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Investigation of Chromosome Number, Sex Mechanisms and Meiotic Behavior of chromosomes in seven species of suborder Heteroptera from Bahir Dar

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BY

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Bahir Dar, Ethiopia

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INVESTIGATION OF CHROMOSOME NUMBER, SEX MECHANISMS AND MEIOTIC BEHAVIOR OF CHROMOSOMES IN SVEN SPECIES OFSUBORDER HETEROPTERA FROM BAHIR DAR

A THESIS SUBMITTED TO COLLEGE OF SCIENCE, DEPARTMENT OF BIOLOGY, BAHIR DAR UNIVERSITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY (GENETICS)

BY

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AUGUST, 2020

BAHIR DAR, ETHIOPIA

DECLARATION

I declare that this thesis is my original work in partial fulfillment for the requirements for the degree of Master of Science in Biology (Applied Genetics). All the sources of the materials used for this thesis and all people and institutions who gave support for thesis work are fully acknowledged.

NAME: YIHUNIE FENTA EMIRIE

Signature: _____

Date: _____

APPROVAL SHEET

As a thesis research advisor, I hereby certify that I have read and evaluated this thesis prepared under my supervision, by Yihunie Fenta entitled as"Investigation of Chromosome Number, Sex Mechanisms and Meiotic Behavior of chromosomes in some species of Heteroptera from Bahir Dar". I recommended the paper to be submitted as fulfilling the requirement for the Degree of Master of Science in Biology (Applied Genetics).

_____	_____	_____
Advisor	Signature	Date

As members of the board of examiners for the MSc thesis open defense examination, we certify that we have read and evaluated the thesis prepared by Yihunie Fenta and examined the candidate. We recommended the thesis to be accepted as fulfillment for the requirement of the Degree of Master of Science in Biology (Applied Genetics).

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Chair Person	Signature	Date
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Internal Examiner	Signature	Date
_____	_____	_____
External Examiner	Signature	Date

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LIST OF ACRONMYS

CSA Central Statistical Agency

DPX Distyrene Plasticizer Xylene

EMA Ethiopian Meteorology Agency

Mb Mega base

M-Chromosome Micro Chromosome

ABSTRACT

This study was intended to investigate the chromosome number, sex mechanisms and meiotic behavior of seven species of suborder Heteroptera collected from Bahir Dar at different localities by hand picking and insect net. Live adult male specimens were dissected under stereoscope microscope (B3040) in 0.67% saline solution and fixed in freshly prepared Carnoy's fixative. The slides were stained with carbol fuchsin, mounted on a microscope and well spread stages were photographed. In the present study four species of Pentatomidae, two species of Coreidae and one species of Reduviidae were cytologenetically investigated. Out of the four Pentatomid species, a male diploid number of 14=12A+XY was found in *Rhacognathus punctatus* and *Halyomorpha annulicornis* while 16=14A+XY was observed in *Podisus nigrispinus* and *Banasa dimidiata.* Male diploid number of Coridae was found to be 18=14A+2m+X_1X_2O in *Cletus borealis* and 17=16A+XO in *Acanthocephala femorata,* while male diploid number of Reduviidae was 22= 20A+XY in *Androcluspictus.* Microchromosomes were present in one species of Coreidae (*Cletus borealis*) and absent in the second species of Coreidae, all Pentatomidae and Reduviidae of this study. Cytogenetic observation of all the seven species is the first in Ethiopia and those of *Rhacognathus punctatus* (Pentatomidae) and *Acanthocephalafemorata* (Coreidae) was the first for the cytogenetic world. Certain characteristics are common in the three species of Heteroptera such as fusion of the X and Y to form a heteropycnotic body at diffuse stage, and their behaviour at diakinesis and metaphase II. Sex chromosomes, X1 and X2, remain fused throughout meiosis in *Cletus borealis.*

Key words: Heteroptera, Pentatomidae, Meiosis, chromosomes

1. INTRODUCTION

1.1. Background of the study

The Suborder Heteroptera constitutes one of the most important insect groups because most of its species are plant feeders during both nymphal and adult stages, and cause severe damage on many plants of economic importance. With approximately 37, 000 nominal species the suborder comprises eight infraorders, five of which include agronomically important species (Triplehorn and Johnson, 2005). The superfamily Pentatomoidea includes 1080 genera and 5907 species belonging to 16 families. Cydnidae, Pentatomidae, Scutellerid ae and Tessaratomidae are the most important since 94% of the species belong to them (Schaefer and Panizzi, 2000). One of the largest families within Heteroptera is Pentatomidae including 4112 species (Schaefer and Panizzi , 2000). They are called stinkbugs because they produce a disagreeable odor by means of scent glands that open in the region of the metapleuras. Schuh and Slater (1995) include eight subfamilies in this family:Asopinae, Cyrtocorinae, Discocephalinae, Edessinae, Pentatominae, Phyllocephalinae, Podopinae and Serbaninae. Packauskas and Schaefer (1998) most economically important phytophagous species belong to the subfamilies Edessinae and Pentatominae, and the latter contains the majority of species that are crop pests. The species belonging to Asopinae are predators, and some of them are important agents of biological control (Schaefer and Panizzi, 2000).

Cytogenetic reports on Pentatomoidea refer to 391 species belonging to only nine families: Acanthosomatidae (12 species), Cydnidae (14 species), Dinidoridae (12 species), Plataspidid ae (16 species), Tessaratomidae (nine species), Thaumastellidae (two species), Scutelleridae (27 species), Urostylididae (five species), and Pentatomidae, which is by far the more studied (294 species) (Ueshima 1979; Manna and Deb-Mallick, 1981; Manna, 1984; Dey and Wangdi, 1988; Satapathy and Patnaik, 1988, 1989; Satapathy *et al.,* 1990; Gonza´

lezGarcı´a *et al*., 1996; Rebagliati *et al*., 2001, 2002, 2003; Kerzhner *et al.*, 2004, Kaur and Semahagn 2010, Kaur and Bansal 2012, Kaur and Gaba 2018).

There is little information on the evolution of chromosomes in organisms with holocentric chromosomes because of the difficulty of detecting structural variation, due to the lack of a morphologically differentiated centromere and the scarcity of longitudinal chromosomal differentiation. For these reasons chromosome rearrangements, such as inversions and reciprocal translocations, are rarely reported in Heteroptera (Papeschi and Mola, 1990).

Regardless of this rich biodiversity, cytogenetic work on African Heteroptera is meagre. Only a handful studies from Ghana and South Africa have been released for the scientific world (Jacobs and Groneveld, 2002). Cytogenetic study of heteroptera in Ethiopia only done by Kaur and Semahagn (2010) for the first time on three Hereoptera species of two of the species of *Paracritheus trimaculatus* and *Eurydema pulchrum* belong to Pentatomidae while the other, *Cletus punctulatus*from family Coreidae. In the present study to investigate chromosome number, sex mechanisms and meiotic behavior of four species of the family Pentatomidae, two species of the family Coreidae and one species of the family Reduviidae included in the study in Ethiopia for the first time.

1.2. Statement of the problem

Chromosome studies of Heteroptera are not commonly organized and implemented in Ethiopia. Regardless of this rich Heteropteran biodiversity, cytogenetic work on Africa Heteroptera is meager. Handful studies from Ghana and South Africa have been released for the scientific world (Jacobs, 2004). Therefore, the intension of this study was to full fil some gap of limited studies on Ethiopian Heteropterans and documenting the result for the comparison of previous and possible works in the future.

1.3. Objective of the study

1.3.1. General objective

The general aim of this study was to conduct laboratory investigation on chromosome number, sex mechanisms and meiotic behavior of some species of suborder Heteroptera collected from Bahir Dar.

1.3.2 Specific objectives

This work was done to accomplish the following specific objectives:

- To investigate chromosome number of collected Heteroptera species in the study area.
- To analyse the meiotic behavior of chromosome of collected Heteroptera species in the study area.
- To study the sex mechanisms of collected Heteroptera spices.

1.4. Significance of the study

The Heteroptera, or true bugs, are diversified group of insects displaying number of unusual and sometimes unique cytogenetic characters such as holokinetic chromosomes, M-chromosomes, multiple sex chromosome systems, sex chromosome post reduction and occasionally prereduction in male meiosis, variation in the presenceor absence of chiasmata in spermatogenesis, different types of a chiasmate meiosis and others.Therefore, this study could be important in the following aspects. It could generate valuable cytogenetic information on chromosome number, sex determination mechanism and meiotic behavior of Heteroptera. It would provide a base line information and direction for those who want further investigation in the field. In addition, as certain groups of Heteroptera were predators , understanding their genetics may give a clue for selecting them as biological control agents.

2. REVIEW OF RELATED LITERATURE

2.1. Taxonomy of studied species

The suborder Heteroptera is separated into seven infraorders, two of which are primarily aquatic (Gerromorpha and Nepomorpha), one semi aquatic (Leptopodomorpha) and the remaining terrestrial (Enicocephalomorpha, Dipsocoromorpha, Cimicomorpha and Pentatom omorpha).Pentatomomorpha is the second largest infraorder in species diversity, the first being the Cimicomorpha (Schuh *et al*., 2009).

Heteroptera comprises 89 families with more than 42,300 described species (Henry, 2015). Members of Heteroptera range from less than 1 mm to 10 cm in length, and have four or five segmented antennae, two or three ocelli and well developed compound eyes. At rest, wings cross over one another to lie flat over the insect's back. The wing bases do not cover a visible triangle at the center of back which is called scutellum. Mouthparts are of the piercing or sucking type. The mandibles and maxillae form two pairs of piercing stylets and are contained in a flexible sheath derived from the labium. Metamorphosis is gradual with immature usually appearing like the adults but wingless (Leraut, 2003).

The family Coreidae, often called leaf-footed bugs, pod bugs or squash bugs, includes 2200 species belonging to 500 genera (Dursun and Fent, 2009). Coreids are worldwide in distribution but are more abundant in the tropics and sub-tropics; they are divided in to 4 subfamilies: Coreinae,Pseudophloeinae, Meropachydinae and Agriopocorinae (Schuh and Slater, 1995). Of these 4 subfamilies, cytogenetic data is available for 127 species in only 2 subfamilies, Coreinae and Pseudophloeinae (Kaur and Bansal, 2012a; Yang *et al*., 2012). Homocentric chromosomes, post reductional division of sex chromosomes and a pair of micro chromosomes and absence of a Y chromosome (Bressa *et al*., 2008).

Reduviidae is the largest family of the predaceous land Heteroptera and includes about 6500 species in 930 genera and 22 subfamilies. Peiratinae is one of the most important predaceous subfamilies of Reduviidae distributed worldwide with 32 genera and over 300 described species (Jande, 1959a, b, Ueshima, 1979; Manna and Deb-Mallik, 1981;Ambrose,2006; Ambrose *et al*., 2007, Panizzi 2000).

