

# Introduction

Biological diversity refers to the variety and variability of living species from a wide range of environments, including terrestrial, marine and other aquatic ecosystems. It gives information on the present status of species evolution and other ecological processes in connection to the ecosystem. Biological diversity contributes an essential role in various aspects of ecosystem stability (Peterson *et al.*, 1998). Biodiversity study is recognized as a vital parameter to assess the global and local environmental changes and sustainable development (Jana *et al.*, 2006). The world is currently facing massive biodiversity loss due to climate change, human overpopulation, pollution, habitat destruction, the effects of invasive species, harvesting, excessive recreational use and natural ongoing geological processes (Wagner and Van Driesche, 2010). Human conflicts with nature mainly cause biodiversity loss, environmental pollution and continuous exploitation of ecological resources. Ecosystem services such as productivity and nutrient retention in the soil suffer when a community's diversity and composition deteriorate (Loreau *et al.*, 2001). The most commonly used operational unit to prevent environmental changes and further biodiversity loss is multicellular species (Purvis and Hector, 2000).

Recording the information of species diversity, distribution, species richness, biogeography, ecology, biology and evolution in an ecosystem is a key step towards identifying the biodiversity hotspots and great importance in designing effective conservation strategies to prevent the loss of biodiversity. Currently, biodiversity conservation is an important focus for scientists and decision-makers because biodiversity is a foundation of ecosystem function and provides the life-support system of the earth (Waters *et al.*, 2016).

Insects are the most abundant and diversified faunal group of the dynamic ecosystem by providing multiple services (Miller, 1993). Insects have been employed as landmark studies in biomechanics, developmental biology, climate change, evolution, ecology, physiology and genetics. The public is familiar with them because of their various and varied characters and conservation is a challenge (Jalali *et al.*, 2015). Insects are essential to

the diversity, distribution and quantity of plants and birds in an ecosystem. Species richness and abundance of many groups of insects have been threatened due to various environmental factors (Didham *et al.*, 1996). Insect outbreaks may significantly affect invertebrate and vertebrate diversity, either directly by altering food availability or indirectly by altering their habitat suitability.

Order Orthoptera is one of the largest and most diverse groups of insects belonging to the monophyletic suborders Caelifera called short-horned Orthopterans it includes the family Acrididae, Pyrgomorphidae and Tetrigidae; Ensifera called long-horned Orthopterans it has family Tettigoniidae, Gryllidae, Haglidae and Gryllotalpidae. The name of the order “Orthoptera” refers to primitive or generalized winged insects and is derived from the Greek word “Ortho” means straight and “Pteron” means wing. Order Orthoptera constitutes of 22,500 species (Ghosh and Sengupta, 1982) worldwide, out of which 1,750 species, 400 genera and 21 families are known from India (Tandon and Hazra, 1998). There are currently 27,260 Orthoptera fauna species known to exist in the world (Eades *et al.*, 2016). Orthopterans are one of the most well-known invertebrate groups in terrestrial environments. These are found in all kinds of natural and anthropogenic habitats and are an essential component of both healthy and disturbed ecosystems as well as considered to be of both ecological and conservation importance (Latchininsky *et al.*, 2011). Orthopterans are distributed throughout the physiographic zones of the world but their distribution largely relies on the vegetation like grasslands, agricultural fields and forests (Thakkar *et al.*, 2015). Grasshopper biodiversity is a result of grassland ecosystem evolution and this richness, in turn, impacts the structure and function of grassland ecosystems (Guo *et al.*, 2006). Grasshoppers account for more than half of the total arthropod biomass in the above-ground grass layer in grassland ecosystems (Gillon, 1983).

