RESEARCH PAPER

A Review on Diversity, Ecological Significance and Molecular Insights Of Family Gryllidae And Tettigoniidae (Orthoptera: Ensifera)

Neenu Daroch[1], Neha Choudhary[2] and Neha Katnoria[3*]

[1,2]M.Sc., Ph.D. Pursuing., Department of Biosciences, Division Zoology, Career Point University, Hamirpur, Himachal Pradesh, India-176041.

[3]M.Sc., M. Phil., Ph.D., Assistant Professor in Department of Biosciences, Division Zoology, Career Point University, Hamirpur, Himachal Pradesh, India-176041.

***Corresponding Author -** [3*]Neha29katnoria@gmail.com*

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ABSTRACT

Order Orthoptera consists of medium to large sized land insects including grasshoppers, locusts, crickets and wetas. These insects are globally distributed with the highest diversity found in tropical regions. Order Orthoptera is categorized into two suborders: Caelifera and Ensifera. Suborder Caelifera which include short horned grasshoppers and suborder Ensifera which comprises long horned grasshoppers, crickets, katydids and wetas. Suborder Ensifera is notable for species that communicate via sound and members typically feature long and multi segmented antennae. Ensiferans species serve significant ecological roles in food webs and as indicators of ecosystem health. This suborder contains two infraorders; Gryllidea and Tettigoniidea. The Gryllidae family classified under the infraorder Gryllidea and is known for its diverse species including field crickets that produce sound through stridulation. In contrast, the Tettigoniidae family belonging to the infraorder Tettigoniidea and consists of katydids which are recognized for their leaf like appearance and intricate mating behaviors. Both families have a global distribution with significant biodiversity in India and Himachal Pradesh where numerous species have been identified. This is a detailed review covering the diversity, ecological significance, molecular work and distribution of species of family Gryllidae and Tettigoniidae. Cytochrome c Oxidase Subunit 1 (COI) and 16S rRNA are the most common mitochondrial genes used for species identification. The COI gene is commonly used in DNA barcoding because its variation within and between species allows for precise taxonomic identification. This review explores the diversity and ecological role of order Orthoptera highlighting the molecular tools of studying their taxonomy and evolution.

Keywords: Orthoptera, Caelifera, Ensifera, Grylloidea, Tettigonioidea, Diversity, Mitochondrial DNA, COI gene, 16S rRNA gene and Molecular work.

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INTRODUCTION

Insecta is the largest class of phylum Arthropoda. Insects have distinct head, thorax and abdomen (Rockstein, 1973). They are known for having one pair of antennae on their head, two pairs of wings, three pair of legs that bend at the joints, three body parts and a tough outer shell (LaDouceur et al., 2021). More than 40% of insect species are declining because of habitat loss caused by intensive farming, land development, pesticide use, invasive species and climate change (Goulson, 2019). According to IUCN (2024), out of 12,568 insect species assessed for extinction risk, a quarter are classified as near threatened, threatened or extinct. Additionally, one third of insect species with recorded population trends are experiencing declines (Dirzo et al., 2014). The decline in insect biomass can cause harmful impacts on food webs and ecosystem services (Hallmann et al., 2017; Yang & Gratton, 2014). Insect extinctions will affect the whole ecosystem because insects play many important roles, a risk that is being increasingly recognized globally (Cardoso et al., 2020).

ORDER ORTHOPTERA

The Orthoptera comes from the Greek words *orthos* meaning "straight" and *pteron* meaning "wings." (Tidame and Yasmeen, 2025). The order Orthoptera is a significant insect group comprising approximately 30,000 species worldwide and plays a crucial role in food webs by serving as a food source for both vertebrates and arthropod predators. Their strong association with vegetation structure and specific microhabitat preferences makes them excellent indicators of ecosystem health, vegetation dynamics and microclimatic variations on a fine scale (Stefanidis et al., 2025). The order Orthoptera includes mostly medium to large terrestrial insects including long-horned and short-horned grasshoppers, crickets, locusts & wetas. Order Orthoptera are found worldwide but are most

**Author for Correspondence: Neha29katnoria@gmail.com*

diverse in tropical regions. They are well known for their strong jumping legs and the singing of males and sometimes females in many species (Rentz, 1978). Order Orthoptera is categorized into two suborders: Caelifera and Ensifera. Suborder Caelifera consists of short horned grasshoppers while the suborder Ensifera includes well known singing insects like long horned grasshoppers, crickets, katydids and wetas. This group contains 12,000 species across 2,000 genera.

The Orthoptera Species File (OSF) presently recognizes 9 geographic regions namely North America including Mexico, Africa, Central and South America, Tropical Asia, Temperate Asia, Australia, Antarctica, Pacific & Europe. When examine the order orthoptera and its suborders carefully, some common trends were found to be emerged. According to OSF, diversity of order Orthoptera in Central and South America represents (22%) followed by Africa (20%), Tropical Asia (21%) and Temperate Asia (17%). North America does not have as many different species as some other places. It represents only 8% of all the described species. In the suborder Ensifera, Tropical Asia or Central and South America is most species rich region (Table 1). The diversity of suborder Ensifera in Tropical Asia representing (25%) followed by Africa (15%), Central and South America (24%), Australasia (8%), North America (6%), Pacific (5%), Europe (4%) and Temperate Asia (3%). Insects of suborder Ensifera are most abundant in tropical areas where there are lots of different species. In the suborder Caelifera, Africa and Temperate Asia is the most species rich region. The diversity of suborder Caelifera in Africa representing (25%) followed by Temperate Asia (23%), Central and South America (20%), Tropical Asia (16%), North America (9%), Australasia (4%) Europe (2%) and Pacific (1%) (Eades et al., 2015; Song, 2018) (Table 1).