2.2. Life cycle of Heteroptera

The Heteroptera do not undergo complete metamorphosis during their life cycle but instead typically have five wingless nymph instars before becoming reproductive, and typically winged, adults (Cárdenas *et al*., 2001). However, flexibility in the number of larval stages has been observed in the New Zealand species *Nysius huttoni*. In this species, the number of larval instars ranges from three to seven, though individuals with five instars still form the majority. In the laboratory, variation in the number of instars is affected by both temperature and photoperiod, with lower numbers of instars more frequent at lower temperatures (Wei, 2010). This suggests that flexibility in the number of larval instars may aid survival under changing environmental conditions (Wei, 2010). *Nysius huttoni* normally have three generations per year in their natural range and overwinter as adults, therefore a shorter nymph stage could potentially be beneficial to enable individuals to reach adulthood during poor, shorter summers. So far, this is the only species found to show this flexibility in nymph instars within the Lygaeidae; however it is well documented in other insect groups (Sandre *et al.*, 2007).

2.3. Economic importance

The Heteroptera, or true bugs, include many species of economical and medical importance. Among them, the subfamily Triatominae is particularly relevant because most of their membe rs are vectors of the protozoan *Trypanosoma cruzi*, causative agent of Chagas disease or Ame rican Trypanosomiasis. This parasitic disease is one of the most important in the tropics and subtropics of the Americas, with about 200,000 cases per year (Carpintero, 2002). Lygaeidae species are of economic importance due to their status as pests (Sweet; 2000, Summers *et al*., 2010). Turning to feeding, as one of the common names suggests, many, though not all, species of Heteroptera feed on seeds (Sites *et al*., 2000). As pests to attack grasses including grain crops, sugar, grasses and grasses used for lawns and playing fields (Sweet, 2000). *Anoplocnemis curvipes* is aspecies of sap sucking insect in the genus *Anoplocnemis*. They are native to subSaharan Africa where considered as major pest of many types of agricultural plants such as trees and shrubs, including legumes this has earned them the name leaf wilt (Schabel, 2006).

2.4. Chromosome study of Heteroptera

A chromosome is adeoxyribonucleic acid (DNA) molecule with part or all of the genetic material (genome) of an organism. Most eukaryotic chromosomes include packaging proteins which, aided by chaperone proteins, bind to and condense the DNA molecule to prevent it from becoming an unmanageable tangle (Hammond *et al*., 2017).Chromosomes are the nuclear components of special organization, individuality and function. They are capable of self-reproduction and play a vital role in heredity, mutation, variation and evolutionary development of the species (Verma *et al*., 2007).

Cytogenetically Coreidae is characterized by the presence of a pair of micro chromosomes and absence of Y chromosome (Kuar*et al*., 2012). Chromosome studies have been used for species differentiation (Pérez *et al*., 1992), detection of intra specific variation (Panzera*et al*., 1992) and to establish the evolutionary relationships of several species (Panzera *et al*.,1995; 1997; Pérez *et al*., 2002).Cytogenetically,Pentatomidae is characterized by the absence of mi crochromosomes and XY sex mechanism (Rebagliati *et al*., 2005). Regardless of rich biodiversity, cytogenetic reports on Pentatomidae refer to less than 400 species (Ueshima, 1979; Manna & Deb-Mallick, 1981; Nuamah, 1982; Manna, 1984; Satapathy and Patnaik, 1988;1989; Satapathy*et al*.,1990; Gozàlez-Garcia *et a*l., 1996; Rebagliati, 2000; Rebagliati*et al*. 2001, 2002, 2003, 2005; Lanzone, 2003; Kerzhner *et al*., 2004; Kaur&Semahagn 2010 a & b; Rebagliati&Mola, 2010a and Bizuayehu Kerisew, 2011).

2.4.1. Holokinetic chromosomes

Holokinetic chromosomes sometimes designated as holocentric occur in certain scattered groups of plants and animals, being particularly widespread in insects, including the Heteroptera (Kuznetsova *et al*., 2002, Lukhtanov and Kuznetsova, 2009). The chromosomes have no primary constriction, the centromere that are considered nonlocalized or diffuse formed by a large kinetochore plate extending all or most of the length of a chromosome (Ver ma *et al*., 2007). Although variations in chromosome number of related species are probably due to both fissions and fusions of holokinetic chromosomes, fusions are suggested to be more common. The point is that a chromosome, be it holokinetic or monocentric, has to display two functional telomeres in order to survive a mitotic cycle. The fusion chromosome always displays functional telomeres originated from the ancestral chromosomes, where as a fission chromosome has to be able to develop a functional telomere (Nokkala *et al*., 2007; Marques *et al*., 2016).

2.4.2. M-chromosomes

The term “m-chromosomes” has been introduced by Wilson (1905), for a pair of very minute autosomes, which were first discovered in the Coreidae species *Anasatristis* in which these peculiar chromosomes behaved differently from both autosomes and sex chromosomes during male meiosis (Ituarte, 2004). Microchromosomes is a type of very small chromosome which i s a typical component of the karyotypes of birds, some reptiles, fish and amphibians; they tend to be absent in mammals (Fillon, 1998). Micro chromosomes measure less than 20 Mb in size ; chromosomes greater than 40 Mb in size are as macro chromosomes, while those between 2 0 and 40 Mb as intermediate Chromosomes (Hederson *et al.*, 2014).

Micro chromosomes are characteristically very small and often cytogenetically indistinguisha ble in karyotypes. Because of this, it is estimated that the majority of genes are located on micro chromosomes(Burt, 2002). However, due to the difficulty in physically identifying micro chromosomes and the lack of micro satellite markers, it has been difficult to place genes on specific microchromosomes (Groenen *et al*., 2000).

Replication timing and recombination rates have been found to differ between microchromosomes and macro chromosomes in chickens. Micro chromosomes replicate earli er in the S phase of inter phase than macrochromosomes (McQueen *et al*., 1998). Recombinat ion rates have also been found to be higher on microchromosomes *(Henderson et al*., 2014). As a rule, m-chromosomes are extremely small while in some species they might be of approximately the same size as the autosomes (Grozeva *et al*., 2009). M-Chromosomes are a characteristic of several Heteroptera families. M-chromosomes behave as univalent during diakinesis and, as a rule, are negatively heteropycnotic over meiotic division (Grozeva and Nokkala, 2003).

2.4.3. Sex chromosome system in Heteroptera

Heteroptera, the XX/XY sex determination is of commonest occurrence, although XX/X0 and multiple sex chromosome systems (X_n0, X_nY, and XY_n) as well as rare neo-XY systems do occur (Ueshima, 1979). The most common sex chromosome system is XY (Kaur *et* al., 2006). Particular type of testicular follicle called a harlequin lobe, which produces abnormal spermatozoa, has been described in the family Pentatomidae, mainly in the subfamily Discocephalinae (Ueshima, 1979; Rebagliati *et al*., 2001).

2.5. Meiotic Behavior of Heteroptera

As a rule; the sex chromosomes in Heteroptera undergo post-reductional meiosis in males. Species with an XY sex-system, the meiotic metaphases are generally radial and the sex chromosomes are found in the center of the meiotic plate, during the first division, the sex chromosomes are univalent, while during telophase I or metaphase II the X and Y-chromosomes form a pseudobivalent that segregates in a post-reductional manner in male second meiotic division (Banho *et al.,* 2016).

In most Heteropteran males, autosomal bivalents are chiasmate whereas sex chromosomes have no chiasmata, however in a number of families male meiosis is completely a chiasmate (Kuznetsova and Grozeva, 2010). The first paper to describe the a chiasmate meiosis within the Heteroptera was that of Nokkala (1983). Meiosisin Heteropterais pre-reductional for the autosomes and post-reductional for the sex chromosomes, the autosomes segregate reductionally while the sex chromosomes equationally during the first meiotic division and the reverse happens during the second meiotic division (Ueshima, 1963; Papeschi, 1994; Grozeva and Nokkala, 2001; Papeschi *et al.,* 2003). Inaddition Ueshima (1979), Nokkala (1985), Perez*etal* .(1997) and Cattani*etal.* (2004), in their study of different groups of Heteroptera, showed autosomal bivalents to be chiasmatic where as sexchromosomes to be a chiasmatic during meiosis (Kaur *et al*., 2006)

3. MATERIALS AND METHODS

3.1. General description of the study area

This study was conducted in Bahir Dar**.** Bahir Dar is the capital city of Amhara National Regional State of Ethiopia. The town is situated at 578 km North -West of Addis Ababa, the capital of Ethiopia. In its absolute location, it is found 11033'15" and 11036'53" north latitude and 37021'11" and 37025'49" east longitude. The town has been the capital of Amhara National Regional State since 1991. Currently the town covers a total area of 256.4 km². It is a rapidly expanding town with commercial centers, small industries and residences in all sectors of the town. The total population of Bahir Dar was 313,997 inhabitants (CSA, 2017).

Bahir Dar lies on a very gentle slope with elevations ranging between 1783 m and 1889 m above sea level. It occupies the head stream of the Blue Nile basin. The town is situated at the southern shore of Lake Tana, a freshwater lake, with weak seasonal fluctuation. The town experiences a tropical climate with annual average rainfall of 1409 mm and average temperature of 21.30. The area receives a maximum rainfall during the summer season (June to August) and short rainfall in the spring season (September and October). The rainy season accounts for nearly over 96% of the total annual rainfall (EMA, 2009).