Orthopterans play a critical role in the environment, making it a safer and more efficient place for plants and other animals to thrive. Orthopterans are proposed as important bioindicators of threatened environments because of their unique microhabitat preferences, functional importance in ecosystems, sensitivity to changes in biotic and abiotic factors of their habitats and ease of sampling (Samways, 1997; Soliman *et al.*, 2017). It is one of the most important invertebrate groups for environmental monitoring and assessment. As pests, grasshoppers cause severe damage to crops and rangelands (Lockwood and Lockwood,

2008). Only a few species of Acrididae are important pests and the vast majority are not. Some species act as a biological control for weeds (Oberholzer and Hill, 2001). However, this group plays a significant role in terrestrial food webs because they are important primary herbivores and provide an abundant amount of protein to their predator's diets such as amphibians, birds, small reptiles and mammals, thereby maintaining the ecosystem balance; therefore, their scarcity may disturb an ecosystem trophic structure (Soliman *et al.*, 2017). Grasshopper population dynamics will be affected by resource management strategies at different trophic levels in the food chain (Capinera *et al.*, 1997). Orthopterans have also been successfully used in biomonitoring the effects of changes in land management regimes (Gardiner *et al.*, 2002, O'Neill *et al.*, 2003) and identifying the grasslands conservation status (Andersen *et al.*, 2001).

The grasshopper benefits human and the ecosystem in general by facilitating plant regrowth and decomposition. The death and moulting of a grasshopper provide extremely valuable minerals that can be naturally returned to the soil; microbes in the soil easily break down nitrogen-rich bodies, fertilizing the soil and helping carbohydrate-rich plants to grow (Jonas *et al.*, 2015). Grasshopper manure is an excellent soil amendment capable of increasing soil quality and their excrete is also highly beneficial for enriching the soil and promoting plant growth. The reduced size of the grasshopper feces allows microorganisms can break it down more quickly and fertilize the soil faster, thereby helping nutrient cycling into the ecosystem (Das *et al.*, 2010). Scientists utilize these insects as model frameworks for contemplating bioacoustics, life history characteristics, synthetic environment, transformative nature, physiology, neurobiology and speciation (Branson *et al.*, 2006).

Insect-plant interactions are important building blocks of terrestrial food webs in natural ecosystems and play an important role in ecological community function. These organisms are closely connected as insects have several beneficial behaviors such as defense and pollination, whereas plants provide food, shelter and oviposition. These three factors are required for insect breeding (Panda and Khush, 1995). Depending on plant-insect interactions, vegetation may affect community structure in different ways. If the insects are monophagous, their presence will rely on a particular host plant to survive. However, if insects are polyphagous, the plant species composition becomes more essential (Sirin *et al.*, 2010). Among phytophagous Orthopteran taxa, polyphagy is more widespread than

monophagy (Bernays and Mikenberg, 1997; Szövényi, 2002). Closely related plants support closely related insects (Price, 1997). Host plant availability is a critical factor for the fecundity of insects at both individual and population levels (Branson, 2006).

Orthopterans are exclusively phytophagous and they feed on a variety of vegetations. Their food resources are dependent on the quality and quantity of plant tissue. Plant tissue quality is dependent on chemical constituents, the physical qualities of the tissue and the water content in relation to the state of hydration of the individual (Chapman, 1990; Bernays and Bright, 1993). Plant leaves are of higher quality than stems and young tissue is of higher quality than older tissue. Stems and older tissue have lower protein levels and more indigestible tissue (Field, 1983; Anten *et al.*, 1998). The host plant range of the polyphagous grasshopper includes multiple plant families or specific species. The host plant range of the oligophagous grasshopper is limited to a single plant family. The monophagous grasshopper only feeds on the same plant genus as their host plants (Chapman, 1990). Moreover, the bivory group feeds on broad leaves herbs, the graminivory group feeds on Gramineacea grasses and the mixed herbivory group feeds on both forbs and herbs (Gangwere, 1991).

Most of the grasshoppers are oligophagous and show defined host preferences (Mulkern, 1967). Grasshoppers have chewing mouthparts and are often associated with foliage, but they also feed on flowers, seeds, fruits, stems, heads and other aerial plant parts. Differences in vegetation characteristics and associated heterogeneity strongly affect insect richness, abundance and taxonomic composition (Meyer *et al.*, 2002).