Table 1: Worldwide diversity of order Orthoptera as per Orthoptera Species File in different geographical regions (Eades et al., 2015; Song, 2018).

	Africa	North America	Central & South America	Temperate Asia	Tropical Asia	Australasia	Europe	Pacific
Orthoptera	20%	8%	22%	17%	21%	6%	3%	3%
Ensifera	15%	6%	24%	3%	25%	8%	4%	5%
Caelifera	25%	9%	20%	23%	16%	4%	2%	1%

SUBORDER CAELIFERA

The suborder Caelifera represents short horned grasshoppers. They usually have shorter antennae as compared to other insects and includes grasshoppers and locusts. The main features of this suborder includes strong antennae with less than 30 segments, asymmetric mandibles with a strong tooth for grinding mostly visible sides of the middle part of the body, three or fewer segments on the feet and sound producing organs on the abdomen (Song, 2018). The suborder Caelifera consists of 11700 species representing 9 superfamilies, 28 families, and 2477 genera (Table 2) (Song et al., 2015).

Table 2: Worldwide distribution of species of suborder Caelifera (Song et al., 2015).

Order	Suborder	Superfamily	Family	Subfamilies	Genera	Species
Orthoptera	Caelifera	9	28	102	2477	11700

SUBORDER ENSIFERA

The suborder Ensifera consists of long horned grasshoppers, crickets, katydids and wetas. Many biologists find Ensifera fascinating because several of its members communicate through sounds (Zhou et al., 2017). This suborder is identified by its antennae which consist of numerous segments usually more than 30 and are often equal to or greater in length than the body. Ensiferans can be found in almost all terrestrial habitats (Gillot, 2005; Resh & Carde, 2003; Tan & Kamaruddin, 2014). This group plays a crucial ecological role serving as key indicators of terrestrial ecosystem health within the food web and is also important in various human activities. Some species serve as prey for larger animals like birds while others are consumed by smaller insects. Some play a vital role in maintaining ecosystem balance as keystone species while others act as indicators of environmental health (Erawati et al., 2004; Rentz, 2010; Samways, 1997). Additionally, certain species are widely bred and distributed globally for use as fish bait and as a food source for laboratory animals. Others are considered pests and can contribute to plant diseases. The suborder Ensifera consists of 14,313 species pertaining to 11 families, 2111 genera and 7 superfamilies (Table 3) (Song et al., 2015).

Table 3: Worldwide distribution of species of suborder Ensifera (Song et al., 2015).

Order	Suborder	Superfamily	Family	Subfamilies	Genera	Species
Orthoptera	Ensifera	7	11	74	2111	14313

The suborder Ensifera consists of two infraorder: - Gryllidea and Tettigoniidea. The infraorder Gryllidea including two superfamilies and these are Grylloidea and Gryllotalpoidea. The superfamily Grylloidea consists of only one single family i.e. Gryllidae while the superfamily Gryllotalpoidea consists of three families i.e. Gryllotalpidae, Mogoplistidae and Myrmecophilidae. The family Gryllidae consists of 21 subfamilies, 605 genera and 4900 species worldwide. The infraorder Tettigoniidea including five superfamilies and these are Schizodactyloidea, Rhaphidophoroidea, Hagloidea, Stenopelmatoidea and Tettigonioidea. The superfamily Schizodactyloideak consists of one family i.e. Schizodactylidae. The superfamily Rhaphidophoroidea

consists of one family Rhaphidophoridae. The superfamily Hagloidea consists of one family Prophalangopsidae. The superfamily Stenopelmatoidea consists of three families and these are Anostostomatidae, Gryllacrididae and Stenopelmatidae. The superfamily Tettigonioidea consists of single family Tettigoniidae. The family Tettigoniidae including 23 subfamilies, 1228 genera and 7163 species worldwide (Song, 2018).

SUPERFAMILY GRYLLOIDEA

Superfamily Grylloidea is a highly diverse group within the order Orthoptera comprises 5,555 living species. This superfamily includes various crickets such as true crickets, beetle crickets, sword tailed crickets and scaly crickets.Some of these crickets are well known for their sounds. Crickets can be found in nearly all land habitats ranging from treetops to deep underground. They are most active at night and staying hidden during the day under rocks, stones, leaf litter and dense vegetation. They can be either dull or brightly colored. They undergo incomplete metamorphosis progressing through three stages: egg, nymph and adult. Female crickets deposit their eggs either in the soil or on plants (Alexander, 1962). Superfamily Grylloidea is now divided into 4 families; Gryllidae, Phalangopsidae, Mogoplistidae and Trigonidiidae. The taxonomic classification of the superfamily Grylloidea has changed over time (Ma et al., 2018). Family Gryllidae is distinguished by their long antennae, a typically square shaped pronotum, tegmina that lie flat across their back, elongated cerci and a slender needle shaped ovipositor. In many species, males produce musical sounds by rubbing the scrapers on their left forewing against the stridulatory files on their right forewing. In most crickets, sound production occurs when the right forewing moves over the left (Kevan, 1982). The Gryllidae family is known for its significant diversity comprising 21 subfamilies, 605 genera and 4,900 species found worldwide (Song et al., 2015) (Fig 1). Chandra et al. (2010) documented 231 species of the Gryllidae family in India distributed across 13 subfamilies and 72 genera. Shishodia et al. (2009) identified 28 species belonging to 13 genera of the Gryllidae family in Himachal Pradesh, India (Fig 1).

