	13.3	14.7
MW490473.1_*O coerulescens* Germany	33.3	19.1	31.3	16.4	34	9.3	53.6	3.3	25	17.3	26.7	31.3	41	30.7	13.3	14.7
KC122236.1_*O pruinosum* Mizoram	35.5	18.6	30.4	15.5	42	6.6	51.0	.7	23	18.7	26.7	31.3	41	30.7	13.3	14.7
MN961328.1_*O melania melania* Japan	34.6	19.7	30.4	15.3	39	8.6	51.7	.7	23	20.0	26.0	30.7	41	30.7	13.3	14.7
MN609568.1_*O japonicum* South Korea	31.3	20.4	31.7	16.6	32	8.6	55.0	4.0	20	22.0	26.7	31.3	41	30.7	13.3	14.7
MW490175.1_*O brunneum* Germany	33.5	19.7	30.4	16.4	38	7.9	51.0	3.3	21	20.7	26.7	31.3	41	30.7	13.3	14.7
AB781568.1_*O triangulare* Malaysia	34.8	19.1	30.8	15.3	40	6.6	53.0	.7	23	20.0	26.0	30.7	41	30.7	13.3	14.7
KU496890.1_*O chrysis* Malaysia	33.5	21.1	29.0	16.4	36	12.6	47.7	3.3	23	20.0	26.0	31.3	41	30.7	13.3	14.7
MZ882339.1_*C cerinorubellum* Kerala	19.8	7.9	63.7	8.6	18	2.6	76.3	3.3	17	7.9	59.6	15.2	25	13.2	55.0	7.3
Avg.	33.4	18.5	32.5	15.6	37	7.4	53.3	2.4	23	18.5	28.5	30.3	40	29.7	15.7	14.3

21) Phylogenetic analysis of the genus *Palpopleura*

Phylogenetic analysis of the genus *Palpopleura* based on COI was conducted by using the sequence of *Palpopleura sexmaculata*, four more sequences of the corresponding genus retrieved from GenBank and sequence of the damselfly *Agriocnemis splendidissima* as out group. A total of 6 COI sequences were used to resolve the phylogeny (Table 4.4.101; Figure 4.4.45).

Table 4.4.101: Details of COI gene sequences involved in the phylogenetic analysis of genus *Palpopleura*

Sl No.	**Accession Number**	**Scientific Name**	**Product size**
1	OK083552.1	*Palpopleura sexmaculata*, Kerala	581bp
2	MN159179.1	*Palpopleura sexmaculata*, Punjab	638bp
3	MN345066.1	*Palpopleura jucunda*, Malawi(Africa)	658bp
4	MN345612.1	*Palpopleura vestita*, Madagascar	658bp
5	MN344115.1	*Palpopleura lucia*, Malawi (Africa)	407bp
6	MN850441.1	*Agriocnemis splendidissima*, Kerala	647bp

Phylogeny of six sequences including four *Palpopleura* species and one out group was resolved with bootstrap values ranging from 71-99%. *Palpopleura sexmaculata* samples from Kerala and Punjab form sister clades, well supported by 99% bootstrap which authenticated the morphologic identity of this species. *Palpopleura sexmaculata* was closest to *Palpopleura jucunda* and then to *Palpopleura lucia. Palpopleura vestita* was diverged from the common ancestor at an earlier stage.

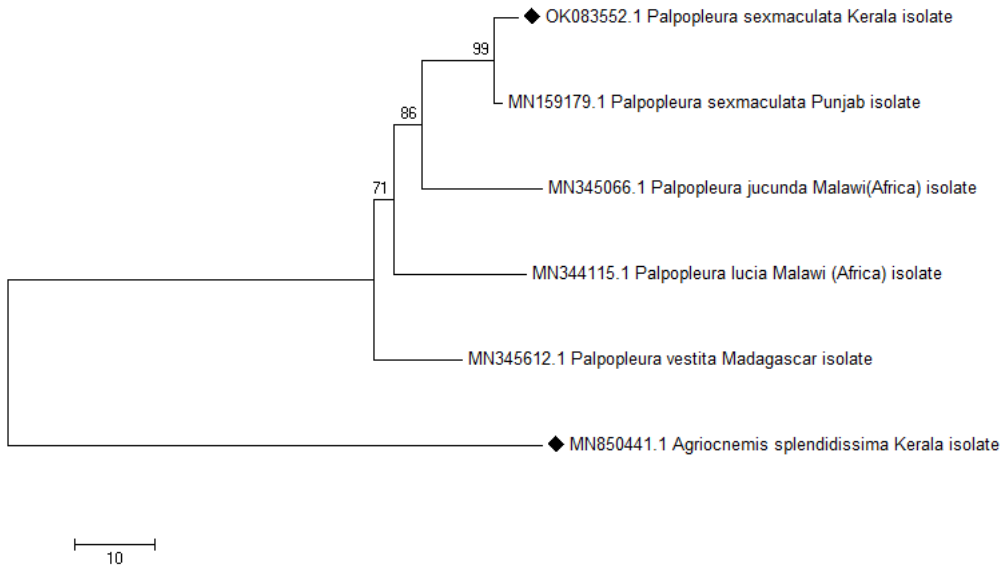

Figure 4.4.45: Inferred phylogenetic tree of the genus *Palpopleura*, rooted by outgroup

Intraspecific and interspecific divergence

The calculated intraspecific divergence between *Palpopleura sexmaculata* specimens from Kerala and Punjab was 1.3%. The interspecific divergence values ranged from 8.2% to 12.2% (Table 4.4.102).

Nucleotide composition

The nucleotide frequencies of 6 nucleotide sequences were 32.62 % (A), 33.83% (T/U), 16.56 % (C) and 17.00 % (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The base composition of *Palpopleura sexmaculata* was T=37.5%, C=18.4%, A= 26.3%, G=17.8% with an AT content of 63.8% and GC content of 36.2% (Table 4.4.103).

Table 4.4.102: Estimates of genetic divergence between COI gene sequences of genus *Palpopleura*

	Species	**1**	**2**	**3**	**4**	**5**
1	OK083552.1_*Palpopleura_sexmaculata*_Kerala					
2	MN159179.1_*Palpopleura_sexmaculata*_Punjab	0.013				
3	MN345066.1_*Palpopleura_jucunda*_Malawi(Africa)	0.089	0.082			
4	MN345612.1_*Palpopleura_vestita*_Madagascar	0.089	0.082	0.122		
5	MN344115.1_*Palpopleura_lucia*_Malawi_(Africa)	0.109	0.102	0.109	0.095	
6	MN850441.1_*Agriocnemis_splendidissima*_Kerala	0.431	0.424	0.428	0.405	0.434

Table 4.4.103: Nucleotide base composition of COI gene sequence of genus *Palpopleura*

Species																
	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
OK083552.1 *Palpopleura sexmaculata* Kerala	37.5	18.4	26.3	17.8	43	31.4	9.8	15.7	44	5.0	43.6	7.9	26	18.8	25.7	29.7
MN159179.1 *Palpopleura sexmaculata* Punjab	37.5	17.8	26.6	18.1	43	30.4	10.8	15.7	44	5.0	43.6	7.9	26	17.8	25.7	30.7
MN345066.1 *Palpopleura jucunda* Malawi	35.9	19.1	27.0	18.1	43	30.4	10.8	15.7	39	8.9	45.5	6.9	26	17.8	24.8	31.7
MN345612.1 *Palpopleura vestita* Madagascar	38.5	16.1	26.6	18.8	43	30.4	10.8	15.7	46	1.0	44.6	8.9	27	16.8	24.8	31.7
MN344115.1 *Palpopleura lucia* Malawi	35.9	18.1	27.0	19.1	43	30.4	10.8	15.7	39	5.9	45.5	9.9	26	17.8	24.8	31.7
MN850441.1 *Agriocnemis splendidissima* Kerala	17.8	9.9	62.2	10.2	25	14.7	50.0	9.8	13	3.0	79.2	5.0	15	11.9	57.4	15.8
Avg.	33.8	16.6	32.6	17.0	40	27.9	17.2	14.7	37	4.8	50.3	7.8	24	16.8	30.5	28.5

22) Phylogenetic analysis of the genus *Rhodothemis*

Phylogeny of the genus *Rhodothemis* based on partial COI gene sequence was resolved by using the sequence of *Rhodothemis rufa*, four sample sequences of conspecifics downloaded from GenBank and sequence of the damselfly *Aciagrion approximans krishna* as out group. Sequences of non-conspecifics were not available in the databases hence five sequences of single species and out group was incorporated in phylogenetic reconstruction (Table 4.4.104; Figure 4.4.46).

Table 4.4.104: Details of COI gene sequences involved in the phylogenetic analysis of genus *Rhodothemis*

Sl No.	**Accession Number**	**Scientific Name**	**Product size**
1	OK083604.1	*Rhodothemis rufa,* Kerala	640bp
2	KX281843.1	*Rhodothemis rufa*, Malaysia	658bp
3	MH019983.1	*Rhodothemis rufa,* Bangladesh	641bp
4	MF774531.1	*Rhodothemis rufa*, Pakistan	643bp
5	KJ873228.1	*Rhodothemis rufa*, Austria	510bp
6	MW246065.1	*Aciagrion approximans krishna*, Kerala	670bp

Phylogeny of 5 COI sequence samples of geographically different *Rhodothemis rufa* individuals has been resolved and the result suggested that specimens from Kerala, Bangladesh and Austria were highly similar. Specimen from Malaysia also showed close resemblance with 100% bootstrap. Specimen from Pakistan was distantly placed was found as paraphyletic to them.

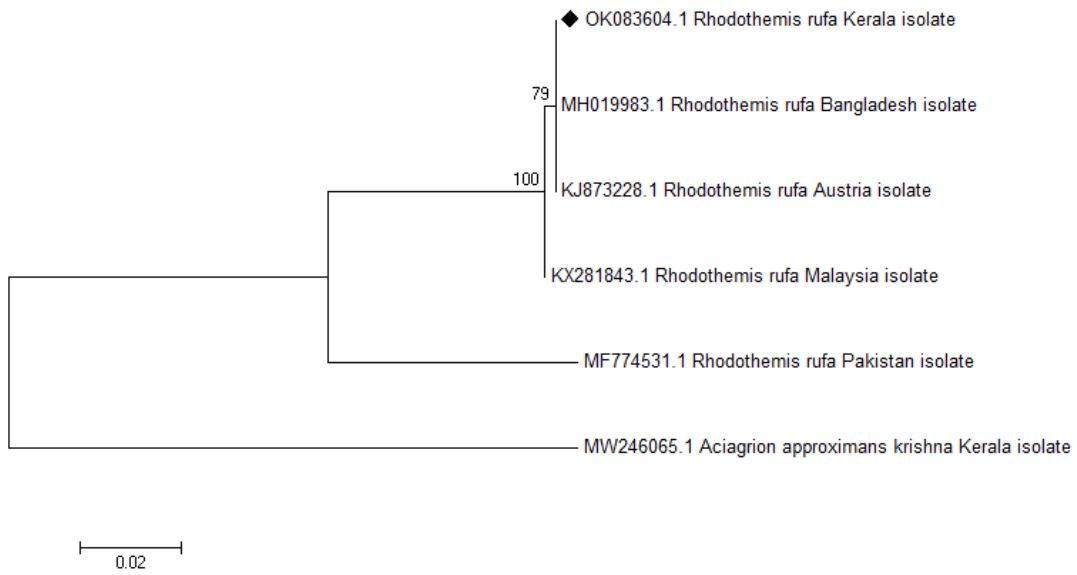

Figure 4.4.46: Inferred phylogenetic tree of the genus *Rhodothemis*, rooted by outgroup

Intraspecific and interspecific divergence

The intraspecific divergence between *Rhodothemis rufa* specimens of Kerala, Bangladesh, Austria and Malaysia was ranged from 0% -0.2%. But the specimen from Pakistan showed a high percentage of divergence ranged from 8.5% to 8.8% from the other specimens. More samples from Pakistan is required to be analysed to confirm the authenticity of the same. Interspecific divergence between *Rhodothemis rufa* and *Aciagrion approximans krishna* was calculated (Table 4.4.105).