Furthermore, human activities in grasslands, such as grazing and farming, can alter the vegetation's properties, as well as the geographic structure and composition of grasshopper groups (Guo *et al.*, 2006). Grazing in various ways can result in a variety of changes in flora and grasshopper biodiversity (Kang, 1995; Kang, 1997). Changes in agricultural land use methods, cropping patterns and expansions are primarily due to urbanisation and industrialisation, which has an impact on grasshopper seasonal distribution patterns (Pareek *et al.*, 2017). Characteristics of soil are highly important on grasshopper's populations as they effect on oviposition, embryonic development and egg survival. Grasshopper mostly prefers porous soil for egg-laying; eggs are lay in pods (Bhusnar, 2015).

Understanding the proportional contributions of numerous elements in population and community processes is a long-standing topic in ecology because both intrinsic and extrinsic factors influence insect abundances and community structure (Turchin, 2003). Intrinsic impacts from biotic interactions can modify the pattern of species abundance and coexistence by acting through several density and frequency-dependent feedbacks (Brook and Bradshaw, 2006). Individual grasshopper performance is influenced by extrinsic environmental factors such as weather and land use, which impacts population dynamics in density-dependent ways (Pitt, 1999). Interactions between biotic and abiotic factors can lead to significant seasonal variation in the reproductive landscape of many insects.

Species diversity mostly depends on climatic conditions like temperature, rainfall and humidity. The abiotic factors such as climate and weather as well as yearly seasonal patterns severely affect the populations density, abundance, breeding, digestive efficiency and reproductive success of grasshoppers in various ways (Skinner and Child, 2000 and Karpakakunjaram *et al.*, 2002). Furthermore, the temperature has an impact on grasshopper life history features as clutch size and egg mass (Hassall *et al.*, 2007) and extreme temperatures may be associated with demographic changes that could lead to grasshopper outbreaks (Olfert and Weiss, 2006). Weather can also indirectly modify resource availability (White, 1993), habitat structure and the strength of species interactions (Ovadia and Schmitz, 2004).

Grasshoppers are a good model group for studying the effects of climate change on phenology because of their sensitivity to thermal conditions (Joern and Gaines, 1990). The thermal environment is extremely vital to grasshoppers because temperature affects all biological functions and ultimately fitness. At cool body temperature, the grasshopper moves very slowly and are unable to feed, whereas at high body temperatures, the grasshopper suffers an enzyme denature and death occurs (Chappell and Whitman, 1990). Rainfall stimulates the population by increasing the growth of food plants. However, high rainfall reduces the population. Humidity also stimulates the development of eggs (Köhler *et al.*, 1999).

The study of grasshoppers is needed because a large number of grasshoppers species are under the risk of extinction which is an indication of environmental influence. Therefore, the conservation of grasshoppers is highly needful in ecosystem balance. Once

ecosystems are disturbed, the population of a particular group of animals may increase or decrease. Low population of grasshopper's effect on food chain and high population of grasshopper's effect on rapid loss of vegetation. In a balanced ecosystem, have the self-ability to maintain a suitable level of population. Therefore, biodiversity conservation has become a major environmental challenge worldwide (Bhusnar, 2015).

Morphometrics is the measurements of morphological changes during the life of an insect. Investigations of body shape have been central to the biological sciences in order to understand systematics, taxonomy and evolution. Morphometric studies have contributed the main role in resolving the taxonomic problem. Morphometrics is defined as the quantitative description analysis and interpretation of the shape and variation of the structure in biology (Rohlf, 1990). Both hereditary and environmental factors influence insect morphology; variation in morphometric features may reveal important information about insect life in various ways (Adams *et al.*, 2004).

Morphometric features of grasshopper have been widely used to study the evolution of body size, life history and color patterns (Ahnesjö and Forsman, 2003). Morphometric approaches have proven to be useful in differentiating morphologically similar groups in the absence of any other diagnostic features (Claridge *et al.*, 1983 & 1985). Among Orthoptera, this technique has been widely used to distinguish new species and subspecies in groups of closely related taxa (Barrientos, 1988 & 1998).

