REVIEW OF LITERATURE

Heteroptera is a highly diverse insect taxon with approximately 42,300 described species worldwide, separated into 7 infraorders and 89 families (Henry, 2009). Their body size ranges from less than 1 mm to 10 cm. Heteropterans live in virtually all terrestrial and aquatic ecosystems from Antarctic birds’ nests to rainforest canopies, from the open surface of the ocean to torrential and stagnant rivers, from ephemeral rain pools and phytotelmata to large lakes, and in aphotic caves and man-made buildings. They feed on a variety of resources (e.g., haemolymph of insects, blood of endotherms, algae, fungi, mosses, ferns, monocotyledons and dicotyledons). Long evolutionary history and apparent adaptability of the Heteroptera have resulted in great biological and structural diversity (Schuh and Slater, 1995).

A. Chromosomal complement and meiosis in Heteroptera

a. International level

Heteroptera is a diversified group of insects displaying unique cytogenetic characters such as holokinetic chromosomes, presence of microchromosomes in some families, multiple sex chromosome systems and inverted meiosis for sex chromosomes. The pioneer investigator of true bug cytogenetics is Henking (1891) who deserves the credit for the discovery of a relationship between chromosomes and sex determination in the firebug *Pyrrhocoris apterus* (Pyrrhocoridae). The cytogenetic information on Heteroptera continued with the works of Montgomery (1901a, b, 1904, 1906), Wilson (1905a, b, c, 1906, 1907a, b, c, 1909a, b, c, 1910, 1911, 1912, 1913, 1932), Foot and

b. National level

Significant cytogenetic work pertaining to diploid chromosome complement and course of meiosis has been done on Indian Heteroptera. Manna (1950, 1951, 1956, 1957, 1958, 1962, 1982, 1983, 1984) is the pioneer and major researcher in Indian Heteroptera cytogenetics. The work was continued by his associates and other workers and more and more Indian species were investigated for chromosome number, sex mechanism and
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B. Chromosome complement and meiosis in Coreidae

Coreidae is divided into 4 subfamilies: Coreinae, Pseudophloeinae, Agriopocorinae and Meropachydinae (Schuh and Slater, 1995). Cytogenetic data for only 2 subfamilies i.e. Coreinae and Pseudophloeinae is available.

a. Coreinae

Coreinae is divided into 31 tribes but cytological data is available for 18 tribes only.

1) Coreini

International level
Tribe Coreini is the most widely studied tribe of the subfamily Coreinae. A total of twenty one taxa have been studied till date. The most common complement is $2n=22$ shown by eight out of twenty one. Initial cytogenetic studies pertaining to the course of meiosis in Coreini were done by Montgomery (1901a, 1904, 1906), Wilson (1905a, b, c, 1906, 1907a, b, c, 1909a, b, c, 1911), Schachow (1932), Geitler (1939b), Yosida (1944) and Xavier (1945). Nokkala (1986) explained the mechanisms behind the regular segregation of the m-chromosomes in *Coreus marginatus*. Papeschi and Mola (1990a) described the behavior of univalents in desynaptic individuals of *Acanonicus hahni*. Souza *et al.* (2007a) investigated cytogenetical aspects of testicular cells in *Sphicyttus fasciatus*, *Anasa bellator* and *Zicca annulata*. Souza *et al.* (2009) studied meiosis, spermatogenesis and nucleolar behavior in the seminiferous tubules of *Hypselonotus fulvus* and *Catorintha guttula*.

**National level**

No cytogenetic data is available.

2) **Mictini**

**International level**

Eleven species of Mictini have been studied so far. Diploid number ranges from 15 to 26. Sands (1982a) gave detailed information on male meiosis in five Malaysian species *viz.*, *Anoplocnemis phasiana*, *Ochrochira rubrotincta*, *Derepteryx chinai*, *Mictis gallina* and *Mictis longicornis*. Takenouchi and Muramoto (1970b) studied diploid chromosome complement and course of meiosis in *Molipteryx fuliginosa*.

**National level**
Indian works on Mictini include that of Manna (1951), Parshad (1957a, d), Jande (1959b) and Dey and Wangdi (1985, 1990). Manna (1951) and Parshad (1957d) described the course of meiosis in *Elasmomia granulipes* and *Ochrochira albiditarsis*. Jande (1959b) and Dey and Wangdi (1985) reported diploid chromosome number and sex mechanism in *Ochrochira* sp. and *Derepteryx hardwicki*.

3) Gonocerini

**International level**

Sixteen species have been studied so far. The diploid number ranges from 17 to 22. Earlier studies include those of Schachow (1932), Toshioka (1935) and Xavier (1945) on *Cletus hoplomachus*, *Cletus rusticus*, *Cletus trigonus*, *Gonocerus acuteangulatus* and *Gonocerus juniperi* var. *triquetricornis*. Sands (1982a) provided detailed information on male meiosis of two Malaysian species viz., *Cletus trigonus* and *Cletus punctiger*. Kaur and Semahagn (2010a) and Yang *et al.* (2012) described male meiosis in *Cletus punctulatus* from Ethiopia and *Cletus graminis* from China respectively.