Nucleotide composition

The nucleotide composition of 6 nucleotide sequences were 29.24 % (A), 35.28% (T/U), 18.40 % (C) and 17.08 % (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The base composition of *Rhodothemis rufa* were T=36.0%, C=17.9%, A=29.0%, G=17.1% with a high AT content (65%) over GC content (35%). The values are given in Table 4.4.106.

Table 4.4.105: Estimates of genetic divergence among COI gene sequences of genus *Rhodothemis*

	Species	**1**	**2**	**3**	**4**	**5**
1.	OK083604.1_*Rhodothemis_rufa*_Kerala					
2.	KX281843.1_*Rhodothemis_rufa*_Malaysia	0.002				
3.	MH019983.1_*Rhodothemis_rufa*_Bangladesh	0.000	0.002			
4.	MF774531.1_*Rhodothemis_rufa*_Pakistan	0.088	0.085	0.088		
5.	KJ873228.1_*Rhodothemis_rufa*_Austria	0.000	0.002	0.000	0.088	
6.	MW246065.1_*Aciagrion_approximans_krishna*_Kerala	0.188	0.185	0.188	0.190	0.188

Table 106: Nucleotide base composition of COI gene sequence of genus *Rhodothemis*

Species																
	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
OK083604.1 *Rhodothemis rufa* Kerala	36.0	17.9	29.0	17.1	26	15.6	26.9	31.3	42	29.4	12.5	16.3	40	8.8	47.5	3.8
KX281843.1 *Rhodothemis rufa* Malaysia	36.0	17.9	29.2	16.9	26	15.6	26.9	31.3	42	29.4	12.5	16.3	40	8.8	48.1	3.1
MH019983.1 *Rhodothemis rufa* Bangladesh	36.0	17.9	29.0	17.1	26	15.6	26.9	31.3	42	29.4	12.5	16.3	40	8.8	47.5	3.8
MF774531.1 *Rhodothemis rufa* Pakistan	34.4	20.0	29.0	16.7	27	16.9	27.5	28.8	42	29.4	12.5	16.3	34	13.8	46.9	5.0
KJ873228.1 *Rhodothemis rufa* Austria	36.0	17.9	29.0	17.1	26	15.6	26.9	31.3	42	29.4	12.5	16.3	40	8.8	47.5	3.8
MW246065.1 *Aciagrion approximans krishna* Kerala	33.1	18.8	30.4	17.7	24	16.3	29.4	30.6	42	29.4	12.5	16.3	34	10.6	49.4	6.3
Avg.	35.3	18.4	29.2	17.1	26	15.9	27.4	30.7	42	29.4	12.5	16.3	38	9.9	47.8	4.3

23) Phylogenetic analysis of the genus *Tetrathemis*

Five partial COI gene sequences were used for the phylogenetic reconstruction of genus *Tetrathemis*. Along with the current COI sequence of *Tetrathemis platyptera*, 3 COI sequences of conspecifics and non-conspecifics were retrieved from GenBank and the sequence of damselfly *Dysphaea ethela* was included as out group (Table 107; Figure 4.4.47).

Table 4.4.107: Details of COI gene sequences involved in the phylogenetic analysis of genus *Tetrathemis*

Sl No.	Accession Number	Scientific Name	Product size
1	MZ092924.1	*Tetrathemis platyptera*, Kerala	688bp
2	KC122235.1	*Tetrathemis platyptera*, Mizoram	669bp
3	MN344139.1	*Tetrathemis platyptera*, Thailand	307bp
4	KJ873236.1	*Tetrathemis irregularis*, Austria	576bp
5	MN882704.1	*Dysphaea ethela*, Kerala	677bp

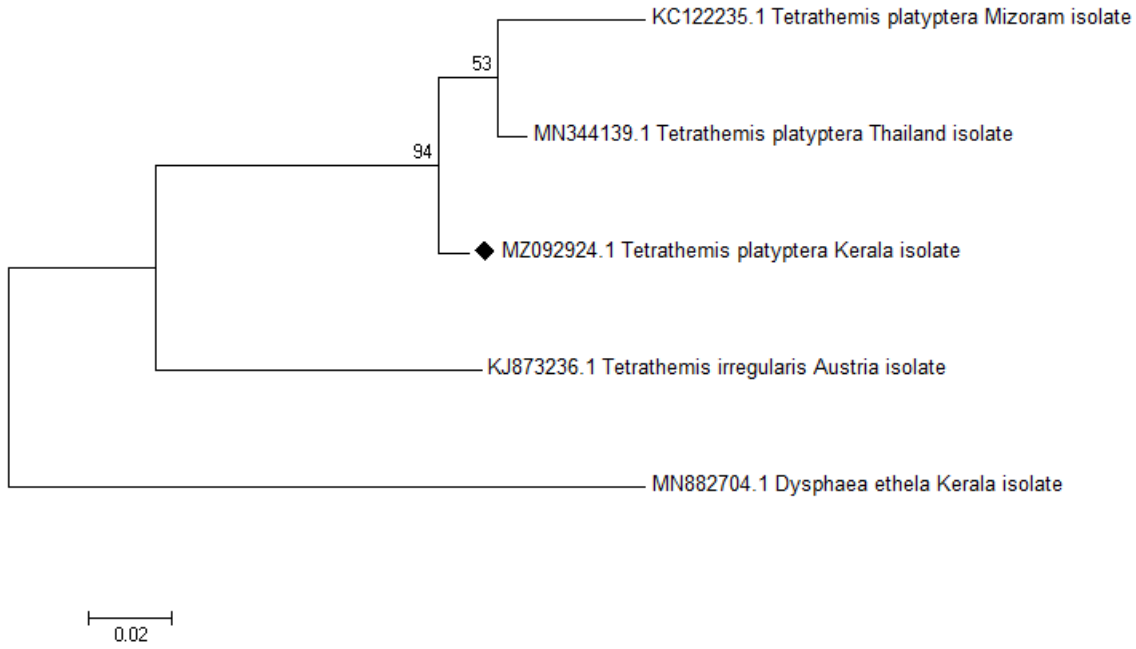

Figure 4.4.47: Inferred phylogenetic tree of the genus *Tetrathemis*, rooted by outgroup

Monophyly was observed between three *Tetrathemis platyptera* samples from Kerala, Mizoram and Thailand with 94% bootstrap support. Samples from Mizoram and Thailand form sister clade with each other. Variations existed among the three specimens which denoted that change has occurred in the gene sequence of *Tetrathemis platyptera* specimens as a result of geographic isolation. *Tetrathemis irregularis* was paraphyletic to *Tetrathemis platyptera*.

Intraspecific and interspecific divergence

The calculated intraspecific divergence values of *Tetrathemis platyptera* ranged from 2.8% to 5.5%. The reason for this elevated intraspecific divergence values may be revealed after a detailed study. The interspecific values ranged from 13.8% to 15.2% (Table 4.4.108).

Nucleotide composition

The nucleotide frequencies of the 5 nucleotide sequences were 32.41 % (A), 33.38% (T/U), 19.45 % (C) and 14.76 % (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The base composition of *Tetrathemis platyptera* was T=35.2%, C=17.9%, A=32.4%, G=14.5%. High AT bias was observed with an AT content of 67.6% and GC content of 32.4% (Table 4.4.109).

Table 4.4.108: Estimates of genetic divergence among COI gene sequences of genus *Tetrathemis*

	Species	**1**	**2**	**3**	**4**
1	MZ092924.1_*Tetrathemis_platyptera*_Kerala				
2	KC122235.1_*Tetrathemis_platyptera*_Mizoram	0.055			
3	MN344139.1_*Tetrathemis_platyptera*_Thailand	0.028	0.041		
4	KJ873236.1_*Tetrathemis_irregularis*_Austria	0.138	0.152	0.152	
5	MN882704.1_*Dysphaea_ethela*_Kerala	0.221	0.221	0.221	0.221

Table 4.4.109: Nucleotide base composition of COI gene sequences of genus *Tetrathemis*

Species																
	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
MZ092924.1 *Tetrathemis platyptera* Kerala	35.2	17.9	32.4	14.5	20	24.5	34.7	20.4	48	22.9	8.3	20.8	38	6.3	54.2	2.1
KC122235.1 *Tetrathemis platyptera* Mizoram	34.5	20.0	31.0	14.5	22	24.5	32.7	20.4	48	22.9	8.3	20.8	33	12.5	52.1	2.1
MN344139.1 *Tetrathemis platyptera* Thailand	33.8	19.3	31.0	15.9	20	24.5	34.7	20.4	48	22.9	8.3	20.8	33	10.4	50.0	6.3
KJ873236.1 *Tetrathemis irregularis* Austria	35.2	17.9	31.7	15.2	22	22.4	34.7	20.4	48	22.9	8.3	20.8	35	8.3	52.1	4.2
MN882704.1 *Dysphaea ethela* Kerala	28.3	22.1	35.9	13.8	22	24.5	34.7	18.4	48	22.9	8.3	20.8	15	18.8	64.6	2.1
Avg.	33.4	19.4	32.4	14.8	22	24.1	34.3	20.0	48	22.9	8.3	20.8	31	11.3	54.6	3.3

24) Phylogenetic analysis of the genus *Tholymis*

In addition to the current COI sequence of *Tholymis tillarga*, 7 more COI sequences of conspecifics and non-conspecifics were downloaded from GenBank for phylogenetic reconstruction of the corresponding genus. Sequence of the damselfly *Copera vittata* was included as out group. The sequence data was comprised of 9 COI sequences (Table 4.4.110; Figure 4.4.48).