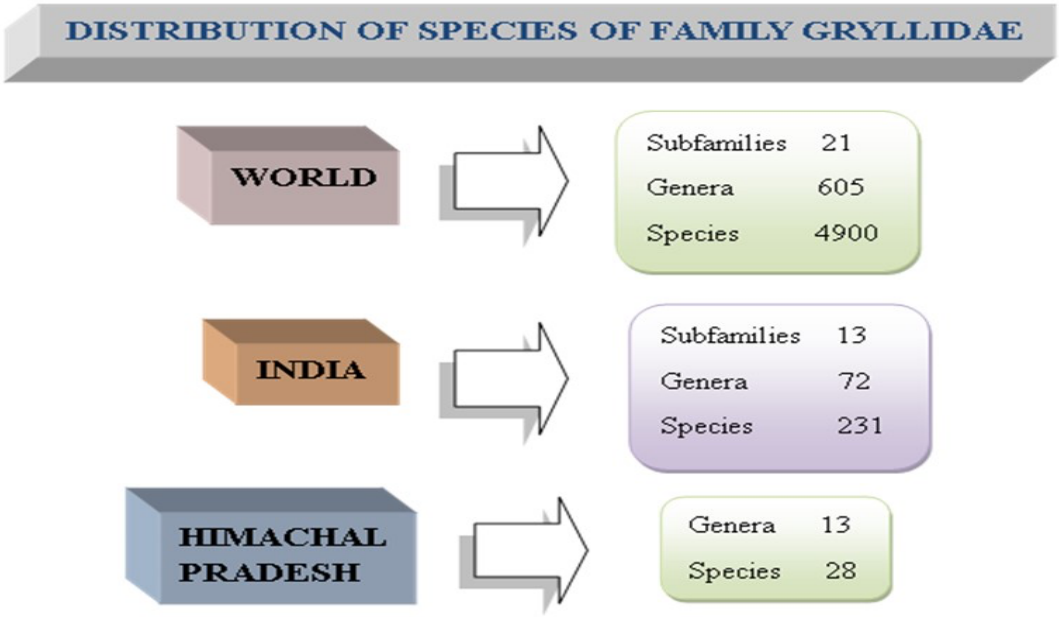

Fig. 1: Distribution of species of family Gryllidae (Orthoptera: Ensifera).

Among the 21 recognized subfamilies, four exhibit a widespread global distribution extending across mild, warm and equatorial regions on every continent. These four subfamilies are; Gryllinae, Nemobiinae, Oecanthinae and Trigonidiinae. Subfamily Gryllinae commonly known as field crickets including 1121 species, subfamily Nemobiinae commonly known as ground crickets including 327 species, subfamily Oecanthinae commonly known as tree crickets including 169 species and subfamily Trigonidiinae commonly known as trigs or sword tail crickets including 635 species (Table 4). Some subfamilies are present in both the Old World and the New World but are restricted to warm and equatorial regions. These encompass 141 species of subfamily Landrevinae, 70 species of subfamily Pentacentrinae commonly known as silent litter crickets, 802 species of subfamily Podoscirtinae, 280 species of subfamily Luzarinae, 121 species of subfamily Paragryllinae, 958 species of subfamily Phalangopsinae commonly known as spider crickets and 340 species of subfamily Eneopterinae commonly known as bush crickets. In the Old World, exclusive subfamilies include Gryllomiminae, Itarinae, Gryllomorphinae, Sclerogryllinae, Phaloriinae, Pteroplistinae and Euscyrtinae whereas Hapithinae is the only subfamily found solely in the New World (Song, 2018; Cigliano et al., 2021) (Table 4).

Table 4: Worldwide distribution of species of family Gryllidae (Cigliano et al., 2021; Song, 2018)

Family	Subfamilies	Genera	Species
Gryllidae	Gryllinae (field crickets)	92	1121
	Nemobiinae (ground crickets)	73	327
	Trigonidiinae (trigs or sword tail crickets)	51	635
	Phalangopsinae (spider crickets)	39	958
	Gryllomorphinae	6	28
	Itarinae	2	63
	Landrevinae (bark crickets)	40	141
	Eneopterinae (bush crickets)	10	340
	Pentacentrinae (silent litter crickets)	8	70
	Sclerogryllinae (stiff winged crickets)	2	5
	Euscyrtinae	10	43
	Podoscirtinae	3	802
	Oecanthinae (tree crickets)	25	169

ECONOMIC IMPORTANCE OF FAMILY GRYLLIDAE

The family Gryllidae holds significant economic importance due to its diverse roles in human and ecological systems. Crickets are widely used as a high protein food source for humans and as nutritious feed for poultry, fish and pet animals. They also serve as useful biological indicators for assessing environmental health. Although a few species can cause minor damage to crops, seedlings and stored products many contribute positively to agriculture by aiding in soil aeration and decomposition of organic matter. Crickets are also valuable in scientific research, particularly in studies of behavior, communication and genetics making them important both economically and scientifically.

