ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF BIOLOGY

KARYOTYPE STUDY OF SOME SPECIES OF RODENTS FROM LOCALITIES AROUND ADDIS ABABA CITY AND HURUTA TOWN, ETHIOPIA

A THESIS PRESENTED TO THE SCHOOL OF GRADUATE STUDIES, ADDIS ABABA UNIVERSITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY (APPLIED GENETICS)

By

ASHEBIR SIMRET

October, 2010
Addis Ababa

KARYOTYPE STUDY OF SOME SPECIES OF RODENTS FROM LOCALITIES AROUND ADDIS ABABA CITY AND HURUTA TOWN, ETHIOPIA

By

ASHEBIR SIMRET

A Thesis Presented to the School of Graduate Studies, Addis Ababa University, in Partial Fulfillment of the Requirement for the Degree of Master of Science in Biology (Applied Genetics)

ACKNOWLEDGEMENTS

I am very grateful to my advisor Dr. Kifle Dagne for his constant advice, critical comments and innumerable helps. His helps have been, often, far beyond the responsibility of an advisor. I would like to express my gratitude to Prof. Afework Bekele for providing me literatures and sharing his knowledge. I am grateful to Dr. Tilaye Wube for his valuable advices and practical suggestions that I received at the beginning of this study. Special thanks to Ato Ketema Amberbir for his willingly help in specimens identification and for providing me a field guidebook of mammals. My deepest gratitude and sincere thanks go to my friend and classmate, Tesfaye Dilebo for his kindly and patiently help in laboratory works and in sharing his valuable experiences. I am also greatly indebted to my friend and classmate, Birhanu Kumbi for his kindly and patiently cooperation and help in the collection of specimens. I wish to acknowledge Biology Department of Addis Ababa University for admitting me in postgraduate program and for providing me financial and materials support for this study. I would like also to acknowledge Finfinne Forest Enterprise/Subba-Sebbeta Forest; and Ethiopian Heritage Trust for their willingness and cooperation during the collection of specimens in their forest site. Special thanks go to my parents for their constant encouragement and support. Specially, my father, Ato Simret Abebe has played important roles in my education and career, since my early ages up to this level.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

In this study, live specimens of six rodent species were captured from localities around Addis Ababa by using Sherman live-trap. Somatic metaphase chromosomes were prepared by bone marrow method and the diploid number of chromosomes (2n) and the number of autosomal arms (FNa) were recorded and karyotypes constructed for these species. The karyological results were recorded as follows: *Mastomys natalensis* (2n = 32 and FNa = 54), *Arvicanthis abyssinicus* (2n = 62 and FNa = 64), *Stenocephalemys albipes* (2n = 46 and FNa = 50), *Rattus rattus (*2n = 38 and FNa = 58), *Mus musculus* (2n = 40 and FNa = 38) and one unidentified species (designated as *ARSI-X*) (2n = 32 and FNa =50*)*. These karyological results were compared with previous reports on the same species from different localities of Ethiopia and abroad; and found that they were largely in agreement. The unidentified species, *ARSI-X,* morphologically looked liked *Mastomys* species but with distinctly different karyotype. It was suggested that this specimen, could probably be a sibling species or a new cryptic species in the genus *Mastomys,* but it requires further detail morphological and cytological studies in order to determine its proper taxonomic status.

Key words: Chromosome, Ethiopia, karyotype, morphology, rodent.

1. INTRODUCTION

Rodents (Rodentia) belong to the class Mammalia. Mammals comprise 4600-4800 extant species classified into three subclasses, Monotremata (egg-laying mammals), Marsupialia (marsupials) and Eutheria (placentals). Eutherians comprise more than 3700 species in 19 extant orders to which the vast majority of living species belong (Serov *et al.*, 2005). The distribution of species richness among these orders in highly skewed, rodents representing approximately half of the species while other orders have only few extant species (Purvis and Hector, 2000).

All rodents have one conspicuous characteristic in common: a pair of long and prominent teeth (incisors), which are firmly inserted in both upper and lower jaws. Rodents are one of the most successful groups and are distributed almost throughout the world (Vaughan *et al*., 2000). Rodents consume all sorts of plant material. They also consume insects and other invertebrates. Some are partially carnivorous. Most rodents produce large litters within a short gestation period (Nowak, 1999).

Among mammals, rodents are the most noxious with regard to their pest and vector status. Rodents such as rats bite humans inflicting disease, anxiety and terror. Each year, they destroy billions of dollars' worth of property worldwide. They cause innumerable fire hazards by gnawing insulation from electrical wiring (Canby, 1997).

On the other hand, rodents play important roles in various ecosystems that range from carbon and energy flow to nutrients recycling (Kingdon, 1997; Davis, 2002). Several species of rodents are important in biological experimentation in a wide variety of research areas. Some rodent species are valued for their fur and some are popular house pets. In some regions of the world rodents are important food sources for humans (Davis, 2002).

In Africa, 386 species of rodents have been listed (Musser and Carleton 1993) and more than 70 species of rodents have been recorded from Ethiopia (Afework Bekele and Corti, 1997).

Knowledge of African rodent taxonomy has been largely influenced by the history of colonization and by independent expeditions organized by Natural History museums of Europe and USA, during the early 20th century. Thus, these museums have collections of most species types so serving as a unique reference for correct taxonomic identification and classification. During the later part of 20th century, new disciplines such as genetics, cytogenetics, molecular genetics and morphometrics have been developed and employed in the taxonomic studies of African rodents, often in collaboration with African institutions.

However, much of the information on African rodents is scattered over wide areas. In addition, sibling and cryptic species have been occurred. As a result, the taxonomic revision of many genera is far from completion (Corti *et al*., 2005).

Many scholars on African rodents have indicated that the taxonomy and systematics of several genera of African rodents is in chaotic state and follow opposite trends of cyclic lumping and splitting (Afework Bekele *et al.*, 1993; Capanna *et al.*, 1996; Ducroz *et al.*, 1997; Lavrenchenko *et al.*, 1998; Fadda *et al.*, 2001; Corti *et al.*, 2005).

The occurrence of several new sibling/cryptic species have been recorded (Lavrenchenko *et al.*, 1998; Fadda *et al.*, 2001; Corti *et al.*, 2005) resulting from speciation processes leading to an increment in genetic diversity linked with little morphological variation (Fadda *et al.*, 2001). Morphological and cytogenetic characters are important for identification of most species; and karyotypic analysis is very essential for cryptic/sibling species (Geise *et al.*, 1998).

The intraspecific chromosome variation of African rodents which sometimes occurs within populations indicated the requirement of careful cytogenetic studies. It is generally accepted that the great diversity in rodents is related to their fast rate of chromosomal rearrangements. As a result, karyotype descriptions constitute the primary tool for rodent species identification (Fadda *et al.*, 2001; Corti *et al.*, 2005).

The present study is aimed at generating cytological data and analyzing chromosomal variation among some species of rodents from around Addis Ababa city and Huruta town.

2. LITERATURE REVIEW

2.1. GENERAL DESCRIPTION OF RODENTS

2.1.1. Taxonomy and Diversity of Rodents

Rodents are classified as:

Class: Mammalia

Subclass: Eutheria (Placentals)

Order: Rodentia

There are more species of rodents than of any other mammalian order. The order Rodentia comprises 29 families and 443 genera with over 2000 species (Musser and Carleton, 2005), accounting, approximately, for 45% of the extant mammalian species (de Villena, 2005). Rodents were recorded first from the late Paleocene epoch, about 55 million years ago (Futuyma, 2005). However, rodents of the family Muridae, which comprise more than half of all rodent species, did not appear until 6 million years ago (Kingdon, 1997). Though murids are believed to be late comers of African rodents, they have become widespread and dominant species by progressively replacing other types of rodents and by relegating more conservative groups to the status of relict (Kingdon, 1997).

Musser and Carleton (1993) listed 386 species of African rodents. However, researchers on African rodents taxonomy and systematics suggest that the biodiversity is much larger than previously estimated so that the list will increase rapidly (Corti *et al*., 2005).

Until 1997, in Ethiopia, more than 277 mammalian species have been recorded and 11% of these species are endemic (Yalden and Largen, 1992; Afework Bekele and Corti, 1997). The small mammal fauna is particularly diverse. About 70 species of rodents have been recorded from Ethiopia, and of these 21% are endemic; and rodents represent about 50% of the entire Ethiopian endemic mammals. Among the nine families of rodents that are found in Ethiopia, the family Muridae comprises 57 species, thereby, representing 84% of the total rodent species and 93% of the endemic rodents (Afework Bekele and Corti, 1997).

Rodents have been adapted to diverse habitats. They are widely distributed throughout the world. They dwell in various habitats. Some species are aquatic, some are terrestrial, and some live in burrows in the ground. Some are arboreal, and about 35 species are semiaerial, gliding from one tree to another (Nowak, 1999; Vaughan *et al*., 2000).

The success of rodents as a group is no doubt. They combine three adaptations to thrive: ability to produce large litters in a short period of gestation, ability to adapt quickly to environmental changes and they are relatively small animals, which can easily hide from predators (Vaughan *et al*., 2000).

2.1.2. Common Features of Rodents

Rodents as members of the mammalian group, share all the main characteristics common to all mammals and in addition have their own distinct features not shared by other mammalian groups. All rodents have one conspicuous characteristic in common: a pair of long and prominent teeth (incisors). These chisel-like, broad, sharp-edged incisors are firmly inserted in both the upper and lower jaws; grow throughout life; and are extremely effective for gnawing. Rodents use these teeth to gnaw into their food and to cut through any obstacles in their path. The front surface of each incisor is composed of enamel while the hind surface is composed of soft dentine, which wears away during the process of gnawing, so that the teeth are constantly kept sharp (Nowak, 1999).

2.1.3. Food and Feeding Habits of Rodents

The diets of rodents are diverse. Rodents consume all sorts of plant material; primarily seeds, but also stems, fruits, flowers and roots. They also consume insects and other invertebrates (Nowak, 1999). There tend to be specialized structures depending on their feeding habits. Insectivores tend to have sharp-cusped molars and a slender muzzle, while herbivores have broad incisors, mill-like grinding teeth and a stout skull; and omnivores tend to be intermediate (Kingdon, 1997).

2.1.4. Fecundity of Rodents

Their high fecundity is one of the factors that contribute to the success of rodents. Most rodents reproduce rapidly. They produce large litters quickly (Nowak, 1999; Vaughan *et al*., 2000). For example, *Mastomys* species have been reported to be exceptionally fecund (Kingdon, 1997). Rodents, such as rats are extremely prolific, breeding 1 to 13 times a year and producing 1 to 22 young in a litter. These rodents multiply so rapidly that a pair could have more than 15,000 descendants in a year's life span (Canby, 1997).

2.1.5. Importance of Rodents

Rodents are economically injurious, destroying crops and stored foods. They destroy approximately 20% of all food crops planted worldwide (Canby, 1997). Several agricultural pest species of rodents such as *Mastomys natalensis, Arvicanthis dembeensis* and *Tatera robusta* occur in Ethiopia (Workneh Gebresilassie *et al.*, 2004). In central Ethiopia, *Arvicanthis dembeensis* and *Mastomys erythroleucus* were reported to be the major pest species, whereas *Mus mahomet* and *Tatera robusta* are minor pest species (Afework Bekele *et al.*, 2003).

Some species of mouse and rats cause serious hazard to human health. For example, *Mastomys* species have been well known as dangerous disease vectors (Kingdon, 1997). Around the world, rats and their abundant parasites spread more than twenty kinds of diseases, such as typhus, trichinosis and the deadly Lassa fever. In Africa, Asia and America people die of plague. The rats carried the fleas that caused the plague of Europe (Black Death) that destroyed about a quarter of the medieval population (Canby, 1997).

On the other hand, rodents play important roles in various ecosystems. They prune vegetations or their parts; they aerate the soil through their digging and burrowing activities; they spread seeds and pollens, they involve in competition; and they serve as food for various predatory mammals, birds and reptiles. Thus, their roles within various ecosystems range from carbon and energy flow to nutrients recycling (Davis, 2002).

Hamsters and albino strains of the mouse and rat are important in biological experimentation. White mice and white rats have been used as laboratory animals in a wide variety of research areas such as cancer, drug toxicity, immunodeficiency diseases, and allergic reactions to cosmetic products. The house mouse has long been of importance for genetic studies (Sumner, 2003). The mouse genome is the most valuable to use and understand human biology. This makes mouse as the preeminent model mammalian organism in genetics. A great deal is known about the mouse genome and more can be investigated using knockout studies that would be impossible to conduct in humans (Filipski and Kumar, 2005).

Some rodent species such as, the muskrat and beaver are valued for their fur. In addition to their use as laboratory animals; white mice, white rats, gerbil and guinea pig are popular house pets. In some regions of Africa and Asia, rodents serve as important food sources for humans (Davis, 2002). In Ethiopia, the Gumuz indigenous people are reported to feed on rodents (Tadesse Habtamu, 2005).

2.2. CYTOGENETIC STUDY AND CHROMOSOME FEATURES

Chromosomes are complex structures with distinct structural features that play important roles in the processes of replication, transcription, and regulation of gene expression. Cytogenetics focuses on structure and genetic organization of chromosomes. It combines the methods and findings of cytology and genetics. Thus, it helps to analyze behavior of chromosomes in the organization and transmission of genetic information, evolutionary pathway and mechanism of variation (Griffiths, 1996).

In a diploid organism, each somatic cell has two copies of each chromosome type. During most of the life of a cell, the chromosomes are not condensed and thus invisible. However, during cell division, particularly at metaphase, the chromosomes condense and become visible under light microscope. Therefore, mitotic chromosomes offer a unique opportunity to observe the nuclear genome by microscope, allowing analysis of its components individually, as well as globally (the karyotype) (Dobigny *et al*., 2004). Cytogeneticists frequently use chemical dyes to stain the chromosomes. After staining, the visible metaphase chromosomes can be photographed and sorted by size and shape to obtain the karyotype represented by ideogram or karyogram (Stace, 2000). Thus, karyotype is the visual description of the complete set of chromosomes of a typical somatic cell of an eukaryotic organism. Usually, in the construction of karyotypes, autosomes are arranged in order of decreasing size and the sex chromosomes, when distinguished, placed at the end of the sequence (de Villena, 2005).