**National level**

Significant cytogenetic contribution was made on Indian Gonocerini in the mid-century but afterwards it was almost neglected. Manna (1951), Parshad (1957d), Dutt (1957), Banerjee (1958) and Satapathy and Patnaik (1989) gave detailed information on diploid chromosome number and course of meiosis in *Cletomorpha hastata*, *Cletus bipunctatus*, *Cletus punctulatus*, *Cletus trigonus*, *Cletus pugnator* and *Cletus* sp. Parshad
(1958) studied behavior of the long chromosomes and structure of the heteropteran kinetochore in *Cletus punctiger*.

4) **Homoeocerini**

**International level**

Seventeen species belonging to two genera *viz.*, *Homoeocerus* (sixteen species) and *Anacanthocoris* (one species) have been studied so far. The diploid number ranges from 18 to 21. Toshioka (1934, 1935) investigated diploid male complement of *Anacanthocoris concolaratus*, *Homoeocerus dilatus* and *Homoeocerus unipunctatus*. Takenouchi and Muramoto (1967) described course of meiosis in *Homoeocerus dilatatus*. Sands (1982a) provided detailed information on male meiosis of three Malaysian species *viz.*, *Homoeocerus serrifer*, *Homoeocerus limbatipennis* and *Homoeocerus angulatus*. Yang *et al.* (2012) carried out meiotic studies in *Homoeocerus bannaensis* by DAPI staining.

**National level**

In India, Parshad (1957a) and Manna and Deb Mallick (1981a) reported diploid chromosome number and sex determination mechanism of *Homoeocerus simidus*, *Homoeocerus indus*, *Homoeocerus* sp. and *Homoeocerus signatus*. Parshad (1957d) and Dutt (1957) described diploid chromosome complement and course of meiosis in *Homoeocerus prominulus*, *Homoeocerus indus*, *Homoeocerus* sp., *Homoeocerus lacertorsus*, *Homoeocerus borealis* and *Homoeocerus* (*Omanocoris*) *variabilis*. Muramoto (1978b) investigated male diploid autosome number in *Homoeocerus pallidulus*. 20
5) Acanthocorini

International level

Six species have been studied so far. Diploid chromosome number ranges from 19 to 26. Yosida (1944, 1946, 1947) investigated diploid chromosome number and sex determination mechanism in *Acanthocoris sordidus*. Sands (1982a) provided detailed information on male meiosis in three Malaysian species *viz.*, *Acanthocoris scabrator*, *Physomerus parvulus* and *Physomerus grossipes* while Yang *et al.* (2012) carried out similar studies in *Acanthocoris scaber*.

National level

Indian works on Acanthocorini include that of Manna (1951) and Satapathy *et al.* (1990).

6) Daladerini

International level

No cytogenetic data is available.

National level

For Daladerini, chromosome information is available only for one species (*Dalader planiventris*) from India which has been provided by Dey and Wangdi (1985). The diploid chromosome number is $2n=21$ with X0 sex chromosome system.

7) Colpurini

International level
So far, for Colpurini, three species have been cytogenetically worked upon. Japanese species viz., *Hygia opaca* and *Pachycephalus* sp. possess a diploid chromosome complement of 2n=21 and 2n=22 respectively (Yosida, 1950; Muramoto, 1973a, 1979).

**National level**

Indian works pertain to only one species *i.e.* *Hygia touchi* (2n=17) investigated by Manna and Deb Mallick (1981a).

8) **Cloresmini**

**International level**

Four species belonging to a single genus (*Notobitus*) have been studied so far. All the four species possess 2n=21 and X0 sex mechanism for males. Sands (1982a) gave a detailed account of behavior and metaphase arrangement of chromosomes during meiosis in *Notobitus affinis* and *Notobitus* sp.

**National level**

Indian works pertain to two species *viz.* *Notobitus excellens* and *Notobitus meleagris* investigated by Dey and Wangdi (1990) and Kaur (2007) respectively.

9) **Petascelini**

**International level**

No cytogenetic data is available.

**National level**

Cytological reports for this family are available only from India. So far, chromosome number of three species of Petascelini has been given which include *Petillopsis patulicollis*, *Petillopsis calcar* and *Trematocoris notatipes* (Manna, 1951;
Parshad, 1957d; Dey and Wangdi, 1988 respectively). All the three species were studied under the genus *Petillia* and possess 28 chromosomes with 24 autosomes, a pair of microchromosomes and $X_1X_20$ sex mechanism.