DIVERSITY OF FAMILY GRYLLIDAE IN HIMACHAL PRADESH, INDIA

Shishodia et al. (2003) identified seven species of the Gryllidae family in the Pong Dam located in the Kangra district of Himachal Pradesh, India. These seven species are- *Teleogryllus testaceus*, *Teleogryllus occipitalis*, *Plebeiogryllus guttiventris*, *Loxoblemmus detectus*, *Pteronemobius fascipes*, *Gryllus bimacultus* and *Modicogryllus confirmatus.* Shishodia and Gupta (2009) documented 28 species of family Gryllidae from Himachal Pradesh, India. These are- *Tarbinskiellus portentosus*, *Gymnogryllus kashmirensis*, *Acheta domesticus*, *Gryllus bimaculatus*, *Modicogryllus blennus*, *Modicogryllus confirmatus*, *Modicogryllus facialis*, *Modicogryllus tikadari*, *Plebeiogryllus guttiventris*, *Teleogryllus mitratus*, *Teleogryllus occipitalis*, *Platygryllus brunneri*, *Platygryllus melanocephalus*, *Turanogryllus babaulti*, *Turanogryllus histrio*, *Turanogryllus jammuensis*, *Turanogryllus rufoniger*, *Loxoblemmus detectus*, *Loxoblemmus equestris*, *Loxoblemmus macrocephalus*, *Loxoblemmus taicoun*, *Velarifictorus dehradunensis*, *Velarifictorus lambai*, *Gryllodes sigillatus*, *Pteronemobius csikii*, *Pteronemobius fascipes*, *Pteronemobius pantelchopardorum* and *Pteronemobius taprobanensis.* Sharma and Mattu (2010) reported 10 species of family Gryllidae from Nalagarh valley of Himachal Pradesh, India. Singh (2013) documented four species belonging to the Gryllidae family in Khajjiar Lake situated in the Chamba district of Himachal Pradesh, India. The identified species include *Acheta domesticus*, *Loxoblemmus equestris*, *Gryllus bimaculatus*, and *Teleogryllus occipitalis*. Yadav et al. (2022) recorded one species of family Gryllidae (*Teleogryllus emma*) from Maharashtra, India. Das (2023) identified two species of family Gryllidae from Baksa district, Assam, India. These two species are- *Gryllus bimaculatus* and *Acheta domestica.* Bhanger et al. (2024) reported nine species of family Gryllidae (Gryllinae: Orthoptera) from Sindh Pakistan. These are- *Acheta domesticus*, *Gryllus bimaculatus*, *Gryllus campestris*, *Gryllodes sigillatus*, *Gryllodes supplicans*, *Callogryllus ovilongus*, *Callogryllus saeedi*, *Teleogryllus occipitalis* and *Modicogryllus sindhensis*

SUPERFAMILY TETTIGONIOIDEA

The superfamily Tettigonioidea is comprised of a single family Tettigoniidae which is the most diverse and extensive group within the suborder Ensifera. Tettigoniidae family often referred to as katydids or bush crickets with over 7,200 species distributed across the globe (Eades et al., 2016). These katydids are recognized by their wings which are arranged to form a shelter or canopy like covering above their abdomen. Male Tettigoniids have a subgenital plate with two appendages while females have a blade like ovipositor and tarsi divided into four segments. They produce sound by scraping their left tegmen across the right. Tettigoniidae family contains highest number of species within order Orthoptera (Song, 2018). Many katydids have forewings that look like leaves and a recent study found that this leaf like appearance has emerged independently multiple times within this family (Mugleston et al., 2013). This discovery suggests that leaf like katydids may have evolved because of their close relationship with insects and plants. Katydids exhibit a variety of feeding habits with many species feeding on herbs and grasses as herbivores. There are several predatory groups among katydids including the Bradyporinae, Listroscelidinae, Hexacentrinae and

Saginae (Bailey and Rentz, 1990). Certain species of subfamily Phasmodinae and Zaprochilinae specialized in eating flowers, nectar and pollen (Rentz, 1996). Besides their sounds katydids are also well known for their mating behavior. During mating males produce protein rich spermatophylaxes which they offer as a gift to females. This gift supports the effective transfer of spermatophores (Gwynne, 2001). This behavior relates to sexual selection where females choose males based on quality of their reproductive gift. In some cases producing the spermatophylax is highly costly for males. In these cases males become the choosy ones and females compete for the protein rich gift (Gwynne, 1993). The family Tettigoniidae known for its wide variety comprises 23 subfamilies, 1228 genera and 7163 species worldwide (Song et al., 2015) (Fig 2). In India, Chandra et al. (2010) identified 160 species belonging to 9 subfamilies under 68 genera. Shishodia et al. (2009) reported 21 species under 16 genera from Himachal Pradesh, India (Fig 2).

