INTRODUCTION

The class Insecta is the most diverse group of all animal taxa. To date, over one million species have been described which is more than all other animal groups combined. Insects may be found in nearly all environments on the planet. They exhibit extreme variations in their feeding habits and range from scavengers to pests, predators and parasites. Insecta is divided into 31 orders which include some economically important groups such as Coleoptera, Diptera, Hemiptera, Hymenoptera, Orthoptera, Odonata etc.

The order Hemiptera comprises about 80,000 species which are divided into two suborders: Homoptera and Heteroptera. Approximately 37,000 species distributed in 77 families belong to the Heteroptera (McGavin, 1999). Members of the suborder Heteroptera are known as "true bugs". They have very distinctive front wings, called hemelytra in which the basal half is leathery and the apical half is membranous. At rest, these wings cross over one another to lie flat along the insect's back. These insects also have elongate, piercing-sucking mouthparts which arise from the ventral (hypognathous) or anterior (prognathous) part of the head capsule. The mandibles and maxillae are long and thread-like, interlocking with one another to form a flexible feeding tube (proboscis) that is no more than 0.1 mm in diameter yet contains both a food channel and a salivary channel. These stylets are enclosed within a protective sheath (the labium) that shortens or retracts during feeding.

The Heteroptera includes a diverse assemblage of insects that have become adapted to a broad range of habitats: terrestrial, aquatic and semi-aquatic. Terrestrial species are often associated with plants and they feed on vascular tissues or nutrients stored within seeds. Some terrestrial species are scavengers living in the litter or underground in caves or ant nests. Some species are predators on a variety of small
arthropods while a few species even feed on the blood of vertebrates. Members of the family Cimicidae live exclusively as ectoparasites on birds and mammals (including humans). Aquatic heteropteran species are found on the surface of both fresh and salt water, near shorelines or beneath the water surface in nearly all freshwater habitats. With only a few exceptions, these insects are predators of other aquatic organisms. Economically, plant feeding heteropterans are important pests of many crop plants. They may cause localized injury to plant tissues or may weaken plants by sucking sap, and in the process may transmit plant pathogens. Predatory species are generally regarded as beneficial as they attack primarily lepidopteran and coleopteran larvae which are important pests in agro systems. Some heteropteran species received attention as potential biological control agents for agricultural pests (Costello et al., 2002).

Insect chromosomes were among the first chromosomes to be investigated and first data on insect chromosomes was published late in the 19th century. Since then, insects have had an important place in cytogenetics. Insect studies were instrumental in proving that genes are on chromosomes and that spindle fibers exist in living cells and are not fixation artifacts. Karyological studies provide important information on the genetic structure, life cycle and ecological characteristics, evolution, taxonomy and phylogeny of insects. Thus, insects have been ideal model organisms for basic cytogenetic research.

In Heteroptera, cytogenetic studies date from 1891 with Henking’s morphological study on the spermatogenesis of the bug Pyrrhocoris apterus. Subsequently a series of publications by Wilson (1905a, b, 1906, 1907a, b, 1911) and Montgomery (1901a, b, 1904, 1906) laid the foundation of heteropteran cytogenetics. Then after, heteropteran cytogenetics has been dealt with exhaustively because of
peculiar features of their chromosomes both in morphology and behavior.

One of the most important cytogenetic characteristics of Heteroptera is the holokinetic nature of their chromosomes (the presence of diffuse kinetic activity) which contrasts with most of the insect taxa which are characterized by monocentric (localized) chromosomes (Schrader, 1935, 1947; Hughes-Schrader and Ris, 1941; Hughes-Schrader and Schrader, 1961). All organisms with holokinetic chromosomes show the same mitotic behavior: chromosomes orientate at the metaphase plate with their long axes perpendicular to the spindle axis and sister chromatids migrate parallel to each other. However, the meiotic behavior presents differences in different groups of organisms (White, 1973). It has been suggested that in holokinetic, systems fusion and fragmentation are the most frequent chromosome rearrangements that cause variations in diploid number (Rebagliati et al., 2005).

