STUDIES ON MOLECULAR CHARACTERISATION AND PHYLOGENETIC ANALYSIS OF DRAGONFLIES AND DAMSELFLIES (ODONATA: INSECTA) OF SELECTED HABITATS OF KERALA

Thesis submitted to the University of Calicut for the award of the Degree of

DOCTOR OF PHILOSOPHY IN ZOOLOGY

By

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Under the supervision of **Dr. Francy K Kakkassery**

and

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DECLARATION

I hereby declare that the thesis entitled **"STUDIES ON MOLECULAR CHARACTERISATION AND PHYLOGENETIC ANALYSIS OF DRAGONFLIES AND DAMSELFLIES (ODONATA: INSECTA) OF SELECTED HABITATS OF KERALA"** submitted to the University of Calicut for the award of Doctor of Philosophy in Zoology is a bonafide research work done by me under the supervision and guidance of Dr. Francy K Kakkassery, Associate Professor and Head (Retd.), Research and Postgraduate Department of Zoology and co-guidance of Dr. C F Binoy, Dean of science, St. Thomas' College (Autonomous), Thrissur.

I also declare that the findings presented in this thesis are original and do not form the basis for the award of any other degree, diploma or other similar titles of any other university.

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I hereby certify that this is the revised version of the thesis entitled "STUDIES ON MOLECULAR CHARACTERISATION AND PHYLOGENETIC ANALYSIS OF DRAGONFLIES AND DAMSELFLIES (ODONATA: INSECTA) OF SELECTED HABITATS OF KERALA" submitted by Nitha Bose C under my guidance after incorporating the necessary corrections or suggestions made by the adjudicators. The content of the CD is the same as in the hard copy.

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Nitha Bose C

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ABSTRACT

Odonata is a primitive order of class Insecta. The order is divided into three suborders- Anisoptera, Zygoptera and Anisozygoptera. Anisoptera is the suborder of dragonflies and damselflies belonging to the suborder Zygoptera. Anisozygoptera is a living fossil and has only 3 species representatives globally. Odonates are hemimetabolous insects and both larval and adult forms are predators. They are amphibiotic; egg and larval development take place in water and adults are aerial. Odonates are naturally considered as biocontrol agents and bioindicators.

Various habitats of high land, midland and low land regions of 5 districts of Kerala were selected for the study. A total of 73 locations were observed in the Thrissur, Ernakulam, Palakkad, Wayanad and Idukki districts of Kerala. The observed habitats include streams, rivers, ponds, lakes, paddy fields, ditches and estuaries.

A total of 71 species (33 species of damselflies and 38 species of dragonflies) were observed during the study. They belong to 10 families and 43 genera. The Western Ghats endemic species recorded during the work were *Aciagrion approximans krishna, Agriocnemis keralensis, Pseudagrion indicum* and *Protosticta gravelyi*. The rarely found species *Paracercion malayanum* is the first record from central and northern Kerala. Taxonomic keys for the observed 71 species of odonates were prepared.

Traditionally organisms were classified based on morphological features. Many research workers pointed out the limitations of traditional taxonomy. These challenges can be overcome by molecular taxonomic approaches. The results of phylogenetic analysis become more convincing when multiple marker genes are included in the study. So, the present work focused on mitochondrial COI gene and nuclear 18S rRNA gene for the molecular characterisation and phylogenetic assessment of selected odonates of Kerala.

Molecular characterisation of 34 species belonging to 28 genera was done. Partial COI gene, 18S rRNA gene sequences and translated protein sequences were generated. Of these, twelve COI gene sequences and twenty three 18S rRNA gene sequences are the first worldwide GenBank records. Mitochondrial COI sequences

can be used for precise and faster identification of odonate species and phylogenetic studies. Nuclear 18S rRNA gene sequences are beneficial in higher level phylogenetic analysis.

Phylogenetic analyses of two suborders and selected families, based on partial COI and 18S rRNA gene sequences were carried out and genetic divergence values among odonates were estimated. The efficiency of partial COI and 18S rRNA marker genes in resolving relationships was studied. 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.

Phylogenetic analyses of the 27 genera were done based on COI gene sequences. The results of the analyses and the calculated genetic divergence values provided insights into intraspecific and interspecific genetic variation of odonates across large geographic distances. The majority of odonates selected for the study showed low genetic variability over long distances (different countries and continents) except for eight species. When comparing the results, genetic variability was lesser in damselflies and considerably high in dragonflies.

The estimated interspecific divergence values within each genus showed that maximum and minimum interspecific divergence values were possessed by genus *Tholymis* and genus *Dysphaea* respectively.

Another finding of the study was the close genetic similarity observed between morphologically dissimilar and geographically distant species.

സംഗ്രഹം

ജീവികളിൽ ഏറ്റവും പ്രധാനപ്പെട്ട ഒരു വംശമാണ് ഷഡ്പദങ്ങൾ. ഈ വംശത്തിലെ ഒരു പ്രാചീന ഗണമാണ് ഒഡോണേറ്റ. ഇവ അനൈസോപ്റ്റീറ, സൈഗോപ്റ്റീറ, അനൈസോസൈഗോപ്റ്റീറ എന്നിങ്ങനെ മൂന്ന് ഉപഗണങ്ങളായി വിഭജിക്കപ്പെട്ടിരിക്കുന്നു. അനൈസോപ്റ്റീറ കല്ലൻതുമ്പികളുടെയും സൈഗോപ്റ്റീറ സൂചിത്തുമ്പികളുടേയും ഉപഗണങ്ങളാണ്. എന്നാൽ അനൈസോസൈഗോപ്റ്റീറ കല്ലൻതുമ്പികളുടെയും സൂചിത്തുമ്പികളുടേയും സവിശേഷതകൾ ഉള്ള മൂന്നാമത്ത അപൂർവ്വ ഉപഗണമാണ്.

തുമ്പികളുടെ ജീവിതചക്രം അപൂർണ രൂപാന്തരീകരണത്തിനുദാഹരണമാണ് (Hemimetabolous type of Metamorphosis). ഇവയുടെ ലാർവകളും മുതിർന്ന തുമ്പികളും ചെറുപ്രാണികളെ ആഹാരമാക്കുന്ന മാംസഭുക്കുകളാണ്. പൊതുവെ ഇവ ഉഭയജീവിതകളാണ്. അവയുടെ ജീവിതചക്രത്തിന്റെ ആദ്യഘട്ടം (മുട്ട, ലാർവ) ശുദ്ധജലത്തിലും രണ്ടാംഘട്ടം (പൂർണവളർച്ചയെത്തിയ മുതിർന്ന തുമ്പികൾ) വായുവിലും പൂർത്തിയാക്കപ്പെടുന്നു. പ്രകൃതിയിൽ തുമ്പികൾ വ്യത്യസ്ത ശുദ്ധജല ആവാസവ്യവസ്ഥകളിൽ ജൈവ നിയന്ത്രണ പ്രവർത്തകരായും (Bio control agents) ജൈവസൂചികകളായും (Bio Indicators) കണക്കാക്കപ്പെട്ടിരിക്കുന്നു.

കേരളത്തിലെ 5 ജില്ലകളിലെ മലനാടുകളിലെയും ഇടനാടുകളിലെയും തീരപ്രദേശങ്ങളിലെയും വ്യത്യസ്തജല ആവാസ വ്യവസ്ഥകൾ ഈ ഗവേഷണ പഠനത്തിനായി തിരഞ്ഞെടുത്തു. തൃശൂർ, എറണാകുളം, പാലക്കാട്, വയനാട്, ഇടുക്കി ജില്ലകളിലായി ആകെ 73 സ്ഥലങ്ങൾ നിരീക്ഷിച്ചു. ഈ ആവാസവ്യവസ്ഥകളിൽ അരുവികളും ചാലുകളും കായലുകളും ഉൾപ്പെടുന്നു.

ഈ പഠനഗവേഷണത്തിന്റെ ഭാഗമായി മേൽപറഞ്ഞ സ്ഥലങ്ങളിൽ ആകെ 71 ഇനം തുമ്പികളെ (33 ഇനം സൂചിത്തുമ്പികളും 38 ഇനം കല്ലൻ തുമ്പികളും) നിരീക്ഷിച്ചു. അവ 10 കുടുംബങ്ങളിലും 43 ജനുസ്സുകളിലും ഉൾപ്പെട്ടിരിക്കുന്നു. ഇതിൽ നീലചിന്നൻ (*Aciagrion approximans krishna*) പത്തിപുൽചിന്നൻ (*Agriocnemis keralensis*) മഞ്ഞവരയൻ പൂത്താലി (*Pseudagrion indium*) പുള്ളിനിഴൽത്തുമ്പി (*Protosticta gravelyi*) എന്നിവ പശ്ചിമഘട്ടത്തിലെ സ്ഥാനീയ തുമ്പികളാണ്. അപൂർവ്വമായി കണ്ടുവരുന്ന

ഇനമായ മലയൻ താമരത്തുമ്പിയെ (*Paracercion malayanum*) മധ്യകേരള ത്തിൽ നിന്നും വടക്കൻ കേരളത്തിൽ നിന്നും ആദ്യമായി കണ്ടെത്താൻ കഴി ഞ്ഞു. നിരീക്ഷിച്ച 71 ഇനം തുമ്പികളുടെ വർഗ്ഗീകരണ സൂചികകൾ (Taxonomic keys) തയ്യാറാക്കിയിട്ടുണ്ട്.

ജീവജാലങ്ങളെ സാധാരണയായി പരമ്പരാഗത രീതിയിലാണ് വർഗ്ഗീക രണം നടത്തികൊണ്ടിരുന്നത്. പല ഗവേഷകരും പരമ്പരാഗത വർഗ്ഗീകരണ വ്യവസ്ഥയുടെ പരിമിതികൾ ചൂണ്ടിക്കാട്ടിയിട്ടുണ്ട്. ഈ വെല്ലുവിളികളെ മറിക ടക്കാൻ തൻമാത്രാ വർഗ്ഗീകരണ വ്യവസ്ഥ (Molecular taxonomy) ഉപയോ ഗിച്ചുകൊണ്ട് സാധിക്കുമെന്ന് അടുത്ത കാലങ്ങളിൽ ശാസ്ത്രീയമായി തെളി യിക്കപ്പെട്ടിട്ടുണ്ട്. തന്മാത്രാ വർഗ്ഗീകരണത്തിൽ ഒന്നിലധികം ജീനുകൾ ഉൾപ്പെടുത്തുമ്പോൾ വംശ-ജനിതക വിശകലനത്തിന്റെ (Phylogenetic analysis) ഫലങ്ങൾ കൂടുതൽ ബോധ്യപ്പെടും. അതിനാൽ ഈ ഗവേഷണ പഠ നത്തിൽ കേരളത്തിലെ തിരഞ്ഞെടുത്ത തുമ്പികളുടെ തന്മാത്രാ സ്വഭാവ നിർണയത്തിനും (Molecular Characterisation) വംശജനിതക വിശകലന ത്തിനും കോശമൈറ്റോ കോൺഡ്രിയയിലെ COI ജീനും കോശമർമ്മത്തിലെ 18S rRNA ജീനും ഉപയോഗിച്ചിരിക്കുന്നു.

പഠനത്തോടനുബന്ധിച്ച് 28 ജനുസ്സുകളിൽപ്പെടുന്ന 34 ഇനം തുമ്പിക ളുടെ തന്മാത്രാ സ്വഭാവ നിർണയം നടത്തി. COI ജീൻ, 18S rRNA ജീൻ എന്നിവയുടെ ഭാഗിക ശ്രേണികളും COI ജീനിൽ നിന്നും ഉരുത്തിരിയുന്ന മാംസ്യശ്രേണികളും രൂപപ്പെടുത്തി. ഇവയെ ആഗോള ജനിതകബാങ്കിൽ (GenBank) നിക്ഷേപിച്ചു. ഇവയിൽ പന്ത്രണ്ട് COI ശ്രേണികളും ഇരുപത്തി മൂന്ന് 18S rRNA ജീൻ ശ്രേണികളും ലോകത്തിലെ തന്നെ ആദ്യ ജെൻബാങ്ക് രേഖകൾ ആണ്. തുമ്പികളുടെ കൃത്യവും വേഗത്തിലുള്ളതുമായ തിരിച്ചറിയി ലിനും വംശജനിതക പഠനത്തിനും COI ശ്രേണികൾ ഉപയോഗിക്കാം. ഉയർന്ന തലത്തിലുള്ള വംശജനിതക വിശകലനത്തിന് 18S rRNA ജീൻ ശ്രേണികൾ പ്രയോജനകരമാണ്.

COI, 18S rRNA ജീനുകളുടെ ഭാഗിക ശ്രേണികൾ അടിസ്ഥാനമാക്കി തുമ്പികളിലെ രണ്ട് ഉപവിഭാഗങ്ങളുടെയും (കല്ലൻതുമ്പികളും സൂചിത്തുമ്പി

കളും) തിരഞ്ഞെടുത്ത കുടുംബങ്ങളുടെയും വംശജനിതക വിശകലനങ്ങൾ നടത്തി. തുമ്പികളിലെ ജനിതക വ്യതിയാന മൂല്യങ്ങളും (Genetic divergence) കണക്കാക്കി തുമ്പിയിനങ്ങൾ തമ്മിലുള്ള ബന്ധങ്ങൾ വിവേചിച്ചറിയുന്നതിൽ COI, 18S rRNA ജീനുകളുടെ കാര്യക്ഷമത പഠിച്ചു. ഇരുജീനുകളും അടിസ്ഥാനമാക്കിയുള്ള വംശജനിതക വൃക്ഷങ്ങളുടെ (Phylogenetic Trees) താരതമ്യം 18S rRNA ജീനിനേക്കാൾ COI ജീനിന്റെ കൂടിയ കാര്യക്ഷമത വെളിപ്പെടുത്തി.

COI ജീൻ ശ്രേണികളെ അടിസ്ഥാനമാക്കി 27 ജനുസ്സുകളുടെ വംശജനിതക വിശകലനങ്ങൾ നടത്തി. വിദൂരസ്ഥലങ്ങളിലെ തുമ്പിയിനങ്ങൾക്കിടയിലും തുമ്പിയിനങ്ങൾക്കുള്ളിലും ഉള്ള ജനിതക വ്യതിയാനങ്ങളെക്കുറിച്ച് (*Interspecific and Intra specific genetic divergence*) ഉൾക്കാഴ്ച നൽകാൻ പ്രസ്തുത വിശകലന ഫലങ്ങൾക്കും ജനിതക വ്യതിയാന മൂല്യങ്ങൾക്കും സാധിച്ചു. 8 ഇനങ്ങൾ ഒഴികെയുള്ള ഭൂരിഭാഗം തുമ്പിയിനങ്ങളും കുറഞ്ഞ ജനിതക വ്യതിയാനം കാണിച്ചു. ജനിതക വ്യതിയാനം താരതമ്യേന സൂചിത്തുമ്പികളിൽ കുറവും കല്ലൻ തുമ്പികളിൽ കൂടുതലുമാണ് എന്ന് ശാസ്ത്രലോകത്തിന് വെളിപ്പെടുത്തി നൽകുവാൻ ഈ പഠനത്തിന് കഴിഞ്ഞു.

തുമ്പിയിനങ്ങൾക്കിടിയിലുള്ള ജനിതകവ്യതിയാനം ഏറ്റവും കൂടുതൽ തൊളിമിസ് (*Tholymis*) ജനുസ്സിലും ഏറ്റവും കുറവ് ഡിസ്ഫേയിയ (*Dysphaea*) ജനുസ്സിലും ആണ് കാണപ്പെട്ടത്.

പഠനത്തിന്റെ മറ്റൊരു കണ്ടെത്തൽ രൂപസാദൃശ്യമില്ലാത്തതും വിദൂരസ്ഥലങ്ങളിൽ കാണപ്പെടുന്നതുമായ തുമ്പികൾ തമ്മിലുള്ള അടുത്ത ജനിതക സമാനതയാണ്.

CHAPTER 1
GENERAL INTRODUCTION

1. GENERAL INTRODUCTION

1.1 General introduction to insects

Insects are the most diverse group on earth, with more than one million described species (Bybee et al., 2016; Weisser and Siemann, 2008). They are primitive organisms that evolved 450 million years ago. They have the expertise to live in all kinds of habitats and persist as a crucial part in the proper functioning and maintenance of the earth's ecosystems. The high diversity, global distribution, and a complicated evolutionary history make them unique organisms of the planet (Speight et al., 2005). They also play different roles in the ecosystem, such as agricultural pests, pollinators, disease vectors, parasites, predators of other insects, and indicators of ecosystem health (Price et al., 2011; Danks, 1992). They are closely linked to human beings and exert influence on us both positively and negatively. While some show remarkable ability for survival and existence, others are under threatened category and some have been extirpated. Studies on insects remain incomplete because of the vast diversity and distribution. It is essential to boost research works on insects as the studies on insect ecology, behaviour, and distribution throw light on our ecosystems' current condition and are beneficial to overcome the present predicament of nature (Foottit and Adler, 2009).

Insecta is the largest class of phylum Arthropoda, the largest animal phylum. The body of all insects is divided into three- head, thorax, and abdomen; head bears a pair of antennae and a pair of compound eyes; thorax has three pairs of legs and two pairs of wings, are some of the characteristics of insects. Several features make insects a specialised taxon.

The class Insecta is divided into two subclasses, viz., Apterygota (wingless insects) and Pterygota (winged or secondarily wingless insects), and comprises 30 orders.

Based on metamorphosis, insects are classified as holometabolous and hemimetabolous insects. The lifecycle of holometabolous insects comprises four stages- egg, larva, pupa and adult. In hemimetabolous type, metamorphosis is incomplete and has three stages: egg, nymph and adult.

1.2 Order: Odonata

Odonata is an order of class Insecta which comprises dragonflies and damselflies. Fossil records suggest that Odonata, the ancient order has history from the Lower Permian. (Wooton, 1981). The largest insect *Meganeuropsis permian*, having a wingspan of 70 cm, belonged to this order (Kalkman et al., 2008). The word Odonata was derived from the word "odonto" (Greek word, meaning- tooth) with reference to the toothed mandibles of the members of this order.

The order is divided into three suborders- Anisoptera, Zygoptera and Anisozygoptera. Anisoptera is the suborder of dragonflies. Anisozygoptera is considered as a living fossil and has only three species globally and only one species in India. Zygoptera is the suborder of damselflies. Dragonflies are robust-bodied, agile fliers. They keep wings horizontally during flight and also at rest. Damselflies are fragile-bodied and weak fliers. During rest, they keep wings parallel to the body. Anisoptera comprises more species than Zygoptera. However, Zygoptera fetches a higher level of taxonomic diversity than Anisoptera and comprises more families. The families Libellulidae and Coenagrionidae evolved recently and are the dominating ones. Species of these families show capability for migration and have extended distribution (Rehn, 2003).

Although there are three suborders, some recent studies consider the two suborders, Anisoptera and Anisozygoptera, together and name the group formed by merging these suborders as Epiprocta (Rehn, 2003; Kalkaman et al., 2008).

1.3 History of Odonatology

Corbet (1991) has given a detailed description on the history of odonatology. It began with the advent of binomial nomenclature by Carl Linnaeus in 1758 with a description of 18 odonate species under the genus Libellula. J.C Fabricius, in 1793 assigned dragonflies under an order named 'Odonata'. He also added descriptions of 69 species. Baron Michel Edmond de Selys-Longchamps contributed a significant advancement in odonate classification in 1820. He found wing venation as a suitable character for odonate classification. His contributions include a description of 700 species and 134 genera (Wasscher and Dumont, 2013).

When knowledge on odonates reached a substantial level, the works were based on a compilation of data on different aspects like ecology, behaviour and physiology of odonates. R J Tillayard was considered the most crucial figure in this field because of his outstanding works (Tillyard, 1917). Another illustrious personality was Corbet, whose contributions include publications enclosing ecology, biology and odonate behaviour (Corbet, 1962; 1980). Another book (Corbet, 1999) encompasses all the aspects of odonatology and still has great value among odonate researchers. Yet another relevant publication in this field was a book by Córdoba-Aguilar (2008), which contains 20 chapters by different authors describing developments in classification, evolutionary biology, ecology, behaviour and conservation biology.

The subsequent development was the launching of journals for the development of odonatology. The journal Odonatologica was established by the foundation Societas Internationalis Odonatologica (S.I.O) in 1972 for the intercommunication of odonate information. The Journal of Odonatology, a quarterly publishing peer-reviewed journal for promoting odonate-based research, was launched in 1998. Many conferences are conducted at regular intervals by different organisations to encourage odonate research and interchanging information. The International Congress of Odonatology was conducted by the Worldwide Dragonfly Association for the presentation of research papers in this field and to discuss the current status of odonatology, the threats faced by odonates and measures for protection (Khelifa et al., 2017).

A large number of Odonate studies on different aspects have been conducted worldwide, and presently they have a significant role in ecological and evolutionary research. However, there remain unresolved areas in this field and a number of odonates are still waiting for description (Froufe et al., 2014).

1.4 Odonate diversity

The diversity of dragonflies relies on mainly two components- geological and ecological. The geological components are the key factors in determining family and genus level composition. The distribution of dragonflies is directly linked with the climatological zones. A steep rise in dragonfly diversity can be observed when moving from the poles to the equator. Although temperature facilitates this

gradation, precipitation makes irregularities as low precipitation areas show decreased diversity. This is also called gaps in diversity. Tropical forests always show rich diversity of dragonflies, especially in montane regions (Oppel, 2005; Orr, 2006). Forest habitats are relatively more stable and this supports the occurrence of habitat specialists and rich diversity. (Kalkman et al., 2008). Most species of odonates are habitat specialists and they act as indicators of the quality of the wetland ecosystem (Subramanian and Babu, 2018).

Wetlands of tropical forests especially in hilly areas support a rich diversity of odonates. The relatively stable tropical forest habitat might support specialist species. The diversity of odonates is higher in the tropics. Both the species diversity and the family level diversity. Out of the 31 families, 12 are confined to streams of tropical forests. (Kalkman et al., 2008).

The odonate diversity of the world is about 6371 species (Paulson et al., 2022).The number of odonate species in India reached 496 in 153 genera and 17 families. The 7 families of Anisoptera found in India are Aeshnidae, Gomphidae, Chlorogomphidae, Cordulegastridae, Corduliidae, Libellulidae and Macromiidae. One representative of the family Epiophlebiidae is found in India. Lestidae, Synlestidae, Platystictidae, Calopterygidae, Chlorocyphidae, Euphaeidae, Philogangidae, Coenagrionidae, Platycnemididae are the 9 families of suborder Zygoptera found in India (Kalkman et al., 2020; Subramanian et al., 2020).

India has 195 endemic species referable to 69 genera (Subramanian and Babu, 2020). Species of the family Gomphidae and genera like Protosticta, Macromia and Idionyx are mostly endemic. Endemic odonates of India are concentrated mainly in southern Western Ghats, Eastern Himalayas, Western Himalayas and Andaman and Nicobar islands (Subramanian and Babu, 2017). From the Western Ghats, 207 species have been reported so far, including 80 endemic species (Nair et al., 2021)

One hundred seventy-four species of odonates have been reported so far from Kerala, from 14 families, including 65 endemic species of the Western Ghats (Gopalan et al., 2022). Seven families of damselflies viz. Lestidae(11 species), Platystictidae (12 species), Calopterygidae(4species), Chlorocyphidae (3 species), Euphaeidae (4 species), Platycnemididae (16species), Coenagrionidae (24 species)

and five families of dragonflies viz. Aeshnidae (9 species), Gomphidae (20 species), Chlorogomphidae (2 species), Macromiidae (10 species), Corduliidae (1 species), Libellulidae (50 species) and 8 species are considered *incertae sedis* as they are not placed in any families are found in Kerala (Kalkman et al., 2020; Gopalan et al., 2021).

1.5 Behaviour

Odonates are amphibiotic in nature. As they use freshwater habitats for breeding and larval development, they are always associated with freshwater bodies. Diversity is high in vegetated wetlands. Odonates species show specificity in their aquatic habitats. The aquatic habitats of different species of odonates are diverse, from torrential streams to stagnant pools. While most species can sustain only in freshwater, a few species can tolerate brackish water. Certain species are seen in urban areas and breed in manmade water reservoirs and canals and even in slightly polluted water. However, some species, especially endemic ones, can survive in undisturbed pristine habitats. Rich diversity is observed in forested streams and rivers and a majority of endemic species are also confined to these habitats. High diversity and endemism can always be observed in wetlands of hilly areas and forested habitats (Subramanian, 2009; Subramanian et al., 2011; Subramanian and Babu, 2017). While generalist species can be observed in paddy fields, ponds, canals and marshes (Subramanian, 2005; Subramanian et al., 2020). The human interference in forest habitats results in the replacement of habitat specialists with generalist species.

Odonates possess excellent vision and flight ability among insects. The eye of odonates is the largest of all known insects, covers almost the entire head and also has the capacity to visualise all regions except the part just behind the head (Corbet, 1999). The compound eyes show the highest development. The number of ommatidia may vary from 10,000 to 28,000. Certain species of dragonflies have up to 30,000-40,000 ommatidia in each eye at maturity. Aeshnides have the highest number of ommatidia of all the known insects (Land and Nilsson, 2012). They can rotate the head 180^0 sideward, 70^0 backward 40^0 forward and downward which makes a vision of nearly 360^0 possible (Andrew et al., 2008).

Odonates show amazing flight capacity due to their uncoupled wings and dynamic thoracic muscles. Wings of odonates are transparent in most of the species, some species have coloured and opaque wings (eg: *Neurothemis fulvia, Rhyothemis variegata*). A strong network of veins makes them robust. There is a pigmented spot called pterostigma in each wing at the leading edge, weighing only 0.1% of the total body weight of a dragonfly. Despite the small size, it contributes well in increasing flight speed by 10-25% (AkeNorberg, 1972). Hindwings are broader than forewings. Certain species, particularly migrating ones, have broader forewings than nonmigratory species, a modification for gliding flight (Huang et al. 2020).

Forewings and hind wings can work independently and the centre of gravity of wing bases is in control so that they exhibit a variety of flight skills. They fly backwards, straightly upward, hover and turn 180^0 also do prey capture and feeding during flight. But damselflies show some exceptions as they are weak fliers. Flight skills vary between species of dragonflies. While some species spend their whole life in the vicinity of water bodies or closer to the ground (e.g., *Acisoma panorpoides, Diplacodes trivialis*), some others fly high and rarely come down (e.g, *Tramea limbata*) and some others travel miles for migration (e.g., *Pantala flavescens*). There is always a direct relationship between flight capacity and geographic distribution. Strong fliers are always widely distributed (Subramanian and Babu, 2017).

1.5.1 Migration

Certain species of odonates possess migratory behaviour. The shape and characteristics of dragonfly wings directly relate to migratory behaviour. The dragonfly species with higher migratory capacity have long and narrow wings, and the anal region is also large. In contrast, the species with a lower ability for migration possess wide and short wings with a small anal region. Although the fore wing characteristics and shape have significance in phylogeny and interspecific relationships, hindwings do not have such kind of phylogenetic role. There are species-specific morphological changes found in dragonfly wings (Huang et al., 2020).

Certain species of odonates, especially dragonflies, exhibit capacity for migration over long distances, e.g., *Anax junius, Tramea lacerata* and *Pantala*

flavescens. Pantala flavescens is a well known migrant found in Kerala. Prevailing winds help in the free wandering of adults and the long periods of gliding with minimal effort is possible by their broader hind wings. There are reports regarding the migration of this species during night time (May, 2013).

1.6 Feeding

Both the larval and imaginal forms of odonates are predators in nature. Mosquito larvae, aquatic beetles, tadpoles etc. become the food of the larvae and adults feed on small to medium sized insects including odonates. Some species show cannibalistic behaviour e.g., *Orthetrum sabina* (Iswandaru, 2018). Adult odonates capture prey during flight. The foraging flights of most species occur during daytime. Some species forage during dusk (e.g., *Gynacantha millardi*). Food consumption also takes place during flights. The legs are modified to hold prey and transfer it to the mouth (Subramanian, 2005).

The predatory mode of lifestyle makes the odonates a key component in the food chain and ecosystem function. Adults can be considered as effective biocontrol agents as they feed on insects including noxious insects like mosquitoes. By feeding on harmful pests, odonates also proved their value in agriculture (Subramanian and Babu, 2018).

1.7 Odonate Biology

1.7.1 Morphology of odonates

Like other members of class Insecta, the body of odonates is divided into three- head, thorax and abdomen.

Head- Two large compound eyes are prominently seen on head, suitable for its predatory behaviour. They meet medially in dragonflies and separated in damselflies. Three tiny ocelli and a pair of short filiform antennae are also present on head. The other parts of head are labrum, labium, clypeus, frons, vertex and occiput. The mouth parts are adapted for biting and chewing type of lifestyle. The head is freely movable over the neck (Fraser, 1933).

Thorax- The thorax has two parts- a prothorax and a synthorax. The synthorax is made by the fusion of meso and metathorax. The prothorax is

composed of anterior, middle and posterior lobes. The fusion of thorax resulted in the forward shifting of three pairs of legs. The first pair of legs are prothoracic legs and the last two pairs are synthoracic legs. The wings are also attached to the synthorax; forewings to mesothorax and hindwings to metathorax (Saha, 2015). The legs along with the spines form a basket which is used for prey capturing (Mitra, 2006). The forwardly placed legs are well adapted for this purpose and perching and not for walking.

Abdomen- The slender elongated abdomen of odonates comprises 10 complete segments. In Zygoptera, all abdominal segments are almost equal in diameter except the basal and apical ends which show slight dilation. In Anisoptera, a variety of dilations can be seen in middle segments particularly closer to the basal ends. The abdominal tip bears the anus. In males, the genital pore is located on the ventral side of the ninth segment and the copulatory organ is in the second segment. The genital pore of the female is visible at the ninth segment ventrally. Well-developed gonapophyses can be seen in damselflies while reduced in some dragonflies. A pair of anal appendages are present at the 10^{th} segment of odonates both in males and females called superior anal appendages. In male dragonflies an additional median appendage is present ventral to these while in male damselflies two inferior appendages are present. In male odonates these appendages are adapted for holding females during tandem flights (Whedon, 1918).

1.7.2 Lifecycle

The life cycle of odonates comprises egg, larval and adult stages as they are hemimetabolous insects.

Egg - The procedure of egg laying and the habitat selected for it is species-specific. Mostly dragonflies lay their eggs by flying over water bodies or perching on any substratum. They select a variety of habitats such as torrential streams, ponds, lakes, rivers, manmade water reservoirs, wet soil near water bodies, water stored in tree holes or even the temporary water accumulation during the rainy season. Generally, eggs hatch after one week or take more time due to several factors (Miller, 1992). Within 7-30days the eggs hatch (Andrew et al., 2008).

Larva - Larval stages are also specially adapted for the predatory mode of lifestyle by their cryptic colouration and sharp eyesight. They feed on small aquatic organisms including mosquito larvae, beetles, tadpoles and even small fishes.

Duration of larval stages varies from a few weeks to 7 years (Kalkman et al., 2008). Also, the number of larval instars vary from 9-15. At the time of emergence they crawl up on nearby vegetation and the moulting process starts. They pump haemolymph into wings to spread them out. The entire procedure takes place during night and the teneral emerges before dawn. As body of the emerged tenerals is wet and fragile, they wait till the sunrise and become dried up and vigorous and fly away (Subramanian and Babu, 2017).

The larva is equipped with a well modified labium with the ability for fast movement for prey capture. The larvae of damselflies make use of two or three caudal gills as respiratory apparatus while dragonfly larvae rely on rectal gills for respiration (Kalkman et al., 2008).

Generally, species of tropical habitats have two or more generations while higher altitude species have only one generation yearly (Corbet, 1999).

Adult- Dragonflies generally take two weeks for maturation, while damselflies need roughly three weeks (Subramanian and Babu, 2017). In most species, immature males resemble females in colouration. Males return to waterbodies mainly for territory establishment and mating. Females also return back to water for mating and oviposition (Kalkman et al., 2008).

1.7.3 Reproduction

Odonates exhibit a unique mechanism of sperm transfer. The sperms are produced in the gonads situated in the last abdominal segment and transferred to the secondary genitalia in the second abdominal segment before copulation. After the process of sperm transfer, a territory is formed by male. Odonates exhibit territorial behaviour. The duration and strength of territoriality are species and individual specific (Corbet, 1999). Territoriality helps to reduce the risk factors like predation, damage and energy loss due to individual fights and makes the accessibility of female easier (Suhonen et al., 2008). Certain species exhibit courtship behaviour. Generally, courtship of damselflies is more prominent (Andrew et al., 2008). The first step of reproduction process is tandem formation. Upon finding a female, the male holds her neck using anal appendages (claspers) and this posture is called as tandem position. The structure of clasper is species specific and helps to prevent interspecific mating. In the next step female bends her abdomen to join the genitalia with male's accessory genitalia which results in the formation of a wheel

and it is called the mating wheel. The duration of mating also varies between species. Especially dragonfly mating lasts for a short duration (few seconds) and damselfly mating extends to several hours. After receiving the sperm the female prepares for oviposition (Mitra, 2006). The copulatory organ in male is also used to remove the already deposited sperm by another male in the female's oviduct.

Odonates show different methods of oviposition. Some species lay eggs within plant tissue by burrowing it (endophytic oviposition), some others lay on the surface of plants (epiphytic oviposition)and some others directly lay eggs as clutches into water or in damp soil (exophytic oviposition) (Corbet, 1999).

Endophytic oviposition is commonly seen in damselflies. The ovipositors of female damselflies are specially adapted for burrowing plant tissue. Some species use only specific plant species for oviposition (Subramanian and Babu, 2017). Endophytic oviposition is advantageous as it prevents water loss to some extent, reduces mortality rate and gives protection from enemies like parasitoids (Capinera, 2008). The majority of damselflies and some species of dragonflies (Aeshnides) use endophytic method of oviposition. Rest of dragonfly species use exophytic method in which risk factors are more. This is compensated by the increased number of eggs in each clutch. i.e. endophytically ovipositing odonates lay less than 300 eggs during oviposition and exophytically ovipositing species lay 1500 or more eggs per clutch (Corbet, 1999).

After mating male guards females during oviposition to prevent disturbances from conspecific males and sperm removal. Three types of mate-guarding behaviour are observed in odonates. 1) Non guarding 2) Non-contact guarding and 3) Contact guarding. In non-contact guarding the male guards the female without direct contact. In contact guarding male and female are in tandem at the time of oviposition (tandem oviposition; Corbet, 1999). Females are more protected in contact guarding than non-contact guarding. But in tandem oviposition, both male and female are under the same risk (Rehfeldt, 1995), while in non-contact guarding only female is under the risk (Schenk et al., 2004).

1.8 Economic importance

Odonates exhibit their economic importance through two roles – as biocontrol agents and as bioindicators. Odonates can be considered as an effective

biocontrol agent. They feed on noxious insects like mosquitoes and other disease vectors. So, they are potent control agents of these insects. By destroying agricultural pests, they have proven their significance in agriculture too.

Odonates also play a crucial role as bioindicators. As each species of odonates have specific habitat preferences, even a slight change in ecosystem quality affects their distribution. Habitat specialists, especially endemic species, are confined to pristine forest habitats. Any slight change in these habitats leads to the disappearance of these species from that ecosystem. (Subramanian, 2009). The increased appearance of generalist species in forest habitats also indicates the disturbance in the ecosystem.

1.9 Conservation

The increasing loss of biodiversity is a huge problem today. Odonates are threatened by habitat destruction or alteration, dams for irrigation and hydroelectric projects, plantation crops, use of pesticides, and climate change (Subramanian et al., 2020). Awareness programs and conservation projects yield little solace from the current condition. But the benefits of conservation programs are not evenly distributed to all organisms. Ordinarily, these projects are always restricted to three vertebrate groups, viz. mammals, birds, and amphibians (Stuart et al., 2004). The invertebrate taxa are usually ignored. Therefore, the invertebrates are more threatened and their extinction rate and diversity loss are not assessed perfectly (Thomas et al., 2004). Particularly insects whose smaller size and vast diversity make the task challenging and laborious (Clausnitzer et al., 2009). So, the biased assessment of diversity loss may not give a correct picture of the status of the world's biodiversity (Clausnitzer, 2009). As insects have an inevitable role in the ecosystem, a proper assessment of global insect diversity is required for the management and well-being of society (Speight et al., 2005).

Odonates have a widespread distribution except in Antarctica. The diversity and abundance are in peaks in tropical forests (Kalkman et al., 2008; Subramanian, 2009; Subramanian et al., 2011). Odonate larvae are sensitive to water quality and aquatic habitat, including aquatic vegetation type and the bottom substrate. The diversity of adults also relies on vegetation structure and shade cover (Bose et al., 2021). So, they show high sensitivity to habitat alteration or destruction. Generalist

species predominate in disturbed or temporary water bodies. Habitat specialists are restricted to undisturbed forest streams, rivers, or swamps. The ecological requirements of each species vary according to the distributional abilities. This particularity makes odonates suitable for evaluating the quality of the ecosystem. The presence of generalist species in forested habitats indicates the increasing disturbances of that habitat. Assessment of ecosystem health using odonates is a convenient method as they are known as indicators of the aquatic ecosystem. (Corbet, 1999; Kalkman et al., 2008; Khelifa, et al., 2017; Clausnitzer, 2009). Many of the odonate species, especially the endemics, are under threat. So, it is important to conserve our freshwater habitats along with other types of habitats for the conservation of odonates. The protection of odonates also supports the existence of other organisms in the freshwater ecosystem (Knight et al., 2005; Taylor, 2006).

It is clear that for the assessment of worldwide conservation status, a complete record of the distribution of odonates is required. There are many gaps in records, and a good number of species are under the data deficient category. Extensive field surveys are required to assess the current odonate status and distribution and mitigate data insufficiency (Clausnitzer, 2009).

Conservation programs should be conducted by giving special emphasis to tropical species. Pollution and habitat destruction are more harmful to habitat specialists (Clausnitzer, 2009).

It is impossible to implement conservation programs specific to each threatened species. Instead, implementing a group of measures that are effective for the conservation of almost all threatened species is commonly practicable. As tropical forest species mainly exhibit a narrow range of habitat tolerance, conservation of forest habitat leads to the protection of these species. Clausnitzer (2009) described the measures for the protection of odonates which includes; 1) Conservation of the existing forests by avoiding further degradation. 2) Promote afforestation with native trees which is also useful for preventing natural calamities like floods and landslides. 3) Proper management of forest fire. 4) Holding up native riparian vegetation of not less than 20m on both sides of streams or rivers in non-forested lands. 5) Accepting measures for the mitigation of water pollution including

the restriction of insecticides and pesticides. 6) Conservation of water catchment areas.

1.10 Significance of the study

Odonates have an inevitable role in aerial and freshwater ecosystems. Alterations in the quality and health of these ecosystems negatively affect the species richness and abundance of odonates. The present altered environmental conditions, including climate change due to anthropogenic activities, is a major threat to the existence of odonates; especially for habitat specialists (Samways and Steytler, 1996; Clark and Samways, 1996). It is essential to document the odonate diversity frequently to understand environmental changes and to formulate suitable conservation strategies. Effective conservation initiatives are only possible with a solid understanding of odonate taxonomy, ecology, and phylogeny. Preparation of taxonomic keys for odonates and the assessment of odonate fauna of different human-inhabited habitats are the aims of the present work.

Phylogeny is an advanced tool for analyzing the evolutionary and taxonomic status of any group of organisms. Even though there are numerous taxonomic studies on Kerala's odonates, phylogenetic studies are inadequate and meagre. The representation of odonates in global databases is seriously deficient relative to the diversity (Cameron, 2014). For accurate molecular phylogenetic assessment, sufficient sequence data of various species of organisms is necessary. The current work aims to contribute the COI and 18S rRNA sequence data of odonate species into the global molecular databases and to delineate the phylogenetic relationships among them. The present research work also compares the efficiency of COI and 18S rRNA genes in the resolution of relationships at different taxonomic levels.

By analysing intraspecific and interspecific divergence values, it is possible to determine the variation among individuals. Intraspecific divergence provides information about the variation that occurred as a result of geographic isolation or other environmental factors.

1.11 Objectives of the proposed work

- To strengthen the classification of odonates of Kerala using molecular taxonomy with COI and 18S rRNA gene sequences.
- To compare the efficiency of COI gene and 18S rRNA gene sequences in discriminating higher level relationships.
- To estimate the intraspecific and interspecific divergence values and scrutinize the genetic variability.

CHAPTER 2

MORPHOLOGY AND MOLECULAR CHARACTERISATION

2.1 INTRODUCTION

2.1.1 Taxonomy

Systematics and taxonomy are two closely related branches of science. Systematics deals with the reorganisation of evolutionary events of taxa and the results are shown in the form of trees (cladograms). Taxonomy is the branch of nomenclature, description and classification of organisms (Komarek and Beutel, 2006).

Conventionally morphological information has remained as the basis of odonate taxonomy. Especially wing venation was the focal point of most taxonomic works (Polhemus, 1997; Trueman, 1996; Carle and Kjer, 2002; Rehn, 2003; Bybee et al., 2008). Till the recent past, wing venation was a popular tool for odonate classification, and priority was given to morphological features more than any other sources of data (Fraser, 1957; Hennig, 1969; Hennig et al., 1981; Pfau, 1991; Trueman, 1996). Homoplasy is the main drawback of these characters. The reliability of plesiomorphic traits in classification is not sufficient. (Vick, 2000; Dijkstra and Vick, 2006). As wing venation is evolved many times, its effectiveness is also reduced. The convergence phenomenon raises challenges in a taxonomic grouping. Protoneurines and Disparoneurines were mistakenly grouped in some taxonomies due to reduced wing venation (Carle et al., 2008). Wing venation data often create misleading hypotheses because of the convergence. Members of Calopterygoidea possess dense patterns, but these are also seen in other damselflies. Many damselflies show sparse venation patterns similar to that seen in Coenagrionidae and Lestidae. The flight style also caused homoplasy in wing venation. Anal vein regions of dragonfly gliders have widened as an adaptation to decrease energy consumption (Corbet, 1999). So, these kinds of characteristics may mislead or complicate the taxonomic studies (Ballare and Ware, 2011).

For the assignment of an organism to a particular genus, only some basic systematic knowledge is considered. The requirement of thorough knowledge of phylogenetics is generally ignored. New species descriptions based on poorly developed species concepts may result in misleading conclusions. Certain variations possessed by existing species may be wrongly identified as features of new species (Komarek and Beutel, 2006).

The inadequacies of morphology-based classification was revealed by modern research. The scarcity of taxonomic experts and the vast diversity of insects make the species identification and description complicated. The advent of integrative taxonomy has alleviated the problem to some extent. It is the application of data from different sources, i.e. genetic, morphological geographical and ecological, to make the species description and identification more accurate. The application of molecular techniques is an efficient and convenient method among the different approaches of integrative taxonomy (Wang et al., 2016). Although odonate systematics has a long history, relevant improvements in this field have occurred through molecular phylogenetic studies (Ware et al., 2007; Fleck et al., 2008; Dijkstra et al., 2014; Khelifa et al., 2017).

Despite the large size and noticeable colours, studies on odonate diversity and taxonomy are not easy as we think. The larval stages and presence of cryptic species remain huge hindrances in exploring odonates. Although lots of studies have been conducted on Odonates, most of them were confined to adults, particularly to their flying period (Bried et al., 2012; Solano et al., 2018; Galimberti et al., 2021, Maggioni., 2021). The data on other development stages are scarce. These gaps can be filled by an integrated approach of morphology and molecular technics. This method is well-accepted in other insect orders -Lepidoptera (Mikkola and Stahls, 2008), Coleoptera (Smith et al., 2006 a) and Hymenoptera (Polaszek et al., 2004). There are many successful works in odonates (Bybee et al., 2008; Caesar and Wenzel., 2009).

2.1.2 Molecular Taxonomy and DNA Barcoding

Precise identification of taxa and reliable assessment of biodiversity is still a challenging field of biology. The high diversity of insects always makes taxonomic studies complicated. Molecular methods alleviate this problem by recognising the interrelationship between different insect taxa through the proper understanding of variations and similarities along with evolutionary descriptions. Various molecular markers are used for this according to the nature of the study. Analysis using molecular markers can precisely discriminate between adult and larval forms, males and females, individuals of different castes in social insects and polymorphic individuals (Danforth et al., 2005).

For the assignment of organisms in higher taxa, a strong phylogenetic concept is inevitable. This led to the advent of evolutionary taxonomy, in which phylogenetic relationships are used as the basis for classification (Mayr, 1981). Classifications without the involvement of phylogeny fail to connect with historic evolutionary processes and are not confirmable. Evolutionary taxonomy is always predictable (Komarek and Beutel, 2006).

The application of molecular techniques in systematics evolved as additional information to increase the accuracy of traditional methods. But in some cases, molecular taxonomy has failed to synchronise with traditional taxonomy (Misof et al., 2001; Saux et al., 2003). While certain works agree with the traditional taxonomies (Kjer et al., 2006; Dijkstra et al., 2007; Ware et al., 2007; Bybee et al., 2008; Carle et al., 2008; Ballare and Ware, 2011).

DNA barcoding is a method of application of a short standardised gene sequence in species identification (Hebert et al., 2003). It varies from molecular phylogeny in that the primary purpose is to identify an unknown sample in terms of a known classification rather than to determine classification. DNA barcoding eliminates the requirement of taxonomic experts in identification to some extent. For animal DNA barcoding, COI gene is conventionally used. Its increased insertion-deletion rate and nucleotide substitution rate are suitable for distinguishing cryptic species. Universal primers of COI are robust. DNA barcoding requires databases having sequences of almost all species. Any specimen can be identified by DNA barcoding; for this, the specimen is sequenced first and compared with the sequences that exist in the database. (De Mandal et al., 2014). There is a considerable difference between DNA taxonomy and DNA barcoding. In DNA taxonomy, evolutionary species concepts are implemented for the portrayal and confinement of species, while the latter emphasises sequence similarity for deducing species circumscription (Vogler and Monaghan, 2007; Rach et al., 2008). DNA barcoding is the most suitable method for species identification. But for species discovery, implementation of DNA barcoding alone is not appropriate (Vogler and Monaghan, 2007). For this purpose, DNA barcoding should be used with additional data to form an integrated taxonomy (Rubinoff, 2006; Rach et al., 2008). The significance of DNA barcoding in the identification of organisms is well proven today. Use of short DNA sequences of particular regions called marker genes in barcoding has resolved

many current problems like confirmation of certain specimens into species, assignment of different species into higher taxa like genus, subfamilies and families and identification of cryptic species (Hebert et al., 2003; Smith et al., 2006 b). The complete database for CO I identification will definitely be a solution for the identification problems facing today (Hebert et al., 2003)

However, molecular identification techniques also face some problems. Sequence submission of wrongly identified specimens to the public databases is the major problem. These kinds of practices diminish the efficacy of the molecular approach and make the species delineation more complicated (Vilgalys, 2003; de Mendonca et al., 2011). The importance of specialised taxonomists is not reduced completely, as the reference databases require precisely identified sequences. Only with error-free databases, the accuracy of DNA based identification possible (Salvi et al., 2020). The use of holotype for DNA extraction and sequencing is a solution to this problem but it is not practical in all cases as the holotype is preserved for future reference or maybe obsolete (Wang et al., 2016).