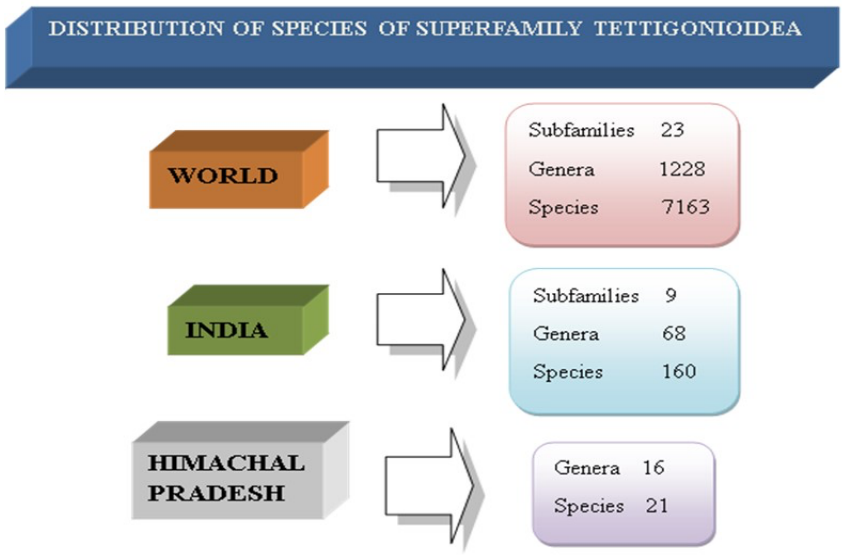

Fig. 2: Distribution of species of superfamily Tettigonioidea (Orthoptera: Ensifera).

Table 5: Worldwide distribution of species of family Tettigoniidae (Cigliano et al., 2021; Song, 2018).

Family	Subfamilies	Area of occurrence	Genera	Species
Tettigoniidae	Austrosaginae	Australia	5	30
	Bradyporinae	Southest, Europe, West & Central Asia	28	170
	Conocephalinae	Global	168	1142
	Hetrodinae	Africa	14	63
	Hexacentrinae	Pantropical, Especially Asia	13	58
	Lipotactinae	Asia	2	6
	Listroscelidinae	Americas, Australia	22	82
	Meconematinae	Global	107	567
	Mecopodinae	South America, Africa, Southest Asia	57	182
	Microtettigoniinae	Australia	1	7
	Phaneropterinae	Global	85	2060
	Phasmodinae	Australia	1	3
	Phyllophorinae	Australasia	11	67
	Pseudophyllinae	Global	232	262
	Pterochrozinae	Central & South America	14	96
	Saginae	North America, Africa, Europe	5	45
	Tettigoniinae	Global	164	938
	Tympanophorinae	Australia	1	14
	Zaprochilinae	Australia	4	18

Among the 23 recognized subfamilies, subfamily Conocephalinae and Phaneropterinae are the only ones that have a worldwide range spanning mild, warm and equatorial regions. Subfamily Conocephalinae including 1197 species and subfamily Phaneropterinae including 2471 species. Four subfamilies have a global distribution. Subfamily Meconematinae including 811 species inhabit warm and equatorial zones while subfamily Hexacentrinae including 48 species and subfamily Mecopodinae including 150 species are restricted to tropical areas. Subfamily Tettigoniinae including 893 species are found only in temperate climates. Five subfamilies; Austrosaginae, Phasmodinae, Microtettigoniinae, Zaprochilinae and Tympanophorinae are native to

Australia whereas Pterochrozinae and Polyancistrinae are unique to the New World. Most other subfamilies are distributed throughout the Old World (Table 5) (Song, 2018).

ECONOMIC IMPORTANCE OF FAMILY TETTIGONIIDAE

The family Tettigoniidae has notable economic importance due to its ecological and scientific roles. While some katydid species feed on crops, fruits and leaves occasionally causing agricultural losses many others help maintain ecosystem balance by acting as predators of harmful insects. Their presence supports natural pest control in farming systems. Tettigoniids also contribute to nutrient cycling through plant and detritus feeding. In addition, they are valuable in research related to bioacoustics, ecology and evolutionary biology because of their diverse communication systems and behaviors. Overall, they play both positive and negative economic roles with their ecological benefits often outweighing their pest impacts.