10) **Dasynini**

**International level**

Chromosome work is reported only for one species of this tribe. Yang *et al.* (2012) described the diploid chromosome number and male sex chromosome system of *Paradasynus longirostris*.

**National level**

No cytogenetic data is available.

11) **Acanthocerini**

**International level**

So far, four species of Acanthocerini have been cytogenetically investigated by Wilson (1907c, 1909c), Piza (1956), Cattani *et al.* (2004), Bressa *et al.* (2005) and Souza *et al.* (2007a). All the species show a chromosome number of $2n=21$.

**National level**

No cytogenetic data is available.

12) **Nematopodini**

**International level**
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For Nematopodini, six species have been analyzed so far by Wilson (1905a, 1907c, 1909c, 1911, 1932), Piza (1946) and Papeschi et al. (2003). Five species show a chromosome complement of $2n=15$ while one has $2n=17$.

National level

No cytogenetic data is available.

13) Anisoscelini

International level

So far, fourteen species of Anisoscelini have been cytogenetically investigated. The diploid chromosome complement has been described by Wilson (1907c, 1909c, 1911) and Piza (1945, 1946, 1956). More recently, Souza et al. (2007a) described cytogenetic aspects of testicular cells in *Leptoglossus zonatus*, *Leptoglossus gonagra*, *Dallocoris pictus* and *Dallocoris obscura* and Toscani et al. (2008) carried out meiotic studies in *Holhymenia rubiginosa*.

National level

No cytogenetic data is available.

14) Discogastrini

International level

Chromosome work is reported only for one species (*Carlisis wahlbergi*) of this tribe by Fossey and Liebenberg (1995) which is found to have a diploid chromosome number of $2n=20$ and $X_1X_20$ male sex chromosome system.

National level

No cytogenetic data is available.
15) **Chariesterini**

**International level**

Only two species *viz.* *Chariesterus antennator* and *Chariesterus armatus* have cytogenetically worked upon by Montgomery (1901a), Wilson (1905a), Piza (1957) and Souza *et al.* (2007a).

**National level**

No cytogenetic data is available.

16) **Chelinideini**

**International level**

Chromosome work is reported only for one species (*Chelinidea vittiger*) of this tribe by Wilson (1907c) which possesses a diploid chromosome number of 2n=21 and X0 male sex chromosome system.

**National level**

No cytogenetic data is available.

17) **Acanthocephalini**

**International level**

For the tribe Acanthocephalini, three species belonging to a single genus, *Metapodius* (*M. terminalis, M. granulosus* and *M. femoratus*) have been studied by Montgomery (1901a, 1906) and Wilson (1907b, 1909c, 1910). The tribe represents an exception in possessing a number of supernumeraries seen in all the species.

**National level**

No cytogenetic data is available.
18) Spartocerini

**International level**

Only two species belonging to the genus *Spartocera* have been studied by Cattani and Papeschi (2004) and Franco *et al.* (2006) and both possess the same chromosome number of $2n=24$.

**National level**

No cytogenetic data is available.

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b. Pseudophloeinae

**International level**

For Pseudophloeinae, cytogenetic data for two tribes is known. From Pseudophloeini, three species have been investigated by Schachow (1932), Xavier (1945) and Takenouchi and Muramoto (1964). Diploid chromosomal complement of *Clavigralla spinofemoralis* of the tribe Clavigrallini has been described by Muramoto (1978b).

**National level**

No cytogenetic data is available.

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**C-banding**

Heterochromatin has been defined by Heitz (1928) as the nuclear material that remains condensed throughout the cell cycle. It is characterized by high proportion of repetitive sequences, late S-phase replication and lack of recombination. It has been
argued that heterochromatin is a dynamic component of the chromosome and is liable to both qualitative and quantitative variations. Furthermore, the assortment of heterochromatin and euchromatin into different chromosomal domains appears to play an important role in chromosomal structure (Wallrath, 1998). C-banding technique has made it easier to assess the changes in constitutive heterochromatin and has revealed the prevalence of remarkable degree of variations in C-banding pattern in different species (King and John, 1980; Lopez-Fernandez and Gosalvez, 1981).

