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Molecular studies on Hemipteran bugs (Hemiptera: Heteroptera) based on mitochondrial genes: A review

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Abstract
Molecular studies are recognized as a useful tool in species identification and sequence disparity. Mitochondrial genome is most extensively studied genomic system in insects. Nuclear region and mitochondrial genome has been analysed and studied comprehensively all over the world. In this review, we describe various molecular works that have been done on hemipteran bugs based on mitochondrial genes. It can be concluded that, the molecular studies focused more on COI, Cyt b and 16S gene region and rest of the molecular markers are overlooked.

Keywords: mitochondrial DNA, hemipteran bugs, COI

Introduction
Hemiptera is a very large and diverse order. The hemipterans have evolved an astonishing diversity of morphological structures and their ecological diversity is formidable. The diversity in ecological requirements and their specificity to particular habitats or host plants make them suitable ecological (Di Giulio *et al.*, 2001) [17] and biodiversity indicators (Dueli and Obrist, 2003; Virolainen *et al.*, 2000) [18, 62] as well as indicators for climate change (Musolin, 2007) [50]. It is further divided into five suborders: Heteroptera, Cicadomorpha, Fulgoromorpha, Sternorrhyncha and Coelorrhyncha (Schuh and Slater, 1995) [56]. Out of these five suborders, Heteroptera is a highly diverse taxon with more than 42,000 species in over 5800 genera and 140 families described worldwide (Henry, 2009; Park *et al*., 2011) [27, 51]. Mitochondrial DNA is widely used as a valuable molecular marker for phylogenetic studies in animals, because of its simple genomic structure (Avise, 1994) [5]. It is small, circular DNA with length ranging from 14,000 to 17,000 bp that comes predominantly from maternal sources (Lessinger *et al*., 2000; Cameron *et al*., 2007) [36, 12]. The molecule comprises approximately 37 genes including 13 protein coding genes, 22 transfer RNA genes and two ribosomal RNA genes (12S rRNA and 16S rRNA), it also contains a major non-coding region called the control region or A+T rich region (Wolstenholme, 1992; Simon *et al.*, 1994; Boore, 1999) [66, 94, 9]. It is widely used due to its unique properties, including maternal inheritance, orthologous genes and lack of the substantial intermolecular recombination (Zhang *et al*., 1995; Avise *et al*., 1987; Dellaporta *et al.*, 2006) [69, 6, 16]. It is considered as a reliable marker for studying evolutionary aspects ranging from population genetics to phylogenetics among distantly related species. Due to the high rate of substitution occurring in the third codon positions (wobble positions) of protein coding genes, the DNA sequences of protein coding genes have frequently been used for species level or population level phylogeny (Hwang and Kim, 1999) [31]. In contrast, because its amino acid sequences are much more conserved than nucleotide sequences, it has been applied to relatively higher levels such as in families or rarely among orders (Liu and Beckenbach, 1992; Frati *et al*., 1997; Hwang and Kim, 1999) [44, 21, 31]. In this article an attempt has been made to review the molecular research work that has been conducted on different species of Hemipteran bugs based on mitochondrial genes.

Molecular studies on Hemipteran bugs based on mitochondrial genes
The phylogenetic relationships among 8 species of genus *Triatoma* were analysed using the

Mitochondrial DNA sequences (12S, 16S and COI) by Gracia and Powell (1998) [22]. Borges *et al.* (2000) [10] used RAPD PCR method to compare genetic variability within and between three populations of *Triatoma brasiliensis* from North East Brazil. Muraji *et al.* (2001) [48] analysed the nucleotide sequences of the mitochondrial 16S rRNA, Cyt b and COI genes in order to evaluate the usefulness of each mtDNA sequence for the estimation of the anthocorid species relationships. Brenière *et al.* (2003) [11] analysed RAPD profiles for three species of reduviid bug *Triatoma longipennis*, *T. pallidipennis* and *T. picturata*, which are sympatric in limited areas of their distribution ranges. Arensburger *et al.* (2004) [2] studied the phylogeny of 14 species of *Cicada* (Hemiptera) using four mitochondrial genes (COI, COII, 12S and 16S subunits) and one nuclear gene (EF-I). PCR RFLP technique was used for the discrimination among the species of genus *Orius* (Hemiptera: Anthocoridae) by Muraji *et al.* (2004) [49]. Hebsgaard *et al.* (2004) [26] studied the phylogeny of the true water bugs (Heteroptera) based on 16S and 28S rDNA. Xie *et al.* (2005) [67] used 18S rDNA to study the evolutionary relationships among the infraorder Pentatomorpha (Heteroptera) using 26 representative species, which belonged to 13 different families and studied their phylogenetic relationship. Kavar *et al.* (2006) [35] studied the genetic variation in southern stink bug *Nezara viridula* from eleven geographically separated sampling locations using RAPD markers and sequencing of 16S, 28S rDNA, Cytochrome b and Cytochrome C oxidase subunits. The use of molecular markers in insects also included RAPD as a good and reliable marker was reviewed by Behura (2006) [8] and Jain *et al.* (2010) [32]. Valenzuela *et al.* (2007) [61] identified 25 aphid species (Hemiptera: Aphididae) from Australia using RFLP method. The results suggested that COI based RFLP profiles provided a useful tool for identifying aphid species. RAPD PCR technique was used to estimate genetic structuring of *Dalbulus maidis* (Hemiptera: Cicadellidae) among five geographically distant localities and to estimate gene flow between populations by Martins de Oliveira *et al.* (2007) [47]. The phylogenetic relationship of family groups in Pentatomoidea based on morphology and DNA sequences (18S rRNA, 16S rRNA, 28S rRNA and COI gene) has been studied by Grazia *et al.* (2008) [23]. The phylogeny of the semiaquatic bugs (Heteroptera) was analysed using mitochondrial (COI, COII and 16S rRNA) and the nuclear gene 28S rRNA by Damgaard (2008) [15].