Table 4.4.110: Details of COI gene sequences involved in the phylogenetic analysis of genus *Tholymis*

Sl No.	Accession Number	Scientific Name	Product size
1.	MZ127380.1	*Tholymis tillarga*, Kerala	700bp
2.	KJ499454.1	*Tholymis tillarga*, Mizoram	675bp
3.	KT957512.1	*Tholymis tillarga*, Thailand	657bp
4.	KX055060.1	*Tholymis tillarga*, France	658bp
5.	MH019978.1	*Tholymis tillarga*, Bangladesh	630bp
6.	MF774556.1	*Tholymis tillarga*, China	601bp
7.	MF358751.1	*Tholymis citrina,* China	680bp
8.	KJ994784.1	*Tholymis citrina*, Austria	686bp
9.	MZ895506.1	*Copera vittata*, Kerala	691bp

The result depicted the monophyly among the *Tholymis tillarga* samples from 6 different locations with 99% bootstrap support. Of the 6 samples, samples from Kerala, Bangladesh, Mizoram and Thailand were closely similar. However, samples from France and China showed considerable sequence diversion. The two *Tholymis citrina* samples formed monophyletic clade.

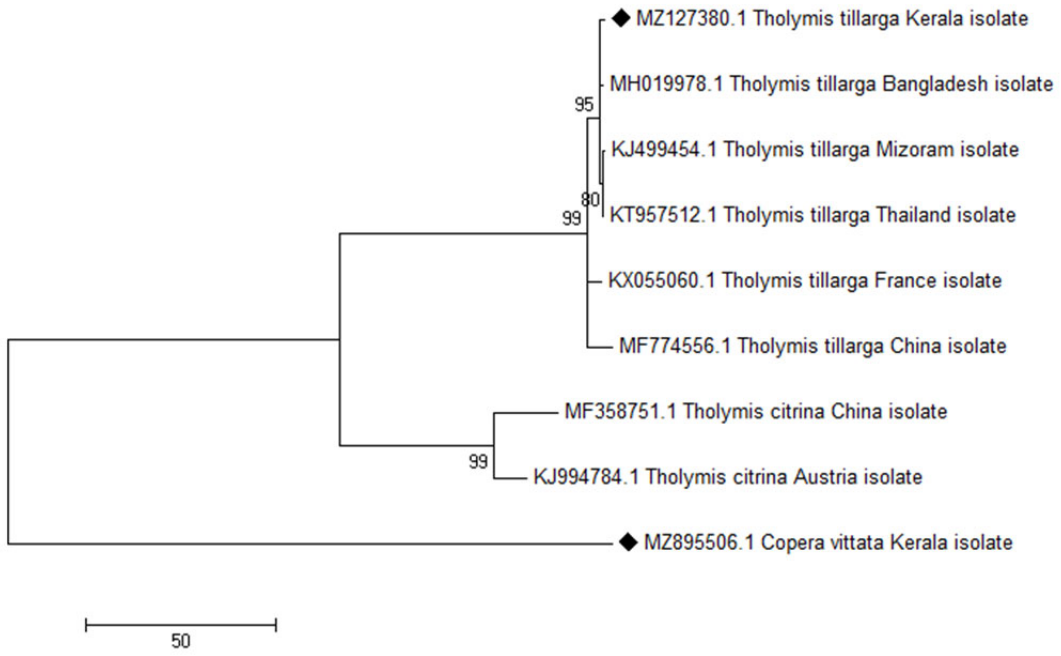

Figure 4.4.48: Inferred phylogenetic tree based on COI gene sequences of *Tholymis* species and out group

Intraspecific and interspecific divergence

The intraspecific divergence values of *Tholymis tillarga* ranged from 0.4% to 4.2%. Higher percentage of divergence was showed by France and China specimens. The intraspecific divergence between *Tholymis citrina* specimens was 6.0%. The interspecific divergence ranged from 39.7% to 47.2% (Table 4.4.111).

Nucleotide composition

The nucleotide frequencies (Table 4.4.112) of the 10 nucleotide sequences were 34.03 % (A), 31.41% (T/U), 17.99 % (C) and 16.58 % (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The base composition of *Tholymis tillarga* was T=33.3%, C=17.9%, A=30.9%, G=17.9% with a high AT content (64.2%) over GC content(35.8%).

Table 4.4.111: Estimates of genetic divergence among COI gene sequences of genus *Tholymis*

	Species	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**
1.	MZ127380.1_*Tholymis_tillarga*_Kerala								
2.	KJ499454.1_*Tholymis_tillarga*_Mizoram	0.005							
3.	KT957512.1_*Tholymis_tillarga*_Thailand	0.005	0.004						
4.	KX055060.1_*Tholymis_tillarga*_France	0.018	0.020	0.020					
5.	MH019978.1_*Tholymis_tillarga*_Bangladesh	0.005	0.007	0.007	0.020				
6.	MF774556.1_*Tholymis_tillarga*_China	0.039	0.038	0.042	0.040	0.042			
7.	MF358751.1_*Tholymis_citrina*_China	0.445	0.443	0.443	0.443	0.439	0.472		
8.	KJ994784.1_*Tholymis_citrina*_Austria	0.403	0.401	0.401	0.406	0.397	0.428	0.060	
9.	MZ895506.1_*Copera_vittata*_Kerala	0.269	0.263	0.269	0.255	0.266	0.281	0.391	0.355

Table 4.4.112: Nucleotide base composition of COI gene sequence of genus *Tholymis*

Species																
	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
MZ127380.1 *Tholymis tillarga* Kerala	33.3	17.9	30.9	17.9	39	23.8	17.5	20.1	39	12.8	41.0	7.4	22	17.1	34.2	26.2
KJ499454.1 *Tholymis tillarga* Mizoram	33.3	18.3	30.7	17.7	39	24.3	17.5	19.6	39	13.3	40.4	7.4	22	17.1	34.2	26.2
KT957512.1 *Tholymis tillarga* Thailand	33.3	18.3	30.7	17.7	39	24.3	17.5	19.6	38	13.3	41.0	7.4	23	17.1	33.7	26.2
KX055060.1 *Tholymis tillarga* France	33.2	17.9	31.4	17.6	39	23.8	17.5	19.6	38	12.8	42.6	6.9	22	17.1	34.2	26.2
MH019978.1*Tholymis tillarga* Bangladesh	33.3	18.1	31.2	17.4	39	24.3	17.5	19.6	39	12.8	42.0	6.4	22	17.1	34.2	26.2
MF774556.1 *Tholymis tillarga* China	33.0	17.9	31.2	17.9	38	24.9	18.5	19.0	39	11.2	42.0	7.4	22	17.6	33.2	27.3
MF358751.1 *Tholymis citrina* China	37.4	16.0	33.3	13.3	41	21.7	20.1	17.5	43	13.8	38.8	4.3	28	12.3	41.2	18.2
KJ994784.1 *Tholymis citrina* Austria	37.9	16.3	32.1	13.7	43	21.7	18.0	17.5	43	14.4	38.8	4.3	28	12.8	39.6	19.3
MZ895506.1 *Copera vittata* Kerala	22.5	12.2	54.4	10.9	20	16.4	46.4	17.3	29	12.8	53.2	4.6	18	7.3	63.6	10.9
Avg.	33.5	17.4	32.7	16.4	38	23.3	19.6	19.1	39	13.2	41.1	6.6	23	15.5	37.3	23.8

25) Phylogenetic analysis of the genus *Tramea*

For resolving phylogeny of genus *Tramea*, along with the current sequence of *Tramea limbata*, 7 COI sequences of conspecifics and non-conspecifics of the corresponding genus were retrieved from GenBank and the sequence of damselfly *Agriocnemis pieris* was included as out group. A total of 9 species were involved in the phylogenetic analysis (Table 4.4.113; Figure 4.4.49).

Table 4.4.113: Details of COI gene sequences involved in the phylogenetic analysis of genus *Tramea*

Sl No.	Accession Number	Scientific Name	Product size
1.	MZ076547.1	*Tramea limbata*, Kerala	671bp
2.	KX055147.1	*Tramea limbata*, France	658bp
3.	KX055146.1	*Tramea limbata*, France	658bp
4.	KY947461.1	*Tramea abdominalis*, Brazil	658bp
5.	KC122231.1	*Tramea basilaris*, Mizoram	645bp
6.	JF839443.1	*Tramea lacerata*, Canada	658bp
7.	LC365693.1	*Tramea transmarina*, Japan	451bp
8.	AB709204.1	*Tramea loewii*, Japan	451bp
9.	MN850440.1	*Agriocnemis pieris*, Kerala	627bp

The result indicated that, *Trama limbata* was monophyletic to *Tramea transmarina* and *Tramea loewii* with 93% bootstrap support. *Tramea limbata* from Kerala showed diversion from samples of France. This indicated the variations occurred in the gene sequence due to geographical isolation. *Tramea basilaris* diverged from the common ancestor earlier. *Tramea abdominalis* and *Tramea lancerata* formed sister clades.

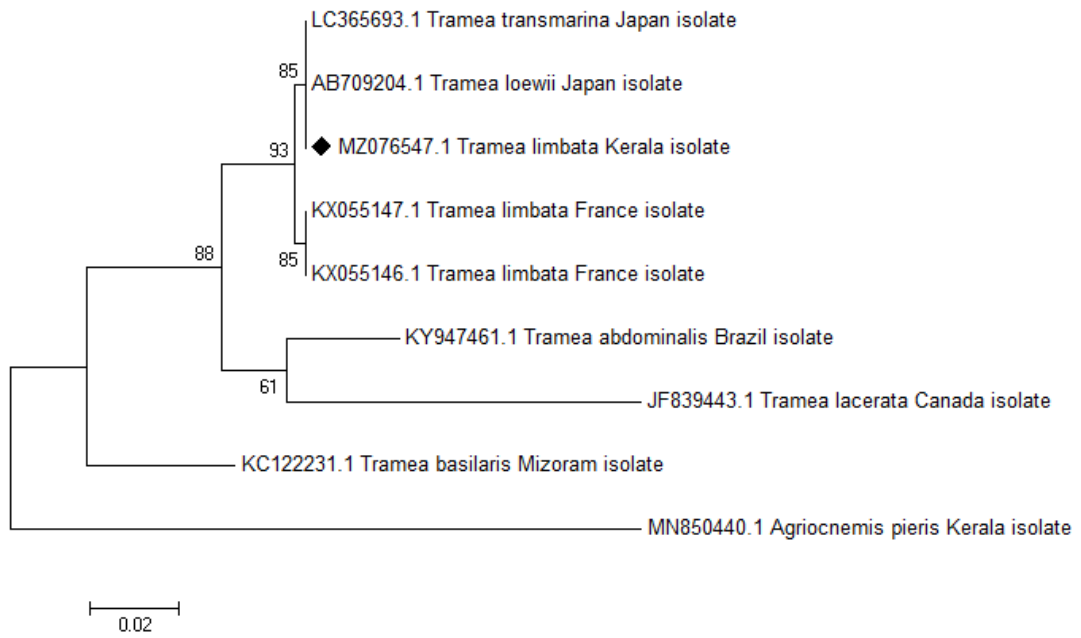

Figure 4.4.49: Inferred phylogenetic tree based on COI gene sequences of *Tramea* species and out group

Intraspecific and interspecific divergence

The calculated intraspecific divergence values (Table 4.4.114) showed a close similarity of *Tramea limbata* Kerala specimen with *Tramea transmarina* and *Tramea loewii* (0% divergence). 0.5% divergence could be observed between Kerala and France specimens of *Tramea limbata*. The monophyly of these species in the phylogenetic tree supported the divergence values. The maximum value of interspecific divergence was found between *Tramea lancerata* and *Tramea basilaris* (13.4%).