2.1.3 Molecular Markers

The search for the most suitable DNA marker gene has still not ended. The mitochondrial DNA and nuclear rDNA are consistently sequenced in insect systematics. Gene sequences showing faster evolution are essential for analysing recent divergences. In contrast, deep relationships can be studied by conserved gene sequences (Chippindale, 1999). Although many marker genes are available today, no single gene exists that comprises all the essential qualities of an ideal marker gene. For example, ribosomal genes are far better than protein coding genes in the case of informative sites, but the alignment of these genes(ribosomal) is not easy (Cruickshank, 2002). The currently using marker genes come under the following categories.

a) Non-coding regions

Non-coding regions are suitable for species level studies, especially for closely related species, due to their high evolutionary rate. The regions coming under this category are control region of mitochondrial genes, introns and ITS regions of nuclear genes (Zhang and Hewitt, 1997). ITS1 and ITS2 (first and second internal transcribed spacer region of the nuclear ribosomal gene cluster) are commonly used

markers under this category. Since these regions are spliced out after transcription and have no other functions these are under weak selection pressure and have increased substitution rate. Because of these characteristics, ITS markers are generally used for intraspecific studies or studies of closely related species (Weekers et al., 2001; Cruickshank, 2002; Hovmoller and Johansson, 2004; Dumont et al., 2005, Kiyoshi and Sota, 2006).

b) Mitochondrial markers

There are certain qualities of mitochondrial DNA that make it a popular marker gene for phylogenetic studies. The mitochondrial genes are maternally transmitted and the rate of mutation is very high due to a weak repair system and also have conserved regions (Brown et al., 1979). These features are advantageous in the development of universal primers. The different regions vary in evolutionary rate and the increased copy number facilitates trouble-free isolation from degraded or scanty samples. The absence of recombination and rarely occurring gene rearrangements are the other advantages of mtDNA in phylogenetics. Sometimes nuclear mitochondrial DNA is formed when the mtDNA sequence invades into nucleus this sequence can be utilized for distinguishing between insect species (Richly and Leister, 2004; De Mandal et al., 2014). These genes are known as mitochondrial pseudogenes or numts (nuclear mitochondrial DNA). Certain studies have reported that these genes may interfere during amplification and sequencing and lead to inaccurate results (Cruickshank, 2002).

Mitochondrial genome of animal phyla is a double-stranded molecule enclosing 37 genes of large and small subunit ribosomal RNAs, 22 tRNAs for the translation of the protein coding genes and 13 protein coding genes required for oxidative phosphorylation process. A regulatory component, AT rich region significant in the initiation process of translation and replication is also present. The crucial mitochondrial genes are highly conserved in animals. Yet insects are exceptional and show variability among different orders. The two kinds of mitochondrial genes, the ribosomal and the protein-coding genes are used for phylogenetic studies (De Mandal et al., 2014).

The ribosomal RNA genes seen in insect mitochondria are, 12S rDNA and 16S rDNA. Internal transcribed spacers are not present between these genes. There

are 13 mitochondrial protein coding genes seen in insects. According to Zardoya and Meyer (1996), based on the effectiveness in phylogenetic studies these genes can be grouped into three:- good performers (ND4, ND5, ND2, COI and COII), medium performers (COB, COIII, ND1 and ND6) and poor performers (ATPase 6, ND3, ATPase 8 and ND4L).

Cytochrome oxidase subunit I (COI) is the most accepted marker gene. Cytochrome c oxidase is a crucial enzyme of mitochondrial electron transport system. Three genes are coding for the cytochrome oxidase subunit of mitochondria. Among these COI is the biggest one and is 894bp long approximately. COI and COII are widely used in the resolution of a wide range of taxonomic levels in insects from species level to families or orders. Different regions of COI are sequenced for various studies (De Mandal et al., 2014). The evolution of COI is slower than other protein coding mitochondrial genes (Patwardhan et al., 2014). 658 bp at the 5' end of Mitochondrial cytochrome c oxidase subunit I gene has been widely used (Maggioni et al., 2021). The next most frequently used gene sequences are 16S and 12S rRNA (Caterino et al., 2000; Misof et al., 2000; Kambhampati and Charlton, 2002; Saux et al., 2003). CO II is widely used and homologous sequences of almost all insect orders are obtainable in databases. Works based on COIII, NADH dehydrogenase 1 (Rach et al., 2008), ND2, ND4, ND5 and cytochrome b are not so common (Caterino et al., 2000).

12S rDNA is suitable for distinguishing higher level taxa like phyla as it is highly conserved (De Mandal et al., 2014). 16S rDNA is less conserved than 12S rDNA and it can be used for the classification of genera or families. The percentage of conservation among mitochondrial genes varies in this order: 12S rDNA> 16S rDNA> Cytochrome b> control region (CR). When moving from 12S rDNA to CR variability increases (Arif and Khan, 2009). Chippindale et al. (1999), Artiss et al. (2001), Lin et al. (2010), Yong et al.(2016), Zhang et al. (2017) and Cai et al. (2018) are relevant studies using mitochondrial marker genes.

c) Nuclear ribosomal genes

Ribosomal RNA is ubiquitous and comprises both highly conserved and variable regions and it is an appropriate tool for phylogenetic studies. Ribosome is composed of ribosomal RNAs and proteins. Ribosome have 2 subunits- a small subunit (SSU)

and a large subunit (LSU) in all organisms. In eukaryotes the SSU comprises a single RNA- 18S rRNA and LSU consists of three RNA species viz. 5S, 5.8S and 28S rRNAs. As the evolution of rRNA genes is slower when compared to that of the protein encoding genes, rRNAs are the right tool for studying distant relationships (Moritz et al., 1987; Patwardhan et al., 2014). 18S and 28S rRNAs are considered as the robust tool for analyzing deeper relationships (Cruickshank et al., 2002, Dumont et al., 2010).

Nuclear protein-coding genes also used for resolving phylogenies. Paralogy is the main problem with these genes. Duplications frequently happen and the probability of sequencing same copy of the gene in all taxa is low (Cruickshank et al., 2002). From this category of genes Elongation factor 1 alpha (EF1α) is generally analyzed in insects. Because of the intron/exon structure nuclear genes are applicable in the resolution of low taxonomic levels too (Cruickshank et al. 2002). Nuclear protein-coding genes show different intragenic and intergenic substitution rates and are easy to align. Because of these advantages, these are also good tools for insect phylogeny (Friedlander et al., 1992; 1994; Danforth et al., 2005).

Although a variety of mitochondrial and nuclear DNA markers are currently used for phylogenetic analysis, it is very important to select appropriate marker genes for the study purpose. As the marker genes are different in various aspects like conserved regions a wrongly selected marker gene can mislead the result Sunnucks (2000). Therefore, proper planning is crucial for every molecular work (De Mandal et al., 2014).

2.2 REVIEW OF LITERATURE

Odonata is a relatively well studied order when compared to other insect orders. A great deal of literature on odonates is available worldwide today, handling various aspects. Some relevant works related to the current topic are discussed here.

2.2.1 Taxonomic works on odonates

Among the earliest literature on world odonata, book written by Tillyard (1917) describing the anatomy, morphology, embryology, taxonomy, geographical distribution and collection and preservation techniques still draws attention. Another detailed account on odonate behaviour, life cycle and ecology was provided by Philip S Corbet (Corbet, 1962; 1980; 1999) enclosing reproductive behaviour, oviposition, stages of the life cycle, foraging behaviour, the physical environment, microhabitat and also interaction with human beings. A detailed description of North American odonates including classification and morphological features, collection and preservation techniques was prepared by Needham (1975) and he also published a book for the identification of odonates of North America (Needham et al., 2000). Dragonfly diversity of Great Britain and Ireland was studied by Hammond (1983). d'Aguilar, et al. (1986) provided a field guide to the odonates of Britain, continental Europe and North Africa. An elaborated description comprising evolutionary history, zoogeography, collection and preservation methods, breeding and rearing procedures, detailed checklist, descriptions and keys for larvae and adults of New Zealand dragonflies were prepared by Rowe (1987). A field guide was prepared by Watson et al. (1991) for the identification of Australian odonates. Dunkle (2000), prepared a field guide to the dragonflies of North America. An elaborated account encompassing all the aspects of dragonflies of Europe including a checklist of 124 species was published by Askew (2004). Theischinger and Hawking (2006) published a field guide to the entire species of Australia. A detailed record on odonates including conservation and a list of threatened species odonates of South Africa was published by Samways (2008). Dijkstra has made crucial contributions to the world odonate literature. Dijkstra and Kalkman (2012) reviewed odonate literature of Europe and generated summarized phylogenies. A detailed revision of the suborder Zygoptera was done, synonymized a number of well-established genera and described new families and subfamilies (Dijkstra and Kalkman, 2013; Dijkstra

et al., 2014). An elaborated description of odonate morphology and ultrastructure along with phylogeny, taxonomy, biology, ecology and behaviour, collection and sampling methods was done by Suhling et al. (2015). Garrison and Von Ellenrieder (2019) published of a list of synonyms of new world odonates as a revision of the old volume Garrison and Von Ellenrieder (1991). Two volumes of field guide to the dragonflies of Britain and Europe were also published (Dijkstra, 2006; Dijkstra and Schröter, 2020).

A detailed monograph on odonates of South Asia by Kalkman et al. (2020), presented a checklist of odonates of Bangladesh, Bhutan, India (including Andaman and Nicobar Islands), Nepal, Pakistan and Sri Lanka. The monograph documented 559 species of odonates including 251 single country endemic species. Most of the recent findings in odonate literature were compiled in the monograph. The species *Enallagma parvum* was placed under genus *Amphiallagma* (May, 2002). All the species of the genus *Cercion*, except *Cercion lindenii* was brought under the genus *Paracercion. Cercion dyeri* was synonymized to *Paracercion calamorum* (Weeker and Dumont, 2004). Specimens which were considered as *Ischnura aurora* from India are now regarded as *Ischnura rubilio* (Papazian et al., 2007). The genus *Onychargia* which was formerly placed under Family Coenagrionidae was shifted to Family Platycnemididae according to the studies of Dijkstra et al. (2014) and Orr and Dow (2015). *Gynacantha millardi* was wrongly considered as a synonym of *Gynacantha bayadera*. According to Priyadarshana et al. (2015), while *Gynacantha millardi* is distributed in India and Sri Lanka, *Gynacantha bayadera* is restricted to Northeast India. *Vestalis submontana* and *Vestalis nigrescens* were raised from subspecies level to species level. The three species *Vestalis nigrescens, Vestalis submontana* and *Vestalis apicalis* are endemic species. *Vestalis nigrescens* is endemic to Sri Lanka while the other two are endemic to India. *Vestalis submontana* is restricted to the Eastern and Western Ghats (Hamalainen, 2011; 2016). The South Indian species *Indosticta deccanensis* was formerly placed in genus *Platysticta* which is confined to Sri Lanka the morphological examination and molecular study confirmed the independent existence of genus *Indosticta* (Bedjanic et al. 2016). Among the two subspecies of *Lestes praemorsus* i.e. *Lestes praemorsus praemorsus* and *Lestes praemorsus decipiens*, only the latter one is seen in the Indian subcontinent. One more subspecies *Lestes praemorsus sikkima* was described from

Sikkim. The genus *Rhinocypha* was divided into *Aristocypha, Calocypha, Heliocypha* and *Rhinocypha*. *Libellago indica* was promoted to species level from the subspecies position of *Libellago lineata*. The former is endemic to peninsular India while the latter is widely distributed in southeast Asia (Hamalainen, 2016). Specimens which were considered as *Aciagrion hisopa* from India are now identified as *Aciagrion approximans* while *Aciagrion approximans krishna* is an endemic species of the Western Ghats and *Aciagrion approximans approximans* is distributed in northeast India (Joshi et al., 2016). *Ceylonolestes* is considered as the synonym of *Indolestes*; *Lestes umbrina* and *Lestes thoracica* are synonymized to *Lestes concinnus* (Dumont et al., 2017). *Anaciaeschna donaldi* and *Anaciaeschna kashmirense* are synonyms of *A. martini* (Conniff et al., 2019; Kalkman et al., 2020).

In India, the odonate studies were pioneered by Linnaeus (1758) through the description of the damselfly *Neurobasis chinensis*. But the site where he found the specimen was outside of India. In fact, *Rhyothemis variegata* was the first species described from India (Linnaeaus, 1763). Drury(1770; 1773), Fabricius (1775-1798), Selys-Longschamps (1840-1891) and Rambur (1842) were the initial contributors by whom several species from India were described (Subramanian and Babu, 2017).

Laidlaw's works in the Western Ghats and Eastern Himalayas (1914-1932) are noteworthy even in the modern world. F. C. Fraser can be considered a legend in this field because of his remarkable contributions, including publications (1918-1935) and a book series Fraser (1933; 1934; 1936). These books still have great value among odonate researchers and are being used as inevitable guides for identification. The earlier notable works in odonate literature are Singh and Baijal (1954), Singh (1955; 1963), Kumar (1971;1980;1984), Kumar and Prasad (1977), Lahiri (1977;1979;2003), Ram et al.(1983), Prasad and Ghosh (1988), Lahiri and Sinha (1991), Srivastava and Sinha (1993), Ram and Prasad(1999).

Odonate fauna of central India was well documented by Mitra (1986), who reported 11 new species. Further studies recorded 39 species from Central India (Mitra, 1988). A checklist of Indian odonate species with larval description was prepared by Prasad and Varshney (1995) with an allusion on *Epiophlebia laidlawi.*

Mitra recorded odonate fauna of different states of India such as 69 species from Orissa (Mitra, 2000), 92 from Arunachal Pradesh (Mitra, 2006), 13 species from Rajasthan (Bose and Mitra, 1975) and 32 species from Nicobar islands (Mitra, 2002 a). Mitra (2002 b) prepared list of endemic odonates of India. The high rate of endemism is observed in India due to its separation from the whole world by the Thar desert, Himalayas, oceans and seas. Odonate fauna of Rajastan Thar desert national park was studied by Prasad (2004). A systematic list and description of Indian odonates were prepared and presented a hand book (Mitra, 2006). 30 species of odonates were recorded from the moist deciduous forest surrounding Dholbaha dam, Hoshiarpur, Punjab, India (Sharma and Joshi, 2007). The impact of riparian land use on diversity and distribution of odonates was investigated by Subramanian et al. (2008). Andrew et al. (2008) prepared a field guide with detailed notes on 45 species, 32 species of dragonflies and 13 species of damselflies found in Nagpur. Subramanian (2009) published a checklist of Indian odonates. He has listed 463 species of odonates of India and has also given notes on species newly described, new species reported, species synonymized from India after 1995 and also a list of species removed from the checklist. 367 species of odonates were recorded by Mitra et al. (2010) from Eastern Himalayas of which 4 species are coming under the Threatened category. A review on the Indian species of the families Platycnemididae and Coenagrionidae was done by Mitra and Babu (2010). Rangnekar et al. (2010) recorded 66species of odonates from Goa with first records of 34 species from the state. The study raised the odonate diversity of Goa from 39 to 74. Through another study Rangnekar and Naik (2014), 13 more species were newly recorded from the same state with 5 endemic species. Thirty six species of odonates were recorded from Kanha National Park, Madhya Pradesh (Tiple et al., 2011). Odonate surveys were carried out at Tropical Forest Research Institute, Madhya Pradesh by Tiple et al. (2012) and reported 48 species of odonates referable to 32 genera of nine families including 8 new records to Madhya Pradesh. Tiple and Chandra (2013) documented 106 species of Odonata with 14 new records from Chhattisgarh and Madhya Pradesh. Studied the role of environmental variables such as canopy cover, area of water spread on transect, and altitude on the diversity and species composition of odonates. Studies conducted by Ashish D Tiple and Pankaj Koparde concentrated mainly on odonate fauna of Maharashtra. Tiple et al. (2013) documented 82 species from Vidarbha , Maharashtra by compiling survey results and other authenticated

records. Of the 82 species 13 were new to Vidarbha and 6 new records for Maharashtra. Seasonal distribution, habitat and significance of catchment land use on odonate diversity were studied by Kulkarni and Subramanian (2013). Tiple and Koparde (2015) compiled their survey results with previous records to generate a checklist of 134 species of odonates from Maharashtra. Checklist of odonates of India was prepared by Subramanian (2009) and Subramanian and Babu (2017). Subramanian and Babu (2018) compiled the previous works on the odonate diversity of the Himalayas and found 257 species including 23 endemics. Eastern Himalayas showed the highest diversity. The least diversity was observed in cold deserts. The study pointed out the threats faced by odonates in this region like dams, hydroelectric projects, agricultural activities, tourism and pollution. There is no recent reports of the living fossil *Epiophlebia laidlawi* from India. Subramanian and Babu (2019) described odonates of India including keys to adult and larval stages, habitat, life cycle, conservation status, economic importance and collection and preservation. Diversity and distribution along with the checklist of Northeast Indian odonates were recorded by Subramanian et al. (2020). By the study, it was revealed that streams and rivers of montane regions possess rich odonate diversity. Recently some new species descriptions and additions to the Indian Odonate checklist were done by Joshi and Sawant (2019; 2020), Joshi and Kunte (2017) and Joshi et al., 2016; 2017; 2020; 2022).

Odonate fauna of northeast India was explored by Prosenjith Dawn. A study was conducted in Kolkata and Howrah and documented 80 species of odonates, among them 4 are new records to West Bengal. The water bodies used for fish culture showed low odonate diversity due to manmade disturbances like the destruction of aquatic vegetation and the use of pesticides Dawn (2014). Joshi and Kunte (2014) documented 69 species from Nagaland including 43 new additions. Dawn, (2016) published a review work on larval studies in India and pointed out the insufficiency of larval and exuvial studies and also the need to improve such studies. He found that larval stages of only 20% odonates of India were brought under study. A bulk of data still remains unexplored. So, the studies on odonates should be extended not only to adults but also to larval stages and exuviae. A checklist comprising 239 species of 114 genera and 17 families was prepared from West Bengal (Dawn, 2021). 8 species were newly recorded from the state and two of them

Lyriothemis mortoni Ris, 1919 and *Cephalaeschna triadica* Lieftinck 1977, were the first records from India. He has also given brief notes on species wrongly identified and reported previously from West Bengal.

The Western Ghats, one of the 36 Biodiversity hotspots of the world, has high biodiversity and endemism. The Western Ghats, one of the most favoured places for diversity studies, and the exploration of odonate diversity of this region is still going on. The Western Ghats is always an ideal study location for odonate researchers. Odonates of western Ghats have been being studied by researchers and some prominent works such as Fraser, 1918 to 1936, 1938, 1939, 1946, 1953; Kimmins, 1958; Laidlaw, 1914-1917,1930; Lieftinck, 1960; Mitra, 1994; Prasad and Varshney, 1995; Tyagi, 1997 (Subramanian et al., 2018).

Subramanian (2007) studied about the endemic odonates of the Western Ghats and documented 68 species of endemics out of 176 odonates of the Western Ghats. He described the habitat distribution and conservation of endemic species of the Western Ghats. The seasonal and habitat distribution of odonate species at different land-use types in river basins of the Western Ghat region of Maharashtra was investigated by Kulkarni and Subramanian (2013). Subramanian et al. (2013) gave a note on the current distribution of the genus *Idionyx* of the WG and described a new species *Idionyx gomantakensis* from Goa(WG). Checklist of Odonates of Karnataka was prepared by Emiliyamma and Subramanian (2013) reported 137 sps of which 41 were endemics. Endemics are restricted to hill streams and forests of the WG. Koparde et al. (2014) documented 64 species of odonates from the WG region of Maharashtra, including 7 new records to the study area and 4 new records to Maharashtra. Adarsh et al. (2015) documented odonate diversity of the Chinnar Wildlife Sanctuary in the southern WG. Subramanian et al. (2018) studied the geographical distribution of odonates of different zones of Western Ghats comprising Coorg-Wayanad Nilgiri complex, Anamalai-Palani-Kodaikanal, Periyar and Aghastyamalai landscapes, prepared distribution maps and analysed the patterns of distribution of Western Ghats odonate species. Libellulidae, Gomphidae and Coenagrionidae are the predominating families of the Western Ghats. The representatives of these families occupy 58% of the total odonates species and 36% of endemics. 22 species of gomphids, 4 species of coenagrionids and only one species of libellulids were endemic to the study location. Most of the endemic

odonates are from Platystictidae, Platycnemididae, Gomphidae, Macromidae and Synthemistidae families. All representatives of the families Platystictidae and Chlorogomphidae and genera *Euphaea, Esme, Burmagomphus, Megalogomphus, Merogomphus, Microgomphus, Idionyx* are endemics to the Western Ghats. Hill streams and rivers of pristine riparian forests are the reservoirs of rich diversity and endemism. In contrast, low diversity and endemism can be seen in paddy field, marsh, lake and pond ecosystems. 2 species of genus *Euphaea* are recently described from the Western Ghats region of Maharashtra – *Euphaea thosegharensis* and *Euphaea pseudodispar* by Bhakare et al. (2021). First record of *Gynacantha khasiaca* from the Western Ghats was provided by Koli et al. (2021). The latest work revealed that the number of odonate species of the Western Ghats is 207, out of which 80 species are endemic (Nair et al., 2021).

In addition to the work of Fraser, the initial odonate studies of Kerala include the following works. Rao and Lahiri (1982) studied the odonate diversity of Silent Valley and New Amarambalam reserved forests and Mathavan and Miller(1989) conducted odonate survey of the Periyar National Park. Prasad and Kulkarni (2002) reported 34 specimens from Eravikulam National Park. A systematic account on 27 species of odonates of Thiruvananthapuram district was prepared by Emiliyamma and Radhakrishnan (2002). Odonate fauna of Kottayam District was recorded by Emiliyamma (2005). An authentic record of 31 species of odonates from Kottayam district was made by Emiliyamma (2005). 52 species of odonates were recorded from the Kerala Agricultural University (KAU) campus, Thrissur by Adarsh et al.(2014). The presence of agricultural fields and vegetated water bodies supported the rich diversity of this area. *Heliogomphus promelas* and *Indothemis carnatica* were also reported by this study which are belonging to the near threatened category in the IUCN Red List. The study also pointed out the significance of university campuses in the conservation of biodiversity. Emiliyamma and Radhakrishnan (2000; 2014) documented 39 species of odonates from Parambikulam wild life sanctuary. The photographic field guide by Kiran and Raju (2013) having records of 154 odonate species is still having great value among researchers. Varghese et al. (2014) recorded 82 species of odonates from the vicinity of Salim Ali Bird Sanctuary, Thattekkad including 21 endemic species and also some near threatened and vulnerable species. Endemic species were mostly confined to forested streams

and river ecosytems. Rivers and streams flowing through non forested habitats possessed less species richness and endemism. The study also revealed the occurrence of species coming under IUCN near threatened category (*M. hannyngtoni*) and vulnerable species (*P. deccanensis* and *P. sanguinostigma*). Forty-four odonate species were recorded from Kannur (Nair, 2014). Adarsh et al. (2015) reported 48species of odonates from Chinnar Wild Life Sanctuary, Idukki. Five different habitats were selected for study viz. scrub jungle, dry deciduous forest, moist deciduous forest, riparian forest and montane shola forest. The highest species richness was observed in the riparian forest. Two endemic species of the WG, *Protosticta gravelyi* and *Esme mudiensis* were also recorded. Three species namely *Gynacantha dravida*, *Esme mudiensis* and *Dysphaea ethela* which are categorized under Data Deficient IUCN Red List category were reported from Chinnar. Bose and Kakkassery (2019) documented the odonate diversity of Thrissur district. 59 species of odonates were recorded from Wayanad (Susanth and Anooj, 2020). Of these, four species are coming under IUCN data deficient category namely *Esme mundiensis*, *Pseudagrion indicum*, *Hylaeothemis indica* and *Macrogomphus wynaadicus*. 44 species of odonates were recorded from the Kole wetlands of central Kerala (Chandran et al., 2021).

Riparian diversity of odonates were studied by Vincy et al.(2016) at Meenachil river basin, Kottayam district and documented 36 species of odonates. Of these 9 species were newly added to the checklist of Kottayam district. Riparian odonate diversity of midstream Chalakkudy river was studied by Bose et al.(2021) and documented 25 species of odonates from the riparian habitat. Five endemic species were recorded of these *Pseudagrion indicum* is endemic to the WG and 4 species viz., *Vestalis apicalis, Libellago indica, Dysphaea ethela* and *Heliocypha bisignata* are endemic to India. The study pointed out the significance of abundant native riparian vegetation in odonate diversity and the adverse effects of habitat alteration and tourism activities on the existence of odonates. Although disasters like floods can cause an immediate drop in species richness and abundance, they will be bounced back to the normal level.

Recent studies added up the odonate diversity of Kerala to 174. Emiliyamma et al. (2012) *Microgomphus souteri* was recorded for the first time in Kerala. Emiliyamma et al. (2013) newly added *Lyriothemis acigastra* to the species list of

Kerala. *Protosticta ponmudiensis* was newly described from Ponmudi hills of the Agasthyamalai region of the WG region of Trivandrum district Kerala Kiran et al.(2015). *Protosticta monticola* was described from shola forests of Idukki district (Emiliyamma and Palot, 2016). Rangnekar et al.(2019) described a new species *Cyclogomphus flavoannulatus* from the Western Ghats region of Kerala and Goa. *Protosticta cyanofemora* was described from WG region of Kollam (Joshi et al., 2020). *Platylestes kirani* was described from the northern coastal region of Kerala (Emiliyamma et al., 2020). *Platylestes platystylus* was added to the checklist of Kerala (Rison and Chandran, 2020). *Protosticta rufostigma* from the Western Ghats region of Kerala (Sadasivan and Palot, 2021). Sadasivan et al. (2021) rediscovered *Anaciaeschna martini* from the WG region of peninsular India(also from Idukki dist.). *Bradinopyga konkanenesis* was recently reported from Kasaragod district of Kerala (Haneef et al., 2021). According to Nair et al. (2021) odonate list of Kerala is extended by the addition of species such as *Amphiallagma parvum*, *Ceriagrion chromothorax, Pseudagrion australasiae, Crocothemis erythraea, Protosticta sholai, Zygonyx torridus, Paracercion malayanum, Indothemis limbata, Indolestes pulcherrimus* and *Anax indicus* and reported the total species richness as 181. However, Gopalan et al. (2022) and Chandran and Sherif, (2022) confined the number of odonate species to 174.

Taxonomic characters of dragonfly exuviae can also be used for diversity studies of odonates. This method is advantageous because it does not harmfully affect the odonate population (Paul and Kakkassery, 2013; Adambukulam and Kakkassery, 2013).

2.2.2 Molecular taxonomy

Morphology is the cornerstone of taxonomy; however, sometimes, data from other areas of biology become inevitable for species identification and phylogenetic assessment. Identification based only on morphological features may lead to wrong classification (Herrera et al., 2010). The worldwide acceptance of integrative taxonomy has been increasing consistently because of the reliability contributed to taxonomy. It mainly integrates ecological, geographical, morphological, behavioural, developmental and molecular data to taxonomy to produce highly refined results Dayrat (2005). Since the development of molecular techniques,

molecular data have been regularly used in taxonomic works for species delineation and the construction of more reliable species hypotheses (Pimenta et al., 2019). Even though there are lots of studies by combining data from different branches with morphological data, application of molecular data is the most accepted, reliable and convenient method. The application of molecular techniques in phylogenetic studies has led to more reliable results.

While classical phylogeny depends on morphological traits for identifying the evolutionary relationships, application of nucleotide or protein sequences in analysing the relationships between organisms or genes is the base of molecular phylogeny (Patwardhan et al., 2014). The diversity is not confined to the morphological features but spread across the structural, biochemical and molecular frameworks. Organisms which show morphological resemblance may vary greatly in their biochemical and molecular characters. Both methods, i.e. classical and molecular phylogeny, have advantages and disadvantages and are equally significant as the morphological traits are determined mainly by the gene sequences. Thus, a combination of classical and molecular phylogeny yields a better resolution of relationships (Patwardhan et al., 2014). A perfect resolution of relationships occurs when molecular and morphological sources are congruent with each other and with geographical and ecological patterns (Dijkstra and Kalkman, 2012). Species identification becomes more accurate and objective when a combination of molecular based and traditional morphology based identification is applied (Tallei et al., 2017). Generally, when molecular and morphological pieces of evidence are in agreement, often in synchrony with geographical or ecological patterns, relationships are resolved most convincingly.

Molecular method of phylogeny is advantageous over morphological method because the former can be acquired easily. The gaps in fossil records can be filled by molecular method and is also free from sampling bias (Patwardhan et al., 2014). Among different molecular methods, the most accepted one is that which comprises DNA isolation, PCR amplification and sequencing and using these sequences for phylogenetic analysis. The identification based on marker genes becomes the most reliable method of taxonomy nowadays.

Molecular methods like DNA barcoding are accurate and nondiscriminatory tools for the assessment of taxonomy (Pfenninger et al., 2007). DNA barcoding is a highly efficient tool for taxonomic identification using a universal marker gene. Dr. Paul Hebert is called the father of DNA barcoding as he first applied this technique for taxonomic identification in 2003. This method has many advantages and the most important one is that the identification process is not affected by the stages of life cycle of organisms and the damage occurs for the specimen. The principle behind this method is comparing a particular marker gene sequence of unknown organism with the barcode library of the same gene for the precise identification of that organism.

Traditional methods of taxonomic identification of organisms, especially insects are time consuming and require the help of experts. Identification of immature or partially deformed forms is another hindrance in this field. Although the molecular methods are more accurate and reliable, the public databases (GenBank and BOLD) require more completely identified sequences. That means the sequences available in these databases are still inadequate, and many of the sequences are identified only up to the higher taxonomic ranks. The effectiveness of molecular taxonomy can be fulfilled only by the sufficient number of DNA barcode sequences identified up to the species rank (Porter et al., 2014).

Although species identification and species discovery are considered as the two important applications of DNA barcodes, only the former one is more appropriate. In species identification process DNA sequences are used. Make use of DNA sequences as markers of already described species is happened in the process of species identification. Instead of sticking on to a single species concept, DNA barcoding is congruent with any species concept that used for the establishment of a named species (Rach et al., 2008). Sometimes the traditional taxonomy fails to describe new species identified through molecular methods. Species discovery is more complicated and it is closely related to taxonomy. So, DNA barcoding alone cannot be used for this purpose. This is not a matter only for DNA barcoding but applicable to morphological, ecological and behavioural attributes. A single data type is not sufficient for species discovery process. It should be done with the aid of a species concept and verification is also needed (DeSalle et al., 2005; Rach et al., 2008). The genera *Stenocypha, Matticnemis* and *Spesbona* were newly identified by

molecular methods after that they were well described by morphological characters (Dijkstra, 2013).

A great deal of literature based on molecular taxonomy on odonates is available in the modern world. In the earlier time taxonomic studies were conducted based on single marker genes e.g., based only on 12S rRNA gene (Saux et al., 2003) or 16S rRNA gene (Misof et al., 2000). But presently a wide variety of marker genes are used both as single and in combination. The mitochondrial and nuclear marker genes have been predominantly used. The relevant works based on multiple marker genes are Chippindale et al. (1999) [mitochondrial Cytochrome b, Cytochrome oxidase II, and 12S ribosomal DNA]; Artiss et al. (2001) [mitochondrial COI and 16S rRNA]; Hasegawa and Kasuya (2006) [nuclear 28S rRNA and mitochondrial 16S rRNA]; Ware et al. (2007) [mitochondrial 16S rRNA and nuclear 28S rRNA]; Dumont et al. (2010) [nuclear ribosomal genes 5.8 S, 18S, and ITS1 and 2]; Froufe et al. (2014) [mitochondrial COI and nuclear ITS-1]; Guan et al. (2013) [mitochondrial COI and ITS]; Carle et al. (2015) [nuclear EF-1α and Histone H3 genes and mitochondrial COI and COII]. In other insect orders also the molecular identification methods are effective e.g., Insecta: Psocodea, based on 16S rRNA gene and COI gene (Yang et al., 2013).

2.3 MATERIALS AND METHODS

2.3.1 Study area

Selected habitats of Kerala state of India including high land, mid land and low land regions were selected for the present study. Kerala is located between 10.8505° N latitude and 76.2711° E longitude. Different types of habitats from 5 districts of central northern Kerala were randomly selected which include Wayanad, Palakkad, Thrissur, Ernakulam and Idukki (Plate 1). The observed habitats cover a variety of ecosystems including aquatic habitats near forests, agricultural lands including paddy fields and other human inhabited villages and urban areas, except the habitats of protected areas. Details of the locations selected for observation are given in Table 2.4.1. As the odonates can be easily found near water bodies the observations were mainly concentrated to the vicinity of water bodies including forest streams, rivers, ponds, paddy fields, lakes, canals, ditches and estuaries. The field study was continued in all seasons and the locations were randomly selected. Most of the observations were done between 9 AM and 1PM because majority of odonates are active during this period. Limited number of observations were done after 5 PM to observe the crepuscular species. Some of the observed habitats were given in Plate 2.

2.3.2 Collection, Identification and Preservation

Visual encounter survey method (Heyer et al., 1994; Arunima and Nameer, 2021) was used for the assessment of odonate species richness. The samples were collected using hand sweeping nets and kept in collection bottles. The samples were identified with the help of photographs, keys and descriptions given in the literature (Fraser 1933, 1934, 1936; Kiran and Raju, 2013; Joshi et al., 2022). After identification, the samples were kept in storage vials having 70% ethanol at 0^0C temperature in freezer. The vials were labeled with scientific name of the species, gender, date and location of collection.

2.3.3 Photographic documentation

The observed odonates were photographed using Nikon D3400 camera. Photographs showing the identification features clearly were documented.

2.3.4 Molecular Characterisation

Out of the total 71 odonate species observed, 34 species were selected for mitochondrial COI and nuclear 18S rRNA gene sequencing and for molecular

PLATE 1 - MAP OF KERALA SHOWING THE DISTRICTS SELECTED FOR THE STUDY

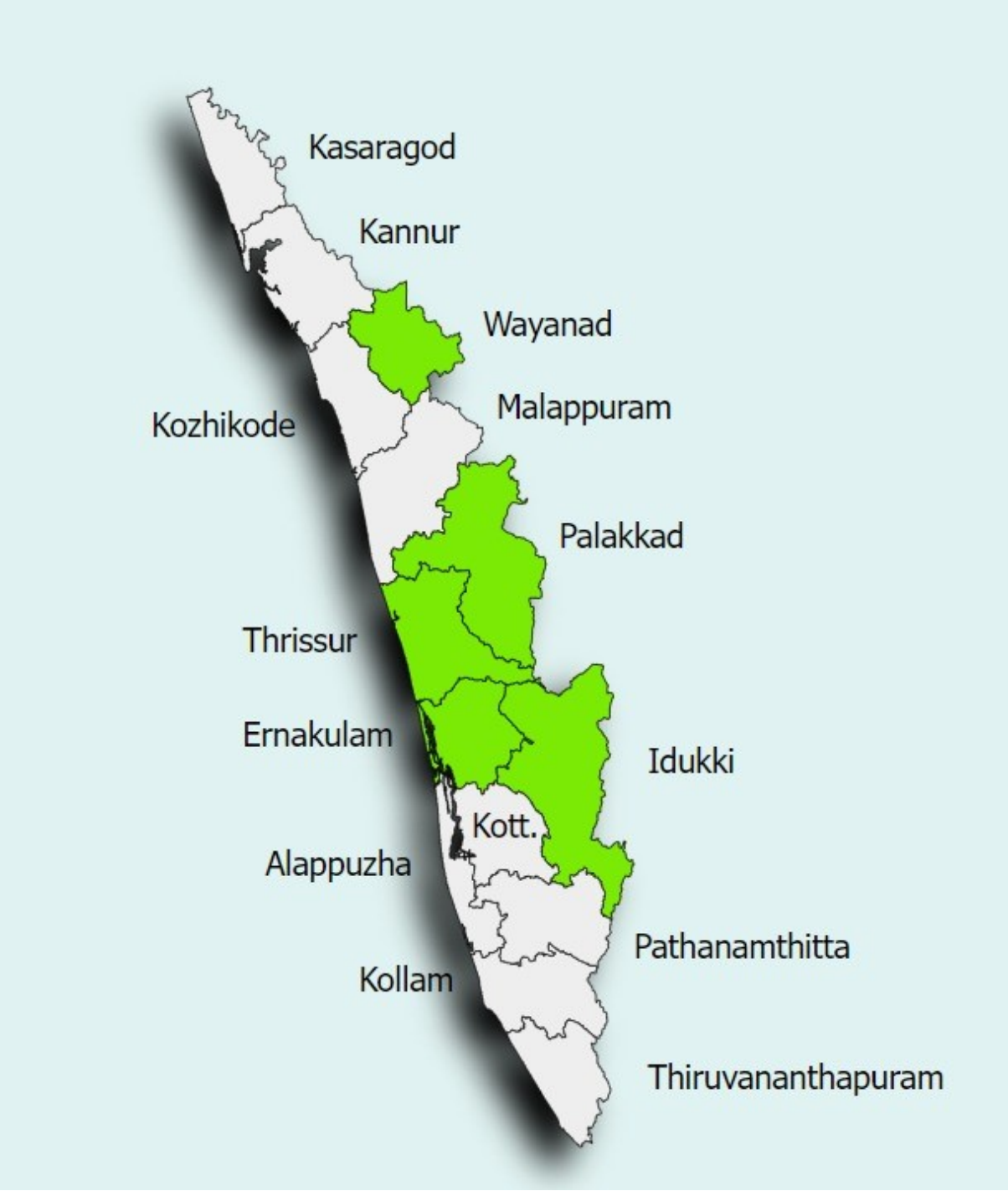

Figure 1A: Map of Kerala showing the districts selected for the study

PLATE 2 - STUDY LOCATIONS

Figure 2A: Mundakai (Wayanad)

Figure 2B: Ambalavayal (Wayanad)

Figure 2C: North Paravur (Ernakulam)

Figure 2D: Ottapalam (Palakkad)

Figure 2E: Kappithottam (Idukki)

Figure 2F: Chalakkudy (Thrissur)

phylogenetic analysis. The odonate species which were sequenced and analyzed in previous study in Kerala (Krishnan, 2018) were excluded from the current molecular study. From the remaining species, one or two representatives of each genus were selected. 34 species belonging to 28 genera, 10 families and 2 sub orders were selected for the present study.

2.3.4.1 Sample Preparation

For sample preparation, specimens kept in 70% ethanol were used. One of the thoracic legs of each dragonfly specimen and 3-4 thoracic legs of each specimen of damselfly were collected using forceps. Samples collected from each species were ground separately using mortar and pestle and used for DNA isolation and PCR amplification. The remaining samples were kept as voucher specimens at - 20°C in the repository of Laboratory of the Research and Post Graduate Department of Zoology, St. Thomas' College (Autonomous), Thrissur.

2.3.4.2 Isolation of genomic DNA

NucleoSpin® Tissue Kit (Macherey-Nagel) was used to extract genomic DNA from tissue samples.

1. Proteinase K (25 µl, 10mg/ml) and T1 buffer (180 µl) were added to the tissue kept in microcentrifuge tube (1.5ml), gently mixed and incubated for complete lysis in water bath at 56°C for 10 minutes.
2. RNase A was added (5 µl, 100 mg/ml) and incubated for 5 minutes at room temperature.
3. This was followed by incubation at 70^0C for 10 minutes after adding B3 buffer (200 µl).
4. Added 100% ethanol (210 µl) and mixed thoroughly.
5. Lysates were transferred to the NucleoSpin® Tissue column attached to a collection tube in the kit and centrifuged at 11000 x g for one minute.
6. The filtrate was discarded; to the remaining mixture added BW buffer (500 µl).
7. Then B5 buffer (600 µl) was added into the column and centrifuged at 13,500*g* for one minute.

8. The column was removed, transferred to a fresh receptacle, 50 µl of BE buffer was introduced to the centre of the column and centrifuged at 13,500 *g* for one minute.
9. DNA was stored at -20°C for further use.

2.3.4.3 Determination of quality of DNA

The quality of the extracted genomic DNA was assessed by agarose gel electrophoresis. The procedure adopted was as follows. 0.8 percent gel with long combed wells was prepared in 0.5X TBE, with 1.5 µl ethidium bromide (0.5µg/ml). One µl of 6 X gel loading buffer (bromophenol blue (0.25%) and sucrose (30%) in TE buffer pH-8.0) was mixed to 5 µl of DNA sample taken in a PCR tube and loaded into the large well created in the gel. Electrophoresis was done at 75 Volts using 5X TBE as tank buffer, until the migration of the dye was upto 2/3rd length of the gel. The gel was visualized and the image was recorded using Gel Doc EZ imager (Bio Rad).

2.3.4.4 Amplification of COI gene

Primers to amplify COI gene were selected from published reports of Folmer et al., 1994. The primers selected were custom synthesized from Sigma, diluted to a concentration of 10 pM/µl and used in PCR to amplify COI gene. The primer sequences and details were given in the Table 2.3.1. The PCR reaction mix and cyclic conditions used are given in Table 2.3.2 & 2.3.3. The PCR conditions were optimised by using different concentrations of reagents and different temperatures ranging from 54-66ºC. The PCR amplification was performed in a PCR thermal cycler (Veriti 96 well Thermal Cycler, Applied Biosystems).

Table 2.3.1 Details of primers used for the amplification of COI gene

Marker gene	Name of Primer	Direction	Sequence in 5' → 3' direction	Reference
COX1	LCO	Forward	GGTCAACAAATCATAAAGATATTGG	Folmer et al., 1994
	HCO	Reverse	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al., 1994

Table 2.3.2 PCR mix for the amplification of COI gene

Sl. No.	Constituents	Quantity
1	2X Phire Master Mix	5µL
2	Distilled water	4µL
3	Forward Primer	0.25µL
4	Reverse Primer	0.25µL
5	Template DNA	1µL
	Total	10.5 µL

Table 2.3.3 PCR conditions optimised for amplification of COI gene

Sl No.	Step	Temperature (^{0}C)	Time	No. of cycles
1.	Initial denaturation	98^{0}C	30 sec	1
2.	Denaturation	98 ^{0}C	5 sec	10
3.	Annealing	45 ^{0}C	10 sec	
4.	Extension	72 ^{0}C	15 sec	
5.	Denaturation	98 ^{0}C	5 sec	30
6.	Annealing	50 ^{0}C	10 sec	
7.	Extension	72 ^{0}C	15 sec	
8.	Final extension	72 ^{0}C	60 sec	1
9.	Hold	4 ^{0}C	∞	

2.3.4.4 Amplification of 18S rRNA gene

Primers to amplify 18S rRNA gene were selected from published reports by Giribet et al. 1996. The primers selected were custom synthesized from Sigma and were diluted to a concentration of 10 p*M*/µl. The primer sequences used for amplification of18S rRNA gene is listed in Table 2.3.4. The PCR was standardized

for different gradients of temperatures. The PCR conditions were optimised by using different temperatures ranging from 54-66ºC. The PCR amplification was performed in a PCR thermal cycler (Veriti 96 well Thermal Cycler, Applied Biosystems). The PCR reaction mix and cyclic conditions used are given in Table 2.3.5 and 2.3.6.

Table 2.3.4 Details of primers used for the amplification of 18S rRNA gene

Marker gene	**Name of the Primer**	**Direction**	**Sequence in 5' → 3' direction**	**Reference**
18S	1F	Forward	TACCTGGTTGATCCTGCCAGTAG	Giribet *et al*. 1996
	4R	Reverse	GAATTACCGCGGCTGCTGG	Giribet *et al*. 1996

Table 2.3.5 PCR mix for the amplification of 18S rRNA gene

Sl No.	**Constituent**	**Quantity (20 µL reaction)**
1.	5X Phire reaction buffer	4µL
2.	10mM dNTPs	0.4 µL
3.	Forward primer (10pM/ µL)	1µL
4.	Reverse primer (10pM/ µL)	1µL
5.	Template DNA	1µL
6.	DMSO	0.6 µL
7.	Phire Hot Start II DNA Polymerase	0.4 µL
8.	Distilled water	11.6 µL

Table 2.3.6 PCR conditions optimised for amplification of 18S rRNA gene

Sl No.	Step	Temperature (^{0}C)	Time	Cycles
1.	Initial denaturation	98	30Sec	1
2.	Denaturation	98	5 Sec	40
3.	Annealing	54	10 Sec	
4.	Extension	72	15 Sec	
5.	Final Extension	72	60 Sec	1
6.	End/Hold	4	∞	-

2.3.4.6 Agarose Gel electrophoresis

The amplified PCR products were checked in 1.2% agarose gel in 0.5X TBE buffer with ethidium bromide 1.5 µl (0.5 µg/ml). A volume of 5 µL of PCR product was mixed with 1µL of 6 X DNA loading dye and loaded in well along with 2-log DNA ladder (NEB) as the molecular standard. Electrophoresis was set at 75 V and after 45 minutes gel was documented using gel documentation system (Bio-Rad).

2.3.4.7 Sequencing

Representative PCR products were sequenced commercially at Rajiv Gandhi Centre for Biotechnology, Trivandrum by Sanger sequencing technique using automated DNA sequencer. In all cases both forward and reverse sequences were sequenced and final complete sequences were obtained in FASTA format along with respective ABI files containing chromatogram.

2.3.4.8 Sequence Analysis

After sequencing, the obtained sequences were processed using various bioinformatics tools. The reverse complement of the reverse sequence was generated using the Reverse complement bioinformatic tool. The reverse sequence was used along with the forward sequence in Emboss merger, which merged two overlapping nucleic acids into one (Bell and Kramvis, 2013).

The NCBI Basic Local Alignment Search Tool [BLAST] (Johnson et al., 2008) was used to check the sequence similarity of the resultant sequence with other sequences in the database. The COI sequences were translated to amino acid sequences by using the Expasy translate tool (Ramasubramanian, 2016) to identify the premature stop codons occurring through sequencing errors. The edited sequences were submitted to GenBank through the submission portal and received accession numbers.

2.4 RESULTS

Variety of habitats including paddyfields, ponds, streams, rivers, estuaries, canals, lakes, ditches and puddles of human occupied regions covering high land, mid land and low land were involved in the present study, except the habitats of protected areas and reserved forests. A total of 73 locations were observed for the study. The details of the study locations were given in Table 2.4.1.