Each species has a characteristic chromosome complement - the species karyotype (Ferguson-Smith and Trifonov, 2007). Generally, the karyotypes of closely related species are more similar to each other than they are to the karyotypes of distantly related species (Sumner, 2003).

The chromosome number, the total length of the chromosome complement, the absolute and relative sizes of chromosomes, the symmetry of each chromosome as dictated by the position of the centromere on each chromosome, the number and positions of satellites associated with the nucleolar-organizer regions, and the distribution of heterochromatic segments are the main features of metaphase chromosomes that describe the karyotype of a species (Levin, 2002). Some of these chromosomal features are briefly discussed below.

2.2.1. Chromosome Number

The chromosome number is species specific for most species, i.e., all members of the species share the same chromosome number and members of different species may have different numbers. However, there are some exceptions to this rule. For example, in some insects, the males are XO and the females are XX, resulting in numerical difference of a single chromosome between the two sexes (Ferguson- Smith and Trifonov, 2007).

Somatic chromosome number among species ranges from one to over 600 pairs. A single chromosome pair has been reported for the ant, *Myrmecia pilosula* (Crosland and Crozier, 1986). At the other extreme, the fern, *Ophioglossum reticulatum,* has a diploid number of over 1260 chromosomes (Otto and Whitton, 2000). Among mammals, chromosome numbers vary from 2n = 6 in the Indian muntjac (*Muntiacus muntjack)* to 2n=102 in the South American rodent - viscacha rat (*Tympanoctomys barrerae)* (Ferguson-Smith and Trifonov, 2007).

The analysis of chromosome numbers represents an important approach in the studies of genetic variation, phylogeny, taxonomy and evolution. It is also important in studies on the structure and diversity of genomes (Jara-Seguel *et al.*, 2006). Different aspects of chromosome number still remain important taxonomic tools as they have ever been earlier. The systematic community is well served by catalogues of chromosome number. Chromosome number and homology determine pairing behavior, fertility and breeding behavior and patterns of variation. Thus, knowledge of chromosome number is fundamental to achieve a classification that reflects evolution (Stace, 2000).

Studies on the distribution of diploid and fundamental numbers of 1170 mammalian species showed that both distributions are almost symmetric and centered on the mean (de Villena and Sapienza, 2001). The mean diploid number for all mammals is 43.3, ranging from 2n = 6 in the Indian muntjac to 2n = 102 in the viscacha rat. This wide range of diploid numbers indicates that, since the last common ancestor, diploid number has undergone considerable changes in some mammalian species. The diploid number of the hypothetical ancestral placental mammals is estimated to range from 2n = 44 to 2n = 50, which is remarkably similar to the mean diploid number among placental mammals (2n = 44.7). However, about 90% of mammalian species have different diploid numbers from the mean, indicating that in the majority of placental mammals, the diploid number has changed; and similar proportions of extant species have lower and higher diploid numbers than their common ancestor (de Villena, 2005). The mean fundamental numbers of autosomes is 61.8, ranging from FNa = 8 to FNa = 198, showing also a wide variability of fundamental number in mammals (de Villena and Sapienza, 2001).

2.2.2. Centromeric Position and Chromosome Shape

Centromere is a constricted region of an eukaryotic chromosome to which spindle fibers attach during cell divisions. Centromeres mediate the faithful segregation of chromosomes during mitosis and meiosis (de Villena, 2005).

Chromosome shape is determined by its length and the positions of centromere (primary constriction), and the nucleolus organizing region (secondary constriction) (Sumner, 2003). The position of the centromere is the most important feature defining chromosome morphological variation (Stace, 2000).

Morphologically, chromosomes can be classified into four groups. Telocentric chromosomes have centromere at one end and thus they are uniarmed. Acrocentrics have centromere near one end of the chromosome, so that it contains one long arm and one very short arm. Submetacentrics have centromere nearer to one end of the chromosome than the other, so that the two arms are distinctly unequal but less so than in acrocentrics. Metacentric chromosomes have the centromere at or near to the middle of the chromosome, so that the two arms are nearly or quite equal in length (Stebbins, 1971). Originally telocentric chromosomes were defined as chromosomes with a strictly terminal centromere produced by centromere misdivision or breakage within the centromere. After the discovery of the organization and behavior of telomere, such chromosomes have been suggested to be unstable (Sumner, 2003). However, mouse chromosomes, in which the centromeric DNA is linked directly to the telomere sequences (Kipling *et al.*, 1991), can be regarded as telocentric (Sumner, 2003). On the other hand, in rodents cytogenetics, some authors use the term acrocentric, instead of telocentric, for uniarmed chromosomes (Lavrenchenko *et al*., 1998; Fadda *et al.*, 2001; Corti *et al.*, 2005).

Chromosome shape can also be defined in terms of the arm ratio (Levan *et al*., 1964). The arm ratio (**r = l/s**) is calculated as the length of the longer arm (**l**) divided by the length of the shorter arm (**s**). Based on this, chromosomes are designated as **M**, centromere at the median point (r = 1); **m**, centromere in the median region (r = 1.0–1.7); **sm,** centromere in the submedian region (r = 1.7–3.0); **st**, centromere in the subterminal region (r=3.0–7.0); **t**, centromere in the terminal region (r = 7.0 - ∞); and **T**, centromere at the terminal point (r = ∞).

2.2.3. Secondary Constriction and Satellite

The number and position of any secondary constrictions is one of the important features used to characterize chromosomes (Stace, 2000). They might play a major role on identifying closely related species (Affonso *et al.,* 2007). The nucleolus organizer regions (NORs) are the sites of origin of nucleoli, meaning the region of DNA that produces ribosomal RNA, and are known as rDNA sites. They remain undercondensed at metaphase and thus form a secondary constriction, i.e., a conspicuous chromosomal structure that can be recognized as apparent breaks by light microscopy on metaphase chromosomes (Sumner, 2003). NORs can occur in a variety of locations on chromosomes, but positions are normally constant for a given chromosome of a species. In many species, frequently, they are subterminal in position, so that, distally, they delimit a short and well-stained chromosome region. This distal region is known as a satellite and the chromosomes bearing them as satellited chromosomes (Stace, 2000). The number, size and position of satellites are important morphological features of chromosomes. Up to eight numbers of satellited chromosomes (NORs) per somatic cells are reported (Kifle Dagne and Heneen, 1992; Kifle Dagne, 1995). Their appearance in mitotic spread is variable and it is not always possible to get the same results. Their appearance possibly depends on their level of metabolic activity at the precise moment of observation as well as whether or not they have participated in organizing the nucleolus during the preceding interphase (Stace, 2000).

2.3. STRUCTURAL REARRANGEMENTS AND CHANGES IN CHROMOSOME MORPHOLOGY

2.3.1. Types of Chromosome Structural Rearrangements

Karyotype evolution can be defined as the accumulation of a particular set of chromosome rearrangements in a specific lineage. The impact of rearrangements on diploid and fundamental numbers is considered significant because of the evolutionary trend correlating these numbers (White, 1973; King, 1993; de Villena, 2005).

The karyotypes of animals display a great diversity in number and morphology of chromosomes. Rearrangement of chromosomal segments into different combinations in the course of speciation explains much of the observed diversity in species karyotypes (Ferguson-Smith and Trifonov, 2007). Chromosome rearrangements due to inversions, translocations, duplications, deletions, fusions and fissions are well understood and excellently explain the origin of the diversity of karyotypes (Stace, 2000).

For instance, the subterranean rodents genus *Ctenomys* (tuco-tucos) contains more than 56 species. It presents one of the most diverse karyotypic ranges in mammals and its karyotypic diversity is ranging from 2n=10 to 2n=70. This diversity has been postulated to be the result of chromosomal speciation, which has been facilitated by a population structure that, like the *Mus musculus* races, consists of small, isolated demes (Reig *et al*., 1990; cited in O'Neill *et al.,* 2005). Neotropical rodents (Cricetidae, particularly Sigmodontinae) present chromosomal variation within and among populations, species and genera (Geise *et al.*, 1998). The association of chromosomal variation and species diversity in rodents indicates that karyological diversity is related to differentiation of populations and constitutes an adaptive component of evolution (Corti and Rohlf, 2001).

Studies on karyotype evolution may provide important information about the factors that influence genome instability, because chromosome rearrangements are the building blocks of karyotype evolution (de Villena, 2005). Following, brief accounts of the main types of chromosome structural changes are presented.

2.3.1.1. Robertsonian translocations/centric fusions and fissions

Robertsonian translocations are fusions and fissions in which the rearrangements are involving whole chromosome arms (de Villena, 2005). In Robertsonian fusion (named after US geneticist W. R. B. Robertson), the centromeric regions of two acro- or telocentric chromosomes fuse to from a single meta- or submetacentric chromosome. On the other hand, Robertsonian fission involves the splitting of a meta- or submetacentric chromosome horizontally at centromere to form two telocentrics (Sumner, 2003). Robertsonian translocations are the most easily recognized and probably the most intensively studied rearrangements in mammals and there is a general agreement that they have played a key role in the mammalian karyotype evolution (Qumsyieh, 1994).

When a Robertsonian fusion and fission become fixed, the fundamental number remains the same, while the diploid number either increases (fission) or decreases (fusion) by two units. Therefore, if a karyotype is only evolved through Robertsonian translocations, the fundamental number in the ancestor determines all possible derived karyotypes.

Though Robertsonian translocations alone cannot account for full diploid number diversity, evidence based on natural populations showed that they play a key role in diploid number diversity of mammals (de Villena, 2005).

For instance, Robertsonian fusion is occurring extraordinarily and continually in different populations of house mouse. More than a dozen species that diverged from the *Mus musculus* lineage between 1.5 and 5 million years ago are included in the genus *Mus.* About 750,000 years ago, several subspecies diverged from each other within the *Mus musculus*. Several chromosomal races diverged from the *Mus musculus domesticus* branch 5,000–10,000 years ago (Britton-Davidian *et al.*, 1989; Nachman *et al.*, 1994). Most populations have a karyotype consisting of 40 acrocentrics, but numerous populations exist, often in isolated places such as alpine valleys, in which the chromosome number has been reduced to as low as 2n=22. In these populations all the autosomes, except one pair are fused to from metacentrics. Each race is characterized by its own combinations of telocentrics to from metacentrics. Therefore, it can be concluded that the ancestral karyotype was 40 telocentric chromosomes and the later races are the derived situation (Nachman and Searle, 1995).

The karyotypes of more than 25% of the extant mammalian species have been described (de Villena, 2005). Comparisons of diploid and fundamental number among mammals indicate a wide diversity and most mammals had undergone opposite evolutionary trends, 50% of the species towards larger diploid numbers, mostly uniarmed, and 50% toward lower diploid number, mostly biarmed (de Villena, 2005). Since fissions increase diploid numbers and fusions decrease diploid numbers, it is possible to conclude that both types of chromosome rearrangements have been common processes operating during the evolution of mammalian karyotype (Qumsyieh, 1994).

2.3.1.2. Reciprocal translocations

A reciprocal translocation happens when single breaks, in each of two nonhomologous chromosomes produce an exchange of chromosome segments between them. If the exchanged segments are of equal size, then there may not be a change in the morphology of the chromosomes involved. However, unequal translocations can alter the position of the centromeres (arm ratio) and the relative size of chromosomes. Successive unequal translocations progressively increase differences in the relative size of chromosomes and may even cause loss of the centromere of the chromosome, from which important genetic information would be translocated to the other chromosome, thereby reducing the chromosome number (Stebbins, 1971). Individuals, heterozygous for reciprocal translocations produce unbalanced gametes during meiosis and are associated with strong fitness reductions (de Villena, 2005).

2.3.1.3. Inversions

Inversion involves breaking a chromosome segment, rotating it 180 degrees, and then reinserting it in its original location. An inversion that includes the centromere is called pericentric and that excludes the centromere is called paracentric. Inversions have been common in many mammalian lineages (Goureau *et al.*, 2001). In heterozygous condition, both types may cause reduction in fertility depending on how often chiasma is formed within the inversion loop. In inversion heterokaryotypes, fertility is reduced because many of the gametes become genetically unbalance if chiasma occurs within the inversion loop. Pericentric inversions can change the chromosome morphology by shifting the position of centromeres. For example, the karyotypes of *Arvicanthis abyssinicus* and *Arvicanthis dembeensis* differ only by one chromosome pair, which is submetacentric in the former and telocentric in the later; and this difference was suggested to be due to pericentric inversion (Capanna *et al*., 1996; Corti *et al*., 1996). Similar cases were reported in different populations of the Ethiopian endemic species *Stenocephalemys albipes* (Corti *et al.,* 2005).

2.3.1.4. Duplications and deletions

Duplications and deletions of chromosome segments cause a change in the sizes of chromosomes (Stebbins, 1971). They can also affect the morphology of the chromosomes, depending on the length and the position of the affected region (Sumner, 2003). Several factors can give rise to duplications and deletions. One factor that simultaneously produces both aberrations is unequal crossing over. Unequal crossing over (unequal exchange) can occur between two homologous sequences or chromosomes that are not perfectly aligned. This produces tandem duplication on one recombination product and a deficiency on the other chromosome. The length of the affected region may range from a single base pair to a large segment of chromosome (Futuyma, 2005). The distribution of duplicated segments in the chromosome is non-random, concentrating in the pericentric and subtelomeric regions (Bailey *et al.*, 2001).

Duplications are not usually as detrimental as deficiencies; and even sometimes, they could be advantageous. It has given rise to many gene families, and it has been extremely important in the evolution of greater numbers of functional genes and of total DNA (Futuyma, 2005).

Unlike duplication, a deficiency is rarely ever advantageous for an organism. Deletions result in an overall loss of genetic information and typically produce a detrimental phenotype (Futuyma, 2005).

