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.