Nucleotide composition

The nucleotide composition of nine sequences were 29.57 % (A), 35.48 % (T/U), 18.27 % (C) and 16.68% (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The base composition of *Tramea limbata* was T=36.0%, C=18.1%, A=28.9%, G=16.9%. High AT bias was found with an AT content of 64.9% and GC content of 35% (Table 4.4.115).

Table 4.4.114: Estimates of genetic divergence among COI gene sequences of genus *Tramea*

	Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1.	MZ076547.1_*Tramea_limbata*_Kerala								
2.	KX055147.1_*Tramea_limbata*_France	0.005							
3.	KX055146.1_*Tramea_limbata*_France	0.005	0.000						
4.	KY947461.1_*Tramea_abdominalis*_Brazil	0.053	0.057	0.057					
5.	KC122231.1_*Tramea_basilaris*_Mizoram	0.076	0.072	0.072	0.091				
6.	JF839443.1_*Tramea_lacerata*_Canada	0.098	0.098	0.098	0.098	0.134			
7.	LC365693.1_*Tramea_transmarina*_Japan	0.000	0.005	0.005	0.053	0.076	0.098		
8.	AB709204.1_*Tramea_loewii*_Japan	0.000	0.005	0.005	0.053	0.076	0.098	0.000	
9.	MN850440.1_*Agriocnemis_pieris*_Kerala	0.179	0.179	0.179	0.177	0.169	0.205	0.179	0.179

Table 4.4.115: Nucleotide base composition of COI gene sequence of genus *Tramea*

Species																
	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
MZ076547.1 *Tramea limbata* Kerala	36.0	18.1	28.9	16.9	39	7.9	47.1	6.4	26	15.7	27.9	30.0	43	30.9	11.5	14.4
KX055147.1 *Tramea limbata* France	35.8	18.4	29.1	16.7	38	8.6	47.9	5.7	26	15.7	27.9	30.0	43	30.9	11.5	14.4
KX055146.1 *Tramea limbata* France	35.8	18.4	29.1	16.7	38	8.6	47.9	5.7	26	15.7	27.9	30.0	43	30.9	11.5	14.4
KY947461.1 *Tramea abdominalis* Brazil	36.5	17.7	29.8	16.0	39	7.9	50.0	3.6	28	14.3	27.9	30.0	43	30.9	11.5	14.4
KC122231.1 *Tramea basilaris* Mizoram	34.8	17.9	30.3	16.9	34	7.1	51.4	7.1	27	15.7	27.1	30.0	43	30.9	12.2	13.7
JF839443.1 *Tramea lacerata* Canada	33.7	20.0	30.1	16.2	34	11.4	50.0	4.3	24	17.9	28.6	30.0	43	30.9	11.5	14.4
LC365693.1 *Tramea transmarina* Japan	36.0	18.1	28.9	16.9	39	7.9	47.1	6.4	26	15.7	27.9	30.0	43	30.9	11.5	14.4
AB709204.1 *Tramea loewii* Japan isolate	36.0	18.1	28.9	16.9	39	7.9	47.1	6.4	26	15.7	27.9	30.0	43	30.9	11.5	14.4
MN850440.1 *Agriocnemis pieris* Kerala	34.6	17.7	31.0	16.7	35	6.4	52.9	5.7	26	17.1	27.9	29.3	43	29.5	12.2	15.1
Avg.	35.5	18.3	29.6	16.7	37	8.2	49.0	5.7	26	16.0	27.9	29.9	43	30.8	11.7	14.4

26) Phylogenetic analysis of the genus *Urothemis*

Phylogeny of genus *Urothemis* was resolved based on 6 COI partial gene sequences. In addition to the current COI sequence of *Urothemis signata*, 4 COI sequences of the conspecifics and non-conspecifics of the corresponding genus were downloaded from GenBank and the sequence of damselfly *Ischnura rubilio* was included as out group. (Table 4.4.116, Figure 4.4.50).

Table 4.4.116: Details of COI gene sequences involved in the phylogenetic analysis of genus *Urothemis*

Sl No.	Accession Number	Scientific Name	Product size
1.	MZ895798.1	*Urothemis signata*, Kerala	688bp
2.	MN345156.1	*Urothemis signata signata*, Thailand	658bp
3.	KU566464.1	*Urothemis venata*, Gabon(Africa)	658bp
4.	KU566469.1	*Urothemis venata*, Sierra Leone (Africa)	658bp
5.	MN345375.1	*Urothemis signata signata*, Sri Lanka	371bp
6.	MN850442.1	*Ischnura rubilio*, Kerala	670bp

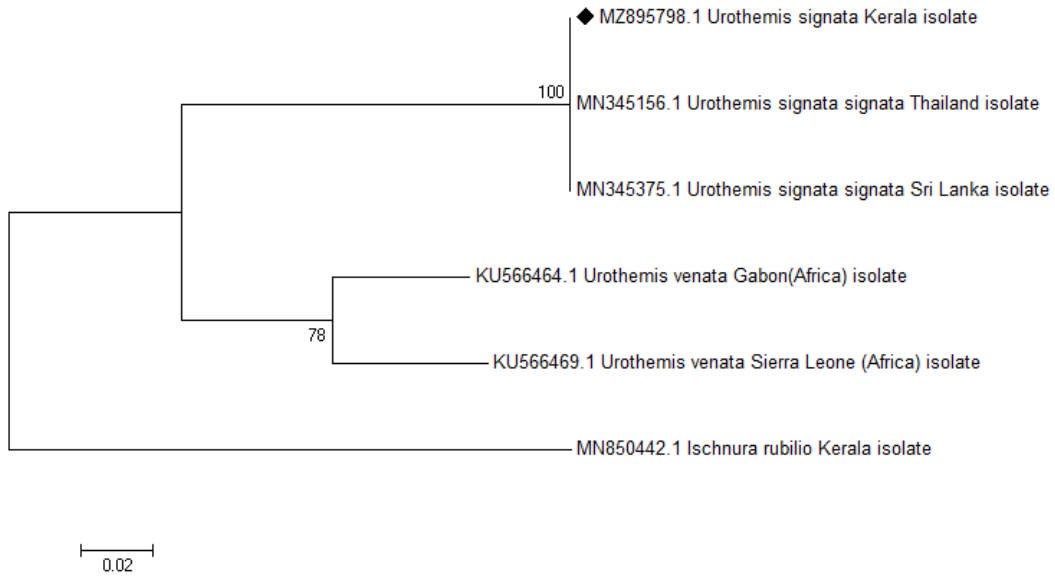

Figure 4.4.50: Inferred phylogenetic tree based on COI gene sequences of *Urothemis* species and out group

The authenticity of the species *Urothemis signata* was confirmed by the monophyly formed between samples from Kerala, Thailand and Sri Lanka with a bootstrap value of 100. *Urothemis venata* is paraphyletic to *Urothemis signata* and formed a separate clade.

Intraspecific and interspecific divergence

No intraspecific divergence was observed among the conspecifics of *Urothemis signata* from three different geographical regions (Kerala, Sri Lanka and Thailand). This value along with the phylogenetic tree result corroborated that no significant change has occurred in the gene sequence of this species by geographical isolation. The intraspecific divergence between *Urothemis venata* specimens was 7.5%. The interspecific divergence values ranged from 15.4% to 16.9% (Table 4.4.117).

Nucleotide composition

The nucleotide frequencies of the 6 sequences were 30.93 % (A), 32.81% (T/U), 18.29% (C) and 17.97% (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The base composition of *Urothemis signata* was T=32.8%, C=19.5%, A=29.5%, G=18.2%. The observed AT content was 62.3% over GC content of 37.7% (Table 4.4.118).

Table 4.4.117: Estimates of genetic divergence between COI gene sequences of genus *Urothemis*

	Species	**1**	**2**	**3**	**4**	**5**
1.	MZ895798.1_*Urothemis_signata*_Kerala					
2.	MN345156.1_*Urothemis_signata_signata*_Thailand	0.000				
3.	KU566464.1_*Urothemis_venata*_Gabon(Africa)	0.154	0.154			
4.	KU566469.1_*Urothemis_venata*_Sierra_Leone_(Africa)	0.169	0.169	0.075		
5.	MN345375.1_*Urothemis_signata_signata*_Sri_Lanka	0.000	0.000	0.154	0.169	
6.	MN850442.1_*Ischnura_rubilio*_Kerala	0.254	0.254	0.232	0.219	0.254

Table 4.4.118: Nucleotide base composition of COI gene sequence of genus *Urothemis*

Species																
	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
MZ895798.1 *Urothemis signata* Kerala	32.8	19.5	29.5	18.2	25	14.8	37.7	23.0	32	26.6	16.9	24.2	41	17.1	34.1	7.3
MN345156.1 *Urothemis signata signata* Thailand	32.8	19.5	29.3	18.4	25	14.8	36.9	23.8	32	26.6	16.9	24.2	41	17.1	34.1	7.3
KU566464.1 *Urothemis venata* Gabon	32.2	20.3	28.7	18.7	26	15.6	34.4	23.8	31	27.4	17.7	23.4	39	17.9	34.1	8.9
KU566469.1 *Urothemis venata* Sierra Leone	33.3	19.5	28.7	18.4	29	13.1	34.4	23.8	31	28.2	16.9	24.2	41	17.1	35.0	7.3
MN345375.1 *Urothemis signata signata* Sri Lanka	32.8	19.5	29.5	18.2	25	14.8	37.7	23.0	32	26.6	16.9	24.2	41	17.1	34.1	7.3
MN850442.1 *Ischnura rubilio* Kerala	29.5	16.6	39.3	14.6	22	14.0	43.3	20.4	28	24.8	27.4	19.7	38	10.8	47.1	3.8
Avg.	32.1	19.0	31.2	17.6	25	14.5	37.7	22.8	31	26.6	19.2	23.2	40	15.9	36.9	6.9

27) Phylogenetic analysis of the genus *Zyxomma*

For the phylogenetic reconstruction of genus *Zyxomma*, 5 partial COI gene sequences were used which include the current sequence of *Zyxomma petiolatum*, 3 partial COI gene sequences of the corresponding genus were retrieved from GenBank and the sequence of damselfly *Pseudagrion indicum* as out group (Table 4.4.119; Figure 4.4.51).