2.3.1.5. Heterochromatin changes

Heterochromatin is an important feature of the genomes. It is composed of repetitious DNA sequences that remains condensed for most of the cell cycle. It is late to replicate, and it is usually transcriptionally inactive (Sumner, 1990). Its location and amount may vary at the species, population, and/or individual level. It has clear biological and evolutionary importance and can have phylogenetic value (Dobigny *et al.*, 2004). Variations in the quantity, position and properties of heterochromatin are very common among related species (White, 1973; Sumner, 1990; King, 1993). Among many rodents, heterochromatic short arms of chromosomes could occur in some species and not in others, with a corresponding difference in the total amount of nuclear DNA (Sumner, 2003). Many of the differences between rodent species are due to that they share a library of DNA sequences, and some of which may be amplified to form a block of heterochromatin in one species while different sequences might be amplified in other species (Fry and Salser; 1977 cited in Sumner, 2003).

2.3.2. Rate of Chromosome Rearrangement

Rate of chromosome rearrangement is defined as the number of chromosome rearrangements that had occurred per unit of evolutionary time. Evolutionary times are estimated by using fossil records and molecular divergence (de Villena, 2005).

Rate of rearrangement may vary from extremely slow, that have remained unmodified for about 200 million years (Bickham, 1981), to extremely rapid that is observed in some populations of tropical rodents segregating at high frequencies of multiple fission/fusion combinations (Koop *et al.*, 1983).

Among mammals, there is no particular rate of karyotype evolution. Generally, chromosomal rearrangements occur in rodents at a particular high rate. Within rodents, high rates of karyotype evolution are found in Muroid rodents (Trifonov *et al.*, 2002) and slow rates are found in Sciuridae family (Richard *et al.*, 2003; Stanyon *et al.*, 2003). Murid rodents (mice and rats) are one of the most rapidly radiating groups. These highly diverse groups arose in the Miocene and took about 1.98 million years to double in species number. The rate implies that, without extinction, each rodent species would speciate, on average, within about 2 million years (assuming that each species split into two "daughter" species) (Stanley, 1979; cited in Futuyma, 2005).

Murphy *et al.* (2001) proposed two modes of karyotype evolution rates: an ancestral slow rate (one or less change per 10 million years) and higher rates. However, closer investigation within groups has suggested that at different times the rates of evolution as well as the type of undergoing rearrangement have varied greatly (Ferguson-Smith and Trifonov, 2007). Drawing general conclusion on lineage-specific rates of chromosome rearrangements needs caution. For example, based on the presence of fast rates in mouse and rat, it was assumed that fast rates of rearrangements had to be typical in rodents. But slow rates have been reported in squirrels (Richard *et al.*, 2003; Stanyon *et al.*, 2003).

In addition, within lineages, there are variations in rates overtime. Studies suggested that in at least some lineages, karyotype evolution involves long period of stability followed by short intervals of dramatic change, resembling the punctuated equilibrium hypothesis (Eldredge and Gould, 1972; cited in Gregory, 2005).

The evolutionary time since the divergence of mouse and rat up to present can be classified into three periods. During the first 7 million years since divergence, rate of translocation has been estimated as one change per million. In the next 5 million years no rearrangements can be detected. In the last 10,000 years, some populations of mouse underwent as many translocations as in the previous 12 million years, indicating a dramatic variation of rates of rearrangements within lineage overtime. At least 14 translocations occurred in the 10-20 million years since rat and mouse diverged, indicating fast rate of genomic rearrangement between them (Stanyon *et al*., 1999).

2.3.3. Role of Chromosome Rearrangements in Speciation

Most chromosomal rearrangements are deleterious when heterozygous, while having normal fitness when homozygous (White, 1978). Rearrangements that do not affect fitness behave as neutral mutation (Hedrick, 1981). The fixation of rearrangements in the karyotype must be considered as significant evolutionary features that are important for phylogenetic inferences (Dobigny *et al*., 2004).

Speciation requires the establishment of reproductive isolation. Karyotype is one of the few characters that can contribute to the formation of postzygotic isolation between biological species. Studies, on the role of chromosome rearrangements in speciation, generally indicate that their importance lie in producing post-mating reproductive isolation through inviability or infertility of the heterokaryotypes (Coluzzi, 1982).

When species with different karyotypes mate, they produce hybrids that are heterozygous for chromosomal rearrangements fixed between parental species. These hybrids have reduced fertility due to mis-segregation of homologous chromosomes during the first meiosis. If the effect on hybrid fitness is less severe, chromosomal rearrangements, together with genetic differences, may impede gene flow between chromosomal races (Kowalczyk, *et al.,* 2008). The type of mutation/rearrangement involved greatly determines the extents of the effect on hybrid fertility (King, 1993; Noor *et al*., 2001). Generally, the extent of karyotype divergence between parental taxa and hybrid fertility are negatively correlated (White, 1973). The biological impact of each rearrangement on fertility is directly measured in heterozygotes and/or hybrids. The greater the underdominance, the more likely a specific rearrangement will play a role in the speciation process either by initiating or reinforcing reproductive isolation (King, 1993; Noor *et al*., 2001; Rieseberg, 2001; Delneri *et al*., 2003). Therefore, chromosomal rearrangements have a direct role in the final stage of speciation (Dobzhansky, 1937; 1940; cited in Kandul, 2007; White, 1973).

Earlier comparative studies showed a positive correlation between karyotypic diversity and species richness (Wilson *et al.,* 1975; Bush *et al*. 1977; cited in Kandul, 2007); and several models of speciation that are accompanied by structural rearrangements of the karyotype have been described (White, 1978; King, 1993). In less vagile and/or relatively small interbreeding organisms, chromosome rearrangements might become fixed by random genetic drift and promote the isolation and differentiation of the gene pool involved. This type of chromosomal speciation pattern is well documented in various organisms (White, 1973; 1978; King, 1993).

Gross chromosomal changes are not always a requirement for speciation as there are groups in which there is remarkable similarity of chromosomes between species (Sumner, 1990; King, 1993). For example, in some families of mammals, such as in the family Felidae, certain species underwent only a few chromosomal rearrangements during some 11 million years of their evolution (Johnson *et al.*, 2006). On the other hand, in a few mammal genera, species show extreme interspecific karyotypic diversity. For instance, the genus *Muntiacus* (Mammalia: Cervidae) has species with different karyotypes ranging from n = 3 to n = 23 (Yang *et al*., 1997) and the genus *Sigmodon* (Mammalia: Cricetidae) has an interspecific karyotypic diversity ranging from n = 11 to n = 26 (Zimmerman, 1970; cited in Kandul *et al*., 2007). These types of genera are very important to examine the potential role of chromosome rearrangements in animal speciation, because such types of karyotypically diverse genera tend to have many morphologically similar species (Kandul *et al*., 2007).

Before reaching fixation, however, the chromosomal variants resulting from chromosomal rearrangements exist as polymorphism in the population. As a result, variation between individuals of the same species is not uncommon in mammals. Karyotypic variation within species is a remarkable characteristic and some species show high level of chromosome polymorphism. One of the most described examples is *Mus musculus* (Anderson, 2004).

2.4. IMPORTANCE OF CYTOGENETIC DATA IN SYSTEMATICS

Chromosomal data have the potential to reveal both structural and functional homologies among taxa, which can be utilized in phylogenetic and taxonomic investigations (Dobigny *et al*., 2004). Morphological and cytogenetic characters are important for identification of most species; and particularly, karyotypic analysis is very essential for the identification of cryptic/sibling species, i.e., morphologically similar/identical species that never interbreed (Geise *et al.*, 1998).

Being as discrete hereditary units of mutation responsible for the transmission of the nuclear genome, chromosomes bear a direct evidence of evolutionary history of extant lineages (Dobigny *et al.*, 2004). Due to their Mendelian patterns of inheritance, it is possible to detect synapomorphies (characters that are shared due to common ancestry) and identify sister-group relationships among taxa (Hennig, 1966; cited in Dobigny *et al.*, 2004). These characteristics make chromosomal structural differences powerful markers in modern phylogenetic investigations. According to O'Brien *et al*. (1999), chromosomal changes are discrete events and they offer "a large cadre of cladistic characteristics which combine the advantages of previous molecular and morphological evolutionary tracks."

At present, the taxonomic status of many taxa is unresolved. This is true even for large and obvious animals. Taxonomic uncertainties result mainly from inadequate data. Many species' descriptions are usually based on information obtained from a limited number of morphological traits of unknown genetic bases (Frankham *et al.*, 2004). Morphological definitions of species may have limited connection to genetics or evolution. Some groups of individuals initially appear morphologically indistinguishable but are composed of two or more distinct (cryptic or sibling) species, on the basis of their genetic analysis. Thus, the taxonomy of particular groups of populations can be resolved with sufficient morphological, reproductive and genetic data (Frankham *et al.*, 2004). This would reduce the problems of incorrect lumping of several distinct species into one recognized species and the converse splitting of one species into two or more recognized taxa (Frankham *et al.*, 2004).

Studies have shown that some genera of rodents were incorrectly classified as monospecific genus. This problem was clarified by karyological studies, which revealed sibling or cryptic species (Ortells *et al*., 1988). Various characteristics of chromosome are used as a measure to explain karyoevolutionary trends in a given taxa and chromosomal studies are important to clarify the nature and distribution of chromosomal variants within a species and to determine their taxonomic rank (Granjon, 1996).

Chromosomal change can be a partial or complete barrier to interspecific gene exchange; and chromosomal traits may provide clues to the relationships of species. Due to these major reasons, the diversity of chromosomes within genera has been of major interest in evolutionary studies, and thus many chromosomal studies of genera involve the description of species' karyotypes (Levin, 2002). Karyotype evolution operating in mammals has been known for decades. This knowledge was based on the high diversity of diploid and fundamental numbers among mammalian species; and the existence of similar karyotypes, sparkled with few and distinctive chromosome rearrangements, among closely related species. Comparative analysis based on the two most widely described parameters, the diploid number and fundamental number, are most useful to identify evolutionary trends (de Villena, 2005).

The modern developments of molecular cytogenetics are providing a wealth of new data of enormous taxonomic and evolutionary importance. However, these modern techniques will have to be fully integrated with conventional cytological data in order to exert their full impact. The basic information of chromosomal number and morphology coupled with simple banding techniques and light microcopy analyses are still useful to explain a variety of subjects ranging from cytotaxonomy to karyotype evolution (Affonso *et al.,* 2007).

The results of several molecular and morphological studies on mammalian phylogeny have proven to be in agreement with the results that are obtained from chromosomal phylogeny works (Dobigny *et al*., 2004). For example, the results of molecular and chromosomal phylogeny studies of great apes, family Bovidae, genus *Gazella,* and the African rodent sibling species complexes, *Arvicanthis* and *Acomys,* were found to be congruent (Dobigny *et al*., 2004).

Results obtained from several empirical data clearly show the importance of chromosomal characters for inferring phylogenies (Dobigny *et al.*, 2004), and sometimes, chromosomes may be vitally important characters in cases when morphological and molecular data fail to resolve evolutionary relationships, and particularly so in recently differentiated sibling species (Volobouev *et al*., 2002).

2.5. CYTOLOGY OF RODENT GENERA INCLUDED IN THE PRESENT STUDY

The taxonomy of several taxa of African rodents is chaotic; and the taxonomic revision of many genera is far from completion (Corti *et al.*, 2005). The situation is not different in Ethiopia. Several genera of Ethiopian rodents are also a matter of controversy. Some chromosomal, morphological and molecular studies have been used in Ethiopia to clarify the systematics of such controversial groups and even to describe new species (Afework Bekele *et al.*, 1993; Capanna *et al.*, 1996; Corti *et al.*, 1996; Ducroz *et al.*, 1997; Lavrenchenko *et al.*, 1998; Bulatova and Lavrenchenko, 2005; Corti *et al.*, 2005). Karyological identification studies of rodents in Ethiopia have mainly focused on the highland areas, which harbors high biodiversity and endemism in the country. Even preliminary chromosome analysis has given rich empirical information for the correction of current systematic definitions, interpretation of endemic taxa and evaluation of their possible relationships with the already known karyotypic forms in Ethiopia and abroad (Bulatova and Lavrenchenko, 2005).

In this work, species of rodents belonging to five genera of the family Muridae and one taxonomically yet unidentified rodent specimen are included. The literature available on the chromosome studies of these taxa are briefly reviewed below.

2.5.1. ***Arvicanthis*** (Lesson, 1842)

The genus *Arvicanthis*, the unstriped grass rat, is very common to the South and East of the Sahara and North of the Zambezi (Kingdon, 1974; cited in Corti, *et al*., 1996). It is one of the most successful rodent genera (Afework Bekele *et al*., 1993; Kingdon, 1997). Being as a major agricultural pest and a reservoir for various tropical infections, it is an important genus (Poulet and Poupon, 1978; cited in Ducroz *et al.*, 1997). However, very little is known about the systematics of the group (Afework Bekele *et al*., 1993; Ducroz *et al.*, 1997). Its taxonomy is chaotic, involving cyclic lumping and splitting (Afework Bekele *et al*., 1993). Over the last one or two decades, however, much attention has been given and the number of recognized species has risen to seven (Corti *et al.*, 2005).

Yalden *et al*. (1976) recognized *A. abyssinicus, A. dembeensis, A. blicki* and *A. somalicus,* as four distinct species of *Arvicanthis* occurring in Ethiopia.

The available karyological data for the genus are as follows: *A. somalicus*, 2n = 62 and FNa = 62-63 (Ethiopia; Baskevich and Lavrenchenko, 2000). *A. abyssinicus,* 2n = 62 and FNa = 64 (Ethiopia; Corti *et al.*, 1996). *A. dembeensis,* 2n = 62 and FNa = 62 (Ethiopia; Corti *et al.*, 1996; Tesfaye Dilebo, 2009). *A. blicki,* 2n = 48 and FNa = 64 (Ethiopia; Corti *et al.*, 1996). *A. neumanni*, 2n = 54 and FNa = 62 (Tanzania; Fadda *et al.*, 2001). *A. nairobae*, 2n = 62 and FNa = 78 (Tanzania; Fadda *et al.*, 2001). *A. ansorgei*, 2n = 62 and FNa = 74/76 (Senegal, Mali, Burkina Faso; Volobouev *et al.*, 2002). "*A. niloticus"* complex, 2n = 62 and FNa = 62/64 (Egypt, Sudan, Ethiopia, N. Senegal, N. Burkina Faso, S. Mauritania, Mali, Niger, Chad; Volobouev *et al.*, 1998; 2002; Philippi, 1994; Ducroz *et al.,* 1997; Civitelli *et al.,* 1995; cited in Corti *et al.*, 2005). *Arvicanthis* ANI-6, 2n = 60 and FNa = 72 (Zeway, Ethiopia; Corti *et al.*, 2005).