Many heteropteran species have a minute chromosome pair with a special meiotic behavior. The term micro-chromosomes (m-chromosomes) was assigned to them by Wilson (1905b) and they are found only in Heteroptera (Ueshima, 1979). These m-chromosomes are generally observed unpaired during early meiotic prophase and no chiasma is detected between them. The mode of origin of this chromosome pair is uncertain and even their function in the genetic system of the species possessing them is still to be ascertained. It has been suggested that these chromosomes were present in the ancestral karyotype of Heteroptera as they are present in the primitive Infraorder Dipsocoromorpha (Grozeva and Nokkala, 1996).

Another cytogenetic characteristic of heteropteran species is the meiotic behavior of the chromosomes. It has been observed that the meiotic behavior of autosomal bivalents, sex chromosomes and m-chromosomes is slightly different. As a rule, autosomal bivalents are chiasmatic whereas sex chromosomes and m-
chromosomes are achiasmatic (Ueshima, 1979; Manna, 1984; Gonzalez-Garcia et al., 1996; Suja et al., 2000). When autosomal bivalents possess only one chiasma located terminally, they orientate axially at metaphase I with their long axes parallel to the polar axis and they segregate reductionally during meiotic division I and equationally during meiotic division II. Bivalents with two terminal chiasmata orientate equatorially and two different behaviors have been described. In the first type, one chiasma is released first and an axial orientation is finally achieved. In the second type, alternative sites of kinetic activity become functional and no telokinetic activity is observed (Mola and Papeschi, 1993; Papeschi et al., 2003). On the other hand, sex chromosomes are achiasmatic and act as univalents in male meiosis I segregating post-reductionally. Similarly, the m-chromosomes are achiasmatic but associate at first meiotic division segregating pre-reductionally (Mola and Papeschi, 1993).

Heteropteran insects are also characterized by the presence of a special stage of prophase called the diffuse stage during which the autosomes undergo decondensation while the sex chromosomes remain condensed. The presence of the diffuse stage is a frequent meiotic phenomenon among heteropterans although its duration and the degree of chromosome decondensation seems to vary between species, ranging from a high degree of decondensation to partial decondensation up to cases of species whose chromosomes do not decondense at all and which consequently do not have a diffuse stage (Solari, 1979; Ueshima, 1979; Nokkala and Nokkala, 1984; Camacho et al., 1985; Papeschi and Mola, 1990; Rebagliati et al., 1998, 2001; Jacobs and Liebenberg, 2001). The meaning of the diffuse stage has not yet been completely established, although Stack and Anderson (2001) have suggested a model by which the chromosomes change from meiotic to mitotic organization during the diffuse stage which corresponds to a final G2 phase in which the
decondensed chromosomes are actively transcribed. Data obtained from most heteropterans agree with Stack and Anderson’s model which represents a mechanism of general chromosome organization found in most organisms. It is possible that the differences in the duration and levels of decondensation of the diffuse stage correspond to variations in the interval of disintegration between the meiotic and mitotic chromosome structure among different organisms (Lanzone and Souza, 2006a).

Another cytogenetic characteristic of heteropteran species is the presence of “inverted meiosis” for the sex chromosomes i.e. the sex chromosomes divide equationally during the first meiotic division and reductionally during the second meiotic division (post-reductional meiosis) wherein division of sister chromatids takes place using the plane of replication between them as division plane followed by the disjunction of homologous chromosomes using the pairing plane as division plane. The presence of “inverted meiosis” has been considered as an evolutionary adaptive modification to accomplish a successful meiosis (Hughes-Schrader, 1948). In this type of meiosis, the kinetic activity is restricted to a pair of telomeric regions of the bivalent, and thereby they can be considered as telokinetic chromosomes. Both telomeric regions of a chromosome are kinetically active during meiosis I but become inactive during the second one (Nokkala, 1985; Albertson and Thomson, 1993; Gonzalez-Garcia et al., 1996).