Table 4.4.119: Details of COI gene sequences involved in the phylogenetic analysis of genus *Zyxomma*

Sl No.	Accession Number	Scientific Name	Product size
1.	MZ093432.1	*Zyxomma petiolatum*, Kerala	677bp
2.	MK534739.1	*Zyxomma petiolatum,* India	609bp
3.	AB709240.1	*Zyxomma petiolatum*, Japan	451bp
4.	AB709239.1	*Zyxomma obtusum*, Japan	451bp
5.	MN882703.1	*Pseudagrion indicum*, Kerala	649bp

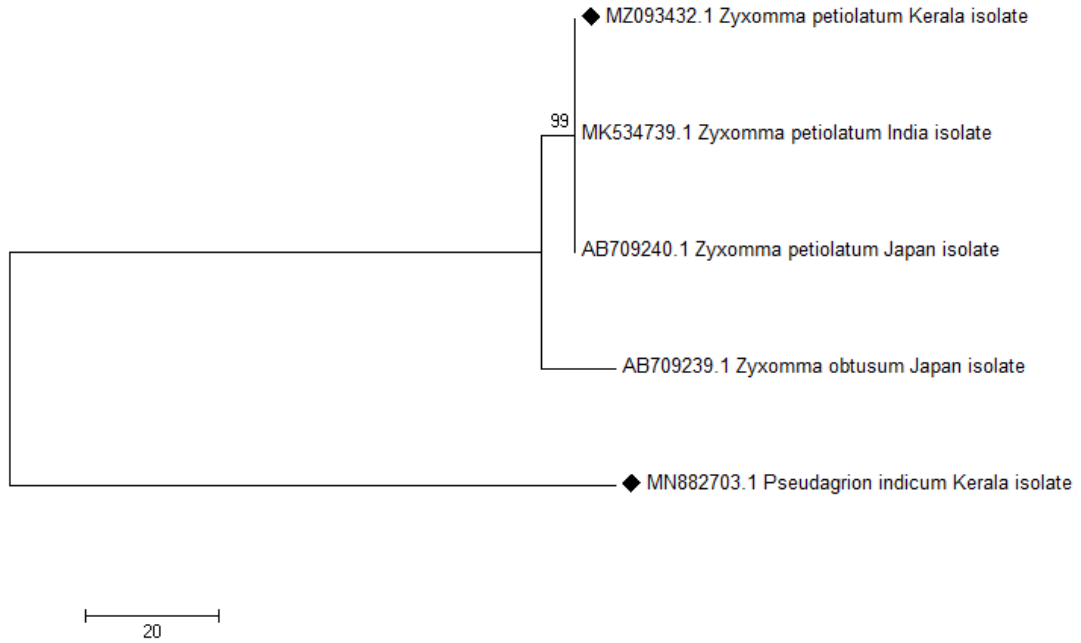

Figure 4.4.51: Inferred phylogenetic tree based on COI gene sequences of *Zyxomma* species and out group

From the result, it was clear that morphologic identity of *Zyxomma petiolatum* was well supported by the monophyly with 99% bootstrap support observed between the samples from differerent geographic locations. *Zyxomma obtusum* was in paraphyletic relationship with *Zyxomma petiolatum*. The species authenticity of *Zyxomma petiolatum* was well supported by the phylogenetic tree.

Intraspecific and interspecific divergence

Intraspecific divergence among the specimens of *Zygxomma petiolatum* from three different locations was 0%. This authenticated the identity of this species. The interspecific divergence between *Zyxomma petiolatum* and *Zyxomma obtusum* was 3.6% (Table 4.4.120) .

Nucleotide composition

The nucleotide composition of 5 sequences were 40.27 % (A), 27.87% (T/U), 16.76 % (C) and 15.10% (G). Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated. The base composition of *Zyxomma petiolatum* was T=30.8%, C=18.2%, A=34.6%, G=16.4%. High AT bias was observed with AT content of 65.4% and GC content of 34.6% (Table 4.4.121).

Table 4.4.120: Estimates of genetic divergence among COI gene sequences of genus *Zyxomma*

	Species	**1**	**2**	**3**	**4**
1	MZ093432.1_*Zyxomma_petiolatum*, Kerala				
2	MK534739.1_*Zyxomma_petiolatum*, India	0.000			
3	AB709240.1_*Zyxomma_petiolatum*, Japan	0.000	0.000		
4	AB709239.1_*Zyxomma_obtusum*, Japan	0.036	0.036	0.036	
5	MN882703.1_*Pseudagrion_indicum,* Kerala	0.396	0.396	0.396	0.409

Table 4.4.121: Nucleotide base composition of COI gene sequence of genus *Zyxomma*

Species																
	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
MZ093432.1 *Zyxomma petiolatum* , Kerala	30.8	18.2	34.6	16.4	26	9.4	63.1	2.0	26	14.9	27.7	31.8	41	30.4	12.8	15.5
MK534739.1 *Zyxomma petiolatum,* India	30.8	18.2	34.6	16.4	26	9.4	63.1	2.0	26	14.9	27.7	31.8	41	30.4	12.8	15.5
AB709240.1 *Zyxomma petiolatum,* Japan	30.8	18.2	34.6	16.4	26	9.4	63.1	2.0	26	14.9	27.7	31.8	41	30.4	12.8	15.5
AB709239.1 *Zyxomma obtusum* , Japan	30.8	18.0	33.9	17.3	26	8.1	61.1	4.7	25	15.5	27.7	31.8	41	30.4	12.8	15.5
MN882703.1*Pseudagrion indicum,* Kerala	16.2	11.2	63.6	9.0	14	6.0	75.2	4.7	10	13.5	60.8	15.5	24	14.2	54.7	6.8
Avg.	27.9	16.8	40.3	15.1	23	8.5	65.1	3.1	22	14.7	34.3	28.5	38	27.2	21.2	13.8

Among the 34 species selected for the present work intraspecific divergence of 25 species could be calculated, as conspecific sequences of the remaining 9 species were not available in databases. Out of 25 species, 11 showed divergence of less than 1% (Table 4.4.122), 6 possessed 1-2% divergence and 8 species have divergence values more than 2%. Under suborder Zygoptera, 3 species viz. *Dysphaea ethela, Ceriagrion cerinorubellum* and *Ishnura rubilio* have intraspecific divergence values more than 2%. *Ceriagrion cerinorubellum* possessed maximum intraspecific divergence (8.8%) followed by *Dysphaea ethela* (2.3%) and *Ischnura rubilio* (2.1%). While considering families, species of family Coenagrionidae have maximum value of intraspecific divergence followed by family Euphaeidae.

More than 2% divergence was observed in 5 species of suborder Anisoptera (*Ictinogomphus rapax* (3.5%), *Orthetrum luzonicum* (5.1%), *Rhodothemis rufa* (8.8%), *Tetrathemis platyptera* (5.5%), *Tholymis tillarga* (4.2%)). Highest divergence was observed in genus *Rhodothemis* followed by *Tetrathemis* and *Orthetrum.* Among the three families of the present work species of family Libellulidae showed maimum intraspecific divergence values and family Gomphidae was the second most.

Interspecific divergence values within 27 genera were calculated and given in Table 4.4.123. The maximum value of interspecific divergence was exhibited by genus *Tholymis*. Divergence of 47.2% was observed between *Tholymis tillarga* and *Tholymis citrina*. The second most diverged genus was *Prodasineura*. Least divergence was observed between members of genus *Dysphaea*.

Table 4.4.122: Calculated intraspecific divergence values of selected species

	Name of species	**Intra specific divergence values**
1.	*Lestes praemorsus*	1.3%
2.	*Neurobasis chinensis*	0-0.5%
3.	*Heliocypha bisignata*	0-0.2%
4.	*Libellago indica*	0-0.7%
5.	***Dysphaea ethela***	**2.3%**
6.	*Copera vittata*	0.4%
7.	*Prodasineura verticalis*	1.5%
8.	*Agriocnemis pieris*	1.1%
9.	*Agriocnemis splendidissima*	0.4%
10.	***Ceriagrion cerinorubellum***	**0.3- 8.8%**
11.	***Ischnura rubilio***	**0-2.1%**
12.	*Paracercion calamorum*	1.1%
13.	*Pseudagrion indicum*	0.3%
14.	***Ictinogomphus rapax***	**1.6- 3.5%**
15.	*Diplacodes nebulosa*	1.1%
16.	*Hydrobasileus croceus*	0%
17.	*Orthetrum glaucum*	0.4%
18.	***Orthetrum luzonicum***	**5.1%**
19.	*Palpopleura sexmaculata*	1.3%
20.	***Rhodothemis rufa***	**8.8%**
21.	***Tetrathemis platyptera***	**2.8-5.5%**
22.	***Tholymis tillarga***	**0.4- 4.2%**
23.	*Tramea limbata*	0.5%
24.	*Urothemis signata*	0%
25.	*Zyxomma petiolatum*	0%

Table 4.4.123: Calculated Interspecific divergence within genera

Sl No.	Name of genus (Zygoptera)	Maximum Inter specific divergence	Sl No.	Name of genus (Anisoptera)	Maximum inter specific divergence
1.	*Lestes*	13.3	16.	*Gynacantha*	12.3
2.	*Protosticta*	20.9	17.	*Ictinogomphus*	14.2
3.	*Neurobasis*	15.7	18.	*Diplacodes*	17.5
4.	*Heliocypha*	13.1	19.	*Hydrobasileus*	8
5.	*Libellago*	16.1	20.	*Orthetrum*	15.5
6.	*Dysphaea*	0.4	21.	*Palpopleura*	12.2
7.	*Copera*	16.2	22.	*Rhodothemis*	8.8
8.	*Prodasineura*	21.6	23.	*Tetrathemis*	15.2
9.	*Aciagrion*	21.2	24.	*Tholymis*	47.2
10	*Agriocnemis*	19.8	25.	*Tramea*	13.4
11	*Archibasis*	5.3	26.	*Urothemis*	16.9
12	*Ceriagrion*	14	27.	*Zyxomma*	3.6
13	*Ischnura*	14.9			
14	*Paracercion*	10.2			
15	*Pseudagrion*	20			

4.5 DISCUSSION

Phylogeny is the study of evolutionary relationships between organisms. In ancient periods morphological features were used for phylogenetic studies. Wing venation was a popular feature for phylogenetic study in odonates (Carle, 1982; 1995; Bechley,1996; Carle and Kjer, 2002; Rehn, 2003). By the advent of molecular taxonomy more reliable results could be generated. The mitochondrial COI gene was used in initial studies and a variety of other marker genes are now commonly used. Phylogenetic analysis involving multiple marker genes provide more precise and reliable results, particularly marker genes having different origin. This is the basis of the present work, by using mitochondrial and nuclear marker genes for better resolution, and is the first phylogenetic work in Kerala on odonates, using a dual gene approach.

This chapter deals with the study of phylogenetic relationships and genetic divergence among odonates and the efficiency of partial COI and 18S rRNA marker genes in resolving relationships.