Table 2.4.1 Details of study locations

Sl No.	LOCATION	LATITUDE	LONGITUDE	LOCATION TYPE
THRISSUR DISTRICT				
1.	Thrissur	10.5524° N	76.2272° E	Pond in urban area
2.	Chembuthara	10.5569° N	76.3169° E	Rocky stream
3.	Poomala	10.6096° N	76.2340° E	Dam reservoir
4.	Palakkal	10.4731° N	76.2125° E	Paddy field
5.	Kanimangalam	10.4861° N	76.2088° E	Pond with vegetation
6.	Nedupuzha	10.4867° N	76.1929° E	Kole field
7.	Kodannur	10.4665° N	76.1842° E	Kole field
8.	Pullu	10.4575° N	76.1523° E	Kole field
9.	Marottichal	10.4768° N	76.3435° E	Rocky stream
10.	Mannamangalam	10.4878° N	76.3435° E	Pond and stream
11.	Athirappilly	10.2908° N	76.5156° E	River near forest
12.	Vettilappara	10.2922° N	76.5149° E	River near forest
13.	Ayyampuzha	10.2279° N	76.6182° E	River near forest
14.	Thumboormuzhy	10.2956° N	76.4614° E	River near forest
15.	Nellayi	10.3941° N	76.2863° E	Pond near paddy field
16.	Chalakudy	10.3070° N	76.3341° E	Pond with vegetation
17.	Vellikulangara	10.3604° N	76.4115° E	Stream near rubber plantation
18.	Mala	10.2403° N	76.2631° E	Ditch near mangrove
19.	Poopathi	10.2188 °N	76.2649° E	Pond with minimum vegetation
20.	Kodungallur	10.2277° N	76.1971° E	Paddy field
21.	Valappad	10.3997° N	76.1160° E	Ditch and river
22.	Thoomanam	10.6678° N	76.2708° E	Waterfalls

23.	Kallamparachola	10.6254° N	76.2478° E	Waterfalls
24.	Varantharappilly	10.4255° N	76.3304° E	Stream near rubber plantation
25.	Elinjipra	10.3235 °N	76.3561° E	Pond and stream
26.	Kunnamkulam	10.6508° N	76.0694° E	Paddy field
27.	Chettuva	10.5242° N	76.0479° E	Mangove
ERNAKULAM DISTRICT				
28.	Moothakunnam	10.1902° N	76.2002° E	Ditch near estuary
29.	Sathar Island	10.1894° N	76.1914° E	Ditch near estuary
30.	North Paravur	10.1446° N	76.2273° E	Pond with shoreline vegetation
31.	Malayattur	10.1955° N	76.4968° E	River with shoreline plants
32.	Illithode	10.2032° N	76.5117° E	Vegetated ditch near brick factory
33.	Kodanad	10.1813 ° N	76.5150° E	Small stream with polluted water
34.	Kuruppampady	10.1112 ° N	76.5112° E	Vegetated small stream
35.	Nedumbassery	10.1679°N	76.3978° E	Ditches with shoreline grass
36.	Puthussery	10.1663°N	76.4097°E	Vegetated ditch
37.	Puthenvelikkara	10.1934°N	76.2456°E	Paddy field
38.	Kunnukara	10.1558°N	76.2902°E	Paddy field
39.	Thruthippuram	10.1975° N	76.2218° E	River
WAYANAD DISTRICT				
40.	Nellimunda	11.5397° N	76.1316° E	Rocky stream
41.	Elimbileri	11.5449° N	76.1060° E	Forest stream
42.	Cholamala	11.5397° N	76.1167° E	Rocky river
43.	Puthumala	11.5033° N	76.1431° E	Ditch with scarce vegetation
44.	Mundakai	11.4875° N	76.1556° E	Waterfalls
45.	Attamala	11.5021° N	76.1714° E	Stream near tea plantation
46.	Kuzhivayal	11.6003° N	76.14248° E	Ditch with shoreline plants
47.	Karapuzha	11.6181° N	76.1809° E	Dam reservoir

48.	Kalladi	11.5120° N	76.1330° E	Rocky stream
49.	Meppadi	11.5576° N	76.1317° E	River near tea plantation
50.	Kappamkolli	11.5636 ° N	76.1222° E	Ditch near banana plantation
51.	Palavayal	11.5727° N	76.1213° E	Ditch near brick factory
52.	Chembothara	11.5705° N	76.1258° E	Stream with thick vegetation
53.	Ambalavayal	11.6360° N	76.2037° E	Pond with emergent vegetation
54.	Palakkara	11.6430° N	76.3141° E	Tapioca plantation
55.	Kalpetta	11.5962° N	76.0868° E	Ditch
56.	Sulthan Battery	11.6629° N	76.2570° E	Tapioca plantation
57.	Thirunelli	11.9081° N	75.9971° E	Forest stream
58.	Vythiri	11.5517° N	76.0403° E	Small stream
59.	Choondale	11.5721° N	76.0580° E	River with grassy shore
60.	Moopainad	11.5359° N	76.1711° E	Ditch with shoreline plants
61.	Thomattuchal	11.5695° N	76.2197° E	Small stream
62.	Kuruva Island	11.8217° N	76.0922° E	Forest river & streams
63.	Panamaram	11.7381° N	76.0740° E	Paddy field
64.	Soochippara	11.5111° N	76.1643° E	Waterfalls
PALAKKAD DISTRICT				
65.	Govindhapuram	11.0083° N	79.4674° E	VegeTable plantation
66.	Parali	10.7977° N	76.5626° E	River
67.	Kollamkodu	10.7952° N	76.6630° E	Pond
68.	Vadakkumcherry	10.6008° N	76.4904° E	Grassland
69.	Ottappalam	10.7767° N	76.3759° E	Pond
70.	Puthuppariyaram	10.8597° N	76.6229° E	Waterfalls
71.	Nelliampathy	10.5349 ° N	76.6932 ° E	Vegetated streams
IDUKKI DISTRICT				
72.	Thodupuzha	9.8951° N	76.7237° E	Banana plantation
73.	Kappithottam	9.8892° N	76.7237° E	Paddy field

As a result of the study, a total of 71 species (33 species of damselflies and 38 species of dragonflies) were observed, belonging to 10 families and 43 genera. The systematic account of the observed species is given below.

2.4.1 Systematic account of order Odonata

Order: Odonata

Suborder: Zygoptera

Superfamily: Lestoidea

I. Family- Lestidae

1) Genus- *Lestes*

1. *Lestes elatus*
2. *Lestes praemorsus*

Superfamily: Platystictoidea

II. Family- Platystictidae

2) Genus- *Protosticta*

3. *Protosticta gravelyi*

Superfamily: Calopterygoidea

III. Family- Calopterygidae

3) Genus- *Neurobasis*

4. *Neurobasis chinensis*

4) Genus- *Vestalis*

5. *Vestalis apicalis*
6. *Vestalis gracilis*

IV. Family: Chlorocyphidae

5) Genus: *Heliocypha*

7. *Heliocypha bisignata*

6) Genus*: Libellago*

8. *Libellago indica*

V. Family: Euphaeidae

7) Genus: *Dysphaea*

9. *Dysphaea ethela*

Superfamily: Coenagrionidea

VI. Family: Platycnemididae

8) Genus: *Copera*

10. Copera marginipes

11. Copera vittata

9) Genus*: Onychargia*

12. Onychargia atrocyana

10) Genus: *Prodasineura*

13. Prodasineura verticalis

VII. Family: Coenagrionidae

11) Genus: *Aciagrion*

14. Aciagrion approximans krishna

15. Aciagrion occidentale

12) Genus: *Agriocnemis*

16. Agriocnemis keralensis

17. Agriocnemis pieris

18. Agriocnemis pygmaea

19. Agriocnemis splendidissima

13) Genus: *Archibasis*

20. Archibasis oscillans

14) Genus: *Ceriagrion*

21. Ceriagrion cerinorubellum

22. Ceriagrion coromandelianum

23. Ceriagrion rubiae

15) Genus: *Ischnura*

24. Ischnura rubilio

25. Ischnura senegalensis

16) Genus: *Paracercion*

26. Paracercion calamorum

27. Paracercion malayanum

17) Genus: *Pseudagrion*

28. Pseudagrion australasiae

29. Pseudagrion decorum

30. Pseudagrion indicum

31. Pseudagrion malabaricum

32. Pseudagrion microcephalum

33. Pseudagrion rubriceps

Suborder: Anisoptera

Superfamily: Aeshnoidea

VIII. Family: Aeshnidae

18) Genus: *Anax*

34. Anax guttatus

35. Anax immaculifrons

19) Genus: *Gynacantha*

36. Gynacantha dravida

37. Gynacantha millardi

Superfamily: Gomphoidea

IX. Family: Gomphidae

20) Genus: *Ictinogomphus*

38. Ictinogomphus rapax

X. Family: Libellulidae

21) Genus: *Acisoma*

39. Acisoma panorpoides

22) Genus: *Aethriamanta*

40. Aethriamanta brevipennis

23) Genus: *Brachydiplax*

41. Brachydiplax chalybea

42. Brachydiplax sobrina

24) Genus: *Brachythemis*

43. Brachythemis contaminata

25) Genus: *Bradinopyga*

44. Bradinopyga geminata

26) Genus: *Crocothemis*

45. Crocothemis servilia

27) Genus: *Diplacodes*

46. Diplacodes nebulosa

47. Diplacodes trivialis

28) Genus: *Hydrobasileus*

48. Hydrobasileus croceus

29) Genus: *Lathrecista*

49. Lathrecista asiatica

30) Genus: *Neurothemis*

50. Neurothemis fulvia

51. Neurothemis tullia

31) Genus: *Onychothemis*

52. Onychothemis testacea

32) Genus: *Orthetrum*

53. Orthetrum chrysis

54. Orthetrum glaucum

55. Orthetrum luzonicum

56. Orthetrum pruinosum

57. Orthetrum sabina

33) Genus: *Palpopleura*

58. Palpopleura sexmaculata

34) Genus: *Pantala*

59. Pantala flavescens

35) Genus: *Potamarcha*

60. Potamarcha congener

36) Genus: *Rhodothemis*

61. Rhodothemis rufa

37) Genus: *Rhyothemis*

62. Rhyothemis variegata

38) Genus: *Tetrathemis*

63. Tetrathemis platyptera

39) Genus: *Tholymis*

64. Tholymis tillarga

40) Genus: *Tramea*

65. Tramea limbata

41) Genus: *Trithemis*

66. Trithemis aurora

67. Trithemis festiva

68. *Trithemis pallidinervis*

42) Genus: *Urothemis*

69. Urothemis signata

43) Genus: *Zygonyx*

70. Zygonyx iris

44) Genus*: Zyxomma*

71. Zyxomma petiolatum

2.4.2 Detailed systematic account of order Odonata

Order Odonata

I. Suborder Zygoptera

The head of Zygopterans or damselflies is transversely elongated in shape. Eyes are well separated. All wings are almost identical in shape. During rest wings are kept closed over the abdomen and parallel to it. There are 2 pairs of anal appendages which are present at the end of the 10^{th} segment- a pair of superior anal appendages and a pair of inferior anal appendages. A highly developed ovipositor is seen in female.

Suborder Zygoptera is divided into 4 superfamilies – Lestoidea, Platystictoidea, Calopterygoidea and Coenagrionidea (Kalkman et al., 2020). In Kerala, there are 7 families coming under Zygoptera. Representatives of all families were recorded during the study.

1) **Family Lestidae** Calvert, 1907

The members of this family are known as spreadwings as they keep their wings wide open during rest. Damselflies having small to medium sized body. The colours on body are iridescent or non-iridescent. Most species move their abdomen up and down during rest.

Out of 12 species of 3 genera found in Kerala, 2 species belong to genus *Lestes* were recorded.

1. **Genus *Lestes*** Leach, 1815

They are small to medium sized damselflies. Petiolation starts just before the level of *ac*. The position of *ac* is about half way between two antenodal nervures. Pterostigma is twice in length than its breadth. Discoidal cells of fore and hind

wings are closely similar. Metallic markings on head, thorax and abdomen is present in some species.

2) **Family Platystictidae** Kirby, 1890

They are slender and long bodied damselflies. Generally black or brown coloured body having white or blue markings. Transparent wings with pointed tips. Length of abdomen is twice or more than twice of hind wing length.

All species of this family, found in Kerala are endemic to the Western Ghats. 12 species belonging to 2 genera are found in Kerala as representatives of this family. One species was recorded by the current study.

2. **Genus *Protosticta*** Selys, 1885

This genus comprises very slender elongated damselflies, known as 'reed tails' and they are found in untouched forest streams. Of the 11 representatives reported from Kerala, only one was recorded during the study.

3) **Family Calopterygidae** Selys, 1850

These are large sized and iridescent coloured damselflies also known as 'glories'. Head is broad and eyes are round and very prominent. Hindwings are broad and with rounded tips. Wings are transparent or opaque with iridescent colouration. Abdomen is more elongated than hindwing (Subramanian, 2008).

Out of 4 species of 2 genera found in Kerala, 3 species coming under both genera were recorded.

3. **Genus *Neurobasis* Selys, 1853**

In males, fore wings are transparent, hind wings are opaque and coloured with metallic blue or green. Pterostigma is absent. All wings are transparent and with false pterostigma in females. Only one species of *Neurobasis* is reported from India (Kalkman et al., 2020).

4. **Genus *Vestalis*** Selys, 1853

Wings are rounded at tips. Wings are transparent or with blackish brown tips. Pterostigma is absent. Discoidal cell has the same length of basal space and has a number of nervures. Legs are thin and elongated. Abdomen is long and has cylindrical shape. The ground colour of body is metallic blue or green.

4) **Family Chlorocyphidae,** Cowley, 1937

They are damselflies, also known as 'stream jewels' having small sized body, prominent bulbous eyes and protruding face. Thorax is short and stout. Wings are transparent or partially opaque with iridescent colours. Cylindrical shaped abdomen and shorter than hindwing. Forested streams are preferred breeding habitats.

Three species belonging to 3 genera are reported from Kerala. During the present study 2 species were recorded.

5. **Genus *Heliocypha*** Fraser, 1949

This is the genus of small damselflies with iridescent coloured wings. These are commonly found in forested streams (Subramanian, 2009). The genus *Heliocypha* has only a single representative in Kerala.

6. **Genus *Libellago*** Selys, 1840

Fore wings of males are black at the apices in most species of this genus. The end point of petiolation is proximal to the first antenodal nervure. *Riii* begins far distal to node. Mesothoracic triangle is reduced and without bright colours. Slender and elongated legs. Abdomen is considerably shorter than wings and tapering towards segment 10.

5) **Family Euphaeidae** Yakobson & Bainchi, 1905

Members of this family are having large body size and large round eyes. Wings are transparent, tinted with brown or having iridescent markings. Hindwings are broad and rounded and shorter than forewings. Length of abdomen is more than that of wings in males. In females it is shorter than wings or of the same length (Subramanian, 2008).

Four species coming under 2 genera are reported from Kerela out of them only one species was recorded by the current study.

7. **Genus *Dysphaea*** Selys, 1853

Wing petiolation is completely absent. Narrow and long pterostigma is present in wings. Thorax is robust. Anal appendages are longer than segment 10, simple and homogenous.

6) **Family Platycnemididae** Yakobson & Bainchi, 1905

They are small and slim bodied damselflies. The body is black in colour and marked with red, yellow or blue. Wings are transparent and narrow with rounded tips. Length of abdomen is more than that of hind wing (Subramanian, 2008).

In Kerala the representatives of this family are 16 species belonging to 9 genera. Four species of 3 genera was encountered during the study.

8. **Genus *Copera*** Kirby, 1890

Damselflies having small or medium size. Length of abdomen is less than twice the wing length. The 2nd segment of antennae is equal in length or more than 3rd segment. Transparent wings with moderately rounded tips. Anal appendages in males are less homogenous.

9. **Genus *Onychargia*** Selys, 1865

Small or medium sized damselfies with ground colour of black or bronzed purple and marked with citron yellow. Markings are absent in old adults. Short, broad and transparent wings. Pterostigma is half as long as broad.

10. **Genus *Prodasineura*** Cowley, 1934

This is the genus of slender, elongated black damselflies. Genus prodasineura has a single representative in Kerala, which is endemic to India.

7) **Family Coenagrionidae** Kirby, 1890

This is the largest family of damselflies in Kerala. The smallest damselflies in Kerala belong to this family. These are small to medium sized damselflies and found in variable non-iridescent colours. The wings are transparent with rounded tips. Abdomen is very slim and slightly longer than wings.

The representatives of this family in Kerala are found in 9 genera and 24 species. In the present study a total of 20 species of 7 genera were recorded.

11. **Genus *Aciagrion*** Selys, 1891

They are slender, small sized damselflies. Non metallic. The ground colour of body is blue or violaceous and have black markings. Wings are narrow and transparent. The size and shape of pterostigma vary in fore and hind wings. In forewings it is diamond shaped and has double the size of that in hindwings. Head

is narrow with triangle shaped or elongated postocular spots. Slim thorax and short legs. Anal appendages vary between species and very small sized.

12. **Genus *Agriocnemis*** Selys, 1877

These are very slender and smallest damselflies. Non metallic colours. Black with bluish markings or greenish or bluish with black markings and with orange coloured last abdominal segments. Pterostigma is small and diamond shaped, similar or dissimilar in fore and hind wings. Head is narrow and the frontal ridge is absent. Coloured postocular spots are present. Short and robust thorax. Slender and cylindrical abdomen is dilating towards last segments. Legs are short.

13. **Genus *Archibasis*** Kirby, 1890

These are slender medium sized damselflies. Colours are non metallic and bluish with black markings. Wings are narrow and transparent. Pterostigma is similar in all wings and subquadrate shaped. Head is small, postocular spots are present or absent. Thorax is moderately robust. Legs are short and robust. Superior anal appendages have the same length of segment 10. Rounded or slightly notched end. Inferiors are cone shaped and half the length of superiors.

14. **Genus *Ceriagrion*** Selys, 1876

Slender medium sized damselflies with non metallic colours. Generally, with ground colour yellow, olivaceous or orange. Wings are transparent with lozenge shaped, narrow pterostigma. Narrow head with a prominent frontal ridge. Postocular spots are absent. Long narrow thorax. Slender, cylindrical abdomen is twice the length of hind wings. Superior anal appendages are short and hook shaped. Inferiors are longer and conical in shape.

15. **Genus *Ischnura*** Charpentier, 1840

These are small, slender damselflies. Colours are non metallic. Generally blue or green with black markings. Females are polychromatic. Transparent wings and the pterostigma varies in shape and size in fore and hind wings. Head is narrow and the frontal ridge is absent. Postocular spots are present. Abdomen is robust and moderately short. Anal appendages vary greatly between species.

16. **Genus *Paracercion*** Weeker & Dumont, 2004

They are small sized slender damselflies. Colours are non metallic. Generally blue with black markings. Females vary from males in colour. Pterostigma is small and similar in all wings. Head is narrow and frontal ridge is absent. Coloured postocular spots are presents. Thorax is robust. Abdomen is slender and cylindrical slightly dilating on both ends. Anal appendages vary between species. Superiors are longer than inferiors.

17. **Genus *Pseudagrion*** Selys, 1876

These are slender and medium sized damselflies. Colours are non metallic. Generally bright bluish with black markings. Wings are transparent. Pterostigma is narrow, lozenge shaped and similar in all wings. Head is narrow and having coloured, triangular postocular areas. Slender thorax and abdomen. Anal appendages are variable. Superiors are forked or notched at ends and have same length or shorter than segment 10. Inferiors are shorter and conical in shape.

II. **Suborder Anisoptera** Selys, 1854

Head of Anisopterans or dragonflies is globular and compact. Eyes are confluent on vertex but in some genera they are separated. Wings are dissimilar in venation and shape. Hind wing is broad at the base. Discoidal cell is divided into two triangular cells- a superior hypertrigone and an inferior discoidal cell. Pterostigma is present and vary in length. Wings are kept horizontally perpendicular to the body or downwards during rest. Tenth abdominal segment has a pair of superior and a single inferior anal appendages.

In Kerala suborder Anisoptera is represented by 6 families viz. Aeshnidae, Gomphidae, Chlorogomphidae, Macromiidae, Corduliidae, Libellulidae and some species are considered *Incertae sedis* as it is not confirmed to which family they belong. Out of these representatives of 3 families were recorded during the study.

8) **Family Aeshnidae** Leach, 1815

Family of crepuscular or diurnal species. Dragonflies having large to medium sized body. Body colouration is non-iridescent. Eyes are widely contiguous at their inner margins. Transparent wings often lightly tinted with brownish yellow. The basal part of abdomen is swollen in most species.

In Kerala 9 species of Aeshnidae belonging to 3 genera are found (Nair *et al*. 2021). Out of them 4 species coming under 2 genera viz. *Anax* and *Gynacantha* were recorded during the study.

18. **Genus *Anax*** Leach, 1815

They are large sized dragonflies and robustly build body. Transparent wings with yellow or pale brownish tint partially. Large and globular head and the eye borders are widely contiguous. Occiput is small. Robust thorax and legs. Long and broad wings having pointed tips. Long narrow and braced pterostigma. Basal segments of abdomen are tumid and there is constriction at segment 3. Superior anal appendages are broadly lanceolate with rounded tips and have a small spine. Inferiors are shorter and quadrate shaped.

19. **Genus *Gynacantha*** Rambur, 1842

They are large sized and robust build dragonflies having crepuscular nature. Ground colour is dull brown or green generally. Head is large and globular in shape. Eyes are widely contiguous. Small thorax and short legs. Long, broad and transparent wings with close reticulation and the pterostigma is moderately long, narrow and braced. Basal segments of abdomen are tumid with or without constriction on segment 3. Long slender anal appendages and inferiors are narrowly triangular.

9) **Family Gomphidae** Rambur, 1842

Gomphids are large sized dragonflies. The body colour is generally black with yellow markings or yellow with brown or black markings. Wings are transparent. The last abdominal segments are enlarged to form a club shape. So, the dragonflies belong to this family is called as clubtails. Out of 22 species of 17 genera found in Kerala (Nair *et al.* 2021), 1 species was recorded by the present study.

20. **Genus *Ictinogomphus*** Rambur, 1842

They are large and robust bodied dragonflies. Black coloured body marked with citron yellow or greenish yellow. Large triangular head. Robust thorax and legs. Wings with close reticulation. Abdomen dialated at both ends and middle segments are narrow.

10) **Family Libellulidae** Leach, 1815

They are the most diverse and most abundant group of dragonflies. They can be found in a variety of size, shape and in non-iridescent colouration. Inner margins of eyes are meeting widely. Size, shape, colouration and transparency of wings vary greatly between species. Most of them are globally distributed and generalist species. They breed in a wide range of aquatic habitats including estuaries and polluted waters.

In Kerala this family was represented by 52 species of 31 genera (Nair et al. 2021). 33 species of 24 genera have been recorded as the representatives of this family by the current study.

21. **Genus *Acisoma*** Rambur, 1842

Small sized dragonfly having body colour blue with black markings. Head is small. Eyes join at a point. Narrow and small thorax. Wings are short with open reticulation and large pterostigma. Abdominal segments 1-5 are widely dilated and tapering from segments 6 to 10.

22. **Genus *Aethriamanta*** Kirby, 1889

One of the small sized dragonflies. Head is large in size. Eyes are meeting broadly at their inner margins. Thorax is smaller in size with long and robust legs. Transparent wings are coloured at the base. Abdomen is very short and fusiform.

23. **Genus *Brachydiplax*** Brauer, 1868

Dragonflies with medium sized body and small head. Eyes are broadly contiguous at their inner margin. Robustly built thorax with slender long legs and transparent wings. Abdominal segments are broader at the base and gradually tapering to apical end.

24. **Genus *Brachythemis*** Brauer, 1868

Small or medium sized dragonflies. Medium sized head with broadly contiguous eyes. Body colour is yellow with brown markings. Thorax and legs are robustly built. Transparent wings having yellowish or orange patches. Short and stout abdomen with segments tapering to the apical end.

25. **Genus *Bradinopyga*** Kirby, 1893

Dragonflies with medium body size. Ground colour is black with white or grey markings. Eyes are meeting broadly at their inner margins. Robust thorax with short slender legs and transparent wings. Abomen is slender with slightly dilated base.

26. **Genus *Crocothemis*** Brauer, 1868

Dragonflies with medium sized body and uniform red colour. Medium sized head. Eyes are contiguous to a short area. Thorax is robustly built with short robust legs and transparent wings. Abdomen is broad and the last segments are tapering to the end.

27. **Genus *Diplacodes*** Kirby, 1889

Small sized dragonflies. Greenish yellow ground colour with black markings or black coloured body with pruinescence. Eyes are contiguous in a short area. Narrow thorax with slender legs. Transparent wings are with or without black patches. Abdomen is slender but the basal segments are dilated.

28. **Genus *Hydrobasileus*** Kirby, 1889

Large sized dragonflies with ground colour ochreous of ferruginous. Large head with broadly contiguous eyes. Thorax is robust and the legs are long and slender. Transparent wings with coloured patches. Basal abdominal segments are broader and narrows gradually towards the base.

29. **Genus *Lathrecista*** Kirby, 1889

Dragonflies with moderately larger size. Medium sized head and broadly contiguous eyes. Thorax is robust and bronze coloured with yellow markings. Legs having moderate length and the wings are transparent. Abdomen is slender and reddish.

30. **Genus *Neurothemis*** Brauer, 1867

Medium sized dragonflies with medium sized head and shortly contiguous eyes. Robust thorax with slender legs. Broad wings are entirely or partially coloured. Abdomen is short with broad base and gradually tapers to the end.

31. **Genus *Onychothemis*** Brauer, 1868

Dragonflies with large sized dark metallic coloured body and yellow markings. Head is small sized and eyes are confluent at a short area. Robust thorax with long legs. Wings are transparent. Abdomen is broad and robust with tapering end and a high mid dorsal carina.

32. **Genus *Orthetrum*** Newman, 1893

Dragonflies with moderately large sized body. Eyes are confluent in a shorter or wider area. Robust thorax with short and robust legs. Wings are transparent. Abdomen is long and shape varies between species.

33. **Genus *Palpopleura*** Rambur, 1842

One of the genera of smallest sized damselflies. Large head with moderately confluent eyes. Robust thorax with slender moderately elongated legs. Wings are short and transparent with coloured patches. Abdomen is short and fusiform and pruinosed blue in matured adults.

34. **Genus *Pantala*** Hagen, 1861

Dragonflies with large body size. Gound colour of body is ochreous or reddish orange. Large head with broadly contiguous eyes. Robust thorax with slender legs. Broad elongated wings are transparent. Abdomen is dialated at base and have a constriction at segment 3 and tapering to the apical end.

35. **Genus *Potamarcha*** Karsch, 1890

Medium sized dragonflies having blackish brown body colour with yellow markings. Eyes meeting widely along inner margins. Robust thorax with slender legs. Slender abdomen is slightly wider at the base.

36. **Genus *Rhodothemis*** Ris, 1909

Large sized dragonflies with red body colour. Small sized head and eyes contiguous at a point. Robust thorax. Legs are long and robust. Wings are transparent with a basal coloured patch. Basal part of abdomen is slightly dilated and tapers to apical end.

37. **Genus *Rhyothemis*** Hagen, 1867

Dragonflies having medium body size, metallic body colour and entirely or partially coloured opaque wings. Small head with widely confluent eyes. Small thorax with long slender legs. Abdomen is short.

38. **Genus *Tetrathemis*** Brauer, 1868

Dragonflies having small body size and the abdomen is shorter than wings. Black body colour with citron yellow markings. Medium sized head with widely confluent eyes. Small and slender thorax and the legs are elongated and slim. Wings are transparent. Abdomen is short.

39. **Genus *Tholymis*** Hagen, 1867

Large sized dragonflies with robustly build body. Body colour is ochreous or reddish. Large head having widely confluent eyes. Robust thorax with long slender legs. Transparent wings with coloured patches. Abdomen has a broader base and tapering end.

40. **Genus *Tramea*** Hagen, 1867

Large sized dragonflies. Head is large and eyes are contiguous in a moderate area. Robust thorax with long slender legs. Wings are transparent and coloured and opaque at the base. Abdomen is slender with a slightly broader base.

41. **Genus *Trithemis*** Brauer, 1868

Dragonflies with medium sized body. Colour and shape vary between species. Medium sized head and the eyes are confluent moderately. Thorax is slim. Characteristics of legs also vary between species. Transparent wings with coloured base. Size and shape of abdomen also vary between species.

42. **Genus *Urothemis*** Brauer, 1868

Dragonflies with moderately large sized body. Large head with widely confluent eyes. Robust thorax with slender long legs. Transparent wings with partially coloured base. Abdomen with slightly dilated base and tapering apical end.

43. **Genus *Zygonyx*** Hagen, 1867

Dragonflies with large body size. Body colour is dark metallic with yellow markings. Head is large. Eyes are widely contiguous. Robust thorax with robust and

elongated legs. Wings are transparent. Abdomen is long and narrow and shorter than wings.

44. **Genus *Zyxomma*** Rambur, 1842

Slender crepuscular dragonflies having medium size. Large globular head and broadly confluent eyes. Thorax is small with long slender legs. Wings are transparent. Abdomen is well dilated at segments 1-3 and abruptly tapers to form very narrow till the end.

Detailed description of species recorded

Suborder Zygoptera

1. *Lestes elatus* Hagen in Selys, 1862

Size: Male: Abdomen- 34-36mm Hind wing- 23-24mm

Female: Abdomen- 34mm Hind wing- 24mm

Description: Male: Labium is white in colour. Labrum, cheeks, anteclypeus and eyes are turquoise blue. Bronze coloured triangular spot bordered with black is present at the inner side of each eye above. Thorax is dark reddish brown and pruinosed on sides. A pair of J shaped metallic green stripes expanding outwardly is present on dorsal side. This is a crucial distinguishing feature of this species. Legs are pale greenish yellow having a black stripe on femoral outer surface. Wings are transparent with black pterostigma. Abdomen pale bluish green having metallic green or bronze markings dorsally. Anal appendages are completely black in colour (Fraser, 1936).

Female: Almost similar to male with prominent markings but the ground colour is pale brown.

Behaviour and habitat: It is commonly found near ponds, streams and paddy fields. Weak flies and rests by keeping wings open and occasionally moving abdomen up and down. Usually lays eggs on wet grass surfaces (Kiran and Raju, 2013).

2. *Lestes praemorsus* Hagen in Selys, 1862

Size: Male: Abdomen: 32-35mm Hind wing: 21-22mm

Female: Abdomen: 30-32mm Hind wing: 20-21mm

Description: Male: Yellow coloured labium. Labrum, anteclypeus and cheeks are turquoise blue. Eyes are sapphire blue in colour. Thorax is black dorsally with a pair of dark green metallic antehumeral stripes having scalloped outer borders. Thorax is blue or greenish yellow laterally having irregular spots. In pruinosed forms these spots and antehumeral stripes are completely covered by pruinescence. Wings are transparent with dark reddish brown or blackish brown pterostigma. Legs are black in colour. Pale blue coloured abdomen is marked dorsally with bronzed green or coppery metallic. Segment 9 is marked with a lateral blue spot and segment 10 with a small ventro lateral spot.

Female: Similar to male but shows some differences. Labrum and cheeks are olivaceous. Thorax is yellowish or pale greenish blue laterally. Legs are ochreous instead of black.

Behaviour and habitat: It can be found commonly near ponds and marshes. Rests by keeping wings open and shows upward and down ward movement of abdomen similar to that seen in *Lestes elatus*. Robust fliers than other *Lestes* damselflies. Emergent grass surfaces are usually selected for egg laying.

3. *Protosticta gravelyi* (Laidlaw, 1915)

Size: Male: Abdomen: 46-49mm Hind wing: 20-22mm

Female: Abdomen: 33-35mm Hind wing: 19-23mm

Description: Male: Brownish black coloured labium. Labrum and clypeus are turquoise blue in colour. Frons, vertex and occiput are glossy black coloured. Eyes are dark bottle green above and light green below. A black triangle shaped marking is present at the middle of creamy white prothorax. Metallic black thorax with two creamy white lateral stripes on each side. Creamy white legs with darker knees. Transparent wings with black pterostigma. Abdomen is black coloured with white and turquoise blue markings. Half of the basal segment is turquoise blue. On dorsal side the apical black extends as a triangle into the basal blue. Segments 9 and 10 are without any markings.

Female: Females are almost similar to male in appearance but abdomen is shorter and stout. Segment 8 is black with basal large white spot on each side.

PLATE 3 – ODONATES OBSERVED DURING THE STUDY

Figure 3A*: Lestes elatus*

Figure 3B: *Neurobasis chinensis*

Figure 3C:*Vestalis apicalis*

Figure 3D: *Heliocypha bisignata*

Figure 3E: *Libellago indica*

Figure 3F*: Dysphaea ethela*

Behaviour and habitat: They are found among rocks and ferns of forested streams with dark shade cover. They are weak fliers. Usually found as small groups and lay eggs in forest streams.

4. *Neurobasis chinensis* (Linnaeus, 1758)

Size: Male: Abdomen: 45-50mm Hind wing: 32-38mm

Female: Abdomen: 44-50mm Hind wing: 36-40mm

Description: Male: Labrum turquoise blue in colour having a large black triangle marking. Eyes are blackish brown with white bottom. Thorax is bright metallic green with blackish brown humeral and antero-lateral stripes. Legs are elongated and black. Wings are without pterostigma. Fore wings are transparent with yellowish green tint. Hind wings are shorter than forewings. Hind wings are opaque and bright metallic green or peacock blue coloured and at the apex blackish brown in colour. Abdomen is considerably longer than wings and metallic green coloured.

Female: The major difference from male is the absence of coloured opaque wings. Wings are transparent tinted with pale brown. A creamy white spot is present at the nodes of all wings. Creamy white pterostigma is also present only in hind wings.

Behaviour and habitat: Commonly found in streams and rivers of forested habitats. Males show courtship behaviour through displaying the bright colouration of wings. Submerged decaying woods are used for depositing eggs (Subramanian, 2009).

5. *Vestalis apicalis* Selys, 1873

Size: Male: Abdomen: 49-55mm Hind wing:36-39mm

Female: Abdomen: 46-50mm Hind wing: 38-40mm

Description: Male: Head is metallic emerald green except labium, labrum, cheeks, bases of mandibles and bases of antennae which are yellow coloured. Eyes are brown above and pale yellow below. Thorax is metallic emerald green with black mid dorsal carina and fine pale yellow stripes. Legs are dark brown in colour. Wings are transparent. Distal portion of all wings about 5mm is tipped with

blackish brown. Pterostigma absent. Abdomen metallic emerald green having pale yellow markings and black intersegmental nodes.

Female: Females are similar to male with paler shades. Colour on wing tip is also paler. Coppery tint is prominent on abdomen and less metallic.

Behaviour and habitat: Commonly found in forested streams and rivers. Emergent plant parts or wet rocks are selected for egg deposition (Kiran and Raju, 2013)

6. *Vestalis gracilis* (Rambur, 1842)

Size: Male: Abdomen: 45-56mm Hind wing: 34-38mm

Female: Abdomen: 43-50mm Hind wing: 36-39mm

Description: Male: Labium, labrum, bases of mandibles, anteclypeus, cheeks and antennal bases are yellow in colour. The remaining portions of head are metallic green. Eyes are brown above and greenish yellow below. Thorax is metallic emerald green with fine black mid dorsal carina and fine yellow stripes. Legs are brown coloured with yellow tibial flexor surface and femoral extensor surface. Transparent wings tinted with greenish yellow and having iridescent blue shades. Abdomen metallic green above and black below.

Female: Closely similar in appearance with male. Metallic colours are dull.

Behaviour and habitat: Commonly found in forest areas but rarely seen in non-forest areas also. They are found as groups along with *Vestalis apicalis*. Submerged plant parts and logs are used as substratum for oviposition.

7. *Heliocypha bisignata* (Hagen in Selys, 1853)

Size: Male: Abdomen: 20mm Hind wing: 24-26mm

Female: Abdomen: 16mm Hind wing: 22mm

Description: Male: Head is black with dark brown eyes. Prothorax is black with a prominent large pink spot. Thorax is black with a rose pink coloured mesothoracic triangle and two larger spots of same colour on both sides of the former spot. Thorax also has yellow markings laterally. Black legs having white shades. Wings are transparent in basal part and tinted with yellow. Forewings have bright coppery colour at apical fourth part. Apical region of hind wing is opaque

and blackish brown coloured and glow with coppery or violaceous reflex from the vitreous spots of this region. Pterostigma is black. Black abdomen with yellow markings.

Female: The rose pink coloured markings on thorax is absent in females. The thorax is black and have yellow markings. Wings are transparent. Pterostigma is black with a central creamy patch.

Behaviour and habitat: Very common in forest habitats. Rarely found in some non-forested areas. Lays eggs on floating and decaying logs and submerged vegetation.

8. *Libellago indica* (Fraser, 1928)

Size: Male: Abdomen: 14-16mm Hind wing: 15-18mm

Female: Abdomen: 13-17mm Hind wing: 17-20mm

Description: Male: Head is black with ochreous labium and brownish yellow labrum. Eyes are brown above and pale grey below. Eyes have a bluish metallic luster. Thorax is black with yellow markings. Legs are black with pruinosed white shades. Transparent wings with amber tinted bases. Apices of forewings have black patch. Abdomen is black and yellow coloured. Last segments are almost black in colour.

Female: Females are dull coloured and stout bodied. Wings are transparent and amber tinted. Pterostigma creamy white bordered with black. Abdomen yellow with black markings.

Behaviour and habitat: Commonly seen in forested habitats and also found in non-forested regions. Territorial fights can be observed between males. Floating logs and submerged vegetation are selected for oviposition.

9. *Dysphaea ethela* (Fraser, 1924)

Size- Male: Abdomen:38mm Hind wing: 33mm

Female: Abdomen : 32mm Hind wing: 33mm

Description: Male: Head is velvety black. Eyes are black above and grayish below. Velvet black thorax having fine reddish yellow stripes. These are obscured in completely matured adults. Legs are black. Transparent wings are tinted with

brown. Pterostigma is black and elongated covering 7-8 cells. Abdomen is black and have brownish yellow apical annules and lateral stripes.

Female: Eyes are olivaceous brown above and bluish grey below. Black thorax has prominent and broad yellow markings. Yellow markings on abdomen are also extensive. A large triangle shaped yellow spot is present on both sides of 9^{th} segment.

Behaviour and habitat: Found in fast flowing rivers and streams of forested habitats. Usually perch on rocks or twigs in streams.

10. *Copera marginipes* (Rambur, 1842)

Size- Male: Abdomen: 28-31mm Hind wing: 16-18mm

Female: Abdomen: 29-30mm Hind wing: 20mm

Description- Male: Pale brown labium. Labrum, bases of mandibles, genae, ante and post clypeus are pale greenish yellow. Eyes are black above and pale greenish yellow below with a fine black equatorial band. Bronzed black thorax with bluish yellow stripes and markings. Markings on lateral sides are yellowish. Legs are yellowish orange. Transparent wings with yellow framed brown pterostigma. Abdomen is bronzed black upto 8^{th} segment. Segment 9 is bluish white or white on dorsum and black ventrally. Segment 10 is white or bluish white. Teneral individuals have white abdomen with fine black markings. Anal appendages are white and the inferiors have black tips. The length of superiors is half the length of 10^{th} segment. Inferiors are four times longer than superiors.

Female: Females are dull coloured. Markings of thorax are pale brown in colour. They have stout and cylindrical abdomen. Pale brown pterostigma.

Behaviour and habitat: Common in ponds, canals, streams, rivers and ditches of forests and non-forests. Always fly in closer distances to ground. Breed in marshes and streams.

11. *Copera vittata* (Selys, 1863)

Size- Male: Abdomen: 28-34mm Hind wing: 16-18mm

Female: Abdomen: 28-30mm Hind wing: 18mm

PLATE 4 - ODONATES OBSERVED DURING THE STUDY (CONT.)

Figure 4A*: Copera marginipes*

Figure 4B*: Copera vittata*

Figure 4C*: Onychargia atrocyana*

Figure 4D*: Prodasineura verticalis*

Figure 4E*: Aciagrion approximans krishna*

Figure 4F*: Aciagrion occidentale*

Description- Male: Labium reddish brown and labrum greenish yellow in colour. Eyes are brown above and olivaceous below with a fine black equatorial line. Thorax is bronzed black dorsally and have bluish white and pale brown markings. Markings on lateral sides are more yellowish. Legs are reddish yellow coloured. Wings are transparent with black long pterostigma. Abdomen is black dorsally and laterally upto segment 9 and segment 10 is entirely bluish white. Segment 9 has a bluish white patch on dorsal side. Anal appendages are pale blue or white. The length of superiors is equal to that of segment 10. Inferiors are twice longer than superiors.

Female: Females are dull coloured and more brownish in appearance. They have stout and cylindrical abdomen.

Behaviour and habitat: Commonly found in ponds, streams, puddles, canals and rivers. Fly in closer distances to the ground.

12. *Onychargia atrocyana* (Selys, 1865)

Size- Male: Abdomen: 23mm Hind wing: 17mm

Female: Abdomen: 23mm Hind wing: 18mm

Description: Male: Head is entirely black in fully matured adults. Eyes are black above and pale blue below. Thorax is velvety bluish black coloured. Fine citron yellow stripes can be seen in tenerals and subadults. These are obscured by pruinescence in fully matured individuals. Legs are black. Transparent wings with olivaceous yellow pterostigma. Abdomen black with pale blue basal rings.

Female: Females are similar in appearance of subadult males. Velvet black thorax with citron yellow stripes. Abdomen is black dorsally with yellow basal rings.

Behaviour and habitat: Rarely found in marshes, ponds and streams of forested and non-forested habitats. They have the ability to fly high and migrate to longer distances (Kiran and Raju, 2013)

13. *Prodasineura verticalis* (Selys, 1860)

Size- Male: Abdomen: 29mm Hind wing: 19mm

Female: Abdomen: 30mm Hind wing: 20mm

Description- Male: Velvety black head with pale brown labium. Labrum, bases of mandibles and genae are in reddish brown colour. Eyes are black above and brown below. Velvety black thorax having blood red and yellow stripes. Legs are black with reddish and ochreous shades. Transparent wings with dark reddish brown pterostigma. Abdomen black and have paired baso-dorsal spots are present in segments 3-7.

Female: Eyes are dark brown above and pale yellow below. Females have similar thoracic markings of males but markings are yellowish in colour.

Behaviour and habitat: Commonly found in streams, ponds and rivers. Perch on overhanging vegetation closer to water surface. Eggs are laid in running water or on plant parts closer to water (Kiran and Raju, 2013).

14. *Aciagrion approximans krishna* (Fraser, 1921)

Size- Male: Abdomen: 24-25mm Hind wing: 15mm

Female: Abdomen: 24-25mm Hind wing: 15-16mm

Description: Male: A black transverse band is present on pale labium and labrum. Black head with bright blue postocular spots connected by a blue stripe. Eyes are dark brown above and pale green below. Thorax is broadly black on dorsum with violaceous blue antehumeral stripes. Lateral sides are also violet blue. Legs have blue and black shades. Transparent wings with dark violet or black pterostigma. Violet blue abdomen is blackish on dorsum upto the 7^{th} segment. Segment 8 and 9 are violaceous blue in colour. Segment 8 is marked with lateral black stripe on each side. Segment 10 is black dorsally.

Female: Thoracic antehumeral stripes are pale bluish green in colour. Pterostigma dirty brown coloured.

Behaviour and habitat: Found in ponds, marshes and canals with slow moving water of high altitude regions. Floating or aquatic vegetation in stagnant water habitats are selected for oviposition (Joshi *et al.* 2016).

15. *Aciagrion occidentale* (Laidlaw 1919)

Size- Male: Abdomen: 23-24mm Hind wing: 15-16mm

Female: Abdomen: 24mm Hind wing: 16mm

Description: Male: Bluish white labium and labrum. Elongated blue postocular spots are connected by a fine blue line. Eyes are black above and pale blue below. Thorax is black dorsally and pale azure blue laterally. Fine greenish yellow ante humeral stripes are present. Pale blue legs with black shades. Transparent wings with blackish grey pterostigma. Slender abdomen is dorsally black upto the 7th segment. 8th and 9th segments are blue. Segment 8 has a narrow triangle shaped black marking on dorsum. Segment 10 is blue with x shaped black marking dorsally.

Female: Markings on thorax and abdomen are exactly similar to males except in segments 8-10. Segment 8 has a broad black marking dorsally. Segment 9 has a black patch on dorsal side at the basal end. Segment 10 is wholly blue. The antehumeral stripes are pale yellow.

Behaviour and habitat: Seen in marshes, canals and ponds of forests and non-forests. They have the ability to migrate. Breed in stagnant fresh water.

16. *Agriocnemis keralensis* (Peters, 1981)

Size- Male: Abdomen: 13mm Hind wing: 8mm

Female: Abdomen: 14mm Hind wing: 9mm

Description: Male: Labium, labrum, anteclypeus, postclypeus and bases of mandibles are pale yellowish green in colour. Two yellowish green postocular spots are present at the back of head. Green eyes are capped with black. Thorax is black on dorsum with apple green antehumeral stripes. Thorax is green on lateral sides. Legs are creamy white with black spines. Transparent wings with ochreous pterostigma. Abdomen is greenish in segments 1 and 2 changes to orange from 3 to 10 and all segments are marked with black. 2nd segment has a peculiar marking in the shape of cobra's hood.

Female: Head, prothorax and thorax are exactly similar with male. Abdomen is greenish yellow in colour with dorsal broad black stripe. Heteromorphic females can be seen with orange and pale brown ground colour.

Behaviour and habitat: Commonly seen in aquatic grasses of marshes and paddy fields, particularly in plains. (Koli *et al*., 2021)

17. *Agriocnemis pieris* (Laidlaw, 1919)

Size- Male: Abdomen: 16- 18mm Hind wing: 9-10mm

Female: Abdomen: 18mm Hind wing: 11mm

Description: Male: Labium is white in colour and labrum, bases of mandibles, genae and clypeus are pale azure blue. Eyes are black above and pale blue below. Black occiput has coma shaped white postocular spots and a fine median band between them. Thorax is black dorsally and pale blue laterally. Fine pale blue coloured antehumeral stripes are present. White legs with black markings. Transparent wings and the pterostigma is pale greyish white. Pale blue coloured abdomen having black markings upto the segment 7. Segment 8 is unmarked or having thin black basal annule. Segment 9 and 10 are unmarked.

Female: Females are similar to male but more bluish and have more extensive black markings on abdomen. Broad black markings are present on 1-9 segments. Segment 8 and 9 are black in most of the dorsolateral surfaces. Segment 8 has a lateral blue stripe extending from the basal end on each side. Segement 10 has a black marking on basal end. Immature females have orange ground colour.

Behaviour and habitat: Commonly found in forested and non-forested habitats. Fly among grasses and closer to the ground. Marshes, rivers and ponds are the breeding habitats.

18. *Agriocnemis pygmaea* (Rambur, 1842)

Size- Male: Abdomen: 16-17mm Hind wing: 9-10mm

Female: Abdomen: 18mm Hind wing: 11-12mm

Description: Male: Pale yellow coloured labium and bright metallic blue labrum. Black occiput with pale greenish round shaped postocular spots. Pale green eyes are capped with black. Apple green thorax marked dorsally with black and have apple green antehumeral stripes. Legs are yellow with black markings. Transparent wings. In forewings, pterostigma is yellow coloured and in hind wings it is black coloured. Abdomen is pale grrenish yellow upto 7^{th} segment and orange in segments 8-10 and anal appendages. Abdomen is broadly marked with black on dorsum upto 8^{th} segment. Pruinosed males are also found.

PLATE 5 - ODONATES OBSERVED DURING THE STUDY (CONT.)

Figure 5A*: Agriocnemis pieris*

Figure 5B*: Agriocnemis pygmaea*

Figure 5C*: Agriocnemis splendidissima*

Figure 5D*: Archibasis oscillans*

Figure 5E*: Ceriagrion cerinorubellum*

Figure 5F*: Ceriagrion coromandelianum*

Female: Different colour morphs are found in females. In red morphs, head, thorax and abdomen are dark brick red coloured. Thorax is marked with a dorsal broad black stripe. Terminal abdominal segments are suffused with black.

In androchrome forms, thorax is apple green and have dorsal balck stripe and bluish brown antehumeral stripes. Abdomen is almost similar with that of males.

Behaviour and habitat: They are common in all kinds of habitats like forests, plains and so on. Fly among grasses and closer to the ground. Breeds in ponds, marshes and rivers.

19. *Agriocnemis splendidissima* (Laidlaw 1919)

Size- Male: Abdomen: 18mm Hind wing: 10mm

Female: Abdomen: 17-18mm Hind wing: 12-13mm

Description: Male: Labium yellow coloured. Labrum, bases of mandibles, genae and ante clypeus are bluish. Comma shaped blue coloured postocular spots are present in black occiput. Geen eyes are capped with black. Thorax is black on dorsum and blue on sides. Black dorsal portion of thorax is marked with pinkish or pale bluish narrow stripes. Legs are black with bluish pruinescence. Transparent wings with black pterostigma. Abdomen is blue coloured with black markings. Superior anal appendages are peculiar with their narrow and curved hook like apperence.

Female: Females are similar to male but the ground colour is brownish or greenish yellow and stout abdomen.

Behaviour and habitat: They are not so common. Their distribution is more in higher altitudes than in plains. Ordinarily found among aquatic grasses of rivers, ponds and canals.

20. *Archibasis oscillans* (Selys, 1877)

Size- Male: Abdomen: 30-32mm Hind wing: 21-23mm

Female: Abdomen: 35mm Hind wing: 28mm

Discription: Male: Labium white and labrum turquoise blue. Eyes are dark blue above and pale blue below. Large triangular azure blue postocular spots are present. Thorax is black on dorsum with azure blue stripes. Lateral sides are also

azure blue in colour. Legs are creamy white with black markings. Wings are transparent and pterostigma is black, subquadrate shaped and framed in pale brown. Abdomen is black dorsally and laterally upto 7th segment. Segments 8-10 are azure blue and have apical black markings.

Female: Females have stouter abdomen and paler colours. 8th segment has a large dorsal black spot. 9th segment has two small black spots on dorsum. 10th segment is entirely azure blue.

Behaviour and habitat: Rarely found in streams and canals, particularly in forests or in habitats near to forests. Lay eggs on submerged vegetation.

21. *Ceriagrion cerinorubellum* (Brauer, 1865)

Size- Male: Abdomen: 31-33mm Hind wing: 20-21mm

Female: Abdomen: 31-35mm Hind wing: 20-21mm

Description: Male: White coloured labium and dark olivaceous labrum. Eyes are pale bluish above and greenish below. Thorax is greenish on dorsum, bluish on sides and yellowish beneath. Yellowish legs with black spines. Transparent wings and pterostigma amber coloured. Abdomen is orange coloured in segments 1, 2 and basal half of 3, apical half 7 and segments 8-10 are reddish orange. The central segments are black dorsally.

Female: Females are with paler colours and stouter abdomen.