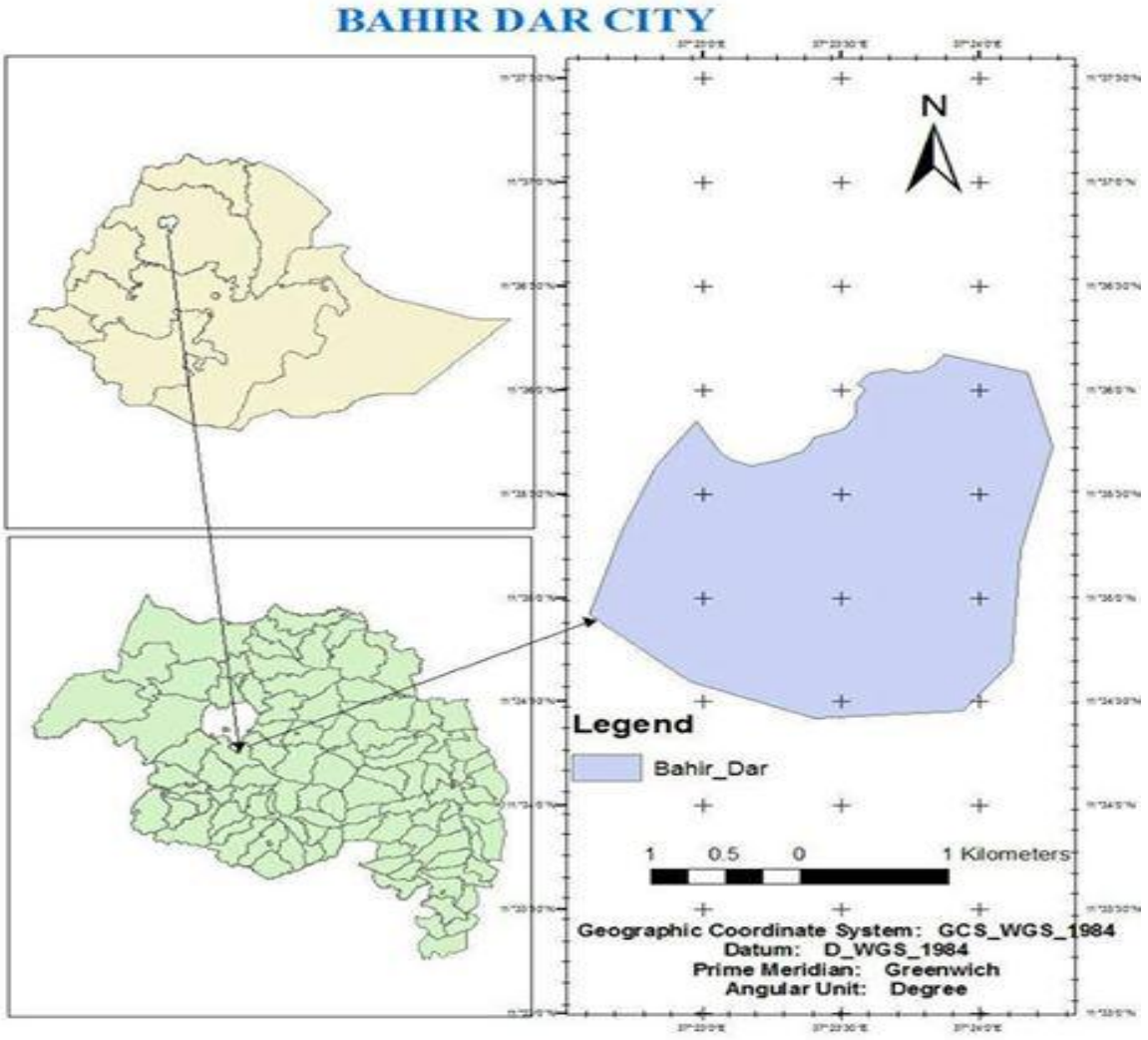

Figure 1. Map of the study area

3.2 Study design

The study was carried out by using experimental study at the laboratory to determine chromosome number, meiotic behavior and sex mechanisms of Heteroptera species in the study area.

3.3. Specimenes for the investigation

One species of the family Reduviidae, four species of Pentatomidae and two species of Coreidae all belonging to the sub order Heteroptera collected from different localities of Bahir Dar were used as the materials for the investigation of chromosome number, sex mechanisms and meiotic behavior of this research.The investigations in this thesis have been carried out in two parts. The first one was the fieldwork for collecting the specimenes and the second one was the laboratory work.

3.4. Collection of the specimens

The specimens were true bugs belonging to the sub-order Heteroptera. The adults range in size from very small to bigger ones, Collection attempts were made all year around from January 2019-June 2020 for two years. However, 75% of the results were obtained from these male adult bugs collected during the period from April to May. Most of them were collected by aspirators and some of them collected by insect net.

3.5. Identification of Specimens

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 awooden box provided with naphthalene balls. Information about localities of collection, collection date, family level, and hostplant, if any, of each species were labeled. Specimens were identified by Dr. Bizuayehu Kerisew with the help of relevant literature, Heteroptera classification guide(Schuh and Slater, 1995) and effort with his experience.

3.6. Preparation of slides

Live adult male specimens were dissected under stereoscope microscope (B3040) in 0.67% saline (NaCl in distilled water) solution and the gonads were extracted. The testes were 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 forceps and then the slides were air-dried and the prepared slides were kept in refrigerator at until use (Hayes *et al*., 2000).

3.7. Conventional staining

Air-dried slides were stained with carbol-fuchsin for two to three hours according to the methodology suggested by Kim (2013). The slides were rinsed in distilled water air dried and mounted under a 22 mm by 50 mm cover slip in DPX mounting medium.

3.8. Study of slides

Prepared slides were scanned under the microscope. Initial scanning was done under 40X objective and the readings of selected stages were noted down. Already studied stages were re-observed under the immersion oil (100X) to study the details of chromosomal behavior during cell division. Cells containing complete and well spread chromosome complement were photographed with a total magnification of 1000× using a camera-fitted microscope (Olympus fully DP73 fluorescent automated microscope).

Table 1: Details of collected species and parameters studied.

№	Family	Species	Area of collection	Period of collection	Host of species	Mode of collection	Diploid chromosome	References
1	Pentatomidae	*Halyomorha annulicoris*	Bahirdar University	April-May	Cabbage	Hand picking	14=12A+XY	Signoret,1858
2	Pentatomidae	*Podisus nigrispinus*	Zenzelima	April-May	Grass	Hand picking	16=14A + XY	Dallas,1851
3	Pentatomidae	*Rhacognathus punctatus*	Zenzelima	August-September	Tomato	Hand picking	14=12A+XY	(Linnaeus, 1758)
	Pentatomidae	*Banasa* dimidiata	Bahirdar Kebele 13	August – September	Mango	Hand picking	16=14A + XY	Say, 1832
5	Coreidae	*Cletus borealis*	Bahirdar University	April-May	Grass	Hand picking	18=14A+2m +X1X2O	Blöte, 1935
6	Coreidae	*Acanthocephala femorata*	Bahirdar University	May-June	Grass	Hand picking	17=16A+XO	Fabricius, 1775
7	Reduviidae	*Androchus pictus*	Bahirdar University	April-May	Grass	Insect net	22= 20A+XY	Jande,1959

4. RESULTS

4.1 Pentatomidae

4.1.1 *Podisus nigrispinus*

The first meiotic metaphase plate shows seven autosomal bivalents and two sex chromosomes. This implies that *Podisus nigrispinus* species has a male diploid chromosome number of 2n=16=14A+XY. Two large, four medium-sized and one small autosomal pairs were distinguished, the X chromosome was similar in size to the smallest autosomes and the Y chromosome was the smallest member of the complement Fig 2(5).

At diffuse stage the sex chromosomes, X and Y are fused together and form a very darkly stained heteropycnotic body located on one side of the nucleus while the autosomes were moderately decondensed Fig 2(1). At diplotene, three large autosomal bivalents show one or two chiasmata which may be terminal, subterminal or interstitial while small autosomal bivalents show one terminal, subterminal or interstitial chiasma each. Sex chromosomes, X and Y were fused together and form a very darkly stained heteropycnotic body located on center of the nucleus. Three autosomal chromosomes seen clearly and satin darkly Fig 2(2).

At diakinesis, all the autosomes appear as rod shape bivalents with single terminal chiasma in each while sex chromosomes, X and Y are arranged side by side Fig 2(3). At metaphase I, all the seven autosomal bivalents arrange themselves in a ring shape and the sex chromosomes, X and Y are located at their center side-by-side arrangement Fig 2(4). Anaphase I, is reductional for autosomes and equational for sex chromosomes Fig 2(6). Durin g metaphase II, polar view autosomal chromosomes form a ring, while sex chromosomes of X and Y that associate terminally to form a pseudo bivalent, lie within the ring Fig 2(7).

During anaphase II, the sex chromosomes divide reductionally and the X and Y are seen moving to the opposite poles ahead of the autosomes Fig 2(8). At telophase II, Sex

chromosomes divide reductionally during the second division so that the X chromosome goes to one pole and the Y- chromosome to the other forming four types of nuclei Fig 2(9).

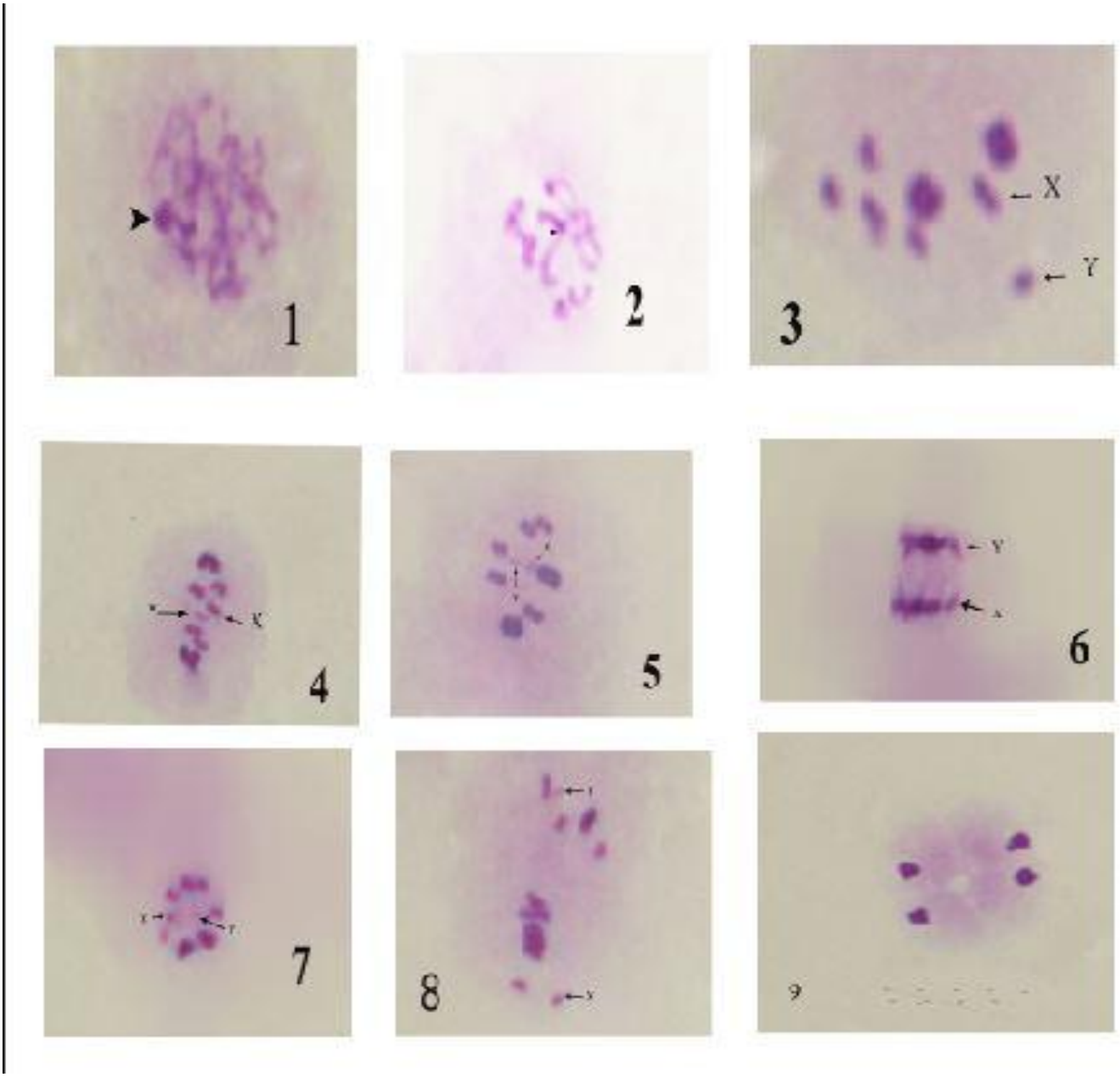

Figure 2. *Podisus nigrispinus*

1- Diffuse stage shows fused sex chromosomes of X and Y. *2*- Diplotene. 3- Diakinesis. 4- Metaphase I, polar view. 5- Metaphase I side view. 6 - Anaphase I. 7- Metaphase II, 8- Anaphase II. 9- Telophase II. Arrow heads indicate fused sex chromosomes; arrows indicate sex chromosomes. Scale bar = 10 μm.