relationship between C-bands and DNA content in four species of family Belostomatidae. Dey and Wangdi (1990) made an attempt to distinguish karyotypes of six species of Heteroptera viz., Petilopsis patulicollis, Ochrochira granulipes, Anoplocnemis phasiana (Coreidae), Leptocorisa acuta (Alydidae), Iphita limbata (Largidae) and Nezara icterica (Pentatomidae) on the basis of C-banding pattern. Panzera et al. (1992) analyzed C-heterochromatin polymorphism in Triatoma infestans (Reduviidae). Papeschi (1995) discussed correspondence between C-bands and AgNORs in Belostoma oxyurum (Belosmatidae). Nokkala and Grozeva (1997) gave a detailed account of C-banding pattern in Berytinus minor and Metatropis rufescens (Berytidae). Perez et al. (2000) and Panzera et al. (2000) compared C-banding patterns of Triatoma infestans and Triatoma brasiliensis (Reduviidae) while Grozeva and Nokkala (2001) compared C-banding pattern of thirteen species of family Tingidae. Cattani et al. (2004) and Ituarte and Papeschi (2004) described the localization of C-heterochromatin in Camptischium clavipes (Coreidae) and Tenagobia sp. (Micronectidae) respectively. Grozeva et al. (2004) and Angus et al. (2004) compared patterns of C-banding in four species of Nabidae and Notonectidae respectively. Bressa et al. (2005), in a comparative study on different families, analyzed the amount, composition and location of heterochromatin in three species of Coreidae (Leptoglossus impictus, Atheta mustus haematicus and Phthia picta), one species of Rhopalidae (Jadera sanguinolenta) and one species of Largidae (Largus rufipennis). Waller and Angus (2005) compared C-banding pattern in the chromosomes of four species of Corixa viz., Corixa punctata, Corixa iberica, Corixa dentipes, Corixa affinis and Corixa panzeri. Franco et al. (2006) described the
distribution of constitutive heterochromatin in *Spartocera batatas* (Coreidae). Lanzone and Souza (2006b) discussed the localization of constitutive heterochromatin in three species of *Antiteuchus* namely *Antiteuchus mixtus*, *Antiteuchus sepulcralis* and *Antiteuchus macraspis* (Pentatomidae). Grozeva et al. (2006) studied the localization of constitutive heterochromatin in *Macrolophus costalis* (Miridae) and reported heavy interstitial C-bands. Kuznetsova et al. (2007) and Bressa et al. (2008) analyzed C-banding pattern in *Arachnocoris trinitatus* (Nabidae) and *Holhymenia rubiginosa* (Coreidae) respectively. Kaur et al. (2010) provided a comparative account of C-banding pattern in three species of *Dieuches*, *Dieuches insignis*, *Dieuches uniguttatus* and *Dieuches coloratus* (Lygaeidae) and revealed C-heterochromatic heterogeneity in autosomes and sex chromosomes of the three species.

**Fluorescent Banding**

Fluorescent DNA-banding dyes of different base specificity have made it possible to characterize heterochromatin regions in a much more precise manner. These fluorochromes have been used to display A-T rich and G-C rich DNA (Schweizer, 1976, 1981).

Bressa et al. (1999) effectively used DAPI staining for the chromosomes of *Dysdercus albofasciatus* (Pyrrhocoridae) to identify the neo-XY chromosome. Perez et al. (2000), Bressa et al. (2001) and Rebagliati et al. (2003) applied fluorescent dyes to characterize constitutive heterochromatin in species of Reduviidae (*Triatoma infestans*), Rhopalidae (*Jadera haematoloma* and *Jadera sanguinolenta*) and Pentatomidae (*Edessa*...
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meditabunda and E. rufomarginata) respectively. Grozeva et al. (2004) and Kuznetsova et al. (2007) performed fluorescent banding on nabid species and reported C-heterochromatin to be A-T rich in all the species. Cattani et al. (2004) studied localization of A-T and G-C rich regions in Camptischium clavipes (Coreidae) and found all chromosomes to be stained uniformly with fluorochromes DAPI and CMA3 except for the largest autosomal bivalent and the X chromosome. Ituarte and Papeschi (2004) found C-heterochromatin to be both A-T and G-C rich in Tenagobia sp. (Micronectidae). Lanzone and Souza (2006b), Severi-Aguiar et al. (2006) and Grozeva et al. (2006) studied localization of A-T and G-C rich regions in the chromosomes of Antiteuchus mixtus, Antiteuchus sepulcralis and Antiteuchus macraspis (Pentatomidae), Triatoma vitticeps (Reduviidae) and Macrolophus costalis (Miridae). Morielle-Souza and Azeredo-Oliveira (2007) compared fluorescent banding pattern in three species of Reduviidae (Panstrongylus megistus, Rhodnius pallescens and Triatoma infestans). Bressa et al. (2008) applied fluorochromes to the chromosomes of Holhymenia rubiginosa (Coreidae) to characterize heterochromatin. Similar studies were carried out by Kaur et al. (2010) in two species of Lygaeidae (Dieuchus uniguttatus and Dieuchus insignis) and by Rebagliati and Mola (2010b) in three species of Pentatomidae (Acledra kinbergii, Acledra modesta and Acledra bonariensis).

Silver Banding

Silver ion impregnation, known for almost a century, is another technique frequently employed in cytogenetics. It has been used to display the Nucleolar Organiser
Regions (NORs) in the chromosomes that house the genes which code for ribosomal RNA. RNA is synthesized and processed into pre-ribosomes in the nucleolus. Under acidic conditions, the proteins that join the 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, these mostly disperse but some remain attached to the NORs (Goodpasture and Bloom, 1975).