Behaviour and habitat: Very common in ponds, canals, marshes, paddy fields and rivers of forested and non-forested habitats. Found among grasses.

22. *Ceriagrion coromandelianum* (Fabricius, 1798)

Size- Male: Abdomen: 28-30mm Hind wing: 18-20mm

Female: Abdomen: 29-32mm Hind wing: 20mm

Description: Male: Labium, labrum, clypeus, genae, bases of mandibles and frons are citron yellow coloured. Olive green eyes changes to greenish yellow below. Thorax is olive green on dorsum pale greenish yellow on sides. Legs are citron yellow with black spines. Wings are transparent and the pterostigma is golden yellow in colour. Abdominal segments are evenly citron yellow coloured.

PLATE 6 - ODONATES OBSERVED DURING THE STUDY (CONT.)

Figure 6A*: Ceriagrion rubiae*

Figure 6B*: Paracercion calamorum*

Figure 6C: *Paracercion malayanum*

Figure 6D: *Pseudagrion malabaricum*

Figure 6E*: Pseudagrion rubriceps*

Figure 6F*: Pseudagrion indicum*

Female: Females are large and stout bodied and with dull colours. Thorax is olivaceous brown and abdomen is golden yellow or brownish yellow.

Behaviour and habitat: Very common in distribution. Found in ponds, canals, ditches, rivers and lakes and backwaters. Breed in marshes and stagnant ponds with aquatic grass and vegetation.

23. *Ceriagrion rubiae* (Laidlaw, 1916)

Size- Male: Abdomen: 26-29mm Hind wing: 17-18mm

Female: Abdomen: 30-31mm Hind wing: 20mm

Description: Male: Pale yellow coloured labium. Labrum, clupeus and frons are ochreous coloured. Eyes are dark olivaceous above and paler below. Thorax is bright orange on dorsum and yellowish on sides. Legs are brownish yellow and having black spines. Wings are transparent with amber coloured perostigma. Abdomen is bright orange in colour.

Female: Females are robust bodied and dull coloured. The orange tint of thorax and abdomen is replaced by olivaceous.

Behaviour and habitat: Rarely found in forested habitats or in regions not far from forests. Breed in marshes and ponds with grasses and aquatic vegetation.

24. *Ischnura rubilio* (Selys, 1876)

Size- Male: Abdomen: 16-20mm Hind wing: 10-12mm

Female: Abdomen: 18-20mm Hind wing: 14-15mm

Description: Male: White coloured labium and citron yellow coloured labrum. Small round, azure blue coloured postocular spots are present. Eyes are olive green, darker above and paler beneath with a small cap of black. Thorax is broadly bronzed black on dorsum with grass green stripes. Lateral sides are grass green in colour. Pale yellow legs are marked with black. Transparent wings. Pterostigma vary in size and shape in fore and hind wings. In fore wings it is kite shaped and rose red in proximal half and transparent in remaining portion. In hind wing it is smaller and pale greyish in colour. Abdomen is bright yellow upto the segment 7 and the remaining segments are azure blue in colour. A dorsal diamond shaped spot

is present near the apical end of segement 6. Segment 7 is bronzed black dorsally. Segment 10 has a dorsal broad black spot.

Female: Thorax is pale green laterally. The azure blue colour of last segments is absent in females. Abdomen is pale yellow or pale brownish and a dorsal broad black stripe is present on all abdominal segments.

Behaviour and habitat: Commonly found in forested, non-forested habitats and also in brackish water habitats. Ordinarily seen in ponds, rivers, marshes, canals, lakes and back waters.

25. ***Ischnura senegalensis*** **(Rambur, 1842)**

Size- Male: Abdomen: 21-23mm Hind wing: 13-15mm

Female: Abdomen: 20-24mm Hind wing: 14-16mm

Description: Male: Pale yellow coloured labium and pale blue coloured labrum. Eyes are green coloured above, yellowish beneath and capped with black. Thorax is broadly black dorsally with citron yellow or pale green stripes and pale green on sides. Black legs are marked with yellow. Wings are transparent and pterostigma varies in fore and hind wings. In forewings it is black with white costal border and in hindwings it is pale brown framed with black. Abdomen is dorsally black in all segments except segment 8. Remaining portion of segments 1 and 2 are azure blue and pale green coloured. Segements 3-7 are yellow on sides. Segment 8 is azure blue. Segment 9 is azure blue on sides and segment 10 is yellow laterally.

Female: Females are found in different forms. Generally, they are dull coloured and blue colour on abdomen is completely absent. They are red or brown tinted. But in androchrome forms they look exactly like males.

Behaviour and habitat: Very common in high altitude forests, plains and brackish water habitats. Found in marshes, ponds, rivers, streams, lakes and wet grasslands. Stagnant ponds and marshes are the oviposition sites.

26. ***Paracercion calamorum*** **(Ris, 1916)**

Size- Male: Abdomen: 22-23mm Hind wing: 16mm

Female: Abdomen: 21-23mm Hind wing: 15-16mm

Description: Male: Head, thorax and legs are pruinosed in matured adults. Vertex and occiput are black. Postocular spots are blue coloured. In old adults these are obscured by pruinescence. Eyes are brown above and yellowish green below. Thorax is black on dorsum and blue on sides, entirely hidden by bluish white pruinescence in old adults. Pale bluish legs with black markings obscured by pruinescence. Transparent wings with pale yellow pterostigma framed in black. Abdomen azure blue broadly marked with black on segments 1-7. Segments 8-10 are azure blue. Segments 8 and 9 are marked with fine black apical fringe. Segment 10 with a narrow black stripe along the mid dorsal carina.

Female: Females are with ground colour yellowish green. All abdominal segments are marked broadly with black on dorsum.

Behaviour and habitat: Rarely found in paddy fields and weedy ponds. Perch on floating leaves.

27. *Paracercion malayanum* (Selys, 1876)

Size- Male: Abdomen: 22mm Hind wing: 15mm

Female: Abdomen: 20mm Hind wing: 15mm

Description: Male: Black coloured vetex and occiput with bluish green postocular spots. Other parts of head is azure blue coloured. Eyes are deep blue coloured. Thorax is black on dorsum with broad azure blue stripes. Lateral sides are also blue in colour. Pruinescence on thorax is absent. Pale bluish legs with black markings and black spines. Transparent wings and pterostigma is pale yellow with black frame. Azure blue coloured abdomen is marked dorsally with broad black stripe upto segment 7. Segment 2 has a peculiar thistle head shaped black coloured marking on dorsum. Segments 8-10 are azure blue and segments 8 and 9 with narrow black apical fringe. There is a fine black stripe on the mid dorsal carina of segment 10.

Female: Females are with yellowish green ground colour. Greenish yellow thorax marked with black. Abdominal segments are broadly black on dorsum with pale green rings.

Behaviour and habitat: Rarely found in aquatic grasses and floating vegetation of ponds and paddy fields.

28. *Pseudagrion australasiae* Selys, 1876

Size- Male : Abdomen: 30-32.5mm Hind wing: 20-21mm

Female: Abdomen: 29mm Hind wing: 20mm

Description: Male: Pale yellow coloured labium. Labrum, bases of mandibles, clypeus, genae and frons are pale greenish blue. Postocular spots are large and bluish in colour. Eyes are blue coloured capped with black. Bright azure blue coloured thorax with broad black stripes on dorsum. Pale bluish legs with black markings. Wings are transparent and the pterostigma is dark brown in colour. Abdomen is azure blue in colour marked with black broadly on dorsum upto segment 7. Segments 8-10 are azure blue. Segments 8 and 9 are with fine black apical rings. There is a broad X shaped marking on segment 10 dorsally. Superior anal appendages are bifid at the apex and length is half that of segment 10.

Female: Thorax is pale greenish blue coloured with black stripes. Abdomen is pale blue with broad dorasal stripe of black upto segment 9.

Behaviour and habitat: Found in forests or habitats not far from forests. Breeds in weedy ponds and marshes.

29. *Pseudagrion decorum* (Rambur, 1842)

Size- Male: Abdomen: 28-30mm Hind wing: 18-20mm

Female: Abdomen: 31mm Hind wing: 20mm

Description: Male: Labium whitish. Labrum, genae, bases of mandibles, clypeus, frons and vertex are coloured pale bluish green. Postocular spots are deep azure blue and triangle shaped. Colour of eyes changes from blue, bluish green to pale green from top to bottom and also with a small cap of black. Thorax is bluish green dorsally and azure blue on sides. Thorax has three peculiar fine mid dorsal lines. Pale bluish legs are with black markings. Transparent wings. Pterostigma whitish brown and diamond shaped. Abdomen azure blue and marked dorsally with black except segments 8-10. Segments 8-10 are azure blue marked with black apical rings.

Female: Females with greenish yellow ground colour. Markings on thorax is similar with that of males. Dorsal black abdominal markings are present upto segment 9.

Behaviour and habitat: Rarely found in weedy ponds, lakes and marshes. Perch on emergent grasses. Exhibit migratory behaviour.

30. *Pseudagrion indicum* (Fraser, 1924)

Size: Male: Abdomen: 34mm Hind wing: 22mm

Female: Abdomen: 32mm Hind wing: 22mm

Description: Male: white coloured labium. Pale yellowish green labrum and cheeks. Eyes are green coloured and with black cap. Thorax is broadly black on dorsum with broad grass green stripes. Lateral sides are azure blue in colour. Pale blue legs with black stripes. Transparent wings with blackish brown pterostigma. Abdomen is azure blue coloured with a broad dorsal stripe of black upto segment 7. Segments 8 and 9 are entirely azure blue and having broad black apical rings.

Female: The blue colour on lateral sides of thorax is replaced with pale yellowish green. Ground colour of abdomen is blue with black stripe, similar to male. Segments 8 and 9 are broadly black on dorsum and segment 10 is blue with narrow black basal ring.

Behaviour and habitat: This species is endemic to the Western Ghats. These are found in streams, rivers and ponds of forested habitats and regions near to forests and hills.

31. *Pseudagrion malabaricum* (Fraser, 1924)

Size- Male: Abdomen: 33mm Hind wing: 20mm

Female: Abdomen: 32mm Hind wing: 22mm

Description: Male: Labium whitish and labrum is azure blue coloured. Turquoise blue eyes are capped with black. Azure blue coloured thorax is marked dorsally with three broad black stripes. Legs are pale blue with black markings. Transparent wings with dark brownish pterostigma. Abdomen is azure blue marked with black dorsally on segments 1-7. Segments 8-10 are azure blue. Segments 8 and 9 have narrow apical black coloured rings. Segment 10 has a dorsal broad black marking. Superior anal appendages are black and slightly shorter than segment 10. These are not bifid and curled inward.

Female: Thorax is pale greenish blue coloured. Stripes on thorax are similar to male. Pale blue coloured abdomen. The dorsal black stripe is present upto segment 9.

Behaviour and habitat: Not a common species. Found in forests or in regions near to forests. Breeds in weedy ponds and marshes.

32. *Pseudagrion microcephalum* (Rambur, 1842)

Size- Male: Abdomen: 27mm Hind wing: 17mm

Female: Abdomen: 29mm Hind wing: 20mm

Description: Male: Labium, labrum and genae are pale blue in colour. Postocular spots are very large and azure blue coloured. Eyes are pale blue beneath and azure blue above with a small brown cap. Azure blue coloured thorax with broad black stripes on dorsum. Pale blue legs with black stripes. Transparent wings. Pterostigma grey coloured and framed with black. Abdomen azure blue coloured and marked with black. Segments 1-7 are marked dorsally with broad black stripes. Segment 2 has a goblet shaped marking. Segment 8 has thick annule and segment 9 has a thin annule apically. Segment 10 is marked broadly on dorsum with black. Superior anal appendages are black with inner side blue and same length of segment 10 and bifid at the apex.

Female: Differs from male in colour and markings. Thorax with bluish green ground colour, dorsally suffused with orange and narrow black stripes. Pterostigma pale brown coloured. Pale blue coloured abdomen with black broad dorsal stripe upto segment 9. Segment 2 has a dumbbell shaped marking. Markings are absent on Segment 10.

Behaviour and habitat: This species is common in plains. Found in ponds, canals, marshes rivers and paddy fields. Breeds in marshy and vegetated aquatic habitats. Exhibits migratory behavior (Kiran and Raju, 2013).

33. *Pseudagrion rubriceps* (Selys, 1876)

Size- Male: Abdomen: 29mm Hind wing: 18-20mm

Female: Abdomen: 29mm Hind wing: 21mm

PLATE 7 - ODONATES OBSERVED DURING THE STUDY (CONT.)

Figure 7A*: Pseudagrion microcephalum*

Figure 7B*: Gynacantha dravida*

Figure 7C*: Ictinogomphus rapax*

Figure 7D*: Acisoma panorpoides*

Figure 7E*: Aethriamanta brevipennis*

Figure 7F*: Brachydiplax chalybea*

Description: Male: Bright orange colour of face is the peculiar character of this species. Eyes are orange above and golden yellow beneath. Large dark bluish triangle shaped postocular spots are present. Thorax is golden olivaceous green dorsally with black stripes. Lateral sides are azure blue coloured. Pale yellow coloured legs with black shades. Wings are transparent and the perostigma is diamond shaped and reddish brown coloured. Abdomen is azure blue with broad dorsal black stripe on segments 1-7. Segment 8 has a triangle shaped apical dorsal marking. Markings are absent on segments 9 and 10.

Female: Bright orange colour is replaced with yellowish green on face, thorax and eyes. Lateral sides of thorax and abdomen is pale blue in colour. Black dorsal broad marking on abdomen is extended upto segment 9. Segment 10 is unmarked.

Behaviour and habitat: Commonly found in streams, rivers, ponds and streams of forested and non-forested habitats. Seen in groups of 3-4.

Suborder Anisoptera

34. *Anax guttatus* (Burmeister, 1839)

Size- Male: Abdomen: 56-62mm Hind wing: 50-54mm

Female: Abdomen: 56-58mm Hind wing: 52-54mm

Description: Male: Labium and labrum are ochreous coloured. Eyes are greenish or greenish blue in colour. Thorax is unmarked and pale green coloured. Legs are black coloured. Wings are transparent. Central portion of hind wings have large brownish yellow patch. Pterostigma is ferruginous coloured. Abdominal segments 1 and 2 are pale greenish, segment 2 is turquoise blue on dorsum and segment 3 has a pair of triangle shaped turquoise blue markings dorsally. 3 pairs of orange coloured spots are present on segments 4-7. Segments 8 and 9 have fewer spots and segment 10 is yellow with a basl reddish brown annule.

Female: Females show close similarity to males but colours are duller. The brownish yellow patch on hind wing is absent.

Behaviour and habitat: Common species of forested and non-forested habitats. Found in weedy ponds, lakes, marshes. It is diurnal species and attracted by light during night.

35. *Anax immaculifrons* (Rambur, 1842)

Size- Male: Abdomen: 52-55mm Hind wing: 55mm

Female: Abdomen: 56mm Hind wing: 58-60mm

Description: Male: Labium ochreous and labrum greenish yellow coloured. Eyes are sapphire blue in colour. Pale bluish green thorax with broad black lateral stripes. Legs are black. Transparent wings are tinted with brownish yellow. Colour of perostigma varies from ochreous to reddish brown. Abdomen with a ground colour of pale reddish brown and black markings.

Female: Closely similar to male. But instead of the turquoise blue colour, greenish yellow is the ground colour of thorax and base of abdomen.

Behaviour and habitat: Common species in forested and non-forested habitats. Found in rivers, canals and estuaries. Lays eggs by piercing submerged plant parts.

36. *Gynacantha dravida* (Lieftinck, 1960)

Size- Male: Abdomen: 50-58mm Hind wing: 43-50mm

Female: Abdomen: 48-55mm Hind wing: 44-50mm

Description: Male: Olivaceous brown face with peculiar T shaped marking on frons. Eyes are brownish blue. Brown coloured thorax. Legs are reddish brown. Transparent wings are tinted with reddish brown and having reddish brown pterostigma. Pale brown coloured abdomen with dark brown markings. Segment 3 is constricted. In fully matured adults bright bluish or greenish colours appear on thorax and first abdominal segments.

Female: Females are similar to males with duller colours.

Behaviour and habitat: This is a crepuscular species. Rests among dark vegetation during day time. Attracted by light during night. Found around weedy ponds, streams and marshes of forested and non-forested areas. Oviposit on soil adjacent to water bodies and the eggs reach in water during rain.

37. *Gynacantha millardi* Fraser, 1920

Size- Male: Abdomen: 46mm Hind wing: 44mm

Female: Abdomen: 45mm Hind wing: 43-45mm

Description: Male: Labium and labrum are pale yellowish. Eyes are greyish above and yellowish green below. Thorax is bright olive green or grass green. Yellowish brown legs. Wings are transparent with ochreous pterostigma having fine black frame. Abdomen is pale brown dorsally with dark brownish and black markings. Segments 1-3 are grass green laterally. Segment 3 is not constricted and this characteristic helps to distinguish from the similar species *Gynacantha bayadera*.

Female: Exactly similar to male. Colours are duller in females.

Behaviour and habitat: This is a crepuscular species. During day time they rest on vegetation. Rarely found around marshes and weedy ponds surrounded by thick vegetation.

38. *Ictinogomphus rapax* (Rambur, 1842)

Size- Male: Abdomen: 52mm Hind wing: 40mm

Female: Abdomen: 50mm Hind wing: 42-44mm

Description: Male: Labium and labrum are yellow with black frame. Bluish grey coloured eyes. Black coloured thorax with yellow markings. Legs are black in colour and marked with yellow. Transparent wings. Pterostigma black. Abdomen black with yellow markings.

Female: Females show close similarity with males with stouter abdomen and extensive yellow markings.

Behaviour and habitat: It is a commonly found species. Strong, agile flier. Both running and still water is used for breeding.

39. *Acisoma panorpoides* (Rambur, 1842)

Size- Male: Abdomen: 15-18mm Hind wing: 16-21mm

Female: Abdomen: 15-18mm Hind wing: 17-22mm

Description: Male: Labium and labrum pale yellowish. Pale blue coloured face. Eyes are bright blue coloured and have black spots. Azure blue coloured thorax is marbled with black. Legs are black with pale yellow stripes. Wings are transparent and the pterostigma is pale yellow in colour. Abdomen is also azure blue with black markings. Segments 1-5 are well dilated and the remaining

segments are slender. This is an identifying feature of this species. Segments 8-10 are entirely black and the anal appendages are white coloured.

Female: Shows similarity with males in shape and markings of thorax and abdomen. Eyes are greenish in colour and the ground colour of body is greenish yellow.

Behaviour and habitat: It is a common species of plains and weak flier. Found always closer to water among aquatic grasses and reeds. Breeds in marshes, vegetated ponds and paddy fields.

40. *Aethriamanta brevipennis* (Rambur, 1842)

Size- Male: Abdomen: 17-20mm Hind wing: 23-26mm

Female: Abdomen: 16mm Hind wing: 23mm

Description: Male: Small sized dragonfly. Eyes are dark reddish brown above and paler beneath. Thorax is dark chocolate brown in colour. Legs are black and having a bright red spot at the extensor surface of the distal ends of hind femora. Transparent wings with dark golden amber coloured tint at the base. Pterostigma is dark brown coloured. Abdomen is bright reddish. Anal appendages are red.

Female: In females the ground colour is yellow. Eyes are reddish brown above and greyish beneath. The red colour of femoral spot is replaced with citron yellow. Thorax and abdomen are golden olivaceous with black markings.

Behaviour and habitat: This species is commonly found in rivers, ditches, canals and weedy ponds. They exhibit obelisk posture in hot sunny days.

41. *Brachydiplax chalybea* (Brauer, 1868)

Size- Male: Abdomen: 21-25mm Hind wing: 26-30mm

Female: Abdomen: 21-23mm Hind wing: 27-29mm

Description: Male: Eyes are dark brown above and greyish beneath. Thorax is grey dorsally due to pruinescence and ochreous on sides. Legs are black with ferruginous shades. Wings are transparent and the bases are tinted with dark brown. Pterostigma is yellow coloured. Abdomen dorsally black with white pruinescence. Anal appendages are black.

Female: Thorax and abdomen are ochreous with black markings.

Behaviour and habitat: Common dragonfly found in estuaries, marshes, ponds, ditches, paddy fields and rivers.

42. *Brachydiplax sobrina* (Rambur, 1842)

Size- Male: Abdomen: 20-24mm Hind wing: 25-28mm

Female: Abdomen: 16-22mm Hind wing: 22-26mm

Description- Male: Eyes are reddish brown above and pale bluish beneath. Thorax is olivaceous brown in colour and dorsally pruinosed with bluish white. Black legs. Transparent wings having pale yellowish pterostigma. Abdomen is black in colour and segments 1-7 have bluish white pruinescence. Anal appendages are black.

Female: Thorax and abdomen are yellow in colour and marked with black.

Behaviour and habitat: It is not commonly seen. Generally found in paddy fields, marshes and ponds.

43. *Brachythemis contaminata* (Fabricius, 1793)

Size- Male: Abdomen: 18-21mm Hind wing: 20-23mm

Female: Abdomen: 18-20mm Hind wing: 22-25mm

Description: Male: Small dragonfly. Eyes are brown above and greyish beneath. Thorax is olivaceous brown with reddish brown stripes on sides. Legs are ochreous with brownish or blackish shades. Transparent wing with red venation and a bright orange patch extending from wing base to pterostigma. Pterostigma is rust red in colour. Abdomen is ochreous red with brown stripes. Anal appendages are rusty.

Female: Thorax is yellow coloured with black and brown markings.

Behaviour and habitat: This is a commonly found species especially in non-forested areas. Stagnant or slowly flowing water bodies including weedy ponds, paddy fields, marshes, canals, rivers and ditches are the breeding habitats. They can survive in habitats having polluted water. Presence of this species in pristine habitats indicates the increased disturbance of that habitat.

44. *Bradinopyga geminata* (Rambur, 1842)

Size- Male: Abdomen: 26-29mm Hind wing: 33-36mm

Female: Abdomen: 26-29mm Hind wing: 32-36mm

Description: Eyes are brownish above and greyish below. Thorax is ashy grey marbled with black. Legs are greyish with pruinescense. Transparent wings and the pterostigma is bicoloured, centrally black and whitish on both ends. Abdomen is black marbled with pale dirty yellow. Anal appendages are creamy white in colour.

Female: Females are closely similar to male. They can be differentiated through sexual characters.

Behaviour and habitat: Common species of forested and non-forested habitats. Small sized stagnant water bodies such as rock pools, overhead tanks, garden tanks, wells and ponds are the preferred habitats. They are used as mosquito control agent in some countries.

45. *Crocothemis servilia* (Drury, 1770)

Size- Male: Abdomen: 24-35mm Hind wing: 27-38mm

Female: Abdomen: 25-32mm Hind wing: 31-37mm

Description: Male: Eyes are blood red in colour and paler below. Thorax is blood red coloured. Legs are reddish ochreous. Transparent wings with amber yellow patch at the base. Pterostigma is dark ochreous. Blood red abdomen with a fine black mid dorsal carina. Anal appendages are blood red.

Female: Females vary widely in colouration from males. Eyes are brown above and pale yellowish green below. Thorax is olivaceous brown. Abdomen ochreous with black mid dorsal carina.

Behaviour and habitat: It is common particularly in non-forested habitats like paddy fields, weedy ponds, marshes, ditches and canals.

46. *Diplacodes nebulosa* (Fabricius, 1793)

Size- Male: Abdomen: 15-17mm Hind wing: 17-19mm

Female: Abdomen: 14-15mm Hind wing: 18mm

PLATE 8 - ODONATES OBSERVED DURING THE STUDY (CONT.)

Figure 8A*: Brachythemis contaminata*

Figure 8B*: Bradinopyga geminata*

Figure 8C: *Crocothemis servilia*

Figure 8D*: Diplacodes trivialis*

Figure 8E: *Hydrobasileus croceus*

Figure 8F: *Lathrecista asiatica*

Description: Male: Eyes are brownish above and greyish yellow beneath. Thorax, legs and abdomen are black. In old adult, body is slightly pruinosed. Transparent wings are tipped with blackish brown. Pterostigma is black.

Female: Thorax and abdomen of females are yellow with black markings.

Behaviour and habitat: This is not a commonly found species. Seen in weedy ponds and marshes. Flies in short heights.

47. *Diplacodes trivialis* (Rambur, 1842)

Size- Male: Abdomen: 19-22mm Hind wing: 22-23mm

Female: Abdomen: 18-20mm Hind wing: 22-24mm

Description: Male: Small sized dragonfly. Eyes are bluish brown above and pale blue beneath. Thorax is greenish yellow and dorsally it is violaceus brown with fine black markings. In old adults the thorax is covered with blue pruinescence. Legs are black marked with yellow. Wings are transparent. Pterostigma is dark greyish or black. Abdominal segments 1-7 are greenish yellow and marked with black. Segments 8-10 are entirely black and the anal appendages are yellow. In old adult, abdominal segments are also pruinosed with blue.

Female: Eyes are brownish above and yellow beneath. Abdomen and thorax are greenish yellow with black markings.

Behaviour and habitat: It is a common species of forested and non-forested habitats. Found around ponds, paddy fields, canals and streams. Always flies in short heights, closer to the ground and seen in gardens, play grounds, meadows and pathways.

48. *Hydrobasileus croceus* (Brauer, 1867)

Size- Male: Abdomen: 29-33mm Hind wing: 40-42mm

Female: Abdomen: 28-34mm Hind wing: 42-48mm

Description: Male: Large sized dragonfly. Eyes are reddish brown above and olivaceous beneath. Thorax is olivaceous brown in colour. Legs are ochreous. Transparent wings with pale brownish tint. The base of hind wing at the posterior end is marked with a broad reddish brown patch. Pterostigma is rusty brown anteriorly and ochreous posteriorly. Abdominal segements are ochreous at the

initial segments and reddish or ochreous at the last segments are have black markings. Anal appendages are reddish brown coloured.

Female: Females are stouter but similar to male in all respects.

Behaviour and habitats: This is not a common species. Found around weedy ponds, marshes and lakes. Genrally flies in heights.

49. *Lathrecista asiatica* (Fabricius, 1798)

Size- Male: Abdomen: 27-32mm Hind wing: 33-37mm

Female: Abdomen: 27-32mm Hind wing: 34-36mm

Description: Male: Eyes are reddish brown above and bluish grey below. Thorax is dark coppery brown dorsally and yellow laterally with brown stripes. Legs are dark reddish brown with yellow shade on anterior femora. Transparent wings are enfumed at tips and with reddish brown pterostigma. Abdomen is bright crimson red upto segment 8. Segments 9 and 10 and anal appendages are wholly black in colour.

Female: Females are almost similar to males. The colour of abdomen is olivaceous brown. Greenish yellow mid dorsal carina is present which is bordered with black.

Behaviour and habitat: It is common in forested and non forested habitats including ponds and marshes.

50. *Neurothemis fulvia* (Drury, 1773)

Size- Male: Abdomen: 21-26mm Hind wing: 27-32mm

Female: Abdomen: 20-24mm Hind wing: 26-32mm

Description: Male: Eyes are reddish brown above and brownish beneath. Prothorax and thorax are reddish brown in colour. Legs are rusty coloured. Wings are dark reddish brown except at tips. Pterostigma is reddish brown. Abdomen is reddish brown with ferruginous anal appendages.

Female: Head, thorax and abdomen of females are rusty brown coloured. Wings are amber yellow coloured.

PLATE 9 - ODONATES OBSERVED DURING THE STUDY (CONT.)

Figure 9A: *Neurothemis fulvia*

Figure 9B*: Neurothemis tullia*

Figure 9C: *Onychothemis testacea*

Figure 9D: *Orthetrum chrysis*

Figure 9E: *Orthetrum luzonicum*

Figure 9F: *Orthetrum pruinosum*

Behaviour and habitat: Found around ponds, rivers, streams and canals of forested and non forested habitats.

51. *Neurothemis tullia* (Drury, 1773)

Size- Male: Abdomen: 16-20mm Hind wing: 19-23mm

Female: Abdomen: 16-19mm Hind wing: 20-23mm

Description: Male: Eyes are dark brown above and olivaceous beneath. Thorax is black and having a pale yellowish mid dorsal carina. Legs are black. Basal half of wing is black which is bordered with milky white patch at the distal end and transparent at the tips. Pterostigma is dirty ochreous. Abdomen is black and having creamy white mid dorsal carina. Anal appendages are creamy white with black tips.

Female: Thorax is greenish yellow with a yellow mid dorsal stripe and two broad black stripes. Basal half of wing is tinted with amber yellow bordered with brownish black patch at the distal end followed by an area of pale yellow reticulation. The wing tips are opaque and brownish black. Abdomen is yellowish with two broad black stripes.

Behaviour and habitat: Common in a wide variety of habitats including paddy fields, ponds, canals, lakes, ditches, streams, rivers and marshes with short and weak flights.

52. *Onychothemis testacea* (Laidlaw, 1902)

Size- Male: Abdomen: 34-36mm Hind wing: 40-42mm

Female: Abdomen: 36mm Hind wing: 42-44mm

Description: Male: Eyes are bottle green in colour. Dark metallic bluish thorax with citron yellow mid dorsal carina and lateral stripes. Legs are black. Transparent wings with black pterostigma. Abdomen is black with citron yellow spots. Anal appendages are black and slim.

Female: Females are similar to males but with stouter abdomen.

Behaviour and habitat: It is not common. Found in streams and rivers of forested habitats.

53. *Orthetrum chrysis* (Selys, 1891)

Size- Male: Abdomen: 28-33mm Hind wing: 31-38mm

Female: Abdomen: 25-30mm Hind wing : 31-36mm

Description: Male: Brownish eyes and reddish face. Thorax is dark rusty brown. Legs are reddish black. Transparent wings and the wing base has an amber coloured patch and dark reddish brown pterostigma. Abdomen is bright blood red.

Female: Females are with brownish thorax and bright ochreous abdomen. The patch at the wing base is absent. There are expansions on segment 8 bordered broadly with black.

Behaviour and habitat: Common species of paddy fields, ponds, rivers, ditches, marshes,,lakes streams and canals. They can tolerate polluted water to some extent.

54. *Orthetrum glaucum* (Brauer, 1865)

Size- Male: Abdomen: 29-35mm Hind wing: 33-40mm

Female: Abdomen: 30mm Hind wing: 36mm

Description: Male: Face is olivaceous brown in younger and glossy black in older individuals. Eyes are dark green in colour. Thorax is dark dull blue with pruinescence. Legs are black. Transparent wings have dark amber coloured small patch at the base. Pterostigma is dark ochreous finely framed with black. Abdomen is pale blue with pruinescence at segments 1-8 and segments 9 and 10 are black. Anal appendages are black.

Female: Thorax is olivaceous in colour with dark reddish brown stripes. Abdomen is reddish brown and has yellowish stripes.

Behaviour and habitat: Common in forested habitats but rare in non forested areas. Found around marshes, streams and canals.

55. *Orthetrum luzonicum* (Brauer, 1868)

Size- Male: Abdomen: 28-30mm Hind wing: 30-32mm

Female: Abdomen: 28-32mm Hind wing: 30-32mm

Description: Male: Eyes are bluish green with brown spots. Face and frons are pale blue or pale greenish yellow in colour. Thorax is pale olivaceous green with lateral reddish brown stripes. Legs are blackish brown with bright yellow markings. Transparent wings with black bordered ochreous pterostigma. Abdomen is pale azure blue with pruinescence and very thin black markings. Anal appendages are blackish brown.

Female: Thorax of females are greenish yellow with brown mrkings. Abdomen is greenish yellow marked with black stripes.

Behaviour and habitat: Common species of forested and non forested habitats. Found around marshes, streams, ponds, ditches and paddy fields.

56. *Orthetrum pruinosum* (Burmeister, 1839)

Size- Male: Abdomen: 28-31mm Hind wing: 32-36mm

Female: Abdomen: 30mm Hind wing: 37mm

Description: Male: Face is ochreous or pale brownish. Eyes are bluish black above and bluish grey beneath. Thorax is brownish and with dull purple pruinescence. Legs are black. Transparent wings are having pale brownish tint at the apices and the pterostigma is reddish brown coloured. Abdomen is bright red which changes to purplish red when pruinosed. Anal appendages are red.

Female: Thorax is reddish brown or ochreous with brown stripes. Thorax is not pruinosed. Abdomen is ochreous with fine black markings on sutures and borders.

Behaviour and habitat: Commonly found in ponds, rivers, streams and ditches. They ganeraly perch on ground or shrubs.

57. *Orthetrum sabina* (Drury, 1770)

Size- Male: Abdomen: 30-36mm Hind wing: 30-36mm

Female: Abdomen: 32-35mm Hind wing: 31-35mm

Description: Male: Pale greenish eyes with black spots. Thorax is greenish yellow with black markings. Black coloured legs with yellow markings. Wings are transparent and the pterostigma is black. Abdomen pale greenish yellow with black

markings. Segments 7-9 are wholly black. Creamy white anal appendages have the same length of segment 9.

Female: The colouration and markings of females are similar to males.

Behaviour and habitat: Very common in forested and non forested habitats. Seen in ponds, rivers, ditches, streams, lakes, estuaries, marshes and paddy fields.

58. *Palpoleura sexmaculata* (Fabricius, 1787)

Size- Male: Abdomen: 14-16mm Hind wing: 15-21mm

Female: Abdomen: 13-14mm Hindwing: 18-21mm

Description: Male: Eyes are brown above and bluish grey beneath. Face is creamy yellow coloured. Greenish yellow coloured thorax is reddish brown on dorsum and has black and brown markings. Bright yellow coloured legs are marked with black. Wings are transparent having black markings. Hind wings have yellowish tint except at the tips. Pterostigma black with white patch. Abdomen is pale bluish and pruinosed. Anal appendages are black.

Female: Yellowish thorax is dorsally rich ochreous and with brown and black markings. Abdomen bright ochreous with a black mid dorsal stripe and broader black subsubdorsal stripes.

Behaviour and habitat: This is one of the smallest dragonflies found in Kerala. This is not commonly found. Seen around marshes and streams.

59. *Pantala flavescens* (Fabricius, 1798)

Size- Male: Abdomen: 29-35mm Hind wing: 38-40mm

Female: Abdomen: 30-33mm Hindiwng: 39-41mm

Description: Male: Eyes are reddish brown above and bluish grey beneath. Frons is bright golden yellow or orange. Thorax is olivaceous or rusty with yellow hairs. Legs are black with yellowish markings. Wings are transparent. Hind wing has golden yellow coloured base and a small brownish spot at the posterior border of the wing tip. Pterostigma is bright reddish brown. Abdomen is bright ochreous with dorsal reddish tint and black mid dorsal markings.

PLATE 10 - ODONATES OBSERVED DURING THE STUDY (CONT.)

Figure 10A: *Orthetrum sabina*

Figure 10B: *Palpopleura sexmaculata*

Figure 10C: *Potamarcha congener*

Figure 10D:*Rhodothemis rufa*

Figure 10E: *Rhyothemis variegata*

Figure 10F *: Tholymis tillarga*

Female: Female shows similarity with male except in some characteristics. Wings are evenly enfumed and the brownish spot is absent. The dorsal red colour of abdomen is absent.

Behaviour and habitat: This is one of the most common dragonflies. Found as swarms in open areas like paddy fields, play grounds, highways, rivers etc. This species possess highly efficient migratory capacity. Generally migrate with monsoon winds in huge numbers.

60. *Potamarcha congener* (Rambur, 1842)

Size- Male: Abdomen: 29-32mm Hind wing: 33-35mm

Female: Abdomen: 29-31mm Hind wing: 33-37mm

Description: Male: Greyish yellow coloured eyes are capped with reddish brown. Black coloured thorax is pruinosed to impart a bluish grey appearance. Legs are black. Transaprent wings, brownish at the extreme apices. Pterostigma is dark reddish brown. Abdomen is dark brown or black with medial and lateral yellow markings. Thorax and abdomen is completely pruinosed in old adults.

Female: Thorax and abdomen is dark brown in colour and marked with yellow. Segment 8 has lateral dilations.

Behaviour and habitat: This is a common species. Weedy ponds and marshes are the breeding habitats.

61. *Rhodothemis rufa* (Rambur, 1842)

Size- Male: Abdomen: 22-29mm Hind wing: 32-37mm

Female: Abdomen: 25-29mm Hind wing: 32-37mm

Description: Male: Eyes are brown capped bright red. Reddish brown coloured thorax. Legs are reddish black. Transparent wings with ochreous pterostigma. Abdomen and anal appendages are bright red.

Female: Thorax is dark brownish. Abdomen golden yellowish brown. A yellow mid dorsal stripe is present extending from prothorax to the segment 4 of abdomen.

Behaviour and habitat: Commonly found in paddy fields, rivers, ponds and estuaries.

62. *Rhyothemis variegata* (Linnaeus, 1763)

Size- Male: Abdomen: 23-25mm Hind wing: 33-36mm

Female: Abdomen: 20-22mm Hind wing: 28-37mm

Description: Male: Eyes are brownish above and grey beneath. Thorax is metallic green and the legs are black. Wings are tinted with yellow. Blackish brown patches are present at the wing tips, nodes and central area of wings. Hind wing has a large patch of yellow with blackish brown markings at the base. Abdomen is black in colour.

Female: Females vary greatly in the colour of wing from male. A large opaque area of yellow and blackish brown is present at the basal half of forewing. In hind wing opaque area of yellow and blackish brown extends the entirely except at the wing tip.

Behaviour and habitat: Common dragonfly of forested and non forested habitats. Found around paddy fields, ponds, canals, rivers and marshes. Occasionally found as small groups.

63. *Tetrathemis platyptera* (Selys, 1878)

Size- Male: Abdomen: 15-18mm Hind wing: 18-21mm

Female: Abdomen: 14-16mm Hind wing: 19-24mm

Description: Male: This is a small sized dragonfly. Eyes are bluish green coloured. Thorax is metallic black with citron yellow stripes. Legs are black. Transparent wings are tinted with amber yellow at the base of forewing and basal half of hind wing. Pterostigma black coloured. Abdomen black with citron yellow markings.

Female: Females are closely similar to males but stouter. Amber tint of wings is deeper.

Behaviour and habitat: Common species of forested and non forested habitats. Found in ponds, weedy wells and ditches. Females lay eggs during flight. Eggs are deposited on overhanging vegetation which are washed down duing rain.

64. *Tholymis tillarga* (Fabricius, 1798)

Size- Male: Abdomen: 28-33mm Hind wing: 33-37mm

Female: Abdomen: 27-31mm Hind wing: 31-37mm

Description: Male: Reddish olivaceous eyes are capped with brown. Thorax is yellowish or olivaceous laterally and bright reddish dorsally. Legs are ferruginous in colour. Wings are transparent and the hind wings have a golden brown patch and a cloudy white patch at the central portion. Abdomen is bright rusty red in colour.

Female: Head, thorax and abdomen of females are olivaceous in colour. The white patch on hind wings are absent.

Behaviour and habitat: This is a crepuscular species. Commonly found in ponds, paddy fields, streams and rivers.

65. *Tramea limbata* (Desjardins, 1832)

Size- Male: Abdomen: 33-35mm Hind wings: 44-46mm

Female: Abdomen: 32mm Hind wing: 43-46mm

Description: Male: Eyes are dark brown above and olivaceous below. Thorax is dark reddish olivaceous. Legs are black and have reddish brown shades. Transparent wings with reddish venation at the base. Hind wing has blackish brown patch at the base. Pterostigma drak brown coloured. Abdomen reddish with black rings.

Female: Females are similar to males with extensive black markings on abdomen.

Behaviour and habitat: Commonly found in forested and non forested habitats, especially around weedy ponds and marshes. Fly high during day time.

66. *Trithemis aurora* (Burmeister, 1839)

Size- Male: Abdomen: 21-29mm Hind wing: 24-34mm

Female: Abdomen: 19-27mm Hind wing: 24-31mm

Description: Male: Eyes are crimson red coloured. Reddish brown coloured face. Thorax is red in colour with purple pruinescence. Legs are black with rusty

markings. Wings are transparent with red reticulation. Wings base has an amber yellowish patch. Pterostigma is dark reddish brown. Abdomen is violaceous red in colour.

Female: Female differs from male in shape and colour. Thorax and abdomen are ochreous with black markings. Wings are brownish at apices.

Behaviour and habitat: Commonly found in forested and non forested habitats like ponds, streams, rivers, canals, marshes etc.

67. *Trithemis festiva* (Rambur, 1842)

Size- Male: Abdomen: 22-28mm Hind wing: 26-32mm

Female: Abdomen: 21-24mm Hind wing: 24-29mm

Description: Male: Bluish grey eyes are capped with purplish brown. Thorax is black and pruinosed with purple. Black coloured legs. Transparent wings. There is an opaque dark brown patch at the base of hind wing. Pterostigma is black. Abdomen is black in colour with fine yellow markings and with blue pruinescence.

Female: Thorax and abdomen are yellowish with black markings.

Behaviour and habitat: Commonly found in habitats like streams, canals and rivers. Generally, perch on rocks and twigs.

68. *Trithemis pallidinervis* (Kirby, 1889)

Size- Male: Abdomen: 28-32mm Hind wings: 30-36mm

Female: Abdomen: 26-28mm Hind wing: 30-32mm

Description: Male: Bluish grey eyes are with reddish brown cap. Frons is metallic purple on upper surface. Thorax is bright olivaceous and dorsally olivaceous brown with grey hairs. Thorax is marked with black. Legs are black and long. Transparent wings with reddish reticulation. There is an amber coloured patch at the base of wing. Pterostigma is black bordered with white at both ends. Abdomen is olivaceous and marked with black.

Female: Females show close resemblance with males. Upper surface of frons is ochreous.

PLATE 11 - ODONATES OBSERVED DURING THE STUDY (CONT.)

Figure 11A: *Tramea limbata*

Figure 11B: *Trithemis aurora*

Figure 11C: *Trithemis festiva*

Figure 11D: *Urothemis signata*

Behaviour and habitat: Commonly found in lakes, marshes and paddy fields of plains.

69. *Urothemis signata* (Rambur, 1842)

Size- Male: Abdomen: 27-28mm Hind wing: 34-37mm

Female: Abdomen: 25-27mm Hind wing: 34-36mm

Description: Male: Eyes are bright reddish above and reddish brown laterally. Thorax is blood red in colour. Legs are reddish brown or ochreous. Wings are transparent and with reddish reticulation. The base of forewing has an amber coloured small patch. The basal patch on hind wing is broader and darker. Abdomen is blood red and has dorsal black markings at the last segments.

Female: Females are yellowish coloured. All abdominal segments are dorsally marked with black.

Behaviour and habitat: Commonly found in habitats like marshes, lakes, streams, rivers, ponds and paddy fields.

70. *Zygonyx iris* (Selys, 1869)

Size- Male: Abdomen: 40-42mm Hind wings: 46-48mm

Female: Abdomen: 40-43mm Hind wing: 47-49mm

Description: Male: Eyes are dark brown above and greyish beneath. Metallic blue coloured frons. Thorax is dark metallic bluish with citron yellow markings. Legs are black. Transparent wings with blackish brown pterostigma. Abdomen in black in colour with narrow yellow markings. A large yellow spot is present at segment 7 dorsally.

Female: Females show close similarity with males but the yellow markings on abdomen are more prominent.

Behaviour and habitat: This species is common in forested habitats. Perches rarely. Found always flying over streams and brooks.

71. *Zyxomma petiolatum* (Rambur, 1842)

Size- Male: Abdomen: 37-42mm Hind wings: 32-35mm

Female: Abdomen: 37-42mm Hind wings: 32-38mm

Description: Male: Eyes are bright emerald green. Thorax is chocolate brown dorsally and paler on sides. Legs are reddish brown or ochreous. Transparent wings are tipped with brown at apices and with blackish brown pterostigma. Abdomen is chocolate brown with black rings.

Female: Females show exact resemblance with males.

Behaviour and habitat: This species is crepuscular in nature. Common in forested and non forested habitats such as ponds, wells and stagnant pools.

2.4.3 Photographic documentation of observed odonates

Phototgraphs of the observed odonates, showing the identification features were documented. The recorded photographs were displayed in Plates 3-11.

2.4.4 Molecular characterisation of selected families of odonates

Out of the observed 71 species of odonates, 34 species were selected for molecular characterisation. The selected species were coming under 10 families and 28 genera. One or two representatives of each genus were chosen for the study. Species which were characterised in previous studies (Jisha, 2018) were excluded from the present work. DNA was isolated from the specimens, the partial coding sequence of the COI gene and the partial sequence of the 18S rRNA gene were amplified using the primers given in Table 2.3.1 and 2.3.4 and they were sequenced. The sequence results were submitted to the NCBI GenBank database and received accession numbers. The accession numbers and product size are given in Table 2.4.2. The translation products of the obtained COI gene sequences were generated by using Expasy translate tool and presented in Table 2.4.3.