4.1.2. *Banasa dimidiata*

The diploid complement of *Banasa dimidiata* was found to be 2n=16=14A+XY. At the diffuse stage, single darkly stained heteropycnotic bodies representing the fused X and Y was found at the center of the nucleus while the autosomes appeared as diffused chromatin and one bivalent autosome was seen clearly Fig 3(1).

At diplotene, the sex chromosomes remained associated and heteropycnotic. Three largest bivalents existed as a ring with two sub-terminal chiasmata Fig 3(2). During early metaphase, seven autosomal bivalent chromosomes and sex chromosomes of X and Y were clearly seen. Y- Chromosome was very lightly stained, size degradation occurred in autosomes and sex chromosomes, from the seven autosomal bivalent chromosomes two large, three medium and two small sizes similar to X chromosome were seen clearly .Y- chromosome was the smallest of the complement in the plate Fig 3(3).

At metaphase I, all the seven autosomal bivalents arranged themselves in a ring shape and the sex chromosomes, X and Y are located at their center. Y chromosome was found near to the larger auotosomal bivalents. From the seven autosomal bivalents two large, three medium and two small sizes similar to X chromosome, while Y chromosome is the smallest complement of the plate Fig 3(4).

At metaphase II, the autosomal univalents arranged in a circle while the X and Y pseudobivalent lied in the center by side-by-side arrangement. The autosomal chromosomes had size degradation there were two large, two medium and three small sized autosomal univalents were seen sex chromosomes had partilly equal size but, X chromosome located near to one of the larger univalent autosomes Fig 3(6).

At telophase II, four nuclei were formed Fig.3.(7).

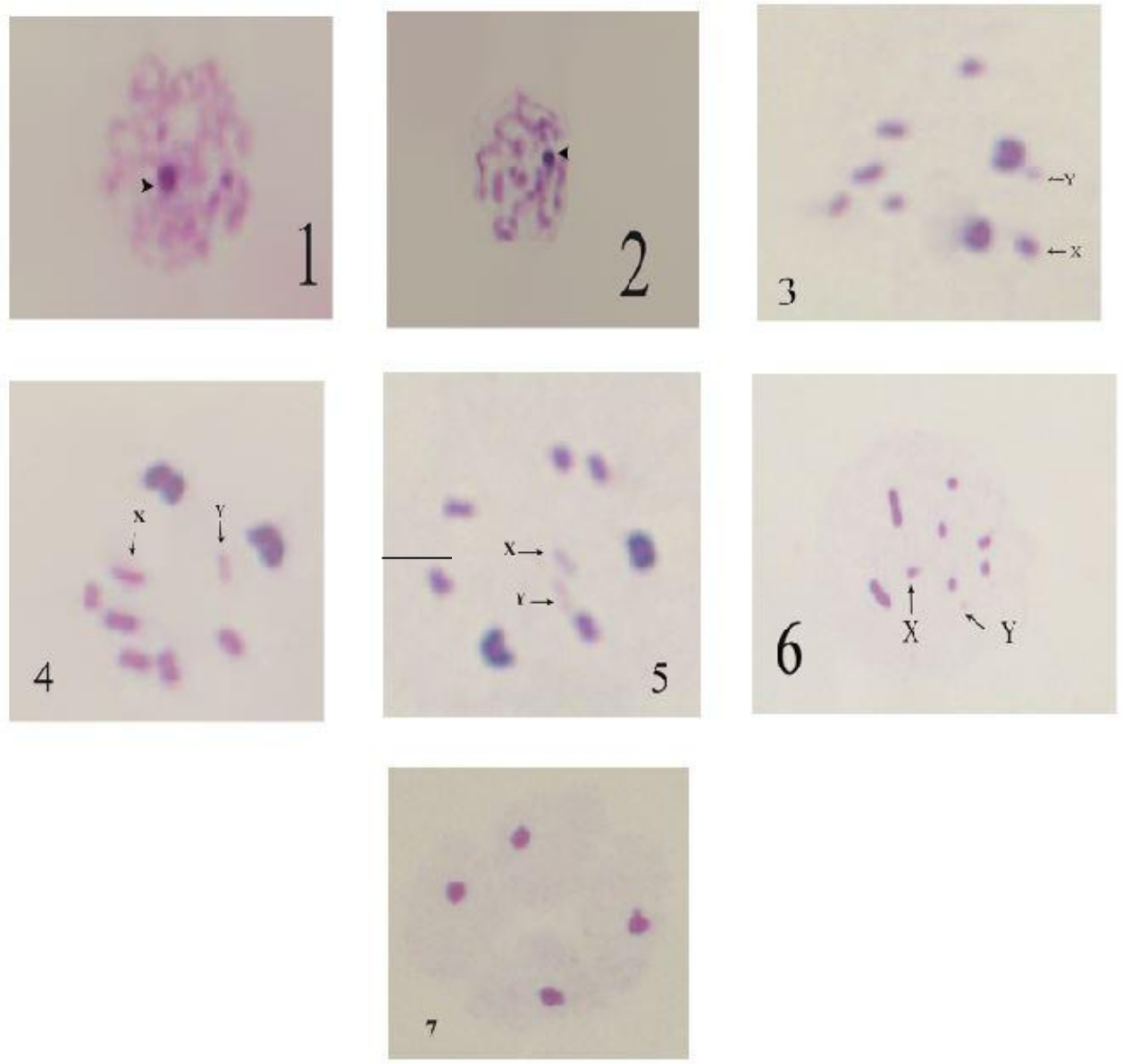

Figure 3.*Banasa dimidiata*

1- Diffuse stage shows fused sex chromosomes of X and Y at the center of the nucleus. . 2- Diplotene showing two ring bivalents and fused sex chromosomes of X and Y. 3- Early Metaphase. 4- Metaphase I polar view.5- Metaphase I side view .6- Metaphase II. 7- Telophase II. Arrow heads indicate fused sex chromosomes; arrows indicate sex chromosomes. Scale bar = 10 μm.

4.1.3 *Rhacognatus punctatus*

The diploid chromosome complement of *Rhacognatus punctatus* was observed as 2n=14=12A+XY. At the diffuse stage, the sex chromosomes were fused together to form darkly stained heteropycnotic body located in the periphery of the nucleus while the autosomes were highly decondensed Fig 4(1).

During diplotene, three autosomal pairs were ring bivalents while four autosomal bivalents present single terminal chiasma each. Sex chromosomes remain fused Fig 4(2). At diakinesis, the sex chromosomes were still fused together. Four of the autosomes were rod bivalents while two were ring bivalents Figs 4(3). At metaphase I, different arrangements of autosomal bivalent chromosomes were seen. Metaphase I, polar view showed that five autosomal bivalent chromosomes and sex chromosomes form ring one autosomal bivalent chromosomes were located on the center of the ring .All autosomal bivalents were rod shaped Figs 4 (4).

At metaphase I, side view showed that seven autosomal bivalent chromosomes and sex chromosomes of X and Y arranged side by side Figs 4 (5). Metaphase II showed the typical arrangement of chromosomes of five autosomal univalents and sex chromosomes of X and Y form a ring and one autosomal univalents lies in the center of the plate. At this stage very slight size difference between X and Y-chromosomes was seen in the plate. Five autosomal univalent chromosomes were large and two autosomal univalents medium in sized Fig 4(6). At anaphase II, the sex chromosomes segregate reductionally Fig 4 (7). At telophase II, f our nuclei are formed Fig 4 (8).

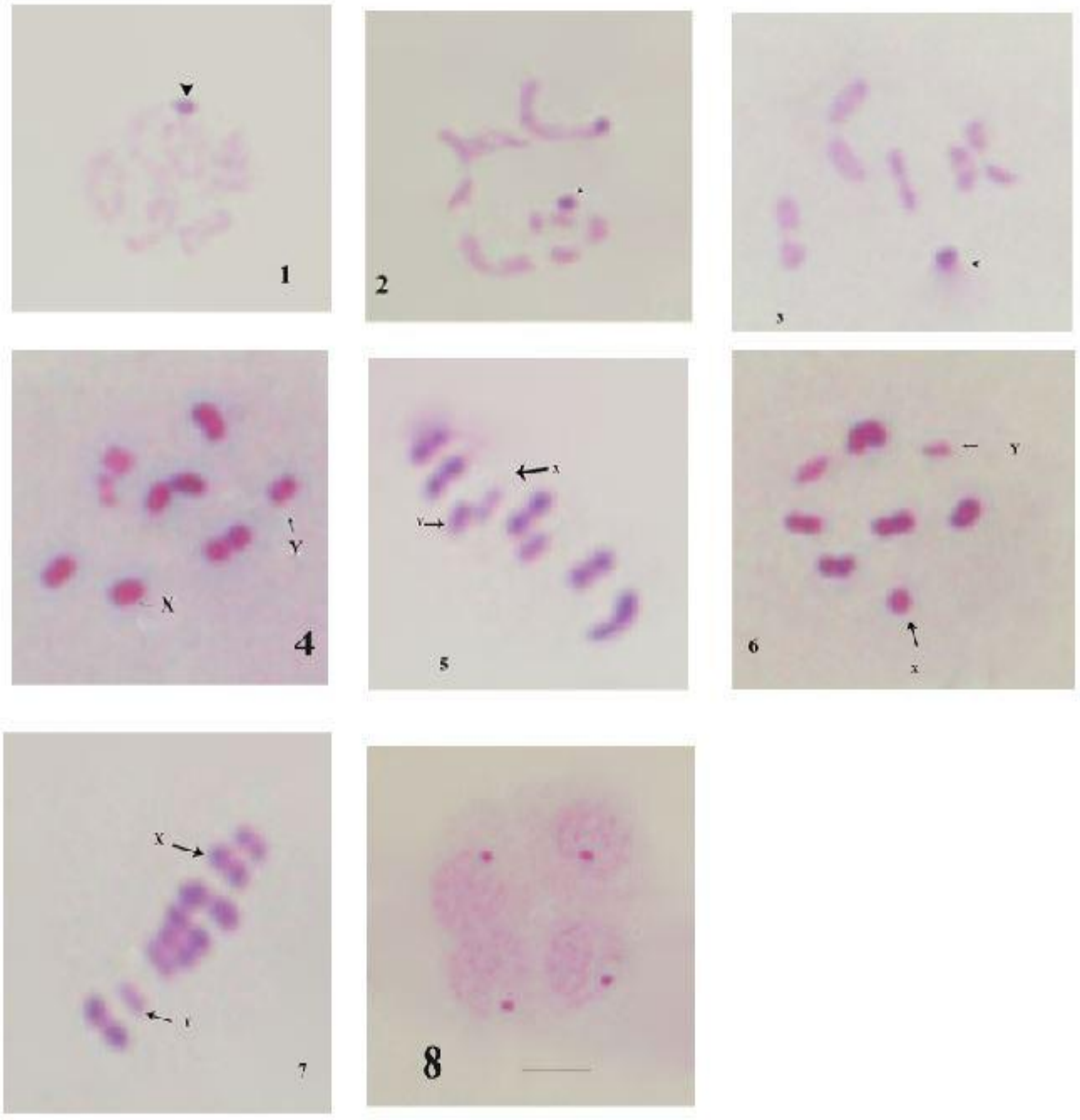

Figure 4. *Rhacognatus punctatus*

1- Diffuse stage. *2*- *Diplotene*. 3- Diakinesis. 4- 5- Metaphase I. 6-Metaphase II. 7- Anaphase II. 8- Telophase II. Arrow heads indicate fused sex chromosomes; arrows indicate sex chromosomes. Scale bar = 10 μm.