2.5.2. *Mastomys* (Thomas, 1915)

*Mastomys***,** the multimammate rat, is widespread across sub-Saharan Africa (Lavrenchenko *et al*., 1998). It is a serious problem of agriculture and human health (Singleton *et al*., 1999). The genus was mentioned as comprising eight species (Kingdon, 1997), and later on Lavrenchenko *et al*. (1998) described a new species, *M. awashensis,* from Awash Valley, Ethiopia. The taxonomy of species of this genus has been a matter of controversy for a long time and the situation was reported to be the same in Ethiopia. All Ethiopian *Mastomys* were initially lumped under *M. natalensis,* and later on three different species, *M. natalensis, M. erythroleucus* and *M. awashensis,* were reported to occur in Ethiopia (Lavrenchenko *et al.*, 1998).

The available cytogenetic data on this genus are as follows. *M. natalensis,* 2n=32 and FNa=52-54 and this is the most representative species occurring in sub-Saharan Africa (Lavrenchenko *et al.*, 1998; Fadda *et al.,* 2001; Corti *et al.,* 2005; Tesfaye Dilebo, 2009). *M. huberti*, 2n = 32 and FNa = 44 - 46, and its range is restricted to Mauritania, Mali, Burkina Faso and Senegal (Granjon *et al.,* 1997). *M. coucha,* 2n = 36 and FNa = 52-54 (Southern Africa; Lyons *et al.,* 1980; Green *et al.,* 1980; cited in Corti *et al.,* 2005). *M. erythroleucus,* 2n = 38 and FNa = 40-60, and it ranges from Senegal to Ethiopia and Uganda (Corti *et al.,* 2005). *M. shortridgei,* 2n = 36 and FNa = 50, occurs in the extreme N-W of Botswana and in N-E Namibia (Gordon, 1995; cited in Corti *et al.,* 2005). *M. hildebrandtii,* 2n = 32 and FNa = 50-54 (Kenya; Qumsiyeh, 1990; cited in Corti *et al.,* 2005). *M. awashensis,* 2n=32 and FNa=50-54 (Ethiopia; Lavrenchenko *et al.*, 1998; Corti *et al.,* 2005).

2.5.3. ***Stenocephalemys*** (Frick, 1914)

Taxonomically, the genus *Stenocephalemys* has been considered as a confused group. It was included in the genera of *Praomys, Mastomys, Hylomyscus, Colomys* and as a separate genus, *Stenocephalemys.* At present, the genus includes the three Ethiopian endemic species *S. albocaudata, S. griseicauda* and *S. albipes* (Musser and Carleton, 2005).

The available karyological data for *S. albocaudata,* 2n = 54 and FNa = 60, *S. griseicauda,* 2n = 54 and FNa = 54 (Corti *et al.,* 2005) and *S. albipes,* 2n = 46 and FNa varying from 50 to 53 across different localities of Ethiopia (Corti *et al.,* 2005; Tesfaye Dilebo, 2009).

2.5.4. ***Mus*** (Linnaeus, 1758).

The genus *Mus,* a very variable group of mice, comprises 20 species. They can be found almost in all vegetation types and all altitudes (Kingdon, 1997). Within the *Mus musculus* species, several subspecies diverged from each other; and several chromosomal races diverged from the *Mus musculus domesticus* branch (Nachman *et al*., 1994).

Recently available karyotypic data for *Mus musculus domesticus* are 2n=40 and FNa=38 (Turkey**:** Gozcelioglu *et al*., 2005; Iran**:** Yigit *et al.*, 2006). *Mus mahomet,* 2n=36 and FNa=34 (Ethiopia**:** Tesfaye Dilebo, 2009).

Subgenus *Nannomys* (Peters, 1876)

The subgenus *Nannomys* (*Leggada*) represents one of the major taxonomic puzzles. The African species of this subgenus are also known by their fast rate of speciation often associated with chromosomal rearrangements (Corti *et al.,* 2005). The 19 species, which are listed in the subgenus by Musser and Carleton (2005), is likely to increase due to the occurrence of cryptic and chromosomal species (Corti *et al.,* 2005). The ancestors of these monophyletic groups are thought to have migrated from Asia through Iraq, Iran, and Saudi Arabia to Ethiopia (Jotterand, 1972; cited in Corti *et al.,* 2005).

The recognition of *Nannomys* as a separate genus is far from being resolved, though the dichotomy between *Nannomys* and *Mus* is no doubt (Corbet, 1990; cited in Corti *et al.,* 2005).

The available karyotypes are as follow: *Nannomys sp.* Rongai-A, 2n=22 and FN=36 (Kenya); *Nannomys sp.*, 2n=25 and FN=36 (Zambia); *Nannomys procondon,* 2n=36 and FNa=34 (Ethiopia); *Nannomys emesi*, 2n=36 and FN=36 (Kenya); *Nannomys sp.* Rongai-B, 2n=36 and FNa=34 (Kenya) (Corti *et al.,* 2005).

3. OBJECTIVES OF THE STUDY

3.1. GENERAL OBJECTIVE

To investigate the karyology of some species of murid rodents from around Addis Ababa city and Huruta town.

3.2. SPECIFIC OBJECTIVES

The specific objectives are:

i. To document the chromosome number and the autosomal fundamental number (FNa) of the species.
ii. To characterize and describe the karyotypes of the species.
iii. To investigate karyotypic differences and/or similarities among the species.
iv. To compare the karyotypes of the species with karyotypes reported for them and for their close relative species by previous studies.

4. MATERIALS AND METHODS

4.1. SPECIMENS COLLECTION

In this study, a total of 20 rodent specimens (12 males and 8 females) were collected from Arsi-Huruta, Menagesha-Suba, Holeta-Gunteta, Entoto, Sebeta town and Addis Ababa.

The localities were selected based on the available budget and facility for the study and their convenience for transportation. Their proximity to AAU is safe for bringing the animals alive to Genetics Laboratory, at Science Faculty of AAU.

All specimens were live collected by using Sherman live traps (baited with peanut butter mixed with barley flour) and transported to AAU, Science Faculty, Genetics Laboratory, for chromosome preparation and taxonomic identification.

Table 1. Number of rodent specimens collected and their collection sites

Sites of Collection	Number of Specimens Collected	Male	Female
Menagesha-Suba …. Forest	**4**	**3**	**1**
Holeta (Gunteta) …. Near Farmland	**4**	**2**	**2**
Entoto …. Forest	**4**	**1**	**3**
Arsi (Huruta) …. Riverine-Forest	**2**	**2**	**-**
Sebeta …. Store	**3**	**2**	**1**
Addis Ababa …. Residence House	**3**	**2**	**1**

4.2. SOMATIC METAPHASE CHROMOSOME PREPARATION

4.2.1. Pretreatments and Air-dry Slide Preparation

Metaphase chromosomes were prepared from the bone marrow, following Hsu and Patton (1969), with the injection of colchicine (*in vivo*).

- Each live animal was intraperitoneally injected with 0.05 % colchicine at 0.01 ml/gram of body weight to arrest the cell division at the metaphase stage (Ford and Hamerton, 1956).
- After about an hour, the animal was killed by overetherization and the femur bone was dissected out and cleared from flesh.
- Then, the bone was chipped with a bone cracker to release the bone marrow cells into about 3ml of 0.075M KCL hypotonic solution in a Petri dish. Then the cell suspension was pipetted into a centrifuge tube and incubated for about 20 minutes.
- The cell suspension was centrifuged for about 5 minutes at 1000 rpm. The supernatant fluid was discarded and the cell pellet resuspended in 3ml of freshly prepared fixative (3:1 methanol/glacial acetic acid) and allowed to fix for about 20min.
- Again the cell suspension was centrifuged for about 5 minutes and the supernatant decanted, cells resuspended in 3ml of the fixative and allowed to fix for about 20 minutes and centrifuged for 5 minutes. This was repeated one or more times to obtain satisfactory fixation of the chromosomes.
- After the final centrifugation, the pellet was resuspended in 1ml of the fixative, a portion was drawn with a pipette, and two or three drops were splashed onto a clean glass slide from a height of about half a meter. While the drops were splashed, the slide was inclined to about 45 degrees to allow cells to spread by running down the slide.
- The slides were allowed to air dry until ready for staining. The air-dried slides were stained for about 30 minutes in Giemsa's solution in a phosphate buffer (pH = 6.8).
- The stained slides were rinsed in distilled water, air dried, and mounted with a 22 x 50mm cover slip using DEPEX.
- An average of 10 slides were prepared for each specimen, and well–spread metaphase cells from each preparation were photographed by using camera fitted microscope with a magnification of x1000 (x100 objective and x10 eyepiece).

4.2.2. Chromosome Analysis

The morphology of chromosomes, the diploid number (2n) and the number of autosomal arms (FNa) were determined by examining photograph of the chromosomes. The karyogram was constructed on the basis of at least three enlarged photographic copies of each animal. Chromosomes were classified as biarmed and uniarmed; and to assist in the description of karyotypes, arm ratios have been determined for biarmed chromosomes based on measurements of enlarged chromosome prints. In designating chromosomes as metacentric, submetacentric, acrocentric and telocentric, Levan *et al.* (1964) was followed with some modifications. In this study, contrary to the reverse usage of the terms acrocentric and telocentric by some rodents cytogeneticists, the term acrocentric refers to chromosomes with the centromere close to one end, so that one arm is distinctly shorter than the other; and the term telocentric is used for chromosomes with the centromere at the end, so that there is no cytologically detectable second arm. In addition, in the counts of FNa; each biarmed chromosome is counted as two; and each telocentric is counted as one.

4.3. SPECIMENS IDENTIFICATION

During chromosome preparations, after the femur bone was dissected out, the skins were prepared and treated with 0.8% formaldehyde solution for the purpose of species identification and for future deposit in the Zoological Natural History Museum of Addis Ababa University.

Specimens were identified taxonomically by comparing their skin with voucher specimens deposited in the Zoological Natural History Museum of Addis Ababa University. This was further confirmed by karyological analysis and comparison with karyological data available in the literature.

5. RESULTS

5.1. THE TAXONOMICALLY IDENTIFIED SPECIMENS

All the specimens of the present study, except for the one from Arsi-Huruta, were identified to species level. Accordingly, five species belonging to five different genera of the family Muridae have been identified (Table 2).

In the present study, the specimen from Arsi-Huruta was not identified to species level and designated as *ARSI-X*.

Table 2. The taxonomically identified specimens and their sites of collection

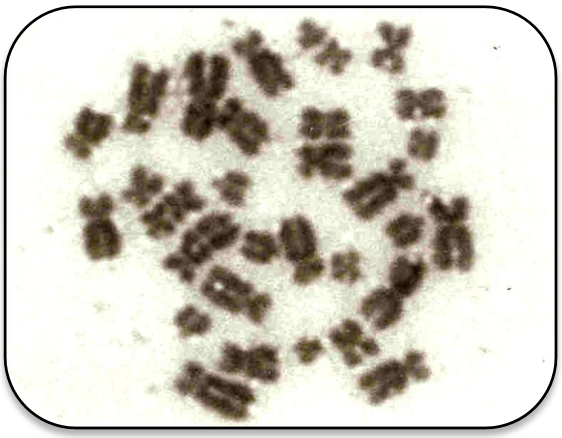
Genera	Species	Collection Site
Stenocephalemys	***S. albipes***	**Menagesha-Suba**
Arvicanthis	***A. abyssinicus***	**Entoto**
Mastomys	***M. natalensis***	**Holeta /Gunteta**
Rattus	***R. rattus***	**Sebeta**
Mus	***M. musculus***	**Sebeta and Addis Ababa**

5.2. KARYOLOGICAL RESULTS

Following, the karyological results of the present study are presented.

5.2.1. *Mastomys natalensis*

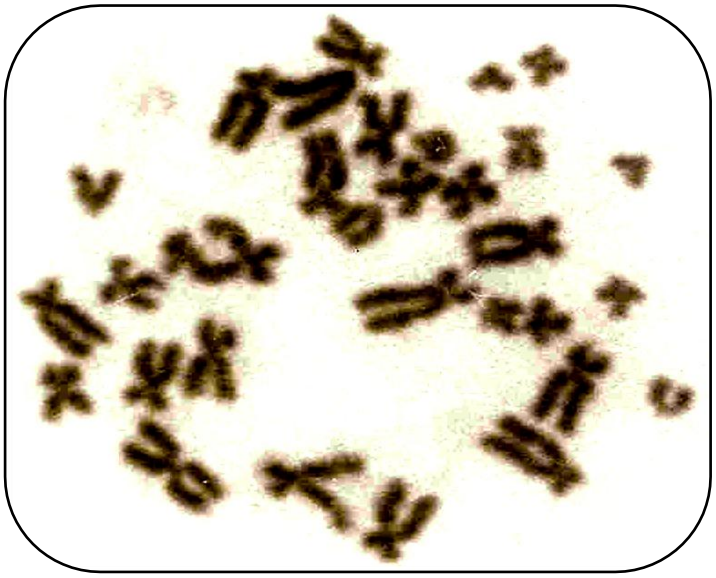
The diploid number is 2n = 32 and FNa = 56. The karyotype is consisting of 12 pairs of biarmed autosomes of gradually decreasing size, plus a very small pair (pair 15) with tiny second arm and two pairs of small telocentrics (Figure 1 and 2). Among the 12 pairs of biarmed autosomes, the first seven pairs are large to medium sized submetacentrics followed by five pairs, consists of two pairs of medium sized and three pairs of small sized metacentrics. The smallest chromosome pair (pair № 15), with a very short second arm, apparently looks like submetacentric in the female (Figure 1B) and subtelocentric in the male specimen (Figure 2B). As shown in Figure 1(female) and Figure 2 (male), the X-chromosome is a large metacentric and the Y-chromosome (Figure 2) is a large telocentric. Arm ratio data for the biarmed chromosomes are presented in Table 3.