Hua *et al.* (2008) [30] sequenced fifteen mitochondrial genomes of the suborder Heteroptera to investigate the phylogenetic relationships among the superfamilies of Pentatomomorpha and stated that mitochondrial genome is a powerful molecular marker for resolving phylogeny at the level of the superfamily and family. The nucleotide sequences of 16S rDNA and 28S rDNA have been analysed in family Decaptophalinae (Hemiptera) by Huai *et al.* (2008) [30]. Foottit *et al.* (2008) [20] studied 300 species of aphids belonging to 130 genera and found that most (96%) species were well differentiated using DNA Barcoding region. Hua *et al.* (2009) [29] studied the phylogenetic analysis of the true water bugs (Heteroptera) using 16S rDNA and 18S rDNA sequences and showed that it is an effective data source for resolving intraordinal phylogenetic problems at the superfamily level within Heteroptera. Schuh *et al.* (2009) [57] studied phylogenetic relationships within the Cimicomorpha (Heteroptera) using 16S rDNA, 18S rDNA, 28S rDNA and COI gene sequences. The complete nucleotide sequence of the mitochondrial genome of *Geisha distinctissima* (Hemiptera) was studied by Song and Liang (2009) [60] indicating that the gene content and arrangement were similar to other hemipteran insects. Ashfaq *et al.* (2010) [3] used PCR-RFLP as a tool for invasive mealybug (Hemiptera) species damaging cotton in Pakistan showing its species characterization and composition. Li *et al.* (2010) used 16S rRNA, COI and Cytochrome b genes to study the phylogeographic relationships of the southern green stinkbug *Nezara viridula* (Hemiptera: Pentatomidae) and to understand *N. viridula* in the worldwide historical range and dispersal pattern. A comparative analysis of insect mitochondrial DNA representing five insect orders (Diptera, Lepidoptera, Hemiptera, Ephemeroptera and Coleoptera) consisting of 24 different species was performed by Habeeb *et al.* (2011) [25]. They used COI, 16S rRNA and 12S rRNA sequences to construct a phylogenetic tree to determine the relationship among these insect orders. Park *et al.* (2011) [51] has used COI gene to study the sequence divergence pattern in order Heteroptera. They showed that COI gene revealed less than 2% intraspecific divergence in 90% of the taxa examined and indicated that COI barcoding provided a useful identification tool for order Heteroptera. Two species of *Eurydema* (Hemiptera: Pentatomidae), *E. dominulus* and *E. gebleri* were distinguished using DNA barcording as molecular identification tool by Jung *et al.* (2011a) [33]. Jung *et al.* (2011b) [34] tested the effectiveness of a COI barcode to identify true bugs from 139 species collected from Korea and adjacent regions (Japan, North Eastern China and Far East Russia) and suggested that COI barcodes could reveal new cryptic true bug species and were able to contribute for the exact identification of the true bugs.

The development of Heteropteran systematics during the past 25 years was reviewed by Weirauch and Schuh (2011) [65]. Lis *et al.* (2011a) [41] investigated the mitochondrial gene sequences (12S and 16S) of the Heteropteran Infraorder Pentatomomorpha and provided the analyses of their nucleotide composition, nucleotide divergence and evaluated their phylogenetic utility from 46 Pentatomid species. Lis *et al.* (2011b) [43] performed molecular studies on museum specimens of five families of Pentatomoid bugs (Hemiptera) as a preliminary approach to molecular phylogenetic analyses of these families using mitochondrial gene sequences. Lis *et al.* (2012a) [40] studied the systematic position of two families, i.e. Dinidoridae and Tessaratomidae, within the superfamily Pentatomoidea (Heteroptera) based on the analysis of the mitochondrial DNA sequences. They suggested a close affinity of both families, usually identifying the Dinidoridae, as a sister group of Tessaratomidae. Lis *et al.* (2012b) [42] used the mini barcode as a marker for phylogenetic analyses in family Pentatomidae. Their study showed that DNA mini-barcodes could be employed not only for species identification, but also to draw the phylogenetic inferences. Li *et al.* (2012) used maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) with multiple genes (18S rDNA, 28S rDNA, 16S rDNA and COI) to estimate phylogenetic relationships among the infraorders of order Heteroptera. The genetic differences in COI sequences among 12 populations of *Helopeltis antonii* (Hemiptera: Miridae) collected from different geographic locations in India were examined by Ashokan *et al.* (2012) [4]. Phylogenetic analyses in *Aphis gossypii* and *Myzus persicae* (Hemiptera: Aphididae) using COI, COII and nuclear elongation factor-1 (EF-1) was performed by Rebijith *et al.* (2012) [54]. Cui *et al.* (2013) [14] studied the first phylogenomics of Hemiptera (Insecta: Paraneoptera) based on mitochondrial genomes and showed the close relationship