Table 2.4.2 Details of molecular characterization

Sl No.	Scientific Name	COI		18S rRNA	
		Accession Number	Product size	Accession Number	Product size
1.	*Lestes praemorsus*	MZ074000.1	671bp	MZ068299.1	893bp
2.	*Protosticta gravelyi*	MN974377.1	593bp	MZ882296.1	912bp
3.	*Neurobasis chinensis*	MW931875	642bp	MW931850.1	881bp
4.	*Heliocypha bisignata*	MW940786.1	676bp	MW940775.1	900bp
5.	*Libellago indica*	MW309318.1	585bp	MZ098271.1	907bp
6.	*Dysphaea ethela*	MN882704.1	677bp	MZ817954.1	925bp
7.	*Copera vittata*	MZ895506.1	691bp	MZ895795.1	905bp
8.	*Prodasineura verticalis*	MZ081640.1	701bp	MZ081546.1	902bp
9.	*Aciagrion approximans krishna*	MW246065	670bp	MZ098107.1	896bp
10.	*Agriocnemis pieris*	MN850440	627bp	OK083599.1	878bp
11.	*Agriocnemis splendidissima*	MN850441	647bp	MZ803194.1	911bp
12.	*Archibasis oscillans*	MW309421.1	617bp	MZ127377.1	909bp
13.	*Ceriagrion cerinorubellum*	MZ882339.1	690bp	MZ882369.1	906bp
14.	*Ceriagrion rubiae*	OK148120.1	346bp	OK105141.1	905bp
15.	*Ischnura rubilio*	MN850442.1	670bp	MZ809355.1	889bp
16.	*Paracercion calamorum*	MW940750.1	668bp	MZ220521.1	296bp
17.	*Paracercion malayanum*	MZ700177.1	689bp	MZ882306.1	908bp
18.	*Pseudagrion decorum*	MZ254912.1	628bp	MZ220525.1	537bp
19.	*Pseudagrion indicum*	MN882703.1	649bp	MZ817953.1	855bp
20.	*Gynacantha dravida*	MK990607.1	631bp	MZ678639.1	911bp
21.	*Gynacantha millardi*	MW649897.1	615bp	MZ145224.1	920bp
22.	*Ictinogomphus*	MW945399.1	582bp	MW940949.1	896bp

	rapax				
23.	*Diplacodes nebulosa*	MZ254913.1	555bp	MZ081547.1	904bp
24.	*Hydrobasileus croceus*	MW965658.1	671bp	MW945405.1	903bp
25.	*Onychothemis testacea*	MN803150.1	632bp	MZ803139.1	898bp
26.	*Orthetrum glaucum*	MZ087263.1	696bp	MZ081550.1	892bp
27.	*Orthetrum luzonicum*	MZ092847.1	692bp	MZ092846.1	909bp
28.	*Palpopleura sexmaculata*	OK083552.1	581bp	MZ092848.1	907bp
29.	*Rhodothemis rufa*	OK083604.1	640bp	OK083605.1	896bp
30.	*Tetrathemis platyptera*	MZ092924.1	688bp	MZ092849.1	907bp
31.	*Tholymis tillarga*	MZ127380.1	700bp	MZ093144.1	909bp
32.	*Tramea limbata*	MZ076547.1	671bp	MZ076516.1	906bp
33.	*Urothemis signata*	MZ895798.1	688bp	MZ895802.1	893bp
34.	*Zyxomma petiolatum*	MZ093432.1	677bp	MZ093372.1	912bp

Table 2.4.3 Translation products of the obtained COI gene sequences

Sl No.	Scientific Name	Translation Product
1.	*Lestes praemorsus*	>*Lestes praemorsus* isolate LPN22 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/223AA YIGTLYLMFGAWAGMVGTALSMLIRVELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLML GAPDMAFPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSILGAI NFITTTINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAGGGDPILYQHLFW
2.	*Protosticta gravelyi*	>Protosticta gravelyi isolate PGN9 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/197AA STNHKDIGTLYLMFGAWAGMVGTALSMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMI GGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGSIAHAGGS VDLTIFSLHLAGVSSILGAINFITTTINMKSPGMKMDQMPLFVWAVVITAVLLLLSLPVLAGA
3.	*Neurobasis chinensis*	>Neurobasis chinensis isolate NCN16 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/214AA LTLYLLFGAWAGMVGTALSMIIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWL VPLMLGAPDMAFPRLNNMSFWLLPPALTILMASSLVESGAGTGWTVYPPLASGLGHPGGSVDLTIFSL HLAGVSSILGAINFITTTINMKTPGMKMDQMPLLVWAVLITAVLLLLSLPVLAGAITMLLTDRNMNTSF FDPAGGGDPI
4.	*Heliocypha bisignata*	>Heliocypha bisignata isolate HBN18 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/224AA HKDIGTLYLLFGAWAGMAGTALSMLIRVELGQPGTLIGDDQIYNVVVTAHAFIMIFFMVMPIMIGGFGNWLVPL MLGAPDMAFPRMNNMSFWLLPPSLTLLLSSSLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIFSLHLAGVSSILG AINFITTTINMKPPGMKMDQLPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAGGGDPILYQHLF
5.	*Libellago indica*	>Libellago indica isolate LIN12 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/194AA TALSMLIRVELGQPGTLIGDDQIYNVVVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMN NMSFWLLPPSLSLLLSSSLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIFSLHLAGVSSILGAINFITTTI NMKAPGMKMDQMPLFVWAVIITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAG
6.	*Dysphaea ethela*	>Dysphaea ethela isolate DEN8 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/225AA IGTLYLMFGAWAGMVGTALSMLIRIELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWL VPLMLGAPDMAFPRLNNMSFWLLPPSITLLLTSSLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIFSLH LAGVSSILGAINFISTTINMKTPGMKMDQMPLFVWAVVITALLLLLSLPVLAGAITMLLTDRNINTSFFD PAGGGDPILYQHLFWFFG

7.	*Copera vittata*	>Copera vittata isolate CVN33 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/230AA HKDIGTLYLMFGAWAGMVGTALSMLIRIELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFG NWLVPLMLGAPDMAFPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGAIAHSGGSVDLTI FSLHLAGVSSILGAINFITTTINMKSPGMSMDQMPLFVWAVIITAVLLLLSLPVLAGAITMLLTDRNINTS FFDPAGGGDPILYQHLFWFFGHW
8.	*Prodasineura verticalis*	>Prodasineura verticalis isolate PVN24 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/233AA STNHKDIGTLYLMFGAWAGMVGTALSMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMI GGFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGSIAHAGGS VDLTIFSLHLAGVSSILGAINFITTTINMKSPGMKMDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTD RNINTSFFDPAGGGDPILYQHLFWFFGHP
9.	*Aciagrion approximans krishna*	>Aciagrion approximans krishna isolate AAKN11 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/223AA YLMIGAWAGLVGTALSMLIRIELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLM LGAPDMAFPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLASVIAHAGASVDLTIFSLHLAG VSSILGAINFITTTINMKSPGMNMDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAG GGDPILYQHLFWFFGHP
10.	*Agriocnemis pieris*	>Agriocnemis pieris isolate APN3 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/208AA KDIGTLYLMFGAWAGMVGTALSMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFG NWLVPLMLGAPDMAFPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGAIAHGGGFVDLT IFSLHLAGVSSILGAINFITTTINMKSPGMKLEQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINT SFF
11.	*Agriocnemis splendidissima*	> Agriocnemis splendidissima isolate ASN4 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/215AA IGTLYLMFGAWAGMVGTALSMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNW LVPLMLGAPDMAFPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLASAIAHAGASVDLTIFSL HLAGVSSILGAINFITTTINMKSPGMKMEQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFF DPAGGGDPI
12.	*Archibasis oscillans*	>Archibasis oscillans isolate AON13 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/205AA GMVGTALSMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMA

		FPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIFSLHLAGVSSILGAIN FITTTINMKSPGMKMDQMPLFVWAVIITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAGGGDPILY
13.	*Ceriagrion cerinorubellum*	>Ceriagrion cerinorubellum isolate CCN32 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/221AA HKDIGTLYLMFGAWAGMVGTALSMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGF GNWLVPLMLGAPDMAFPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGAIAHAGGSVDL TIFSLHLAGVSSILGAINFITTVINMKSPGMKLDQLPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNIN TSFFDPAGGGDPILYQHLFWFFGHQ
14.	*Ceriagrion rubiae*	>Ceriagrion rubiae isolate CRN36 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/115AA IGSPPPAGSKNDVLMFRSVNNMVMAPANTGNDNNNNTAVITTAHTNSGNWSSFIPGDFMLITVVMKLI APKIEDTPAKCNEKIVSSTDPPACAIAPASGGYTVQPVPAPLSTKLL
15.	*Ischnura rubilio*	>Ischnura rubilio isolate IRN5 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/223AA HKDIGTLYLMFGAWAGMVGTALSMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGF GNWLVPLMLGAPDMAFPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGVIAHAGASVDL TIFSLHLAGVSSILGAINFITTTINMKSPGMNMDQLPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNI NTSFFDPAGGGDPILYQHL
16.	*Paracercion calamorum*	>Paracercion calamorum isolate PCN17 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/222AA YIGTLYLMFGAWAGMVGTALSMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGN WLVPLMLGAPDMAFPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIF SLHLAGVSSILGAINFITTTINMKSPGMKMDQLPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTS FFDPAGGGDPILYQHLF
17.	*Paracercion malayanum*	>Paracercion malayanum isolate PMN15 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/229AA NKDIGTLYLMFGAWAGMVGTALSMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGF GNWLVPLMLGAPDMAFPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGAIAHAGGSVDL TIFSLHLAGVSSILGAINFITTTINMKSPGMKMDQLPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNI NTSFFDPAGGGDPILYQHLFWFFGH
18.	*Pseudagrion decorum*	>Pseudagrion decorum isolate PDN21 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/209AA TLYLMFGAWAGMVGIALSMLIRVELGQPGSLIGGDQIYNVVVTVHAFVMIFFMFMLIMIGGFGNWVV

		PLMLGAPDMAFPRLNNMSFWLLPPSLMLLLASSLVESGAGMGWTVYPPLAGAVAHAGGSVNLTVFS LHLGGVSSILGAINCITTTINMKSPGMKMDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDGNINTS FFDPAGV
19.	*Pseudagrion indicum*	>Pseudagrion indicum isolate PDN21 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/216AA YIGTLYLMFGAWAGMVGTALSMLIRVELGQPGSLIGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFGN WLVPLMLGAPDMAFPRLNNMSFWLLPPSLTLLLASSLVESGAGTGWTVYPPLAGAIAHAGGSVDLTIF SLHLAGVSSILGAINFITTTINMKSPGMKMDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTS FFDPAGGGDPI
20.	*Gynacantha dravida*	>Gynacantha dravida isolate GDN1 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/209AA RSTNHKDIGTLYFLFGAWAGMVGTALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIG GFGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSLTLLLAGSMVESGAGTGWTVYPPLAGAIAHAGAS VDLTIFSLHLAGVSSILGAINFITTTINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTD RNIN
21.	*Gynacantha millardi*	>Gynacantha millardi isolate GBN14 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/205AA AWAGMVGTALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPD MAFPRLNNMSFWLLPPSLTLLLAGSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAGVSSIL GAINFITTTINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAGGGDP
22.	*Ictinogomphus rapax*	>Ictinogomphus rapax isolate IRN19 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/193AA VGTALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRM NNMSFWLLPPSLTLLLASSMVESGAGTGWTVYPPLAGAIAHARGSVDFTIFSIHLAGVSSILGAINFITT TINMKFPGMSMEQMPLFVWAVLITAVLLMLSLPVLAGAITMLLTDRNLNTSFFD
23.	*Diplacodes nebulosa*	>Diplacodes nebulosa isolate IRN19 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/184AA LYLIFGAWAGMIGTALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPLM LGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLAG VSSILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPV
24.	*Hydrobasileus croceus*	>Hydrobasileus croceus isolate IRN19 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/223AA KDIGTLYLIFGAWAGMVGTALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNW LVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFS LHLAGVSSILGAINFITTVINMKSPGMTLDQLPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFF

		DPAGGGDPILYQHLF
25.	*Onychothemis testacea*	>Onychothemis testacea isolate OTN2 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/210AA QQNHKDIGTLYLIFGAWAGMIGTALSVLIRVELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGG FGNWLVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLSSSLVESGAGTGWTVYPPLAGAIAHAGASVD LTIFSLHLAGVSSILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRN INTS
26.	*Orthetrum glaucum*	>Orthetrum glaucum isolate OGN26 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/232AA HKDIGTLYLIFGAWAGMIGTALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNW LVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFS LHLAGVSSILGAINFITTVINMKSPGMKLDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSF FDPAGGGDPILYQHLFWFFGHPEV
27.	*Orthetrum luzonicum*	>Orthetrum luzonicum isolate OLN27 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/230AA HKDIGTLYLIFGAWAGMIGTALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNW LVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLTSSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFS LHLAGVSSILGAINFITTVINMKSPGMKMDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTS FFDPAGGGDPILYQHLFWFFGHP
28.	*Palpopleura sexmaculata*	>Palpopleura sexmaculata isolate PSN28 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/193AA GQQNHKDIGTLYLIFGAWAGMIGTALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGG FGNWLLPLMLGPPDMAFPRVNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGGIAHAGASVD LTIFSLHLASVSSILGAINFITTVITMKSPGMKLDQLPLFVWAVLITAVLLLLSLP
29.	*Rhodothemis rufa*	>Rhodothemis rufa isolate RRN34 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/213AA WTLYLVFGAWAGMVGTALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLV PLMLGAPDMAFPRLNNMSFWLLPPSFTLLLSSSLVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHL AGVSSILGAINFITTVINMKSPGMKLDQLPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDP AGGGDP
30.	*Tetrathemis platyptera*	>Tetrathemis platyptera isolate TPN29 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/229AA YIGTLYLIFGAWAGMVGTALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWL VPLMLGAPDMAFPRMNNMSFWLLPPSFTLLLASSIVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSL

		HLAGVSSILGAINFITTVINMKSPGMKMDQLPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFF DPAGGGDPILYQHLFWFFGHPG
31.	*Tholymis tillarga*	>Tholymis tillarga isolate TTN30 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/233AA NHKDIGTLYFIFGAWAGMIGTALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGN WLVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGGIAHAGASVDLTI FSLHLAGVSSILGAINFITTVINMKSPGMKMDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNIN TSFFDPAGGGDPILYQHLFWFFGHPEV
32.	*Tramea limbate*	>Tramea limbata isolate TLN23 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/223AA LTLYLIFGAWAGMIGTALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGNWLVPL MLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTIFSLHLA GVSSILGAINFITTVINMKSPGMSIDQLPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTSFFDPAG GGDPILYQHLFWFF
33.	*Urothemis signata*	>Urothemis signata isolate USN35 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/229AA HKDIGTLYLIFGAWAGMVGTALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPIMIGGFGN WLVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTI FSLHLAGVSSILGAINFITTVINMKSPGMSLDQLPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNINTS FFDPAGGGDPILYQHLFWFFGH
34.	*Zyxomma petiolatum*	>Zyxomma petiolatum isolate ZPN31 cytochrome c oxidase subunit I (COX1), partial(mitochondrial)/225AA KDIGTLYLIFGAWAGMVGTALSVLIRIELGQPGSLIGDDQIYNVIVTAHAFVMIFFMVMPVMIGGFGN WLVPLMLGAPDMAFPRLNNMSFWLLPPSFTLLLASSMVESGAGTGWTVYPPLAGAIAHAGASVDLTI FSLHLAGVSSILGAINFITTVINMKSPGMKMDQMPLFVWAVVITAVLLLLSLPVLAGAITMLLTDRNLN TSFFDPAGGGDPILYQHLFWL

2.4.5 NOVEL RECORDS OF THE PRESENT WORK

Of the 34 species characterised, COI gene sequence records of 12 species and 18S rRNA gene sequence records of 23 species were the novel GenBank records. While considering the remaining records, COI gene sequences of 8 species and 18S gene sequences of 6 species are the first records from India. The pioneer records from Kerala are twelve COI and five 18S rRNA gene sequences. Sequences of *Onychothemis testacea* are the first GenBank records of the corresponding genus of both genes. The details of the novel records are given in Table 2.4.4.

Table 2.4.4: Novel GenBank records of the present work

Sl No.	Name of species	GenBank	India	Kerala
1.	*Lestes praemorsus*	18S rRNA	COI	
2.	*Protosticta gravelyi*	COI & 18S rRNA		
3.	*Neurobasis chinensis*			COI & 18S rRNA
4.	*Heliocypha bisignata*	18S rRNA		
5.	*Libellago indica*	COI & 18S rRNA		
6.	*Dysphaea ethela*	18S rRNA		COI
7.	*Copera vittata*		18S rRNA	COI
8.	*Prodasineura verticalis*	18S rRNA		COI
9.	*Aciagrion approximans krishna*	COI & 18S rRNA		
10.	*Agriocnemis pieris*	COI & 18S rRNA		
11.	*Agriocnemis splendidissima*	COI & 18S rRNA		
12.	*Archibasis oscillans*	18S rRNA		
13.	*Ceriagrion cerinorubellum*			COI & 18S rRNA
14.	*Ceriagrion rubiae*	COI & 18S rRNA		
15.	*Ischnura rubilio*		18S rRNA	
16.	*Paracercion calamorum*		COI	18S rRNA
17.	*Paracercion malayanum*		COI & 18S rRNA	
18.	*Pseudagrion decorum*	COI		18S rRNA
19.	*Pseudagrion indicum*	COI & 18S rRNA		
20.	*Gynacantha dravida*	COI & 18S rRNA		
21.	*Gynacantha millardi*	COI & 18S rRNA		
22.	*Ictinogomphus rapax*	18S rRNA	COI	
23.	*Diplacodes nebulosa*	18S rRNA		COI
24.	*Hydrobasileus croceus*		COI & 18S rRNA	
25.	*Onychothemis testacea*	COI & 18S rRNA		
26.	*Orthetrum glaucum*		18S rRNA	COI
27.	*Orthetrum luzonicum*		COI	18S rRNA
28.	*Palpopleura sexmaculata*	18S rRNA		COI
29.	*Rhodothemis rufa*	18S rRNA	COI	
30.	*Tetrathemis platyptera*	18S rRNA		COI
31.	*Tholymis tillarga*	18S rRNA		COI
32.	*Tramea limbata*	18S rRNA		COI
33.	*Urothemis signata*	18S rRNA	COI	
34.	*Zyxomma petiolatum*		18S rRNA	COI

2.5 DISCUSSION

The odonate fauna of Kerala is well studied. The number of people interested in odonate studies is increasing day by day and the contributions of odonatologists along with other scientific works nourish the odonate literature of Kerala. Although most of the works are sporadic, the works of Kiran and Raju (2013), Nair et al. (2021) and Gopalan et al. (2022) have provided a summarized picture on odonates of Kerala. According to Gopalan et al. (2022), there are 174 species in Kerala (74 damselflies and 100 dragonflies), in comparison to 71 species (33 species of damselflies and 38 species of dragonflies), recorded in the present study which was confined to 5 districts of Kerala and excluded the protected areas. Diversity is highest at pristine forested habitats especially in montane forests. Most of the endemics are restricted to the streams and rivers of untouched forests (Subramanian and Babu, 2007; Subramanian et al., 2008; Sureshan et al., 2022). As the present study was concentrated mainly on human inhabited habitats the species list was dominated by generalist species. The Western Ghats endemic species recorded during the study are *Aciagrion approximans krishna, Agriocnemis keralensis, Pseudagrion indicum* and *Protosticta gravelyi.* The rarely encountered species *Paracercion malayanum* is the first record from central and northern Kerala. Nair et al. (2021) has reported this species as personal record of Bo Nielson from Agasthyamalais landscape only.

During the present survey species richness of dragonflies was more than that of damselflies. A total of 38 species of dragonflies were recorded, which constitute 54% of the total species recorded during the study. Number of damselfly species found was 33 which comprises 46% of the total species found. Findings of other workers (Adarsh et al., 2014; Kulkarni and Subramanian, 2013; Tiple et al., 2013) match with this result that, dragonflies are more diverse and dominant over damselflies. Dragonflies are highly specialized for wide range habitat tolerance and possess high dispersal ability (Williams,1997; Lawler, 2001; Suhling et al.,2004; 2005; Saha, 2015). This can be the reason for increased species richness and abundance of dragonflies. In contrast to this, fragile body, weak flight capacity, limited dispersal ability and requirement of shade cover may be the reason for decreased abundance of damselflies (Weir, 1974; Williams, 1997; Clark and Samways, 1996).

The locations for the survey were randomly selected to include various habitats ranging from sea level to hilly areas. The habitats selected for the present study included ponds, rivers, lakes, canals, paddy fields, coastal marshes and streams. A total of 71 species belonging to 44 genera and 10 families were recorded during the survey. 33 species of damselflies of 17 genera which are the representatives of 7 families of Zygoptera viz. family Lestidae, Platystictidae, Calopterygidae, Chlorocyphidae, Euphaeidae, Platycnemididae, Coenagrionidae were recorded. The most dominant family and genus of damselflies observed were Coenagrionidae and *Pseudagrion* respectively. The rarely found species were *Protosticta gravelyi, Paracercion malayanum* and *Ceriagrion rubiae* which were found only once in single location. 38 species of dragonflies belonging to 27 genera and 3 families (Aeshnidae, Gomphidae and Libellulidae) were encountered during the survey. Members of the genus *Orthetrum* were dominant during the study. Representatives of family Libellulidae were abundantly seen. The dominance of families Coenagrionidae and Libellulidae was supported by a good number of literature (Arunima and Nameer, 2021; Bose et al., 2021; Chandran et al., 2021; Subramanian et al., 2008; Kulkarni and Subramanian, 2013).

The observed species richness was high in vegetated ponds, lakes and streams and was minimum in unvegetated habitats. Native vegetation supported maximum odonate diversity (Bose et al., 2021; Lozano et al., 2022). Habitats with rich shade cover showed maximum damselfly diversity and this finding was in agreement with Arulprakash & Gunathilagaraj (2010). While dragonflies were abundantly found in sunny biotopes which were in congruence with the result of Remsburg (2008), Corbet (1999), Samways and Steytler (1996).

Out of the observed 71 species, 34 species were selected for molecular characterisation. Previously studied species were excluded from the present study (Krishnan, 2018). Partial mitochondrial COI gene sequence and nuclear 18S rRNA gene sequence of 34 species of odonates were sequenced and submitted to the GenBank database. Out of the 34 COI gene sequences, 12 are the first GenBank records of the corresponding species, 8 are the pioneer records from India and 12 are the first records from Kerala. While considering the 34 sequences of 18S rRNA gene, 23 are pioneer records of GenBank, 6 are first records from India and 5 are

first from Kerala. The partial COI and 18S rRNA gene sequences of *Onychothemis testacea* are the first GenBank records of the corresponding genus.

Most of the COI sequences have good product sizes of more than 600bp length and 18S rRNA sequences have more than 850bp length. All the generated amino acid sequences did not have internal stop codons and the quality of the sequences was confirmed by an uninterrupted open reading frame. Mitochondrial COI sequences can be used for precise and faster identification of odonate species and phylogenetic studies. Nuclear 18S rRNA gene sequences will be useful in phylogenetic analysis at different taxonomic levels. (Carle et al., 2008; Dumont et al., 2010; Huang et al., 2020).

CHAPTER 3
TAXONOMIC KEY

3. TAXONOMIC KEY

INTRODUCTION

Taxonomic keys are used for the taxonomic identification of organisms, in biological sciences. A key is formed as a series of couplets, each couplet having two opposing features of an organism. Users can select the feature that best fits the unidentified organism, and this leads to another couplet or to a specific taxon. All of the features that were not selected are instantly rejected. Since the key is formed of pairs of contrasting characteristics, it is denoted as a dichotomous key.

Sometimes, identification can be easily done using pictures in field guides. However, the reliability of identification is corroborated only with the help of a taxonomic key.

A good number of taxonomic keys are available in the literature for the identification of odonates. Most of them were constructed using complicated morphological features like wing venation. Only a person who has a thorough knowledge of odonate morphology including wing venation features can use these keys for identification.

During the present study, taxonomic keys were prepared for the 71 species recorded from both suborders. Keys were prepared using simple morphological features, which are easily perceivable for an interested layman. However, since Libellulidae is one of the most diverse families, simple venation characteristics had to be used for the preparation of the key to genera of this family and it is the modified version of the existing key (Fraser, 1936).

Key to suborders of Odonata

1. Fore and hind wings are similar in shape and width and are petiolated. Abdomen is very slender. During rest wings are kept closed over the body parallel to it. ……..…. Suborder **Zygoptera** (Damselflies)

   Fore and hind wings vary in shape and venations. Wings are not petiolated. Robust bodied. During rest wings are kept open and perpendicular to the body. ……..… Suborder **Anisoptera** (Dragonflies)

Key to the families of suborder Zygoptera

1. Wings are kept nearly wide open at rest ….…… Family **Lestidae**

   Wings are closed during rest ……..…… **(2)**

2. Abdomen shorter than hind wing

   Bulbous eyes and protruding face……. Family **Chlorocyphidae**

Abdomen twice or more than twice the length of hind wing

Wings with slightly pointed tips …….. Family **Platystictidae**

Abdomen longer than hind wing but never have twice the length of hind wing ……..…….. **(3)**

3. Iridescently coloured body or wings ……...… **(4)**

   Non iridescently coloured body and wings ……. **(5)**

4. Body iridescent green in colour

   Wings are iridescently green or blue or transparent with amber tint; often tipped with black …… Family **Calopterygidae**

   Iridescently coloured body with red, black or blackish brown colours

   Wings are transparent, brownish tinted or with black tips ……... Family **Euphaeidae**

5. Variously coloured non metallic damselflies. Short discoidal cell, the anterior side much shorter than the basal, the distal end very acute …… Family **Coenagrionidae**

   Body black coloured, with blue, red, yellow markings. Discoidal cell elongated, the costal or anterior side slightly shorter than the basal, the distal end subacute …… Family **Platycnemididae**

Key to the species of genus *Lestes*

1. Thorax with metallic green antehumeral stripes having scalloped outer borders. Pale blue coloured abdomen is marked dorsally with black. Segment 9 marked with a lateral blue spot and segment 10 with a small ventro lateral spot ……..…… ***Lestes praemorsus***

   Thorax with metallic green 'J' shaped antehumeral stripes with expanding outer borders. Abdomen is pale brown laterally with broad dorsal metallic green stripe. Segment 9 is dirty white at the apical half and segment 10 is entirely dirty white …….. ***Lestes elatus***

Key to the genera of Family Calopterygidae

1. Forewings are transparent. Hind wings in male are metallic coloured and opaque. In males, pterostigma is absent. In females false pterostigma is present or absent…….. Genus ***Neurobasis***

   Wings are transparent with or without blackish brown patch at wing tips. Pterostigma is absent ……. Genus ***Vestalis***

Key to the species of Genus *Vestalis*

1. Transparent wings with broad blackish brown patch on tips…… ***Vestalis apicalis***

   Transparent wings without any markings …… ***Vestalis gracilis***

Key to the genera of family Chlorocyphidae

1. Wings are transparent at the basal half. Apical half is opaque, coloured and with bright coppery and violaceous reflex ……..……. Genus ***Heliocypha***

   Transparent wings with amber tinted bases. Apices of forewings have black patch…… Genus ***Libellago***

Key to the genera of family Platycnemididae

1. Anal appendages are homogenous and black in colour; Cylindrical abdomen is broader at the anal and basal ends……. **(2)**

   Superior anal appendages are shorter than inferiors, white or bluish white in colour; Abdomen cylindrical and of about even thickness throughout ……..…… Genus ***Copera***

2. Pterostigma nearly half as long again as broad; body metallic black or bronzed purple marked with citron yellow …….…….. Genus ***Onychargia***

   Pterostigma small 1 ½ times as long as broad, diamond shaped; Black coloured body with red, yellow or blue markings…….. Genus ***Prodasineura***

Key to the species of genus *Copera*

1. Inferior anal appendages are 4 times elongated than superiors …… ***Copera marginipes***

   Inferior anal appendages have double the length of superiors ……..…….. ***Copera vittata***

Key to the genera of the family Coenagrionidae

1. Post ocular triangular coloured areas or spots present …… **(2)**

   Post ocular triangular coloured areas or spots absent. Body yellow, orange or olivaceous coloured, rarely marked with black …… Genus ***Ceriagrion***

2. Smallest damselflies with abdomen not more than 18mm in length …… Genus ***Agriocnemis***

   Damselflies with abdomen more than 20 mm in length …….. **(3)**

3. Pterostigma dissimilar in fore and hind wings…… **(4)**

   Pterostigma similar in fore and hind wings ……. **(5)**

4. Blue or violaceous coloured damselflies marked with black ……. Genus ***Aciagrion***

   Blue, green and yellow coloured damselflies marked more or less with black …… Genus ***Ischnura***

5. Pterostigma diamond shaped …… **(6)**

   Pterostigma subquadrate shaped ……. Genus ***Archibasis***

6. Black marked with blue or blue marked with black coloured damselflies with abdomen having the legth of around 22 mm …… Genus ***Paracercion***

   Bright blue coloured damselflies marked with black or orange, green etc. with abdomen having the length of around 30 mm …….. Genus ***Pseudagrion***

Key to the species of genus *Aciagrion*

1. Blue and black coloured damselfly; segment 8 with a narrow dorsal triangle of black …….. ***Aciagrion occidentale***

   Violaceous blue coloured damselfly marked with black; segment 8 with a short narrow black stripe on each side …… ***Aciagrion approximans krishna***

Key to the species of genus *Agriocnemis*

1. Damselfly having a cobra hood marking on the dorsal side of segment 2 …….. ***Agriocnemis keralensis***

   Damselfly without a cobra hood marking on the dorsal side of segment 2 …….. **(2)**

2. Labrum metallic blue …… ***Agriocnemis pygmaea***

   Labrum nonmetallic ……..…….. **(3)**

3. Superior anal appendages are black, narrow, elongated and slightly curved downward; bluish coloured body with extensive black markings ……. ***Agriocnemis splendidissima***

   Superior anal appendages are pale blue or pinkish, broadly triangular and obtusely pointed; body bluish white marked with black ……..…….. ***Agriocnemis pieris***

Key to the species of genus *Ceriagrion*

1. Abdomen single coloured ……. **(2)**

   Abdomen multicoloured; Abdomen bright red at base and anal ends , black on dorsum in between …… ***Ceriagrion cerinorubellum***

2. Abdomen bright citron yellow, without markings …….. ***Ceriagrion coromandelianum***

   Abdomen bright reddish orange, without markings ……...…….. ***Ceriagrion rubiae***

Key to the species of genus *Ischnura*

1. Abdominal segments 3-6 citron yellow; abdomen having the length of less than 20mm…… ***Ischnura rubilio***

   Abdominal segments 3-6 black on dorsum; abdomen having the length of more than 20 mm ……..…… ***Ischnura senegalensis***

Key to the species of genus *Paracercion*

1. Yellowish green eyes are brownish above. Thorax is dorsally black without any stripes and covered with greyish pruinescence …… ***Paracercion calamorum***

2. Bluish eyes. Thorax dorsally black with broad blue stripes and without any pruinescence …… ***Paracercion malayanum***

Key to the species of genus *Pseudagrion*

1. Medium sized blue damselfly with black markings on body. Orange shade is present at the front portion of head…….. ***Pseudagrion rubriceps***
   Medium sized blue damselfly with black markings on body, without orange shade at the front portion of head……. **(2)**
2. Thorax with a broad black median line…… **(3)**
   Bluish green thorax with three fine black median lines ……. ***Pseudagrion decorum***
3. Black median line of thorax is accompanied with lateral broad blue stripes……. **(4)**
   Black median line of thorax with broad greenish yellow stripes on adjacent sides…… ***Pseudagrion indicum***
4. Black band on 8th abdominal segment is broad……..*****Pseudagrion microcephalum***
   Black band on 8th abdominal segment is narrow (thread like) …….. **(5)**
5. Superior anal appendages are about half the length of segment 10 ……. ***Pseudagrion australasiae***
   Superior anal appendages are slightly shorter than segment 10 …….. ***Pseudagrion malabaricum***

Key to the families of suborder Anisoptera

1. Eyes widely separated. Abdomen bulbous at the end ……..……..…… Family **Gomphidae**
   Eyes broadly touch each other on face. Abdomen not bulbous at the end ……..…….. **(2)**
2. Dragonflies with large size and non iridescent body color; abdomen tumid at the base …….. Family **Aeshnidae**
   Dragonflies with bright body colour; found in small, medium and large body sizes; Abdomen with a variety of shapes ……. Family **Libellulidae**

Key to the genera of family Aeshnidae

1. Dragonflies having homogenous body colouration of dull brown or green. Long and narrow anal appendages, long hairs are present at the inner side of apical half. Inferiors are triangular shaped ……. Genus ***Gynacantha***

   Dragonflies having variably coloured body with yellow, blue and black. Superior anal appendages are lanceolate with bluntly rounded apices having a small spine. Inferiors are quadrate shaped ……. Genus ***Anax***

Key to the species of genus *Anax*

1. Thorax is unmarked and pale greenish. Abdominal segments 1 and 2 are pale greenish, segment 2 is turquoise blue on dorsum and segment 3 has a pair of triangle-shaped turquoise blue markings dorsally ……. ***Anax guttatus***

   Bluish green thorax with broad black lateral stripes. Abdomen with a ground colour of pale reddish brown and black markings …….. ***Anax immaculifrons***

Key to the species of genus *Gynacantha*

1. Thorax and abdomen is brown in colour with dark brown markings. Segment 3 is constricted…… ***Gynacantha dravida***

Thorax and segments 1-3 of abdomen are grass green in colour. Segment 3 is not constricted …….. ***Gynacantha millardi***

Key to the genera of family Libellulidae

1. Discoidal cell base of hind wing is broadly distal to level of arc; forewing discoidal cell is markedly angulated at the coastal side, anal loop is absent or present in small size having up to 6 cells; discoidal field is started by 1 row of cells …….. Genus ***Tetrathemis***

   Hind wing discoidal cell base at level of arc; forewing discoidal cell is not angulated at the costal side; elongated anal loop with more than 6 cells; 2 or more rows of cells present at the beginning of discoidal field ……. **(2)**

2. Hooks are absent in claws. Metallic coloured thorax ……..……..……. Genus ***Onychothemis***

   Length of claw hooks same as that of claws, bifid in appearance. Metallic coloured thorax ……..…. Genus ***Zygonyx***

   Claw hooks are shorter in length that of claws and originating from about middle of latter. Thorax is metallic rarely…… **(3)**

3. Anal loop borders are meeting at the posterior of wing (open) …..... **(4)**

   Anal loop borders converging and meeting before posterior wing border, anal loop with closed apex ..... **(5)**

4. Abdomen with wider base, and gradually narrowing to the end; hind wing has white patch at the centre..... Genus ***Tholymis***

   Abdomen is dialated at the base, then abruptly tapered and narrow and cylindrical to the end; wing tips are dark brownish and white patch is absent in wings ..... Genus ***Zyxomma***

5. Forewings with complete distal antenodal nervures..... **(6)**

   Forewings with incomplete distal antenodal nervures..... **(7)**

6. Prothorax with large lobe and long hairs..... **(8)**

   Prothorax with small lobe, inconspicuous and without hairs …… **(9)**

7. Prothorax with large lobe and long hairs …….. **(11)**

   Prothorax with small lobe, inconspicuous and without hairs ……..…….. **(12)**

8. Frons metallic coloured above…….. Genus ***Brachydiplax***

   Frons non metallic above …… **(10)**

9. Subtrigone is single celled in forewing …… Genus ***Aethriamanta***

   Subtrigone is 3-celled in fore wing …….. Genus ***Urothemis***

10. Forewing with 6 antenodal nervure; abdomen is dilated at segments 1 to 6, narrow and cylindrical at segments 7 to 10 ……. Genus ***Acisoma***

    Forewing with 12 or more antenodal nervures; variable shaped abdomen; but never resembling the last ……..…….. Genus ***Orthetrum***

11. Cuii in hindwing is widely separated from posterior angle of discoidal cell; eyes meeting at a single point; row of 3 cells at the beginning of discoidal field and then continued as rows of 2 cells …….. Genus ***Rhodothemis***

    Cuii in hind wing, arising from posterior angle of discoidal cell; eyes are broadly or narrowly contiguous; discoidal field variable ……..…….. **(13)**

12. Sectors of arc in fore wing separated and diverging at origin; Dark metallic coloured body; Wings are broadly opaque with bluish black or black and golden amber ……..…. Genus ***Rhyothemis***

    Sectors of arc in fore wing arising from a common and rather long stalk …… **(14)**

13. Eyes contiguous narrowly; discoidal cell in hind wing entire; forewing with straight coastal border; frons non metallic above; discoidal field in fore wing beginning with a row of 2 cells ……. Genus ***Diplacodes***

    Eyes are broadly contiguous; hind wing discoidal cell is traversed; costal border of fore-wing sinuous near base; frons metallic above; discoidal field beginning with at least 3 rows of cells ……...……. Genus ***Palpopleura***

14. Discoidal field with borders converging strongly at wing margin …… **(15)**

    Discoidal field with borders parallel or widely divergent at wing margin …… **(16)**

15. Forewing discoidal cell is narrow, its coastal side only about one fourth to one third the length of basal; in between Rii and Riii, a prominent

supplementary nervure (IRii) is present…….
Genus ***Pantala***

Wider discoidal cell is present in fore wing, its costal side about one half the length of basal; in between Rii and Riii, no supplementary nervure(IRii) is present ……..…….. Genus ***Trithemis***

16. Elongated genital hamules; projecting and prominent in profile; broad based hind wing is tapered at apex; straight rows of closely packed narrow cells are present at base of hind wing; short pterostigma with unequal size in fore and hind wings ……..…… **(17)**

    Small genital hammules are inconspicuous in profile; hind wing not markedly broad at base and apex not markedly tapered; no closely packed straight rows of cells are present at base of hind wing; pterostigma variable but equal sized in fore and hind wing……. **(18)**

17. Riii markedly wavy; equal sized pterostigma; distal and apical angles of anal loop equal …….……. Genus ***Hydrobasileus***

    Riii evenly curved, not wavy; smaller pterostigma in hind wing than in forewing; apical angle of anal loop much more acute than distal …….…….. Genus ***Tramea***

18. Bicolorous pterostigma, black at centre and white on ends; 2 rows of cells are present between IRiii and Rspl ……. Genus ***Bradinopyga***

    Unicolourous pterostigma; 1 or rarely 2 rows of cells are present between IRiii and Rspl …… **(19)**

19. Wings are coloured and opaque at the basal half or more boadly…….……..…. Genus ***Neurothemis***

    Transparent wings usually uncoloured or having a yellow patch at the hind wing base…… **(20)**

20. Body red or ochreous in colour with ochreous or orange patch at the base or centre …….…… **(21)**

Body colour is variable and darker, never with a reddish or ochreous tint ……..…. **(22)**

21. Wings with ochreous patch at the base; eyes are narrowly contiguous; face and frons red……..…….. Genus ***Crocothemis***

    Wings with orange patch at the centre; face and abdomen never red; eyes are widely contiguous …….……. Genus ***Brachythemis***

22. Arc is present between the second and third antenodal nervures; 1 row of cells between IRiii and Rspl …….…….. Genus ***Lathrecista***

    Arc is present between the first and second antenodal nervures; 2 rows of cells between IRiii and Rspl …… Genus ***Potamarcha***

Key to the species of genus *Brachydiplax*

1. Wings are transparent tinted with brown at the base ……..…… ***Brachydiplax chalybea***

   Wings are transparent without any colouration ……..…… ***Brachydiplax sobrina***

Key to the species of genus *Diplacodes*

1. Wings with blackish brown tips ……..… ***Diplacodes nebulosa***

   Transparent wings …… ***Diplacodes trivialis***

Key to the species of genus *Neurothemis*

1. Eyes, thorax and abdomen reddish brown; Wings are reddish brown except at the apical end …….. ***Neurothemis fulvia***

2. Thorax and abdomen black coloured with yellow mid dorsal carina; Basal half of wings are black with milky white border …..…… ***Neurothemis tullia***

Key to the species of genus *Orthetrum*

1. Abdomen reddish coloured ……..…… **(2)**
   Abdomen not reddish in colour …….…….. **(3)**
2. Thorax is dark brown with purple pruinescence; Abdomen vermilion red in colour …..…….. ***Orthetrum pruinosum***
   Thorax is reddish brown. Abdomen blood red coloured ……..…… ***Orthetrum chrysis***
3. Abdomen is slim, with enormously swollen base and laterally compressed end; body greenish yellow with black markings ……..…….. ***Orthetrum sabina***
   Abdomen with dorso-ventrally dilated base; pruinosed pale blue in colour …….. **(4)**
4. Transparent wings are tinted with dark amber yellow at the base; last two segments of abdomen are black …….…. ***Orthetrum glaucum***
   Transparent wings; Abdomen with pale blue pruinescence upto the last segment …….. ***Orthetrum luzonicum***

Key to the species of genus *Trithemis*

1. Elongated legs; bicolourous pterostigma; yellow coloured body with black markings ……. ***Trithemis pallidinervis***
   Legs with ordinary length; unicolourous pterostigma; variable body colour …… **(2)**
2. Body black coloured with purple pruinescence; a small dark brown spot at the extreme base of hind wing; venation is black ……..……. ***Trithemis festiva***
   Body violaceous red in colour; a reddish brown spot at the base of hind wing; venation is red ……. ***Trithemis aurora***

PLATE 12 – WING VENATION OF DRAGONFLY

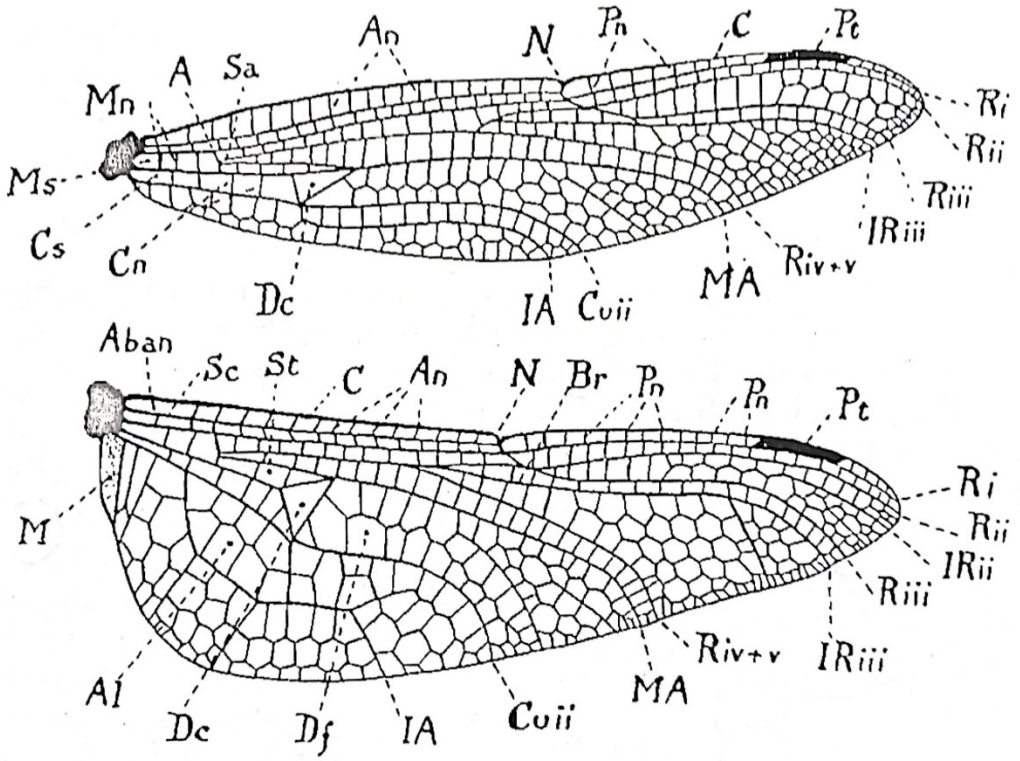

Figure 13 A: Fore and Hind wings of a dragonfly © Fraser (1933). C- Costa; Sc- Subcosta; Ri- Radius; Rii, IRii, Riii, Riv+v -Branches and intercalated branches of radius; MA- Anterior median; Cui- First cubitus; Cuii- Second cubitus; IA- First anal; Cuii+IA- Cubito-anal; A' -Second anal

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

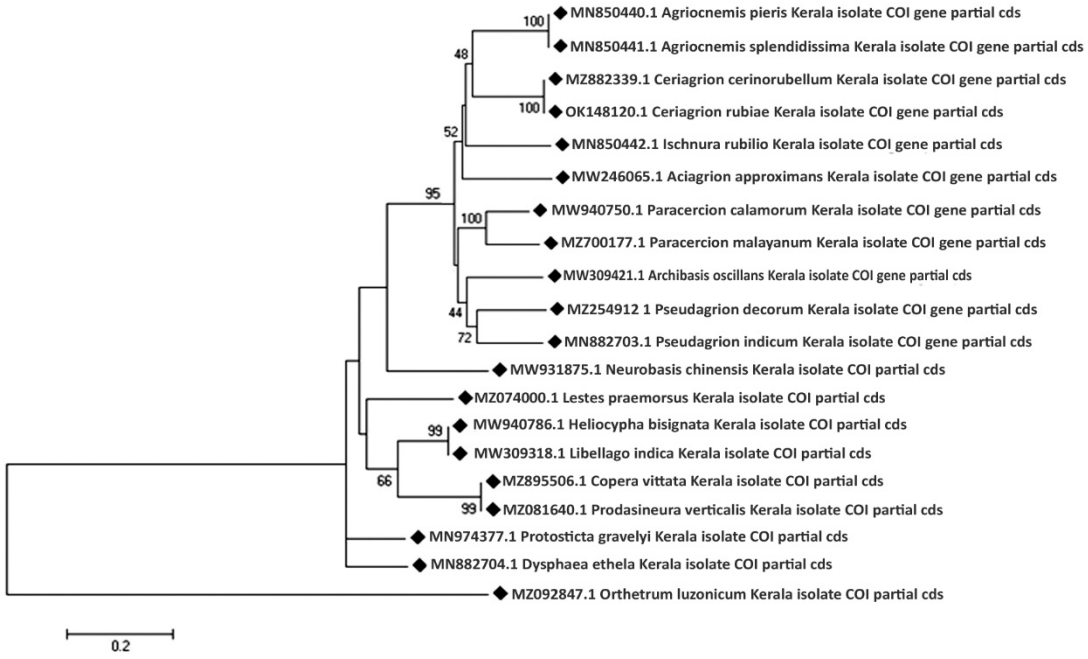

Figure 4.4.1: Inferred phylogenetic tree based on COI gene sequences of suborder Zygoptera

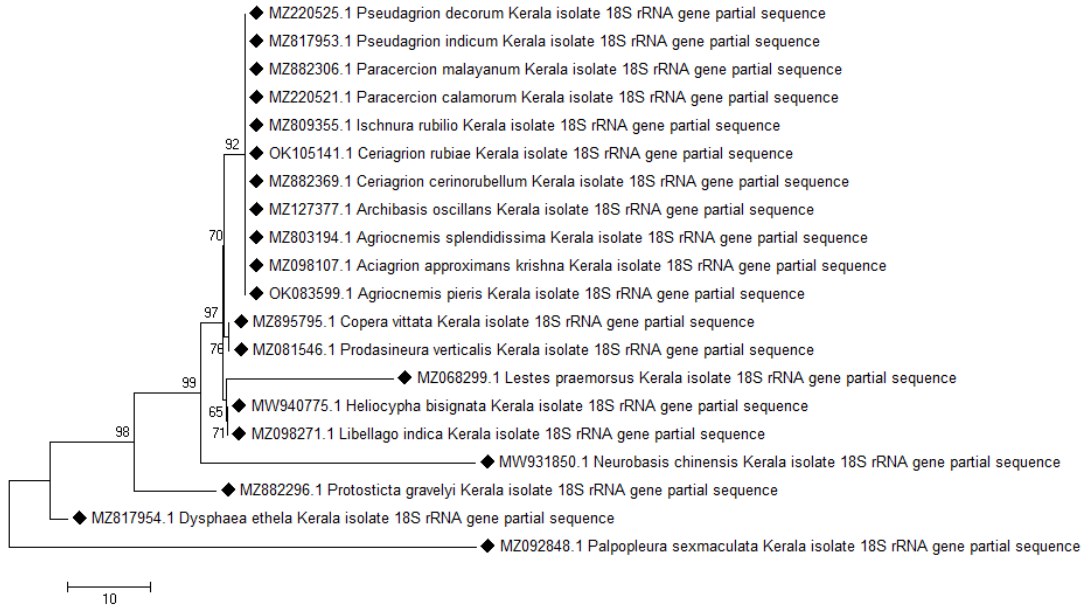

Figure 4.4.2: Inferred phylogenetic tree based on 18S rRNA gene sequences of suborder Zygoptera

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

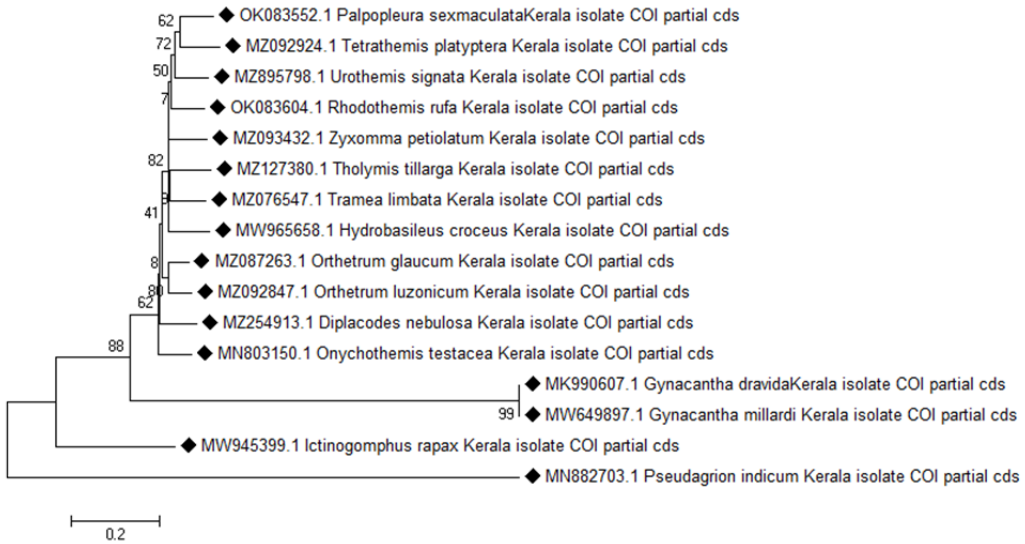

Figure 4.4.3: Inferred phylogenetic tree based on COI gene sequences of suborder Anisoptera

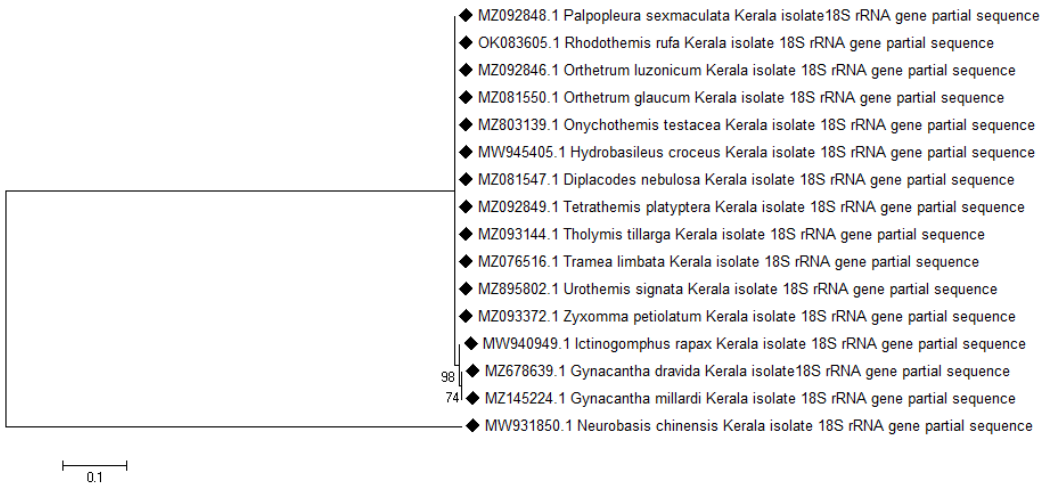

Figure 4.4.4: Inferred phylogenetic tree based on 18S rRNA gene sequences of suborder Anisoptera

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

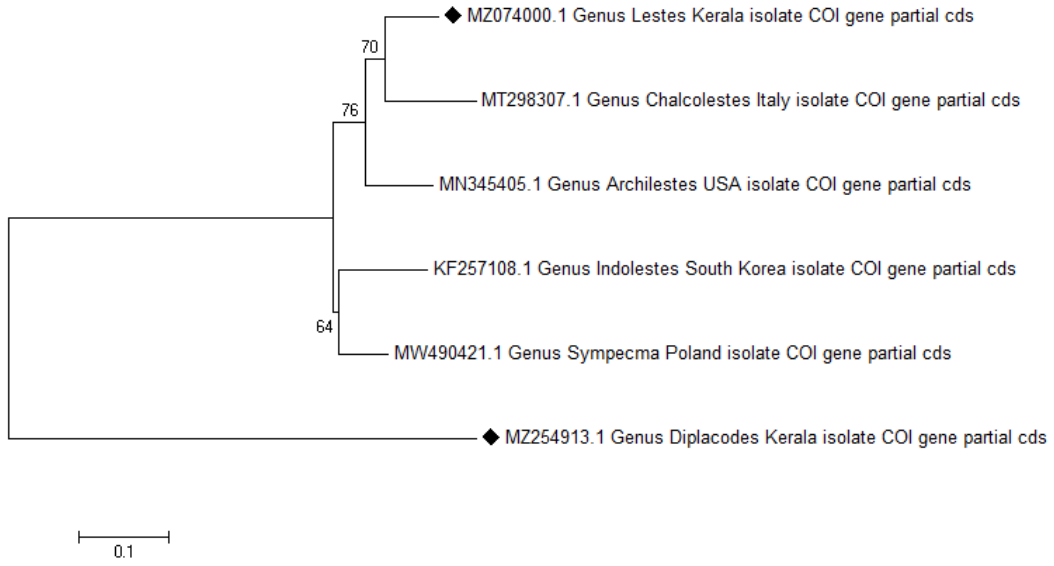

Figure 4.4.5: Inferred phylogenetic tree based on COI gene sequences of family Lestidae, rooted by outgroup

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).