Insect morphometric traits are very useful in population and community research and are used to identify nymph phases, sexual dimorphism features and ecological studies of populations and groups. Body size is an essential physiological characteristic that affects many aspects of an individual's function and performance, including foraging, growth rate and survival (Belovsky, 1997; Ovadia and Schmitz, 2002). Body size variation in natural populations has crucial implications for understanding the dynamics and stability of ecological systems (Filin and Ovadia, 2007). Insects exhibit morphometric variances interrelated with the environment that may be the result of phenotypic and genotypic variation (Adis *et al.*, 2008).

Geometric morphometrics is a powerful tool for evaluating the correlation between the shape of species. In general, geometric morphometric analyses the shape differences of

morphological features using either homologous markers or the outline of the structure (Adams *et al.*, 2004).

Landmark-based geometric morphometric technique is one of the most rigorous methods for morphological analysis. Landmark points provide an accurate geometric description of the differences in the shape of a structure (Sendaydiego *et al.*, 2013). It is gradually used to explore systematic, biological, developmental and pathological differences among and between species (Manimegalai *et al.*, 2009). This technique effectively identifies numerous insect species and analyzes external morphology related to phenotypic variation between organisms (Roth and Mercer, 2000). Geometric morphometrics is not only useful for insect identification but also provides a convenient way to study phylogeny (Güler, 2006) and the division of sibling species (Villemant *et al.*, 2007).

Geometric morphometrics is a powerful mechanism for capturing the shape structure of several morphological features such as head, wings and genitalia. Wings are a suitable structure for studying morphological variation in insects because their 2D flattened shape bears some useful landmarks (Zelditch *et al.*, 2004) and the metric properties of the wing provide exact quantitative data for insect identification (Villegas *et al.*, 2002). Wing shape morphology using geometric morphometrics has been extensively studied in the field of entomology to clarify the relationship between closely related taxa and helps in identifying population within and between species of insects (Aytekin *et al.*, 2007; Tuzun, 2009). Therefore, several studies use geometric morphometric as an effective mechanism to differentiate morphological variations specifically wing morphology (Benitez *et al.*, 2011; Kiyoshi and Hikida, 2012). The importance of studying variation in wing geometry in acridid populations relies on the fact that it can provide insight into the population structure, ecology and even the taxonomic identity of the Orthopteran insects.

Orthopterans have distinct physical characteristics that are used in taxonomic keys to identify particular species. Furthermore, phenotypic differences may or may not be related to genotypic differences and accurate correlations might be difficult to establish. Molecular-based identification can resolve various problems encountered during morphology-based identification, especially of morphologically close species. Recently, the use of DNA has substantially increased the speed and accuracy of recognising species and determining their

molecular relationships. DNA barcoding is one of the most essential and powerful methods for taxonomic studies, allowing short genetic markers to be used to identify organisms. The molecular technique is a powerful tool for the study of insect population ecology and insect systematics (Hebert *et al.*, 2003).

DNA barcoding is an attractive proposition and offers an opportunity for biologists to obtain a measure of biodiversity rapidly and easily. DNA barcoding, as a generic word, contributes significantly to the global creation of biodiversity data (Taberlet *et al.*, 2012; Nagy *et al.*, 2013). DNA barcoding is gaining a lot of attention in the scientific community because of its copious benefit in identification (Hebert *et al.*, 2004). The basic scientific advantage of DNA barcoding is that it allows quick and accurate species identification at any stage of life or in any part of an organism and simplifies species explorations (Janzen *et al.*, 2005). DNA sequence distinguishes the species based on interspecific and intraspecific variations (Matz and Nielsen, 2005). Barcoding has aided in resolving cryptic species complexes (Burns *et al.*, 2007; Deng *et al.*, 2012) and performing ecological research on a variety of animal phyla (Valentini *et al.*, 2009). The generated data is also being used to create barcode reference libraries in order to identify unknowns by matching sequences with the recognized species (Guralnick and Hill, 2009).