Nokkala and Nokkala (1984) used silver staining in *Coreus marginatus* (Coreidae) and reported the Ag-NOR banding results in holokinetic chromosomes to be different from those of monokinetic chromosomes. Papeschi and Bidau (1985) applied silver staining to recognize NORs in four species of Belostomatidae (*Belostoma elegans, Belostoma cummingsi, Belostoma dentatum* and *Belostoma oxyurum*) and found the localization of NORs to be different in four species. Camacho *et al.* (1985) studied nucleolar organizer regions by silver impregnation in chromosomes of *Nezara viridula* (Pentatomidae) and found NORs to be associated with the largest autosomal bivalent. Fossey and Liebenberg (1995) described nucleolar structures in *Carlisis wahlbergi* (Coreidae). Rebagliati *et al.* (2003) discussed the behavior of NORs during the meiotic cycle in *Edessa meditabunda* (Pentatomidae). Cattani *et al.* (2004) applied Ag-NOR banding on *Camptischim clavipes* (Coreidae) and used NOR location as a cytological marker to confirm earlier hypothesis on the alternate kinetic activity of telomeric regions of autosomes during meiosis. Cattani and Papeschi (2004) used silver staining to confirm whether an unknown extra element observed during meiotic process of *Spartocera fusca* (Coreidae) was a B chromosome or a nucleolus. Grozeva *et al.* (2004) discussed the
localization of NORs in four species of Nabidae (*Nabis indicus, Nabis viridis, Himacerus mirmicoides* and *Prostemma guttula*) and found Ag-NOR banding pattern to be specific for each species. Severi-Aguiar and Azeredo-Oliviera (2005) applied silver banding to three species of *Triatoma* (*Triatoma planensis, Triatoma protacta* and *Triatoma tibiamaculata*) while Severi-Aguiar et al. (2006) used fluorescent in situ hybridisation to study nucleolar activity in *Triatoma vitticeps* (Reduviidae). Lanzone and Souza (2006b) studied nucleolar organiser regions in three species of *Antiteuchus* namely *Antiteuchus mixtus, Antiteuchus sepulcralis* and *Antiteuchus macraspis* (Pentatomidae) and found them to be located on the largest autosomal pair. Localization of nucleolar organiser regions has been studied in *Macrolophus costalis* (Miridae) by Grozeva et al. (2006), *Nysius californicus* (Lygaeidae) by Souza et al. (2007c), *Arachnocoris trinitatus* (Nabidae) by Kuznetsova et al. (2007) and *Holhymenia rubiginosa* (Coreidae) by Bressa et al. (2008). Souza et al. (2009) discussed the nucleolar behavior in the seminiferous tubules of five species viz., *Hyalymenus* sp., *Neomegalotomus pallescens* (Alydidae), *Catorhintha guttula*, *Hypselonotus fulvus* (Coreidae) and *Niethrea sidae* (Rhopalidae). Castanhole (2009) described the nucleolar behavior in the aquatic heteropteran families, Gerridae (*Brachymetra albinevra, Cylindrosthetus Palmaris, Halobatopsis platensis* and *Limnogonus aduncus*), Notonectidae (*Martarega* sp.) and Veliidae (*Rhagovelia* sp. and *Rhagovelia whitei*). Souza and Itoyama (2010) described the nucleolar behavior in testicular lobes of *Euschistus heros* (Pentatomidae).
MATERIALS AND METHODS

The work was carried out along the following lines:

1. Collection of specimens
2. Identification
3. Preparation of slides
4. Study of slides

1. Collection of specimens

Coreids are phytophagous, feeding on both cultivated and wild plants. So, for collection of bugs, collection tours were conducted during the period extending from March to May and August to October and vegetative areas, both cultivated and wild falling in the states of Punjab, Himachal Pradesh and Uttarakhand were surveyed. Collection was done by hand picking and light trap methods. Only Homoeocerus lacertorsus was collected from light trap while the rest were collected by hand picking. During the survey, it was found that many species of Coreidae are host specific as reported by Schaefer and Mitchell (1983). Two species of Cletomorpha, C. hastata and C. raja, were found exclusively on prickly chaff flower plants. Homoeocerus signatus was found on kikar plants. Homoeocerus borealis and Anhomoeus nepalensis were found on shisham plants. Clavigralla scutellaris was found on pod plants. Notobitus affinis was found on bamboo plant.

In all, 23 coreid species referable to 12 genera have been collected which include 22 of the subfamily Coreinae and 1 of the subfamily Pseudophloeinae.