4.1.*4 Halyomorpha annulicornis*

Spermatogonial metaphase shows a diploid chromosome number of 2n=14=12A+XY. One pair of large, three pairs of medium and two pairs of small autosomes were seen. Sex chromosomes X and Y were smaller than autosomes, the Y chromosome being the smallest member of the complement Fig 5(1). At diffuse stage, darkly stained single heteropycnotic body representing fused X and Y was located on one side of the nucleus, while autosomal bivalents were highly condensed Fig 5 (2). At diplotene at least three of the autosomal pairs existed as ring bivalents, the sex chromosomes remained fused Fig 5 (3).

At diakinesis, the sex chromosomes became isopycnotic and their bipartite appeared and seen clear. Four autosomal bivalents were rod shaped with single terminal chiasma each and two autosomal bivalents were ring shaped Fig 5(4). At metaphase I, autosomal bivalents and sex chromosomes partially formed a ring, while two large autosomal bivalent chromosomes were located near to the center of the ring. Size degradation occurs between autosomal bivalents, two large and four medium size autosomal bivalent chromosomes seen on the plate. X chromosome larger than Y chromosome and Y chromosome is the smallest complement in the plate. X chromosome is isopycnotic to the autosomal bivalents while Y chromosome heteropycnotic to the autosome and X chromosome Fig 5(5).

At metaphase II, side view four autosomal univalent chromosomes and two sex chromosomes of X and Y form ring. While two autosomal univalent chromosomes located outside the ring Fig 5(6). At metaphase II, polar view five autosomal univalent chromosomes and sex chromosomes of Y form a ring. One autosomal univalent chromosome and X chromosome located inside the ring Fig 5(7). At anaphase II, autosomes divide equationally while the sex chromosomes divided reductionally to form two types of nuclei at telophase II, one with 6A+X and the other with 6A+Y Fig 5(8).

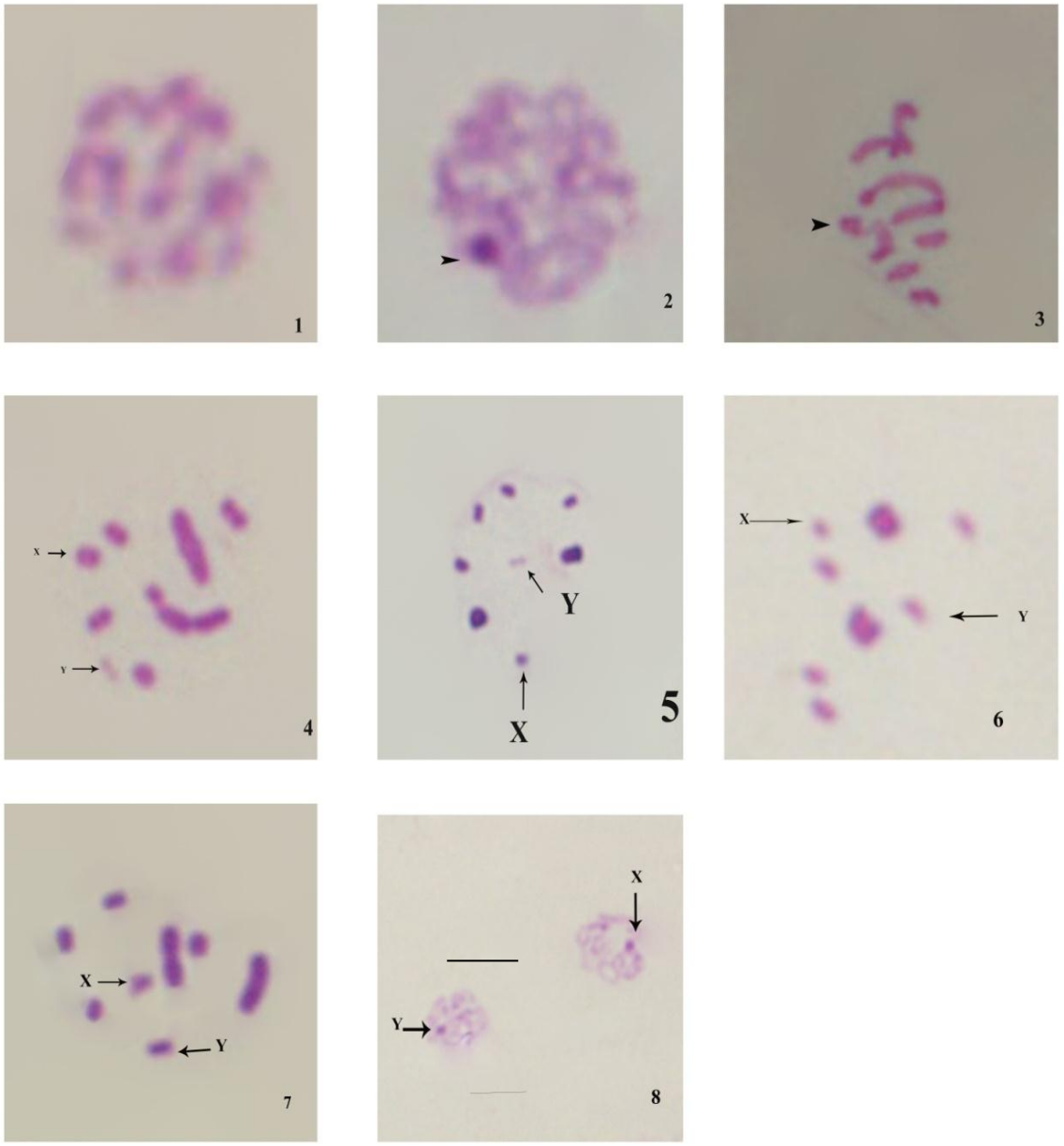

Figure 5. *Halyomorpha annulicornis*

1 - Spermatogoonial metaphase. *2*- Diffuse stage. *3*- Diplotene. 4- Diakinesis. 5- Metaphase I. 6-7- Metaphase II. 8- Telophase II. Arrowheads indicate fused sex chromosomes; arrows indicate sex chromosomes. Scale bar = 10 μm.

4.2 Coreidae

4.2.1 *Cletus borealis*

The diploid chromosome complement of Cletus borealis was 18=14A+2m+X_1X_2O. At the diffuse stage, darkly stained single heteropycnotic body representing fused X1 and X2 lied on one side of the nucleus Fig 6 (1). During early diplotene, three or four autosomal bivalent existed as aring bivalent with two terminal chiasmata.The sex chromosomes mainta in their association and appear as a single dark body Fig 6(2).

At diplotene, three autosomal bivalents were ring bivalents which were terminal or sub-terminal. The microchromosomes were lightly stained and lied far apart from each other. X1 and X2 were fused Fig 6(3). During diakinesis, the autosomal bivalents became distinct all showing single terminal chiasma. The sex chromosomes of X_1 and X2 werefused, but microchromosomes lied close to each other around one autosomal bivalent chromosome Fig 6(4).

At metaphase I, polar view showed six autosomal bivalents and fused X1and X2 sex chromosomes form ring. Microchromosomes lied at the center of the ring and stain heteropycnotic from autosomes and sex chromosomes of fused X1 and X2 chromosomes. Size degradation of autosomal chromosome seen, these were two large and rod shaped autos omal bivalent chromosomes and five medium sized autosomal chromosomes were observed Fig 6(5). During metaphase II, autosomes and fused X1 and X2 sex chromosomes formed a ring. Microchromosomes lied inside the ring Fig 6(6). At telophase II, autosomes divide equationally while the sex chromosomes divide reductionally and four types of nuclei were formed Fig 6(7).

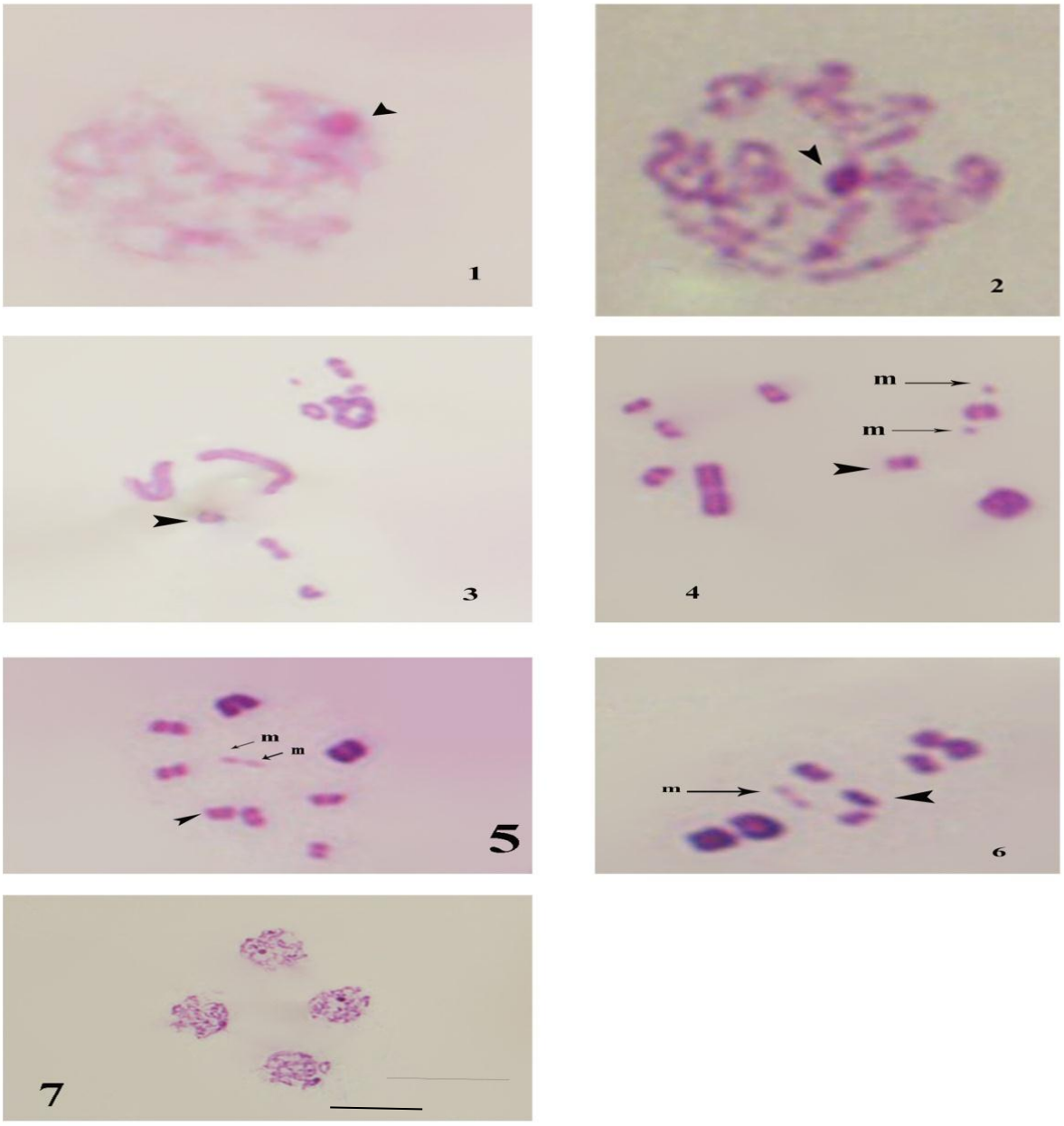

Figure 6.*Cletus borealis*

1-Diffuse stage. 2- Early diplotene. 3- Diplotene. 4- Diakinesis. 5- Metaphase I. 6- Metaphase II. 7- Telophase II. Arrowheads indicate fused sex chromosomes; arrows indicate micro chromosomes. Scale bar = 10 μm.