Heteropterans show several different types of sex chromosome mechanisms. 71.4% of cytogenetically analyzed species have simple system of XX females and XY males (XX/XY), 14.7% are with XX females and X0 males (XX/X0), 13.5% show multiple systems (X_nX_n/X_nY, X_nX_n/X_n0, XX/XY_n) , while the rest 0.4% have neo-XY system (Ueshima, 1979; Bressa et al., 1999; Nokkala and Nokkala, 1999; Jacobs,
2004; Papeschi and Bressa, 2006). Different views have been given for the evolution of various sex mechanisms in Heteroptera. Ueshima (1979) suggested that the XY system is derived from ancestral X0 that is commonly encountered in primitive taxa and in phylogenetically related homopteran species. On the other hand, Nokkala and Nokkala (1983, 1984) and Grozeva and Nokkala (1996) based on the presence of a Y chromosome in very primitive Heteropteran species, suggested that the X0 system is a derived condition from the ancestral XY that is present in the majority of the species cytogenetically analyzed. The probable origin of the multiple sex chromosome system was suggested to be through fragmentation of the ancestral X or Y (Papeschi, 1996). The origin of the neo-sex chromosome mechanism is thought to be due to a complex system of fusion between the sex chromosome and an autosome (Chickering and Bacorn, 1933; Schrader, 1940b; Jacobs, 2004).

The suborder Heteroptera is divided into seven infraorders (Stys and Kerzhner, 1975; Schuh and Slater, 1995). Pentatomorpha is one of the infraorders which constitutes the most recognizable groups of true bugs, and includes Pentatomoidea, Coreoidea, Lygaeoidea and Aradoidea as the major superfamilies (Cassis and Gross, 2002). The Pentatomoidea is the most diverse superfamily of Pentatomorphan bugs and includes 14 families (Schuh and Slater, 1995). The Pentatomidae is the largest family of Pentatomoidea numbering about 642 genera and 4112 species (Schaefer and Panizzi, 2000). Pentatomids are mostly ovoid in shape, although a few species are elongate. The body is dorsally flattened and ventrally convex. The antennae are usually 5 segmented. The scutellum is usually triangular and large with few species having it greatly enlarged and shield like. They are called stinkbugs because they produce a disagreeable odour by means of scent glands that open in the region of the metapleuras.
Cytogenetic reports on Pentatomoidea refer to 391 species belonging to only nine families: Acanthosomatidae, Cydinidae, Dinidoridae, Plataispididae, Tessaratomidae, Thaumastellidae, Scutelleridae, Urostylidae and Pentatomidae. About 294 species belonging to 121 genera of Pentatomidae have been cytogenetically studied. Cytogenetic reports show that pentatomid species have a diploid number of $2n = 14$ (85% of cytogenetically reported species), a sex chromosome system of XX/XY (except three species) and without a pair of m-chromosomes (Rebagliati et al., 2005). In addition to sharing the major cytogenetic characteristics of Heteroptera, some members of the Pentatomidae show regular presence of a unique lobe of the testes. In these species, one of the lobes is of the harlequin type that differs from the other lobes by showing spermatogonial cells with meiotic pairing, non specific association of autosomal bivalents, anomalous chromosome segregation and cell fusion resulting in the production of spermatozoa with an abnormal and highly variable chromosome number which will affect the individual fertility. There are reports of this type of lobe in 22 species belonging to 15 genera in three Pentatomidae subfamilies: Discocephalinae, Edessinae, Pentatominae (Bowen, 1922a, b; Schrader, 1945a, b, 1946a, b; 1960a, b; Martin 1953; Srivastava, 1957; Ansley, 1958; Rebagliati et al., 2001).