A

B

Figure 1. Metaphase chromosome spread (**A**) and karyotype (**B**) of a female *M. natalensis*.



A

B

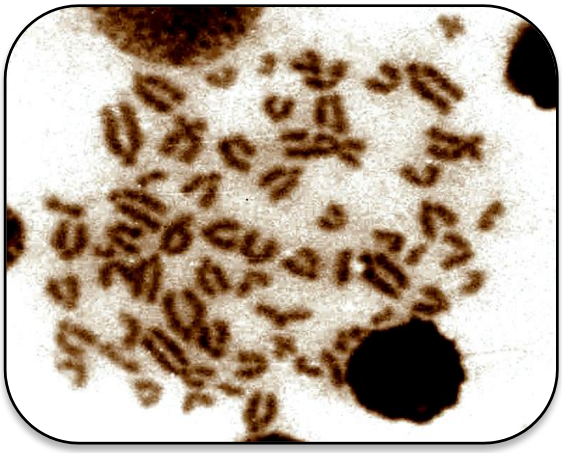
Figure 2. Metaphase chromosome spread (**A**) and karyotype (**B**) of a male *M. natalensis.*

Table 3. Arm ratio measurements of biarmed chromosome pairs of *M. natalensis.*

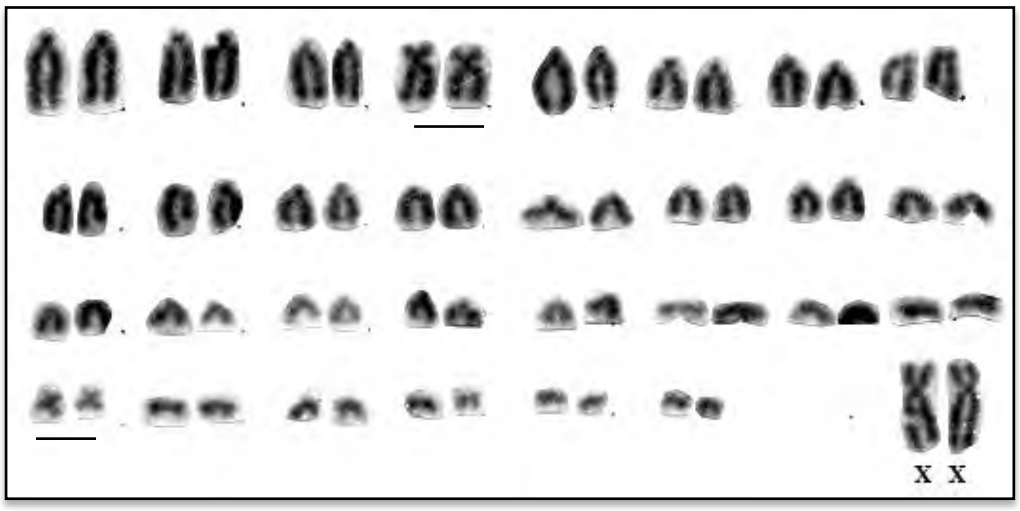
Pair №	1	2	3	4	5	6	7	8	9	10	11	12	X
Arm Ratio (l/s)	2.67	2.50	2.33	2.17	2.00	1.83	2.00	1.17	1.00	1.20	1.00	1.00	1.09
Nomenclature	sm	sm	sm	sm	sm	sm	sm	m	m	m	m	m	m

5.2.2. *Arvicanthis abyssinicus*

The diploid number is 2n=62 and FNa=64. The karyotype is composed of 28 pairs of uniarmed and two pairs of biarmed autosomes. The size of the autosomes is gradually decreasing from a large to small size chromosomes. As shown in Figure 3, except for chromosome pair № four, which is a medium sized submetacentric; and chromosome pair № 25, which is a small sized metacentric; all the rest of the autosomes are telocentrics. The X-chromosome is a large submetacentric. The biarmed chromosomes have arm ratios of 2.60, 1.00 and 2.57, which designate them as **sm**, **m** and **sm**, respectively. The high arm ratio of 2.60 and 2.57 places these chromosomes (pair 4, and X-chromosomes, respectively) closer to acrocentric classes.



A



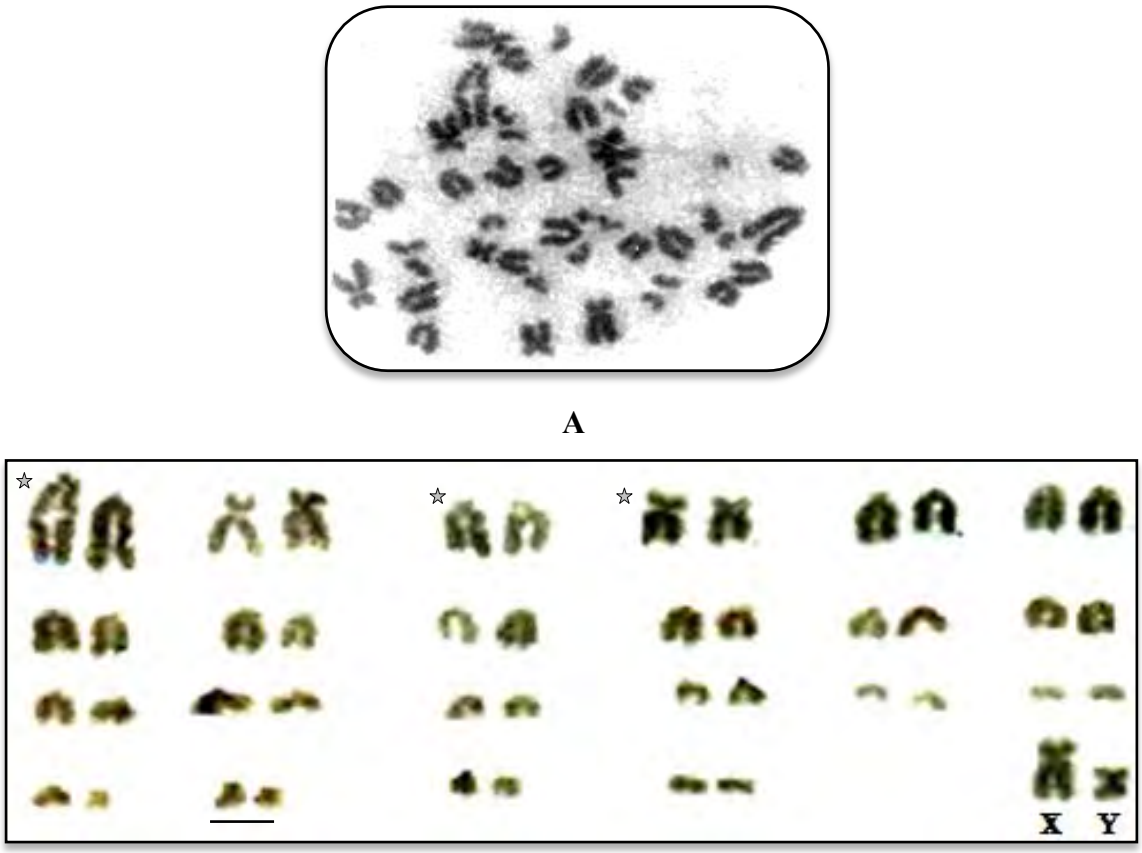
B

Figure 3. Metaphase chromosome spread (**A**) and karyotype (**B**) of a female *A. abyssinicus*.

Biarmed autosomal chromosomes are underlined.

5.2.3. *Stenocephalemys albipes*

The diploid number is 2n=46 and FNa=52. The karyotype of a male specimen is presented in Figure 4. The 22 pairs of autosomal chromosomes are gradually decreasing in size from large to very small. The autosomes are consisting of 18 pairs of telocentrics and these include the largest pair in the complement. The biarmed chromosomes are consisting of two pairs of submetacentric chromosomes of medium size (pair 2 & 4), and one pair of small metacentric (pair 20) chromosomes. In addition, pair № three appears subtelocentric types of biarmed chromosome. Both sex chromosomes are biarmed, with the X-chromosome being a large submetacentric and the Y being a small metacentric chromosome.



A

B

Figure 4. Metaphase chromosome spread (**A**) and karyotype (**B**) of a male *S. albipes*.

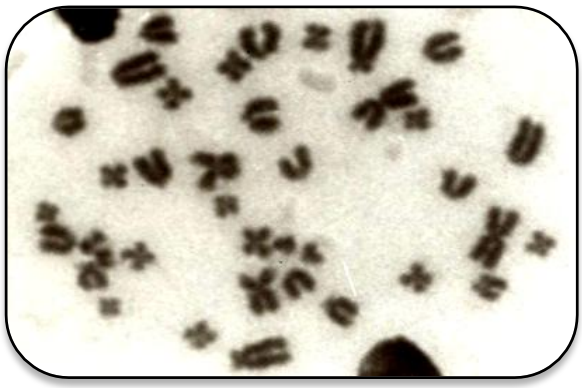
The underlined chromosome is a small metacentric pair.Chromosome pairs that differ from previous reports are indicated by an asterisk.

Table 4. Arm ratio measurements of biarmed chromosome pairs of *S. albipes*.

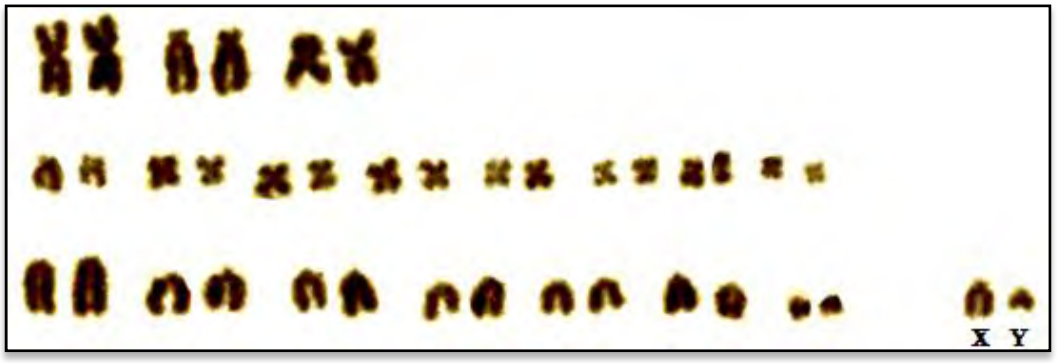
Pair №	2	3	4	20	X	Y
Arm ratio (l/s)	2.20	5.50	2.00	1.00	2.20	1.00
Nomenclature	sm	st	sm	m	sm	m

5.2.4. *Rattus rattus*

The chromosomes of a male specimen are presented in Figure 5. The diploid number is 2n=38 and FNa=58. On the basis of centromeric position, the chromosomes are consisting of three groups – metacentrics, acrocentrics and telocentrics. The metacentrics include the largest pair of the complement (pair № 1) a medium size chromosome (pair № 3) and seven pairs of small chromosomes (pairs 5-11). Two pairs of acrocentrics are present which consists of a medium sized pair (pair 2) and small sized (pair 4) chromosomes. The telocentrics are consisted of seven pairs of medium to small size (pairs 12-18) chromosomes. Both sex chromosomes are small sized telocentrics, with the X-chromosome being relatively larger than the Y-chromosome.



A



B

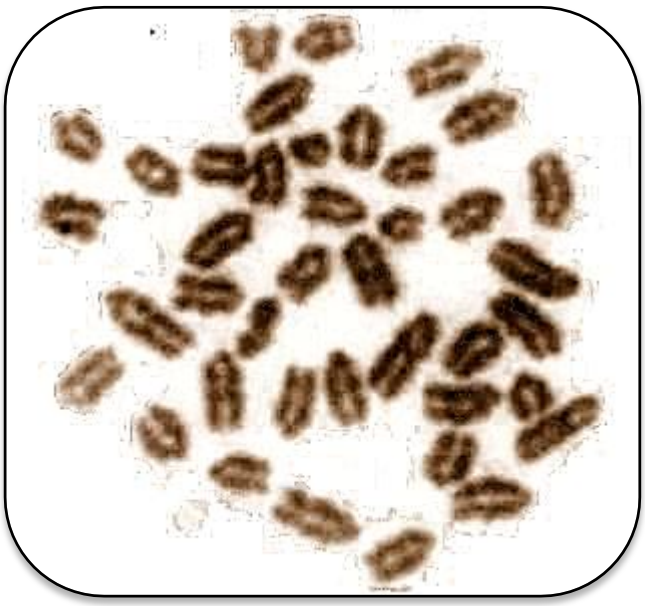
Figure 5. Metaphase chromosome spread (**A**) and karyotype (**B**) of a male *Rattus rattus*

Table 5. Arm ratio measurements of biarmed chromosome pairs of *Rattus rattus*

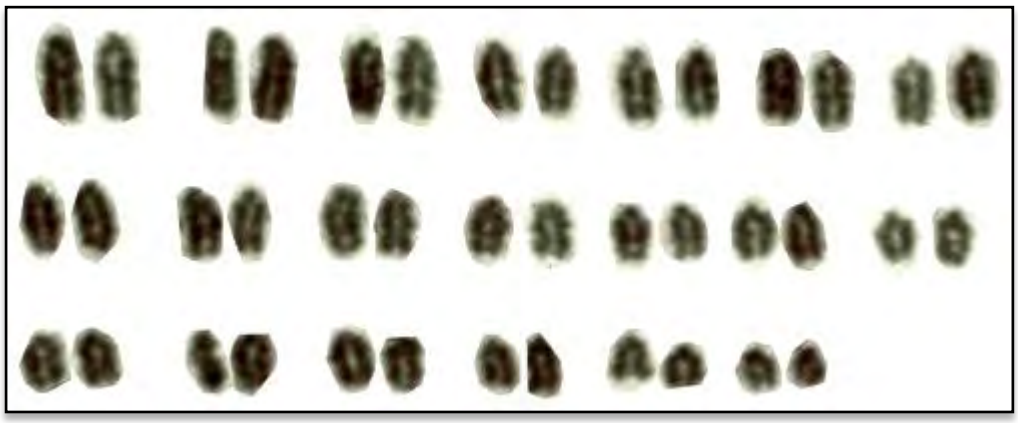
Pair №	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Arm ratio (l/s)	1.10	3.25	1.17	3.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Nomenclature	m	ac	m	ac	m	m	m	m	m	m	m

5.2.5. *Mus musculus*

The diploid number is 2n=40 and FNa=38. The entire chromosome complement is composed of telocentric chromosomes. As shown in Figure 6 for a female and Figure 7 for a male specimen, the chromosomes show gradual gradation in length. As could be seen from a male karyotype, there is no easily distinguishable heteromorphic pair of chromosomes. A pair that apparently looks like heteromorphic, i.e. sex chromosomes pair is placed at the right-hand corner of Figure 7.