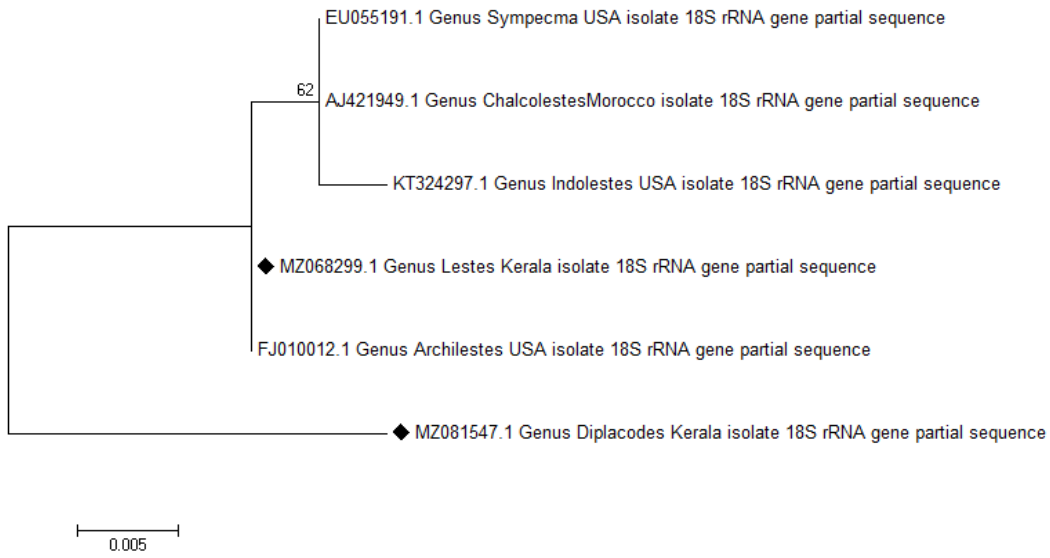

Figure 4.4.6: Inferred phylogenetic tree based on 18S rRNA gene sequences of family Lestidae, rooted by outgroup

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

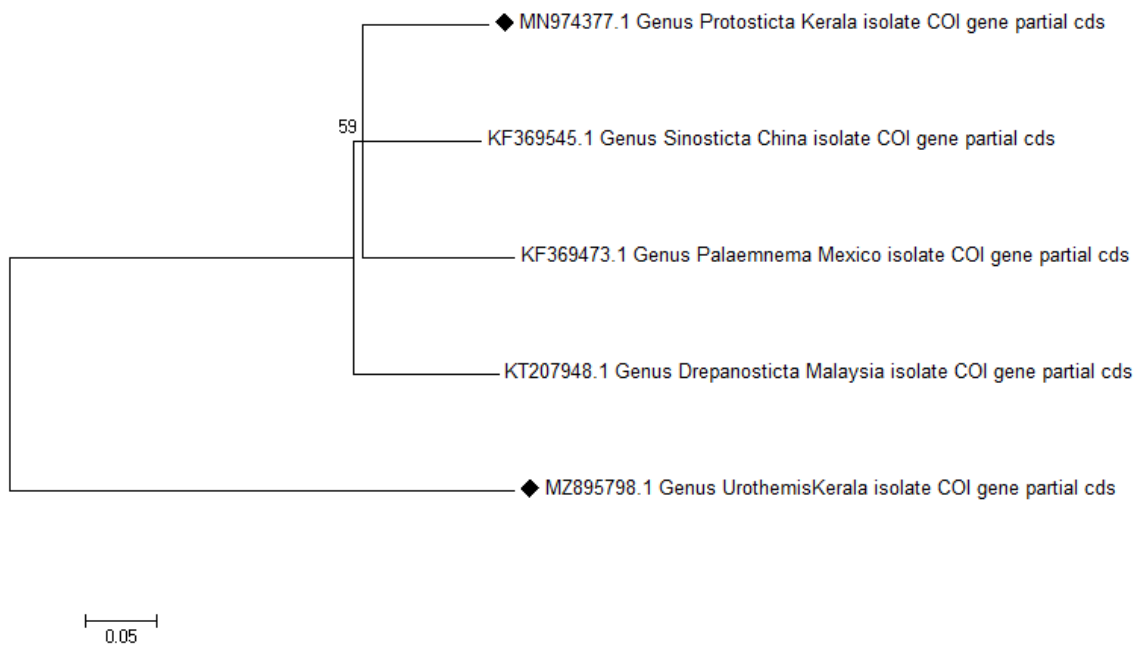

Figure 4.4.7: Inferred phylogenetic tree based on COI gene sequences of family Platystictidae, rooted by outgroup

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

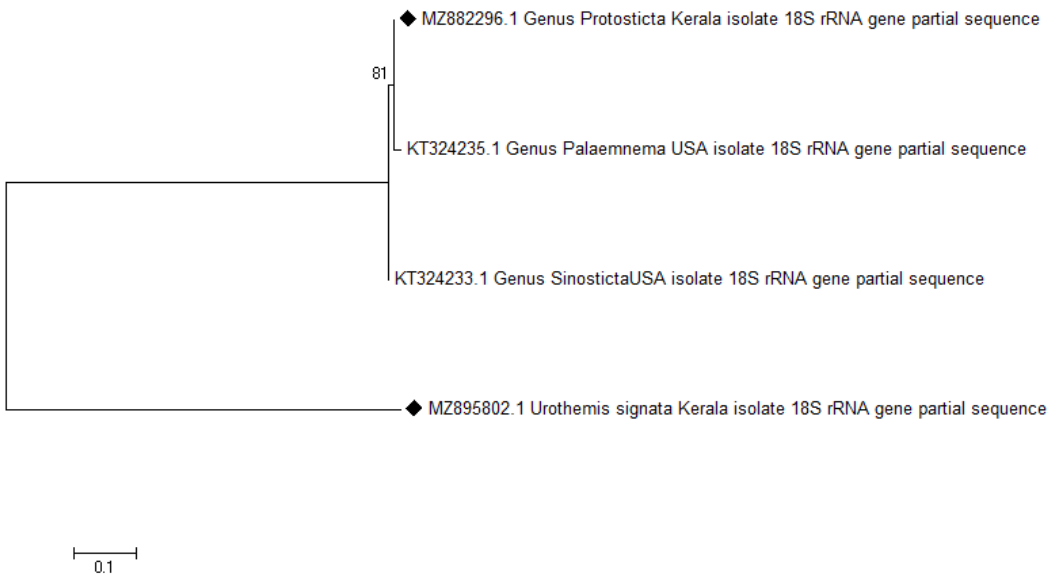

Figure 4.4.8: Inferred phylogenetic tree based on 18S rRNA gene sequences of family Platystictidae, rooted by outgroup

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

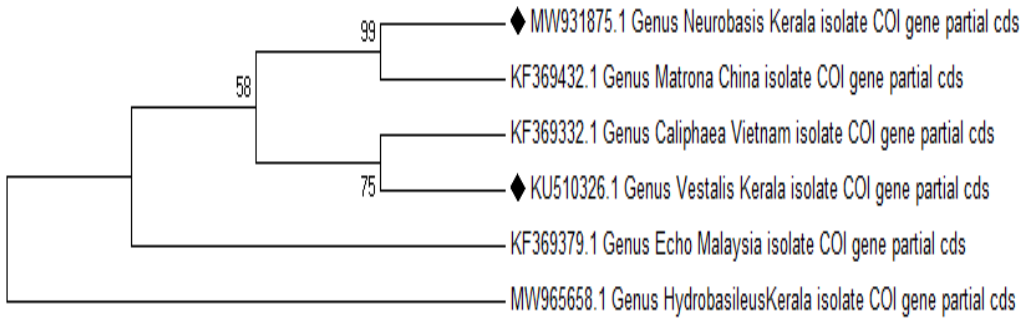

Figure 4.4.9: Inferred phylogenetic tree based on COI gene sequences of family Calopterygidae, rooted by outgroup

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

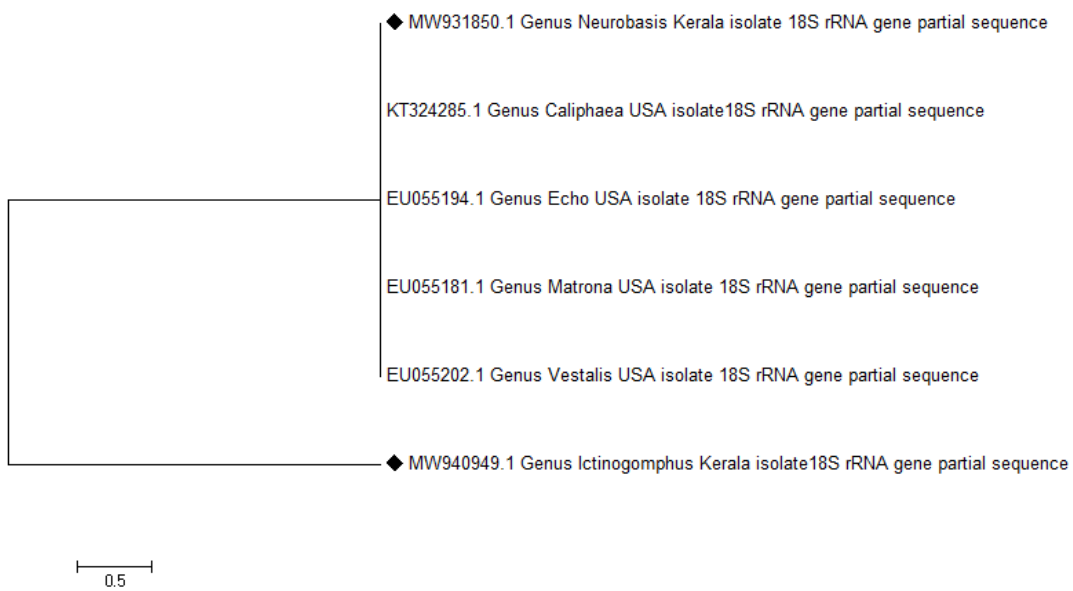

Figure 4.4.10: Inferred phylogenetic tree based on 18S rRNA gene sequences of family Calopterygidae, rooted by outgroup

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

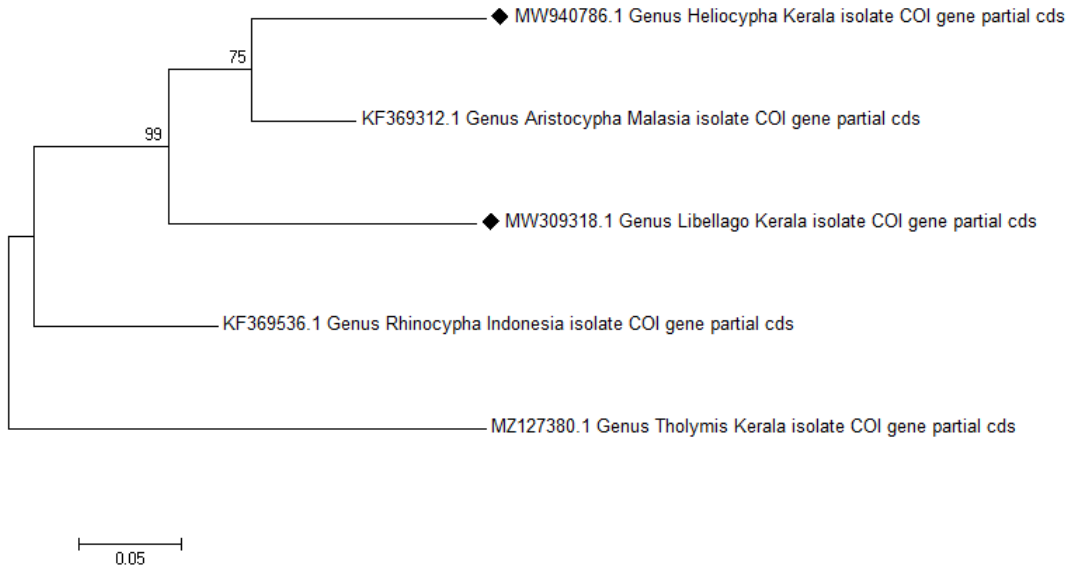

Figure 4.4.11: Inferred phylogenetic tree based on COI gene sequences of family Chlorocyphidae, rooted by outgroup

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).

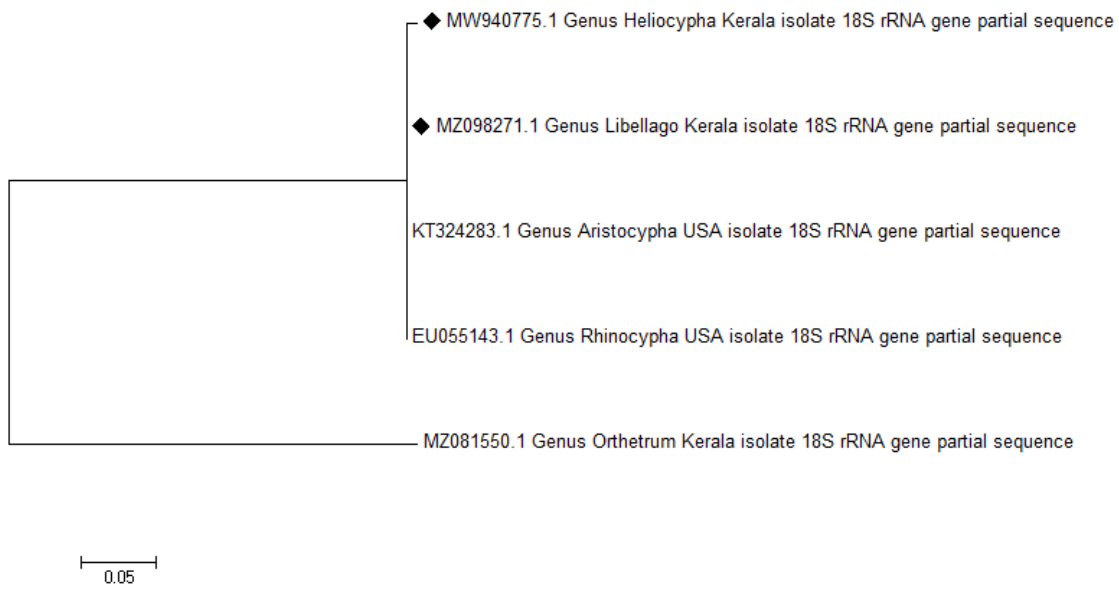

Figure 4.4.12: Inferred phylogenetic tree based on 18S rRNA gene sequences of family Chlorocyphidae, rooted by outgroup

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

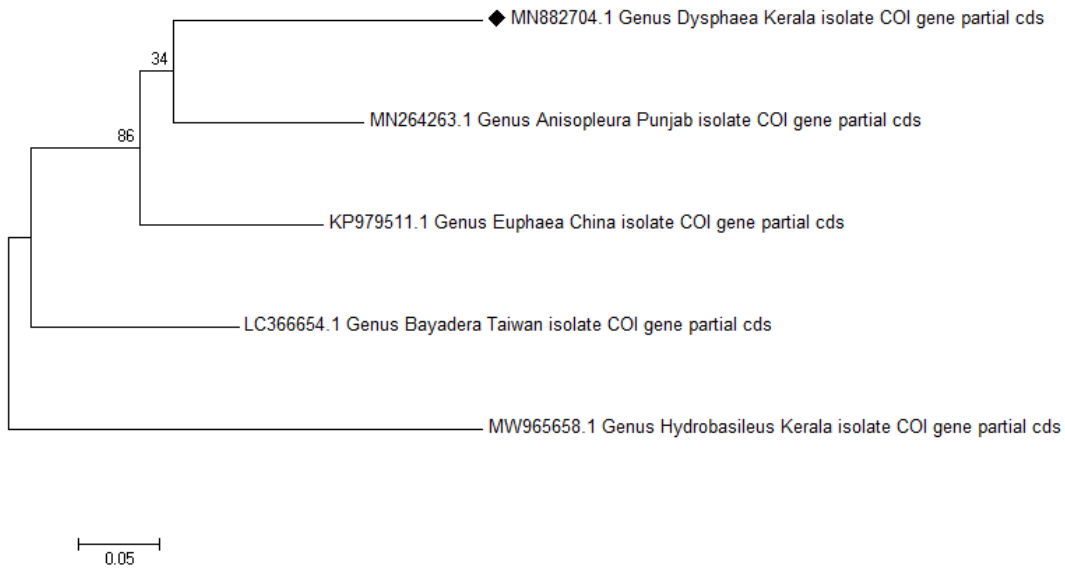

Figure 4.4.13: Inferred phylogenetic tree based on COI gene sequences of family Euphaeidae, rooted by outgroup

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

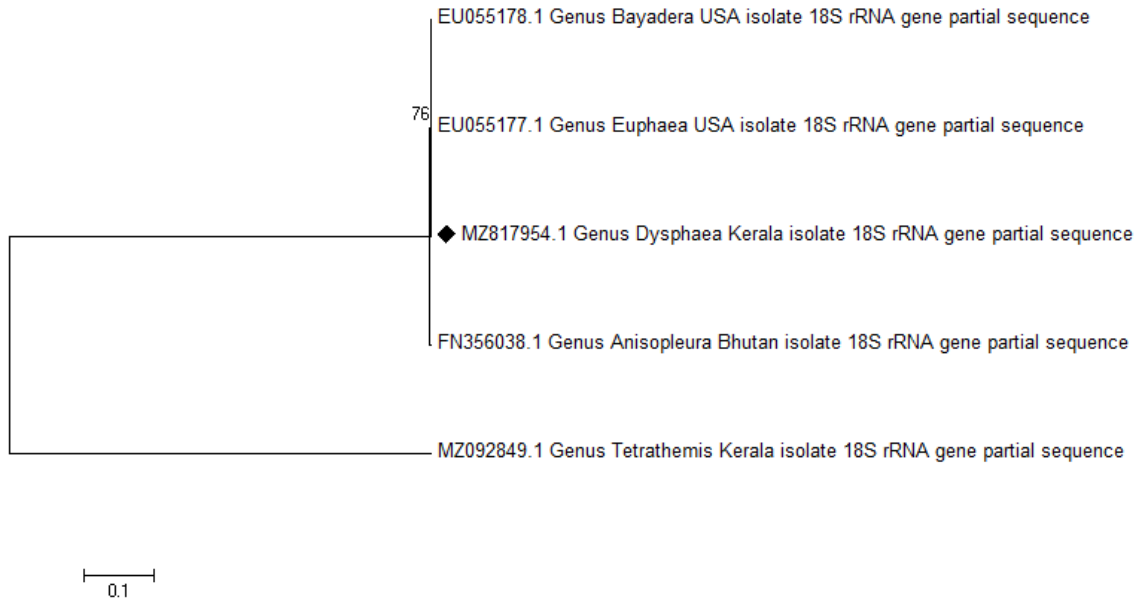

Figure 4.4.14: Inferred phylogenetic tree based on 18S rRNA gene sequences of family Euphaeidae, rooted by outgroup

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

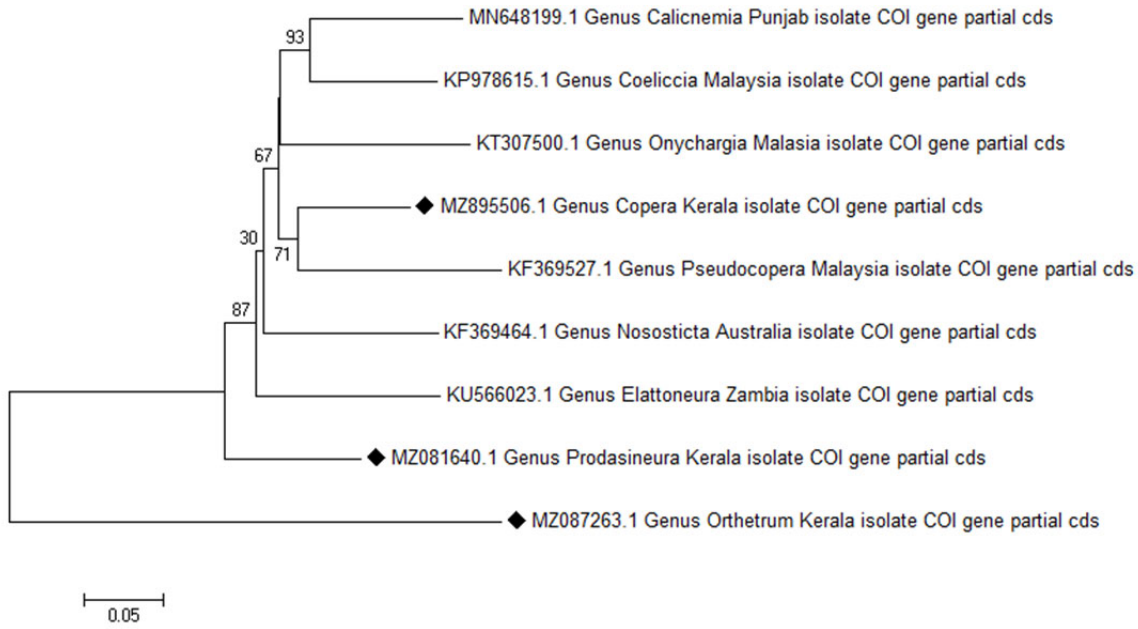

Figure 4.4.15: Inferred phylogenetic tree based on COI gene sequences of family Platycnemididae, rooted by outgroup

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

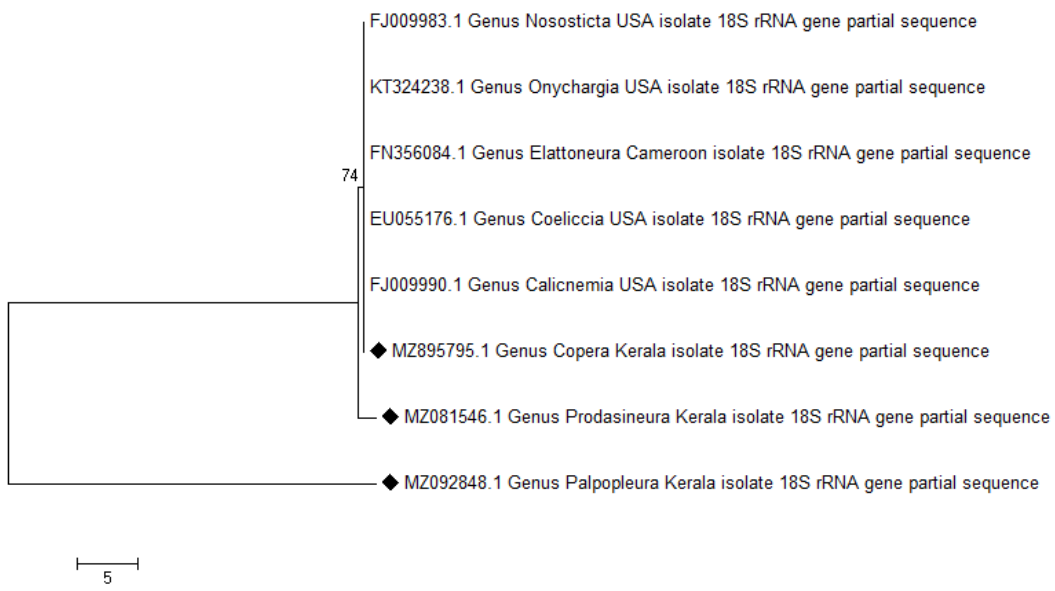

Figure 4.4.16: Inferred phylogenetic tree based on 18S rRNA gene sequences of family Platycnemididae, rooted by outgroup.

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

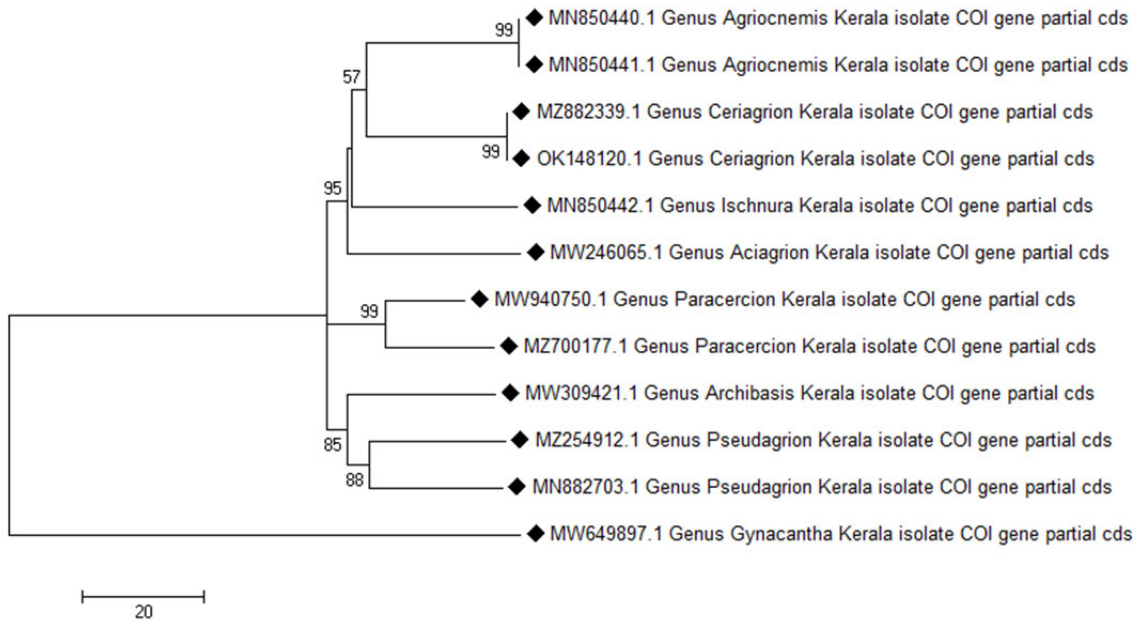

Figure 4.4.17: Inferred phylogenetic tree based on COI gene sequences of family Coenagrionidae, rooted by outgroup

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

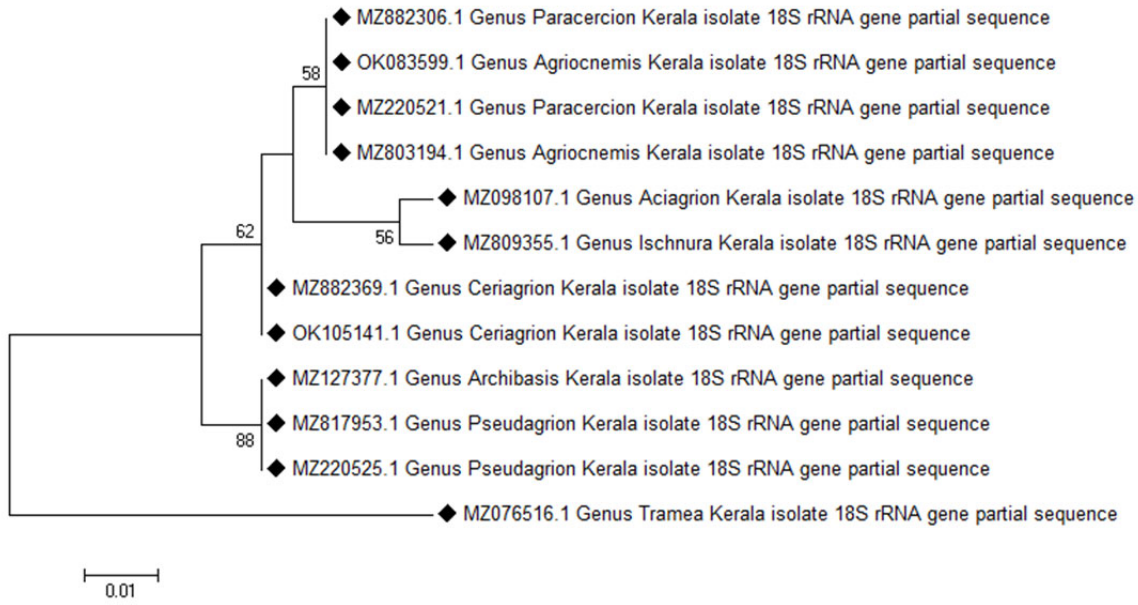

Figure 4.4.18: Inferred phylogenetic tree based on 18S rRNA gene sequences of family Coenagrionidae, rooted by outgroup.

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

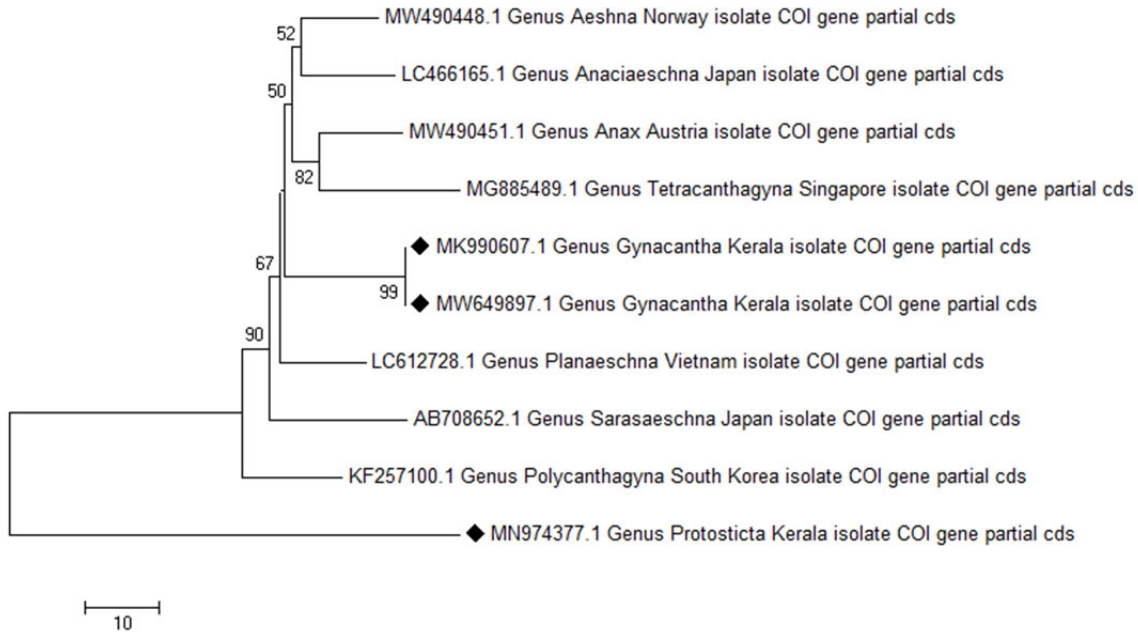

Figure 4.4.19: Inferred phylogenetic tree based on COI gene sequences of family Aeshnidae, rooted by outgroup.

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

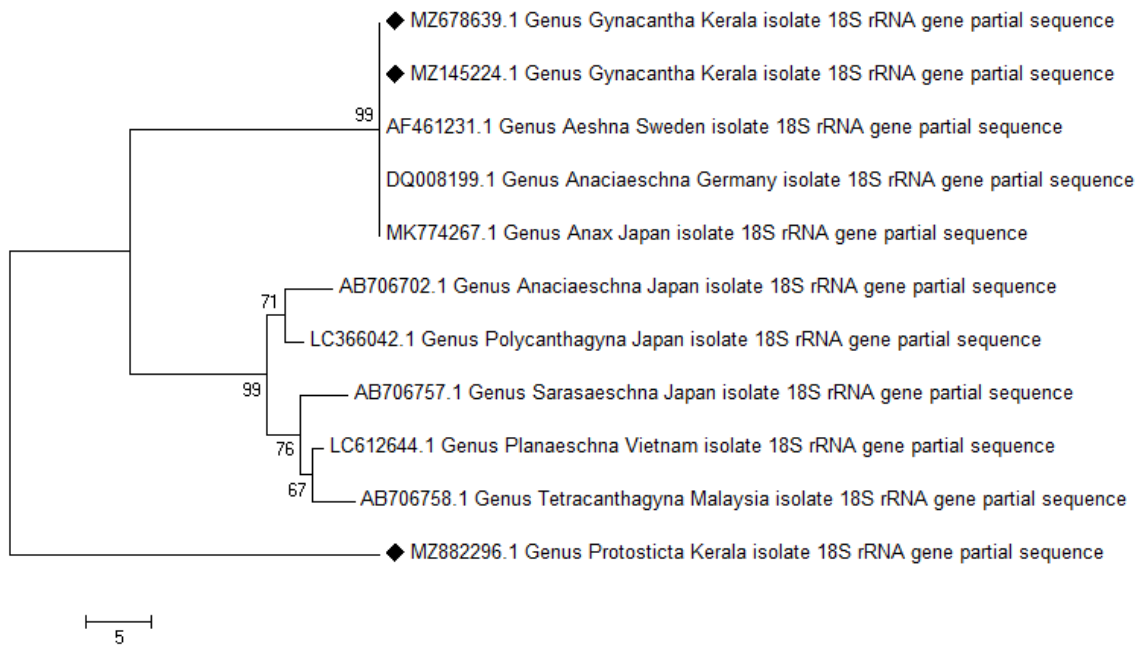

Figure 4.4.20: Inferred phylogenetic tree based on 18S rRNA gene sequences of family Aeshnidae, rooted by outgroup.

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

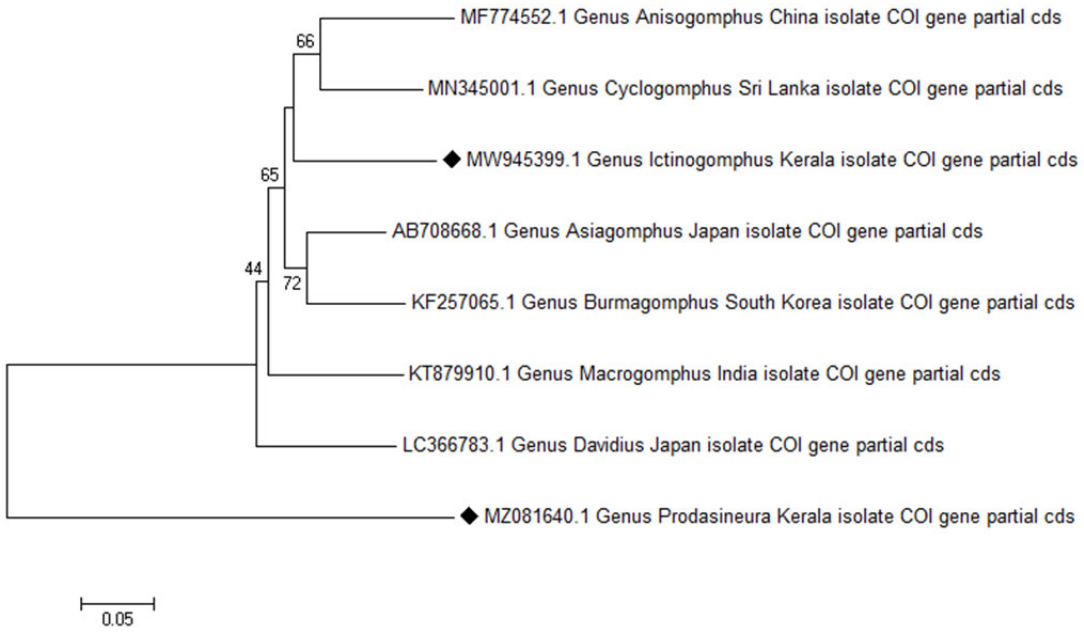

Figure 4.4.21: Inferred phylogenetic tree based on COI gene sequences of family Gomphidae, rooted by outgroup.

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

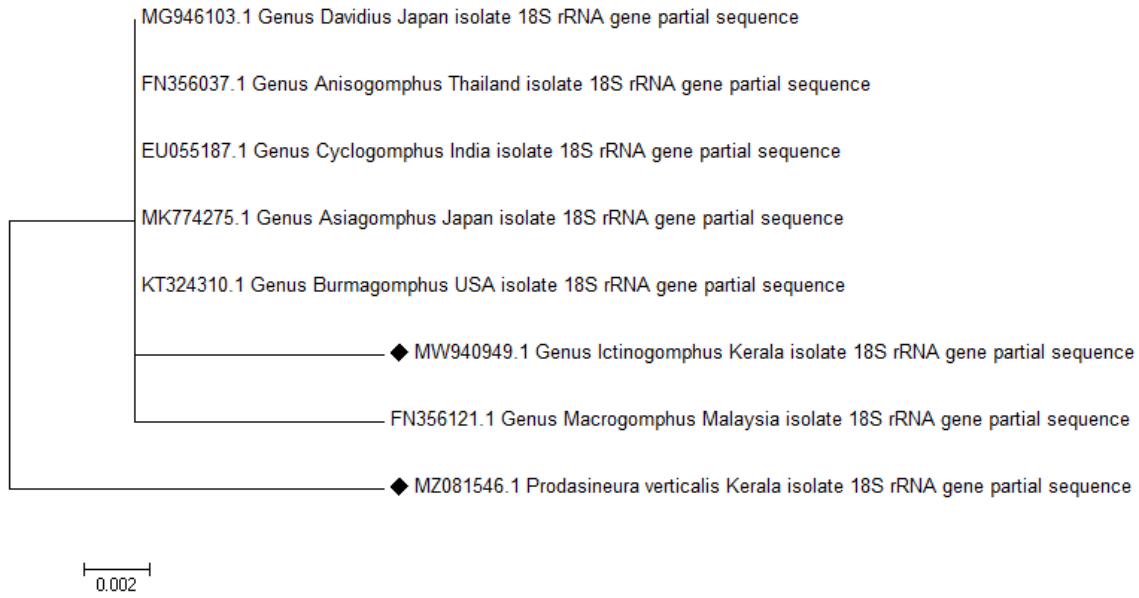

Figure 4.4.22: Inferred phylogenetic tree based on 18S rRNA gene sequences of family Gomphidae, rooted by outgroup.

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

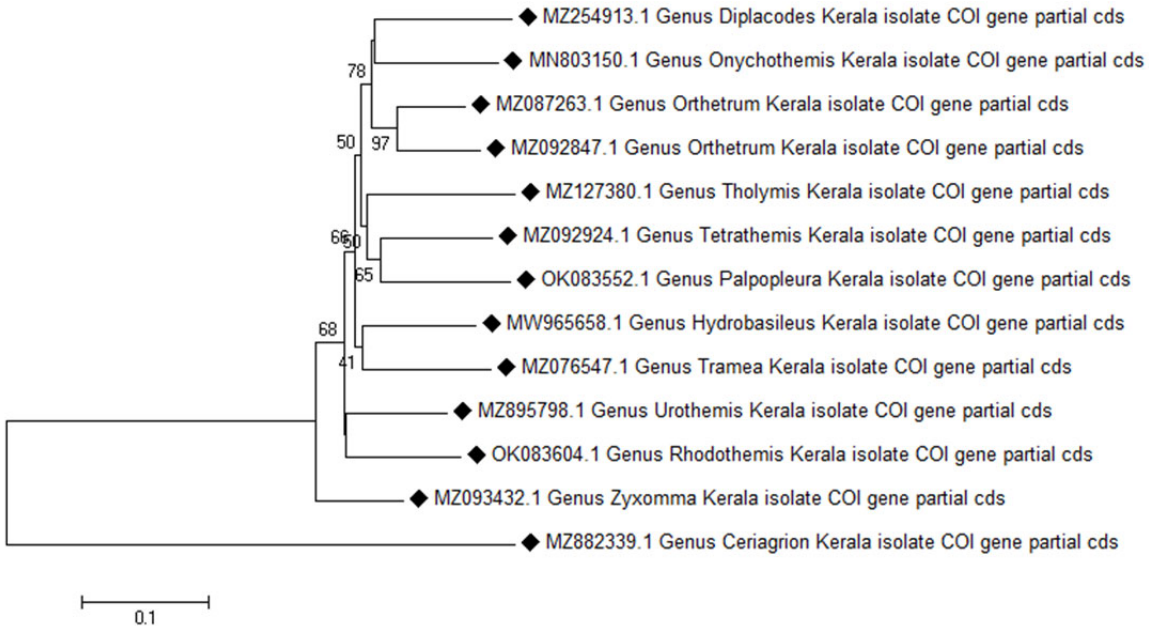

Figure 4.4.23: Inferred phylogenetic tree based on COI gene sequences of family Libellulidae, rooted by outgroup.

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*.

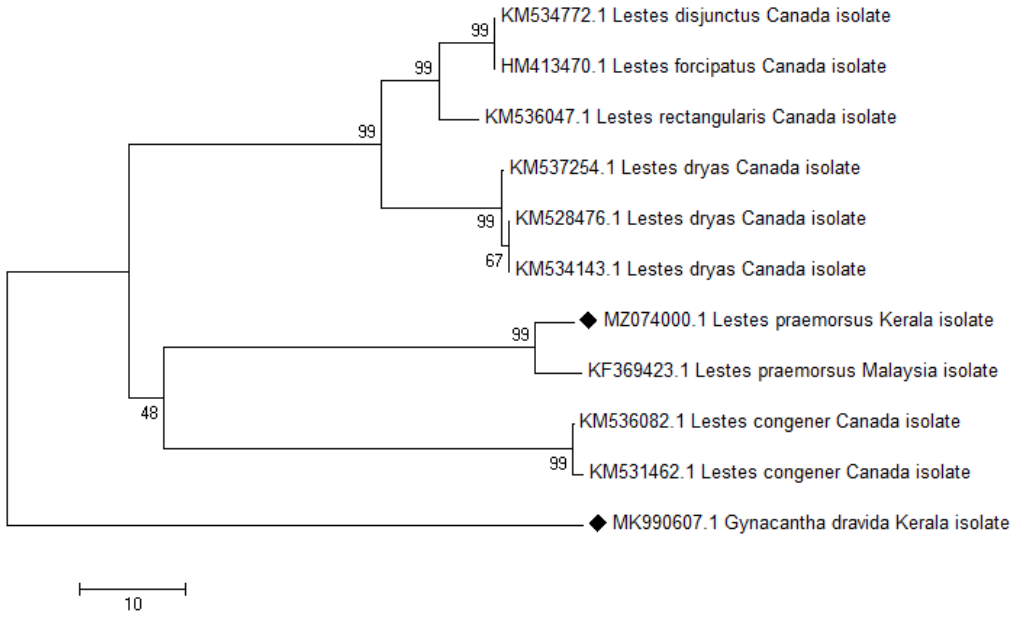

Figure 4.4.25: Inferred phylogenetic tree of the genus *Lestes*, rooted by outgroup.

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

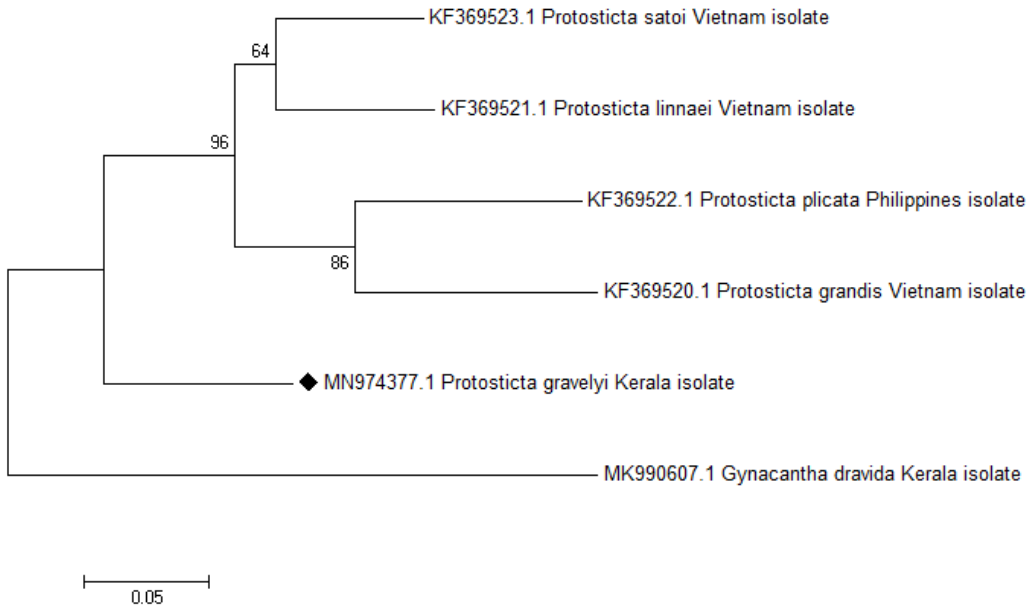

Figure 4.4.26: Inferred phylogenetic tree of the genus *Protosticta*, rooted by outgroup.