DIVERSITY OF FAMILY TETTIGONIIDAE IN HIMACHAL PRADESH, INDIA

Shishodia et al. (2003) described four species of Tettigoniidae family in the Pong Dam located in the Kangra district of Himachal Pradesh, India. These species are: *Mecapoda elongata, Ducetia japonica, Holochlora indica* and *Letana megastridula.* Barman (2003) reported total 18 species of family Tettigoniidae from Sikkim representing 15 different genera. These species are: *Letana linearis, Elimaea securigera, Holochlora indica, Euhexacentrus annulicornis, Mecapoda elongata, Euconocephalus incertus, Conocephalus (Xiphidion) maculatus, Sathrophyllia rugosa, Sathrophyllia femorata, Sanaa regalis, Sanaa imperialis, Parasanaa donovani, Tegra viridivitta, Tegra novaehollandiae viridinotata, Climacoptera venosa, Pseudophyllus ligatus, Chloracris brunneri and Phyllozellus siccus*. Shishodia and Gupta (2009) documented 21 species within the Superfamily Tettigonioidea from Himachal Pradesh, India. These include *Conocephalus maculatus, Euconocephalus nasutus, Euconocephalus pallidus, Neoconocephalus palustris, Ducetia japonica, Elimaea (Orthelimaea) securigera, Hexacentrus unicolor, Himertula kinneari, Holochlora indica, Pseudorhynchus sp, Ruspolia lineosus, Isopsera pedunculata, Isopsera stylata, Letana despecta, Letana linearis, Letana megastridula, Phaneroptera gracilis, Phaneroptera myllocerca, Mecopoda elongata, Paramorsimus robustus* and *Onomarchus* species. Bhumi et al. (2015) reported a total of nine species belonging to the family Tettigoniidae in their study on the diversity of Orthoptera fauna in South Gujarat, India. These species are: *Paracaedicia sp, Neoconocephalus velox, Euthystira brachyptera, Hexacentrus sp, Amblycorypha rotundifolia, Holochlora sp, Scuddderia furcata, Sathrophyllia sp and Ceuthophilus sp.* Das et al. (2020) identified 13 species of family Tettigoniidae from West Bengal, India consisting of *Conocephalus maculatus, Euconocephalus pallidus, Euconocephalus indicus, Mecopoda elongata, Khaoyaiana ambigua, Letana linearis, Letana gracilis, Letana pyrifera, Letana atomifera, Ducetia japonica, Holochlora indica, Elimaea subcarinata and Phaneroptera gracilis.* Paulson et al. (2020) documented six species of the Tettigoniidae family from Vadodara, Gujarat, India. These species include *Amblycorypha rotundifolia, Mecopoda elongata, Neoconocephalus velox, Sathrophyllia sp, Scudderia furcuta* and *Trigonocorypha unicolor sp.* Singh et al. (2021) recorded four species of the family Tettigoniidae in the Parvati Aranga Bird Sanctuary located in Gonda District, Uttar Pradesh, India. The identified species are *Trigonidium humbertianum, Conocephalus longipennis, Conocephalus maculates* and *Elimaea securigera.* Yadav et al. (2022) documented five species of the family Tettigoniidae from the Indapur (Pune) and Phaltan (Satara) Tehsils in Maharashtra, India. These five species are- *Microcentrum rhombifolium, Tettigonia viridissima, Letana intermedia, Conocephalus maculatus* and *Pterophylla camellifolia.* Das (2023) documented two species belonging to the family Tettigoniidae from Baksa District, Assam, India. These are- *Mecopoda elongata* and *Ruspolia baileyi.* Dabhi et al. (2024) reported three species from the Tettigoniidae family in Jabugam, Gujarat, India. The subfamily Conocephalinae includes *Euconocephalus incetus,* the subfamily Hexacentrinae comprises *Hexacentrus unicolor* & the subfamily Pseudophyllinae includes *Sanaa regalis.*

MITOCHONDRIAL DNA

Mitochondrial DNA is a circular molecule composed of two strands. It contains around 16000 base pairs of nucleotide data (Copeland, 2008). Mitochondrial DNA varies in length from 14 to 39 kilobases and contains a primary non-coding region often referred to as the regulatory or Adenine+Thymine rich region (Boore, 1999; Breton et al., 2014). The length of this region can range from tens to multiple thousand base pairs (Lewis et al., 1995; Shao et al., 2001). This region of mtDNA is highly variable due to frequent nucleotide substitutions, insertions and deletions along with differences in the number of tandem repeat elements (Duenas et al., 2006; Zhang and Hewitt, 1997). Mitochondrial DNA contained a fundamental group of 37 genes which included 13 genes encoding proteins, 2 genes for rRNA and 22 genes responsible for tRNA (Boore, 1999; Breton et al., 2014). It has been extensively applied in research on population dynamics, geographical genetic variation and evolutionary relationships across various taxonomic groups (Fernandes-Matioli et al., 2001; Moore, 1997). The mitochondrial genome has been frequently used in phylogenetic studies as it can offer abundant information for phylogenetic analysis (Fenn et al., 2008). DNA barcoding is an important classification technique that employs short genetic sequences to assist in identifying organisms (Muhammedali et al., 2017). In 2003, Paul Hebert proposed COI gene as the barcode region due to its variations both within and between species. Most common mitochondrial genes used for species identification COI gene and 16S rRNA gene (Fig 3).