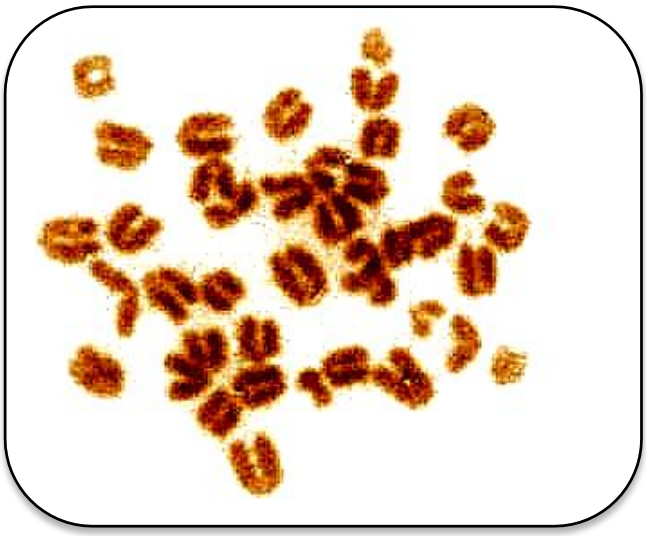


A

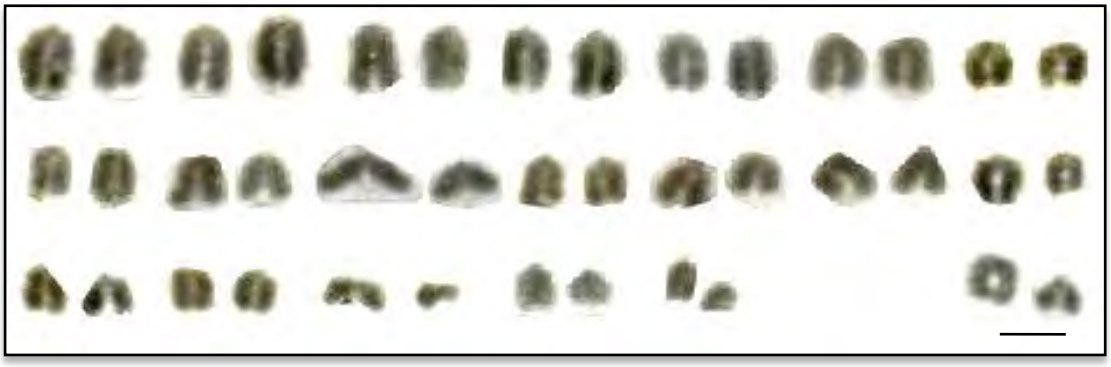


B

Figure 6. Metaphase chromosome spread (**A**) and karyotype (**B**) of a female *M. musculus.*



A



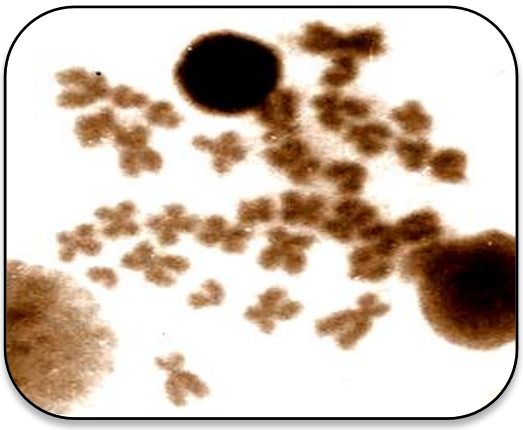
B

Figure 7. Metaphase chromosome spread (**A**) and karyotype (**B**) of a male *Mus musculus.*

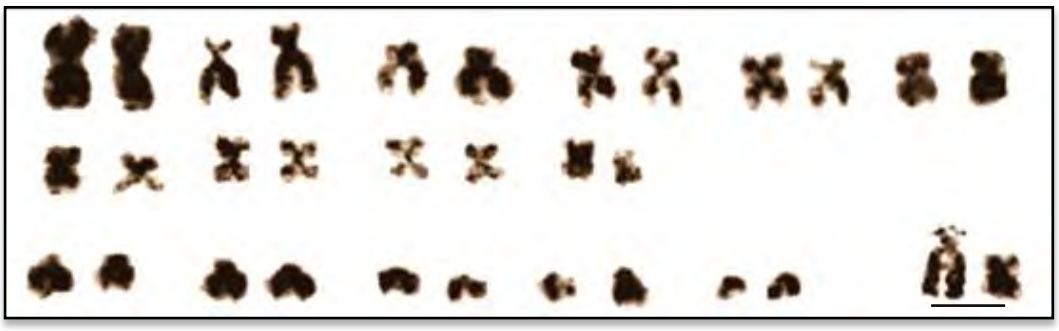
The underlined chromosomes are presumed to represent heteromorphic sex chromosome pair.

5.2.6. *ARSI-X specimen*

This male specimen was captured near Huruta town in Arsi region. The diploid number is 2n=32 and FNa=50. The autosomes are composed of 10 pairs of biarmed and five pairs of telocentric chromosomes (Figure 8). Among the biarmed, the largest pair of the complement (pair 1) plus seven pairs of medium to small-sized chromosomes (pairs 4-10) are metacentrics. The remaining two pairs of biarmed chromosomes (pair № 2 and3) are submetacentrics. The telocentrics make five pairs (pairs 11-15) of small-sized chromosomes. The heteromorphic pair, which is supposed to be the sex chromosomes, consists of a medium sized submetacentric/acrocentric and a small metacentric chromosomes. Possibly, the former is the X, and the latter is the Y-chromosome.



A



B

Figure 8. Metaphase chromosome spreads (**A**) and karyotype (**B**) of a male specimen tentatively designated as *ARSI-X.*
The heteromorphic sex chromosome pair is underlined**.**

Table 6. Arm ratio measurements of biarmed chromosome pairs of *ARSI-X specimen.*

Pair №	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**X**	**Y**
Arm ratio (l/s)	**1.17**	**1.86**	**2.20**	**1.25**	**1.67**	**1.67**	**1.14**	**1.00**	**1.00**	**1.00**	**3.00**	**1.20**
Nomenclature	**m**	**sm**	**sm**	**m**	**m**	**m**	**m**	**m**	**m**	**m**	**sm**	**m**

Table 7. Summary of chromosome cytology data of rodent species of the present study.

Species	Chromosome Numbers		Number of Chromosomes in Each Class of Centromeric Position					Centromeric Position of Sex Chromosomes	
	2n	FNa	m	sm	ac	st	t	X	Y
M. natalensis	32	54/56	10	14/16	-/2	-	4	m	t
A. abyssinicus	62	64	2	2	-	-	56	sm+sm	-
S. albipes	46	52	2	4	-	2	36	sm	m
R. rattus	38	58	18	-	4	-	14	t	t
M. musculus	40	38	-	-	-	-	38	t	t
ARSI-X	32	50	16	4	-	-	10	sm/ac	m

N. B. **m** = metacentric, **sm** = submetacentric, **ac** = acrocentric, **st** = subtelocentric and **t** = telocentric.

N. B. The present:

m (r = 1.00 – 1.70) is equivalent to Levan's *et al.* (1964) **M** and **m**.

sm (r = 1.70 – 3.0) is equivalent to Levan's *et al.* (1964) **sm**.

ac (r = 3.00 – 5.00) and **st** (r = 5.00 – 7.00) are equivalent to Levan's *et al.*, (1964) **st.**

t (r = 7.00 - ∞) is equivalent to Levan's *et al.* (1964) **t** and **T**.

6. DISCUSSION

Mastomys natalensis (Smith, 1834)

The available cytogenetic data for *M. natalensis* show 2n=32 and the FNa varying from 52 to 54 across the distribution range of sub-Saharan Africa. Corti *et al.* (2005) reported a karyotype of 2n=32 and FNa=52/54 (FNa=54 being the common), for specimens of different countries of East Africa: Kenya, Tanzania and Zambia. The most common type of karyotype, 2n=32 and FNa=54, has also been reported for specimens from Tanzania by Fadda *et al.* (2001) and from Ethiopia by Lavrenchenko *et al.* (1998); and Tesfaye Dilebo (2009). These studies agreed that the autosomes are consisting of 12 pairs of biarmed chromosomes of decreasing size and three pairs of small acrocentrics [telocentrics]. However, none of them reported an FNa =56.

In the present study, the karyotype of *M. natalensis* (Figure 1 and 2) was found to contain 2n = 32 and FNa = 56 (Figure 1 and 2). The metacentric X and the telocentric Y- chromosomes are both large and are in agreement with previous reports. However, in the present study, the smallest chromosome pair (pair № 15) has a short second arm which can be described as submetacentric/acrocentric. The second arm is better distinguishable in the karyotype of the female specimen (Figure 1). Lavrenchenko *et al.* (1998) also mentioned the presence of very short arms in this chromosome pair, but did not consider the second arm in the calculation of FNa. Relative to the total chromosome size, we think that the second arm of this pair is large enough to be considered when FNa is calculated. Therefore, the present result of the autosomal fundamental number, FNa =56, was found to differ from the previous reports of FNa =54.

Arvicanthis abyssinicus (Ruppell, 1842).

In this study, the karyotype of *A. abyssinicus* was found to contain the diploid number of 62 and the autosomal fundamental numbers of 64 (Figure 3). This karyotype is in agreement with the karyotype description of a specimen of *A. abyssinicus* from Sululta by Corti *et al*. (1996). In addition, the karyotype of *Arvicanthis abyssinicus* was found to be similar to the karyotype description of *Arvicanthis dembeensis* by Corti *et al*. (1996) and Capanna *et al*. (1996), except for chromosome pair number 4 (Figure 3), which is submetacentric in *Arvicanthis abyssinicus* and telocentric in *Arvicanthis dembeensis.* According to Corti *et al*. (1996), this difference was originated due to a pericentric inversion.

Capanna *et al*. (1996) and Corti *et al*. (1996) reported that the X-chromosome of *A. abyssinicus* shows variability in size and shape and can have either submetacentric or subtelocentric configurations. In the present study, the X-chromosome was found to be a large submetacentric.

Stenocephalemys albipes (Ruppell, 1842)

Corti *et al.(*2005) reported *Stenocephalems albipes* (from Mugo-Ethiopia) to contain diploid number 2n = 46, and FNa = 50-53. According to this report, all the karyotypes are composed of four pairs of large biarmed, one pair of small biarmed and 17 pairs of uniarmed autosomes. Tesfaye Dilebo (2009) for a female specimen from around Hossena town reported a karyotype with 2n=46 and FN=54.

The same diploid number of 2n=46 and the autosomal fundamental number FNa=52, which is within the range of the previously described (50-53), was found in the current study (Figure 4). However, contrary to Corti *et al. (*2005), who reported the presence of four pairs of large biarmed chromosomes, in the present study, only three pairs of large biarmed autosomal chromosomes were found (pair № 2 - 4).

Furthermore, the karyogram representations of *S. albipes* by Corti *et al. (*2005) and Tesfaye Dilebo (2009) showed chromosome pair № 4 as being metacentric. Instead, this was found to be submetacentric in the present study, indicating the presence of polymorphism for this pair in addition to the polymorphism previously reported for pairs 1 and 3.

According to Corti *et al. (*2005), the chromosome pair numbers 1 and 3 are polymorphic, i.e. they are found either as telocentric or submetacentric because of a pericentric inversion. In the present study, only the telocentric form of chromosome № 1 was found. Regarding chromosome pair № 3, short second arm was observed which could be considered as a subtelocentric type. This shows that there exist three polymorphic forms of this pair – submetacentric, subtelocentric and telocentric.

Moreover, the karyogram provided by Corti *et al. (*2005) showed heteromorphism of pair № 1, which consisted of a telocentric and a submetacentric chromosomes. In the present study size heteromorphism rather than morphological heteromorphism was observed, which was also shown in the work of Tesfaye Dilebo (2009). However, further studies need to be done to verify whether the observed size difference is true polymorphism or the result of different condensation of homologous chromosomes.

With regard to the sex chromosomes, the X-chromosome was found to be a large submetacentric and the Y-chromosome is a small size metacentric of about half the size of the X-chromosome. This is in agreement with the work of Corti *et al.* (2005).

To sum up the comparison, in the work of Corti *et al.* (2005), pair № 1 showed morphological polymorphism, while it showed size polymorphism in the present study. Corti *et al.* (2005) reported that pair № 3 is polymorphic, being either [telocentric] or submetacentric and it was also found as submetacentric in the work of Tesfaye Dilebo (2009) but, in the present study it was a subtelocentric type. Chromosome pair № 4 was found being metacentric in both the works of Corti *et al.* (2005) and Tesfaye Dilebo (2009), while being submetacentric in the present study. This implies that, in this species, not only pair № 1 and 3 but pair № 4 is also polymorphic with regard to morphology.

Rattus rattus (Linnaeus, 1758)

We have not found previous reports on the karyotype of *Rattus rattus* specimens from Ethiopia, except that of Tesfaye Dilebo's (2009) for a specimen from Hosanna for which a diploid number of 2n = 38 and FNa = 60 were reported. Yigit *et al*. (1998) reported a specimen from Turkey with the diploid number 2n = 38 and FN = 60. The result of the present study (Figure 5), 2n =38 and FNa = 58, agreed with the results of Yigit *et al*. (1998). However, the autosomal fundamental number of the present study was found to be different from that of Tesfaye Dilebo (2009). Both the sex chromosomes are telocentrics, with X slightly larger than Y, which is in agreement with the report of Tesfaye Dilebo (2009) and Yigit *et al*. (1998).