Manna (1958, 1962) and Banerjee (1958) have established the interrelationship between the various groups of Heteroptera based on their cytological studies and have pointed out that Pentatomidae is the most primitive family from which all other families of Heteroptera have evolved. Thus, a detailed cytogenetic study of the family Pentatomidae may help in a better understanding of the evolutionary pathways through which the various families have evolved.

Schuh and Slater (1995) included 8 subfamilies in the family Pentatomidae:
Asopinae, Cyrtocorinae, Discocephalinae, Edessinae, Pentatominae, Phyllocephalinae, Podopinae and Serbaninae.

The **Asopinae** is a cosmopolitan subfamily of predatory bugs comprising 63 genera and 357 species (Schuh and Slater, 1995). They are called “soldier” bugs and are of moderate to large size ranging in length from 7 to 25 mm. The modal diploid chromosome number of the asopin species is $2n = 14$ with XX/XY sex determining system (Rebagliati *et al.*, 2005). The **Pentatominae** is the most diverse subfamily of heteropteran bugs and comprises 404 genera and 2771 species (Schuh and Slater, 1995). Most of the phytophagous plant pests are included under this subfamily. Cytologically, it is the most studied subfamily with a diploid chromosome number of $2n = 14$ and a sex determining mechanism of XX/XY except few species which show multiple sex chromosome mechanism and neo-XY systems (Wilson, 1911; Schrader, 1940a; Hughes-Schrader and Schrader, 1956). The **Podopinae** is a cosmopolitan phytopagous subfamily comprising 64 genera and 255 species worldwide (Schuh and Slater, 1995). All the chromosomally investigated podopin species except one show a diploid chromosome number of $2n = 14$ and a stable sex chromosome system of XX/XY. The **Discocephalinae** comprises 71 genera and 263 species which are most diverse in the neotropics (Schuh and Slater, 1995). Members of this subfamily exhibit a diploid chromosome number of $2n = 14$ with XX/XY sex determining mechanism. The **Edessinae** comprises 4 genera and 263 species which are found in the Western Hemisphere, predominantly in South America (Schuh and Slater, 1995). They show a constant diploid number and sex determining system of $2n = 14$ and XX/XY respectively. The **Phyllocephalinae** comprises 31 genera and 175 species which are restricted to the Eastern Hemisphere. Only a single species (*Macrina juvenca*) has been cytogenetically analyzed from this subfamily with a diploid chromosome
number of $2n = 14$ and XX/XY sex determining mechanism (Nuamah, 1982). No cytogenetic work has been reported from the subfamilies **Cyrtocorinae** and **Serbaninae**.

The routine methods (conventional staining methods) are essential from two major perspectives. In the first, they are used to study all aspects of individual chromosomes such as the type of centromere, the absolute and relative length of chromosomes (or area in case of holocentric chromosomes) and the presence of secondary constrictions and the nature of the telomeres. Secondly, these methods are important to analyze the features of the karyotype as a whole which include the chromosome number, the sex determining mechanism and the presence and position of chiasmata in autosomal bivalents.

In addition to conventional staining, methods of differential staining revealing specific segments of chromosomes according to their structure and base composition were introduced in the comparative cytogenetic studies of insects in 1970s and are rapidly developing these days. These techniques are used to selectively stain the constitutive heterochromatin (high-repeat DNA sequences) and the nucleolar organizer regions which are highly important to be used as cytogenetic markers Zoshcuk et al. (2003). It is generally presumed that constitutive heterochromatin is inert material. However, there is abundant and increasing evidence that constitutive heterochromatin can have important functions in chromosome pairing and segregation, position effect variegation and can even contain genes and other functional DNA sequences (Sumner, 1972). Structurally, the constitutive heterochromatin is composed of highly repetitive DNA found in and near centromeres, telomeres and other heterochromatic regions (Hoy, 1994). This type of DNA exhibits special characteristic of stability under extreme conditions of heat and
chemical exposure. This property of tightly condensed heterochromatin can be exploited to produce a unique banding pattern (C-banding) in which constitutive heterochromatin stains darkly and rest of the chromatin remains pale. C-banding is primarily of value in the identification of the gene coding potential of various segments of the genome and for the study of chromosomal polymorphisms (Moore and Best, 2001).