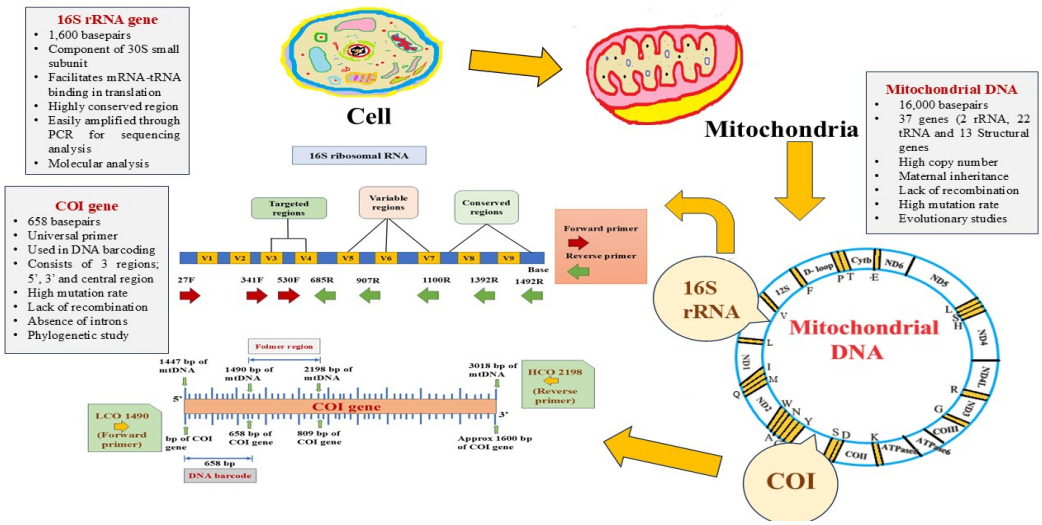

Fig. 3: Mitochondrial DNA and its genetic markers: 16S rRNA and COI genes

COI GENE

COI gene codes for a mitochondrial protein that resides in the inner membrane and serves as an essential enzyme in the electron transport system. It is crucial for the metabolic processes of eukaryotic organisms that rely on oxygen for energy production. This protein is composed of multiple subunits with subunit 1 responsible for catalysis encoded within the mitochondrial genome (Hebert et al., 2003b). COI gene is the most widely used for species identification (Machida et al., 2017). In 2003, Paul Hebert proposed COI gene as a basis for species identification considering its variations within and between species (Hebert et al., 2005). It serves as a valuable method for classifying species and recognizing different organisms. COI gene is an universal primer and are used to amplify a 658 base pairs segment (Hebert et al., 2003; Vrijenhoek et al., 1994). COI gene is widely recognized as an essential indicator in genetic population analysis and evolutionary research due to its status as one of the most preserved protein-coding genes in animal mitochondrial DNA (Hebert et al., 2003). A 658 base pair segment of COI gene was amplified utilizing the primer set LCO1490 and HCO2198 and widely recognized as most suitable mtDNA locus for DNA barcoding in various insect species (Kurata et al., 2024).

The COI gene has some features that make it especially useful for studying evolution and these are:-

- The dimensions and composition of this mitochondrial gene seem to remain consistent in oxygen dependent organisms (Saraste, 1990).
- It consists of multiple functional domains each exhibiting distinct substitution patterns (Erpenbeck et al., 2006; Lunt et al., 1997) (Fig 3).

16S RIBOSOMAL RNA GENE

16S rRNA gene consists of about 1600 base pairs of nucleotide data and is used for genus and species identification (Janda et al., 2007; Patel, 2001). It is large enough and has enough specific variations to allow for accurate and reliable identification. It includes two regions conserved regions and variable regions. The variable region between these conserved areas is used for comparative taxonomy (Chen et al., 1989; Clarridge, 2004; Relman, 1999). The 16S rRNA gene has highly conserved regions mixed with nine variable regions (V1 to V9) (Janda et al., 2007; Woese et al., 1990). Conserved region of this gene serves as a binding site for primers (Baker et al., 2003) (Fig 3).

Table 6: Comparative table of commonly used molecular markers.

Sr. No.	Marker	Genome Type	Typical Length	Evolutionary Rate	Variation Pattern	Common Applications	Advantages	References
1	**COI (Cytochrome c oxidase subunit I)**	Mitochondrial DNA	~658bp (Folmer region)	Moderate to high	High interspecific variation; low intraspecific variation	DNA barcoding, species delimitation, population genetics, phylogeography	Universal primers available; high amplification success; easy cross-taxon comparison	Hebert et al., 2003; Machida et al., 2017; Vrijenhoek et al., 1994
2	**16S rRNA gene**	Mitochondrial DNA	~1,550–1,600 bp	Moderate	Conserved regions flanking variable regions (V1–V9)	Deep-level phylogenetics, species/genus identification, bacterial classification	Highly conserved primer-binding sites; good for degraded DNA	Chen et al., 1989; Clarridge, 2004; Janda et al., 2007; Patel, 2001; Relman, 1999; Woese et al., 1990