4.2.2 *Acanthocephala femorata*

At spermatogonial, first metaphase *Acanthocephala femorata* had a male diploid chromosome number of 17=16A+XO Fig 7(1). During diffuse stage, the sex chromosomes were associated to form a single darkly stained heteropycnotic body lying on the center of the nucleus while the autosomes were moderately decondensed appearing as chromatin threads. One bivalent autosomes were clearly seen and eight autosomes are decondensed Fig 7(2).

At diakinesis, the sex chromosomes found near to the center and have rod shaped. Six of the autosomes were rod bivalents while two were ring bivalents Figs 7(3). During early metaphase, sex chromosomes and autosomes were clearly seen. From eight autosomal bivalents, seven bivalents had rod shaped and one autosomal bivalent formed a partial ring Figs 7(4). During metaphase I, seven autosomal bivalents formed a ring and X chromosome and one of the autosomal bivalents lied in the center of the ring. All autosomal bivalents had one rod shaped chiasma for each autosomal bivalent Fig 7(5).

During anaphase I, autosomes divided reductionally and X chromosome divided equationally. Each pole received eight autosomes and one X chromosome Fig 7(6). During metaphase II, eight autosomal univalents formed a ring while the sex chromosome was located in the center. Two autosomal univalents had large size while the other six autosomal univalents had slightly similar size Fig 7(7) and 7(8).

At anaphase II, the autosomal univalents were divided equationally while the sex chromoso me divided reductionally Fig 7(9). At telophase II, two sets of chromosomes were observ ed, one with 2n=8A+ X and the other with 2n = 8A + O Fig 7 (10).

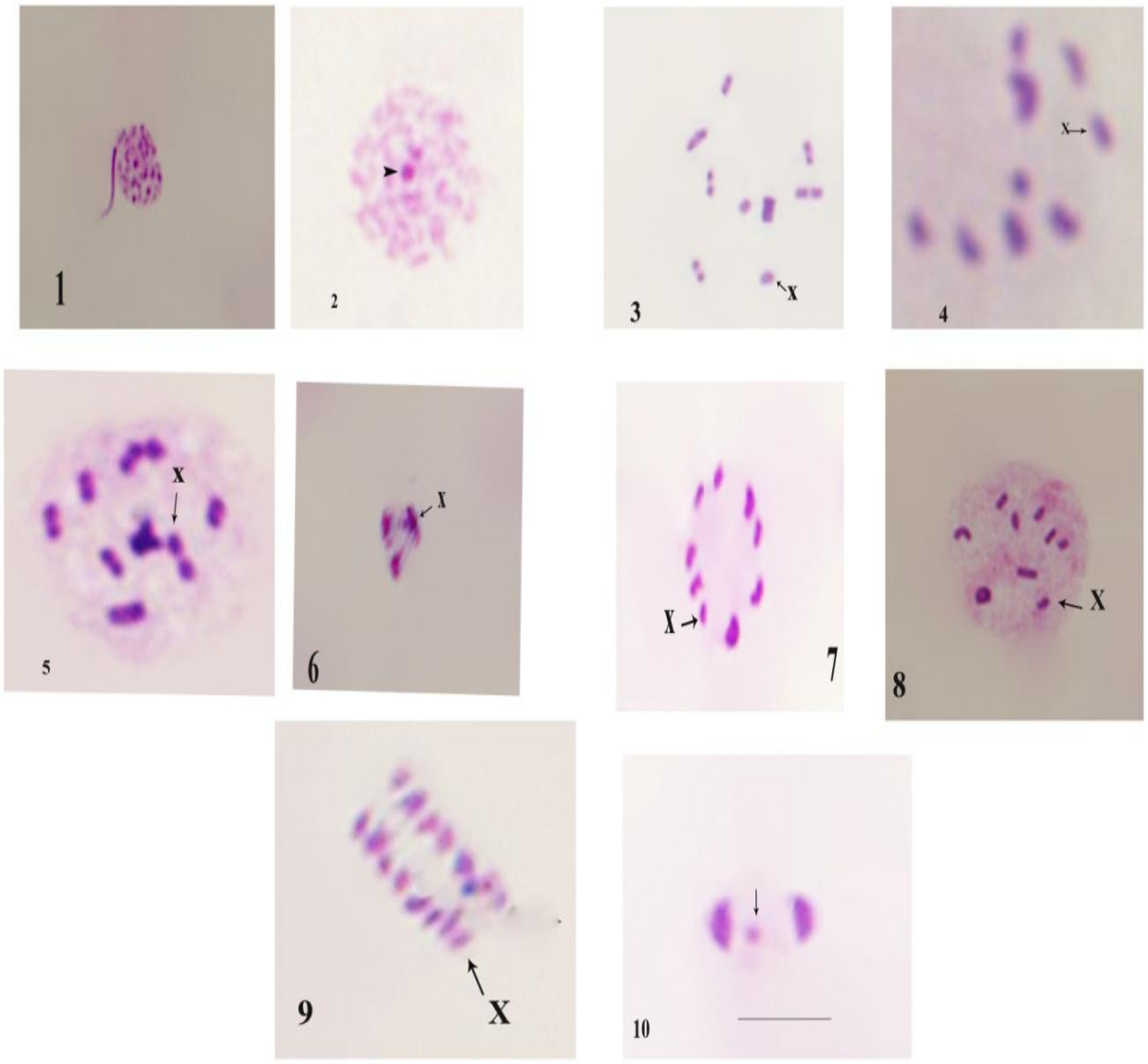

Figure 7. *Acanthocephala femorata*

1- Spermatogonial metaphase. 2- Diffuse stage. 3- Diakinesis. 4- Early metaphase. 5- Metaphase I. 6- Anaphase I. 7- 8- Metaphase II polar view and side view. 9- Anaphase II. 10- Telophase II. Arrow heads indicate fused sex chromosomes; arrows indicate sex chromosomes. Scale bar = 10 μm.

4.3 Reduviidae

4.3.1 *Androchus pictus*

The diploid chromosome complement of *Androchus pictus* is 22= 20A+XY.At diffuse stage, darkly stained single heteropycnotic body representing fused X and Y, that located on one sid e of the nucleus, while autosomal bivalents were highly condensed Fig 8(1). At diplotene, at least six of the autosomal bivalents existed as ring bivalents and two autosomal bivalents were seen clearly, the sex chromosomes remained fused Fig 8 (2).

During diakinesis, autosomal bivalents became more condensed and the sex chromosomes lied to different sites of the plate Fig 8 (3). At metaphase I, seven autosomal bivalents and X chromosome formed a ring. Three autosomal bivalents lied in the circle close to each other Fig 8 (4). During metaphase I, polar view showed six autosomal bivalents; X and Y chromo somes fomed a ring and four autosmal bivalent chromosomes were found in the center of the ring Fig 8 (5).

During metaphase II, nine autosomal univalents and the X chromosome formed a ring, while Y chromosome and on autosomal univalent lied in the ring Fig 8(6). The sex chromosomes divided reductionally during the second meiotic division as a result of which the X and the Y chromosomes moved to opposite poles and two types of nuclei were formed at telophase II, one with ten autosomes and the X- chromosome and the other with ten autosomes and the Y-chromosome Fig 8 (7).

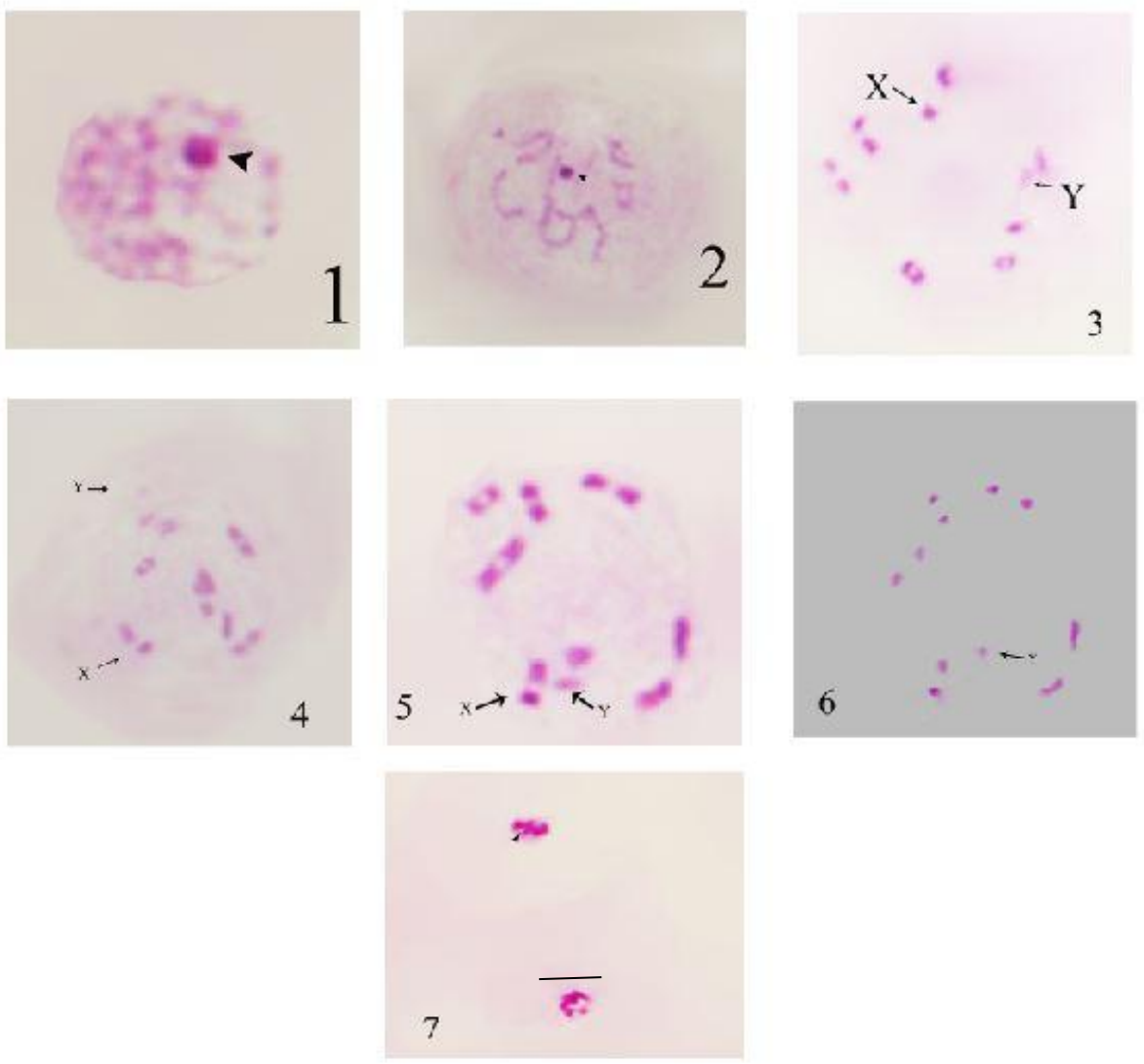

Figure 8.*Androchus pictus*

1- *Diffuse* stage showing fused sex chromosome of X and Y that lies on one side of the nucleus. *2*- Diplotene showing fused sex chromosomes and ring autosomal bivalent chromosomes. 3- Diakinesis showing well separated X and Y sex chromosomes. 4- 5- Metaphase I plate showing two different arrangements of the chromosome. 6- Metaphase II showing a ring of autosomal univalents and sex chromosomes. 7- Telophase II showing reductional divided sex chromosomes. Arrowheads indicate fused sex chromosomes; arrows indicate sex chromosomes. Scale bar = 10 µm.