In the first part suborder trees based on COI and 18S rRNA genes were constructed. The result of phylogenetic analysis of the suborder Zygoptera strongly supported the monophyly of family Coenagrionidae by both marker genes (COI-95% bootstrap and 18S rRNA-92% bootstrap). The species of family Platycnemididae clustered together to form a monophyletic clade with 99% (COI) and 76% (18S rDNA) bootstrap support. Both analyses supported the monophyly of Coenagrionidae, Calopterygidae, Lestidae, Chlorocyphidae and Platycnemididae and the polyphyly of Platystictidae and Euphaeidae. In COI analysis result, family Platycnemididae and family Chlorocyphidae are sister clades (Bootstrap=66). In 18S rRNA analysis Chlorocyphidae form sister clade with family Lestidae (Bootstrap= 65) and, Platycnemididae formed sister clade with Coenagrionidae (97%).

A number of studies pointed out the sister group relationship of family Lestidae with all other Zygopteran families (Bybee et al., 2008; Carle et al., 2008; Davis et al., 2011; Dumont et al., 2010, Dijkstra et al., 2013). Such a relationship was not observed in the present work. Platystictidae is sister to the remaining families (Bybee et al., 2008, Davis et al., 2011, Van tol et al., 2009, Dijkstra, 2013) however the present result showed that Platystictidae was sister to all other

Zygopteran families except Euphaeidae. Both COI and 18S analyses were congruent with the above findings. The monophyly of Platystictidae (Bybee et al., 2008; Davis et al., 2011; Dumont et al., 2010; Van tol, 2009), Calopterygidae, Chlorocyphidae, Euphaeidae (Bybee et al., 2008; Dumont et al., 2010, Rehn, 2003) was also observed in the both analyses.

Coenagrionidae was found to be monophyletic. Although Bybee et al. (2008) found this family as non-monophyletic it is because of non-Indian species were included in that study. The genera selected for the current study were found to be monophyletic in Bybee's work too. After a few years the monophyly of Coenagrionidae was confirmed by Kim et al. (2014) with the help on concatenated mitochondrial and nuclear genes. Both COI and 18S analyses results were congruent in most of relationships and supported the current taxonomy of Zygoptera which substantiated the efficiency of both in discriminating family level relationships.

The phylogenetic tree of suborder Anisoptera based on partial COI gene sequences depicted distinct clades for each family. The monophyly of Libellulidae (Dumont et al., 2010, Ware et al., 2007), Gomphidae (Rehn, 2003) and Aeshnidae were well supported (Bybee et al., 2008, Carle et al., 2008, Davis et al., 2011, Fleck et al., 2008, Dijkstra, 2013). According to COI analysis, family Aeshnidae and family Gomphidae were in polyphyletic relationship with family Libellulidae. The finding was supported by the works of Dumont et al. (2010) based on the nuclear ribosomal genes 5.8 S, 18S, and ITS1 and ITS2 and Bybee et al. (2008) based on mitochondrial (12S, 16S and COII) and nuclear (18S, 28S, H3) genes. 18S analysis provided a contrasting result, family Aeshnidae formed a monophyletic clade and the other families, Gomphidae and Libellulidae were polyphyletic to the former one. The 18S analysis didn't resolve the lower relationships well.

The taxonomic relationships within selected families were analysed based on COI and 18S rRNA gene sequences. 7 families of Zygoptera and 3 families of Anisoptera were involved in the analysis.

The result of 18S phylogeny of family Lestidae was in agreement with Dumont et al. (2010) Gyulavári et al. (2011) and Dijkstra and Kalkman (2012), according to them *Chalcolestes, Sympecma* and *Indolestes* have a recent common ancestor and *Lestes* is paraphyletic to these genera. The 18S analysis of the present

study supported this relationship with a bootstrap value of 68%. The result was based on nuclear ribosomal ITS region and COI gene by Gyulavari et al. (2011), based on nuclear ribosomal genes 5.8S, 18S and ITS1 and ITS2 by Dumont et al. (2010). But the COI analysis provided a contrasting result. In which *Chalcolestes* is distantly placed from *Sympecma* and *Indolestes* without an immediate common ancestor. Sister clade relationship of genus *Lestes* and *Archilestes* has been observed in both 18S and COI analysis. So here 18S rRNA gene analysis has proven to be more successful in discriminating relationships within the family Lestidae.

There is no adequate number of sequence records of genera coming under the family Platystictidae in global databases. So available 18S rRNA gene sequences of 3 genera and COI sequences of 4 genera were used for the phylogenetic study of the family. Both analyses were congruent with each other and strongly supported the close relationship and monophyly of the three genera, *Protosticta, Sinosticta* and *Palaemnema*. Genus *Drepanosticta* was paraphyletic.

Phylogeny of family Calopterygidae based on partial COI gene sequences resolved the phylogenetic relationships well. The monophyly of *Neurobasis* with *Matrona* as sister group was supported by a bootstrap value of 99%. The result is in agreement with Dumont et al. (2005; 2010). The paraphyly of *Echo* is also supported by Dumont et al. (2005), but the position of *Caliphaea* and *Vestalis* is contrasting. Which are paraphyletic according to Dumont et al. (2005) but in the current study they are sister clades with 75% bootstrap support. In 18S rRNA gene analysis all the Calopterygid members are monophyletic to each other. The variation between the 18S rRNA gene sequences may be too small to found any grouping among the species. The highly conserved regions in 18S rRNA gene sequence may be a reason for the non-discrimination of relationships. So, in this case, the COI analysis well resolved the relationships between genera when compared to 18S analysis.

Phylogeny of 4 genera of the family Chlorocyphidae has been resolved using 18S rRNA and COI gene sequences. COI analysis has clearly discriminated the relationship between genera. Genus *Heliocypha* and *Aristocypha* are found as sister clades. This relationship is congruent with Dijkstra et al. (2014), which depicts the sister clade relationship between these two genera. Genus *Libellago* was closer to

Heliocypha and *Aristocypha* and formed monophyletic clade of three and genus *Rhinocypha* is paraphyletic. The 18S analysis has grouped the four into a monophyletic clade so exact relationship has not been depicted.

Phylogenetic analysis of family Euphaeidae based on COI gene showed that the genus *Dysphaea, Anisopleura* and *Euphaea* are monophyletic and they are paraphyletic to genus *Bayadera*. *Dysphaea* and *Anisopleura* were sister clades. The monophyly of the three is in agreement with Ji *et al.* (2019) but *Anisopleura* and *Euphaea* were found to be closer. The close resemblance among *Dysphaea, Euphaea* and *Anisopleura* is supported by Dumont *et al.* (2010). According to the 18S analysis *Anisopleura* was paraphyletic and the remaining three were monophyletic.

In the phylogenetic reconstruction of the family Platycnemididae, both COI and 18S analysis showed the paraphyly of genus *Prodasineura*. Both analyses placed the genus *Prodasineura* as paraphyletic to the other Playcnemididae members in the current study. The paraphyletic relationship between *Elattoneura* and *Prodasineura* is supported by Dumont et al. (2010). According to COI analysis *Calicnemia* and *Coeliccia* formed sister clades and *Onychargia* was paraphyletic to them. *Copera* and *Pseudocopera* were another monophyletic sister clades and *Nososticta* was closer to them then *Elattoneura* was closer. The relationship between the genera except *Prodasineura* was not clearly resolved by 18S analysis.

Eleven species of family Coenagrionidae sequenced during the current study have been used for 18S and COI phylogenetic resolution. The results of both analyses are congruent with the present taxonomy of family Coenagrionidae (Kalkman et al. 2020). All the genera were assembled into separate groups. The species of genus *Agriocnemis, Ceriagrion, Paracercion* (bootstrap 99%) and *Pseudagrion* (bootstrap 88%) have clustered into distinct monophyletic clades in COI analysis. *Ischnura* and *Aciagrion* are polyphyletic to *Ceriagrion. Archibasis* is paraphyletic to *Pseudagrion*. The resultant phylogeny of 18S analysis showed some variations from that of COI analysis. Species of genera *Paracercion* and *Agriocnemis* are found to be monophyletic. *Aciagrion* and *Ischnura* are monophyletic each other. In the COI analysis the common ancestor gives rise to three main clades, the first clade is formed by the monophyly of *Agriocnemis,*

Ceriagrion, Ischnura and *Aciagrion;* the second one is a cluster of *Paracercion* species and the last one is a cluster of *Pseudagrion* and *Archibasis*. In contrast to this, the 18S analysis result presents a tree with two main clades. One is formed by the grouping of *Pseudagrion* and *Archibasis*, which resembles the clade in COI analysis. The second clade is formed by clustering the remaining Coenagrionid genera.

Phylogenetic analysis of family Aeshnidae based on COI and 18S rRNA gene sequences resolved the phylogeny well. According to the COI analysis *Aeshna, Anaciaeshna, Anax* and *Tetracanthagyna* were found in a monophyletic clade and *Gynacantha* as a separate clade. The monophyly of *Aeshna, Anaciaeshna* and *Anax* and the polyphyly of *Gynacantha* and *Polycanthagyana* observed in COI analysis are in agreement with Mehmood et al. (2021). In 18S analysis *Aeshna, Anaciaeshna, Anax* and *Gynacantha* were clutered together and *Tetracanthagyna* more distantly placed. Both analyses strongly supported the clustering of *Aeshna, Anaciaeshna* and *Anax*.

The COI analysis better resolved the relationship among the members of family Gomphidae. The analysis indicated close relationship of *Anisogomphus + Cyclogomphus* and *Asiagomphus+ Burmagomphus* and the paraphyly of *Ictinogomphus.* This also pointed out that *Macrogomphus* and *Davidius* are more distantly placed than other genera. The 18S analysis showed the resemblance among Gomphid members but the resolution between genera was vague.

While considering the phylogeny of family Libellulidae the COI analysis indicated the close relationship of *Tramea* and *Hydrobasileus.* Although the bootstrap is only 41%, it is in harmony with Ware *et al.* (2007). The monophyly of *Orthetrum* species (bootstrap 97%) has also been reported in Ware's work. 18S analysis provided a contrasting result to that of COI analysis. In COI analysis *Zyxomma* was paraphyletic to the other genera but in 18S analysis *Rhodothemis* showed paraphyly.

The divergence values of 18S sequences were not efficient in discriminating between genera, as these are highly conserved variation was too meager to distinguish the relationships. In contrast to this COI sequences clearly displayed the genetic divergence between genera. A detailed comparison of trees based on both

marker genes revealed the efficiency of COI over the 18S rRNA gene in resolving family and suborder trees. In 50% of analyses both genes provided congruent and reliable results. But in majority COI yielded better resolution than 18S rRNA gene. However analysis using longer 18S rRNA gene sequences may produce more reliable results. Longer gene sequences can provide better resolution in phylogeny (Lee et al., 1996; Thomassen et al., 2003).