In eukaryotic organisms, ribosome synthesis largely takes place in a specialized nuclear domain- the nucleolus. This organelle is the site of rDNA transcription, pre-rRNA processing and modification and initial steps of pre-ribosome assembly (Thiry and Lafontaine, 2005). Ribosomal genes are organized into highly repeated gene families, one that codes for the major ribosomal subunit composed of 28S, 18S and 5.8S rRNA and the other that codes for the minor ribosomal subunit composed of 5S rRNA. The first gene family corresponds to nucleolar organizer regions (NORs) and is identified with silver staining (Deiana et al., 2000). Ag-NORs are not rDNAs themselves but a complex of acidic residual proteins associated with the nucleolar fibrillar center, and they are used to investigate rDNA expression. Under acid conditions, the proteins that joined recently transcribed rRNA are stained by this technique because they are able to reduce silver. At interphase, these proteins are located in the nucleolus and during cell division they are specifically located in the NORs (Goodpasture and Bloom, 1975). The determination of the number and localization of rDNA/Ag-NOR loci, which are species specific, makes them an important chromosomal marker in phylogenetic analysis.

Chromosomes of most insects lack euchromatic bands but they may be identified by using DNA specific fluorochromes to reveal nature of their heterochromatic bands. Two types of DNA binding fluorochromes which have the
capacity to discriminate in their binding to base pairs are currently being used for chromosome identification. The first type includes the A-T specific DAPI (4′-6-diamidino-2-phenylindole). Solution interaction studies of these fluorochromes in the presence of DNA have shown that binding of the fluorochrome to DNA is accompanied by a fluorescence enhancement which is directly related to the A-T content of the DNA (Weisblum and de Haenssler, 1974; Latt and Stetten, 1976). The chromosome banding pattern produced by these fluorochromes can then be rationalized in terms of the distribution of A-T base pairs along the length of the chromosomes. The second type of fluorochromes consists of the G-C specific CMA₃ (Chromomycin A₃). CMA₃ exhibits enhanced fluorescence when bound to DNA which is directly related to the G-C content of the DNA (Van de Sande et al., 1977).

Differential staining of chromosome regions by DAPI and CMA₃ is thus of great importance to identify the base composition of the heterochromatic regions in Heteroptera.

All these chromosomal characteristics are essentially morphological, and therefore can be analyzed in approximately the same way as other morphological features. Moreover, some characters of a karyotype (such as the number of chromosomes, chromosome arms, nucleolar organizers and heterochromatic blocks) can possess discrete values, allowing one to recognize easily most cases of the intraspecific chromosomal polymorphism as well as hybridization between forms with different chromosome number. In addition, methods of chromosomal analysis relatively allow vast material to be examined in a short space of time. Due to these reasons, chromosome studies have some obvious advantages over other methods used in taxonomic studies.

Regardless of immense economic importance and unique nature of their
genetic system, cytogenetic investigations on Heteroptera in general and on Pentatomidae in particular are meager. More so, most of the cytogenetic studies are based on conventional preparations. In India too, in spite of rich biodiversity, only a small fraction of heteropteran/pentatomid population has been cytogenetically analyzed. Bearing this in mind, the present study was undertaken to provide a detailed characterization of chromosomal complement in species of the family Pentatomidae by the use of different cytogenetic techniques. Routine staining method has been used to study the chromosome number, behavior and sex determining mechanisms; C-banding, Ag-NOR and sequence specific banding techniques have been used to get further insight into the chromatin organization in terms of amount, composition and location of constitutive heterochromatin and nucleolar organizer regions in different pentatomid species. Cytogenetic features of 23 species belonging to 3 subfamilies have been described and through a comparative analysis, certain cytological markers have been identified.