5. DISCUSSION

Karyologically, a diploid number of 2n=14=12A+XY possessed by about 83% (243 from 294) species has been considered as the modal number of this family (Ueshima, 1979; Dey and Wangdi, 1988; Satapathy and Patnaik, 1988). In the rest of the 17 % species, the diploid n umber varies from only 6 in *Rhytidolomia senlis* (Wilson, 1913;) to 28 in *Thyanta calceata* (Wilson, 1911).

In the present study, four species of Pentatomidae were investigated (*Halyomorha annlicornis* and *Rhacognathus punctatus*) species had a diploid chromosome number of 2n=14 while two *Podisus nigrispinus* and *Banasa dimidiata* had 2n=16 and all the four presently investigated species had XX/XY (female/male) sex chromosome determining system. This type of sex determining system is the most commone mechanism in Pentatominae present in 226 species out of 228 species studied so far. Deviations were seen in *Rhytidolomiasenilis w*ith 2n=6 and Neo X-Neo Y as the sex mechanism (Wilson, 1913) and *Thyantacal ceata* with 2n=27 and X1X2Y as the sex mechanism (Wilson, 1911).

The general course of meiosis in all the presently studied species is fairly uniform and behavior of chromosomes is typical of the Heteroptera. In all of them, the autosomes divide reductionally while the sex chromosomes divide equationally during the first meiotic division and just the reverse happens during the second meiotic division. One important variation is in the diffuse stage. The diffuse stage has been a common meiotic feature in Heteropteran species during which autosomes are decondensed, sex chromosomes are condensed and the cell size increases (Ueshima, 1979). Among different species of Heteroptera, the degree of decondensation of the a utosomes during the diffuse stage varies from those with high degree of decondensation to species whose chromosomes do not decondensed at all and diffuse stage is absent (Lanzone and Souza, 2006).

In the present study the degree of decondensation of autosomes during diffuse stage showed varation between Pentatomidae species such that high decondensation in *Halyomorha annulicor nis* and *Rhacognathus punctatus* species and moderate decondensation of autosome in *Podisus nigrispinus* species and slight decondensation in *Banasa dimidiate* species of Pentatomidae which agrees with different authors (Rebagliati *et al*., 2001; Lanzone and Souza, 2006; Kaur and Semahagn, 2010; Kaur and Sharma, 2015; Bizuayehu Kerisew, 2011). In Pentatomidae, sex chromosomes usually associate during diffuse stage have some variation with respect to degree of association between X and Y among different species (Rebagliati *et al*., 2001; Lanzone and Souza, 2006; Viera *et al.,* 2009).

In the present study, X and Y chromosomes remained associated to form a single heteropycnotic body throughout the diffuse stage in all four species (*Podisus nigrispinus, Banasa dimidiate, Halyomorha annulicornis* and *Rhacognathus punctatus*). These meiotic behavior of Pentatomidae was reported by other researchers and confirm the meiotic behavior of sex chromosome (Manna, 1951; Satapathy and Pataik, 1988; Rebagliati *et al.,* 2001, Kaur and Semahagn, 2010; Bizuayehu Kerisew, 2011; Gabriela *et al*., 2013; Bardella *et a*l., 2016). In Heteroptera, there is a predominance of one chiasma per bivalent (Satapathy&Patnaik 1988; Gonza`lez- Garcia *et al*., 1996; Grozeva & Nokkala 1996; Nokkala &Nokkala 1999; Lanzone & Souza, 2006).

The present observations substantiate the viewpoint of Rebagilati & Mola (2010) that the presence of ring bivalent is common rather than exceptional in Pentatomidae. In *Podisus nigrisp inus* and in *Banasa dimidiata*) autosomal bivalents are arranged in a ring on the metaphase I plate in the centre of which lie X and Y as has been reported in most of the pentatomidae species (Manna 1951; Satapathy and Patnaik 1988; Rebagilati*et al*., 2001; Lanzone and Souza, 2006). However, in *Halyomorha annulicornis*, chromosome arrangement deviates from this typical pattern. Here, X- chromosomes have been peripheral forming a part of the ring. This behavior is

less common and has earlier been reported only in 7 species viz., I (Kaur et al., 2006; Kaur and Semahagn, 2010), *Mormidae paupercula* (Rebagliati and Mola, 2010), *Acledra kimber gii* (Rebagliati and Mola 2010), Eysarcoris rosaceous,*Halys seregera*, *Plautia fimbriata* and *Priassus exemptus* (Bizuayehu Kerisew, 2011). Deviations from the typical metaphase I arrangement have also been reported earlier in two species of subfamily Pentatominae viz., *Carbulasocia* (Satapathy and Patnaik, 1988) and *Eurydema pulchrum* (Kaur et al., 2006; Kaur and Semahagn, 2010) where all the chromosomes are randomly arranged on the metaphase plate. In *Podisus nigrispinus* and *Banasa dimidiate*, at metaphase II, autosomes form a ring in the centre of which lies the XY pseudobivalent, a behavior commonly observed in Pentatomidae (Parshad 1957; Satapathy and Patnaik, 1988; Rebagliati *et al.*, 2001; Kaur and Semahagn 2010 and Bizuayehu Kerisew, 2011).

In *Halyomorpha annulicoris* at metaphase II, polar view five autosomal univalent chromosomes and sex chromosomes of Y form ring. While one autosomal univalent chromosome and X chromosome located inside. It is supported by other researchers so far by Rebagliati and Mola (, 2010); Bizuayehu Kerisew, 2011).The family Coreidae, often called leaf-footed bugs, pod bugs or squash bugs, includes 2200 species belonging to 500 genera (Dursun and Fent, 2009). Cytogenetic data pertains merely to 134 species, cytogenetically, Coreidae is characterized by holocentric chromosomes, post-reductional division of sex chromosomes, a pair of micro-chromosomes and absence of Y chromosome. The most common diploid number of the subfamily is 21 observed in 48 species (Schuh and Slater 1995; Papeschi and Bressa, 2006, Yang *et al*., 2012; Kaur and Bansal, 2012, Bansal and Kaur, 2013). The diploid number of the family ranges from 13 to 29, the most common sex chromosome system of the family is X0/XX (male/female).

In the present study, two species of Coreidae have been cytogenetically investigated. Male diploid chromosome number was 2n=18=14A+2m+X1X2 in *Cletus borealis* species and 17=16A+Xo in *Acanthocephala femorata* species. Sex determination mechanism was X0 in *Acanthocephala femorata* species and X1X2O in *Cletus borealis* species. sex determination mechanism of X1X2O was observed earlier in 8 species of *Cletus* by Toshioka (1935), Manna (1951), Dutt (1957), Parshad (1957a, d, 1958), Banerjee (1958), Sands (1982a), Satapathy and Patnaik (1989) and Kaur and Semahagn (2010a). The diploid chromosome number was 2n=18=14+2m+X1X20 in *Cletus borealis* as was also reported earlier in *Cletus bipunctatus*, *Cletus hoplomachus*, *Cletus pugnator*, *Cletus punctulatus*, *Cletus rusticus*, *Cletus trigonus* and *Cletus* sp. by Toshioka 1935; Dutt, 1957; Banerjee,1958 and Kaur and Semahagn (2010).

The diploid chromosome number of 2n=17=16A+xo in *Acanthocephala femorata* in the present investigation does not support the report of other authors that investigated the genus *Acanthocephala*. In the present study of *Cletus borealis* species the fourteen autosomes can be assigned two size groups. Two pairs of autosomes were extremely large and five pairs were small, while in *Acanthocephala femorata* species showed two pair of large and six pair of similar size autosomes are investigated. But Similar size grouping has been reported in other species of Coreidae by Kaur and Semahagn (2010) and Yang *et al*. (2012). Microchromosomes were present in the currently studied species of *Cletus borealis*. Their presence had earlier been reported in all other species of *Cletus* and *Cletomorpha* (Toshioka, 1935; Dutt, 1957; Parshad, 1957 ; Banerjee, 1958 ; Satapathy and Patnaik, 1989; Kaur *et al*., 2006; Kaur and Semahagn, 2010; Yang *et al*., 2012). In the present study sex chromosomes of *Cletus borealis* species X1 and X2 fused together, that does not agree with the report by Banassal and Kuar,(2013) who reported that sex chromosomes of X1 and X2 are well separated and X1 is slightly bigger and X2 is smaller than the smallest autosomes. Reduviidae reveals a chromosome diploid number that varies from 10 to 34, with both simple and multiple sex chromosome systems (XY/XX,

X0/XX, and XnY/XnXn) (Ueshima, 1979; Poggio *et al.,* 2007; Kaur *et al.,* 2009; Panzera *et al.,* 2010). In the present study, *Anrdrochus pictus* shows the diploid chromosome number of 2n=22=20A+XY.This result agrees with the previous studies of sex determination system and meiotic behaviour of Reduviidae (Grozeva *et al.,* 2010, Poggio *et al*., 2007). During the diffuse stage, a common meiotic feature of Heteroptera, is condensed sex chromosomes which usually fuse or associate closely. In the present study, the, X and Y-chromosomes associated closely to form a single heteropycnotic body during the diffuse stage but variations in their association pattern have been observed in the subsequent stages. X and Y chromosomes fused at diplotene and well separated at diakinesis in *Anrdrochus pictus* it is confirm the meiotic behaviour of Red uviidae. The sex chromosomes divided reductionally during the second meiotic division as a result of which the X and the Y chromosomes moved to opposite poles and two types of nuclei were formed at telophase II, one with ten autosomes and the X- chromosome and the other with ten autosomes and the Y-chromosome (Panzera *et al*., 2010; Kaur *et al*., 2009; Kaur and Patial, 2012; Kaur and Kaur 2013). In Heteroptera, there is predominance of one chiasma per bivalent (Lanzone and Souza, 2006; kaur, 2011). In general, the autosomal bivalents show a single chiasma terminally located (rod bivalents) and orientate at metaphase I with their long axes parallel to the polar axis. During both meiotic anaphases only their ends are able to show kinetic activity leading the chromosome/chromatid segregation to opposite poles (pre-reductional division) (Viera *et al*., 2009). On the other hand, the sex chromosomes are achiasmatic and behave as univalents during meiosis I. Most sex chromosomes segregate their chromatids equationally at anaphase I and reductionally at anaphase II (post-reductional division) (Suja *et al*., 2000 andViera*et al*., 2009). The present study supports the above report. The first meiotic division is reductional and the second one is equational for the m-chromosomes (Papeschi and Bressa, 2006). In the present study microchromosomes were not observed in *Anrdrochus pictus* species of family Reduviidae.