Phylogenetic relationships within selected genera were resolved based on partial COI gene sequences and estimated interspecific and intraspecific genetic divergence values. 27 genera were included in the analyses. Genus *Onychothemis* was excluded as sequences of the same genus were unavailable in GenBank database.

The phylogenetic analysis of different genera based on COI gene sequence results indicated the variation occurred in the gene sequence of conspecifics due to geographical isolation. While considering the phylogenetic tree of Genus *Lestes*, *Lestes praemorsus* from Kerala showed close similarity with Malaysia specimen with a bootstrap value of 99%. The other species such as *Lestes dryas* and *Lestes congener* also clustered with the conspecifics and formed distinct monophyletic clades (bootstrap 99%). The divergence values also supported the tree result. The taxonomic identity of *Lestes praemorsus* was well corroborated by the phylogenetic analysis and evolutionary divergence values. 1.3% divergence was found between the *Lestes praemorsus* specimens. *Lestes praemorsus* was closest to *Lestes congener* and were monophyletic to each other. The phylogenetic tree result along with the evolutionary divergence percentage authenticated the taxonomic identity of this species.

Protosticta gravelyi is an endemic and rare species of the Western Ghats. All the species of the genus *Protosticta* found in Kerala are endemic to the Western Ghats (Nair et al. 2021). The phylogenetic tree result showed that *Protosticta gravelyi* was formed as a distinct clade and separated from other species. The other species were clustered into monophyletic clade well supported by bootstrap value of 96% and *Protosticta gravelyi* was paraphyletic to them. According to the divergence values *Protosticta satoi* showed least divergence (15.5%) from

Protosticta gravelyi. Maximum divergence (20.9%) was observed between *Protosticta grandis* and *Protosticta gravelyi*.

Neurobasis chinensis is the only species of the corresponding genus found in Kerala (Nair et al. 2021). All the six specimens of *Neurobasis chinensis* were clustered into monophyletic clade with 100% bootstrap. The specimen from Kerala showed closest resemblance to the specimen from Tamil Nadu. There was no intraspecific divergence existed between them. This corroborated the taxonomic authenticity of this species. *Neurobasis longipes* was more closely related to *Neurobasis chinensis(11.7%). Neurobasis ianthinipennis* was the most distant with a divergence of 14.7%.

In the present analysis of genus *Heliocypha*, out of the 11 members involved, 5 belong to *Heliocypha bisignata*. The sequence with accession number KM675769 is found as *Rhinocypha bisignata* in GenBank records. *Heliocypha bisignata* was previously known as *Rhinocypha bisignata* in the Indian subcontinent (Kalkman et al., 2020). So here it can be considered as *Heliocypha bisignata* because of the high sequence similarity observed. There was no evolutionary divergence was observed between these species which provided supplementary support for the phylogenetic tree. All the *Heliocypha bisignata* members were grouped as a monophyletic clade with a bootstrap value of 100. The divergence values were 0.2% or less. Only slight changes were observed between *Heliocypha bisignata* members from Kerala and Punjab. So, it was confirmed that no significant variation has occurred in the *Heliocypha bisignata* species from Kerala and Punjab. The other members of the genus viz. *Heliocypha perforata*, *Heliocypha biforata* and *Heliocypha fenestrata* have formed separate clusters for each and were polyphyletic. *Heliocypha perforata* had the highest sequence diversion from *Heliocypha bisignata*. Phylogenetically *Heliocypha bisignata* was closer to *Heliocypha biforata*. Conspecifics of *Heliocypha biforata* and *Heliocypha perforata* did not exhibit any evolutionary divergence. The present work authenticated the taxonomic integrity of *Heliocypha bisignata* and provided molecular identification ID for faster and more precise identification and phylogenetic resolution of the species.

The phylogenetic tree suggested that the conspecifics of *Libellago lineata* showed 0% divergence from each other. The *Libellago indica* specimen from Kerala

collected during the study showed only 0.7% divergence from the Punjab specimens of *Libellago lineata*. Hamalainen (2016) has raised the taxonomic position of *Libellago indica* from subspecies level to the species level. The common ancestor of both species has recently diverged to form two different clades thus supporting the finding of Hamalainen (2016) and Kalkman et al. (2020). Kalkman et al. (2020) recorded *Libellago lineata* widespread in Southeast Asia including India. According to Nair et al. (2021), *Libellago lineata* is not found in Kerala and *Libellago indica* endemic to the Western Ghats.

Dysphaea ethela is the only species of genus *Dysphaea* found in Kerala. This species is endemic to India (Kalkman et al., 2020; Bose et al., 2021). As per the phylogenetic tree *Dysphaea ethela* specimen from Kerala formed a separate clade from the Punjab specimen. This was supported by the divergence values and denoted the variations occured in the gene sequence of *Dysphaea ethela* due to geographical isolation. From the calculated divergence values, it was clear that the intraspecific divergence between Kerala and Punjab specimens of *Dysphaea ethela* was 2.3%. There was no divergence between Punjab specimens. Increase in divergence percentage might be the result of geographical variation. *Dysphaea dimidiata* formed a distinct monophyletic clade well supported by bootstrap.

From the tree result it was clear that *Copera vittata* was monophyletic. There was a slight variation was observed between Kerala and Punjab specimens and found as sister clades. This was supported by evolutionary divergence values. 0.4% divergence was existed between Kerala and Punjab specimens. *Copera vittata* and *Copera marginipes* are the two species found in Kerala that belong to the genus (Raju and Kiran, 2013). Only minute morphological dissimilarities exist between the two. Although *Copera vittata* shows close morphological resemblance with *Copera marginipes,* both were phylogenetically distant with interspecific divergence of 12.1 to 12.6%. The phylogenetic tree supported the same.

Genus *Prodasineura* has only one representative in Kerala, *Prodasineura verticalis*. In the current phylogenetic resolution, all specimens of *Prodasineura verticalis* along with *Prodasineura humeralis* were grouped into a monophyletic clade well supported by bootstrap 98%. According to Lok (2008), *Prodasineura verticalis* is known in the name of *Prodasineura humeralis* in Singapore and this

supported the current finding. The phylogenetic tree branches were congruent with evolutionary divergence values.

Aciagrion approximans krishna is an endemic species of the Western Ghats (Kalkman et al., 2020). The sequences generated during the present work are the first GenBank records of this species. The phylogenetic tree indicated close similarity with *Aciagrion migratum* from India (Kerala) with accession number MW812349.1. As this species is absent in India, and 0% divergence value was observed, this can be considered as conspecific to the former one and this was wrongly submitted to GenBank in the name of *Aciagrion migratum*. However, *Aciagrion approximans krishna* showed only 0.5-0.7% divergence from *Aciagrion migratum* from Japan. Lieftinck et al. (1984) considered record of *Aciagrion hisopa* from China (Needham, 1930) as *Aciagrion migratum* (Wilson, 2000). *Aciagrion hisopa* and *Aciagrion approximans* show close morphological similarity (Joshi et al., 2016). Despite there being a number of records on *Aciagrion hisopa*, distribution of the same still needs confirmation (Kalkman, 2020). Another *Aciagrion* species found in Kerala *Aciagrion occidentale* showed similarity with *Aciagrion borneese* and formed a monophyletic clade. *Aciagrion pallidum* was paraphyletic.

Agriocnemis pieris and *Agriocnemis splendidissima* are the commonly found damselflies in Kerala (Kiran and Raju, 2013). In the phylogenetic analysis *Agriocnemis pieris* from Kerala clustered with its conspecific from Punjab with a bootstrap of 100%. With a divergence percentage of 1.1%, the species authenticity was confirmed by the present study. *Agriocnemis splendidissima* from Kerala formed monophyletic clade with the specimen from Punjab (boot strap 100%) and possessed a divergence percentage of 0.4% each other. The morphological identity of *Agriocnemis splendidissima* was strongly confirmed by the close relationship with Punjab sample. *Agriocnemis keralensis* which is endemic to the Western Ghats showed resemblance with *Agriocnemis forcipata* from Africa. There was no genetic divergence observed between both specimens. However, they are morphologically distinct species. *Agriocnemis pieris, Agriocnemis splendidissima* and *Agriocnemis keralensis* the species found in Kerala were found as paraphyletic.

Archibasis oscillans is the only representative of the genus *Archibasis* in India. The sequence recorded by the current study is the first record of this species in

GenBank. The phylogenetic tree indicated that the other species of *Archibasis* genus were diverged from the ancestor of *Archibasis oscillans* at an earlier stage. *Archibasis oscillans* showed marked sequence diversion from the other two species in the phylogenetic tree and was paraphyletic to the other two.

Ceriagrion rubiae is not so common in Kerala habitats and the sequence record of *Ceriagrion rubiae* generated by the present work is the first record of this species in GenBank. So, no intraspecific study could be carried out. *Ceriagrion cerinorubellum* is a common damselfly. In the phylogenetic analysis, this species showed close resemblance with its conspecific from India with 100% bootstrap support. However, the Indian specimens markedly diverged from Malaysia and Bangladesh specimens (8.5% to 10.7% divergence). As the observed intraspecific divergence values were considerably high, a detailed study will surely throw light on the species authenticity of the same. All the nodes of the tree except two were supported by >60 boot strap values. The present finding is in agreement with Guan et al. (2013), in which the paraphyly of *Ceriagrion glabrum* and the monophyly of *Ceriagrion fallax*, *Ceriagrion coromandelianum, Ceriagrion cerinorubellum* and *Ceriagrion olivaceum* were depicted. Dumont et al., (2010) presented the close relationship between *Ceriagrion fallax* and *Ceriagrion olivaceum*. The monophyly found between *Ceriagrion olivaceum, Ceriagrion fallax* and *Ceriagrion coromandelianum* in their work resembled the current tree. The paraphyly of *Ceriagrion glabrum* was also supported by the same.

According to the result, a high similarity could be observed between *Ischnura aurora, Ischnura delicata* and *Ischnura rubilio* and they are monophyletic with 99% bootstrap support. Zero percentage divergence was observed among these three species. However, *Ischnura aurora* from Kerala showed a divergence of 2.1%. *Ischnura delicata* is synonymised to *Ischnura aurora (*Babu, 2017). *Ischnura aurora* in Indian subcontinent is now considered as *Ischnura rubilio* (Kalkman et al., 2020). The current phylogeny results substantiated the literature and confirmd that the species names *Ischnura delicata* and *Ischnura aurora* are the synonyms of *Ischnura rubilio*. The resultant phylogenetic tree is in agreement with Blow et al. (2021) in which the phylogeny of Genus *Ischnura* is mainly consists of 3 clades resembling to the current tree. One clade comprises (bootstrap 99%) *Ischnura senegalensis, Ischnura elegans, Ischnura rufostigma* and *Ischnura nursei*. The second clade is

composed of (bootstrap 99%) *Ischnura kellicotti* and *Ischnura verticalis*. The third one is the clade of *Ischnura taitensis, Ischnura rubilio* and the synonyms (bootstrap 91%). *Ischnura taitensis* is closest to *Ischnura rubilio* and paraphyletic to it.