The list of collected species, place of collection, mode of collection, period of collection, host plants, if any, and cytogenetic techniques applied are shown in Table.1.
2. Identification

A few specimens of each collected species were killed using ethyl-acetate and were stretched, pinned and dried. The pinned specimens were mounted on a thermocol sheet placed in a wooden box provided with naphthalene balls. Information about place of collection, collection date and host plant, if any, of each species were specified. Specimens were identified in the department with the help of relevant literature and with the guidance of Prof. (Dr.) Devinder Singh, Department of Zoology and Environmental Sciences, Punjabi University, Patiala. Some of the species were identified by Prof. (Dr.) Harbhajan Kaur by comparing with specimens lying in the Natural History Museum, London.

3. Preparation of Slides:

Live adult male specimens (at least 5 for each species) were dissected in 0.67% saline. The gonads were taken out and fixed in freshly prepared Carnoy’s fixative (3:1:: absolute alcohol: glacial acetic acid) for 15 minutes followed by a second change of fresh Carnoy’s fixative for another 15 minutes. The fixed material was tapped on clean slides with the help of fine forceps. The tapped slides were allowed to air dry and were stored in refrigerator at 4°C. They were used for different parameters as and when required. The various parameters for which the slides were processed are as follows:

(A) Conventional Staining

Conventional staining was done to study the diploid chromosomal complement and course of meiosis. Air-dried slides were stained with Carbol-fuchsin for three to four hours followed by differentiation in N-butyl alcohol in three
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consecutive changes. The slides were allowed to air dry and mounted in DPX (Carr and Walker, 1961). Stain was prepared as follows:

(i) Stock Solution A:
   Basic Fuchsin                =  3 g
   70% Ethanol                 = 100 ml

(ii) Stock solution B:
   Stock solution A            = 10 ml
   5% phenol in distilled water = 90 ml

(iii) Working solution:
   Stock solution B            = 95 ml
   Glacial acetic acid         = 12 ml
   40% Formaldehyde            = 12 ml

(B) C-banding

C-banding was performed on the slides to study the localization of constitutive heterochromatin in the chromosomes. Technique suggested by Sumner (1972) was followed with slight modifications. Air-dried slides (one to two weeks old) were treated with 0.2N HCl for one hour at room temperature and were then washed with deionized water to remove the acid completely. Then the slides were kept in freshly prepared aqueous solution of Ba(OH)\textsubscript{2} at 50\degree C for 5-7 minutes. After rinsing in deionized water, the slides were incubated for 1-2 hours in 2x SSC. Again the slides were rinsed briefly with deionized water followed by staining in 10% Giemsa for 20 minutes and finally the slides were rinsed in water, blotted, allowed to dry and mounted in DPX.

Preparation of Solutions

(i) 0.2 N Hydrochloric acid (0.2N HCl)
   Concentrated hydrochloric acid = 2 ml
   Distilled water                = 98 ml
(ii) Barium hydroxide solution $[\text{Ba(OH)}_2]$  
Barium hydroxide = 5 g  
Distilled water = 100 ml  

(iii) 2 x SSC  
Sodium citrate = 0.882 g  
Sodium chloride = 1.755 g  
Distilled water = 100 ml  

(iv) Sorrensen's buffer  
Stock solution A  
(a) Disodium hydrogen phosphate = 1.876 g  
(b) Distilled water = 100 ml  

Stock Solution B  
(a) Potassium dihydrogen phosphate = 0.907 g  
(b) Distilled water = 100 ml  

Working solution (pH 6.8)  
(a) Stock solution A = 50.8 ml  
(b) Stock solution B = 49.2 ml  

(v) Giemsa Stain  
Giemsa stock  
(a) Giemsa = 380 mg  
(b) Glycerol = 25 ml  
(c) Methanol = 25 ml  

Working Giemsa Stain  
(a) Giemsa stock = 10 ml  
(b) Sorrensen's buffer (pH 6.8) = 90 ml
(B) Sequence-specific staining

Sequence specific staining was performed to characterize constitutive heterochromatin of the chromosomes in terms of base specificity. A-T rich regions give bright fluorescence with the fluorochrome 4'-6-Diamidino-2-Phenylindole (DAPI) and G-C rich with Chromomycin A₃ (CMA₃). The methodology suggested by Schweizer (1976) was employed with minor modifications:

The refrigerated slides were brought to room temperature and stained with Methyl-Green solution for 20 minutes. Then the slides were treated with DAPI solution for 15 minutes, rinsed sequentially with distilled water, McIlvaine buffer and distilled water, and were air dried. Then, one drop of CMA₃ solution was poured on each slide which was covered with a cover slip and was kept for half an hour at room temperature. The slides were then rinsed sequentially with distilled water, McIlvaine buffer and distilled water, and were air dried. Finally the slides were mounted in a solution consisting of 9 ml of glycerol mixed with 1 ml of McIlvaine buffer (pH 6.8) containing 10 mM MgCl₂. Slides were kept in incubator at 60°C for 72 hours before being examined under the microscope.