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

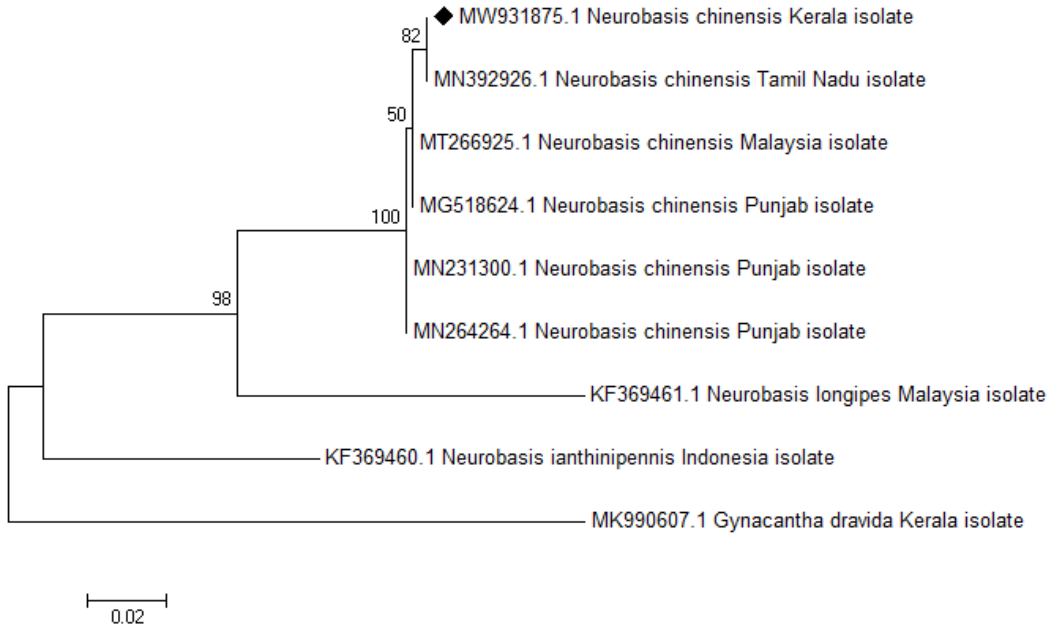

Figure 4.4.27: Inferred phylogenetic tree of the genus *Neurobasis*, rooted by outgroup.

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

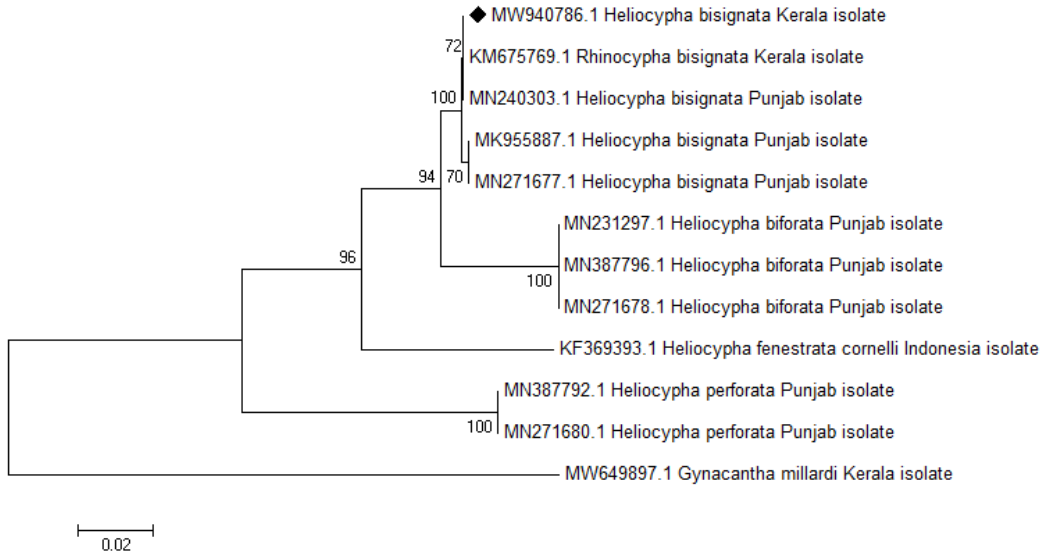

Figure 4.4.28: Inferred phylogenetic tree of the genus *Heliocypha*, rooted by outgroup.

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

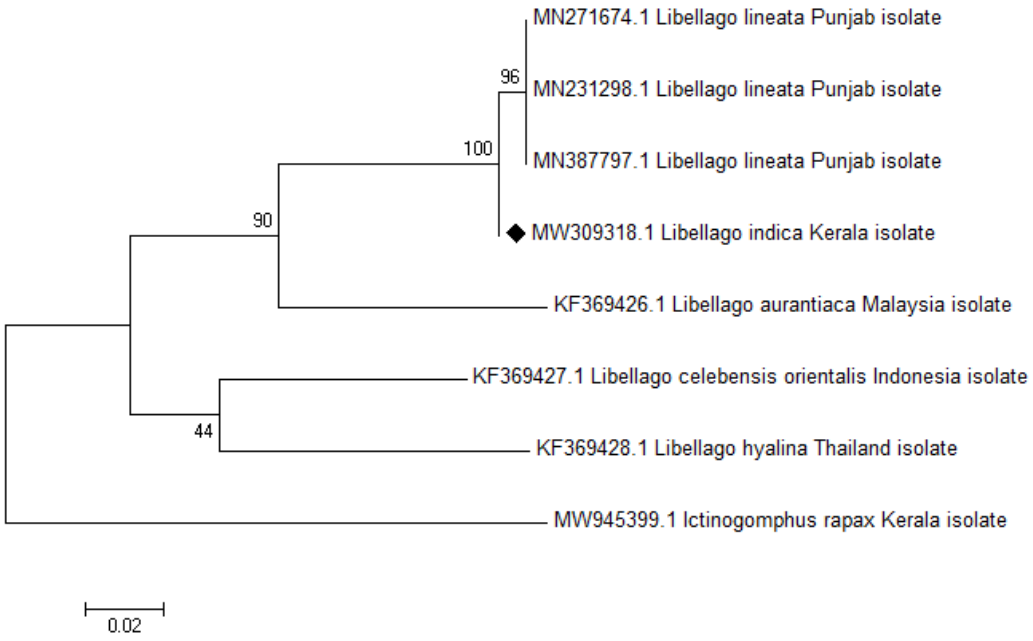

Figure 4.4.29: Inferred phylogenetic tree of the genus *Libellago*, rooted by outgroup.

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.

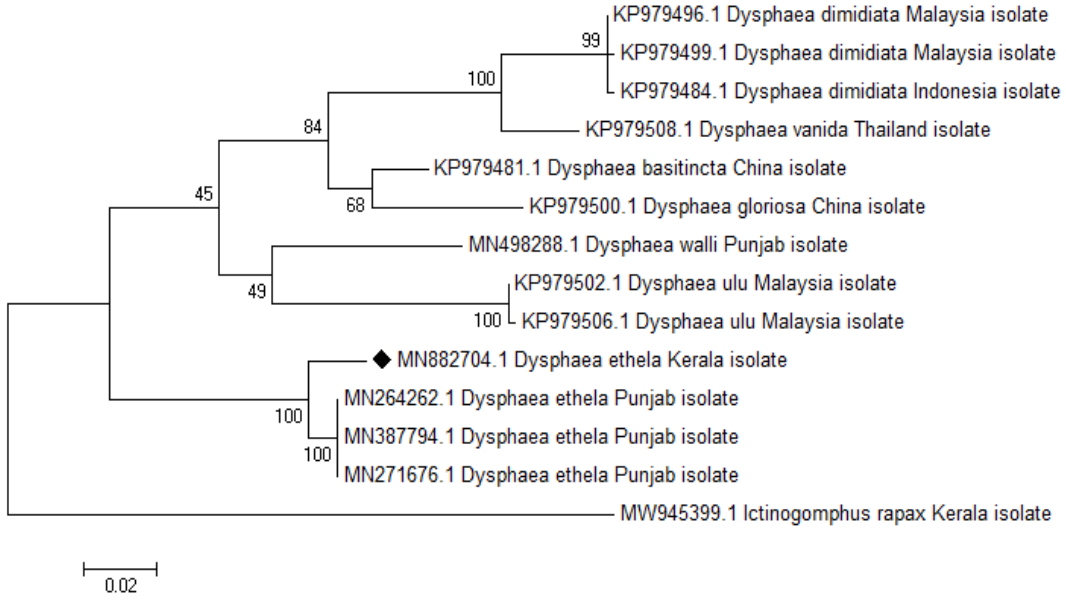

Figure 4.4.30: Inferred phylogenetic tree of the genus *Dysphaea*, rooted by outgroup.

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.

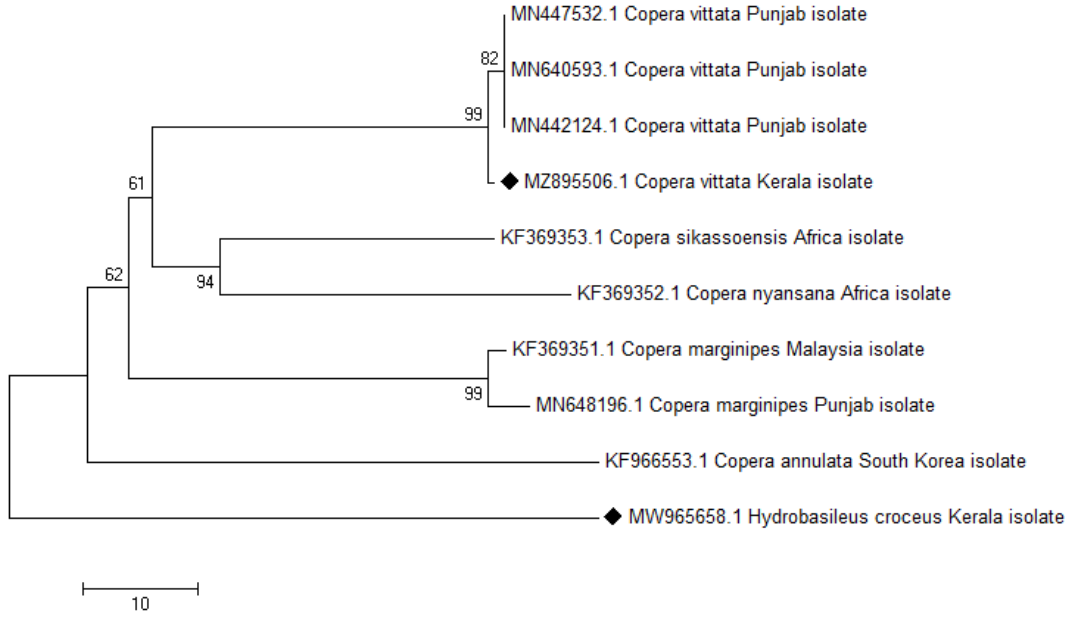

Figure 4.4.31: Inferred phylogenetic tree of the genus *Copera*, rooted by outgroup.

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*.

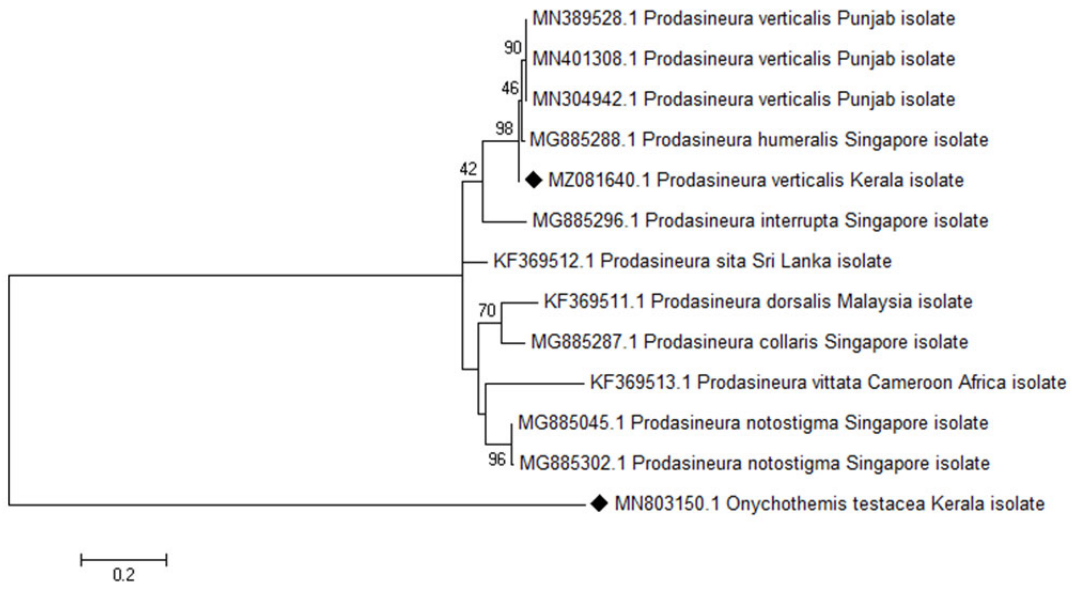

Figure 4.4.32: Inferred phylogenetic tree of the genus *Prodasineura*, rooted by outgroup.

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

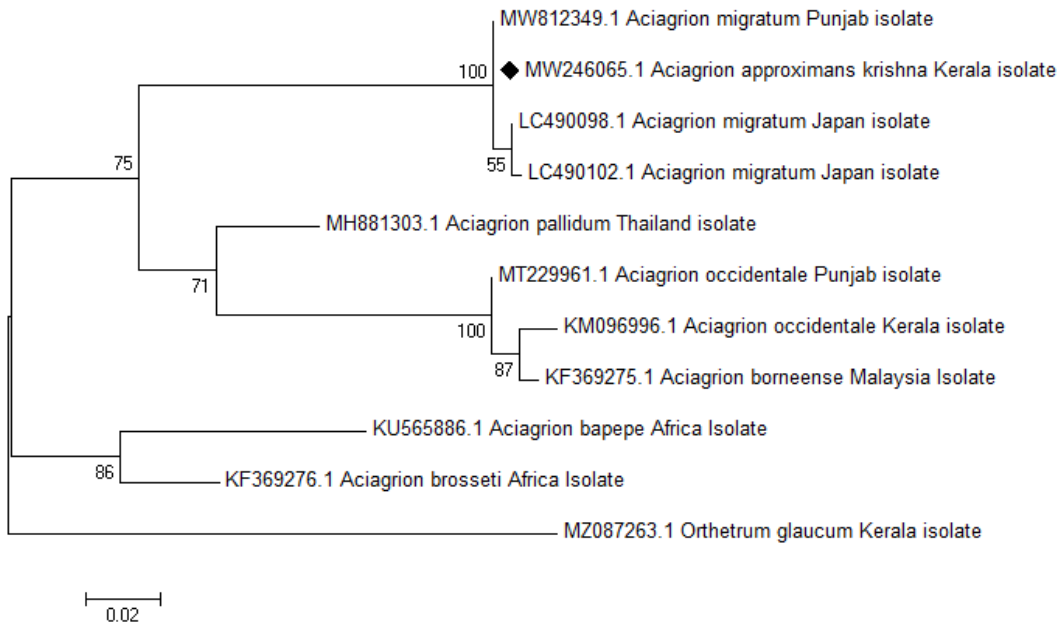

Figure 4.4.33: Inferred phylogenetic tree of the genus *Aciagrion*, rooted by outgroup.

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.

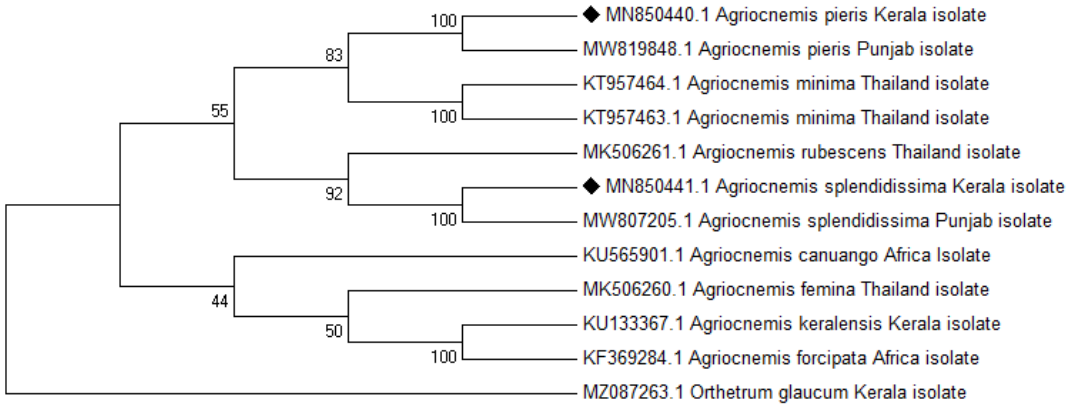

Figure 4.4.34: Inferred phylogenetic tree of the genus *Agriocnemis*, rooted by outgroup.

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

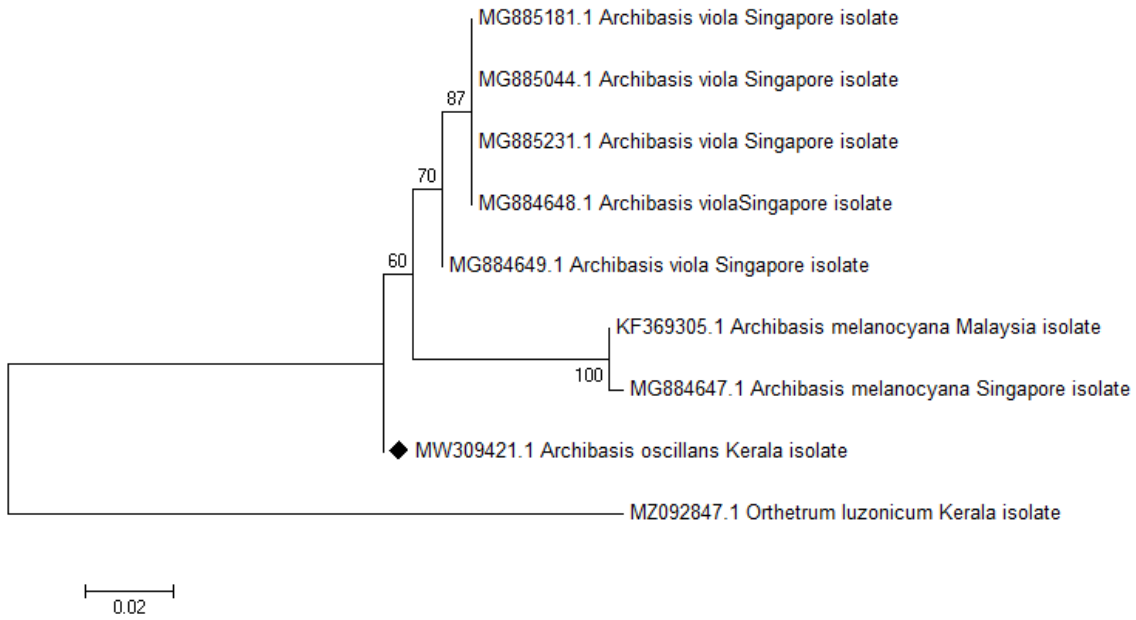

Figure 4.4.35: Inferred phylogenetic tree of the genus *Aciagrion*, rooted by outgroup.

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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

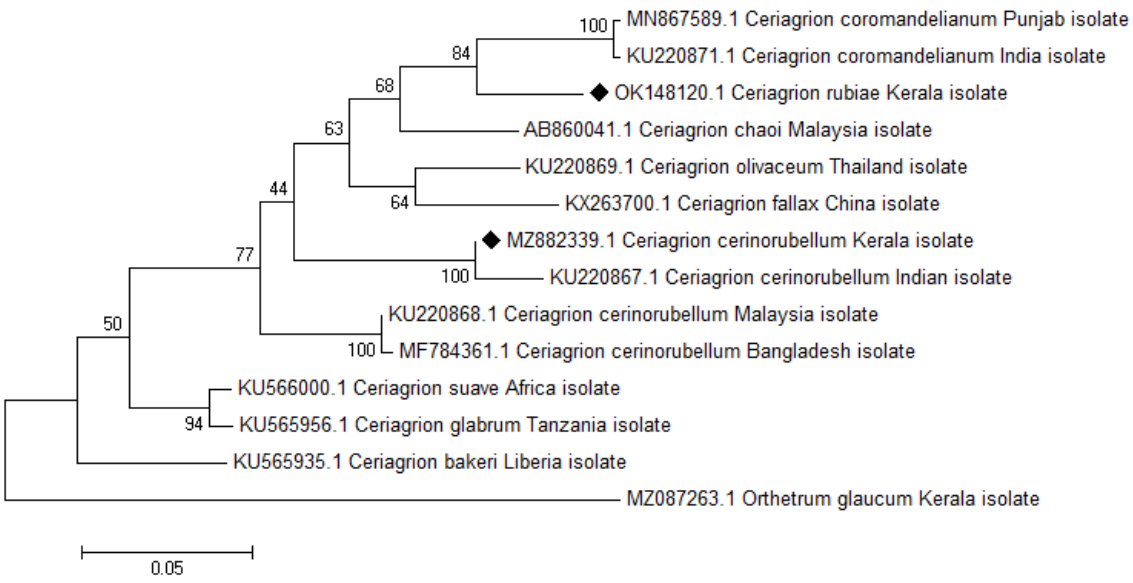

Figure 4.4.36: Inferred phylogenetic tree of the genus *Ceriagrion*, rooted by outgroup.

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%.

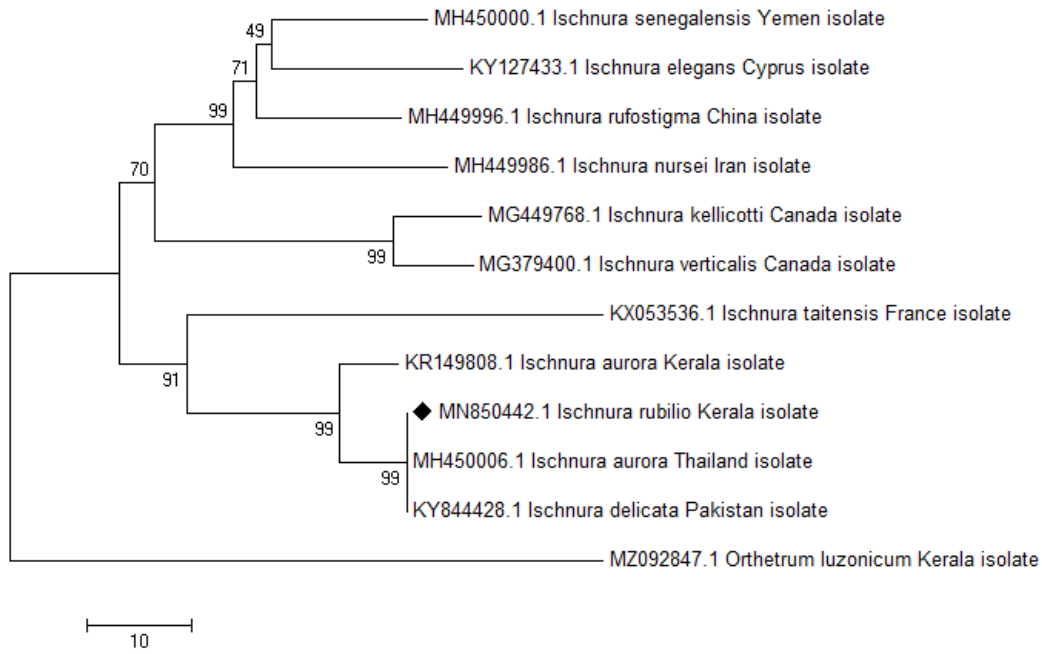

Figure 4.4.37: Inferred phylogenetic tree of the genus *Ischnura*, rooted by outgroup.

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.

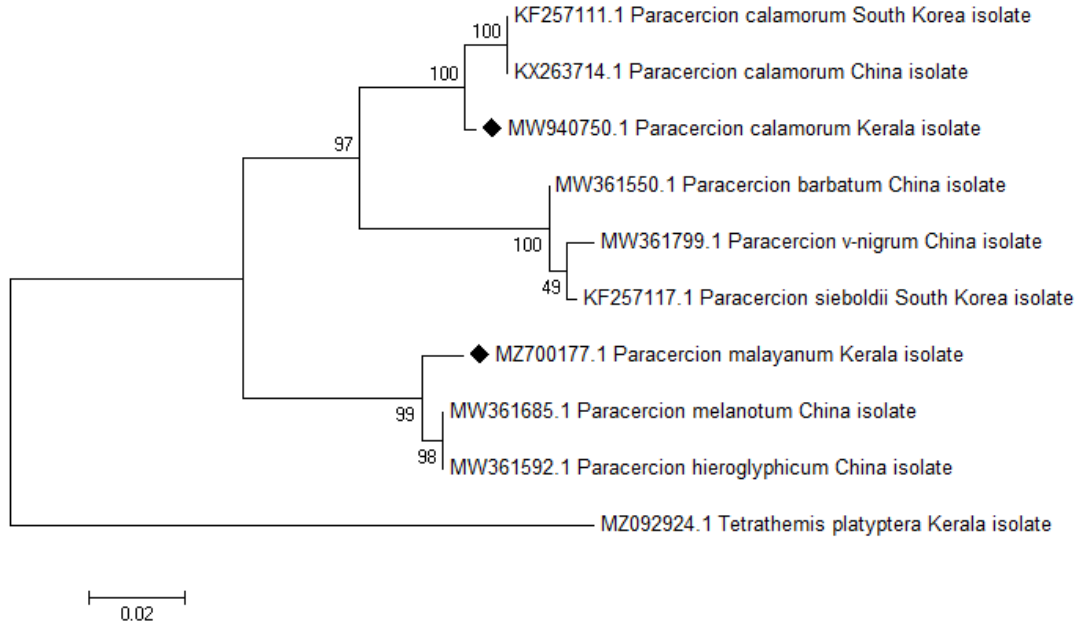

Figure 4.4.38: Inferred phylogenetic tree of the genus *Paracercion*, rooted by outgroup.

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.

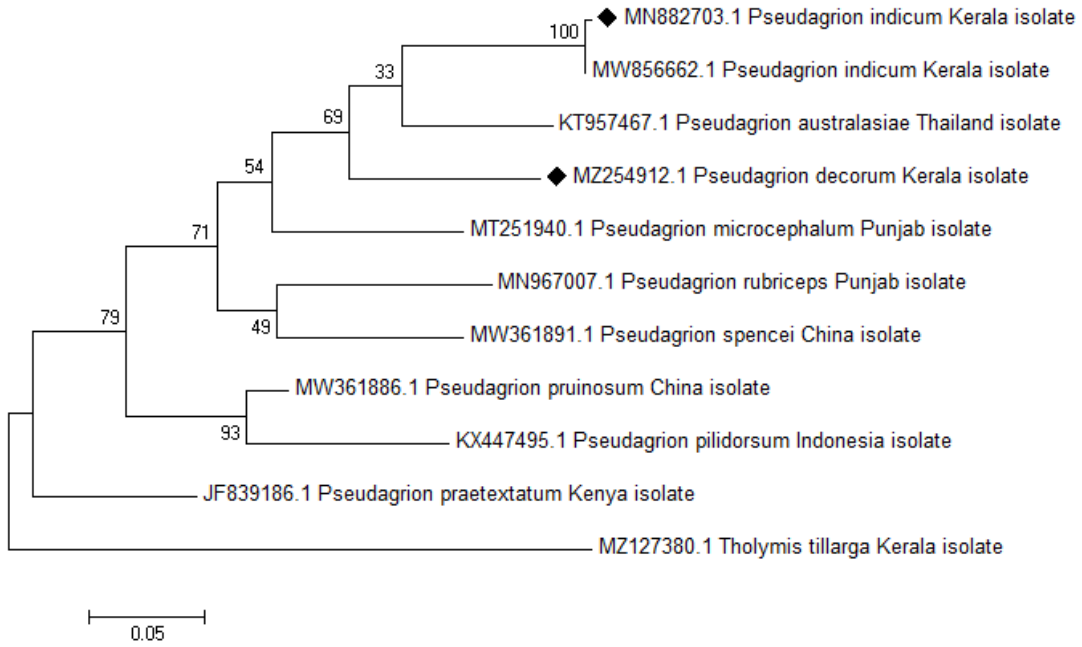

Figure 4.4.39: Inferred phylogenetic tree of the genus *Pseudagrion*, rooted by outgroup.

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%).

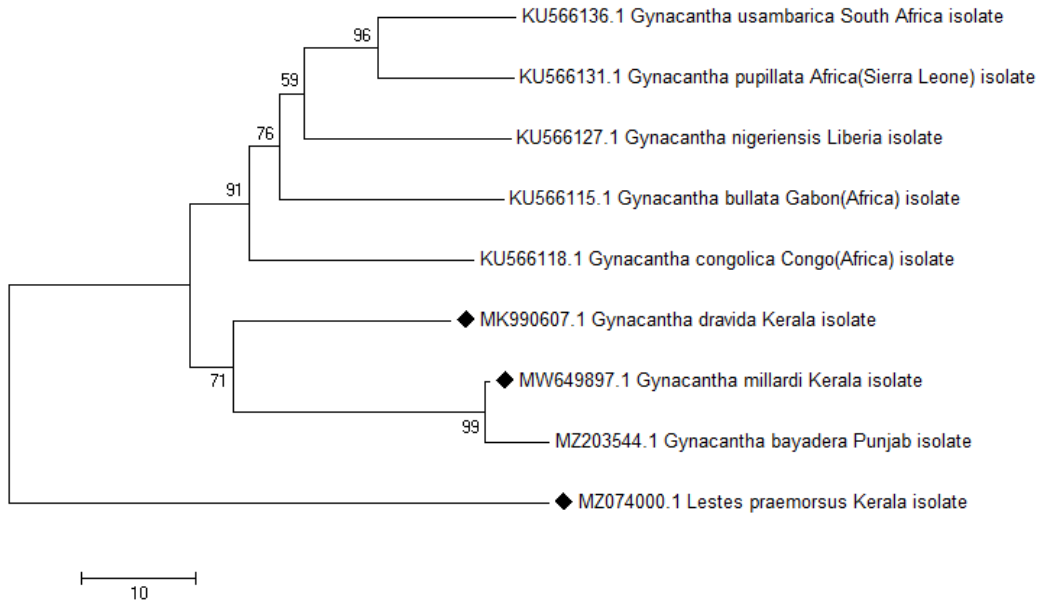

Figure 4.4.40: Inferred phylogenetic tree of the genus *Gynacantha*, rooted by outgroup.

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).

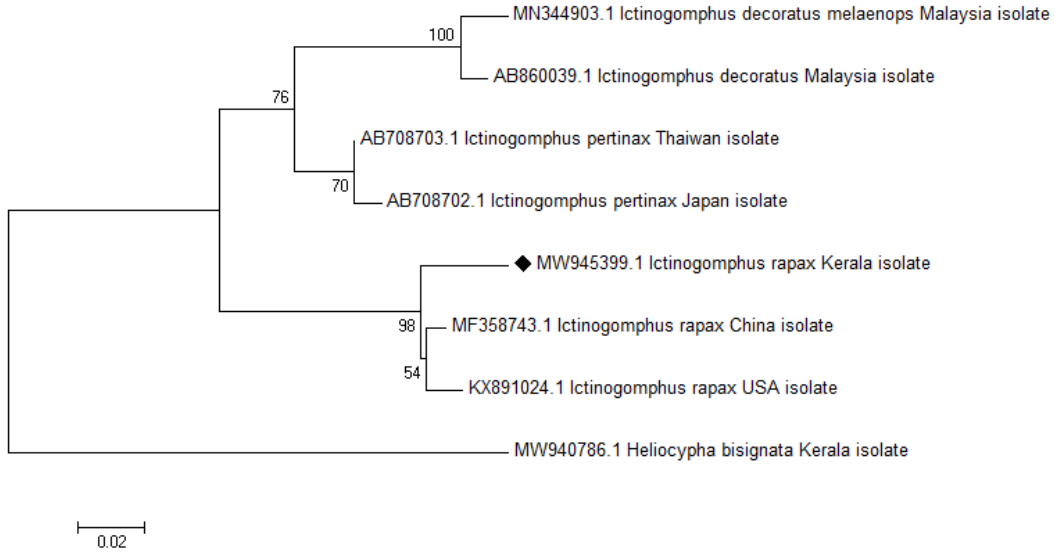

Figure 4.4.41: Inferred phylogenetic tree of the genus *Ictinogomphus*, rooted by outgroup.

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

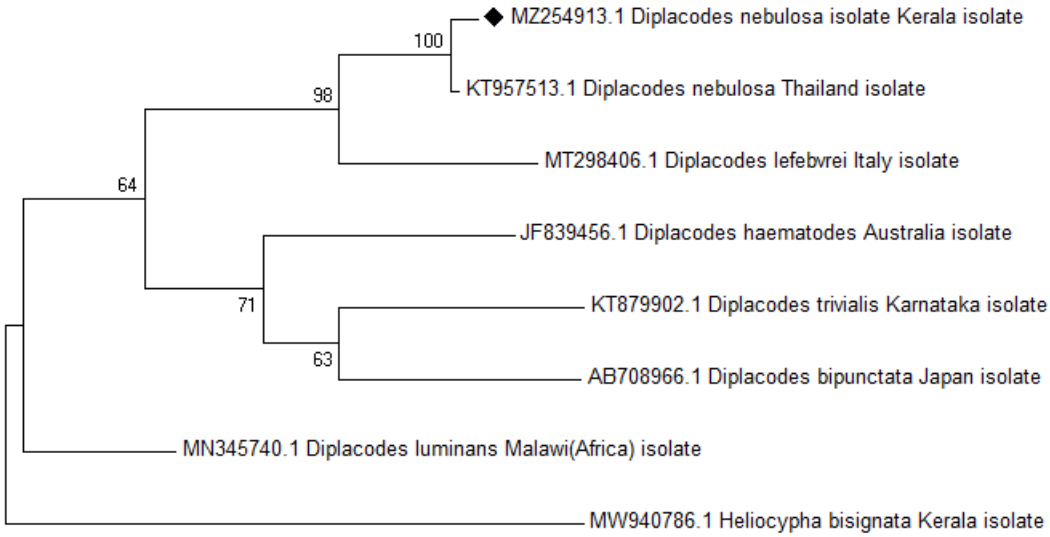

Figure 4.4.42: Inferred phylogenetic tree of the genus *Diplacodes*, rooted by outgroup

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

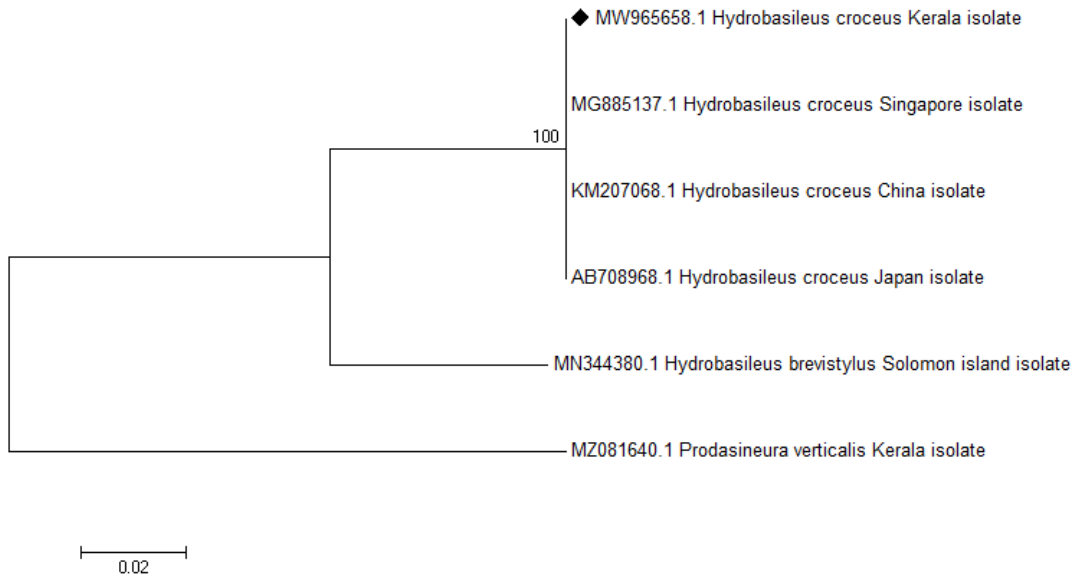

Figure 4.4.43: Inferred phylogenetic tree of the genus *Hydrobasileus*, rooted by outgroup

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

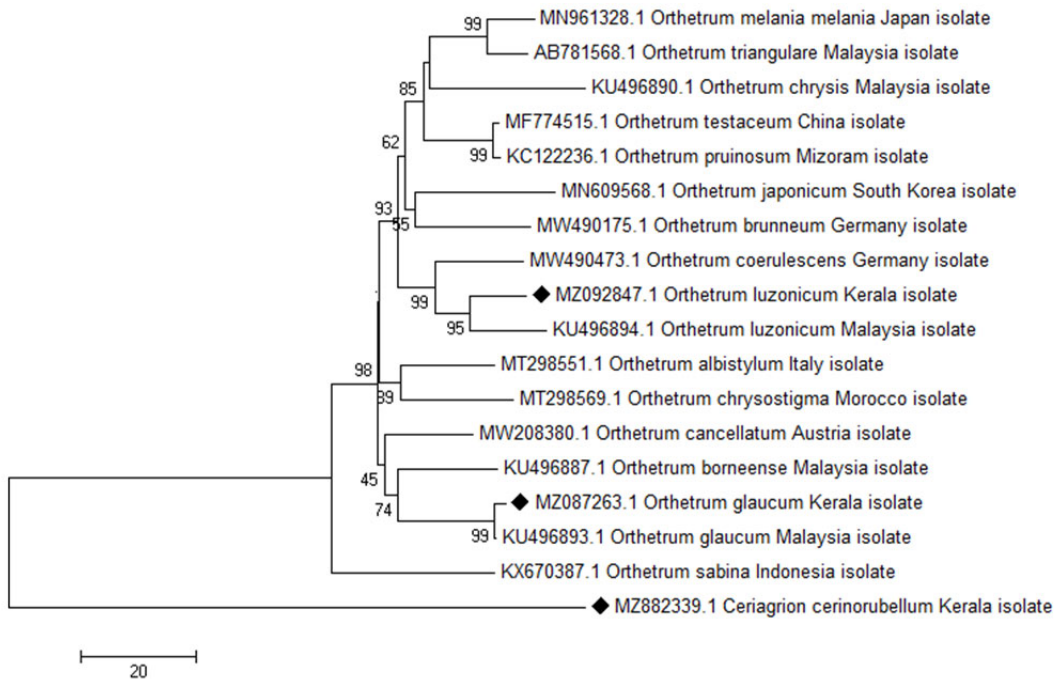

Figure 4.4.44: Inferred phylogenetic tree of the genus *Orthetrum*, rooted by outgroup

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.

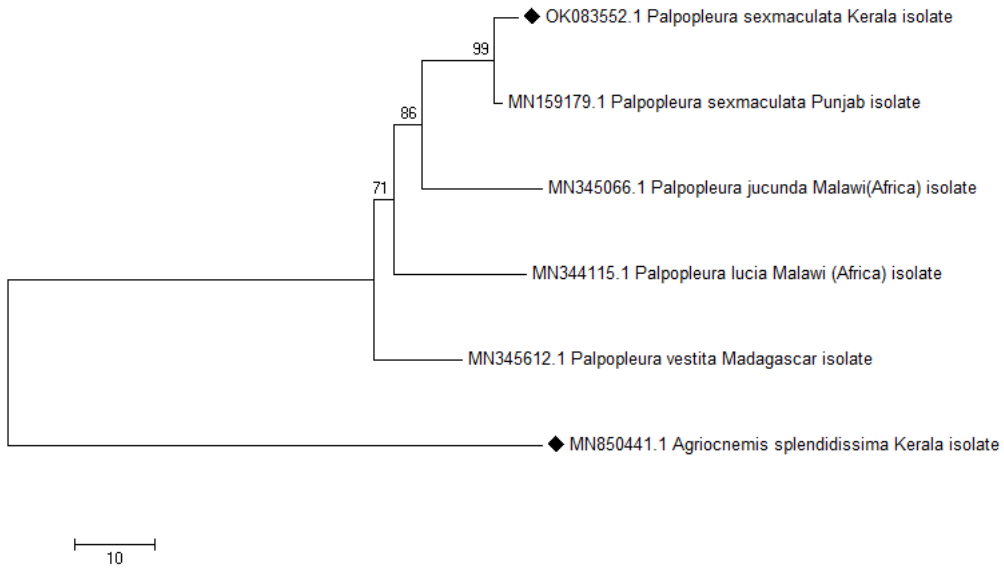

Figure 4.4.45: Inferred phylogenetic tree of the genus *Palpopleura*, rooted by outgroup

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.

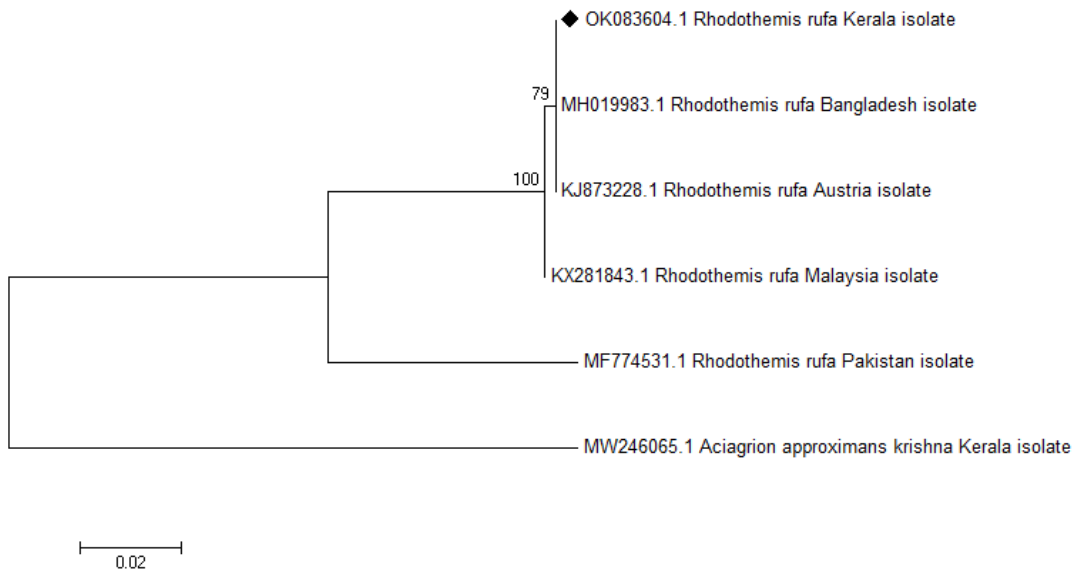

Figure 4.4.46: Inferred phylogenetic tree of the genus *Rhodothemis*, rooted by outgroup

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

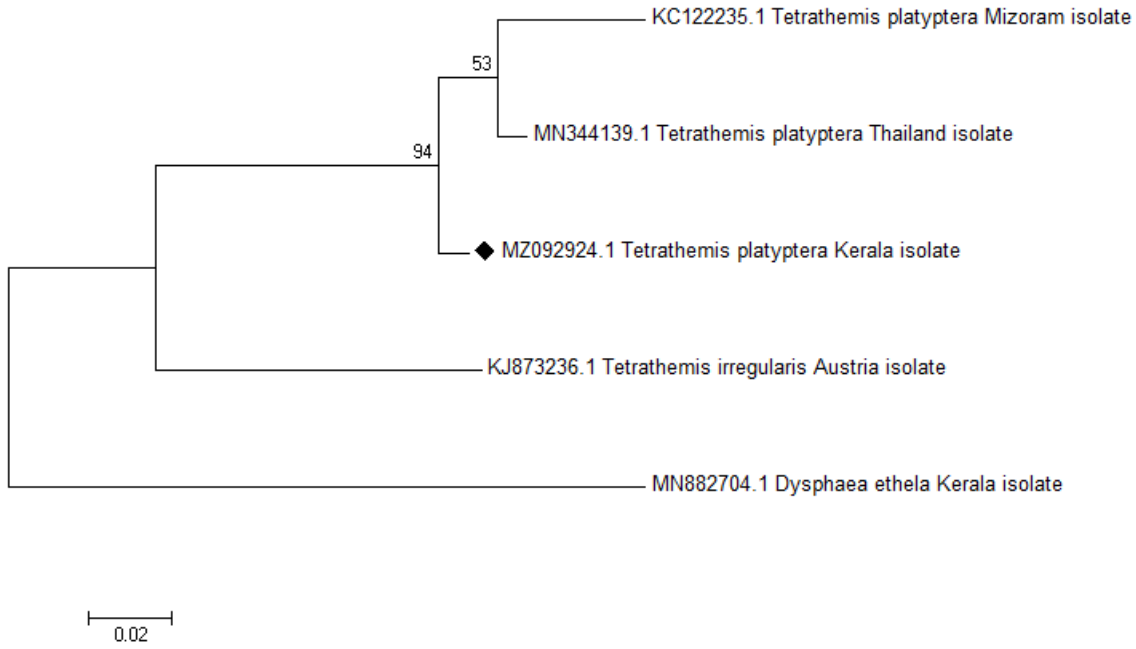

Figure 4.4.47: Inferred phylogenetic tree of the genus *Tetrathemis*, rooted by outgroup

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.

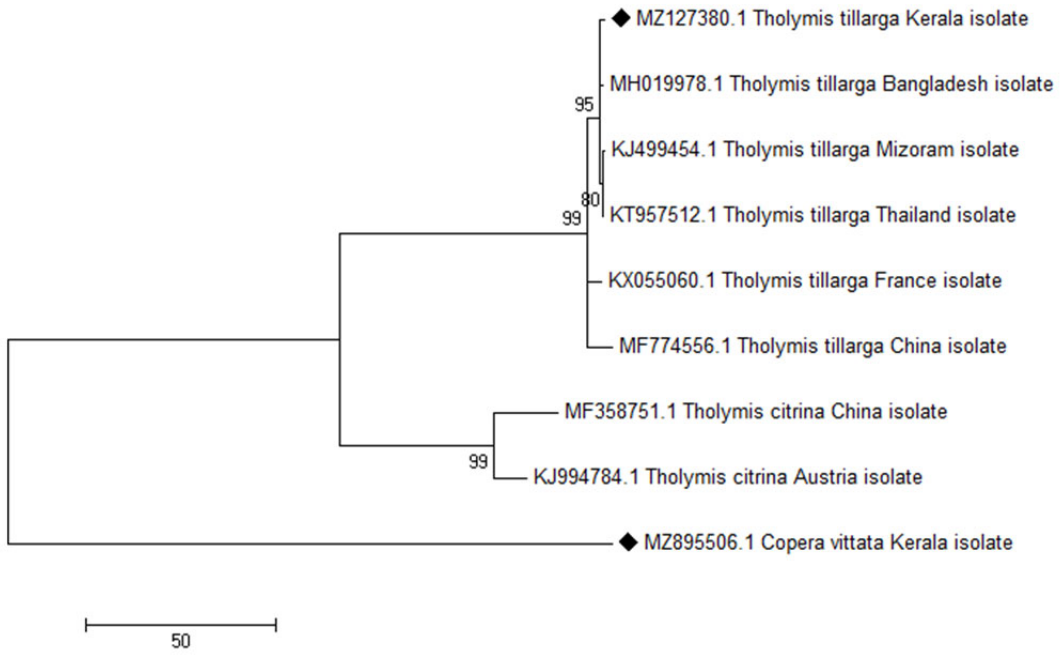

Figure 4.4.48: Inferred phylogenetic tree based on COI gene sequences of *Tholymis* species and out group

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.