Paracercion species are not so common in Kerala, particularly *Paracercion malayanum*. The present study recorded the same as first report from central and northern Kerala. The present partial COI gene sequence records of *Paracercion calamorum* and *Paracercion malayanum* are the first records from India. The monophyly observed among the three specimens of *Paracercion calamorum* confirmed the species authenticity of the same. *Paracercion malayanum* is found as *Paracercion melanotum* in GenBank records as it was synonymized to the later (Zang et al., 2021; Paulson et al., 2022). The divergence value between *Paracercion malayanum* and *Paracercion melanotum* was 1.3% and this supported the synonymy of the two. Although the sister clade relationship of *Paracercion v-nigrum* and *Paracercion sieboldii* was not well supported (bootstrap 49%) in the present work, this relationship was strongly supported by the work of Dumont et al. (2010) and Ning et al. (2016). The monophyly of *Paracercion barbatum, Paracercion v-nigrum* and *Paracercion sieboldii* and the monophyly of *Paracercion malayanum, Paracercion melanotum* and *Paracercion hieroglyphicum* were congruent with the finding of Zang et al. (2021). The divergence between *Paracercion melanotum* and *Paracercion hieroglyphicum* was 0% and this also was in agreement with the finding of Zang et al. (2021). They have accepted data from ITS and morphological characters which is found as more reliable in that case and confirmed the existence of both as two distinct species.

The phylogeny of genus *Pseudagrion* indicated the common ancestry of all *Pseudagrion* species (except *Pseudagrion malabaricum*) found in Kerala. *Pseudagrion indicum* is a Western Ghats endemic. The partial COI sequence of *Pseudagrion indicum* showed high similarity with another sequence sample from Kerala. The morphological identity of *Pseudagrion indicum* was well supported by this close relationship and the genetic divergence values (0.3%). *Pseudagrion decorum* was polyphyletic to *Pseudagrion indicum*. According to Dumont et al. (2010) the three species *Pseudagrion decorum, Pseudarion rubriceps* and *Pseudagrion spencei* are monophyletic and *Pseudagrion pruinosum* is paraphyletic.

Here also the monophyly among the three species was observed with 71% bootstrap support and also the paraphyly of *Pseudagrion pruinosum*.

Gynacantha millardi morphologically shows high resemblance with *Gynacantha bayadera.* The constriction on abdominal segment 3 is absent in *Gynacantha millardi*. The close similarity was also observed in phylogenetic analysis result. They were found as sister clades with 99% boot strap support. A divergence value of 1.2% was observed between them. *Gynacantha dravida* was genetically closer to both and grouped to form monophyletic clade.

Ictinogomphus rapax is the single representative of the genus in Kerala. *Ictinogomphus rapax* specimens from 3 countries were clustered to form a monophyletic clade. Specimens from China and USA showed high resemblance and the percentage of divergence was 1.6. They showed divergence of 3% to 3.5% from Kerala specimen. *Ictinogomphus pertinax* and *Ictinogomphus decoratus* was closely similar with their conspecifics.

Diplacodes nebulosa specimens from Kerala and Thailand were found to be monophyletic with 100% bootstrap support and the percentage of divergence was 1.1%. This substantiated the morphologic identity of the species. *Diplacodes luminans* was paraphyletic to the remaining members of the genus. *Diplacodes lefebvrei* was closer to *Diplacodes nebulosa* with 98% boot strap value. *Diplacodes trivialis* closely related to *Diplacodes bipunctata* and *Diplacodes haematodes* was paraphyletic (71% boot strap).

Hydrobasileus croceus is the only representative of the genus in Kerala. All the 4 specimens of *Hydrobasileus croceus* from different geographical regions showed high similarity and no divergence was observed between them. This strongly corroborated the morphologic identity of this species. From the result it was clear that *Hydrobasileus croceus* has not undergone any significant change in gene sequence by the effect of geographic variation.

Genus *Orthetrum* in Kerala was represented by 7 species (Gopalan et al., 2022). Out of them, 6 species were included in the analysis. Most of the nodes of the tree were supported by >60 boot strap values. The 6 species of *Orthetrum* found in Kerala were polyphyletic and they were distantly placed in phylogenetic tree. *Orthetrum sabina*, the common cannibalistic dragonfly (Iswandaru, 2018) was

paraphyletic to the other members of the genus. *Orthetrum luzonicum* specimens of Kerala and Malaysia clustered together to form sister clades but the divergence value was high (5.1%). They were phylogenetically closest to *Orthetrum coerulescens*. *Orthetrum cancellatum, Orthetrum borneense* and *Orthetrum glaucum* are monophyletic to each other. *Orthetrum glaucum* from Kerala and Malaysia showed close similarity with 99% bootstrap support and a divergence of 0.4%. This corroborated the taxonomic identity of this species. This phylogenetic relationship among *Orthetrum* species in this study match with the results of the study conducted by Yong et al. (2014).

Genus *Palpopleura* has only single representative in Kerala, *Palpopleura sexmaculata*. The specimen from Kerala was highly resemble to the Punjab specimen supported by 99% boot strap.1.3% evolutionary divergence was found between them. *Palpopleura jucunda* was paraphyletic to this species. The other two species were polyphyletic to *Palpoleura sexmaculata*.

Genus *Rhodothemis* in Kerala has only single representative, *Rhodothemis rufa*. Phylogeny of the conspecifics of *Rhodothemis rufa* indicated the monophyly among the specimens from Kerala, Bangladesh, Austria and Malaysia and they were highly similar. Divergence values were ranged from 0% to 0.2%. Specimen from Pakistan was paraphyletic with a divergence of 8.5% to 8.8%.

Tetrathemis platyptera is a small sized damselfly and not common in occurrence. The phylogenetic analysis showed the monophyly of three samples of *Tetrathemis platyptera* and the paraphyly of *Tetrathemis irregularis*. The phylogenetic tree and the estimates of evolutionary divergence revealed the existence of variations among the three samples from geographically distant locations. This indicated the changes occurred in the gene sequence of *Tetrathemis platyptera* due to geographical isolation. Kerala sample was closer to Thailand sample than Mizoram sample.

Six samples of *Tholymis tillarga* from different geographical areas were monophyletic. However, samples from France and China diverged considerably from the other four. The intraspecific divergence values supported the finding. The observed genetic variation might be the result of geographical changes. *Tholymis citrina* formed a separate monophyletic clade (bootstrap 99).

Samples of *Tramea limbata* formed monophyletic clade with *Tramea transmarina* and *Tramea loewii*. *Tramea limbata* from Kerala showed close similarity with *Tramea transmarina* and *Tramea loewii* and it showed diversion from its conspecifics from France. The evolutionary divergence values also supported the same. There was no genetic divergence observed among the species.

Samples of *Urothemis signata* from three different geographical areas showed close resemblance with 0% evolutionary divergence (bootstrap 100%). This indicated that no variation exists among the gene sequences of *Urothemis signata* samples due to geographical variation. *Urothemis venata* samples formed a separate monophyletic clade with 78% bootstrap support.

Zyxomma petiolatum is a common crepuscular dragonfly. The result of phylogenetic analysis showed that three samples *of Zyxomma petiolatum* from geographically different areas showed close similarity (99% bootstrap) and 0% evolutionary divergence. This denoted the taxonomic integrity of the species. The position of *Zyxomma obtusum* was paraphyletic to the former.

The calculated genetic divergence values provided insights into intraspecific and interspecific genetic variation of selected species of odonates across large geographic distances. According to Hebert et al. (2003), the intraspecific divergence values are generally less than 1%, however, in rare instances it raises above 2% (Tallei et al., 2017). Intraspecific divergence of 25 species was estimated, the remaining 9 species were excluded because of the unavailability of conspecific sequences in databases. Of the species investigated, 11 showed intraspecific divergence less than 1% (Table 4.4.122). Six species have divergence of 1-2% and 8 species showed intraspecific divergence values above 2%. A good number of literature supported the genetic constancy of odonates (Haring et al., 2020; Kohli et al., 2018; Kim et al., 2007; Christudhas and Mathai, 2014). The majority of odonates selected for the study showed low genetic variability over long distances (different countries and continents) except the eight species. A rapid increase in population after a genetic bottleneck or gene flow due to wide dispersion might be the reason for the genetic homogeneity. Dragonflies are active dispersers over large geographic distances (Corbet, 1999; May and Matthews, 2008). Despite the low dispersal ability of Zygoptera, there is no significant variation in the gene structure of conspecifics

(Haring et al., 2020). The passive dispersal capacity of Zygoptera with seasonal winds to long distances was recorded by Corbet (1999), May and Matthews (2008)and Haring et al. (2020). These might be the explanation behind the genetic homogeneity of both suborders.

Higher genetic variability was observed in 8 species. Among these, 6 possessed intraspecific divergence above 3%. When comparing the results, genetic variability was lesser in Zygopterans. Under Zygoptera, *Dysphaea ethela, Ceriagrion cerinorubellum* and *Ischnura rubilio* showed higher divergence, and two of them have values almost closer to 2%, i.e. 2.3% and 2.1% for *Dysphaea ethela* and *Ischnura rubilio* respectively. *Ceriagrion cerinorubellum* showed a value of 8.8%. Of the 7 families studied, genetic variability of >2% was observed in members of the family Euphaeidae and family Coenagrionidae. The intraspecific divergence was considerably high in dragonfly species. The species showed divergence values above 3% were *Ictinogomphus rapax* (1.6- 3.5%), *Orthetrum luzonicum* (5.1%), *Rhodothemis rufa* (8.8%), *Tetrathemis platyptera* (2.8-5.5%) and *Tholymis tillarga* (0.4-4.2%). Out of the 3 Anizopteran families studied, members of family Libellulidae showed high divergence values. The highest divergence value of possessed by species of genus *Rhodothemis*, followed by *Tetrathemis* and *Orthetrum*. According to Low et al. (2017) more research is needed to determine whether the high genetic variability is due to geographical influence or the sensitivity of marker genes. Islam et al. (2018a, 2018b) observed the increased genetic variability as a result of mutations occurred in the gene sequences of odonates under family Libellulidae and Gomphidae. These studies indicated that, occurrence of intraspecific divergence can be because of their highly sensitive gene sequences.

The estimated interspecific divergence values within each genus showed that maximum inter specific divergence was possessed by genus *Tholymis*. 47.2% divergence was observed between species *Tholymis tillarga* and *Tholymis citrina*. Minimum interspecific divergence was found in the genus *Dysphaea* (Table 4.4.123).

Another finding of the study was the close genetic similarity between *Agriocnemis keralensis*, endemic species of Western Ghats and *Agriocnemis*

forcipata from Africa. 0% genetic variation was observed and the phylogenetic tree result supported the same. Both are morphologically dissimilar.

Phylogenetic analysis of the genus *Tramea* pointed out the close resemblance among the three species- *Tramea limbata*, *Tramea transmarina* and *Tramea loewii* with 0% genetic variation.