Mus musculus (Linnaeus, 1758)

Since early studies, it has been known that metaphase chromosomes of the house mouse are very difficult for detailed analysis due to their small size, gradual decrement in size and strictly terminal centromeres. Because of this, arranging chromosomes into pairs during karyotype construction are prone to errors or mismatches (Bennett, 1965). In this study, we have also faced these difficulties in arranging chromosomes in pairs and in identifying the sex chromosomes of the male animal, since a morphological dimorphism pair is not evident. A pair that apparently looks like a heteromorphic chromosome pair, i.e. sex chromosomes, is placed at the lower right-hand corner of (Figure 7B).

Previous works on the karyotype of specimens of Ethiopian *Mus musculus* was not also found. The karyological reports on specimens from Turkey by Gozcelioglu *et al.* (2005) and from Iran by Yigit *et al.* (2006), have both showed that 2n=40, FNa=38, FN=40; and the sex chromosomes are both telocentrics, X being larger than Y. These results are in agreement with an entirely telocentric chromosome complement description of the present study (Figure 6 and 7). Despite the abundant reports on their rapid karyotype evolution; and the presence of wide karyotype diversity, these results showed karyotype conservations across wide geographic areas.

ARSI-X specimen

For most of the specimens used in the present study, taxonomic identification was done to the species level by comparing the skin of the specimens with voucher specimens deposited in the Zoological Natural History Museum of Addis Ababa University, and the chromosome data with published karyotypes. However, one specimen, which was captured from Arsi (Huruta) in a riverine forest habitat, was not possible to identify as its karyotype does not match with any of the karyotypes reported for Ethiopian murids, and it was tentatively designated as *ARSI-X*.

Morphologically, the specimen looked like the voucher specimens of *Mastomys* species. Like members of this genus, it has soft, short fur, dark grey upper part, pale grey (white) lower side, and 8.5cm of body length and 8cm of tail length. But, the analysis of its karyotype (Figure 8) showed that it has a different karyotype from what have been described for Ethiopian *Mastomys* species: *M. natalensis* (2n=32, FNa=54), *M. erythroleucus* (2n=38, FNa=52-53) and *M. awashensis* (2n=32, FNa=54), whereas *ARSI-X* specimen has 2n=32 and FNa=50.

When compared, the karyotypes of *ARSI-X* specimen and *M. erythroleucus* differ from each other both in 2n number and autosomal fundamental number.This specimen has the same 2n=32 as *M. natalensis* and *M. awashensis*, but differs from both by FNa as well as by the ratios and types of biarmed chromosomes. Both the karyotypes of *M. natalensis* and *M. awashensis* are composed of FNa=54, though they differ in chromosomes morphology (Lavrenchenko, *et al.,* 1998). They both have 12 pairs of biarmed autosomes with decreasing size from large to medium size and three pairs of small uniarmed autosomal chromosomes. The karyotypes of *M. natalensis* and *M. awashensis* are distinguished from each other by: (1) the prevalence of large metacentrics in the karyotype of *M. awashensis*, while submetacentrics are prevalent in *M. natalensis.* (2) The Y-chromosome is a small submetacentric in *M. awashensis*, while it is a large telocentric in *M. natalensis* (Lavrenchenko, 1998). When the karyotype of *ARSI-X* specimen compared with those of *M. awashensis* and *M. natalensis*, *ARSI-X* specimen has 10 pairs of biarmed and five pairs of telocentric autosomes, whereas the karyotypes of *M. natalensis* and *M. awashensis* contain 12 pairs of biarmed chromosomes and three pairs of telocentrics. This specimen has less number of biarmed and more number of telocentrics than the two species. Even among the biarmed chromosomes, in *M. natalensis,* seven pairs are large submetacentrics and five pairs are metacentrics of medium to small size. In *M. awashensis,* two pairs of large and four pairs of medium size chromosomes are submetacentrics, four pairs of large to medium size and one pair of small size chromosomes are metacentrics; and one pair is a small subacrocentric. In the case of the *ARSI-X* specimen, eight pairs are metacentrics of large to small size and two pairs are large submetacentrics.

With regard to sex chromosomes, X is a large metacentric in both *M. natalensis* and *M. awashensis;* and Y is a large telocentric in the former, and a small submetacentric in the latter. In *ARSI-X* specimen, however, as in *M. awashensis* both sex chromosomes are biarmed consisting of a large submetacentric/acrocentric chromosome (possibly X) and a small metacentric (possibly Y), where most probably the former is X and the latter is Y chromosome. Since we did not have a female specimen, it is not possible to distinguish the X and Y-chromosomes. However, on the basis that, generally, in eutherian mammals the X-chromosome is larger than Y (Sumner, 2003), it can be tentatively concluded that the larger chromosome of the heteromorphic pair is X.

To sum up, metacentrics and telocentrics are more prevalent in *ARSI-X* specimen, whereas the total biarmed autosomes in general and submetacentrics in particular are prevalent in *M. natalensis* and *M. awashensis,* with the associated clear difference in FNa.

The taxonomy of the African genus *Mastomys* has been controversial for a long time (Corti *et al*., 2005) and the need of a careful taxonomic revision has been suggested (Musser and Carleton, 1993). They are distinguished from each other by chromosomal and biochemical characters and the data on their relationships and peripheral distribution are heavily uncertain (Lavrenchenko *et al.,* 1998). The four closely related and weakly separable species: *M. natalensis, M. erythroleucus, M. hildebrandtii* and *M. coucha* are phylogenetically closely related, and constitute a presumably a monophyletic group called the *M. natalensis* species complex, while the taxonomical and systematic position of other taxa of the genus is still unresolved (Granjon *et al.,* 1997; Lavrenchenko *et al.,* 1998; Lecompte *et al*., 2002).

At present three species of *Mastomys* are recognized to occur in Ethiopia: *M. natalensis, M. erythroleucus* and *M. awashensis.* The taxonomic situation of Ethiopian *Mastomys* is also a matter of discussion (Lavrenchenko *et al.,* 1998). Morphological and cytogenetic studies on *Mastomys* have shown the possibility of the occurrence of sibling and/or new cryptic species (Lavrenchenko *et al.,* 1998; Volobouev *et al*., 2001; 2002).

As described above, this taxonomically unidentified specimen shares a large similarity both morphologically and karyotypically with the identified species of the genus *Mastomys*, and yet shows karyotypic distinctness. On these bases, it can tentatively be concluded that the *ARSI-X* specimen belongs to the genus *Mastomys.* It can also be suggested that this specimen could probably be a new sibling or cryptic species. However, it is obvious that there is a need for further morphological and cytogenetic studies to determine its taxonomic position with certainty.

7. CONCLUSION AND RECOMMENDATIONS

7.1. CONCLUSION

The results of the present study showed diploid chromosome numbers ranging from 2n=32 (*Mastomys natalensis* and *ARSI-X* specimen) to 2n=62 (*Arvicanthis abyssinicus*) and autosomal fundamental number that ranged from FNa=38 (*Mus musculus*) to FNa=64 (*Arvicanthis abyssinicus*). The chromosome morphologies have also shown high variation from being all telocentrics in *Mus musculus*; predominantly being telocentrics in *Stenocephalemys albipes* and *Arvicanthis abyssinicus;* being almost partially biarmed and partially uniarmed in *Rattus rattus*; to predominantly being biarmed in *Mastomys natalensis* and *ARSI-X* specimen. These show high karyotypic diversity among Ethiopian murid rodents.

The karyotypes of most of the species of the present study showed similarity with what have previously reported for the species from different localities of Ethiopia, and even from abroad, across a wide geographic area.

The present study has also described a new karyotype which implies the existence of the yet unrecognized (cryptic) species of rodents in the Ethiopian rodents fauna.

The autosomal fundamental number and the morphology of autosomal chromosomes found for *Stenocephalemys albipes* and *Mastomys natalensis,* in the present study, showed some differences from what have previously reported for them from different localities; and this can be taken as evident example for the phenomenon that, in rodents, chromosome variation is common within species. For *Stenocephalemys albipes* species chromosome pair 3 was found being subtelocentric type, indicating the existence of third type of polymorphic configuration, in addition to the previously reported submetacentric and telocentric forms. Chromosome pair 4 was also found to be polymorphic for this species.

Species identification is more reliable if a combination of both karyotypic and morphological characters are used as would be evident from the case of *ARSI-X* specimen, which would have been taken as one of the recognized species of *Mastomys* had there been no chromosomal data were available.

7.2. RECOMMENDATIONS

- Combination of morphological and cytogenetic characters are important to make reliable taxonomic identification of most rodent species, particularly cryptic species. Therefore, it is recommended that, as much as possible, studies on rodent taxonomy and systematics should include karyotype analysis.
- To resolve the current taxonomic problems of several genera of Ethiopian rodents, as well as uncover cryptic species, large scales cytological studies are needed.
- For better utilization of chromosome cytological data in taxonomic and systematic studies of rodents, conventional cytogenetic techniques should be supplemented with advanced techniques such as chromosome banding and molecular cytogenetics.
- Further detailed morphological and cytogenetic studies are recommended for *ARSI-X* specimen in order to determine its proper taxonomic status.

8. REFERENCES

Afework Bekele and Corti, M. (1997). Forest blocks and altitude as an indicators of *Myomys albipes* (Ruppell 1842) (Mammalia Rodentia) distributed in Ethiopia. *Tropical Zoology* **10:** 287-293.

Afework Bekele, Capanna, E., Corti, M., Marcus, L.F. and Schlitter, D.A. (1993). Systematics and geographic variation of Ethiopian *Arvicanthis* (Rodentia, Muridae). *J. Zool. Land.* **230:** 117-134.

Afework Bekele, Leirs, A. and Verhagen, R. (2003). Composition of rodents and damage estimates on maize farms at Zeway, Ethiopia. **In:** *Rats, Mice and People: Rodent Biology and Management. ACIAR Monograph* **96:** 262-263.

Affonso, P. R. A. M, Miranda, V. S., Medrado, A. S., Jacobina, U. P, Bitencourt, J. A., Almeida, J. S. and Carneiro, P. L. S. (2007). Chromosomes in focus: basic cytogenetics, light microscopy and the case of Neotropical fish. **In:** *Modern Research and Educational Topics in Microscopy,* pp.370-377 (Méndez-Vilas, A. and Díaz, J. eds). FORMATEX, Bahia, Brazil.

Andersson, A-C. (2004). Post glacial population history of the common shrew (*Sorex araneus*) in Fennoscandia. Molecular studies of recolonization, sex-biased gene flow and formation of chromosome races. **In:** *Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 986.* Acta Universitatis Upsaliensis, Uppsala, Sweden.

Bailey, J.A., Yavor, A.M., Massa, H.F., Trask, B.J. and Eichler, E.E. (2001). Segmental duplications: organization and impact within the current human genome project assembly. *Genome Research* **11:** 1005–1017.

Baskevich, M. I. and Lavrenchenko, L. A. (2000). Review of karyological studies and the problems of systematics of Ethiopian *Arvicanthis* Lesson, 1842 (Rodentia: Muridae). *Bonn. Zool. Monogr.* **46:** 209-215.

Bennett, D. (1965). The karyotype of the mouse, with identification of a translocation. *Proc. Natl. Acad. Sci. U S A.* **53(4):** 730–737.

Bickham, J. W. (1981). Two-hundred-million-year- old chromosomes: Deceleration of the rate of karyotypic evolution in turtles. *Science* **212:** 1291- 1293.

Britton-Davidian, J., Nadeau, J.H., Crosset, H. and Thaler, L. (1989). Genic differentiation and origin of Robertsonian populations of the house mouse (*Mus musculus domesticus* Rutty). *Genetics Research* **53:** 29–44.

Bulatova, N.S. and Lavrenchenko, L.A. (2005). Possible karyological affinities of small mammals from north of Ethiopian plateau. **In:** *African Biodiversity,* pp. 315-319 (Huber, B.A. ed). Springer, Netherlands.

Canby, T.Y. (1997). *The Rat: Lapdog of the Devil*. National Geographic Society, U.S.A.

Capanna, E., Afework Bekele, Capula, M., Castiglia, R., Civitelli, M.V., Codjia, J.T.C., Corti, M. and Fadda, C. (1996). A multidisciplinary approach to the systematics of the genus *Arvicanthis* Lesson, 1842 (Rodentia, Muridae). *Mammalia* **60:** 677-696.

Coluzzi, M. (1982). *Mechanisms of Speciation*. Alan R. Liss Inc., New York, pp 143-153.

Corti, M. and Rohlf, F.J. (2001). Chromosomal speciation and phenotypic evolution in the house mouse. *Biological Journal of the Linnean Society* **73:** 99-112.

Corti, M., Castiglia, R., Colangelo, P., Capanna, E., Beolchini, F., Afework Bekele, Oguge, N. O., Makundi, R. H., Sichilima, A. M., Leirs, H, Verheyen, W. and Verhagen, R. (2005). Cytotaxonomy of rodent species from Ethiopia, Kenya, Tanzania and Zambia. *Belg. J. Zool*. **135(S):** 197-216.

Corti, M., Civitelli, M.V., Castiglia, R., Afework Bekele and Capanna, E. (1996). Cytogenetics of the genus *Arvicanthis* (Rodentia, Muridae). 2. The chromosomes of three species from Ethiopia: *A. abyssinicus, A. dembeensis* and *A. blicki. Z. Saugetierkunde* **61:**339-351.

Crosland, M.W.J. and Crozier, R.H. (1986). *Mymecia pilosula*, an ant with only one pair of chromosomes. *Science* **231:** 1278.

Davis, G. (2002). *African Forest Biodiversity*: A Field Survey Manual for Vertebrates. Earth Watch, Cambridge.

de Villena, F. P-M. (2005)**.** Evolution of the mammalian karyotype**. In:** *Mammalian Genomics*, pp.317-348 (Ruvinsky, A. and Graves, J.A.M. eds). Biddles Ltd, King's Lynn., CABI Publishing, UK.

de Villena, F. P-M. and Sapienza, C. (2001). Female meiosis drives karyotypic evolution in mammals. *Genetics* **159:** 1179–1189.

Delneri, D., Colson, I., Grammenoudi, S., Roberts, I.N., Louis, E.J. and Oliver, S.G. (2003). Engineering evolution to study speciation in yeasts. *Nature* **422:**68-72.