Mitochondrial DNA (mtDNA) sequencing data has been frequently used to elucidate the intraspecific and interspecific evolutionary relationships of insects (Simon *et al.*, 1994; Huang *et al.*, 2000) using the polymerase chain reaction (PCR). At the species level, the use of polymerase chain reaction (PCR) and universal mitochondrial primers revolutionised biodiversity research (Wilson *et al.*, 1985; Saiki *et al.*, 1988). PCR is used to amplify and sequence genes and then the nucleotide data is used for phylogenetic analysis and the development of evolutionary links between grasshopper species. Mitochondrial DNA haplotype data has proven to be a strong tool for studying species evolution and informing on a wide range of evolutionary and ecological challenges (Avise, 2004).

The insect mtDNA is typically a circular molecule between 14 and 19 kilobase in length that encodes 37 genes: 13 protein-coding genes (PCGs), two ribosomal RNA (rRNA) genes, 22 transfer RNA (tRNA) genes and a large non-coding region (A+T-rich region), which contains some initiation sites for transcription and replication of the genome

(Wolstenholme, 1992). Mitochondrial genes are regarded as superior markers to nuclear genes because of their quantity (1000's copies per cell), lack of recombination, maternal inheritance, absence of introns and quick evolutionary rate. Mitochondrial DNA has been frequently employed to identify cryptic species, reconstruct phylogenetic trees and phylogeographic patterns and infer molecular evolution for a variety of animal taxa (Avice, 2009; Cameron, 2014).

The cytochrome c oxidase subunit I (COI), COII, cytochrome b, 16S rDNA and other markers based on mtDNA sequence were widely used in phylogenetic studies. Fragments of two mitochondrial genes, Cytb and COI, have been used as phylogenetic markers in insects (Nai-Xin *et al.*, 2008). DNA barcoding has been promoted as a valid method for identifying species in a number of invertebrate and vertebrate taxa based on genetic variation within a defined region of the Cytochrome c oxidase subunit I (COI) gene (Hebert *et al.*, 2003). A 658-bp fragment of COI is amplified using universal primers, known as Folmer primers, which efficiently amplify this region from metazoan mitochondria (Folmer *et al.*, 1994; Hebert *et al.*, 2003). Cytochrome c oxidase (CO) is the final enzyme of the electron transport chain and performs a vital role. In eukaryotes, the subunits I, II and III are encoded by mitochondrial genes (COI, COII and COIII) and they are the only coexistent homologous subunits both in eukaryotic and prokaryotic organisms, showing their important function (Li and Huang, 2001). The COI gene is the largest of the three mitochondrial markers and its protein sequence contains highly conserved functional domains and variable areas that are frequently used in evolutionary studies (Murugan *et al.*, 2016). Cytochrome c gene is one of the largest protein-coding genes in the mitochondrial genome of invertebrates. The cytochrome c gene is well suited to comparative studies of species within the same genus or family due to its quick evolutionary rate.

The aim of the present study is to contribute novel information regarding the diversity and population dynamics of Orthoptera fauna in relation to seasonality, meteorological parameters, host plant as well as to understand their molecular evolutionary history and morphometric variation among different sites of Coimbatore, Tamil Nadu, India.

In view of the above, the current investigation was undertaken with the following objectives.

1. To study the species diversity and species richness of Orthoptera fauna among various seasons as well as geographical regions of Coimbatore.
2. To evaluate the Orthoptera faunal richness in relation to plant species diversity and vegetation structure.
3. To examine the geographical and seasonal variation effects on population dynamics of acridids and their correlation with meteorological parameters.
4. To analyze the intra- and inter-specific morphometric variation in body size of acridids and their wing shape variability using landmark-based geometric morphometrics.
5. To determine the molecular taxonomic analysis of acridids using mitochondrial gene Cytochrome C Oxidase subunit I (COI).
6. To predict phylogenetic relationships to study the evolutionary lineage of selected species of acridids.