MOLECULAR WORK

National and International molecular work on family Gryllidae

Gray et al. (2006) explored the molecular differences between *Gryllus rubens* and *Gryllus texensis* which are closely related species of field crickets within the family Gryllidae by analyzing sequences of CO1 and cytb genes. Weissman et al. (2009) explored Gryllus assimilis a field cricket species identifying two newly discovered sister species within the family Gryllidae of order Orthoptera and analyzed the molecular phylogeny by mitochondrial gene of CO1 and 16S Rrna. Jaiswara et al. (2012) examined species boundaries within the *Itaropsis* genus of field crickets (Orthoptera: Grylloidea) from India using acoustic, structural, and genetic information. The molecular analysis included a portion of the nuclear gene elongation factor EF1-α approximately 990 base pairs along with four sections of mitochondrial DNA. These included CO1 gene around 690 base pairs, Cyt b about 365 base pairs and two ribosomal RNA subunits: the large 16S rRNA gene about 500 base pairs and the small 12S rRNA gene around 400 base pairs. Mandal et al. (2014) explored the use of mitochondrial markers for identifying and studying the phylogeny of insects in Mizoram, India. These mitochondrial markers have become more widely adopted for molecular phylogenetic research. Different species of insects are studied using various markers such as those for 16S rRNA, 12S rRNA, ND (genes 1-6), ATPase and control regions. He et al. (2015) conducted molecular cloning, functional analysis and expression profiling of an Ecdysone Receptor homolog in *Teleogryllus emma*, a species belonging to the Gryllidae family. Chintauan-Marquier et al. (2016) established the foundation for evolutionary and taxonomic studies in crickets by conducting an extensive examination utilizing various genetic markers including 18S, 28SD, 28SA, H3, 16S rRNA, 12S and Cytb. Ma et al. (2019) examined the full mitochondrial DNA of *Xenogryllus marmoratus* a bush cricket from the Gryllidae family by analyzing the COX1 gene sequences. Yang et al. (2021) studied the mitochondrial genome of *Gryllodes sigillatus* a cricket of Gryllidae family by analyzing sequences of COX1 and cytb. They examined how this information could help us understand the relationships between different species. Chang et al. (2022) delved into an examination of the growth and genetic diversity of two spotted cricket of species *Gryllus bimaculatus* from South Korea by analyzing sequences of CO1 gene. Zheng et al. (2023) conducted a thorough taxonomic revision of *Stephoblemmus Saussure* employing both morphological characteristics and molecular phylogeny by analyzing sequences of COX1 gene, 18S and 28S rRNA gene with a specific focus on the Grylloidea superfamily within the Gryllidae family of order Orthoptera. He et al. (2024) conducted molecular phylogenetic analysis and systematic reassessment of *Eurepini* crickets of family Gryllidae leading to the identification of two new genera alongside comprehensive taxonomic revisions. Yu et al. (2024) utilized mitochondrial genomes to establish the evolutionary relationships within the infraorder Gryllidea providing insights into the structural arrangement of dorsal spines and subapical spurs on back legs.

National and International molecular work on family Tettigoniidae

Jost and Shaw (2006) studied three ribosomal loci 18S rRNA, 28S rRNA & 16S rRNA to examine the evolutionary relationships within suborder Ensifera (Hexapoda: Orthoptera) offering important findings on the evolution of sound based communication. Snyder et al. (2009) investigated the molecular phylogeny of genus Neoconocephalus within Tettigoniidae family and exploring the adaptation of life history traits in temperate regions. Zhou et al. (2010) analyzed the phylogenetic relationships within order Orthoptera using mitochondrial DNA and extensively described the mitochondrial genome of *Elimaea cheni* of Tettigoniidae family. Kaya et al. (2011) examined the morphology, singing and 16S rDNA phylogeny of sp. *Anterastes davrazensis* of family Tettigoniidae. Mashhoor et al. (2012) investigated the molecular phylogenetic position of *Microcentrum rhombifolium* using a segment of the mitochondrial COI gene from India within the Tettigoniidae family. Arya et al. (2015) documented four species of family Tettigoniidae from Nanda Devi Biosphere Reserve in the Western Himalayas of India. Muhammedali (2017) employed DNA barcoding specifically targeting the Cytochrome Oxidase Subunit I gene to identify *Conocephalus dorsalis* (Tettigoniidae) specimens collected from Northern Kerala. Mugleston et al. (2018) have detailed study of the Tettigoniidae family which showed significant similarities in ecological forms and many taxonomic inconsistencies through analysis of five molecular markers including 18S rDNA gene, COII gene, 28S rDNA gene, wingless and Histone 3. Tan et al. (2020) investigated the classification and sound-based communication of the subfamily Meconematinae belonging to the Tettigoniidae family from Laguna, Luzon, Philippines. Chang et al. (2021) identified three newely classified genera and five species belonging to the subfamily Meconematini (Tettigoniidae: Meconematinae) on the basis of COI gene in Southwestern China. Zhao et al. (2022) developed a DNA barcode database for a range of insects, including katydids, cave crickets and leaf rolling crickets utilizing the COI Barcode region from Zhejiang Province, China. Pang et al. (2024) investigated the mitochondrial genomics and its evolutionary implications of nine species of subfamily Meconematinae of family Tettigoniidae family utilizing 16S rRNA.

Scope

This review covers the diversity, ecological importance and molecular studies of crickets (Gryllidae) and katydids (Tettigoniidae). It highlights their classification, roles in the environment and the latest genetic research used to understand their identification and evolution.

Objectives

1. To describe the diversity and main features of family Gryllidae and Tettigoniidae.

2. To explain their ecological roles in natural ecosystems.

3. To summarize their distribution and habitat preferences.

4. To highlight important behavioral and acoustic traits.

5. To review recent molecular techniques used for species identification and evolutionary studies.

6. To point out gaps in current knowledge and suggest areas for future research.

COLLECTION AND PRESERVATION TECHNIQUES FOR SUPERFAMILIES GRYLLOIDEA AND TETTIGONIOIDEA SPECIES

Species belonging to the superfamilies Grylloidea and Tettigonioidea will be collected from various locations in Himachal Pradesh using the sweep net method. During fieldwork, collected specimens will be preserved in glass vials containing absolute alcohol. After collection, these samples will be stored in a deep freezer maintained at –20°C for later DNA extraction. Additionally, selected specimens will be carefully spread and pinned inside fumigated wooden boxes. Each specimen will be properly labeled with detailed information such as collection site, locality, date of capture and the collector's name to ensure accurate identification.

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Authors contributions

Conception and design of the study, acquisition of data, analysis and/or interpretation of data, and drafting of the manuscript: Neenu Daroch, Neha Choudhary. Critical revision of the manuscript for important intellectual content and approval of the final version to be published: Neha Katnoria.

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