Preparation of Solutions

(i) McIlvaine buffer Stock

(a) Solution A

\[
\begin{align*}
\text{Disodium hydrogen phosphate} & = 3.5 \text{ g} \\
\text{Distilled water} & = 125 \text{ ml}
\end{align*}
\]

(b) Solution B

\[
\begin{align*}
\text{Citric acid} & = 2.4 \text{ g}
\end{align*}
\]
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Distilled water = 125 ml

(c) Working McIlvaine buffer (pH 6.8)
   (a) Solution A = 90 ml
   (b) Solution B = 10 ml

(ii) 10 mM Mg Cl₂
   (a) Magnesium chloride = 5.07 g
   (b) Distilled water = 25 ml

(iii) Methyl Green
   (a) Methyl Green = 1 g
   (b) Distilled water = 100 ml

(iv) DAPI
   (a) DAPI = 4 µg
   (b) McIlvaine buffer = 1 ml

(v) CMA₃
   (a) CMA₃ = 0.4 mg
   (b) Distilled water = 1 ml

(D) Silver nitrate (AgNO₃) staining

Silver nitrate staining was done to study the localization of Nucleolar Organizer Regions (NORs). The methodology suggested by Howell and Black (1980) was employed on the air-dried slides as follows:

Two drops of colloidal developer and four drops of aqueous silver nitrate were pipetted onto the surface of air-dried slides containing chromosomai preparations. The solutions were mixed and covered with coverglass. The slides were placed onto the surface of a slide warmer stabilized at 70°C and were removed from the slide warmer
the moment the solution turned golden brown. The slides were then washed under running deionised water, dried, mounted in DPX and were examined under the microscope.

**Preparation of Solutions**

(i) **Colloidal Developer**

(a) Gelatin powder = 2 g  
(b) Deionized water = 100 ml  
(c) Formic acid = 1 ml

(ii) **Aqueous silver nitrate solution**

(a) Silver Nitrate = 4 g  
(b) Deionized water = 8 ml

4. **Study of Slides:**

The prepared slides were scanned under the Nikon microscope. Initial scanning was done under 40X and the readings of the selected stages were noted down. The selected stages were re-observed under the immersion oil (100X) to study the details of chromosomal behavior during division. Slides stained with fluorochromes were studied under Nikon fluorescent microscope using UV filter for DAPI and BV filter for CMA<sub>3</sub>. Well spread stages were photographed using Nikon digital camera (DXM 1200C).
### Materials and Methods

#### Table-1: List of species collected and cyto-techniques applied

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Taxa</th>
<th>Place of Collection</th>
<th>Period of Collection</th>
<th>Chromosome complement (2n)</th>
<th>Techniques applied</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CS</td>
<td>C</td>
</tr>
<tr>
<td>1.</td>
<td><em>Anoplocnemis compressa</em></td>
<td>Himachal Pradesh</td>
<td>July-August</td>
<td>15=14A+X0</td>
<td>√</td>
</tr>
<tr>
<td></td>
<td>(Dallas, 1852)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2.</td>
<td><em>Anoplocnemis phasiana</em></td>
<td>Jammu and Kashmir</td>
<td>September-October</td>
<td>15=14A+X0</td>
<td>√</td>
</tr>
<tr>
<td></td>
<td>Fabricius, 1781</td>
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<tr>
<td>3.</td>
<td><em>Anoplocnemis binotata</em></td>
<td>Himachal Pradesh</td>
<td>July-August</td>
<td>15=14A+X0</td>
<td>√</td>
</tr>
<tr>
<td></td>
<td>Distant, 1918</td>
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<tr>
<td>4.</td>
<td><em>Ochrochira nigrorufa</em></td>
<td>Himachal Pradesh</td>
<td>June-July</td>
<td>21=18A+2m+X0</td>
<td>√</td>
</tr>
<tr>
<td></td>
<td>(Distant, 1889)</td>
<td></td>
<td></td>
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<tr>
<td>5.</td>
<td><em>Ochrochira aberrans</em></td>
<td>Himachal Pradesh</td>
<td>July-August</td>
<td>21=18A+2m+X0</td>
<td>√</td>
</tr>
<tr>
<td></td>
<td>(Walker, 1871)</td>
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<td></td>
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<tr>
<td>6.</td>
<td><em>Prionolomia sp.</em></td>
<td>Himachal Pradesh</td>
<td>August-September</td>
<td>27=24A+2m+X0</td>
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</tbody>
</table>