6. CONCLUSION AND RECOMMONDATION

6.1. Conclusion

Results obtained from this study showed that chromosome number, sex determining mechanisms and meiotic behavior of Heteroptera. In the present study, the observed chromosome numbers reveal that there are variable basic chromosome numbers, different sex determination and meiotic behaviour among the three families: Pentatomidae (*Podisus ni grispinus, Rhacognathus punctatus* and *Halyomorha annulicornis),* Coreidae (*Cletus borealis , Acanthocephala femorata)* and Reduviidae (*Anrdrochus pictus).* The results of this cytological investigation showed that the diploid chromosome in the four Pentatomidae sepecies, *Halyomorha annulicoris* and *Rhacognathus punctatus* was 2n=14=12A+XY and *Ba nasa dimidiata, Halyomorha annulicornis* was 2n=16=14A+XY and that of *Anrdrochus pictus (Reduviidae)* was 2n=22=20A+XY, while 2n=17=16A+XY, 2n=18=14A+2m+X1X2 O in *Acanthocephala femorata* and *Cletus borealis* respectively in Coreidae. In the present, study from the seven Heteroptera species *Acanthocephala femorata (*Coreidae) and *Rhacog-nathus punctatus* (Pentatomidae) was not investigated earlier.

In all species of Pentatomidae, Coreidae and Reduviidae analyzed here sex chromosomes associated closely to form a single heteropycnotic body during diffuse stage. At metaphase I, all the seven autosomal bivalents arrange themselves in a ring shape and the sex chromosomes, X and Y are located at their center side-by-side arrangement in *Banasa dimidiata* and *Podisus nigrispinus* while microchromosomes found only in *Cletus borealis (Coreidae).* At telophase II, Sex chromosomes divide reductionally during the second division and forming four types of nuclei in four species, while two nuclei formed in three species.

6.2. Recommendation

Based on the results obtained in this study, the following recommendations are forwarded:

- The cytogenetics of few species of Heteroptera from Ethiopia had been investigated. This cytogenetic survey suggests that if comprehensive studies are conducted more cytogenetic features of the Ethiopian Heteroptera could have been identified.
- Further chromosomal number study of many other species should be done for better understanding of cytogenetics of the families.
- Heteroptera species used as biological control mechanism, while the species of Heteroptera in Ethiopia was not taxonomically identified.The present species were identified by Dr.Bizuayehu Kerisew so, Entomologists need to identify Heteroptera species to use as biological control and Phylogenetic studies of species for future.
- Cytological techniques combined with molecular techniques are recommended to produce more information about the genetic diversity and Phylogenetic relationships within the families.

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8. APPENDIXES

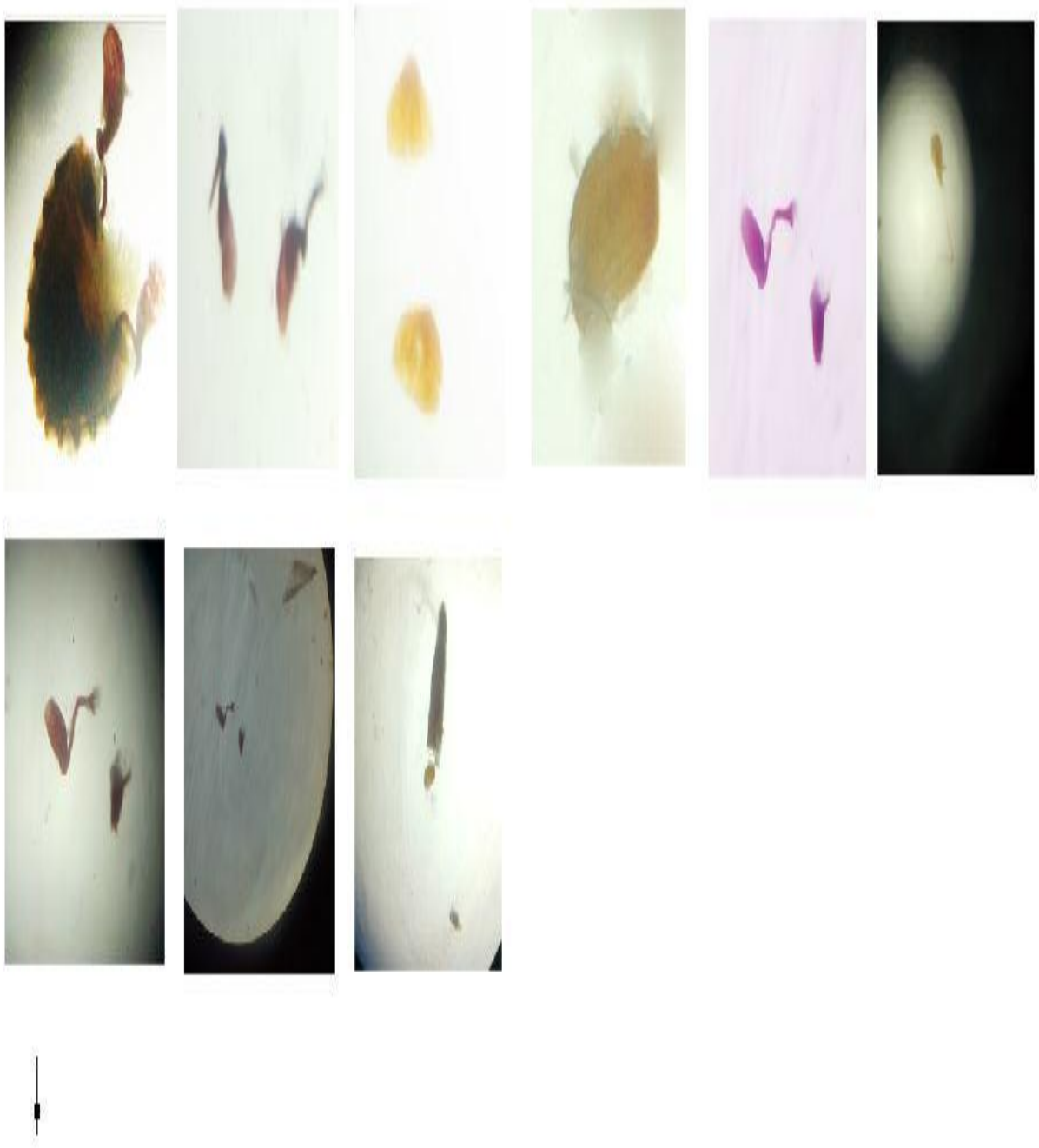

Figure 9. Samples of testis during experiment

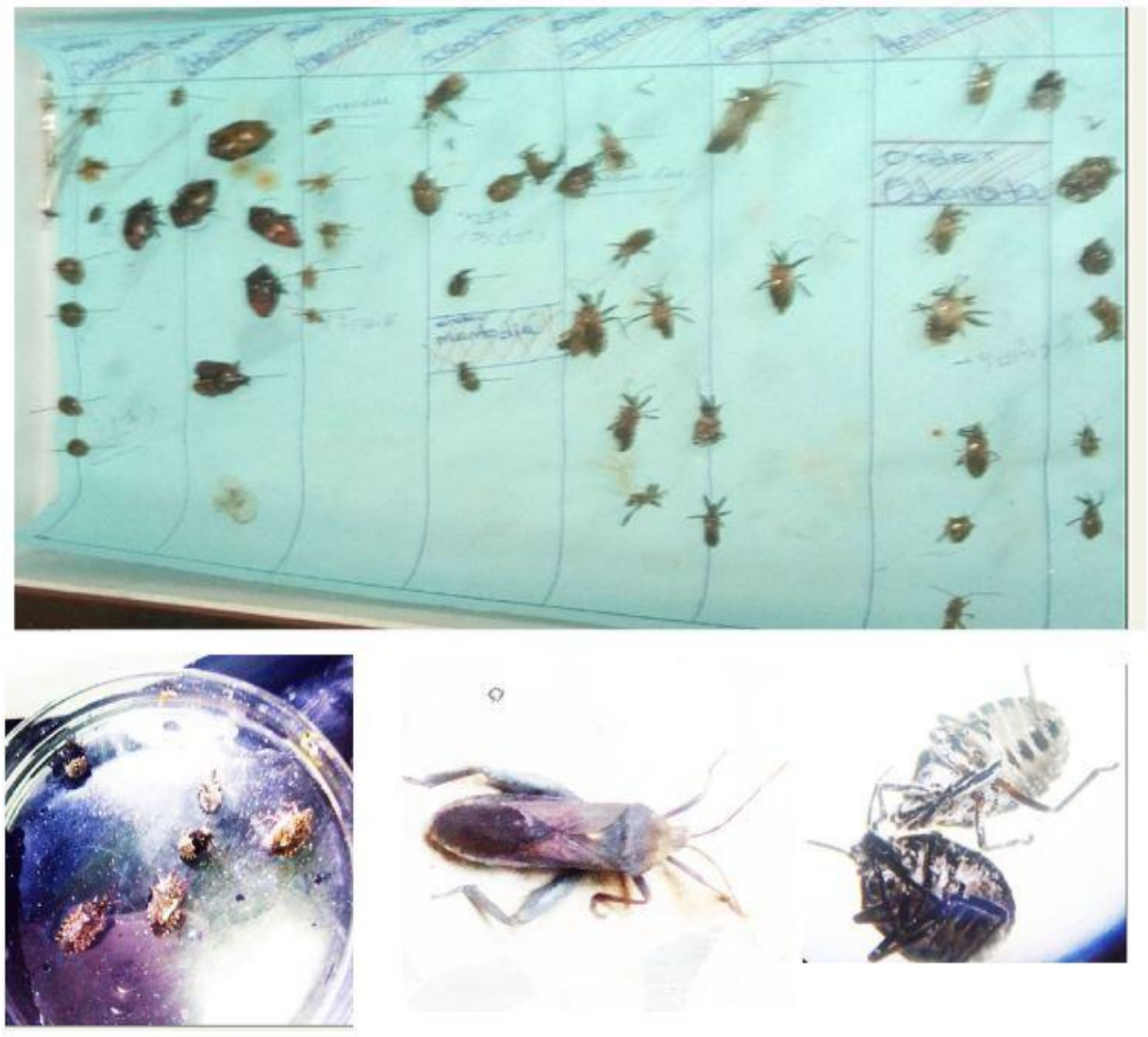

Figure 10. Samples of the studedspesies in wood box and petridish

Figure 11. Olympus microscope, dissecting microscope ,preparid slide and observation