Dobigny, G., Ducroz, J.F., Robinson, T. J. and Volobouev, V. (2004). Cytogenetics and cladistics. *Syst. Biol*. **53(3):** 470-484.

Ducroz, J. F., Granjon, L., Chevret, P., Duplantier, J. M., Lombard, M. and Volobouev, V. (1997). Characterization of two distinct species of *Arvicanthis* (Rodentia, Muridae) in West Africa: cytogenetic, molecular and reproductive evidence. *J. Zool. Lond*. **241:**709-723.

Fadda, C., Castiglia, R., Colangelo, P., Corti, M., Machang'u, R., Makundi, R., Scanzani, A., Tesha, P., Verheyen, W. and Capanna, E. (2001). The rodent fauna of Tanzania: a cytotaxonomic report from the Massai Steppe. *Rend. Fis. Acc. Lincei* **12(9):**29-49.

Ferguson-Smith, M.A. and Trifonov, V. (2007). Mammalian karyotype evolution. *Nature Reviews: Genetics* **8:** 950-962.

Filipski, A. and Kumar, S. (2005). Comparative genomics in eukaryotes. **In:** *The Evolution of the Genome*, pp.521-583 (Gregory, T.R. ed). Elsevier Inc., USA.

Ford, C.E. and Hamerton, J.L. (1956). A colchicine hypotonic citrate squash sequence for mammalian chromosome. *Stain Tech.* **31:** 247-251.

Frankham, R., Ballou, J.D. and Briscoe, D.A. (2004). *A Primer of Conservation Genetics*. Cambridge University Press, USA, New York, pp.101-115.

Futuyma, D. J. (2005). *Evolution*. Sinauer Associates, Inc., Massachusetts, U.S.A.

Geise, L., Canavez, F.C. and Seua´nez, H.N. (1998). Comparative karyology in *Akodon* (Rodentia, Sigmodontinae) from Southwestern Brazil. *J. Heredity* **89:** 158–163.

Goureau, A., Garrigues, A., Tosser-Klopp, G., Lahbib-Mansais, Y., Chardon, P. and Yerle, M. (2001). Conserved synteny and gene order difference between human chromosome 12 and pig chromosome 5. *Cytogenetics and Cell Genetics* **94:** 49–54.

Gozcelioglu, B., Colak, R., Colak, E. and Yigit, N. (2005). A study on *Mus domesticus* Rutty, 1772 and *Mus macedonicus* Petrov and Ruzic, 1983 (Mammalia: Rodentia) distributed along the line of Ankara, Bolu and Zonguldak. *Turk. J. Zool.* **29:** 133-140.

Granjon, L., Duplantier, J.M., Catalan, J. and Britton-Davidian, J. (1997). Systematics of the genus *Mastomys* (Rodentia: Muridae): a review. *Belg. J. Zool.* **127:**7-18.

Granjon, L., Duplantier, J.M., Catalan, J., Britton-Davidian, J. and Bronner, G.N. (1996). Conspecifity of *Mastomys natalensis* (Rodentia, Muridae) from Senegal and South Africa: Evidence from experimental crosses, karyology and biometry. *Mammalia* **60:** 697-706.

Gregory, T.R. (2005).*The Evolution of the Genome*. Elsevier Inc., California, USA.

Griffiths, A.J.F., Miller, J.H., Suzuki, D.T., Lewontin, R.C. and Gelbart, W.M. (1996). *An Introduction to Genetic Analysis*. W.H. Freeman and Company, New York, USA.

Hedrick, P.W. (1981). The establishment of chromosomal variants. *Evolution* **35:** 322–332.

Hsu, T. C. and Patton, J. L. (1969). Bone marrow preparation for chromosome studies. **In:** *Comparative Mammalian Cytogenetics*, pp. 454- 460, (Benirischke, K. ed). Springer-Verlag, New York.

Jara-Seguel, P., Romero-Mieres, M. and Palma-Rojas, C. (2006). Chromosome numbers of Chilean pteridophytes: first contribution. *Gayana Bot.* **63(1):** 115-118.

Johnson, W. E., Eizirik, E., Pecon-Slattery, J., Murphy, W. J., Antunes, A., Teeling, E., and O'Brien, S. J. (2006). The late Miocene radiation of modern Felidae: a genetic assessment. *Science* **311:**73–77.

Kandul, N. P., Lukhtanov, V. A. and Pierce, N. E. (2007). Karyotypic diversity and speciation in *Agrodiaetus* butterflies. *The Society for the Study of Evolution.* 546-559. http://www.blackwell-synergy.com/doi/abs/10.1111/j.1742-4658.2007.00046.x.

Kifle Dagne (1995). Karyotypes, C- banding and nucleolar numbers in *Guzotia* (Compositae). *Pl. Syst. Evol.* **195:** 121-135.

Kifle Dagne and Heneen, W.K. (1992). The karyotype and nucleoli of *Guzotia abyssinica* (Compositae). *Hereditas* **117:** 73-83.

King, M. (1993). *Species Evolution*. *The Role of Chromosome Change*. Cambridge University Press, Cambridge, UK.

Kingdon, J. (1997). *The Kingdom Field Guide to African Mammals.* Natural World Academic Press, San Diego, California, USA.

Kipling, D., Ackford, H.E., Taylor, B.A. and Cooke, H.J. (1991). Mouse minor satellite DNA genetically maps to the centromere and is physically linked to the proximal telomere. *Genomics* **11:** 235–241.

Koop, B. F., Baker, R. J. and Genoways, H. H. (1983). Numerous chromosomal polymorphisms in a natural population of rice rats (Oryzomys: Cricetidae). *Cytogenet. Cell Genet.* **35:**131-135.

Lavrenchenko, L.A., Likhnova, O.P., Baskevich, M.I. and Afework Bekele (1998). Systematic and distribution of *Mastomys* (Muridae, Rodentia) from Ethiopia, with the description of new species. *Z. Saugetierkunde* **63:**37-51.

Lecompte, E., Granjon, L. Peterhans, J.K. and Denys, C. (1997). Cytochrome b-based phylogeny of the *Praomys* group (Rodentia:Murinae): a new African radiation. *Biologies* **325:** 827-840.

Levan, A., Fredga, K. and Sandberg, A. (1964). Nomenclature for centromeric position in chromosomes. *Hereditas* **52:** 201–220.

Levin, D. A. (2002). *The Role of Chromosomal Change in Plant Evolution.* Oxford University Press, Inc., New York.

Murphy, W.J., Stanyon, R. and O'Brien, S.J. (2001). Evolution of mammalian genome organization inferred from comparative gene mapping. *Genome Biol.* **2:**1–8.

Musser, G.G. and Carleton, M.D. (1993). Family Muridae. **In:** *Mammal Species of the World: A Taxonomic and Geographic Reference*, (Wilson, D.E. and Reeder, D.H. eds). Smithsonian Institute Press, Washington D.C.

Musser, G.G. and Carleton, M.D. (2005). Superfamily Muroidea. **In:** *Mammal Species of the World: A Taxonomic and Geographic Reference*, 3rd edition (Wilson, D.E. and Reeder, D.H. eds). John Hopkins University Press, Baltimore, USA.

Nachman, M.W. and Searle, J.B. (1995) Why is the house mouse karyotype so variable? *Trends in Ecology and Evolution* **10:** 397–402.

Nachman, M.W., Boyer, S.N., Searle, J.B. and Aquadro, C.F. (1994). Mitochondrial DNA variation and the evolution of Robertsonian chromosomal races of house mouse, *Mus musculus*. *Genetics* **136:** 1105–1120.

Noor, M. A. F., Grams, K. L., Bertucci, L. A. and Reiland, J. (2001). Chromosomal inversions and the reproductive isolation of species. *Proc. Natl. Acad. Sci*. **98:**12084–12088.

Nowak, R.M. (1999). *Walker's Mammals of the World*, 6th ed., Vol. II. John Hopkins University Press, Baltimore and London.

O'Brien, S.J., Menotti-Raymond, M., Murphy, W.J., Nash, W.G., Wienberg, J., Stanyon, R., Copeland, N.G., Jenkins, N.A., Womack, J.E. and Graves, J.A.M. (1999). The promise of comparative genomics in mammals. *Science* **286:** 458–481.

O'Neill, R.J., Ferreri, G.C. and O'Neill, M.J. (2005). Elements and mechanisms of genome change. **In:** *Mammalian Genomics*, pp. 279-299 (Ruvinsky, A. and Graves, J.A.M. eds). Biddles Ltd, King's Lynn., CABI Publishing, UK.

Ortells, M. O., Reig , O. A., Brum-Zorrilla, N. and Scaglia, O. A. (1988). Cytogenetics and karyosystematics of phyliotine rodents (Cricetidae, Sigmodontinae). I. Chromosome multiformity and gonosomai-autosomal translocations in *Reithrodon. Genetica* **77:** 53-63.

Otto, S.P. & Whitton, J. (2000). Polyploid incidence and evolution. *Annual Review of Genetics* **34:** 401–437.

Purvis, A. and Hector, A. (2000). Getting measure of biodiversity. *Nature,* **405:**212–219.

Qumsyieh, M.B. (1994). Evolution of number and morphology of mammalian chromosomes. *Journal of Heredity* **85:** 455–465.

Richard, F., Messaoudi, C., Bonnet-Garnie, A., Lombard, M. and Dutrillaux, B. (2003). Highly conserved chromosomes in an Asian squirrel (*Menetes bermorei*, Rodentia: Sciuridae) as demonstrated by ZOOFISH with human probes. *Chromosome Research* **11:**597–603.

Rieseberg, L. H. (2001). Chromosomal rearrangements and speciation. *Tr. Ecol. Evol.* **16:**351-358.

Serov, O.L., Chowdhary, B., Womack, J.E. and Graves, J.A.M. (2005). Comparative gene mapping, chromosome painting and the reconstruction of the ancestral mammalian karyotype. **In:** *Mammalian Genomics,* pp.349-392 (Ruvinsky, A. and Graves, J.M. eds)**.** Biddles Ltd, King's Lynn., CABI Publishing, UK.

Singleton, G.R., Hinds, L.A., Leirs, H. and Zhang, Z. (1999). *Ecologically Based Management of Rodent Pes*t. Australian Center for International Agricultural Research, No.59, Canberra, Australia.

Stace, C. A. (2000). Cytology and cytogenetics as a fundamental taxonomic resource for the 20th and 21st centuries. *Taxon* **49:** 451-477.

Stanyon, R., Stone, G., Garcia, M. and Froenicke, L. (2003). Reciprocal painting shows that squirrel, unlike murid rodents, have highly conserved genome organization. *Genomics* **82:** 245–249.

Stanyon, R., Yang, F., Cavagna, P., O'Brien, P.C.M., Bagga, M., Ferguson-Smith, M.A. and Weinberg, J. (1999). Reciprocal painting shows that genomic rearrangements between rat and mouse proceeds ten times faster than between humans and cats. *Cytogenetics and Cell Genetics* **84:** 150–155.

Stebbins, G.L. (1971). *Chromosomal Evolution in Higher Plants*. Edward Arnold Publishers Ltd., London.

Sumner, A.T. (1990). *Chromosome Banding*. Unwin Hyman, London.

Sumner, A.T. (2003). *Chromosomes Organization and Function*. Blackwell Science Ltd, Blackwell Publishing Company, UK.

Tadesse Habitamu (2005). *The Study of Diversity, Distribution, Relative Abundance and Habitat Association of Small Mammals* in Alatish Proposed National Park, North Ethiopia. M. Sc. Thesis, Addis Ababa University, Addis Ababa.

Tesfaye Dilebo (2009). *Chromosome Study of Some Ethiopian Rodent Species* from Southern Nations, Nationalities and Peoples Region. M. Sc. Thesis, Addis Ababa University, Biology Department, Addis Ababa.

Trifonov, V.A., Perelman, P.L., Kawada, S.I., Iwasa, M.A., Oda, S.I. and Graphodatsky, A. S. (2002). Complex structure of B-chromosomes in two mammalian species: *Apodemus peninsulae* (Rodentia) and *Nyctereutes procyonoides* (Carnivora). *Chromosome Research* **10:**109–116.

Vaughan, T.A. Rayan, J.M. and Czaplewiski, N.J. (2000). *Mammalogy*, 4th ed., Saunders College Publishing, Philadelphia.

Volobouev, V., Aniskin, V., Lecompte, E. and Ducroz, J. F. (2002). Patterns of karyotype evolution in complexes of sibling species within three genera of African murid rodents inferred from the comparison of cytogenetic and molecular data. *Cytogenet. Genome Res.* **96:**261- 275.

White, M.J.D. (1973) *Animal Cytology and Evolution*. Cambridge University Press, Cambridge, UK.

White, M.J.D. (1978). *Modes of Speciation*. CA: W.H. Freeman and Co, San Francisco.

Workneh Gebreslassie, Afework Bekele, Gurja Belay and Balakrishnan, M. (2004). Microhabitat choice and diet of rodents in Maynugus irrigation field, northern Ethiopia. (2004). *Afr. J. Ecol.* **42:** 315-321.

Yalden, D.W. and Largen, M.J. (1992). The endemic mammals of Ethiopia. *Mammal Review* **22:**115-150.

Yang, F., Obrien, P. C. M., Wienbberg, J., Neitzel, H., Lin, C. C. and Ferguson-Smith, M. A. (1997). Chromosomal evolution of the Chinese muntjac (*Muntiacus reevesi*). *Chromosoma* **106:** 37–43.

Yigit, N., Colak, E., Sozen, M. and Ozkurt, S. (1998). The taxonomy and karyology of *Rattus norvegicus* (Berkenhout, 1769) and *Rattus rattus* (Linnaeus, 1758), in Turkey. *Tr. J. Zool.* **22:** 203-212.

Yigit, N., Gharkheloo, M. M., Colak, E., Ozkurt, S., Bulut, S., Kankilic, T. and Colak, R. (2006). The karyotypes of some rodent species (Mammalia: Rodentia) from Eastern Turkey and Northern Iran with a new record, *Microtus schidlovskii* Argyropulo, 1933, from Eastern Turkey. *Turk. J. Zool.* **30:** 459-464.