between Cicadomorpha and Heteroptera. Li *et al.* (2013) studied the complete mitochondrial genome of the stalk eyed bug *Chauliops fallax* Scott, the most destructive insect pest of soybean and rice fields in Asia *Leptoglossus occidentalis*, a leaf footed bug, (Hemiptera) was reported for the first time from Korea by Ahn *et al.* (2013) [1] based on COI gene sequences. Mani *et al.* (2013) [46] reported the mealybug, *Pseudococcus jackbeardsleyi* (Hemiptera: Pseudococcidae) for the first time on papaya in India by both morphological and molecular methods using COI gene sequences. Zeiodinni *et al.* (2013) collected 46 species from 37 genera and 17 subfamilies from 10 families of Heteroptera and used COI gene sequences to study the divergence among the dominant species among them. Rebijith *et al.* (2013) [55] discriminated 142 individual aphids (Hemiptera: Aphididae) representing 32 species collected from various host plants in South India using COI barcoding and revealed that the intraspecific and interspecific distances ranged from 0.0 to 3.8% and 2.31 to 18.9%, respectively. Babu and Livingstone (2014) [7] identified the bug, *Scutellera nobilis* (Fabricius) (Hemiptera: Pentatomoidae), which is a pest of a biodiesel plant *Jatropha curcas* using mitochondrial COI gene sequence. Wang *et al.* (2014) [64] studied the complete mitochondrial genome of bug, *Apolygus lucorum* (Hemiptera: Miridae), an important cotton pest which is presently known as the smallest in Heteroptera. Raupach *et al.* (2014) [53] presented a comprehensive DNA barcode analysis of 1742 specimens representing 457 species comprising 39 families of the true bugs (Hemiptera: Heteroptera) from Germany and showed DNA barcoding as an efficient method for the identification of species. Parveen *et al.* (2015) [52] studied all the life stages of *Tessaratoma javanica* (Hemiptera) using DNA barcoding region of COI gene fragment. DNA barcode library for about 45% of the recognized species of order Hemiptera from Canada was provided by Gwiazdowski *et al.* (2015) [24]. Ebong *et al.* (2016) [19] created DNA barcodes for 188 specimens of Heteroptera and discussed the importance of integrative taxonomy including morphological and molecular approaches. Chen *et al.* (2017) [13] studied the complete mitochondrial genome of *Gonopsis affinis* (Heteroptera: Pentatomidae), which is an important pest in China. Their results confirmed the monophyly of each family of Pentatomoidea with high node support. Singh and Kaur (2017) [59] compared three different methods for DNA extraction from bugs of family Pentatomidae (Hemiptera) for PCR and the effect of killing methods and storage conditions for extraction and purification of intact DNA in Pentatomid bugs. Wang *et al.* (2017) [62] studied the complete mitochondrial genome of *Graphosoma rubrolineata* belonging to subfamily Podopinae (Hemiptera: Pentatomidae). *G. rubrolineata* was from the subfamily Podopinae mixed with species from the subfamily Pentatominae. This suggested that Pentatominae might not be monophyletic or Podopinae may not be an effective taxonomic category. Zhao *et al.* (2017a) [71] studied the complete mitochondrial genome of *Eurydema maracandica* (Hemiptera: Pentatomidae) and their phylogenetic analysis indicated that the species of family Pentatomidae formed a solid monophyletic group. Zhao *et al.* (2017b) [70] examined COI sequences of 203 specimens of genus *Eurydema* collected from 16 provinces of China and showed that DNA barcoding was a useful and effective method for enhancing morphological data and resolving taxonomic problems in family Pentatomidae (Hemiptera). Mitochondrial COI, COII and Cyt b and nuclear EF-1α markers were sequenced from 383 individuals from 38 populations to study Niche divergence of two closely related *Carbula* species (Insecta: Hemiptera: Pentatomidae) by Liu *et al.* (2018) [45].

Conclusion

An attempt has been done to provide data for molecular studies conducted on hemipteran bugs. On the basis of literature surveyed it is quite clear that a lot of molecular work has been done on COI (barcoding region), Cyt b and 16S rRNA gene region while few studies have been conducted on other mitochondrial genes. An important limitation of molecular work is that researchers have focused more on barcoding region thereby neglecting rest of the molecular markers which could also prove valuable in determining the other vast and diverse objectives of the research work.

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