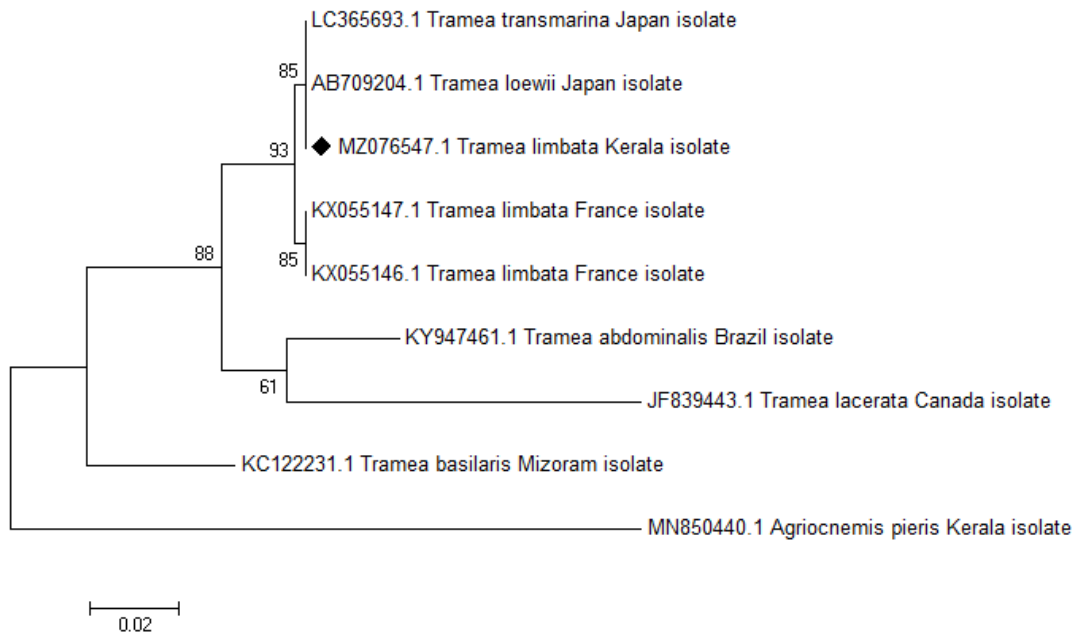

Figure 4.4.49: Inferred phylogenetic tree based on COI gene sequences of *Tramea* species and out group

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

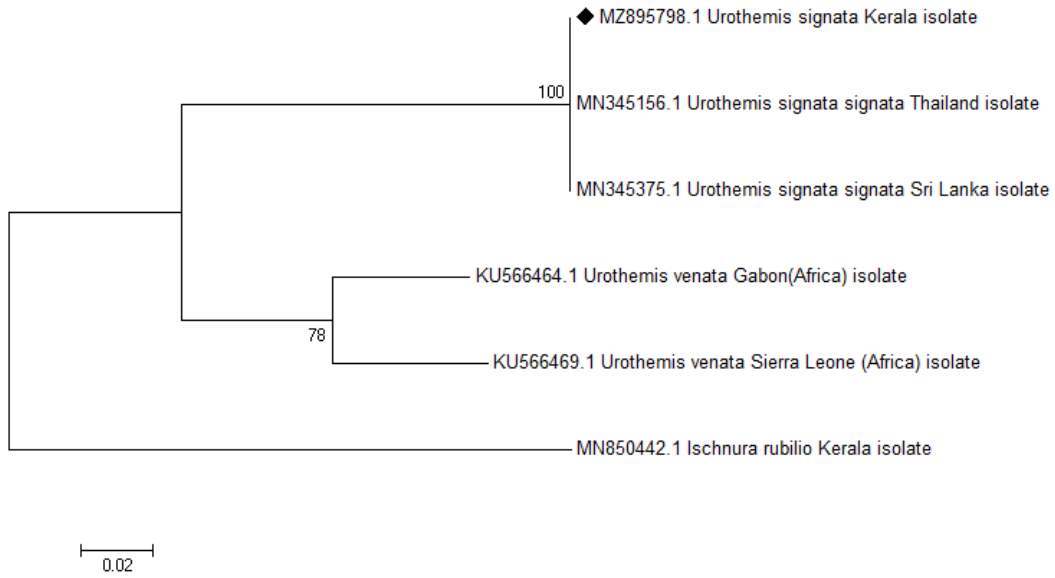

Figure 4.4.50: Inferred phylogenetic tree based on COI gene sequences of *Urothemis* species and out group

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

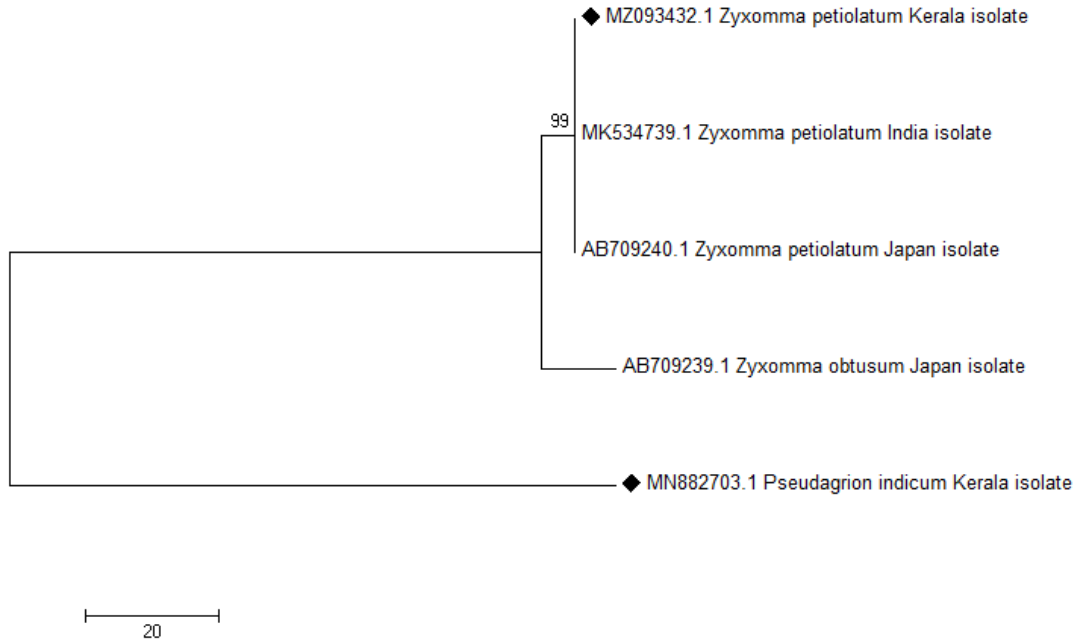

Figure 4.4.51: Inferred phylogenetic tree based on COI gene sequences of *Zyxomma* species and out group

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.

5. CONCLUSION

Five districts of Kerala encompassing the high land, midland and low land regions were selected for the observation of odonates. Seventy three different habitats of Thrissur, Ernakulam, Palakkad, Wayanad and Idukki districts of Kerala were observed that including streams, rivers, ponds, lakes, paddy fields, ditches and estuaries. The reserved forests and protected areas were excluded from the study.

During the study period, a total of 71 species belonging to 43 genera and 10 families were recorded. Of these 33 species were damselflies and 38 species were dragonflies. Four Western Ghats endemic species viz. *Aciagrion approximans krishna, Agriocnemis keralensis, Pseudagrion indicum* and *Protosticta gravelyi* were recorded. *Paracercion malayanum* was recorded as the first report from central and northern Kerala. The observed species richness was more in dragonflies than in damselflies. Coenagrionidae and Libellulidae were the most dominant families of damselflies and dragonflies respectively. The observed species richness was high in vegetated ponds, lakes and streams and minimum in unvegetated habitats. Taxonomic keys for the observed 71 species of odonates were prepared.

Representing 28 genera, molecular characterisation of 34 species was done. Partial COI gene, 18S rRNA gene sequences and translated protein sequences were generated. The sequences were deposited in the GenBank database and received accession numbers. Of these twelve COI gene sequences and twenty three 18S rRNA gene sequences are the first records in worldwide databases. The obtained COI gene sequences are useful for precise and faster species level identification and phylogenetic analyses while the 18S gene sequences are beneficial in higher level phylogenies.

Phylogenetic analyses of two suborders and selected families were carried out based on both marker genes. The effectiveness of both genes in discriminating relationships was compared. A detailed comparison of trees revealed the efficiency of COI over the 18S rRNA gene in resolving family and suborder trees. In the majority of analyses, COI yielded better resolution than 18S rRNA gene.

COI based phylogenetic trees were constructed for selected 27 genera and estimated intraspecific and interspecific divergence values. Low genetic variability

was exhibited by the majority of odonates analysed except eight species. According to the literature, the occurrence of intraspecific divergence can be because of their highly sensitive gene sequences. Interspecific divergence values were also estimated and found maximum and minimum values in genus *Tholymis* and *Dysphaea* respectively.

Close genetic similarities without any divergence were observed between different species of geographically distant regions was another significant finding of this work.

RECOMMENDATIONS

- The present research work has revealed the baseline data of order Odonata in different habitats of Kerala, which will encourage further investigations by research scholars and amateurs regarding the species richness, abundance and diversity of rare and endemics of this group in this region.
- Further research studies can generate odonate diversity register (peoples' biodiversity register) at the local administrative level (Panchayath level) to record the distribution of odonates in various habitats.
- Information recorded on the distribution of rare and endemic odonates in this region will help to formulate strategies for conserving different valuable habitats.
- This can be achieved by conducting awareness programmes for local people such as seminars and providing posters and brochures to apprise them about the relevance of odonate habitat conservation.
- Proper understanding of rare and endemic odonate distribution will attract national and international odonatologists. This will eventually promote tourism activities.
- This group can be considered as model organisms for further research in detail, on genetic variability in different geographic locations of the world.
- Only 37% of the total odonates of Kerala were sequenced and studied so far. Molecular taxonomy becomes successful only with a complete database. So, future studies can be focused to fill these gaps in databases to make them complete.
- Studies on the classification and phylogeny of rare and endemic odonate species of the Western Ghats are sparse. Hence works based on this concept are also encouraged.

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COMMUNICATION

On the diversity and abundance of riparian odonate fauna (Insecta) of the midstream Chalakkudy River, Kerala, India

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Abstract: The riparian Odonate insect diversity of the midstream Chalakudy River at six locations assessed from February 2018 to January 2019 has revealed the occurrence of 25 species of odonates. Among them,10 species are dragonflies belonging to seven genera of the family Libellulidae and the remaining 15 species are damselflies belonging to six families and 11 genera. Five endemic damselfly species have been recorded. *Pseudagrion indicum* is endemic to the Western Ghats, while the remaining four species, *Vestalis apicalis, Libellago indica, Dysphaea ethela*, and *Heliocypha bisignata*, are endemic to India. Diversity indices of the odonates in all the six locations were analyzed and it showed less abundance at sites where tourist activities are more and with thin native riparian vegetation. Further, the study has unequivocally revealed that thick native riparian vegetation is essential for their perching and existence. By and large, the uncontrolled tourism activities and habitat alteration interfere with the density and diversity of these endemic species.

Keywords: Damselflies, dragonflies, endemism, odonates, tourism, Western Ghats.

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Author contributions: NBC— data collection, data analysis and interpretation, drafting the article, editing; CFB—final approval of the version; FKK—conception or design of the work, critical revision of article, editing, final approval of the version.

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INTRODUCTION

Kerala has a comprehensively documented odonate fauna. The relevant works among them include that of Rao & Lahiri (1982), Mathavan & Miller (1989), Radhakrishnan (1997), Emiliyamma & Radhakrishnan (2002), Emiliyamma (2005), Palot et al. (2005), Adarsh et al. (2014), Varghese et al. (2014), Nair (2017), and Susanth & Anooj (2020). Recent works further added up the rich odonate diversity of Kerala to 174 species (Emiliyamma et al. 2020; Joshi et al. 2020). The seasonal and habitat distribution of Odonata diversity of riparian habitats such as Mula and Mutha river basins in Maharashtra was studied by Kulkarni & Subramanian (2013). Species turn over and abundance of the odonates of riparian zones depends on season and land use types. Endemics and habitat specialists are restricted to undisturbed riverine ecosystems as they possess a narrow range of habitat tolerance. Conservation of riparian zone results in the conservation of endemics of odonates (Subramanian 2007; Subramanian et al. 2008). The present study investigated the odonate diversity and abundance of midstream Chalakkudy river giving special reference to endemics.

METHODS

The survey was conducted once a month from February 2018 to January 2019 by conventional random sampling. Six locations of midstream Chalakkudy River were randomly selected for the observation of odonates. The river is 13.5 km (approximately) long from the first location to last one (Bachan 2003). The details of the study localities are given in Table 1. All the six locations are with rocky river bed and evergreen and semi evergreen forest vegetation. *Madhuca neriifolia, Syzigium occidentale, Humboldtia vahliana, Elaeocarpus*, and *Homonoia riparia* are the dominant species of flowering plants in these locations (Bachan 2010). The selected locations have been confronted with anthropogenic disturbances such as habitat alteration due to tourism activities including resorts & commercial establishments, oil palm plantations, and activities of local people. The odonates were documented and identified with the help of photographs, keys, and descriptions given in the literature (Fraser 1933, 1934, 1936; Kiran & Raju 2013). The species richness and abundance were recorded and Simpson & Shannon diversity indices and eveness values were calculated using PAST software. The observed species of odonates were categorized as VC—Very common (180–240 sightings), CO—Common (120–180 sightings), OC—Occasional (60–120 sightings), and RA—Rare (1–60 sightings)) depending upon their occurrence during the survey (Palot et al. 2005; Tiple et al. 2012).

Table 1. Odonate collection localities.

	Sample collection sites	Latitude	Longitude	Altitude (m)
L1	Ezhattumugham	10.295	76.451	39
L2	Chiklayi	10.294	76.470	46
L3	Ayyampuzha	10.292	76.478	47
L4	Vettilappara	10.289	76.512	64
L5	Athirappilly	10.285	76.558	86
L6	Athirappilly waterfalls	10.284	76.569	116

RESULTS

During the study period, 2,186 individuals of 25 species were observed. Out of these, 10 species were dragonflies of the suborder Anisoptera, belonging to seven genera and the family Libellulidae. The remaining 15 species were damselflies under the suborder Zygoptera and they come under 11 genera in six families (Tables 1, 2). Libellulidae is the only anisopteran family, which has been observed among the odonates in the present survey. *Orthetrum sabina* a well-known cannibalistic dragonfly, has been found to be very common. On the other hand, *Onychothemis testacea* was encountered very rarely during the present survey. Members of the family Coenagrionidae (6 species) were dominating in the order Zygoptera succeeded by Calopterigidae (3 species) and Platycnemididae (3 species). *Vestalis apicalis* and *Prodasineura verticalis* were common but *Aciagrion occidentale* was observed only sporadically in this region. Out of the 25 species recorded, five species are endemics and they belong to the suborder Zygoptera. But *Pseudagrion indicum* is endemic to Western Ghats, while *Vestalis apicalis* is endemic to southern and central India, *Libellago indica* is endemic to peninsular India, whereas *Dysphaea ethela* and *Heliocypha bisignata* are endemic to India (Kalkman et al. 2020). The most dominant endemic species observed in the present survey was *Dysphaea ethela* and *Heliocypha bisignata*, which exhibited a minimum level of occurrence. The percentage distribution of each endemic species is as follows: *Pseudagrion indicum* 9%, *Vestalis apicalis* 26%, *Libellago indica* 28%, *Dysphaea ethela* 34%, and *Heliocypha bisignata* 3%. The first location Ezhattumugham (L1) harboured as many as 536

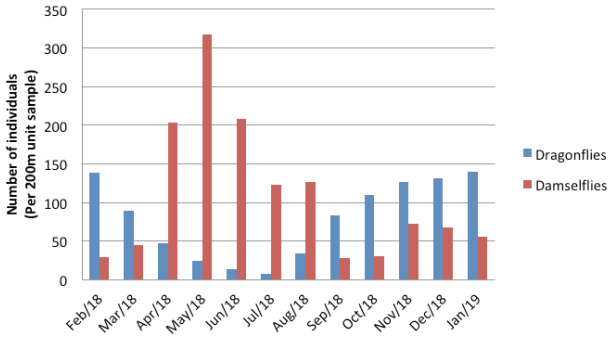

Figure 1. Abundance of dragonflies and damselflies in the Chalakudy River.

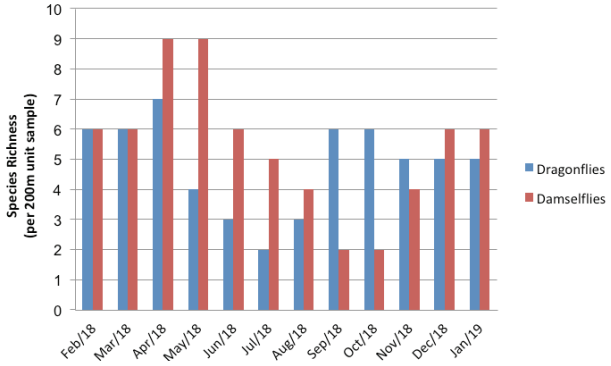

Figure 2. Species richness of dragonflies and damselflies in the Chalakudy River.

individuals in 21 species. *Vestalis apicalis* was the most abundant, and endemic species. *Onychothemis testacea* and *Zygonyx iris* were recorded only from this location. The highest number of endemics were also recorded from here. Inspite of the disturbances from tourists, this location showed a good quantity of native vegetation including emergent vegetation and shade cover and that perhaps resulted in the collection of a maximum number of individuals. The second location, Chiklayi (L2) yielded a maximum observation of 363 individuals of 17 species. *Orthetrum sabina* was the common species but *Libellago indica* was the prevalent endemic of this location. The habitat is rocky in nature with moderate shade cover and prominent emergent vegetation. Tourists' activities are appreciably low and the native vegetation is limited by oil palm plantation. Maximum value of diversity indices was shown by location. The third location, Ayyampuzha (L3) was polluted by the activities of local people and tourists to some extent. But the oil palm plantation ousted the native vegetation. From this location having traces of shoreline plants, limited shade cover, boulders and rocks, 284 individuals of 15 species were recorded of which, *Trithemis aurora* was dominant with the endemic species *Libellago indica.* Vettilappara (L4) is yet another location having least human interference with appreciable shade cover and riparian vegetation. But the native riparian vegetation is narrowed into a thin belt by the plantation crops. *Libellago indica* (endemic) and *Pseudagrion rubriceps* were the commonly found species during the study period. A total of 501 individuals belonging to 17 species were encountered in Vettilappara. Athirappilly (L5) is slightly polluted by human activities (tourism and nearby construction works) with minimum shade cover and moderate emergent vegetation. Eighteen species were recorded during the survey. *Orthetrum sabina* and *Prodasineura verticalis* were the common species

Table 2. List of dragonflies recorded from Chalakudy River.

	Scientific name (Family: Libellulidae)	Abundance	IUCN status
1	*Diplacodes trivialis* (Rambur, 1842)	O	LC
2	*Neurothemis tullia* (Drury, 1773)	O	LC
3	*Onychothemis testacea* (Laidlaw, 1902)	R	LC
4	*Orthetrum chrysis* (Selys, 1891)	R	LC
5	*Orthetrum pruinosum* (Burmeister, 1839)	R	LC
6	*Orthetrum sabina* (Drury, 1770)	VC	LC
7	*Pantala flavescens* (Fabricius, 1798)	O	LC
8	*Trithemis aurora* (Burmeister, 1839)	VC	LC
9	*Trithemis festiva* (Rambur, 1842)	C	LC
10	*Zygonyx iris* (Selys, 1869)	R	LC

VC—Very common | CO—Common | OC—Occasional | RA—Rare | EN—Endemic.

found along with the frequently encountered endemic damselfly, *Libellago indica*. Athirappilly waterfalls (L6) is another beautiful location where the tourists activities are significantly high and endowed with rocky habitat and riparian vegetation. But the presence of macrophytes and overhanging vegetation is scanty due to tourists disturbances. As a result, the numerical abundance of species recorded from this location was very less. However, the endemic dragonflies, *Dysphaea ethela* and *Vestalis apicalis* were the dominating species of this location.

Effect of flood

During the month of August of the study period, heavy down pour at Kerala led to a deluge and it badly affected the study areas. Riparian vegetation was totally destroyed. Natural soil texture was lost, soil accumulation could be found in river and river banks. As a consequence, a sudden drop in damselfly diversity

Table 3. List of damselflies recorded from Chalakudy River.

	Scientific name (Suborder: Zygoptera)	Abundance	IUCN Red List status
	Family: Calopterygidae		
1	*Neurobasis chinensis* (Linnaeus, 1758)	R	LC
2	*Vestalis apicalis* (Selys, 1873)	VC & EN	LC
3	*Vestalis gracilis* (Rambur, 1842)	C	LC
	Family: Chlorocyphidae		
4	*Libellago indica* (Fraser, 1928)	C & EN	LC
5	*Heliocypha bisignata* (Hagen in Selys, 1853)	R & EN	LC
	Family: Coenagrionidae		
6	*Aciagrion occidentale* (Laidlaw, 1919)	R	LC
7	*Agriocnemis pieris* (Laidlaw, 1919)	R	LC
8	*Agriocnemis pygmaea* (Rambur, 1842)	R	LC
9	*Ischnura rubilio* (Brauer, 1865)	R	LC
10	*Pseudagrion indicum* (Fraser, 1924)	O & EN	DD
11	*Pseudagrion rubriceps* (Selys, 1876)	C	LC
	Family: Euphaeidae		
12	*Dysphaea ethela* (Fraser, 1924)	VC & EN	LC
	Family: Platycnemididae		
13	*Copera marginipes* ((Rambur, 1842)	R	LC
14	*Copera vittatta* (Selys, 1863)	R	LC
15	*Prodasineura verticalis* (Selys, 1860)	VC	LC

was noticed just after the flood. Only two species of damselflies were recorded in the first two months after the flood, i.e., September and October 2018. But dragonfly diversity was not much affected. In the succeeding months the species richness and abundance were observed to have rebounded.

Simpson & Shannon diversity indices and evenness values of the six locations were calculated (Table 4). Maximum species richness and abundance were found at Location 1. Simpson and Shannon diversity indices (0.9197 and 2.628, respectively) were found to be equally high for location 2, while the least values were shown by Location 6 (0.8694 and 2.191, respectively). Maximum value of evenness (0.8257) was recorded at Location 3 and a minimum at Location 1.

DISCUSSION

The current study points out the role of native riparian vegetation and the impact of human interference such as habitat alteration by tourism, construction works and plantations on the density and diversity of odonate fauna. Studies revealed that riparian vegetation promotes the occurrence of invertebrates including insects and facilitates suitable habitat for insects by providing food, resting and hiding places for emergent adults and substratum for egg laying. Also the shade cover regulates water temperature and overall quality of the stream (Knight & Bottorff 1981; Ober & Hayes 2008). Moreover, the prey insects are attracted by flowering plants, which in turn form ideal food for odonates. Therefore, these conditions become more pertinent for the carnivorous odonates. The hanging plants and emergent macrophytes furnish perching sites and structures for egg laying and emergence of adults. Literature delineates the role of macrophytes and shoreline structures in oviposition, formation of larval microhabitat, emergence support and adult perching site (Samways & Steytler 1996; Schindler et al. 2003).

In the present study 15 species of damselflies and 10 dragonflies were recorded. As the damselflies are weak fliers, they may depend on their own microhabitat for food and reproduction. But the agile fliers, dragonflies are free to move to more extensive habitats according to their preferences. This is a factor of variation in species richness between the two suborders. The most commonly encountered dragonfly was *Orthetrum*

Table 4. Community structure of odonates.

Parameters/ Indices	L1 Ezhattumugham	L2 Chiklayi	L3 Ayyampuzha	L4 Vettilappara	L5 Athirappilly	L6 Athirappilly waterfall
Species richness	21	17	15	17	18	12
No. of individuals (per 200m unit sample)	536	363	284	501	377	125
Simpson 1-D	0.8983	0.9197	0.9091	0.9121	0.9064	0.8694
Shannon H	2.518	2.628	2.517	2.561	2.545	2.191
Evenness	0.5907	0.8142	0.8257	0.7617	0.7079	0.7456

Image 1. *Dysphaea ethela*

Image 2. *Pseudagrion indicum*

Image 3. *Libellago indica* (male)

Image 4. *Libellago indica* (female)

Image 5. *Vestalis apicalis*

Image 6. *Heliocypha bisignata*

sabina, which predate on other insects and exhibits cannibalistic behavior too (Iswandaru 2018). Further, adequate quantities of reeds support the occurrence of damselflies than dragonflies (Fulan et al. 2008). In the present study, L1, L2, L4 and L5 locations showed the maximum species richness, abundance and diversity. Despite the human disturbances, L1 showed the highest value of species richness and abundance. Presence of comparatively abundant native vegetation including emergent macrophytes supported the diversity in L1. Moreover, in L2, L3 and L4 sites, the native riparian vegetation is narrowed by the plantation crops. Vegetation in location L5 was destroyed as a result of resort construction. Pristine habitat loss results in the loss of odonate diversity (Rodrigues et al. 2016). But the presence of a modest percentage of riparian vegetation could hold up the diversity in these locations to some extent. Although L6 is devoid of plantation crops, the prominent disturbances from tourists have destroyed the emergent macrophytes and overhanging vegetation. This has led to the least diversity indices on species richness and abundance in L6. Another observation noticed in the present study was on the high abundance of endemic species in L1 and minimum distribution at L6. *Dysphaea ethela* and *Heliocypha bisignata* were reported to be respectively the common and rarely occurring endemic species.

As per the literature, undisturbed riparian forests are typically rich with the presence of endemics (Subramanian et al. 2008). Destruction of riparian flora and fauna could be attributed to damming, tourists activities, construction works and expanding the area for agricultural plantations leading to the declined number of species. For instance, it is evident that the fish fauna of Chalakkudy river is highly threatened by damming, deforestation and pesticide pollution (Raghavan et al. 2008). Habitat alteration interferes with the abundance of endemic odonates and supports the occurrence of generalist species like libellulids (Kalkman et al. 2008; Subramanian et al. 2008), and that is evident in the present study. Research work delineates the resilience capacity of organisms to flood (Death 2008; Golab & Sniegula 2012; Raghavan 2019). Inspite of the destructive flood during the current study, odonates showed a tendency to bounce back to pre-flood conditions within a very short time. Further studies are required to authenticate the same.

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New Additions to the Odonate Fauna of Thrissur District, Kerala With Their Ecological Notes

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ABSTRACT

Thrissur district encompasses the central region of Kerala state, with its cross section including a variety of ecosystems such as forest lands, plain lands, kole wetlands and seashore ecosystems comprising a rich diversity of Odonate fauna. Odonates from different habitats of Thrissur District were observed from January 2017 to August 2018. Selected habitats include forested landscapes, plains, paddy fields etc. A total of 60 species of odonates could be encountered during the present study. Out of these , 16 are newly recorded from Thrissur district. *Paracercioncalamorum*, which is rarely found in Kerala has been recorded from Poomala dam reservoir, perching on lotus leaves. *Onychothemistestacea*, *Aciagrionoccidentale*, *Agriocnemissplendidissima*, *Pseudagrion decorum*, *Pseudagrionmalabaricum* and *Protostictagravelyi*are the other rarely found odonates which is newly recorded by the present study from Thrissur district. As a result of the present study, the number of odonates from Thrissur district reaches 70 from the existing 54. This makes 45% of the total diversity of Kerala.

KEYWORDS: Kole wetlands, *Paracercioncalamorum*, *Onychothemistestacea*, *Protostictagravelyi.*

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INTRODUCTION

Odonata is the order of dragonflies and damselflies and it is one of the ancient orders of insects. About 6000 species of Odonates are found all over the world. Odonata fauna of India was revealed first through Fraser(1933,1934 and 1936).Subramanian(2009) has made a checklist of 473 species of odonates of India.

A lot of studies and surveys on odonate fauna have been conducted in Kerala also. Peters (1981) reported 26 species of Odonates from Thiruvananthapuram district. An addition of 17 species to this record was done by Emiliyamma & Radhakrishnan (2002). Mathavan & Miller(1989) recorded 36 species of odonates from Periyar National Park. From the Parambikulam Wildlife Sanctuary 25 species were recorded (Emiliyamma & Radhakrishnan 2000). A record of 31 species of odonata from Kottayam district was presented(Emiliyamma ,2005).Odonate diversity of Kerala was well documented by Kiran & Raju (2013) and prepared a checklist of 154 species of Odonates. Odonates of Southern Western ghats were studied and 169 species of odonates were reported. (Emiliyamma, 2014).A total of 82species were recorded from Salim Ali Bird Sanctuary, Thattekkad (Varghese et. al. 2014). Odonate diversity of Kerala Agricultural University campus, Thrissur was well documented Adarshet. al. (2014) and 52 species of odonates were recorded. Odonate diversity of Chinnar wildlife sanctuary was recorded as 48 species (Adarsh et. al. 2015). Dragonfly diversity of Irinjalakkuda was studied by Gigi et. al. (2016) and reported 12 species of dragonflies. 36 species of Odonates were reported from a riparian ecosystem, Meenachilriver basin, Kottayam (Vincy et. al.,2016). 68 species of odonates were reported from Varadoor, Kannur (Nair, 2017).

Study area

The study was conducted in different habitats of Thrissur district, situated in the central Kerala (10.52^0N – 76.21^0E). This district spans an area of 3032Km^2. The land of Thrissur district slopes down from the Western ghats to Arabian sea. The habitats such as forested landscapes, mountain streams, inland waterbodies, paddy fields and marshes etc are selected for the survey. The period of observation was from January 2017 to July 2018. This period of observation includes pre monsoon, monsoon and post monsoon season.

MATERIALS AND METHODS

The Odonate survey was conducted in different localities of Thrissur district such as Kodungallur,Valappad, Mala, Chalakkudy, Nellayi, Mannamangalam, Marottichal, Athirappilly, Kanimangalam, Valiyalukkal, Nedupuzha,Kodannur,Manakkodi, Alappad, Thrissur, Poomala,Peechi,Wadakkancherry, Kunnamkulam.

Field observations were done between 9 am and 1 pm when the odonates are more active.Odonates were caught using insect net , identified with the help of photographic field guides (Kiran&Raju, 2013; Subramanian, 2009) and released to conserve biodiversity.

1. Onychothemistestacea(Laidlaw, 1902)

Description: Male: Black and yellow face with green eyes. Thorax is dark metallic green with yellow stripes. Black legs and transparent wings.Black and stout abdomen having yellow spots dorsally and laterally.

Female: Similar to male.

Ecological notes: Major habitats are forested streams. It is a fast flying dragonfly,chases other odonates aggressively. During the present study, it was located at Athirappilly and Thumboormuzhy perching on twigs overhanging streams.

2. *Zygonyx iris* (Selys,1869)

Description: Male: Eyes are reddish brown above and pale grey below. Metallic greenish black thorax with yellow spots. Abdomen is metallic black with yellow rings. 7^{th} segment has a broad dorsal yellow spot. Black legs and transparent wings.

Female: Similar to male. Thorax have more prominent yellow stripes.

Ecological notes: It was encountered as hovering over fast flowing streams inAthirappilly and Thumboormuzhy. Breeds in forested streams.

3. Aciagrionoccidentale(Laidlaw, 1919)

Description: Male: Eyes are sky blue below and black above. A blue line is present behind the head by connecting both eyes. Dorsal side of thorax is black with two bluish green stripes. Ventral side is sky blue. Legs are pale blue with black outer side. Transparent wings. Abdomen is black with blue rings. Ventral side and last three segments of abdomen are sky blue. 8^{th} and 10^{th} segments have a black spot on dorsal side.

Female: Resembling male in size and shape. But the colour is greenish blue instead of sky blue. Stripes of thorax are yellow coloured.

Ecological notes: It is not very common. Found in marshes, ponds and streams. During the survey it was located at Mannamangalam, Marottichal and Kanimangalam darting among grasses and shrubs.

4. Agriocnemissplendidissima(Laidlaw, 1919)

Description: Male: Eyes are black above and apple green below. Thorax is black with two pale blue dorsal stripes. Ventral side of thorax is also pale blue. Bluish black legs.Transparent wings.Abdomen pale blue with extensive black markings.Hook shaped anal appendages.

Female: Resembling male but reddish brown thorax instead of black.

Ecological notes: It is not a common damselfly. Seen in marshes and weedy ponds of forested landscapes.Perches on vegetation close to water. It was found during the survey, darting among emergent vegetation in marshes of Poomala dam reservoir.

5. *Paracercioncalamorum*(Ris, 1916)

Description: Male: Eyes are olivaceous green with brownish black dorsal side. Head and thorax are pruinosed , they are bluish grey in colour. Thorax with fine hairs.Femora of leg is also pruinosed. Transparent wings. Abdomen is black but last segments are sky blue. Base of abdomen is also pruinosed.

Female: Similar to male but abdomen is brownish orange in colour with dorsal black marking.

Ecological notes: It is rarely reported in Kerala. During the study it was located in the marshes near Poomala dam reservoir, perching on lotus leaves. Eggs are inserted well in lotus leaves.

6. *Protostictagravelyi (Laidlaw, 1915)*

Description: Male: Blackish brown eyes. Thorax is metallic black with creamy white stripes. Pale white legs.Transparent wings.Black abdomen with creamy white rings at the end of segments.
Female: Abdomen is shorter and stouter than that of male.

Ecological notes: It is a rarely found damselfly. Males were found in shaded streams of Marottichal waterfalls and females on tree bark near water bodies.

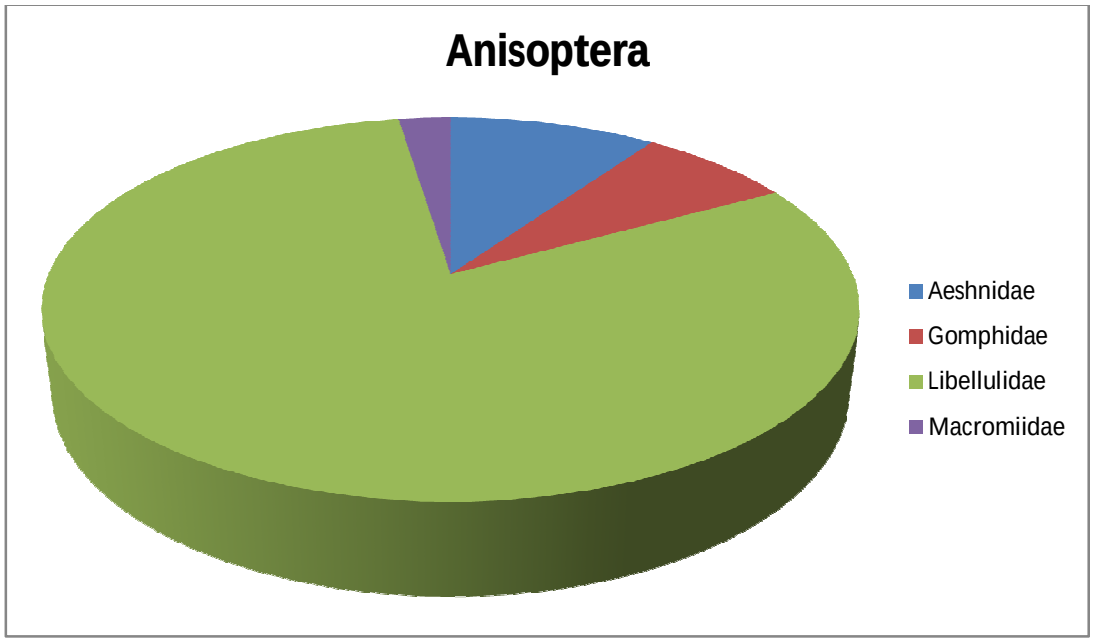

Figure 1: Family wise contribution of Dragonflies in Thrissur district.

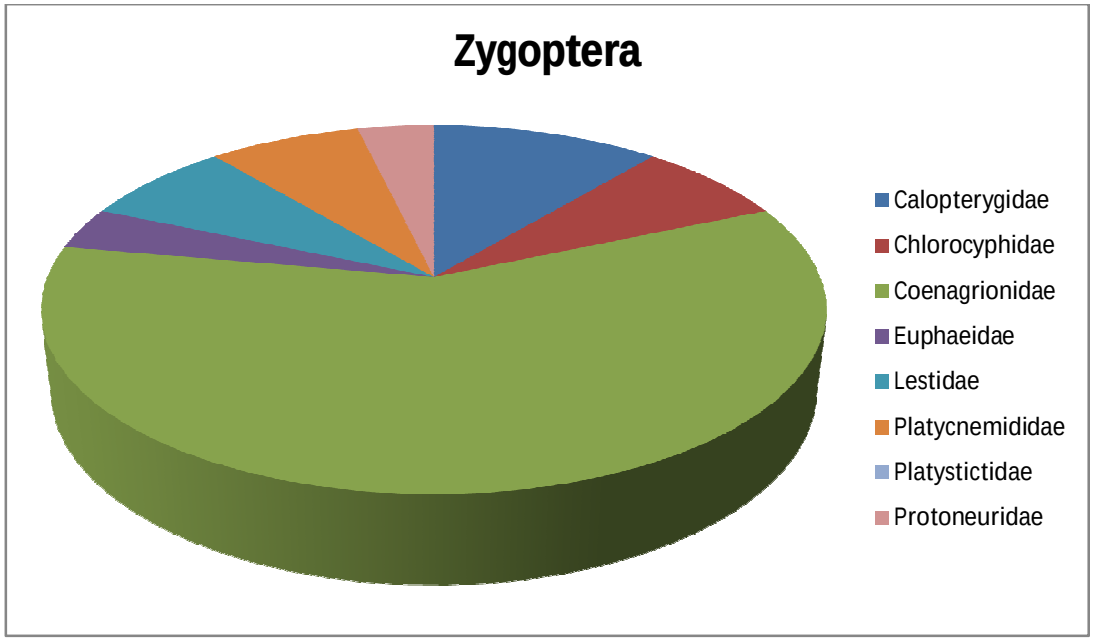

Figure 2: Family wise contribution of Damselflies in Thrissur district

Table 1: List of odonates observed during the survey

Sl No.	Scientific Name	**Common Name**	**Locations**	**Status**
	Suborder :Anisoptera			
	Family: Aeshnidae			
1.	*Anaxguttatus (Burmeister, 1839)**	Blue Tailed Green Darner	Mannamangalam	C
2.	*Anaximmaculifrons (Rambur, 1842)*	Blue darner		C
3.	*Gynacanthabayadera (Selys, 1891)*	Parakeet darner		U
4.	*GynacanthadravidaLieftinck, 1960*	Brown darner	Common	
	Family: Gomphidae			
5.	*Heliogomphuspromelas (Selys, 1873)*	Spotted Lyretail		U
6.	*Ictinogomphusrapax (Rambur, 1842)*	Common Clubtail	Common	C
7.	*Paragomphuslineatus (Selys, 1850)*	Common Hooktail		U
	Family: Libellulidae			
8.	*Acisomapanorpoides (Rambur, 1842)*	Trumpet Tail	Common in plains and paddy fields	C
9.	*Aethriamantabrevipennis (Rambur, 1842)*	Scarlet Marsh Hawk	Common in all habitats	C
10.	*Brachydiplaxchalybea (Brauer, 1868)*	Rufous Backed Marsh Hawk	Common in plains	C
11.	*Brachydiplaxsobrina (Rambur, 1842)*	Little Blue Marsh Hawk	Mala	U
12.	*Brachythemiscontaminata (Fabricius, 1793)*	Ditch Jewel	Common in plains	C
13.	*Bradinopygageminata (Rambur, 1842)*	Granite Ghost	Common in all habitats	C
14.	*Crocothemisservilia (Drury,1770)*	Ruddy Marsh Skimmer	Common in plains	C
15.	*Diplacodesnebulosa (Fabricius, 1793)*	Black Tipped Ground Skimmer	Nellayi, Poomala	U
16.	*Diplacodestrivialis (Rambur,1842)*	Ground Skimmer	Common in all habitats	C
17.	*Hydrobasileuscroceus (Brauer, 1867)*	Amber Winged Marsh Glider	Kanimangalam, Poomala	U
18.	*Indothemiscarnatica (Fabricius, 1798)*	Black Scrub glider		R
19.	*Lathrecistaasiatica (Fabricius, 1798)*	Asiatic Blood Tail	Common in all habitats	C

20.	*Neurothemisfulvia (Drury, 1773)*	Fulvous Forest Skimmer	Marottichal	C
21.	*Neurothemistullia (Drury, 1773)*	Pied Paddy Skimmer	Common in all habitats	C
22.	*Onychothemistestacea (Laidlaw, 1902)**	Stellate River Hawk	Athirappilly	U
23.	*Orthetrumchrysis (Selys, 1891)*	Brown Backed Red Marsh Hawk	Common in all habitats	C
24.	*Orthetrumglaucum (Brauer, 1865)*	Blue Marsh Hawk	Marottichal	C
25.	*Orthetrumluzonicum (Brauer, 1868)*	Tricoloured Marsh Hawk	Mannamangalam	C
26.	*Orthetrumpruinosum (Burmeister, 1839)*	Crimson- Tailed Marsh Hawk	Athirappily	C
27.	*Orthetrum Sabina (Drury, 1770)*	Green Marsh Hawk	Common in all habitats	C
28.	*Pantalaflavescens (Fabricius, 1798)*	Wandering Glider	Common in all habiats	C
29.	*Potamarcha congener (Rambur, 1842)*	Yellow Tailed Ashy Skimmer	Kodungallur, Mannamangalam, Nellayi	C
30.	*Rhodothemisrufa (Rambur, 1842)*	Rufous Marsh Glider	Nellayi	C
31.	*RhyothemisVariegata (Linnaeus, 1763)*	Common Picturewing	Common in all habitats	C
32.	*Tetrathemisplatyptera*	Pigmy Skimmer	Mannamangalam, Kanimangalam	C
33.	*Tholymistillarga*	Coral Tailed Cloud Wing	Kanimangalam, Mala, Nellayi, Kodungallur	C
34.	*Tramealimbata (Desjardins, 1832)*	Black Marsh Trotter	Mannamangalam, Kunnamkulam	C
35.	*Trithemis aurora (Burmeister, 1839)*	Crimson Marsh Glider	Common in forests and plains	C
36.	*Trithemisfestiva (Rambur, 1842)**	Black Stream Glider	Common in forested landscapes	C
37.	*Trithemispallidinervis (Kirby, 1889)*	Long Legged Marsh Glider	Poomala, Kole fields	C
38.	*Urothemissignata (Rambur, 1842)*	Greater Crimson Glider	Common in all habitats	C
39.	*Zygonyx iris (Selys, 1869)**	Iridescent Stream Glider	Athirappilly	C
40.	*Zyxommapetiolatum (Rambur, 1842)*	Brown Dusk Hawk	Common in all habitats	C
	Family: Macromiidae			
41.	*Epophthalmiavittata (Burmeister, 1839)*	Common Torrent Hawk		
	Suborder: Zygoptera			
	Family: Calopterygidae			
42.	*Neurobasischinensis (Linnaeus, 1758)**	Stream Glory	Athirappilly	C
43.	*Vestalisapicalis (Selys, 1873)*	Black Tipped Forest Glory	Mannamangalam, Marottichal, Athirappilly	C
44.	*Vestalisgracilis (Rambur, 1842)*	Clear Winged Forest Glory	Athirappilly, Mannamangalam, Marottichal	C
	Family: Chlorocyphidae			
45.	*Libellagolineata (Burmeister, 1839)*	River Heliodor	Athirappilly, Marottichal	C
46.	*Rhinocyphabisignata (Hagen in Selys, 1853)**	Stream Ruby	Athirappilly, Marottichal	C
	Family: Coenagrionidae			
47.	*Aciagrionoccidentale (Laidlaw, 1919)**	Green Striped Slender	Kanimangalam, Mannamangalam	U
48.	*Agriocnemiskeralensis (Peters, 1981)**	Kerala Dartlet	Common in plains	C
49.	*Agriocnemispieris (Laidlaw,1919)*	White Dartlet	Common in all habitats	C
50.	*Agriocnemispygmaea (Rambur, 1842)*	Pygmy Dartlet	Common in all	C

			habitats	
51.	*Agriocnemissplendidissima (Laidlaw, 1919)**	Splendid Dartlet	Poomala	U
52.	*Ceriagrioncerinorubellum*	Orange Tailed Marsh Dart	Common in all habitats	C
53.	*Ceriagrioncoromandelianum*	Coromandel Marsh Dart	Common in plains	C
54.	*Ceriagrionrubiae (Laidlaw, 1916)*	Orange Marsh Dart		
55.	*Ischnura aurora (Brauer, 1865)*	Golden Dartlet	Common in all habitats	C
56.	*Ischnurasenegalensis (Rambur, 1842)**	Senegal Golden Dartlet	Kodungallur, Nellayi	C
57.	*Paracercioncalamorum (Ris, 1916)**	Dusky Lily Squatter	Poomala	R
58.	*Pseudagrion decorum (Rambur, 1842)**	Green Striped Grass Dart	Poomala	R
59.	*Pseudagrionindicum (Fraser, 1924)**	Yellow Striped Grass Dart	Athirappilly	U
60.	*Pseudagrionmalabaricum (Fraser, 1924)**	Jungle Grass Dart	Poomala	R
61.	*Pseudagrionmicrocephalum (Rambur, 1842)*	Blue Grass Dart	Common in plains	C
62.	*Pseudagrionrubriceps (Selys, 1876)*	Saffron Faced Grass Dart	Peechi, Athirappilly	C
	Family: Euphaeidae			
63.	*Dysphaeaethela (Fraser, 1924)*	Black Torrent Dart	Athirappilly	U
	Family: Lestidae			
64.	*Lesteselatus (Hagen in Selys, 1862)*	Emerald Spreadwing		C
65.	*Lestespraemorsus (Hagen in Selys, 1862)*	Sapphire Eyed Spreadwing		U
	Family: Platycnemididae			
66.	*Coperamarginipes (Rambur, 1842)*	Yellow Bush Dart	Common in all habitats	C
67.	*Coperavittatta (Selys, 1863)**	Blue Bush Dart	Common in forested landscapes	C
	Family: Platystictidae			
68.	*Platystictadeccanensis (Laidlaw, 1915)*	Saffron Reedtail		
69.	*Protostictagravelyi (Laidlaw, 1915)**	Pied Reedtail	Marottichal	C
	Family: Protoneuridae			
70.	*Prodasineuraverticalis (Selys, 1860)*	Black Bambootail	Athirappilly, Marottichal	C

RESULTS& DISCUSSION

Up to 60 species of odonates could be recorded from different habitats of Thrissur district as a result of this survey. Out of these, 35 species belong to 26 genera and 3families and come under suborder Anisoptera. The remaining 25 species belong to 15genera and 6 families and come under suborder Zygoptera.

Nine species i.e. *Lesteselatus, Lestespraemorsus, Ceriagrionrubiae, Anaximmaculifrons, Gynacanthabayadera, Heliogomphuspromelas, Paragomphuslineatus, Epophthalmiavittata and Indothemiscarnatica*reported earlier by Adarsh et. Al (2014) , were not encountered during the survey. *Platystictadeccanensis*reported by Subramanian et. al. (2011) was also not seen during the present survey. Addition of these species with the present data results in a total of 70 species from Thrissur district.

Family Libellulidae with 33 species is found as the dominant family of dragonflies, followed by families Aeshnidae (4 species), Gomphidae (3 species) and lastly family Macromiidae (1 species).

In the case of damselflies, Family Coenagrionidae with 16 species is the dominant one followed by families Calopterygidae (3 species), Chlorocyphidae (2 species), Lestidae (2 species), Platycnemididae (2species), Platystictidae (2species), Euphaeidae (1 species) and Protoneuridae (1 species) respectively.

*Brachydiplaxsobrina, Diplacodesnebulosa, Hydrobasileuscroceus, Onychothemistestacea*are the rarely encountered dragonflies during the study. The rarely found damselflies are *Aciagrionoccidentale, Agriocnemissplendidissima, Paracercioncalamorum, Pseudagrion decorum, Pseudagrionmalabaricum, Protostictagravelyi* etc.

Although the IUCN status of *Paracercioncalamorum*is Least concern, it is rarely found in Kerala. During the present study it is encountered near the water reservoir of Poomala dam, perching on lotus leaves.

More number of species could be observed in forested landscapes like Athirapally ,Marottichal, Mannamangalam and Poomala. Majority of rare species are also found there. Although increased number of odonates could be found in kole wet lands of Kanimangalam, Nedupuzha, Manakkodi, Pullu etc. number of species found was moderate.

*Neurothemistullia, Rhyothemisvariegata, Orthetrum Sabina, Brachythemiscontaminata*etc. are the common dragonflies in all seasons. Large swarms of *Pantalaflavescens*was seen mainly in Post monsoon season. Commonly found damselflies are Ceriagrioncoromandelianum, Ceriagrioncerenorubellum and Agriocnemispygmaea. Along with common form, a pruinosed form of Agriocnemispygmaeawas encountered in Athirappilly.

45% of total odonates of Kerala can be reported by this study. Further studies in this topic is significant as a lot of odonate species have to be revealed to explore the biodiversity of Thrissur district.

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