**Coreinae**

**Mictini**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Taxa</th>
<th>Place of Collection</th>
<th>Period of Collection</th>
<th>Chromosome complement (2n)</th>
<th>Techniques applied</th>
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</thead>
<tbody>
<tr>
<td>7.</td>
<td><em>Cletus punctiger</em></td>
<td>Himachal Pradesh</td>
<td>August-September</td>
<td>18=14A+2m+X₁X₂₀</td>
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<tr>
<td></td>
<td>(Dallas, 1852)</td>
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<td>8.</td>
<td><em>Cletus borealis</em></td>
<td>Punjab</td>
<td>August-September</td>
<td>18=14A+2m+X₁X₂₀</td>
<td>√</td>
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<tr>
<td></td>
<td>Blote, 1935</td>
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<tr>
<td>9.</td>
<td><em>Cletus pallescens</em></td>
<td>Uttarakhand</td>
<td>July-August</td>
<td>18=14A+2m+X₁X₂₀</td>
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<tr>
<td></td>
<td>Walker, 1871</td>
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<tr>
<td>10.</td>
<td><em>Cletomorpha hastata</em></td>
<td>Punjab</td>
<td>August-September</td>
<td>22=18A+2m+X₁X₂₀</td>
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<tr>
<td></td>
<td>(Fabricius, 1887)</td>
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<tr>
<td>11.</td>
<td><em>Cletomorpha raja</em></td>
<td>Uttarakhand</td>
<td>August-September</td>
<td>20=16A+2m+X₁X₂₀</td>
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<tr>
<td></td>
<td>Distant, 1901</td>
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**Gonocerini**

<table>
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<th>Chromosome complement (2n)</th>
<th>Techniques applied</th>
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<tbody>
<tr>
<td>12.</td>
<td><em>Homoeocerus borealis</em></td>
<td>Punjab</td>
<td>August-September</td>
<td>21=18A+2m+X0</td>
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<tr>
<td></td>
<td>Distant, 1918</td>
<td></td>
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<tr>
<td>13.</td>
<td><em>Homoeocerus lacertorsus</em></td>
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<td></td>
<td>Distant, 1889</td>
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<td>14.</td>
<td><em>Homoeocerus signatus</em></td>
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<tr>
<td></td>
<td>Walker, 1871</td>
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<td>15.</td>
<td><em>Homoeocerus macula</em></td>
<td>Uttarakhand</td>
<td>July-August</td>
<td>21=18A+2m+X0</td>
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<tr>
<td></td>
<td>Dallas, 1852</td>
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**Homoeocerini**

<table>
<thead>
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<th>Period of Collection</th>
<th>Chromosome complement (2n)</th>
<th>Techniques applied</th>
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<tr>
<td>16.</td>
<td><em>Acanthocoris anticus</em></td>
<td>Uttarakhand</td>
<td>July-September</td>
<td>24=22A+X₁X₂₀</td>
<td>√</td>
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<tr>
<td></td>
<td>Walker, 1871</td>
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<td>17.</td>
<td><em>Acanthocoris sp.</em></td>
<td>Jammu and Kashmir</td>
<td>August-September</td>
<td>24=22A+X₁X₂₀</td>
<td>√</td>
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<tr>
<td></td>
<td>Walker, 1871</td>
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<tr>
<td>18.</td>
<td><em>Petalocnemis obscura</em></td>
<td>Punjab</td>
<td>August-September</td>
<td>26=22A+2m+X₁X₂₀</td>
<td>√</td>
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<tr>
<td></td>
<td>(Dallas, 1852)</td>
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**Acanthocorini**
### Materials and Methods

<table>
<thead>
<tr>
<th>Anhomoeini</th>
<th>Uttarakhand</th>
<th>September-October</th>
<th>21=18A+2m+X0</th>
<th>√</th>
<th>√</th>
<th>√</th>
</tr>
</thead>
<tbody>
<tr>
<td>19. <em>Anhomoeus nepalensis</em> (Distant, 1908)</td>
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<tr>
<td>20. <em>Anhomoeus sulcatus</em> (Distant, 1908)</td>
<td>Punjab</td>
<td>July-September</td>
<td>21=18A+2m+X0</td>
<td>√</td>
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<table>
<thead>
<tr>
<th>Cloresmini</th>
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</thead>
<tbody>
<tr>
<td>21. <em>Notobitus affinis</em> (Dallas, 1852)</td>
<td>Himachal Pradesh</td>
<td>September-October</td>
<td>21=18A+2m+X0</td>
<td>√</td>
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<table>
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<thead>
<tr>
<th>Pseudophloeinae Clavigrallini</th>
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</thead>
<tbody>
<tr>
<td>23. <em>Clavigralla scutellaris</em> (Westwood, 1842)</td>
<td>Punjab</td>
<td>July-September</td>
<td>13=10A+2m+X0</td>
<td>√</td>
<td>√</td>
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</tr>
</tbody>
</table>

CS= Conventional staining, C= C-banding, F= Fluorescent banding, S=Silver staining